Applications of High Resolution Mass Spectrometry in Forensic Sexicology

ck Kye Po Palk

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- Board/Committee/Advisory Board:

-Clinical and Laboratory Standards Institute (CLSI)

-American Board of Clinical Chemistry

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- Expenses: Thermo Fisher Scientific
- Intellectual Property/Royalties: None



Expected Outcomes

Participants will be able to:

- Cite common applications of High Resolution LC-MS
- Relate advantages of Full Scan Spectra acquisition during Toxicology Screening
- Identify utilities of mzCloud[™] software for Unknown Toxicology Screening



Forensic Toxicology Drugs of Abuse

- Prevalent in Society
- Commonly used by Teenagers and Adults











Drug Abuse

Dangers:

- Decreased Mental Capacity / Test Scores
- Miscarriage during Pregnancy
- Decreased Immune Response, † Illness
- Risky Behavior → Accidents, Disease
- Tolerance \rightarrow Addiction \rightarrow Overdose \rightarrow Death



Factoids

- The United States consumes 99% of the World's hydrocodone production, and 80% of the World's opiate production
- Hydrocodone is the most highly prescribed drug in the United States (for past 8-9 years)
- Use of PCP increased 400% in the USA from 2005-2011
- Of 22.5 million Americans surveyed, 8.7 self-reported <u>past</u> <u>month</u> illicit drug use (SAMHSA 2012)
- Abuse of synthetic designer drugs has increased 500% since 2010



Current Illicit Drug Use





SAMHSA 2013

Drugs of Abuse







Immunoassay Screening



False-negatives:

Concentration Below Cutoff Dilute Urine New Drug Formulations



Immunoassay Screening



Immunoassay Screening

Compound	Siemens	Roche
6-Acetylmorphine	435	386
Morphine	300	300
Codeine	306	224
Dihydrocodeine	291	510
Hydrocodone	247	1,086
Hydromorphone	498	1,425
Meperidine	> 15,000	> 100,000
Nalbuphine	> 100,000	?
Oxycodone	1,500	> 75,000
Tramadol	> 100,000	?

*Siemens EMIT II Plus Opiate Assay using 300 ng/mL cutoff. **Roche Cobas Opiates II assay using 300 ng/mL cutoff.



Why HRAM LC-MS/MS?

- Drug/Analyte extraction into solvent not required
- Accurate mass (0.0001) is more specific
- OrbitrapTM Technology (Thermo ScientificTM)
 - Supports Spectral Matching (like EI GC-MS)
 - Allows Retrospective Analysis of Previously-injected Samples



Opiates Confirmation using HRAM LC-MS/MS

Mobile Phase: A) 0.1% Formic Acid (in water) B) 0.1% Formic Acid in Acetonitrile

Initial: 5% Acetonitrile
 Increase to 50% Acetonitrile over 5.5 min
 Increase to 90% Acetonitrile over 0.5 min
 Hold at 90% Acetonitrile for 4 min
 Return to 5% Acetonitrile over 2 min
 Re-equilibrate with 5% Acetonitrile for 2 min



Sample Preparation

- Combine: 0.5 mL urine, β-glucuronidase, 2 M Sodium Acetate (pH 4.5), D²-Internal Standard Mix
- Incubate for 30 minutes at 65°C
- Centrifuge for 5 minutes at 8000 rpm (in microfuge)
- Dilute 1/10
- Inject 10 µL onto LC-MS/MS

Analytical Column: 2.1x100 mm Phenyl



Mass Transitions @ 35% CE 6-MAM: 328.1543 \rightarrow 165.0697, 211.0754, 193.0646 Codeine: **300.1594** → 215.1064, 199.0751, 183.0802 Dihydrocodeine: **302.1750** → 199.0751, 227.1063 Hydrocodone: **300.1594** → 199.0759, 241.0865, 213.0917 Hydromorphone: $286.1437 \rightarrow 185.0597, 227.0704, 157.0648$ Morphine: **286.1437** → 201.0914, 185.0601, 165.0701 Norcodeine: **286.1467** → 165.0701, 181.0654 Oxycodone: $316.1543 \rightarrow 241.1091$, 256.1328, 298.1433 Oxymorphone: **302.1386** → 227.0937, 242.1173, 284.1277



Mass Transitions @ 35% CE 6-MAM: $328.1543 \rightarrow 165.0697, 211.0754, 193.0646$ Codeine: **300.1594** → 215.1064, 199.0759, 183.0802 Dihydrocodeine: **302.1750** → 199.0751, 227.1063 Hydrocodone: $300.1594 \rightarrow 199.0759, 241.0865, 213.0917$ Hydromorphone: **286.1437** → 185.0601, 227.0704, 157.0648 Morphine: **286.1437** → 201.0914, 185.0601, 165.0701 Norcodeine: **286.1467** → 165.0701, 181.0654 Oxycodone: **316.1543** → 241.1091, 256.1328, 298.1433 Oxymorphone: **302.1386** → 227.0937, 242.1173, 284.1277









- LLOD = 3 ng/mL
- LLOQ = 10 ng/mL
- Specific (HRMS fragments)
- Complex Precision (2x/day for 10 days) Morphine 4.6 CV% Codeine 11.6 CV%

Linear Response 10 - 2500 ng/mL (tested)



Codeine Triplicate Cal Curves 10 - 2,500 ng/mL (R2 = 0.9966)





Benzodiazepines Confirmation using HRAM LC-MS/MS

- 7-aminoflunitrazepam
- Alprazolam
- α-Hydroxyalprazolam
- α-Hyroxymidazolam
- Clonazepam
- Desalkylflurazepam
- Diazepam
- Estazolam
- Etizolam
- Flunitrazepam

- Flurazepam
- Lorazepam
- Midazolam
- Nordiazepam
- Oxazepam
- Temazepam
- Triazolam
- Zaleplon
- Zolpidem
- Zopiclone



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Thermo ScientificTM TraceFinderTM software



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Comparative View	9 9		27	74 CC	Sp	e 9	-		Noroxycodone	4.49	-0.01	302.1387	-0.1279	24832914	611.6		10	0.2802 ppm	0.8722 ppm	N/S
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QAQC	A	AA: 1 AH: 7 SN: 1	980259.54 53285.10 14.58	sity		sity		AA: 18220558.40 AH: 6274054.48 SN: 3184.99	ath		sity	RT: 43 AA: 69	20 106528.64	Al atta	AA: 3697871.05 AH: 1345669.05 SN: 1965.13	Al a		E
Groups	100 100 100 100		Δ	5 100 5 80	Λ	e Inten	80-	Δ	5 100 9 80		00 g 100 9 80	3 ^{SN: 54}	558.63	ug 100 100 100	A	9 80 RT:	ur J	
Intel Seq	1081-00- 82 40-		1	100- 8 40-	RT: 3.68	Relativ	60- 40-		1989 60- 40-	RT: 4.12 AA: 111341.07	10 10 10 10 10 10 10 10 10 10 10 10 10 1	9,	Δ	151 60- 15198 40-		11 60 AA 11 60 AH: 12 40 SN:	8079.49 52496.32 53.89	
Library Settings	20-		6	20-	AH: 166571.01 SN: 94.34		20-		20-	AH: 56912.56 SN: 32.94	20	1/		20-	\square	20	R	
Reports		3.4 R	3.6 (min)		3.6 3.8 RT(min)		3.6	3.8 4.0 RT(min)	40	0 4.2 RT(min)		4.0 R1	4.2 4.4 F(min)		4.2 4.4 RT(min)	4.2	4.4 4 RT(min)	
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	Real time	e status																↓ # ×
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	Acquisit	ion Inst	rument Qu	eues														
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Acquisition															No Acqu	isition in Progre	SS.	
Analysis	Item	Value																
Method Development																		

Thermo ScientificTM TraceFinderTM software



Recent Illicit Drugs seized in Mississippi

- 1. Marijuana +/- PCP
- 2. Opiates/Opiods
 - Hydrocodone, Heroin, Oxycontin
- 3. MDMA (Ecstasy, Molly)
- 4. Synthetic Cannabinoids (K2, Spice)
- 5. Carisoprodal/Meprobamate



WARTE 'Spice' overdose cases now up to 45 at UMMC, another 40 overdose cases treated at other **Mississippi Hospitals** April 9, 2015

©CBS NEWS Synthetic marijuana leads to nationwide spike in hospitalizations May 8, 2015

Ehe New Hork Eimes33 Suspected of Overdosing onSynthetic Marijuana in BrooklynNYJuly 12, 2016



- Compounds often sprayed onto plant material and then smoked.
- Vape Pens & eCigarettes recently used
- Can produce 10x intense effects than THC because many analogues are full CB1 agonists (THC is a partial agonist)
- JWH-018 = 5x activity of THC





△9-THC

JWH-018



MAB-CHMINACA





2015 MAB-CHMINACA



Physiology/Symptoms

- GABA inhibition → paranoia, agitation, anxiety, seizures
- **B1** Agonist \rightarrow hypertension, tachycardia, palpitations
- Psychosis reported in some individuals
- Renal failure in some individuals



- No formal Toxicity Testing performed
- Some 5x 10x more potent than THC
- No Quality Control
- Compounds Sprayed -or- Soaked onto leaf material → Variable Concentrations
- Multiple compounds often in a single batch
- Attractive packaging may lure youngsters















Use of HRAM LC-MS/MS

Advantages

- Less Sample Preparation than GC-MS
- Differentiate isobaric compounds via chromatography and/or fragmentation
- Linear response
- Full Scan MS/MS Spectral Matching
- Identify new Synthetic Designer Drugs



Targeted Screening using HRAM LC-MS/MS

Instrument: Q-Exactive Focus (Thermo ScientificTM)

Detection: ddMS/MS (90-600 m/z)

Library: HRMS Maurer/Wissenbach/Weber (Wiley)

Also Incorporated:

>150 most commonly prescribed medications

- >30 Synthetic Cannabinoids
- >25 "Bath Salts"
- >20 Other abused Drugs (Mytraginine, etc)

Targeted Screening using HRAM LC-MS/MS

Sample Preparation

- Add internal standard mix to 0.5 mL urine
- Centrifuge for 5 minutes at 8000 rpm (in microfuge)
- Dilute 1/20 into 0.1% Formic Acid
- Inject 10 µL onto LC-MS/MS

Mobile Phase: 5% B to 90% B over 15 minutes Total Analytical Run Time: 20 minutes



- 27 y/o Male transported to ED after "running in traffic, barking at cars" and assaulting a pedestrian. Altered mental status noted.
- DOA Immunoassays: THC Positive
- THC Confirmation (GC-MS): Positive for THC-COOH





Caffeine Theobromine PCP

LC-MS/MS Caffeine Cotinine Nicotine Theobromine PCP MAB-CHMINACA -Metabolite



File View Tools Help																		Real time	status L	Jser: Thermo (•
□ ⊠ ¶⊜li ⊟lei Elle mé	* • V & F W																286CX	20	Acqui	ring	
Analysis 🗸 🕈	Data Review - Targeted Drug Sci	een [Screer	ning]																		
 Batch View 	Samples + # ×	Compounds	T																		₩ Ū ×
Samples	PT 182c	• I F	RT -=	MZ 👳	IP -=	abase FI – 👳	LS -	Flag	Compound Name	⊣⊨ m/z (Ap	pex) 👳	RT (Expected)	-10 RT (0	Delta) 👳	sotopic Pattern : 👍 Libr	ary Score (%) 😑 Fr	ragment 1 (De 👒	Fragment 2 (+ Frag	gment 3 (👍	
🗶 Data Basiaw			•	•	•	•	•	•	<u>A</u> a •	Aa	•	= •	-	•		▼ <u>A</u> a	· •	<u>A</u> a •	Aa	•	E
Data Review		1	•	•	•	•		A	Acetaminophen	15	52.0706	4	4.10	0.03	100	56	-0.0189	1.3	297	N/S	
Target Screening		2	•	•	•	•	•	•	Caffeine	19	95.0877	5	5.20	-0.01	100	100	-1.9059	-0.7	306	-0.6672	
Report View		3	.	•	•	•	•		Cotinine MAR-CHMINACA Metal	17	77.1022	3	3.00 3.40	0.02	100	100	-0.292	-0.5	001	0.4471	
		5	•	•	•	•	•	•	Nicotine	1	163.123	2	2.40	-0.01	100	100	-0.0999	1.	127	N/S	
 Local Method 		6	•	•	•	•	•	•	Phencyclidine	2	244.206	7	7.70	0.04	100	100	0.0752	-0.0	302	0.7592	
Acquisition		7	•	•					4-MethoxyAmphetamin	e 16	66.1227	5	5.00	-0.19	100	N/A	N/S	1	v/s	N/S	
Target Screening		8	•	٠					4-MethoxyPhencyclidin	e (4 27	74.2162	8	3.00	-0.17	100	N/A	N/S	- I	v/s	N/S	
Target Screening		9	•	٠				A	Alpha-Pyrrolidinovalero	ph 23	32.1696	6	5.50	-0.01	2	20	N/S	1	v/s	N/S	
Processing		10	•	•				A	Butylone	22	22.1125	5	5.40	0.21	100	N/A	N/S	1	v/s	N/S	
Peak Detection		11	•	•	•			A	Carisoprodal	26	61.1808	8	3.65	-0.12	100	10	N/S		v/s	N/S	
Reports		12	•	•				<u> </u>	Cimetidine	2	253.124	3	3.80	-0.10	51	N/A	N/S		V/S	N/S	
		13				÷.,	а.	<u>.</u>	Desmethylselegiline	17	74.1277	5	5.60	-0.04	100	N/A	N/S		V/S	N/S	
		14			- 21-	÷.,	а.		Desmethyltapentadol	20	08.1695	-	5.90	0.03	100	N/A	N/S		V/S	N/S	
		15	1.1		÷.	÷.	а.		Gabanentin	23	72 1 3 3 1	/	130	-0.08	100	N/A	N/S		u/s	N/S	
	Chromatogram	10		_	-	-	-	_	Gubapentin	- U	X Sne	ctrum		0.10		17/6	14/3		w/0	14/3	- 1 ×
	M+H										Sp	ectrum	otopes 100	0% (4 of 5)	Fragments (2 of 2)	Library (3 matches)					
	PT 182cx1 MAB-CHMINACA Metabol	te 1 NL: 1.5	3E7 m/z:	387.2371	- 387.241	1					PT	182c×1 #: 34	88 RT: 8	.40		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,					
	F: FTMS + c Full ms [90.0000-650.000	0]		DT: 0	40						E	FTMS + c ESI	Full lock m	ns (90.0000	-650.0000]	297 220	00			,	NL:
				AA: 5	40 6541871.8	17						¹⁰⁰				367.239	90			1	1.60E7 MAB-
	100 -			AH: 1	5269266.2	25						90-								c	CHMIN
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	[∞] 40−										e c	30-	135.1	1015	314.23	27					
				/	· · ·	\						20-1			257.2223	342.2172	38.2425				
	20			/				0.57				10-	1.1	180.1594	217.1073 262.1649	370.2123	432 2971				
	0-18.03	8.26			·		1	10.91	8.66 8.70	8.75		₀᠋᠇ᢆᡰᡰ᠇ᡰᡰᠠᡰ	┟ _╋ ╸┢┧╍╾╽┫┫┧╴┡╽	^I IV→I+I++++++++++++++++++++++++++++++++	$\psi_{1}, \psi_{1}, $	\cdots	1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-	1,1915 53	9.1796 ******	T.T.T.T.T.T.T	
	8.1 8	2	8.3	R	8.4 (T(min)	8	.5		8.6 8.7			100	150	200	250 300	350 40 m/z	00 450	500	550	600	
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Analysis	Real time status Instrument Controls																				
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Thermo ScientificTM TraceFinderTM software



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Analysis 👻 🖣	Data Review - Targeted Drug Sc	reen [Scree	ning]														
Batch View	Samples 👻 🕂 🗙	Compound															▲ ù ×
Samples	Targeted Drug Screen PT1	9 1 Ær	Targeted So	reening H	IRAM Datab	ase IS	Elac	Compound Name	m/z (Anex)	RT (Expected)	RT (Delta)	Isotopic Pattern	Librany Score (%)	Fragment 1 (De	Fragment 2 (agment 3 (Â
Samples		8- 6	•	-	•	• •	- 110g	Aa -	Aa •	= •	= •	= •	= •	Aa 🗸	Aa ▼ <u>A</u> a	•	=
▼ Data Review >		1	•	•	•	• •		Acetaminophen	152.070	5 4.10	0.03	100	56	-0.0189	1.3297	N/S	
Target Screening		2	•	•	•	• •	•	Caffeine	195.087	7 5.20	-0.01	100	100	-1.9059	-0.7306	-0.6672	
Report View		3	-	•	•	••••	•	Cotinine MAR CHMINACA Mataba	177.102	2 3.00	0.02	100	100	-0.292	-0.5001	0.4471	
		5						Nicotine	163.12	3 2.40	-0.01	100	100	-0.3233	-1.0014	N/S	
 Local Method 		6	•	•	•	• •	•	Phencyclidine	244.20	5 7.70	0.04	100	100	0.0752	-0.0302	0.7592	
Acquisition		7	•	•	•		▲ (4-MethoxyAmphetamine	166.122	7 5.00	-0.19	100	N/A	N/S	N/S	N/S	
Target Screening		8	•	•			A	4-MethoxyPhencyclidine	(* 274.216	2 8.00	-0.17	100	N/A	N/S	N/S	N/S	
Processing		9		•	÷	: :		Alpha-Pyrrolidinovaleropl Butylope	h 232.169	5 6.50	-0.01	2	20 N/A	N/S	N/S	N/S	
Peak Detection		10		•	-			Carisoprodal	261.180	3 8.65	-0.12	100	10	N/S	N/S	N/S	
Paparte		12	•	•				Cimetidine	253.12	4 3.80	-0.10	51	N/A	N/S	N/S	N/S	
Reports		13	•	•	•		L 🔺	Desmethylselegiline	174.127	7 5.60	-0.04	100	N/A	N/S	N/S	N/S	
		14	•	٠	•		I ▲	Desmethyltapentadol	208.169	5 5.90	0.03	100	N/A	N/S	N/S	N/S	
		15	•	•			▲	Diphenhydramine	256.169	3 7.90	-0.08	100	N/A	N/S	N/S	N/S	
	Characterization	16	••	•	-			Gabapentin	1/2.133	L 4.30	-0.16	6/	N/A	N/S	N/S	N/S	
	M+H								T A B	Spectrum Spectrum	pes 100% (4 of 5)	Eragments (2 of 2)) library (3 matche	ac)			• + ^
	PT 182cx1 MAB-CHMINACA Metabo	ite 1 NL: 1.	53E7 m/z:	387.2371	- 387.241					Scan #: 3470-3509 RT: 8.30 - 8.52 AV: 14 Score: 100							
	F: FTMS + c Full ms (90.0000-650.000	0]		DT 0/	10					Multi-Isotopes F: FTMS + c ESI Full lock ms [90.0000-650.0000]							
				AA: 56	541871.87					#1: 387.23907	100-	*1					
	100			AH: 15	109200.25					#2: 388.23619							
	l ≩ 80-			1	$\langle \rangle$					#3: 388.24243	50						
	Inter									#4: 389.24558		207.44	200 05 ^{*3}	200.11	'4	*5	
	at 10-									#5: 390.24819	_E_	#1	366.05	508.112			-
	10 H										100	Ē.					
	-										50-						
	20-					\ \							#2,-#3		#4		
	0-18.03	8.2	<u>b</u>	·			8.57	8.66 8.70 8.	.75		04-		- granderege		hegenner		-
	8.1 8	.2	8.3	R	8.4 T(min)	8.5		8.6 8.7			387.0	D 387.5	388.0 388.	i 389.0 m/z	389.5 39	10.0	
Acquisition	Real time status																
Analysis	Real time status Instrument Controls																
Method Development	Acquisition Instrument Queue	5															

Thermo ScientificTM TraceFinderTM software



File View Tools Help															Real time status	User: Thermo	•
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Analysis 👻 🎙	Data Review - Targeted Drug Scr	een [Screeni	ng]														
 Batch View 	Samples	Compounds															- ₽ ×
Samples	PT 182c	- 1 I	RT 😑 MZ	g HRAM Dat	FI → +=	LS 👳	Flag 👒	Compound Name	m/z (Apex)	RT (Expected)	RT (Delta) 👳	Isotopic Pattern 🖙 🗠 Li	brary Score (%) 🐵 Frag	ment 1 (De 👍 Fra	igment 2 (👍 🖡	ragment 3 (🛛 🕁	
▼ Data Review >			• •	•	•	•	•	<u>A</u> a •	<u>A</u> a 🔻	= •	= •	= - =	▼ <u>A</u> a	▼ <u>A</u> a	• <u>A</u>	• •	=
Target Screening		1			•	:	•	Acetaminophen Caffeine	152.070	6 4.10 7 5.20	-0.01	100	56 100	-0.0189 -1.9059	1.3297 -0.7306	-0.6672	
Report View		3	• •	•	•	•	٠	Cotinine	177.102	2 3.00	0.02	100	100	-0.292	-0.5001	0.4471	
Report view		4			•	•	•	MAB-CHMINACA Metabol	163.12	9 8.40	0.00	100	100	-0.5235	-1.0014	N/S	
 Local Method 		6	• •	•	•	•	•	Phencyclidine	244.20	6 7.70	0.01	100	100	0.0752	-0.0302	0.7592	
Acquisition		7	• •			•		4-MethoxyAmphetamine	166.122	7 5.00	-0.19	100	N/A	N/S	N/S	N/S	
Target Screening		8	• •			•		4-MethoxyPhencyclidine (4	274.216	2 8.00	-0.17	100	N/A	N/S	N/S	N/S	
Deservation		9	• •			•	A	Alpha-Pyrrolidinovaleroph	232.169	6 6.50	-0.01	2	20	N/S	N/S	N/S	
Processing		10	• •			•	<u> </u>	Butylone	222.112	5 5.40	0.21	100	N/A	N/S	N/S	N/S	
Peak Detection		11	•	•		÷	<u></u>	Carisoprodal	261.180	8 8.65	-0.12	100	10	N/S	N/S	N/S	
Reports		12		- 2 -	- C - C	2.1		Cimetidine	255.12	4 5.80	-0.10	51	N/A	N/S	N/S	N/S	
		15		- 2 -	÷.	2.1	1	Desmethylselegiline	208 160	/ 3.00	-0.04	100	N/A	N/5	N/S	N/S	
		14		- 2 -	- C - C	а.	1	Dishophydramino	206.109	3 3.90	0.05	100	N/A	N/5	N/S	N/S	
		15		- 21	÷.	÷.,	1	Gabanentin	172.133	1 4.30	-0.16	67	N/A	N/S	N/S	N/S	-
	Chromatogram					-	-		- 4 × S	pectrum							- 4 ×
	M+H								1	Spectrum 🕒 Isoto	pes 100% (4 of 5)	Fragments (2 of 2)	Library (3 matches)				
	PT 182cx1 MAB-CHMINACA Metaboli	te 1 NL: 1.53	E7 m/z: 387.23	371 - 387.241	1					All Fragments Minimum # of fragments needed: 1							
	F: FTMS + c Full ms [90.0000-650.000	D]								+1, 257 1 2940	PT 182cx1	1 #: 3504 RT: 8.44	00@bcd35.00.050.0000-/	15 00001			
			AA RT	: 8.40 : 56541871.8	37					+2: 275 12010	2,056	J ["1	50@ncd55.00 [50.0000-	10.0000			
	100 -		AH	: 15269266.2	25					+2:273.15910		-					
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	0.1 0.	2	0.5	RT(min)	0.5	,		0.0 0.7				230 200	202 204 .	m/z	210 212	2/4	
Acquisition	Real time status	_	_												_		→ ‡ ×
Analysis	Real time status Instrument Controls								_							_	
Method Development	Acquisition Instrument Queues																

Thermo ScientificTM TraceFinderTM software



File View Tools Help															Real time status	User: Thermo	8
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Analysis 👻 🖣	Data Review - Targeted Drug Sci	een [Screeni	ng]														
Batch View	Samples 💌 म 🗙	Compounds															▲ 廿 ×
Camples	Targeted Drug Screen PT 182c	8 1 Ta	argeted Scree	ning HRAN	1 Database	- 19	Fine	Compound Name	m/z (Anav)	PT (Expected)	PT (Dalta)	Inotonic Pattern	Library Score (%)	Fragment 1 (De	Fragment 2/	agment 2 (Â
Samples		PU				- L -	Tiag .	Aa •	Aa 🔻	= •	= -			Aa 🗸	Aa 🗸 Aa	iginenco (⊸	_
▼ Data Review >		1	•	• •	•		A	- Acetaminophen	152.070	6 4	.10 0.03	100	56	-0.0189	1.3297	N/S	
Target Screening		2	•	• •	•	٠	٠	Caffeine	195.08	7 5	.20 -0.01	. 100	100	-1.9059	-0.7306	-0.6672	
Report View		3	•	• •	•	•	•	Cotinine	177.10	2 3	.00 0.02	100	100	-0.292	-0.5001	0.4471	
		4						MAB-CHMINACA Metabol	387.2	19 8 12 2	40 0.00	100	100	-0.5235	-1.0014	N/S	
Local Method		6						Phencyclidine	244.20	.5 2 16 7	.70 0.04	100	100	0.0752	-0.0302	0.7592	
Acquisition		7	•	• •				4-MethoxyAmphetamine	166.12	.7 5	.00 -0.19	100	N/A	N/S	N/S	N/S	
Target Screening		8	•	• =				4-MethoxyPhencyclidine (4	274.21	2 8	.00 -0.17	100	N/A	N/S	N/S	N/S	
rarget screening		9	•	• =				Alpha-Pyrrolidinovaleroph	232.16	6 6	.50 -0.01	. 2	20	N/S	N/S	N/S	
Processing		10	•	• •				Butylone	222.11	5 5	.40 0.21	. 100	N/A	N/S	N/S	N/S	
Peak Detection		11	•	• •	-		<u> </u>	Carisoprodal	261.18	8 8	.65 -0.12	100	10	N/S	N/S	N/S	
Reports		12		•		- 21	<u>.</u>	Cimetidine	253.1	4 3	.80 -0.10	51	N/A	N/S	N/S	N/S	
		13				- 21	<u></u>	Desmethylselegiline	1/4.12	·/ 5	.60 -0.04	100	N/A	N/S	N/S	N/S	
		14				- 21		Desmethyltapentadol	208.16	כ כו	.90 0.03	100	N/A	N/S	N/S	N/S	
		15				- 21		Gabapentin	172 13	1 /	30 -0.08	100	N/A	N/S	N/S	N/S	-
	Chromatogram	10				_		Cooperation	- 4 ×	- ·					140		- 4 ×
	M+H									Spectrum Spectrum	topes 100% (4 of 5	5) Fragments (2 of)	2) 🔍 Library (3 matche	es)			
	PT 182cx1 MAB-CHMINACA Metabol	te 1 NL: 1.53	E7 m/z: 38	7.2371 - 38	7.241					M+H #1: MAB-CHMINACA Metabolite 1 100 M+H #1: MAB-CHMINACA Metabolite 1 Score: 100 Rank: 1 of 3 Id: 4634							
	F: FTMS + c Full ms [90.0000-650.000	0]		DT 0 40						#3504 F:FTMS + c ESI d Full ms2 387.2390@hcd35.0							
				AA: 56541	871.87					#3: Testosterr	ine 17-isocanroate	10 = 100 -		257.1283 [°] ľ	257.1283		
	100 -			AH: 15269	266.25						ine 27 isocaprodic	ueut					
					\							-E 50-			275.1388		
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Thermo ScientificTM TraceFinderTM software



- 37 y/o Female jumped from her attic and attacked family members, then found naked on the scene by police. History of cocaine, methamphetamine, and ecstasy (MDMA) use.
- BP 128/113 (1), Resp 20, Temp 98.9
- Ethanol: Negative



<u>GC-MS</u>

Nicotine Cotinine Cocaine Benzoylecgonine **Ecgonine Methylester** Levamisole PCP Tramadol Tramadol Metabolite

LC-MS/MS Nicotine Cotinine Cocaine Benzoylecgonine Levamisole Naproxen PCP Tramadol

Ziprasidone

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

- A 19 y/o Male presented to the PER with altered mental status (delusions, hallucinations).
- Ethanol: Neg
- DOA Immunoassays: Amphetamines Pos Benzodiazepines - Pos





Caffeine Nicotine Cotinine Diphenhydramine **Diphenhydramine** Metab Methamphetamine Olanzapine

LC-MS/MS Caffeine Nicotine Cotinine Diphenhydramine Alprazolam + Metab Ketamine Norketamine Methamphetamine Olanzapine



Unknown Screening using m/zCloud[™] Software

- <u>WWW.mzCloud.org</u>
- Online MS searches in the Cloud
- Database updated daily
- Other ID Functions:
 - -Structure
 - -Tree (Molecular Fragments)
 - -MSn



Unknown Screening using m/zCloud[™] Software

- 21 y/o Male presented to the ED with incessant vomiting. Admitted smoking "Spice"
- Leafy material found in bag in pocket.
- GC-MS (methanolic extraction): ∆9-THC, MAB-CHMINACA, and large unknown Peak



















$m/zCloud^{TM}$ Software

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Summary

Advantages of HRMS LC-MS/MS Analysis

- Only one HRMS fragment required for drug confirmation
- Less Sample Preparation vs GC-MS
- Specific Compound Identification
- HRMS MS/MS Full Scan spectra allow users to identify compounds using multiple databases (User's Library, Commercial Libraries, m/zCloud[™], m/zVolt[™])



Questions?





The University of Mississippi Medical Center

minutes

