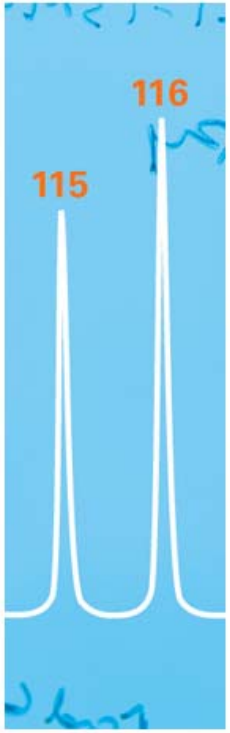


Using LC-MS/MS and Advanced Software Tools to Screen for Unknown and Non-targeted PPCP in Environmental Samples

Stacy Tremintin and André Schreiber
AB SCIEX

Outline

- Pharmaceuticals and Personal Care Products (PPCP), Endocrine Disrupting Compounds (EDC), Pesticides
  - Screening for PPCP in water samples
    - Comprehensive screening using Multi-MRM methods
    - Direct injection of water with LOD < 10ppt
    - Hybrid triple quadrupole linear ion trap systems (QTRAP®)
- Compound Identification
  - MRM ratio
  - MS/MS library searching
  - Multi-Target Screening (MTS) and Quantitation
- General Unknown Screening (GUS)
  - Non-targeted peak finding algorithm
  - Statistical data analysis (Principal Components Analysis PCA)
  - Automatic data reporting

LC-MS/MS analysis of EDC and PPCP

- Screening for hundreds or possibly thousands of compounds covering a wide range of chemical properties in environmental samples (water, soil, biota…)
- Minimum sample cleanup without time consuming derivatization and generic LC setup
- LC-MS/MS ideal for analysis of medium polar, polar and ionic compounds in trace levels
- Positive and negative ionization with different ion sources (ESI, APCI, APPI)
- Quantitation and identification at ng/L (ppt) levels
- Screening for unknown environmental pollutants

Screening strategies using LC-MS/MS

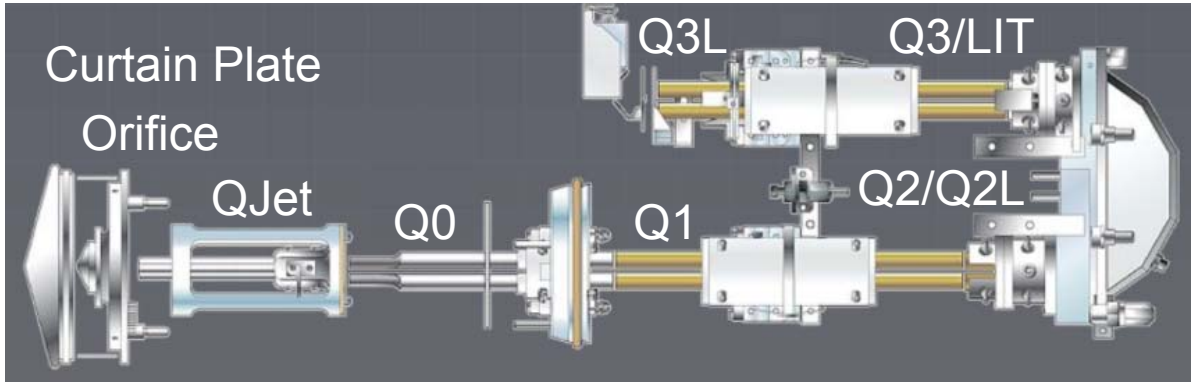
- Screening using full scan (high resolution) spectra lacks in selectivity and sensitivity causing a high risk of false positive and false negative results
- Fragment ions are needed for identification
- 2 Multiple Reaction Monitoring transitions per compound
  - MRM ratio for identification [1]
  - Scheduled MRM for extended screening [3-4]
- Information Dependent Acquisition (IDA) of MS/MS
  - Multi Target Screening: MRM to trigger MS/MS [2]
  - Unknown Screening: Full scan MS to trigger MS/MS [5]

[1] Christopher Borton et al.: at ASMS conference (2006) Seattle, WA
[2] Jim Krol et al.: at AOAC annual meeting (2007) Anaheim, CA
[3] André Schreiber et al.: at ASMS conference (2008) Denver, CO
[4] Rolf Kern et al.: at Pittcon (2009) Chicago, IL
[5] André Schreiber et al.: at LC/MS/MS workshop (2010) Barcelona, Spain

Experimental

- Sample preparation
  - Direct injection of drinking water samples
  - SPE cleanup of waste water and sea water
- HPLC separation
  - Shimadzu Prominence UFLC XR system
  - Different RP columns with fast gradients of water/methanol and volatile buffer, such as (ammonium acetate, formate, formic acid)
- MS/MS detection
  - New QTRAP® 5500 LC-MS/MS system with Turbo V™ source and Electrospray Ionization (ESI) probe
  - MRM with *Scheduled* MRM™ algorithm for targeted quantitation
  - Enhanced MS (EMS) for non-target screening
  - Enhanced Product Ion (EPI) CE=35 with CES=15V for compound identification using MS/MS library searching

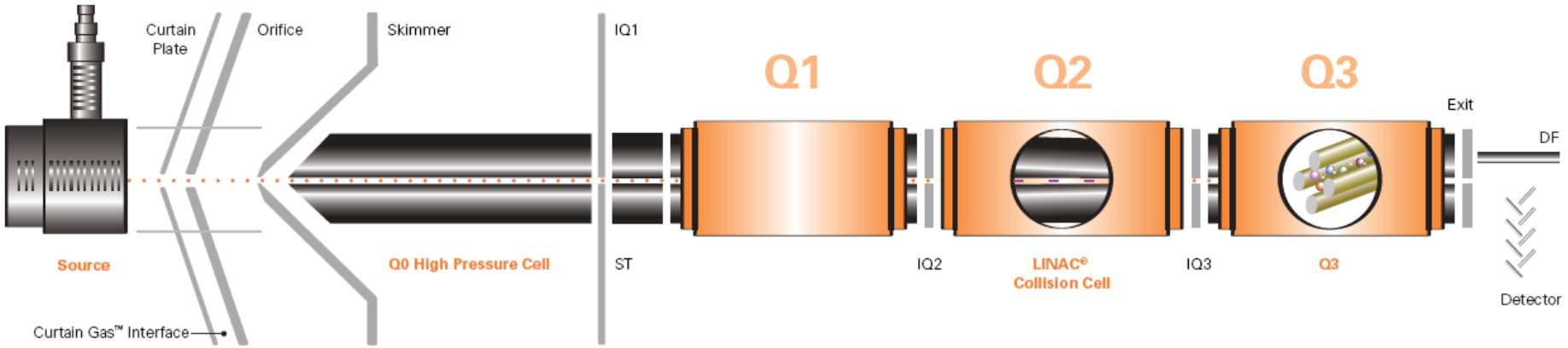
New Technology in the 5500 QTRAP® Systems



- TurboV™ source for highest ionization efficiency, ruggedness and robustness with low carry-over
- Ceramic interface and QJet® 2 ion guide for best ion transmission
- Space saving design of collision cell with Linear Accelerator fields
- Faster electronics for fast MRM (2ms dwell time) and polarity switching in 50ms without loss in S/N
- Linear Accelerator Q3 for fastest QTRAP® scanning (20,000Da/s)

Multi Target Screening and Quantitation

MRM offers highest Selectivity and Sensitivity



Ion production

Ion filtering

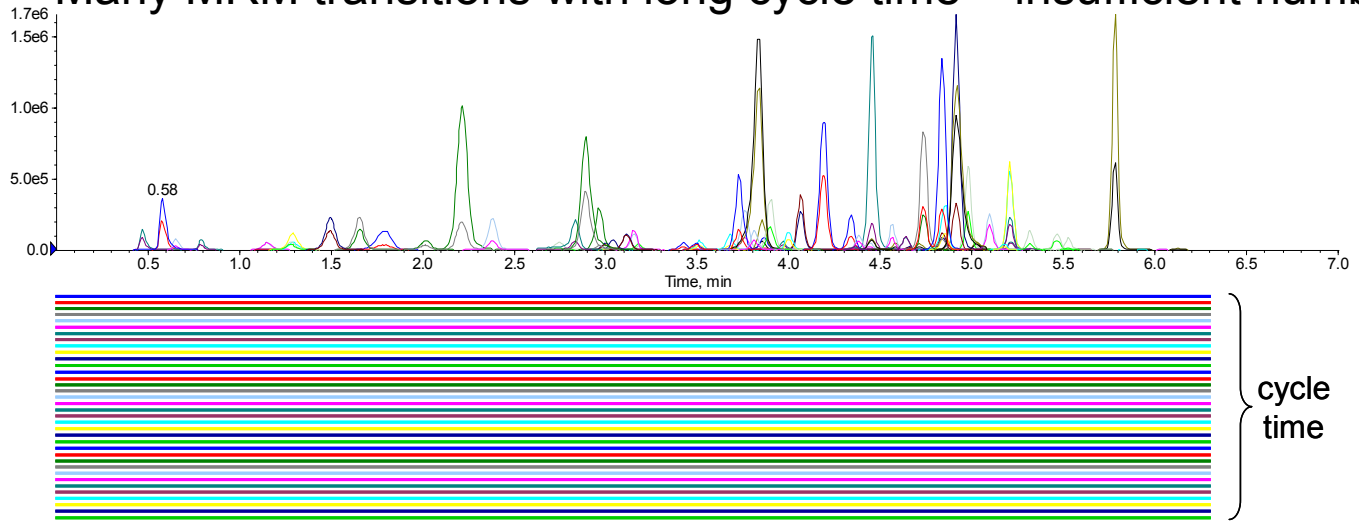
Ion filtering

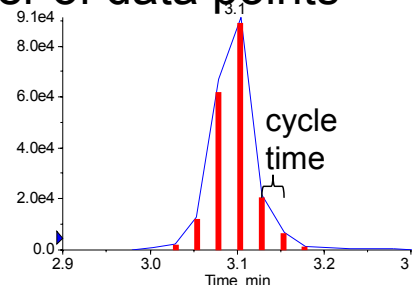
Ion transport

Fragmentation

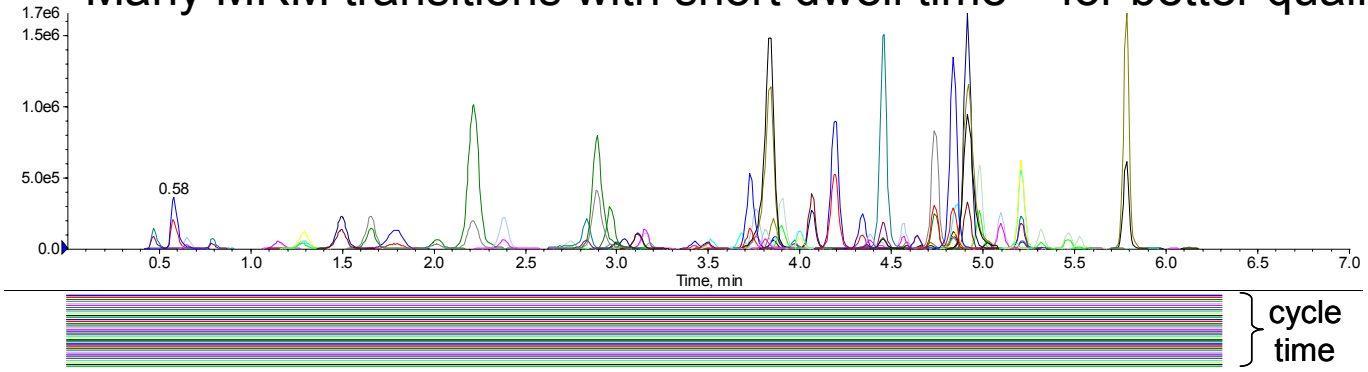
MRM in Multi-Target Analysis

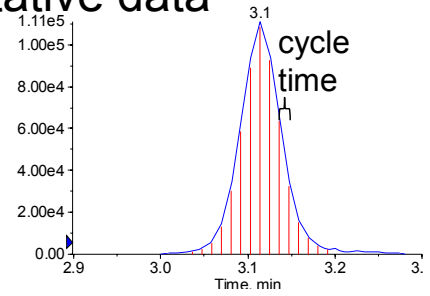
Many MRM transitions with long cycle time – insufficient number of data points



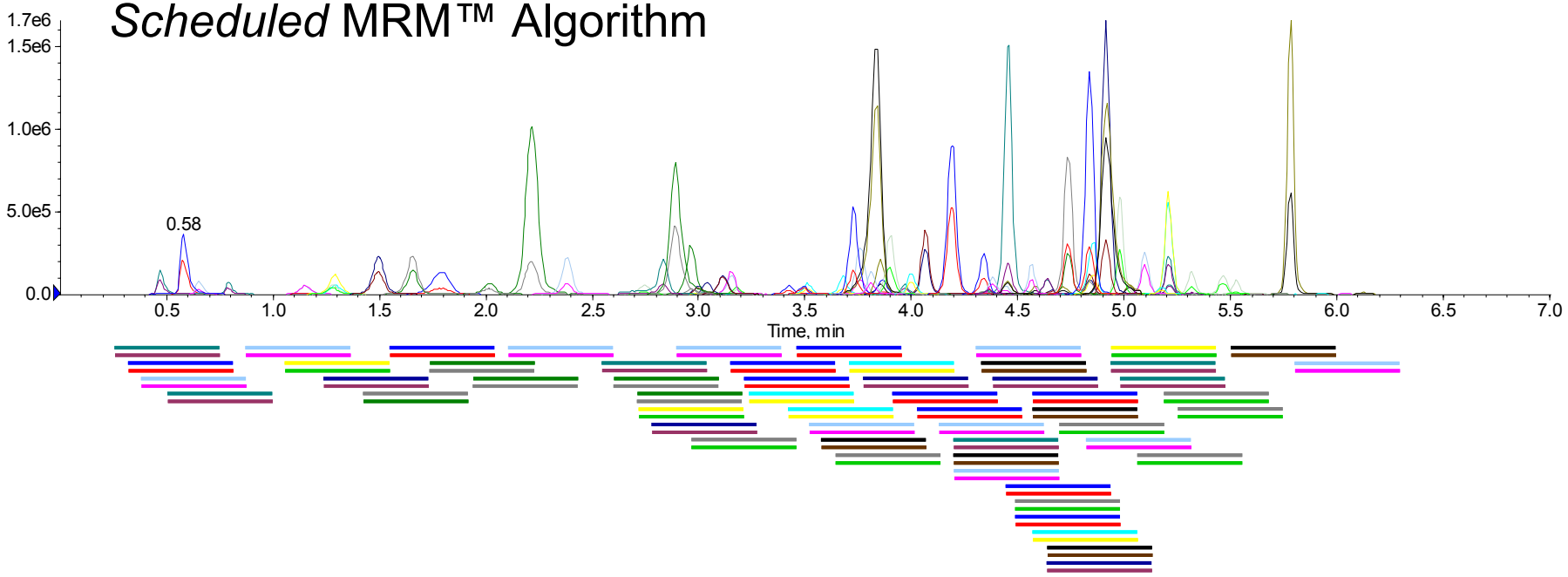


Many MRM transitions with short dwell time – for better qualitative data





The Key for Multi-Target Analysis – Smart Software

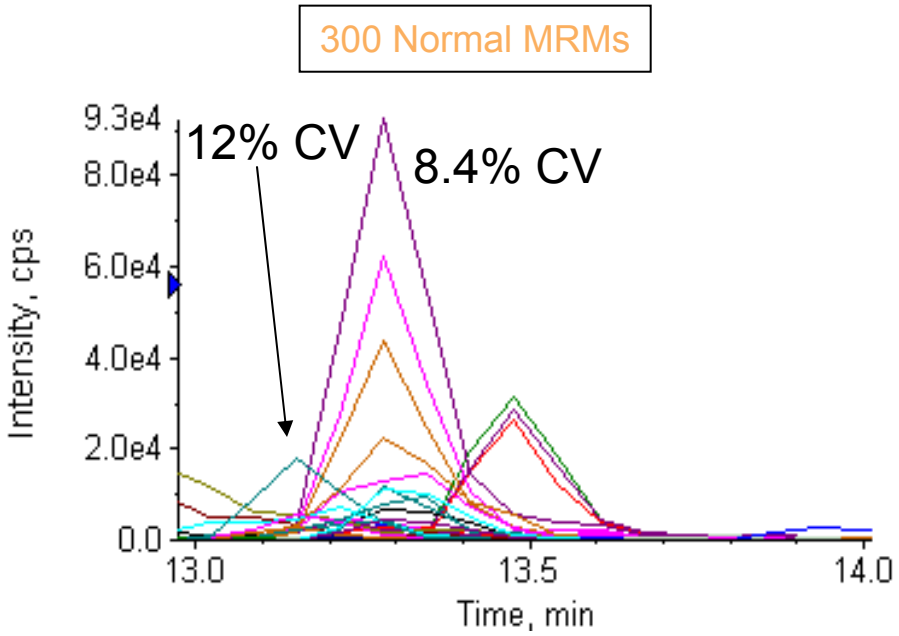


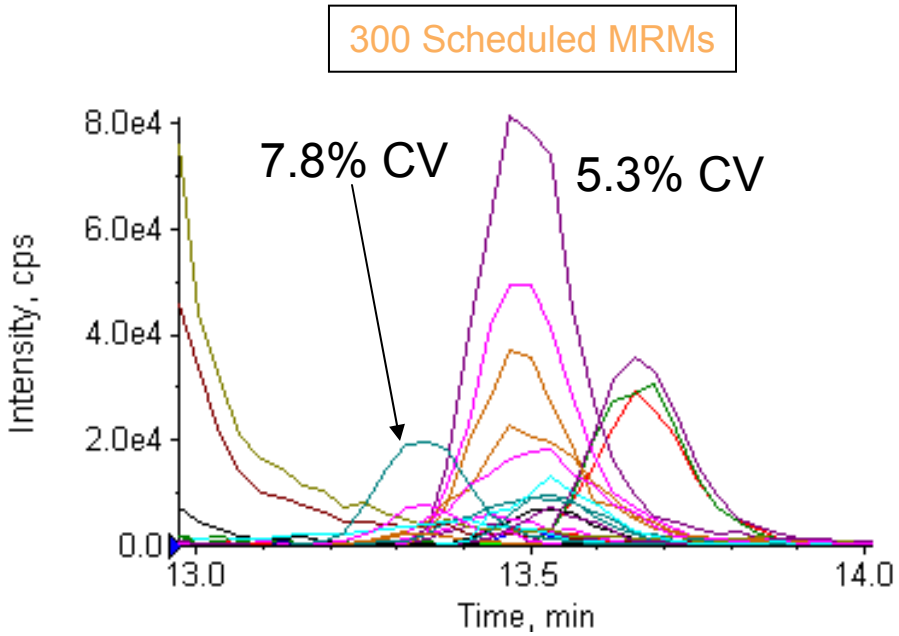
- Adjusts detection windows automatically depending on retention time
- Optimizes dwell times for each analyte and cycle time
  - → Allows detecting many more MRM transitions
  - → Allows using UHPLC

Maintaining Quantitative Accuracy

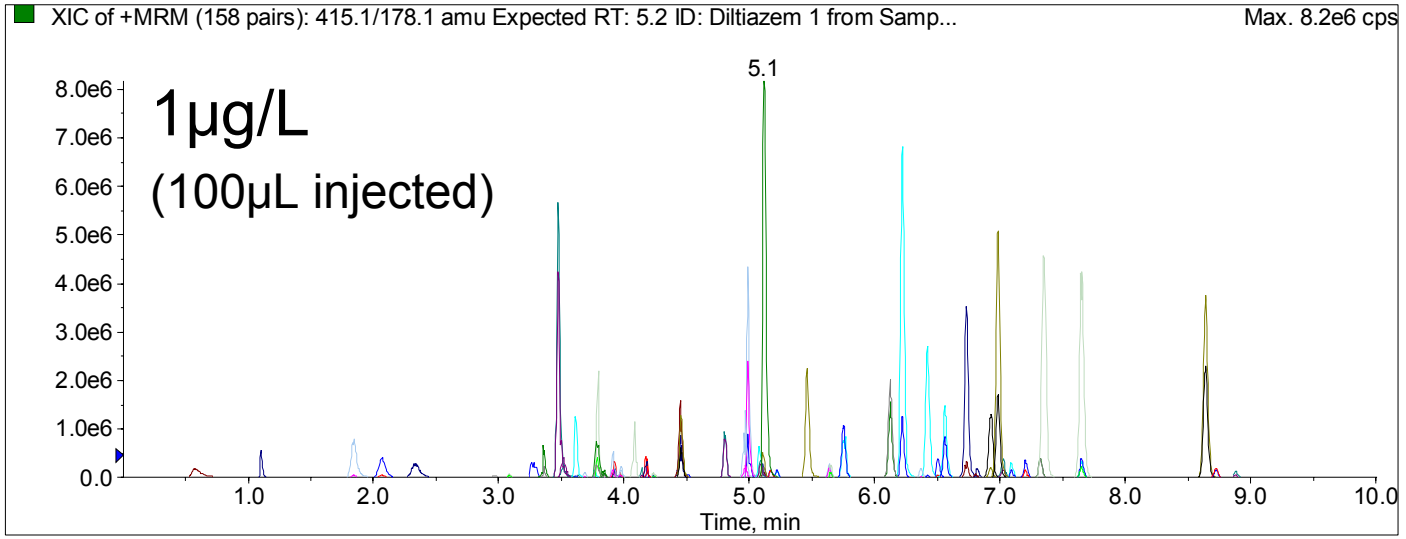
Maximizing Effective Duty Cycle

- Poor peak sampling makes robust peak integration very difficult (left)
- Proper cycle time provides good peak sampling and increases assay reproducibility



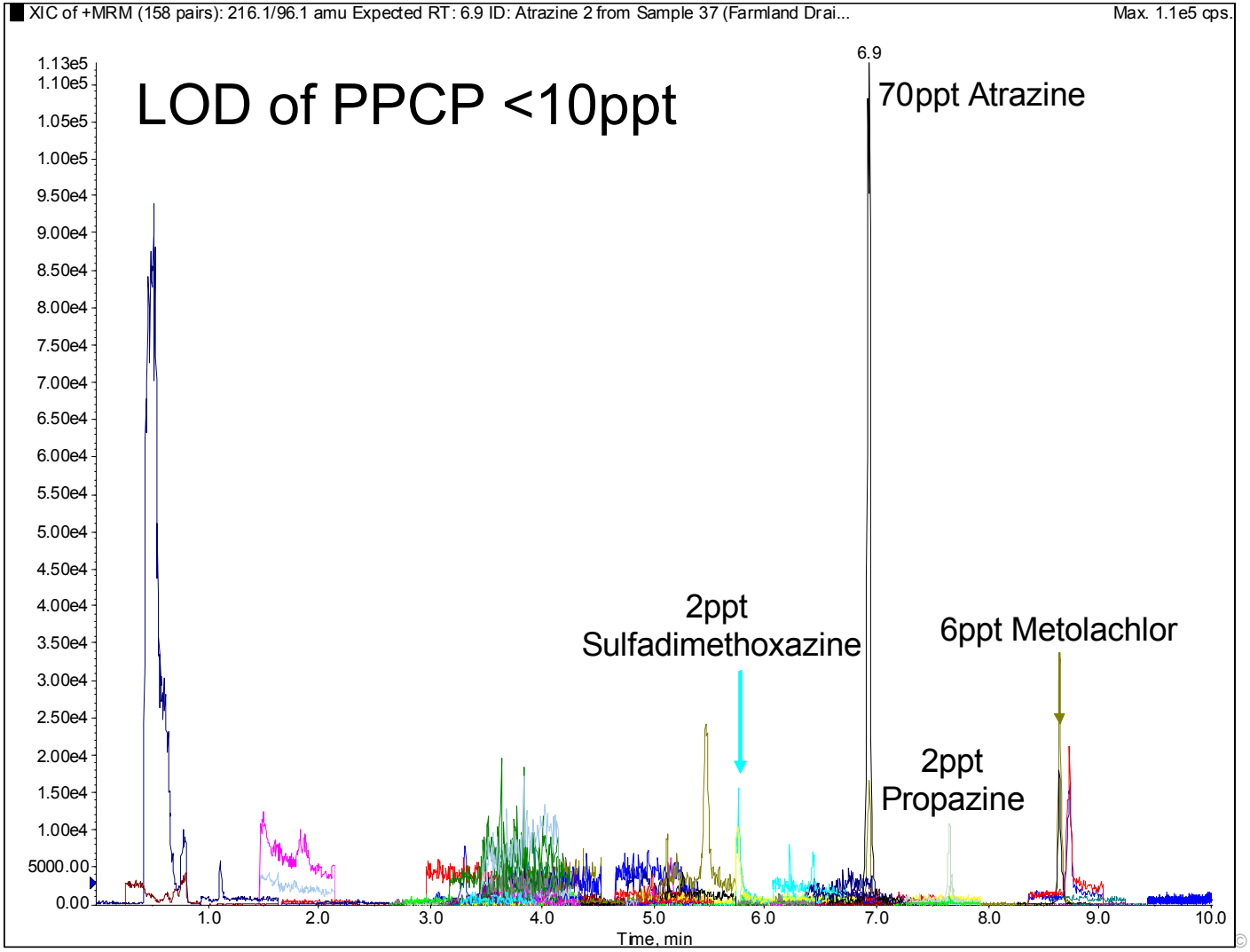


LC-MS/MS Screening – Direct Injection of Water

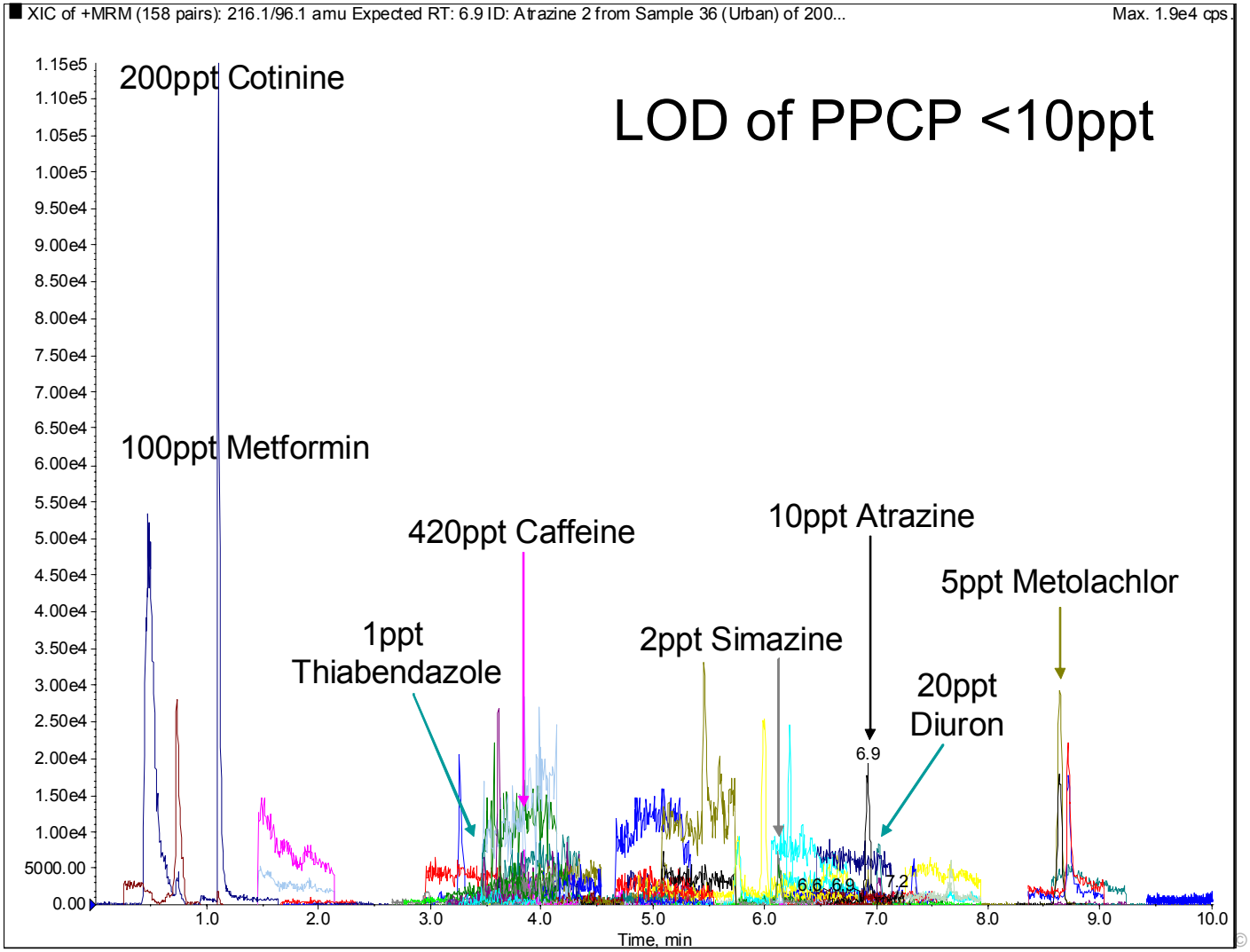


- 158 *Scheduled* MRM™ transitions to screen for PPCP (EPA 1694)
- Injection of 100µL into QTRAP® 5500 LC/MS/MS system
- Phenomenex Luna C18 HST (100x3mm 2.5µm) and gradient of water/acetonitrile with 0.1% formic acid

LC-MS/MS Screening – Farmland Drain Sample



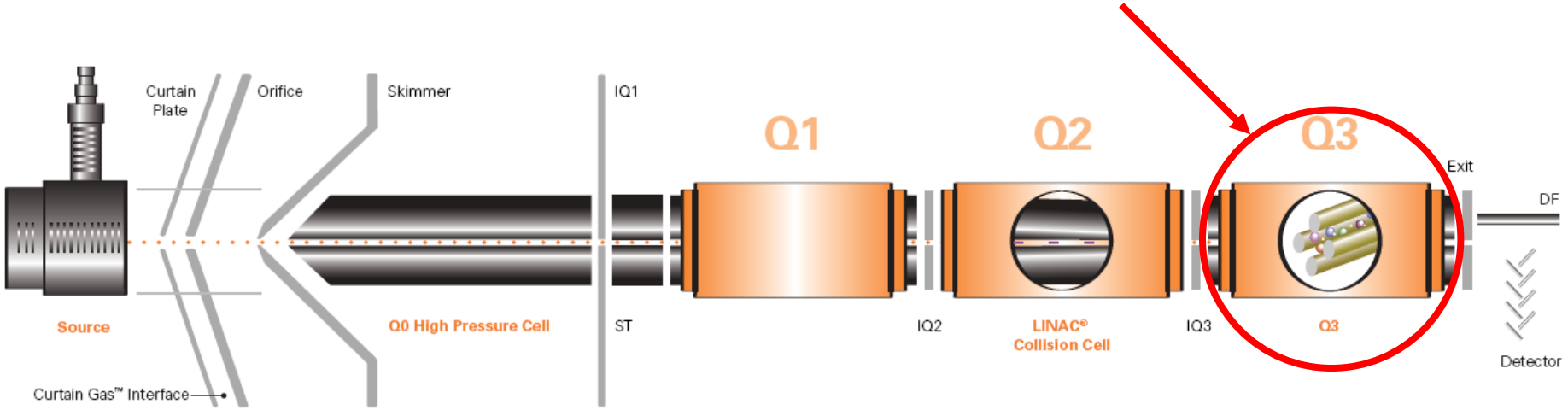
LC-MS/MS Screening – Urban Water Sample



Multi-Target Screening with MS/MS Library Searching

An Alternative to MRM Ratios – Full Scan MS/MS

Q3 of a QTRAP® System can be used as quadrupole or as linear ion trap



Ion trapping

Ion production

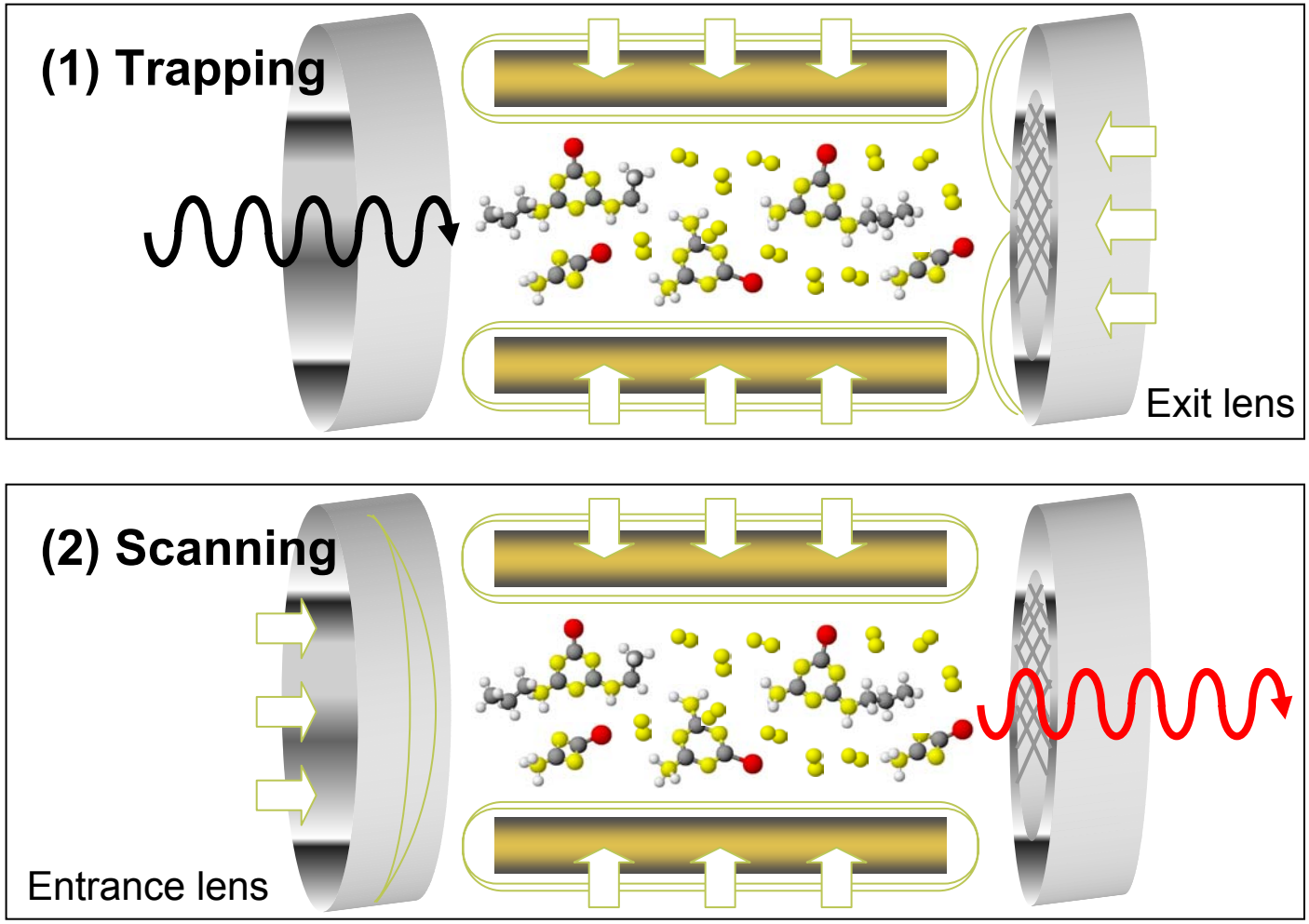
Ion filtering

Ion filtering

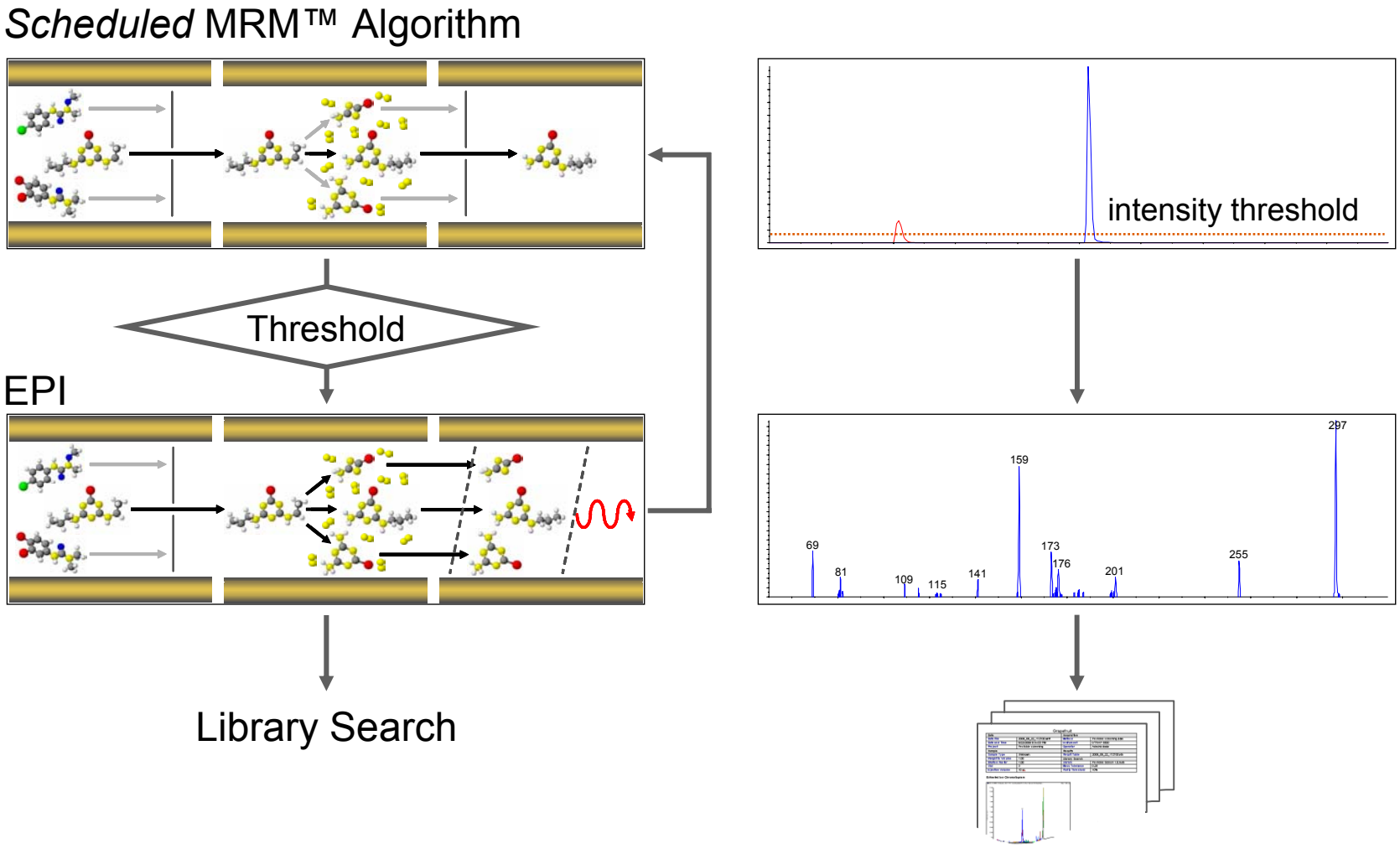
Ion transport

Fragmentation

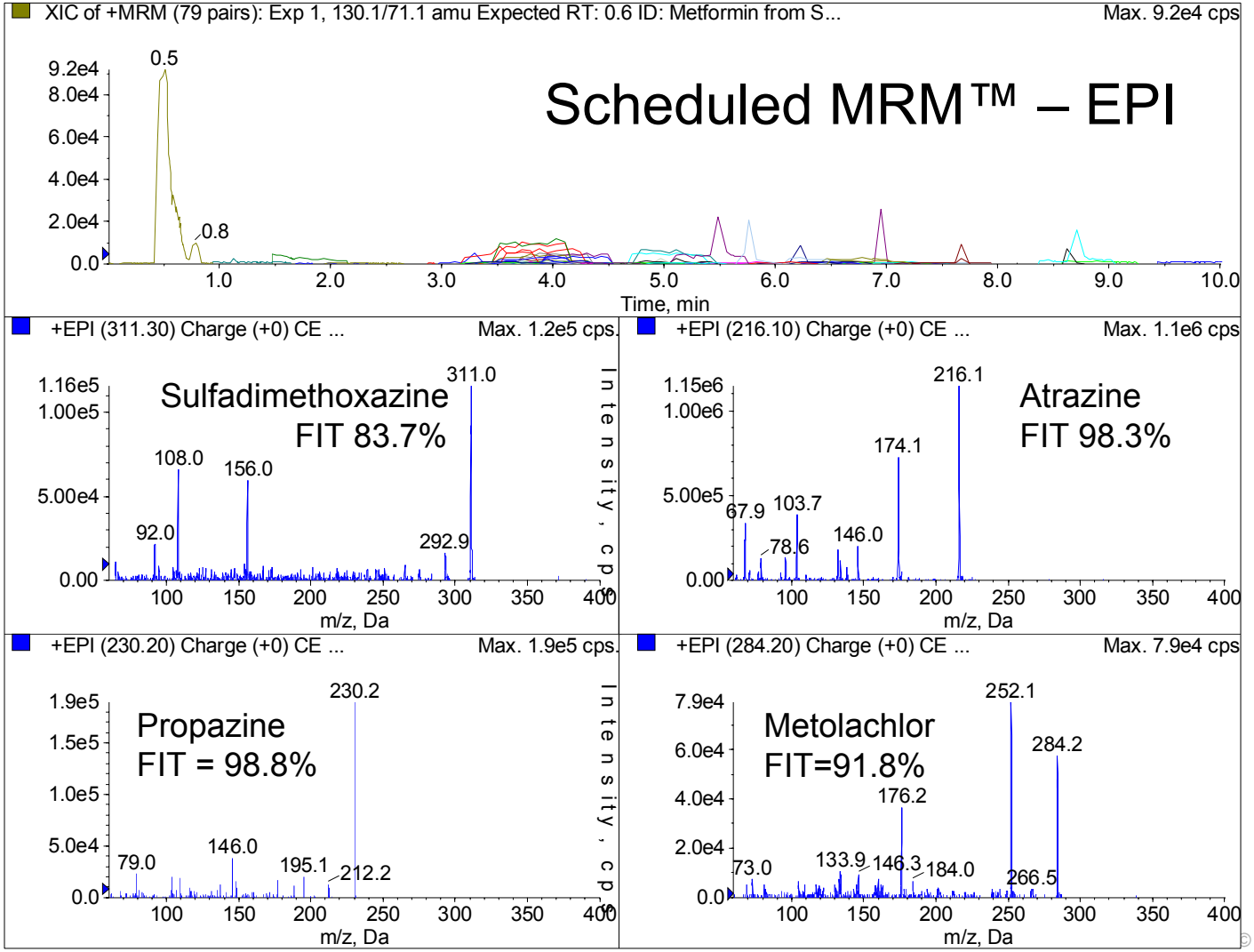
Trapping and Scanning in a Linear Ion Trap (Q3)



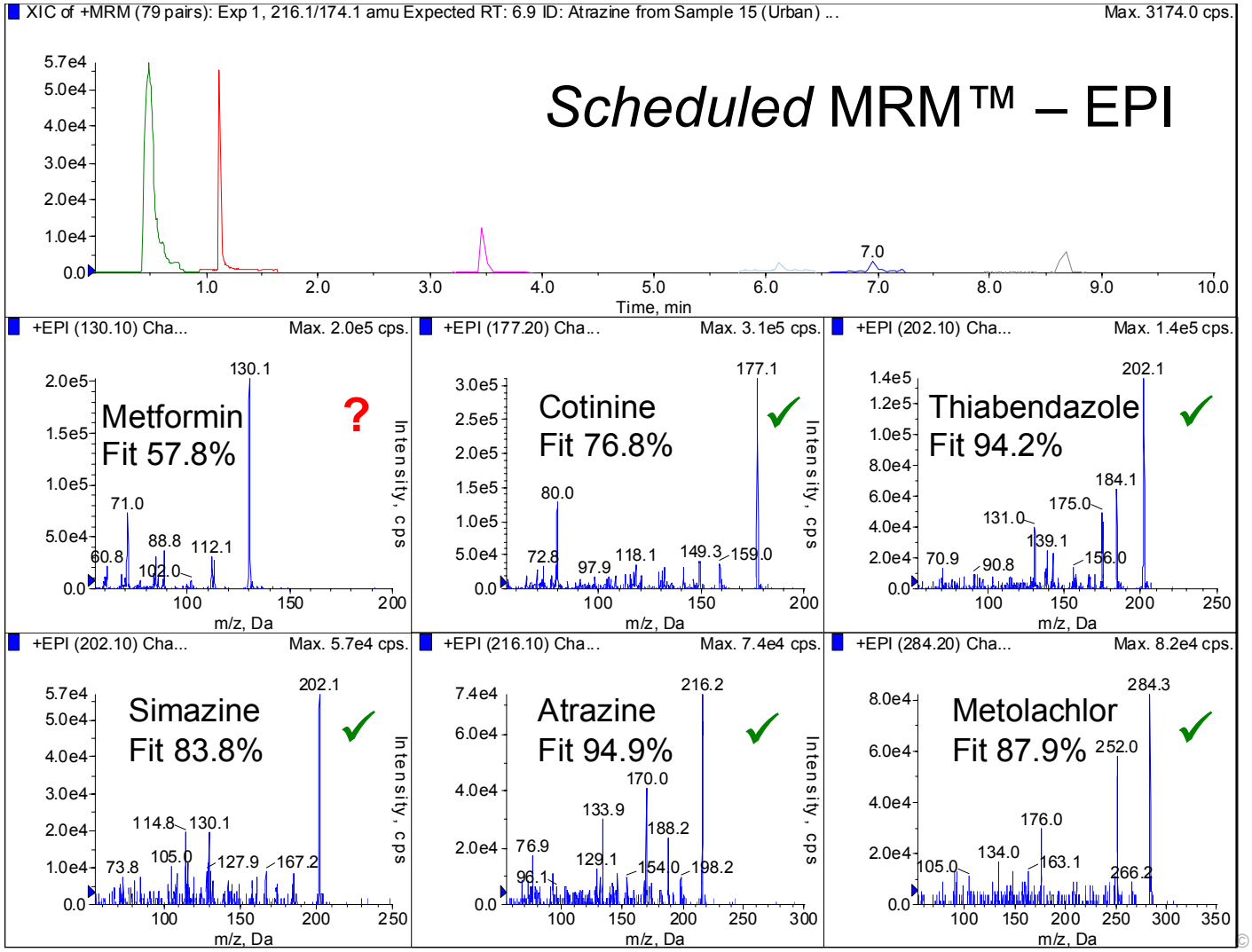
Multi-Target Screening with MS/MS Library Search



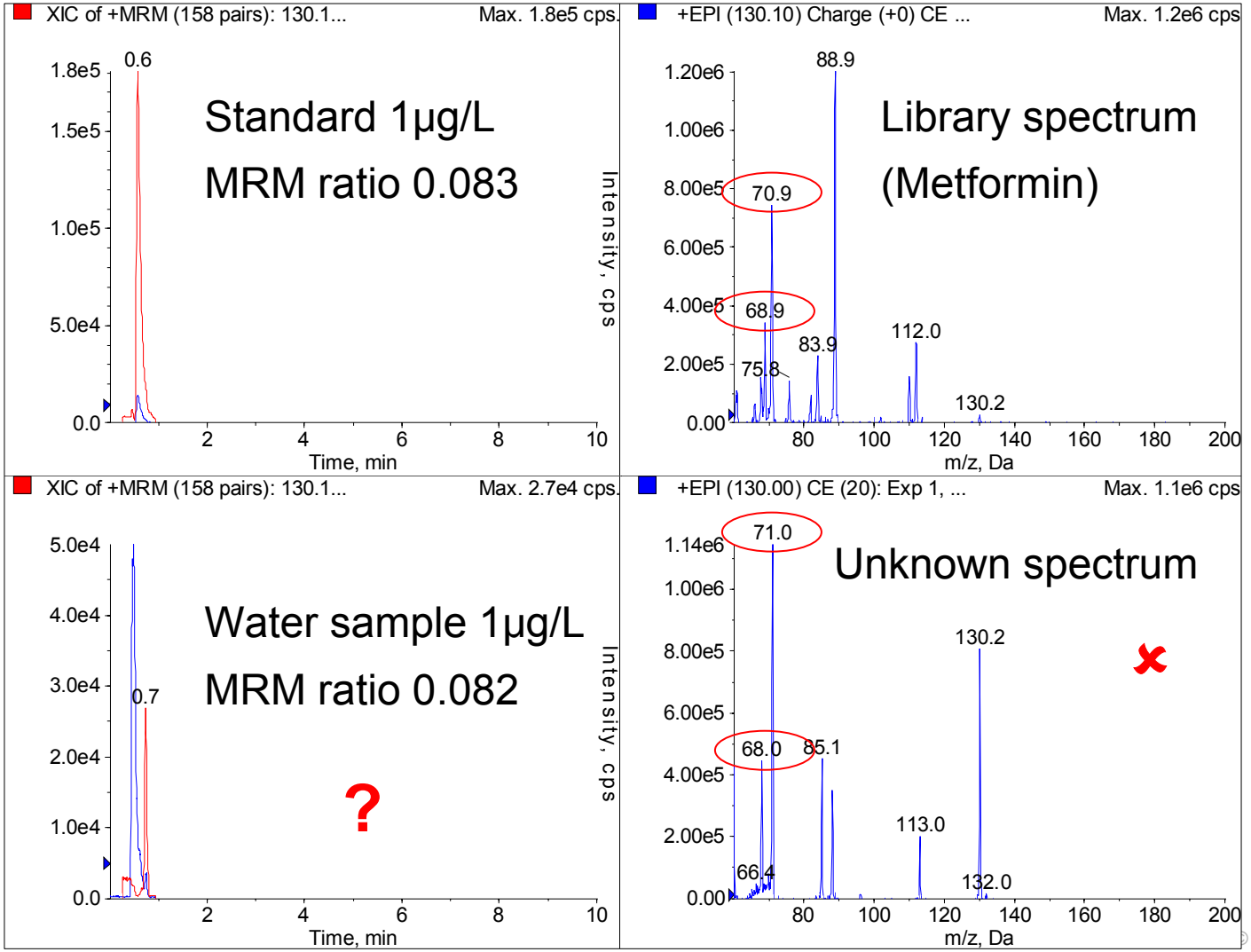
LC-MS/MS Screening – Farmland Drain Sample



LC-MS/MS Screening – Urban Water Sample

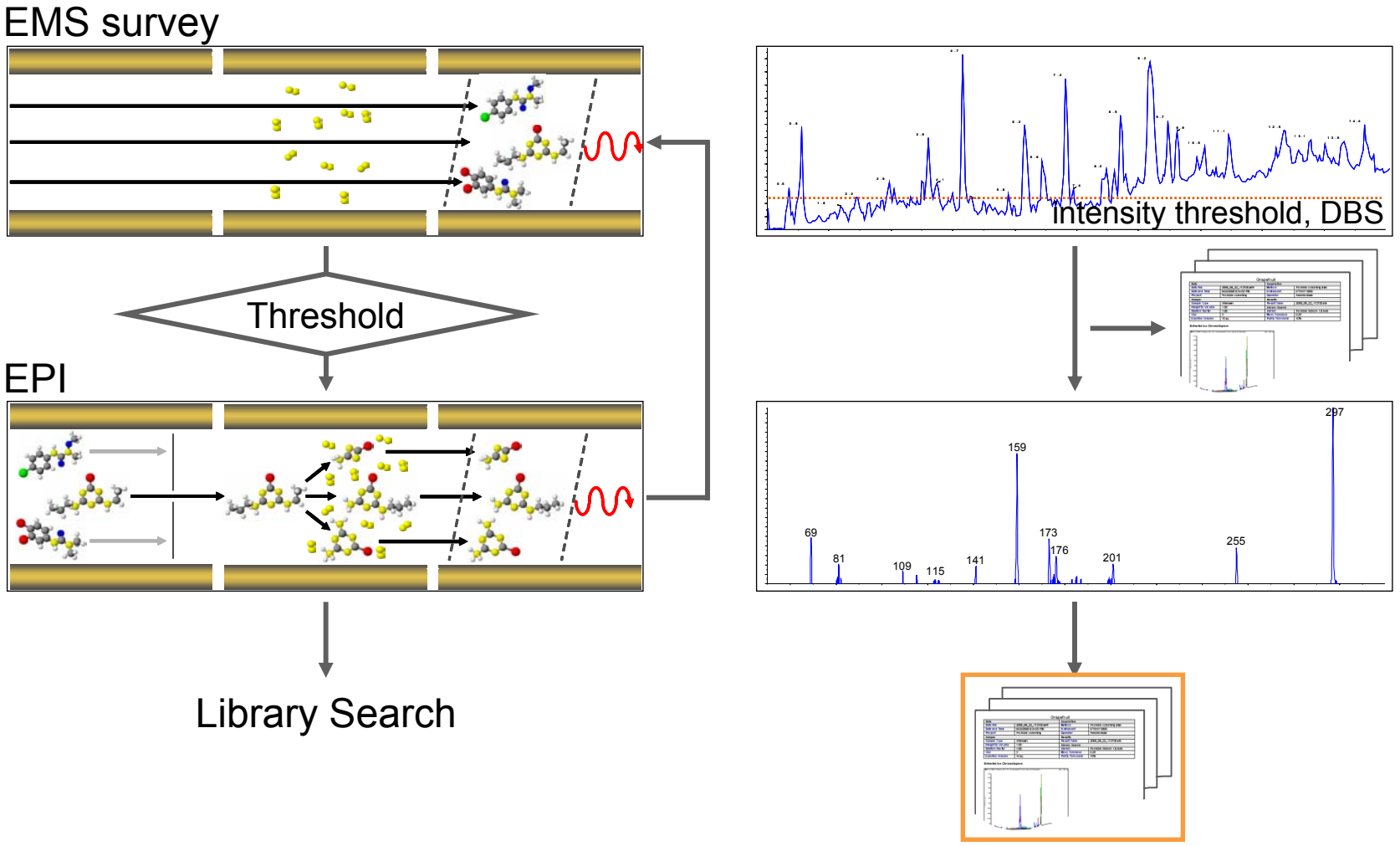


LC-MS/MS Screening – MRM vs. EPI Identification



General Unknown Screening and Quantitation

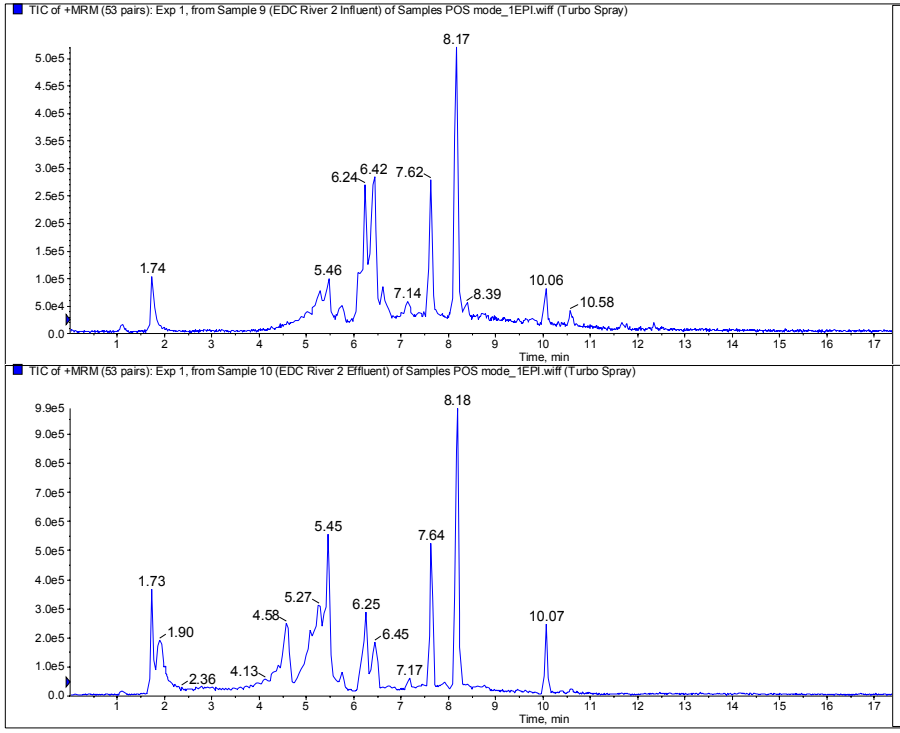
General Unknown Screening using a Non-Target Peak Finding Algorithm in Cliquid® Software



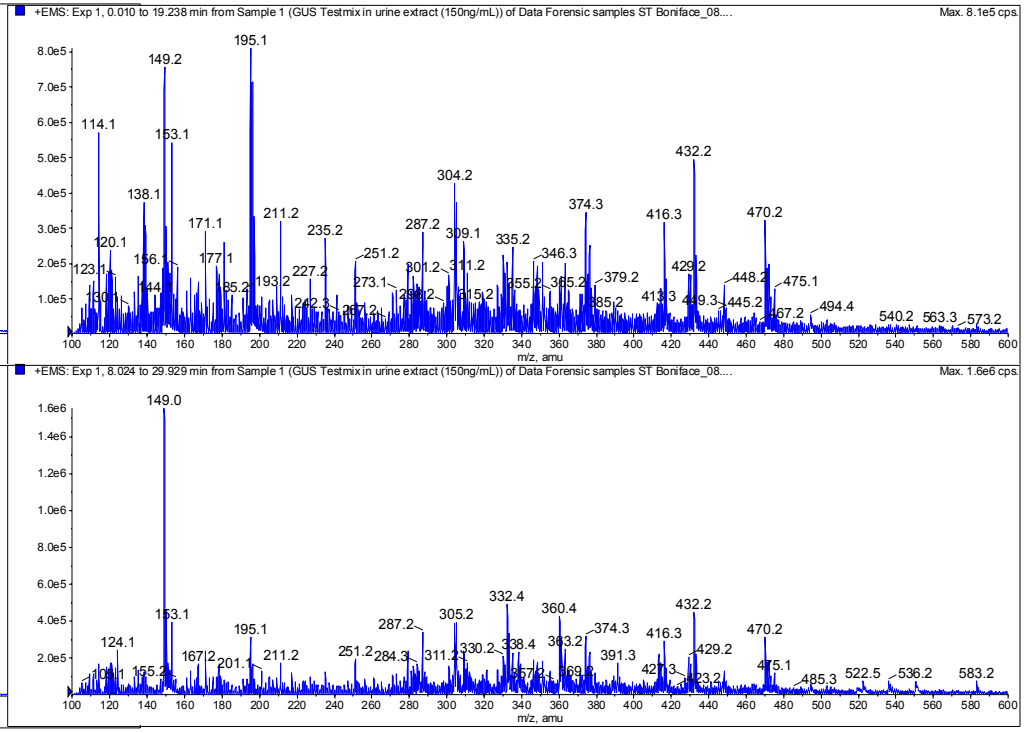
Comparison of chromatograms and mass spectra

2 unknown samples

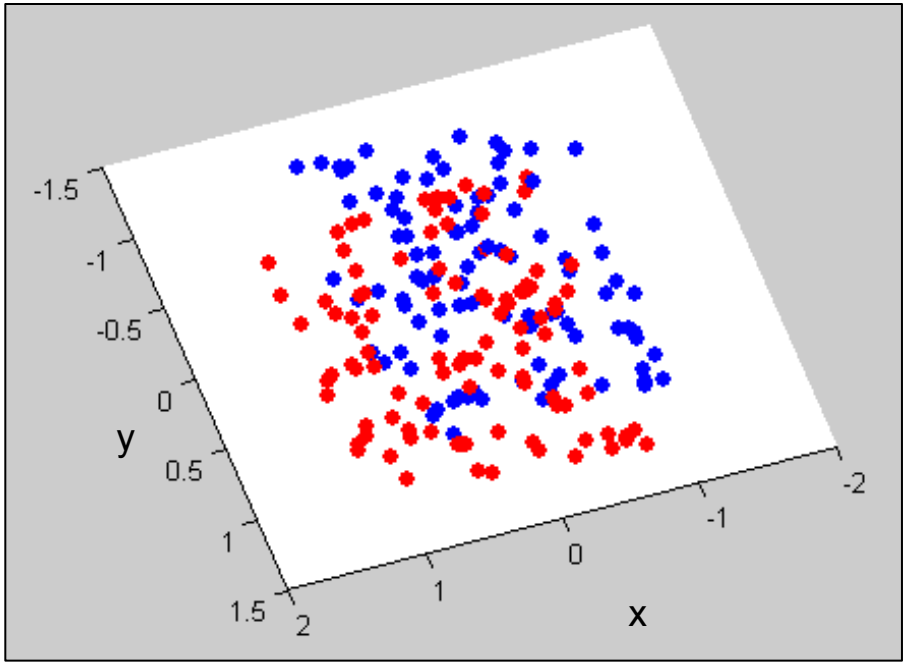
Chromatograms (TIC)



Mass spectra

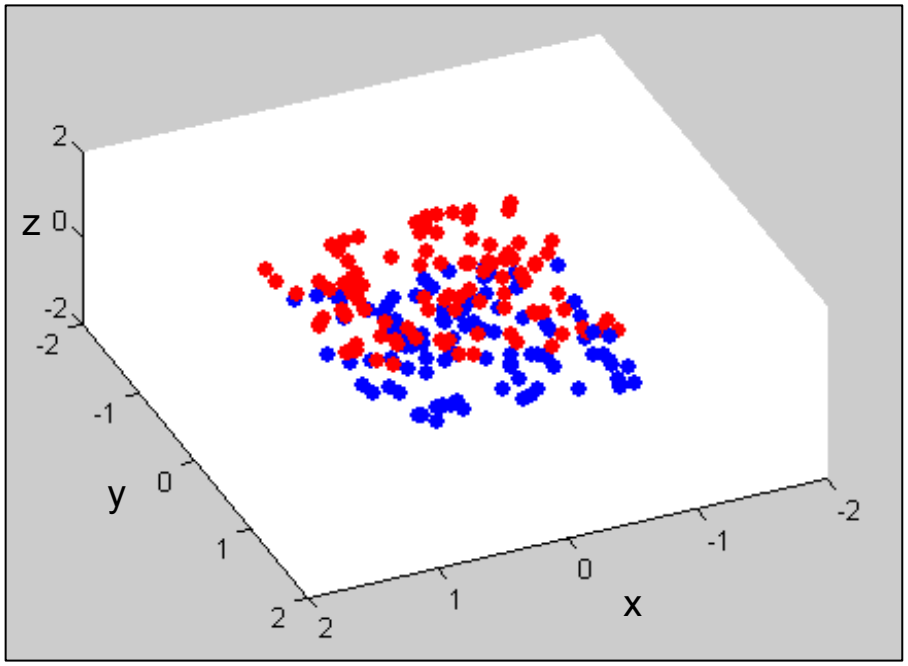


PCA Explanation 1



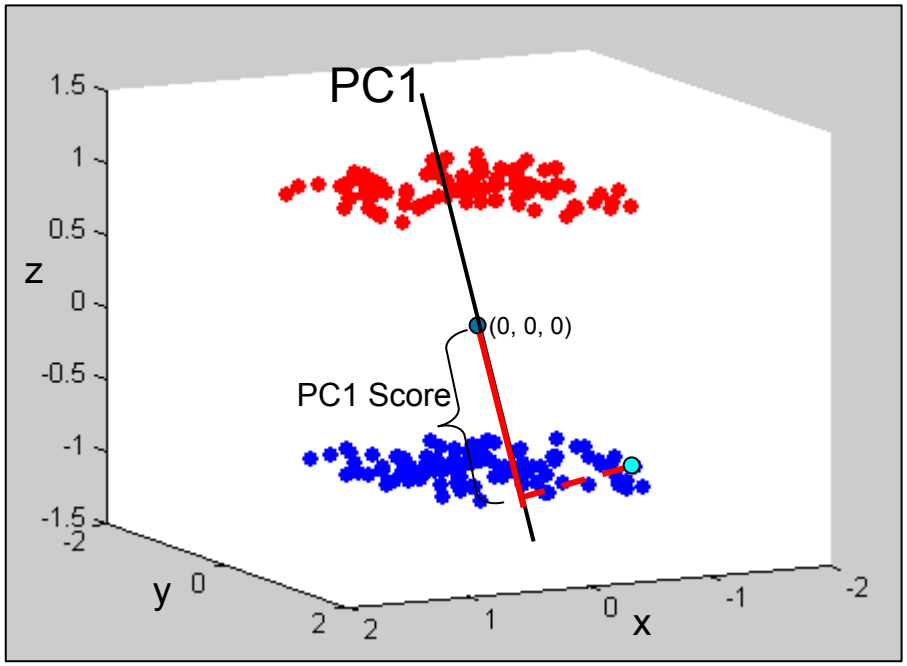
2 dimensional plot

PCA Explanation 2



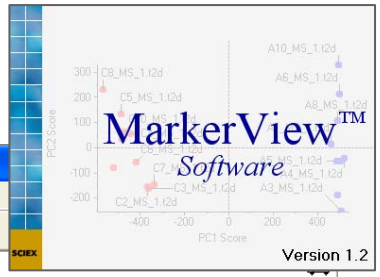
3 dimensional plot

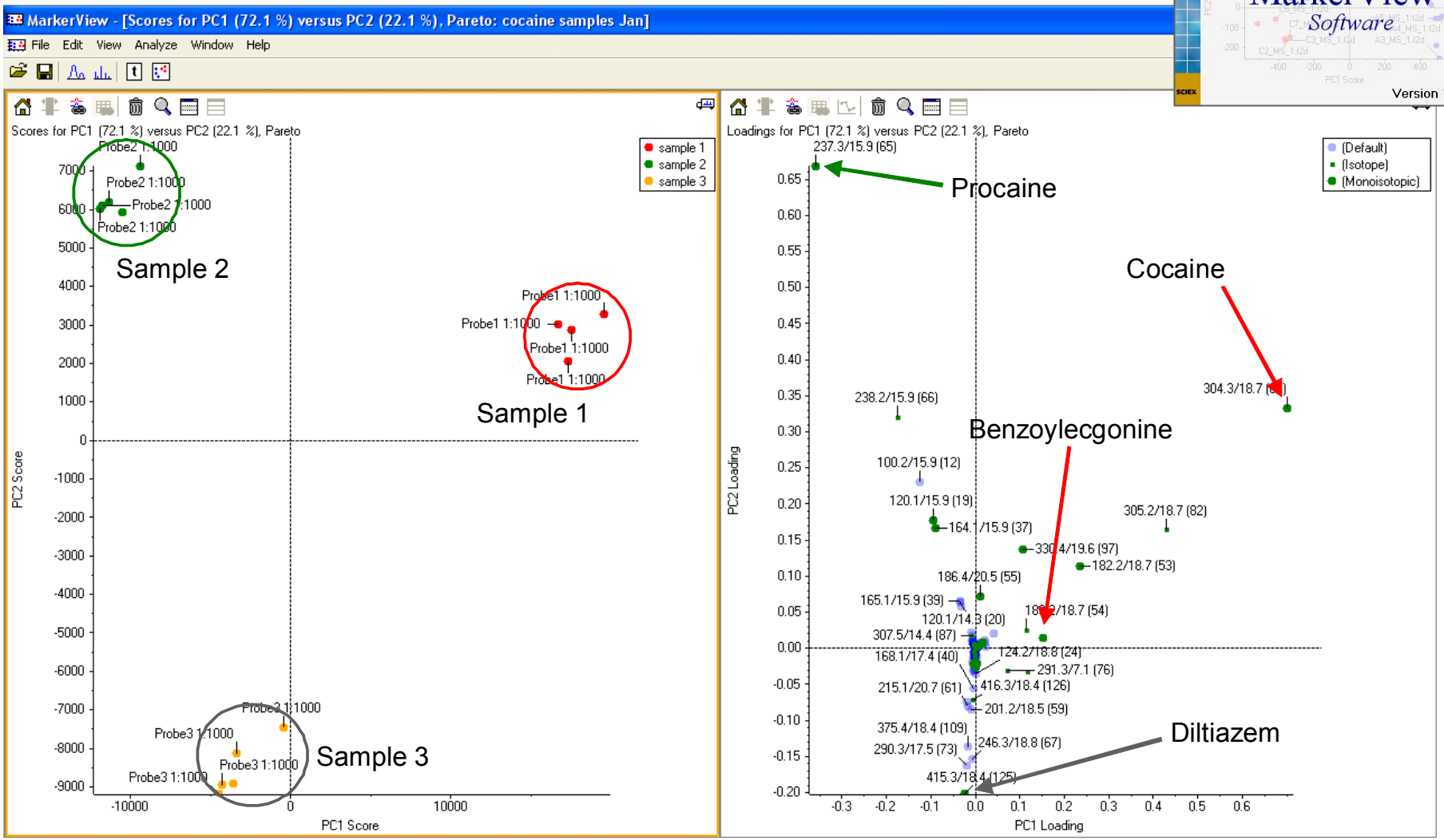
PCA Explanation 3



$$PC1 = 0.1\,\vec{x} + 0.3\,\vec{y} + 0.95\,\vec{z}$$

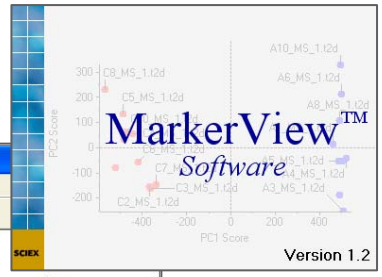
Analysis of “Street Cocaine” Samples

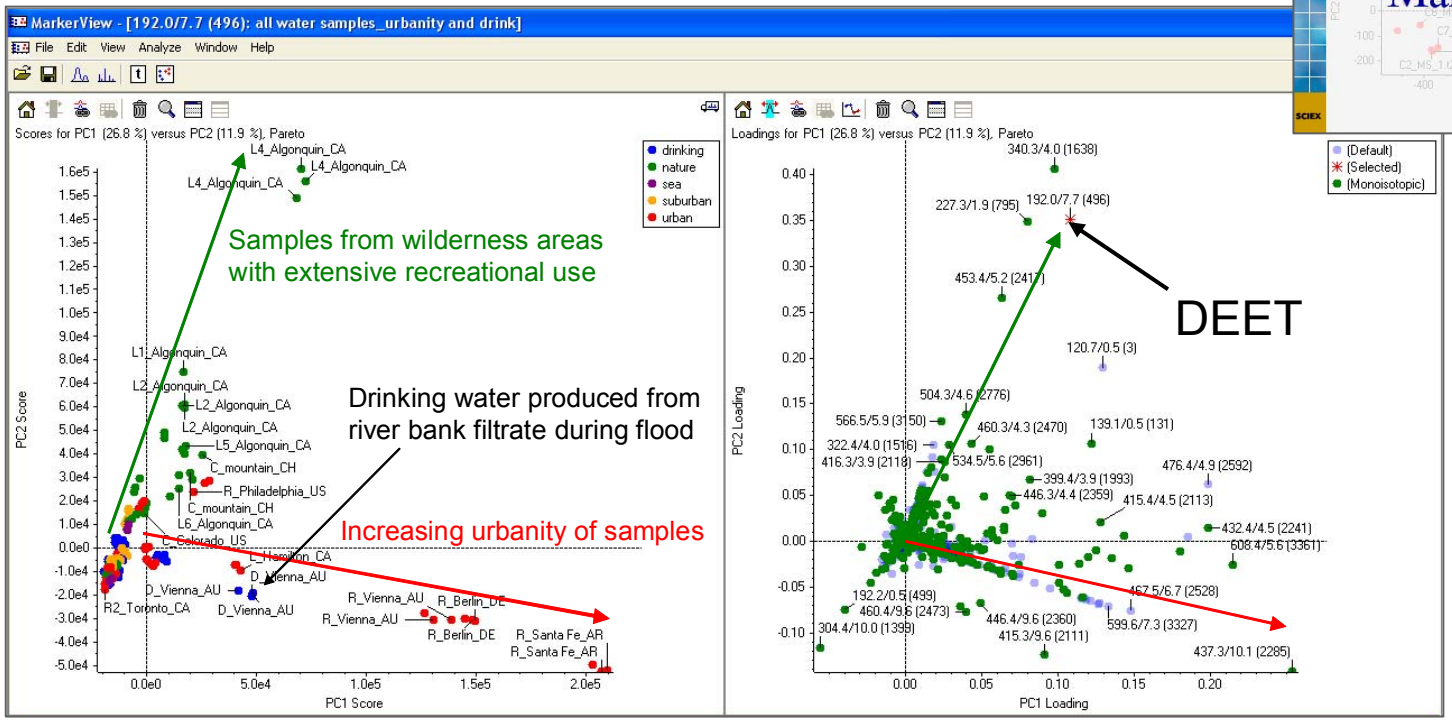


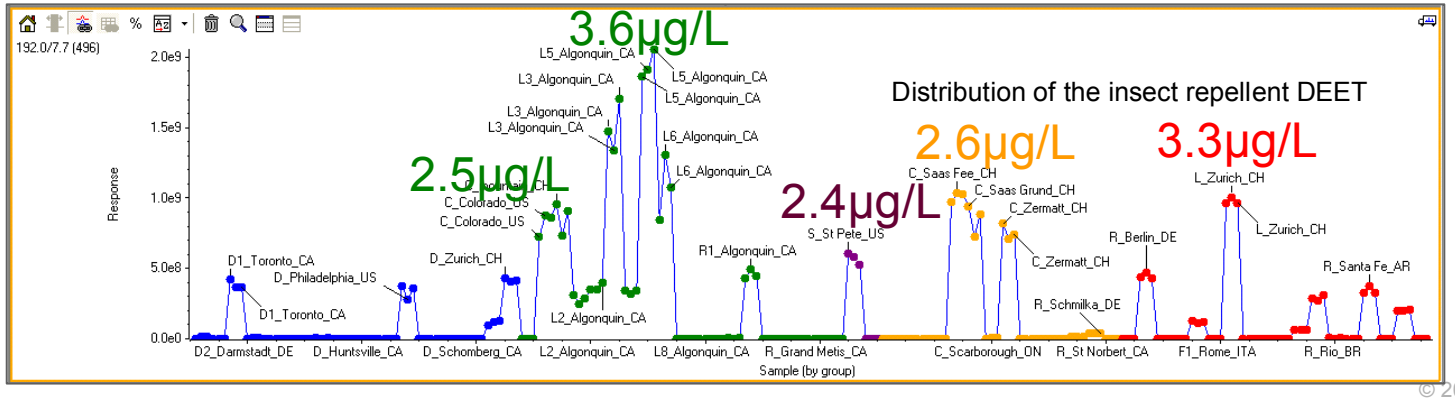


Principal Components Analysis (PCA) – scores and loadings plot

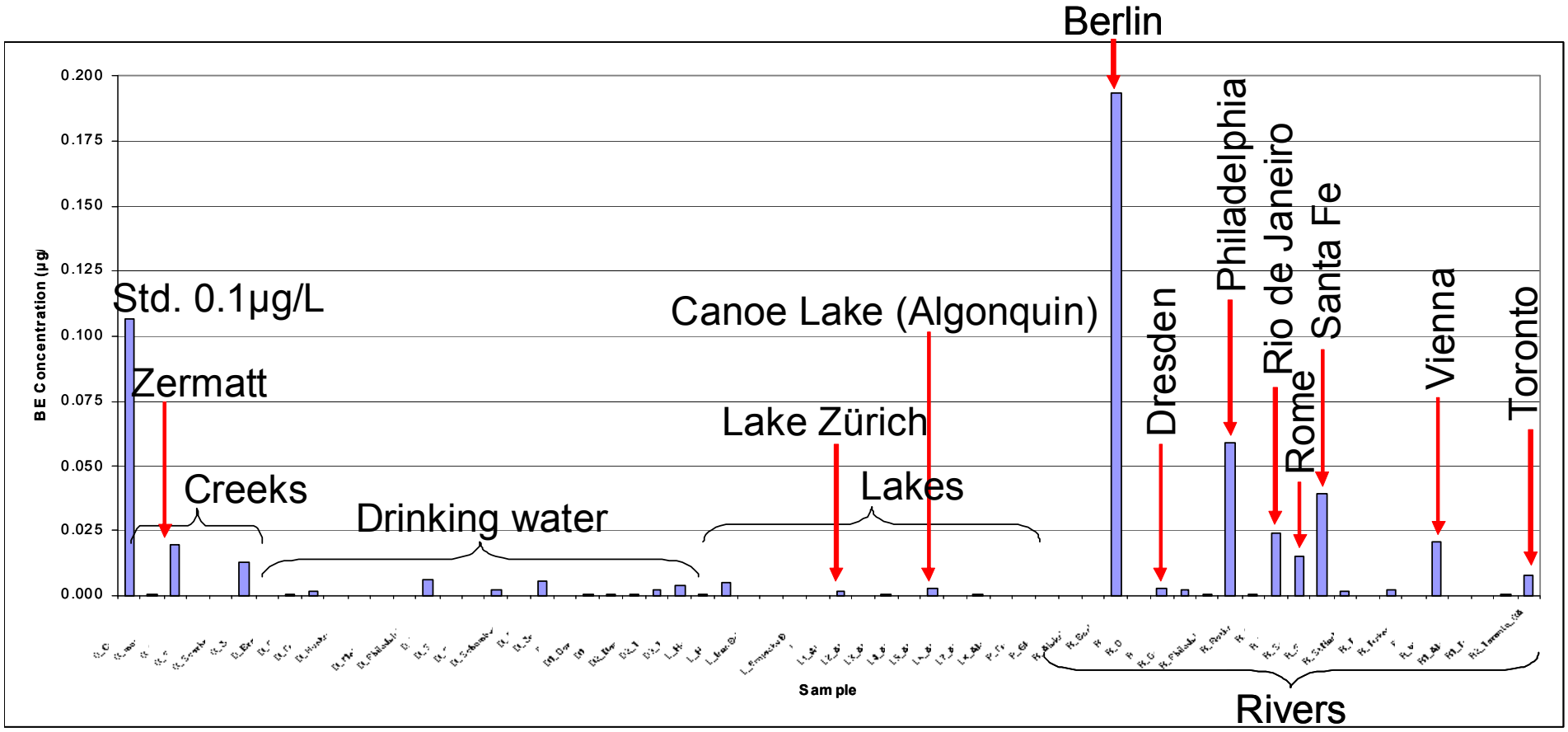
Screening for Unexpected PPCP







Quantitation of Benzoylecgonine (Metabolite of Cocaine) in 70 Water Samples



Cliquid® – General Unknown Screening

Cliquid™ Software for Routine Forensic Toxicology

Cliquid™
Software for Routine Forensic Toxicology

Run Screening

Home | Help | Log Out

Step 1
Choose test

Step 2
Build sample list

Step 3
Customize report

Step 4
Submit samples

Choose a test

- Drug screen
- Drug screen
- Drug Screen
- Drug Screen

Cancel

Instrument Panel

Stop | Standby | Restart | Live View | Reset

Standby

Mass Spec
Standby

Pump
Standby

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General Unknown Screening Matching Hits (CES)

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Data		Acquisition	
Data File	extrafos unknowns screening on 6500.wiff	Method	Extrafos Scheduled EMS-EPI_fixal.dam
Date and Time	11/15/2008 6:52:51 AM	Instrument	Unknown:22
Project	AS GUG (pesticides)	Operator	TAG4888\Administrator
Sample		Results	
Sample Type	Unknown	Result Table	2009_11_27_154809_0020.mdb
Weight to Volume	0.00	Library Search	
Dilution Factor	10.0	Library	Drug Screen 1.0.mdb
Vial	41	Mass Tolerance	0.40
Injection Volume	10 µL	Purity Threshold	30%

Extracted Ion Chromatogram

Summary

RT (min)	Mass	
1.02	285.16	Caffeine
1.48	104.10	Atrazine
1.84	116.14	Hydroxy
2.38	142.22	Cyclopen
3.17	191.24	Escitalo
3.35	413.36	Benzetha
3.49	191.16	Escitalo
3.85	180.24	Phenylep
4.27	385.26	Yohimbin
4.51	181.22	Methamf
4.84	280.20	Reproter

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5.47	328.27	Stanozolol	85.9	95.9	86.3
5.47	137.18	Nonlamide	75.4	84.6	78.9
5.88	337.38	Oxmetidin	48.9	67.4	72.8
5.19	137.18	Nonlamide	75.4	84.6	78.9
5.90	317.38	Halofuginone ethoxyethyl ester	47.1	67.4	69.8
6.02	295.26	Escitalo	39.2	85.3	42.9
6.29	384.42	Dihydroergotamine	41.0	53.3	76.9
7.27	437.55	Bezitramide	47.3	47.3	100.0
7.32	375.53	Prochlorperazine	45.1	62.7	72.0
7.32	346.67	Mebendazole acetate	33.9	42.1	80.4
7.32	367.47	Topotecan	53.0	68.2	71.2
7.41	317.09	Haloperidol	41.4	49.0	84.4
7.41	379.50	Escitalo	34.7	85.5	38.5
7.46	339.88	Dimethrin	42.8	55.3	77.4
7.46	337.55	Acebutolol	51.6	72.3	71.4
7.50	353.81	Trifluoperazine	30.8	66.7	47.0
7.69	313.64	Lorazepac	53.4	61.5	86.8
7.93	375.89	Prochlorperazine	37.9	62.7	60.4
8.02	407.62	Escitalo	31.9	82.9	35.9

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Retention Time: 3.79 minutes
m/z: 191.8 - 192.2 Da
Area: 2220000000 counts

Date: 11/15/2008 5:17:44 AM

Collision Energy = 35 ± 15 eV

Acquired

Library

	Compound Name	Purity(%)
1	Caribendazole	73.9

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Retention Time: 5.45 minutes
m/z: 136.4 - 137.9 Da
Area: 5820000000 counts

Date: 11/15/2008 6:52:51 AM

Collision Energy = 35 ± 15 eV

Acquired

Library

	Compound Name	Purity(%)
1	Nonlamide	75.4
2	Inosine	82.8
3	Diclanoline	82.8
4	Allopurinol	51.0
5	Methfinoxam	46.9

Summary

- LC-MS/MS is a powerful tool for the analysis of Pharmaceuticals and Personal Care Products in environmental samples
  - The combination of high resolution LC separation and high sensitivity MS/MS is the most powerful tool to screen and quantify targeted compounds.
  - Direct injection of water samples
  - MS/MS fragmentation required to identify compounds
  - MRM transitions to quantify targeted contaminants
  - MRM ratios or MRM to trigger MS/MS spectra (EPI) for compound identification with high confidence
  - GUS coupled with library searching and PCA is a powerful research tool for any laboratory

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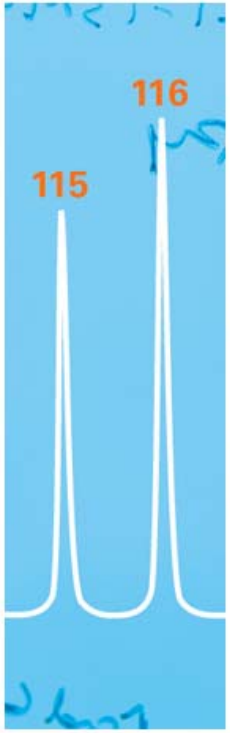

Thank you for listening!

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