



WorkPlace PlusSM

Enhanced Occupational Drug Screening using High Resolution Accurate Mass LCMS

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*Setting standards
in analytical science*

Overview

- Accurate Mass – Why/How does it work
- The ‘Legal High’ Issue
- Traditional (Current) Workplace Testing
- Enhanced Workplace Testing – WorkPlace PlusSM
- City Centre work
- Synthetic Cannabinoids

Low Resolution



High Resolution



High Resolution accurate detail



Selectivity of nominal mass

<u>Compounds – all different elemental compositions</u>	<u>Nominal Mass</u>
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 ($C_7H_{14}O_6$)	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

Selectivity of accurate mass

<u>Compound</u>	<u>Accurate Mass</u>
Aminacrine (C ₁₃ H ₁₀ N ₂)	194.08385
Aminohippuric acid (C ₉ H ₁₀ N ₂ O ₃)	194.06859
Anthranol (C ₁₄ H ₁₀ O)	194.07262
Butylparaben (C ₁₁ H ₁₄ O)	194.09375
Caffeine (C ₈ H ₁₀ N ₄ O ₂)	194.07983
Dimethyl phthalate (C ₁₀ H ₁₀ O ₄)	194.05736
Galacturonic acid (C ₆ H ₁₀ O ₇)	194.04210
Hexylresorcinol (C ₁₂ H ₁₈ O ₂)	194.13103
Methylglucoside (C ₇ H ₄ O ₆)	194.07849
Depreton (C ₈ H ₁₀ N ₄ S)	194.06207
Phenocoll (C ₁₀ H ₁₄ N ₂ O ₂)	194.10498
Solanone (C ₁₃ H ₂₂ O)	194.16652
Temozolamide (C ₆ H ₆ N ₆ O ₂)	194.05467

Basic Concept

- Accurate mass measurement gives much higher degree of selectivity to full scan mass spectrometric detection.
- Selectivity can be utilised to re-designed screening protocols and change the way the lab works.
- Two elements:
 - Resolution
 - Accurate mass measurement

Mass Resolution

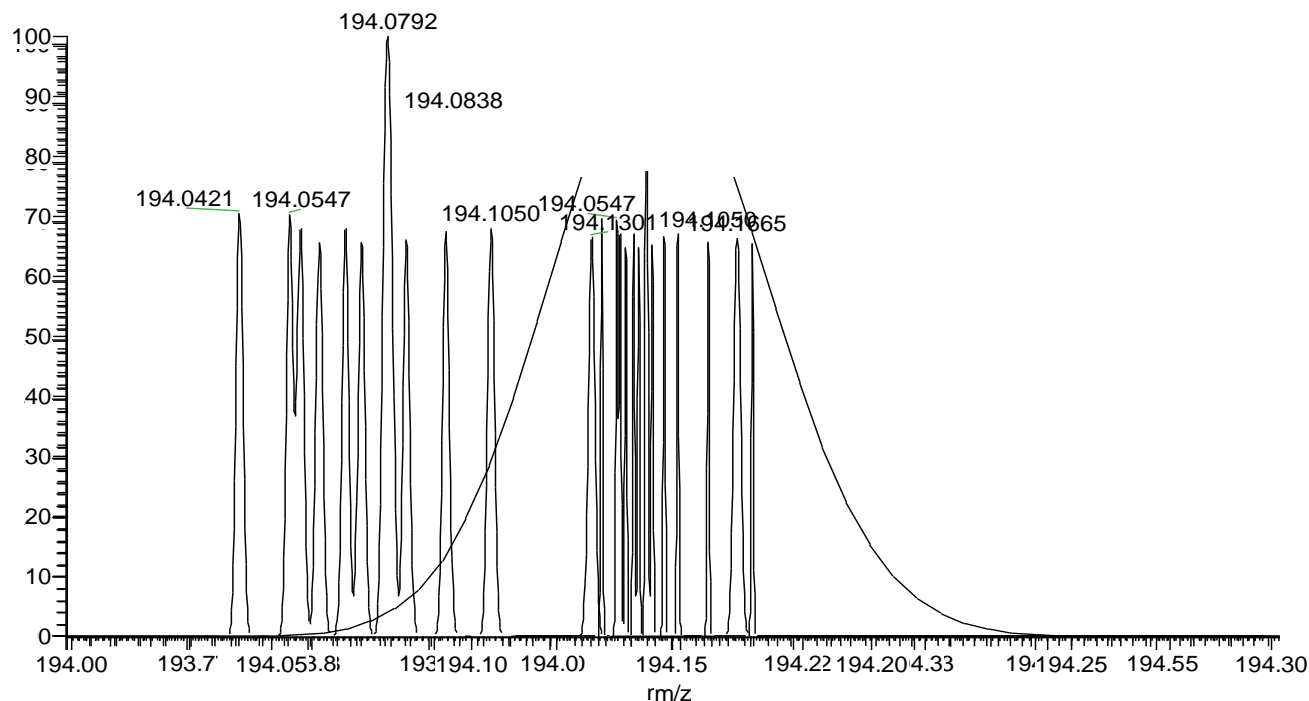
- Measure of the ability of a mass spectrometer to separate ions (MS peak sharpness)
- Defined as:- Mass (Da)/peak width at half height (Da).
- So for peak of mass 200 Da with a width of 0.5 Da at half peak height the resolution would be

$$200/0.5 = 400$$

- Often quoted as FWHM (full width half height maximum)

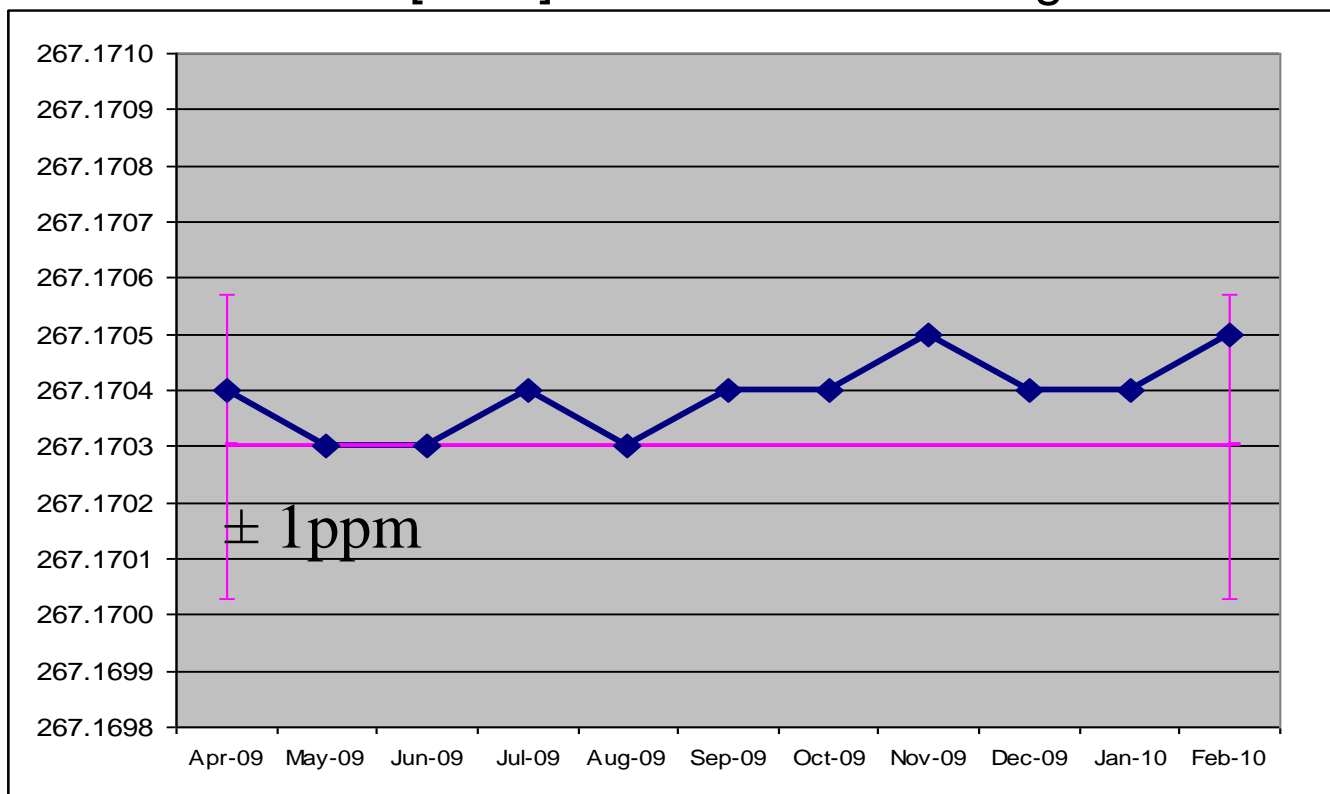
Selectivity through resolution and accurate mass measurement

$C_6H_6N_6O_2 \cdot 1.00 + C_{13}H_{10}N_2 \cdot 1.00 + C_{14}H_{10}O \cdot 1.00 + C_{14}H_{10}O \cdot 1.00$



Mass stability / Mass accuracy

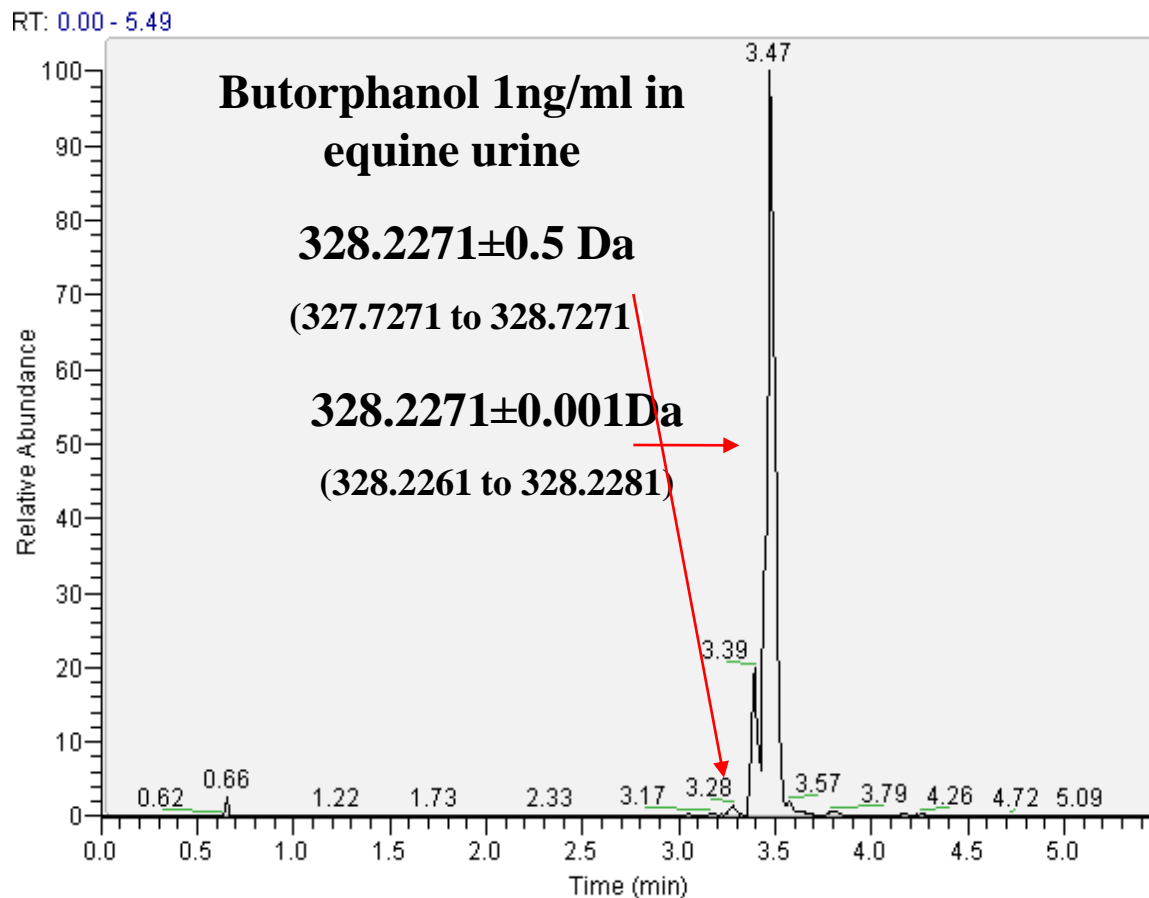
Atenolol $[M+H]^+ = 267.1703 - 200\text{ng/ml}$



Accurate Mass for screening

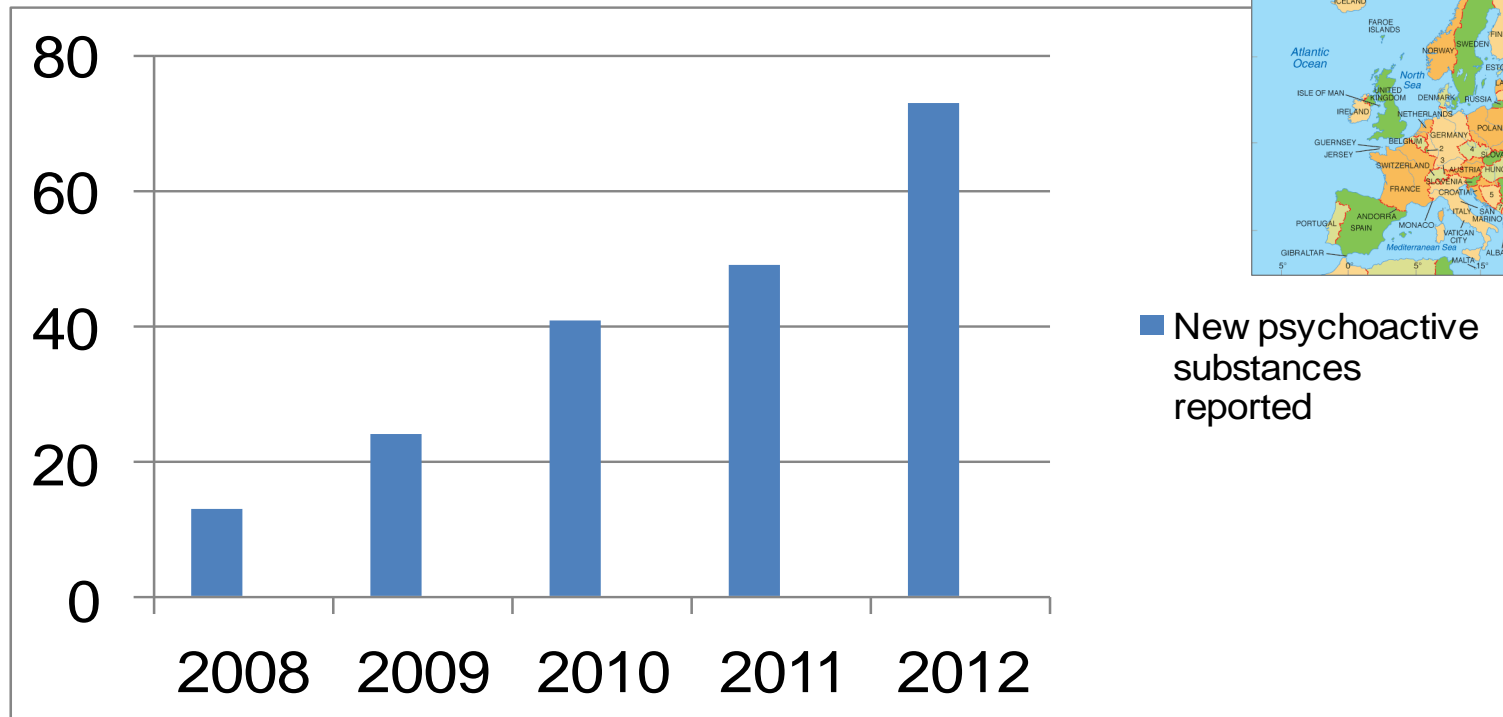
- Full scan analysis
- Combination of ability to separate ions and then measure them accurately gives a high degree of selectivity.
- Use high selectivity in full scan to give very broad drug coverage.
(MRM, even when scheduled, can maybe give coverage for 100-200 analytes.)
- Do not need reference materials to set up screen.
- Full scan accurate mass can realistically cover thousands of compounds in a run if we combine mass with retention time.

Example – selectivity of full scan accurate mass



Workplace Drug Testing - The Issue

- New psychoactive substances reported are increasing (EMCDDA)



New Drug Categories listed by EMCDDA (July 2013)



- **Phenethylamines** – 59 - amphetamine, MDMA, fluroephedrine, 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe)
- **Tryptamines** -28 alphamethyltryptamine
- **Piperazines**- 13 benzylpiperazine, trifluoromethylphenylpiperazine
- **Cathinones** – 45 mephedrone, pentedrone, methcathinone
- **Narcotic analgesics** – 5- O-desmethyl tramadol
- **Synthetic cannabinoids**- 86 - JWH-018, AM-694, AM2201
- **Others** – 65 - ketamine, methoxetamine, 6-APB, methiopropamine

Clinical Analyser Coverage

- **Opiates**, including morphine, codeine, dihydrocodeine, 6-monoacetylmorphine
- **Amphetamines**, including MDMA, methamphetamine and any other cross reactors
- **Benzodiazepines**, including nordiazepam, diazepam, lorazepam, oxazepam, temazepam,
- **Cannabis** as Carboxy THC
- **Cocaine** as benzoylecgonine
- And, on request, specific substances such as ketamine, PCP, LSD, methadone, propoxyphene, barbiturates, buprenorphine.



What about new drugs ?



- **Doesn't** cover: most cathinones, most phenethylamines, tryptamines, piperazines, synthetic cannabinoids and most of the 'other' category.
- So....
- A large number of the currently available and used illicit drugs can't be detected using traditional workplace testing

A New Approach – WorkPlace PlusSM

- Use different technology platform
- High resolution accurate mass (HRAM) LCMS to cover much broader range of analytes.
- Easy to update analyte coverage
- But how to cover constantly evolving drug situation

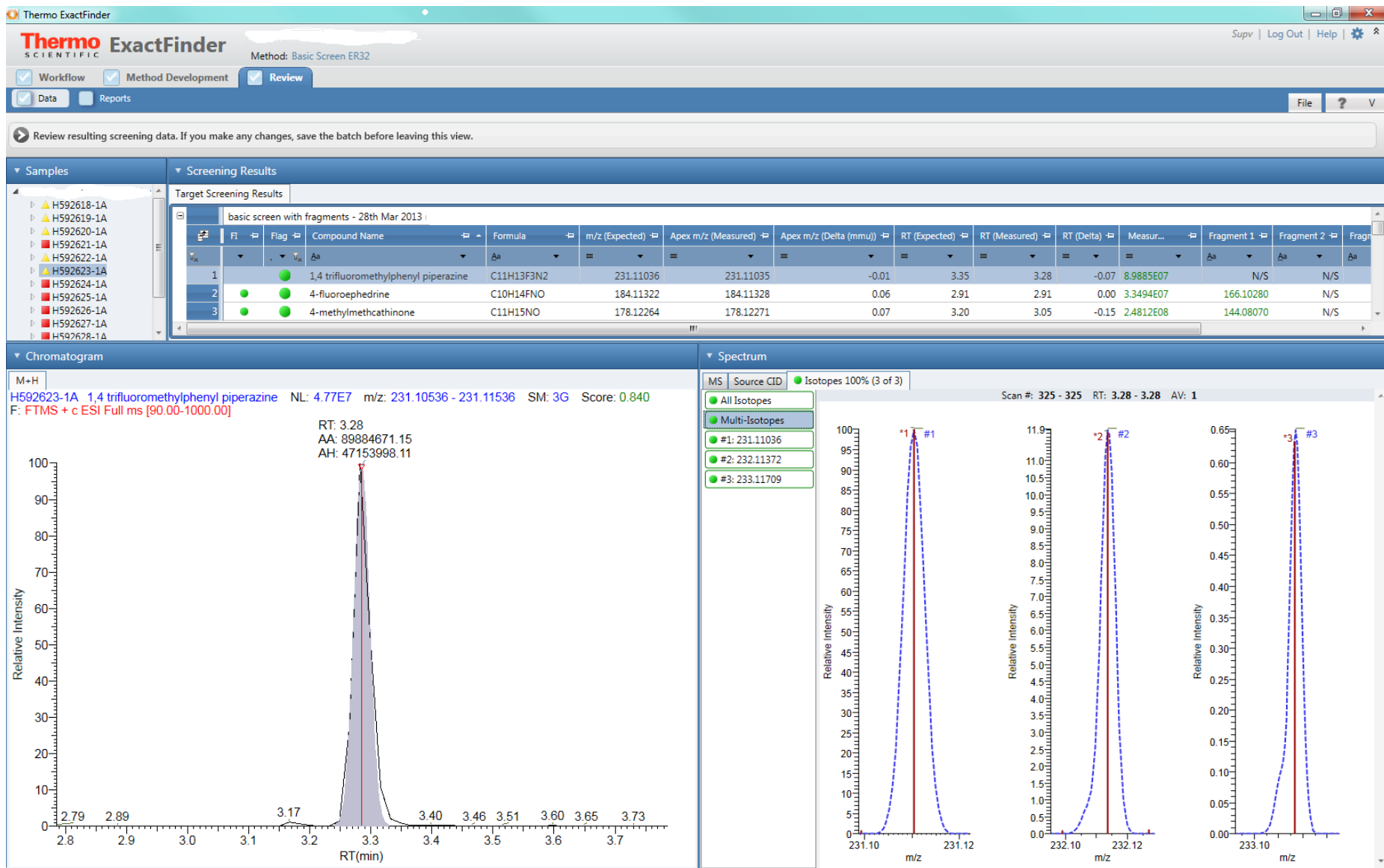


Analysis

- Samples prepared by SPE
- Full scan analysis @ 30000 resolution with in source fragmentation
- Data processing against accurate mass database with fragment ion qualifiers where known



Data Processing



Workplace Testing – Old vs WorkPlace PlusSM

<u>Sample Number</u>	<u>Conventional Workplace Test</u>	<u>WorkPlace PlusSM</u>
Sample 1		4 methylmethcathinone/fluorephedrine
Sample 2		negative
Sample 3	Cocaine	pentedrone/benzoylecgonine
Sample 4	Cocaine	pentedrone/benzoylecgonine
Sample 5		4-methylmethcathinone/fluorephedrine/trifluoromethylphenylpiperazine/benzylpiperazine
Sample 6	Cannabis	4-methylmethcathinone
Sample 7		methylhexanamine
Sample 8		negative
Sample 9		negative
Sample 10		4-methylmethcathinone
Sample 11	Cocaine	Pentedrone/trifluoromethylphenylpiperazine/benzoylecgonine
Sample 12	4-methylmethcathinone	4 methylmethcathinone/4-methyl ethcathinone/fluorephedrine/trifluoromethylphenylpiperazine/benzylpiperazine
Sample 13		pentedrone

Drug monitoring in the general population



- What drugs are being used in the night time economy of city/town centres? Studies to date rely on 'self reporting'
- Samples taken from urinals (pooled). Night club and city centre
- Sample prepared and analysed using WorkPlace PlusSM
- Processed using databases regularly updated with latest findings



Setting standards
in analytical science

Approx 150 compounds

1,4 methoxyphenylpiperazine	chlordiazepoxide	gabapentin	morphine	propranolol
4 methylethcathinone	chloroquine	guaifenesin	Nandrolone	pyrimethamine
4-ethylmethcathinone	chlorpheniramine	HMMA	naproxen	quetiapine
4-fluoroephedrine	Chlorthalidone	hordenine	nefopam	quinine
4-methylmethcathinone	cimetidine	hydrochlorthiazide	nevirapine	ranitidine
5/6-APB	citalopram	Ibuprofen	nicotine	risperidone
Ambroxol	clenbuterol	isometheptene	niflumic acid	salbutamol
Amisulpiride	clobazam	ketamine	nordazepam	sertaline
Amitriptylline	clomipramine	Ketoprofen	nortriptyline	sildenafil
Amphetamine	clozapine	lamotrigine	noscaphine	sotalol
Ampyrone	codeine	lansoprazole	Olanzapine	Stanozolol
Arecoline	cotinine	Lidocaine	omeprazole	sulpiride
atenolol	cyclobenzaprine	MDA	opipramol	tamoxifen
Benzocaine	DEET	MDMA	oripavine	temazepam
Benzoylcegonine	desloratidine	Meclofenamic acid	orphenadrine	tetracycline
Benzydamine	dextromethorphan	mefenamic acid	oxazepam	tetramisole
bisoprolol	dextrorphan	methadone	oxprenolol	theobromine
bromhexine	Diazepam	methamphetamine	oxycodone	theophylline
Buprenorphine	diclofenac	methcathinone	oxytetracycline	tramadol
buprenorphine-nor	dihydrocodeine	methiopropamine	papaverine	Trazodone
Caffeine	dihydromorphone	methoxetamine	paracetamol	trenbolone
Camphor	diphenhydramine	methylhexaneamine	paroxetine	trifluoromethylphenylpiperazine
Capsaicin	dipyridamole	methylphenidate	pentedrone	trimethoprim
Carbamazepine	doxycycline	metoclopramid	pheniramine	vardenafil
Carboxy THC	enalapril	metoprolol	Phenylpropanolamine	venlafaxine
Cathine	ephedrine/pseudoephedrine	metronidazole	Pholcodine	xylometazalone
Cathinone	ethylphenidate	midazolam	pipradrol	Yohimbine
celecoxib	Etoricoxib	minoxidil	pregabalin	zolpidem
cetirizine	fluoxetine	mitragynine	prilocaine	
chlorcylizine	furosemide	modafinil	Propoxyphene	

Drugs with abuse potential not detected by current workplace tests

1,4 methoxyphenylpiperazine	chlordiazepoxide	gabapentin	morphine	propranolol
4 methylethcathinone	chloroquine	guaifenesin	Nandrolone	pyrimethamine
4-ethylmethcathinone	chlorpheniramine	HMMA	naproxen	quetiapine
4-fluoroephedrine	Chlorthalidone	hordenine	nefopam	quinine
4-methylmethcathinone	cimetidine	hydrochlorthiazide	nevirapine	ranitidine
5/6-APB	citalopram	Ibuprofen	nicotine	risperidone
Ambroxol	clenbuterol	isometheptene	niflumic acid	salbutamol
Amisulpiride	clobazam	ketamine	nordazepam	sertaline
Amitriptylline	clomipramine	Ketoprofen	nortriptyline	sildenafil
Amphetamine	clozapine	lamotrigine	noscapine	sotalol
Amprone	codeine	lansoprazole	Olanzapine	Stanozolol
Arecoline	cotinine	Lidocaine	omeprazole	sulpiride
atenolol	cyclobenzaprine	MDA	opipramol	tamoxifen
Benzocaine	DEET	MDMA	oripavine	temazepam
Benzoylcegonine	desloratidine	Meclofenamic acid	orphenadrine	tetracycline
Benzydamine	dextromethorphan	mefenamic acid	oxazepam	tetramisole
bisoprolol	dextrorphan	methadone	oxprenolol	theobromine
bromhexine	Diazepam	methamphetamine	oxycodone	theophylline
Buprenorphine	diclofenac	methcathinone	oxytetracycline	tramadol
buprenorphine-nor	dihydrocodeine	methiopropamine	papaverine	Trazodone
Caffeine	dihydromorphine	methoxetamine	paracetamol	trenbolone
Camphor	diphenhydramine	methylhexaneamine	paroxetine	trifluoromethylphenylpiperazine
Capsaicin	dipyridamole	methylphenidate	pentedrone	trimethoprim
Carbamazepine	doxycycline	metoclopramid	pheniramine	vardenafil
Carboxy THC	enalapril	metoprolol	Phenylpropanolamine	venlafaxine
Cathine	ephedrine/pseudoephedrine	metronidazole	Pholcodine	xylometazoline
Cathinone	ethylphenidate	midazolam	pipradrol	Yohimbine
celecoxib	Etoricoxib	minoxidil	pregabalin	zolpidem
cetirizine	fluoxetine	mitragynine	prilocaine	
chlorcyclizine	furosemide	modafinil	Propoxyphene	

Synthetic Cannabinoids in blood

- Microcosm of the whole NPS situation
- Ever evolving list of analytes
- Most methods based on LCMSMS
- New compounds very hard to detect in biological matrices as LCMSMS only detects what it's looking for!
- Methodology constantly evolving, aided by collaborative work



New Zealand Serum Study



- 90 samples from hospital admissions following reported consumption of herbal smoking mixtures.
- Analysis by HRAM LCMS using 250 known compound database to process data.
- Identified many synthetic cannabinoids including BB-22, AM2233, PB-22, PB22F, JWH-250, AM-694, JWH-073, JWH-018, MAM2201, EAM2201, AKB-48F, JWH-022, JWH-122, JWH-081, SDB-001.
- Novel compounds are slowly being located and identified and the data set reprocessed.

Detection of unknowns/new compounds

- Data acquisition – scan and MS scan with HCD AIF fragmentation
- Look for indicative fragments of synthetic cannabinoids
- Determine monoisotopic mass of compound and then elemental composition
- Propose structures based on elemental composition and MS/MS data

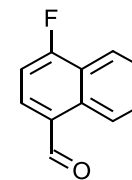
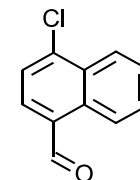
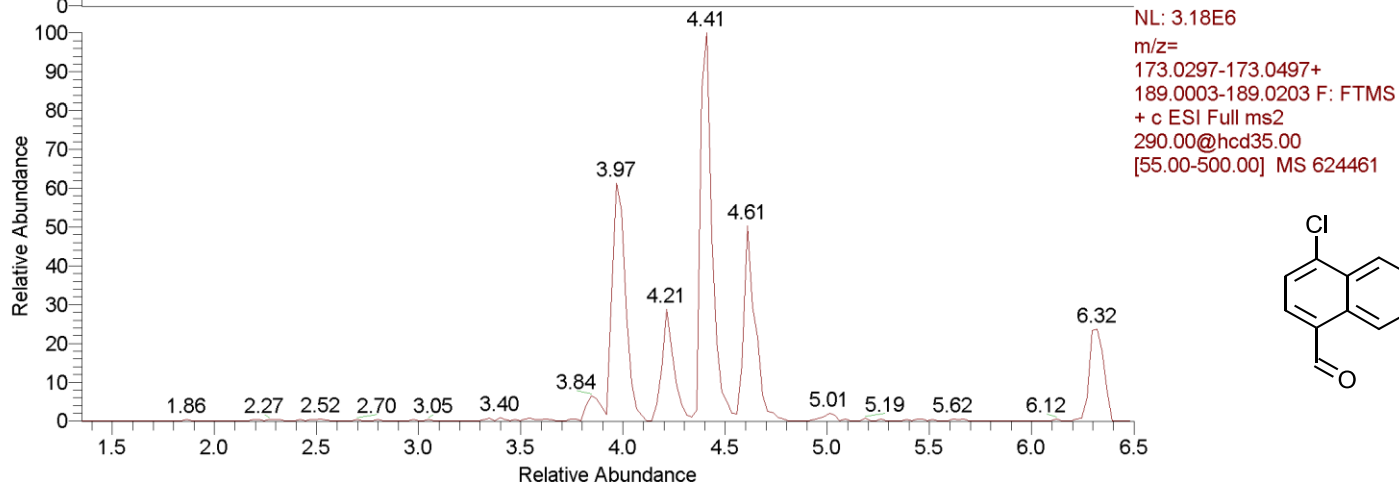
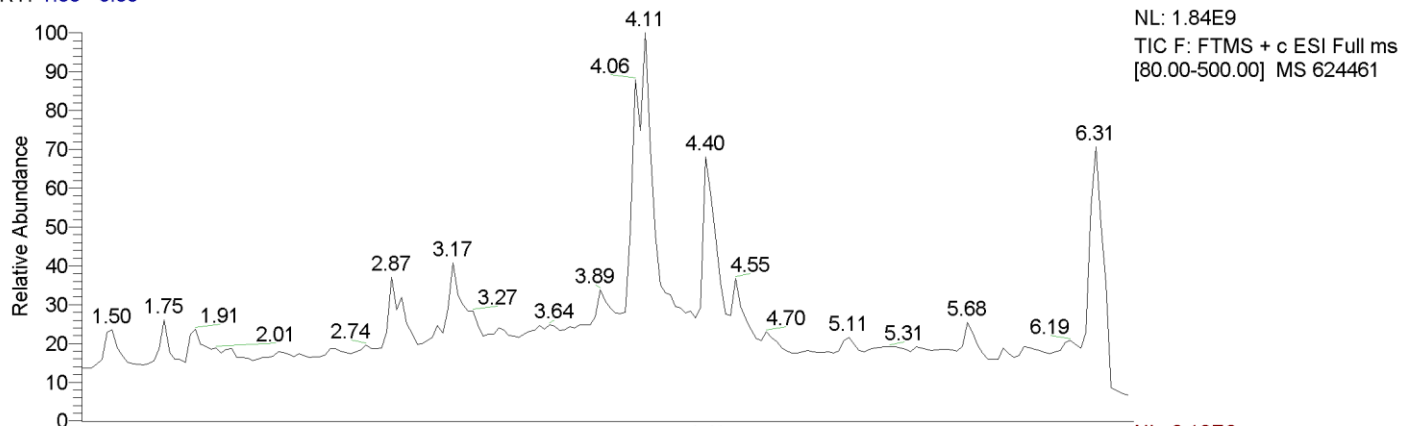


Unknowns

X:\Data\...\Leo Schep\rep\624461
Synthetic cannabinoid - blood extract

15/08/2013 23:24:52

RT: 1.35 - 6.50



WorkPlace Plus SM - Summary

- Much wider coverage than traditional workplace test
- Ability to adapt rapidly with intelligence
- Full screen combines
 - LCMS for over 1700 analytes
 - GCMS for 40 anabolic steroids
- Additional add on for synthetic cannabinoids
 - Recent Findings
 - mephedrone, pentedrone, fluoroephedrine, norfluoroephedrine, TFMPP, Benzylpiperazine, Methylhexaneamine, Methiopropamine, 1,4-methoxyphenylpiperazine, 1-(3-chlorophenyl) piperazine, methylone, MDEA, tramadol



Conclusion

- Ever increasing numbers of designer drugs and 'legal' highs.
- Many drugs that should be detected by workplace testing regimes aren't.
- WorkPlace Plus SM utilising HRAM LCMS offers greater coverage and flexibility for workplace testing than existing protocols.
- WorkPlace Plus SM backed by research collaborations.