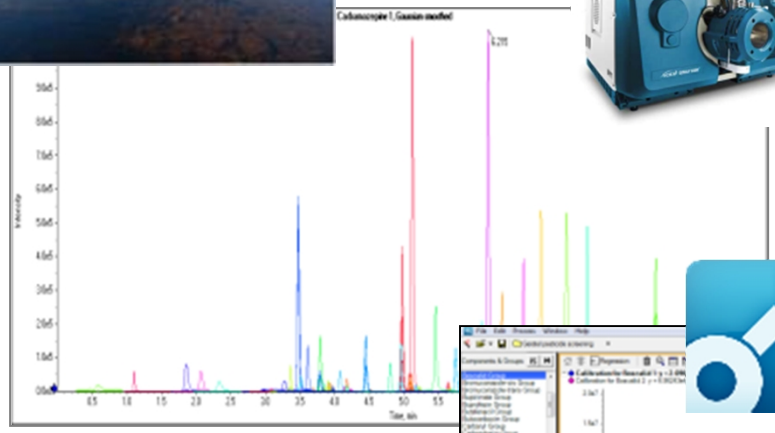
Detection of PPCP, Drugs of Abuse and Unexpected Environmental Pollutants

Jennifer Krone & André Schreiber
SCIEX, Concord, Ontario (Canada)

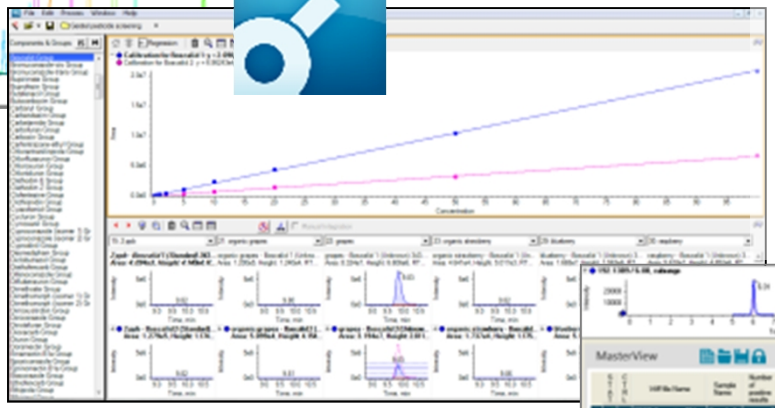
SCIEX

Outline

1. Sampling and sample preparation



2. Target quantitation and identification LC-MS/MS



3. Unknown identification accurate mass LC-MS/MS

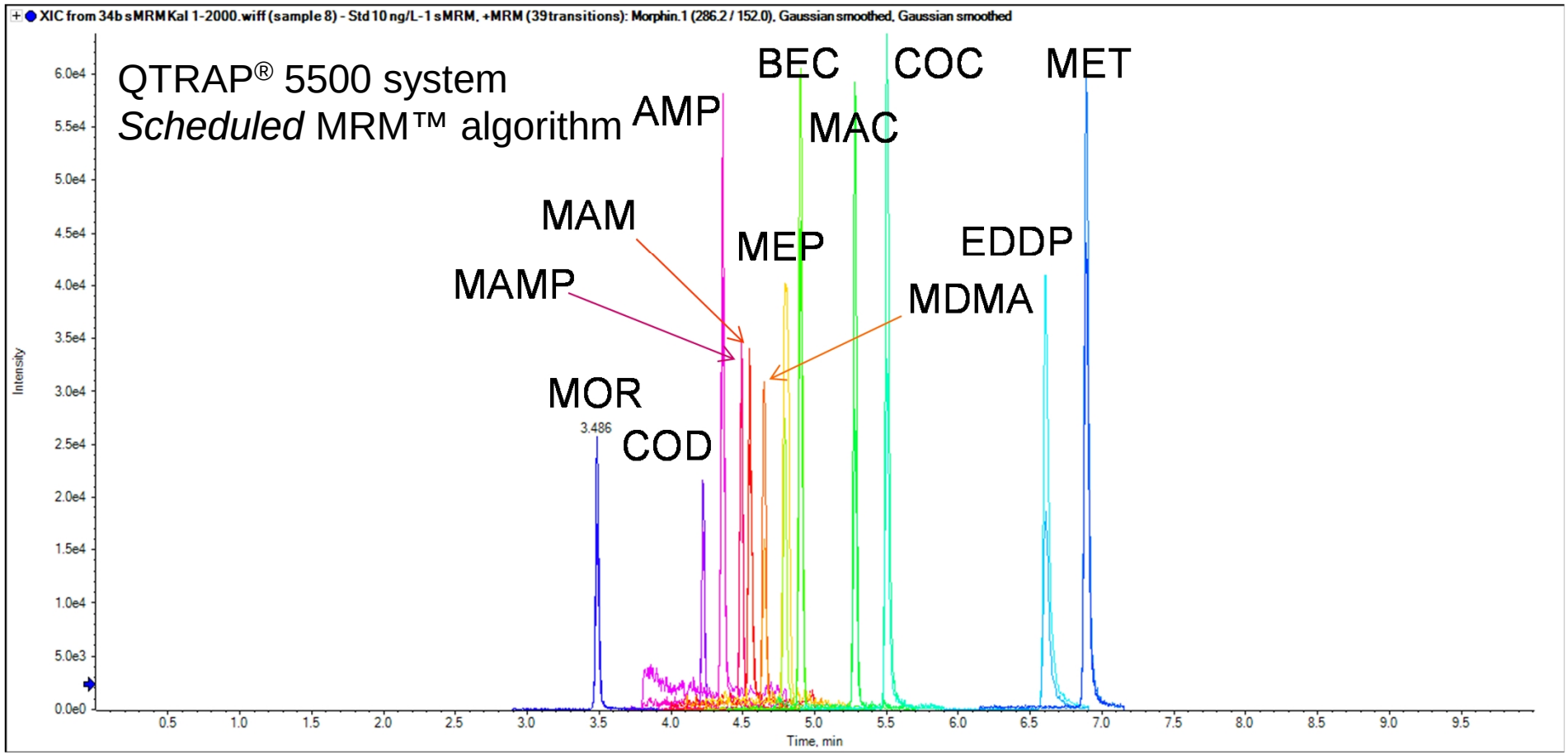
LC-MS/MS in Environmental Analysis

Considerations on Sampling, Extraction and Separation

1. Statistical sampling needed for unknown screening
   - "The more samples the better"
2. Direct injection of (filtered) water
   - Easiest and most cost efficient way to handle "clean" water samples, i.e. drinking water, surface water, and ground water
   - Best for multi-target and non-target analysis
   - Modern LC-MS/MS systems allow low-ppt detection limits
3. (Automated online) extraction and cleanup
   - Cleanup of "dirty' environmental samples, i.e. waste water, biosolids etc.
4. Dilution to minimize ion suppression
5. Generic reversed phase UHPLC setup
   - C18 or polar modified C18, small particle size or core-shell particle columns

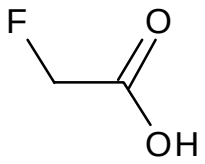
10 ng/L Drugs of Abuse and Metabolites

Generic LC Conditions for Screening Method

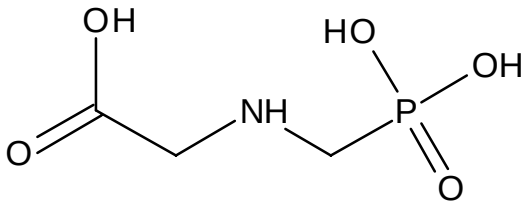


LC: Phenomenex Kinetex C18 5 µm (100 x 4.6 mm)
water / acetonitrile + 0.1% formic acid + 2 mM ammonium formate
Injection of 100 µL

Generic LC does not work for all analytes… 2 exam



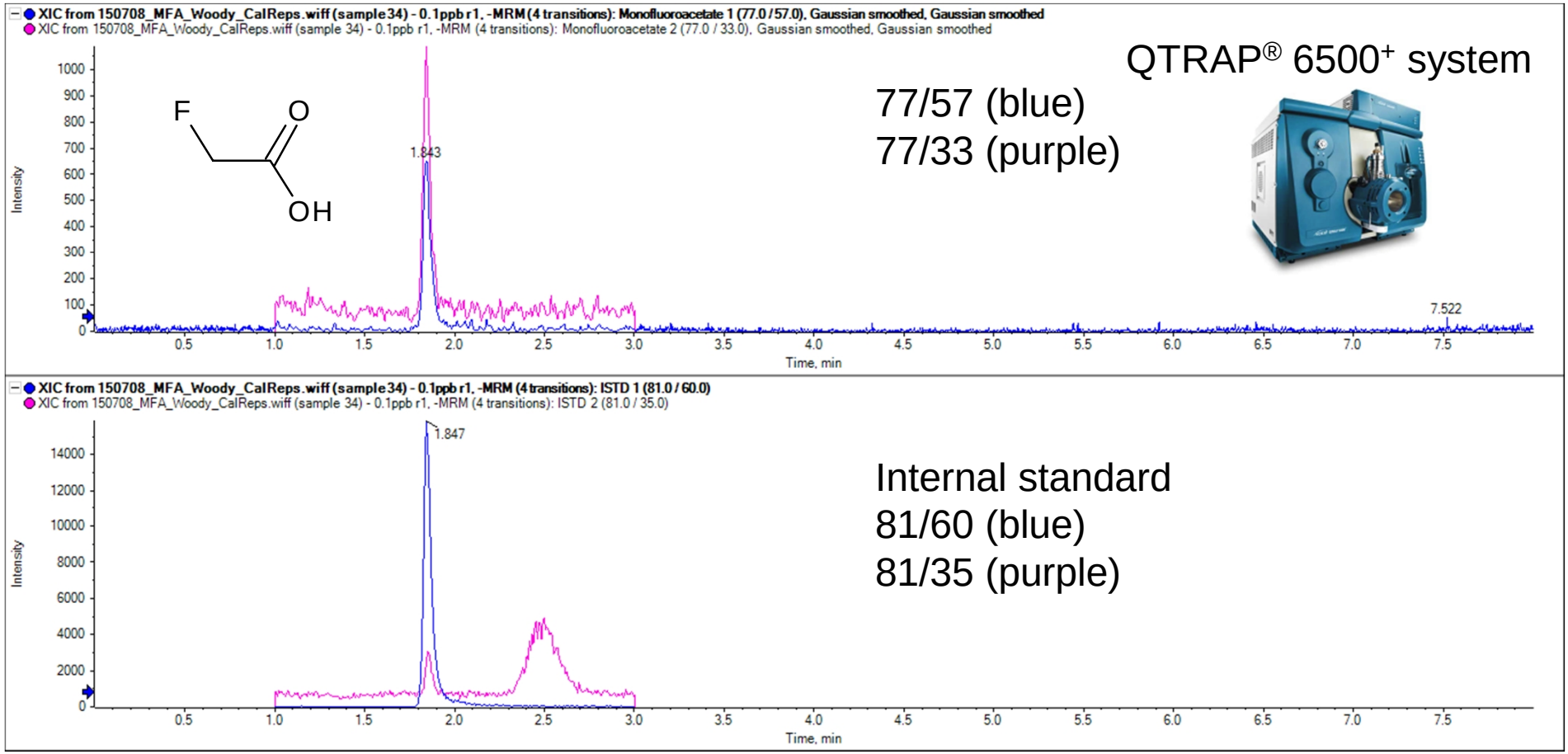
Pesticide 1080



Glyphosate

0.1 µg/L of Pesticide 1080 (Mono Fluoroacetate)

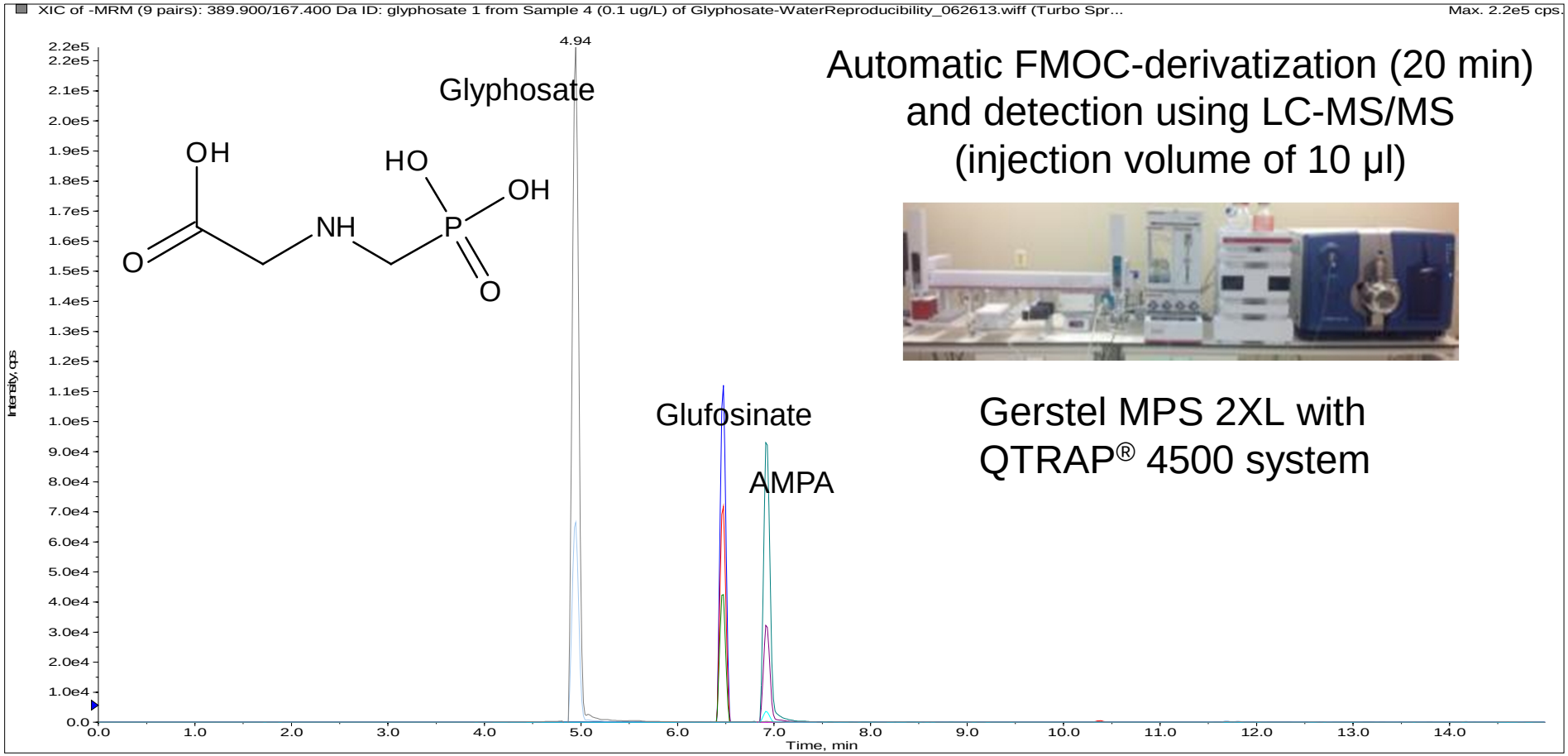
High Polarity Requires Different LC Setup – Normal Phase HILIC



LC: BEH Amide 1.7 µm (100 x 2.1 mm)
acetonitrile / water + 5 mM ammonium formate and 0.01% formic acid

0.01 µg/L of Glyphosate and Co.

High Polarity Requires Derivatization (FMOC)



Automatic FMOC-derivatization (20 min) and detection using LC-MS/MS (injection volume of 10 µl)

Gerstel MPS 2XL with QTRAP® 4500 system

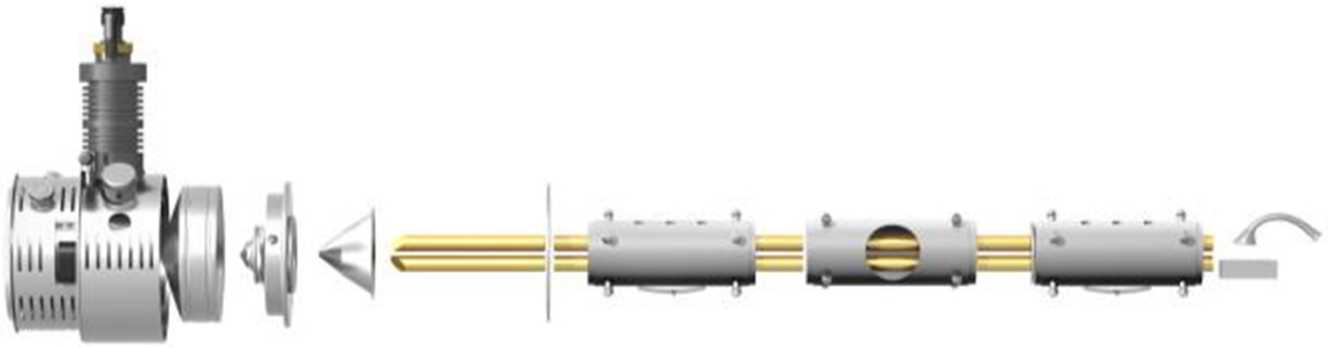
Automatic FMOC-derivatization (20 min) and direct injection (10 µL) into LC-MS/MS

Derivatization-free analysis possible (but tricky) using HILIC or Hypercarb

MS/MS in Environmental Analysis

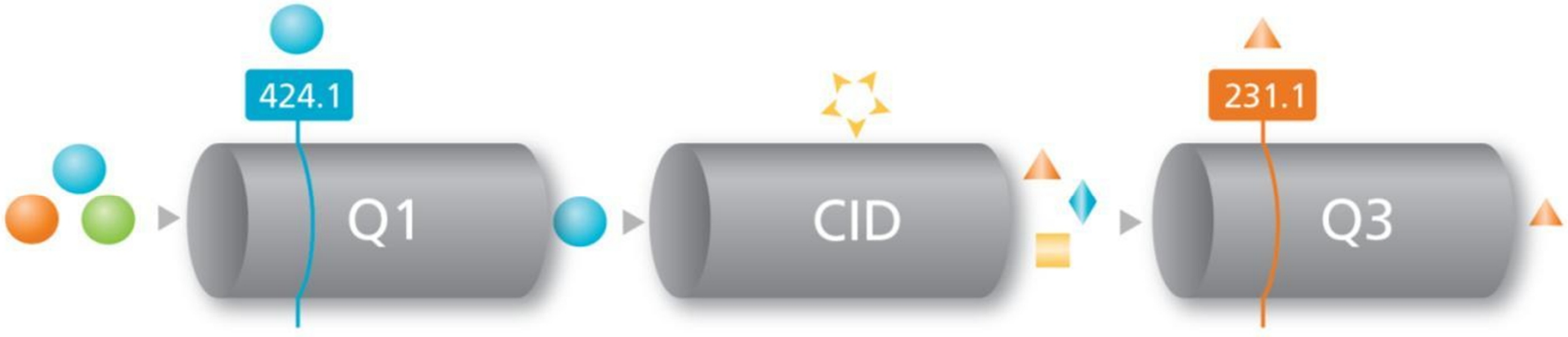
Considerations on Targeted Detection

- Electrospray Ionization (ESI) and APCI or APPI for low polarity analytes
- Highest selectivity and sensitivity in MRM using triple quadrupole instruments, with *Scheduled* MRM™ algorithm for multi-target methods
- MRM ratio (two fragment ions) for compound identification
- QTRAP® MS/MS spectra with MRM like sensitivity for compound identification using library searching



Triple Quadrupole – Multiple Reaction Monitoring (MRM)

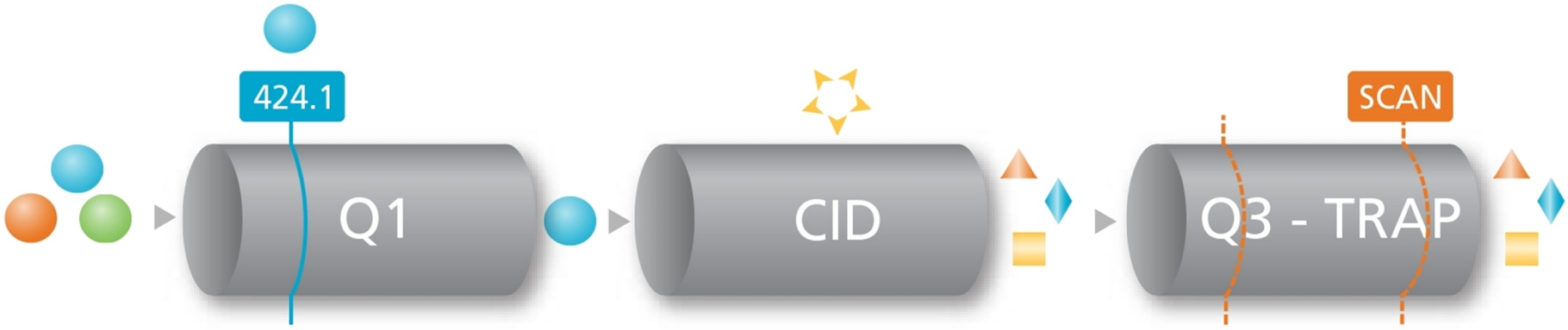
Highest Selectivity and Sensitivity for Screening and Quantitation



Filter Fragment Filter

QTRAP® System – MS/MS Full Scan and Library Searching

High Selectivity and Fast MS/MS Scan to Increase Confidence

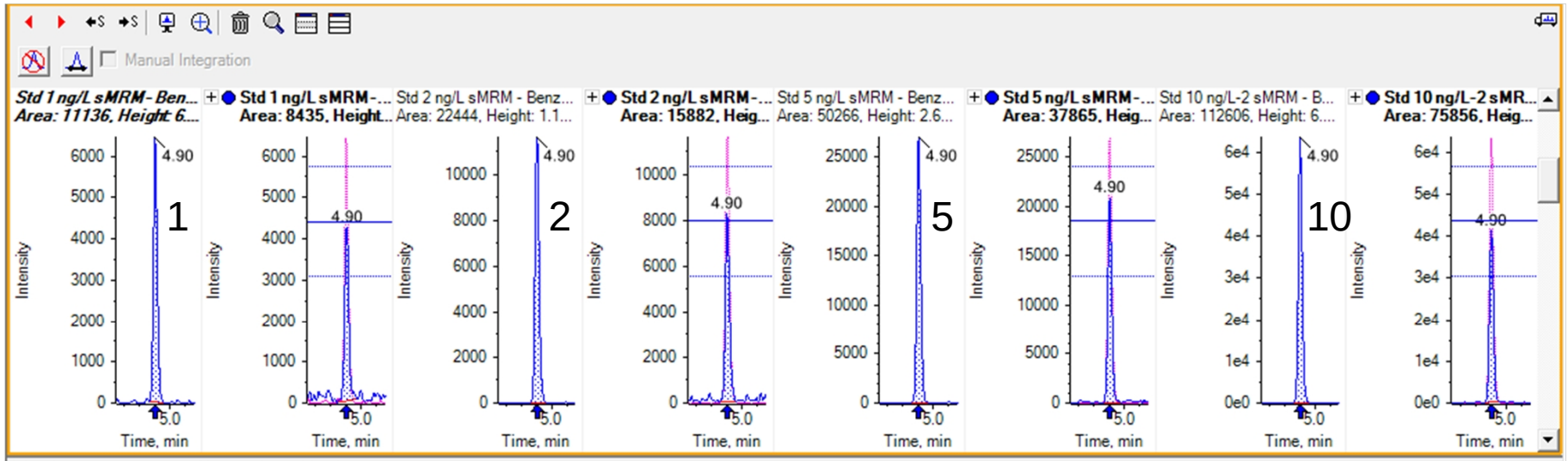


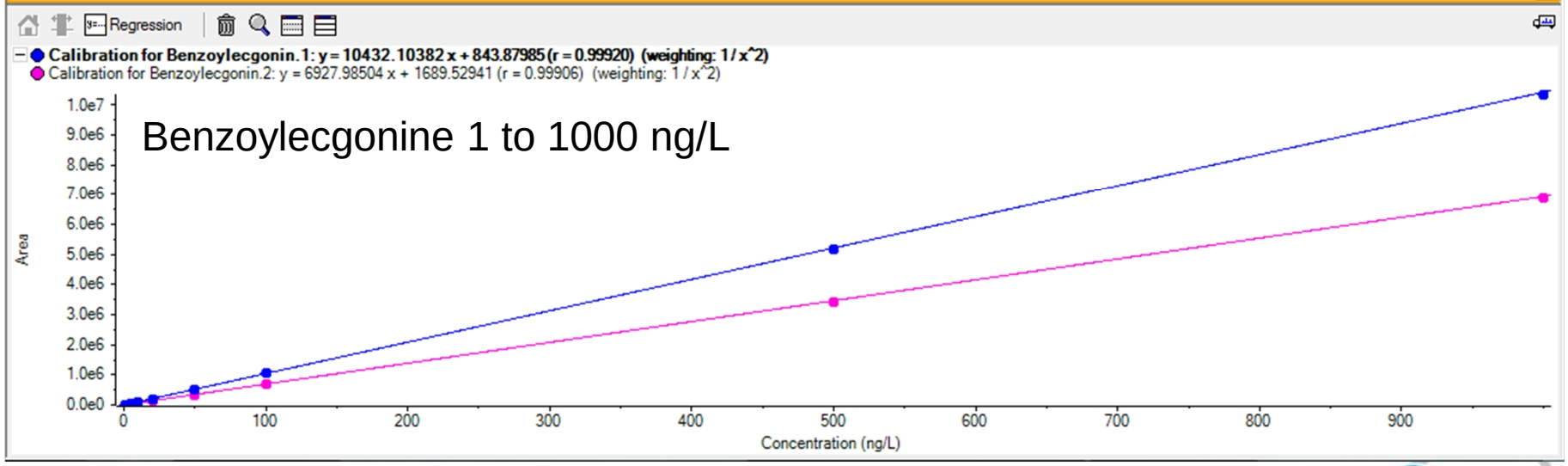
Filter Fragment Trap and Scan

MRM Quantitation and QTRAP® Full Scan MS/MS can be combined in Information Dependent Acquisition (IDA) methods for simultaneous quantitation and Identification

Quantitation and Identification using MRM Ratios

Benzoylecgonine with Ion Ratio Tolerance of 30% (SANCO12571)

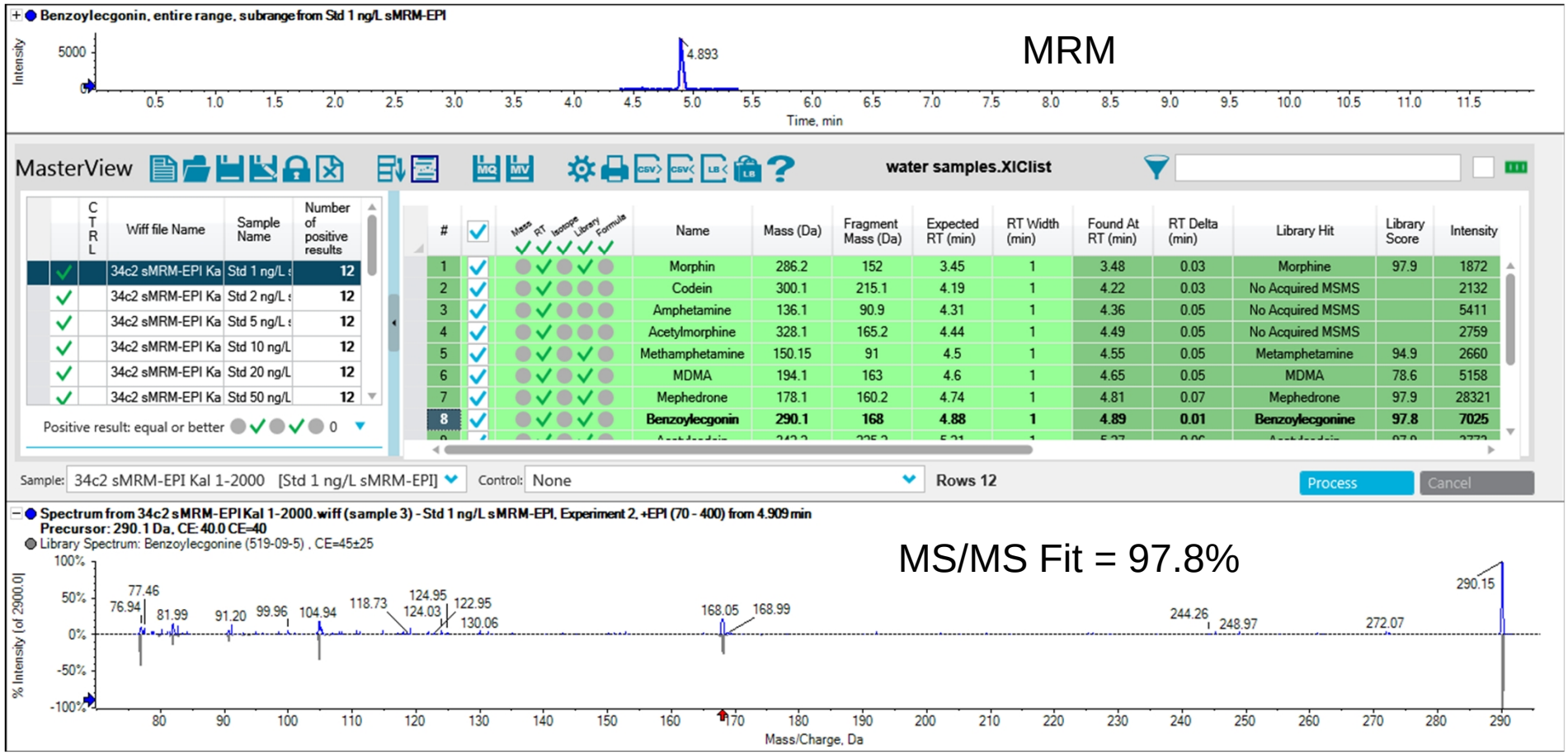




MultiQuant™ software

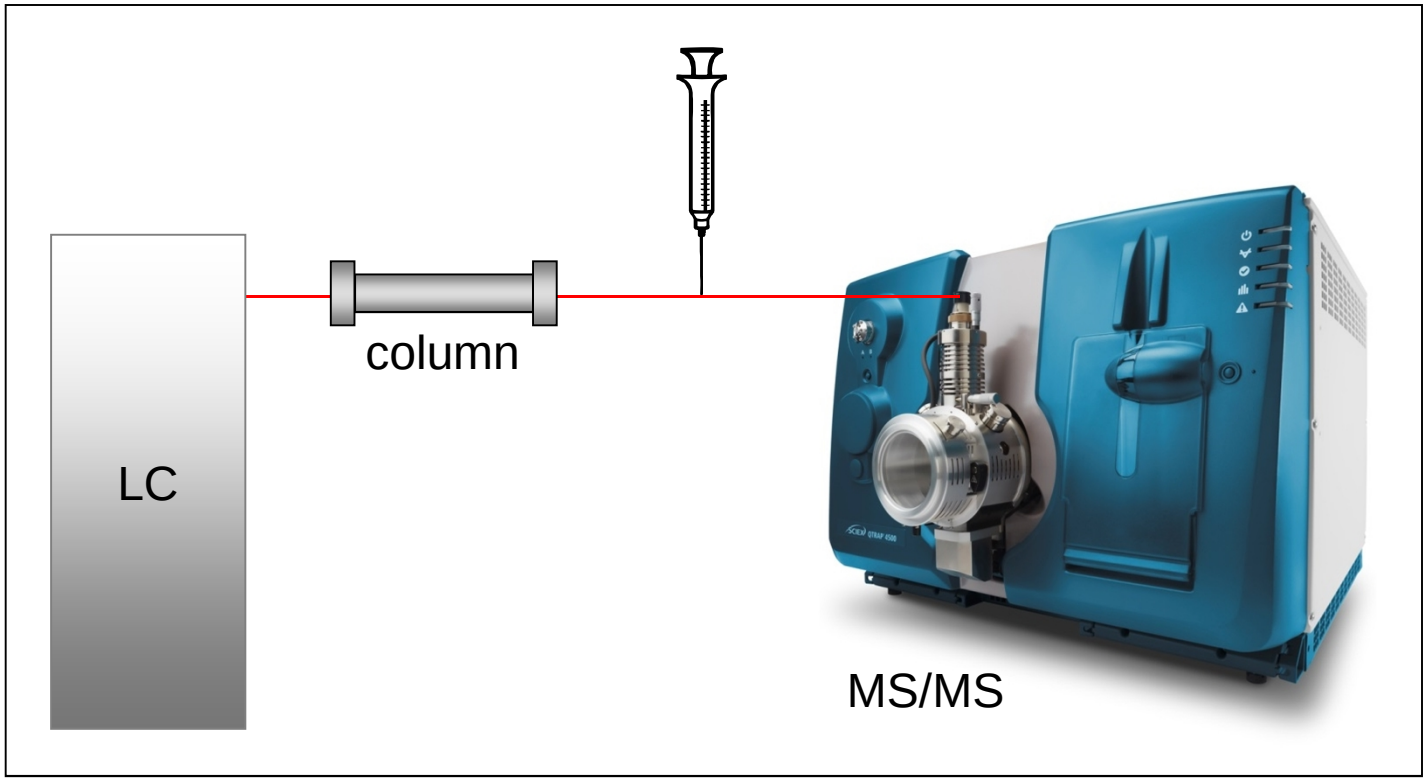
QTRAP® MS/MS Library Searching

Identification of Benzoylecgonine



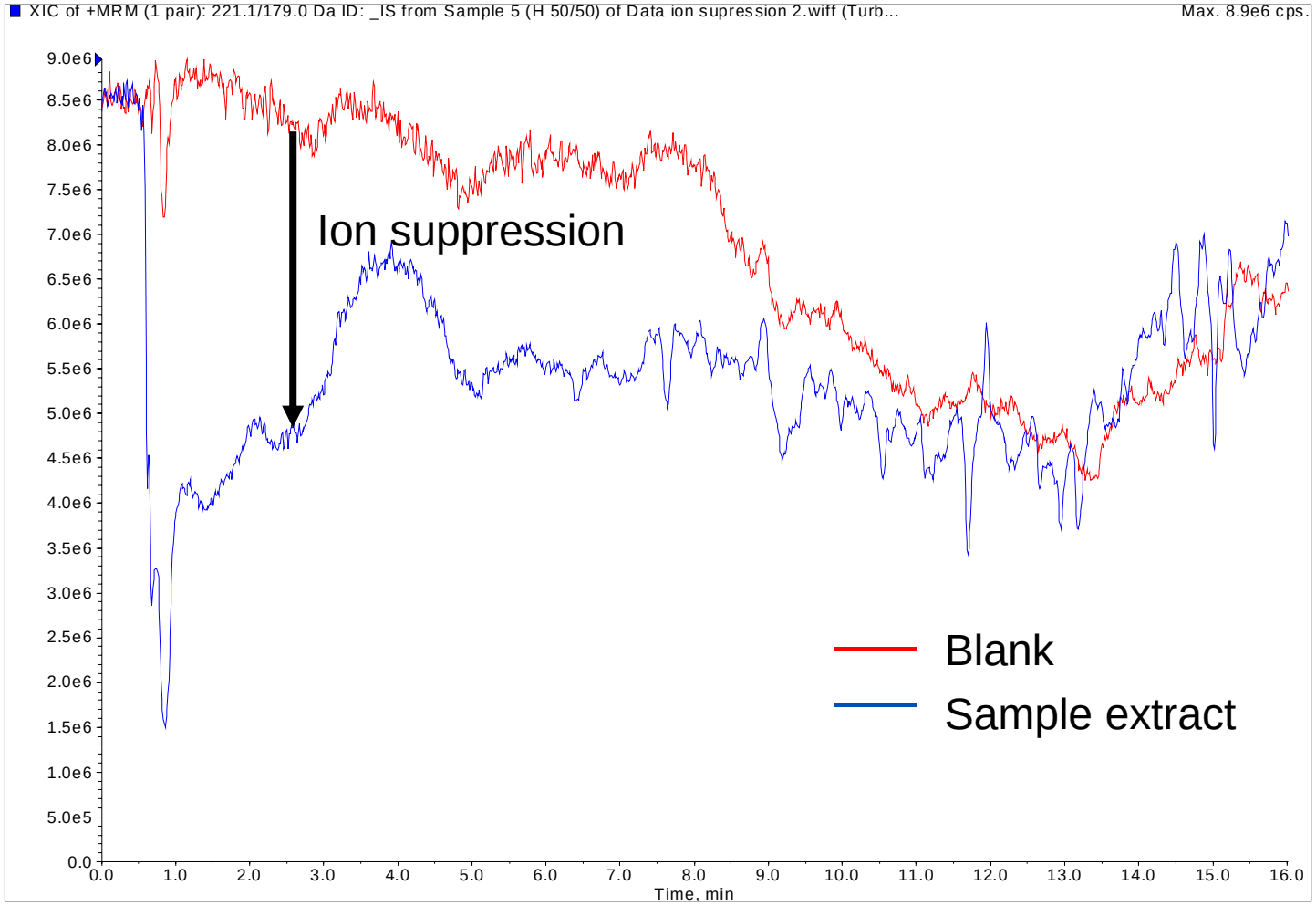
MasterView™ software

Post-Column Infusion to Study Matrix Effects



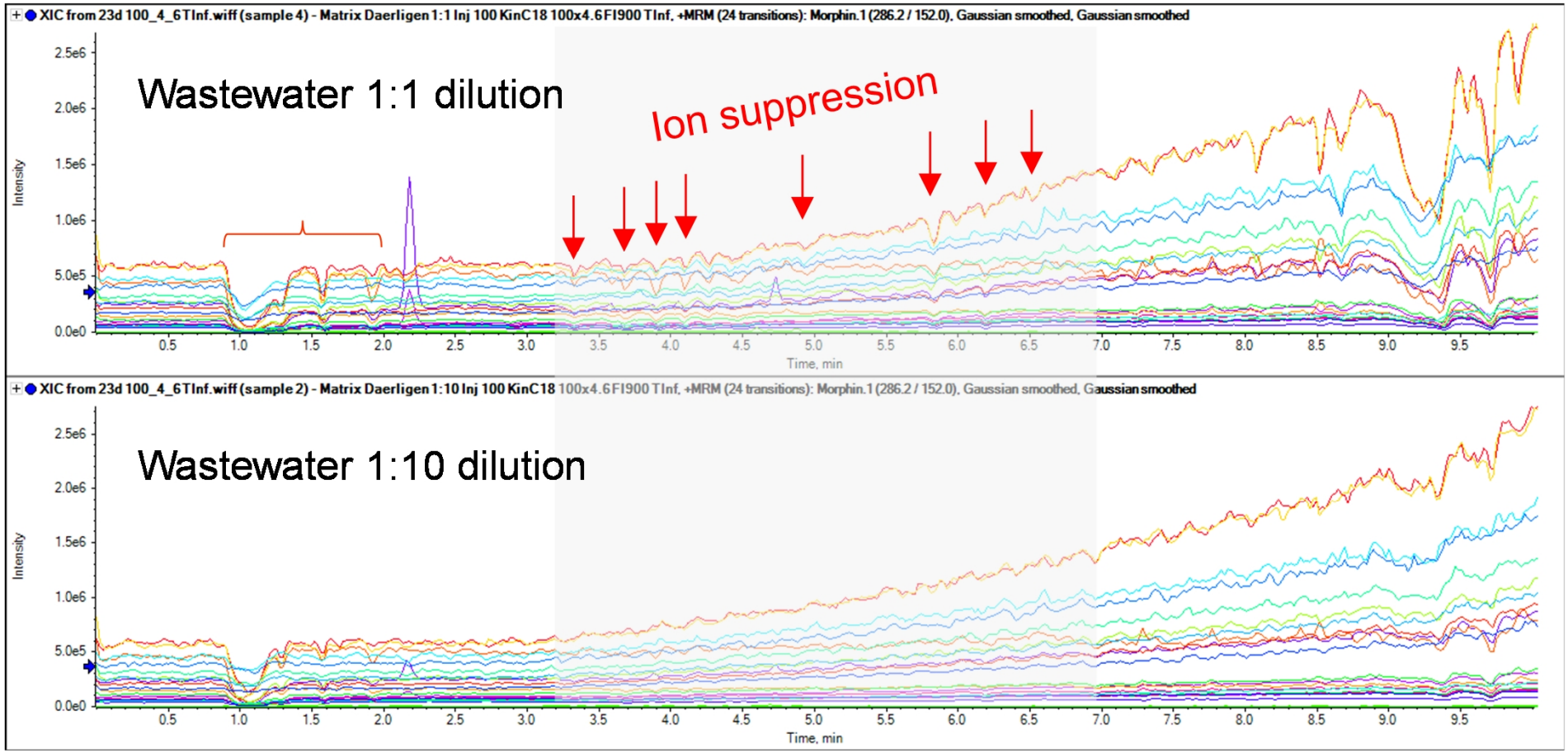
Infuse standard post-column (high level signal detected).
Injection of matrix sample, matrix components will be separated by LC…
Reduced signal indicates ion suppression.

Post-Column Infusion to Study Matrix Effects – Results



Dilution of Wastewater Sample to Reduce Ion Suppression

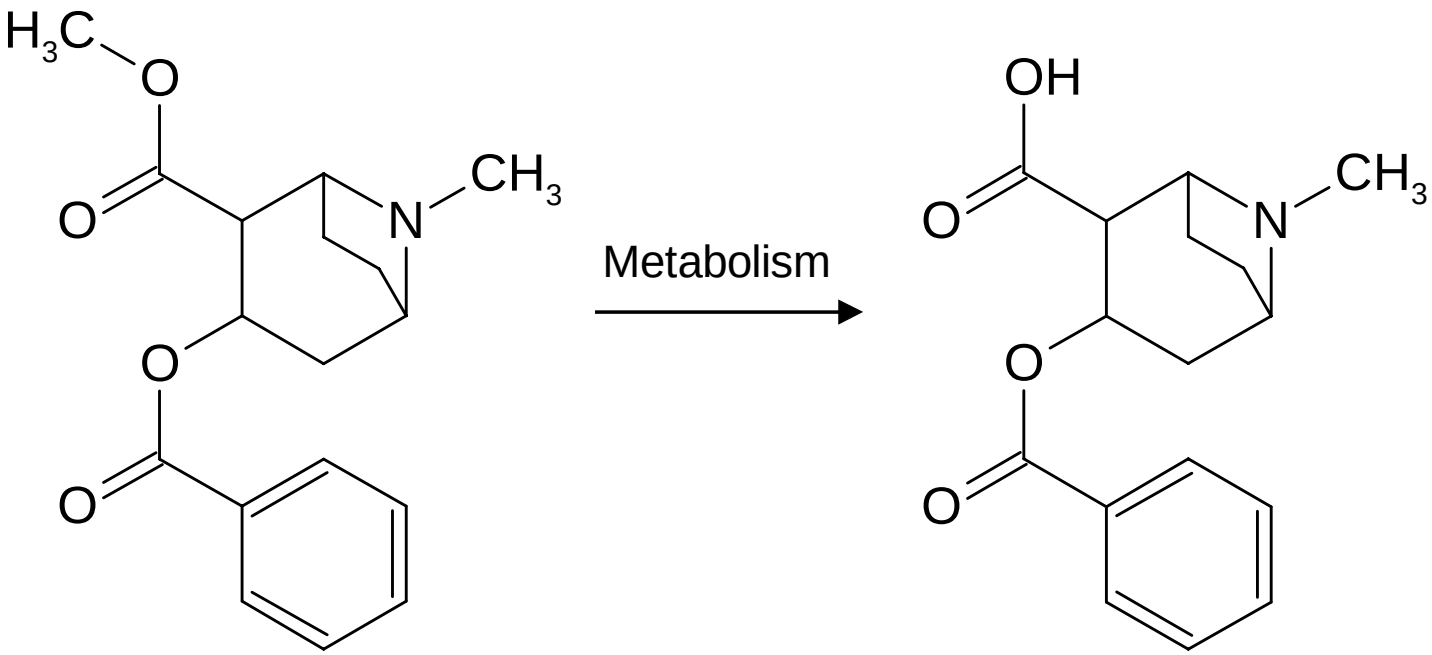
(Grey Box Indicates Area of Elution of Compounds of Interest



Screening of Water Samples for PPCP

Benzoylecgonine – a Marker for Cocaine Consumption

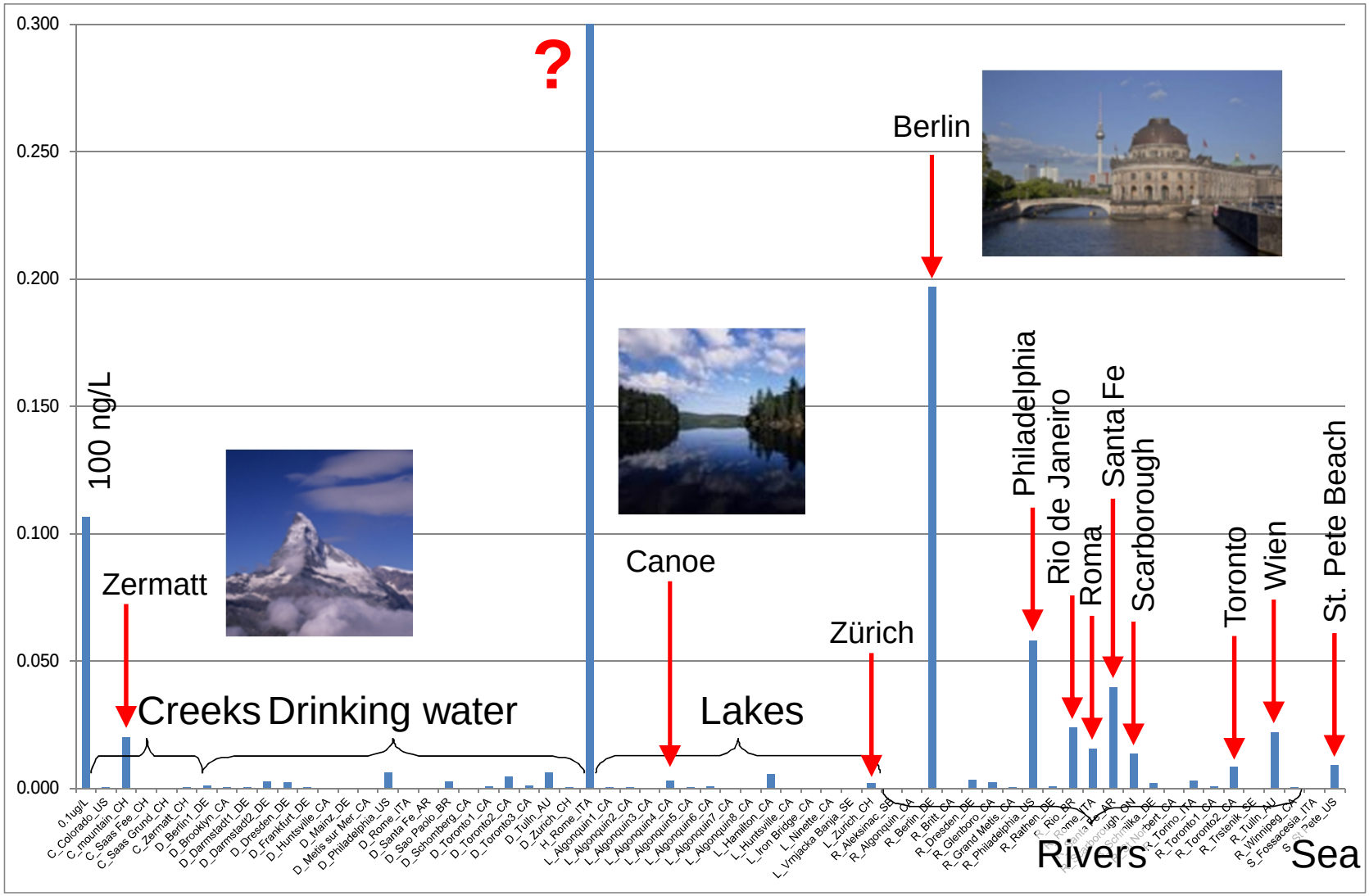
Detection of BE Indicates Consumption of Cocaine



Cocaine

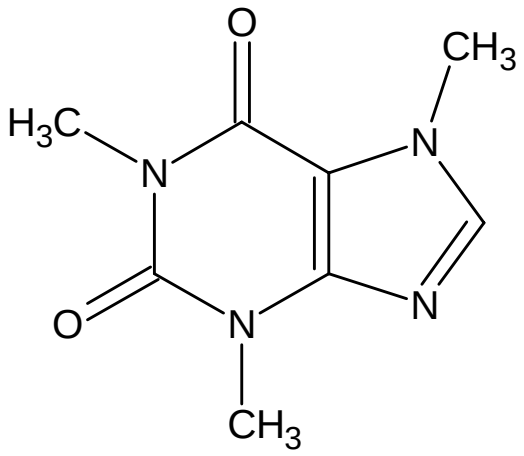
Benzoylecgonine (BE)

Benzoylecgonine in Different Water Samples



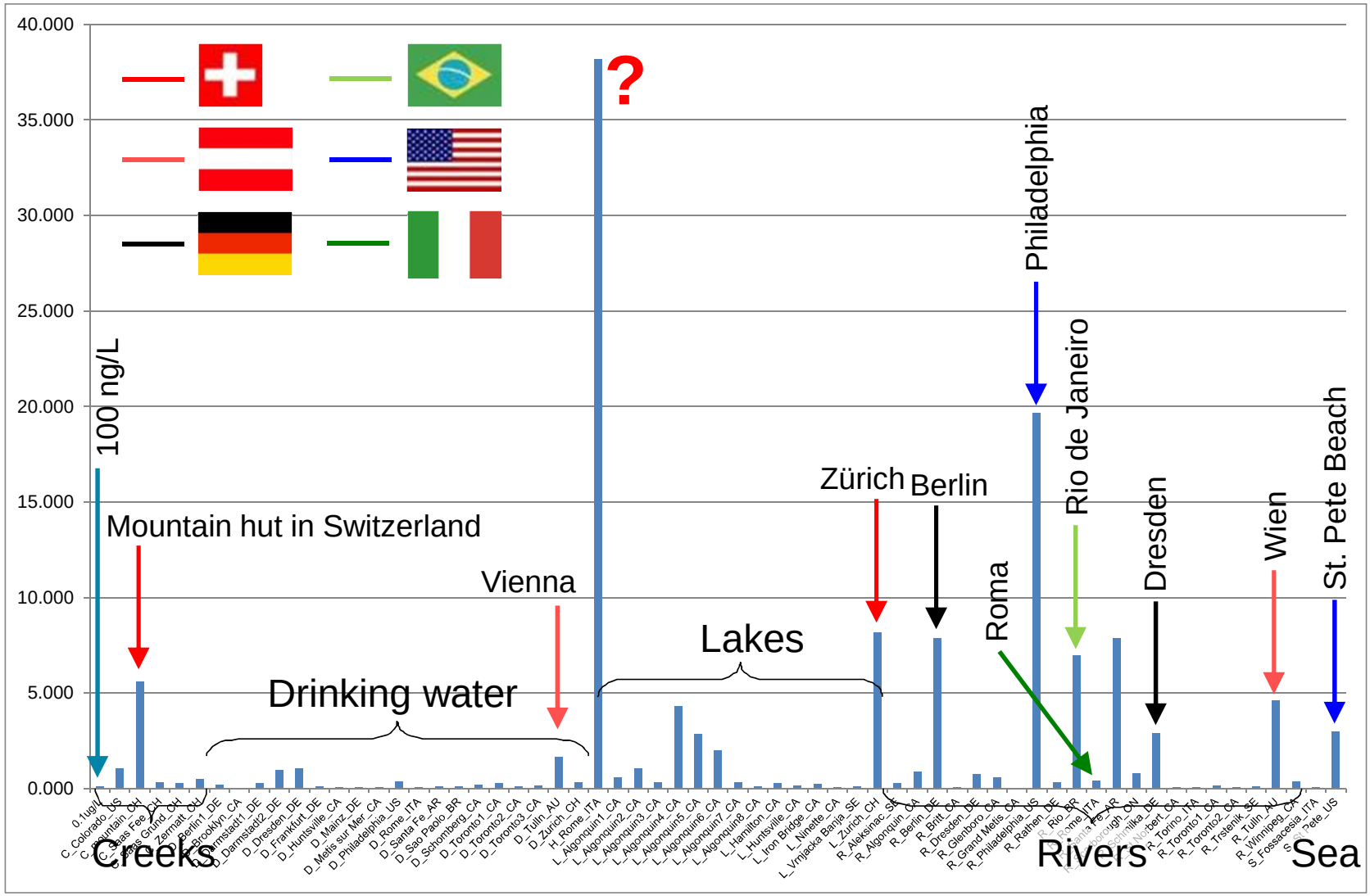
BE and other drug metabolites are detected in rivers in major cities, concentration can be used to estimate consumption.

Caffeine – A Marker for Human Activity (Coffee Consumption and Pain Medication)



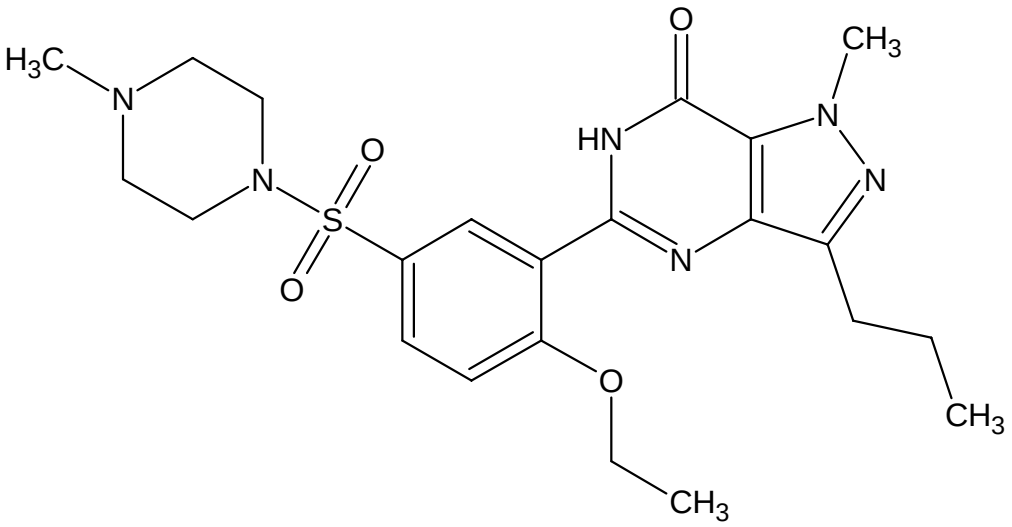
Caffeine

Caffeine in Different Water Samples

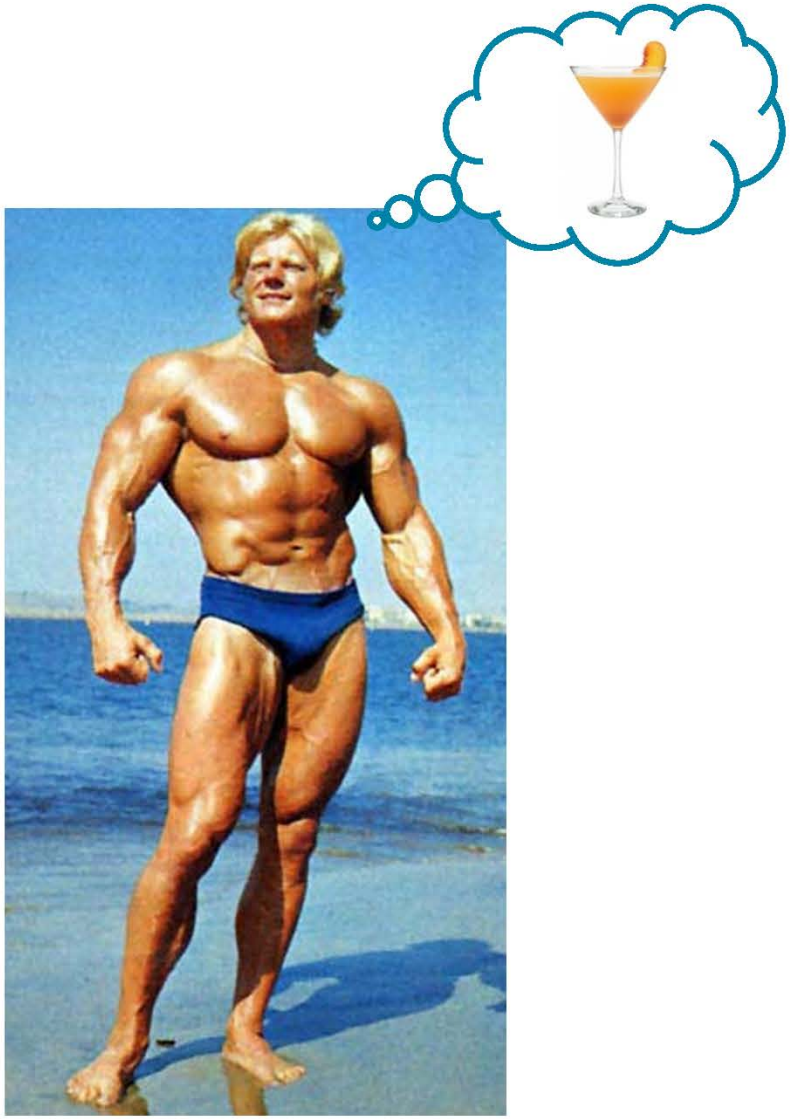


Caffeine or other compounds consumed in large amounts are markers for human activity, i.e. artificial sweetener

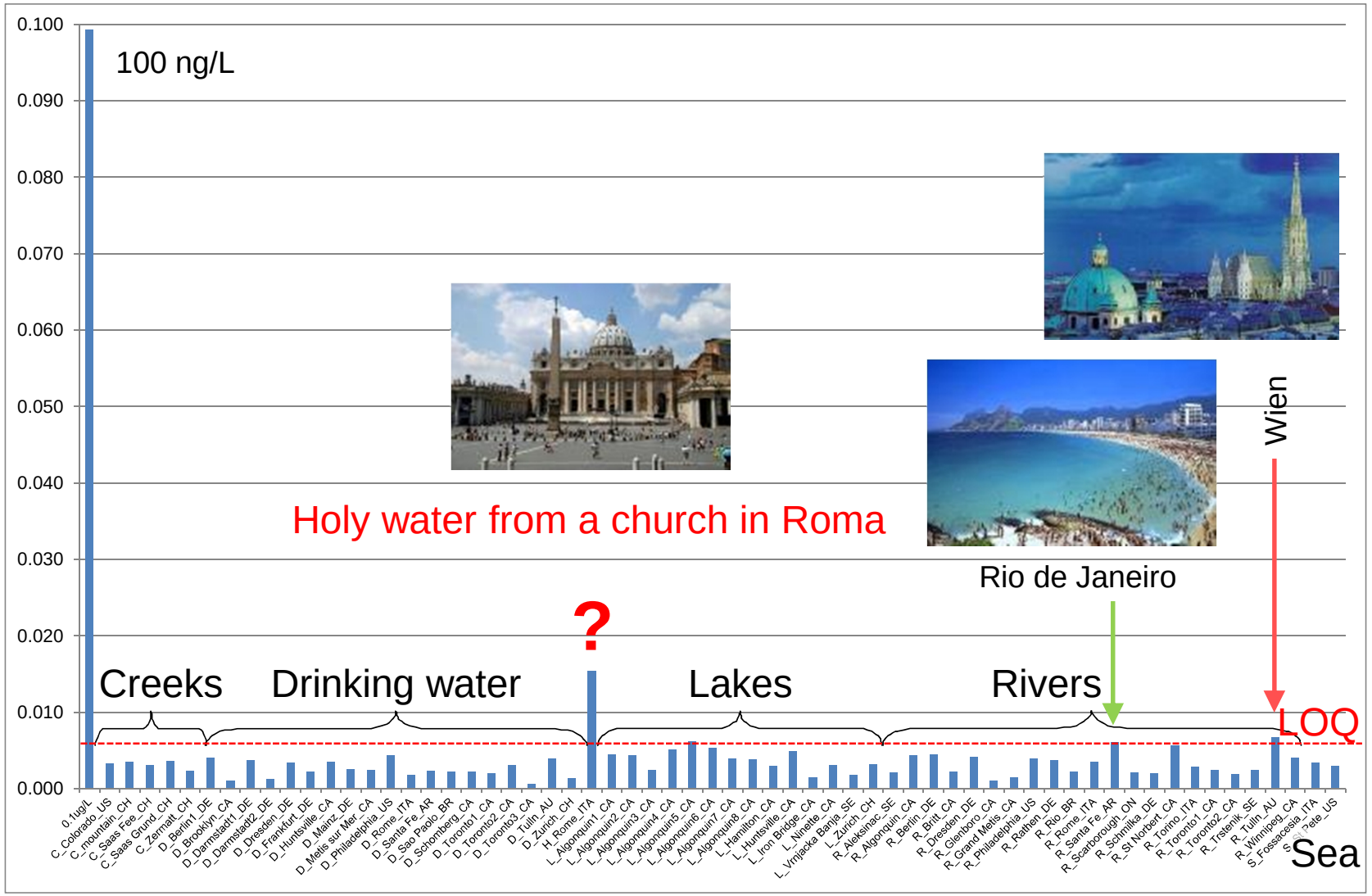
Sildenafil – Viagra (Virility Regulator)



Sildenafil



Sildenafil in Different Water Samples



Concentration of sildenafil detected were very low (<10 ppt), one exception!

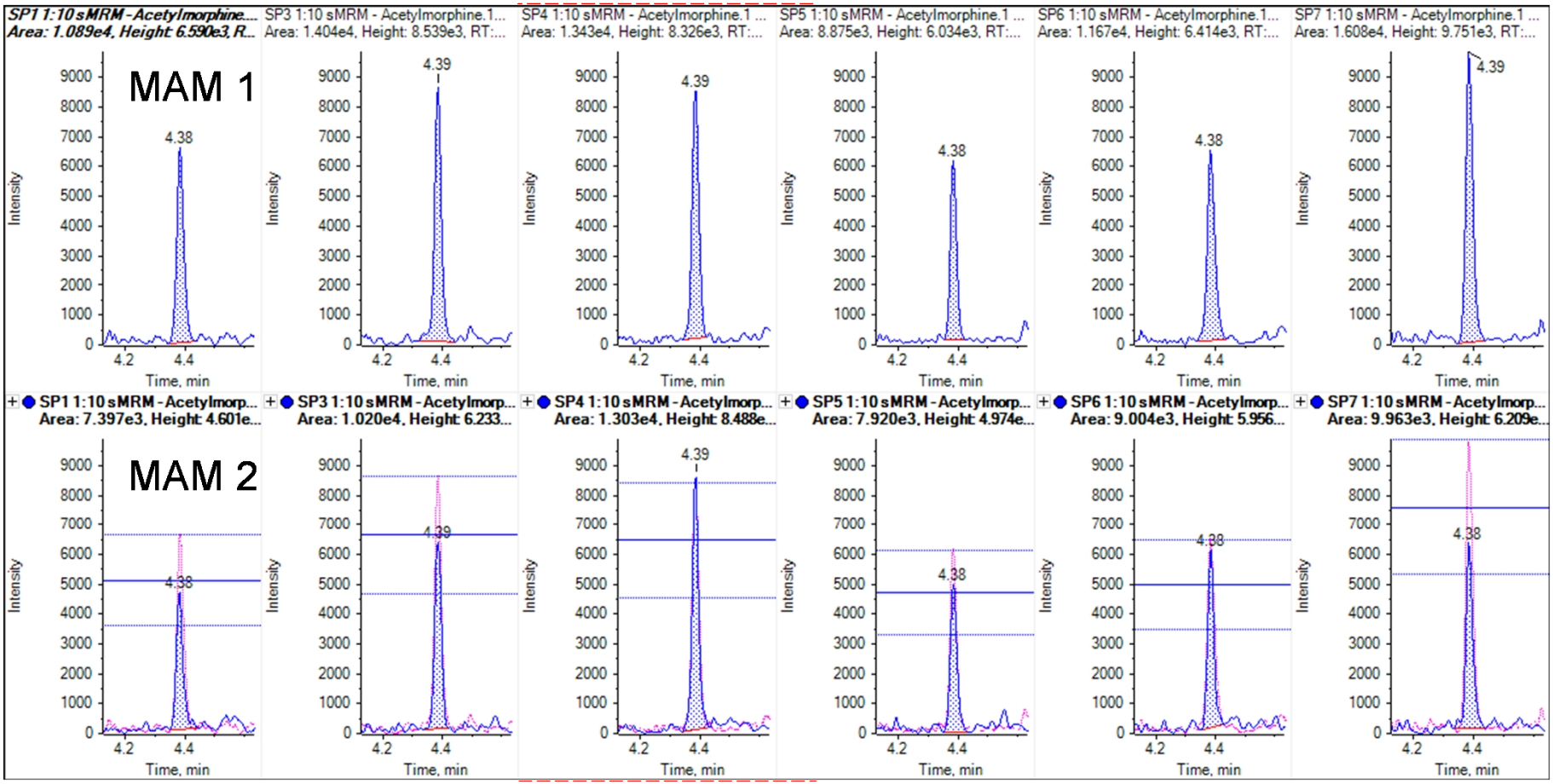
Data of Holy Water (Direct Injection 100 µL)

Compound	µg/L	LOQ (µg/L)
Acetaminophen	9.1	0.010
Benzoylecgonine (metabolite of cocaine)	0.47	0.001
Caffeine	38	0.010
Carbamazepine	0.21	< 0.001
Codeine	0.050	0.001
Dextromethorphan	0.021	0.001
Diazepam	0.003	0.001
EDDP (metabolite of methadone)	0.001	0.001
Erythromycin	1.7	0.050
Morphine	0.15	0.005
Sildenafil	0.015	0.005
Thiabendazole	0.016	< 0.001

Determination of Drugs of Abuse and Metabolites during the “Zürich Street Parade”

Monoacetylmorphine, Benzoylecgonine, and Ecstasy in Waste Water Samples

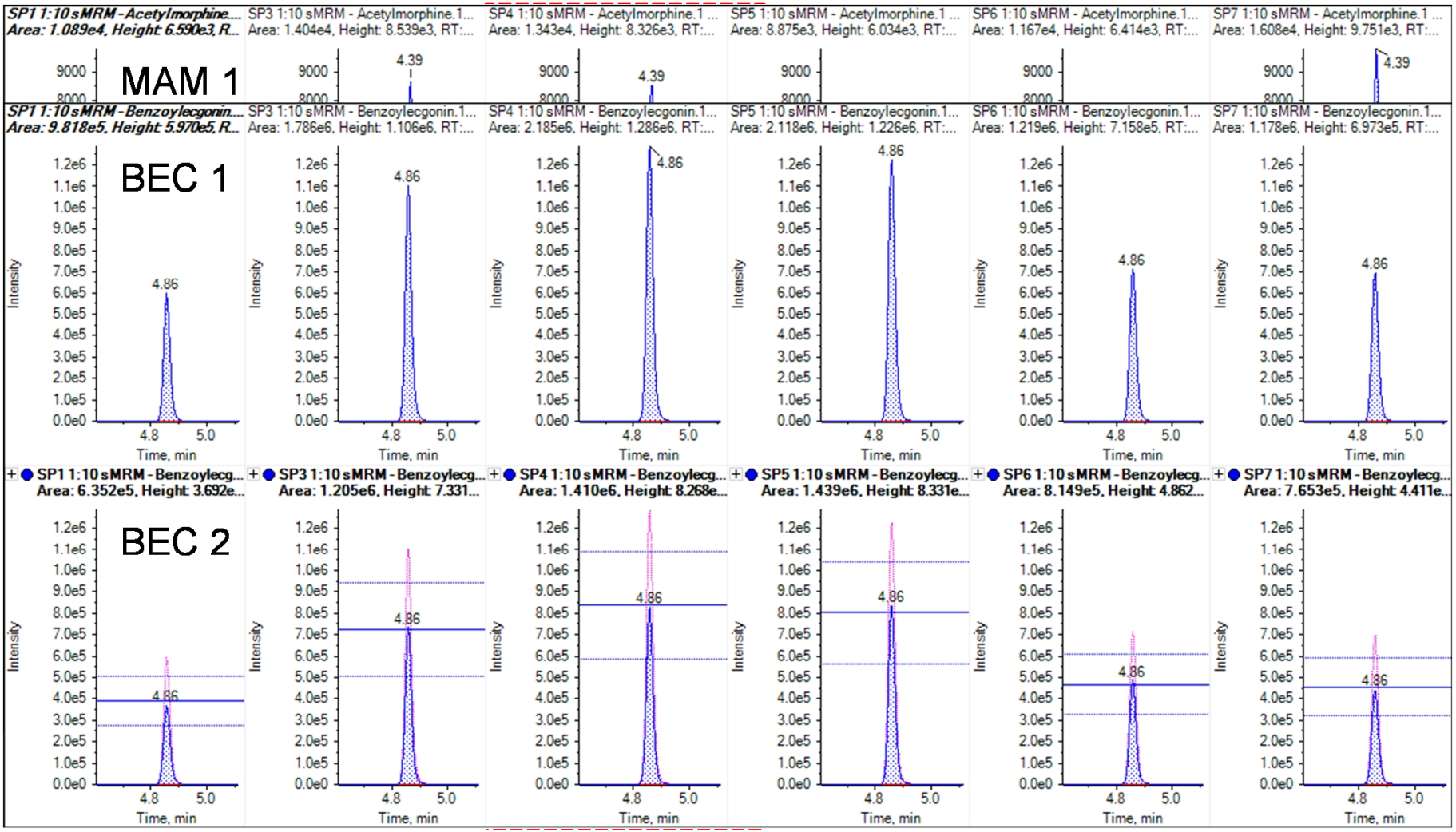
Day 4 = Street Parade Zürich



Monoacetylmorphine is the indicative metabolite for heroine consumption, concentration in wastewater did not change during Street Parade.

Monoacetylmorphine, Benzoylecgonine, and Ecstasy in Waste Water Samples

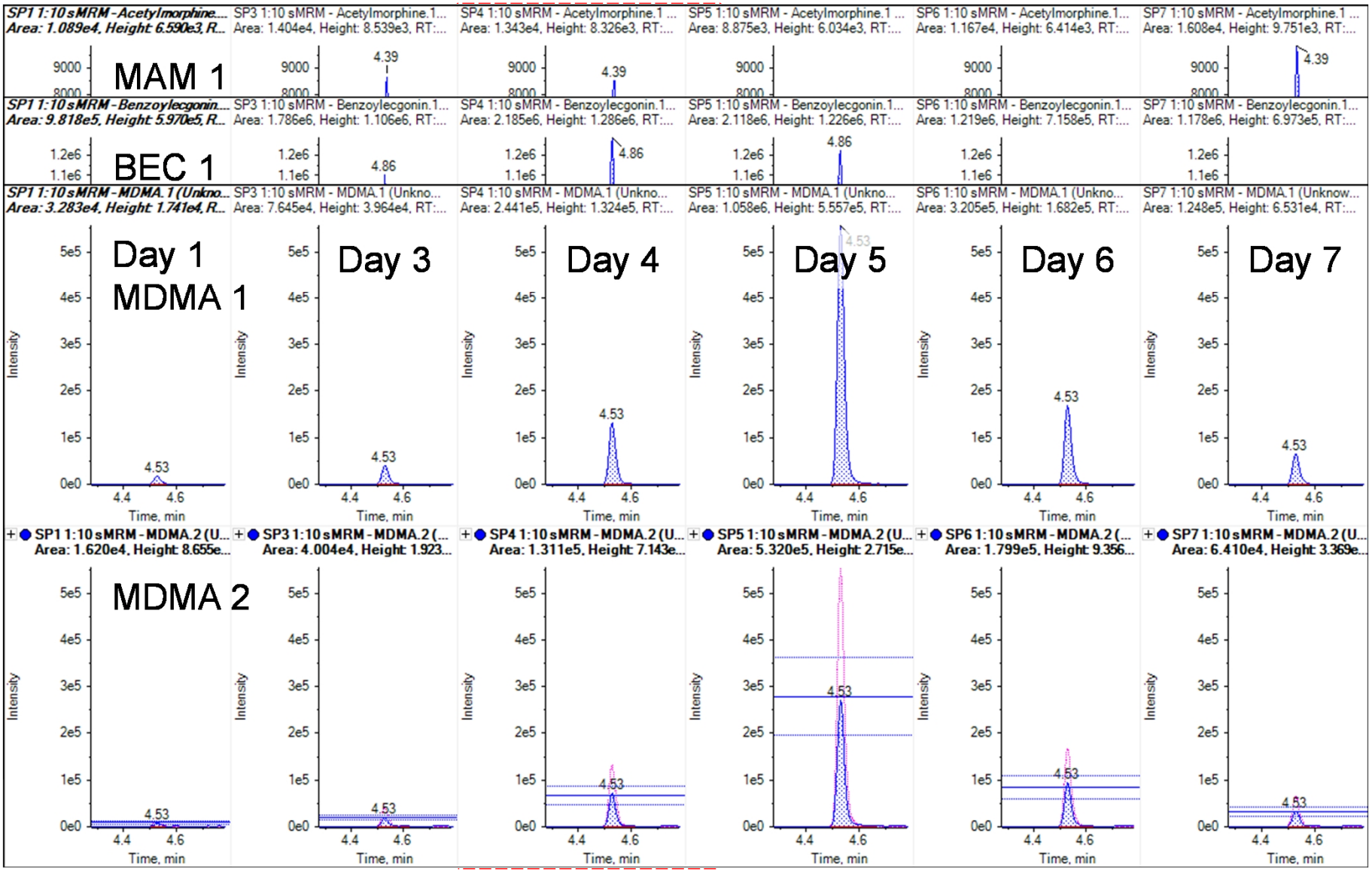
Day 4 = Street Parade Zürich



Increase in BEC concentration in wastewater = increase in cocaine consumption during Street Parade.

Monoacetylmorphine, Benzoylecgonine, and Ecstasy in Waste Water Samples

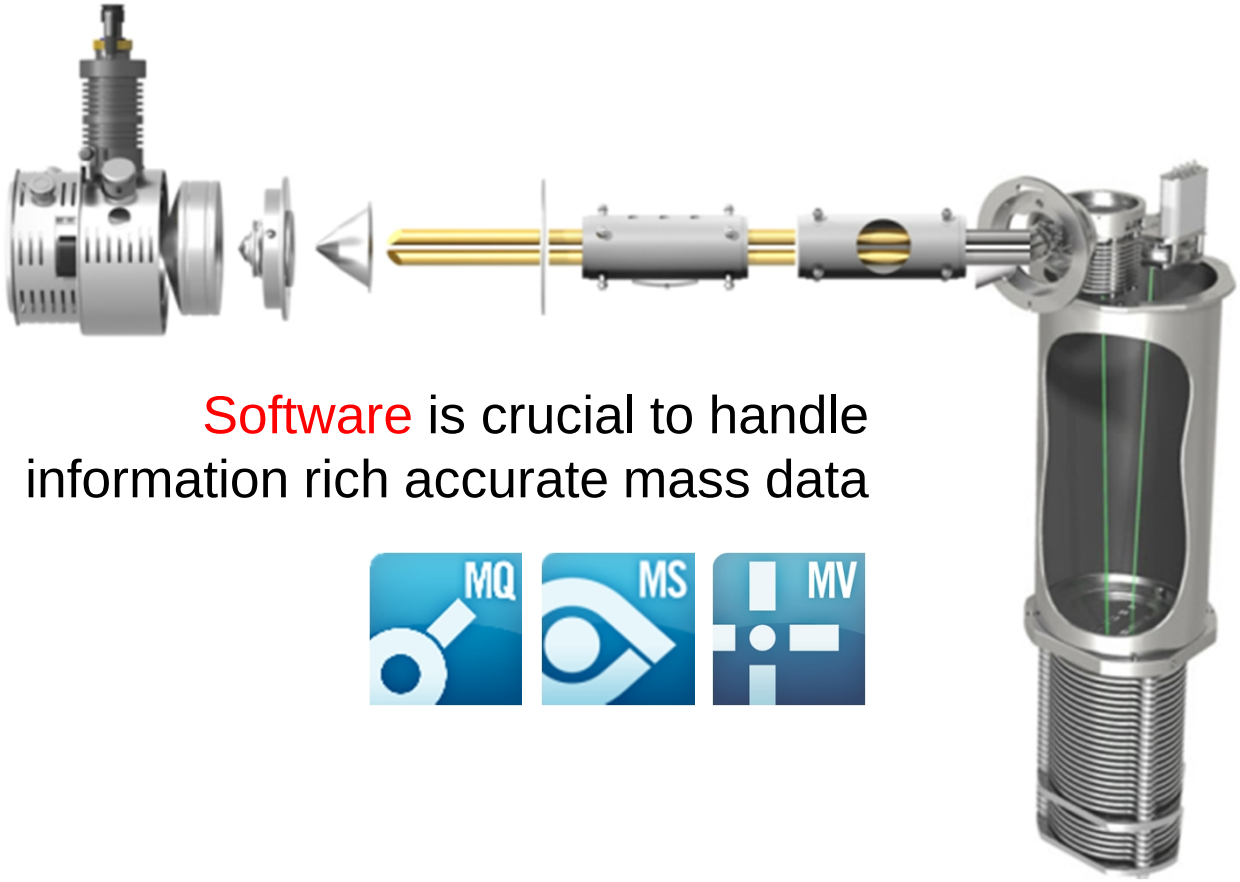
Day 4 = Street Parade Zürich



HR-MS/MS in Environmental Analysis

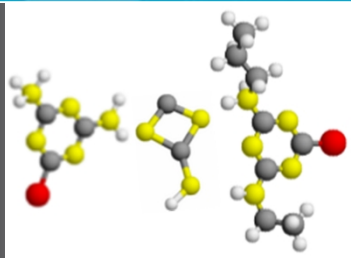
Considerations on Non-Targeted (Unknown) Detection

- High resolution and accurate mass TripleTOF® system
  - MRM like quantitation using narrow extracted ion chromatograms
  - Identification of known and unknown compounds with high confidence using MS and MS/MS information



Software is crucial to handle information rich accurate mass data

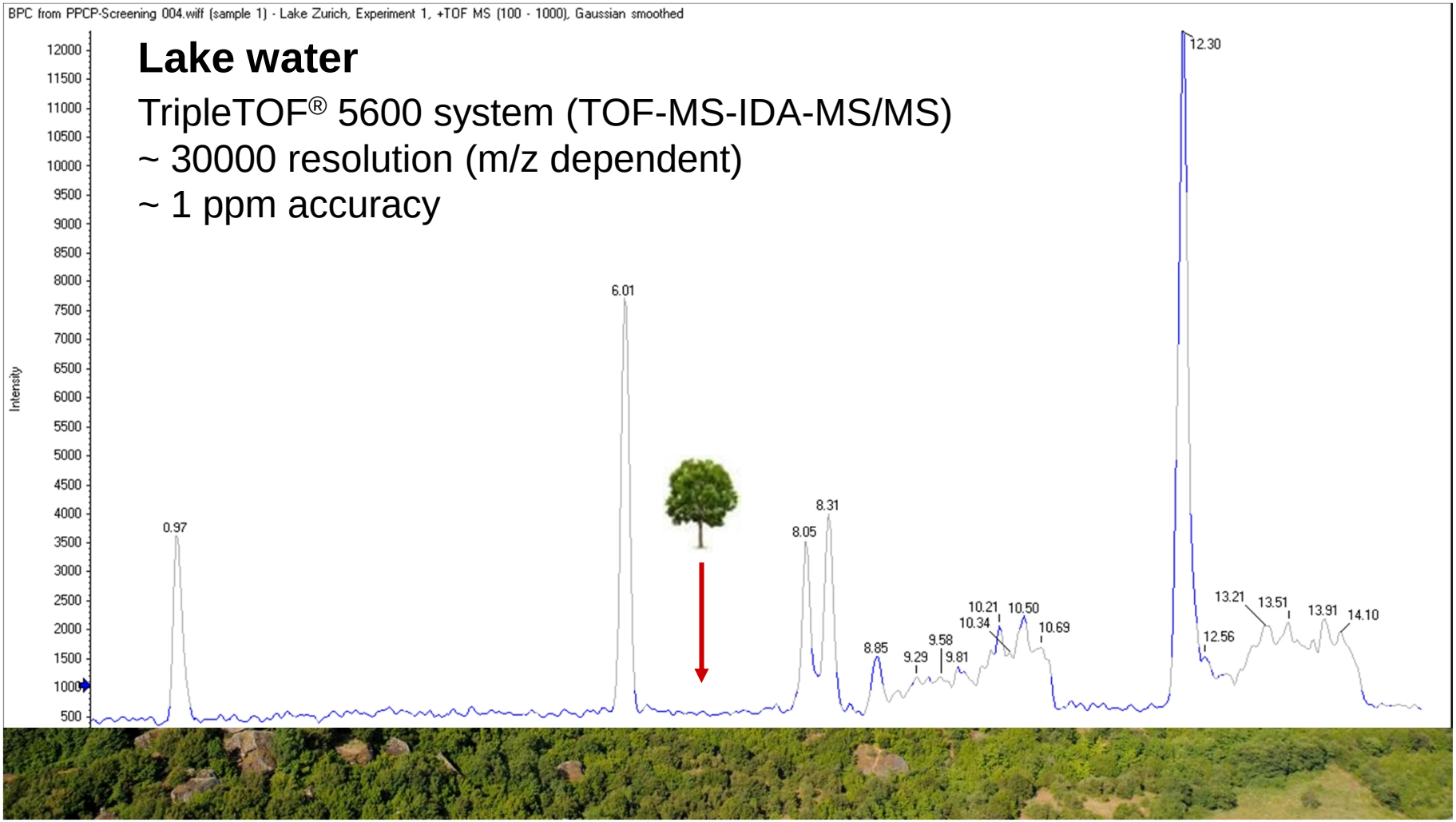
Principle of TOF Mass Spectrometry



- Ions are pulsed and accelerated into the TOF analyzer
- Separation of ions (m/z) based on time to travel through the flight path (measured on a picosecond to nanosecond time scale)
  - Small m/z faster than big m/z
  - It's like in sports: the big guy will finish last

Finding Unexpected Environmental Pollutants

It's Like Finding a Tree in a Forest (of LC-MS Peaks)

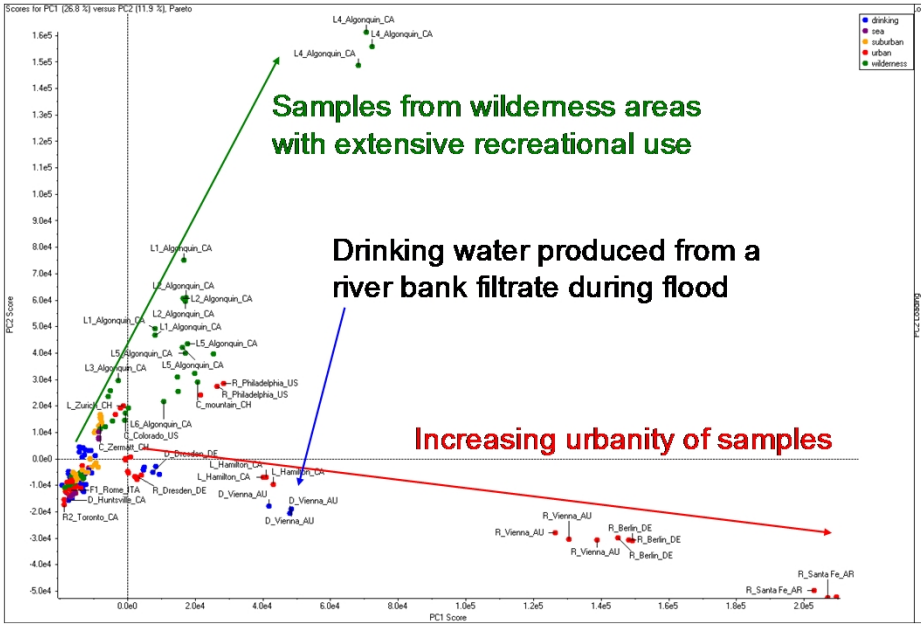


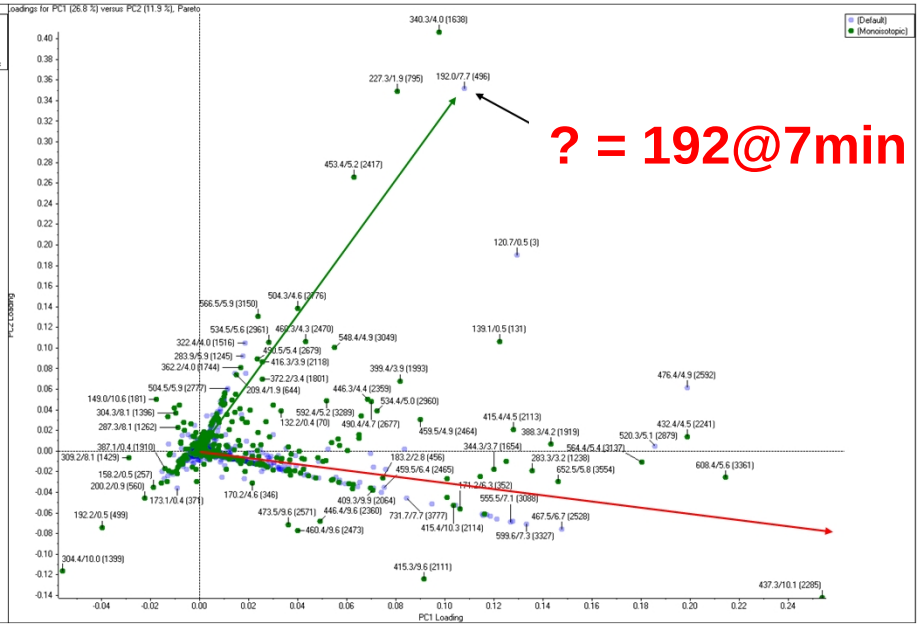
Find the Unexpected with Statistical Analysis

Principal Components Analysis (PCA) Shows Trends

1. Unknown Screening (PCA)

2. Identification





Scores-plot

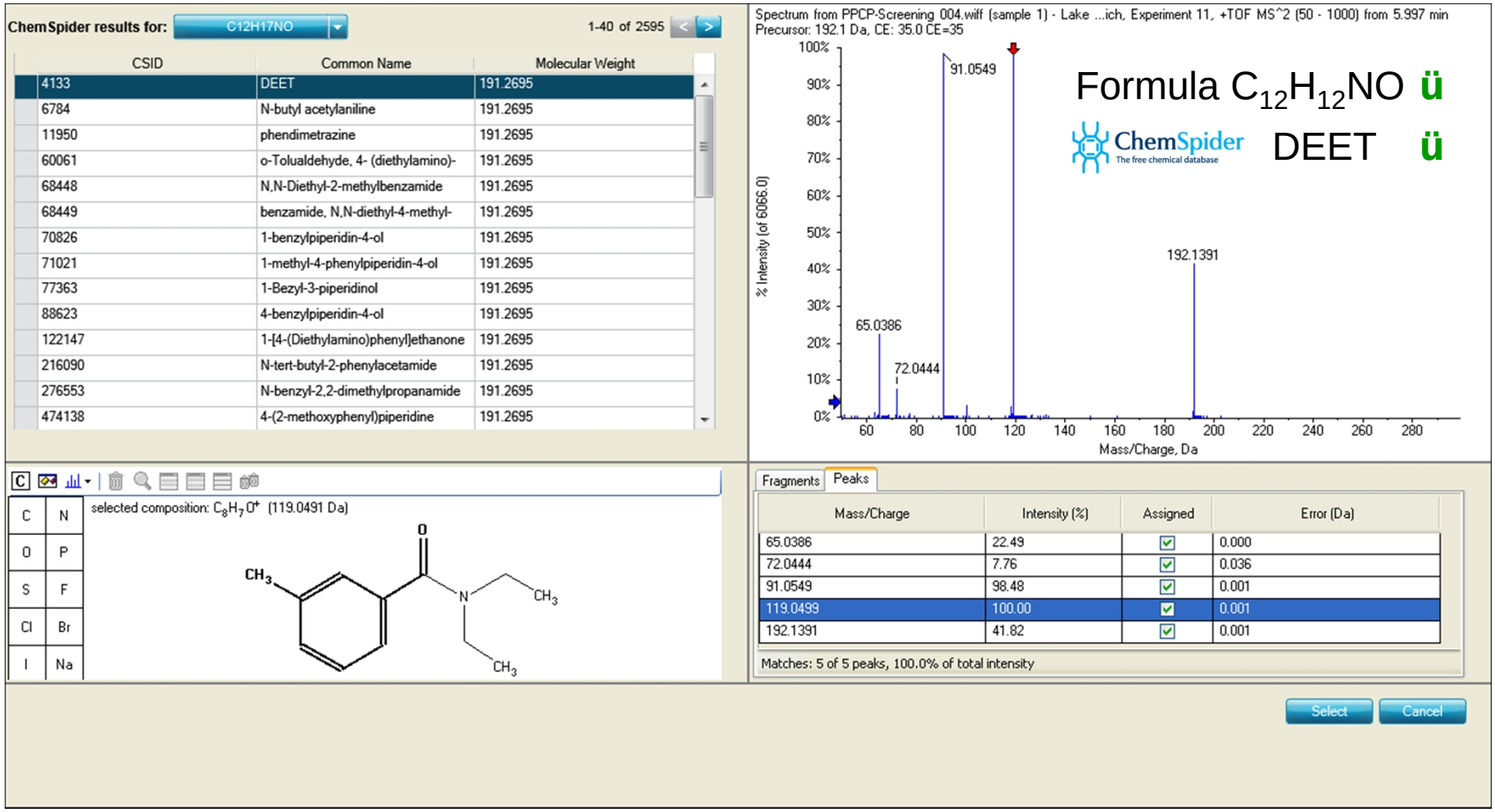
Loadings-plot

What sample is important?

What is in this sample?
RT and m/z

Identification of “Known Unknown Compounds”

Formula Finding and ChemSpider Searching (HR-MS and MS/MS)

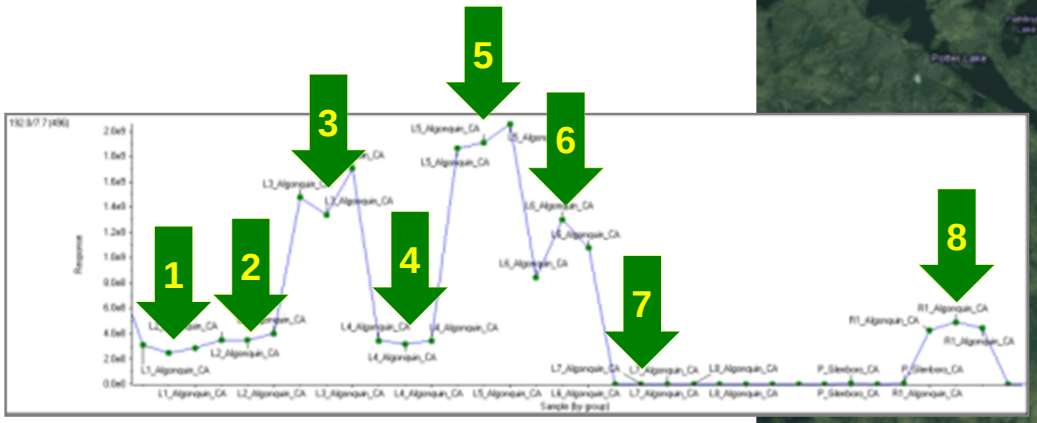


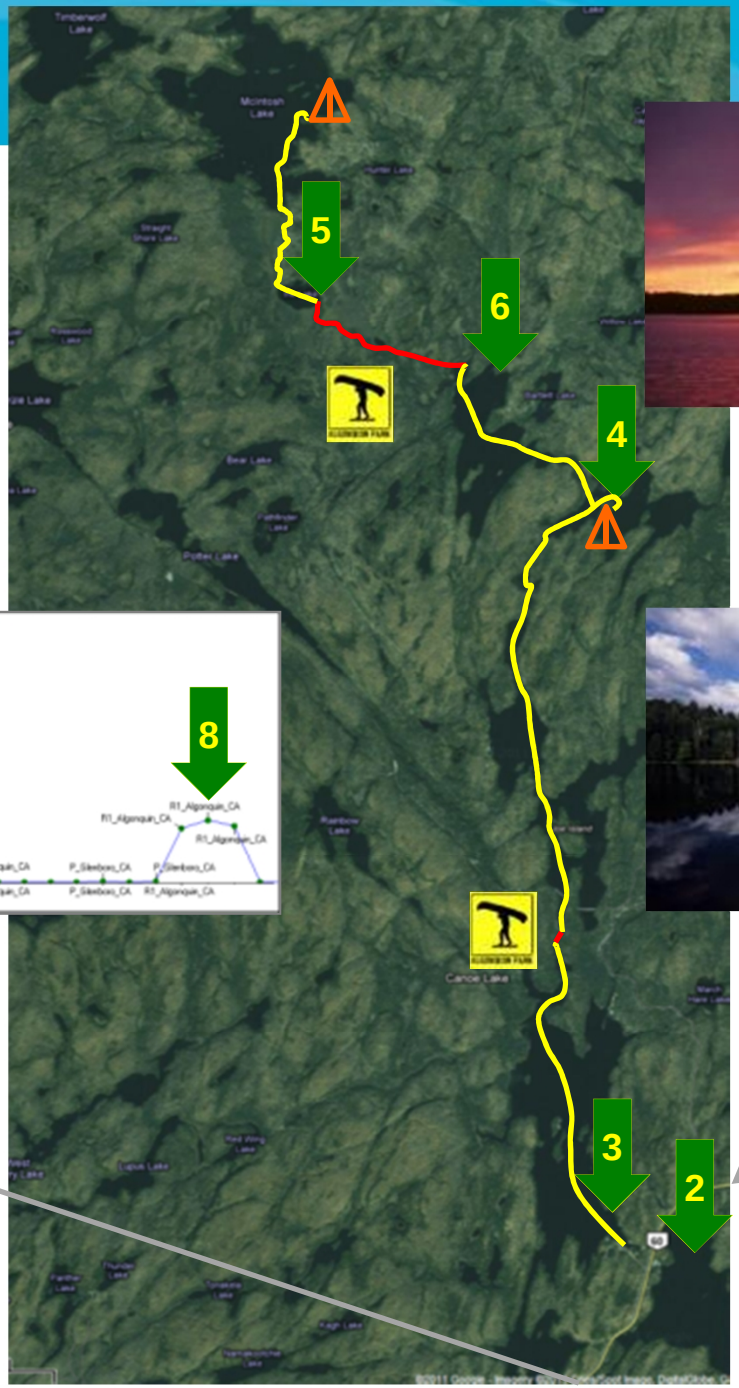
Automatic identification in MasterView™ software

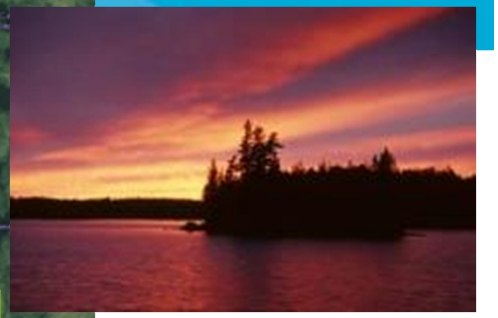
Quantitation of DEET

Water Samples of Algonquin Provincial Park, ON (Canada)

DEET concentration changes with touristic activities







Summary

- LC-MS/MS strategies for monitoring of emerging environmental pollutants
  - Statistical sampling for unknown screening
  - Direct injection vs. extraction
  - Generic LC separation
  - MS/MS detection
    - Triple quadrupole MRM
    - *Scheduled* MRM™ algorithm for multi-target methods
    - MRM ratio and QTRAP® full scan for identification
    - Accurate mass TripleTOF® MS and MS/MS for quantitation and identification of known and unknown compounds

Trademarks/Licensing

The author likes to thank

- Jean-Daniel Berset (Water and Soil Protection Laboratory, Bern, Switzerland)
- Colleagues of SCIEX for collecting water samples all over the world during their vacation or while attending conferences.

Thank you for your attention!

Morning mist over Mink Lake in Algonquin Provincial Park, ON (Canada)