



Screening of Emerging Contaminants in Water by HRAM MS

Tarun Anumol¹; Jerry Zweigenbaum¹; Linda Kennedy²

¹Agilent Technologies Inc.

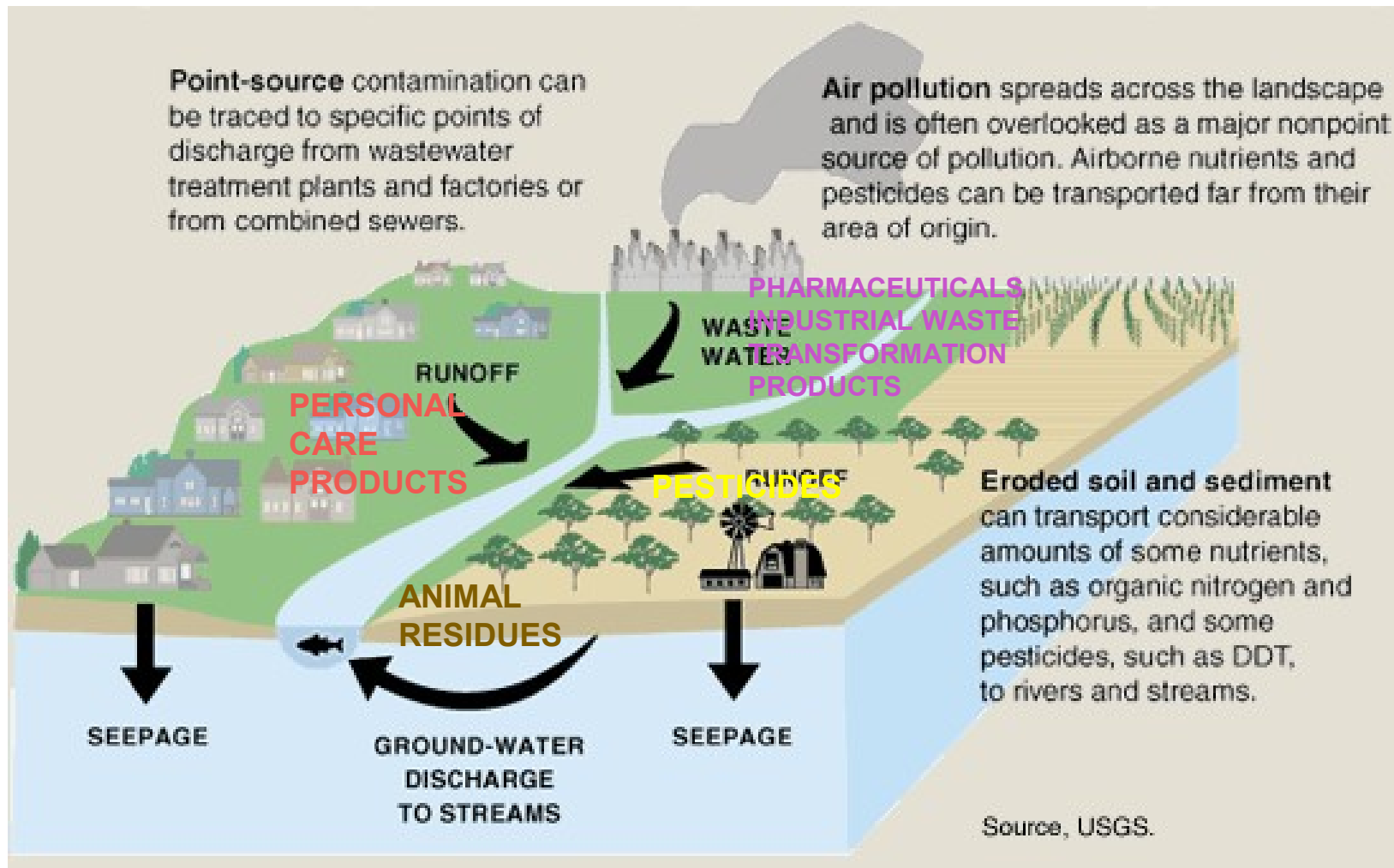
²Mansfield University

August 19, 2016

Confidentiality Label

1

WATER CONTAMINATION



Water Contamination

Media and Public Awareness

More health news on  **NBC NEWS**.com

Pharmaceuticals lurking in U.S. drinking water

AP probe found traces of meds in water supplies of 41 million Americans

Energy and Environment

The Washington Post

Pesticides are polluting our waters — and we often don't know it

Fracking Chemicals Detected in Pennsylvania Drinking Water

By NICHOLAS ST. FLEUR MAY 4, 2015

The New York Times

Fears About Water Supply Grip Village That Made Teflon Products

Water Contaminants



Pain Points for Analysis



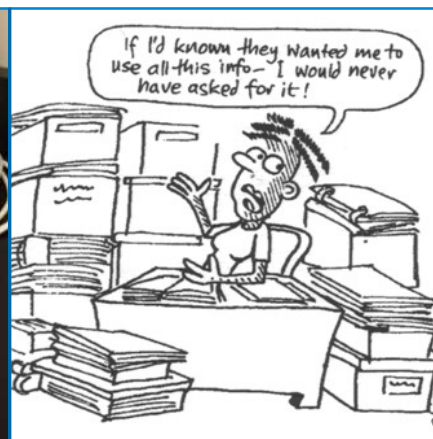
Compounds
to monitor



Sample
Preparation

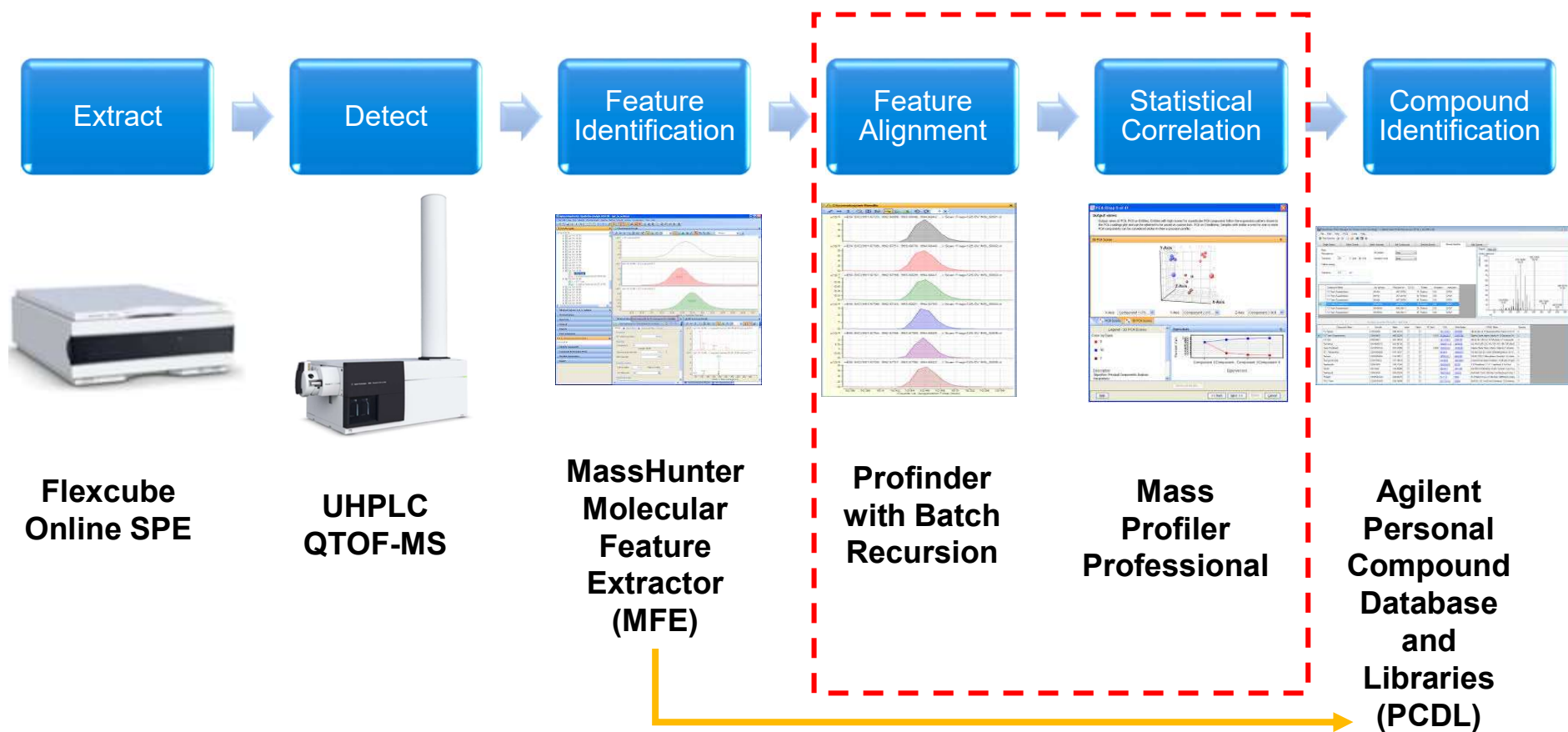


Standard
Preparation

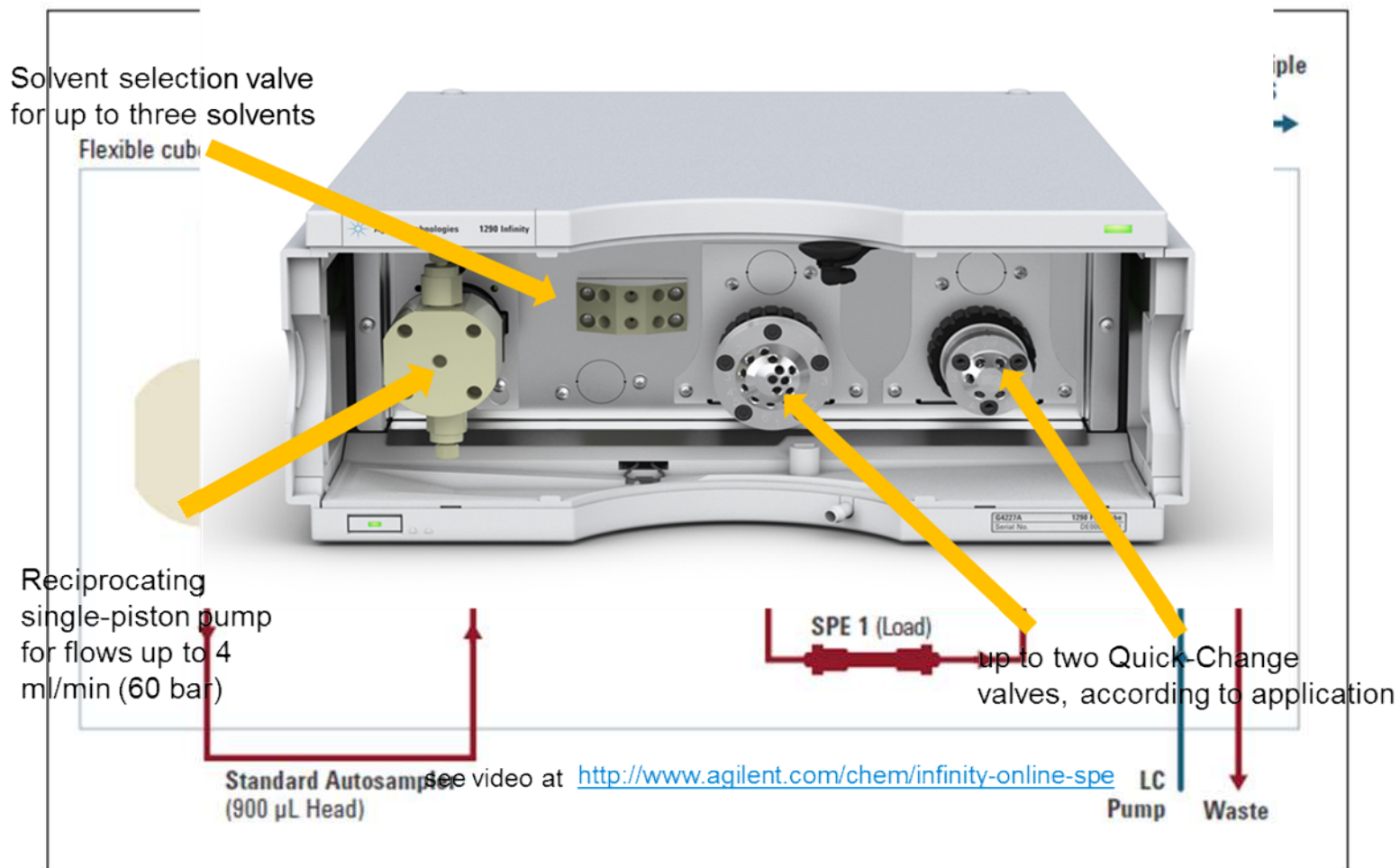


Data
Analysis and
Interpretation

Sample Analysis - Workflow

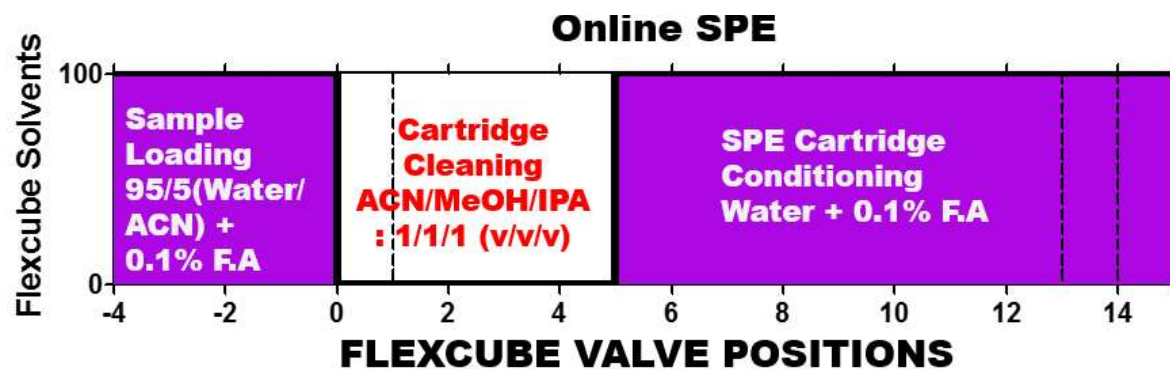


Automated Solid Phase Extraction Flexcube



Method Parameters

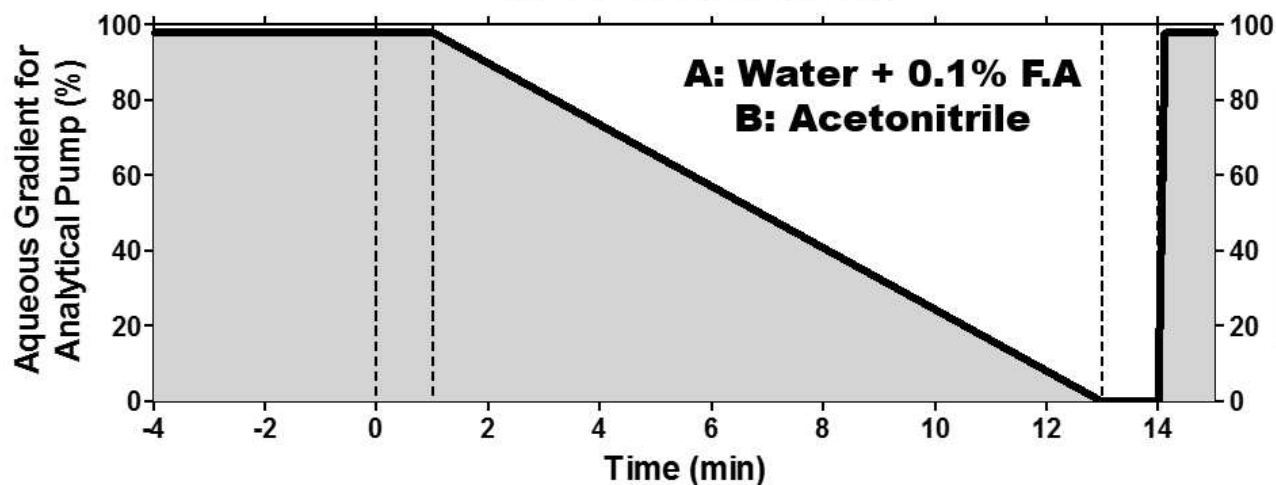
Flexcube + LC



**SPE Cartridge: PLRP-s,
2.1x12.5 mm
Flowrate: 1 mL/min**



HPLC SEPERATION



**Column: Poroshell EC
120 C-18, 2.1x100 mm
(2.7 μ m)
Flowrate: 0.35 mL/min
Injection Volume: 900
 μ L**

Method Parameters

6550 QTOF-MS



Parameter	Condition
Instrument	Agilent 6550 Accurate-Mass QToF-MS with iFunnel technology
Ionization Mode	Positive electrospray ionization with dual jet stream
Instrument Mode	2 GHz extended dynamic range
Instrument Tune Range	Classic tune (m/z: 50-1700)
Mass Range	m/z: 50-1000
Drying Gas Temperature	130 °C
Drying Gas Flow	16 L/min
Sheath Gas Temperature	375 °C
Sheath Gas Flow	11 L/min
Nebulizer Gas	35 psi
Fragmentor	365 V
Capillary	3500

Agilent 6550 LC-QTOF MS

iFunnel Technology

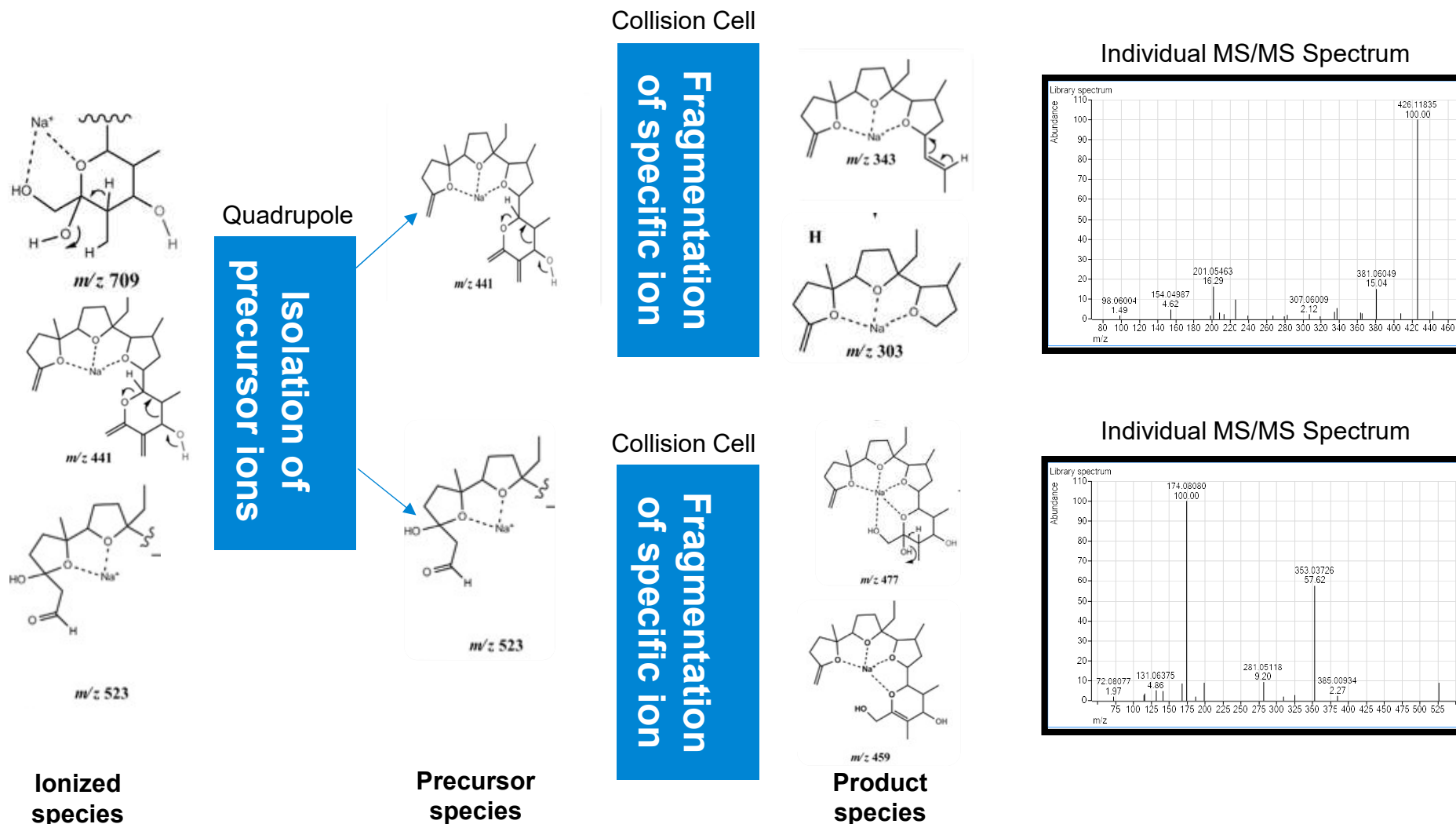


- **5-10x increase in ions sampled**
- **Enhanced sensitivity**



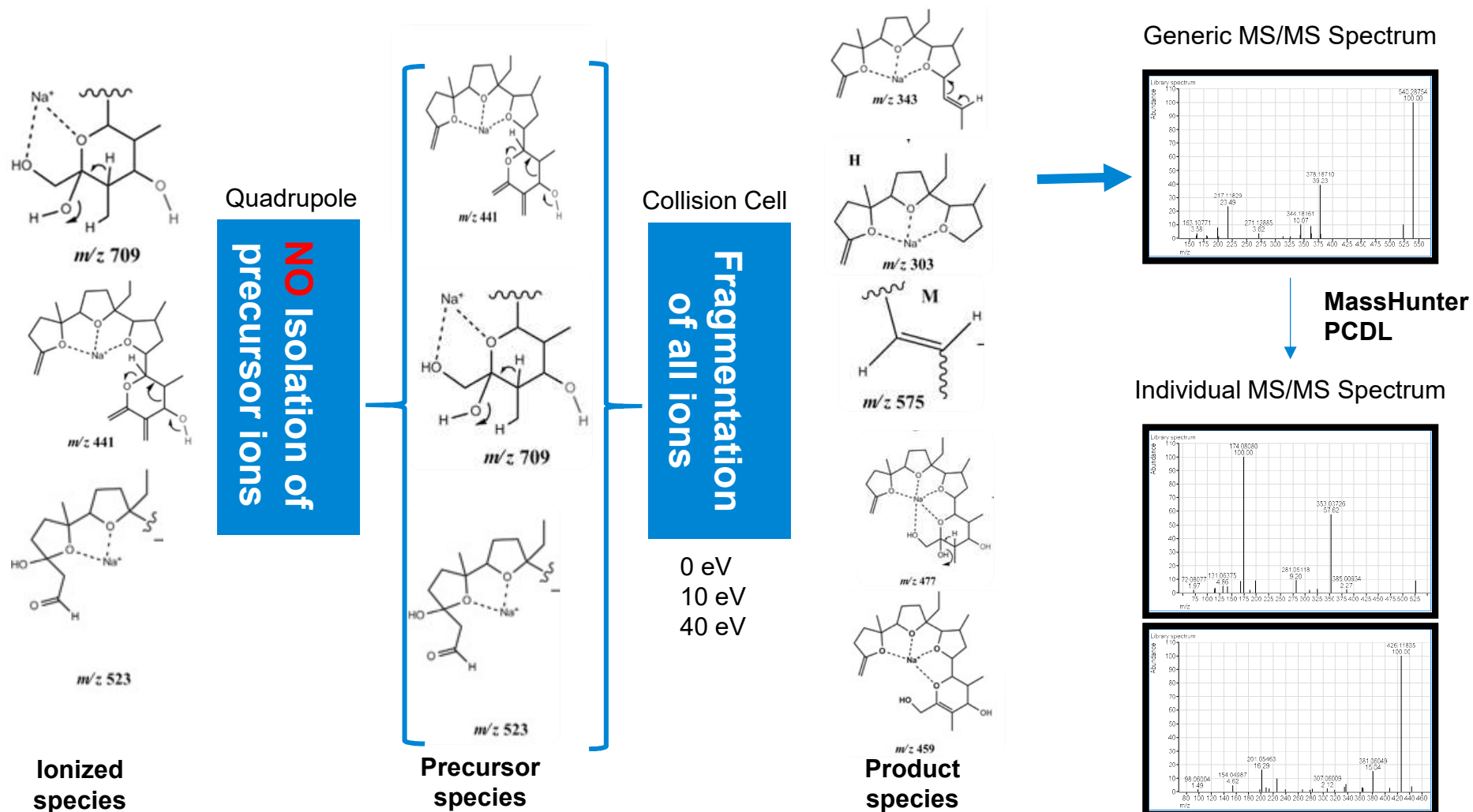
Analysis of Emerging Compounds

Traditional MS/MS



Analysis of Emerging Compounds

All Ions MS/MS



Agilent Technologies

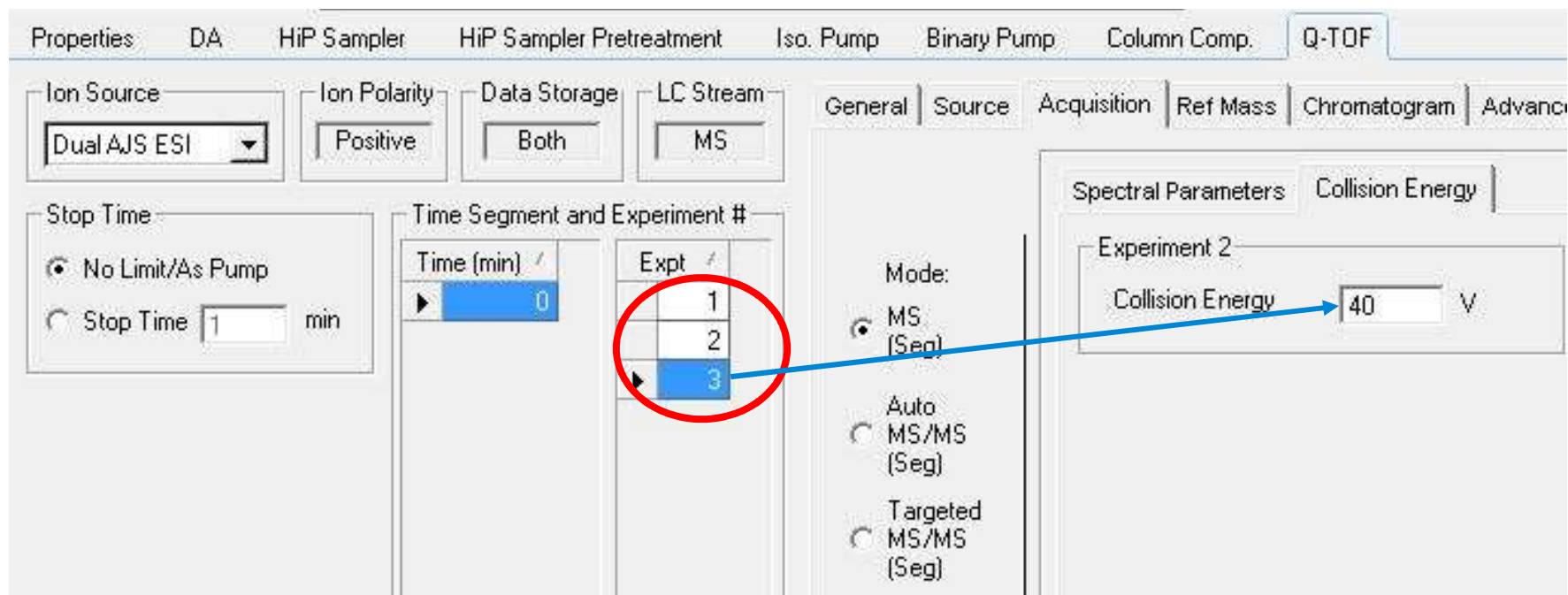
Confidentiality Label

19 August 2016

12

Analysis of Emerging Compounds

All Ions MS/MS Data Acquisition



- ❑ Three Collision energy experiments performed simultaneously at 0, 10 & 40 eV
- ❑ Spectral acquisition at 4.5 spectra/sec

All Ions MS/MS

Workflow – Find By Formula

Method Editor: Find Compounds by Formula - Options

Find Compounds by Formula | Method Items

Scoring | Results | Result Filters | Fragment Confirmation

Formula Source | Formula Matching | Positive Ions | Negative Ions

Source of formulas to confirm

☐ These formulas:
(type a comma-separated list of formulas, e.g., "C6H6, CH4")

☐ Compound exchange file (.CEF):

☒ Database / Library
D:\MassHunter\PCDL\WetDrugs_Std.cdb

☐ Worklist

Matches per formula

Maximum number of matches: 1

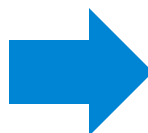
☒ Automatically increase for isomeric compounds

Values to match

☐ Mass

☒ Mass and retention time (retention time optional)

☐ Mass and retention time (retention time required)



Method Editor: Find Compounds by Formula - Options

Find Compounds by Formula | Method Items

Formula Source | Formula Matching | Positive Ions | Negative Ions | Results | Result Filters | Fragment Confirmation

Search fragment ions

☒ Confirm with fragment ions

☐ Molecular ion optional

Fragment ion source

☒ Use spectral library only

☐ Use average fragment spectrum if spectral library not available

Number of most specific ions from spectral library: 5

Number of most specific ions from average fragment spectrum: 7

Fragment ion EIC qualification settings

RT difference +/-: 0.10 min. of expected RT

☒ S/N ratio >=: 900

Coelution score >=: 90

Fragment ion confirmation criteria

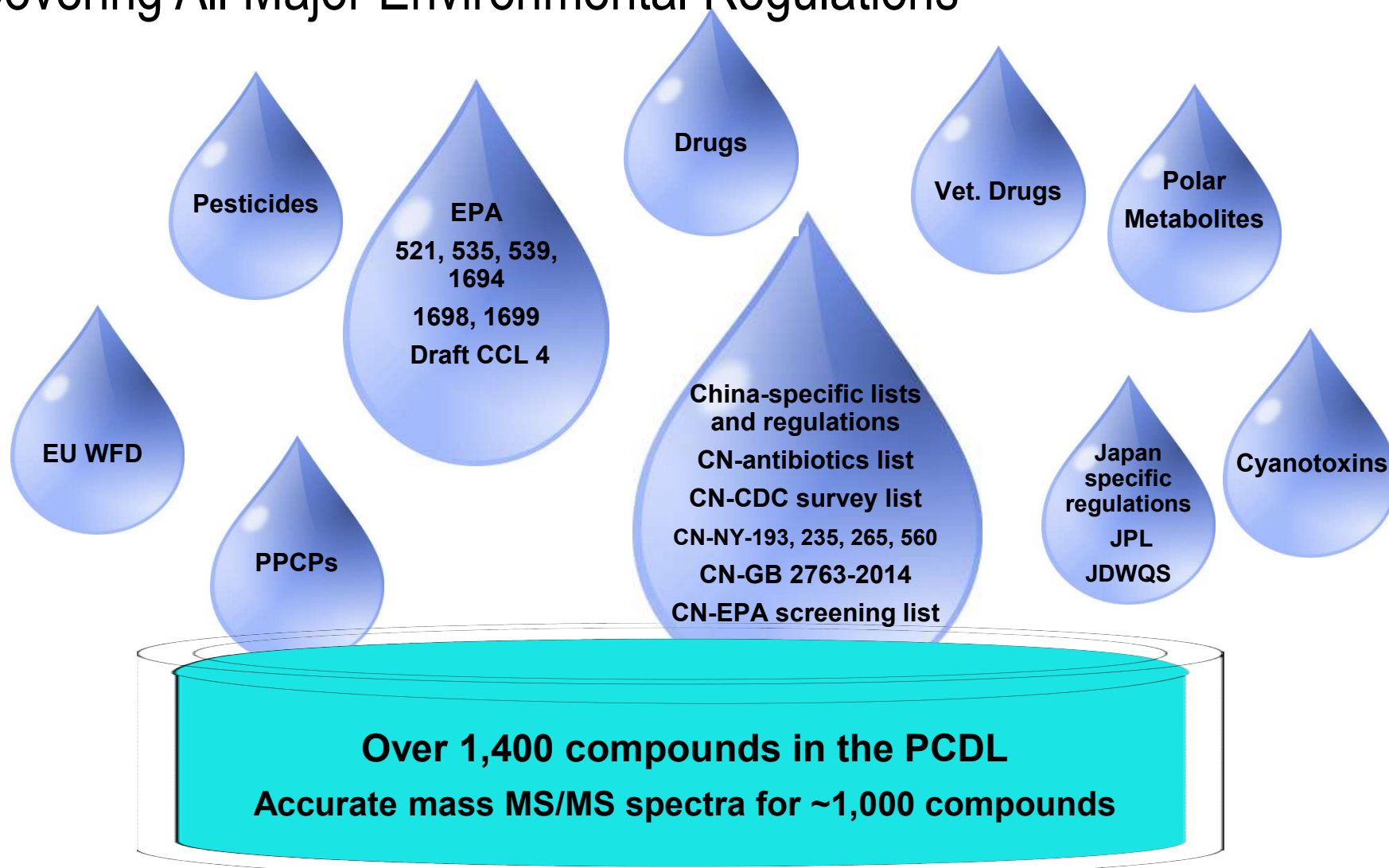
☒ Minimum number of qualified fragments: 1

☐ Minimum percent of qualified fragments: 75



Water Screening PCDL

Covering All Major Environmental Regulations



Analysis of Emerging Contaminants

Personal Compound Database and Library (PCDL)

MassHunter PCDL Manager for Forensics and Toxicology - C:\MassHunter\PCDL\VetDrugs_AM_PCDL.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Mass
Precursor ion:
Tolerance: 200 ppm mDa
Ion polarity: (Any)
Ionization mode: (Any)
Collision energy
Tolerance: 2.0 eV

Spectra for compound: Sulfamethizole

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization
Sulfamethizole	(M+H)+	271.03179	10	Positive	ESI
Sulfamethizole	(M+H)+	271.03179	20	Positive	ESI
Sulfamethizole	(M+H)+	271.03179	40	Positive	ESI
Sulfamethizole	(M-H)-	269.01724	10	Negative	ESI
Sulfamethizole	(M-H)-	269.01724	20	Negative	ESI
Sulfamethizole	(M-H)-	269.01724	40	Negative	ESI

Library spectrum

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Spectra
Acetazolamide	C4H6N4O3S2	221.98813				59-66-5	1909	N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide	6
Bufexamac	C12H17NO3	223.12084				2438-72-4	2372	2-(4-Butoxyphenyl)-N-hydroxyacetamide	6
Nabumetone	C15H16O2	228.11503				42924-53-8	4256	4-(6-Methoxy-2-naphthyl)-2-butanone	6
Naproxen	C14H14O3	230.09429				22204-53-1	137720	(2S)-2-(6-Methoxy-2-naphthyl)propanoic acid	6
Nalidixic acid	C12H12N2O3	232.08479				389-08-2	4268	1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridi...	6
Methazolamide	C5H8N4O3S2	236.00378				554-57-4	3958	N-[(2Z)-3-Methyl-5-sulfamoyl-1,3,4-thiadiazol-2(3H...	6
Amino-mebendazole	C14H11N3O	237.09021				52329-60-9	36839	(2-amino-1H-benzimidazol-6-yl)(phenyl)methanone	6
Salbutamol (Albuterol)	C13H21NO3	239.15214				18559-94-9	1999	2-(Hydroxymethyl)-4-(1-hydroxy-2-[(2-methyl-2-pro...	6
Mefenamic acid	C15H15NO2	241.11028				61-68-7	3904	2-[(2,3-Dimethylphenyl)amino]benzoic acid	6
Oxibendazole	C12H15N3O3	249.11134				20559-55-1	4461	Methyl (5-propoxy-1H-benzimidazol-2-yl)carbamate	6



Agilent Technologies

Confidentiality Label

19 August 2016

16

Analysis of Veterinary Drugs in Meat

All Ions MS/MS Method Conditions



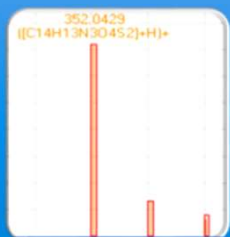
Find by Formula

- Database/Library Search
- Water Contaminant PCDL



Precursor Identification

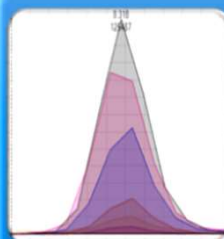
- Mass Tolerance: 10 ppm
- Retention Time Tolerance: 0.2 min
- Minimum Absolute Area Counts: 10,000



Molecular-Ion Assessment

- Mass accuracy
- Isotope abundance
- Isotope spacing

• Warn if <85.0 Reject if <50.0



Fragment Confirmation

Min. Number of fragments req.: 1
Minimum S/N: 9.0
Coelution score: >90.0



Agilent Technologies

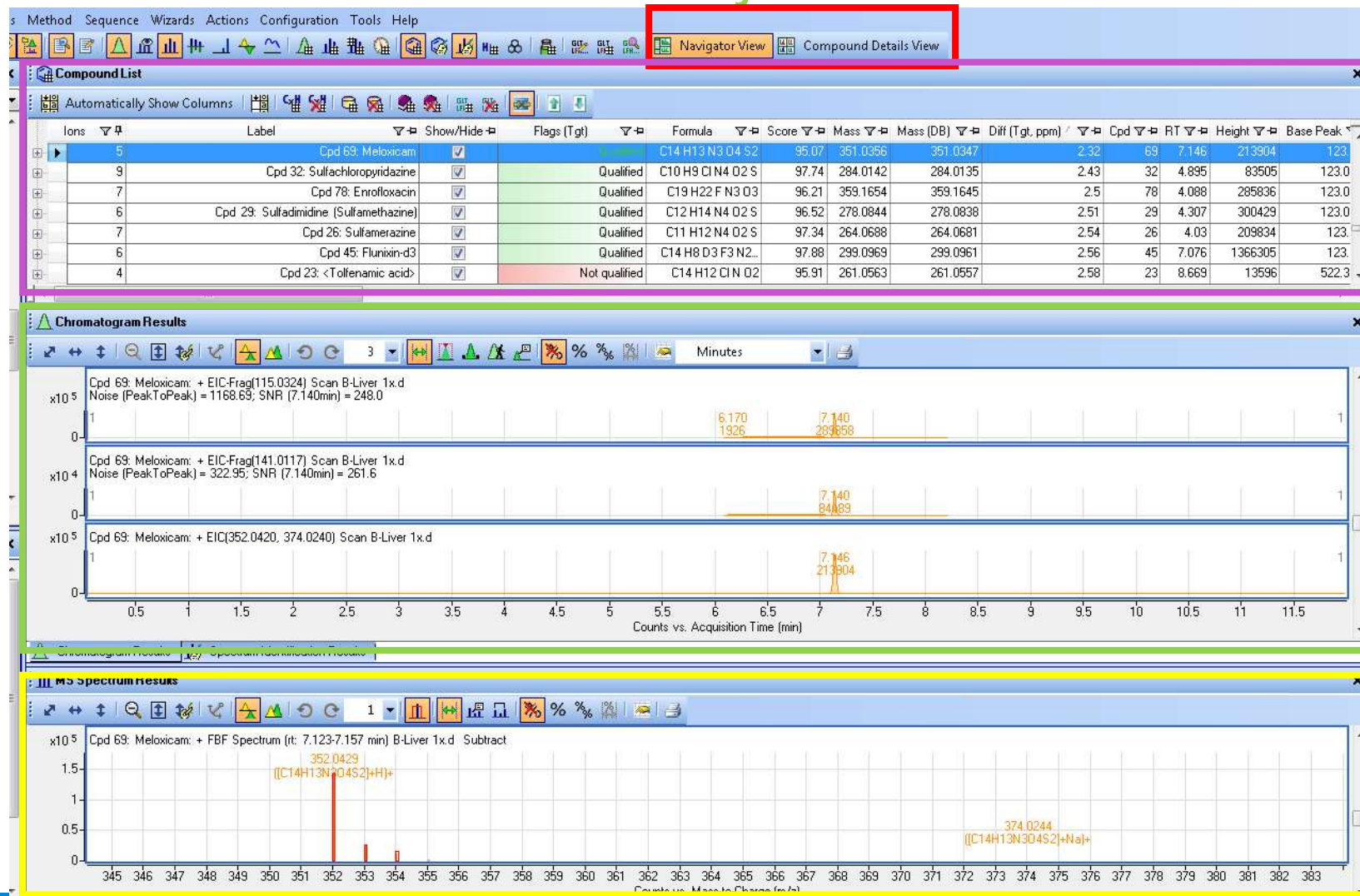
Confidentiality Label

19 August 2016

17

Analysis of Water Contaminants

All Ions MS/MS Data Analysis



Agilent Technologies

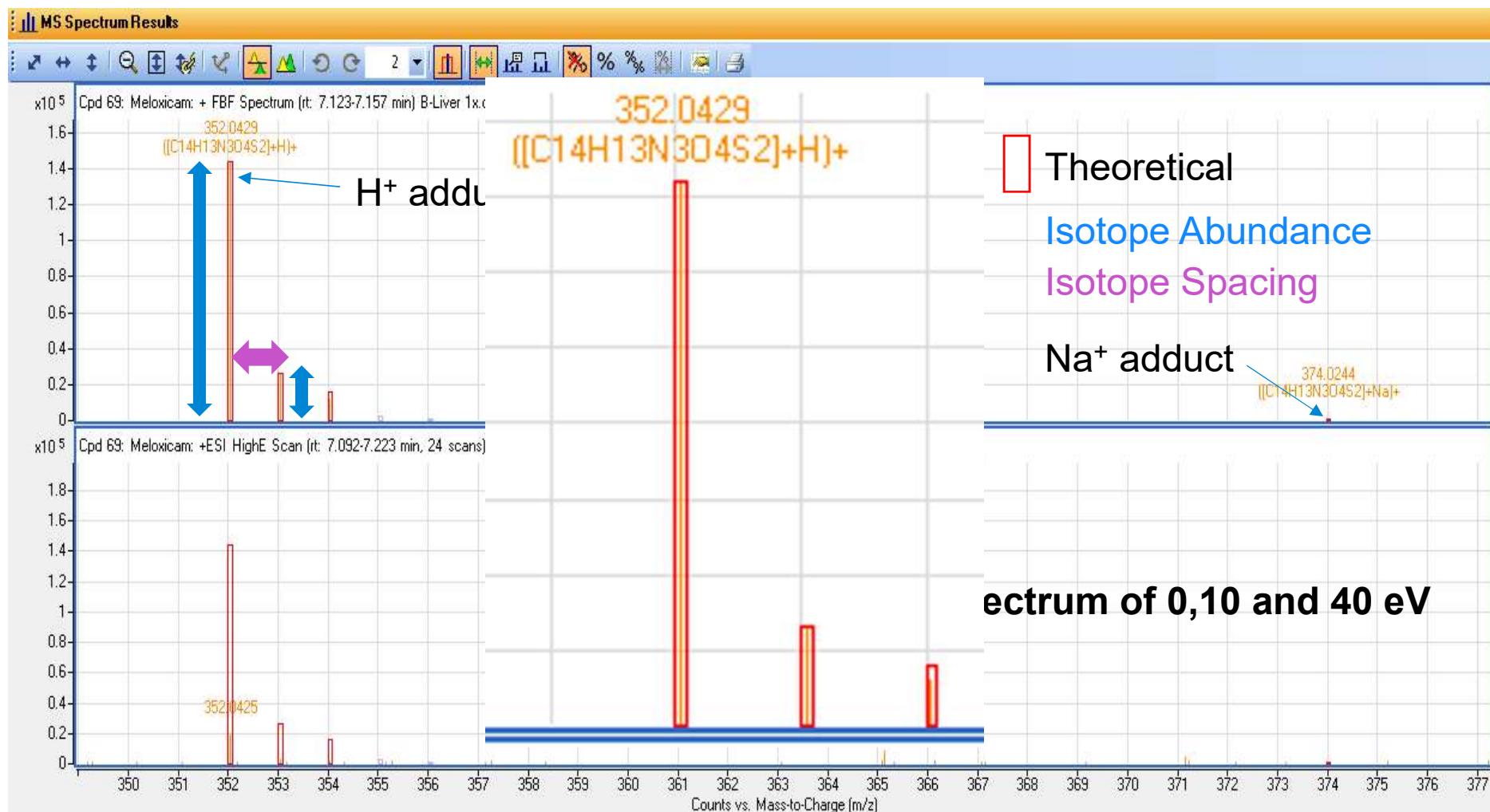
Confidentiality Label

19 August 2016

18

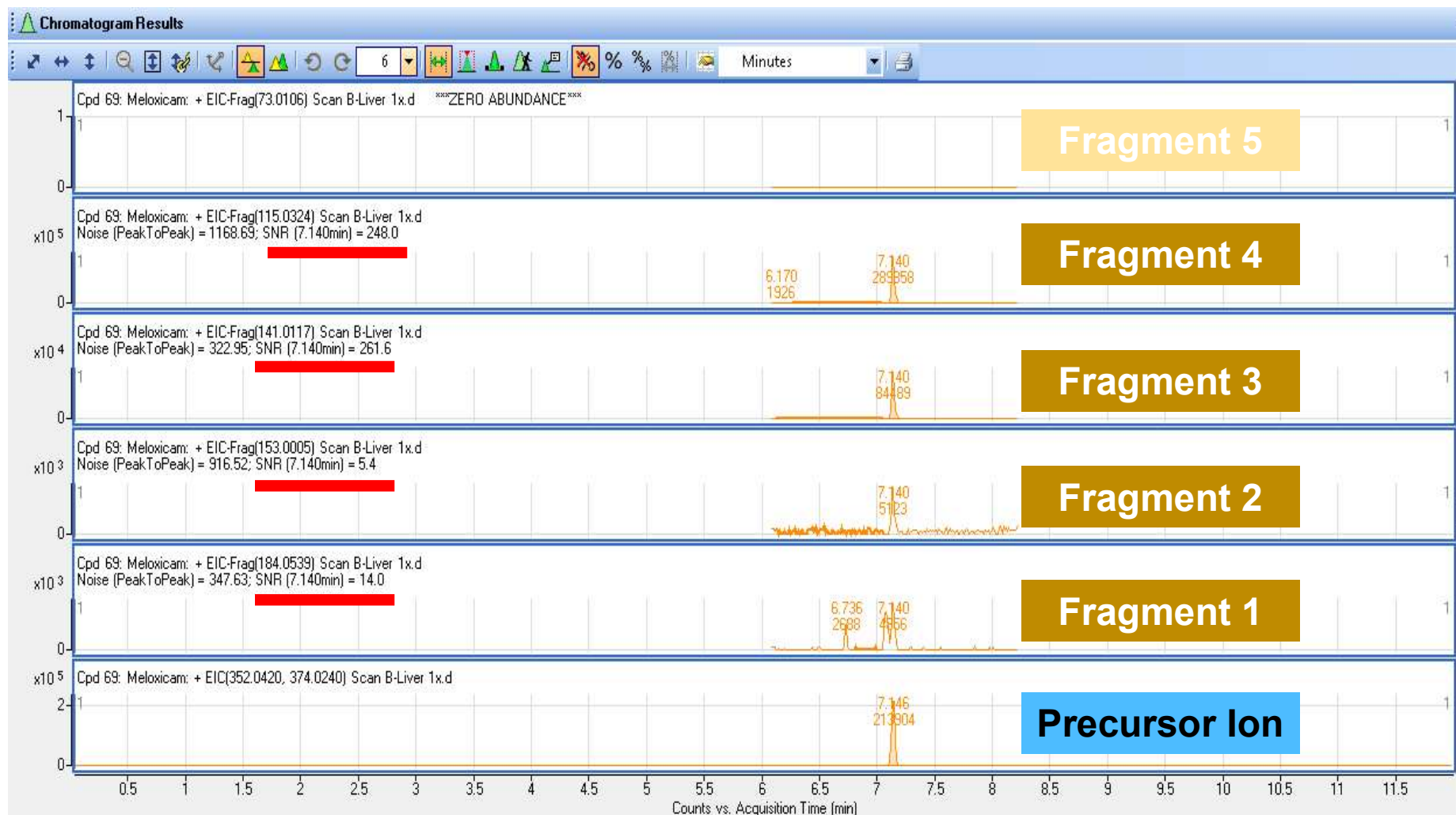
Analysis of Water Contaminants

All Ions MS/MS Data Analysis



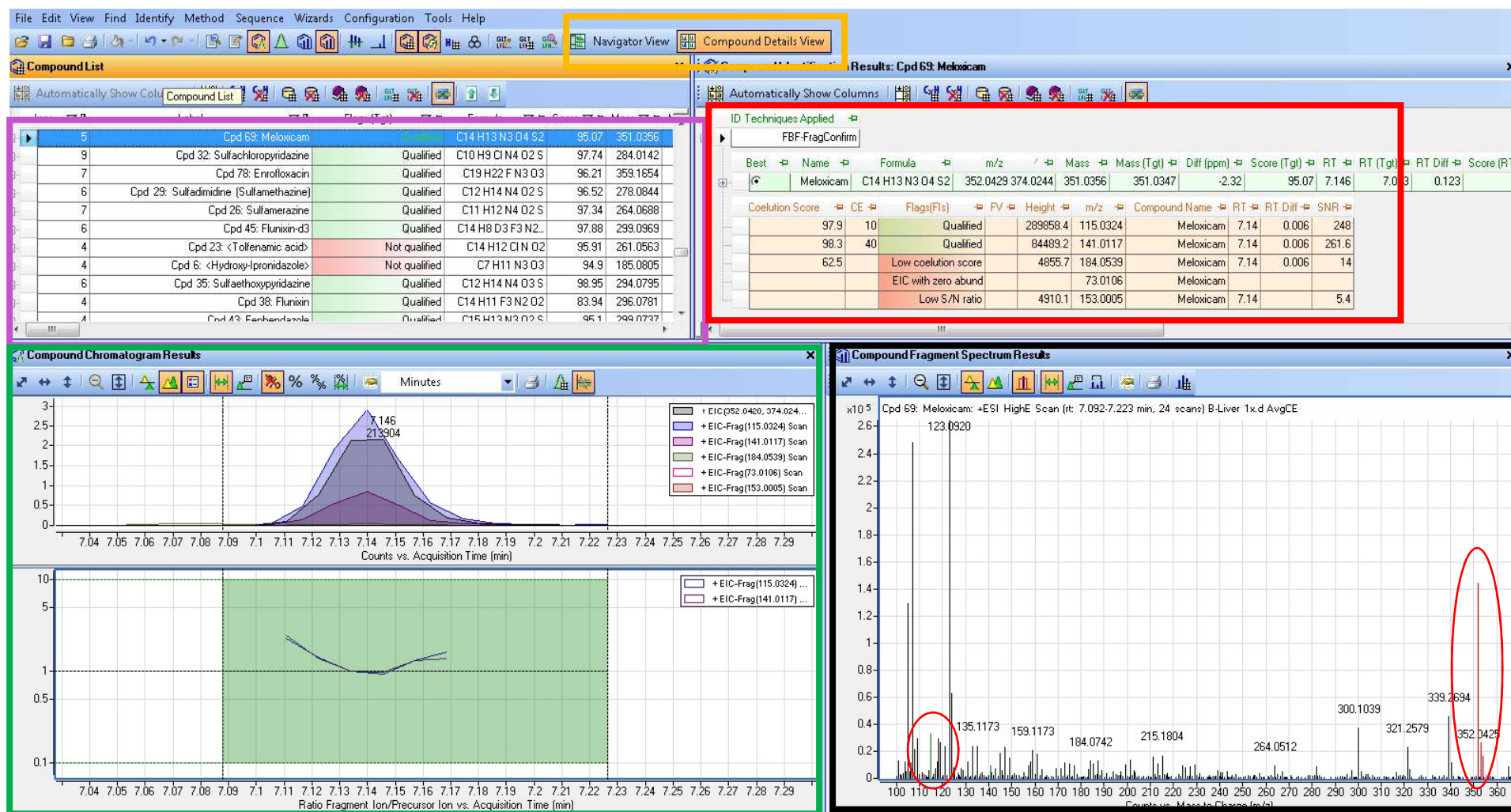
Analysis of Water Contaminants

All Ions MS/MS Data Analysis



Analysis of Water Contaminants

All Ions MS/MS Data Analysis



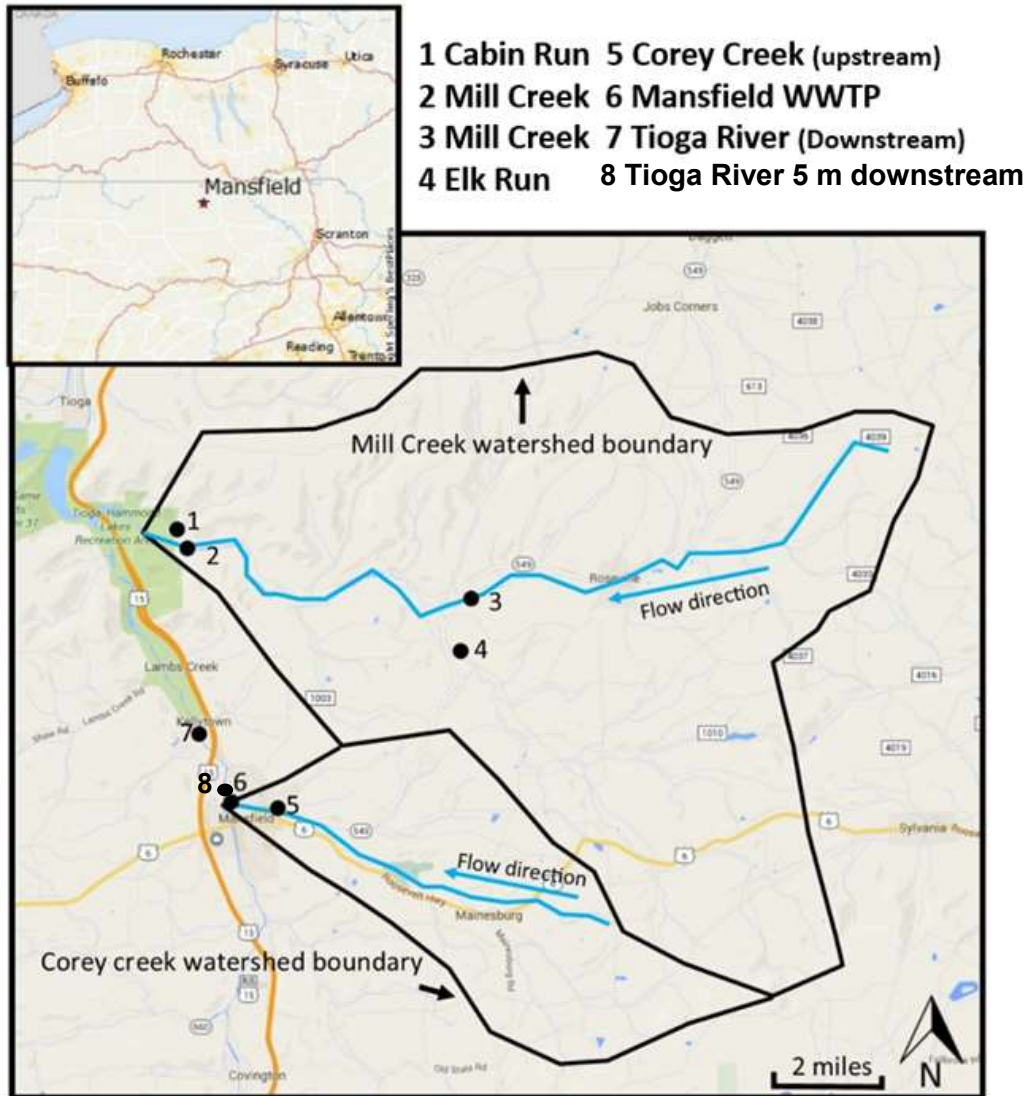
Agilent Technologies

Confidentiality Label

19 August 2016

21

Sampling Locations



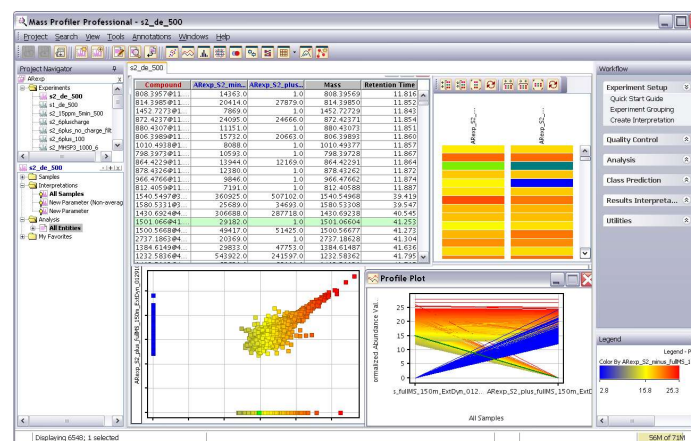
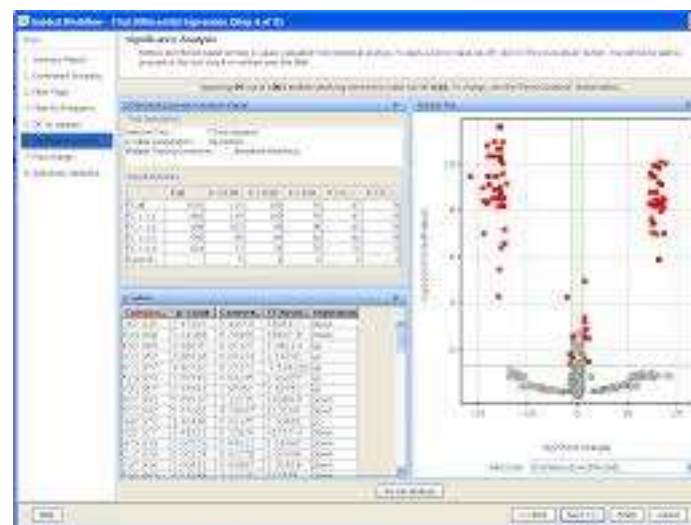
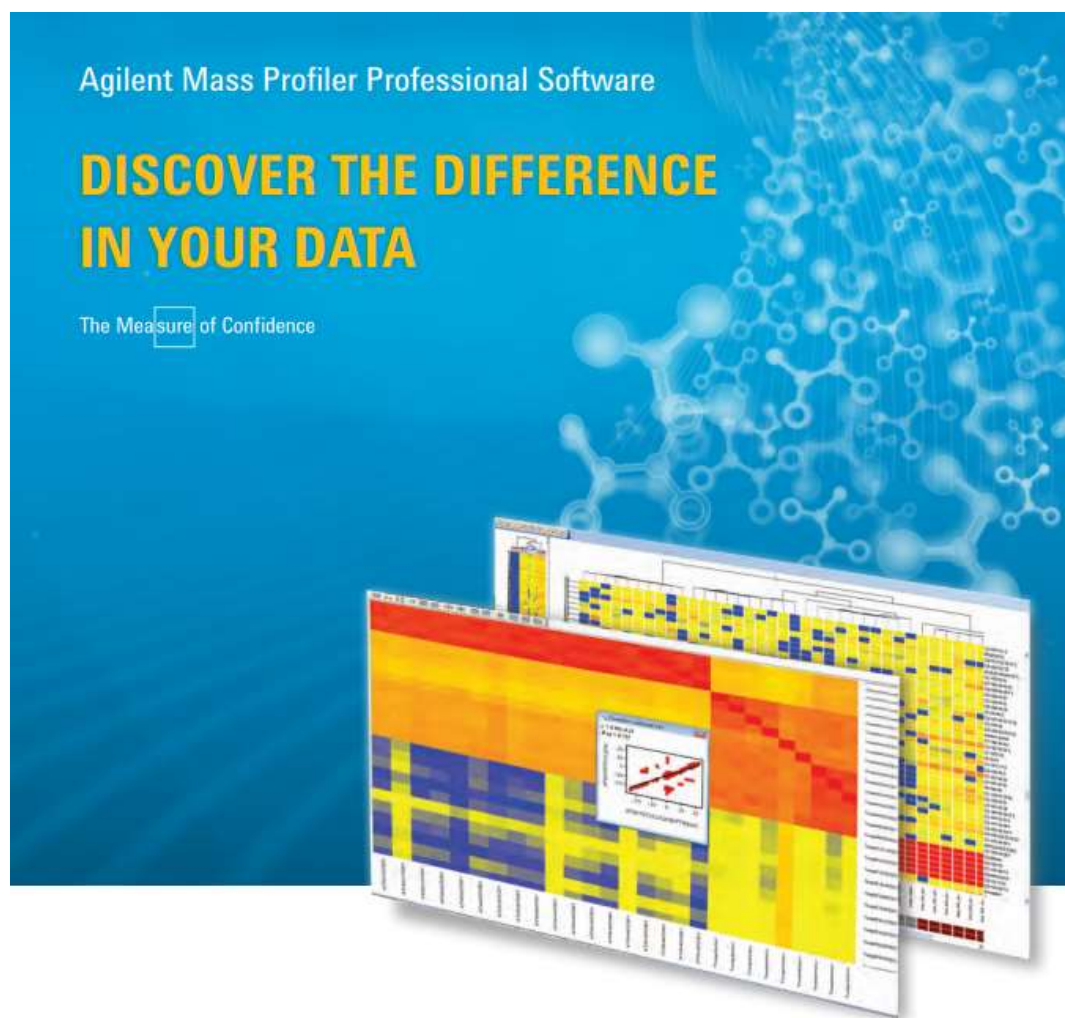
Mill Creek (1-4) and Corey Creek (5) are tributaries of the Tioga River (7) which discharges into the Susquehanna River. The River serves as a source of drinking water to 4.1 million people in NY, PA and MD.

SAMPLE COLLECTION

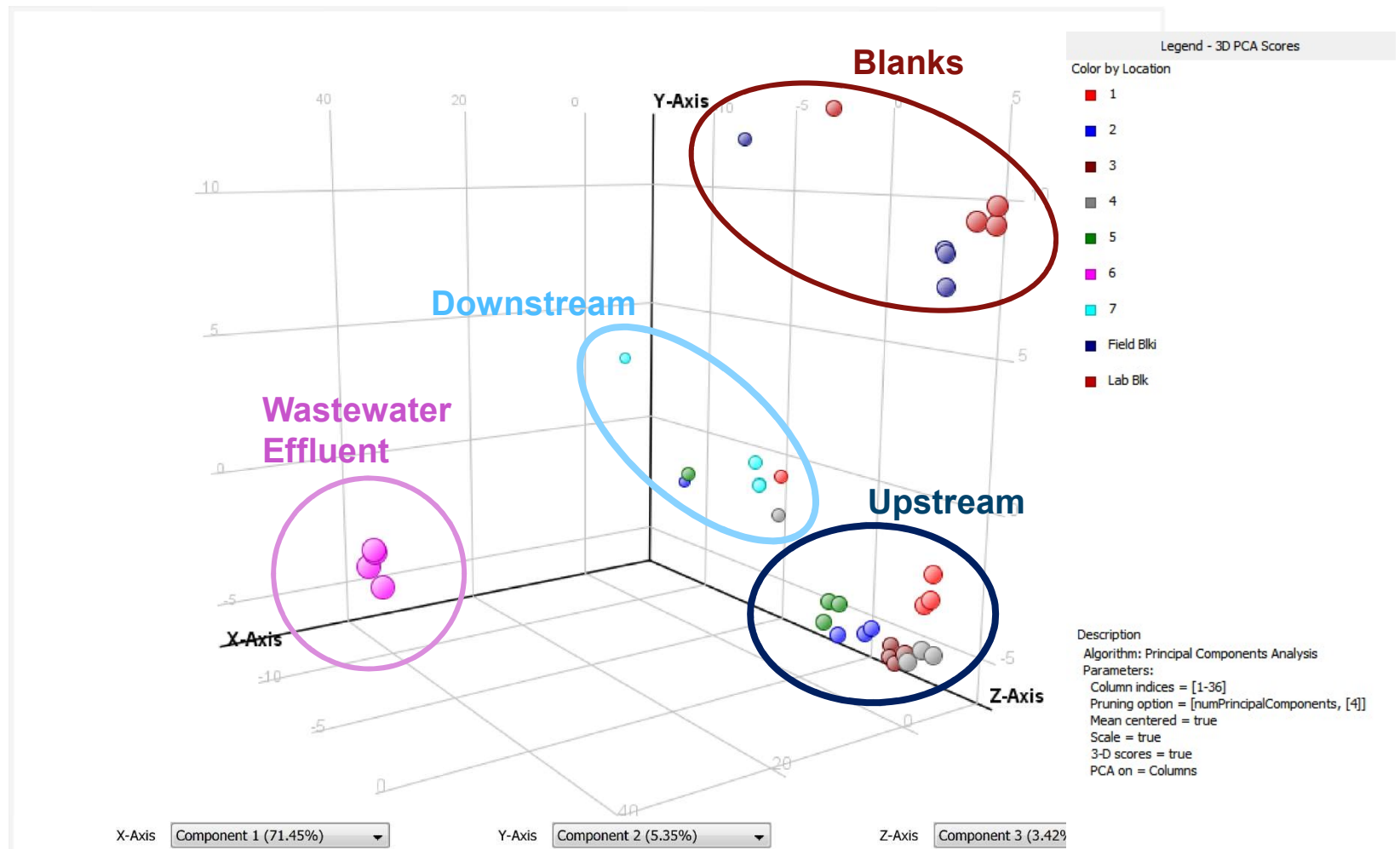
40 mL samples collected in amber glass vials and shipped to the lab on ice within 24 hours.

Statistical Differentiation of Samples

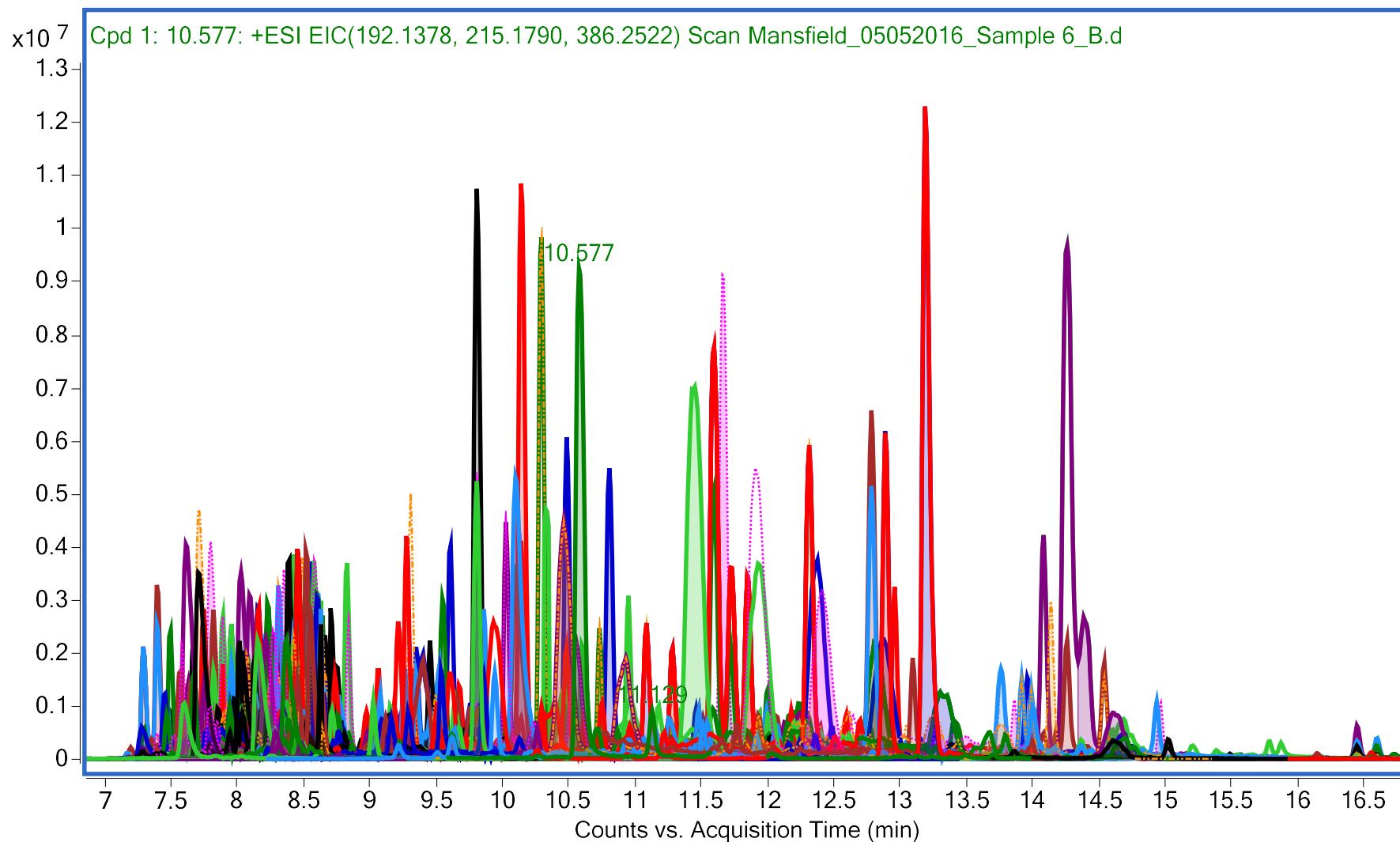
Mass Profiler Professional



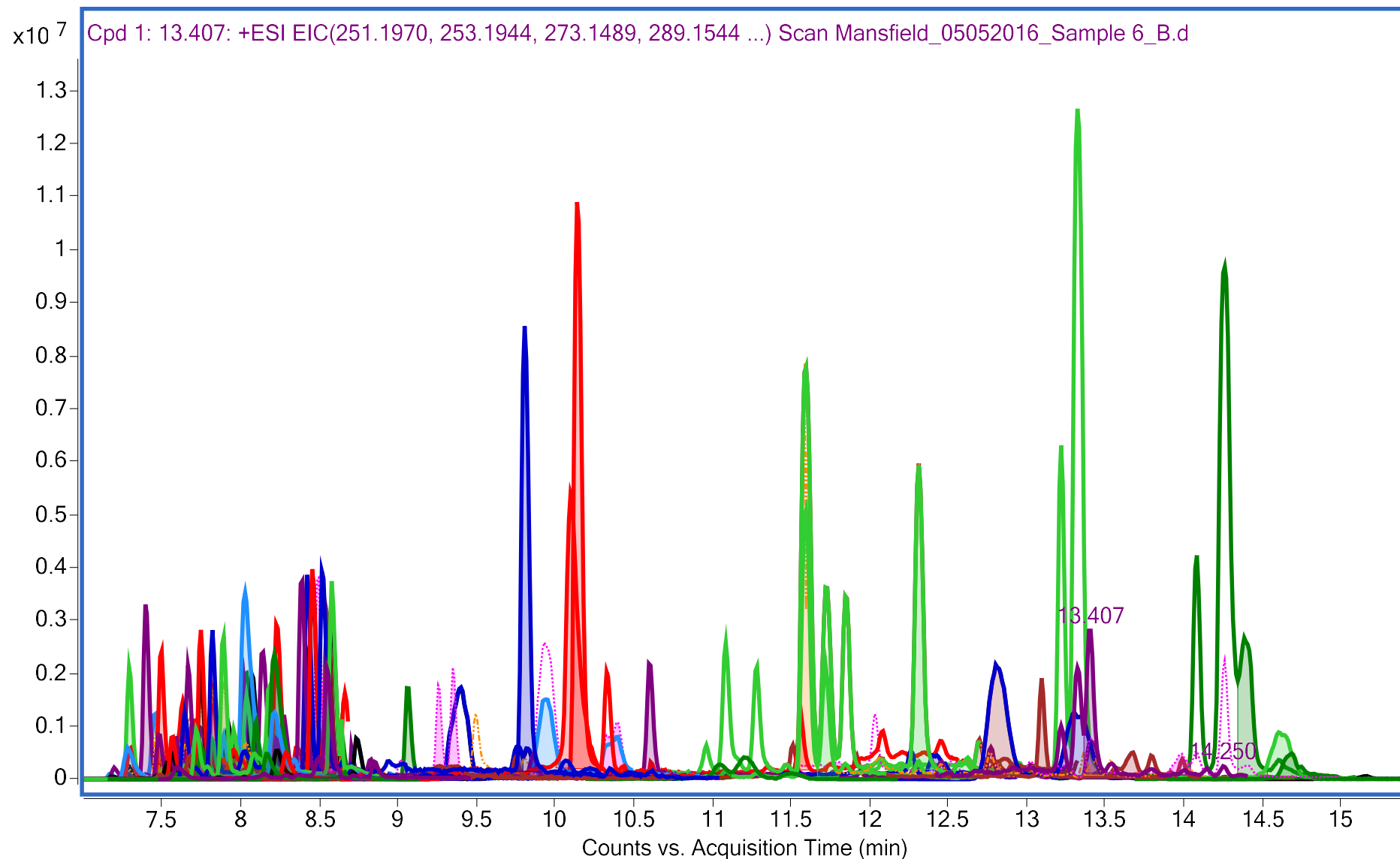
PCA Scores Plot



Tukeys HSD 746 Entities Location 6 vs Controls



115 Compounds in Wastewater that are absent upstream



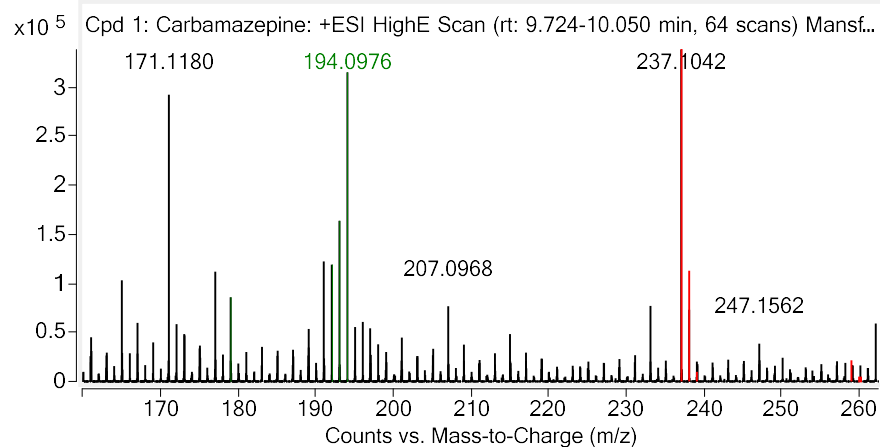
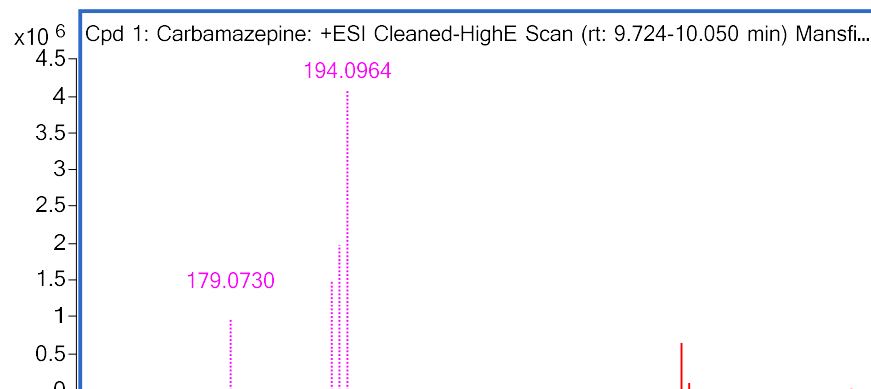
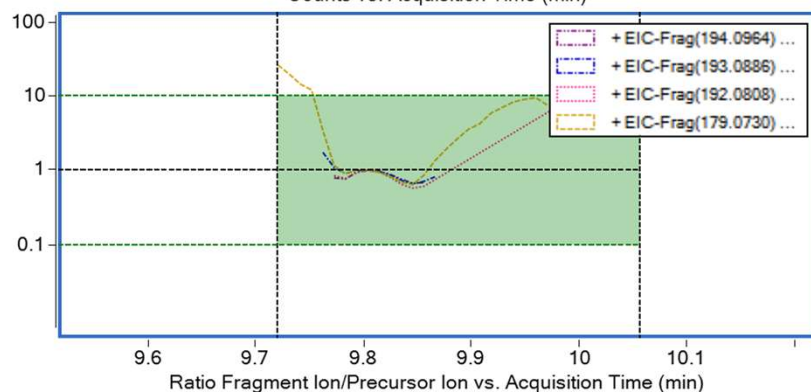
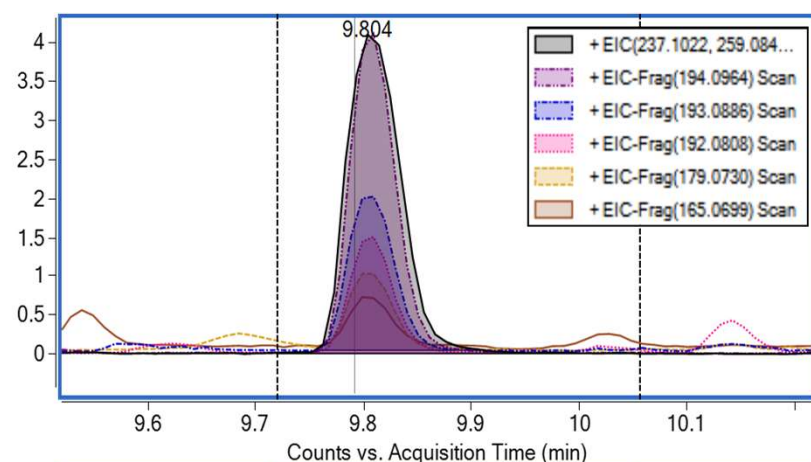
Database Search of Location 6 Only Entity List (115)

Cpd	Label	Name	Formula	Score	Mass	RT
38	Cpd 38: Valethamat; C19 H32 N O2; 9.273	Valethamat	C19 H32 N O2	99.86	306.2436	9.273
63	Cpd 63: trans-10,11-Dihydroxy-10,11-dihydroca...	trans-10,11-Dihydroxy-10,11-dihydroca...	C15 H14 N2 O3	53.99	270.0956	8.167
56	Cpd 56: Sulmepride; C14 H21 N3 O4 S; 7.749	Sulmepride	C14 H21 N3 O4 S	87.87	327.1231	7.749
66	Cpd 66: Pyridarone; C13 H9 N O; 10.117	Pyridarone	C13 H9 N O	97.72	195.0693	10.117
78	Cpd 78: Pipebuzone; C25 H32 N4 O2; 7.888	Pipebuzone	C25 H32 N4 O2	72.96	420.2559	7.888
65	Cpd 65: Oxcarbazepine; C15 H12 N2 O2; 9.000	Oxcarbazepine	C15 H12 N2 O2	65.54	252.0848	9
26	Cpd 26: Irbesartan; C25 H28 N6 O; 10.137	Irbesartan	C25 H28 N6 O	99.18	428.2329	10.137
61	Cpd 61: Fenretinide; C26 H33 N O2; 7.766	Fenretinide	C26 H33 N O2	9.86	391.2311	7.766
23	Cpd 23: Dehydroepiandrosterone; C19 H28 O2; 13.367					
113	Cpd 113: Carbamazepine; C15 H12 N2 O; 9.810					
34	Cpd 34: Cannogenin; C23 H32 O5; 14.660					
43	Cpd 43: Benderizine; C28 H34 N2 O2; 8.014	Benderizine	C28 H34 N2 O2	68.19	430.2665	8.014
22	Cpd 22: 3-(4-methylbenzylidene)-Camphor; C1...	3-(4-methylbenzylidene)-Camphor	C18 H22 O	58.54	254.1662	14.274
15	Cpd 15: 17 a-Estradiol; C18 H24 O2; 14.100	17 a-Estradiol	C18 H24 O2	96.94	272.1783	14.1
17	Cpd 17: 17 a-Estradiol; C18 H24 O2; 12.390	17 a-Estradiol	C18 H24 O2	85.62	272.1785	12.39
1	Cpd 1: C11 H24 N2 O4; 11.602		C11 H24 N2 O4	95.26	248.1733	11.602
2	Cpd 2: C32 H17 N2; 8.603		C32 H17 N2	93.34	429.1394	8.603
3	Cpd 3: C21 H31 N O2 S2; 9.640		C21 H31 N O2 S2	87.13	393.1802	9.64
4	Cpd 4: C18 H20 N; 13.417		C18 H20 N	83.16	250.1592	13.417
5	Cpd 5: C16 H24 O2; 12.329		C16 H24 O2	96.9	248.1786	12.329
6	Cpd 6: C10 H24 N3 O5; 12.635		C10 H24 N3 O5	83.74	266.1713	12.635
7	Cpd 7: C9 H24 N4 O2 S; 12.036		C9 H24 N4 O2 S	72.88	252.1616	12.036
8	Cpd 8: C14 H38 N4 O3 P5; 9.639		C14 H38 N4 O3 P5	87.1	465.163	9.639

Identification of Unknowns- Data Independent Analysis

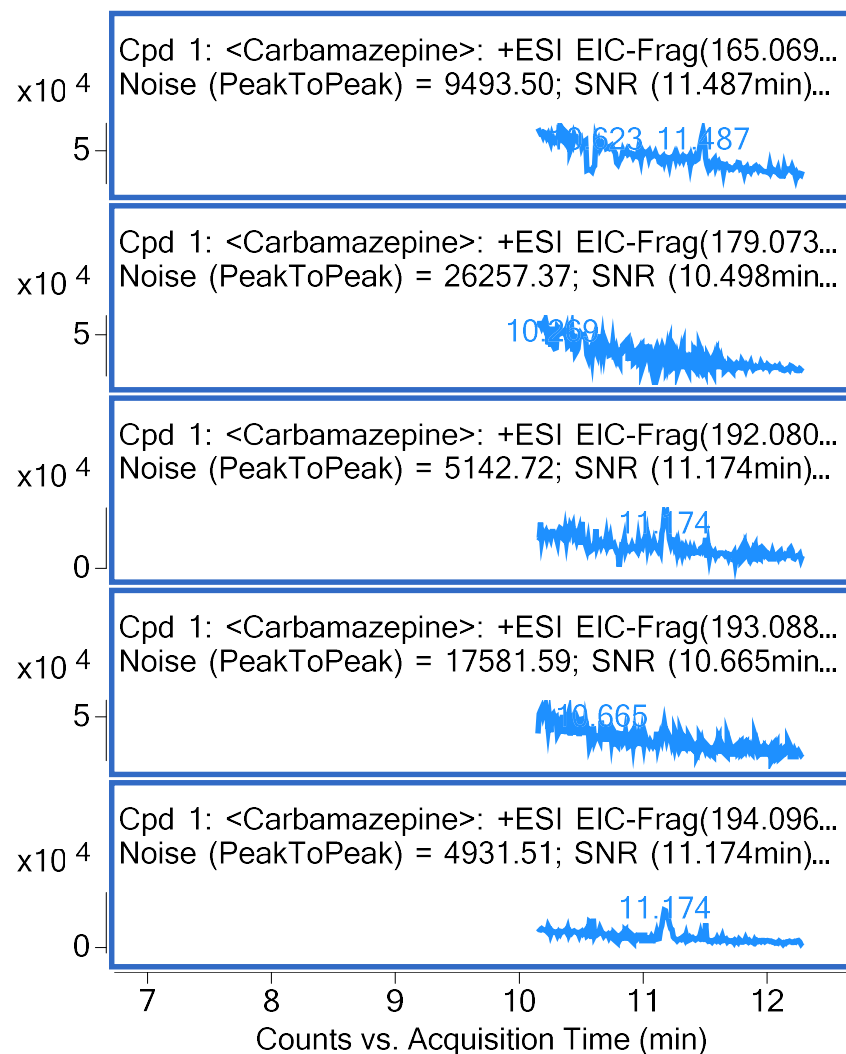
All Ions MS/MS

Coelution Score	CE	Flags(FIs)	FV	Height	m/z
98.8	10	Qualified		4092627	194.0964
98.6	40	Qualified		1973776.7	193.0886
97.1	40	Qualified		1485877.2	192.0808
96.4	40	Qualified		972295.9	179.073
89.6		Low coelution score		633932.9	165.0699

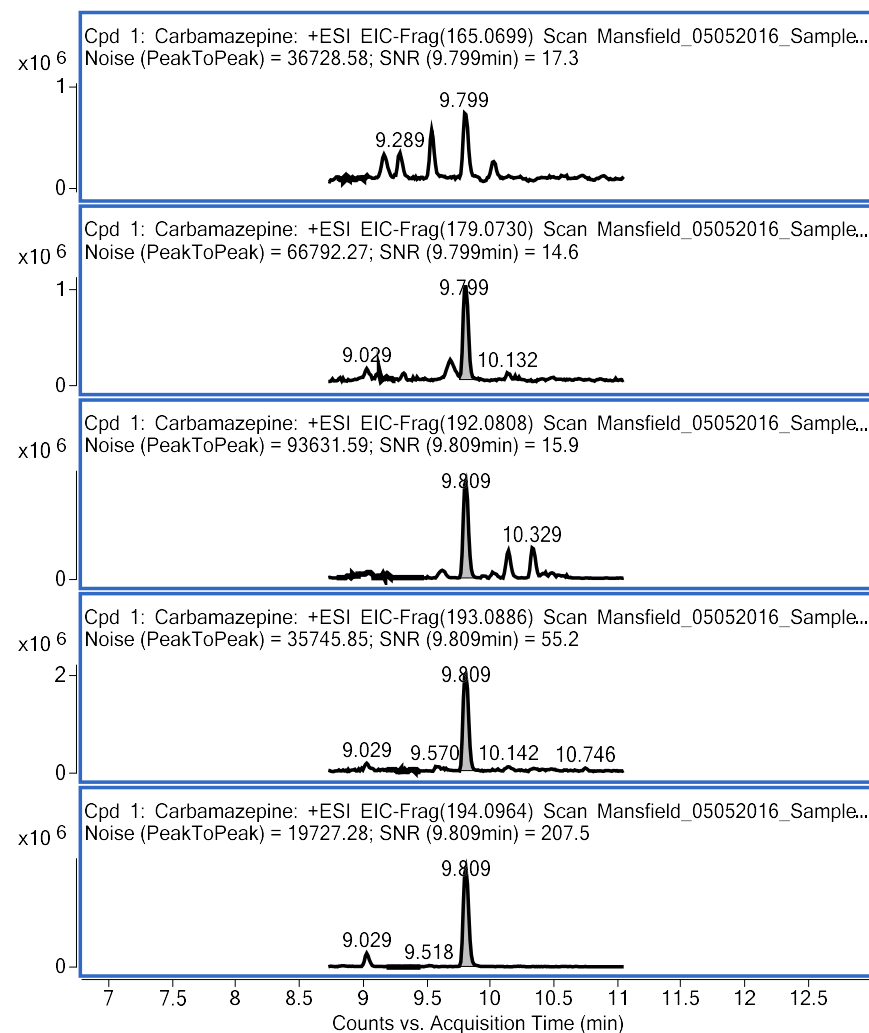


Carbamazepine

Sample Location 5



Sample Location 6



Pain Points for Analysis



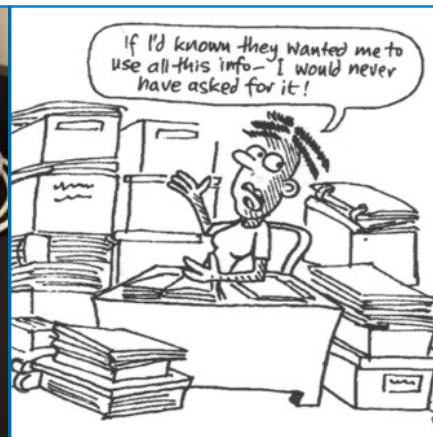
Compounds
to monitor



Sample
Preparation



Standard
Preparation



Data
Analysis



Agilent Technologies

64th ASMS WOC-410

June 8, 2016

30

Analysis of Water Contaminants

Summary

- The current LC-QTOF MS systems can offer rapid screening of several hundred emerging compounds in real water samples
- Online Automated SPE allows users to analyze samples rapidly and with very little water sample while maintaining sensitivity
- All Ions MS/MS workflow allows for additional verification tools in suspect screening with fragment confirmation and retention time through personal compound database and libraries (PCDLs)
- Several contaminants are detected in water; statistical analysis to choose SIGNIFICANT ones are essential





Agilent Technologies

For more information please visit:
[**www.agilent.com/en-us/solutions/environmental**](http://www.agilent.com/en-us/solutions/environmental)

Speaker contact: tarun.anumol@agilent.com



Agilent Technologies