Analysis of Hexabromocyclododecane Diastereomers and Tetrabromobisphenol-A Using a Novel Method Combining Supercritical Fluid Chromatography with Tandem Mass Spectrometry

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Overview

- Supercritical fluid chromatography (SFC) applicability to hexabromocyclododecane (HBCDD) and tetrabromobisphenol-A (TBBPA) analysis
- Chromatography and MS/MS method development
- Sample analyses results
- New/preliminary results: Chiral separations and additional flame retardants
- Conclusions

What is a Supercritical Fluid?

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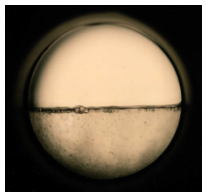
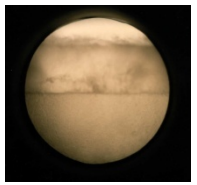


> temp and pressure



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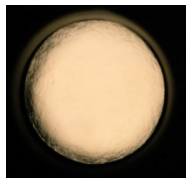
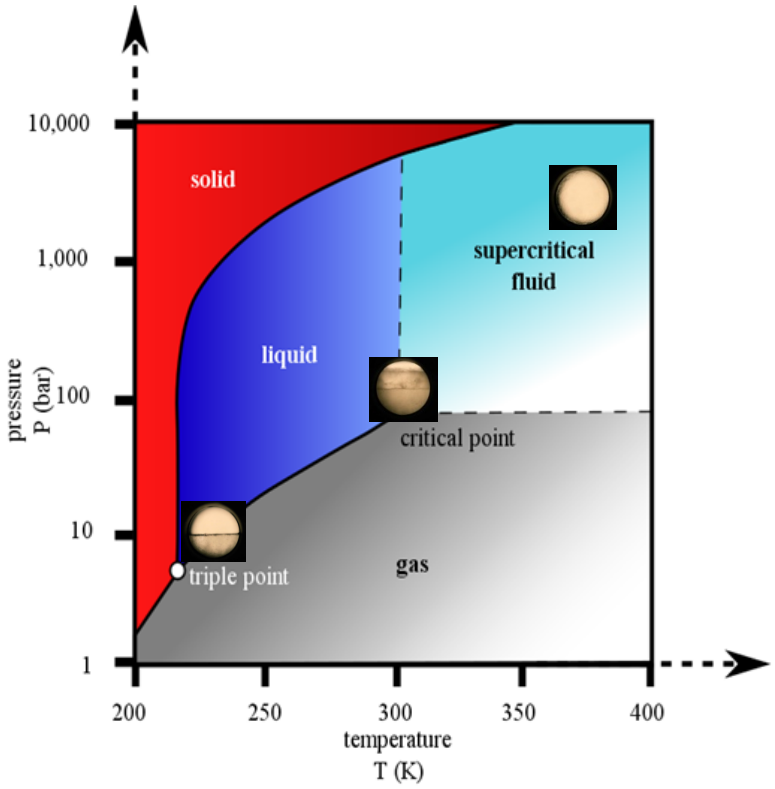


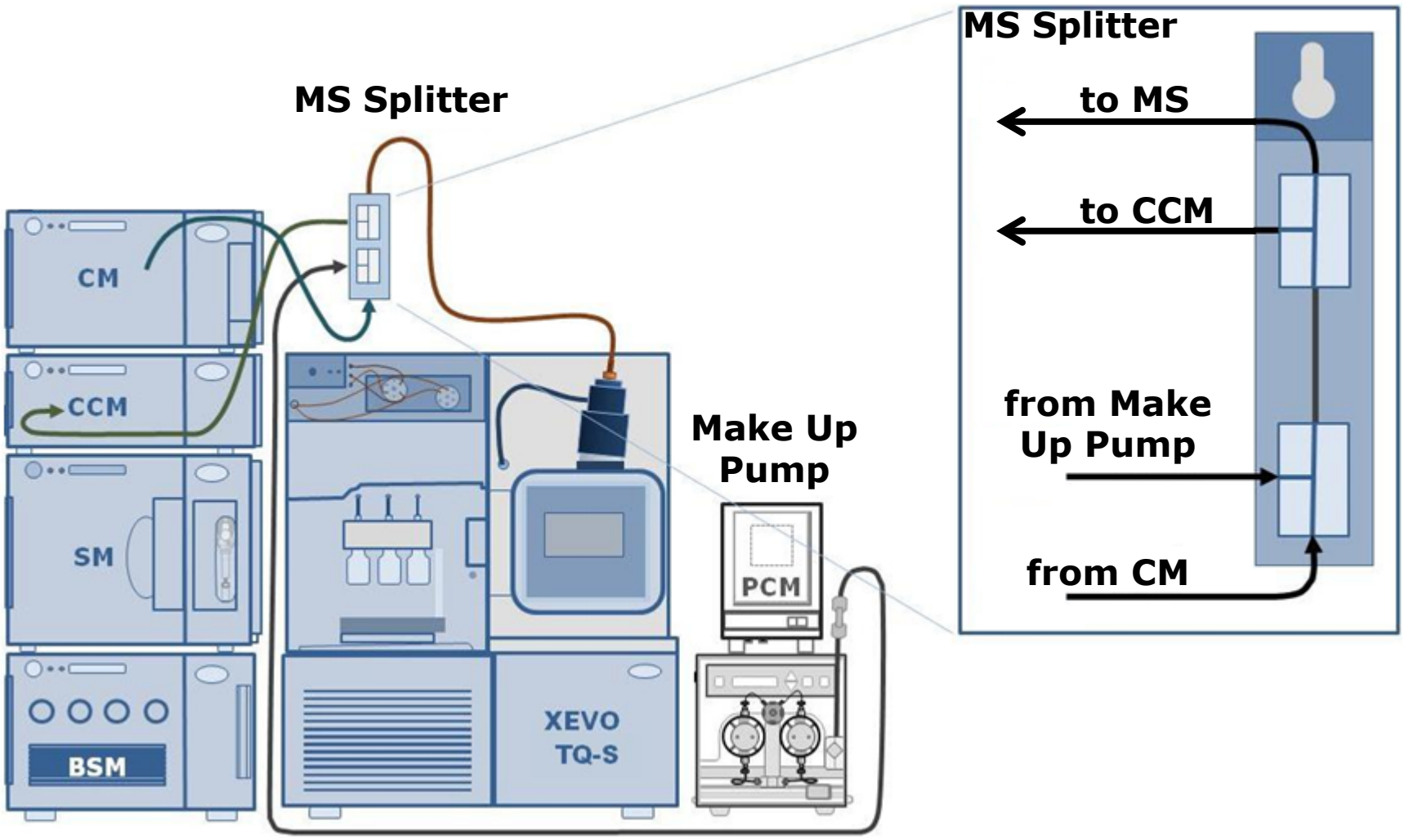
Liquid/gas **Critical point** **Supercritical fluid**

	Diffusivity (cm^2/s)	Viscosity (g/cm x s)
Gas	10^{-1}	10^{-4}
Supercritical Fluid	10^{-4} - 10^{-3} Liquid Like	10^{-4} - 10^{-3} Gas Like
Liquid	< 10^{-5}	10^{-2}

High diffusivity, low viscosity = fast, efficient chromatography



UPC² TQ-S



HBCDD Isomeric Separation

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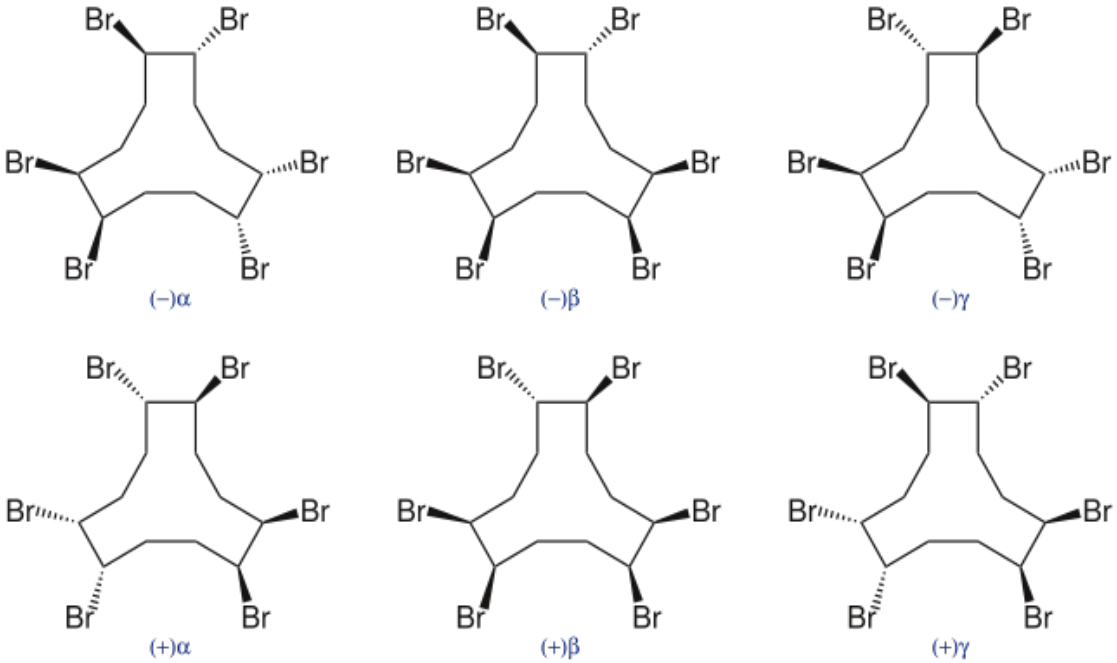
- HBCDD added to Stockholm Convention list of Persistent Organic Pollutants (POPs)
- Predominant HBCDD diasteromers and enantiomers



- Proportion dependent on sample type

Chromatographic Efficiency: 3 min separation of HBCDD and TBBPA

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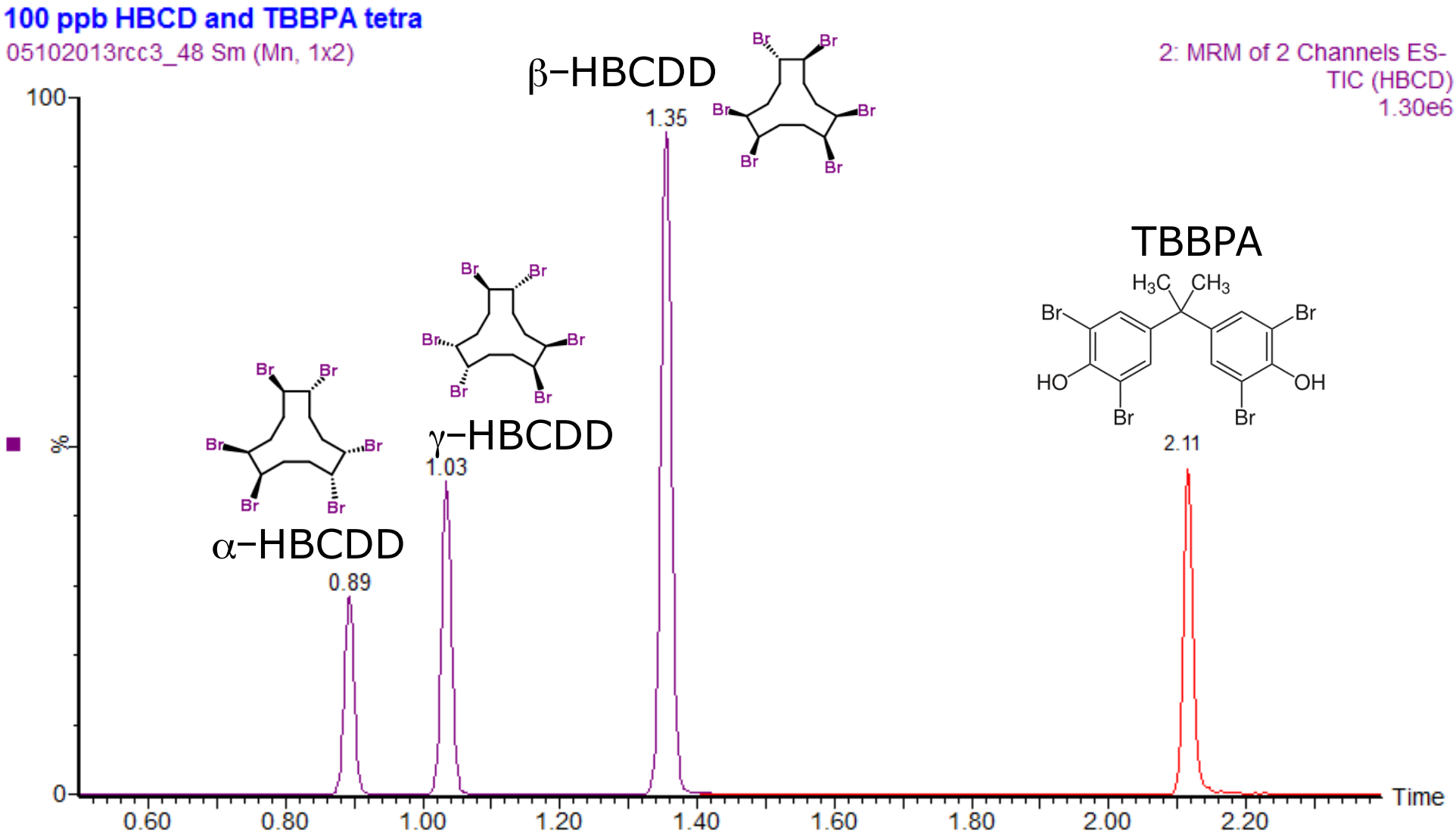

Faster Analysis Time and Chromatographic Orthogonality

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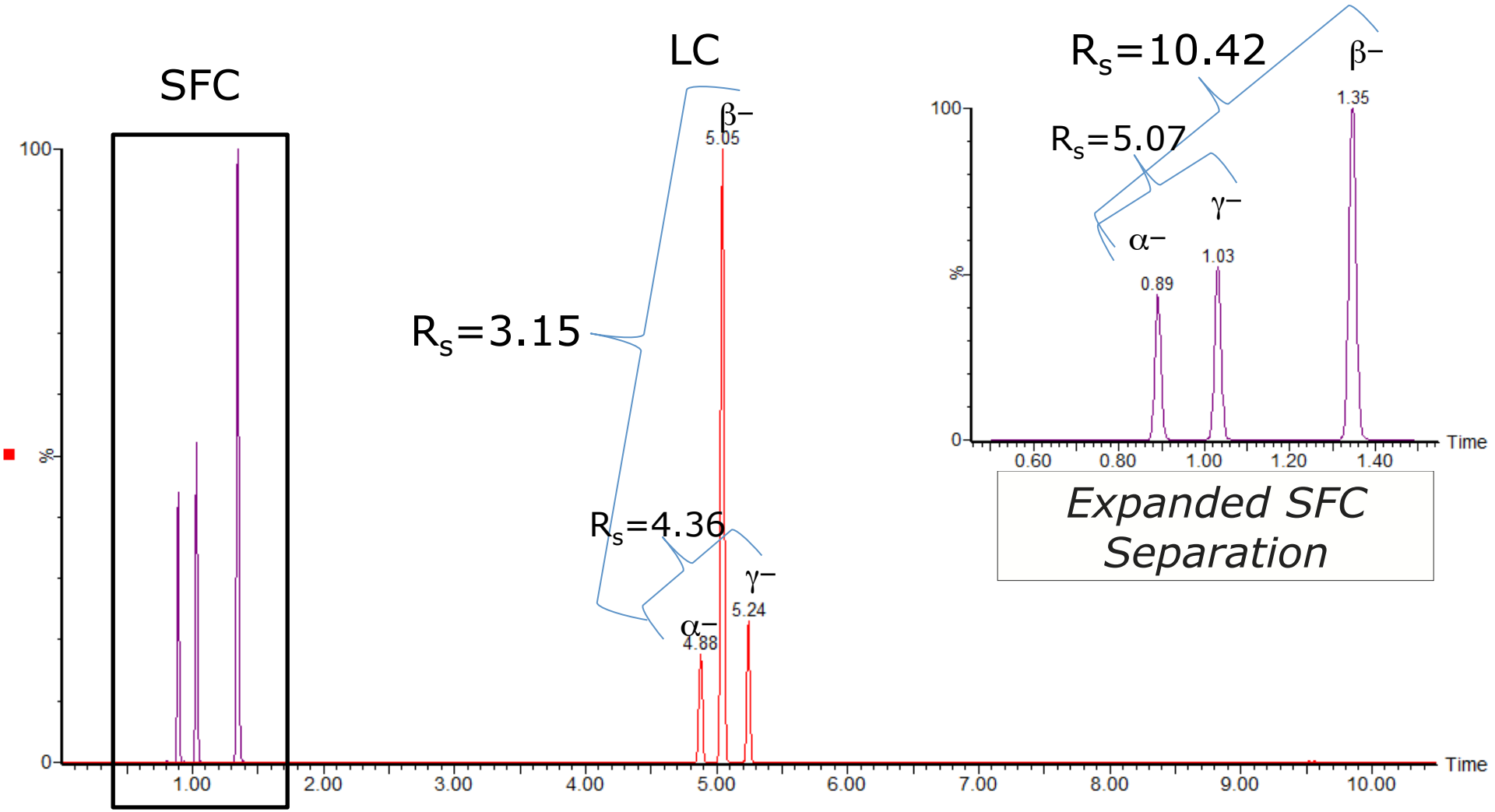

Expanded SFC Separation

Method Development

UPC² TQ-S

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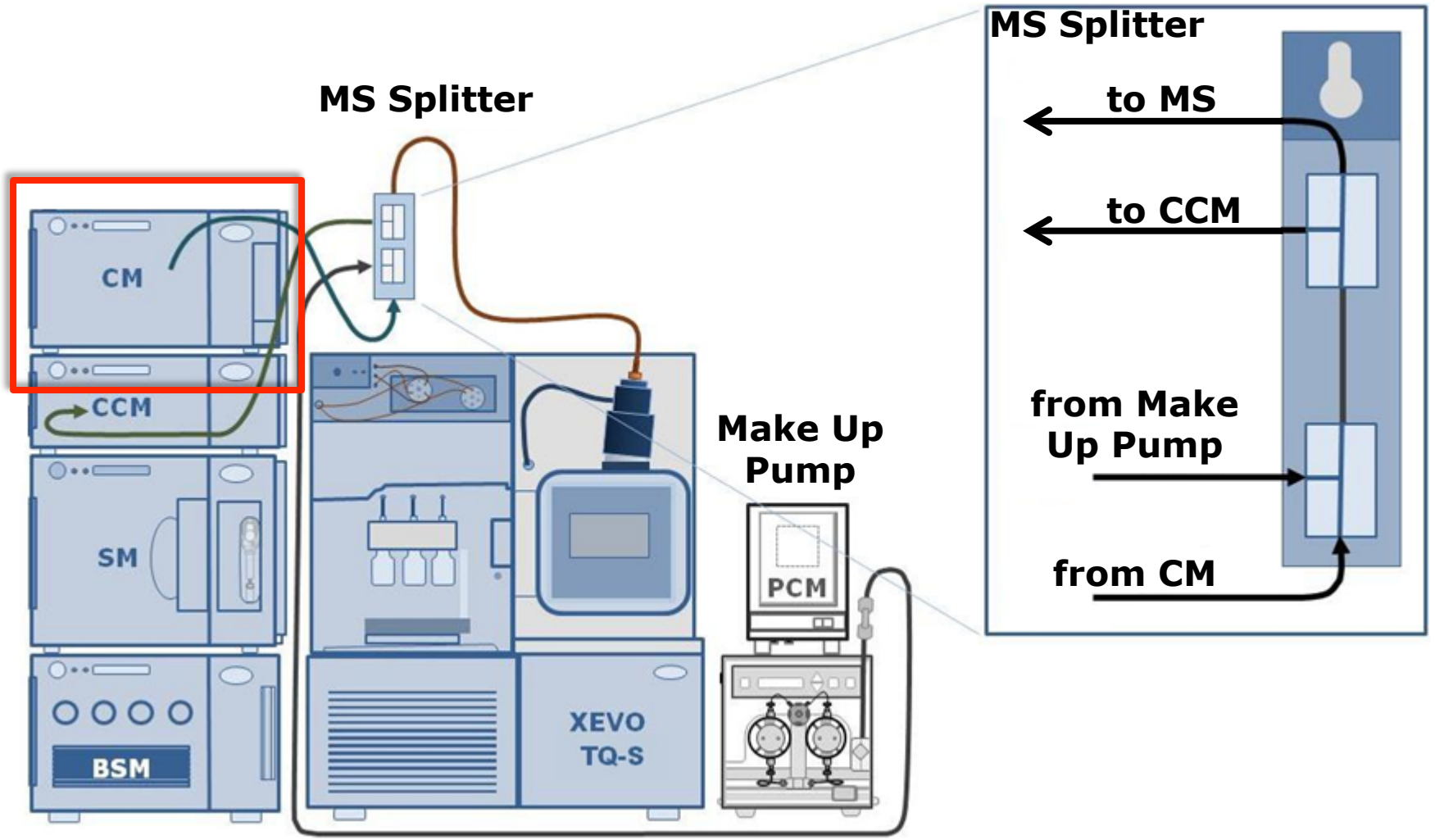

Chromatography Optimization

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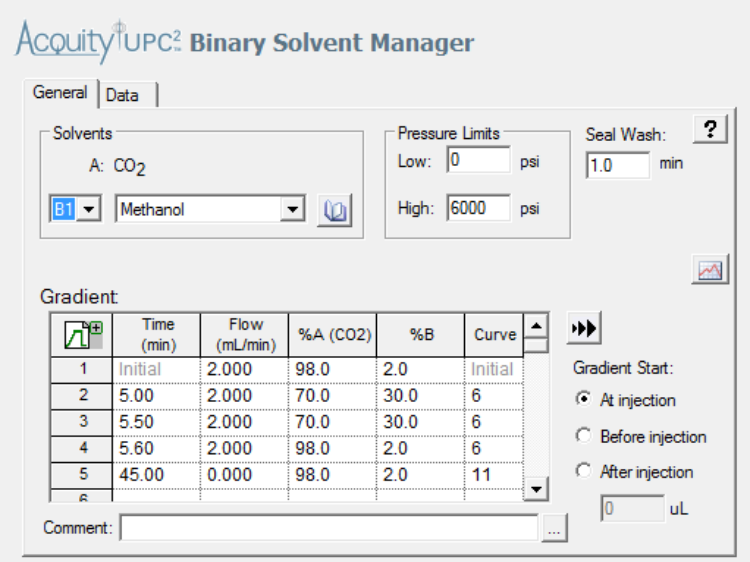
	α–HBCDD		β–HBCDD		γ–HBCDD		TBBPA	
	gradient	isocratic	gradient	isocratic	gradient	isocratic	gradient	isocratic
HSS C18 SB 1.8 µm 3.0x100 mm	●	●	●	●	●	●	●	●
BEH 1.7 µm 3.0x100mm	●	NA	●	NA	●	NA	●	NA
BEH 2-EP 1.7 µm 3.0x100mm	●	●	●	●	●	●	●	●

● = peak detected, baseline separation

● = peak present, but not baseline separation

● = peak not detected



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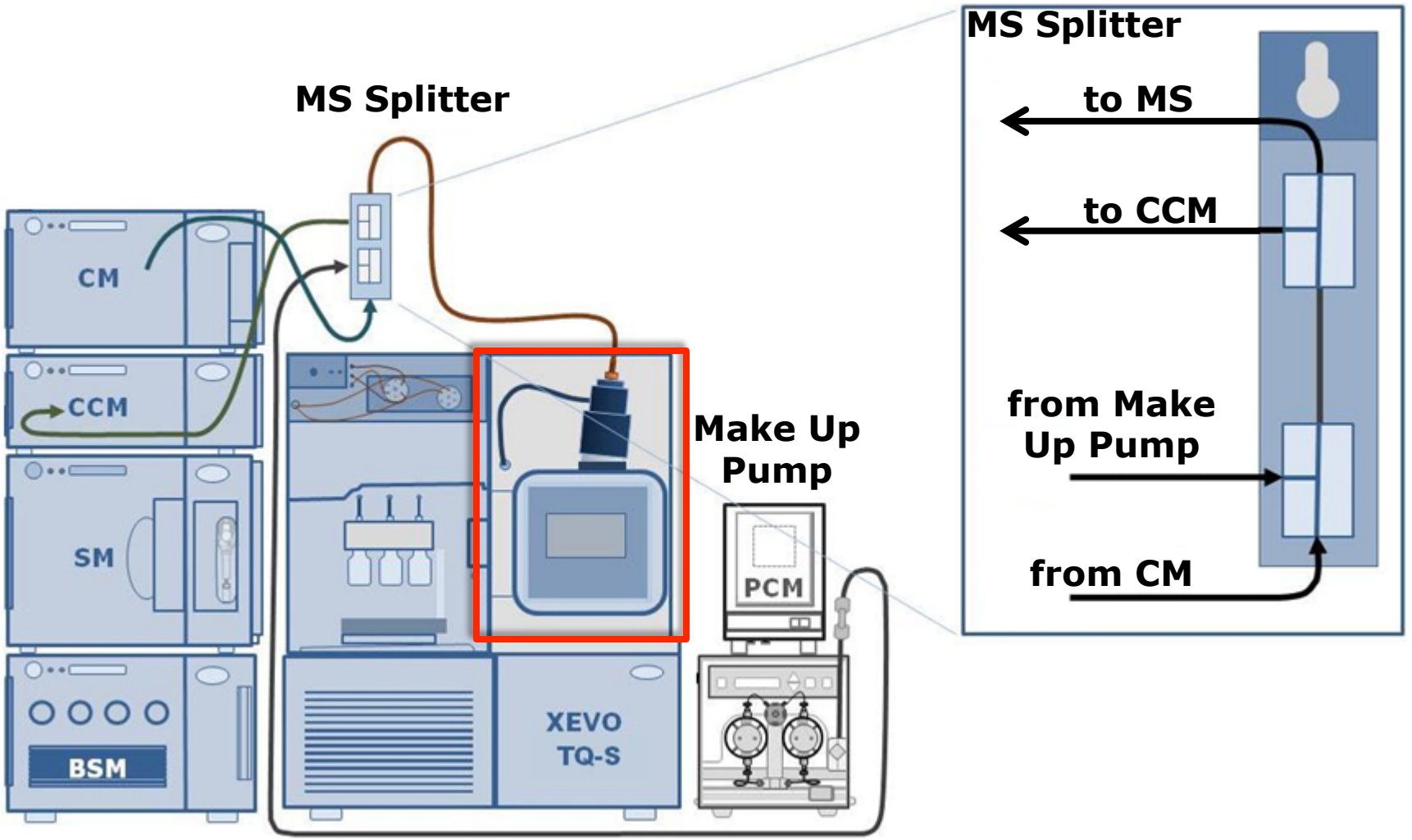

Ionization Type Comparison

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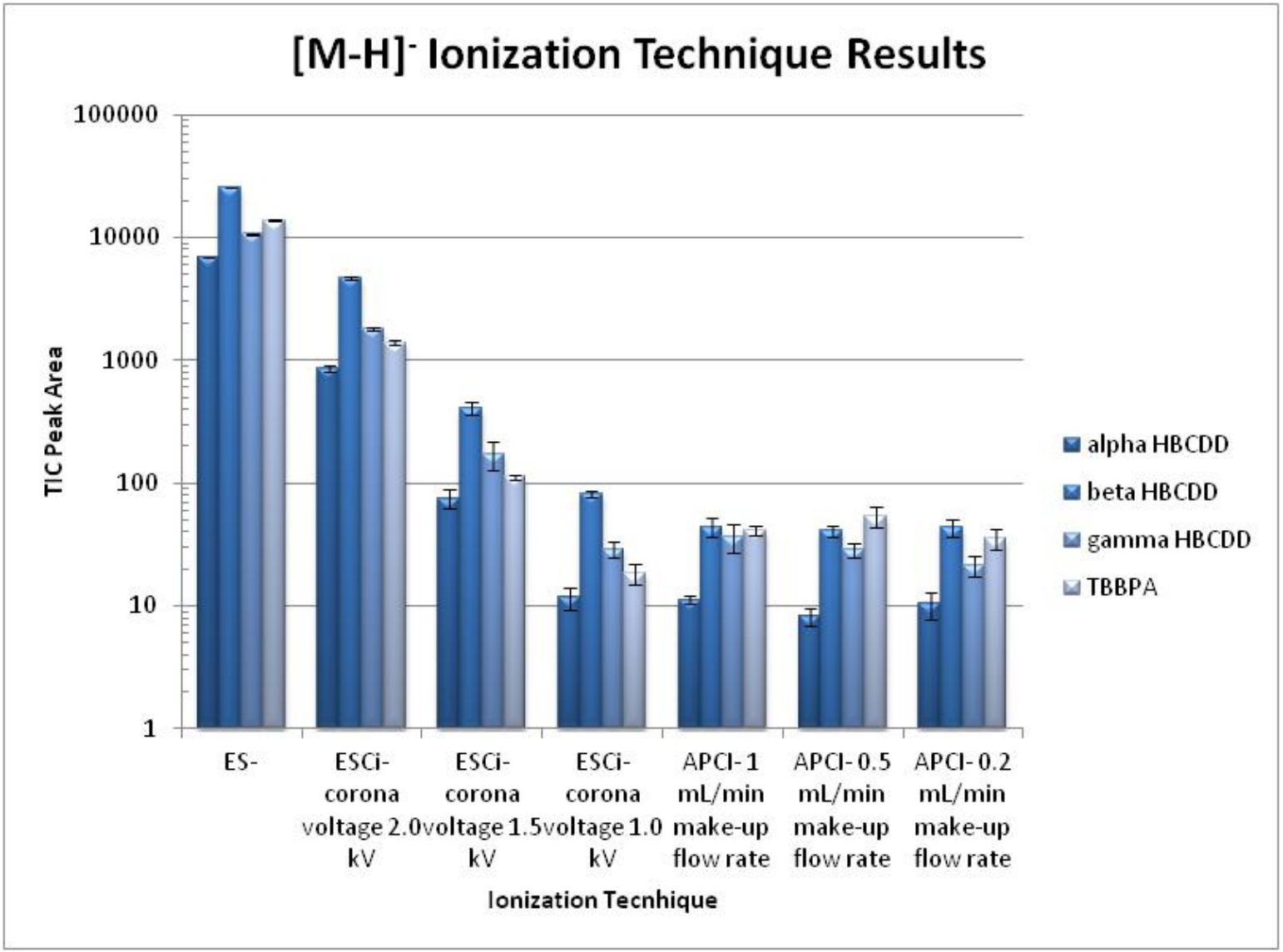
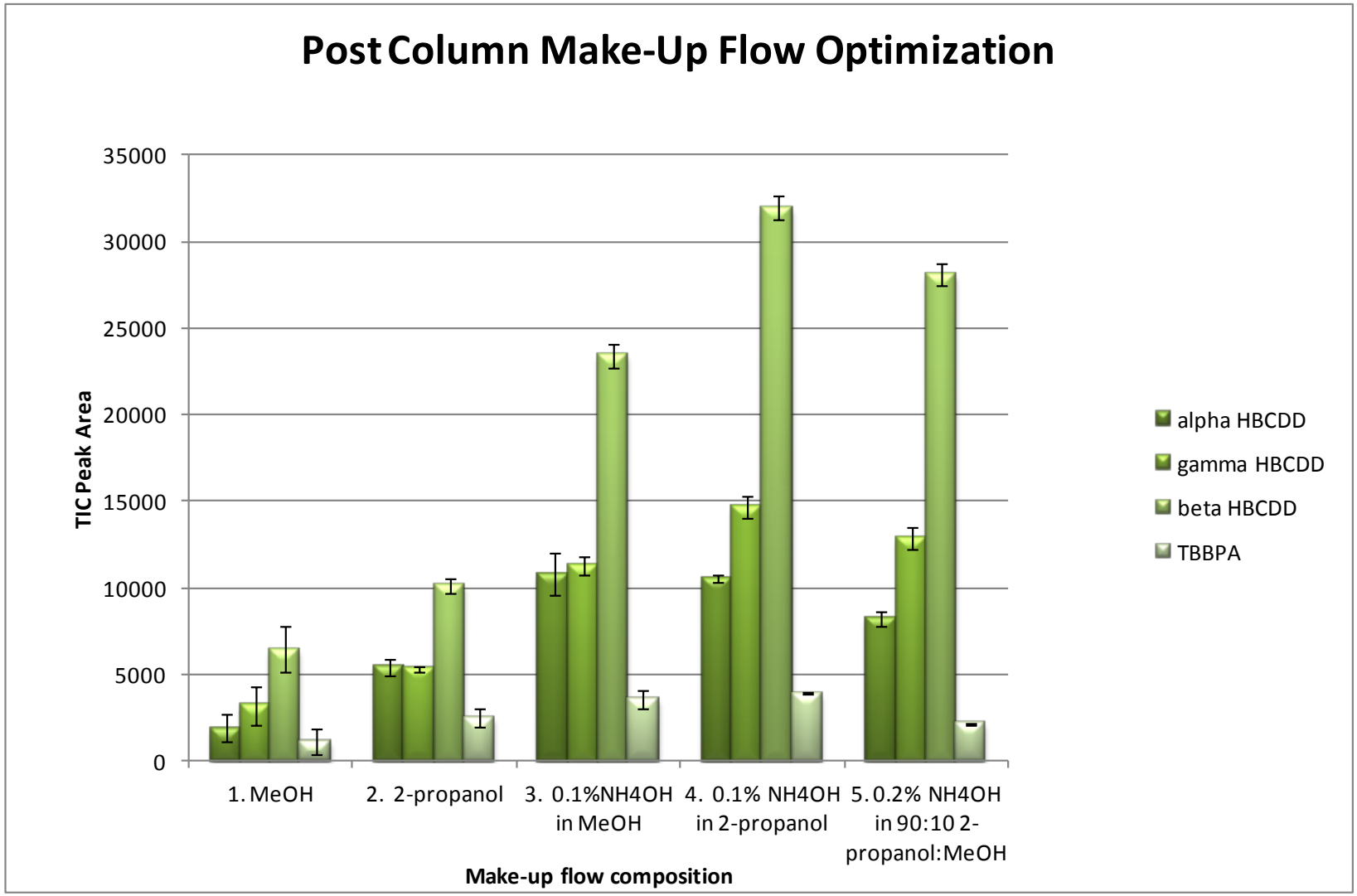


Make-up Flow Composition



Method Limit-of-detection (LOD) and quantification (LOQ)

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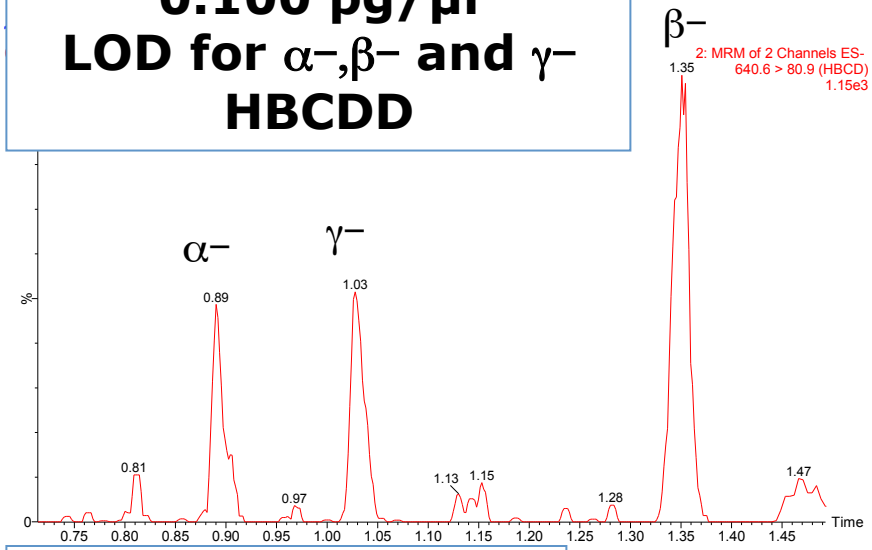
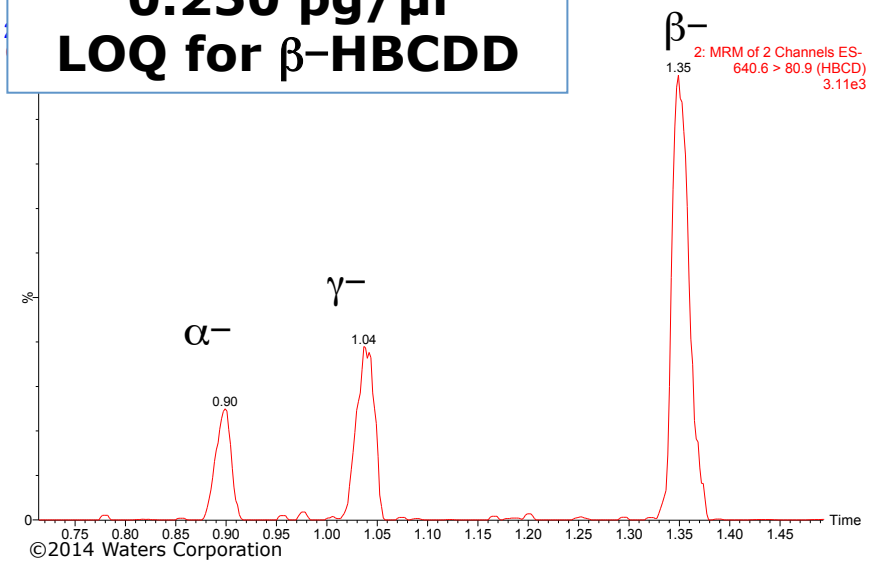
0.100 pg/µl
LOD for α–,β– and γ– HBCDD



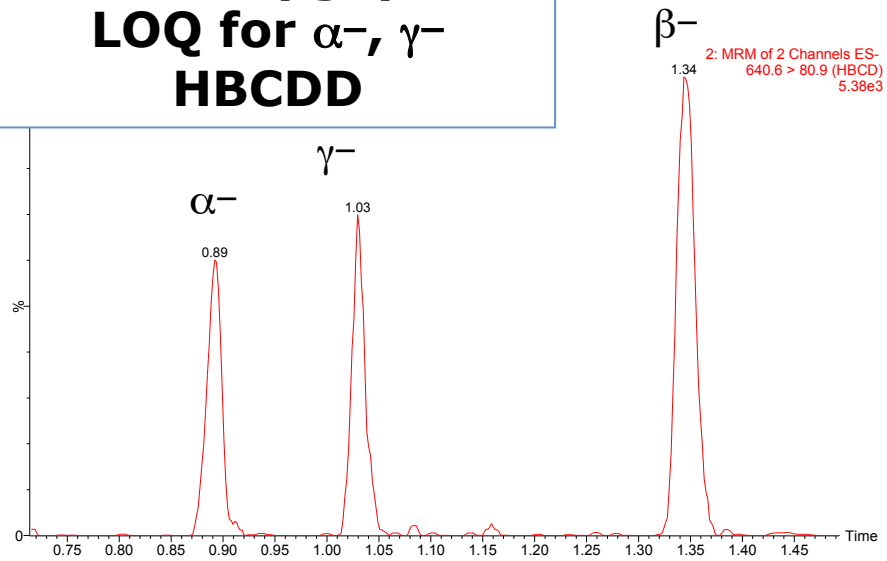
LOD
Peak-to-peak S/N >3

LOQ
Peak-to-peak S/N >10

0.250 pg/µl
LOQ for β–HBCDD



0.500 pg/µl
LOQ for α–, γ– HBCDD



ES$^-$ MS/MS conditions

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	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	HBCD	640.6000	78.9000	☑	0.050	30	20	☐	
2	HBCD	640.6000	80.9000	☑	0.050	30	20	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	TBBPA	542.5000	78.9000	☑	0.050	30	50	☐	
2	TBBPA	542.5000	80.9000	☑	0.050	30	50	☐	
3	TBBPA	542.5000	419.5000	☑	0.050	30	40	☐	

- Capillary Voltage: 2.0
- Cone Voltage: 30
- Source Temperature: 150 °C
- Desolvation Temperature: 500 °C
- Desolvation Gas: 1000 L/hr
- Cone Gas: 150 L/hr
- Points per peak: 15

UPC² Gradient and Conditions

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Time (min.)	% A	% B	Flow Rate (mL/min.)
Initial	98	2	2
2	90	10	2
2.01	90	10	2.5
2.3	98	2	2.5
2.31	98	2	2

- Column: HSS C18 SB (1.8µm 3.0 x 100 mm)
- Column Temperature: 40 °C
- Sample Temperature: 25 °C
- Phases: CO_2 (A) and methanol (B)
- Injection Volume: 1 µL
- Make-up flow composition: 0.1% NH_4OH in 2-propanol
- Make-up flow rate: 0.2 mL/min

Application to Biological Matrices

Sample Analyzed

- Can method separate and detect HBCDD isomers in complex matrices?
- Wide solvent compatibility with SFC so final extract used without solvent exchange
- Extracts of human plasma and whale blubber were donated from prior GC analyses

Human Plasma Detection: Low Level

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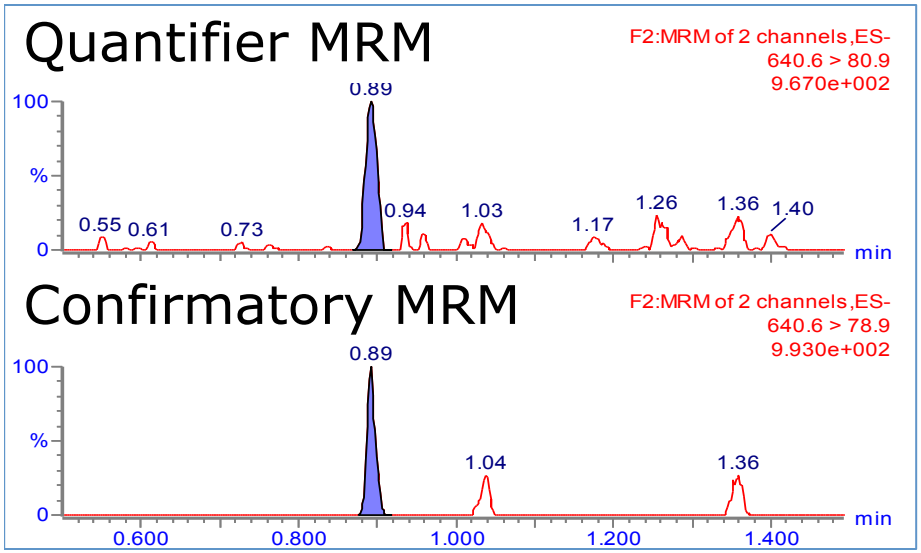
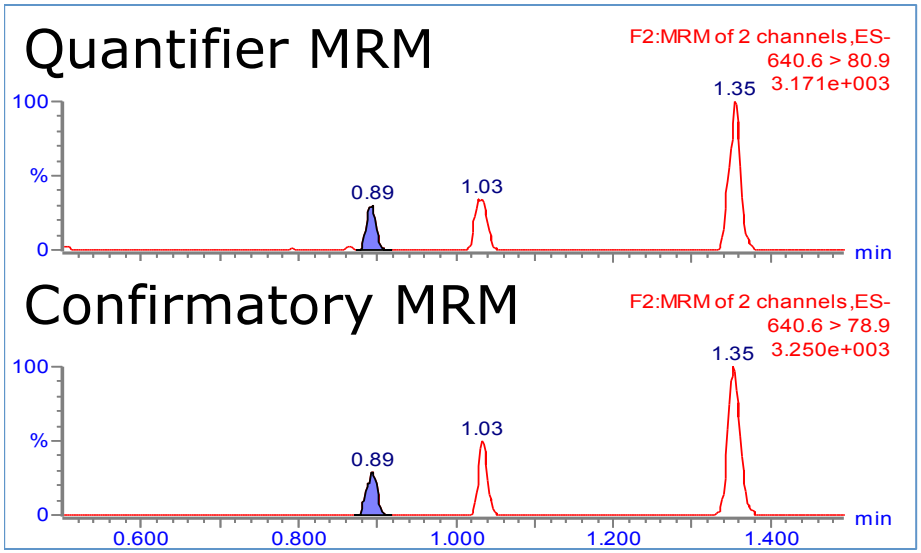


SAMPLE



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	Calculated Concentrations ng/g lipid weight		
Sample	α-HBCDD	β-HBCDD	γ-HBCDD
HS1	4.21	<LOD	<LOD

Calibration Curve Results

	%RSDs (n=5)		
Concentration (pg/µl)	**α-HBCDD**	**β-HBCDD**	**γ-HBCDD**
0.25	<LOQ	7.9	<LOQ
0.5	5.6	11.6	16.7
1	12.6	4.0	13.1
5*	6.8	2.3	5.3
10	3.0	2.2	3.7
25	5.5	2.0	1.6
50	2.3	2.2	1.8
100	3.7	1.3	1.0
Fit	Linear	Linear	Linear
Weighting	1/x	1/x	1/x
R2	0.998	0.999	0.999
*n=4			

Whale Blubber Detection: High Level

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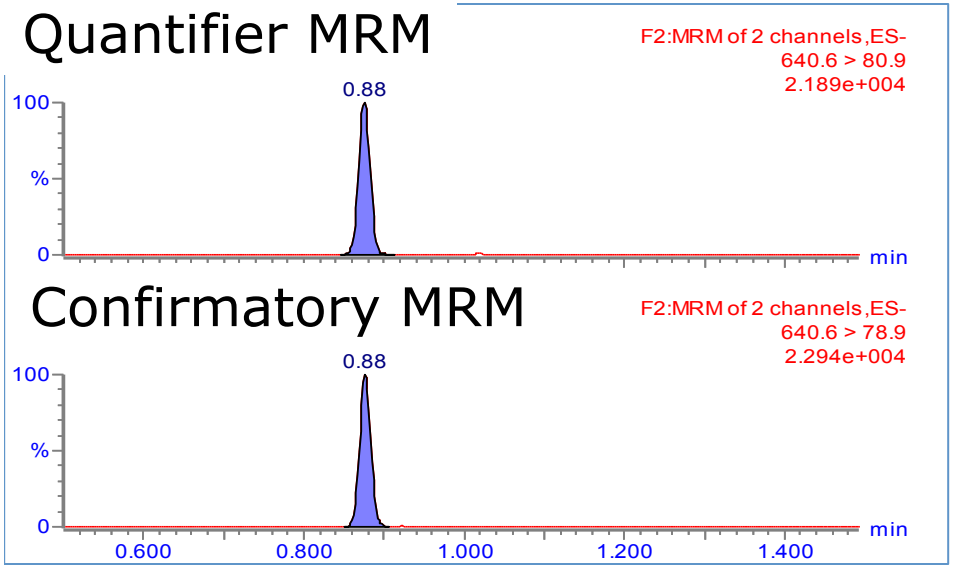
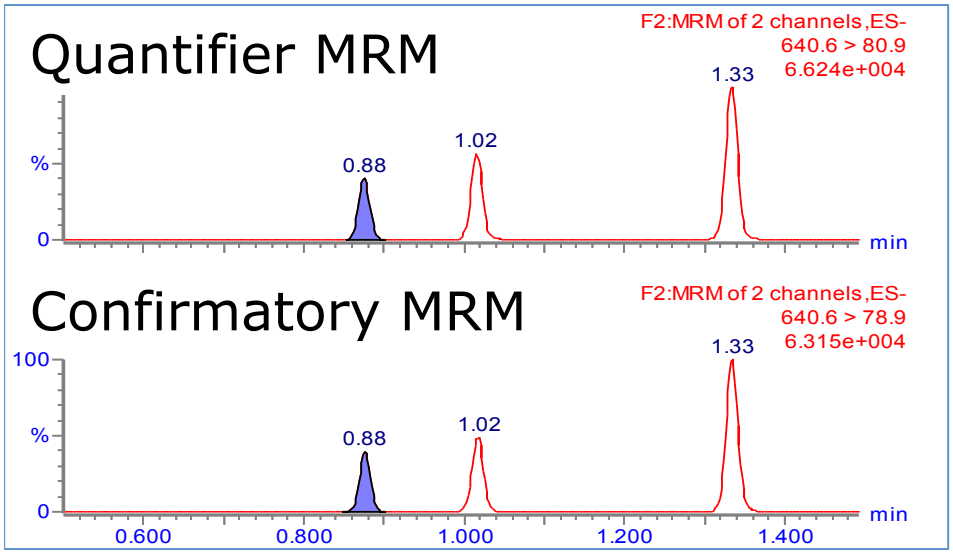
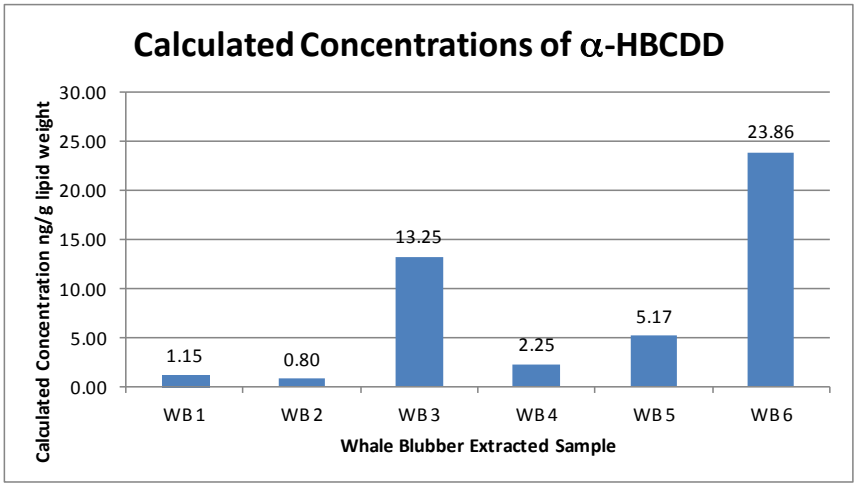


SAMPLE AND





Calibration Curve Results

	%RSDs (n=5)		
Concentration (pg/µl)	α–HBCD	β–HBCD	γ–HBCD
0.25	<LOQ	11.7	<LOQ
0.5	9.8	6.4	15.1
1	16.8	9.4	5.7
5	4.3	1.0	2.3
10	5.4	1.0	4.4
25	3.4	2.2	3.4
50	1.6	0.6	1.3
100	3.4	2.6	3.5
Fit	Linear	Linear	Linear
Weighting	1/x	1/x	1/x
R^2	0.998	0.999	0.999

Ion Ratio Confirmation

					Ion Ratios	
Calculated Concentrations pg/µl				α-HBCDD	β-HBCDD	γ-HBCDD
Human Serum Samples	α-HBCDD	β-HBCDD	γ-HBCDD	[1.123]	[1.097]	[1.11]
HS 1	0.575	ND	ND	1.342	ND	ND
Whale Blubber Samples				[1.112]	[1.163]	[1.127]
WB 1	12.88	ND	ND	1.108	ND	ND
WB 2	8.33	ND	ND	1.109	ND	ND
WB 3	47.7	ND	ND	1.117	ND	ND
WB 4	19.8	ND	ND	1.16	ND	ND
WB 5	45.5	ND	ND	1.088	ND	ND
WB 6	85.9	ND	ND	1.003	ND	ND

- Ion Ratio = Quantifier MRM area/Confirmatory MRM area
- Pass = +/- 20% of expected ion ratio
- Expected ratio determined by averaging all points' ion ratio in calibration series

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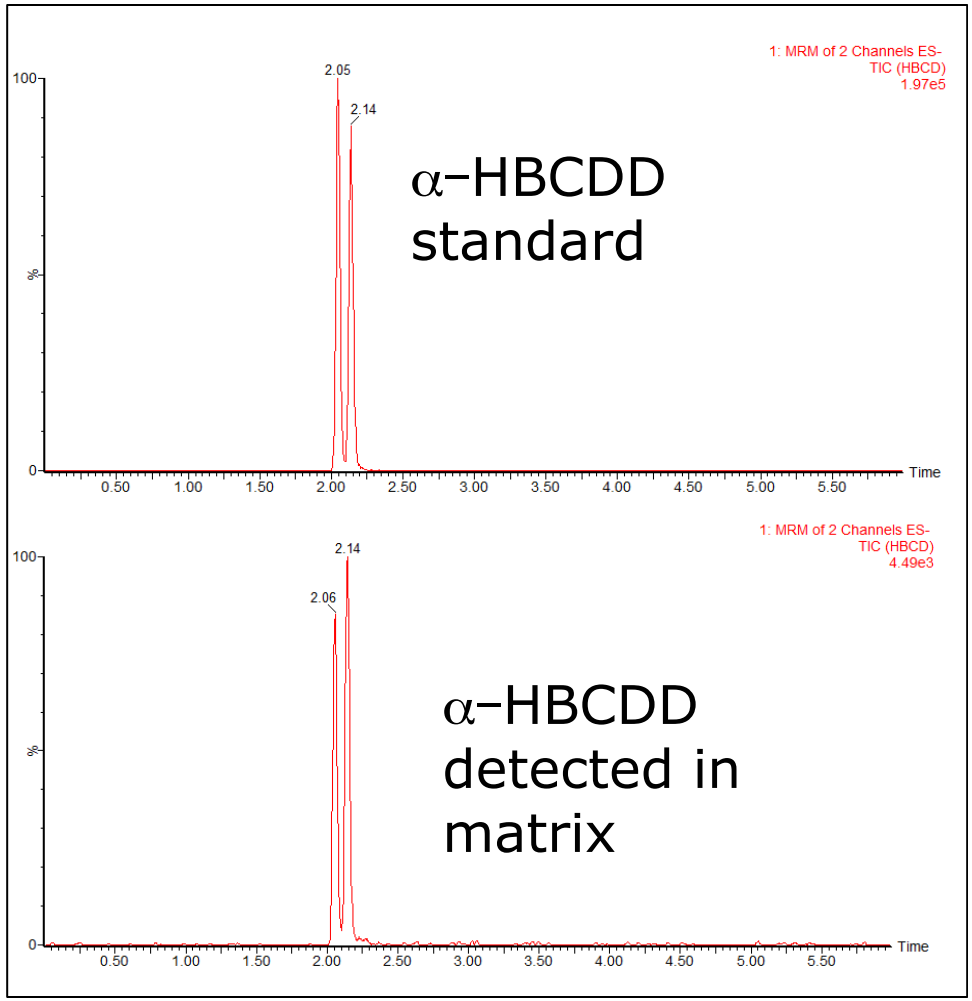
Chiral Separations
Preliminary Results

Chiral HBCDD Separation

- Prior LC method 4.0 x 200mm 5µm PM-β-CD column with >15 min run
- UPC² prototype cellulose 2.1 x 150mm 2.5µm column on standards
- (+/-) α– and γ– separated using same co-solvent, (+/-) β– with different co-solvent
- Further method optimization underway

Chiral Separation
EtOH co-solvent



(+/-) α-HBCDD in Whale Blubber

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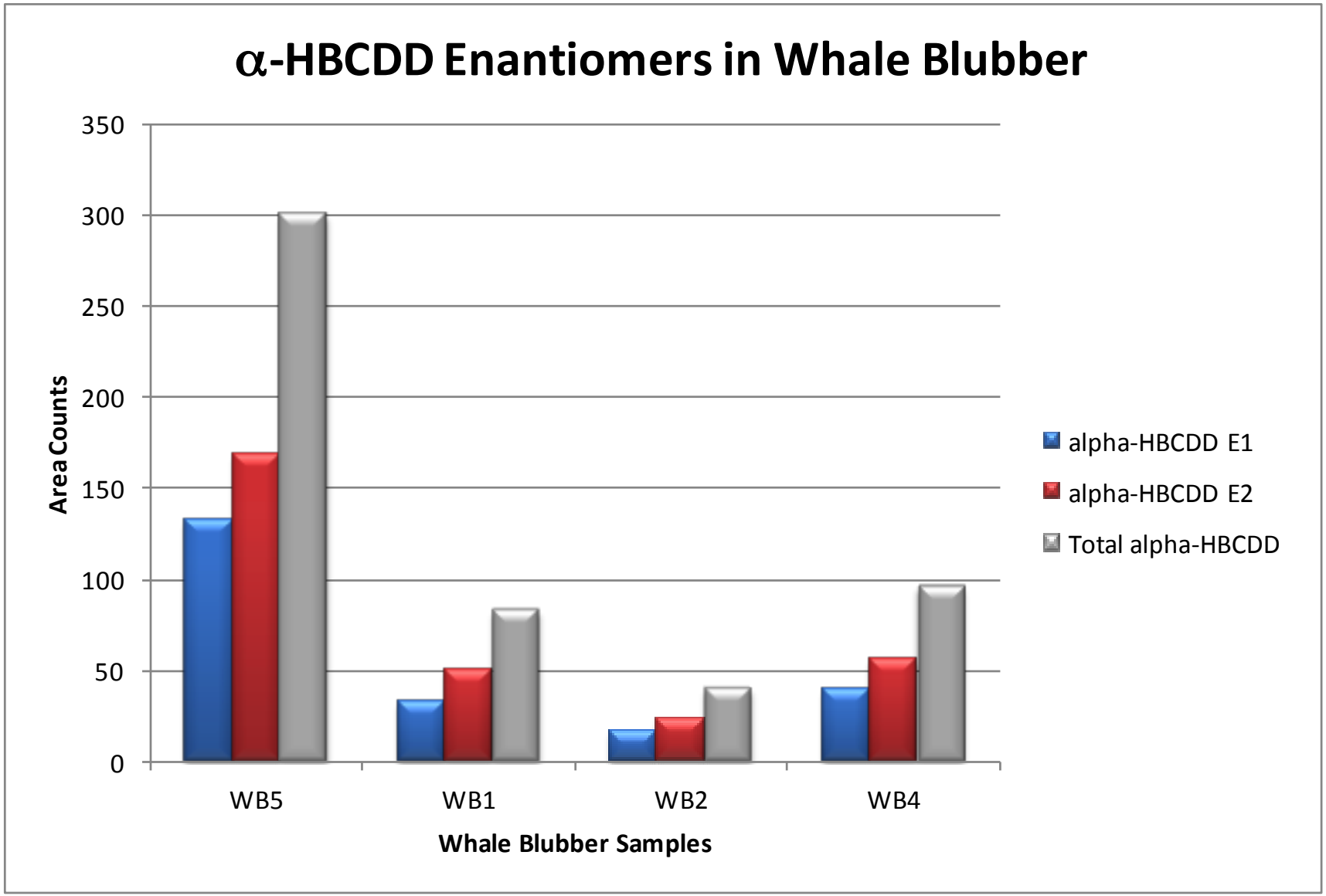

Additional Flame Retardants
Preliminary Results

Investigation of Traditionally GC-Amenable Flame Retardants

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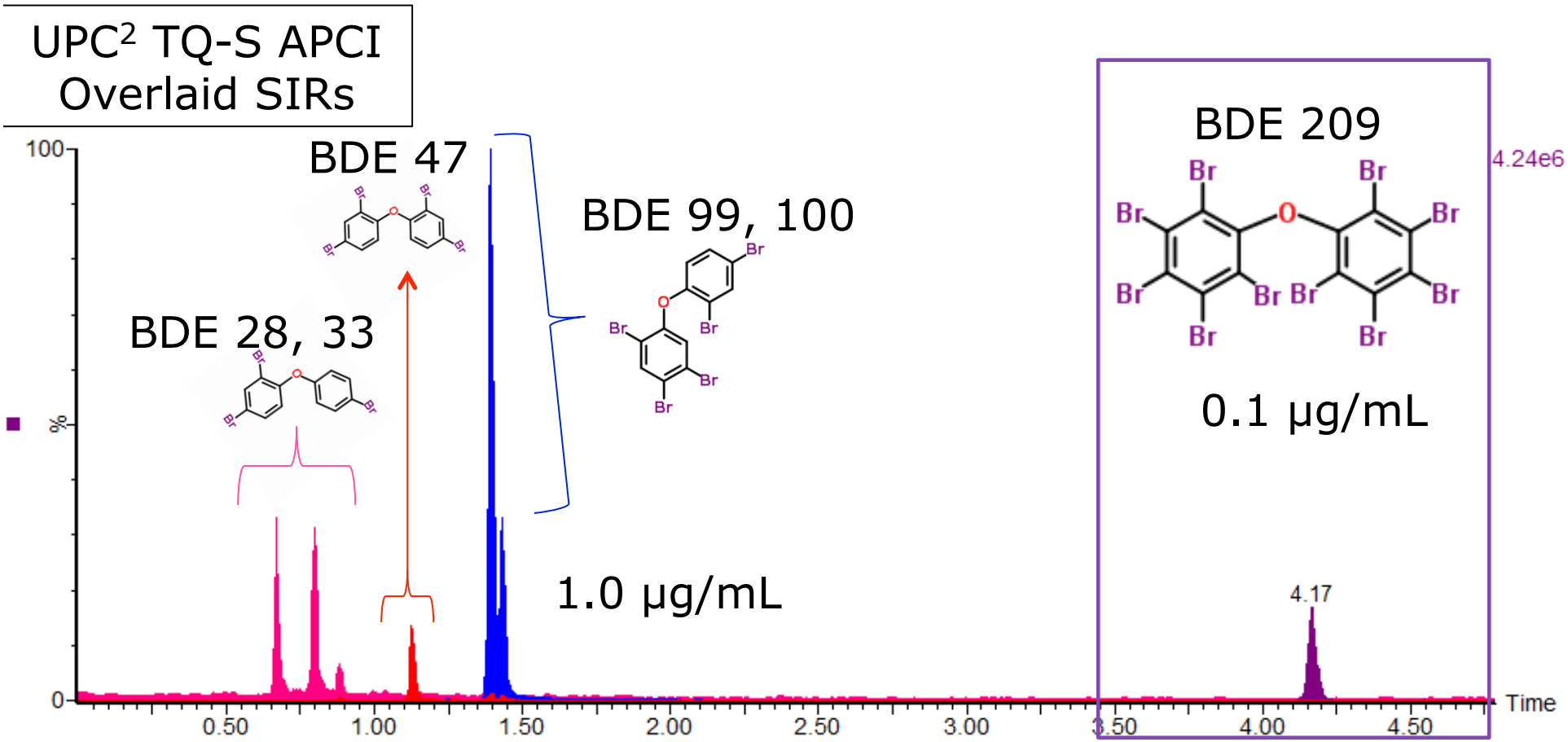
- Wide variety of FRs monitored in environment and biota
- Polybrominated diphenyl ethers (PBDEs) traditionally require GC-MS



Diverse Flame Retardants on Single Platform: Preliminary Detection

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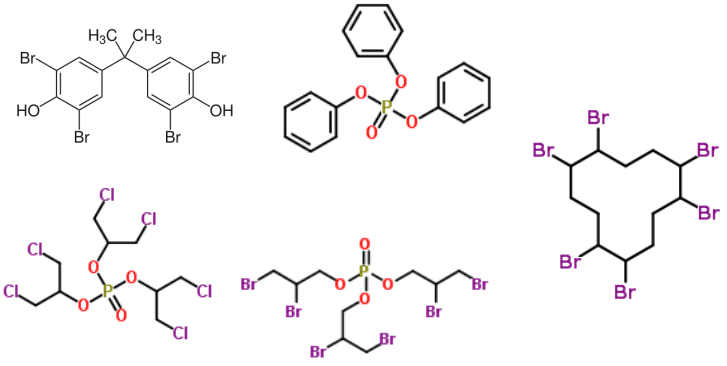
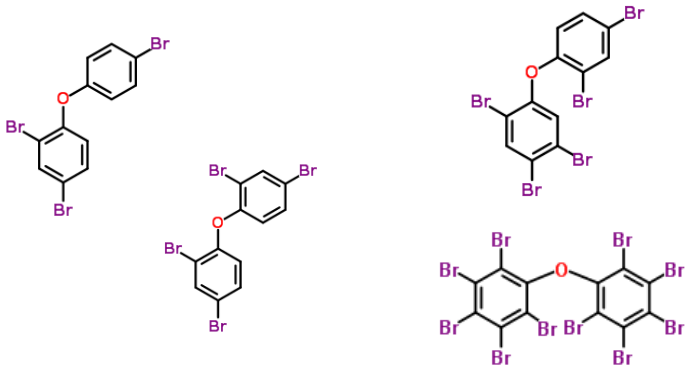
Flame Retardant	Current Chromatographic Technique	Optimum Ionization Technique (UPC^2)	Predominantly Observed Ion	Retention Time (min.)
triphenylphosphate	LC	ESI^+	$[M+H]^+$	1.01
α–,β–,γ–HBCDD	LC	ESI^-	$[M-H]^-$	0.88,1.01,1.33
TBBPA	LC	ESI^-	$[M-H]^-$	2.11
tris (1,3-dichloro-2-propyl) phosphate	GC and LC	ESI^-	$[M-H]^-$	0.62
tris (2,3-dibromopropyl) phosphate	GC and LC	ESI^-	$[M-H]^-$	1.11
BDE 28	GC	$APCI^-$	$[M-H]^-$	0.88
BDE 33	GC	$APCI^-$	$[M-H]^-$	0.67
BDE 47	GC	$APCI^-$	$[M-Br+O]^-$	1.12
BDE 99	GC	$APCI^-$	$[M-Br+O]^-$	1.4
BDE 100	GC	$APCI^-$	$[M-Br+O]^-$	1.43
BDE 209	GC	$APCI^-$	$[M-C6Br5]^-$	4.19





Conclusions

- Rapid and efficient 3 min separation of α–,β– and γ–HBCDD demonstrated effective for complex samples
- Ability to inject wide range of solvents eliminates need for solvent exchange required for RP-LC
- Chiral separation method development underway for HBCDD enantiomers
- UPC^2 shows potential for single chromatographic platform for GC and LC amenable flame retardants

Acknowledgements

- Samira Salihovic (human serum extracts) and Anna Rotander (whale blubber extracts), MTM Research Centre, Örebro Universitet, Örebro Sweden

- Waters Corp - Baiba Cabovska, Mike Jones, Ken Fountain and Mark Baynham, Chris Hudalla

- Reference for APCI Ionization of PBDEs:

S. Zhou, E. Reiner, C. Marvin, P. Helm, N. Riddell, F. Dorman, M. Misselwitz, L. Shen, P. Crozier, K. MacPherson and I. Brindle, *Analytical and Bioanalytical Chemistry, 2010, 396, 1311-1320.*