Synthetic Cannabinoids, Forensic & Legal Aspects

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NIDA NATIONAL INSTITUTE ON DRUG ABUSE

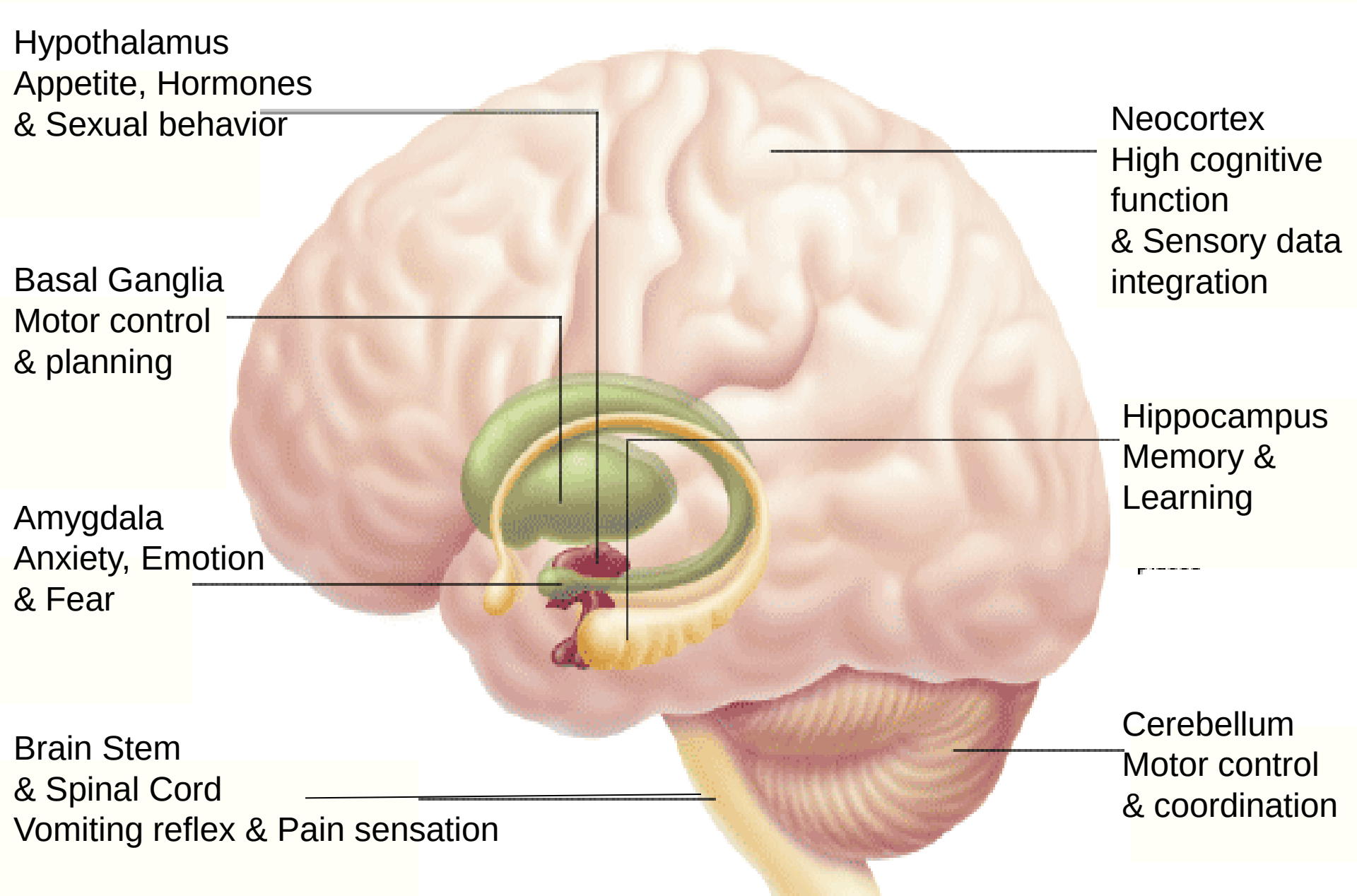
Synthetic Cannabinoid Overview

- Cannabinoid pharmacology
- Chemistry of synthetic cannabinoids
- Metabolism of synthetic cannabinoids
- Controlled drug administration studies
- Analytical methods for the identification of synthetic cannabinoids in biological & non-biological matrices
- Current legal status of synthetic cannabinoids

Cannabis Mechanisms of Action

- THC binds to cannabinoid receptors & modulates endogenous cannabinoid & other neurotransmitter systems
  - CB-1 receptors primarily in central nervous & cardiovascular systems
  - CB-2 receptors primarily in immune system
  - Non-CB1, non-CB2 receptors
  - G-protein receptors discovered & cloned in late 1980's
  - Endogenous cannabinoids include anandamide, 2-AG, virodhamine, N-arachidonyl dopamine (NADA), oleamide, 2-arachidonyl glyceryl ether (noladin ether) & others

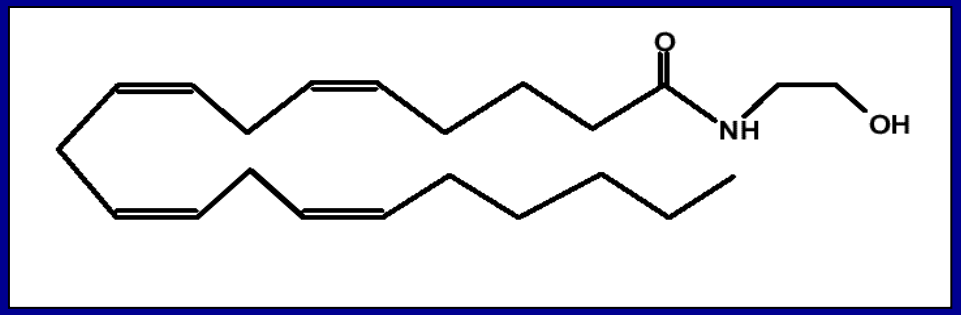
High CB1 Receptor Density



Cannabinoid Mechanisms of Action

- Receptor distribution in brain correlates with areas involved in physiological, psychomotor & cognitive effects
  - High density in caudate nucleus & cerebellum: motor behavior
  - Significant binding in striatum, cerebral cortex, & hippocampus: perception, cognition, memory, learning, endocrine function, food intake & regulation of body temperature
- Synthetic cannabinoids bind more avidly to receptors than THC; hijack endogenous cannabinoid system

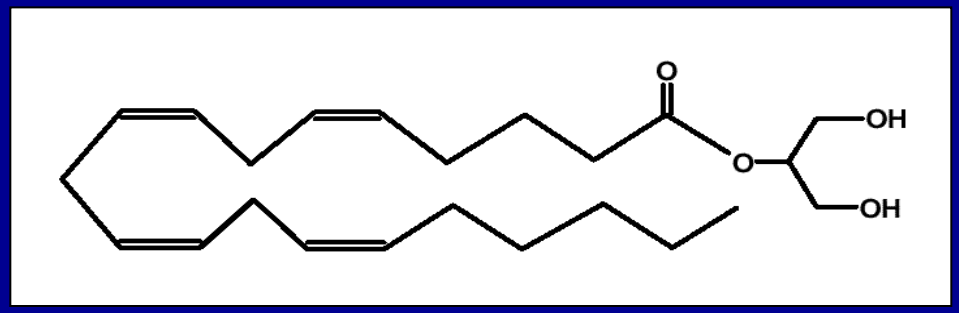
Endogenous Cannabinoids



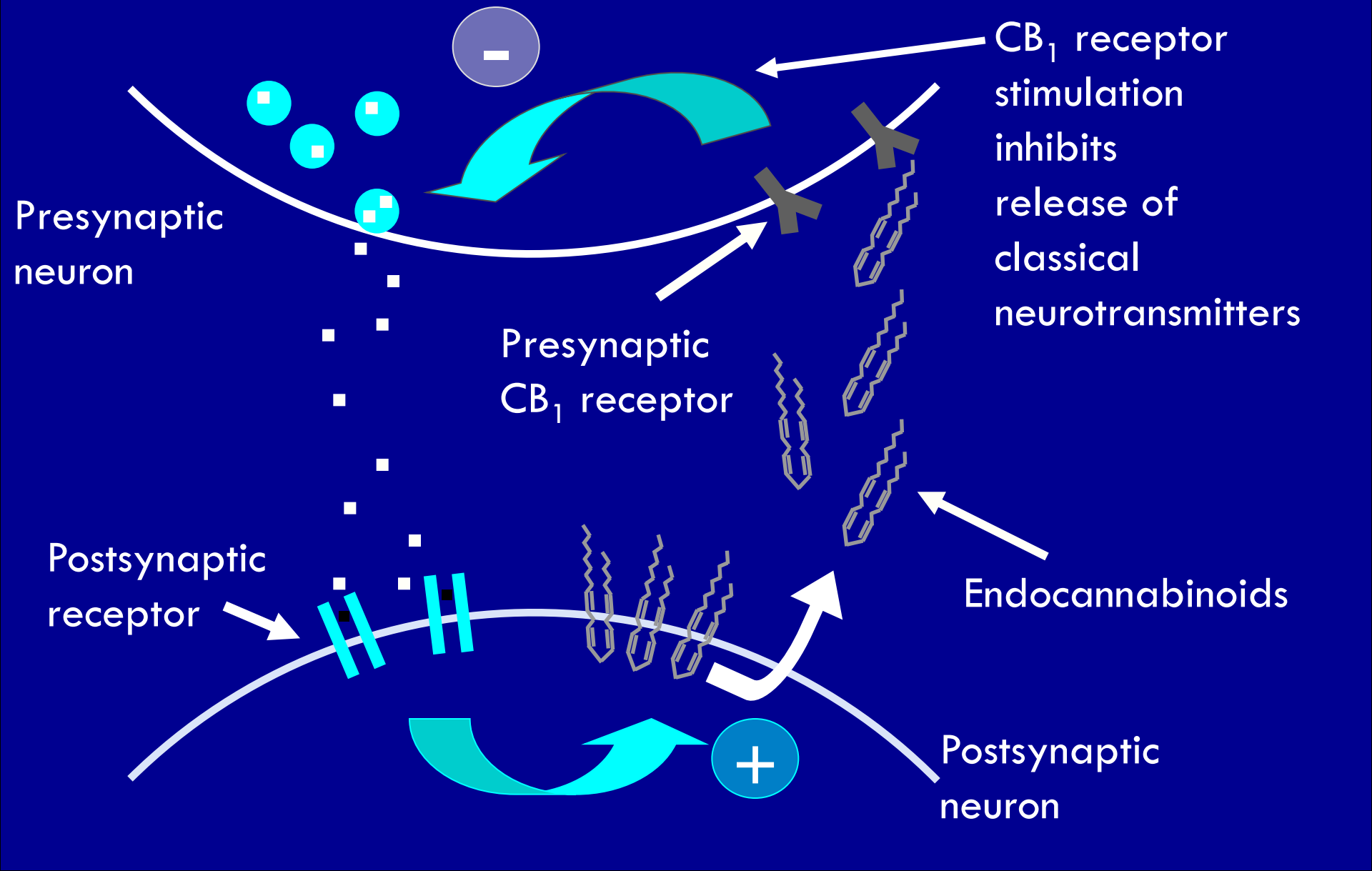
Anandamide (AEA)

- Different routes of synthesis
- Different modes of degradation (FAAH & MAGL)
- Different efficacy

2-Arachidonyl glycerol (2AG)



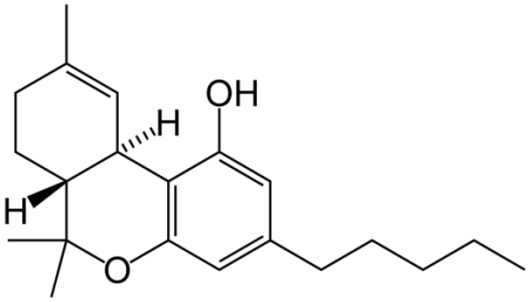
Endocannabinoid signaling



Synthetic Cannabinoids

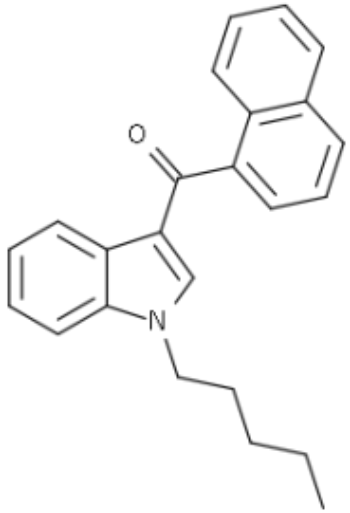
- Chemically diverse structures
- 1st synthesized as investigational drugs in 1980's
- Since 2000's designer drugs of abuse
- Sold as legal highs, incense, potpourri in head shops & on internet
- State on package "not for human consumption

Chemical Structures



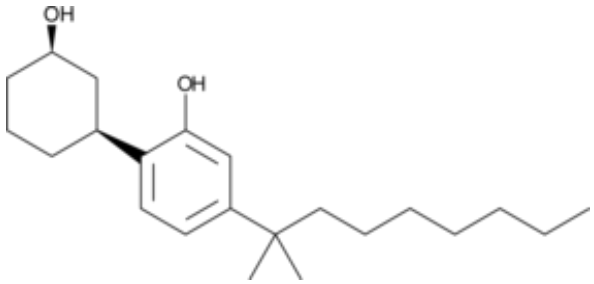
THC
Classical

HU-210/HU-211
JWH-133



JWH-018
Naphthoylindoles

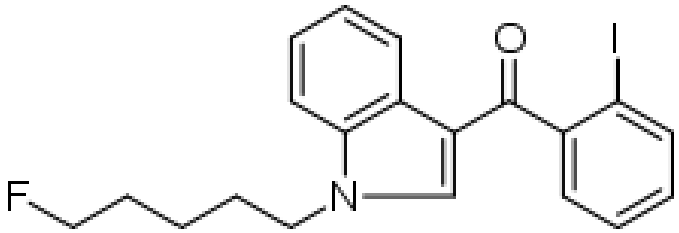
JWH-073
JWH-081
JWH-122
JWH-200
JWH-210
JWH-398
WIN55,212-2



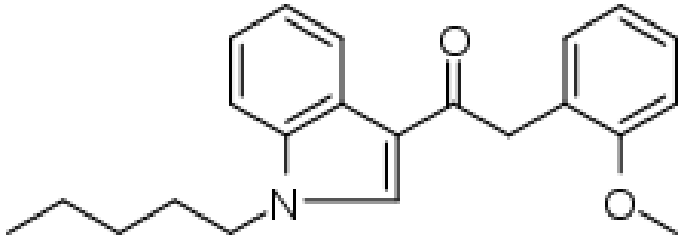
CP47,497(C8)
Non-Classical

CP47,497(C7)
CP55,940
HU-308

Chemical Structures



AM-694
Benzoylindoles



JWH-250
Phenylacetylindoles

AM-630
AM-1241

JWH-203
JWH-251

Reference Materials

- Manufacturers/Suppliers
  - Cayman Chemicals
  - Toronto Research Chemicals
  - Cerilliant
  - Sigma
  - BOC Sci
  - Tocris

K2 Phenomenon

- **Millard South Shooter Positive for Synthetic Marijuana**
- Omaha, NE - Tests discovered a controversial, yet legal drug, in Robert Butler Juniors' body the day he killed Doctor Vicki Kaspar, then himself.
- "The confusion, the delirium, people can hurt themselves, they can hurt others, they can hurt other people," says Doctor Ron Kirschner with the Nebraska Regional Poison Center.

K2 Phenomenon

- Woman Pleads Guilty In Hit, Run That Killed Passenger
- 1/12/2011 6:00 am
- … emergency workers took Watlington to Moses Cone Hospital where she died. Also, Prosecutors said that SBI Lab results revealed Neal had cocaine and … K2 or synthetic marijuana in her system

K2 Phenomenon

- ROCK SPRINGS, WY -- Rock Springs police reported several cases of teens apparently overdosing on synthetic marijuana
- Memorial Hospital of Sweetwater County says six people have been treated since Jan. 1 for symptoms believed to have been caused by ingesting or smoking fake pot
- Three people ages 15, 16 and 17 who went to the hospital Jan. 7 were charged with violating a city ordinance against inhaling toxic vapors. Three Rock Springs High School students, all age 15, were charged Jan. 11 with the same offense after at least one teen suffered adverse symptoms.

Current Analytical Scope

- AM-694
- AM-2201
- CB-25
- CB-52
- CP47,497 (C7)
- CP47,497 (C8)
- CP55,940
- HU-210
- HU-211
- HU-308
- HU-331
- JWH-015
- JWH-018
- JWH-019
- JWH-073
- JWH-081
- JWH-122
- JWH-133
- JWH-200
- JWH-203
- JWH-210
- JWH-250
- JWH-251
- JWH-398
- RCS-4
- RCS-8
- WIN 55,212-2
- WIN 55,212-3

Identification of Synthetic Cannabinoids in Solid Dose Materials

NMS Solid Material Preparation

- Qualitative Identification
  - ~ 30 mg botanical specimen
  - Can ID on residues in pipes
  - Acid/Base extraction
  - Multiple aliquots for different analyses

- Quantitative Analysis
  - Grind entire sample for homogeneous specimen
  - 15-30 mg botanical material in 1mL MeOH
  - 4 calibrators & 2 controls
  - 3 replicates + 1 sample with fortified standards

NMS Methods of Identification

- Presumptive Tests
  - Macro/Microscopic Analysis
  - Duquenois reagent –Levine
  - Thin Layer Chromatography (TLC)
  - High Performance Liquid Chromatography (HPLC)
    - Diode-Array Detection (DAD)

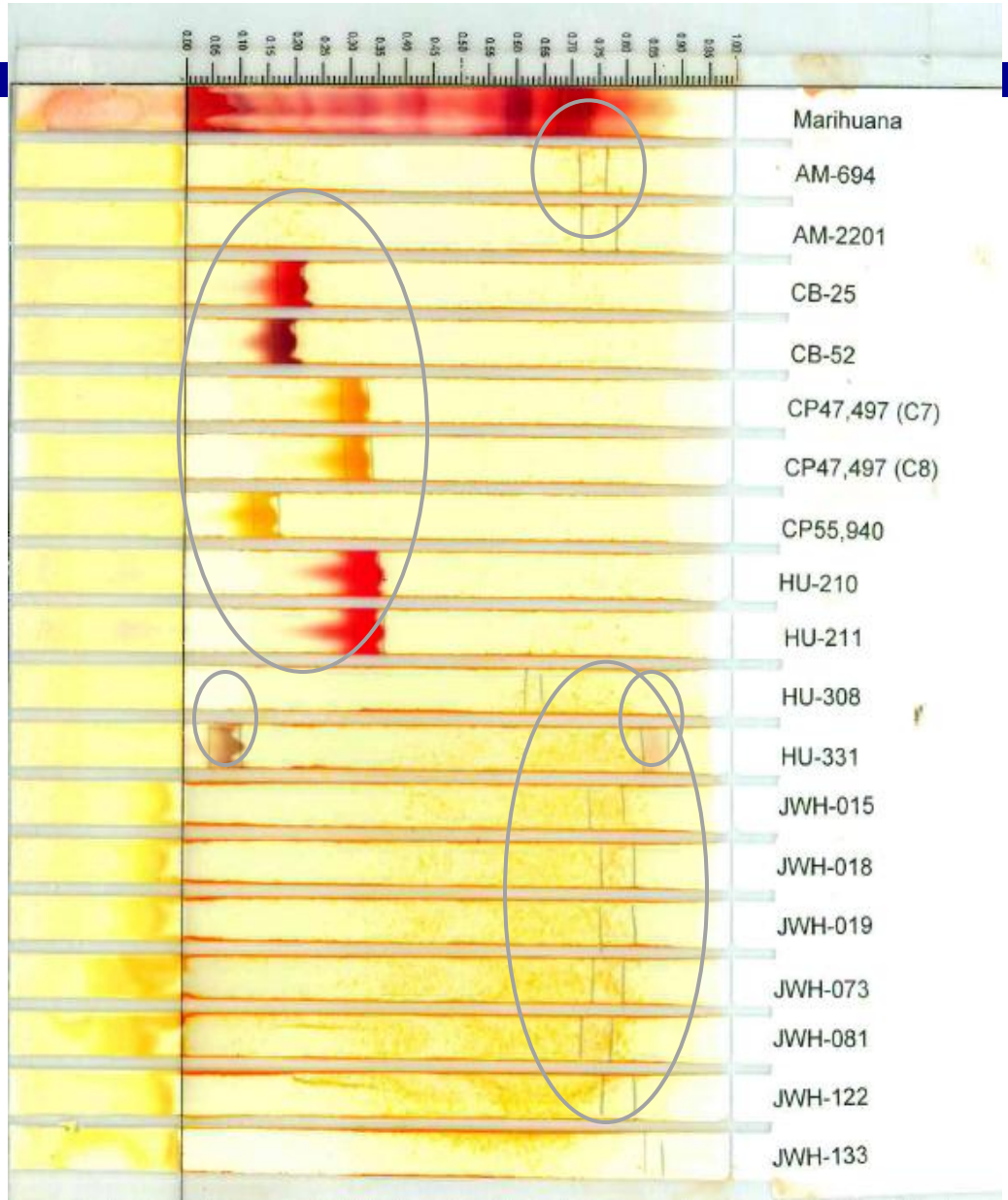
Macro/Microscopic Analysis

- Clearly different macroscopically from Cannabis
- No identifiable microscopic hairs as for Cannabis
- Variable matrix

Duquenois-Levine Color Test

Duquenois-Levine Color Test

NMS Thin Layer Chromatography



Solvent System:
9:1 Toluene:Diethylamine

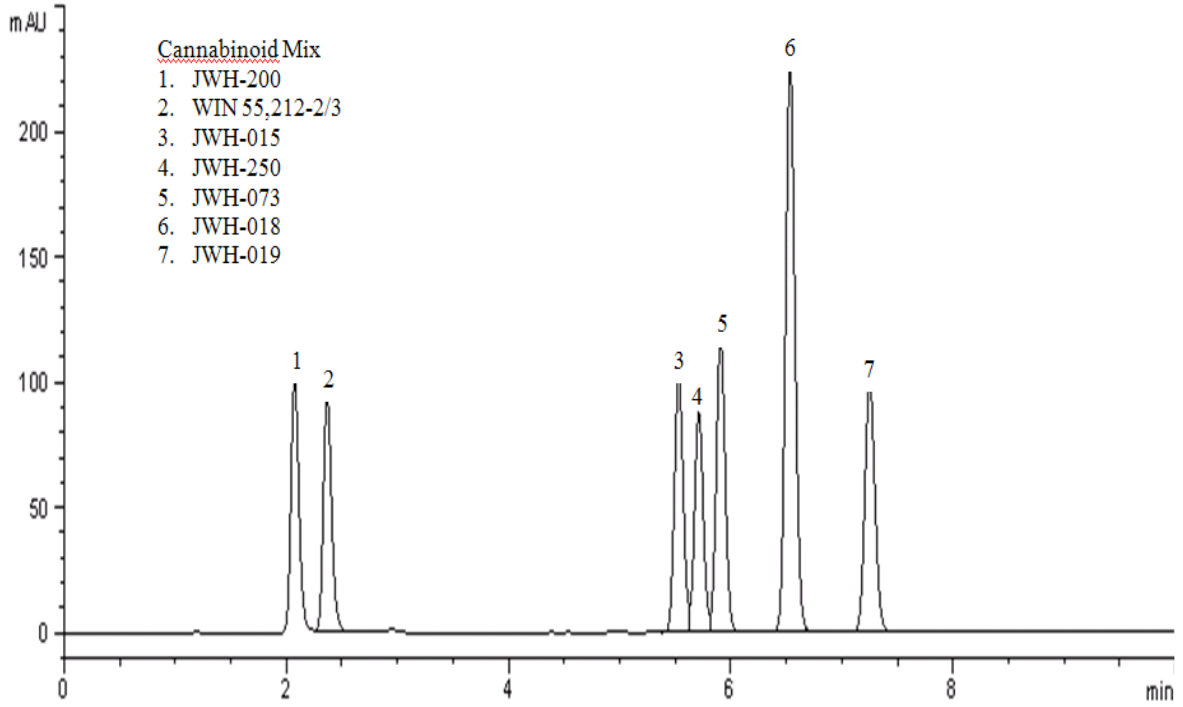
Visualization Spray:
1.5 g Dianiside Tetrazotized in 50:50 MeOH:DI Water
(Fast Blue B Spray)

Distinct red/orange bands for Cannabinoids, CP & most HU compounds

Obvious 254 nm UV absorbance for AM, JWH & WIN compounds

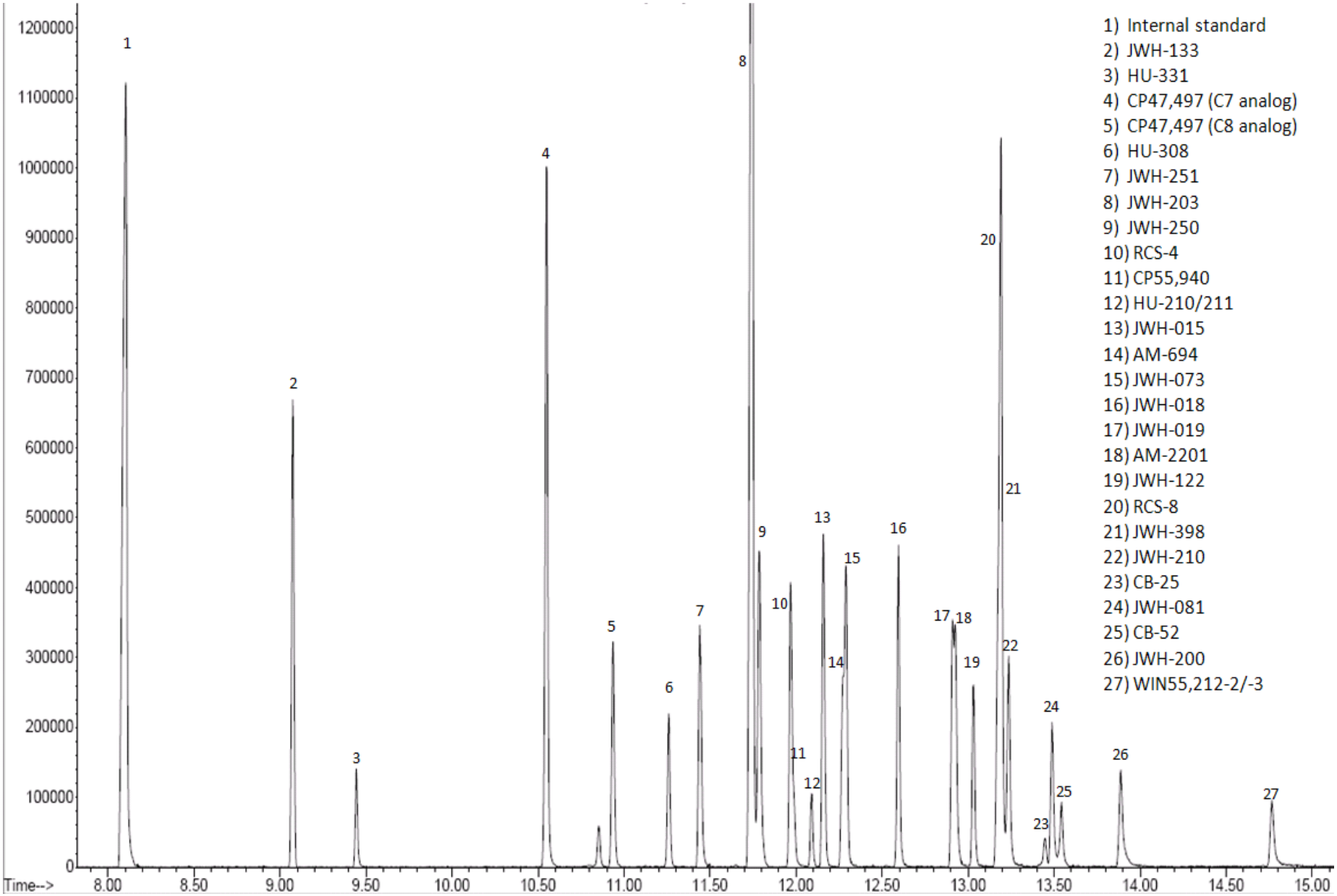
High Performance Liquid Chromatography Diode Array Detection

- HP 1100 Series HPLC with a UV DAD
- 5 µm Aquasil C18 4.6 x 100 mm Column
- 70:30 ACN:Water + 0.1% TFA
- 1.0 mL/min flow
- 40°C
- 316 nm



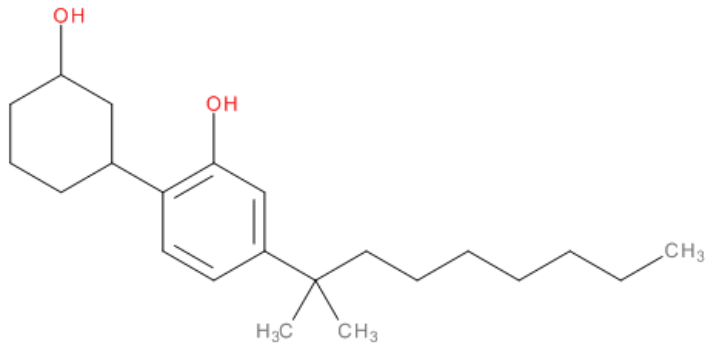
NMS Methods of Identification

- Confirmatory Tests
  - Gas chromatography/mass spectrometry (GCMS)
    - BSTFA derivatization
  - Liquid chromatography tandem mass spectrometry (LCMSMS)
  - Accurate-Mass Time-of-Flight Liquid Chromatography/ Mass Spectrometry (LC-TOF)

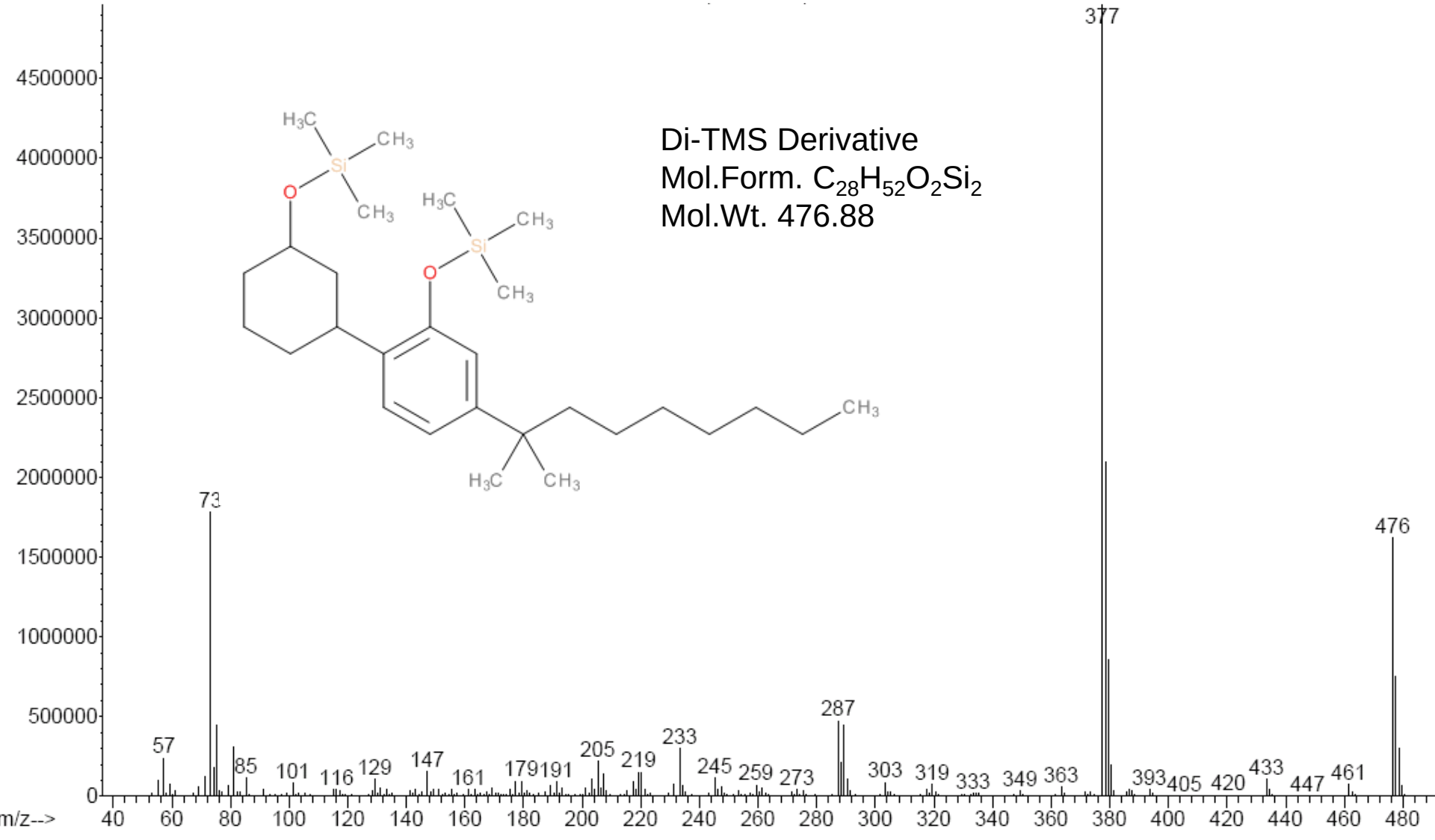

1) Internal standard
2) JWH-133
3) HU-331
4) CP47,497 (C7 analog)
5) CP47,497 (C8 analog)
6) HU-308
7) JWH-251
8) JWH-203
9) JWH-250
10) RCS-4
11) CP55,940
12) HU-210/211
13) JWH-015
14) AM-694
15) JWH-073
16) JWH-018
17) JWH-019
18) AM-2201
19) JWH-122
20) RCS-8
21) JWH-398
22) JWH-210
23) CB-25
24) JWH-081
25) CB-52
26) JWH-200
27) WIN55,212-2/-3
Time-->
8.00
8.50
9.00
9.50
10.00
10.50
11.00
11.50
12.00
12.50
13.00
13.50
14.00
14.50
15.00
0
100000
200000
300000
400000
500000
600000
700000
800000
900000
1000000
1100000
1200000

CP47,497 (Cannabicyclohexanol)

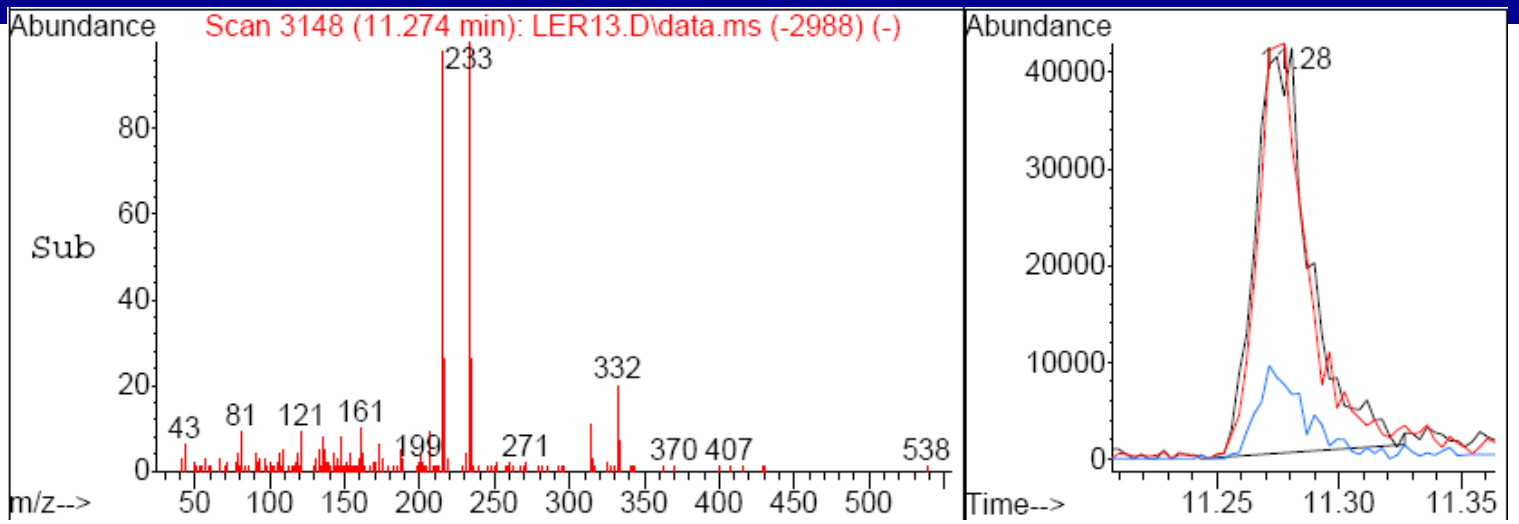
CAS# 70434-92-3
Formal Name: 5-(1,1-dimethyloctyl)-2-[(1R,3S)-3-hydroxycyclohexyl]-phenol
Mol.Form. $C_{22}H_{36}O_2$
Mol.Wt. 332.27153



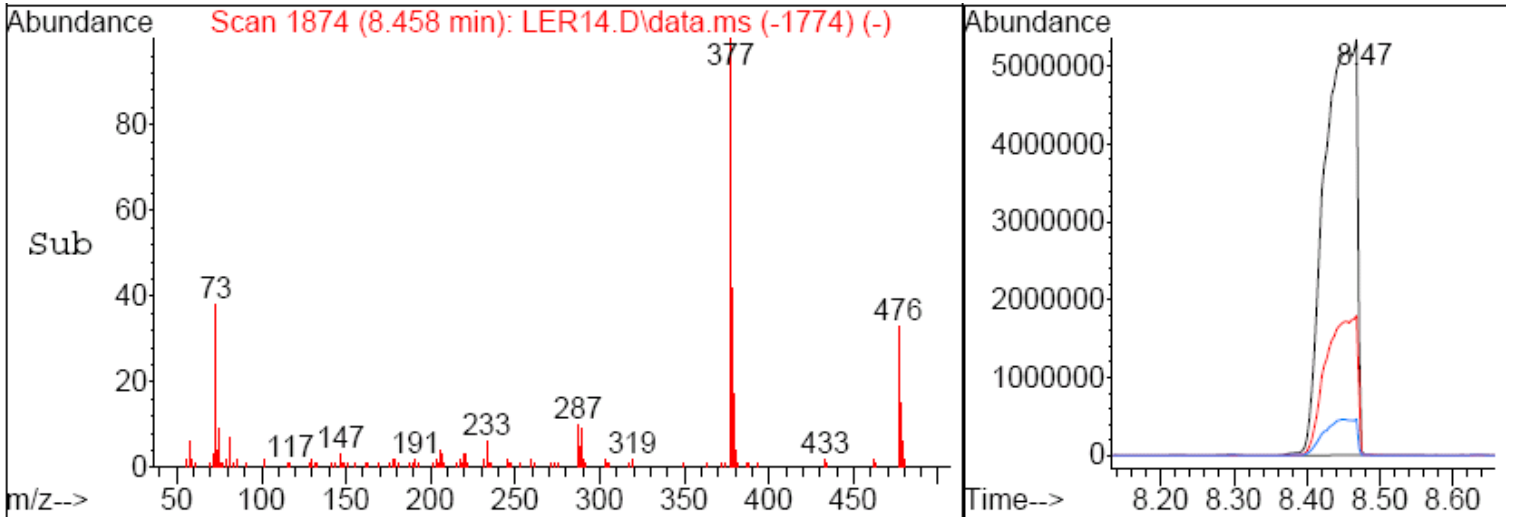
CP47,497 (Cannabicyclohexanol)



0.02 µg/mL CP47,497 (C8)



Underivatized



Derivatized

LCMSMS

Strong Ionization	Good Ionization	Weak Ionization
JWH-015	AM-2201	CP47,497(C7)
JWH-018	JWH-081	CP47,497(C8)
JWH-019	JWH-200	CP55,940
JWH-073	JWH-251	HU-210
JWH-122	JWH-398	HU-211
JWH-210		JWH-133
JWH-250		WIN55,212-2
RCS-4		WIN55,212-3
RCS-8		
~ 0.01 ng/µL	~ 0.1 ng/µL	~ 1 ng/µL

K2 Latte

Analytical Technique	JWH-018	JWH-073	JWH-250
TLC	Positive	Positive	Positive
GCMS	Positive (~16 mg/g)	Positive (~0.03 mg/g)	Positive (~14 mg/g)
LC-TOF	Positive	Positive	Positive

Solid Dosage Results Results

Controlled	Non-Controlled	Non-Cannabinoid
JWH-018	AM-694	5-MeO-DALT
JWH-073	AM-2201	Mitragynine
JWH-200	JWH-081	MDPV
CP47,497(C8 analog)	JWH-122	Methylone
	JWH-210	Mephedrone
	JWH-250	Butylone
	RCS-4	4-MEC
	RCS-8	

Challenges

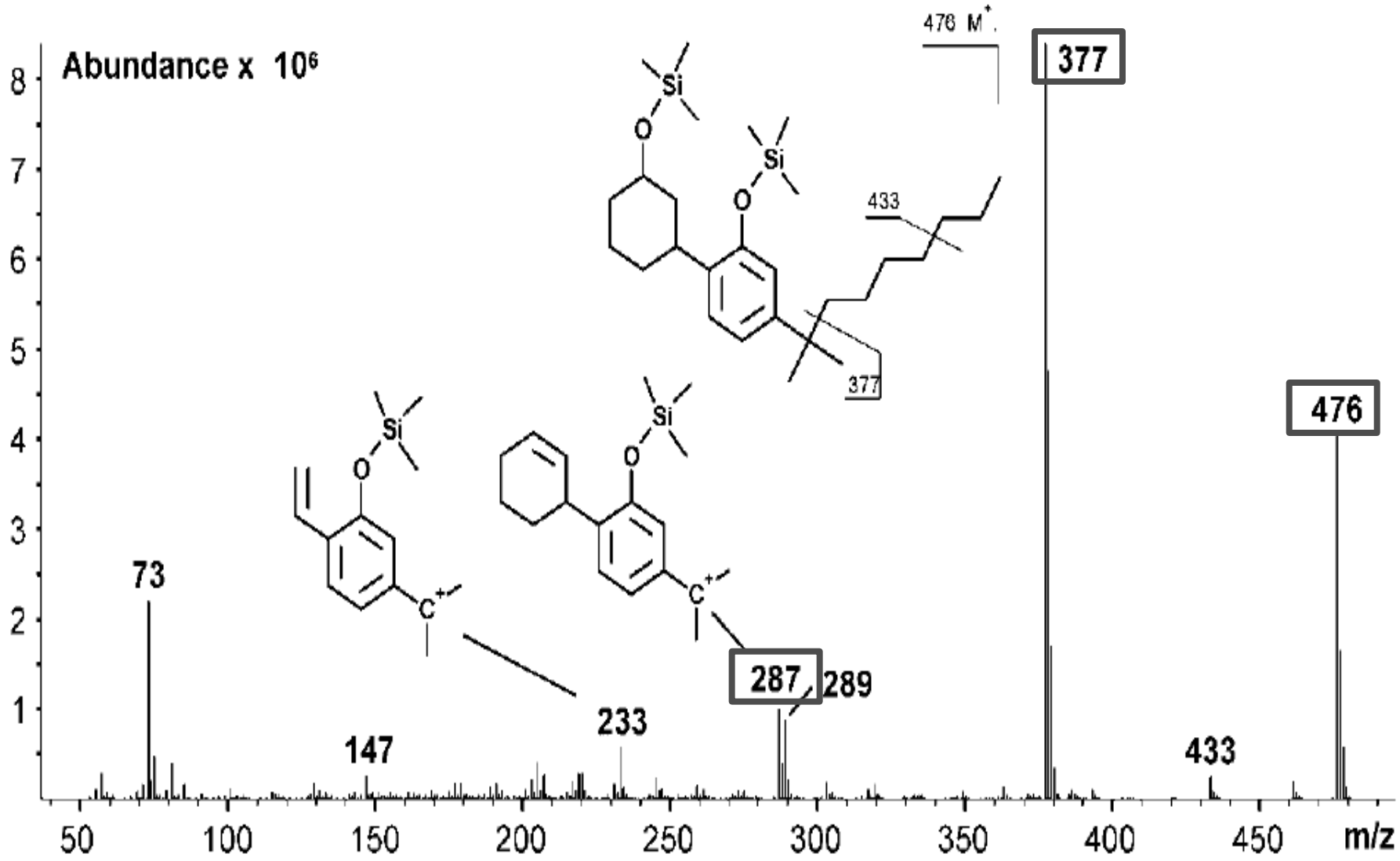
- Constantly changing market
  - Clandestine labs constantly developing new products
  - Reference materials not available quickly
  - Black market changes more quickly than testing/standards can be developed legitimately
  - Legal challenges for illicit drugs based on analog law
  - Different packages of same brand contain highly different drug concentrations

Identification of Synthetic Cannabinoids in Blood/Plasma/Serum

CP47497-C8 Auwärter et al. JMS Letter, 2009

- Spice Diamond drugs identified by GCMS, TLC, UV, NMR
- Authors smoked 0.3 g Spice Diamond to document pharmacological effects & collect specimens
- Physiological & psychological effects began by 10 min & peaked at 30 min after smoking
- Reddened conjunctivae, increased pulse, mood & perception alterations
- C18 SPE, TMS derivatization & GC-EI-MS

Auwärter et al. JMS Letters 2009



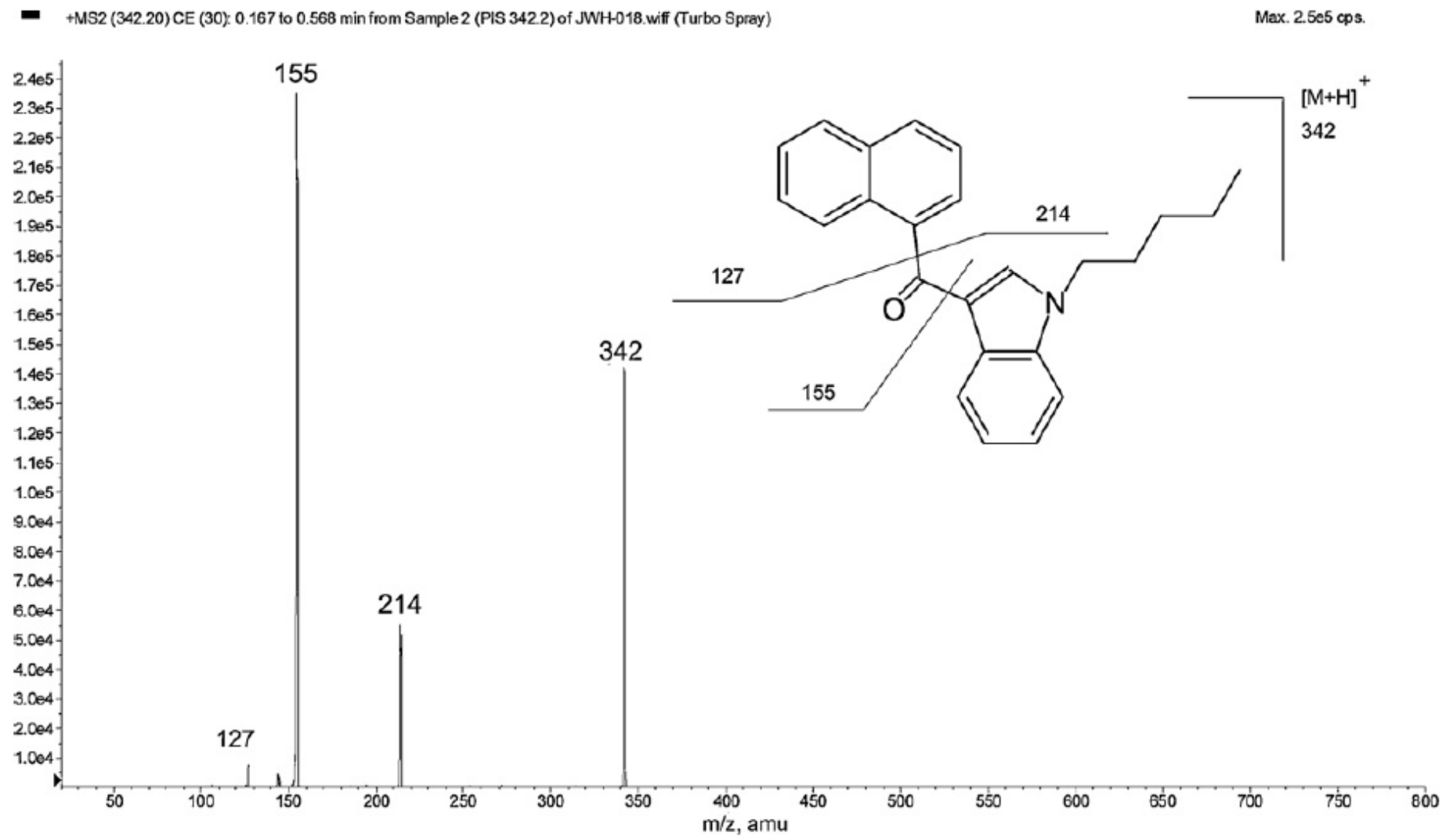
TMS derivative of CP47497

Teske et al., J Chromatogr B 2010

- 2 individuals smoked JWH-018 & reported tachycardia, little change in blood pressure, sickness, sedation, hot flushes, burning eyes & thought disruption, followed by tiredness & exhaustion attenuating 6-12 h after smoking
- LCMSMS JWH-018 serum quantification, d5-diazepam IS

Post-smoking time	Volunteer 1 Concentration [ng/ml]	Volunteer 2 Concentration [ng/ml]
5 min	8.1	10.2
15 min	4.6	6.1
1 h	1.7*	1.8
3 h	0.41	0.25
6 h	0.16**	0.13**
24 h	p.	p.
48 h	p.	n.p.

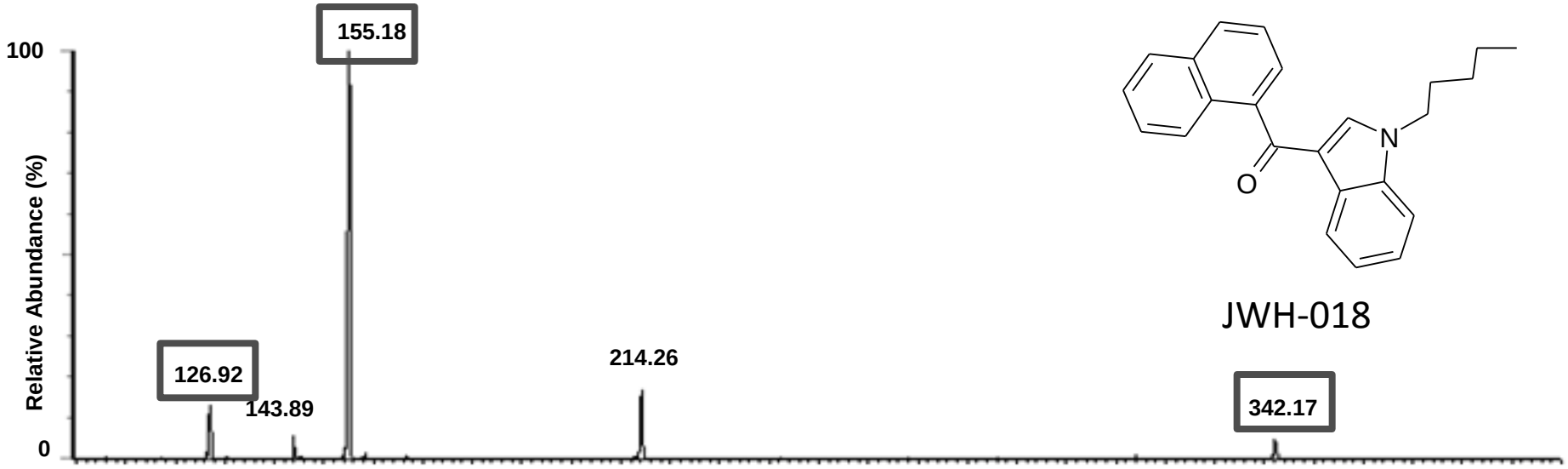
Teske Product Ion Spectrum JWH-018



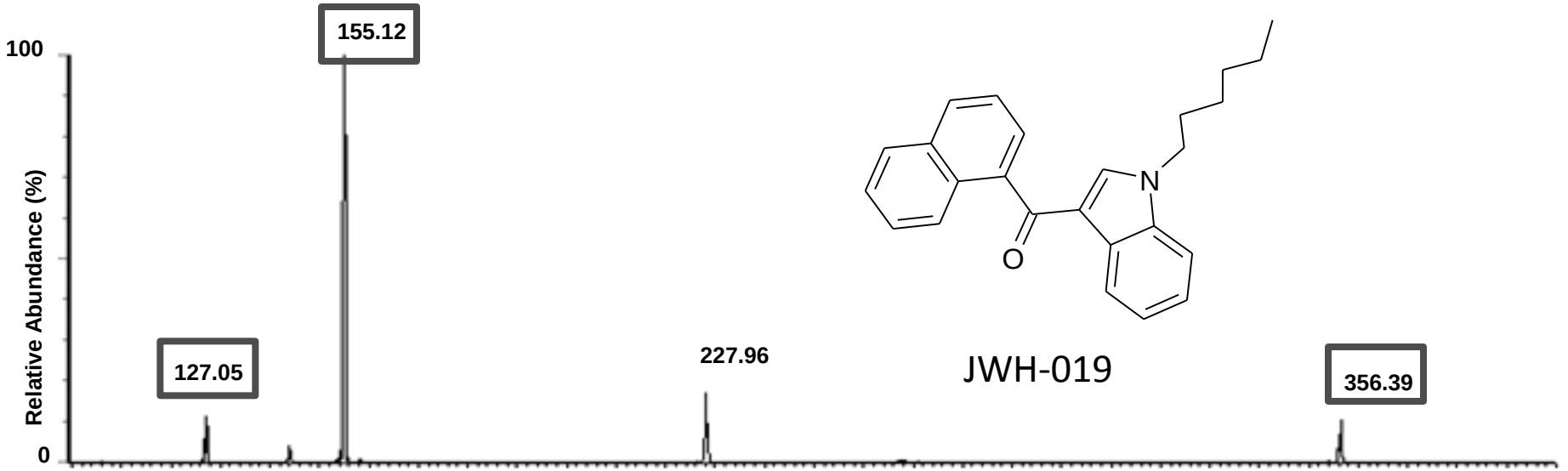
Kacinko et al. JWH-018, 019, 073 & 250 Quantification in Whole blood JAT

- Single step liquid-liquid extraction
- Internal standards: d9-JWH-018 & d9-JWH-073
- LC-positive ion ESI – MSMS
- 0.2 mL sample + saturated sodium bicarbonate + saturated NaCl
- 1% ethyl acetate in hexane, evaporate, reconstitute
- Acquity HHS T3 column, Waters Premier instrument
- Formic acid/methanol gradient
- Linear Range: 0.1 – 20 ng/mL

Kacinko et al. JWH-018, 019, 073 & 250 Quantification in Whole blood JAT

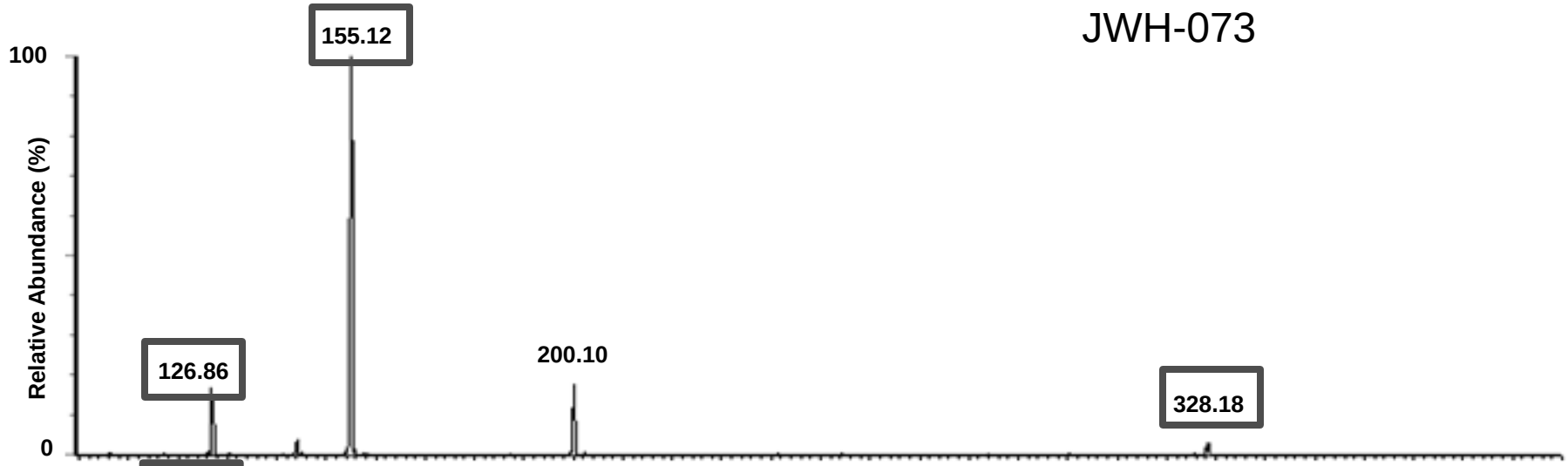


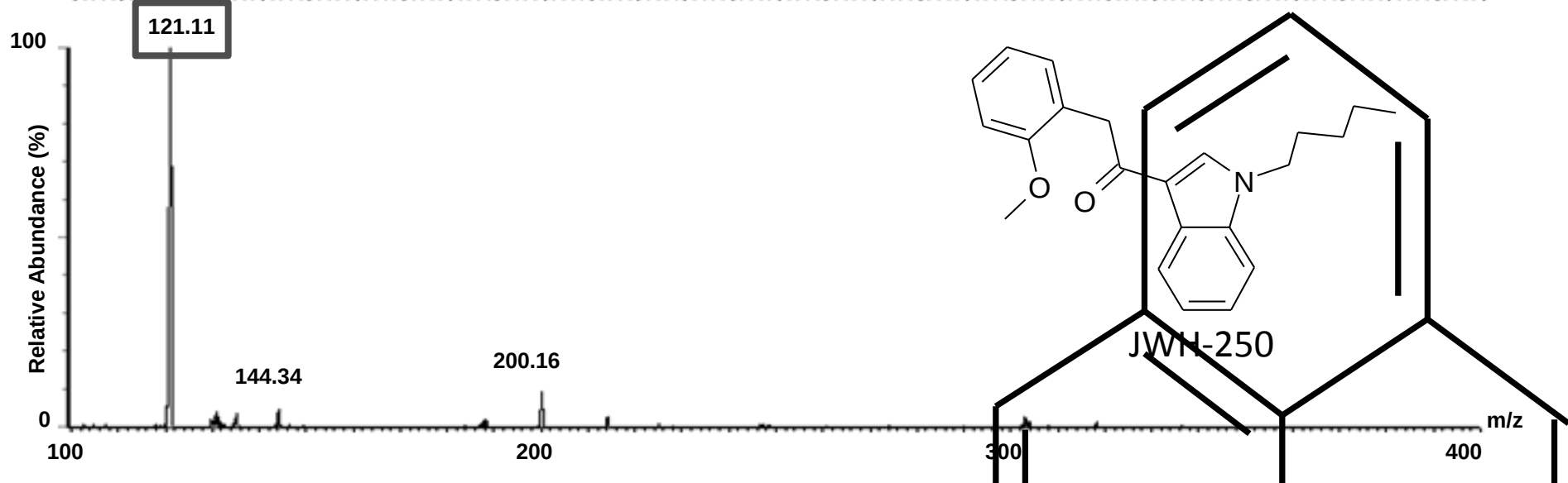
JWH-018



JWH-019

Kacinko et al. JWH-018, 019, 073 & 250 Quantification in Whole blood JAT





NMS Laboratory Whole Blood Concentrations

- JWH-018: 0.12 – 20 ng/mL
- JWH-073: 0.11 – 1.6 ng/mL
- JWH-250: 0.23 – 8.8

Synthetic Cannabinoids: A Controlled Administration Study University of Central Missouri

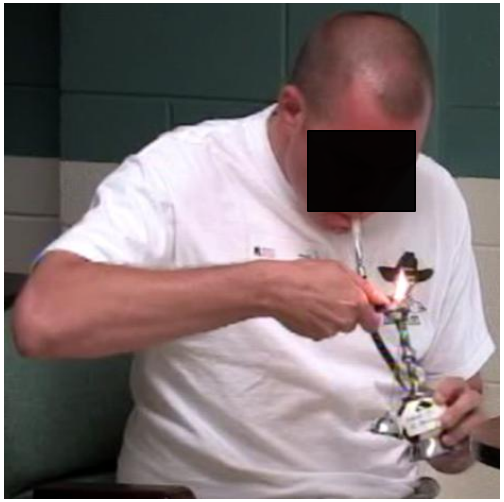
Barry K Logan, PhD
National Medical Services Laboratory

K2 Synthetic Cannabinoid Concentrations

	K2 Standard	K2 Citron	K2 Summit	Herbal Blend
JWH-018 (mg/g)	9	10	11	-
JWH-073 (mg/g)	9	10	9	-
CP47,497 (C7) (mg/g)	-	-	-	6

University of Central Missouri
K2 Controlled Administration Study

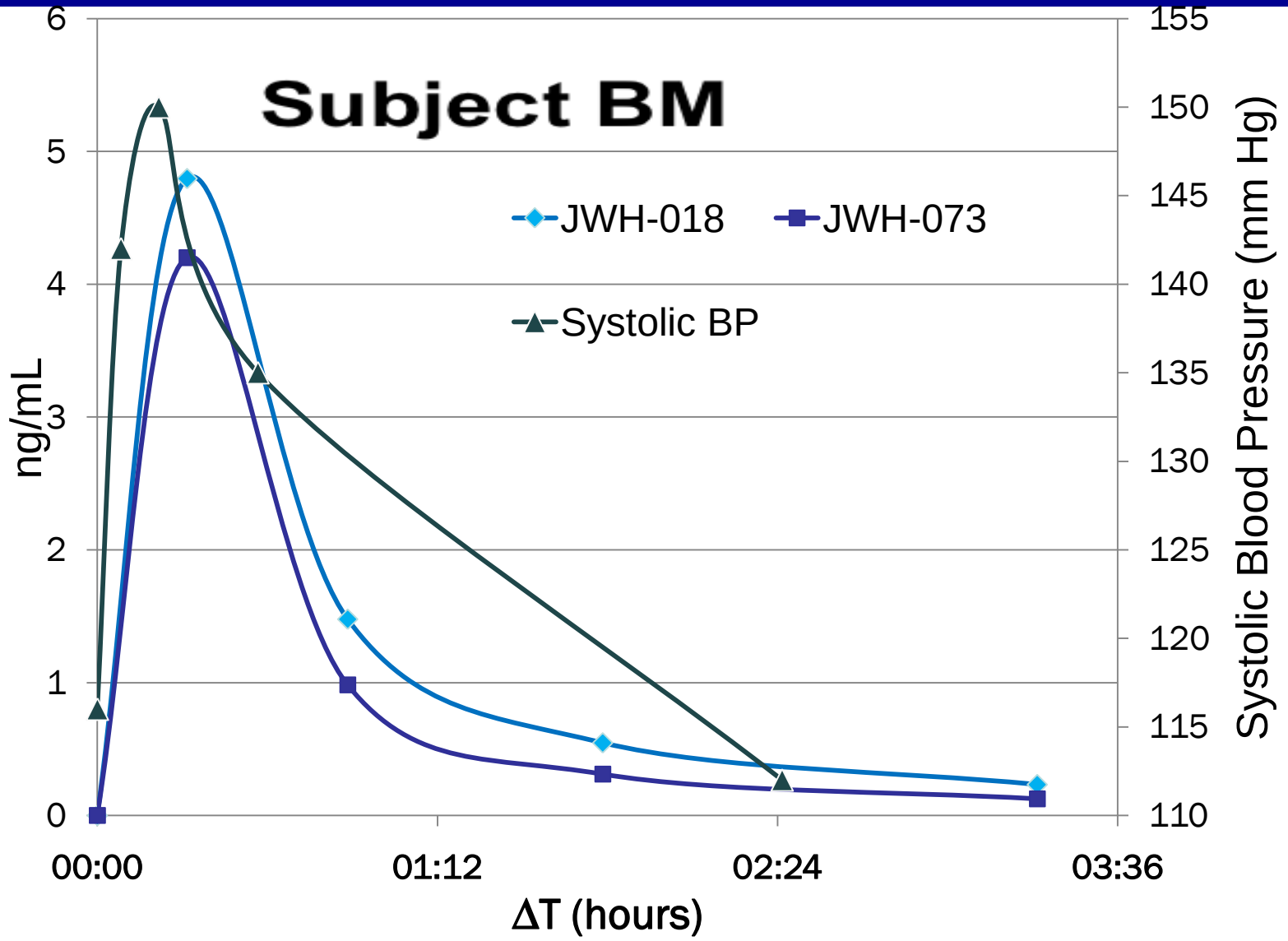
- 2010 DRE training: 6 subjects smoked K2 products containing JWH-018, JWH-073 &/or CP-47,497
- NMS blood, oral fluid & urine
- IRB approved protocol
  - 0.3 g, 1-3 puffs per subject in 30 min
  - Medical staff on-site
  - 8 h specimen collection
  - Field sobriety tests, DRE & eye exams, cognitive tests
  - Subjective effects onset ~2-3 min, peak 5-10 min



University of Central Missouri K2 Study

- Psychomotor effects highly variable
- DRE Exam & SFST's
- Increased pulse & blood pressure
- Lack of convergence, no HGN, or VGN
- Pupils normal, muscle tone normal
- 3-4 inches of sway, leg body tremors
- Loss of balance & motor coordination

University of Central Missouri
K2 Controlled Administration Study



Authors	Sobolevsky	Teske	Auwater	Elsohly	UCMO					
					1	2	3	4	5	6
Parameter	**JWH-018 Active intoxication 3 subjects**	**2 subjects smoked Spice containing JWH-018**	**2 subjects smoked Spice JWH-018 & Cannabi-cyclohexanol**	**THC**	**K2 Std**	**K2 Citron**	**K2 Std**	**K2 Summit**	**K2 Citron**	**K2 Summit**
Red eyes	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Burning eyes	-	Yes	-	-	-	Yes	-	-	-	-
Dry mouth	-	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes
Increased pupils	-	Yes	-	Yes	-	-	-	-	-	-
Tachycardia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Anxiety	Yes	-	-	Yes	Yes	-	Yes	-	Yes	Yes
Hallucination	Yes	-	-	Yes	-	-	-	-	-	-
Paranoia	Yes	-	-	Yes	-	-	-	-	Yes	Yes

Authors	Sobolevsky	Teske	Auwater	Elsohly	UCMO					
					1	2	3	4	5	6
Parameter	JWH-018 Active intoxication 3 subjects	2 subjects smoked Spice containing JWH-018	2 subjects smoked Spice JWH-018 & Cannabi-cyclohexanol	THC	K2 Std	K2 Citron	K2 Std	K2 Summit	K2 Citron	K2 Summit
Sickness	-	Yes	-	-	-	-	-	-	-	-
Sedation	-	Yes	-	Yes	Yes	-	Yes	-	-	Yes
Changes in perception/mood	-	-	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes
Loss of concentration	-	Yes	-	Yes	Yes	Yes	-	-	Yes	Yes
Impaired sense of time	Yes	-	-	Yes	Yes	Yes	Yes	-	-	Yes
Exhaustion	-	6-12 hours	6-24 hours	-	Yes	Yes	Yes	-	-	-
Self assessed impairment	-	-	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes

Research Findings

- Subjects conservatively dosed with commercially available K2 products
- Effects qualitatively similar to cannabis, additional anxiety/paranoia
- Subjects reported hangover effect
- No adverse events reported
- Blood synthetic cannabinoid <1ng/mL within 2 h

NMS Controlled JWH-018 & JWH-073 Administration Study: Human Blood

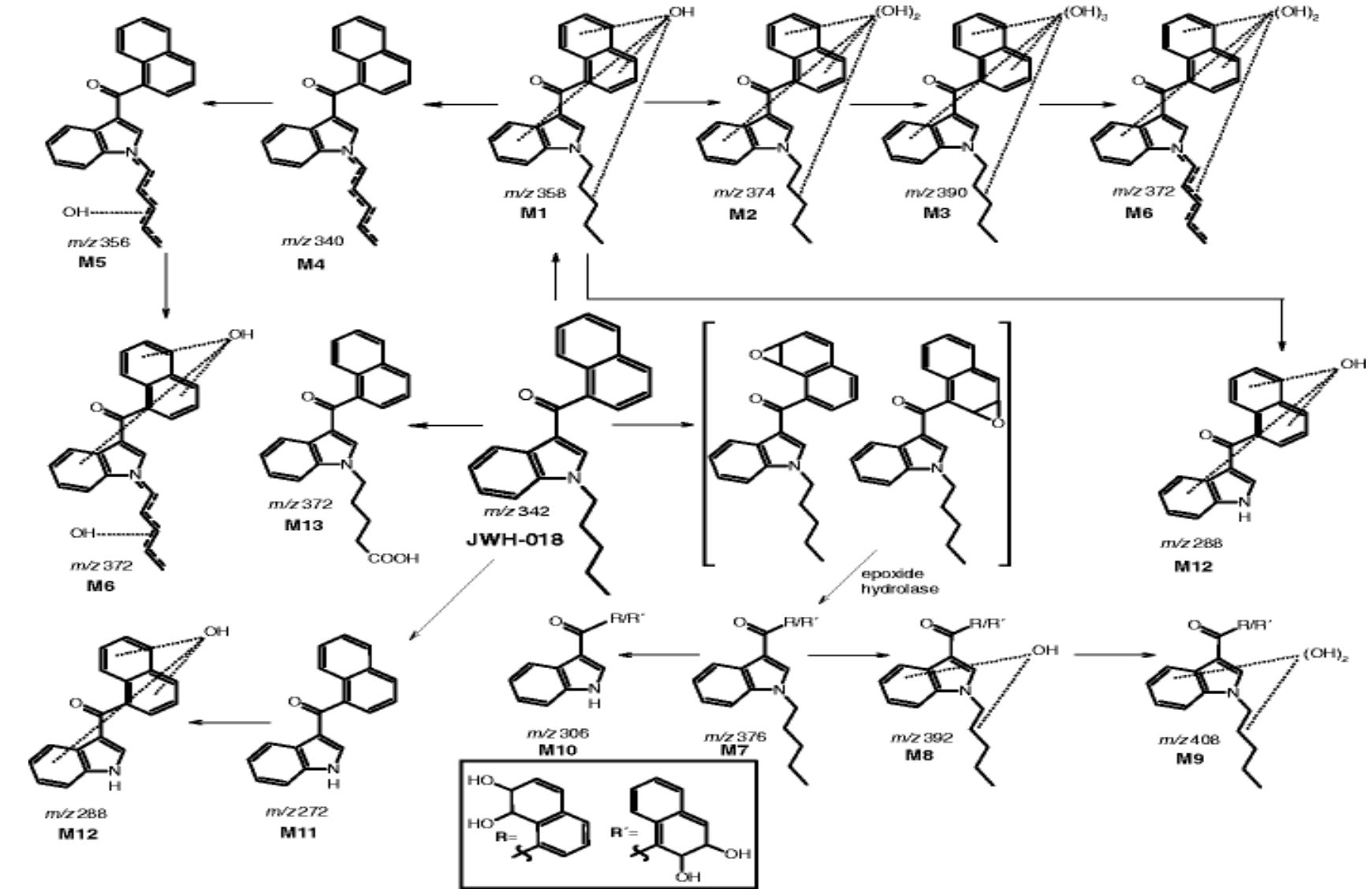
- LCMSMS
  - Parent
  - Mono-hydroxy
  - Di-hydroxy
  - Tri-hydroxy
  - Mono-hydroxy desalkyl
  - Di-hydroxy desalkyl
  - Carboxylic acid

Determining Appropriate Urinary Analytes from Rat & Human Liver Microsome Studies

Zhang, et al. Rat Liver Microsomes

- WIN 55212-2 Drug Metab & Disp 2002
  - LCMS, LCMSMS & NMR to elucidate metabolite structures
  - Similarities in major metabolic pathway -Dihydroxylation via epoxide intermediary
- AM-694 J Mass Spec 2004
- JWH-015 Analytical Bioanalytical Chem 2006

Wintermeyer et al Anal Bioanal Chemistry, 2010
Incubated JWH-018 & Human Liver Microsomes



NMS In Vivo Rat Studies

- Intraperitoneal injection (10 mg/kg)
- Blood:
  - 1 h parent only
  - 3 h mono-hydroxy desalkyl metabolites (prominent)
  - Other metabolites mono-, di-, & tri-hydroxy metabolites; carboxy-, reduced di-hydroxy
- 5 h urine contained mono-hydroxy-desalkyl prominent, tri-hydroxy, mono-hydroxy, dihydroxy, & carboxy trace

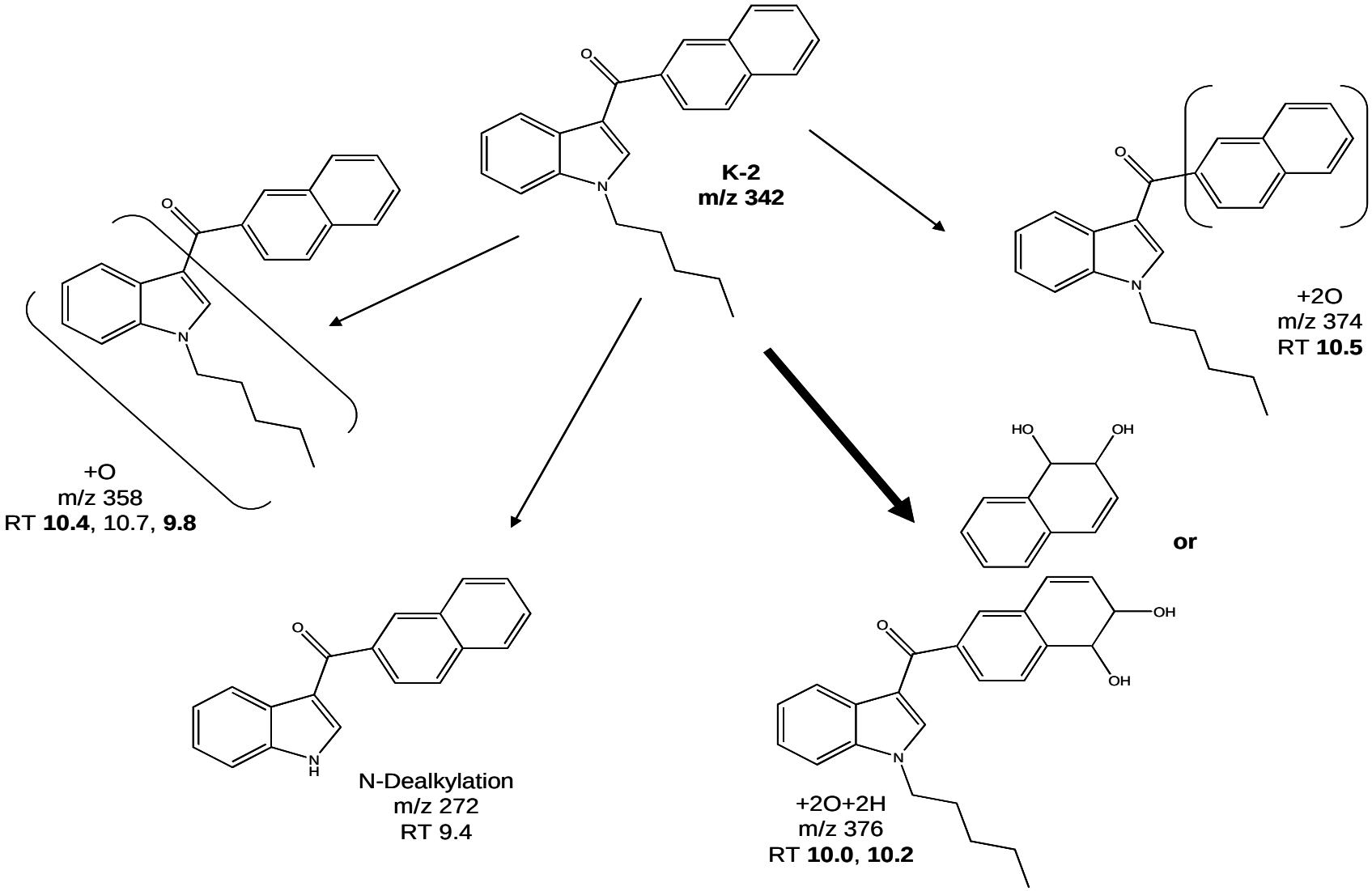
Missouri K2 Controlled Administration Study

Subject BM - Urine

Time	JWH-018	*Mono-OH*	*Di-OH*	*Tri-OH*	*Glucuronides*
Pre-dose	-	-	-	-	-
1:15	-	+	+	-	+
2:07	-	+	+	+	+
2:40	-	+	+	+	+

Time	JWH-073	Mono-OH	Di-OH	Tri-OH	Glucuronides
Pre-dose	-	-	-	-	-
1:15	-	+	+	-	+
2:07	-	+	+	+	+
2:40	-	+	+	+	+

NMS Human Liver Microsome Studies



Determination & Quantification of Urinary Synthetic Cannabinoid Metabolites

- Identify metabolites without benefit of standards & deuterated internal standards
- Only a few unauthorized self-administration studies
- Few case reports from police & poison control
- In vivo rat, & in vitro rat & human microsome studies
- One approved controlled administration study
- New synthetic cannabinoid drugs constantly appearing on the market

Redwood Toxicology Urinary Synthetic Cannabinoid Metabolites: Suman Rana

- What is the most common metabolic pathway for elimination: hydroxylation, carboxylation or de-alkylation/Hydroxylation?
- Hydroxylation can occur at multiple sites - which one is the most common?
- Is parent drug eliminated in urine?

Redwood Toxicology Urinary Synthetic Cannabinoid Metabolites

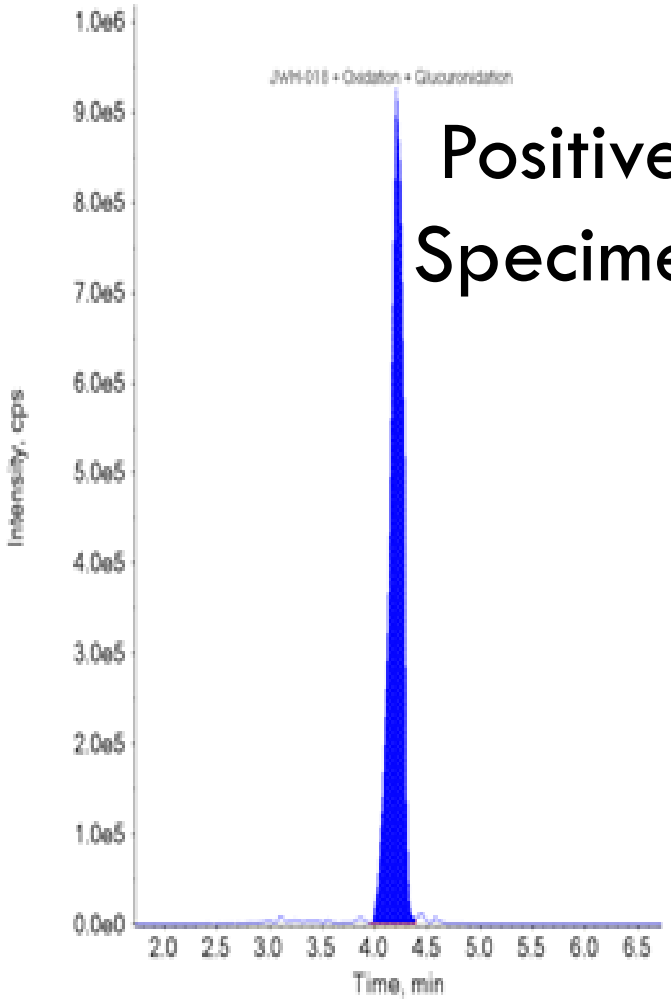
- Urine specimens collected from:
  - 1 individual at 12, 24 & 48 hr after smoking spice
  - 2 individuals who admitted smoking 24 h
  - 13 urine specimens over 4 days collected from 1 individual after oral 5 mg JWH-018 dose
  - Window of detection evaluated in all 3 cases
  - 7077 unknown specimens tested for JWH-018 & 073
  - 2335 positives – 33% positivity
  - 200 positives selected for further study

Redwood Method – HPLC &MS Parameters

- Mobile phase:
  - A: Water + 0.1% Formic acid and 0.2% ammonium formate
  - B: ACN+ 0.1%formic acid and 0.2% ammonium formate
  - Column: Pinnacle DB Biphenyl; 5u 100x2.1mm; flow rate: 0.5mL/min
- 4000 QTRAP instrument
  - MRM-IDA-EPI scans for targeted ID
- Sample Prep: Dilute and shoot

ANALYTE	MRM TRANSITION	CHARACTERISTIC IONS	RETENTION TIME
THCCOOH D3 (IS)	348/302	348, 330, 302, 196, 187	5.12
JWH-018 (Parent)	342/155	342, 214, 155, 127	6.98
JWH-018 + Oxidation + Glucuronidation	534/358	534, 358, 155, 127	4.15
JWH-018 +Oxidation 1	358/155	358, 155, 145, 127, 117	4.94
JWH-018 + Oxidation 2	358/127	358, 155, 145, 127, 117	5.33
JWH-018 + Carboxylation 1	372/155	372, 356, 155, 145, 127	4.82
JWH-018 + Carboxylation 2	372/127	372, 356, 155, 145, 127	4.86
JWH-018 + Carboxylation + Glucuronidation	548/372	548, 372, 155, 127	4.22
JWH + N-dealkyl + Oxidation + Hydrogenation	288/155	288, 155, 145, 127	4.18
JWH-073 (Parent)	328/155	328, 200, 155, 127	5.81
JWH-073 + Oxidation 1	344/155	344, 216, 155, 145, 127, 117	4.64
JWH-073 + Oxidation 2	344/127	344, 216, 155, 145, 127, 117	5.16
JWH-073 + Oxidation + Glucuronidation	520/344	520, 344, 216, 155, 127	3.93

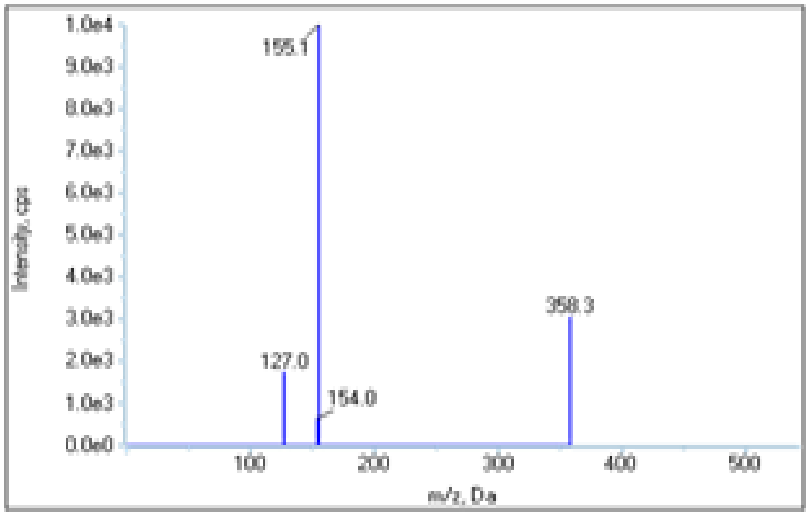
Retention Time: 4.21 minutes Q1/Q3: 534.100/358.000 Da Fit (%) 56.5 RFit (%) 89.2	Exp RT: 4.23 minutes Analyte Peak Name (MRM Transition): JWH-018 + Oxidation + Glucuronidation	Date: 10/7/2010 5:22:26 PM



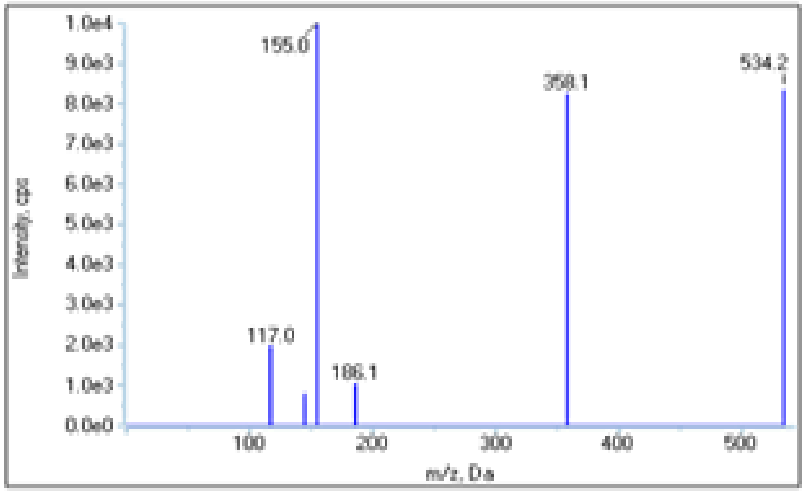
Positive Specimen

Collision Energy = 35 ± 15 eV

Acquired Spectrum



Library Spectrum



	Compound Name	Peak Area	Purity(%)	Visual Check?
1	JWH-018 + Oxidation + Glucuronidation	7.92e+006	50.4	

Negative Specimen

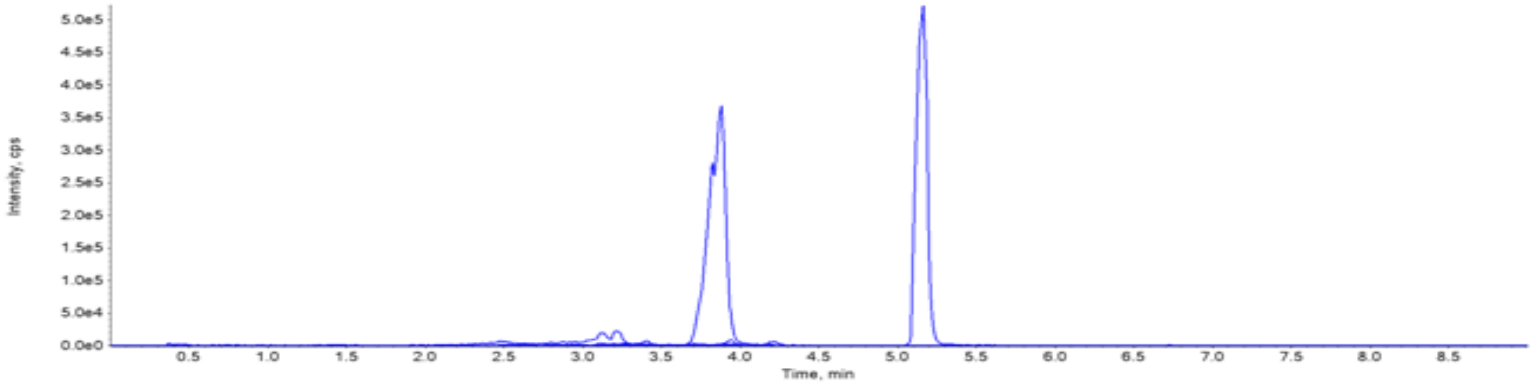
REDWOOD TOXICOLOGY LABORATORY

LCMSMS #11 Report
Printed: 08/10/2010 10:46:57 AM

10/6 Q145

Data		Acquisition	
Data File	SC100710.wiff	Method	K2.dam
Date and Time	10/7/2010 6:20:06 PM	Instrument	4000 Q TRAP AR25591006
Project	K2_Spice\10-07-10	Operator	\lcmslab@redwoodtoxicology.com
Sample		**Results**	
Sample Type	Unknown	Result Table	SC100710A.rdb
Weight to Volume	0.00	**Library Search**	
Dilution Factor	1.00	Library	Redwood Comp Panel.mdb
Vial	10	Mass Tolerance	0.50
Injection Volume	20 µL	Purity Threshold	10%

Extracted Ion Chromatogram



Summary

RT (min)	Exp RT (min)	Compound Name	Peak Area	Purity (%)
5.16	5.15	THC-COOH-D3 (IS)	2.56e+006	86.0

Results for Known Urine Specimens

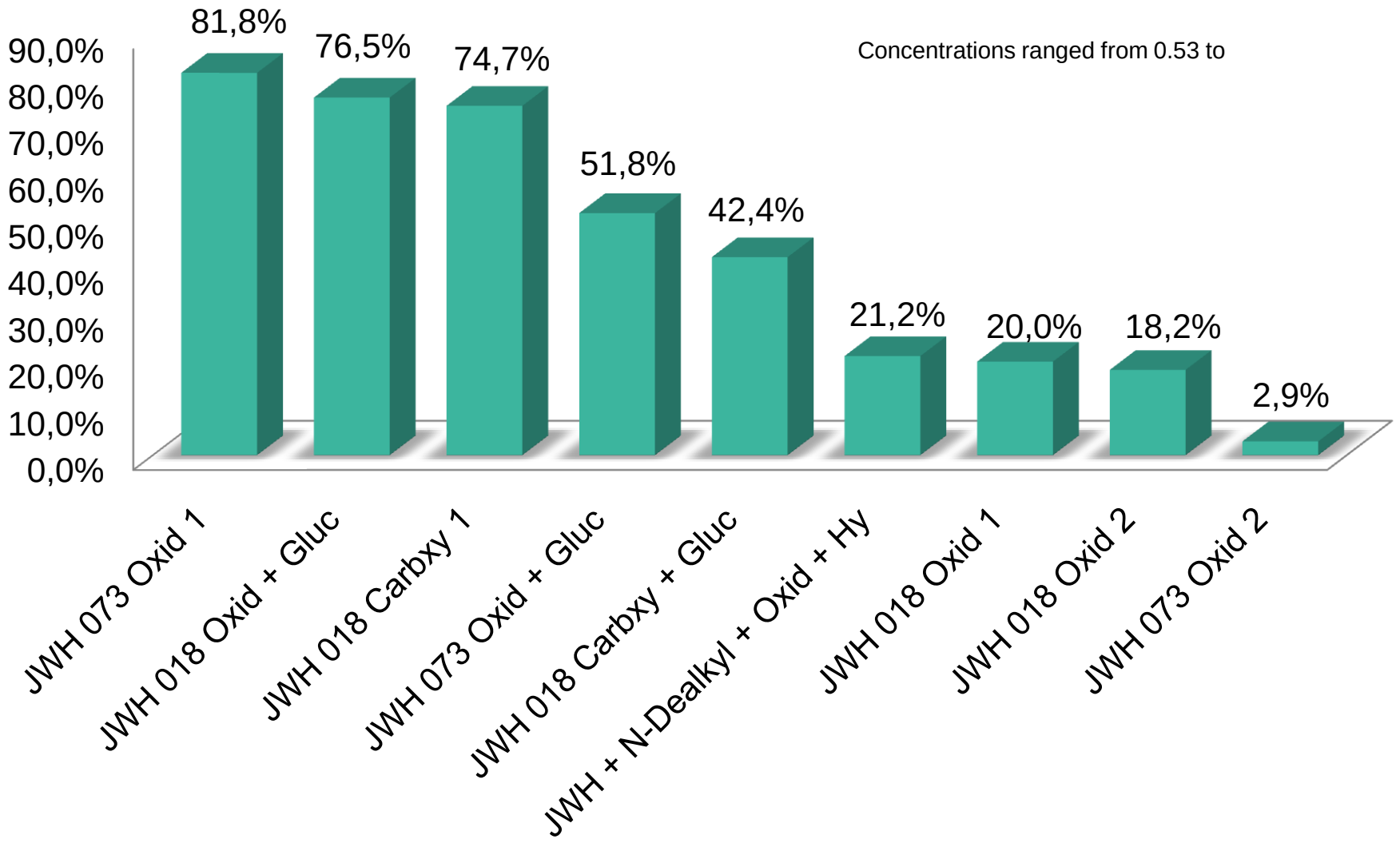
- Case 1: specimens 12, 24 & 48 h after smoking K2
  - No parent drug
  - JWH-018 & JWH-073 hydroxy metabolites in 48 h urine
  - JWH-018 carboxy metabolite in 48 h urine
- Cases 2 & 3: specimens 24 h after smoking K2
  - No parent drug
  - JWH-018 & JWH-073 hydroxy metabolites in 24 h urine
  - JWH-018 carboxy metabolite in 24 h urine

Results for Known Urine Specimens

- Cases 4-16: 13 specimens after 5mg oral JWH-018
  - No parent drug detected
  - JWH-018 & JWH-073 hydroxyl metabolites up to 72 h
  - JWH-018 carboxy metabolites up to 72 h
- Material ingested was only JWH-018; no JWH-073
- Hydroxy JWH-073 major metabolite detected after ingesting JWH-018 due to demethylation
- Presence of hydroxy JWH-073 is not indicative of JWH-073 ingestion

Metabolite Frequency in Unknown Urine Specimens

33% of 7077 specimens positive



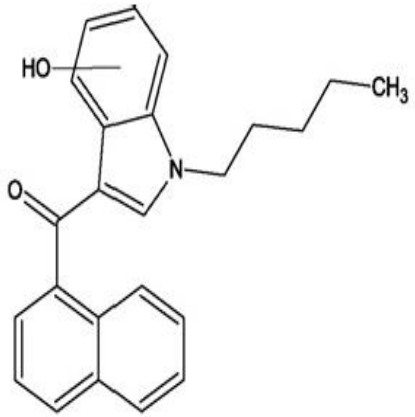
Conclusions

- Parent compounds rare in urine (0.17%), detection relies on monitoring free & glucuronidated alkyl-hydroxylated & alkyl- carboxylated metabolites
- JWH 018 N-(5- hydroxypentyl) metabolite most frequently observed, next JWH 018 N-pentatonic acid metabolite
- Hydroxy JWH073 metabolite not conclusive indication of JWH-073 ingestion
- Detection windows JWH-018 & 073 metabolites in urine ~72 h for occasional use

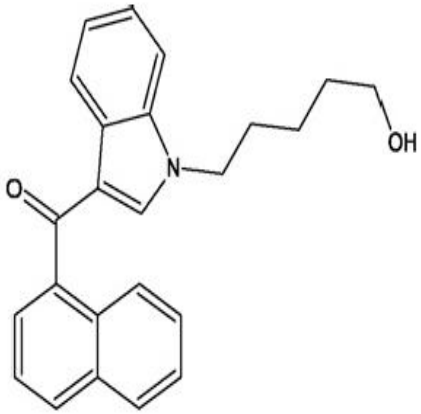
Sobolevsky et al FSI 2010

- Detection of JWH-018 metabolites in urine after smoking
- Urine samples collected from 3 intoxicated individuals seized by police
- Hydrolyzed urine analyzed by GCMSMS & LCMSMS
- Similar behavioral effects to cannabis
- Reddening of eyes, tachycardia, anxiety, paranoia & hallucinations
- Short-term memory defects & impaired sense of time

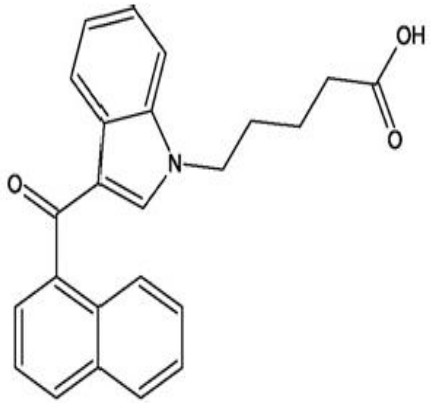
13 Urinary Metabolites of JWH-018



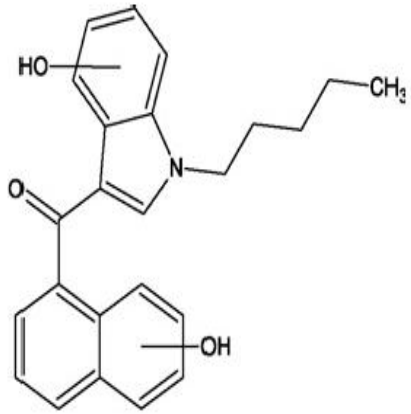
M1, M3



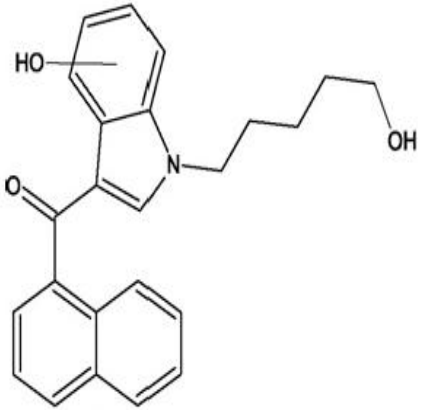
M2



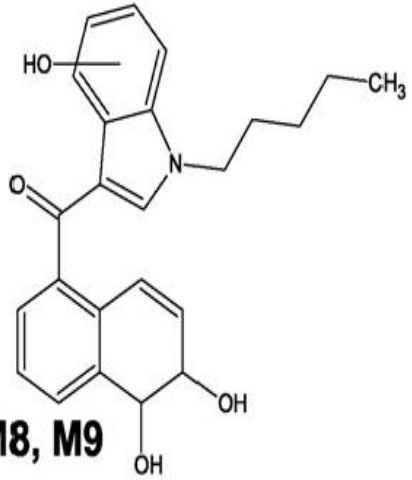
M4



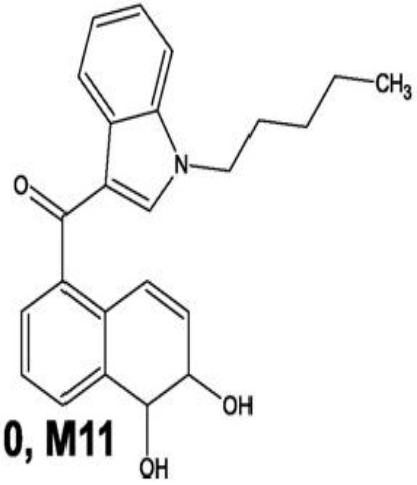
M6



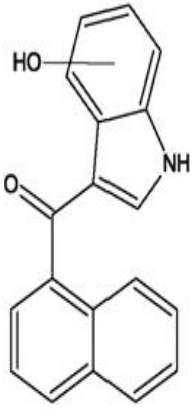
M7



M8, M9



M10, M11



M12, M13

NMS Urine Test Development

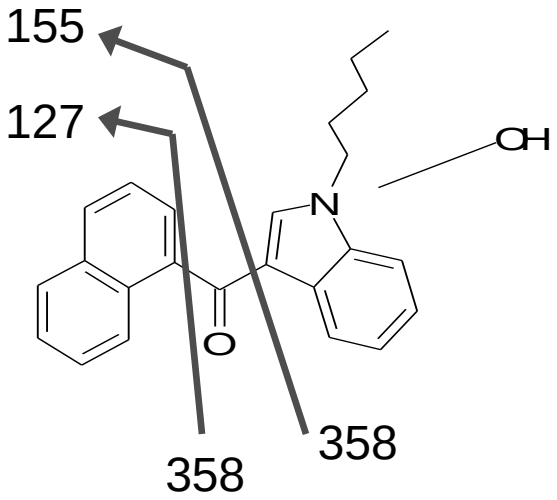
- Authentic human urine collected in controlled JWH-018 & JWH-073 study
  - LC-TOF identified predicted metabolite exact mass
  - LCMSMS for MRM studies to verify structure
- LC-APCI-MSMS for intact glucuronide of mono-hydroxy as a direct injection screening test
- LC-ESI-MSMS following enzymatic hydrolysis
  - Monitored all mono-, di- & tri-hydroxy metabolites

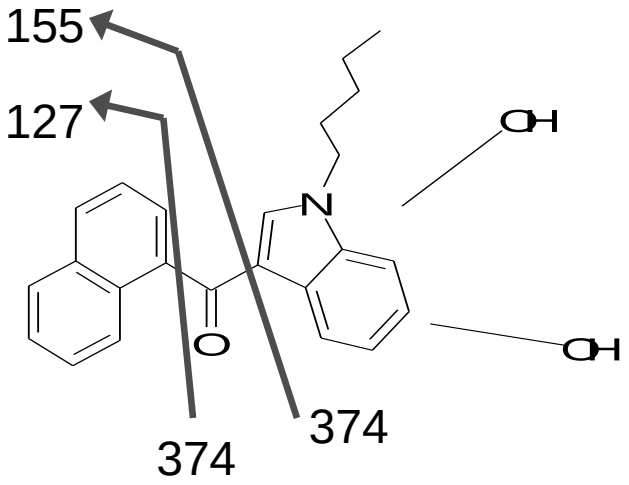
NMS Urine Test Development

Confirmation Method		
	Precursor Ion	Product Ions
OH-JWH-018	358	155 127 284 186
2OH-JWH-018*	376 374	214 171 155 127
3OH-JWH-018	374	189 171
OH-JWH-073	344	155 127
2OH-JWH-073*	362 360	200 171 155 127
3OH-JWH-073	378	189 171

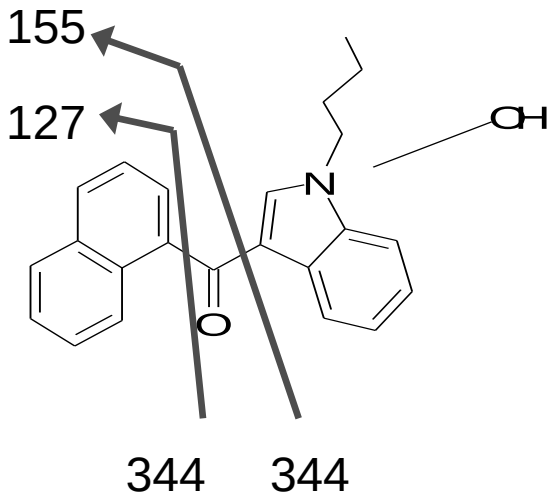
Final Monitored Transitions

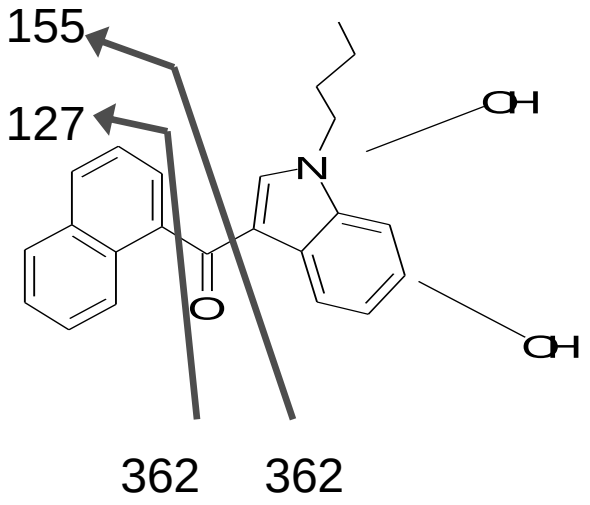
JWH-018





JWH-073





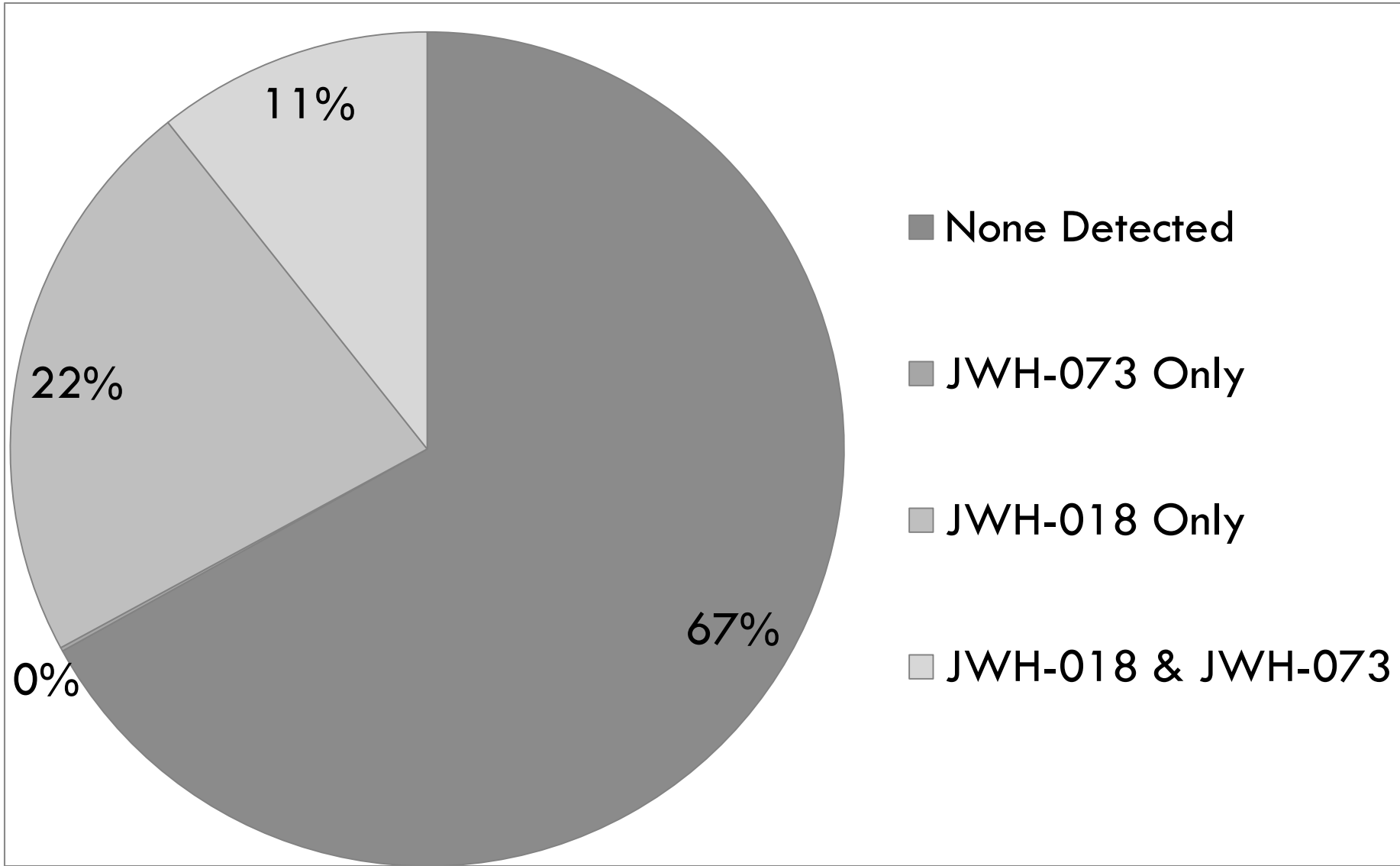
NMS Urine Test Development

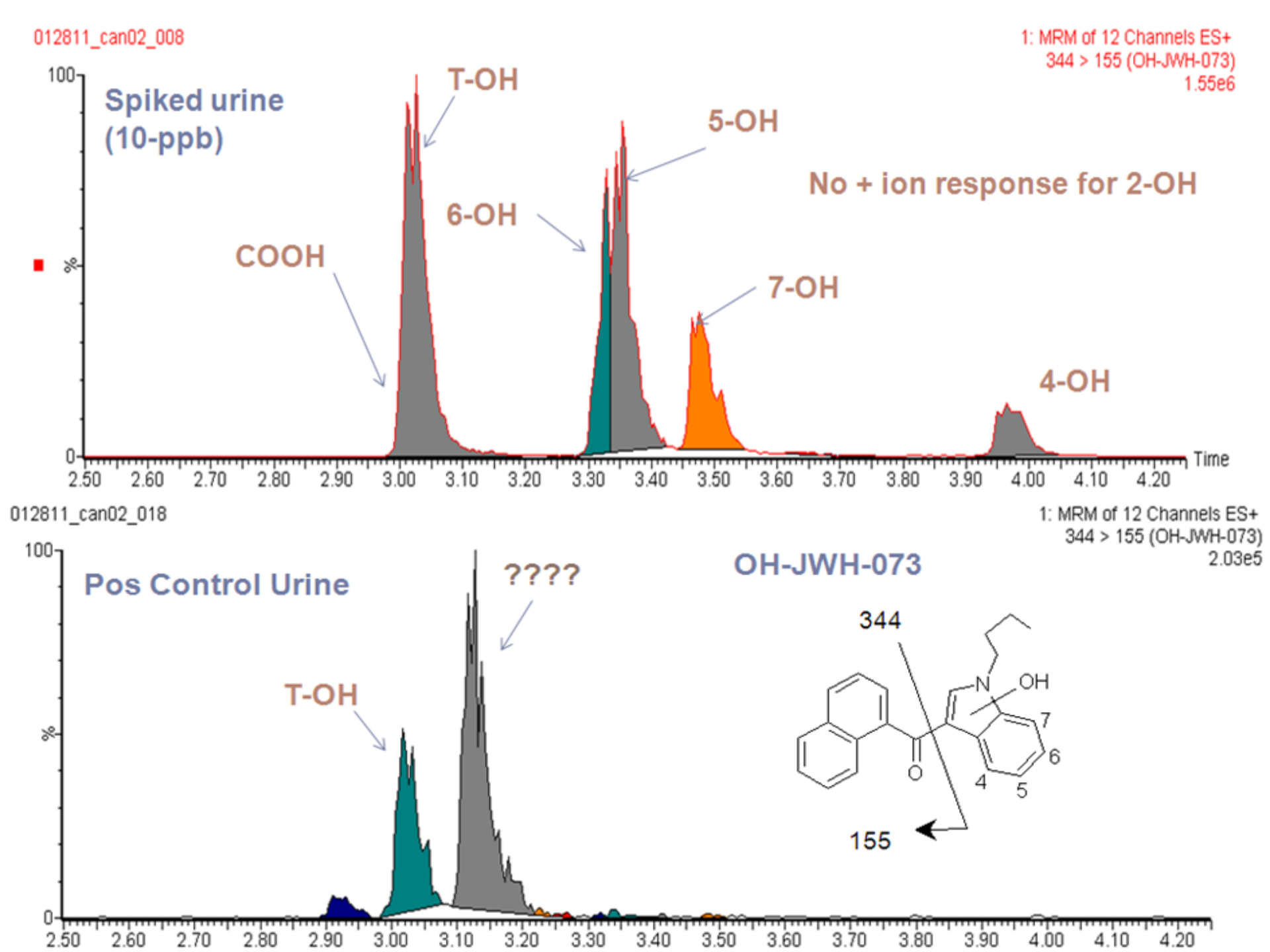
- 1 mL urine + JWH-018 (d9)
- Enzymatic hydrolysis for 1 hour at 60°C
- 10.4 Borax Buffer, extract MTBE
- Evaporate & reconstitute
- XBridge C18 3.5 um, 2.1 x 100mm
- Formic acid / ACN gradient
- Waters TQD MS with an ACQUITY UPLC
- Positive & Negative control pools

Acceptance Criteria

- Negative control must be negative
- Positive control must be positive
- Retention Time ±2% of the positive control
- Signal to noise ratio of each transition must be at least 10:1
- Transition ratios +/- 30% of positive control
- Detection of both mono-hydroxy & di-hydroxy metabolites
- Stable for at least 30 days

Urine Distribution (n=1404)




012811_can02_008
1: MRM of 12 Channels ES+
344 > 155 (OH-JWH-073)
1.55e6
100
%
0
Spiked urine
(10-ppb)
T-OH
5-OH
6-OH
COOH
7-OH
4-OH
No + ion response for 2-OH
Time
2.50 2.60 2.70 2.80 2.90 3.00 3.10 3.20 3.30 3.40 3.50 3.60 3.70 3.80 3.90 4.00 4.10 4.20
012811_can02_018
1: MRM of 12 Channels ES+
344 > 155 (OH-JWH-073)
2.03e5
100
%
0
Pos Control Urine
????
T-OH
OH-JWH-073
344
155
N
OH
O
7
6
4
5
2.50 2.60 2.70 2.80 2.90 3.00 3.10 3.20 3.30 3.40 3.50 3.60 3.70 3.80 3.90 4.00 4.10 4.20

US Laboratory Synthetic Cannabinoid Tests

- Redwood Laboratories 1st to offer synthetic cannabinoid metabolites in urine
- NMS, MedTox & others offer testing in urine & some in oral fluid
- Identify different numbers of metabolites, patterns of metabolites, & concentration levels
- High sensitivity LCMSMS instruments are needed
- Synthetic cannabinoids & metabolites do not cross-react with THC & metabolite immunoassays

Oral Fluid Synthetic Cannabinoid Tests

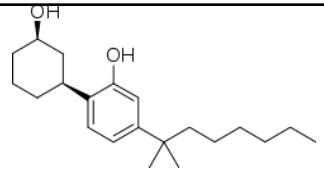
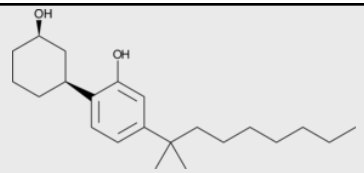
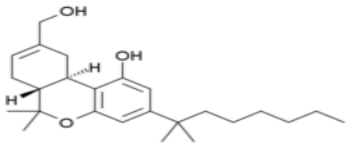
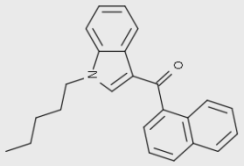
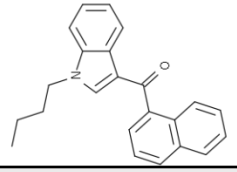
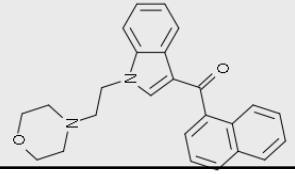
- Parent synthetic cannabinoids found in oral fluid
- Research with rat & human liver microsomes, & controlled drug administration studies not needed
- Much easier to add new synthetic cannabinoids to an existing assay
- Time course of synthetic cannabinoids in oral fluid is not yet known; ~72 h in urine depending upon the dose & sensitivity of assay (Redwood Laboratory)
- Controlled drug administration studies needed for detection of synthetic cannabinoids

Legal Status

- Multiple EU countries scheduled individual synthetic cannabinoids; constantly catching up with new drugs
- In US, individual synthetic cannabinoids emergency scheduled; differences across states
- March 2011, DEA announced temporary emergency scheduling of 6 synthetic cannabinoids of different chemical structures
- New cannabinoids will be prosecuted as analogs
- DEA has 1 year to obtain adequate scientific data from NIDA & FDA to permanently schedule

Legal Status in the US

DEA Schedule I –approval for Temporary Scheduling

CP47,497 (C7 analog) (non-classical)	
CP 47,497 (C8 analog) – Cannabicyclohexanol (non-classical)	
HU-210 (classical)	
JWH-018 (non-classical)	
JWH-073 (non-classical)	
JWH-200 (non-classical)	

US Military Policy

- The Army Substance Abuse Program, prohibits use of substances, including naturally occurring substances, used for the primary purpose of inducing excitement, intoxication or stupefaction of the central nervous system.
- Use or possession of SPICE is a violation of AR 600-85, and a direct violation of Article 92 of the Uniform Code of Military Justice (Failure to Obey an Order or Regulation).

NIH & Department of Defense (DoD)

- Interagency agreement to determine feasibility of testing for synthetic cannabinoids in urine &/or oral fluid
- Legal defensibility including passive contamination & knowing use of illicit substance
- Feasibility for screening samples
- Feasibility & time required to add new drugs as needed

Acknowledgements

- Contributors to this presentation
  - Barry Logan, PhD, National Medical Services
  - Suman Rana, MS, Redwood Laboratories
  - US Drug Enforcement Agency
  - European Monitoring Centre for Drugs & Drug Addiction

Thank You for Your Attention
& Wonderful Hospitality