


Advances in sample preparation for semi-volatile analysis per EPA Method 625

Agilent Technologies, Inc.

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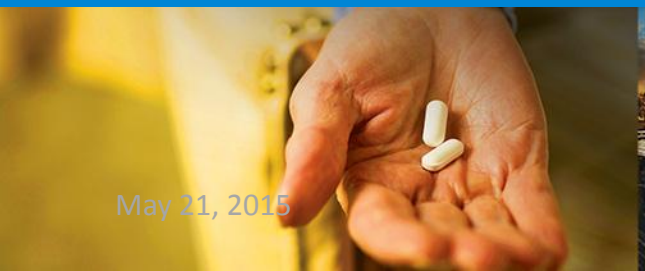


Outline

- Introduction – ILI Committee SPE Study for EPA625
- Solid Phase Extraction
- Round Robin Study – Phase II
- Future Work
- Summary and Conclusions

Revision to US EPA Method 625
ILI Committee Overview

Independent Laboratory Institute (ILI)

- a non-profit, 501(c)(3), multi-disciplined, member-driven scientific educational organization affiliated with American Council of Independent Laboratories (ACIL)
- coordinated EPA round robin study to evaluate application of solid phase extraction (SPE) to meet sample preparation requirements for EPA 625

Industry representatives

- Manufacturers of instrumentation, chemical standards and sample preparation product

Environmental testing laboratories

- Providers of contract analytical services involving EPA methodology

Revision to US EPA Method 625
Purpose of Study

Demonstrate efficacy of solid phase extraction (SPE) for inclusion in EPA625

Multi-laboratory, multi-vendor participation with EPA

Answer the following questions…

- Do the SPE products that failed in Phase I actually give acceptable results in the Phase I matrix?
- Do the SPE products give acceptable results in a second, challenging matrix
- Does the new suite of surrogates give a good indication of the measurement system performance?

Revision to US EPA Method 625
Round Robin Study

Manufacturers worked with laboratories to prepare samples and analyze per EPA 625

Test Criteria:

- Performance test in synthetic wastewater (triplicate analysis)
- Performance test in drinking water (i.e., Aquafina bottled water; triplicate analysis)
- LCS in laboratory reagent water (quadruple analysis)
- Wastewater, bottled water, and reagent water blanks
- MDL/LOQ check at three levels in reagent water

Twenty-five commercial, government, and academic laboratories participated in this study, many of which had no prior experience with Solid Phase Extraction or with the use of the particular SPE product that they were using to analyze the samples

Revision to US EPA Method 625 Target Analytes

Base/Neutrals [1]	µg/L
Acenaphthene	10 to 200
Acenaphthylene	10 to 200
Anthracene	10 to 200
Benzo(a)anthracene	10 to 200
Benzyl butyl phthalate	50 to 200
Benzo(b)fluoranthene	20 to 200
Benzo(k)fluoranthene	20 to 200
Benzo(g,h,i)perylene	10 to 200
Benzo(a)pyrene	10 to 200
4-Bromophenyl-phenylether	20 to 200
bis(2-Chloroethoxy)methane	20 to 200
bis(2-Chloroethyl)ether	20 to 200
bis(2-Chloroisopropyl) ether[18]	30 to 200
Bis(2-ethylhexyl) phthalate	20 to 200
4-Chlorophenyl-phenylether	20 to 200
2-Chloronaphthalene	20 to 200
Chrysene	10 to 200
Dibenzo(a,h)anthracene	20 to 200
Dibenzofuran	30 to 200
1,2-Dichlorobenzene	20 to 200
1,3-Dichlorobenzene	20 to 200

Base/Neutrals [1]	µg/L
1,4-Dichlorobenzene	20 to 200
3,3'-Dichlorobenzidine	50 to 200
Diethyl phthalate	50 to 200
Dimethyl phthalate	50 to 200
Di-n-butyl phthalate	40 to 200
2,4-Dinitrotoluene	20 to 200
2,6-Dinitrotoluene	20 to 200
Di-n-octyl phthalate	30 to 200
Fluoranthene	30 to 200
Fluorene	10 to 200
Hexachlorobenzene	20 to 200
Hexachlorobutadiene	50 to 200
Hexachlorocyclopentadiene	50 to 200
Hexachloroethane	50 to 200
Indeno(1,2,3, cd)pyrene	30 to 200
Isophorone	20 to 200
2-Methylnaphthalene	20 to 200
Naphthalene	20 to 200
Nitrobenzene	20 to 200
N-Nitrosodimethylamine	75 to 200
N-Nitroso-di-n-propylamine	30 to 200
N-Nitrosodiphenylamine	30 to 200
Phenanthrene	10 to 200
Pyrene	10 to 200
1,2,4-Trichlorobenzene	20 to 200

Acids [1]	µg/L
4-Chloro-3-methylphenol	30 to 200
2-Chlorophenol	30 to 200
2,4-Dichlorophenol	30 to 200
2,6-Dichlorophenol	30 to 200
2,4-Dimethylphenol	40 to 200
2,4-Dinitrophenol	100 to 200
2-Methyl-4,6-Dinitrophenol	40 to 200
2-Methylphenol (o-Cresol)	40 to 200
4-Methylphenol (p-Cresol) [14]	50 to 200
2-Nitrophenol	50 to 200
4-Nitrophenol	100 to 200
Phenol	100 to 200
Pentachlorophenol	40 to 200
2,4,5-Trichlorophenol	30 to 200
2,4,6-Trichlorophenol	30 to 200

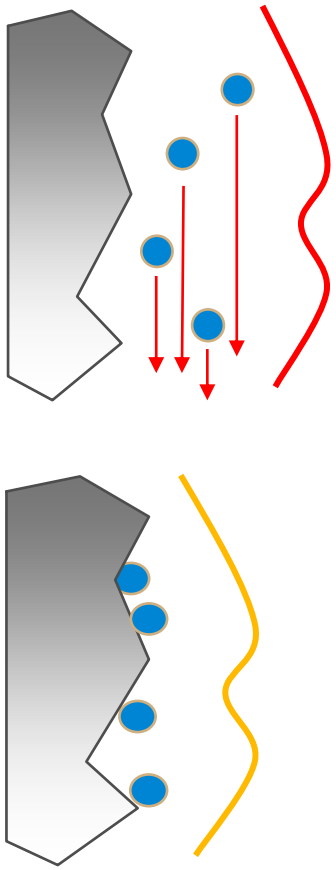
Surrogates	Conc
Acenaphthylene-d8 Anthracene-d10 Benzo(a)pyrene-d12 Bis-(2-chloroethyl)ether-d8 4-Chloroaniline-d4 2-Chlorophenol-d4 2,4-Dichlorophenol-d3 Dimethylphthalate-d6 4,6-Dinitro-2-methylphenol-d2 Fluorene-d10 4-Methylphenol-d8 Nitrobenzene-d5 2-Nitrophenol-d4 4-Nitrophenol-d4 Phenol-d5 Pyrene-d10 N-Nitrosodimethylamine-d6 (NDMA-d6)	50 µg/L

Solid Phase Extraction (SPE)
Basic Chromatography Theory

SPE is not much different than HPLC, it is still chromatography and the same rules apply.

If the analyte has a higher affinity for the mobile phase than the stationary phase, the analyte will migrate through the cartridge with little interaction with the stationary phase (no retention).

If the analyte has increased affinity for the stationary phase, it will adsorb to the cartridge depending on the extent of the interaction (retention).



Solid Phase Extraction (SPE)
Relationship Between SPE and HPLC

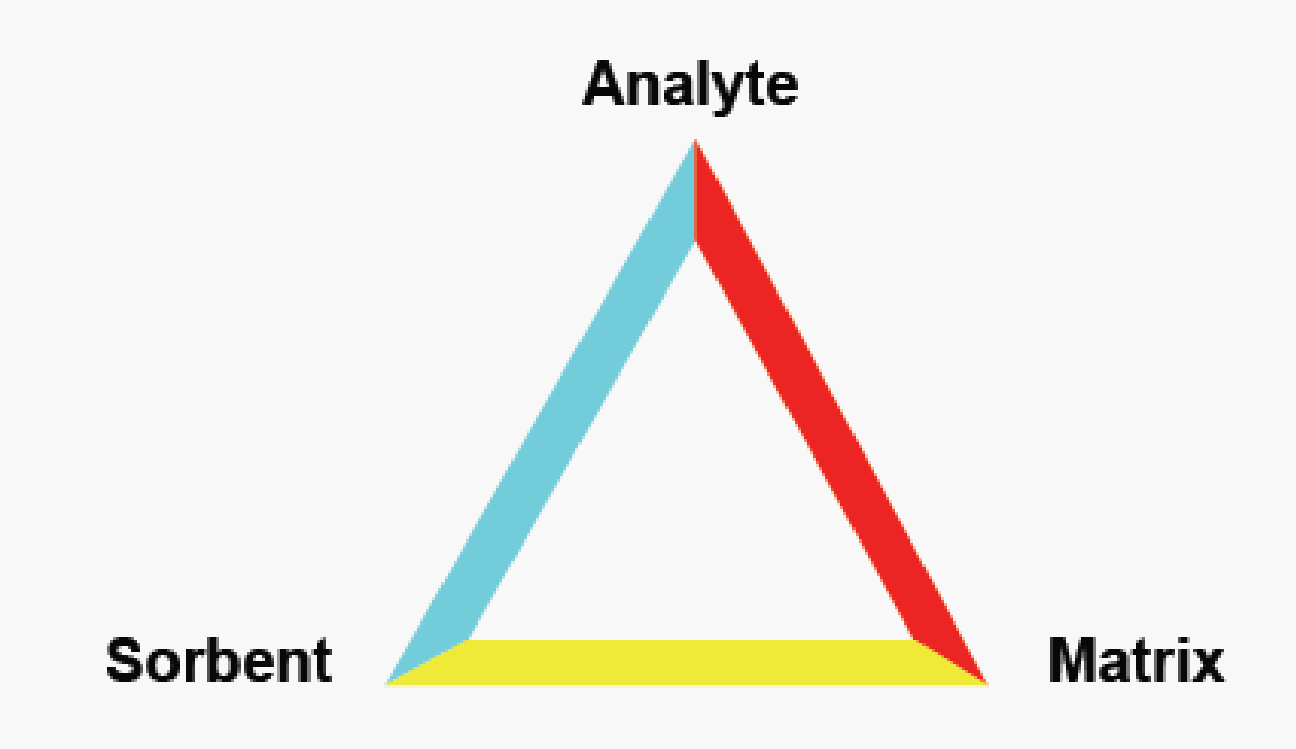
Elution chromatography is defined by the equation

$$0.2 << K << 20$$

Digital chromatography lies outside this range:

Retention	ON	K = [stationary phase]/[matrix] > 1000
Elution	OFF	K = [stat. phase]/[elution solvent] < 0.001

Solid Phase Extraction (SPE)
The SPE Triangle



Often, both the the analyte and the matrix are known, it is sorbent choice which is the critical component

Solid Phase Extraction (SPE)
Why it works

SPE works because:

- Oil And Water Do Not Mix, Like Dissolves Like
  - Reversed phase
    - Van der Waals or hydrophobic interactions
  - Normal Phase
    - Polar interactions (hydrogen bonding and dipole-dipole)
- Opposites Charges Attract
  - Ion Exchange
    - Electrostatic interactions

Phase selection is determined by these rules

Solid Phase Extraction (SPE)
Mechanisms of SPE

Hydrophobic

- Non-polar phases (reversed phase)

Polar

- Polar phases (normal phase)

Electrostatic

- Ion exchange phases

Mixed mode

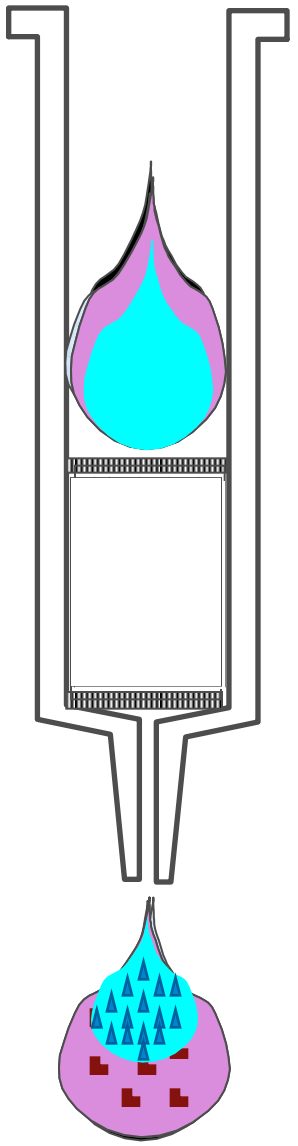
- Can also introduce secondary interactions

Solid Phase Extraction (SPE)
Four Steps of SPE

- **Conditioning**: Preparation of the sorbent prior to sample addition
- **Retention**: Analytes of interest and other interferences adsorb onto the surface of the sorbent during sample addition
- **Washing**: Elimination of undesired interferences
- **Elution**: Selective desorption and collection of desired analytes from the sorbent

Solid Phase Extraction (SPE)
The SPE Sequence

- Wet the cartridge (Step 1)
- Apply sample (e.g. food extract, water, plasma) (Step 2)
- Some compounds “retain”
- First wash of the cartridge, interference removal (Step 3a)
- Second wash of the cartridge, additional interference removal (Step 3b)
- Apply a different liquid to “elute” (Step 4)
- The extract is cleaner, in a different liquid and usually concentrated

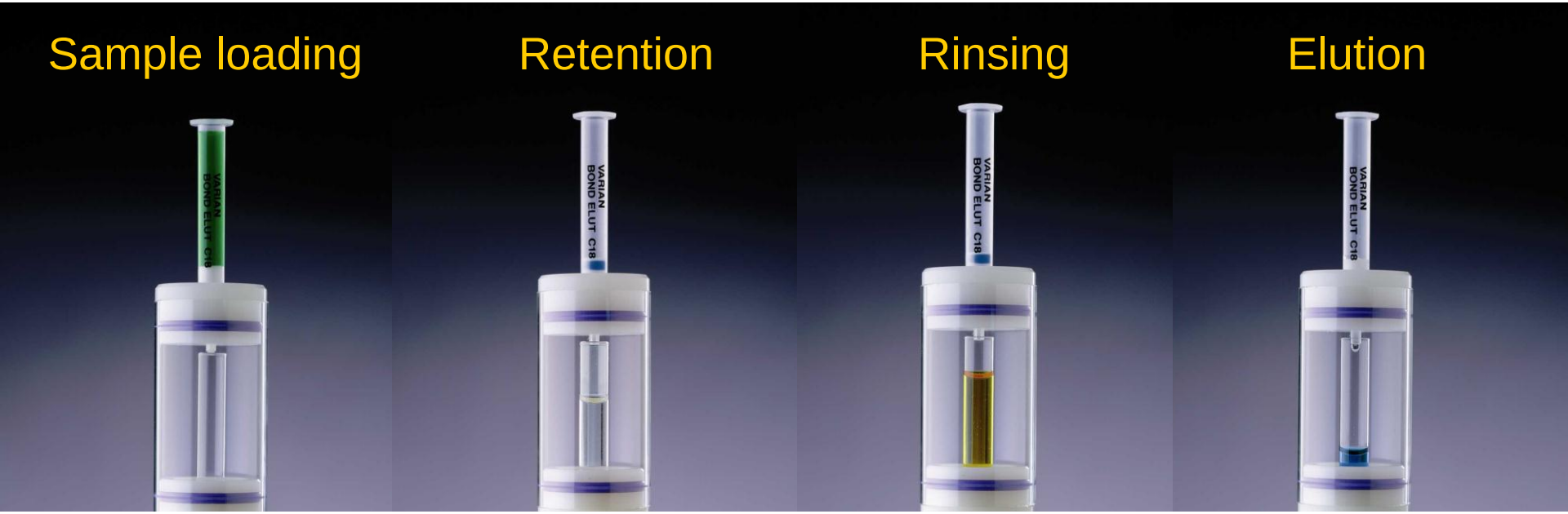


Four Steps of SPE
Selective Elution

Green = Blue and Yellow

Blue is more non polar than yellow

Blue is retained



EPA 625.1 Update
Sample Preparation

Sample Pretreatment: 250 mL TCLP acetate buffer solution; bring to pH 2 with sulfuric acid.

↓

SPE: Bond Elut ENV – 6 mL, 500 mg
Bond Elut C18 – 6 mL, 1000 mg

↓

Condition: 5 mL DCM, 5 mL MeOH, 5 mL water

↓

Load: Pass through cartridge bed at 10 mL/min; dry for 2 min

↓

Elution: 2 x 4 mL DCM, 1 x 4 mL ethyl acetate; dry over sodium sulfate

↓

Evaporation and Reconstitution: Reconstitute in 0.5 mL DCM

GC Parameters

Working to get specific conditions.

EPA 625: Base/Neutrals and Acids by GC/MS

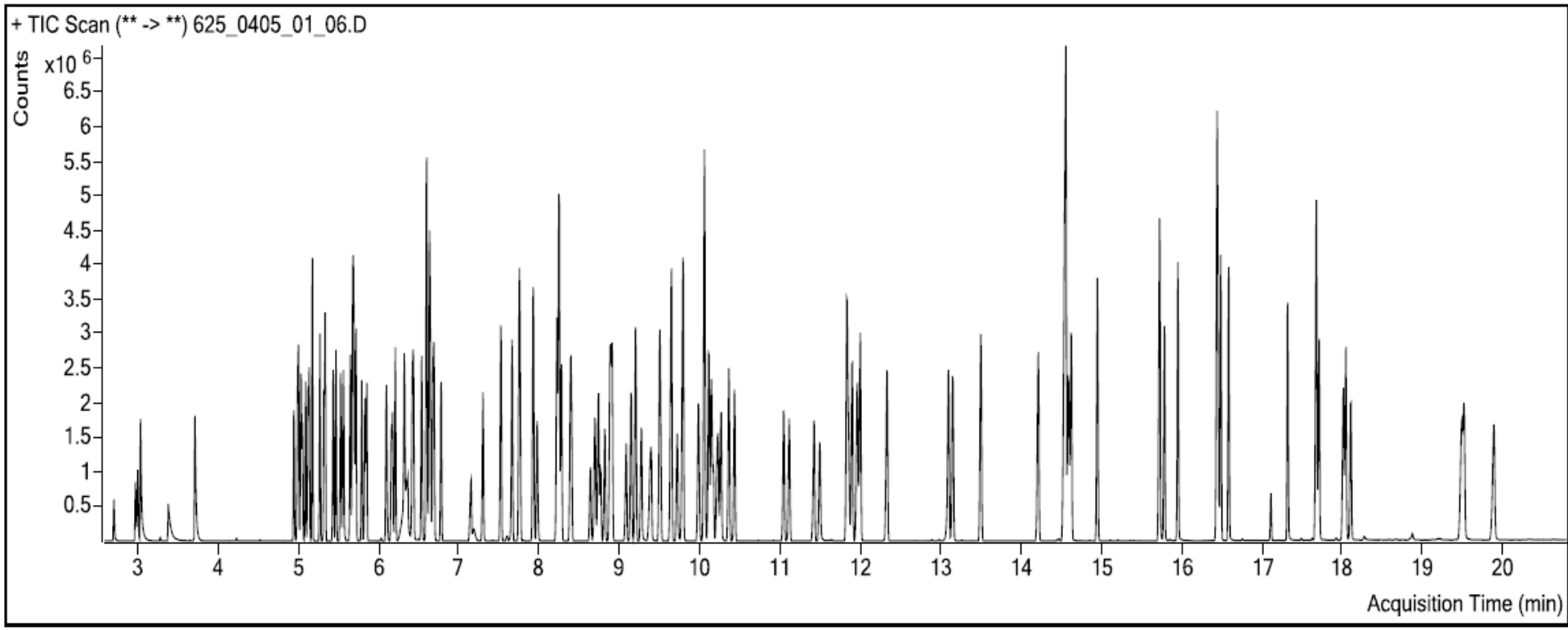
Run on Agilent 7000B GC/QQQ in MRM mode

- Multi-mode injector
- 2mm dimpled liner (PN: 5190-2296)
- Dual 15 m DB-5 columns with mid-point backflush

Offline SPE evaluation of 2 products

- Bond Elut C-18, 6 mL, 1000 mg (silica)
- Bond Elut ENV, 6 mL, 500 mg (polymeric)

Revision to US EPA Method 625
GC-MS (SIM) Chromatogram



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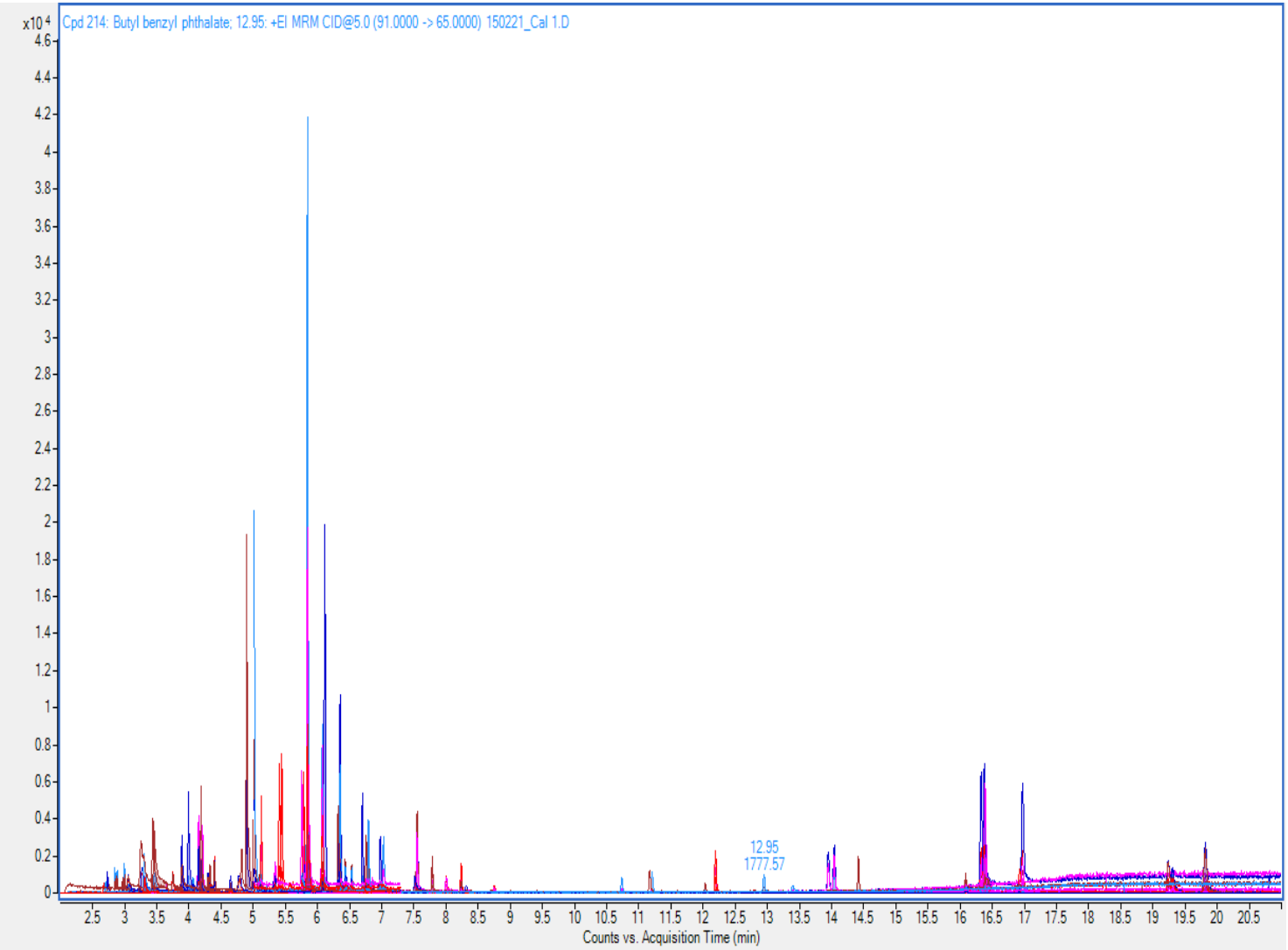
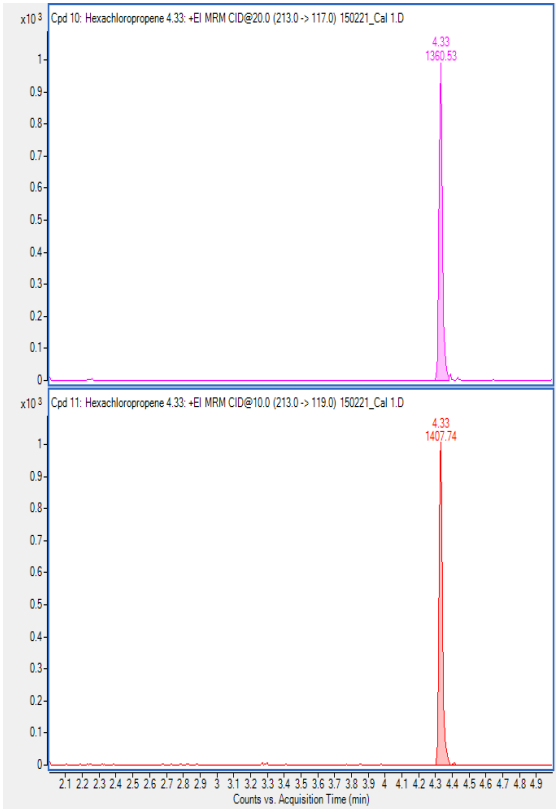
Chromatograms – Cal 1 @ 78 ng/mL

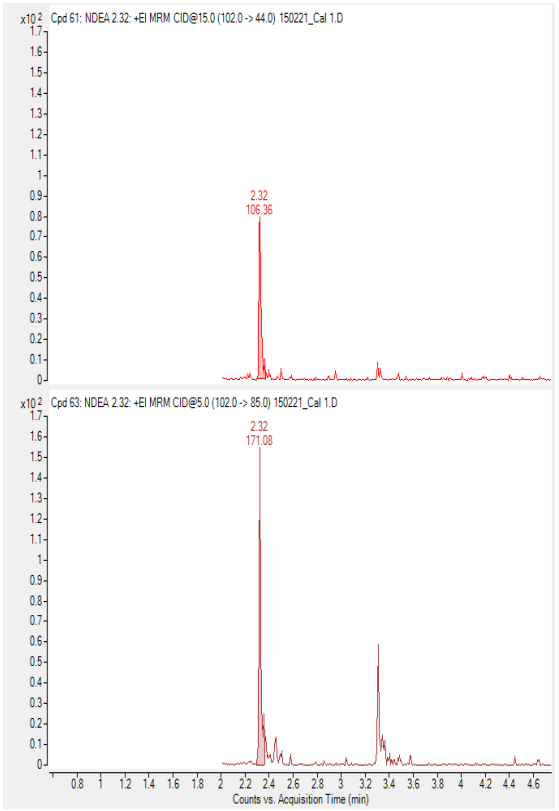


Revision to US EPA Method 625

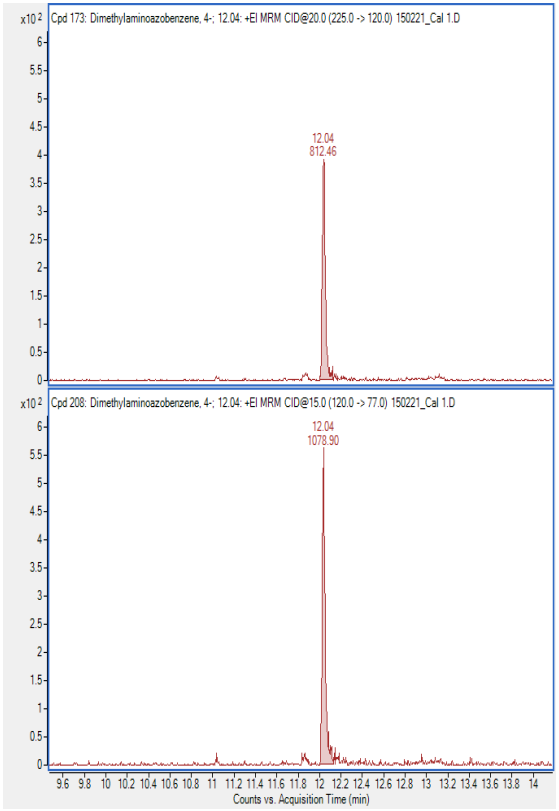
Chromatograms – Cal 1 Compounds @ 78 ng/mL



Hexachloropropene



N-Nitrosodiethylamine



Dimethylaminobenzene

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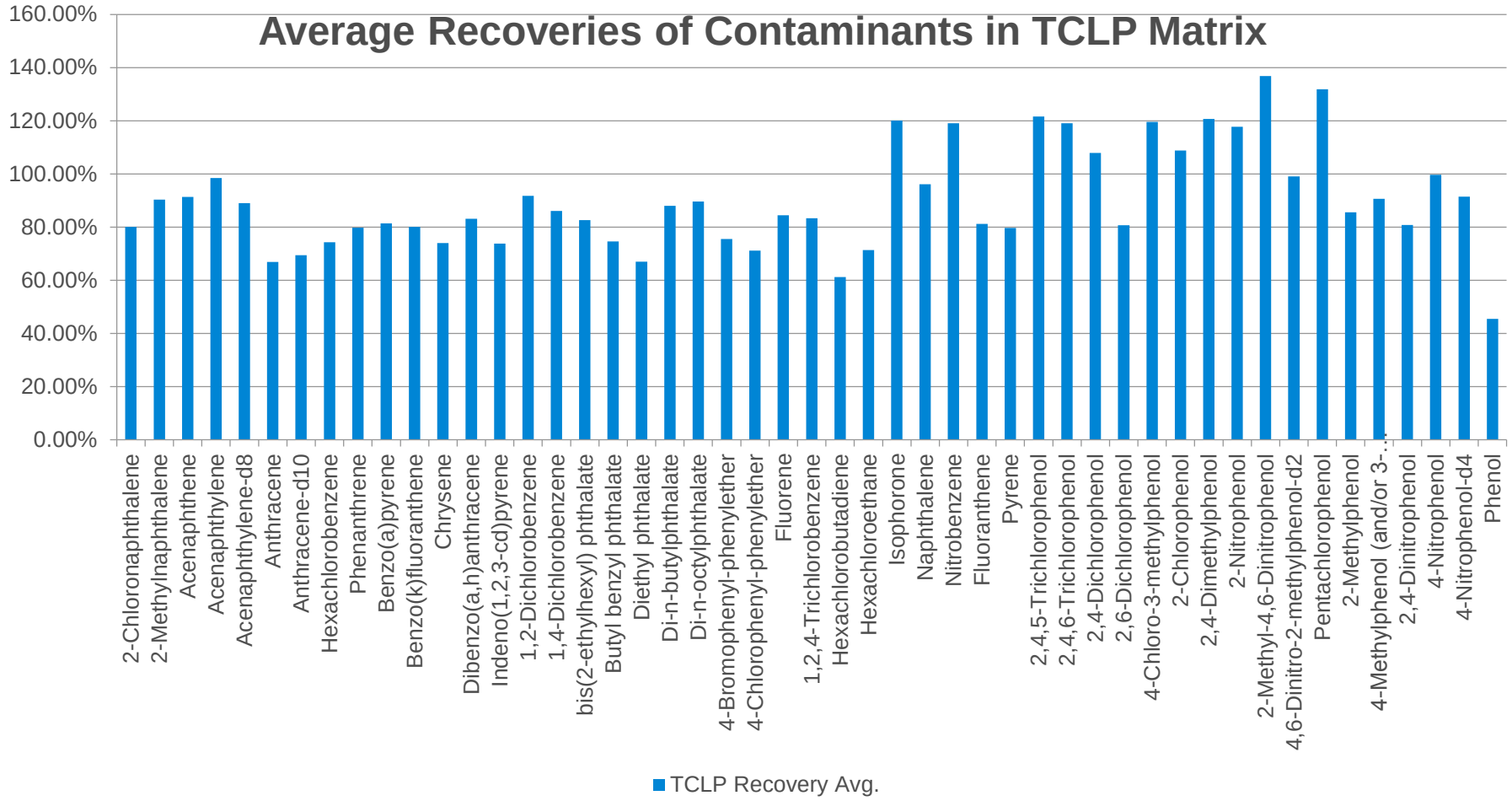
Recovery by Analyte, Bond Elut ENV – Laboratory Average



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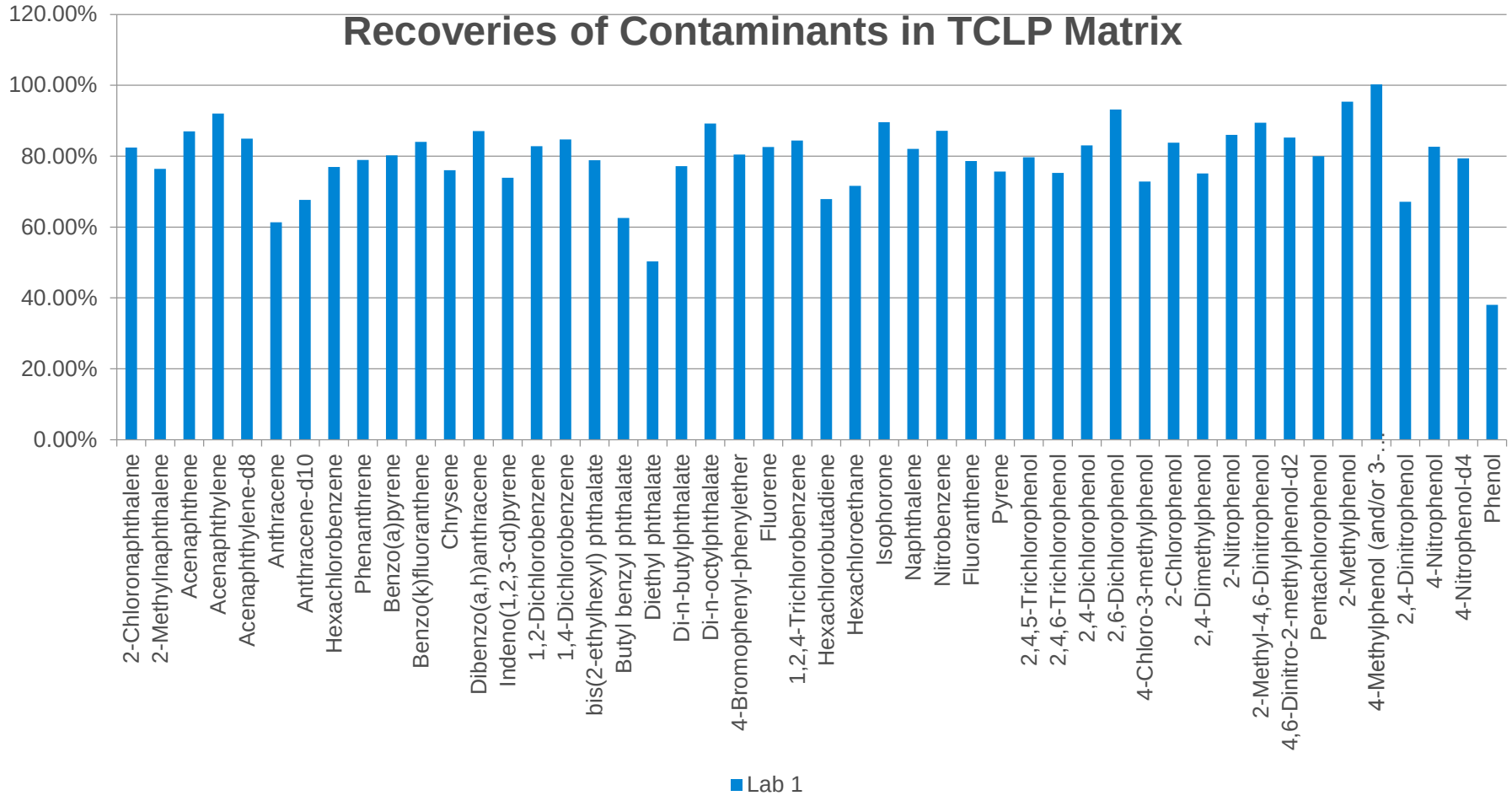
Recovery by Analyte, Bond Elut ENV – Laboratory 1 (n = 3)



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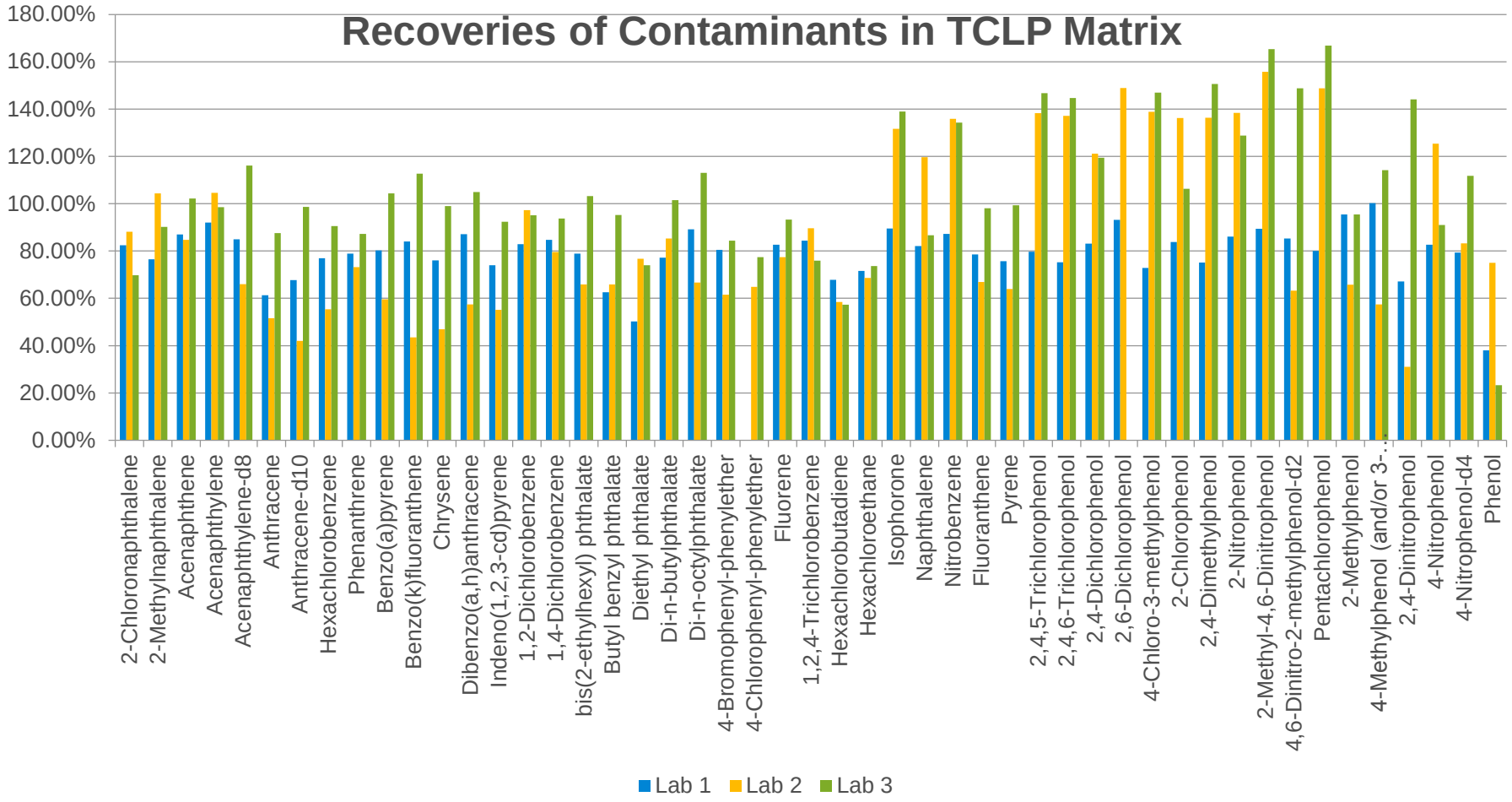
Recovery by Analyte, Bond Elut ENV – By Laboratory (n = 3)



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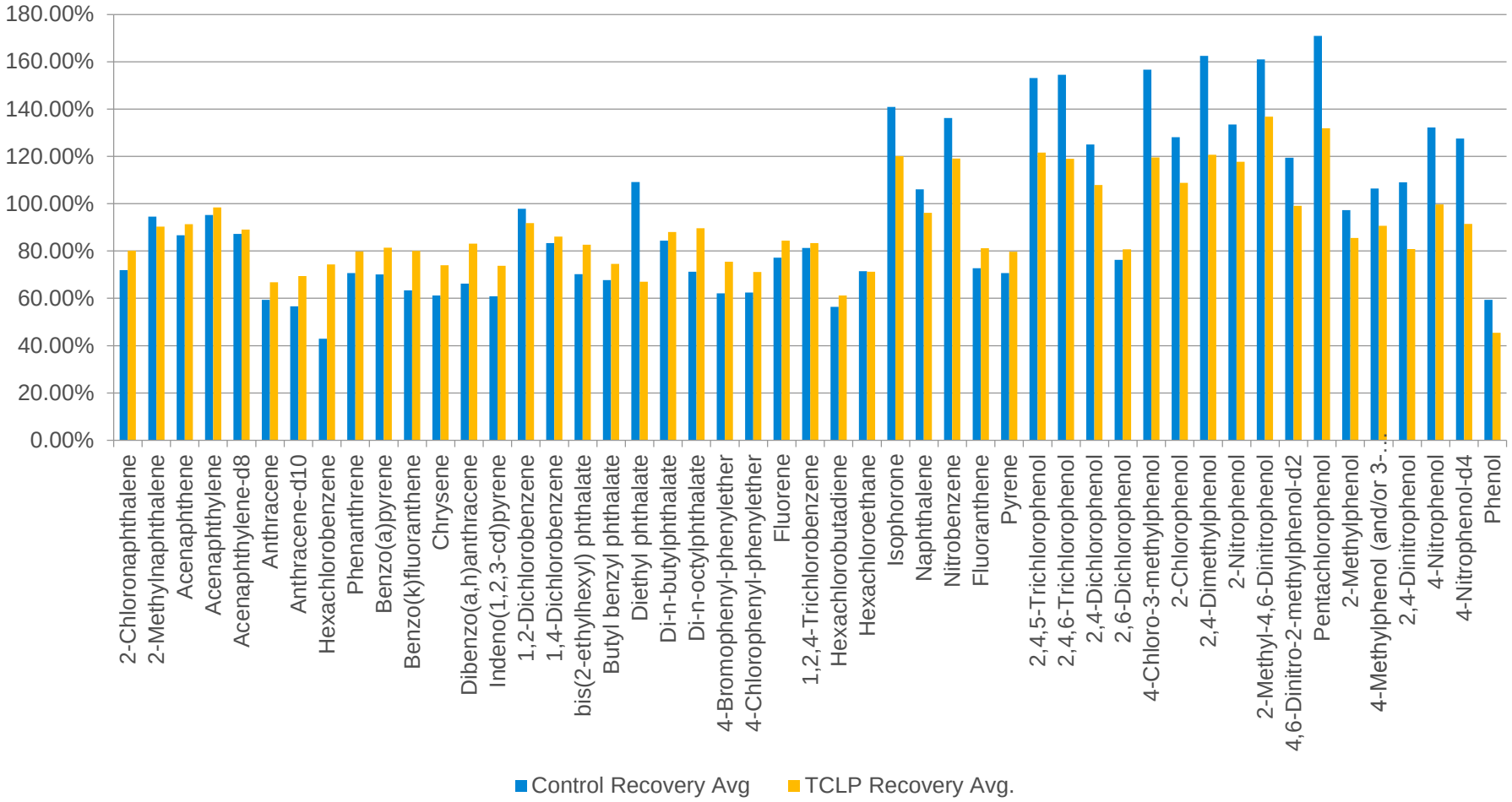
Recovery by Analyte, Bond Elut ENV – LCS versus TCLP Matrix



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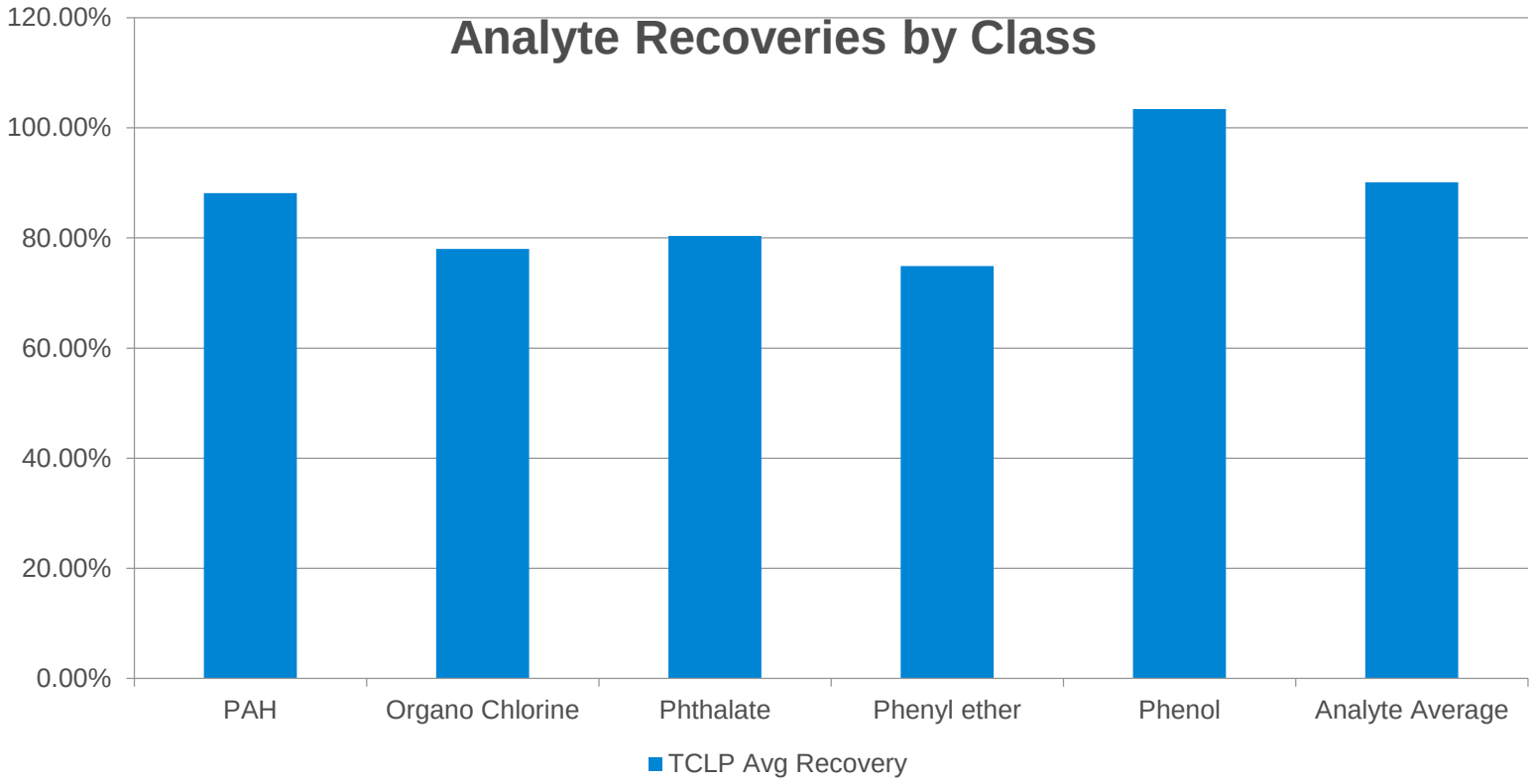
Recovery by Class, Bond Elut ENV – Laboratory Average



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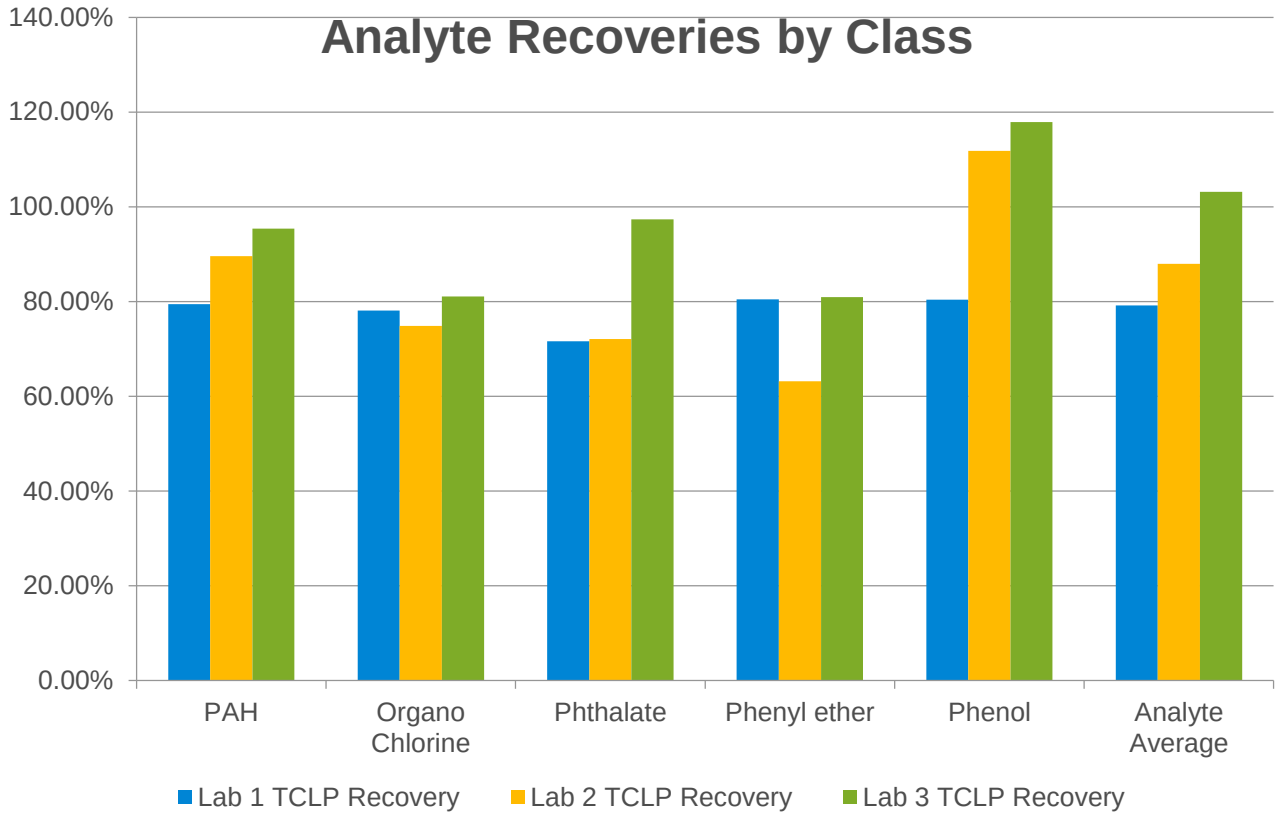
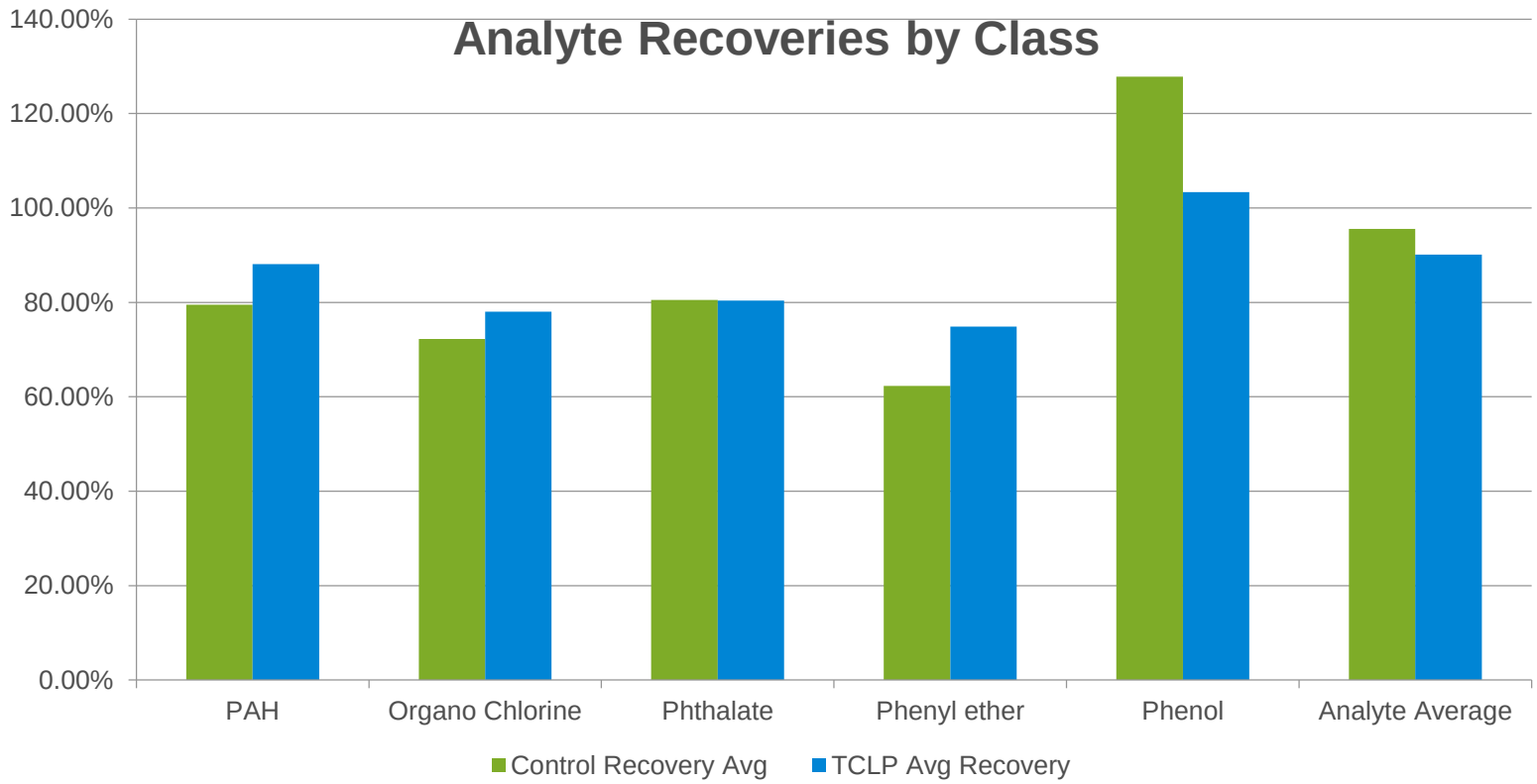
Recovery by Class, Bond Elut ENV – By Laboratory



Recovery by Class, Bond Elut ENV – Control Avg. Versus TCLP Avg.



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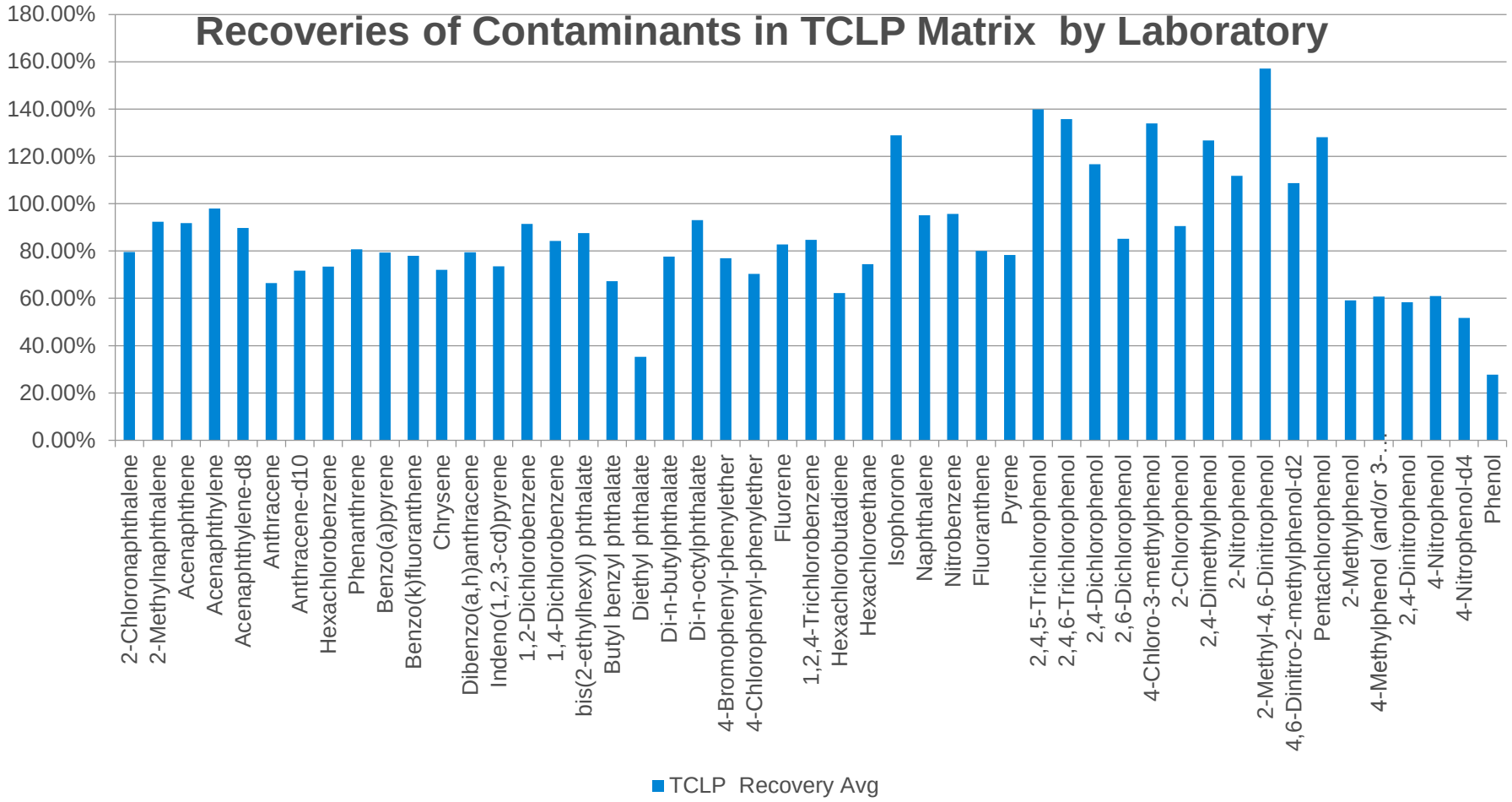
Recovery by Analyte, Bond Elut C18 – Laboratory Average (n = 3)



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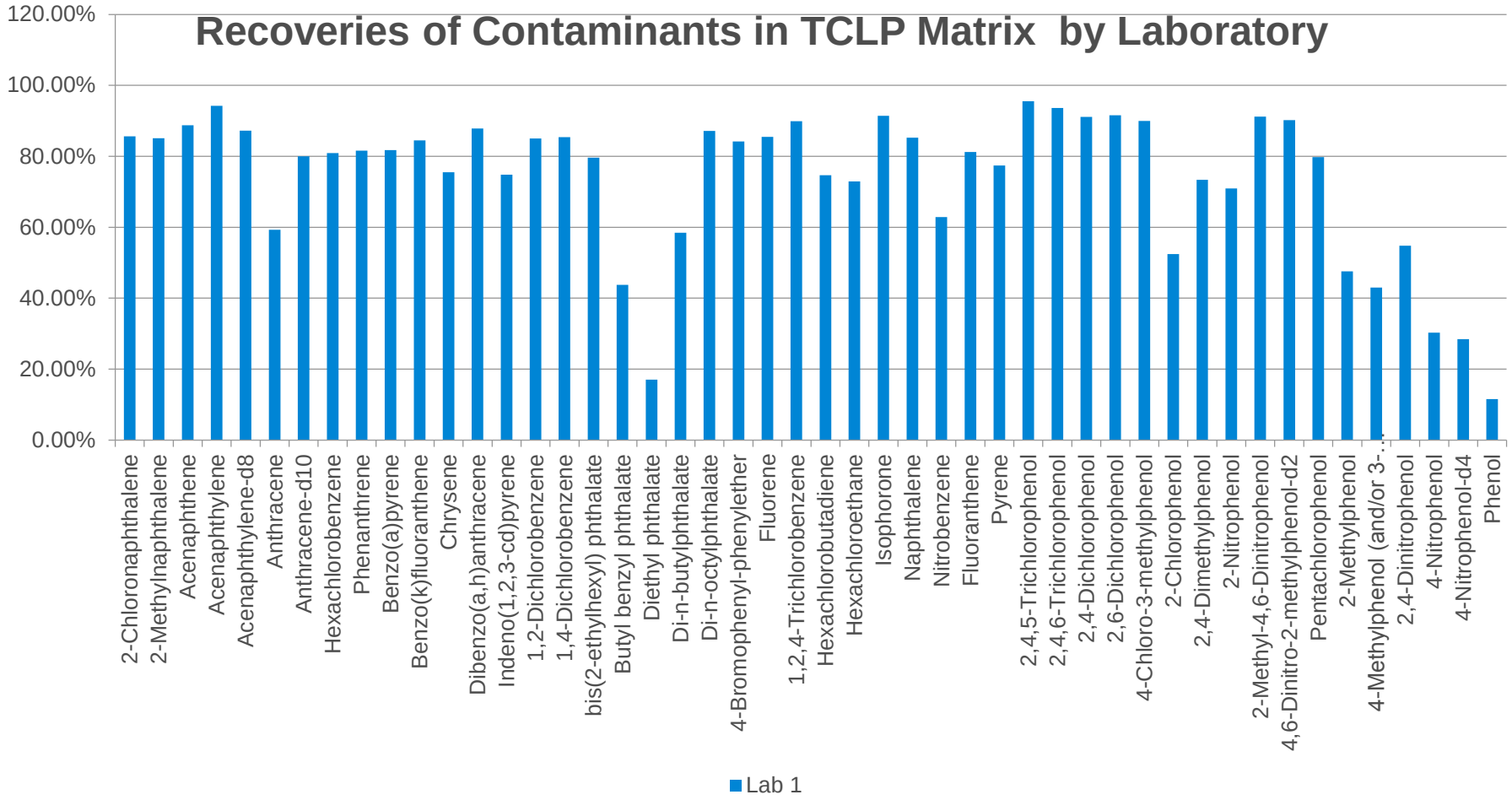
Recovery by Analyte, Bond Elut C18 – Laboratory 1 (n = 3)



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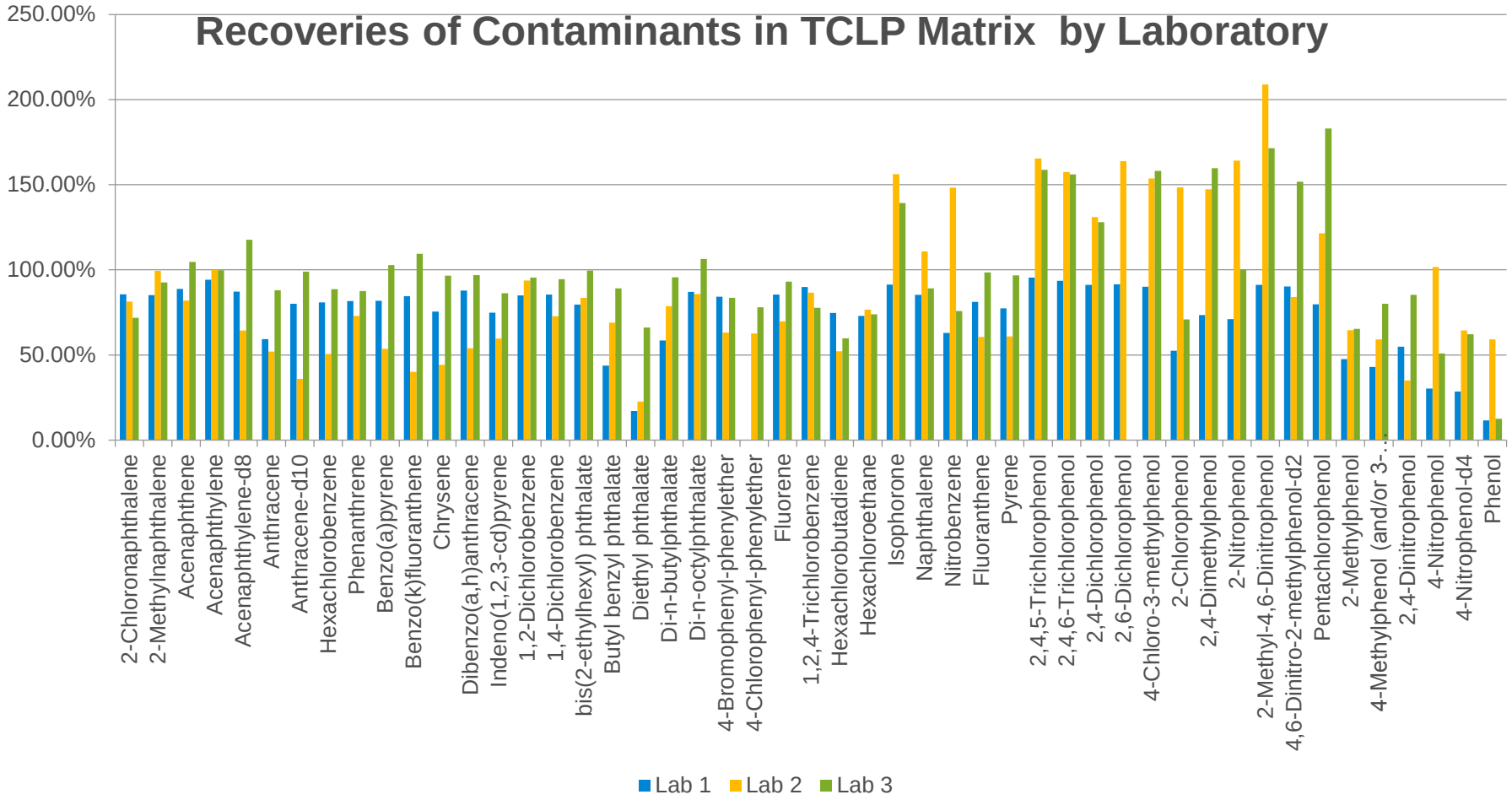
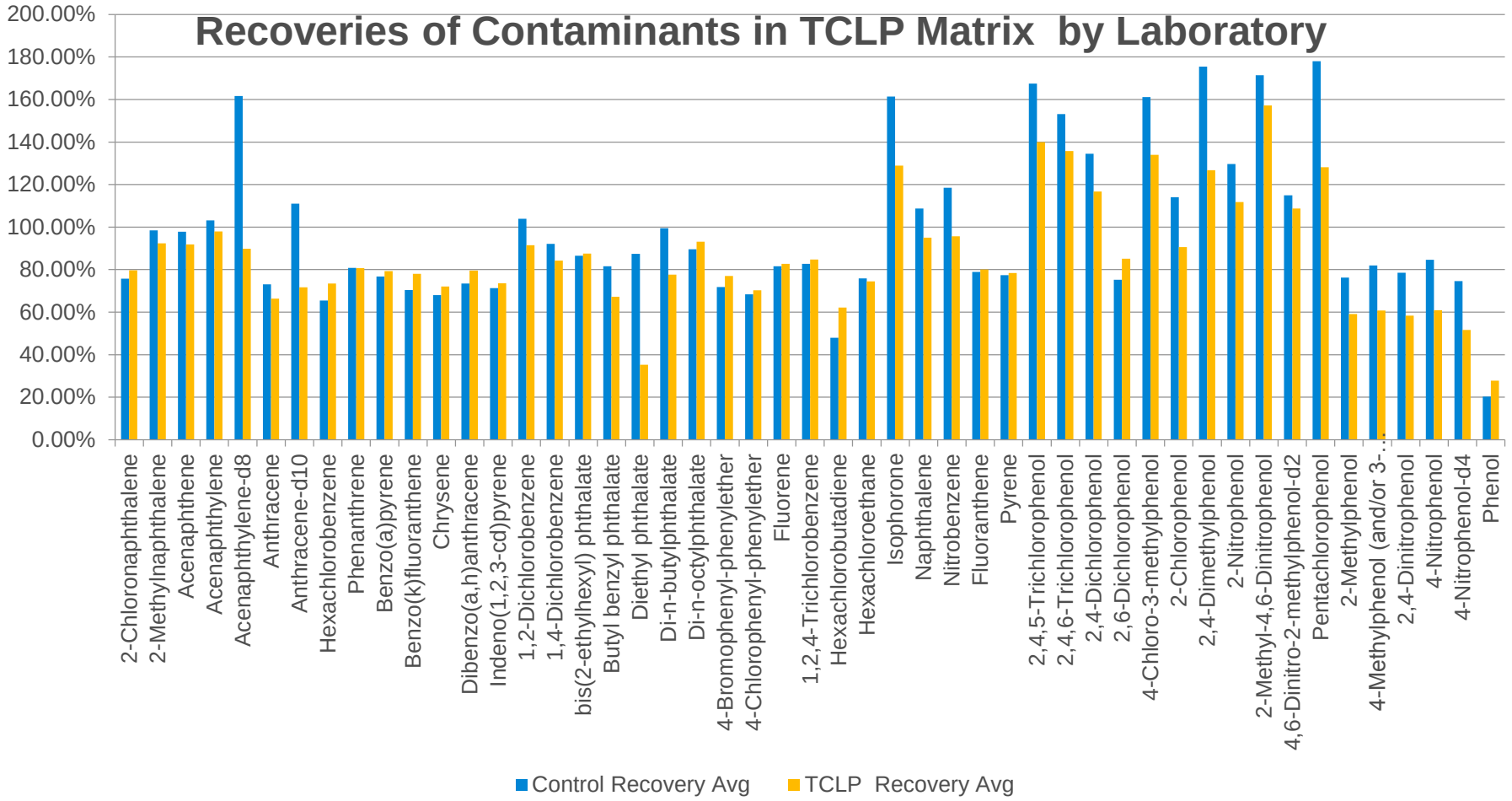
Recovery by Analyte, Bond Elut C18 – By Laboratory (n = 3)



Analyte Recovery by Analyte, Bond Elut C18 – Laboratory Control versus TCLP Matrix



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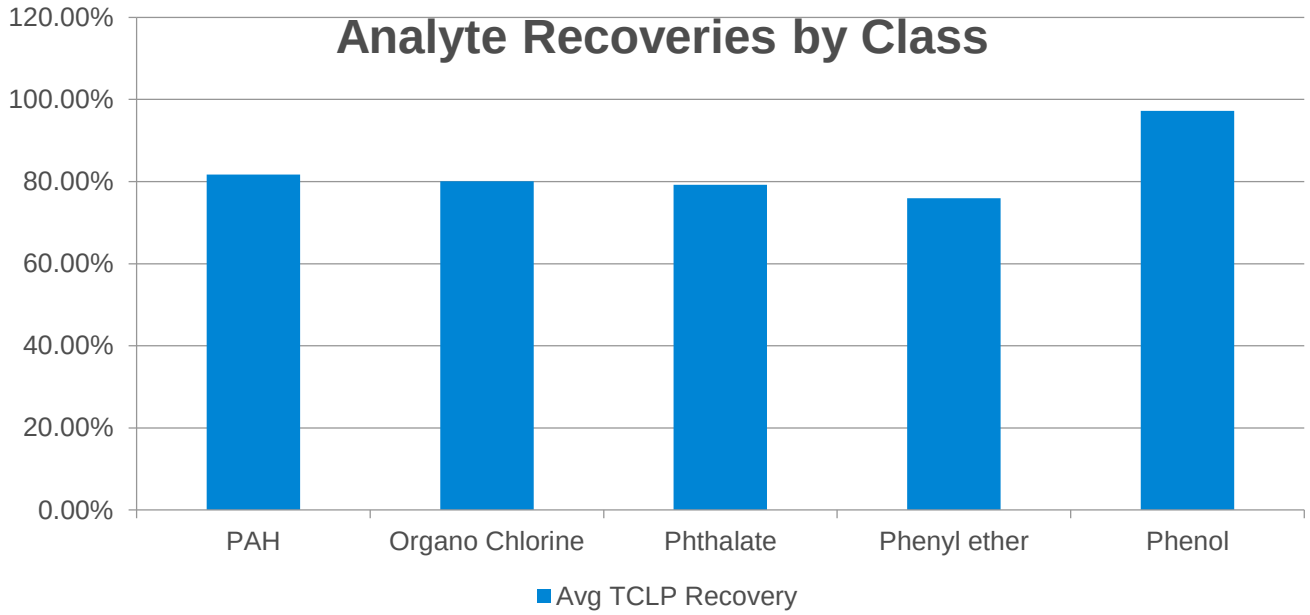
Recovery by Class, Bond Elut C18 – Laboratory Average



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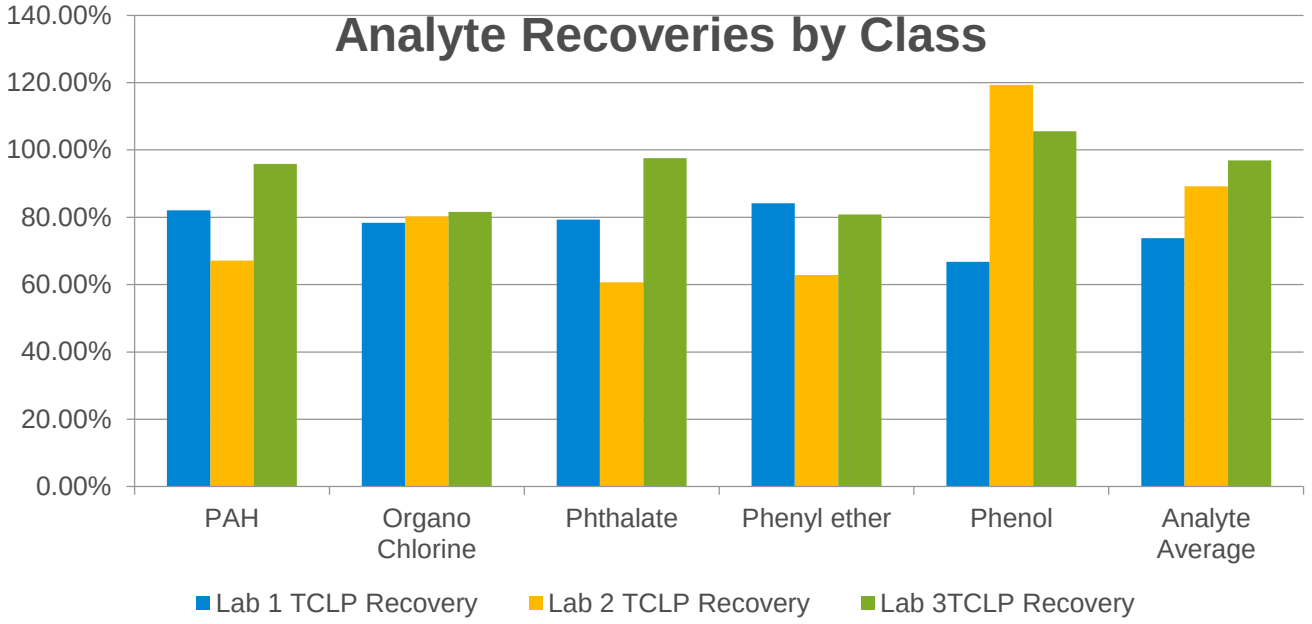
Recovery by Class, Bond Elut C18 – By Laboratory



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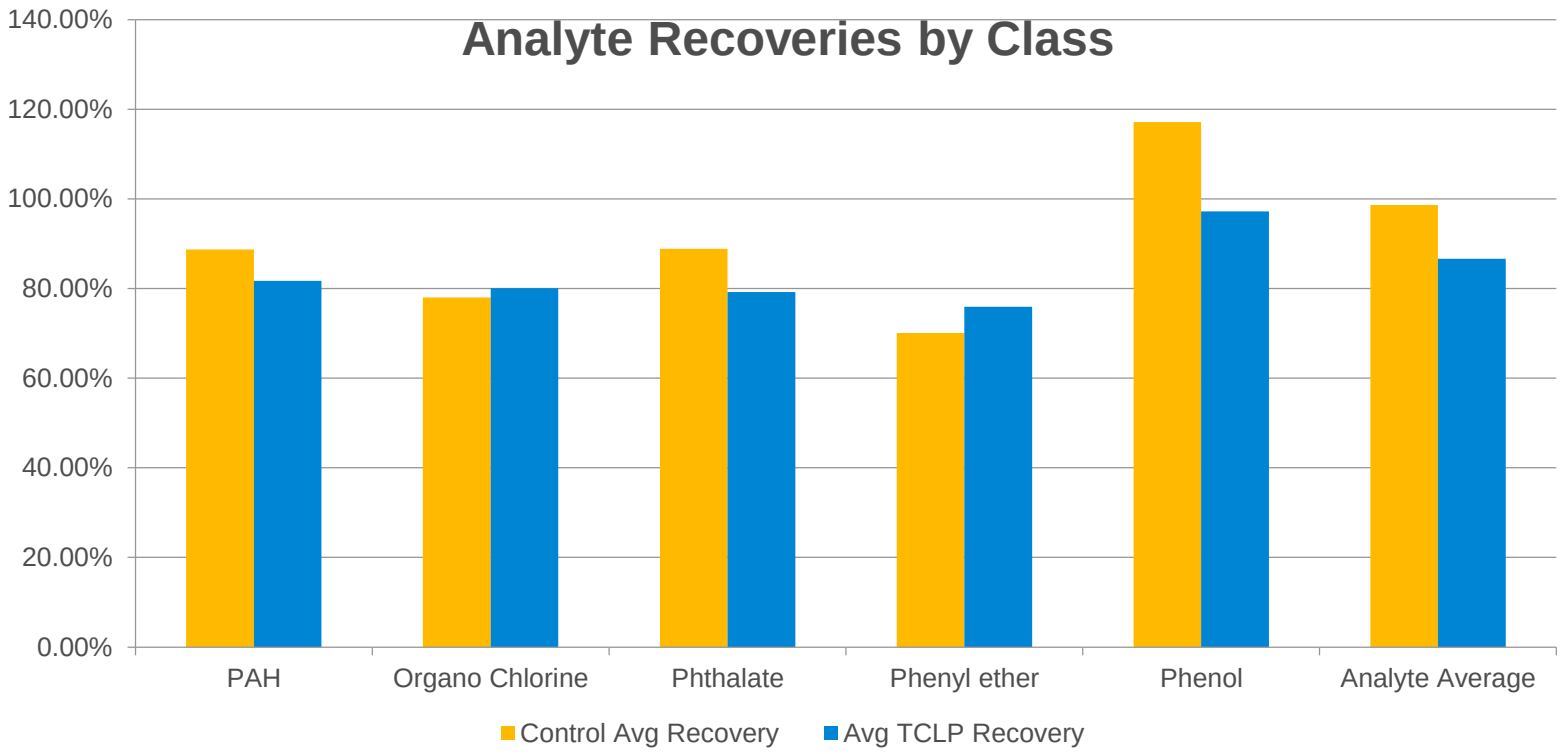
Recovery by Class, Bond Elut C18 – Control Avg. Vs TCLP Avg.



Revision to US EPA Method 625
Conclusions

In ASTM Synthetic Wastewater samples and for the TCLP extract matrix, the analyte recoveries for all tested analytes were well within the acceptance limits established by EPA in Method 625.1 and by TNI for their laboratory performance testing studies.

The use of SPE yields analytical results that are comparable to those obtained using the conventional liquid-liquid extraction process even when employed by laboratory personnel that have not had extensive training in the technology and its use.

The study encompassed the use of six different SPE products from five manufacturers without optimization of the particular SPE products for the types of aqueous matrix being used in the study or of the particular types of analytes that were spiked into the test samples. This aspect of the program further demonstrates the ruggedness of the SPE protocol and of the SPE technique itself.

Twenty-five commercial, government, and academic laboratories participated in this study, many of which had no prior experience with Solid Phase Extraction or with the use of the particular SPE product that they were using to analyze the samples. This demonstrates the ruggedness of the SPE protocol.

Thank you

Let's Continue the Conversation

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APPENDIX

Solid Phase Extraction (SPE)
Agilent SPE Offering

- Reliable SPE with a 30 year history
- Agilent offers the most comprehensive set of phases, sizes and formats of any SPE provider.
- Includes packed bed silica and polymeric phases, and monolithic silica phases

Bond Elut Silica and polymer SPE

Bond Elut AccuCAT
Bond Elut Alumina (AL-A)
Bond Elut Alumina (AL-B)
Bond Elut Alumina (AL-N)
Bond Elut NH_2
Bond Elut C1
Bond Elut C2
Bond Elut C8
Bond Elut C18 .....
..... **40 phases**

Bond Elut Plexa polymer SPE

Bond Elut Plexa
Bond Elut Plexa PCX
Bond Elut Plexa PAX

SampliQ SPE

Multiple phases

SPEC monolithic silica disk SPE

SPEC C2
SPEC C8
SPEC C18
SPEC C18AR
SPEC PH
SPEC NH2
SPEC CN
SPEC Si
SPEC PSA
SPEC SAX
SPEC SCX
SPEC MP1
SPEC MP3

OMIX monolithic silica tip SPE

OMIX C18
OMIX MP1
OMIX SCX

Bond Elut Quechers Dispersive SPE

Multiple kit configurations for multiple food types