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The Detection and Identification of Unknown Contaminants During ToF Screening and Structural Elucidation for Pesticides in River Water Using an Integrated Software Approach

Chemical Analysis Business Operations
Waters Corporation
Milford MA
Email: ken_rosnack@waters.com

Streamlined Screening Workflow

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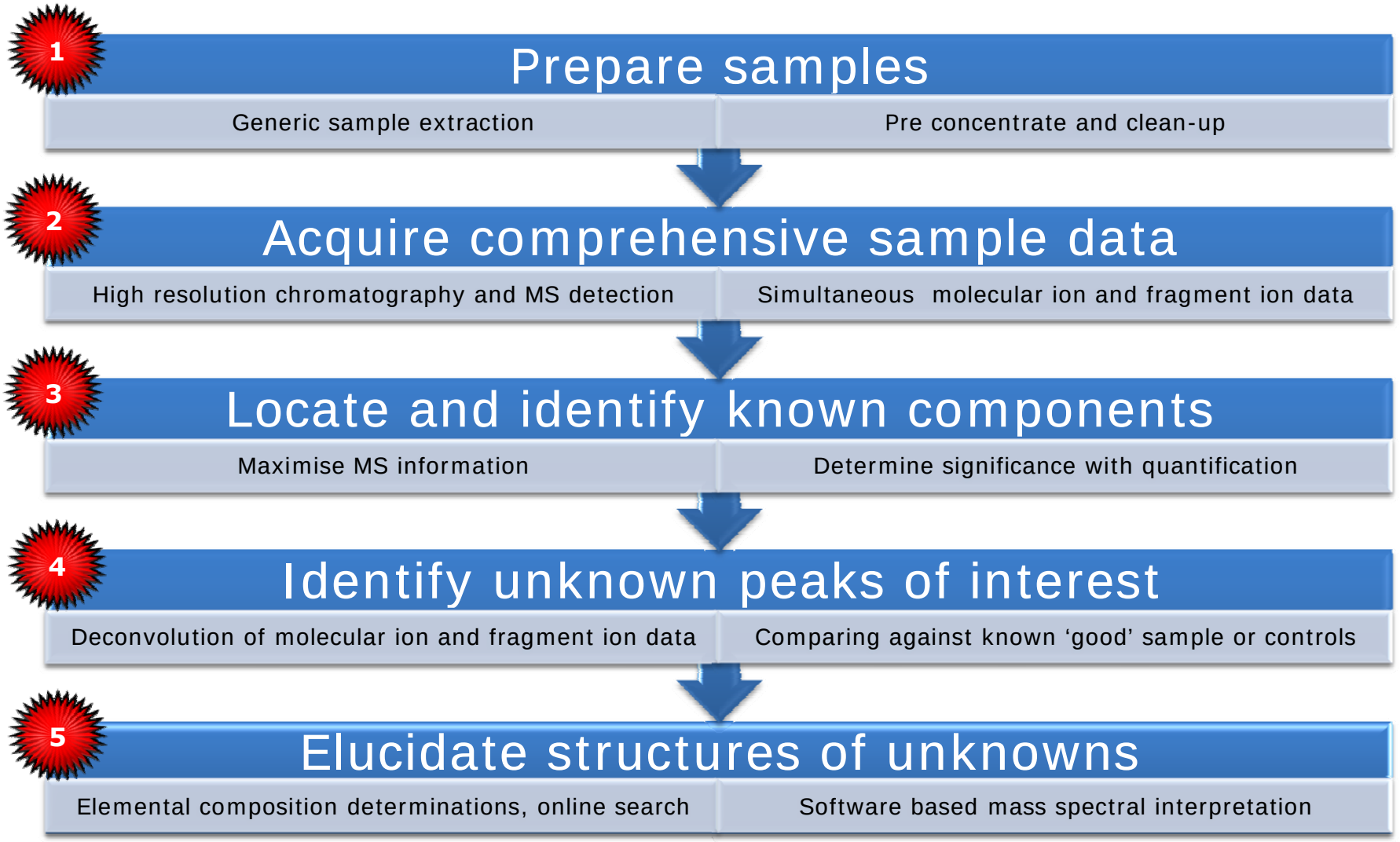

Screening Enabling Technologies

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- Oasis HLB SPE
  - Efficient water extraction
- Xevo G2 QTof
  - QuanTOF technology
  - 20,000 resolution
  - MS^E enabled
- POSI±IVE workflow software
  - Targeted screening using compound database
- ChromaLynx XS, MassFragment
  - Locate and identify non-targeted unknown components

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Rapid and Comprehensive data acquisition

Speed AND Resolution
MS^E with rapid chromatography

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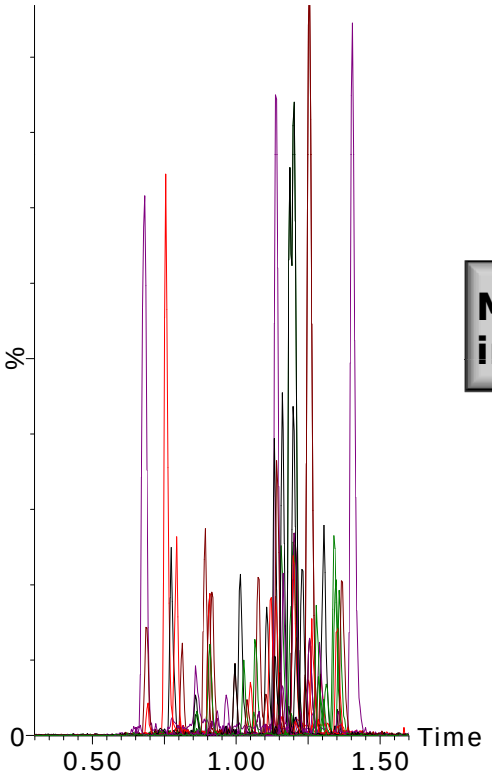
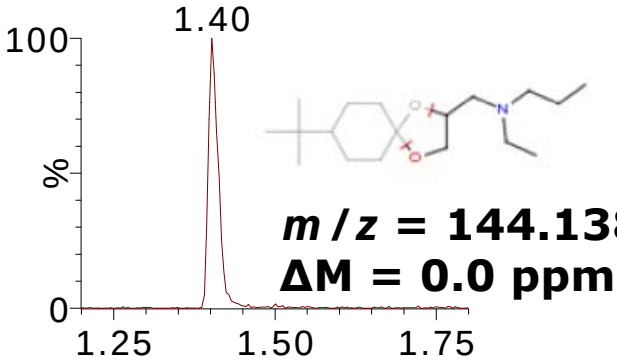
28 datapoints across spiroxamine peak



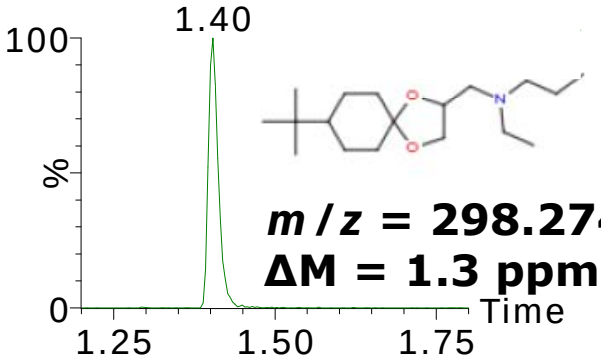
Hundreds of Pesticides, <85 seconds

MS^E = All the masses in ONE injection

Fragments = 14 data points



***m / z* = 144.1388**
ΔM = 0.0 ppm



***m / z* = 298.2746**
ΔM = 1.3 ppm

Precursors = 14 data points

Speed AND Resolution
MS^E with rapid chromatography

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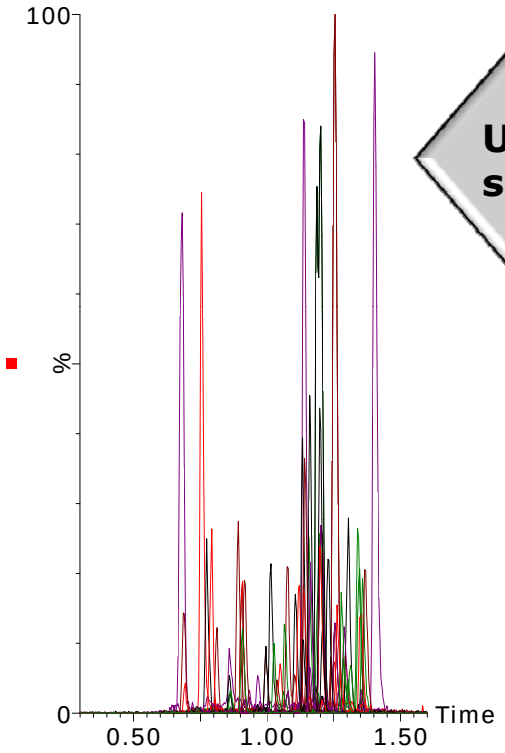
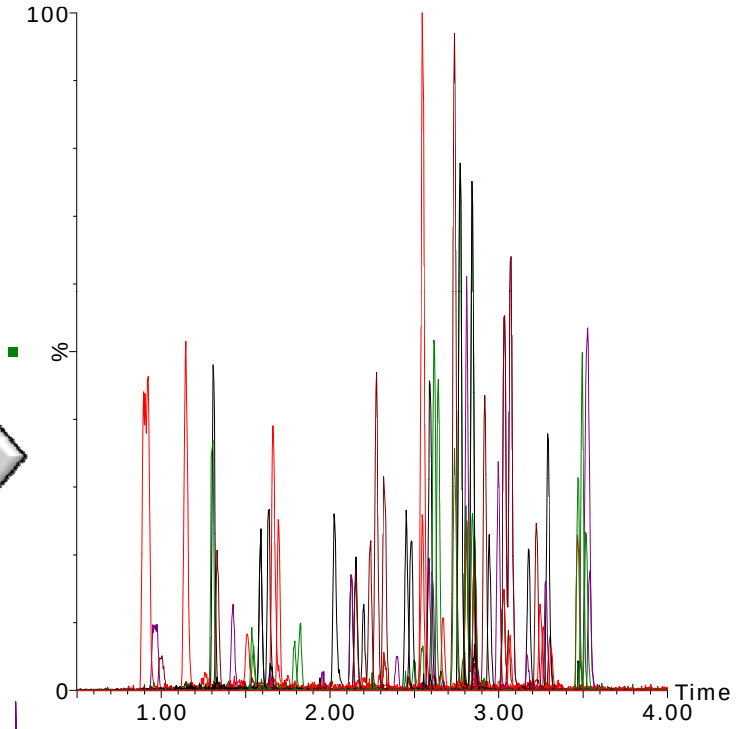
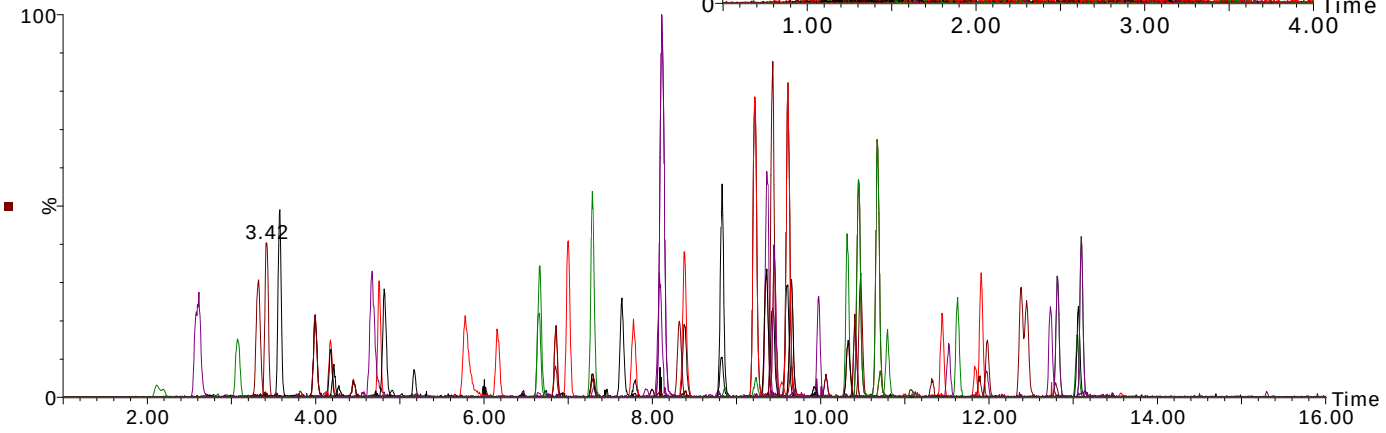


Ultra fast for simple matrices

Medium speed for 'normal' matrices



Maximum resolution for the most complex matrices



Comprehensive Data Acquisition - UPLC/MS^E

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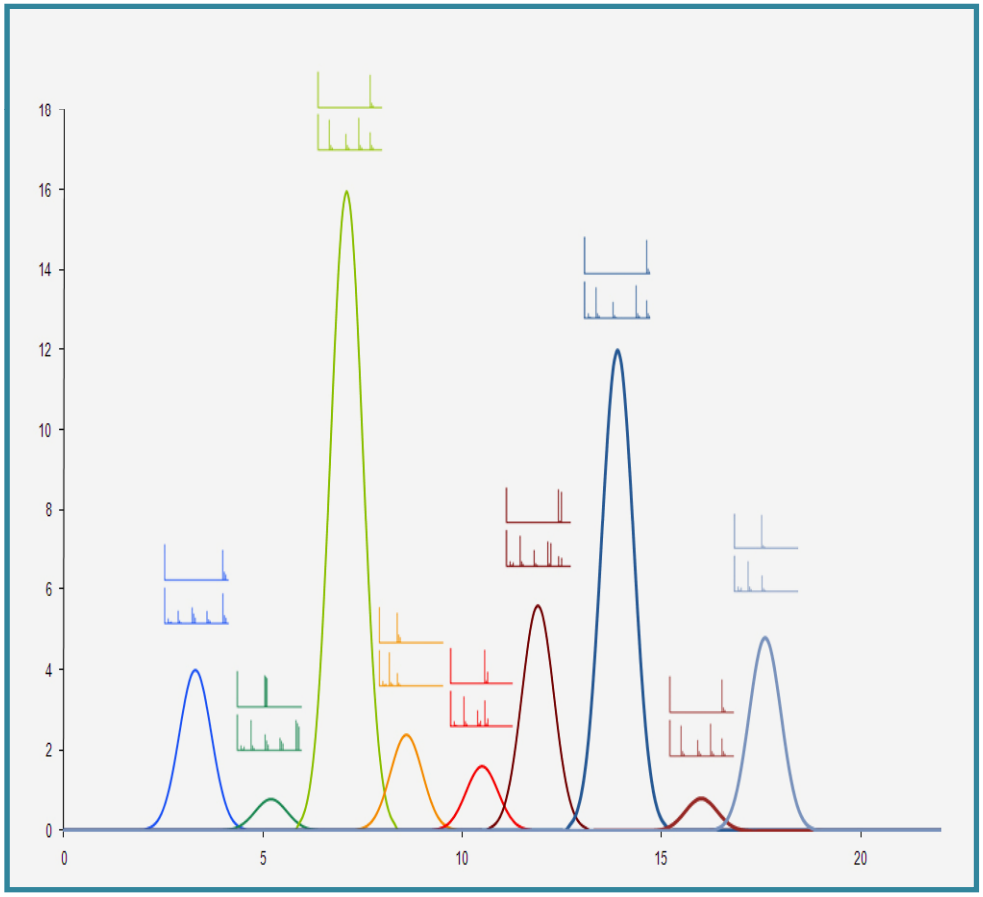
- Simple, patented method for unbiased data acquisition
  - Catalogs complex samples in a single analysis
  - Comprehensive record of precursor and fragment ion data



Comprehensive Fragment ion data @ High Speeds

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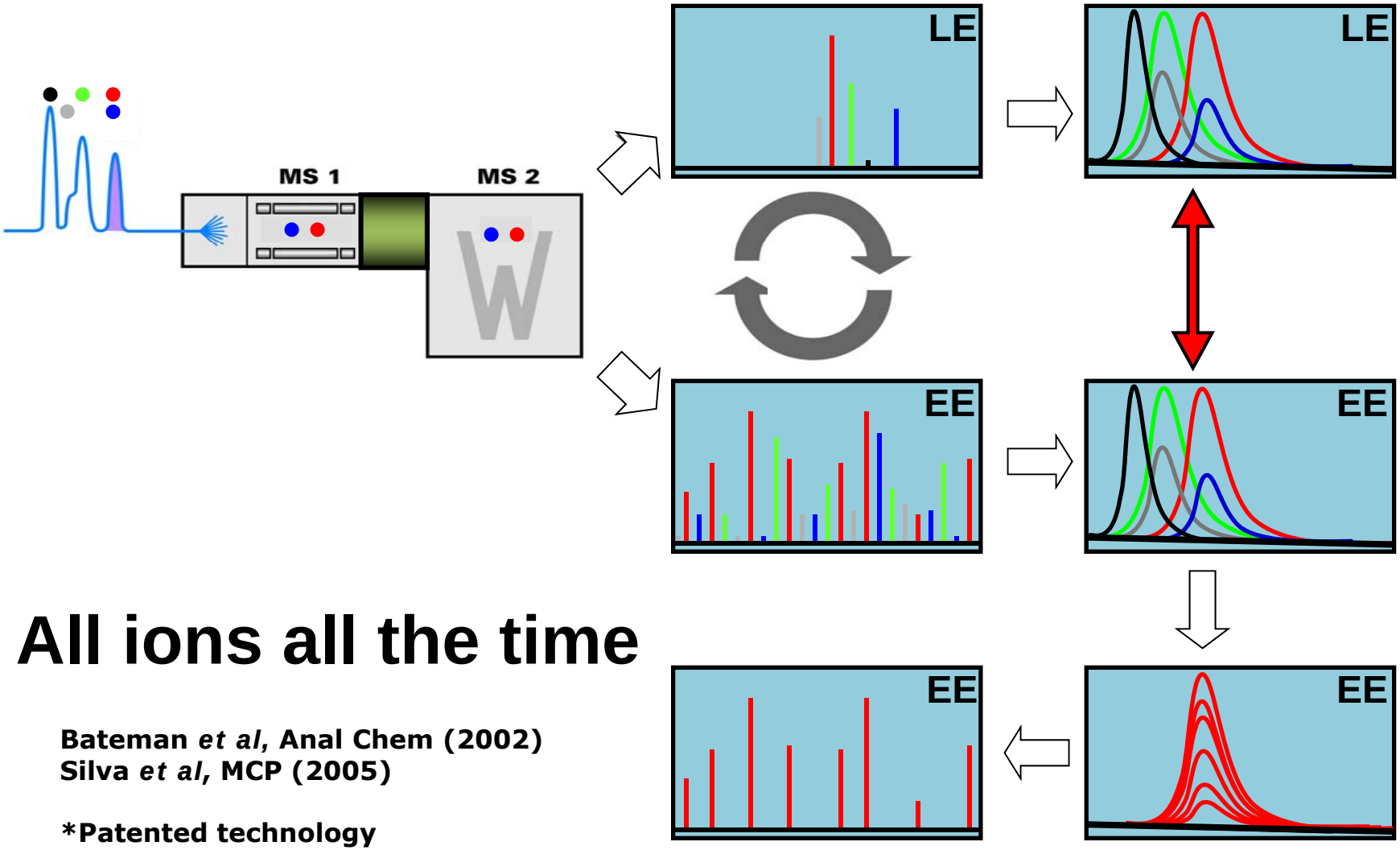

All ions all the time

Bateman *et al*, Anal Chem (2002)
Silva *et al*, MCP (2005)

***Patented technology**

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Matrix Tolerance

In Spectrum Dynamic range - Matrix Tolerance

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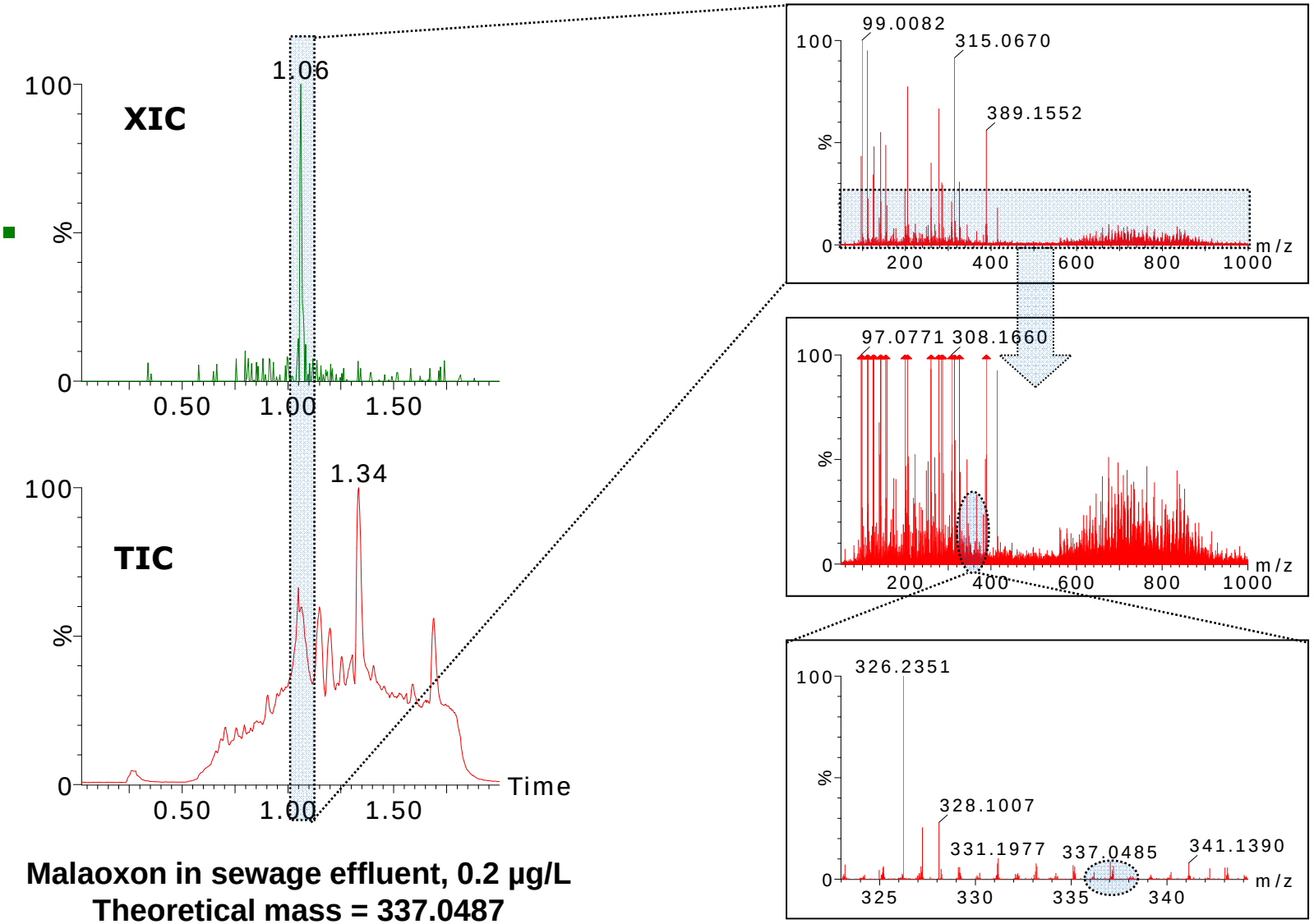

Malaoxon in sewage effluent, 0.2 µg/L
Theoretical mass = 337.0487

Stability and Selectivity

Mass Stability
Essential for High Quality Data

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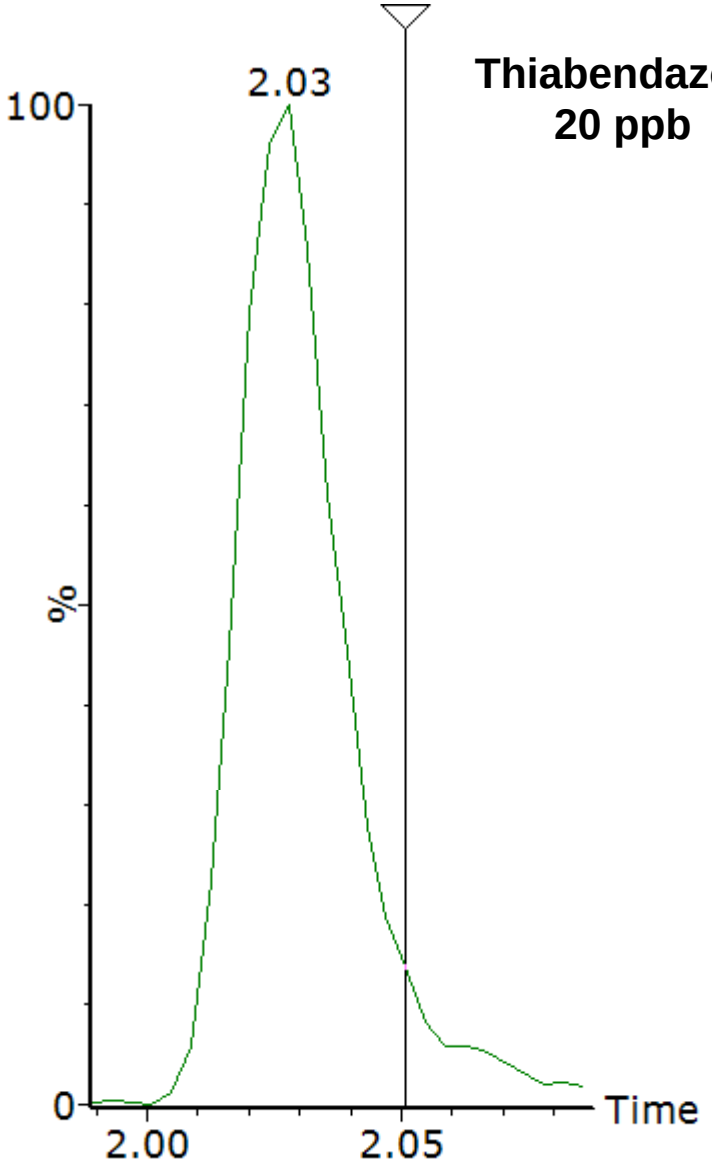
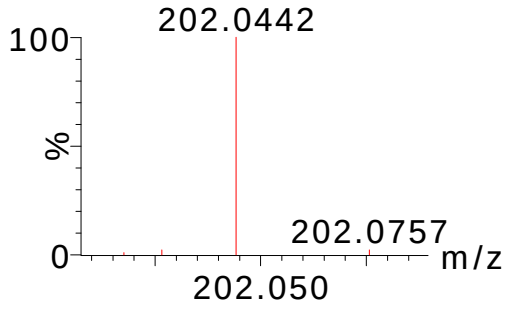


Scan No.	Measured Mass	ΔM (mDa)	ΔM (ppm)
505	202.0434	-0.50	-2.47
506	202.0439	0.00	0.00
507	202.0436	-0.30	-1.48
508	202.0442	0.30	1.48
509	202.0437	-0.20	-0.99
510	202.0440	0.10	0.49
511	202.0439	0.00	0.00
512	202.0435	-0.40	-1.98
513	202.0441	0.20	0.99
514	202.0435	-0.40	-1.98
515	202.0433	-0.60	-2.97
516	202.0442	0.30	1.48
	RMS =	**0.30**	**1.50**



Excellent mass accuracy across peak

Targeted Screening
Narrow XIC Windows

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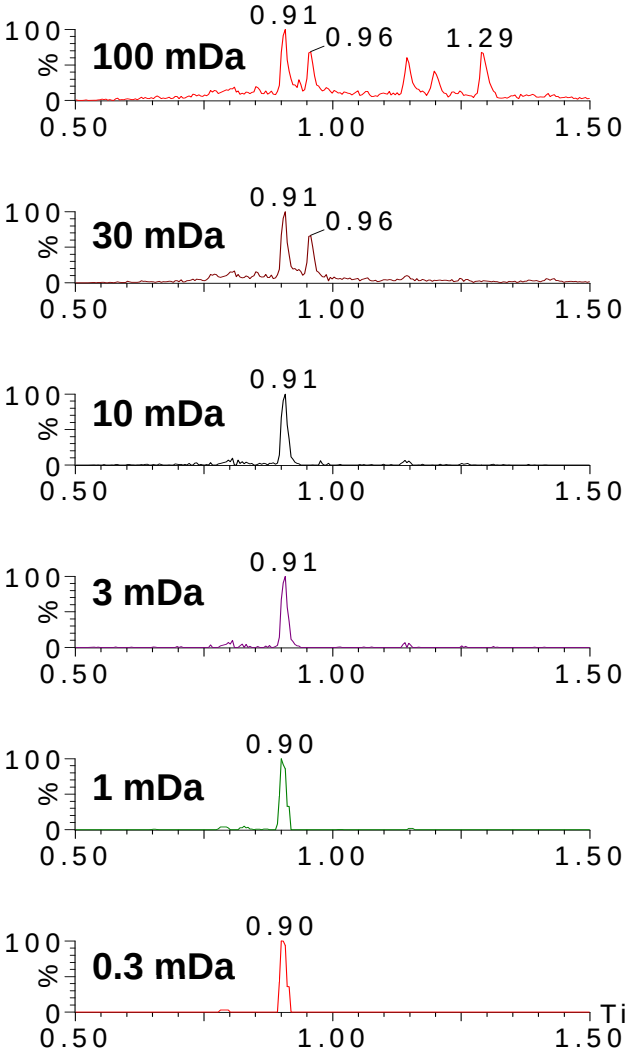
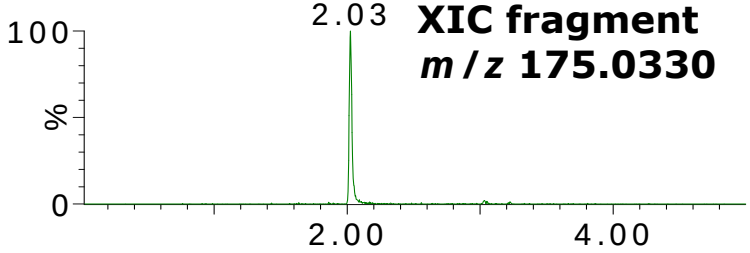
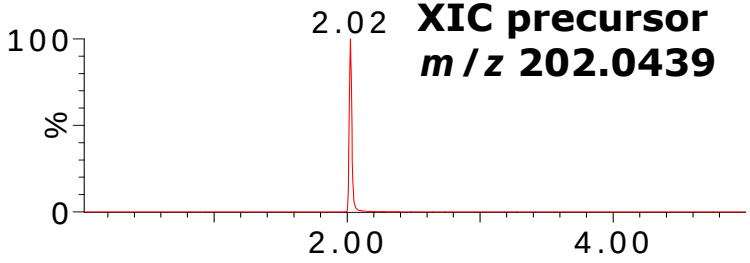
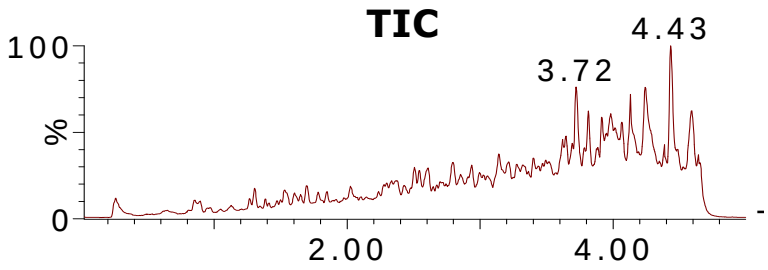


Fenuron m/z 165.1028

Mass accuracy + stability permits use of narrow XICs







Thiabendazole

High selectivity for precursors and fragments with MS^E

Reducing False Positives
Narrow XIC Windows

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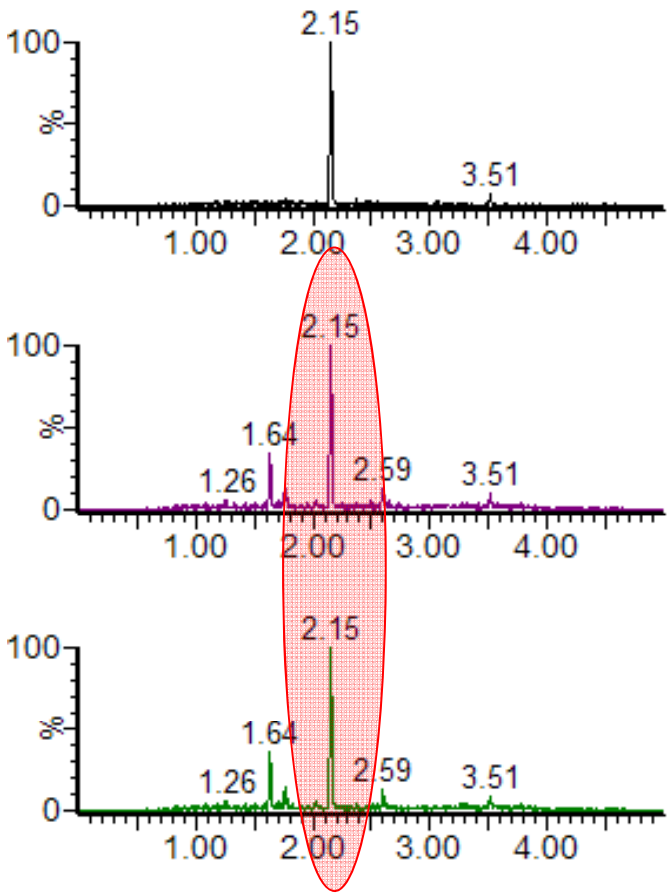
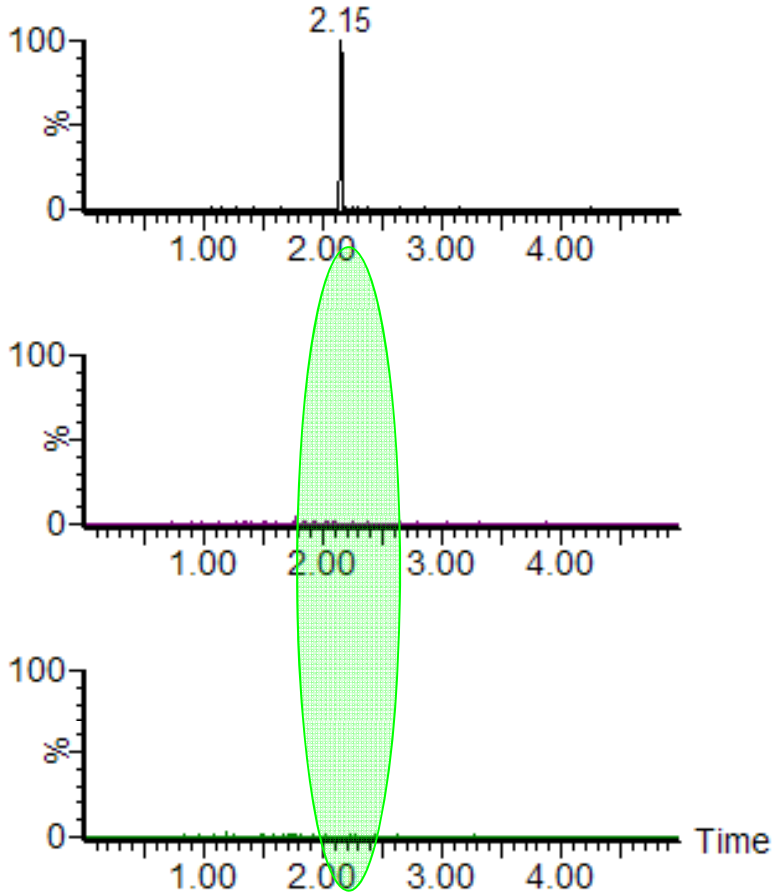


Monuron
***m / z* 199.0638**

Fenazox
***m / z* 199.0871**

Cymoxamil
***m / z* 199.0831**



50 mDa **Mass extraction window** **3.0 mDa**

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Selectivity gains using high resolution fragment ions

Comprehensive Data Acquisition - Resolve Isobaric Precursors with MS^E

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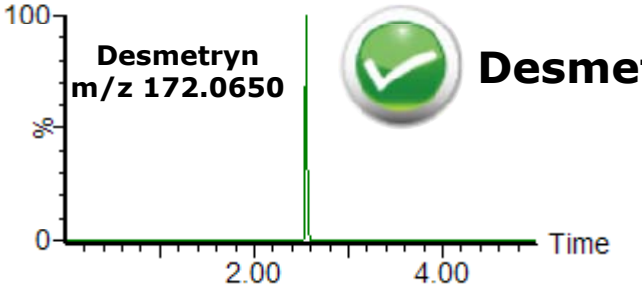
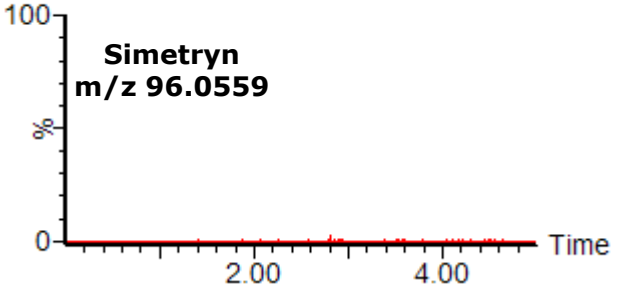
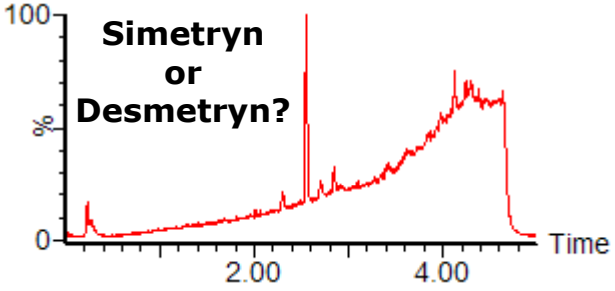
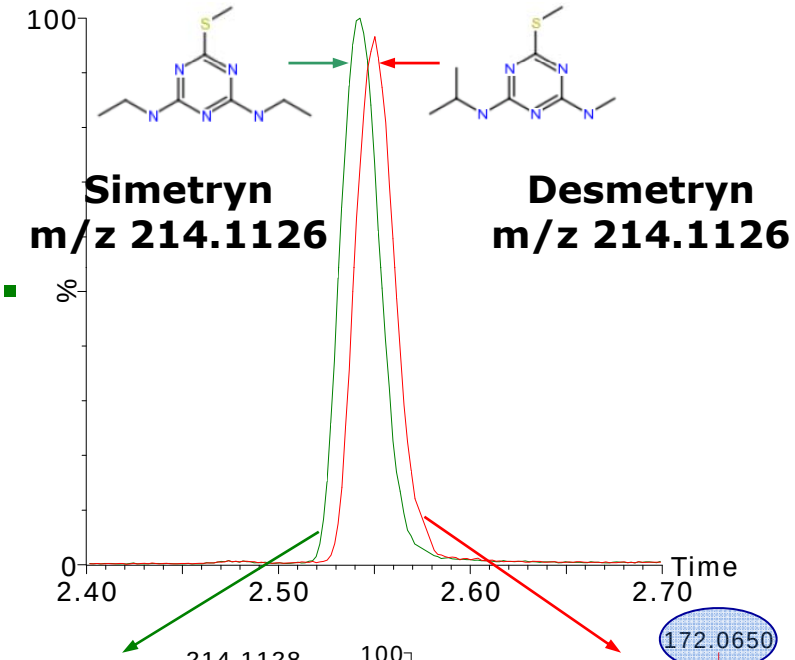
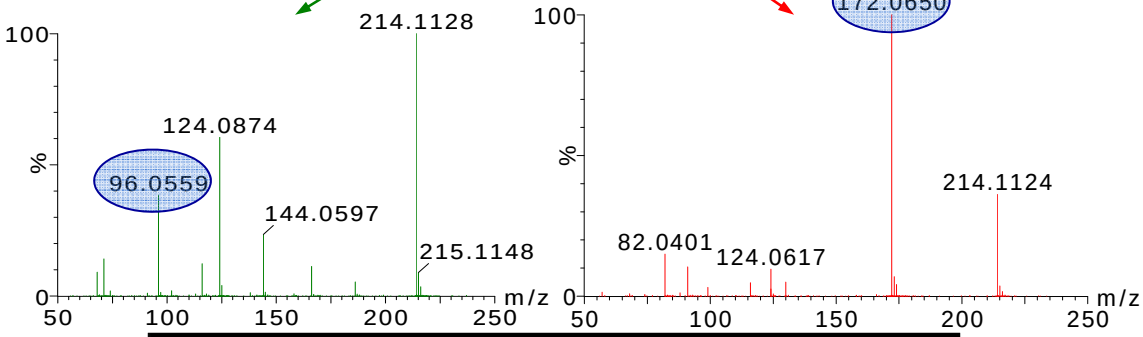


Desmetryn









Selective MS^E fragment in standards

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Screening Against a Target List of Compounds

Experimental

- 200 mL UK River Water
- Oaisis HLB Cartridge (200x concentration)
- ACQUITY UPLC System
  - Runtime: 5 min
  - Flow Rate: 0.6 mL/min
  - Injection: 3.0 µL
  - Column: ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 50 mm (45 °C)
  - Mobile Phase: A) Ammonium Acetate in Water. B) Ammonium Acetate in Methanol

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.60	98	2	0
0.10	0.60	98	2	6
3.75	0.60	1	99	6
4.25	0.60	1	99	6
4.26	0.60	98	2	11
5.00	0.60	98	2	6

Experimental Continued

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- **MS system:** Xevo G2 QTof (Figure 1)
- **Ionization mode:** ESI positive
- **Analyser:** Resolution mode (>20K resolution)
- **Scan time:** 0.1 s
- **Capillary voltage:** 1.0 kV
- **Sampling cone:** 30.0 V
- **Source temp:** 120 ^{o}C
- **Desolvation temp:** 550 ^{o}C
- **Mass range:** *m/z* 50 – 1000
- **MS^{E} Low energy:** 6.0 V
- **MS^{E} High energy:** 25.0 - 30.0 V
- **LockSpray solution:** Leucine enkephalin
- **LockSpray masses:** *m/z* 556.2771 and *m/z* 278.1141

Screening against a Target List

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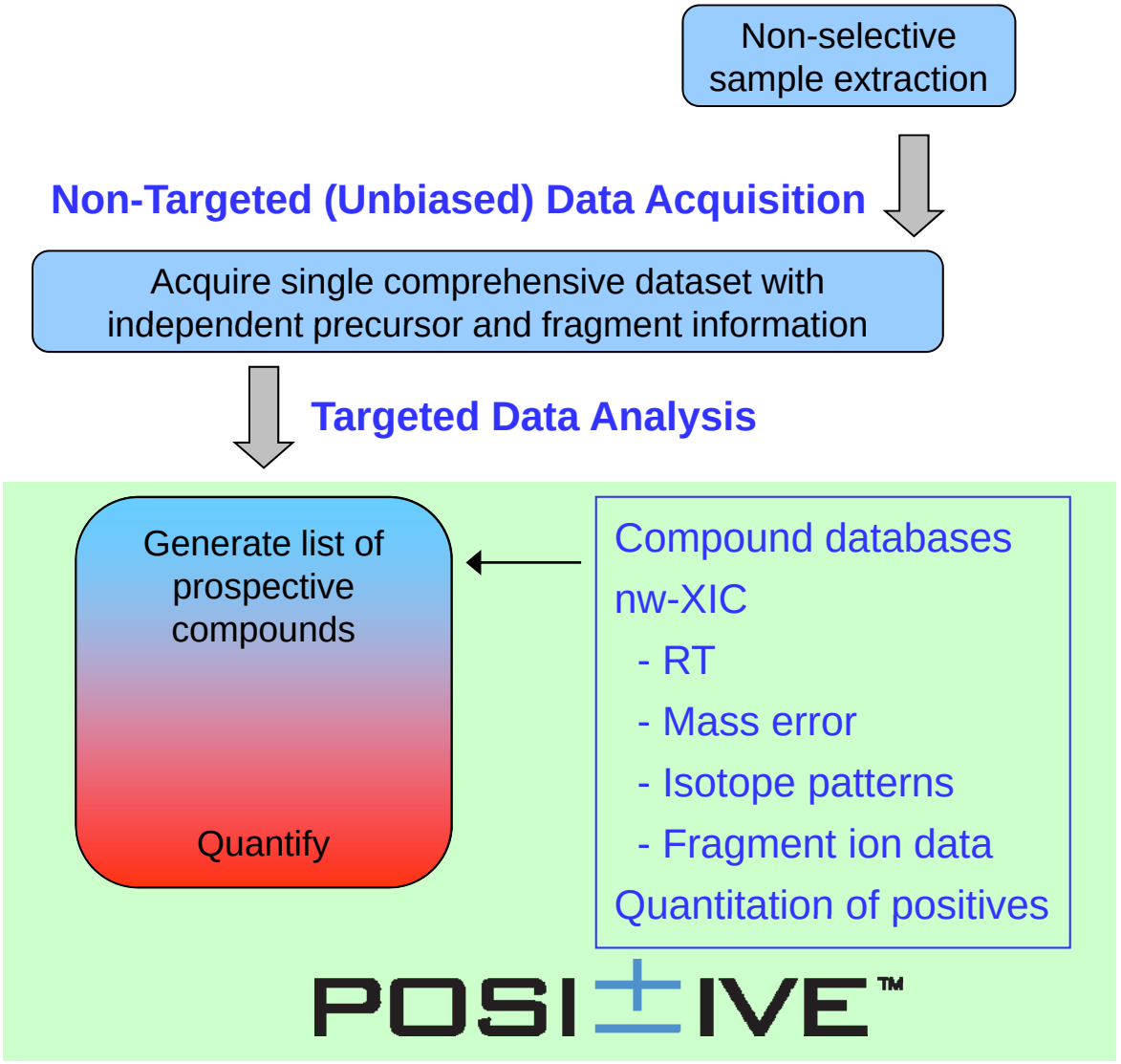

Benefits

- Utilise U-HPLC speed
- Precursors & Fragments in single injection
- Good selectivity from UPLC & MS resolution, mass accuracy & stability, use of fragment ions
- Reduced false positives
- Required sensitivity for regulatory use & discovery of unknowns
- Works with complex matrices
- Screen & Quantify

Targeted Screening
River Water

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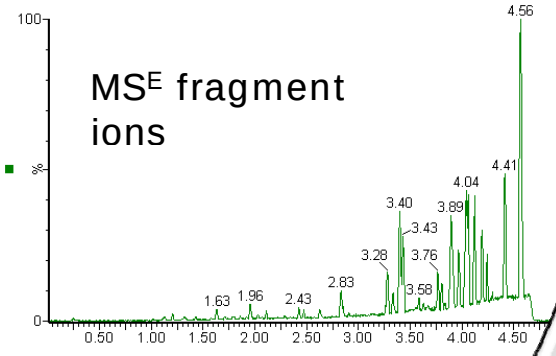
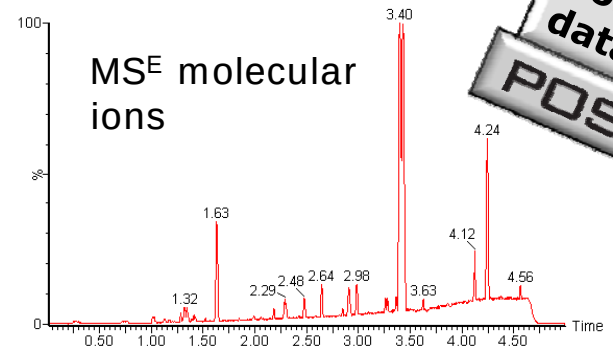
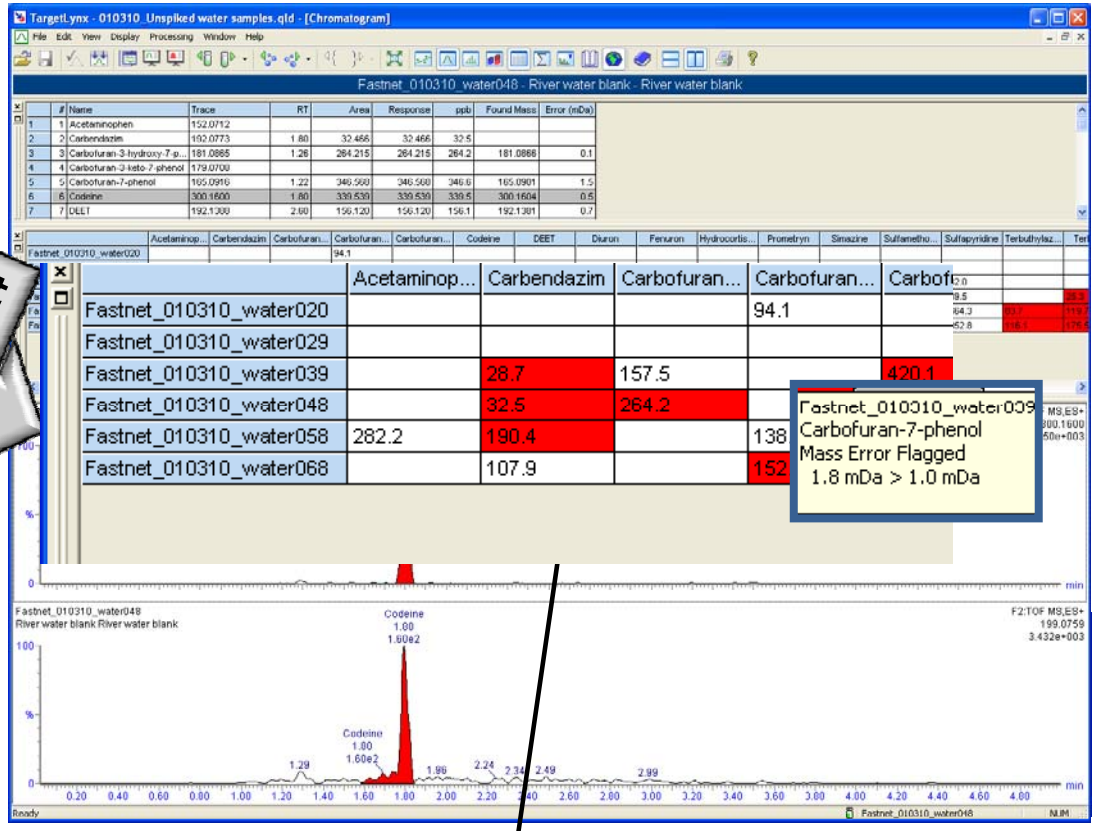




Contaminant database

POSITIVE™

590 compounds targeted, 5 detected within tolerances



Overview of all detected compounds in all samples

River Water Screening
Codeine Detection

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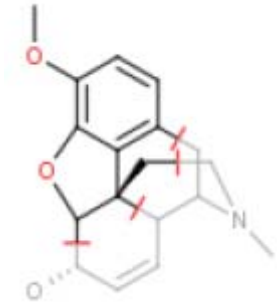
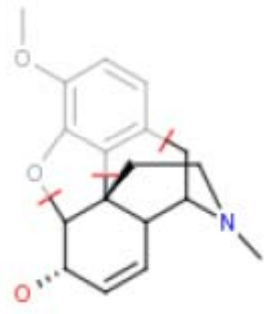
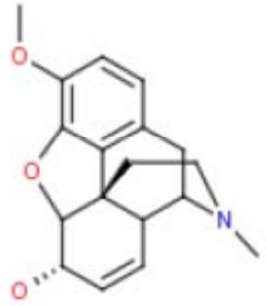


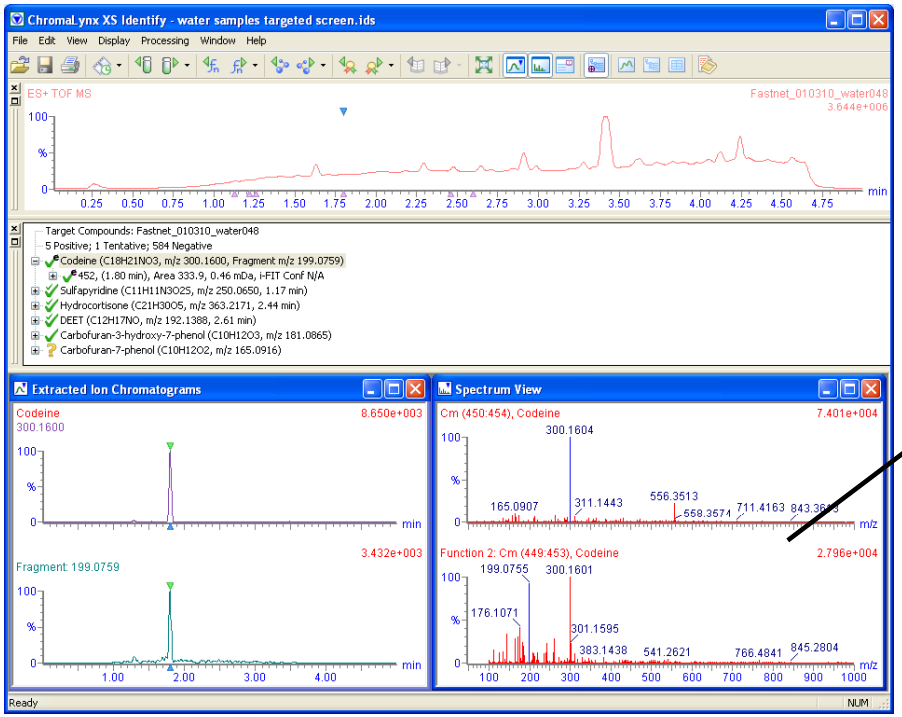
ΔM: +0.4 mDa

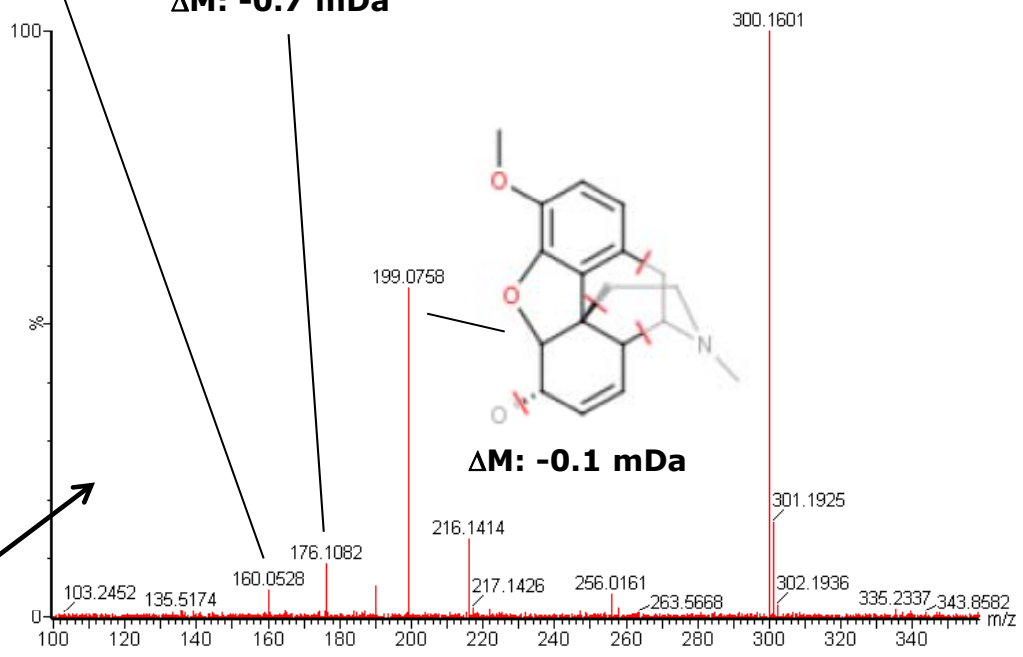


ΔM: -0.7 mDa



ΔM: +0.1 mDa





ΔM: -0.1 mDa

MassFragment

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Discovery & Identification of "Unknown" Residues

"Unknown" Discovery and Identification

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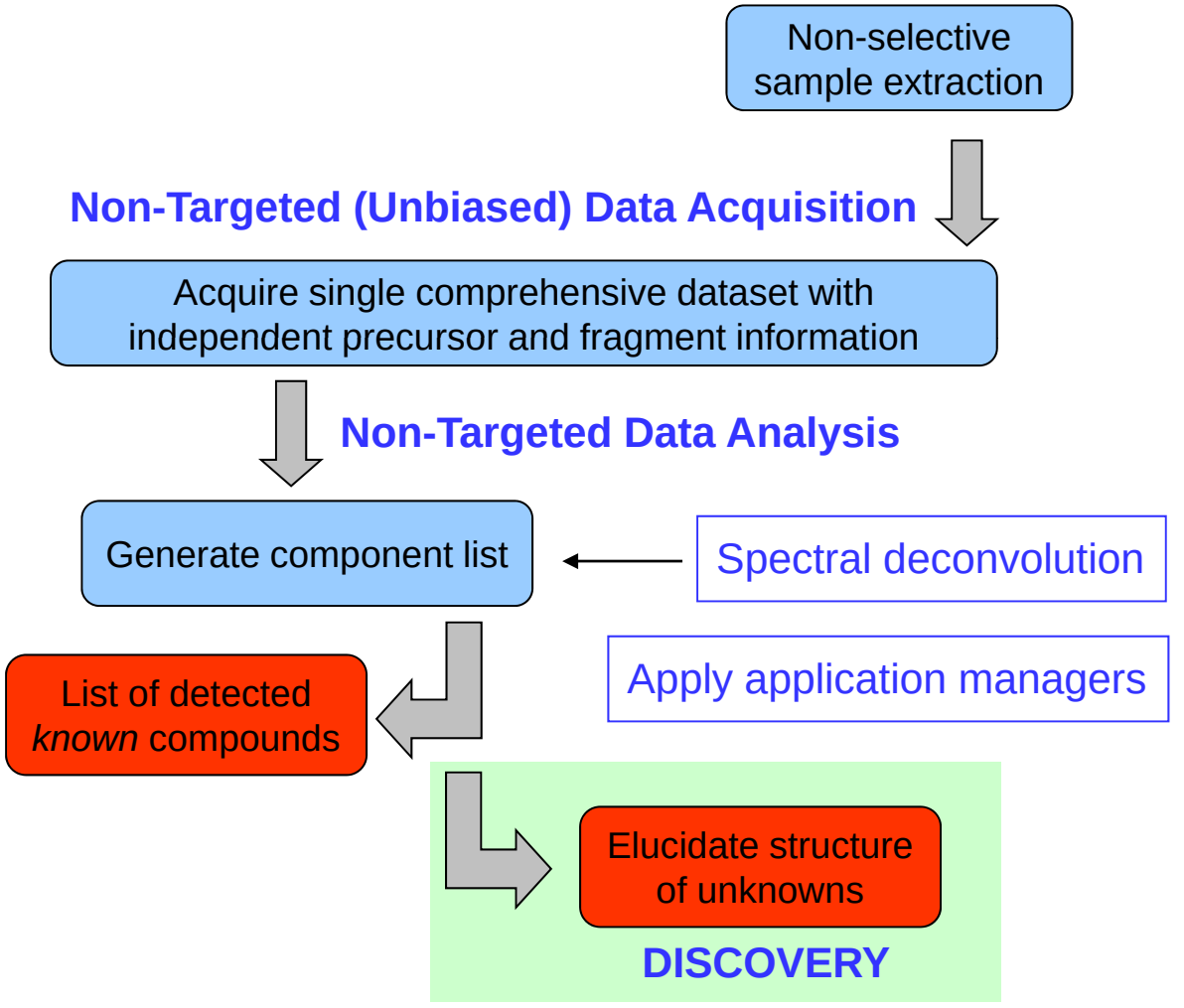

Application Managers

- Identify using spectral libraries
- Compare

- Detect and identify transformation products

- Detect and identify markers of sample differentiation

- Apply structural elucidation tools
- Confirm identity

Example Workflow

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Acquire single comprehensive dataset with independent precursor and fragment information

Non-Targeted Data Analysis

DISCOVERY

- **Spectral deconvolution**
- **Compare samples**
- **Use Acc Mass and isotope profile filters**
- **Search database for candidates**
- **Provide structure and fragment ion spectra to "Massfragment"**

Component List

1. **Detect "Unknown"**
2. **Propose Elemental Composition**
3. **Propose Candidate Structure**
4. **Test Candidate Structure**

5. **Confirm Candidate (Standard + MS/MS)**

Non-Targeted Components
Deconvoluting river water sample

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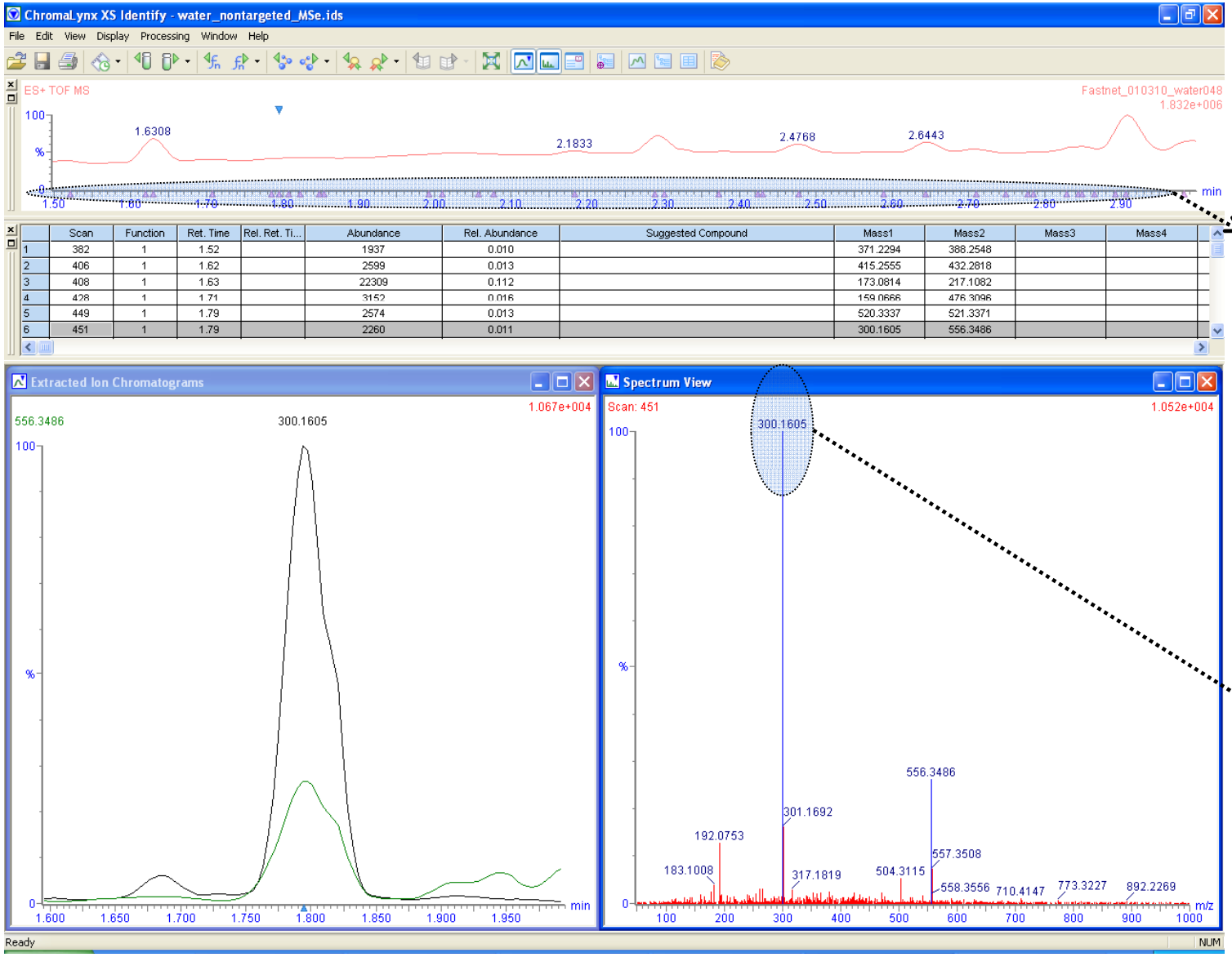

	Scan	Function	Ret. Time	Rel. Ret. Ti...	Abundance	Rel. Abundance	Suggested Compound	Mass1	Mass2	Mass3	Mass4
1	382	1	1.52		1937	0.010		371.2294	388.2548		
2	406	1	1.62		2599	0.013		415.2555	432.2818		
3	408	1	1.63		22309	0.112		173.0814	217.1082		
4	428	1	1.71		3152	0.016		159.0666	476.3096		
5	449	1	1.79		2574	0.013		520.3337	521.3371		
6	451	1	1.79		2260	0.011		300.1605	556.3486		

1

deconvoluted non-targeted components

Codeine detected in non-target mode

Non-Targeted Components
Comparison with control sample

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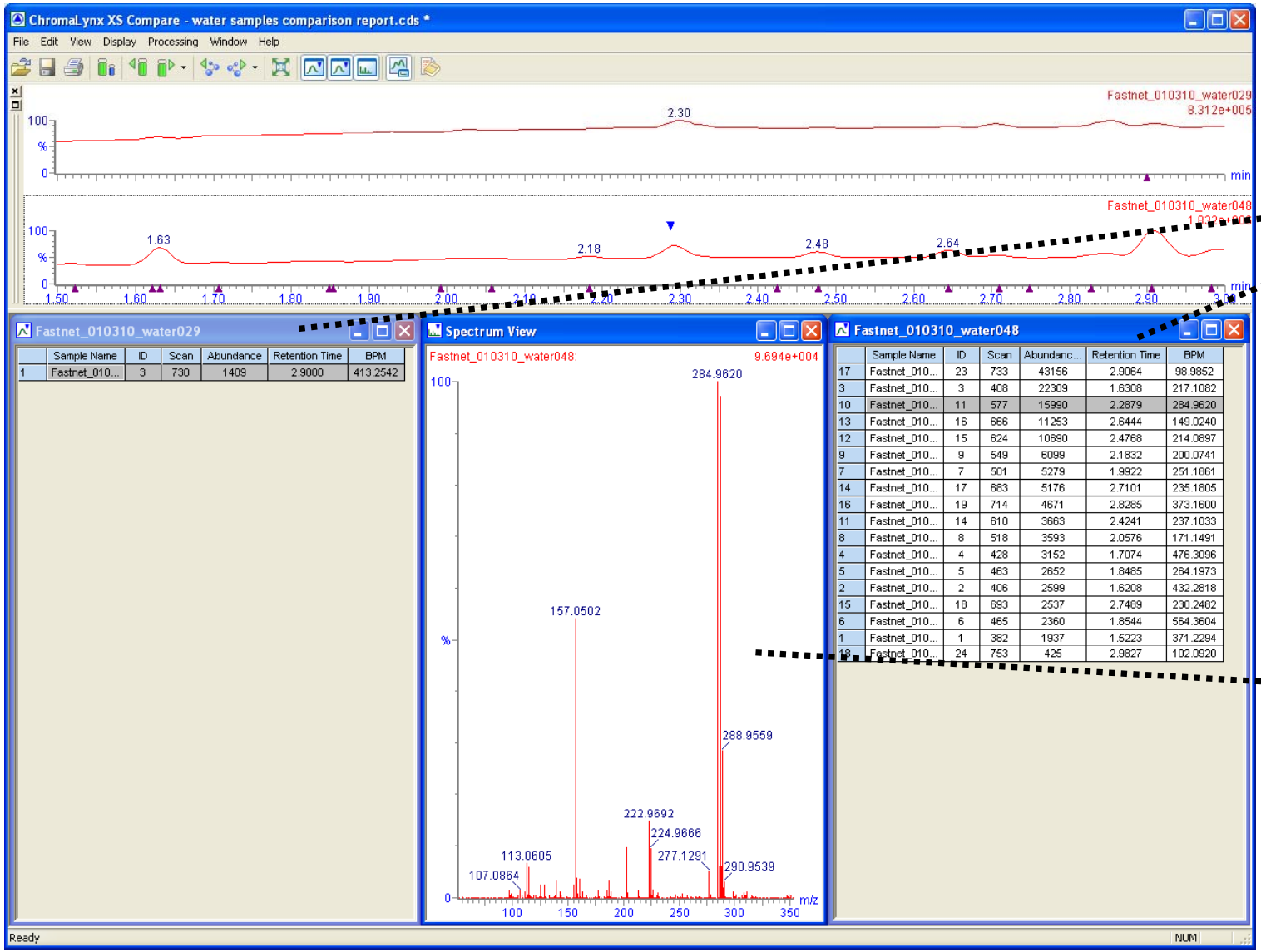

1

Unique components in each sample

Unusual Chlorinated component 3rd highest abundance

Non-Targeted Component
Elucidating structure of unknown

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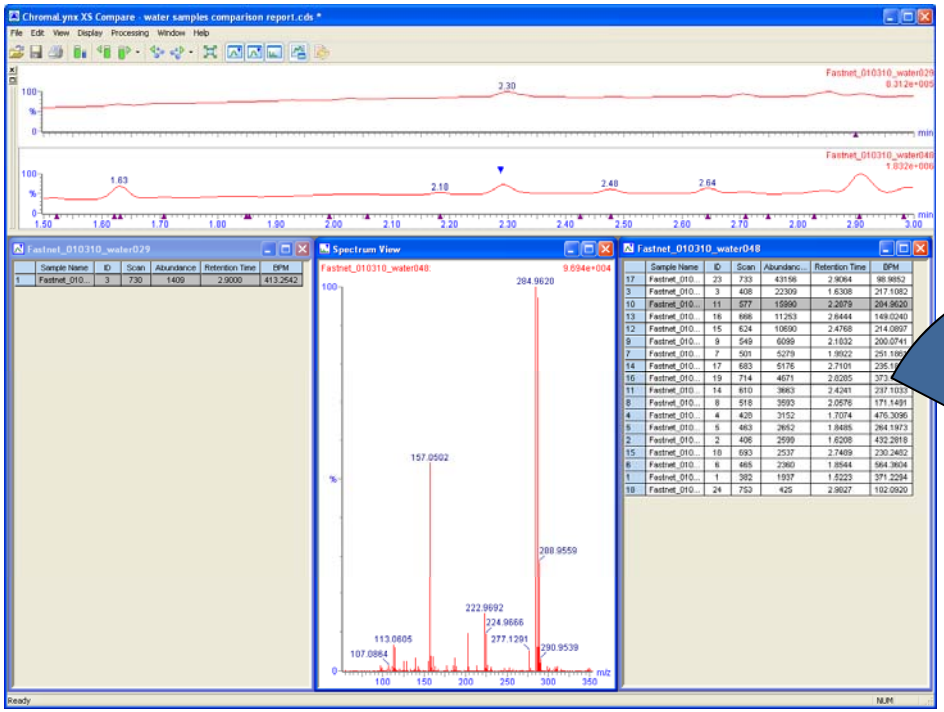
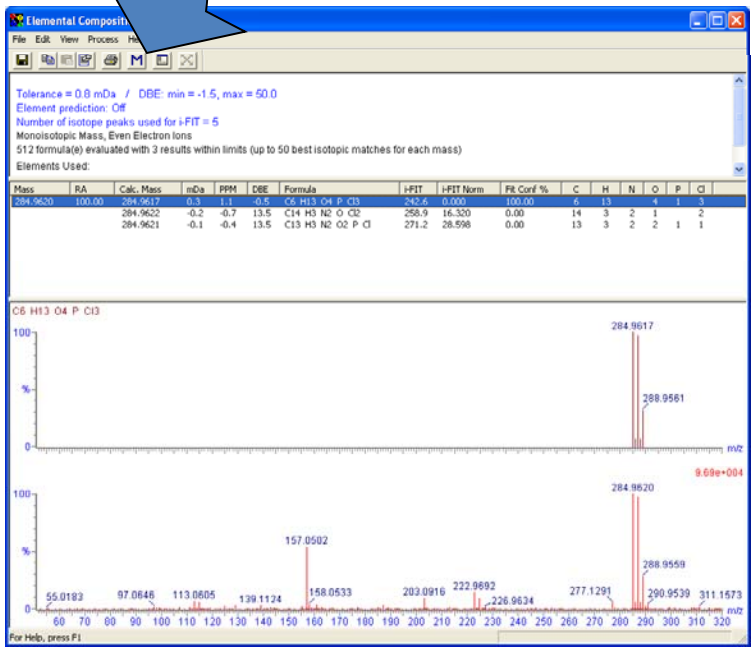


Unknown component RT 2.28min, m/z 284.9620

2



Elecomp and i-Fit proposed $C_6H_{13}O_4PCl_3$ as candidate

Non-Targeted Component
Elucidating structure of unknown

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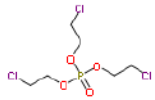
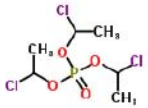
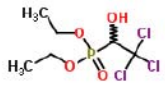
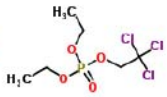
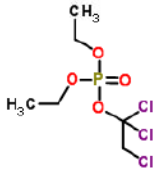
3

ID	Structure	Empirical Formula	Molecular Weight	# of Data Sources	# of References
7994		$C_6H_{12}Cl_3O_4P$	285.4898	32	80
2283616		$C_6H_{12}Cl_3O_4P$	285.4898	10	12
83190		$C_6H_{12}Cl_3O_4P$	285.4898	9	14
107417		$C_6H_{12}Cl_3O_4P$	285.4898	6	6
19979868		$C_6H_{12}Cl_3O_4P$	285.4898	1	1

2

$C_6H_{12}O_4PCl_3$ entered into Chemspider search ($[M+H]^+$)

ChemSpider proposed a number of structures Tris(2-chloroethyl) Phosphate is a commonly used flame retardant and the top hit

Non-Targeted Component MassFragment and MS^E

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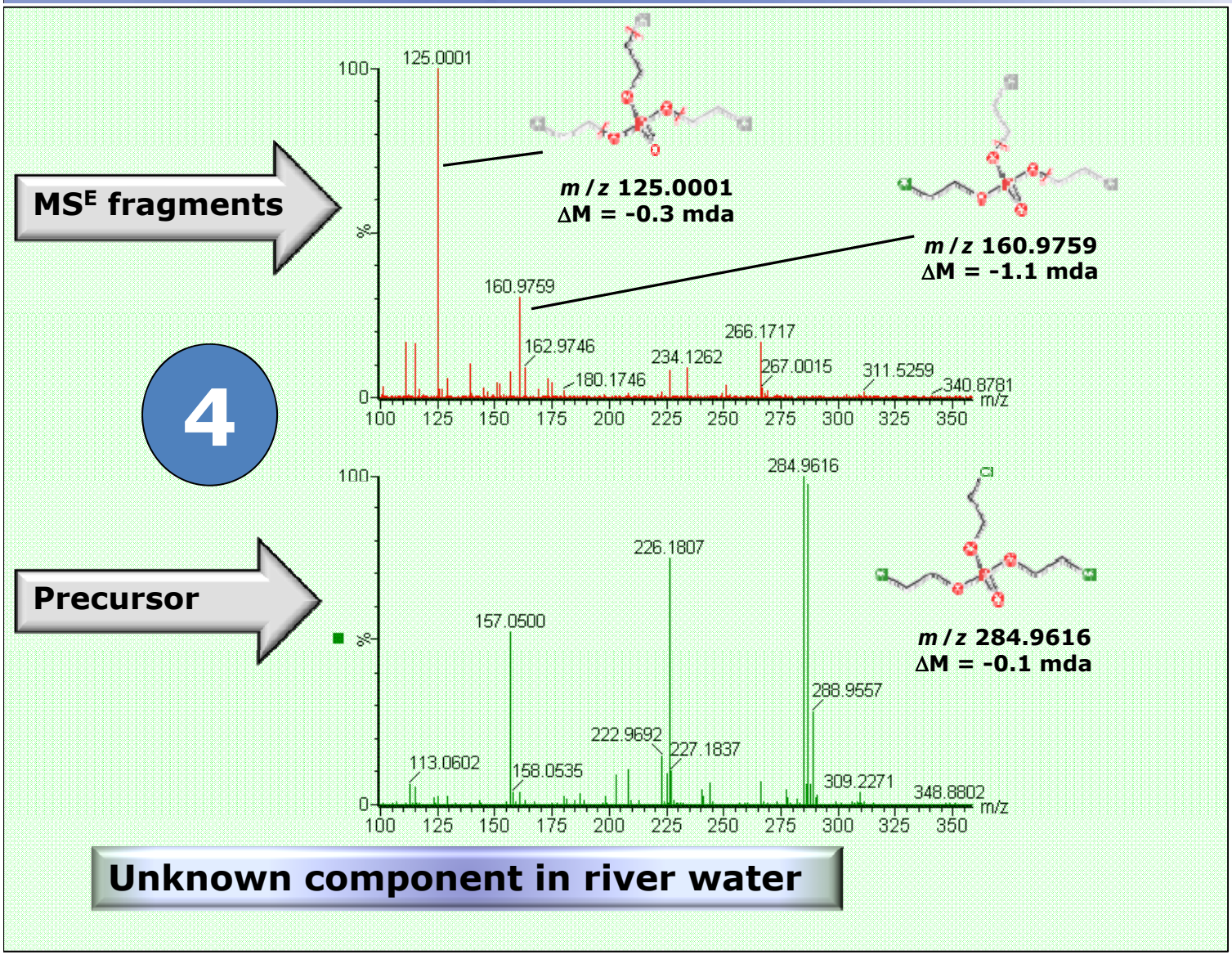

Non-Targeted Component Isotope Model Comparison

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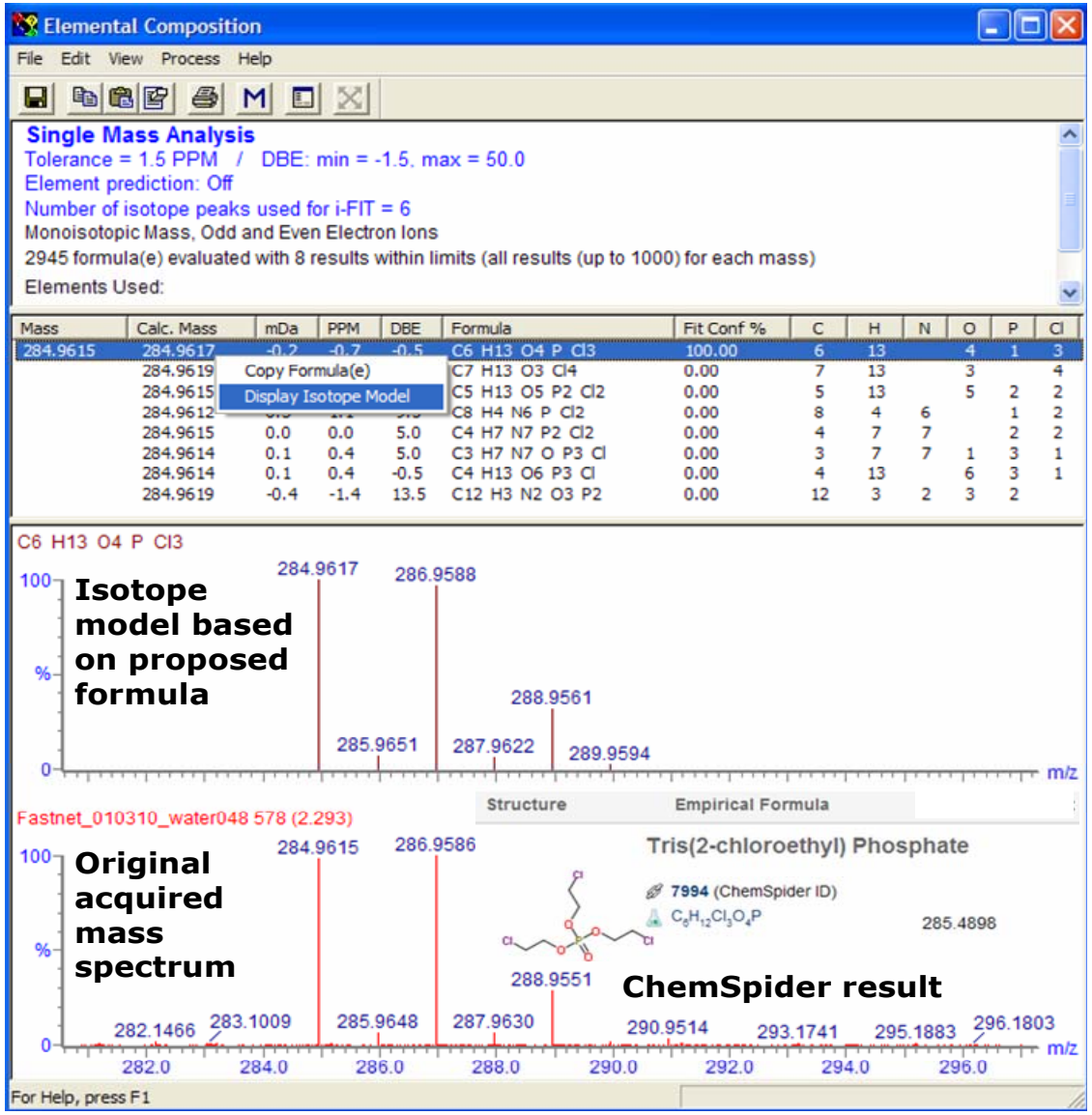

Non-Targeted Components
Comparison with control sample

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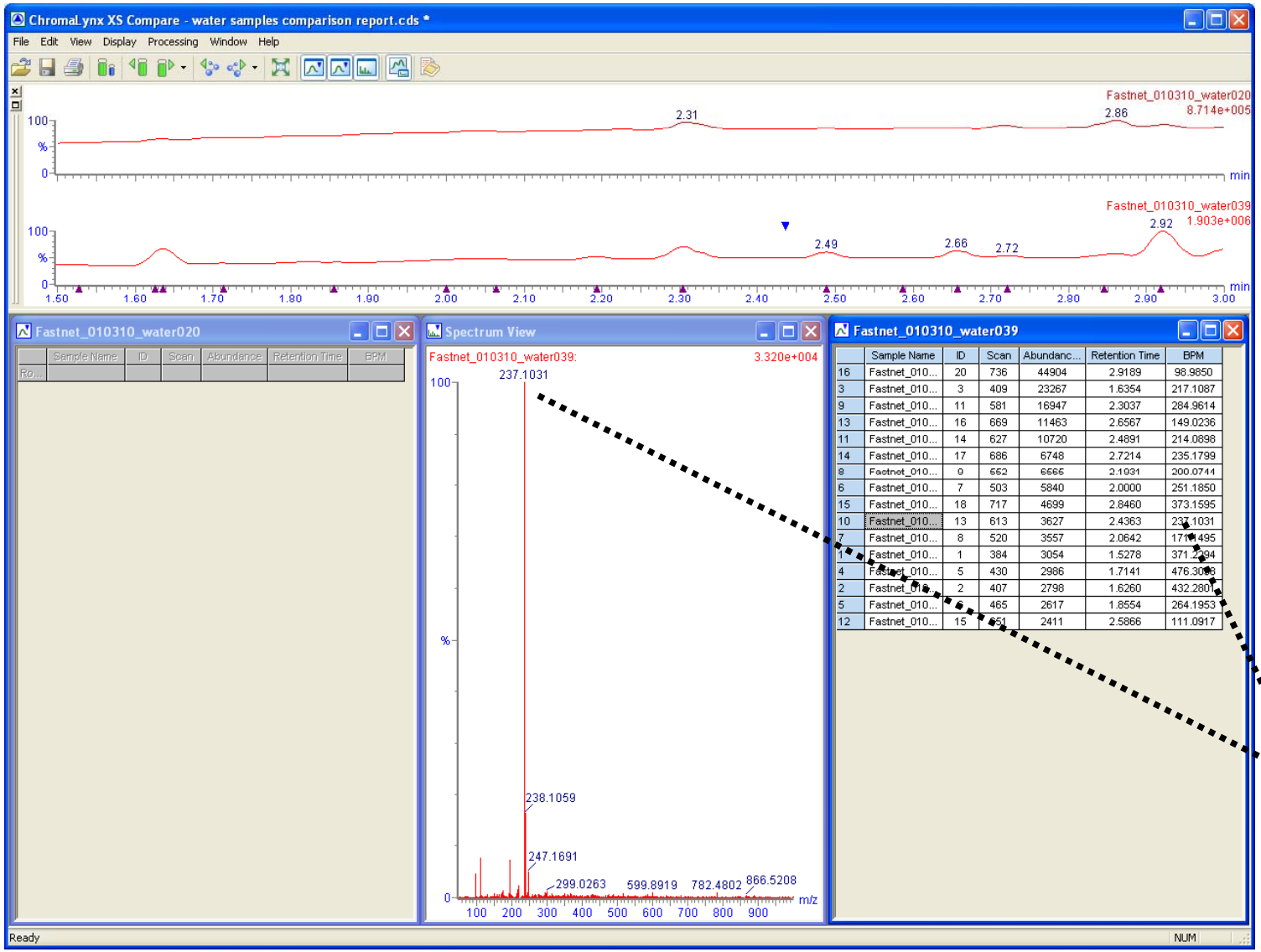
1



Another unusual compound not detected in the control

Non-Targeted Component
Emerging contaminants in river water

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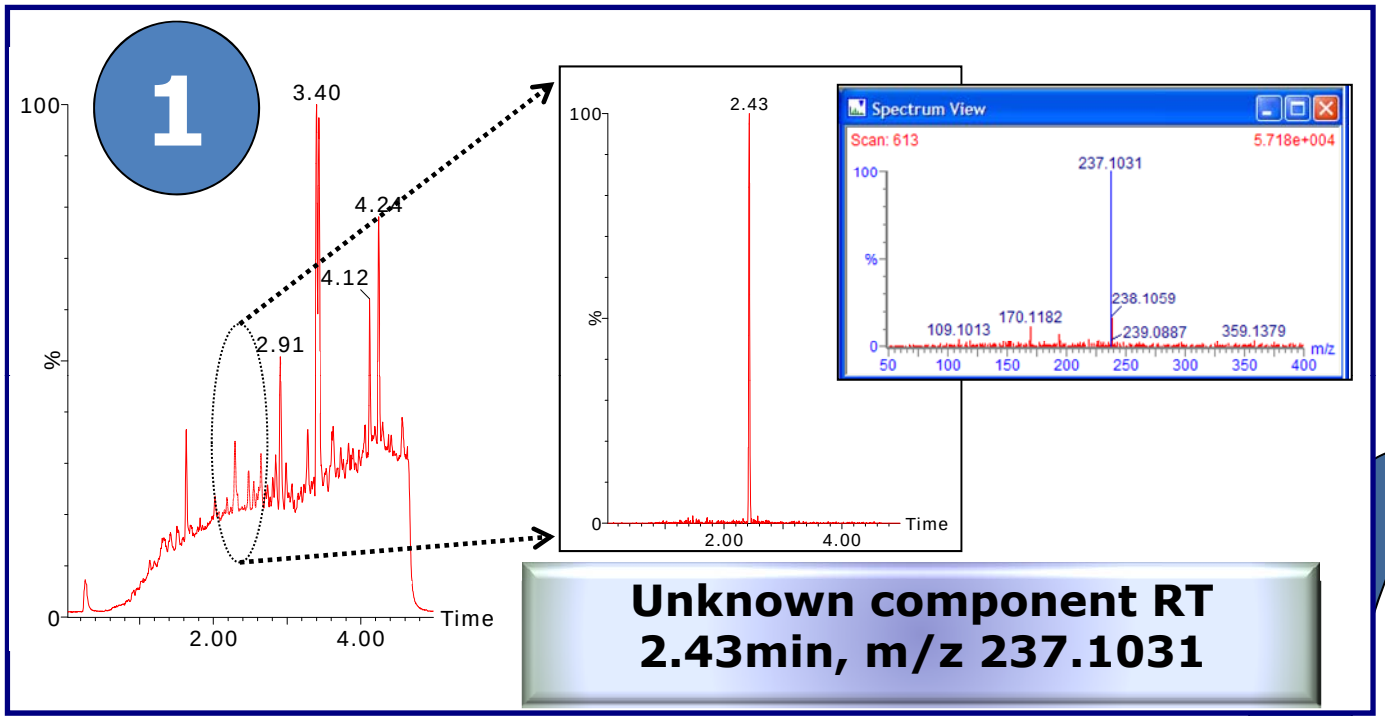

2

Elecomp and i-Fit proposed $C_{15}H_{13}N_2O$ as candidate

3

ChemSpider proposed Carbamazepine

Non-Targeted Component MassFragment and MS^E

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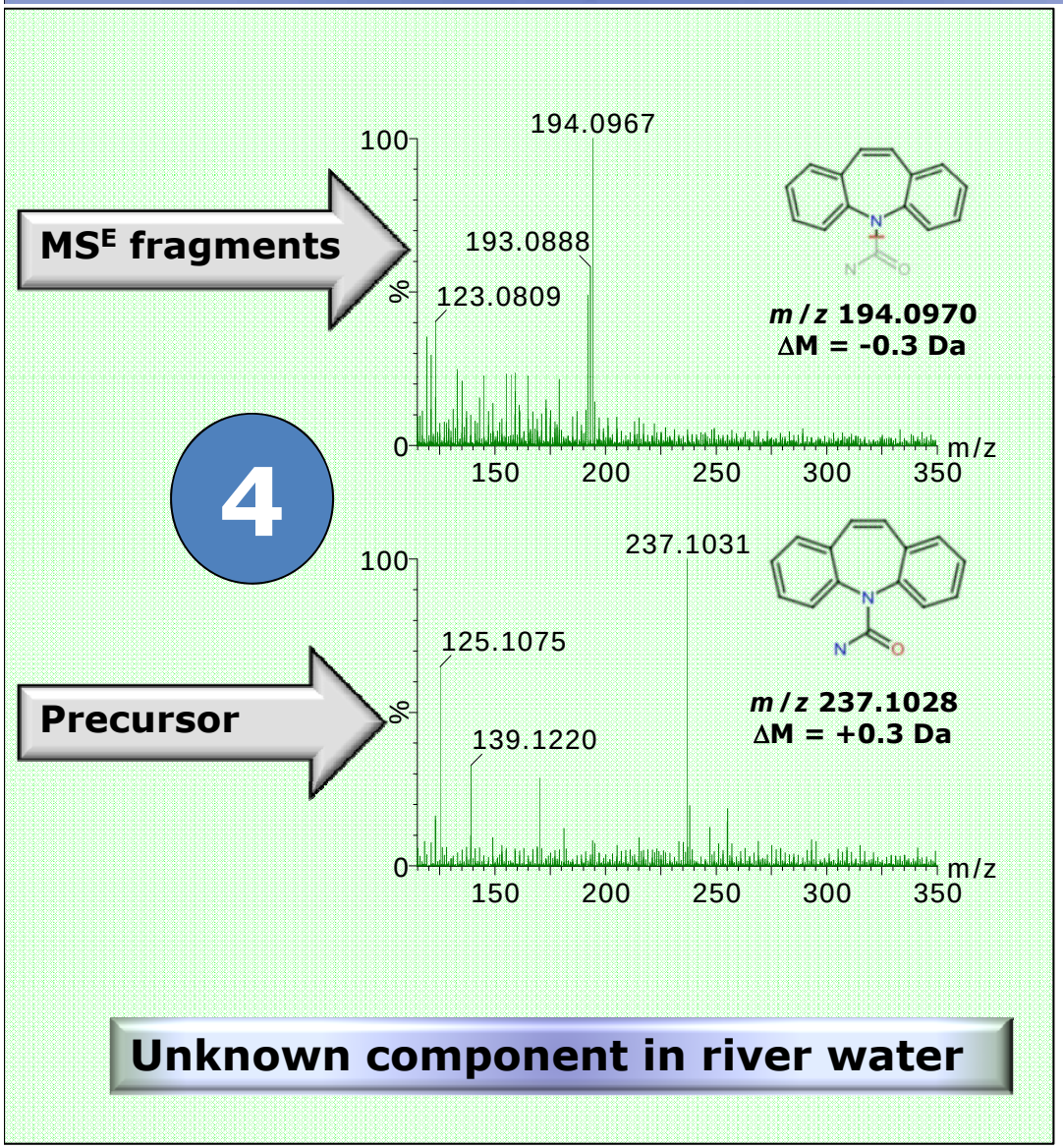

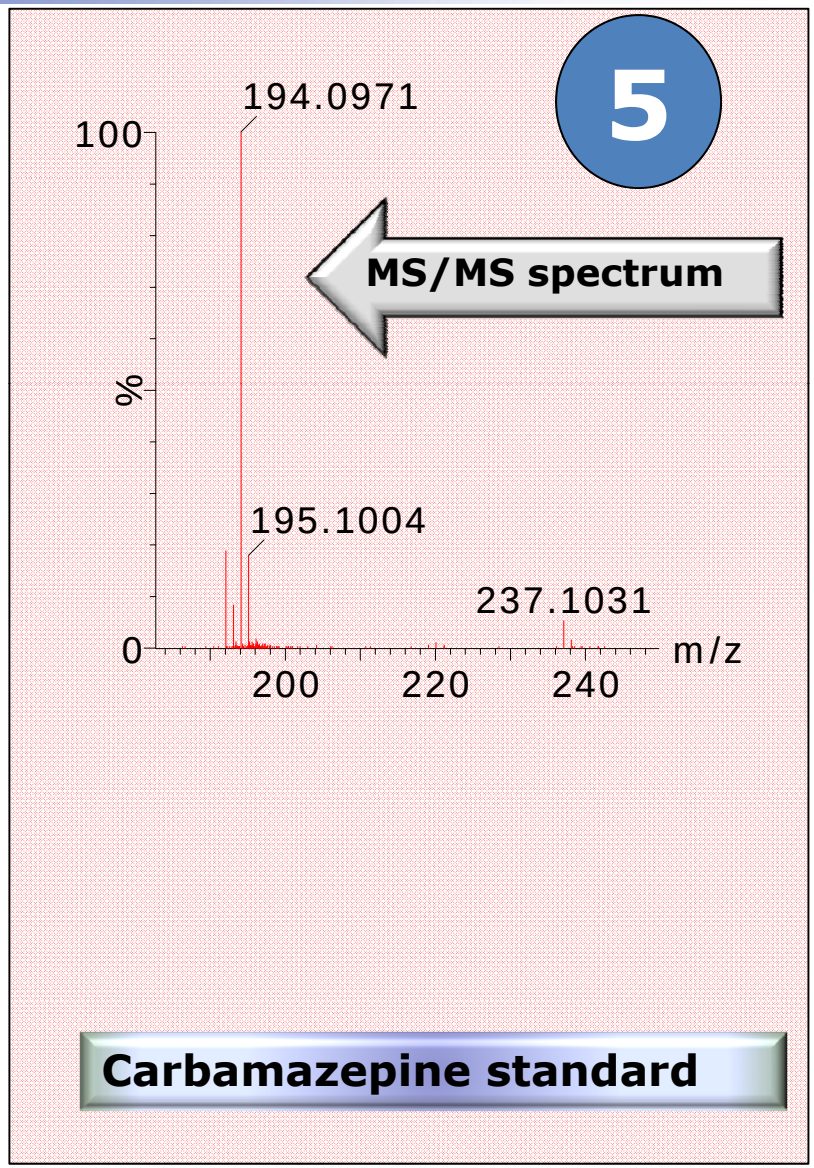

Conclusions

- Compounds as diverse as the anticonvulsant mood stabilizing drug carbamazepine, the analgesic compound codeine and the flame retardant tris(2-chloroethyl) phosphate were discovered in the river water blank using this non-targeted structural elucidation workflow approach.
- The MS^E functionality enables the acquisition of both low energy (precursor ion) and high energy (fragment ion) data in a single rapid screening run.
- MS^E fragment ion data can be used to help with unequivocal identification of detected compounds.
- The compare functionality, elemental composition, and mass fragment strategies in the workflow provide powerful tools for screening, detection, and identification of true non-targeted species in environmental matrices.

Many Thanks

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QUESTIONS?

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