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Simultaneous Extraction of PAHs and PCBs from Tissue and Soil Samples Using a New Accelerated Solvent Extraction (ASE) Procedure

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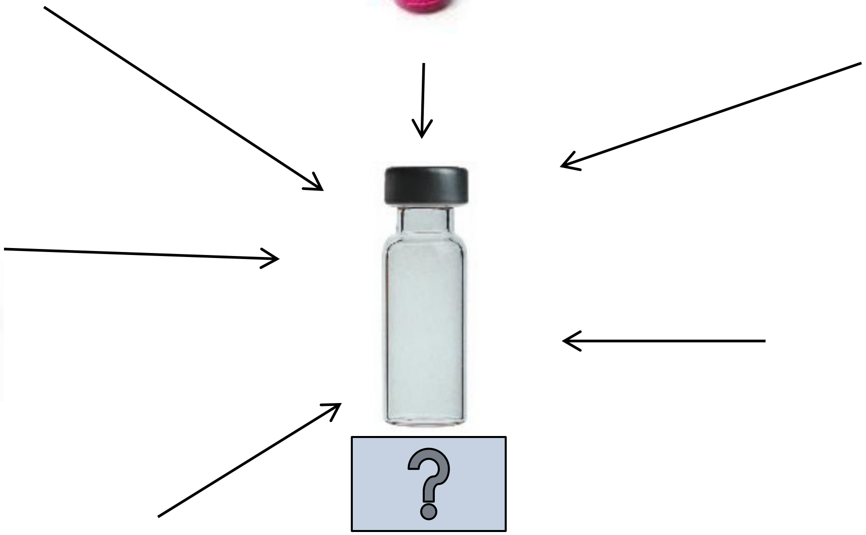
The Challenge for Analysis



1.5 mL GC / LC Vial

How do we get analytes out of these samples?

The Answer is Sample Preparation

- Extraction
  - Removes analytes from the sample
  - Eliminates compounds that interfere with the analysis (Clean Up)

- Evaporation
  - Concentrates extracted analytes for analysis
  - Evaporates extracted samples for re-constitution

- Most time consuming part of analytical procedure (>60%)*

- Single largest source of errors in the workflow (>30%)*

*Majors, R.E. *LC-GC*, **1995**, *13*, 742-749, and **Majors, R.E. *LC-GC*, **1999**, *17*, S8 - S13

Importance of Sample Preparation

"Eighty Percent of the Variance in an Assay Usually Arises from the Sample Prep."

R. Stevenson, "Pittcon® '98: Part 3 Sample Prep: The Place to Make a Difference" American Laboratory, Vol. 30, No. 14 p.21, 1998

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Analysis Techniques!

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Sample Preparation…

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The Important Parameters for Sample Prep

- Solvent Use
  - Amount of solvent consumed for the extraction
  - Solvents are expensive; reducing use reduces costs
- Extraction Time
  - Amount of time required for each extraction to occur
  - Reducing extraction time increases lab throughput
- % Recovery
  - Amount of analyte recovered following the extraction
  - Low % recovery yields poor analytical results
- % Relative Standard Deviation (RSD)
  - Measure of reproducibility between extractions
  - Extraction results have greater reproducibility with lower %RSDs

These Parameters Evaluate SP Techniques

Thermo Scientific Dionex Sample Prep Product Line

Thermo Scientific™ Dionex ™ASE™ 150 and 350 Accelerated Solvent Extractor

Thermo Scientific Dionex AutoTrace™ 280 Solid-Phase Extraction (SPE) Instrument

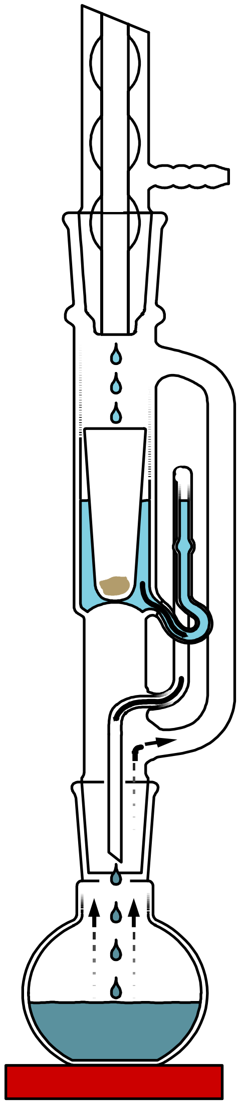
Thermo Scientific Dionex SolEx™ SPE Cartridges

Genevac Rocket™ Evaporator

Novel & Innovative Solutions

In the Beginning, There Was Soxhlet . . .

- 1879 Franz von Soxhlet
- *de facto* standard for solvent extraction
- Slow, high solvent usage



Key Parameters for Liquid-Solid Extraction

- Temperature
  - Increases analyte solubility
  - Increases diffusion rate
  - Decreases solvent viscosity
- Solubility
  - Increases as temperature increases
  - E.g. Anthracene solubility increases 13 fold in DCM (50^{o}C to 150^{o}C)
- Viscosity
  - Decreases as temperature increases
  - Allows solvents to migrate through the matrix easier
- Surface Tension
  - Increased temperature decreases solvent surface tension
  - Lower surface tension allows the solvent to better cover the matrix

Now . . . Accelerated Solvent Extraction

- Automates sample preparation for solid and semisolid samples using solvents at elevated temperatures and pressure.
- Operates above the boiling point of extraction solvents by using sealed extraction cells.
- Pressure is used to keep solvents liquid during extraction (1500 psi).
- Well established and proven technique that is superior to Soxhlet and approved for U.S. EPA Method 3545A.

Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system

ASE® Technology

- Patented flow-through design allows:
  - In-line filtration
  - In-cell cleanup
  - Fractionation or selectivity
- Control of temperature and pressure for each individual sample:
  - Requires small quantities of solvent and short periods of time
  - 50 mL vs. hundreds of mL
  - 15 min vs. several hours
- Multiple cell sizes support a wide range of sample sizes (1–100 g).
- Ability to extract up to 24 samples in a single batch.

How Does Accelerated Solvent Extraction Work?

Cell loading

↓

Fill, heat, equilibrate (min) 5 to 9 — dynamic extraction

↓

Static extraction 0 to 99 — static extraction

↓ (cycle ↑)

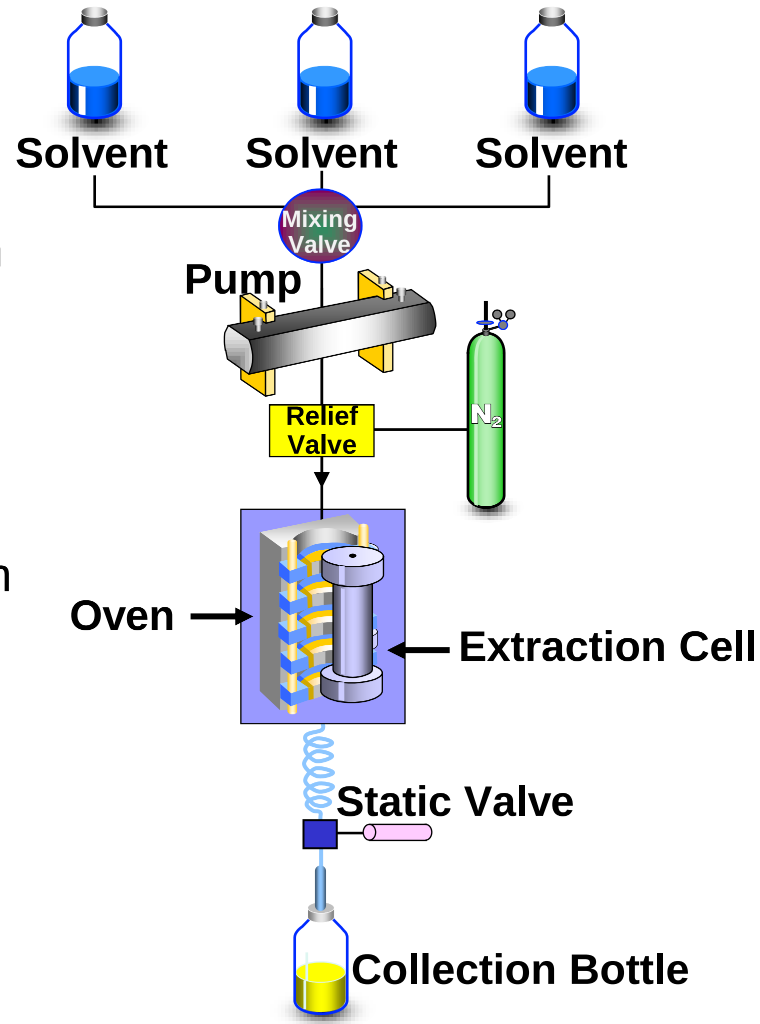
Rinse with fresh solvent 0.5 — dynamic extraction

↓

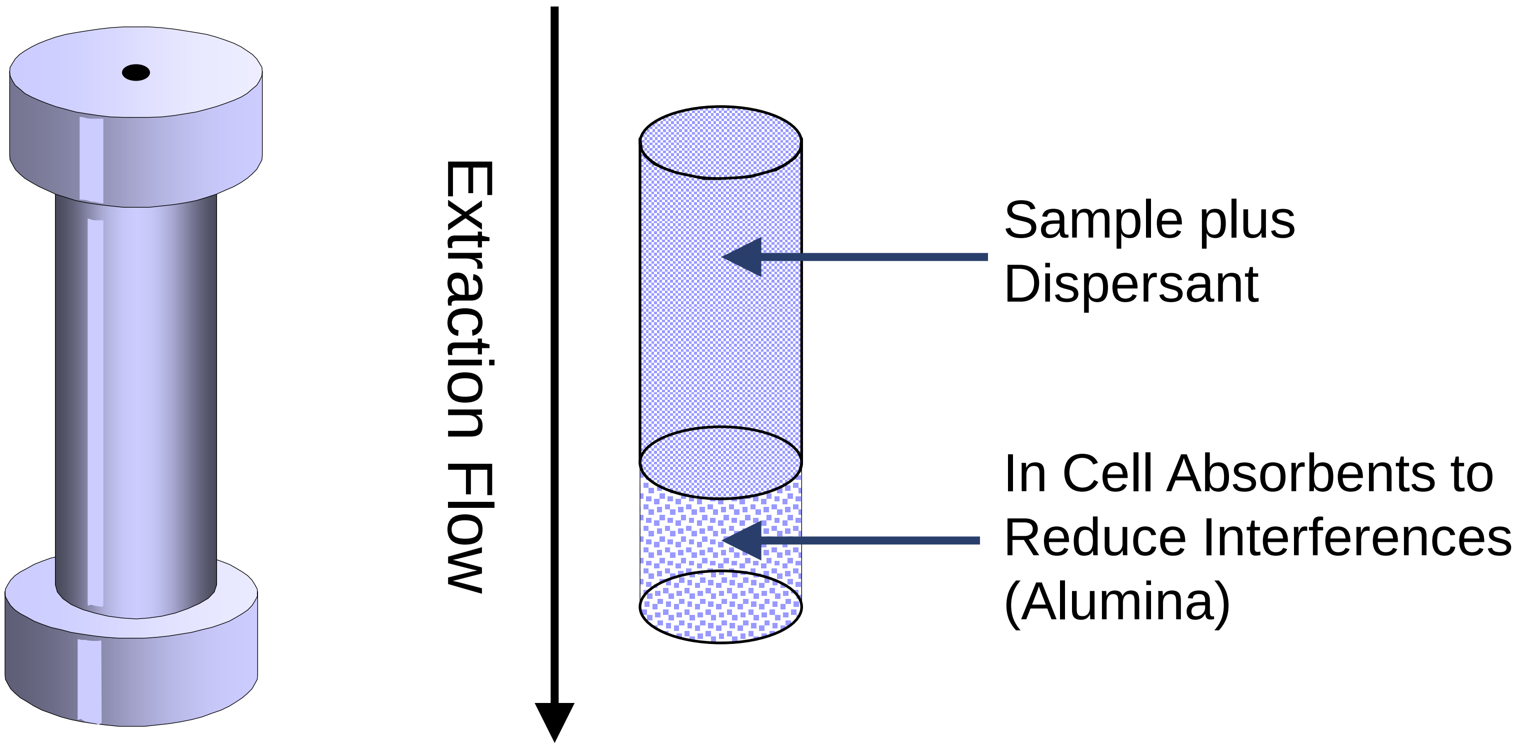
Purge with nitrogen 1–2

↓

Extract ready — Total (min) 12–20



Schematic of Selective Extraction Using ASE®



Simultaneous PAH and PCB Extraction with ASE

- The ability to perform a simultaneous extraction of PAHs and PCBs in a single method was evaluated using Accelerated Solvent Extraction.
- Extractions were performed on both spiked mussel and spiked soil samples to evaluate the % recovery and %RSD for PAHs and PCBs.
- Analytical determinations were made using GC-MS for the PAHs and GC-ECD for the PCBs.

Accelerated Solvent Extraction Conditions

Table 1. Accelerated solvent extraction conditions.

	Method 1	Method 2
System Pressure	10 MPa (1500 psi)	10 MPa (1500 psi)
Oven Temperature	125 °C	100 °C
Sample Size	5 g	5 g
Oven Heatup Time	6 min	5 min
Static Time	6 min	4 min
Static Cycles	4	5
Rinse Volume	40 mLs (60% of extraction cell volume)	40 mLs (60% of extraction cell volume)
Solvent	Dichloromethane	Dichloromethane
Nitrogen Purge	300 s	300 s
Extraction Time	30 min	25 min
Cell Size	66 mLs	66 mLs

Sample Preparation

- Both commercially purchased mussel and contaminated soil were spiked with PAH, Base/Neutral, PCB, and Arochlor surrogates.
  - PAH & Base/Neutral Surrogates = 5.0 µg/g
  - PCB = 30 ng/g
  - Arochlor = 2 mg/g
- Both samples were mixed with 10 g of DE dispersant, homogenized, and added to a 66 mL extraction cell.
- 20 g of acidic alumina were added to each cell prior to sample introduction.

GC-ECD / GC-MS Conditions

GC-MS Conditions	
Column:	5% Diphenyl Capillary Column 30 m × 0.25 mm i.d., d_f = 0.5 µm
Injection Port Temperature:	280 °C
Injection Mode:	Splitless
Column Flow Rate:	1.4 (mL/min) constant flow
Oven Temp.:	50 °C (hold for 1 min) to 320 °C at 6 °C/min (hold for 10 mins)

GC-ECD Conditions	
Column:	Capillary Column 40 m × 0.18 mm i.d., d_f = 0.18 µm
Injection Port Temperature:	250 °C
Injection Mode:	Splitless
Purge Time:	1.00 min
Makeup Gas:	Nitrogen
Column Flow Rate:	1.5 (mL/min) constant flow
Oven Temp.:	100 °C (hold 1 for min) to 200 °C at 30 °C/min to 320 °C at 2 °C/min (hold 2 min)

Extraction Method 1 – Mussel & Soil

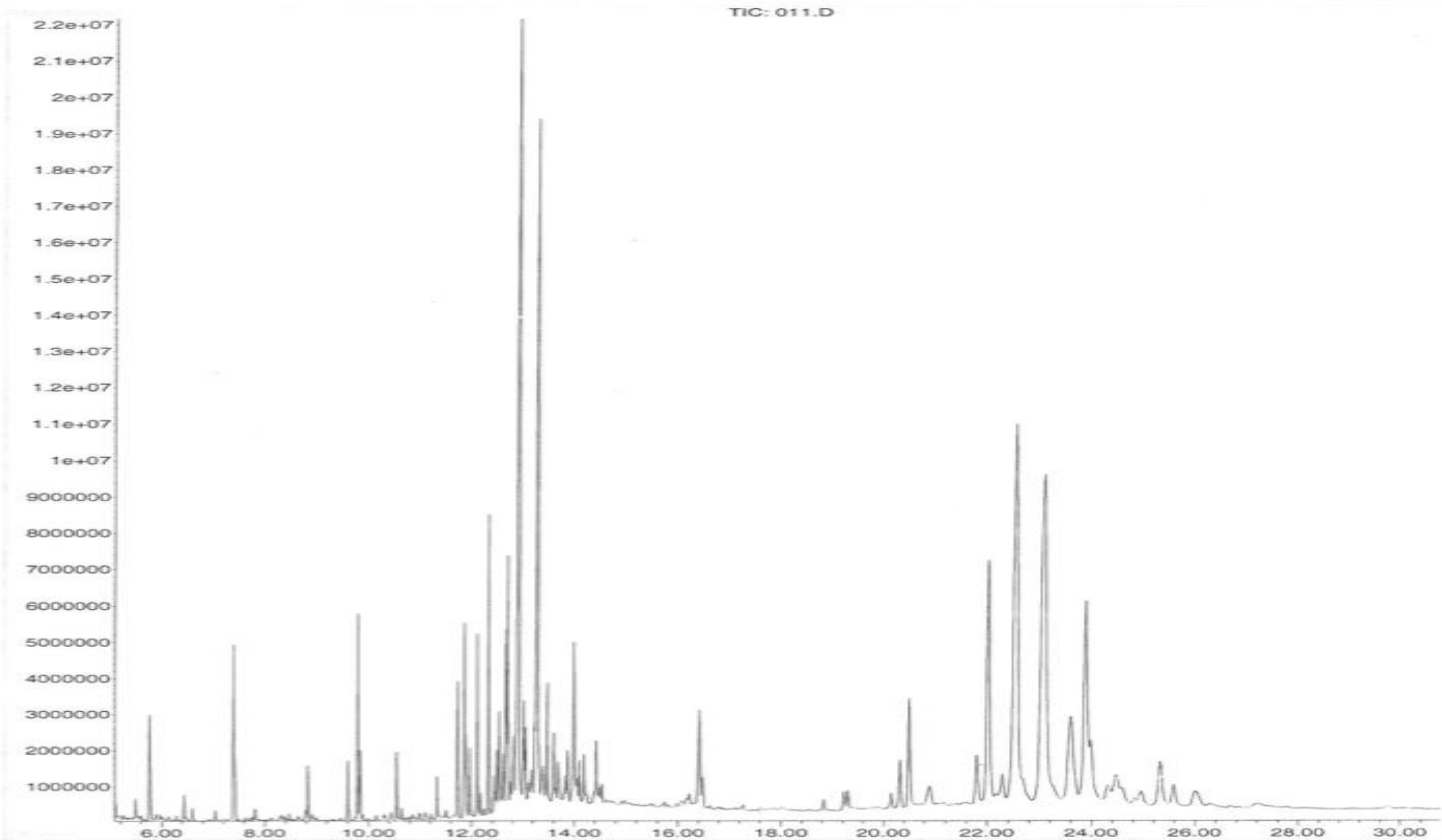
Table 2. Data for mussel and soil samples extracted by Method 1.

PAH Recoveries – Mussel (N = 6)				PAH Recoveries – Soil (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**	**Compound**	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	83.3	0.54	13.05	Nitrobenzene-d5**	94.6	0.81	17.20
2-Fluorobiphenyl**	95.1	0.43	9.13	2-Fluorobiphenyl**	101.2	0.25	4.87
p-Terphenyl-d4**	91.4	0.27	5.92	p-Terphenyl-d4**	102.1	0.10	1.94
Naphthalene	89.1	0.28	6.33	Naphthalene	79.0	0.47	6.29
Acenaphthylene	101.2	0.30	5.91	Acenaphthylene	76.3	0.21	5.44
Acenaphthene	98.3	0.28	5.65	Acenaphthene	102.9	0.33	6.40
Fluorene	107.5	0.46	8.65	Fluorene	80.3	0.21	5.31
Phenanthrene	104.6	0.30	5.70	Phenanthrene	114.8	0.37	6.39
Anthracene	100.1	0.29	5.77	Anthracene	91.4	0.51	11.19
Fluoranthene	97.1	0.30	6.24	Fluoranthene	103.6	0.12	2.23
Pyrene	88.9	0.24	5.31	Pyrene	97.4	0.14	2.90
Benzo(a)anthracene	85.4	0.21	4.85	Benzo(a)anthracene	99.0	0.17	3.35
Chrysene	95.5	0.27	5.66	Chrysene	91.2	0.09	1.90
Benzo(b)fluoranthene	91.7	0.31	6.72	Benzo(b)fluoranthene	96.3	0.14	2.82
Benzo(k)fluoranthene	88.3	0.20	4.43	Benzo(k)fluoranthene	92.8	0.13	2.70
Benzo(a)pyrene	89.9	0.28	6.29	Benzo(a)pyrene	83.0	0.23	5.52
Benzo(ghi)perylene	94.1	0.31	6.60	Benzo(ghi)perylene	82.4	0.13	3.22
Dibenzo(a,h)anthracene	92.3	0.28	6.06	Dibenzo(a,h)anthracene	78.9	0.15	3.68
Indeno(1,2,3-cd) pyrene	91.1	0.31	6.72	Indeno(1,2,3-cd) pyrene	84.6	0.11	2.65
PCB Recoveries – Mussel (N = 6)				**PCB Recoveries – Soil (N = 6)**			
Compound	**% Recovery**	**SD**	**% RSD**	**Compound**	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	93.1	0.48	5.21	2,4,5,6-tetrachloro-m-xylene**	86.7	1.2	4.72
Aroclor 1254	95.9	0.06	3.26	Aroclor 1254	101.6	0.19	3.15

**Surrogate Spike

Recovery Ranges from 83 – 107 %

Extraction Method 1 – Mussel Tissue



Interference due to Co-Extractable Compounds

Extraction Method 2 - Mussel Tissue

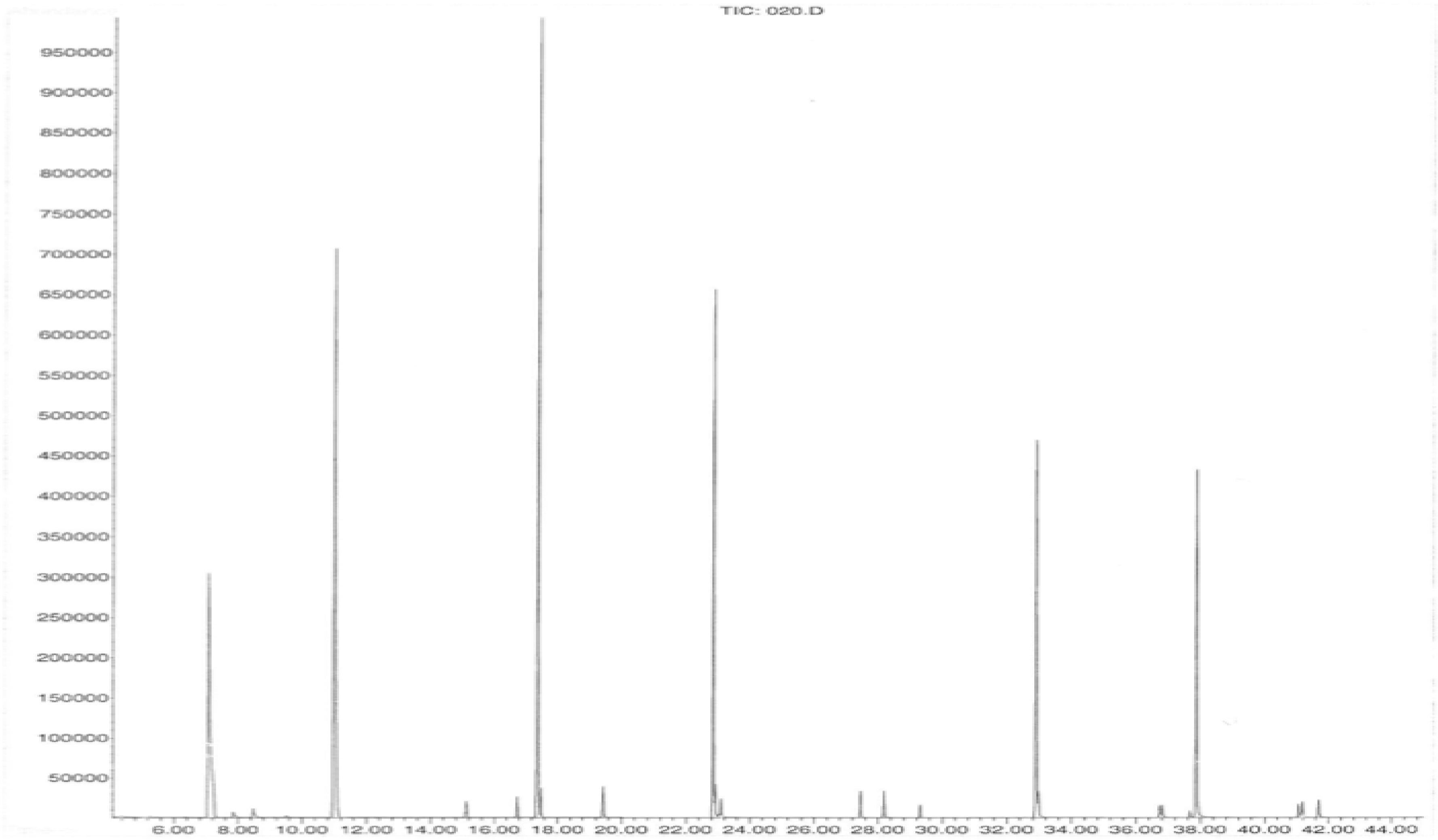
Table 3. Data for mussel samples extracted by Method 2.

PAH Recoveries - Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	84.8	0.11	12.46
2-Fluorobiphenyl**	112.3	0.06	5.12
p-Terphenyl-d4**	105.8	0.10	9.09
Naphthalene	72.5	0.08	10.85
Acenaphthylene	82.3	0.09	10.50
Acenaphthene	81.2	0.07	9.20
Fluorene	79.5	0.06	7.41
Phenanthrene	95.3	0.06	6.49
Anthracene	85.2	0.07	8.01
Fluoranthene	90.8	0.08	8.43
Pyrene	86.2	0.07	7.82
Benzo(a)anthracene	84.7	0.09	10.48
Chrysene	114.0	0.11	9.99
Benzo(b)fluoranthene	89.2	0.07	7.97
Benzo(k)fluoranthene	84.7	0.05	5.33
Benzo(a)pyrene	77.7	0.08	10.39
Benzo(ghi)perylene	87.5	0.14	16.46
Dibenzo(a,h)anthracene	77.7	0.08	10.85
Indeno(1,2,3-cd) pyrene	83.5	0.07	7.97
PCB Recoveries – Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	94.67	3.75	3.96
Aroclor 1254	85.68	1.87	2.18

**Surrogate Spike

Recovery Ranges from 83 – 114%

Extraction Method 2 – Mussel Tissue



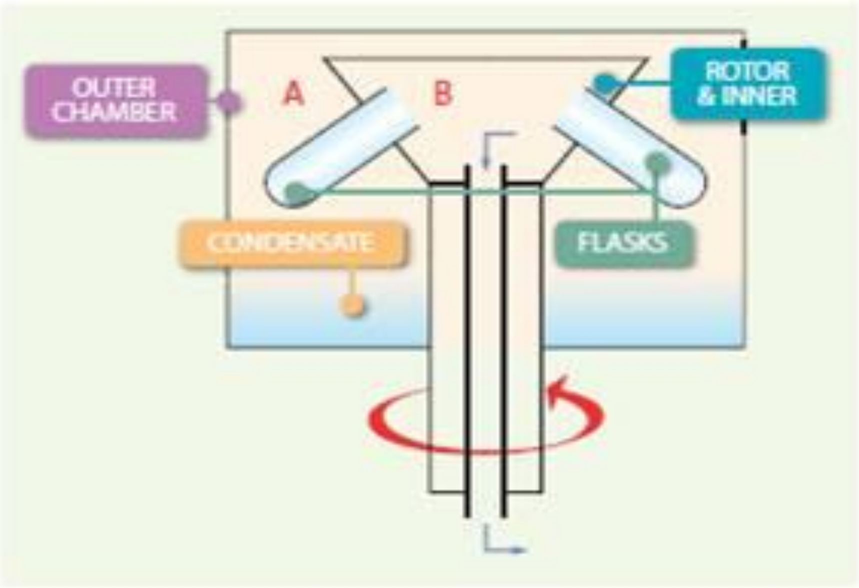
No Interferences from Co-Extracting Compounds

System Overview

Working principle

- Developed to handle larger numbers or higher volume samples
- Dries or concentrate up to 6 x 450 mL flasks or 18 x 60 mL ASE tubes
- Sample Genie Flasks that evaporate or concentrate into multiple vial sizes
- **Why spend time on evaporation when it can be used for science?**

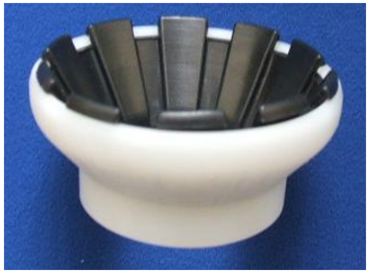
Working Principle



- Samples are loaded into the rotor and placed under vacuum (B);
- Low pressure steam is used to heat the samples (A);
- The steam condenses on the flasks/tubes, which are cold due to the solvent(s) boiling inside them (A);
- Condensate is thrown off the spinning flask and recycled (A).

Sample Genie For Evaporating or Concentrating

- Dries sample directly into storage vial
- Works with vials from 12 – 28 mm diameter & up 70 mm height



- Concentrates sample into GC autosampler vials
- Vial is insulated so that only solvent in flask evaporates

60 mL ASE Vials for Direct Transfer

- Pucks accept 60 mL ASE vials
- Each puck accepts 3 vials
  - Total capacity per system is 18 vials
- Working volume is 55 mL each

60 mL ASE Vial Flip Flop

- GC Vial is inserted into the flip flop funnel
- GC vial is insulted to prevent boiling of solvent
- ASE vial is inserted directly into the Rotor
- GC vial is placed directly into autosampler once complete

How Fast Does it Go?

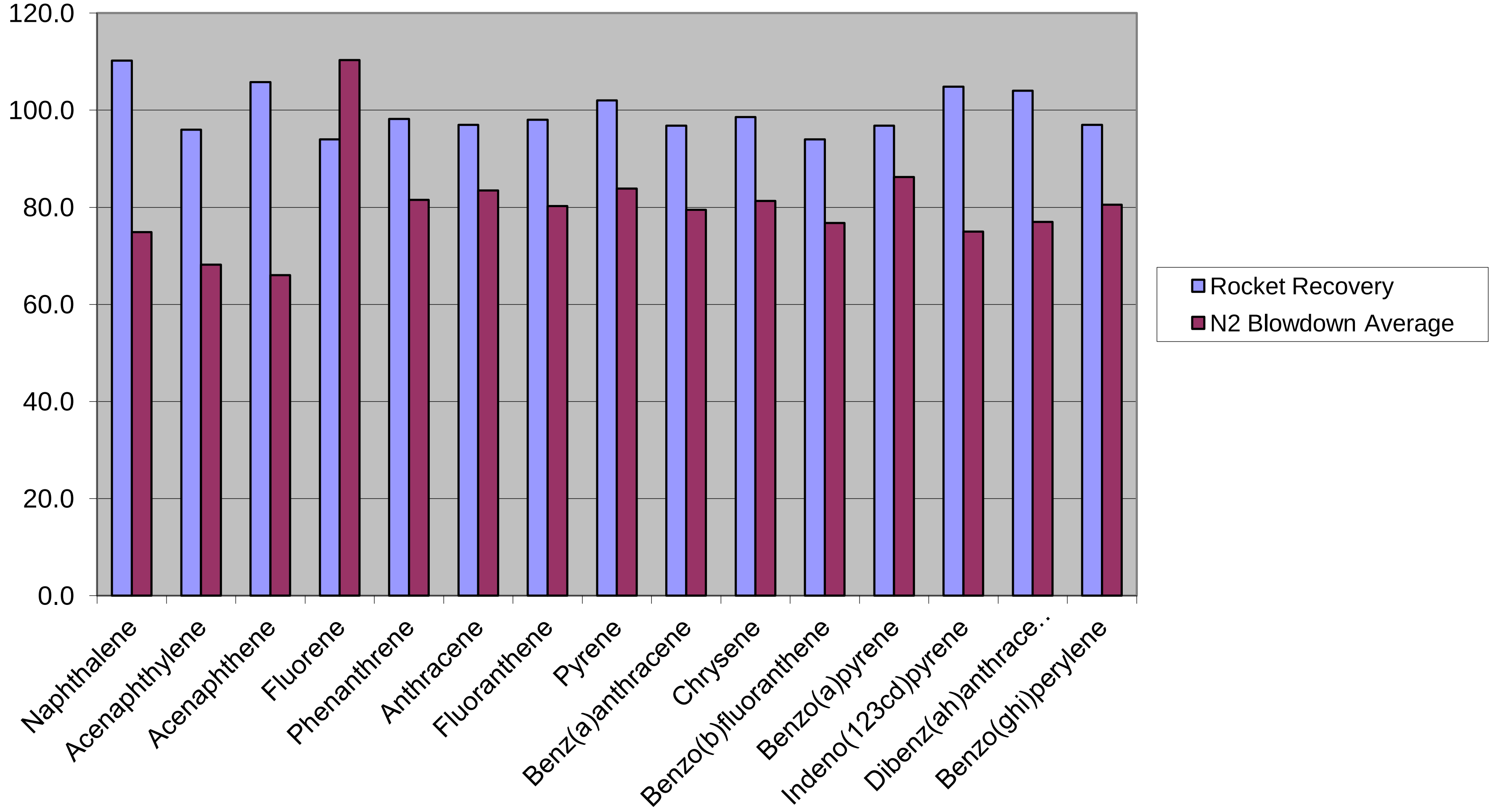
	100 mL	250 mL	450 mL
DCM (dichloromethane)	10 min	20 min	35 min
Methanol	20 min	45 min	1.5 h
DMF	30 min	1 h	2 h
Water	35 min	1.5 h	2 h
Water/ACN (1:1)	1 h	1.5 h	3 h

Times are given for complete dryness for 6 flasks simultaneously evaporated

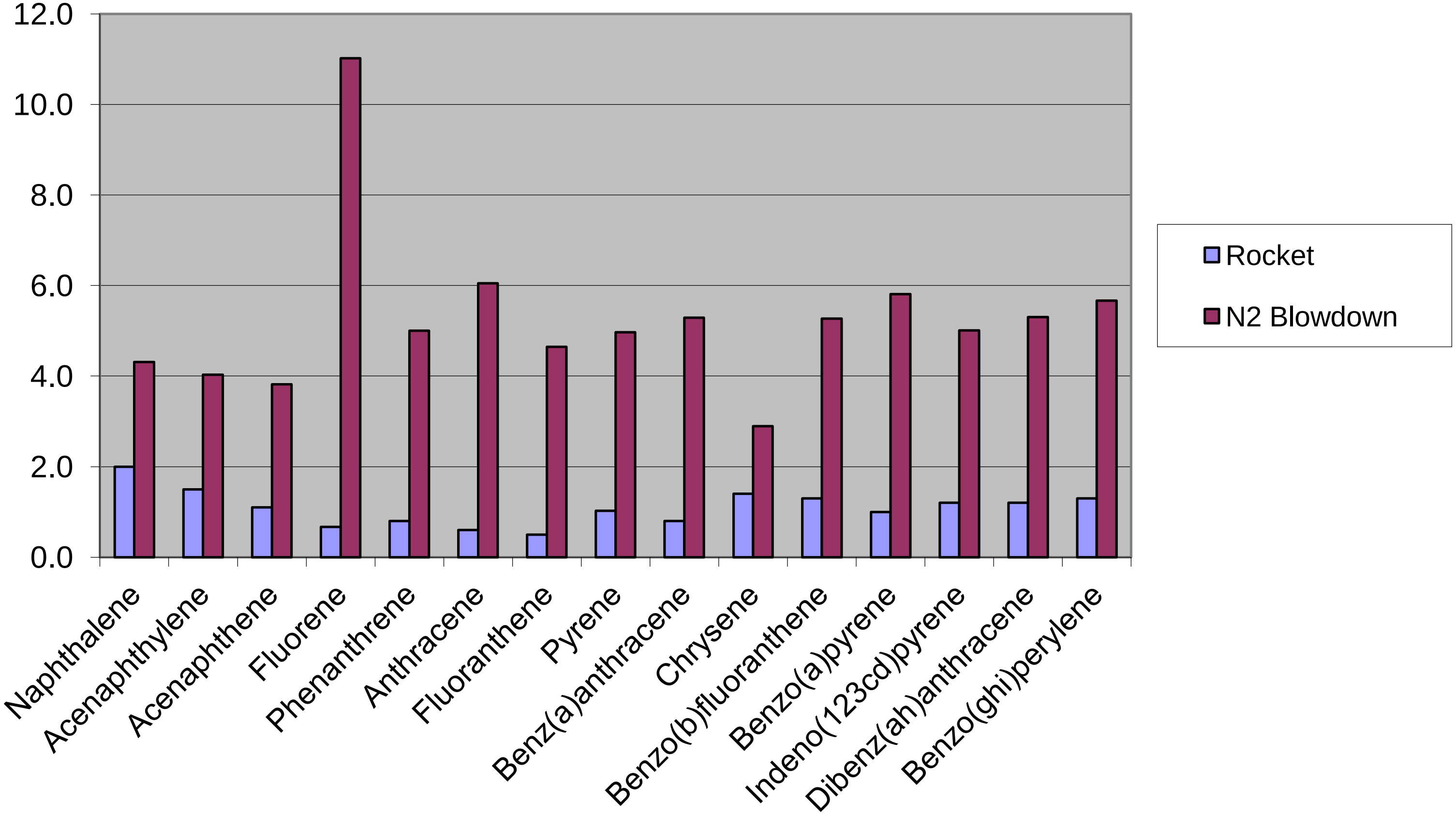
What About Solvent Recovery?

Solvent	Recovery
DCM	80%
DMF	99%
Ethanol	99%
Methanol	98%
Water	99%
Water/acetonitrile	98%

PAH Recoveries: Rocket v. Nitrogen Blow down



PAH Recoveries: Standard Deviations



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Conclusions

- The use of Accelerated Solvent Extraction increases extraction efficiency of PAHs and PCBs from two different environmental matrices.
- Both classes of compounds can be extracted under a single set of conditions within the same run in less than 45 min.
- The ASE Method is optimized to remove co-extracting compounds that cause interferences in the analysis.
- The use of alumina for in-cell clean up eliminates the need to GPC clean up prior to analysis.