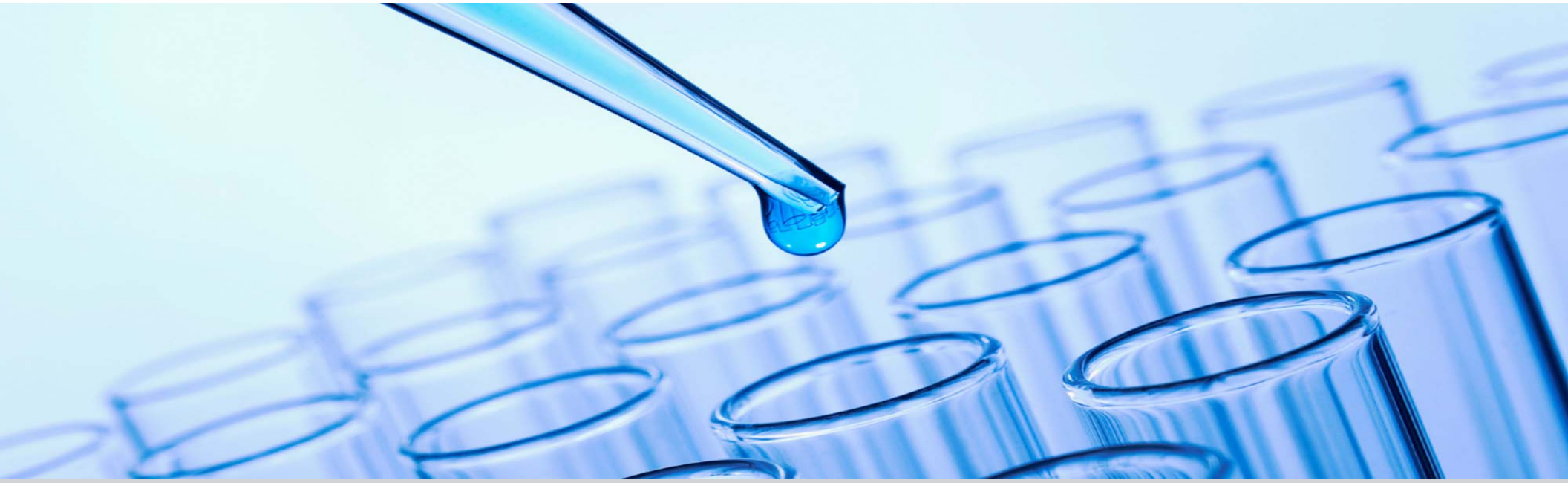


On-line sample concentration coupled with LC-MS/MS Orbitrap HRMS for the EU Water Framework Directive: Target quantitation and non-targeted screening of PFAS and other priority environmental contaminants in water

John Quick[1], Ed George[2], Neville Llewellyn[2], Olaf Scheibner[2], Cristina Jacob[2], and Martin Jech[2]

[1]ALS - Coventry UK; [2]ThermoFisherScientific

Outline

- Introduction to ALS
- Background to UK Chemical Investigation Programme (CIP)
- Past experience with Exactive-Plus for CIP (phase 2)
- Development of a new method for CIP (phase 3) with Q Exactive Focus and Exploris 120
- Non-Target Screening
- Conclusions & future plans

ALS Global locations

50+ Countries

300+ Locations

40+ Years of strong business performance

13,000+ Staff worldwide

20+ million Processed samples per year

$1.2+ billion Global revenues

ALS Life Sciences in UK

- **Food and Pharma (1000 staff)**
  - Chatteris – HUB laboratory
  - Carlisle
  - Mirfield
  - Ely
  - Shrewbury
  - Rotherham
  - Trowbridge
  - Sittingbourne
  - Newton Abbot
  - Clonmel (Ireland)
- **Environmental – Water (500 staff)**
  - Coventry – HUB laboratory
  - Wakefield
  - Otterbourne
  - Glasgow
  - Dublin (Ireland sales office)
- **Environmental – Land (250 staff)**
  - Chester/Hawarden – HUB laboratory
  - Aberdeen

ALS – Background

- ALS Environmental
  - Busy, high throughput commercial environment
  - Efficient, robust methods required
- 2015 – Set of low level methods developed to meet requirements of phase 2 of the UK Chemical Investigation Program (CIP)
  - Ultra-trace level work – low pg/L levels required for some compounds
  - Investment in modern instrumentation including Exactive-Plus
- 2016/2018 – CIP methods extended to fully cover WFD suite and matrices
  - Accredited to 17025
- 2019 – Successful completion of CIP2 project
- 2020 – Start of phase 3 of CIP
  - New compounds added
  - Larger numbers of more “challenging” matrix types
  - Purchase of Thermo QE Focus

UK Chemical Investigation Program

- The UKWIR Chemicals Investigation Programme (CIP) is the UK water Industry's response to current and emerging legislation on trace substances in the water environment. It brings together the water and wastewater companies in England and Wales with the various regulators in a collaborative programme.
- Phase 1 – 2010 to 2014
  - £25 million, 46 chemicals, effluent screening, process sampling, sewerage catchment sampling.
- Phase 2 – 2015 to 2020
  - £140 million, 74 chemicals, 600 STW sites, over 3 million determinations
  - Pharmaceuticals, pesticides, personal care products, EDCs, flame retardants & industrial chemicals.
  - Focus on effluent impact assessment.
- Phase 3 – 2020 to 2022
  - Builds on CIP2, more new and emerging compounds, trend monitoring, microplastics and AMR also included.
  - Split into 14 work packages (CHEM1 to CHEM14)
  - Start delayed until September 2020 due to COVID.

CIP 2 (2015) METHOD – EXACTIVE PLUS/EQUAN

- Analytes
  - PFOS, PFOA, DEHP, Triclosan
- Matrices
  - Surface waters, sewage effluents
- Method- RSH EQuan 850 Autosampler for On-line Sample Preparation
  - Load 5mls of sample onto Hypersil Gold AQ column (20 x 2.1mm)
  - Trapping column to reduce PFOA background
  - Backflush onto Phenylhexyl analytical column (100 x 2.1mm)
  - Mobile phase – Methanol/Water with formic acid and ammonium formate
  - Gradient elution, 12 minute runtime
  - Exactive Plus – Simultaneous pos/neg acquisition @ 70,000 resolution
- Performance
  - All targets for uncertainty and LODs were met.
  - Sub-ppt LODs for PFOS/PFOA obtained
  - Method ran robustly throughout the duration of the project

What is TriPlus RSH EQuan 850 Autosampler?

Automated high-throughput LC-MS solution for the analysis of contaminants in environmental water, drinking water and beverages at low ng/L levels

- **Online sample pre-concentration**
  - 2 LC pumps: Loading and Eluting
  - 2 injection valves and 1 switching valve
  - 2 LC columns
- **High injection volumes**
  - 1 – 20 mL
- **Standard injection volumes**
  - 1–100 µL

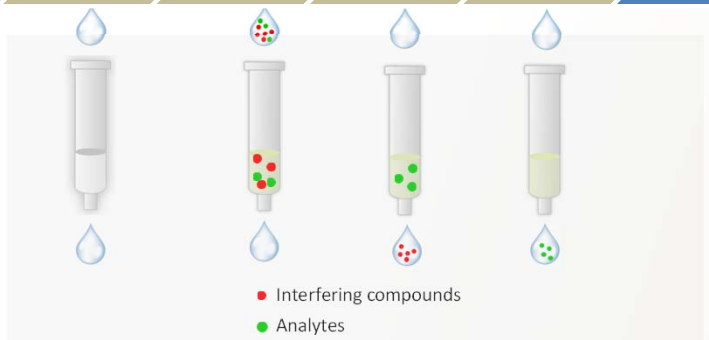
Find out more at **thermofisher.com/EQuan850**

Offline *vs.* Online Sample preparation

Conventional Method: Offline Sample Preparation

~ 2 days

Filtration → Condition → Load sample → Wash → Elution → Evaporation to dryness → Reconstitution → LC-MS/MS



TriPlus RSH EQuan 850: Online Sample Preparation

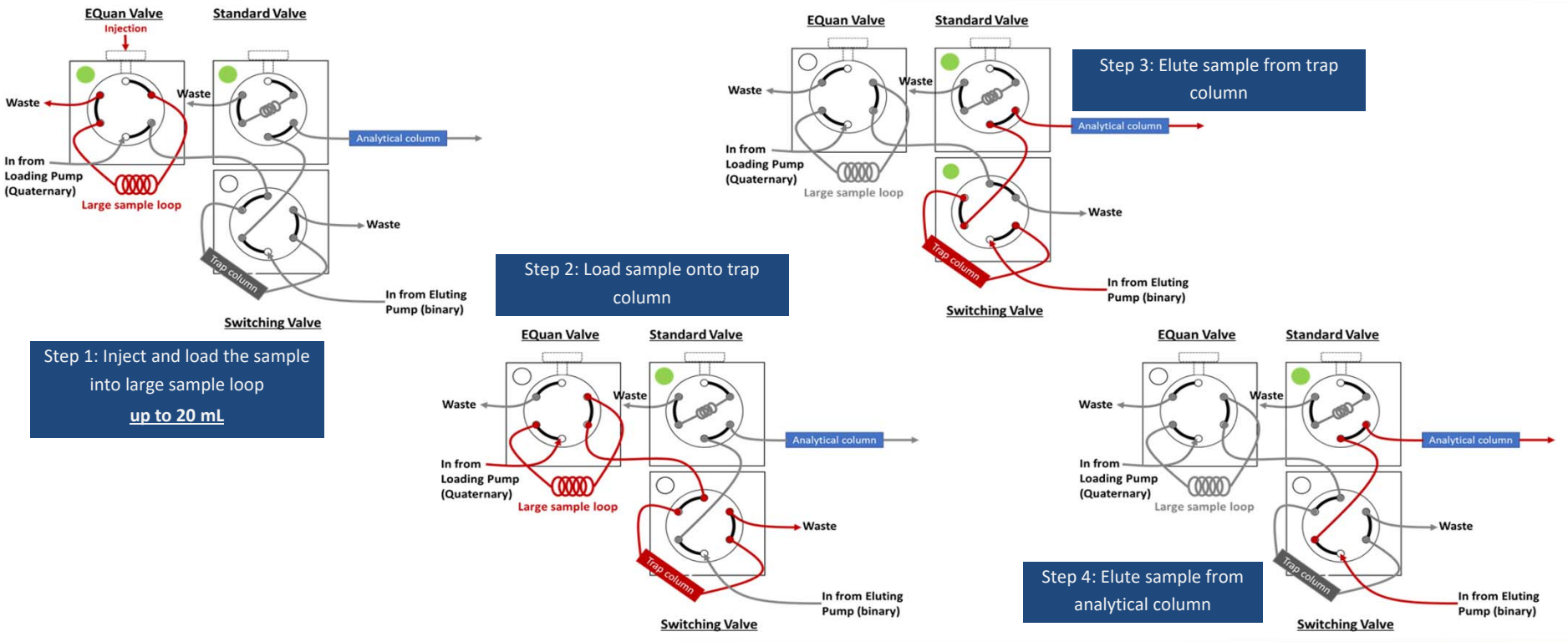
~ 30 minutes

Preconcentration → LC-MS/MS

Analysis time is reduced from hours to minutes

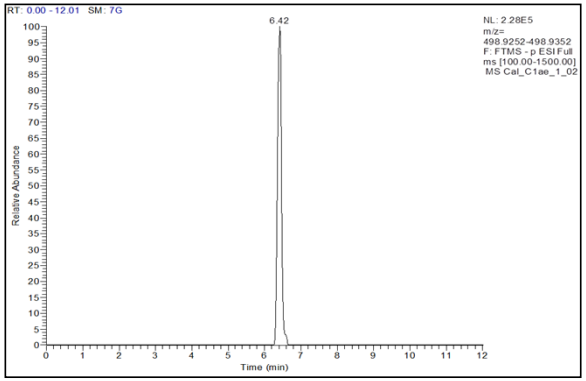
Online Sample Pre-Concentration: How it Works?

EQuan Injection Mode: Red Flow Path

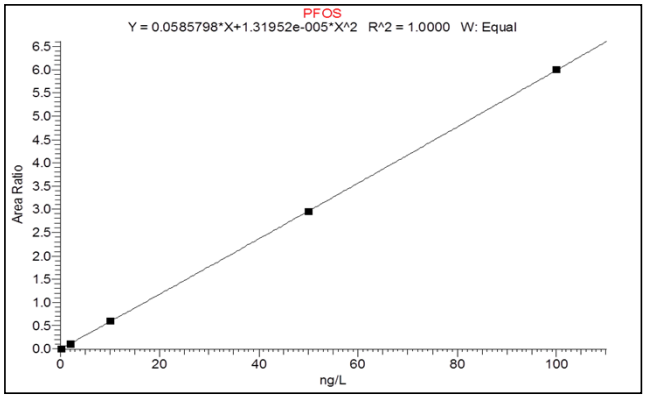


CIP2 – PFOS on EXACTIVE-PLUS

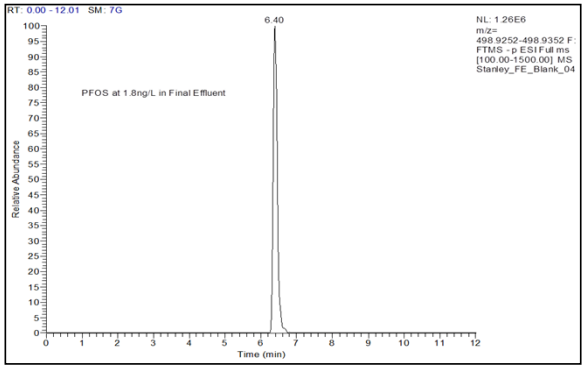
Sensitivity at 0.2ng/L



Calibration curve – 0.2 – 100.0ng/L



PFOS in Sewage Effluent – 1.8ng/L



Performance Statistics

ANALYTE	PFOS	PFOA
UNITS	ng/L	ng/L
Method LOD	0.0676	0.0539
Method MRL	0.09	0.09
EFFLUENT		
Spike Recovery	96.9%	94.8%
Spike RSD	4.15%	2.38%
Uncertainty	11.41%	10.00%
RIVERINE		
Spike Recovery	94.4%	95.9%
Spike RSD	5.53%	5.17%
Uncertainty	16.65%	14.47%

CIP – Phase 3

- CHEM 14 work package – new and emerging contaminants
- Matrices – surface waters, sewage effluents & sewage influents
- LC–MS amenable compounds shown below

		Required LOD (ng/L)	
Name	**CAS**	**STW Effluent**	**STW Influent**
Ranitidine	66357-35-5	100	1000
Benzotriazole	95-14-7	100	1000
Imidacloprid	138291-41-3	1	10
Bisphenol S	80-09-1	10	100
Climbazole	38083-17-9	10	100
Perfluorooctanoic acid	335-67-1	0.1	1
Perfluorooctanesulfonic acid	1763-23-1	0.1	1
Perfluoropentanoic acid	2706-90-3	1	10
Perfluorobutanesulfonic acid	375-73-5	1	10
Perfluorohexanoic acid	307-24-4	1	10
GEN-X	62037-80-3	1	10
Perfluoroheptanoic acid	375-85-9	1	10
Perfluorohexanesulfonic acid	355-46-4	1	10
6:2 Fluorotelomer Sulfonamide Alkylbetaine (6:2 FTAB)	34455-29-3	1	10
6:2 Fluorotelomer Sulfonate (6:2 FTS)	27619-97-2	1	10
Fipronil	120068-37-3	10	100
Triclocarban	101-20-2	100	1000

Q EXACTIVE FOCUS

- Justification
  - Development of extended method for CIP phase 3 required
  - Options to improve sensitivity and selectivity over Exactive Plus – targeted SIM, PRM
  - Demonstrated reliability of Exactive Plus during CIP2
  - Possibility for developing a wide ranging environmental screening method.
- Purchase
  - QE Focus purchased January 2020
  - Method for the analysis of the LC-MS amenable compounds for the CHEM14 work package of CIP 3 developed in conjunction with Thermo.

Q EXACTIVE FOCUS

QE Focus Method Targets- Goals

- Meet LOD and uncertainty of measurement targets as defined in the CIP technical specification.
- Single method if at all possible
- Robust operation across different matrix types
- On-line sample preparation (EQUAN)

QE Focus Method Summary

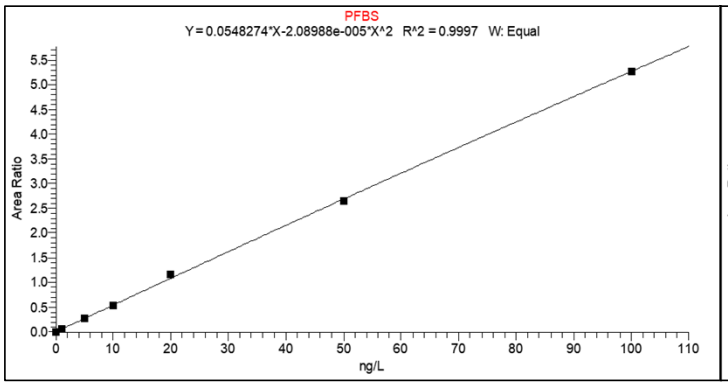
- Analytes
  - 17 compounds as listed – includes PFAS compounds, pesticides industrial chemicals and pharmaceuticals.
  - Isotope labelled surrogates available for all except 1 (6:2FTAB)
- Matrices
  - Surface waters, sewage effluents and influents
- Sample preparation
  - On-line trace enrichment using EQUAN
  - Centrifugation to remove particulates
  - Sample volume – 5ml
  - Loading column – Thermo Scientific Hypersil Gold aQ pre-concentration column (20x2.1, 12µm)

QE Focus Method Summary – continued

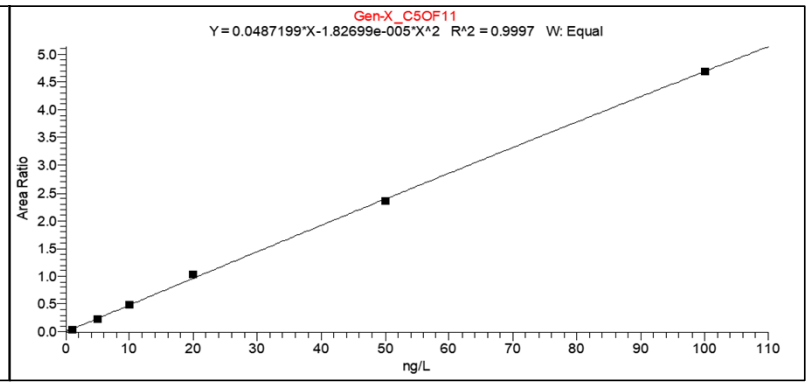
- HPLC
  - Column – Thermo Scientific Acclaim RSLC PolarAdvantage 2.1x150mm. Particle size 2.2um.
  - Mobile Phase – Water, 5mM Amm.Form, 1% Formic Acid / Methanol, 5mM Amm.Form, 1% Formic Acid
  - Run – Gradient, 20 minutes
- MS
  - Full Scan MS
    - Pos/neg switching
    - Scan range – 110 – 600 m/z
    - Resolution – 70,000 (QE Focus), 120,000 (Exploris 120)
    - AGC target – 1e6
  - t-SIM
    - Isolation window – 1.0 m/z
    - Resolution – 70,000 (QE Focus), 120,000 (Exploris 120)
    - AGC target – 2e5
- Quantification
  - Full MS used for all compounds apart from 6:2FTAB (t-SIM)

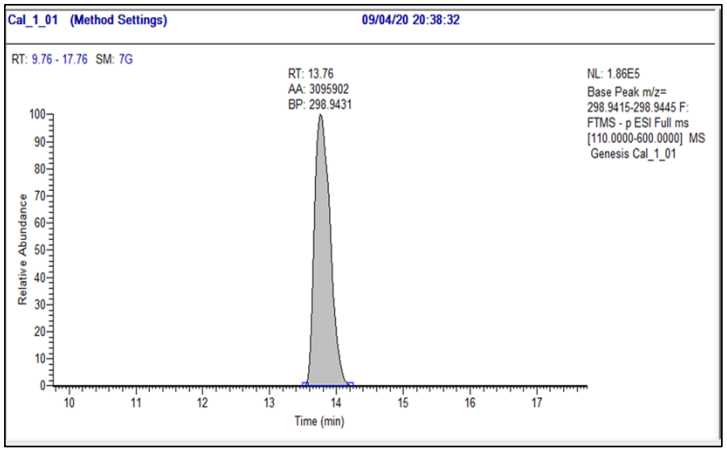
Sensitivity and Linearity

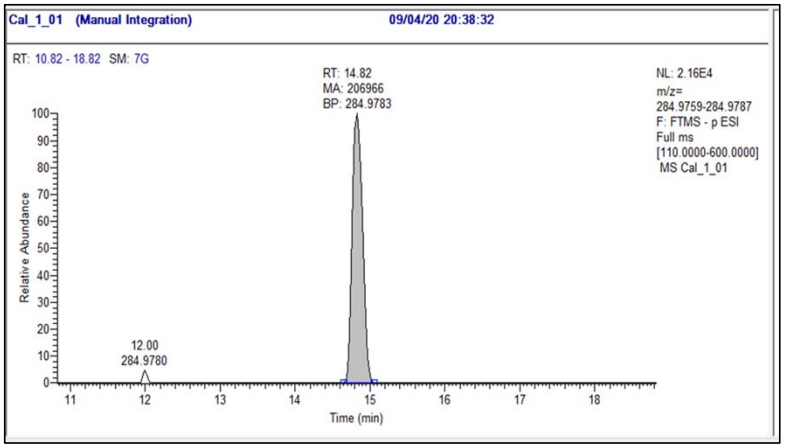
Pentafluorobutane Sulfonate 1 -> 100ng/L



GenX 1 -> 100ng/L

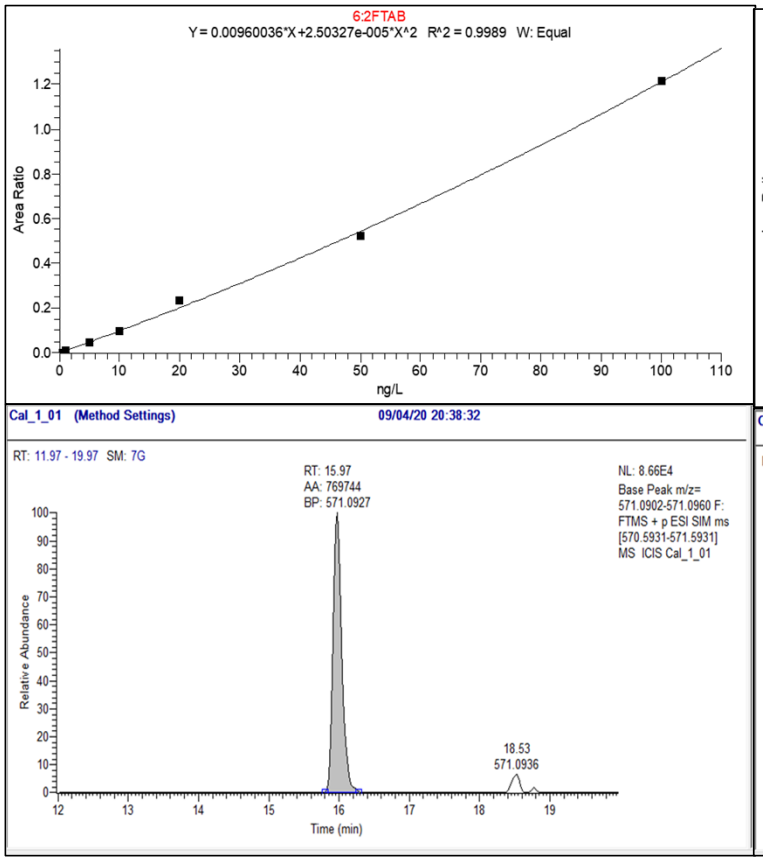




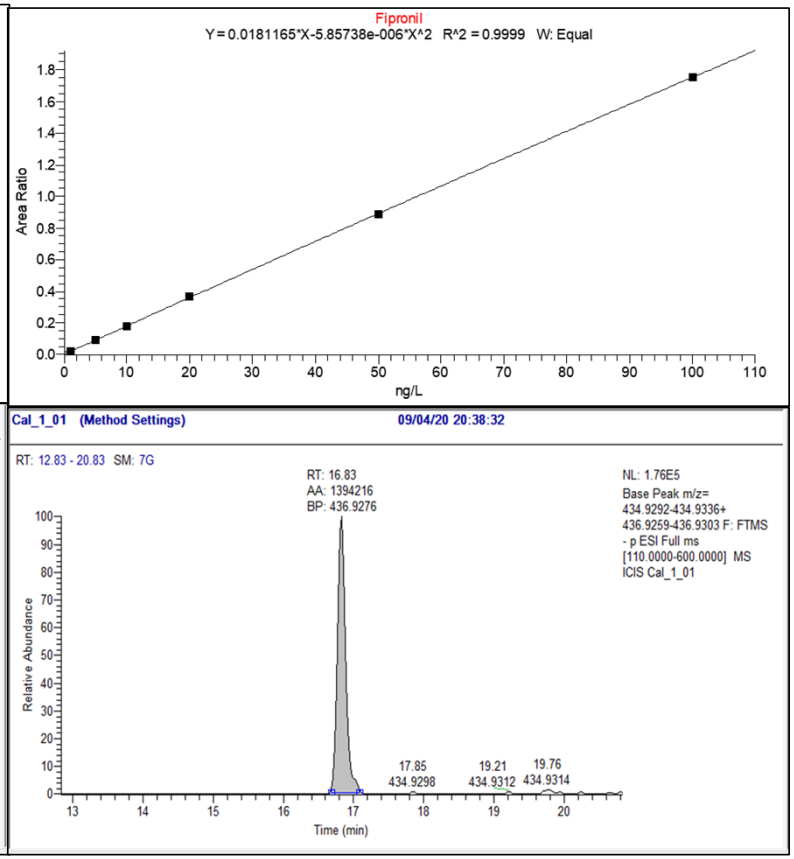


Sensitivity and Linearity

6:2 FTAB (SIM) 1 -> 100ng/L



Fipronil 1 -> 100ng/L



Method Validation Results

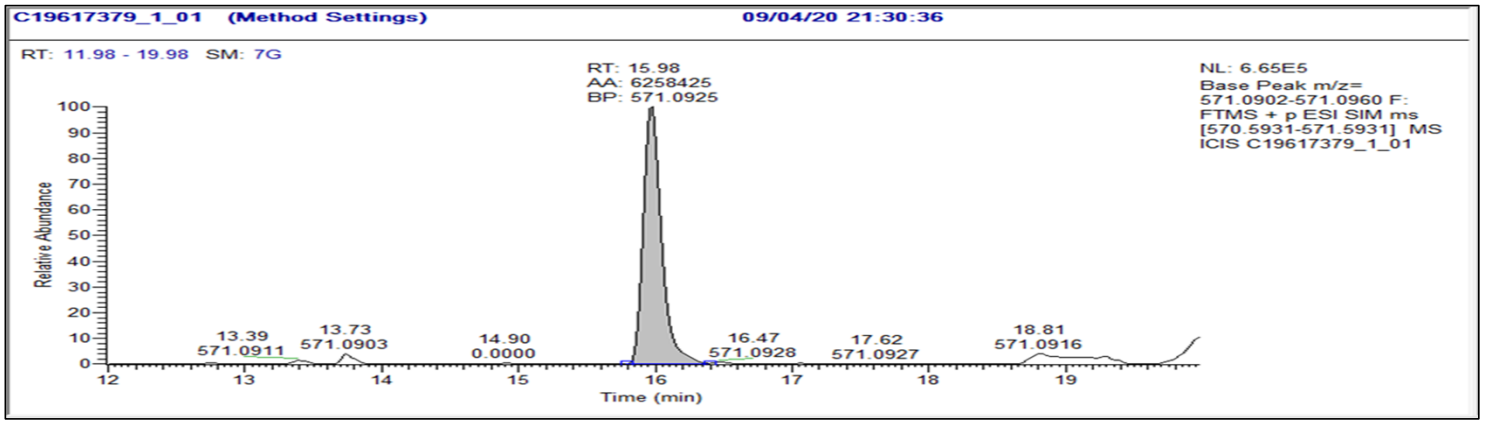
Compound	LOD, ug/L				MRL	Low Std		High Std	
	Treated Sewage	Surface Water	Ground Water	Crude Sewage	ug/L	%RSD	%Bias	%RSD	%Bias
Ranitidine	0.00890	0.00230	0.000804	0.0225	0.1	3.10	-2.89	2.87	-0.63
Benzotriazole	0.944	0.0140	0.00399	0.361	0.1	5.43	-1.24	2.26	-0.59
Imidacloprid	0.00205	0.000462	0.000293	0.00644	0.001	4.57	-2.81	1.53	-0.43
Bisphenol S	0.00426	0.00243	0.00284	0.0233	0.01	4.52	-3.87	2.30	-0.57
PFPeA	0.00191	0.000623	0.000332	0.00467	0.001	4.11	-2.35	2.13	-0.22
Climbazole	0.00297	0.00170	0.00149	0.0110	0.1	3.91	-1.39	1.98	-0.29
PFBS	0.000426	0.0000973	0.000179	0.00176	0.001	2.37	-2.52	2.21	-0.48
PFHxA	0.00126	0.000292	0.000192	0.00159	0.001	4.24	-2.99	1.32	-0.70
GenX	0.00129	0.000334	0.000147	0.00816	0.001	4.07	-2.81	1.13	-1.87
PFHpA	0.000591	0.000230	0.000158	0.00137	0.001	2.37	-2.81	2.21	-0.35
PFHxS	0.000424	0.0000788	0.000107	0.00112	0.001	2.37	-3.63	1.94	-0.94
6:2FTAB	0.00535	0.000507	0.000995	0.0221	0.001	8.26	0.76	8.76	-4.30
6:2FTS	0.00249	0.000326	0.000176	0.00176	0.001	2.21	-1.73	2.21	1.39
PFOA	0.00108	0.000232	0.0000668	0.000594	0.00009	2.37	-4.04	2.01	-0.38
PFOS	0.00830	0.000151	0.0000233	0.0200	0.00009	10.01	-3.95	4.45	-0.77
Fipronil	0.000939	0.000216	0.000234	0.00227	0.01	6.47	-3.14	2.03	0.28
Triclocarban	0.00132	0.00110	0.00115	0.00736	0.1	4.83	-1.99	1.63	0.30

Method Validation Results – continued

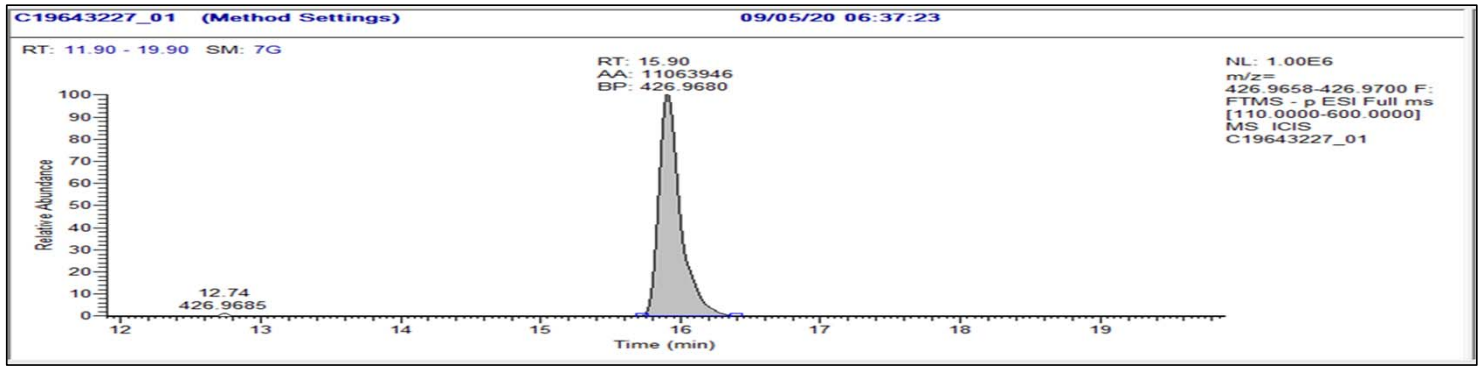
Compound	Treated Sewage 10%		Treated Sewage 60%		Surface Water 60%		Crude Sewage 60%	
	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec
Ranitidine	3.99	94.7	4.91	101	3.98	98.5	6.93	99.3
Benzotriazole	10.61*	106.0*	9.61*	95.7*	4.26	103.1	5.49	104.4
Imidacloprid	3.14	96.9	1.61	101	1.64	99.6	6.95	98.8
Bisphenol S	3.09	97.1	3.03	99.8	2.61	99.3	6.18	97.7
PFPeA	6.46	100	3.43	102	2.18	102	6.62	99.7
Climbazole	2.84	96.5	3.73	101	2.35	101	7.37	98.7
PFBS	2.93	96.8	2.24	99.2	1.34	99.1	6.60	98.4
PFHxA	2.33	97.1	3.33	101	2.28	100	6.38	97.7
GenX	4.78	95.9	3.04	99.9	1.58	101	6.92	98.5
PFHpA	2.29	95.7	2.42	99.2	1.49	99.1	7.52	97.8
PFHxS	3.46	94.8	2.68	96.8	1.08	97.3	6.73	96.1
6:2FTAB	18.19	93.9	9.73	98.2	9.70	128	12.50	109
6:2FTS	2.60	95.3	2.32	99.1	1.69	101	6.95	94.9
PFOA	4.54	84.0	5.70	105	2.53	102	2.48	102
PFOS	*	*	5.38	118	2.92	101	11.27	118
Fipronil	2.55	95.5	2.79	100	1.77	101	5.83	99.2
Triclocarban	5.28	96.2	3.93	98.5	2.49	98.9	7.25	98.2

Real samples

6:2 FTAB in sewage effluent – 11ng/L

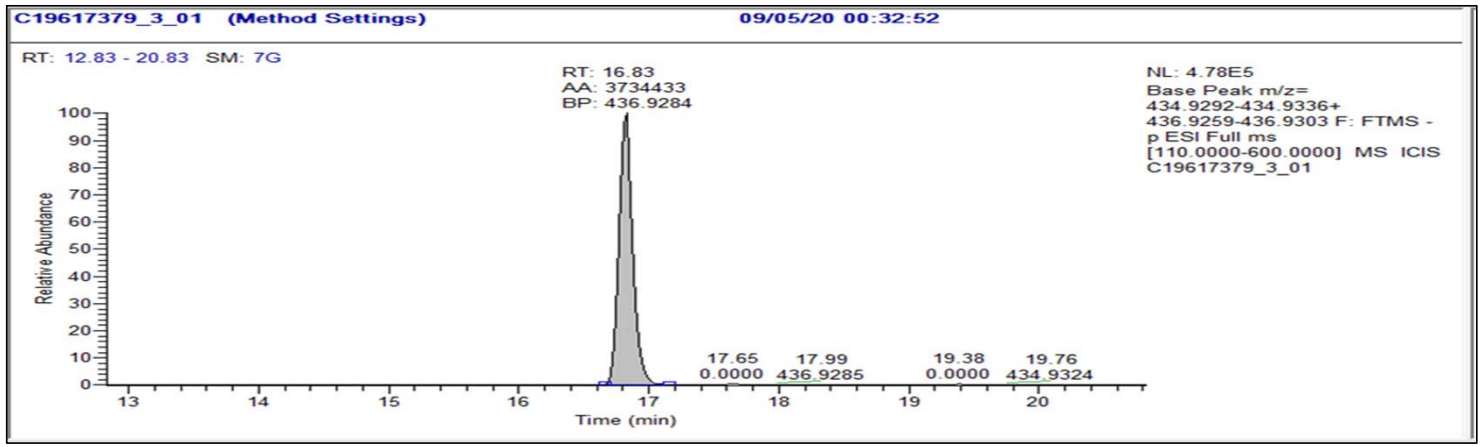


6:2 FTS in sewage effluent – 10ng/L

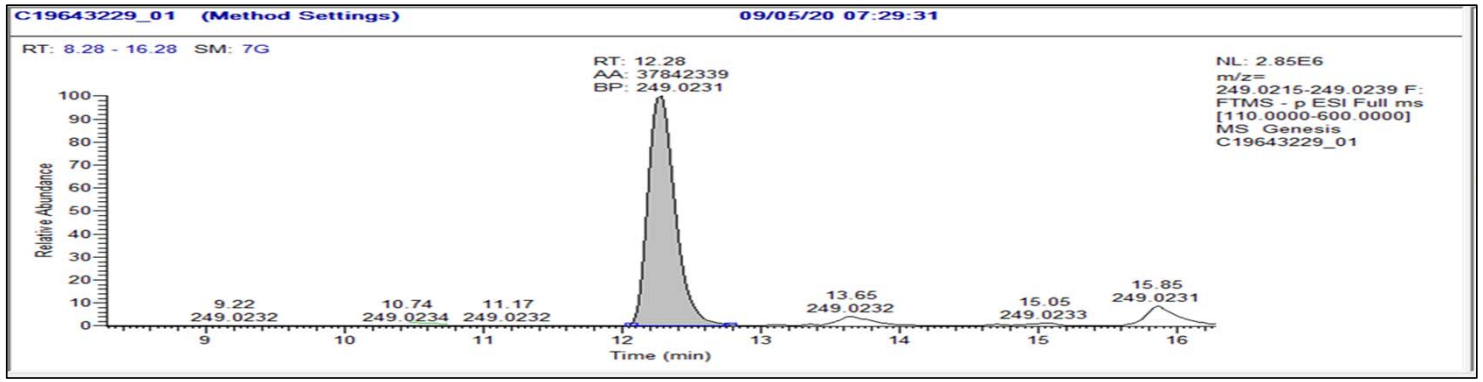


Real samples

Fipronil in sewage effluent – 57ng/L



Bisphenol S in sewage effluent – 750ng/L

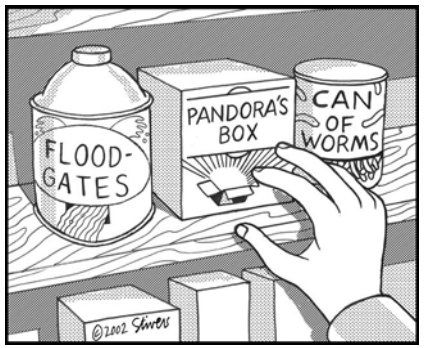


Non Target Screening

Food for thought……

- Typically an Orbitrap acquisition is Full Scan with a range of 100–1000
- We can routinely acquire to 1 ppm mass accuracy
- If we have a targeted list of 500 compounds obtained from Extracted Ion chromatograms – the this leaves 99.95% of data unused…….and this is just at one snapshot of the retention time axis.

So what can we learn from the rest of the data and how do we use it?



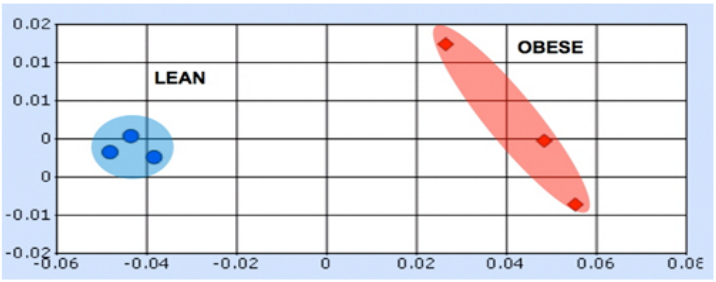
Non Target Screening

What else can we get out of our Orbitrap data?

- Identification of Unknowns and emerging compounds?

- Food and Drink Authenticity

- Extractables and Leachables?



- Identification of Pesticide Metabolites?

- Identify Markers for Environmental states or catchment change?

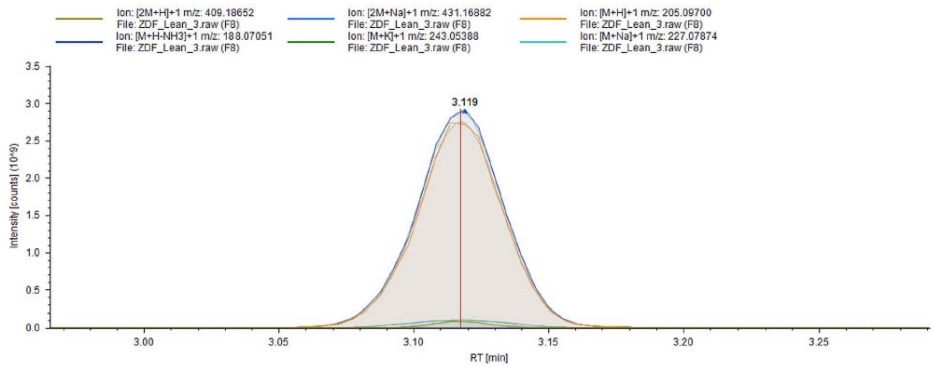
Non Target Screening – *Peak Picking*

m/z Features to Compounds to Answers

For the questions raised above, the answers lie in our Full Scan MS1 and related MS2 data.

Close examination of the TIC time-axis will show a highly complex picture of 'm/z features' with some exhibiting chromatographic behaviour.

Are m/z features with the same chromatographic profile, at the same retention time, related? For the same Rt – are they the same m/z feature in other related samples?



XIC overlay of tryptophan from Compound Discoverer software showing associated ion species. Several ions detected in positive polarity at the same retention time are assembled to reflect one compound within one sample injection of rat plasma extract. Associated ions include $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M+H-NH_3]^+$, $[2M+H]^+$, $[2M+Na]^+$.

Peak Picking: If we can 'assemble' these features into compounds both within the sample and those common to related samples;

- and if we can assign elemental composition, we can begin to move forward –

How?

Non Target Screening – *Peak Annotation*

Thermo Scientific™ Compound Discoverer™ software solves this challenge.

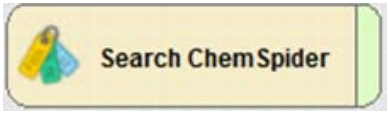
Using:

- Sophisticated feature assembly algorithm.
- Comprehensive integrated set of libraries, databases: mzCloud, ChemSpider
  Custom mass lists
  Compound Class Scoring
- Tools to assist Structural Identification: FISh, mzCloud Slimilarity, mzLogic
  Fine Isotope Trace, Mass Defect.
- Integrated Statistical analysis tools: Differential analysis, PCA, PLS-DA, ANOVA,
  Volcano, box-whisker, and trend-line plots
- Template-based and Customisable node-based workflows

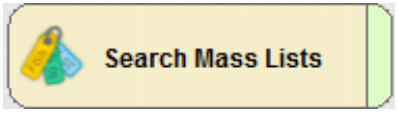
…..The software streamlines the identification of unknowns; the determination of real differences between samples; and the elucidation of degradation and biological pathways.

Non Target Screening – *Peak Annotation*

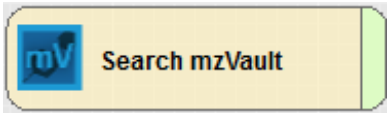
Online spectral library
> 19,251 Compounds with MS2



Chemical structure database with >480 data sources, 59 million structures



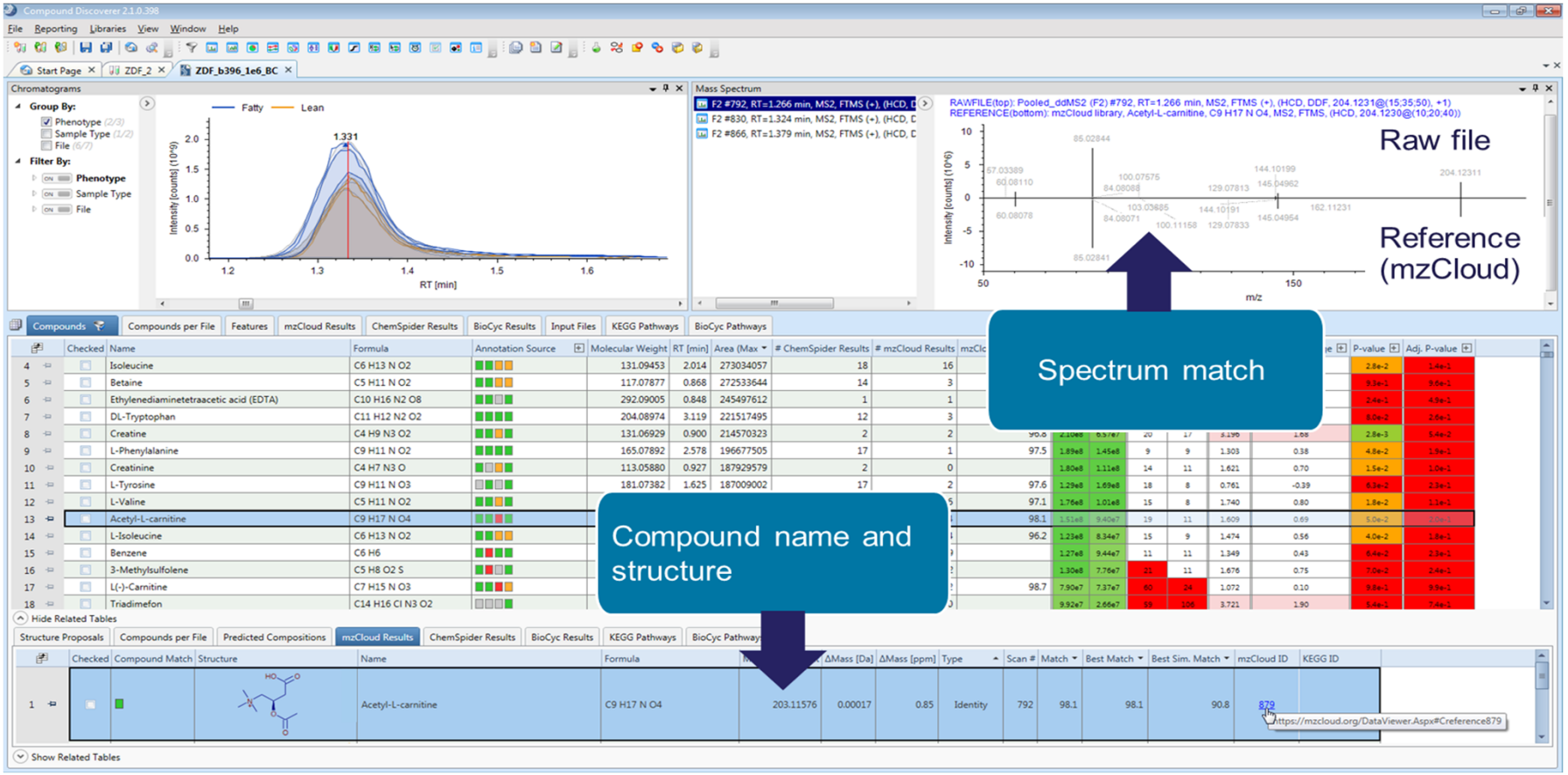
Local spectral libraries



Local databases e.g MassBank

Non Target Screening

mzCloud ID Search Results in Compound Discoverer



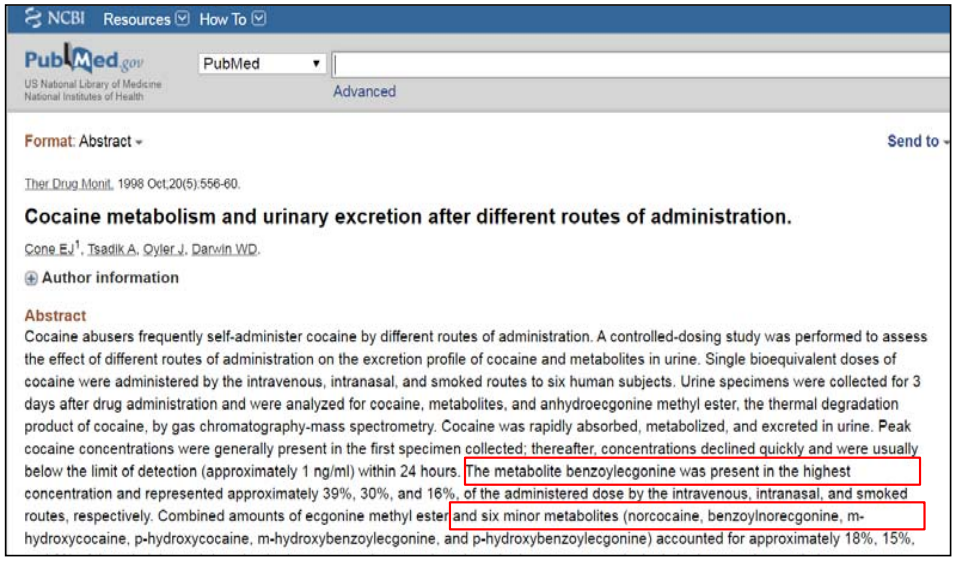
Non Target Screening

Metabolomic Tools for Authenticity and Environmental state Investigation

- Volcano plots, PCA, PLS-DA ,… are interactive
- Results are always directly linked to raw data.

Non Target Screening – Illicit Drugs

- Compound Discoverer used to detect parent compounds as well as related metabolites, to investigate different metabolism pathways that shed light on the original source of illicit drugs detected in wastewater effluent.
- Here we look at effluent data collected from six UK sewage plants, where cocaine and related metabolites were detected. We will focus on Benzoylecgonine, a dominant metabolite in urinary excretion.



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Ther Drug Monit. 1998 Oct;20(5):556-60.

Cocaine metabolism and urinary excretion after different routes of administration.

Cone EJ[1], Tsadik A, Oyler J, Darwin WD.

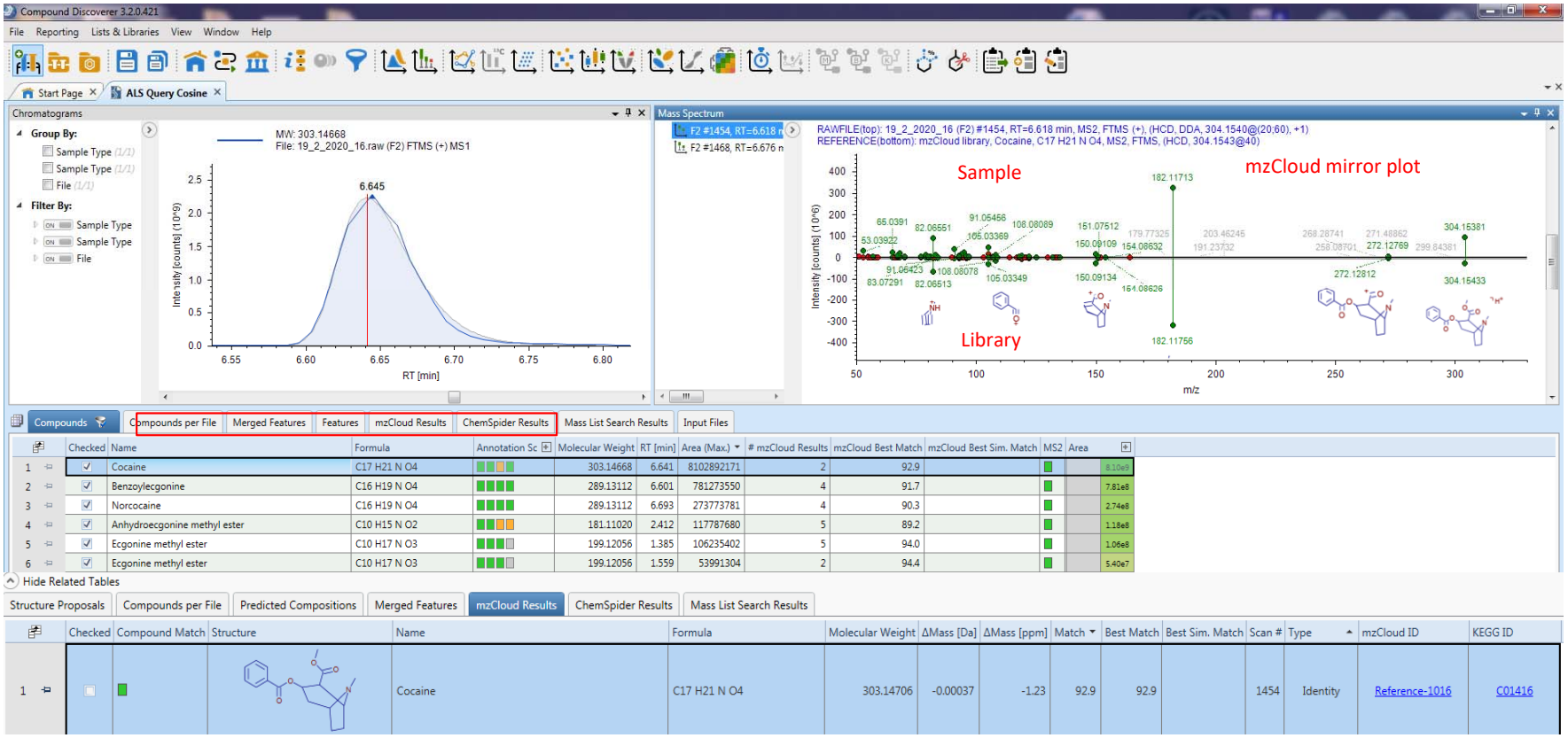
Author information

Abstract

Cocaine abusers frequently self-administer cocaine by different routes of administration. A controlled-dosing study was performed to assess the effect of different routes of administration on the excretion profile of cocaine and metabolites in urine. Single bioequivalent doses of cocaine were administered by the intravenous, intranasal, and smoked routes to six human subjects. Urine specimens were collected for 3 days after drug administration and were analyzed for cocaine, metabolites, and anhydroecgonine methyl ester, the thermal degradation product of cocaine, by gas chromatography-mass spectrometry. Cocaine was rapidly absorbed, metabolized, and excreted in urine. Peak cocaine concentrations were generally present in the first specimen collected; thereafter, concentrations declined quickly and were usually below the limit of detection (approximately 1 ng/ml) within 24 hours. The metabolite benzoylecgonine was present in the highest concentration and represented approximately 39%, 30%, and 16%, of the administered dose by the intravenous, intranasal, and smoked routes, respectively. Combined amounts of ecgonine methyl ester and six minor metabolites (norcocaine, benzoylnorecgonine, m-hydroxycocaine, p-hydroxycocaine, m-hydroxybenzoylecgonine, and p-hydroxybenzoylecgonine) accounted for approximately 18%, 15%,

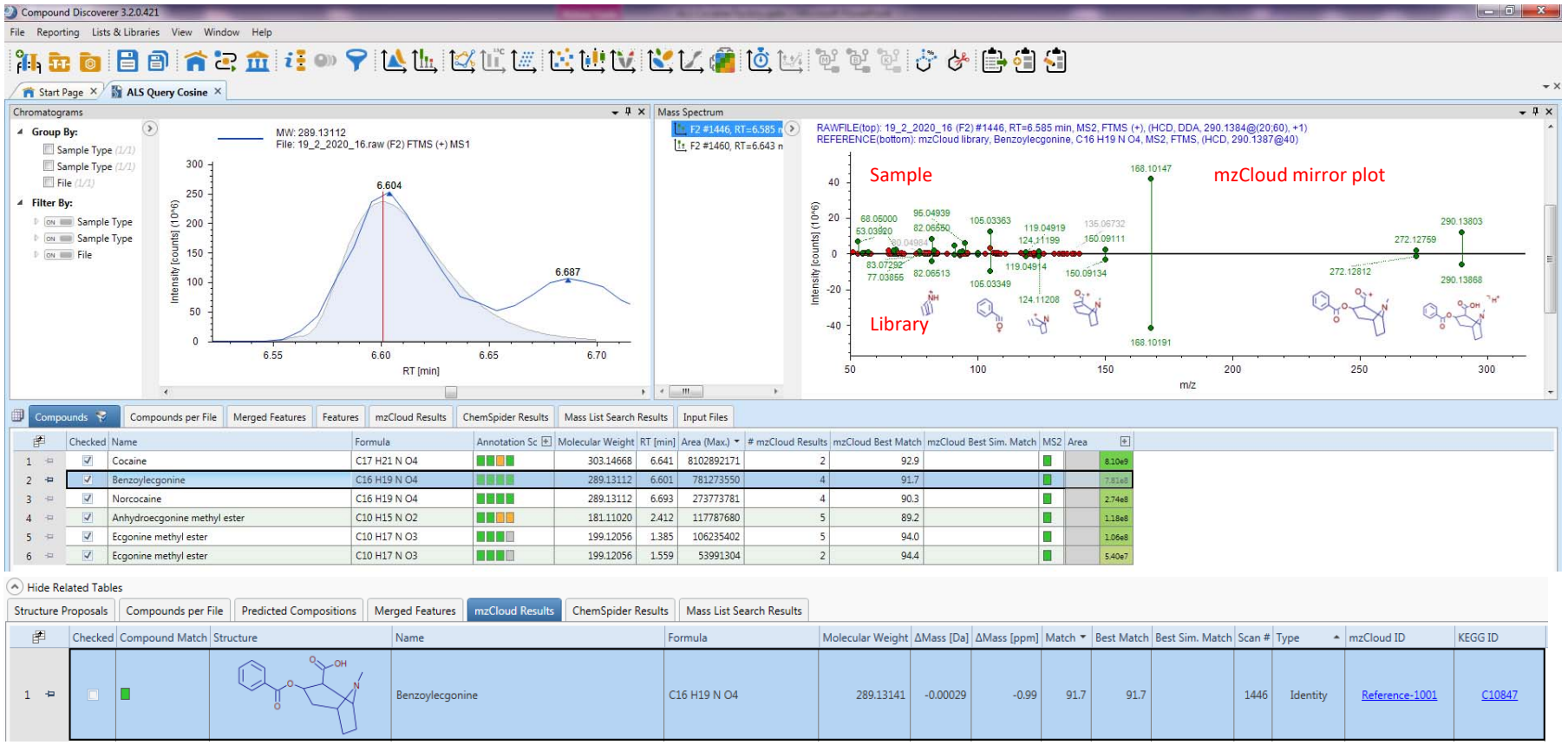
Non Target Screening

Example of Cocaine detection showing mzCloud mirror plot



Non Target Screening – Illicit Drugs

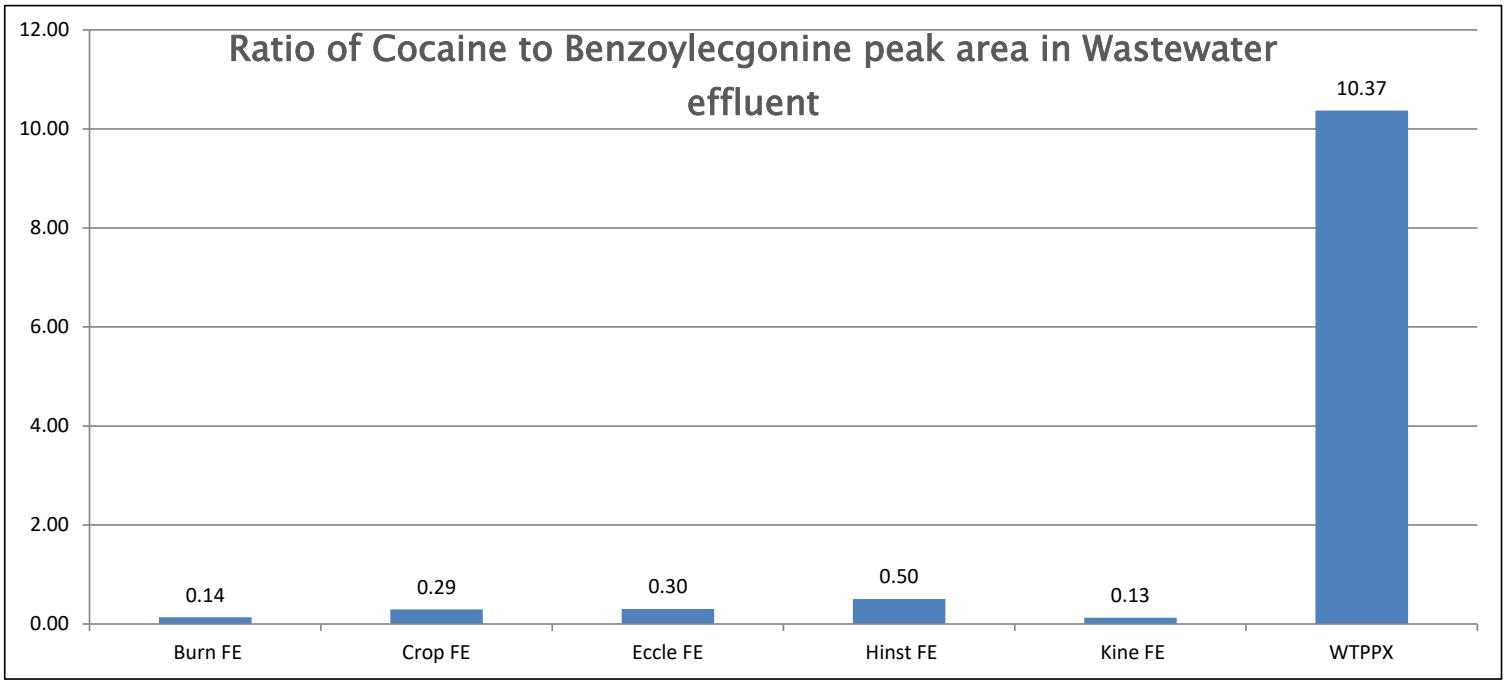
Example of Benzoylecgonine detection showing mzCloud mirror plot



Non Target Screening – Illicit Drugs

- For the six effluent samples, the ratio of Cocaine with respect to Benzolylecgonine was plotted below.

- The ratio in WTPPX clearly shows cocaine dominating, which suggests that the drugs were not from an excretionary route but rather likely from direct disposal.

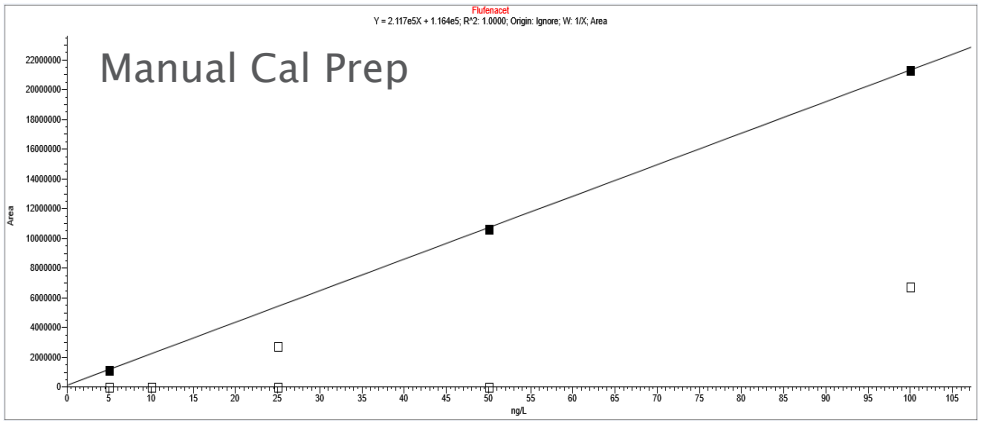


Conclusions / Further Work

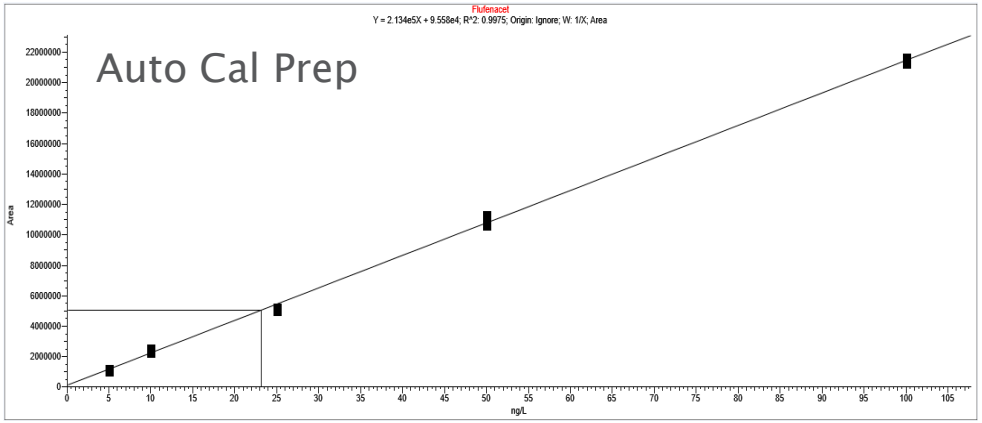
- A method has been developed using HRMS orbitrap with on-line trace enrichment for the low level analysis of a suite of pollutants spanning multiple compound classes in environmental waters using a sample volume of just 5mL.
- Low limits of detection – in many cases <1ng/L – were obtained together with excellent precision and bias statistics.
- Software tools and libraries are critical aspects in looking at non-targeted workflows as shown in the previous examples
- Next steps will focus on the development of a wide ranging, low level screening method covering a large number of pesticides, pharmaceuticals, PFAS compounds and other chemicals of environmental concern.
- Some new features of the Equan 850 will help simplify and automate the method even further

Future work- Equan850 Exploris120

Equan 850 Direct and On-line SPE and Automatic Calibration Preparation



Problem on instrument, so limited points



Each Level ran three times

Acknowledgements

- Sam Towers – ALS Environmental
- David Evans – ALS Environmental
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Any Questions?