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Scaling Liquid-Liquid Sample Preparation: Moving from Separatory Funnels to a Vial-Based Extraction for Semivolatile Organics Analysis by GC-MS/MS

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and
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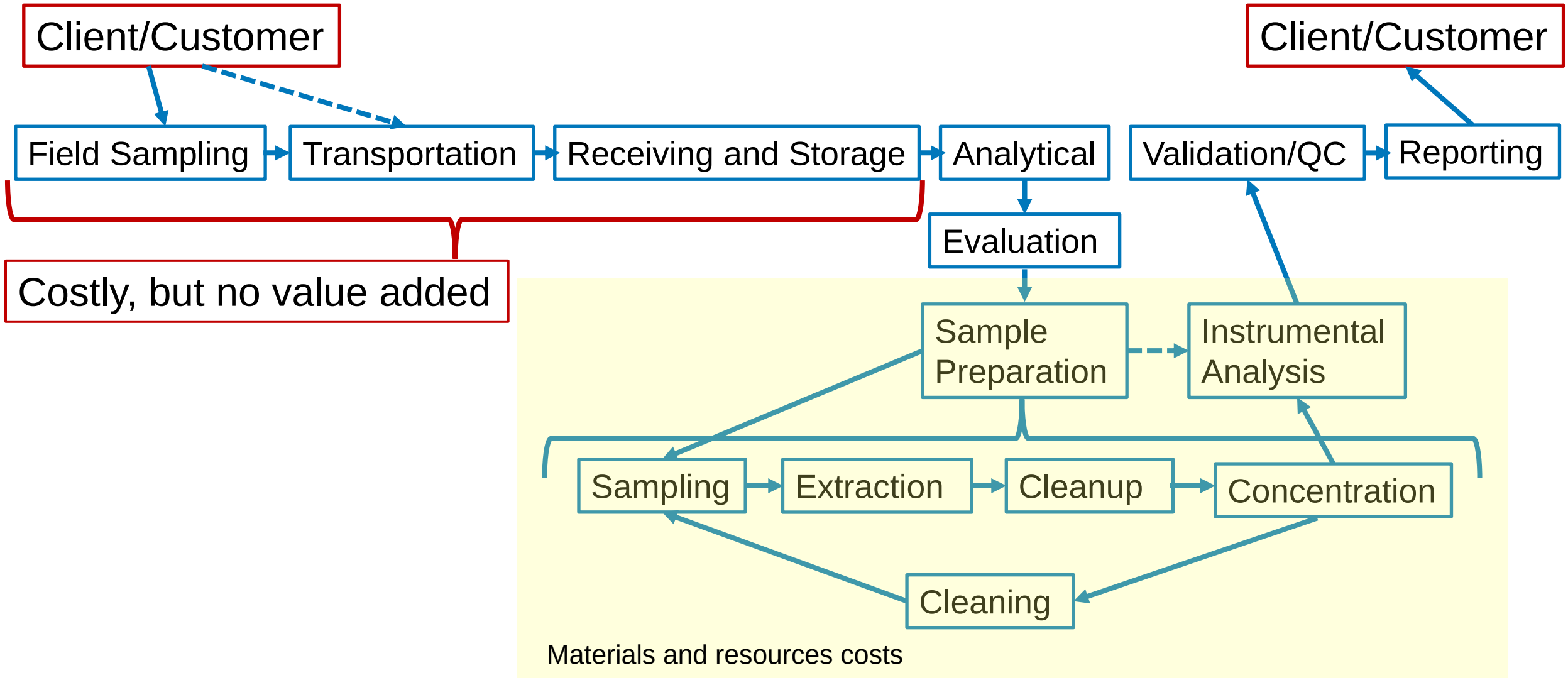
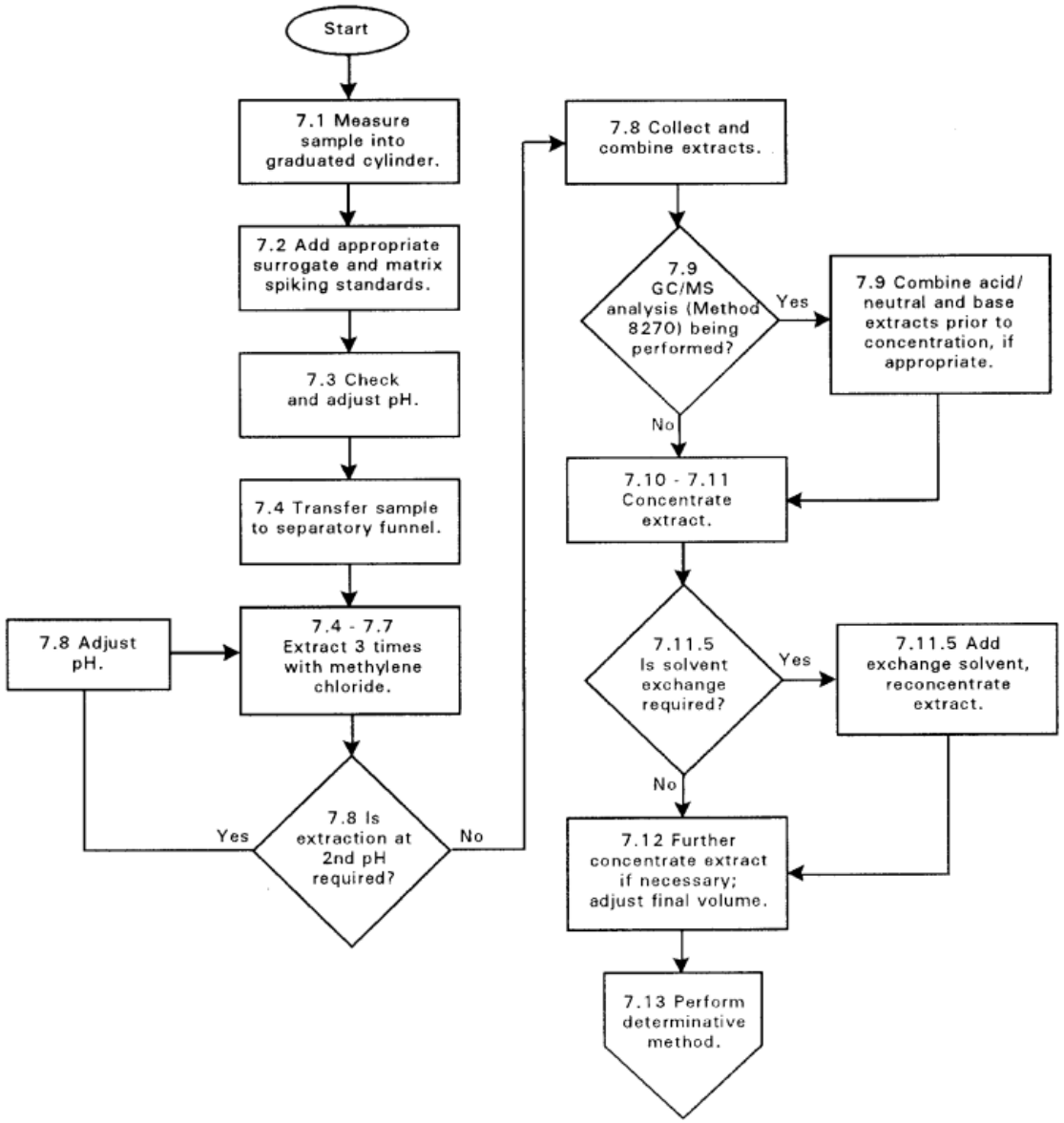
NEMC: Monday July 31st 11:00AM

The Environmental Laboratory (Extractable Organics)



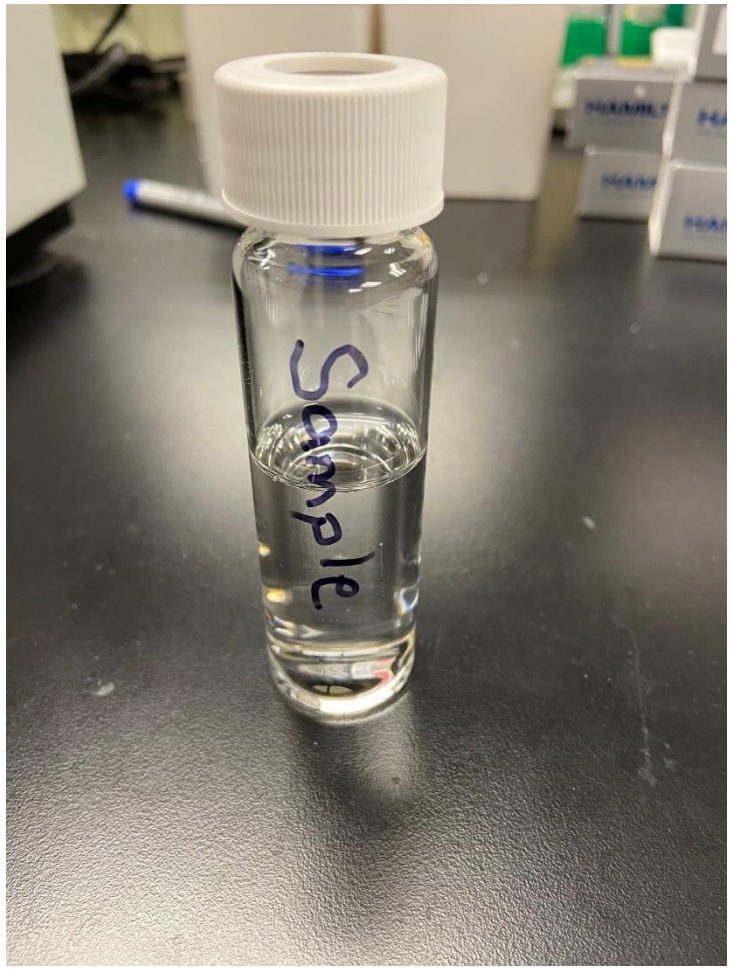
Original Sample Prep



- Based on EPA 3510C Separatory Funnel Liquid-Liquid Extraction https://www.epa.gov/sites/default/files/2015-12/documents/3510c.pdf
- Starting quantity: 1 L of water
- Extraction Solvent: Methylene Chloride
  - 3 x 60 mL of methylene chloride extracts at pH 11
  - 3 x 60 mL of methylene chloride extracts at pH 2
- Extract is concentrated from ~360 mL to 1.0 mL for injection
- Uses a lot of consumables and reusable glassware!

Scaled Sample Prep

- Starting quantity: 20 mL of water
- Extraction Solvent: Methylene Chloride
  - 3 x 2 mL of methylene chloride extracts at pH 11
  - 3 x 2 mL of methylene chloride extracts at pH 2
- No concentration
- Disposable glassware – 40 mL VOA vials and glass pipettes



Scaled Sample Prep Procedure – proof of concept (This retains a similar volume ratio of aqueous to organic)

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Load 20 mL of Water Sample to 40 mL VOA vial

↓

Adjust the pH to 10 with Sodium Hydroxide

↓

Add 2 mL of Methylene Chloride for Extraction

↓

Sample and Methylene Chloride Mixed and Vortexed for ~2 min

↓

Methylene Chloride Extract Removed from the Sample Vial

↓

Repeat Methylene Chloride Extraction 2 x for 6 mL total at pH 10

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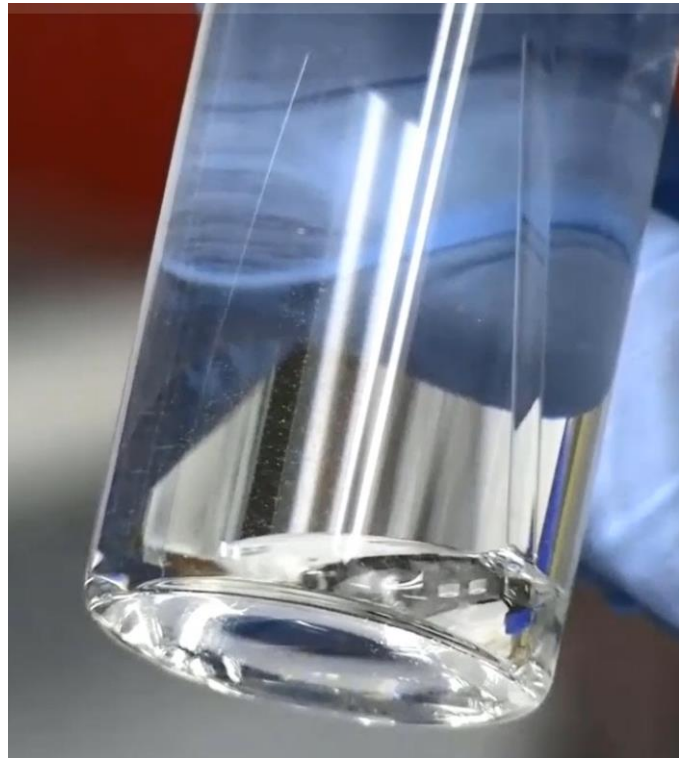
Adjust the pH to 2 with Sulfuric Acid

↓

Extract with Methylene Chloride Extraction 3 x for 6 mL total at pH 2

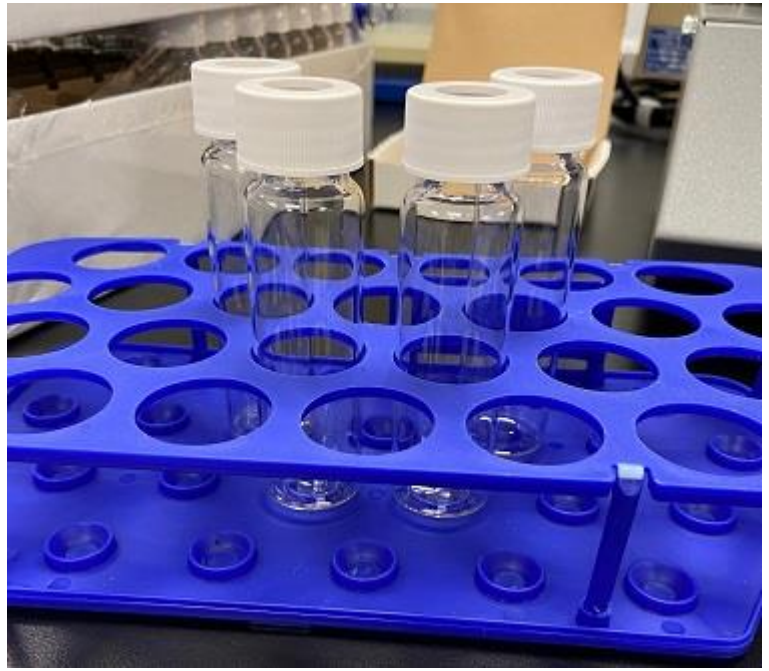
↓

Combine Extracts and Dry with Sodium Sulfate



Scaled Sample Prep Test

- 20 mL of water loaded into VOA vials
- Spiked Samples were prepared at 50 pg/µL with the Restek 8270MegaMix Standard (Catalog No. 31850)
  - 8 replicate spiked samples were prepared to test recovery
  - The MegaMix includes 76 SVOC compounds of varying chemistry
  - Internal standards (Restek Catalog No 31885) were added prior to injection on the GC-MS/MS
- A calibration curve from 3- 500 pg/µL was prepared in methylene chloride for sample quantitation



Experimental: Xevo TQXS MS Parameters

- Ionization: API^{+}
- Corona pin: 2.0 μA
- Source temp: 120 °C
- Cone gas flow: 250 $l.h^{-1}$
- Auxiliary gas: 200 $l.h^{-1}$

Experimental: GC Method (N_2 Carrier Gas)

- Column: Rxi-SVOCms 20m x 0.15mm ID, 0.15 µm film thickness
- Transfer line temperature: 320°C
- Make-up gas: 300 $ml.min^{-1}$ (nitrogen)
- Injection Port Temperature: 280°C
- Injection Volume: 1 µL (10:1 split)
- Pressure Program:

Rate (psi/min)	Value (psi)	Hold (min)
Init.	10.182	0
90	30	0.1
99	10.29	0
1.4	28	0

- Temperature Program:

Ramp (°C/min)	Temp (°C)	Hold (min)	Total Time (min)
Initial	45	0.6	
16.8	100	0	
20.9	270	0	
8.3	310	1.75	
20.8	320	3	22.058

Chromatographic Performance: N_2 Carrier Gas, 20m Column

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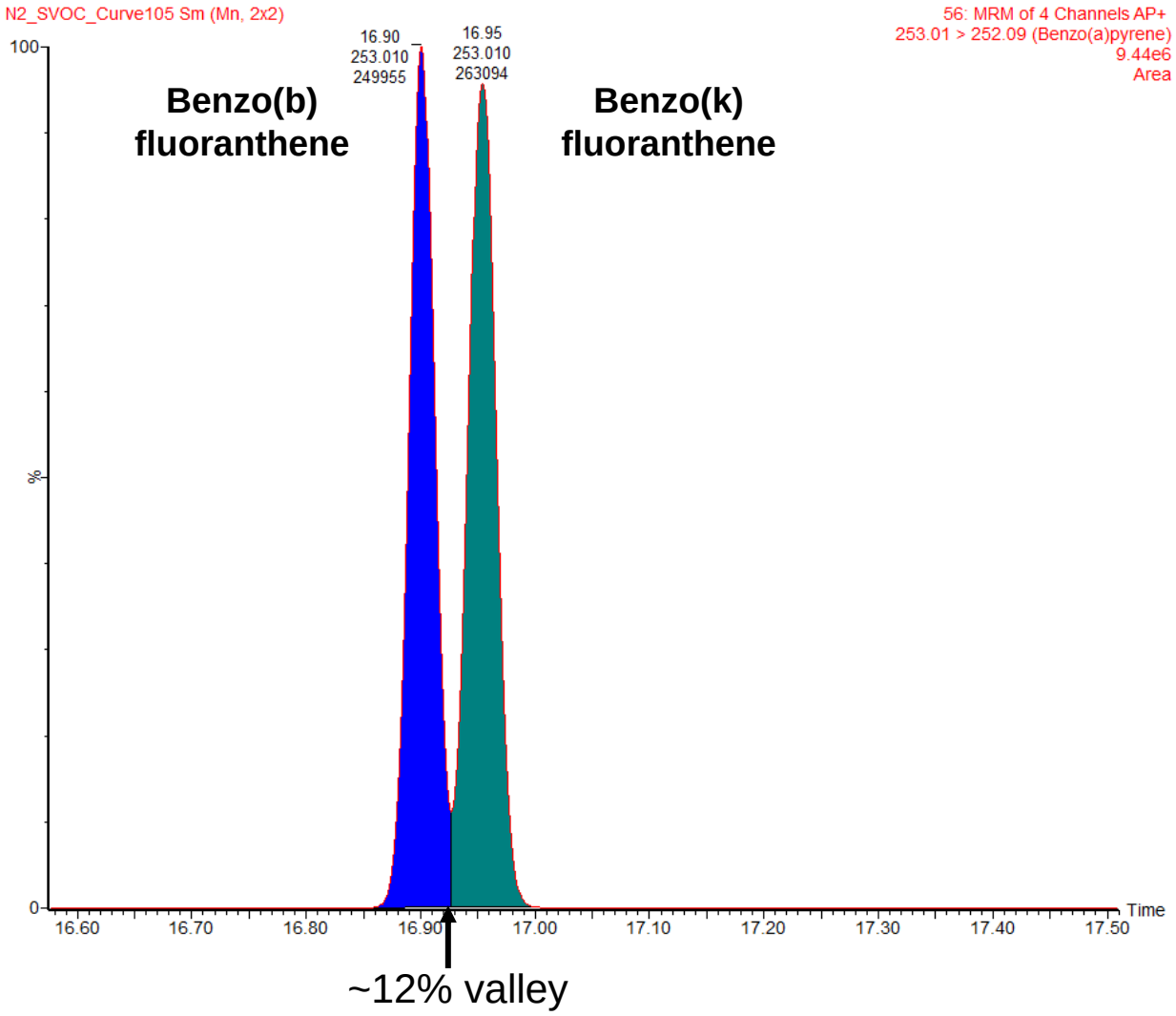
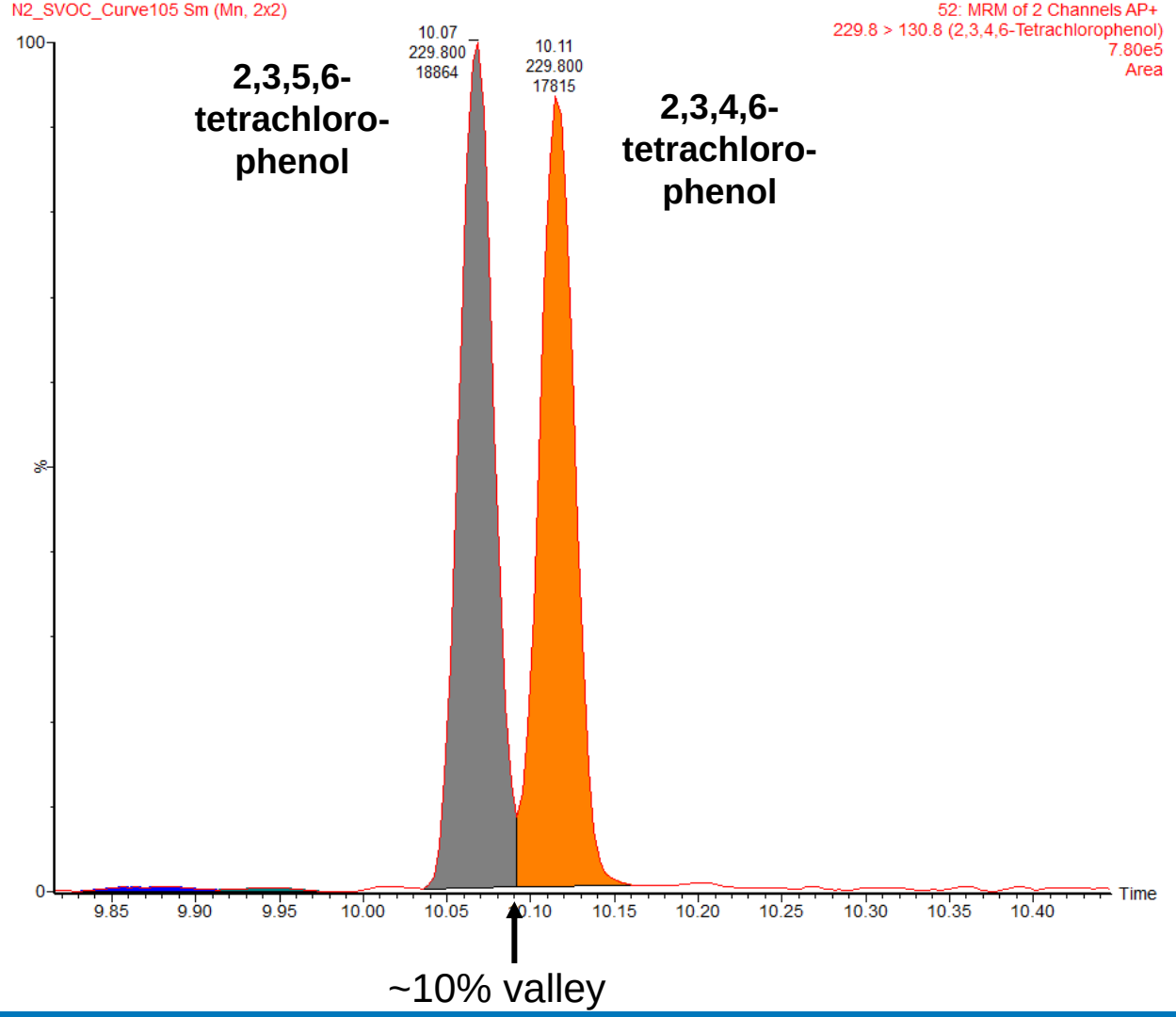
Benzo(b,k)fluoranthene



Tetrachlorophenols



Chromatographic Performance: N_2 Carrier Gas, 20 m Column

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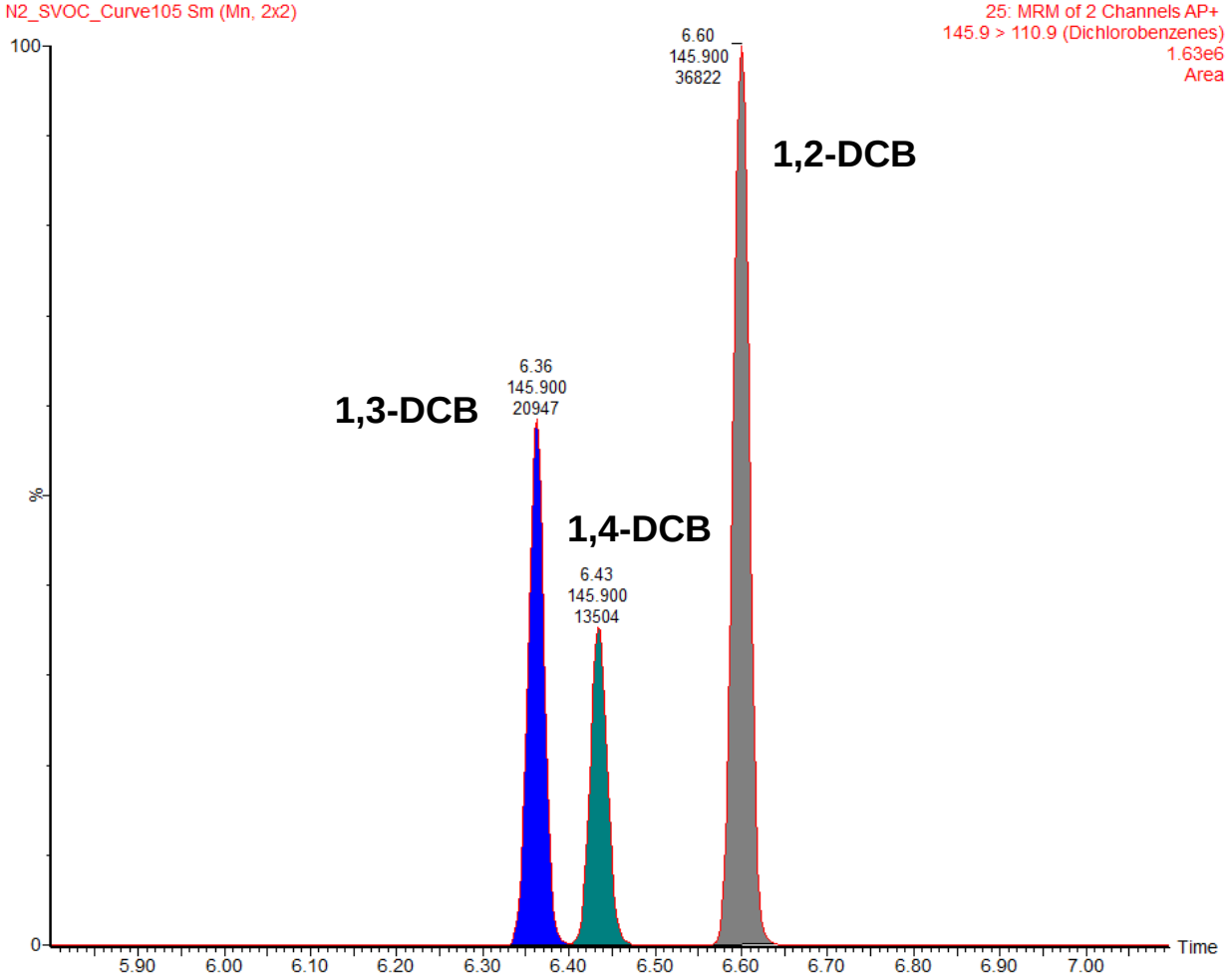
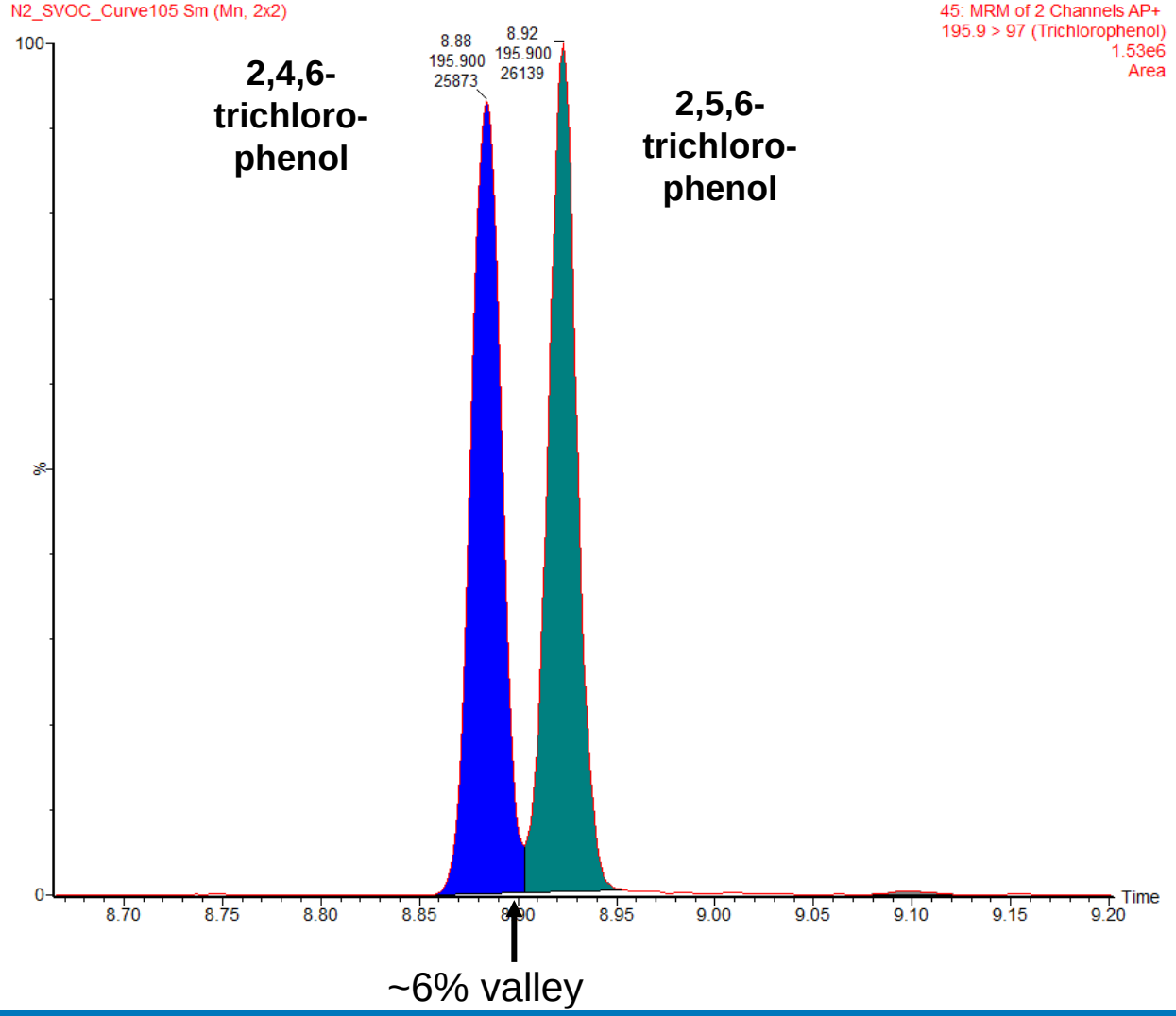
Dichlorobenzenes



Trichlorophenols



Sensitivity of the APGC-MS/MS Method

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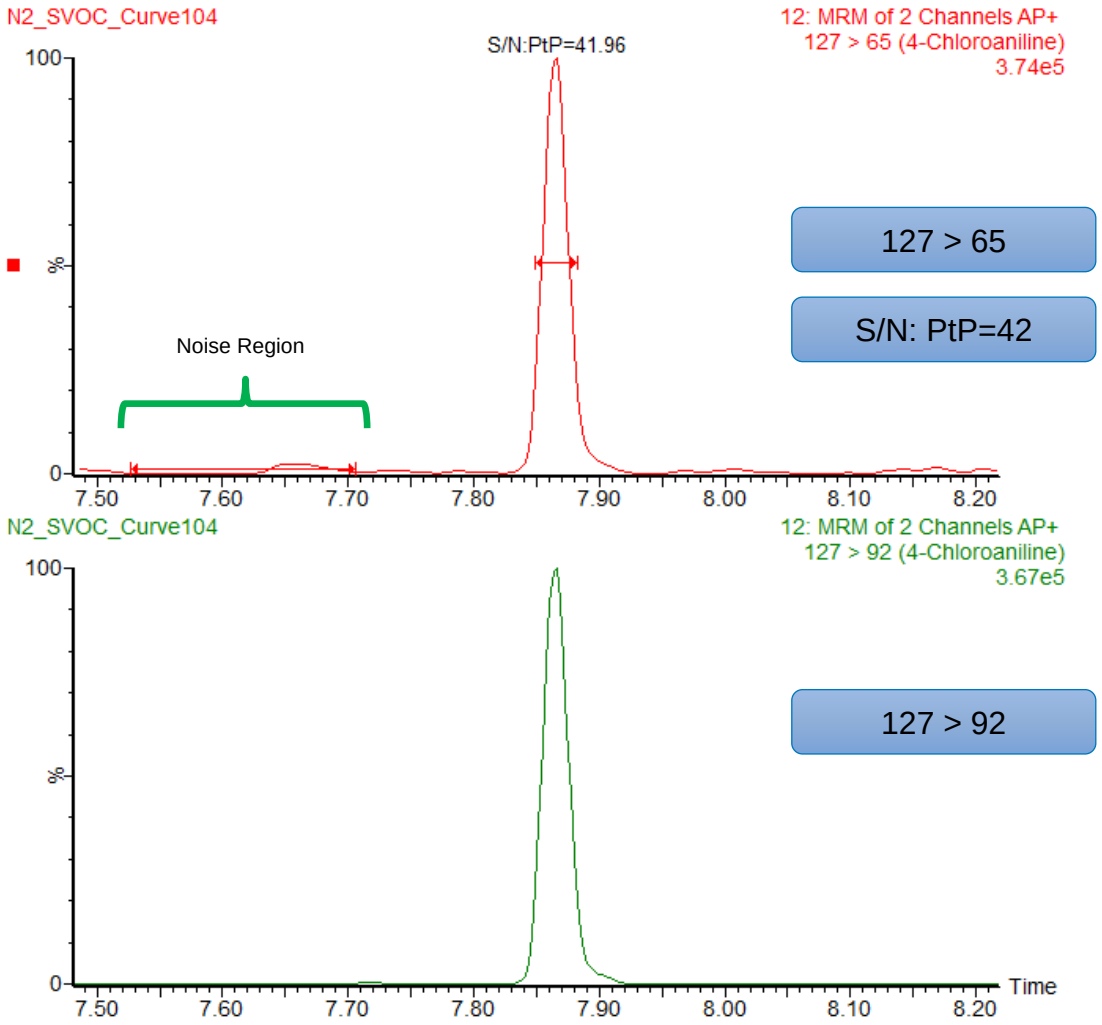
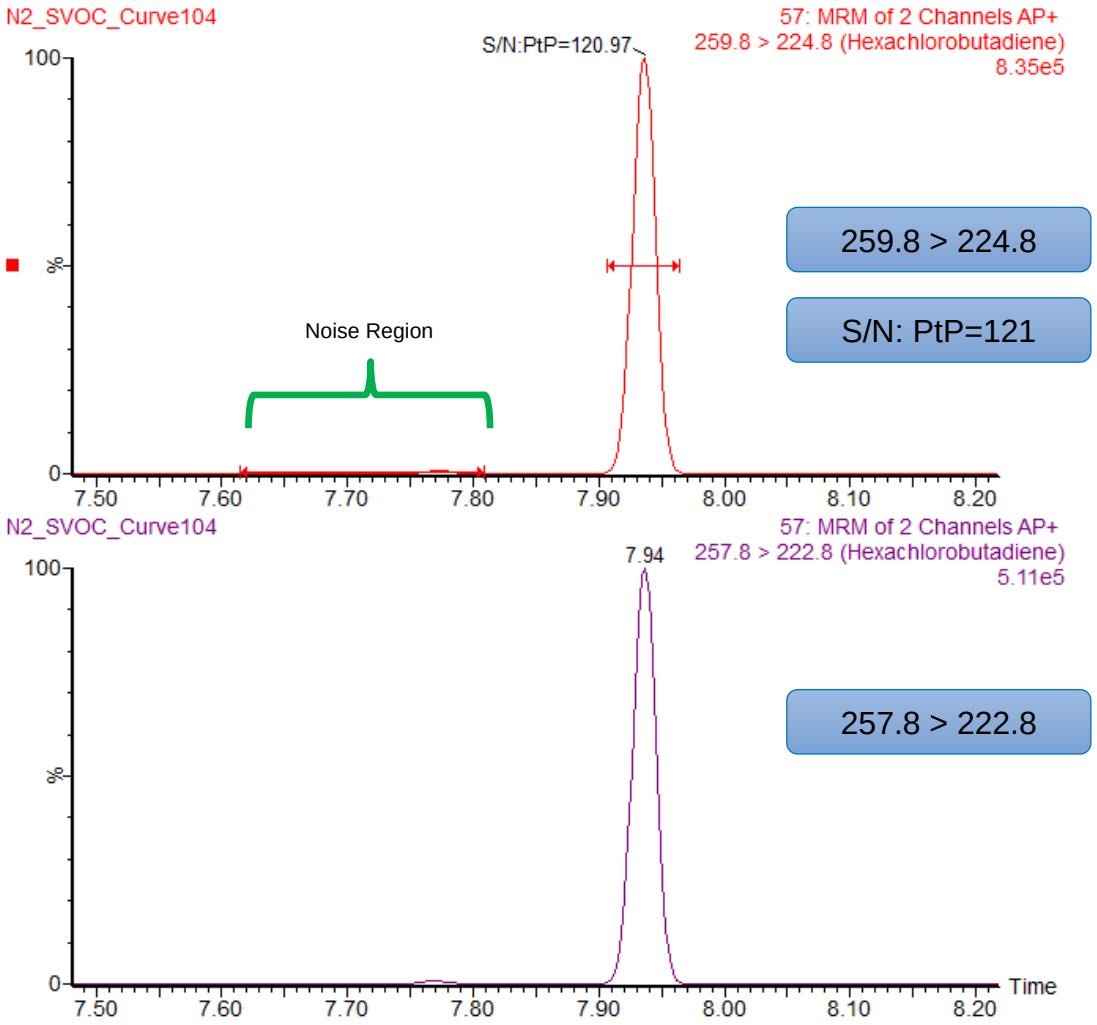
4-Chloroaniline at 5 pg/µL



Hexachlorobutadiene at 5 pg/µL



EPA 8270 SVOCs Analysis

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Analysed the Restek 8270 MegaMix Standard (cat.# 31850), containing 76 components, spiked with the SV Internal Standard Mix (cat.# 31206). Compounds fall into three categories:

Charge Transfer Only

1,2,4-Trichlorobenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, **1,4-Dichlorobenzene-d4**, 1-Methylnaphthalene, 2,3,4,6-Tetrachlorophenol, 2,3,5,6-Tetrachlorophenol, 2,4-Dichlorophenol, 2,4-Dimethylphenol, 2,4-Dinitrophenol, 2,4-Dinitrotoluene, 2-Chloronaphtalene, 2-Chlorophenol, 2-Methylnaphthalene, 2-Methylphenol, 2-Nitroaniline, 2-Nitrophenol, 3-Methylphenol, 3-Nitroaniline, 4-Bromophenyl phenyl ether, 4-Chloro-3-methylphenol, 4-Chloroaniline, 4-Chlorophenyl phenyl ether, 4-Methylphenol, 4-Nitroaniline, 4-Nitrophenol, Acenapthylene, Aniline, Benzyl alcohol, Bis(2-chloroethyl)ether, Carbazole, Dibenzofuran, Dimethyl phthalate, Hexachlorobenzene, Hexachlorobutadiene, Hexachlorocyclopentadiene, Hexachloroethane, Pentachlorophenol, Phenol, Pyridine, Trichlorophenol

Both Modes

Acenaphthene
Acenaphthene-d10
Anthracene
Benzo(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo[g,h,i]perylene
Chrysene, **Chrysene-d12**
Dibenz(a,h)anthracene
Fluoranthene, Fluorene
Indeno[1,2,3-cd]pyrene
Naphthalene, **Naphthalene-d8**
Perylene-d12
Phenanthrene
Phenanthrene-d10
Pyrene

Protonation Only

1,3-Dinitrobenzene
1,4-Dinitrobenzene
2,2'-oxybis(1-chloropropane)
2,6-Dinitrotoluene
4,6-Dinitro-2-methylphenol
Bis(2-chloroethoxy)methane
Bis(2-ethylhexyl) phthalate
Bis(2-ethylhexyl)adipate
Diethyl phthalate
Di-n-butyl phthalate
Di-n-octyl phthalate
Isophorone
Nitrobenzene
N-Nitroso-di-n-propylamine

Low Level Detection of Phenols

1 µl of 5 pg/µL standard = 500 fg on column

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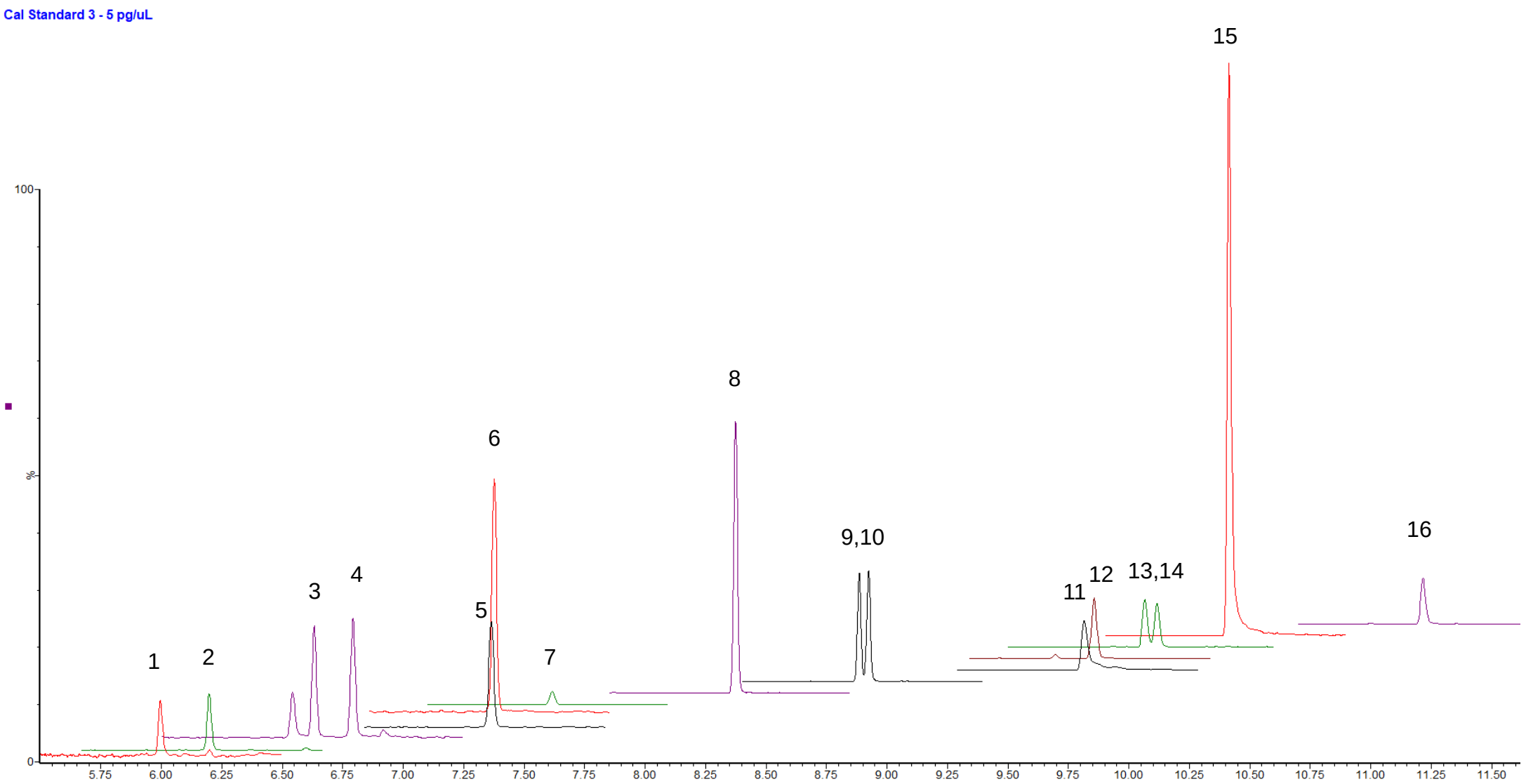
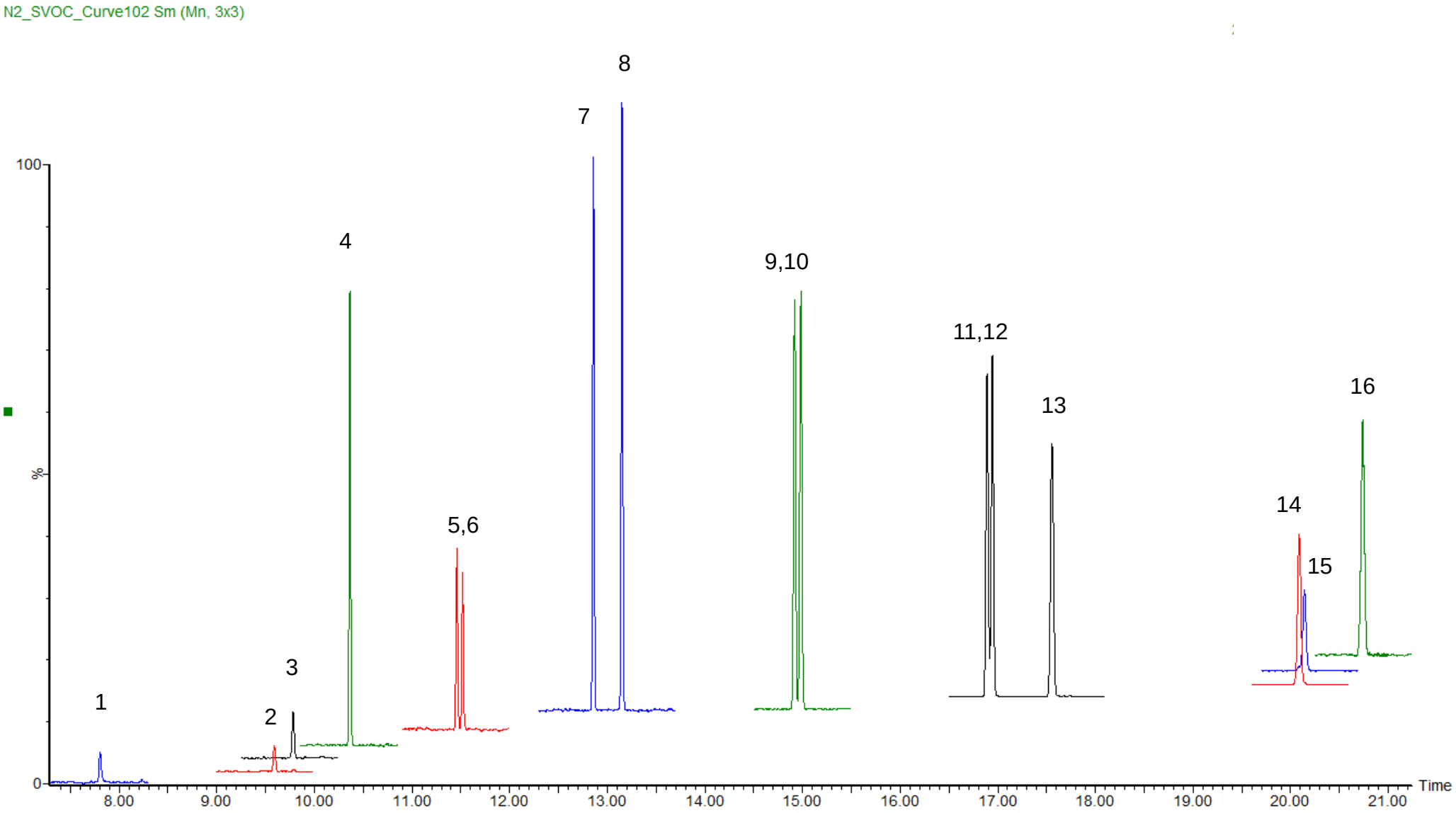


1	Phenol
2	2-Chlorophenol
3	2-Methylphenol
4	3-Methylphenol & 4-Methylphenol
5	2-Nitrophenol
6	2,4-Dimethylphenol
7	2,4-Dichlorophenol
8	4-Chloro-3-methylphenol
9	2,4,6-Trichlorophenol
10	2,4,5-Trichlorophenol
11	2,4-Dinitrophenol
12	4-Nitrophenol
13	2,3,5,6-Tetrachlorophenol
14	2,3,4,6-Tetrachlorphenol
15	4,6-Dinitro-2-methylphenol
16	Pentachlotophenol

Low Level Detection of PAHs

1 µl of 1 pg/µL standard = 100 fg on column



1	Naphthalene
2	Acenapthylene
3	Acenapthene
4	Fluorene
5	Phenanthrene
6	Anthracene
7	Fluoranthene
8	Pyrene
9	Benzo(a)anthracene
10	Chrysene
11	Benzo(b)flouranthene
12	Benzo(k)flouranthene
13	Benzo(a)pyrene
14	Indeno[1,2,3-cd] pyrene
15	Dibenz(a,h)anthracene
16	Benzo[g,h,i]perylene

Example Calibration Curves for Naphthalene

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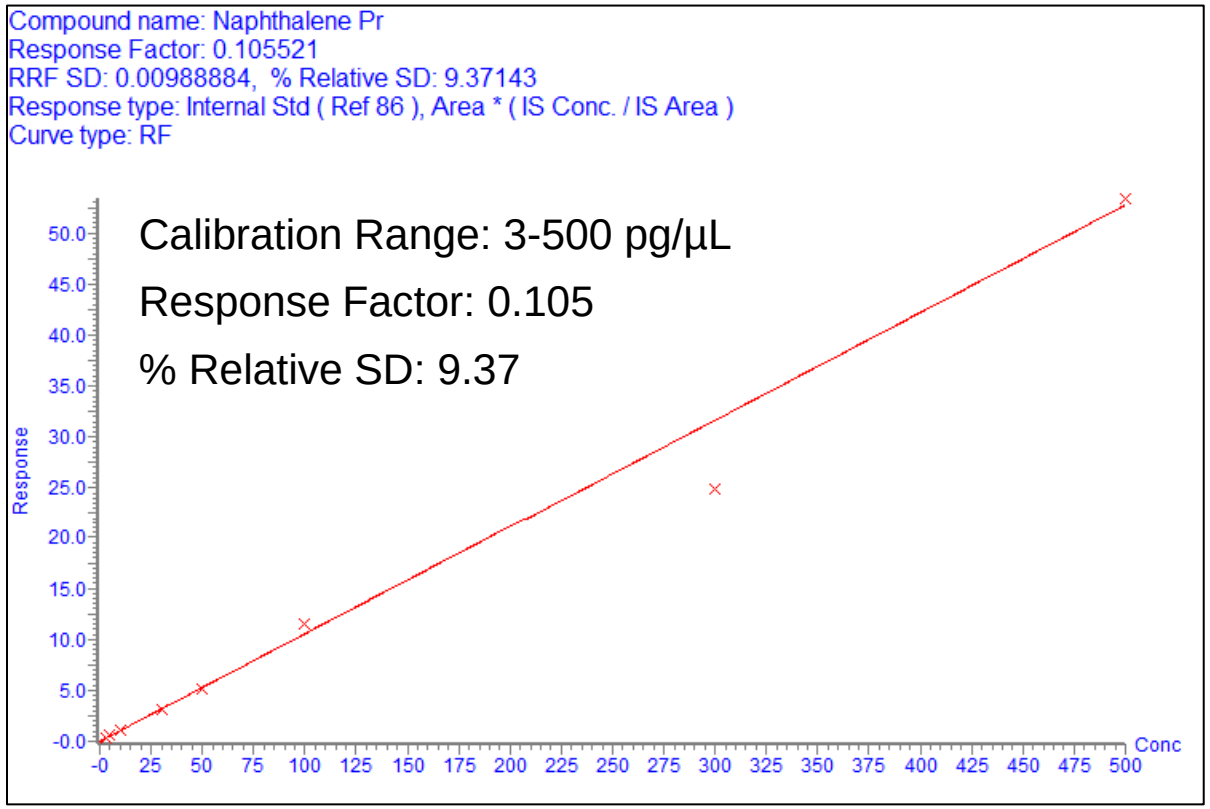
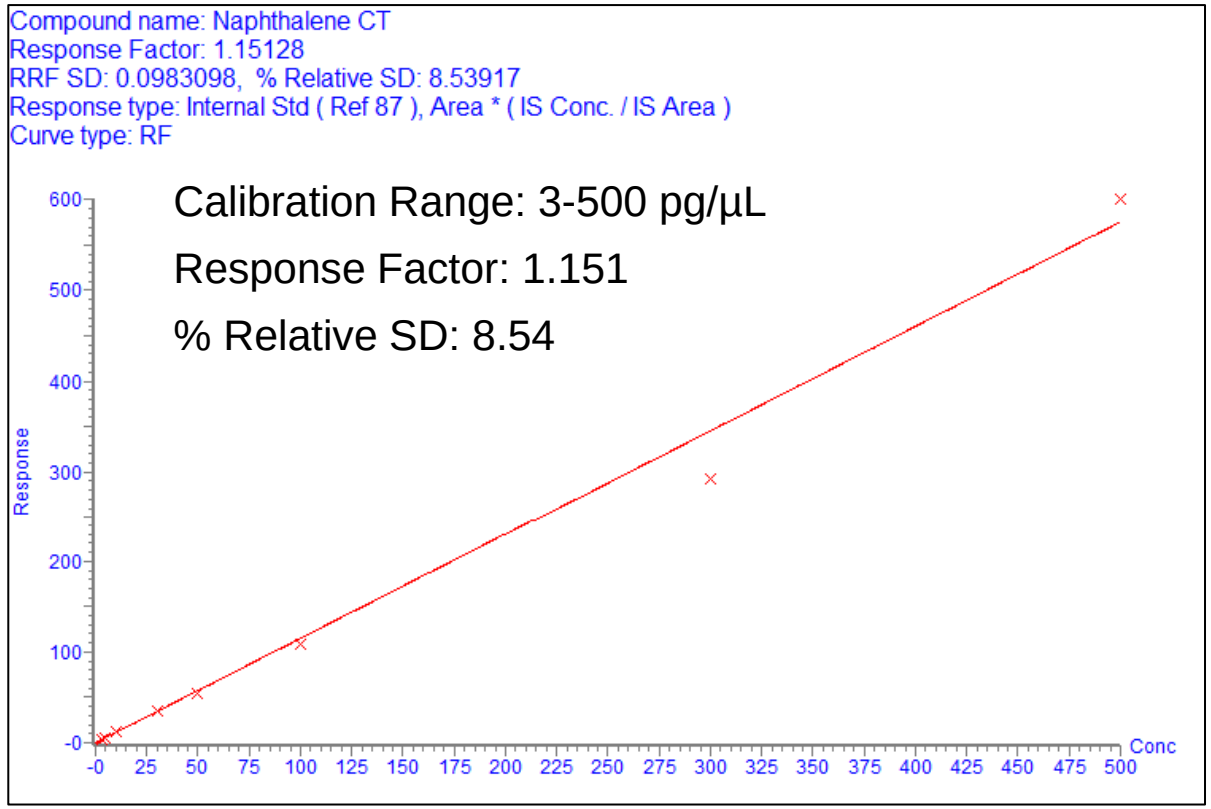
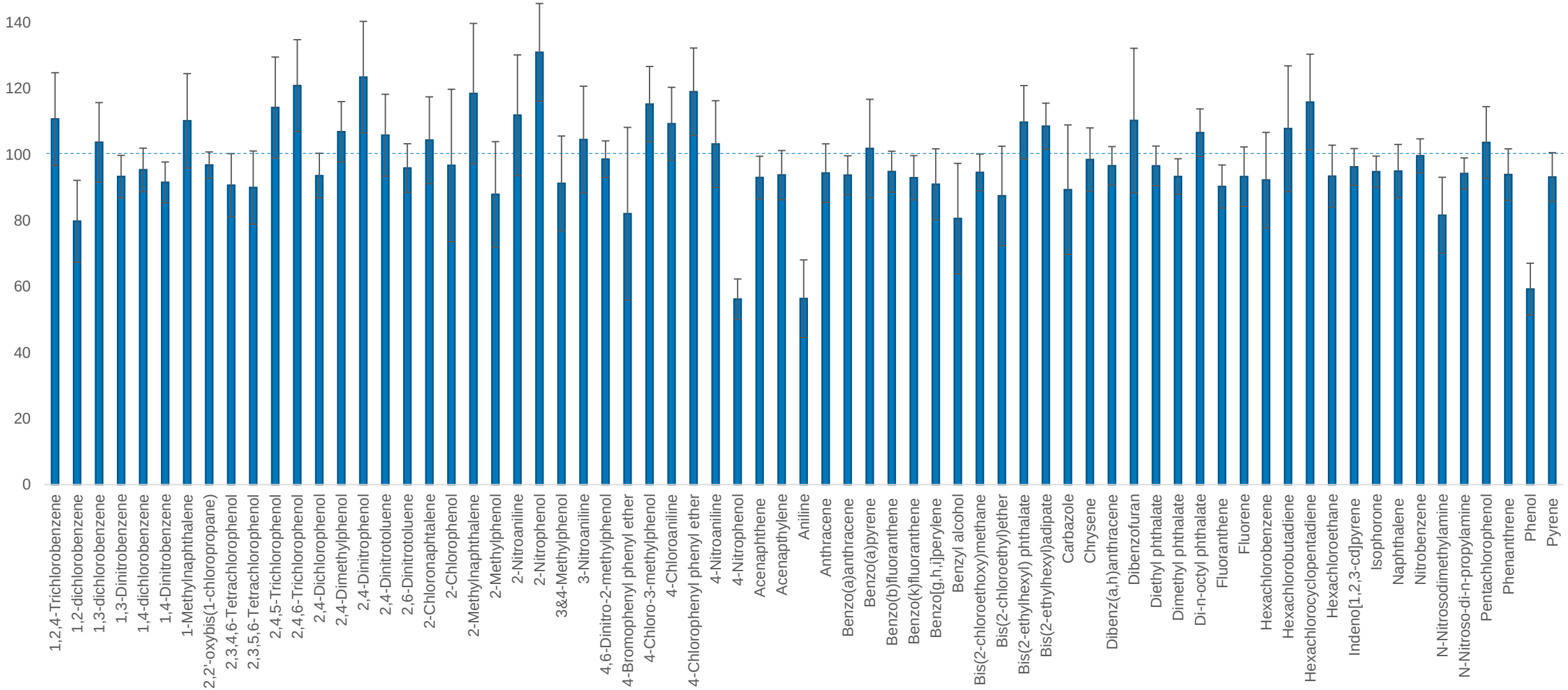
Naphthalene Protonation



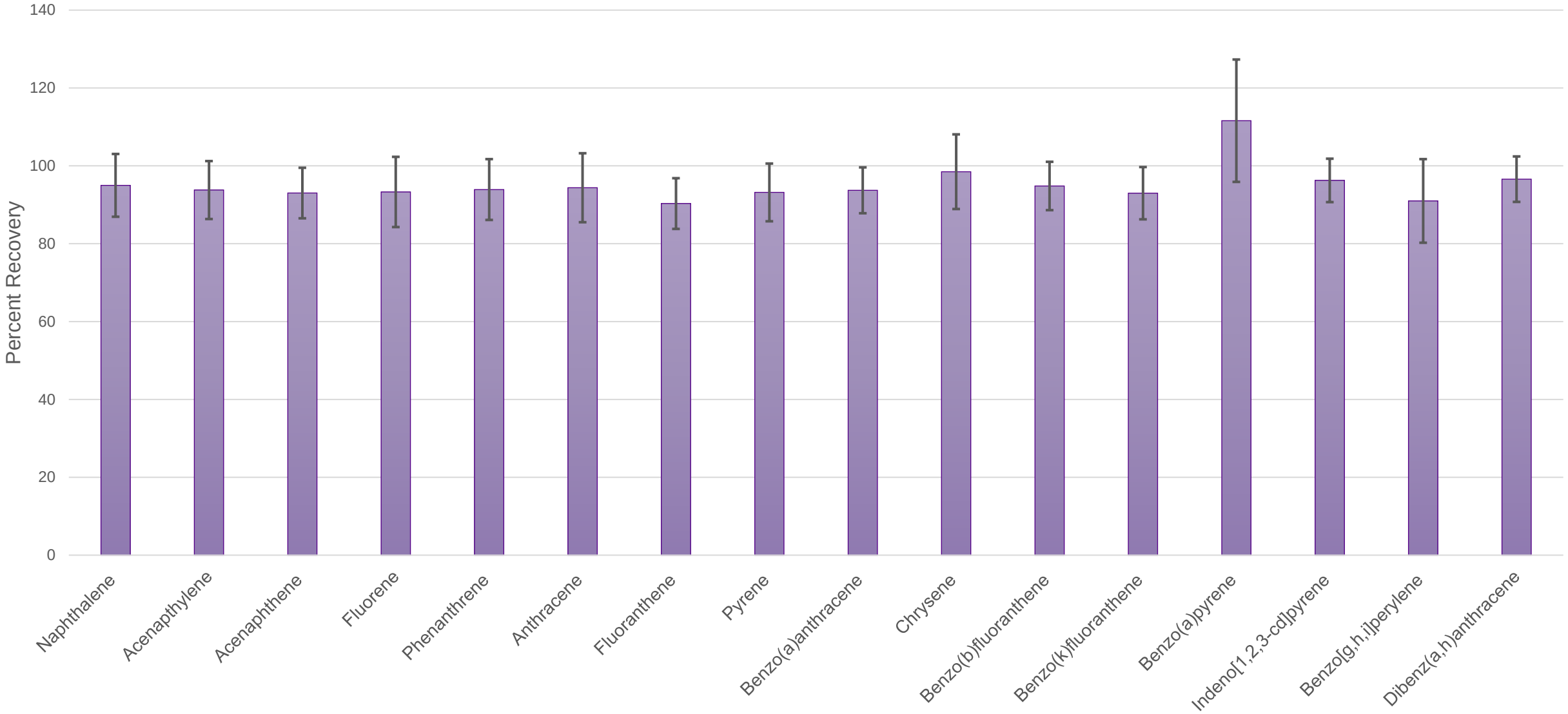
Napthalene Charge Transfer



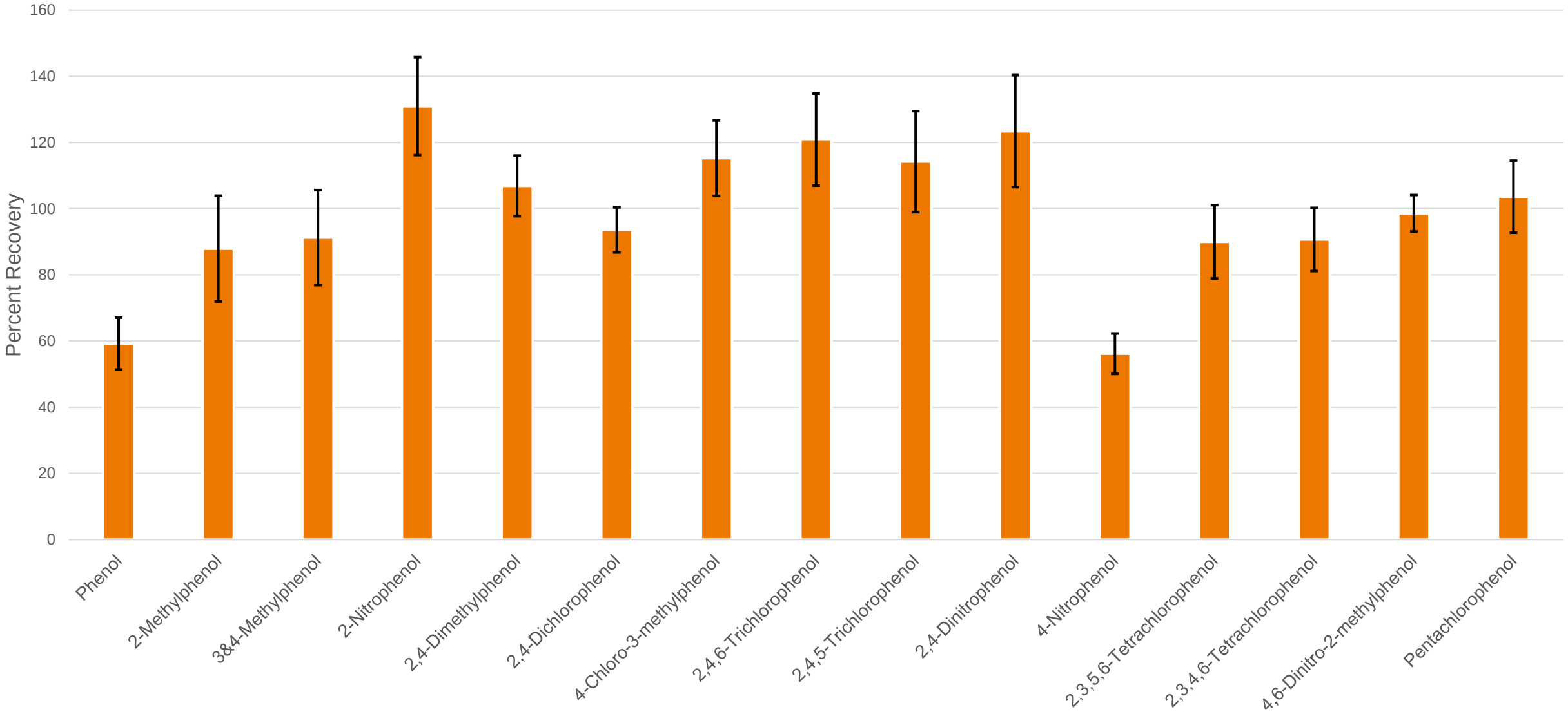
Recoveries – All but dibutyl phthalate (laboratory contaminant)



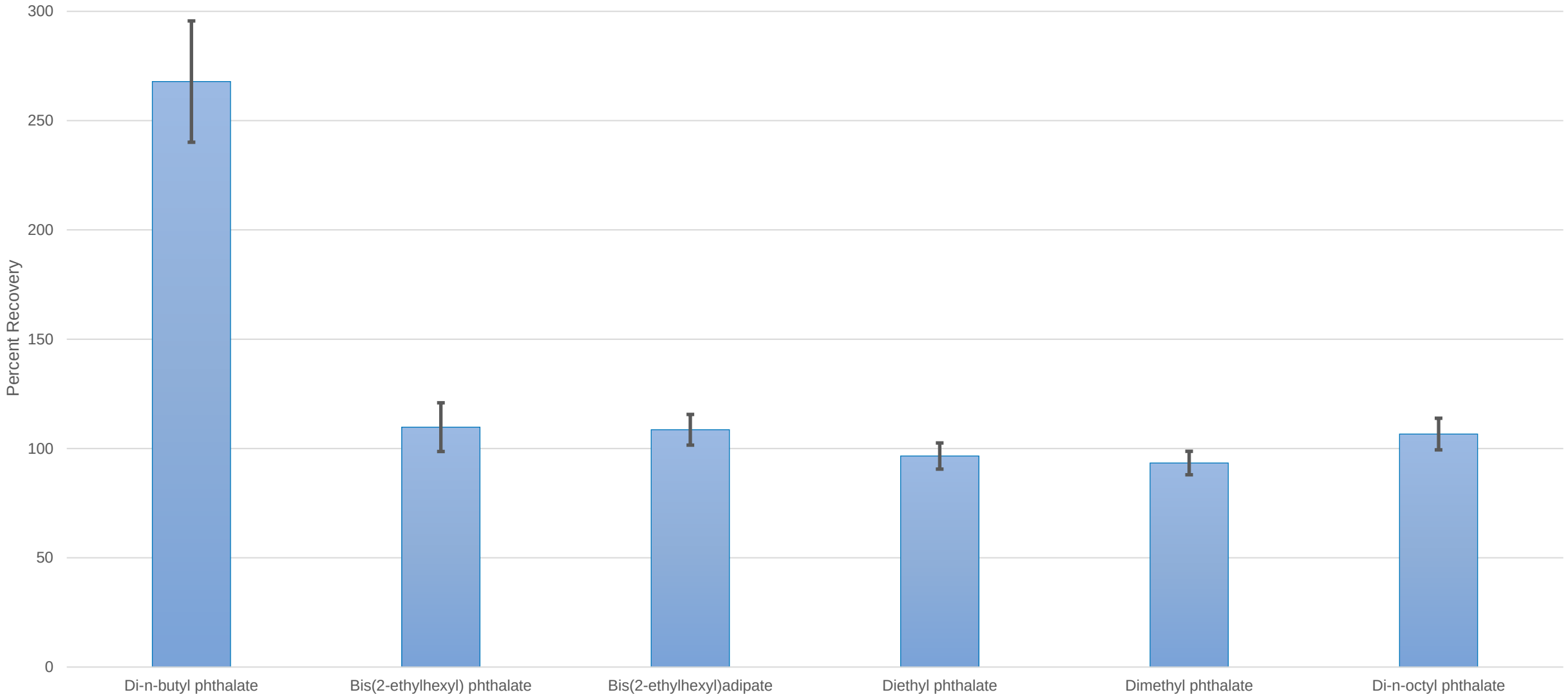
Recoveries: PAHs



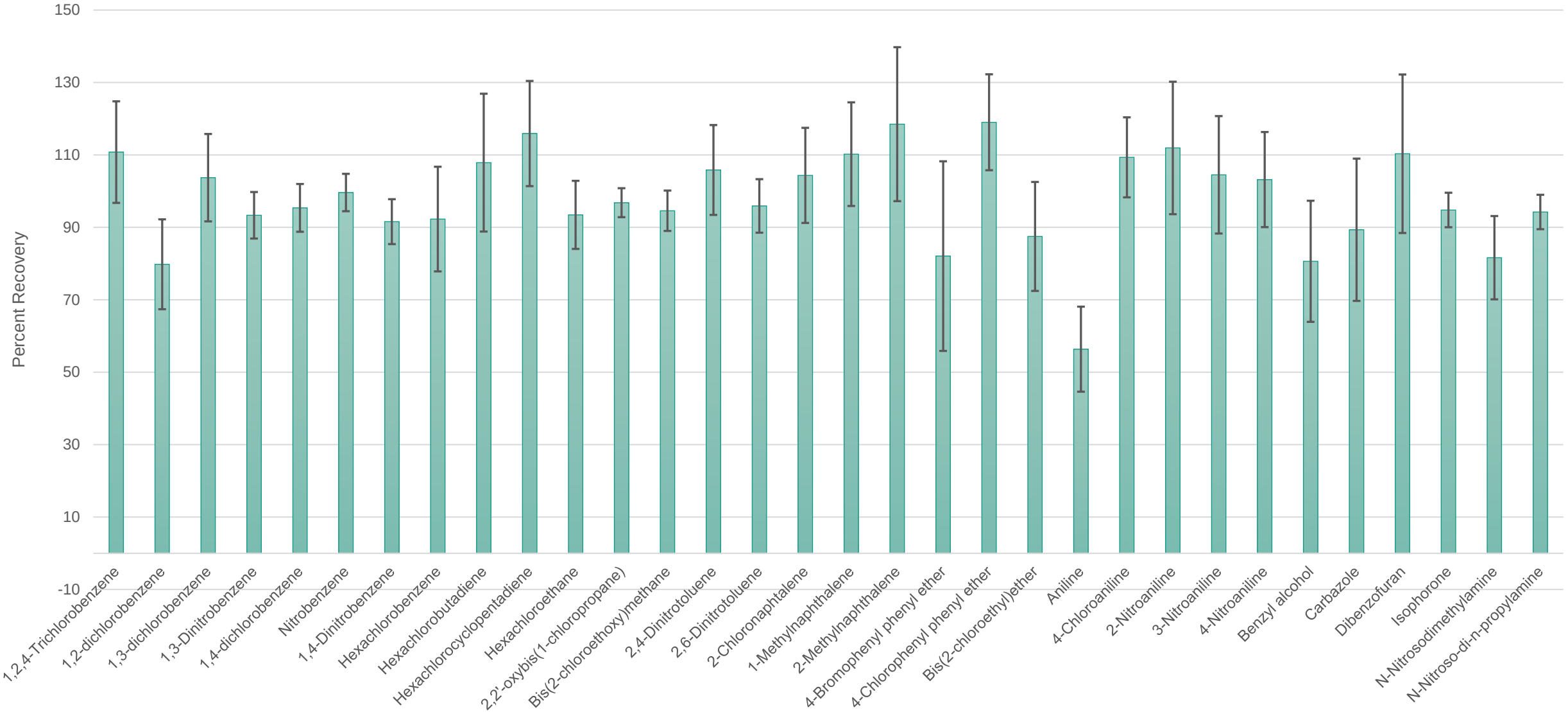
Recoveries: Phenols



Recoveries: Phthalates



Recoveries: All Other Compounds



Compounds with Average % Recovery <80% or >120%

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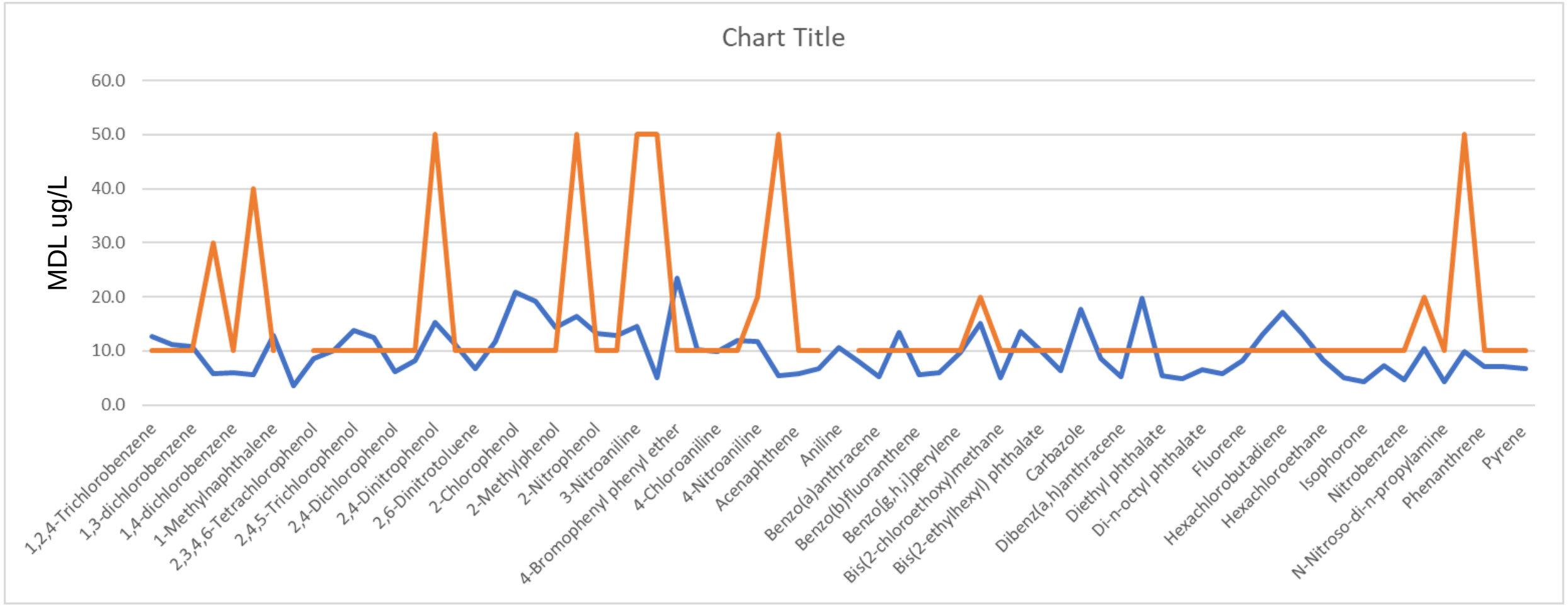
Compound Name	RRF %RSD	Average % Recovery	% Recovery Std Dev
Aniline	9.94	56.4	11.7
Phenol	17.59	59.2	7.8
1,2-Dichlorobenzene	23.47	79.8	12.4
2,4,6-Trichlorophenol	19.84	120.9	13.9
2,4-Dinitrophenol	18.15	123.4	16.9
2-Nitrophenol	19.3	131.0	14.8
Di-n-butyl phthalate	11.64	267.9	27.7

Method Detection Limit (MDL) Calculated



- Blue is Calculated Value
- Orange is USEPA Method 8270 Reference Value

Summary and Future Work

- The scaled sample prep showed a promising initial proof of performance
  - All compounds were detected in the n=8 replicate extracts
  - Only 7 of 73 compounds were outside the range of 80-120% recovery

- APGC can use N_2 as a carrier gas without seeing a loss in performance.
  - Scaled column dimensions allowed for a 22 min chromatographic method
  - The Rxi-SVOCms column had nice peak shape for phenolic compounds
  - Chromatographic performance was good enough to separate closely eluting isomers

- Additional testing will see if the sample preparation method can be improved:
  - Fewer extraction steps
  - Test with a single pH for extraction

Thank You for Your Attention

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