Qualitative Identification of Synthetic Cannabinoids using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF/MS)

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Quality Forensic Toxicology: Who we are

First Independent, Full Service Forensic Toxicology Lab in Texas

- Sixty years of combined forensic toxicology experience
 - Human performance (DUI/DWI)
 - Postmortem
 - Workplace
 - Probation
 - Pain management/Compliance
- Staff represents experience from multiple different laboratories
- Accredited by the American Association for Laboratory Accreditation
- ISO 17025
- Certified by Texas Forensic Science Commission



Certificate#4197.01



What we can do for you

- Forensic Toxicology Analysis
 - Alcohols
 - Prescription Drugs
 - Illicit Drugs
 - Cannabinoids
 - Synthetic Cannabinoids (K2, Spice)
 - "Bath Salts"
 - Z-Drugs
 - Others
 - Multiple matrices- Blood, urine, oral fluid







Quality Forensic Toxicology: Our Team

- Jeff Walterscheid, PhD, F-ABFT-FT, Laboratory Director, Forensic Toxicologist
- **Greg Jellick**, MSFS, D-ABFT-FT, Quality Manager, Forensic Toxicologist
- Michael Frontz, MSFS, D-ABFT-FT, D-ABC, Technical Manager, Forensic Toxicologist
- Natalie Alvarez, BS, MA, Lab Supervisor, Forensic Toxicologist
- Janine De King, BS, D-ABFT-FT, Senior Analyst, Forensic Toxicologist
- Stephanie Troupe, BS, Analyst, Forensic Toxicologist
- Lance Escobedo, BS, Analyst
- Amanda Moore, BS, MS, Analyst, Forensic Toxicologist
- *Crystal Garcia, AS,* Analyst
- Kendra Araujo, BS, Lab Technician







Synthetic Cannabinoids

- Known as synthetic marijuana, Spice, K2, and fake weed
- Act on the CB1 and CB2 receptors to elicit mind altering effects
- Marketed as a "legal high"
- Compounds sprayed on dried plant material
- E-cigarette liquid





LC-QTOF/MS

 Agilent 6530 Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometer (LC-QTOF/MS)





LC-QTOF/MS

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Objective

Validate screening method for synthetic cannabinoids in blood using LC/QTOFMS

- Obtain Retention Times
- Create a Personal Compound Database and Library (PCDL)
- Determine Qualitative Decision Point for each compound
- Reproduce the Qualitative Decision Point
- Determine LOQ for each compound
- Stability
 - Unprocessed
 - Processed
- Carryover
- Proficiency Testing Samples







Instrument Conditions

Optimized using 8 representative compounds: AB FUBINACA, 5F-APINACA, 5-fluoro MDMB-PINACA, 5-fluoro AMB, MMB-FUBINACA, THJ-018, UR-144, and XLR-11

LC

Column: InfinityLab Poroshell 120 EC -C18

3.0x 50mm, 2.7 Micron

Mobile Phase A: H₂O with 0.05% FA and 5mM Ammonium Formate

Mobile Phase B: Methanol with 0.05% FA

Time	Α	B
0.00	30	70
4.30	5	95
5.30	2	98
7.00	2	98
7.10	30	70

QTOF/MS

- Positive ion mode
- Mass range: 50-600 m/z
- Acquisition rate: 1 scan/sec
- Lock mass ions: 121.0509, 922.0090



Synthetic Cannabinoid Screening Library

- Regional Survey
- 370 SC Standards
 - Parents and isomers
 - 10 mM solution in DMSO
- 12 Additional SC Standards
 - Parents and metabolites

7 SC Groups

- Dibenzopyrans (classical cannabinoid)
- Cyclohexylphenols
- Napthoylindoles
- Tetramethylcyclopropanoylindoles
- Indazole-3-carboxamides
- Phenylacetylindoles
- Benzoylindoles



Dibenzopyrans

Cyclohexylphenols



Naphthoylindoles

Phenylacetylindoles



Tetramethylcyclopropanoylindoles Benzoylindoles



Indazole-3-carboxamides



Creating a PCDL

Personal Compound Database and Library (PCDL)
Standards made at approximately 1,000 ng/mL (10 mM)
Molecular formula and retention time





Validation Sample Preparation Procedure

Preparation of blood samples

- Add 50 µL deuterated internal standards mix in glass tubes
 - Target ISTD concentration 100 ng/mL for AB-PINACA-D9, JWH-122-D9, and XLR-11-D9
- Add validation mix at targeted concentrations
- Evaporate with nitrogen
- Add 0.5 mL of whole blood



Extraction Procedure

Liquid-liquid extraction

- Add 0.5 mL LC/MS grade water
- Vortex briefly
- Add 2 mL extracting solvent- 80:20 hexane/ethyl acetate
- Vortex for approximately 30 seconds
- Centrifuge at 4400 rpm for 5 minutes
- Transfer supernatant to freshly labeled tubes
- Evaporate supernatant to dryness using Cerex 48 manifold Nitrogen stream
- Reconstitute with 200 µL of Mobile Phase Mix- 30:70



Determining Qualitative Decision Point

- Blood samples spiked at approximately 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, and 50 ng/mL
- Actual values varied per compound based on millimolar concentrations and compound molar mass
- Once analytical QDP determined...
 - Over 3 days
 - 3 replicates each at QDP, less than QDP, and greater than QDP



Stability Study-Unprocessed

Storage: Refrigerator

- Mock blood samples prepared at 10 ng/mL, grey top tubes
- Analyzed 3 replicates on Day 0
 - Liquid-Liquid Extraction
- Stored @ -4 C°
- Analyzed 3 replicates on Day 5
 - Liquid-Liquid Extraction

Storage: Freezer

- Mock blood samples prepared at 10 ng/mL, grey top tubes
- Analyzed 3 replicates on Day 0
 - Liquid-Liquid Extraction
- Stored @ -20 C°
- Analyzed 3 replicates on Day 5
 - Liquid-Liquid Extraction



Stability Study-Processed

Blood samples spiked at 10 ng/mL and 100 ng/mL

- Liquid-liquid extraction performed
- Samples reinjected 3x each day for 5 days



Carryover Study

- Samples prepared each day for 3 days
- Blood samples spiked at approximately 500 ng/mL
- Blank blood samples injected after 500 ng/mL samples
- Liquid-liquid extraction performed



Proficiency Test Samples

- Proficiency tests in urine, not blood
- College of American Pathologists Synthetic Cannabinoids/Designer Drugs
- CAP 2016 SCDD-A, CAP 2016 SCDD-B
- CAP 2017 SCDD-A, CAP 2017 SCDD-B



Results – Decision Points and LOQs

SC Group	Example Compound	LOD (ng/mL)	LOQ (ng/mL)*
Dibenzopyrans	HU-210	4.8	9.7
Cyclohexylphenols	CP 47,497	****	****
Naphthoylindoles	JWH-018 JWH-073	0.9 0.8	4.3 4.1
Phenylacetylindoles	JWH-167	0.8	3.8
Tetramethylcyclopropanoylindoles	UR-144 XLR-11	0.8 0.8	3.9 4.1
Benzoylindoles	RCS-4	0.8	4.0
Indazole-3-carboxamides	AB-FUBINACA MAB-CHMINACA	0.9 0.9	4.6 4.6

*LOQ within 20% for bias (accuracy) and precision, over three days, n=9



Results – Unprocessed Stability (Refrigerated)

Unprocessed Samples - Refrigerated Storage Stability



Results – Unprocessed Stability (Frozen)

Unprocessed Samples - Frozen Storage Stability



Results – Processed Stability



Results – Processed Stability



* JWH-018 and XLR-11 not included in graph

Results – Carryover

- Criteria: Blank blood samples after 500 ng/mL sample injection does not exceed 10% of signal of lowest calibrator
- Carryover was not detected



Results – Proficiency Tests

CAP	Expected Test Panel Result	TOF Result
16 SCDD-A 01	AKB-48 N-pentanoic acid metabolite	Detected
16 SCDD-A 02	AB-CHMINACA	Detected
16 SCDD-A 03	AB-PINACA N-pentanoic acid UR-144 N-pentanoic acid metabolite THJ-2201	Detected Detected Detected
16 SCDD-B 04	AKB-48 N-pentanoic acid metabolite	Detected
16 SCDD-B 05	UR-144 N-pentanoic acid metabolite	Detected
16 SCDD-B 06	None	N/D



Results – Proficiency Tests

CAP	Expected Test Panel Result	TOF Result
17 SCDD-A 01	None	N/D
17 SCDD-A 02	AB-CHMINACA 3-methyl butanoic acid	Detected
17 SCDD-A 03	UR-144 N-pentanoic acid metabolite 5-F AMB Metabolite 7	Detected Detected
17 SCDD-B 04	None	N/D
17 SCDD-B 05	None	N/D
17 SCDD-B 06	AB-PINACA N-pentanoic acid AKB-48 N-pentanoic acid metabolite	Detected Detected



Conclusions

382 Synthetic Cannabinoids evaluated

- Successful qualitative Identification for 370 SCs of various classes
- Cyclohexylphenols compounds problematic
 - Unable to identify 12 of 13 included
 - Ionization difficulties due to structure
- Necessary sensitivities obtained for expected SC blood values
- Stability of SCs vary greatly
- Able to qualitatively identify all PCDL compounds present in CAP SCDD Proficiency Tests



Future Studies

- Adding the "Q" at CE 10v, 20v, 40v
- More SWGTOX validation components?
 - Carryover at >500 ng/mL
- More detailed stored stability study
- More metabolite RTs
 - As the illicit market continues to grow
- Pyrolysis products



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Questions?

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