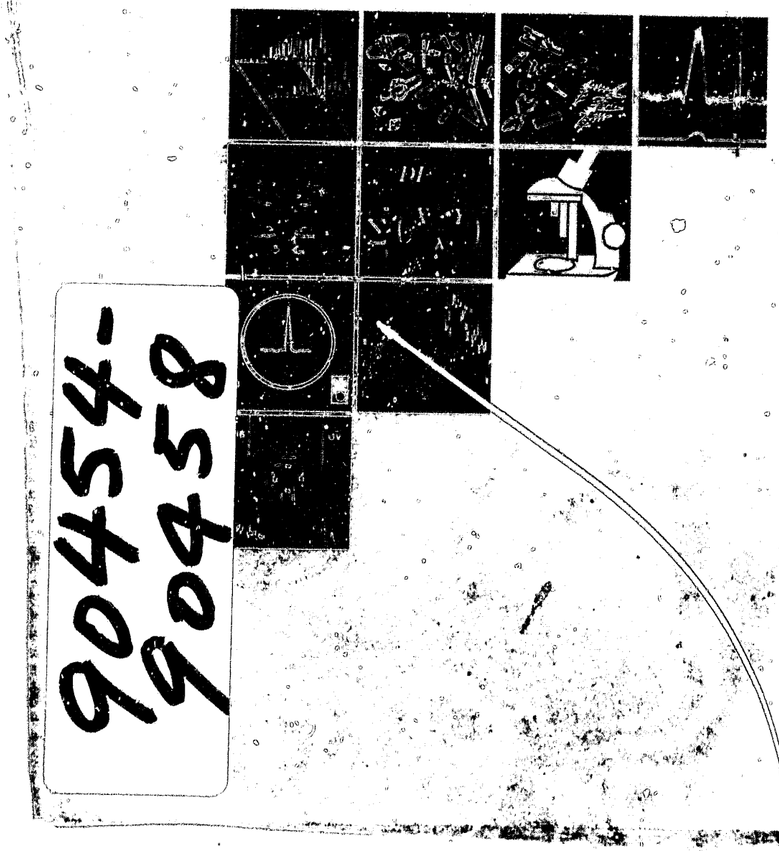
Instrumental Applications in Forensic Drug Chemistry

United States Department of Justice
Drug Enforcement Administration
Office of Science and Technology
Forensic Sciences Division
Special Testing and Research Laboratory

Proceedings of the International Symposium, May 29-30, 1978



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Edited by:

Michael Klein
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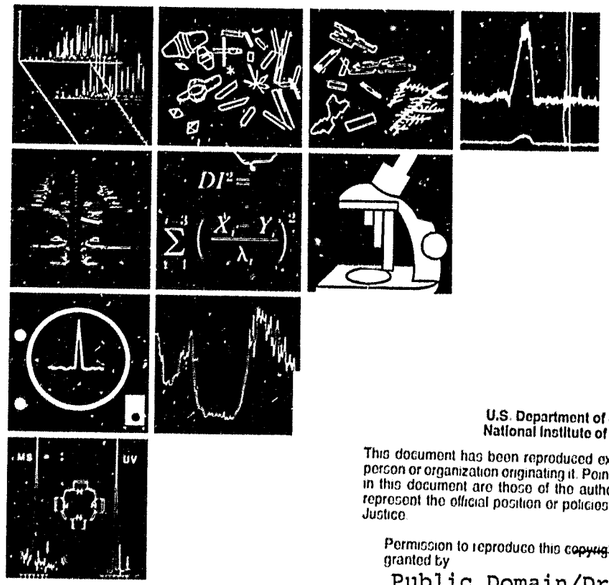
Stanley P. Sobol



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Letter From the Administrator

The Drug Enforcement Administration hosted a two-day International Symposium on Instrumental Applications in Forensic Drug Chemistry in Arlington, Virginia on May 29 and 30, 1978. The Symposium focused on the increasing reliance of the forensic scientist on modern instrumentation and computers which make possible the rapid accumulation of accurate data.

The Symposium consisted of presentations from internationally known invited speakers who covered current methodology and approaches, and attempted to project future needs and developments.

The purpose of the Symposium was to provide significant contributions to the forensic sciences in the field of Forensic Drug Chemistry, highlighting advances in instrumental and computer applications to drug analysis. Leading scientists in both Europe and the USA presented papers to an international audience of scientists from government, university, and law enforcement laboratories.

The Drug Enforcement Administration was honored to be able to sponsor this Symposium and provide a forum for the sharing of scientific information which can be applied to forensic work on a worldwide scale. This Symposium should expand knowledge in the forensic sciences and support international law enforcement.

This volume presents the papers at the International Symposium. By publishing the proceedings, it is our hope that the widest possible international audience will be reached. Thus, it will contribute to one of DEA's primary responsibilities—

> "coordination and cooperation with other Federal, State, and local agencies and foreign governments in programs designed to the training of foreign officials, and the encouragement of knowledge and commitment against drug abuse."

I wish to express my appreciation to members of the DEA staff who were involved in preparing this Symposium. Also, a warm word of praise and thanks is offered to those most responsible for the success of the Symposium—the speakers. The interest they aroused is a testimony to their professional competence.

Peter B. Bensinger
Administrator
Drug Enforcement Administration

Foreword

The Drug Enforcement Administration of the U.S. Department of Justice was created in 1973 by Presidential reorganization to enforce the U.S. Drug Laws and to bring to justice those organizations and principal members of organizations involved in illicit drug activities. DEA has recognized science and technology as an integral part of its law enforcement efforts. Furthermore, our policy from the beginning has been to share our knowledge with our colleagues in police agencies and laboratories on an international basis. The Symposium and its proceedings were one more attempt by DEA to provide a means to share scientific information which will help meet not only today's needs but also to address advances in new instrumental techniques to help solve future problems.

The 1973 reorganization which led to the formation of DEA combined the function and assets of the Bureau of Narcotics and Dangerous Drugs, the narcotic enforcement segment of the U.S. Customs Service, the Office of Drug Abuse Law Enforcement (ODALE), the Office of National Narcotics Intelligence (ONNI) and a segment of the White House Office of Science and Technology.

We are concerned with illicit activities which involve the cultivation, manufacture, or distribution of drugs appearing in or destined for the U.S. illicit market. DEA also supports non-enforcement programs aimed at combating the drug traffic at home and abroad.

To accomplish this mission we have a total of 4,200 employees. Half of these employees are criminal investigators who carry the title "Special Agent". As of October 1, 1978 we will consolidate our present 12 domestic regions into 5 regions. Overseas we have 3 regions, with 64 offices in 39 countries manned by 200 Special Agents.

Control of narcotics and dangerous drugs is an international and multifaceted endeavor and requires not only the full efforts of DEA, but the cooperation of many agencies including our foreign police colleagues, the United Nations, Interpol, Department of State, U.S. Customs Service, Federal Bureau of Investigation, Immigration and Naturalization Service, Internal Revenue Service, U.S. Coast Guard, and State, County and Local Police.

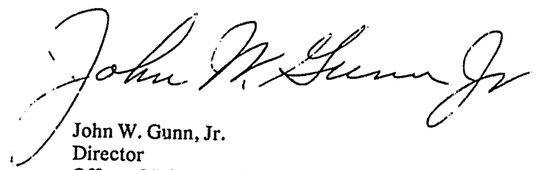
Besides investigative and enforcement efforts, drug control requires the application of regulatory efforts and the effective use of science and technology. In our Office of Science and Technology we have a staff of over 250, with specialists in the fields of chemistry, mathematics, engineering, electronics, psychology and other disciplines.

The office has three divisions. The Research and Engineering Division conducts feasibility studies on various aspects of technology, produces actual equipment for use by our Special Agents in the field and conducts behavioral and social research such as the study of the effects of drugs on crime.

The Technical Operation Division manages the National UHF Radio System, the Single Side Band Radio System, the National Secure Teletype System and the investigative equipment program.

Our eight forensic laboratories are managed by the Forensic Sciences Division. As in other crime labs, DEA labs examine evidence and present evidence in court when necessary. They also conduct research, method development, training of other forensic chemists and distribute various scientific and technical publications.

I want to personally thank each of the Symposium participants for contributing to the Symposium and helping us provide a worthwhile scientific sharing experience. It takes many people to stage an event such as this, and I would like to recognize some of these now for their outstanding work: Drs. Michael Klein and Alice V. Kruegel the individuals who first proposed the idea of holding the Symposium. I would also like to thank Clayton McNeill, Irene Armstrong, Lani Hidalgo and, of course, Stanley P. Sobol, the General Chairman.



John W. Gunn, Jr.
Director
Office of Science and Technology
Drug Enforcement Administration

Preface

The papers collected in this volume were presented at the International Symposium on Instrumental Applications in Forensic Drug Chemistry held in Arlington, Virginia on May 29 and 30, 1978.

The Symposium consisted of four sessions: Spectroscopy, Computer Applications, Chromatographic Advances, and Special Topics. It covered current methodology and approaches and also projected future needs and developments.

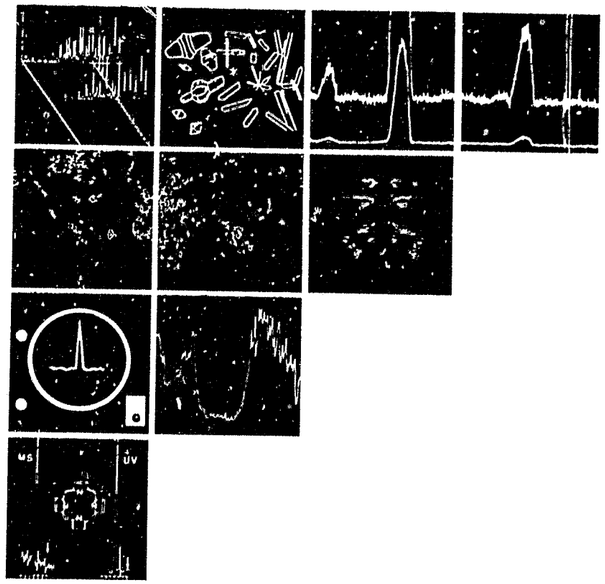
The Symposium presented 24 experts from 8 countries highlighting current state-of-the-art of instrumental advances encompassing spectrometry, computers, chromatography, and such topics as drug standards, scanning electron and light microscopy, immunoassays and toxicology. We heard review papers in mass spectrometry and chemical identification processes. We examined the advantages of using stable isotopes in the quantification of drugs by GC-MS-COM systems, as well as the current interest in negative ion mass spectrometry. Techniques of FT-IR and NMR were discussed, and their possible future applications to the forensic sciences. The use of mass spectral computer systems in drug identification, and some computer programs in Federal, State and Overseas facilities were described.

Many and varied applications of high pressure liquid chromatography and gas chromatography, as well as improvements and capabilities of the instruments used in these techniques were covered. Also, derivatization techniques, detection systems and a new approach to the optimization of chromatographic systems were discussed.

We wish to express our appreciation to the staff of the Drug Enforcement Administration Special Testing and Research Laboratory, Office of Science and Technology and Administrative Services for their contributions to the Symposium and publication of the proceedings. It was the consensus of the participants and attendants that forensic drug chemistry is a unique, continually developing and intellectually stimulating applied science. We hope that those who read the following manuscripts will agree.

The Editors
Washington, D.C.

U.S. International Narcotics Policy



U.S. International Narcotics Policy

by

Charles O'Keeffe

The White House
Washington, D.C.

It is a pleasure to be here today to talk about the international narcotics policy of the United States. Before I move specifically to our international policy, let me say that this Administration has two broad policy objectives in the field of drug abuse prevention and control: first, to reduce the health and social consequences of drug abuse; and second, to reduce all illicit consumption of psychoactive drugs on the assumption that, regardless of the presence or absence of health hazards, the interests of society are not served by the widespread use of illicit drugs.

Drug abuse is a reality of modern life, and we cannot promise to make it disappear. In the United States in 1978, drug abuse has evolved from an epidemic to a chronic problem affecting every segment of our society.

There are three important arenas for Federal action to deal with the drug abuse phenomenon as it exists today.

The first is the world of international agriculture, economics, and human needs—ancient traditions often colliding abruptly with modern values and crises.

The second is the world of crime and criminals, people who build networks of illicit traffic to reap huge profits and thwart the laws and customs of many societies.

And the third is the world of millions of people, young people and working people, affluent and poor, for whom life is a complex of frustrations too often compounded by easily available drugs.

These worlds seem remote and unrelated to the daily lives of most Americans, and that is an unfortunate misconception. The quality of our lives and the promise of our future is affected by what goes on in a Burmese village, a Mexican border town, a clinic in Portland, a shooting gallery in New York, a schoolroom in Atlanta, and a drugstore in Los Angeles.

The programs that we have in these three arenas—our international narcotics control program, domestic drug law enforcement, and domestic treatment and prevention efforts, are the three important components of our entire drug abuse prevention and control program. Each is important, and the international program is especially so, as it contributes to this Administration's emphasis on international cooperation to address the problems of basic human needs worldwide.

I'd like to take just a few minutes to describe our international program which has four major parts: 1. efforts to reduce supplies of illegal narcotic source materials worldwide; 2. cooperation with international organizations in a variety of endeavors; 3. U.S. cooperation with foreign narcotics enforcement agencies; and, 4. international drug abuse prevention initiatives.

The U.S. role in reducing supplies of illegal narcotics source materials is complicated, and we hope will produce long-term lasting results.

The plants that produce some of the drugs we are most concerned with (heroin, cocaine, and to a large degree marijuana) generally grow outside of U.S. borders. The laboratories for processing drugs are likewise beyond our boundaries. The flow of drugs into this country passes through many nations, and increasingly, the people of other countries are experiencing drug problems of their own.

There are ten countries in the world which we view as top priority targets for diplomatic, economic, and technical initiatives designed to reduce the cultivation and production of drugs. More than a hundred other nations figure significantly in our international drug initiatives, but these ten are the major sources of "problem drugs" and we intend to concentrate our energies there:

- **Burma** produces some 400 tons of opium every year.
- **Thailand** and **Laos** each grow about 50 tons of opium a year.
- **Mexico** grows about 40 tons of opium, which is converted to about 4 tons of heroin, most of which makes its way into the United States.
- The **Afghanistan-Pakistan** region of South Asia is now the world's largest producer of illicit opium with production far in excess of 500 tons annually.
- **Bolivia** and **Peru** have the potential to produce over 50,000 tons of coca leaf, making 15–20 tons of cocaine available to the illict U.S. market in the course of a year.
- **Ecuador** and **Colombia** are major processing and trafficking countries for the cocaine flow to the United States.

In most of these countries, the growth and consumption of opium or coca leaves is an old tradition, an integral part of the culture and economics of the nation. Only Mexico, among the opium-growing nations, has no history of opium use. For the poor farmers of the remote regions of these countries these crops are the *only* cash crops they know, providing them with a bare livelihood.

The United States supports a broad range of approaches in partnership with other countries and multilateral institutions. Of these approaches, crop eradication has proven to be the most successful way to reduce bulk cultivation of illegal drugs. Eradication is unfortunately only appropriate in some countries, and in every instance must be a cooperative effort, supported by the host government.

Mexico provides an example of a successful eradication program. Opium is not an indigenous crop in Mexico; it is not part of Mexican history or culture. In 1972 Turkey effectively banned opium cultivation, and criminal entrepreneurs had turned to Mexico to plant and process their poppies. This action concerned both the governments of Mexico and the United States. The Mexicans were concerned for two reasons. First, the vast profits in the illegal enterprise were undermining their legitimate market economy in favor of a drug-based economy. Second, the Mexican government was fearful that, given a ready supply of heroin, use would spread in Mexico with enormous health and social consequences. The United States was concerned over the amount of heroin flooding across the 2,000-mile border, bringing violence, crime and death to our cities.

The opium eradication program was started in late 1973 as a cooperative U.S.–Mexican effort. The U.S. provided technical assistance and equipment for the opium eradication. The program slowly began to have results. In 1975, almost 1,800 people died of heroin overdose. Two years later in 1977, 584 died, and this year, if projections bear out, only about 400 people will die, a decrease since 1975 of 78%, and a prevention of 1,400 deaths. Heroin availability today is at the lowest level in seven years, with a national average retail purity of 5% and a price of $1.65 per milligram pure.

But the program remains centrally and operationally a Mexican government initiative. In matters of *how* the government carries out its crop eradication programs, U.S. policy will be to encourage effective alternatives where we find a trouble spot and continue to support the primary role of the Mexican authorities in dealing with that country's illicit drug production.

Crop eradication is only the first phase of a

program to reduce supplies of illicit drugs at their source. It must be followed by efforts to provide alternative sources of income for the farmers who are engaged in cultivating illicit drugs.

Rural development is a long-term strategy to reduce the cultivation of illegal drugs, but it is likely to have lasting results. Farmers will not stop growing opium or coca until they have an alternate income source to support themselves and their families.

This is a difficult and long-range proposition, far more than simply telling a farmer, "Yesterday you grew poppies, tomorrow it will be lentils." The growers are generally cut off from central governments; agriculture is often primitive; lines of communication and transportation are much more secure between the individual farmer and the buyer of his crop than between the farmer and the government. And the tradition of growing and using both the poppy and coca is an ancient one in many areas, and the use of these plant-drugs is integrated into local culture and medicine.

Instead of single-crop substitution efforts, we are attempting to work out rural and agricultural development programs for the regions of the countries where the crops are produced. This will have to include attention to such matters as land-use planning; irrigation and harvesting; transportation; economic projections; political liaison between and among the United States, the host government, and the individual farmer and his community.

Programs of this scope and complexity will require the full-scale cooperation of the host government, various international agencies, and our government. Some modest experiments are already underway in selected countries:

- Income and crop substitution projects in Bolivia and Pakistan are being assisted by the Department of State.
- The United Nations Fund for Drug Abuse Control has projects underway in Thailand and Pakistan, and is developing one in Burma.
- U.S. Department of Agriculture research projects are underway in Thailand and Pakistan.

The U.S. Government is prepared to provide funds and technical assistance for some of these and other well-designed projects in rural development. But the initiative for carrying out the long-term economic renaissance in remote farming regions must come from the host government and the people of the country. Coordinated development assistance is one way we can help put these forces in motion.

The difficulty in carrying out crop substitution programs is sharply drawn in analyses prepared by the leading international financial institutions. They point to high incomes from poppy cultivation; margins of profit which will allow the price of opium to go up without disrupting flow; and the political isolation of many of the poppy-growing regions.

Nevertheless, U.S. policy is to encourage rural development loans to drug-producing countries, and to influence the actions of global financial agencies in their lending. Institutions like the World Bank, International Monetary Fund, and the Regional Development Banks all have an important role to play in strengthening the economies of countries where new crops might be substituted for narcotic-producing crops.

It is very important to remember that the primary responsibility for these efforts to reduce the supply of illegal drugs rests with the governments of other countries. We are asking our allies and neighbors to join us as partners, and our role thus becomes one of assisting and supporting the leadership those governments show. Drug abuse and the drug problem is not just an "American disease" as it has been styled; but there is a world of difference between our perceptions of heroin, for example, and those of a Thai farmer or a Mexican campesino. Our strategy will be to respect those differences, close the gap, and seek international cooperation. In summary, we have three strategies to reduce the supply of illegal narcotic drugs: eradication, rural development, and international cooperation.

The United States has long advocated multinational cooperation to solve truly global problems. In no area is this emphasis more important, or more urgent, than in health and drug abuse prevention, treatment, rehabilitation, and control. While there are many immediate gains from one-to-one partnerships with other countries, as with crop eradication or income substitution programs, the long-term resolution to international drug problems requires systematic, shared collaboration with many partners—and world organizations are the proper forum for these efforts.

The United States is a participant in nearly a dozen international organizations, groups and networks dedicated to world health, narcotics control, and human needs. These include the U.N. family of agencies that deal with drug abuse or health issues: Interpol for the sharing of enforcement information, the Customs Cooperation Council for the sharing of smuggling information and a variety of regional and interregional collaborations.

Our cooperation with foreign narcotics agencies is another major part of our international narcotics program.

By 1978, the United States will have been involved in cooperative overseas narcotics enforcement assistance for 66 years, dating from the Hague Opium Convention of 1912. This country has tried to provide two kinds of assistance: first, to cooperate with governments in identifying and shutting down major producers and traffickers of illegal drugs; and second, to strengthen the enforcement capabilities of host country police and narcotics-related agencies. We have long believed that successful control of trafficking and use of illicit drugs in this country depends on the cooperation and assistance of other countries. Our strategy is to emphasize efforts to apprehend traffickers and reduce drug supplies as close to their base of operation and place of origin as possible. This strategy maximizes our effectiveness by identifying bulk traffickers and large amounts of drugs before they are dispersed in a more diffused distribution system. There is continuing evidence that this strategy works, such as the 1,123 pounds of heroin seized domestically vs. 2,695 seized overseas by foreign officials, and the recent seizure of 674 tons of marijuana near an airport in Colombia.

Our role in overseas enforcement is changing, as is our perception of the comprehensive mission of cultural, economic, and technical planning in assisting victim countries deal with drugs. In 1976, the "Mansfield Amendment" specifically prohibited involvement of U.S. law enforcement officers in direct police actions or arrests in foreign countries. This has meant a continual evolution in the role of the U.S. agencies assigned to strengthen foreign enforcement.

There are several activities in which U.S. agencies are now involved in support of international narcotics enforcement. Intelligence is one of the most critical activities.

The Federal Government cannot carry out an effective international narcotics program without adequate intelligence about producers, crop production, financing, trafficking and the other elements of the networks involved in drug distribution. But the government is equally concerned that the intelligence-

gathering functions be performed sensitively, thoughtfully, and with specific purpose. This Administration will emphasize the importance of evaluating and sharing sensitive and timely information which helps to support policy and pinpoint targets for enforcement.

Although U.S. enforcement personnel take no active part in foreign police actions, U.S. agents overseas do carry out undercover and other intelligence-gathering activities where force will not be a factor. They handle and develop informants, evaluate intelligence generated by several sources, and cooperate with foreign police and enforcement agencies in handling special surveillance assignments.

The flow of illicit drugs into the United States has been significantly reduced as a result of U.S. assistance to foreign law enforcement agencies. Other governments are as interested as we are in shutting down criminal networks and conspiracies and preventing the distortion of their economies by huge illegal profits. Our participation means that they will be able to multiply the impact of limited enforcement personnel and resources. In Thailand and Ecuador, for example, U.S. Customs narcotics advisors are helping the local governments improve border control procedures. This will mean better enforcement of anti-smuggling laws at the border, and improved overall customs procedures in those countries —which translates into more revenue for the host countries and more resources available for narcotics control.

In general, U.S. Government personnel assist foreign law enforcement agencies with support services aimed at identifying and stopping criminal networks and major narcotics violators as close to their base of operations as possible.

Training is also an important part of our assistance to foreign enforcement.

The immediate goal for these training projects is to strengthen the capabilities of host country police institutions and build a self-development potential in their enforcement systems.

In a single year, 272 foreign enforcement officers from approximately 50 countries came to the United States for narcotics enforcement training. The course work ranged from management and supervisory techniques, to forensic chemistry for lab technicians who examine drugs as evidence, to the use of dogs to detect narcotics in shipments of cargo.

Abroad, the Drug Enforcement Administration concentrates on joint training with officers from the host country, offering work in narcotics intelligence, specialized subjects, and manpower development. Customs foreign training emphasizes border control procedures and techniques, search and seizure approaches, and drug concealment. In a year, the two programs combined reached 1,794 foreign narcotics officers.

Finally, there is the important area of financial disclosures and compliance.

Narcotics production and trafficking is a multi-billion dollar industry. Enormous sums of money are routinely generated by the sale of illicit drugs. The economies of many small countries are seriously distorted by the ebb and flow of illicit drug-related funds; the economies of many urban centers in the United States and other large countries are seriously weakened by drug profiteering.

A first initiative in this area is the enforcement of the provisions of the Bank Secrecy Act, which enable U.S. officials to monitor more closely the transactions in foreign banks that might be serving as cover for large narcotics financing and profiteering.

U.S. agencies can, and do, share narcotics financial information between and among themselves. DEA, IRS, Customs, and the Office of Law Enforcement in the Department of the Treasury all collect and monitor certain kinds of financial information, dealing with currency transactions and apparent violations of financial records-keeping requirements. The agencies cooperate as appropriate in the sharing and collaborative analysis of this information. A special financial intelligence working group has been formed by the Strategy Council to facilitate this cooperation.

There are a number of ways the U.S. can exchange financial data with foreign countries relative to narcotics trafficking. Mutual Assistance Treaties between nations allow sharing of data and information to help enforce the domestic laws of each country. Similar income tax treaties allow exchanges of tax data. The Single Convention on Narcotic Drugs of 1961 allows governments to exchange financial data on traffickers. And a United Nations Resolution in 1976 urged all governments to make narcotics financing a crime, and to exchange information on all such criminals.

The Government of the United States can underscore the importance of financial disclosures by aggressively applying these statutes and provisions, and by seeking full penalties for violations.

The final part of our international policy involves cooperation in drug abuse prevention initiatives.

Americans are not the only people in the world who have a drug problem. Misuse and abuse of licit and illicit drugs have made serious inroads in the health of several nations, particularly countries with rapidly changing social customs or emerging technological/industrial crises. There are few, if any, areas of the world that have managed to escape involvement with the drug problem.

It was once argued that the United States had no role, and no business, in trying to deal with addiction and abuse outside our own borders. We could not, it was claimed, intervene in treatment or prevention in other countries, on at least two bases: (1) since we had not "solved" our drug problem, we had no right to tell others what to do; and (2) we needed all our money and resources to apply to domestic programs and approaches. A third factor was international indifference to U.S. assistance; many governments categorically denied they had a drug problem in the first place. (Some still do.)

But the world has changed in the last several years. Abroad, the leaders of many nations have identified their own drug problems. At home, we have begun to realize the importance of trying to cope with drug misuse and abuse wherever it appears, and of addressing the global realities of an international crisis.

There are several important reasons for U.S. Government support of global initiatives to treat and prevent drug misuse and abuse:

a. Millions of people around the world are suffering from drug involvement, and we cannot ignore the health and stability of the world's people.

b. Our help with another nation's drug problem can lead to that country's participation in broader programs of international narcotics control.

c. A viable and consistent U.S. foreign policy is jeopardized by erosion of the quality of life in other countries—and serious drug abuse clearly erodes that quality.

d. The continued presence of a market for illicit drugs in any country confounds our attempts to reduce or eliminate production; the illegal flow of drugs will cross any border and follow any flag.

These and other factors encourage us to participate in international drug abuse programs. Our goal is to assist host governments in identifying and carrying out useful prevention and treatment programs, in the context of overall social health policy for each nation with which we are associated.

In summary, the international programs of the United States Government will, more and more, reflect our emphasis on developing a host country's own capacity—and motivation —to take effective action against narcotics production and trafficking.

In rural and economic development programs, our intention is to encourage narcotics-producing nations to take the initiative in planning and financing the agricultural and cultural transition from narcotics to non-narcotics as principal cash crops.

In prevention and treatment, our goal is to strengthen what the host countries themselves are prepared to do to begin to bring about the lifestyle changes that will alter the ways drugs affect the people of the country.

In the multi-national arena of global organizations, the U.S. will play an active participatory role, not seeking to dominate the agenda of any organization but seeking, instead, to contribute to all meaningful international efforts.

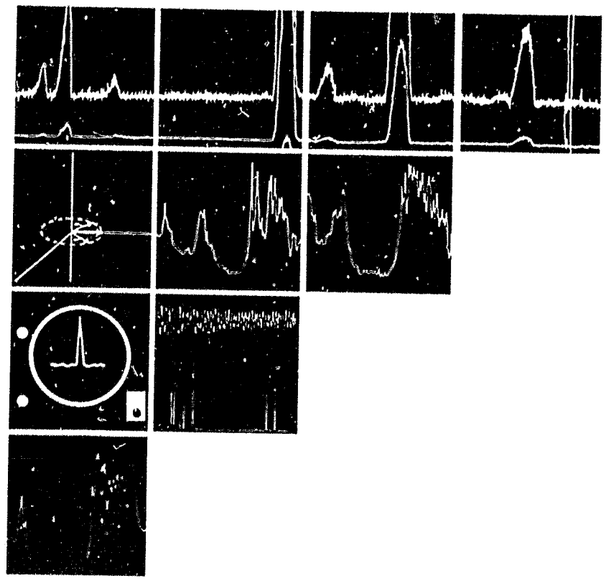
And in cooperation with narcotics enforcement agencies abroad, U.S. personnel and resources will be used to buttress what host country police and enforcement agencies are already doing, and to strengthen the country's capacity to deal with its own enforcement priorities.

We will emphasize the importance of the world arena as a critical threshold in our attempts to deal with illegal drugs, and cut down the flow of drugs to our streets. We depend on mutual respect and cooperation for all our overseas drug initiatives, and we place high priority on continuing to build trust, confidence, and support for this international endeavor.

Spectroscopy Session

I. Mass Spectrometry

II. Infrared and Nuclear Magnetic Resonance



Development of Mass Spectrometry as a Tool in Forensic Drug Analysis—Review

by
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I. INTRODUCTION

A. General

Although this Symposium is organized according to the individual disciplines—*Spectrometry, Computer Applications, Chromatographic Advances,* and *Special Topics*—these categories are, in fact, interrelated. Development of improved analytical techniques in the determination of potential or established "drugs of abuse" requires the fast, accurate and precise, highly sensitive and specific, qualitative and quantitative determination of increasingly lower levels of drugs, drug impurities and drug metabolites. The compound being analyzed may be in the medium of the drug sample itself, or in extracts of biological fluids (blood, urine, sweat, saliva, milk, cerebrospinal and synovial) from individuals who have received the drug. Optimum utilization of the spectrometric analysis may be dependent upon interactive computer systems, chromatographic analysis, and chemical techniques, e.g., derivatization and extraction procedures.

By introduction of compounds eluting from a gas chromatographic (GC) column into the mass spectrometer (MS), spectral data collected on each peak makes possible their positive identifications. GC is the most suitable method currently available for resolving into individual components the highly complex mixtures of compounds encountered in drug or biological specimens. (57) Of course, recent articles describing the development of the liquid chromatograph (LC-MS) interface point out advantages over GC-MS in the analysis of thermally unstable molecules. (9) (28) Development of sophisticated instrumentation for GC and MS has made it possible to use these tools separately or in conjunction with a computer (COMP). The methodology can be improved by the incorporation of digital computers into the system to allow automatic collection and analysis of mass spectra. (4)

The detection by GC-MS-COMP of specific compounds, whether introduced exogenously or arising from a known disease state, has been developed to the point such that the system is relied upon as a routine method in the laboratory. The advantages of speed, reliability, and dependability of this technique for the analysis of volatile and non-volatile materials will be emphasized in this review.

Most common instrumental methods do not provide the necessary levels of sensitivity, as well as specificity, for the identification and quantification of drug components. Fluorometry, radioimmunoassays, thin layer chromatography (TLC) and many spectrophotometric methods often may not provide the adequate sensitivity and/or necessary specificity. When high pressure liquid chromatography (HPLC) is limited because of low molar absorptivity of a chromophore group with ultraviolet (UV) detection, the required sensitivity for analysis would be lacking. The hazards and ethical responsibilities associated with measurement of radiolabeled drugs limit this application. Colorimetric procedures provide adequate sensitivity, but lack the specificity of mass spectrometric analysis. (69)

Even gas chromatographic methods with the flame ionization detector (FID) seldom meet the sensitivity requirements. The maximum usable sensitivity of GC (FID) would be around 0.05 μg with biological samples. Under these conditions, the flame tends to be somewhat noisy, however, and interfering peaks limit sensitivity. In addition, peaks of interest may be masked (or only partially resolved) by biological components present in the sample. (49)

Nitrogen detectors or electron capture detectors (ECD) meet sensitivity requirements, but adequate chromatographic separation of structurally similar drugs is difficult. Another disadvantage of ECD-GC is that no structural information would be associated with a response, and slight differences in conditions may effect that response. These disadvantages have led in the past to mistakes both in identity and quantification of material. (31)

Even mass fragmentographic methods cause problems when ions of sufficient intensity but of low specificity are measured, i.e., when the most intense fragment ions are of low mass or formed from structurally similar molecules. However, the advantage of using GC-MS assays with chemical ionization (CI) is that the most characteristic ion of the compound, i.e., the parent ion, would be available for quantification. (70)

Only the GC-MS method has been shown overall to be sensitive and specific enough for monitoring concentrations of various drugs in whole blood or other media, even after a single oral dose of 50 or even 25 mg. (15) In addition, since serum constituents can interfere with many photometric determinations, often quite tedious extraction procedures are necessary to minimize these effects. By using mass fragmentography, losses in extraction or chromatographic absorption can be allowed for by use of the appropriate deuterium-labeled standard. (5)

B. Applications of Mass Spectrometry in Forensic Drug Analysis

In forensic analysis, several specific examples can be cited to illustrate modern applications of mass spectrometry in the laboratory. These include the following:

1. Identification of the Illicit Drug Sample

a. Routine analysis of samples of drugs, diluents, and other major constituents. GC-MS techniques are increasingly used as a tool for drug identification. Standard techniques for the analysis of street drugs are still based upon infrared (IR) and UV in conjunction with specific chemical tests. These methods often do not provide conclusive results in analyses of drug mixtures. Methaqualone, phendimetrazine and phenmetrazine found by GC-MS in a confiscated illicit street drug was discussed. (7) The incorrect identification of phendimetrazine and phenmetrazine, possible by other techniques, could be dangerous due to the toxic nature of the latter.

b. Drugs difficult to analyze. Lysergic acid diethylamide (LSD) is generally considered to be one of the more difficult illicit preparations to analyze. This is because of the numerous forms and sizes of the preparations, and the presence of highly colored excipients. Therefore, the development of a single standardized extraction sequence has been difficult. In addition to the different forms of LSD preparations, differences in the chemical composition of the ergot content occur, including small amounts of the stereoisomeric iso-LSD, LSD degradation products and other ergot alkaloids. Recently, an analytical scheme with the use of MS for the unequivocal confirmation of LSD in forensic samples was described. (66)

c. Identification of impurities and diluents for evidence comparison. The comparison of evidence is an important function of the forensic laboratory. The

examinations are based upon the comparison of an exhibit with either a reference collection or another specific exhibit. If correlations among drug exhibits can be made, then specific information regarding the history of the drug samples can be developed. The usual means of comparing drug exhibits is to identify the impurities which are present in the material and then determine their relative concentrations. (63)

Identification of heroin and its diluents by chemical ionization mass spectroscopy has been shown. (12) The procedure required no sample preparation or prior chromatographic treatment. Its sensitivity permitted a direct and rapid identification of microgram quantities of illicit preparations, by solid probe. Isobutane was the reagent gas of choice, since it has been demonstrated as yielding relatively simple CI mass spectra.

Leaders in the area of field ionization (FI) mass spectrometry emphasize that its unique characteristics make it a potentially powerful tool in such diverse fields as medicine, criminalistics, and environmental research. (1) Recent work has described two important areas of application—analysis of complex multicomponent mixtures without preseparation and isotope dilution analysis by use of multilabeled molecular tracers.

Impurities in commercially available chlorpheniramine were analyzed by a combination of separation techniques, followed by electron impact, chemical ionization and field desorption mass spectrometry to determine whether the drug was safe for human consumption. (72) Several methods of ionization were required for determination of various impurities, and each furnished unique and supporting MS data.

The stimulant methamphetamine has been obtained both by diversion of legitimately manufactured material and by synthesis in illicit or clandestine laboratories. Methamphetamine produced in clandestine laboratories often contains impurities arising from incomplete reaction and inadequate purification of intermediates and the final product of the synthesis. As the synthesis proceeds, various impurities (reactants, by-products, and intermediates, as well as contaminants within reagents themselves) are produced. The identification of these impurities are elucidated with the aid of mass spectrometry (GC-MS) as well as other instrumental methods, to further support the structural assignments, e.g., nuclear magnetic resonance spectroscopy (NMR), IR, etc., as well as chemical synthesis. In this case, identifying impurities may provide significant information about the manufacturing process, distinguishing between samples of licit and illicit manufacture. (3) (45)

2. Verification of the Analytical Approach

The partial thermal decomposition of drugs in the injection port of a gas chromatograph can affect the accuracy of analysis. The decomposition products of methylphenidate were identified by GC-MS as methyl phenylacetate and a tetrahydropyridine. The extent of decomposition was found to be a function primarily of the injector temperature, and this resulted in considerable variability. After identification by MS and subsequent determination of the problem, an improved analytical method, eliminating this thermal decomposition, was accomplished by derivatization with trifluoroacetic anhydride. (18)

3. Identification of Drugs in Body Fluids by Mass Spectrometry

a. Rapid identification of a drug in emergency overdoses. Phencyclidine (PCP) concentrations in blood, when associated with intoxication are generally low. In addition, the limited quantity of blood available from living individuals necessitated the development of specific and sensitive assays. Sensitive and specific GC-MS methods have been developed for the rapid determination of PCP. (56)

b. Rapid identification of drug metabolites. A combination of GC and MS has been shown to be useful in the identification of small amounts of mecloqualone metabolites in cases of poisoning, particularly when several other drugs have been taken at the same time. Identification of these metabolites was performed by comparing physico-chemical and mass spectral data of synthetically prepared compounds with those of the isolated metabolites. (67)

4. Quantitative Methods—Mass Fragmentography

Combined GC-MS has become an established method for detecting and quantifying very low levels of compounds in complex mixtures. The technique of mass fragmentography is one of the most sensitive detection systems known. Mass fragmentography, also known as single or multiple ion mass detection, is the simultaneous monitoring of one or more fragment ions rather than the scanning of the total ion spectrum as in conventional mass spectrometry. (20) (23) The use of mass fragmentography as a single or multiple ion monitoring for GC has been applied in the qualitative and quantitative analysis of low levels of endogenous compounds, drugs, drug metabolites, biological samples, pesticides, and environmental pollutants. The ion-detecting device of the mass spectrometer is set to monitor, as a function of time, a fragment ion of relatively high abundance in the spectrum of the compound of interest. The resulting record is that of a gas chromatogram performed with a selective detector. Depending on the ions chosen, a limited number of peaks will be observed at the retention time of each compound being analyzed. Therefore, the chromatogram is relatively free of interferences from other compounds in the sample, and the method often offers sensitivity greater than that obtainable with conventional GC detectors.

Due to monitoring of specific mass ions of the compound being analyzed, a preceding separation of other drugs is not required. Quantitative GC requires that the compound being analyzed and the internal standard be completely resolved, but this is not needed in mass fragmentography. (54)

By focusing on a fixed m/e ratio, the mass spectrometer behaves as a highly specific gas chromatography detector, only responding to compounds that, on fragmentation, yield ions at the m/e ratio upon which it is focused.

a. Single ion monitoring (SIM). Single ion monitoring usually requires selection of an appropriate derivative of a component which has at least one unique ion of high abundance at a reasonably high m/e ratio so that interference is minimized. Calibration curves must be obtained to determine the losses in extraction, purification and derivatization, as well as losses in column absorption and variation in instrumental response. This is often time-consuming and unreliable.

The sensitivity of detection over that of conventional GC methods, e.g., electron

capture, flame detection and thermal conductivity, is 10^3 to 10^4. (23) High resolution can be used when more than one fragment ion with a similar nominal m/e ratio is present. When a number of compounds in a sample and an internal standard produce on ionization an ion fragment with the identical m/e ratio, but have different GC retention times, quantitation of more than one compound is performed. The mass spectrometer is acting as specific detectors of certain structural features in a mixture and this is referred to as functional group analysis.

b. Multiple ion monitoring (MIM). The mass spectrometer focuses on several unique prominent fragment ions. MIM is a powerful method for the simultaneous quantitation of drugs and their metabolites. Maximum sensitivity results from monitoring a single ion. As more ions are selected, each will be monitored for a shorter period with subsequent decrease in signal-to-noise ratio and increased contribution from random electrical noise. However, MIM has the added advantage of greater specificity, by focusing on more than one characteristic fragment. One application of MIM can be for detection of drugs and metabolites containing elements with more than one naturally-occurring stable isotope (e.g., Cl and Br).

In carrying out quantitative estimations, the ideal standard should be as chemically similar to, but in some way distinguishable from the compound under investigation. ^{2}H-, ^{13}C- and ^{15}N-labeled compounds are the best suited, and should be added prior to extraction and analysis. Generally, labeling with ^{13}C would be preferred over deuteration since it would be likely to exchange; in addition, the isotope effect of ^{13}C is extremely small. However, the natural abundance of ^{13}C is much greater than that of ^{2}H and this would decrease the sensitivity of the method. (71) In addition, the deuterated compounds are less expensive.

Since the chemical properties of labeled drugs are very similar to those of the parent compound, this procedure overcomes problems caused by incomplete extraction and derivatization and also minimizes absorptive losses encountered on GC. (24) In addition, they show similar fragmentation patterns to the unlabeled compound. Due to its extra mass, the resultant fragments will have different m/e ratios, distinguishing them from the unlabeled fragments. Suitably labeled compounds are therefore useful for quantifying the compound. One disadvantage is that multiply-labeled standards can show GC or HPLC properties slightly different from those of the compound of interest. This approach will be discussed in greater detail by Dr. Horning later in this Symposium. (35)

When compounds other than stable-isotope-labeled analogs are used as internal standards, it is necessary to determine a response factor, which is the ratio of the peak area per microgram of drug divided by the peak area per microgram of internal standard. The response factors should be constant over the range of concentrations measured. In the case of unavailable standards, a hydrocarbon or drug with suitable GC properties can be added as an internal standard. (34)

c. Therapeutic drug monitoring.

i. Pharmacokinetic determination—determination of proper drug dose. Drug monitoring is useful in the management of patients undergoing chronic therapy, especially if the therapeutic concentration range of the drug is narrow. Determination of plasma levels of several drugs is important for rational therapy. Patients treated for grand mal epilepsy have been significantly improved by adjusting plasma levels of diphenylhydantoin to the therapeutic level of 10–20 μg/ml. The method applied must be sensitive since the therapeutic dose is low. Specificity is especially important because of the multiple drug nature of anticonvulsant therapy and extensive metabolism of the drug. (51) Carbamazepine is used for the treatment of convulsive disorders and trigeminal neuralgia. In order for its pharmacokinetics to be determined in man, a specific and sensitive analytical method was needed. (54) A variety of techniques was used to eliminate endogenous material which may interfere with the assay. (60)

Preliminary studies on the metabolism and pharmacology of chemotherapeutic drugs have been carried out using radioactively labeled compounds. Investigations in humans over extended periods of time during long-term chemotherapy have been difficult because the concentration at which toxicity occurs and the minimum concentration required for therapy have not been determined. It is also not known if the concentration of the drug within individuals decreases with chronic therapy as a consequence of enhanced drug metabolism. The CI-MS assay currently is being used to measure concentrations of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in the plasma of patients who are undergoing a schedule of BCNU treatment for brain tumor. With the exception of mass fragmentography, no analytical method could provide sufficiently accurate measurements in patients. Therefore, individual pharmacokinetic parameters were not previously determined. (69)

ii. Limited sample size. Mass fragmentography was useful for monitoring theophylline, a bronchodilator, in newborns and pediatric cases. There are risks of toxicity associated with the use of the drug and, therefore, it is generally recommended that its concentrations be monitored in the blood. Chromatographic interference from other drugs, metabolites and organic material found in serum or blood usually occurs. In addition, several such procedures require large volumes of blood or serum for analysis and therefore cannot be used routinely to monitor theophylline concentrations in newborns or small children. (62) Mass fragmentography has been used to study the transfer of drug from the mother's circulation to the fetus and to amniotic fluid and breast milk. (33)

Etorphine, 6,14-endo-etheno-7-[1-(*R*)-hydroxy-1-methylbutyl]-6,7,8,14-tetrahydroripavine, an analgesic approximately 1000 times as potent as morphine, and which can be taken sublingually, may become a future threat in the drug abuse field. Its study in biological systems in hindered since it cannot be detected in urine by the usual analytical approaches (GC), after administration of pharmacologically effective doses. The SIM method was developed for the urine analysis with (tritiated) $^3H_2$-etorphine as the internal standard. (39)

iii. Determination of Alternate Clinical Methods. Although blood is the usual sample for drug monitoring, saliva samples have also been used. The concentra-

tion of most drugs in saliva corresponds to unbound plasma drug concentrations, and that value may be a more meaningful value for considerations of pharmacological activity or toxicity than a value that reflects both bound and unbound drugs. In addition, saliva can be obtained by noninvasive techniques and this is helpful when multiple serial samples are needed and in monitoring drug concentrations in children. Furthermore, most therapeutic agents transfer rapidly from plasma to saliva, and the concentration of drugs in saliva is proportional to the concentration in plasma. (33)

iv. Potential overdoses. Methods have been developed for monitoring the concentration of tricyclic antidepressants in plasma to be able to successfully manage patients with depression. There is an increased prevalence of toxic overdose as a result of the ready availability of the tricyclic drugs to the depressed patient. Overdose symptoms may include grand mal seizures, cardiac failure, coma, and eventual death. It is important to monitor low therapeutic concentrations in cases where the drug identity is known, and to be able to screen for toxic concentrations in cases where the drug identity is not known. (70)

v. Monitoring patients on methadone maintenance. Recently, chemical-ionization mass spectrometry and deuterium-labeled morphine were used to monitor the possible abuse of narcotics by methadone maintenance patients, as well as low level quantitation of methadone in biological samples. The latter method permits more accurate measurement of low methadone levels present in adolescent heroin addicts on methadone maintenance therapy who are receiving lower dosages (5–40 mg/day) and in newborn infants of mothers who are maintained on methadone. This method will also permit studies in animals in which plasma methadone levels are lower and turnover rates are much faster than in humans. (24)

vi. Drug assays. Mass fragmentographic assays are among the most selective analytical methods available to date. The elucidation of the biochemical pharmacology of a drug has been predicated upon the availability of assays capable of measuring the drug in plasma and/or serum. (60) (61)

vii. Indirect measurement of enzyme activity (inborn errors of metabolism). In phenylketonuria, one of the most common inborn errors of human metabolism, phenyl-4-monooxygenase, the liver enzyme which converts phenylalanine into tyrosine, shows no, or only partial, activity. In order to evaluate the remaining enzyme activity or to determine an increase in the enzyme activity after therapeutic treatment, an indirect *in vivo* measurement of phenyl-4-monooxygenase was done by SIM MS. The specificity and reliability of other determinations are disputable. (71) Quantitative FD-MS has also been applied in medical research studies. When phenylalanine-d_7 was administered to patients suffering from phenylketonuria, the determination of the ratio of tyrosine-d_0 to tyrosine-d_6 also gave a measure of the enzymatic reactivity still available. (47)

viii. Amino acid analysis. GC-MS has been used for amino acid analysis because of the high sensitivity and specificity in mass spectrometric detection. (19)

II. *ANALYTICAL METHODS BY IONIZATION TECHNIQUE*

A. *General*

Often the success of a mass spectrometric analysis depends upon the type of ion source chosen. Often, electron impact mass spectrometry (EI-MS) of the compounds does not provide the necessary level of confidence needed to make molecular assignments. Therefore, alternative (CI, FI and FD) ionization techniques can be performed to further substantiate the results and thereby obtain a high degree of confidence. (72) These alternative methods of ionization most often provide simpler mass spectra dominated by a few highly intense ions. In addition, FD ionization processes allow the analysis of nonvolatile compounds to be achieved without derivatization.

The mass spectra of some biologically important compounds, e.g., amino acids, triglycerides, have been measured using field ionization (FI) and field desorption (FD) means of ionization and comparing the results to the more common approaches of electron impact (EI) and chemical ionization (CI). (17) The mass spectra obtained suggested that neither of these techniques is superior to the others, but that the method of choice in any analytical problem depends on the type of compound involved.

B. *Electron Impact (EI) Ionization*

Several derivatization techniques have been studied with respect to their effect on gas chromatography and resultant electron impact mass spectral characteristics. These studies have been critical with respect to analysis of cannabinoids. Since the major function of derivatization is to enhance volatility and thermal stability, efforts have been devoted to the development of new derivatives which also improve the compatibility of various chromatographic and mass spectral techniques. With respect to improvement of MS techniques, our desire is for greater structural information from mass spectrometry and improved detectability with GC detectors or with GC-MS selected ion detection.

1. Applications of Improved Derivatization Procedures in EI Mass Spectral Analysis

The gas chromatographic separation of major and minor components of marihuana and hashish extract has been described. Mass spectrometric data have been produced for these components. Some of the minor components of cannabis resin are in fact isomers and homologues of the major cannabinoids: cannabidiol, $\Delta^{1,2}$-tetrahydrocannabinol and cannabinol. (65) These components include cannabidivarol (the cannabidiol-C_3 homologue), cannabicyclol, and the C_5-homologue of $\Delta^{1,6}$-and $\Delta^{1,2}$-tetrahydrocannabinol, etc. More recently, the use of improved derivatization techniques has provided better gas chromatographic separation of additional cannabinoids and, in some cases, has increased mass spectral capabilities in structural elucidation of minor components. Fairly good separations have been obtained using trimethylsilyl (TMS) derivatives of cannabis extracts and a 3% SE-30 column. However, some poorly resolved peaks were present. These corresponded to mixtures of mono- and dihydroxy compounds and separation of them was achieved by variation of the derivatives—i.e., substitution of the TMS group with silyl moieties containing higher alkyl substituents. This resulted in the production of longer retention times for cannabis diols, thus shifting their respective signals away from the other constituents while still retaining good gas chromatographic properties and being stable.

The tri-*n*-alkylsilanes included triethyl-, tri-*n*-propyl-, tri-*n*-butyl, and tri-*n*-hexylsilanes. (26) Derivatives with alkyl groups greater than C_4 required, however, undesirably high elution temperatures. By increasing the molecular weight of the silyl moiety, however, SIM and MIM work was improved due to lack of background ions at the higher masses. In general, the mass spectral characteristics of the higher alkylsilyl derivatives were similar to the TMS derivatives.

Fresh samples of *Cannabis sativa* L. usually contain cannabinoids in the form of their carboxylic acid derivatives (Δ^1-tetrahydrocannabinolic acid). These acids cannot be examined directly by gas chromatography since they decarboxylate upon heating, even to some extent, as the TMS ethers. Preparation of cyclic alkylboronate derivatives (alkyl=methyl or butyl) has been shown to be suitable for GC-MS studies on these types of compounds. In addition, isomeric acids in which the phenolic hydroxyl was *para* to the carboxylic acid have been reported to be present in cannabis. Since they are incapable of forming cyclic alkylboronates, this method of derivatization provided an ideal means of distinguishing between the two isomers. (25) The methylboronates had retention times comparable with those of the TMS derivatives, whereas the *n*-butylboronates had longer retention times. A major advantage over TMS derivatization was the reduction in molecular weight obtained for all of the alkylboronate derivatives as the result of replacing two TMS groups with the relatively low mass boronate moiety. In addition, the mass spectral characteristics observed for the alkylboronate derivatives included positive charge localization away from the boronate ring systems onto the heterocyclic oxygen atom. The spectra of the Δ^1-THC-acid boronate derivatives exhibited molecular ions as the most abundant in the spectra, a marked improvement over the TMS derivatives.

Additional new derivatives examined for GC analytical chemistry (steroids and nucleosides) included *tert*-butyldimethylsilyl (TBDMS), cyclotetramethyleneisopropylsilyl (TMIPS), and cyclotetramethylene-*tert*-butylsilyl (TMTBS) derivatives. (59) These derivatives offered several advantages over TMS, including greater stability for TLC and isolation of standards, better separations by GC and structural information by EI-MS.

Silylation has proved to be one of the most effective derivatization methods for a wide variety of compounds. Due to steric crowding, these bulky groups have decreased susceptibility to nucleophilic attack. The large silyl ethers have greater stability towards hydrolysis than do TMS ethers, and, therefore, offer much greater utility as protecting groups in synthesis. Like TMS ethers, the bulky silyl ethers offer good volatility and thermal stability and some other characteristic properties make them valuable for identifying fragment ion types and deducing fragmentation pathways in mass spectrometry. The high mass of the bulky ether derivatives in the case of polyhydroxy compounds may be a disadvantage when using some models of mass spectrometers with low mass limits. However, it may be an additional advantage in GC-MS-SIM, where the increased mass will place important fragment ions in a region free from GC bleed peaks.

The mass spectra of these ethers are usually dominated by peaks arising from initial siliconium formation, rationalized by elimination of a stable, branched alkyl radical, thus relieving steric crowding in the silyl group. Ions of these derivatives are much more prominent in the important high mass region than those in the spectrum of the TMS ethers.

Siliconium ion centers can interact with sterically accessible electron dense groups in a molecule, often resulting in cyclic transition states which subsequently rearrange. These fragmentation processes have provided important structural information useful in distinguishing between isomers, specifically between derivatives of deoxynucleosides and of ribonucleosides. Even when the molecule does not contain the sterically accessible electron dense function, the derivative may be useful for isomer differentiation. One example of this is the difference between the mass spectra of the epimeric steroids androsterone and epiandrosterone, where the intensity of the $[M-(R\text{-}HX_2SiOH)]^+$ ion depends upon the stereochemistry of ring A.

2. Mass Spectral Analysis of Compounds by EI-MS

a. Mass spectral analysis of opiate derivatives. Several recent articles have utilized GC-MS, high resolution MS, along with supporting instrumental and chemical methods to identify atypical or unusual morphine or heroin trace impurities. These have included β-chloromorphide, (29) 3,6,17 -triacetyl*nor*morphine, (42) and 0^3-monoacetylmorphine (43) (52)—as well as acetylcodeine and 0^6-monoacetylmorphine. (42) In addition, synthesis of deuterated heroin analogs and mass spectral analysis has allowed differentiation between the isomers, 0^3- and 0^6-monoacetylmorphines, which led to their identification and confirmation in illicit heroin samples. (41)

The levels of normorphine and norcodeine in the urine of individuals (patients or opiate addicts) who take massive doses of morphine have been confirmed by CI- and EI-MS (approximately 5% normorphine and >0.1% norcodeine relative to the administered morphine dose). The difficulty in detecting normorphine was attributed to its instability in acidic and alkaline media, its poor solubility in water immiscible solvent systems and/or to its limited sensitivity to potassium iodoplatinate, the spray reagent commonly used for alkaloids in TLC examination. The presence of normorphine and norcodeine in urine of individuals who are chronic users or have received a single large dose of morphine suggested that morphine metabolizes to a limited extent to normorphine and to codeine and norcodeine. Codeine formation and consequent norcodeine formation seem to appear after prolonged heroin abuse, and is possibly attributed to changes in hepatic functions frequently observed in opiate addicts. (6)

A sensitive assay for the determination of etorphine in urine was developed. The sensitivity of the method was about 5 ng/ml, which compared favorably with a sensitivity of 100 ng/ml by GC analysis. The procedure included repeatedly basifying the urine of animals, extraction into butyl chloride, and reacidification. A final organic extract was derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide to form the TMS derivatives. $^3H_2$-Etorphine was added to control urine and treated by the identical procedure. The mass spectrum of etorphine showed a molecular ion at m/e 483. The spectrum of tritiated etorphine showed a molecular ion at m/e 487($^3H_2$) and ions at m/e 483($^3H_0$) and 485($^3H_1$). Samples were extracted, derivatized and analyzed on the GC-MS

focused at m/e 483 and 487. The method could possibly be twice as sensitive with a fully deuterated standard, although this was not available. Using a radioisotope, however, enabled each step of the analytical procedure to be monitored. Using tritiated compounds in the mass spectrometer posed no problem with safety procedures, e.g., proper exhaustion from the vacuum lines and proper precautions when oil was changed in the vacuum pumps. (39)

b. Determination of LSD. The analysis began by prior extraction of an ammoniated LSD sample with 1,2-dichloroethane, followed by re-extraction into 0.5*N* H_2SO_4. This latter aqueous solution was then divided into two portions. The LSD in one half was again basified, re-extracted with 1,2-dichloroethane, and recovered. The second half was scanned from 210–360 nm to give a broad absorption band with the maximum at approximately 313 nm. After scanning, the solution in dilute sulfuric acid was irradiated with long wave ultraviolet light for five minutes, re-scanned to show a shift to the broad absorption maximum to approximately 294 nm. The reaction product was then re-extracted by the usual method. The shift in UV absorption maximum, due to a photochemically induced hydration reaction at the $C_{9,10}$ double bond, was previously too non-specific to provide unequivocal confirmation of the presence of LSD. The EI mass spectral analyses of the non-irradiated and the irradiated products provided the specificity required for forensic purposes, as well as proving that the hypothesized hydration product was formed. Observation of a molecular ion at m/e 341 confirmed the addition of water to LSD (mol. wt. = 323). (66)

c. Analysis of methamphetamine impurities. The impurities identified in illicit methamphetamine include N-formylmethamphetamine (N-methyl-N-(α-methylphenethyl)formamide), N-α,α′-trimethyldiphenethylamine and α-benzyl-N-methylphenethylamine. Other laboratory exhibits have been shown to contain formic acid, methylamine, N-formylamphetamine, and dibenzylketone. They were identified by UV, IR, NMR and combined GC-MS techniques. The methods used to isolate and identify each compound as well as the manner in which each can occur in methamphetamine samples were described. (3) (45) An impurity detected in illicit amphetamine has been identified as α-benzylphenethylamine. (44) The above combined instrumental approaches were used in determining the substance. This contaminant was cited for its reported toxicity (CNS stimulation, increase in blood pressure, hypotensive action, respiratory difficulties).

d. Identification of mecloqualone metabolites. Mecloqualone (2-methyl-3-(2-chlorophenyl)-4-(3H)-quinazolinone) is a non-barbituric hypnotic structurally similar to methaqualone (2-methyl-3-*o*-tolyl-4-(3H)-quinazolinone). After oral ingestion, unmetabolized mecloqualone is excreted in very small amounts (2–3%), and the majority (95%) undergoes hydroxylation, followed by glucuronide formation. Hydrolysis with 20% hydrochloric acid or β-glucuronidase yields the free hydroxy compounds.

Eight synthetic monohydroxy compounds (including four with a hydroxy group on the chlorophenyl moiety and four others with a hydroxy group on the quinazolinone moiety) were examined mass spectrometrically, as were their TMS derivatives. Depending on the site of hydroxylation, distinct differences between the mass spectra of these compounds were observed. Compounds hydroxylated on the quinazolinone moiety exhibited fragment ions at m/e 111 and 152 (both containing the chlorine atom). M/e 152 ion shifted to m/e 168 in the mass spectra from the compounds hydroxylated at the chlorophenyl moiety. However, no fragment at m/e 127 resulting from a shift of the m/e 111 fragment ion was observed. This difference provided a means of differentiation between the two groups. However, differences among the individual members of the same group were less clear. A fragment at m/e 160, present in the MS of only the 8-hydroxy compound, was used to identify one of the major metabolites.

The mass spectra of the compounds silylated in the position 5, 6, 7 or 8 (quinazolinone) exhibited m/e 111 and 152. These fragment ions were not present in the mass spectra of the compounds substituted in the chlorophenyl nucleus (3′, 4′, 5′ or 6′) which, however, showed characteristic fragments at m/e 143, 117 and 116. Again the presence or absence of the chlorine isotopes facilitated identification of some typical fragments.

No distinct differences could be observed among the compounds silylated on positions 3′, 4′, 5′ or 6′. There were, however, characteristic differences in the mass spectra of compounds silylated in positions 5, 6, 7, or 8. The 6-TMS compound produced a molecular ion. The base peak in the mass spectrum of the 7-TMS compound was at m/e 323, and for the other compounds at m/e 343. This allowed for identification of the 7-hydroxy compound in the metabolite mixture.

After acid hydrolysis, four metabolites could be isolated and identified in urine (GC). Combined GC-MS enabled determination of the chemical structures of all four metabolites: 2-methyl-3-(2-chloro-3-hydroxyphenyl)-4(3H)-quinazolinone; 2-methyl-3-(2-chloro-4-hydroxyphenyl)-4(3H)-quinazolinone; 2-methyl-3(2-chlorophenyl)-7-hydroxy-4(3H)-quinazolinone; and 2-methyl-3(2-chlorophenyl)8-hydroxy-4(3H)-quinazolinone. The other four hydroxy isomers were not detected in the isolated metabolite mixtures. (67)

e. Measurement of enzyme activity. A specific method for quantitation of deuterated and non-deuterated phenylalanine and tyrosine in human plasma by the GC-MS-SIM technique was developed to measure phenylalanine-4-monooxygenase activity. (71) The N- and N,O-trifluoroacetyl methyl ester derivatives provided sensitivity measurements as small as ca. 2.5 ng/ml; coefficient of variation ca. 1.6% (phenylalanine) and 3.0% (tyrosine). The chosen derivatives show characteristic and intensive signals typical for tyrosine, or phenylalanine. Subjects were administered deuterated L-phenylalanine-d_5 and resulting deuterated L-tyrosine-d_4 and residual L-phenylalanine-d_5 were measured.

f. Analysis of amino acids. A method for GC-MS analysis of amino acids hydrolysates containing low picomole quantities of oligopeptides was described. (19) The method utilized GC-MS-SIM of their trifluoroacetyl n-butyl ester derivatives. Analysis of amino acids released by hydrolysis of picomole quantities of oligopeptides generally requires stringent precautions to prevent introduction of

amino acid or peptide containing contaminants into the sample. This has been avoided by use of vacuum line and microtechniques involving solution and reaction mixture volumes of ca. 1 μl. The GC column was protected by means of a solvent/reagent vent valve from reactive reagents present in the amino acid derivatization reaction mixture. Techniques were developed which permitted analysis of 25–50 pmol of a decapeptide based on GC-MS, hydrolysis, derivatization and amino acid analysis. Two critical problems were overcome: (1) avoiding introduction of amino acids or proteins into the sample during processing, and (2) maintaining high performance characteristics of the column despite the necessity of injecting highly reactive reagents (trifluoroacetic acid, anhydride). The instrumental techniques used to avoid column degradation involved use of a bypass valve and a precolumn to remove solvent and reagents prior to adsorption onto the chromatographic column. To avoid contamination with extraneous amino acids, specialized microtechniques were developed including vacuum line reagent and solvent transfer methods.

Methodology for hydrolysis of oligopeptides and derivatization of amino acid mixtures using reaction solution volumes of 1 μl was developed. Vacuum line transfer of solvents and reagents and sealed capillaries as reaction vessels significantly reduced amino acid (or protein) contamination and allowed amino acid analysis of picomole quantities of oligopeptides.

g. Mass fragmentography. Mass fragmentography was applied to the analysis of the anti-epileptic drug, diphenylhydantoin (DPH). (49) The internal standard was 5-(p-methylphenyl)-5-phenylhydantoin (MPPH). Both compounds were chromatographed as their 1,3-dimethyl derivatives following quantitative on-column methylation with trimethylanilium hydroxide. MPPH reacted similarly, in both methyl derivative formation and in recovery from plasma, because of its chemical similarity to DPH.

The most abundant ion common to both DPH and the internal standard was at m/e 118. Although monitoring at m/e 118 produced the greatest sensitivity, the relatively low m/e ratio of that fragment increased the possibility of interference from other components. Larger ions common to both compounds are at m/e 203 and m/e 194. M/e 194 was chosen to be monitored due to its greater intensity in the MPPH spectrum. The retention times of the 1,3-dimethyl derivatives of DPH and MPPH were 6.0 and 7.7 min. by GC-MS. DPH was quantified down to 0.2 ng (with 3:1 signal-to-noise ratio). The detection limit was 0.05 ng.

A mass fragmentographic method for the quantitative determination of small amounts of carbamazepine in plasma, using 10,11-dihydrocarbamazepine as the internal standard, was accomplished by monitoring the intensities of the molecular ions of carbamazepine (m/e 236) and dihydrocarbamazepine (m/e 238). (54) When carbamazepine is injected into a GC, decomposition to iminostilbene occurs. The decomposition is not reproducible, depending on several factors. To compensate for the variability of decomposition, yield of extraction, and injection volume, choice of an internal standard as chemically similar to carbamazepine was critical. 10,11-Dihydrocarbamazepine has properties almost identical to carbamazepine with respect to decomposition, i.e., loss of HNCO. It was possible, therefore, to quantitate carbamazepine down to 50 ng/ml using 0.5 ml plasma.

The illegitimate use of phencyclidine (PCP), 1-(1-phenylcyclohexyl)-piperidine, has increased drastically throughout the USA, partially due to the relative ease with which it may be synthesized. PCP preparations are self-administered by smoking, insufflation, oral ingestion and injection.

A procedure was developed for identifying and measuring PCP in blood with internal standard, 1-(1-phenyl-[$^2H_5$]-cyclohexyl) piperidine (pentadeuterophencyclidine). (56) A known quantity of this internal standard was added to a measured amount of blood, and to aqueous PCP solutions of appropriate concentrations. Each sample was then carried through a three-step separation. The resulting extract was taken to dryness, reconstituted and two aliquots subjected to analysis, one for quantitation and the other for positive identification. The PCP was quantitated by monitoring the ions at m/e 205 ([$^2H_5$]-phencyclidine) and 200 (phencyclidine). The concentration of PCP was calculated by normalizing the area of the response at m/e 205 to 100%. Applying the factor obtained to the area of the response at m/e 200 yielded the concentration of PCP in μg/liter. A second injection of a similar volume was used to confirm the presence of PCP. The ions at m/e 243 (molecular ion), 242, 200 and 186 were monitored and the calculated ratios of the responses compared to that of a standard sample.

The use of SIM can be limited by contribution of unresolved components which give ions at the same nominal mass as those compounds being analyzed. The majority of GC-MS work is performed on low resolution instruments. The use of high resolution GC-MS-SIM would allow for quantification of a specific fragment in the presence of other fragments with the identical nominal mass.

The application has been demonstrated in SIM quantitation of dimethylnitrosamine, a potential carcinogen in tobacco smoke condensates, by monitoring the molecular ion at m/e 74.0480. (14) The samples, of course, are extremely complex and some of the ions with identical nominal masses which interfered with the component being analyzed included $C_3H_6O_2^+$ (74.0368), $^{13}CC_2H_5O_2^+$ (74.0401), $CH_4N_3O^+$ (74.0354) and $C_3H_8NO^+$ (74.0606). However, the use of high resolution avoided interference by other components.

C. Chemical Ionization (CI).

CI-MS, a "softer" method of ionization, often provides a larger fraction of ions related to the molecular weight of the sample under analysis. Other advantages are that the ion source can take most or all of the column effluent, and the carrier gas can be used as the reagent gas. CI-MS is best utilized in conjunction with EI-MS, in order to complement molecular weight information with mass spectral structural information. (22) The occurrence of both proton transfer and addition reactions on the same compound can often provide further evidence for the molecular weight assigned. This results in the quasimolecular ion, $(MH)^+$, an even electron species which, if unstable, can further fragment to lose a neutral molecule. One would expect more fragmentation as the proton affinity of the reagent gas decreases. Proton abstraction $(M-H)^+$ can occur mostly with compounds of low proton affinity, e.g., alkanes. Addition reactions be-

tween functional groups and the reagent gas can also be observed.

SIM analysis using CI for various compounds (e.g., morphine) has been reported to achieve comparable sensitivity with a specificity not equalled by immunoassay or hemagglutination analyses. Because of its high sensitivity and specificity, SIM analyses allow quantitation of nanogram or picogram amounts of several drugs and drug metabolites in a single run. Chemical ionization is now frequently used for the quantitation of drugs and drug metabolites. Compounds which can not be derivatized easily or which show poor chromatographic properties can be quantitated using the CI-MS direct insertion-SIM method.

The degree of interference depends on the uniqueness of the ion masses monitored, and, therefore, CI offers significant advantages over the more commonly used electron impact ionization due to the high abundance of quasimolecular ions. The ion current from the compound being analyzed is concentrated in a relatively few high m/e values where background contributions are low. CI has the additional advantage of eliminating the need for a separator between the gas chromatograph and the mass spectrometer. The separator can be a major source of sample loss, owing to adsorption, decomposition, or diffusion. CI-MS offers the advantage in determining isotope ratios on compounds obtained from biological sources by improving the uniqueness of a particular ion and thus minimizing interferences from ions due to natural biological contaminants. (24) Several disadvantages of CI-MS are possible; these include sensitivity to temperature changes, which affect the extent of fragmentation, and as the ion source gets contaminated, gradual deterioration of linearity and precision can be expected. (70)

1. Reagent Gases

Methane and isobutane have been used as reagent gases since their "reactive activities" cover a wide range. A reagent gas can be used to identify basic groups in an unknown molecule. In addition, if the functional groups present in a molecule are known, a reagent gas can be selected which will selectively protonate one of these, and, therefore, provide fragmentations which are initiated by and are specific for that particular functional group. Hydrogen (H_2), methane (CH_4) and isobutane (i-C_4H_{10}) all enhance the MH^+ ion and can be used in GC analyses. In general, more fragmentation is observed with H_2 and less with i-C_4H_{10}. Water has been shown useful in direct determination of organic compounds in aqueous solution down to 1 ppm, producing typical ions, MH^+ and $M+H_3O^+$. Deuterium oxide (D_2O) can additionally be used in the determination of the number of active hydrogens in a compound. Amide hydrogens can be exchanged, whereas hydrogens α- to carbonyl groups are exchanged only in amounts less than 15%. (22)

Ammonia (NH_3) and alkylamines ($R-NH_2$) selectively protonate amines, amides, and α,β-unsaturated ketones and form addition products $(M+NHR)^+$ from ketones, aldehydes, acids and esters. When ammonia was used as the reagent gas, the CI-MS of triglycerides produced peaks which corresponded to quasimolecular ions. When isobutane was the reagent gas, quasimolecular ions were usually not recorded. With ammonia as the reagent gas, the $(M+NH_4)^+$ ion was the base peak. (53) An alternative to CI-MS with ammonia is isobutane modified with ethanolamine and ethylenediamine, in which less fragmentation is obtained than with isobutane used alone.

Another method of selective detection by CI-MS is the reactant ion monitoring technique. (27) As each component in a sample elutes from the GC into the source of the mass spectrometer, there is a decrease in the ion current of the reactant ions, because of the reactions of these ions with the added sample. Following this GC trace is called reactant ion monitoring and it is equivalent to a plot of total sample ion current vs. time.

Selective detection of various classes of compounds is obtained by CI-MS, because each class should react at a different rate with a given reactant ion. An example is that t-$C_4H_9^+$ reacts with alcohols at a much greater rate than with hydrocarbons. In addition, it reacts much more slowly with primary alcohols than with secondary or tertiary alcohols; therefore, structural information may be obtained from relative rate constants for reactions. This method can help eliminate large peaks on a GC-FID trace which cover up minor peaks. Another example of applicability is the qualitative analysis of complex mixtures. Also, protonated acetone can distinguish among primary, secondary, and tertiary alcohols. Proton transfer from $C_3H_7O^+$ to ketones should be rapid and proton transfer from $C_3H_7O^+$ to aromatic hydrocarbons should be slow. Therefore, ketones and aromatic hydrocarbons can be distinguished by reactant ion monitoring with $C_3H_7O^+$.

a. Clonazepam and its 7-amino metabolite. A sensitive GC, ammonia chemical ionization mass spectrometry, ^{15}N isotope dilution assay was developed to measure the antiepileptic drug clonazepam and its 7-amino metabolite in blood or plasma. (51) The method was used to measure both compounds in the blood of one subject administered a single 2-mg dose of clonazepam, and in the plasma of thirteen subjects on a clonazepam oral dosing regimen. The assay developed involves a simple extraction of plasma or blood, followed by GC-MS analysis of the residue after evaporation of the extraction solvent. Specificity was obtained by monitoring the MH^+ ions of clonazepam and its 7-amino metabolite, generated by ammonia CI. Assay accuracy was insured by the use of stable isotope analogs of clonazepam and the metabolite as internal standards. The ions monitored were m/e 286 (MH^+ of 7-amino metabolite), m/e 287 (MH^+ of ^{15}N-7-amino metabolite), m/e 316 (MH^+ of clonazepam) and m/e 317 (MH^+ of ^{15}N-clonazepam). The limit of detection of the method, 1 ng/ml for clonazepam and 2 ng/ml for the 7-amino metabolite, was sufficient for measuring clonazepam and its metabolite in most subjects on the chronic dosing regimen, and in many subjects following administration of a single dose of clonazepam.

2. Charge Exchange Gases

Charge exchange (CE) reactions arise when the ionized reagent gas cannot donate a proton. The ionization potential of the molecule must be less than the recombination energy of the ionized gas. The degree of fragmentation will depend on the energy difference between the two parameters. Combined charge exchange-chemical ionization occurs when a second gas containing hydrogen is used with the CE gas. The second gas, once ionized, can then ionize the sample molecule. The spectra of molecules ionized in this way will present a situation between that obtained for true EI and CI spectra. (22)

Generally, CE gases (He, Ar, N_2) yield spectra which do not differ significantly from those obtained by EI. Mass spectra ob-

tained with nitric oxide, however, show relatively abundant $(M+NO)^+$ ions with molecules containing *pi*-electrons. Therefore, alkenes can be distinguished from alkanes and cycloalkanes since only the former give $(M+NO)^+$ ions.

Nitric oxide CI can also differentiate between primary, secondary, and tertiary alcohols. Primary alcohols give spectra in which (M−2), (M−3) and (M−2+NO) ions are present. Secondary alcohols give major ions (M−1), (M−17) and (M−2+NO), whereas M−17 ions only are produced from tertiary alcohols. The mass spectra of twelve morphine and tropane alkaloids obtained by using N_2/NO as the reagent gas all showed the M^+ as the base peak. (37) In general, acetoxy, hydroxy, carbonyl and aromatic groups will not be involved in CE since their ionization potentials are greater than the recombination energy of the NO^+ ion. Therefore, fragmentations characteristic of these groups will not occur to the same extent as in the EI spectra.

EI was shown to be four times more sensitive than CI and twenty times more sensitive than CE by measuring the relative intensity of the MH^+ molecular ion of heroin by SIM. The use of mixtures of CI and CE gases also appears to offer some advantages. Mixtures of Ar/H_2 and Ar/CH_4 have been shown to distinguish between the two isomeric compounds amobarbital and pentobarbital mass spectrometrically. The major CE gases are Ar (combined with NO, H_2O or CH_4), He (combined with H_2O or NO), N_2 (combined with NO), NO and O_2. (22)

3. Direct CI-MS Analysis

Multi-drug mixtures have been applied directly to the probe of the CI mass spectrometer, in order to identify compounds rapidly by abundant and unique quasimolecular ions. Therefore, this method of direct analysis is useful in cases of drug overdose in which rapid identification is critical. The disadvantages, however, are the inability to differentiate between some pairs of drugs, relatively large quantities of material are needed, and, of course, the presence of non-drug components could be falsely interpreted.

a. Illicit heroin. The CI-isobutane spectrum of illicit heroin and other samples clearly indicated the presence of a number of common drugs by this approach. (12) Heroin, acetylcodeine and O^6-monoacetylmorphine were easily identified, although O^3-monoacetylmorphine was not distinguished from the latter compound. Diluents which were easily distinguished by this method included caffeine, methapyrilene, quinine and procaine, owing to relatively strong and characteristic molecular and fragment ions. The isomeric hexahydric alcohols of mannitol and sorbitol were not differentiated by this method, nor were the monosaccharides, glucose, fructose, galactose and mannose. The disaccharides, sucrose and lactose, exhibited spectra similar to those of the monosaccharides; this was attributed to their decomposition in the CI source prior to ionization. In addition, identical CI spectra were produced by a compound in both salt and free-base form. No attempt was made to quantitate the constituents based on mass fragmentation other than providing a potentially useful fingerprint.

b. Quantitation of quinidine. Direct analysis has been shown improved to GC method for quantitation of quinidine (m/e 325) and the impurity dihydroquinidine (m/e 327). The ion doublet observed for each compound allows ready identification and quantitation with incorporation of the internal standard, a deuterated dihydroquinidine, m/e 329. (32)

c. Quantitation of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). BCNU is an effective chemotherapeutic agent used in the treatment of brain tumors. A direct insertion CI-MS method for the analysis of BCNU in biological samples, with the use of a stable octadeuterium-labeled BCNU internal standard was used for determining drug concentrations in the plasma of experimental animals and in humans undergoing chemotherapy. (69) The direct insertion method for determination of BCNU pharmacokinetics was required because of the drug's labile chemical nature. The internal standard was added to blood, plasma, water, or enzyme preparations, after which hexane/ether extraction and mass spectrometric analysis of the extract are performed at low temperatures, with isobutane as the carrier gas. SIM of the protonated molecular ions of the drug and internal standard yielded ion intensity ratios, from which the concentration of the drug was calculated. The protonated molecular ions of BCNU occurred at m/e 214, 216, and 218, with a ratio close to 9.3/6.2/1.0, (characteristic of a molecule containing two chlorine atoms in the ratio of their natural abundance). A similar pattern was observed from BCNU-$^2H_8$ at m/e 222, 224, and 226. The ions 214, 216, 222 and 224 were monitored in the course of each sample analysis. The ratios remained constant during the probe evaporation. Both the 214/222 and the 216/224 ratio gave satisfactory quantitative results. The sensitivity limit for the mass spectrometer used in these analyses was about 10^{-13} mole; the lower limit for peak ratio measurements of pure BCNU and BCNU-$^2H_8$ mixture was 10^{-11} mole.

4. Gas Chromatography—CI Mass Spectrometry (GC-CI-MS)

The CI mass spectrometer is more easily combined than its EI counterpart with the gas chromatograph. The ability of the CI source to handle high gas loads decreases the pressure differential between the two instruments. When reagent gases (H_2O, NO, O_2) that may adversely affect the GC stationary phase are used, these should be introduced as far as possible from the GC interface.

a. Phencyclidine (PCP). PCP has been quantitated in body fluids and the structural elucidations of two of its metabolites were achieved by GC-CI-MS. Blood and urine samples from individuals intoxicated with PCP on extraction gave only unchanged drug. The metabolites were present as conjugates and could be extracted after enzymatic hydrolysis with β-glucuronidase.

Quantitation of PCP was achieved by SIM using 1-(1-phenyl-($^2H_5$)-cyclohexyl)-piperidine as the internal standard. Levels of 1 ng/ml in body fluid could be measured. It was established that 1-phenylcyclohexane, previously reported as a metabolite, actually resulted from the thermal degradation in the injection port of the GC even at temperatures as low as 150°C. Molecular weight (CI methane MS), data from EI spectra, and comparison with reference standard allowed confirmation of the structures of the PCP metabolites. (38)

b. Methadone. A CI-MS-SIM method for monitoring methadone maintenance individuals was an improvement over previous GC analyses that did not provide re-

liable quantitation less than 10 ng/ml with high precision. The internal standard was $^2H_5$-methadone, and body fluids analyzed included plasma and urine. Isotope ratios were obtained using isobutane CI and by monitoring the protonated molecular ions at m/e 310 and 315 for methadone and $^2H_5$-methadone, respectively. This procedure was an improvement over previous EI ion monitoring methods, in which the $(M-15)^+$ m/e 294 ion was used for quantitation and its relative abundance was low. This procedure used one of the most intense ions in CI mass spectrum of methadone (m/e 310, $(MH)^+$). The sensitivity was also about a factor of 15–20 better than GC procedures which also required a larger volume of plasma. (24)

c. Heroin. Several CI-MS-MIM methods are available for monitoring heroin in patients on methadone maintenance. This is accomplished by determining morphine (the heroin metabolite) in blood and urine. Two methods reported utilized a stable isotope internal standard—N-trideuteromethyl-morphine—which was derivatized in one case with trifluoroacetic anhydride (TFA) (16) and in the other, trimethylsilylated with N,O-bis(trimethylsilyl)acetamide. (13) The previous case was a study of heroin hydrolysis in blood plasma and therefore also included determination of O^6-monoacetylmorphine with its respective deutero-TFA derivative as internal standard. (16) With the TFA derivative, m/e 364 (protio compound) and m/e 367 (deutero compound) were chosen to be monitored by MIM because they were high molecular weight fragments to insure specificity, and showed a high relative total intensity to insure sensitivity. The same fragments, m/e 364 and m/e 367, were monitored for determination of O^6-monoacetylmorphine. Prior to the GC-MS run of the plasma samples, the standards, trideuteromorphine and trideutero-O^6-monoacetylmorphine were added to the sample, and therefore subjected to identical chemical treatment as the substrate being analyzed. In the other morphine determination, in which the TMS derivatives were analyzed, four masses were monitored: m/e 340, 343, 414 and 417. Although it was only necessary to monitor one pair of masses, monitoring an additional pair provided convenient corroborative information.

d. Secondary and tertiary tricyclic antidepressants. The tricyclic antidepressants are used extensively in the treatment of depression. The common tricyclic tertiary amines include amitriptyline, imipramine and doxepin, and the common secondary amines include nortriptyline, desipramine and desmethyldoxepin. In order to correlate laboratory results with the clinical effect, both the primary drug and demethylated metabolite were measured.

The assay is performed in two parts: (a) extraction and direct injection of the extract, for analysis of the tertiary amines, and (b) derivatization and re-injection, for analysis of the secondary amines. To determine the tertiary tricyclic antidepressant amines by GC-MS, amitriptyline was measured at m/e 278, imipramine at m/e 281, and doxepin at m/e 280; clomipramine, the internal standard, was measured at m/e 317, the $(M+2)^+$ isotope peak. To determine the derivatized (TFA) secondary tricyclic antidepressants by GC-MS, desmethyltrimipramine-TFA (internal standard) was monitored at m/e 377, desipramine-TFA at m/e 363, desmethyldoxepin-TFA at m/e 363 and nortriptyline-TFA and protriptyline-TFA at m/e 360. (70)

D. *Other Ionization Techniques*

1. Field Ionization (FI)

FI techniques minimize fragmentation and also provide molecular ions of high intensity. Therefore, their mass spectra are potentially useful in the analysis of mixtures that are often encountered in biological samples. (17) Field ionization of gaseous sample molecules occurs when the molecule is brought near a metal surface in the vicinity of a high electric field (a field anode held at a highly positive potential).

Quantum mechanical tunneling of an electron from a molecule into the metal surface provides a positive ion. Since the ion has very little internal energy in the form of electronic or vibrational excitation, it is usually detected as a highly stable molecular ion. However, the efficiency and the sensitivity of FI is much less than that of EI or CI methods ($^1/_{10}$ to $^1/_{100}$), and ionization components are fragile. (10) (46)

In many cases, EI does not produce an intense (or any) molecular ion, even in the case of derivatization. Many compounds (e.g., amino acids) do not possess an adequate vapor pressure and therefore produce only the quasimolecular ions, (M+H) or (M−H), in their mass spectra. This is sufficient for indicating their molecular weights, but due to the absence of any fragment peaks in the spectra, structural information must be obtained by another method. FI, on the other hand, may show an intermediate degree of fragmentation, as well as an intense molecular ion. (17)

Field ionization, in general, may be used with the same types of samples and inlet systems as electron impact ionization. In addition, elemental composition determinations have been determined by FI on a high-resolution mass spectrometer on compounds which do not produce electron impact molecular ions. (11)

It has also been shown that alcoholic dehydration is not necessarily thermal in nature; it occurs with the FI source, producing the $(M-18)^+$ intense fragment. (17) Dual field ionization-electron impact, and dual field ionization-field desorption sources have been constructed. (10) (68) (2) The advantages of FI and CI over EI are obvious in that there will be an indication of the resulting molecular ions. However, at higher source temperatures (owing to reduced volatilities), protonated molecular ions are not observed.

2. Field Desorption (FD)

The utility of EI-, FI- and CI-MS for identification or structural elucidation of salts and highly polar, thermally labile, organic molecules is restricted because samples must be in the gaseous state prior to ionization and analysis. For these types of compounds, the energy required to disrupt intermolecular bonds is often greater than the required energy to break bonds within the molecule. Therefore, heating of the sample to volatilize it will decompose it. (36)

FD-MS does not, however, have the disadvantage that the substance be volatile, and many non-volatile solids have been analyzed by this technique. In addition, its resulting mass spectra can be expected to lead to minimal fragmentation. (17) In FD-MS, the sample is ionized from the adsorbed state from the field anode on which it is deposited. These anodes consist of 10 μm tungsten wires covered with a large number of carbon microneedles approximately 30 μm in length. Application of an external field lowers the potential for

migration of an electron from the sample molecule to the wire electrode. By increasing the temperature of the wire anode, ionization of the sample occurs under mild conditions and repulsion between the resulting positive ion and the emitter drives the ion into the gas phase. It is estimated that the total thermal energy involved in this process may be two or three times smaller than that required for direct thermal vaporization. (36)

As the excess thermal energy is very small, highly polar compounds of low volatility can be analyzed without the decomposition expected on thermal volatilization. Although FD has been shown to give spectra of many physiological compounds (without prior derivatization), the nature of the method prohibits GC introduction. (46) This may not, however, necessarily limit its general use to pure compounds.

Other problems with the method include an uncertainty in the precise moment of volatilization, irregular volatilization processes, instrument dependent heating rates, sample decomposition prior to ionization and excessive ion source contamination. (64) Since FD allows formation of molecular ions of molecules with low volatility, due to better control of emitter current, it many times will produce mass spectra which contain intense protonated molecular ions at these lower temperatures.

FD may give comparable results to FI, although differences in the intensity of the molecular ion by this latter technique may be dependent upon the solubility of the sample in the solvent on which it is adsorbed on the anode, and the resulting temperature at which field desorption will occur. Sample loading can be by the emitter dipping technique, or by use of an ultra-microsyringe in solvents, including deionized water, methanol, ethanol, or dimethylsulfoxide (DMSO).

Many of the thermally labile and nonvolatile compounds which have been analyzed by FD-MS include the alkali metal salts of acetic acid, nucleotides, amino acids, sodium salts of glucose phosphates and glucuronides and quaternary ammonium salts, potassium salts of some alkyl sulfates and cyclohexylphenyl sulfate, sodium salts of stilbestrol glucuronide, cortisone sulfate, deoxycholic acid, taurodeoxycholic acid, dehydrocholic acid, glycocholic acid, taurocholic acid, taurochenodeoxycholic acid and cyclic adenosine monophosphate, methotrexate and folic acid analogs, gentamicins, ionic dyes, and alkali elements. The spectra were obtained from solutions of the salts in water, with the addition of some methanol. Ions were produced which not only identified the molecular ions, but, in some cases, structurally useful fragment ions were also obtained. (21)

FD-MS has been shown to be uniquely useful for identifying sub-milligram quantities of ionic dyes. Identification of ionic dyes is generally difficult because molecular weights of the dye salts cannot be obtained by ionization methods which require vaporization of the dyes. Molecular weight information and characteristic fragment ions, for some dyes, were observed. (50)

In most cases, the potassium salts all showed quasimolecular $(M+K)^+$, and the sodium salts showed $(M+Na)^+$ quasimolecular ions. Other prominent ions could include the doubly-charged ion $(M+2Na)^{2+}$ and in the sodium salts of alkyl hydroxy-carboxylic acids $(M+Na-18)^+$ was produced. In the case of compounds containing a glycosidic linkage, CI spectra exhibit some fragmentation, although FD conditions produce the

$(M+H)^+$ ions with little glycosidic cleavage. In addition, FD has been shown to be useful in studies on underivatized sugars, as well as dissaccharides. (55) Related compounds present in mixtures as trace impurities may also be identified by their $(M+H)^+$ ions. In typical FD mass spectra, the molecular ion or quasimolecular ion $(M+H)^+$ was usually the base peak. The most frequent fragment ions of alcohols were due to $(M-OH)^+$ or $(M-H_2O)^+$. Many times, acids produced peaks at $(M+23)^+$ due to cationization by sodium present as an impurity in either the sample or the solvent. Disodium salts of dicarboxylic acids generally produced the more prominent ion due to $(M+Na)^+$, although other ions produced were attributed to $(M+2Na)^{2+}$, $(M-(ONa)_2)^+$ and $(2M-Na+H)^{2+}$. (40)

FD, as well as FI, techniques have also been used for SIM in drug analysis. (20)

It had been proposed that ions from each major component in a sample mixture could be used to afford a more rapid and precise method of quantification than those available for thermally labile compounds. Ions from each major component at $(M+H)^+$ could be monitored and related compounds as trace impurities could be identified by their highly characteristic $(M+H)^+$ ions. (55) Until recently, however, no analytical application of FD-MS had been reported. Experiments by H. R. Schulten, *et al.*, (48) explored the sensitivity and precision of FD-MS in the SIM mode for quantitative studies of alkali ions to show the potential of this technique for trace analysis. The sensitivity of the FD method for alkali ions exceeded the sensitivity for organic compounds. The sensitivity was of the same order of magnitude as the sensitivity of EI-MS for the detection of organic compounds. One of the reasons for this phenomenon is that the particles that are detected are already present as positive ions on the emitter surface. Field desorption of these monovalent ions is much more efficient than field desorption of organic compounds. In the case of organic compounds, the processes of thermal decomposition and evaporation of neutral molecules that do not undergo ionization compete effectively with the ionization. However, this does not occur for field desorption of alkali cations.

Levels of cesium were estimated in a variety of media: in spectrograde solvents, in body fluids such as saliva and blood, and in environmental samples, e.g., drinking water, seawater and a natural aerosol. The determination of cesium in sample sizes of 0.2 to 1 μl containing 0.3 to 100 pg of the element was achieved with precision and accuracy of $\pm 10\%$ and $\pm 20\%$, respectively. A linear emitter heating current programmer was used for the desorption of the samples, and the evaporation profiles for $(Cs)^+$ were obtained. From the peak areas of the evaporation profiles obtained in a standard, the unknown amount of the alkali element present in the sample was calculated. The results showed the potential of estimation by FD-MS of alkali elements in very small amounts of untreated biological and environmental samples. (48)

The quantitative FD data was in good agreement with the EI measurements and demonstrated the utility of FD-MS as a quantitative technique in biomedical research. In selecting internal standards for quantitative FD-MS, homologous compounds and extensively deuterated compounds are poor choices since their desorption behavior differs strongly. However, substances labeled with only a few deuterium atoms or ^{13}C, ^{15}N, ^{18}O are good internal standards since no fractionated desorption has been observed. (47)

3. Atmospheric Pressure Ionization (API)

The sample is introduced in a solvent and swept along by nitrogen carrier gas. The sample undergoes a complex series of ion molecule reactions in a chamber containing a ^{63}Ni foil or a corona discharge, as a source of electrons. Positive or negative sample ions are produced. These pass through a small aperture (25 μm diameter) to be analyzed in the mass analyzer. The reported limits of detection are in the femtogram range (10^{-15}g). Although no structural information is available, the simplicity of such spectra permits detection of known compounds with a separation stage. (45)

The system is very versatile; the source is able to accept a variety of gases and solvents. The sample may be introduced in the gas phase without solvents, by probe injection, or in the effluent stream from a gas chromatograph. Samples have been introduced in the liquid phase in solvents by injection, or in the effluent stream by HPLC, or in solution by syringe injection. (30)

4. Negative Ion Formation

In conventional ion sources, negative ions which are produced are normally trapped. A reversal of the potentials of the ion repeller and accelerator voltage plates can result in the acceleration and focusing of these negative ions.

Many compounds which show a response to electron capture detection (ECD) can also be determined mass spectrometrically by the resultant negative ions formed which lead to that response. The use of ECD-GC in the analysis of compounds present in low concentrations in highly complex samples is effective as a selective detector. Typical compounds which respond to ECD are halogen-containing drugs, derivatized compounds (with halogen-containing derivatizing agents—pentafluoropropionic anhydride, heptafluorobutyric anhydride, trifluoroacetic anhydride, etc.), substituted phenols, barbituric acid derivatives, diphenylhydantoin, biphenyls, polycyclic aromatics and heteroaromatics. (31) Additionally, oxygen as a CE gas appears to be particularly useful for negative CI studies on polyaromatic and polyhalogenated compounds. A negative ion mass spectrum can provide direct knowledge of the chemical events occurring under ECD conditions.

It also provides far less structural information than a positive ion mass spectrum. However, in quantitative procedures for selected molecules, electron capture currents can exceed their corresponding positive ion currents by two or three orders of magnitude. (58) The basic route of formation of a negative ion may be either by direct attachment of an electron (electron capture) or by reaction with a negative reactant ion. Further applications will be discussed in greater detail by Dr. Brandenberger in this Symposium. (8)

III. CONCLUSION

Within the past few years, many reviews have been written describing various aspects of mass spectral applications. These summaries have included applications in clinical chemistry, analytical systems based on mass spectrometry, mass fragmentography in biological and drug research, and chemical ionization methods in metabolite studies. This is the result of the increasing interest in modern scientific methods in solving many of today's problems. As such, this review has attempted to cover applications in forensic drug analysis, as well as related areas in which the techniques applied may make a significant future contribution to the science.

For any review to be of value to the reader, it should do more than merely compile, abstract and organize past accomplishments of researchers. In addition, it should attempt to project future developments in the "state-of-the-art." The more common applications of EI and CI mass spectrometry have been covered. In addition, one can anticipate increased usage of FI, FD, and API methods, and instrumental development of multiple ionization sources. With respect to sample handling prior to ionization in the mass spectrometer, more selective derivatizing techniques, the development of more applications of stable isotope internal standards in the quantification of drugs, and increased usage of the liquid chromatograph-mass spectrometer interface can be expected. Other methods of improvement in selective detection of sample molecules can be anticipated by increased application of SIM with high resolution mass spectrometers, further development of reagent gas mixtures in CI, and more common use of negative ion MS.

REFERENCES

1. Anbar, M. and Aberth, W. H., *Anal. Chem.*, 46 (1), 59A–64A (1974).
2. Anbar, M. and St. John, G. A., *Anal. Chem.*, 48 (1), 198–203 (1976).
3. Barron, R. P., Kruegel, A. V., Moore, J. A., and Kram, T. C., *J. AOAC*, 57 (5), 1147–58 (1974).
4. Baty, J. D. and Wade, A. P., *Anal. Biochem.*, 57, 27–37 (1974).
5. Bjorkhem, I., Blomstrand, R., and Svensson, L., *Clin. Chem. Acta*, 54, 185–93 (1974).
6. Boerner, U., Roe, R. L., and Becker, C. E., *J. Pharm. Pharmac.*, 26, 393–98 (1973).
7. Boyd, P. M., Moses, P. J., and Bowman, L., *Spectroscopy Letters*, 7, (4 & 5), 223–27 (1974).
8. Brandenberger, H., presented at the International Symposium on Instrumental Applications in Forensic Drug Chemistry, Arlington, VA May 29–30, 1978.
9. Carroll, D. I., Dzidic, I., Stillwell, R. N., Haegele, K. D., and Horning, E. C., *Anal. Chem.*, 47 (14), 2369–73 (1975).
10. Chait, E. M., *Anal. Chem.*, 44 (3), 77A–91A (1972).
11. Chait, E. M., Shannon, T. W., Amy, J. W., and McLafferty, F. W., *Anal. Chem.*, 40 (4), 835–37 (1968).
12. Chao, J.-M., Saferstein, R., and Manura, J., *Anal. Chem.*, 46 (2), 296–98 (1974).
13. Clarke, P. A. and Foltz, R. L., *Clin. Chem.*, 20 (4), 465–69 (1974).
14. Compson, K. R., Evans, S., Hazelby, D., and Moore, L. E., presented at the Twenty-Fifth Annual Conference on Mass Spectrometry and Allied Topics, Washington, DC, May 29–June 3, 1977.
15. Dubois, J.-P., Kung, W., Theobald, W., and Witz, B., *Clin. Chem.*, 22 (6), 892–97 (1976).
16. Ebbighausen, W. O. R., Mowat, J. H., Stearns, H., and Vestergaard, P., *Biomed. Mass Spectrom.*, 1, 305–11 (1974)
17. Fales, H. M., Milne, G. W. A., Winkler, H. U., Beckey, H. D., Damico, J. N., Barron, R., *Anal. Chem.*, 47 (2), 207–19 (1975).

18. Flamm, B. L. and Gal J., *Biomed. Mass Spectrom.*, 2, 281–83 (1975).

19. Frick, W., Chang, D., Folkers, K., and Daves, G. D., Jr., *Anal. Chem.*, 49 (8), 1241–45.

20. Frigerio, A. and Ghisalberti, E. L., "Mass Fragmentography (Single and Multiple Ion Monitoring) in Drug Research," in *Mass Spectrometry in Drug Metabolism* (A. Frigerio and E. L. Ghisalberti, Eds.), p. 131–49, Plenum Press, New York, 1977.

21. Games, D. E., Games, M. P., Jackson, A. H., Olavesen, A. H., Rossiter, M., and Winterburn, P. J., *Tetrahedron Letters*, 27, 2377–80 (1974).

22. Ghisalberti, E. L., "Chemical Ionization Mass Spectrometry in the Identification of Drugs and Drug Metabolites," in *Mass Spectrometry in Drug Metabolism* (A. Frigerio and E. L. Ghisalberti, Eds.), p. 291–324, Plenum Press, New York, 1977.

23. Gordon, A. E. and Frigerio, A., *J. Chromatogr.*, 73, 401–17 (1972).

24. Hachey, D. L., Kreek, M. J., and Mattson, D. H., *J. Pharm. Sci.*, 66 (11), 1579–82 (1977).

25. Harvey, D. J., *Biomed. Mass Spectrom.*, 4 (2), 88–93 (1977).

26. Harvey, D. J. and Paton, W. D. M., *J. Chromatogr.*, 109, 73–80 (1975).

27. Hatch, F. and Munson, B., *Anal. Chem.*, 49 (6), 731–33 (1977).

28. Henion, J. D. presented at the Twenty-Fifth Annual Conference on Mass Spectrometry and Allied Topics, Washington DC, May 29–June 3, 1977.

29. Herman, G. J. and Kan, M.-N., *Biomed. Mass Spectrom.*, 1 (5), 350 (1974).

30. Horning, E. C., Carroll, D. I., Dzidic, I., Haegele, K. D., Lin, S.-N., Oertli, C. U., and Stillwell, R. N., *Clin. Chem.*, 23 (1), 13–21 (1977).

31. Horning, E. C., Carroll, D. I., Dzidic, I., Lin, S.-N., Stillwell, R. N., and Thenot, J.-P., *J. Chromatogr.*, 141, 481–95 (1977).

32. Horning, E. C., Horning, M. G., Carroll, D. I., Dzidic, I., and Stillwell, R. N., in *Advances in Biochemical Psychopharmacology*, Vol. 7 (E. Costa and B. Holmstedt, Eds.), p. 15, Raven Press, New York, 1973.

33. Horning, M. G., Brown, L., Nowlin, J., Lertratanangkoon, K., Kellaway, P., and Zion, T. E., *Clin. Chem.*, 23 (2), 157–64 (1977).

34. Horning, M. G., Nowlin, J., Butler, C. M., Lertratanangkoon, K., Sommer, K., and Hill, R. M., *Clin. Chem.*, 21 (9), 1282–87 (1975).

35. Horning, M. G., Nowlin, J., Lertratanangkoon, K., and Thenot, J.-P., presented at the International Symposium on Instrumental Applications in Forensic Drug Chemistry, Arlington, VA, May 29–30, 1978.

36. Hunt, D. F., Shabanowitz, J., Botz, F. K., Brent, D. A., *Anal. Chem.*, 49 (8), 1160–63 (1977).

37. Jardine, I. and Fenselau, C., *Anal. Chem.*, 47, 730 (1975).

38. Jenden, D. J. and Cho, A. K., *Annu. Rev. Pharmacol.*, 13, 371 (1973).

39. Jindal, S. P. and Vestergaard, P., *J. Pharm. Sci.*, 67 (2), 260–61 (1978).

40. Kirk, M. C., Coburn, W. C., Jr., and Piper, J. R., *Biomed. Mass Spectrom.*, 3, 245–47 (1976).

41. Klein, M., "Analysis of Impurities in Illicit Heroin," in *Mass Spectrometry in Drug Metabolism* (A. Frigerio and E. L. Ghisalberti, Eds.), p. 449–63, Plenum Press, New York, 1977.

42. Klein, M., presented at the International Symposium on Mass Spectrometry in Drug Metabolism, Milan, Italy, June 1976.

43. Klein, M. and Moore, J. M., presented at the Twenty-Fifth Annual Conference on Mass Spectrometry and Allied Topics, Washington, DC, May 29–June 3, 1977.

44. Kram, T. C., *J. Pharm. Sci.*, 66 (3), 443 (1977).

45. Kram, T. C. and Kruegel, A. V., *J. Forensic Sci.*, 22 (1), 40–52 (1977).

46. Lawson, A. M., *Clin. Chem.*, 21 (7), 803–24 (1975).

47. Lehmann, W. D., Beckey, H. D., and Schulten, H.-R., presented at the Twenty-Fifth Annual Conference on Mass Spectrometry and Allied Topics, Washington, DC, May 29–June 3, 1977.

48. Lehmann, W. D. and Schulten, H.-R., *Anal. Chem.*, 49 (12), 1744–46 (1977).

49. Lehrer, M. and Karmen, A., *Biochem. Med.*, 14, 230–37 (1975).

50. McEwen, C. N., Layton, S. F., and Taylor, S. K., *Anal. Chem.*, 49 (7) 922–26 (1977).

51. Min, B. H. and Garland, W. A., *J. Chromatogr.*, 139, 121–33 (1977).

52. Moore, J. M. and Klein, M., *J. Chromatogr.*, 154, 76–83 (1978).

53. Murata, T. and Takahashi, S., *Anal. Chem.*, 49 (6), 728–31 (1977).

54. Palmer, L., Bertilsson, L., Collst, P., and Rawlins, M., *Clin. Pharm. and Therapeutics*, 14 (5), 827–32 (1973).

55. Parfitt, R. T., Games, D. E., Rossiter, M., Rodgers, M. S., and Weston, A., *Biomed. Mass Spectrom.*, 3, 232–34 (1976).

56. Pearce, D. S., *Clin. Chem.*, 22 (10), 1623–26 (1976).

57. Politzer, I. R., Dowty, B. J., and Laseter, J. L., *Clin. Chem.*, 22 (11), 1775–88 (1976).

58. Prescott, S. R., Campara, J. E., and Risby, T. H., *Anal. Chem.*, 49 (11), 1501–04 (1977).

59. Quilliam, M. A. and Westmore, J. B., *Anal. Chem.*, 50 (1), 59–68 (1978).

60. Rosenfeld, J., Bowins, B., Roberts, J., Perkins, J., and MacPherson, A. S., *Anal. Chem.*, 46 (14), 2232–34 (1974).

61. Rosenfeld, J. and Taguchi, V. Y., *Anal. Chem.*, 48 (4), 726–29 (1976).

62. Sheehan, M., Hertel, R. H., and Kelly, C. T., *Clin. Chem.*, 23 (1), 13–21 (1977).

63. Sobol, S. P. and Sperling, A., presented at the Forensic Science Symposium, ACS National Meeting, Atlantic City, NJ, Sept. 1974, *ACS Symposium Series*, 18 (13), 170–82 (1975).

64. Soltmann, B., Sweeley, C. C., and Holland, J. F., *Anal. Chem.*, 49 (8), 1164–65 (1977).

65. Stromberg, L., *J. Chromatogr.*, 96, 179–87 (1974).

66. Urich, R. W., Bowerman, D. L., Wittenberg, P. H., McGaha, B. L., Schisler, D. K., Anderson, J. A., Levisky, J. A., and Pflug, J. L., *Anal. Chem.*, 47 (3), 581–83 (1975).

67. Van Boven, M., Janssen, G., and Daenens, P., *Mikrochimica Acta*, 603–10 (1974).

68. Wanless, G. G. and Glock, G. A., Jr., *Anal. Chem.*, 39 (1), 2–13 (1967).

69. Weinkam, R. J., Wen, J. H. C., Furst, D. E., and Levin, V. A., *Clin. Chem.*, 24 (1), 45–49 (1978).

70. Wilson, J. M., Williamson, L. J., and Raisys, V. A., *Clin. Chem.*, 23 (6), 1012–17 (1977).

71. Zagalak, M. J., Curtius, H.-Ch., Leimbacher, W., and Redweik, U., *J. Chromatogr.*, 142, 523–31 (1977).

72. Zarembo, J. E., Killmer, L. B., Berkoff, C. E., Rivard, D., and Kirkpatrick, D., presented at the Twenty-Fifth Annual Conference on Mass Spectrometry and Allied Topics, Washington, DC, May 29–June 3, 1977.

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The Use of Stable Isotopes in the Quantification of Drugs

by

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I. INTRODUCTION

Recently, interest in monitoring blood levels of drugs, particularly those drugs which are administered chronically, has increased significantly. Many methods for quantifying drugs in plasma, urine, and saliva are available based either on gas chromatography (GC), high performance liquid chromatography (HPLC), competitive protein binding (RIA and EMIT), spectrophotometric methods or gas chromatographic-mass spectrometric computer (GC-MS-COM) procedures. From an analytical point of view, two types of procedures are needed; (a) simple, rapid, and reasonably precise methods for clinical laboratories and (b) reference procedures with high precision and accuracy that can be used to validate the clinical procedures. The reference methods frequently involve several complicated and time-consuming steps and consequently are not suitable for routine use in clinical chemistry laboratories.

The most satisfactory reference procedures at the present time are those based on the use of GC-MS-COM systems. The analyses are carried out by selected ion monitoring using magnetic or electrical field instruments operated in either the electron impact (EI) or chemical ionization (CI) mode. These reference methods are usually based on the use of stable isotope-labeled internal standards which are essential for high precision and accuracy.

II. SAMPLE PREPARATION

For GC-MS-COM analysis sample preparation involves the transfer of the drug(s) from an aqueous into an organic matrix. The stable isotope-labeled internal standard should be added to the biological sample as early as possible and preferably before isolation is initiated. It is important that the initial measurements of the biological sample and the internal standard be done very carefully because any error in either measurement will decrease the precision and accuracy of the final determination. Pipetting mistakes can be a major source of error.

If the labeled internal standard is added to the plasma, urine, or saliva prior to isolation, it is necessary to carry out only one extraction with an organic solvent, thus saving time. Although subsequent extractions will increase the total amount of drug extracted, the ratio of the drug to internal standard will remain unchanged and in the GC-MS-COM analyses, it is the ratio of drug to internal standard that is measured. If the internal standards are homologs or analogs, it may be necessary to carry out several extractions, depending on differences in the partition coefficients of the drug and internal standard.

Adding the internal standard to the biological sample before isolation provides an additional advantage. Subsequent loss of part of the sample, for whatever reason, will not affect the precision or the accuracy of the analysis; the ratio of drug to internal standard will not be altered by the loss.

The stability of solutions of labeled internal standards (and also unlabeled internal standards) must be checked frequently. This is accomplished most readily by carrying out quantitative mass spectrometric comparisons of the old solution and a freshly prepared solution of labeled internal standard with a reference solution of the unlabeled drug.

III. DERIVATIZATION

In our studies, the extracted drugs and internal standards were converted into derivatives

for GC-MS-COM analysis. Methylation with diazomethane or ethylation with diazoethane followed by silylation with bis-trimethylsilyl-acetamide and pyridine was the preferred procedure (1). However, many other types of derivatives can be used (2). The chief considerations governing the choice of derivatives for quantitative work are adsorption behavior and stability. When adsorption is minimal, symmetrical GC peaks are obtained resulting in improved quantification, and losses in the analytical system are reduced resulting in increased sensitivity of detection. Most derivatives used in gas phase analytical methods have sufficient stability for quantitative work. Precautions may be needed for relatively labile compounds; for example, N-silyl derivatives are silylating agents and trimethylsilyl esters of carboxylic acids are easily hydrolyzed.

IV. GC-MS-COM ANALYSIS

When quantification is carried out with a GC-MS-COM system, the mass spectrometer is used as a specific ion detector. This technique has been called "mass fragmentography" (3) but the term "selected ion detection" (SID) is used more frequently today. The detection process involves monitoring the appearance of preselected ions which are characteristic of the drugs under study and the labeled internal standards. The mass spectrometer can be operated in either the electron impact (EI) or the chemical ionization (CI) mode. A comparison of the EI spectrum of diphenylhydantoin (N-methyl derivative) (Figure 1) with the CI (methane spectrum) of the same compound (Figure 2) shows why CI techniques are useful in quantitative work. Only a few ions are obtained under CI conditions and the major ionic product usually corresponds to MH^+, the protonated molecular ion. For some drugs and drug derivatives, however, the major ion may be a characteristic fragment ion; under these conditions the fragment ion or the fragment ion and MH^+ can be monitored. If CI conditions are used with nitrogen, argon, or helium as the carrier gas, the resulting spectra resemble EI spectra taken under 15–20 eV conditions. This form of ionization may be useful in some drug analyses.

V. INTERNAL STANDARDS

In our studies, internal standards labeled with ^{13}C, ^{15}N and ^{2}H (deuterium) have been used. Many labeled drugs are available from commercial sources.[1] However, if the drugs under study contain methyl, ethyl, or isopropyl groups, it may be easier and cheaper to synthesize the deuterated analog of the drug in the laboratory by the use of the appropriate deuterated alkyl iodide. It is advisable to have at least three heavy atoms in the labeled internal standard to avoid any overlap with ions at M+1 and M+2 due to the natural abundance of heavy isotopes of carbon, hydrogen, sulfur, chlorine, bromine, or silicon which may be present in the drug or its derivative. For example, in Figure 1 ions at M+1 in the spectrum of unlabeled Dilantin are due to the natural abundance of ^{13}C. Regardless of the source of the labeled internal standard, it is necessary to check its isotopic composition and purity.

The isotopic composition can be checked by mass spectrometric analysis. An example is shown in Figure 2; the chemical ionization spectrum of diphenylhydantoin-2,4,5-^{13}C (MH^+=270) is compared to the chemical ionization spectrum of the unlabeled diphenylhydantoin (MH^+=267) obtained as an analytical

[1] *Stable isotope labeled drugs can be obtained from Merck, Sharp and Dohme, Canada, Ltd.; Pointe Claire-Dorval, Quebec, Canada; and Kor Isotopes, Cambridge, Massachusetts.*

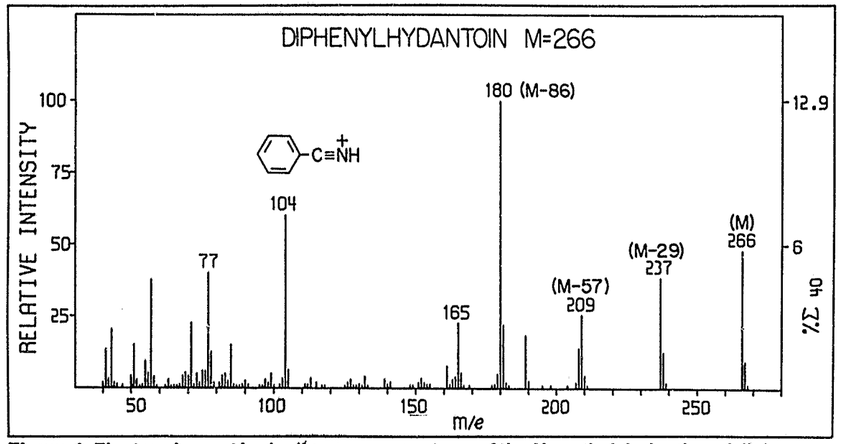

Figure 1. Electron impact ionization mass spectrum of the N-methyl derivative of diphenylhydantoin; molecular ion (M) is present at *m/e* 266.

standard from Applied Science Laboratories. From the chemical ionization spectrum it is obvious that the labeled internal standard contains an appreciable amount (20%) of diphenylhydantoin containing only two ^{13}C atoms in the molecule (MH^+=269). Thus for every milligram of the labeled standard added only 0.8 milligram is present as the species quantified by mass spectrometry at MH^+=270. This correction factor must be included in the calculations of plasma concentrations.

The purity of the labeled standard must also be evaluated. The CI spectrum of the labeled diphenylhydantoin is identical with the corresponding spectrum of analytical standard of diphenylhydantoin (Figure 2). However, in some samples impurities eluting with a different retention time in a GC-MS analysis or nonvolatile substances (salts) may contaminate labeled standards. Mass spectral analysis (SID) can be used to check the purity. The quantitative response of the labeled internal standard and the analytical standard should be the same (microgram/microgram) after correction for the isotopic composition of the labeled internal standard has been made. In our experience, the commercial stable isotope-labeled standards have been very satisfactory.

If the internal standard has been labeled with deuterium, an additional source of error in quantification may be introduced because the properties of the deuterated internal standard may differ significantly from those of the unlabeled drug. For example, the solubility, partition coefficients and hydrogen bonding of the two species may not be identical. These effects are illustrated in Figures 3 and 4.

In Figure 3, caffeine and *tri*-trideuteromethylxanthine (d_9-caffeine) apparently were eluted as one symmetrical gas chromatographic peak. In Figure 4, the same sample was ana-

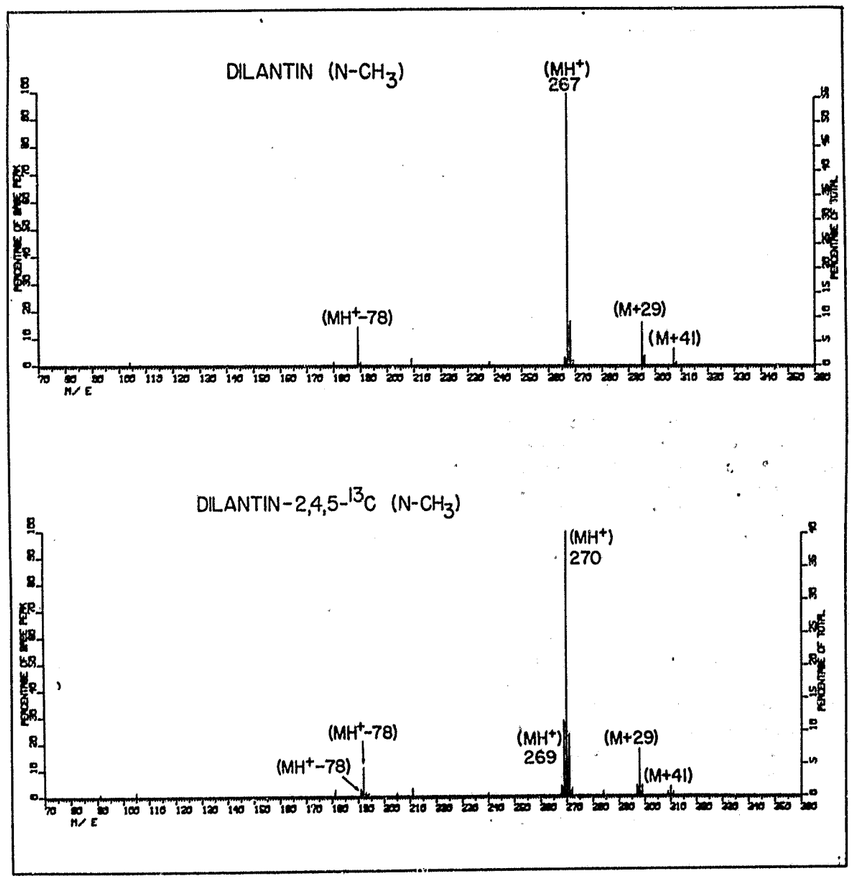

Figure 2. Chemical ionization (methane) mass spectra of the N-methyl derivatives of diphenylhydantoin (Dilantin) and the stable isotope-labeled standard, 2,4,5,-^{13}C-diphenylhydantoin. The major ions at 267 and 270 correspond to the protonated molecular ions, MH^+.

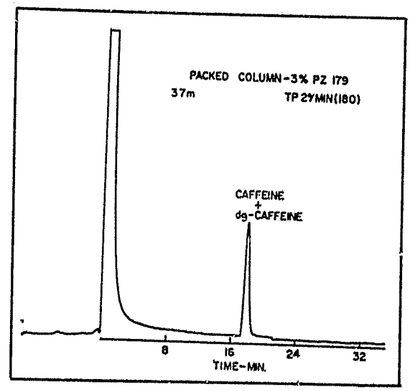

Figure 3. Gas chromatographic analyses of a mixture of caffeine and 1,3,7-*tri*-trideuteromethylxanthine (d_9-caffeine). The analysis was carried out by temperature programming at 2°/min from 180°C using a 3.7 m glass column packed with 3% PZ-179 (Poly-S 179, Applied Science Laboratories).

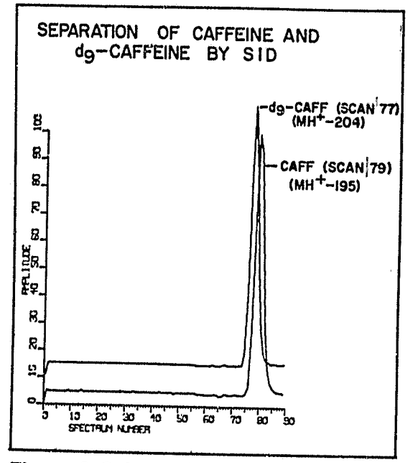

Figure 4. Selected ion detection chart for the analysis of caffeine and d_9-caffeine; using a GC-MS-COM system operated in the CI mode with methane as the carrier gas. The ions monitored were *m/e* 204 (MH^+, d_9-caffeine) and *m/e* 195 (MH^+, caffeine).

lyzed by selected ion monitoring with a GC-MS-COM system operated in the CI mode. The protonated molecular ions at *m/e* 195 (MH^+, d_0-caffeine) and 204 (MH^+, d_9-caffeine) were monitored. It is obvious that d_0-caffeine is eluted from the column (scan 77) before d_9-caffeine (scan 79). If the caffeine had been quantified on the basis of the amount of d_9-caffeine present in scan 79 rather than scan 77, a considerable error would have been introduced. Similar results have been obtained with several other deuterium-labeled drugs.

VI. PRECISION AND ACCURACY

The precision and accuracy of quantitative analyses of biological samples carried out with a GC-MS-COM system using stable isotope-labeled internal standards should be 2–6% for the overall analysis. If the biological sample (plasma, saliva, urine) and the internal standard are measured by weight rather than by volume, the precision and accuracy are improved significantly. The overall precision of GC-MS methods under these conditions can be as low as 0.33% and the precision of the mass spectrometric analysis as low as 0.17% (4).

We have compared the precision and accuracy of GC-MS-COM procedures for monitoring anticonvulsant drugs with methods based on HPLC and EMIT. Plasma standards supplied by the National Bureau of Standards con-

taining several anticonvulsant drugs were used. The GC-MS-COM procedure involved four steps: extraction, concentration of the sample, derivatization, and instrumental analysis; the HPLC procedure involved three steps: extraction, concentration of the sample, and instrumental analysis; EMIT involved only instrumental analysis. Ten analyses were made of each sample by each procedure. The mean and standard deviation were calculated by the use of a computer program. The results are summarized in Tables I–III.

In Table I quantification of phenytoin by EMIT and HPLC are compared. The standard deviation (SD) for the EMIT analyses was 0.28 μg/ml and for HPLC the standard deviation was 1.12 μg/ml. The mean found by both methods was 7.3 μg/ml; the value supplied by NBS was 7.3 μg/ml. In Table II, the results of HPLC and GC-MS-COM analyses are compared using another NBS plasma sample. The SD for the HPLC analyses of phenytoin was 0.76 μg/ml and the SD for the GC-MS-COM analyses was 0.51 μg/ml. The mean of the HPLC analyses was 22.8 μg/ml and the mean of the GC-MS-COM analyses was 22.3 μg/ml. The value provided by NBS for this sample was 23.8 μg/ml. The precision and accuracy of the three methods for these samples were excellent.

In Table III the analyses of phenobarbital by GC-MS-COM, EMIT, and HPLC are compared. The SD was 1.5 μg/ml, 1.4 μg/ml, and 1.5 μg/ml for the GC-MS-COM, HPLC, and EMIT analyses, respectively. The mean of the GC-MS-COM determinations was 33.7 μg/ml; the mean obtained by HPLC was 37.2 μg/ml; and the mean obtained by EMIT was 37.9 μg/ml. The NBS value for this sample was 36.3 μg/ml. The precision of the three methods was essentially the same but the results found by the GC-MS-COM method were less accurate than the HPLC and EMIT determinations. Since the precision of the GC-MS-COM analyses was excellent and since the precision and accuracy of the GC-MS-COM determinations for the phenytoin set in Table II was excellent, these results suggest that a slight error in the addition of the internal standard and/or the sample occurred.

Table IV illustrates one of the difficulties encountered with the EMIT procedure. Two different sets of plasma samples with low, medium, and high concentrations of phenobarbital were analyzed by GC-MS and EMIT. At high plasma concentrations, the EMIT method was not very reliable and dilution of the sample usually did not improve the accuracy. The GC-MS-COM method was accurate at the high concentrations; the GC-MS method was also precise and accurate at concentrations much lower than can be measured by EMIT.

It is not possible to define the accuracy to be expected from GC-MS-COM methods in all applications, but in most work the accuracy should approach the precision. This assumes that appropriate precautions have been taken in sample preparation and that sources of error have been identified and eliminated. The sensitivity and selectivity of detection coupled with the precision and accuracy that GC-MS-COM methods provide when stable isotope-labeled internal standards are used, make these the method of choice for validation of other analytical procedures.

Table I

PHENYTOIN-7.3 μg/ml (NBS VALUE)

METHOD	MEAN* μg/ml	SD μg/ml	AC
EMIT	7.3	0.28	0.0
HPLC**	7.3	1.12	0.0

*Ten determinations
**Based on peak area

Table II

PHENYTOIN-23.8 μg/ml (NBS VALUE)

METHOD	MEAN* μg/ml	SD μg/ml	AC
GC-MS-COM	22.3	0.51	−1.5
HPLC	22.8	0.76	−1.0

*Ten determinations

Table III

PHENOBARBITAL-36.3 μg/ml (NBS VALUE)

METHOD	MEAN* μg/ml	SD μg/ml	AC
GC-MS-COM	33.7	1.5	−2.6
EMIT	37.9	1.4	+1.6
HPLC**	37.2	1.5	+0.9

*Ten determinations
**Based on peak area

Table IV

PHENOBARBITAL

	SET 1	GC-MS* μg/ml	EMIT* μg/ml	NBS-VALUE μg/ml
I	(Low)	8.65	9.0	8.5
II	(Medium)	22.0	22.2	22.7
III	(High)	110.6	100.0	112.2
	SET 2			
I	(Low)	8.6	9.0	8.3
II	(Medium)	35.4	37.8	36.3
III	(High)	110.6	95.0	107.0

*Average of duplicate determinations

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REFERENCES

1. Harvey, D. J., Glazener, L., Johnson, D. G., Butler, C. M., and Horning, M. G., *Drug Metab. Dispos.*, 5, 527 (1977).
2. Horning, E. C., Thenot, J-P., and Horning, M. G., "Quantitative Mass Spectrometry in Life Sciences," p. 1. Elsevier Scientific Publishing Company, Amsterdam, 1977.
3. Hammar, C-G., Holmstedt, B., and Ryhage, R., *Anal. Biochem.*, 25, 532 (1968).
4. Schaffer, R., White, E., Sniegoski, L. T., and Cohen, A., "Trace Organic Analysis: A New Frontier in Analytical Chemistry," presented at the 9th Materials Research Symposium, Gaithersburg, MD, April 10–13, 1978; Special Publication 591, National Bureau of Standards.

Negative Ion Mass Spectrometry—a New Tool for the Forensic Toxicologist

by

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I. INTRODUCTION

A. Negative Ions by Electron Impact

Conventional mass spectrometry by electron impact (EI-MS) is essentially a positive ion method. Table I lists a selection of negative ions which are produced. Most of them belong to the low mass range and have little analytical value. Among the few exceptions are some molecular range anions yielded by alcohols and acids. But even those have not much significance since the anion yields are more than 4 magnitudes of 10 lower than the cation yields.

B. Negative Ions by Methods related to Chemical Ionization

Table II lists the approaches of some research groups which have successfully applied techniques related to chemical ionization (CI) to negative ion formation. In all cases, reagent gases such as argon, nitrogen, oxygen and others are used to transmit the negative charge to the substrate molecules. The chamber pressure is usually kept high, at 0.1 to 1 torr (analogous to positive CI) (2, 5) or even at atmospheric pressure (3, 4). Good yields are reported, but in most cases, the investments were high, both with respect to instrumentation and time. Special ion sources or even custom-made instruments were constructed for the negative ion work: a Duoplasmatron mass spectrometer by v. Ardenne (1), two external atmospheric pressure chambers by Horning, one with a radio-Ni-foil (3), the other with a Corona source (4), a Townsend discharge source by Hunt, who has also built an instrument for simultaneous positive and negative ion recording (5).

Table I

Negative ions by electron impact (at 70 eV and near 10^{-6} torr)

Aliphatics: $[H]^-$, $[C_2]^-$, $[C_2H]^-$, $[C_x]^-$, $[C_xH]^-$

Aromatics: $[C_2H]^-$, $[C_4H]^-$, $[C_6H]^-$

Oxygen compounds: $[O]^-$, $[OH]^-$, $[C_2HO]^-$
—alcohols little $[M-1]^-$ and $[M-3]^-$
—acids some $[M-1]^-$

Nitrogen compounds: $[CN]^-$, $[C_xN]^-$

Halogen compounds: $[F]^-$, $[Cl]^-$, $[Br]^-$, $[I]^-$

Negative ions yield ≦ 10^{-4} of positive ion yield

II. OUR STUDIES WITH ANION MASS SPECTROMETRY

A. Instrumentation

When we decided to start with negative mass spectrometry by CI, we chose a very simple approach. In collaboration with Dr. R. Ryhage from the Karolinska Institute for Mass Spectrometry, 2 units of commercially available magnetic sector instruments with combined EI/CI source (LKB-2091) were equipped with new power supplies which permit reversion of magnet current, accelerating voltage and repeller voltage by shifting a single switch. The modified instruments are capable of recording anion or cation spectra. The switch-over time is less than a minute (6, 7, 8).

B. General Aspects of Anion Spectra

We have now dealt with anion MS by CI for about one year. We have tried over 10 different reagent gases (6, 7), optimized some of the conditions and made a few systematical studies (9, 10). Even though the field is still new and a great deal of development work remains to be done, anion CI-MS has already become—in our forensic laboratory in Zurich—one of the most powerful analytical tools in the daily toxicological service work (8, 11). The following examples are chosen in order to outline—point by point—the advantages of the technique as we use it.

Table II

Negative ion formation by methods related to chemical ionization

Research group	Instrumentation and principle	Chamber pressure	First paper	Ref.
v. Ardenne	Argon Duoplasmatron, electron attachment	10^{-2} torr	1958	(1)
Dougherty	CI-MS with CH_4 and CH_2Cl_2, cluster formation	1 torr	1968	(2)
Horning	External reaction chamber with radio-Ni-foil	atm. (API)	1973	(3)
Horning	External reaction chamber with Corona source	atm. (API)	1974	(4)
Hunt	Townsend discharge source, simultaneous positive and negative ion recording	1 torr	1976	(5)

Figure 1 gives the cation EI and the anion CI mass spectra of etonitazene, which is probably the most potent analgetic ever synthesized. We realize that anion CI-MS is a soft ionization technique which quite often permits recognition of the molecular mass. In 1966, a batch of this substance was sold on the European black market, and we had to identify it. It took us—with the help of UV, IR, NMR and MS, and with all the necessary purification steps—6 weeks (12). Anion MS would have been useful, especially in combination with GC. It shows the molecular mass 396 at first sight, it indicates the presence of a nitro group by the anions with mass 46 and $[M-16]^-$, and it points to the loss of a side chain with 100 mass units (side chain abstraction is typical for anion MS by CI).

Figure 2 shows that the molecular anion may lose hydrogen, often one (as in the case of codeine), sometimes two (as in the case of morphine), and in some cases even more. It is interesting to note that dihydrocodeinone, which does not possess a hydroxy group, gives a stable molecular anion.

Figure 3 contains the EI-spectrum and the cation and anion CI-spectra of a saturated barbiturate. The anion in the molecular range is $[M-1]^-$, an even electron ion. It is also the base anion, just like the quasimolecular ion

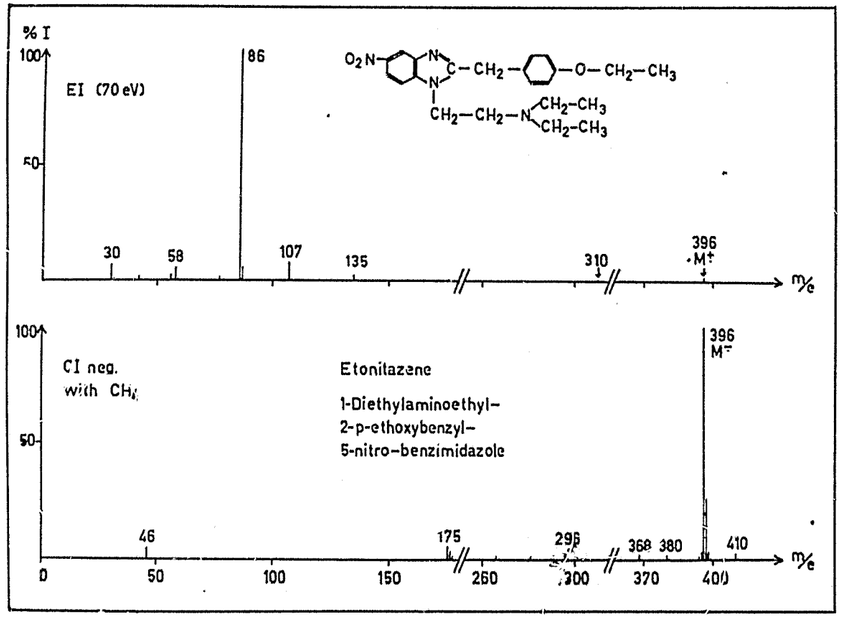


Fig. 1. EI and anion CI spectra of the narcotic Etonitazene.

$[M+1]^+$ in cation CI. Two additional facts are important:

1. Anion mass spectra are often simple, but still highly characteristic. This helps in the interpretation. Barbiturates show, besides proton abstraction, sidechain abstraction (anions M−29 and M−57) and an anion with mass 42.
2. While cation CI—apart from pointing out the molecular mass—does not furnish much more information than EI-MS, anion CI is a complementary method. It does not duplicate the EI-results; it supplements them. That is one of the most important points.

Figure 4 shows the corresponding spectra of an allyl barbituric acid. Allyl abstraction is favored over proton abstraction; $[M-41]^-$ is the base anion. This holds for all allyl barbituric acids. Again, we would like to point out that cation EI and anion CI are complementary. Often, the sum of the base ions of the 2 methods gives the molecular mass of the compound investigated. For identification work we recommend using—side by side—cation EI and anion CI mass spectrometry.

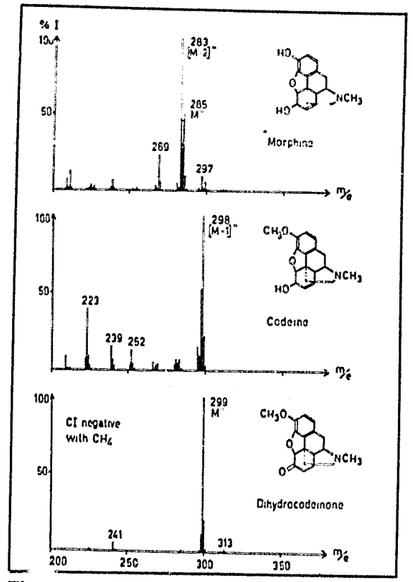


Fig. 2. Anion CI spectra of Morphine, Codeine and Dihydrocodeinone

C. Reagent Gases and Flexibility

Anion MS by CI is an extremely flexible method. It is possible to guide the fragmentation process by proper choice of the reagent gas. So far, we have worked with over 10 different reagents. Figure 5 gives the anion mass spectra for 8 of them.

Noble gases, nitrogen, and essentially also methane do not yield negative ions on electron impact. They act primarily as moderators for the production of low energy electrons which can convert substrate molecules to anions by electron attachment and dissociative electron attachment.

For the analysis of compounds with low electron affinity, reagents which readily form negatively-charged ions are often better choices. The negative charge is transmitted from the reagent anion to the substrate molecule by charge exchange or by anion cluster formation. Methylene chloride has been used for this purpose (2). We prefer Freons. Since they are gases, dosage is easier. The chloride and fluoride ions are good cluster-forming agents. SF_6^- was initially our choice as a charge exchange agent, since it is formed easily

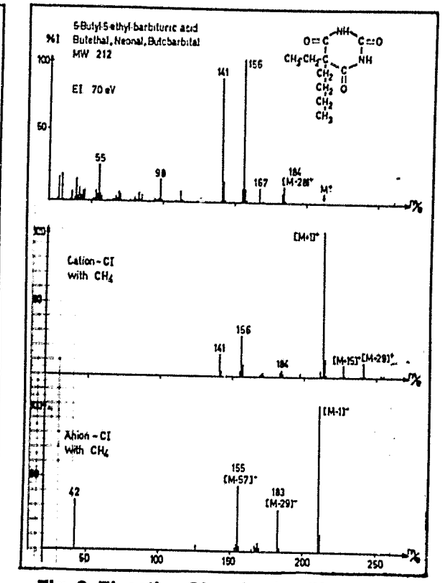


Fig. 3. EI, cation CI and anion CI spectra of a saturated barbiturate

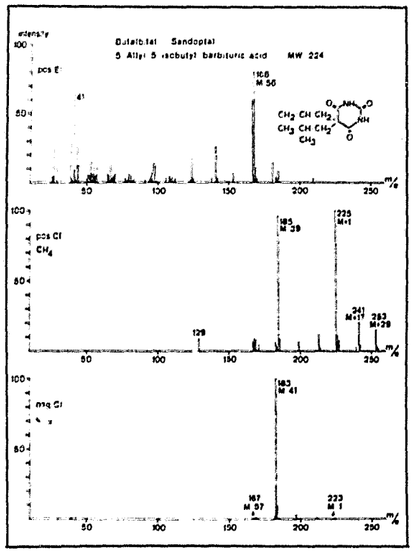


Fig. 4. EI, cation CI and anion CI spectra of an allyl barbituric acid

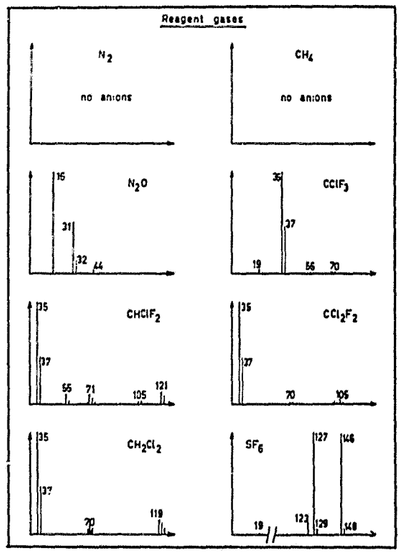


Fig. 5. Anion spectra of 8 reagent gases used for anion CI

on electron impact; however, it also produces fluoride cluster anions.

Nitrous oxide yields O^- on electron impact and permits oxidations in the ion source, in presence of the heated filament. Other authors have used $[O_2]^-$, which has to be produced from oxygen for this purpose (3, 4). This necessitates a special ion source.

The anion spectra in Figures 1 to 4 have been obtained by electron attachment and dissociative electron attachment, using methane as reagent. Figure 6 shows the anion spectra of 3 hypnotics obtained with nitrous oxide. The strong base O^- is a powerful charge exchange agent. Proton abstraction leads to the base ions $[M-1]^-$. The anions which exceed the molecular masses by 15 or 30 mass units are oxygen substitution products. By using deuterated compounds, we could verify that 1 or 2 hydrogens are replaced by oxygen.

Figure 7 illustrates the importance of the choice of reagent gas. For a large number of tricyclic psychoactive drugs such as Desipramine, the reagent methane yields mainly $[M-1]^-$ with only a small contribution of an anion representing the tricyclic ring system. With nitrous oxide, $[M-1]^-$ disappears and the ring anion (R^-) becomes the base peak.

D. Sensitivity

Table III gives an idea of the sensitivity of

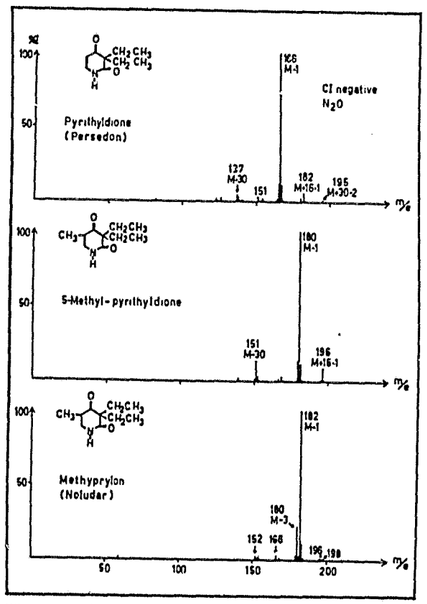


Fig. 6. Anion CI spectra of 3 hypnotics with piperidine-structure with N_2O as reagent gas

anion MS by CI. If we measure, in GC-MS combination, the positive and the negative total ion current of a peak (we have to run identical chromatograms for this purpose) a fairly good comparison results. With saturated barbiturates, the total ion currents are not too different, the sensitivities are comparable (see figures for barbital). With allyl barbiturates, however, anion CI can be over a factor of 10 more sensitive than cation EI and cation CI (see figures for allobarbital).

Compounds with fluoro or nitro groups give very intense anion spectra. Electron attachment leads to an abundant molecular anion. Figure 8 shows such an example, flunitrazepam, which is on its way to becoming one of the most dangerous drugs of abuse. The anion current is 40 times larger than the cation current. The molecular ion dominates the anion spectrum. The fluoro group (m/e 19) and the nitro group (m/e 46, M−16, M−32) are visible.

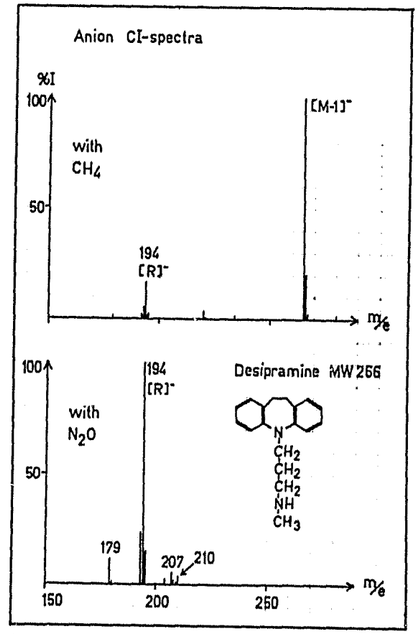


Fig. 7. Anion CI spectra of Desipramine with CH_4 and N_2O as reagents

Figure 9 contains the corresponding spectra of a similar benzodiazepine, this time chloro-substituted diazepam. Again the anion current is much larger than the cation current.

Table III

Relative total ion current and base peaks of 4 barbiturates (Barbital, Phenobarbital, Hexobarbital, Allobarbital) using 5 different ionization techniques.

R_1	R_2	mode	reagent	rel. TIC	base peak	% of TIC
$-CH_2-CH_3$	$-CH_2-CH_3$	EI pos.	–	500	$[M-28]^{+\cdot}$	21
		CI pos.	CH_4	80	$[M+1]^+$	49
		CI neg.	CH_4	45	$[M-1]^-$	33
		CI neg.	CH_4+SF_6	1100	$[M-1]^-$	50
		CI neg.	N_2O	460	$[M-1]^-$	61
$-CH_2-CH_3$		EI pos.	–	190	$[M-28]^{+\cdot}$	21
		CI pos.	CH_4	70	$[M+1]^+$	52
		CI neg.	CH_4	360	$[M-77]^-$	55
		CI neg.	CH_4+SF_6	120	$[M-1]^-$	60
		CI neg.	N_2O	360	$[M-77]$	30
$-CH_3$ with $N-CH_3$		EI pos.	–	440	$[M-15]^+$	16
		CI pos.	CH_4	530	$[M-79]^+$	32
		CI neg.	CH_4	180	m/e 42	26
		CI neg.	CH_4+SF_6	520	$[M-1]^-$	67
		CI neg.	N_2O	390	$[M-1]^-$	51
$-CH_2-CH=CH_2$	$-CH_2-CH=CH_2$	EI pos.	–	430	m/e 41	10
		CI pos.	CH_4	310	$[M+1]^+$	19
		CI neg.	CH_4	6700	$[M-41]^-$	89
		CI neg.	CH_4+SF_6	2000	$[M-41]^-$	84
		CI neg.	N_2O	1250	$[M-41]^-$	88

But the molecular anion is not stable. Chloride is the base anion. The chloride ions make up the largest part of the total anion current. Bromo-substituted compounds give very similar results.

E. Mass Specific Trace Detection in GC

All compounds with high electron affinity show high intensity anion spectra. The qualitative information may be somewhat one-sided, since most of the total ion current results from one or a few anions. Identification must be carried out in conjunction with cation mass spectrometry using EI. For trace analysis by mass specific detection, however, the situation is ideal. An anion mass spectrometer can be a more sensitive GC detector than a conventional electron-capture system. It is, of course, much more specific and flexible. We believe that it will become the ECD of the future.

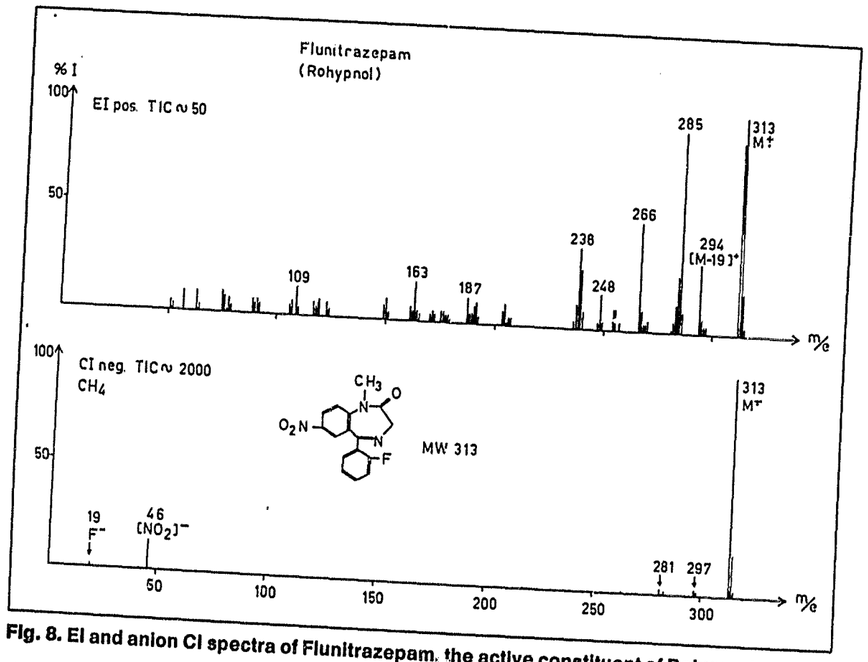


Fig. 8. EI and anion CI spectra of Flunitrazepam, the active constituent of Rohypnol

Figure 10 gives an example. It shows a chromatogram with bromide anion detection. Each peak represents about 20 pg of a bromo sedative; one is bromadal and the other bromisoval. Both are among the most heavily abused drugs in Europe. Their identification is assured by the retention times and the intensity ratio of the 2 isotopic bromide anion signals. A packed column was used. The base lines of the GC tracings would permit higher amplification which is possible with our detection system. Sub-pg-detection is feasible, as long as adsorption effects and background problems can be handled.

F. The Source Pressure

In Table IV, the anion intensities of the hypnotic methaqualone are tabulated along with the corresponding ion source pressures. Nitrous oxide is the reagent gas.

In contrast to the U.S. investigators (2–5), we work with much lower pressure, in the range of 10^{-3} to 10^{-2} torr. That is quite an ad-

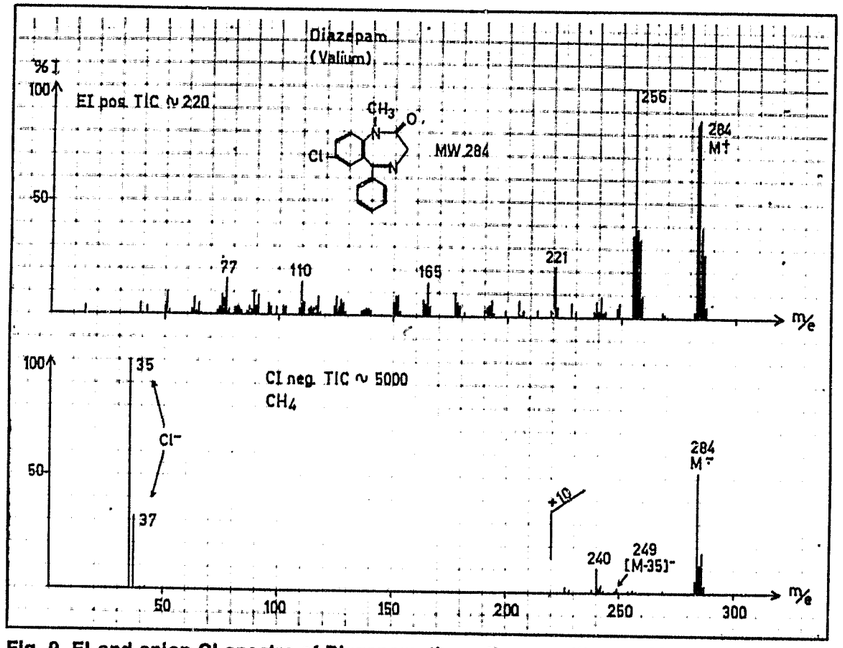


Fig. 9. EI and anion CI spectra of Diazepam, the active constituent of Valium

vantage. The ion source stays clean.

In our working range, the pressure dependence of the anion CI-spectra is tolerable. $[M-1]^-$ and the fragment anions are not much affected by the pressure change, nor is the oxygen substitution product $[M+15]^-$. $[M+30]^-$ is probably the cluster ion $[M+NO]^-$, since it is influenced by the pressure. In our recent work, we therefore tend to avoid cluster ion formation.

Table V shows that in positive CI it is not possible to work with as low chamber pressures as in the negative mode, since EI-like spectra are produced. Even in the usual working range between 0.1 and 1 torr the pressure dependence is quite pronounced.

III. TEN POSITIVE ASPECTS OF NEGATIVE ION MS BY CI

What have we learned from our present work with anion CI-MS?

1. It can be carried out at much lower source-pressure than cation CI-MS.

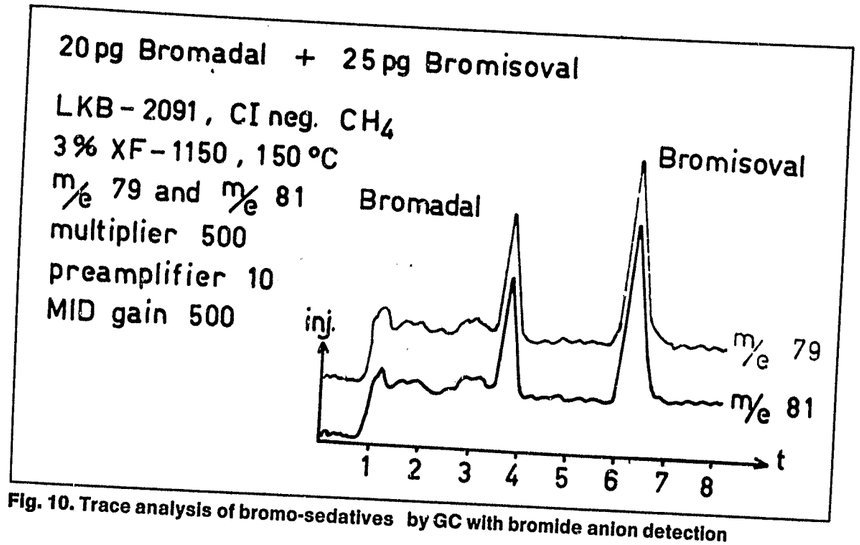


Fig. 10. Trace analysis of bromo-sedatives by GC with bromide anion detection

2. The pressure dependence in our working range (10^{-3} to 10^{-2} torr) is moderate.

3. It is a soft ionization method which usually permits the recognition of the molecular mass.

4. The anion CI-spectra are often simple which makes interpretation relatively easy.

5. Anion CI-spectra are complementary to cation EI-spectra.

6. The method is flexible. By proper choice of reagent gases we can favor electron attachment, negative charge exchange, or negative cluster ion formation.

7. Oxygen substitution in the conventional ion source is possible by using nitrous oxide as reagent which produces O^-.

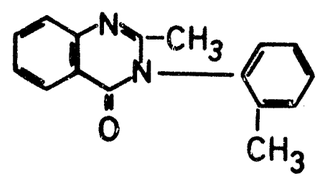
8. It is often more sensitive than cation CI-MS and EI-MS.

9. It is specifically suited for trace detection of substances with high electron affinity by GC with selected anion recording (the ECD of the future).

10. Compounds with similar structures can be detected simultaneously by GC with specific anion recording of a common structural unit such as a halogen or a ring anion.

Table IV

Anion CI of Methaqualone. Relative anion intensities at different pressures of the reagent gas N_2O.



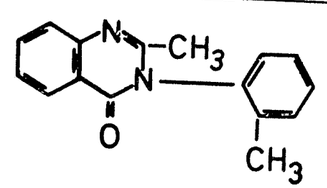
m Torr	135	159 M-91	233 M-17	247 M-3	248 M-2	249 M-1	261 M+11	265 M+15	280 M+30
1	6.6	3.6	26.1	17.7	51.1	100	0.9	63.6	-
3	7.9	4.4	25.4	19.7	55.7	100	4.4	67.2	0.7
5	8.4	4.3	25.1	18.9	56.8	100	6.2	67.6	0.8
7	8.1	2.7	25.7	21.6	59.5	100	9.1	67.6	1.4
9	7.7	4.6	26.2	20.0	58.5	100	13.8	67.7	3.1

REFERENCES

1. v. Ardenne, M., *Kernenergie,* 1, 1029 (1958); v. Ardenne, M., and Steinfelder, K., *Kernenergie,* 3, 717 (1960); v. Ardenne, M., Steinfelder, K., and Tümmler, R., "Elektronenanlagerungs-Massenspektrographie organischer Substanzen", Springer, Berlin, 1971.
2. Dougherty, R. C., and Weisenberger, C. R., *J. Am. Chem. Soc.,* 90, 6570 (1968); Dougherty, R. C., Dalton, J., and Biros, F. J., *Org. Mass Spectrometry,* 6, 1171 (1972).
3. Horning, E. C., et al., *Anal. Chem.,* 45, 036 (1973); *J. of Chrom.,* 142, 481 (1977).
4. Horning, E. C., et al., *J. Chromatogr.,* 99, 13 (1974); *Anal. Chem.,* 47, 2369 (1975).
5. Hunt, D. F., et al., *Anal. Chem.,* 48, 2098 (1976).
6. Brandenberger, H., and Ryhage, R., "4th Intern. Symp. on Mass Spectrometry in Biochemistry & Medicine", June 1977, Riva del Garda; A. Frigerio (Ed.), "Recent Developments in Mass Spectrometry in Biochemistry & Medicine", Plenum Publ. & Co., New York, 1978.
7. Brandenberger, H., and Ryhage, R., "Intern. Bioanalytical Forum", September 1977, Guildford; E. Reid (Ed.), "Assay of Drugs and other Trace Compounds in Biological Fluids", Vol. II, North-Holland Publ. Co., Amsterdam, in press.
8. Brandenberger, H., and Ryhage, R., *Mass Fragments,* 1, 1 (1978).
9. Frangi-Schnyder, D., and Brandenberger, H., *Z. Anal. Chem.,* 290, 153 (1978).
10. Ryhage, R., and Brandenberger, H., submitted for publication in *Biom. Mass Spectrometry.*
11. Brandenberger, H., Amsler, U., and Frangi-Schnyder, D., "8th Annual Symposium on the Analytical Chemistry of Pollutants", Geneva, 1978.
12. Brandenberger, H., *Deutsche Lebensmittel-Rundschau,* 70, 31 (1974).

Table V

Cation CI of Methaqualone. Relative cation intensities at different pressures of the reagent gas CH_4.



Torr	91	233 M-17	235 M-15	250 M	251 M+1	265 M+15	279 M+29	291 M+41	307 M+57
0.008	27.0	26.5	100	69.8	27.0	3.7	1.3	-	-
0.010	27.1	21.5	100	68.4	38.1	4.7	2.5	-	-
0.013	21.7	23.6	100	92.9	96.4	6.6	9.3	-	-
0.180	6.6	3.0	12.9	18.1	100	1.9	17.7	5.2	0.6
0.220	7.0	3.0	11.3	17.2	100	2.2	18.8	5.8	0.8
0.300	5.6	2.5	8.0	14.3	100	2.3	20.5	7.2	1.0

Applications of Fourier Transform Infrared Spectrometry in Forensic Analysis

by

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I. INTRODUCTION

Right at the outset of this paper it can safely be said that, in spite of its popularity for general analytical work, infrared (IR) spectrophotometry has not been accepted as widely as other instrumental techniques of forensic drug analysis. The very fact that this paper is being given by workers in an academic laboratory which does not even have a license to handle drugs of abuse testifies to the truth of this statement! Most analyses of abused drugs are currently made using some type of chromatography, in particular thin-layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC). However the retention characteristics of chromatographic separations do not provide completely unambiguous identifications of each "peak", and subsequent confirmation of the identity is often necessary through the use of some ancillary technique, e.g., the interface of a mass spectrometer with a gas chromatograph (GC-MS).

Where then should IR spectrophotometry fit into the overall picture of forensic drug analysis? Certainly the identification of materials through their IR absorption spectrum gives an unambiguous identification of the material. In addition, IR spectrometry has the important property of allowing great versatility in the type of samples which can be investigated, and spectra of samples in the solid, liquid, and vapor phases can all be readily measured. However, conventional IR grating spectrometers are generally less sensitive than many other instruments used in the forensic laboratory, and it is probably this lack of sensitivity rather than any other single factor which has led to the unpopularity of IR spectrometry in this context.

Since the commercial development of Fourier transform infrared (FT-IR) spectrometers, detection limits for samples measured by infrared spectrometry have been substantially reduced. These instruments show a practical improvement in sensitivity of at least an order of magnitude when compared with conventional IR spectrometers (1), and this improved sensitivity can be traded off for even greater reductions in the time required to measure high quality spectra. (The scan time required for the measurement of medium resolution spectra is typically less than 1 second). When a liquid nitrogen cooled mercury cadmium telluride (MCT) photodetector is substituted for the more commonly used room temperature triglycine sulfate (TGS) pyroelectric bolometer, a further increase in sensitivity of up to an order of magnitude is obtained. Additionally, all modern FT-IR spectrometers are equipped with a general purpose data system which enables the analyst to perform arithmetic operations on digitally stored spectra, thereby further increasing the versatility of these instruments. The theory, instrumentation and chemical applications of FT-IR spectrometry have been summarized in several books and review articles (2–7), and will not be discussed in detail in this paper.

II. ABSORBANCE SUBTRACTION

Perhaps the most important application of FT-IR spectrometers in the analytical laboratory is one where the high sensitivity of the instrument is secondary to the presence of a versatile data system. Spectra can be added, subtracted, divided, raised to a power, logged and antilogged. The most commonly used arithmetic operation is the so-called "absorbance subtraction" routine. In this procedure, the absorbance spectrum of a mixture and the spectra of one or more of the individual components are measured. The absorbance spectrum of the component is multiplied by a suitable scaling factor which is chosen so that, on subtraction of the scaled spectrum from the original spectrum of the mixture, all the bands due to the component disappear. This procedure may be repeated several times for multicomponent mixtures so that intense bands due to major components may be eliminated from the spectra of mixtures, enabling weaker bands caused by minor components to become evident.

One example of absorbance subtraction procedures to the analysis of samples of forensic interest is the detection and identification of a shampoo on hair several days after application. Attenuated total reflection (ATR) spectra of hair three days after shampooing (Fig. 1A) and six days after the shampoo (Fig. 1B) were measured, and the result of subtracting the two spectra is shown in Fig. 2. Measurement of the ATR spectra of fibers requires that several fibers are wrapped around the internal reflection element, but another technique has been developed for a similar analysis using only a fraction of a single hair. The hair is placed between two diamond anvils of a high pressure cell (8) and compressed until the thickness is suitable for infrared analysis. Although diamonds usually totally absorb in the region between 2250 and 1850 cm^{-1}, little significant chemical information is found in this region and a useful spectrum can be measured (see Fig. 3).

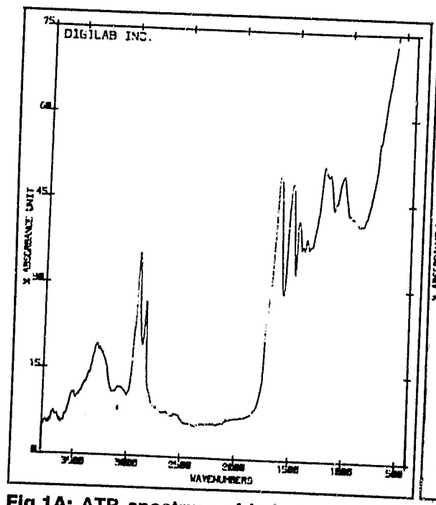

Fig.1A: ATR spectrum of hair three days after shampooing.

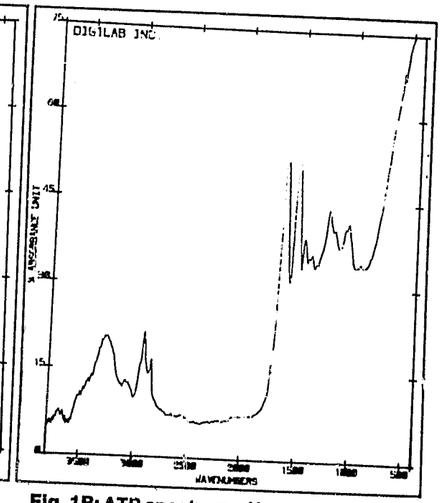

Fig. 1B: ATR spectrum of hair six days after shampooing.

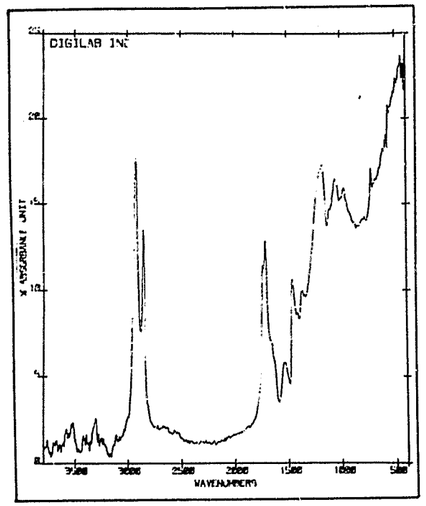

Fig. 2: Difference spectrum resulting from the scaled subtraction of the spectrum shown in Fig. 1B from that in Fig. 1A, showing the nature of the shampoo.

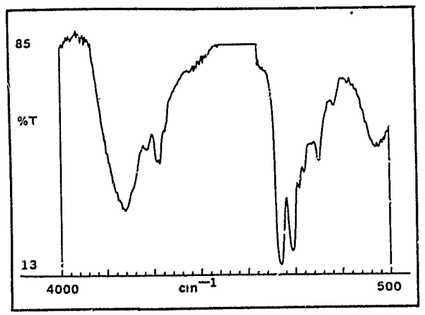

Fig. 3: Transmittance spectrum of a single hair in a diamond cell.

III. DIFFUSE REFLECTANCE SPECTROSCOPY

The examples discussed above are certainly rather nonroutine analyses, and we believe that it is in the areas which are not readily studied by other analytical methods that FT-IR spectroscopy will prove to have the greatest application. For example, no instrumental method may currently be used for the *direct* identification of street drugs, controlled substances, prescription pharmaceuticals, and over-the-counter (OTC) pharmaceuticals. Therefore any technique which permits the rapid, unequivocal identification of the active major ingredient and other active trace components in samples believed to contain physiologically active materials should find extensive application in the forensic drug laboratory.

At least two infrared techniques have been previously proposed for this purpose. In one method, the active component must be extracted from the tablet and prepared as a KBr disc prior to measurement of its spectrum (9). After the spectrum is measured, the sample can be classified into one of nine groups of controlled substances (such as barbiturates, amphetamines, etc.) by an easily followed flow chart based on the presence or absence of characteristic absorption bands. After classification, the active component may be identified by comparison with the small number of reference spectra of drugs in its class. This method is simple and elegant but not fast, since the active ingredient(s) must be separated from the excipients prior to the preparation of the KBr disc. In addition, all the measurements in this study were performed using a grating spectrophotometer, so that at least two hours were required between receipt of the sample and identification of the active ingredient.

Sadtler Research Laboratories, Inc., developed a method for reducing sample preparation time. Tablets were subjected to extractions in acidic and basic chloroform, after which a film of the extracted drug was cast on a salt window, and its absorption spectrum was then measured. Once again in this work a grating spectrophotometer was used for the spectral measurement, although it may be noted that Sadtler now uses an FT-IR spectrometer for the measurement of all their infrared reference spectra. The extracted drugs could then be identified by comparison with a compilation of reference spectra of samples prepared in the same way (10). Although this method certainly reduced sample preparation time compared to the previous method, over an hour expires between receipt of the sample and identification of the drug. For drug overdose cases where several tablets may have to be rapidly identified, this time lag may prove to be too long and an even more rapid technique is needed.

We have developed a novel method for identifying drug tablets in which the time lag between receipt of the sample and initiation of the spectral measurement can be less than one minute. The method is based on the measurement of the diffuse reflectance spectrum of the ground sample, either as received or diluted (but not pressed) with an alkali halide, usually KCl (11). Diffuse reflectance infrared spectrometry has not been considered to be a useful analytical technique because of the low energy of the diffusely reflected radiation, but we have found that a combination of an FT-IR spectrometer with an MCT detector and efficient ellipsoidal collecting mirror gives such intense signals that we have to screen out much of the radiation passing through the interferometer to avoid digitization noise. The spectra that are measured are single-beam traces, and must be ratioed against the single-beam spectrum from a "100%" diffuse reflector. We generally use KCl powder ground to less than 10μm particle size to approximate a perfect reference material. Diffuse reflectance spectra of tablets (either whole or crushed) can be measured directly (see Fig. 4). However, we often dilute one part of the ground sample with about 20 parts of KCl to increase the information content of the spectrum. Provided that the proportion of excipient in the tablets is not high, diffuse reflectance spectra measured in this way may generally be used to identify the active ingredient very rapidly (see Fig. 5). On a spectrometer operating at the current state-of-the-art in sensitivity and computing speed, less than 3 min. would be required between receipt

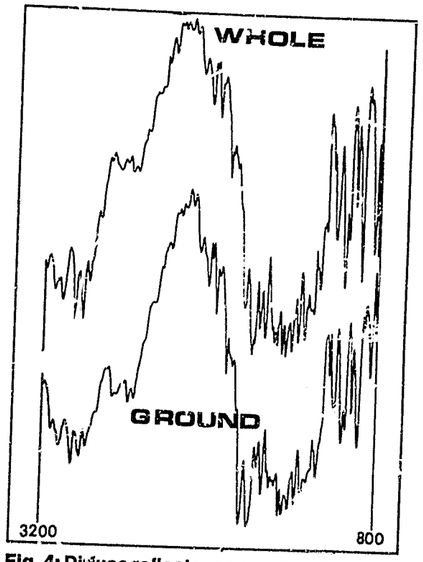

Fig. 4: Diffuse reflectance spectra, linear in R_∞, of a tablet of Empirin (an APC tablet) measured (A) as the whole tablet, and (B) after grinding for one minute.

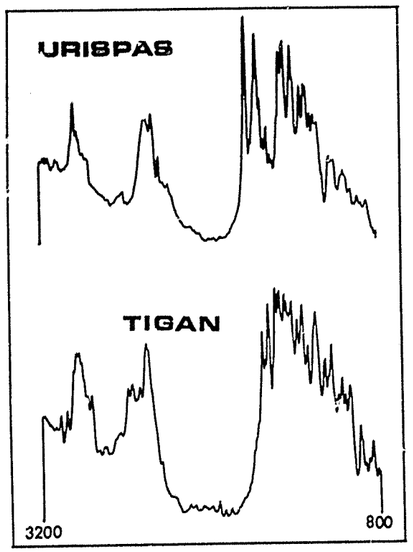

Fig. 5: Kubelka-Munk plots of the diffuse reflectance spectra of Tigan (a capsule to counteract nausea, especially morning sickness) and Urispas (a tablet to counteract bladder infections), after grinding each for one minute.

of the sample and display of the final spectrum on an oscilloscope. Small libraries of spectra (typically less than 1000 curves) could be stored on 10 megabyte moving head discs, facilitating a rapid limited spectral search to provide the identity of the tablet.

In the same way that absorption bands in absorbance spectra can be related to concentration through Beer's Law, the diffuse reflectance of a sample at infinite depth (about 3 mm or more), R_∞, can be related to sample concentration, c, by the expression first developed by Kubelka and Munk (12, 13):

$$f(R_\infty)=\frac{(1-R_\infty)^2}{2R_\infty}=\frac{k\,a\,c}{s}$$

where a is the absorptivity, s is a scattering factor, and k is a constant of proportionality. Spectra of neat, finely ground powders plotted as $f(R_\infty)$ are closely similar to the absorbance spectra of KBr discs of the same materials (11).

We have found that in practice $f(R_\infty)$ is accurately proportional to c provided R_∞ is fairly high, typically greater than about 30%. Thus, provided that no strong bands are present in the

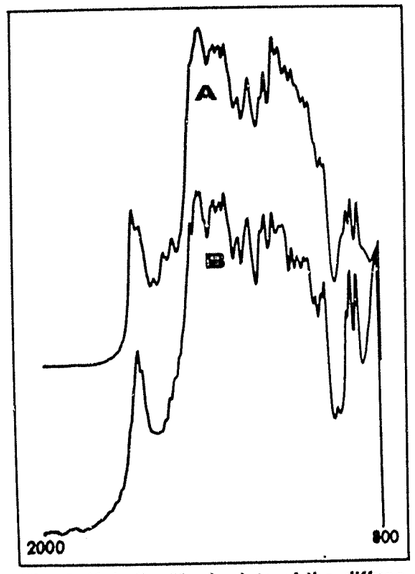

Fig. 6: Kubelka-Munk plots of the diffuse reflectance spectra of (A) a crushed tablet of Valium containing 1% Valium and 99% lactose and (B) pure lactose.

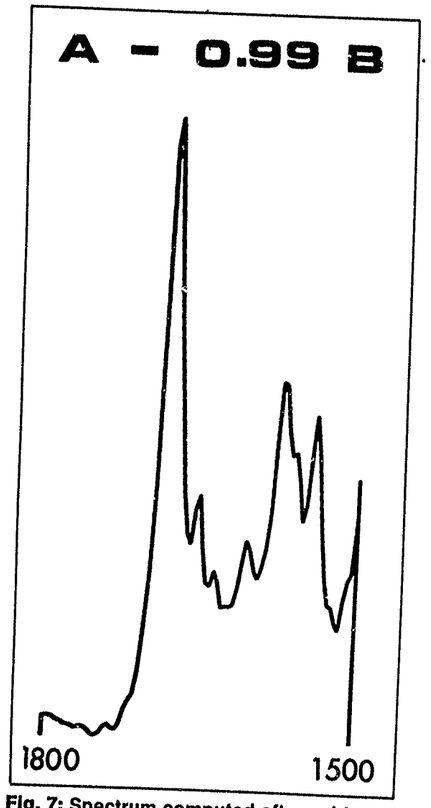

Fig. 7: Spectrum computed after subtracting 99% of the lactose spectrum in Fig. 6B from the spectrum of the Valium tablet. All the bands in this spectrum may be assigned to Valium. The reflectance in the region between 1500 and 1100 cm^{-1} is so low that useful analytical information cannot be obtained in this region.

spectrum, one should be able to perform subtraction operations on diffuse reflectance spectra stored as $f(R_\infty)$ in the same way that absorbance subtraction routines can be applied to absorption spectra. For example, the spectrum of lactose may be subtracted from the spectrum of a tablet of Valium, which contains about 99% lactose, to yield several weak bands of Valium (see Figs. 6 and 7).

Another example of difference measurements may be seen for the $f(R_\infty)$ spectra of two

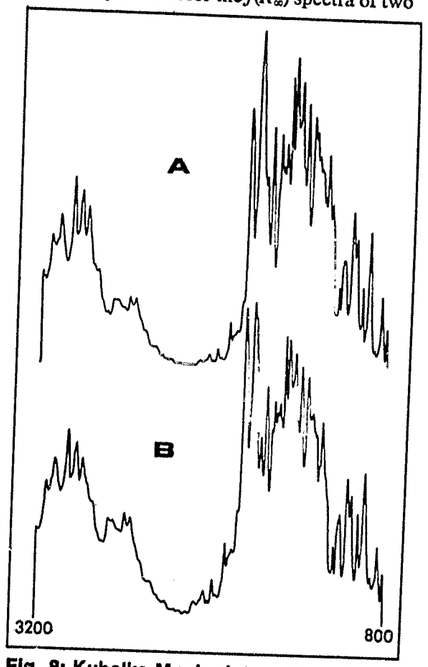

Fig. 8: Kubelka-Munk plots of the diffuse reflectance spectra of two APC tablets from different manufacturers.

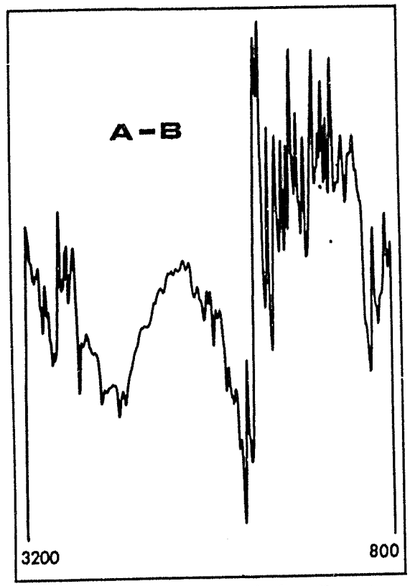

Fig. 9: Spectrum computed when the spectrum in Fig. 8B is subtracted from the spectrum in Fig. 8A. Upward-going bands are primarily due to phenacetin and downward-going bands are primarily due to aspirin.

aspirin-phenacetin-caffeine (APC) tablets with the same specified composition (3.5 gr aspirin, 2.5 gr phenacetin, 0.5 gr caffeine) but from different manufacturers. The two spectra are shown in Fig. 8, and the unscaled difference spectrum is shown in Fig. 9. It can be seen that the tablet from manufacturer A contains more phenacetin, while the one from manufacturer B contains more aspirin. Although this example uses OTC pharmaceuticals, the application of difference techniques to the analysis of forensic samples, e.g. for the presence of strychnine in heroin, is immediately apparent.

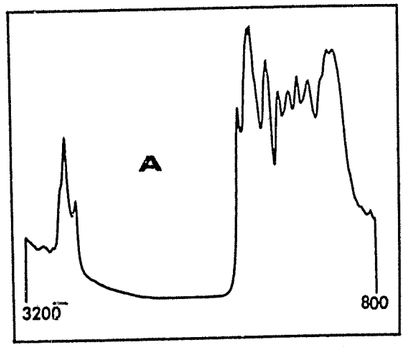

Fig. 10A: Kubelka-Munk plot of the diffuse reflectance spectrum of a dried leaf after spraying with a commercial Paraquat spray. The leaf was ground with KCl and the spectrum was ratioed against a KCl reference spectrum.

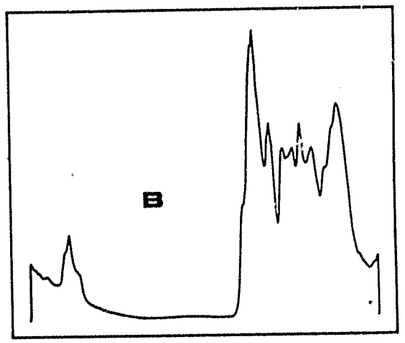

Fig. 10B: Kubelka-Munk plot of the diffuse reflectance spectrum of an untreated leaf from the same plant, which was cut and allowed to dry naturally. The sample was prepared identically to the sample in Fig. 10A.

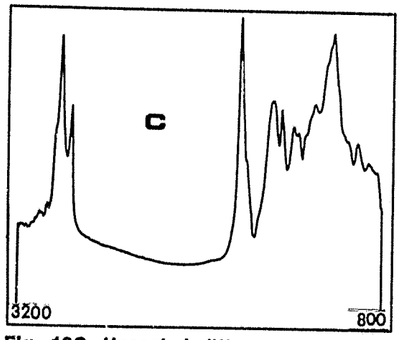

Fig. 10C: Unscaled difference spectrum produced when the spectrum in Fig. 10B was subtracted from the spectrum in Fig. 10A.

In the past few weeks a crisis has arisen on all university campuses with the discovery that much of the marijuana being purchased is contaminated with the herbicide, Paraquat. We attempted to prepare simulated samples by spraying a common local broadleaf weed, Polygonaceae Rumex, with a commercially available Paraquat spray ("Ortho" Spot Weed and Grass Killer) which contained only 0.44% Paraquat. The weed was allowed to die, at which time it was ground with powdered KCl and its diffuse reflectance spectrum was measured (see Fig. 10A). An untreated plant was cut and also allowed to die after which it was treated in an identical fashion (see Fig. 10B). On subtraction of the spectra of the treated and the untreated plants, several bands were apparent in the difference plot (see Fig. 10C). Although none of the bands in the difference spectrum may be assigned directly to Paraquat, frequencies of the more intense bands corresponded to the frequencies of the bands in the spectrum of a sample prepared by spraying the contents of the can directly into water, separating off the aqueous phase, evaporating off the water from the extract, and mixing the residue with ground KCl (see Fig. 10D). Although our primary goal of developing an analytical method for the *in situ* determination of Paraquat was not reached, this method did provide an indirect indication of the presence, and the experiment again gives an indication of the tremendous versatility of FT-IR spectrometry.

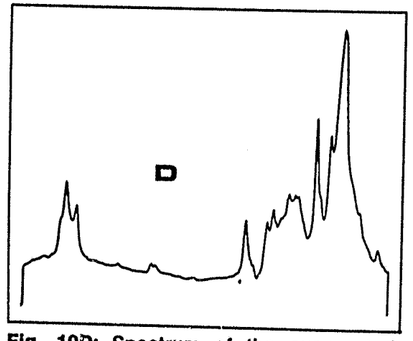

Fig. 10D: Spectrum of the evaporated water extract of the Paraquat spray used in this work.

IV. CHROMATOGRAPHIC APPLICATIONS

A. GC-IR

Another common application of FT-IR spectrometry in general organic analysis is the interface of the spectrometer to a gas chromatograph for the on-line identification of eluting samples (GC-IR) (14–16). This experiment makes use of the short scan time of a rapid-

scanning interferometer, which typically requires less than 1 second per scan to generate the information for a medium resolution infrared spectrum. The GC effluent is passed through a long, narrow gas cell, light-pipe, through which the infrared beam is also passed. Data is collected while each component is in the light-pipe, and after each peak elutes the signal-averaged interferograms are stored in the spectrometer memory until the end of the chromatogram, at which time all the spectra are calculated.

We have not encountered any examples of GC-IR in forensic drug analysis, and to our knowledge GC-IR is not being used in a routine fashion in any forensic laboratory. In view of GC-IR detection limits (between 25 ng and 1 μg of sample injected into the chromatograph), it is not likely that GC-IR will supersede GC-MS in the forensic laboratory. However, GC-IR can produce useful confirmatory evidence in cases where mass spectrometry does not provide a definite identification.

B. LC-IR

While GC-IR is, to a certain extent, a competitive technique to GC-MS, no such well accepted instrument as a mass spectrometer is routinely used for the on-line identification of peaks eluting from a liquid chromatograph. Some work has been published showing how an FT-IR spectrometer can be used to measure the spectra of HPLC peaks (LC-IR) as they pass through a flow-cell (17–19), but in general submicrogram detection limits are not obtained, primarily because of strong absorption by the solvent in some frequency regions. We believe that the best way to perform LC-IR measurements is to eliminate the solvent prior to measuring the spectrum. Preliminary work in this area has led us to believe that the best way to achieve this end is to evaporate the effluent from the chromatograph on powdered KCl. After each peak elutes, the diffuse reflectance spectrum of the solute may be rapidly measured. This work is currently at a very early stage, but feasibility studies indicate that submicrogram detection limits are possible.

C. TLC-IR

Fourier spectrometers may also be used to measure the infrared spectra of separated "spots" directly on TLC plates (TLC-IR). One method involves depositing the adsorbent layer on an infrared transmitting substrate, such as a sheet of AgCl, and measuring the transmittance spectrum through the adsorbent layer either without treatment (20) or after treatment with an infrared mulling oil to decrease scattering (21). TLC-IR measurements are optimized by combining the high sensitivity of FT-IR with the high efficiency of programmed multiple development TLC. In this case detection limits of 10 ng of sample may be obtained (22). These methods have the disadvantage that the analyst must prepare his own TLC plates, since AgCl plates are not available commercially. To circumvent this problem we have studied the possibility of using diffuse reflectance spectroscopy to identify materials on aluminum foil backed TLC plates (which are commercially available) and, although the early results are promising (11), more work is required before the usefulness of this technique for TLC separations of drugs may be determined.

V. *SUMMARY*

The increased sensitivity of FT-IR over conventional IR spectrometers not only permits the detection limits of conventional infrared sampling methods to be decreased, but also allows an even greater variety of samples to be investigated. The biggest advantage of FT-IR spectrometry over other non-infrared techniques used in the forensic laboratory is probably due to its versatility and specificity rather than any inherent sensitivity advantage over other instrumental methods.

REFERENCES

1. Griffiths, P. R. Sloane, H. J., and Hannah, R. W., *Appl. Spectrosc.*, 31, 485 (1977).
2. Bell, R. J., *Introductory Fourier Transform Spectroscopy*, Academic Press, New York (1972).
3. Griffiths, P.R., *Chemical Infrared Fourier Transform Spectroscopy*, Wiley-Interscience, New York (1975).
4. Ferraro, J. R., and Basile, L. J. (eds.), *Fourier Transform Infrared Spectroscopy–Applications to Chemical Systems*, Academic Press, New York (1978).
5. Griffiths, P. R. (ed.), *Transform Techniques in Chemistry*, Plenum Publishing Corp., New York (1978).
6. Griffiths, P. R., *Anal. Chem.*, 46, 645A (1974).
7. Koenig, J. L., *Appl. Spectrosc.*, 29, 293 (1975).
8. Lippincott, E. R., Weir, C. E., Van Valkenburg, A., and Bunting, E. N., *Spectrochim Acta*, 16, 58 (1960).
9. Hannah, R. W., and Pattacini, S. C., Perkin-Elmer Infrared Applications Study No. 11, Perkin-Elmer Corp., Norwalk, CT (1972).
10. *Infrared and Ultraviolet Spectra of Commonly Abused Drugs*, Sadtler Research Laboratories, Inc., Philadelphia, PA (1972).
11. Fuller, M. P., and Griffiths, P. R., *Anal. Chem.* (submitted for publication, 1978).
12. Kubelka, P., and Munk, F., *Z. Tech. Phys.*, 12, 593 (1931).
13. Kubelka, P., *J. Opt. Soc. Am.*, 38, 448 (1948).
14. Kizer, K. L., *Amer. Lab.*, 5(6), 40 (1973).
15. Coffey, P., Mattson, D. R., and Wright, J. C., *Amer. Lab.*, 10(5), 126 (1978).
16. Griffiths, P. R., in *Fourier Transform Infrared Spectroscopy–Applications to Chemical Systems* (J. R. Ferraro and L. J. Basile, Eds.), p. 143, Academic Press, New York (1978).
17. Kizer, K. L., Mantz, A. W., and Bonar, L. C., *Amer. Lab.*, 7(6), 85 (1975).
18. Vidrine, R. W., and Mattson, D. R., *Appl. Spectrosc.* (in press, 1978).
19. Gomez-Taylor, M. M., Kuehl, D., and Griffiths, P. R., *Env. Analyt. Chem.*, 5, 103 (1978).
20. Percival, C. J., and Griffiths, P. R., *Anal. Chem.*, 47, 154 (1975).
21. Gomez-Taylor, M. M., Kuehl, D., and Griffiths, P. R., *Appl. Spectrosc.*, 30, 447 (1976).
22. Gomez-Taylor, M. M. and Griffiths, P. R., *Appl. Spectrosc.*, 31, 528 (1977).

Nuclear Magnetic Resonance: Recent Developments in Techniques and their Potential Application to Forensic Research

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I. INTRODUCTION

Nuclear magnetic resonance (NMR) has long been recognized as an extremely valuable technique for the structure elucidation and analysis of organic compounds. For more than 25 years NMR has played a major role in many types of organic chemistry. Yet, there are other areas of chemistry—most forensic research and analysis included—where NMR has had little impact. Probably the principal reason for the failure of NMR to make these inroads is its limited sensitivity relative to that of other spectroscopic methods. But NMR sensitivity has been steadily improving over the years. While it still cannot compete in sensitivity with mass spectroscopy, fluorescence, and neutron activation analysis, NMR is now applicable to many types of problems where it was once deemed useless. Thus, the advantages of NMR in terms of a readily understood theory, rather easily interpretable spectra, and remarkable versatility can be more widely exploited.

In this paper we shall examine the present state of the NMR art regarding sensitivity, sample size, and quantitative analytical capabilities. We shall not discuss specific applications to forensic problems nor shall we go into the interpretation of NMR spectra, information on which is widely available elsewhere (1).

II. IMPROVEMENTS IN SENSITIVITY

During the last decade there have been major advances in NMR sensitivity. Four separate factors can be identified as the principal contributors, as follows:

1. *Better electronic design and better electronic components.* A more thorough understanding has been developed of the various parameters that govern the magnitudes of both the signal and the noise emanating from the radio frequency receiver coil of an NMR spectrometer. This has spawned improvements in the geometry of NMR probes and has led also to the design of more sophisticated electronic circuits that amplify the weak NMR signal while discriminating against unwanted noise from many sources. Coupled with design improvements has been the availability of the markedly better electronic circuit components found in today's integrated circuit technology.

2. *Use of higher magnetic fields.* During the last 20 years the magnetic field strengths used for high resolution NMR have increased by a factor of six. The principal impetus for development of ever higher field spectrometers is the increased dispersion available, since chemical shift differences expressed in frequency units are directly proportional to the strength of the applied field. However, an important secondary advantage is the increased sensitivity that arises from the more favorable Boltzmann distribution of nuclei among the magnetic energy levels. The fundamental limitation on NMR signal strength comes from the fact that radio frequency absorption is unavoidably accompanied by induced emission. Since we can measure only the difference between these two processes, it is actually only the *difference* between the populations of the magnetic energy levels that contributes to the net absorption signal. For protons in a magnetic field of 1.4 tesla (a frequency of 60 MHz) the difference is only about 1 out of 200,000 nuclei; for a field of 8.5 tesla (360 MHz) it is six times as great.

3. *Use of Fourier transform methods.* In the following section we shall describe the rationale for the use of Fourier transform (FT) methods and illustrate the very significant gains in sensitivity derived thereby.

4. *Use of Microcells.* This, too, is a topic that we shall take up in detail in a later section.

Table I illustrates the remarkable improvements in sensitivity that have resulted from factors (1) and (2) above. It is conventional to express the sensitivity of proton NMR spectrometers by specifying the signal/noise ratio obtained in a single scan (or for FT, in a single pulse) for a particular resonance line in a sample of ethylbenzene at a concentration of 1% (by volume) in a suitable solvent. The Varian Model A-60 spectrometer was the first really widely-used NMR instrument. Introduced in 1961, it had a typical signal/noise ratio of about 6. By the mid-1960's improvements in probe design and the availability of higher magnetic fields led to substantially better sensitivity, as indicated for the Model HA-100. The HR-220 was the first spectrometer to employ a superconducting magnet, rather than an electromagnet. The greatly increased field strength provided another significant increase in S/N, which ran about 80 for the HR-220 that we purchased at NIH in 1969. Note, however, how much further improvement there has been in the last decade; the XL-200 (with essentially the same field strength as our old HR-220) has just been introduced with a specified S/N that equals or exceeds 300. Finally, the highest field high resolution NMR spectrometer operating today has a proton frequency of 360 MHz. With recent improvements in probe design, that instrument provides a signal/noise of about 800 (in one case a value of 1200 has been obtained).

Table I

Signal/Noise for Various Spectrometers*

Year	Spectrometer Model	S/N
1961	A-60	6
1965	HA-100	30
1969	HR-220	80
1978	XL-200	300
1978	WH-360	800

*Proton specification—1% ethylbenzene, single scan/single pulse

Thus, during the last 15 years or so, the "state of the art" in NMR sensitivity has improved by at least a factor of 100, just on the basis of the use of higher magnetic field strengths and of improvements in electronics and probe design. This dramatic enhancement in sensitivity has opened up many new areas to NMR, especially those in the biological field, where sample size and concentration are often severely limited. However, another concurrent but independent development has been equally important in improving the effective sensitivity of NMR. That development is the introduction of Fourier transform methods, coupled with the principles of time averaging.

III. FOURIER TRANSFORM METHODS

Whatever the sensitivity of a particular spec-

troscopic method, there are occasions when it must be pushed to the limit. It has long been recognized that the effective sensitivity of the method can be improved by spending more time in acquiring the data. Infrared, Raman, visible, and ultraviolet spectra are usually obtained by rather slow scans, with suitable electronic filtering to reduce noise. In NMR it has usually been more feasible, for several reasons, to scan spectra more rapidly but to repeat the scan several times and coherently add the data in a digital computer. Such "time averaging" of n spectra provides a signal n times as great as in a single scan, but the noise (which is not coherent) is only $n^{1/2}$ as large, so that S/N is improved by $n^{1/2}$. With typical scan times of 5–10 minutes, it is feasible to add the data from about 100 scans in order to increase S/N by a factor of 10, but beyond that the expenditure of time rapidly becomes prohibitive. Clearly, the time spent on each scan must be drastically reduced if very large numbers of repetitions are to be employed. Fortunately, that is just what can be done with Fourier transform (FT) methods.

There are several different techniques for obtaining NMR spectra rapidly, all of which employ a Fourier transformation at some stage in processing the data. The most commonly used method at present is the use of a short radio frequency pulse to excite a spectrum, and it is only this pulse-FT method that we shall discuss here.

In an NMR experiment the macroscopic nuclear magnetization can be thought of as a vector that resides initially along the z axis parallel to the applied magnetic field, and it is tipped toward or into the xy plane by the action of the radio frequency magnetic field that is applied. In a conventional, continuous wave NMR experiment, a small amount of radio frequency power is applied continuously, but at a gradually changing frequency, to cause the magnetization corresponding to each different resonant frequency to tip *in turn* through a small angle. The component thus generated in the xy plane induces a small current in the receiver coil placed along the y axis, which we detect and display as an NMR line. In a pulse experiment, on the other hand, a large amount of radio frequency power (larger by a factor of at least 10^4 than that typically used in cw NMR) is applied for a short time—only a few microseconds—and at a single, fixed frequency. As shown in Figure 1, the pulse causes the magne-

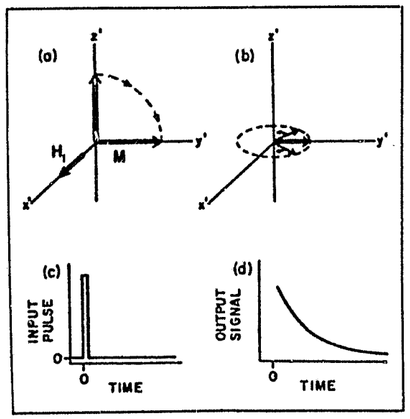


Figure 1. Behavior of the macroscopic magnetization on application of a radio frequency pulse, and resultant signal. (a) The radio frequency field H_1, applied along the x' axis rotates the magnetization M from its equilibrium position to the y' axis. (b) M decreases as individual magnetic moments dephase due to spin-spin relaxation and magnetic field inhomogeneity effects. (c) Input signal, a short pulse. (d) Exponential free induction decay resulting from process (b).

tization to tip into the xy plane, where it induces a large signal in the receiver coil. The signal decays exponentially with a time constant T_2^*, the magnitude of which is determined by the nuclear spin-spin relaxation time and the magnetic field inhomogeneity. Because the radio frequency power is so high, nuclei over a range of resonance frequencies of several kilohertz are affected, so that the magnetization vectors of nuclei at all chemical shifts tip *simultaneously*. Each provides an exponentially decaying signal at its own resonance frequency. In the detection process all of these signals beat with each other and with the reference frequency of the pulse itself. The resultant time response, the "free induction decay," typically looks like that in Figure 2. Although it is not interpretable by inspection in the same way as a normal NMR spectrum, it does indeed contain all the same information and requires only a second or so for data acquisition, rather than several minutes for a typical slow scan. Fortunately, the information is easily converted to a recognizable form by carrying out a Fourier transformation and some other simple mathematical processing. These operations are rapidly and easily carried out in a small computer, which normally forms an integral part of the NMR spectrometer. With the pulse-FT method it is possible to make many pulse repetitions, sometimes tens or hundreds of thousands, coherently add the free induction decays, and then carry out the mathematical processing on the co-added data with its enhanced S/N. In later sections we shall see some examples of FT spectra and also explore the limitations on the pulse repetition rate resulting from spin-lattice relaxation.

Fourier transform methods often improve the sensitivity in a given time by a factor of about 10–20 and permit the study of ^{1}H NMR of samples in standard 5 mm diameter sample

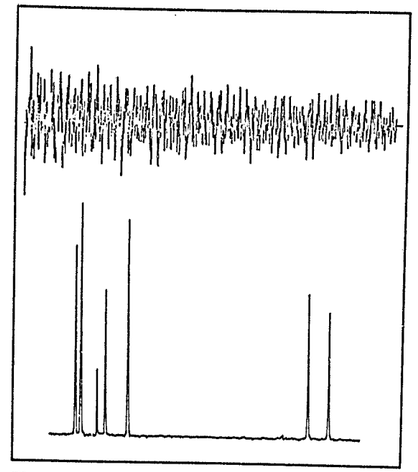


Figure 2. Free induction decay (top) and Fourier-transformed spectrum (bottom) of ^{13}C in 3-ethylpyridine.

tubes at concentrations as low as 10 μM with overnight runs. But the area in which FT methods become really essential is the study of nuclei other than hydrogen, which have lower inherent sensitivity and often lower isotopic abundance. The most significant in organic chemical problems is ^{13}C, with a sensitivity only about $^1/_{64}$ as great as that of ^{1}H and a natural abundance of only 1.1%. To overcome the combined adverse factor of nearly 6000 has required the development of superior instruments and experimental techniques, but as we shall see in the next section it is possible to obtain good ^{13}C spectra with remarkably small amounts of sample. Figure 3 illustrates a rough but very useful guide to the relative signals obtained for ^{1}H and ^{13}C—*if a good ^{1}H spectrum can be obtained with a single pulse*

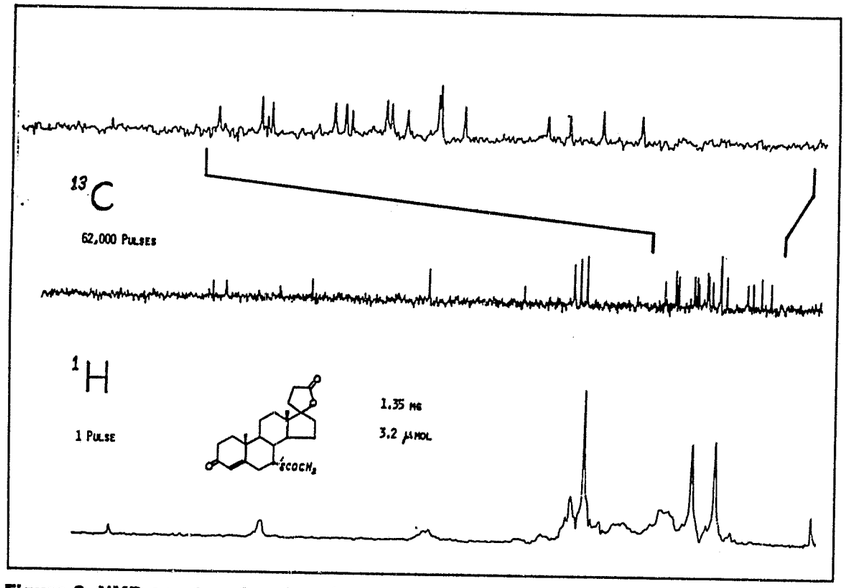


Figure 3. NMR spectra of spironolactone. Bottom: ^1H spectrum, taken with a single pulse. Middle: ^{13}C spectrum resulting from 62,000 pulses (17 hour experiment). Top: Expansion of part of the ^{13}C spectrum. (R. J. Highet, private communication.)

for a particular sample, an acceptable ^{13}C spectrum can be obtained in an overnight run.

IV. MICROCELLS

Since the earliest days of high resolution NMR, such spectra have been obtained with samples in 5-mm diameter sample tubes. Initially this was the maximum size sample tube that could be accommodated in the magnets and probes that were available. During the last few years it has become possible to utilize much larger tubes (up to at least 30-mm diameter) for studies of very dilute solutions of studies of nuclei of very low sensitivity. On the other hand, when concentration is not a problem but the total amount of sample is limited, there is nothing to be gained by using a large sample tube and a correspondingly large amount of solvent. In fact, studies have shown that it is far more effective to restrict the volume of solution that is used. This can be done in two ways: (1) With probes and inserts designed for use with 5-mm diameter sample tubes, the total length of the sample solution itself can be limited to that just needed to fill the receiver coil. A volume as small as 25–50 μl can be attained, but there may be some degradation of resolution. (2) Microprobes have been designed with very small diameter receiver coils that accommodate 1.7–2 mm diameter sample tubes (similar to conventional melting point capillaries). This approach is generally superior and permits the use of sample volumes as small as 5–10 μl. Resolution is usually excellent.

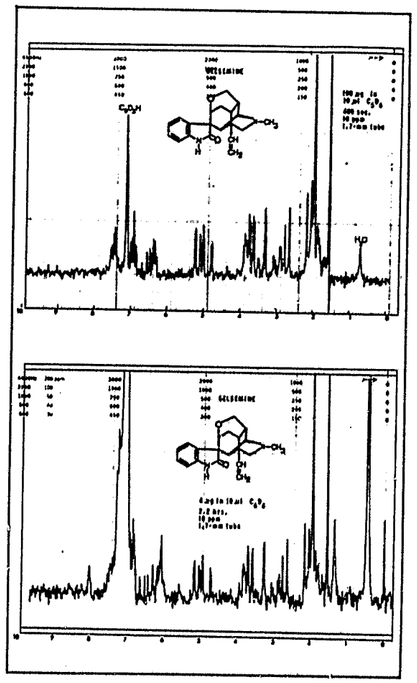


Figure 4. Proton NMR spectra of gelsemine in capillary-type microcells. Shoolery (2).

The use of 1.7-mm diameter capillary tubes and a microprobe for obtaining ^1H and ^{13}C spectra has recently been reviewed by Shoolery (2). We present here two examples from that review. Figure 4 shows the ^1H spectrum of gelsemine, a natural product of molecular weight 322. The upper spectrum was obtained with 100 μg of sample in 10 μl of C_6D_6 solvent. With this amount of material a good spectrum, showing all essential features, was obtained with only 400 sec. of data acquisition. The lower spectrum comes from only 4 μg of gelsemine. It required 8000 pulses and took 2.2 hours to acquire the data. While the noise level is higher than that of the upper spectrum, all important features show up clearly except those obscured by peaks due to solvent impurities. The 0.2% C_6D_5H isotopic impurity in the C_6D_6 gives by far the largest line in the spectrum, with a small amount of water providing the second strongest line. For proton NMR the real limitation in sample size now comes, not from instrument sensitivity, but from problems in handling samples and in avoiding proton-containing impurities in the solvent.

Let us turn now to the ^{13}C spectrum of this same substance. As we have seen, ^{13}C sensitivity is expected to be more than three orders of magnitude poorer than that of proton, so a correspondingly larger sample is needed. Figure 5 shows the proton-decoupled ^{13}C spectrum of 1.0 mg of gelsemine in 6.5 μl of C_6D_6. In this case 76,000 pulses were used for a total experimental time of 17 hours. This spectrum is of rather good quality. Accumulation of data for a longer period than overnight is usually not worthwhile, since there is relatively little improvement in S/N. In some cases submilligram quantities have provided good ^{13}C spectra, but with current technology the limit

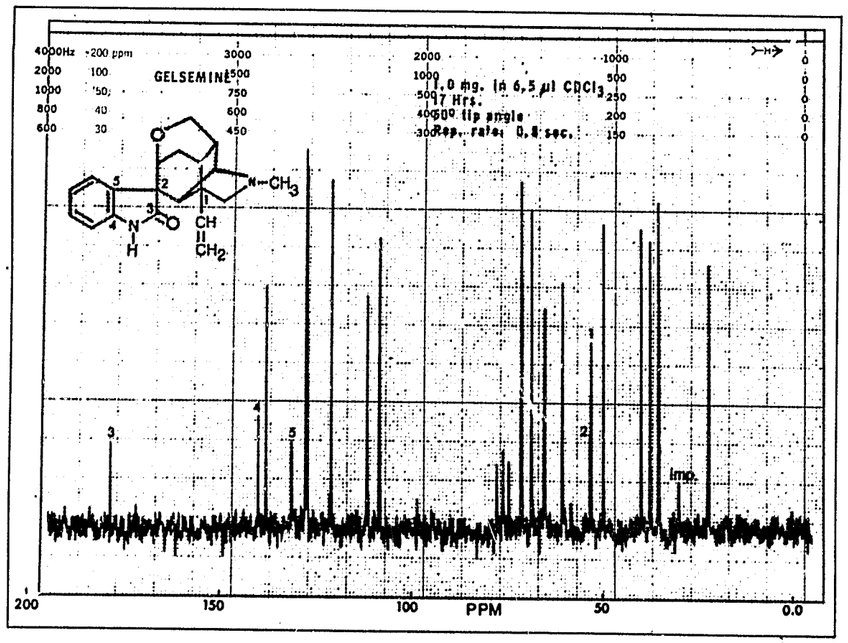


Figure 5. ^{13}C NMR spectrum of gelsemine (proton decoupled) in a capillary-type microcell. Shoolery (2).

lies somewhere in the vicinity of a milligram of a typical organic sample of molecular weight 300–400.

V. *QUANTITATIVE ^{13}C NMR MEASUREMENTS*

NMR is, in some respects, a nearly ideal quantitative technique. In principle the area under a line is proportional to the number of nuclei contributing to it, without differential extinction coefficients that enter into optical and infrared spectroscopy. Lines are usually sharp and well-separated, so that overlap problems are minimized. Line assignments are usually made rather easily, so that identifications are facilitated. On the other hand, there are some serious drawbacks in the quantitative use of NMR, as we discuss in the next paragraph. For proton NMR the difficulties have been largely overcome (3), but ^{13}C NMR has been widely regarded as being almost incapable of use for quantitative analysis. Only recently has work in a few laboratories begun to dispel that myth.

The principal problems in the use of ^{13}C NMR for quantitative purposes stem from the following factors:

1. Because of inherently low sensitivity and natural abundance, S/N is often lower than desired. These properties of ^{13}C cannot really be overcome completely, but instrumental improvements, as discussed previously, have alleviated the problem. Clearly, the poorer the signal/noise, the larger the random error in the quantitative determination.

2. Long spin-lattice relaxation times reduce signals below the expected levels when pulses are repeated too quickly (the analog of "saturation" in continuous wave NMR). Although this problem affects ^1H NMR also, it is usually more severe for ^{13}C NMR because of the wide disparity in T_1's for carbon atoms in different environments. We comment below on some means for overcoming the difficulty.

3. The nuclear Overhauser effect (NOE) that accompanies the proton decoupling usually carried out while measuring ^{13}C spectra is, like relaxation times, dependent on the environments of the individual carbon atoms. Thus, the areas under the various lines do not necessarily reflect the numbers of carbon atoms giving rise to the lines. Fortunately, as we shall see, it is possible to suppress the NOE, but at some loss in signal/noise.

Probably the best systematic treatment of ^{13}C NMR problems is contained in a recent review by Shoolery (4). Acenaphthene was chosen as a test compound, since T_1's of its carbon nuclei vary by a factor of at least 50, as indicated in Figure 6. The spectrum at the top of Figure 6 is a proton decoupled ^{13}C spectrum taken under customary conditions, in which

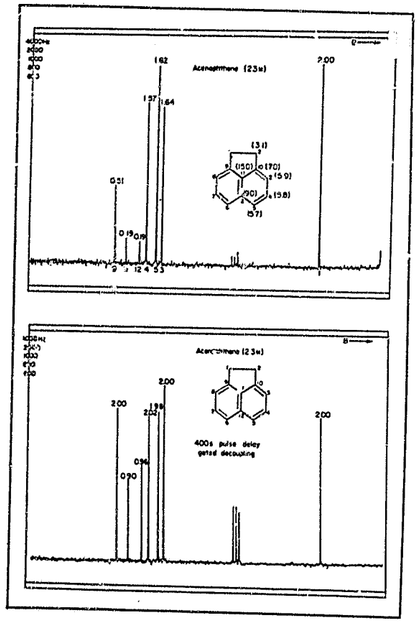


Figure 6. ^{13}C NMR spectra of acenaphthene (proton decoupled). Top: Obtained under routine conditions for optimization of overall signal/noise (20° flip angle, 1.0 sec repetition time, 640 pulses). Measured values of T_1 are given in parentheses on the structural formula. Bottom: Conditions for quantitative measurements (90° flip angle, 400 sec repetition time, decoupler gated off except during data acquisition). Shoolery (4).

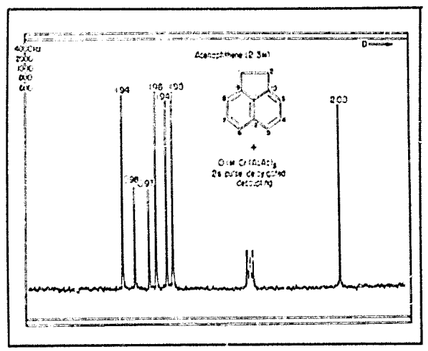


Figure 7. ^{13}C NMR spectrum of acenaphthene. Solution is also 0.1 M in chromium acetylacetonate. Conditions for quantitative measurements (20° flip angle, 1.0 sec acquisition time, decoupler gated off during 2.0 sec delay time following the acquisition time). Shoolery (4).

the magnetization flip angle and pulse repetition rate are selected so as to optimize overall S/N in the minimum time. If the area under the line due to C-1 is arbitrarily set equal to 2.00 (to reflect the two equivalent carbons, 1 and 2), then the other lines should have areas of either 1.00 or 2.00. As indicated in Figure 6 those due to the singly-protonated carbons 3, 4, and 5 are low by about 20%, while those due to the three non-protonated carbons are 75–80% low!

The effects of long T_1's can be eliminated by simply waiting long enough between pulse repetitions, while the NOE can be suppressed by gating the decoupler off during the long waiting periods and turning it on only during the brief periods of data acquisition following each pulse. The spectrum at the bottom of Figure 6 shows the results of incorporating both of these features. The areas are now much closer to their expected values, with the remaining discrepancies attributable to the very long T_1's, for which even a 400-sec. delay is insufficient. Unfortunately, such long waiting periods increase the length of the experiment to a nearly intolerable level. An alternative procedure, illustrated in Figure 7, is to shorten the T_1's by adding to the solution a paramagnetic "relaxation reagent." The most commonly used reagent is chromium acetylacetonate; as indicated in Figure 7, a low concentration of this substance is sufficient to bring all areas to within 3% of theoretical values with only a 2-sec. pulse delay. When such adulteration of the sample can be tolerated, the use of a relaxation reagent is by far the most effective way of obtaining quantitative ^{13}C data.

VI. FUTURE TRENDS IN NMR

NMR is a dynamic field, with versatile new methods constantly being developed. One of the most exciting areas is the study of "high resolution" spectra in solids. The techniques and instrumentation needed are, so far, rather specialized, but within a few years such studies should be rather widespread. Meanwhile, for conventional liquid samples, we can expect improvements in higher field systems, in sample handling capabilities and in computer support for NMR spectrometers. Without a major conceptual breakthrough it seems unlikely that sensitivity, the major subject of this paper, can be improved really substantially, but further small increments will doubtless occur.

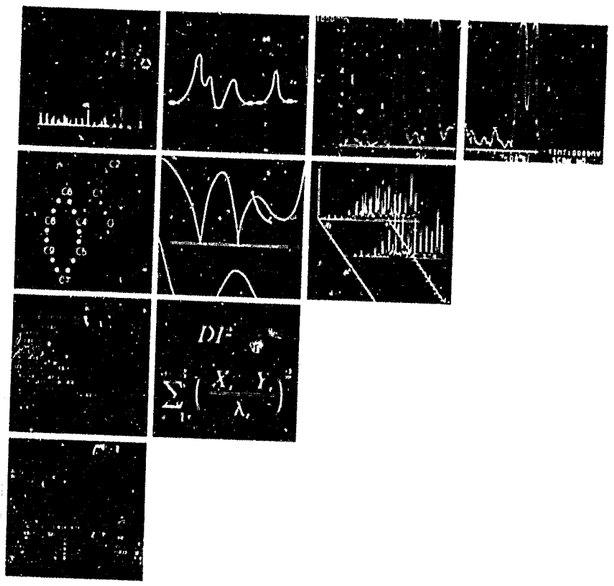
REFERENCES

1. See, for example, Becker, E. D., "High Resolution NMR." Academic Press, New York, 1969.
2. Shoolery, J. N., "Microsample Techniques in ^{1}H and ^{13}C NMR Spectroscopy." Varian Associates, Palo Alto, Calif., 1977.
3. Kasler, F., "Quantitative Analysis by NMR Spectroscopy." Academic Press, New York, 1973.
4. Shoolery, J. N., *Progr. NMR Spectry. 11*, 79 (1977).

Computer Applications Session

I. Drug Identification

II. Computer Systems in Government Facilities



A Brief Review of the Computerized Identification of Known Compounds and the Elucidation of Unknown Structures

by

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I. INTRODUCTION

To present a thorough review of the literature in this field is impossible in the time available. In the past six years alone, there have been twenty-five articles on the search and retrieval of infrared (ir) spectra using comparison methods by many authors. There have been thirteen articles using pattern recognition techniques, mainly from two groups of workers, Jurs at Penn State (1) and Isenhour at North Carolina (2). In addition, twelve interpretation programs have been reported. The use of computerized methods for mass spectra (ms) (3) is even larger, while proton magnetic resonance (pmr) (4) and carbon-13 magnetic resonance (cmr) (5) spectra have only recently been computerized. Thus, I must restrict this discussion to systems that use combined data. The ACS Symposium series # 54 on "Computer-Assisted Structure Elucidation" published last year (6) is a useful starting point for the beginner.

A difficult part of developing a search or interpretation system is obtaining the data files. This is especially true of the older types of spectra, ir and ms. Often the file entries are miscoded or redundant; perhaps they do not have some of the now useful data coded at all. It would cost too much to go back and extract the intensity data for the old ir files. The NIH-EPA[1] Chemical Information System (CIS) (6c) has cleaned up its ms files and is now collecting a new file of vapor phase ir spectra that are obtained under the strict conditions recommended by the Coblentz Society. We shall see how these files affect the retrieval process later.

[1] *NIH = National Institutes of Health, EPA = Environmental Protection Agency.*

II. RETRIEVAL SYSTEMS FOR KNOWN COMPOUNDS

A. The NIH-EPA Chemical Information System

The largest system in use at present is the NIH-EPA CIS, whose name has recently been changed to Structure and Nomenclature Search System (SANSS). It is available for use on a commercial system, the ADP-Cyphernetics Computer network. The system was developed by Heller, Milne and Feldmann and a description was published in *Science* last year (7, 6c). The latest version is shown in Figure 1. SANSS contains 42 files of data that can be searched for names, structures or substructures, Chemical Abstracts Service (CAS) registry number and molecular weight. The system also includes the enormous bibliographic files of the National Library of Medicine (NLM) and the LOCKHEED literature system that includes over 15,000,000 records.

The oldest part (1971) of CIS is the Mass Spectral Search System (MSSS), which has a carefully checked file of 31,700 mass spectra. As Dr. Milne will discuss the system later, I will not describe it further, except to point out that MSSS includes more than searching and identification facilities. One program, LABDET, which was written in my laboratory (8), calculates the mole fractions and % isotopic label incorporation of deuterated, C-13 or N-15 labeled compounds using the experimental mass spectra of the labeled and unlabeled compounds.

CIS also includes a file of x-ray powder diffraction data (PDAS), but perhaps more useful is the Cambridge Crystal File (CRYST) (9), a data base of about 15,000 entries containing published crystallographic data, mostly on organic compounds. These can be searched by CAS number, name, formula, molecular weight, space group and unit cell parameters. CAMSEQ is a program developed at Case-Western that uses the CRYST structural data as a starting point to calculate the conformations of molecules in solution by a combination of empirical and quantum mechanical techniques.

For those interested in toxicities, the files of NIOSH's[2] Registry of Toxic Effects of Chemical Substances (RTECS) can be searched. An

[2] *NIOSH = National Institute of Occupational Safety and Health.*

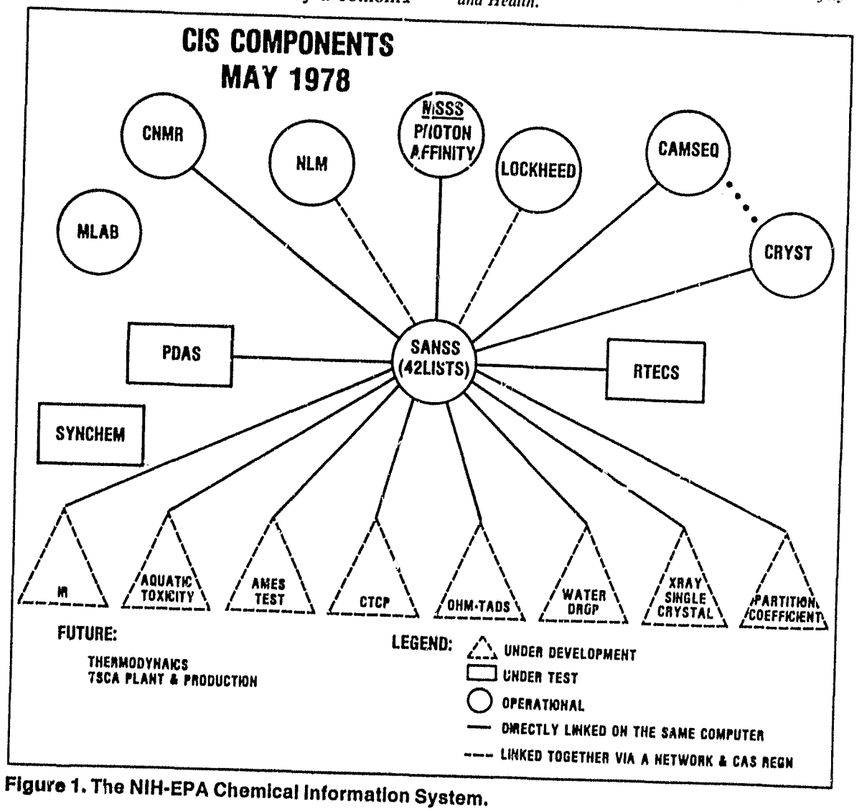
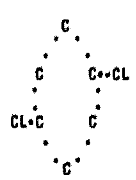


Figure 1. The NIH-EPA Chemical Information System.

example of a RTECS search for para-dichlorobenzene is shown in Figure 2.

Finally I would like to illustrate the carbon-13 nuclear magnetic resonance (CNMR) file that contains 3800 spectra. The file can be searched, as in Figure 3, for specific peaks at a specific chemical shift ± an error and given multiplicity of the nondecoupled cmr spectrum. CNMR found 37 entries at δ=197.5±.5(S). The operator decided to add different peaks at 26.0±.5(Q) and at 137.0±.5(S). This time only 1 match was found and was then listed. Then compound #300 was requested, Figure 4 and the entire assigned spectrum was printed for acetophenone. Note that the structure is given with carbon numbers as well as other particulars of the compound and conditions that were used to obtain the spectrum. The file can be searched for a particular type of substructural carbon, e.g., a methyl group γ to a nitrogen atom has a mean shift of 11.7±0.2 ppm based on 7 entries in the file (10).

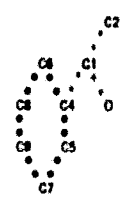
```
                                  NIH/EPA
                        SUBSTRUCTURE SEARCH SYSTEM
LATEST NEWS GENERATED ON 4/16/77
COMPLETE DATA BASE SELECTED
ENTER NEW SELECTION (H FOR HELP).32
DATA BASE SELECTED 32
OPTION? RING
OPTION? ALTBD  1 2
OPTION? ABRAN 1 AT 1 1 AT 4
OPTION? SATOM 7 8
SPECIFY ELEMENT SYMBOL = CL
OPTION? IDENT
TOTAL PROTON LOUNT FOR THIS STRUCTURE IS
     (P FOR PROGRAM ESTIMATE) 4
FILE 1. THIS STRUCTURE IS CONTAINED IN 1 COMPOUNDS.
STRUCTURE     1 CAS REGISTRY NUMBER 106-46-7
NIOSH RTECS:  CZ45500
                                                  C6H4Cl2
```



```
Benzene, 1,4-dichloro- (9CI)
Benzene, p-dichloro- (8CI)
p-Chlorophenyl chloride
p-Dichlorobenzene
Di-chloricide
Evola
Paradi
Paradow
OPTION? TSHOW 106-46-7
YOU ARE NOW IN THE RTECS RETRIEVAL SYSTEM
LAST NEWS UPDATE 24-MAR-78
CAS NUMBER =  106467                    NIOSH NUMBER =  CZ45500
 ORL-HMN  TDLO:    300 MG/K    TFX UNS    PCOC**    -,851,-
 ORL-RAT  LD50:    500 MG/K    TFX:       WRPCA2    9,119,70
 IPR-RAT  LD50:   2500 MG/K    TFX:       JAPMA8    38,124,49
 ORL-MUS  LD50:   2950 MG/K    TFX:       GUCHAZ    6,183,73
 ORL-GPG  LDLO:   2800 MG/K    TFX:       14CYAT    -,1338,63
 Benzene, 1,4-dichloro- (9CI)
 C6H4Cl2
```

Figure 2. A RTECS Search on the CIS.

```
Option SPEC
ID # 300
# 300
```



```
Ethanone, 1 phenyl-
REGN =       98862    MW = 120.06
C8 H8 O1
L.F. JOHNSON, W.C. JANKOWSKI, C-13 NMR SPECTRA. JOHN WILEY&SONS, NY (1972)
Solvent   CHLOROFORM

SHIFT   MULT   INTENS   ASSIGN
197.8    S       9        1
137.1    S       9        4
132.9    D      50        9
128.4    D      44        7
128.4    D      44        8
128.2    D      42        5
128.2    D      42        6
 26.3    Q      46        2
```

Figure 3. NIH-EPA C-13 NMR Search System—Version 3.3.

```
Latest NEWS dated 14 JULY 1977
To list options, type  OPT

Option: SH

Enter SHIFT, DEVIATION, MULT   197.5, .5, S
     37 matches using 197.5

L(ist), Q(uit), or next SHIFT, DEVIATION, MULT:  26.0, .5, Q
     3 matches using 197.5  26.0

L(ist), Q(uit), or next SHIFT, DEVIATION, MULT.:  137.0, .5, S
     1 matches using 197.5  26.0  137.0

L(ist), Q(uit), or next SHIFT, DEVIATION, MULT.: L

     ID#              NAME

     300        Ethanone, 1-phenyl-
                CAS REG#   98862        MF: C8 H8 O1
```

Figure 4. Spectrum Search for Acetophenone (from Figure 3).

B. The Yugoslavian COSMOSS System

Zupan *et al.* (11) have recently published data on a combined retrieval system containing ir, ms and cmr data. Their concept was that "it should work as a black box: using only the spectra as input data." The data base consists of 92,000 ir spectra based mainly on the ASTM-WYANDOTTE collection (12), which includes the 35,000 spectra of Sadtler (13) and 13,000 of the DMS collection (14). The ms file is made up of the 16,000 spectra of the Aldermaston collection (15). While many of the spectra of these collections are doubles and are wrongly coded, no attempt was made to weed out the data base, as was done on the CIS system. The cmr file contained about 1600 good spectra. The search can be made on a single or multiple file basis. The authors reported data for 100 test searches for each of the data bases. Accuracy was defined as the fraction of examples in which the correct compound was found in the top five choices listed. Searches of this type invariably give several possibilities. However, a user is more interested in the number of correct first choices. For ir, only 77% first choices were correct, for ms, 91%, and cmr, 95%. These results are probably a reflection of the quality of the various data bases.

The user hopes that when a spectrum not present in the data base is searched, the retrieved examples will be close to the correct structure. This can be true only when the choices picked are reliable. Interpretive methods are actually better for the cases of true unknowns. McLafferty (6a) has suggested that the performance of a search system should include a recall factor, a reliability factor and a false positive factor. When the spectrum being searched is not a part of the data base, then correct matches are only those relevant to the structure of the unknown. Thus a search system must be judged by both the correct or near-correct matches and the misleading false positives found.

C. Other Retrieval Systems

Since the advent of gas chromatography-mass spectrometry instruments, there have been a large number of search routines developed. As Drs. Milne and Biemann will describe the use of their systems, I will move on to mention briefly two other groups concerned with the problem. Clerc of Switzerland has separately published search routines for ms (16), ir (17) and cmr (18). It is interesting that he has not yet put them all together.

Clerc's ms-search algorithm is used in the Associated Electronics Industries (A.E.I.) ms-data systems.

Finally, McLafferty's (6a) Probability Based Matching System (PBM) and Self-Training Interpretive and Retrieval System (STIRS) represent fairly successful attempts to identify complete structures or 179 substructural parts of an unknown system. A quick review of both is given in the ACS Symposium volume (6a). Both of these programs are available for use on the Cornell-TYMNET computer system. A recent comparison on the same data base of STIRS with Isenhour's K-Nearest Neighbor (KNN) algorithm (19), which is a pattern recognition procedure, has shown STIRS to be generally superior (20).

While several individual spectral systems have been devised, it seems to me that all possible data should be used. This leads us to the next major topic.

III. *COMBINED SYSTEMS FOR THE ELUCIDATION OF UNKNOWNS USING INTERPRETIVE TECHNIQUES*

Four systems using combined data for the structure determination of unknown compounds are currently being developed:

1. *CHEMICS-F* by Sasaki *et al.* in Japan (6h)
2. *CASE* by Munk *et al.* at Arizona State University (6g)
3. *DENDRAL* and *CONGEN* by Smith and Carhart *et al.* at Stanford University (6i, 21)
4. *STREC* by Gribov and Elyashberg *et al.* in Russia (22).

Since the ACS Symposium volume (6) discusses the first three of these systems, I will only mention some of their characteristics and then describe the Russian system in a little more detail.

One of the major problems in any system is deriving all the possible structures without redundancies. The Japanese system derives all possible structures that fit a given molecular formula, then eliminates the various structures on the basis of automatically acquired and interpreted ir, pmr, cmr, ultraviolet (uv) and ms data. The original work was done only on C, H and O compounds, but more recently nitrogen has been added (23b).

The Stanford Group (6i, 21c) has developed a highly operator-interactive heuristic program, CONGEN, that derives the possible nonredundant structures that are consistent with a molecular formula and any constraints the operator wants to impose. These constraints may be derived from known chemical reactions, such as the number of moles of periodate used up, or from spectral data. The CONGEN approach allows the practicing chemist to be intimately involved in the process. Much more difficult structures can thus be studied and the likelihood of missing possible structures is reduced. When no constraints are used, the program is similar to that used by Sasaki.

The Arizona State group (6g) has also developed a highly sophisticated interactive program, CASE, that attempts to parallel the natural product chemist's reasoning process. The molecule assembler uses both operator data and automatically interpre ed ir and cmr data. Possible structures are then checked by calculation of the cmr spectrum. CASE has been shown to be quite effective on real-world problems.

Finally, let us look at the Russian system (22), which follows a somewhat different approach. Figure 5 shows a simplified and compressed block diagram of STREC. STREC sequentially identifies various structural groups present in the vibrational spectrum (ir and Raman) that are consistent with the elements present in the empirical formula. These are combined into sets of fragments and then assembled into structural formulas under the constraints of the identified fragments plus any chemical information provided by the operator. The structures are then double checked against the vibrational data, previously identified fragments and forbidden fragments (the latter corresponds to the BAD LIST used in CONGEN [21c]). The spectral filter process is then repeated using pmr, ms and uv data. The various fragment libraries include the indicated number of fragment possibilities. Only the 20 most intense peaks are used from their low-resolution mass spectral data. The vibrational spectrum of each compatible structure is then calculated and compared to the unknown spectrum. The result, along with the performance of chemical functional analysis (which was not identified in the paper), provides a measure of the reliability of the answers. Several examples of C, H, N and O containing compounds with molecular weights up to 202 were given.

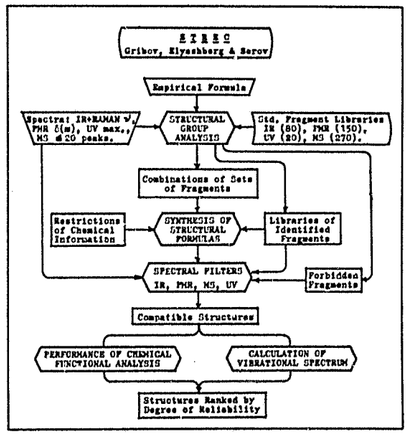


Figure 5. Condensed Block Diagram of STREC. (22).

All of these approaches are promising. The Japanese and Russian systems cannot yet attempt highly complex structures, but the operator also does not have to be an expert, versatile, natural products chemist. All the data interpretation is done by the computer. The two American systems, on the other hand, require qualified operators. Both, however, have been used successfully on quite complex structures and are indeed an aid to the structural chemist. Both are also expandable to include new algorithms that will eventually reduce the knowledge required by the operator.

I have been unable to determine how much interaction between the various interpretive routines is allowed. In real life, the approach one uses to interpret spectral data depends completely on the type of spectra available. Generally, the spectral data are highly complementary and not specific to only one type. As far as I can determine, the systems described above consider each type separately. In addition, all these systems use a molecular formula or empirical formula as input data, but do not describe how it is obtained.

IV. *MOFO, A PROGRAM FOR THE DETERMINATION OF MOLECULAR FORMULAS*

Several years ago, we devised a general program, MOFO, to assist us in determining the molecular formula of an unknown using whatever data were available (24). As we have only a medium-resolution mass spectrometer, we could make only crude mass measurements. The block diagram of MOFO is shown in Figure 6. Data inputs include the mass and accuracy of measurement of the parent molecular ion and the intensities of the molecular ion cluster including any P-H, H_2 or H_3 ions. The program has a subroutine, MSMS, that can calculate the mass of an ion using distance or time measurements from known standard ions, such as perfluorokerosene or perfluorotri-*n*-butylamine. The operator then enters the upper and lower limits for the number of atoms of C, H, N, O, S, P, Si, F, Cl, Br, I and any one additional heteroatom. The heteroatom can be any element; we have used Se, Mg, Cd and Fe. Subroutine CHECK then generates all possible formulas within the limits specified and eliminates those corresponding to fragment species (points of unsaturation=n+0.5) and computes the exact mass for each. If pro-

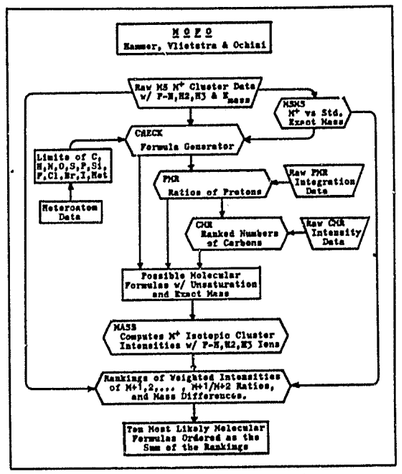


Figure 6. Block Diagram of MOFO.

ton integration data are available, then the formulas are further pruned to conform to the correct ratio of protons; if cmr data are available, a further pruning is done. In either case, no interpretation by the operator is required.

The molecular ion cluster of each remaining formula is then computed with the associated P-H, H_2, H_3 effects found in the experimental spectrum. The calculated spectrum is then compared to the M+1, 2, . . . ions of the experimental spectrum, as well as the (M+1)/(M+2) ratio and exact mass, if available. These comparisons are then ranked and the ten (or fewer) most likely formulas are listed with the best formula first.

When only unit resolution ms data are available, the correct formula is chosen first about 75% of the time. The addition of pmr or cmr data improves the accuracy to about 95%. When both cmr and pmr data are used, or when high resolution ms data are used, MOFO rarely misses. Both our undergraduate and graduate students have been using MOFO for several years; thus the operator does not have to be highly experienced.

ACKNOWLEDGMENTS

I wish to thank Dr. G. W. A. Milne for the first four figures used here and Prof. Sasaki of Miyagi University of Education in Sendai, Japan, for his hospitality when part (cmr) of MOFO was being developed.

REFERENCES

1. Jurs, P. C., *et al., Appl. Spectrosc.*, 27. 371 (1973); *Anal. Chem.*, 46, 520 (1974); *ibid.*, 46, 2126.

2. Isenhour, T. L., *et al., Anal. Chem.*, 46, 150 (1974); *ibid.*, 47, 1126 (1975); *ibid.*, 47, 2027; *Appl. Spectrosc.*, 29, 226 (1975); *J. Chem. Inf. Comput. Sci.*, 15, 212 (1975); *Anal. Chem.*, 48, 1027 (1976); *ibid.*, 48, 1513; *Appl. Spectrosc.*, 30, 213 (1977); *ibid.*, 31, 18 (1977).

3a. To mid-seventies, Pesyna, G. M., and McLafferty, F. W., in "Determination of Organic Structures by Physical Methods," Vol. 6, F. C. Nachod, J. J. Zuckerman, and E. W. Randall, Eds. Academic Press, New York, 1976, pp. 91–155.

b. Burlingame, A. L., Kimble, B. J., and Derrick, P. J., *Anal. Chem.*, 48(5), 374R (1976).

c. Burlingame, A. L., Shackleton, C. H. L., Howe, I., Chizhov, O. S., *ibid.*, 50(5), 352R (1978).

4a. Jones, R. T., *Proc. Soc. Anal. Chem.*, 10, 165 (1973).

b. Wasson, J. R., and Johnson, D. K., *Anal. Chem.*, 46(5), 317R (1974).

c. Wasson, J. R., and Lorenz, D. R., *ibid.*, 48(5), 248R (1976).

d. Yamasaki, T., and Sasaki, S., *Jpn. Anal.*, 213 (1975).

5a. Ochiai, S., Hirota, Y., Kudo, Y., and Sasaki, S., *Jpn. Anal.*, 22, 399 (1973).

b. Brunner, T. R., Williams, R. C., Wilkins, C. L., and McCombie, P. J., *Anal. Chem.*, 46, 1978 (1974).

c. Jezl, B. A., and Dalrymple, D. L., *Anal. Chem.*, 47, 203 (1975).

d. Schwarzenbach, R., Meili, J., Könitzer, H., and Clerc. J. T., *Org. Mag. Res.*, 8, 11, (1976).

e. References 6e, 6f, 6g, and 6h, below.

f. Dalrymple, D. L., Wilkins, C. L., Milne, G. W. A., and Heller, S. R., *Org. Mag. Res.*, in press (1978).

6. Smith, D. H., Ed., "Computer-Assisted Structure Elucidation," ACS Symposium Series # 54, American Chemical Society, Washington, D.C., 1974.

a. McLafferty, F. W., *et al.*, "Computer-Assisted Identification of Unknown Mass Spectra," pp. 1–17.

b. Beinann, K., *et al.*, "Identification of the Components of Complex Mixtures by GC-MS," pp. 18–25.

c. Milne, G. W. A., and Heller, S. R., "The NIH-EPA Chemical Information System," pp. 26–45.

d. DeHaseth, J. A., and Isenhour, T. L., "An Information Approach to the Determination of the Secondary Structures of Globular Proteins," pp. 46–57.

e. Schwenzer, G. M., and Mitchell, T. M., "Computer Assisted Structure Elucidation Using Automatically Acquired ^{13}C NMR Rules," pp. 58–76.

f. Surprenant, H. L., and Reilley, C. N., "Computerized Structural Predictions from ^{13}C NMR Spectra," pp. 77–91.

g. Munk, M. E., *et al.*, "Interactive Structure Elucidation," pp. 92–107.

h. Sasaki, S., *et al.*, "CHEMICS: A Computer Program System for Structure Elucidation of Organic Compounds," pp. 108–125.

i. Carhart, R. E., *et al.*, "Computer Assistance for the Structural Chemist," pp. 126–145.

7a. Heller, S. R., Milne, G. W. A., and Feldmann, R. J., *Science*, 195, 253 (1977).

b. Reference 6c.

8. Hammer, C. F., and Vlietstra, A. J., unpublished results; see references 6c (p. 42) and 7a for brief descriptions.

9. Kennard, O., Watson, D. G., and Town, W. G., *J. Chem. Doc.*, 12, 14 (1972).

10. Milne, G. W. A., Zupan, J., Heller, S. R., and Miller, J. A., Spectra-Structure Relationships in Carbon-13 Nuclear Magnetic Resonance Spectroscopy. Results from a Large Data Base, *Org. Mag. Res.*, submitted (1978).

11. Zupan, J., Penca, M., Hadžl, D., and Marsel, J., *Anal. Chem.*, 49, 2141 (1977).

12. Codes and Instructions for WYANDOTTE-ASTM, American Society for Testing Materials, 1916 Race St., Philadelphia, Pa. (1964).

CONTINUED

1 OF 4

13. Sadtler Standard Spectra, Sadtler Research Laboratories, Philadelphia, Pa.

14. DMS, Documentation of Molecular Spectroscopy. Verlag Chemie, Weinheim, and Butterworth, London.

15. Aldermaston Mass Spectrometry Data Collection, Aldermaston, Reading, U.K.

16. Clerc, J. T., and Erni, F., *Top. Curr. Chem.*, 39, 91 (1973).

17. Clerc, J. T., Knutti, R., Koenitzer, H., and Zupan, J., *Fresenius' Z. Anal. Chem.*, 283, 177 (1977).

18. Schwarzenbach, R., Meili, J., Koenitzer, H., and Clerc, J. T., *Org. Mag. Res.*, 8, 11 (1976).

19. Justice, J. B. Jr., and Isenhour, T. L., *Anal. Chem.*, 46, 223 (1974).

20. Lowry, S. R., Isenhour, T. L., Justice, J. B. Jr., McLafferty, F. W., Dayringer, H. E., and Venkataraghavan, R., *ibid.*, 49, 1720 (1977).

21a. Masinter, L. M., Sridharan, N. S., Lederberg, J., and Smith, D. H., *J. Am. Chem. Soc.*, 96, 7702 (1974).

b. Masinter, L. M., Sridharan, N. S., Carhart, R. E., and Smith, D. H., *ibid.*, 96, 7714, (1974).

c. Carhart, R. E., Smith, D. H., Brown, H., and Djerassie, C., *ibid.*, 97, 5755 (1975).

22. Gribov, L. A., Elyashberg, M. E., and Serov, V. V., *Anal. Chim. Acta*, 95, 75 (1977) and references therein.

23a. Sasaki, S., Abe, H., Ouki, T., Sakamoto, M., and Ochai, S., *Anal. Chem.*, 40, 2220 (1968).

b. Miyashita, Y., Ochiai, S., and Sasaki, S., *J. Chem. Inf. Comput. Sci.*, 17, 228 (1977).

24. Hammer, C. F., Vlietstra, A. J., and Ochiai, S., unpublished results.

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The Use of Mass Spectrometry for Drug Identification

by

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I. INTRODUCTION

In 1969, using facilities of the (then) National Heart and Lung Institute, of the National Institutes of Health, the Suburban Hospital of Bethesda, MD., in collaboration with NIH scientists, undertook a pilot study designed to investigate the possible utility of mass spectrometry in problems of drug identification. As a typical suburban community hospital, Suburban Hospital receives a small, but continuing number of patients who have consumed excessive amounts of drugs, and it was felt that rapid and accurate identification of the drug or drugs involved in each case would facilitate treatment.

The pilot study (1) permitted the conclusions that drugs could in fact be rapidly and accurately identified by gas chromatography-mass spectrometry and that, in the view of a majority of the attending physicians, the resulting information was of value in the clinical handling of drug overdoses. Accordingly, in 1971, support was sought from NIH by Suburban Hospital for the purchase of a gas chromatograph-mass spectrometer system which was to be installed at the hospital and which, it was hoped, would permit the hospital to continue to offer this service independently of the National Heart and Lung Institute's laboratories. A grant from NIH was secured, the gas chromatograph-mass spectrometer was installed at Suburban Hospital in 1972, and the service has been in use since then.

The purpose of this paper is to describe the results that have been obtained in the period since then.

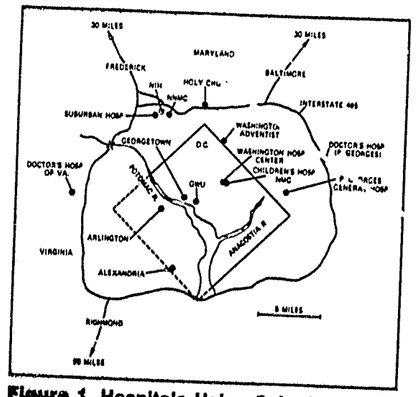

Figure 1. Hospitals Using Suburban Hospital's Emergency Drug Indentification Service.

II. DISCUSSION

A. Service Area

The major user of the drug identification service is, of course, Suburban Hospital itself. During the seven-year period however, increasing numbers of samples from other hospitals have been received at Suburban. Currently, thirteen of the approximately fifteen major hospitals in the Greater Washington Area, shown in Figure 1, send samples to Suburban on a regular basis.

In 1976, Suburban Hospital instituted, for the first time, a charge of approximately $75 for each gas chromatography-mass spectrometry (GC-MS) analysis completed. At the same time, major health insurance schemes agreed

to accept this expense. As a result of these two events, usage of the service offered by Suburban had dropped slightly and at the present time, about 580 cases are handled per year. This averages to about 1.5 cases per day which is a load that can be carried by the Special Chemistry Laboratory at the hospital without any major re-staffing. The $75 charge covers the cost to Suburban of the service.

The cases that are submitted to the service are usually those in which drug involvement is suspected, but the drug or drugs in question cannot be identified by other, simpler means such as information from the patient or others, or techniques such as thin-layer or gas chromatography. A substantial number of samples received contain no drugs, and in many others, alcohol is the only drug found. In many of these cases, the suspicion that drugs was involved was ill-founded and this result is of value in treatment. Where drugs are involved, their identity is determined and reported and this information is also of value in treatment. In this way, the service permits the physician to reduce the number of variables that must be considered in diagnosis and treatment and consequently GC-MS appears to have a legitimate role in the functions of a Clinical Chemistry

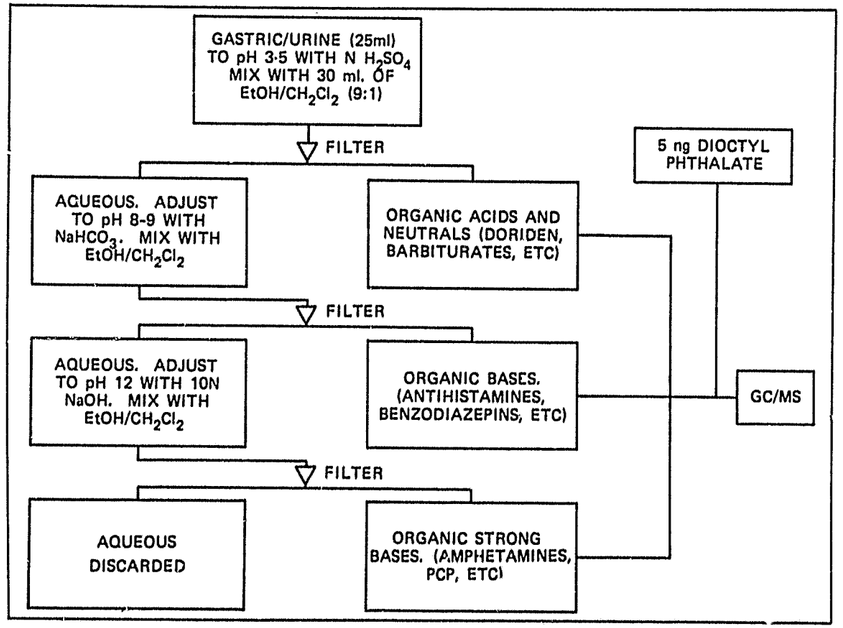

Figure 2. Protocol For Drug Identification by GC-MS.

Laboratory.

A member of the laboratory staff is on call at all times to handle drug identification samples. Those from other hospitals are usually delivered to Suburban by car or taxi. The handling time, once the sample is received at Suburban is between one and three hours. The result is then relayed by telephone and a written record follows.

B. Methodology

The most readily available samples that can be obtained for drug identification are blood, urine, and gastric contents. Our experience with blood has indicated that it is the least useful of the three for a number of reasons and it is not used unless no other sample can be obtained. Gastric contents frequently contain detectable amounts of orally ingested drugs, unless too much time has elapsed since ingestion. A number of drugs, ingested in large amounts, can be detected unchanged in urine many hours after ingestion. Consequently, it is requested that, if possible, both gastric contents and urine be submitted. When these are received at Suburban, they are combined, and the mixture is worked up according to the procedure shown in Figure 2. This procedure is designed to extract from the sample all organic acids, bases and neutrals. No effort is made to separate these classes, because the gas chromatographic step makes this unnecessary. An internal standard of dioctyl phthalate is added to the extract before gas chromatography. This is used to standardize the temperature program of the gas chromatograph.

Two mass spectrometric approaches have been used in this work. The method of choice, which is used routinely at Suburban, employs chemical ionization (CI) mass spectrometry (2), a method that has been shown (3) to be readily applicable to problems in drug identification. In the other method, which is used as a second-string approach, the standard electron ionization (EI) mass spectrometric technique is used. This is also known (1) to be quite applicable to the problem, but, because it gives more complex spectra (4), is not used routinely.

The CI GC-MS analysis is carried out using the system that is shown in Figure 3. The sample, after having been processed as shown in Figure 2, is injected into the gas chromatograph shown in Figure 3. As compounds are eluted from the chromatograph, they are admixed with a reagent gas, typically methane, and the methane/drug mixture passes into the source of the mass spectrometer. Here, chemical ionization takes place and the CI mass spectrum of each component of the mixture is recorded as the substance emerges from the chromatograph, which is temperature programmed.

The mass spectra that are obtained in this way are very simple. The spectrum of chlordiazepoxide (Librium) is shown in Figure 4, and from this it can be seen that the CI mass spectrum consists mainly of the protonated

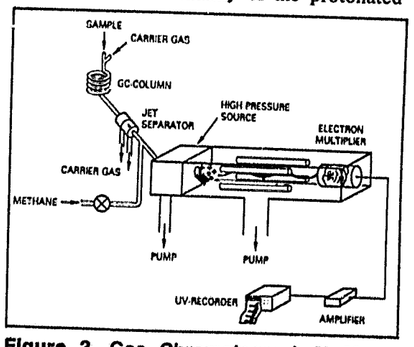

Figure 3. Gas Chromatograph-CI Mass Spectrometer Combination.

molecular ion at an m/e value of (M+1), where M is the molecular weight of the substance. The molecular weight of the substance can thus be determined directly from such a spectrum, and the identity of the compound usually follows immediately. A secondary check on this identification is afforded by the retention time, or elution temperature of the compound in the gas chromatograph. The only important drugs with the same molecular weight as each other are pentobarbital and amobarbital (M=226). Distinction between these, when necessary, is made possible by examination of either the gas chromatogram or the respective EI mass spectra.

The second method employs EI mass spectrometry and can be accomplished using any of a variety of commercial GC-MS systems. These differ from the system shown in Figure 3 principally in that the source must be operated at high vacuum, and so, rather than adding a reagent gas to the stream emerging from the gas chromatograph, a separator is built into the system to remove much of the helium carrier gas before the stream enters the mass spectrometer source. The mass spectrometer need not be a quadrupole, of course; magnetic sector machines are used very commonly.

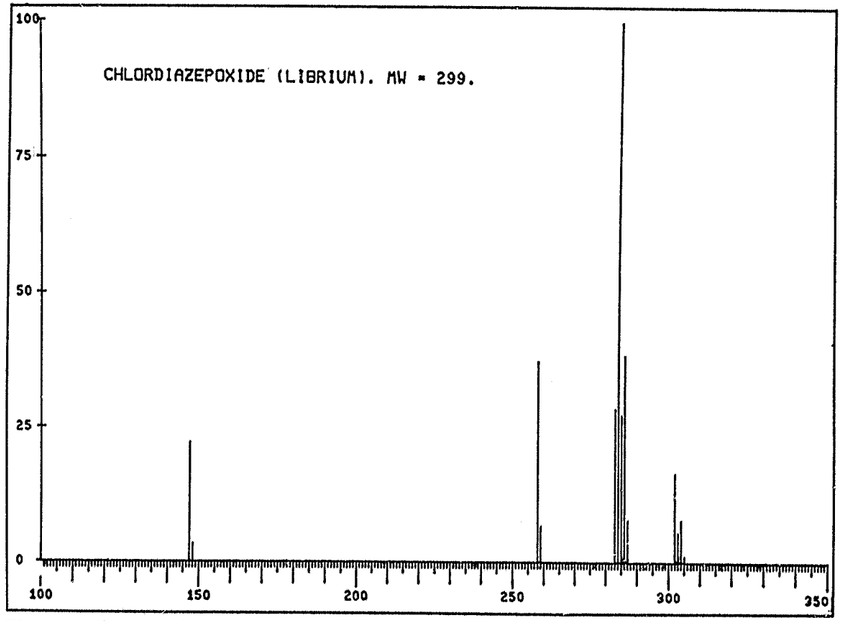

Figure 4. Methane CI Mass Spectrum of Chlordiazepoxide (Librium).

The EI mass spectra of drugs are usually more complex than the corresponding CI mass spectra. A typical EI mass spectrum, that of phenobarbital, is shown in Figure 5. From this, it can be seen that a great deal of fragmentation is occurring, and the molecular ion, at m/e 232, is not the most intense ion in the spectrum. Identification of substances from such spectra is therefore not always easy and may require the use of a computer search system such as the NIH-EPA Mass Spectral Search System. An example of the use of this system is given in Figure 6. The computer uses the two major peaks of the EI mass spectrum (m/e 204, intensity between 100%, and m/e 232, intensity between 20% and 30%) to identify phenobarbital unequivocally from the data base of 30,000 mass spectra.

Using either CI or EI mass spectrometry, in conjunction with gas chromatography, identification of drugs contained in urine or gastric contents has become a standard procedure and

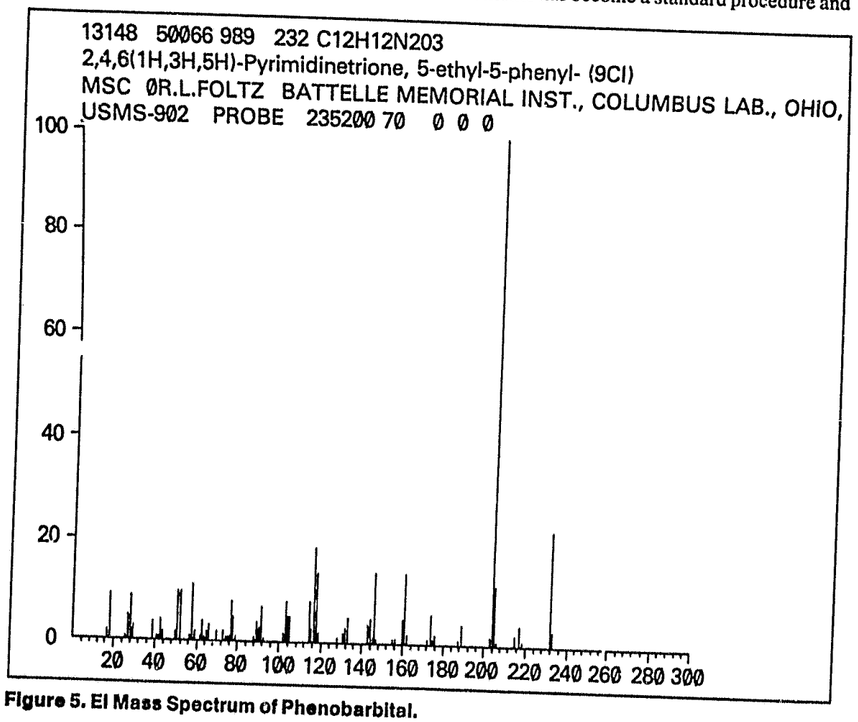

Figure 5. EI Mass Spectrum of Phenobarbital.

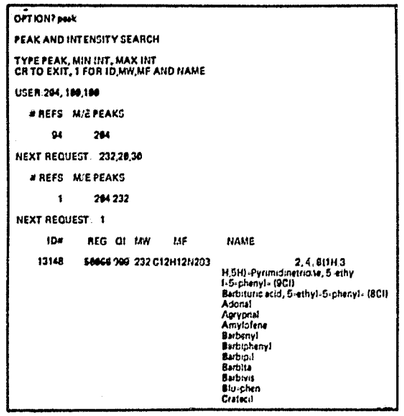

Figure 6. Identification of Phenobarbital From Its EI Mass Spectrum By Computer Library Searching.

has led to the results that are discussed in the next section.

III. RESULTS

A. Case load

The number of cases handled by the drug identification service can be seen in Figure 7. These data suggest that the rapid growth in the use of the service during 1973–1976 has ceased and perhaps that a steady state may soon be reached in which between 500 and 600 cases per year are involved. This presumably may be taken as a rough approximation to the total number of serious drug overdoses per year in the Greater Washington area and, if correct, suggests that a single GC-MS facility is easily adequate to deal with the medical drug identification problems that arise in such a community.

In the same Figure, it can be seen that the number of drugs identified in each case has remained very close to 2 on average. The number of cases in which no drugs were found has been decreasing steadily for three years, in spite of the increase in the total number of cases. This is in part because of minor changes in reporting; alcohol, for example, has been reported as a drug only since 1975 The decrease is also due in part to the fact that the laboratory has been steadily gaining experience and now recognizes a larger number of drugs than was the case in earlier years.

B. Drugs Encountered

A total of approximately 90 different drugs have been encountered in the seven years that the service has been in existence. As can be

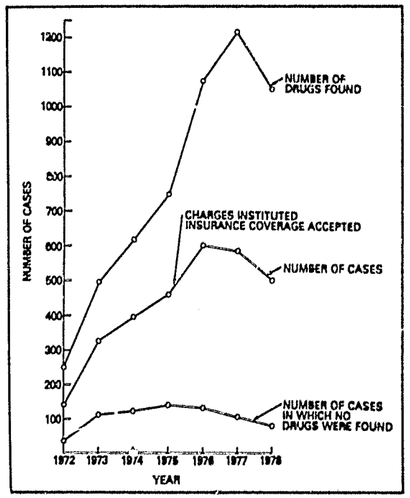

Figure 7. Seven Year Summary of Drug Identification Program.

seen from Figure 8, the most commonly encountered substance, by far, is caffeine, which is found in about 25% of all cases. Nicotine, though somewhat less common, is also found very frequently. The most commonly found 'drug' is aspirin, which is found in perhaps 15% of all cases. Aspirin alone is rarely responsible for admission to a hospital, except where small children are involved. The most commonly abused lethal drug appears to be secobarbital, which has held this dubious position throughout the entire seven years of this work. In contrast to aspirin, secobarbital is found both alone and in admixture with other drugs, such as aspirin.

Valium, or diazepam, has been the most commonly implicated tranquilizer, being found consistently more frequently than chlordiazepoxide (Librium), amitriptyline (Elavil), or meprobamate (Miltown).

The drugs in Figure 8 are those that have been found most frequently and it can be seen at a glance that the classes represented are but three; sedatives, analgesics, and tranquilizers. This comes as little surprise to students of the abuse of legitimate drugs, and eliminates the possibility that stimulants, for example, are more abused than other drugs.

The stimulants that have been found are cited in Figure 9, and it can be seen that, neglecting caffeine and cotinine, which is a nicotine metabolite, the use of stimulants does not appear to contribute to the case load substantially. In fact, of the 24 positive identifications of amphetamine or methamphetamine that have been made in seven years, probably half were not associated with an overdosed patient, but were submitted as material samples by

DRUG	7 YEAR TOTAL	1977
CAFFEINE	605	165
ACETYL SALICYLIC ACID	391	116
NICOTINE	282	112
SECOBARBITAL	206	31
DIAZEPAM	186	32
PHENOBARBITAL	179	46
DEXTROPROPOXYPHENE	178	36
AMOBARBITAL	139	20
METHAQUALONE	135	15
CHLORDIAZEPOXIDE	119	19
PENTOBARBITAL	119	29
MEPROBAMATE	118	22
ACETOPHENETIDIN	107	15

Figure 8. Most Commonly Encountered Drugs.

legal authorities.

In Figure 10 are shown the data concerning sedatives, certainly the most lethal group from the perspective of drug overdoses. It is clear from Figure 10 that barbiturates dominate the class with only occasional appearances of non-barbiturate sedatives such as methaqualone, ethchlorvynol and glutethimide. As was mentioned above, sedatives, particularly barbiturates, frequently are the only drug involved in an overdose. Mixtures of barbiturates are, however, noted and a very common occurrence is the identification in a patient of a barbiturate sedative and a tranquilizer, for

Stimulant	1972	1973	1974	1975	1976	1977	1978	7 Year Total
Caffeine	15	17	49	116	162	165	81	605
Cotinine (Nicotine metabolite)	–	–	–	1	13	14	16	44
Amphetamine, methamphetamine	0	3	5	1	2	8	5	24
Mescaline	0	0	1	2	0	1	1	5

Figure 9. Stimulants Encountered in Drug Identification Program.

Drug	1972	1973	1974	1975	1976	1977	1978	7 Year Total
Secobarbital	23	37	30	29	40	31	16	206
Phenobarbital	11	22	20	17	45	46	18	179
Amobarbital (Amytal)	11	30	29	16	29	20	4	139
Methaqualone (Quaalude)	19	38	24	19	15	15	5	135
Pentobarbital	12	14	19	19	19	29	7	119
Ethchlorvynol (Placidyl)	5	4	19	14	27	20	7	96
Methyprylon (Noludar)	7	7	13	16	16	18	9	86
Glutethimide (Doriden)	9	4	25	12	10	10	6	76
Chlorpromazine (Thorazine)	2	4	2	2	16	13	7	46
Meperidine (Demerol)	0	3	5	3	17	11	6	45
Butalbital (Sandoptal)	0	3	1	10	9	11	2	36
Carbromal (Nyctal)	0	14	2	2	2	11	3	34
Butabarbital (Butisol)	0	2	4	2	5	8	2	23
Mephobarbital (Mebaral)	0	2	1	1	5	9	4	22
Vinbarbital (Delvinal)	0	0	0	0	10	10	2	22
Barbital	0	1	1	2	3	5	2	14
Aprobarbital (Alurate)	0	0	0	0	1	5	3	9
Hexobarbital (Evipal)	0	0	1	2	1	1	0	5
Allobarbital	0	0	0	1	2	1	0	4
Talbutal (Lotusate)	0	0	0	0	1	1	1	3
Alphenal	0	0	0	0	0	2	0	2
Cyclobarbital	0	0	0	0	1	1	0	2
Benzocaine (Et Aminobenzoate)	0	0	0	0	0	1	0	1
Methohexital (Brevital)	0	0	0	0	0	0	1	1

Figure 10. Sedatives Encountered in Drug Identification Program.

example, secobarbital and diazepam. A sedative which has caused great concern in the past is glutethimide. Overdoses of this drug are difficult to handle because the compound is susceptible to enterohepatic re-circulation. In 1973 and 1974, it was clear from trends such as those in Figure 11, that abuse of glutethimide was threatening to become a serious problem, and so the stricter controls that were placed on sale of the drug in 1974 seem, in retrospect, to have been appropriate. It is not clear that steady decline in abuse of glutethimide noted since then has resulted only from these controls, but the decline is clear and is appreciated by physicians. Interestingly, abuse of methaqualone (quaalude) has experienced a quite similar history in the same time period. The reasons for this are less clear, but methaqualone has received increasing attention from legal authorities in the last four years and, perhaps as a result, has become less popular with young people.

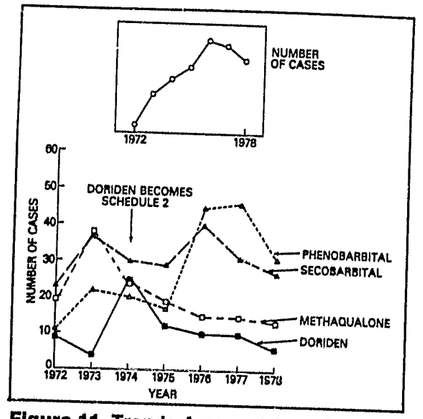

Figure 11. Trends Amongst Sedatives Encountered in Drug Identification Program.

The tranquilizers that have been encountered in overdose cases are identified in Figure 12. The first three—diazepam, chlordiazepoxide, and meprobamate—were, between 1972 and 1976, consistently the most commonly found drugs in this class. Since 1976, however, abuse of chlordiazepoxide seems to have been diminishing and at the same time, there has been a sharp increase in the appearance of amitriptyline (Elavil) and flurazepam (Dalmane). As a result, it is likely that there will be basic re-ordering of the drugs in this category during 1978.

It can be noted from Figure 12 that phencyclidine (PCP), which is widely regarded to be a drug that is abused by the young, is not encountered frequently in life-threatening overdose situations. Moreover, the frequency with which this substance is found in such a setting seems not to be changing much from year to year.

Some of the trends involving tranquilizers can be seen in Figure 13. In this Figure, it is interesting that the curve for Valium is the only one that seems related to the curve for the total number of cases, shown in the inset. The decrease in the occurrence of Librium in overdoses has been noted, as has the increase for Elavil. The recent, sharp drop in the curve for Dalmane defies explanation and clearly more data are needed before any commentary on such trends is justified.

Beyond the major classes of sedatives, tranquilizers, and analgesics and the trivial cases of alcohol, nicotine, and cotinine, other classes of drugs that are involved in overdoses are relaxants and anticonvulsants, of which diphenylhydantoin is most frequently observed, and antihistaminics, a group headed by diphenhydramine. In addition to these a number of miscellaneous compounds have been found in the course of this work. These include nicotine

Drug	1972	1973	1974	1975	1976	1977	1978	7 Year Total
Diazepam (Valium)	12	21	25	39	35	32	22	186
Chlordiazepoxide (Librium)	14	12	30	15	21	19	8	119
Meprobamate (Miltown)	10	9	21	17	29	22	10	118
Amitriptyline (Elavil)	2	4	5	13	21	25	18	88
Flurazepam (Dalmane)	3	7	6	6	17	30	10	79
Phencyclidine (PCP)	2	1	6	7	7	5	4	32
Imipramine (Tofranil)	0	0	1	8	3	9	4	25
Doxepin (Sinequan)	1	1	2	4	5	5	6	24
Tranxene	0	0	0	0	2	10	6	18
Thioridazine (Mellaril)	0	5	1	2	1	2	1	12
Desipramine (Norpramin)	0	2	4	0	2	2	1	11
Trifluoperazine (Selazine)	0	0	0	0	1	2	1	4
Haloperidol (Haldol)	0	0	0	0	0	1	0	1
Mesoridazine (Serentil)	0	0	0	0	0	0	1	1
Prochloroperazine (Compazine)	0	0	0	0	0	0	1	1

Figure 12. Tranquilizers Encountered In Drug Identification Program.

and alcohol, as well as the local anesthetic xylocaine, the preservatives, methyl, ethyl and propyl p-benzoate, the anorexics phenmetrazine and phendimetrazine and the narcotic analgesic, methadone. Apart from nicotine and alcohol however, substances in this general group are found rather rarely, i.e. less frequently than four times per year.

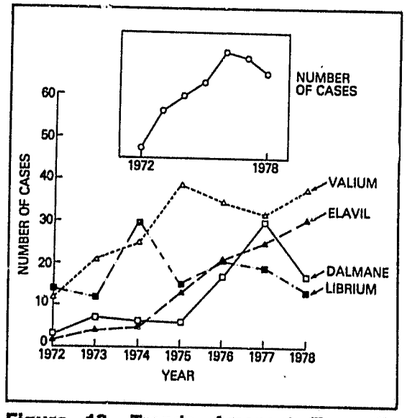

Figure 13. Trends Amongst Tranquilizers Encountered In Drug Identification Program.

IV. SUMMARY

The system described in this paper handles the drug identification work connected with overdoses that occur in the Greater Washington area. The use of mass spectrometry in this sense can be regarded as an example of 'high technology' but such a designation is not supported by the cost of the service, which is $75 per sample.

Rather, in a regional setting, it represents a practical solution to the problems of identifying not only the well-known drugs of abuse, but also less frequently-encountered drugs involved in overdose situations.

REFERENCES

1. Law, N. C., Aandahl, V. A., Fales, H. M., and Milne, G., W. A., *Clin. Chim. Acta, 32,* 221, (1971); Law, N. C., Fales, H. M., and Milne, G. W. A., *Clin. Tox., 5,* 17, (1972).

2. Munson, M. S. B., and Field, F. H., J. *Amer. Chem. Soc., 88,* 2621, (1966).

3. Fales, H. M., Milne, G. W. A., and Axenrod, T., *Anal. Chem., 42,* 1432, (1970). Milne, G. W. A., Fales, H. M., and Axenrod, T., *Anal. Chem., 43,* 1815, (1971). Chao, J.-M., Saferstein, R., and Manura, J., *Anal. Chem., 46,* 296, (1974).

4. Complex mass spectra, such as EI spectra can be used for identification of compounds by library searching techniques. See, for example, Heller, S. R., *Anal. Chem., 44,* 1951, (1972); Heller, S. R., Fales, H. M., and Milne, G. W. A., *Org. Mass Spectrom., 7,* 107, (1973).

The Identification of Drugs and Related Substances by Computer-Aided Mass Spectrometry

by

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The combination of a gas chromatograph which is capable of separating very small quantities of rather complex mixtures with a mass spectrometer, an instrument of high sensitivity producing data which are overabundantly correlated to the structure of organic compounds, has emerged as one of the most powerful tools in organic analytical chemistry. The two methods are highly compatible with respect to sample properties, both being very sensitive and operating with samples in the gas phase, albeit at quite different pressures. The chief limitations, volatility and thermal stability, can often be overcome by suitable chemical derivatization.

It is therefore no wonder that the gas chromatograph-mass spectrometer has found wide application in organic, bio- and clinical chemistry, particularly for the identification and quantification of drugs and their metabolites. This group is well suited because most drugs are organic molecules of a size (generally ranging in molecular weight between mass 150 and 500) which present no problem for even relatively simple mass spectrometers and they are usually sufficiently volatile, especially if their polar functional groups are properly derivatized, to be amenable to gas chromatography and therefore also to mass spectrometry..

In a previous paper, M. G. Horning (1) has outlined the use of this technique for the quantification of various components of body fluids for clinical diagnosis, chiefly in the newborn and infants. In contrast to the quantitation of compounds known to be present in a system, the identification of unknown substances has somewhat different requirements. For that purpose, one has to record complete mass spectra during the entire gas chromatogram in a continuous, fast, and repetitive mode to insure that every compound emerging from the gas chromatograph can be characterized. In earlier days the identification was attempted by scanning the mass spectrometer at the maximum of each gas chromatographic peak or shoulder. The tediousness of this approach, particularly the transformation of the raw data to an interpretable format, as well as the need to record mass spectra at many sections of the gas chromatogram to avoid missing a crucial but perhaps minor component, led to the automation of the recording step through digital computers (2). Continuous recording of mass spectral data during the entire gas chromatogram assured that no information remained unrecorded. This, in turn, caused a complex data handling problem because one was suddenly faced with hundreds of mass spectra generated within the time span of a gas chromatogram, a period of perhaps thirty minutes. A number of computer approaches were developed to efficiently digest the information content of these data and G. W. A. Milne has summarized these (3). Once the techniques for the automatic recording of the data and computer-based identification of the resulting mass spectra had been developed, it became the logical next step to fully automate this process, namely to generate a gas chromatogram in which all fractions are automatically identified and the information available at the end of the experiment.

We were prompted to develop this system in the course of a program which involved the collaboration of our laboratory with various hospitals in the Greater Boston area to determine drugs and related substances in the body fluids of comatose patients who had ingested, accidentally or on purpose, a large amount of one or more drugs or other toxic substances. In these cases the results had to be available in as short a time as possible and the frequency of

these cases (sometimes a few a day) made automation mandatory. To improve our own efficiency, and for use in laboratories where very experienced mass spectrometrists were not available, it was desirable to automate the procedures so as to require nominal decision making by the analyst. Figure 1 shows a simplified flow diagram of the steps involved.

The operation and function of the gas chromatograph-mass spectrometer have been outlined previously by M. G. Horning (1) and the comparison technique of unknown mass spectra with the collection of known spectra ("Library Search") has also been discussed by G. W. A. Milne (3). In our laboratory we use a simple matching routine which compares the relative intensities of the two most intense peaks in consecutive 14 amu sections of the mass spectrum of the unknown with the same set of data from each one of the known substances in the library. A weighted average of the intensity ratios of the matching pairs (or its inverse if the value is larger than 1) is computed and serves as an indication of the degree of identity of the mass spectrum of the unknown and each particular known spectrum (this is the "Similarity Index") (4). If this comparison had to be done for all of the presently available 20,000 to 30,000 known mass spectra with each of the few hundred mass spectra recorded during the gas chromatogram the process would be too slow to allow comparison for each one of these spectra. Two factors help to reduce this time to a tolerably short period in the case of the identification of drugs in body fluids. First, one needs to identify only compounds that are likely to be present, particularly drugs and their metabolites and other toxic substances. The number of these is well below one thousand. Secondly, one can make use of the one piece of information which the gas chromatograph provides, namely the retention time of the substance in question. Since this parameter is characteristic and reproducible for each compound under identical conditions or, if converted to the retention index (5), even under non-identical conditions, one can eliminate from the com-

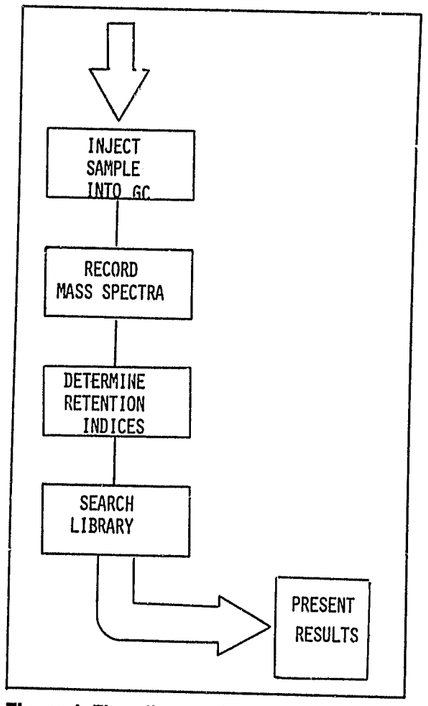

Figure 1. Flow diagram for automatic identification of components of complex mixtures.

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parison those which differ in retention index by a preselected value and therefore cannot possibly be identical. To make use of this concept in real-time applications, however, it is necessary to automatically and concurrently determine the retention index of the unknown material emerging from the gas chromatograph in order to have this information available for use in the spectral comparison process. Such a technique was developed (6) based on the automatic, mass spectrometric identification of three standard hydrocarbons co-injected with the sample. From the position of these hydrocarbons in the gas chromatogram a retention index scale is established and a retention index is associated with each consecutively recorded mass spectrum.

Using two restrictions, a limited library containing only the known spectra of compounds of possible interest and the correlation of retention indices, the time required for the comparison of each consecutive mass spectrum is less than it takes to print the name of the most similar compound on a line printer. Thus one achieves an identification of the materials emerging from the gas chromatograph at intervals corresponding to the scan time of the mass spectrometer, in our case 4.0 seconds (7). As an illustration Figure 2 shows the gas chromatogram of a methylene chloride extract of a blood sample from a patient brought to Massachusetts General Hospital after first being ad-

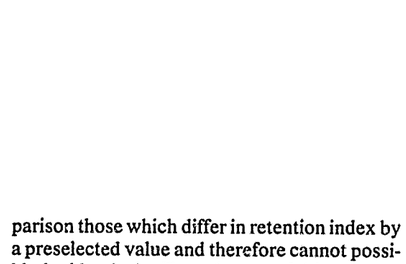

Figure 2. Total ionization plot (gas chromatogram) of a methylene chloride extract of a blood sample.

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75			NO FINDS
76			NO FINDS
77			NO FINDS
78			NO FINDS
79	0.486	147	MEPERIDINE
80	0.694	147	
81	0.687	147	MEPERIDINE
82	0.662	147	MEPERIDINE
83	0.543	147	MEPERIDINE
84	0.614	147	MEPERIDINE
85	0.522	147	MEPERIDINE
86	0.482	147	MEPERIDINE
87	0.462	147	MEPERIDINE
88	0.420	147	MEPERIDINE
89	0.444		MEPERIDINE
90	0.376		MEPERIDINE
91	0.*		MEPERIDINE
92	0.		MEPERIDINE
93	0.		MEPERIDINE
94	0.*	7	MEPERIDINE
95	0.4	47	MEPERIDINE
96			NO FINDS
97			NO FINDS
98	0.362	129	N-ALKANE
99	0.270	129	N-ALKANE
100	0.178	129	N-ALKANE
101	0.059	147	MEPERIDINE
102	0.107	147	MEPERIDINE
103	0.122	147	MEPERIDINE
104	0.144	147	MEPERIDINE
105			NO FINDS
106			NO FINDS
107			NO FINDS
108			NO FINDS
109			NO FINDS
110			NO FINDS
111	0.027	130	STEARIC ACID
112	0.031	92	EMYLCAMATE
113	0.042	92	EMYLCAMATE
114			NO FINDS
115			NO FINDS
116			NO FINDS
117			NO FINDS
118			NO FINDS
119			NO FINDS
120			NO FINDS
121			NO FINDS
122			NO FINDS
123			NO FINDS
124			NO FINDS
125	0.270	225	METHAQUALONE
126	0.554	225	METHAQUALONE
127	0.305	225	METHAQUALONE
128	0.187	225	METHAQUALONE
129			NO FINDS
130			NO FINDS

Figure 3. Section of printout of the results of the automatic identification of each consecutive mass spectrum recorded during the gas chromatogram shown in Figure 2. For details see text.

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mitted to a suburban hospital. Figure 3 shows a portion of the above mentioned printout of the results of the automated identification program.

In order to render this listing visually more comparable to the gas chromatogram, the printing of the name of the identified substance is displaced to the right by a number of blank spaces related to the intensity of the spectrum. Thus a profile resembling that of the original gas chromatogram is obtained, along which the name of the emerging compound is printed. Below a certain level of intensity and similarity with any of the substances in the library, the comment "no finds" is printed. The values below each line represents the consecutive scan index number, the similarity index, and the code number of the compound in the library. In this section of 55 consecutive spectra, meperidine elutes as the major component followed by one of the hydrocarbon standards, a trace of stearic acid, and finally methaqualone. The next peak in the gas chromatogram (not shown in this figure) was identified in the same manner as diazepam. It is worth noting that this patient had apparently a blood level of meperidine of twice the lethal dose and his surprising survival was due to the efforts and expertise of the attending personnel at MGH. This example also prints out one of the major problems in the analysis of body fluids, namely that the ingestion of multiple drugs is becoming more and more common. Their unambiguous detection and identification require methods that are very specific and free from cross interference.

The recording of mass spectra at repetitive fixed time intervals during the gas chromatogram not only assures that one or more mass spectra are taken from each compound present in the mixture but it also adds a third dimension to the data accumulated, namely time. In the examples discussed above, only the mass spectrum itself and the computer analog of the gas chromatogram (the total ionization plot, which is generated by summing all intensities in each one of the consecutive mass spectra and plotting that summed intensity versus time) were utilized. Alternatively, one can plot the relative abundance of a particular mass in the course of the gas chromatogram, thus generating a plot that is identical to the one which would have been obtained if the mass spectrometer had remained focused at that particular mass rather than having scanned the entire spectrum. This plot can be generated after the experiment from the complete set of the mass spectra by selecting any particular mass and looking at its intensity versus time. Since all data have been recorded, one can generate a plot of this type for any and therefore all masses.

Figure 4 shows a graphic representation of this principle: The x, y plot at the origin of the z axis represents time (x) versus summed intensity (y), i.e. the gas chromatogram. The same x, y coordinate along the z axis (mass scale) represents the relative intensity of that particular mass during the course of the gas chromatogram (a so-called mass chromatogram) (8). Finally the z, y axis represents mass (z) versus intensity (y), i.e. the mass spectrum at any given point along the x axis.

Such mass chromatograms have turned out to be extremely useful for a variety of purposes. For example, they can be used to increase the apparent resolution of a gas chromatogram and at the same time to remove the contributions of adjacent or unresolved gas chromatographic fractions to the spectrum of the component of interest (9). The principle of this approach is shown in Figure 5 which shows a section of a poorly resolved gas chromatogram (heavy solid line) and superimposed, the mass chromatograms of m/e 179, 310, and 256, all of which maximized in that general area. It is immediately obvious that the component

that gives rise to a sharp peak in the mass chromatogram of mass 310 is not resolved from the front of the very broad peak due to a substance exhibiting an ion of m/e 256. Furthermore, the component that shows a peak at mass 179 trails into the one responsible for the maximum of m/e 310. It is clear that all other mass chromatograms of masses associated with the mass spectrum of one of those three compounds must have exactly the same shape and one can therefore generate the "pure" mass spectrum by plotting only those masses which maximize at the same scan index number versus relative intensity.

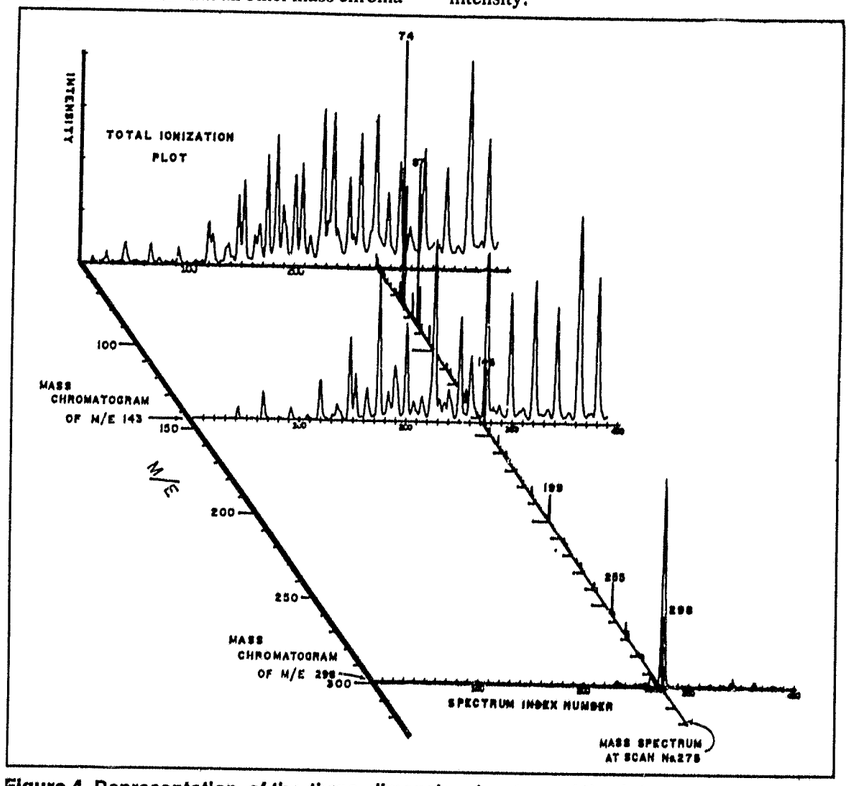

Figure 4. Representation of the three dimensional nature of the information content of GC-MS-data.

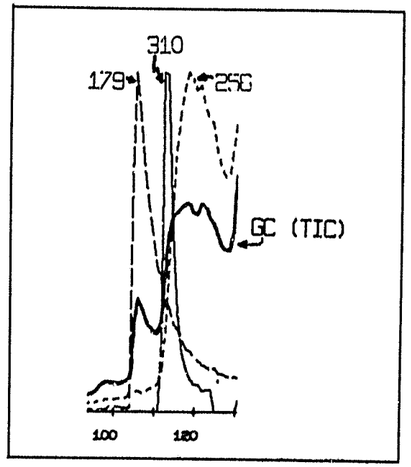

Figure 5. Overplot of three mass chromatograms characteristic of three partially resolved components to illustrate the principle of "reconstruction" of mass spectra (see text).

This procedure automatically eliminates the contribution of leading or tailing edges of unresolved peaks to the central component. For example, mass 179 will not show up in the "reconstructed" mass spectrum of the component giving rise to the peak at mass 310 and mass 256 will also be eliminated. This approach represents a very sophisticated method of "background" subtraction, far more efficient than that which one would have obtained by subtracting the background spectrum obtained at a place in the gas chromatogram where the signal approaches the base line. Equally important is the fact that plotting the sum of the maximizing intensities rather than all intensities results in a plot in which the peaks appear to be extremely sharp, their widths being one or two scan intervals.

Figure 6 compares the conventional total ionization plot (gas chromatogram) with this "mass resolved gas chromatogram". It should also be noted that along the x axis is plotted not only the spectrum index number representing a time scale (time = spectrum index number times 4.0 seconds) but also the retention index associated with each scan, derived from the three internal hydrocarbon standards as mentioned previously. This treatment of the data not only leads to an apparent improvement in the gas chromatographic resolution but, as described earlier, also generates "cleaner" mass spectra. Needless to say, the reliability of the comparison of such spectra with the library of authentic mass spectra leads to a better correlation because the contributions of incompletely resolved components have been eliminated. As an example, the results of the library search of scan 181 of Figure 6 is shown (Figure 7) both for the original chromatogram where there is a barely discernible shoulder and the mass resolved gas chromatogram where this shoulder has been converted to a discernible small peak. Comparison of the original mass spectrum with the library of drugs and related substances lists a number of methaqualone metabolites as well as two diphenylhydantoin derivatives. The similarity index, shown in the right hand column, to those authentic spectra is extremely low, indicating a very questionable assignment. However, comparison of the reconstructed mass spectrum at this point in the gas chromatogram shows a significantly high similarity to a metabolite of diazepam, the correct identification (Figure 7).

One of the most unique aspects of our data processing system is the method of display and permanent storage of all data. Rather than the experimenter using display terminals on the

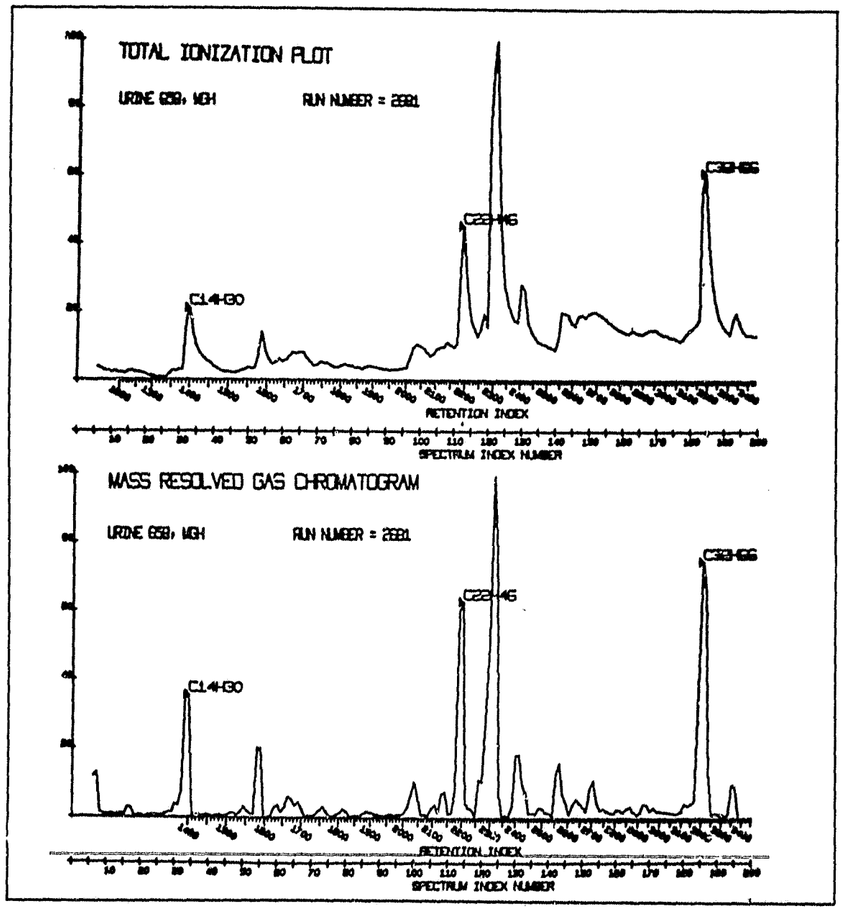

Figure 6. Top: Original total ionization plot (gas chromatogram); Bottom: "mass resolved gas chromatogram" generated from reconstructed mass spectra, illustrating the apparent improvement in resolution.

computer to inspect the data in a dialogue mode by requesting the computer to display the pertinent set of data such as a mass spectrum, a mass chromatogram, etc., all data are immediately and consecutively displayed on a cathode ray tube and the image permanently recorded on 16 mm microfilm (10). The entire operation is under computer control and is carried out while the raw data recorded during the experiment are converted to their final form, namely mass spectra and mass chromatograms. In this manner, the experimenter has all the primary data available on microfilm within about 15 minutes of the completion of the experiment because the film is developed in an automatic film developer (Prostar, Eastman Kodak and Company). The microfilm is inspected using a microfilm reader and copies of any image can be obtained from a hard copy unit. The mass resolved gas chromatogram and the reconstructed mass spectra are part of the microfilmed data set if so desired. In this way the experimenter can interpret and inspect all the data at his or her leisure independent of the computer which therefore is available to others for the accumulation of the next data set.

This microfilming technique has made it possible to treat a much larger volume of data in a comprehensive manner than otherwise possible. One of these involves the simultaneous inspection and interpretation of more than one set of GC-MS data. Generally, it is extremely cumbersome to compare the data of one such experiment with that of another because they

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URINE 658, MGH                                  181   RUN NO. = 2681

RESULTS                                         ID.       SIM.

METHAQUALONE MTB 2                              216       0.028
5,5-DIPHENYLHYDANTOIN 1,3-DIETHYL DERIV.        426       0.026
METHAQUALONE MTB 4                              307       0.020
DIPHENYLHYDANTOIN 1,3-DIMETHYL DERIV.           372       0.018
METHAQUALONE MTB 3                              306       0.013
METHAQUALONE MTB 5                              308       0.010
METHAQUALONE MTB 5 (GC)                         309       0.003

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URINE 658, MGH (RECONSTRUCTED)                  181   RUN NO. = 2681

RESULTS                                         ID.       SIM.

*DIAZEPAM MTB 1                                 448       0.454
 MEDAZEPAM                                      242       0.132
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Figure 7. Library search results of scan 181 in Figure 6 before (top) and after (bottom) "reconstruction" of the mass spectrum free of interference from adjacent components and background.

have to be inspected consecutively. The microfilming system permits one, however, to interleave or merge a number of experiments in such a fashion that the mass chromotograms of the same mass number of the various experiments are displayed together on one microfilm frame for easier comparative inspection. It is useful to examine a number of GC-MS data sets for the presence of certain compounds, expected or unexpected, which could serve to differentiate one family of samples (for example patients, drugs of various origins, etc.) from another. The identity of the pattern of the mass chromatograms of certain m/e values within each group but differing from the other group which in turn is characterized by a family of similar mass chromatograms serves this purpose.

As a very simplified example, the survey of urine specimens containing phencyclidine and codeine and their differentiation from those free of the drug will be discussed. Figure 8 shows the mass spectrum of phencyclidine which is characterized by an abundant ion of m/e 200, as well as others at m/e 86, 91, 243, etc. The mass chromatograms of m/e 200 of

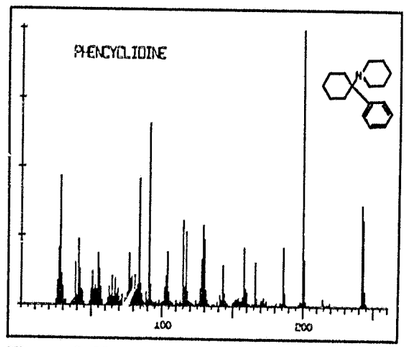

Figure 8. Mass spectrum of phencyclidine.

five emerged GC-MS runs are shown in Figure 9 which dramatically reveals that four of the five samples do contain the drug as indicated by the sharp maximum at the same spot in the gas chromatogram (normalized scan number 103) at the retention time appropriate for PCP while the fifth one does not show this maximum. Since all are plotted on the same scale, one can quickly make an estimate of the relative amounts of the drug present. Not shown are the mass chromatograms at other characteristic mass numbers which show the same pattern, thus supporting the conclusion.

Another example is the search for codeine in the same five samples. This substance shows an abundant peak for the molecular ion m/e 299 (Figure 11). Its mass chromatogram for the same five merged sets is shown in Figure 10. It is clear that two of the specimens do contain codeine while the other three are free of it. In these experiments all mass chromatograms (for mass 30 to mass 450) were merged and microfilmed for visual inspection. One could, however, just as well merge only those mass chromatograms of masses which are expected to be significant.

This approach is very useful for the intercomparison of large numbers of GC-MS data sets by convenient visual inspection. Presentation of the data on microfilm takes the load off the computer because the merging of all the data requires a large amount of primary and secondary data storage capability. The number of data sets that can be merged is almost unlimited because the microfilm reader displays consecutive frames continuously and at any desired speed. Even though only five or six mass chromatograms are visible at one time on the screen, inspection of ten to twenty consecutive mass chromatograms is still quite convenient.

The data processing and evaluation tech-

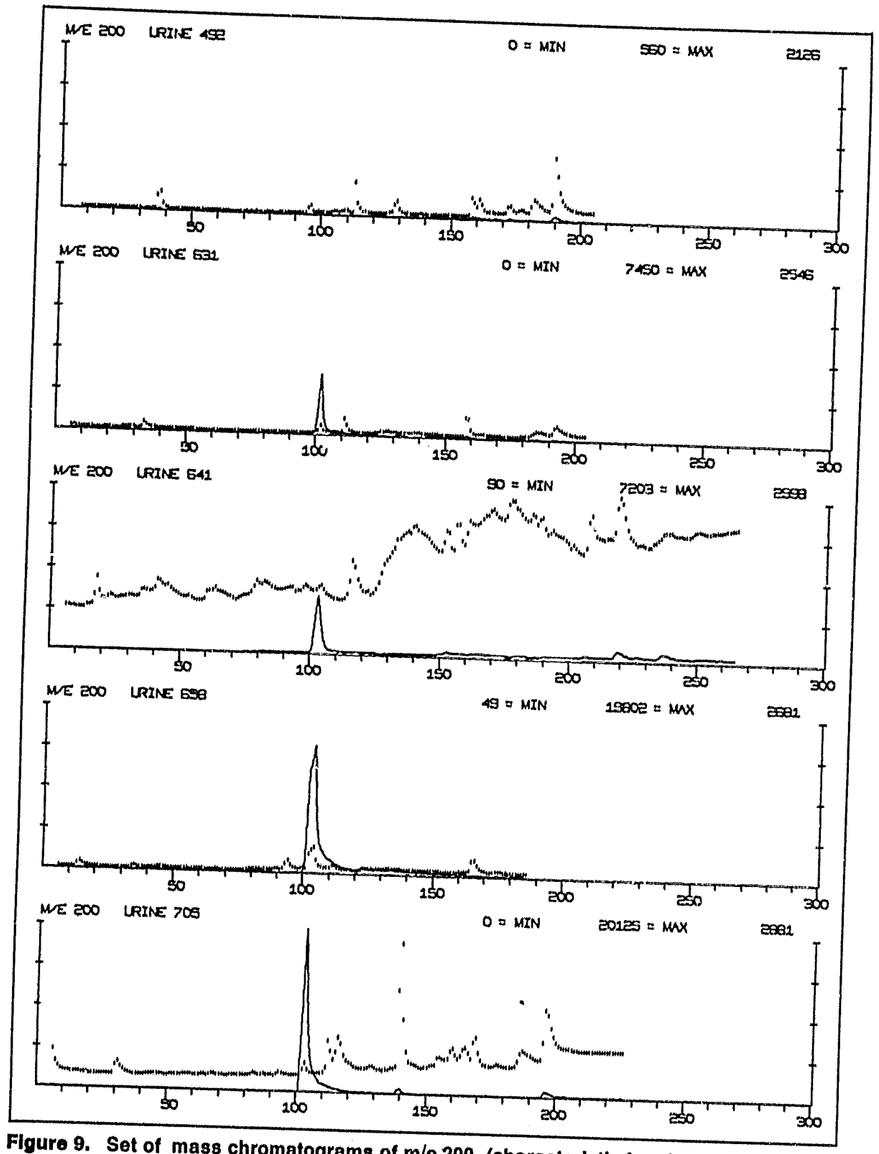

Figure 9. Set of mass chromatograms of m/e 200 (characteristic for phencyclidine) obtained by merging the data from five GC-MS analyses of urine samples showing that four contain phencyclidine but one does not.

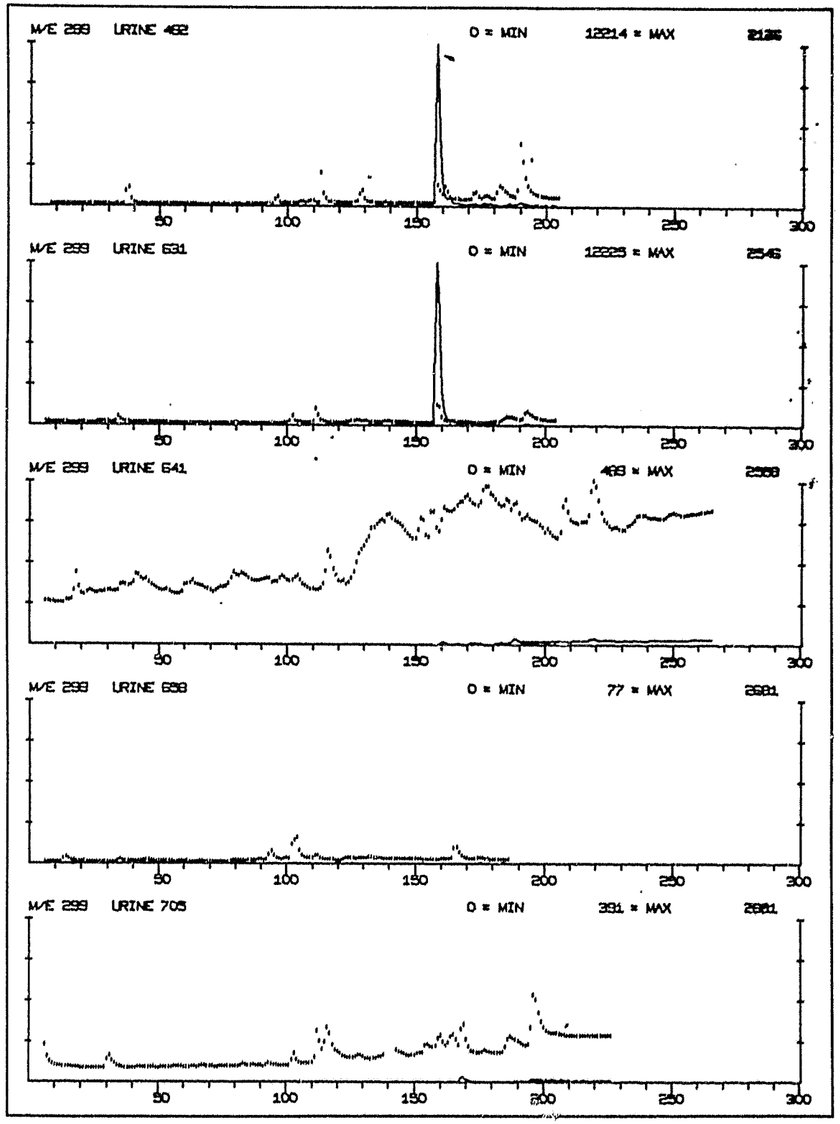

Figure 10. Set of mass chromatograms of m/e 299 (characteristic of codeine) obtained by merging the data from the same set of five samples shown in Figure 9, indicating that the one free of phencyclidine contains codeine and one of them contains both.

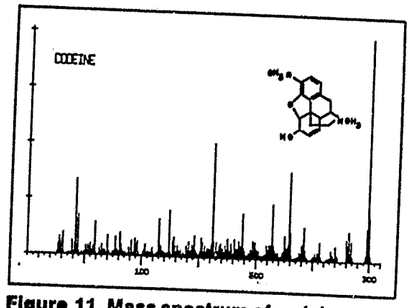

Figure 11. Mass spectrum of codeine.

niques which we have developed in our laboratory and are describing in this paper have proven very useful for the handling of the very large amount of data which are generated by a gas chromatograph-mass spectrometer system coupled to a computer and used for specific identifications or general surveys of complex mixtures of biological origin.

The availability of all original data on 16 mm microfilm, one of the most widely used information storage media, lends itself well to distribution and interchange of data. For example, the entire set of results can be mailed to the laboratory where the sample originated and used and interpreted there on a microfilm reader rather than requiring a computer compatible to the one which generated the data.

The permanency, completeness, and authenticity of the microfilmed data should make it a particularly useful record for forensic problems where the result may become part of legal proceedings.

REFERENCES

1. M. G. Horning, Proceedings of the International Symposium on Instrumental Applications in Forensic Drug Chemistry, Arlington, Virginia, May 29–30, 1978.
2. R. A. Hites and K. Biemann, *Anal. Chem.*, 40, 1217 (1968).
3. G. W. A. Milne, Proceedings of the International Symposium on Instrumental Applications in Forensic Drug Chemistry, Arlington, Virginia, May 29–30, 1978.
4. H. S. Hertz, R. A. Hites and K. Biemann, *Anal. Chem.*, 43, 681 (1971).
5. E. Kovats, *Helv. Chim. Acta*, 41, 1915 (1958).
6. H. Nau and K. Biemann, *Anal. Letters* 6, 1071 (1973).
7. C. E. Costello, H. S. Hertz, T. Sakai and K. Biemann, *Clin. Chem.*, 20, 255 (1974).
8. R. A. Hites and K. Biemann, *Anal. Chem.*, 42, 855 (1970).
9. J. E. Biller and K. Biemann, *Anal. Letters*, 7, 515 (1974).
10. J. E. Biller, H. S. Hertz and K. Biemann, Paper F2, Presented at the Nineteenth Annual Conference of Mass Spectrometry and Allied Topics, Atlanta, Georgia, May, 1971.

Development of a National Criminalistics Laboratory Information System

by

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I. INTRODUCTION AND BACKGROUND

A Criminalistics Laboratory Information System (CLIS) is being established as a service to all law enforcement agencies—local, state, and Federal. CLIS will be a computerized laboratory information system for the collection and dissemination of forensic science material for law enforcement laboratories. Through it, forensic science data will be identified, collected, and stored for on-line retrieval by forensic scientists across the country.

Late in 1969 and during 1970, the Law Enforcement Assistance Administration sponsored Project Search (System for Electronic Analysis and Retrieval of Criminal Histories). The purpose of this project was to demonstrate the feasibility of an interstate exchange of criminal history data by means of a computerized system.

In July, 1973, Project Search (now Search Group, Inc.) as part of its ongoing program of facilitating the application of advanced technology to the administration of criminal justice, formed a committee consisting of 16 authorities in the forensic sciences to address itself to three topics: (1) information needs of criminalistics laboratories throughout the United States; (2) conceptual design of an automated information storage and retrieval system; and (3) creation of a plan for implementating the system. This study was completed in December, 1974.

Two primary questions emerged from the study: (1) What agency will host CLIS? (2) What telecommunications network will provide communications services for CLIS? The study concluded that responsiveness of CLIS to its users and high priority development at the national level are more likely if the national data bases and the processing are maintained by a functional crime laboratory with wide forensic experience and operations.

On September 8, 1976, the Attorney General of the United States granted permission to the FBI to implement a prototype and full CLIS system. The FBI Laboratory will host the data base and FBI National Crime Information Center (NCIC) telecommunications lines will be used to access it.

II. NCIC TELECOMMUNICATIONS

The idea of the National Crime Information Center was conceived in the early 1960's, when it appeared that advances in computer technology could answer law enforcement's growing need for rapid dissemination of vital information. The FBI, in conjunction with the Advisory Group to the Committee on Uniform Crime Records and International Association of Chiefs of Police (which group is made up of law enforcement representatives from local, state, and Federal agencies) recognized the advantages a computerized index of information could offer criminal justice agencies. The officer on the street would have a wealth of information concerning crime and criminals at his command at all times.

In January, 1967, NCIC became operational. This Center serves as a nucleus of a vast communications network which includes local, state, and Federal criminal justice agencies throughout the United States, Canada, and Puerto Rico. Designed to complement the development of metropolitan and state-wide information systems, the NCIC in a matter of seconds makes available information essential to the effective performance of law enforcement personnel.

The NCIC computer equipment is located at FBI Headquarters in Washington, D.C. The equipment includes rapid access storage units with a capability of accommodating records representing an index of fugitives, missing persons, and stolen property. In a matter of seconds, stored information can be retrieved through the telecommunications network. Connecting terminals are located throughout the country in police departments, sheriff's offices, state police facilities, Federal law enforcement agencies, and other criminal justice agencies. The NCIC computers are linked to many state-wide and metropolitan area computer systems, thus providing a large number of criminal justice agencies with access to NCIC files.

CLIS will function as one file of NCIC. CLIS messages will be received via telecommunications lines by the NCIC computer. Immediately upon receiving the message the NCIC computer will acknowledge receipt of the message. The CLIS message then will be written on an input queue for later processing.

III. MANAGEMENT OF CLIS

In developing management guidelines for CLIS, provision for maximum input by the American Society of Crime Laboratory Directors (ASCLD) on all matters has been made since the Society is representative of the crime laboratory community in the United States. The ASCLD Board of Governors had adopted the following resolution:

> *The Governing Board of the American Society of Crime Laboratory Directors (ASCLD) resovles that in the implementation of a Criminalistics Laboratory Information System (CLIS) that a CLIS Operating Committee be established to (1) review and consider rules, regulations and procedures for operation of CLIS; (2) set standards for participation by crime laboratories; (3) select laboratories for participation in the prototype and full CLIS system; and (4) make determinations as to the files that will be implemented in CLIS. The Board further resolves that the CLIS Operating Committee should be composed of seven members, four to be nominated by the ASCLD Governing Board from among ASCLD member laboratories for approval by the Director, FBI; and three to be appointed by the Director, FBI; one each from the FBI, Drug Enforcement Administration and Bureau of Alcohol, Tobacco, and Firearms Laboratories."*

Although the CLIS Operating Committee has overall responsibility for developing policy for CLIS, the FBI Laboratory has direct responsibility for the implementation of the system which includes file development and quality control. In addition, a representative of the FBI Laboratory is required to furnish a semiannual status report on CLIS to the NCIC Advisory Policy Board.

IV. SELECTION OF DATA FILES FOR PROTOTYPE SYSTEM

In October, 1976, the CLIS Operating Committee held its first meeting. The primary issue resolved was the selection of data files for the prototype system. First priority was a recommendation to establish a General Rifling Characteristic File consistent with the ability to determine the possible make and model of a firearm from the general rifling characteristics present on a fired bullet and the possible make of a firearm on the basis of markings present on fired cartridge cases. The recommendation was based on the perception of the widespread need for this file with respect to crimes against persons, the unavailability of the file from commercial sources, and the large number of law enforcement agencies requiring this service for firearms-related matters.

Second priority was a recommendation to establish effective standard procedures for identification of unknown compounds by the

use of infrared spectrophotometry. The data file should be capable of being referenced through a manually coded data format of the infrared spectrum. This recommendation was based on usage by the largest proportion of laboratories since the forensic community utilizes this universal technique for a wide variety of physical evidence examinations. Infrared spectrophotometry is a versatile method of analysis and is generally used in laboratories ranging from the highest to the lowest level of sophistication.

Third priority was a recommendation that in the event of successful implementation of the first and second priorities, consideration be given to establishing a Mass Spectral Data File.

The priorities were developed based upon information available from the initial study by the CLIS Committee of Search Group, Inc. Additionally, input was provided in the areas of firearms, instrumental analysis, computer science, and data management by advisors to the CLIS Operating Committee.

V. *SELECTION OF PROTOTYPE LABORATORIES*

A profile of potential CLIS user laboratories was developed by the CLIS Committee of Search Group, Inc. in its 1974 study. This profile was developed based upon responses to detailed questionnaires received from 168 laboratories supplemented by on-site staff interviews with a representative sampling of these laboratories. This material was made available to the CLIS Operating Committee to assist in the selection process of the prototype laboratories. In order to be considered for selection as a prototype laboratory, a laboratory must have responded to the questionnaire in the 1974 study. Additionally, other selection criteria included geography, laboratory size, caseload, availability of high speed circuits to NCIC and utilization of infrared spectrophotometry in compound identification.

Laboratories which appeared to meet the necessary criteria were sent a questionnaire to obtain additional information and to verify that they did, in fact, meet the required criteria.

On July 11, 1977, the CLIS Operating Committee selected 43 laboratories to participate in the prototype development of CLIS.

VI. *CURRENT DEVELOPMENTAL STATUS OF CLIS*

The present objectives are to implement a prototype CLIS consisting of the participating laboratories, to train personnel who will be involved in the CLIS and to provide user operating and training manuals. The prototype system is expected to become completely operational in 1978 with a General Rifling Characteristics File and an Infrared Spectral Data File. Currently our scientific personnel have accumulated and encoded data from over 15,000 bullets and cartridge cases which is the basis of the General Rifling Characteristics File.

The General Rifling Characteristics File has been brought on-line in the FBI Laboratory. This prototype laboratory is serving to debug the system. It was originally planned to bring each prototype laboratory on-line on an individual basis over a period of time. However, it is now felt that the training of a firearms examiner who will use the system from each of the prototype laboratories can most effectively be provided if all are trained as a group.

Accordingly, a CLIS Users Meeting is scheduled for May 30–31, 1978.

Following the development of the prototype CLIS, implementation of the complete system will begin consisting of an expanded data base and additional participating laboratories.

VII. *SUMMARY*

The FBI has been designated by the Attorney General of the United States to develop and implement a nationwide Criminalistics Laboratory Information System (CLIS). This will be a computerized laboratory information system for the collection and dissemination of forensic science material for law enforcement throughout the United States. Through it, forensic science data will be identified, collected and stored for on-line retrieval by forensic scientists across the country.

The forensic science data will be stored centrally at FBI Headquarters and transmitted over the National Crime Information Center (NCIC) telecommunications lines. The FBI Laboratory will maintain the files and perform related quality control tasks.

The model for a CLIS was developed by Search Group, Incorporated. This portion of the project which included the conceptual design was completed in December, 1974. The prototype CLIS and complete system will be developed by the FBI.

Forty-three laboratories will participate in the prototype CLIS. The objectives of this phase are to implement a Rifling Class Characteristics File and an Infrared Spectral Data File and train personnel who will be involved in CLIS.

Following the development of the prototype CLIS, implementation of the complete system will begin consisting of an expanded data base and increased number of participating laboratories.

90456

Development and Use of Computer Systems in the Virginia Bureau of Forensic Science

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I. HISTORY

In 1972, the Virginia General Assembly enacted legislation to establish the Division of Consolidated Laboratory Services. This Division consists of four Bureaus—Forensic Science, Environmental Science, Microbiological Science, and Product Testing. The Bureau of Forensic Science was designed to provide comprehensive laboratory services from more than 350 law enforcement agencies. Presently, the Bureau of Forensic Science consists of a central laboratory in Richmond and regional laboratories in Northern Virginia, Roanoke, and Norfolk.

On April 13, 1973, Computer Sciences Corporation was awarded a contract by Commonwealth of Virginia, Division of Automated Data Processing, Richmond, Virginia, to perform an engineering study to develop a configuration, cost, and implementation schedule for a Laboratory Data Acquisition System for the Consolidated Laboratory in Richmond, Virginia.

A. The Objectives of the Study.

1. Gather Detailed Information on Laboratory Instrumentation and Operation.

   This includes type and number of instruments, instrument characteristics, number of analyses, problems associated with each analysis, location of instruments, etc. In this regard, discussions were held with cognizant laboratory personnel.

2. Define Data Acquisition, Computation, Control, Reporting, and Display Requirements.

   This task determined the work load for the laboratory data acquisition system. A document was prepared that identified each instrument to be scanned, scan frequency, foreground and background processing requirements, type and number of reports to be generated, working and bulk memory requirements, and operator interface. Schematics were prepared, as required, to show the various hardware elements in the system including instrument detector and internal controls, electrical signal conversion and conditioning elements, and computer input/output (I/O) conversion equipment.

3. Define Functional Capability of Computer Hardware and Software.

   The functional capability of computer hardware and software needed for the laboratory application was described. The various software modules were identified, including systems, applications, and support routines. The computer hardware requirements were identified, covering Central Processing Unit (CPU) speed, working memory type and size, bulk memory type and size, analog/digital input/output subsystem characteristics, telecommunications, operator input/output devices, and programmer station.

4. Develop Alternative System Configurations.

   Certain design differences were incorporated into each system including:

   a. Technique used to gather and reduce analytical data (*e.g.*, peak picking vs. peak integration).
   b. Dedicated minicomputers to handle the same type of lab instrument vs. general purpose (large) computer to handle all the instruments.
   c. Data acquisition and reduction performed on minicomputer-based system and subsequent report generation, statistical analysis, etc., performed on a larger central computer system.

   The reason for investigating alternative system configurations was to insure that the system eventually selected will have the best cost-to-performance ratio consistent with the input/output processing requirements.

5. Conduct Survey of Vendor Hardware/Software Packages.

   A survey of existing computer hardware/software packages that included an instrument data collection program was conducted. Major computer vendors with proven hardware/software were included in the survey.

6. Determine Cost and Performance for each Alternative System.

   A cost estimate was developed for each alternative system. The cost was broken down into several categories, including:

   a. Computer hardware and software.
   b. System design and engineering.
   c. Field installation, start-up and training.

   The anticipated performance of each system was evaluated. Performance factors included the following:

   a. Reliability.
   b. Developmental risks.
   c. Ease of operator use.
   d. Ease of maintenance.
   e. Future expandability.

   Using the cost estimates and performance ratings developed above, the optimum system configuration was selected.

II. CURRENT CONFIGURATION AND CAPABILITIES

The system configuration for the Laboratory Data Acquisition System for the Bureau of Forensic Science in Richmond (Fig. 1) is comprised of two Hewlett-Packard 2100A minicomputers. System No. 1, with a 32K core memory and Real Time Executive (RTE)-II operating system is interfaced to low-data-rate instruments such as gas chromatographs. It acquires data from each instrument in real-time, stores and processes the data, generates analysis reports, provides "background" capability to enable compilation and debugging of new programs and nontime critical analysis of reduced data on-line, and permits communication with System No. 2 computer and telecommunications with a computer located at the State Data Processing Center.

System No. 2, with 16K core memory and Disk Operating System (DOS)-III operating system, is interfaced to the high-data-rate instruments, such as a mass spectrometer. It acquires data from these instruments in real-time, generates analysis reports, plots analysis re-

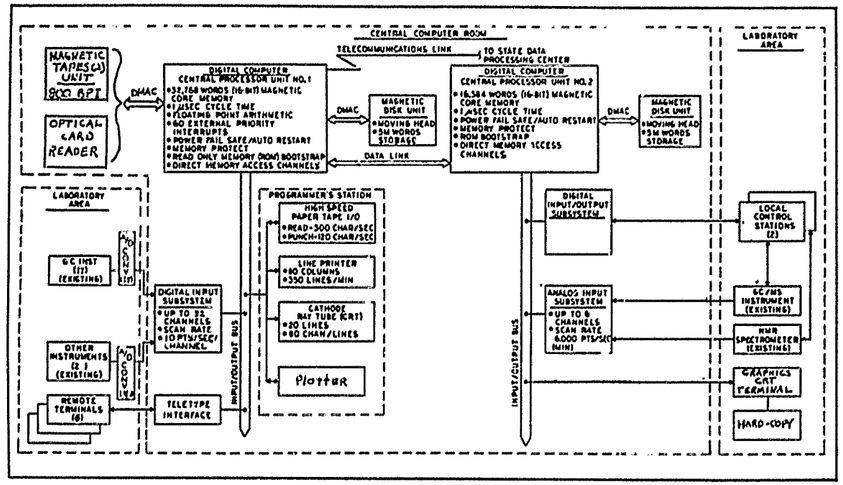

Figure 1. Computer configuration for laboratory automation system.

sults on a cathode ray tube (CRT), and provides storage for a library of spectral data and the capability to search the library for qualitative identification of unknowns. Both systems, with disk storage capacity of five million bytes, have been operational for approximately three years.

A. Functions Performed by Processor No. 1.

1. Real-time Data Acquisition for Low-Data-Rate Instruments with Scan Rates Up to 10 Points per Second per Instrument.

2. Analysis Reports Generated Locally for Gas Chromatographs.

3. Background Capability to Enable Compilation, Debug, or Non-Time Critical Analysis of Reduced Data.

4. Provides Storage for a Library of Data for Use in Searches.

5. Communicates with the "High-Data-Rate" Computer System (CPU #2).

6. Telecommunicates Reduced Data and Results to IBM-370 Located at State Data Processing Center.

7. Programmer's Station Includes a Line Printer and a CRT to Permit Chemist/Scientist to Print Out/Display Raw Data or Reduced Data and to Efficiently Develop Analytical and Statistical Routines.

B. Functions Performed by Processor No. 2.

1. Real-time Data Acquisition for High-Data-Rate Instruments with Scan Rates up to 3,000 Points/Sec. per Instrument.

2. Analysis Reports Generated and Analysis Results Plotted for the Gas Chromatograph/Mass Spectrometer (GC/MS).

3. Provides Storage for a Library of Spectral Data for Use in Searches.

4. Provides Search Capability.

5. Communicates Data to the Low-Data-Rate Computer System (CPU #1).

6. CPU #2 Includes a Graphics CRT with a Hard-Copy Unit to Assist the Scientist to Quickly Analyze Raw Data and Reduced Data.

C. Gas Chromatograph Data Reduction.

Time constraints preclude a detailed discussion of all of the capabilities of this computer system. However, one of the most widely used functions of the computer

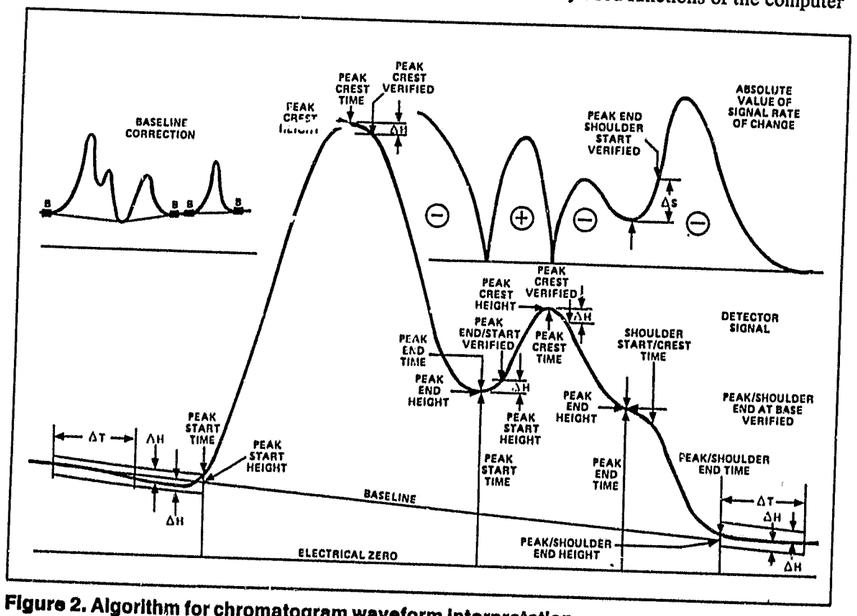

Figure 2. Algorithm for chromatogram waveform interpretation.

system is its data reduction capability used with gas chromatographs.

1. Chromatograph Signal Monitoring.
   During the period that the injected sample's constituents are eluting through the column and passing over the detector, the computer monitors the detector output signal. It processes the signal "on the fly", recognizing baseline, peaks, and "shoulders" and stores data relating to the sample for further processing at the end of the run.

   Chromatograph signals are amplified and digitized at the instrument and transmitted serially to the computer's central processor. Scan rate is up to 10 samples/second. Both hardware and software filtering are provided to control noisy signals.

   The algorithm for detection of baseline, peak, and shoulders operates on the absolute value of signal rate of change (Fig. 2).

   For each detected peak or shoulder, the following data are stored for processing by the analysis report routine:

   a. Peak Area.
   b. Time of peak start, crest, and end.
   c. Height of peak start, crest, and end.
   d. Flags indicating the character of the peak shape (e.g., peak starts on baseline and ends on shoulder.)

2. Analytical Procedure

   On-line analyses are performed according to analytical method specifications retained in the central processor's core memory. The analytical method is the instruction that the chemist gives to the computer to tailor it to a particular analysis.

   The first step in the analytical procedure is creation of analytical methods which consists of inputting information to indicate the following:

   a. Duration of run.
   b. Concentration of internal or external standard.
   c. Names to apply to identified peaks.
   d. Response factor to be applied to convert peak area ratios to concentration.

Methods are established, altered, deleted, or listed via on-line console terminal (e.g., ASR-33 teletypewriter) without affecting the scanning of detector output signals. The chemist performs these tasks in a conversational mode wherein the computer prompts and checks all conversational mode entries, assuring that none destroy system functioning.

Initialization of analysis and calibrate mode are also performed via the console keyboard. Lights and pushbuttons at the interface unit serve to indicate the chromatograph's state and enable the operator to start, abort, or end an analysis.

The second step of the analytical procedure is the calibration run. A standard of known concentration is injected with the method interfaced in the calibrate mode. At the end of the run, a new response factor is calculated from the peak area ratios (supplied by the computer) and the standard concentration (supplied by the analyst). This new response factor replaces the old response factor in the method, ready to be used for the analysis of samples of unknown concentration.

The third step of the analytical procedure is the analysis of actual samples. The method is interfaced in the analytical mode, and the run is started. At the end of the run, the concentration is calculated from the peak area ratios (supplied by the computer), and the response factor (supplied by the method). The resulting concentration, along with other information, is printed out on the teletypewriter for the analyst.

When more than one component is present in the sample, the relative retention time is used to distinguish between peaks. In the calibration mode, a response factor is calculated for each component the analyst includes in the method, and this response factor is stored back into the method in association with the relative retention time of the peak. In the analysis mode, the relative retention time of each peak is compared to the relative retention time of each component included in the method, and, when a match occurs, the corresponding response factor is used to calculate the concentration of that compound.

A special case occurs when the gas chromatograph is used to identify an unknown substance, rather than to calculate the unknown concentration of a known substance. Here, the objective is to measure the retention time of an unknown substance and compare it to the retention time of known compounds. A match in the retention time indicates a possible identity for the unknown. It would be desirable to have the computer search a library of known compounds to see if there are any matches to help identify unknown substances, and such an option is available.

Again, the procedure involves three steps. First, a method is entered to obtain raw data for a set time. Second, a library of known compounds is constructed, and third, unknown samples are analyzed. In the library building mode, a known compound is injected, and the method gathers the retention time relative to some starting time (usually the onset of the solvent front). The analyst enters a name to correspond to this retention time. This is repeated until a library of up to 99 compounds per gas chromatographic column is created. Each new entry is automatically inserted into the library according to increasing retention time. A "window" of plus-or-minus X number of seconds is defined for the library. After the library is completed, analysis of unknown samples or mixtures can begin.

In the library search mode, the method gathers the retention time and the peak area of each peak, and, at the end of the run, gives this data to the library search program. The search program lists all matches within the limits defined by the "window" for each peak in the run, printing out the name, retention time, and peak area for each match. If no matches are found, the retention time and area of each unmatched peak is printed out.

An update mode is also available, which will recalculate the retention time for each compound in the library, based on the change in retention time for any specified compound in the library. Additions and deletions to the library can be easily made at almost any time.

D. Information and Retrieval Functions

This computer system is also used for several information storage and retrieval functions. These information storage programs run in the background partition of CPU No. 1, in a non-real-time mode. Such background programs appear to be running simultaneously with the real-time data acquisition programs, but actually run in between cycles of data acquisition. For example, test results from some 26 centers that send samples for urinalysis are

entered into the computer each month. Approximately 3,000 samples per month are analyzed, resulting in an equal number of entries in the data-file. From this data a monthly report is generated, which contains valuable information for planning, monitoring trends, and even billing some centers. Data from several months, or even a whole year, can be retrieved to make quarterly, semiannual, and yearly reports. A considerable savings in man-hours was realized in switching from a manual data base to a computerized data base in this program, and the reduction in computational errors alone was significant.

II. ANTICIPATED CAPABILITIES

In conclusion, the Division utilizes a central computer to perform real-time data processing in support of various scientific instruments used in chemical analysis of compounds as a part of the Division's major task of processing cases and samples. For a variety of reasons it is desirable to accomplish this real-time data processing with a distributed network of minicomputers (Fig. 3) each closer to the laboratory than a central computer can be; it is also very desirable to accomplish a new type of data processing within the Division's capability—

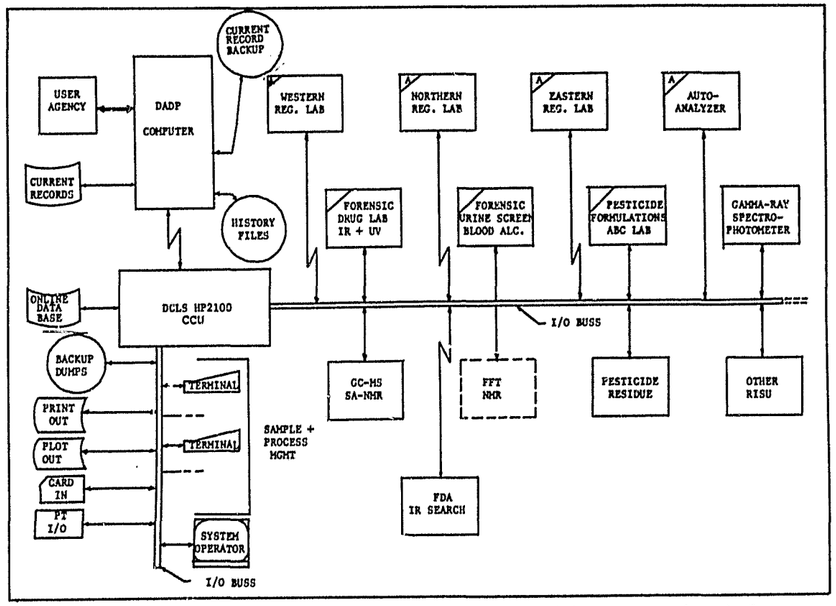

Figure 3. Distribution network of minicomputers in relation to laboratory and central computer.

that of the storage and retrieval of management information related to the operation of the Division.

Initially an information system centered around the Division's prime operation of processing cases and samples is anticipated; additional information systems may well be added later based on other operational functions of the Division. This information system aspect of our future data processing requirements represents an entirely new area of data processing from those previously done on the existing equipment. This part of the Division's data processing must meet the formal information requirements of operations and all levels of management and provide scheduled reports and inquiry capability for decision making. Some areas will require on-line access to data; other areas will need only batch oriented scheduled and ad hoc reports.

The Laboratory Automation System is being completed by the Virginia Polytechnical Institute and State University (VPI&SU) under contract. As designed, this portion of the Laboratory Automation and Data Management System (LADMS) consists of Texas Instrument 980B minicomputers connected in a distributive network. Each minicomputer will support up to sixteen instruments near its location and is known as a Remote Instrument Service Unit (RISU). The RISUs in the network will be able to communicate (transmit data) with all other RISUs on the network and a central control unit (CCU) computer located in the Richmond laboratories. The use of a distributive network of minicomputers will allow for future expansion of the number of instruments supported, the addition of different types of automation applications, enhanced capabilities, and the automation of the three regional Forensic laboratories.

90457

The Development and Applications of the Computer System of the Home Office Central Research Establishment

by

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I. INTRODUCTION

The Home Office Central Research Establishment (HOCRE) got its first computer in 1972. It was bought with the idea of using it for instrument control, data processing and information storage. It was soon realized that it was unsatisfactory to use the same computer for all purposes and other computers have been introduced for instrument control and some of the data processing requirements. These computers are usually referred to as dedicated computers. Now, the aim is to dedicate small process units to each sophisticated piece of scientific equipment and to develop separate computer units for use in storage and retrieval of literature and analytical data.

Manufacturers of scientific apparatus often select one type of computer for use with their equipment and they develop software packages for particular applications. In these circumstances it is often convenient to buy the pieces of scientific equipment, the computer hardware, and the software as a single package, or, if this is too costly, to buy these in stages. In our experience, the disadvantage of this approach is that it lacks flexibility for the development of new ideas and for applications not connected with large equipment. Nevertheless, it is costly for an organization to develop its own software and in modern forensic science laboratories there is a place for both dedicated and non-dedicated computers.

Curry and Kazyak (1) and Kazyak (2) reported a number of computer applications in toxicology and several authors have described applications to other branches of forensic science (3-6).

In this paper we shall describe how a variety of information files of forensic significance have been transcribed into a form suitable for computer storage and retrieval. Most of the applications that will be described will be in the field of drug chemistry and toxicology but the HOCRE computer is used for storage and retrieval of data on most of the major evidence types encountered in forensic science.

II. EQUIPMENT

The main computer at HOCRE, which was installed in 1972, is a Hewlett-Packard 2100A. The original specification was 8K core, a paper tape reader, a magnetic tape drive and a teletype. This has been enhanced almost yearly and the equipment configuration now consists of 16K core, a magnetic tape drive, a cartridge disc drive, a teletype, a paper tape reader and punch, a line printer, a visual display unit (VDU) and an "optical mark-card" reader. There are also two terminals, one in an operational forensic science laboratory, the other in the Enquiry Centre at HOCRE. Programming is normally done in Fortran IV.

III. COMPUTER APPLICATIONS AT HOCRE

The main applications of the computer can conveniently be divided into five recognizable areas:

(i) storage and retrieval of forensic science literature;

(ii) data banks on evidence types;

(iii) analytical and reference data;

(iv) statistics and data processing;

(v) general "housekeeping" programs.

A. Storage and Retrieval of Forensic Science Literature

The computer is now an integral part of the total information system provided for forensic scientists in the UK. The computer is particularly useful for storage and retrieval of information relating to papers, reports and books.

The papers for indexing are obtained in four main ways:

(i) by careful screening of about 100 selected journals at HOCRE;

(ii) by running tailored profiles on the UK Chemical Information Service (UKCIS) computer (which uses a computer readable version of Chemical Abstracts—Chemical Condensates);

(iii) by scanning Current Contents (Life Sciences);

(iv) by contact with other Government Establishments.

In this way about 300 papers each month are collected and indexed.

Since 1971 subject retrieval from the accumulated store of literature has been by a keyword system on a computer. By 1975 there was a growing awareness that improved methods of retrieval were necessary for the names of authors and other bibliographic details and this led to the addition of a further retrieval system known as the "Author Title Index" (ATI).

The present literature system utilizes the keyword and ATI retrieval systems as complementary search procedures. The keyword system contains over 27,000 records which can be handled through about 20 different programs. The ATI system comprises some 14 different computer programs handling a file of about 7000 bibliographic references dating from 1975. The ATI is prepared each month from the records added to the computer. An annual index of the monthly ATI is being prepared each January.

With both the ATI file and the keyword file the accent is on the need to get the information coded and stored very quickly. The articles for storing are chosen by the appropriate specialist within HOCRE. Once the items have been selected the bibliographic details can be coded and recorded immediately using clerical grade staff. The keywording takes longer since the keywords are chosen by Information Division scientific staff after careful reading of selected material. Typically, material appearing in the literature in a particular month will go into the ATI file in that same month or the next; it will be entered in the keyword file three to six months after it first appeared. Ways of speeding up the keyword process are being researched.

1. The ATI file

Each paper accessed by HOCRE for computer is given a five figure reference number or "accession" number. The information is entered into the first of five available lines of the ATI record entry as shown in Figure 1. The remainder of the first line is then available for author details. The title of the article is entered on the second line, and also the third and fourth if necessary. The last line is used to code the article by discipline (two character spaces available); to indicate whether the item has been sent out in hard

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26453 Drayton J V
Mass Spectrometry in Forensic Science
. C Chem Br 1975, 11, 439
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Figure 1. Sample of the Author Title Index (ATI)

copy form to the operational laboratories in the UK (third character position); to present bibliographic reference details of the paper (the rest of the last line).

The ATI produced at HOCRE is sent to centres in several countries where it is copied and distributed to authorized forensic science laboratories.

2. The keyword file

The keyword file can be searched using a thesaurus of about 4000 keywords and is useful because many papers contain important work which is not sufficiently well described by the title alone. For example a paper entitled "The mass spectrometry of drug metabolites" gives relatively little away about the content. Indeed there are 11 papers with this or a very similar title in the last 7,000 records. In the 27,000 forensic science records on the keyword file, there are almost 1,000 records on mass spectrometry. Not all of these deal with drugs, of course. Most are keyworded under mass spectrometry and a particular drug name and are easily retrievable, although others, which deal with generic classes of drugs, the benzodiazepines for example, present difficulties for the keyworder.

Apart from drugs, the other references on the keyword file at HOCRE deal with the other evidence types encountered in forensic science: glass, blood, paint and so on.

As will be described later, apart from literature, details on analytical data, and information from casework are stored on the computer. The advantage to the operational forensic scientist is that he has a wealth of information of direct relevance to him at his fingertips. For drugs, some of this information is available via commercial information systems but it is often necessary to make enquiries of several commercial systems to obtain the required information and the process can be time consuming and costly. Additionally, the same or similar information is often required by several laboratories so that obtaining information from an outside organization can become unnecessarily costly. In the UK it is preferable to maintain the information at a centralized facility. For many evidence types, of course, no commercial systems exist for the provision of information.

The main advantage of the keyword system is the speed of use of the file and the small amount of file storage space needed. But the keywording process itself is time consuming. The HOCRE is investigating the possibility of replacing the keyword system by title enrichment, i.e., by the addition of selected words to the title in the ATI system. It is also possible that by the end of 1979 or 1980, brief paper summary details will be added to the bibliographic file as a separate feature. All the papers are stored in hard copy form and on microfilm.

B. Data Banks on Evidence Types from Casework

Ten operational forensic science laboratories in the UK submit details to HOCRE of cases that have been examined. Information is submitted about evidence types such as tires, glass (household and vehicle), paint (household and vehicle), fibers, blood, and toxic substances. The information is usually recorded on optical cards but sometimes on forms, depending on the evidence type. At HOCRE the information is stored and indexed in the computer.

To take one example, the collection of case details from toxicological examinations carried out in the UK Forensic Science Service was initiated by information scientists at HOCRE in 1967. Operational toxicologists provided HOCRE with details of levels, type, and combinations of drugs together with some general background information by filling in prepared forms and sending them to HOCRE. The data were compiled to form the "Registry of Human Toxicology". Because of the complexity of the steps in the preparation of the document it was only available at infrequent intervals.

Not surprisingly, therefore, other methods of data collection, collation and retrieval were sought as the collection of such data was considered to be important and toxicologists were anxious for it to be continued.

In 1974 it was decided to store the data on the HOCRE computer, and a specially designed optical card, tailored to meet the needs of the UK Forensic Science Service, was designed so that the relevant data could be accessed. In addition to duplicating the earlier function of the "Register of Human Toxicology" the optical card enables more detailed information to be used to:

(i) monitor extraction and identification techniques used by toxicologists in an attempt to highlight areas of difficulty;
(ii) survey cases involving drugs and driving;
(iii) follow changes in the pattern of drug abuse; and
(iv) monitor and interpret trends in cases of multiple drug intoxication.

The project to collect and store these data from casework began in 1977 and, to date, there are about 1,400 records on file. These records contain information such as:

CASE DETAILS
DRIVING, NOT DRIVING
COMPOUND(S) FOUND
INGESTED PREPARATION
SAMPLE (BLOOD, LIVER, ETC)
EXTRACTION TECHNIQUE
IDENTIFICATION TECHNIQUE
LEVEL

The data are presented in tabular form to the operational scientists at six-month intervals and specific queries are run at any time on request (7).

C. Analytical and Reference Data Maintained at HOCRE

Since the inception of HOCRE, the Establishment has obtained and stored selected analytical and reference data of interest to forensic scientists. Data are available on analytical and reference information such as:

Analytical Data
IR SPECTRA
UV SPECTRA
MASS SPECTRA
TLC;GLC
X-RAY DATA

Reference Data
HEADLAMPS
SIDE LAMPS
TYPESTYLES

1. Mass spectrometry

One of our main areas of compound identification by the computer is from a collection of mass spectrometric data. The steps we take in attempting to identify a mass spectrum are shown in Figure 2.

The main collection used contains details of names, molecular weight, and the eight largest peaks for each compound. (There are 1,900 compounds in the collection at present.) The collection is used for computer searching using a discrepancy index (DI) (Figure 3), and also for the production of manual indexes, indexed by name, molecular weight, and base peak, which are distrib-

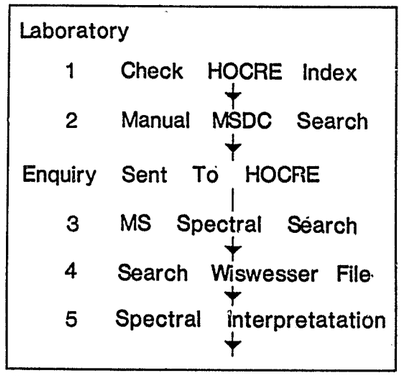


Figure 2. A model procedure for the identification of a mass spectrum in a UK Home Office Forensic Science Laboratory. (*MSDC = Mass Spectrometry Data Center*)

Peaks 44 91 65

Amphetamine								MW	DI
44	91	45	65	42	43	41	51	135	0.274
Amphetamine									
44	103	91	65	42	104	45	102	135	0.548
Ethyl Amphetamine									
72	44	91	73	65	42	56	0	163	1.644

Figure 3. Part of a mass spectral search using peaks at 44, 91 and 65. (*DI = Discrepancy Index.*)

uted to the UK forensic science laboratories (8). These actions are summarized in step 1 of Figure 2. Steps 2 and 3 involve searching the available large commercial collections of spectra. If we still have not identified the compound, we have, on our computer, a file of about 8,000 compounds each coded with name, molecular formula, molecular weight, and Wiswesser line notation (WLN) (Step 4). This enables us to attempt to identify compounds with a computer search (9) on combinations of

(i) substructural fragments;
(ii) molecular weight range;
(iii) molecular formula;
(iv) atoms present;
(v) numbers of atoms of specific elements, i.e., Cl, Br, F, S. P.

As with the mass spectral collection, manual indexes of our Wiswesser file (molecular weight and molecular formula) are produced for the UK forensic science laboratories. If the unknown is still unidentified, then, if the case demands it, spectral interpretation may have to be carried out (Step 5). Spectral processing on the Micromass 16F low resolution mass spectrometer at HOCRE is done on the computer via a Carrick interface and an off-line magnetic-tape deck. The programs for this have been developed at HOCRE.

D. Statistics and Data Processing

The discriminating power of a chromatographic system is defined as the probability that two compounds selected at random would be separated by that system (10). Two compounds are regarded as having been separated if the difference between their chromatographic values exceeds a certain critical limit termed the error factor. Compounds that are not separated are assumed to be chromatographically similar, i.e., they form a matched pair. Discriminating power (*DP*) can be expressed by the equation

$$DP = 1 - \frac{2M}{N(N-1)}$$

Where M = the number of matched pairs from all compounds recorded;
N = the number of chromatographic values recorded.

Discriminating power measurements can be applied to any chromatographic system or combination of systems. At HOCRE this approach has been used, for example, to select Thin-Layer Chromatography (TLC) systems for the separation of basic, neutral, and acidic drugs (11).

The number of paired comparisons is extremely large when many systems are investigated and these comparisons and the *DP* calculation are carried out on the HOCRE computer. The best system (or combination of systems) is the one which produces the highest *DP* value. For example, for basic drugs, three TLC systems were chosen as most suitable for the separation of the 100 basic drugs most commonly encountered in forensic science (12). The systems are: cyclohexane-toluene-diethylamine; chloroform-methanol; acetone.

Unknown basic drugs can be identified by running them on the three TLC systems together with reference compounds (13). The data obtained for the reference compounds are used to correct those for the unknown drug. The data for the unknown are then compared against data held on the computer for the 100 most commonly encountered basic drugs. Clearly, exact agreement between the data for the unknown drug and any of the data on file for the three systems run is most unlikely and a statistical approach is used to produce a Discrepancy Index.

Where $DI^2 = \sum_{i=1}^{i=3} \left(\frac{X_i - Y_i}{\lambda_i} \right)^2$

$X_i = Rf$ for the unknown drug on system i;

$Y_i = Rf$ for the any one of the 100 drugs on system i;

λ_i = the standard deviation of the system as determined by inter-laboratory experiments (this depends upon X_i).

The comparison is repeated for each of the three TLC systems used and the index is computed between the data for the unknown drug and the data for each drug held on file. The computer then prints out the names of the drugs producing the smallest *DI*. The approach can be extended to include other parameters besides TLC data, for example other chromatographic properties, and spectrometric data (Figure 4).

The approach is now being tried on an interlaboratory basis for the identification of drugs. The level of succe s has been such that data for other types of drugs are to be added to the file (A. C. Moffat, personal communication).

Similarly, data processing programs are being used for comparison of bullet striation patterns, handwriting characteristics, color data, as well as for multi-element data from analytical measurements on paint, glass, and hair. Programs are run for the processing of radioimmunoassay data.

E. General

The remaining computer applications are quite simply of the "housekeeping" type and small individual and non-specific requirements.

	T1	T2	T3	GC	UV	SH	DI
Benzphetamine	67	70	70	1850	257	PPT	2.6
Meclozine	58	79	70	3050	230	0	9.5
Diethylpropion	62	63	64	1485	253	-5	18.4

Figure 4. Part of a computer output using the Compound Identification program. (*T1, T2, T3 = Thin Layer Chromatography Systems, GC = Gas Chromatography Retention Index, DI = Discrepancy Index.*)

IV. USE OF THE HOCRE COMPUTER BY OPERATIONAL FORENSIC SCIENCE LABORATORIES

The information held on the computer at HOCRE is used by staff at the UK forensic science laboratories. Queries from these laboratories are dealt with by telephone or telex. The officer in charge of the computer accepts the inquiry, carries out the necessary work and then passes the information back to the inquirer. At the moment the Enquiry Centre of the Information Division at HOCRE handles over 200 queries each month.

On-line facilities have been considered but have been rejected because of the age and limitations of the present computer. Experimental acoustic coupler-computer links have been successful for some of the UK forensic science laboratories but depend upon the quality of the telephone lines.

V. FUTURE DEVELOPMENT

Because of the successful way computer usage has developed at HOCRE, plans are well advanced to replace the HP 2100A with another computer in 1979. The existing HP 2100A will probably be "dedicated" to an organic mass spectrometer already in service. The new computer will be required to support on-line terminals in the UK forensic science laboratories for:

(i) an interactive literature retrieval system;

(ii) an interactive retrieval project for the collection of data from cases examined in forensic science laboratories;

(iii) an interactive information retrieval project for the collection of analytical and reference data on various materials for identification purposes;

(iv) the running of a selection of statistical and data processing activities;

(v) a general purpose computing facility in support of research and development in forensic science.

The new mini-computer will have on-line terminal facilities, 128K of store, a disc storage facility (80M bytes), and a similar range of peripherals to the existing machine. The configuration of the present HOCRE computer is shown in Figure 5 while Figure 6 shows the configuration of the proposed machine.

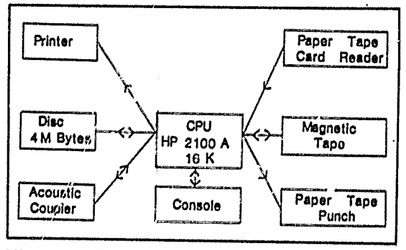


Figure 5. Present Home Office Central Research Establishment computer configuration. (*CPU = Central Processing Unit.*)

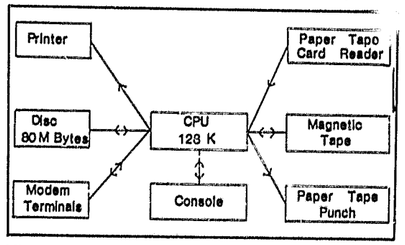


Figure 6. Proposed Home Office Central Research Establishment computer configuration. (*CPU = Central Processing Unit.*)

VI. CONCLUSION

A computer-based system for the storage and retrieval of forensic science literature including drugs has been described. The HOCRE computer system also involves a co-operative scheme of information exchange for drugs and toxic materials encountered in forensic science. Analytical and reference data are stored and "unknown" drugs can be identified by use of a discrepancy index. Data on other evidence types of interest to forensic scientists are also coded and stored.

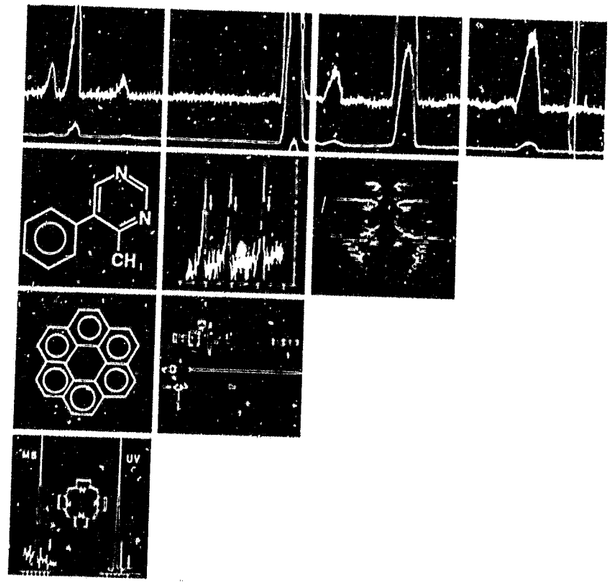
The system is to be developed in the UK into an interactive on-line system involving operational forensic science laboratories. It is hoped that the on-line system will be operational in late 1979 or early in 1980.

REFERENCES

1. Curry, A. S., and Kazyak, L., Sixth International Meeting of the Association of Forensic Sciences, Edinburgh, September 1972, *Int. Mic. J. of Leg. Med.*, 8 (1973).
2. Kazyak, L., *J. Forensic Sci.*, 19, 147 (1974).
3. Bryan, J. L., Icove, D. V., *Fire Journal*, 20 (1977).
4. Gardner, G. Y., Carnahan Conference on Crime Countermeasures, p 149, College of Engineering, University of Kentucky, May 1977.
5. Blaisdell, B. E., *Anal. Chem.*, 49, 180 (1977).
6. Heller, S. R., *Science*, 195, 253 (1977).
7. Owen, G. W., and Brown, C., Home Office Central Research Establishment, Report No 253, (Restricted Circulation) HOCRE, Baughurst Rd., Aldermaston, Berkshire, England, 1977.
8. Ardrey, R. E., and Brown, C., Home Office Central Research Establishment, Report No 194A, (Restricted Circulation), HOCRE, Baughurst Rd., Aldermaston, Berkshire, England, 1976.
9. Ardrey, R. E., and Brown, C., *J. Forensic Sci. Soc.*, 17, 63 (1977).
10. Moffat, A. C., Smalldon, K. W., and Brown, C., *J. Chromatogr.*, 90, 1 (1974).
11. Owen, P., and Moffat, A. C., Eighth International Meeting of the Association of Forensic Sciences, Wichita, May 1978, Int. Mic. J. of Leg. Med., (to be published).
12. Moffat, A. C., Home Office Central Research Establishment, Collection of Analytical Data for Drugs, HOCRE, Baughurst Rd., Aldermaston, Berkshire, England, 1976.
13. Moffat, A. C., *J. Chromatogr.*, 110, 341 (1975).

Chromatographic Advances Session

I. Instrumentation
II. Methodology
III. Drug Analysis



Applications of HPLC to the Analysis of Drugs

by

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I. INTRODUCTION

During the 1970's there was an amazingly rapid development in high performance liquid chromatography (HPLC). This arose partly from the needs of the pharmaceutical industry for new analytical techniques and partly as the result of the efforts of instrument manufacturers, mainly in the United States, to satisfy these demands and also create new products for their factories.

Although we commenced work with HPLC in 1971, it soon became clear that the factors noted above had resulted in equipment which was not particularly well suited for use in forensic science laboratories. For instance, in Table I are listed some of the major differences in analytical requirements between these laboratories and those of the pharmaceutical industry.

Prior to the advent of HPLC, thin layer chromatography (TLC) and gas chromatography (GC) were the principle methods of analysis for drugs. The method of operation of both these techniques had an important bearing on the design of HPLC instruments since, in general, it was the manufacturers of TLC and GC equipment who extended their activity to include HPLC.

A comparison of the three methods is shown in Table II.

The three final points are the ones which have most influenced the development of HPLC in our laboratory and need to be elaborated. The solvent used in HPLC plays a very important part in a separation (unlike the carrier gas in GC) and slight compositional changes are often accompanied by large changes in the elution volumes of particular compounds. Instrumentally it is inconvenient and time consuming to change solvents, and to avoid this problem it is necessary either to use

Table I

Analytical requirements of pharmaceutical and forensic laboratories

PHARMACEUTICAL INDUSTRY	*FORENSIC LABORATORY*
1) High throughput of samples of the same type	1) Widely differing range of sample type, and numbers
2) The components in a sample are usually known and the levels should be within well defined limits	2) Wide ranging differences in composition, particularly with illicit preparations
3) Analysis carried out by a specialist group	3) Analysis to be carried out by individual reporting officer or their assistants
4) Quantitative analysis of primary importance	4) Most emphasis on quailtative analysis, although quantitation is important in toxicology cases and for comparative drugs analysis

each instrument to carry out one particular analysis, or to select different analyses which can be achieved with the same column and solvent system. The high cost of commercial instruments would make this approach very expensive.

The sequential nature of HPLC is another aspect of the technique which has had a marked influence on the way in which we use it. For many years TLC has provided the most convenient method of screening for drugs in a forensic laboratory, and it seems logical to use analogous conditions to achieve separations by HPLC. In practice, however, most TLC methods use a silica adsorbent and a non-polar solvent and these are probably the worst type of conditions for ensuring reproducible analyses with a sequential method such as HPLC. It does not matter in TLC whether polar components (e.g., water) introduced with the sample deactivate the adsorbent, for the plate is used once only and many samples can be run simultaneously. With HPLC, however, the introduction of such a sample would lead to a continuous deactivation of the column, making it difficult to ensure long-term reproducibility of elution volume. Emphasis has therefore concentrated on developing column separations in which the eluting solvent is itself highly polar. Thus, our strategy for HPLC (1) can be summarized as follows: to develop low cost systems, for specific analyses based on the use of polar eluting solvent, avoiding complications such as gradient elution. Furthermore, the HPLC would be used for those applications where existing separation methods, particularly GC, could not be employed to supplement the information obtained from the initial screening of samples by TLC. Before considering some examples of such analyses it is appropriate to detail some areas where considerable savings in the cost of equipment can be achieved while still maintaining adequate performance.

II. LOW COST EQUIPMENT

For routine analysis based on an isocratic

Table II

Comparison of TLC, GC and HPLC as analytical methods

TLC	*GC*	*HPLC*
1) Applicable to volatile and involatile compounds	1) Applicable only to volatile compounds	1) As for TLC
2) Difficult to quantitate	2) Readily quantitated	2) Readily quantitated
3) Versatile	3) One instrument readily used for a variety of analyses	3) Solvent specificity makes instruments less flexible
4) Simultaneous analysis	4) Sequential analysis	4) Sequential analysis
5) Low cost	5) Moderate cost	5) Moderate to high cost

(i.e., the same solvent system throughout the analysis) elution the equipment for an HPLC system can be greatly simplified and can consist of:

		Commercial cost	*Our cost*
a)	pump	up to £2500	£250–300
b)	injector	up to £500	£15–25
c)	column	up to £150	£15–20
d)	detector	up to £2000	commercial detectors mainly used

Thus, savings of close to £3000 per system can be achieved. It should however be emphasized that this is on equipment for routine analysis. It is advantageous for method development to use the more expensive pumps, although we would still use these in conjunction with low cost injectors and columns.

On the other hand, commercial instruments have a number of disadvantages compared with laboratory assembled units which arise because of the influence of GC design concepts. These can be summarized thus:

1) The design of liquid chromatographs with all the equipment housed in a single box rather like a gas chromatograph. This tends to be inconvenient (particularly if solvent leaks occur) and adds to expense.

2) The success of temperature programming in GC led to the mistaken assumption that gradient elution could be equally effective in HPLC. In fact, gradient elution imposes considerable design and operating difficulties in many instances.

3) The role of temperature was overemphasized in the early liquid chromatographs. There is no need for elaborate thermostatting of columns.

4) The way in which substituting a liquid mobile phase for a gaseous one reduces the flexibility of an instrument was not appreciated. A single GC can be used for many different analyses, whereas HPLC equipment is best used in a dedicated mode.

Cumulatively these "mistakes" led to the development of equipment of high cost, occupying much space and having a performance that was often much better than required. This point becomes more apparent when the components of the HPLC are considered in detail.

A. Pumps

The key features of most of the pumps specifically developed for HPLC are:

1) A virtually pulse free solvent delivery.

2) A wide range of pressure capacity—typically from 0–6000 psi.

3) Variable and constant delivery rate from 0–10 ml/min.

4) Low internal dead volume, useful when changing solvents or carrying out gradient elution.

In practice for analysis using ¼" o.d. columns packed with microparticulate packing material, much of a pump's performance is superfluous, for most chromatographers use a flow rate of 1–2 ml/min with a pressure requirement of about 500–2000 psi. Hence, pumps with a performance specification well below that of a commercial HPLC pump can be of value. Pulse free flow is important because many of the detectors used in HPLC are sensitive to solvent pulsing, but it is important to appreciate that pulsing must be absent from the solvent stream as it emerges from the column not necessarily from the pump. It is comparatively simple to remove much pump induced pulsing by incorporating a Bourdon tube gauge into the solvent line between pump and column. Moreover, the packed bed of the column also provides a smoothing effect. It has been our experience that the flow from single piston reciprocating pumps can be smoothed sufficiently to cause the minimum of disturbance to many commercially available HPLC detectors. Fortunately for the "do-it-yourself" chromatographer such pumps are readily available at low cost as they have industrial uses and have been employed for many years for fluid metering. Although these pumps often have a large internal dead volume this does not detract from their value for routine analysis where solvent of the same composition may be pumped for weeks or years at a time.

B. Injectors

The introduction of a sample to the top of the chromatographic column is one of the key areas in HPLC, for a badly designed injector can lead to appreciable loss in the separating power of a column. Some excellently engineered injectors have been developed to allow a syringe injection to be made with no drop in pressure in the column. Ironically, despite their high cost such injectors rarely give the best performance since appreciable band spreading occurs as the sample leaves the injector and enters the column. Again, the "do-it-yourself" chromatographer is in luck for the low compressibility of liquids can be used to good effect if stop-flow injection is made. Thus, by stopping the flow in the column, allowing the pressure to drop to atmospheric and making a direct syringe injection to the top of the packing material bed, followed by resumption of pumping, highest column efficiency can be obtained with a simple TEE joint, as shown in Figure 1. (B. B. Wheals, submitted for publication *J. Liquid Chromat.*)

The method of injection is crucial for highest performance. Thus, injection directly onto a metal frit at the top of the column as in many commercial instruments (Figure 2A) gives low efficiencies because of stagnant solvent on either side of the capillary inlet. Similarly, injection onto a metal mesh on top of the stationary phase with or without a metal holding collar (Figures 2B and 2C) is unsatisfactory since the injected solution back diffuses into the solvent above the column.

We have found that the best method is to pack the column with a layer of 80–100 mesh glass beads and inject the sample into the center of these using a hypodermic syringe (Figure 2D). The sample, which can be up 10–15 μl, is thereby confined to a small volume well away from the walls of the column and efficiencies are greatly improved. Figure 3 shows chromatograms obtained with a 25 cm column packed with 5 μm silica modified with C_{18}

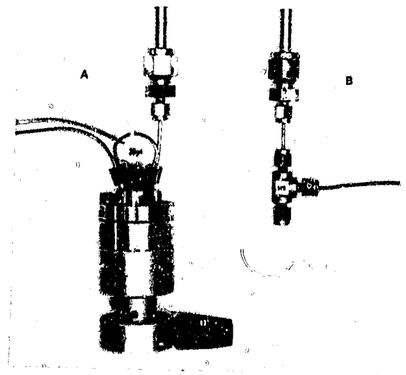


Figure 1. HPLC injectors. A, commercial valve injector; B, simple 1/10" tee for stop-flow injection.

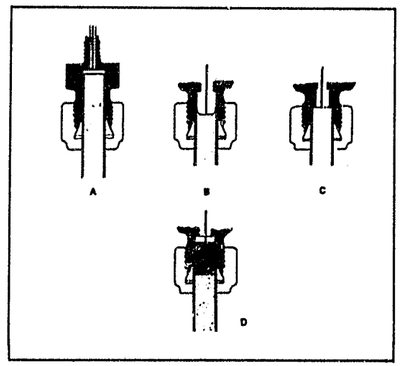


Figure 2. Modes of injection. A, onto frit at top of column; B, onto mesh resting on column packing; C, onto mesh held by metal collar; D, into glass beads at top of column.

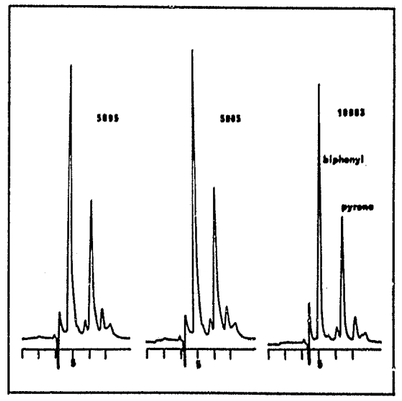


Figure 3. Column performance resulting from different injection modes. Figures indicate plate numbers for same column with injections indicated.

groups on the surface. The use of glass beads doubles the plate value of the column.

C. Columns

Much mystique has been built around the columns used in HPLC since special slurry techniques are necessary for their packing.

Without going into details it is true to say that with a pneumatic amplifier pump costing less than the price of a single commercial column it is possible to pack high quality columns. The ability to do this opens up the whole field of HPLC for the chromatographer to then begin to experiment to produce packing materials appropriate to his special analytical problems. We have never used a commercially packed column in our laboratory but have instead taken a small particle size silica, e.g., Partisil 6 μm (Reeve Angel, Maidstone, England) and graded this by sedimentation to improve the particle size range (2). In this way efficiencies of up to 18,000 plates have been obtained with a 25 cm column.

It is also possible to modify the silica quite easily by treatment with reagents such as octadecyl trichlorosilane so that reverse phase chromatography can be carried out (see 3). A particularly interesting development in this respect is the use of vinyl trichlorosilane to introduce reactive — $CH{=}CH_2$ groups onto the silica. These can then be used as the sites for addition of other vinyl monomers, such as acrylonitrile or diethylaminoethyl acrylate (4)

D. Detectors

There are a variety of these based on different principles of operation, e.g., refractive index, moving-wire, etc., some of which are shown in Table III.

Because of its simplicity and reliability we have based our HPLC machines on ultra-violet (UV) absorption using an inexpensive variable

Table III
Detectors for Liquid Chromatography

DETECTOR	*TYPE*	*DETECTION LIMIT (g)*
UV	SELECTIVE	10^{-8}
FLUORIMETRIC	SELECTIVE	10^{-11}
MOVING WIRE/FID	UNIVERSAL	10^{-5}
REFRACTIVE INDEX	UNIVERSAL	10^{-5}

wavelength UV spectrometer, e.g., Cecil Instruments C212. This limits analyses to those compounds which have UV absorption but the restriction is not as narrow as would be expected since many drugs have such chromophores.

The alternative means of detection which we selected was fluorescence. For this either a commercial spectrofluorimeter (Perkin-Elmer MPF 2A) with a laboratory constructed capillary cell (5) or a single home made filter-based fluorimetric detector (3) is used. The UV and fluorescent detectors can also be coupled in series to monitor the effluent from the column.

III. APPLICATIONS

The analysis of drugs and their metabolites by HPLC has recently been reviewed by Wheals and Jane (6). What follows hereafter is an indication of the uses to which the technique has been put in our laboratory.

A. Drugs Arising From Illicit Possession Cases

Most of the applications have been of this kind, one of these being in the screening of lysergic acid diethylamide (LSD) microdots for the active ingredient.

1. Lysergic acid diethylamide.

LSD can be readily separated from other fluorescent ergot alkaloids and related compounds on a column containing 5 μm silica eluted with an ammoniacal methanol/water solvent (Figure 4). The separation mechanism is not clearly understood but seems to involve siloxane groups on the silica surface rather than silanol groups. The same type of conditions are used here for many basic drugs and it has the advantage of being a chromatographic system that is stable and the silica is obviously not in a form which leads to poisoning by the addition of water, which is the most common contaminant for columns based on adsorption with a non-aqueous eluent.

In the case of LSD the fluorescent detector gives a high level of specificity to the analysis as well as the sensitivity to cope with the low level of doses associated with LSD (i.e., 100–150 μg per microdot). Only a portion of the whole tablet is required and is merely crushed with the eluting solvent.

LSD can also be analyzed when present in blood, urine (7) and viscera (J. M. Wiles, J. Hughes, J. Christie & M. White, to be published *J. Chromatogr.*); the extraction procedures used convert some of the LSD to iso-LSD. Hence, there is a second peak in the chromatogram to reinforce an identification made on the retention volume of the LSD peak. Similar HPLC procedures including monitoring the column effluent by

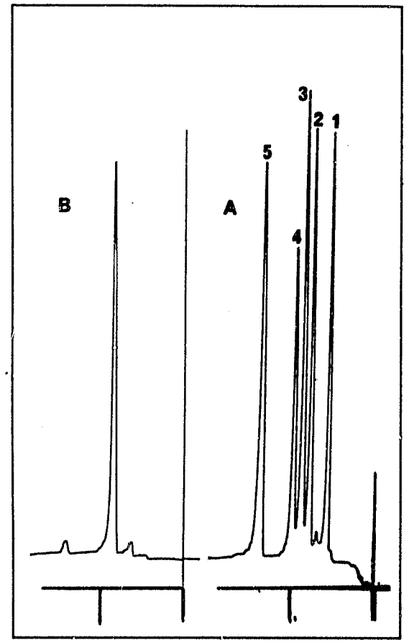


Figure 4. Separation of ergot alkaloids. A. Synthetic mixture. B. Extract from LSD microdot. Column 15 cm ×4.9 mm I.D. packed with Partisil 5 (7 μm) Solvent, methanol: 0.2% ammonium carbonate (3:2) Flow 1 ml min^{-1} at 1600 psi. Fluorimetric detector.

1. Lysergic acid
2. Lysergamide
3. LSD
4. Lysergol
5. Iso-LSD

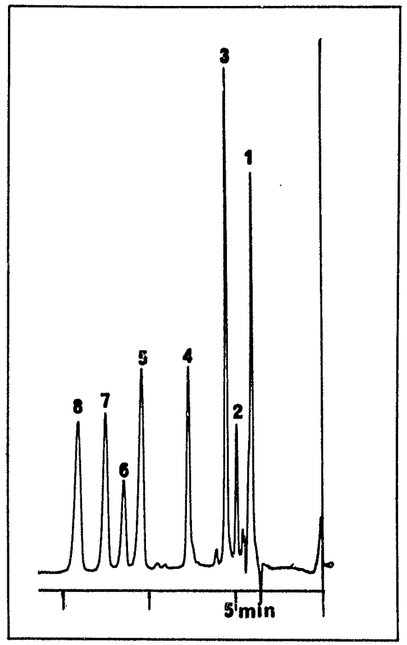


Figure 5. Separation of amphetamine type drugs. Column 25 cm ×4.9 mm I.D. packed with Partisil 5 (7 μm) Solvent methanol: 2N ammonium hydroxide: 1N ammonium nitrate (27:2:1) Flow 1 ml min^{-1} at 1500 psi. Detector UV at 254 nm.

1. Benzphetamine
2. Phendimetrazine
3. Phenmetrazine
4. Amphetamine
5. N-methylephedrine
6. Ephedrine
7. Methylamphetamine
8. Methphentermine

immunoassay techniques have also been reported (8).

2. Amphetamine type drugs

Figure 5 shows that the separation of many amphetamine type drugs can be accomplished rapidly by isocratic elution on a silica column (1, 2).

3. General basic drugs

One of the advantages of a UV detector is that monitoring the column effluent at different wavelengths enables a wider range of drugs to be analyzed than with other detectors. This is illustrated in Figure 6 for example, wherein ephedrine, amphetamine, and methylamphetamine are detected by their absorption at 254 nm whereas papaverine, thebaine and dihydrocodeine are monitored better at 278 nm. The analyses are carried out with a 25 cm silica column. Further applications of such general analyses are listed in references 1 and 2.

4. Opiates

The opiate drugs are readily separated on a 25 cm silica column, Figure 7, and this system has also been used for the quantitative analysis of the so-called "Chinese" heroin preparations which were in circulation in London a year or so ago (1). The latter, in addition to heroin and monoacetylmorphine or monacetylcodeine, contained caffeine and strychnine. The procedure involved HPLC analysis of the original material followed by a second analysis after the sample had been hydrolyzed with alkali to give morphine and codeine since the monoacetyl derivatives of these were not separated in the initial chromatogram.

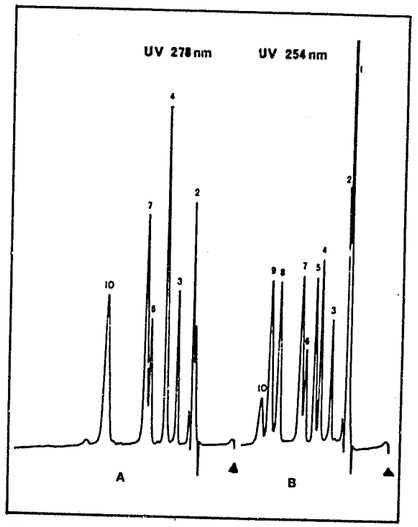


Figure 6. Separation of basic drugs, showing effect of different detector wavelengths A,278; B,254 nm. Other conditions as in Figure 5.

1. Benzphetamine
2. Papaverine
3. Protopine
4. Thebaine
5. Amphetamine
6. Codeine
7. Morphine
8. Ephedrine
9. Methylamphetamine
10. Dihydrocodeine

5. Psilocin and psilocybin

These substances are the active principles in *psilocybes* mushrooms which grow quite readily in the U.K. Unfortunately, the En-

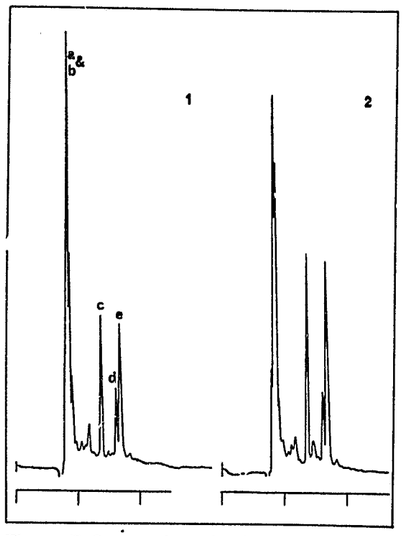


Figure 7. Separation of opium alkaloids on silica: use in opium comparison. Column 25 cm×4.9 mm I.D., packed with Partisil 5. Chromatographic conditions as in Figure 5. Samples 1 and 2 are different opium extracts: (a) papaverine, (b) narcotine, (c) thebaine, (d) codeine, (e) morphine

glish law is at present not clear as to whether or not their possession is illegal but nevertheless samples have been submitted for analysis by HPLC. A methanolic extract from the mushrooms is redissolved in methanol / chloroform (9:1) for injection onto the column. A ready separation can then be achieved with 25 cm column packed with 5 μm Partisil-5 silica using methanol, water, 1N ammonium nitrate (240: 50: 10) eluent which has been adjusted to pH 9.7 with 0.88 ammonia. The eluted compounds are monitored at 254 nm.

6. Diconal

This material, which contains cyclizine and dipipanone, is becoming increasingly prominent in cases submitted to our laboratory. The components are not resolved under the normal conditions for the separation of basic drugs on silica and elute almost at the solvent front. By treating the silica with (3-mercaptopropyl)-trimethoxysilane it is possible to attach mercaptopropyl groups to the silica and carry out elution with the same solvent system as that used for the basic drugs (B. B. Wheals, to be published, *J. Chromatogr.*). An excellent separation is obtained, Figure 8.

7. Cannabis

Surface modification of the silica with octadecyl-trichlorosilane is necessary in order to achieve the best separations of the cannabinoid constituents, Figure 9, most of which have been identified by comparison of their chromatographic behavior with authentic specimens or by measuring the mass spectra of the eluted peaks (9).

HPLC is especially useful for the detection of the thermally labile acidic cannabinoids and provides a convenient method for the comparison of samples in order to establish common origin (10), particularly if detection is carried out at two wavelengths (Figure 10). Quantitation can be carried out quite satisfactorily as is shown (11) by the data in Table IV.

Because of the reliability of quantitation it is also possible to carry out studies on the rise and fall in concentration of various components as the sample ages (Figure 11).

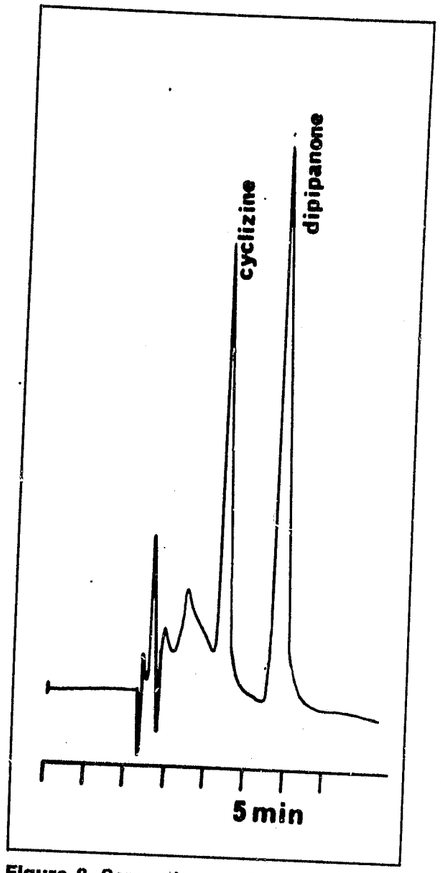


Figure 8. Separation of diconal constituents. Column 25 cm×4.9 mm I.D., packed with 5 μm silica modified with mercaptopropyl groups. Other conditions as in Figure 5.

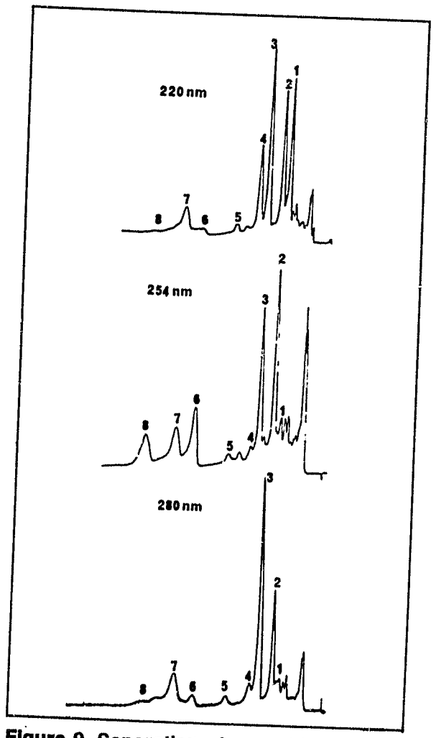


Figure 9. Separation of cannabinoid constituents. Column 25 cm×4.9 mm I.D. packed with 5 μm silica modified with C_{18} groups on the surface. Solvent methanol: 0.02N sulphuric acid 86:14. UV detector at A,220; B, 254; C,280 nm

1. Cannabidiol CBD
2. Cannabidiolic acid CBDA
3. Cannabinol CBN
4. Δ^9tetrahydrocannabinol Δ^9THC
5. Cannabichromene CBCh
6. Cannabinolic acid CBNA
7. Δ^9-tetrahydrocannabinolic acid Δ^9THCA
8. Cannabichromenic acid CBChA

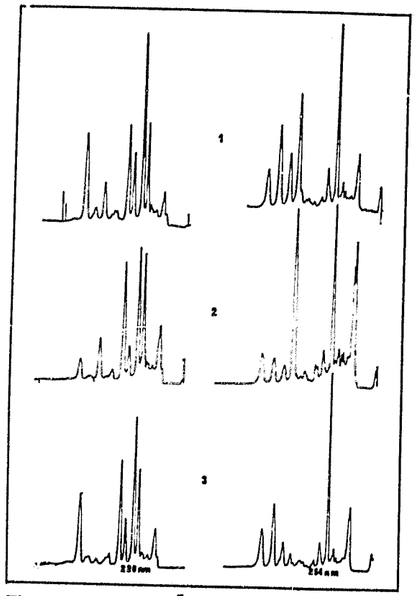


Figure 10. Comparison of different Pakistani cannabis resins. Conditions as for Figure 9, but with UV detector at A,220; B,254, nm

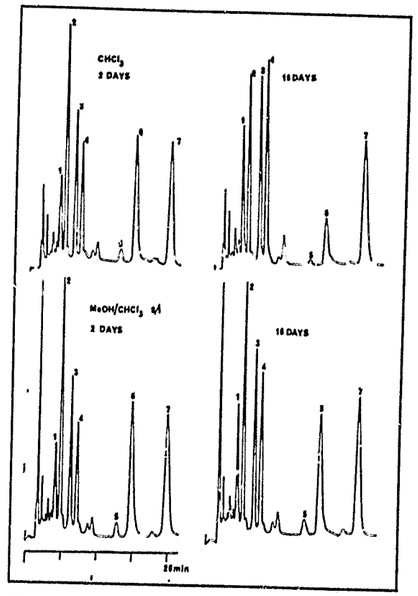


Figure 11. Aging of cannabis extracts. Conditions as for Figure 9.

1. **Cannabidiol CBD**
2. **Cannabidiolic acid CBDA**
3. **Cannabinol CBN and cannabigerolic acid CBGA**
4. **Δ^9tetrahydrocannabinol Δ^9THC**
5. **Cannabinolic acid CBNA**
6. **Δ^9-tetrahydrocannabinolic acid Δ^9THCA**
7. **Dibutylphthalate (internal standard)**

B. Toxicological Applications

1. Naturally fluorescing materials

HPLC is less easy to use for toxicological analysis because of the relatively low sensitivity of the UV detectors, which in general will respond only to concentrations corresponding to massive overdoses of drugs. On the other hand, fluorescence is inherently more sensitive and it is possible to exploit this property (see Table III). Occasionally the substance is naturally fluorescent as is the case with LSD (7). Warfarin is another such substance.

2. Chemical modification of the drug

In the absence of fluorescence or where it is only weak, as is the case with morphine, it is necessary to resort to chemical treatments to produce more strongly fluorescing derivatives. For example, morphine can be oxidized with alkaline ferricyanide to pseudomorphine, which is strongly fluorescent. This reaction has been applied to the estimation of morphine in urine, but since the process is very dependent on the co-extractives present, it is necessary to add dihydromorphine as an internal standard (13). Figure 12 illustrates this and levels of morphine as low as 0.01 μg/ml can be detected quantitatively in urine.

Table IV

Quantitative HPLC of Cannabis

CANNABINOID	*PERCENTAGE IN RESIN*	*PERCENTAGE STD DEVIATION*
Tetrahydrocannabinol	3.9	2.1
Cannabidiol	0.7	1.2
Cannabinol	0.4	6.8
Tetrahydrocannabinolic acid	4.8	1.9
Cannabidiolic acid	1.5	2.0
Cannabinolic acid	0.4	1.7
	23 Analyses	

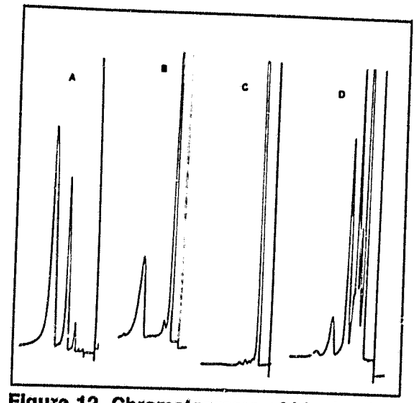


Figure 12. Chromatograms of blank urine and morphinized urine (2 μg/ml). A, 2 μl blank urine extract; B, 2 μl blank urine extract plus 2 μl ferricyanide; C, 2 μl morphinized urine extract; D, 2 μl morphinized urine extract plus 2 μl ferricyanide reagent. Column 25 cm×4.6 mm I.D. packed with Partisil 5 (7 μm). Solvent methanol: 2N ammonium hydroxide: 1N ammonium nitrate: 30:20:10. Fluorescent detector λ_{exc} 320 nm λ_{emis} 436 nm.

3. Derivatization

The use of fluorigenic reagents to attack functional groups in a drug and thereby attach a fluorescent label to the substance is not a new one. The methods are not yet of routine application even though reagents such as fluorescamine, NBD chloride (4-chloro-7-nitrobenzofurazan), dansyl chloride (5-dimethylamino-1-naphthalenesulphonyl chloride) and o-diphthalaldehyde are available. We have had some success with EDTN (2,4-dichloro-6-(4-ethoxy-1-

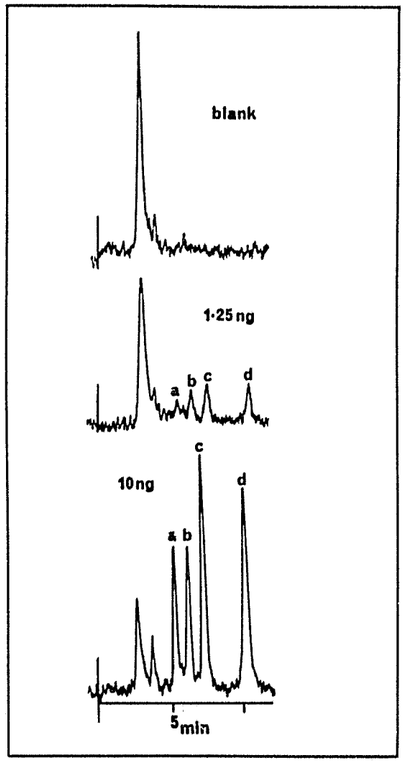


Figure 13. HPLC of dansyl derivatives. Column 25 cm ×4.9 nm I.D. packed with Partisil 5 surface modified with C_{18} groups. Solvent methanol: water:85:15. Fluorescent detector λ_{ex}, 339 nm; λ_{em}, 420 nm.
a norephedrine
b ephedrine
c amphetamine
d methylamphetamine

naphthyl)-s-triazine) as a labelling reagent and it is possible to prepare variants of this by reacting cyanuric chloride with other aromatic moieties.

An example of the use of dansyl chloride is given in Figure 13, wherein is shown the chromatograms obtained by injection onto the column of 1.25 ng and 10 ng quantities of norephedrine, ephedrine, a.nphetamine and methylamphetamine after dansylation (14).

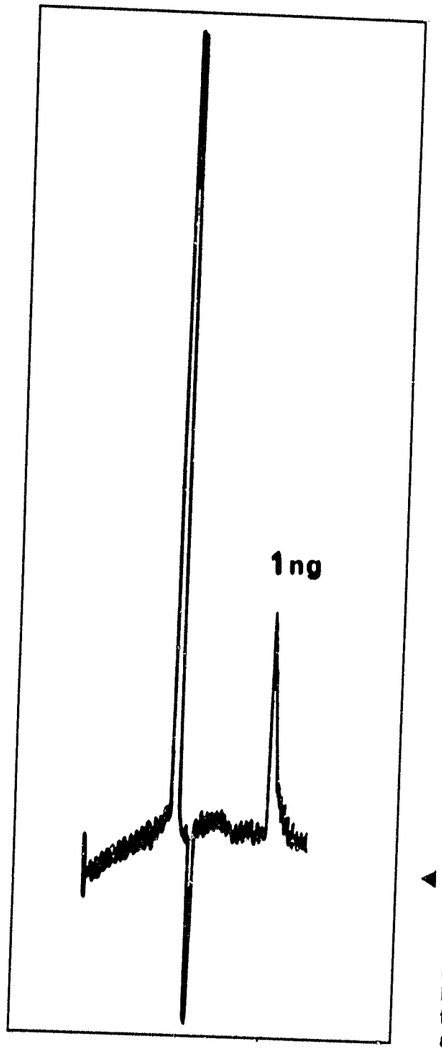
4. Electrochemical detection

Quite recently we have been examining the possibility of electrochemical detectors based on glassy carbon electrodes (15). A prototype cell has been constructed and shows considerable promise for detection of morphine in blood or urine. Figure 14 shows the peak produced by injection of a sample of urine extract containing 1 ng morphine onto the column. The sensitivity is already fairly good and can be improved considerably as only a small proportion of the morphine is being oxidized at the electrode's surface.

C. Conclusion

Although there is a wide range of commercial equipment available, HPLC is still at a stage where there is ample scope for the chemist to exercise his talents. Apparatus to suit particular analytical problems can be put together quite easily and inexpensively. It can provide reliable results for many months before the columns need repacking.

It is also possible by the exercise of skill and chemical ingenuity to bring the analytical problem well within the scope of this powerful technique.



◀ Figure 14. Electrochemical detection of morphine. Column 25 cm ×4.9 mm I.D., packed with 5 μm silica. Solvent methanol: 2M ammonium hydroxide: 1M ammonium nitrate: 27:2:1. Flow rate 1 ml min^{-1}. Detection, oxidation at glassy carbon electrode (+0.6 V versus Ag/AgCl reference) Ag auxilliary electrode.

D. Acknowledgment

We should like to acknowledge the valuable contributions made by all our co-workers, especially I. Jane, R. N. Smith and M. Whitehouse.

REFERENCES

1. Wheals, B. B., *J. Chromatogr.*, 122, 85 (1976).
2. Jane, I., *J. Chromatogr.*, 111, 227 (1975).
3. Wheals, B. B., Vaughan, C. G., and Whitehouse, M. J., *J. Chromatogr.*, 106, 109 (1975).
4. Wheals, B. B., *J. Chromatogr.*, 107, 402, (1975).
5. Vaughan, C. G., Wheals, B. B., and Whitehouse, M. J., *J. Chromatogr.* 78, 203 (1973).
6. Wheals, B. B., and Jane, I., *The Analyst*, 102, 625 (1977).
7. Christie, J., White, M. W., and Wiles, J. M., *J. Chromatogr.*, 120, 496 (1976).
8. Ratcliffe, W. A., Fletcher, S. M., Moffat, A. C., Ratcliffe, J., Harland, W. A., and Levitt, T. A., *Clin. Chem.*, 23, 169 (1977).

9. Smith, R. N., *J. Chromatogr.*, 115, 101 (1975).
10. Wheals, B. B., and Smith, R. N., *J. Chromatogr.*, 105, 396 (1975).
11. Smith, R. N., and Vaughan, C. G., *J. Chromatogr.* 129, 347 (1976).
12. Smith, R. N, and Vaughan, C. G., *J. Pharm. Pharmac.*, 29, 286 (1977).
13. Jane, I., and Taylor, J. F., *J. Chromatogr.*, 109, 37 (1975).
14. Wheals, B. B., in "High Pressure Liquid Chromatography in Clinical Chemistry" (P. F. Dixon, C. H. Gray, C. K. Lim, and M. S. Stoll, Eds.) p.211, Academic Press, London, 1976.
15. Lankelma, J., and Poppe, H., *J. Chromatogr.*, 125, 375, (1976).

On-line Liquid Chromatography Mass Spectrometry: the Monitoring of HPLC Effluents by a Quadrupole Mass Spectrometer and a Direct Liquid Inlet Interface (DLI).

by

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SUMMARY

On-line coupling of HPLC to a mass spectrometer is a promising technique as a detection system for HPLC. It provides good sensitivity, a wide range of applications, and is also an identification method capable of analyzing nanogram amounts of pure substances eluted from the column.

The method for the direct introduction of liquid solution from the HPLC column into the source block of a mass spectrometer through a "Direct Liquid Inlet" interface is discussed. The technique does not extend to all of the different aspects of modern liquid chromatographic methods; however, it is well adapted to reverse phase chromatography on chemically bonded stationary phases or on carbon adsorbents.

The potential and the future of the technique are presented with respect to recent developments of MS techniques which make many nonvolatile substances amenable to mass spectrometric analysis.

I. INTRODUCTION

Liquid chromatography/mass spectrometry (LC/MS) is a recent technique; after a few preliminary attempts, it effectively began in the years 1973–74 with the publication of the results obtained by E. C. Horning, P. P. W. Scott, and F. W. McLafferty (see Table I). During the following two years only a few papers appeared in the literature. As LC/MS is a costly and difficult research topic, a considerable amount of work was achieved by only a very small number of research teams. Their work is beginning to appear now; users of commercially available machines are showing interest, and new methods are being suggested. Today one may write that LC/MS is alive and opens to a vast field of promising developments. Despite its relative youth, many review articles on LC/MS have already been published (1–5).

The advantages of an instrument which would combine a separation method such as high pressure liquid chromatography (HPLC) to an identification method such as MS are obvious to any analytical chemist. However, such a combined technique has been regarded for many years as utopia, because the two techniques appear fundamentally non-compatible. Mass spectrometry requires a high vacuum and ionization of molecular species in the gas phase, whereas liquid chromatography is intended to analyze those substances which lack sufficient volatility to be analyzed by gas chromatography (GC).

Thus, even today, some authors consider that off-line techniques (6–8), such as collection of liquid fractions eluted from the LC column, evaporation of the solvent, and transfer of the solute onto the solid probe of a mass spectrometer are the only realistic LC/MS techniques. An evaluation of off-line LC/MS was reported by Huber *et al.* (8) It is true that this procedure may be simplified and partly automated. Often by dipping the tip of the MS probe into the collected fraction enough solute is transferred, and the complete evaporation of the solvent may take place during the introduction of the probe through the MS vacuum locks.

Off-line LC/MS coupling may use for the MS analysis new techniques recently developed

for the analysis of non-volatile molecules. These methods often require the desorption of the solute from a surface introduced into the MS source block and include field desorption (FD) (9), laser assisted FD (10), electron impact/desorption (EI/D), (11, 12) and the very simple method of chemical ionization/desorption (CI/D) (13) in which no high electrical field or sharp needles on the emitter tip are required to obtain results which previously could be obtained only by conventional FD. Other methods, such as direct chemical ionization (14), rapid evaporation from inert surfaces (15–17), laser enhanced vaporization (10, 18, 19), pyrolysis FD/MS (20), plasma desorption induced by Californium-252 fission fragments (21–25), and electrohydrodynamic ionization mass spectrometry (26–28) have been suggested for off-line LC/MS methods.

On the other hand, the manual collection of fractions from LC systems is long and tedious. It may be automated, but the following step, the introduction of the solute alone into the mass spectrometer, is more difficult to achieve (see, for instance, the work of Lovins *et al.*, 29–30). The collection of fractions is impossible to achieve in the case of fast eluting peaks, such as those now encountered in modern high performance liquid chromatography (HPLC): columns with plate number as high as 50,000 may be produced (31), but extracolumn effects are of such dramatic importance (32–34) that the analyst is often unable to take full advantage of these high performances. Off-line coupling requires that a greater amount of solute be injected, and it requires also that a conventional HPLC detector be used to monitor the fraction being collected to reduce the number of fractions further examined by MS. The detector in HPLC systems is a very critical part of the instrument, and most of the detectors used suffer from some limitations. (4) Clearly, on-line LC/MS avoids all of these problems. In addition, we believe that MS might be the only detector which offers a broad field of application to organic molecules, good sensitivity (10^{-12} g), and a wide dynamic range (10^5 to 10^6) suitable for modern HPLC. Therefore, the rest of this text will deal exclusively with on-line LC/MS techniques.

II. A DIFFICULT PROBLEM

The detection system in HPLC is of such paramount importance that it has even been suggested that a cheap mass spectrometer, with a low mass resolution, could be used. We feel that, with the technology now at hand, this is unlikely to happen. Even a simple quadrupole mass spectrometer with a very low mass resolution, used only as a low mass filter eliminating the solvent ions from the solute ions, or as a total ion integrator for the solute ions, will always be a relatively expensive detector. This is because mass spectrometry requires precisely machined parts, high vacuum, and sophisticated electronic controls. An LC/MS interface should be an accessory as part of a general multipurpose mass spectrometer combining different iniet systems for solid, gas, and liquid samples, and for GC and LC introduction.

It is a simple matter to define what the ideal interface should be:

1. The interface must not pose limitations on the operating conditions of the LC; it must tolerate different solvent systems under different flow conditions.
2. The interface also must not restrict the operating conditions of the mass spectrometer. Electron impact ionization (EI) and CI, under controlled pressure and selected nature of the reagent gas, must be available.

3. The interface, when regarded as a post-column dead volume, should have a time constant less than one second so that fast eluting peaks are not broadened (34) during their transfer through the interface.
4. The lower sensitivity limit should be less than 1 nanogram, and the dynamic range should be better than 10^4.
5. The interface should carry into the mass spectrometer a high percentage of the solute eluted from the LC column.
6. The transfer of the solute through the interface should be quantitative, independent of the nature of the solute and the solvent system used during the LC separation.
7. The solute should not be degraded (chemically altered or pyrolyzed) during its passage through the interface.

Consideration of the results obtained so far show that none of the described interfaces is perfect; at least one, and often many, of these idealistic definitions had to be sacrificed. The methods are listed in Table I.

It is not the purpose of this paper to review and evaluate the different methods now offered for LC/MS. The reader is referred to the original papers, with a specific emphasis on the atmospheric pressure ionization source of Horning *et al.*, (35–41) the moving wire of Scott (4, 42, 43) and the moving belt of McFadden. (44–47)

The LC/MS interface, referred to here as the interface with "Direct Liquid Introduction" (DLI), was originally developed in 1973 in the laboratory of Prof. F. W. McLafferty at Cornell University. Preliminary work (48) was on dilute solutions of peptides in hexane or liquid ammonia, kept in small glass vials with one end partially sealed until a small aperture restricted the flow of liquid out of the vial. It was then introduced through the solid probe inlet of a mass spectrometer model AEI MS 902 modified for CI work. CI spectra resulting from solvent/solute quadrupole mass spectrometer were used.

Table I

List of the systems proposed for LC/MS

A) Systems with complete elimination of the solvent before entering the mass spectrometer:	
—modified solid probe	
Lovins	**29, 30**
—moving wire	
Scott	**42, 43**
—moving belt	
McFadden	**44–47**
—membrane	
Jones	**78**
—collimated molecular beam	
Takeuchi	**50**
Vestall	**69**
Futrell	**70**
B) Systems which let the solution enter the mass spectrometer:	
—DLI interface and EI	
Tal'Rose	**79, 80, 82**
—DLI interface and CI	
McLafferty	**49–54**
Arpino	**58, 66**
Henion	**55, 56**
Melera	**57**
—API source and plasma chromatograph	
Karasek	**81**
—API source and quadrupole MS	
Horning	**35–41**

III. THE DLI INTERFACE

A. Principle

The principle of an interface based on DLI is simple. The major portion of the solution eluted from the HPLC column, which is compatible with the vacuum pumping of the MS, is sucked into the MS. The amount of liquid introduced is controlled by the liquid pressure drop across the DLI interface; it is convenient to place a restriction in the interface to lower its flow permeability. The pressure conditions in the mass spectrometer depend on the amount of solution introduced, on the pumping equipment, and on the vacuum conductances of the different elements of the MS.

A quadrupole MS requires operating pressures of 10^{-4} t in the source envelope, and 10^{-5} t in the analyzer. For CI conditions in the source block, the vacuum conductance of the source block is adjusted so that inside source pressures from 0.2 to 1 torr are obtained during LC/MS operations. Most commercial quadrupole MS designed for GC/MS work include fast vacuum pumps and a differentially pumped source. They may accept the direct introduction of about 1 to 10 μl/min. of solution from a HPLC column, and good LC/MS data, using only oil diffusion pumps for evacuating the solvent, have been previously presented. (49, 55, 56) The use of very large diffusion pumps, with opening size larger than 20 cm, is not recommended as they are expensive, bulky, and have a high level of oil vapor back diffusion. The alternative method to increase the vacuum pumping speed is cryogenic pumping (50, 57).

B. The cryopump

Most solvent molecules, from solvent systems used for HPLC, have a condensation coefficient equal to one when they are adsorbed on a surface at the temperature of boiling liquid nitrogen; there is not back desorption of the molecule. Thus, the pumping speed of the cryotrap is controlled by the surface of the trap, and by the conductance of the pathway between the source block and the trap (59, 60). The pumping speed is even faster when the partial pressure of the solvent vapor in the vicinity of the surface is close to 10^{-3} t, as viscous flow takes place (60). Such is the case in our prototype, which includes a large hemicylinder, chilled by liquid nitrogen, with an inner surface of 326 cm^2 directly in line of sight with the source block, and within 2 cm distance from the source block. Up to 100 μl/min. of acetonitrile may be introduced over periods of many hours (5 to 10), without lowering the vacuum pumping speed of the cryotrap. However, under such high flow rates, the pressure between the ion exit slit and the entrance aperture of the quadrupole rods is too high. This causes a mass discrimination for ions greater than m/e=300; thus, we routinely introduce 30 to 50 μl/min. of solution of polar solvents. With this liquid flow rate, we observe a source envelope pressure of 4×10^{-5} t and an analyzer pressure of 4×10^{-6} t, as read without correction on hot cathode type high vacuum gauges.

The cryotrap may be regenerated within 30 min. after isolation of the oil diffusion pumps, introduction of dry nitrogen in the vacuum envelope of the MS up to atmospheric pressure, warming up of the cold surface, and pumping down to vacuum by the roughing pumps. This procedure is repeated two or three times. Other procedures which allow the solvent vapors to go through the oil diffusion pump, even when they are turned off, are not recommended.

C. The source block

In the source block, enough thermal energy is supplied to the liquid solution for its complete vaporization. In our instrument, as in most of the other instruments of the same type, (55–57) the thermal energy is taken from the heated source block. In one case, a laser beam has been used (54) to supply this energy. As the solution expands in the source block, a pressure of about 0.5 t is reached, this pressure being practically equal to the partial pressure of the solvent molecules. Ionization of the solvent molecules to produce a plasma of primary reactant solvent ions is induced by the interaction of the solvent molecules with an electron beam of 100 eV energy. The electron beam is obtained from a conventional heated rhenium filament. A plate with a pinhole was placed between the filament and the source block to focus the electron beam, and to hide the filament from the solvent vapors which exit from the source block. This protects the filament which does not burn when oxygen containing solvents, such as water or methanol, are used; its average life time is one month.

D. The mass spectrum

The mass spectrum results from chemical ionization between the plasma of the solvent ions in the mass range from m/e=100 to the higher mass limit of the mass spectrometer. Usually the MS records the ions in the solute mass range, thus discarding solvent contribution to the mass spectra. On the other hand, the monitoring of one of the reactant ions from the solvent may give a useful LC chromatogram (61, 62).

Different solvents have already been tested in view of their use in a DLI system: pentane, hexane, tetrahydrofuran, chloroform, acetonitrile, methanol, and water. All of these solvents perform well under DLI conditions (49–51). Pure alkane solvents, such as n-pentane or n-hexane, yield CI reaction with hydride abstraction, so that the quasimolecular ion of the solute appears at m/e=M−1, (M being the molecular weight of the solute). Chloroform, when void of traces of the methanol used as a stabilizer, yields a mixture of hydride abstraction and protonation. All polar solvents (tetrahydrofuran, acetonitrile, methanol, water) give chemical ionization with protonation, so that the quasimolecular ion of the solute appears at m/e=M+1. It is one of the most attractive features of a DLI interface to match well the requirement of reverse phase HPLC, the most widely used and the most promising of the HPLC techniques (63–65). LC/MS operations, using chemically bonded silica (58) or graphitized thermal carbon black (66) as the stationary phase, have been run in our laboratory. Melera has shown (57) that a buffered aqueous solution of volatile inorganic salts, such as ammonium acetate (up to 0.1 M in water), may be continuously introduced in a cryopumped quadrupole.

The quadrupole mass spectrometer may be operated in the negative ion mode (67), and detailed studies of the behavior of the commonly used solvent systems under such an operating mode should be performed, but preliminary results (57) appear very promising.

E. The liquid/gas interface

Another critical part of the instrumentation in a DLI system is the restriction in the interface which limits the amount of liquid introduced into the mass spectrometer. In the original model (49–51, 53), a long (30 cm) thick wall glass capillary tube (6.35 mm OD×0.075 mm ID) was used. Excess glass at the end introduced in the source block was removed by dissolution in HF, and a pinhole of about 0.010 mm was obtained by glassblowing. The same technique has been used by others (55, 56). The same permeability is more easily prepared by inserting a thin metallic wire inside the capil-

lary tube, starting at the end normally placed inside the MS source block (58). Adjustment of the permeability to the required value is easily obtained, and the tube is rapidly cleaned if it becomes plugged. However, any DLI interface which makes use of such a long and narrow capillary tube, either with a pinpoint restriction or with a regularly decreasing pressure drop as with the wire model, suffers from a very severe limitation when solutions of a nonvolatile solute are percolated through the interface (58, 68). Because of the viscosity of the polar solvent, the capillary forces opposing the flow of liquid through the tube, and the range of pressure of liquid one may develop across the interface (0–100 bars), the speed of the solution through the tube is about 10 cm/sec. This is too low to insure the transport of the liquid into the vacuum of the mass spectrometer without vaporization of the solvent inside the capillary tube, as the liquid reaches the end of the capillary tube under vacuum. Thus, nonvolatile solutes and high molecular weight impurities, present in either the solvent or resulting from degradation of the chemical bonding of the HPLC column, accumulate inside the glass tube and plug it. Heating the end of the glass tube, either by thermal convection from the source block, or by a laser beam, does not bring any improvement, as it simply moves the liquid/gas transition zone deeper within the capillary tube. Such a problem is avoided when a water-cooled diaphragm is used instead of a long capillary tube (57) or when the physical parameters at the end of the capillary tube are such that a supersonic molecular beam of solution molecules, expanding in a jet, is produced (69–71). In a freely expanding jet a skimmer may be installed to discard the outer layers, which contain solvent molecules, and to inject only the central part of the beam, which contains the solute molecules, into the mass analyzer. Vestal (69) and Futrell (70) have recorded on their instruments either pure EI spectra, or CI spectra from nonvolatile molecules, such as nucleosides. However, their prototypes which include many differentially pumped zones and a laser (69), or a sonicating horn (70) to break the liquid into well homogenized droplets, are far more complex instruments than the modified quadrupole MS equipped with DLI interfaces (56–58).

Finally, the main theoretical advantages of a DLI interface have not been obtained yet. Assuming that the conclusions from a theoretical study done by Giddings *et al.* (72) are valid, we may expect that the breakage of the solution during a rapid expansion leaves the solute molecule in the gas phase during a very short (10^{-6} sec.) period of time, before solute molecules recombine to form a solid crystal. Thus, one should try to ionize the solute molecules in the gas phase during this brief period of time. The demonstration of possible isolation of macroions in the gas phase was realized by Dole *et al.*, (73) but none of the described LC/MS systems have been able to provide such an effect.

F. The chromatographic system

The HPLC instrument should be considered as a full part of the interface, and be optimized to provide the best LC/MS operations. Most analytical HPLC columns utilize small particles (5 to 10 μM) and column inside diameters of 2 to 6 mm. The output flow rate should be 0.5 to 2 ml/min, which is 10 to 40 times greater than tolerated by a cryopumped quadrupole MS. On the other hand, microcolumns (74–76) with internal tube diameter of 0.5 to 1 mm have an output flow rate of 5 to 50 μl/min, which matches well the requirements of a DLI interface. Different authors, including ourselves, have used such microcolumns in conjunction with LC/MS systems (55, 58, 71). Unfortunately, their chromatographic performances are poor, compared to the separating power of larger diameter columns. We have tried to adjust the injection parameters in a 4 mm column, using a divided flow injection mode (32). Then only the central part of the column was transferred through the DLI interface. A considerable enrichment was observed for non-retained solute, such that 10% of outlet solution in the central zone contained 60% of the injected solute. Unfortunately, no enrichment was observed for retained peaks with $K' \geq 1$, which sets a severe limitation on the practical advantages of the procedure.

Research work on improved microcolumns, and on on-line solvent concentrators may result from efforts to adjust the HPLC system to the DLI interface for LC/MS.

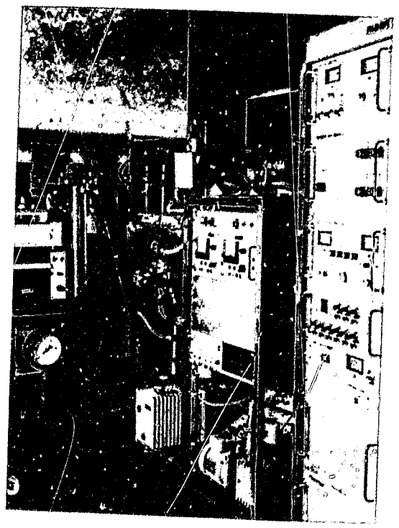
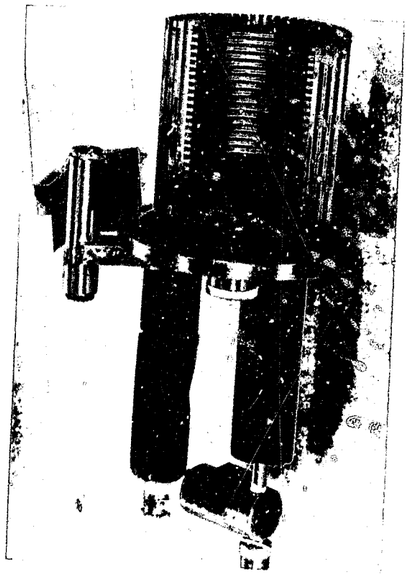


Photo 1



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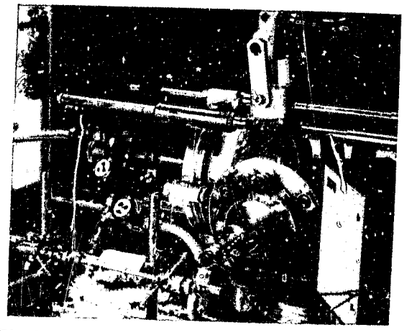

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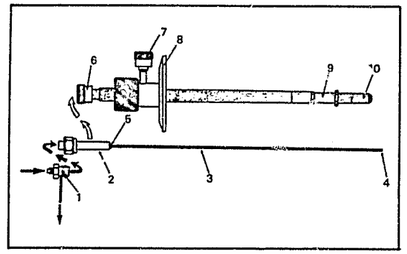

Figure 1. Schematic drawing of the DLI interface: (1) modified Swagelok® reducing union for stream splitting; (2) brass cylinder holding the glass tube; (3) glass capillary tube (2 mm od×0.1 mm id); (4) glassblown restriction, or thin metallic wire; (5) epoxy resin seal; (6) compression fitting making the seal on part (2); (7) compression fitting attached to a roughing pump line; (8) flange making seal on a gate valve; (9) movable metallic probe shaft; (10) insulated tip (Vespel®).

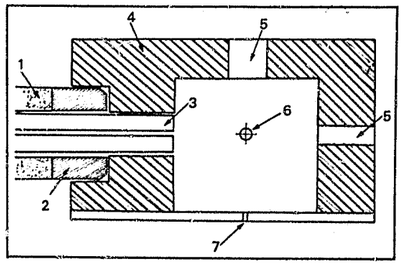

Figure 2. Schematic drawing of the MS source block with the DLI interface being connected: (1) movable metallic probe shaft; (2) insulated tip; (3) end of the capillary glass tube; (4) MS source block; (5) vents; (6) electron beam for primary ionization of solution vapors; (7) ion exit slit.

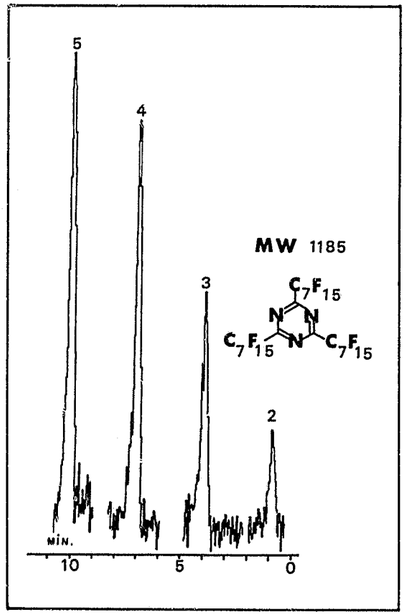

Figure 3. LC/MS detection of 2 to 5 micrograms of a triazine derivative injected onto a HPLC column. Solvent: acetonitrile at 1 ml/min. and an inlet pressure of 30 bars; column: 15 cm long×4 mm id filled with 5 μ C_{18} bonded silica; flow rate of solution introduced into the MS: 30 μl/min; electron energy: 100 eV. A total ion integrator synchronized with the MS scan was used for tracing the chromatogram; integrated mass range 800–1200 amu in 1.5 sec.

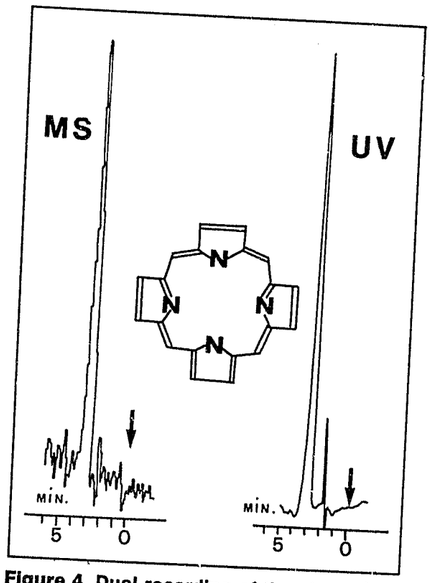

Figure 4. Dual recording of the signal obtained from a conventional UV detector, and from the LC/MS during the analysis of 1 μg of porphine. HPLC and MS conditions are the same as in Figure 3; integrated mass range 130–500 amu in 3 sec.

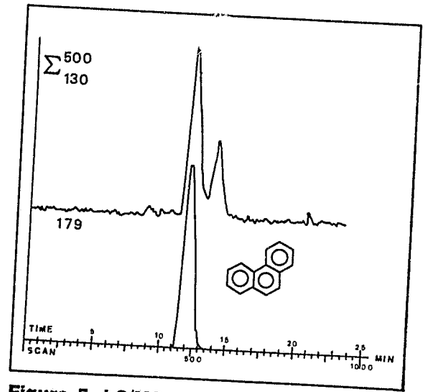

Figure 5. LC/MS analysis of 400 ng of a phenanthrene/chrysene mixture analyzed on a micro LC column directly coupled to the MS. Top trace: reconstructed LC chromatogram from ions in the mass range 130–500 amu. Bottom trace: mass chromatogram of m/e 179 (MH^+ of phenanthrene). Column: 43 cm long×1 mm id filled with 5μ C_{18} bonded silica. Solvent: acetonitrile at 30 μl/min. and an inlet pressure of 40 bars. Plate number for the phenanthrene peak: 1100. The data were processed by a Ribermag 400 data system; MS scan: 130–500 amu in 1.5 sec.

IV. RESULTS

The advantages of a DLI interface may appear more clearly after the presentation of some of the results obtained on the instrument assembled at the Ecole Polytechnique, which is shown in Photo No. 1. The HPLC pump is a gas pressurized coil, seen on the left end; the quadrupole is in the center, and the electronic controls are on the right. Photo No. 2 shows the cryotrap; it is a hollow hemi-cylinder which surrounds the CI source block of the mass spectrometer. Details of the DLI interface appear in Photo No. 3. The injector head of the HPLC is directly mounted on the column; the LKB detector monitors the major part of the eluent left after it exits the interface. Figure 1 is a schematic representation of the interface and of its holder; Figure 2 represents the cut section of the CI source block with the inter-

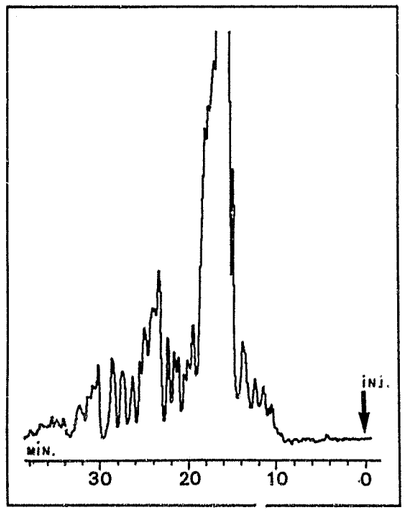

Figure 6. LC/MS analysis of 1 μl of lemon essential oil. Column: 14 cm long×4 mm id filled with 5μ C_{18} bonded silica. Solvent: acetonitrile at 0.5 ml/min. and an inlet pressure of 20 bars. Integrated mass range 100–700 in 3 sec. Other MS conditions are the same as in Figure 3.

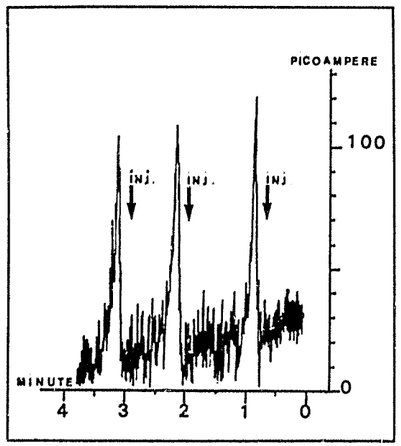

Figure 7. Single ion recording of m/e 203 (MH^+) during the introduction of 10 pg of fluoranthene through the DLI interface into the MS. No HPLC column was used. Dead volume between the injection point and the MS source block: 7 μl. Flow rate of acetonitrile: 33 μl/min. Retention time of the solute: 12.6 sec. Volume of the solution injected: 1 μl.

face in running position.

The LC/MS analysis of a high molecular weight substance is presented in Figure 3. Increasing amounts of a triazine derivative (from 2 to 5 μg) were injected on the HPLC column; the signal is the integrated ion currents of ions in the mass range m/e 800–1200. Figure 4 shows the dual parallel recording of the signal seen by the MS and by the UV detector, after injection of 1 μg of porphine.

It is possible to connect directly a micro LC column to the mass spectrometer, with the introduction of the total of the output solution into the mass spectrometer. Figure 5 shows an example of such a coupling for the analysis of a polyaromatic hydrocarbon mixture (phenanthrene and chrysene); only 1100 plates were measured for the phenanthrene peak, which is selectively displayed on the computer generated ion chromatogram for mass 179 ($M+H^+$).

Better results are obtained with a conventional large bore HPLC column, as shown in Figure 6. It is the total ion chromatogram for ions in the mass range m/e 120–500, after injection of 1 μl of lemon oil on a C-18 reverse phase HPLC column.

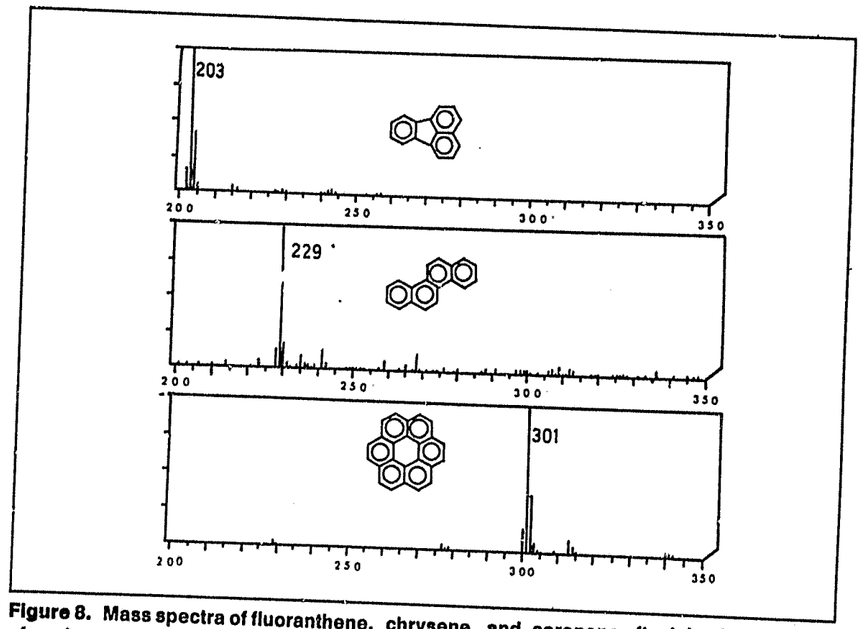

Figure 8. Mass spectra of fluoranthene, chrysene, and coronene after injection of 100 ng of each sample. LC conditions are the same as in Figure 7. Data were acquired by a Ribermag 400 data system. Mass range 150–500 amu in 1.5 sec.

The sensitivity of the method is illustrated in Figure 7: 10 pg of fluoranthene is introduced into the CI source block, while recording the output signal of the quasimolecular ion ($M+H^+$=203). The dynamic range of detection was shown to be 10^6 for this analysis.

Further work is needed to obtain the higher limit of volatility a solute should present to be analyzed by LC/MS with a DLI interface. Selected test substances should be run by the different research groups who use similar machines. Polyaromatic hydrocarbons, with increasing number of aromatic rings, or the series of the oligomers of polystyrene polymer could serve as valuable test probes. As an example, we have demonstrated that benzene, naphthalene, phenanthrene, and chrysene are transferred without significant peak broadening. However, coronene and pentacene are partly retained due to adsorption of the solute on the glass tube of our DLI interface (see Figures 8 and 9). This arises from the fact

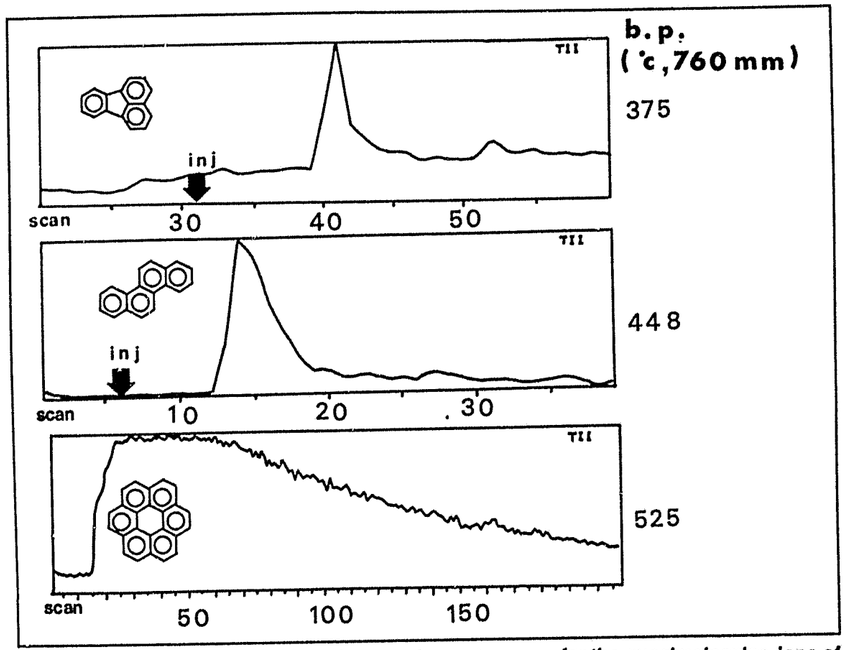

Figure 9. Computer reconstructed mass chromatograms for the quasimolecular ions of the aromatic hydrocarbons during the analysis in Figure 8. Fluoranthene and chrysene peaks are eluted from the DLI interface without peak broadening, whereas coronene is strongly adsorbed on the glass of the interface.

that the solvent evaporates inside the glass tube before the solution reaches the CI source block. We are now working on a rather different system, compared to the model shown in Figures 1 and 2, which keeps the DLI concept for a more efficient transfer of the liquid solution into the CI source block.

CONCLUSION

The direct introduction of liquid solution inside the CI source block of a mass spectrometer appears to be a simple and reliable method to achieve on-line LC/MS coupling. The sensitivity of the detection and the range of solutes amenable to the device make the mass spectrometer the most sensitive and universal HPLC detector, although it is a costly and complex detector. Among the results, which we assume now to be well-established, there is the absence of problems posed by the routine introduction of liquid solvents into the vacuum of the MS. No long term degradation effects have been observed, and routine LC/MS operations are possible on a general multi-purpose instrument. As most of the problems around the DLI interface have been solved, we may now concentrate our efforts on the interface, itself, to find the design with optimal performance.

ACKNOWLEDGMENTS

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REFERENCES

1. Bollet, C., *Analusis*, 5, 157 (1977).
2. Arpino, P. J., *La Recherche*, 6, 769 (1975).
3. Dawkins, B. G. and McLafferty, F. W., in "GLC and HPLC Analysis of Drugs. Vol. 1" (K. Tsuji and W. Morozowich, eds.), Marcel Dekker, New York, N.Y., 1977.
4. Scott, R. P. W., in "Journal of Chromatography Library Vol. 11. Liquid Chromatography Detectors" p. 29, Elsevier Scientific Publishing Co., Amsterdam and New York, 1977.
5. Kenndler and Schmid, E. R., in "Instrumentation for High Performance Liquid Chromatography" (J.F.K. Huber, ed.), Elsevier Scientific Publishing Co., Amsterdam and New York, 1978.
6. Elbert, S., Gruhn, B., Wipfelder, E., and Heusinger, H., *Anal. Chem.*, 48, 1270 (1976).
7. Majors, R. E., and Wilson, B., Greewood, H., and Snedden, W., *Biochemical Society Transactions*, p. 867 (1975).
8. Huber, J. F. K., Urk-Schoen, A. M. van, and Sieswerda, Z., *Anal. Chem.* 264, 257 (1973).
9. Schulten, H. R. and Beckey, H. D., *J. Chromatogr.*, 83, 315 (1973).
10. Schulten, H. R., Lehman, W. D., and Haaks, C., presented at the 26th ASMS meeting, paper No. TA-9, St. Louis, May 28–June 2, 1978.
11. Holland, J. F., Soltmann, B., and Sweeley, C. C., *Biomed. Mass Spectrom.*, 3, 340 (1976).
12. Soltmann, B., Sweeley, C. C., and Holland, J. F., *Anal. Chem.*, 49, 1164 (1977).
13. Hunt, D. F., Shabanowitz, J., Botz, F. K., and Brent, D. A., *Anal. Chem.*, 49, 1160 (1977).
14. Baldwin, M. A. and McLafferty, F. W., *Org. Mass Spectrom.*, 7, 1353 (1973).
15. Beuhler, R. J., Flanigan, E., Greene, L. J., and Friedman, L., *Biochem.*, 13, 5060 (1974).
16. Buehler, R. J., Flanigan, E., Greene, L. J., and Friedman, L., *J. Amer. Chem. Soc.*, 96, 3990 (1974).

17. Beuhler, R. J., Flanigan, E., Greene, L. J., and Friedman, L., *Biochem. Biophys. Res. Commun.*, 46, 1082 (1972).

18. Mumma, R. O. and Vastola, F. J., *Org. Mass Spectrom.*, 6, 1373 (1972).

19. Bingham, R. A., *Anal. Chem.* 48, 1735 (1976).

20. Schulten, H. R. and Beckey, H. D., *Anal. Chem.*, 45, 2358 (1973).

21. Torgerson, D. F., Skowronski, R. P., and McFarlane, R. D., *Biochem. Biophys. Res. Commun.*, 60, 616 (1974).

22. McFarlane, R. D. and Torgerson, D. F., *Science*, 191, 920 (1976).

23. McFarlane, R. D. and Torgerson, D. F., *Int. J. of Mass Spectrom. and Ion Physics*, 21, 81 (1976).

24. Krueger, F. R., *Pharmazeut, Zeitung*, 121, 190 (1976).

25. Krueger, F. R., *Chromatographia*, 10 151 (1977).

26. Colby, B. N. and Evans, C. A., Jr., *Anal. Chem.*, 45, 1884 (1973).

27. Simons, D. S., Colby, B. N., and Evans, C. A., Jr., *Int. J. of Mass Spectrom. and Ion Physics*, 15, 291, (1974).

28. Stimpson, B. P. and Evans, C. A., Jr., *Biomed. Mass Spectrom.*, 5, 52 (1978).

29. Lovins, R. E., Craig, J., Thomas, F., and McKinney, C. R., *Anal. Biochem.*, 47, 539 (1972).

30. Lovins, R. E., Ellis, S. R., Tolbert, G. D., and McKinney, C. R., *Anal. Chem.*, 45, 1553 (1973).

31. Kraak, J. C., Poppe, H., and Smedes, F., *J. Chromatogr.*, 122, 147 (1976).

32. Kirkland, J. J., Yau, W. W., Stoklosa, H. J., and Dilks, C. H., Jr., *J. Chromatogr. Sci.*, 15, 303 (1977).

33. Martin, M., Eon, C., and Guiochon, G., *J. Chromatogr.*, 108, 229 (1975).

34. Sternberg, J. C., in "Advances in Chromatography, Vol. 2" (J. C. Giddings and R. A. Keller, eds.), Marcel Dekker, New York, p. 205 (1966)

35. Horning, E. C., Horning, M. G., Carroll, D. I., Dzidic, I., and Stillwell, R. N., *Anal. Chem.*, 45, 936 (1973).

36. Carroll, D. I., Dzidic, I., Stillwell, R. N., Horning, M. G., and Horning, E. C., *Anal. Chem.*, 46, 706 (1974).

37. Horning, E. C., Carroll, D. I., Dzidic, I., Heagele, K. D., Horning, M. G., and Stillwell, R. N., *J. Chromatogr. Sci.*, 12, 725 (1974).

38. Horning, E. C., Carroll, D. I., Dzidic, I., Haegele, K. D., Horning, M. G., and Stillwell, R. N., *J. Chromatogr.*, 99, 13 (1974).

39. Carroll, D. I., Dzidic, I., Stillwell, R. N., Haegele, K. D., and Horning, E. C., *Anal. Chem.*, 47, 2369 (1975).

40. Horning, E. C., Carroll, D. I., Dzidic, I., and Stillwell, R. N., *Pure and Applied Chemistry*, 50, 113 (1978).

41. Horning, E. C., Carroll, D. I., Dzidic, I., Haegele, K. D., Shen-Nan, Lin, Oertli, C. J., and Stillwell, R. N., *Clin. Chem.* 23, 13 (1977).

42. Scott, R. P. W., Scott, C. G., Munroe, M., and Hess, J., Jr., *J. Chromatogr.*, 99, 395 (1974).

43. Scott, R. P. W., Scott, C. G., Munroe, M., and Hess, J., Jr., in "The Poisoned Patient, the Role of the Laboratory" CIBA Foundation Symposium 26, Publ. Associated Scientific Publishers, p. 155, Amsterdam, 1974.

44a. McFadden, W. H., U.S. Patent 4,055,987, Nov. 1, 1977, App. 664,058, March 4, 1976, 4 pp.

b. McFadden, W. H., Schwartz, H. L., and Evans, S., *J. Chromatogr.*, 122, 389 (1976).

45. Dark, W. A., McFadden, W. H., and Bradford, D. L., *J. Chromatogr. Sci.*, 15, 454 (1977).

46. McFadden, W. H., Bradford, D. L., Eglinton, G., Hajibrahim, S., Dark, W. A., and Nicholaides, N., presented at the 26th ASMS meeting, paper No. RE-3, St. Louis, May 28–June 2, 1978.

47. McFadden, W. H., Bradford, D. L., Games, D. E., and Gower, J. L., *Amer. Lab.*, 9, 55 (1977).

48. Baldwin, M. A. and McLafferty, F. W., *Org. Mass Spectrom.*, 7, 1111 (1973).

49. Arpino, P. J., Baldwin, M. A., and McLafferty, F. W., *Biomed. Mass Spectrom.*, 1, 80 (1974).

50. Arpino, P. J., Dawkins, B. G., and McLafferty, F. W., *J. Chromatogr. Sci.*, 12, 574 (1974).

51. McLafferty, F. W., Knutti, R., Venkataraghavan, R., Arpino, P. J., and Dawkins, B. G., *Anal. Chem.*, 47, 1503 (1975).

52. Arpino, P. J., and McLafferty, F. W., in "Determination of Organic Structures by Physical Methods, Vol. 6" (F. Nachod, J. J. Zuckerman, and E. W. Randall, eds.), Academic Press, New York, p. 1 (1975).

53. Dawkins, B. G., Arpino, P. J., and McLafferty, F. W., *Biomed. Mass Spectrom.*, 5, 1 (1978).

54. Lory, E. R., Dawkins, B. G., Arpino, P. J., Hoffman, P., and McLafferty, F. W., presented at the 26th ASMS meeting, paper No. RE-4, St. Louis, MO, May 28–June 2 (1978).

55. Henion, J. D., presented at the 26th ASMS meeting, paper No. RE-2, St. Louis, MO, May 28–June 2, 1978.

56. Henion, J. D., in "Advances in Mass Spectrometry, Vol 7." (N. R. Daly, ed.), p. 885, Heyden & Son, London (1978).

57. Serum, J. W. and Melera, A., presented at the 26th ASMS meeting, paper No. RP-2, St. Louis, MO, May 28–June 2, 1978.

58. Arpino, P. J. and Krien, P., presented at the 26th ASMS meeting, paper No. RE-6, St. Louis, MO, May 28–June 2, 1978.

59. Hands, B. A., *Vacuum*, 26, 11 (1975).

60. Davey, G., *Vacuum*, 26, 17 (1975).

61. Hatch, F. and Munson, B., *Anal. Chem.*, 49, 169 (1977).

62. Hatch, F. and Munson, B., *Anal. Chem.*, 49, 731 (1977).

63. Colin, H. and Guiochon, G., *J. Chromatogr.*, 141, 289 (1977).

64. Colin, H. and Guiochon, G., *J. Chromatogr.*, to be published in "Advances in Chromatography 1978"

65. Colin, H., Ward, N., and Guiochon, G., *J. Chromatogr.*, 149, 169 (1978).

66. Arpino, P. J., Colin, H., and Guichon, G., Proceedings of the 25th ASMS meeting, Washington, DC, May 29–June 3 (1977), p. 189.

67. Hunt, D. F., McEwen, C. N., and Harvey, T. M., *Anal. Chem.*, 47, 1730 (1975).

68. Melera, A., private communication.

69. McAdams, M. J., Blackley, C. R., and Vestal, M. L., presented at the 26th ASMS meeting, paper No. RE-1, St. Louis, MO, May 28–June 2, 1978.

70. Udseth, H. R., Orth, R. G., and Futrell, J. H., presented at the 26th ASMS meeting, paper No. RP-4, St. Louis, MO, May 28–June 2, 1978.

71. Takeuchi, T., Hirata, Y., and Okumura, Y., *Anal. Chem.*, 50, 659 (1978).

72. Giddings, J. C., Myers, M. N., and Wahrhaftig, A. L., *Int, J. Mass Spectrom. and Ion Physics*, 4, 9 (1970).

73. Dole, M., Mack, L. L., Hines, R. L., Mobley, R. C., Ferguson, L. D., and Alice, M. B., *J. Chemical Physics*, 49, 2240 (1968).

74. Ishii, D., Hibi, K., Asai, K., and Nagaya, M., *J. Chromatogr.*, 152, 341 (1978).

75. Ishii, D., Asai, K., Hibi, K., Jonokuchi, T., and Nagaya, M., *J. Chromatogr.*, 144, 157 (1977).

76. Tsuda, T., and Novotny, M., *Anal. Chem.*, 50, 271 (1978).

77. Knox, J. H., Laird, G. R., and Raven, P. A., *J. Chromatogr.*, 122, 129 (1976).

78. Jones, P. R. and Yang, S. K., *Anal. Chem.*, 47, 1000 (1975).

79. Tal'Rose, V. L., Skurat, V. E., Gorodetskii, I. G., and Zolotoi, N. B., *Russ. J. Phys. Chem.*, 46, 456 (1972).

80. Tal'Rose, V. L., Grishin, V. D., Skurat, V. E., and Tantsyrev, G. D., in "Recent Developments in Mass Spectrometry" (K. Ogata and T. Hayakawa, eds.), University Park Press, Baltimore, MD, USA, p. 1218 (1970).

81. Karasek, F. W. and Denney, D. W., *Analyt. Letters*, 6, 993 (1973).

82. Tal'Rose, V. L., Gorodetskii, I. G., Zolotoi, N. B., Karpov, G. V., Skurat, V. E., and Maslennikova, V. Ya., in "Advances in Mass Spectrometry Vol. 7" (N. R. Daly, ed.), p. 858, Heyden & Son, London (1978).

A New Approach to the Optimization of Chromatographic Systems and the Use of a Generally Accessible Data Bank in Systematic Toxicological Analysis

by

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I. INTRODUCTION

Systematic toxicological analysis, *i.e.* the undirected search for a potentially harmful substance whose presence is unsuspected, represents a most difficult analytical problem. This applies to both forensic and clinical toxicology, regardless whether the sample is a biological fluid or tissue, a drug formulation, or some stuff traded in the streets. To a large extent this is due to the almost infinite number of drugs that have to be taken into account, either as single substances or as mixtures.

During recent years, chromatographic techniques, such as thin-layer chromatography (TLC), gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC), have gained general acceptance as basic tools for toxicological analysis. At first sight, when looking into the literature one may get the impression that a large number of systems is already available. Yet, most of these systems were developed for a limited number of substance classes and/or individual components. However, in order to be suitable for systematic toxicological analysis (STA), it is necessary that chromatographic systems be evaluated with a large number of substances, representing a variety of classes. Moreover, as obviously no single separation system will be adequate, combinations of systems have to be tested. The latter may comprise two or more systems based on the same technique (*e.g.* two TLC-systems), or based on different techniques (TLC and GLC; GLC and HPLC). Evaluation of separation systems combined with other analytical methods such as spectrophotometry, mass spectrometry, and color reactions may also be envisaged.

Despite their crucial importance for STA, these aspects do not seem to have received much attention. This may be due to the large amount of work that is necessary for this type of research and/or the lack of suitable criteria for adequate evaluation and optimization. In recent years, major contributions came from three groups: Moffat *et al.* (1–6) classified PC-, TLC- and GLC-systems on the basis of their discriminating power (DP); Massart *et al.* (7–10) did the same for TLC-systems, using the concept of information content (IC), and Müller *et al.* (11) evaluated TLC-systems using separation quotients (q_s), in combination with color reactions on the plate (12).

All three approaches are system-directed, in that they provide information on the efficacy of single systems and combinations of systems. Though quite helpful in selecting proper systems, they give little or no information on individual substances, for example if substances *a* and *b* can be separated in system *z* or if substance *c* can be unequivocally identified in the presence of substances *d*, *e*, *f*, *g* . . . , etc. Therefore, we have developed a new approach which is both system- *and* substance-directed. Utilizing the concept of identification power (IP), systems can be evaluated and substances can be identified by means of their chromatographic retention behavior, provided adequate consideration is given to reproducibility and spot or peak size.

This paper reports the first results obtained with this concept. It has been applied to a selection of ninety basic drugs which were chromatographed in eight TLC-systems. Four of them were "normal" systems in which the drugs migrated as bases; the other four represented ion pair adsorption systems in which the

drugs migrated as ion pairs. The latter was done to get an insight into the efficacy in STA of the newly developed ion pair adsorption systems (13) as compared to that of the normal systems.

II. THIN LAYER CHROMATOGRAPHY

A. Choice of TLC-Systems

It is well known that identification of a drug by using a combination of chromatographic systems can best be achieved if the Rf-values for compounds in one system are independent of their values in another system, *i.e.*, the Rf-values should have low inter-system correlation (2, 14). This may be achieved by selecting widely different developing solvents, or by applying a different physico-chemical separation principle. An example of the latter can be found in the TLC of barbiturates. When using a neutral solvent, *e.g.* chloroform-ether (75+25), barbiturates migrate as undissociated acids, but with an alkaline solvent such as chloroform-isopropanol-conc. ammonia (45+45+10) the substances migrate as anions (15). Unfortunately, this principle cannot be applied to basic drugs. With alkaline or neutral solvents they migrate as undissociated bases, but if an acidic solvent is used, the resulting protonated base BH^+ usually becomes too polar to migrate on silica gel. Yet, we recently introduced simple TLC-systems in which basic drugs are converted to ion pairs according to the equations

$$B + H^+ \rightleftharpoons BH^+$$
$$BH^+ + X^- \rightleftharpoons BHX$$

in which X^- represents a suitable, negatively charged counter ion, usually bromide or perchlorate (13). As the basic drugs now migrate as ion pairs we were interested to see if such systems would be of advantage for STA.

In order to be able to properly study the effect of the ion pair formation on the chromatographic retention we tried to select our systems in such a way that, preferably, the ion pair TLC-system differed in only one aspect (the presence of the counter ion) from a corresponding, normal system. A second factor in selecting our systems was that we wanted to achieve optimum reproducibility for reasons that will be explained later. As we had observed that the presence of basic components such as NaOH, KOH, ammonia, and diethylamine usually lowered reproducibility, without being necessary to let basic drugs migrate as bases (13), we tried to circumvent the use of these basifying components, which could be realized in three of the normal systems. This resulted in the selection of the eight solvent systems listed in Table I. All further chromatographic conditions were as described previously (13).

B. Substances

Ninety basic drugs were investigated. They all belonged to the selection of 100 substances evaluated by Moffat *et al.* (2, 4, 5), the remaining 10 substances not being available at the time. The bases were each dissolved in methanol to give solutions containing about 1 mg/ml, of which 2–5 μl were spotted. After development, drugs were localized under UV light of 254 nm and by spraying with acidified iodoplatinate spray.

C. Reproducibility of the TLC-Systems

In order to be able to apply the concept of IP, it is necessary to know for each system the standard deviation of its Rf-values. This was assessed by measuring the Rf-values of 10 substances in six repetitive experiments on six different days. The 10 compounds were selected so that they were evenly spread across the plate in each system: benzocaine, bromodiphenhydramine, dimethoxanate, mepyramine nikethamide, nortriptyline, pethidine, pipamazine, promazine, and strychnine. It could be shown that the variance in Rf-values was essentially constant across the plate..

D. Correction of Rf-values

In order to correct for systematic changes (*e.g.*, geometry of the chamber, relative humidity, temperature, quality of sorbent and solvent) and/or to allow inter-laboratory comparisons, we corrected Rf-values according to Galanos and Kapoulas (16), using defined substances as reference compounds to convert observed Rf-values to corrected Rf-values (Rf^c-values). It has been shown that this approach results in a remarkable increase in inter-laboratory reproducibility with both single- and multicomponent solvent systems (17, 18). The original method uses two reference substances t and u for which corrected

TABLE I

Normal TLC-systems and ion pair adsorption TLC-systems for basic drugs as used in the study*

Normal systems	*Ion pair adsorption systems*
System 1 **Solvent: Methanol** **Unsaturated chamber**	***System 2*** **Solvent: Methanol, 0.1 *M* in NaBr** **Unsaturated chamber**
System 3 **Solvent: Methanol-Butanol (60+40)** **Unsaturated chamber**	***System 4*** **Solvent: Methanol-Butanol (60+40), 0.1 *M* in NaBr** **Unsaturated chamber**
System 5 **Solvent: Chloroform-Methanol (90+10)** **Saturated chamber**	***System 6***** **Solvent: Chloroform-Methanol (90+10), saturated with NaBr** **Saturated chamber**
System 7 **Solvent: Ethyl acetate-Cyclohexane-Methanol-conc. NH_4OH (70+15+10+5)** **Saturated chamber**	***System 8***** **Solvent: Ethyl acetate-Cyclohexane-Methanol (70+15+15), saturated with NaBr** **Saturated chamber**

** All plates were Silica gel 60 F 254 (E. Merck, Darmstadt, G.F.R.)*
*** These solvent systems do not allow dissolution of 0.1 M NaBr so that plates had to be impregnated with a 0.1 M solution of NaBr in methanol prior to development.*

values have already been established. The substances t and u are then run together with the substance to be determined, p, and the following corrections are applied:

$$(Rf^c)_p = a\,Rf_p + b$$

$$a = \frac{(Rf^c)_t - (Rf^c)_u}{Rf_t - Rf_u}$$

$$b = (Rf^c)_t - a\,Rf_t$$

It should be observed that this method works best if $Rf_t < Rf_p < Rf_u$ and if the differences between Rf_t, Rf_p, and Rf_u are small. Therefore, in our investigation where we are dealing with a large number of substances, we have increased the number of reference compounds to 3 and have also used two fixed reference points, namely the starting point S with Rf-value 0 and the position of the solvent front F after developments with Rf-value 1. The correction method then works as follows:

$0 < Rf_p < Rf_t$: use references S and t
$Rf_t < Rf_p < Rf_u$: use references t and u
$Rf_u < Rf_p < Rf_v$: use references u and v
$Rf_v < Rf_p < 1$: use references v and F

For reasons of simplicity we arbitrarily selected codeine (t), nikethamide (u), and benzocaine (v) as reference substances for all systems. Their respective Rf^c-values were determined by averaging Rf-values of 9 repetitive measurements.

III. THE CONCEPT OF IDENTIFICATION POWER

A. General

The IP of an analytical methodology is the number of substances that can be unequivocally identified out of a large population of compounds. The methodology may be based on a single parameter obtained in one system, or on a set of parameters obtained in more than one system or with more than one technique.

In this work we have evaluated 8 TLC-systems. In order to be able to adequately handle the large amount of data, we developed a computer program TOXIP (19), written in Pascal, and made use of the CYBER 74-18 computer at the University Computer Center in Groningen. Pascal has the advantages over Fortran that it allows more compact statements, that more efficient use is made of memory capacity, and that it is faster. In the following sections the mathematical-statistical approach to IP is given for these TLC-investigations, but the principles are equally well applicable to other systems and techniques.

B. Mathematical Approach

Given are N different TLC-systems j, $j \in \{1, \ldots, N\}$ and M different substances i, $i \in \{1, \ldots, M\}$. For each system j, a number of Rf-observations were carried out for each substance i in order to calculate Rf^c-values and mean Rf^c-values.

If we are to identify an unknown component belonging to the population M, we can analyze this substance in k of the N systems and we want to know how good that set of k systems $\{j_1, \ldots, j_k\}$ will be for this purpose, *i.e.*, which system or set will have the highest IP.

If Rf^c-values have been determined for M substances in N systems and if $(x_{ij})_1, \ldots (x_{ij})_n$ represent the outcomes of the n independent determinations, the mean Rf^c-value a_{ij} of substance i in system j will be

$$a_{ij} = \frac{1}{n}\sum_{k=1}^{n} x_{ijk}$$

This will result in a total of M×N mean Rf^c-values, representing the matrix $\{a_{ij}\}_{i=1,\ldots,M;j=1,\ldots,N}$.

C. Definitions

It will be clear that in order to distinguish between two substances p and q in a system j, there has to be a minimum distance between p and q, the discriminating distance d_j. This leads to the following definitions:

Definition 1. Substances p and q are discernible in system j if and only if:

$$|a_{pj} - a_{qj}| > d_j$$

(For $p,q \in \{1, \ldots, M\}, j \in \{1, \ldots, N\}$)

Definition 2. Substances p and q are discernible in a set of systems $\{j_1, \ldots, j_k\}$ if and only if p and q are discernible in at least one system j of this set.

Definition 3. Substance p can be identified in set $\{j_1, \ldots, j_k\}$ if and only if p is discernible from all other substances $p \neq q$ in this set.

Definition 4. The identification power (IP) of set $\{j_1, \ldots, j_k\}$ is the number of substances p ($p \in \{1, \ldots, M\}$) that can be identified in that set. From Definitions 1 and 2, it follows that p and q are discernible in set $\{j_1, \ldots, j_k\}$ if and only if there exists at least one system j ($j \in \{j_1, \ldots j_k\}$) in which $|a_{pj} - a_{qj}| > d_j$.

D. The Discriminating Distance d_j

The discriminating distance is dependent on the standard deviation of the system j and a statistical factor:

$$d_j = c_j \sigma_j$$

The standard deviation for each system was found as described in II.C., by using 10 substances in 6 repetitive experiments. The factor c_j is defined as:

$$c_j = 2\,t_\alpha = 2\,u_\alpha \sqrt{1 + \frac{1}{n}}$$

in which n is the number of observations and u_α the excentricity factor for a probability α. For 10 substances and 6 observations u has 10(6-1)=50 degrees of freedom and can be found in tables for t-distributions. For a probability of 95%, u=1.64; for a probability of 99%, u=2.33. The factor 2 has been introduced to take into account the sizes and shapes of the spots as well as the fact that sizes and shapes may change with the amount of substance present.

Thus, $|a_{pj} - a_{qj}| > d_j$ guarantees that the observation of a certain Rf^c-value is correctly appropriated to substance p and not to q if a choice has to be made between these two.

E. Computer Calculations

In these investigations, for N systems, M substances and $N \times M$ Rf^c-values $\{a_{ij}\}_{i=1,\ldots,M;j=1,\ldots,N}$, and for N discriminating distances $\{d_j\}_{j=1,\ldots,N}$ calculations were made for:

a. The IP's of the single systems $j=1, \ldots, N$
b. The IP's of combinations $\{j_1, \ldots, j_k\}$ of k systems among the N given systems, k being 2, 3, or 4.

These calculations were carried out for 95% and 99% probability, respectively, for comparison purposes. It will be obvious that in practice one should work with 99% probability.

IV. RESULTS AND DISCUSSION

Excellent ion pair adsorption chromatography could be obtained with bromide as counter ions, provided that the counter ion concentration applied to the systems was in the order of 0.1 M. With the more polar systems 1–4, this can be easily achieved by dissolving the corresponding amount of NaBr or KBr in

TABLE II

Rf-values x 100 for the 90 basic compounds in 8 TLC-systems.

Substance *System:*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
ACETOPHENAZINE	33	41	18	32	13	17	34	9
AMETAZOLE	5	41	4	42	0	0	8	11
AMETHOCAINE	39	46	30	39	25	17	66	22
AMITRIPTYLINE	23	50	17	51	21	31	73	31
AMPHETAMINE	11	71	8	75	5	5	43	45
ANTAZOLINE	7	67	4	66	4	14	47	23
ATROPINE	6	27	4	28	2	12	22	9
BENZOCAINE	82	83	87	87	56	58	71	95
BROMODIPHENHYDRAMINE	24	54	17	48	18	25	73	24
BUPHENINE	30	84	29	83	11	24	57	72
BUTACAINE	42	76	43	76	27	39	76	65
BUTETHAMINE	45	61	36	55	34	34	77	35
CAFFEINE	63	65	53	55	49	52	46	59
CARBETAPENTANE	18	58	12	49	12	33	12	23
CARBINOXAMINE	11	22	6	16	6	11	56	7
CHLORCYCLIZINE	35	50	28	52	33	35	71	48
CHLORDIAZEPOXIDE	78	79	73	77	43	47	43	80
CHLORPHENIRAMINE	10	23	5	21	5	18	58	10
CHLORPROMAZINE	21	45	15	45	18	32	72	39
CINCHONINE	19	55	13	61	13	41	45	52
CLEMIZOLE	69	67	73	73	56	52	76	9
COCAINE	29	43	22	30	29	16	76	14
CODEINE	20	26	13	22	16	18	31	10
CYCLIZINE	35	50	28	52	31	40	72	42
CYCLOPENTAMINE	6	67	4	68	12	1	39	28
DESIPRAMINE	7	69	0	71	10	34	44	54
DIAMORPHINE	26	29	17	33	23	33	52	23
DIAZEPAM	81	81	84	85	66	67	70	95
DIMETHOXANATE	21	43	14	38	15	20	71	23
DIPHENHYDRAMINE	25	52	17	50	21	28	72	30
DIPHENYLPYRALINE	18	44	12	48	18	38	68	36
EPHEDRINE	9	63	6	64	0	0	31	27
ETHOHEPTAZINE	12	41	8	41	11	29	59	21
ETHOPROPAZINE	24	60	17	55	16	37	79	43
FLUPHENAZINE	42	57	27	49	17	23	48	26
GUANETHIDINE	0	22	1	30	0	0	0	4
HYDROXYZINE	54	71	50	65	37	26	55	29
HYOSCINE	47	48	32	33	23	12	45	17
IMIPRAMINE	18	48	13	47	17	34	73	35
IPRONIAZINE	70	67	66	69	28	28	29	53
ISOCARBOXAZID	81	84	87	86	63	64	68	95
ISOTHIPENDYL	20	40	14	35	15	27	71	19
LEVALLORPHAN	35	74	32	73	15	34	72	58
LIGNOCAINE	68	73	69	69	58	37	74	39
LYSERGIDE	59	63	52	59	35	30	55	37
MECLOZINE	79	78	87	88	67	66	82	95
MEPIVACAINE	58	67	52	60	34	20	63	21

Substance *System:*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
MEPYRAMINE	15	40	9	33	8	20	68	15
METHADONE	14	62	10	60	11	31	78	37
METHAPYRILENE	12	32	7	24	9	19	52	10
METHAQUALONE	79	75	84	84	68	70	71	94
METHOTRIMEPRAZINE	28	53	20	49	25	39	77	38
METHYLAMPHETAMINE	9	63	6	63	9	22	44	32
MORPHINE	16	24	11	23	5	7	11	9
NAPHAZOLINE	4	54	3	52	3	2	32	13
NIALAMIDE	71	69	62	64	18	16	2	25
NICOTINE	36	33	24	22	22	11	61	6
NICOTINYLALCOHOL	70	70	70	69	25	24	32	50
NIKETHAMIDE	70	66	65	67	49	49	52	55
NITRAZEPAM	83	78	85	86	48	44	51	93
NORTRIPTYLINE	9	68	5	71	7	30	56	37
ORPHENADRINE	23	51	18	49	18	27	73	29
PAPAVERINE	72	72	75	74	64	58	62	72
PERPHENAZINE	36	48	23	40	17	21	42	20
PETHIDINE	27	42	19	40	21	32	80	28
PHENELZINE	29	53	18	82	9	0	72	96
PHENINDAMINE	38	55	30	49	29	33	74	35
PHENIRAMINE	14	34	10	26	9	20	60	11
PHENMETRAZINE	25	45	18	45	23	37	70	45
PHENYLPROPANOLAMINE	12	74	8	75	5	9	25	46
PHENIPAMIDOL	78	82	85	86	43	34	66	82
PIPAMAZINE	41	62	29	52	9	10	37	18
PIPERIDOLATE	53	62	47	52	47	32	78	31
PIPEROCAINE	21	60	18	56	20	38	74	30
PRAMOXINE	62	60	58	60	56	47	73	60
PROCAINE	31	52	22	42	12	14	64	17
PROCYCLIDINE	16	70	13	68	17	39	75	52
PROMAZINE	14	37	8	35	13	37	66	28
PROMETHAZINE	26	45	18	44	24	32	69	40
PROPIOMAZINE	36	64	27	52	32	38	72	50
PROTHIPENDYL	12	41	7	29	8	24	66	18
PYRROLBUTAMINE	22	69	18	66	24	45	74	52
QUININE	25	63	16	65	17	41	40	63
STRYCHNINE	7	10	4	11	10	40	33	14
THENYLDIAMINE	19	45	12	36	10	17	68	15
THIORIDAZINE	18	58	13	55	22	44	72	44
THONZYLAMINE	22	40	14	31	15	22	68	18
TRANYLCYPROMINE	34	69	26	67	18	17	59	45
TRIPELENNAMINE	16	41	9	34	7	18	69	15
YOHIMBINE	63	72	57	70	26	15	57	25

the developing solvent. The less polar systems 5 and 6 cannot dissolve 0.1 *M* halide salt and therefore the plates need to be impregnated by dipping in a 0.1 *M* solution of the halide salt in methanol prior to development. Moreover, the solvent must be saturated with the halide salt to prevent a wash-out of bromide during development. The systems 7 and 8, though capable of dissolving 0.1 *M* halide salt in the solvent, give rise to α,β and γ-fronts during development (20) resulting in an uneven distribution of counter ion across the plates. Impregnation of the sorbent and saturation of the solvent, as with systems 5 and 6, circumvent the latter problem.

When applying systems 5–8 in unsaturated chambers it was occasionally observed that the solvent fronts tended to sag somewhat, which was accompanied by an uneven migration of the substances as described by Geiss and Sandroni (21). As this affected reproducibility, it was decided to use saturated chambers for systems 5–8. The tanks were lined with filter paper and filled with solvent 1 hour prior to the introduction of the plate. Systems 1–4 could be used in unsaturated chambers with adequate reproducibility.

The application of the substances to the plate was done as bases, dissolved in methanol. It should be observed that they may equally well be applied as salt in methanol or as halide ion pair in methanol, regardless of the system, as this does not affect Rf-values. Of course, the diameter of the starting spot must be kept as small as possible which may be facilitated by selecting a relatively non-polar dissolution medium. In our case we selected methanol for simplicity and uniformity reasons.

Systems 1–2, 3–4, and 5–6 represent combinations differing only in the presence or absence of halide salts. Thus, differences in migration behavior may be attributed to the occurrence of ion pair phenomena. This is not entirely the case with systems 7–8. When we introduced halide salts and replaced the ammonia by water to obtain an ion pair system, the water caused solvent demixing and an uneven distribution of bromide. Therefore, water had to be omitted, which resulted in the composition of system 8. Migration differences obtained with system 8 as compared with system 7 may thus be due to ion pair phenomena and/or the absence of ammonia and water.

Table II gives the Rf-values for the 90 basic substances investigated. In Table III the standard deviations for the systems are listed, together with the resulting discriminating distances and identification powers for two confidence limits.

Decimal values for d_j, which can be expressed in millimeters or hRf-units, were rounded off to the lowest full unit. For example, if a d_j of 3.8 was calculated, the value 3 was introduced in the computer program. To fulfill $|a_{pj}-a_{qj}| \geq d_j$, a value of 4 will then be taken into account as a search window for the IP-calculations.

It is interesting to note the large differences in reproducibility between the systems (a factor of 3–4 going from system 1 to system 7). The simplest system, plain methanol, has the best reproducibility and the general rule seems to be that the reproducibility decreases with the number of components in the system, be it a second or a third solvent component or the addition of bromide. The deleterious effects on the reproducibility of ammonia as basifying agent is reflected by the standard deviation of system 7, being by far the largest of all systems investigated.

As can be expected, the large differences in reproducibility have a severe impact on the d_j-factors for the various systems and, consequently, on their IP's. For example, with a d_j of 2 for system 1, the computer will apply a search window of 2 hRf-units to see if that system can identify a substance p from the 89 remaining ones. Any substance within a range of 2 hRf-units from substance p will be considered to coincide. Yet, for system 7, with a d_j of 9, a search window of 9 hRf-units will be applied in two directions, thus covering 18% of the total separation distance.

Clearly, with a large selection of substances such as in the present investigation, the IP-values for single systems are of limited value. For 90 substances and a total separation distance of 10 cm, these IP-values will always be very close to zero as only a very limited number of substances will have a chance to migrate to areas in which no other substances are present. An example is given by system 3. From Tables II and III it can be seen that with a search window of 2, Butethamide (hRf=36), Butacaine (hRf=43), Piperidolate (hRf=47), and Nialamide (hRf=62) can be identified with a probability of 95%. Yet, if we would like to identify with 99% probability, a search window of 3 must be applied which means that Piperidolate can no longer be distinguished from Hydroxizine (hRf=50) and Nialamide no longer from Niketamide (hRf=65).

The value of the IP-concept becomes fully visible in the evaluation of combinations of TLC-systems. Table IV gives the IP's of the best combinations of any 2, 3, or 4 systems, whereas in Table V IP's of some less suitable combinations have been given, including the

TABLE III

Standard deviations (σ), discriminating distances (d_j) and identification power (IP) for 8 TLC systems.

System:	1	2	3	4	5	6	7	8
σ_j	0.61	1.40	0.75	0.98	0.89	1.61	2.0	1.38
For α=0.05								
d_j	2	5	2	3	3	6	7	5
IP	1	1	4	2	0	0	0	0
For α=0.01								
d_j	2	6	3	4	4	7	9	6
IP	1	1	2	2	0	0	0	0

As the developing distances were always 10 cm, σ-values and d_j values can be read in millimeters or in hRf-units

TABLE IV

IP's of optimum combinations of TLC-systems for two confidence limits.

	For α=0.01	For α=0.05
Best Two	1+4 IP=35	1+4 IP=40
Best Three	1+4+8 IP=56	3+4+7 IP=60
Best Four	1+2+7+8 IP=65	3+4+5+8 IP=72

TABLE V

IP's of some less suitable combinations of TLC-systems, for confidence limit α=0.01

Normal Combinations		*Ion Pair Combinations*		*Mixed Combinations*	
1+7	IP=26	4+8	IP=12	1+8	IP=28
1+5	IP=16	4+6	IP=11	3+4	IP=20
5+7*	IP= 7	2+6*	IP= 4	5+6*	IP= 2
1+3+7	IP=35	4+6+8	IP=21	1+2+7	IP=53
3+5+7*	IP=29	2+4+6*	IP=14	5+6+7*	IP=10
1+3+5+7	IP=47	2+4+6+8	IP=28	2+6+7+8*	IP=27

** Indicates worst combination in that category.*

worst combination in each category.

As can be seen, there are striking differences in IP-values, emphasizing the pitfalls of randomly combining systems without proper criteria. Even with two "established" systems, such as 5 and 7, only seven compounds can be reliably identified on the basis of the Rf-values, which can be attributed to a large extent to the low reproducibility of the systems involved. Yet, the combination of system 5 with the more reproducible system 1 increases the IP-value to 16. Furthermore, it should be noted that the combinations with the highest IP-values always include at least one ion pair system. Thus, the ion pair adsorption systems can be considered to be a very useful and effective alternative for STA and even better results may be expected by further optimizing the ion pair systems with regard to their reproducibility and separation selectivity. This has not been included in the present investigation, in which we simply added bromide to a suitable normal system. Yet, the resulting systems may not necessarily represent the best basis for ion pair adsorption systems.

The impact of the reproducibility on the IP's of combined systems is demonstrated in Table VI in which a number of relevant combinations are compared with regard to their correlation coefficients, identification power and the d_j's of the system used.

The systems 1+3, which are highly correlated as expected, still have an IP comparable to that of the systems 7+8 with a correlation coefficient of 0.283, while systems 4+7, with the lowest correlation coefficient of 0.146 have an IP of only 14. It is evident that for identification purposes correlation coefficients are of limited importance if the systems have bad reproducibility and, accordingly, large d_j-values. On the other hand, it will be clear that, besides having good reproducibility, the individual TLC-systems must provide a good spread of the substances across the plate.

From the above results it may be concluded that the concept of IP provides a very useful and relevant tool to evaluate and to optimally combine chromatographic systems. Not only does it show how optimum combinations can be achieved, it also indicates that the gain in information decreases again when using combinations of 3 or 4 systems. The results further indicate how dangerous it is to identify a substance on the basis of two or three retention data from randomly selected chromatographic systems, a procedure which is not uncommon in toxicology. With our best two systems, only 35 out of 90 substances can be identified correctly and it should be realized that this number will decrease if a larger population of basic drugs is taken into account.

Although this report deals with TLC-systems only, the IP-concept has almost universal applicability as other separation systems such as GLC, HPLC, and PC can be included as well. Moreover, the computer program also allows incorporation of other physico-chemical parameters, such as UV and IR absorption data, mass spectrometric data including molecular weight, melting and boiling points, color reactions, solubility and extraction behavior, etc., provided that for each system or technique adequate reproducibility data are available.

The present approach is not only suitable to evaluate and optimize systems. As it is substance-directed, it can also be used for the identification of unknown components. For this purpose one measures some relevant parameters as they become available in STA, such as TLC- and GLC-behavior, UV and mass spectrometry. These data are fed into the computer together with the reproducibility factor for each parameter and the computer then sorts out the drugs that fulfill these data. If more than one drug is listed, it will also be possible to ask the computer about the next most suitable technique to discriminate between the listed drugs. If that technique is not available or cannot be applied, the second best choice may be asked, etc. Obviously, the computer will be most useful in the more complicated and/or uncommon intoxications, but it should be emphasized that it can also assist in the seemingly easy cases with the more common drugs by checking if there are other possible drugs which fit a particular analytical behavior, and by providing suggestions how to discriminate against these other possibilities.

The concept of IP together with the TOXIP program thus seems to offer a number of important advantages for STA. Yet it will be clear that a lot of work still remains to be done. With regard to the evaluation and optimization, a great many systems and techniques will have to be tested with relevant selections of drugs. Then, after having selected the most suitable systems and combinations, data collections are to be made for these systems with as large a number of substances as possible. The latter should not only include drugs and other relevant poisons, but also metabolites, endogenous compounds, exogenous interferences such as plasticizers and antioxidants, etc. As it would be unrealistic as well as undesirable to try and carry out this work in a single laboratory, it is hoped that it can be undertaken as a joint effort between various cooperating institutions.

TABLE VI

Correlation coefficients (r) and IP's of combinations of two TLC-systems, in relation to their d_j's (α=0.01)

Combination		*IP*	d_j
1+3	0.985	10	2 and 3
1+8	0.613	28	2 and 6
1+4	0.582	35	2 and 4
7+8	0.283	9	9 and 6
4+7	0.146	14	4 and 9

V. SUMMARY

A new approach, based on the concept of Identification Power, is described to evaluate and to optimally combine chromatographic systems with regard to their applicability in systematic toxicological analysis. Contrary to earlier approaches, which are only system-directed, the present IP-approach is both system- and substance-directed, allowing substance identification by means of chromatographic retention data. Special considerations had to be given to system reproducibility and spot or peak size, whereas data handling and data retrieval were achieved by a special computer program TOXIP, written in Pascal.

The approach was tested in a study comprising 90 basic drugs which were chromatographed in four classical thin layer chromatographic systems and in four ion pair adsorption TLC-systems. Combinations of a normal system with an ion pair system proved to be best suitable for systematic toxicological analysis. Other systems based on paper-, gas-, and high-performance liquid chromatography can be evaluated and optimized in the same way.

A special feature of the present approach is that other physicochemical parameters such as UV- and IR-absorption data, mass spectral properties including molecular weight, color reactions, etc., can also be included, thus providing a data bank with suitable flexibility for the identification of unknown substances.

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REFERENCES

1. Moffat, A. C., Smalldon, K. W., and Brown, C., *J. Chromatogr.* 90, 1 (1974).
2. Moffat, A. C. and Smalldon, K. W., *J. Chromatogr.* 90, 9 (1974).
3. Moffat, A. C., Stead, A. H., and Smalldon, K. W., *J. Chromatogr.* 90, 19 (1974).
4. Moffat, A. C. and Clare, B., *J. Pharm. Pharmacol.* 26, 665 (1974).
5. Moffat, A. C., *J. Chromatogr.* 110, 341 (1975).
6. Moffat, A. C., *J. Chromatogr.* 113, 69 (1975).
7. Massart, D. L., *J. Chromatogr.* 79, 157 (1973).
8. Massart, D. L. and Smits, R., *Anal. Chem.* 46, 283 (1974).
9. Massart, D. L. and De Clercq, H., *Anal. Chem.* 46, 1988 (1974).
10. De Clercq, H. and Massart, D. L., *J. Chromatogr.* 115, 1 (1975).
11. Müller, R. K., Möckel, W., Wallenborn, H., Weihermüller, A., Weihermüller, C., and Lauermann, I., in "Beiträge zur Gerichtlichen Medizin" (W. Holczabek, Ed.), Vol. 34, p. 265. Verlag Franz Deuticke, Vienna, 1976.
12. Lauermann, I. and Müller, R. K., Proceedings, Europ. Meeting TIAFT, 1977 Leipzig, DDR, in press.
13. De Zeeuw, R. A., Van Mansvelt, F. J. W., and Greving, J. E., *J. Chromatogr.* 148, 255 (1978).
14. Connors, K. A., *Anal. Chem.* 46, 53 (1974).
15. De Zeeuw, R. A. and Feitsma, M. T., *Pharm. Weekblad* 101, 957 (1965).
16. Galanos, D. S. and Kapoulas, V. M., *J. Chromatogr.* 13, 128 (1964).
17. Dhont, J. H., Vinkenborg, C., Compaan, H., Ritter, F. J., Labadie, R. P., Verweij, A., and de Zeeuw, R. A., *J. Chromatogr.* 47, 376 (1970).
18. Dhont, J. H., Vinkenborg, C., Compaan, H., Ritter, F. J., Labadie, R. P., Verweij, A., and de Zeeuw, R. A., *J. Chromatogr.* 71, 283 (1972).
19. Schepers, P., to whom requests for further details should be directed.
20. De Zeeuw, R. A., in "Progress in Separation and Purification" (E. S. Perry and C. J. van Oss, Eds.), Vol. 3, p. 40. Academic Press, New York, N.Y., 1970.
21. Geiss, F. and Sandroni, S., *J. Chromatogr.* 33, 201 (1968).

Note added in the final version of the manuscript:
We recently discovered that some erroneous IP-values were given at the oral presentation of this paper in Washington, D.C., for which we apologize. The IP-values in this manuscript are correct to the best of our knowledge.

The Application of Derivatization Techniques in Forensic Drug Analysis

by

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A review of methodology that utilizes derivatization techniques in the analysis of illicit drugs and related substances is presented. Included in the review is a discussion of the interrelationship of the derivatization technique with determinative steps such as crystallography, chromatography, infrared and mass spectrometry. The various applications of these techniques include (a) the use of derivatizing reagents in the determination of the stereochemical composition of illicit drugs, (b) the utilization of derivatization reagents and their isotopic analogues, in the mass spectral characterization of trace quantities of synthetic by-products of illicit drugs, (c) the use of derivatizing reagents in the gas chromatographic quantitation of illicit drugs and their synthetic by-products, and (d) miscellaneous applications of derivatization techniques in forensic drug analysis. The forensic significance of the above applications is discussed in general terms.

The analysis of illicit drugs and related substances invariably involves a qualitative, and often, a quantitative determination. In most instances, these analyses may be accomplished without altering the elemental composition of the drug. In some cases, however, the modification of a drug's structure is desirable, and sometimes essential, to allow for its full characterization. For this purpose, the term "derivatization" is most often used in conjunction with techniques employed in the structural modification of drugs and other substances. Ideally, derivatization procedures should require a minimum number of manipulations and should proceed in rapid one-step reactions to completion. The derivatized drug species can then be characterized by a number of determinative steps, including crystallography, chromatography, infrared and mass spectrometry (MS).

One form of derivatization commonly used is in the presumptive testing of drugs and in the determination of their enantiomeric composition using microcrystallographic analysis. (1) In general, these procedures involve reaction of the drug with heavy metal salts and observing characteristic crystal formation using microscopic techniques. These procedures enjoy the advantages of using small sample sizes as well as speed of analysis. Derivatization techniques are also used to introduce fluorophores into drug structures to allow for their detection at trace levels using spectrofluorometric determinative steps. (2–5) Derivatization has also been used to render drugs and other substances suitable for analysis by thin-layer chromatography (TLC) (6) and high performance liquid chromatography (HPLC) (7).

Undoubtedly, derivatization procedures have been most widely associated with the analysis of drugs by gas chromatography (GC). These chemical derivatization procedures include techniques using gas chromatography-flame ionization detection (GC-FID), gas chromatography-electron capture detection (GC-ECD) and gas chromatography-mass spectrometry (GC-MS). In these chemical derivatization procedures, functional groups are introduced into the drug molecule in order to: (a) render those drugs and other substances, which possess poor chromatographic behavior, less polar and more volatile to allow for their GC quantitation and GC-MS identification; (b) enhance the electrophilic character of the drug to allow for its detection and quantitation at ppb levels using GC-ECD; (c) effect the GC resolution and quantitation of certain drug enantiomers; and (d) allow for the more facile mass spectral characterization of the drug species and related impurities, especially in conjunction with the use of deuterated analogues of the derivatizing reagent.

In chemical derivatization procedures using GC-FID, GC-ECD and GC-MS as determinative steps, the substance to be derivatized usually contains an active hydrogen atom. These active protons are found in functional groups such as RNH_2, R_1R_2NH, RCOOH, ROH, $RCONH_2$, RSH and R=NH. The exchange of these active protons with the appropriate substituent can be accomplished by procedures such as esterification, silylation, acylation, alkylation, perhalogenation, etc. Though some drugs that do not contain functional groups with active protons—such as tertiary amines—can be chemically derivatized, these procedures are not widely used because they involve multiple steps, are often time-consuming and sometimes result in less-than-desirable yields of the derivative. (8, 9)

A detailed discussion of chemical derivatization procedures used in conjunction with GC and GC-MS analyses is not necessary in the present paper, as they are adequately reviewed in the literature. Drozd (10) describes the derivatization of a wide variety of substances using a multiplicity of derivatizing reagents. Included in the article are over 600 references. Ahuja's (11) excellent review of the subject deals primarily with pharmaceutical preparations and includes over 200 references. A recent review by Nicholson (12) describes derivative formation in the GC quantitation of pharmaceuticals. This comprehensive review includes over 450 references. Lochmuller and Souter (13) review the GC analysis of enantiomers using derivatization procedures. Cimbura and Kofoed (14) describe derivatization techniques used in forensic toxicology. Pierce's text on the subject of derivatization deals primarily with silylation techniques. (15) McCloskey, *et al.* (16) report the use of deuterium-labeled trimethylsilyl derivatives in mass spectrometry.

Derivatization techniques are commonly employed in legitimate pharmaceutical analyses. This is due to the abundance of drugs not amenable to direct GC or GC-MS analysis. The analysis of drugs in clinical and toxicological situations routinely use derivatization procedures. In these analyses, trace quantities of drugs and their metabolites in biological materials are often derivatized to allow for their detection and quantitation using sensitive determinative steps such as spectrofluorometry, GC-ECD, GC-MS and mass fragmentography.

The application of derivatization techniques has enjoyed limited use in routine forensic drug analysis. This is because most controlled drug substances are amenable to direct quantitation by GC-FID and identification using GC-MS without prior derivatization; alternately, those drugs not suitable for analysis by GC-MS or GC-FID may be unequivocally identified using infrared spectrometry (IR) and quantitated by ultraviolet spectrometry (UV), HPLC, etc. Additionally, in most cases the sample size is usually sufficient (mg quantities) to allow for the ready identification of the drug using IR and GC-MS techniques and quantitation by UV, or, more often, by GC-FID; this is unlike toxicological examinations in which analyses frequently require derivatization of ng-μg quantities of the drug and/or its metabolite to render it suitable for identification and quantitation using sensitive GC-ECD and mass fragmentographic procedures. Finally, the time limitations imposed upon the forensic drug chemist discourage the use of the more time consuming derivatization procedures.

In the present paper we review derivatization procedures that have applicability in routine forensic drug analysis, as well as report on recent studies that have used derivatization techniques in forensic drug research analysis.

Though most illicit drugs can be successfully

zures for common source determination by detecting trace impurities in these samples. In this study, the samples were subjected to analysis by GC-FID and GC-ECD. Stromberg (52) has also conducted this type of comparison analysis on hashish samples. Holley, *et al.* (53) examined marihuana samples in geographic origin studies. Davis and co-workers (54) utilized GC and paper chromatography in determining the origin of cannabis. Lee and Kim (55) have investigated geographical differences of Korean opium based on the alkaloidal content. Van der Slooten and van der Helm (56) have analyzed illicit heroin samples in-depth for common source determination using GC-FID. Clark and Miller (57) have reported the forensic characterization of dyes in brown heroin samples as an aid in drug comparison studies. In this study, the dyes were characterized using LC techniques. Miller (58) has also studied the GC analysis of trifluoroacetyl derivatives of sugar diluents in illicit heroin preparations for sample comparison purposes. More recently, Clark and Cooper (59) have reported a GC derivatization procedure for the characterization of sugar diluents in illicit drug samples. In their procedure, the sugars are chromatographed as TMS derivatives.

In the procedures described above, none utilized derivatization techniques in the characterization of trace drug impurities associated with clandestine drug manufacture. In our laboratory, we have been concerned primarily with the characterization of manufacturing impurities in illicit cocaine and heroin. In these studies, the development of derivatization methodology was essential in order to: (a) allow for the GC and GC-MS analysis of certain drug impurities that normally exhibit poor chromatographic behavior (e.g., ecgonine and benzoylecgonine in cocaine and morphine and O^6-acetylmorphine in heroin); this derivatization was essential in order to detect and subsequently quantitate these and other impurities by GC-FID at levels as low as 0.2% (based on 50 mg heroin and 2 cc dilution); (b) detect and quantitate impurities in the 10^{-2}–10^{-5}% range (based on 10 mg heroin and 2 to 50 cc dilution); this necessitated the introduction of perhalogenated groups into the drug impurity to allow for subsequent GC-ECD analysis; (c) allow for the GC separation of numerous trace impurities that were not resolvable in underivatized samples; and (d) facilitate in the mass spectral characterization of new trace impurities in illicit drugs; the utilization of the deuterated analogues of certain derivatizing reagents is invaluable in these characterizations.

The selection of the appropriate derivatizing reagent in the characterization of trace drug impurities is critical. Though many and varied derivatizing reagents are available, we have utilized a select few in our studies. These include N,O-bis-(trimethylsilyl)-acetamide (BSA), its deuterated analogue N,O-bis-(trimethyl-d_9 silyl)-acetamide (BSA-d_9), heptafluorobutyric anhydride (HFBA), and acetic anhydride (Ac_2O) and its deuterated analogue, acetic anhydride-d_6 (Ac_2O-d_6).

BSA was selected as one derivatization reagent of choice because:

(a) Its well-recognized TMS-donating properties often allowed for complete derivatization of the drug impurity in usually less than 1 hour at temperatures of 70°C and below; in most instances the use of catalysts was not necessary;

(b) TMS derivatives of the impurities studied invariably exhibited good chromatographic behavior on columns of widely varying polarity; this was essential in the resolution of overlapping peaks and in providing reproducible and accurate quantitative results using

Parker, *et al.* (35) have reported on the derivatization of morphine, codeine, O^3-acetylmorphine and O^6-acetylmorphine followed by GC analysis. In this procedure, TMS derivatives were formed using BSA. Grooms (36) subjects heroin samples to derivatization with BSA for the GC-FID analysis of morphine and O^6-acetylmorphine. Rasmussen (37) quantitates morphine by means of GC with on-column silylation. Brugaard and Rasmussen (38) determine morphine and codeine by GC after on-column acylation. More recently, methodology has been reported for the GC-FID quantitation of morphine and codeine in opium as TMS derivatives. (39)

The analysis of hallucinogenic drugs can usually be accomplished readily without subjecting these drugs to derivatization. The direct GC analysis of LSD is difficult, however, unless subjected first to derivatization. Sperling (40, 41) describes the GC and TLC analyses of LSD as the TMS derivative after reaction with BSA. Radecka and Nigam (42) subject LSD to hydrogenation followed by GC-FID analysis.

The routine forensic analysis of cocaine may be accomplished without subjecting the sample to derivatization. Nonetheless, Hammer, *et al.* (43) describe an improved GC characterization of illicit cocaine by reacting it on-column with trimethylanilinium hydroxide. Recent controversy has arisen concerning the forensic chemist's ability to differentiate cocaine from its diastereoisomers as well as distinguish *d*- from *l*-cocaine. Though cocaine may be differentiated from its diastereoisomers by IR, the determination of its enantiomeric composition poses another problem. Unfortunately, cocaine is not amenable to enantiomeric analysis by derivatization and GC analysis. To resolve this isomer problem Allen and Cooper (44) describe a procedure in which cocaine is reacted with a heavy metal salt, namely gold chloride, and the resultant characteristic crystals examined microscopically. This procedure allows for the differentiation of *d*- and *l*-cocaine.

As previously mentioned, GC-FID, GC-ECD and GC-MS derivatization techniques have limited applicability in routine forensic drug analysis. Their greatest potential is unquestionably in the area of forensic drug research analysis. This research involves the in-depth analysis of illicit drug samples and includes identification and quantitation of active drug components, characterization of trace quantities of their manufacturing by-products and characterization of major and minor diluents as well as other adulterants. The in-depth characterization of illicit drug samples is of importance for forensic and intelligence purposes in that it may allow forensic chemists and law enforcement officials to: (1) compare various drug seizures for common source determination in drug conspiracy and related cases, as well as determine drug distribution routes; (b) determine, in some cases, the precursor chemicals used in the manufacture of the illicit drug; this is of importance in the subsequent monitoring of the distribution of these chemicals; (c) differentiate between illicit drugs manufactured clandestinely and those drugs produced legitimately but diverted from legitimate channels for illicit use; and (d) ascertain in general terms the geographical origin of the drug.

A number of studies have been reported on the in-depth analysis of illicit drug samples. Barron, *et al.* (45) and Kram (46–48) have characterized a number of impurities associated with the clandestine manufacture of illicit methamphetamine. Lomonte, *et al.* (49) have studied manufacturing by-products in illicit amphetamine samples. Bailey and co-workers (50) have investigated impurities associated with illicit methamphetamine. Stromberg (51) has compared various illicit amphetamine sei-

characterized without prior derivatization, there are some that require derivatization to allow for their more facile analysis. These drugs include phenethylamines, barbiturates, cannabis components, opium and coca alkaloids and hallucinogens.

The analysis of amphetamine and methamphetamine samples using various derivatization procedures has been reported. Since the biological activity of these compounds is dependent upon their enantiomeric composition, it is of forensic importance to be able to differentiate between their optical isomers. Wells (17, 18) describes a GC-FID procedure for the resolution of amphetamine enantiomers. In this procedure, a derivative is formed with N-trifluoroacetyl-(1)-prolyl chloride which allows for the GC resolution of *d*- and *l*-amphetamine and subsequent quantitation of these enantiomers. Beckett and Testa (19) have studied similar derivatives of phenylisopropylamines. Ment and Marion (20) differentiate the optical isomers of amphetamine by thermal analysis of their benzoyl derivatives. Choulis (21) resolves *d*- and *l*-amphetamine isomers on alumina, cellulose and silica gel thin-layers in the presence of optically active mandelic and tartaric acids. Eskes (1) describes the differentiation of amphetamine and methamphetamine isomers by derivatization with N-trifluoroacetyl-l-prolyl chloride followed by TLC analysis. Classically, the determination of the enantiomeric composition of amphetamine and methamphetamine has been done by their reaction with heavy metal salts and observing the characteristic crystal formation by means of high-power microscopy. (25) Clark (24) reports on an improved method for the GC-FID analysis of amphetamine. In this procedure, amphetamine is reacted with cyclohexanone to yield a Schiff-base derivative that exhibited good GC behavior.

The direct GC analysis of barbituric acid derivatives is sometimes difficult owing to their poor chromatographic behavior. Brochmann-Hanssen and Olawuyioke (25) report on the GC analysis of barbiturates by flash-heater methylation using trimethylanilinium hydroxide. Venturella, *et al.* (26) describe the use of dimethylformamide dimethylacetal in the derivatization of barbiturates for GC analysis. Hooper, *et al.* (27) assay phenobarbital by GC using on-column butylation. Street (28) describes the characterization of barbiturates and other drugs by the GC analysis of their trimethylsilyl (TMS) derivatives. Heagy (29) describes a rather novel infrared method for distinguishing optical isomers of amphetamine.

Though the major cannabinoid components in marihuana and hashish samples can be chromatographed directly, improved methodology has been reported for their GC analysis as a variety of derivatives. Knaus, *et al.* (30) have characterized cannabinoids in hashish by analyzing their t-butyldimethylsilyl, trimethylsilylacetate and diethylphosphate derivatives. Determinative steps used included HPLC, GC and GC-MS. Harvey and Paton (31) report the use of trimethylsilyl and other homologous derivatives in the analysis of certain cannabinoids by GC-MS. Turner, *et al.*, (32) describe the routine analysis of Cannabis sativa L. by the GC determination of its components as trimethylsilyl derivatives. Harvey (33) reports the GC and GC-MS characterization of cannabinoids as substituted silyl derivatives.

In the forensic analysis of opium constituents and related substances, the characterization of morphine and similar drugs is enhanced by derivatization. Nakamura and Noguchi (34) describe methodology for the GC-FID determination of morphine in opium by analysis of its di-TMS derivative after reaction with N, O-bis-(trimethylsilyl)-acetamide (BSA).

GC-FID; additionally, drug impurities that would not otherwise chromatograph were detected using GC-FID and eventually characterized;

(c) No "clean-up" was necessary prior to derivatization in most drug samples studied; this was an important factor when attempting to minimize sample analysis time; additionally; direct derivatization of highly adulterated samples obviated the use of liquid-liquid partition schemes to isolate the drug matrix from the diluents; this was important when attempting to minimize drug decomposition and to allow for more reproducible and accurate quantitative results necessary in drug conspiracy cases;

(d) No adverse effects were noted when BSA solutions were introduced directly into GC-FID and GC-MS (EI and CI) systems over a prolonged time period (note: BSA solutions do degrade the performance of GC-nitrogen-phosphorous (GC-NPD) and GC-ECD systems);

(e) The TMS derivatives we have studied were stable for several days; additionaliy, samples with relatively high moisture content were usually derivatized in a quantitative manner under mild reaction conditions;

(f) The relatively low volatility of BSA allowed for reaction conditions using μl volumes of BSA at elevated temperatures without significant loss of the reagent; this was an important factor when attempting to minimize dilution effects in the analysis of ultratrace quantities of drug impurities;

(g) The TMS derivatives of some of the drug impurities we have studied were amenable to GLC fraction collection techniques and subsequent solvent treatment with a minimum amount of degradation of the derivative; this was important when attempting to isolate trace drug quantities from bulk drug matrices; and

(h) The formation of TMS derivatives of drug impurities with BSA has proven invaluable in their mass spectral characterization; e.g., TMS derivatives of morphine-related alkaloids often yield characteristic fragmentation patterns; additionally, the availability of BSA-d_9 in pure form has provided considerable structural information which is usually only obtained through high resolution mass spectrometry (HRMS); these factors are especially critical when characterizing impurities at ultratrace levels, where supporting infrared and nuclear magnetic resonance spectroscopic data are often not available.

Though not as versatile as BSA, we have found HFBA invaluable as a derivatization reagent in selected cases. Some of the characteristics of HFBA, as well as HFB derivatives of drug impurities, are given below:

(a) The most obvious advantage of HFBA is its ability to readily introduce HFB group(s) into the drug impurity, which allows for its detection and subsequent quantitation at levels as low as 10^{-5}% (based on 10 mg of heroin and final dilution of 2 cc) using GC-ECD;

(b) Derivatization of certain heroin impurities with HFBA allows for their gas chromatographic resolution not attain-

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able as TMS derivatives (e.g., the chromatographic resolution of O^6- and O^3-acetyl-morphines);

(c) The HFB derivatives studied generally exhibited acceptable gas chromatographic behavior; however, on GC columns that were not well-conditioned, we have observed some interaction of the HFB-derivatized drug impurity and column substrate; these interactions appeared as pre- and post-peak inflections;

(d) Most of the heroin impurities we have investigated were derivatized rapidly and to completion by HFBA in an acetonitrile medium at room temperature;

(e) The HFB esters and amides studied did not hydrolyze readily in weakly basic aqueous media; this allowed for the rapid one-step extraction of the HFB derivative from a sodium bicarbonate-water-acetonitrile solution into an appropriate organic solvent (usually petroleum ether); this eliminated the necessity of removing the HFBA solvent via evaporation procedures; the obvious advantages were in the reduction of analysis time as well as eliminating undesirable side-effects associated with evaporation techniques (such as the application of heat, which may result in undesirable side-products); unlike HFB derivatives, other perhalogenated derivatives, such as trifluoroacetyl esters, apparently hydrolyze when using the extraction procedures mentioned above;

(f) The HFB derivatives of the heroin impurities studied were stable in petroleum ether during the course of a normal working day; however, they were not as stable as the corresponding TMS derivatives, in that hydrolysis was noted after 1–2 days;

(g) Unlike their corresponding TMS derivatives, it was found that in most cases, the mass spectra of HFB derivatives of heroin-related impurities yielded limited structural information; in general, the mass spectra of the HFB derivatives were not as abundant in structurally significant fragment ions as their TMS counterparts.

Acetic anhydride and its deuterated analogue have been used primarily in the characterization of acetylated impurities in illicit heroin. Its use has been limited primarily in the mass spectral analysis of these impurities and is often useful in supporting structural assignments made on the corresponding TMS derivatives. It has not been employed as often as BSA or HFBA because, in general:

(a) Reaction rates were not as rapid nor yields as favorable under mild reaction conditions;

(b) Acetic anhydride has a greater degradative action on GC column substrates when compared to BSA;

(c) The resultant acetyl derivatives did not exhibit significant sensitivity towards ECD detection; and

(d) The chromatographic behavior of acetyl derivatives of the impurities studied was not as good as the corresponding TMS derivatives.

As mentioned above, most of our work utilizing derivatization techniques has been in the characterization of impurities in illicit cocaine and heroin. Cocaine is a widely abused stimulant. It also has legitimate medicinal value as a topical anesthetic. The majority of illicit cocaine is believed produced from the South American coca plant by extraction of the alkaloid from the coca leaf, followed by a series of purification steps. On the other hand, pharmaceutical cocaine is produced by the double esterification of ecgonine. The total synthesis of cocaine can also be achieved as described by Willstatter. (60) Due to differences in manufacture, illicit cocaine contains some impurities not associated with pharmaceutical cocaine. Moore (61) has identified the presence of *cis*- and *trans*-cinnamoylcocaine in over 50% of illicit cocaine samples examined. The cinnamoylcocaines are natural components of the coca leaf and are found in illicit cocaine due to their co-extraction with cocaine from the leaf. In all samples examined to date, when cinnamoylcocaine was detected, it was present as both *cis*- and *trans*-isomers in roughly equal quantities. Most illicit cocaine samples also contain varying quantities of benzoylecgonine and ecgonine, both being acid hydrolysis products of cocaine. While a number of papers have been published on the analysis of ecgonine and benzoylecgonine in biofluids (62) using derivatization techniques, little work has been reported on their analysis in illicit cocaine samples until recently. In our studies, we have reported methodology for the detection of benzoylecgonine and ecgonine in illicit cocaine by the GC-FID analysis of their TMS derivatives. (63) Majlát and Bayer (64) separated benzoylecgonine and ecgonine in pharmaceutical cocaine by paper chromatography. Another impurity present in virtually all illicit cocaine is methylecgonine. (65) It is believed formed primarily as a result of the potassium permanganate oxidation of the cinnamoylcocaines during the cocaine manufacturing process. Methylecgonine can also be detected by GC-FID as a TMS derivative.

In our laboratory, we have developed GC-FID derivatization methodology which allows for the comparative analysis of illicit cocaine samples. Figure 1 illustrates the derivatization of impurities in cocaine samples with BSA and subsequent GC-FID analysis. For comparison purposes, a GC-FID derivatization profile of pharmaceutical cocaine is included in Figure 1. After an analysis of the derivatization method and chromatograms in Figure 1, the following observations can be made: (a) it is possible to make a rapid, yet in-depth, comparison of illicit cocaine seizures for common source determinations; Figure 1 clearly demonstrates that samples A, B and C were not derived from a common batch source; (b) the sample containing the cinnamoylcocaines was probably produced clandestinely from the coca plant; this is of forensic significance when attempting to differentiate between samples of naturally-occurring cocaine and samples containing cocaine produced synthetically; (c) though sample B does not contain detectable quantities of cinnamoylcocaines, the relatively high methylecgonine content would suggest that the cocaine was naturally-occurring rather than synthetically produced; (d) it is possible to relate a cocaine sample that has degraded to an undegraded sample from the original source by doing a total alkaloid analysis; this type of analysis is usually supported by additional comparative data; (e) in many cases, illicit cocaine samples can be distinguished from high-grade pharmaceutical cocaine; due to the manufacturing process used, pharmaceutical cocaine will not contain detectable quantities of methylecgonine or cinnamoylcocaines commonly found in illicit cocaine samples; additionally, the quantities of ecgonine and benzoylecgonine in pharmaceutical cocaine would expectedly be significantly lower than in its illicit counterpart.

The minimum quantities of coca alkaloids and related impurities that can be accurately

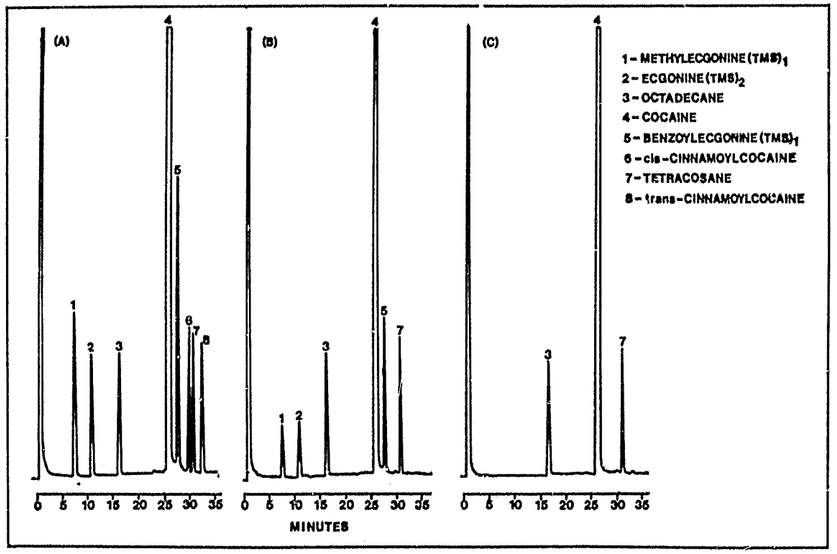


Figure 1. GC-FID Chromatograms of Uncut Cocaine Samples Subjected to Derivatization with BSA

Sample composition: (A) illicit cocaine containing: cocaine–86%, benzoylecgonine–5%, methylecgonine–3%, ecgonine–2%, *cis*-cinnamoylcocaine–2%, *trans*-cinnamoylcocaine–2%; (B) illicit cocaine containing: cocaine–95%, benzoylecgonine–3%, methylecgonine–1%, ecgonine–1%; (C) pharmaceutical cocaine containing: cocaine–100%.

Derivatization procedure: To a tube containing about 50 mg of cocaine is added 0.5 ml of BSA and 0.5 ml of $CHCl_3$ (containing 1 mg/ml of octadecane and tetracosane internal standards); the tube is heated at 75°C for about 15 min.; about 2 μl of the solution is injected into the GC under conditions given below.

GC parameters: Perkin-Elmer 3920 GC equipped with FID detection; 6 ft.×4 mm i.d. glass column packed with 3% OV-1 on GCQ (100–120 M); column is temperature programmed with an initial temperature of 140°C at an initial hold of 8 min. and a program rate of 4°C/min. with a final temperature of 270°C; injector and detector temperature–275°C; air and H_2 flow to FID–500 and 50 ml/min., respectively; N_2 carrier flow–60 ml/min; amplifier sensitivity–64×; chart speed–10 min./in.

quantitated by this derivatization procedure lie in the 0.2–1.0% range using GC-FID (based on 50 mg cocaine and final dilution of 2 cc); this level of sensitivity is adequate for the comparison of many uncut illicit cocaine samples; though not illustrated in Figure 1, many samples contain another cocaine hydrolysis product, namely benzoic acid; using the derivatization procedure described in Figure 1, benzoic acid elutes as a TMS derivative in the solvent front.

In summary, the GC derivatization procedure described above for cocaine was found to be rapid, accurate and of adequate sensitivity for most samples examined. Preliminary results indicate that common cocaine diluents do not interfere with the analysis. These diluents include benzocaine, procaine, lidocaine, caffeine, lactose, and dextrose. It should be noted that a diluent sometimes seen in illicit cocaine, namely boric acid, does inhibit TMS formation of cocaine-related impurities. The obvious limitation of applying the above method to highly adulterated cocaine samples lies in the relatively insensitive GC-FID determinative step.

It should be noted that in the comparative analysis of illicit drugs such as cocaine, a thorough knowledge of the various manufacturing processes is important. Additionally, a statis-

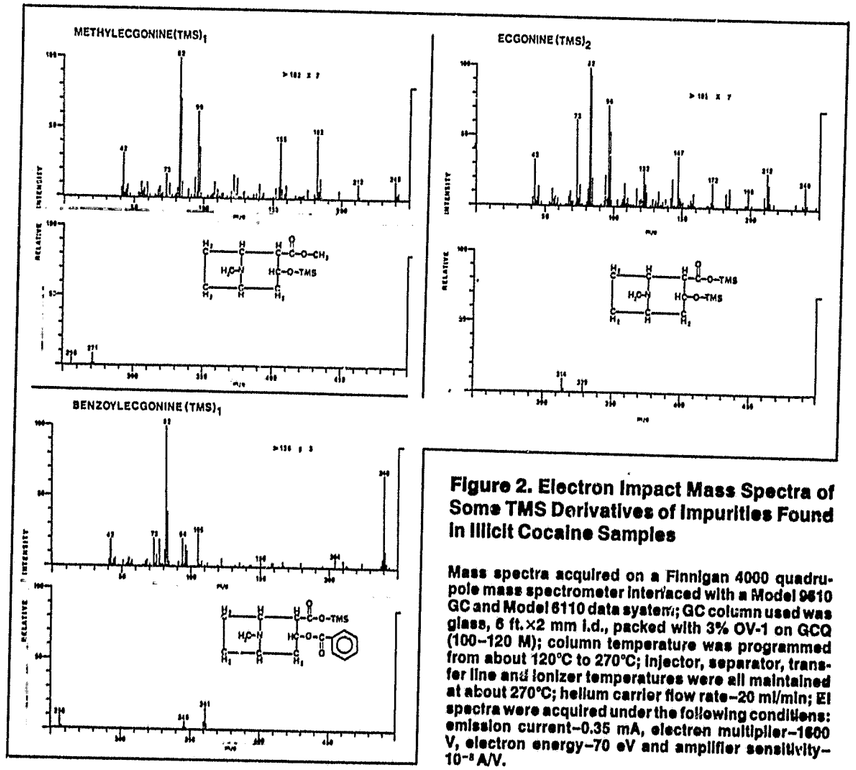


Figure 2. Electron Impact Mass Spectra of Some TMS Derivatives of Impurities Found in Illicit Cocaine Samples

Mass spectra acquired on a Finnigan 4000 quadrupole mass spectrometer interfaced with a Model 9610 GC and Model 6110 data system; GC column used was glass, 6 ft.×2 mm i.d., packed with 3% OV-1 on GCQ (100–120 M); column temperature was programmed from about 120°C to 270°C; injector, separator, transfer line and ionizer temperatures were all maintained at about 270°C; helium carrier flow rate–20 ml/min; EI spectra were acquired under the following conditions: emission current–0.35 mA, electron multiplier–1600 V, electron energy–70 eV and amplifier sensitivity–10^{-8} A/V.

tically sound data base is desirable when attempting comparative analysis on illicit drug samples. Finally, the comparative analysis should be complemented with other intelligence data.

In the forensic comparison of samples such as illicit cocaine, it is desirable to obtain adequate identification of the TMS derivatives of the impurities being quantitated (as well as non-derivatized impurities, such as the cinnamoylcocaines). To accomplish this, the BSA-$CHCl_3$ solution of the cocaine samples may be subjected to direct GC-MS analysis. Figure 2 illustrates the mass spectra of the derivatized cocaine impurities utilized for forensic comparison purposes. Though not illustrated in Figure 2, *cis*- and *trans*-cinnamoylcocaines yield virtually identical EI mass spectra.

Derivatization techniques have also been used extensively in the characterization of illicit heroin impurities. Heroin is produced illicitly by the acetylation of morphine, which is obtained from the opium poppy, Papaver somniferum L. Most all illicit heroin samples contain well-recognized impurities. These include heroin hydrolysis products, namely O^6-acetylmorphine and morphine, as well as codeine alkaloid and its acetylated product, namely O^6-acetylcodeine. Recently, Sobol and Sperling (66) have reported methodology which allows for the comparison of uncut illicit heroin samples for forensic purposes. In this procedure, the heroin samples are subjected to derivatization with BSA in order to form TMS derivatives of morphine, codeine, and O^6-acetylmorphine. The derivatized samples are subjected to GC-FID analysis. Figure 3 illustrates a GC-FID chromatogram of a typical uncut illicit heroin sample. Though acetylcodeine is not derivatized, it is included in the comparison analysis. As with the cocaine profiles, the practical quantitative limitations fall in the 0.2–1.0% range for acetylcodeine and O^6-acetylmorphine (based on 50 mg heroin and final dilution of 2 cc). Highly adulterated heroin samples can also be compared with one another using a modified GC derivatization procedure. Figure 4 represents a GC-FID profile of a derivatized heroin sample adulterated with procaine, mannitol, lactose, and dextrose.

Derivatization techniques have also played an integral role in the characterization of impurities heretofore not detected in illicit heroin. Klein (67) has identified the presence of triacetylnorheroin in illicit heroin samples. This characterization was made possible, in part, by mass spectral analysis of the acetylated and deutero-acetylated derivatives of morphine N-oxide, a substance believed present in crude morphine base.

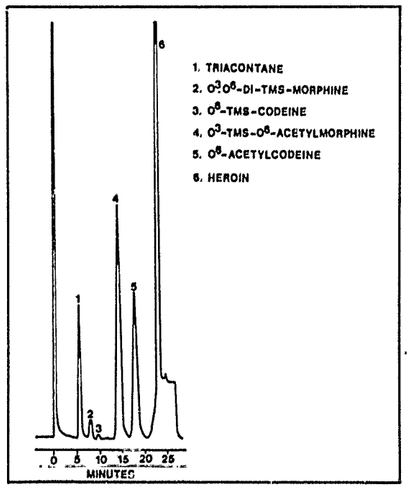


Figure 3. GC-FID Chromatogram of Uncut Illicit Heroin Sample Subjected to Derivatization with BSA

Uncut illicit heroin sample subjected to derivatization with BSA (see Ref. 66) and then analyzed by GC-FID under the following conditions: Perkin-Elmer 900 GC equipped with FID; 6 ft.×4 mm glass column packed with 3% OV-25 on GCQ (100–120 M); column temperature–265°C; injector and detector temperature–275°C; air and H_2 flow to FID–500 and 50 ml/min., respectively; N_2 carrier flow–60 ml/min. (Note: after elution of acetylcodeine, column temperature increased to effect rapid elution of heroin.)

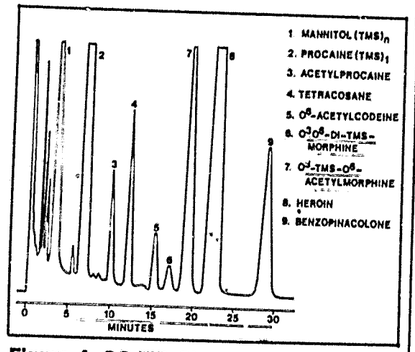


Figure 4. GC-FID Chromatogram of Adulterated Illicit Heroin Sample Subjected to Derivatization with BSA

Adulterated illicit heroin sample subjected to derivatization with BSA and then analyzed by GC-FID under the following conditions: Hewlett-Packard 5840A GC equipped with FID; 6 ft.×4 mm i.d. glass column packed with 3% OV-1 on GCQ (100–120 M); column temperature–230°C; injector and detector temperature–275°C; air and H_2 flow to FID–500 and 50 ml/min., respectively; N_2 carrier flow–60 ml/min.

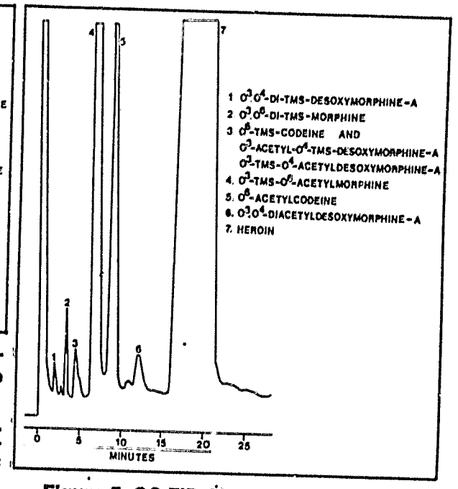


Figure 5. GC-FID Chromatogram of Uncut Illicit Heroin Sample Subjected to Derivatization with BSA and Chromatographed on OV-225

Uncut, illicit heroin sample subjected to derivatization with BSA and then analyzed by GC-FID on OV-225 under the following conditions: Perkin-Elmer 900 GC equipped with FID; 6 ft.×2 mm i.d. glass column packed with 3% OV-225 on GCQ (80–100 M); column temperature–240°C; injector and detector temperature–275°C; air and H_2 flow to FID–500 and 50 ml/min., respectively; N_2 carrier flow–60 ml/min.

During extension of the original work by Sobol and Sperling, additional unidentified peaks were noted in the GC-FID chromatograms of some BSA-derivatized illicit heroin samples using columns of very high polarity. Figure 5 illustrates such a chromatogram on OV-225. Since these new impurities were present in only trace quantities in a rather complex matrix and to minimize problems associated with hydrolysis, it was determined that GC fraction collection of the minor impurities represented in the chromatogram was perhaps the most expedient means of isolating these impurities for mass spectral characterization. The heroin sample was subjected to derivatization in BSA and BSA-d_9 and chromatographed on OV-225. The GC effluent of peaks 1, 3 and 6 (Figure 5) were condensed in a melting point tube at room temperature. The condensates were washed from the tubes with ethyl ether,

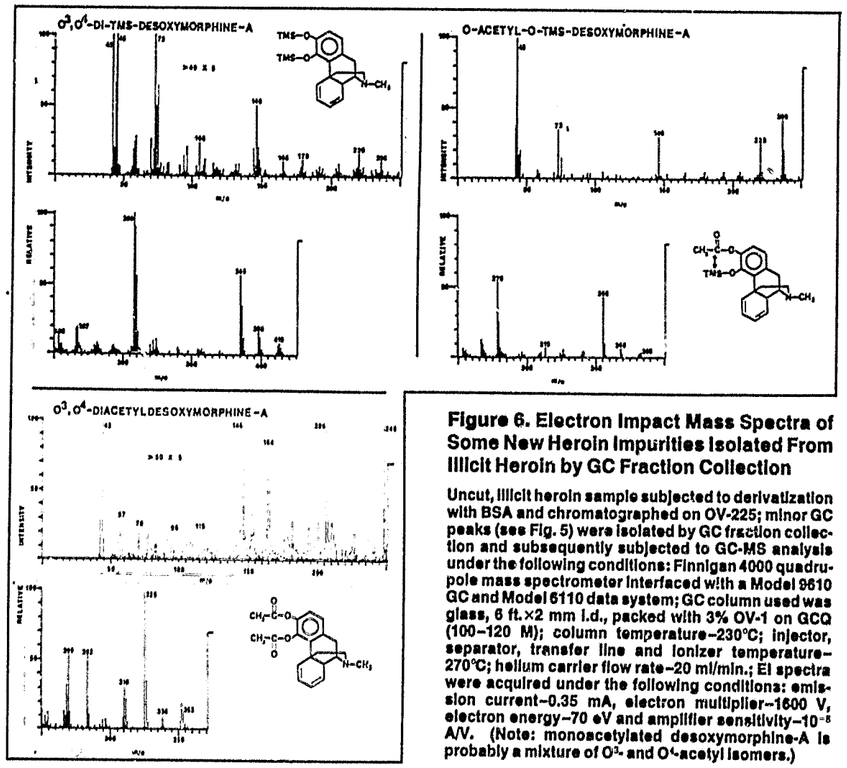


Figure 6. Electron Impact Mass Spectra of Some New Heroin Impurities Isolated From Illicit Heroin by GC Fraction Collection

Uncut, illicit heroin sample subjected to derivatization with BSA and chromatographed on OV-225; minor GC peaks (see Fig. 5) were isolated by GC fraction collection and subsequently subjected to GC-MS analysis under the following conditions: Finnigan 4000 quadrupole mass spectrometer interfaced with a Model 9610 GC and Model 6110 data system; GC column used was glass, 6 ft.×2 mm i.d., packed with 3% OV-1 on GCQ (100–120 M); column temperature–230°C; injector, separator, transfer line and ionizer temperature–270°C; helium carrier flow rate–20 ml/min.; EI spectra were acquired under the following conditions: emission current–0.35 mA, electron multiplier–1600 V, electron energy–70 eV and amplifier sensitivity–10^{-5} A/V. (Note: monoacetylated desoxymorphine-A is probably a mixture of O³- and O⁴-acetyl isomers.)

the ether evaporated and the residues reconstituted in microliter volumes of BSA and BSA-d_0 for mass spectral analysis. The EI mass spectra of the isolated impurities are given in Figure 6. These impurities are desoxymorphine-A, monoacetyl-desoxymorphine-A and diacetyl-desoxymorphine-A. The identification of these impurities was made possible initially by a detailed analysis of silyl and deutero-silyl mass spectra of peak 1 (subsequently characterized as desoxymorphine-A). This analysis was enhanced by observing amu shifts of significant TMS-containing ions after deutero-silylation. Certain ions that did not shift upon deutero-silylation were also used in making structural assignments.

The molecular ion in the di-TMS derivative of desoxymorphine-A occurred at m/e 413 and

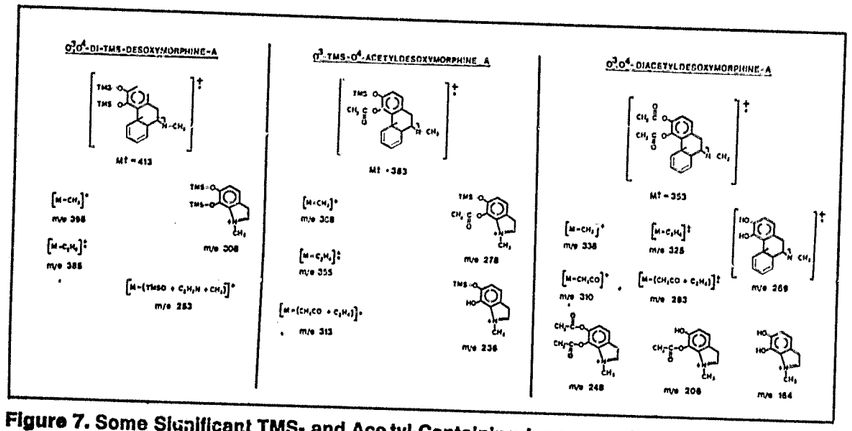
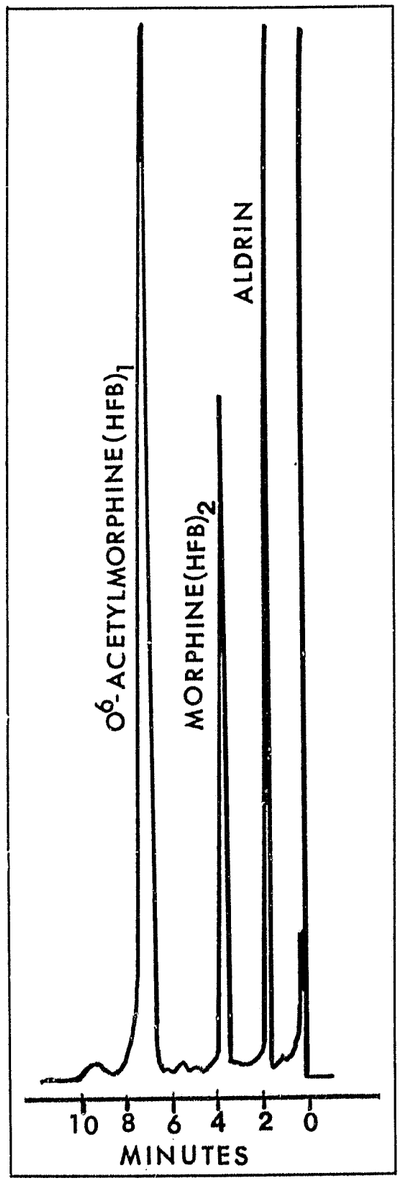


Figure 7. Some Significant TMS- and Acetyl-Containing Ions as well as Other Ions Used in the Electron Impact Mass Spectral Characterization of Desoxymorphine-A and its Acetylated Analogues

Parameters describing isolation and electron impact mass spectral analysis of desoxymorphine-related heroin impurities are given in body of paper and in Figures 5 and 6.

shifted an expected 18 amu upon deutero-silylation. The next most important ion in the spectrum appeared at m/e 308 and represented the loss of the stable phenethyl radical from the molecular ion. This fragment ion also shifted an expected 18 amu upon deutero-silylation. This loss of 105 amu's is prominent in the mass spectra of desoxymorphine and desoxycodeine-related alkaloids and its presence was important in making the proper structural assignment for the impurity. After MS characterization of desoxymorphine-A as its di-TMS derivative, the presence of mono- and diacetyldesoxymorphine-A was confirmed by the analysis of their silyl-deutero-silyl and acetyl-deutero-acetyl mass spectra (note: the monoacetyldesoxymorphine-A isolated from illicit heroin is probably a mixture of O³- and O⁴-acetyl isomers). Figure 7 illustrates some significant TMS- and acetyl-containing ions as well as other ions used in the MS characterization of desoxymorphine-A and its acetylated analogues. A paper describing in detail the GC isolation and MS characterization of these desoxymorphine-A impurities will be published in the near future.

As demonstrated above, the utilization of deutero-derivatization techniques is essential in the MS characterization of impurities using low-to-medium-resolution mass spectrometry. This requirement is amplified in the absence of supporting spectroscopic data provided by IR and NMR. It is also useful to correlate low- to medium-resolution mass spectral data on derivatized impurities with high-resolution data obtained previously on structurally-related non-derivatized compounds. A number of studies have reported



the HRMS analysis of morphine and related compounds. (68–71)

Much of the GC-derivatization work described above was done with FID detection. In trace drug studies, FID detection suffers from an inherent lack of sensitivity. In order to characterize impurities at levels as low as 10^{-5}% (based on 10 mg heroin and final dilution of 2 cc), we have conducted studies utilizing derivatization of the impurities with HFBA and detection using the sensitive GC-ECD determinative step. Moore (72) has recently reported methodology for the GC-ECD quantitation of morphine, codeine and O⁶-acetylmorphine in illicit heroin as HFB derivatives. Figure 8 illustrates a typical sample chromatogram of uncut illicit heroin subjected to HFB derivatization and GC-ECD analysis. Using this procedure, we have been able to quantitate morphine in some heroin samples at levels as low as 10^{-3}–10^{-4}% (based on 10 mg heroin and final dilution of 2 cc) while using a minimum of manipulative steps. The excellent sensitivity enjoyed by this procedure is demonstrated by the results given in Table I which illustrate the minimum detectable quantities of morphine, codeine and O⁶-acetylmorphine as HFB derivatives.

During utilization of the above-mentioned GC-ECD procedure, a number of additional peaks of unknown composition appeared in the chromatograms of illicit heroin samples. One such peak has been recently identified by Moore and Klein (73) as O³-acetylmorphine. Figure 9 illustrates a GC-ECD chromatogram

◀ **Figure 8. GC-ECD Chromatogram of Illicit Heroin Sample Subjected to Derivatization with HFBA for Morphine and O⁶-Acetylmorphine Quantitation**

See Reference 72 for sample analysis and chromatographic parameters.

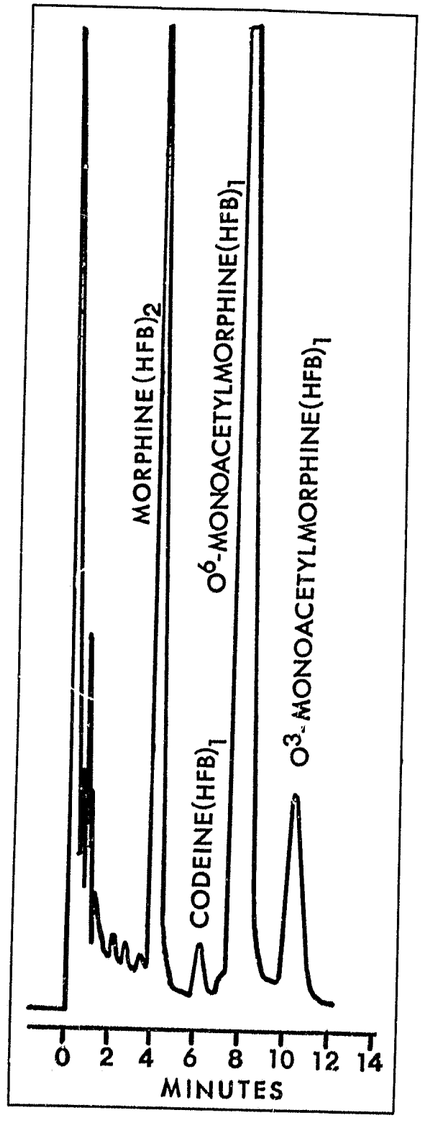


TABLE I

Minimum Detectable Quantities (MDQ) of HFB Derivatives of Morphine, Codeine and O⁶-Acetylmorphine Subjected to GC-ECD Analysis*

COMPOUND	MDQ
Morphine $(HFB)_2$	20 pg
O⁶-Acetylmorphine $(HFB)_1$	100 pg
Codeine $(HFB)_1$	80 pg

*Derivatization and GC-ECD parameters given in Reference 72.

of an HFB-derivatized brown heroin sample containing a significant quantity of O³-acetylmorphine. The identification of this impurity was accomplished by the mass spectral analysis of its HFB derivative. It should be noted that the mass spectrum of O³-acetylmorphine $(HFB)_1$ is significantly different from the mass spectrum of its isomer, O⁶-acetylmorphine $(HFB)_1$. This is illustrated in Figure 10. The presence of O⁶-acetylmorphine in heroin samples is due primarily to the acid hydrolysis of heroin, while the presence of O³-acetylmorphine is due mostly to the incomplete acetylation of morphine.

In addition to O³-acetylmorphine, other late-eluting peaks were noted in the GC-ECD chromatograms of HFB-derivatized heroin samples. Two of these impurities have been recently identified as O³,O⁶-diacetylnormorphine and O⁶acetylnormorphine. Figure 11 il-

◀ **Figure 9. GC-ECD Chromatogram of HFB-Derivatized Illicit Brown Heroin Sample Containing Significant Quantity of O³-Acetylmorphine**

See Reference 73 for sample analysis and chromatographic parameters.

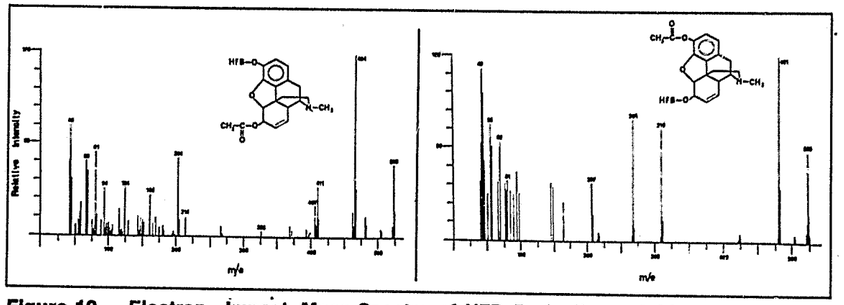


Figure 10. Electron Impact Mass Spectra of HFB Derivatives of O^6- and O^3-Acetylmorphine

See Reference 73 for electron impact mass spectral parameters.

lustrates the GC elution of these compounds as HFB derivatives in an illicit heroin sample using ECD detection. Due to their rather low levels in illicit heroin (10^{-2}–10^{-5}%) GC fraction collection of these peaks for subsequent mass spectral characterization was not practical. They were therefore isolated from the bulk heroin matrix using liquid-liquid partition chromatography. The isolated impurities were subjected to derivatization with BSA, BSA-d_9, and HFBA, and the resultant TMS, TMS-d_9 and HFB derivatives subjected to mass spectral analysis. Figures 12 and 13 illustrate the EI mass spectra of some of these derivatives. As in the case of the desoxymorphine-A impurities, these acetylated normorphine impurities were characterized primarily from significant ion shifts observed in the TMS and TMS-d_9-mass spectra of O^3,O^6-diacetyl-N-TMS-normorphine. Fragment ions at m/e 326, 342, 368 and 385 suggested the presence of acetoxyl groups at the C3 and C6 positions in the morphine-type molecule. All of these ions shifted the expected 9 amu upon deutero-silylation. One of the most significant ions occurred

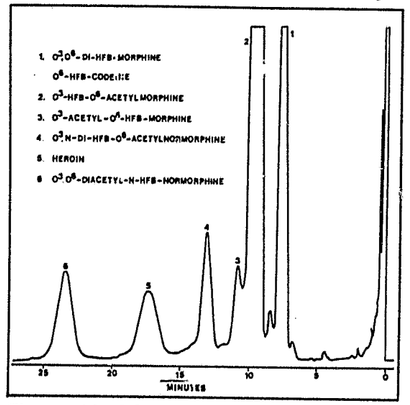


Figure 11. GC-ECD Chromatogram Illustrating Some Late-Eluting Heroin Impurities Found in an HFB-Derivatized Illicit Heroin Sample

Heroin sample is subjected to derivatization with HFBA as described in Reference 72; derivatized sample is subjected to GC-ECD analysis under the following conditions: Perkin-Elmer 990 GC equipped with a ^{63}Ni electron capture detector; 6 ft.×4 mm i.d. glass column packed with 3% OV-1 on GCQ (100–120 M); column temperature–220°C; injector and detector temperature–275°C and 300°C, respectively; N_2 carrier flow–100 ml/min.

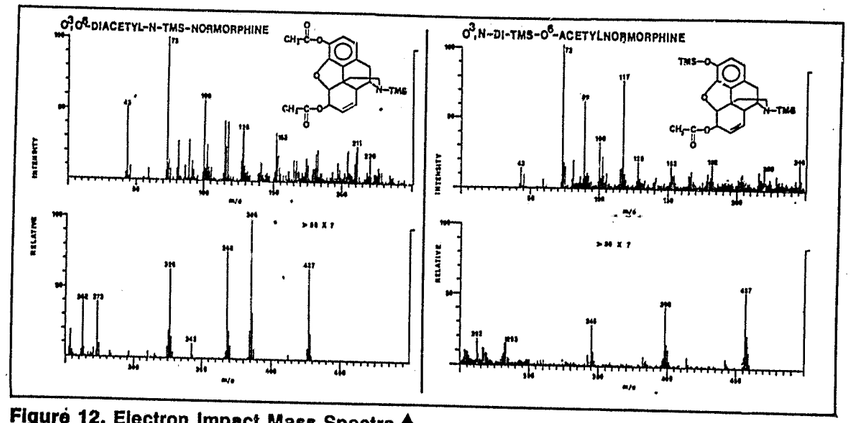
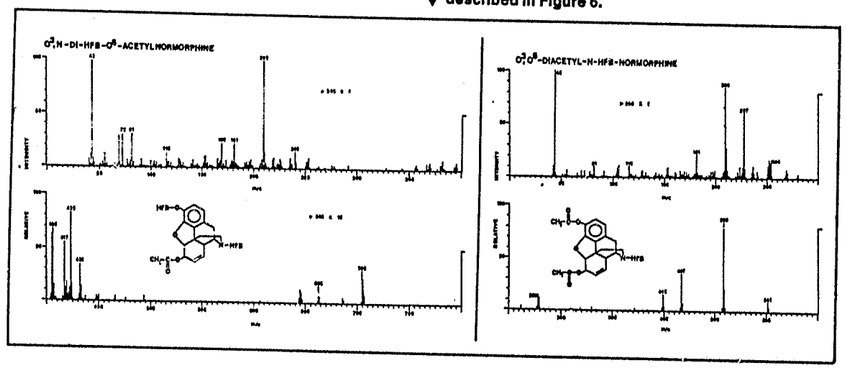


Figure 12. Electron Impact Mass Spectra of TMS derivatives of Acetylated Normorphine Impurities Found in Illicit Heroin

Heroin impurities O^3,O^6-diacetylnormorphine and O^6-acetyl-normorphine were isolated from the bulk heroin matrix by liquid-liquid partition chromatography; the isolated impurities were derivatized with BSA for 30 min. at 75°C; the derivatized impurities were subjected to GC-MS analysis under conditions described in Figure 6.

Figure 13. Electron Impact Mass Spectra of HFB Derivatives of Acetylated Normorphine Impurities Found in Illicit Heroin

Heroin impurities O^6-acetyl-normorphine and O^3,O^6-diacetylnormorphine were isolated from the bulk heroin matrix by liquid-liquid partition chromatography; the isolated impurities were derivatized with HFBA as described in Reference 72; the derivatized impurities were subjected to GC-MS analysis under conditions described in Figure 6.



at m/e 100 and represented in part a TMS-substituted N function. This ion also shifted 9 amu upon deutero-silylation. Figure 14 illustrates some significant TMS- and acetyl-containing ions as well as other ions used in the MS characterization of the acetylated normorphines. As with the desoxymorphine-A impurities, details of the isolation and characterization of the acetylated normorphine impurities will be published soon.

Though the mass spectra of the HFB derivatives of the acetylated normorphine and other impurities were obtained, they were not utilized as fully as the mass spectra of the TMS derivatives for mass spectral elucidation. This is because most of the intense ions represented either HFB fragments or losses of HFB fragments from parent ions. Additionally, the lack of an isotopic analogue of HFBA permitted only limited mass spectral evaluation of the resultant HFB derivative of the impurity. Finally, the lesser stability of the HFB derivatives compared to their TMS counterparts did not allow for their repetitive mass spectral analysis from a single solution.

ACKNOWLEDGMENTS

I would like to thank Dr. Kenner C. Rice, Medicinal Chemistry, National Institutes of Health, Bethesda, Maryland, for supplying standards of desoxymorphine-A and the acetylated normorphines. I would also like to extend my appreciation to Mrs. Jean Nolan for typing the final manuscript.

SUMMARY

The application of derivatization techniques has enjoyed limited use in routine forensic drug analysis, and this situation is not likely to change in the foreseeable future. Derivatization methodology, especially when used in conjunction with GC-FID, GC-ECD, and GC-MS techniques, has significant potential in forensic drug research. It is especially applicable in the in-depth analysis of illicit samples and in the characterization of trace drug impurities for forensic and intelligence purposes.

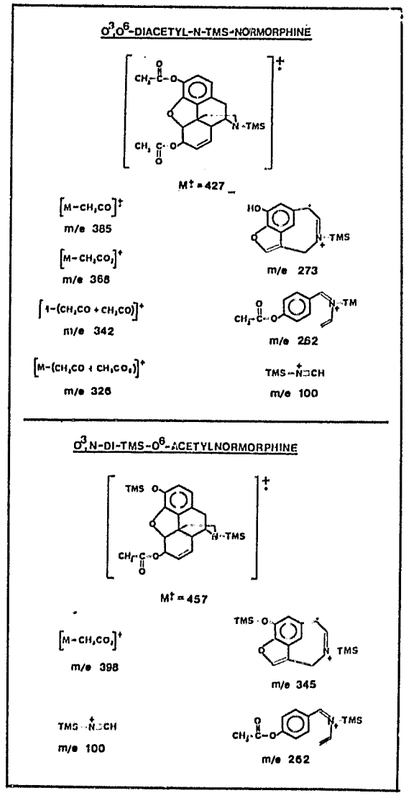


Figure 14. Some Significant TMS- and Acetyl-Containing Ions Used in the Electron Impact Mass Spectral Characterization of O3,O6-Diacetylnormorphine and O6-Acetylnormorphine

Parameters describing isolation, derivatization and GC-MS analysis of acetylated normorphine heroin impurities given in body of paper and in Figure 12.

REFERENCES

1. *Official Methods of Analysis of the Association of Official Analytical Chemists,* Eleventh Ed., pp. 708–21 (1970).
2. Nakamura, H. and Fisano, J. J., *J. Chromatogr.*, Vol. 121, pp. 33–40 (1976).
3. Frei, R. W., Santi, W. and Thomas, M., *J. Chromatogr.*, Vol. 116, pp. 365–77 (1976).
4. Nowicki, H. G., *J. Forensic Sci.*, Vol. 21, No. 1, pp. 154–62 (1976).
5. Hopen, T. J., Briner, R. C., Sadler, H. G. and Smith, R. L., *J. Forensic Sci.*, Vol. 21, No. 4, pp. 842–50 (1976).
6. Stahl, E., *Thin-Layer Chromatography,* 2nd Ed., Springer-Verlag Co., New York (1969).
7. Lawrence, J. F. and Frei, R. W., *Chemical Derivatization in Liquid Chromatography,* Vol. 7, Elsevier Scientific Publishing Co., New York (1976).
8. Vessman, J., Hartvig, P. and Molander, M., *Anal. Letters,* Vol. 6(8), pp. 699–707 (1973).
9. Hartvig, P. and Vessman, J., *Anal. Letters,* Vol. 7 (4), pp. 223–31 (1974).
10. Drozd, J., *J. Chromatogr.*, Vol. 113, pp. 303–56 (1975).
11. Ahuja, S., *J. Pharm. Sci.*, Vol. 65, No. 2, pp. 163–82 (1976).
12. Nicholson, J. D., Analyst, Parts I and II, Vol. 103, pp. 1–10 and pp. 193–222 (1978).
13. Lochmüller, C. H. and Souter, R. W., *J. Chromatogr.*, Vol. 113, pp. 283–302 (1975).
14. Cimbura, G. and Kofoed, J., *J. Chromatogr. Sci.*, Vol. 12, pp. 261–64 (1974).
15. Pierce, A. E., *Silylation of Organic Compounds,* Pierce Chemical Co., Rockford, IL (1968).
16. McCloskey, J. A., Stillwell, R. N. and Lawson, A. M., *Anal. Chem.*, Vol. 40, No. 1, pp. 233–36 (1968).
17. Wells, C. E., *J.A.O.A.C.*, Vol. 53, No. 1, pp. 113–15 (1970).
18. Ibid, Vol. 55, No. 1, pp. 146–48 (1972).
19. Beckett, A. H. and Testa, B., *J. Chromatogr.*, Vol. 69, pp. 285–89 (1972).
20. Ment, W. M. and Marino, V. S., *J.A.O.A.C.*, Vol. 53, No. 5, pp. 1097–99 (1970).
21. Choulis, N. H., *J. Pharm. Sci.*, Vol. 61, No. 8, pp. 1325–26 (1972).
22. Eskes, D., *J. Chromatogr.*, Vol. 117, pp. 442–44 (1976).
23. See Ref. 1.
24. Clark, C. C., *J.A.O.A.C.*, Vol. 58, No. 5, pp. 1174–77 (1975).
25. Brochmann-Hanssen, E. and Olawuyioke, T., *J. Pharm. Sci.*, Vol. 58, No. 3, pp. 370–71 (1969).
26. Venturella, V. S., Gualario, V. M. and Lang, R. E., *J. Pharm. Sci.*, Vol. 62, No. 4, pp. 662–68 (1973).
27. Hooper, W. D., Dubetz, D. K., Eadie, M. J. and Tyrer, J. H., *J. Chromatogr.*, Vol. 110, pp. 206–9 (1975).

28. Street, H. V., *J. Chromatogr.*, Vol. 41, pp. 358–66 (1969).

29. Heagy, J. A., *Anal. Chem.*, Vol. 42, p. 1459 (1970).

30. Knaus, E. E., Coutts, R. T. and Kazakoff, C. W., *J. Chromatogr. Sci.*, Vol. 14, pp. 525–30 (1976).

31. Harvey, D. J. and Paton, W. D. M., *J. Chromatogr.*, Vol. 109, pp. 73–80 (1975).

32. Turner, C. E., Hadley, K. W., Henry, J. and Mole, M. L., *J. Pharm. Sci.*, Vol. 63, No. 12, pp. 1872–75 (1974).

33. Harvey, D . *Chromatogr.*, Vol. 147, pp. 291–').

34. Nakamur , . R. and Noguchi, T. T., *J. Forensic Sci. Soc.*, Vol. 14, pp. 347–53 (1974).

35. Parker, K. D., Wright, J. A., Halpern, A. F. and Hine, C. H. *J. Forensic Sci. Soc.*, Vol. 10, pp. 17–22 (1970).

36. Grooms, J. O., *J.A.O.A.C.*, Vol. 51, No. 5, pp. 1010–13 (1968).

37. Rasmussen, K. E., *J. Chromatogr.*, Vol. 120, pp. 491–95 (1976).

38. Brugaard, G. and Rasmussen, K. E., *J. Chromatogr.*, Vol. 147, pp. 476–80 (1978).

39. Joint Committee of the Pharmaceutical Society and the Analytical Division of the Chemical Society on Pharmaceutical Analysis, *Analyst*, Vol. 103, pp. 268–83 (1978).

40. Sperling, A. R., private communication (1973).

41. Sperling, A. R., *J. Chromatogr. Sci.*, Vol. 12, pp. 265–66 (1974).

42. Radecka, C. and Nigam, I. C., *J. Pharm. Sci.*, Vol. 55, No. 8, pp. 861–62 (1966).

43. Hammer, R. H., Templeton, J. L., and Panzik, H. L., *J. Pharm. Sci.*, Vol. 63, No. 12, pp. 1962–65 (1974).

44. Allen, A. and Cooper, D., private communication (1976).

45. Barron, R. P., Kruegel, A. V., Moore, J. M. and Kram, T. C., *J.A.O.A.C.*, Vol. 57, No. 5, pp. 1147–58 (1974).

46. Kram, T. C. and Kruegel, A. V., *J. Forensic Sci.*, Vol. 22, No. 1, pp. 40–52 (1977).

47. Kram, T. C., *J. Pharm. Sci.*, Vol. 66, No. 3, p. 443 (1977).

48. Kram, T. C., *J. Forensic Sci.*, Vol. 22, No. 3, pp. 508–14 (1977).

49. Lomonte, J. N., Lowry, W. T. and Stone, I. C., *J. Forensic Sci.*, Vol. 21, No. 3, pp. 575–82 (1976).

50. Bailey, K., Boulanger, J. G., LeGault, D. and Taillefer, S. L., *J. Pharm. Sci.*, Vol. 63, No. 10, pp. 1575–78 (1974).

51. Stromberg, L., *J. Chromatogr.*, Vol. 106, pp. 335–42 (1975).

52. Ibid, Vol. 63, pp. 253–58 (1972).

53. Holley, J. H., Hadley, K. W., and Turner, C. E., *J. Pharm. Sci.*, Vol. 64, No. 5, pp. 892–94 (1975).

54. Davis, T. W. M., Farmilio, C. G. and Osadchuk, M., *Anal. Chem.*, Vol. 35, pp. 751–55 (1963).

55. Lee, C. K. and Kim, H. K., *Bulletin on Narcotics*, Vol. 22, No. 2, pp. 41–6 (1970).

56. Van der Slooten, E. P. J. and van der Helm, H. J., *Forensic Sci.*, Vol. 6, pp. 83–8 (1975).

57. Clark, A. B. and Miller, M. D., *J. Forensic Sci.*, Vol. 23, No. 1, pp. 21–8 (1978).

58. Miller, M. D., *J. Forensic Sci.*, Vol. 16, No. 1, pp. 150–63 (1972).

59. Clark, C. C. and Cooper, D. A., private communication (1977).

60. Willstatter, R., Wolfes, O. and Mader, H., *Ann.*, 434 III (1923).

61. Moore, J. M., *J.A.O.A.C.*, Vol. 56, No. 5, pp. 1199–1205 (1973).

62. Mulé, S. J., *COCAINE: Chemical Biological, Clinical, Social and Treatment Aspects*, CRC Press, Cleveland, OH (1976).

63. Moore, J. M., *J. Chromatogr.*, Vol. 101, pp. 215–18 (1974).

64. Majlát, P. and Bayer, I., *J. Chromatogr.*, Vol. 20, p. 187 (1965).

65. Moore, J. M. Unpublished data.

66. Sobol, S. P. and Sperling. A. R., *A.C.S. Symposium Ser.*, No. 13, p. 170 (1975).

67. Klein, M., "Analysis of Impurities in Illicit Heroin by Mass Spectrometry," in *Mass Spectrometry in Drug Metabolism*, Ed. by Frigerio, A. and Ghisalberti, E. L., Plenum Publishing Corp., New York, pp. 449–63 (1977).

68. Audier, H., Fetizon, M., Ginsburg, D., Mandelbaum, and Rüll, Th., *Tetrahedron Letters*, No. 1, pp. 13–22 (1965).

69. Nakata, H., Yoshimasa, H., Tatematsu, A., Tada, H. and Sawa, Y. K., *Tetrahedron Letters*, No. 13, pp. 829–36 (1965).

70. Mandelbaum, A. and Ginsburg, D., *Tetrahedron Letters*, No. 29, pp. 2479–89 (1965).

71. Wheeler, D. M. S., Kinstle, T. H. and Rinehart, K. L., Jr., *J. Amer. Chem. Soc.*, 89:17, pp. 4494–501 (1967).

72. Moore, J. M., *J. Chromatogr.*, Vol. 147, pp. 327–36 (1978)

73. Moore, J. M. and Klein, M., *J. Chromatogr.*, Vol. 154, pp. 76–83, (1978).

Advances in Chemical Signature Analyses of Drugs

by

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INTRODUCTION

The National Laboratory of Forensic Science handles the majority of all analyses for narcotics and dangerous drugs in Sweden (about 8 million inhabitants). This entails transportation problems but ensures a standardized methodology and evaluation of results for the whole country. Also, it enables the narcotic police to maintain an up-to-date intelligence link. The analytical results can thus serve two main purposes, *i.e.*, (i) forming the legal proof for convictions of narcotics peddlers, and (ii) being an instrument for the perfection of the police intelligence network.

At present, the laboratory receives annually over 3,000 cases with more than 25,000 items to be analyzed Each item is identified, described, and weighed. The items within one case are then grouped, based on screening techniques. Samples from each group are analyzed qualitatively for narcotics and other accompanying substances. If positive identification of a narcotic drug has been achieved, a quantitative analysis is carried out for all "hard" narcotics—and for other narcotics and dangerous drugs if the weight of the group exceeds 5 g.

The detailed results of these analyses are reported to the police in two ways: (i) a written report is sent for use in the court procedure and (ii) a computer input sheet is sent in coded form. The latter contains information on the composition of each group. Nowadays, it is not unusual that individual items contain 3, 4 or more different compounds in addition to the narcotic drug.

This composition alone, fed into the central police computer system, allows certain conclusions to be drawn. When seizures at different locations in the country have similar compositions, the drug CID may get interested. In about 100 cases per year, a detailed comparison of chemical signatures of seizures is requested by the police.

This special analysis activity has two main purposes, *i.e.*, (i) the direct comparison of the chemical signatures, and (ii) the possible evaluation of the method of synthesis of clandestinely-prepared narcotics.

Most of the reports that have appeared in the literature have been concerned with the second of these tasks (1–10). These papers are especially concerned with the Leuckart synthesis (11–14) of amphetamine and methamphetamine. A number of impurities have been observed in seized samples of these amines and many of these have been identified.

We have earlier reported signature work on amphetamine, phenmetrazine, morphine, and heroin (15, 16) for the purpose of direct comparison. An even earlier paper describes the comparison between cannabis resin samples where the removal of the main components is not necessary (17).

Our methods have now been used in practical work for several years and have proven their value. Recently, a signature comparison of large seizures of hashish in Norway and Sweden could be carried out when samples were made available to our laboratory by the Norwegian police (Fig. 1). Typical signatures for amphetamine can be found in Figs. 1–2 of an earlier paper (15). Signatures of phenmetrazine, morphine, and heroin appeared in another publication (16).

On some occasions the method established links between amphetamine seized in Sweden with amphetamine seized from cars entering Sweden from Holland.

In connection with questions of "international comparison" of drug seizures, we think

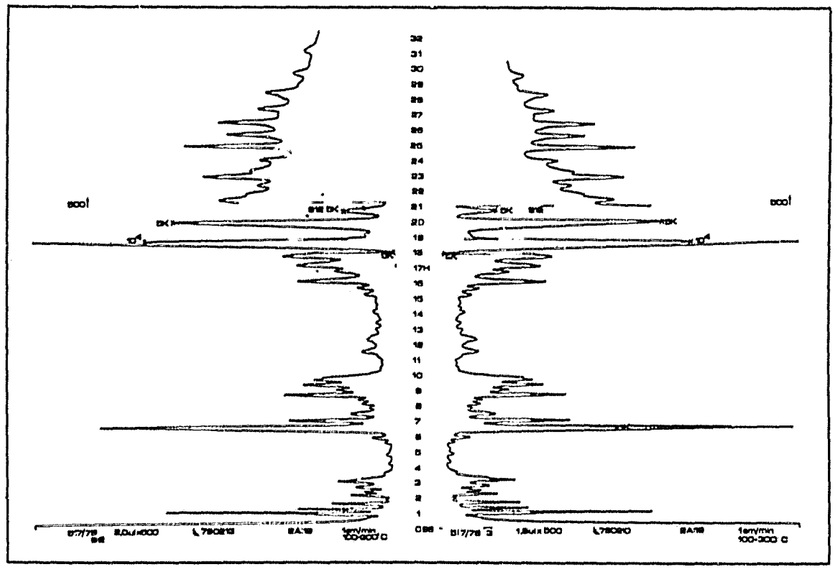


Fig. 1. Chemical FID signatures of hashish samples seized in Norway and Sweden, respectively.

that such analyses may be carried out without exchange of genuine drug samples which often involve intricate legal problems. Only solvent extracts of the impurities need to be exchanged. These extracts contain drugs only in trace amounts and are, therefore, of no interest from the point of view of drug control.

Before going on, it may be useful to consider the origin of the impurities and by-products forming a chemical signature. A somewhat simplified scheme is rendered in Fig. 2. The raw product contains the substances of the A, B, C, D group, *i.e.*, impurities from the equipment and the laboratory (A), contributions from the starting materials (B, the starting materials proper; and b, impurities in these products), from products of side reactions (C), and finally from intermediate compounds (D). The B, C and D compounds which we call key impurities can be useful in determining the method of synthesis used. This is the subject of most of the publications mentioned above (1–10).

The E, F, G . . . . Z substances, on the other hand, stem from the further fate of the raw product obtained at the clandestine laboratory. The contribution of these substances to the chemical signature can also be of value for the police intelligence work. The F, G . . . . Z substances can be subdivided into

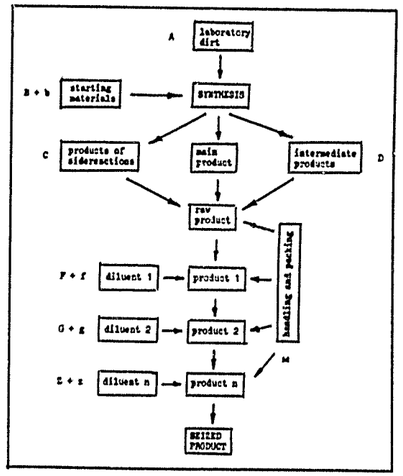


Fig. 2. The origin of the compounds of a chemical signature. The details are explained in the text.

the main adulterants (F, G . . . . Z) and the impurities in these (f, g . . . .z). The former are, in general, easily identified. In seizures of central nervous system stimulating amines, the following adulterants have been observed quite frequently in Sweden: sugars, caffeine, phenazone, ascorbic acid, ephedrine, norephedrine, pseudonorephedrine, barbituric acid derivatives, lidocaine and procaine, as well as mixtures of CNS-stimulating amines. Sugars are the most frequent additives.

Most of these adulterants are themselves pharmacologically active compounds, whereas sugars, alongside with the more sporadically used substances, such as starch or flour, can be regarded as passive diluents.

Finally, handling and packing can introduce another type of contaminant (E).

RESULTS

The earlier signature work on synthetic narcotic drugs has been based on recording simultaneously the signals from an FI- and an EC-detector, using an effluent splitter. Recently we began using a similar technique but replaced the ECD by an NP-detector. Obtaining different types of signatures enhances the possibility of establishing a common origin of different samples.

We were interested to find out to what extent impurities in adulterants might contribute to the total signature pattern. Owing to its widespread occurrence, we started with the sugars (Strömberg, L. and Bergqvist, H., to be published). So far, two kinds of glucose and two kinds of sucrose were investigated, using n-heptane for extracting impurities. The signatures of the sugars, especially the sucroses, were found to be weak, probably due to the high standards of purity requested for food ingredients. The glucose samples showed one or two major peaks in their signatures. The corresponding impurities have been partially identified.

Next the sucrose and dextrose samples were mixed with samples of seized amphetamine sulfate and with amphetamine sulfate synthesized and twice recrystallized at our laboratory. As expected, the addition of sucrose did not noticeably interfere with the signatures of drugs. The stronger signature of the dextrose samples, on the other hand, contributed to that of the specially purified amphetamine. However, the signature of most seized amphetamine samples is so strong that the sugar contribution to the signature is very small. The dilution of drugs with sugars should, therefore, as a rule, not hinder this analytical procedure.

We can now return to the second task of signature work, *i.e.*, identifying key impurities in order to establish the method of synthesis. The major part of the work published in the literature concerns itself with the key impurities in methamphetamine (1, 2, 3, 5, 9, 10), which we have not studied in detail. Amphetamine impurities were investigated by Lomonte *et al.* (4), van der Ark *et al.* (6, 7), and Kram and coworkers (8, 9). All of this work was concerned with the Leuckart method of synthesis (*i.e.*, the reductive amination of phenylacetone by formamide, or modifications thereof). The compounds reported by these authors are listed in Table I.

Using our method of analysis, very rich signatures were obtained, and only a limited number of gas chromatographic peaks have been investigated so far. A typical reconstructed gas chromatogram from a GC/MS experiment (JEOL D100 coupled to a Varian gas chromatograph 2700) is shown in Fig. 3. Some of the major peaks have been identified by mass spectrometry and the mass spectra were compared with those published by other workers (cf Table I). Peak 1 is impurity nr 8 (6), peak 2 is impurity nr 9 (6), peak 3 has not yet been identified, peak 4 is impurity nr 6 (4) and peak 5 is impurity nr 11, observed in methamphetamine preparations by Bailey *et al.* (2) and Barron *et al.* (3), but to our knowledge not reported in amphetamine samples.

The two remaining larger peaks 6 & 7 have identical mass spectra. These spectra are similar to that reported for impurity nr 7. Lomonte *et al.* (4) published a mass chromatogram (m/e=91) where a double peak is visible. The larger (first eluted) peak was shown by them to be tri-(1-phenyl-isopropyl) amine. We postulate that the double peak results from diastereomeric forms of this tertiary amine.

The key impurities so far described are those of Leuckart amphetamine. A second method of amphetamine synthesis which has been used

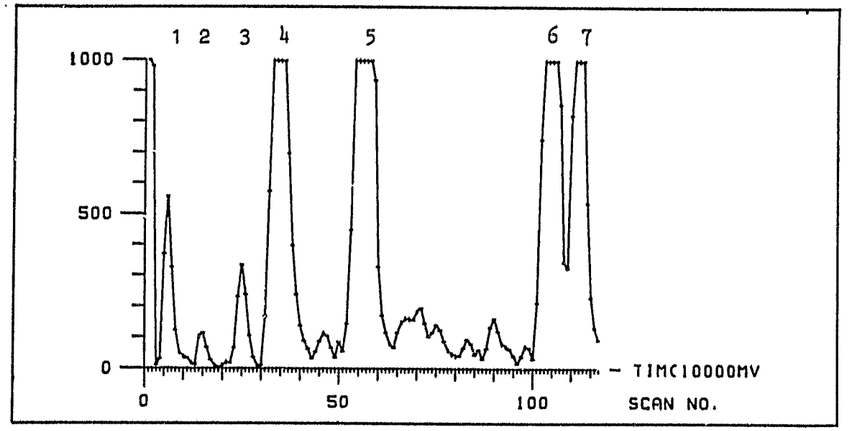
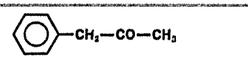
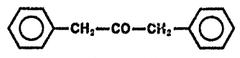
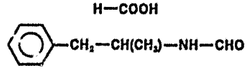
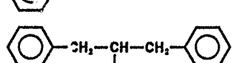
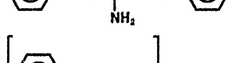
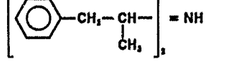
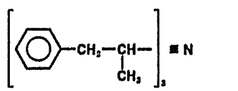
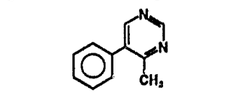
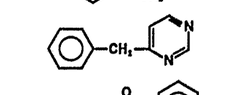
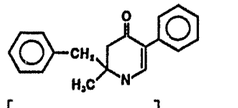
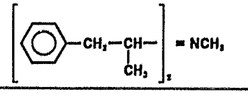


Fig. 3. Reconstructed Total Ionization Chromatogram (TIC) of a Leuckart amphetamine signature. The significance of the peaks numbered 1–7 is explained in the text.

Table I

REPORTED IMPURITIES IN AMPHETAMINE SAMPLES SYNTHESIZED BY THE LEUCKART METHOD

NR.	MOL WT	FORMULA	STRUCTURE	NAME	REMARKS	REF
1.	134.05	$C_9H_{10}O$		methyl benzyl ketone	a)	(9)
2.	210.06	$C_{15}H_{14}O$		dibenzyl ketone	b)	(9)
3.	45.99	CH_2O_2		formic acid	a)	(9)
4.	163.07	$C_{10}H_{13}NO$		N-formyl-amphetamine	c)	(8)
5.	211.11	$C_{15}H_{17}N$		α-benzyl phen-ethylamine	d)	(8)
6.	253.15	$C_{18}H_{23}N$		di-(1-phenyl-isopropyl) amine	d)	(4)
7.	371.22	$C_{27}H_{33}N$		tri-(1-phenyl-isopropyl) amine	d)	(4)
8.	170.06	$C_{11}H_{10}N_2$		4-methyl-5-phenylpyrimidine	d)	(6)
9.	170.06	$C_{11}H_{10}N_2$		4-benzyl-pyrimidine	d)	(6)
10.	276.99	$C_{18}H_{16}NO$		2-benzyl-2-methyl-5-phenyl-1,3-di-hydropyrid-4-one	d)	(7)
11.	267.17	$C_{19}H_{25}N$		N-methyl-di-(1-phenylisopropyl) amine	d)	this paper

a) starting material;
b) impurity in starting material;
c) intermediate product;
d) product of side reaction.

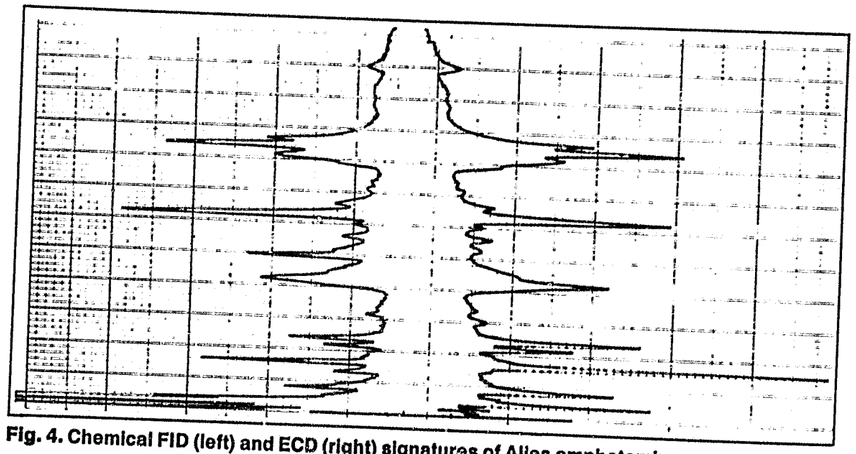


Fig. 4. Chemical FID (left) and ECD (right) signatures of Alles amphetamine.

many times in clandestine laboratories in Sweden during the 1970's is the electrolytic reduction of phenylnitropropene, first described by Alles (18). This method has been subject to preliminary studies at this laboratory. An example of an FID/ECD signature of such amphetamine is shown in Fig. 4.

The reduction probably proceeds stepwise as outlined in Fig. 5. Phenylnitropropene residues can be identified with the FID/ECD

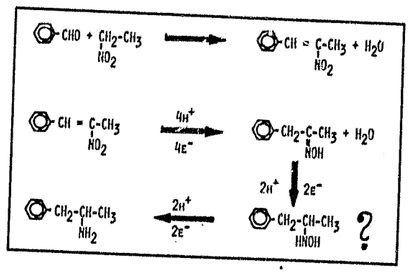


Fig. 5. Stepwise electrolytic reduction of phenylnitropropene (E^- = electron). The actual existence of N-hydroxyamphetamine (a metabolite of amphetamine in humans) in the reaction sequence has not been documented.

combination. Phenylacetoxime and N-hydroxyamphetamine have not been found in any clandestine preparation or reaction mixture of the type concerned. However, there are two indications that formation of phenylacetoxime is an intermediate process. First, the compound has been obtained as the end-product in experiments at this laboratory where the cathode potential was carefully controlled. Second, benzyl methyl ketone was found in a seized reduction mixture of the type concerned (Fig. 6) probably as a result of the hydrolysis of the oxime. The absence of the oxime itself in the seized materials may be explained by the fact that these reductions were carried out with

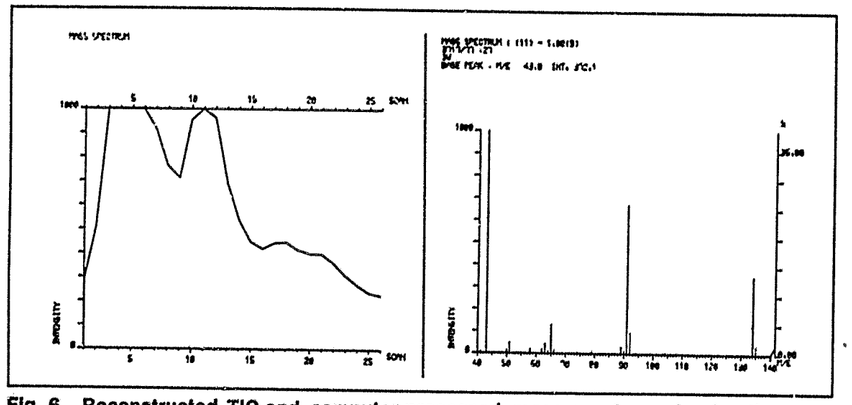


Fig. 6. Reconstructed TIC and computer-processed mass spectrum showing the presence of benzyl methyl ketone in Alles amphetamine. The first "peak" in the TIC is amphetamine, the second benzyl methyl ketone.

uncontrolled extreme potentials, leading directly to amphetamine. The presence of benzyl methyl ketone just mentioned is interesting from another point of view. Evidently, the presence of benzyl methyl ketone residue in illicitly prepared amphetamine does not necessarily point to the Leuckart method of synthesis.

Key impurities are obviously of great interest in the forensic analysis of narcotics. Their recognition among the numerous gas chromatographic peaks which constitute the chemical signature should therefore be an important question for forensic laboratories concerned with the analysis of narcotics. The "classical" method for recognition of an organic compound in a complex mixture is gas chromatography—mass spectrometry. We think, however, that key impurities of drugs may be recognized also by less sophisticated (and less expensive) methods. A gas chromatograph with a dual detector system (19) should have a sufficient discrimination power especially when using a capillary column.

Note added May 17, 1978: Phenylacetoxime was found to be present in a seizure of Alles amphetamine.

REFERENCES

1. LeBelle, M., Sileika, M., and Romach, M., *J. Pharm. Sci.*, 62, 862 (1973).
2. Bailey, K., Boulanger, J. G., Legault, D., and Taillefer, L., *J. Pharm. Sci.*, 63, 1575 (1974).
3. Barron, R. P., Kruegel, A. V., Moore, J. M., and Kram, T. C., *J. Assoc. Off. Anal. Chem.*, 57, 1147 (1974).
4. Lomonte, J. N., Lowry, W. T., and Stone, I. C., *J. Forens. Sci.*, 21, 575 (1976).
5. Kram, T. C., personal communication (1976).
6. Van der Ark, A. M., Theeuwen, A. B. E., and Verweij, A. M. A., *Pharm. Weekblad*, 112, 977 (1977).
7. Van der Ark, A. M., Sinnema, A., van der Toorn, J. M., and Verweij, A. M. A., *Pharm. Weekblad*, 112, 980 (1977).
8. Kram, T. C., *J. Pharm. Sci.*, 66, 443 (1977).
9. Kram, T. C. and Kruegel, A. V., *J. Forens. Sci.*, 22, 40 (1977).
10. Kram, T. C., *J. Forens. Sci.*, 22, 508 (1977).
11. Leuckart, R., *Ber.*, 18, 2341 (1885).
12. Leuckart, R. and Bach, E., *Ber.*, 19, 2128 (1886).
13. Leuckart, R., *Ber.*, 20, 3441 (1887).
14. Leuckart, R. and Janssen, H., *Ber.*, 22, 1409 (1889).
15. Strömberg, L., *J. Chromatogr.*, 106, 335 (1975).
16. Strömberg, L. and Maehly, A. C., *J. Chromatogr.*, 109, 67 (1975).
17. Strömberg, L., *J. Chromatogr.*, 68, 253 (1972).
18. Alles, G. A., *J. Amer. Chem. Soc.*, 54, 271 (1932).
19. Baker, J. K., *Anal. Chem.*, 49, 906 (1977).

Special Problems in TLC, GC and GC-MS Analysis of Heroin

by

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I. PREFACE—DRUG SITUATION IN BERLIN

This is a brief review of the drug situation in Germany, and it is focused on one city in Germany, West Berlin, since this city in some way has become a nerve center in drug trafficking. The reason for this lies in the political and geographical situation of West Berlin. This city is completely surrounded by the German Democratic Republic and the demarcation line between West and East Berlin is semipermeable, at least in the East/West direction; therefore, foreigners arriving at the airport of the eastern part of the city are able to penetrate the border uncontrolled via one of the numerous subway stations in Berlin. No doubt this is one of the ways, maybe the most frequented one, for importing heroin and other drugs from Middle and Far East countries. The knowledge of current availability of sufficient amounts of heroin at a moderate price is drawing large numbers of drug addicts into Berlin. Within a few years, the number of drug addicts has increased in an unbelievable way. In 1977, almost 100 young people died from overdoses of drugs in a city of two million inhabitants; this emphasizes the seriousness of the situation in Berlin.

For this reason, our Crime Laboratory mostly encounters drug analysis cases. In 1977, 2400 of a total number of 3000 requests for analysis concerned drugs and a high percentage of these included heroin.

The following refers to these heroin samples only. Amounts of heroin received range from several hundreds of grams hidden in bags, boxes, and cases, to one or two small crystals coming from a suspect's pocket. In most cases, it is received in so-called "scene-quarters," i.e., amounts of approximately 100 mg folded in tinfoil. After determining the color by comparison, using a light microscope for spotting the type of crystals or starch, etc., TLC is used for a screening procedure. The advantage is that not only is heroin indicated, but also the number of constituents contained in the respective sample.

II. TLC AS SCREENING METHOD FOR HEROIN

A. Preparation of Sample

The preparation of a sample is rather simple. The heroin sample is dissolved in chloroform. According to our tests, the base and the HCl salt are sufficiently soluble in excess chloroform. The solution then is chromatographed in the normal manner.

B. Plates

Commercially available plates (Merck) have proved quite serviceable as, after an activation procedure, the *Rf*-values show satisfactory reproducibility; silica gel is used as a stationary phase.

C. Mobile Phases

The following mobile phases are recommended by an expert board of chemists of the Federal and Länder Crime Laboratories in accordance with tests made under equal conditions:

1.	Benzene	(80)	Ethanol	(20)	Ammonia	(1)
2.	Ethyl acetate	(85)	Methanol	(10)	Ammonia	(5)
3.	Methanol	(100)	Ammonia	(1)		
4.	Cyclohexane	(5)	Chloroform	(4)	Diethylamine	(1)
5.	Chloroform	(9)	Acetone	(1)		

A modified system is being used in Berlin only:

6.	Benzene	(7)	Ethyl acetate	(2)	Diethylamine	(1)

Normally three, but at least two, different systems of mobile phases are used. For comparison purposes, a standard heroin solution is added to each plate. High performance plates show good results in an adequately short time.

D. Methods of Detection

For detection of spots, plates are observed under UV light and sprayed with iodine-platinate, Dragendorff and van Urk reagents and $FeCl_3$-iodine solution.

After completion of this TLC procedure, samples which do not contain heroin are discarded.

III. GC ANALYSIS OF HEROIN

For further investigation of heroin samples, GC is used. All instruments utilized are supplied by Perkin-Elmer, Type F 22.

A. Preparation of Sample

For preparing samples, a method has been adopted which was recommended by the Crime Laboratory of Baden-Württemberg: a few milligrams of a heroin sample are stirred into 10 ml of chloroform with 100 mg of diethylamino-polysterine added with continuous stirring. One μl of the upper layer, without separating the layer, is used for GC analysis. This solution contains an adequate amount of heroin and/or other alkaloids, respectively, in the form of their bases.

B. Qualitative Analysis

Under normal conditions, no difficulties arise for spotting even trace amounts of heroin.

C. Quantitative Analysis

According to German law, it is important for the jury to know whether or not a suspect has been found in possession of a "not small amount" of a drug. Consequently, police laboratories are often requested to determine the concentration of heroin in a specific sample. Various methods have been used in the past, ranging from UV- and IR-spectrometry to titration with perchloric acid, but, of course, best results are gained by GC analysis using procaine hydrochloride as internal standard. A Sigma 10 Integrator (Perkin-Elmer) is a valuable aid.

D. Derivative Analysis

Advantages in analysis of heroin samples, either qualitatively or quantitatively, are gained by a derivative method recommended by the Federal Criminal Investigation Department. A one percent solution of a heroin sample in a mixture of 50 percent MSTFA, 1 percent TMCS, and 49 percent dimethylformamide is heated for 15 minutes in a water bath at 70°C. An aliquot of the solution is used for GC. In addition to heroin, other alkaloids, sugars, and other components of the mixture can be determined or identified by this method of silylation as well. One of the gas chromatographs is equipped with an autosampler, Type AS 41 (Perkin-Elmer), using a capsule technique instead of injection by microsyringe. This method of silylation can be easily transferred in such a way that the reaction takes place within the aluminum capsule. There is another advantage in using these capsules as reaction vessels. It is well known that determination of morphine in biological fluids, especially urine, is a considerable problem. We have added excess acetic anhydride to the extract in the capsule using pyridine as a solvent, thus converting morphine into heroin which can be easily determined by GC.

IV. GC-MS ANALYSIS OF HEROIN

For reasons already discussed during this Symposium, GC-MS is also used for drug analysis. Our experience has been with a quadrupole instrument supplied by Finnigan (Model 4000). The highly specific method of identification needs no further explanation.

A. Preparation of Sample

Preparation of the sample and conditions are the same as with GC.

B. Conditions

Our application of mass spectroscopy has been mainly EI; in only a few cases, CI also has been employed with methane, isobutane or ammonia as reagent gases. For trace amounts of heroin, SID has been used successfully.

C. Methods of Analysis

The identification of heroin by mass spectroscopy is considered to be an absolute proof in German courts, either after gas chromatographic separation from other components of a mixture has been made, or, in fewer cases, by use of the solid probe. GC-MS is likewise used for the identification of metabolized drugs in body fluids or other biological material. Generally, it can be said that heroin types 3 or 4 have been found in almost all cases. This does not exclude the possibility of dealers having added some other ingredients. For instance, the following have been found in samples of illicit heroin in Germany:

1. Opium alkaloids and their derivatives, i.e., acetylmorphine, codeine, acetylcodeine, thebaine, dihydrocodeine, narceine, noscapine, papaverine, and methadone.
2. Anesthetics, i.e., lidocaine, procaine, cocaine.
3. Benzodiazepines, i.e., diazepam.
4. Pyrazolone derivatives, i.e., antipyrine, aminopyrine.
5. Salicylic acid or phenol derivatives, i.e., salicylic acid, salicylamide, acetylsalicylic acid.
6. Hypnotics, i.e., barbital, carbromal, methaqualone.
7. Alkaloids, i.e., caffeine, quinine, strychnine, scopolamine.
8. Sugars.
9. Inorganic compounds, i.e., $CaCO_3$, $NaHCO_3$, $NaClO_3$, $KClO_3$.

A change in heroin itself has been noticed. There is an increasing number of samples containing mixtures of free bases and salts of heroin. Another noticeable change in the by-products is the appearance of papaverine and narcotine.

V. CONCLUSIONS

A. Developments

1. HPLC

In the near future, HPLC is a method that is also going to be adopted. At the Bavarian Police Laboratory, the following isocratic method for determining heroin has been developed, which can be used for qualitative and quantitative determinations[1].

The instrument is a Waters HPLC, a μ-Bondapak C_{18} column is used, the mobile phase is water (156 ml), acetonitrile (140 ml), and 1% aqueous $(NH_4)_2CO_3$ solution (4 ml) with a flow rate of 2 ml/min. 2,3-Dimethylnaphthalene is applied as internal standard. The results are extremely good, and this method is to be used in the Berlin Laboratory, too, employing a Perkin-Elmer HPLC (Model 3).

2. Use of Data Systems

The high rate of requests for investigations, as mentioned in the preface, forces us to apply data techniques, even in our small laboratory unit. Therefore, the GC-MS system is complemented by an Incos Data System, including a library with 23,000 mass spectra. The IR spectrometers have interfaces in order to employ an interdata system. As long as police laboratories remain responsible for fast and accurate analyses, they must be supplied with the necessary technical means. It must be understood, however, that no computer, no data system, not even the most sophisticated one, is able to assume the responsibility of an expert, and none of these systems used in forensic chemistry can see the link between analytical work and human fates.

(1) *Dr. G. Megges and Dr. J. Fehn, Bavarian Crime Lab, Munich. (In press)*

90458

Street Drugs and the Forensic Toxicologist

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A forensic toxicologist is concerned with the analysis of body tissue for drugs and poisons and the interpretation of the results obtained. It is not surprising therefore that he or she will come into contact with so called "Street Drugs", as some of these drugs are used legitimately in medicine while others such as butyl nitrite, methylenedioxyamphetamine (MDA), tetrahydrocannabinol (THC), and phencyclidine (PCP) have widespread illicit use. The analysis of body tissue for many of these non-medicinal drugs represents a considerable challenge for the toxicologist as adequate methodology is often not available. The approach we have tried to follow with street drugs is to incorporate their detection in the general screening procedures we are presently using for the detection of the common prescription and non-prescription drugs. This, of course, is not always possible and important exceptions will be dealt with later.

Screening procedures of one type or another are in use in most toxicological laboratories; these vary from simple inexpensive thin-layer chromatography (TLC) procedures to methods utilizing mass spectrometry. The use of gas chromatography (GC) for screening blood and urine for basic and neutral drugs is a technique in which we have taken a particular interest (1). We would like to describe our latest work in this area as we feel it would be of some interest to the reader.

GC screening of blood and urine for drugs has been used by us and others for a number of years. The differences in the methods used revolve around the extraction procedures and the type of GC liquid phases employed. Using such procedures many drugs are detectable at 0.1 mg/100 ml, a level that is adequate for many overdose situations but inadequate to detect therapeutic levels of some of the common drugs. Flame ionization detectors have generally been used in these procedures although electron capture detectors have found some application (2). The introduction of stable nitrogen phosphorus (NP) detectors marks a major advance in GC screening. These detectors were first used to screen the urine of athletes competing in the 1976 Montreal Olympics (3); the urine was extracted with ether and an aliquot injected directly into the GC. We have used a similar approach for screening blood. The extraction procedure is shown in Fig. 1 and as can be seen it is very simple. The choice of 1 ml of blood and 2 ml of solvent is not critical and was in fact dictated by the volume (2 ml) of the disposable vials used by the automatic liquid injector. Vials with a volume of 100 μl are available and the use of such vials would enable blood volumes of 0.1 ml to be used. The

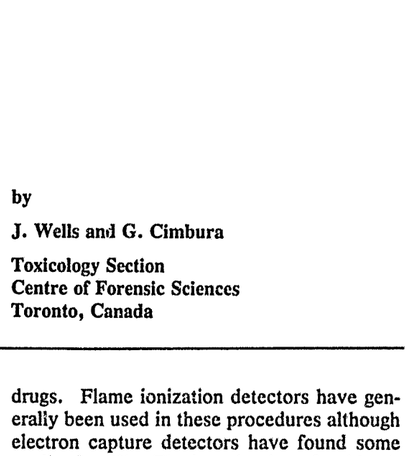


Fig. 1. Extraction procedure for neutral and basic drugs.

Fig. 2. Gas chromatographic equipment used in screening blood and urine for basic and neutral drugs.

choice of solvent for the extraction is limited in the sense that halogenated solvents are not recommended for routine use with NP detectors. We have found toluene to be adequate for most of the drugs of current interest.

The GC system is shown in Fig. 2; the small data system, after suitable calibration, converts retention times into retention indices. The sample (10 μl) is injected twice, once at 200°C and a second time at 240°C. The GC column system (Fig. 3) is a split glass column which we have used previously (4); the combination of OV-1 and Poly A-103 liquid phases enables us to screen for a large range of drugs. Figs. 4–8 illustrate the results; sensitivity is not generally a problem as most drugs are detectable below the 0.05 mg/100 ml level. An additional advantage of the NP detectors is that they do not respond to the lipid material nor-

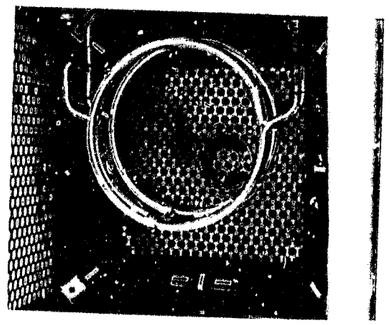


Fig. 3. The split glass column system used with dual NP detectors and packed with 2% OV-1 (Back Column) and 3% Poly A-103 (Front Column) both on 80/100 mesh Chromasorb W.

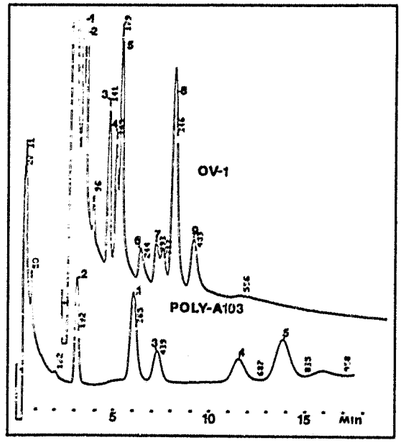


Fig. 4. GC Chromatograms obtained simultaneously (at 200°C) using the split column (Fig. 3) by injecting 10 μl of an acetylated toluene extract of spiked blood obtained as outlined in Fig. 1. The blood contains 1-Caffeine, (not quantitated), and 2-PCP, 3-Methadone, 4-Amitriptyline, 5-Imipramine, 6-Promazine, 7-Codeine, 8-Diazepam, 9-Chlorpromazine, at a concentration of 0.05 mg/100 ml.

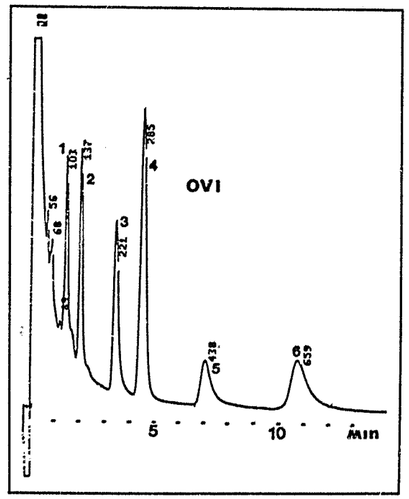


Fig. 5. A GC Chromatogram obtained (at 240°C) by injecting 10 μl of a toluene extract (Fig. 1) of blood (1 ml) containing 1-Codeine, 2-Chlorpromazine, 3-Stelazine, 4-Flurazepam, 5-Haloperidol, 6-Strychnine, all at a concentration of 0.05 mg/100 ml.

mally co-extracted with the drugs, although we suspect that this material does contribute to base line drift which can be a problem during overnight runs.

The use of a simple extraction procedure in combination with the sensitive and specific NP detectors enables the use of relatively inexpensive automatic injectors. This combination has had a dramatic effect on the number of samples that can now be screened; one technician can now screen 25 samples a day compared to 5 samples using our previous system (1).

Although NP detectors represent a considerable advance in GC analysis they do have some disadvantages. Putrefied bases are co-extracted with any drugs that may be present in blood or tissue and can interfere in the analysis of some of the more volatile drugs. Blood stored in tubes closed with rubber stoppers creates a problem as the NP detectors are very sensitive to the phosphate plasticizers found in rubber. Syringe contamination can also occur especially when using automatic injectors. The use of NP detectors is relatively new to forensic toxicology, however, we feel that their impact in specific drug analysis, as well as in

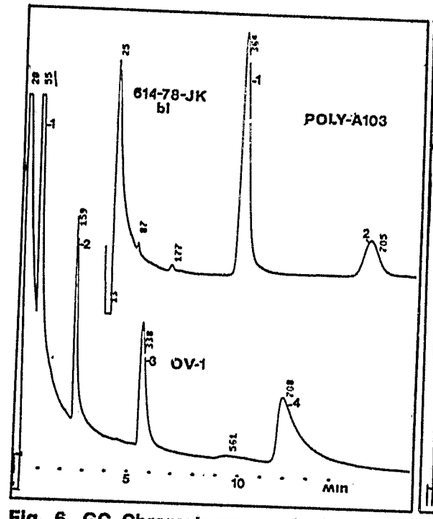


Fig. 6. GC Chromatograms obtained at 200°C from a case blood treated as in Fig. 1 and containing 1-Caffeine, 2-Amitriptyline (0.1 mg/100 ml), 3-Diazepam (0.04 mg/100 ml), 4-Acetylated Nortriptyline.

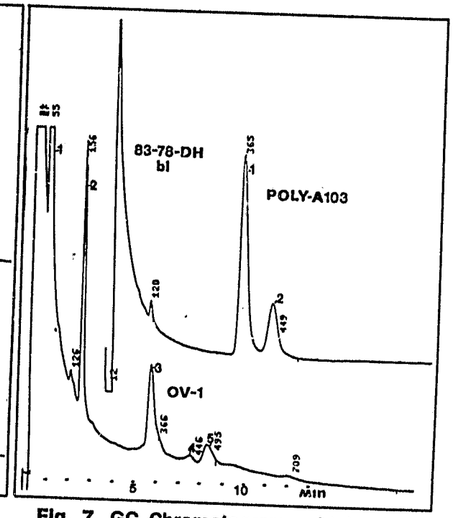
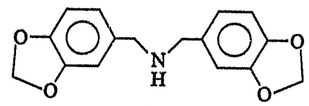


Fig. 7. GC Chromatograms obtained at 200°C from a case blood treated as in Fig. 1 and containing 1-Caffeine, 2-Propoxyphene (0.2 mg/100 ml), 3-Diazepam (0.06 mg/100), 4-N-Desmethyldiazepam, 5-Norpropoxyphenamide.

screening will be considerable.

The use of this GC screening system allows us to screen for many of the common street drugs. In a recent case MDA was suspected in a fatality and was indicated by GC in the blood; however, a second GC peak (of longer retention time) was also noted. GC/MS analysis indicated a structure (a) for this compound. The product (a) was also detected in the stomach



(a)

contents but no MDA was detected in that specimen. In our previous experience with MDA fatalities (5) we did not detect such a compound although we cannot be sure it wasn't present. We presume that product (a) could be formed by reacting the aldehyde (3,4 methylenedioxybenzaldehyde) with ammonia and reducing the condensation product with $LiAlH_4$. The authors are not specialists in street drug synthesis, however, and therefore leave it to those more knowledgeable in this area to rationalize the occurrence of (a).

There are a few street drugs that require special attention in that the usual analytical tech-

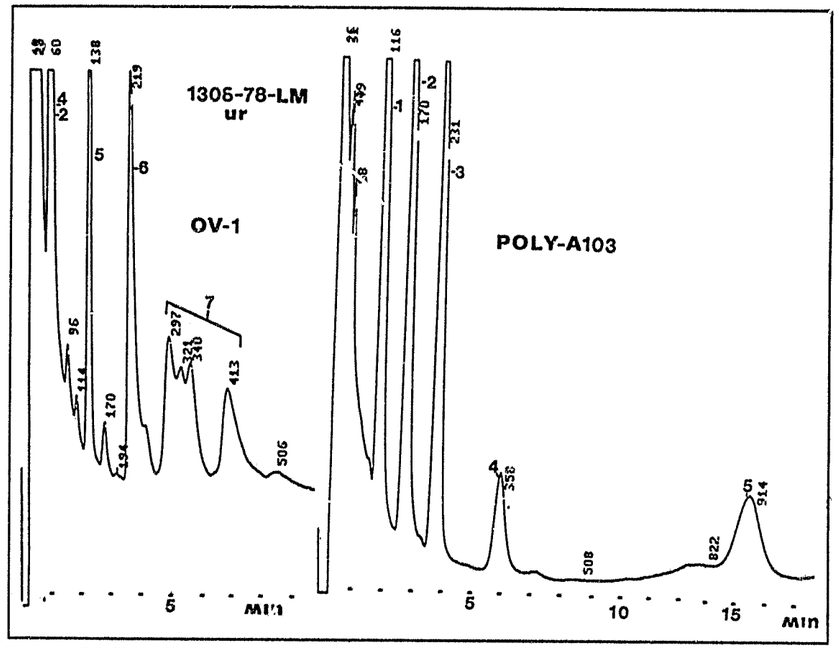


Fig. 8. GC Chromatograms obtained (at 200°C) from a urine sample (1 ml) treated as in Fig. 1 and containing 1-Methyprylon, 2-Diphenhydramine, 3-Methyprylon metabolite (6-Hydroxy), 4-Caffeine, 5-Methaqualone, 6-Acetylated Nordiphenhydramine, 7-Methaqualone metabolites.

niques available to the toxicologist are either inadequate or too cumbersome to be of much value. LSD (lysergic acid diethylamide), cocaine, and marijuana are examples of such drugs.

For a number of years we have used a commercially available radioimmunoassay (RIA) method (6) for the analysis of LSD in urine and blood and found it adequate as far as sensitivity is concerned. A few drugs, e.g., chlorpromazine, can interfere; however, by using a preparative TLC clean-up procedure of the urine extract we were able to increase the specificity of the technique (7).

The use of preparative high pressure liquid chromatography (HPLC) is a distinct improvement over TLC and has been used together with RIA in the detection of LSD in urine (8). A recent publication describes an elegant method for the detection of LSD in urine employing HPLC with a fluorescence detector (9). The detection of LSD using a mass spectrometer has been described and 50 femtograms were reported to be detectable (10). Our own results using mass spectrometry have not been as fruitful and, in fact, we have obtained greater sensitivity employing a GC equipped with NP detectors. Using a 2-ft. glass column (OV-1 and 250°), we have detected less than 1 ng of LSD injected on column. This level of detectability should be adequate for the confirmation of most positive LSD cases where urine levels of 2 ng/ml and greater are the norm.

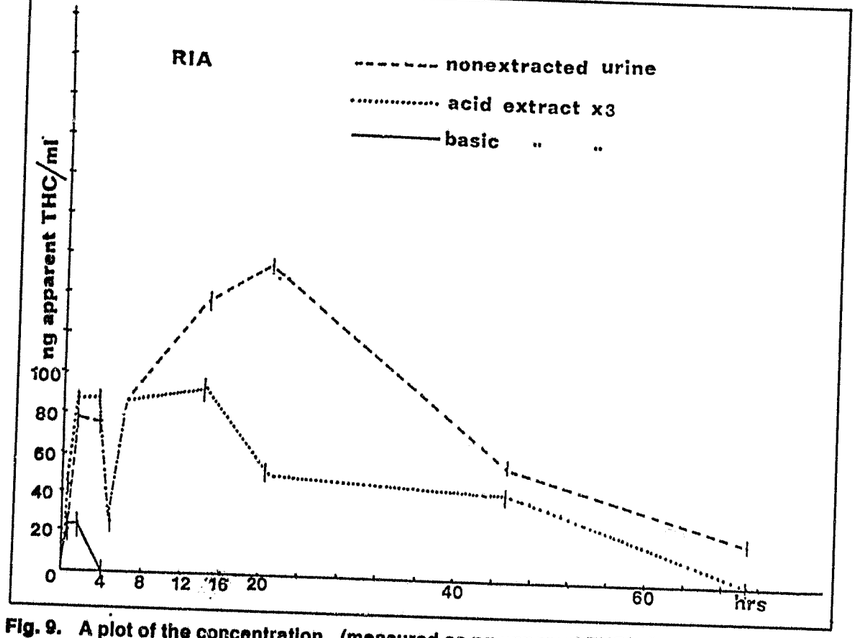


Fig. 9. A plot of the concentration (measured as ng apparent THC/ml) of those cannabinoids (in 1 ml of urine) that react with the THC antibody, against time (in hours) of urine collection, after smoking 12 mg of THC. The intermittent line (---) represents non-extracted urine. The dotted line (.....) represents a Di-Isopropylether (IPE) extract of the urine (1 ml, made strongly acidic) after enzyme hydrolysis. The solid line (_____) represents an IPE extract of the urine (1 ml, made strongly alkaline) after enzyme hydrolysis. Both the acid and basic values are multiplied by a factor of 3.

Benzoylecgonine is the principal metabolite of cocaine and the substance most likely to be found in the urine of cocaine users. We have used a GC method, similar to that described by Wallace *et al.* (11), for the detection of benzoylecgonine in which the metabolite is converted (by methylation) back to the parent cocaine. The method works well, but it is too time-consuming for the routine screening of urine specimens which are perhaps best screened using a RIA method (12).

The detection of cannabinoids in urine and blood is a difficult problem for most toxicologists. In Canada at the present time the author's laboratory is the only forensic laboratory which is reporting cannabinoid analysis on body fluids. There have been numerous methods published (13) for the detection of cannabinoids (mostly THC and metabolites) in urine and plasma; however, as far as we are aware, no method has been published for whole blood.

Our own work in this area* has centered around the use of a commercially available RIA method (6). The antibody was developed for THC; however, it does cross-react with a number of cannabinoids, in particular with 11-hydroxy THC, 8-hydroxy THC and the acid metabolite, Δ9-THC-9-oic acid. Fig. 9 illustrates the kind of results we have obtained in analyzing urine specimens of a smoker who over a period of 20 minutes smoked two "joints" containing a total of 12 mg of THC. The intermittent line represents the cannabinoid content (measured as ng/ml THC) of the non-extracted urine. The dotted line represents the cannabinoid content of an acid extract of the urine after enzyme hydrolysis and the solid line represents the cannabinoid content of a basic extract after enzyme hydrolysis. The interesting point here is that the basic extract is only positive for those urine specimens collected up to 2 hours after smoking and preliminary results indicate that a positive basic extract is associated with a reasonably high THC blood level. Although much more work is required, the positive RIA result of a basic urine extract may be indicative of recent marijuana smoking. It would be interesting to see what kind of results we would obtain if the marijuana smoked contained no THC, but, say a high level of cannabinol (CBN) as it is not known if the THC antibody we are presently using is sensitive to the hydroxy metabolites of CBN or even cannabidiol (CBD).

When MDA and more recently *para*-methoxyamphetamine (PMA) were first implicated in deaths in Ontario (14), we were not aware that such compounds were available on the street. Although the performance of screening procedures is constantly improving, perhaps a more practical step to ensure the detection of street drugs in tissue would be a closer cooperation at the local level between those agencies involved in street drug analysis and those involved in forensic toxicology.

The results of toxicological analysis can play an important role in alerting the public and authorities to the toxicity of street drugs. In Ontario, for example, our discovery of the danger of PMA (14) resulted in massive warnings to the public throughout Ontario and Canada, and was at least partly responsible for the placement of PMA on the list of "Restricted" drugs in Canada.

* *A full account of this work will be published.*

ACKNOWLEDGMENTS

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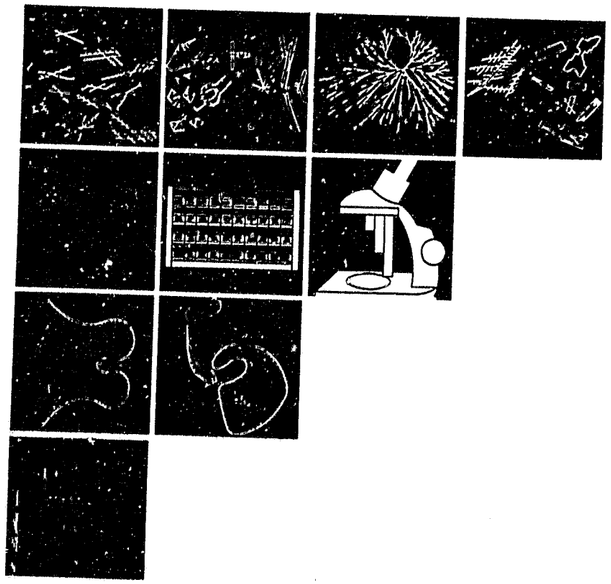
REFERENCES

1. J. Wells, G. Cimbura and E. Koves, *J. For. Sci.*, Vol. 20, 383, (1975).
2. J. A. F. Desilva, I. Bekersky, C. V. Puglisi, M. A. Brooks and R. E. Weinfield, *Anal. Chem.*, 48, 10 (1976).
3. R. Dugal and M. Bertrand, *Canadian Research*, Vol. 9, 17, (1974).
4. J. Wells, *J. Can. Soc. Forensic Sci.*, Vol. 8, 97 (1975).
5. G. Cimbura, *J. For. Sci.*, 17, 329 (1972).
6. *Collaborative Research Inc.*, Waltham, Mass. 02154.
7. G. Cimbura, *Unpublished work.*
8. A. S. Curry, *Private Communication.*
9. J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.*, 12, 496 (1976).
10. D. J. Marshall, B. A. Petersen and P. Vouros, *Biomedical Mass Spec.*, Vol. 5, 243 (1978).
11. J. E. Wallace, H. E. Hamilton, D. E. King, D. J. Bason, H. A. Schwertner, and S. C. Harris. *Anal. Chem.*, 48 (1976).
12. Hoffmann-La Roche Inc., Nutley, N. J. 07110.
13. *"Cannabinoid Assays in Humans"*, Research Monograph, Series 7, National Institute on Drug Abuse, Maryland, 20852.
14. G. Cimbura, *CMA Journal*, VOl. 110, 1263 (1974).

Special Topics Session



Impact of Instruments on Analysis of Drug Purity and Reference Standards

by

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As recent decades have witnessed a torrent of instrumental developments, analysis of chemical purity and reference standards have experienced a resultant flood of applications of instrumental methods. These have become the most important tests in drug purity and reference standard protocols. Each method has a known scope of applicability, and each has generated substantial cumulative experience. Reasonable assessments of the impacts of these methods may be made, therefore, both relative to classical methods and relative to recently instrumented versions of established chemical methods.

Our consideration of the impact of instrumental methods must be postponed momentarily because definitions are in order. The word "purity" is particularly elusive and culturally loaded.

I. PURITY CONCEPT

This treatment must be limited to chemical purity, although it is recognized that microbiological contamination is of some forensic significance. It is based on experience with over 700 different chemical reference standards (USP, NF and FCC). Chemical purity was best formulated in terms of physical chemistry (1) as the extent and sense in which a substance conforms to all known methods of resolving this or similar substances into more than one component. Unresolvable specimens conform to this definition and are, thereby, called "pure". We have a specimen of unqualified purity merely because we have failed to discern another component (impurity). To the forensic or medicinal chemist, therefore, purity is the extent to which all the atoms in a specimen are accounted for by a single component. That component may be defined by a single chemical formula, or perhaps as a single optical or positional isomer, or it may be limited to a single crystal modification.

Separation science, using modern instrumental methods, in particular leaps to mind when this general definition is considered.

A. Reference Standard

Most modern instrumental methods give quantitative and qualitative data relative to concomitant treatment of another sample of the same component, one which is of known composition, that is, a chemical reference standard. This is in contrast to some older methods, called "absolute", in which the equipment or reagents are separately standardized or calibrated by some other means, as in gravimetric or titrimetric methods.

We must define reference standard purity as *known composition with respect to intended use*. Adoption of a chemical reference standard demands a known purity level which is obtained by meaningful experiments. The experimental results are held up against the already known and certain challenges to the standard—the *intended uses* of the reference standard, the reasons that it was called into existence in the first place. Here is the anchor against aimless drifting on seas of arbitrary numericism (two, three or four "9's") or empiricism. In other words, we can say that, at the minimum, a chemical reference standard must be suitable for each intended use. Consider these examples: a chemically pure sample of one polymorph of a drug is unsuited as a reference standard if the intended use is to give positive identification to a different polymorph; on the other hand, highest purity is extraneous if the identification is to be made by thin-layer chromatography or solution spectroscopy.

Finally, a reference standard is a tool. It is a means to an end, not an end in itself. It usually serves rather limited purposes, and usually is consumed in the process.

II. PURITY TESTING AND PURITY PROFILES

Some analytical methods are crucial to drug purity testing because these methods tend to reveal the number and abundance of components (impurities) in a specimen. Purity-indicating methods are those which have demonstrated potential for resolution of a specimen into more than one component. Because compositional, rather than structural, information is sought, the most powerful, the high impact, general-purity methods are chromatographic rather than spectroscopic. This is not to disparage those classic measures of bulk properties which are prerequisites to refined purity testing: titrimetry, moisture, color or clarity of solution, characteristic spectra, elemental composition or physical constants. Optical rotation is an intermediate case. Indeed, nearly all classical methods now are available in labor-and time-savings instrumented versions. Classical methods have not been made obsolete by recent instrumental developments; rather, these are complementary. Our actual test load per sample therefore has increased regularly.

Compositional information given by each method has a characteristic, and limited, scope. A better picture would focus on the results of more than one method. Individual purity-indicating measurements converge to establish a *purity profile*. It is this purity profile (2, 3) alone which is meaningful and which allows decisions as to the "purity" of a specimen, or the scope of suitability of that specimen as a reference standard for certain stated applications. To confuse matters, purity measurements give data in different units, *i.e.*, mass or mole % purity, u.v.-absorptivity, acid-base balance, flame-ionization response, visualization response, etc. These units can be interchanged only when the identities and properties of all impurities are known. Because the identity of the impurity may not be as significant as the degree to which it interferes with measurement of the main component, thorough identification of trace or minor impurities often is unnecessary.

III. IMPACT RANKING

Figure 1 ranks purity-indicating methods by relative impact. It is based on experiences with about a thousand specimens of candidates for adoption as reference standards over a ten-year period. It is not meant to outline a general testing strategy applicable to most, or any particular, specimens. It is offered as an overall appreciation of methods. Quite a different ranking would result if identification, rather than compositional purity, was the objective. Special analyses such as X-ray spectroscopy are not listed as these are critical to only a few drugs where polymorphs are specified. On the other hand, useful bulk tests such as limits of heavy metals or ignition residues are not given as these rarely are violated. As an everyday fact of life in a reference standards program, little can compare to moisture. We live and work in the atmosphere of a wet planet. It can be no surprise that the moisture content of specimens is the foremost variable. Figure 1 does not contain moisture or solvent residues as these are variables which must be controlled

Information[1]	*Scope*[2]	*Overall Impact*[5]
TLC	Elemental, Spectral (IR,UV)	TLC
HPLC	TLC	*HPLC*
GLC	Titrimetry;[3] Classical functional group methods[4]	PSA
PSA	HPLC	Titrimetry; functional groups
Titrimetry;[3] functional groups[4]	Optical Rotation	*GLC*
Optical Rotation	PSA	Optical Rotation
Thermal Analysis (DSC)	GLC	Thermal analysis
Spectral (IR, UV)	Thermal Analysis	Spectral (IR, UV)
Elemental		Elemental

Figure 1. IMPACT RANKINGS

1. A function of potential for resolving specimens into components.
2. A function of proportions of specimens to which the measurement may be meaningfully applied.
3. Includes acid-base balance, aqueous or nonaqueous, chloride, complexes.
4. Includes polarography, preparation of derivatives, gravimetrics, functional group (i.e. ethinyl) titrations.
5. Based on data available to DSL-DRTL. Does not include moisture, loss-on-drying or thermogravimetric analysis.

in every laboratory, *e.g.*, drying instructions should accompany chemical reference standards, and results usually are expressed on the dry basis.

IV. COMMENTS ON INSTRUMENTAL METHODS

Gas-liquid and pressurized liquid chromatographies (GLC and HPLC) are instrumented methods which have obvious potential for resolving a specimen into components. Although not usually used in an instrumented version, thin-layer chromatography (TLC) also is an irreplaceable technique.

1. HPLC

Incorporation of this new development into the existing framework of pharmaceutical-organic analysis has been nothing less than precipitous. The number of published accounts forbids selection of a range of examples. It rapidly became the method of choice for steroids, and is being applied to most other families of drugs. Each year, the impact of HPLC has grown until it is now a mature, routine technique, particularly in laboratories working with drug purity or reference standards. Rapid development resulted from exploitation of existing capabilities: chromatographic know-how about adsorption, partition and ion-exchange; and, from gas chromatography, experience in chromatograph design and data reduction (not to mention inheriting the expectant, prepared market place).

Examples of the specificity achieved by HPLC are the assay of progesterone in the presence of fixed oils (4) and assay and purity evaluation of folic acid (5). These are just two examples of the great impact which HPLC is having on pharmaceutical analysis.

Solid solutions of impurities in the main component are a common feature of steroid chemistry, and often escape accurate assay by calorimetry or solubility analysis. Examples of HPLC resolving such mixtures are estradiol dipropionate (2) and testosterone propionate (acetate as second component).

Our laboratory performs HPLC analyses on about two-thirds of all candidate reference standards, and has examined all steroids during the last seven years.

The main problem in HPLC has been nonuniformity of packings, but some improvements in this sophisticated technology have been made, and the future promises even more useful packings. Limitations on detector scope are also of practical significance. Preparative HPLC presently is too expensive except for limited purposes, but this already is having impact on reference standards programs.

2. GLC

For purity work, low-polarity phases, such as the polysiloxanes, are preferred in order to minimize thermal degradation. Where a distillate is analyzed, GLC is an absolute method; more commonly, it is used to

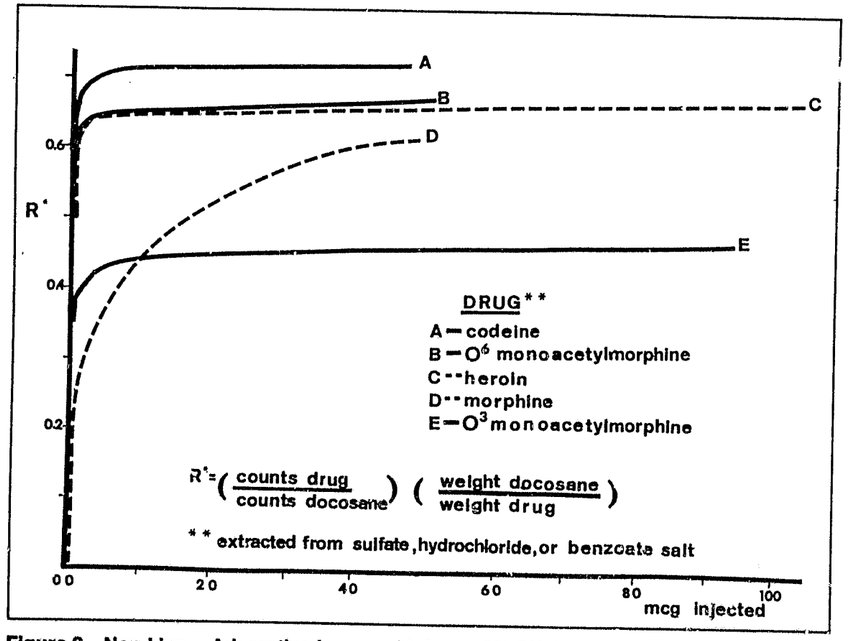

Figure 2. Non-Linear Adsorptive Losses (Column: 3% OV-1/gas chrom Q; He: 50 ml/min; FID)

compare batches of standards. Sources of error (2) are injection-port degradation which gives rise to other distinct peaks, and non-linear adsorptive losses. The latter is particularly important in that minor impurities can be grossly underestimated (see Figure 2). It is important to analyze different sample sizes, up to overload, to measure minor impurities in terms of fractional areas. This is a common practice because authentic samples of all possible impurities needed for "spiking" (i.e., standard addition method) are seldom in hand.

In many cases, GLC and HPLC are complementary, such as in the purity evaluation (3) of heroin. The considerable specificity and sensitivity of GLC also allows assay of small quantities of related drugs, such as atropine and scopolamine (6).

3. TLC (flat-beds)

These methods are so well appreciated and documented that little discussion is warranted. It is a mainstay of purity work. A variety of visualizations, and low cost, makes it clear that TLC cannot be replaced by HPLC. TLC has a characteristic unmatched by GLC or HPLC: all of the components of the applied specimen are somewhere on the plate; at worst, there is a failure to separate or visualize these. This assertion cannot be made for chromatography which detects only *emerging* components.

Recently, instrumented versions have appeared which are helpful in automatic elution of separated spots, or controlled multiple developments. Densitometers have been available for some time, but are generally viewed as unreliable for measuring impurities.

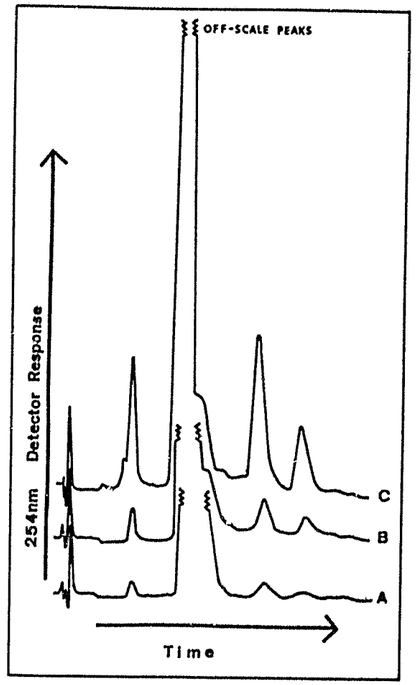

Figure 3. Betamethasone Acetate HPLC—Reversed Phase A=phase purified crystals; B=untreated drug; C=impurity enriched supernatant.

4. Phase-solubility analysis (PSA)

This is a gravimetric technique (1, 7) which has benefited from printout electronic microbalances and electronic computation. Although its importance in purity assessment has declined relative to HPLC and GLC, it is still useful and lends itself to semi-preparative applications. Integration of PSA with chromatography is important in working with minor impurities. Figure 3 shows a chromatogram of betamethasone acetate which is enriched in impurities by a precise factor obtained from previous solubility analysis.

From PSA, as shown in Figure 4, one determines both the solubility of pure drug and the fact that, at 3 to 10 times that solubility value, impurities continue to dissolve into the drug-saturated supernate; therefore, the supernatant liquid, from a system containing solvent and 3 to 10 times the amount of drug which can dissolve, will be enriched in impurities relative to the main component by a precise factor. Another application is to collect the undissolved, purified crystals from a solubility analysis. On a large scale, this is known as swish-purification or isothermal recrystallization, and also is based on a prior PSA.

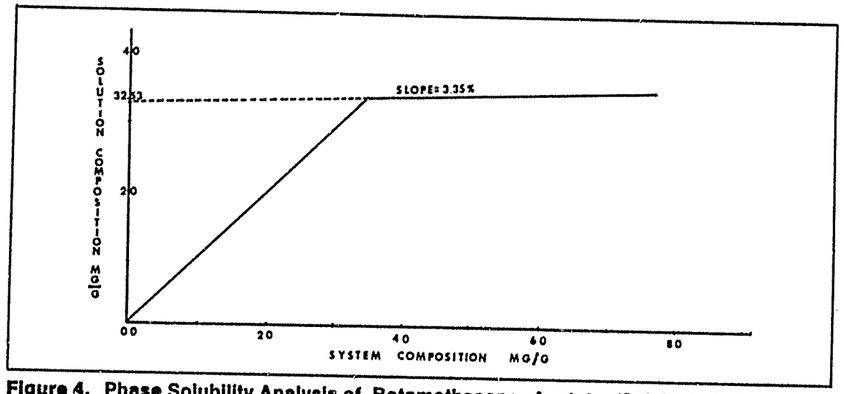

Figure 4. Phase Solubility Analysis of Betamethasone Acetate (Solubility 32.53 mg/g in 70% ethanol in water at 25°C, for 20 days).

REFERENCES

1. Webb, T. J., *Anal. Chem.* 20, 100 (1948).
2. Grady, L. T., Hays, S. E., *et al, J. Pharm. Sci.* 62, 456 (1973).
3. Hays, S. E., Grady, L. T. and Kruegel, A. V., *J. Pharm. Sci.* 62, 1509 (1973).
4. King, R. H., Grady, L. T. and Reamer, J. T., *J. Pharm. Sci,* 63, 1591 (1974).
5. Reif, V. D., Reamer, J. T. and Grady, L. T., *J. Pharm. Sci,* 66, 1112 (1977).
6. Wyatt, D. K., Richardson, W. G., *et al., J. Pharm. Sci,* 65, 680 (1976).
7. Mader, W. J., *CRC Crit. Rev. Anal. Chem.*, 1, 193 (1970).

Drug Analysis by Immunoassays

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I. INTRODUCTION

Analysts seldom recognize immunological techniques as part of their armamentarium. The sensitivity and selectivity of these novel (to most chemists) procedures are significant assets which should recommend them to analysts' attention. Because immunology is not part of the standard curriculum for chemists, they have little or no basic background in this area on which one could build a discussion of the value of immunology to the analytical chemist. Those chemists who are concerned with the analysis of licit or illicit substances have had little or no occasion to become sensitized to immunological procedures because analyses of physical evidence have not been modified to incorporate these techniques. Those concerned with the analysis of biological material may have had some experience in immunological procedures because these are being used more extensively as time goes on. Nonetheless, the field is relatively novel to most chemists and my charge is to develop some background in immunology for the readers so that they will become familiar with these useful and powerful techniques. Furthermore, I will assume that the readers have no knowledge with respect to these immunological procedures.

Since the basic principle of this procedure is based on the reaction between an antigen and an antibody, these entities must be defined. An antigen is an *antibody generator.* It is a chemical (hapten) which, when injected into an experimental animal, provokes an immune response by that animal to that hapten. This results in the production of antibodies in the animal's blood. In order to be effective, a hapten must have a large molecular weight. The substances of concern to chemists seldom have molecular weights greater than 300. This is insufficient to produce an antibody response. Hence it is essential to aggrandize the molecule, and this is done by coupling the drug with a suitable protein such as bovine serum albumin or some other large protein molecule. There are many synthetic pathways whereby this can be accomplished. These are beyond this discussion, but are detailed and referenced in Landon's and Moffat's excellent review article (*Analyst* 101 (1201), p. 224–243, 1976) and will not be repeated here. This coupling can be accomplished in one of several different ways, depending on which active site of the drug the protein is coupled. Each way will produce a slightly different hapten and a slightly different antibody. Thus the specificity of the antibody is a function of the hapten synthesis. This specificity needs careful elaboration so that the limitations of the procedure can be clearly understood.

These few sentences should not be taken as an indication that the described procedures are short and simple. On the contrary, good organic chemists are needed to synthesize the required condensation products (hapten) which will evoke the immune response to produce the desired antibody. This response is elicited by injecting, periodically, the experimental animal with the hapten. It usually takes two or three months before an adequate titer (strength) is developed. Different animals in the same species will develop different titers. The variation from species to species can also be significant. The resulting antibody hopefully is so strong that even a few milliliters will go a long way. Rabbits are generally used as the experimental animals, but rats, sheep, and even horses have been used. Sheep are particularly useful when a large volume of antibody is desired because there is expectation of great

demand for the antibody. These animals are literally worth their weight in gold.

Having successfully generated a sensitive antibody (Ab) how does one use it? Very simply, by reacting it with an antigen (A) according to the general equation shown below:

$$\underset{\text{(antigen)}}{A} + \underset{\text{(antibody)}}{Ab} \rightarrow \underset{\text{(complex)}}{A{\cdot}Ab}$$

If one adds to this system another component which contains a labeled antigen (A_1) it will compete with the unlabeled antigen (A) for the antibody and form some labeled antibody-antigen complex ($A_1 \cdot Ab$). The labeled antigen (A_1) will be distributed in this system—some in the free state (A_1) and some in the bound state ($A_1 \cdot Ab$). This distribution is a function of the unlabeled antigen (drug in sample). Thus, by measuring the amount of labeled products, one may determine the amount of competing antigen (drug) in the sample.

Of the many techniques available for labeling antigens, only two will be discussed—radioimmunoassay (RIA) and homogeneous immunoassay, particularly an enzyme multiplied immunoassay technique (EMIT).

II. RADIOIMMUNOASSAY (RIA)

In radioimmunoassay the antigen is labeled with one of the following radioactive atoms—^{3}H, ^{14}C, or ^{125}I. To achieve an analytical result in this system, one must separate the labeled antigen-antibody complex from the labeled antigen, and count either or both of the separated fractions. The quantitative measurement is based on the ratio of the bound label to the free, the bound to the total, or free to total. The separation of these fractions may be achieved in several different ways, among the most common of which are: (a) precipitations of proteins usually with ammonium sulfate; and (b) adsorption of the labeled antigen on a suitable adsorbent, such as charcoal or silica gel. In (a) the precipitate contains the labeled antigen-antibody complex which, when separated from the supernatant containing the unreacted labeled antigen, can be counted. In (b) the unreacted labeled antigen is adsorbed by the adsorbent, and the supernatant fluid contains the labeled antibody-antigen complex and this may be counted. Actually, each fraction in both procedures may be counted to get a ratio of free/bound; or one may be counted and its ratio to the total count can be used. Labeled products with ^{3}H and ^{14}C require a beta counter and scintillation fluid and special vials, and are more tedious to handle than those using a gamma counter for ^{125}I labeled materials. Once counted and the ratios calculated, then one may quantitate by comparing the ratios obtained from each sample with a standard curve. This curve is obtained by adding known amounts of the drug in question to drug-free specimens, analyzing these simultaneously with the specimens under concern, and plotting the resulting ratios against concentration, or sometimes log concentration.

RIA procedures require a reasonable amount of skill and aptitude from the analyst, particularly in handling small volumes; excellent centrifuges, a "hot" laboratory for radioactive materials (even though those in use for RIA have little radioactive hazard), and expensive counting equipment. The time required to complete one analysis is 60–90 minutes. Reagent costs are high and technician time requirements are moderate. A large number of specimens can be processed simultaneously and the counting can be done automatically, thus reducing the average technician time requirements for an analysis when a large number are to be done simultaneously. If the

demand for a particular assay is not high, then shelf-life of the reagent may be a cost problem because the material may become useless if not used in a reasonable time period.

III. ENZYME MULTIPLIED IMMUNOASSAY TECHNIQUE (EMIT)

In an effort to avoid the tedium of the separation steps required for RIA and the need for a "hot" laboratory, another and very ingenious technique was developed which eliminates these problems (Ullman, E. F., *et al., J. Biol. Chem.*, 251, 472, 1976). Called EMIT, it uses an enzyme to tag the antigen. In this procedure, the drug and the drug-labeled antigen compete for the antibody sites and, as a result of the equilibrium which is established, some of the enzyme from the labeled antigen is released. In the presence of a suitable substrate, a reaction ensues and its rate is a function of the concentration of the drug in the sample specimen (Figure 1). Measuring this reaction rate thus measures the amount of drug in the sample when this rate is compared with those obtained by analyzing samples of known concentration of drug added to drug-free specimens processed simultaneously with the samples in question. No separation of material is required. The entire procedure can be carried out in one vessel, and, thanks to modern engineering, the rate can be measured in less than one minute. Computations have been simplified by the manufacturer who currently markets the most frequently used homogeneous assay procedure (so-named because no separation is required). Syva Corporation provides Probit charts which linearize the standard curve data, but at a price. The middle range permits good precision and accuracy, but this decreases some at either the high or low concentration ranges. Nonetheless, even in these ranges, the CV's obtainable are well within the tolerances acceptable for clinical data. The literature is replete with data attesting to this.

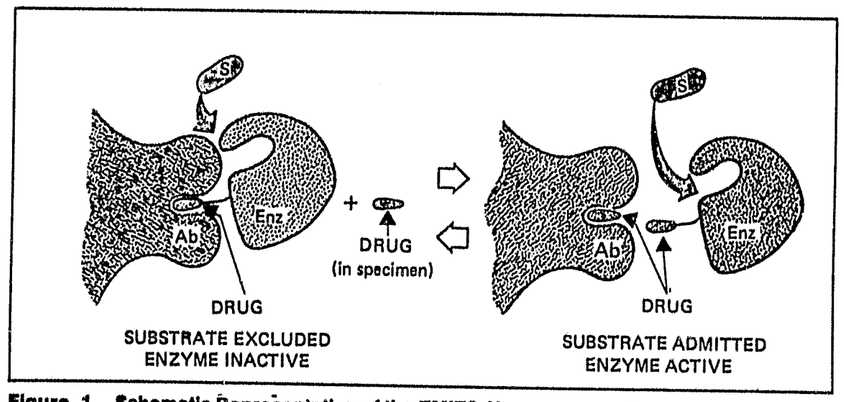

Figure 1. Schematic Representation of the EMIT® Homogeneous Enzyme Immunoassay for Drug Determination.

The manipulative skills required for EMIT analyses are minimal and easily learned. The time to analyze one sample is 40–60 seconds *in toto*. A numerical result ensues, making interpretation simple and objective. The reagents are stable for at least six months. The required bench space for the entire analysis is about 4 feet. If a qualitative analysis is required—positive or negative—then only three determinations are required: a blank, a sample containing the concentration above which a result is considered positive, and the test sample. If other specimens are to be tested within 1–2 hours after the first test sample, it is not essential to repeat the first two tests. Reagent costs are comparable to those for RIA and total cost is lower because less technician time is required and no special vials or scintillation fluid are needed. Costs may be significantly reduced, if many specimens are to be analyzed at one time, by using expensive automatic enzyme analyzers or centrifugal fast analyzers. Their high cost can be amortized in a reasonable period of time if the volume of analyses is high.

IV. IMMUNOLOGICAL PROCEDURES

Having described the two principle methodologies now in common use, some general comments on immunological procedures are in order.

For clinical purposes, when one analyte is involved, this technique compares favorably with many other procedures. However, if one must analyze a specimen for more than one substance, e.g., phenytoin, phenobarbital and primidone, then three separate analyses must be performed in contrast to chromatographic procedures which will determine all three substances in one analysis.

It is very difficult to produce an antibody that is specific for one drug and none of its analogues. To do so is laborious, difficult, and very expensive, although theoretically possible. Hence a positive immunoassay indicates that a drug in a given family of drugs may be present, but does not indicate which one is present. Quantitative data are difficult to achieve because the specific drug in question may not be identified by the immunological technique. If one knows the drug in question, e.g., clinical evaluation of digoxin or the antiepileptic drugs, then reasonably precise and accurate data may be achieved. Table I illustrates this problem with data for the barbiturate assay where the hapten was produced using secobarbital and all the others are less sensitive, barbital being 1/10 that of secobarbital. However, though this may seem to be a liability in some respects, it is an asset in another—i.e., in answering the question of whether or not a given class of drugs is present or absent. This question is frequently posed in the diagnosis of acute poisoning, and if the immunoassay is performed and it is negative, then you can be sure that that group of drugs is *not* present and you must seek elsewhere for the diagnosis.

Another aspect of immunoassays that must

TABLE I

Relative Activity of Five Barbiturates (100 μg/liter Secobarbital Standard)

Secobarbital	**100***
Pentobarbital	**45**
Butabarbital	**45**
Amobarbital	**35**
Phenobarbital	**25**
Barbital	**10**

***Secobarbital equivalents, μg/liter of urine.**

(Reprinted from original source: R. Cleeland, J. Christenson, M. Usategui-Gomez, J. Heveran, R. Davis, and E. Grunberg, Clin. Chem., 22 (6), 712–725, 1976)

be checked is the specificity of the antibody, not only for other drugs related chemically to the antigen, but also for its specificity against the metabolites of the drug. If these metabolites are present and the antibody titer for them is unknown, some disparate data may ensue.

Non-specific reactions to most antibodies occur from many sources. From the data in Table II, one would hestitate to indicate a sensitivity less than 30 μg/l for the ^{125}I assay and less than 50 μg/l for the ^{3}H assay. Similar, but not identical data are demonstrable for all immunoassays and one must select a realistic "low cut off" value to avoid false positive results.

Two other aspects of sensitivity need discussion. If one obtains a positive immunoassay result and has to certify that the result is accurate, then at least a second independent technique is required. To obtain a second chemically or physically different analysis which has the same sensitivity of the immunoassays is quite difficult. GC/MS might do this, by ion monitoring. But as yet, few laboratories have this potential. There are a few, if any, other procedures that will satisfy this requirement. Hence one's ability to certify with certainty that a result obtained is precise and accurate is open to question unless large amounts are present, and these then are detectable by less sensitive methodology. An immunoassay for tetrahydrocannabinol has been developed and it is to be tested on urines of drivers suspected to be "under the influence" to ascertain what role, if any, THC plays in vehicular accidents. In the test program, a non-punitive one, all alleged positive THC results by immunoassay will be checked by a sensitive GC/MS procedure. It will be most interesting to learn what degree of correlation will be obtained in this study. In practical terms, for medico-legal purposes, the probability is great that the correlation will not be high enough to allow one to state "without a reasonable doubt" that the immunoassay result alone is accurate.

TABLE II

Apparent Concentration of Morphine in Urines of 100 Normal Individuals

μg ME/liter*	^{125}I assay %	^{3}H assay %
0–9	90	70
10–19	8	17
20–29	2	6
30–39	0	5
40–49	0	2

*Results expressed as morphine equivalents (ME).

(Reprinted from original source: R. Cleeland, J. Christenson, M. Usategui-Gomez, J. Heveran, R. Davis, and E. Grunberg, Clin. Chem., 22 (6), 712–725, 1976)

V. CONCLUSION

With the resurging demand for increased monitoring of urines for the detection of those drugs which may be abused, immunoassay offers a novel approach. If 300 samples are to be tested, one could pool each of three and analyze the 100 samples thus prepared. As the chart below indicates, even if 10% of the samples were positive, a most unusual situation based on past experience, only 130 analyses need be done instead of 300. This is a significant saving of time, effort, and reagents, and can be done effectively if the sensitivity of three times the "low cut off" is adequate.

% positive	0	3	5	10
No. of analyses	100	109	115	130

TABLE III

Applications of Immunoassays to Drug Analysis

Drug	*References*
Amitriptyline	335, 336
Amphetamines	21, 66, 72, 111, 195, 196, 321, 328, 337, 338
Antibiotics	27
Antipyrine	47, 339
Aprindine	340
Aspirin	30, 85, 298
Atropine	73, 341, 437
Barbiturates	51, 78, 111, 128, 195, 196, 198, 226, 257, 258, 276, 290, 320, 327, 342
Benzoylecgonine	140, 322, 343, 353, 354
Benzodiazepine	324
Caffeine	344
Cannabinoids	48, 93, 101, 180, 181, 270–273, 345–347, 438
Carbamazepine	316, 348–351
Chloramphenicol	109
Chlordiazepoxide	61, 62, 352, 359, 439
Chlorpromazine	134, 179, 360, 440
Clomipramine	361, 362
Clonazepam	358, 363
Clozapine	364
Cocaine	133, 140, 278, 281, 322
Codeine	11, 74, 84, 100, 261, 279, 282, 289, 355, 356
Colchicine	23, 69, 341, 357
Cotinine	156, 158, 283
Debrisoquin	365
Desmethylimipramine	260, 366, 442
Dexamethasone	68, 115, 162, 187
Diazepam	207, 352, 359, 367, 368
Digitalis	104, 251, 252
Digitoxin	16, 64, 83, 108, 150, 203
Digoxin	10, 12, 16, 18, 25, 28, 33, 35, 54, 60, 63, 77, 79, 89, 94–97, 99, 103, 110, 112, 117, 119, 124, 131, 141–147, 150, 160, 161, 172, 176, 199, 202, 203, 204, 209, 216, 242–244, 246, 248–250, 288, 291, 329–332, 369, 370
Dihydrodigoxin	99, 141
Diphenylhydantoin (see phenytoin)	20, 29, 58, 165, 192, 208, 262, 274, 308, 309, 311, 313, 315, 317, 320, 372, 373, 422–426
Ethosuximide	314
Etorphine	91, 220
Fentanyl	114, 275, 374–376, 445
Flupenthixol	377–379
Gentamicin	12, 27, 169, 177, 189
Glutethimide	320
Haloperidol	380
Heroin	1, 11, 12, 37, 39, 90, 166, 237
Indomethacin	381
Loperamide	382
Lysergic acid diethylamide	267, 175, 301, 254
Marijuana	see cannabinoids
Meperidine	287, 383, 384, 409
Meprobamate	320
Mescaline	217, 239
Metanephrine	387
Methadol, α-acetyl	185
Methadone	90, 173, 185, 228, 323, 385, 386
Methaqualone	15, 22, 24, 225, 389, 390
Methotrexate	19, 52, 113, 130, 174, 215, 292, 391–396
Morphine	1, 2, 3, 11, 13, 24, 34, 38–40, 74, 82, 84, 100, 111, 137–139, 166, 167, 193, 196, 197, 230–237, 247, 256, 257, 259, 261, 265, 276, 279, 284, 286, 294, 319, 325, 328, 399–408
Naloxone	14, 443
Nicotine	42, 105, 106, 156, 183, 283, 444
Ouabain	241
Penicillin	27, 87
Penicillamine	5
Pentazocine	298
Perphenazine	179
Phenobarbital	20, 29, 50, 56, 210, 233, 234, 238, 262, 308, 309, 311, 313, 315, 410–422
Phenothiazines	255
Phenytoin	4, 56, 58, 75, 163, 165, 210, 218, 221, 224, 311, 314, 315
Primidone	310, 312, 315, 317, 429
Propranolol	430
Reserpine	431
Serotonin	257
Tetracycline	27, 211
Tetrahydrocannabinol	57, 272, 432–434
Theophylline	59
Thyroxine	143, 333, 334, 435
d-Tubocurarine	123, 184, 436

Applications: An extensive bibliography of immunoassays is attached to this article and is indexed in Table III. Because RIA and EMIT have not been indicated in Table III, a separate Table IV indicates the EMIT applications.

A very novel application of RIA was made by Möller, *et al.* which merits attention. They dropped blood and urine on samples of cotton, wool, and fired clay and stored these dry or under humid conditions for several days. As Table V indicates, using RIA the drug was detectable in the blood stains, but not easily in those from the urine samples. Much more work will be needed to be certain this has general application, but it merits further investigation.

Another interesting application has been developed in the Home Office Research Establishment at Aldermaston. They tested the eluate from an HPLC column with an RIA assay, and thus had a very sensitive detector for this chromatographic technique. Again, further development is needed, but the potential for a new HPLC detector is indicated.

Other novel approaches include the use of fluorescence as a labelling device rather than an enzyme or a radioactive molecule. Applications to opiates and gentamicin have been described (Burd, J. F., *et al., Clin. Chem.*, 23/8, 1402–1408, 1977).

Still other techniques are probable using enzyme systems linked to substrates that develop chromophores. Imagine lining up many different tubes, adding an aliquot of the test sample to each and noting any positive results by the development of a characteristic color in the tube.

This last vision is a fit closing to the rather superficial presentation of a most valuable and novel analytical technique which merits every analyst's attention and consideration. The portent for increased use is great and will be realized as more ingenious investigators apply their talents to this area.

TABLE IV
EMIT Assays

A—THERAPEUTIC DRUGS	*B—"DRUGS" SUBJECT TO ABUSE*
1. Antiepileptic drugs	1. Morphine
a. Phenytoin	2. Methadone
b. Phenobarbital	3. Amphetamines
c. Primidone	4. Benzoyl ecgonine
d. Carbamazepine	5. Barbiturates
e. Ethosuximide	6. Tetrahydrocannabinol
2. Cardiovascular drugs	
a. Digoxin	
b. Lidocaine	
c. Procainamide	
d. N-acetylprocainamide	
e. Quinidine	
f. Propranolol	
3. Respiratory drugs	
a. Theophylline	
4. Anticancer drugs	
a. Methotrexate	

TABLE V
Results of RIA Detection
(Reprinted from original source: M. R. Möller, D. Tausch, and G. Biro, Z. Rechtsmed., 79 (2), 103, 1977.)

		Blood				
	storage	"humid"			"dry"	
	time [day(s)]	7	21	1	7	21
5 ng	cotton	+++	++	+++	+++	++
	wood	–	–	++	+++	++
	fired clay	–	–	?	+++	+
20 ng	cotton	++	+	+++	+++	++
	wood	+++	++	+++	+++	+++
	fired clay	+++	++	++	+++	++

		Urine				
	storage	"humid"			"dry"	
	time [day(s)]	7	21	1	7	21
5 ng	cotton	+	–	–	–	–
	wood	–	–	–	–	–
	fired clay	–	–	–	–	–
20 ng	cotton	++	+	++	++	++
	wood	–	–	–	–	–
	fired clay	–	–	–	–	–

20 ng	+++: 10.0–13.9 ng recovery of morphine	
	++ : 5.0– 7.6 ng	" " "
	+ : 4.3– 5.0 ng	" " "
5 ng	+++: 2.5– 3.1 ng	" " "
	++ : 2.0– 2.5 ng	" " "
	+ : 1.7– 2.0 ng	" " "

REFERENCES

1. Adler, F. L., Lin, C. T., and Catlin, D. H., Immunological studies on heroin addiction. I. Methodology and application of a hemagglutination inhibition test for detection of morphine, *Clin. Immunol. Immunopathol.*, 1, 53, 1972.
2. Adler, F. and Liu, C.-T., Detection of morphine by hemagglutination-inhibition, *J. Immunol.*, 106, 1684, 1971.
3. Aherne, G. W., Marks, V., Morris, B. A., Piall, E. M., Robinson, J. D., and Twycross, R. G., Proceedings: the measurement of serum morphine levels by radioimmunoassay follow oral administration of diamorphine or morphine, *Br. J. Pharmacol.*, 54(2), 228P, 1975.
4. Aherne, G. W., Marks, V., Morris, B. A., and Robinson, J. D., Measurement of serum phenytoin levels by radioimmunoassay following single oral doses proceedings, *Br. J. Clin. Pharmacol.*, 2(4), 382P, 1975.
5. Assem, E. S., Assay of penicillamine in blood and urine, *Curr. Med. Res. Opin.*, 2(9), 568, 1974.
6. Barrett, M. J. and Cohen, P. S., *Clin. Chem.*, 18, 1339, 1972.
7. Bastiani, R., Clinical Study #20, Syva Corp., Palo Alto; Calif., 1975.
8. Bastiani, R., Clinical Study #20, Syva Corp., Palo Alto, Calif., 1976.
9. Bastiani, R. J., Phillips, R. C., Schneider, R. S., and Ullman, E. F., Homogenous immunochemical drug assays, *Am. J. Med. Technol.*, 39(6), 211, 1973.

10. Battaglia, D. J. and Cianci, M. L., Iodine-125-digoxin radioimmunoassay: comparison of commercial kits, *J. Nucl. Med.*, 17(9), 847, 1976.

11. Bennett, R., Critical determinants enabling an antibody to distinguish morphine from heroin, codeine and dextromethorphan, *Immunochemistry*, in press.

12. Berk, L. S., Lewis, J. L., and Nelson, J. C., One-hour radioimmunoassay of serum drug concentrations, as exemplified by digoxin and gentamicin, *Clin. Chem.*, 20(9), 1159, 1974.

13. Berkowitz, B. A., Cerreta, K. V., and Spector, S., The influence of physiologic and pharmacologic factors on the disposition of morphine as determined by radioimmunoassay, *J. Pharmacol. Exp. Ther.*, 191(3), 527, 1974.

14. Berkowitz, B. A., Ngai, S. H., Hempstead, J., and Spector, S., Disposition of naloxone: use of a new radioimmunoassay, *J. Pharmacol. Exp. Ther.*, 195(3), 499, 1975.

15. Berman, A. R., McGrath, J. P., Permisohn, R. C., and Cella, J. A., Radioimmunoassay of methaqualone and its monohydroxy metabolites in urine, *Clin. Chem.*, 21(13), 1878, 1975.

16. Besch, H. R., Jr. and Watanabe, A. M., Radioimmunoassay of digoxin and digitoxin, *Clin. Chem.*, 21(12), 1815, 1975.

17. Bidanset, J. H., Drug analysis by immunoassays, *J. Chromatogr. Sci.*, 12(5), 293, 1974.

18. Boguslaski, R. C. and Dennings, C. E., A column radioimmunoassay method for the determination of digoxin, *Biochem. Med.*, 14(1), 83, 1975.

19. Bohuon, C., Duprey, F., and Boudene, C., Radioimmunoassay of methotrexate in biological fluids, *Clin. Chim. Acta*, 57(3), 263, 1974.

20. Booker, H. E. and Darcey, B. A., Enzymatic immunoassay vs. gas/liquid chromatography for determination of phenobarbital and diphenylhydantoin in serum, *Clin. Chem.*, 21(12), 1766, 1975.

21. Bost, R. D., Sutheimer, C. A., and Sunshine, I., Relative merits of some methods for amphetamine assay in biological fluids, *Clin. Chem.*, 22(6), 789, 1976.

22. Bost, R. D., Sutheimer, C. A., and Sunshine, I., Letters: methaqualone assay by radioimmunoassay and gas chromatography, *Clin. Chem.*, 22(5), 689, 1976.

23. Boudene, C., Duprey, F., and Bohuon, C., Radioimmunoassay of colchicine, *Biochem. J.*, 151(2), 413, 1975.

24. Brattin, W. J. and Sunshine, I., Immunological assays for drugs in biological samples, *Am. J. Med. Technol.*, 39(6), 223, 1973.

25. Brock, A., A radioimmunoassay for digoxin in serum, urine, and myocardial tissue, *Acta Pharmacol. Toxicol.*, 34(3), 198, 1974.

26. Broughton, A. and Ross, D. L., Drug screening by enzymatic immunoassay with the centrifugal analyzer, *Clin. Chem.*, 21(2), 186, 1975.

27. Broughton, A. and Strong, J. E., Radioimmunoassay of antibiotics and chemotherapeutic agents, *Clin. Chem.*, 22(6), 726, 1976.

28. Brown, D. D. and Abraham, G. N., Plasma digoxin levels in normal human volunteers following chronic oral and intramuscular administration, *J. Lab. Clin. Med.*, 82(2), 201, 1973.

29. Brunk, S. D., Hadjiioannou, T. P., Hadjiioannou, S. I., and Malmstadt, H. V., Adaptation of "Emit" technique for serum phenobarbital and diphenylhydantoin assays to the miniature centrifugal analyzer, *Clin. Chem.*, 22(6), 905, 1976.

30. Butler, G. C. et al., *Biochem, J.*, 34, 838, 1940.

31. Butler, H. A. et al., Eds., *Radioimmunoassay Methods*, Churchill Livingstone, Edinburgh, 1971, 635.

32. Butler, V. P., Jr., Drug immunoassays, *J. Immunol. Methods*, 7(1), 1975.

33. Calesnick, B. and Dinan, A., Improved micro-radioimmunoassay of digoxin in serum, with use of ^{125}I-labeled digoxin, *Clin. Chem.*, 22(6), 903, 1976.

34. Capelle, N., Fournier, E., Kuntz, M. O., and Treich, I., Use of radio-immunologic technics in the study of the antigenicity of morphine, *Eur. J. Toxicol. Environ. Hyg.*, 7(3), 147, 1974.

35. Carliner, N. H., Gilbert, C. A., Pruitt, A. W., and Goldberg, L. I., Effects of maintenance digoxin therapy on systolic time intervals and serum digoxin concentrations, *Circulation*, 50(1), 94, 1974.

36. Castro, A. et al., *Res. Commun. Chem. Pathol. Pharmacol.*, 6, 879, 1971.

37. Cate, J., IV, Clarkson, M., Strickland, J., and D'Amato, N. A., Spin immunoassay for opiates in urine—results of screening military personnel, *Clin. Toxicol.*, 9(2), 235, 1976.

38. Catlin, D. H., Urine testing: a comparison of five current methods for detecting morphine, *Am. J. Clin. Pathol.*, 111, 472, 1973.

39. Catlin, D. H., Adler, F. L., and Liu, C.-T., Immunological studies on heroin addiction. II. Applications of a sensitive hemagglutination-inhibition test for detecting morphine to diagnostic problems in chronic heroin addiction, *Clin. Immunol. Immunopathol.*, 1, 446, 1973.

40. Catlin, D. H., Cleeland, R., and Grunberg, E., A sensitive rapid radioimmunoassay for morphine and immunologically related substances in urine and serum, *Clin. Chem.*, 19, 216, 1973.

41. Centeno, E. R. et al., *Int. Arch. Allergy Appl. Immun.*, 37, 1, 1973.

42. Cernosek, S. F. et al., *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 32, 511, 1973.

43. Chambers, R. E., Digoxin radioimmunoassay: improved precision with iodinated tracer, *Clin. Chim. Acta*, 57(2), 191, 1974.

44. Challard, G. et al., *Br. Med. Bull.*, 30, 38, 1974.

45. Chamberlain, D. A. et al., *Br. Med. J.*, 3, 429, 1970.

46. Chang, J. J. et. al., *Clin. Chem.*, 21, 967, 1975.

47. Chang, R. L., Wood, A. W., Dixon, W. R., Conney, A. H., Anderson, K. E., Eiseman, J., and Alvares, A. P., Antipyrine: radioimmunoassay in plasma and saliva following administration of a

high dose and a low dose, *Clin. Pharmacol. Ther.*, 20(2), 219, 1976.

48. Chase, A. R., Kelley, P. F., Taunton-Rigby, A., Jones, R. T., and Harwood, T., Quantitation of cannabinoids in biological fluids by radioimmunoassay, *Natl. Inst. Drug Abuse Res. Monogr. Ser.*, 7, 1, 1976.
49. Cheng, L. T., *FEBS Lett.*, 36, 339, 1973.
50. Chung, A., Kim, S. Y., Cheng, L. T., and Castro, A., Phenobarbital-specific antisera and radioimmunoassay, *Experientia*, 29(7), 820, 1973.
51. Cleeland, R., Christenson, J., Usategui-Gomez, M., Davis, R., Heveran, J., and Grunberg, E., A simple, rapid ^{125}I radioimmunoassay for the detection of barbiturates in biological fluids, *J. Forensic Sci.*, 20(1), 45, 1975.
52. Colburn, W. A., *Steroids*, 25, 43, 1975.
53. Colburn, W. A. and Buller, R. H., *Steroids*, 22, 687, 1973.
54. Coltart, D. J., Gullner, H. G., Billingham, M., Goldman, R. H., Stinson, E. B., Kalman, S. M., and Harrison, D. C., Physiological distribution of digoxin in human heart, *Br. Med. J.*, 4(947), 733, 1974.
55. Cornett, J. C. et al., *J. Clin. Endocrinol.*, 33, 459, 1971.
56. Cook, C. E., Amerson, E., Poole, W. K., Lesser, P., and O'Tauma, L., Phenytoin and phenobarbital concentrations in saliva and plasma measured by radioimmunoassay, *Clin. Pharmacol. Ther.*, 18(6), 742, 1975.
57. Cook, C. E., Hawos, M. L., Amerson, E. W., Pitt, C. G., and Williams, D., Radioimmunoassay of delta-9-tetrahydrocannabinol, *Natl. Inst. Drug Abuse Res. Monogr. Ser.*, (7), 15, 1976.
58. Cook, C. E., Kepler, J. A., and Christensen, H. D., Antiserum to diphenylhydantoin: preparation and characterization, *Res. Commun. Chem. Pathol. Pharmacol.*, 5(3), 767, 1973.
59. Cook, C. E., Twine, M. E., Myers, M., Amerson, E., Kepler, J. A., and Taylor, G. F., Theophylline radioimmunoassay: synthesis of antigen and characterization of antiserum, *Res. Commun. Chem. Pathol. Pharmacol.*, 13(3), 497, 1976.
60. Cree, J. E., Coltart, D. J., and Howard, M. R., Plasma digoxin concentration in children with heart failure, *Br. Med. J.*, 1(851), 443, 1973.
61. Dixon, R., Brooks, M. A., Postma, E., Hackman, M. R., Spector, S., Moore, J. D., and Schwartz, M. A., *N*-Desmethyldiazepam: a new metabolite of chlordiazepoxide in man, *Clin. Pharmacol. Ther.*, 20(4), 450, 1976.
62. Dixon, W. R., Earley, J., and Postma, E., Radioimmunoassay of chlordiazepoxide in plasma, *J. Pharm. Sci.*, 64(6), 937, 1975.
63. Drewes, P. A. and Pileggi, V. J., Faster and easier radioimmunoassay of digoxin, *Clin. Chem.*, 20(3), 343, 1974.
64. Duchateau, A. M., Van Megen, T., and Merkus, F. W., Digitoxin radioimmunoassay. I. Comparison of three assays for the determination of serum digitoxin levels, *Pharm. Weekbl.*, 108(10), 193, 1973.
65. Dumasia, M. C. et al., *Biochem, J.*, 133, 401, 1973.

66. Ebert, M. H., Van Kammen, D. P., and Murphy, D. L., Plasma levels of amphetamine and behavioral response, in *Pharmacokinetics of Psychoactive Drugs*, Gottschalk, L. A. and Merlis, S., Eds., Spectrum Publications, New York, 1976, 1974, 157.
67. Ekins, R. P., *Br. Med. Bull.*, 30, 3, 1974.
68. English, J., Chakraborty, J., Marks, V., and Parke, A., A radioimmunoassay procedure for dexamethasone: plasma and urine levels in man, *Eur. J. Clin. Pharmacol.*, 9(2), 239, 1975.
69. Ertel, N. H., Mittler, J. C., Akgun, S., and Wallace, S. L., Radioimmunoassay for colchicine in plasma and urine, *Science*, 193(4249), 233, 1976.
70. Evenson, M. A., et al., *Clin. Chem.*, 16, 107, 1970.
71. Evered, D. C. et al., *Br. Med. J.*, 3, 427, 1970.
72. Faraj, B. A., Israili, Z. H., Kight, N. E., Smissman, E. E., and Pazdernik, T. J., Specificity of an antibody directed against *d*-methamphetamine. Studies with rigid and nonrigid analogs, *J. Med. Chem.*, 19(1), 20, 1976.
73. Fasth, A., Sollenberg, J., and Sorbo, B., Production and characterization of antibodies to atropine, *Acta Pharm. Suec.*, 12(4), 311, 1975.
74. Findlay, J. W., Butz, R. F., and Welch, F. M., A codeine radioimmunoassay exhibiting insignificant cross-reactivity with morphine, *Life Sci.*, 19(3), 389, 1976.
75. Finley, P. R., and Byers, J. M., III, Assay of phenytoin: adaptation of "EMIT" to the centrifugal analyzer, *Clin. Chem.*, 22(6), 911, 1976.
76. Fish, F. and Wilson, W. C. C., *J. Pharm Pharmacol.*, 21(Suppl), 13355, 1969.
77. Fleckenstein, L., Kroening, B., and Weintraub, M., Assessment of the biologic availability of digoxin in man, *Clin. Pharmacol. Ther.*, 16(3), 435, 1974.
78. Flynn, E. J. and Spector, S., Determination of barbiturate derivatives by radioimmunoassay, *J. Pharm. Exp. Ther.*, 181, 547, 1972.
79. Fraser, E. J., Leach, R. H., Poston, J. W., Bold, A. M., Culank, L. S., and Lipede, A. B., Dissolution and bioavailability of digoxin tablets, *J. Pharm. Pharmacol.*, 25(12), 968, 1973.
80. Freund, J., *Am. J. Clin. Pathol.*, 21, 645, 1951.
81. Galen, R. S. and Forman, D., *Clin. Chem.*, 23, 119, 1977.
82. Gershman, H. et al., *Prostaglandins*, 1, 407, 1972.
83. Giardina, A. C., Ehlers, K. H., Morrison, J. B., and Engle, M. A., Serum digitoxin concentrations in infants and children, *Circulation*, 51(4), 713, 1975.
84. Gintzler, A. R., Mohacsi, E., and Spector, S., Radioimmunoassay for the simultaneous determination of morphine and codeine, *Eur. J. Pharmacol.*, 38(1), 149, 1976.
85. Girard, J. P. et al., *Helv. Med. Acta*, 35, 86, 1969.
86. Glazko, A. J. and Dill, W. A., in *Antiepileptic Drugs*, Woodbury, D. M., Penry,

J. K., and Schmidt, D. P., Eds., Raven Press, New York, 1972, 413.

87. Gleich, G. J. and Stankievic, R. R., *Immunochemistry*, 6, 85, 1969.

88. Glogner, P. et al., *Klin. Wochenschr.*, 51, 352, 1973.

89. Goldman, R. H., The use of serum digoxin levels in clinical practice, *JAMA*, 229(3), 331, 1974.

90. Goldstein, A. and Brown, B. W., Urine testing schedules in methadone maintenance treatment of heroin addiction, *JAMA*, 214, 311, 1970.

91. Gorodetzky, C. W. and Kullberg, M. P., Etorphine in man. II. Detectability in urine by common screening methods, *Clin. Pharmacol. Ther.*, 17(3), 273, 1975.

92. Gorodetzky, C. W. and Kullberg, M. P., Validity of screening methods for drugs of abuse in biological fluids. II. Heroin in plasma and saliva, *Clin. Pharmacol. Ther.*, 15(6), 579, 1974.

93. Grant, J. D., Gross, S. J., Lomax, P., and Wong, R., Antibody detection of marihuana, *Nature New Biol.*, 236(68), 216, 1972.

94. Greenblatt, D. J., Duhme, D. W., Koch-Weser, J., and Smith, T. W., Evaluation of digoxin bioavailability in single-dose studies, *N. Engl. J. Med.*, 289(13), 651, 1973.

95. Greenblatt, D. J. Duhme, D. W., Koch-Weser, J., and Smith, T. W., Comparison of one- and six-day urinary digoxin excretion in single-dose bioavailability studies, *Clin. Pharmacol. Ther.*, 16(5), 813, 1974.

96. Greenblatt, D. J., Duhme, D. W., Koch-Wester, J., and Smith T. W., Intravenous digoxin as a bioavailability standard: slow infusion and rapid injection, *Clin. Pharmacol. Ther.*. 15(5), 510, 1974.

97. Greenwood, H., Howard, M., and Landon, J., A rapid, simple assay for digoxin, *J. Clin. Pathol.*, 27(6), 490, 1974.

98. Greenwood, H. et al., *Clin. Chem.*, 19, 187, 1973.

99. Greenwood, H., Snedden, W., Hayward, R. P., and Landon, J., The measurement of urinary digoxin and dihydrodigoxin by radioimmunoassay and by mass spectroscopy, *Clin. Chim. Acta*, 62(2), 213, 1975.

100. Gross, S. J., Grant, D. B., Wong, R., Schuster, R., Lomax, P., Campbell, D. H., and Bennett, P. D., Critical determinations enabling an antibody to distinguish morphine from heroin, codeine, and dextromethorphan, *Immunochemistry*, in press.

101. Gross, S. J., Soares, J. R., Wong, S. L., and Schuster, R. E., Marijuana metabolites measured by a radioimmune technique, *Nature*, 252(5484), 581, 1974.

102. Grunberg, E., Detection of drugs of abuse by radioimmunoassay: a summary of published data and some new information, *Clin. Chem.*, 22(6), 712, 1976.

103. Gullner, H. G., Stinson, E. B., Harrison, D. C., and Kalman, S. M., Correlation of serum concentrations with heart concentrations of digoxin in human subjects, *Circulation*, 50(4), 653, 1974.

104. Haber, E., The radioimmunoassay of digitalis glycosides, in *Radioassay: Clinical Concepts*, Hunter, O. B., Jr., Ed., Searle, Skokie, Ill., 1974, 53.

105. Haines, C. F., Jr., Mahajan, D. K., Miljkov, C. D., Miljkovi, C. M., and Vessell, E. S., Radioimmunoassay of plasma nicotine in habituated and naive smokers, *Lung*, 91(5), 1083, 1974.

106. Haines, C. F., et al., *Clin. Pharm. Ther.*, 16, 511, 1974.

107. Hales, C. N. and Randle, P. J., *Biochem. J.*, 88, 137, 1963.

108. Hamamoto, H., Takeda, H., Katoh, T., Tokuoka, T., and Kitamura, K., Correlation between myocardial and plasma concentration of digitoxin and its metabolites with special reference to digitoxin radio-immunoassay, *Jpn. Circ. J.*, 40(7), 747, 1976.

109. Hamburger, R. N., *Science*, 152, 203, 1966.

110. Hansell, J. R., Three-years' experience in interlaboratory testing of commercial digoxin kits, *Am. J. Clin. Pathol.*, 66(1), 234, 1976.

111. Harwood, C. T., Radioimmunoassay: its application to drugs of abuse, *Pharmacology*, 11(1), 52, 1974.

112. Hayes, C. J., Butler, V. P., Jr., and Gersony, W. M., Serum digoxin studies in infants and children, *Pediatrics*, 52(4), 561, 1973.

113. Hendel, J., Sarek, L. J., and Hvidberg, E. F., Rapid radioimmunoassay for methotrexate in biological fluids, *Clin. Chem.*, 22(6), 813, 1976.

114. Henderson, G. I., Frincke, J., Leung, C. Y., Torten, M., and Benjamini, E., Antibodies to fentanyl, *J. Pharmacol. Exp. Ther.*, 192(2), 489, 1975.

115. Herbert, V. et al., *J. Clin. Endocrinol. Metab.*, 25, 1375, 1965.

116. Hichens, M. and Hogans, A. F., Radioimmunoassay for dexamethasone in plasma, *Clin. Chem.*, 20(2), 266, 1974.

117. Hobson, J. D. and Zettner, A., Digoxin serum half-life following suicidal digoxin poisoning, *JAMA*, 223(2), 147, 1973.

118. Hoeschen, R. J. and Proveda, V., *Can. Med. Assoc. J.*, 105, 170, 1971.

119. Holtzman, J. L., Shafer, R. B., and Erickson, R. R., Methodological causes of discrepancies in radioimmunoassay for digoxin in human serum, *Clin. Chem.*, 20(9), 1194, 1974.

120. Hooker, S. B. and Boyd, W. C., *J. Immunol.*, 38, 479, 1940.

121. Hooper, W. D., Tyrer, J. H., and Eadie, M. J., Plasma drug concentrations in therapeutics, *Med. J. Aust.*, 1(10), 313, 1976.

122. Horgan, E. G. and Riley, W. J., *Clin. Chem.*, 19, 187, 1973.

123. Horowitz, P. E. and Spector, S., Determination of serum *d*-tubocurarine concentration by radioimmunoassay, *J. Pharmacol. Exp. Ther.*, 185(1), 94, 1973.

124. Huffman, D. H., Relationship between digoxin concentrations in serum and saliva, *Clin. Pharmacol. Ther.*, 17(3), 310, 1975.

125. Hunter, W. M. and Greenwood, F. C., *Nature*, London, 194, 495, 1962.

126. Hurn, B. A. and Landon, J., *Radioimmunoassay Methods*, Kirkham, K. E. and Hunter W. M., Eds., Churchill Livingstone, Edinburgh, 1971, 121.

127. Hyslop, N. E. et al., *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 34, 975, 1975.

128. Jain, N. C. and Cravey, R. H., The identification of barbiturates from biological specimens, *J. Chromatogr. Sci.*, 12(5), 228, 1974.

129. Jaklitsch, A. P. et al., *Clin. Chem.*, 22, 1185, 1976.

130. Jaton, J. C. and Ungar-Waron, H., *Arch. Biochem. Biophys.*, 122, 157, 1967.

131. Jusko, W. J., Gerbracht, L., Golden, L. H., and Koup, J. R., Digoxin concentrations in serum and saliva, *Res. Commun. Chem. Pathol. Pharmacol.*, 10(1), 189, 1975.

132. Karchmer, A. W. et al., *Clin. Res.*, 20, 531, 1972.

133. Kaul, B., Millian, S. J., and Davidow, B., The development of a radioimmunoassay for detection of cocaine metabolites, *J. Pharmacol. Exp. Ther.*, 199(1), 171, 1976.

134. Kawashima, K., Dixon, R., and Spector, S., Development of radioimmunoassay for chlorpromazine, *Eur. J. Pharmacol.*, 32(2), 195, 1975.

135. Kido, Y. et al., *Pharm. Soc.*, Japan, 94, 1290, 1974.

136. Kirkham, K. E. and Hunter, W. M., Eds., *Principles of Competitive Protein-binding Assays*, Lippincott, Philadelphia, 1971.

137. Koida, M., Takahashi, M., and Kancto, H., The morphine 3-glucuronide directed antibody: its immunological specificity and possible use for radioimmunoassay of morphine in urine, *Jpn. J. Pharmacol.*, 24(5), 707, 1974.

138. Koida, M., Takahashi, M., and Kaneto, H., Proceedings: radioimmunoassay of urinary metabolites of morphine, *Jpn. J. Pharmacol.*, 24, 84, 1974.

139. Kokoski, R. J. and Jain, M., Comparison of results for morphine urinalyses by radioimmunoassay and thin-layer chromatography in a narcotic clinic setting, *Clin. Chem.*, 21(3), 417, 1974.

140. Koontz, S., Besemer, D., Mackey, N., and Phillips, R., Detection of benzoyl ecgonine (cocaine metabolite) in urine by gas chromatography, *J. Chromatogr.*, 85(1), 75, 1973.

141. Kramer, W. G., Bathala, M. S., and Reuning, R. H., Specificity of the digoxin radioimmunoassay with respect to dihydrodigoxin, *Res. Commun. Chem. Pathol. Pharmacol.*, 14(1), 83, 1976.

142. Kramer, W. G. Lewis, R. P., Cobb, T. C. Forester, W. F., Jr., Visconti, J. A., Wanke, L. A., Boxenbaum, H. G., and Reuning, R. H., Pharmacokinetics of digoxin: comparison of a two- and a three-compartment model in man, *J. Pharmacokinet. Biopharm.*, 2(4), 299, 1974.

143. Kroening, B. H. and Weintraub, M., Reduced variation of tracer binding in digoxin radioimmunoassay by use of (1251)-labeled tyrosine-methyl-ester derivatives: relation of thyroxine concentration to binding, *Clin. Chem.*, 22(10), 1732, 1976.

144. Kubasik, N. P., Brody, B. B., and Barold, S. S., Problems in measurement of serum digoxin by commercially available radioimmunoassay kits, *Am. J. Cardiol.*, 36(7), 975, 1975.

145. Kubasik, N. P., Hall, J. L., Barold, S. S., Volosin, M. T., and Sine, H. E., Evaluation of the sensitivity of commercially available digoxin radioimmunoassay kits, *Chest*, 70(2), 217, 1976.

146. Kubasik, N. P., Norkus, N. S., and Sine, H. E., Comparison of commercial kits for radioimmonassay. II. The radioimmunoassay of serum digoxin using iodinated tracer, *Clin. Biochem.*, 8(4), 307, 1974.

147. Kubasik, N. P., Schauseil, S., and Sine, H. E., Comparison of commercial kits for radioimmunoassay. I. The radioimmunoassay of serum digoxin using tritium tracer, *Clin. Biochem.*, 7(3), 206, 1974.

148. Kundu, N., *Steroids*, 22, 327, 1973.

149. Kundo, N., *Steroids*, 23, 155, 1974.

150. Kuno-Salai, H., Sakai, H., and Ritzmann, S. E., Radioimmunoassay of digoxin-interference by digitoxin, *Clin. Chem.*, 21(1), 156, 1975.

151. Kupfenberg, H. J., *J. Pharm. Sci.*, 61, 284, 1972.

152. Kupfenberg, H. J., *Clin. Chem. Acta*, 29, 283, 1970.

153. Lader, S. et al., *Eur. J. Clin. Pharmacol.*, 5, 22, 1972.

154. Landon, J. et al., *Br. J. Hosp. Equip.*, 5, 29, 1975.

155. Landon, J. and Moffat, A. C., The radioimmunoassay of drugs. A review, *Analyst*, 101(1201), 225, 1976.

156. Langone, J. J. et al., *Biochemistry*, 12, 5025, 1973.

157. Langone, J. J. et al., *Arch. Biochem. Biophys.*, 164, 536, 1974.

158. Langone, J. J. and Van Vunakis, H., *Res. Commun. Chem. Path. Pharamacol.*, 10, 21, 1975.

159. Langone, J. J. and Van Vunakis, H., Radioimmunoassay for dieldrin and aldrin, *Res. Commun. Chem. Pathol. Pharmacol.*, 10(1), 163, 1975.

160. Larbig, D. and Kochsiek, K., Determination of serum-digoxin concentrations by radioimmunoassay, *Klin. Wochenschr.*, 49(18), 1031, 1971.

161. Larese, R. J. and Mirkin, B. L., Kinetics of digoxin absorption and relation of serum levels to cardiac arrhythmias in children, *Clin. Pharmacol. Ther.*, 15(4), 387, 1974.

162. Lee, Y. S. and Osawa, N., A non-chromatographic radioimmunoassay for plasma dexamethasone, *Endocrinology* Japan, 21(6), 481, 1974.

163. Legaz, M. and Raisys, V. A., Correlation of the "EMIT" antiepileptic drug assay with a gas-liquid chromatographic method, *Clin. Biochem.*, 9(1), 35, 1976.

164. Lesne, M., *Arch. Int. Pharmacodyn Ther.*, 199, 206, 1972.

165. L'Estrange Orme, M., Borgoa, O., Cook, C. E., and Sjoqvist, F., Measurement of diphenylhydantoin in 0.1-ml plasma samples: gas chromatography and radioimmunoassay compared, *Clin. Chem.*, 22(2), 246, 1976.

166. Leute, R. K., Ullman, E. F., and Goldstein, A., Spin immunoassay of opiate narcotics in urine and saliva, *JAMA*, 211, 1231. 1972.

167. Leute, R. K., Ullman, E. F., Goldstein, A., and Herzenberg, L. A., Spin immunoassay technique for determination of morphine, *Nature*, 236, 253, 1971.

168. Levine, L. and Powers, E., *Res. Commun. Chem. Path. Pharmacol.*, 9, 543, 1974.

169. Lewis, J. E., *Antimicrob. Agents Chemother.*, 7, 42, 1975.

170. Lewis, J. E. et al., *Nature* London, 239, 214, 1972.

171. Lindquist, C. A. and Spratt, J. L., *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 32, 719, 1973.

172. Line, W. F., Siegel, S. J., Kwong, A., Frank, C., and Ernst, R., Solid-phase radioimmunoassay for digoxin, *Clin. Chem.*, 19(12), 1361, 1973.

173. Liu, C. T. and Adler, F. L., Immunological studies on drug addition. I. Antibodies reactive with methadone and their use for detection of the drug, *J. Immunol.*, 1973.

174. Loeffler, L. J., Blum, M. R., and Nelson, M. A., A radioimmunoassay for methotrexate and its comparison with spectrofluorimetric procedures, *Cancer Res.*, 36(9), 3306, 1976.

175. Loeffler, L. J. and Pierce, J. V., Radioimmunoassay for lysergide (LSD) in illicit drugs and biological fluids, *J. Pharm. Sci.*, 62(11), 1817, 1973.

176. MacKinney, A. A., Jr., Burnett, G. H., Conklin, R. L., and Wasson, G. W., Comparison of five radioimmunoassays and enzyme bioassay for measurement of digoxin in blood, *Clin. Chem.*, 21(7), 857, 1975.

177. Mahon, L. A. et al., *Antimicrob. Agents Chemother.*, 3, 58, 5, 1973.

178. Manders, W. M. and Dominquez, A. M., Application of radioimmunoassay techniques in support of toxicologic investigations of aircraft accidents, *Aviat. Space Environ. Med.*, 46(10), 1297, 1975.

179. Marks, V. et al., *Br. Med. Bull.*, 30, 80, 1974.

180. Marks, V., Teale, D., and Fry, D., Detection of cannabis products in urine by radioimmunoassay, *Br. Med. J.*, 3(5979), 348, 1975.

181. Marks, V., Teal, J. D., and King, L. J., Radioimmunoassay of cannabis products in blood and urine, in *Marihuana: Chemistry, Biochemistry, and Cellular Effects*, Nahas, G. G. et al., Ed., Springer-Verlag, New York, 1976.

182. Matsukura, S., *Biochem. Biophys. Res. Commun.*, 64, 574, 1975.

183. Matsuyama, H., Tamada, T., Ishiguro, T., and Muranaka, H., Radioimmunoassay of nicotine, *Biochem. Biophys. Res. Commun.*, 64(2), 574, 1975.

184. Matteo, R. S., Spector, S., and Horowitz, P. E., Relation of serum *d*-tubocurarine concentration to neuromuscular blockade in man, *Anesthesiology*, 41(5), 440, 1974.

185. McIntyre, J. A., Armandi, A. E., Risen, L. P., Ling, W., and Haberfelde, G. C., Thin-layer chromatography and enzyme immunoassay of L-alpha-acetyl methadol and methadone metaboiites in urine. *Clin. Chem.*, 21(1), 109, 1975.

186. Meade, R. C. and Kliest, T. J., *J. Lab. Clin. Med.*, 80, 748, 1972.

187. Meikle, A. W., Lagerquist, L. G., and Tyler, F. H., A plasma dexamethasone radioimmunoassay, *Steroids*, 22(2), 193, 1973.

188. Michiels, L. J. M., *Life Sci.*, 16, 937, 1975.

189. Minshew, B. H. et al., *Antimicrob. Agents Chemother.*, 7, 107, 1975.

190. Moffat, A. C., Interpretation of postmortem serum levels of cardiac glycosides after suspected overdosage, *Acta Pharmacol. Toxicol.*, 35(5), 386, 1974.

191. Montgomery, M. R., Holtzman, J. L., and Leute, R. K., Application of electron spin resonance to determination of serum drug concentrations, *Clin. Chem.*, 21(9), 1323, 1975.

192. Montgomery, M. R., Holtzman, J. L., Leute, R. K., Dewees, J. S., and Boltz, G., Determination of diphenylhydantoin in human serum by spin immunoassay, *Clin. Chem.*, 21(2), 221, 1975.

193. Morris, B. A., Robinson, J. D., Piall, E., Aherne, G. W., and Marks, V., Proceedings: development of a radioimmunoassay for morphine having minimal cross-reactivity with codeine, *J. Endocrinol.*, 64(1), 6P, 1975.

194. Morris, H. G. et al., *Steroids*, 22, 445, 1973.

195. Mulé, S. J., Methods for the analysis of narcotic analgesics and amphetamines, *J. Chromatogr.*, 10, 275, 1972.

196. Mulé, S. J., Whitlock, E. and Jukofsky, D., Radioimmunoassay of drugs subject to abuse: critical evaluation of urinary morphine-barbiturate, morphine, barbiturate, and amphetamine assays, *Clin. Chem.*, 21(1), 81, 1975.

197. Mulé, S. J., Methods for the analysis of morphine and related surrogates: current status, *J. Chromatogr. Sci.*, 12(5), 245, 1974.

198. Mulé, S. J., Bastos, M. L., and Jukofsky, D., Evaluation of immunoassay methods for detection, in urine, of drugs subject to abuse, *Clin. Chem.*, 20(2), 243, 1974.

199. Noble, R. J., Rothbaum, D. A., Watanabe, A. M., Besch, H. R., and Fisch, C., Limitations of serum digitalis levels, *Cardiovasc. Clin.*, 6(1), 299, 1974.

200. Nygren, K. G., *Contraception*, 9, 265, (1974).

201. Oliver, G. C. et al., *Am. J. Med.*, 51, 186, 1971.

202. Oliver, G. C., Parker, B. M., and Parker, C. W., Radioimmunoassay for digoxin. Technic and clinical application, *Am. J. Med.*, 51(2), 186. 1971.

203. Oliver, G. C., Jr., Parker, B. M., Brasfield, D. L., and Parker, C. W., The measurement of digitoxin in human serum by radioimmunoassay, *J. Clin. Invest.*, 47, 1035, 1968.

204. O'Malley, K. and Stevenson, I. H., Digoxin levels in neonates, *N. Engl. J. Med.*, 288(12), 635, 1973.

205. Parker, K. D. et al., *Clin Toxicol.*, 3, 131, (1970).

206. Paxton, J. W., Rowell, F. J., and Ratcliffe, J. G., Production and characterization of antisera to diphenylhydantoin suitable for radioimmunoassay, *J. Immunol. Methods.*, 10(4), 317, 1976.

207. Peskar, B. and Spector, S., Quantitative determination of diazepam in blood by radioimmunoassay, *J. Pharmacol. Exp. Ther.*, 186(1), 167, 1973.

208. Pettinger, W. A. et al., *Clin. Pharmacol. Ther.*, 17, 146, 1975.

209. Phillips, A. P., Case experience with digoxin analysis of post mortem blood, *J. Forensic Sci. Soc.*, 14(2), 137, 1974.

210. Pippenger, C. E., Bastiani, R. J., and Schneider, R. S., Evaluation of an experimental homogeneous enzyme immuno-assay for the quantitation of phenytoin and phenobarbitone in serum or plasma, in *Clinical Pharmacology of Anti-epileptic Drugs*, Schneider, H. et al., Eds., Springer-Verlag, Berlin, 1975, 331.

211. Queng, C. T. et al., *J. Allergy*, 36, 505, 1965.

212. Rag, P. N. et al., *Steroids*, 24, 803, 1974.

213. Randall, D. et al., *Clin. Pharmacol. Ther.*, 13(1), 64, 1971.

214. Rao, P. N., *Steroids*, 23, 173, 1974.

215. Raso, V. and Schreiber, R., *Cancer Res.*, 35, 1407, 1975.

216. Ravel, R. and Schail, R. F., Jr., Pitfalls in iodine-125 digoxin measurement, *Anal. Clin. Lab. Sci.*, 6(4), 365, 1976.

217. Riceberg, L. J., Van Vunakis, H., and Levine, L., Radioimmunoassays of 3,4,5-tri-methoxy-phenethylamine (mescaline) and 2,5-dimethoxy-4-methylphenyl-isopropylamine (DOM), *Ann. Biochem.*, 60(2), 551, 1974.

218. Richens, A., Results of a phenytoin quality control scheme, *Clinical Pharmacology of Anti-epileptic Drugs*, Schneider, H. et al., Eds., Springer-Verlag, Berlin, 1975, 293.

219. Robinson, J. D. et al., *J. Endocrinol.*, 64, 7, 1975.

220. Robinson, J. D., Morris, B. A., and Marks, V., Development of a radioimmunoassay for etorphine (preliminary communication), *Res. Commun. Chem. Pathol. Pharmacol.*, 10(1), 1975.

221. Robinson, J. D., Morris, B. A., Aherne, G. W., and Marks, V., Pharmacokinetics of a single dose of phenytoin in man measured by radioimmunoassay, *Br. J. Clin. Pharmacol.*, 2(4), 345, 1975.

222. Robinson, M. G., Howe, R. C., Ream, N. W., Siegel, H. W., and Hegge, F. W., Acute heroin withdrawal in Viet Nam. An immunochemical evaluation of excretion, *Clin. Pharmacol. Ther.*, 16(2), 303, 1974.

223. Royer, M. E. et al., *Steroids*, 23, 713, 1974.

224. Robinson, J. D., Morris, B. A., Aherne, G. W., and Marks, V., Proceedings: development of radioimmunoassays for anticonvulsant drugs, *J. Endocrinol.*, 64(1), 7P, 1975.

225. Rock, N. L. and Moore, R. J., Methaqualone (mandrax) abuse, urine testing, and identification: clinical correlation between a new mass urinalysis test and a military drug abuse program, *Int. J. Addict.*, 11(2), 237, 1976.

226. Roerig, D. L., Lewand, D. L., Mueller, M. A., and Wang, R. I., Comparison of radioimmunoassay with thin-layer chromatographic and gas-liquid chromatographic methods of barbiturate detection in human urine, *Clin. Chem.*, 21(6), 672, 1975.

227. Roerig, D. L., Lewand, D., Mueller, M., and Wang, R. I., Methods of identification and confirmation of abusive drugs in human urine, *J. Chromatogr.*, 110(2), 349, 1975.

228. Roerig, D. L., Wang, R. I., Mueller, M. M., Lewand, D. L., and Adams, S. M., Radioimmunoassay compared to thin-layer and gas-liquid chromatography for detecting methadone in human urine, *Clin, Chem.*, 22(11), 1915, 1976.

229. Rosenthal, A. F., Vargas, M. G., and Klass, C. S., Evaluation of enzyme-multiplied immunoassay technique (EMIT) for determination of serum digoxin, *Clin. Chem.*, 22(11), 1899, 1976.

230. Ross, R., Horwitz, C. A., Hager, H., Usategui, M., Burke, M. D., and Ward, P. C., Preliminary evaluation of a latex agglutination-inhibition tube test for morphine, *Clin. Chem.*, 21(1), 139, 1975.

231. Rubenstein, K. E., Schneider, R. S., and Ullman, E. F., "Homogeneous" enzyme immunoassay. A new immunochemical technique, *Biochem. Biophys. Res. Commun.*, 47, 846, 1972.

232. Russell, A. S. and Ziff, M., *Clin. Exp. Immunol.*, 3, 901, 1968.

233. Sato, H. and Kuroiwa, Y., Characterization of specific antibodies to phenobarbital, *J. Biochem.* Tokyo, 76(6), 1293, 1974.

234. Sato, H., Kuroiwa, Y., Hamada, A. and Uematsu, T., Radioimmunoassay for phenobarbital, *J. Biomech.*, 75(6), 1301, 1974.

235. Scharpe, S. L. et al., *Clin. Chem.*, 22(6), 733, 1976.

236. Schneider, R. S., Bastiani, R. J., Leute, R. K., Reubenstein, K. E., and Ullman, E. F., Use of enzyme and spin labeling in homogeneous immunochemical detection methods, in *Immunoassays for Drugs Subject to Abuse*, Mulé, S. J., Sunshine, I., Braude, M., and Willette, R. E., Eds., CRC Press, Cleveland, 1974, 45.

237. Schneider, R. S., Lindquist, P., Tong-In Wong, E., Rubenstein, K. E., and Ullman, E. F., Homogeneous enzyme immunoassay for opiates in urine, *Clin. Chem.*, 19(8), 821, 1973.

238. Schmidt, D., Measurement of diphenylhydantoin and phenobarbital by enzyme immunoassay and gas-liquid chromatography, *J. Neurol.*, 213(1), 41, 1976.

239. Schnoll, S. H., Vogel, W. H., and Odstrchel, G., The specificity of anti-mescaline antibody produced in rabbits, *Fed. Proc. Abstr.*, 33, 719, 1973.

240. Schwenk, R. et al., *Clin. Chem.*, 2, 1059, 1975.

241. Selden, R. and Smith, T. W., Ouabain pharmacokinetics in dog and man. Determination by radioimmunoassay, *Circulation*, 45(6), 1176, 1972.

242. Shapiro, B., Kollmann, G. J., and Heine, W. I., Pitfalls in the application of digoxin determinations, *Semin. Nucl. Med.*, 5(3), 205, 1975.

243. Shaw, T. R., Raymond, K., Howard, M. R., and Hamer, J., Therapeutic nonequivalence of digoxin tablets in the United Kingdom: correlation with tablet dissolution rate, *Br. Med. J.*, 4(895), 763, 1973.

244. Sheiner, L. B., Rosenberg, B., Marathe, V. V., and Peck, C., Differences in serum digoxin concentrations between outpatients and inpatients: an effect of compliance, *Clin. Pharmacol. Ther.*, 15(3), 239, 1974.

245. Simon, E. J., Dole, W. P., and Hiller, J. M., Coupling of a new active morphine derivative to sepharose for affinity chromatography, *Proc. Natl. Acad. Sci.*, 69, 1835, 1972.

246. Singh, R. B., Singh, V. P., Dube, K. P., Vaish, S. K., and Burma, D. P., Radioimmunoassay for serum digoxin, *J. Assoc. Physicians* India, 23(12), 879, 1975.

247. Sine, H. E., Kubasik, N. P., and Rejent, T. A., Determination of the presence of morphine in urine, *Clin. Biochem.*, 7(2), 102, 1974.

248. Smith, T. W., Digitalis toxicity: epidemiology and clinical use of serum concentration measurements, *Am. J. Med.*, 58(4), 470, 1975.

249. Smith, T. W., Digoxin radioimmunoassay: capabilities and limitations, *J. Pharmacokinet. Biopharm.*, 4(2), 197, 1976.

250. Smith, T. W. et al., *N. Engl. J. Med.*, 281, 1212, 1969.

251. Smith, T. W. and Haver, E., Clinical value of the radioimmunoassay of the digitalis glycosides, *Pharmacol. Rev.*, 25(2), 219, 1973.

252. Smith, T. W. and Haber, E., Digitalis, *N. Engl. J. Med.*, 289(20), 1063, 1973.

253. Sohn, D., Simon, J., Hanna, M. A., Ghali, G. V., Tolba, R. A., and Melkonian, V., Screening for heroin—a comparison of current methods, *Anal. Chem.*, 45(8), 1498, 1973.

254. Solow, E. B. and Green, J. B., *Neurology*, 22, 540, 1972.

255. Spector, S., *The Phenothiazines and Structurally Related Drugs*, Raven Press, New York, 1974, 363.

256. Spector, S., Quantitative determination of morphine in serum by radioimmunoassay, *J. Pharmacol. Exp. Ther.*, 178, 253, 1971.

257. Spector, S., Berkowitz, B., Flynn, E. J., and Peskar, B., Antibodies to morphine, barbiturates, and serotonin, *Pharmacol. Rev.*, 25, 281, 1973.

258. Spector, S. and Flynn, E. J., Barbiturates: radioimmunoassay, *Science*, 174, 1036, 1971.

259. Spector, S. and Parker, C. W., Morphine: radioimmunoassay, *Science*, 168, 1347, 1970.

260. Spector, S., Spector, N. L., and Almeida, M. R., Jr., Radioimmunoassay for desmethylimipramine, *Psychopharmacol Commun.*, 1(4), 421, 1975.

261. Spiehler, V. R., Reed, D., Cravey, R. H., Wilcox, W. P., Shaw, R. F., and Holland, S., Comparison of results for quantitative determination of morphine by radioimmunoassay, enzyme immunoassay, and spectrofluorometry, *J. Forensic Sci.*, 20(4), 647, 1975.

262. Spiehler, V., Sun, L., Miyada, D. S., Sarandis, S. G., Walwick, E. R., Klein, M. W., Jordan, D. B., and Jessen, B., Radioimmunoassay, enzyme immunoassay, spectrophotometry, and gas-liquid chromatography compared for determination of phenobarbital and diphenylhydantoin, *Clin. Chem.*, 22(6), 749, 1976.

263. Sulkowski, T. S., Lathrop, G. D., Merritt, J. H., Landez, J. H., and Noe, E. R., A semiautomated radioimmunoassay for mass screening of drugs of abuse, *J. Forensic Sci.*, 20(3), 524, 1975.

264. Sun, L. and Walwick, E. R., Primidone analyses: correlation of gas-chromatographic assay with enzyme immunoassay, *Clin. Chem.*, 22(6), 901, 1976.

265. Takahashi, M., Koida, M., Kanetor, H., Goto, Y., and Haseba, S., Urinary excretion patterns of morphine in humans followed by a radioimmunoassay method, *Jpn. J. Pharmacol.*, 25(3), 348, 1975.

266. Taubert, K. and Shapiro, W., Serum digoxin levels using an 1251-labeled antigen: validation of method and observations on cardiac patients, *Am. Heart J.*, 89(1), 79, 1975.

267. Taunton-Rigby, A., Sher, S. E., and Kelley, P. R., Lysergic acid diethylamide: radioimmunoassay, *Science*, 191(95), 165, 1973.

268. Taylor, B. B., Kennedy, R. D., and Caird, F. I., Digoxin studies in the elderly, *Age Ageing*, 3(2), 79, 1974.

269. Teale, J. D., et al., *Proc. Soc. Analyt. Chem.*, 11, 219, 1974.

270. Teale, J. D., Clough, J. M., Piall, E. M., King, L. J., and Marks, V., Plasma cannabinoids measured by radioimmunoassay in rabbits after intraveous injection of tetrahydrocannibinol, 11-hydroxy-tetrahydrocannabinol, cannabinol, and cannabidiol, *Res. Commun. Chem. Pathol. Pharmacol.*, 11(2), 339, 1975.

271. Teale, J. D., Forman, E. J., King, L. J., and Marks, V., Radioimmunoassay of cannabinoids in blood and urine, *Lancet*, 2(7880), 553, 1974.

272. Teale, J. D., Forman, E. J., King, L. J., and Marks, V., Production of antibodies to tetrahydrocannabinol as the basis for its radioimmunoassay, *Nature*, 249(453), 154, 1974.

273. Teale, J. D., Forman, E. J., King, L. J., Piall, E. M., and Marks, F., The development of a radioimmunoassay for cannabinoids in blood and urine, *J. Pharm. Pharmacol.*, 27(7), 465, 1875.

274. Tigelaar, R. E. Rapport, R. L., II, Inman, J. K., and Kupferberg, H. J., A radioimmunoassay for diphenylhydantoin. *Clin. Chim. Acta*. 43(2), 231, 1973.

275. Torten, M., Henderson, G. L., Leung, C. Y., Miller, C. H., Eisele, J. H., and Benjamini, E., Proceedings: immunopharmacology of a potent opiate, fentanyl. Development of radioimmunoassay and prevention of effects by immunologic means, *Isr. J. Med. Sci.*, 11(12), 1395, 1975.

276. Usategui-Gomez, M., Heveran, J. E., Cleeland, R., McGhee, B., Telischak, Z., Awdziej, T., and Grunberg, E., Simultaneous detection of morphine and barbiturates in urine by radioimmunoassay, *Clin. Chem.*, 21(10), 1378, 1975.

277. Vaitukaitis, J. et al., *J. Clin. Endocrinol. Metab.*, 33, 988, 1971.

278. Valanju, N. N., Baden, M. M., Valanju, S. N., Mulligan, D., and Verma, S. K., Detection of biotransformed cocaine in urine from drug abusers, *J. Chromatogr.*, 81, 170, 1973.

279. Van der Slooten, E. P. and Van der Helm, H. J., Comparison of the EMIT (enzyme-multiplied immunoassay technique) opiate assay and a gas-chromatographic—mass-spectrometric determination of morphine and codeine in urine, *Clin. Chem.*, 22(7), 1110, 1976.

280. Van der Vijgh, W. J. F., *Clin. Chem.*, 21, 1172, 1975.

281. Van Vunakis, H., *Biochem. Pharmacol.*, 18, 393, 1969.

282. Van Vunakis, H. et al., *Cancer Res.*, 34, 2546, 1974.

283. Van Vunakis, H., Langone, J. J., and Milunsky, A., Nicotine and cotinine in the amniotic fluid of smokers in the second trimester of pregnancy, *Am. J. Obstet. Gynecol.*, 120(1), 64, 1974.

284. Van Vunakis, H., Wasserman, E., and Levine, L., Specificities of antibodies to morphine, *J. Pharmacol. Exp. Ther.*, 180, 514, 1972.

285. Wagner, J. G., Appraisal of digoxin bioavailability and pharmacokinetics in relation to cardiac therapy, *Am. Heart J.*, 88(2), 133, 1974.

286. Wainer, B. H., Fitch, F. W., Rothberg, R. M., and Friend, J., Morphine-3-succinyl bovine serum albumin. An immunogenic hapten protein conjugate, *Science*, 176, 1143, 1972.

287. Wainer, B. H., Wung, W. E., Hill, J. H., Fitch, F. W., Fried, J., and Rothberg, R. M., The production and characterization of antibodies reactive with meperidine, *J. Pharmacol. Exp. Ther.*, 197(1), 163, 1976.

288. Wainer, B. H., et al., *Nature*, 241, 537, 1973.

289. Wal, J. M., and Kann, G., *C. R. Acad. Sci.*, 280, 2405, 1975.

290. Walberg, C. B., Correlation of the "Emit" urine barbiturate assay with a spectrophotometric serum barbiturate assay in suspected overdose, *Clin. Chem.*, 20(2), 305, 1974.

291. Walsh, F. M. and Sode, J., Significance of non-steady-state serum digoxin concentrations, *Am. J. Clin. Pathol.*, 63(3), 446, 1975.

292. Wang, Y., Lantin, E., and Sutow, W. W., Methotrexate in blood, urine, and cerebrospinal fluid of children receiving high doses by infusion, *Clin. Chem*, 22(7), 1053, 1976.

293. Warren, R. J., *J. Endour.*, 62, 605, 1974, o294.

294. Way, E. L. and Adler, T. K., The biological disposition of morphine and its surrogates, *Bull. W.H.O.*, 25, 227, 1961.

295. Weiner, L. M. et al., *J. Immunol.*, 90, 788, 1963.

296. Wendum, A. G., *J. Infect. Dis.*, 70, 173, 1942.

297. Wettrell, G. and Andersson, K. E., Absorption of digoxin in infants, *Eur. J. Clin. Pharmacol.*, 9(1), 49, 1975.

298. Williams, T. A. and Pittman, K. A., Pentazocine radioimmunoassay, *Res. Commun. Chem. Pathol. Pharmacol.*, 7(1), 119, 1974.

299. Wingeleth, D. C. et al., *Clin. Chem.*, 20, 870, 1974.

300. Wisdom, G. B., *Clin. Chem.*, 22(8), 1243, 1976.

301. Yalow, R. S. and Berson, S. A., *J. Clin. Invest.*, 39, 1157, 1960.

302. Zbidnen, G. and Randall, L. V., *Adv. Pharmacol.*, 5, 213, 1967.

303. Rubenstein, K. E., Schneider, R. S., and Ullman, E. F., Homogeneous enzyme immunoassay. A new immunochemical technique, *Biochem. Biophys. Res. Commun.*, 47, 846, 1972.

304. Bastiani, R. J., Phillips, R. C., Schneider, R. S., and Ullman, E. F., Homogeneous immunochemical drug assays, *Am. J. Med. Technol*, 39, 211, 1973.

305. Rowley, G. L., Rubenstein, K. E., Huisjen, J., and Ullman, E. F., Mechanism by which antibodies inhibit haptenmalate dehydrogenase conjugates, *J. Biol. Chem.*, 250, 3759, 1975.

306. Kutt, H. and Penry, J. K., Usefulness of blood levels of antiepileptic drugs, *Arch. Neurol.*, 31, 283, 1974.

307. Legas, M. and Raisys, V. A., Correlation of the EMIT® antiepileptic drug assay with a gas-liquid chromatographic method, *Clin. Biochem.*, 9, 35, 1976.

308. Spiehler, V., Sun, L., Miyada, D. S., Sarandis, S. G., Walwick, E. R., Klein, M. W., Jordan, D. B., and Jessen, B., Radioimmunoassay, enzyme immunoassay, spectrophotometry, and gas-liquid chromatography compared for determination of phenobarbital and diphenylhydantoin, *Clin. Chem.*, 22, 749, 1976.

309. Booker, H. E. and Darcey, B. A., Enzymatic immunoassay vs. gas/liquid chromatography for determination of phenobarbital and diphenylhydantoin in serum, *Clin. Chem.*, 21, 1766, 1975.

310. Sun, L. and Walwick, E. R., Primidone analyses: correlation of gas chromatographic assay with enzyme immunoassay, *Clin. Chem.*, 22, 901, 1976.

311. Brunk, S. D., Hadjiioannou, T. P., Hadjiioannou, S. I., and Malmstadt, H. V., Adaption of EMIT technique for serum phenobarbital and diphenylhydantoin assays to the miniature centrifugal analyzer, *Clin. Chem.*, 22, 905, 1976.

312. Finley, P. R., Williams, R. J., and Byers, J. M., Assay of phenytoin: adaptation of EMIT to the centrifugal analyzer, *Clin. Chem.*, 22, 911, 1976.

313. Bastiani, R., Performance Evaluation of the Emit Diphenylhydantoin and Phenobarbital Assays, Clinical Study #20, Syva Corp., Palo Alto, California, 1975, available upon request.

314. Glasko, A. J. and Dill, W. A., Ethosuximide—chemistry and methods for determination, in *Antiepileptic Drugs*, Woodbury, D. M., Penry, J. K., and Schmidt, D. P., Eds., Raven Press, New York, 1972, 413.

315. Kupferberg, H. J., Quantitative estimation of diphenylhydantoin, primidone, and phenobarbital in plasma by gas-liquid chromatography, *Clin. Chem. Acta*, 29, 283, 1970.

316. Kupferberg, H. J., GLC determination of carbamazepine in plasma, *J. Pharm. Sci.*, 61, 284, 1972.

317. Evenson, M. A., Jones, P., and Darcey, B., Simultaneous measurement of diphenylhydantoin and primidone in serum by gas-liquid chromatography, *Clin. Chem.*, 16, 107, 1970.

318. Solow, E. B. and Green, J. B., The simultaneous determination of multiple anticonvulsant drug levels by gas-liquid chromatography: method and clinical application, *Neurology*, 22, 540, 1972.

319. Way, E. L. and Adler, T. K., The biological disposition of morphine and its surrogates-1, *Bull. WHO*, 25, 227, 1961.

320. Parker, K. D., Crim, M., Elliott, H. W., Wright, J. A., Nomof, N., and Hine, C. H., Blood and urine concentrations of subjects receiving barbiturates, meprobamate, glutethimide, or diphenylhydantoin, *Clin. Toxicol.*, 3, 131, 1970.

321. Beckett, A. H., Couttis, R. T., and Ogunbona, F. A., Metabolism of amphetamines, identification of *N*-oxygenated products by gas chromatography and mass spectrometry, *J. Pharm. Pharmacol.*, 25, 708, 1973.

322. Fish, F. and Wilson, W. C. C., Excretion of cocaine and its metabolites in man, *J. Pharm. Pharmacol.*, 21(Suppl.), 1355, May 1969.

323. Randall, D., Basett, B. S., and Casarett, L. J., Urinary excretion of methadone in man, *Clin. Pharmacol. Ther.*, 13(1), 64, 1971.

324. Zbinden, G. and Randall, L. U., Pharmacology of benzodiazepines: laboratory and clinical correlations, *Adv. Pharmacol.*, 5, 213, 1967.

325. Way, E. L. and Adler, T. K., The biological disposition of morphine and its surrogates-3, *Bull. WHO*, 26, 274, 1962.

326. Schneider, R. S., Bastiani, R. J., Leute, R. K., Rubenstein, K. E., and Ullman, E. F., Use of enzyme and spin labeling in homogeneous immunochemical detection methods, in *Immunoassays for Drugs Subject to Abuse*, Mule, S. J., Sunshine, I., Braud, M., and Willete, R. E., Eds. CRC Press, Cleveland, Ohio, 1974, 45.

327. Walberg, C. B., Correlation of EMIT urine barbiturate assay with a spectrophotometric serum barbiturate assay in suspected overdose, *Clin. Chem.*, 20, 305, 1974.

328. Mulé, S. J., Methods for the analysis of narcotic analgesics and amphetamines, *J. Chromatogr. Sci.* 10, 275, 1972.

329. Bastiani, R., Performance Evaluation of the EMIT™ Digoxin Assay Manual Reagent Kit-Barch Procedure, Syva Co., Palo Alto, California, 1976, available upon request.

330. Chang, J. J., Drowl, C. P., and Schneider, R. S., Homogeneous enzyme-immunoassay for digoxin, *Clin. Chem.*, 21, 967, 1975.

331. Van der Vijgh, W. J. F., Modification of a commercially available digoxin radioimmunoassay kit for higher precision, *Clin. Chem.*, 21, 1172, 1975.

332. Chang, J. Y., Cobb, M. E., Haden, B. H., McNeil, K. G., and Schneider, R. S., Stat procedure for digoxin based upon homogeneous enzyme immunoassay, *Clin. Chem.*, 22, 1185, 1976.

333. Jaklitsch, A. P., Schneider, R. S., Johannes, R. J., Lavine, J. E., and Rosenberg, G. L., Homogeneous enzyme immunoassay for T_4 in serum (abstract), *Clin. Chem.*, 22, 1185, 1976.

334. Galen, R. S. and Forman, D., Enzyme-immunoassay of serum thyroxine on the Autochemist Multichannel Analyzer, *Clin. Chem.*, 23, 119, 1977.

335. Lucek, R. and Dixon, R., Specific radioimmunoassay for amitriptyline and nortriptyline in plasma, *Res. Commun. Chem. Pathol. Pharmacol.*, 18(1), 125, 1977.

336. Robinson, J. U., Aherne, G. W., and Risby, D., A radioimmunoassay for amitriptyline and related compounds in blood, in *Clinical Toxicology*, Duncan, W. A. and Leonard, B. J., Eds. Excerpta Medica, Amsterdam, 1977, 168.

337. Bost, R. O., Sutheimer, C. A., and Sunshine, I., Relative merits of some methods for amphetamine assay in biological fluids. *Clin. Chem.*, 22(6), 789, 1976.

338. Ebert, M. H., Van Kammen, D. P., and Murphy, D. L., Plasma levels of amphetamine and behavioral response, in *Pharmacokinetics of Psychoactive Drugs*, Gottschalk, L. A. and Merlis, S., Eds., Spectrum Publications, New York, 1976, 157.

339. Chang, R. L., Wood, A. W., Dixon, W. R., Conney, A. H., Anderson, K. E., Eiseman, J., and Alvares, A. P., Antipyrine: radioimmunoassay in plasma and saliva following administration of a high dose and a low dose, *Clin. Pharmacol. Ther.*, 20(2), 219, 1976.

340. Lesne, M. and Dolphen, R., Development of a radioimmunoassay for aprindine, *J. Immunol. Methods*, 11(1–2), 189, 1977.

341. Wurzburger, R. J., Miller, R. L., Boxenbau..., H. G., and Spector, S., Radicimmunoassay of atropine in plasma, *J. Pharmacol. Exp. Ther.*, 203(2), 435, 1977.

342. Jain, N. C., Mass screening and confirmation of barbiturates in urine by RIA/gas chromatography, *Clin. Toxicol.*, 9(2), 221, 1976.

343. Mulé, S. J., Jukofsky, D., Kogan, M., De Pace, A., and Verebey, K., Evaluation of the radioimmunoassay for benzoylecgonine (a cocaine metabolite) in human urine, *Clin. Chem.*, 23(5), 796, 1977.

344. Cook, C. E., Tallent, C. R., Amerson, E. W., Myers, M. W., Kepler, J. A., Taylor, G. F., and Christensen, H. D., Caffeine in plasma and saliva by a radioimmunoassay procedure, *J. Pharmacol. Exp. Ther.*, 199(3), 679, 1976.

345. Chase, A. R., Kelley, P. R., Taunton-Rigby, A., Jones, R. T., and Harwood, T., Quantitation of cannabinoids in biological fluids by radioimmunoassay, *Natl. Inst. Drug Abuse Res. Monogr. Ser.*, No. 7, 1, 1976.

346. Teale, J. D., Clough, J. M., Fry, D., Backhouse, C., and Marks, V., The use of radioimmunoassay in the detection of urinary cannabinoids, in *Clinical Toxicology*, Duncan, W. A. and Leonard, B. J., Eds., Excerpta Medica, Amsterdam, 1977, 252.

347. Rodgers, R., Crowl, C. P., Eimstad, W. M., Hu, M. W., Kam, J. K., Ronald, R. C., Rowley, G. L., and Ullman, E. F., Homogeneous enzyme immunoassay for cannabinoids in urine, *Clin. Chem.*, 24(1), 95, 1978.

348. Sun, L. and Szafir, I., Comparison of enzyme immunoassay and gas chromatography for determination of carbamazepine and ethosuximide in human serum, *Clin. Chem.*, 23(9), 1753, 1977.

349 Bartels, H., Oldigs, H. D., and Gunther, E., Use of saliva in monitoring carbamazepine medication in epileptic children, *Eur. J. Pediatr.*, 126(1–2), 37, 1977.

350. Mihaly, G. W., Phillips, J. A., Louis, W. P., and Vajda, F. J., Measurement of carbamazepine and its epoxide metabolite by high performance liquid chromatography, and a comparison of assay techniques for the analysis of carbamazepine, *Clin. Chem.*, 23(12), 2283, 1977.

351. Kleine, T. O., Lowering the cost of the enzyme immunoassay (EMIT) for carbamazepine by its adaptation to a mechanized microliter system, *Clin. Chem. Acta*, 82(1–2), 193, 1978.

352. Stronjny, N., Bratin, K. Brooks, M. A., and De Silva, J. A., Determination of chlordiazepoxide, diazepam, and their major metabolites in blood or plasma by spectrophotodensitometry, *J. Chromatogr.*, 143(4), 363, 1977.

353. Kaul, B., Millian, S. J., and Davidow, B., The development of a radioimmunoassay for detection of cocaine metabolites, *J. Pharmacol. Exp. Ther.*, 199(1), 171, 1976.

354. Decato, L., Jr., Liu, C. T., and Adler, F. L., Immunologic studies on drug addiction. III. Antibodies reactive with cocaine metabolites and their use for drug detection, *J. Immunol. Methods*, 18(3–4), 201, 1977.

355. Findlay, J. W., Butz, R. F., and Welch, R. M., Codeine kinetics as determined by radioimmunoassay, *Clin. Pharmacol. Ther.*, 22(4), 439, 1977.

356. Findlay, J. W., Butz, R. F., and Welch, R. M., Specific radioimmunoassays for codeine and morphine. Metabolism of codeine to morphine in the rat, *Res. Commun. Chem. Pathol. Pharmacol.*, 17(4), 595, 1977.

357. Ertel, N. H., Mittler, J. C., Akgun, S., and Wallace, S. L., Radioimmunoassay for colchicine in plasma and urine, *Science*, 193(4249), 233, 1976.

358. Dixon, W. R., Young, R. L., Ning, R., and Liebman, A., Radioimmunoassay of the anticonvulsant agent clonazepam, *J. Pharm. Sci.*, 66(2), 235, 1977.

359. Dixon, R., Brooks, M. A., Postma, E., Hackman, M. R., Spector, S., Moore, J. D., and Schwartz, M. A., *N*-Desmethyldiazepam: a new metabolite of chlordiazepoxide in man, *Clin. Pharmacol. Ther.*, 20(4), 450, 1976.

360. Wiles, D. H., Kolakowska, T., McNeilly, A. S., Mandelbrote, B. M., and Gelder, M. G., Clinical significance of plasma chlorpromazine levels. I. Plasma levels of the drug, some of its metabolites and prolactin during acute treatment, *Psychol. Med.*, 6(3), 407, 1976.

361. Read, G. F., Riad-Fahmy, D., and Walker, R. F., A specific radio-immunoassay procedure for plasma clomipramine, *Postgrad. Med. J.*, 53(Suppl. 4), 110, 1977.

362. Read, G. F. and Riad-Fahmy, D., Determination of tricyclic antidepressant, clomipramine (Anafranil), in plasma by a specific radioimmunoassay procedure, *Clin. Chem.*, 24(1), 36, 1978.

363. Dixon, R. and Crews, T., An ^{125}I-radioimmunoassay for the determination of the anticonvulsant agent clonazepam directly in plasma, *Res. Commun. Chem. Pathol. Pharmacol.*, 18(3), 477, 1977.

364. Rosenthaler, J., Nimmerfall, F., Sigrist, R., and Munzer, H., Non-equilibrium method for the radioimmunoassay of clozapine in the presence of metabolites, *Eur. J. Biochem.*, 80(2), 603, 1977.

365. Dixon, R., Fahrenholtz, K. E., Burger, W., and Perry, C., Radioimmunoassay of the anti-hypertensive agent Debrisoquin, *Res. Commun. Chem. Pathol. Pharmacol.*, 16(1), 121, 1977.

366. Brunswick, D. J., Needelman, B., and Mendels, J., Radioimmunoassay of imipramine and desmethylimipramine, *Life Sci.*, 22(2), 137, 1978.

367. Gelbke, H. P., Schlicht, H. J., and Schmidt, G., Occurence of diazepam in blood samples of drivers under the influence of alcohol, *Z. Rechtsmed.*, 80(4), 319, 1978.

368. Gelbke, H. P., Schlicht, H. J., and Schmidt, G., Radioimmunological screening and gas chromatographic identification of diazepam in blood and serum, *Arch. Toxicol.*, 38(4), 295, 1977.

369. Greenwood, H., Landon, J., and Forrest, G. C., Radioimmunoassay for digoxin with a fully automated continuous-flow system, *Clin. Chem.*, 23(10), 1868, 1977.

370. Wood, W. G., A test of six commercial ^{125}I-labeled digoxin radioimmunoassay kits, *J. Clin. Chem. Clin. Biochem.*, 15(12), 679, 1977.

371. Stavchansky, S., Ludden, T., Allen, J. P., and Wu, P., Correlation of the E.M.I.T. diphenylhydantoin assay in blood plasma with a G.L.C. and a spectrphotometric method, *Anal. Chim. Acta*, 92(1), 213, 1977.

372. Paxton, J. W., Rowell, F. J., and Ratcliffe, J. G., The evaluation of a radioimmunoassay for diphenylhydantoin using an iodinated tracer, *Clin. Chim. Acta*, 78(1), 81, 1977.

373. L'Estrange, O. M., Borg, A. O., Cook, C. E., and Sjoqvist, F., Measurement of diphenylhydantoin in 0.1-ml plasma samples: gas chromatography and radioimmunoassay compared, *Clin. Chem.*, 22(2), 245, 1976.

374. Schleimer, R., Benjamini, E., Eisele, J., and Henderson, G., Radioimmunoassay of fentanyl: pharmacokinetics in man. *Proc. West. Pharmacol. Soc.* 19, 237, 1976.

375. Schleimer, R., Benjamini, E., Eisele, J., and Henderson, G., Pharmacokinetics of fentanyl as determined by radioimmunoassay, *Clin. Pharmacol. Ther.*, 23(2), 188, 1978.

376. Michiels, M., Hendriks, R., and Heykants, J., A sensitive radioimmunoassay for fentanyl. Plasma level in dogs and man, *Eur. J. Clin. Pharmacol.*, 12(2), 153, 1977.

377. Risby, D and Robinson, J. D., A direct radioimmunoassay for flupenthixol in plasma proceeding, *Br. J. Clin. Pharmacol.*, 4(3), 407, 1977.

378. Risby, D. and Robinson, J. D., The development of a radioimmunoassay for flupenthixol, in *Clinical Toxicology*, Duncan, W. A. and Leonard, B. J., Eds., Excerpta Medica, Amsterdam, 1977, 249.

379. Robinson, J. D. and Risby, D., Radioimmunoassay for flupenthixol in plasma, *Clin. Chem.*, 23(11), 2085, 1977.

380. Clark, B. R., Tower, B. B., and Rubin, R. T., Radioimmunoassay of haloperidol in human serum, *Life Sci.*, 20(2), 319, 1977.

381. Hare, L. E., Ditzler, C. A., and Duggan, D. E., Radioimmunoassay of indomethacin in biological fluids, *J. Pharm. Sci.*, 55(4), 486, 1977.

382. Michiels, M., Hendriks, R., and Heykants, J., Radioimmunoassay of the antidiarrheal loperamide, *Life Sci.*, 21(3), 451. 1977.

383. Wainer, B. H., Wung, W. E., Hill, J. H., Fitch, F. W., Fried, J., and Rothberg, R. M., The production and characterization of antibodies reactive with meperidine, *J. Pharmacol. Exp. Ther.*, 197(3), 734, 1976.

384. Wainer, B. H., Wung, W. E., Hill, J. H., Fitch, F. W., Fried, J., and Rothberg, R. M., The production and characterization of antibodies reactive with meperidine, *J. Pharmacol. Exp. Ther.*, 197(1), 163, 1976.

385. Roerig, D. L., Wang, R. I., Mueller, M. M., Lewand, D. L., and Adams, S. M., Radioimmunoassay compared to thin-layer and gas-liquid chromatography for detecting methadone in human urine, *Clin. Chem.*, 22(11), 1915, 1976.

386. Bartos, F., Olsen, G. D., Leger, R. N., and Bartos, D., Stereospecific antibodies to methadone. I. Radioimmunoassay of *d,l*-methadone in human serum, *Res. Commun. Chem. Pathol. Pharmacol.*, 16(1), 131, 1977.

387. Lam, R. W., Artal, R., and Fisher, D. A., Radioimmunoassay for free and conjugated urinary metanephrine, *Clin. Chem.*, 23(7), 1264, 1977.

388. Inayama, S., Tokunaga, Y., Hosoya, E., Nakadate, T., and Niwaguchi, T., The radioimmunoassay for methamphetamine, *Chem. Pharm. Bull.*, 25(4), 840, 1977.

389. Rock, N. L. and Moore, R. J., Methaqualone (Mandrax) abuse, urine testing, and identification: clinical correlation between a new mass urinalysis test and a military drug abuse program, *Int. J. Addict.*, 11(2), 237, 1976.

390. Bost, R. D., Sutheimer, C. A., and Sunshine, I., Letters: methaqualone assay by radioimmunoassay and gas chromatography, *Clin. Chem.*, 22(5), 689, 1976.

391. Aherne, G. W., Piall, E. M., and Marks, V., Development and application of a radioimmunoassay for methotrexate, *Br. J. Cancer.*, 36(5), 608, 1977.

392. Paxton, J. W. and Rowell, F. J., A rapid, sensitive and specific radioimmunoassay for methotrexate, *Clin. Chim. Acta*, 80(3), 563, 1977.

393. Raso, V., Radioimmunoassays for methotrexate, citrovorum factor, neocarzinostatin, and Actinomycin D, *Cancer Treat. Rep.*, 61(4), 585, 1977.

394. Loeffler, L. J., Blum, M. R., and Nelsen, M. A., A radioimmunoassay for methotrexate and its comparison with spectrofluorimetric procedures, *Cancer Res.*, 35(9), 3306, 1976.

395. Hendel, J., Sarek, L. J., and Hvidberg, E. F., Rapid radioimmunoassay for methotrexate in biological fluids, *Clin. Chem.*, 22(6), 813, 1976.

396. Wang, Y., Lantin, E., and Sutow, W. W., Methotrexate in blood, urine, and cerebrospinal fluid of children receiving high doses by infusion, *Clin. Chem.*, 22(7), 1053, 1976.

397. Gintzler, A. R., Mohacsi, E., and Spector, S., Radioimmunoassay for the simultaneous determination of morphine and codeine, *Eur. J. Pharmacol.*, 38(1), 149, 1976.

398. Lafisca, S., Bolelli, G., Mosca, R., and Zanon, C., Radioimmunoassay of morphine and morphine-like substances in biological fluids and human tissues, *Ric. Clin. Lab.*, 7(2), 179, 1977.

399. van der Slooten, E. P. and van der Helm, H. J., Comparison of the EMIT (enzyme multiplied immunoassay technique) opiate assay and a gas-chromatographic—mass-spectrometric determination of morphine and codeine in urine, *Clin. Chem.*, 22(7), 1110, 1976.

400. Nicolau, G., Van Lear, G., Kaul, B., and Davidow, B., Determination of morphine by electron capture gas-liquid chromatography, *Clin. Chem.*, 23(9), 1640, 1977.

401. Busche, J. and Gelbke, H. P., Determination of morphine in the vitreous and cerebrospinal fluid by radioimmunoassay, *Z. Rechtsmed.*, 79(3), 235, 1977.

402. Moller, M. R., Tausch, D., and Biro, G., Radioimmunological detection of morphine in stains of blood and urine, *Z. Rechtsmed.*, 79(2), 103, 1977.

403. Jain, N. C., Sneath, T. C., Leung, W. J., and Budd, R. D., Mass screening and confirmation of codeine and morphine in urine by radioimmunoassay-GLC, *J. Pharm. Sci.*, 55(1), 66, 1977.

404. De Ferrari, F., Gambaro, V., Lodi, F., Marozzi, E., and Saligari, E., Use of RIA (radioimmunoassay) technics in the identification of narcotics in biological fluids, *Minerva Med.*, 67(35), 2301, 1976.

405. Oellerich, M., Kulpmann, W. R., and Haeckel, R., Drug screening by enzyme immunoassay (EMIT) and thin-layer chromatography (drug screen), *J. Clin. Chem. Clin. Biochem.*, 15(5), 275, 1977.

406. Castro, A. and Malkuś, H., Radioimmunoassays of drugs of abuse in humans: a review, *Res. Commun. Chem. Pathol. Pharmacol.*, 16(2), 291, 1977.

407. Alexander, G. J. and Machiz, S., Simplified radioimmunoassay of urinary drugs of abuse adsorbed on ion-exchange papers, *Clin. Chem.*, 23(10), 1921, 1977.

408. Cleeland, R., Christenson, J., Usategui-Gomez, M., Heveran, J., Davis, R., and Grunberg, E., Detection of drugs of abuse by radioimmunoassay: a summary of published data and some new information, *Clin. Chem.*, 22(6), 712, 1976.

409. Freeman, D. S., Gjika, H. B., and Van Vunakis, H., Radioimmunoassay for normeperidine: studies on the *N*-dealkylation of meperidine and anileridine, *J. Pharmacol. Exp. Ther.*, 203(1), 203, 1977.

410. Spiehler, V., Sun, L., Miyada, D. S., Sarandis, S. G., Walwick, E. R., Klein, M. W., Jordan, D. B., and Jessen, B., Radioimmunoassay, enzyme immunoassay, spectrophotometry, and gas-liquid chromatography compared for determination of phenobarbital and diphenylhydantoin, *Clin. Chem.*, 22(6), 749, 1976.

411. Brunk, S. D., Hadjiioannou, T. P., Hadjiioannou, S. I., and Malmstadt, H. V., Adaptation of "EMIT" technique for serum phenobarbital and diphenylhydantoin assays to the miniature centrifugal analyzer, *Clin. Chem.*, 22(6), 905, 1976.

412. Schmidt, D., Measurement of diphenylhydantoin and phenobarbital by enzyme immunoassay and gas-liquid chromatography, *J. Neurol.*, 213(1), 41, 1976.

413. Schmidt, D., Goldberg, V., Guelen, P. J., Johannessen, S., v.d. Kleijn, E., Meijer, J. W., Meinardi, H., Richens, A., Schneider, H., Stein-Lavie, Y., and Symann-Louette, N., Evaluation of a new immunoassay for determination of phenytoin and phenobarbital: results of a European collaborative control study, *Epilepsia*, 18(3), 367, 1977.

414. Turri, J. J., Enzyme immunoassay of phenobarbital, phenytoin, and primidone with the ABA-100 biochromatic analyzer letter, *Clin. Chem.*, 23(8), 1510, 1977.

415. Finley, P. R., Williams, R. J., Lichti, D. F., and Byers, J. M., Assay of phenobarbital-adaption of "EMIT" to the centrifugal analyzer, *Clin. Chem.*, 23(4), 738, 1977.

416. Pitlick, W., Painter, M., and Pippenger, C., Phenobarbital pharmacokinetics in neonates, *Clin. Pharmacol. Ther.*, 23(3), 346, 1978.

417. Viswanathan, C. T., Booker, H. E., and Welling, P. G., Bioavailability of oral and intramuscular phenobarbital, *J. Clin. Pharmacol.*, 18(2–3), 100, 1978.

418. Oellerich, M., Kulpmann, W. R., Haeckel, R., and Heyer, R., Determination of phenobarbital and phenytoin in serum by a mechanized enzyme immunoassay (EMIT) in comparsion with a gas-liquid chromatographic method, *J. Clin. Chem. Clin. Biochem.*, 15(6), 353, 1977.

419. Viswanathan, C. T., Booker, H. E. and Welling, P. G., Interference by *p*-hydroxyphenobarbital in the ^{123}I-radioimmunoassay of serum and urinary phenobarbital, *Clin. Chem.*, 23(5), 873, 1977.

420. Belfield, A., Duncan, A. M. and Reavey, P. C., Enzyme multiplied immunoassay of phenobarbital by LKB reaction rate analyser: a comparisen with analysis by gas-liquid chromatography, *Ann. Clin. Biochem.*, 14(4), 218, 1977.

421. London, M., Sanabria, D., and Yau, D., Enzyme immunoassay of phenytoin and phenobarbital with the centrifugal analyzer letter, *Clin. Chem.*, 23(7), 1362, 1977.

422. Long, J. P., Enzyme immunoassay, with use of a centrifugal analyzer, of phenytoin, phenobarbital, and theophylline, *Clin. Chem.*, 24(2), 391, 1978.

423. McGregor, A. R., Crookall-Greening, J. D., Landon, J., and Smith, D. S., Polarisation fluoroimmunoassay of phenytoin, *Clin. Chem. Acta*, 83(1–2), 161, 1978.

424. Paxton, J. W., Whiting, B., and Stephen, K. W., Phenytoin concentrations in mixed, parotid and submandibular saliva and serum measured by radioimmunoassay, *Br. J. Clin. Pharmacol.*, 4(2), 185, 1977.

425. Paxton, J. W., Rowell, F., Ratclife, J. G., Lambie, D. G., Nada, R., Melvill, I. D., and Johnson, R. H., Salivary phenytoin radioimmunoassay. A simple method of the assessment of nonprotein bound drug concentrations, *Eur. J. Clin. Pharmacol.*, 11(1), 71, 1977.

426. Finley, P. R., Williams, R. J., and Byers, J. M., Assay of phenytoin: adaptation of "EMIT" to the centrifugal analyzer, *Clin. Chem.*, 22(6), 911, 1976.

427. Loughnan, P. M., Greenwald, A., Purton, W. W., Aranda, J. V., Watters, G., and Neims, A. H., Pharmacokinetic observations of phenytoin disposition in the newborn and young infant, *Arch. Dis. Child.*, 52(4), 302, 1977.

428. Chamberlain, R. T., Stafford, D. T., Maijub, A. G., and Monatt, B. C., High-pressure liquid chromatography and enzyme immunoassay compared with gas chromatography for determining phenytoin, *Clin. Chem.*, 23(9), 1764, 1977.

429. Sun, L. and Walwick, E. R., Primidone analyses: correlation of gas-chromatographic assay with enzyme immunoassay, *Clin. Chem.*, 22(6), 901, 1976.

430. Kawashima, K., Levy, A., and Spector, S., Stereospecific radioimmunoassay for propranolol isomers, *J. Pharmacol. Exp. Ther.*, 196(2), 517, 1976.

431. Levy, A., Kawashima, K., and Spector, S., Radioimmunoassay for reserpine, *Life Sci.*, 19(9), 1421, 1976.

432. Rowley, G. L., Armstrong, T. A., Crowl, C. P. Eimstad, W. M., Hu, W. M., Kam, J. R., Rogers, R., Ronald, R. C., Rubenstein, K. E., Sheldon, B. G., and Ullman, E. P., Determination of THC and its metabolites by EMIT homogeneous enzyme immunoassay: a summary report, *Natl. Inst. Drug Abuse Res. Monogr. Ser.*, No. 7, 28, 1976.

433. Cook, C. E., Hawos, M. L., Amerson, E. W., Pitt, C. G., and Williams, D., Radiommunoassay of Δ^9-tetrahydrocannabinol, *Natl. Inst. Drug Abuse Res. Monogr. Ser.*, No. 7, 15, 1976.

434. Garrett, C. P., Braithwaite, R. A., and Teale, J. D., Unusual case of tetrahydrocannabinol intoxication confirmed by radioimmunoassay, *Br. Med. J.*, 2(6080), 166, 1977.

435. Neese, A. L. and Soyka, L. F., Development of a radioimmunoassay for theophylline. Application to studies in premature infants, *Clin. Pharmacol. Ther.*, 21(5), 633, 1977.

436. Matteo, R. S., Pua, E. K. Khambatta, H. J., and Spector, S., Cerebrospina. fluid levels of *d*-tubocurarine in man, *Anesthesiology*, 46(6), 396, 1977.

437. Fasth, A., Sollenberg, J., and Sorbo, B., Production and characterization of anti-

bodies to atropine, *Acta Pharm. Suec.*, 12(4), 311, 1975.

438. Teale, J. D., Forman, E. J., King, L. J., Piall, E. M., and Marks, V., The development of a radioimmunoassay for cannabinoids in blood and urine, *J. Pharm. Pharmacol.*, 27(7), 465, 1975.

439. Dixon, W. R., Earley, J., and Postma, E., Radioimmunoassay of chlordiazepoxide in plasma, *J. Pharm. Sci.*, 64(6), 937, 1975.

440. Kawashima, K., Dixon, R., and Spector, S., Development of radioimmunoassay for chlorpromazine, *Eur. J. Pharmacol.*, 32(02), 195, 1975.

441. Boudene, C., Duprey, F., and Bohuon, C., Radioimmunoassay of colchicine, *Biochem, J.*, 151(2), 413, 1975.

442. Spector, S., Spector, N. L., and Almeida, M. P., Jr., Radioimmunoassay for desmethylimipramine, *Psychopharmacol. Commun.*, 1(4), 421, 1975.

443. Berkowitz, B. A., Ngai, S. H., Hempstead, J., and Spector, S., Disposition of naloxone: use of a new radioimmunoassay, *J. Pharmacol. Exp. Ther.*, 195(3), 499, 1975.

444. Matsuyama, H., Tamada, T., Ishiguro, T., and Muranaka, H., Radioimmunoassay of nicotine, *Biochem. Biphys. Res. Commun.*, 64(2), 574, 1975.

445. Torten, M., Henderson, G. L., Leung, C. Y., Miller, C. H., Eisele, J. H., and Benjamini, E., Proceedings: immunopharmacology of a potent opiate, fentanyl. Development of radioimmunoassay and prevention of effects by immunologic means, *Isr. J. Med. Sci.*, 11(12), 1395, 1975.

Light Microscopy and Forensic Drug Analysis

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I. INTRODUCTION

The polarizing microscope, by making possible so many physical measurements (crystal angles, optical properties, melting points, etc.), is an excellent microanalytical tool. In the hands of an experienced microscopist it is rapid and dependable. Even in the absence of lengthy training and experience a microscopist can expect to find polarized light microscopy very useful as a confirmatory technique. If, for example, GC/MS reports the presence of seconal it should be a simple matter to compare crystals in that sample directly with known crystals of that substance or with data from the literature for that particular compound.

For any analytical procedure to be useful as a general method for complete unknowns, it is essential that the data bank for that method cover all possible substances that might be encountered. Unfortunately, there are many excellent methods for which the data bank is just not available and this unfortunately includes the polarizing microscope with respect to drugs. Therefore, unless and until someone provides the necessary data bank, the light microscope will be used almost entirely as a confirmatory test or to check for the presence of a small number of specific substances. If, for example, one wishes to know whether a given sample contains amobarbital, heroin, aspirin, or another specific drug, the microscope in the hands of a trained microscopist would be the most rapid method possible.

The identification of drugs with the microscope depends on the measurement or observation of crystallographic properties. This might include morphological and optical properties on crystals of the drug itself or it might be the corresponding properties on either precipitated derivatives of the compound in question or of crystals grown from the melt. There are, then, several different procedures useful in light microscopy for drug identification. The first is based on crystal shape, including interfacial angles, axial ratios and crystal system, and optical properties such as refractive indices and dispersion. This has the advantage that the sample is observed directly without dissolving, vaporizing or otherwise modifying it. The second is based on microcrystal tests and these precipitation reactions are based on the known morphology and optics of crystals obtained after dissolving the compound and obtaining a precipitate through use of a specific reagent. Finally, there are methods based on heating the sample between slide and coverslip and observing the phase changes which occur as a result of heating and subsequent cooling. The latter measurements can be made more accurately using a microscope hot stage based on methods developed by the Köflers. Often the appearance of crystals from the melt will be so distinctive that this will be a sufficient indication of composition. A simple mixed fusion with a known sample will then quickly confirm whether the identification is accurate.

II. CRYSTAL MORPHOLOGY AND OPTICS

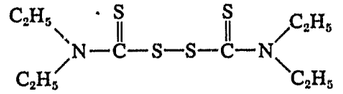
The angles between the crystal faces for a given compound are so distinctive that a scheme has been developed (1) which permits certain identification by measurement of these parameters (for any compound for which data is available). There are, however, many other quantitative measurements that can be made on crystals, especially optical properties (2) (not to mention crystal lattice parameters determined by x-ray diffraction). Most crystals

have three refractive indices measurable to three or even four decimal places and therefore considerably more accurate than capillary melting points. Once determined on known compounds, crystallographic data are quickly and easily checked on possibly identical crystals.

The following description for antabuse, an alcoholism control drug, illustrates the range of crystallographic data available for microanalysis.

A. Crystallographic Data for Antabuse

Antabuse is the drug recently discovered to be of value in the treatment of alcoholism. It is extremely soluble in most organic solvents and good crystals can be obtained from ethyl alcohol, benzene and dioxane. No polymorphism was observed during this study.



Structural formula of antabuse

1. Crystal Morphology

Crystal system. Monoclinic.

Form and habit. Crystallizes from ethyl alcohol in needles elongated parallel to c, and in tablets lying on 010 showing clinopinacoid {010}, orthopinacoid {100}, and clinodome {011}. A variety of other dome, prism and bipyramid forms usually also appears. The simple form shown in Figure 1 is obtained by recrystallization from thymol on a microscope slide.

Axial ratio. $a:b:c=0.870:1:0.545$.

Interfacial angles (polar).
$011 \wedge 0\bar{1}1=47.5°$.

Profile angle. $011 \wedge 0\bar{1}1$ in 100 plane=123°.

Beta angle. 126°.

2. X-Ray Diffraction Data

Cell dimensions. $a=13.84$ A.; $b=15.90$ A.; $c=8.66$ A.

Formula weights per cell. 4.

Formula weight. 296.52

Density. 1.292 (pycnometer); 1.302 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1
9.11	0.16	3.07	0.05
7.97	Very weak	2.98	0.13
7.58	0.40	2.91	0.11
6.38	1.00	2.82	Very weak
6.09	0.38	2.77	0.09
5.53	0.18	2.70	0.04
5.29	Very weak	2.64	0.04
5.09	0.40	2.60	Very weak
4.76	0.07	2.52	0.16
4.54	0.26	2.47	0.15
4.31	0.12	2.43	0.09
4.16	0.69	2.38	Very weak
4.07	Very weak	2.33	Very weak
3.94	0.13	2.28	0.07
3.77	0.09	2.25	0.06
3.60	0.33	2.22	0.13
3.45	0.20	2.19	Very weak
3.38	0.19	2.15	0.09
3.34	Very weak	1.87	0.10
3.25	0.08	1.71	0.11
3.17	0.28		

3. Optical Properties

Refractive indices. (5893 A.; 25°C). $\alpha=1.590\pm0.005$; $\beta=1.67\pm0.01$; $\gamma=1.740\pm0.005$.

Optic axial angle. (5893 A.; 25°C). $2V=84°\pm5°$.

Dispersion. $v>r$.

Optic axial plane. 010.

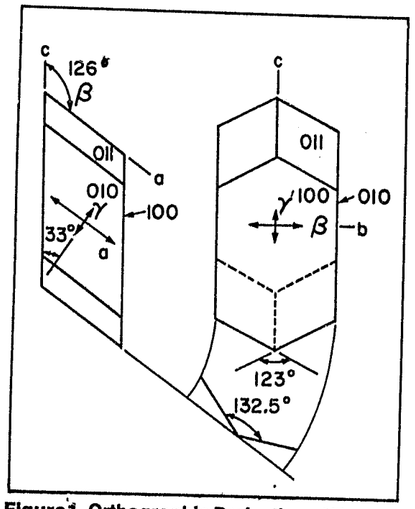


Figure 1. Orthographic Projection of Typical Crystal of Antabuse.

Sign of double refraction. Negative.

Acute bisectrix. $BX_a \Lambda a=3°$ in obtuse β.

Extinction. $\gamma \Lambda c=33°$ in obtuse β.

Molecular refraction. (R) (5893 A.; 25°C). $\sqrt[3]{\alpha\beta\gamma}=1.67$. R(calcd.)=84.2. R(obsd.)=85.0.

4. Fusion Data

Antabuse melts at 69–70°C without sublimation or decomposition. The melt supercools and crystallization usually must be initiated by seeding. The crystals grow rapidly parallel to c and the crystal front in a thymol mixed fusion shows characteristic well-shaped rhombs lying on pinacoid or prism faces. Some crystals may show the characteristic optic axis interference figure.

Although a crystallographer's language is almost entirely incomprehensible even to other microanalysts, a microscopist familiar with these techniques would need no more than five minutes to confirm that a crystallographic substance he has under the microscope is, or is not, identical with a compound as described. In the absence of known data, a known sample will permit an equally speedy and dependable decision. Unfortunately, this data bank covering crystal morphology and optics is very incomplete; therefore, those procedures can only be used for the approximately 40 drugs Winchell describes (2).

III. MICROCRYSTAL TESTS

Since relatively few light microscopists are versed in the use of optical crystallography, they might prefer to use microcrystal tests even though this also requires a certain amount of background study and experience. These methods, as developed by Charles Fulton (3) during a long career with the Food and Drug Administration, are based on the use of reagents such as chloroplatinic acid, potassium iodide or I_2-KI which, when mixed with test drops containing a specific drug, will yield a characteristic crystalline precipitate usually easily remembered as characteristic of a specific substance. I have included here a paragraph from page 150 of Fulton's book covering the results obtained for amobarbital with several different reagents. The book contains descriptions such as this with pictures of specific tests for several hundred individual drugs.

"*Amobarbital:* I_2-KI gives a very sensitive test; immediate precipitation is in little light-colored plates and blades; with some evaporation quite dilute solutions give

larger blades, often in clusters at various angles, PC orange and blue-green colors; the little plates are often 'squares' approaching paddlewheel structure."

There is a second source of microcrystal test data, Behrens-Kley (4) *Organische Mikrochemische Analyse*, translated into English by Richard Stevens. Figures 2–3 show illustrations from this book for a few common drugs. This book has the advantage of simplicity as compared with Fuiton's, but it covers fewer compounds, about 60 in all.

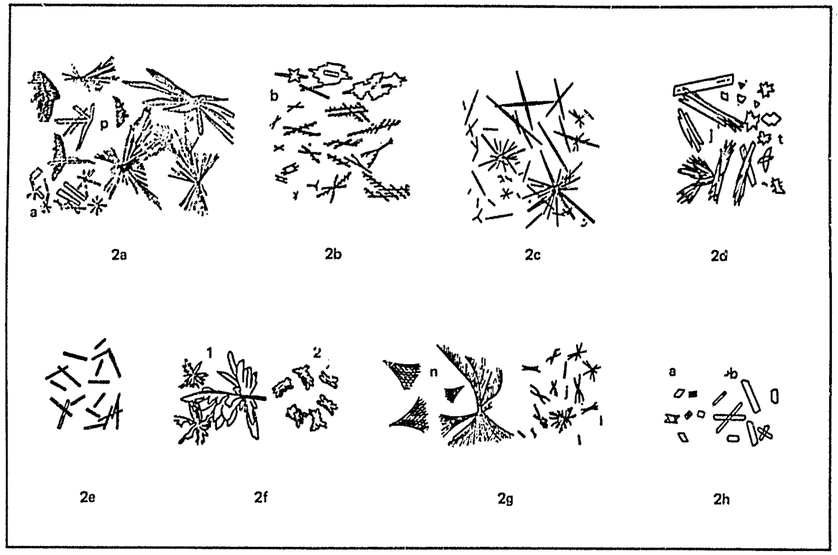


Figure 2. Illustrations from *Organische Mikrochemische Analyse* by Behrens-Kley.

2a. Cocaine Precipitated with Sodium Carbonate (a). Cocaine Chloroplatinate (p). 130:1
2b. Cocaine Chloroaurate; Bromoaurate at (b) from Dilute Solution. 130:1
2c. Atropine Precipitated by NaOH from HCl Solution. 60:1
2d. Hydriodide (j) and Tartrate (t) of Iodoatropine. 130:1
2e. Aconitine-Silver Nitrate. 300:1
2f. Cytisine, (1) Iodoplatinate. 60:1; (2) Bromoaurate. 200:1
2g. Berberine, (n) Nitrate. 130:1; (j) Triiodide. 300:1
2h. Strychnine. Precipitation with Ammonia (a). Precipitation with Sodium Bicarbonate. 60:1

IV. THERMAL METHODS

Ludwig and Adelheid Köfler both taught in the Department of Pharmacognosy of the University of Innsbruck in Austria and this explains why their book (5) includes most of the common drugs. They have published a compilation of methods for the study of compounds and their mixtures using the hot stage microscope. The identification tables they include make it possible to identify any of about eleven hundred compounds in just a few minutes. Table I shows a portion of one of their tables with the data for morphine. Most of their identifications are based on the melting point of the compound itself, as well as melting points of eutectics of that compound with standard second components. They also usually determine

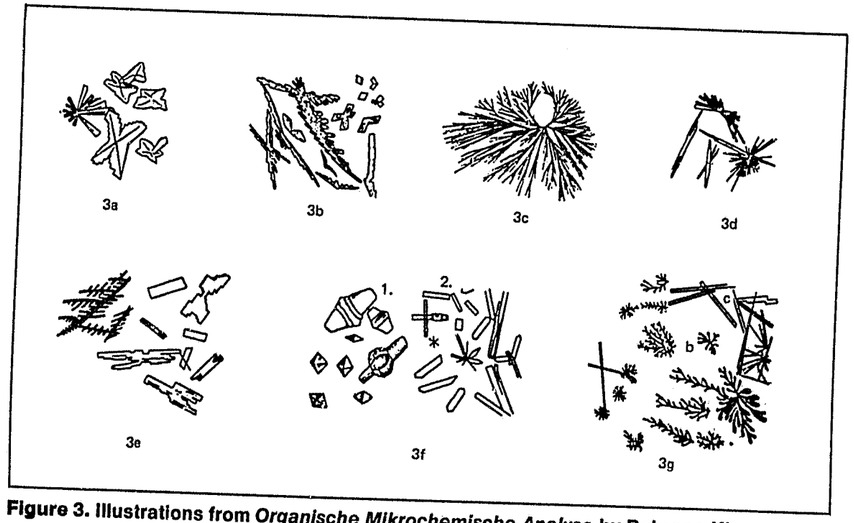


Figure 3. Illustrations from *Organische Mikrochemische Analyse* by Behrens-Kley.

3a. Strychnine Chloroplatinate. 60:1
3b. Strychnine Precipitated with Potassium Ferrocyanide. 90:1
3c. Brucine Precipitated with Ammonia. 60:1
3d. Brucine Chloroplatinate. 60:1
3e. Morphine Precipitated by Ammonium Carbonate from Alkaline Solution. 30:1
3f. Morphine, Precipitated by $NaHCO_3$ (1); Precipitated by Na_2CO_3 from HCl Solution. (1) 90:1; (2) 60:1
3g. Morphine Chloromercurate (c); Bromomercurate (b). 60:1

TABLE I

Kofler Data

Melting Point	*Compound*	*Eutectic Temperature:*		
(°C)		*salophen*	*dicyandiamide*	*phenolphthalein*
244–255	morphine	156	174	255
250–255	dihydrocodeine·HCl	165	131	—
255	α-aminoanthraquinone	176	207	222
256	pentaerythritol	160	174	188

the refractive index of the melt by a reverse immersion method using standard glass powders of known refractive index. Finally, they note any evidence of sublimation, polymorphism, decomposition and so on. Again, the methods are straightforward and simple and the data bank is reasonably complete for drugs.

It is probably worth mentioning, for completeness at least, McCrone's more qualitative procedures (6) which are based on the appearance of the crystals as they grow from the melt. The significant parameters include phase changes, sublimation, decomposition, estimated melting point, degree of supercooling, crystal form, crystal growth rate (as a function of temperature), refractive indices relative to the melt, extinction angles, estimated birefringence, anomalous polarization colors, interference figure, optic axial angle, optic sign and dispersion. The limitation of the method is based on how many compounds one can remember in this way since most of the observations are descriptive rather than quantitative. However, experience has shown that one to two hundred is not too large a number, especially if they are encountered fairly regularly so that one is occasionally reminded of their unique characteristics. Perhaps these procedures are best held for confirmatory tests. If, for example, one felt that he had a sample of sulfamerazine it would take only a minute or two to heat the sample between slide and coverslip to observe the characteristic sublimate and, finally, to melt the sample and observe crystallization from the melt on cooling; the crystal forms and polarization colors are very characteristic of sulfamerazine. If any doubt remained, a few crystals of known sulfamerazine melted at the edge of the coverslip, so that the melt ran under and into contact with the unknown, would then show (by the absence of other crystals in the zone of mixing) that the two were identical or (by some discontinuity of form or behavior in this zone of mixing) that the two were different. This is an ideal identity test, far superior in speed and dependability to a mixed melting point.

V. CONCLUSION

I have tried to avoid overselling light microscopy even though it would always be my personal method of choice. My preference is, of course, based on the fact that my experience lies with the microscope and I am motivated to use it whenever possible. If I were advising any crime lab personnel on how to proceed with drugs I would, on the other hand, recommend GC/MS since it fulfills all of the requirements for a good method and because the training for that method is much less lengthy than for any other method including the microscope. I would, however, point out the possibility of using the microscope and emphasize the possibility that it could be an excellent confirmatory method to give that added degree of confidence when one is going to court.

REFERENCES

1. Porter, R. W. and Spiller, R. C., *The Barker Index of Crystals,* 2 volumes of 3 parts-Heffer, Cambridge (1951).
2. Winchell, A. N., *Optical Properties of Organic Compounds,* 2nd ed.-Academic Press, New York (1954).
3. Fulton, Charles C., *Modern Microcrystal Tests for Drugs,* Wiley-Interscience, New York (1969).
4. Behrens-Kley, *Organische Mikrochemische Analyse,* Leopold Voss, Leipzig (1922); English translation R. E. Stevens-Microscope Publications, London (1969).
5. Kofler, L. and Kofler, A., *Thermomikromethoden,* Wagner, Innsbruck (1954).
6. McCrone, W. C., *Fusion Methods,* Interscience, New York (1956).

Evolution of SEM Utilization in the Crime Laboratory

by

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I. INTRODUCTION

In this paper the scanning electron microscope (SEM) and the associated energy dispersive X-ray analyzer (EDX) will be described. Specifically, the various evolutionary stages in the use of the SEM-EDX in the FBI Laboratory will be presented. Hopefully, this exposure will allow the reader who has not had first-hand experience with the instrument to become familiar with its capabilities, weaknesses, and general use in one crime laboratory.

II. FUNCTIONING OF THE SEM-EDX

The SEM-EDX causes a finely-pointed electron beam to scan, in a raster-like fashion, over the surface of a specimen. The interaction of the beam and the specimen causes various signals (secondary electrons, X-rays, backscattered electrons, etc.) to be produced which can be used to provide information about the specimen's surface. An image is formed by collecting the secondary electrons by a scintillator-photomultiplier chain (SEM). The X-rays are captured by a solid-state crystal and analyzed with respect to their energies (EDX). The result is a spectrum which, after proper interpretation provides an elemental composition of that area of the surface which is being bombarded by the electron beam.

Because of various experimental factors, only elements having atomic number greater than 10 (Ne) can be analyzed. A detailed description of the instrument is omitted but the interested reader is referred to any of several publications on the subject (1, 2).

The specimen is positioned inside the vacuum chamber of the SEM-EDX, thereby placing a restriction on its size and on its vapor pressure (no liquids). For work in criminalistics, very little specimen preparation is required. Usually the small specimen is placed on a carbon flat, examined under a dissecting microscope for color and geometric orientation, and then placed in the chamber.

III. USE OF THE SEM

The instrument was initially used for the comparison of fracture surfaces (3). This research project dealt only with fractures which required high magnifications or where the surface detail was too rough for the limited depth of field of an optical microscope. The SEM's magnification capability (up to 40,000 times) and its large depth of field (about 300 times that of an optical microscope) make it particularly suitable for examining fracture surfaces having these characteristics.

A typical application to case work would be the matching of a broken wire found attached to a piece of stolen electronic gear to another broken wire from the crime scene.

Other applications include recognition of unique morphology associated with microcrystals, tool mark comparisons, and where the small size or depth of field requirements are unable to be satisfied by optical microscopy.

A careful selection of the specimen is required before these types of comparisons are performed. The procedures are time-consuming, requiring great care in the orientation of the specimen. Often days of searching can be spent before completion. Completion does not necessarily mean a positive comparison, but can mean negative or inconclusive results.

If the applications mentioned in this section were all that were possible with the SEM, the instrument would be little used in our laboratory.

IV. USE OF THE EDX

While the work on fracture comparison was being carried out, various samples which were not suitable for analysis by other techniques available in our laboratory were brought to the SEM-EDX. The X-ray fluorescence spectra of these specimens were obtained and a qualitative elemental composition of the specimens was given to the examiner who originally contributed the samples. The number of specimens examined increased with usage and eventually included specimens which could have been adequately run by other types of examination. Two properties of the SEM-EDX caused this phenomenon: fast turn-around time (about 10 minutes for a simple qualitative analysis) and non-destructive qualitative analytical capability. Thus, part of the workload involved specimens which were being given a qualitative "once-over" prior to other analyses (for example, neutron activation analysis) which would provide additional information (quantitative results or increased sensitivity).

Some of the disadvantages of the EDX include a relatively high background signal causing a low sensitivity (0.1 to 1%), ambiguous interpretation of peaks unless secondary peaks are considered, and insensitivity to organic constituents.

A voluntary training program was initiated to introduce the examiners to the use and application of the instrument. The result was an even greater increase in the frequency of examinations requiring the simple qualitative elemental comparison of items in casework. The specimens consist of small chips of paint, metallic fragments, unknown powders, ores, suspected poisons, some drugs, and whatever other specimens enter a crime laboratory.

V. USE OF SEM-EDX

The previous two sections have described two separate instruments: a powerful microscope (section III) and an analytical instrument (section IV). However, the greatest information can be obtained from the specimen by using these two modes simultaneously. That is, a particular microscopic item of an aggregate can be analyzed without requiring the item to be physically isolated from its matrix. A mass sensitivity of 10^{-12} to 10^{-10} grams can be obtained (2). However, in the analysis of a component particle situated in a matrix, the contribution of the matrix to the spectrum cannot be completely eliminated. Common applications of the SEM-EDX are: analysis of individual layers in a multi-layered paint chip; analysis of each grain in an ore sample to determine the distribution of a particular element; analysis for small particles of Zn in bomb explosion debris; and localization of As in a poisoned bread. Another general category of use requires the SEM-EDX to provide the examiner with the two-dimensional distribution of a particular element. That is, the instrument places a heavy dot concentration wherever it finds a previously chosen element. The resultant image is called a "dot pattern" or "elemental distribution pattern".

A typical application was the examination of a knife blade which was suspected to have been used to cut a telephone wire during the commission of a crime. The purpose of the examination was to identify foreign copper smears on the cutting edge. The knife is first examined under an optical microscope for the presence of suspect areas having different light reflecting

properties. Then these areas are analyzed in the SEM-EDX for copper and finally the copper is localized in the fine grooves present on the sharpened edge. Figure 1 shows the sharpened knife edge on the left and, on the right, the "dot pattern" for copper. The copper is localized in particular areas which generally conform with the surface grooves. The dot pattern technique is most often used to localize surface smears as in bullet grazing or whenever a softer substance is brought into forced contact with a harder material.

VI. SUMMARY

The applications of the SEM-EDX in the author's laboratory have been outlined. Most of the casework now being handled involves pure analysis or analysis combined with elemental mapping. The number of work requests are now in excess of 150 cases per year. The main reason for the instrument's use relies on the fact that a quick qualitative non-destructive analysis can be obtained on a microscopic area of the specimen. The disadvantages include

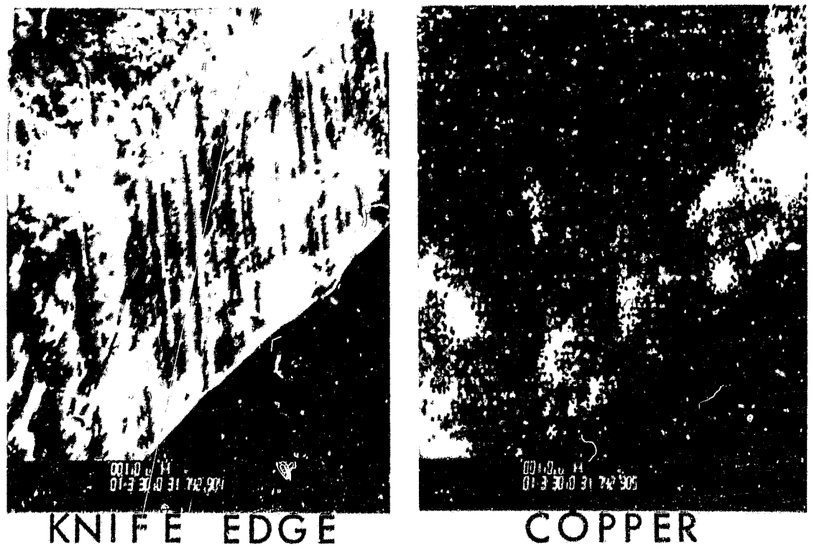

Figure 1. Composite micrograph of a sharpened knife edge and the copper distribution on that same area. The bar at bottom of micrograph corresponds to 0.01 mm.

the cost and complexity of the instrument and the limited size of the specimen which can be accommodated.

The use of the SEM-EDX is still evolving. In our laboratory, research is now being conducted in three general areas: automation of the search for gunshot residue particles (4), quantitative analysis, and signal-to-noise enhancement.

REFERENCES

1. Goldstein, J. I., and Yakowitz, H., eds., *Practical Scanning Electron Microscopy*. Plenum Press, New York, 1975.
2. Beaman, D. R., and Isasi, J. A., *Electron Beam Microanalysis*. A.S.T.M., Philadelphia, 1972.
3. Matricardi, V. R., Clark, M. S., and DeRonja, F. S. *J. Forensic Sci., 20* 507 (1975).
4. Matricardi, V. R., and Kilty, J. W., *J. Forensic Sci., 22*, 725 (1977).

Indistinguishable From Magic—the Threat and the Promise of Laboratory Utopia

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Amongst his many quotable quotes, Winston Churchill once stated: "Never overlook an opportunity to remain silent". This paper, therefore, represents a missed opportunity but the challenge to rationally address the problems, both human and scientific, which accompany laboratory transition from distillation apparatus, retort-stand and evil smelling organic preparation, to the P.C. Board and flashing digital lights of the technological scene, cannot be lightly disregarded. This change, without doubt, represents the most dramatic occurrence in forensic toxicology history, and has introduced an unequalled turmoil of activity during the past five years or so. It demands assessment; the aim of this presentation is then, assessment—inference and cautious proposal.

Any sufficiently advanced technology is indistinguishable from magic. In this one axiomatic statement there is embodied threatening danger, promising hope, and a caution. Certainly the technology of television and modern communication systems is so far beyond the comprehension of a child that it qualifies as magic. But magic is anathema to science, and the replacement of occult mysticism, a belief in gremlins and "Black-box Reverence" by understanding and scientific judgment must always be the first consideration, and is essential if the introduction of computers and other complex instrumentation into the laboratory is not to wreak havoc with the principles and objectives of our Forensic Science responsibility.

Alternatively, there is promise of broadened laboratory capability; improved analytical sensitivity, creation of a wide uniform data base from which to draw reliable inferences, specific identification (perhaps the final touchstone of the analyst), speed and control of high-volume routine work; but all within the cautious context of clear-minded understanding. To select and purchase laboratory instrumentation as if it were the next family car, on the basis of dazzling advertising and sex appeal is to invite the retribution and disappointment of having swapped a Studebaker for an Edsel, (or perhaps a British Airways for an Air France Concorde).

Beginning in the late forties and progressing through the fifties, ultraviolet and infrared spectrophotometry successfully wooed the forensic toxicologist to instrumental analysis by permitting dramatic improvement in sensitivity and characterization of drugs and toxic agents isolated from biological samples. The early sixties saw the birth of the gas chromatography revolution which has continued unabated for well over a decade, until today "optional extras" so abound that there are extras for extras and even the illustrious journal, "Science", publishes an annual "Guide to Scientific Instruments" which in 1977 stretched to more than 300 fine printed pages.

Multiple gas chromatography systems have become the staple of the toxicology laboratory, (a measure of their utility is the fact that even trial lawyers manage to pronounce and spell the name correctly), and are now commonly equipped with mechanized sample injection devices and pyrolysis units, a variety of detectors and peripheral electronics which minimize noise and render ever more beautiful peak shapes. Now, in the seventies, the physicist and electronic engineer with remarkable imagination have mated the gas and liquid chromatographs to mass spectrometers, and in a stroke produced an awesomely powerful analytical tool-and the need for computer control and interactive data reduction systems. Add to this automated immunological testing, including radioimmunoassay, stable isotope techniques, and the demand for very high volume drug control, traffic alcohol and postmortem screening analyses and it is not difficult to appreciate that judiciously applied mechanization is necessary and that computer data-storage, manipulation and calculation provides a solution that cannot be ignored.

The "untouched by human hand" analysis has arrived, making possible an unmanned but productive laboratory by night and little more than a maintenance operation by day. It is kitchen chemistry begone! Enter Utopia! The drudge is an instrument, and the toxicologist a researcher released from harness to expound and expertly opinionate from his experimental data.

The cry of the young bicycle rider, "Look Father. No hands" is all too frequently followed by "Look Father. No teeth!" How then do we preserve the teeth and face of toxicology at a time of bewildering progress and the advent of instruments which many are ill-trained to fully appreciate?

The benefits of saved time, staff management, cost-effectiveness and quality of analytical results make the acquisition of computers, and the laboratory instruments they control, very attractive. But the decision to spend huge sums of precious budget money on equipment, which once indelibly stamped with a government identification number becomes immortal and irreplacable by definition, can induce white elephant nightmares in a toxicologist and send him scurrying to the safety of conservatism. It is an act of considerable courage to buy $100 to $500,000 worth of Utopian hardware, with a percentage of built-in magic. In the United States, at least, there is a general belief in, "you get what you pay for" and, accordingly, at 100 kilo-bucks and above, the scientist expects infallability, and the doctor instant response. The lawyer, of course, simply wants to know how to beat it!

There are, however, some safety rules which can be followed in making this decision. Play a role of careful, critical observer. There is, in my experience, always a year or so of hysteria and hyperbole immediately following the arrival of the new toy in the scientific nursery. The slump of disillusionment follows the realization that it is not a crystal ball, but eventually applied research and development by responsible scientists, and others who bought during the first flush of excitement, brings the equipment into perspective. Now ask questions: Can the instrument fulfill a need in the laboratory which is beyond present capability; or significantly improve a definable inadequacy? Only proceed if the answer is YES. Consider its forensic applicability; it is essential that the function, purpose and data output can be convincingly described and is legally tenable, and that total instrument control (beyond the on/off switch) is in the hands of the scientist. It must be capable of built-in quality control and function testing, and demonstrably require the operator for all variable or judgment steps.

Pay a working visit to a laboratory already experienced and ask knowledgeable colleagues to explain differences, often subtle but very important, between various manufacturer's equipment. Similarly, visit the manufacturer's laboratory for at least a day and analyze test samples typical of your particular case problems. Observe the analysis, perform yourself, and do not accept data generated in your absence.

Ensure that there are adequate training and education courses available, perhaps sponsored by the company or conducted by them. Finally, endeavor to stay with those manufacturers with a proven record in forensic science. These simple precepts can prevent a hardware store-room full of unfulfilled promises of yesteryear.

In any event be assured that the infallible instrument (computer or not) has not arrived and

is not likely to bail you out in the forseeable future. This symposium is about tools; tools of the scientific craftsman, and they perform exactly as commanded—no more and no less. There are dangerous implications for the Forensic Scientist: without an acute sense of realism it is easy to become trapped in the belief that the instrument can overcome deficient analytical technique and apparently produce irrefutable data. It must be remembered that the first rule of GC-MS demands the best chromatography possible as a prerequisite to mass spectrometry, and data is simply a means to an end; it must reflect reality. That reflection can only be seen and described by a mature, experienced scientist. The Forensic Scientist must not permit sacrifice of the slightest scientific integrity in justification of more analyses per hour or a dazzling printout.

The need for quality performance and results is no less important in the small laboratory than in the large, busy, urban county, or state organization. All too often, justification for equipment is based on case-load and staff considerations alone. Development of central resources for complex instruments and reference data banks is essential. It is impressive that there are at least six regional forensic science organizations in the USA and Central Research Laboratories in other countries. Regular meetings and frank discussion have led to the beginnings of real sharing and it is confidently anticipated that teletype terminals and interconnecting lines will eventually allow the common wealth to be used where it is most needed. Similarly, time share computer services are now available at minimal cost and are well-proven as reference libraries, able to search and identify unknown substances. Some private research institutes and a few concerned manufacturers offer services and technical assistance. In short, it is encumbent upon all of us to work together so that the modern technology which is our good fortune can exert the widest possible benefit.

It must be emphasized that there is no single answer to the question of complex instrumentation in scientific affairs For his different purposes, the scientist needs many different structures—some simple, some complex, some exclusive and some comprehensive, but the clamor for a final solution continues. Today we suffer from an almost universal idolatry of computer technology; it is, therefore, necessary to insist upon the virtue and uniqueness of the human brain. Men always seem to need at least two things simultaneously that superficially seem to be incompatible. We always need both freedom and order. In the laboratory this paradox should disappear as the orderly and purposeful application of instrumentation provides time for freedom of thought and a range of experimental testing only dreamed about a generation ago.

If a thoughtful being from another world were to visit us today, what would be the most astonishing—the brilliance of our scientific achievements or the rising crime rates; the progress of our medicine or the overcrowding and cost of our hospitals, the efficiency of our machines or the inefficiency of the system as a whole? The implications of technology in the laboratory can be just as disturbing unless carefully considered. In human terms, increasing complexity may entail a degree of specialization that destroys work satisfaction and produces fragmentary scientists, too narrow to be wise. However, despite this note of guarded reticence, there is real reason for optimism. If the "breakthrough a day keeps the crisis at bay" syndrome can be avoided and application of the wondrous tools at our disposal is made with care and controlled within the human scale to human requirements, the opportunities for creative service in support of the natural harmonies of society through forensic science have surely never been greater. Our work is about service, and the enjoyment that comes to those who know they are involved, at whatever level, in testing scientific capability—about grasping for the apparently unobtainable.

Robert Browning the poet wrote: "But a man's reach should exceed his grasp; Or what's a heaven for?" I trust that there will be many such moments for all those who have the courage to enjoy the challenge of employing modern technological invention in the cause of forensic science.

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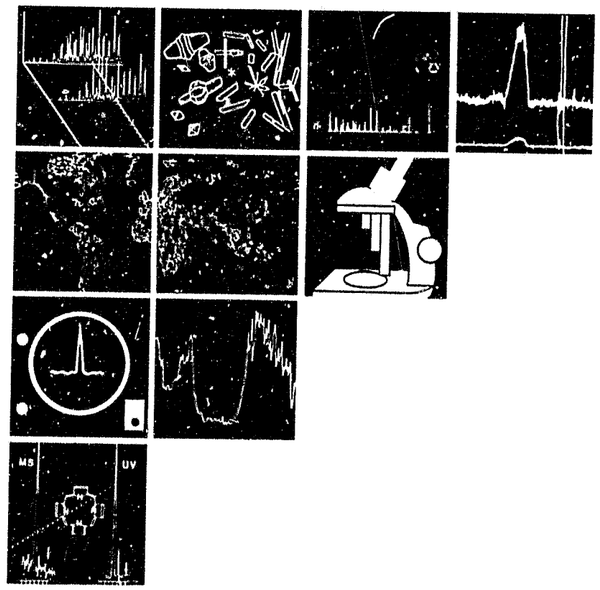

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