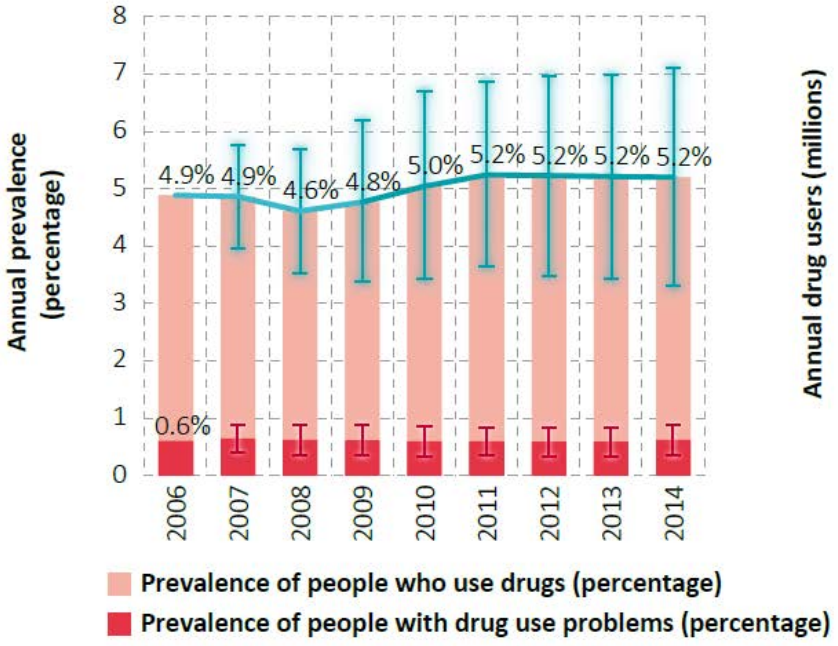
Comprehensive Analytical Tools for the Identification of Emerging Drugs of Abuse

Luis E. Arroyo, Anthony P. DeCaprio, Tom Gluodenis, Ana Michelle Broomes, Melanie Eckberg, Ashley Kimble and Joshua Z. Seither

Dept. of Chemistry & Biochemistry
International Forensic Research Institute
Florida International University
Miami, FL

Global Drug Numbers

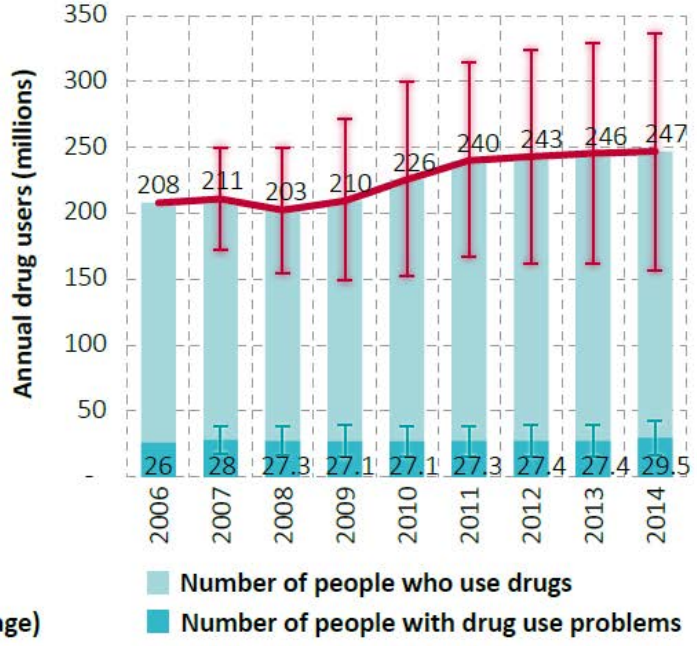
Global trends in the estimated prevalence of drug use, 2006-2014



Source: Responses to the annual report questionnaire.

Note: Estimated percentage of adults (ages 15-64) who used drugs in the past year.

Global trends in the estimated number of people who use drugs, 2006-2014

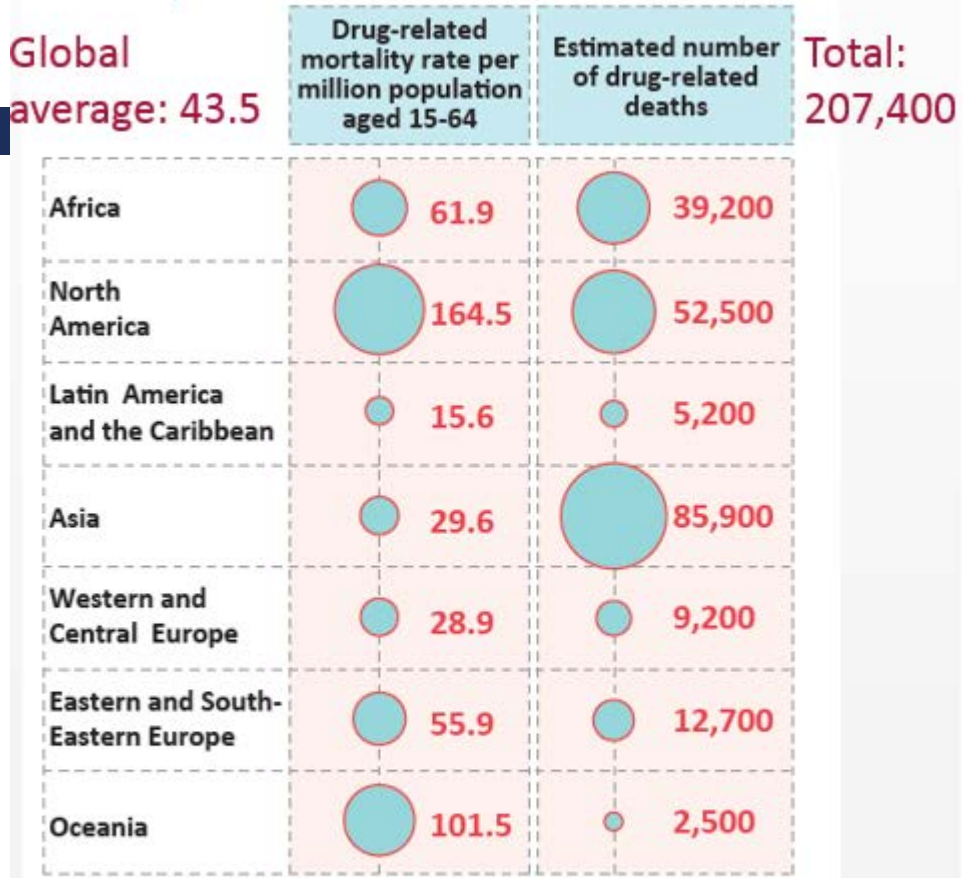


Source: Responses to the annual report questionnaire.

Note: Estimates are for adults (ages 15-64), based on past-year use.

Source: United Nations Office on Drugs and Crime 2016 Report

FIG. 18 Drug-related mortality rate and number of drug-related deaths, by region, 2014



Sources: responses to the annual report questionnaire; Inter-American Drug Abuse Control Commission; and Louisa Degenhardt and others, "Illicit drug use", in *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors*, vol. 1, Majid Ezzati and others, eds. (Geneva, World Health Organization (WHO), 2004), p. 1,109.

WORLD DRUG REPORT 2016

http://www.unodc.org/doc/wdr2016/WORLD_DRUG_REPORT_2016_web.pdf

NPS Worldwide

FIG. 75. Number of new psychoactive substances reported, 2009-2014

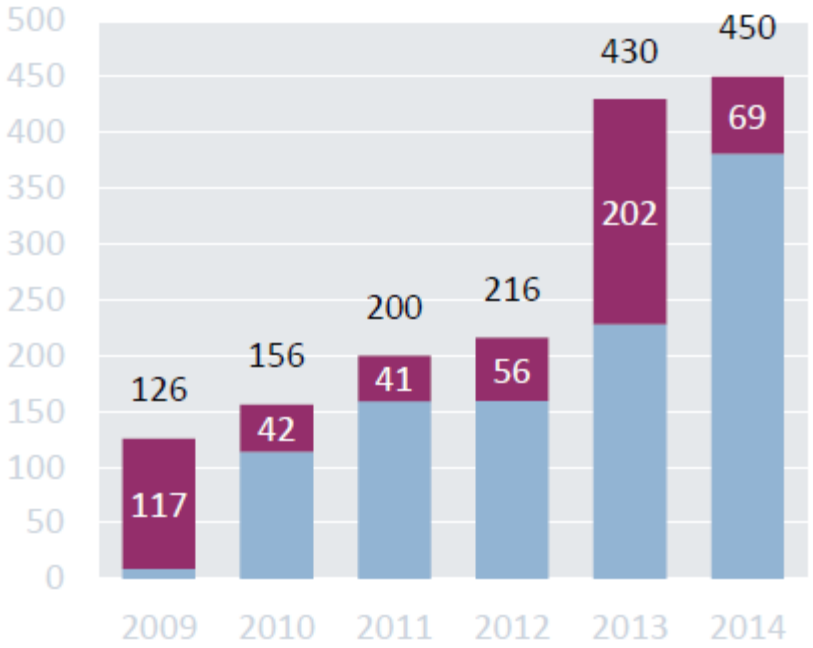
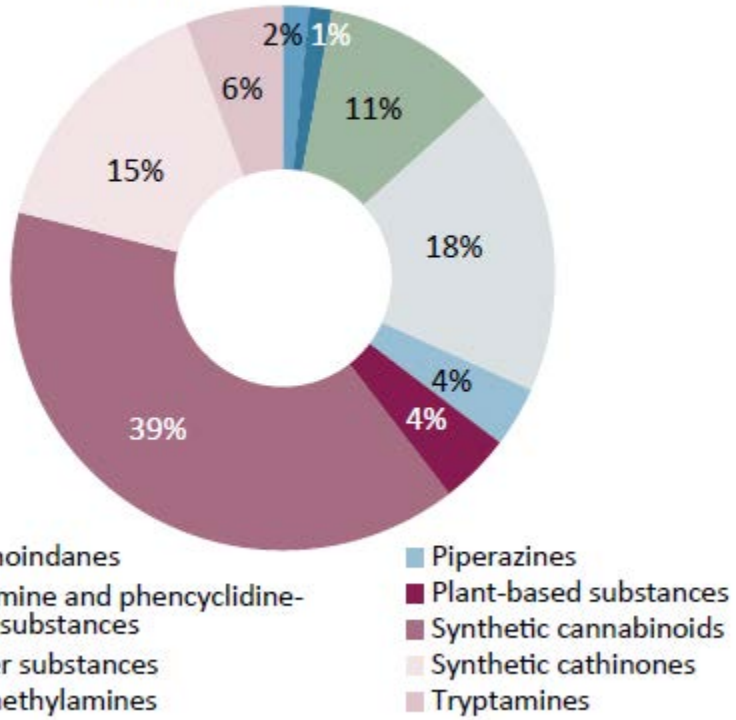


FIG. 76. Number of new psychoactive substances reported, by substance group, 2014



Source: UNODC, early warning advisory on NPS, 2014.

USA Numbers

Table 1.1 NATIONAL AND REGIONAL ESTIMATES FOR THE 25 MOST FREQUENTLY IDENTIFIED DRUGS[1]

Estimated number and percentage of total drug reports submitted to laboratories from January 1, 2014, through December 31, 2014, and analyzed by March 31, 2015

	National		West		Midwest		Northeast		South	
Drug	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Cannabis/THC	437,117	28.92%	50,803	19.61%	144,993	38.42%	75,154	30.36%	166,168	26.49%
Methamphetamine	236,175	15.63%	104,424	40.31%	38,983	10.33%	3,221	1.30%	89,547	14.27%
Cocaine	213,167	14.10%	18,671	7.21%	42,571	11.28%	48,884	19.75%	103,041	16.42%
Heroin	163,600	10.83%	27,418	10.59%	48,950	12.97%	51,924	20.98%	35,308	5.63%
Oxycodone	43,000	2.85%	4,289	1.66%	7,913	2.10%	9,414	3.80%	21,385	3.41%
Alprazolam	40,747	2.70%	3,310	1.28%	7,780	2.06%	5,829	2.36%	23,828	3.80%
Hydrocodone	33,132	2.19%	4,418	1.71%	7,596	2.01%	1,634	0.66%	19,484	3.11%
Buprenorphine	15,209	1.01%	1,251	0.48%	3,014	0.80%	4,539	1.83%	6,405	1.02%
Clonazepam	11,797	0.78%	1,103	0.43%	2,477	0.66%	2,376	0.96%	5,841	0.93%
Amphetamine	11,531	0.76%	1,140	0.44%	3,070	0.81%	1,517	0.61%	5,804	0.93%
XLR11	11,001	0.73%	1,244	0.48%	1,920	0.51%	2,935	1.19%	4,903	0.78%
Morphine	7,620	0.50%	1,191	0.46%	1,797	0.48%	615	0.25%	4,018	0.64%
AB-FUBINACA	6,293	0.42%	249	0.10%	1,647	0.44%	455	0.18%	3,942	0.63%
Noncontrolled, non-narcotic[2]	5,724	0.38%	2,149	0.83%	50	0.01%	580	0.23%	2,946	0.47%
Methadone	5,559	0.37%	837	0.32%	1,077	0.29%	1,237	0.50%	2,407	0.38%
Diazepam	5,446	0.36%	746	0.29%	1,322	0.35%	508	0.21%	2,870	0.46%
Ethylone	5,425	0.36%	310	0.12%	435	0.12%	879	0.36%	3,801	0.61%
Phencyclidine (PCP)	5,004	0.33%	401	0.15%	990	0.26%	1,773	0.72%	1,840	0.29%
AB-PINACA	4,954	0.33%	357	0.14%	1,738	0.46%	496	0.20%	2,363	0.38%
MDMA	4,902	0.32%	1,915	0.74%	1,492	0.40%	421	0.17%	1,074	0.17%
Methylone	4,768	0.32%	679	0.26%	403	0.11%	797	0.32%	2,890	0.46%
Fentanyl	4,642	0.31%	119	0.05%	1,683	0.45%	1,545	0.62%	1,295	0.21%
Hydromorphone	4,629	0.31%	306	0.12%	572	0.15%	155	0.06%	3,597	0.57%
Psilocin/psilocibin	3,965	0.26%	1,319	0.51%	1,223	0.32%	369	0.15%	1,054	0.17%
alpha-PVP	3,905	0.26%	142	0.05%	807	0.21%	673	0.27%	2,283	0.36%
Top 25 Total	1,289,316	85.31%	228,790	88.33%	324,501	85.98%	217,928	88.05%	518,096	82.58%
All Other Drug Reports	221,997	14.69%	30,231	11.67%	52,925	14.02%	29,576	11.95%	109,264	17.42%
Total Drug Reports[3]	1,511,313	100.00%	259,021	100.00%	377,426	100.00%	247,505	100.00%	627,360	100.00%

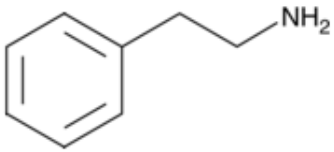
XLR11=[1-(5-Fluoro-pentyl)1H-indol-3-yl],(2,2,3,3-tetramethylcyclopropyl)methanone

AB-FUBINACA=(N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide)

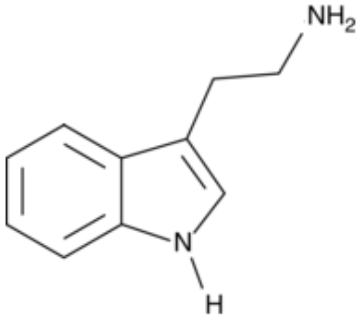
New Psychoactive Substances (NPS)

- Synthetic alternatives to traditional illegal drugs of abuse.
  - Stimulant-like NPS: (e.g. “Bath salts”).
  - Marijuana-like NPS: e.g. “spice”.
  - LSD-like NPS: e.g. “N-bombs”.

Designer Drugs

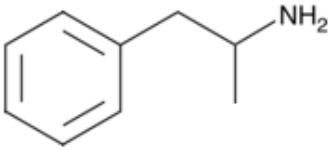


Phenethylamines

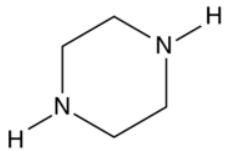


Amphetamines

Tryptamines

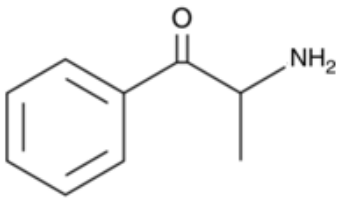


"Analogs or derivatives of illicit drugs with modified structure"

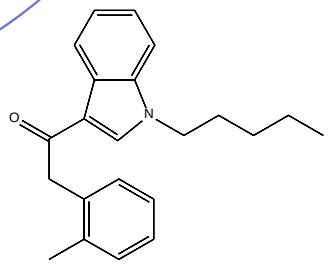


Cathinones

Piperazines



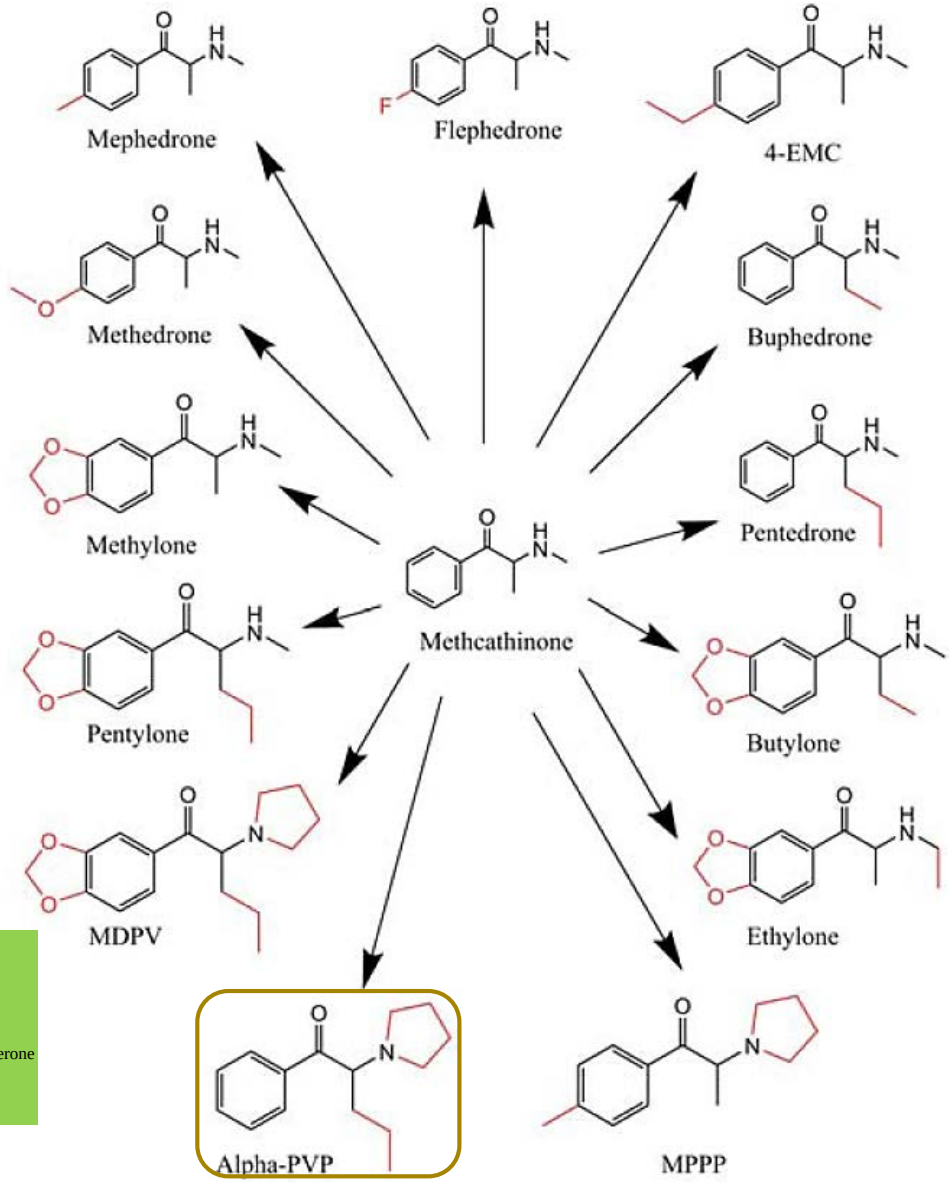
Cannabinoids

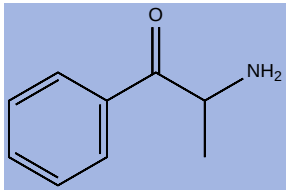


http://www.nj.gov/oag/newsreleases11/pr20110817a.html

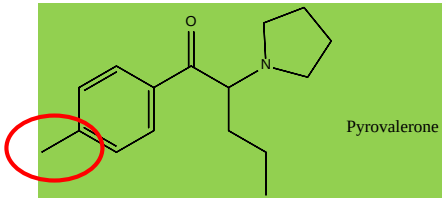
Synthetic Cathinones

- Structurally and pharmacologically related to amphetamine, ecstasy (MDMA), cathinone.
- CNS stimulants.
- Sold in retail stores, internet, “head shops” as batch salts, plant food.





Cathinone



AAPCC: 10,403 human exposures to synthetic cathinones
(from Jan 2011 and Dec 31,2014)

MS-Based Screening of Designer Drugs: Previous Work

- Peters, F.T., et al. (2003). *J.Mass Spectrom.* 38, 659-676. (***18 amphetamines and piperazines***)
- Kölliker, S., and Oehme, M. (2004). *Anal.Bioanal.Chem.* 378, 1294-1304. (***55 phenethylamines***)
- Takahashi, M., et al. (2009). *Talanta* 77, 1245-1272. (***104 analytes***)
- Wohlfarth, A., et al. (2010). *Anal.Bioanal.Chem.* 396, 2403-2414. (***35 analytes***)
- Shanks, K.G., et al. (2012). *J.Anal.Toxicol.* 36, 360-371. (***33 cannabinoids; 26 cathinones/phenethylamines***)
- Ammann, J., et al. (2012). *J.Anal.Toxicol.* 36, 372-380. (***23 cannabinoids***)
- Ammann, D., et al. (2012). *J.Anal.Toxicol.* 36, 381-389. (***25 cathinones and phenethylamines***)
- Guale, F., et al. (2013). *J.Anal.Toxicol.* 37, 17-24. (***32 cannabinoids/cathinones***)
- Scheidweiler KB, Jarvis MJ, Huestis, MA (2015). Anal. Bioanal. Chem. 407, 883-897 (**47 synthetic cannabinoids metabolites in urine**)

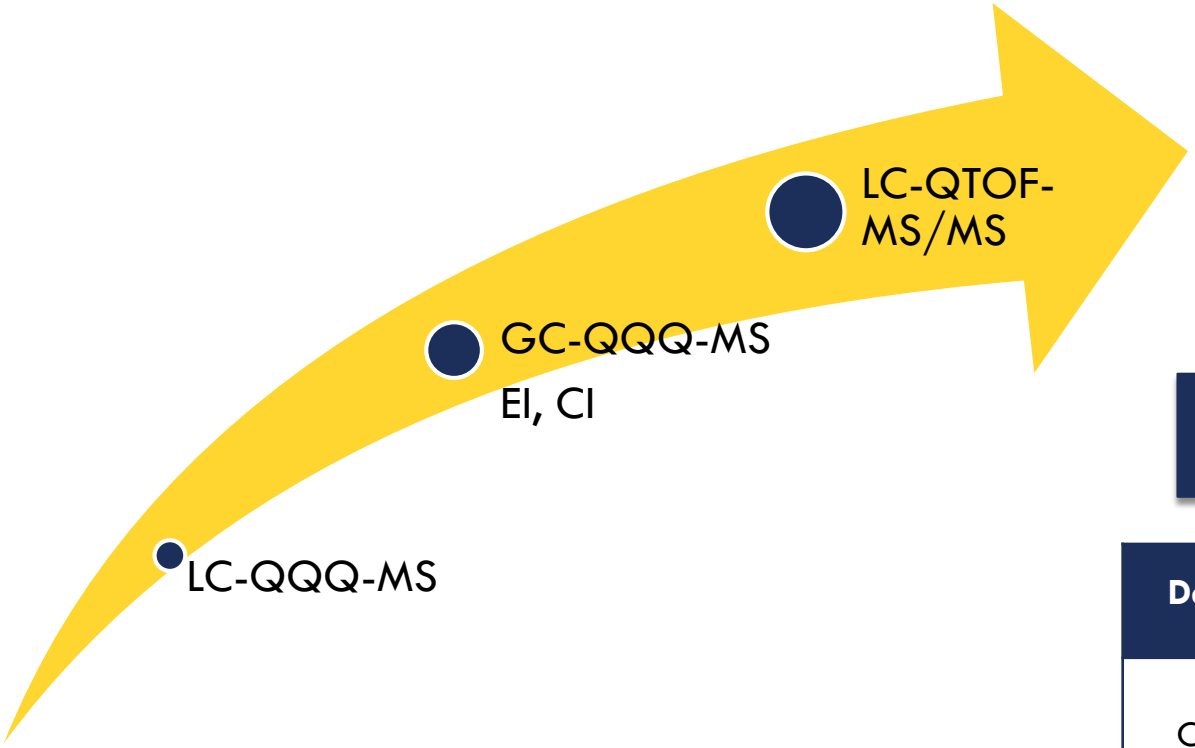
Problem statement:

There is a need for comprehensive methods capable of screening the larger universe of these drugs in clinical and forensic toxicological settings

Research Objective:

To develop a robust, rapid and comprehensive MS-based screening methods for designer drugs and to provide a novel and ample MS spectral database and library.

Evolution of Method Development and Overarching Goal



Screening Analytical Methods Mass Spectral Database (MS/MS and MRM) Libraries

Total of 275 Designer Drugs

Designer Drug Class	Amount in Library
Synthetic Cannabinoids	153
Cathinone	58
Phenethylamine	23
Other	11
Piperazine	8
Tryptamine	7
Indanes	3

Anal Bioanal Chem (2013) 405:1383–1397
DOI 10.1007/s00216-012-6548-8

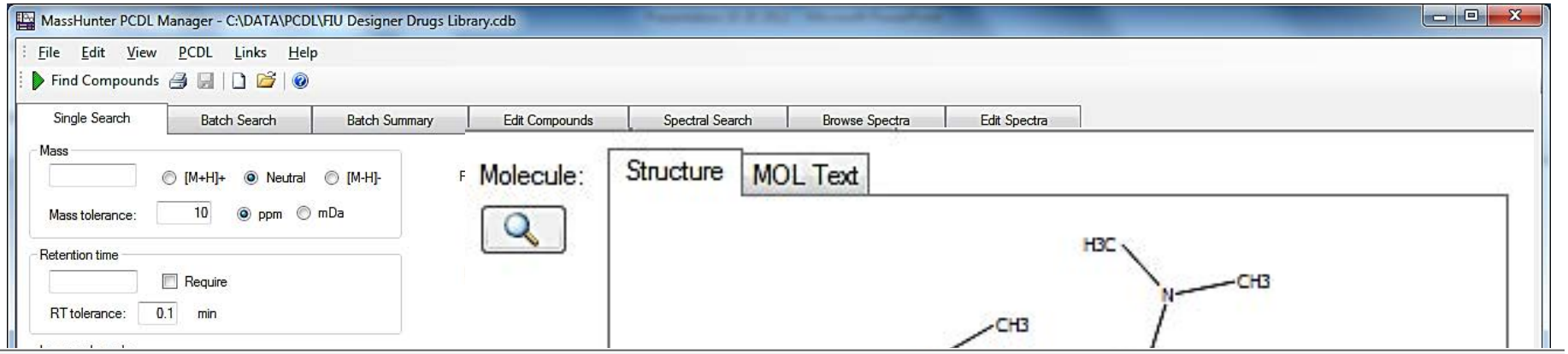
ORIGINAL PAPER

Determination of 32 cathinone derivatives and other designer drugs in serum by comprehensive LC-QQQ-MS/MS analysis

Madeleine J. Swortwood · Diane M. Boland · Anthony P. DeCaprio

Designer Drug "Master List"

- Master List of current or potential designer drugs/metabolites, identified from:
  - Published literature.
  - Government documentation.
  - Commercial standard supplier listings.
  - "PiHKAL" and "TiHKAL" by Alexander & Ann Shulgin.
  - Online drug forums:
    - http://www.bluelight.ru/vb/
    - http://www.drugs-forum.com/index.php
- Currently at 857 unique entries.
- Information collected:
  - Structure.
  - Molecular formula.
  - Accurate mass.
  - IUPAC name.
  - Common name or abbreviation.
  - CAS and Chemspider number (if available).
  - Literature citations.
- Unique ID assigned and data compiled into a Personal Compound Database Library (PCDL; Agilent Technologies).
- Standards available for 275 compounds (Initial Batch of DD).



Single Search Results: 615 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Num Spectra
2C-C	C10H14Cl...	215.07131	☐	☐		88441-14-9	21106221	4-chloro-2,5-dimethoxy-Benzeneethanamine	3
Fencamfamine	C15H21N	215.16740	☐	☐		2240-14-4	13922	N-ethyl-3-phenyl-norboman-2-amine	0
DET	C14H20N2	216.16265	☐	☐		61-51-8	5865	N,N-Diethyl-2-(1H-indol-3-yl)ethanamine	0
MIPT	C14H20N2	216.16265	☐	☐		96096-52-5	21106353	N-methyl-N-(1-methylethyl)-1H-Indole-3-ethanamine	0
4-MePPP	C14H19NO	217.14666	☐	☐		28117-80-8	4936084	1-(4-methylphenyl)-2-(1-pyrrolidinyl)-1-Propanone	0
α-Pyrrolidinobutiophenone	C14H19NO	217.14666	☐	☐		13415-82-2		1-phenyl-2-(1-pyrrolidinyl)-1-butanone	3
PCPr	C15H23N	217.18305	☐	☐			521518	N-(1-phenylcyclohexyl)propanaminee	0
5-MeO-DMT	C13H18N...	218.14191	☐	☐		1019-45-0	1766	2-(5-methoxy-1H-indol-3-yl)-N,N-dimethylethanamine	3
4-MeO-DMT	C13H18N...	218.14191	☐	☐		3965-97-7	23126449	2-(4-methoxy-1H-indol-3-yl)-N,N-dimethyl-ethanami...	3
4-HO-MET	C13H18N...	218.14191	☐	☐		77872-41-4	10513072	3-[2-[ethyl(methyl)amino]ethyl]-1H-indol-4-ol	0
5-HTP	C11H12N...	220.08479	☐	☐		56-69-9	388413	2-amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid	0
MDBZP [1-(3,4-Methylenedioxybenzyl)piperazine]	C12H16N...	220.12118	☐	☐		32231-06-4	85214	1-(benzo[1,3]dioxol-5-ylmethyl)piperazine	0
Dehydronorketamine	C12H12Cl...	221.06074	☐	☐		57683-62-2	142954	6-amino-6-(2-chlorophenyl)cyclohex-2-en-1-one	0
Butylone	C12H15N...	221.10519	☐	☐		802575-1...	21106270	1-(1,3-benzodioxol-5-yl)-2-(methylamino)-1-Butanone	3
bk-MDDMA	C12H15N...	221.10519	☐	☐		765231-5...	7970239	1-(1,3-benzodioxol-5-yl)-2-(dimethylamino)-1-Propa...	0
Ethylone (bk-MDEA)	C12H15N...	221.10519	☐	☐			21106271	(RS)-1-(1,3-benzodioxol-5-yl)-2-(ethylamino)propan...	3
N-Acetyl-3,4-methylenedioxyamphetamine	C12H15N...	221.10519	☐	☐		36209-71-9	262493	N-[1-(1,3-benzodioxol-5-yl)propan-2-yl]acetamide	0
MDPR (3,4-Methylenedioxy-N-propylamphetamine)	C13H19N...	221.14158	☐	☐			10723901	N-[1-(1,3-benzodioxol-5-yl)propan-2-yl]propan-1-a...	0
Norketamine	C12H14Cl...	223.07639	☐	☐		35211-10-0	110322	2-Amino-2-(2-chlorophenyl)cyclohexanone	0
Tiletamine	C12H17N...	223.10308	☐	☐			24714	2-(Ethylamino)-2-(2-thienyl)cyclohexanone	0
4-MTPA	C13H21NS	223.13947	☐	☐		634607-2...		α-methyl-4-(methylthio)-N-propyl-Benzeneethanam...	0
(3,4-DMMA)	C13H21N...	223.15723	☐	☐		58993-77-4		3,4-dimethoxy-N,N,α-trimethyl-Benzeneethanamine	0

MassHunter PCDL Manager - C:\DATA\PCDL\FIU Designer Drugs Library.cdb

File Edit View PCDL Links Help

Find Compounds

Single Search | Batch Search | Batch Summary | Edit Compounds | Spectral Search | Browse Spectra | Edit Spectra

Mass

[M+H]+ Neutral [M-H]-

Mass tolerance: 10 ppm mDa

Retention time

Require

RT tolerance: 0.1 min

Ion search mode

- [x] Include neutrals
- [x] Include anions
- [x] Include cations

Formula:

Name: JWH

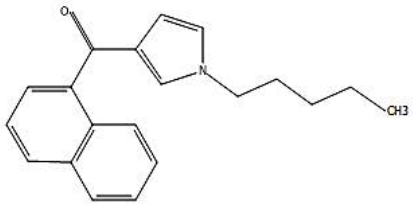
Notes:

IUPAC:

CAS:

ChemSpider:

Molecule: Structure | MOL Text



Notes: FIU_0207

Cayman Chemical Item Number 10831

Single Search Results: 169 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Num Spectra
JWH-030	C20H21NO	291.16231				162934-7...	8147131	1-naphthalenyl(1-pentyl-1H-pyrrol-3-yl)-methanone	3
JWH-031	C21H23NO	305.17796				162934-7...		(1-hexyl-1H-pyrrol-3-yl)-1-naphthalenyl-methanone	3
JWH 133	C22H32O	312.24532					5293702	(6aR,10aR)-3-(1,1-Dimethylbutyl)-6a,7,10,10a-tetr...	0
JWH-072	C22H19NO	313.14666				209414-0...	24629912	1-naphthalenyl(1-propyl-1H-indol-3-yl)-methanone	3
JWH-251 4-methylphenyl isomer	C22H25NO	319.19361				864445-4...		2-(4-methylphenyl)-1-(1-pentyl-1H-indol-3-yl)-ethan...	3
JWH-251 3-methylphenyl isomer	C22H25NO	319.19361						1-(1-pentyl-1H-indol-3-yl)-2-(m-tolyl)ethanone	3
JWH-251	C22H25NO	319.19361				864445-3...	9791472	2-(2-methylphenyl)-1-(1-pentyl-1H-indol-3-yl)-metha...	3
JWH 176	C25H24	324.18780						1-[[(1E)-3-pentylinden-1-ylidine]methyl]naphthalene	0
JWH-015	C23H21NO	327.16231				155471-0...	3480676	(2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenyl-m...	3
JWH-073 2'-naphthyl isomer	C23H21NO	327.16231						(1-butyl-1H-indol-3-yl)(naphthalen-2-yl)-methanone	3
JWH-073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	C23H21NO	327.16231						(1-(tert-butyl)-1H-indol-3-yl)(naphthalen-2-yl)metha...	3
JWH-073 2'-naphthyl-N-(1-methylpropyl) isomer	C23H21NO	327.16231						(1-(sec-butyl)-1H-indol-3-yl)(naphthalen-2-yl)-meth...	3
JWH-073 2'-naphthyl-N-(2-methylpropyl) isomer	C23H21NO	327.16231						(1-isobutyl-1H-indol-3-yl)(naphthalen-2-yl)methanone	3
JWH-073 N-(1,1-dimethylethyl) isomer	C23H21NO	327.16231						(1-(tert-butyl)-1H-indol-3-yl)(naphthalen-1-yl)metha...	3
JWH-073 N-(1-methylpropyl) isomer	C23H21NO	327.16231						(1-(sec-butyl)-1H-indol-3-yl)(naphthalen-1-yl)metha...	3
JWH-073 N-(2-methylpropyl) isomer	C23H21NO	327.16231						(1-isobutyl-1H-indol-3-yl)(naphthalen-1-yl)methanone	3
JWH-073	C23H21NO	327.16231				208987-4...	8647081	(1-butyl-1H-indol-3-yl)-1-naphthalenyl-methanone	3
JWH-175	C24H25N	327.19870				619294-3...		3-(1-naphthalenylmethyl)-1-pentyl-1H-indole	3
JWH 015-d7	C23H14D...	334.20625						(2-methyl-1-propyl-(1,1,2,2,3,3,3-d7)-1H-indol-3-yl)-...	0
JWH 073-d7 (solution)	C23H14D...	334.20625						(1-butyl-1H-indol-3-yl)-1-naphthalenyl-2,2,3,3,4,4,4...	0
JWH-201	C22H25N...	335.18853				864445-4...	23256220	2-(4-methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl)-eth...	3
JWH-302	C22H25N...	335.18853				864445-4...	9668546	2-(3-methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl)-eth...	3

MassHunter PCDL Manager - C:\DATA\PCDL\FIU Designer Drugs Library.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search | Batch Search | Batch Summary | Edit Compounds | Spectral Search | Browse Spectra | Edit Spectra

Mass
Precursor ion:
Tolerance: 200 ppm mDa
Ion polarity:
Ionization:

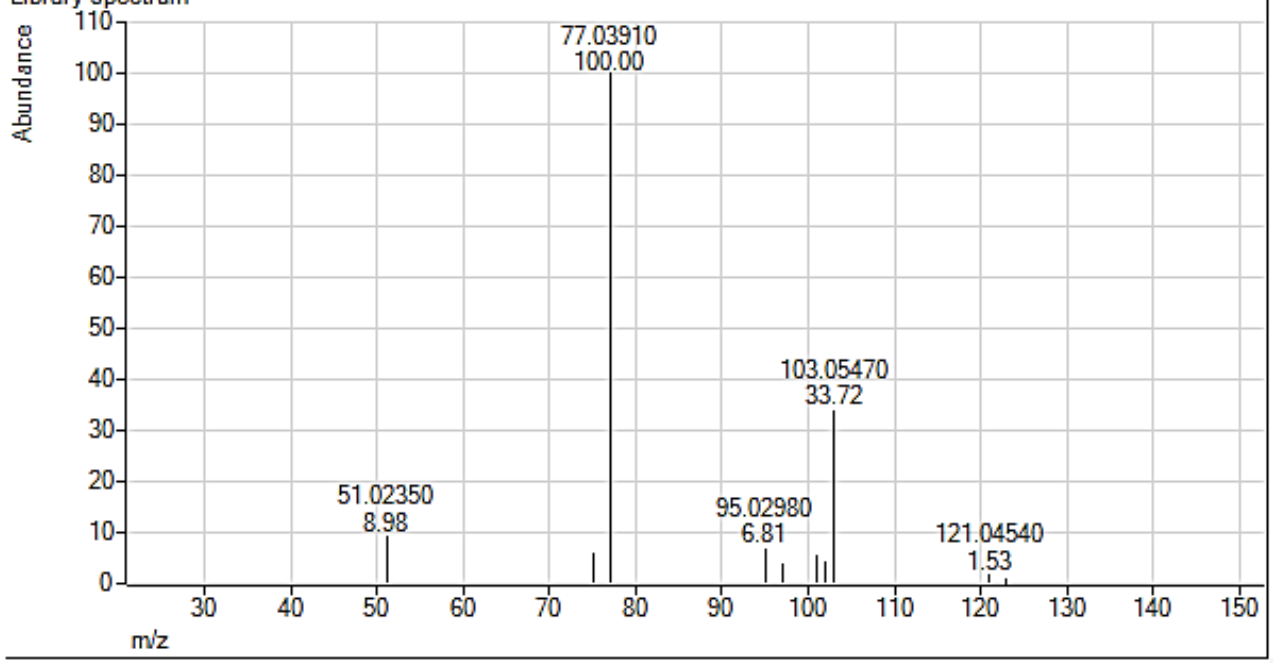
Collision energy
Tolerance: 2.0 eV

Spectra for compound: 4-Fluoroisocathinone

Compound Name	Precursor Ion	Collision Energy
4-Fluoroisocathin...	168.08189	10
4-Fluoroisocathin...	168.08189	20
4-Fluoroisocathin...	168.08189	40

Graphic | Mass List

Library spectrum



Single Search Results: 275 hits

Compound Name	Formula							
Methylhexanamine (DMAA)	C7H17N							
Cathinone	C9H11NO							
3-Fluoroamphetamine (3-FA)	C9H12FN							
2-Fluoroamphetamine (2-FA)	C9H12FN							
4-Fluoroamphetamine (4-FA)	C9H12FN							
Phenylpiperazine (NPP)	C10H14N2							
Methcathinone	C10H13NO							
nor-Mephedrone	C10H13NO	163.09971				31952-47-3		2-amino-1-(4-methylphenyl)-1-propanone	3
Ethylamphetamine	C11H17N	163.13610				457-87-4	9588	N-ethyl-1-phenylpropan-2-amine	3
4-Fluoroisocathinone	C9H10FNO	167.07464				1270532-...		1-amino-1-(4-fluorophenyl)propan-2-one	3
3-Fluoromethamphetamine (3-FMA)	C10H14FN	167.11103				1182818-...		3-fluoro-N,α-dimethyl-Benzeneethanamine	3
2-Fluoromethamphetamine (2-FMA)	C10H14FN	167.11103				1017176-...		2-fluoro-N,α-dimethyl-Benzeneethanamine	3
4-Fluoromethamphetamine (4-FMA)	C10H14FN	167.11103				351-03-1	9919721	1-(4-Fluorophenyl)-N-methyl-2-propanamine	3
Alpha-Methyltryptamine (AMT)	C11H14N2	174.11570				299-26-3	8930	1-(1H-indol-3-yl)propan-2-amine	3
5-APB	C11H13NO	175.09971				286834-8...	8012953	1-(1-Benzofuran-5-yl)-2-propanamine	3
BZP	C11H16N2	176.13135				110475-3...	68493	1-benzylpiperazine	3
MDAI (5,6-Methylenedioxy-2-aminoindane)	C10H11N...	177.07898				132741-8...	111694	6,7-dihydro-5H-cyclopenta[f][1,3]benzodioxol-6-a...	3
4-Methylmethcathinone (4-MMC)	C11H15NO	177.11536				1189726-...	21485694	2-(methylamino)-1-(4-methylphenyl)propan-1-one	3
2-Methylmethcathinone (2-MMC)	C11H15NO	177.11536				1246911-...		2-(methylamino)-1-(2-methylphenyl)-1-Propanone	3
3-Methylmethcathinone (3-MMC)	C11H15NO	177.11536				1246911-...		2-(methylamino)-1-(3-methylphenyl)-1-Propanone	3
Buphedrone	C11H15NO	177.11536				408332-7...	26286946	2-(Methylamino)-1-phenylbutan-1-one	3
Ethcathinone (Ethylcathinone)	C11H15NO	177.11536				18259-37-5	403504	(RS)-2-ethylamino-1-phenyl-propan-1-one	3

Designer Drug Spectral Databases: Current Status

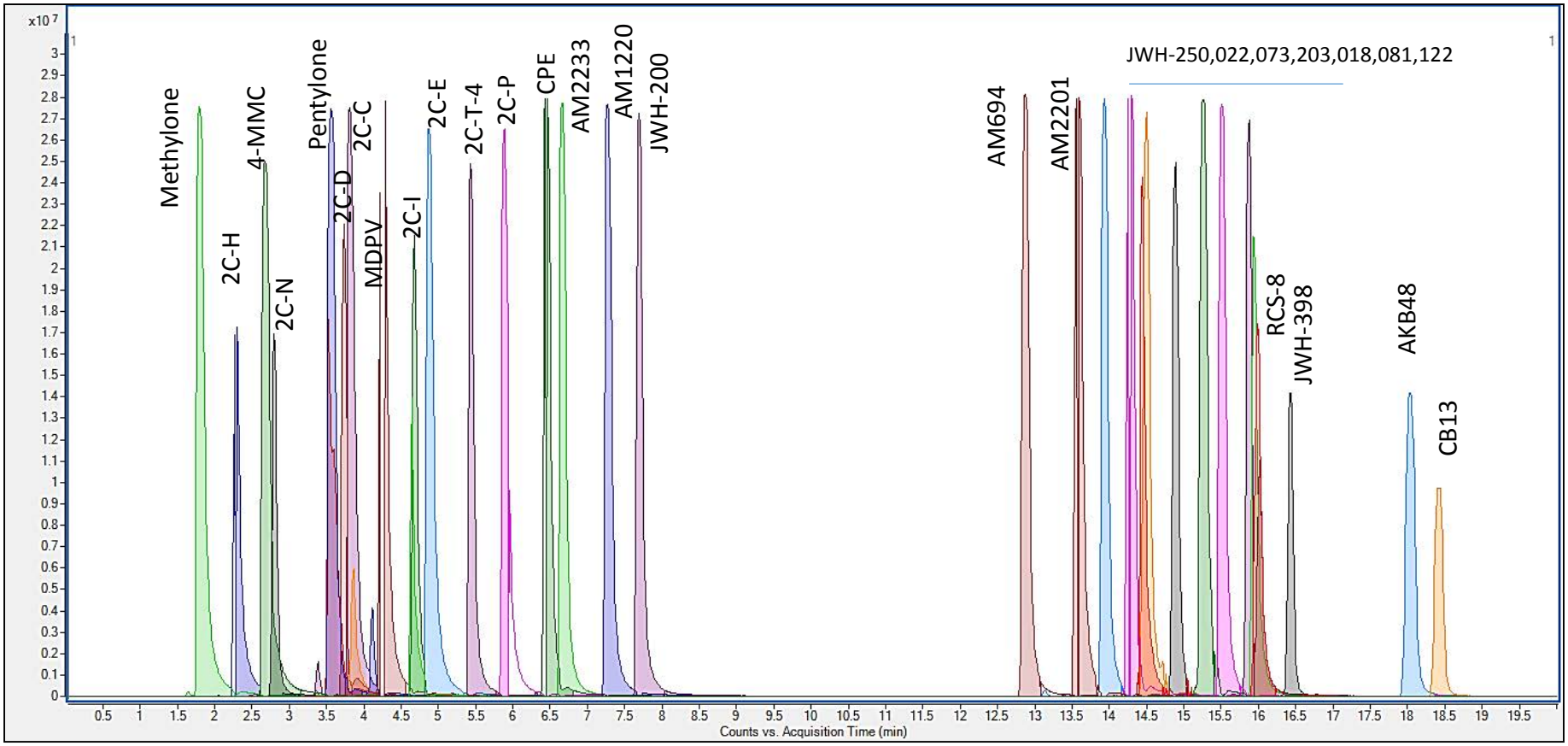
- Collection of LC-QTOF MS/MS data and construction of PCDL completed.
  - Spectra from 263 designer drug standards added to the PCDL.
  - 17 designer drug standards did not produce a 1000-count base peak.
- Collection of LC-QQQ MS/MS data by Dynamic MRM completed for DEA and Japan lists.
- Collection of LC-QQQ MS/MS data by Triggered MRM completed.

"DEA Mix"
4-Methylmethcathinone (4-MMC)
2C-H
2C-D
2C-E
2C-C
2C-P
2C-N
2C-T-2
2C-T-4
MDPV
2C-I
(±)-CP-47,497
RCS-4
JWH-073
(±)-CP-47,497-C8-homolog
JWH-250
JWH-203
JWH-018
JWH-122
JWH-019
AM2201
JWH-081
JWH-398
RCS-8
JWH-200
AM694

"Japan Mix"
4-Methylmethcathinone (4-MMC)
3,4-Dimethylmethcathinone (3,4-DMMC)
Methoxetamine
MDPV
(±)-CP-47,497-C8-homolog
JWH-022 (AM2201 N-(4-pentenyl) analog)
JWH-018
JWH-018 adamantyl carboxamide (2NE1)
AKB48
CB-13
Cannabipiperidiethanone
AM1220
AM2233

"Supermix"
Evolving mix that contains as many designer drugs and metabolites as possible (currently at 275 analytes). Divided into 25 individual calibration mixes for validation purposes (0.01 – 100 ng/mL).

LC Separation of 35 designer drugs including DEA and Japan List using LC-QTOF instrument



3 x 100 mm 1.8u Agilent RRHD Eclipse Plus C-18

A = water 10mM ammonium formate, 0.1% formic acid; B = 90/10 ACN/water, 0.1% formic acid

Summary for Screening and Confirmation of Designer Drugs Using QTOF LC-MS/MS

- Unique Designer Drugs Accurate-Mass Database and Library with 826 compounds, **263** with positive-ion spectra.
- High-resolution LC method on UHPLC column and simple mobile phase.
- Preliminary SPE method for serum using 35 representative drugs from multiple classes:
  - > 60% absolute recovery for 33/35 drugs.
  - 0.5 – 5 ng/mL LODs for 33/35 drugs (others were 10, 25 ng/mL).

Development of a Comprehensive LC-QQQ-MS/MS Designer Drug Spectral Database and Screening Confirmatory Method

Designer Drug LC-QQQ-MS Method Development Work Flow

FIA

- Confirmation of the precursor ion using flow injection analysis in MS2 full scan mode for all standards.

Optimizer/ MRM

- Optimization of fragmentor voltages and collision energies using Mass Hunter MS Optimizer software.
- Acquisition of precursor-to-product ion transitions (4 - 10 for each drug) in dynamic and/or triggered MRM mode.

Chromatography

- On-column separation of designer drug mixes to obtain retention times (Zorbax Eclipse Plus C_{18} column, 2.1 x 100 mm, 1.8 mm).

Designer Drug Library

- Compilation of fragmentation data and retention times to generate MRM database.
- Validation against QTOF database.

"DEA Mix"

4-Methylmethcathinone (4-MMC)
2C-H
2C-D
2C-E
2C-C
2C-P
2C-N
2C-T-2
2C-T-4
MDPV
2C-I
(±)-CP-47,497
RCS-4
JWH-073
(±)-CP-47,497-C8-homolog
JWH-250
JWH-203
JWH-018
JWH-122
JWH-019
AM2201
JWH-081
JWH-398
RCS-8
JWH-200
AM694

"Japan Mix"

4-Methylmethcathinone (4-MMC)
3,4-Dimethylmethcathinone (3,4-DMMC)
Methoxetamine
MDPV
(±)-CP-47,497-C8-homolog
JWH-022 (AM2201 N-(4-pentenyl) analog)
JWH-018
JWH-018 adamantyl carboxamide (2NE1)
AKB48
CB-13
Cannabipiperidiethanone
AM1220
AM2233

"Supermix"

Evolving mix that contains as many designer drugs and metabolites as possible (currently at 275 analytes).

Divided into 25 individual calibration mixes for validation purposes (0.01 – 100 ng/mL).

Flow Injection Analysis Screening (DEA mix)

LC Conditions (FIA)	
Injection volume:	1µl of 1ppm JWH-081 in

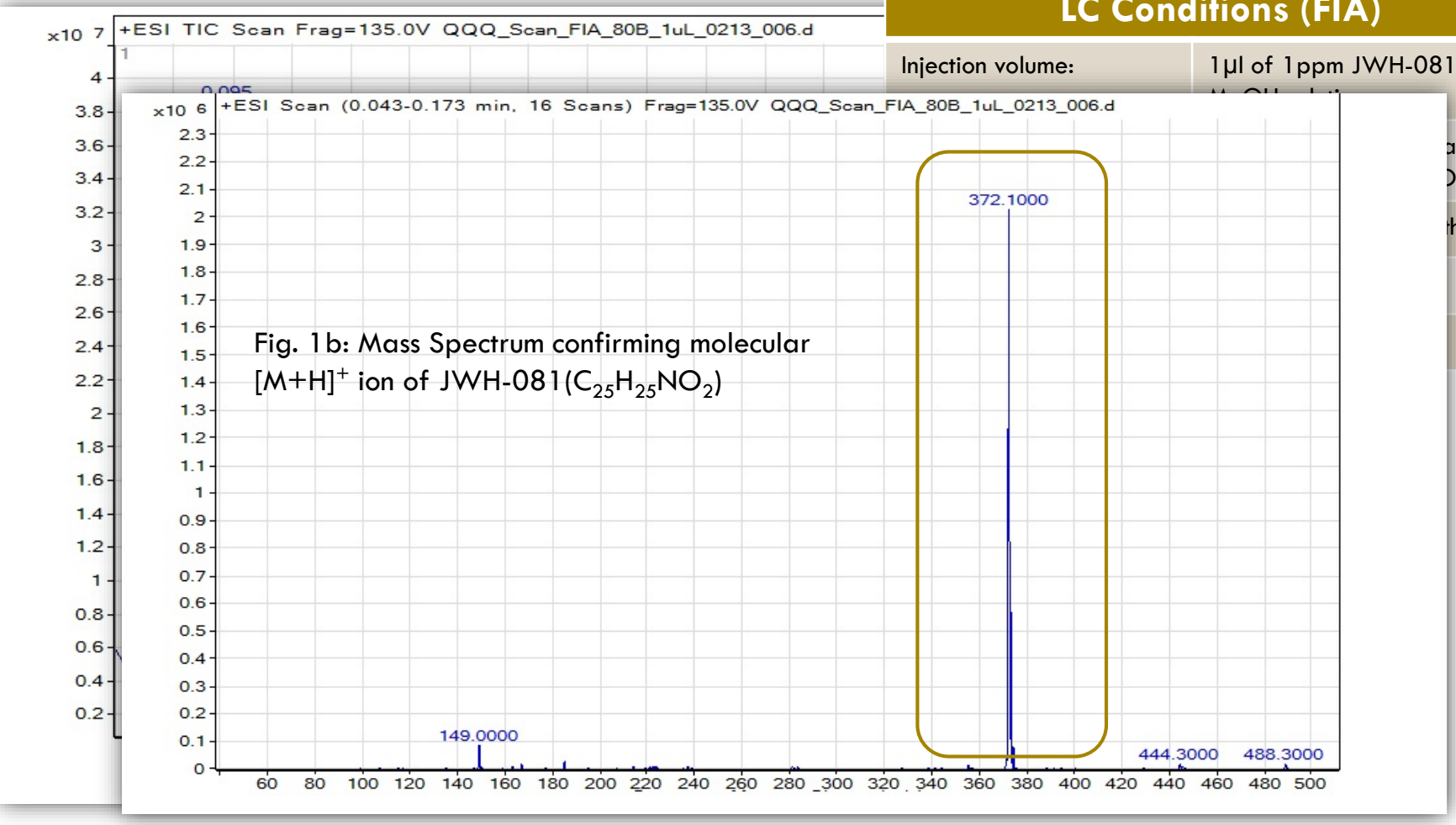


Fig. 1b: Mass Spectrum confirming molecular $[M+H]^+$ ion of JWH-081($C_{25}H_{25}NO_2$)

Flow Injection Analysis Screening (Japan Mix)

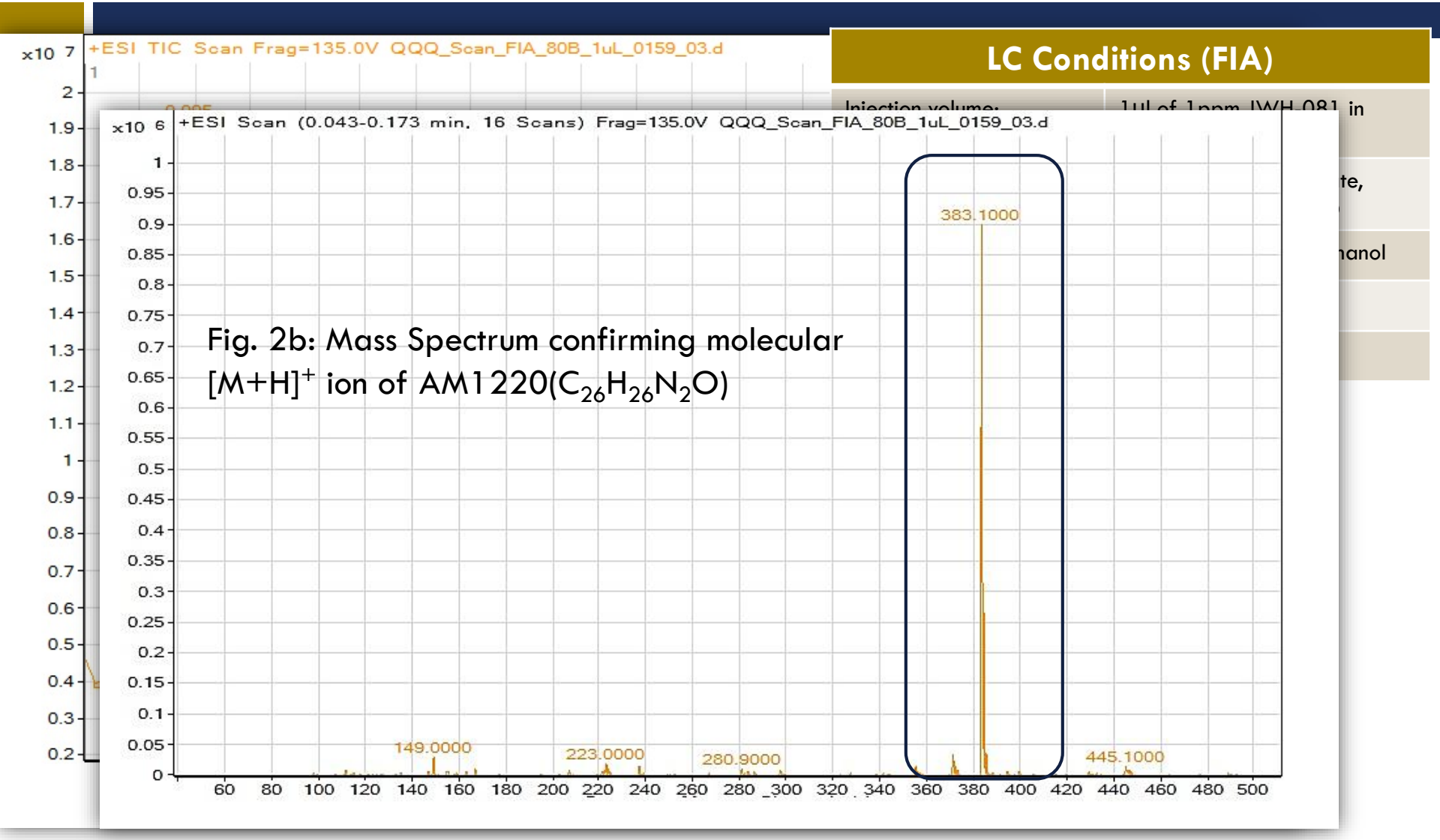


Fig. 2b: Mass Spectrum confirming molecular $[M+H]^+$ ion of AM1220($C_{26}H_{26}N_2O$)

LC Conditions (FIA)

Injection volume:	1uL of 1ppm JWH-081 in
	te,
	nanol

MRM Optimization/Confirmation

	DEA list								
2	Compound Name	Formula	Mass	Precursor	Product	Frag	CE	Abundance	Rel %
3	2C-E	C12H19NO2	209.14	210.1	193.1	75	4	410160	100.0
4	2C-E	C12H19NO2	209.14	210.1	178.1	75	12	162559	39.6
5	2C-E	C12H19NO2	209.14	210.1	91.1	75	44	64133	15.6
6	2C-E	C12H19NO2	209.14	210.1	77.1	75	60	68047	16.6
7	2C-D	C11H17NO2	195.13	196.1	179.1	80	4	27323	100.0
8	2C-D	C11H17NO2	195.13	196.1	164.1	80	16	9980	36.5
9	2C-D	C11H17NO2	195.13	196.1	91.1	80	36	4956	18.1
10	2C-D	C11H17NO2	195.13	196.1	149.1	80	24	5445	19.9
11	2C-C	C10H14ClNO2	215.07	216.1	199.1	65	4	22971	100.0
12	2C-C	C10H14ClNO2	215.07	216.1	184.1	65	16	10235	44.6
13	2C-C	C10H14ClNO2	215.07	216.1	77.1	65	48	4592	20.0
14	2C-C	C10H14ClNO2	215.07	216.1	169.1	65	28	5753	25.0
15	2C-H	C10H15NO2	181.11	182.1	165.1	85	4	10422	100.0
16	2C-H	C10H15NO2	181.11	182.1	150.1	85	16	6593	63.3
17	2C-H	C10H15NO2	181.11	182.1	77.1	85	52	2759	26.5
18	2C-H	C10H15NO2	181.11	182.1	135.1	85	28	2900	27.8
19	JWH-200	C25H24N2O2	384.18	385.2	155	90	16	1059244	100.0
20	JWH-200	C25H24N2O2	384.18	385.2	114.1	90	24	524891	95.7
21	JWH-200	C25H24N2O2	384.18	385.2	127	90	52	502118	24.1
22	JWH-200	C25H24N2O2	384.18	385.2	70.1	90	48	126314	86.0
23	JWH-203	C21H22ClNO	339.14	340.2	125	90	24	451456	100.0
24	JWH-203	C21H22ClNO	339.14	340.2	144	90	40	46283	10.3
25	JWH-203	C21H22ClNO	339.14	340.2	89.1	90	60	66655	14.8
26	JWH-203	C21H22ClNO	339.14	340.2	188.1	90	16	67385	14.9
27	2C-T-4	C13H21NO2S	255.13	256.1	239.1	70	4	312702	100.0
28	2C-T-4	C13H21NO2S	255.13	256.1	197.1	70	16	205668	65.8
29	2C-T-4	C13H21NO2S	255.13	256.1	91.1	70	52	63322	20.2
30	2C-T-4	C13H21NO2S	255.13	256.1	167	70	36	64795	20.7

Mass Hunter Optimizer Software: Optimizer Parameters

Fragmentor Coarse Range	60-210 V
Collision Energy Range	0-60 V
Cell Accelerator Voltage	7

Table 1. Excerpt from Summary Table of Optimization data for DEA list which shows 4 transitions for each compound, optimized fragmentor voltages and collision energies and the abundance of each product ion.

MRM Optimization/Confirmation (DEA Mix)

Table. 2 Optimized transitions of JWH-081

Compound Name	Formula	Mass	Precursor	Product	Frag	CE	Rel %
JWH-081	C25H25NO2	371.19	372.2	185	80	24	100.0
JWH-081	C25H25NO2	371.19	372.2	157	80	44	37.9
JWH-081	C25H25NO2	371.19	372.2	127	80	60	27.7
JWH-081	C25H25NO2	371.19	372.2	214.1	80	20	29.0

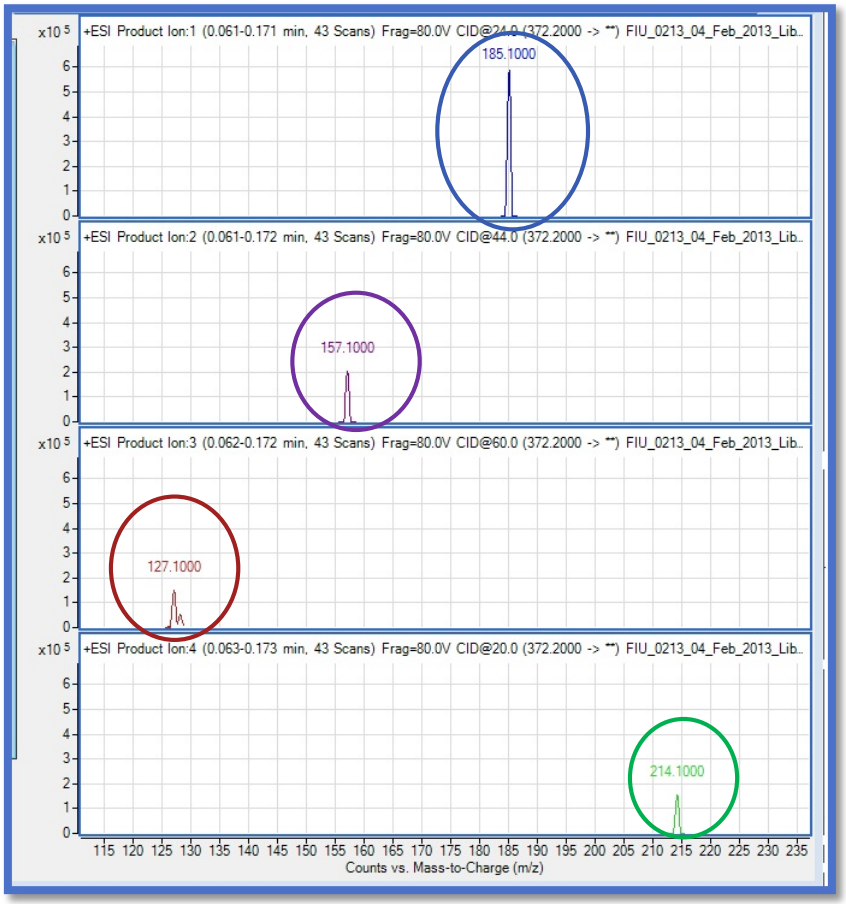


Fig. 3 Shows the correlating product ion peaks produced following CID of $[M+H]^+$ ion during optimization

MRM Optimization/Confirmation (Japan Mix)

Japan List

Compound Name	Formula	Mass	Precursor	Product	Frag	CE	Abundance	Rel %
4-MMC	C11H15NO	177.12	178.1	145.1	70	20	327375	64.8
4-MMC	C11H15NO	177.12	178.1	160.1	70	8	505349	100.0
4-MMC	C11H15NO	177.12	178.1	144.1	70	32	255191	50.5
4-MMC	C11H15NO	177.12	178.1	91.1	70	36	56804	11.2
AM2233	C22H23IN2O	458.09	459.1	98.1	140	32	346271	100.0
AM2233	C22H23IN2O	458.09	459.1	112.1	140	20	213533	61.7
AM2233	C22H23IN2O	458.09	459.1	230.9	140	28	55966	16.2
AM2233	C22H23IN2O	458.09	459.1	70.1	140	60	66737	19.3
JWH-022	C24H21NO	339.16	340.2	127	140	52	127627	87.6
JWH-022	C24H21NO	339.16	340.2	155	140	20	145706	100.0
JWH-022	C24H21NO	339.16	340.2	212.1	140	20	22758	15.6
JWH-022	C24H21NO	339.16	340.2	144	140	40	9884	6.8

Compound Name	Formula	Mass	Precursor	Product	Frag	CE	Rel %
AM-1220	C26H26N2O	382.2	383.2	98.1	85	36	100.0
AM-1220	C26H26N2O	382.2	383.2	112.1	85	20	84.1
AM-1220	C26H26N2O	382.2	383.2	155.1	85	24	40.9
AM-1220	C26H26N2O	382.2	383.2	127.1	85	60	31.0

Cannabipiperidiethanone	C24H28N2O2	376.22	377.2	98.1	75	40	179460	56.3
Cannabipiperidiethanone	C24H28N2O2	376.22	377.2	121.1	75	24	174515	54.8
Cannabipiperidiethanone	C24H28N2O2	376.22	377.2	91.1	75	60	124910	39.2
AKB-48	C23H31N3O	365.25	366.3	135.1	90	16	472795	100.0
AKB-48	C23H31N3O	365.25	366.3	93.1	90	56	64578	13.7
AKB-48	C23H31N3O	365.25	366.3	79.1	90	60	63386	13.4
AKB-48	C23H31N3O	365.25	366.3	107.1	90	48	39198	8.3
AM-1220	C26H26N2O	382.2	383.2	98.1	85	36	270093	100.0
AM-1220	C26H26N2O	382.2	383.2	112.1	85	20	227023	84.1
AM-1220	C26H26N2O	382.2	383.2	155.1	85	24	110348	40.9
AM-1220	C26H26N2O	382.2	383.2	127.1	85	60	83703	31.0

Table. 3 Optimized transitions for AM-1220

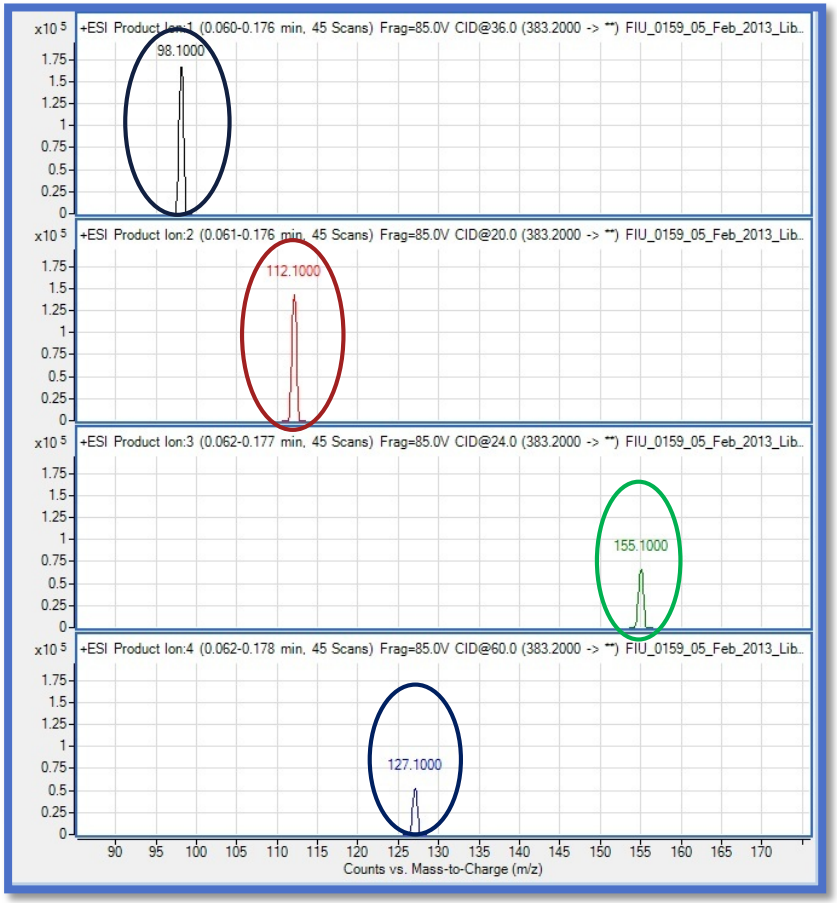


Fig. 4 Shows the correlating product ion peaks produced following CID of $[M+H]^+$ ion during optimization

QTOF Confirmation

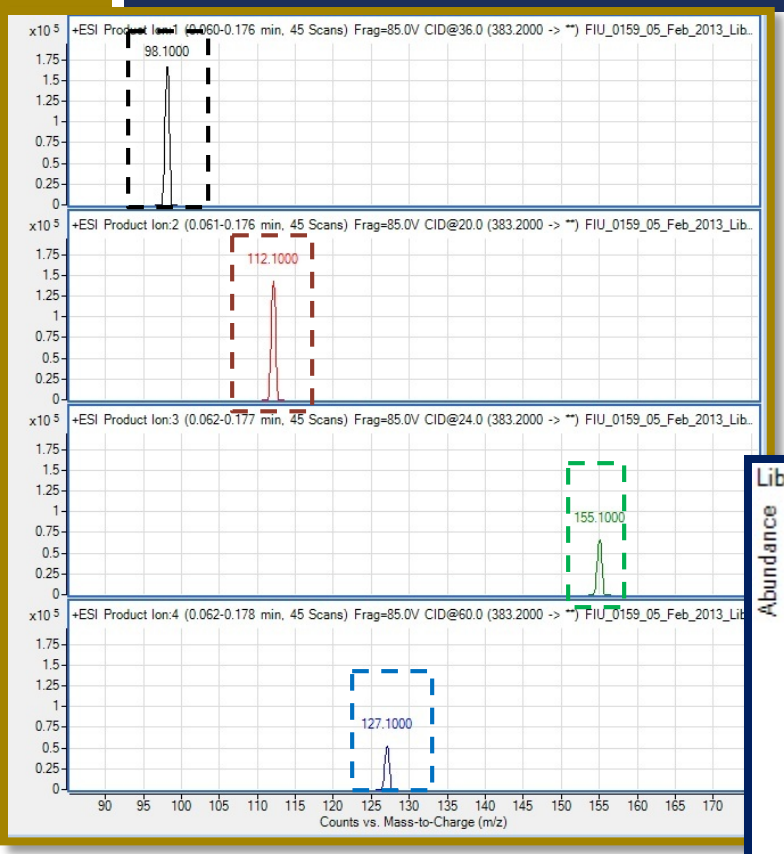


Fig.5a
MH Optimizer product ions of AM-1220

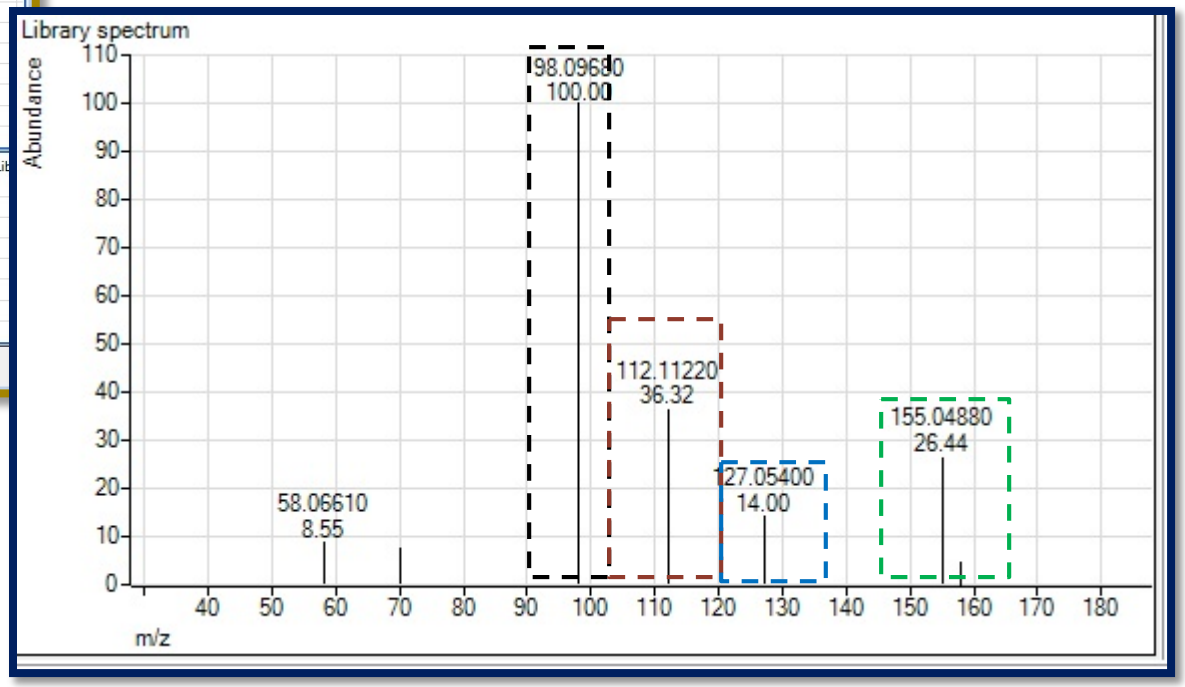


Fig. 5b
QTOF fragmentation pattern at 40eV of AM-1220

Chromatographic Separation (DEA MIX B)

DEA MIX B	
2C-N	JWH 019
2C-I	AM2201
RCS-4	JWH 398
JWH 203	JWH 200
(±)-CP 47,497-C8-homolog	(non-ionizable)

LC Conditions for Column Separation		
Column:		Zorbax Eclipse Plus C18 column, 2.1 x 100mm, 1.8 µm
Injection volume:		20µl of 10ng/ml DEA MIX B solution in H_2O
Gradient:	1.0 min	Mobile phase A (95%) Mobile phase B (5%)
	9.5 min	Mobile phase A (10%) Mobile phase B (90%)
Flow:		0.4ml/min.
Stoptime:		14 mins.
Temperature:		40.00 °C

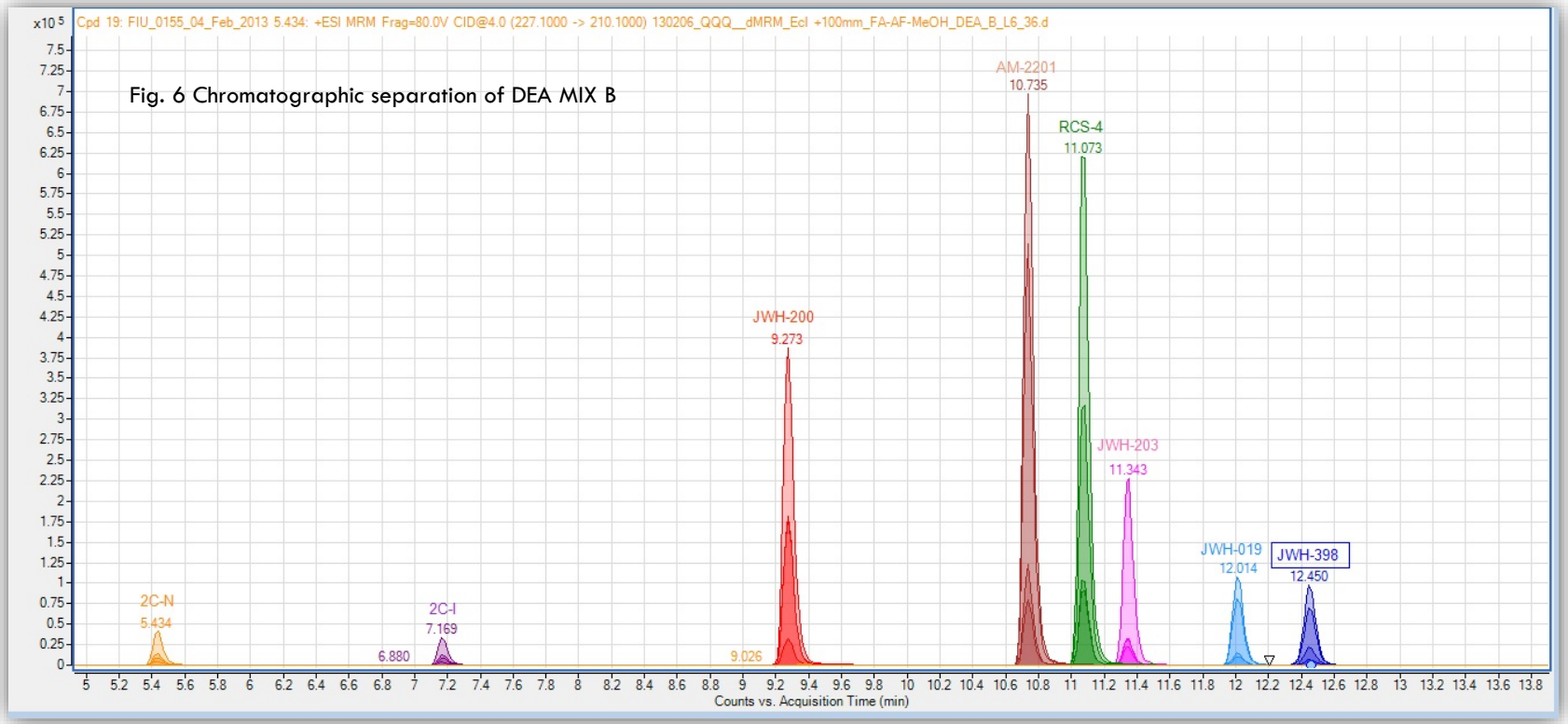


Fig. 6 Chromatographic separation of DEA MIX B

Chromatographic Separation (Dynamic MRM – Complete DEA MIX)

DEA list

Compound Name	Formula	Mass	Precursor	Product	Frag	CE	Rel %	Ret. Times
2C-E	C12H19NO2	209.14	210.1	193.1	75	4	100.0	7.496
2C-E	C12H19NO2	209.14	210.1	178.1	75	12	39.6	
2C-E	C12H19NO2	209.14	210.1	91.1	75	44	15.6	
2C-E	C12H19NO2	209.14	210.1	77.1	75	60	16.6	
2C-D	C11H17NO2	195.13	196.1	179.1	80	4	100.0	6.669
2C-D	C11H17NO2	195.13	196.1	164.1	80	16	36.5	
2C-D	C11H17NO2	195.13	196.1	91.1	80	36	18.1	
2C-D	C11H17NO2	195.13	196.1	149.1	80	24	19.9	
2C-C	C10H14ClNO2	215.07	216.1	199.1	65	4	100.0	6.519
2C-C	C10H14ClNO2	215.07	216.1	184.1	65	16	44.6	
2C-C	C10H14ClNO2	215.07	216.1	77.1	65	48	20.0	
2C-C	C10H14ClNO2	215.07	216.1	169.1	65	28	25.0	
2C-H	C10H15NO2	181.11	182.1	165.1	85	4	100.0	5.561
2C-H	C10H15NO2	181.11	182.1	150.1	85	16	63.3	
2C-H	C10H15NO2	181.11	182.1	77.1	85	52	26.5	
2C-H	C10H15NO2	181.11	182.1	135.1	85	28	27.8	
JWH-200	C25H24N2O2	384.18	385.2	155	90	16	100.0	9.273
JWH-200	C25H24N2O2	384.18	385.2	114.1	90	24	95.7	
JWH-200	C25H24N2O2	384.18	385.2	127	90	52	24.1	
JWH-200	C25H24N2O2	384.18	385.2	70.1	90	48	86.0	
JWH-203	C21H22ClNO	339.14	340.2	125	90	24	100.0	11.340
JWH-203	C21H22ClNO	339.14	340.2	144	90	40	10.3	
JWH-203	C21H22ClNO	339.14	340.2	89.1	90	60	14.8	
JWH-203	C21H22ClNO	339.14	340.2	188.1	90	16	14.9	
2C-T-4	C13H21NO2S	255.13	256.1	239.1	70	4	100.0	7.679
2C-T-4	C13H21NO2S	255.13	256.1	197.1	70	16	65.8	
2C-T-4	C13H21NO2S	255.13	256.1	91.1	70	52	20.2	
2C-T-4	C13H21NO2S	255.13	256.1	167	70	36	20.7	
RCS-4	C21H23NO2	321.17	322.2	135	110	20	100.0	11.072
RCS-4	C21H23NO2	321.17	322.2	77.1	110	56	46.9	
RCS-4	C21H23NO2	321.17	322.2	107.1	110	40	16.2	
RCS-4	C21H23NO2	321.17	322.2	92.1	110	68	16.0	

LC Conditions for Column Separation

Column:		**Zorbax Eclipse Plus C18 column, 2.1 x 100mm, 1.8 µm**
Injection volume:		**20µl of 100ng/ml DEA MIX B solution in H_2O**
Gradient:	**1.0 min**	**Mobile phase A (95%) Mobile phase B (5%)**
	9.5 min	**Mobile phase A (10%) Mobile phase B (90%)**
Flow:		**0.4ml/min.**
Stoptime:		**14 mins.**
Temperature:		**40.00 °C**

Table 4. Summary Table of Optimization data for DEA list which shows 4 transitions for each compound, optimized fragmentor voltages and collision energies, relative abundances reported as percentages and retention times.

Chromatographic Separation (Dynamic MRM – Complete DEA MIX)

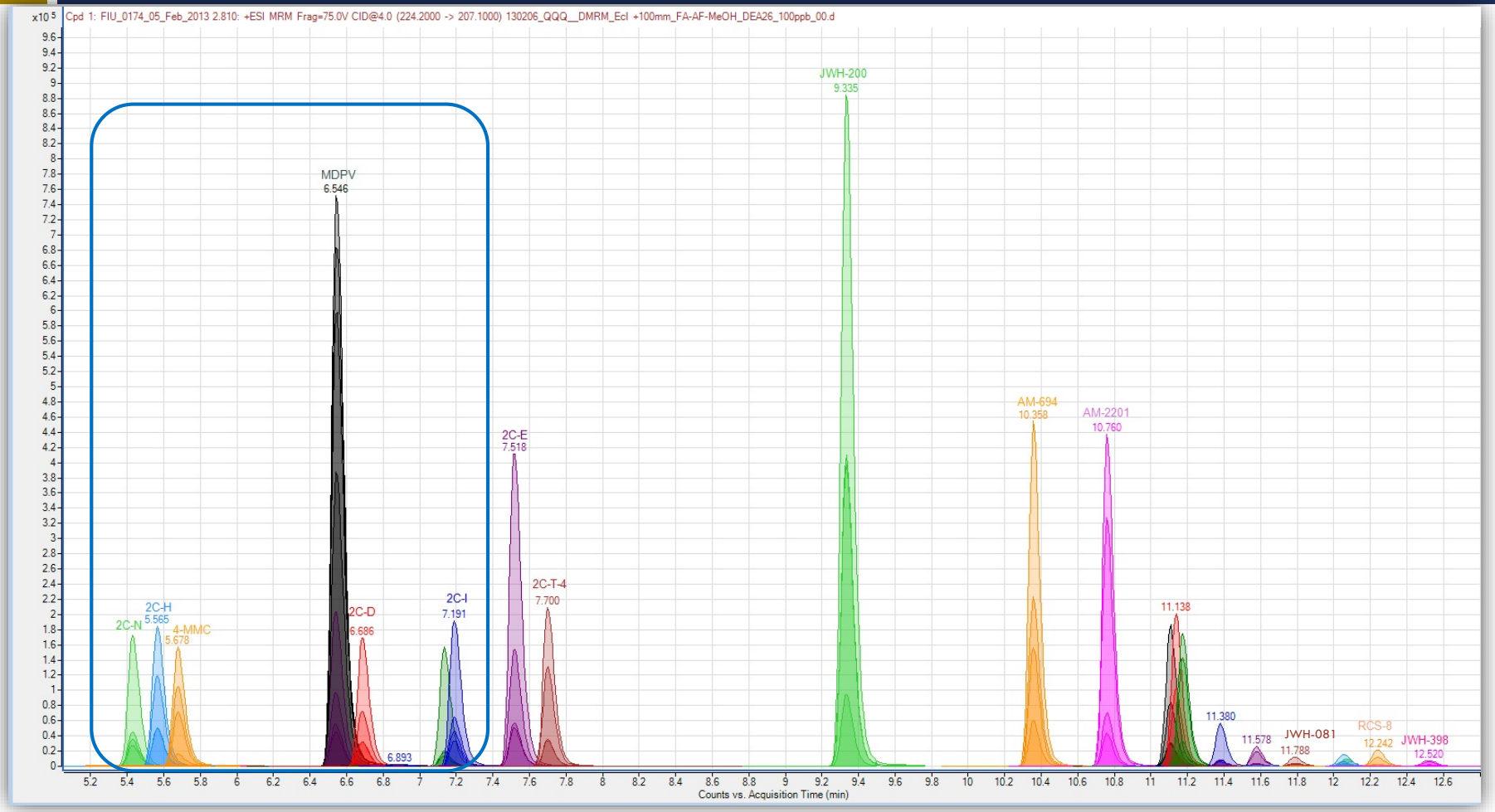


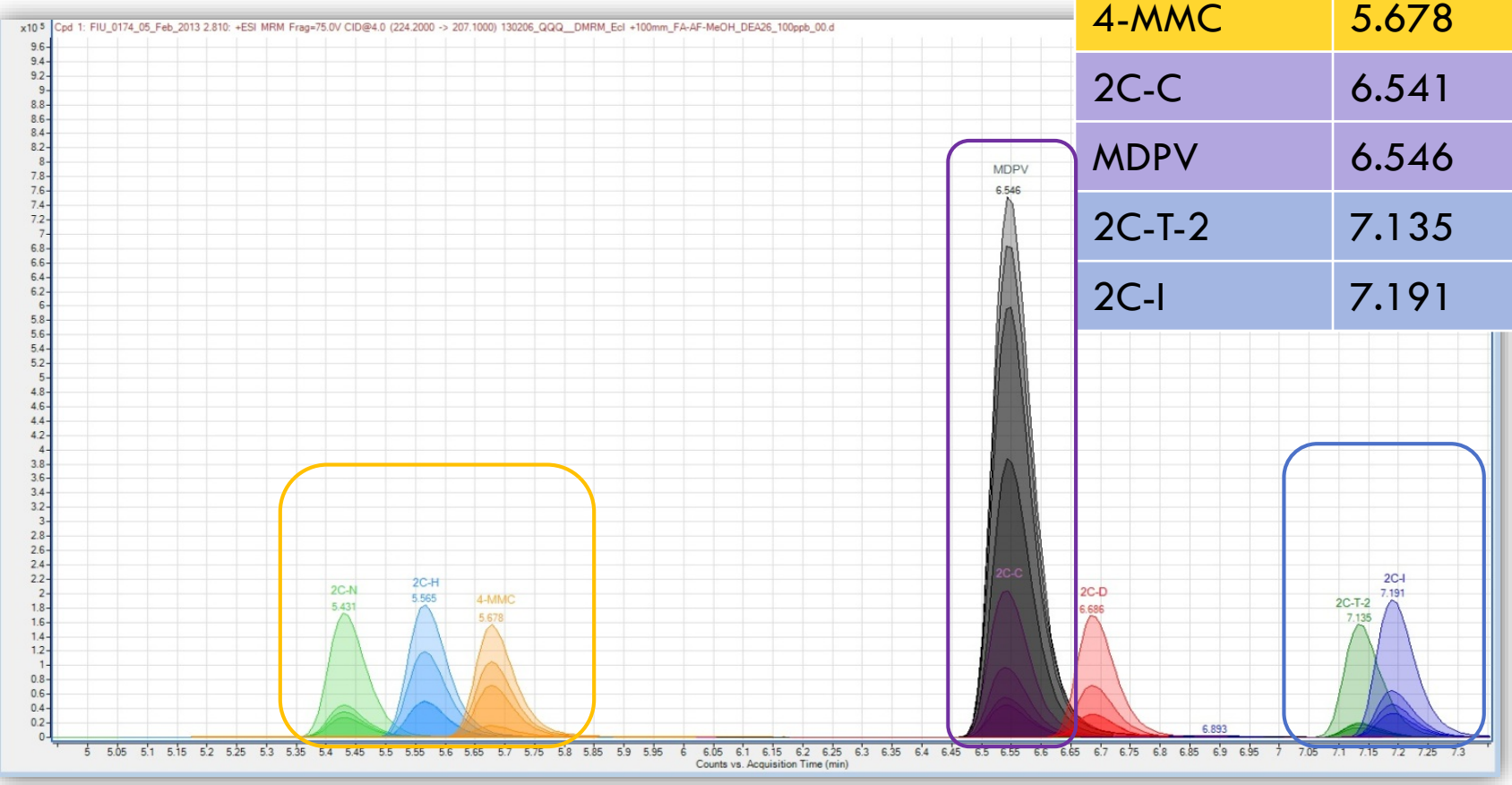
Fig. 7 shows the on-column separation of 23 of 26 compounds recently scheduled by the DEA

NB of the 3 compounds missing: 2 are non-ionizable (CP family) and 1 shows inadequate chromatography

Chromatographic Separation (Dynamic MRM – Complete DEA MIX)

Compounds	Ret. time	$[M+H]^+$
2C-N	5.431	227.1
2C-H	5.565	182.1
4-MMC	5.678	178.1
2C-C	6.541	216.1
MDPV	6.546	276.2
2C-T-2	7.135	242.1
2C-I	7.191	308

Fig. 8 Enlarged view of co-eluting compounds in DEA mix



Chromatographic Separation (Dynamic MRM – Complete DEA MIX)

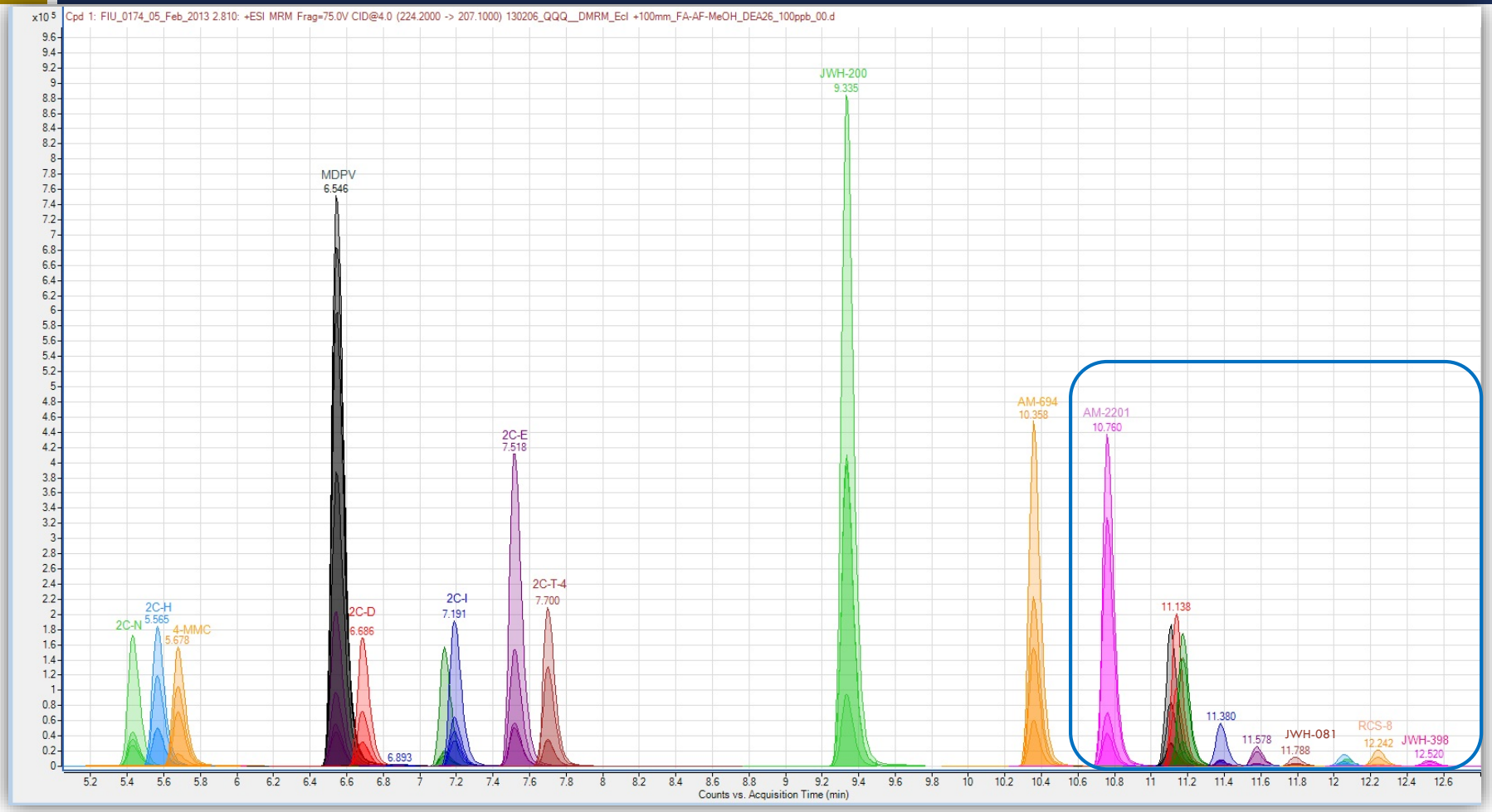
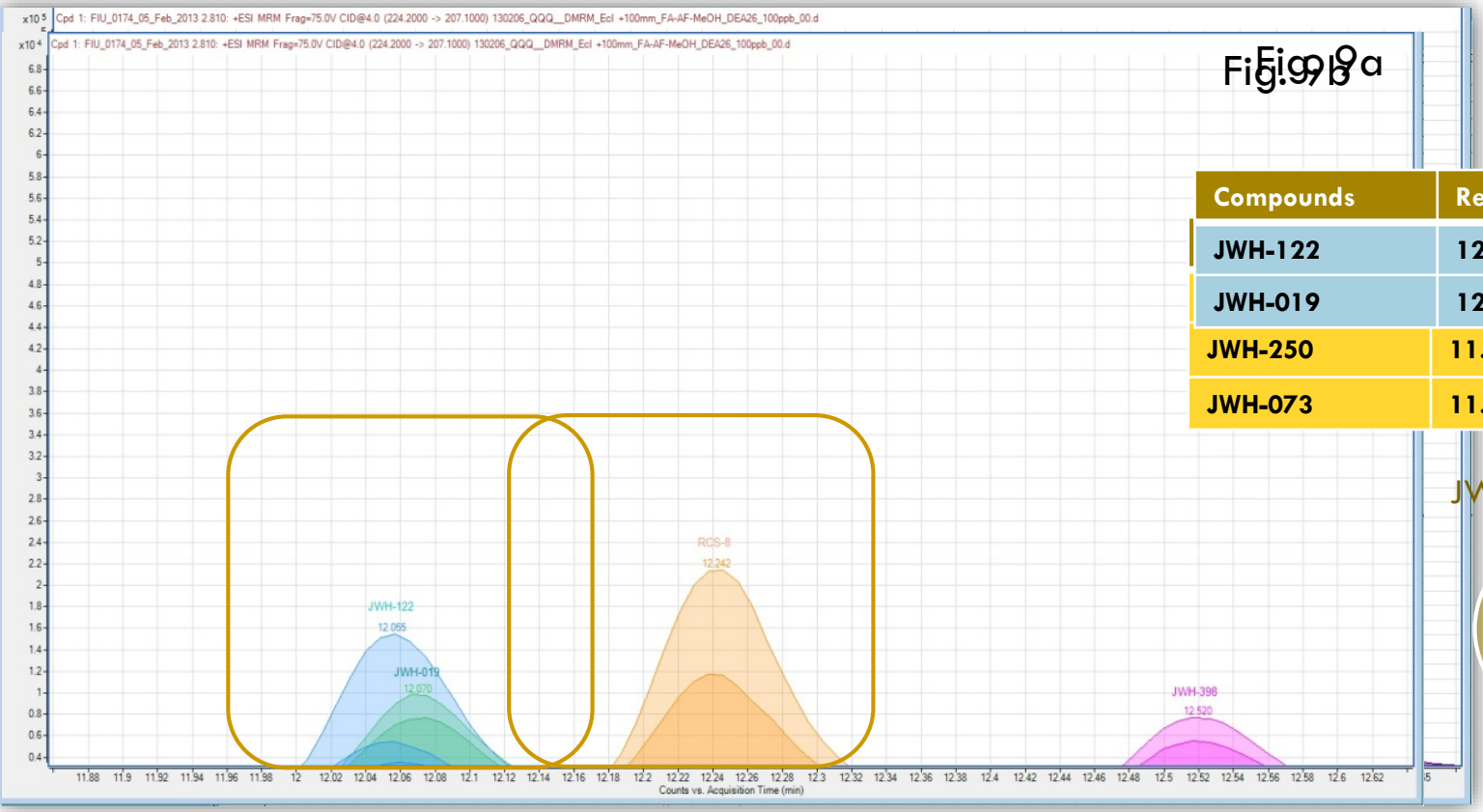


Fig. 7 shows the on-column separation of 23 of 26 compounds recently scheduled by the DEA

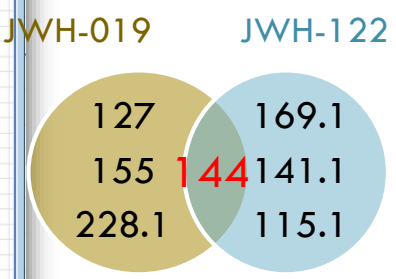
NB of the 3 compounds missing: 2 are non-ionizable (CP family) and 1 shows inadequate chromatography

Chromatographic Separation (Dynamic MRM – Complete DEA MIX)

Figs. 9a and 9b - Enlarged view of co-eluting compounds in DEA mix

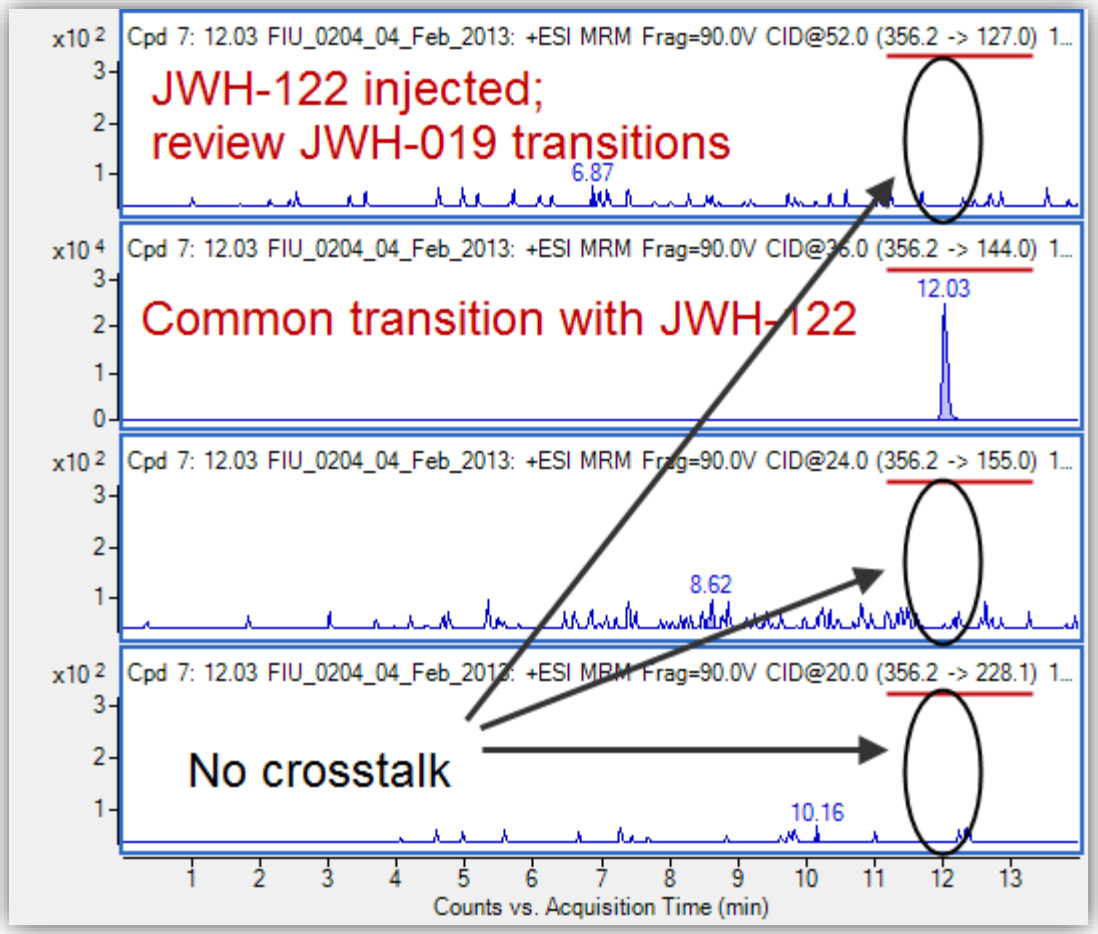


Compounds	Ret. time	$[M+H]^+$
JWH-122	12.055	356.2
JWH-019	12.070	356.2
JWH-250	11.132	336.2
JWH-073	11.174	328.2



Is there cross-talk?

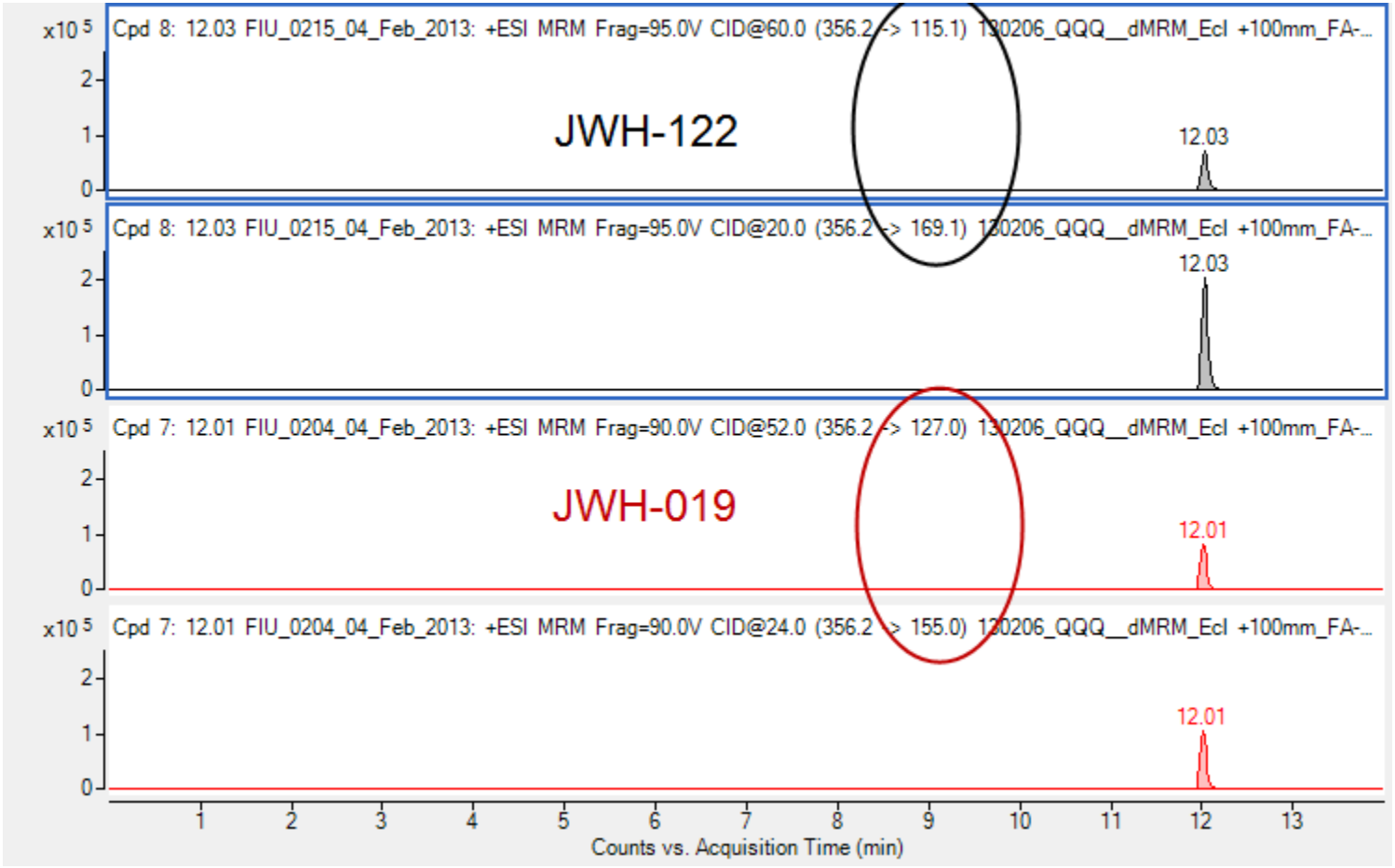
Is There Crosstalk Between JWH-122 and JWH-019?



- Common precursor with no response for unique products = **NO CROSSTALK**

Unique Products for Common Precursor

- Because the main 2 transitions for each of JWH-019 and JWH-022 are unique, the correct identities can be determined even though they co-elute



Chromatographic Separation (Dynamic MRM – Complete Japan MIX)

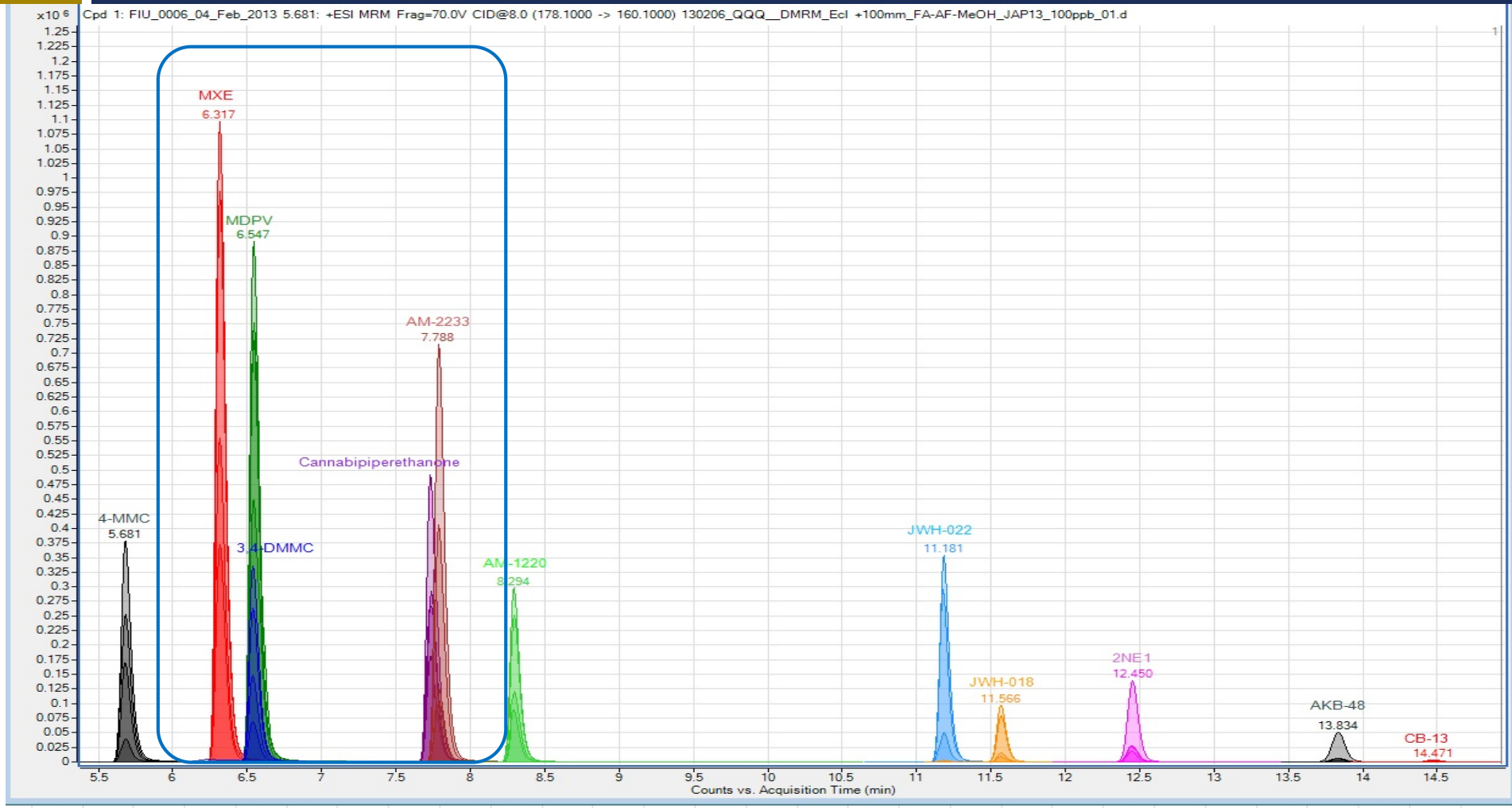


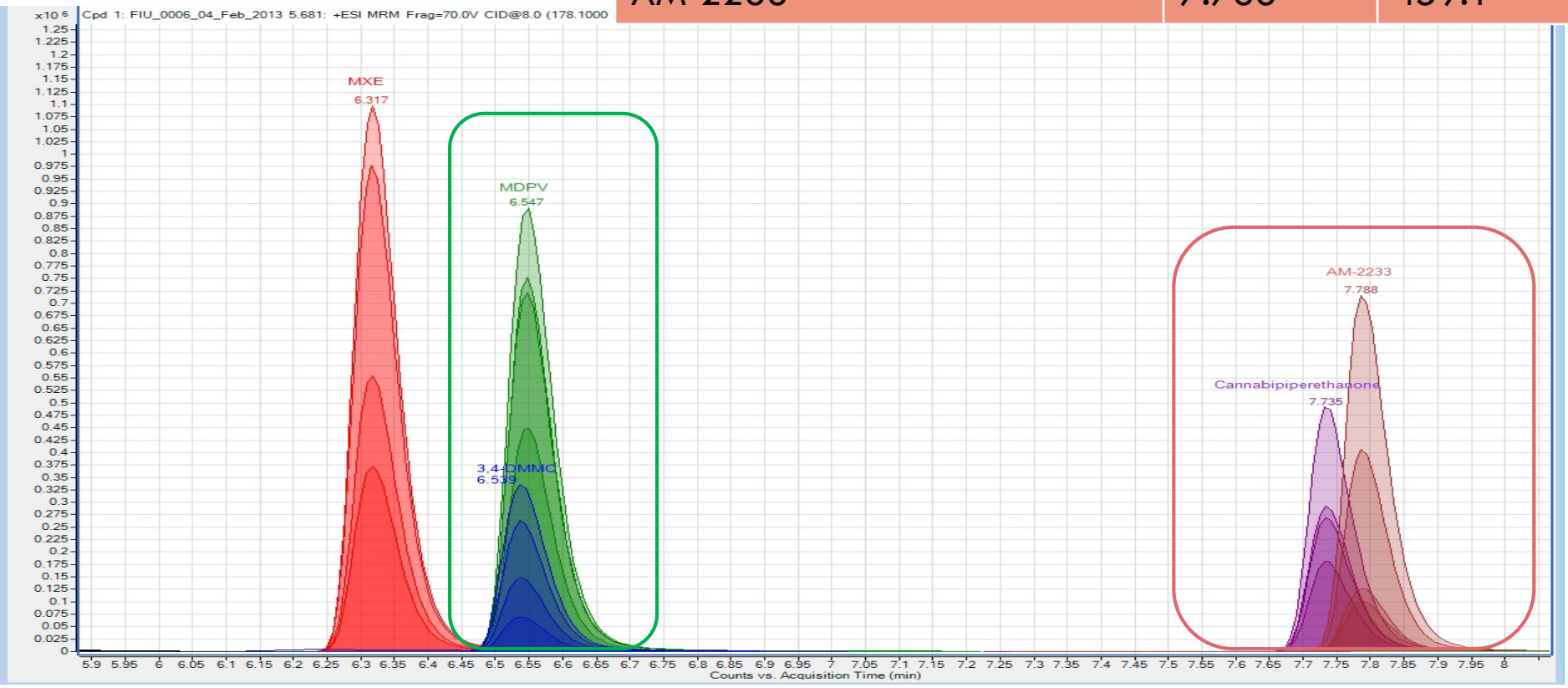
Fig. 10 shows the on-column separation of12 of 13 compounds recently scheduled in Japan

NB the missing compound (1) is non-ionizable (CP family)

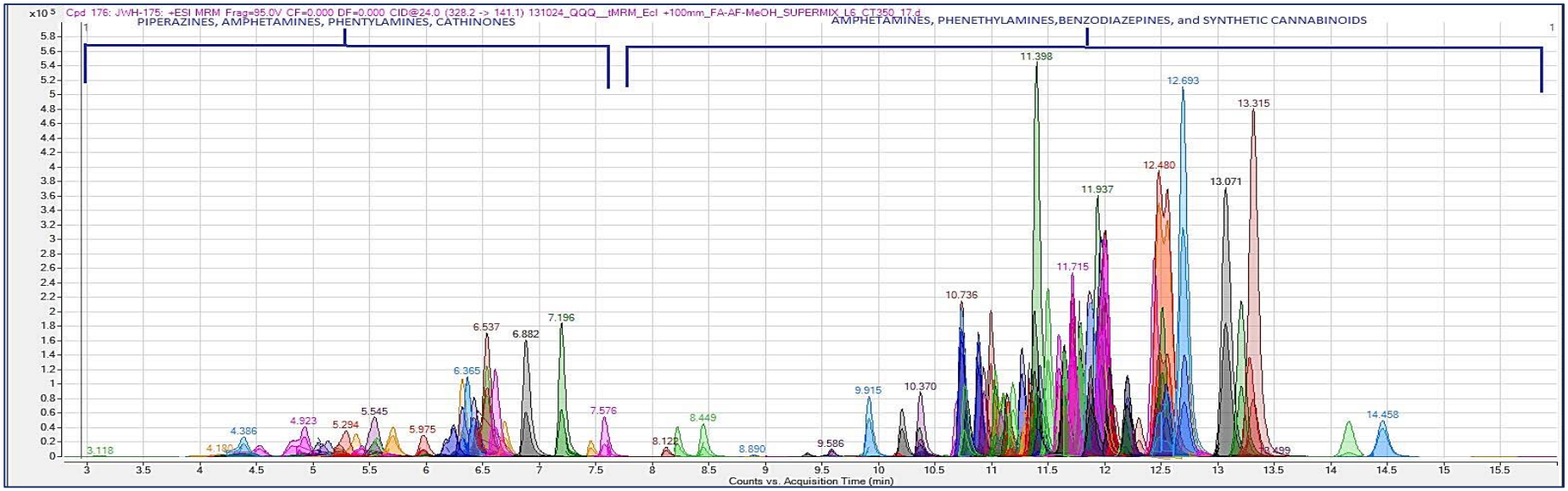
Chromatographic Separation (Dynamic MRM – Complete Japan MIX)

Compounds	Ret. time	$[M+H]^+$
MDPV	6.547	276.2
3,4-DMMC	6.539	192.1
CANNABIPIPERIDIETHANONE	7.735	377.2
AM-2233	7.788	459.1

Fig. 11 - Enlarged view of co-eluting compounds in JAPAN mix



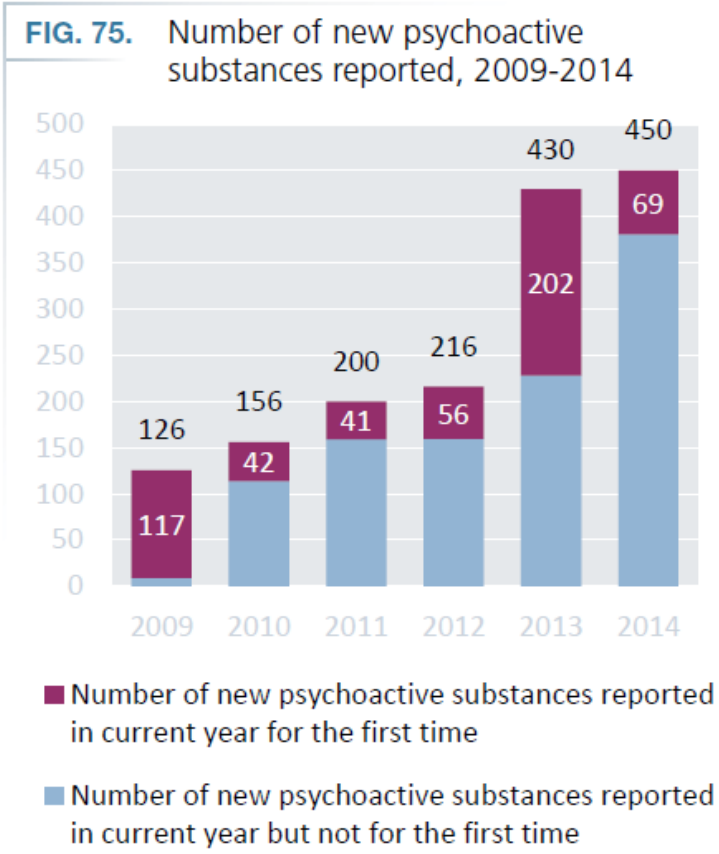
LC-QQQ Separation of 176 Designer Drugs

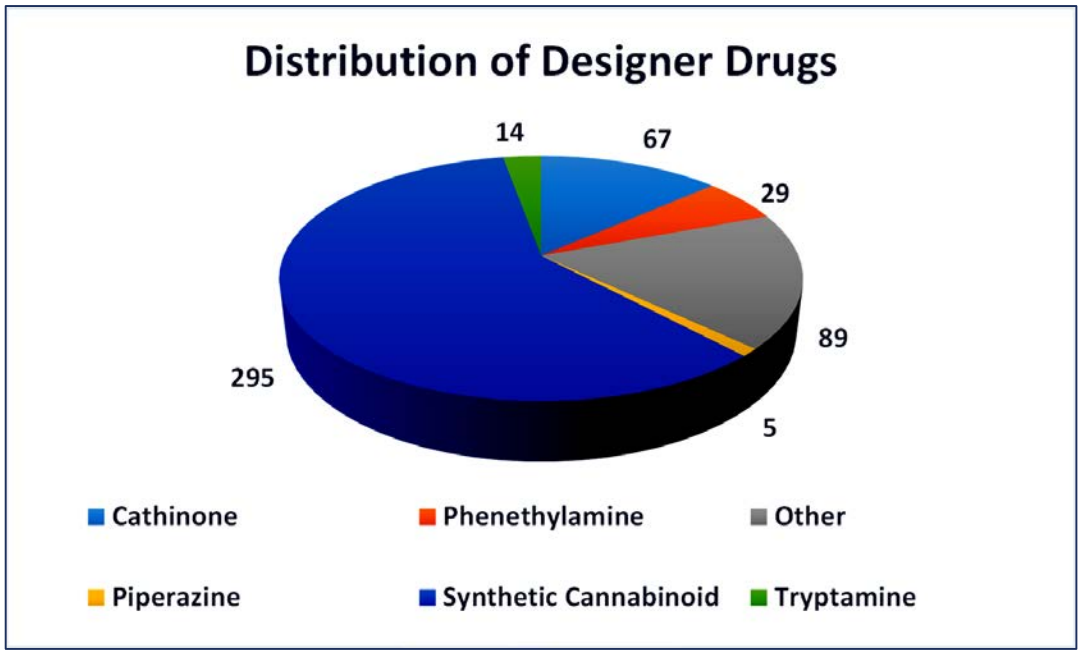


Summary for LC-QQQ- MS/MS Screening

- LC-QQQ-MS is effective in producing characteristic MS/MS spectra and chromatography specific to several hundred designer drugs including DEA and Japan List.
- Quantification data for the majority of the designer drugs studied showed concentrations in the parts per billion range with adequate linearity.
- The use of a triggered MRM database with up to ten transitions for each compound coupled with the characteristic chromatography data obtained allows the LC-QQQ-MS to be a highly discriminatory analytical tool when conducting the analysis of designer drug isomers

Recent efforts





General Conclusions

- A high resolution MS/MS spectral library was created that has spectral data at three different collision energies for **263** designer drugs.
- The Collision Induced Dissociation (CID) fragment spectra is very specific and enable peak identification with high accuracy.
- A compound database that includes structural and chemical information was created for an **additional 550** designer drugs.
- LC-QQQ-MS is effective in producing characteristic MS/MS spectra and chromatography specific to several hundred designer drugs.
- Single quadrupole and triple quadrupole MRM databases are now available for **261** designer drugs.

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