The Challenges of Analytical Method Validation for Hallucinogens and Designer Stimulants in Biological Samples Using LC-TOF

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WHEN YOU NEED TO KNOW

Barry K Logan, Ph.D.,
NMS Labs National Director, Forensic Services

Goals

- The Need:
  - *Accurate, sensitive, versatile tools.*
    - *Identification*
    - *Detection*
    - *Routine analysis*
    - *Novel, complex, esoteric and designer drugs and other analytes in a variety of matrices and sample types.*
- The solution:
  - *LCMS/MS*
  - *LCTOF, LCQTOF*

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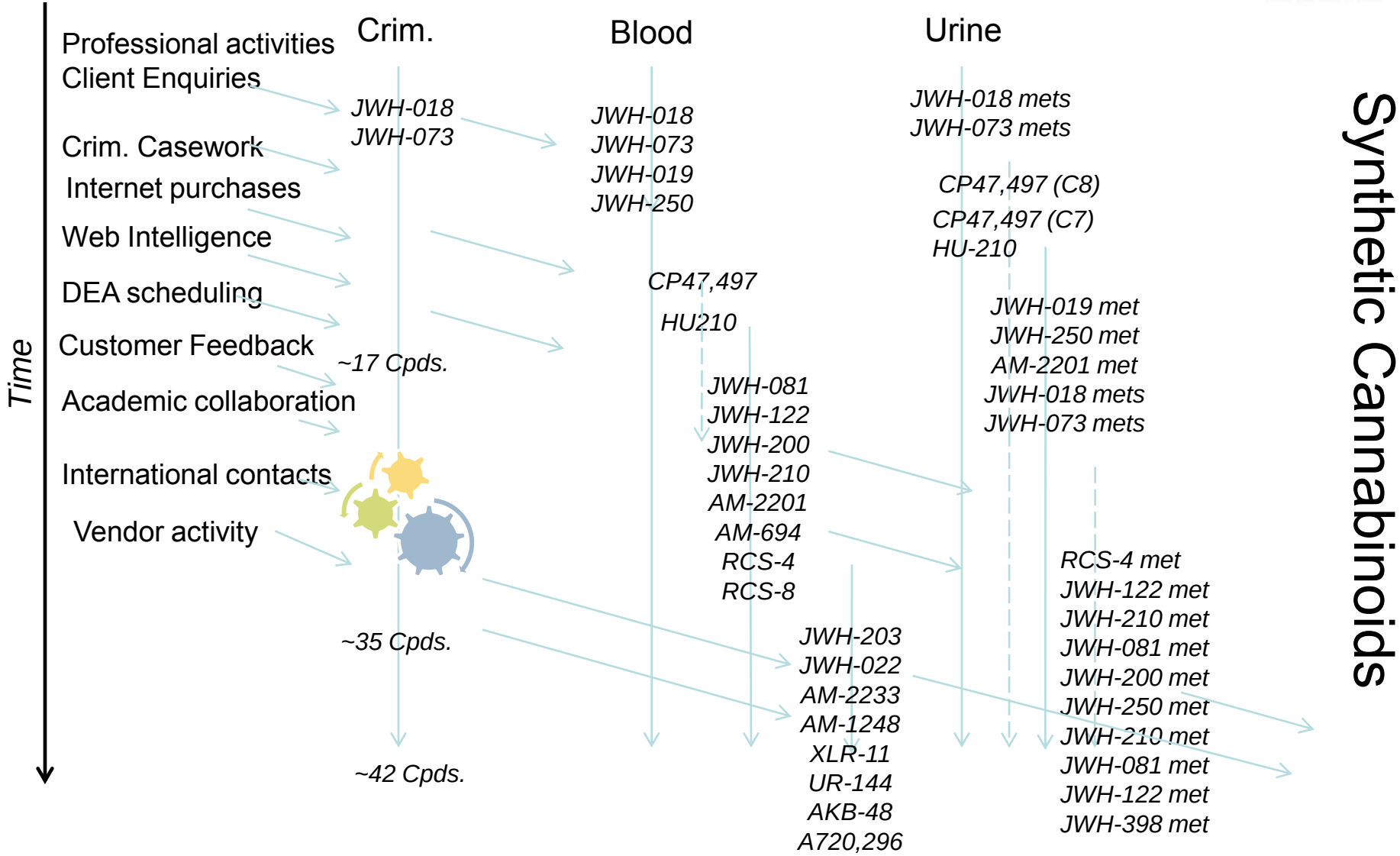
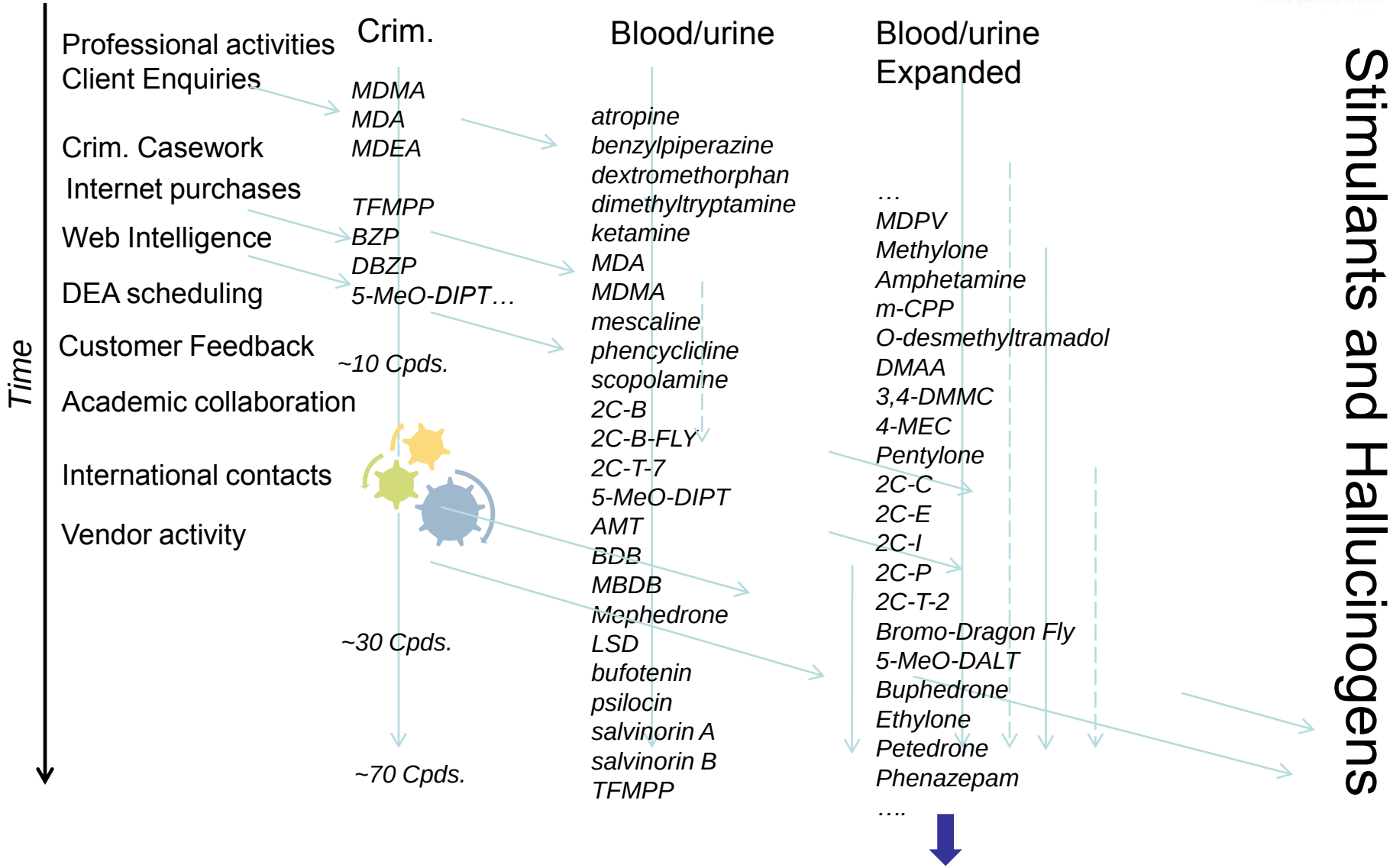


NMS Labs Center for Innovation



The Scope

Bath Salts and Stimulant Designer Drugs – Expanded 43 Compounds

- Classical Stimulants

Amphetamine, Methamphetamine, MDMA, MDEA, DOM, DOB, PMA, Cocaine, Benzoylecgonine

- Cathinones

Cathinone, Mephedrone, Buphedrone, Methylone, Ethylone, Butylone, Pentylone, Mmethcathinone, Methedrone, Naphyrone, 3-FMC, 4-FMC, 4-MEC, 3,4-DMMC,

The Scope

Bath Salts and Stimulant Designer Drugs – Expanded 43 Compounds (Continued)

- 2C Series – Stimulants with Hallucinogenic Properties

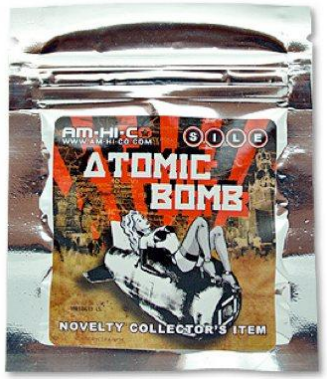
2C-B, 2C-C, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-7

- Benzylpiperazines

BZP, TFMPP, DBZP, MBZP, m-CPP

- Others

Phenazepam, Mitragynine, 7-OH Mitragynine, O-desmethyltramadol, DMAA



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Performance Checklist:

- ✓ High Sensitivity
- ✓ High Performance
- ✓ High Resolution Mass Accuracy (>5ppm)
- ✓ User Friendly and Adaptable Data Reduction Tools
- ✓ Versatility
- ✓ Stable Performance
- ✓ Robust Design
- ✓ Technical Support
- ✓ Value/ROI

The Technology

Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF)

- Agilent 1290 HPLC system
- Agilent 6230 with Jet Stream Technology

- Mass Hunter Software

The Technology

Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF)

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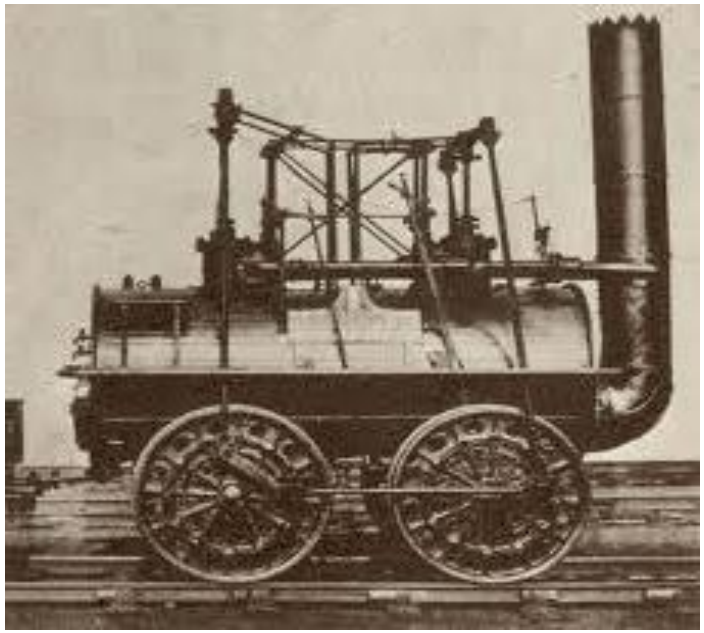
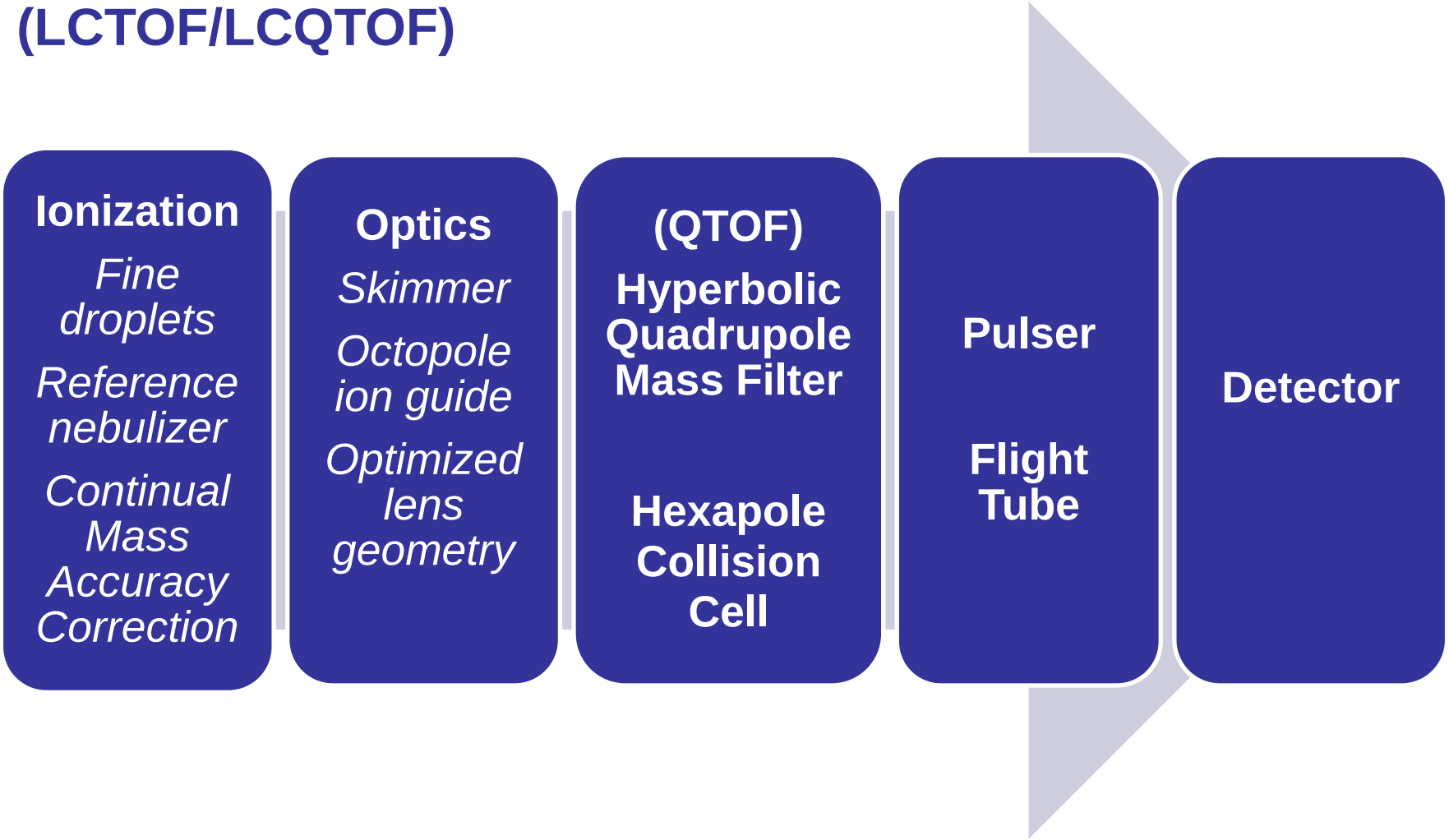
- Agilent 6530 QTOF
- Mass Hunter Software



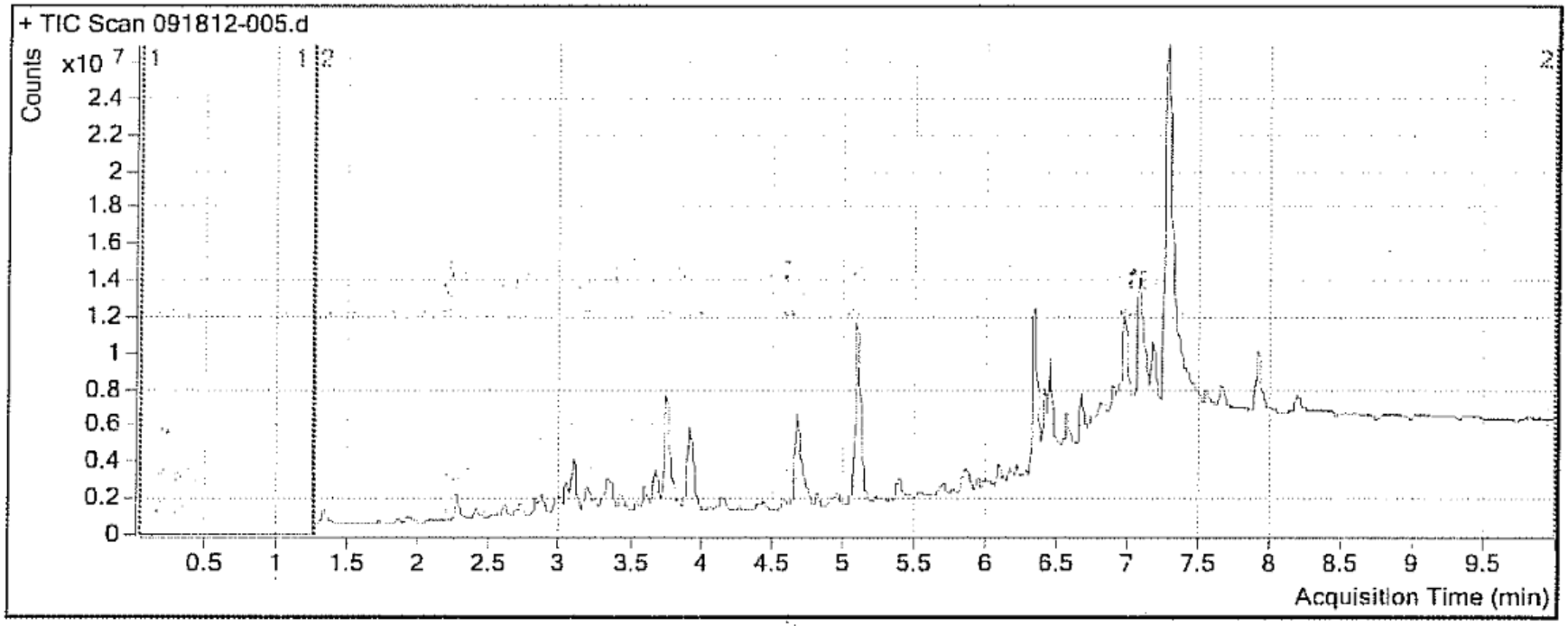
Time of Flight (TOF)

6200/6500 Series Time of Flight Mass Spectrometry (LCTOF/LCQTOF)



Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) - Control

Sample Chromatogram



Establishing Acceptance Criteria

- A sample is considered positive if the following are true:
  - The area is greater than or equal to 25% of the average area of the responses for the two decision point cut off calibrators (10ng/mL).
  - The retention times are within +/- 0.02 min of the average retention time of the decision point cut off calibrators.
  - The Mass Accuracy is within +/- 30ppm

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Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) - Control

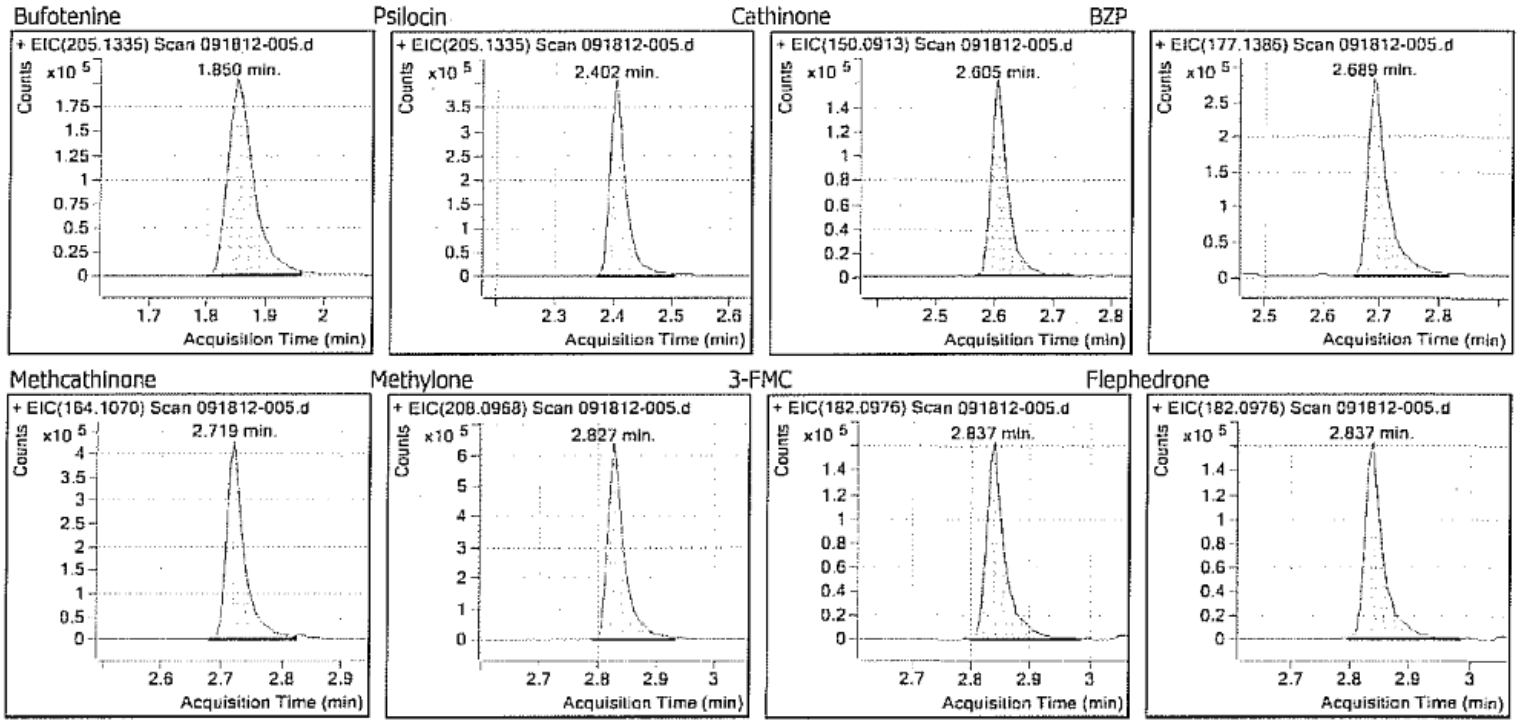
Quantitative Analysis Sample Report

Compound	RT	RT Diff.	Response	Conc. (%)	Mass Acc. (ppm)
PMA	3.209	0.001	452659	99.9	-5.16
2C-N	3.225	0.001	185735	99.6	-7.55
AMT	3.249	0.001	411711	101.3	-13.03
Butylone	3.301	0.001	1521474	100.0	-14.58
2C-H	3.331	0.001	843056	100.0	-19.41
MDEA	3.336	0.001	1515032	100.0	-16.40
ISTD: D3-Atropine	3.344	0.001	107620	101.1	-13.16
Atropine	3.347	0.001	1113319	100.3	-11.48
DET	3.374	0.001	1582414	100.4	-14.83
Mephedrone	3.436	0.001	1402744	97.7	-14.49
MBZP	3.455	0.001	743235	104.9	-14.23
DMAA	3.581	0.000	1679767	94.7	-23.41
MBDB	3.608	0.001	1536053	98.0	-14.97
BDB	3.613	0.002	590746	98.3	-15.73
ISTD: D4-Ketamine	3.664	0.001	1111032	99.2	-11.15
Ketamine	3.679	0.002	993068	97.5	-10.94
ISTD: D4-Norketamine	3.680	0.001	594512	98.9	-12.51
4-MEC	3.688	0.002	1429102	96.3	-14.08

Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF)

Quantitative Analysis Sample Report

Compound Graphics



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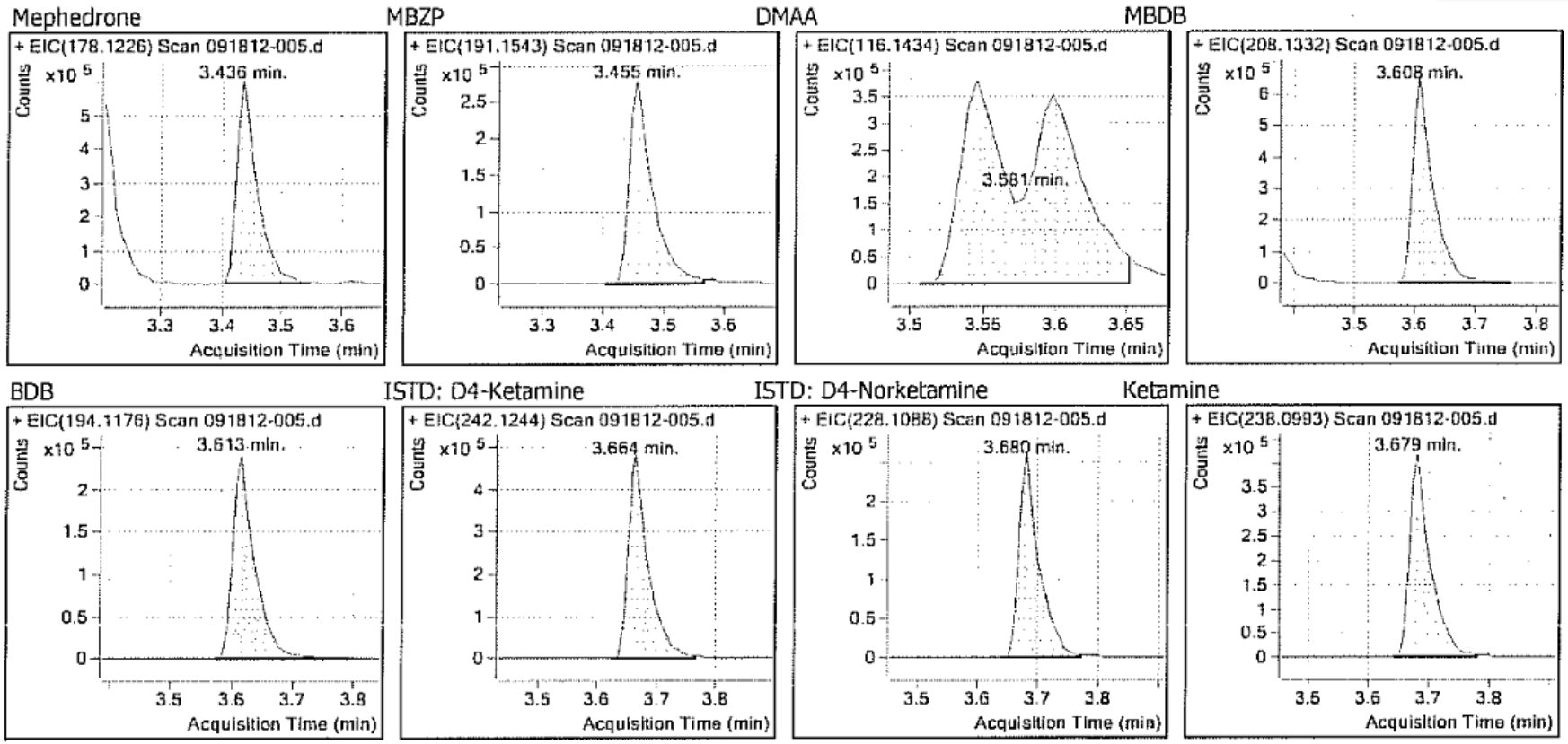
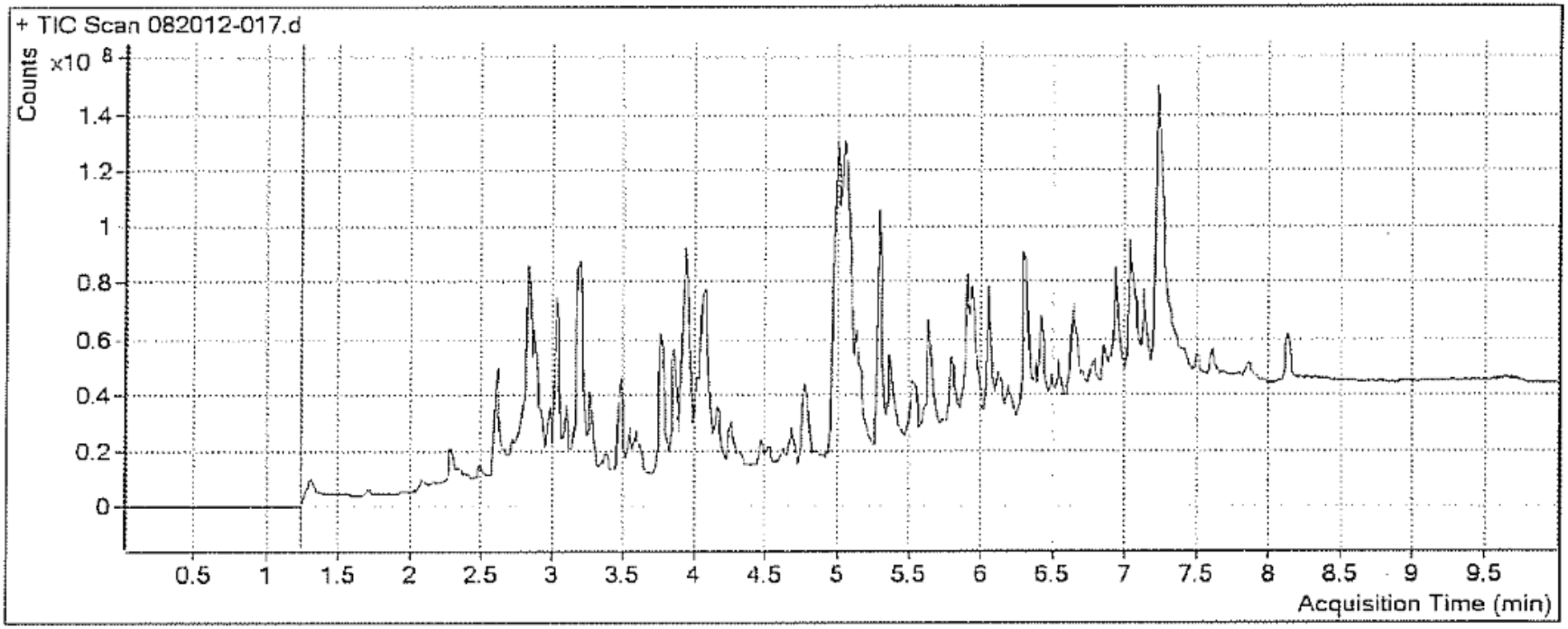


Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) – Case 1

Sample Chromatogram



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Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) – Case 1

Quantitation Results

Compound	RT	RT Diff.	Response	Conc. (%)	Mass Acc. (ppm)
ISTD: D5-MDA	2.973	0.002	1473851	71.7	-8.70
ISTD: D5-MDMA	3.017	0.001	4297762	68.2	-7.93
O-Desmethyltramadol	3.041	0.002	60706092	812.0	-1.30
Butylone	3.208	0.016	2850715	36.0	161.46
Atropine	3.254	0.021	587567	9.3	-0.92
ISTD: D3-Atropine	3.275	0.005	5344126	86.7	-0.25
ISTD: D4-Ketamine	3.593	0.002	3004223	60.3	-0.30
ISTD: D4-Norketamine	3.608	0.002	2194775	81.8	2.68
m-CPP	3.780	0.003	4640588	128.3	0.17
Tramadol	3.856	0.002	81750798	228.0	3.66
Trazodone	4.611	0.001	5973013	13.6	0.30
ISTD: D5-PCP	4.667	0.003	3267090	48.7	-1.96

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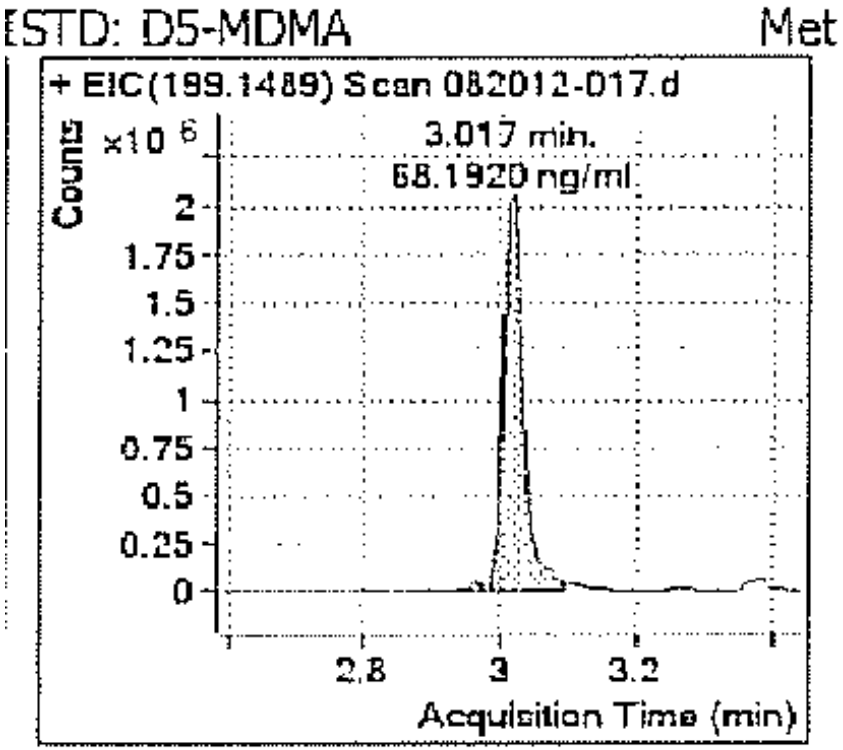

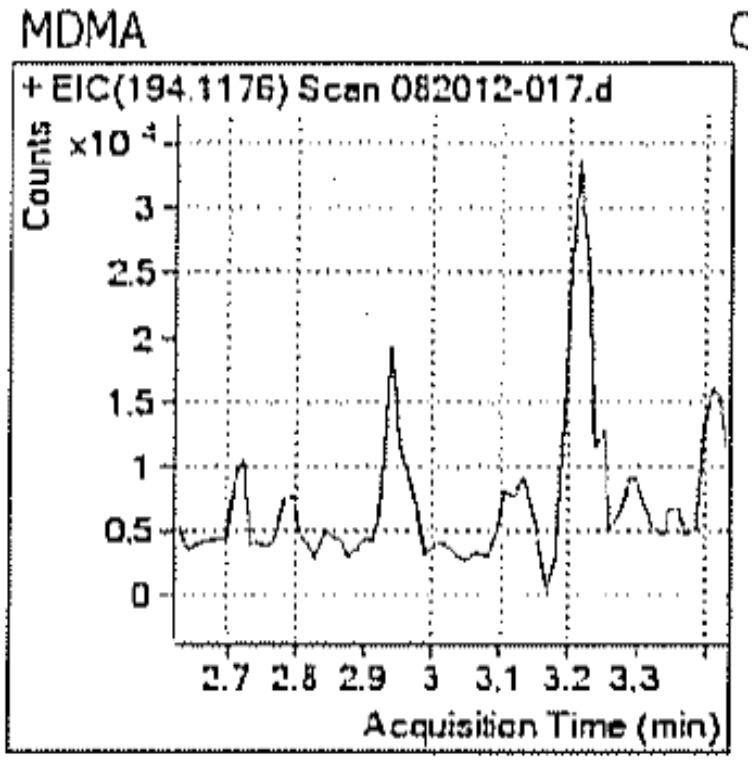

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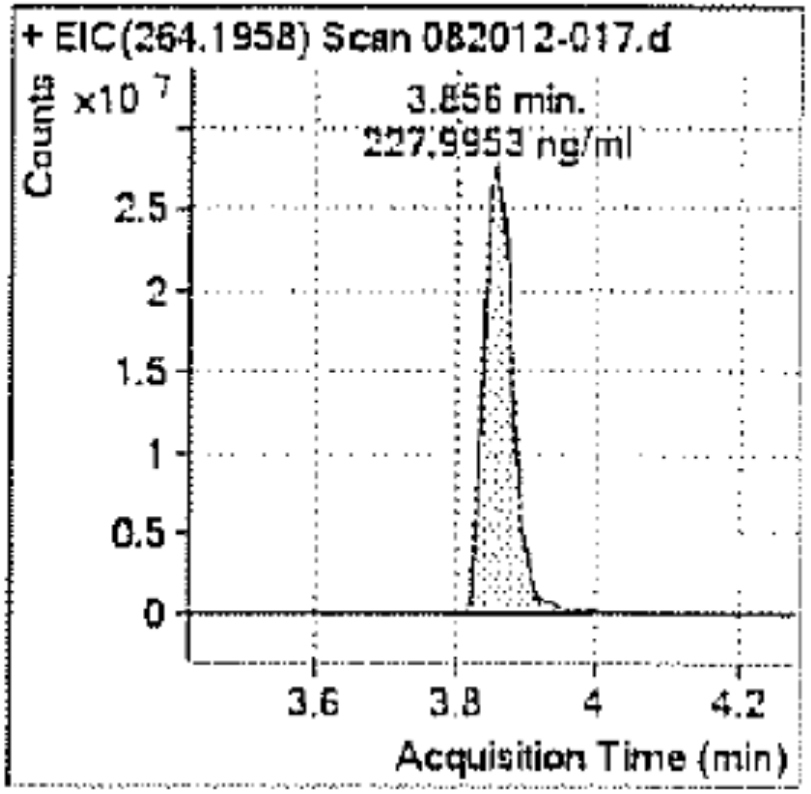
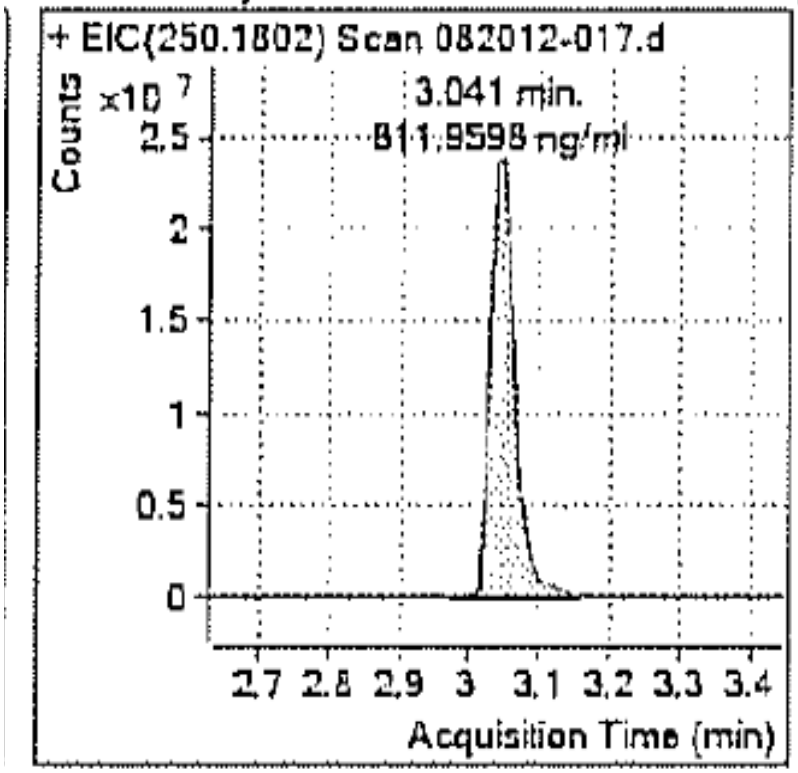
Tramadol



O-Desmethyltramadol



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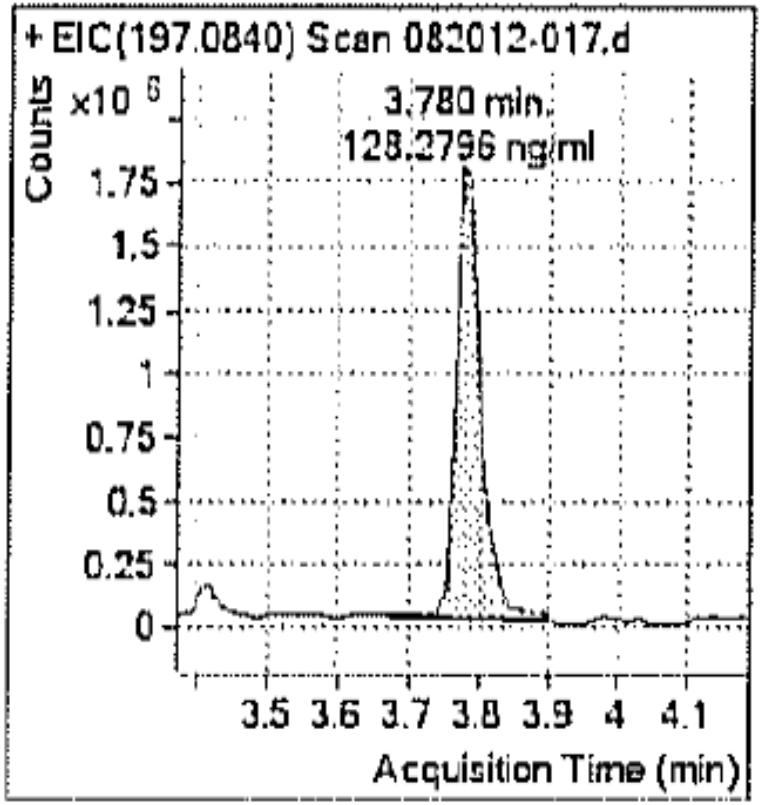
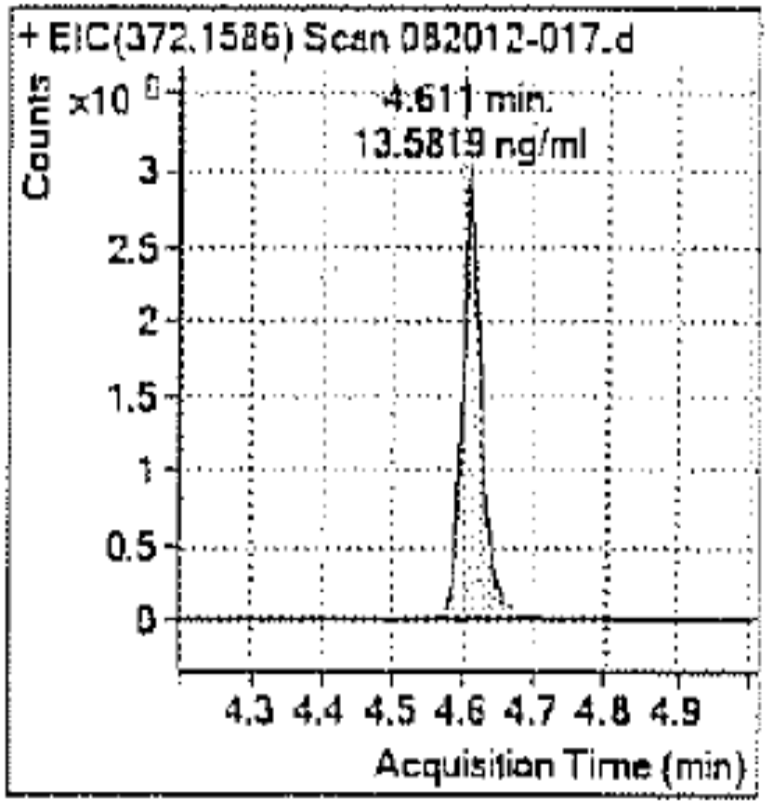
m-CPP



5 Trazodone



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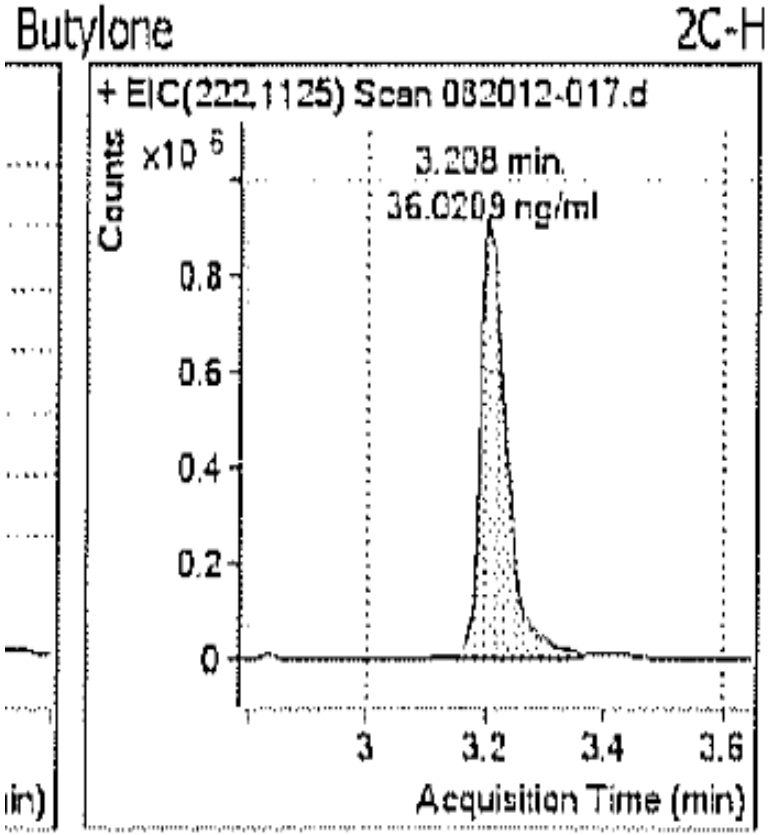

Criterion	Value
Mass Defect	161.46ppm
RT Diff	0.016min
Apparent Conc.	36ng/mL

Not Confirmed ✗

NMS LABS

Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) – Case 2

Quantitation Results

Compound	RT	RT Diff.	Response	Conc. (%)	Mass Acc. (ppm)
Methylone	2.834	0.001	6658897	555.8	-2.50
ISTD: D5-MDA	3.056	0.002	484166	107.5	0.51
ISTD: D5-MDMA	3.100	0.001	1378904	99.6	2.35
Methedrone	3.106	0.002	7572913	571.8	0.28
MDMA	3.106	0.002	7572913	571.8	0.28
Butylone	3.309	0.002	7612872	526.6	-2.65
ISTD: D3-Atropine	3.351	0.002	92844	93.2	-1.48
Mephedrone	3.445	0.002	6712093	503.4	-6.75
ISTD: D4-Ketamine	3.672	0.002	1024182	100.4	1.89
ISTD: D4-Norketamine	3.685	0.001	943740	169.0	-1.09
4-MEC	3.697	0.002	7686210	538.9	-2.68
MDPV	4.167	0.000	8914268	576.5	-0.26
ISTD: D5-PCP	4.748	0.001	1400009	104.0	2.51

NMS LABS

Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) – Case 2

Quantitation Results

Compound	RT	RT Diff.	Response	Conc. (%)	Mass Acc. (ppm)
Methylone	2.834	0.001	6658897	555.8	-2.50
ISTD: D5-MDA	3.056	0.002	484166	107.5	0.51
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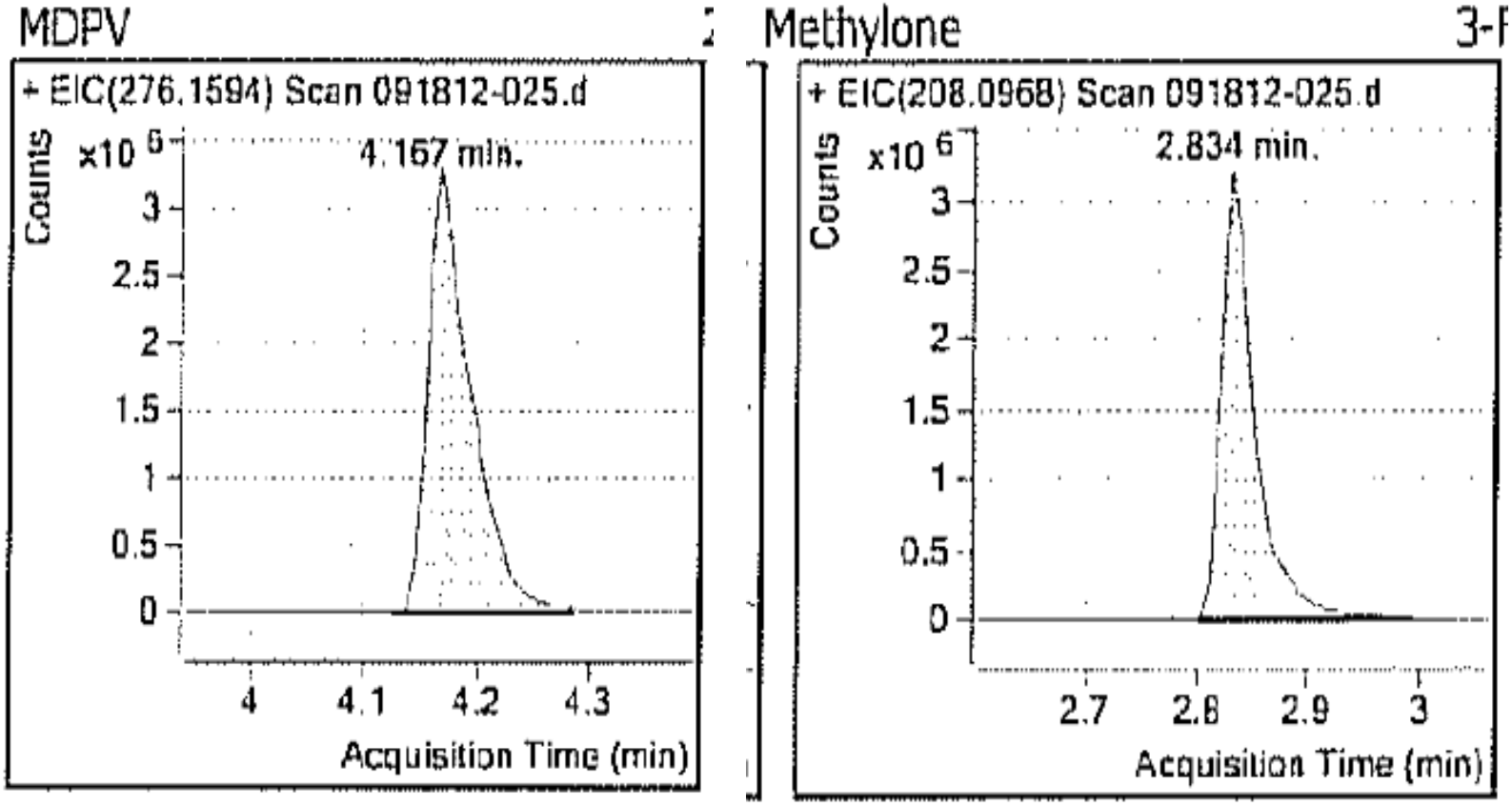
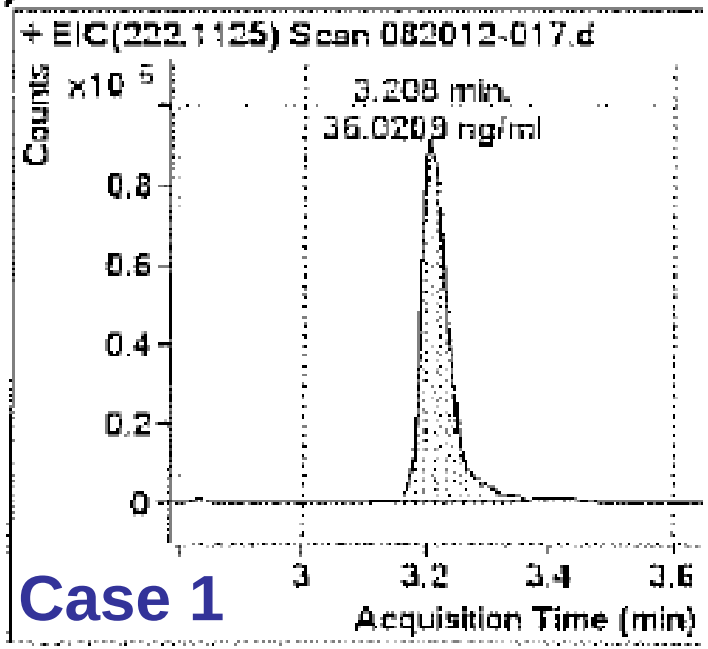


Analysis

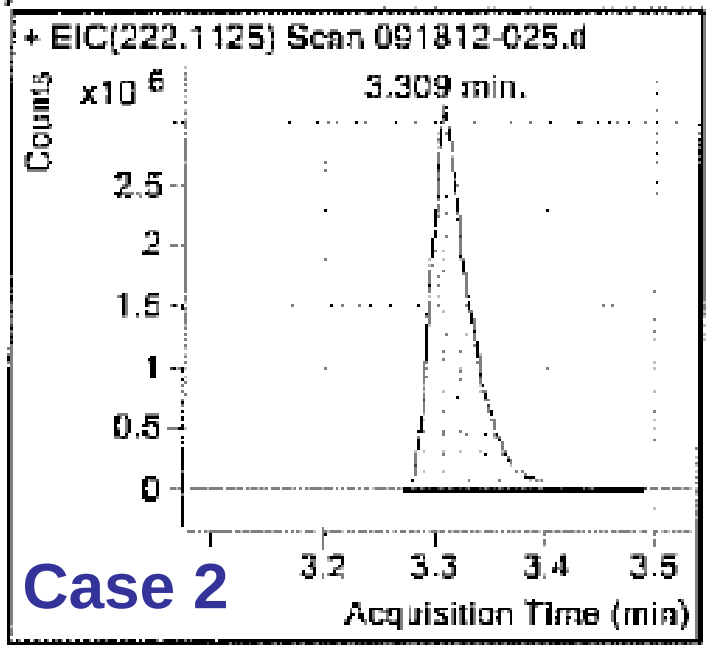
Liquid Chromatography-TOF

Butylone



Case 1

Butylone



Case 2

Criterion	Case 1	Case 2
Mass Defect	161.46ppm	-2.6ppm
RT Diff	0.016min	0.002min
Apparent Conc.	3.6ng/mL	52.6ng/mL
Result	Not Confirmed	Confirmed

Liquid Chromatography-Time of Flight Mass Spectrometry (LCTOF/LCQTOF) – Case 2

Methedrone	3.106	0.002	7572913	571.8	0.28
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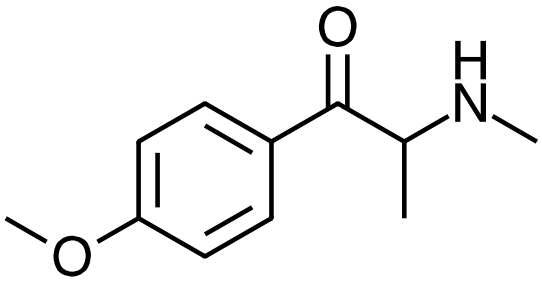
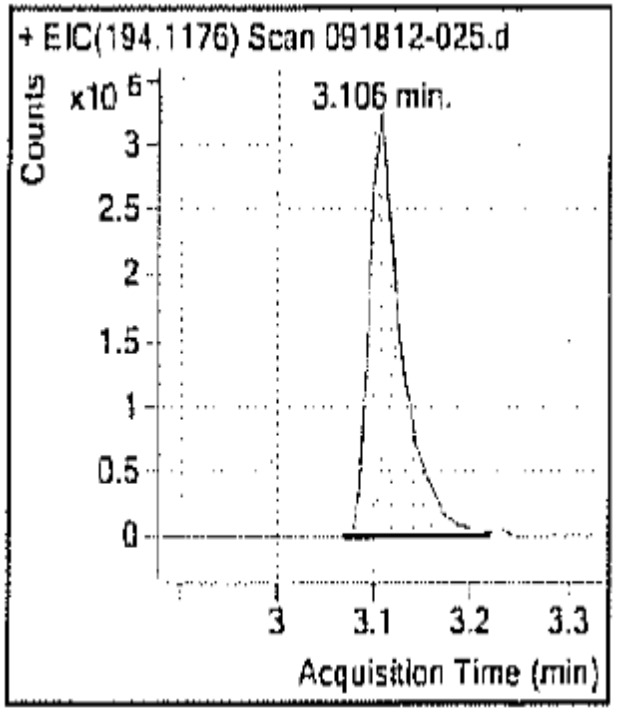
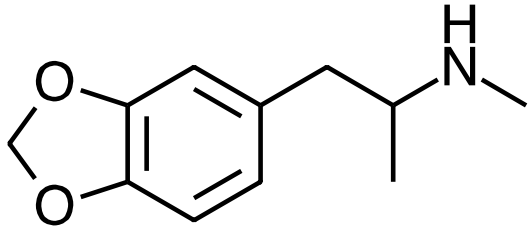


Methedrone
$C_{11}H_{15}NO_2$
193.2423





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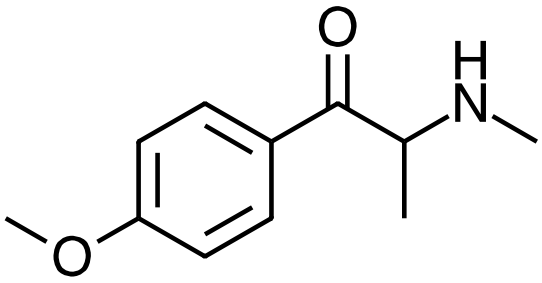

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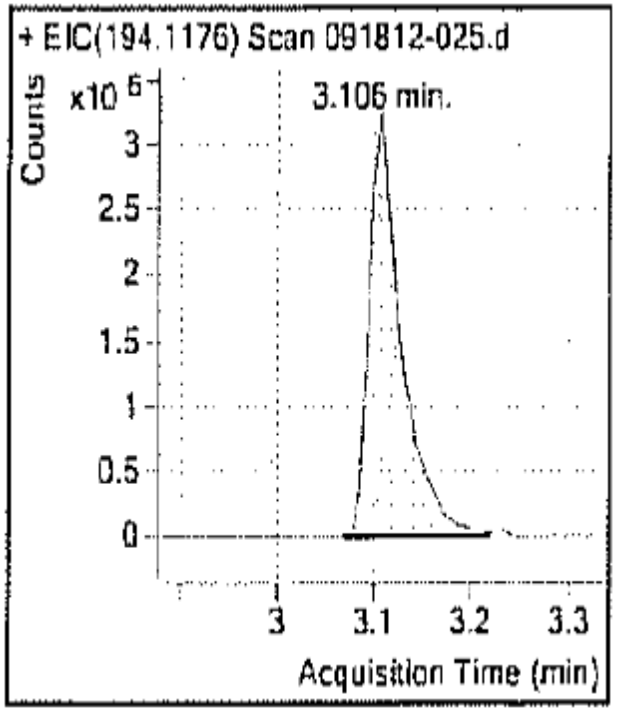
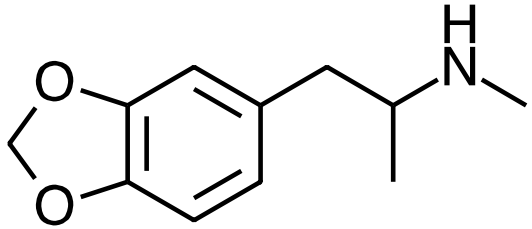




MDMA
$C_{11}H_{15}NO_2$
193.2423

Confirmed by LCMSMS √

Qualitative Validation

- Assay Validation
  - Cut-off Verification
  - Sensitivity and Specificity
  - Interfering Substances
  - Carryover
  - Matrix Matching
  - Stability

Time of Flight

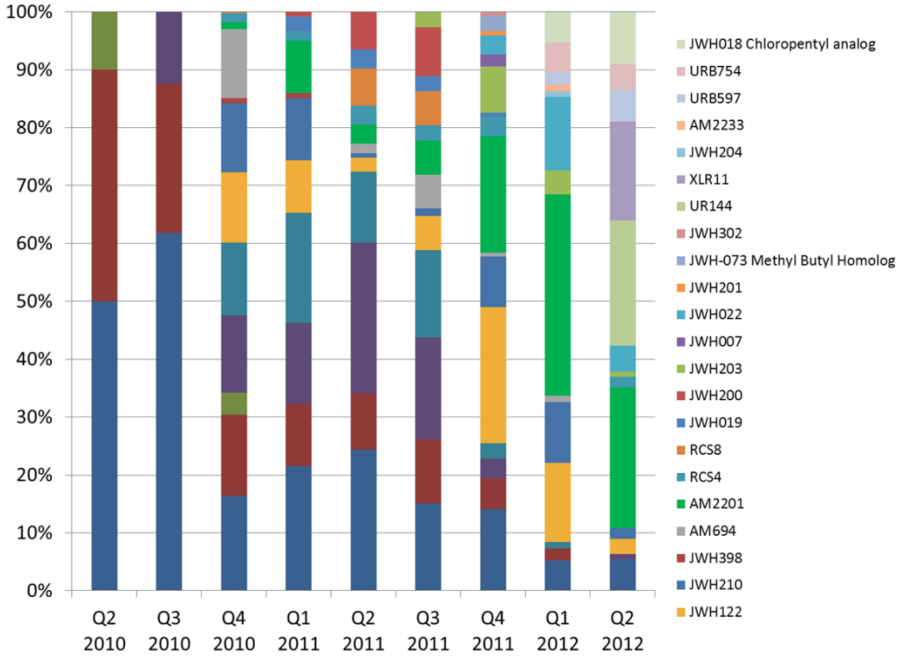
8756B/U Bath Salts and Stimulants Designer Drugs – Expanded

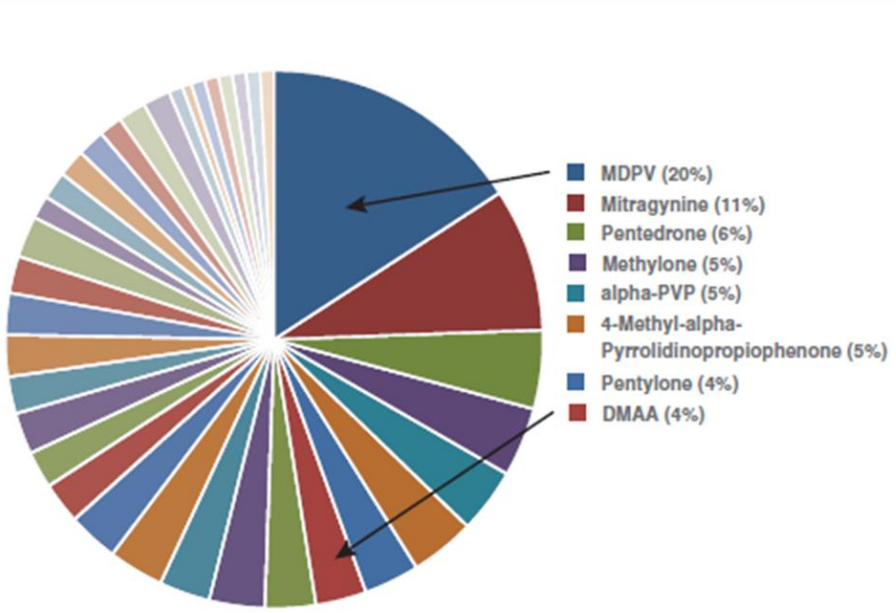
8085B/U Drug Impaired Driving/DRE Forensic Toxicology Bath Salts Add-On

- Test design based on:
- 43 compounds
- Regular updates based on trends
- Compounds found in “bath salts” casework
- Federally Scheduled Drugs
- Covers additional drugs scheduled in many states
- Drugs emerging in Europe

Designer Drugs Trends

NMS Labs Designer Drugs Trends Report August 2012





http://www.nmslabs.com/services-forensic-designer-drug-trends

Compare and Contrast

LCMSMS	LCTOF	LCQTOF
Target Compound Analysis	Comprehensive Screening	Target Compound analysis (Range)
Must set up scanning groups	Acquire all data and query database later	Acquires more limited data in QTOF mode
WYSIWYG	Post hoc identification	Post hoc identification
Compound specific transitions	Molecular formula information	Molecular formula information for fragments
Limited structural confirmation	No structural information	Limited Structural information
May differentiate isobars	TOF does not differentiate isobars	May differentiate isobars
Lower Cost	Higher cost	Even higher cost

Acknowledgements

- **NMS Labs**
- Alex Maggitti
- Sherri Kacinko
- Matthew McMullin
- Fran Diamond

- **Agilent**
- Tom Gluodenis
- Jay Levine
- Mary Cuddrye
- John Hughes