Automated Fractionation of Extractable Petroleum Hydrocarbons (EPH) in Soil Using ISOLUTE® EPH SPE Columns on Biotage® Extrahera™

Introduction

ISOLUTE® EPH SPE columns and associated methodologies have been optimized to efficiently fractionate EPHs into aliphatic and polycyclic aromatic (PAH) fractions (C8–C40 aliphatics, C10–C22 aromatics).

The principle is similar to the approach taken by the Massachusetts Department of Environmental Protection (MADEP) and TPH criteria working group (TPHCWG) methods. However, compared to these methods, the ISOLUTE EPH fractionation column has been significantly reduced in size and the sorbent has been optimized. This allows an automation-compatible method of fractionation which overcomes the common problem of PAH breakthrough into the aliphatic fraction, in addition to reduced solvent volumes.

This application note describes the operating conditions for the automated fractionation of EPH into aliphatic and PAH fractions using the ISOLUTE EPH column in conjunction with the Biotage® Extrahera™ automation system.

Application Analytes

Table 1. Aliphatics: C8-C40.

octane	eicosane	dotriacontane
decane	docosane	tetratriacontane
dodecane	tetracosane	hexatriacontane
tetradecane	hexacosane	octatriacontane
hexadecane	octacosane	tetracontane
octadecane	triacontane	

Table 2. Aromatics: C10-C22

naphthalene	fluoranthene	benzo(a)pyrene
acenaphthalene	pyrene	indeno(123,c,d)pyrene
acenaphthene	benz(a)anthracene	dibenz(ah)anthracene
fluorene	chrysene	benzo(ghi)perylene
phenanthrene	benzo(b)fluoranthene	
anthracene	benzo(k)fluoranthene	

Method Overview

Soil Extraction Procedures

Any of the MADEP or TPHCWG approved soil extraction procedures can be used with the ISOLUTE EPH method. The only requirements for successful fractionation are that the final solvent containing the soil extract should be either pentane or hexane. The presence of any polar modifier in the final extract will compromise the fractionation process. For example, if soil is extracted using dichloromethane, this solvent must be exchanged for hexane or pentane prior to fractionation.

Up to 1 mL soil extract in hexane or pentane can be loaded onto the ISOLUTE EPH columns.

Sample Preparation Procedure

Format

ISOLUTE® EPH 1.45g/3 mL, part number 928-0145-B.

Sample

Soil extract in hexane or pentane.

Column Conditioning

6 mL Hexane (4 x 1.5 mL).

Sample Loading

1 mL Hexane or Pentane extract. Collect column eluate.

Aliphatic Elution

1.5 mL Hexane. Add to column eluate from load step.

Aromatic Elution

4.5 mL DCM (3 x 1.5 mL). Collect in a separate tube to the aliphatic elution.

Post Elution

Gently vortex and homogenize both fractions for each sample and transfer 1 mL of each fraction to separate GC vials for analysis.

GC Conditions

Instrument
Agilent 7890A with QuickSwap

Column
Agilent J&W DB-5, 30 m x 0.25 mm ID x 0.25 µm

Carrier
Helium 1.2 mL/min (constant flow)

Inlet
300 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection
2 µL

Wash Solvents
Methanol and DCM

Oven Temperature
Described in Table 3.

Table 3. Methods for the aliphatic and aromatic hydrocarbon analysis.

Aliphatics	Aromatics
Initial temperature 45 °C, hold for 1 minute	Initial temperature 45 °C, hold for 1 minute
Ramp 35 °C to 115 °C	Ramp 10 °C to 350 °C
Ramp 70 °C to 350 °C, hold for 6.5 minutes	

Post Run
Backflush for 2.4 minutes (3 void volumes)

Transfer Line
300 °C

Mass Spectrometry Conditions

Instrument
Agilent 5975C

Source
230 °C

Quadrupole
150 °C

MSD Mode
SIM

SIM Parameters

Ions were acquired in the Selected Ion Monitoring (SIM) mode.

Aliphatic ions acquired: 41, 43, 57, 71, 85

Aromatic ions acquired displayed in Table 4.

Table 4. Aromatic fraction ions acquired.

SIM Group	Analyte	Target (Quant) Ion	Target (Quant) Ion 1	Target (Quant) Ion 2
1	Naphthalene	128	102	126
2	Acenaphthalene	152	76	150
3	Acenaphthene	153	76	154
4	Fluorene	166	163	165
5	Phenanthrene	178	152	176
5	Anthracene	178	152	176
6	Fluoranthene	202	101	200
6	Pyrene	202	101	200
7	Benz(a)anthracene	228	113	226
7	Chrysene	228	113	226
8	Benzo(b)fluoranthene	252	126	250
8	Benzo(k)fluoranthene	252	126	250
8	Benzo(a)pyrene	252	126	250
9	Indeno(123,c,d)pyrene	276	137	138
9	Dibenz(a,h)anthracene	278	139	
9	Benzo(ghi)perylene	276	137	138

Results

The optimized ISOLUTE® EPH protocol on Biotage® Extrahera™ provided typical recoveries as demonstrated in Figures 1 and 2, with RSD values <10% for all analytes.

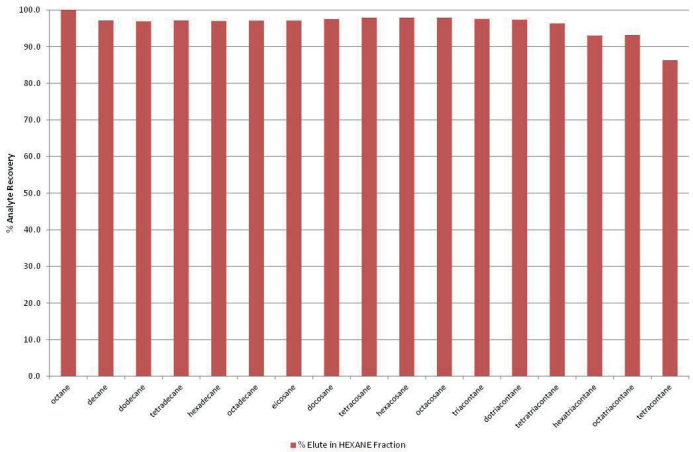


Figure 1. Chart demonstrating typical recoveries of aliphatic analytes in the hexane fraction.

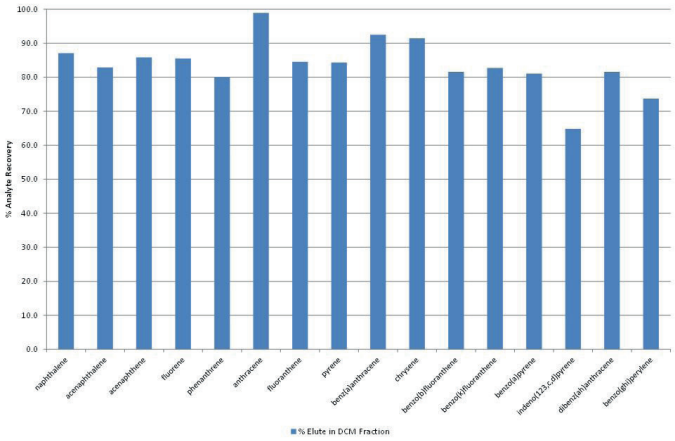


Figure 2. Chart demonstrating typical recoveries of aromatic analytes in the dichloromethane fraction.

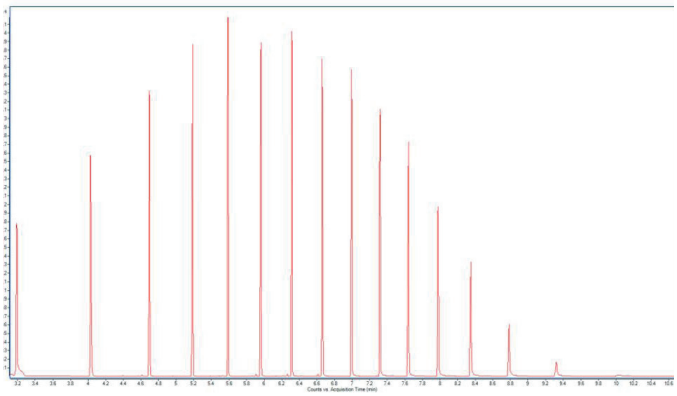


Figure 3. Total Ion Chromatogram of aliphatic analytes in the hexane fraction.

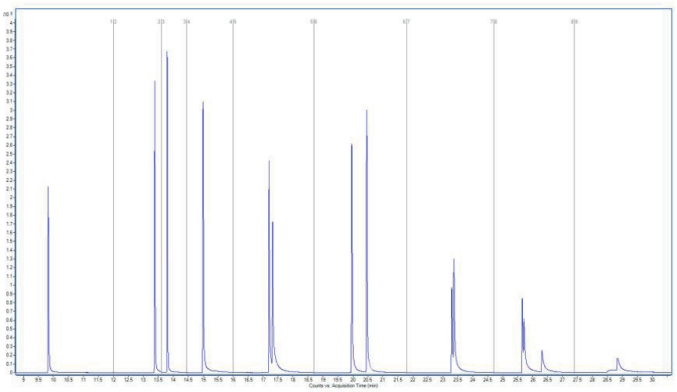


Figure 4. Total Ion Chromatogram of aromatic analytes in the dichloromethane fraction.

Reduction in Solvent Consumption

ISOLUTE® EPH columns allow a significant reduction in solvent consumption compared to the existing MADEP or TPHCWG procedures as is illustrated in the following Table:

Table 5. Solvent consumption of each fractionation method.

Method	Solvent Consumed per Sample During Fractionation Step	
	Hexane/Pentane	Dichloromethane
ISOLUTE EPH	7.5 mL	4.5 mL
MADEP	50 mL	20 mL
TPHCWG	32 mL	30 mL

Additional Information

- All solvents were HPLC grade.
- For fully automated workflow, an additional 12x75mm x 24 position collection rack (part 414511SP) is required to collect separate fractions.

Ordering Information

Part Number	Description	Quantity
928-0145-B	ISOLUTE® EPH 1.45 g/3 mL	50
For Automated Processing		
414001	Biotage® Extrahera	1
414008	Configuration Kit 24 positions	1
414511SP	Collection Rack 12 x 75 mm, 24 positions	1

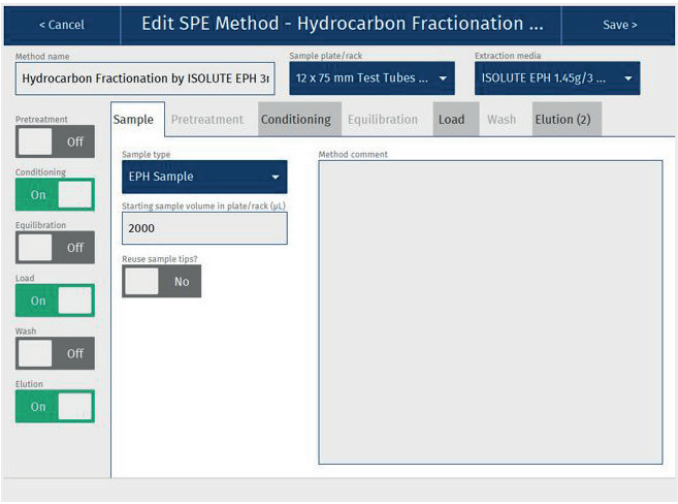
Appendix

Biotage® Extrahera™ Settings

The method described in this application note was automated on the Biotage® Extrahera. This appendix contains the software settings required to configure Extrahera to run this method. An importable electronic copy of this method for Extrahera can be downloaded from www.biotage.com.

When two collection racks are present, sample processing and fractionation can be fully automated and 24 samples can be processed in a total of 42 min 30 secs.

Method Name:	Hydrocarbon Fractionation by ISOLUTE® EPH 1.45 g/3 mL
Sample Plate/Rack:	12 x 75 mm Test Tubes EPH
Extraction Media:	ISOLUTE® EPH 1.45 g/3 mL



Settings

"Sample" Tab

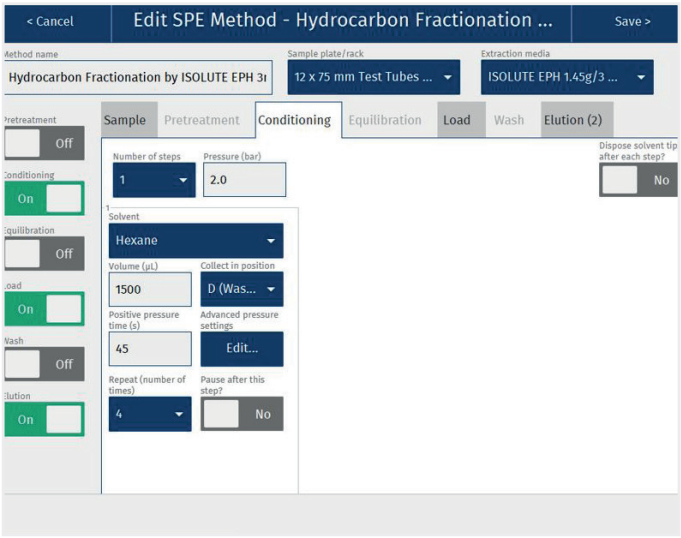
Sample Type:	EPH Sample
Starting Sample Volume (µL)	2000
Reuse sample tips?	No
Method comment:	

Pre-treatment	Not Activated
No. of steps	
Pause after last step	
Dispose tips after last step	

	Solvent
1	
2	
3	
4	

	1	2	3	4
Volume (µL)				
Time				

Screenshot



Settings

	Conditioning	Activated
	No. of steps	1
	Pressure (Bar	2.0
	Dispose tips after last step	No

	Solvent
1	Hexane
2	
3	
4	

	1	2	3	4
Volume (µL)	1500			
Collect in position	D			
Pressure time (s)	45			
Repeat	4			
Pause after this step	No			

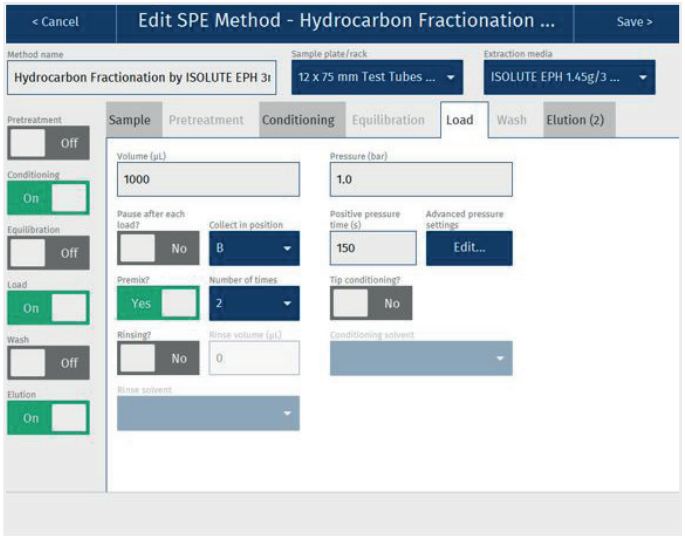
'Advanced Settings'

	Equilibration	Activated
	No. of steps	
	Pressure (Bar	
	Dispose tips after last step	

	Solvent
1	Solvent
2	
3	
4	

	1	2	3	4
Volume (µL)				
Collect in position				
Pressure time (s)				
Repeat				
Pause after this step				

'Advanced Settings'



Load	Activated
Pressure (Bar)	1.0
Pause after each load	No
Volume (µL)	1000
Collect in position	B
Positive pressure time (s)	150
Premix	Yes
Number of times	2
Rinsing	No
Rinse volume (µL)	N/A
Rinse solvent	N/A
Tip Conditioning	No
Conditioning solvent	N/A

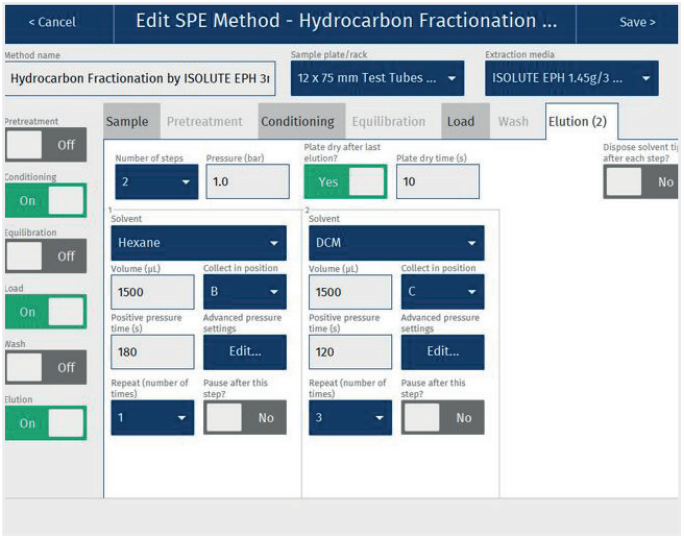
'Advanced Settings'

Wash	Not Activated
No. of steps	
Pressure (Bar)	
Plate dry after last wash	
Plate dry time (s)	
Dispose tips after each step	

	Solvent
1	Solvent
2	
3	
4	

	1	2	3	4
Volume (µL)				
Collect in position				
Pressure time (s)				
Repeat				
Pause after this step				

'Advanced Settings'



	Elution	Activated
	No. of steps	2
	Pressure (Bar)	1.0
	Plate dry after last elution	Yes
	Plate dry time (s)	10
	Dispose tips after each step	No

	Solvent
1	Hexane
2	DCM
3	
4	

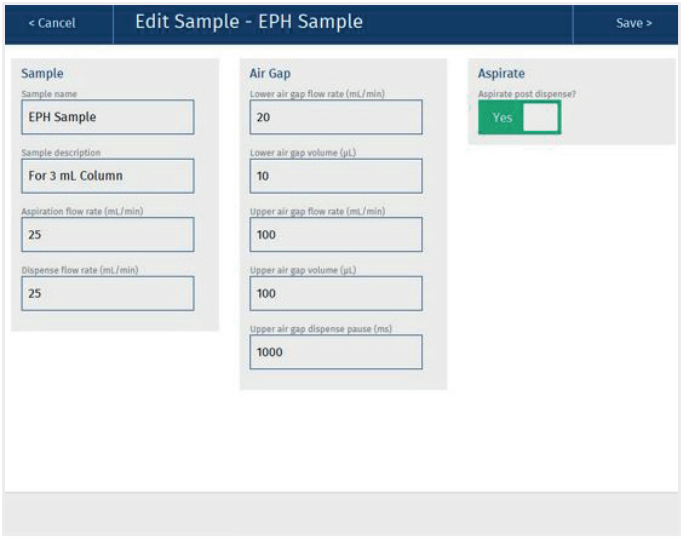
	1	2	3	4
Volume (µL)	1500	1500		
Position	B	C		
Pressure time (s)	180	120		
Repeat	1	3		
Pause after this step	No	No		

	'Advanced Settings'

Solvent Properties

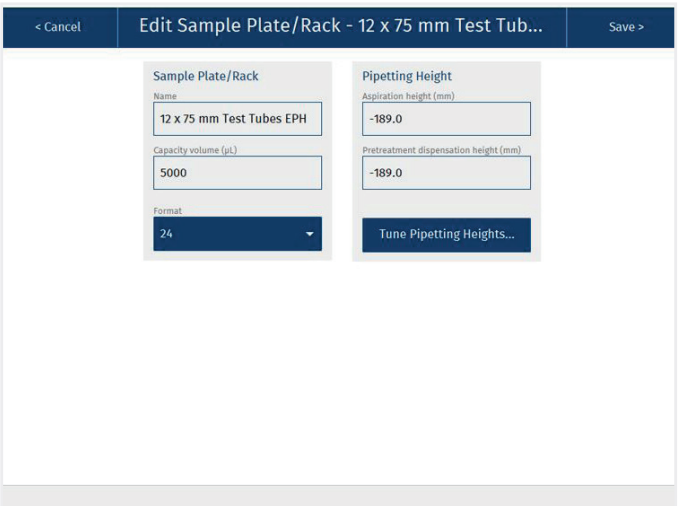
	Solvent Description
1	Hexane
2	DCM
3	
4	
5	
6	
7	
8	
9	
10	

Solvent	1	2	3	4	5	6	7	8	9	10
Reservoir Type	**Refillable**					**Non Refillable**				
Capacity	N/A	N/A	N/A	N/A	N/A					
Aspiration flow rate (mL/min)	10	10								
Dispense flow rate (mL/min)	20	10								
Lower air gap flow rate (mL/min)	20	10								
Lower air gap volume (µL)	5	5								
Upper air gap flow rate (mL/min)	120	120								
Upper air gap volume (µL)	100	100								
Upper air gap dispense pause	300	300								
Conditioning?	Yes	Yes								
Conditioning number of times	3	2								
Conditioning flow rate (mL/min)	20	10								
Conditioning volume (%)	100	100								
Aspirate post dispense	Yes	Yes								
Chlorinated	No	Yes								
Serial dispense	No	No								

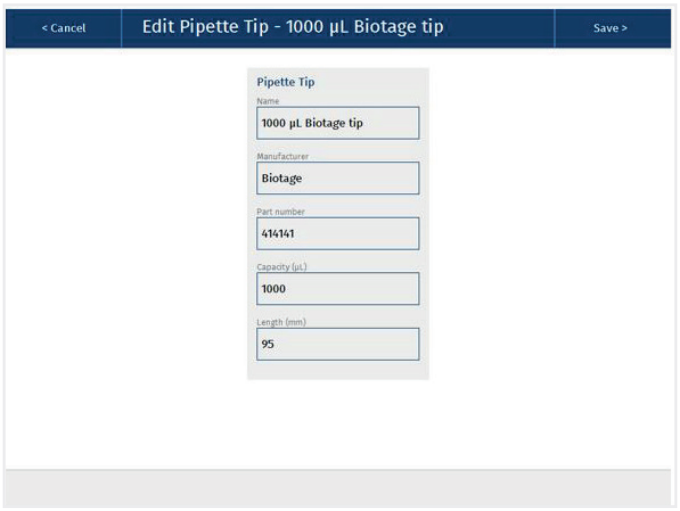


"Sample" Screen	
Sample name	EPH Sample
Sample description	For 3 mL Column
Aspiration flow rate (mL/min)	25
Dispense flow rate (mL/min)	25
Lower air gap flow rate (mL/min)	20
Lower air gap volume (µL)	10
Upper air gap flow rate (mL/min)	100
Upper air gap volume (µL)	100
Upper air gap dispense pause	1000
Aspirate post dispense	Yes

"Extraction Media" Screen	
Name	ISOLUTE EPH 1.45 g/3 mL
Manufacturer	Biotage
Part number	928-0145-B
Capacity volume (µL)	1500
Format	24
Comment	
Solvent dispensation height (mm)	-121.0
Sample dispensation height (mm)	-121.0
Aspiration height (mm)	-124.0



"Sample Plate/Rack" Screen	
Name	12 x 75 mm Test Tubes EPH
Capacity volume (µL)	5000
Format	24
Aspiration height (mm)	-189.0
Pre-treatment dispensation height (mm)	-189.0



"Pipette tip" Screen	
Name	1000 µL Biotage Tip
Manufacturer	Biotage
Part number	414141
Capacity (µL)	1000
Length (mm)	95

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