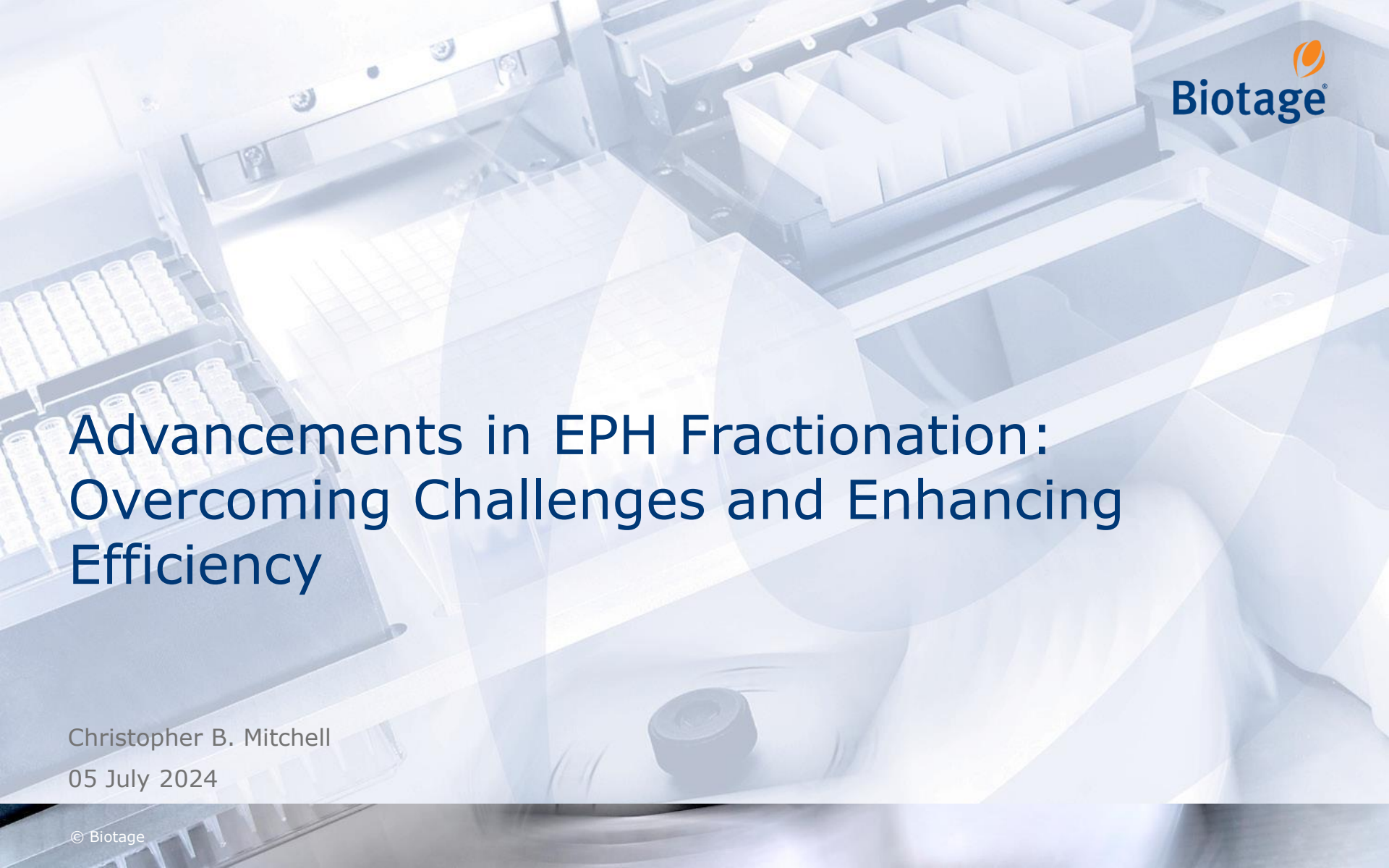

Advancements in EPH Fractionation: Overcoming Challenges and Enhancing Efficiency

Christopher B. Mitchell

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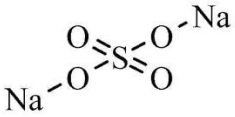
What is EPH and Why does it matter?

- EPH-Extractable Petroleum Hydrocarbons
- Includes analysis of both soil & water samples
- Monitors contamination levels
  - Hydrocarbon materials
    - Fuels, lubricating oils and crude oils
  - Underground storage tanks are possible leakage sources
- Understand toxicological impact
  - Measure the effectiveness of soil remediation

EPH Workflow Step 1 - Extraction

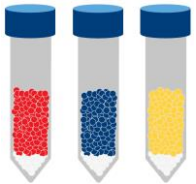
Liquids (LLE or Horizon 5000)

Aqueous (e.g. Surface, Ground & Wastewater)



Sample → Extraction → Drying & Concentration → Fractionation

Solid (e.g. Soils, Biosolids & Solid Waste)



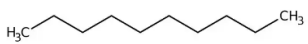
Solid (e.g. Soils, Biosolids & Solid Waste) ASE

Sodium Sulfate + TurbVap® EH

EPH Workflow Step 2 - Fractionation

Fractionation

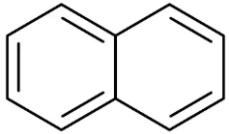
Extrahera™ Classic



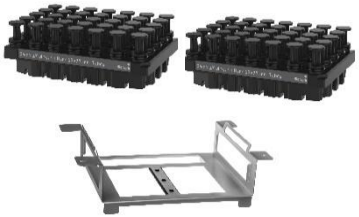
$C_{10}H_{22}$

Aliphatic (Hexane)

Aromatic (DCM)



$C_{10}H_8$



Fractions remain separate

Concentration

TurbVap® EH

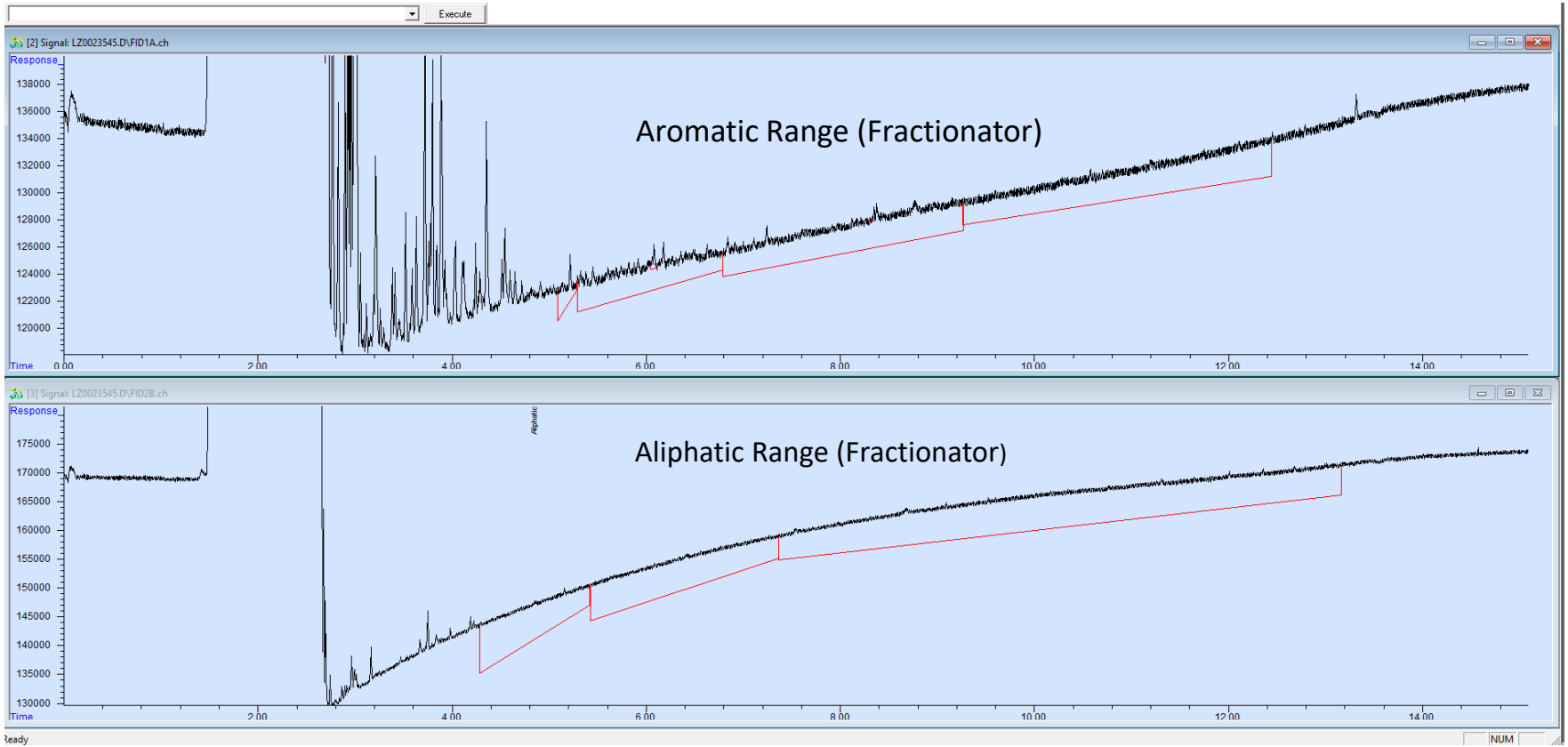
Analysis (GC-FID)

a) Aliphatic sample

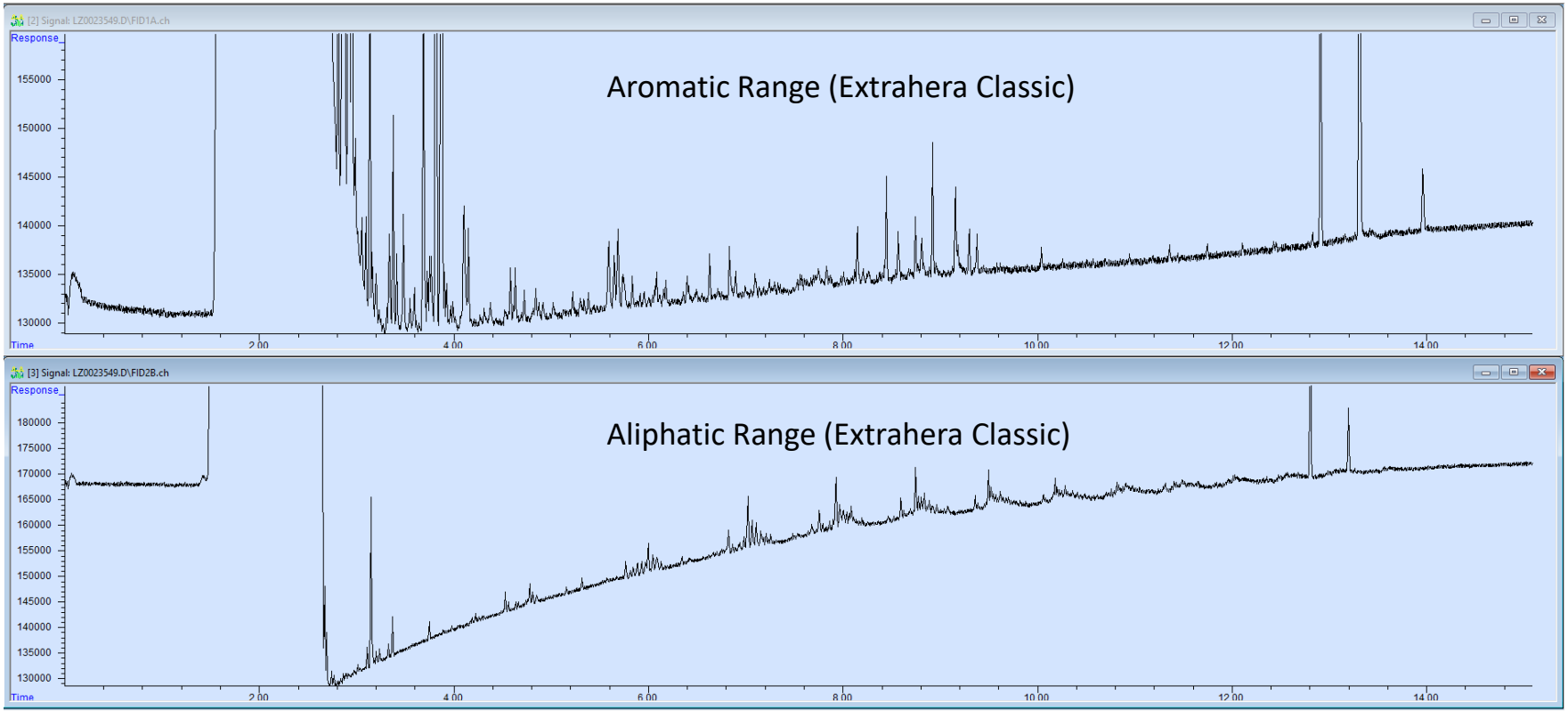
b) Aromatic sample

c) Dual Column Injection

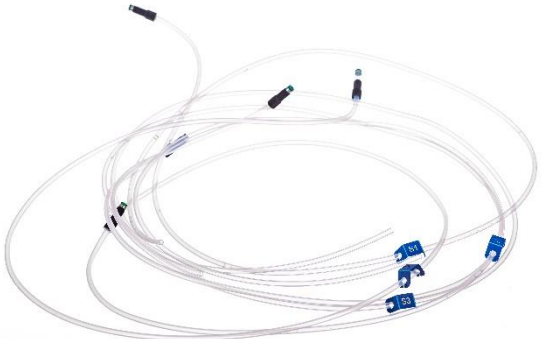
Typical Blank - Aromatic & Aliphatic



Contaminated Blank - Aromatic & Aliphatic



Chemical Resistance of Plastic Components



Workaround Solution-1. Solvent Reservoirs

Potential EPH phthalate Sources	Tested	Detected Background	Isolation Test	DF	Dilution Factor Concentration
Hexane Reservoir	Yes	51ppm (used reservoir) 145ppm (new reservoir)	Rinse reservoir with Hexane squeeze bottle. Manually fill reservoir with 10mL Hexane cover with foil, let sit for 4 hour, concentrate to 1mL, analyze	10	5.1ppm (used reservoir) 14.5ppm (new reservoir)

- **Reservoirs were placed in a closed container of DCM and soaked overnight to allow phthalates to leach off.**
- Tests were done to isolate potential phthalate sources of contamination.
- There were ranges of 5.1-14.5 ppm of leached phthalates that would present themselves after hexane exposure
- In the solvent settings on the Extrahera hexane was identified as a volatile solvent to help minimize contact time with the reservoir.
- We tried covering the reservoirs with foil to run extraction, but the sonic sensor would cause the system to error out.

Workaround Solution-2. Pipette Tips

Potential EPH phthalate Sources	Tested	Detected Background	Isolation Test	DF	Dilution Factor Concentration
Pipette Tips with Hexane		T0 = 3.5 ppm (No tips) T5 = 20.7 ppm (most used tips) T10 = 8.1 ppm (lesser used tips)	Fill 3 beakers with 10mL Hexane, cover beaker #1 with foil. Place 4 tips in beaker #2 and 4 tips in beaker #3 cover with foil as best as you can. After 5 min remove tips for beaker # 2. After 10 minutes remove tips from Beaker #3. Concentrate all 3 down to 1mL and analyze	10	T0 = 0.35 ppm (No tips) T5 = 2.07 ppm (most used tips) T10 = 0.81 ppm (lesser used tips)

- **Sample tips were switched from wide bore to standard 1mL tips and a pre-rinse step with hexane was added.**
- Standard 1mL sample Tips were used for solvents and wide bore tips were used to pipet samples.
- This test was done on standard sample tips since they were reusable consumables.
- Test revealed that at different time points phthalate concentrations varied with concentrations decreasing the longer the contact time.
- Solvent tips would have reduced phthalate influence do to them being reused and their contamination being "rinsed away" with repeated usage.
- Pre-rinse step (Tip Conditioning) was designed to rinse wide bore but contamination persisted.

Other Sources of Contamination

- The plastics were tested in the cartridges but only showed trace amounts of contamination ranging from 0.65-0.72ppm in relation to the other plastics sources.
- The 12x75mm borosilicate collection tubes were also tested using a hexane and DCM drydown on the TurboVap and these results yielded anywhere from 0.1 – 0.8ppm. Glassware was baked at 400°C for 4 hours and retested and results were consistently <0ppm. This was adopted going forward for all testing.

Description	Background
#3: No Bake 12x75mm Glass Vials	0.1 - 0.8ppm Per Vial
#4: Bake 12x75mm Glass Vials @ 400C for 4 Hours	<0ppm

Final Extrahera Method

During the course of these visits, multiple methods were tried and introduced.

It is worth noting that we also tried to do a DCM tip rinse vs hexane. Overall, the hexane performed better with the DCM showing an uptick in non-reported hydrocarbons.

The final method for use was named.“**24_Position_1_mL_EPH_Fractionation-FINAL_20240524**”

This method successfully met RECAP & MADEP aliphatic background criteria

Conditioning flow rate was initially 20mL/min and slowed down to 10mL/min

Solvents

	Solvent 1	Solvent 2	Solvent 3		
Solvent name	DCM Modified2	Hexane2	Hexane Clean		
Solvent description	Adjust Air Gap	Hexane	Hexane		
Aspiration flow rate (mL/min)	10	10	10		
Dispense flow rate (mL/min)	10	20	20		
Lower air gap flow rate (mL/min)	15	20	20		
Lower air gap volume (µL)	10	5	5		
Upper air gap flow rate (mL/min)	120	120	120		
Upper air gap volume (µL)	100	100	100		
Requires tip conditioning	Yes	Yes	Yes		
Conditioning, number of times	5	4	20		
Conditioning flow rate (mL/min)	10	20	10		
Chlorinated	Yes	No	No		
Serial dispensing allowed	No	No	No		
Upper air gap dispense pause (ms)	300	100	100		
Aspirate post dispense	Yes	Yes	Yes		
Highly volatile?	Yes	Yes	Yes		

Conditioning

Number of steps	2
Pressure (bar)	0.5
Dispose solvent tips after each step	No

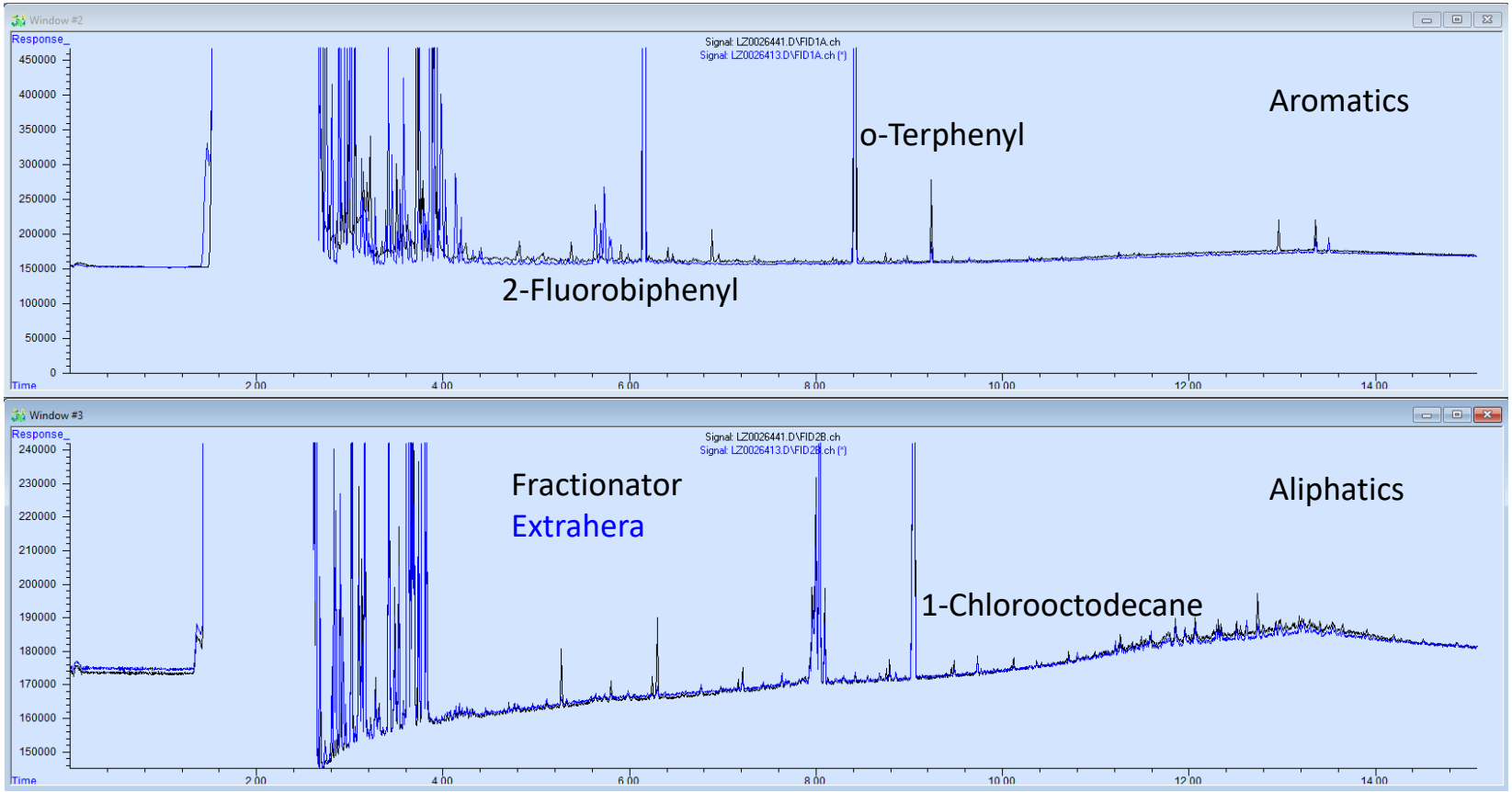
Biotage Workflow Steps

Original vs Modified

1. Obtain dried EPH extracts and samples.
2. Place EPH cartridges into Extrahera.
3. Load Sample (1mL wide bore) and solvent (1mL standard) into instrument.
4. Load 12x75mm collection tubes into 12x75mm collection racks into Position A (Aliphatics) and Position B (Aromatics) on rotating carousel. Place flow through plate into Position D.
5. Load EPH extracts and samples into Extrahera.
6. Select "24_Position_1_mL_EPH_Fractionation" from method list, load and run method.
7. Transfer samples to TurboVap EH and concentrate to 1mL with Nitrogen at 35°C.
8. Transfer to vials and analyze on GC-FID.

1. Prime solvent lines and clean reservoirs
2. Place precleaned solvent reservoirs into Extrahera
3. Obtain dried EPH extracts and samples. (baked)
4. Place EPH cartridges into Extrahera.
5. Load Sample (1mL standard) and solvent (1mL standard) into instrument.
6. Load baked 12x75mm collection tubes into 12x75mm collection racks into Position A (Aliphatics) and Position B (Aromatics) on rotating carousel. Place flow through plate into Position D.
7. Load EPH extracts and samples into Extrahera.
8. Select "24_Position_1_mL_EPH_Fractionation-FINAL_20240524" from method list, load and run method. Updated method to include tip rinsing & eliminated use of wide bore tips.
9. Transfer samples to TurboVap EH and concentrate to 1mL with Nitrogen at 35°C.
10. Transfer to vials and analyze on GC-FID.

Fractionator & Biotage Overlay - Aromatic & Aliphatic



Background Range Summary of Final Method compared to Fractionator

Data File #	Sample ID	Date Acquired	MADEP C9-C18 Aliphatics	MADEP C19-C36 Aliphatics	RECAP C16-C35 Aliphatics	Notes
LZ0030155.D	SB	5/22/2024	3.698	N.D.	1.021	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0030157.D	SB	5/22/2024	4.035	N.D.	1.118	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0030159.D	SB	5/22/2024	3.299	N.D.	1.192	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0030161.D	SB	5/22/2024	3.646	N.D.	0.930	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0029514.D	SBA	5/3/2024	3.092	N.D.		Fractionator A System Blank
LZ0029518.D	SBB	5/3/2024	3.212	N.D.		Fractionator B System Blank

- Overall, the final Biotage method meets background aliphatic range criteria:
  - RECAP (<1.8ppm C16-C35 Aliphatics)
  - MADEP (<5ppm C9-C18 Aliphatics)

Background Individual Hydrocarbon Summary of Final Method compared to Fractionator

Data File #	Sample ID	Date Acquired	C9 Aliphatics	C10 Aliphatics	C12 Aliphatics	C14 Aliphatics	C16 Aliphatics	C18 Aliphatics	Notes
LZ0030155.D	SB	5/22/2024	N.D.	N.D.	0.071	0.061	0.047	0.035	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0030157.D	SB	5/22/2024	N.D.	N.D.	0.072	0.055	N.D.	N.D.	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0030159.D	SB	5/22/2024	N.D.	N.D.	0.050	0.047	0.056	0.050	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0030161.D	SB	5/22/2024	N.D.	N.D.	0.061	0.049	0.040	0.033	Hexane tip rinse changed from 20mL/min to 10mL/min
LZ0029514.D	SBA	5/3/2024	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Fractionator A System Blank
LZ0029518.D	SBB	5/3/2024	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Fractionator B System Blank

- Individual peaks are not reported and only concern is impact to reporting range for RECAP and MADEP.
  - Biotage Extrahera = 0.072 ppm or less
  - Fractionator = N.D.

Demonstration of Criteria

Surrogate Recovery - Surrogates are showing excellent recovery. 2-Fluorobiphenyl has a mean recovery of 97.64%, o-Terphenyl is 102.00% and 1-Chlorooctadecane is 84.58%

When reducing the tip rinse flow rate from 20mL/min to 10mL/min, I observed the following trends:

- MADEP <5ppm - C9-C18 aliphatics for MADEP had a mean concentration change from 4.253 to 3.669 ppm (13.7% reduction)
- RECAP <1.80ppm - C16-C35 RECAP aliphatics had a mean concentration change from 1.558 to 1.065 ppm (31.6% reduction)

Individual Aliphatic Hydrocarbon	Mean Area Counts 20mL/min Tip Rinse	Mean Area Counts 10mL/min Tip Rinse	% Difference
C9	0	0	0
C10	0	0	0
C12	290610.6	136264.5	-53.1
C14	245228.4	123744.8	-49.5
C16	211043.6	77187	-63.4
C18	236294.7	69788.5	-70.5