Summary Overview

Development of Heated Headspace Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry for Chemical Profiling of Marijuana

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Purpose

Statement of the problem

While marijuana is the most widely available and commonly used illicit drug, and remains illegal under the federal law, some states have passed legislation approving the cultivation, possession, and use of marijuana within their respective states [1]. As of February 2017, 29 states, including Washington, D.C., have legislation permitting the use of medical marijuana. Another 16 states have decriminalized marijuana possession, and 8 states have legalized marijuana for recreational use. With such changes in legislation, marijuana and its related products sales increased by 30% between 2015 and 2016. For example, Colorado sold $1.3 billion worth of marijuana in 2016 [2]. According to the World Drug Report 2015, the most recent data also points to an increase in the prevalence of *Cannabis* use in the United States, because of ongoing changes in legislation in some states [3]. Marijuana decriminalization or legalization within a state created a great concern of the diversion of legal marijuana to bordering jurisdictions, or trafficking through border communities. While the knowledge of different varieties of marijuana is growing in the industry, forensic analysis of marijuana evidence in forensic drug laboratories did not keep up with the rapid change of recent new development of marijuana issues. In a routine forensic analysis of marijuana evidence, quantitation of THC (Δ^9-tetrahydrocannabinol) or CBD (cannabidiol) level is not always required to build a case. Current forensic marijuana testing protocol used in most of the crime laboratories did not capture the chemical signatures of marijuana evidence that potentially can differentiate the varieties of marijuana grown under different conditions or from different regions. Medical marijuana (or legal marijuana) may be diverted from its intended use and consumed by people without a doctor's prescription. Regardless the legal status of marijuana in the United States, there is a need to develop an effective, efficient and reliable chemical profiling method and database to determine the variety or source of marijuana evidence for the purpose of law enforcement and forensic intelligence.

Background

Marijuana is currently a Schedule I controlled substance under the federal perspective and the federal government forbids the sale and possession of marijuana and all other forms of *Cannabis*. Because the identification of marijuana and its chemical constituents has been the most

often performed analyses, most forensic analysis of marijuana evidence involves color testing for a qualitative detection of THC and microscopic examination of plant materials in order to accommodate the volume of marijuana cases. In a regional crime laboratory, there is no standardized chemical profiling method for marijuana to create a shared knowledge database for the purpose of criminal investigation. Determination of the origin or varieties of marijuana seizures is often left unsolved at the local community level due to high caseload of marijuana evidence. Several studies have demonstrated the use of chemical profile analysis for the classification of marijuana samples. The chemical profile has been reported as a fingerprint for the source of marijuana. Therefore, we developed heated headspace solid phase microextraction coupled to gas chromatography/mass spectrometry (HHS-SPME-GC/MS) as an analytical platform for the collection of chemical signatures of marijuana evidence. The HHS-SPME-GC/MS methodology can be easily adopted by any crime laboratory because the acquisition cost of hardware for automation are minimal. With the development of automated HHS-SPME-GC/MS, a standard chemical profile method for marijuana samples can be established. A global marijuana chemical database can be shared between crime laboratories.

Rationale

The cannabinoid content of marijuana is associated with the source and varieties of *Cannabis* and their geographic origins [4]. Generally, THC, CBD and CBN (cannabinol) are major cannabinoids found in *Cannabis* and therefore present in marijuana at a higher level [5]. We hypothesize that the headspace chemical profiles (i.e. cannabinoids, additives, impurities) can provide chemical signatures to attribute the source and varieties of marijuana samples. Other acid form of cannabinoids, such as THCA (tetrahydrocannabinolic acid), CBDA (cannabidiolic acid), CBGA (cannabigerolic acid), CBCA (cannabichromic acid) were excluded from this project because those acid forms were not detected by the heated headspace approach.

Research goals and objectives.

Chemical forensics is a nascent field that collects and attributes chemical information of physical evidence to their sources. Ideally, from a chemical forensic analysis, one can identify chemical signatures of physical evidence and use them to classify or trace the source of it. The

development of headspace chemical analysis will provide an effective, faster, more efficient processes for cannabinoids detection in marijuana samples. There is only one step, i.e. putting sample into the vial, with human involvement in the whole process of analysis. Handling of sample, instrumental analysis, data handling, and reporting are automated. The overall goal in this research project was to develop a new forensic analytical system and workflow that could be more efficient and robust to benefit and strengthen the practice of chemical forensics.

Design and Methods

According to the lab manual published by UNDOC (United Nations Office on Drugs and Crime), the use of solid phase microextraction (SPME) approach has been considered to be robust for the analysis of the main cannabinoids. Also, compared to liquid-liquid extraction, headspace SPME (HS-SPME) approach is substantially faster [6]. HS-SPME coupled to GC/MS (HS-SPME-GC/MS) has already been used to profile illicit drugs, such as ecstasy tablets [7]. This analysis technique has also been used to profile the volatile constituents of many different food and plant products [8, 9], and coniferous needles [10]. Moreover, HS-SPME-GC/MS has also been used to determine the geographical origin of several different foodstuffs [11, 12]. Modifications of HS-SPME, such as Headspace-Trap-GC/MS, have also been used to profile volatile compounds in hops [13]. A heated headspace-SPME (HHS-SPME) procedure coupled to a gas chromatography/nitrogen phosphorus detector (GC-NPD) was used to extract organic gunshot residues (GSR) [14]. Extraction of trace amounts of chemical ingredients from a single grain of un-burnt or partially burnt solid gunpowder was made possible from nearly non-destructive headspace chemical analysis. In this project, HHS-SPME-GC/MS method has been optimized for headspace chemical analysis of marijuana plant materials.

Certified reference material containing 10 cannabinoids (THC, Δ8-THC, tetrahydrocannabinolic acid, CBN, CBD, cannabidiolic acid, cannabichromene (CBC), cannabigerol (CBG), cannabigerolic acid and cannabidivarin (CBV)) mixture was obtained from Cayman Chemical (Ann Arbor, Michigan, USA) for the purpose of optimizing chromatography conditions and identifying cannabinoids by the MS detector. To optimize HHS-SPME-GC/MS conditions using reference cannabinoids, 4 µL of 100 µg/mL solutions of standard cannabinoid mixture were placed in a 20 mL headspace vials. The solvent was dried under gentle air stream,

resulting 400 ng of each cannabinoids in the vial, before the subsequent HHS-SPME-GC/MS method development.

The GC-MS used in this experiment was an Agilent 7890B coupled with a 5977A mass selective detector (Santa Clara, CA). The column used for cannabinoids separation was Rxi 35Sil-M3, 15m x 0.25 mm x 0.25 µm obtained from Restek (Bellefonte, PA). Our optimal GC oven separation condition for major cannabinoids was listed in Table 1.

Before HHS-SPME of seized marijuana, the samples were pulverized and sieved by 1 mm mesh as per published recommendation by the United Office Nation on Drug and Crime [33]. Ten milligrams of dried plant material were measured out using an analytical balance and placed into separate 20 mL headspace vials sealed with magnetic caps for HHS-SPME-GC/MS. The fiber used for headspace sampling was a polydimethylsiloxane (PDMS) SPME fiber (23 gauge, 100 µm) obtained from Sigma-Aldrich (St. Louis, MO). It was installed onto a PAL austosampler obtained from Agilent (Santa Clara, CA). Optimal parameters of the PAL sampler for HHS-SPME of marijuana samples are listed in Table 2.

Data Analysis

Qualitative analysis of headspace cannabinoids detected by HHS-SPME-GC/MS approach were processed by MassHunter Workstation Software Qualitative Analysis (Version B.06.00, Agilent). A mass spectrum library of cannabinoids was created using Library Editor (Version B.06.00, Agilent). Cannabinoids were identified by mass spectrum comparison of cannabinoid standards analyzed on the same instrument.

Statement of Results

As shown in Figure 1, 7 cannabinoids (THC, Δ8-THC, CBN, CBD, CBC, CBG, and CBV) could be readily extracted and detected by HHS-SPME-GC/MS from the headspace of 400 ng certified reference material in a 20-mL vial. Because HHS-SPME-GC/MS was capable of extracting sub-micrograms of cannabinoids in a 20-mL headspace vial, ten milligrams of plant

material was selected for this HHS-SPME-GC/MS study. As shown in Figure 2, major cannabinoids could be detected from a marijuana sample. There were also unidentified peaks detected within retention time range between 6 and 12 mins from real marijuana samples. Those peaks potentially could be novel cannabinoids, impurities or thermal break down products during the heating process of HHS-SPME.

Scholarly Products Produced or in Process

Conference presentations

- McDaniel A, Liu F, Yu JCC, Application of headspace solid phase micro extraction in chemical forensics, National Institute of Justice Forensic Science Symposium at Pittcon Conference, Feb 26 - March 1, 2018, Orlando, FL. (Oral Presentation)
- Perry L, Li SY, Yu JCC, Detection of phytocannabinoids from buccal swabs using one vial headspace vaporization derivatization coupled with SPME-GC/MS, the 70th American Academy of Forensic Science Annual Scientific Meeting, Feb 19-28, 2018, Seattle, WA. (Poster)
- Perry L, Yu JCC, Total vaporization of derivatization reagent for in situ headspace derivatization solid phase microextraction, American Chemical Society National Meeting, April 2-6, 2017, San Francisco, CA. (Poster)
- McDaniel A, Perry L, Sweet J, Liu F, and Yu JCC, Detection of marijuana varieties based on heated sample headspace chemical signatures. American Chemical Society National Meeting, April 2-6, 2017, San Francisco, CA. (Oral Presentation)
- McDaniel A, Sweet J, Yu JCC, A comparison of headspace cannabinoid profiles detected from different structures of dried cannabis inflorescences. the 69th American Academy of Forensic Science Annual Scientific Meeting, Feb 13-18, 2017, New Orleans, LA. (Poster)
- Brown A, Sweet J, Yu JCC, A rapid quantitative chemical analysis of cannabinoids in seized Cannabis using heated headspace solid-phase microextraction and gas chromatography/mass spectrometry, American Chemical Society National Meeting, March 13-17, 2016, San Diego, CA. (Oral Presentation)
- Brown A, Sweet J, Yu JCC, Non-destructive sample preparation for cannabinoid profiling in seized marijuana using headspace solid-phase microextraction, American Chemical Society National Meeting, March 13-17, 2016, San Diego, CA. (Poster)

- Winborn J, Sweet J, Yu JCC, Differentiation of seized marijuana samples using automated headspace solid-phase microextraction coupled to gas chromatograph – mass spectrometer/ flame ionization detector and principal component analysis. the 68th American Academy of Forensic Science Annual Scientific Meeting, Feb 22-27, 2016, Las Vegas, NV. (Poster)

- Brown A, Sweet J, Yu JCC, Quantitation of major cannabinoids found in seized marijuana using automated headspace solid-phase microextraction coupled with gas chromatography/mass spectrometry, the 68th American Academy of Forensic Science Annual Scientific Meeting, Feb 22-27, 2016, Las Vegas, NV. (Poster)

- Winborn J, Hanson M, Figueroa L, Konarik A, James D, Chen K, Dassau T, Sweet J, Yu JCC, Analysis of cannabinoids found in seized marijuana using automated headspace solid-phase microextraction coupled with gas chromatography/ mass spectrometry, the 67th American Academy of Forensic Science Annual Scientific Meeting, Feb 16-21, 2015, Orlando, FL. (Poster)

Journal articles

- Franklin T, Perry L, Shih WC, Yu J, Detection of phytocannabinoids from buccal swabs by headspace solid phase microextraction – gas chromatography/mass spectrometry, Anal. Methods, 2018,10, 942-946.

- Winborn J, Yu J, Identification of phytocannabinoids from the headspace of seized marijuana samples, manuscript in process.

- McDaniel A, Perry L, Liu Q, Shih WC, Yu J, Toward the identification of marijuana varieties by headspace chemical forensics, manuscript in process.

Project Findings

The performance of automated HHS-SPME-GC/MS testing method was very satisfactory for chemical profile of cannabinoids from marijuana sample headspace. One of the general concerns of using SPME extraction method in forensic case work is the issue of carryover. In our optimal HHS-SPME-GC/MS condition, we did not observe carryovers of major cannabinoids from our method once the fiber was conditioned at 250 ^{o}C for 10 mins before the next run. Between samples, quality control sample was performed to monitor carryovers. This quality control

procedure is similar to the common syringe-washing step in the everyday operation of a GC autosampler. We found the optimal HHS-SPME-GC/MS was reliable and robust in capturing headspace cannabinoids from small amount of marijuana samples. This analytical process was solvent free, automated, and nearly non-destructive.

Implications for Criminal Justice Policy and Practice

Not all marijuana is created equal. Current forensic marijuana testing protocol used in most of the crime laboratories did not capture the chemical signatures of marijuana evidence that potentially can differentiate the varieties and sources of marijuana grown under different conditions or from different regions. The change of legal status of marijuana at the state level prompt a paradigm shift of marijuana evidence analysis. Medical or legal marijuana may be diverted from its intended use and consumed by people without a legal prescription. Because of the change of legal status of marijuana at the state level, an ideal forensic analysis of marijuana evidence should not only confirm the presence major cannabinoids in the evidence, but also determine the variety or source of marijuana. An improved chemical classification scheme for the determination of marijuana varieties is needed for the criminal justice system.

We have found that the HHS-SPME extraction procedure for headspace chemical analysis of marijuana evidence is rapid, efficient and cost-effective. Major cannabinoids, such as THC, CBD, CBN, CBC, delta 8-THC, THCV and CBG, could be readily extracted and detected from the headspace of marijuana samples by using the HHS-SPME-GC/MS methodology. With almost no sample preparation, the HHS-SPME-GC/MS enables an automated process that efficiently transforms the headspace chemical signature of marijuana evidence into digital data (i.e. GC/MS data). As reported recently by Pawliszyn *et al.*, SPME-GC/MS has been applied to monitor headspace chemicals for metabolomics research [15]. The HHS-SPME-GC/MS approach could

assist the development of an ideal forensic analysis platform to not only confirm the presence of THC or CBD in marijuana evidence, but also capture the chemical signatures from marijuana samples for the purpose of crime investigation and forensic intelligence. The HHS-SPME-GC/MS approach can lead to a more uniformed standard for chemical analysis of marijuana evidence.

For future work, the headspace chemical features captured by HHS-SPME-GC/MS could be processed using machine learning technology. Standard marijuana samples can be used to establish and validate machine learning algorithms for the detection of the varieties of marijuana. Because GC/MS is a common analytical instrument in forensic laboratories, we envision HHS-SPME-GC/MS will meet the need for the new forensic task in marijuana analysis. In this way, the class, or source of marijuana can be reported in a statistical way for forensic purposes. One of the goals in our research laboratory is to apply the HHS-SPME-GC/MS analytical platform and machine learning technology to establish forensic intelligence for physical evidence. In a long-term goal, a marijuana chemical database can be created and shared between crime laboratories.

Appendices

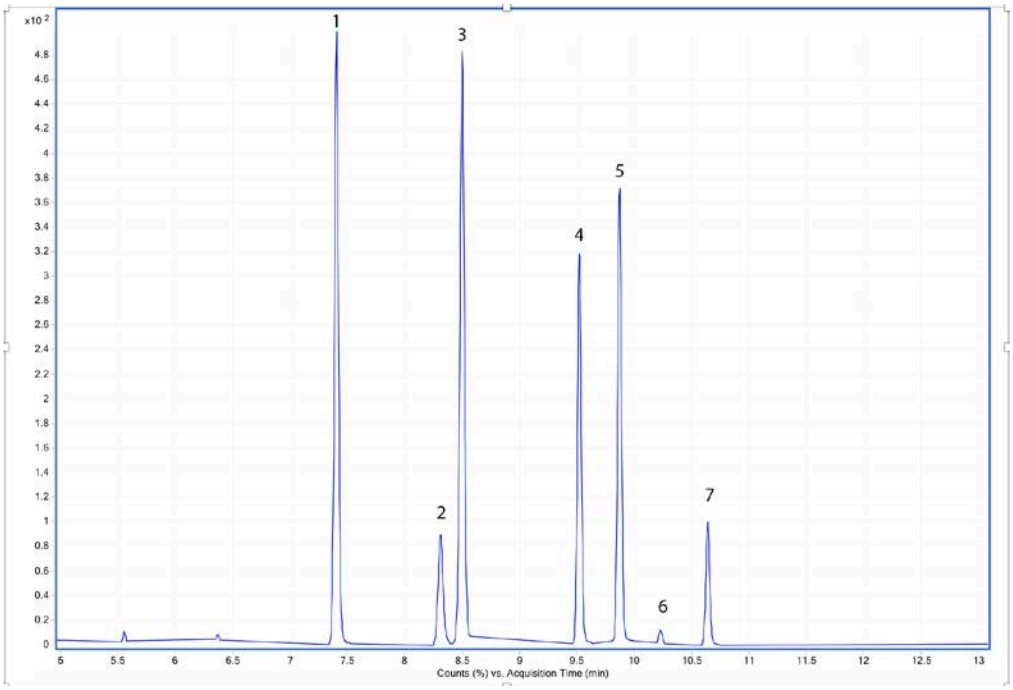

Figure 1 Total ion chromatogram (TIC) of standard cannabinoids detected by HHS-SPME-GC/MS. 1) THCV, 2) CBC, 3) CBD, 4) ∆8-THC, 5) ∆9-THC, 6) CBG, and 7) CBN. During method optimization, 400 ng of each standard cannabinoids was placed in 20 mL headspace vials.

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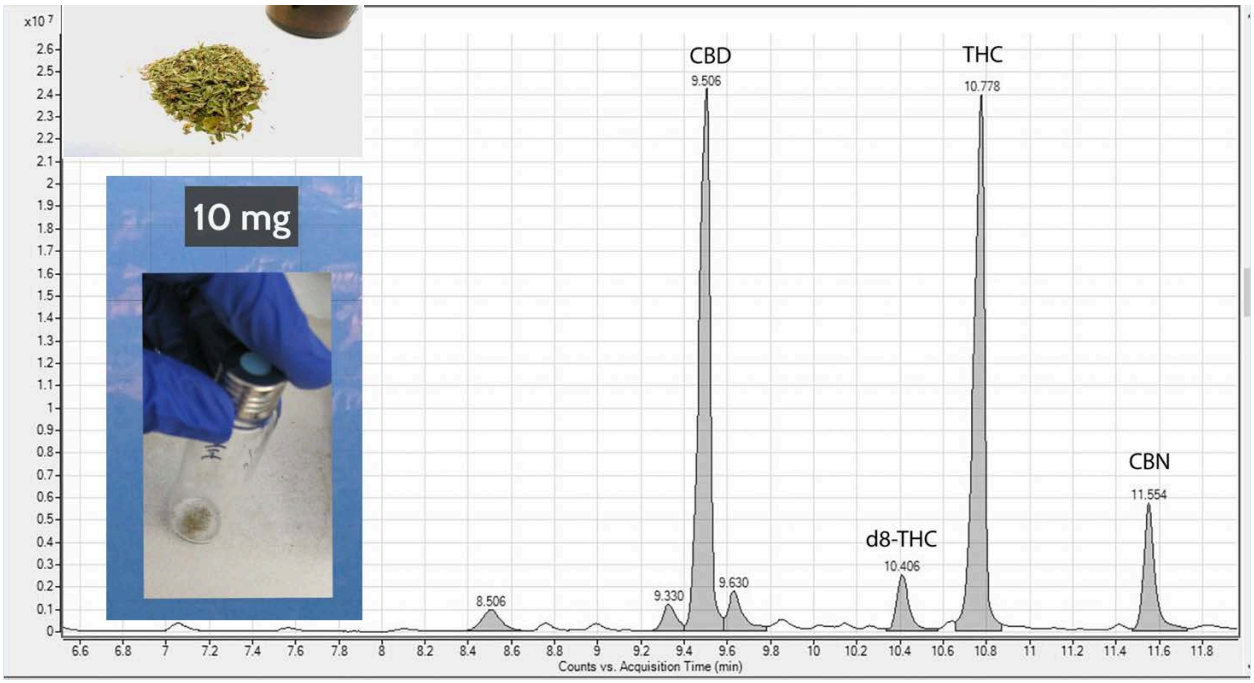

Figure 2. A typical total ion chromatogram (TIC) of HHS-SPME-GC/MS form a standard marijuana sample with 3.8% THC, 6.5% CBD. 10 mg of marijuana plant material was sampled for this test. The optimal HHS-SPME extraction condition can be found in Table 2.

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Table 1. GC oven program for optimal separation of cannabinoids

Oven Program Steps	Condition
GC oven initial temperature	170 °C
Hold time	1 min
Rate #1	15 °C/min
Oven temperature #1	228 °C
Hold time #1	3 min
Rate #2	10 °C/min
Oven temperature #2	250 °C/min
Hold time #2	0 min
Rate #3	5 °C/min
Oven temperature #3	270 °C/min
Hold time #3	1.4 min

Table 2. HHS-SPME automation parameters

HHS-SPME Steps	Condition
Pre-Fiber Conditioning Temperature (°C)	250
Pre-Fiber Conditioning Time (s)	0
Pre-Incubation Time (s)	300
Incubation Temperature (°C)	140
Pre-Incubation Agitator Speed (rpm)	250
Agitator On Time (s)	2
Agitator Off Time (s)	10
Vial Needle Penetration (mm)	11
Vial Fiber Exposure (µl)	12
Extraction Time (s)	150
Desorb to	GC Injection port
Injection Needle Penetration (mm)	32
Injection Fiber Exposure (µl)	12
Desorption Time (s)	30
Post-Fiber Conditioning Temperature (°C)	250
Post-Fiber Conditioning Time (s)	1200
GC Runtime (s)	300

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