Long-term Stability of Synthetic Cannabinoids in Biological Matrices

Final Summary Report

Submitted via Grants.gov to:
U.S. Department of Justice
Office of Justice Programs
National Institute of Justice
810 Seventh St., NW
Washington, DC 20531

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September 30, 2019
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NIJ Award No. 2016-DN-BX-0191
RTI Project No. 0215509.000

Contents

Purpose

The objective of this research was to provide the forensic community with a comprehensive investigation of synthetic cannabinoids of different core structures, and their major metabolites, with respect to their short-term and long-term stability in both urine and whole blood. Results of this research will inform laboratories of the proper handling and storage conditions necessary to preserve the integrity of their samples. This research addressed whether specific classes of synthetic cannabinoids are more susceptible to instability at certain laboratory conditions. This research also attempted to identify any possible major degradants that formed to determine if they could be suitable markers for identification. A secondary objective was to provide laboratories with efficient extraction and analytical methods for synthetic cannabinoids and metabolites.

Project Design

This study validated two liquid chromatography/tandem mass spectrometry (LC-MS/MS) methods for the quantitative identification of 29 synthetic cannabinoids and 25 synthetic cannabinoid metabolites. Boston University validated 17 synthetic cannabinoids in blood (Group 1a) and 10 metabolites in urine (Group 2a). RTI International validated 12 synthetic cannabinoids in blood (Group 1b) and 15 metabolites in urine (Group 2b). UR144 and XLR11 in blood and UR144 5-hydroxypentyl and AB-FUBINACA M2A metabolites in urine were validated at both institutions to evaluate stability for an interlaboratory comparison. Structures for all analytes evaluated during this study are shown in Figure A-1 - Figure A-4.

All analytes in urine and blood were extracted using supported liquid extraction (SLE) followed by LC-MS/MS at each institution (Appendix: SLE Extraction Method for Whole Blood and Urine Samples). LC-MS/MS parameters are in Table A-1. Optimized parameters and

monitored ion transitions for Group 1a and Group 2a are in Table A-2. Optimized parameters for Group 1b and Group 2b are in Table A-3. Linearity, precision, bias, limit of detection (LOD), limit of quantification (LOQ), ion suppression and enhancement, dilution integrity, processed stability, and freeze/thaw stability were assessed during the validation.

This study evaluated the long-term stability of established and emerging synthetic cannabinoids and their relevant metabolites. The goal was to select synthetic cannabinoids with key structural features that may assist laboratories with general knowledge of core structure stability. Stability was evaluated under different storage conditions encountered in routine laboratory procedures. These conditions included storage at room temperature, refrigerator (2°C–8°C), and freezer (−20°C) conditions. At RTI urine samples were stored in plastic screw-top containers typically used for urine collections. Urine samples were stored in 15 mL falcon conical tubes at Boston University. Whole blood samples containing sodium fluoride preservative and potassium oxalate were stored in red top plastic BD vacutainer tubes. Each group of analytes in blood (Group 1a and Group 1b) was separated into three mixes for the stability study. Analytes for each mix were chosen based on dissimilar core structures, ion transitions, and retention times. For example, UR144 and XLR11 were in separate mixes because the only difference in structure is the 5-fluoropentyl moiety for XLR11. Urine mixes were created the same way with metabolites from the same analyte being separated into a separate mix. For example, AB-CHMINACA M2 was in a separate mix from AB-CHMINACA M4. For each matrix mix, a low and high concentration was prepared in triplicate and stored for analysis. Stability was assessed at different time points and compared to the 0-hour time point. There were some variations in time points that were uncontrollable for each of the groups. Time points for Group 1a and Group 2a were 0 hours, 24 hours, 72 hours, 1 week, 3 weeks, 5 weeks, 9 weeks, 17

weeks, 21 weeks, and 35 weeks, and 52 weeks. Time points for Group 1b were 0 hours, 24 hours, 48 hours, 3 weeks, 5 weeks, 9 weeks, 17 weeks, 21 weeks, 35 weeks, and 53 weeks. Time points for Group 2b were 0 hours, 24 hours, 72 hours, 8 days, 3 weeks, 5 weeks, and 9 weeks. The average signal ratio of the analyte area to the internal standard area was compared with the 0-hour time point average ratio. Nontargeted high-resolution MS was used for Group 1b analytes and Group 2b metabolites to evaluate for potential major degradation products.

Results

Interference, Carryover Precision, Bias, LOD, LOQ, and Linearity

Analytes evaluated for commonly encountered interferences are listed in Table A-4 and Table A-5. No interference from commonly encountered drugs analyzed was observed. No interference from the matrix or internal standards was observed. No carryover was observed after the highest calibrator.

In blood for Group 1a analytes (Table A-6), the calibration curve ranged from the LOQ to 25 ng/mL, except for MO-CHMINACA, which ranged to 15 ng/mL. All analytes were weighted 1/x with linear regression with the exception of PB-22, which was quadratic. Precision and bias were based on quality controls (QCs) in triplicate at 1, 3, 7, and 20 ng/mL.

In blood for Group 1b analytes (Table A-7), the calibration curve ranged from the LOQ to 25 ng/mL for all analytes with linear correlations with weighting factors of 1/x for each analyte. Precision and bias were based on QCs in triplicate at 1, 7, and 20 ng/mL, except for 5F-AKB48, which had a QC3 concentration of 3 ng/mL.

For Group 1a and Group 1b, the overall bias in blood ranged from −4.97% to 7.95% with ADB-CHMINACA having the least bias (0.39%) and AMB the highest (7.95%). The overall within-run precision (expressed as percent coefficient of variation) ranged from 2.67% for

UR144 to 9.6% for 5F-AKB48. The overall between-run precision ranged from 4.25% for UR144 to 15.17% for 5F-AKB48. The average correlation coefficient (r^2) was greater than 0.991 for all analytes in blood. LOD ranged from 0.01 to 2 ng/mL with UR144 having the highest LOD.

In urine for Group 2a metabolites (Table A-6), the calibration curve ranged from 5 to 40 ng/mL with linear correlations for each analyte. Precision and bias were based on QCs in triplicate at 15, 25, and 30 ng/mL.

In urine for Group 2b metabolites (Table A-7), the calibration curve ranged from 0.3 ng/mL (LOQ) to 40 ng/mL for all analytes, except for AB-CHMINACA M4, which had an LOQ of 0.5 ng/mL and FUB-PB-22 3-carboxyindole and AB-PINACA pentanoic acid, which had LOQs of 5 ng/mL. All analytes had linear correlation with weighting factors of 1/x, except UR144 5-hdroxypentyl and 5F-AKB48 4-hydroxypentyl, which were quadratic. Precision and bias were based on QCs in triplicate at 1, 15, and 30 ng/mL except UR144 5-hydroxypentyl, 5F-AKB48 4-hydroxypentyl, AB-PINACA pentanoic acid, and FUB-PB-22 3-carboxyindole, which had a medium QC concentration of 25 ng/mL. The low QC was 15 ng/mL for FUB-PB-22 3-carboxyindole and AB-PINACA pentanoic acid.

For Group 2a and Group 2b, the overall bias in urine ranged from −9.83% to 7.16% with 5F-PB-22 3-carboxyindole having the least bias (−0.37%) and 5F-AMB M5 the highest (−9.83%). The overall within-run precision ranged from 2.74% for PB-22 5-hydroxypentyl to 8.09% for 5F-AB-PINACA 4-hydroxypentyl. The overall between-run precision ranged from 3.20% for PB-22 5-hydroxypentyl to 13.65% for 5F-AB-PINACA 4-hydroxypentyl. The average correlation coefficient (r^2) was greater than 0.994 for all analytes in urine except AB-PINACA

pentanoic acid at 0.9877. LOD ranged from 0.05 to 5 ng/mL with AB-PINACA pentanoic acid and FUB-PB-22 3-carboxyindole having the highest at 2 and 5 ng/mL, respectively.

Dilution, Ion Suppression/Enhancement

In blood samples diluted 1:50, precision ranged from 2.39% for XLR11 to 23.01% for 5F-AKB48 (Table A-8 and Table A-9). Bias at 1:50 dilution ranged from −13.15% for 5F-ADB to 25.08% for NM2201. The precision ranged from 2.62% for JWH-250 pentanoic acid to 22.12% for AMB for samples diluted 1:10. Bias at 1:10 dilution ranged from −9.41% for 5F-ADB to 15.25% for NM2201 (Table A-8 and Table A-9). In blood for Group 1a (Table A-10) and Group 1b (Table A-11) analytes, ionization suppression and enhancement at high concentration ranged from −84.20% for UR144 to 33.75% for AB-FUBINACA. AB-FUBINACA was the only analyte to exhibit >25% ionization enhancement whereas 13 other analytes exhibited ionization suppression > 25%. At low concentration, ionization suppression and enhancement ranged from −72.20% for UR144 to 5.7% for AB-FUBINACA. There were 15 analytes that exhibited ionization suppression > 25%.

In urine, at 1:50 dilution, the precision ranged from 2.00% for ADB-PINACA 4-hydroxypenty to 17.72% for FUB-PB-22 3-carboxyindole. Bias at 1:50 dilution ranged from −33.5% for UR-144 5-hydroxypentyl to 9.57% for AB-PINACA pentanoic acid. At 1:10 dilution, the precision ranged from 1.72% for 5F-PB-22 3-carboxyindole to 14.77% for AB-CHMINACA M4. Bias at 1:10 dilution ranged from −19.17% for UR144 5-hydroxypentyl to 4.03% for ADB-PINACA 4-hydroxypentyl (Table A-8 and Table A-9). In urine for Group 2a (Table A-10) and Group 2b (Table A-11) analytes, ionization suppression, and enhancement ranged from −26.18% for JWH-250 pentanoic acid to 36.78% for 5F-AMB M5 at high concentration. The only other analytes at high concentration to exhibit ionization suppression or

enhancement were 5F-AKB48 4-hydroxypentyl (26.62%). At low concentration, ionization suppression and enhancement ranged from −35.59% for 5F-AKB48 4-hydroxypentyl to 51.29% for 5F-AMB M5.

Stability

Processed stability was evaluated by injecting extracted QC samples in triplicate after they were stored for 0, 24, 48, and 72 hours in the autosampler. Blood analytes for Group 1a were evaluated based on average area compared to the average 0-hour area. Most were stable for 72 hours. CUMYL-THPINACA, NM2201, RCS-8, and UR144 were stable for 48 hours. 5F-3,5-AB-PFUPPYCA and 5F-PY-PINACA were stable for 24 hours. Blood analytes in Group 1b were stable in the autosampler for 72 hours. Urine analytes in Group 2a and Group 2b were stable for 72 hours except 5F-ADB M7, which was stable for 48 hours.

Freeze/thaw stability was evaluated over three cycles (24, 48, and 72 hours) except for blood analytes Group 1b, which were evaluated for 24 and 48 hours only because of MS issues at 72 hours. All blood analytes in Group 1b were stable for 48 hours. Most Group 2b urine metabolites were stable for three freeze/thaw cycles.

Blood analytes in Group 1a were spiked at 1.5 and 10 ng/mL (Table A-12) and subjected to freezer, refrigerator, and room-temperature conditions. Analytes were generally more stable in the freezer, then the refrigerator, and were least stable at room temperature in blood preserved with sodium fluoride. Most analytes were generally stable in the freezer for 21 to 52 weeks. The most stable analytes at room temperature were JWH-250, MO-CHMINACA, CUMYL-THPINACA, 5F-PY-PINACA, and UR144. The least stable analytes at room temperature were EMB-FUBINACA (0 hours), 5F-ADB-PINACA (0 hours), 4-cyano CUMYL-BUTINACA (24 hours), XLR11 (24 hours), and MEP-CHMICA (72 hours); however, they were more stable in

the freezer. Urine metabolites in Group 2a were spiked at 10 and 25 ng/mL. They were generally more stable in the freezer and refrigerator than at room temperature. The most stable metabolites at room temperature, refrigerator, and freezer were 5F-MDMB-PICA M7 and 5F-PB-22 3-carboxyindole at 35 weeks.

Blood analytes in Group 1b were spiked at 1.5 and 10 ng/mL (Table A-13). Analytes were generally most stable in the freezer, followed by the refrigerator, and then at room temperature. The least stable analytes at room temperature seemed to be those with a 5-fluoropentyl moiety. In most cases, the analog without the 5-fluoropentyl moiety was more stable. AB-PINACA, AB-FUBINACA, ADB-FUBINACA, AB-CHMINACA, and ADB-CHMINACA were the most stable in each storage condition. Although not the same matrix, storage conditions, or time points, these results are similar to forced stability studies in previous NIJ award 2012-R2-CX-K001 where AB-PINACA, AB-FUBINACA, and ADB-FUBINACA were among analytes that were stable.

UR144 5-hydroxypentyl or a related isomer was observed in the preserved blood mix spiked with XLR11, 5F-AMB, 5F-ADB, and AKB48 at 0-hour, but the area of the UR144 5-hydroxypentyl or related isomer (LC-MS/MS transition 350/125 and 350/230) increased in order of room temperature, refrigerator, and freezer indicating possible formation of a degradant. The accurate mass spectrum showed an [M+H]+ of 328.227 m/z and [M+Na+] 350.209 m/z.

Figure A-5 shows a preserved blood Mix 1 extracted ion chromatogram of fragment ion 145.039 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom at the high collision energy chromatogram. Within Mix 1, 5F-AMB and 5-ADB are shown at 3.87 and 4.89 minutes, respectively. At refrigerator and room temperature conditions potential degradants with this same fragment ion are shown at 2.32, 2.76 and 3.00

minutes. Figure A-6 shows an extracted ion chromatogram of fragment ion 213.102 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom. Again, showing potential degradants with the same fragment ion (213.102 m/z) at 2.32 and 3.00 minutes at refrigerator and room temperature conditions. Evaluation of the high collision energy mass spectrum at 2.32 minutes indicates a potential degradant of 5F-AMB M2 or related isomer with a [M+H]+ 362.208 m/z and [M+Na]+ 384.189 m/z may be present at room temperature and refrigerator conditions, and is confirmed by the presence of a peak at 2.32 min in the high collision energy extracted ion chromatograms for these fragments (Figure A-7 for 362.208 m/z).

Figure A-8 shows a preserved blood Mix 3 extracted ion chromatogram of fragment ion 145.039 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom. AB-PINACA and AB-PINACA-d9 are shown at 3.96 minutes, AB-CHMINACA and AB-CHMINACA-d4 are shown at 4.97 minutes, and AMB is shown at 5.17 minutes. In the refrigerator and room temperature conditions, a potential degradant with this same fragment ion is shown at 4.83 minutes. Figure A-9 shows the same 0-hour and 5 week preserved blood Mix 3 (freezer, refrigerator, room temperature from top to bottom) except with an extracted ion chromatogram at 215.118 m/z. This chromatogram indicates that AB-PINACA, AB-PINACA-d9 and AMB and the potential degradant at 4.83 minutes share the same 215.118 m/z fragment but AB-CHMINICA and AB-CHMINACA-d4 do not.

Urine metabolites in Group 2a were spiked at 10 and 25 ng/mL (Table A-12) and subjected to freezer, refrigerator, and room-temperature conditions. Metabolites were generally more stable in the freezer. The least stable metabolites at room temperature were PB-22 5-hydroxypentyl (3 weeks), ADB-PINACA pentanoic acid (5 weeks), and JWH-250 pentanoic

acid (5 weeks), which were all more stable stored in the freezer. 5F-MDMB-PICA M7 and 5F-PB-22 3-carboxindole were the most stable across all storage conditions.

Urine metabolites in Group 2b were spiked at 1 and 30 ng/mL (Table A-13). Most metabolites were stable up to 9 weeks at each storage condition. The least stable metabolites at room temperature were 5F-AMB-M2 (8 days), UR144 5-hydroxypentyl (8 days), ADB-CHMINACA M1 (3 weeks), and 5F-AKB48 4-hydroxypentyl (5 weeks); however, they were stabilized in refrigerator and freezer conditions.

Scholarly Products

Planned Publications

The following two manuscripts are undergoing internal review prior to submission to the *Journal of Analytical Toxicology*:

Quantitation of Ten Synthetic Cannabinoid Metabolites in Human Urine Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS)
Cassandra Swart, Daniel Lee, Mikayla Caldwell, Nichole Bynum, Megan Grabenauer, Katherine Moore-Bollinger, and Sabra Botch-Jones

Analysis of 17 Synthetic Cannabinoids in Whole Blood by LC-MS/MS
Daniel Lee, Katherine Moore-Bollinger, Courtney Mcgowan, Shawn Foley, Cassandra Swart, Erika Phung, Nicole Bynum, and Sabra Botch-Jones

We plan to publish one manuscript on the urine and blood stability study from Boston University and a publication on the stability study from RTI.

Presentations

E. Phung, D. Lee, C. Swart, K. Moore-Bollinger, N. Bynum, M. Grabenauer, S. Botch-Jones, "Evaluation of the Long-Term Stability of Select Naphthoylindole, Admantoylindole, Quinolinyl, and Carboxamide Synthetic Cannabinoids in Human Whole Blood Using LC-MS/MS," abstract submitted May 2019 for upcoming Society of Forensic Toxicologists Conference, October 2019.

Phung, E., Lee, D., Swart, C., Bynum, N., Moore-Bollinger, Grabenauer, M., Botch-Jones, S. "Evaluation of the Short-Term Stability of Select Naphthoylindole, Admantoylindole, Quinolinyl, and Carboxamide Synthetic Cannabinoids in Human Whole Blood Using LC-MS/MS," paper presented at the Midwest Association for Toxicology and Therapeutic Drug Monitoring, Cleveland, OH, April 2019.

C. Swart, D. Lee, M. Caldwell, K. Moore, N. Bynum, S. Botch-Jones, "The Detection and Quantitation of Ten Synthetic Cannabinoid Metabolites in Human Urine Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)," paper presented at the American Academy of Forensic Sciences, Baltimore, MD, February 2019.

D. Lee, S. Foley, E. Phung, C. Swart, N. Bynum, K. Moore, S. Botch-Jones, "The Detection and Quantitation of 17 Synthetic Cannabinoids in Human Whole-Blood Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Following Supported Liquid Extraction," paper presented at the American Academy of Forensic Sciences, Baltimore, MD, February 2019.

E. Phung, N. Bynum, M. Grabenauer, S. Botch-Jones, K. Moore,"Validation of 15 Synthetic Cannabinoid Metabolites in Urine by LC-MS/MS," paper presented at the Northeastern Association of Forensic Scientist, Bolton Landing, NY, October 2018.

S. Foley, D. Lee, C.K. McGowan, K. Moore, N. Bynum, S. Botch-Jones, "Method Validation for the Detection and Quantitation of Synthetic Cannabinoids in Whole Blood Using Liquid Chromatography-Tandem Mass Spectrometry Following Supported Liquid Extraction," paper presented at The International Association of Forensic Scientists, Ghent, Belgium, August 2018.

Implication for Policy and Practice

The results of this research project address several NIJ Technology Working Group operational requirements for scientific research, technology development, policy or protocol development, assessment and evaluation, and the dissemination and/or training of specific research. Analytical and extraction methods have been made public to the forensic community through presentations at various conferences. As a result of short-term and long-term stability studies, this research informed the community of appropriate collection and handling protocols for samples that contain or are suspected to contain synthetic cannabinoids. By carefully choosing compounds with core structures representative of popular classes of compounds, this information will be useful in the future for new compounds that result from minor modifications producing new structural analogs.

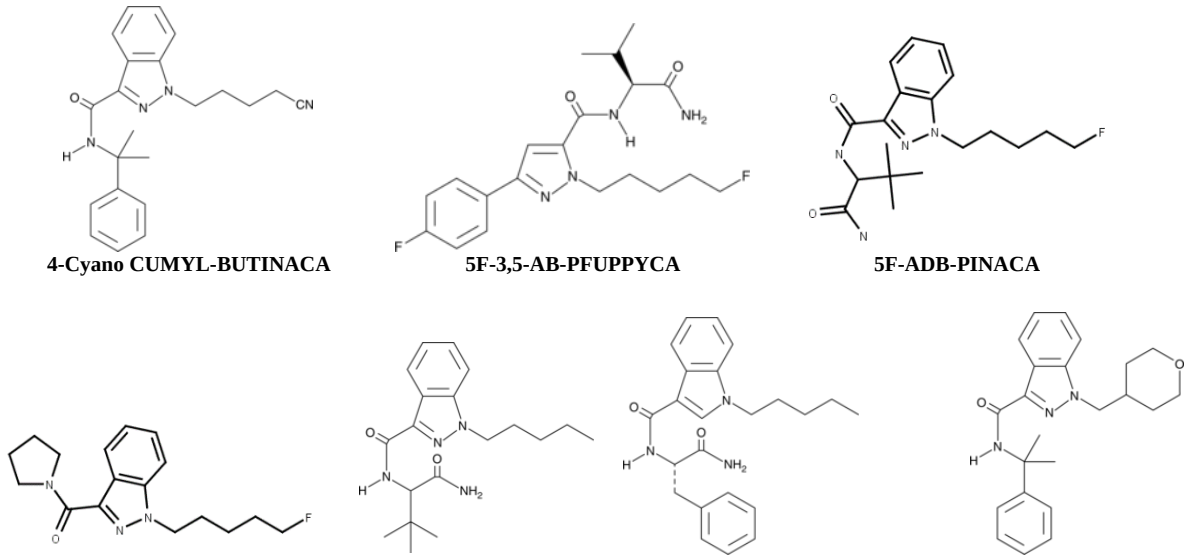
Appendix

Materials and Methods

All synthetic cannabinoid parent drugs, metabolites, and internal standards were purchased from Cayman Chemical (Ann Arbor, MI). All reagents used were of LC/MS grade. Human whole blood was purchased from Biological Specialty Corporation (Colmar, PA), Equitech Enterprises, Inc. (Kerrville, TX), or UTAK Laboratories, Inc. (Valencia, CA). Human urine was purchased from Biological Specialty or collected using approved Boston University School of Medicine Institution Review Board standards and approved methods.

Both RTI and Boston University validated a subset of synthetic cannabinoid parent drugs in blood and metabolites in urine at their respective laboratories. Group 1a (Figure A-1) and Group 1b (Figure A-2) were analytes validated in blood at Boston University and RTI, respectively. Internal standards for Group 1a analytes were UR144-d_5, PB-22-d_9, and XLR11-d_5. Internal standards for Group 1b analytes were AB-PINACA-d_9, AB-CHMINACA-d_4, XLR11-d_5, and ADB-CHMINACA-d_4.

Figure A-1: Chemical structures of Group 1a analytes validated in blood by Boston University.



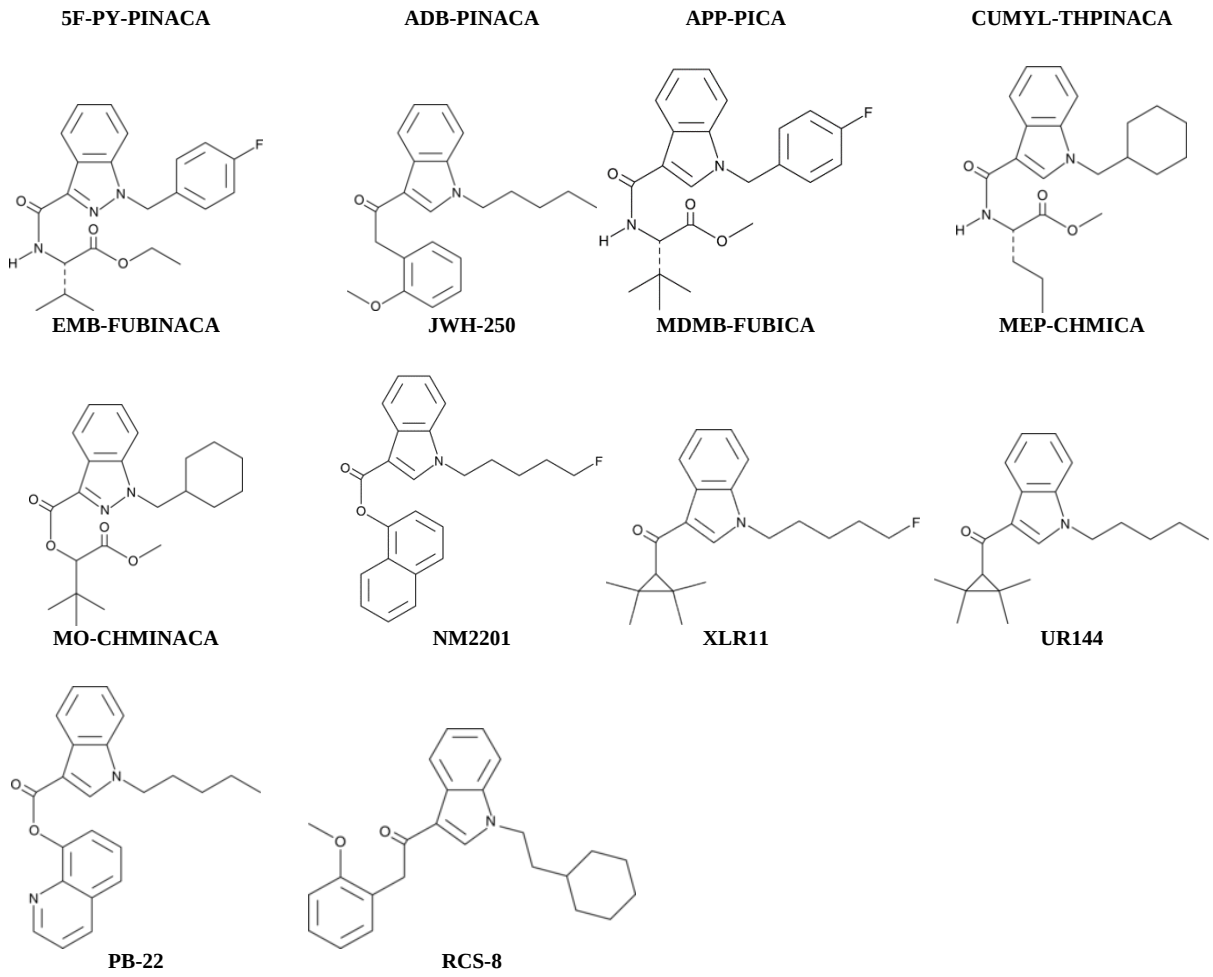
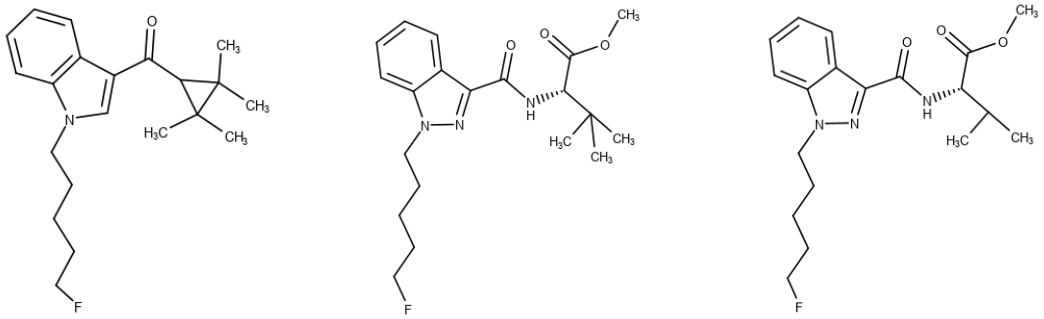
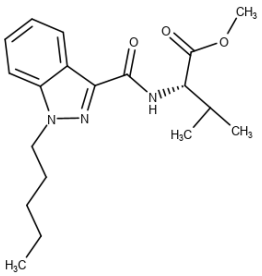
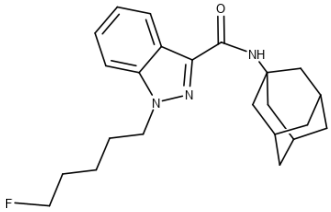


Figure A-2: Chemical structures of Group 1b analytes validated in blood by RTI.

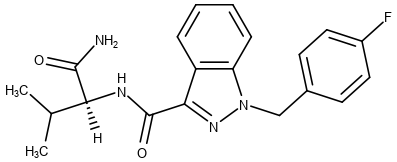




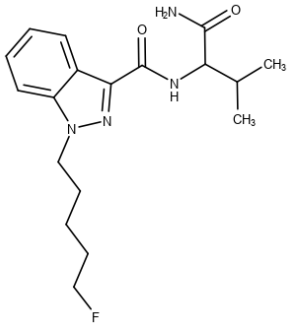
AMB



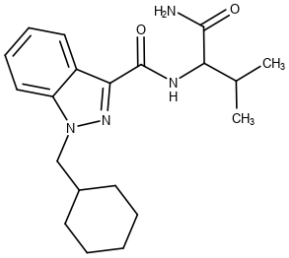
5F-AKB48



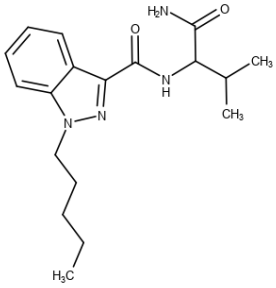
AB-FUBINACA



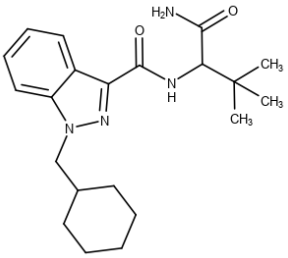
5F-AB-PINACA



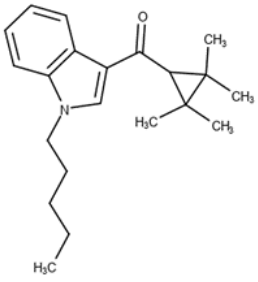
ADB-CHMINACA



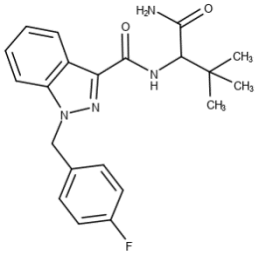
AB-PINACA



AB-CHMINACA



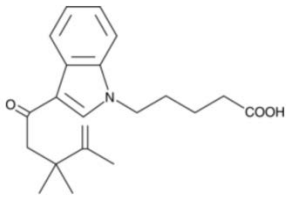
UR144



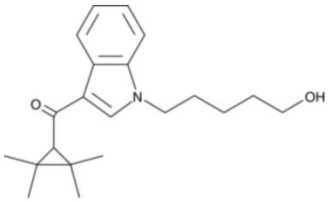
ADB-FUBINACA

Group 2a (Figure A-3) and Group 2b (Figure A-4) were analytes validated in urine at Boston University and RTI, respectively. Internal standards for Group 2a were UR144-d_5, PB-22-d_9, XLR11-d_5, and 5F-PB-22 3-carboxyindole-d_5. Internal standards for Group 2b were AB-CHMINACA M4-d_4, and XLR11 4-hydroxypentyl-d_5.

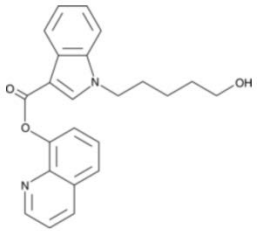
Figure A-3: Chemical structures of Group 2a analytes validated in urine by Boston University.



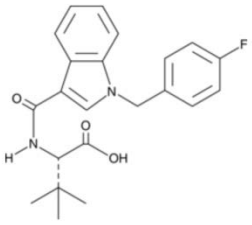
UR144 degradant pentanoic acid



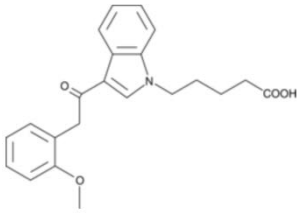
UR144 5-hydroxypentyl



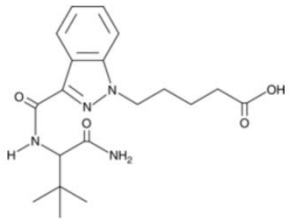
PB-22 5-hydroxypentyl



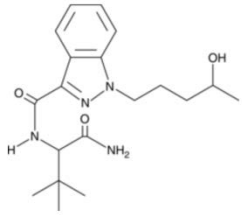
MDMB-FUBICA M3



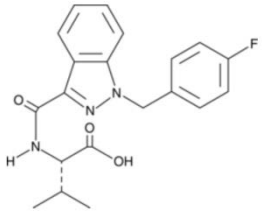
JWH 250 pentanoic acid



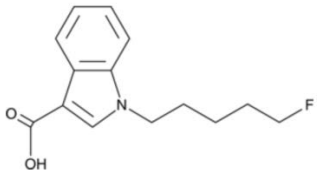
ADB-PINACA pentanoic acid



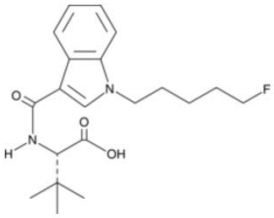
ADB-PINACA 4-hydroxypentyl



AB-FUBINACA M3

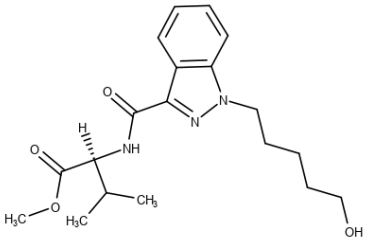


5F-PB-22 3-carboxyindole

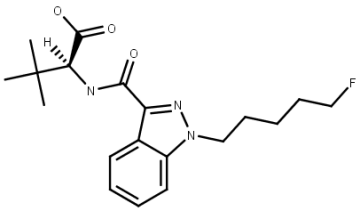


5F-MDMB-PICA M7

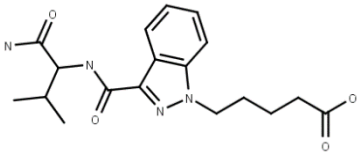
Figure A-4: Chemical structures of Group 2b analytes validated in urine by RTI.



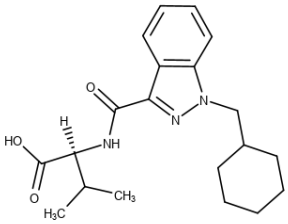
5F-AMB M2



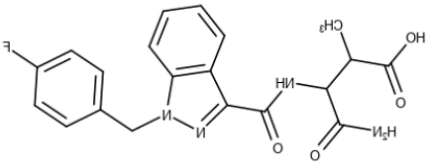
5F-ADB M7



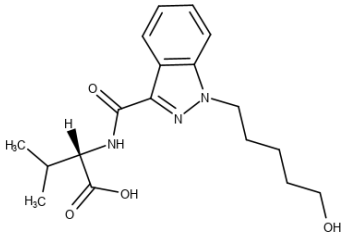
AB-PINACA PENTANOIC ACID



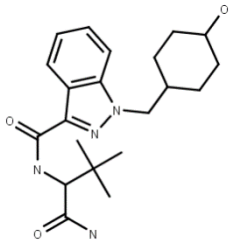
AB-CHIMINACA M2



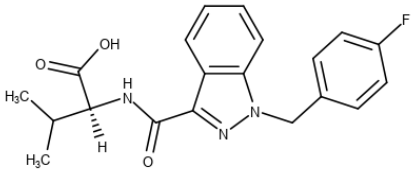
AB-FUBINACAM 2A



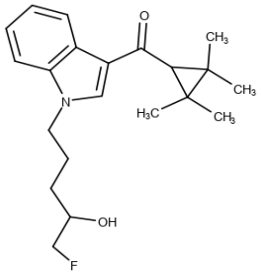
5FAMB M5



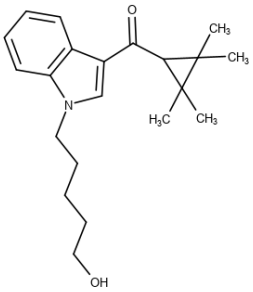
ADB-CHMINACA M1



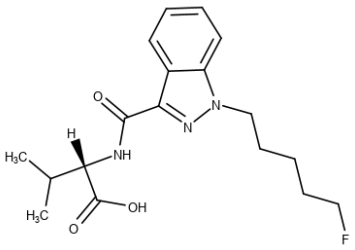
AB-FUBINACA M3



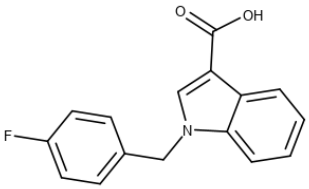
XLR11 4-HYDROXYPENTYL



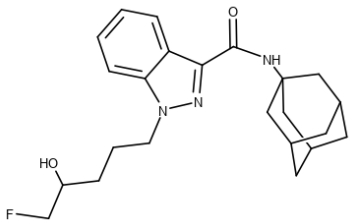
UR144 5-HYDROXYPENTYL



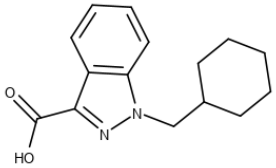
5F-AMB M7



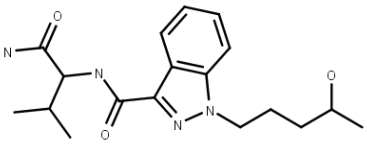
FUB-PB-22 3-CARBOXY



5F-AKB48 4-HYDROXYPENTYL



AB-CHMINACA M4



AB-PINACA 4-HYDROXYPENTYL

SLE Extraction Method for Whole Blood and Urine Samples

Aliquots of human urine or human whole blood (300 µL) were placed into glass conical tubes followed by calibrators or QC working solutions (20 µL) and internal standard working solution (10 µL). The samples were vortexed, and 50 mM ammonium acetate (pH 4,300 µL) was added to each sample. Samples were vortexed, poured onto Isolute SLE+ 1-mL cartridges (Biotage, Charlotte, NC), and allowed to load completely onto the sorbent. After 5 minutes, the analytes were eluted by gravity with ethyl acetate (2.5 mL). After the first elution, 5 minutes passed before eluting by gravity with additional ethyl acetate (2.5 mL). The eluate was dried under nitrogen at 40°C and reconstituted with mobile phase (100 µL) (Table A-1).

LC-MS/MS Methods

Analytes at RTI were analyzed on an Agilent 1290 LC Agilent 6490 MS/MS (Santa Clara, CA) in positive ionization mode. Analytes in urine at RTI (Group 2b) were analyzed using dynamic multiple reaction monitoring, whereas the blood analytes (Group 1b) were acquired with time-segmented multiple reaction monitoring. All analytes at Boston University were acquired on an LC (Shimadzu, Kyoto, Japan) coupled to a 4000 Q-Trap tandem MS (SCIEX, Waltham, MA) in positive ionization mode.

Table A-1: LC-MS/MS analytical method parameters.

	Group 1a and Group 2a	**Group 1b and Group 2b**
Analytical column	Waters XBridge C18 (2.1 × 50 mm, 3.5 μm)	Agilent Poroshell 120 SB-C18 (2.1 × 100 mm, 2.7 μm)
Mobile phase	(A) Water with 0.1% formic acid (FA) (B) Acetonitrile with 0.1% FA	(A) 5 mm ammonium formate with 0.1% FA (B) Methanol with 0.1% FA
Mobile phase gradient	**Time (min)** / **%A** / **%B** 0 / 95 / 5 .5 / 5 / 5 5.5 / 5 / 95 7.5 / 5 / 95 8.0 / 95 / 5 9.0 / 95 / 5	**Time (min)** / **%A** / **%B** 0 / 50 / 50 2.0 / 40 / 60 4.0 / 40 / 60 5.0 / 5 / 95 6.0 / 5 / 95 1 min postrun at initial conditions
Flow rate	0.6 mL/min	
Injection volume	10 μL	4 μL
Column temperature	40°C	55°C
Autosampler temperature	15°C	4°C

Table A-2: Optimized parameters and monitored ion transitions for drugs (Group 1a) and metabolites (Group 2a).

Drug	Precursor Ion (m/z)	Collision Energy 1 Voltage (V)	Product Ion 1 (m/z)	Collision Energy 2 Voltage (V)	Product Ion 2 (m/z)
Group 1a: Boston University Synthetic Cannabinoids in Blood					
4-cyano CUMYL-BUTINACA	361.2	29.7	226.2	33.3	119.2
5F-3,5-AB-PFUPPYCA	393.0	52.6	189.2	89.1	134.2
5F-ADB-PINACA	363.3	14.1	346.3	35.0	233.2
5F-PY-PINACA	304.3	27.5	233.3	50.8	145.1
ADB-PINACA	345.2	13.3	328.3	35.0	215.3
APP-PICA	378.2	14.1	361.3	26.6	214.3
CUMYL-THPINACA	378.2	16.0	260.2	28.7	243.3
EMB-FUBINACA	398.2	21.9	324.3	57.1	109.2
JWH-250	336.3	28.8	121.2	65.1	91.2
MDMB-FUBICA	397.2	21.0	252.3	52.4	109.2
MEP-CHMICA	371.3	20.8	240.4	82.3	55.3
MO-CHMINACA	387.3	22.7	241.3	49.5	145.2
NM2201	376.2	15.3	232.3	52.1	144.3
PB-22	359.2	18.0	214.3	50.9	144.2
PB-22-d9	368.2	18.7	223.3	53.3	145.2
RCS-8	376.2	32.8	121.3	74.6	91.2
UR144	312.3	31.8	125.1	60.2	55.3
UR144-d5	317.3	32.3	125.2	60.9	55.2
XLR11	330.3	33.9	232.3	33.0	125.3
XLR11-d5	335.2	34.4	125.3	66.6	55.2
Group 2a: Boston University Synthetic Cannabinoid Metabolites in Urine					
5F-MDMB-PICA M7	363.2	20.2	232.2	54.5	144.2
5F-PB-22 3-carboxyindole	250.2	22.0	206.3	33.1	118.1
5F-PB-22 3-carboxyindole-d5	255.2	22.6	211.3	34.4	123.2
AB-FUBINACA M3	370.2	19.4	324.3	28.7	253.1
ADB-PINACA 4-hydroxypentyl	361.3	14.8	344.4	22.2	316.4
ADB-PINACA pentanoic acid	375.2	14.6	358.3	22.2	330.3
JWH 250 pentanoic acid	366.2	32.2	121.2	69.5	91.2
MDMB-FUBICA M3	383.2	19.5	252.2	120.6	83.2
PB-22-d9	368.2	18.7	223.3	53.3	145.2
PB-22 5-hydroxypentyl	375.2	19.8	230.3	50.4	144.2
UR144-d5	317.3	32.3	125.2	60.9	55.2
UR-144 5-hydroxypentyl	328.3	38	125.3	61.7	55.3
UR-144 degradant pentanoic acid	342.3	32.1	244.2	73.7	55.3
XLR11-d5	335.2	34.4	125.3	66.6	55.2

Table A-3: Optimized parameters and monitored ion transitions for drugs (Group 1b) and metabolites (Group 2b).

Drug	Precursor Ion (m/z)	Collision Energy 1 Voltage (V)	Product Ion 1 (m/z)	Collision Energy 2 Voltage (V)	Product Ion 2 (m/z)
	Group 1b: RTI Synthetic Cannabinoids in Blood				
5F-AB-PINACA	349.21	16	304.1	24	233.1
5F-ADB	378.22	16	318.3	24	233.1
5F-AKB48	384.25	28	135.3	70	93
5F-AMB	364.21	16	304.1	24	233
AB-CHMINACA-d_4	361.26	28	245.1		
AB-CHMINACA	357.23	16	312.3	28	241.1
AB-FUBINACA	369.17	28	253	56	108.8
AB-PINACA-d_9	340.27	28	224.1		
AB-PINACA	331.22	16	286	28	215.1
ADB-CHMINACA-d_4	375.27	32	245.2		
ADB-CHMINACA	371.25	16	326.2	32	241.2
ADB-FUBINACA	383.19	28	253	64	109
AMB	346.22	20	215.1	44	145
UR144	312.23	12	214	24	125.1
XLR11-d_5	335.26	28	125.1		
XLR11	330.23	12	232	24	125.1
	Group 2b: RTI Synthetic Cannabinoid Metabolites in Urine				
5F-AB-PINACA 4-hydroxypentyl	365.2	16	320.1	28	249.2
5F-ADB M7	364.21	16	318.2	36	213
5F-AKB48 4-hydroxypentyl	400.24	32	135.1	68	93
5F-AMB M2	362.21	16	231.1	48	145
5F-AMB M5	348.19	20	231.1	28	213
5F-AMB M7	350.19	24	233.1	52	145
AB-CHMINACA M2	358.22	16	312.3	44	145.1
AB-CHMINACA M4	259.15	12	241.2	28	145
AB-CHMINACA M4-d_4	263.17	12	245.2		
AB-FUBINACA M2A	399.15	24	253	60	109
AB-FUBINACA M3	370.16	20	253	44	108.9
AB-PINACA pentanoic acid	361.19	36	227.1	36	217.2
ADB-CHMINACA M1	387.24	12	342	52	145
FUB-PB-22 3-carboxyindole	270.14	24	109	70	82.9
UR144 5-hydroxypentyl	328.23	12	230	24	125.1
XLR11 4-hydroxypentyl	346.22	12	248	28	125.1
XLR11 4-hydroxypentyl-d_5	351.25	28	125.2		

LC Quadrupole Time-of-Flight MS Methods and Data Analysis

After LC-MS/MS analysis, nontargeted analyses were acquired on stability samples for analytes in Group 1b and Group 2b. Samples were acquired on a Waters Synapt G2 HDMS quadrupole time-of-flight MS coupled to a Waters Acquity ultraperformance LC (Milford, MA). Data were acquired using the MS^E method during which low-collision and high-collision energy data were collected nearly simultaneously for every m/z. The LC method from the Agilent 1290 was transferred to the Synapt, and the same column was used. The purpose of nontargeted analysis was to determine if there were potential unique degradants that could be used as markers for identification. LC-MS data were mined for potential degradation components through data mining the low-collision and high-collision energy data. This was done by precursor ion search for known fragments of the synthetic cannabinoids and probable modifications of the parent compounds such as metabolite formations.

Validation

Linearity

A minimum of six non-zero calibrators spanning the working range of the analytes in each matrix were prepared and extracted for five separate runs. A linear or quadratic model was applied either as nonweighted or weighted (1/x) and maintained throughout the entire validation.

Precision and Bias

Matrix matched quality control samples at a minimum of three (low, medium, and high) concentrations were extracted in triplicate and analyzed with each of the five linearity runs. These samples were used to determine bias, within-run precision, and between-run precision. Bias was considered acceptable if within ±20% and precision, expressed as coefficient of variation, was acceptable if <20%.

LOD

The LOD was determined using three sources of blank matrix that were fortified in decreasing concentrations of analytes. These samples, along with unfortified blank matrix samples corresponding to each source, were extracted in duplicate, and analyzed over three runs. The LOD was determined as the lowest concentration that had an average area greater than the average area from the blank matrix plus 3.3 times the standard deviation of the area from the blank matrix and met detection and identification criteria (e.g., retention time, peak shape)

LOQ

The LOQ was set at the lowest non-zero calibrator. Over the five linearity runs, the lowest non-zero calibrator was monitored for precision, bias, and qualitative ion-to-quantitative ion ratio.

Carryover

Carryover was assessed by running a blank sample immediately after the high calibrator for each of the five linearity runs. No carryover was observed if the average area at the analyte retention time was less than the LOD area.

Dilution Integrity

Dilution integrity was assessed by preparing 1:10 and 1:50 dilution for each matrix in triplicates. The dilution integrity samples were assessed with the five linearity runs. Overall precision and bias were reported in each matrix with acceptable precision < 20% and acceptable bias within ±20%.

Interference

Matrix interferences were evaluated by analyzing 10 lots of blank matrix. Interferences from internal standards were evaluated by analyzing matrix fortified with internal standards that

was analyzed once in each of the five linearity runs. Commonly encountered analytes were evaluated for potential interferences at 2,000 ng/mL. In all cases, no interference was observed if the area at the analyte retention time was less than the area of the determined LOD.

Table A-4: List of analytes evaluated as potential interferences for Group 1a and Group 2a drugs at Boston University.

25I-NBOMe	Fentanyl
Amphetamine	MDMA
Clonazepam	Methadone
Cocaine	Morphine
Diazepam	Phencyclidine

Table A-5: List of analytes evaluated as potential interferences for Group 1b and Group 2b drugs at RTI.

6-AM	Citalopram	MDA
11-Hydroxy-Δ^9-THC	Clonazepam	MDEA
11-nor-9-Carboxy-Δ^9-THC	Cocaine	MDMA
25I-NBOMe	Codeine	Methamphetamine
7-aminoclonazepam	Diazepam	Methadone
Alpha-hydroxyalprazolam	Ethylone	Morphine
Alpha-PVP	Etizolam	Norcocaine
Amitriptyline	Fentanyl	Oxycodone
Amobarbital	Fluoxetine	PCP
Amphetamine	Hydrocodone	Phenobarbital
Benzoylecgonine	Lidocaine	THC
Butalbital	LSD	Trazadone

Ion Suppression and Enhancement

Two sets of samples were prepared to estimate ionization suppression and enhancement. One set consisted of a neat solution, containing the analytes in mobile phase injected on the instrument six times. The neat solutions were prepared at a high and low concentration. The

second set consisted of 10 lots of matrix each fortified in duplicate at a high and low concentration. These samples underwent extraction following reconstitution with the analyte-fortified neat solution. Below is the calculation used for estimating ionization suppression and enhancement:

$$\left(\frac{Average\ Area\ Set\ 2}{Average\ Area\ Set\ 1} - 1\right) \times 100$$

Stability

In general, analytes were considered stable if the average area ratio between the analyte and internal standard at the time point was within ±20% of the average area ratio response at time point zero. In some cases, it was necessary to evaluate the complete picture of the stability data by reviewing analyte area, concentration, and overall stability data trend between time points at the low and high concentrations. In certain situations, an analyte was considered stable even if specific time points for a concentration were outside the ±20% range. For example, in cases where one concentration at a time point was within the ±20% range and the other concentration fell within the ±30% range the analyte was considered stable overall.

Validation Results

Precision, Bias, LOD, LOQ

Table A-6: Bias, precision, correlation coefficient (r^2), LOD, and LOQ results for 17 drugs in blood (Group 1a) and 10 metabolites (Group 2a) in urine at Boston University.

Drug Analytes	Overall Grand Bias	Overall Between-run Precision	Overall Within-run Precision	Average r^2	LOD	LOQ
Group 1a: Boston University synthetic cannabinoids in blood						
4-cyano CUMYL-BUTINACA	4.32	5.46	3.53	0.9994	0.025	0.5
5F-3,5-AB-PFUPPYCA	3.32	8.46	3.17	0.9987	0.025	0.5
5F-ADB-PINACA	7.66	6.80	3.86	0.9988	0.1	0.5
5F-PY-PINACA	6.83	8.67	3.62	0.9995	0.025	0.5
ADB-PINACA	5.51	4.82	3.37	0.9995	0.5	0.5
APP-PICA	4.29	5.20	3.91	0.9996	0.025	0.1
CUMYL-THPINACA	4.47	4.82	3.72	0.9992	0.025	0.5
EMB-FUBINACA	5.52	6.30	3.10	0.9981	0.025	0.5
JWH-250	4.32	5.21	3.10	0.9996	0.025	0.5
MDMB-FUBICA	4.60	6.73	3.86	0.9983	0.025	0.5
MEP-CHMICA	6.91	4.94	3.76	0.9984	0.025	0.5
MO-CHMINACA	0.84	9.00	3.49	0.9997	0.025	0.5
NM2201	2.49	6.97	5.27	0.9994	0.025	0.1
PB-22	2.53	4.89	3.08	0.9998	0.025	0.5
RCS-8	1.00	5.21	3.18	0.9994	0.025	0.5
UR144	7.43	4.25	2.67	0.9995	0.025	0.5
XLR11	2.02	6.85	3.42	0.9991	0.025	0.5
Group 2a: Boston University synthetic cannabinoid metabolites in urine						
5F-MDMB-PICA M7	5.36	3.94	3.39	0.9977	0.05	5
5F-PB-22 3-carboxyindole	−0.37	6.24	4.88	0.9992	0.3	5
AB-FUBINACA M3	3.40	5.51	4.36	0.9970	0.5	5
ADB-PINACA 4-hydroxypentyl	4.29	6.03	4.50	0.9966	0.3	5
ADB-PINACA pentanoic acid	7.16	5.76	4.49	0.9976	0.5	5
JWH 250 pentanoic acid	1.59	4.53	3.61	0.9971	0.3	5
MDMB-FUBICA M3	4.18	5.70	4.14	0.9971	0.1	5
PB-22 5-hydroxypentyl	4.73	3.20	2.74	0.9963	0.3	5
UR-144 5-hydroxypentyl	5.05	3.80	3.28	0.9977	0.1	5
UR-144 degradant pentanoic acid	3.58	4.43	3.99	0.9973	0.3	5

Table A-7: Bias, precision, correlation coefficient (r^2), LOD, and LOQ results for 12 drugs in blood (Group 1b) and 15 metabolites (Group 2b) in urine at RTI.

* UR144 was evaluated only for qualitative identification.

Drug Analytes	Overall Grand Bias	Overall Between-run Precision	Overall Within-run Precision	Average r^2	LOD	LOQ
Group 1b: RTI Synthetic Cannabinoids in Blood						
5F-AB-PINACA	−4.48	7.26	4.30	0.9986	0.01	0.1
5F-ADB	−4.97	10.08	7.07	0.9947	0.01	0.1
5F-AKB48	5.05	15.17	9.60	0.9910	0.30	0.5
5F-AMB	−1.36	8.22	5.56	0.9970	0.01	0.1
AB-CHMINACA	−0.93	9.27	6.40	0.9952	0.05	0.1
AB-FUBINACA	2.78	7.42	4.67	0.9993	0.01	0.1
AB-PINACA	2.51	5.86	4.55	0.9991	0.01	0.3
ADB-CHMINACA	0.39	10.96	8.27	0.9971	0.05	0.3
ADB-FUBINACA	2.29	7.89	5.12	0.9970	0.01	0.1
AMB	7.95	9.57	8.35	0.9974	0.05	0.3
UR144*					2	
XLR11	−1.71	11.47	8.48	0.9959	0.30	0.3
Group 2b: RTI Synthetic Cannabinoid Metabolites in Urine						
5F-AB-PINACA 4-hydroxypentyl	−6.39	13.65	8.09	0.9969	0.30	0.30
5F-ADB M7	−5.21	6.64	6.61	0.9992	0.05	0.30
5F-AKB48 4-hydroxypentyl	6.17	8.55	7.16	0.9987	0.05	0.30
5F-AMB M2	−3.27	8.78	6.25	0.9975	0.05	0.30
5F-AMB M5	−9.83	8.43	6.39	0.9989	0.10	0.30
5F-AMB M7	−7.24	8.18	6.31	0.9990	0.05	0.30
AB-CHMINACA M2	−0.78	7.26	7.18	0.9982	0.10	0.30
AB-CHMINACA M4	0.82	8.87	6.31	0.9976	0.30	0.50
AB-FUBINACA M2A	−8.27	8.33	6.17	0.9969	0.30	0.30
AB-FUBINACA M3	−7.13	6.75	6.72	0.9992	0.05	0.30
AB-PINACA pentanoic acid	−1.37	9.92	5.54	0.9877	2.00	5.00
ADB-CHMINACA M1	−7.02	8.80	7.31	0.9994	0.30	0.30
FUB-PB-22 3-carboxyindole	−1.55	7.07	5.55	0.9944	5.00	5.00
UR144 5-hydroxypentyl	−6.84	5.92	5.49	0.9993	0.30	0.30
XLR11 4-hydroxypentyl	1.03	5.33	5.26	0.9992	0.10	0.30

Dilution Integrity

Table A-8: Dilution integrity precision and bias 17 drugs in blood (Group 1a) and 10 metabolites (Group 2a) in urine at Boston University.

Drug Analytes	1:50 (2 ng/mL) Precision	1:50 (2 ng/mL) Bias	1:10 (10 ng/mL) Precision	1:10 (10 ng/mL) Bias
Group 1a: Boston University Synthetic Cannabinoids in Blood				
4-cyano CUMYL-BUTINACA	8.46	1.33	8.68	−1.17
5F-3,5-AB-PFUPPYCA	9.04	−4.83	13.42	−4.60
5F-ADB-PINACA	10.11	−4.96	11.98	−5.58
5F-PY-PINACA	10.27	−5.12	5.28	−5.97
ADB-PINACA	8.24	−7.11	7.76	−6.48
APP-PICA	8.67	−11.94	9.84	−8.50
CUMYL-THPINACA	6.90	−4.82	7.67	−6.05
EMB-FUBINACA	8.78	18.57	8.28	11.11
JWH-250	4.79	2.83	2.62	1.77
MDMB-FUBICA	6.01	8.01	4.58	11.18
MEP-CHMICA	6.58	12.50	5.93	8.33
MO-CHMINACA	12.14	−0.08	8.95	−3.08
NM2201	7.25	25.08	12.40	15.25
PB-22	4.60	10.00	3.84	6.95
RCS-8	4.78	12.30	5.62	4.28
UR144	3.29	13.87	3.81	7.54
XLR11	2.39	9.61	6.05	9.98
Drug Analytes	**1:50 (7 ng/mL) Precision**	**1:50 (7 ng/mL) Bias**	**1:10 (35 ng/mL) Precision**	**1:10 (35 ng/mL) Bias**
Group 2a: Boston University Synthetic Cannabinoid Metabolites in Urine				
5F-MDMB-PICA M7	2.31	3.30	3.02	3.38
5F-PB-22 3-carboxyindole	2.36	4.73	1.72	1.96
AB-FUBINACA M3	3.76	6.19	2.35	3.00
ADB-PINACA 4-hydroxypentyl	2.00	6.40	3.11	4.03
ADB-PINACA pentanoic acid	4.64	4.91	4.21	−0.43
JWH 250 pentanoic acid	6.01	3.19	3.68	2.31
MDMB-FUBICA M3	3.83	3.06	4.09	0.95
PB-22 5-hydroxypentyl	4.40	3.39	3.04	1.92
UR-144 5-hydroxypentyl	4.04	3.23	2.78	1.81
UR-144 degradant pentanoic acid	2.33	5.87	2.79	0.46

Table A-9: Dilution integrity for 12 drugs in blood (Group 1b) and 15 metabolites (Group 2b) in urine at RTI.

* UR144 was evaluated only for qualitative identification.

Drug Analytes	1:50 (4 ng/mL) Precision	1:50 (4 ng/mL) Bias	1:10 (20 ng/mL) Precision	1:10 (20 ng/mL) Bias
Group 1b: RTI Synthetic Cannabinoids in Blood				
5F-AB-PINACA	12.19	−10.56	19.51	−8.99
5F-ADB	17.15	−13.15	19.89	−9.41
5F-AKB48	23.01	6.07	21.91	8.02
5F-AMB	10.97	−4.54	17.03	−3.41
AB-CHMINACA	11.83	−1.37	19.10	−1.85
AB-FUBINACA	11.63	0.35	18.41	3.33
AB-PINACA	9.93	−1.02	16.58	−0.04
ADB-CHMINACA	6.38	0.28	11.19	0.00
ADB-FUBINACA	10.45	−1.06	17.18	2.48
AMB	12.92	5.44	22.12	0.83
UR144*				
XLR11	12.71	0.97	16.60	0.51
Drug Analytes	**1:50 (6 ng/mL) Precision**	**1:50 (6 ng/mL) Bias**	**1:10 (30 ng/mL) Precision**	**1:10 (30 ng/mL) Bias**
Group 2b: RTI Synthetic Cannabinoid Metabolites in Urine				
5F-AB-PINACA 4-hydroxypentyl	13.21	−6.4	12.81	−7.63
5F-ADB M7	8.90	−19.48	12.74	−17.24
5F-AKB48 4-hydroxypentyl	14.49	−22.81	9.75	−10.83
5F-AMB M2	12.2	−9.24	10.81	−9.65
5F-AMB M5	9.02	−11.67	12.01	−12.08
5F-AMB M7	9.58	−14.95	11.59	−14.74
AB-CHMINACA M2	13.27	−21.71	9.69	−12.73
AB-CHMINACA M4	13.86	−0.13	14.77	−7.75
AB-FUBINACA M2A	10.53	−6.47	12.26	−9.14
AB-FUBINACA M3	9.26	−14.81	10.86	−15.33
AB-PINACA pentanoic acid	8.10	9.57	10.77	−8.41
ADB-CHMINACA M1	11.56	−16.54	10.43	−14.3
FUB-PB-22 3-carboxyindole	17.72	−6.99	11.38	−9.61
UR144 5-hydroxypentyl	15.93	−33.5	11.71	−19.17
XLR11 4-hydroxypentyl	11.44	−23.95	8.07	−15.11

Ion Suppression and Enhancement

Table A-10: Ion suppression and enhancement of 17 drugs in blood (Group 1a) and 10 metabolites (Group 2a) in urine at Boston University.

Drug Analytes	Ion Suppression and Enhancement 0.5 ng/mL	Ion Suppression and Enhancement 15 ng/mL
Group 1a: Boston University Synthetic Cannabinoids in Blood		
4-cyano CUMYL-BUTINACA	−46.47	−43.71
5F-3,5-AB-PFUPPYCA	2.22	−0.06
5F-ADB-PINACA	−0.63	−5.87
5F-PY-PINACA	−11.38	−28.60
ADB-PINACA	−6.20	−11.68
APP-PICA	−2.94	−15.18
CUMYL-THPINACA	−41.95	−39.71
EMB-FUBINACA	−47.39	−41.20
JWH-250	−53.47	−34.12
MDMB-FUBICA	−25.66	−12.00
MEP-CHMICA	−40.87	−23.07
MO-CHMINACA	−33.70	−4.24
NM2201	−57.78	−58.02
PB-22	−46.80	−17.32
RCS-8	−43.99	−32.52
UR144	−13.99	−10.69
XLR11	−59.03	−54.26
Drug Analytes	**Ion Suppression and Enhancement 10 ng/mL**	**Ion Suppression and Enhancement 30 ng/mL**
Group 2a: Boston University Synthetic Cannabinoid metabolites in Urine		
5F-MDMB-PICA M7	−5.58	−1.67
5F-PB-22 3-carboxyindole	−14.36	−21.95
AB-FUBINACA M3	−18.44	−13.01
ADB-PINACA 4-hydroxypentyl	−29.87	−17.12
ADB-PINACA pentanoic acid	−10.15	−8.93
JWH 250 pentanoic acid	−15.67	−26.18
MDMB-FUBICA M3	−0.80	2.23
PB-22 5-hydroxypentyl	−8.01	−10.95
UR-144 5-hydroxypentyl	−13.06	−9.37
UR-144 degradant pentanoic acid	−13.46	−7.52

Table A-11: Ion suppression and enhancement of 12 drugs in blood (Group 1b) and 15 metabolites (Group 2b) in urine at RTI.

Drug Analytes	Ion Suppression and Enhancement 2 ng/mL	Ion Suppression and Enhancement 10 ng/mL
Group 1b: RTI Synthetic Cannabinoids in Blood		
5F-AB-PINACA	0.12	8.83
5F-ADB	−9.53	−14.7
5F-AKB48	−68.57	−75.08
5F-AMB	−1.03	−7.58
AB-CHMINACA	−5.95	−14.23
AB-FUBINACA	5.70	33.75
AB-PINACA	3.08	5.27
ADB-CHMINACA	−17.38	−10.77
ADB-FUBINACA	−2.34	13.89
AMB	−24.54	−30.24
UR144	−72.20	−84.20
XLR11	−44.15	−48.23
Drug Analytes	**Ion Suppression and Enhancement 5 ng/mL**	**Ion Suppression and Enhancement 25 ng/mL**
Group 2b: RTI Synthetic Cannabinoids in Urine		
5F-AB-PINACA 4-hydroxypentyl	−33.83	−24.34
5F-ADB M7	33.48	13.69
5F-AKB48 4-hydroxypentyl	−35.59	26.62
5F-AMB M2	−30	−17.73
5F-AMB M5	51.29	36.78
5F-AMB M7	38.98	16.79
AB-CHMINACA M2	−1.95	23.19
AB-CHMINACA M4	10.6	4.98
AB-FUBINACA M2A	22.28	4.39
AB-FUBINACA M3	32.99	7.84
AB-PINACA pentanoic acid	−18.74	−8.32
ADB-CHMINACA M1	27.54	23.19
FUB-PB-22 3-carboxyindole	14.18	−19.27
UR144 5-hydroxypentyl	−13.48	9.37
XLR11 4-hydroxypentyl	−14.34	7.1

Stability

Table A-12: Stability of 17 drugs in preserved blood (Group 1a) and 10 metabolites (Group 2a) in urine at Boston University.

Drug Analytes	Room Temperature	Refrigerator	Freezer
Group 1a: Boston University Synthetic Cannabinoids in Blood			
4-cyano CUMYL-BUTINACA	24 hours	3 weeks	52weeks
5F-3,5-AB-PFUPPYCA	24 hours low; 21 weeks high	17 weeks low; 35 weeks high	52 weeks
5F-ADB-PINACA	0 hours	0 hours low; 72 hours high	24 hours low; 21 weeks high
5F-PY-PINACA	35 weeks	52 weeks	35 weeks low; 52 weeks high
ADB-PINACA	21 weeks low; 17 weeks high	52 weeks	52 weeks
APP-PICA	1 week	1 week	1 week high; 24 hours low
CUMYL-THPINACA	52 weeks	17 weeks	17 weeks low; 52 weeks high
EMB-FUBINACA	0 hour	72 hours low; 24 hours high	21 weeks
JWH-250	52 weeks	52 weeks	52 weeks
MDMB-FUBICA	9 weeks low; 1 week high	52 weeks low; 21 weeks high	52 weeks low; 21 weeks high
MEP-CHMICA	72 hours	72 hours	21 weeks
MO-CHMINACA	52 weeks low; 35 weeks high	3 weeks	35 weeks
NM2201	5 weeks low; 3 weeks high	35 weeks	35 weeks low; 17 weeks high
PB-22	3 weeks	52 weeks	52 weeks
RCS-8	21 weeks	35 weeks	35 weeks
UR144	52 weeks	52 weeks	52 weeks
XLR11	24 hours	1 week	21 weeks
Group 2a: Boston University Synthetic Cannabinoid Metabolites in Urine			
5F-MDMB-PICA M7	35 weeks	35 weeks	35 weeks
5F-PB-22 3-carboxyindole	35 weeks	35 weeks	35 weeks
AB-FUBINACA M3	9 weeks	9 weeks	9 weeks
ADB-PINACA 4-hydroxypentyl	9 weeks	9 weeks	9 weeks
ADB-PINACA pentanoic acid	5 weeks	5 weeks	5 weeks low; 9 weeks high
JWH 250 pentanoic acid	3 weeks	17 weeks high; 9 weeks low	17 weeks
MDMB-FUBICA M3	9 weeks	9 weeks	9 weeks
PB-22 5-hydroxypentyl	3 weeks	3 weeks	17 weeks
UR-144 5-hydroxypentyl	9 weeks	17 weeks	17 weeks

Drug Analytes	Room Temperature	Refrigerator	Freezer
UR-144 degradant pentanoic acid	17 weeks	17 weeks	17 weeks

Table A-13: Stability of 12 drugs in preserved blood (Group 1b) and 15 metabolites (Group 2b) in urine at RTI.

Drug Analytes	Room Temperature	Refrigerator	Freezer
Group 1b: RTI Synthetic Cannabinoids in Blood			
5F-AB-PINACA	48 hours	48 hours	53 weeks
5F-ADB	48 hours	48 hours	35 weeks
5F-AKB48	48 hours	48 hours	9 weeks
5F-AMB	48 hours	48 hours	53 weeks
AB-CHMINACA	53 weeks low; 21 weeks high	53 weeks	53 weeks
AB-FUBINACA	35 weeks	53 weeks	53 weeks
AB-PINACA	53 weeks low; 21 weeks high	53 weeks	53 weeks
ADB-CHMINACA	53 weeks	53 weeks	53 weeks
ADB-FUBINACA	53 weeks	35 weeks	35 weeks low; 53 weeks high
AMB	3 weeks low; 48 hours high	9 weeks low; 3 weeks high	9 weeks
UR144	9 weeks	9 weeks	9 weeks
XLR11	48 hours	48 hours	53 weeks
Group 2b: RTI Synthetic Cannabinoids in Urine			
5F-AB-PINACA 4-hydroxypentyl	9 weeks	9 weeks	9 weeks
5F-ADB M7	9 weeks	9 weeks	9 weeks
5F-AKB48 4-hydroxypentyl	5 weeks	9 weeks	9 weeks
5F-AMB M2	8 days	3 weeks low; 5 weeks high	9 weeks
5F-AMB M5	9 weeks	9 weeks	9 weeks
5F-AMB M7	9 weeks	9 weeks	9 weeks
AB-CHMINACA M2	9 weeks	9 weeks	9 weeks
AB-CHMINACA M4	9 weeks	9 weeks	9 weeks
AB-FUBINACA M2A	9 weeks	9 weeks	9 weeks
AB-FUBINACA M3	9 weeks	9 weeks	9 weeks
AB-PINACA pentanoic acid	9 weeks	9 weeks	9 weeks
ADB-CHMINACA M1	3 weeks	9 weeks	9 weeks
FUB-PB-22 3-carboxyindole	9 weeks	9 weeks	9 weeks
UR144 5-hydroxypentyl	8 days	5 weeks	5 weeks
XLR11 4-hydroxypentyl	9 weeks	9 weeks	9 weeks

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

Figure A-5: Preserved blood Mix 1 extracted ion chromatogram of fragment ion 145.039 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom.

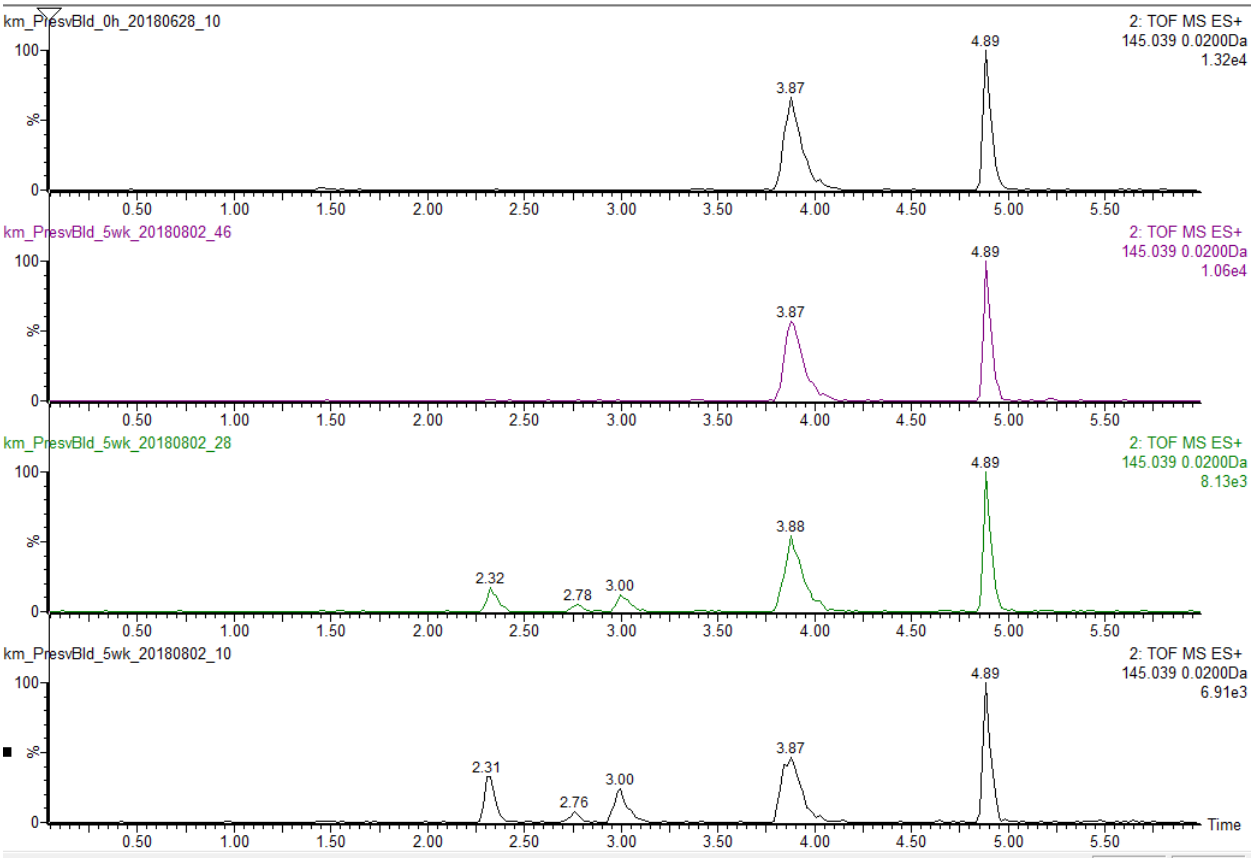


Figure A-6: Preserved blood Mix 1 extracted ion chromatogram of fragment ion 213.102 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom.

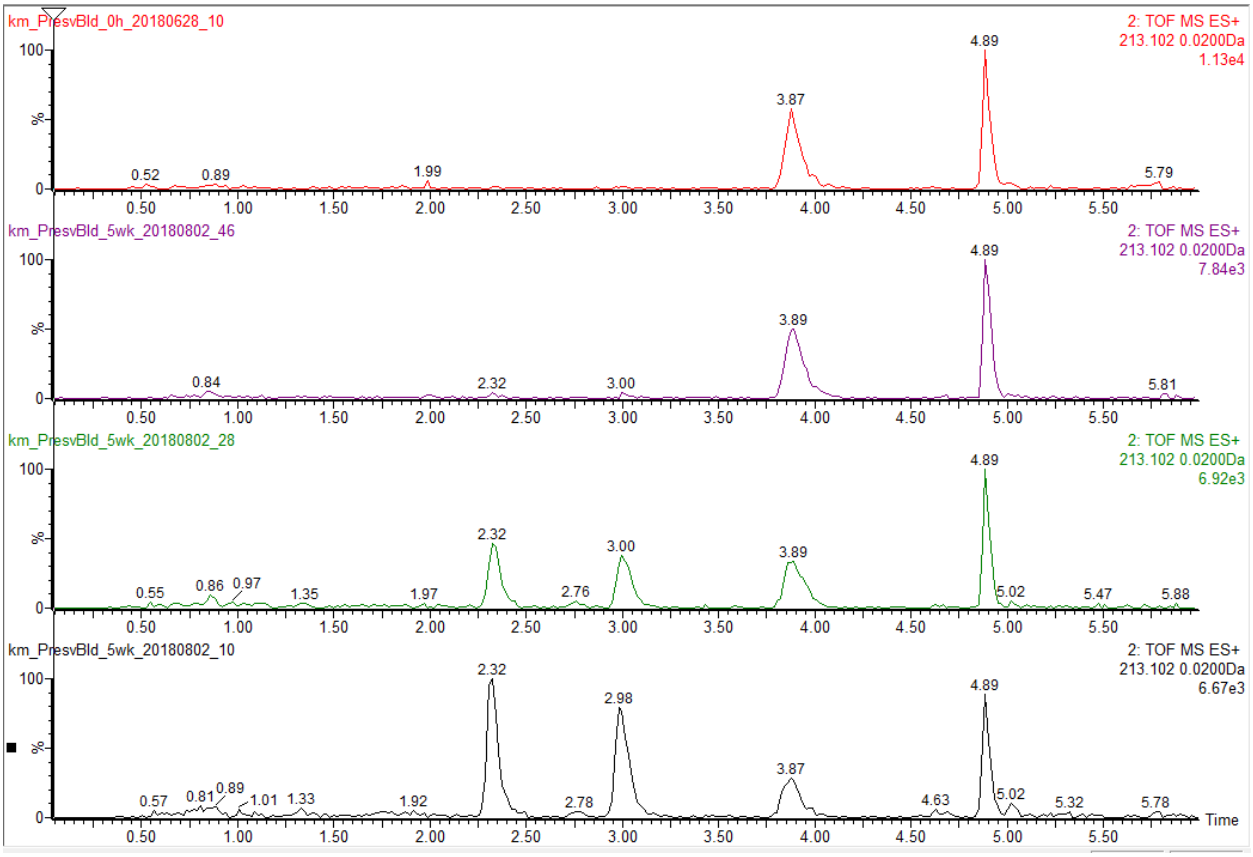


Figure A-7: Preserved blood Mix 1 extracted ion chromatogram at 362.208 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom.

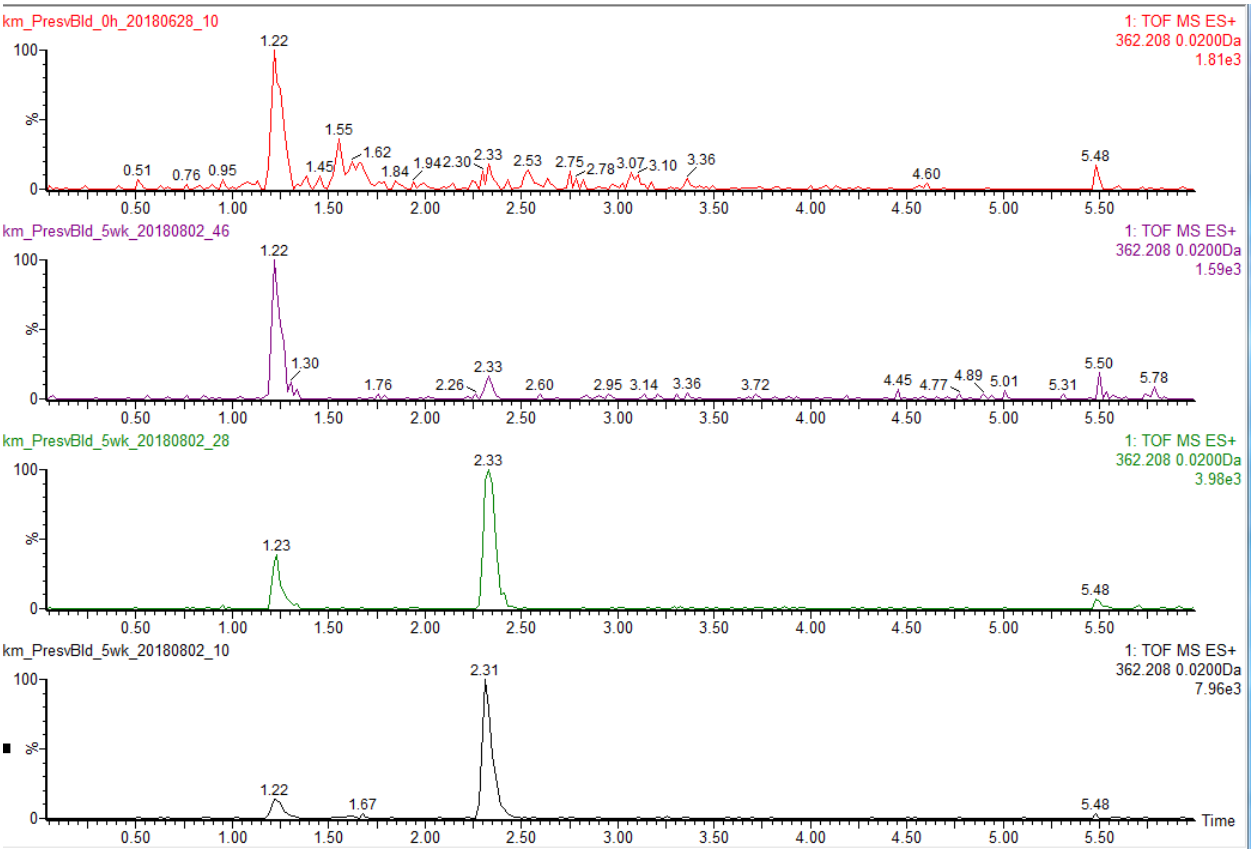


Figure A-8: Preserved blood Mix 3 extracted ion chromatogram of fragment ion 145.039 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom.

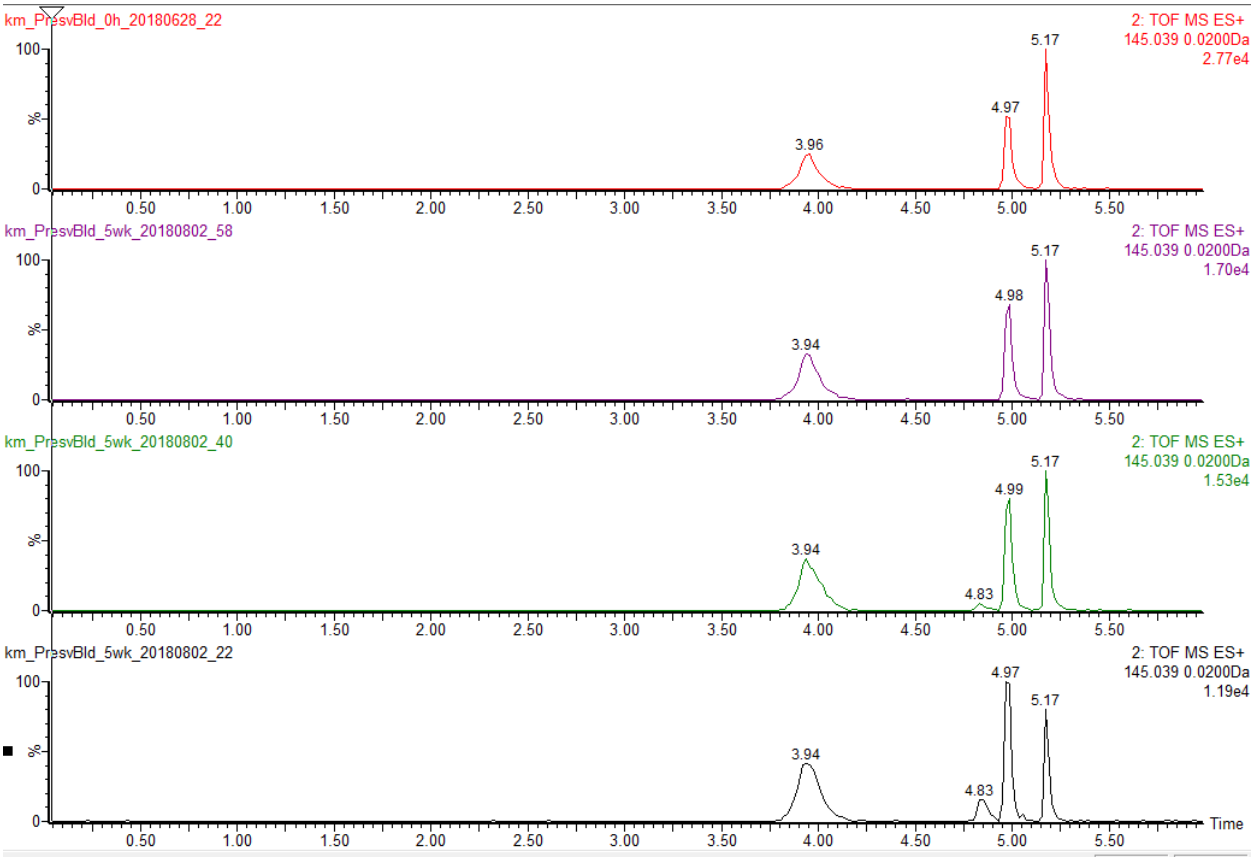


Figure A-9: Preserved blood Mix 3 extracted ion chromatogram of fragment ion 215.118 m/z in 0-hour, 5 week freezer, 5 week refrigerator and 5 week room temperature from top to bottom.