Trying to stay one-step ahead.

How High Resolution Accurate Mass LCMS has enabled early detection of fentanyl analogues (including carfentanil) and synthetic cannabinoids in the UK.

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Sport and Specialised Analytical Services

- Originally operating as Horseracing Forensic Laboratory, HFL, HFL Sport Science and now part of LGC.
- Background in doping and medication control in Equine, Canine and Human sport and also leaders in nutritional supplement testing though ‘Informed Sport’ and ‘Informed Choice’.
- More recently involved in NPS and have applied HRAM LCMS to forensic toxicology and coroners work for customers in the UK.

Outline

1. High Resolution Accurate Mass (HRAM) LCMS
2. Fentanyl analogues
3. Synthetic Cannabinoid Receptor agonists (SCRAs)
4. Summary

High Resolution Accurate Mass (HRAM) LCMS

Thermo Scientific™ Orbitrap™

- First looked at HRAM LCMS in 2006
- Implemented in doping control screening in 2008 – 2 x Thermo Scientific™ LTQ Orbitrap XL™ ETD Hybrid Ion Trap-Orbitrap Mass Spectrometer – removed 10 x GCMS and several LC-MS/MS instruments.
- 2012 – Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer
- 2014 – The Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap MS
- 2016-2019 – 3 Q Exactives and 1 Q Exactive Focus

Thermo Scientific Q Exactive Orbitrap

- High Resolution Accurate Mass (HRAM) LCMS
- 7 minute LC cycle time
- Over 2000 compounds

Selectivity of accurate mass – nominal mass

Compounds – all different elemental compositions	Nominal Mass
Aminacrine ($C_{13}H_{10}N_2$)	194
Aminohippuric acid ($C_9H_{10}N_2O_3$)	194
Anthranol ($C_{14}H_{10}O$)	194
Butylparaben ($C_{11}H_{14}O$)	194
Caffeine ($C_8H_{10}N_4O_2$)	194
Dimethyl phthalate ($C_{10}H_{10}O_4$)	194
Galacturonic acid ($C_6H_{10}O_7$)	194
Hexylresorcinol ($C_{12}H_{18}O_2$)	194
Methylglucoside ($C_7H_4O_6$)	194
Depreton ($C_8H_{10}N_4S$)	194
Phenocoll ($C_{10}H_{14}N_2O_2$)	194
Solanone ($C_{13}H_{22}O$)	194
Temozolamide ($C_6H_6N_6O_2$)	194

Mass scale of elements

Carbon – mass 12.00

Oxygen – mass 15.995

Nitrogen – mass 14.003

Hydrogen – mass 1.008

Selectivity of accurate mass

Compound	Accurate Mass
Aminacrine ($C_{13}H_{10}N_2$)	194.08385
Aminohippuric acid ($C_9H_{10}N_2O_3$)	194.06859
Anthranol ($C_{14}H_{10}O$)	194.07262
Butylparaben ($C_{11}H_{14}O$)	194.09375
Caffeine ($C_8H_{10}N_4O_2$)	194.07983
Dimethyl phthalate ($C_{10}H_{10}O_4$)	194.05736
Galacturonic acid ($C_6H_{10}O_7$)	194.04210
Hexylresorcinol ($C_{12}H_{18}O_2$)	194.13103
Methylglucoside ($C_7H_4O_6$)	194.07849
Depreton ($C_8H_{10}N_4S$)	194.06207
Phenocoll ($C_{10}H_{14}N_2O_2$)	194.10498
Solanone ($C_{13}H_{22}O$)	194.16652
Temozolamide ($C_6H_6N_6O_2$)	194.05467

Accurate masses of elements

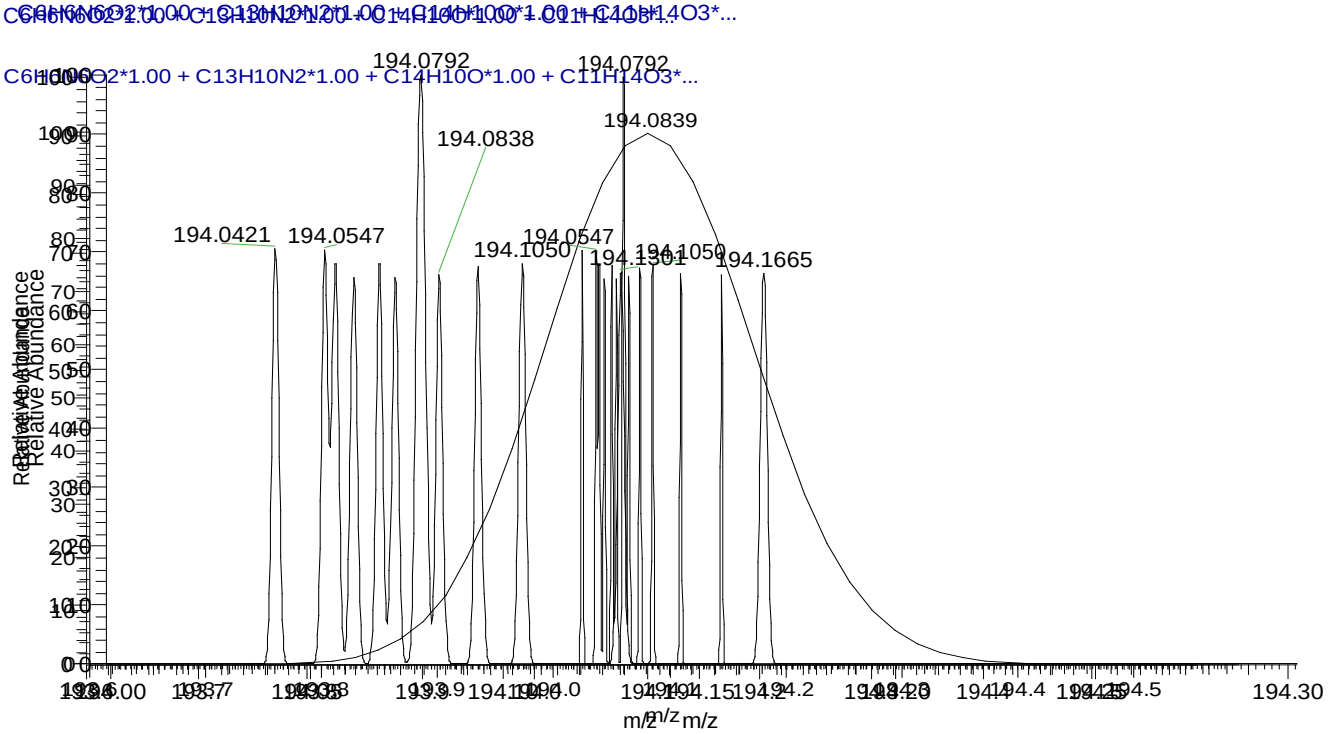
Carbon – mass 12.00

Oxygen – mass 15.995

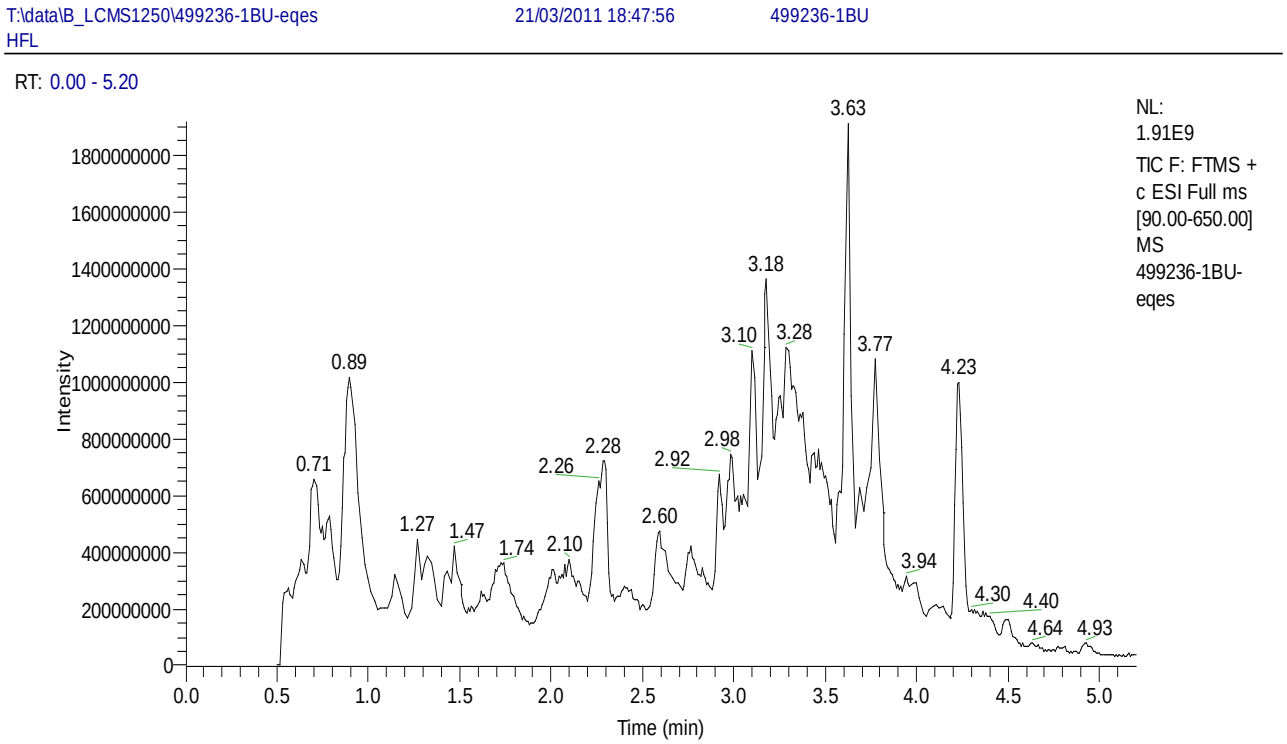
Nitrogen – mass 14.003

Hydrogen – mass 1.008

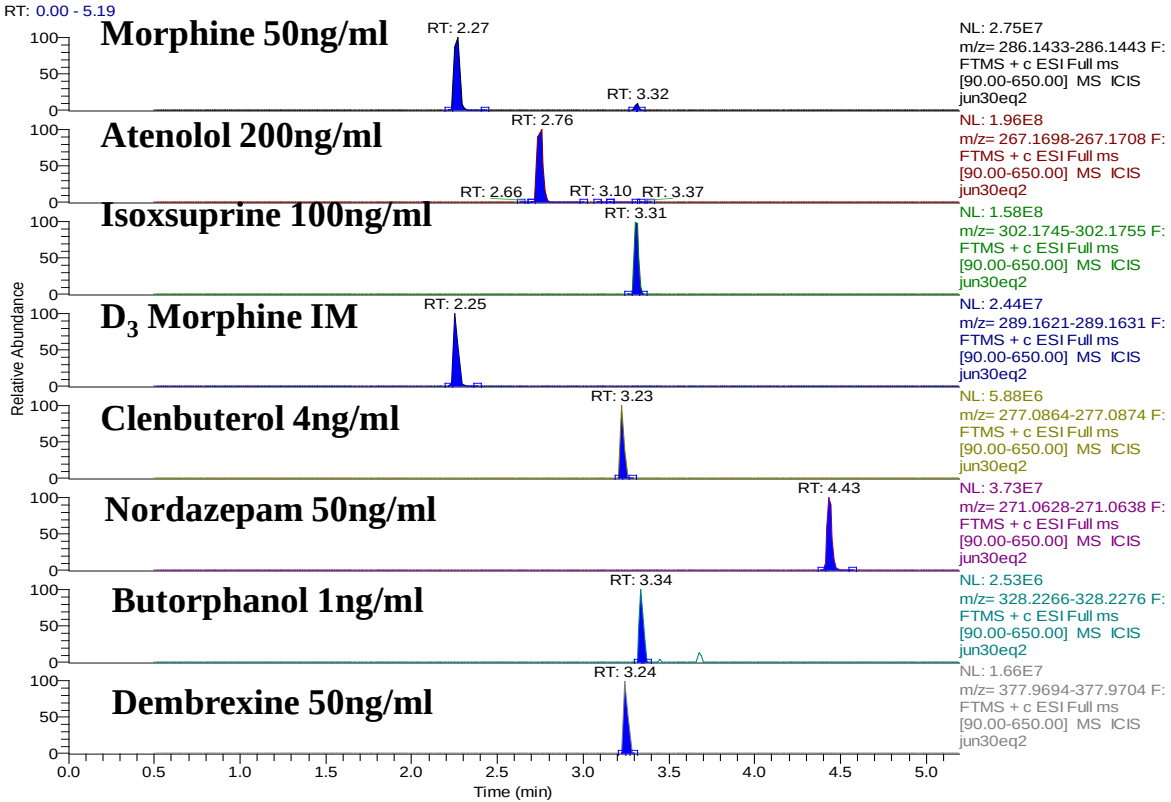
Selectivity through resolution and accurate mass measurement



Equine urine control sample post SPE – full scan

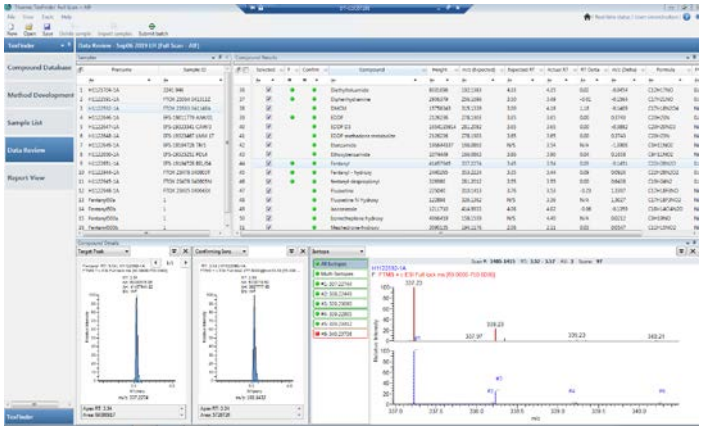


Equine urine control post SPE. EIC with 0.001 amu mass window

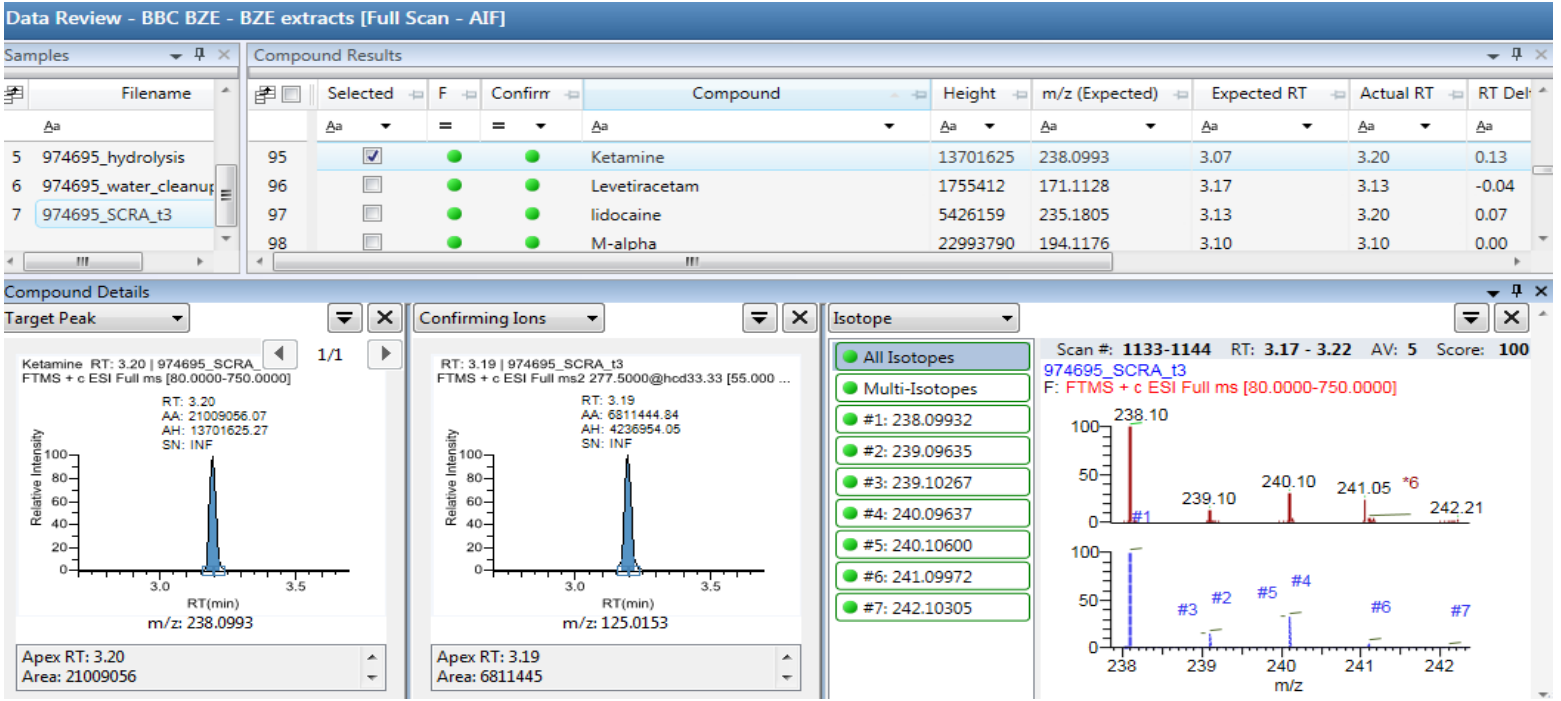


Sample analysis – General Drug Screen

- LC-HRAM-MS
- LC method - acetic acid/acetonitrile gradient, 7 minute cycle time (5 minute acquisition time)
- +ve ESI with full scan and full scan All Ion Fragmentation (AIF) searched against database of over 2000 analytes
- Data dependent (dd) MS^2 scans initiated from inclusion list of around 1000 analytes (a subset of the database) with a tolerance +/- 2mDa.
- Data processing with ToxFinder



Sample analysis – General Drug Screen



Accurate mass processing database

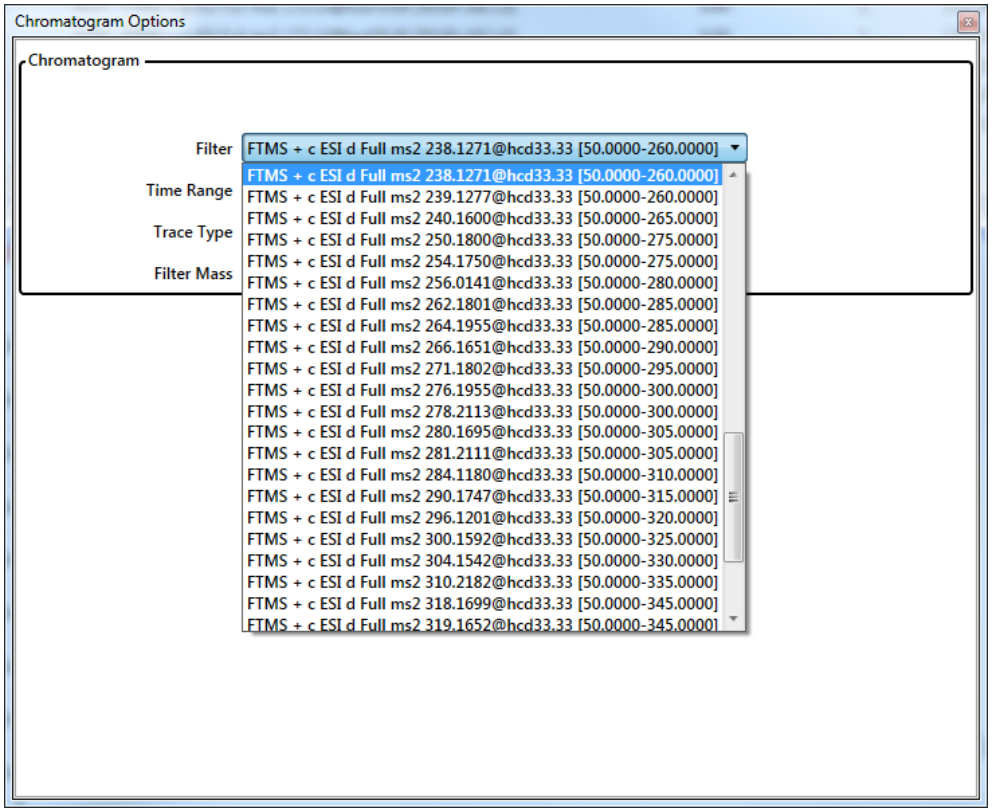
Compound Database - T3 database [Full Scan - AIF]*

	Compound Name	Molecular Formula	Extracted m/z	Polarity	Adduct	Charge State	RT (min)	RT Window (sec)	Relative RT	Internal Standard	Area Threshold	Height Threshold	Isotope Score	Fragment 1
	ketamine													
1	Ketamine-N-ethyl	C14H18ClNO	252.11497	Positive	Hydrogen	1	3.20	24.00	1	BDPA Rt Marker	1000000	1000000	35	125.0151
2	Ketamine	C13H16ClNO	238.09932	Positive	Hydrogen	1	3.07	30.00	0.959375	BDPA Rt Marker	300000	100000	35	125.0153
3	ketamine - dehydronor	C12H12ClNO	222.06802	Positive	Hydrogen	1	3.08	24.00	0.9625	BDPA Rt Marker	1000000	1000000	35	205.0415
4	Ketamine - nor	C12H14ClNO	224.08367	Positive	Hydrogen	1	3.10	24.00	0.96875	BDPA Rt Marker	1000000	1000000	35	207.0571
5	ketamine - hydroxynor	C12H14ClNO2	240.07858	Positive	Hydrogen	1	2.61	36.00	0.815625	BDPA Rt Marker	1000000	1000000	35	223.052
6														

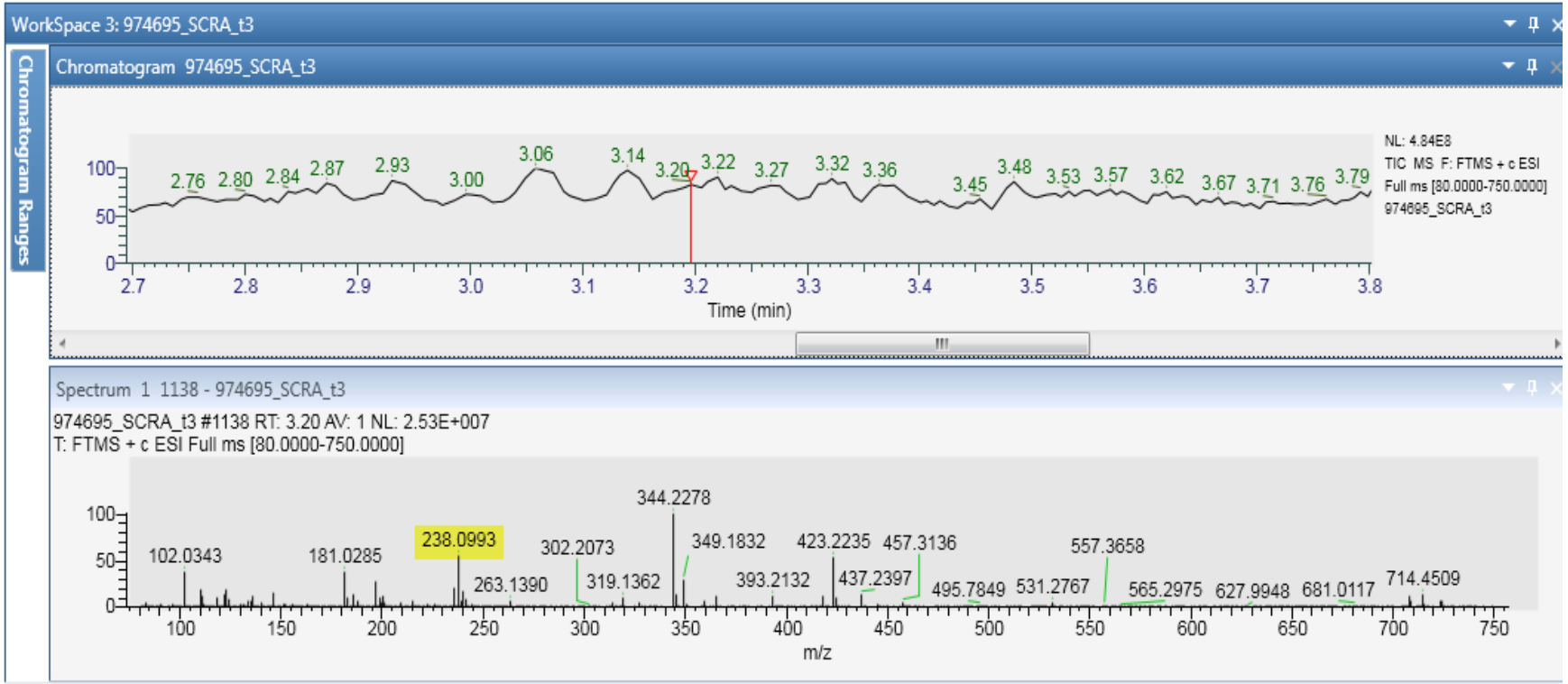
Inclusion list – over 1000 analytes

1210	142.159	C9H19N	Chemical	H	1	Positive	2.99	3.39		NCE	N=Isometheptene; F=C9H19N; A=H; T=SRM
1211	158.1539	C9H19NO	Chemical	H	1	Positive				NCE	N=Isometheptene hydroxy; F=C9H19NO; A=H; T=SRM
1212	138.0662	C6H7N3O	Chemical	H	1	Positive	1.03	1.83		NCE	N=Isoniazid; F=C6H7N3O; A=H; T=SRM
1213	212.1281	C11H17NO3	Chemical	H	1	Positive	1.28	2.08		NCE	N=Isoprenaline; F=C11H17NO3; A=H; T=SRM
1214	354.2666	C23H33N2O	Chemical	H	1	Positive	3.2	3.7		NCE	N=Isopropamide; F=C23H33N2O; A=H; T=SRM
1215	357.1495	C18H26Cl2N2O	Chemical	H	1	Positive	3	4		NCE	N=isopropyl-U-47700; F=C18H26Cl2N2O; A=H; T=SRM
1216	262.1802	C16H23NO2	Chemical	H	1	Positive	2.8	3.8		NCE	N=isopropylphenidate; F=C16H23NO2; A=H; T=SRM
1217	246.1601	C14H19N3O	Chemical	H	1	Positive	2.87	3.37		NCE	N=isopyrine; F=C14H19N3O; A=H; T=SRM
1218	475.2122	C22H30N6O4S	Chemical	H	1	Positive	3	5		NCE	N=isosildenafil; F=C22H30N6O4S; A=H; T=SRM
1219	286.1372	C16H19N3S	Chemical	H	1	Positive	3.21	3.71		NCE	N=Isothipendyl; F=C16H19N3S; A=H; T=SRM
1220	302.1751	C18H23NO3	Chemical	H	1	Positive	3.05	3.55		NCE	N=Isoxsuprine; F=C18H23NO3; A=H; T=SRM
1221	318.17	C18H23NO4	Chemical	H	1	Positive	3	3.5		NCE	N=Isoxsuprine ring OH; F=C18H23NO4; A=H; T=SRM
1222	439.1864	C24H26N2O6	Chemical	H	1	Positive	0	5.1		NCE	N=JTE-907; F=C24H26N2O6; A=H; T=SRM
1223	357.1961	C24H24N2O	Chemical	H	1	Positive	0	5.1		NCE	N=JWH-018 carboxamide derivative; F=C24H24N2O; A=H; T=SR
1224	385.1911	C25H24N2O2	Chemical	H	1	Positive	3.09	3.49		NCE	N=JWH-200; F=C25H24N2O2; A=H; T=SRM
1225	238.0993	C13H16ClNO	Chemical	H	1	Positive	2.82	3.32		NCE	N=Ketamine; F=C13H16ClNO; A=H; T=SRM
1226	222.068	C12H12ClNO	Chemical	H	1	Positive	2.88	3.28		NCE	N=ketamine - dehydronor; F=C12H12ClNO; A=H; T=SRM
1227	240.0786	C12H14ClNO2	Chemical	H	1	Positive	2.31	2.91		NCE	N=ketamine - hydroxynor; F=C12H14ClNO2; A=H; T=SRM
1228	224.0837	C12H14ClNO	Chemical	H	1	Positive	2.9	3.3		NCE	N=Ketamine - nor; F=C12H14ClNO; A=H; T=SRM
1229	252.115	C14H18ClNO	Chemical	H	1	Positive	3	3.4		NCE	N=Ketamine-N-ethyl; F=C14H18ClNO; A=H; T=SRM
1230	369.1001	C20H17ClN2O3	Chemical	H	1	Positive	4.31	4.81		NCE	N=Ketazolam; F=C20H17ClN2O3; A=H; T=SRM
1231	248.1645	C15H21NO2	Chemical	H	1	Positive	2.82	3.32		NCE	N=ketobemidone; F=C15H21NO2; A=H; T=SRM
1232	255.1016	C16H14O3	Chemical	H	1	Positive	4.12	4.62		NCE	N=ketoprofen; F=C16H14O3; A=H; T=SRM
1233	256.0968	C15H13NO3	Chemical	H	1	Positive	4.1	4.6		NCE	N=Ketorolac; F=C15H13NO3; A=H; T=SRM
1234	310.126	C19H19NOS	Chemical	H	1	Positive	3.15	3.65		NCE	N=Ketotifen; F=C19H19NOS; A=H; T=SRM
1235	329.186	C19H24N2O3	Chemical	H	1	Positive	3.14	3.64		NCE	N=Labetalol; F=C19H24N2O3; A=H; T=SRM
1236	251.139	C13H18N2O3	Chemical	H	1	Positive	0.5	6.5		NCE	N=Lacosamide; F=C13H18N2O3; A=H; T=SRM
1237	230.0594	C8H11N3O3S	Chemical	H	1	Positive	1.85	2.35		NCE	N=lamivudine; F=C8H11N3O3S; A=H; T=SRM
1238	256.0151	C9H7Cl2N5	Chemical	H	1	Positive	2.87	3.37		NCE	N=Lamotrigine; F=C9H7Cl2N5; A=H; T=SRM
1239	370.0832	C16H14F3N3O2S	Chemical	H	1	Positive	3.83	4.49		NCE	N=Lansoprazole; F=C16H14F3N3O2S; A=H; T=SRM
1240	328.2873	C22H35N2	Chemical	H	1	Positive	3.89	4.39		NCE	N=laurolinium; F=C22H35N2; A=H; T=SRM
1241	226.159	C16H19N	Chemical	H	1	Positive	3.08	3.58		NCE	N=lefetamine; F=C16H19N; A=H; T=SRM
1242	139.0978	C6H10N4	Chemical	H	1	Positive	3.26	3.76		NCE	N=leptazol; F=C6H10N4; A=H; T=SRM

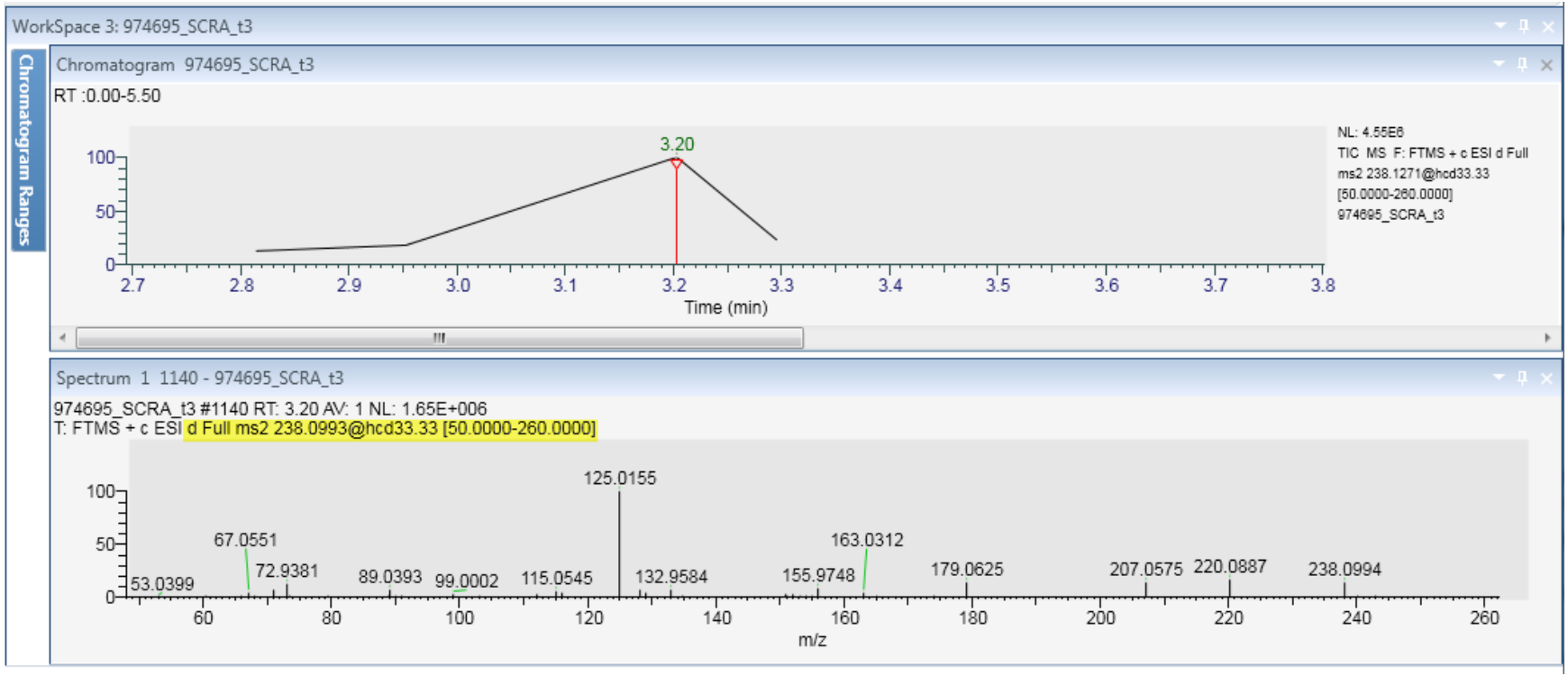
Multiple MS2 events initiated



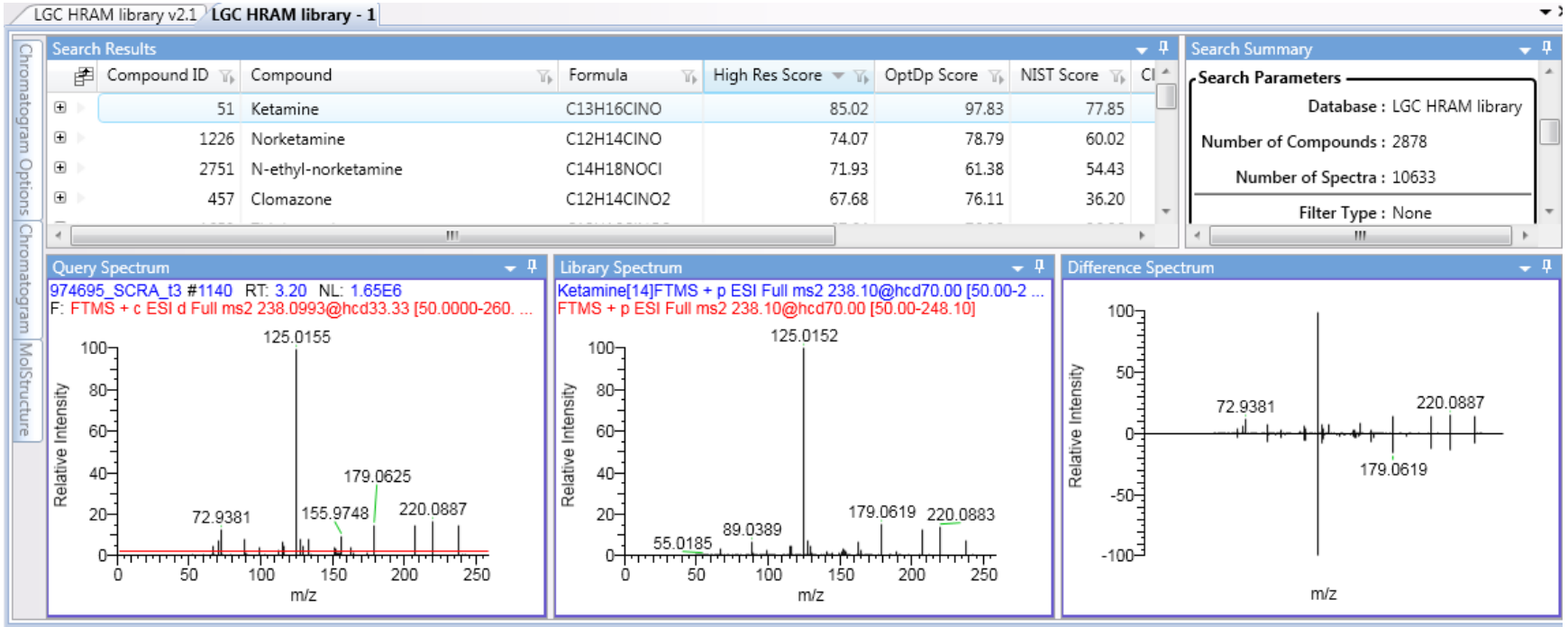
Ion identified in full scan spectrum for MS^2 from inclusion list



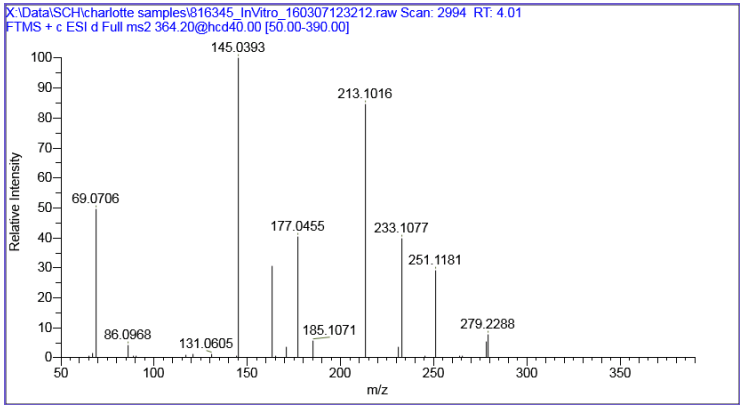
Data dependent MS^2 triggered by m/z 238.0993



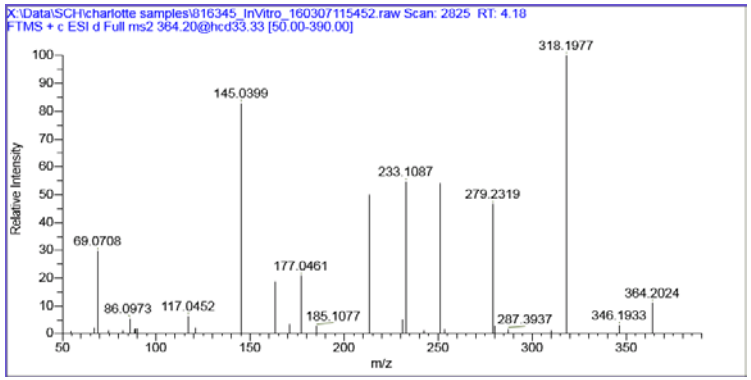
Confirmation of finding using MS2 spectrum generated ‘on the fly’ then matched in library – either mzcloud™ or mzvault ™



MS2 spectra – single CE or stepped CE

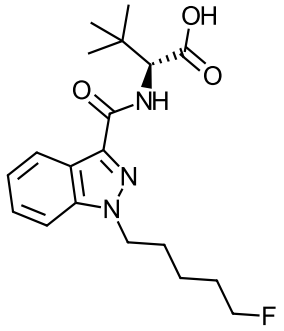


CE 40



CE 15,35,50

5F-ADB metabolite



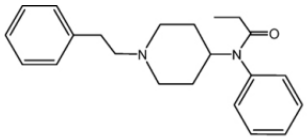
Keeping up to date – need to know what to look for (without reference materials)

- Problems presented by incorporation of new NPS into testing
  - No definitive information on metabolism
  - No reference materials initially for parent drug or metabolites
- Database
  - Updated as new drugs reported through forensic networks
  - Updated as new drugs detected in seizures
  - Updated as new drugs come on to the market or are found in sports drug testing
  - Updated as cases seen with metabolites or from *in-vitro* metabolism
- Libraries
  - Updated as new drugs are detected in lab from seizures or biological samples
  - Updated as cases seen with metabolites or from *in-vitro* metabolism
  - Use of mzcloud which is updated daily

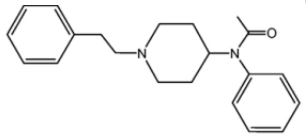
Fentanyl Analogues

Nomenclature

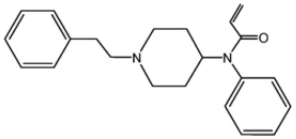
- Fentanyl is a potent opioid drug 100x stronger than morphine
- ‘Fentanyls’ is a generic term used to cover all the compounds of similar structure that are often termed ‘designer’ fentanyls
- A fentanyl can in this context be any of the ‘fentanyl’ group of drugs



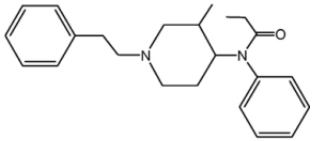
Fentanyl



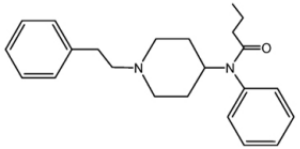
Acetylfentanyl



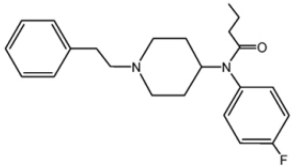
Acryloyfentanyl



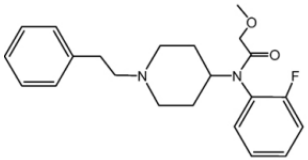
3-Methylfentanyl



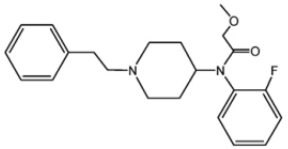
Butyrylfentanyl



4-Fluorobutyrylfentanyl



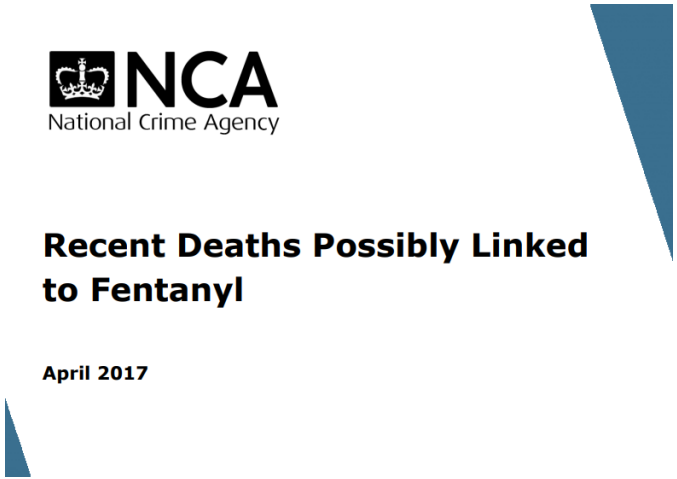
Furanylfentanyl



Ocfentanil

Detection of fentanyl analogues in the UK by LGC

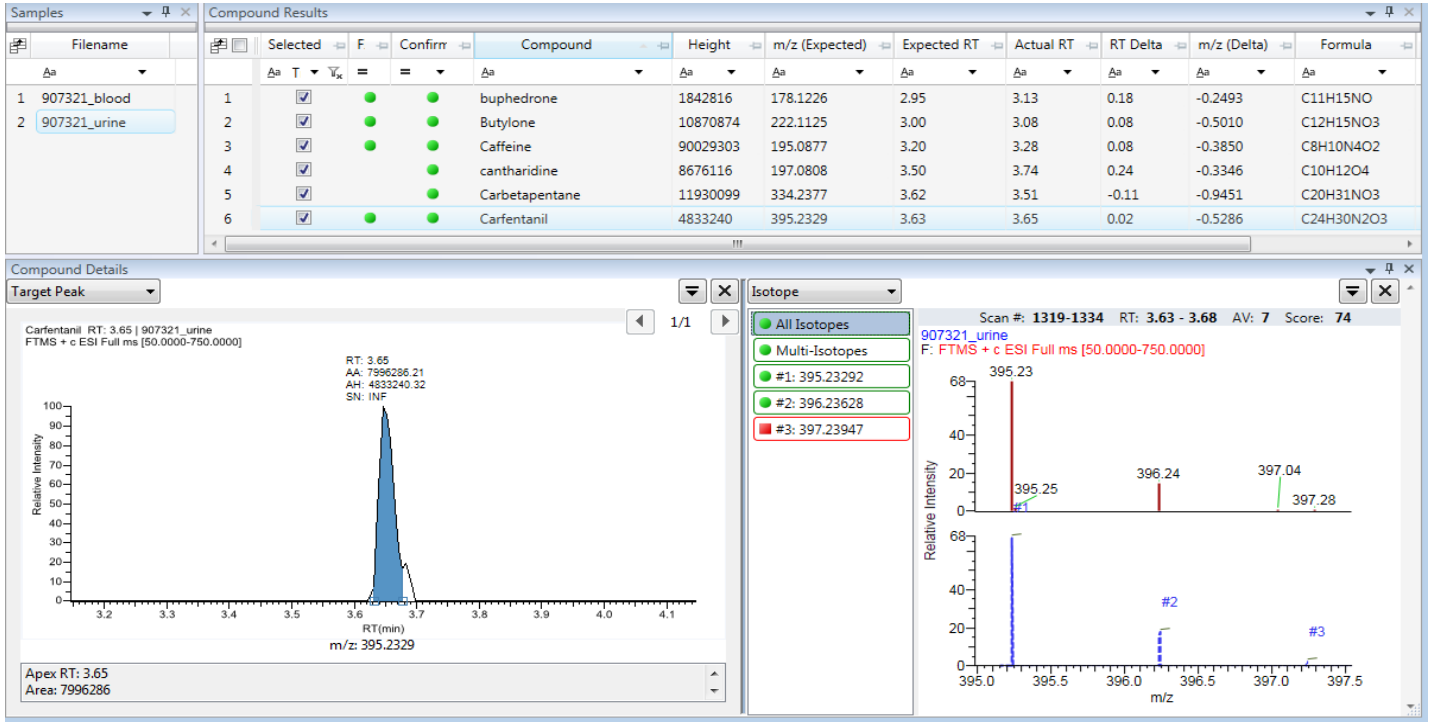
- Up to 2017, a handful of fentanyl, alfentanil and fluorofentanyl findings
- 2017 started with a few fentanyl, fluorofentanyl and fluorobutyryl fentanyl findings
- Late March 2017, detected carfentanil in a urine and blood sample.



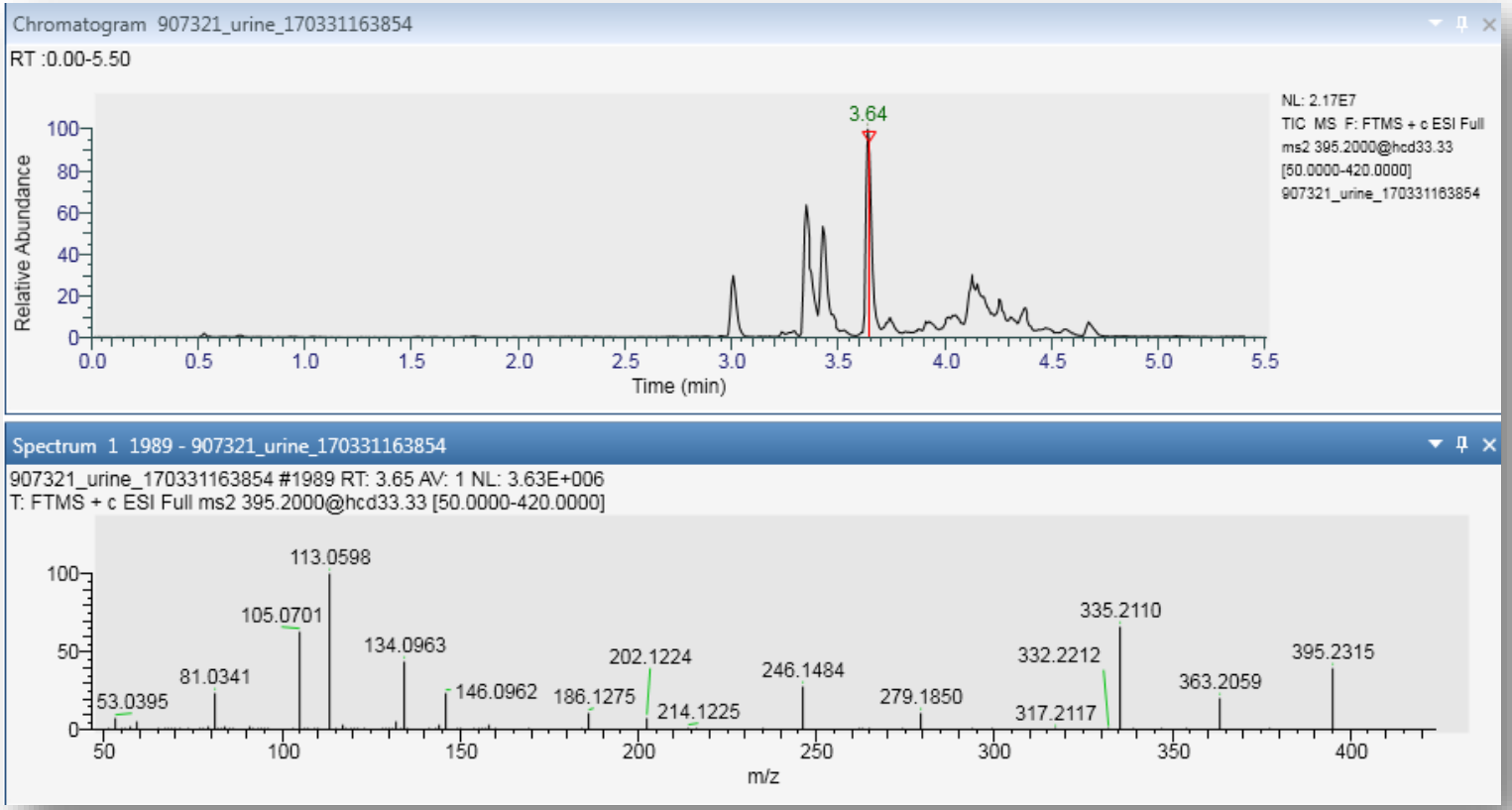
There has been a recent increase in deaths attributed to heroin in the North East of England. Some of these deaths have shown signs of being very sudden, potentially indicating an immediate and fatal overdose. Recent results from toxicology samples submitted for specific fentanyl screening generated 'additional substance' detections.

First carfentanil finding Mar 2017

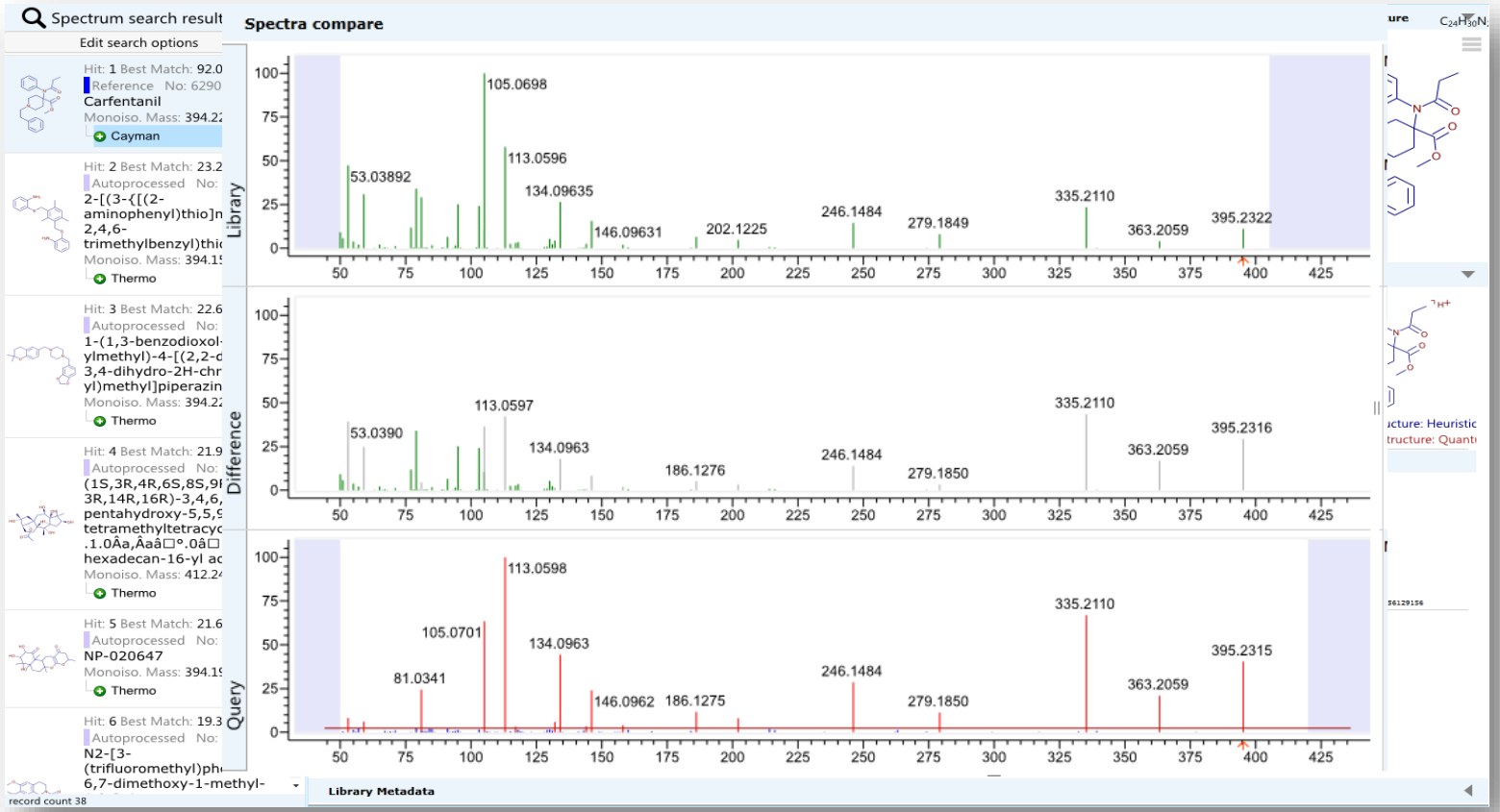
- Carfentanil in accurate mass database from work in animal sport



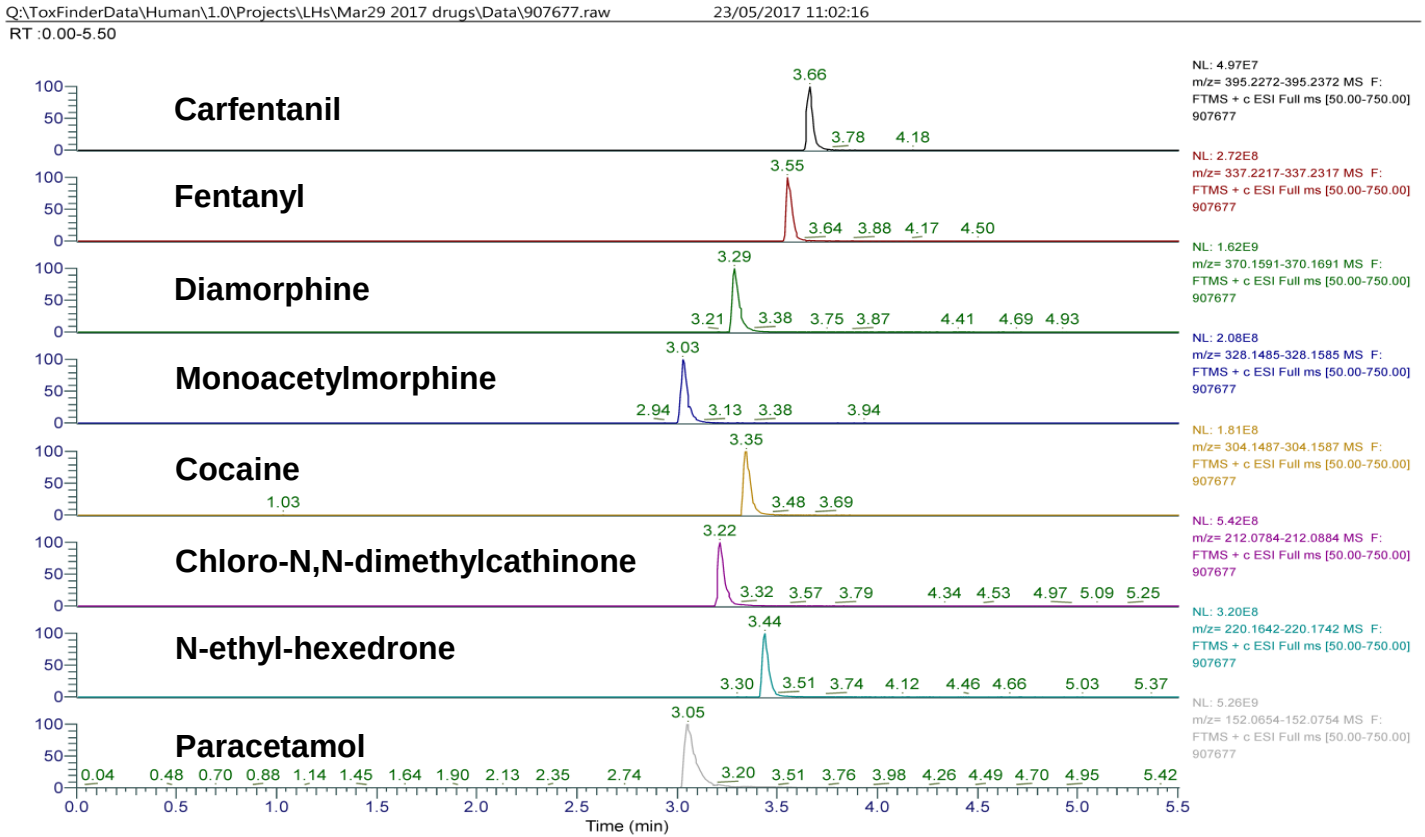
Re-analysed to generate MS^2 data as not in inclusion list



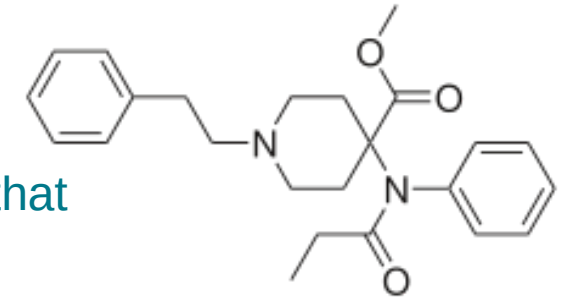
Confirmed ID as carfentanil using mzcloud



Carfentanil in heroin seizure



Over 3 days, identified carfentanil in blood, urine and heroin seizure



- Quantitative potency approximately 10,000 times that of morphine and 100 times that of fentanyl
- Active in humans at 1µg. Believed to be lethal at 20µg
- Being added to heroin
- Other drugs present
- Level seen at LGC – 0.2% carfentanil in heroin – could lead to dose of 200µg assuming a 100mg dose of heroin

Selected coroners cases

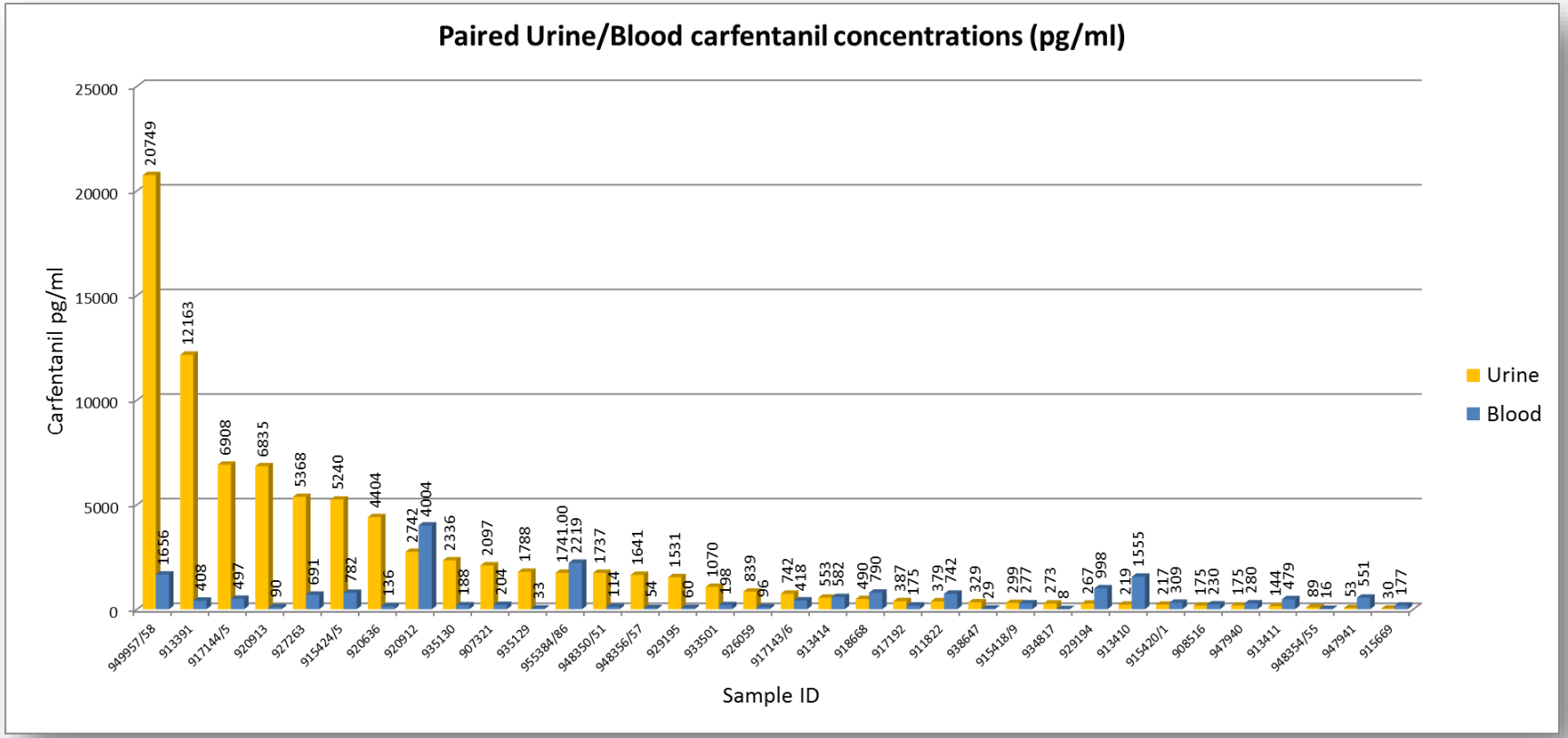
M/F	Age	Circumstances	Synthetic fentanyls	Other drugs
M	44	Ex drug-abuser. Started using again last 4-5 months. Found in foetal position on floor, uncapped syringe present.	Carfentanil 230 pg/mL blood 175 pg/mL urine Fentanyl = <0.1 ng/mL Norfentanyl = <0.1 ng/mL	Morphine (free) = 18 ng/mL Morphine (total) = 21 ng/mL Noscapine and papaverine metabolites detected Mirtazapine = 15 ng/mL Diazepam = 68 ng/mL Nordiazepam = 29 ng/mL Paracetamol = <5 mg/L
M	44	Discovered on living room floor of flat deceased.	Carfentanil 96 pg/mL blood 839 pg/mL urine Fentanyl = <0.1 ng/mL Norfentanyl = <0.1 ng/mL	Duloxetine = 92 ng/mL Paracetamol = 16 mg/L
M	40	Found on waste ground, kneeling position, drug paraphernalia at side of body.	Carfentanil 4004 pg/mL blood 2742 pg/mL urine Fentanyl = <0.1 ng/mL Norfentanyl = <0.1 ng/mL	Morphine (free) = 71 ng/mL Morphine (total) = 94 ng/mL 6-monoacetylmorphine detected Noscapine and papaverine metabolites detected Ethanol = 10 mg/100mL (PB) Ethanol = 22 mg/100mL (PU) Paracetamol = 11 mg/L Pregabalin = 11 mg/L THC-COOH = 7 ng/mL

Selected coroners cases

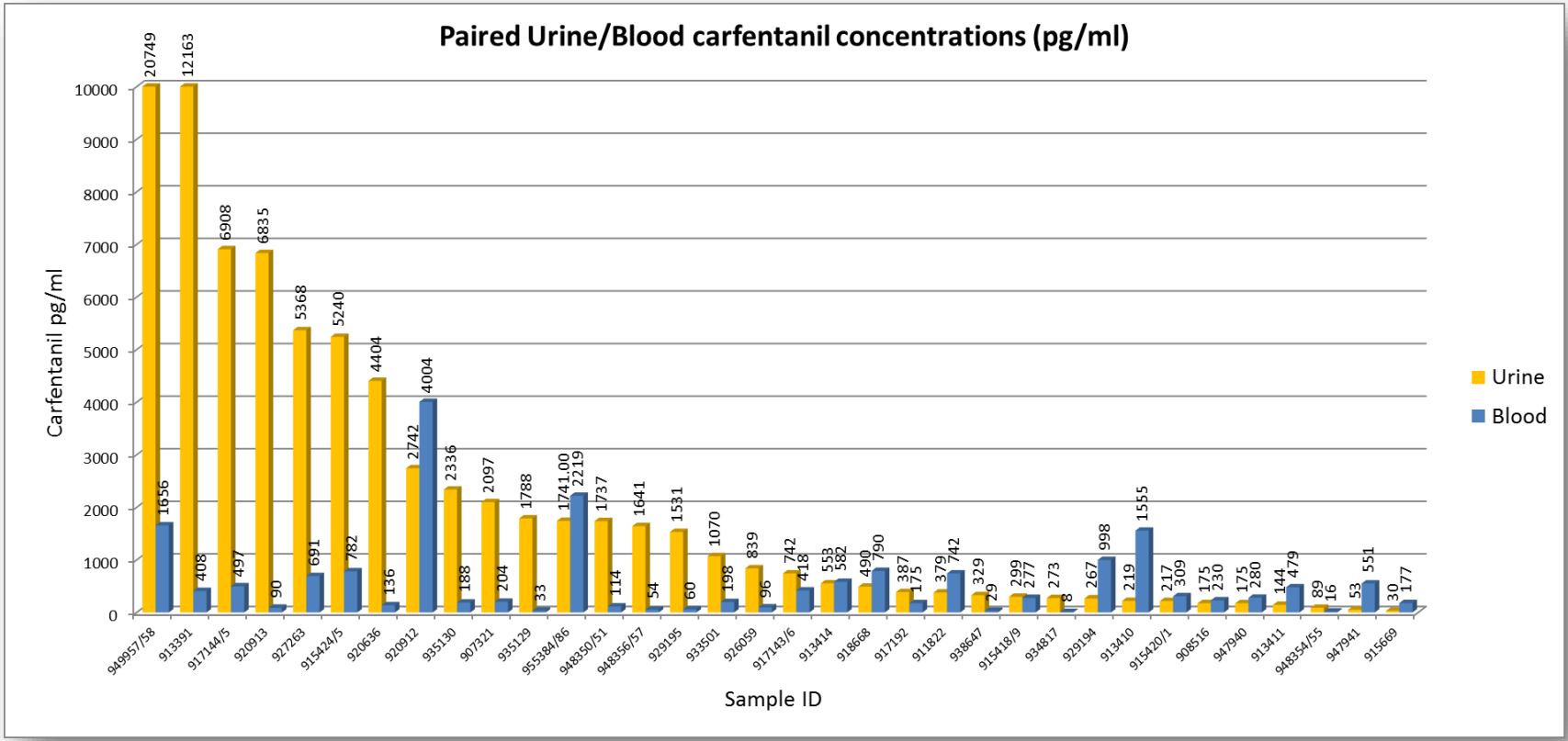
M/F	Age	Circumstances	Synthetic fentanyls	Other drugs
M	31	IV drug abuser. Slumped in bathroom. Syringe in left hand and belt around right arm. Prescribed co-codamol, methadone and mirtazapine.	Carfentanil 479 pg/mL blood, 144 pg/mL urine Butyryl fentanyl 4F-butyryl fentanyl	**Synthetic cannabinoid:** **5F-ADB** Ethanol = 10 mg/100mL (PB) Ethanol = 22 mg/100mL (PU) Morphine (free) =16 ng/mL Morphine (total) = 17 ng/mL Paracetamol = <5 mg/L Diazepam = 558 ng/mL Nordiazepam = 458 ng/mL Temazepam = 16 ng/mL Oxazepam = <5 ng/mL Pregabalin = 4.0 mg/L Methadone = 498 ng/mL EDDP = 50 ng/mL Cocaine metabolite (benzoylecgonine = 19 ng/mL) Mirtazapine = 128 ng/mL

Carfentanil Blood/Urine findings

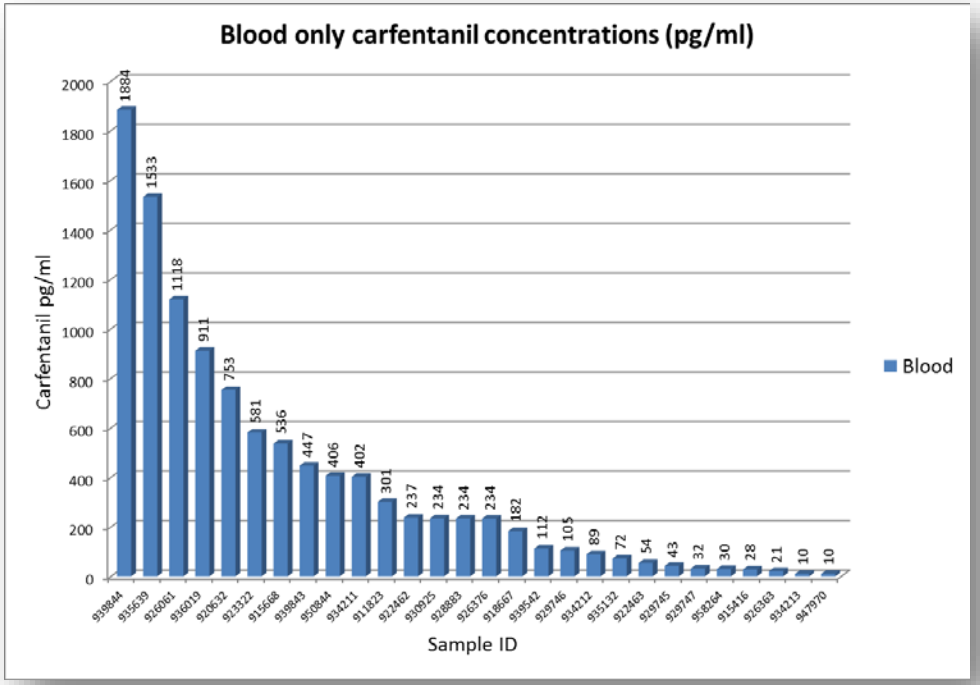
- LOD approximately 5pg/ml (5ppt)
- Highest blood level – 4004 pg/ml
- Highest urine level – 20749 pg/ml

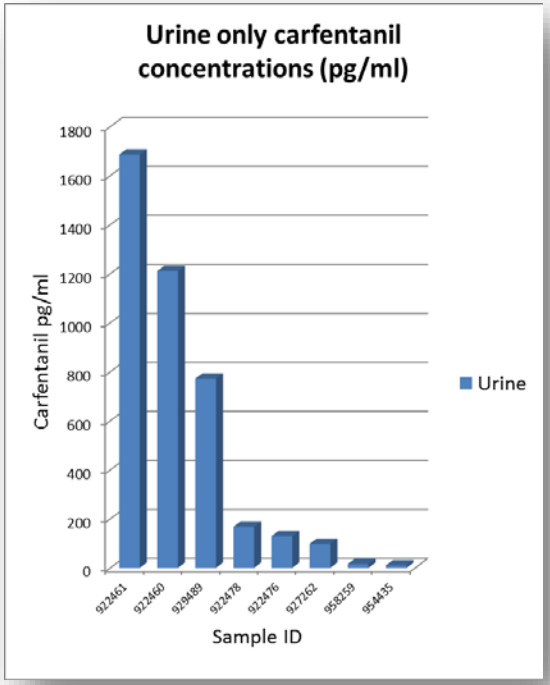


Carfentanil Blood/Urine findings (displayed to 10000 pg/ml)



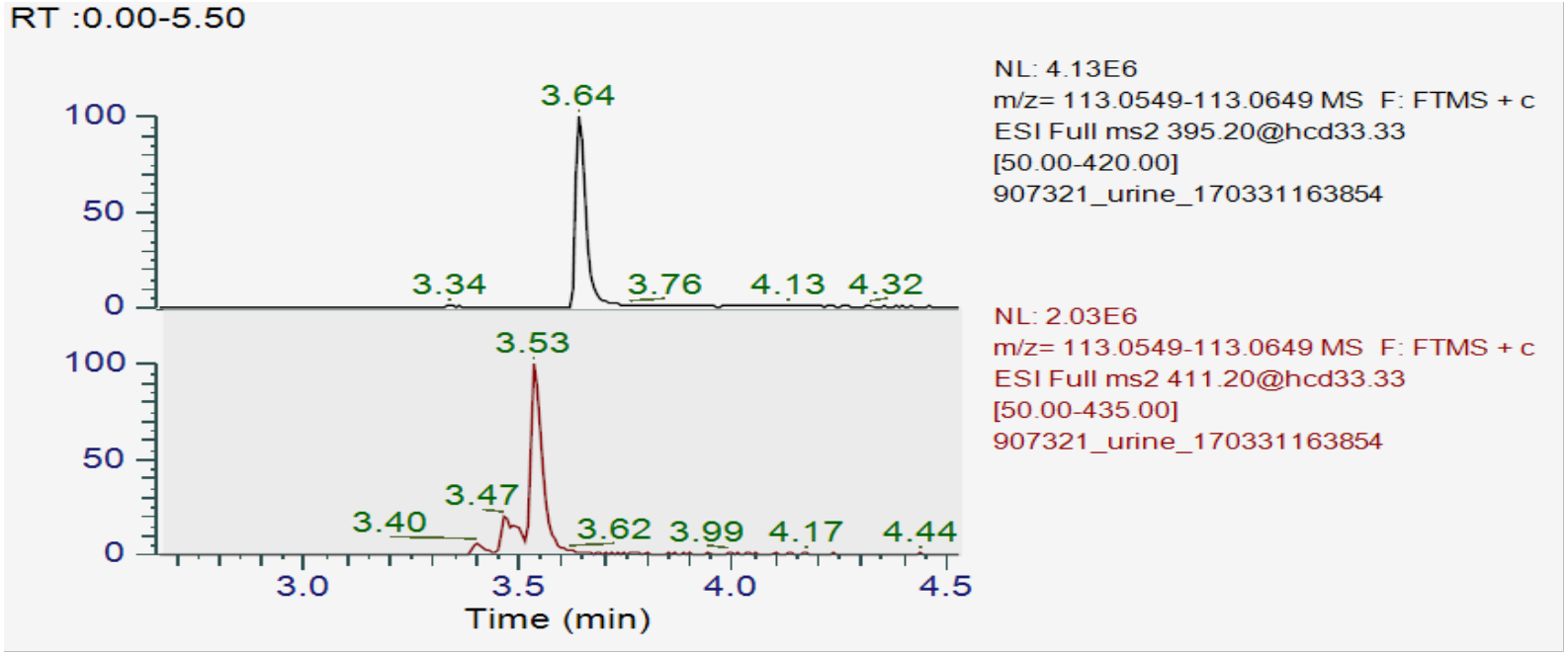
Carfentanil Blood/Urine findings



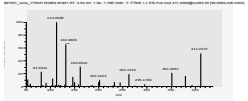


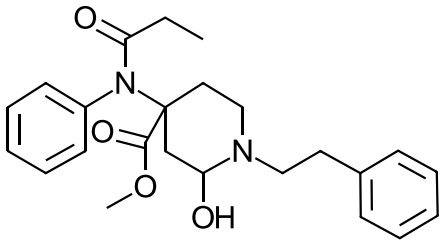
What about metabolites?

- Parent drug and hydroxylated metabolites in urine.
- Added to databases/libraries

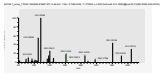


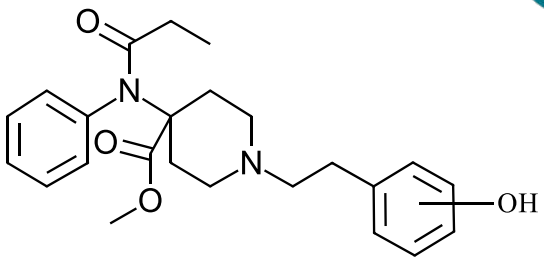
Generally the major hydroxy metabolite

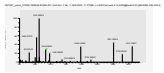


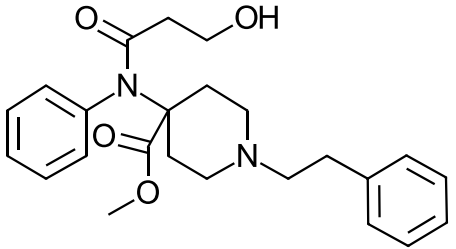


Additional hydroxy metabolites

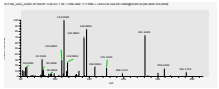


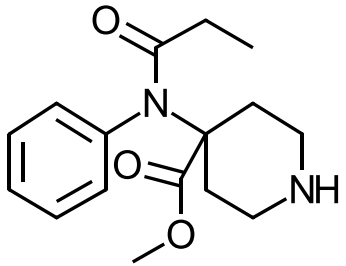






Desalkyl metabolite (but also a metabolite remifentanil)





Fentanyl analogues since Mar 2017

- 70 cases have contained carfentanil (sometimes with other ‘fentanyls’
- Most recent positive carfentanil findings from cases from June 2017 – i.e problem gone.
- Other fentanyls seen over same period included fentanyl, alfentanil, fluorofentanyl, furanylfentanyl, butyrylfentanyl, 4F isobutyrylfentanyl
- Since this period – cyclopropylfentanyl, methoxyacetylfentanyl, fentanyl, alfentanil and remifentanil

West Yorkshire men jailed for selling drugs on the dark web

(L-R) Jake Levine, Mandy Lowther and Lee Childs

Case Study – Operation Tanite

- Selling Carfentanil and other related fentanyls from warehouse through Royal Mail
- Large quantities of cutting agents found namely paracetamol, caffeine and mannitol
- Drugs were being mixed in a blender
- Perpetrators used filtered face masks and strong gloves
- Approximately 650g of carfentanil seized which is equivalent to around 300million to 600million doses
- Case worked on by pairs of analysts one of whom was trained in naloxone antidote administration
- Three defendants were sentenced to a total of 42 years for supply of a Class A drug in January 2019
- Although not confirmed as being a direct result of this gang over 100 people died of overdoses with carfentanil present

Synthetic Cannabinoid Receptor Agonists (SCRAs)

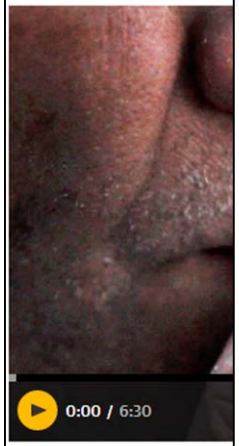
Manchester Spice Zombies

UK Prisons

2014 2015 2016 2017

Synthetic ca
health probl

Prisoners increasingly u
numbers of users in hos



Synthetic cannabis knov
UK prisons with serious
inspector of prisons, Ni

Warning
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Inspectors reveal d
synthetic drug con

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emergencies involving inm

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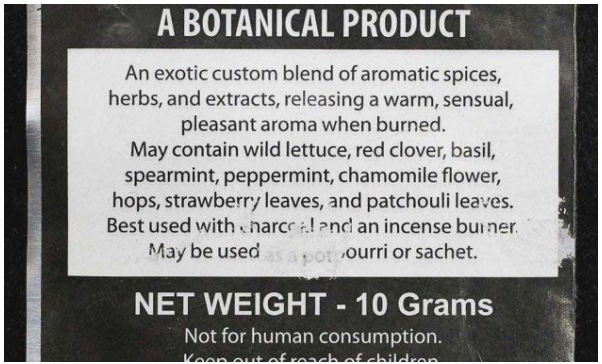
Chief inspector of priso
cannabis substitutes 'sp

Two people die in suspected spice-related incidents

Prisoner dies of suspected overdose at HMP Forest Bank in Salford, and Somerset police investigate death of man in Taunton

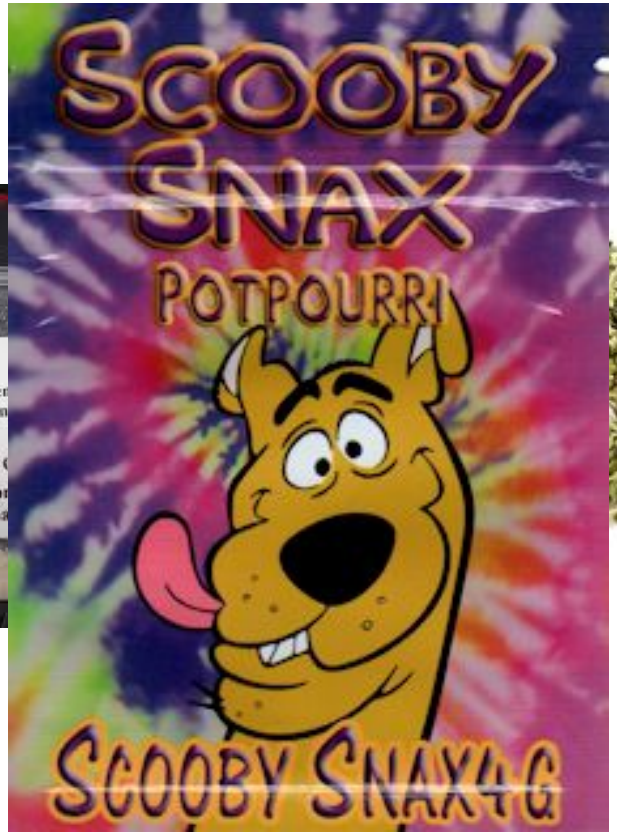
The deaths will heighten concerns that problems relating to spice are growing both inside and outside prison. Photograph: Leon Neal/AFP/Getty Images

Spice – what is it?



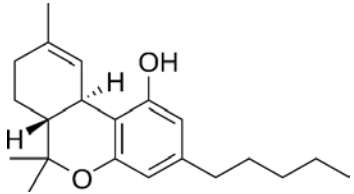
- Available on Internet and from specialist shops from at least 2006
- Advertised as an ‘exotic incense blend that releases a rich aroma’
- Also marked ‘not for human consumption’
- When smoked it’s reported to have effect similar to cannabis
- Listed ingredients are all plant material
- Has been associated with deaths around the world

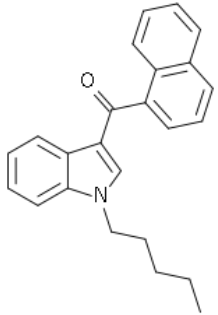
What is ‘Spice’



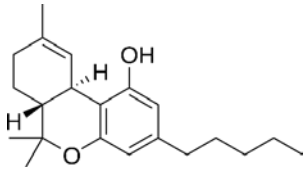
JWH-018 – first SCRA

- First synthesized in 1995 by John W Huffman's group in Clemson University, South Carolina to study structure-receptor relationships
- Never tested in humans
- JWH-018 approximately 5x more potent than THC
- Structurally dissimilar to THC
- Now illegal in many countries
- As of 13th June 2019, 198 different SCRAs have been reported in Europe

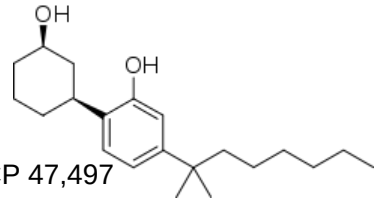




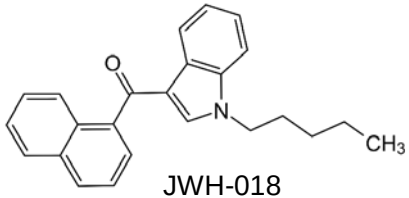
Synthetic Cannabinoid Receptor Agonists - Variety of structures



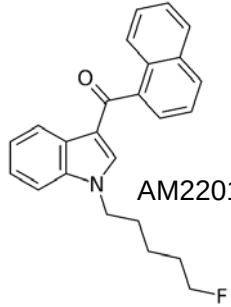
THC



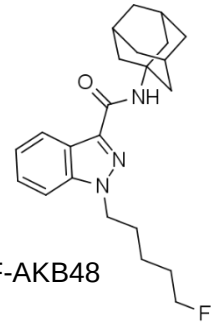
CP 47,497



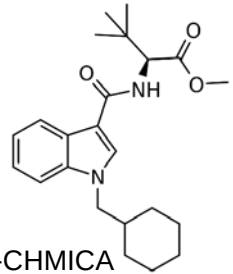
JWH-018



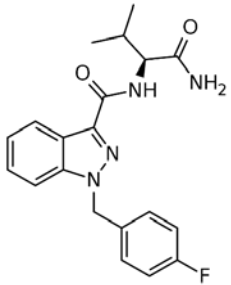
AM2201



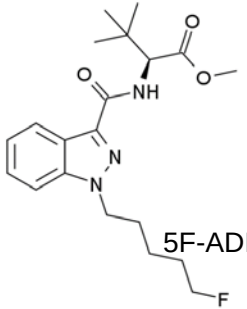
5F-AKB48



MDMB-CHMICA



AB-FUBINACA



5F-ADB

198 different compounds detected since first identifications in 2008

So where have these compounds come from?

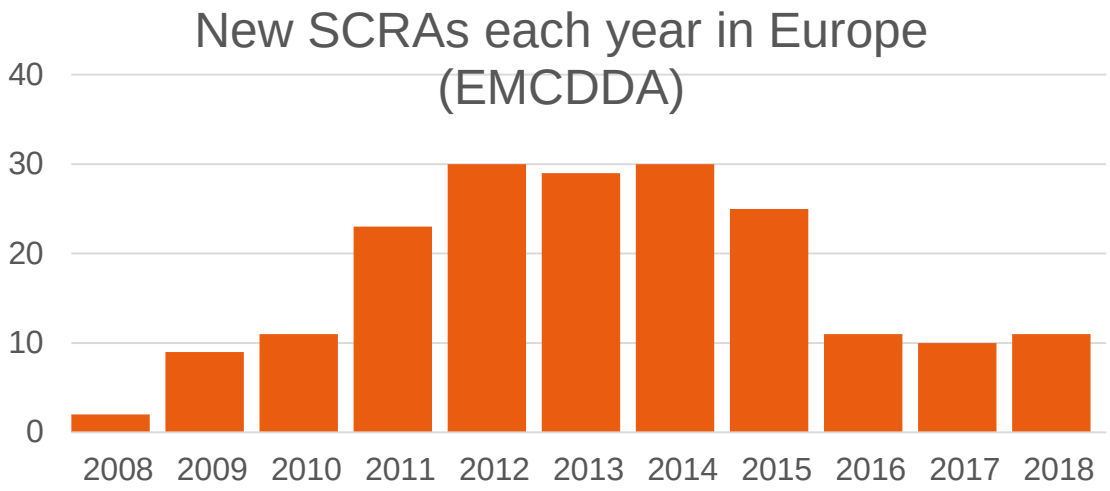
- Scientific literature – e.g. Huffman's work on drug/cannabinoid receptor interaction as investigative tools for the endocannabinoid system
- Filed patents – much work has been done on trying to mimic the pain relieving property of cannabis without the psychoactive effect.
- More recently, attempts to evade legislation by structural modification

Hijacking of Basic Research: The Case of Synthetic Cannabinoids

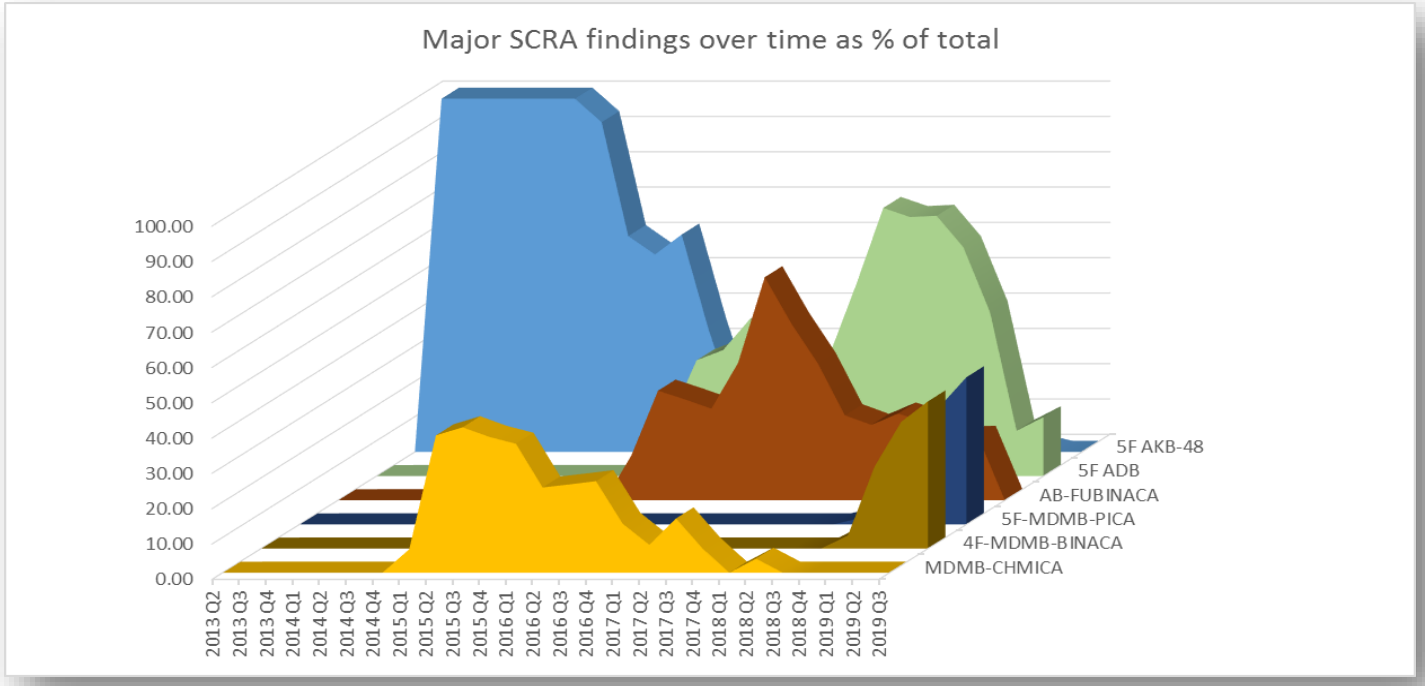
Jenny L. Wiley, PhD, Julie A. Marusich, PhD, John W. Huffman, PhD, Robert L. Balster, PhD, and Brian F. Thomas, PhD

Gathering and communicating knowledge are important aspects of the scientific endeavor. Yet presentation of data in public forums such as scientific meetings and publications makes it available not only to scientists, but also to others who may have different ideas about how to use research findings. A recent example of this type of hijacking is the introduction of synthetic cannabinoids that are sprayed on herbal products and subsequently smoked for their marijuana-like intoxicating properties. Originally developed for the legitimate research purpose of furthering understanding of the cannabinoid system, these synthetic

New SCRAs in Europe



Change in major SCRA findings at LGC over time 2013-2019



Testing Methodology - SCRAs

- Herbals etc prepared in methanol
- LLE sample prep of blood or pre-treated urine
- Analysis on Q Exactive MS
- Full scan + AIF + dd MS2
  - Full scan/AIF – broad screen and new compounds
  - DD MS2 from 800+ analyte inclusion list to confirm actual finding. Mass window to trigger inclusion list MS2 – 5 mmu with 4 second dynamic exclusion

4F-MDMB-BINACA

4F-MDMB-BINACA- [EU EWS FORMAL NOTIFICATION]: methyl 2-(1-(4-fluorobutyl)-1H-indazole-3-carboxamido)-3,3-di...

4F-MDMB-BINACA_FR_Reporting_Form_Nov_2018.doc
211 KB

EU-EWS-RCS-FN-2018-0049_4F-MDMB-BINACA.pdf
61 KB

Action Items + Get more apps

Subject: [EU EWS FORMAL NOTIFICATION]: methyl 2-(1-(4-fluorobutyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate (4F-MDMB-BINACA)

EU EARLY WARNING SYSTEM FORMAL NOTIFICATION

Subject: Formal notification of methyl 2-(1-(4-fluorobutyl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate (4F-MDMB-BINACA) by France as a new psychoactive substance under the terms of Council Decision 2005/387/JHA

RCS ID: EU-EWS-RCS-FN-2018-0049
Issued by: EMCDDA
Date Issued: 20 November 2018
Transmitted by: Action on New Drugs Sector, EMCDDA

1. Read me first

This document provides formal notification of the analytical identification of methyl 2-(1-(4-fluorobutyl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate (4F-MDMB-BINACA) for the first time in Europe.
Please report any additional data you have on this substance to: ews@emcdda.europa.eu

2. Data use restrictions

As with all formal notifications issued by the EU EWS remember that they may contain information that could be regarded as sensitive. Should you provide some of the information in this notification to other groups we would ask that you exercise your best judgment on what information needs to be provided. If you have any questions in this respect, please contact us.

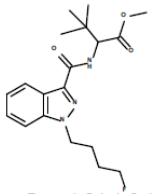
3. Names of substance and other identifiers

- IUPAC name: methyl 2-(1-(4-fluorobutyl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate
- Chemical names: methyl 2-[[1-(4-fluorobutyl)indazole-3-carbonyl]amino]-3,3-dimethyl-butanoate, methyl 2-{[1-(4-fluorobutyl)-1*H*-indazol-3-yl]formamido}-3,3-dimethylbutanoate
- Common name: 4F-MDMB-BINACA
- Other names: none

3. Names of substance and other identifiers

- IUPAC name: methyl 2-(1-(4-fluorobutyl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate
- Chemical names: methyl 2-[[1-(4-fluorobutyl)indazole-3-carbonyl]amino]-3,3-dimethyl-butanoate, methyl 2-{[1-(4-fluorobutyl)-1*H*-indazol-3-yl]formamido}-3,3-dimethylbutanoate
- Common name: 4F-MDMB-BINACA
- Other names: none
- Chemical formula: $C_{19}H_{26}FN_3O_3$
- Molecular weight: 363.43
- CAS Registry number: not registered
- InChIKey: GZGKSDAMWRWYOZ-UHFFFAOYSA-N

Molecular structure



Praça Europa 1, Cais do Sodré, 1249-289 Lisbon, Portugal
Tel. (351) 211 21 02 00 | Fax (351) 211 21 03 80 | ews@emcdda.europa.eu | emcdda.europa.eu

4. Substance classification

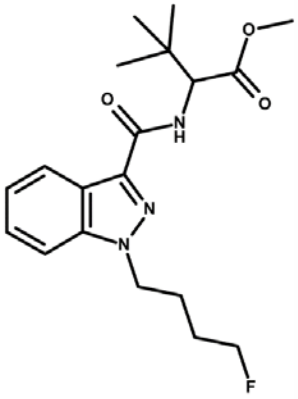
Synthetic cannabinoid

Add to accurate mass database

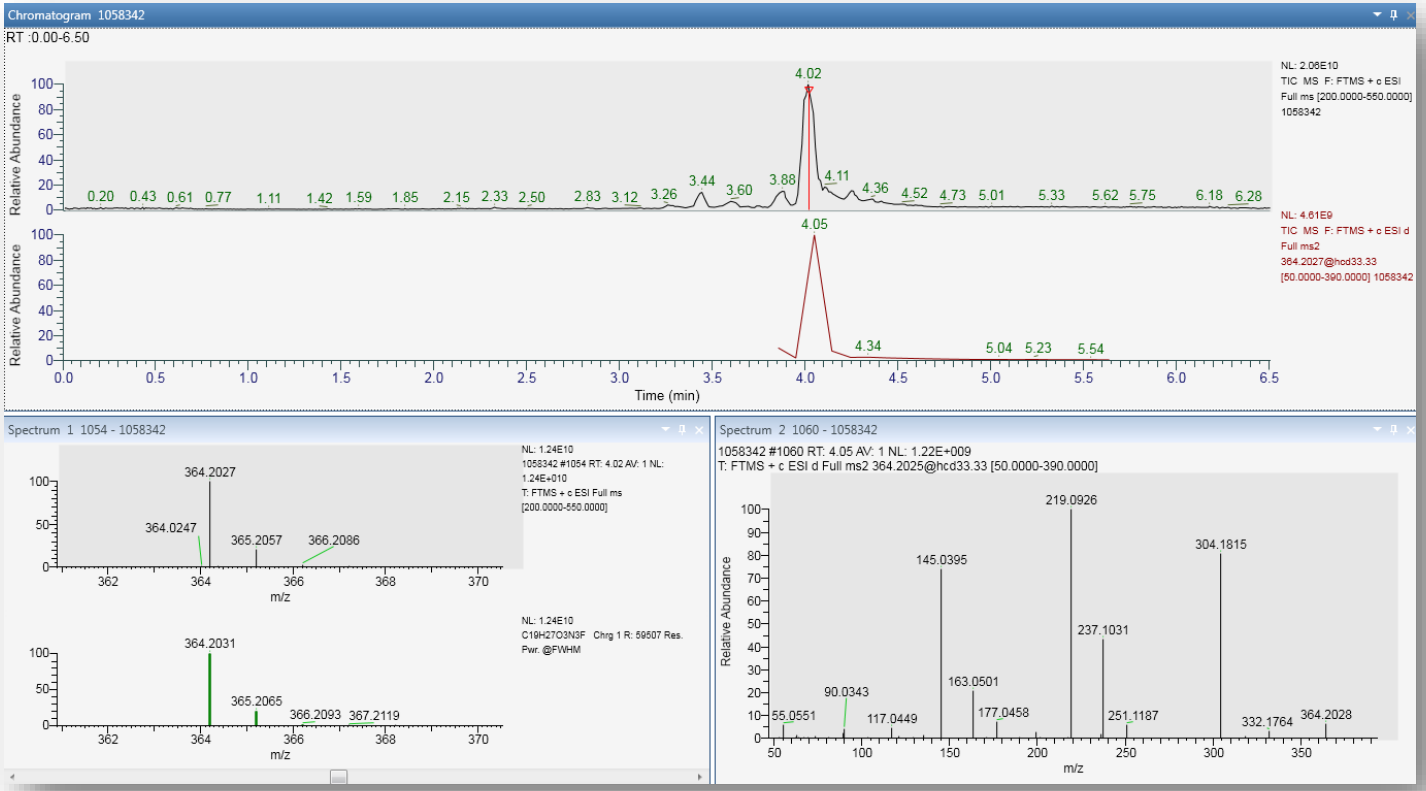
Compound Database - SCRA Master [Full Scan - AIF]

	Compound Name	Molecular Formula	Extracted m/z	Polarity	Adduct	Charge State	RT (min)	RT Window (sec)	Relative RT	Internal Standard	Area Threshold	Height Threshold	Isotope Score	Fragment 1
939	APP-BINACA-desamino-hydroxy	C21H23N3O3	366.18122	Positive	Hydrogen	1	4.64	30.00	1.198966408	d5 hydroxypenty	1400000	100000	35	201.1018
940	4F MDMB-BINACA	C19H26FN3O3	364.2031	Positive	Hydrogen	1	4.10		1.059431525	d5 hydroxypenty	1400000	100000	35	
941	4F-MDMB-BINACA-desmethyl	C18H24FN3O3	350.18745	Positive	Hydrogen	1	3.97	30	1.025839793	d5 hydroxypenty	1400000	100000	35	219.0926
942	APP-BINACA-hydroxy 2	C21H24N4O3	381.19212	Positive	Hydrogen	1	3.16	30.00	0.816537468	d5 hydroxypenty	1400000	100000	35	235.1071
943	APP-BINACA-hydroxy 3	C21H24N4O3	381.19212	Positive	Hydrogen	1	3.32	30.00	0.857881137	d5 hydroxypenty	1400000	100000	35	201.1017
944	APP-BINACA-hydroxy 4	C21H24N4O3	381.19212	Positive	Hydrogen	1	3.64	30.00	0.940568475	d5 hydroxypenty	1400000	100000	35	217.0969
945	APP-BINACA-dihydroxy 1	C21H24N4O4	397.18703	Positive	Hydrogen	1	2.87	30.00	0.741602067	d5 hydroxypenty	1400000	100000	35	235.1074
946	APP-BINACA-dihydroxy 2	C21H24N4O4	397.18703	Positive	Hydrogen	1	3.18	30.00	0.821705426	d5 hydroxypenty	1400000	100000	35	217.0969
947	4F-MDMB-BINACA-desfluoro-hydroxy	C19H27N3O4	362.20743	Positive	Hydrogen	1	3.60	30.00	0.930232558	d5 hydroxypenty	1400000	100000	35	235.1077
948	4F-MDMB-BINACA-desmethyl (-2H)	C18H22N3O3F	348.1718	Positive	Hydrogen	1	3.30	30.00	0.852713178	d5 hydroxypenty	1400000	100000	35	237.1019

Identified in the UK in a ‘Spice’ product – Dec 2018



$C_{19}H_{26}FN_3O_3$



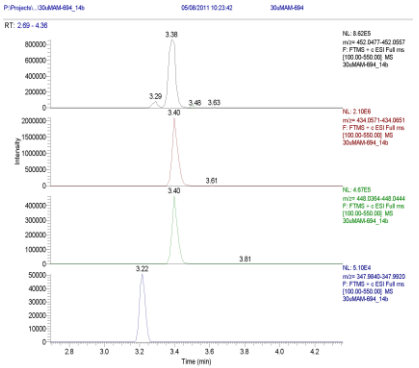
Add Rt and fragment information to accurate mass database

Compound Database - SCRA Master [Full Scan - AIF]

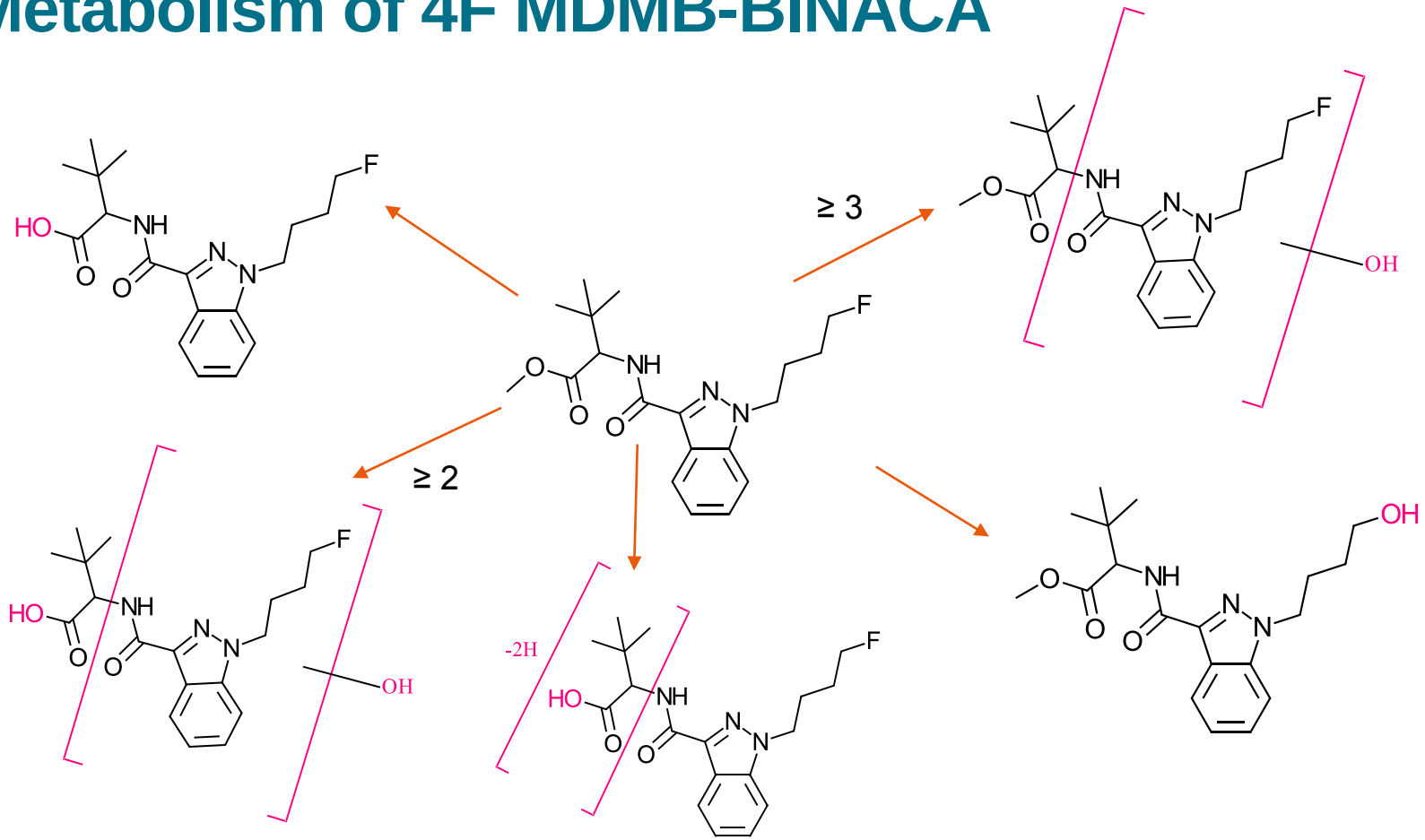
	Compound Name	Molecular Formula	Extracted m/z	Polarity	Adduct	Charge State	RT (min)	RT Window (sec)	Relative RT	Internal Standard	Area Threshold	Height Threshold	Isotope Score	Fragment 1
939	APP-BINACA-desamino-hydroxy	C21H23N3O3	366.18122	Positive	Hydrogen	1	4.64	30.00	1.198966408	d5 hydroxypenty	1400000	100000	35	201.1018
940	4F MDMB-BINACA	C19H26FN3O3	364.2031	Positive	Hydrogen	1	4.10	30.00	1.059431525	d5 hydroxypenty	1400000	100000	35	219.0926
941	4F-MDMB-BINACA-desmethyl	C18H24FN3O3	350.18745	Positive	Hydrogen	1	3.97	30	1.025839793	d5 hydroxypenty	1400000	100000	35	219.0926
942	APP-BINACA-hydroxy 2	C21H24N4O3	381.19212	Positive	Hydrogen	1	3.16	30.00	0.816537468	d5 hydroxypenty	1400000	100000	35	235.1071
943	APP-BINACA-hydroxy 3	C21H24N4O3	381.19212	Positive	Hydrogen	1	3.32	30.00	0.857881137	d5 hydroxypenty	1400000	100000	35	201.1017
944	APP-BINACA-hydroxy 4	C21H24N4O3	381.19212	Positive	Hydrogen	1	3.64	30.00	0.940568475	d5 hydroxypenty	1400000	100000	35	217.0969
945	APP-BINACA-dihydroxy 1	C21H24N4O4	397.18703	Positive	Hydrogen	1	2.87	30.00	0.741602067	d5 hydroxypenty	1400000	100000	35	235.1074
946	APP-BINACA-dihydroxy 2	C21H24N4O4	397.18703	Positive	Hydrogen	1	3.18	30.00	0.821705426	d5 hydroxypenty	1400000	100000	35	217.0969
947	4F-MDMB-BINACA-desfluoro-hydroxy	C19H27N3O4	362.20743	Positive	Hydrogen	1	3.60	30.00	0.930232558	d5 hydroxypenty	1400000	100000	35	235.1077
948	4F-MDMB-BINACA-desmethyl (-2H)	C18H22N3O3F	348.1718	Positive	Hydrogen	1	3.30	30.00	0.852713178	d5 hydroxypenty	1400000	100000	35	237.1019

Getting more information – *In-Vitro* Metabolism

- Knowledge of breakdown in the body is required – metabolism
- Many drugs only leave detectable concentrations of metabolites and not parent drug
- Metabolite information for many NPS are not available
- Able to mimic metabolism in a test tube using the drug and enzymes from liver cells
- Information is added to databases to use in testing process



Metabolism of 4F MDMB-BINACA



Addition of metabolite information

Compound Database - SCRA Master [Full Scan - AIF]

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945	APP-BINACA-dihydroxy 1	C21H24N4O4	397.18703	Positive	Hydrogen	1	2.87	30.00	0.741602067	d5 hydroxypenty	1400000	100000	35	235.1074
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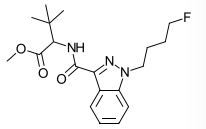
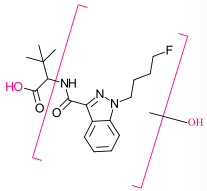
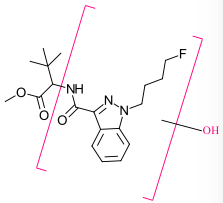
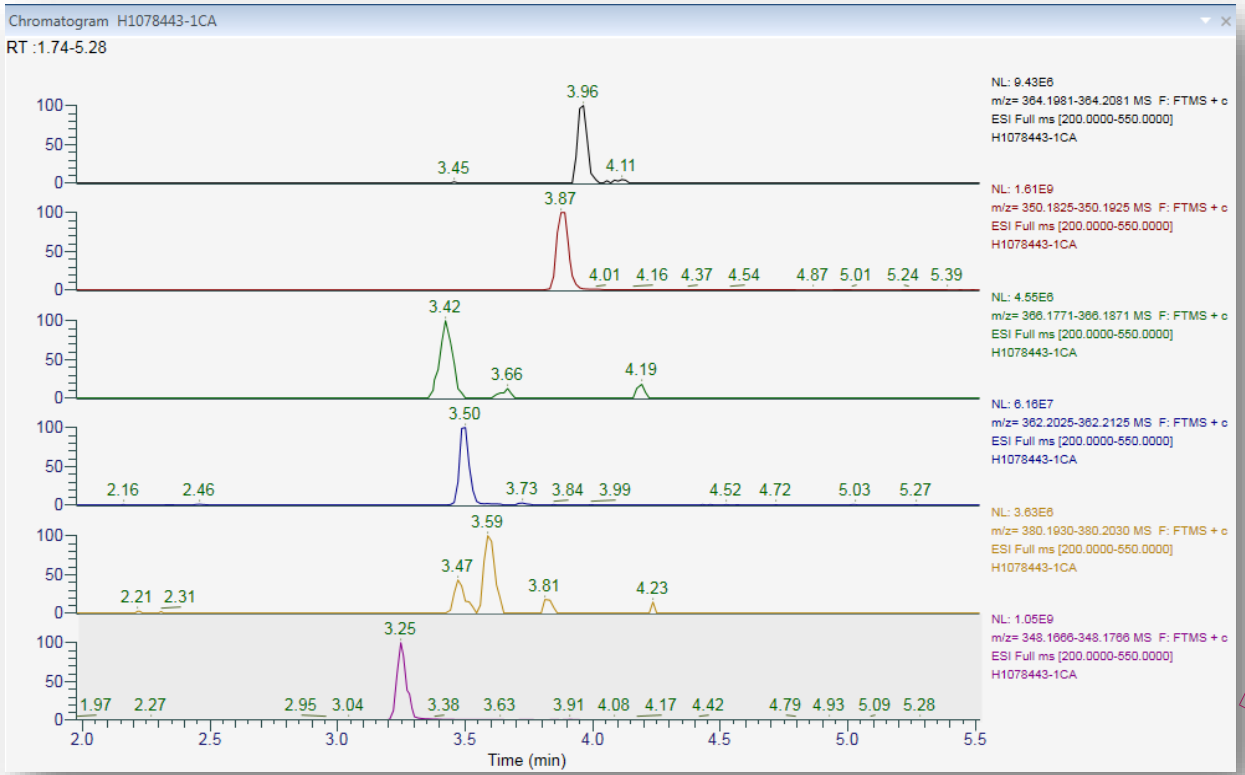
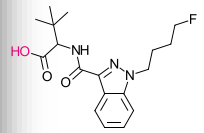
Metabolites identified in post mortem sample – Jan-2019

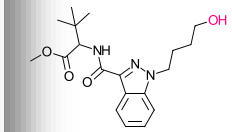


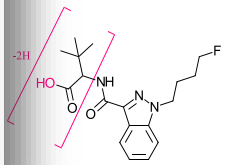




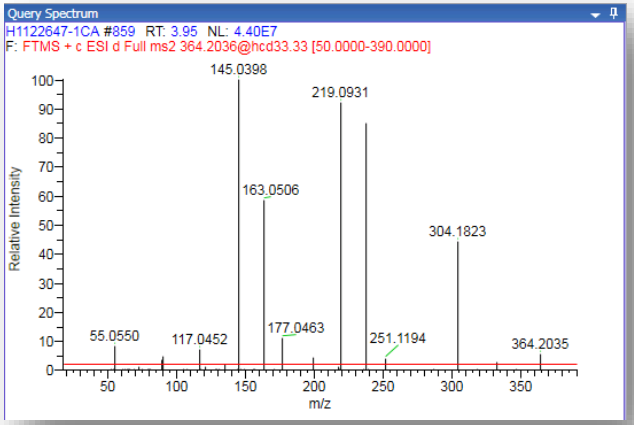


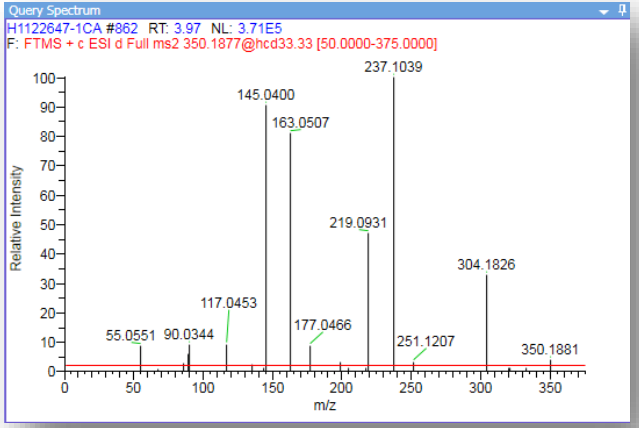


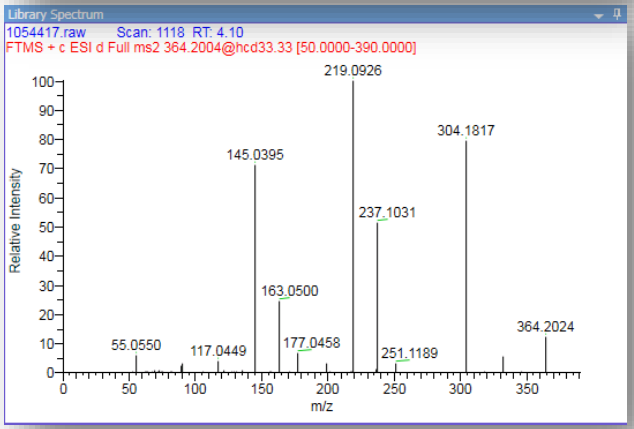


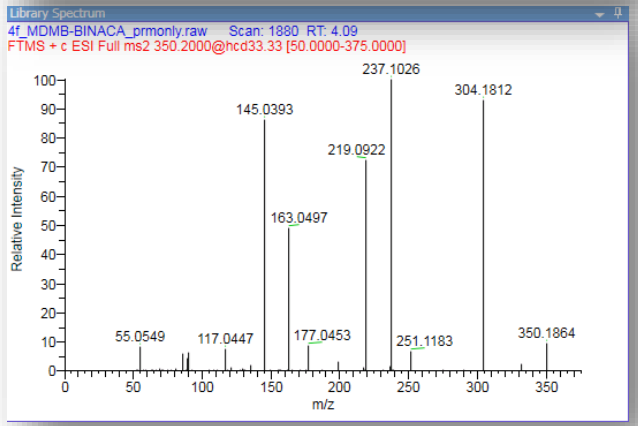


4F MDMB-BINACA parent and desmethyl metabolite matched against in vitro data.



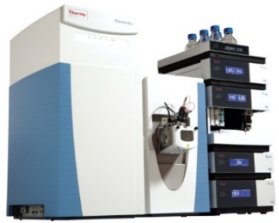






First Study of NPS use in UK prisons

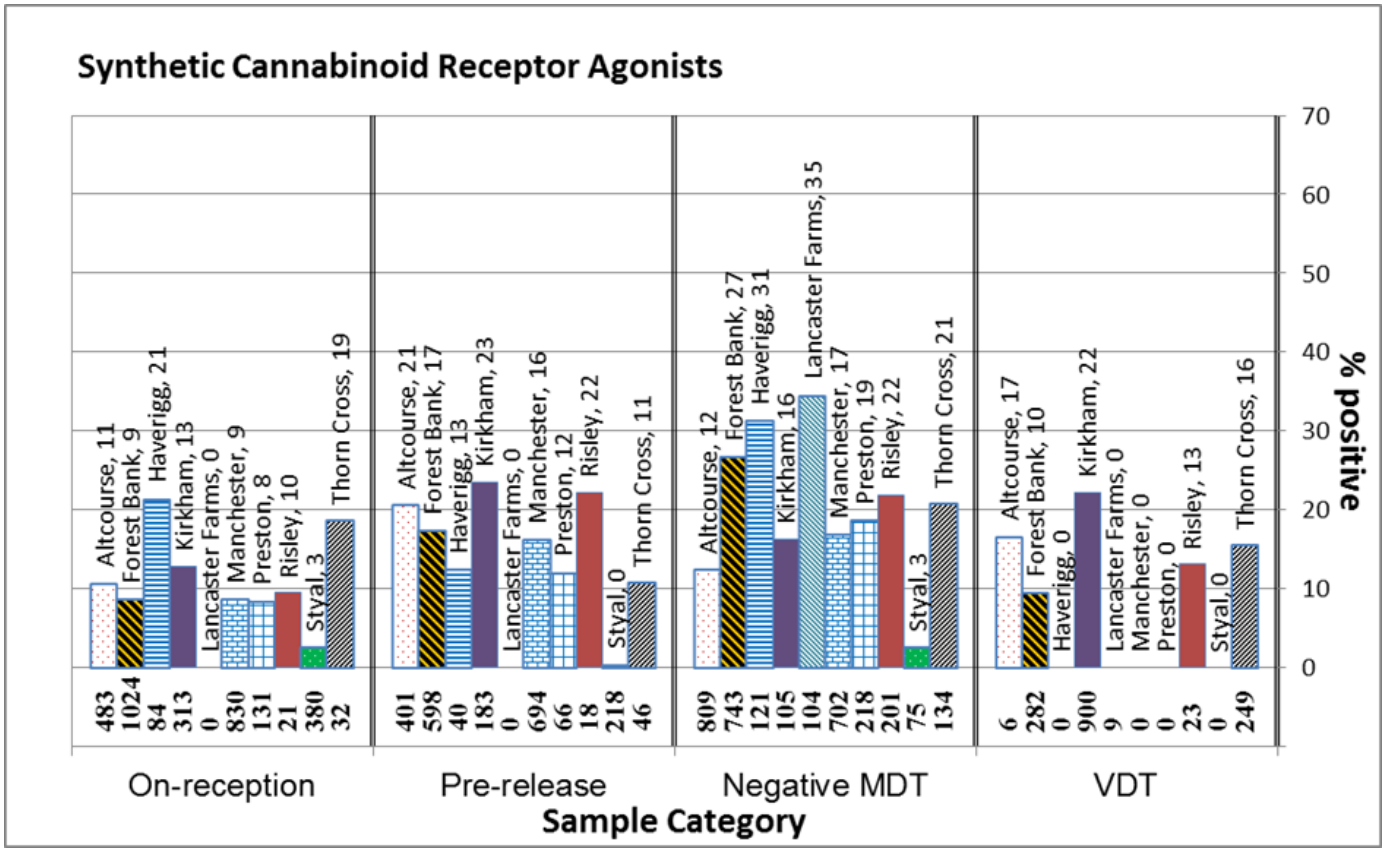
- LGC approached by the National Offender Management Service (NOMS) in August 2014 to look at ‘Spice’ use in prisons
- Agreed a broad screening approach to cover all NPS and ‘traditional’ DoA, prescription medications and ‘over the counter’ products.
- Phase I - 10 prisons in North West (Dec 2014- May 2015) – HRAM-LCMS
- Phase II – 10 prisons in North West and 20 in South East (May 2015-Jul 2015) – HRAM-LCMS and GCMS – (negative MDT samples only)
- Analysis of drug seizures in participating prisons



UK prisons study 2015 (12000 urine samples)

- **SCRAs**
  - 19 different SCRAs were detected
  - Overall % of urines positive was 19.4%
  - 1873 samples contained one of more SCRA
  - Positive rate in some prisons > 30%
  - Very low use in female prisons

UK prisons study 2015 (12000 urine samples)

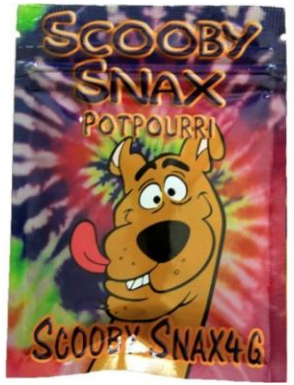


Latest Findings – SCRAs

- **December 2018**
  - Spice material from Eurofins (ex LGC) forensics
  - Analysed and found 4F MDMB-BINACA and APP-BINACA
  - Added to HRAM database along with theoretical metabolites
  - Data supported by NMR
  - Reported to EMCDDA
- **January 2019**
  - In vitro metabolism performed on extract from ‘Spice’
  - Major metabolites identified and confirmed in/added to database and MS2 data added to library
  - Positives seen for 4F MDMB-BINACA and APP-BINACA in blood and urine from coroners cases.
- **Since January 2019**
  - 45 x 4F-MDMB-BINACA findings, 42 x 5F-MDMB-PICA findings

Summary

- Numbers of new NPS detections are reducing. Old ones occasionally re-emerge.
- Still remains a challenge.
- It is possible to keep up with new compounds if labs are pragmatic about the initial data that they use to start screening with.
- Strategies presented here enable us to keep as up to date as possible with NPS evolution



simon.hudson@lgcgroup.com