A Simple, Quick & Low Cost Semi-Automated Clean-Up and Concentration Solution for the Analysis of 209 PCB Congeners and other POPs

Fluid Management Systems
Watertown MA

Introduction (1)

- **POPs (PCDD/Fs, PCBs) continue to attract interest around the world due to strict regulations enforced in many countries**
- **Rapid and quality sample clean up and analysis is needed for many laboratories processing samples**
- **Processing times and cost are important considerations**
- **In the US, EPA methods SW-846, 1613, 1668 and 8082A are used for PCBs and PCDD/Fs work**

Introduction (2)

- **Sample extracts in DCM, hexane or toluene**
- **Cleanup for analysis of all 209 PCBs in common in North America - extracts are often in toluene after Soxhlet Extraction**
- **Dioxins and furans can also be run**
- **Extractable Petroleum Hydrocarbons are analyzed in soils for aliphatics and aromatics (PAHs)**
- **Individual states in US have methods available**

Challenges of POPs Sample Prep

- Labor intensive, prone to error
- Compliance with regulatory procedures and accreditation (lengthy method validation)
- Strict QA/QC requirements
- Sample matrix complexity
- Native background and interferences (can be orders of magnitude higher than analytes)
- Pico/femto-gram analyses require ultra pure extract and excellent instrument sensitivity

Automated Sample Prep

- **Advantages of Automated Sample Prep**
  - Rapid Turn Around Time: 30 to 45 Minutes for 6 Samples
  - Cleaner Background Interferences: Closed Loop System
  - Quality Results: Certified Pre-packaged Columns
  - Green Technology: Lower solvent and power use
  - QA/QC & Accreditation Requirements: Easier to Manage
  - Computerized Method: Instrumentation based prep

Manual Sample Prep

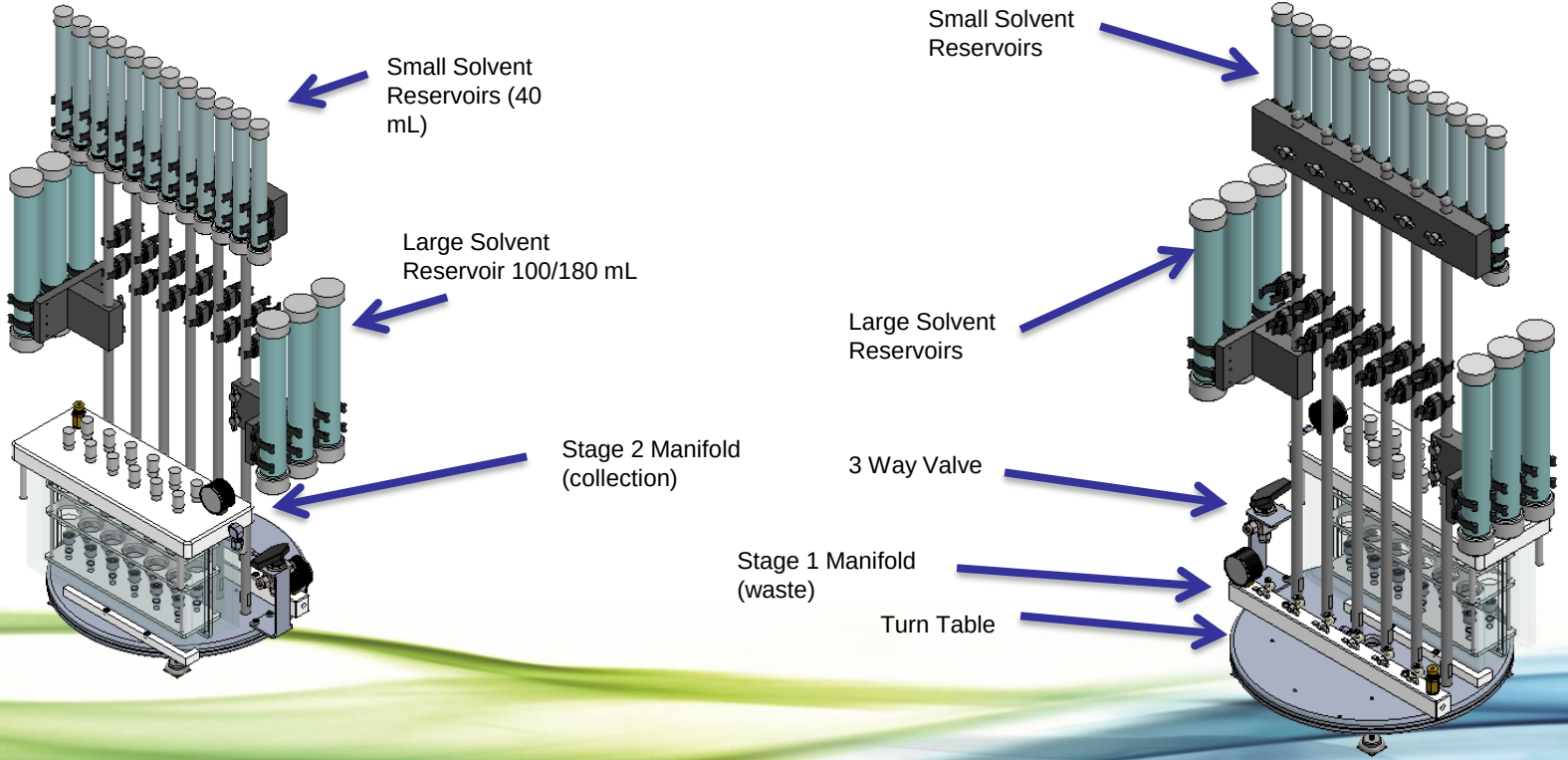
- **Advantages of Manual Sample Prep**
  - Most labs use a Manual Methods for the following reasons:
    - No electronics or mechanical components to fail
    - No down time due to the system failure
    - No service contract
    - No capital equipment cost

Semi-Automated System

Specification:

- Simple to run, no computerized instrumentation
- Fast: 30 to 45 min
- Closed loop system to give a clean background, low level detection
- Use certified pre-packaged columns
- Green technology, only vacuum pump uses power
- Low solvents, as low as 160 mL for serum
- Economical column kits, choice of low fat and high fat column kits
- No capital equipment cost
- No electronics or mechanical equipment to fail
- No downtime

Characteristics of Semi-Automated System (EZPrep)

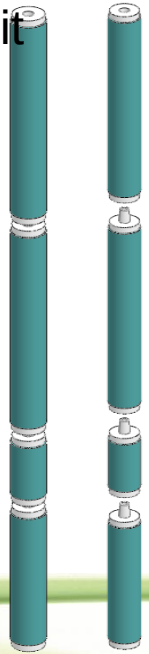


Columns (1)

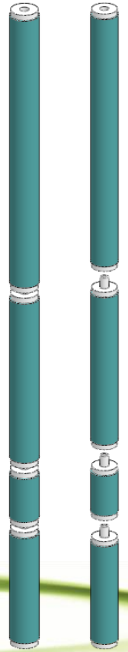

Carbon
Alumina 4g
Alumina 6g
Basic/Neutral
Classical Acid
Mini ABN Acid
Classical Plus Acid
High Capacity Acid
XL Capacity
Acid

Columns (2)

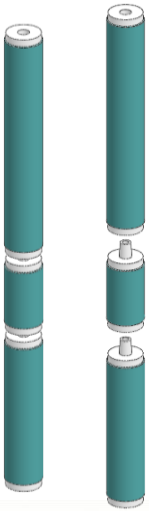
Classical Kit



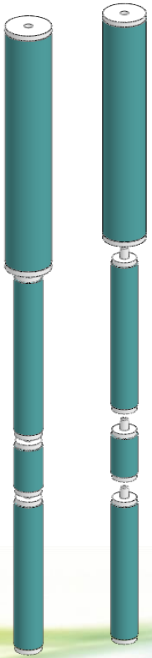
Classical Plus Kit



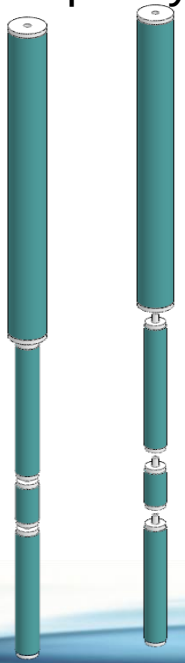
Mini Kit



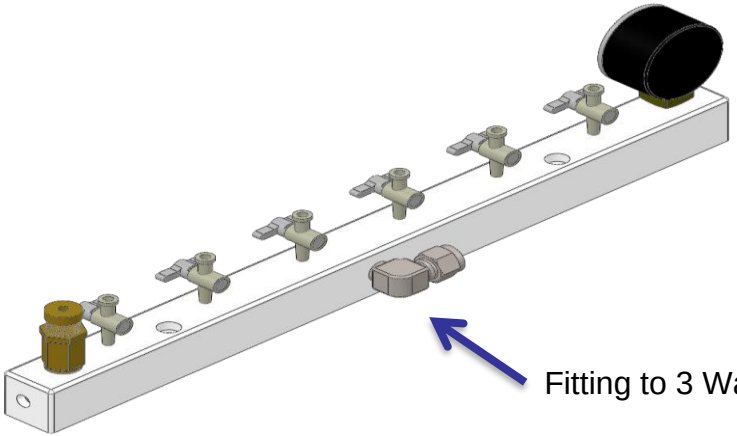
High Capacity Kit



XL Capacity Kit

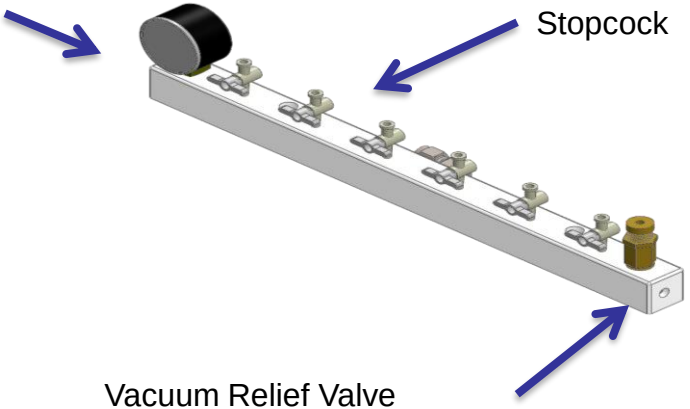


Stage 1 Manifold



Fitting to 3 Way Valve

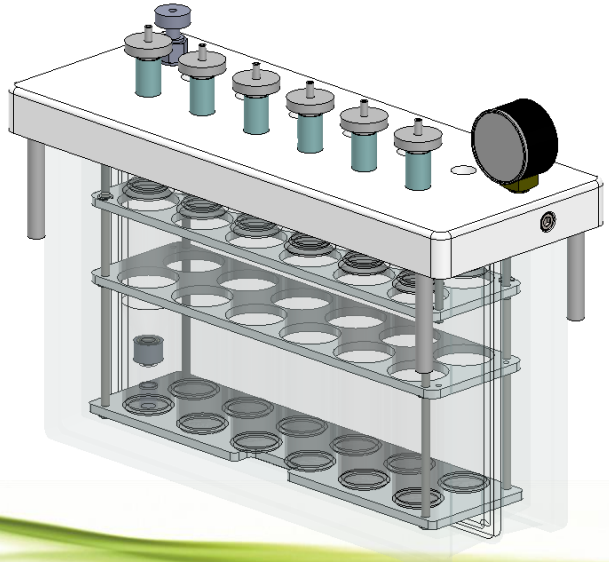
Vacuum Gauge



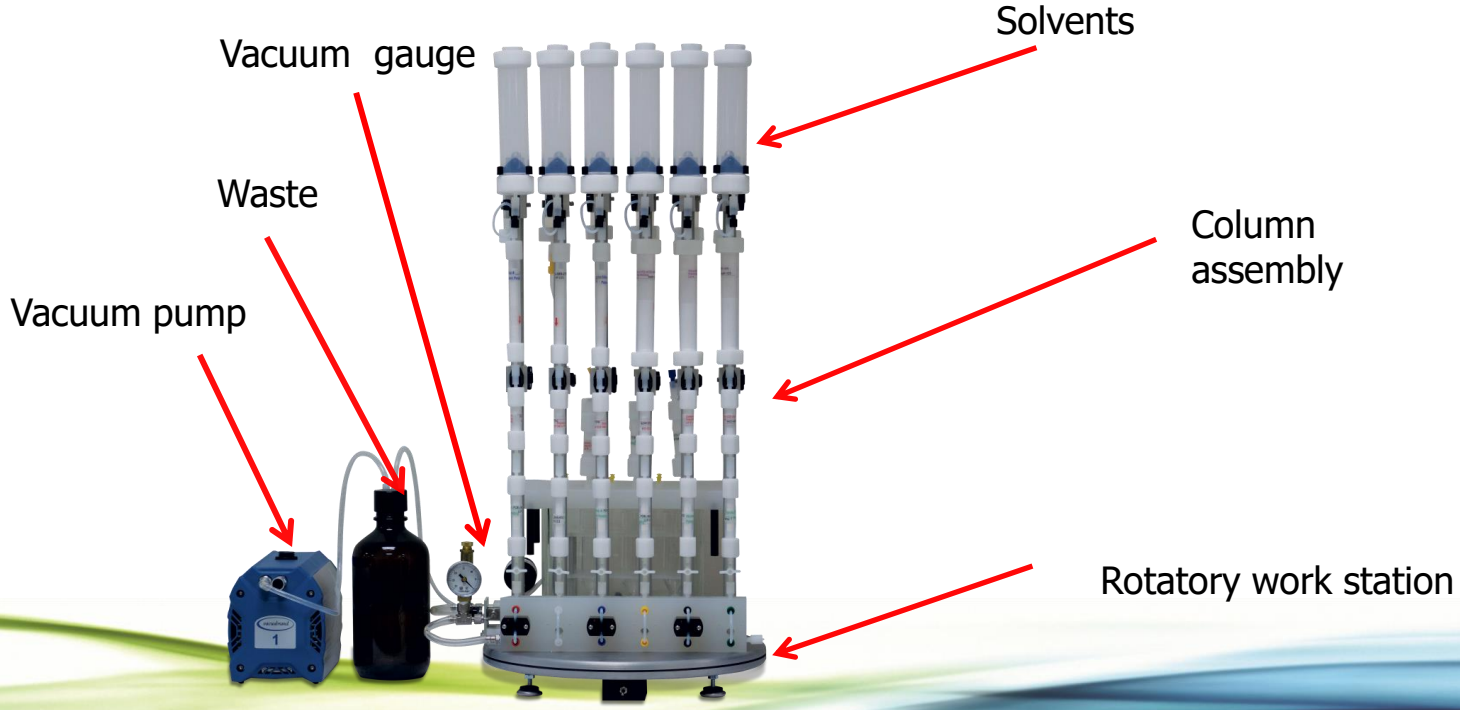
Stopcock

Vacuum Relief Valve

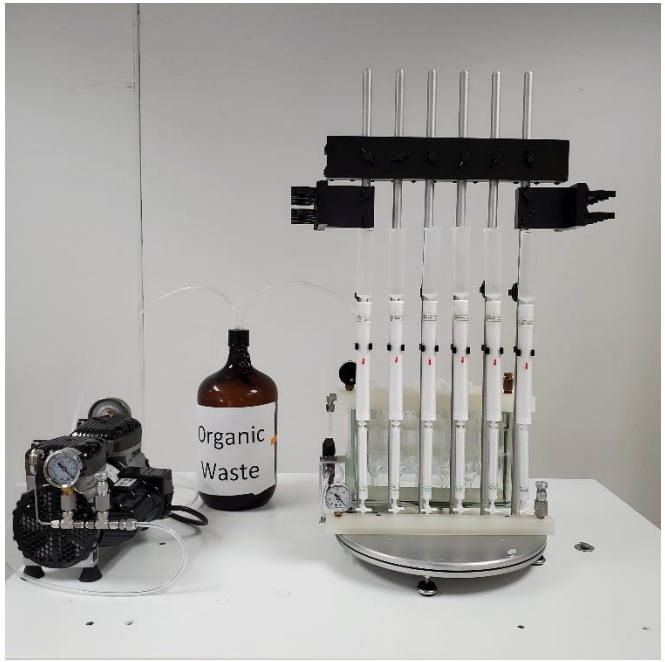
Stage 2 Manifold



Stage 1: to waste



EZPrep Stage 1

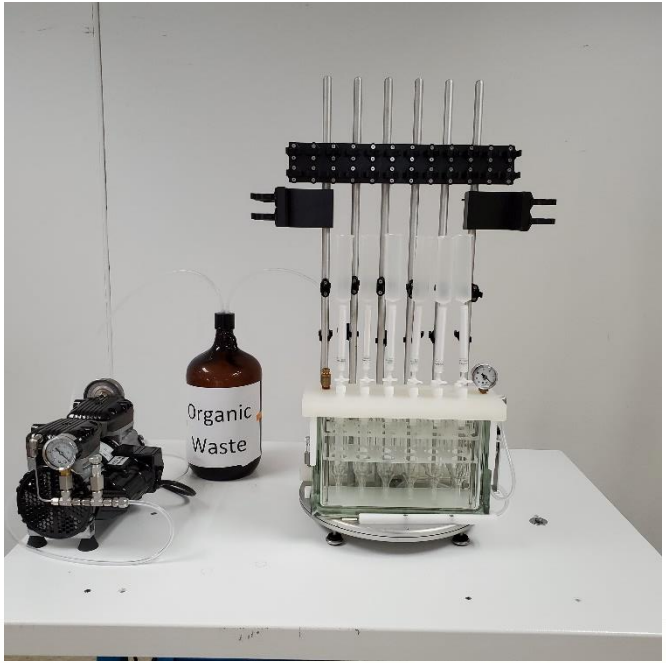


Stage 2: collect

Columns ready for elution

Manifold with collection tubes

EZPrep Stage 2 Fractions



Attributes

- **Closed loop system:**
  - **Eliminates background contaminants**
  - **No washing needed.**
  - **Capped solvent reservoirs**
- **Optimized for solvent reduction while obtaining highest possible recoveries**
- **Easy sample loading on top of silica column via injection or syringe vial**
- **Columns connect easy with SNAP connections**

Extracts in hexane - PCBs

- **Stage 1: Connect High Capacity Acid Silica and Alumina (no Carbon) and condition with 60 mL of hexane (vacuum, waste)**

- **Stage 2: Load sample (in hexane, collect Fraction # 1), rinse loading vials with hexane, elute with 160 mL hexane (collect Fraction # 1), remove acid silica, elute alumina with 50 mL dichloromethane (collect Fraction # 1)**

- **All 209 PCBs are now in Fraction # 1**

SuperVap 6 Concentrator 250 mLs

SuperVap Concentration/Evaporation

- **System pre-heated to 55-60 ^{o}C.**
- **Samples evaporated at stable T under 5-6 psi nitrogen.**
- **1 mL extract vial transferred to GC vial (can have direct-to-vial feature).**
- **Recovery standards added (nonane/dodecane).**
- **Extract taken to 10 uL volume with a gentle stream of nitrogen at ambient temperature.**

SuperVap 24 position GC vial Concentrator

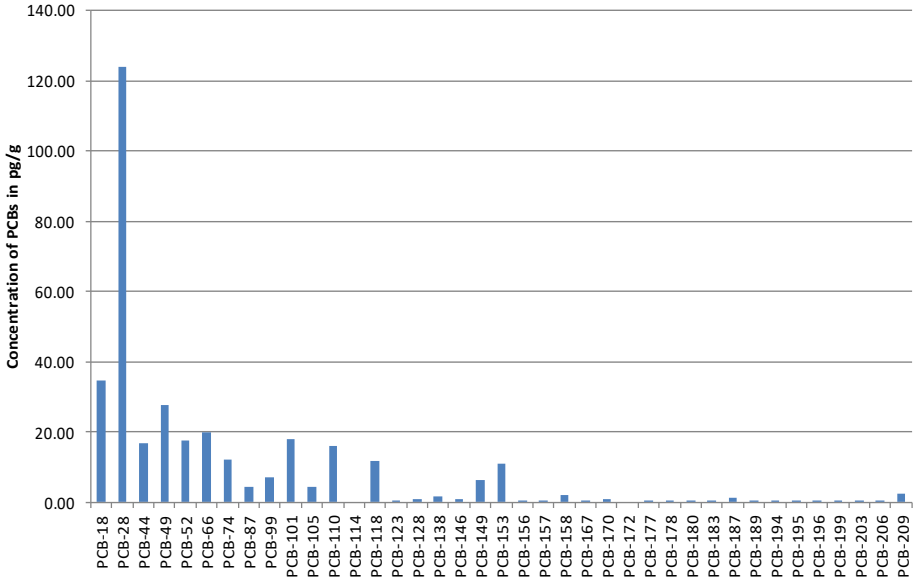
Direct-to-Vial

GC vial

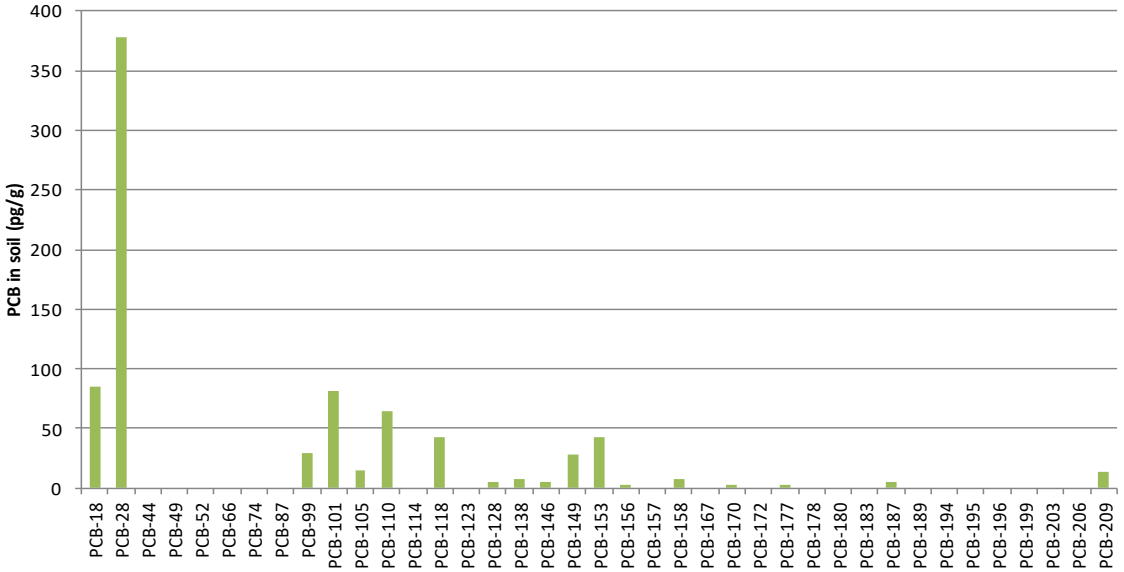
FMS
Fluid Management Systems

DFS HRGC/HRMS

Native PCBs in Serum extract



Native PCBs in Soil extract



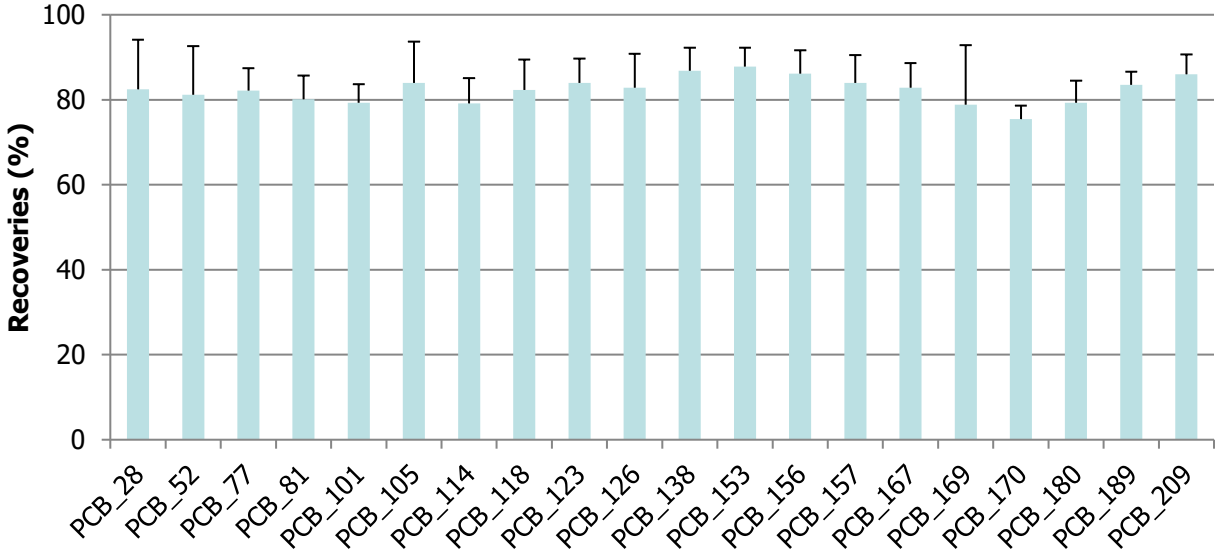
Extracts in toluene -PCBs

- Stage 1: Connect High Capacity Acid Silica and Alumina (no Carbon) and condition with 60 mL of hexane (vacuum, waste)

- Stage 2: Load sample (in 2-10 mL toluene, collect Fraction # 1), rinse loading vials with hexane, elute with 60 mL hexane (collect Fraction # 1), remove acid silica, elute alumina with 50 mL dichloromethane (collect Fraction # 1)

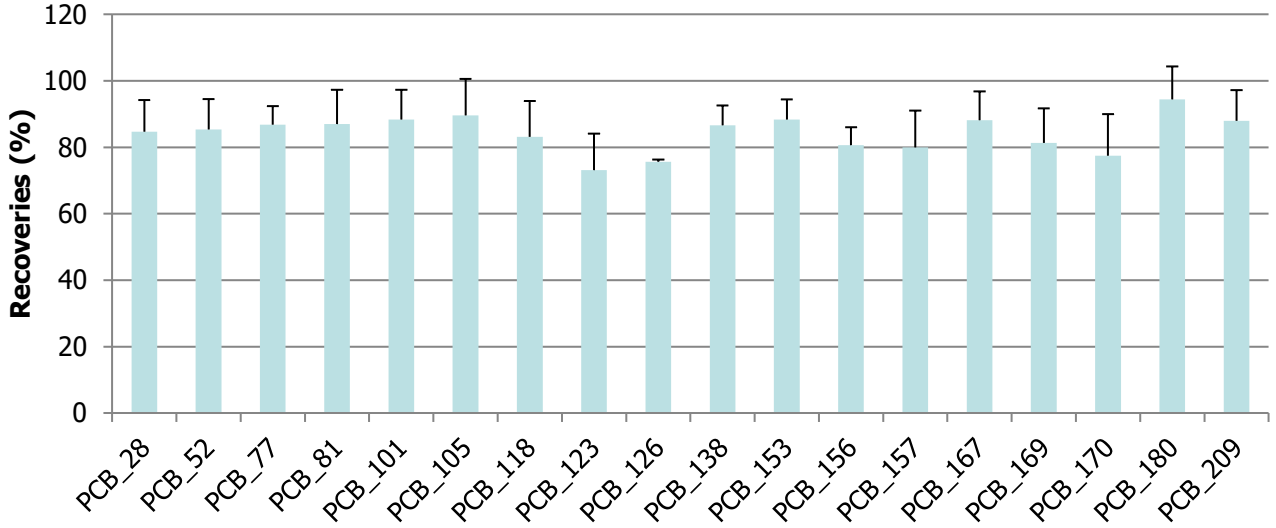
- All 209 PCBs are now in Fraction # 1

13C recoveries PCBs soil



10 g soil in toluene, n=6

13C recoveries PCBs salmon

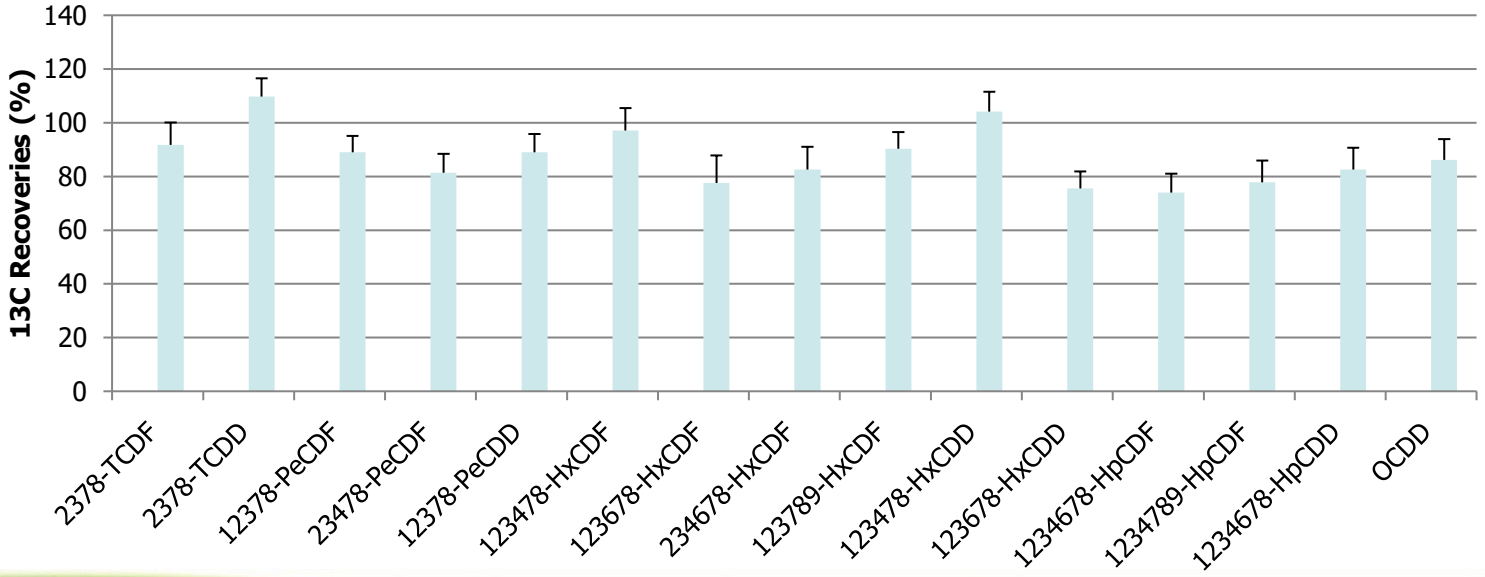


2 g salmon in toluene, n=6

Extracts in hexane - PCDD/Fs

- **Stage 1: Connect High Capacity Acid Silica and Alumina (no Carbon) and condition with 60 mL of hexane (vacuum, waste)**
- **Stage 2: Load sample (in hexane, collect Fraction # 1), rinse loading vials with hexane, elute with 160 mL hexane (collect Fraction # 1), remove acid silica, elute alumina with 30 mL 10% dichloromethane in hexane (collect Fraction # 1)**
- **All 209 PCBs are now in Fraction # 1**
- **Stage 1 Attach carbon column to alumina, elute alumina-carbon with 50 mL dichloromethane (vacuum, waste), all PCDD/Fs now on carbon**
- **Stage 2 Discard alumina, elute carbon in reverse with 50 mL toluene, collect Fraction # 2 with all PCDD/Fs**

13C Recoveries PCDD/Fs - Water

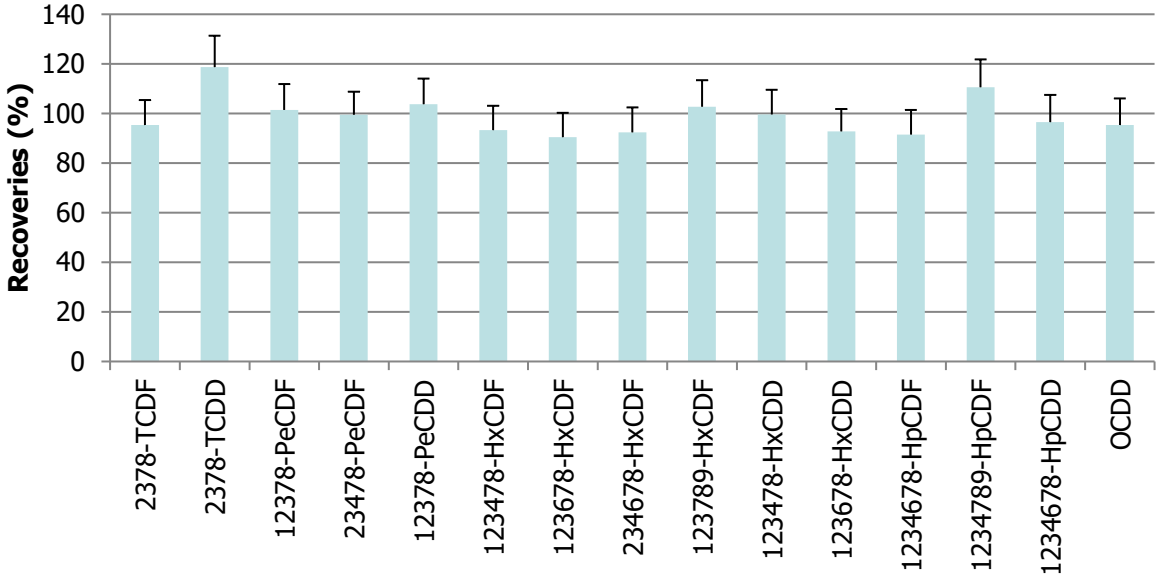


1 L water SPE , transfer to hexane, n=6

Extracts in Toluene - PCDD/Fs

- **Stage 1: Connect High Capacity Acid Silica and Alumina (no Carbon) and condition with 60 mL of hexane (vacuum, waste)**
- **Stage 2: Load sample (in 2-10 mL toluene, collect Fraction # 1), rinse loading vials with hexane, elute with 60 mL hexane (collect Fraction # 1), remove acid silica, elute alumina with 30 mL 10% dichloromethane in hexane (collect Fraction # 1)**
- **All 209 PCBs are now in Fraction # 1**
- **Stage 1 Attach carbon column to alumina, elute alumina-carbon with 50 mL dichloromethane (vacuum, waste), all PCDD/Fs now on carbon**
- **Stage 2 Discard alumina, elute carbon in reverse with 50 mL toluene, collect Fraction # 2 with all PCDD/F**

13C recoveries PCDD/Fs fish



10 g fish in toluene, n=6

Conclusions (1)

- **Samples in toluene (environmental, food): 2-10 mL toluene, separate PCBs and PCDD/Fs completely using hexane and 10% DCM/hexane, followed by DCM and toluene**
- **Reduced hexane volume needed for silica column because of presence toluene**
- **Alternative for samples in toluene: use hexane, DCM and toluene to have mono- and di-ortho PCBs in one fraction, PCDD/F/co-planary PCBs in other fraction**
- **Works also for samples in hexane but more hexane needed in that case for silica elution ("toluene effect" not present)**

Column Kits with various fat removal capacities for samples in hexane

		STAGE 1			STAGE 2		
					PCBs	Dioxins	
	Fat Removal	Hexane	Hexane	Hexane	DCM	Toluene	
Column kits	Capacity	conditioning (mL)	sample volume (mL)	Elute Silica (mL)	Alumina-carbon (mL)	Reverse Carbon (mL)	Time (min)
Classical Plus	1.0 g	20	30	100	50	50	50
High Capacity	2.5 g	40	30	160	50	50	70
Extra high Capacity	5.0 g	60	30	180	50	50	80

Conclusions (2)

- **EZPrep suitable for environmental and food analyses in toluene as solvent. Also suitable for samples in hexane**
- **Can keep PCBs and PCDD/Fs completely separate if so desired. Alternatively have co-planary PCBs in with PCDD/Fs**
- **High sample throughput → 18 samples/hour**
  - **6 samples in parallel per station**
  - **3 stations fit in one hood**
- **System gives excellent recoveries for PCDD/F and PCBs comparable to automated systems**
- **Use of certified pre-packaged columns guarantees low native background**

Conclusions (3)

- **No worries about breakdown or downtime**
- **No washing needed**
- **No cross-contamination**
- **Low cost**

EPH Sample Prep and Analysis

- Soil contamination from diesel fuel, gasoline, heating oil, jet fuel leaks, kerosene or spills is a common occurrence and a global environmental concern.
- EPA 8015B: Total Petroleum Hydrocarbon (TPH) with GC/FID (semi-volatiles)
- Petroleum has > 250 compounds, complex matrix

EPH Sample Prep and Analysis

- Extractable Petroleum Hydrocarbons (EPH): Massachusetts method
- Toxicological approach: evaluate aliphatic and aromatic compounds in extracts
- Semi volatiles evaluated: C_9-C_{36} aliphatics
- Also range of seventeen aromatics (PAHs)

Features of the MA EPH method (1)

- Method quantitates aliphatics within two ranges, $C_9 - C_{18}$ and $C_{19} - C_{36}$
- PAHs are quantitated within $C_{11} - C_{22}$ range
- Collective data reporting
- Method can determine health hazards
- Also used by other states and some Canadian provinces

Features of the MA EPH method (2)

- **Uses neutral silica cartridges or columns to separate aliphatics from aromatics in extract**
- **Aliphatics eluted with hexane, aromatics with dichloromethane**
- **Surrogates used to determine quality of separation between aliphatics and aromatics**
- **Breakthrough of naphthalene and 2-methyl naphthalene into aliphatic fraction is regulated**
- **Samples analyzed with GC/FID**

Semi-Automated approach

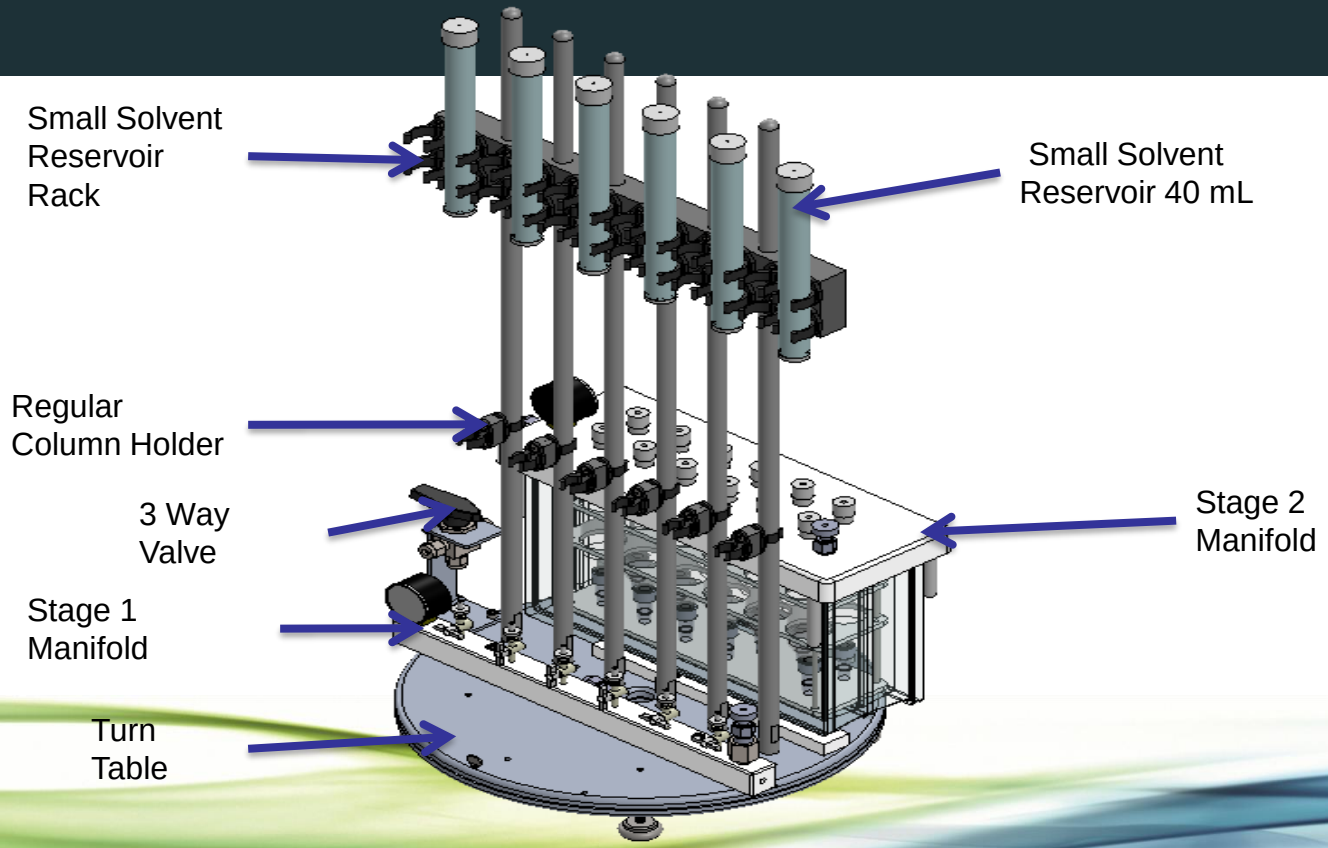
- Manual method is labor intensive, prone to error
- Certified 6 g neutral silica columns can be used with very low native background
- Less interferences in analysis
- Less glassware and solvent use

Semi-Automated System for EPH Analysis

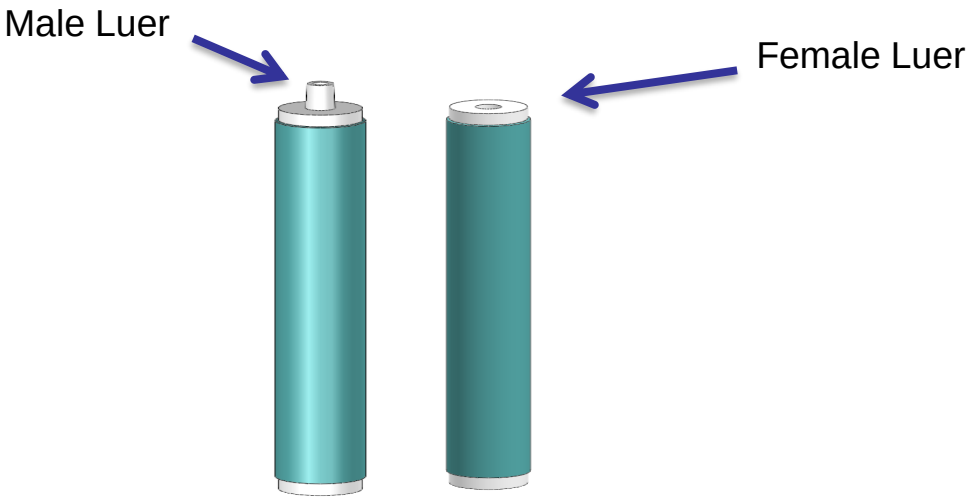
Specification:

- Simple to run, no computerized instrumentation
- Fast: 20 min
- Closed loop system to give a clean background, low level detection
- Use certified columns
- One column per sample
- No capital equipment cost
- No electronics or mechanical equipment to fail
- No downtime

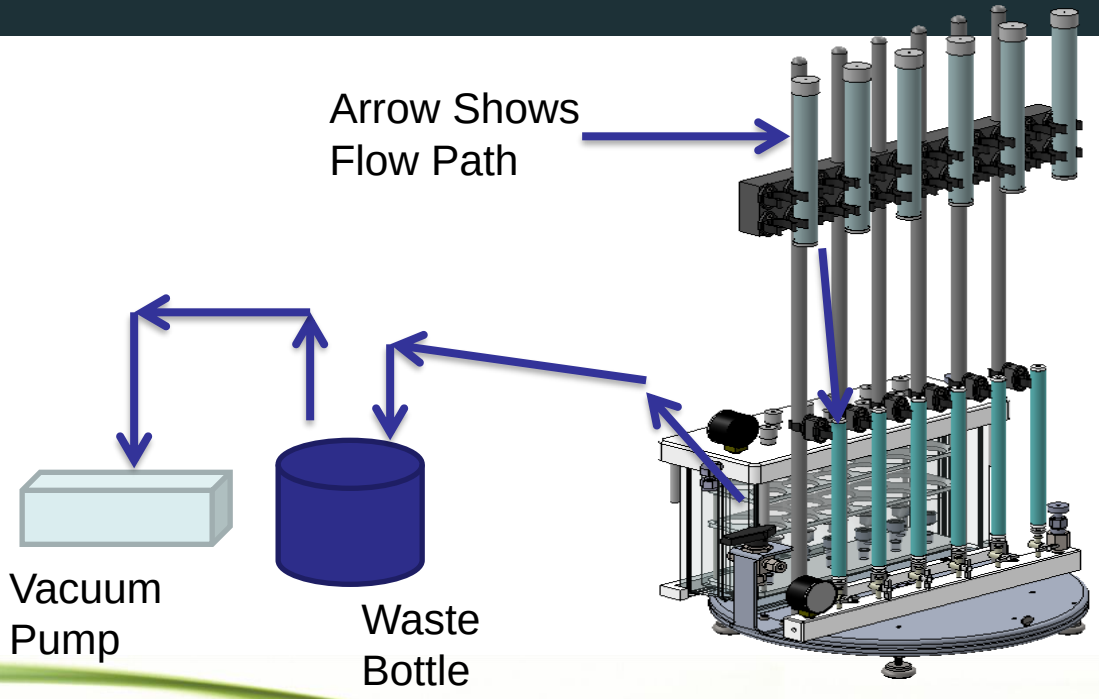
Semi-Automated EZEPH System for EPH



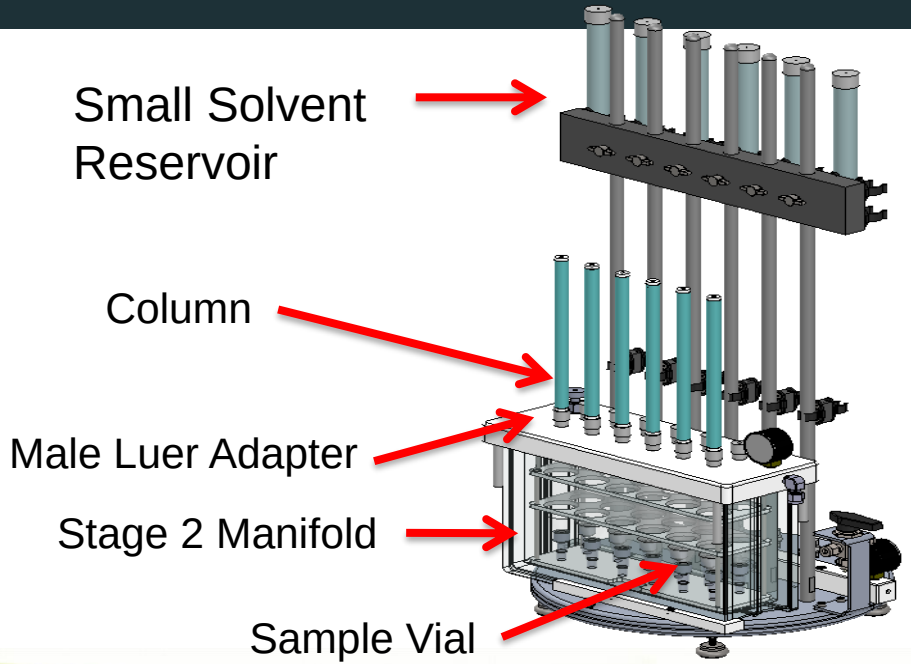
Neutral silica columns



Flow thru system (Stage 1)



Collection (Stage 2)



EZEPH Procedure Stage 1

- **Stage 1:**
- **Assemble silica column with EZPrep set-up**
- **Syringe vial at top is used for conditioning and sample loading**
- **Condition silica column with 30 mL hexane (vacuum, waste)**

FMS
Fluid Management Systems

EZEPH

EZEPH Procedure Stage 2

- **Stage 2:**
- **Dilute sample extract to 9 mL hexane and spike surrogate compounds (dissolved in 1 mL hexane) into sample extract**
- **Load sample extract onto silica column**
- **Elute column with 10 mL hexane, collecting aliphatic fraction**
- **Elute column with 35 mL dichloromethane, collecting aromatic fraction**

EZEPH

SuperVap 12 50 mLs

Evaporation and Analysis

- **System pre-heated to 30 ºC.**
- **Samples evaporated at stable T under 5-6 psi nitrogen.**
- **1 mL extract vial transferred to GC vial (can have direct-to-vial feature)**
- **Analyze on Agilent GC/FID**
- **Samples (hexane) were spiked with 2.5, 12.5 or 25 ug/mL aliphatic and aromatic standards and surrogates before cleanup**

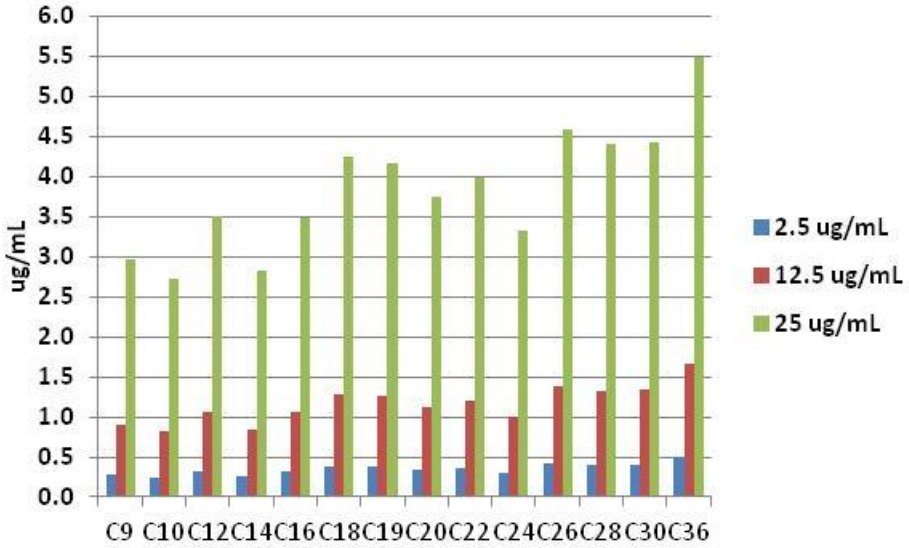
Aliphatic Recoveries (25 ug/mL)

	Average Recoveries (%)	RSD (%)	EPA Limit (%)
Nonane (C9)	74.7	7.3	30-130
Decane (C10)	78.6	8.4	40-140
Dodecane (C12)	80.9	4.5	40-140
Tetradecane (C14)	87.0	5.0	40-140
Hexadecane (C16)	81.4	3.9	40-140
Octadecane (C18)	85.6	3.3	40-140
Nonadecane (C19)	88.6	3.5	40-140
Eicosane (C20)	91.5	4.1	40-140
Docosane (C22)	92.6	4.9	40-140
Tetracosane (C24)	93.2	4.9	40-140
Hexacosane (C26)	93.2	4.8	40-140
Octacosane (C28)	92.4	4.7	40-140
Triacontane (C30)	92.9	4.5	40-140
Hexatriacontane (C36)	98.0	3.9	40-140

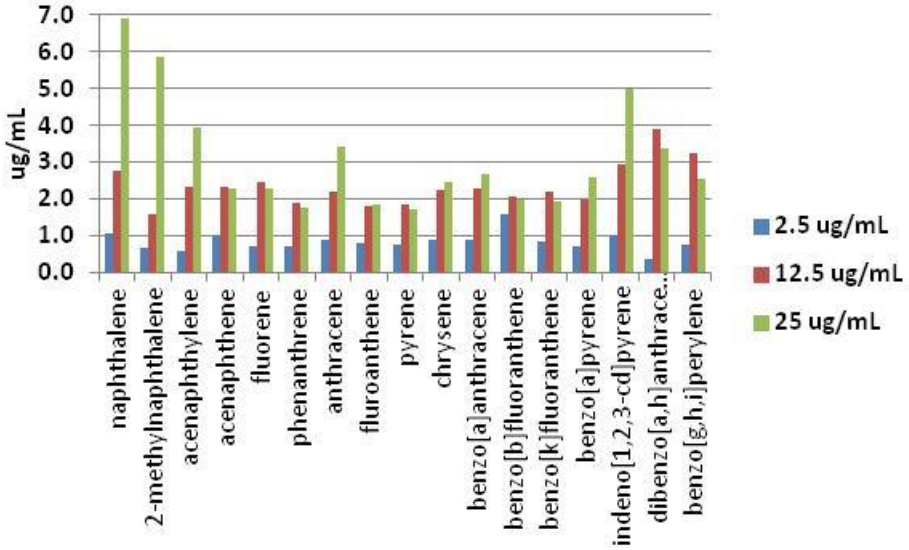
Aromatic Recoveries (25 ug/mL)

	Average Recoveries (%)	RSD (%)	EPA Limit (%)
naphthalene	110.5	6.7	40-140
2-methylnaphthalene	104.2	6.3	40-140
acenaphthylene	94.4	3.5	40-140
acenaphthene	99.3	2.5	40-140
fluorene	107.4	1.8	40-140
phenanthrene	109.0	1.9	40-140
anthracene	103.1	2.4	40-140
fluroanthene	104.8	1.8	40-140
pyrene	103.0	1.7	40-140
chrysene	97.1	2.2	40-140
benzo[a]anthracene	109.6	2.9	40-140
benzo[b]fluoranthene	111.9	1.9	40-140
benzo[k]fluoranthene	109.0	2.3	40-140
benzo[a]pyrene	98.0	2.3	40-140
indeno[1,2,3-cd]pyrene	111.6	3.1	40-140
dibenzo[a,h]anthracene	96.1	2.9	40-140
benzo[g,h,i]perylene	103.7	3.5	40-140

Method Detection Limit Aliphatics



Method Detection Limit Aromatics



Breakthrough

- Average naphthalene breakthrough in aliphatic fraction < 0.02 (limit is < 0.05)
- Average 2-methyl naphthalene breakthrough in aliphatic fraction < 0.01 (limit is < 0.05)

Surrogates (limit 40-140%)

- 1-chloro-octadecane: 83% ± 12%
- O-terphenyl: 89% ± 14%
- 2-bromonaphthalene: 70% ± 13%
- 2-fluorbiphenyl: 104% ± 8%

Comparison lab X vs EZPrep EPH

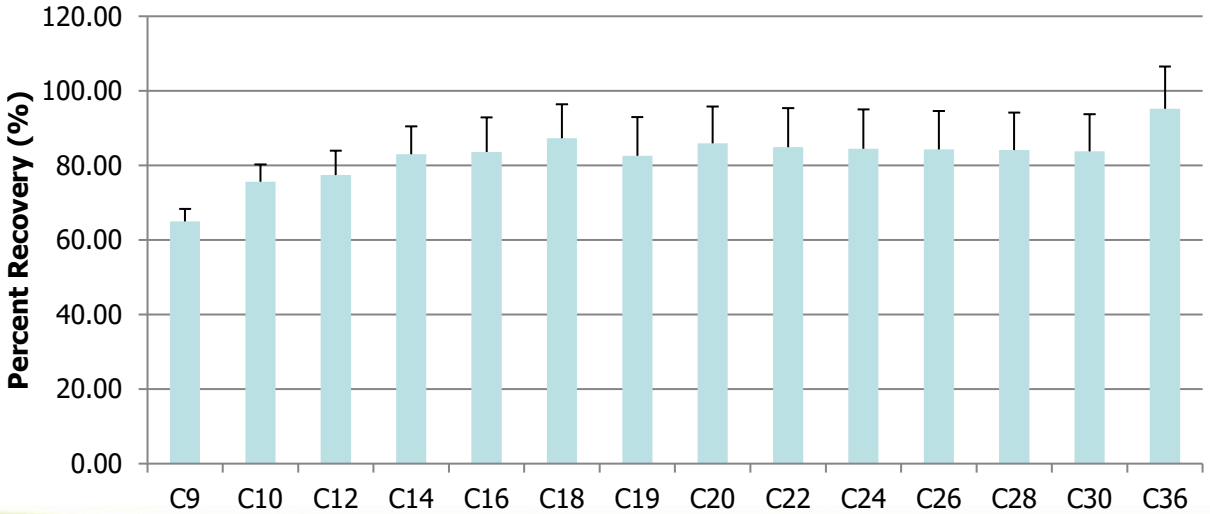
	C9-C18 Aliphatic		C19-C36 Aliphatic		C11-C22 Aromatic	
	Other Lab	Toxic Report	Other Lab	Toxic Report	Other Lab	Toxic Report
Sample 1	17	16	144	115	191	176
Sample 2	410	292	4314	3925	1313	1019
Sample 3	185	136	2335	2222	797	412
Sample 4	33	58	57	41	88	85
Sample 5	28	46	50	68	171	163
Sample 6	82	58	188	89	140	178
Sample 7	16	30	183	171	241	226
Sample 8	22	22	152	138	181	204
Sample 9	27	44	119	93	213	215
Sample 10	2931	2167	1232	1574	a	a
Sample 11	171	128	89	64	113	110
Sample 12	19	20	38	40	33	73
Sample 13	245	135	198	100	682	340
Sample 14	61	69	364	240	334	252
Sample 15	113	91	447	200	860	740
Sample 16	39	51	17	28	23	25

Extracts from commercial lab vs Toxic Reports lab Data in ug/mL

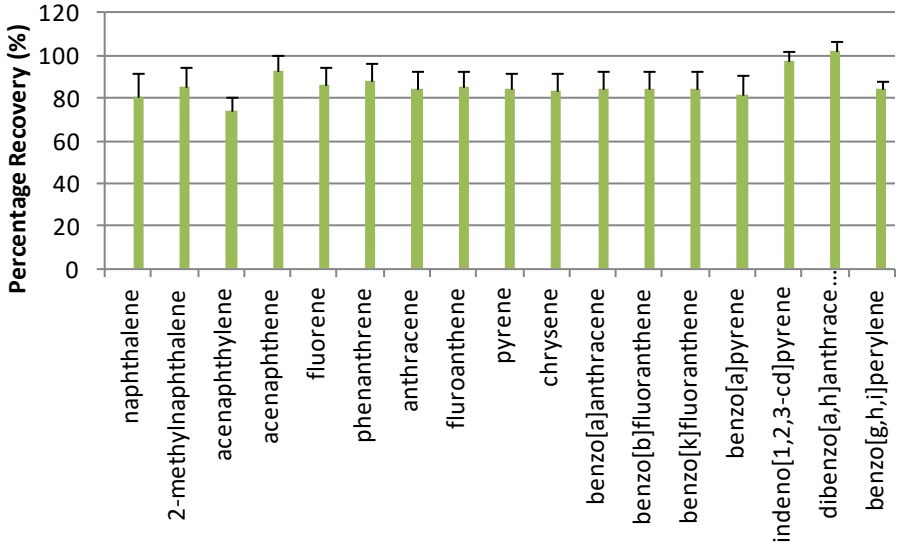
Washington State Method

- **Stage 1: As before but condition 6 g neutral silica column with 10 mL pentane**
- **Stage 2: Spike surrogate compounds in 1 mL pentane or sample extract**
- **Load sample extract onto silica column**
- **Elute column with 15 mL pentane, collecting aliphatic fraction**
- **Elute column with 40 mL dichloromethane, collecting aromatic fraction**

Washington State aliphatics



Washington State aromatics



Conclusions

- Excellent recoveries for aliphatics and aromatics with low RSDs
- All well within MA and WA method windows
- Very good MDL data
- Breakthrough of naphthalene's well within limits
- All surrogates give very good recoveries
- Comparison semi-automated method with manual method for commercial lab samples shows very good agreement
- Can process 6 samples in parallel in 20 min

FMS
Fluid Management Systems

Questions