Coupling Large Volume Injection for Aqueous Samples and Organic Extracts with LC-Tandem Mass Spectrometry

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Outline

- **Large Volume Injection (LVI)** – a revival
- **Myth Busters**: SPE and LVI
- **Applications**
  - **Aqueous samples & Organic extracts**
    - Illicit drugs in wastewater influent (1,800 mL)
    - Oil dispersant (surfactants) in seawater (1,800 mL)
    - Fluorochemicals in groundwater, landfill leachates, and papers & textiles (900 mL)
- **Advantages and Disadvantages**
- **Concluding Remarks**

Alumbaugh et al. 2004, J. Chrom A, 1042: 89., Schultz et al. 2004, ES&T, 38, 1828; Schultz et al. 2006, ES&T, 40, 7350; Schultz et al. 2006, ES&T, 40, 289; Huset et al. 2008, ES&T, 42, 6369, Chiaia et al. ES&T, 2008, ES&T, 42, 8841

Place et al. J Sep. Sci, 2012,00–,1;Isaacson et al. Anal. Chem, 2007, 79, 9091

Backe et al. 2011, Anal. Chem., 83, 2622; Backe and Field, 2012, ES&T, 46, 6750

History of LVI

- Large volume injection (LVI) initially developed and utilized for non-MS/MS applications (e.g. UV-Vis)
- Emergence of LC-MS/MS, the flow rate cannot support large volume sample injection-LVI replaced with SPE
- Instrumentation supports larger flow rates
  - SPE entrenched in regulation and many standard operating procedures
- LVI re-emerges with some instrument modification

Busetti, F., et al. (2012). "Trace analysis of environmental matrices by large-volume injection and liquid chromatography-mass spectrometry." Analytical and Bioanalytical Chemistry **402**(1): 175-186.

Myth Busters: SPE and Mass Spectrometry

- SPE is necessary to concentrate analytes from environmental samples
  - Load a 100 mL sample onto cartridge, elute in 1 mL = 100 x concentration
  - Mass delivered to detector in 10 µL extract (1% of extract volume, labor costs) = 1 mL of sample (LVI)
- Protect expensive analytical equipment from "column killers" and non-volatile salts
- Reduce sample matrix complexity

Why Not Just Use SPE?

- Filtration and SPE = positive and negative artifacts for fluorochemicals
  - Fluorochemicals (e.g., PFOA) used as filter wetting agents (positive artifacts)
  - PFOS removed from water during filtration (negative artifacts)
  - Manifolds (PTFE seals) and SPE media contain PFOA
- SPE is costly (time and materials)
- SPE materials vary by lot
- ‘Fuss factor’ (especially true if not automated)
- And, it turns out, SPE is ‘chemically redundant’ with LC

Chemically-Redundant Processes

SPE	Large-volume Injection
Sample loading (5 – 1,000 mL)	Aqueous sample loading (LVI) onto reverse-phase LC packing
Wash (removes matrix)	LC gradient 'tuned' to wash column
Elution (μL to mLs of total solvent)	Gradient elutes analytes from column
Extract concentration (e.g., N_2)	No equivalent
Injection small portion of final extract (10-20 μL)	No equivalent

SPE Disadvantages

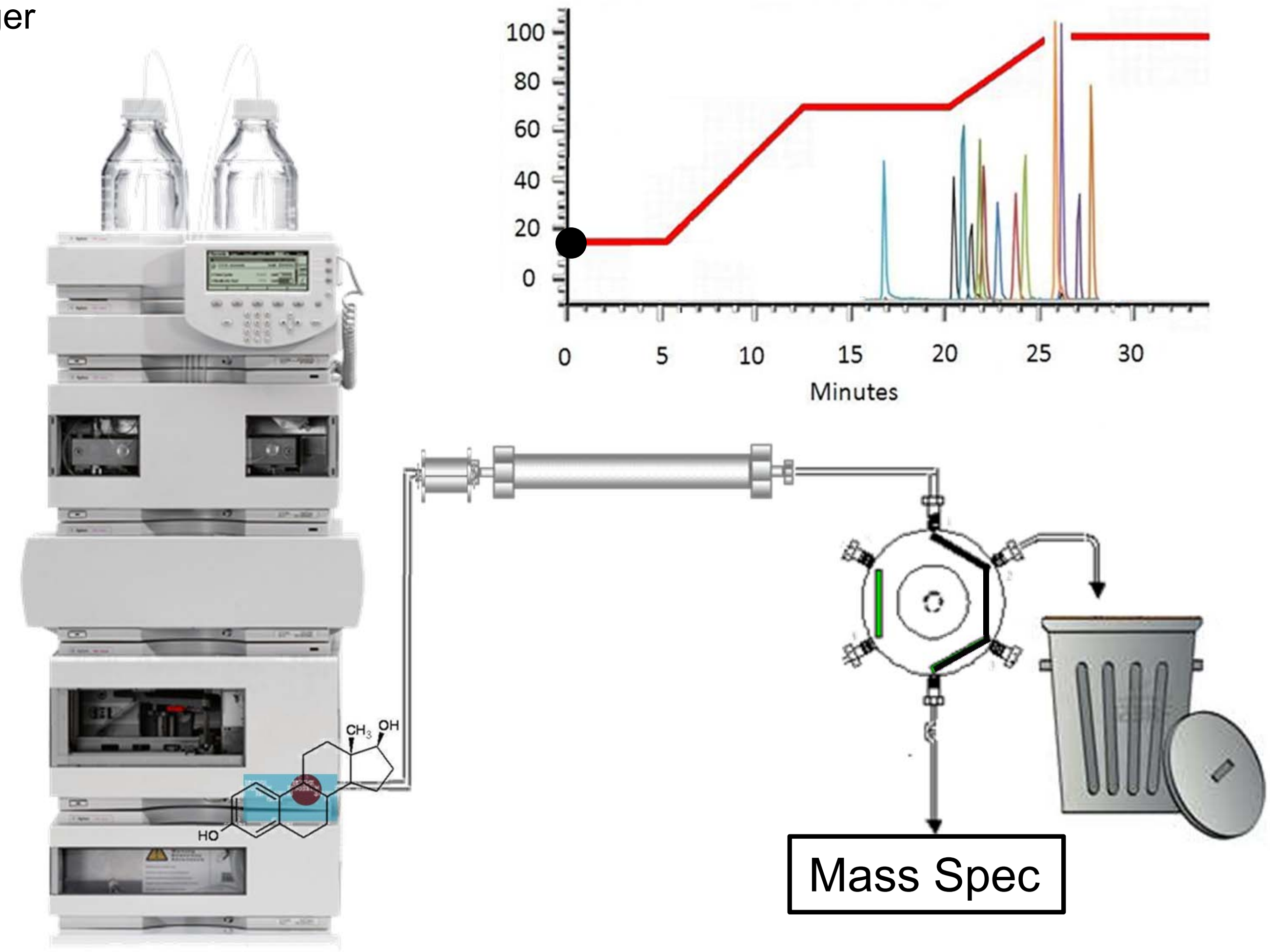
- Cost - cartridge or disk
- Time to manipulate

LC Advantages

- It is already used in the method
- Only minutes added = cost effective

Redundant Chemistry

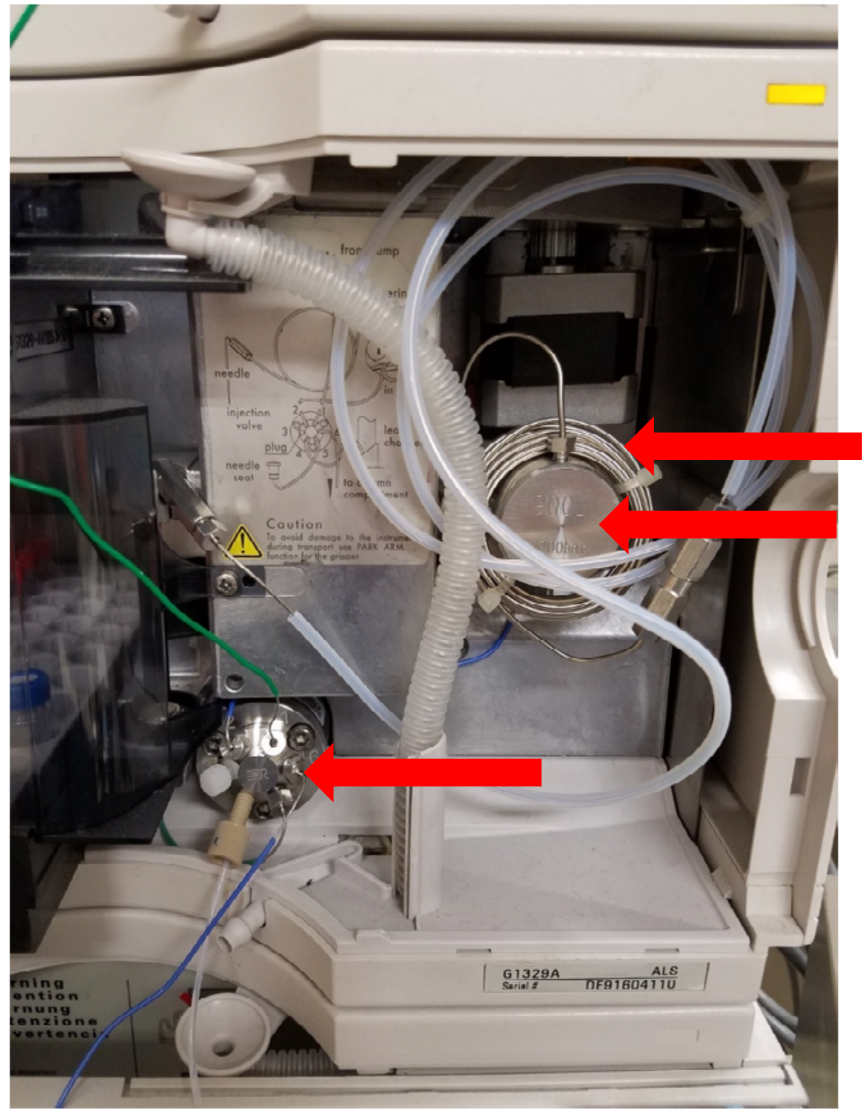
"To increase reproducibility, sample cleanup procedures should be automated and –if possible- be integrated into the LC separation process."
-Klaus K. Unger



Anal. Chem., **2011**, 83 (7), pp 2622–2630

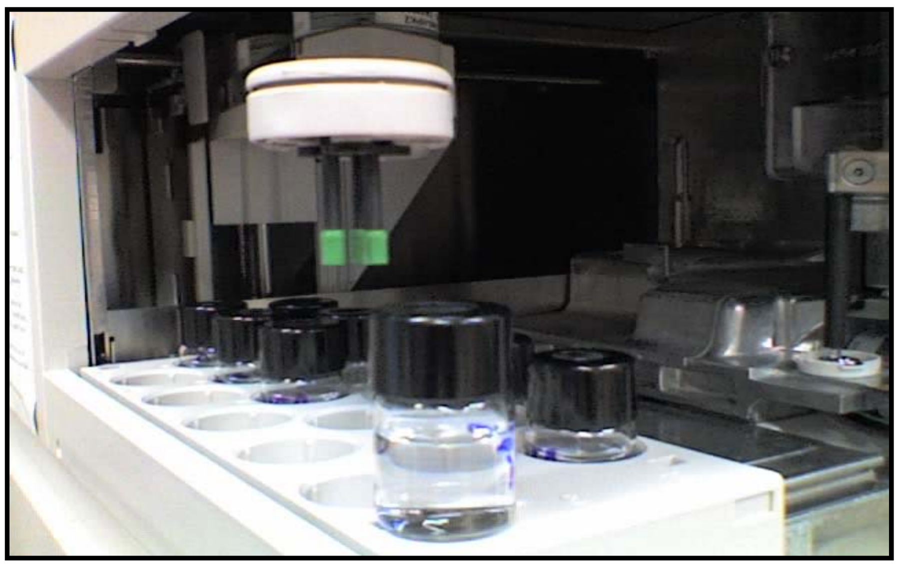
Large Volume Injection–Visual Aid

- Modifications: 900µL loop, analytical head, use of mainpass/bypass
- Worked closely with Doug Martin (Agilent) to understand/use bypass function and for setting flows
- Agilent offers a multi-draw injection kit for volumes up to 5,800 mL!
  - ✓ although commercially available, few units in use (most likely for prep-LC)
- Nothing 'magical' about 900 µL
  - ✓ Only limited by phase-to-volume ratio & breakthrough volumes!



Application 1: LVI for Illicit Drugs in Wastewater

- A tool for community drug surveillance
- 24 hr, flow-normalized composites of raw WWTP influent
- HDPE bottles, frozen
- Centrifuge
- Spike with stable-isotope internal standards



6 mL autosampler vial

1800 µL Injection LC-ESI/MS/MS

System:

Agilent 1100 LC interfaced to a Quattro Micro MS/MS (ESI interface)

LC injector: 1,800 µL

Columns:

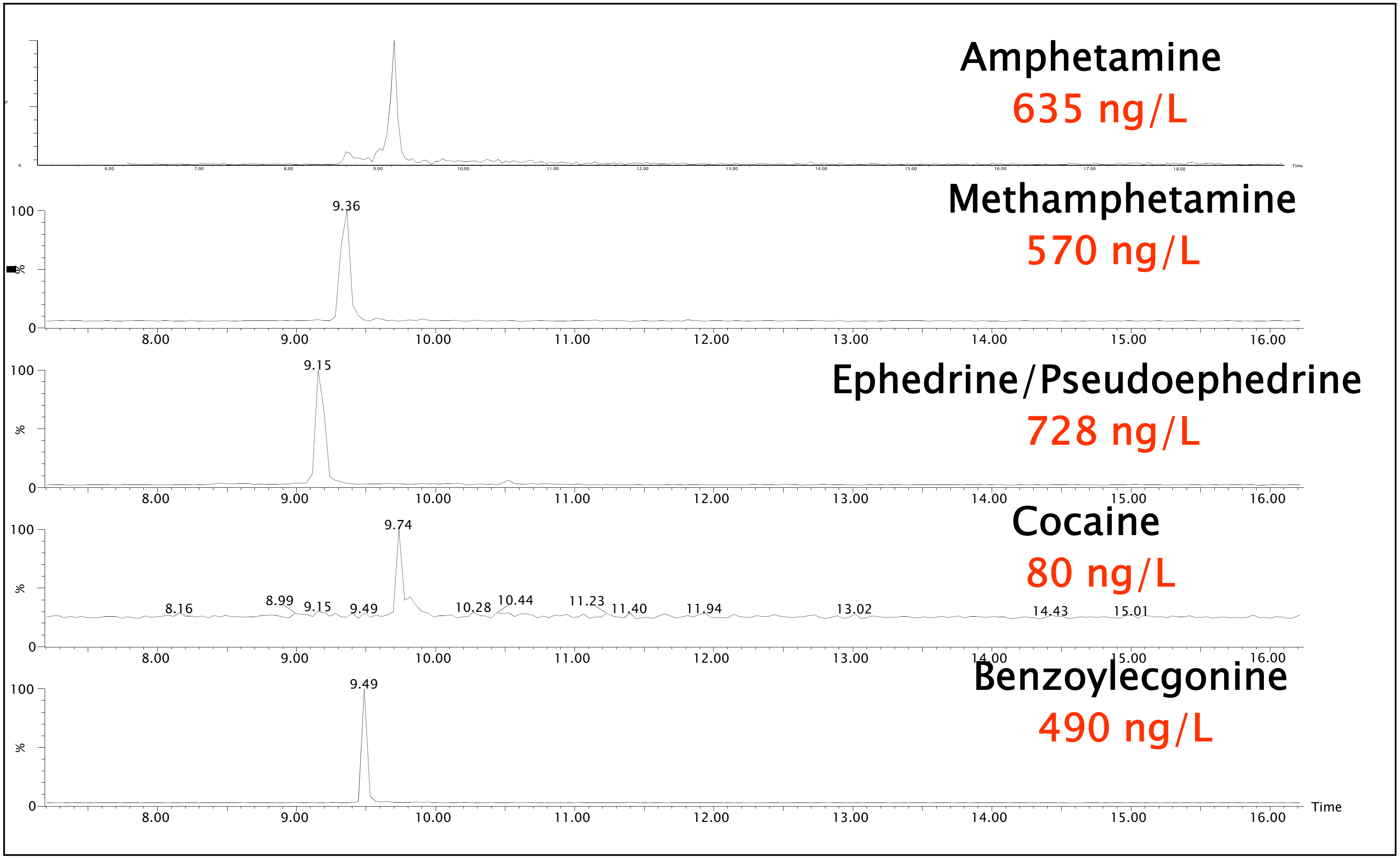
4.0 x 2 mm C_{18} guard column (Phenomenex) + 100 mm x 2.1 mm Hypersil Gold C_{18} with 3µm particle size (Thermo Electron)

Flow Rate: 500 µL/min

MS/MS:

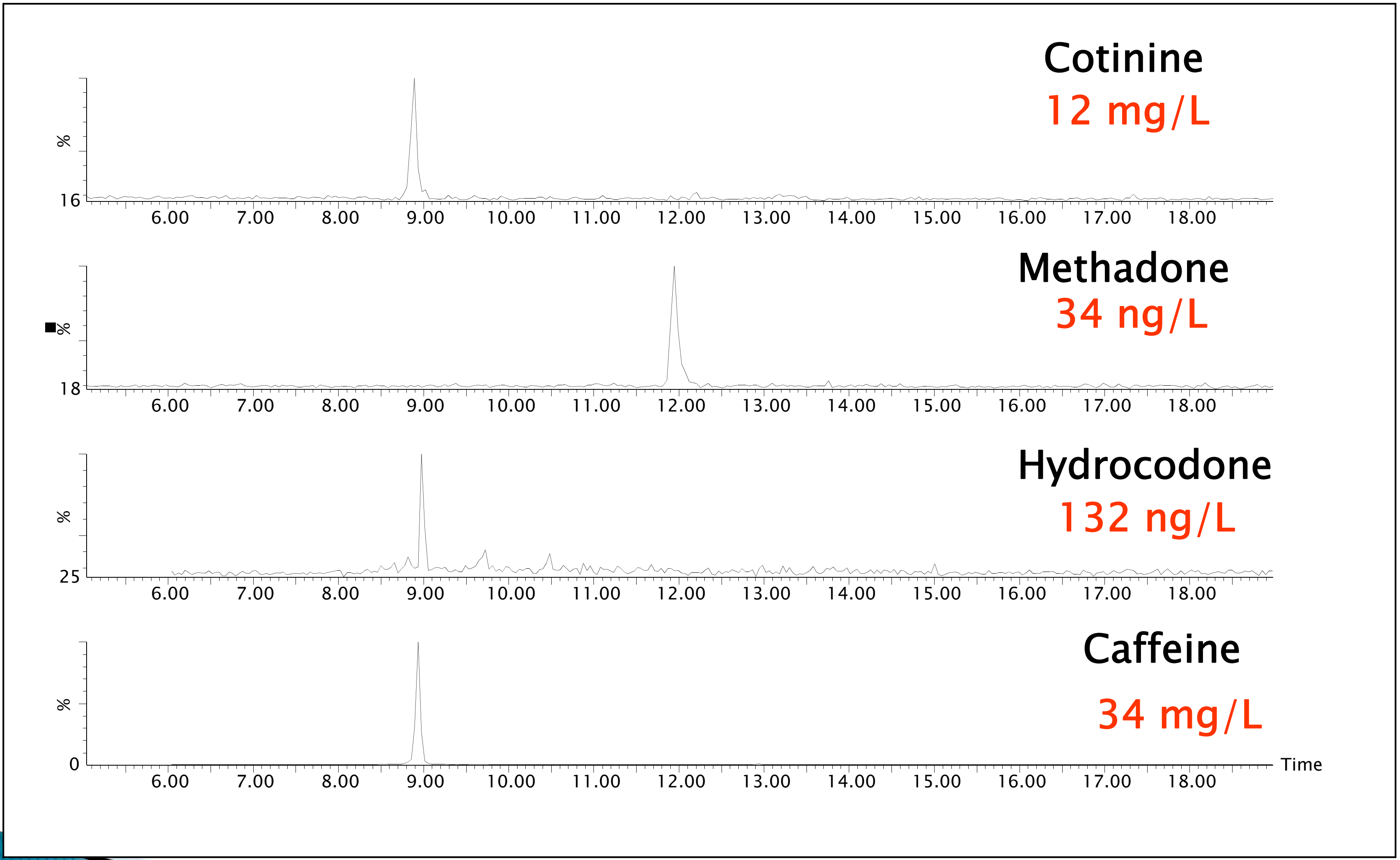
positive mode, 60 msec dwell time, increased desolvation (450 ºC) and source (150 ºC) temp

Illicit Drugs in Raw Wastewater



Time line: 7.35 min 'extraction', 9 min separation; 3 min re-equilibration

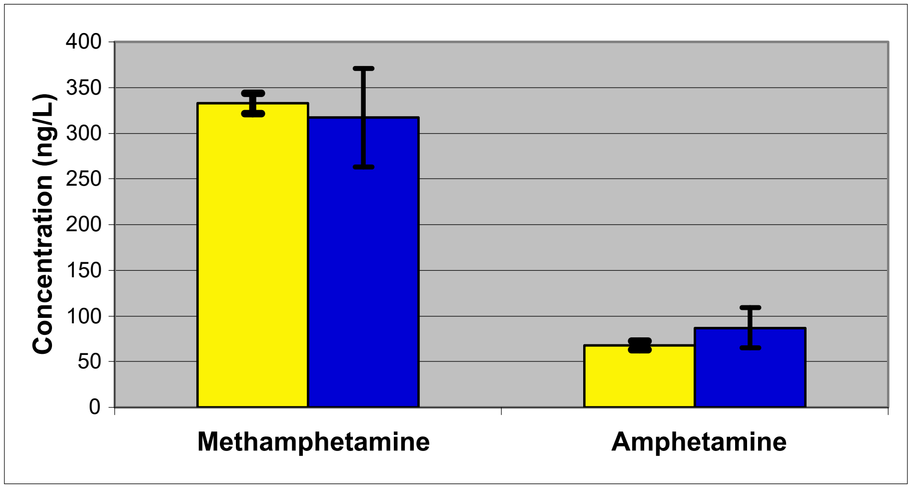
Illicit Drugs in Raw Wastewater

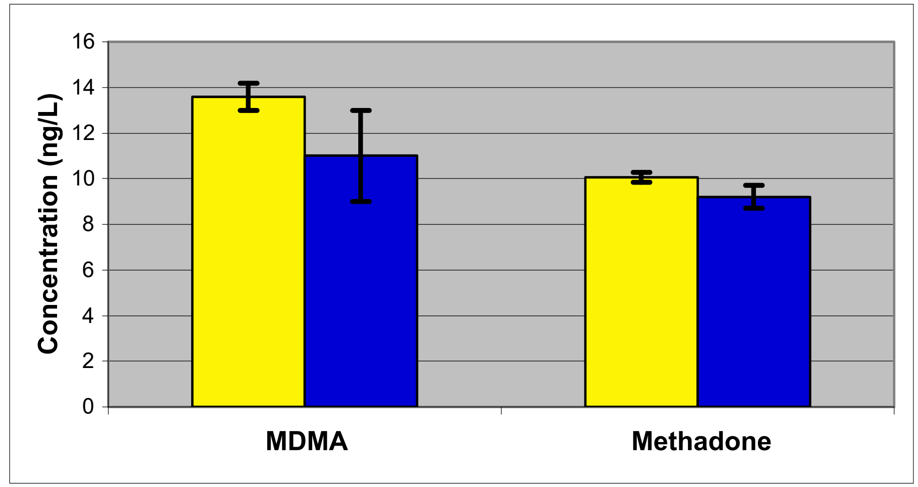


Time (min)

What About Matrix Effects?

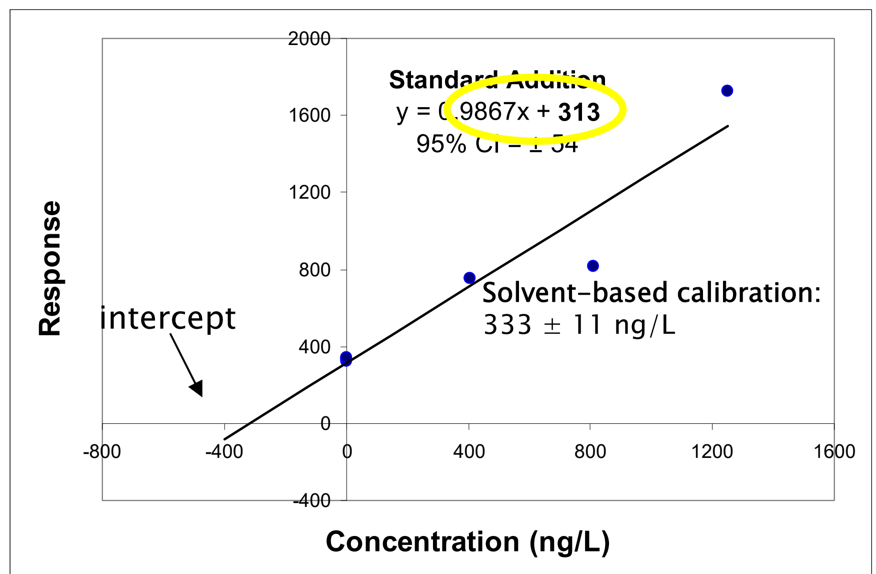
There are matrix effects but stable-isotope standard compensate!





Solvent-based calibration curve
Standard addition

Accuracy: Concentrations from solvent-based curves equivalent to standard addition at 95% CI
Precision: 2-4% RSD



Method Performance

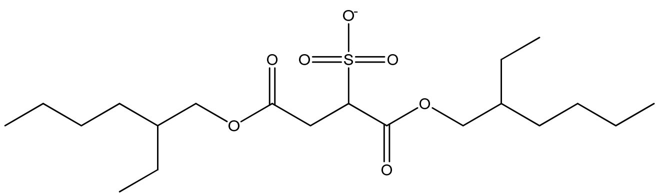
Illicit Drugs and Human urinary biomarkers

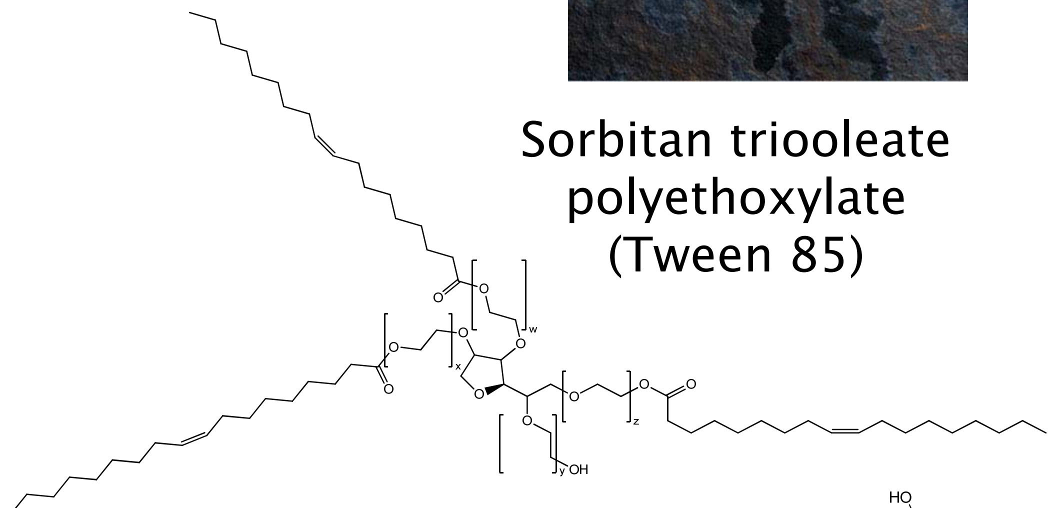
	Large-Volume Direct Injection	SPE-based Methods
Sample Prep/Cost	20 min 'on-line'	SPE material ($) and analyst time
Sample size/ Injection volume (μL)	1.8 mL (1,800 μL)	50[c]–100[a,b] mL (25 mL)[b]
Run time (min)	20 min	20–22 min[a,b]
LOQ	2.5–50 ng/L	0.2–5 ng/L[a] 10–50 ng/L[b] 0.6–8.7 ng/L [c]
Accuracy	Concentrations from solvent-based curves equivalent (95% CI) to standard addition	50–112%[a] 65 – 120%[b] 51–107%[c]
Precision (RSD)	2–4%	1–8%[a], 1.4–8.64%[c]

[a]Huerta-Fontela (2007), [illegible]mel (2006), [c]Castiglioni (2006)

Application 2: Corexit Oil Dispersant in Seawater

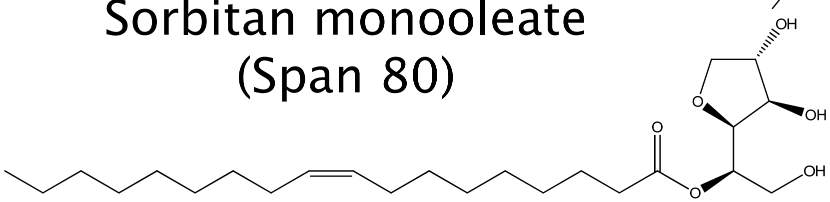
bis-(2-ethylhexyl) sulfosuccinate (DOSS)



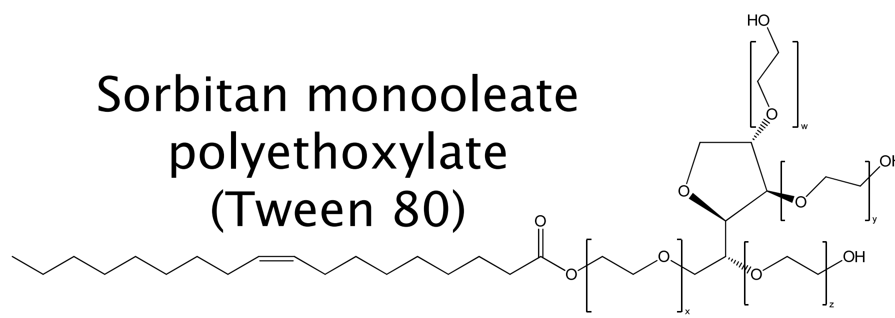


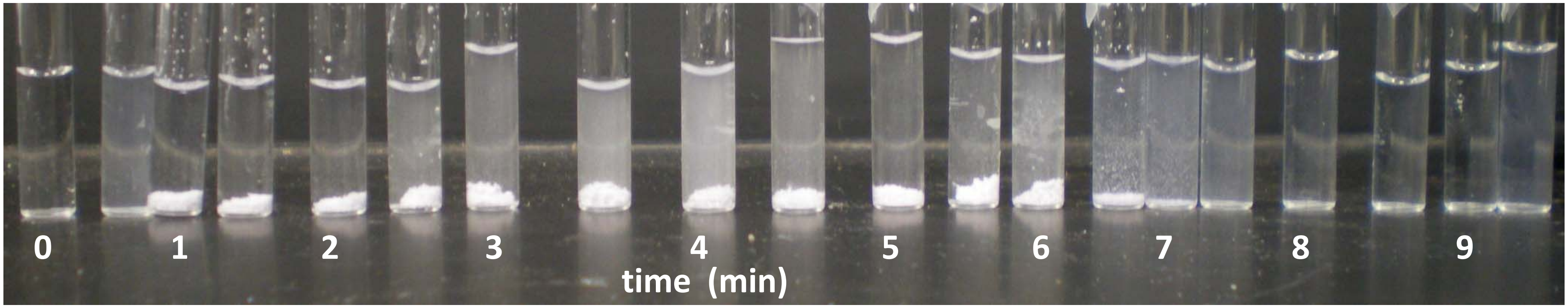
Sorbitan triooleate polyethoxylate (Tween 85)

Sorbitan monooleate (Span 80)



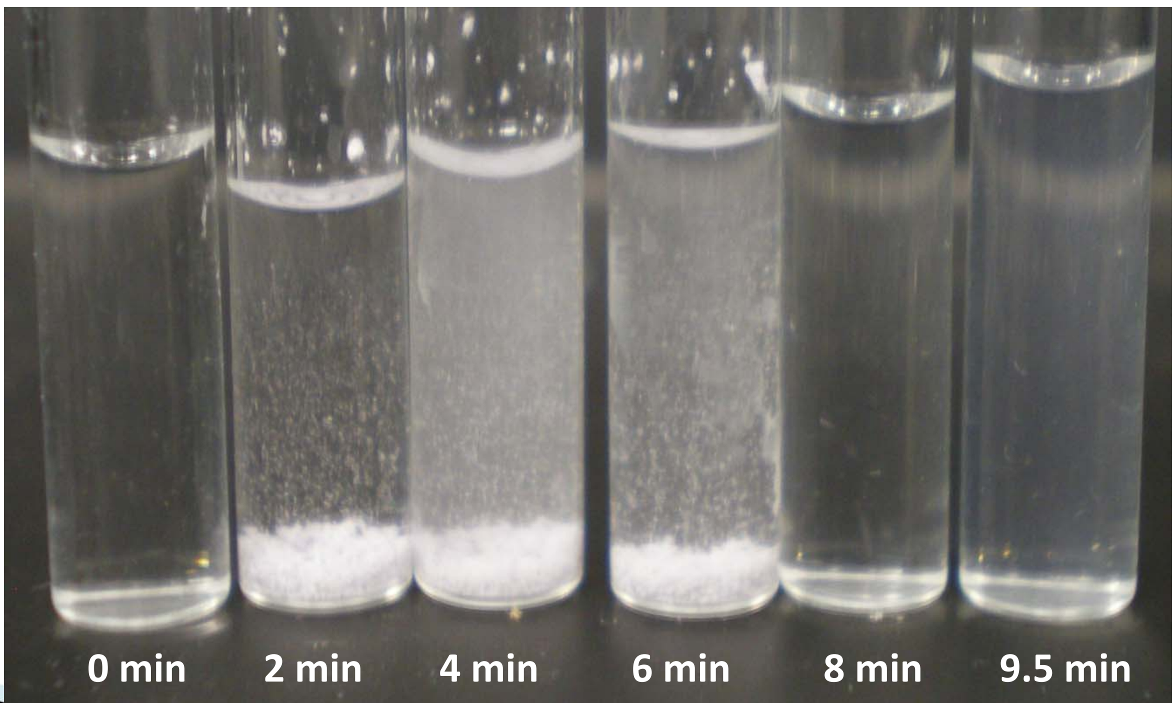
Sorbitan monooleate polyethoxylate (Tween 80)





To Waste

To MS



Collected 30 sec eluents (0.25 mL)

AgNO3/HNO3

LVI and Seawater

Sample Preparation

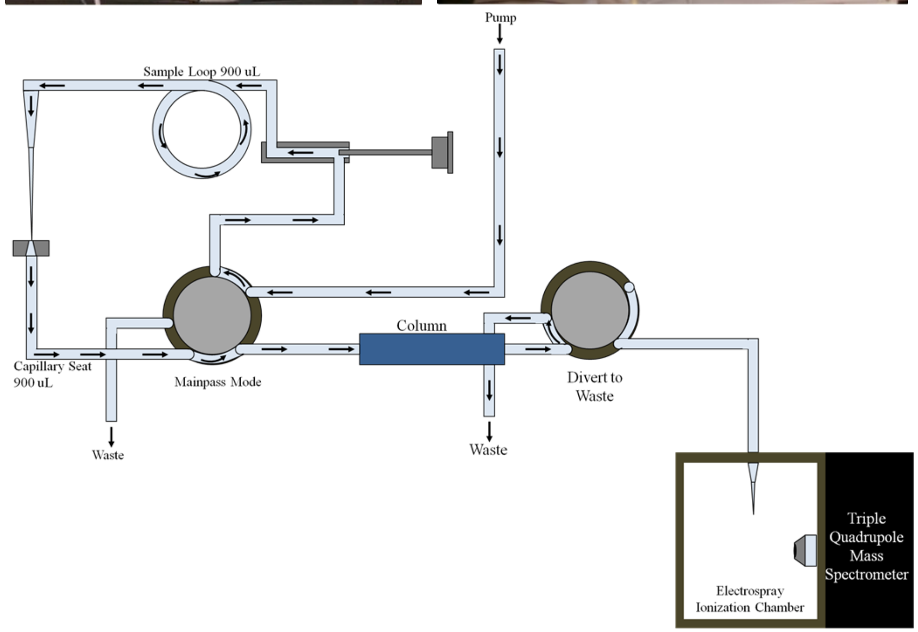
- 25% IPA (v/v) added
- Calibration in Instant Ocean ®

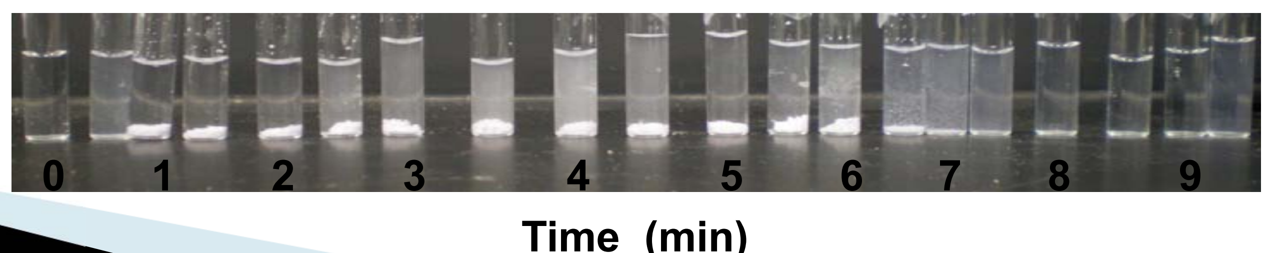
Agilent 1100 HPLC

- 1,800 μL direct injection of seawater with 25% IPA
- C18 guard column + C18 column separation

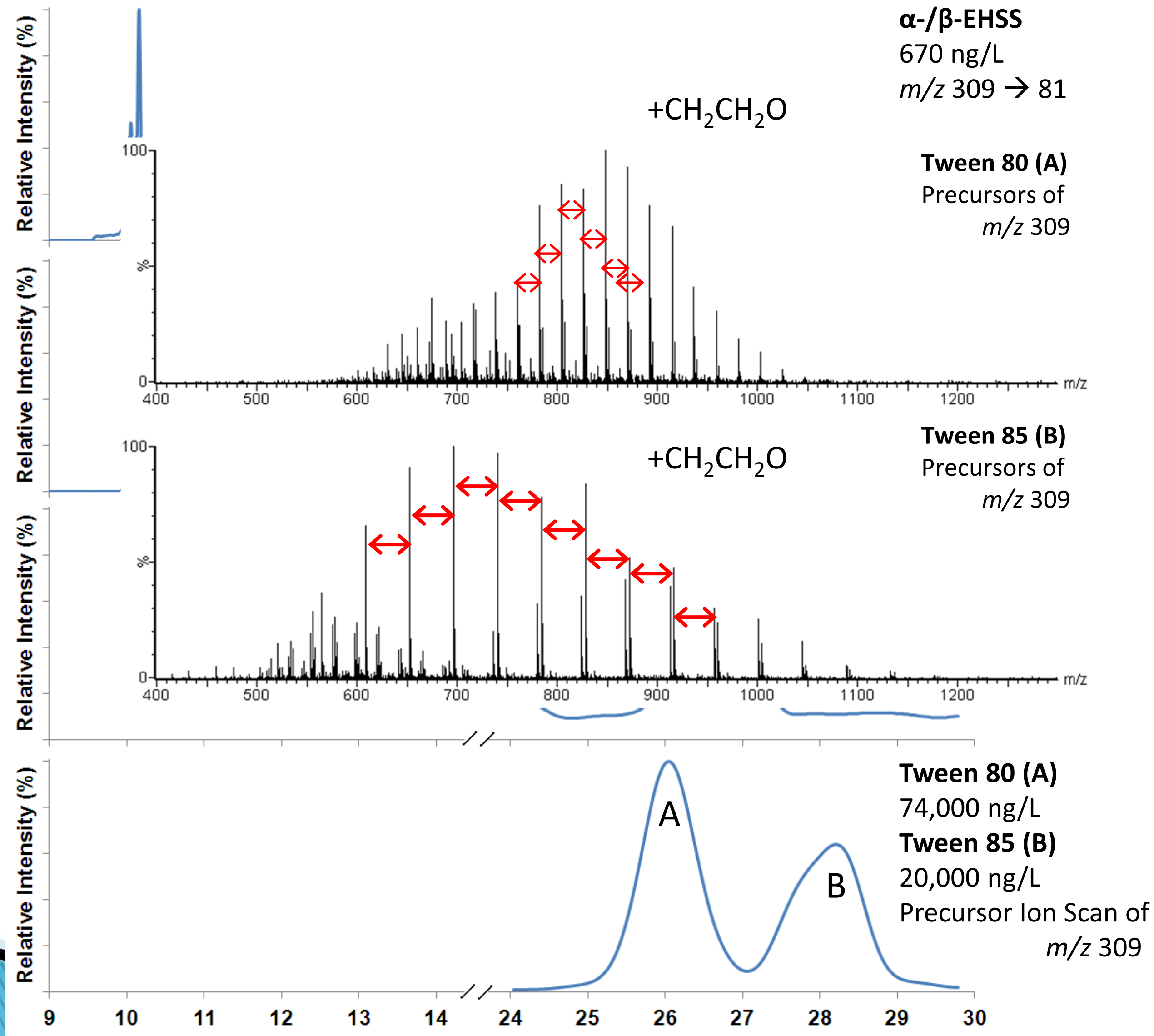
Waters Acquity TQD (ESI)

- MRM mode: DOSS, EHSS, Span 80
- Precursor ion scan mode: Tween 80 and Tween 85





Corexit Analytical Method



Method Parameters

- Standards were prepared in Instant Ocean® seawater.
  - The high salt, buffered solution mimicked the ionization suppression of actual seawater
- Accuracy and Precision experiments were performed using Oregon Coast seawater as the model matrix.
- DOSS LOD/LOQ are equal to accommodate for high variability (130% RSD) of background contamination.

Compound	LOD (ng/L)	LLOQ (ng/L)	ULOQ (ng/L)	Recovery (% ± 95% CI)	RSD (%)	Quantification Method
DOSS	67*	67	34,000	88 ± 10	10	ISC: $^{13}C_4$-DOSS
α-/β-EHSS	1†	49†	25,000	86 ± 11	11	ISC: $^{13}C_4$-DOSS
Span 80	1,250	3,000	60,000	91 ± 21	23	Ext. Cal
Tween 80	987	2,700	400,000	119 ± 13	10	Ext. Cal
Tween 85	99	700	150,000	106 ± 20	17	Ext. Cal

* DOSS LOD is equal to DOSS LOQ due to background variability.
† α-/β-EHSS concentrations were determined assuming equal molar response as that of DOSS.
ISC: 13C4-DOSS - internal standard calibration using 13C4-DOSS as internal standard,
Ext. Cal. - external standard calibration

Application 3: Organic Extracts for Per- and Polyfluoroalkyl Substances in Groundwater

Extraction options:

Direct Aqueous Large-Volume Injection

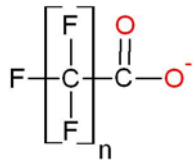
- long-chain PFAS stratify in aqueous solution, adsorb to glass (autosampler vials)
- manageable with labeled internal standards, problematic for others without analytical standards

Solid-Phase Extraction

- laborious & expensive
- contamination (PTFE)
- analyte loss (breakthrough esp. for short-chain PFAS)

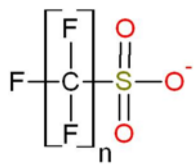
Analyte List

Perfluoroalkyl Carboxylates



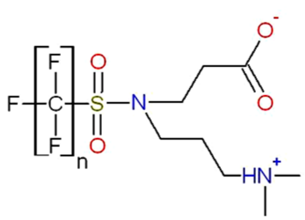
PFBA	n = 4
PFPeA	n = 5
PFHxA	n = 6
PFHpA	n = 7
PFOA	n = 8
PFNA	n = 9
PFDA	n = 10
PFUdA	n = 11
PFDoA	n = 12
PFTrA	n = 13
PFTeA	n = 14

Perfluoroalkyl Sulfonates



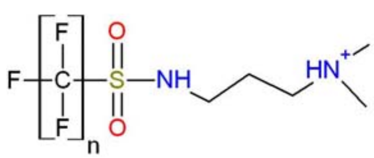
PFBS	n = 4
PFHxS	n = 6
PFHpS	n = 7
PFOS	n = 8
PFDS	n = 10

Perfluoroalkyl Sulfonamide Amino Carboxylates



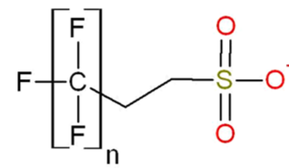
PFBSaAmA	n = 4
PFPeSaAmA	n = 5
PFHxSaAmA	n = 6
PFOSaAmA	n = 8

Perfluoroalkyl Sulfonamido Amines



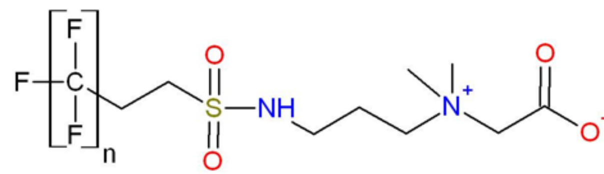
PFBSaAm	n = 4
PFPeSaAm	n = 5
PFHxSaAm	n = 6
PFOSaAm	n = 8

Fluorotelomer Sulfonates



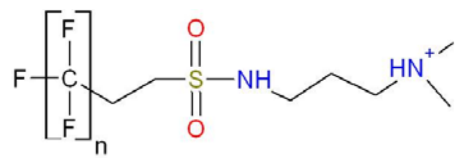
4–2 FtS	n = 4
6–2 FtS	n = 6
8–2 FtS	n = 8

Fluorotelomer Sulfonamido Betaines



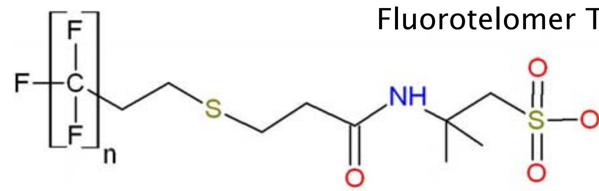
6–2 FtSaB	n = 6
8–2 FtSaB	n = 8
10–2 FtSaB	n = 10
12–2 FtSaB	n = 12

Fluorotelomer Sulfonamido Amines



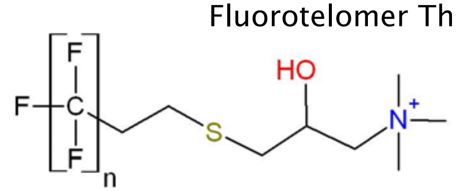
6–2 FtSaAm	n = 6
8–2 FtSaAm	n = 8

Fluorotelomer Thio Amido Sulfonates



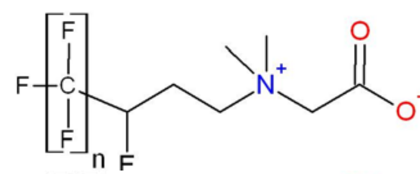
4–2 FtTAoS	n = 4
6–2 FtTAoS	n = 6
8–2 FtTAoS	n = 8

Fluorotelomer Thio Hydroxy Ammonium

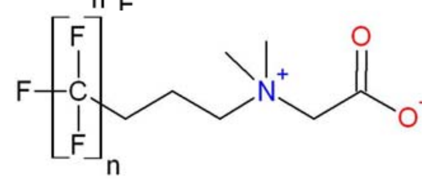


6–2 $FtTHN^+$	n = 6

Fluorotelomer Betaines



5–1–2 FtB	n = 5
7–1–2 FtB	n = 7
9–1–2 FtB	n = 9



5–3 FtB	n = 5
7–3 FtB	n = 7
9–3 FtB	n = 9

Micro Liquid-Liquid Extraction into Organic Solvent

1) Add 10 μL 6M HCl to 3mL Groundwater Sample

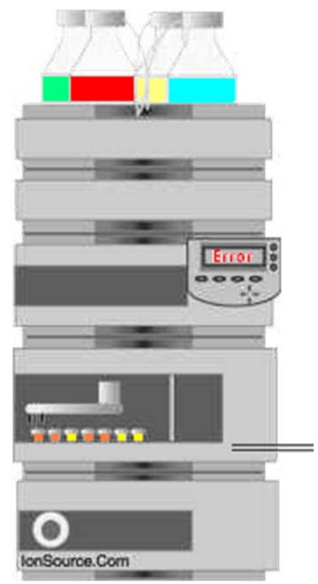
2) Saturate Groundwater with NaCl (~ 1 g)

3) Spike Internal Standards

4) Extract 3x
10% TFE/90% EtOAc (1 mL)

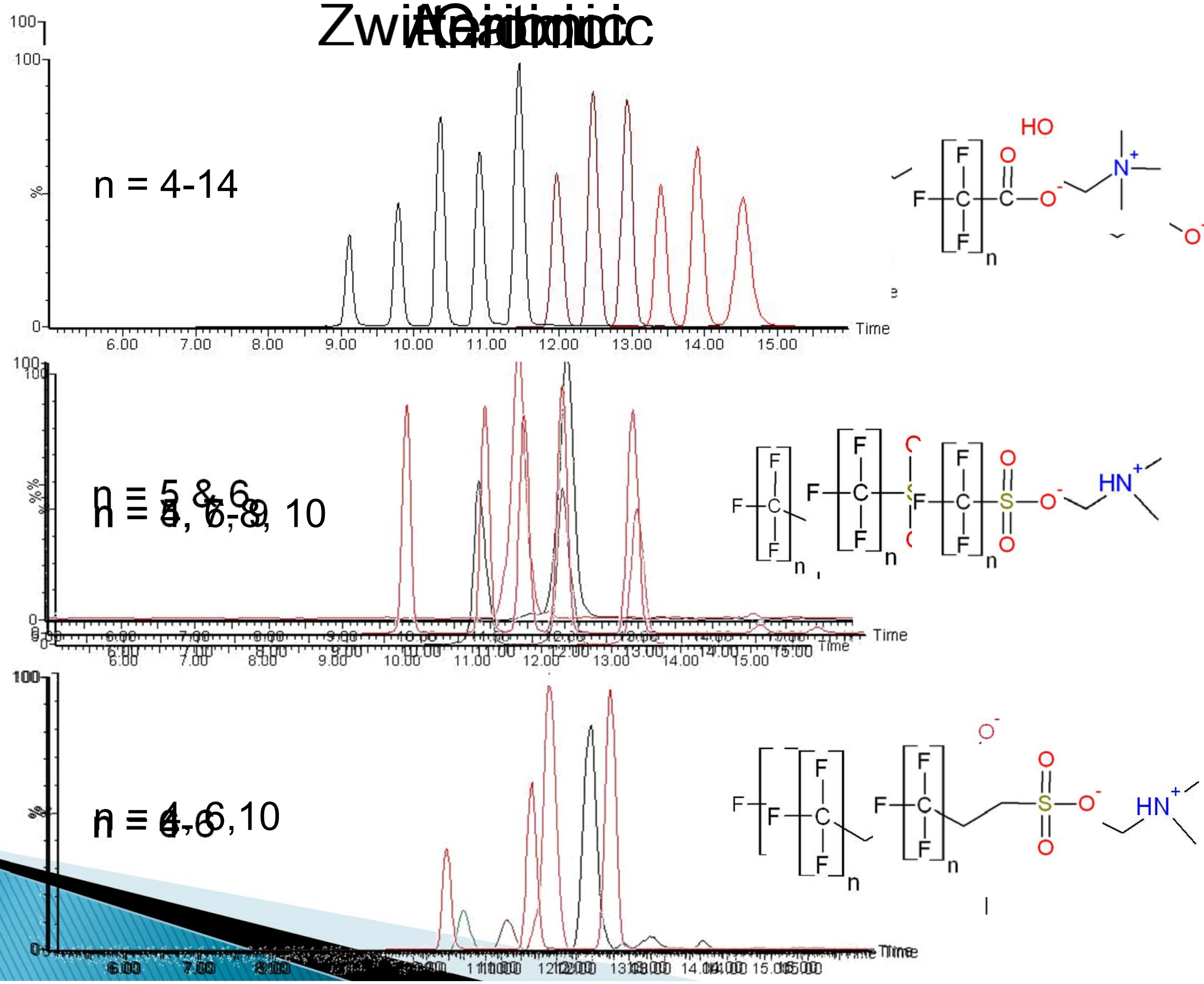
4) Bring to 1.5 mL with MeOH

5) Inject 900 μL by LVI



Chromatograms

Zwitterionic
Anionic
Cationic



Method Performance

Analyte	LOD (ng/L)	Accuracy (%)	Precision (% RSD)
PFBA	4.1	106	9.4
PFPeA	1.1	102	4.8
PFHxA	1.4	101	4.2
PFHpA	1.8	106	11
PFOA	1.5	107	8.5
PFNA	1.0	99	7.8
PFDA	0.94	105	8.4
PFUdA	0.93	104	9.1
PFDoA	1.0	103	6.4
PFTrA	1.2	103	7.1
PFTeA	1.7	106	5.9
PFBS	1.2	98	11
PFHxS	1.7	96	3.7
PFHpS	0.88	100	11
PFOS	0.81	104	6.3
PFDS	0.71	103	2.8

Analyte	LOD (ng/L)	Accuracy (%)	Precision (% RSD)
4-2 FtS	1.6	105	12
6-2 FtS	0.84	99	11
8-2 FtS	1.9	106	10
5-3 FtB	4.6	101	15
5-1-2 FtB	3.6	144	10
7-3 FtB	7.9	96	10
7-1-2 FtB	5.9	128	11
9-3 FtB	6.1	78	13
9-1-2 FtB	8.7	103	11
6-2 FtSaB	23	131	11
6-2 FtSaAm	67	117	8.2
6-2 FtTAoS	2.6	107	9.1
6-2 FtTHN+	5.0	101	5.6

Application 3b: Modified Organic Extracts Per- and Polyfluoroalkyl Substances in Groundwater

Allred, B. M.; Lang, J. R.; Barlaz, M. A.; Field, J. A., Orthogonal zirconium diol/C18 liquid chromatography-tandem mass spectrometry analysis of poly and perfluoroalkyl substances in landfill leachate. *J. Chromatogr. A* **2014,** *1359*, 202-211.

Lang, J. R.; Allred, B. M.; Field, J. A.; Levis, J. W.; Barlaz, M. A., National Estimate of Per- and Polyfluoroalkyl Substance (PFAS) Release to US Municipal Landfill Leachate. *Environ. Sci. Technol.* **2017,** *51*, (4), 2197-2205.

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Robel, A. E.; Marshall, K.; Dickinson, M.; Lunderberg, D.; Butt, C.; Peaslee, G.; Stapleton, H. M.; Field, J. A., Closing the Mass Balance on Fluorine on Papers and Textiles. *Environ. Sci. Technol.* **2017,** *51*, (16), 9022-9032.

Summary

- Many LC-MS/MS analyses simply don't require SPE (chemically redundant)
- Eliminating SPE by LVI saves time and money by reducing steps and waste
- Divert valves offer control over matrix components (salts in seawater)
- LVI does not have to result in shorter analytical column lifetimes or dirtier mass spectrometers

Acknowledgments

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- EPA STAR Program
- NELAC Institute