Solid Phase Extraction (SPE) for the Analysis of Semi-volatile Organic Pollutants According to EPA Method 625

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MELA
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Overview

Introduction

- Why Solid Phase Extraction for EPA 625?
- Multi-lab SPE study for revision to method 625

Method

- Sample preparation and Instrumental Protocols

Results

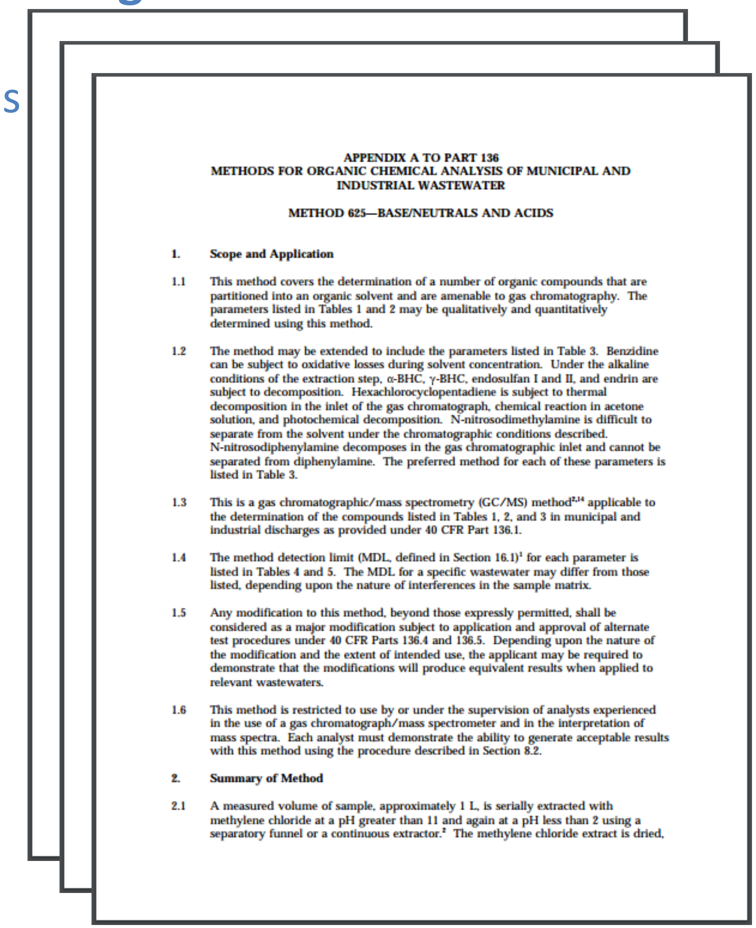
- SPE Performance

Summary and Conclusions

EPA Method 625

Used to measure acidic, basic and neutral semi-volatile organic compounds in municipal and industrial wastewater using GC-MS.

- Polynuclear aromatic hydrocarbons
- Chlorinated hydrocarbons and pesticides
- Phathalate esters
- Organophosphate esters
- Nitrosamines
- Haloethers
- Aldehydes
- Ethers
- Ketones
- Anilines
- Pyridines
- Quinolines
- Nitro aromatics
- Phenols



APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 625—BASE/NEUTRALS AND ACIDS

1. Scope and Application

1.1 This method covers the determination of a number of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography. The parameters listed in Tables 1 and 2 may be qualitatively and quantitatively determined using this method.

1.2 The method may be extended to include the parameters listed in Table 3. Benzidine can be subject to oxidative losses during solvent concentration. Under the alkaline conditions of the extraction step, α-BHC, γ-BHC, endosulfan I and II, and endrin are subject to decomposition. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition. N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. The preferred method for each of these parameters is listed in Table 3.

1.3 This is a gas chromatographic/mass spectrometry (GC/MS) method[2,14] applicable to the determination of the compounds listed in Tables 1, 2, and 3 in municipal and industrial discharges as provided under 40 CFR Part 136.1.

1.4 The method detection limit (MDL, defined in Section 16.1)[1] for each parameter is listed in Tables 4 and 5. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 Any modification to this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5. Depending upon the nature of the modification and the extent of intended use, the applicant may be required to demonstrate that the modifications will produce equivalent results when applied to relevant wastewaters.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph/mass spectrometer and in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 L, is serially extracted with methylene chloride at a pH greater than 11 and again at a pH less than 2 using a separatory funnel or a continuous extractor.[2] The methylene chloride extract is dried,

Revision to US EPA Method 625
Purpose of Study

Objectives

- Demonstrate efficacy of SPE for EPA 625: multi-lab, multi-vendor and EPA
- Does SPE sample prep provide acceptable results?
- Reproducible recovery from a challenging matrix?
- Does surrogate recovery accurately measure system performance?

Revision to US EPA Method 625
Round Robin Study

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

Benefits of SPE Versus LLE

SPE

- Minimal solvent usage/waste.
- Amenable to high-throughput workflows and automation.
- Sample Concentration
- Cheap and disposable one time use tubes/discs.

LLE

- Large amount of organic solvent required.
  - Increases solvent costs, hazardous waste, and evaporation time.
- Requires ample time for sample preparation using and specialized glassware/ capital equipment.
- Large solvent volumes require increased evaporator time/size.
- Emulsions

Benefits of SPE Versus LLE

SPE

LLE

Revision to US EPA Method 625

Target Analytes

Base/Neutrals	µg/L
Acenaphthene	10 to 200
Acenaphthylene	10 to 200
Anthracene	10 to 200
Benzidine	200 to 1000
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	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
1,4-Dichlorobenzene	20 to 200

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

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

Loading

- Analytes of interest and other interferences adsorb onto the surface of the sorbent during sample addition

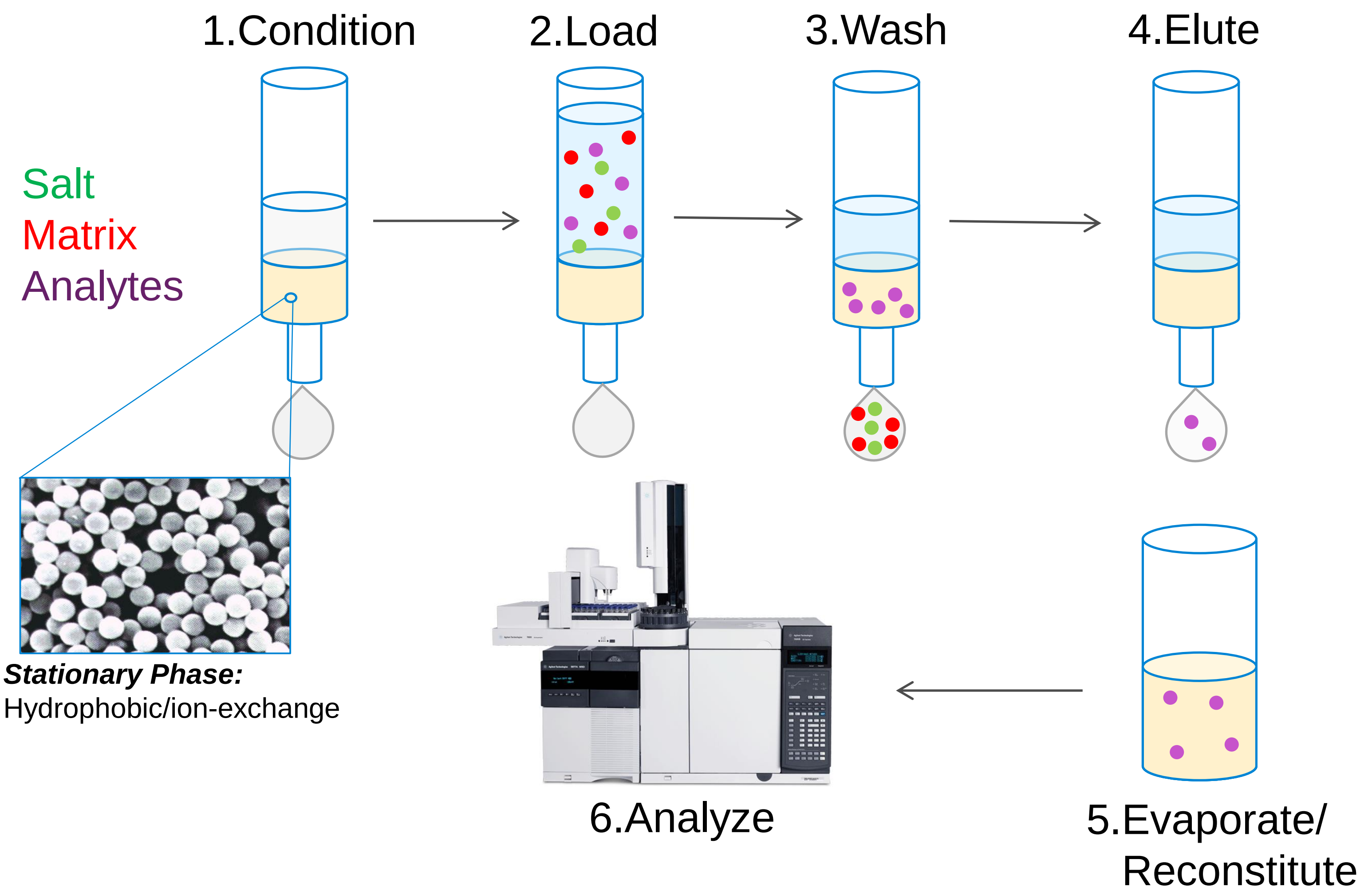
Washing

- Elimination of undesired interferences

Elution

- Selective desorption and collection of desired analytes from the sorbent

Solid Phase Extraction



Solid Phase Extraction (SPE)
Basic Chromatography Theory

Choose the right SPE:

Like Dissolves Like

- Reversed phase
  - Van der Waals or hydrophobic interactions
- Normal Phase
  - Polar interactions (hydrogen bonding and dipole-dipole)

Opposite Charges Attract

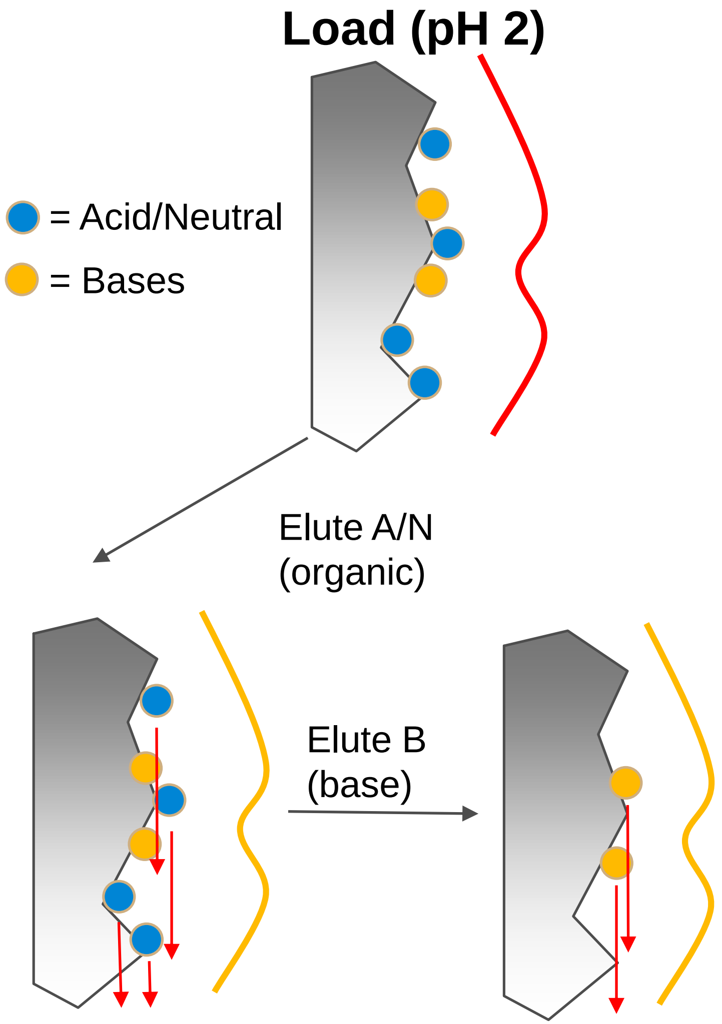
- Ion Exchange
  - Electrostatic interactions

Select Phase based on these rules

Solid Phase Extraction (SPE)
Basic Chromatography Theory

Mechanism of Action

- Similar to HPLC, its chromatography
- Higher affinity for the mobile phase, no retention
- Increased affinity for the stationary phase, retained by stationary phase



Agilent Solutions

Sample Preparation

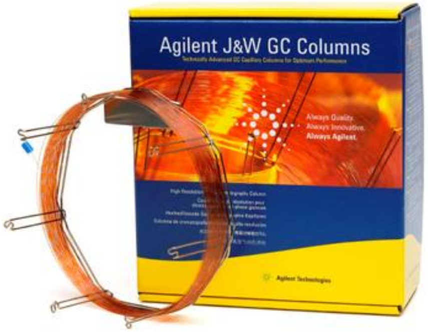
Bond Elut ENV
Polymeric SPE tube

Bond Elut Plexa PCX
Cation Exchange SPE tube

Bond Elut SPECS
Cation Exchange SPE Disk

GC Consumables

Agilent GC Columns
Ultra Inert DB-5ms

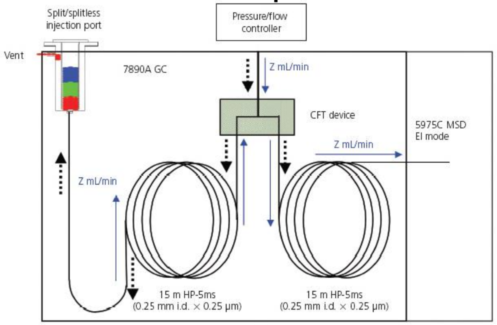


Ultra Inert Liners
2 mm dimpled

Analysis

Agilent 7890 GC
5977 MSD

Column Backflushing
15 x 15 m midpoint



625 Round Robin Study – Synthetic Waste Water Matrix

Recipe:

- 0.400 g flour
- 2.000 g ocean salt
- 0.080 g Kaolin
- 0.024 g Triton™ X-100 surfactant
- 120 mL beer
- Dilute to 2 L with reagent grade water.

Based on ASTM D5905

EPA 625.1 Update – Sample Preparation

Sample Pretreatment: Spike sample (100 mL to 250 mL) and adjust to pH 2 with 6 M HCl.

SPE: Bond Elut ENV – 6 mL, 500 mg

Condition: 5 mL DCM, 5 mL MeOH, 5 mL H_2O (pH 2)

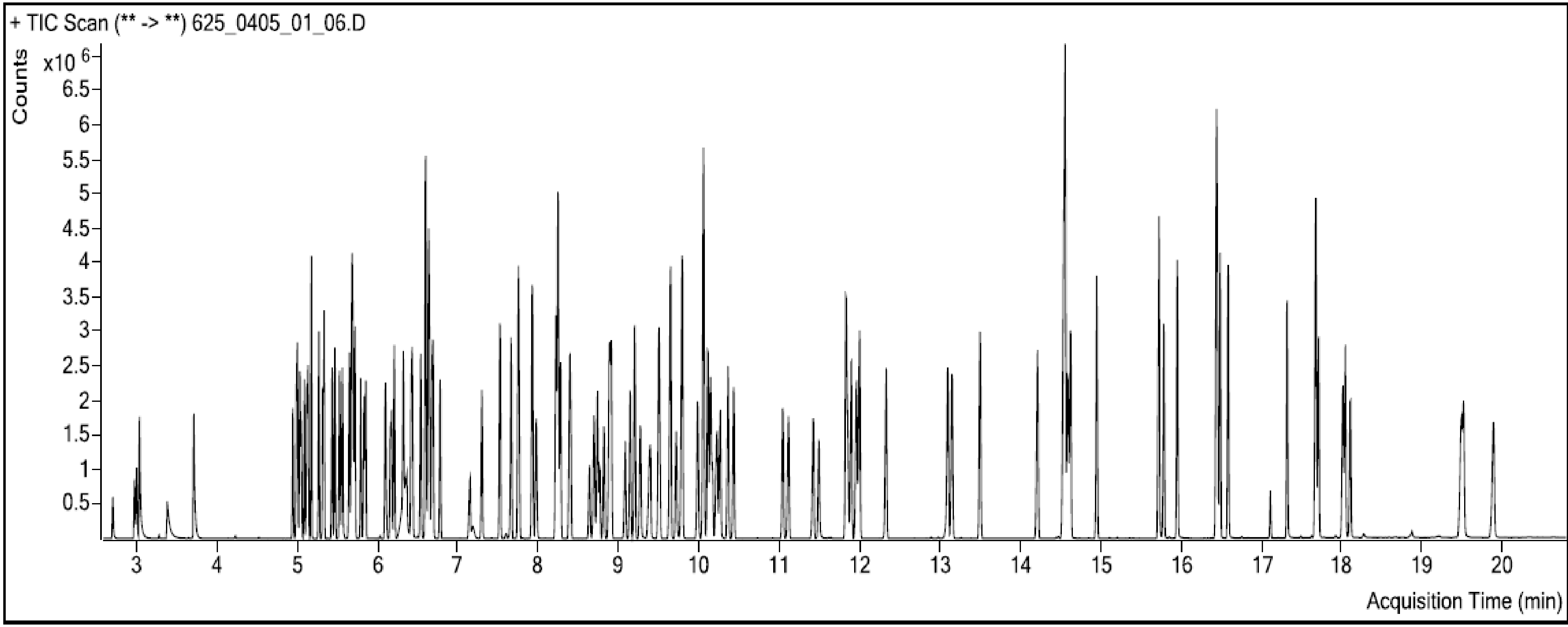
Load: Pass through cartridge bed at 10 mL/min; dry for 2 min

Wash: Pass through 5 mL H_2O

Elution: 2 x 5 mL DCM, 1x 5 mL 1% ammonium hydroxide, 1 x 5 mL ethyl acetate

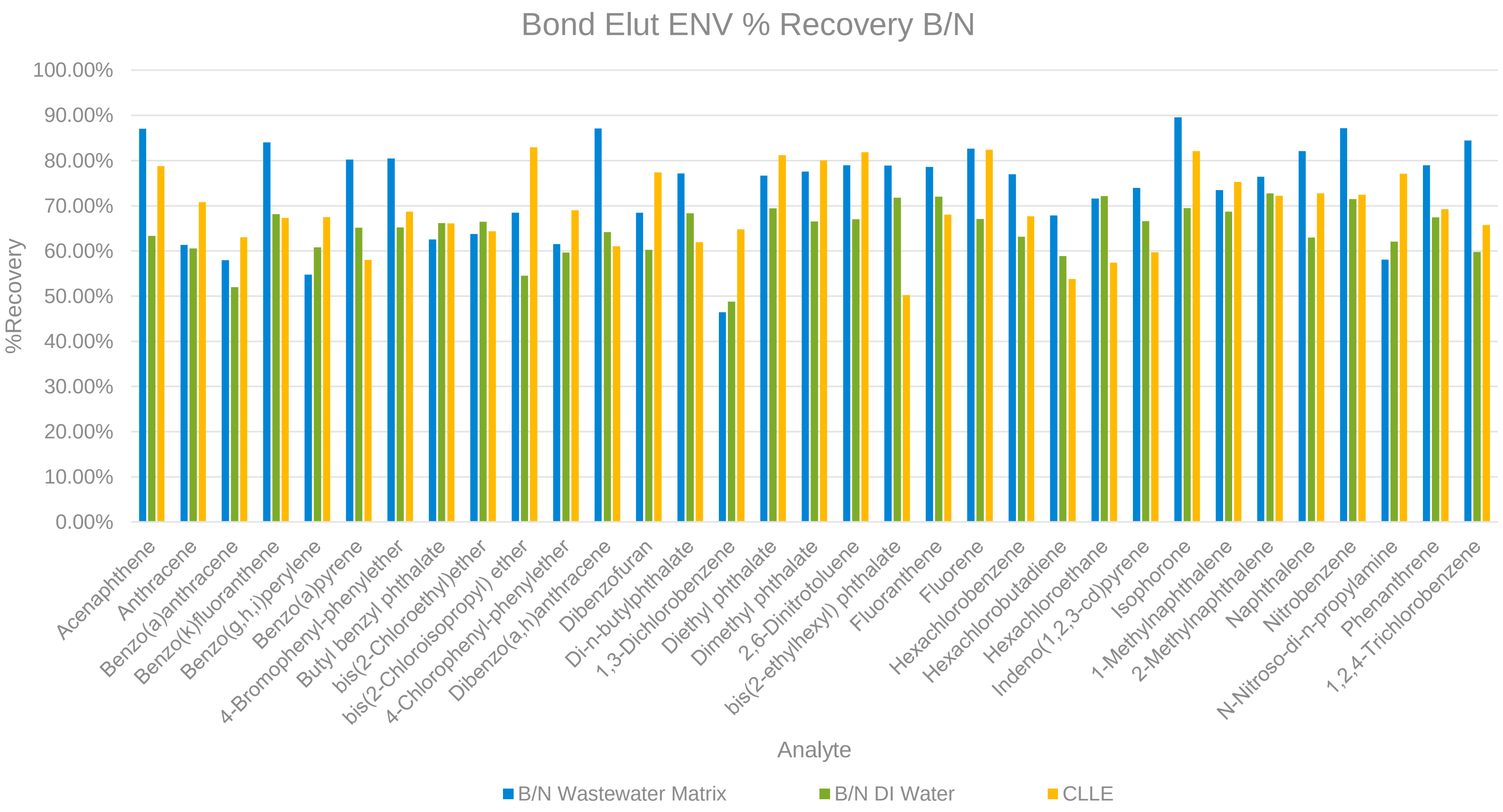
Evaporation/reconstitution: 0.5 mL DCM

Revision to US EPA Method 625
GC-MS (SIM) Chromatogram – 50 µg/L

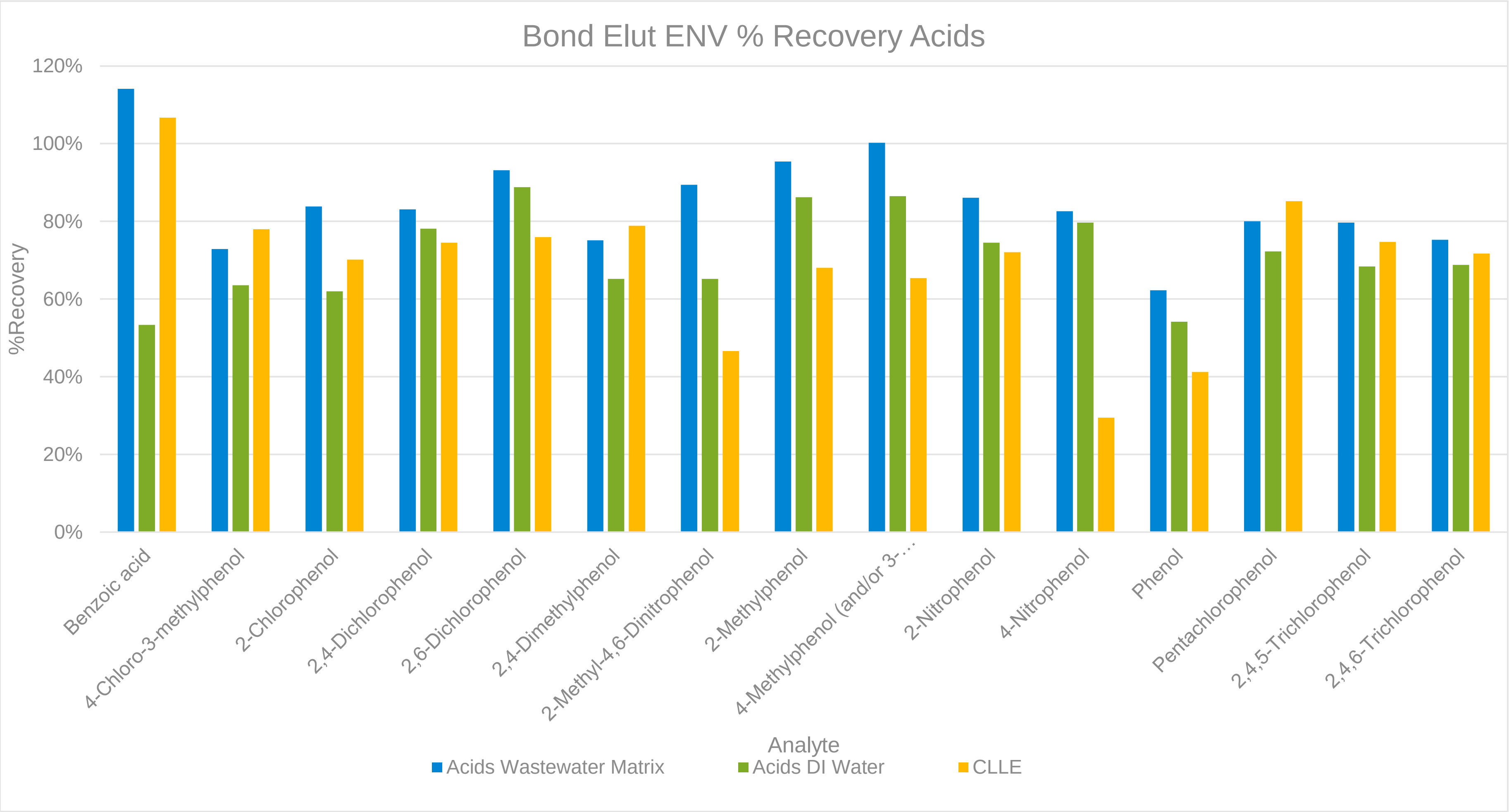


Revision to US EPA Method 625

Recovery by Analyte, Bond Elut ENV – Bases/Neutrals



Revision to US EPA Method 625
Recovery by Analyte, Bond Elut ENV - Acids



Surrogate Recovery and A/B/N Summary

	%Recovery	
Surrogates	**Waste Water**	**DI Water**
Acenaphthylene-d8	85.0	66.8
Anthracene-d10	67.7	60.8
Benzo(a)pyrene-d12	84.7	69.7
Bis-(2-chloroethyl)ether-d8	84.8	82.8
2-Chlorophenol-d4	83.4	76.2
2,4-Dichlorophenol-d3	83.8	75.5
Dimethylphthalate-d6	78.9	69.1
4,6-Dinitro-2-methylphenol-d2	85.3	75.4
Fluorene-d10	82.6	65.8
4-Methylphenol-d8	95.4	83.3
Nitrobenzene-d5	66.5	62.3
2-Nitrophenol-d4	86.1	69.2
4-Nitrophenol-d4	79.4	71.7

	BE ENV Waste Water	BE ENV DI Water	LLE
B/N Avg. %Recovery	**73.8%**	**64.6%**	**69.4%**
Acids Avg. %Recovery	**84.9%**	**71.1%**	**69.2%**

Revision to US EPA Method 625
Conclusions

Recovery

- Analyte recovery from ASTM Synthetic Wastewater samples within the acceptance limits for EPA Method 625.1 and TNI lab performance tests

Applicability

- SPE yields analytical results comparable to conventional liquid-liquid extraction.

Ruggedness

- Use of SPE products without prior optimization of the particular SPE products for the types of aqueous matrix or particular types of analytes demonstrates ruggedness of SPE technique itself.

Ease of Use

- Three commercial laboratories, with no prior experience with SPE, successfully prepared and analyzed

EMR-Lipid – Lose The Fat, Not Analytes

Launch Applications:

- *Veterinary Drugs in Beef Liver*

  5991-6098EN

- *Pesticides in Avocado (LC-MS/MS)*

  5991-6096EN

- *Pesticides in Avocado (GC-MS/MS)*

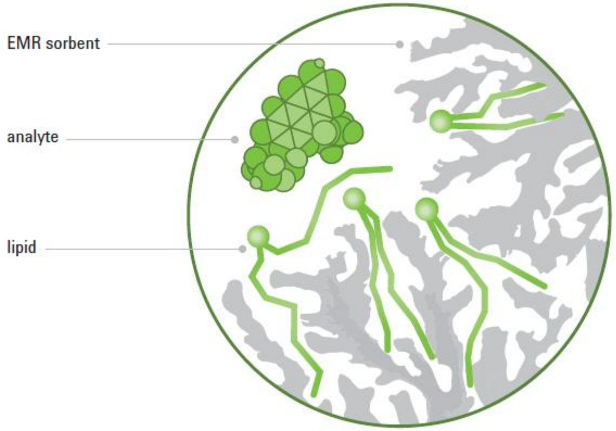
  5991-6097EN

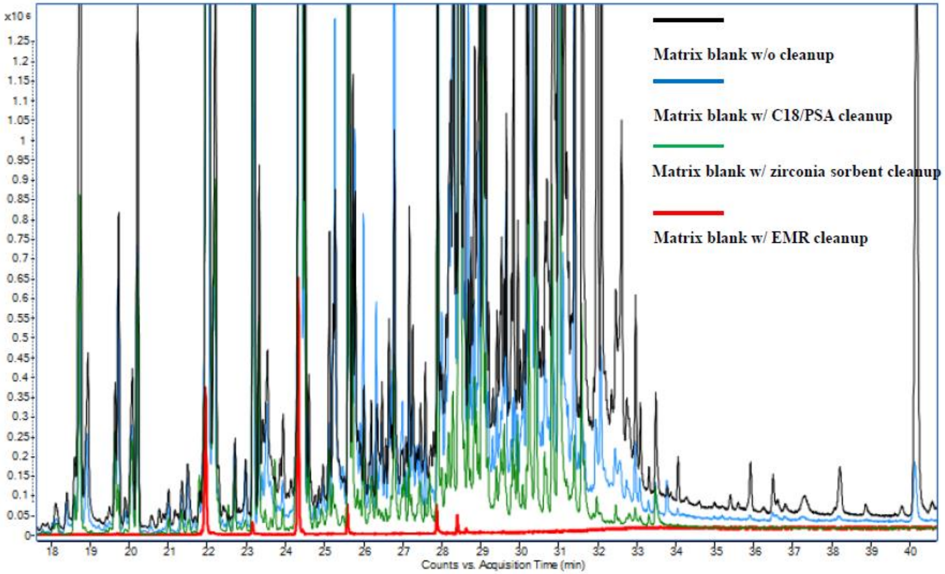
- *PAHs in Salmon*

  5991-6088EN

Coming Soon…

- *Mycotoxins in Infant Formula*
- *Mycotoxins in Peanuts*





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APPENDIX

Revision to US EPA Method 625

Target Anal

Surrogates
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
NDMA-d6

Base/Neutrals	eutrals	Acids	Surrogates
Chrysene	adiene	4-Chloro-3-methylphenol	Acenaphthylene-d8
Dibenzo(a,h)anthrace	lopentadiene	2-Chlorophenol	Anthracene-d10
Dibenzofuran	ane	2,4-Dichlorophenol	Benzo(a)pyrene-d12
1,2-Dichlorobenzene	cd)pyrene	2,6-Dichlorophenol	Bis-(2-chloroethyl)ether-d8
1,3-Dichlorobenzene		2,4-Dimethylphenol	4-Chloroaniline-d4
1,4-Dichlorobenzene	thalene	2,4-Dinitrophenol	2-Chlorophenol-d4
3,3'-Dichlorobenzidine	Naphthalene	2-Methyl-4,6-Dinitrophenol	2,4-Dichlorophenol-d3
Diethyl phthalate	Nitrobenzene	2-Methylphenol (o-Cresol)	Dimethylphthalate-d6
Dimethyl phthalate	N-Nitrosodimethylamine	4-Methylphenol (p-Cresol) 14	4,6-Dinitro-2-methylphenol-d2
Di-n-butyl phthalate	N-Nitroso-di-n-propylamine	2-Nitrophenol	Fluorene-d10
2,4-Dinitrotoluene	N-Nitrosodiphenylamine	4-Nitrophenol	4-Methylphenol-d8
2,6-Dinitrotoluene	Phenanthrene	Phenol	Nitrobenzene-d5
Di-n-octyl phthalate	Pyrene	Pentachlorophenol	2-Nitrophenol-d4
Fluoranthene	1,2,4-Trichlorobenzene	2,4,5-Trichlorophenol	4-Nitrophenol-d4
Fluorene			Phenol-d5
Hexachlorobenzene			NDMA-d6

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

Outline

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

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

Solid Phase Extraction (SPE)
Relationship Between SPE and HPLC

Elution chromatography is defined by the equation

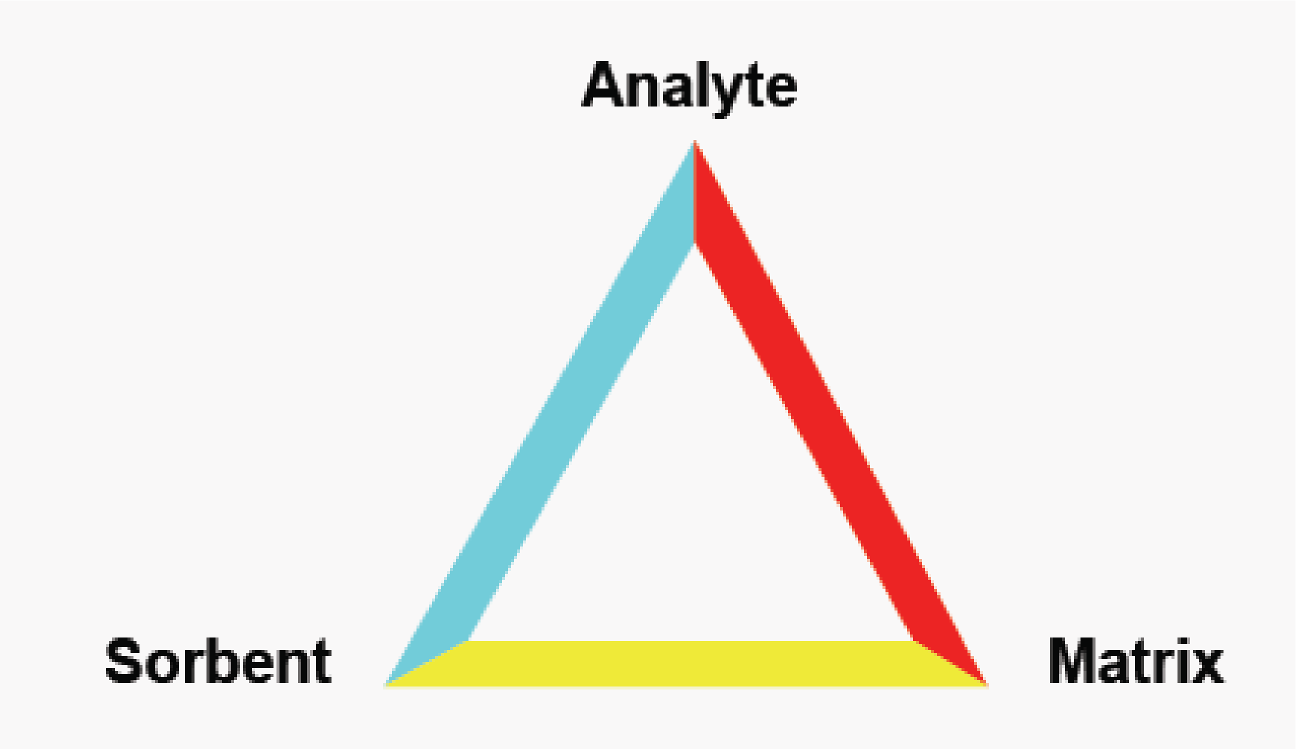
$$0.2 \ll K \ll 20$$

Digital chromatography lies outside this range:

Retention ON K = [stationary phase]/[matrix] > 1000

Elution OFF K = [stat. phase]/[elution solvent] < 0.001

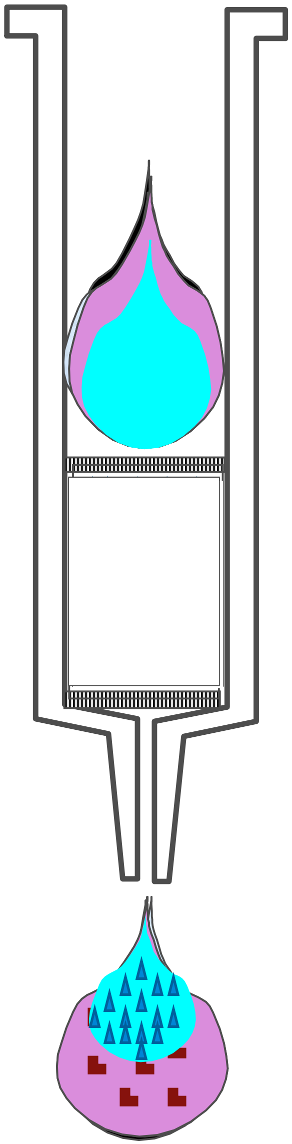
Solid Phase Extraction (SPE)
The SPE Triangle



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

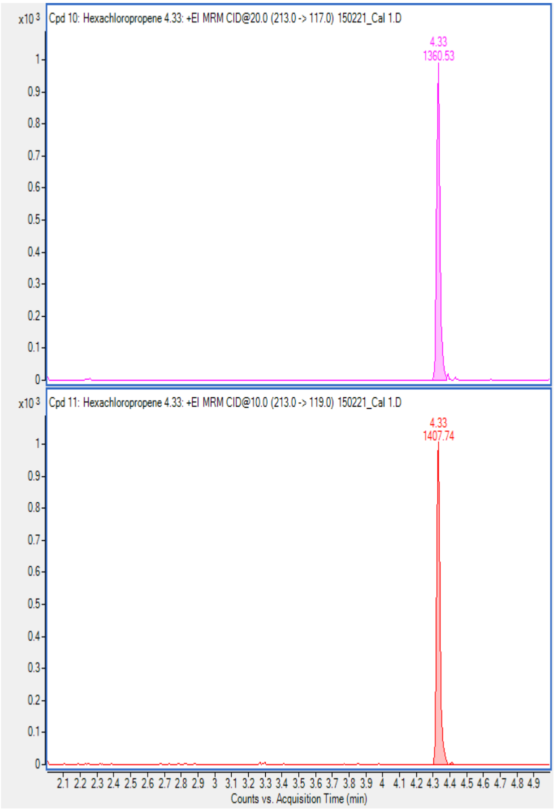
Solid Phase Extraction (SPE)
The SPE Sequence

- 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

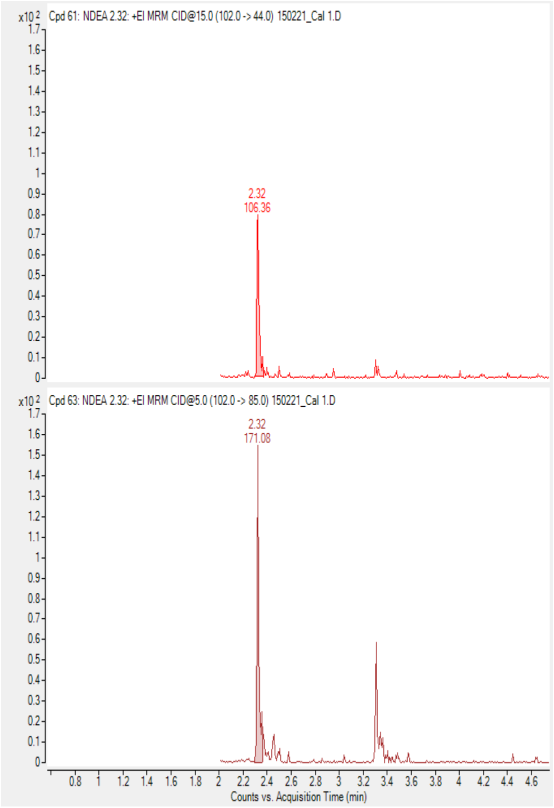


Revision to US EPA Method 625

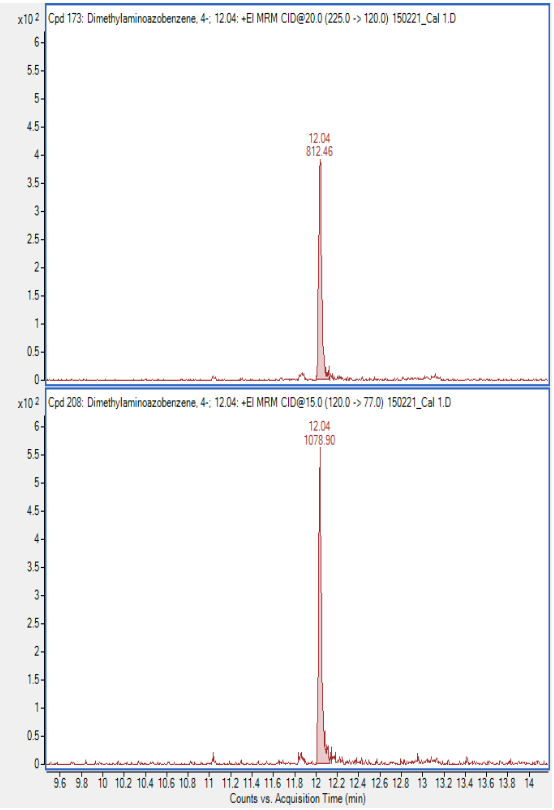
Chromatograms – Cal 1 Compounds @ 78 ng/mL



Hexachloropropene

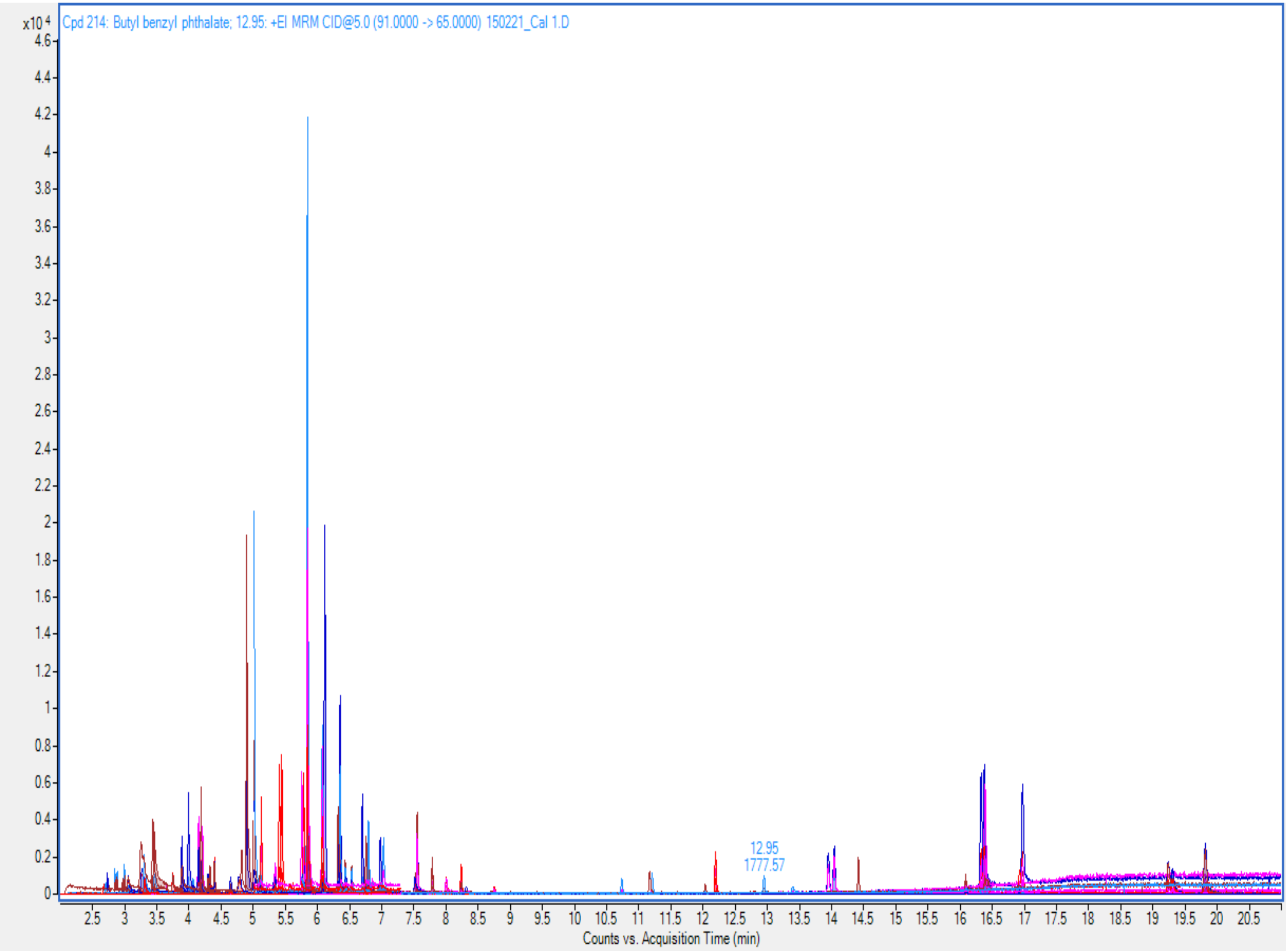


N-Nitrosodiethylamine

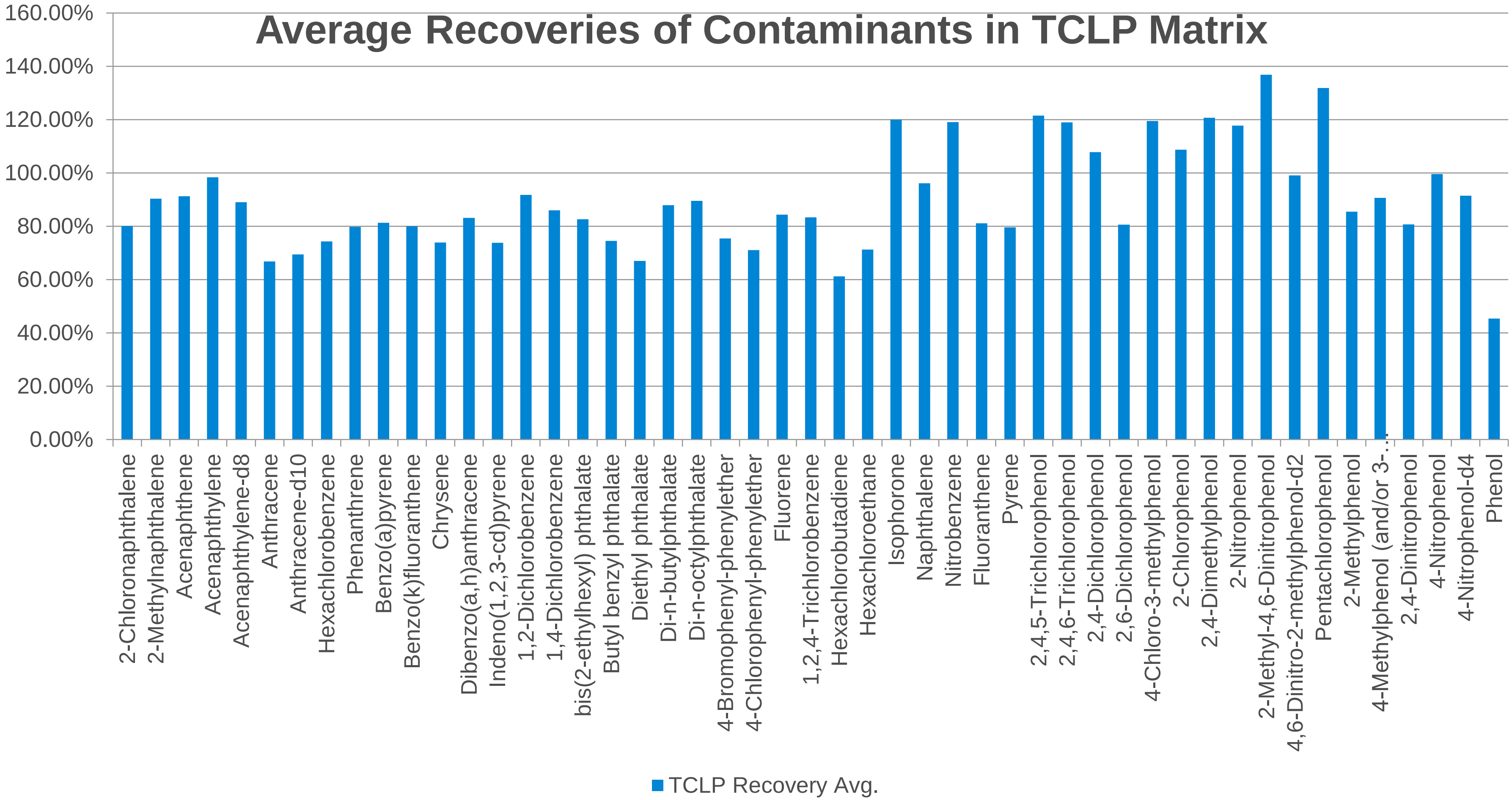


Dimethylaminobenzene

Revision to US EPA Method 625
Chromatogram – Cal 1 @ 78 ng/mL

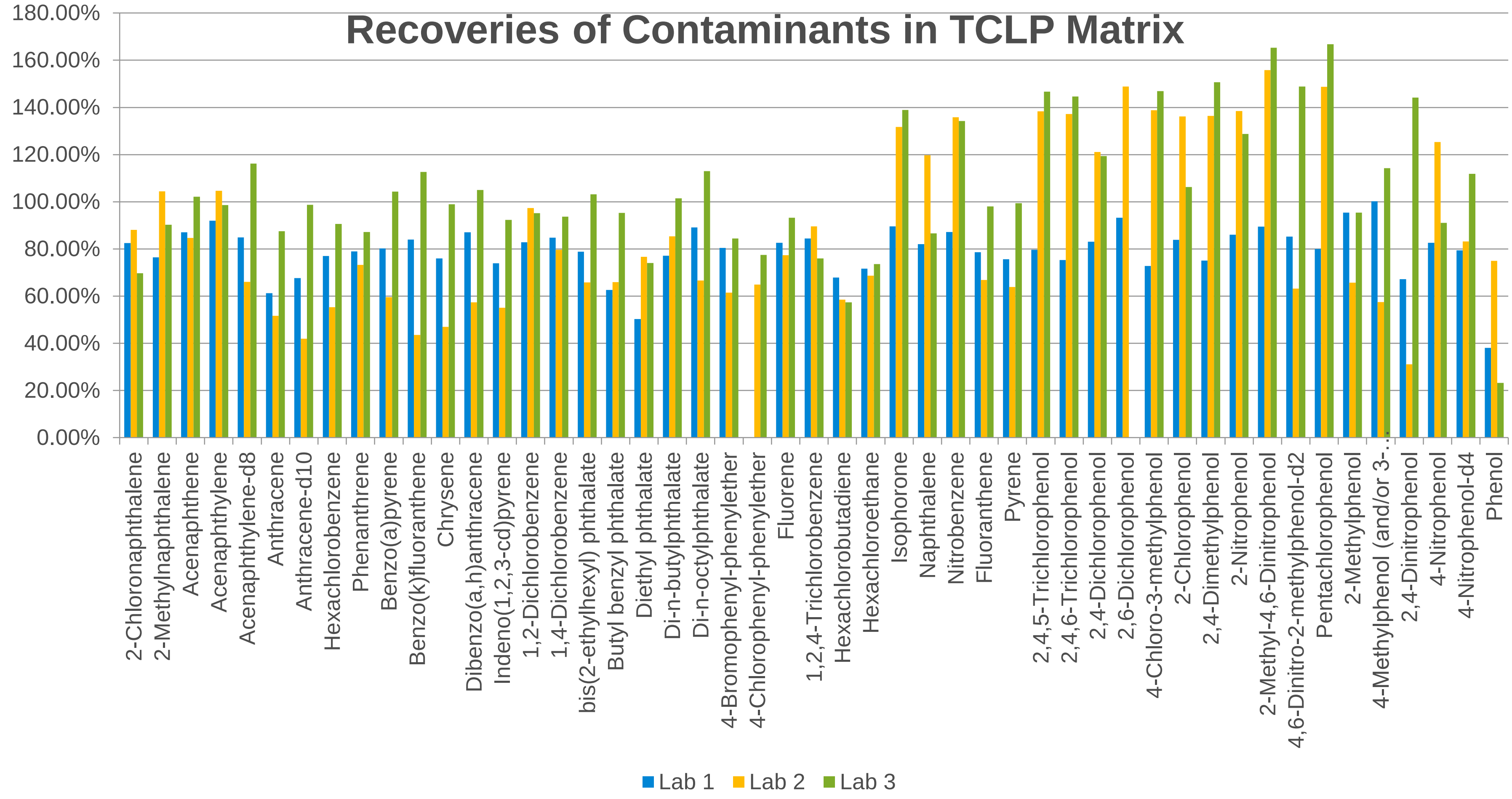


Revision to US EPA Method 625
Recovery by Analyte, Bond Elut ENV – Laboratory Average

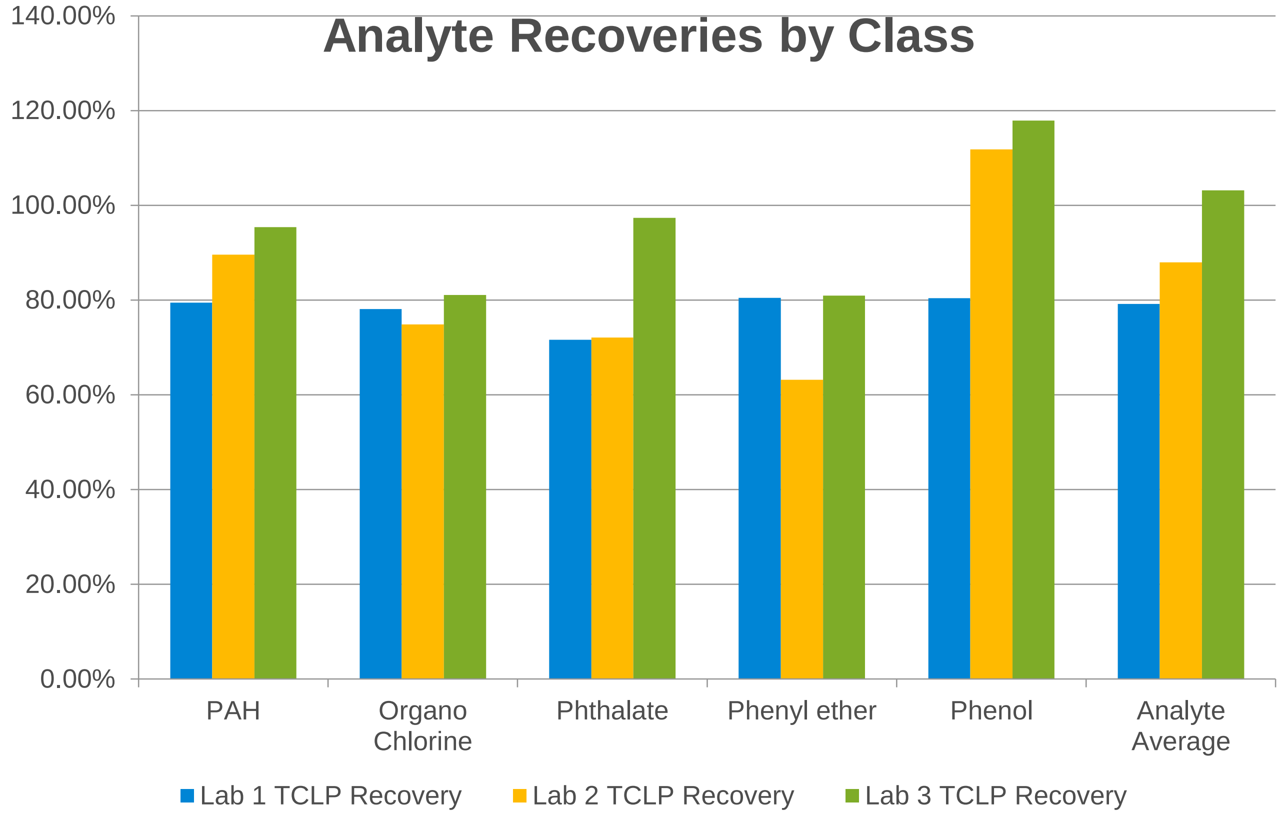


Revision to US EPA Method 625

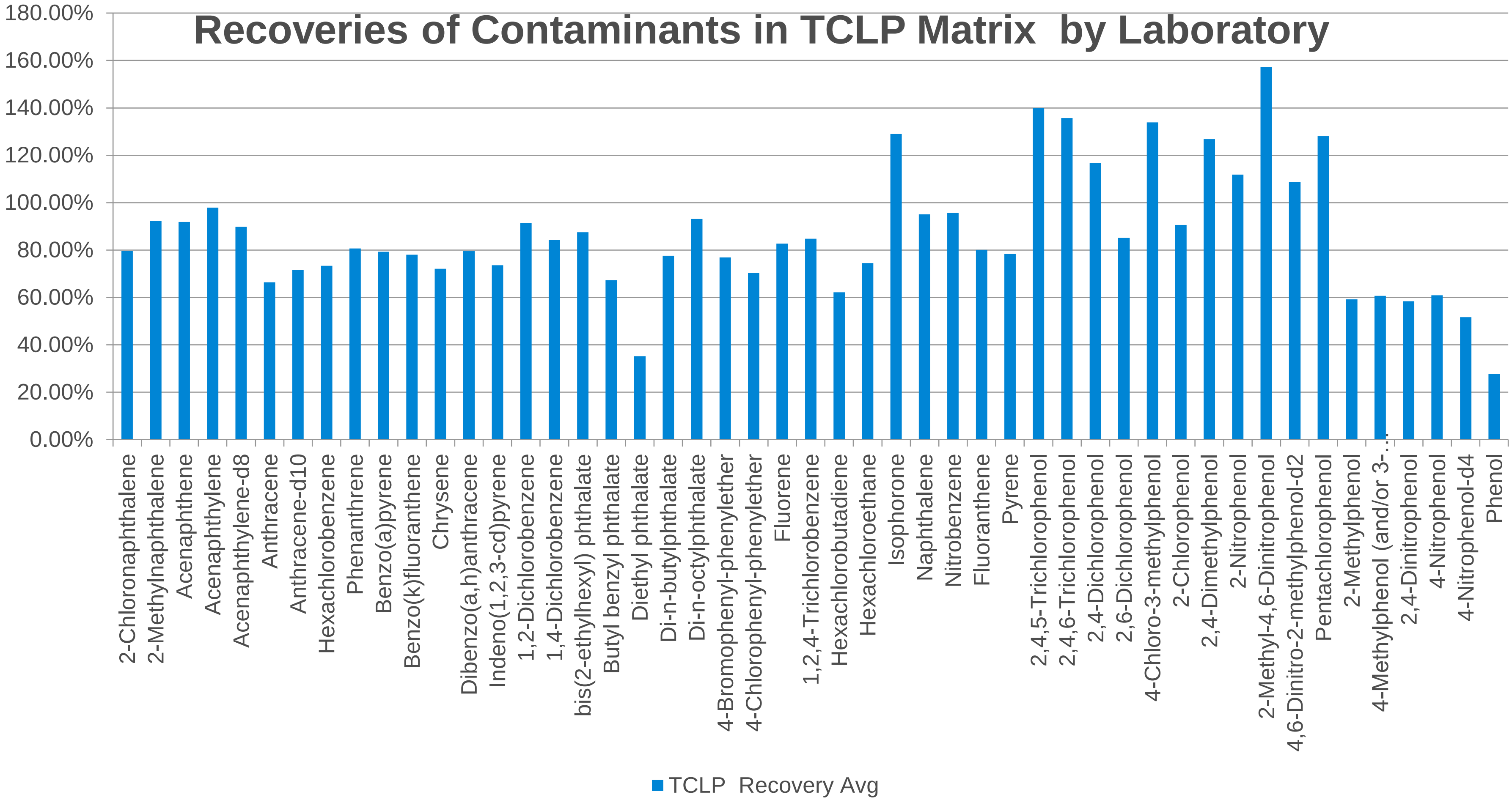
Recovery by Analyte, Bond Elut ENV – By Laboratory (n = 3)



Revision to US EPA Method 625
Recovery by Class, Bond Elut ENV – By Laboratory

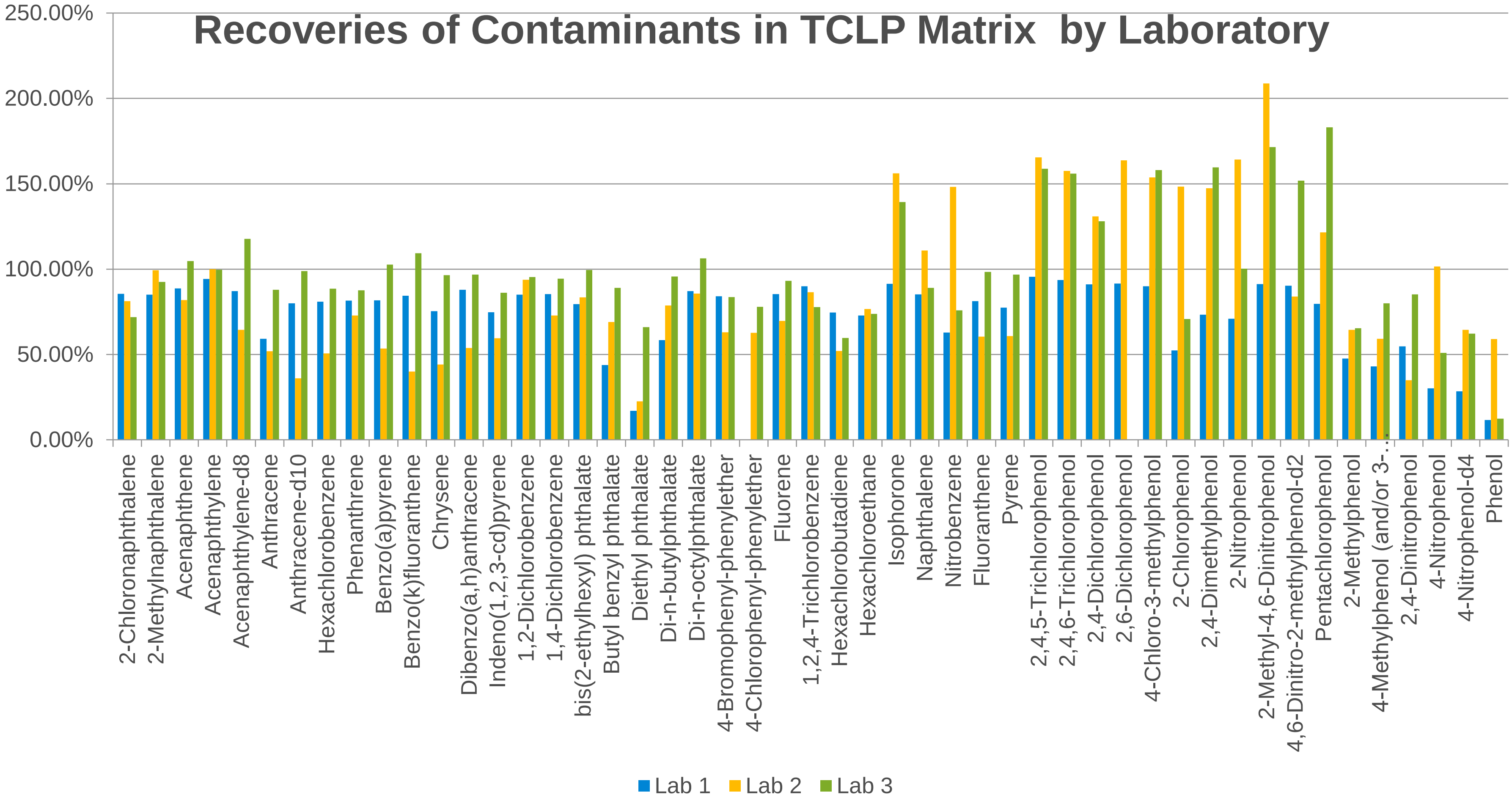


Revision to US EPA Method 625
Recovery by Analyte, Bond Elut C18 – Laboratory Average (n = 3)



Revision to US EPA Method 625

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



Revision to US EPA Method 625

Recovery by Class, Bond Elut C18 – By Laboratory