Fractionation of Extractable Petroleum Hydrocarbons (EPH) Using a Novel Automation Platform for Column-based Extraction Prior to GC/MS Analysis

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Introduction

Characterization of hydrocarbon environments in soil and sediment can be an extremely cumbersome and time-consuming process. Separation of aliphatic and aromatic fractions is not always straightforward. The Massachusetts Department of Environmental Protection (MADEP) and TPH criteria working group (TPHCWG) methods rely on the use of large volumes of solvent to achieve this end. As part of our environmental goals, we are all looking to reduce solvent consumption wherever possible. This poster presents column-based methodologies allowing the efficient fractionation of EPHs into aliphatic and polycyclic aromatic hydrocarbons using drastically reduced solvent consumption. Increased sample throughput was achieved by method transfer to a dedicated automated SPE platform performed.

Experimental

Reagents

EPH standards were purchased from Thames Restek (Bucks, UK) or LGC Standards (Teddington, UK). All solvents were HPLC grade from Rathburn Chemical Ltd (Scotland, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

ISOLUTE® EPH was used in the 3 mL capacity column format (P/N 928-0145-B) using a dedicated fractionation procedure, as shown in ***Table 1***. Initial method optimization was performed using a 48-position positive pressure system, PPM48. Sample loading and aliphatic elution steps were combined for analysis while the aromatic elution was collected separately.

Table 1. *Optimized Extraction Protocols.*

Step	Solvent	Volume
Column conditioning	Hexane	4 x 1.5 mL
Sample Loading	Hexane	1 mL
Aliphatic Elution	Hexane	1.5 mL
Aromatic Elution	DCM	3 x 1.5 mL

Post extraction: Fractionated extracts were gently vortex mixed and 1 mL transferred to GC vials for analysis.

Biotage® Extrahera™ Automated Sample Preparation Platform

The optimized extraction protocol was transferred to an automated sample preparation platform, available in two options: Extrahera Classic, equipped with a 4/8 channel 1 mL pipetting head; High-volume HV-5000 equipped with a 4 channel 5 mL pipetting head. For maximum throughput samples were processed using the tabless column format (P/N 928-0145-BG) to allow simultaneous processing of 48 samples with the positive pressure processing functionality. The Extrahera™ HV-5000 platform is shown in ***Figure 1***.

***Figure 1**. Biotage® Extrahera™ HV-5000 automated sample preparation platform.*

GC Conditions

GC: 7890A GC (Agilent Technologies Inc.)
Column: Restek Rxi-5ms, 30 m x 0.25 mm ID x 0.25 µm
Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: 250 °C, Splitless, purge flow: 50 mL/min at 1.0 min
Injection volume: 1.3 µL
Oven conditions: Initial temperature 45 °C, hold for 1 minute.
Aliphatic: Ramp 20 °C/min to 115 °C, 70 °C/min to 350 °C
Aromatic: Ramp 10 °C/min to 340 °C
Backflush: 3 void volumes (2.4 mins)
Transfer Line: 300 °C

MS Conditions

MS: 5975C MSD (Agilent Technologies Inc.).
Source Temperature: 230 °C
Quadrupole Temperature: 150 °C
Monitored Ions: EI signals were acquired using selected ion monitoring (SIM) mode. Monitored ions for each panel available on request.

Results

Soil extraction procedures are well characterized and have not been investigated in these studies. The pre-requisite for this method is that samples must be partitioned into hexane/pentane prior to successful fractionation. Hexane was spiked with an EPH mix containing: aliphatics (C8-C40) octane to tetracontane and PAHs (C10-C22), naphthalene up to benzo(ghi)perylene. ***Table 2.*** illustrates solvent volume usage of the optimized column-based fractionation method compared to the MADEP and TPHCWG suggestions.

Table 2. *Solvent usage comparison.*

Method	Solvent Consumption per sample	
	Hexane/Pentane	***DCM***
ISOLUTE® EPH	*7 mL*	*4.5 mL*
MADEP	50 mL	20 mL
TPHCWG	32 mL	30 mL

Aliphatic fractionation is detailed in ***Figures 2-4***. for manual positive pressure processing, automated methods using the Extrahera™ Classic and HV-5000 systems, respectively. No cross contamination was observed as shown by the DCM fraction recoveries.

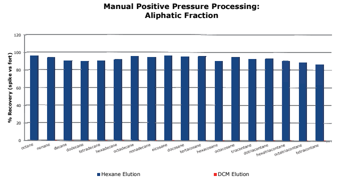

Figure 2. *Aliphatic recovery profiles using positive pressure processing.*

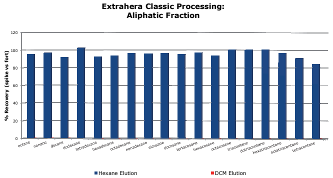

Figure 3. *Aliphatic recovery profiles using Extrahera™ classic automated processing.*

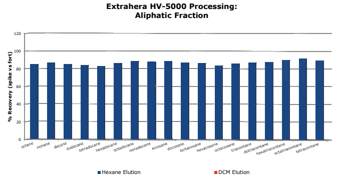

Figure 4. *Aliphatic recovery profiles using Extrahera™ HV-5000 automated processing.*

Corresponding RSDs comparing method performance are demonstrated in ***Figure 5.***

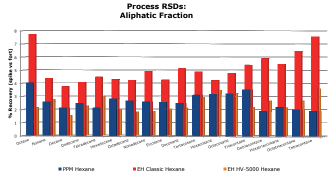

Figure 5. *Aliphatic fraction processing RSD comparison.*

Aromatic fractionation is detailed in ***Figures 6-8***. for manual positive pressure processing, automated methods using the Extrahera Classic and HV-5000 systems, respectively.

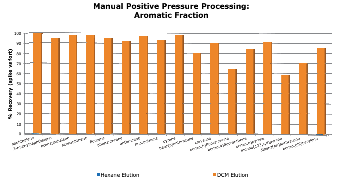

Figure 6. *Aromatic recovery profiles using positive pressure processing.*

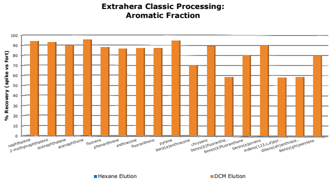

Figure 7. *Aromatic recovery profiles using Extrahera™ classic automated processing.*

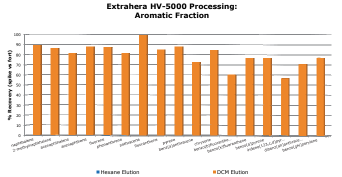

Figure 8. *Aromatic recovery profiles using Extrahera™ HV-5000 automated processing.*

Corresponding RSDs comparing method performance are demonstrated in ***Figure 9.***

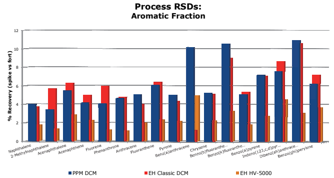

Figure 9. *Aromatic fraction processing RSD comparison.*

Both automation platforms were compared for method performance along with optimized processing and throughput. ***Table 3.*** details throughput comparison, which is ultimately based on speed of aliquoting samples using a 1 mL pipetting head for the Classic and 5 mL for the HV-5000.

Table 3. *Automation timing comparison.*

	Extrahera Automation Timing	
Format	***Classic***	***HV-5000***
48 x B Columns	1 hour 45 minutes	1 hour 6 minutes

Final chromatography is shown in ***Figures 10 and 11.*** for the aliphatic and aromatic fractions respectively.

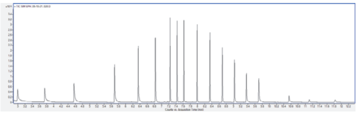

Figure 10. *Aliphatic fraction chromatography.*

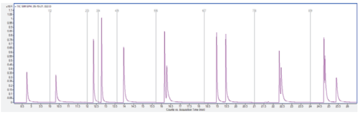

Figure 11. *Aromatic fraction chromatography.*

Conclusion

- This poster demonstrates an automation compatible method for the fractionation of Extractable Petroleum Hydocarbons (EPH).
- Reliable fractionation, eliminating aliphatic/aromatic cross contamination is achieved while increasing throughput and substantially reducing solvent consumption compared to the MADEP and TPHCWG methods.
- Using the Extrahera HV-5000 48 samples were successfully fractionated in 1 hour 6 minutes while demonstrating lowest RSDs of all processing options.

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