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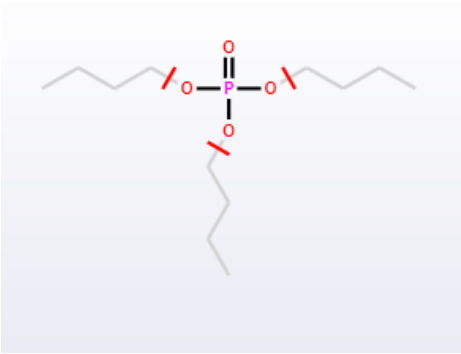

# Method Development of a 2D LC-HRMS Extraction and Detection Method for Organophosphorus Flame Retardants in Environmental Water Samples


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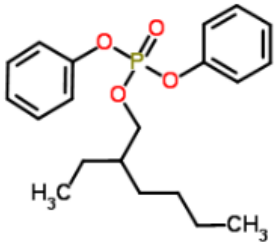
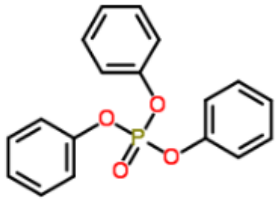
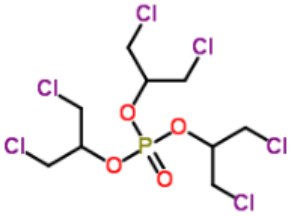
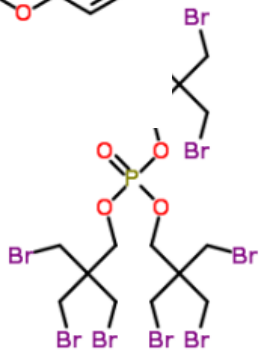
# Overview

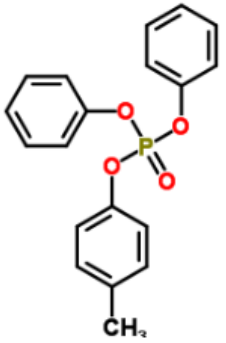
- Introduction
- 2D LC QTof overview
- Method optimization
- Sample results

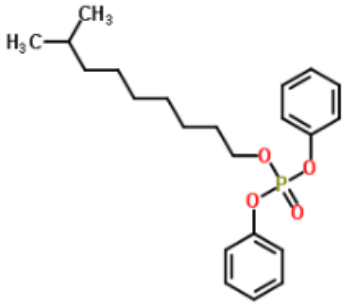


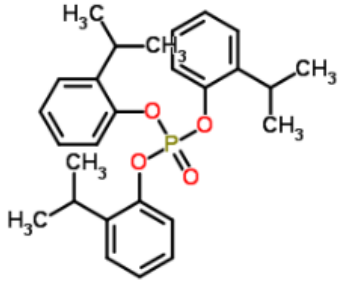


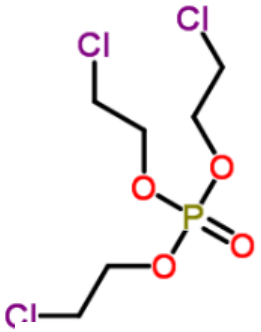


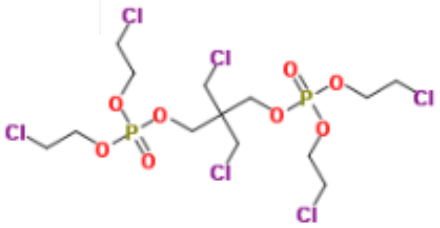


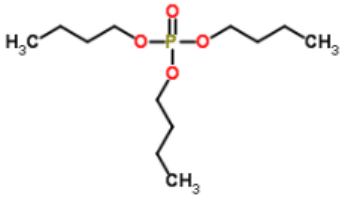












# Introduction

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- Compounds used as flame retardants and plasticizers in various materials
  - $K_{ow}$ ranges 1.71 to 9.0
  - Non-covalently bound to products = potential for environmental occurrence

| Full Name | CAS # | Abbrev. | Formula |
|---|---|---|---|
| Triphenyl phosphate | 115-86-6 | TPHP | $C_{18}H_{15}O_4P$ |
| tris(1,3-Dichloro-2-propyl) phosphate | 13674-87-8 | TDCPP | $C_9H_{15}Cl_6O_4P$ |
| tris(2-Isopropylphenyl) phosphate | 64532-95-2 | IPPP | $C_{27}H_{33}O_4P$ |
| tetrakis(2-Chloroethyl)dichloroisopentyl diphosphate | 38051-10-4 | V6 | $C_{13}H_{24}Cl_6O_8P_2$ |
| Cresyl diphenyl phosphate | 26444-49-5 | DCP, CDP | $C_{19}H_{17}O_4P$ |
| 2-Ethylhexyldiphenyl phosphate | 1241-94-7 | EHDP | $C_{20}H_{27}O_4P$ |
| tris(2-chloroethyl) Phosphate | 115-96-8 | TCEP | $C_6H_{12}Cl_3O_4P$ |
| Isodecyl Diphenyl Phosphate | 29761-21-5 | IDPP | $C_{22}H_{31}O_4P$ |
| tris(Tribromoneopentyl) Phosphate | 19186-97-1 | TBNPP | $C_{15}H_{24}Br_9O_4P$ |
| Tributyl Phosphate | 126-73-8 | TBP | $C_{12}H_{27}O_4P$ |

- Sample analysis incorporating various sample preparation approaches
  - Dry down and solvent exchange common
  - Targeted

- Goal: Implement direct injection of organic solvents + utilize full spectrum acquisition with accurate mass data to identify targeted and non-targeted OPFRs

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# System Overview

# System Layout

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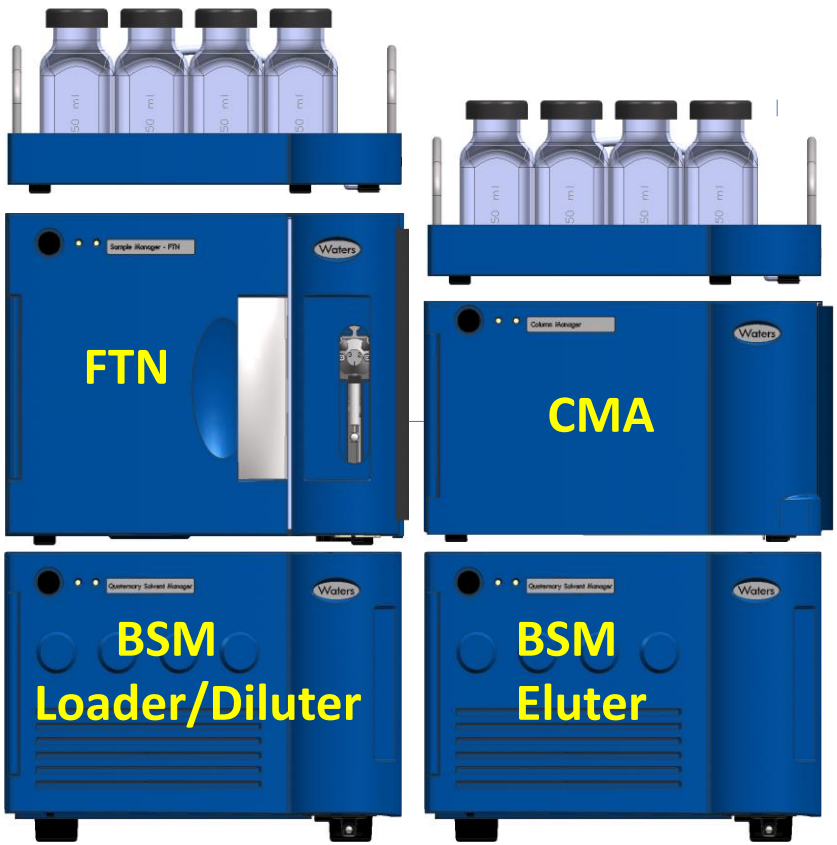


# At-Column Dilution (ACD) Flow Path

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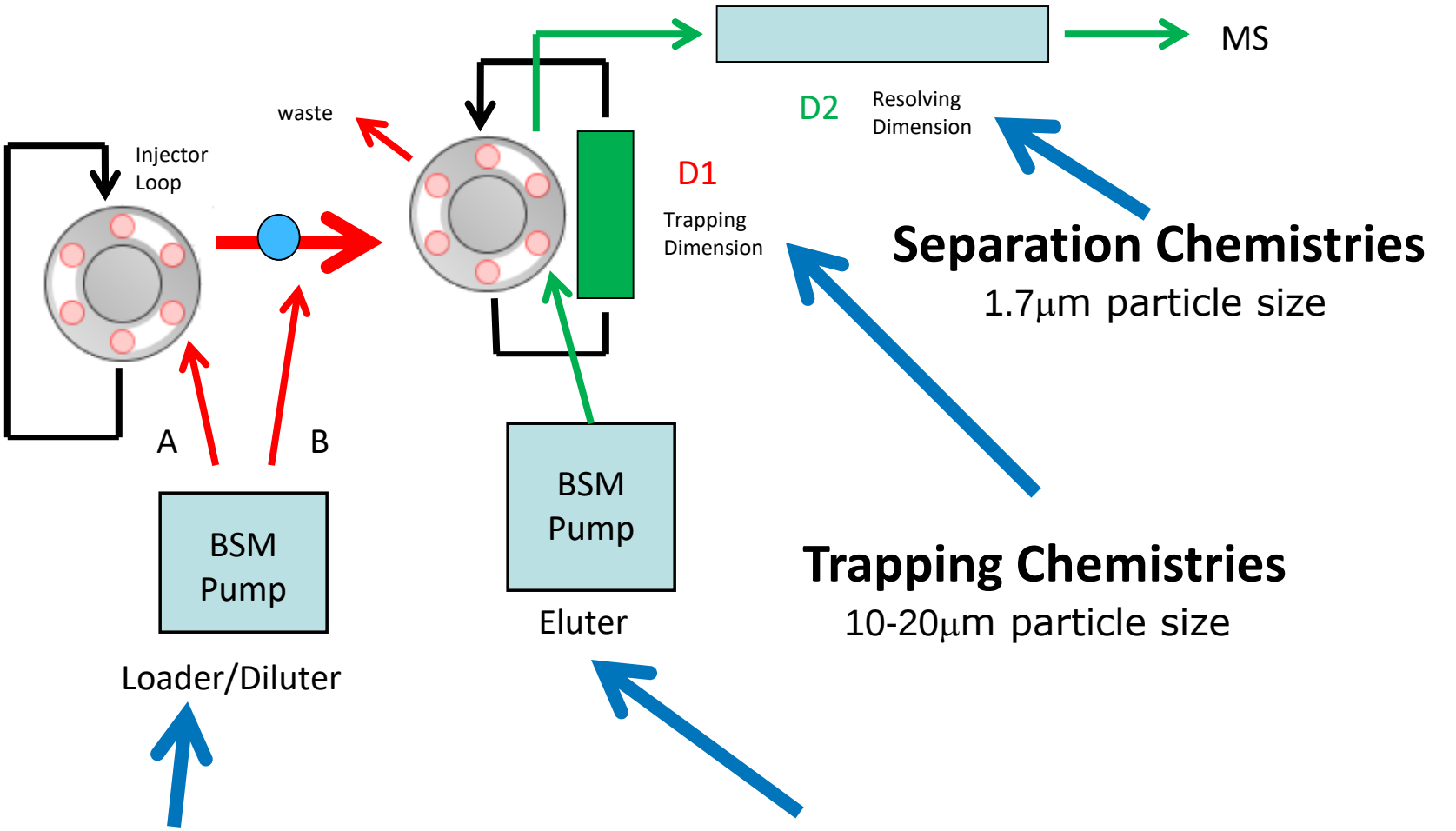

# Why QTof?
## QQQ Vs HRMS

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# Analyte Selectivity:
## Triple Quad vs TOF

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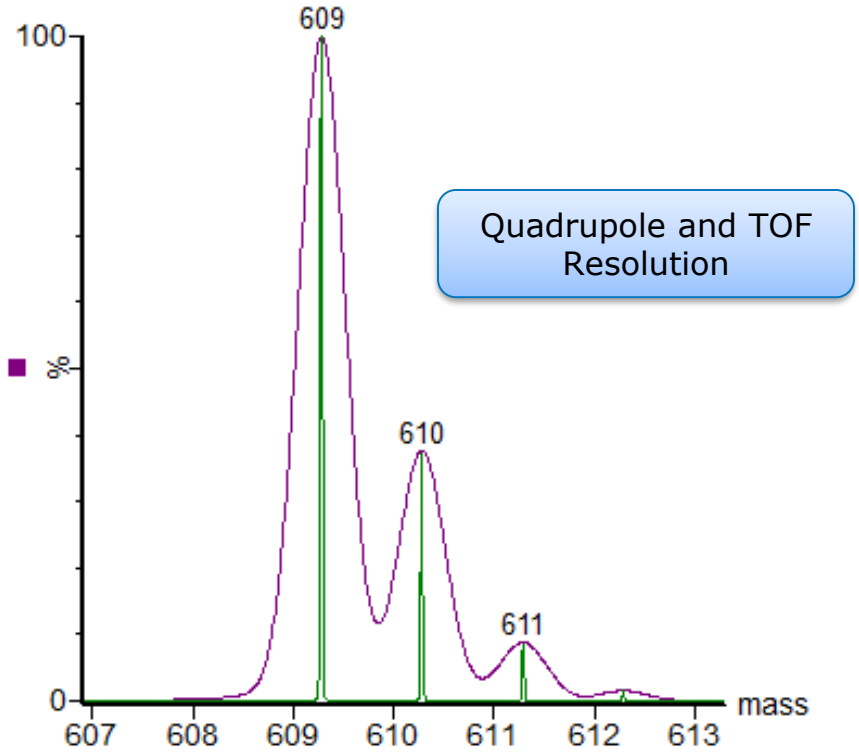
- Triple Quad MRM/SRM
  - Precursor to Fragment ion transmission
  - Compound specific transition
  - Analytes defined in method development
  - Methods redeveloped for new compounds
- TOF
  - Selectivity from mass resolution
  - Data can be re-interrogated



# Xevo G2-XS QTof

## MS$^E$ Acquisition Mode

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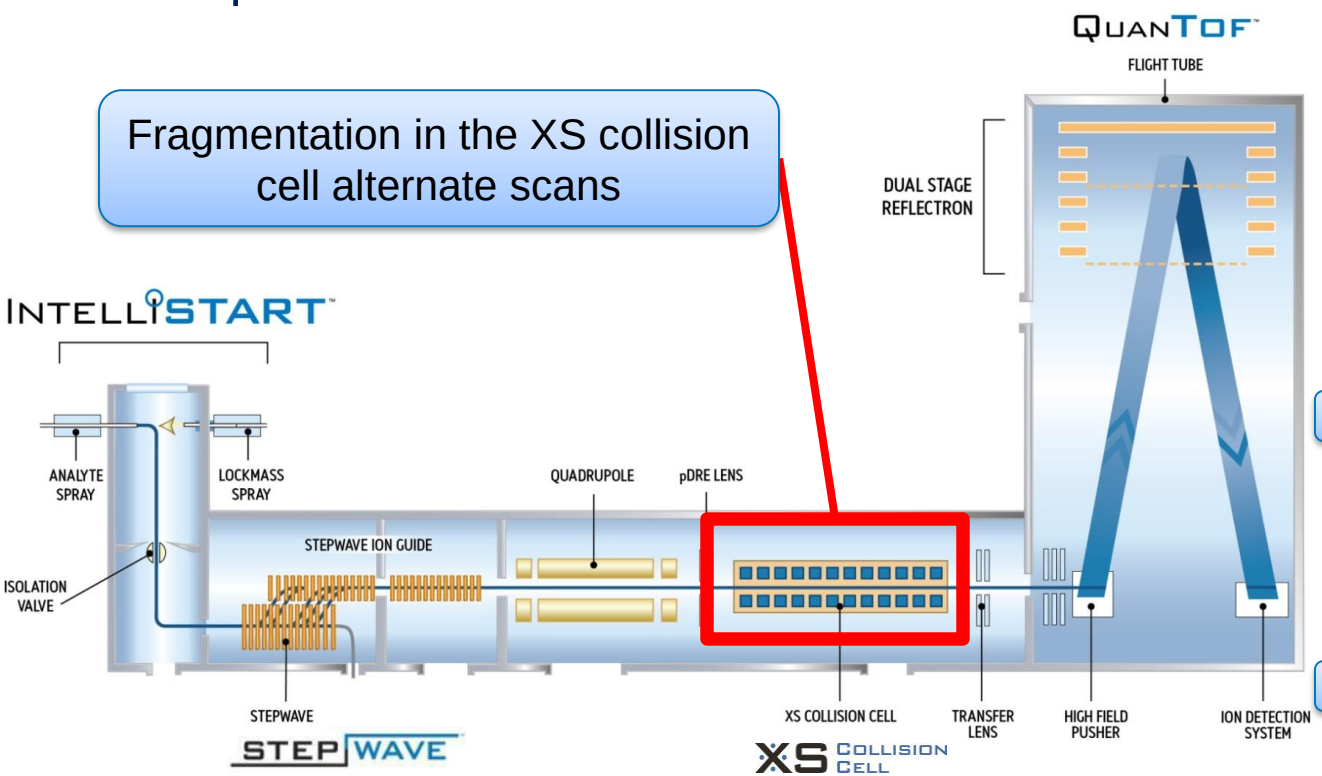
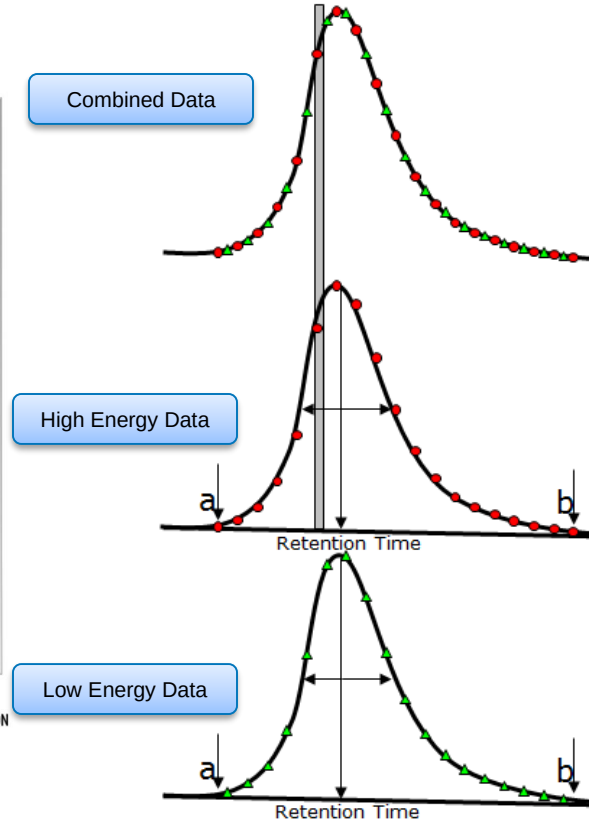
Fragmentation in the XS collision cell alternate scans




Acquisition Conditions

Ionization Mode: ESI$^+$
Acquisition Range: 50-1200 m/z
Acquisition Rate: 8 spectra/sec
Capillary: 0.80 kV
Source Temp: 120 ° C
Desolvation Temp: 550 ° C
Cone Gas: 50 L/hr
Desolvation Gas: 1000 L/hr
Cone Voltage: 20 V

Collision Energy (LE): 4 V
Collision Energy (HE Ramp): 10 to 45 V
Lockspray: Leu Enk (556.2771)

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# Method Development

# 2D Optimization Scheme

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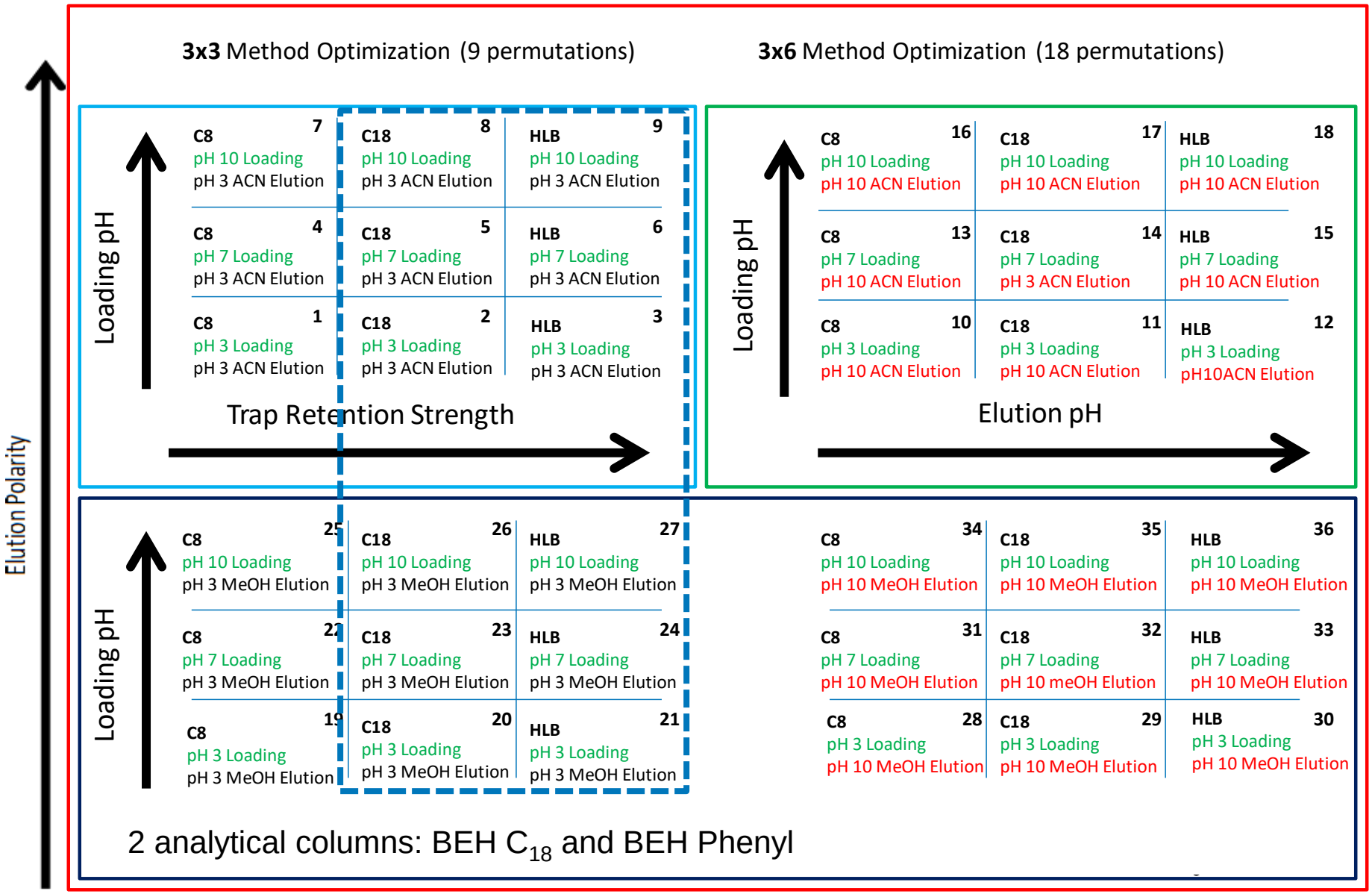

# Chromatography Results: Trap Retention Strength and Elution Polarity

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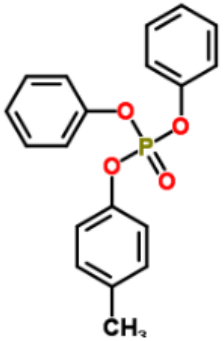
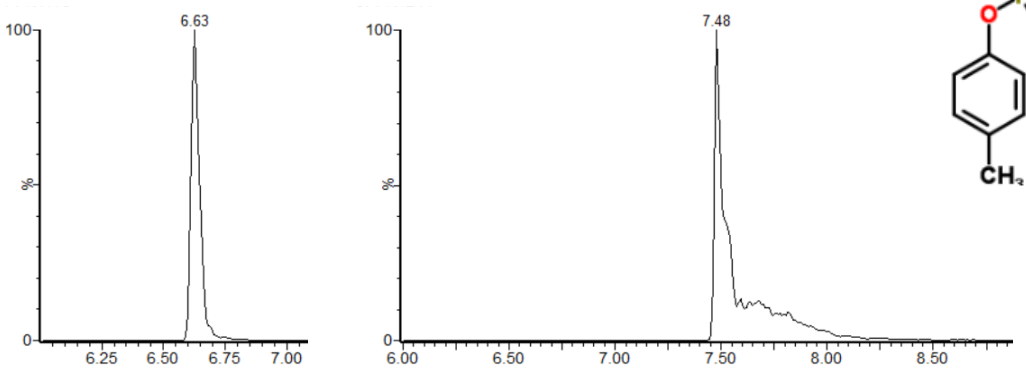
- Tailing with increasing retention strength of trap when MeOH is used for organic mobile phase
- Ex. Cresyl diphenyl phosphate (DCP) MW: 340.31

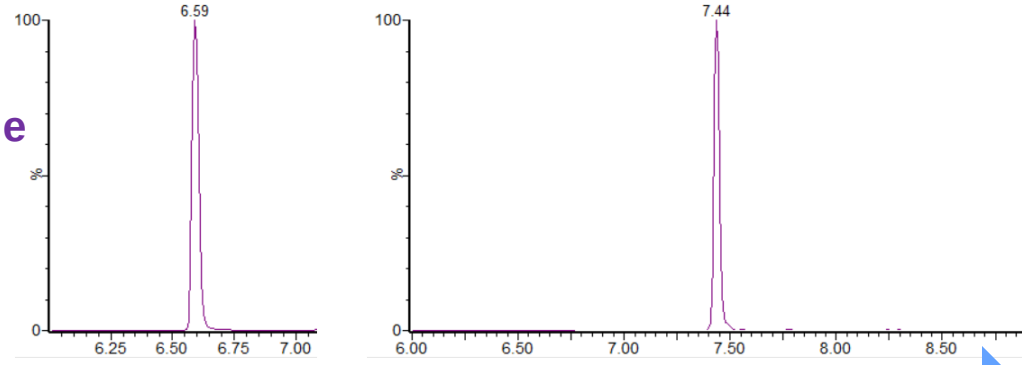



Impacts Earlier Eluters: TCEP, V6, TCDPP, TPhP and TBP

**Methanol**




**Acetonitrile**




$C_{18}$ → HLB

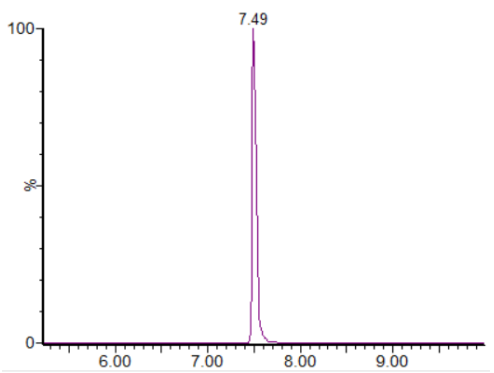
pH 3 loading

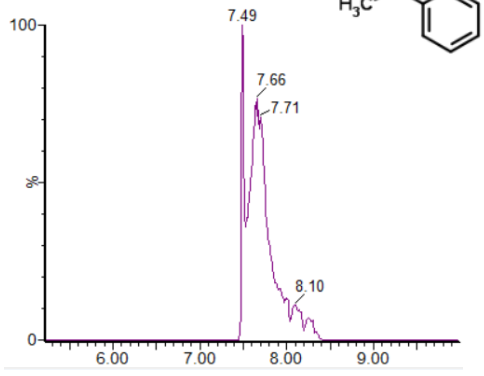
# Chromatography Results: Loading pH Impact

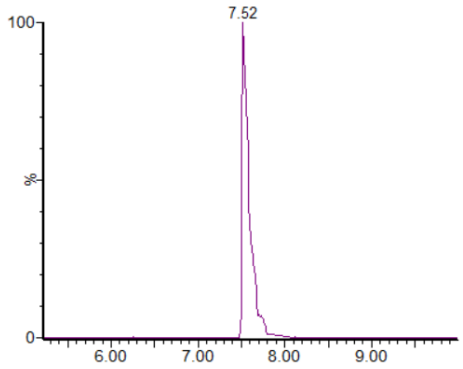
- Tailing with pH 10 loading (organic mobile phase independent)
- Ex. Tris (2-isopropyl phenyl) phosphate (IPPP) MW: 452.52

Impacts Later Eluters: EDHP, IDPP, TBNPP

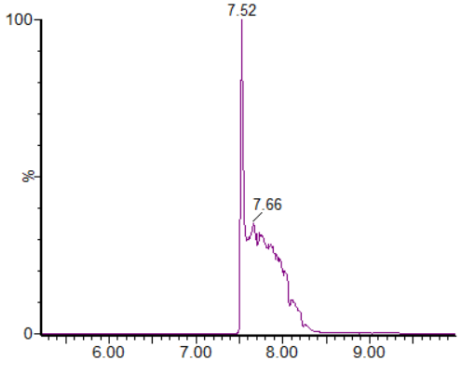
**Acetonitrile**



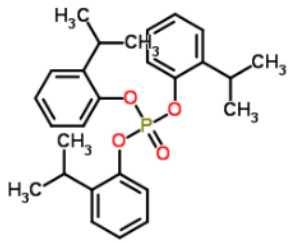




pH 3 loading




pH 10 loading




# Analyte Response Variation by 2D Method

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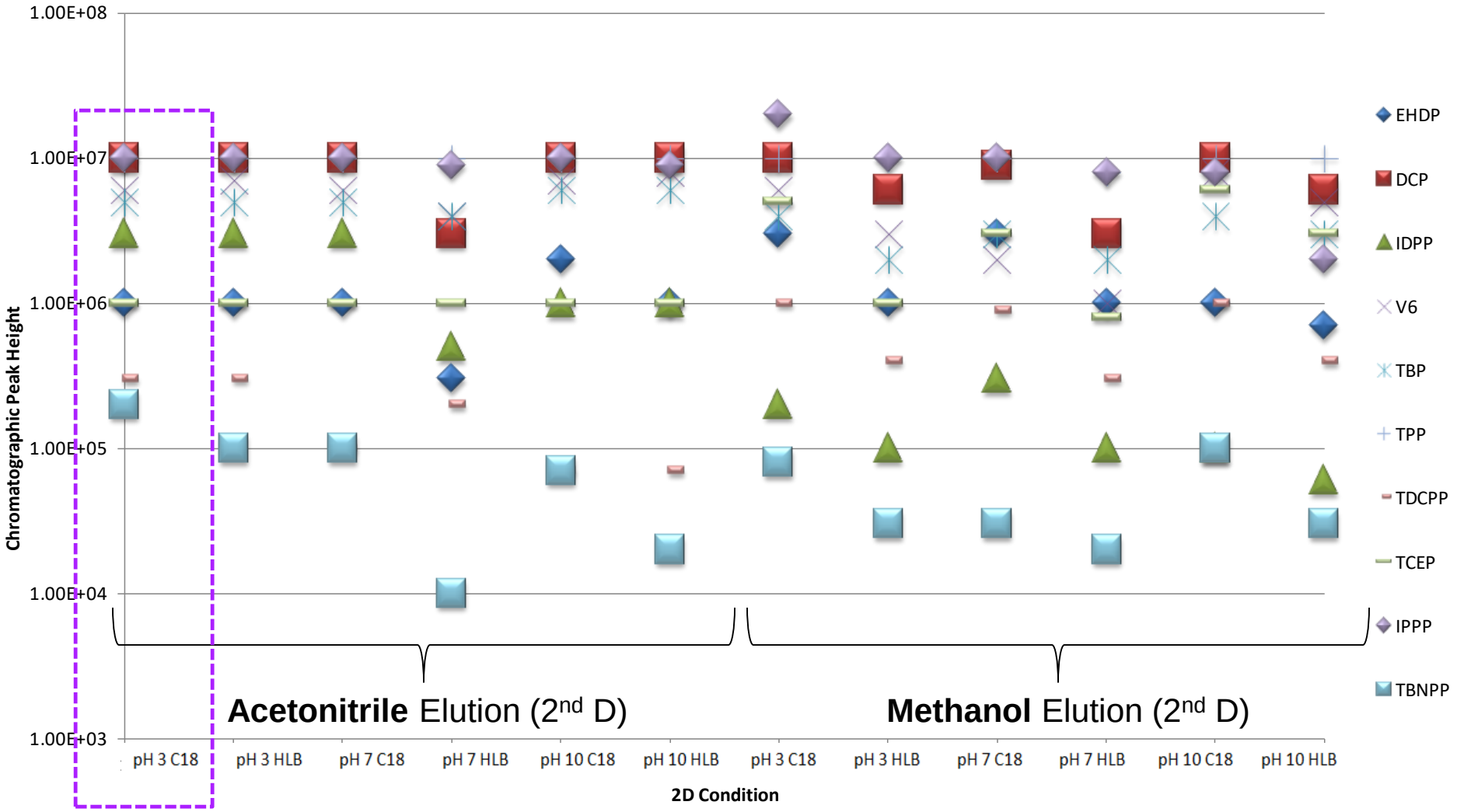

# Solvent $Na^+$ Adducting Trends: Higher for Methanol MP B

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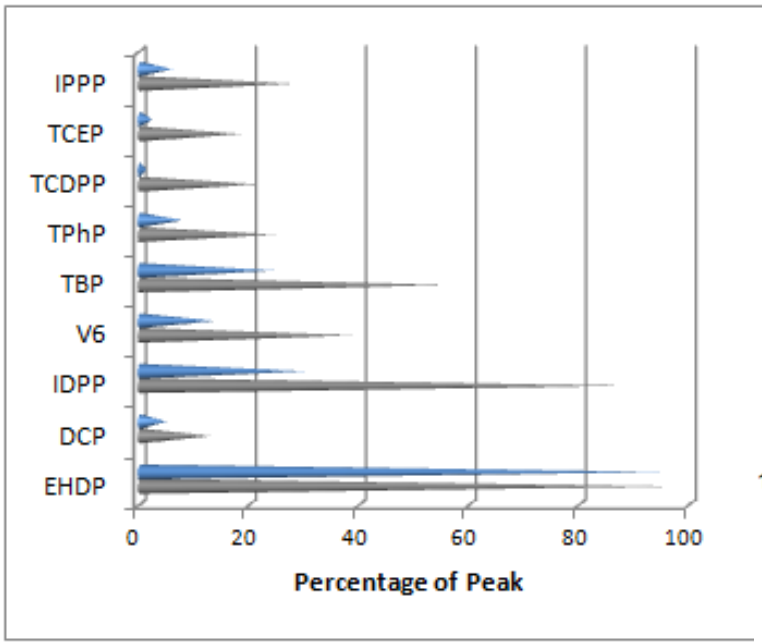
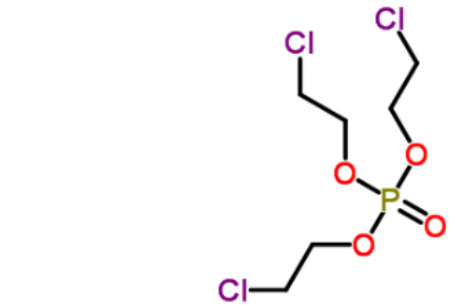
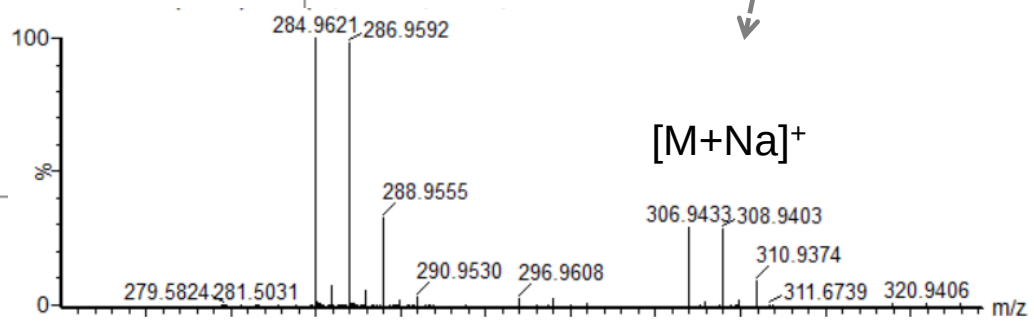


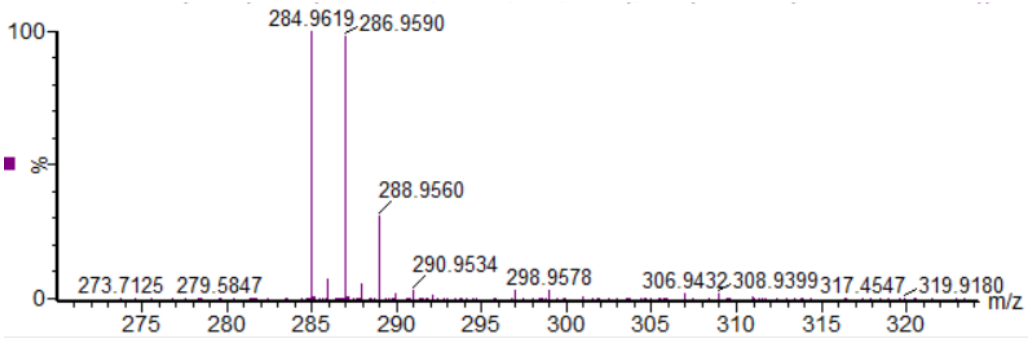

$[M+H]^+$

$[M+Na]^+$

Methanol MP B




Acetonitrile MP B



# Optimum Chromatography Method Results

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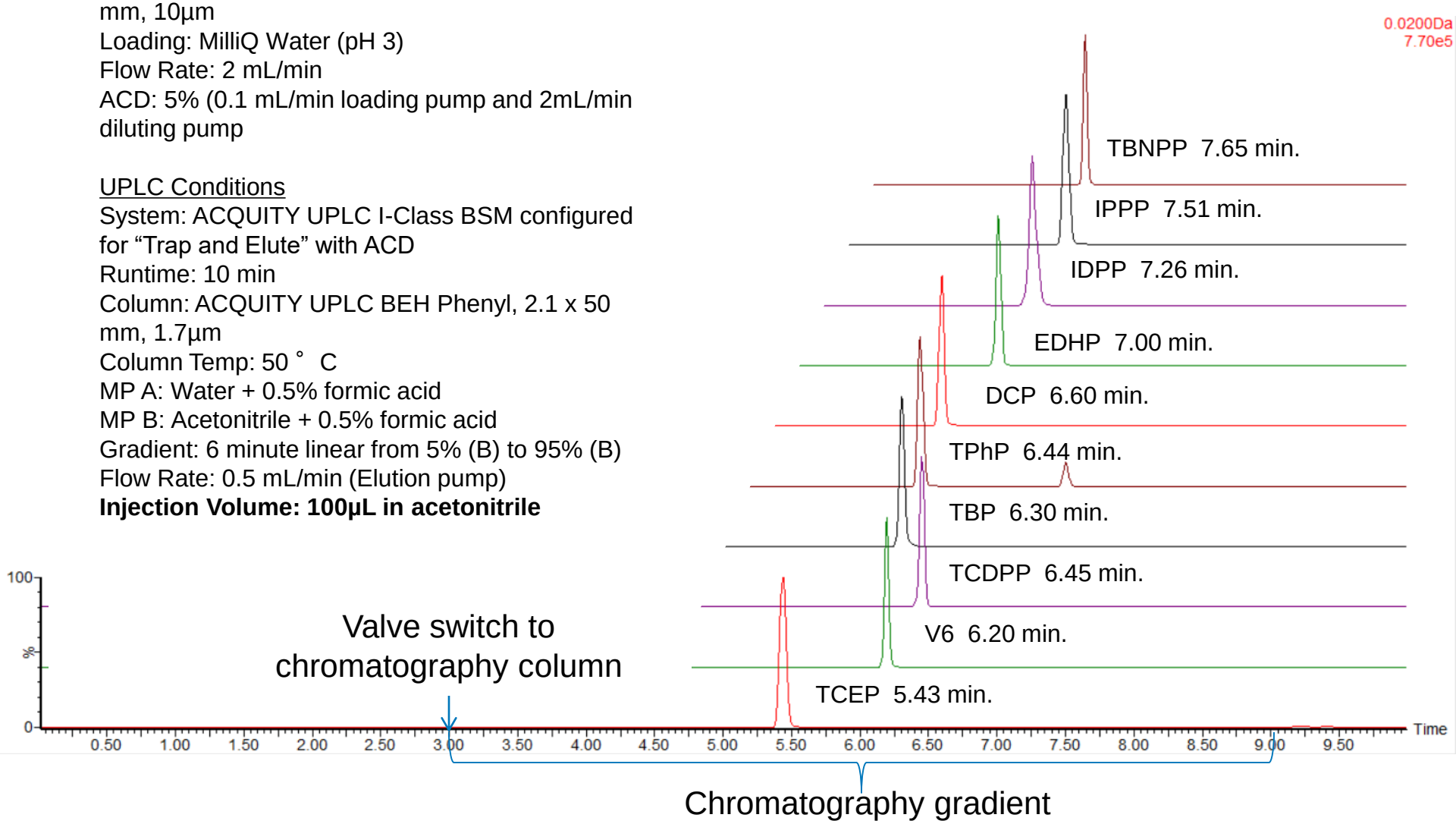
Loading Conditions
Column: XBridge $C_{18}$ Direct Connect HP, 2.1 x 30 mm, 10µm
Loading: MilliQ Water (pH 3)
Flow Rate: 2 mL/min
ACD: 5% (0.1 mL/min loading pump and 2mL/min diluting pump

UPLC Conditions
System: ACQUITY UPLC I-Class BSM configured for "Trap and Elute" with ACD
Runtime: 10 min
Column: ACQUITY UPLC BEH Phenyl, 2.1 x 50 mm, 1.7µm
Column Temp: 50 ° C
MP A: Water + 0.5% formic acid
MP B: Acetonitrile + 0.5% formic acid
Gradient: 6 minute linear from 5% (B) to 95% (B)
Flow Rate: 0.5 mL/min (Elution pump)
**Injection Volume: 100µL in acetonitrile**



# MS$^E$ fragments

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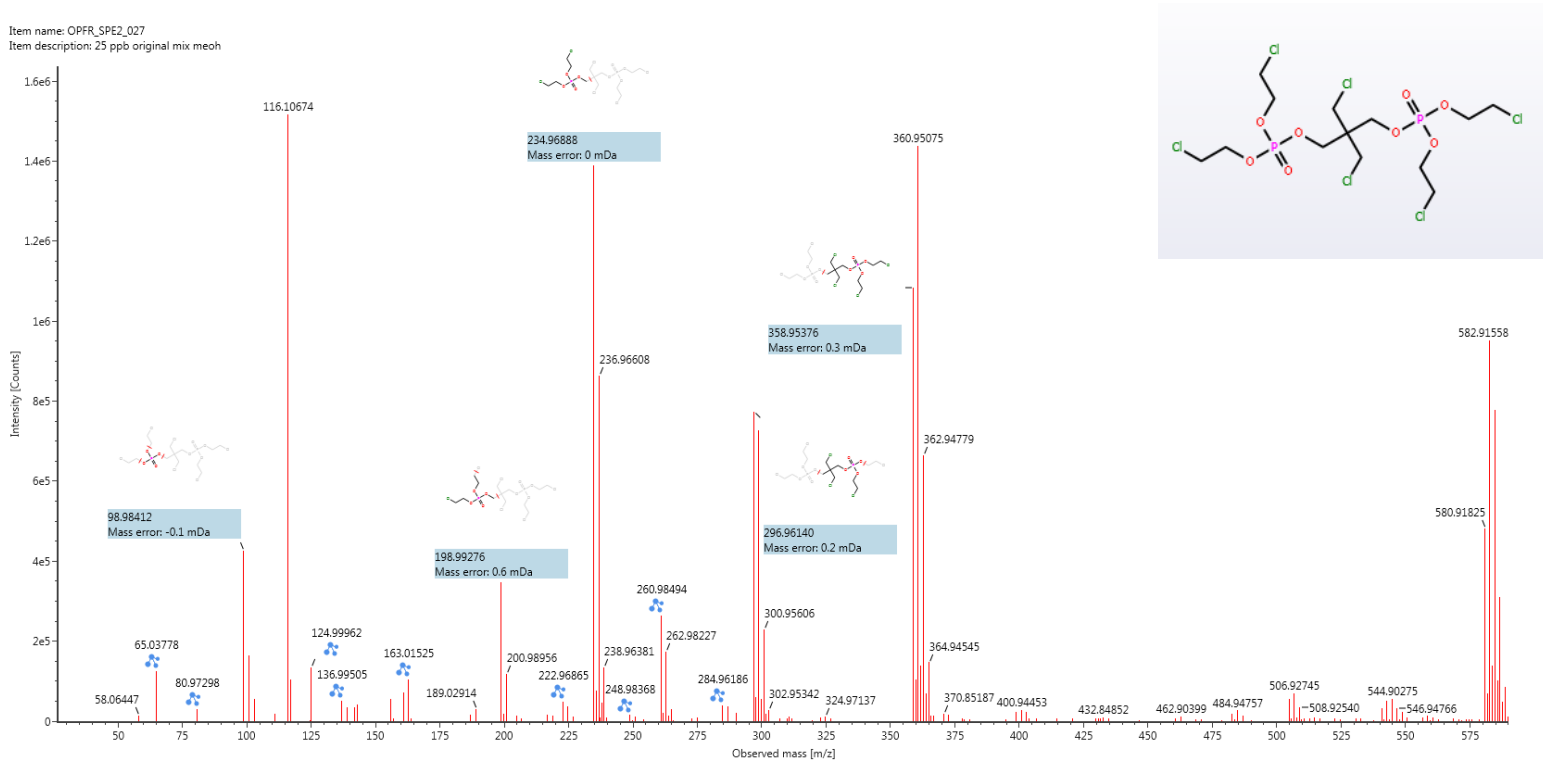




| Compound | F1 | Formula | F2 | Formula2 | F3 | Formula3 | F4 | Formula4 |
|---|---|---|---|---|---|---|---|---|
| TCEP | 98.9842 | $H_4O_4P$ | 222.9688 | $C_4H_{10}Cl_2O_4P$ | 124.9998 | $C_2H_6O_4P$ | 160.9765 | $C_2H_7ClO_4P$ |
| V6 | 358.0953 | $C_6H_{16}Cl_4O_4P$ | 234.9688 | $C_5H_{10}Cl_2O_4P$ | 296.9612 | $C_7H_{13}Cl_3O_4P$ | 260.9845 | $C_7H_{12}Cl_2O_4P$ |
| TDCCP | 98.9842 | $H_4O_4P$ | 208.0953 | $C_3H_8Cl_2O_4P$ | 318.9222 | $C_6H_{12}Cl_4O_4P$ | | |
| TPhP | 251.0468 | $C_{12}H_{12}O_4P$ | 233.0362 | $C_{12}H_{10}O_3P$ | | | | |
| TBP | 98.9842 | $H_4O_4P$ | 80.9736 | $H_2O_3P$ | | | | |
| DCP | 91.0542 | $C_7H_7$ | 247.0519 | $C_{13}H_{12}O_3P$ | 265.0624 | $C_{12}H_{14}O_4P$ | 251.0468 | $C_{12}H_{12}O_4P$ |
| EHDP | 251.0468 | $C_{12}H_{12}O_4P$ | 152.0597 | $C_5H_{13}O_3P$ | 77.0386 | $C_6H_5$ | | |
| IDPP | 251.0468 | $C_{12}H_{12}O_4P$ | 152.0597 | $C_5H_{13}O_3P$ | 77.0386 | $C_6H_5$ | | |
| IPPP | 327.0781 | $C_{18}H_{16}O_4P$ | 369.1250 | $C_{21}H_{22}O_4P$ | 411.1720 | $C_{24}H_{28}O_4P$ | 233.0362 | $C_{12}H_{10}O_3P$ |
| TBNPP | 144.9653 | $C_5H_6Br$ | 224.8914 | $C_5H_7Br_2$ | 304.8176 | $C_5H_8Br_3$ | | |

# Limit-of-Detection/Quantification

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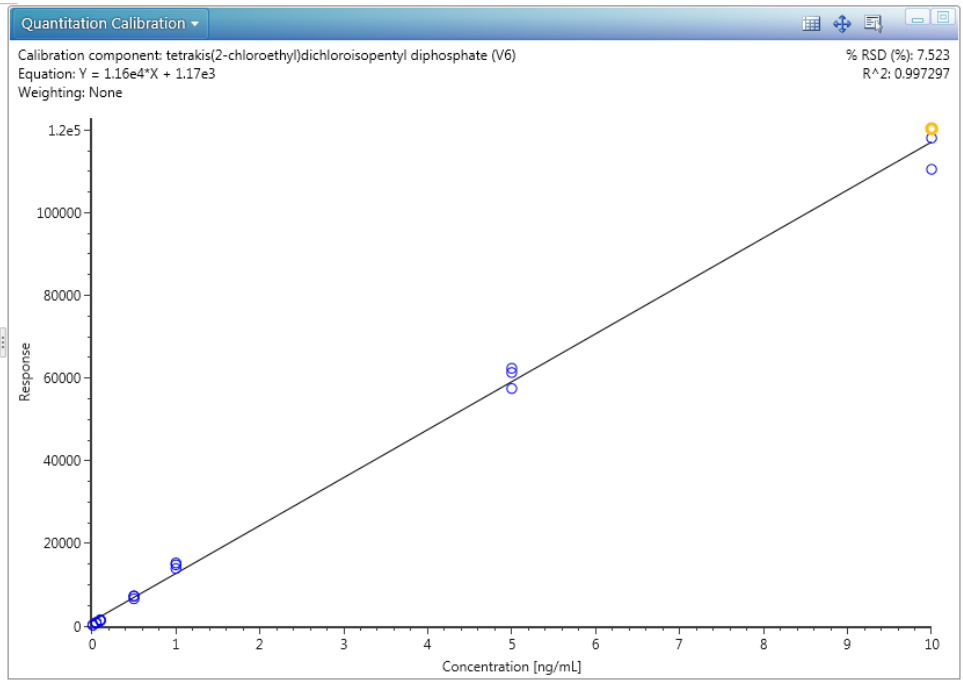


| | LOD (ng/mL) | LOQ (ng/mL) |
|---|---|---|
| TCEP | 0.1 | 0.5 |
| V6 | 0.01 | 0.05 |
| TBP | 0.1 | 0.1 |
| EDHP | 0.1 | 5.0 |
| IDPP | 0.05 | 0.1 |
| IPPP | 0.01 | 0.01 |
| TBNPP | 2.0 | 10.0 |

- LOD S/N PtP 3:1
- LOQ S/N PtP 10:1
- Both: at least 1 fragment ion confirmation
- TPP, TDCPP and DCP excluded as levels observed in blank at lowest cal. point

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# Sample Introduction

# Sample Preparation: SPE

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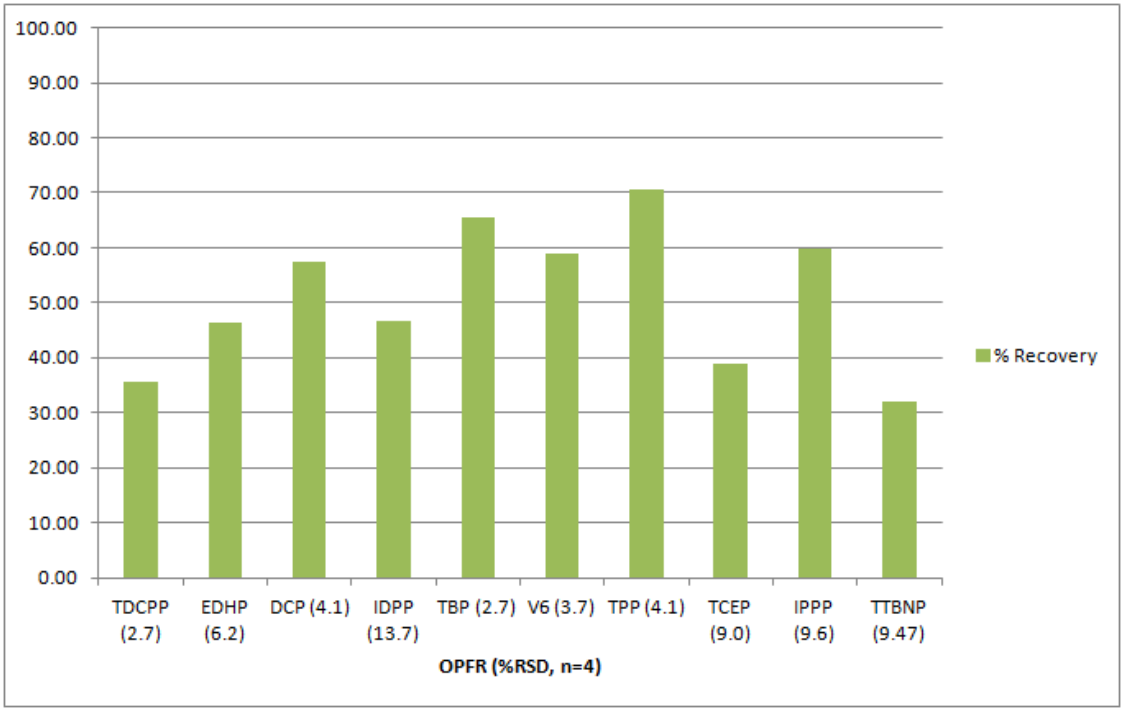
- Previous methodologies for various OPFRs have utilized SPE successfully in environmental water samples
- Simplified version adopted in with dry-down step eliminated

- Steps:
  - Rinse needles with methylene chloride
  - Affix Oasis HLB cartridge (6cc 150 mg) to extraction manifold
  - Wash 5mL MeOH, 5mL water
  - **Load sample (100mL)**
  - Elute 5mL pH 3 acetonitrile
  - Inject 100uL acetonitrile extraction



# Targeted Identification

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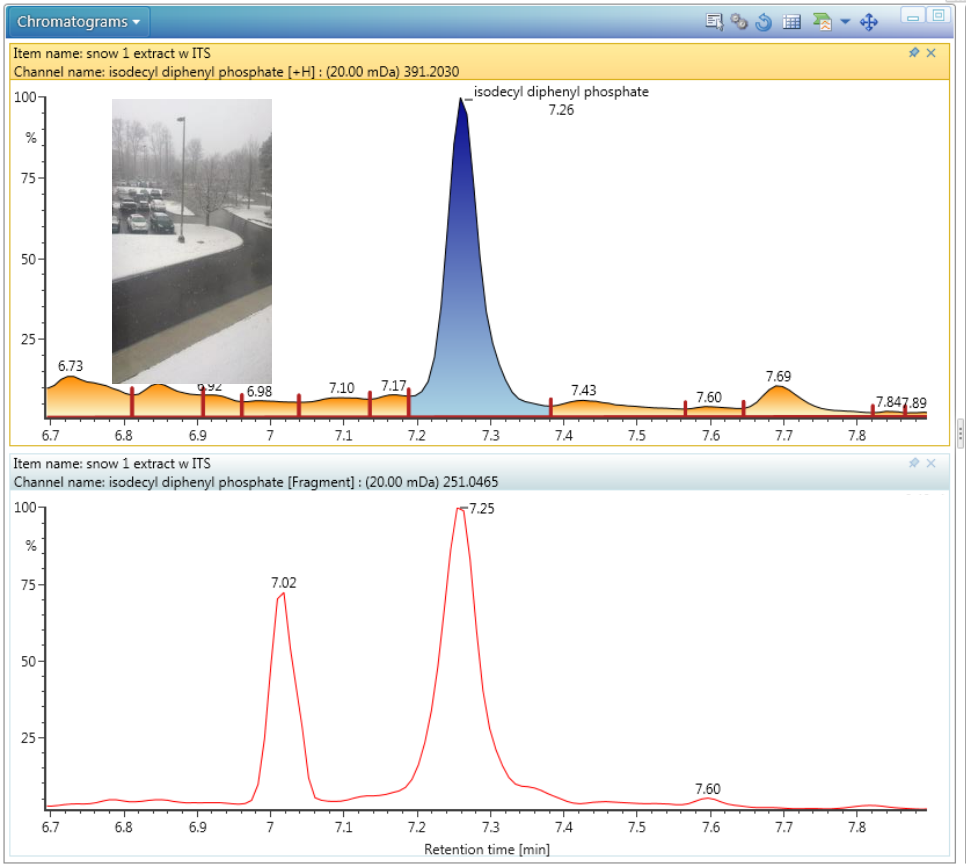
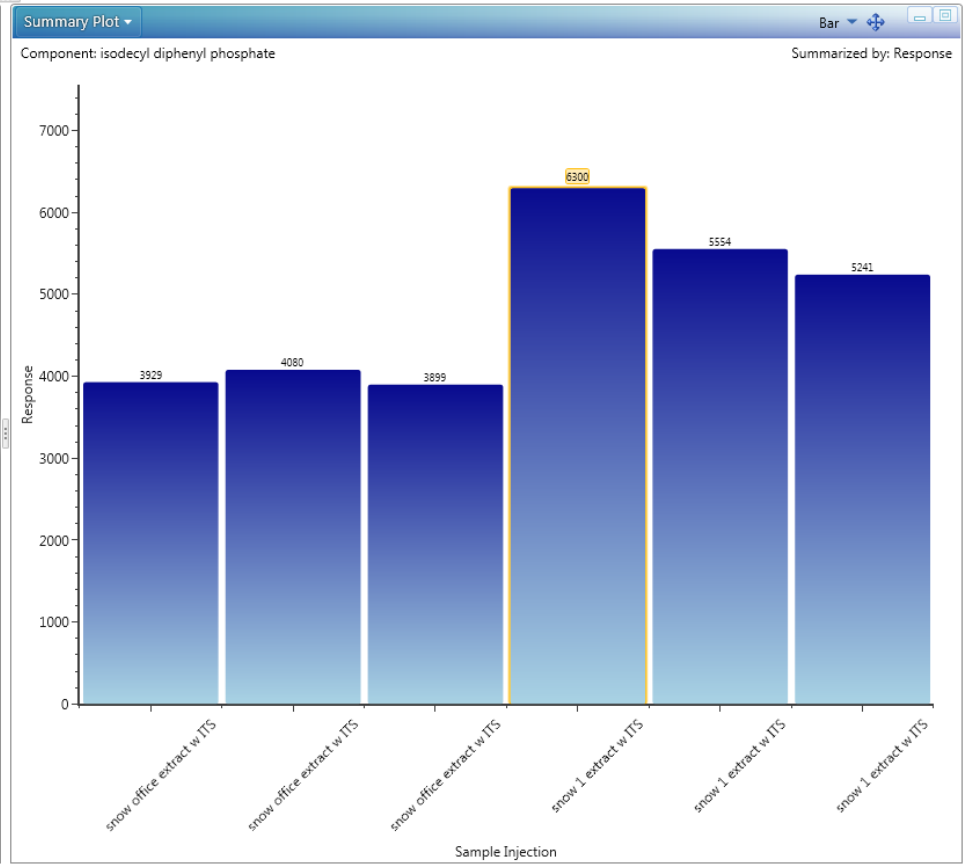
- IDPP, in both snow samples
- Identification criteria
  - Mass error: +/- 5 ppm mass error
  - Fragment mass confirmation

Component Summary

| | Component name | Formula | Expected RT (min) | Retention Time Error (min) | Mass error (ppm) | Response | Adducts | Expected Fragments Count | Expected Fragments Found |
|---|---|---|---|---|---|---|---|---|---|
| 3 | isodecyl diphenyl phosphate | C22H31O4P | 7.26 | 0.00 | -0.8 | 6300 | +H | 3 | 1 |



# Identifying Non-Targeted Compounds: Common Fragments

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- Possibility of other OPRFs and plasticizers
  - Full spectral acquisition=non-targeted acquisition for low and high collision energy
- -$PO_4$ (+4H) common structure and other common fragments

Common Fragment

Enable Common Fragment search

Tolerance: 2.00 mDa

Display

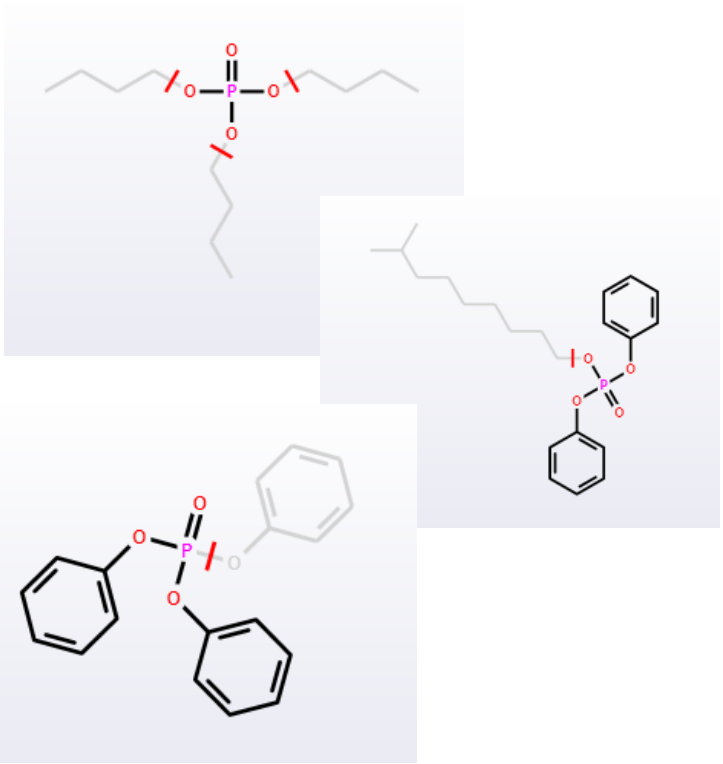
Include Label in results

Include Formula in results

Result output: m/z (Label [Formula])

Add Remove Import

| | m/z | Formula | Label | Charge |
|---|---|---|---|---|
| 1 | 98.98417 | H4O4P | Phosphate Group | Positive |
| 2 | 251.04677 | C12H12O4P | | Positive |
| 3 | 233.03621 | C12H10O3P | | Positive |



# Non-targeted identifications

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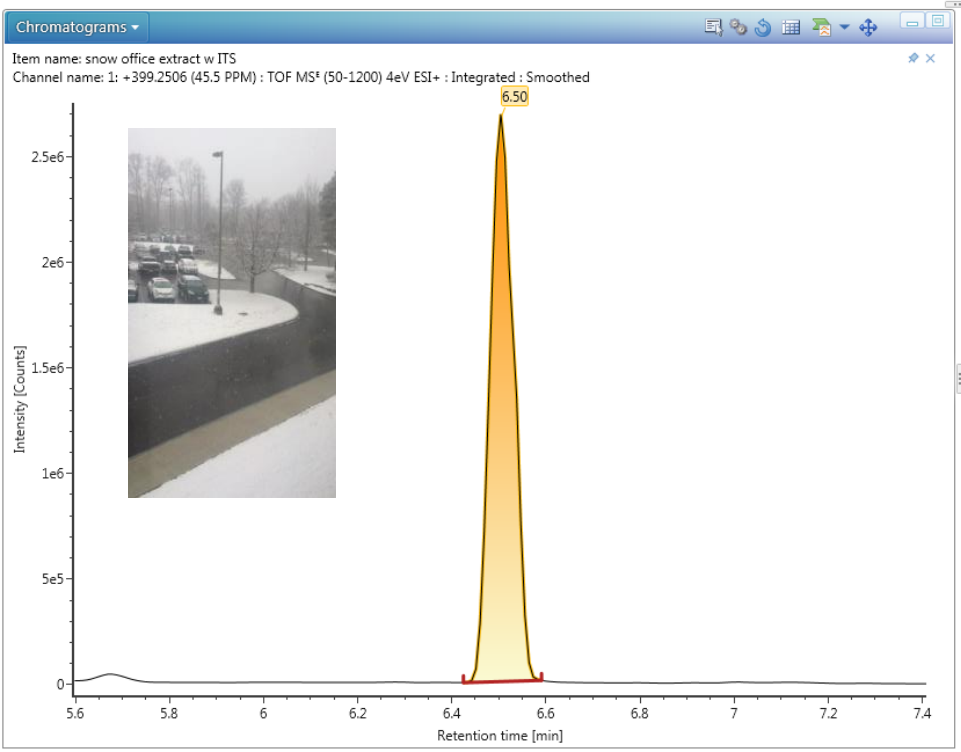
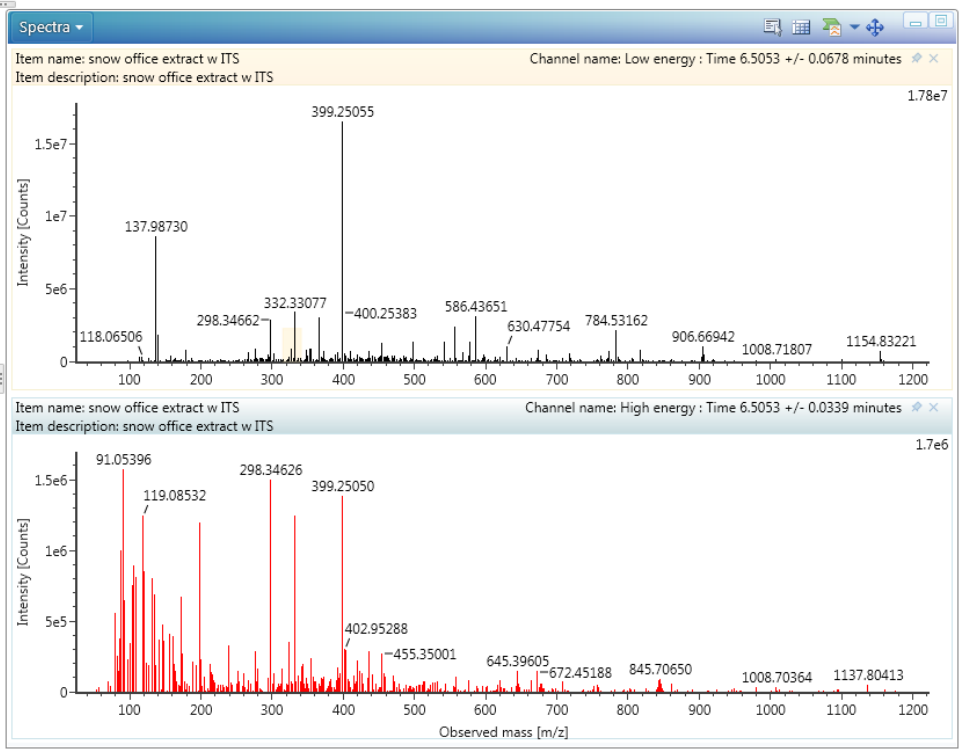
- Additional compounds found with common fragments, ordered by most intense response

Component Summary

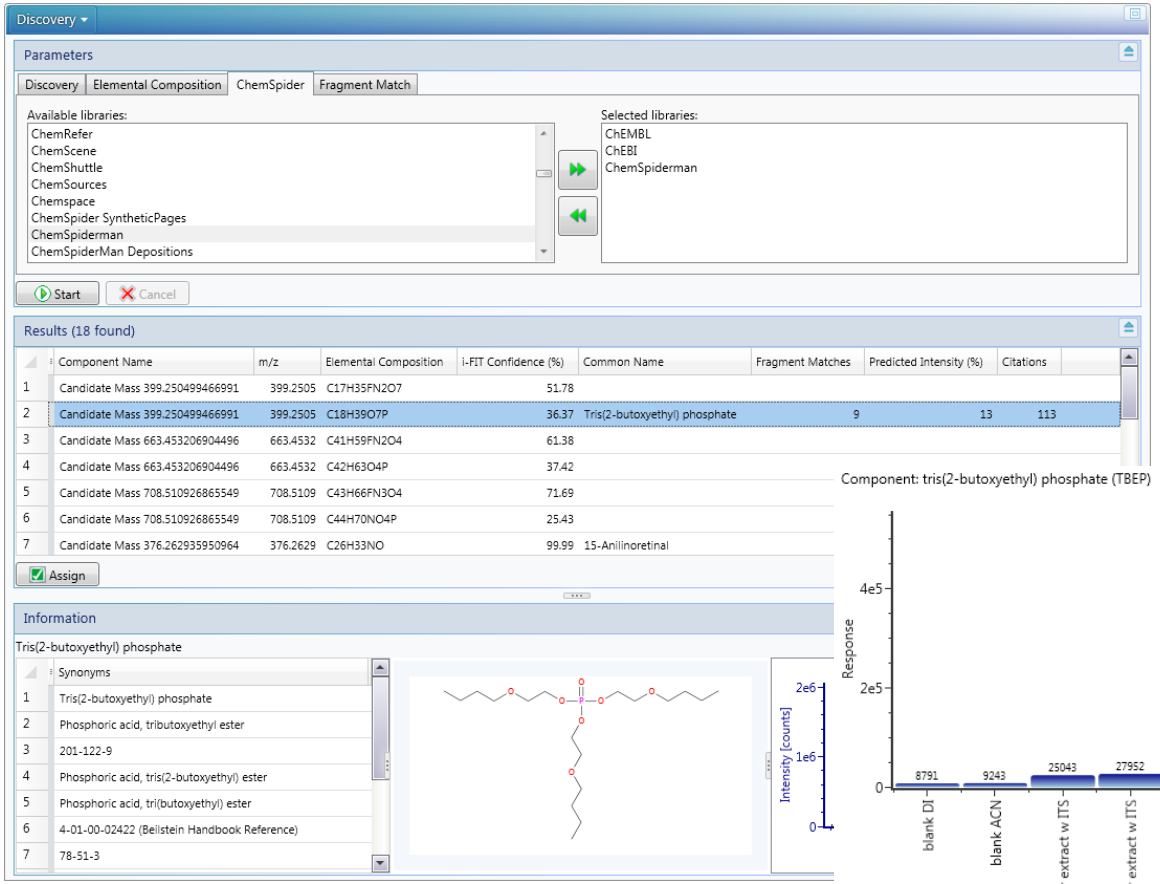
| | Component name | Formula | Expected RT (min) | Retention Time Error (min) | Mass error (ppm) | Response | Adducts | Common Fragment Ions Count | Common Fragment Ions Found |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Candidate Mass 399.2506 | | | | | 423393 | | 1 | 98.98417 (Phosphate Group [H |
| 2 | Candidate Mass 663.4533 | | | | | 320357 | | 2 | 251.04677 [C12H12O4P], 233.0 |
| 3 | Candidate Mass 708.5110 | | | | | 272784 | | 2 | 251.04677 [C12H12O4P], 233.0 |
| 4 | Candidate Mass 376.2629 | | | | | 253989 | | 1 | 251.04677 [C12H12O4P] |

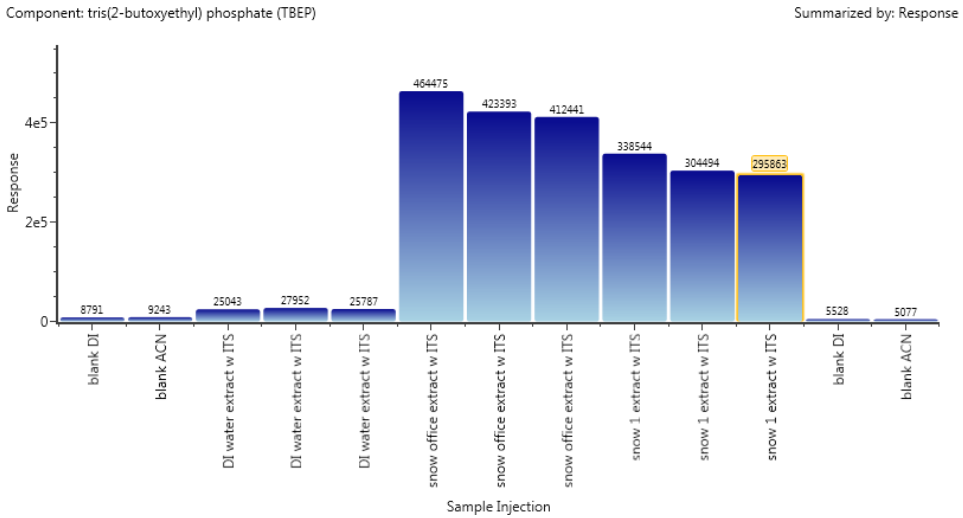


# Database Search for ID

- Tris(2-butoxyethyl) phosphate (TBEP)



# Conclusions

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- 2D LC technology provides ability to inject large volumes of organic solvents without peak shape compromise
- Sensitive detection of OPFRs is possible with this large volume injection approach combined with Qtof detection
- Non-targeted data acquisition coupled with accurate mass measurement affords the ability to search for additional constituents in samples
- Future Direction
  - Revisit sample prep method
  - Biological and other environmental sample analysis

# References and Acknowledgements


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**Thank You!!**