

The Role of LC and LC/MS in the Environmental Laboratory:

An Overview of Recent Technology Trends

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Outline

- Recent trends
- Comparison of GC and LC
- Examples of technological advantages with emerging environmental pollutants

Analytical Trends – An Overview

UCMR 3 (Unregulated Contaminant Monitoring Rule) Proposed Contaminants and Corresponding Analytical Methods

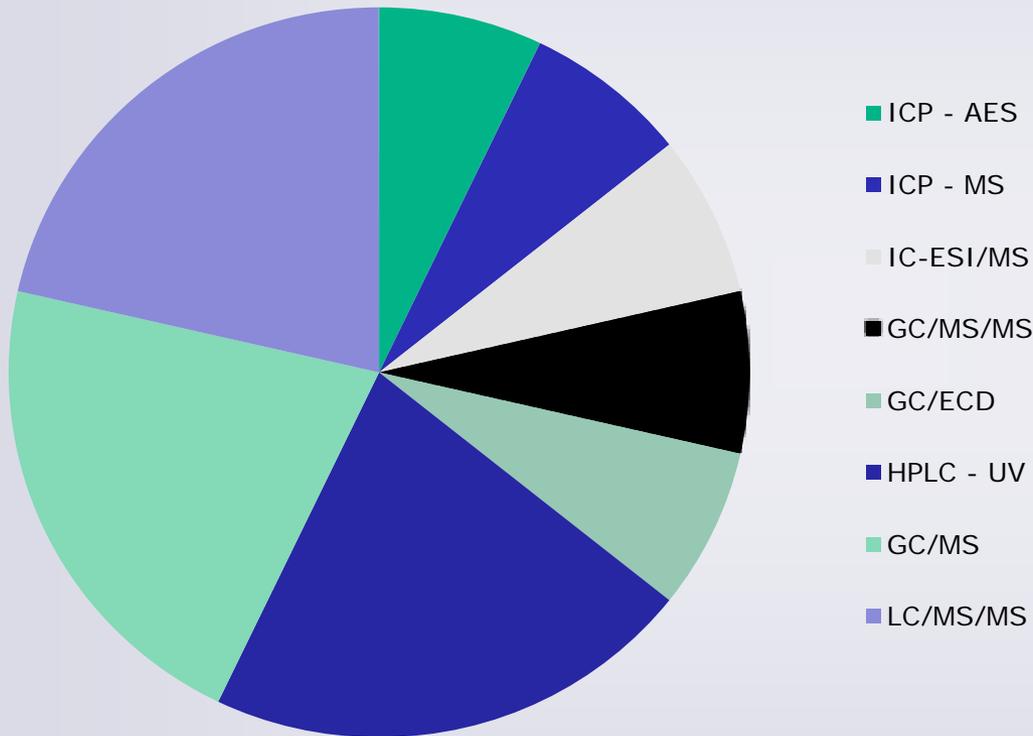


METHOD 539: DETERMINATION OF HORMONES IN DRINKING WATER BY SOLID PHASE EXTRACTION (SPE) AND LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (LC-ESI-MS/MS)

METHOD 537. DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Analytical Trends – An Overview

New Methods for Drinking Water



- 20 % of new methods from ODW are LC/MS/MS, same percentage as GC/MS

- 8 LC/MS/MS methods have been identified in the Standardized Analytical Methods for the Environmental Restoration Following Homeland Security Events (SAM)

Analytical Trends – An Overview

ChromaBLOGraphy

Topical and timely insights from top chromatographers.

LC-MS/MS the Future of New Pesticide Analysis According to Speakers at FPRW

July 22nd, 2011 by [Jack Cochran](#)

Two representatives from companies who make crop protection/science chemicals (pesticides, etc.) spoke at the [Florida Pesticide Residue Workshop](#) this week. Tom Gould of [Bayer Crop Science](#) gave the presentation **Conversion of Three Old Style Analytical Methods to LC/MS-MS Technology Utilizing Isotopic Internal Standards** and Jim Stry of [DuPont Crop Protection](#) talked on **Trends in Residue Method Development at DuPont Crop Protection**. Both indicated that their companies' new chemicals, today and tomorrow, would NOT be GC-able, so the trend towards pesticide residue determinations via LC-MS/MS continues. To keep from being a complete dinosaur in the lab, I'll have to continue looking over Sharon Lupo's shoulder. Sharon is our LC-MS/MS guru, except she does rely on me when there's any problem with the sample loop...

Analytical Comparison

Analytical Step	Advantages of Gas Chromatography	Advantages of Liquid Chromatography
Sample Extraction		<ul style="list-style-type: none"> • Can reduce sample preparation steps and extraction times • Can lessen the need for derivitization (<i>Example 1 - phenoxy acid herbicides</i>)
Sample Introduction	<ul style="list-style-type: none"> • Analyte solubility and compatibility 	<ul style="list-style-type: none"> • Not prone to thermal degradation (<i>Example 2 - BFRs</i>)
Analyte / Matrix Separation	<ul style="list-style-type: none"> • High efficiency and peak capacity – significant for multi-component analyses 	<ul style="list-style-type: none"> • Can analyze a wider range of chemical species (<i>Example 3 - PPCPs</i>) • UHPLC is lessening run times (<i>Example 4 - PAH</i>) • Multiple class analysis is common (<i>Example 5 - Multi pesticides</i>) • Alternate separation mechanisms (<i>Example 6 - PAH and Iodated X-ray media</i>)
Analyte Ionization / Detection	<ul style="list-style-type: none"> • Robust and reproducible electron ionization (EI) and available libraries • Relatively inexpensive 	<ul style="list-style-type: none"> • Specific and sensitive detection with LC/MS/MS and MRM - significant for multi-component analyses

Sample Preparation - Derivatization

METHOD 515.4 DETERMINATION OF CHLORINATED ACIDS IN DRINKING WATER BY LIQUID-LIQUID MICROEXTRACTION, DERIVATIZATION, AND FAST GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

- 11.2.1.2 Add a sufficient amount of MtBE (approximately 7 mL) to tube 1 to cover the first impinger. Add 10 mL of MtBE to the collection vial. Set the nitrogen flow at 5-10 mL/min. Add 4-mL Diazald solution (Sect. 7.1.13) and 3 mL of 37% KOH solution (Sect. 7.1.11) to the second impinger. Connect the tubing as shown and allow the nitrogen flow to purge the diazomethane from the reaction vessel into the collection vial for 30 minutes. Cap the vial when collection is complete and maintain at 0-5 °C. When stored at 0-5 °C, this diazomethane solution may be used over a period of 72 hours.
- 11.2.5 Remove any unreacted diazomethane by adding 0.1 g of silica gel. Effervescence (evolution of nitrogen) is an indication that excess diazomethane was present. Allow the extracts to sit for 0.5 hour.
- 11.2.4 Add 250 uL of the diazomethane solution prepared in Section 11.2.1 to each vial. The contents of the vial should remain slightly yellow in color indicating an excess of diazomethane. Additional diazomethane may be added if necessary. Let the esterification reaction proceed for 30 minutes.

5.4 Diazomethane is a toxic carcinogen which can explode under certain conditions.

Sample Preparation - Derivatization

METHOD 555

DETERMINATION OF CHLORINATED ACIDS IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH A PHOTODIODE ARRAY ULTRAVIOLET DETECTOR

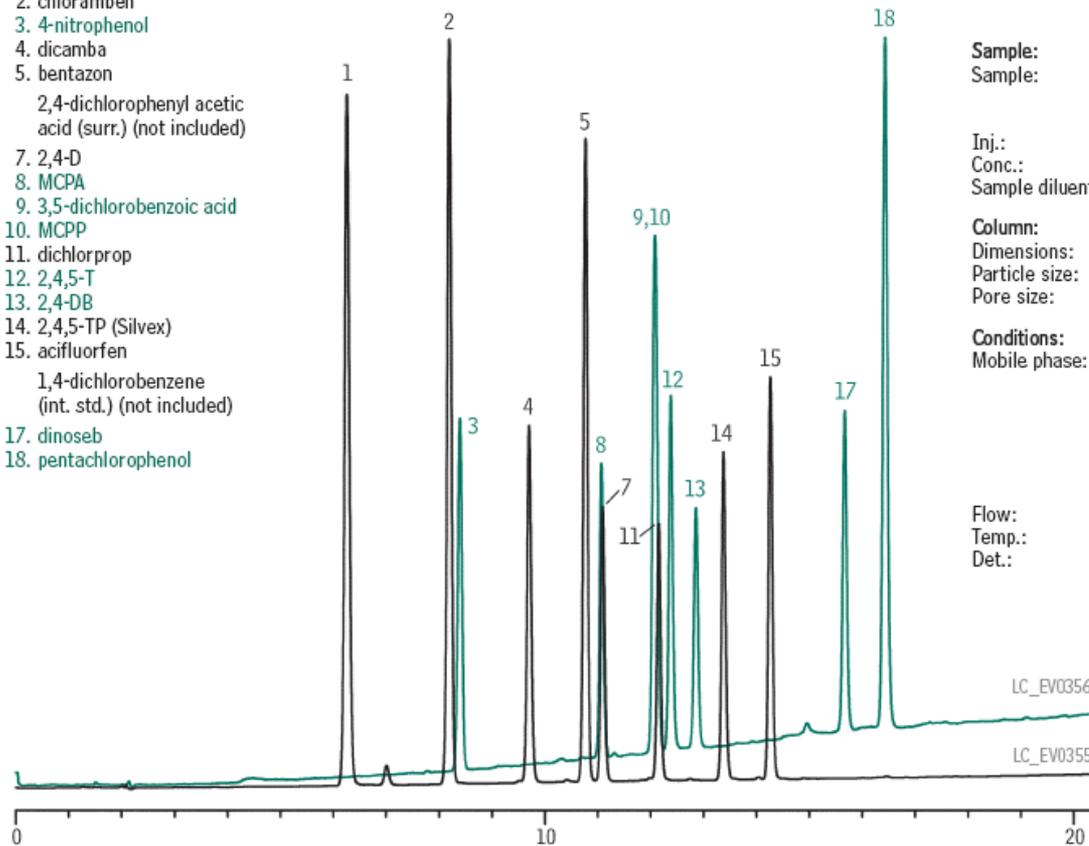
- 11.1.2 Add 1.7 mL of 6 N NaOH to the sample, seal, and shake. Check the pH of the sample with pH paper; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 6 N NaOH. Let the sample sit at room temperature for one hour, shaking the sample bottle and contents periodically.
- 11.1.3 Add 2 mL of concentrated H_3PO_4 to the sample, seal, and shake to mix. Check the pH of the sample with pH paper; if the sample does not have a pH less than or equal to two, adjust the pH by adding more H_3PO_4 .
- 11.1.4 From the homogeneous sample, remove a 20 mL aliquot for analysis. Filter the aliquot through a 0.45 μm filter into a graduated cylinder or other convenient graduated container. Using an HPLC pump (or HPLC reagent delivery pump), pump the 20 mL aliquot through the on-line concentrator column at a flowrate of 5.0 mL/min (See Figure 1). The use of a liquid-solid extraction disk is perfectly acceptable providing all QC criteria in Section 9.0 are met or exceeded. After passing the sample through the concentrator column, follow with an additional 10 mL of the aqueous mobile phase (0.025 M H_3PO_4).

Sample Preparation - Derivatization

Phenoxyacid Herbicides on Ultra Aqueous C18

Mix A and Mix B

1. picloram
2. chloramben
3. 4-nitrophenol
4. dicamba
5. bentazon
- 2,4-dichlorophenyl acetic acid (surr.) (not included)
7. 2,4-D
8. MCPA
9. 3,5-dichlorobenzoic acid
10. MCPP
11. dichlorprop
12. 2,4,5-T
13. 2,4-DB
14. 2,4,5-TP (Silvex)
15. acifluorfen
- 1,4-dichlorobenzene (int. std.) (not included)
17. dinoseb
18. pentachlorophenol



Sample:

Sample: Chlorinated Acids by HPLC, Mix A (cat.# 32431), or Chlorinated Acids by HPLC, Mix B (cat.# 32430)
1000µg/mL each component in acetonitrile
Inj.: 10µL
Conc.: 10ppm each analyte
Sample diluent: acetonitrile

Column:

Ultra Aqueous C18 (cat.# 9178565)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 100Å

Conditions:

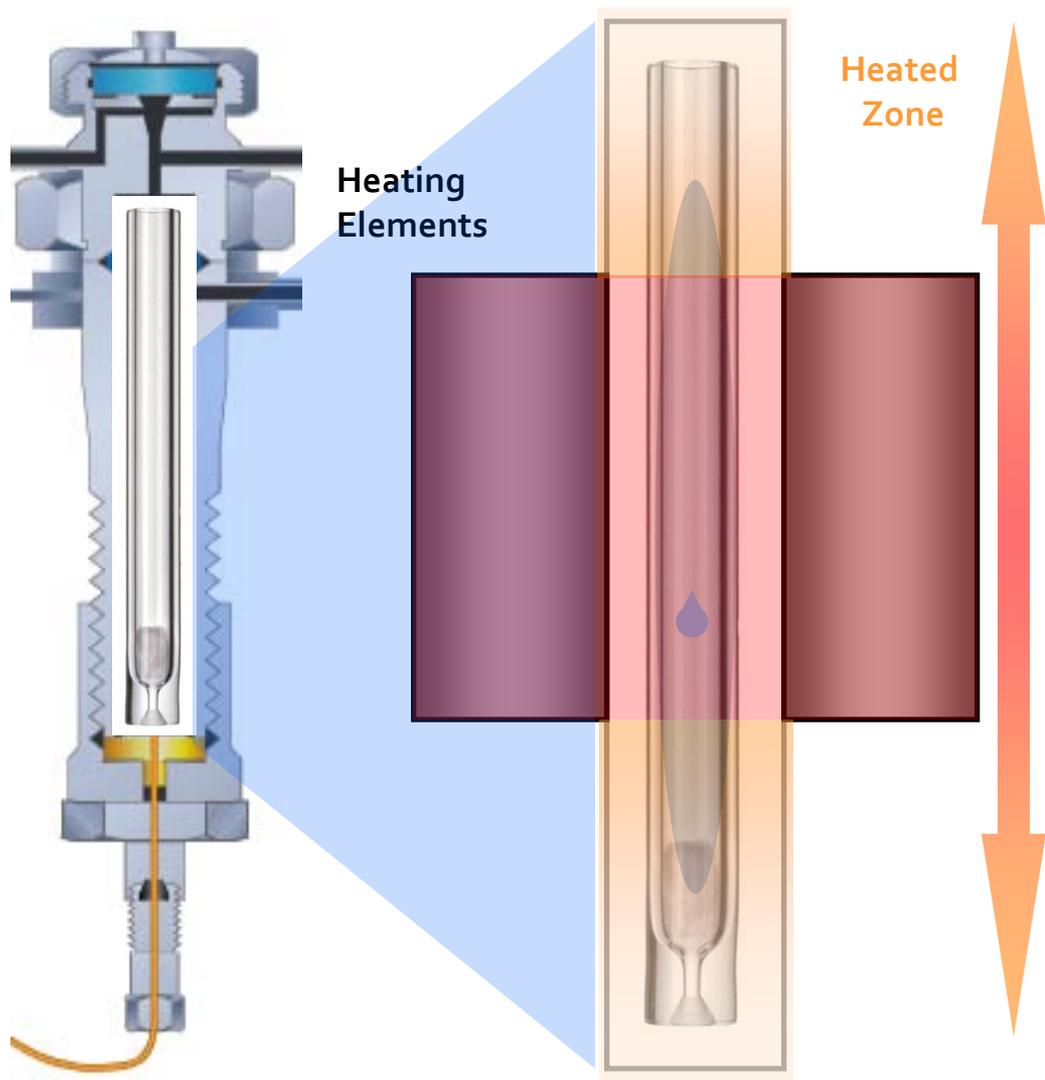
Mobile phase: A) 0.05% H₃PO₄; B) acetonitrile

Time (min.)	%B
0	20.0
28	80.0
33	90.0
34	20.0

Flow:

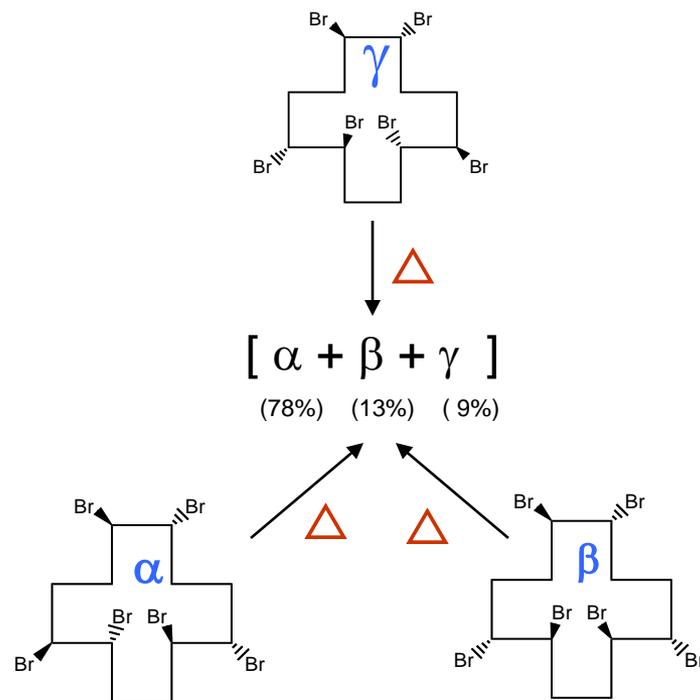
1.0mL/min.
Temp.: ambient
Det.: UV @ 225nm

Sample Introduction - Injection Port Discrimination



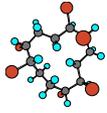
GC/MS is a suitable technique for the determination of TOTAL Hexabromocyclododecane (HBCD)

Problem with HBCD isomers undergoing thermal isomerization in the elevated temperatures of a GC injector port



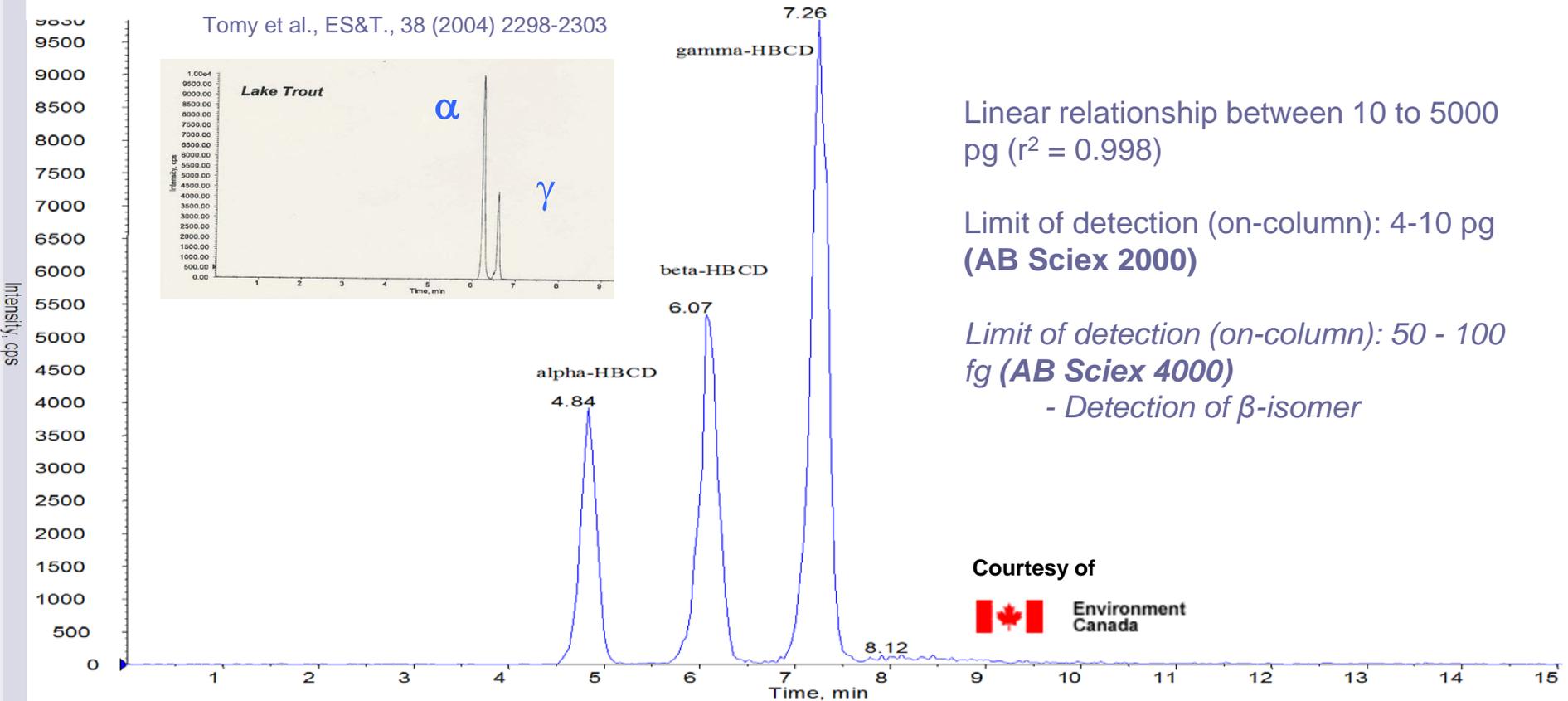
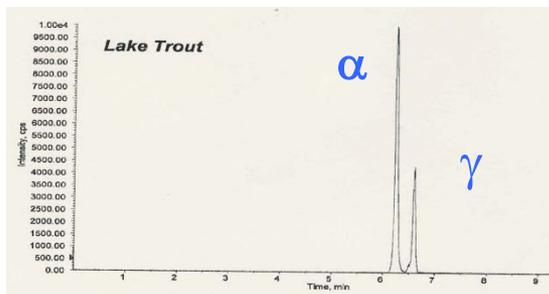
Separation of HBCD diastereoisomers

Viva C₁₈ wide pore (300Å) column (5µm 100mm x 2.1mm id).



HBCD isomers accumulate differently in biota compared to sediment

Tomy et al., ES&T., 38 (2004) 2298-2303



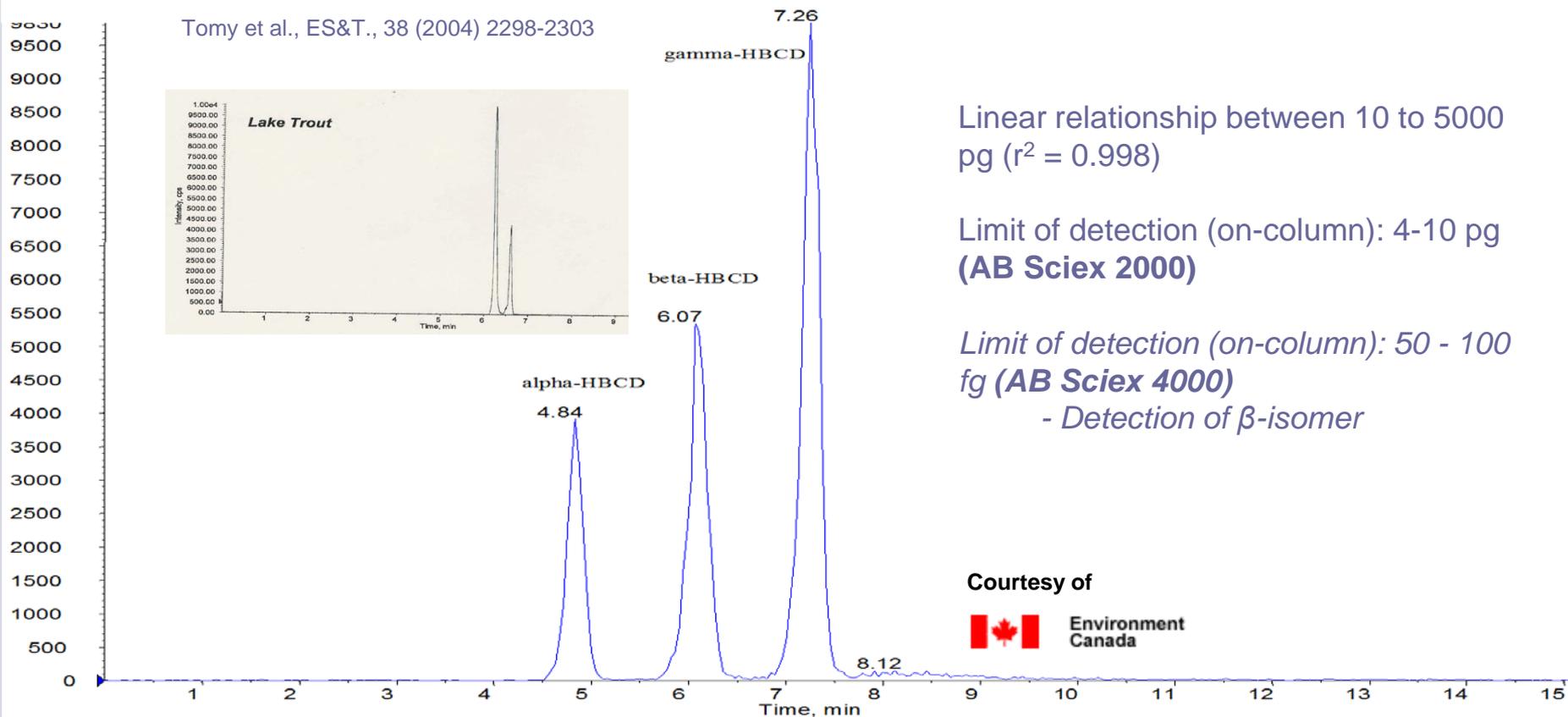
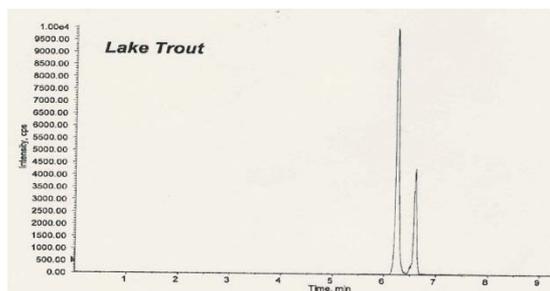
Separation of HBCD diastereoisomers

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HBCD isomers accumulate differently in biota compared to sediment

Tomy et al., ES&T., 38 (2004) 2298-2303



Linear relationship between 10 to 5000 pg ($r^2 = 0.998$)

Limit of detection (on-column): 4-10 pg (AB Sciex 2000)

Limit of detection (on-column): 50 - 100 fg (AB Sciex 4000)
- Detection of β -isomer

Courtesy of



Environment
Canada

Analysis of Halogenated Flame Retardants

Liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish

Simon Ningsun Zhou^{a,b,*}, Eric J. Reiner^b, Chris Marvin^c, Terry Kolic^b, Nicole Riddell^d, Paul Helm^e, Frank Dorman^{f,g}, Michelle Misselwitz^f, Ian D. Brindle^a

Table 1: Halogenated flame retardants ion(s) in APCI source for LC-APCI-MS/MS analysis

Compound	Abbreviation	Ion(s) in source [*]
hexabromocyclododecane	HBCD	[M-H] ⁻ , [M+O ₂] ⁻
2,2',4,4'-tetrabromodiphenyl ether	BDE-47	[M-Br+O] ⁻ , [M-HBr+O ₂] ⁻
2,2',4,4',5-pentabromodiphenyl ether	BDE-99	[M-Br+O] ⁻ , [M-HBr-Br+O] ⁻
2,2',4,4',6-pentabromodiphenyl ether	BDE-100	[M-Br+O] ⁻ , [M-HBr-Br+O] ⁻
2,2',4,4',5,5'-hexabromodiphenyl ether	BDE-153	[M-Br+O] ⁻ , [M-HBr-Br+O] ⁻
2,2',4,4',5,6'-hexabromodiphenyl ether	BDE-154	[M-Br+O] ⁻ , [M-HBr-Br+O] ⁻
2,2',4,4',5,5'-hexabromobiphenyl	BB-153	[M-Br+O] ⁻
dechlorane plus	DP	[M-Cl+O] ⁻ , [M+O ₂] ⁻ , [M-H] ⁻
hexabromobenzene	HBB	[M-Br+O] ⁻
pentabromoethylbenzene	PBEB	[M-Br+O] ⁻
hexachlorocyclopentadienyl-dibromocyclooctane	HCDBCO	[M+O ₂] ⁻
2,2',3,4,4',5',6-heptabromodiphenyl ether	BDE-183	[M-Br+O] ⁻ , [M-HBr-Br+O] ⁻
2-ethylhexyl-2,3,4,5-tetrabromobenzoate	EHTeBB	[M-Br+O] ⁻
1,2-bis(2,4,6-tribromophenoxy) ethane	BTBPE	C ₆ Br ₃ H ₂ O ⁻
2,3,3',4,4',5,5',6-octabromodiphenyl ether	BDE-205	[M-Br+O] ⁻ , [M-HBr-Br+O] ⁻
2,2',3,3',4,4',5,5',6,6'-nonabromo-4'-chlorodiphenyl ether	4PC-BDE208	C ₆ Br ₅ O ⁻ , C ₆ Br ₄ ClO ⁻ , [M-Br+O] ⁻
2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether	BDE-209	[M-Br+O] ⁻ , C ₆ Br ₅ O ⁻
Bis(2-ethyl-1-hexyl)tetrabromophthalate	BEHTBP	[M-Br+O] ⁻ , [M-C ₈ H ₁₇ +H-Br] ⁻
octabromotrimethylphenylindane	OBIND	[M-Br+O] ⁻ , [M-HBr+O ₂] ⁻ , [M-HBr-Br+O] ⁻
3,3',5,5'-tetrabromobisphenol A	TBBP-A	[M-H] ⁻ , [M-Br ₂] ⁻
allyl 2,4,6-tribromophenyl ether	ATE	C ₆ Br ₃ H ₂ O ⁻
2-bromoallyl 2,4,6-tribromophenyl ether	BATE	C ₆ Br ₃ H ₂ O ⁻ , C ₃ H ₆ Br
2,3-dibromopropyl 2,4,6-tribromophenyl ether	DPTE	C ₆ Br ₃ H ₂ O ⁻

* Order with decreasing intensity if there was more than one ion generated in the APCI source.

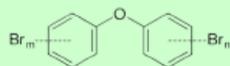
Chemical	On-column IDL (pg)	LOQ (ng/g)
ATE	4	0.037
BTBPE	0.5	0.011
BDE-17	40	3.20
BATE	2	0.32
BDE-47	4	0.049
BDE-66	1	0.016
BDE-71	4	0.036
BDE-77	2	0.011
PBEB	2	0.018
DPTE	20	0.11
EHTeBB	1	0.0061
HBB	0.5	0.0045
BDF-23478	0.5	0.0060
BDE-100	0.5	0.0063
BDE-99	1	0.0045
BDE-126	0.5	0.0027
TBBP-A	0.5	0.012
BB-153	1	0.036
HCDBCO	20	0.39
BDE-138	1	0.0062
BDE-154	0.5	0.020
BDE-153	0.5	0.0031
a-DP	4	0.11
s-DP	20	0.37
α-HBCD	10	0.048
β-HBCD	4	0.051
γ-HBCD	10	0.19
BEHTBP	0.5	0.042
BDE-183	0.5	0.0041
BDE-197	2	0.054
BDE-205	0.5	0.0098
OBIND	1	0.0088
BDE-206	0.5	0.0096
4PC-BDE208	2	0.082
BDE-209	4	0.086
DBDPE	4	0.020

Analysis of Brominated Flame Retardants

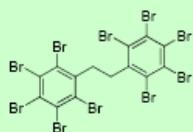
Comprehensive LCMS Method for the Analysis of Commonly found Brominated Flame Retardants

Michelle N. Misselwitz, Frank L. Dorman, PhD., Julie A. Kowalski, PhD., Richard J. Lake.

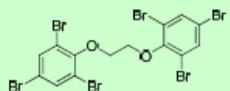
Brominated diphenyl ethers (BDE)



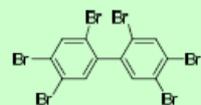
Decabromodiphenylethane (DBDPE)



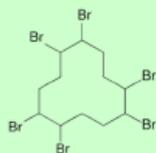
Bis(2,4,6-tribromophenoxy)ethane (BTBPE)



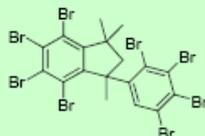
2,2',4,4',5,5'-hexabromobiphenyl (BB 153)



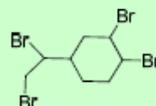
Hexabromocyclododecane (HBCD)



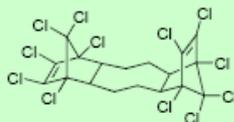
Octabromotrimethylphenylindane (OBIND)



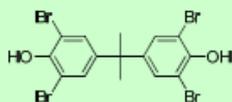
Tetrabromoethylcyclohexane (TBECH)



Dechlorane plus (DP)



Tetrabromobisphenol A (TBBPA)



Some compounds like BDE 99 and BDE 100 have the same ionization patterns and therefore must be chromatographically separated

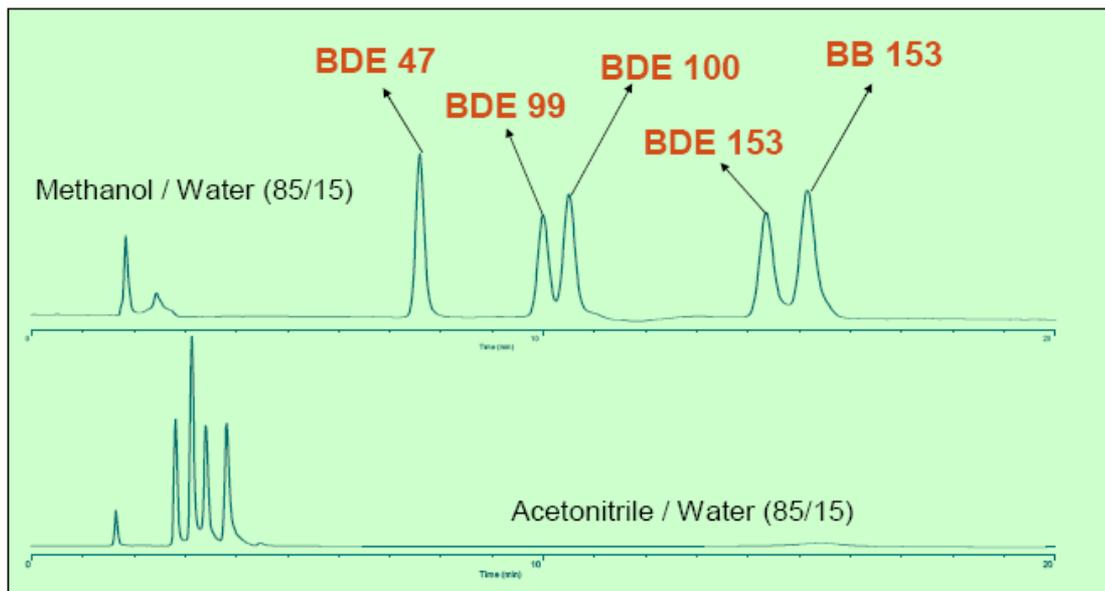
BDE 209 and DBDPE are troublesome compounds when it comes to solubility. Decabromodiphenyl ether has <0.1 µg/L solubility in water [1]. These compounds are typically soluble in toluene, which is not readily amenable to LCMS analysis. Compounds such as HBCD and its individual diastereoisomers degrade rapidly in acetonitrile [2].

1. Agency for Toxic Substances and Disease Registry (ATSDR). **2004**. "Toxicological profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers." Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
2. Tomy, Gregg; Halldorson, Thor; Danell, Robert; Law, Kerri; Arsenault, Gilles; Alaei, Mehran. "Refinements to the diastereoisomer-specific method for the analysis of hexabromocyclododecane." *Rapid Commun. Mass Spectrom.* **2005**;19: 2819-2826

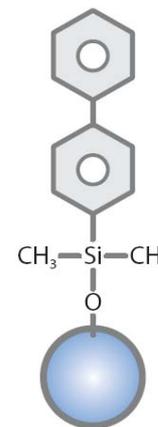
Solvent Compatibility – Methanol vs. Acetonitrile

Comprehensive LCMS Method for the Analysis of Commonly found Brominated Flame Retardants

Pinnacle DB Biphenyl 3 μ m 150mm x 4.6mm



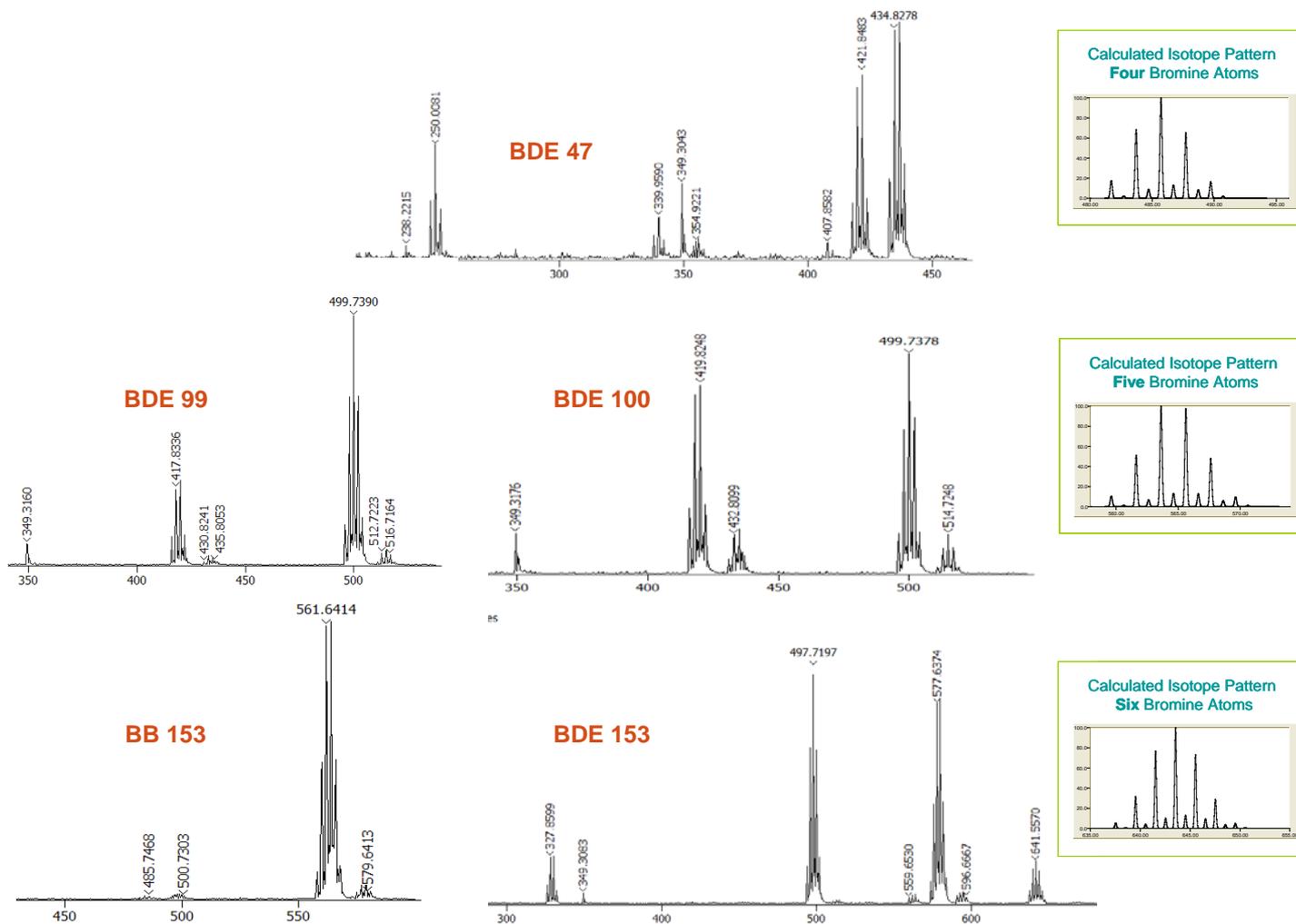
Methanol greatly enhances the retention and selectivity of aromatic, conjugated, and compounds containing electron withdrawing functionality



Biphenyl

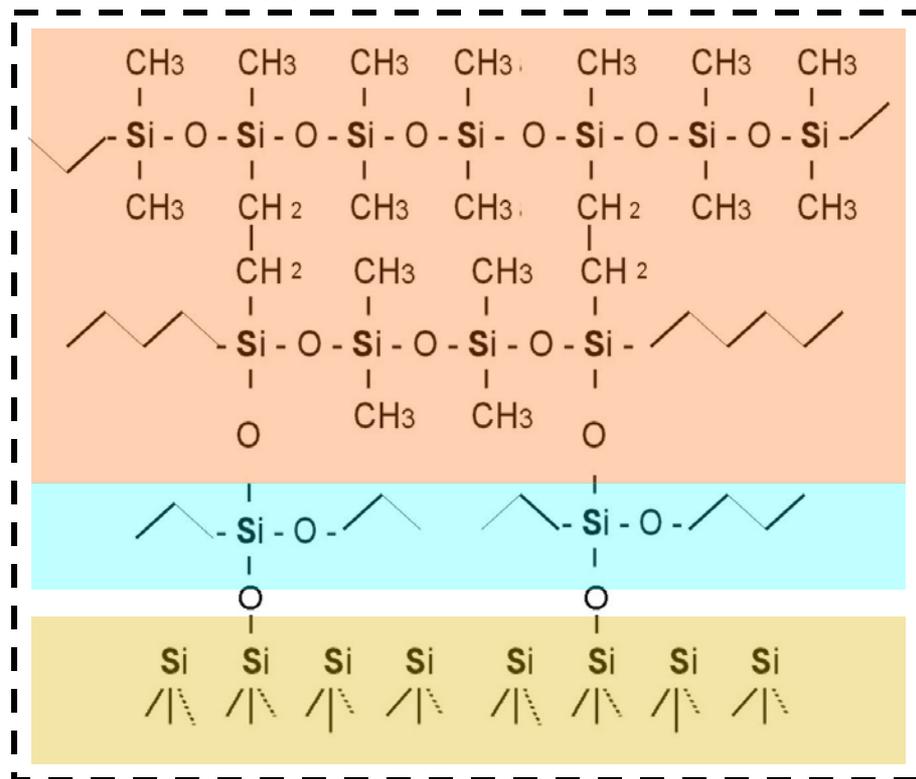
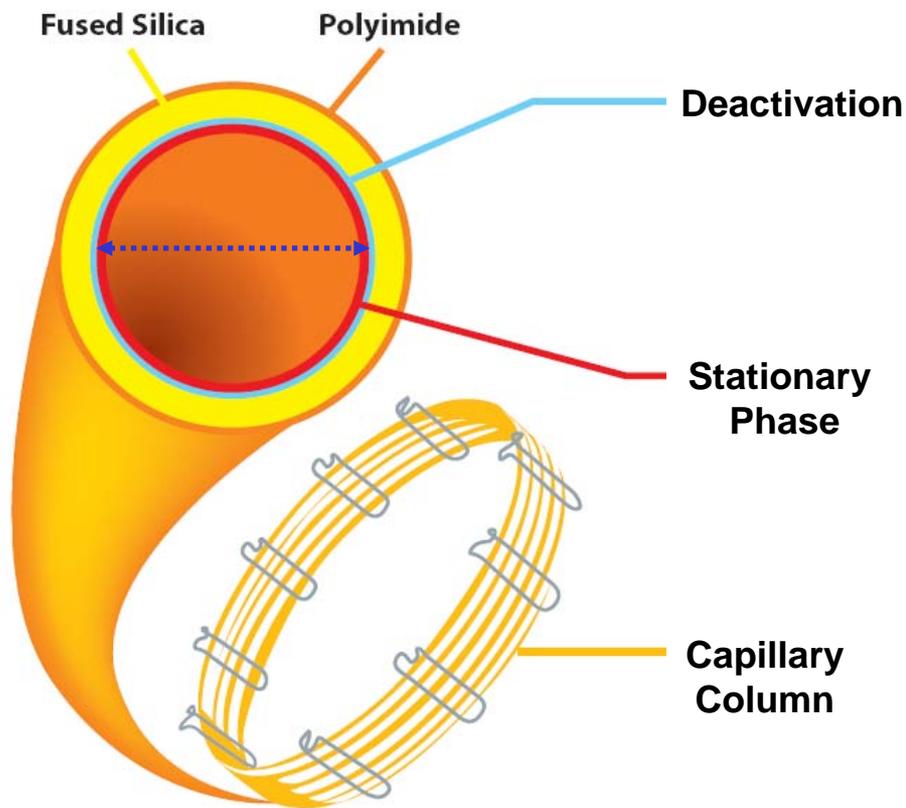
1. Min Yang , Steven Fazio, David Munch and Patrick Drumm , *Journal of Chromatography A* 1097 (2005) 124

Comprehensive LCMS Method for the Analysis of Commonly found Brominated Flame Retardants



Comparison of Separations – Gas - Liquid

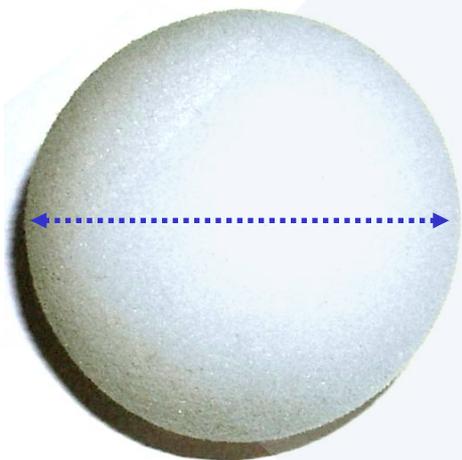
Increase Efficiency by decreasing column inner diameter (ID)



Gas – Liquid diffusion is relatively fast, highly temperature dependent, and utilizes “thick”

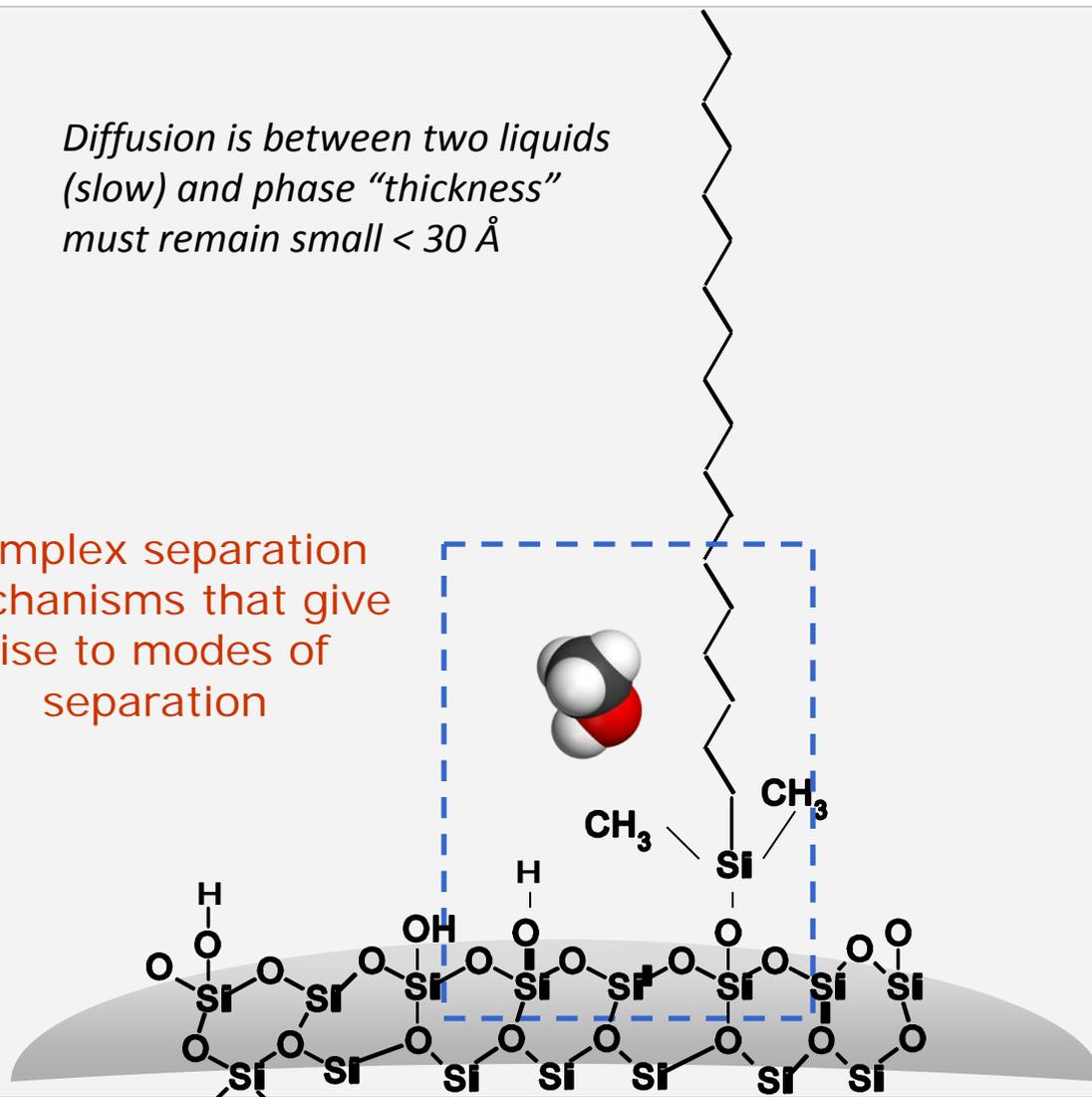
Comparison of Separations – Liquid- Liquid

Increase Efficiency by decreasing column particle diameter (d_p)



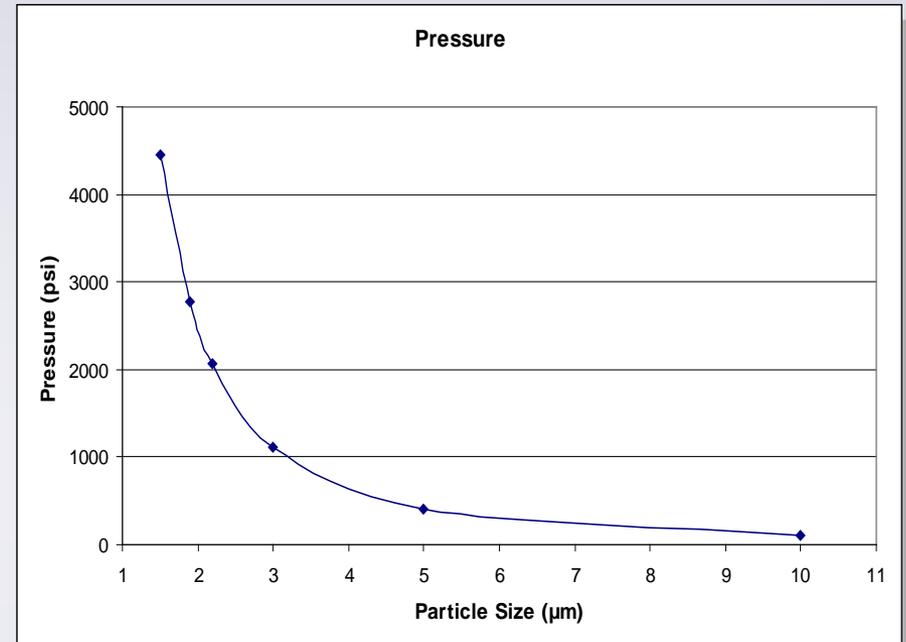
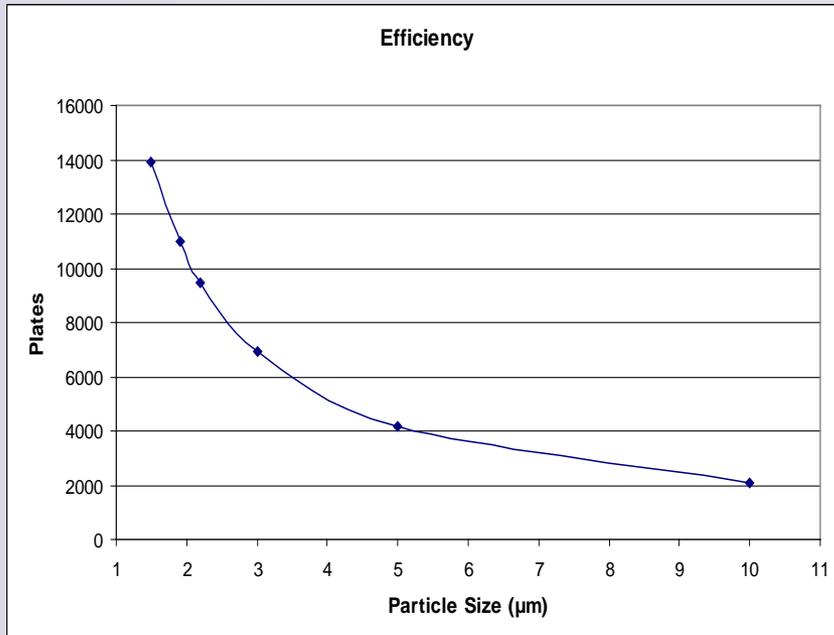
Complex separation mechanisms that give rise to modes of separation

Diffusion is between two liquids (slow) and phase "thickness" must remain small $< 30 \text{ \AA}$



Ultra High Performance Liquid Chromatography

Theoretical Plot of Efficiency and Pressure versus Particle Size

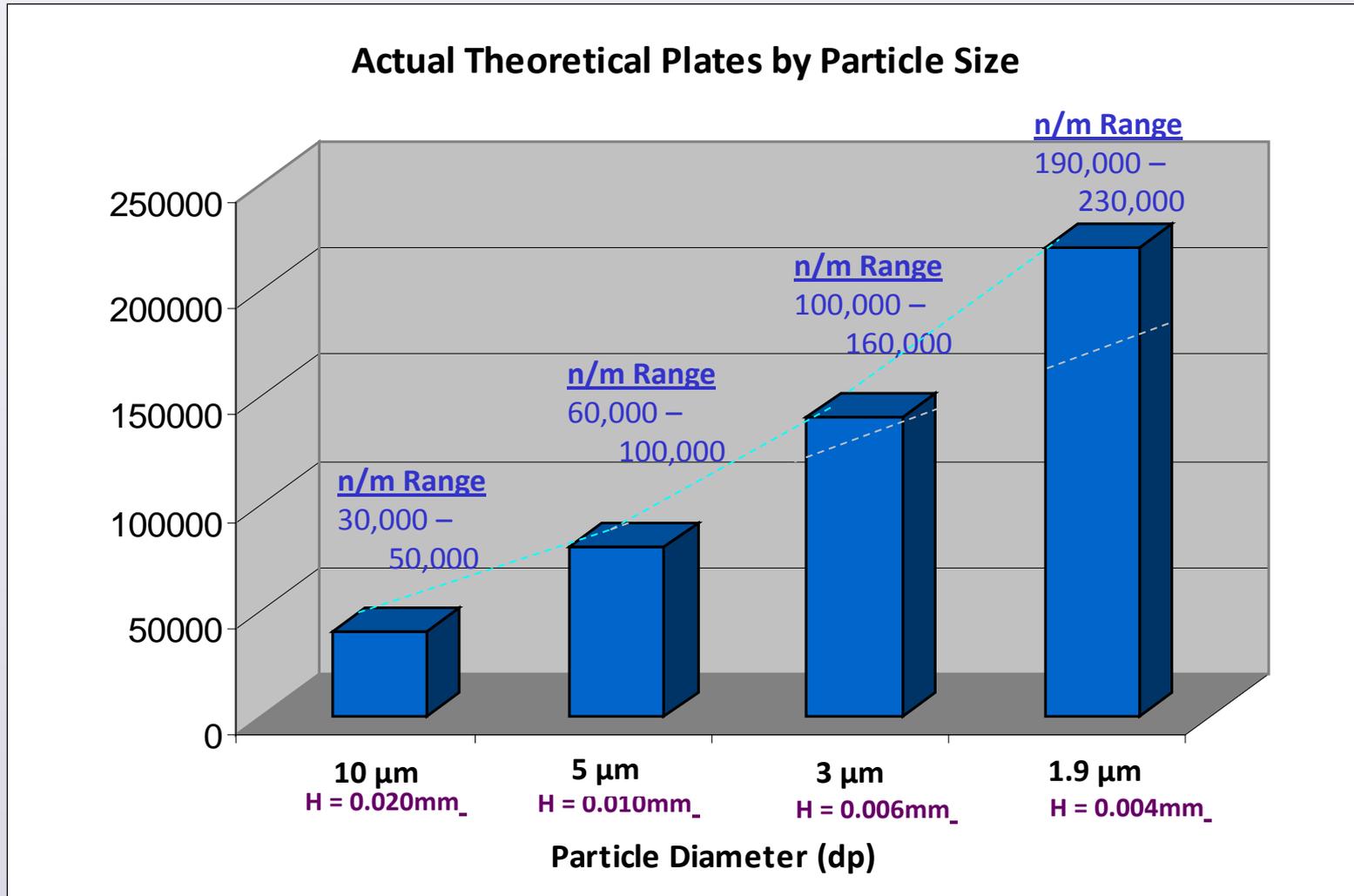


$$N \propto \frac{L}{d_p}$$

$$P = \frac{\Phi L \eta F}{d_p^2 d_c^2}$$

1. L. R. Snyder and J. J. Kirkland "Introduction to Modern Liquid Chromatography" John Wiley & Sons, Inc. (1979) p.37

Comparison of Particle Size Efficiencies



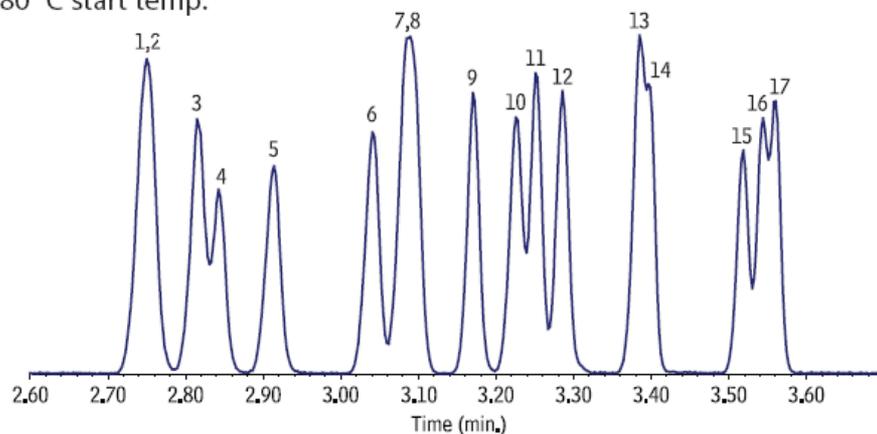
Efficiency Comparison

Rxi®-5Sil MS 30m x 0.25mm x 0.25µm

PC = 9

Split (10:1)

80 °C start temp.



Peak capacity (Pc) is the number of peaks that are resolved within a given retention time window.
Isocratic peak capacity :

$$Pc = (t_2 - t_{r1}) / W [2]$$

Where:

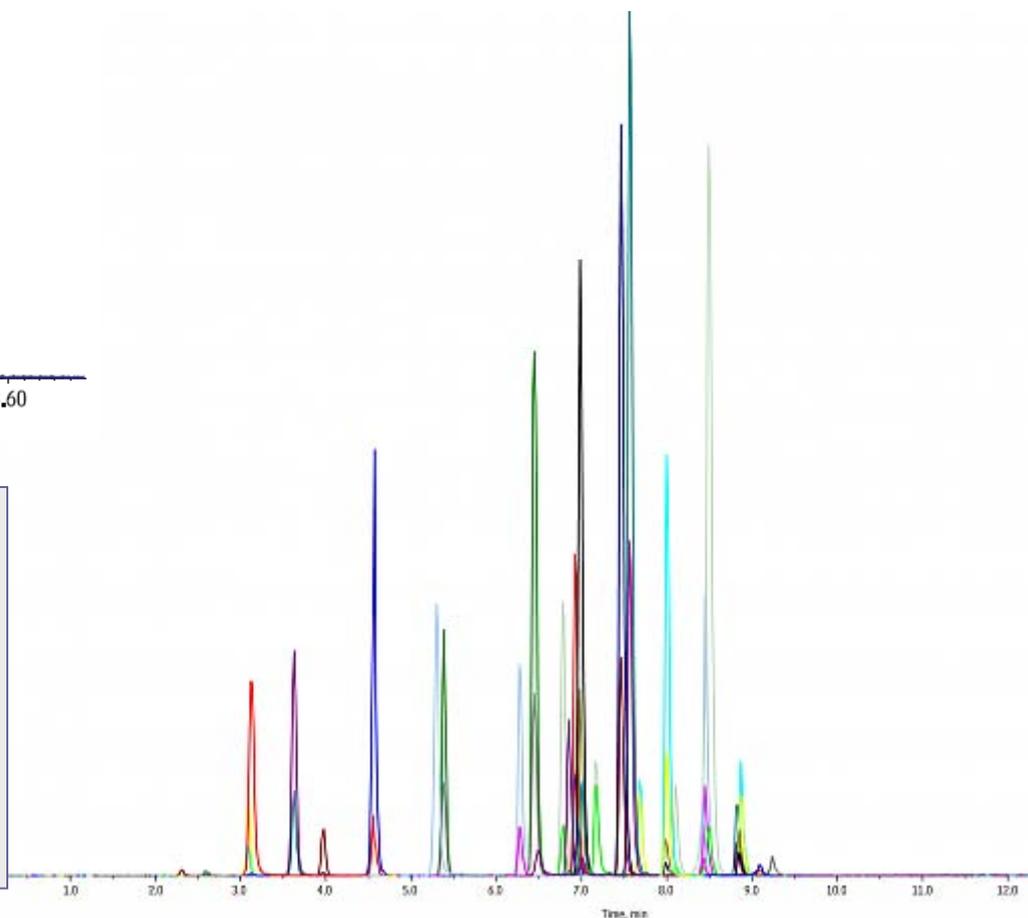
t_2 and t_{r1} = retention time of last and first eluting peaks

W = average peak width at baseline

Pinnacle® DB Aqueous C18

50 mm x 2.1 mm ID, 1.9 µm

PC = 5

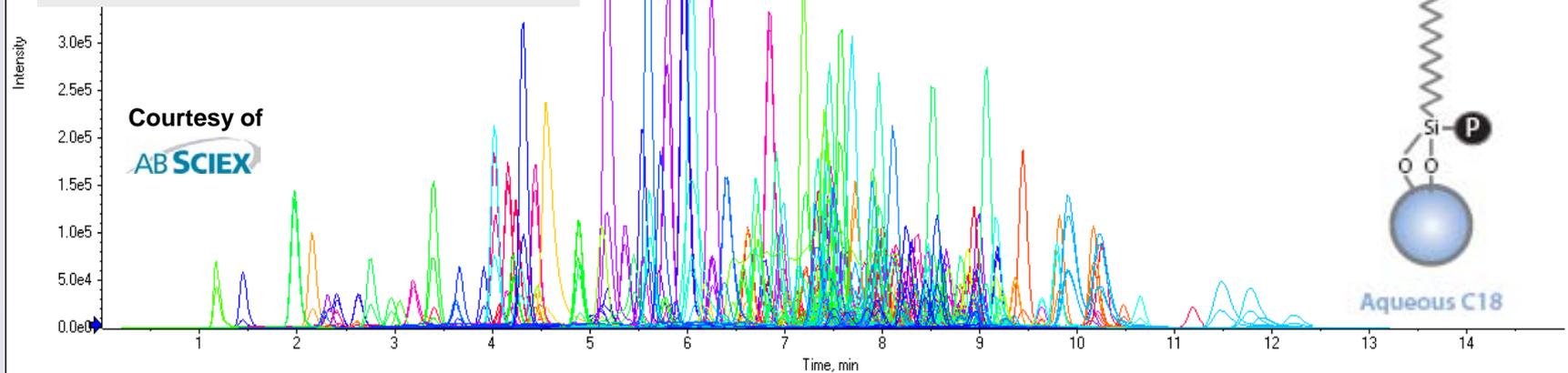


Multi Pesticide Analysis

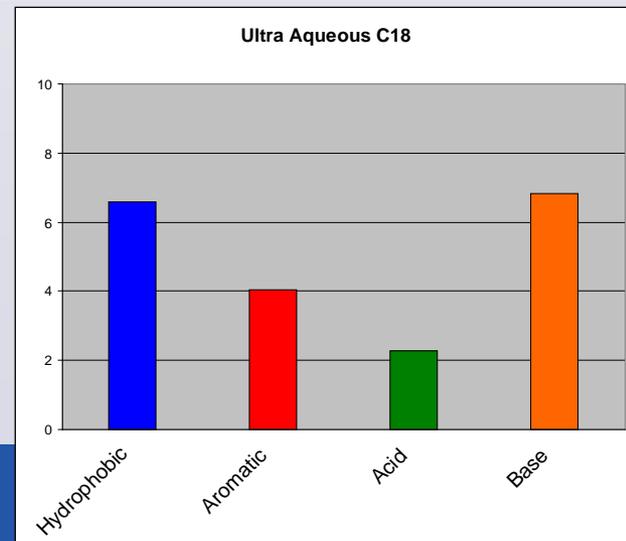
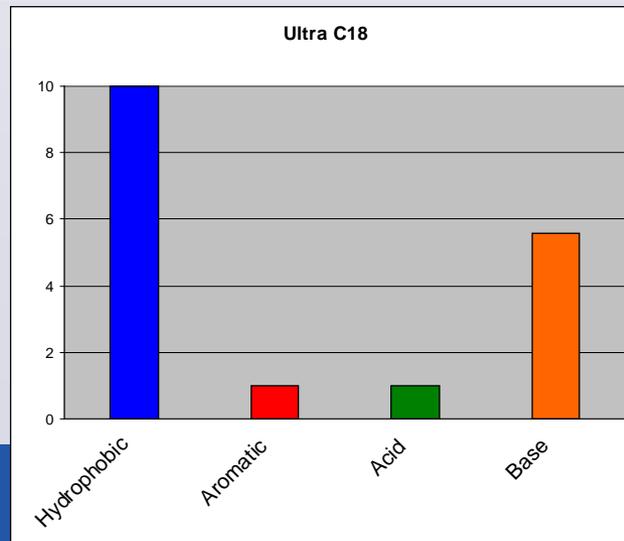
XIC from Data overnight quant. wiff (sample 6) - 1, +MRM (590 transitions): Thiocloprid 1, Gaussian smoothed

Separation of ~ 300 pesticides on a polar modified reversed phase column with a generic gradient of water/methanol + volatile buffer (total run time 20 min)

Restek Ultra Aqueous C18 (100 x 2.1mm) 3µm
Water/methanol + 10mM ammonium formate



Comparison of Retention profiles using the Hydrophobic Subtraction Model



Analysis of PPCPs

Compound	CAS	Q1 Ions	Group		
Acetaminophen	103-90-2	152.2	1	Positive ESI	Acid Extract
Caffeine	58-08-2	195	1		
1,7-dimethylxanthine	611-59-6	181.2	1		
Carbamazepine	298-46-4	237.4	1		
Codeine	76-57-3	300	1		
Cotinine	486-56-6	177	1		
Erythromycin	114-07-8	734.4	1		
Fluoxetine	54910-89-3	310.3	1		
Sulfamethoxazole	723-46-6	254	1		
Thiabendazole	148-79-8	202.1	1		
Trimethoprim	738-70-5	291	1		
Gemfibrozil	25812-30-0	249	3	Negative ESI	
Ibuprofen	15687-27-1	205.1	3		
Naproxen	22204-53-1	228.9	3		
Triclosan	3380-34-5	286.8	3		
Albuterol	18559-94-9	240	4	Positive ESI	Base Extract
Cimetidine	51481-61-9	253.1	4		
Ranitidine	66357-35-5	315	4		

Solvent Preparation

Groups 1 & 4

A: 0.3% Formic Acid, 0.1% Ammonium Formate in water

B: Acetonitrile:Methanol (1:1)

Group 3

A: 0.1% Ammonium Acetate, 0.1% Acetic Acid in water

B: Acetonitrile:Methanol (1:1)

Extract Solvent

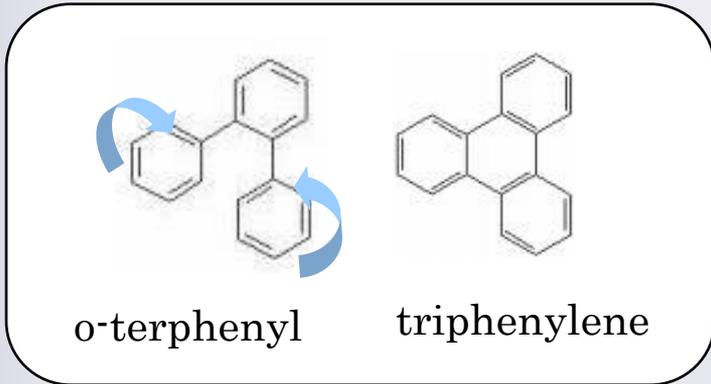
0.1% Formic Acid in Methanol: Water (75:25)

Standard Preparation

25 µg/mL in Extract Solvent

Selectivity Towards Shape

α o-terphenyl / triphenylene (Tanaka)



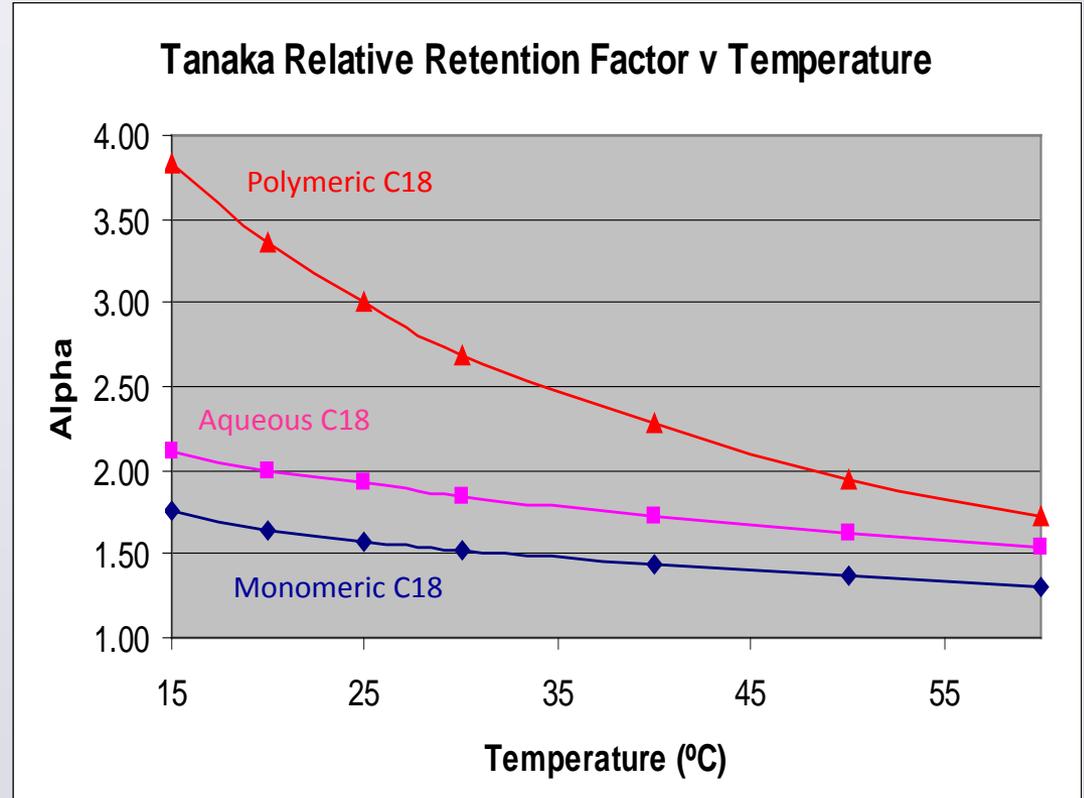
Column Dimensions: 150 x 4.6mm 5 μ m

MPA = Water, MPB = Methanol,

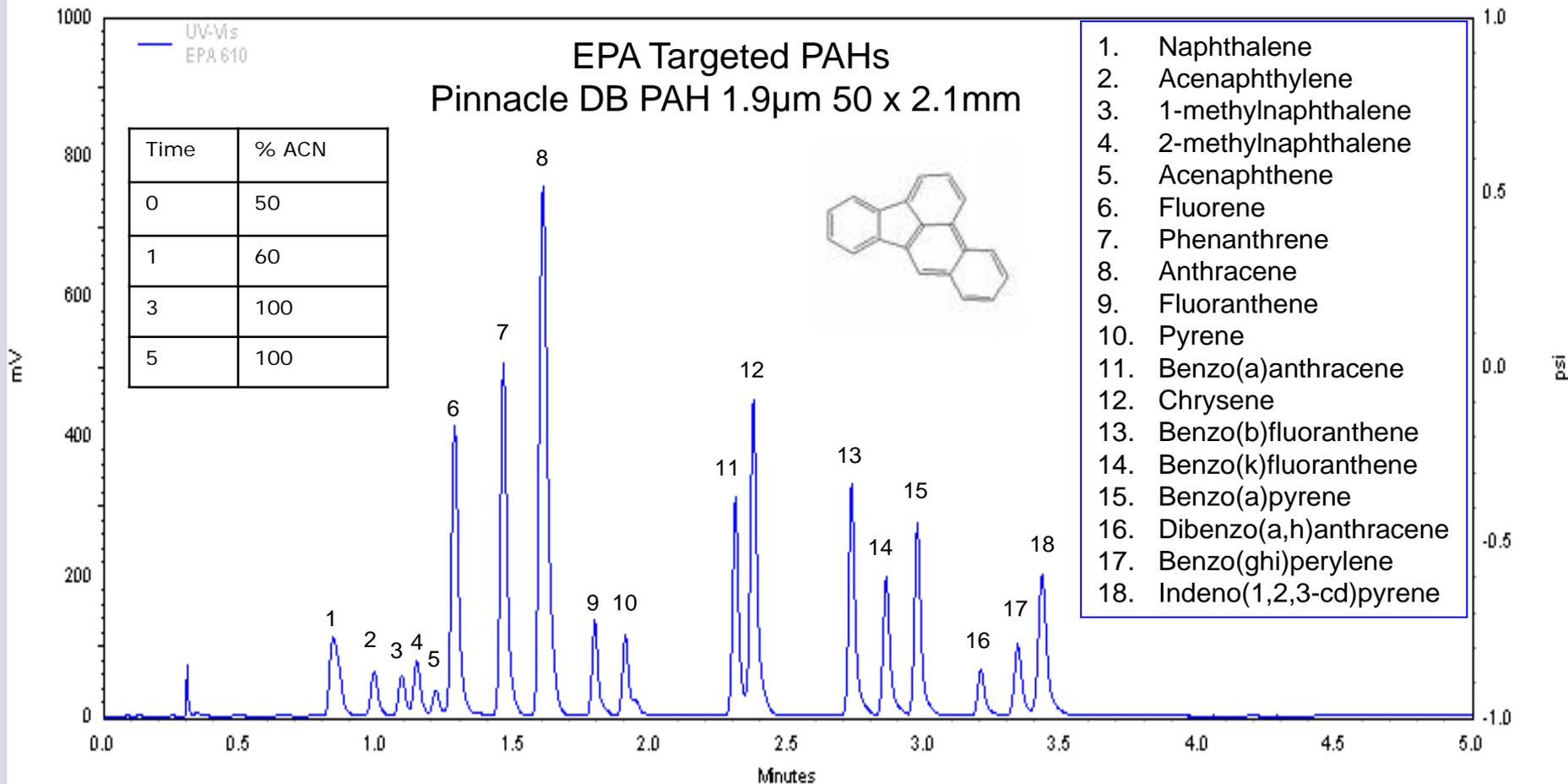
80%B Isocratic

Flow: 1.0mL/min, 5 μ L injection,

254nm,



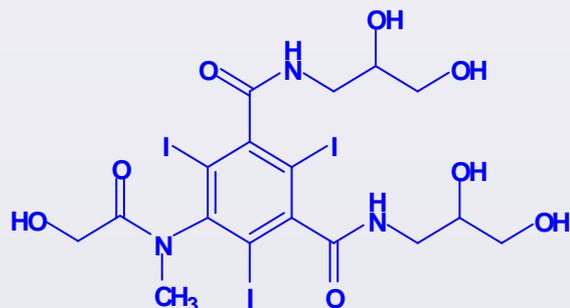
Specifically Designed Phase – L/B ratio



Mobile phase: Water/Acetonitrile, Flow: 0.6 mL/min, Temp: 30°C, UV detector: 254nm

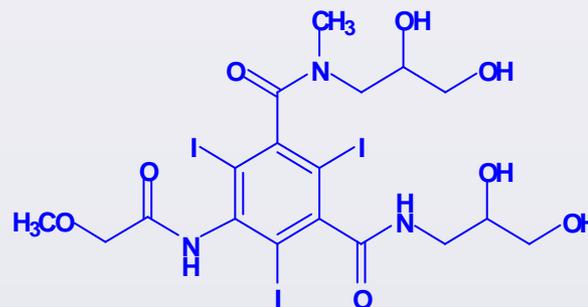
Analysis of X-Ray Agents in Wastewater

Chemical structures of X-ray contrast media



Chemical Formula: C₁₇H₂₂I₃N₃O₈
Exact Mass: 776.85

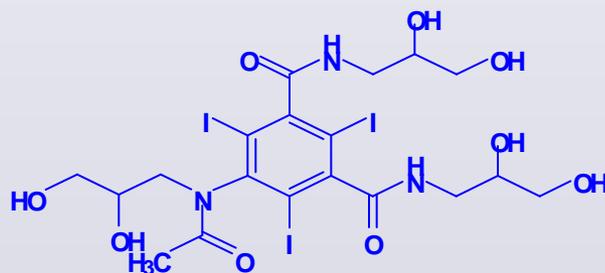
Iomeprol



Chemical Formula: C₁₈H₂₄I₃N₃O₈
Exact Mass: 790.87

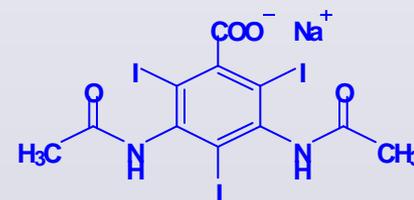
Iopromide

Iopamidol and Iomeprol are positional isomers and difficult to separate



Chemical Formula: C₁₉H₂₆I₃N₃O₉
Exact Mass: 820.88

Iohexol



Chemical Formula: C₁₁H₈I₃N₂NaO₄
Exact Mass: 635.75

Diatrizoate sodium

Structure of Iopamidol – isomer of Iomeprol

Iopamidol and iomeprol are positional isomers - need chromatographic separation

Iomeprol, iopromide and iohexol have chiral centers - need to sum LC peaks

LC/MS/MS Separation Method Parameters

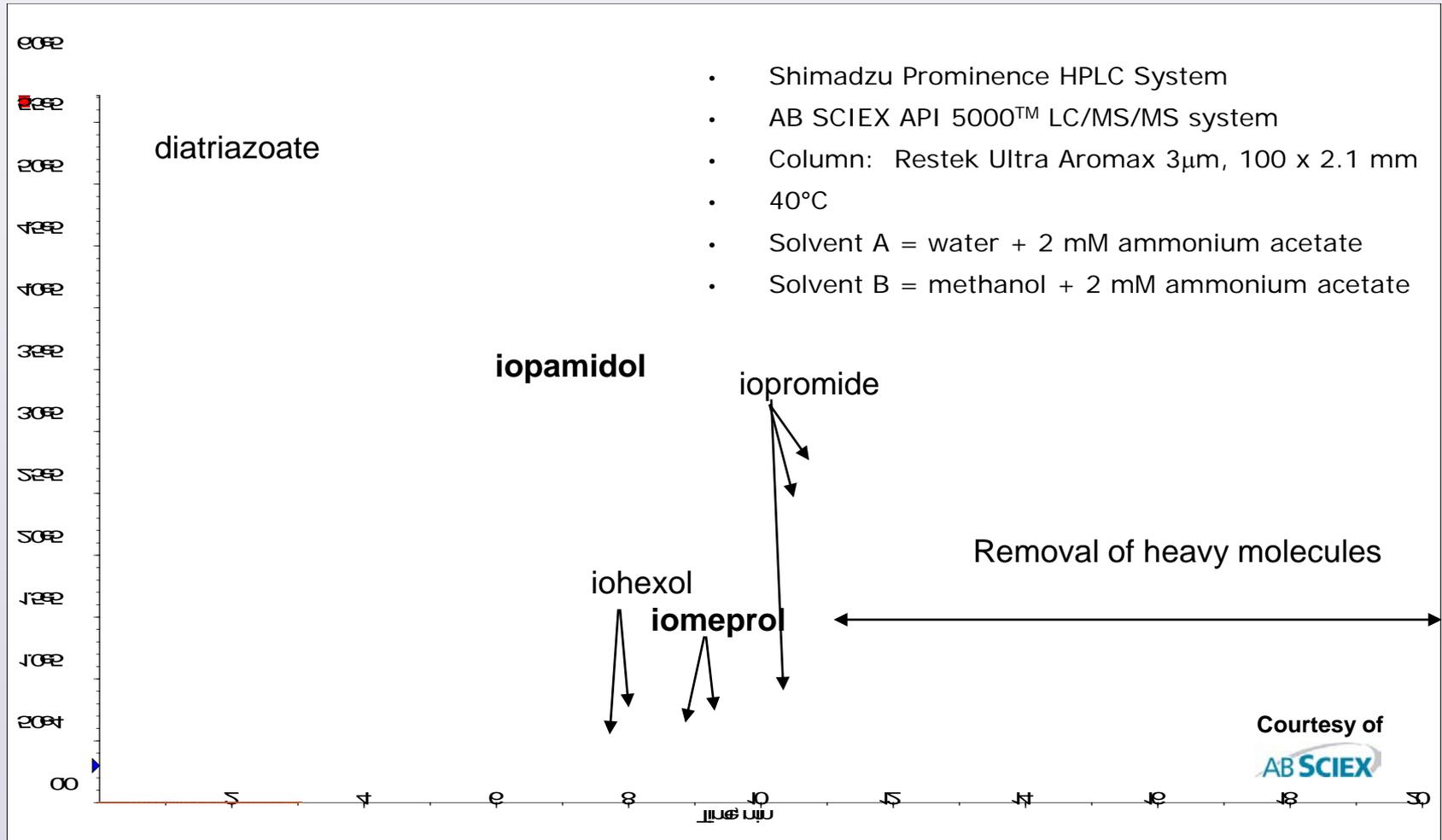
- Shimadzu Prominence HPLC System
- AB SCIEX API 5000™ LC/MS/MS system
- Column: Restek Ultra Aromax 3 μ m, 100 x 2.1 mm
- 40°C
- Solvent A = water + 2 mM ammonium acetate
- Solvent B = methanol + 2 mM ammonium acetate

Time (min)	%A	%B
0.01	98.0	2.0
2.00	98.0	2.0
18.00	2.0	98.0
18.50	98.0	2.0
30.00	stop	

- Flow rate = 0.2 mL/min
- 20 μ L injection volume
- All the analytes eluted within 12 minutes
- Additional time added to remove any heavy residues that may be present in sewage water

The HPLC Separation:

iohexol, iomeprol and iopromide result in 2–3 peaks each



Multiple reaction monitoring (MRM) Table

<i>Q1</i> (<i>m/z</i>)	<i>Q3</i> (<i>m/z</i>)	<i>Dwell</i> (<i>msec</i>)	<i>ID</i>	<i>CE</i> (<i>V</i>)	<i>Retention Time</i> (<i>min</i>)
614.8	361.0	50	diatriazoic acid	28.3	3.41
631.8	361.0	50	diatriazoic acid + NH ₄	37.0	
777.9	558.7	50	iopamidol	32.0	7.30
794.9	558.7	50	iopamidol+ NH ₄	35.8	
777.9	686.9	50	lomeprol	30.0	9.00 + 9.56
794.9	686.9	50	lomeprol + NH ₄	36.0	
791.9	558.9	50	lopromide 1	38.5	10.69 + 10.81
791.9	572.9	50	lopromide 2	34.9	
821.9	803.9	50	lohexol	37.0	8.00 + 8.36
838.9	803.9	50	lohexol + NH ₄	35.3	

IS=5000V; GS1=60; GS2=40; Temp=680C

Summary

- UHPLC and LC/MS/MS in particular are adding a powerful tool to multiple industries
- Liquid Chromatography offers separation and detection advantages
- Liquid Chromatography is well suited for emerging pollutants

Acknowledgements

Special thanks to our collaborating researchers:

- Andre Schreiber and Takeo Sakuma - *AB Sciex*
- Chris Marvin, Simon Zhou Eric Reiner - *Environment Canada, Burlington, Ontario*
- Nicole Riddell – *Wellington Laboratories*
- Frank Dorman – *Pennsylvania State University*