Breaking Through the EPH Fractionation Bottleneck with Automation and Multiple Cartridge Formats

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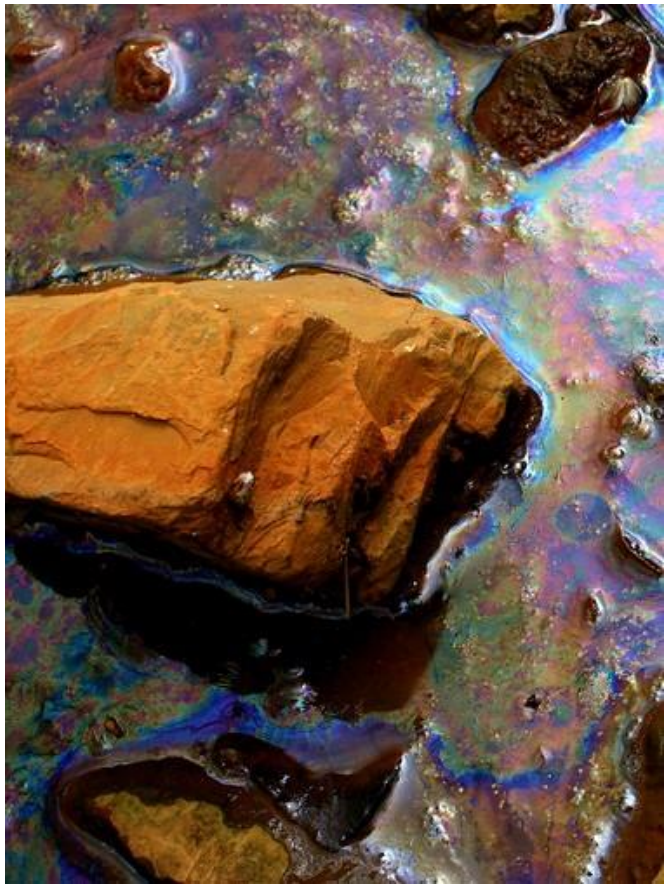
Todays Topics

1. Brief History of EPH.
2. Traditional fractionation and concentration techniques
3. EPH method flexibility overview
4. EPH cartridge format options
5. Why consider automating EPH fractionation
6. Extract concentration- Batch processing options
7. Addressing EPH fractionation Concerns
    1. Background and Contamination Sources
    2. Aromatic Breakthrough
8. Streamlining EPH workflows
9. Look into analytical results: With SPE extraction

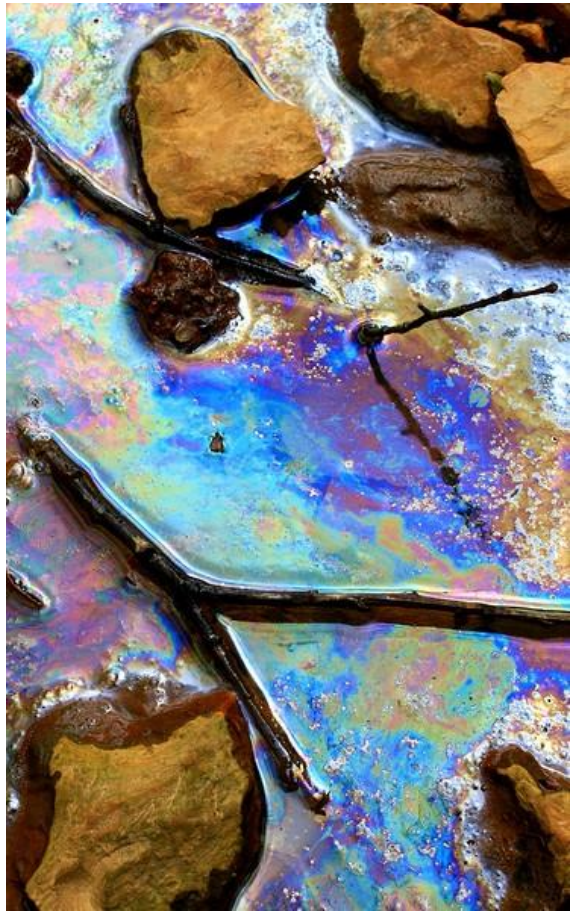
Extractable Petroleum Hydrocarbons

- Analysis of both soil & water
- Monitor contamination levels
  - » Hydrocarbon materials such as fuels, lubricating oils and crude oils
  - » Leaking underground storage tanks
  - » Understand the toxicological impact
  - » Measure the effectiveness of soil remediation



Origins of EPH Analysis

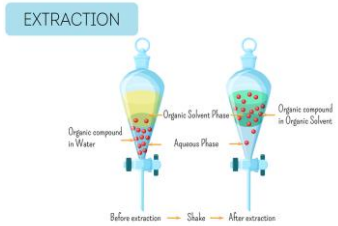
- Massachusetts Dept of Environmental Protection (MADEP)
  - First to use a petroleum fraction approach in characterising and evaluating health risks associated with petroleum contaminated sites
- Other organisations / countries / states have subsequently developed approaches based on these methods
  - CTETPH, NJEPH, TX 1005/1006, FL PRO, AK102/103, NWTPH, etc..



Traditional EPH Workflow

Sample

Extraction



Extract drying & solvent exchange

Fractionation

Concentration
Twice the extracts

Analysis by GC/FID

EPH Fractionation

- MADEP or TPHCWG approved soil or aqueous extraction procedures can be used
- Final solvent containing the sample extract should be hexane
  - Polar solvent will compromise process
- Fractionation is the process of separating the aromatic (DCM) and aliphatic (hexane) compounds into distinct fractions
- Typically performed via SPE on a manual manifold
- End up with two extracts per sample to concentrate!

Manual Fractionation Method

Manual Fractionation Method Overview	
SPE Column	ISOLUTE EPH-M, 5g/25 mL 5g/15mL
Sample	Soil or Aqueous extract in hexane
Conditioning	Load column with 20 mL hexane. Allow to flow under gravity until last of the solvent reaches the top frit. At this point place aliphatic fraction collection vial under column.
Sample Loading	Apply hexane extract. Allow to flow under gravity until it reaches the top frit.
Aliphatic Elution	12 mL hexane. Allow to flow under gravity. Collect aliphatic fraction.
Aromatic Elution	20 mL DCM. Allow to flow under gravity. Collect aromatic fraction.

EPH Method Facts

- EPH Extractions can be done manually or by automated systems based on allowable methods.
- It's recommended to follow method initial volumes for best practices. Alternate volumes can be used as long as comparable RLs are achieved.
- It is recommended to use Silica Gel (5 - 10 grams), either prepared and packed by the laboratory, or purchased in 5 g/15-mL cartridges from a commercial vendor.
- 5g of silica is recommended for samples with up to 5mg of EPH.

EPH Concerns

- Is there a problem with naphthalene breakthrough?
- How disruptive is re-fractionating a sample to our workflow?
- How consistent are the solvent volumes from batch to batch of fractionation cartridges?
- How much solvent do we use during fractionation?
- How can we reduce the time associated with EPH extractions?

What is Breakthrough?

- Loss of naphthalene & fractionation surrogate into the aliphatic fraction of sample extract
  - Result of too much solvent used, moisture in cartridge, or overloading of cartridge
- If observed, re-fractionation must be performed
- Naphthalene is the main indicator of fractionation efficiency

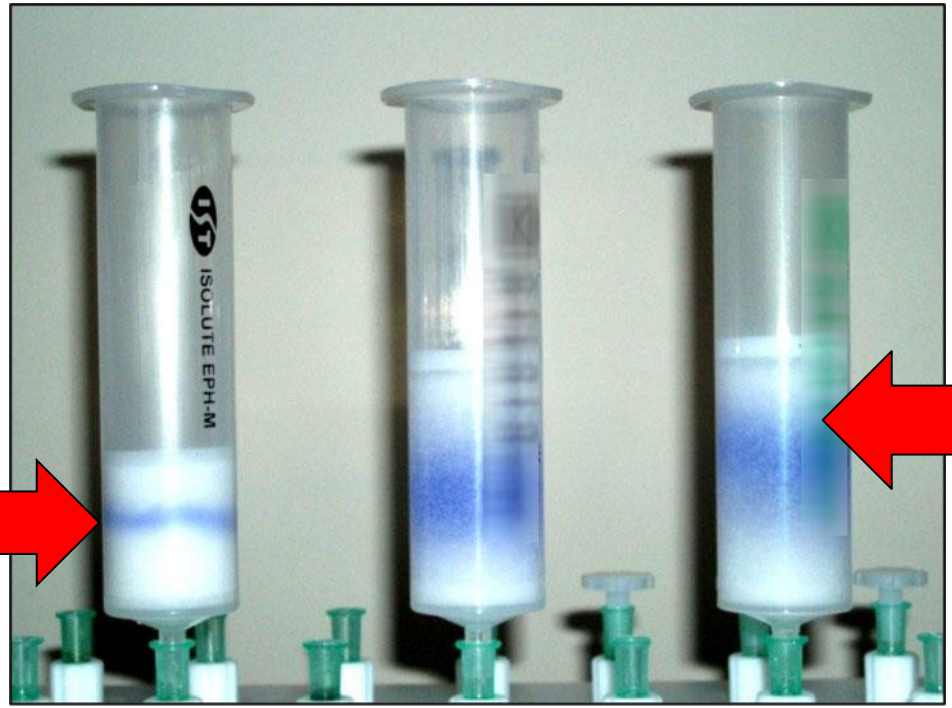
Naphthalene Retention Test

- Utilizes proprietary blue dye to mimic behavior of naphthalene on cartridge media
- 25 mL Isolute EPH Manual Test
  - Compares 25 mL cartridge vs competitor
  - Performed using gravity to elute cartridges
- 3 mL Isolute EPH Automated Test
  - Compares 3 mL cartridge vs competitor
  - Performed using automated system

Naphthalene Retention Test

Biotage

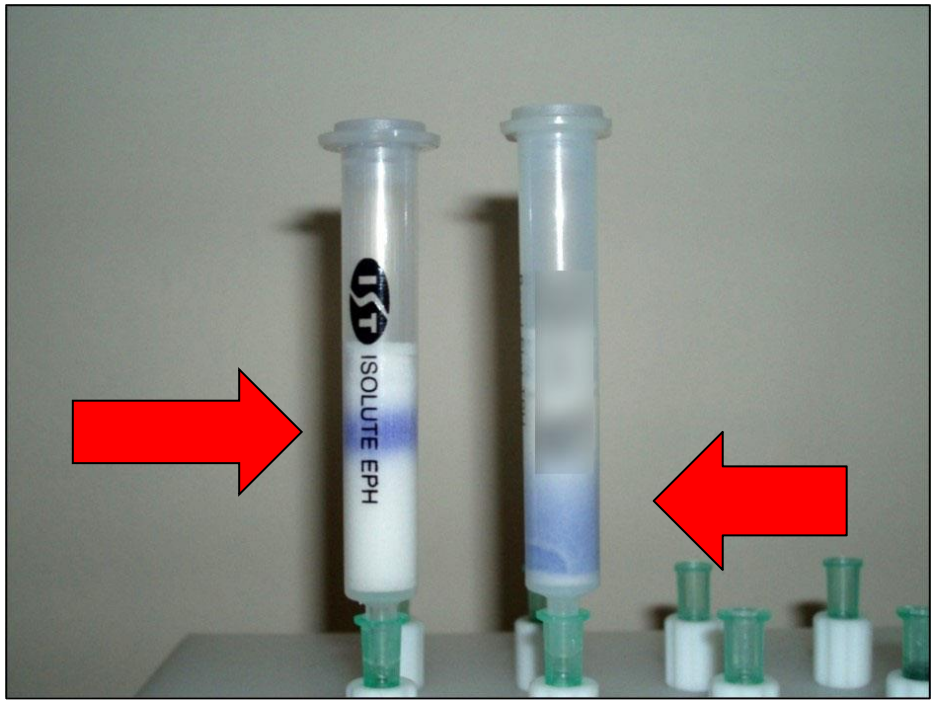
Sharp, well retained, and tight blue band indicates good naphthalene retention and fractionation efficiency



Diffuse band indicates poor naphthalene retention and fractionation efficiency

Naphthalene Retention Test

Sharp, well retained, and tight blue band indicates good naphthalene retention and fractionation efficiency



Diffuse band indicates poor naphthalene retention and fractionation efficiency

Isolute Cartridge EPH Overview

- Optimised, proprietary blend of silica
- 1.45g-3mL and 5g-25mL option
- 3 mL cartridge has been optimized to significantly reduce size without sacrificing reliability
  - Further reductions in solvent
  - Allows for automated fractionation

Cartridge QA Report- Look at Tests

Aromatic / Aliphatic Fractionation	**PASS**
Column Purity Test (GC)	**PASS**
Frit Purity Test (GC)	**PASS**
Material Weight Check	**PASS**
Extraction Residue (%)	**<0.1**
U.V. Abs MeOH Extract @254nm	**<0.1**

- QA report highlights the two most important QC tests relevant to this application:
  - Aromatic/aliphatic fractionation check
  - Column and frit purity checks

Advantages of Automating EPH Fractionation

- Improve productivity
  - Can fractionate 24/48 extracts
- Fractionation consistency
- Dramatic reduction in solvent use
- Reduce evaporation time
- Improved method performance
- Reduce consumable costs

Extrahera™ Classic

- Automated Extraction System
- Plate or cartridge based 3 and 6mL
- Supports the following techniques:
  - **Solid Phase Extraction (SPE)**
- Can easily be configured to handle a variety of different extractions
- 3mL and 6mL format with EPH
- Ability to collect multiple fractions
- Positive pressure- Total control!

Extrahera™ HV-5000

- 4 channel pipetting head reduces processing times by parallel transfer of samples or solvents from 0.25 to 5 mL
- Three channel waste segregation will allow separation of organic, aqueous, or chlorinated waste reducing waste disposal costs
- Utilize 2 pipette channels in parallel to process columns in either 10 or 15mL sizes
- Utilize 4 pipette channels in parallel to process columns in either 3-, 6-mL sizes to run 24 or 48 samples at once

Processing Formats and Supporting Consumables

Biotage

LV-200

Pipette Range: 5 µL –900 µL
Sample Range: 20 µL - 400 µL

Biotage® Extrahera™ LV-200

- 96 well plates
- 96 positions, 1 mL (A)

Concentrations: pg/mL - mg/mL

Extrahera™

Pipette Range: 50 µL - 1000 µL
Sample Range: 50 µL >2000 µL

Biotage® Extrahera™

- 96 well plates & 96 x 1 mL (A)
- 48 well plates & 48 x 3 mL (B)
- 24 position columns 1-, 3-, 6 mL (A, B, and C)

Concentrations: ng/mL - <mg/mL

HV-5000

Pipette Range: 250 µL - 5000 µL
Sample Range: 250 µL >5000 µL

Biotage® Extrahera™ HV-5000

- 48 position column 3 mL tables (DB) only
- 24 position columns 3- (B) & 6-mL tabless (DC)
- 12 position columns 10-, and 15-mL tubes (G/H, and DG)

Concentrations: ng/mL - >mg/mL

Automated Fractionation Method

Automated Method Overview	
SPE Column	ISOLUTE® EPH 1.45g/3 mL
Sample	Soil or Aqueous extract in hexane
Conditioning	6 mL Hexane (4 x 1.5 mL)
Sample Loading	Apply 1 mL Hexane 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.

Automation Reduces Solvent

Method	Fractionation solvent volumes	
	Hexane	Dichloromethane
Isolute EPH-M (manual)	32 ml	20 ml
Isolute EPH (automated)	7.5 ml	4.5 ml
MADEP	50 ml	20 ml
TPHCWG	32 ml	30 ml

- Solvent volumes are significantly reduced via automation
  - Saves cost
  - Reduces waste
  - Reduces time investment

High Throughput EPH Fractionation Workflow!

» 12,24 or 48 samples fractionated at one time is possible!

That’s A Lot of Fractions to Evaporate!

- Each EPH sample will yield two samples to evaporate to 1.0mL
  - If you have 24 samples that’s 48 samples to concentrate to 1.0mL!
- Sounds like a bottleneck situation in sample prep! OH NO!!

Introducing High Throughput Evaporation!

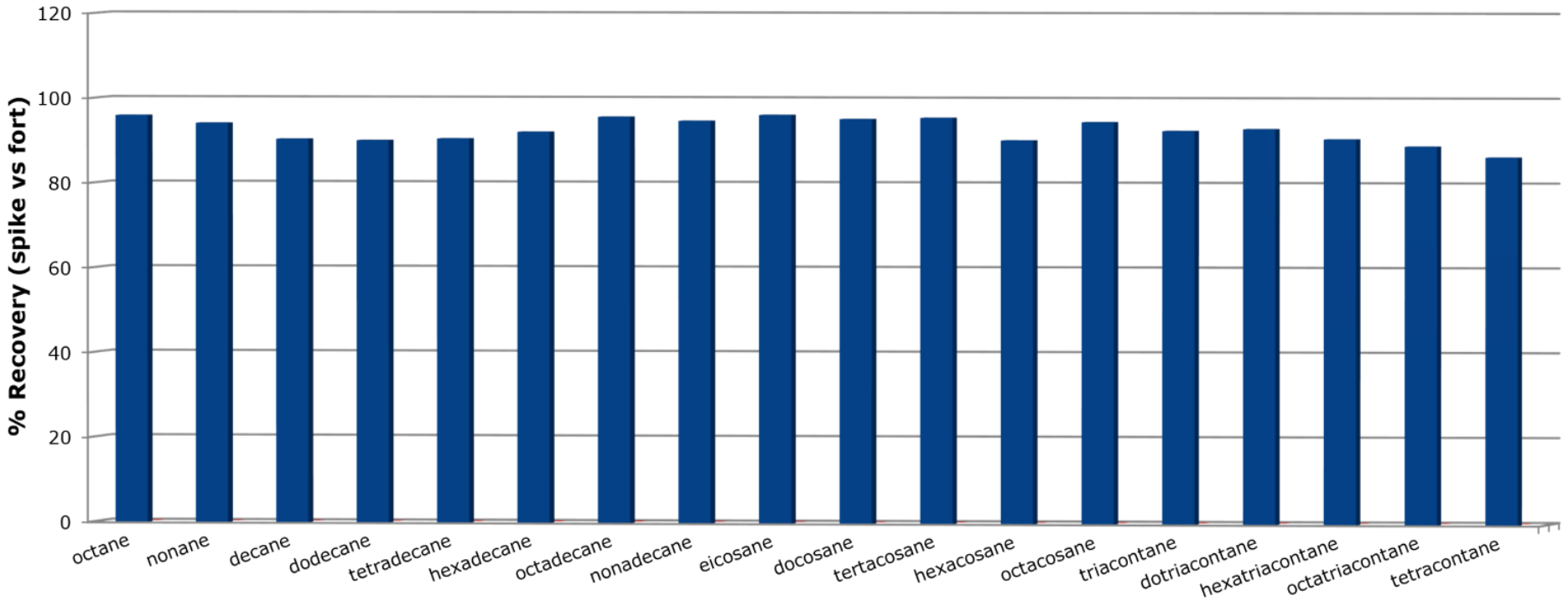
- Batch to batch evaporation!
- Just take fractions off the Extrahera and place all 24 fractions in at one time!
- Easy reconfiguring from a standard II or LV

EPH Quantitative Fractionation and Analysis

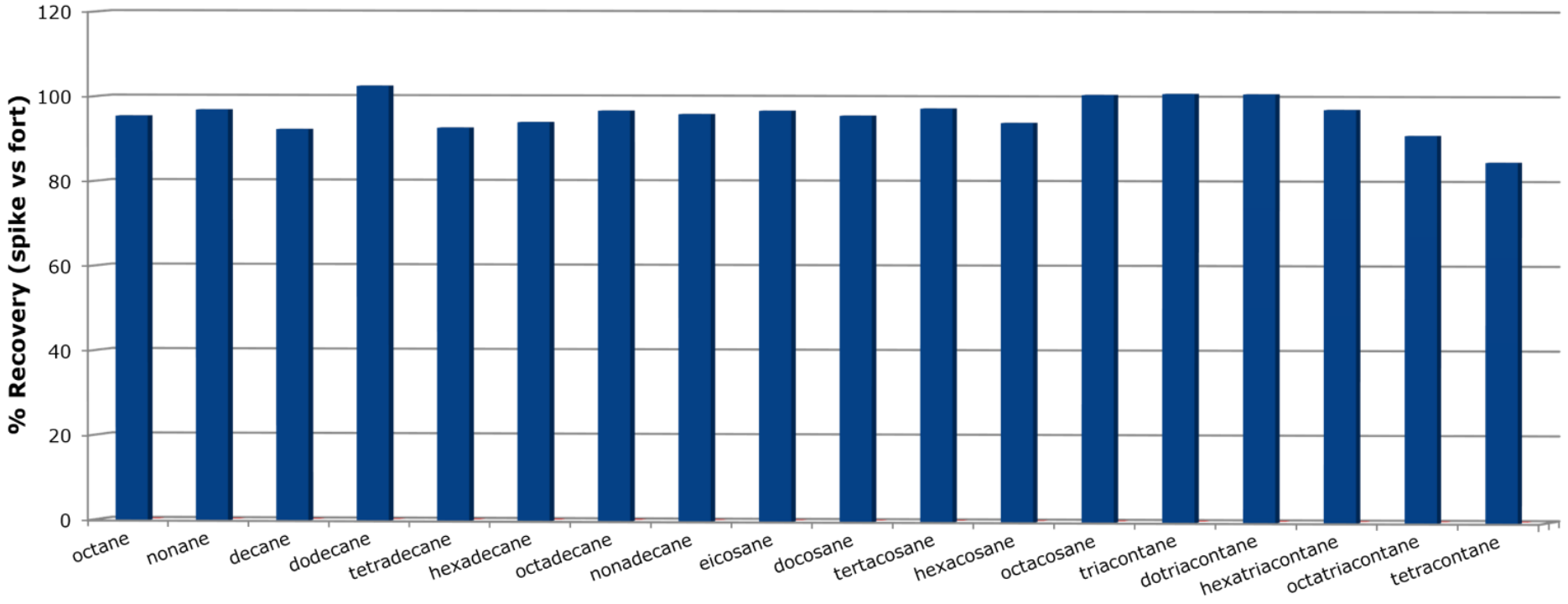
- Six Samples fractionated with each platform at LCS concentrations
- Demonstrates recovery and RSD vs all platforms with 1.45g 3mL option.
- Sample Fractions analysed by Dual GC-FID (Flame Ionization Detector)

Processing System	Processing Time 24 Samples
PRESSURE+ 48	50 minutes
Extrahera™ HV-5000	48 minutes
Extrahera™ Classic	65 minutes

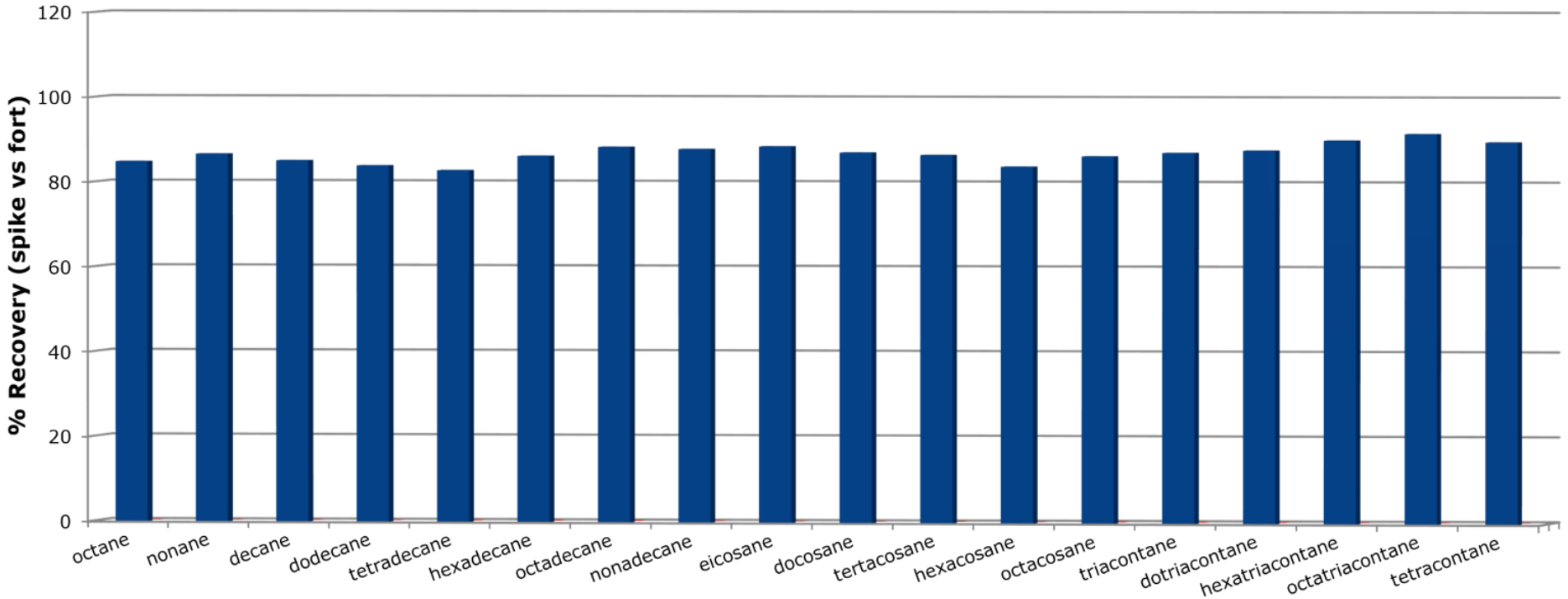
Manual Positive Pressure Processing Aliphatic



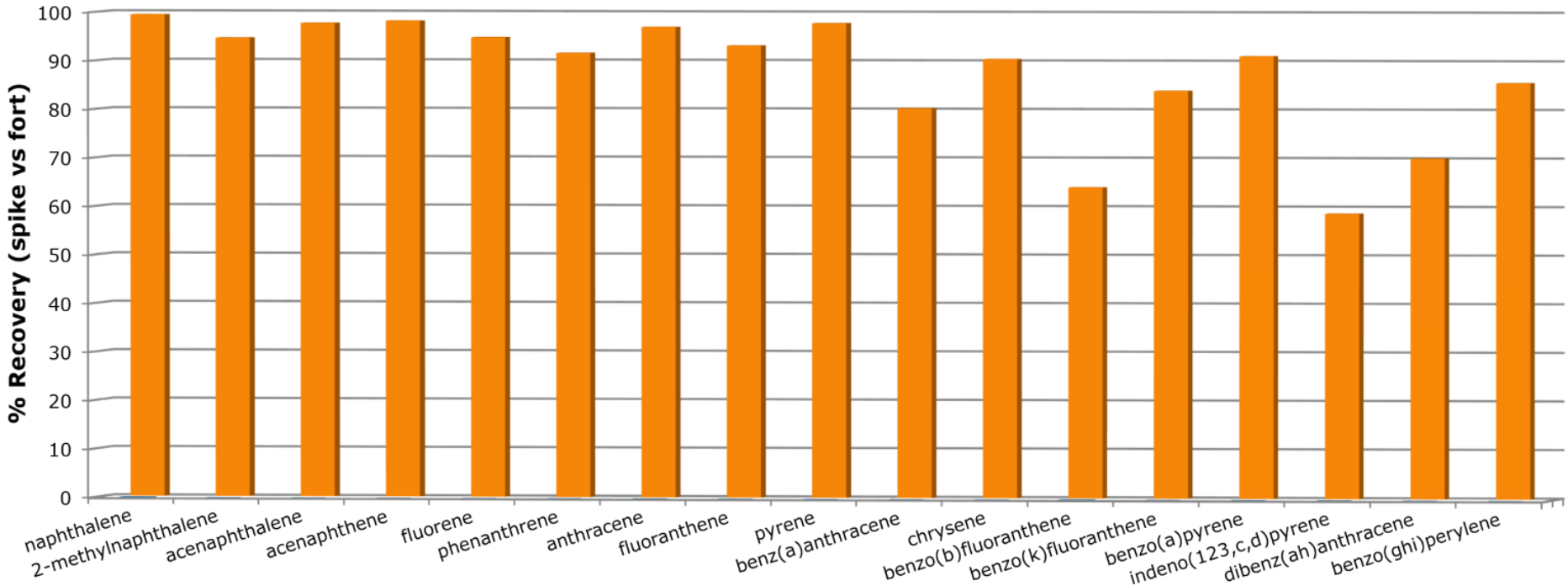
Extrahera Classic Processing Aliphatic



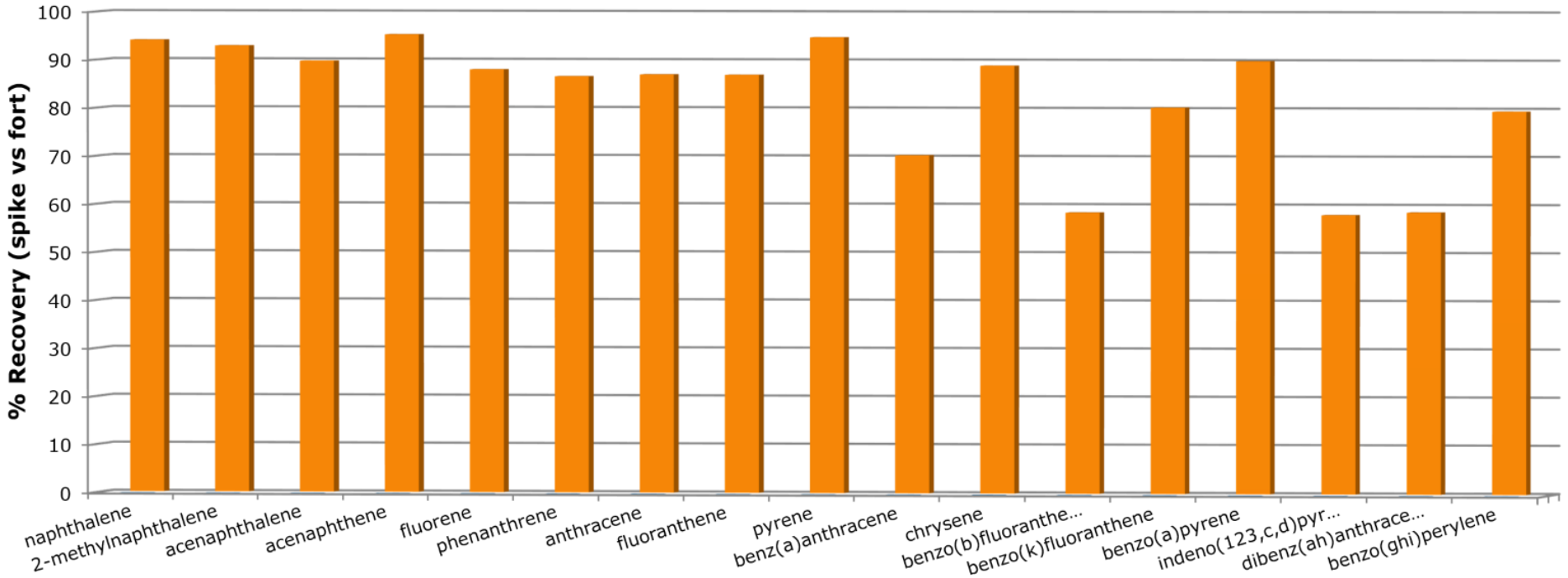
Extrahera HV-5000 Processing Aliphatic



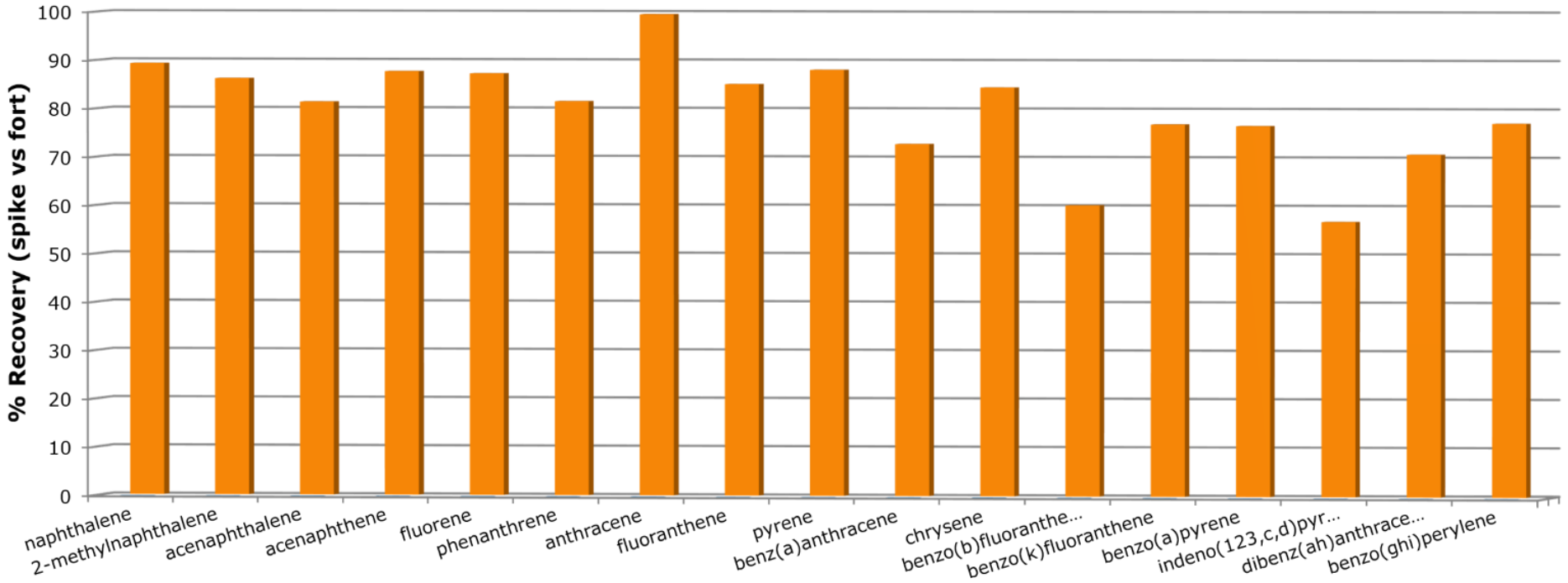
Manual Positive Pressure Processing Aromatics



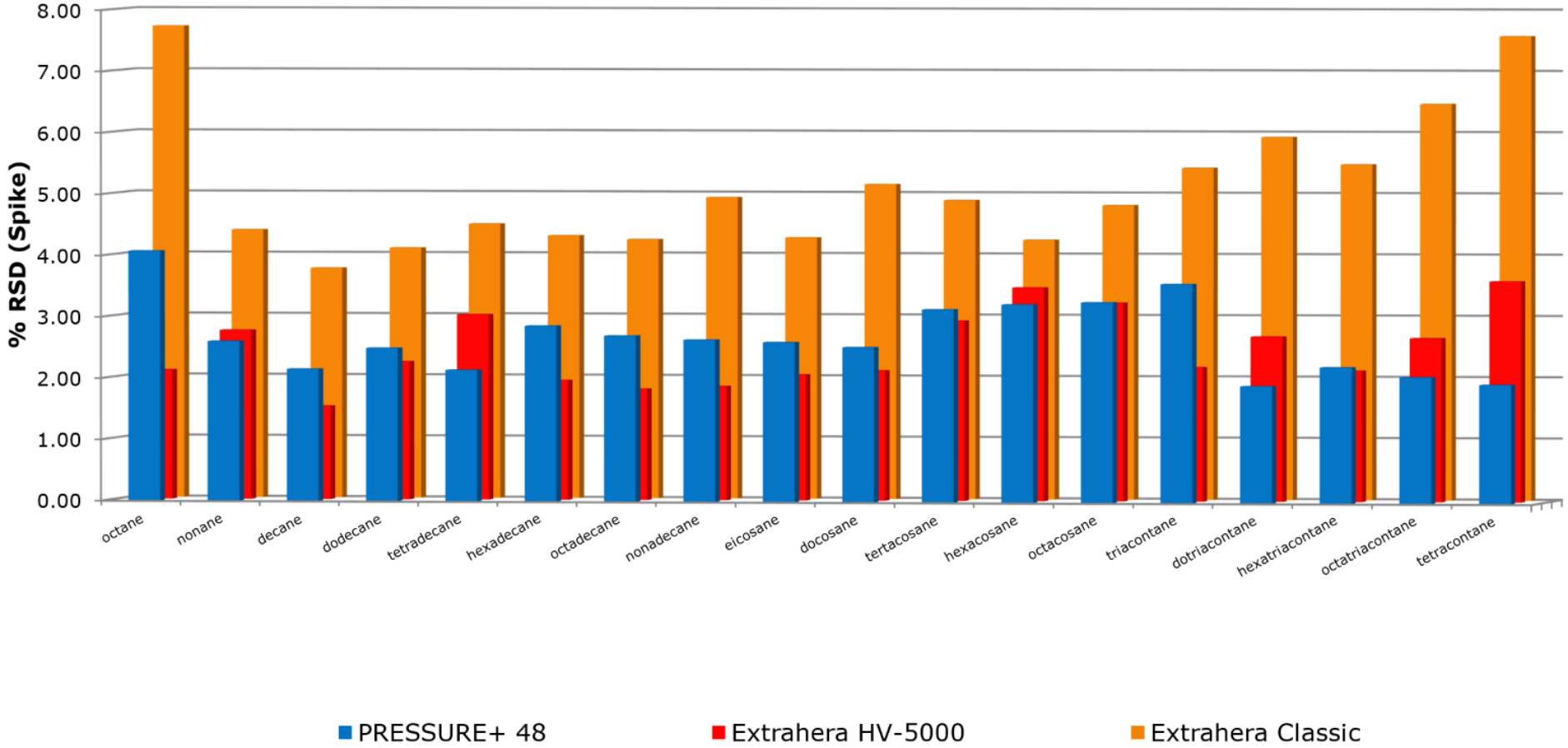
Extrahera Classic Processing Aromatics



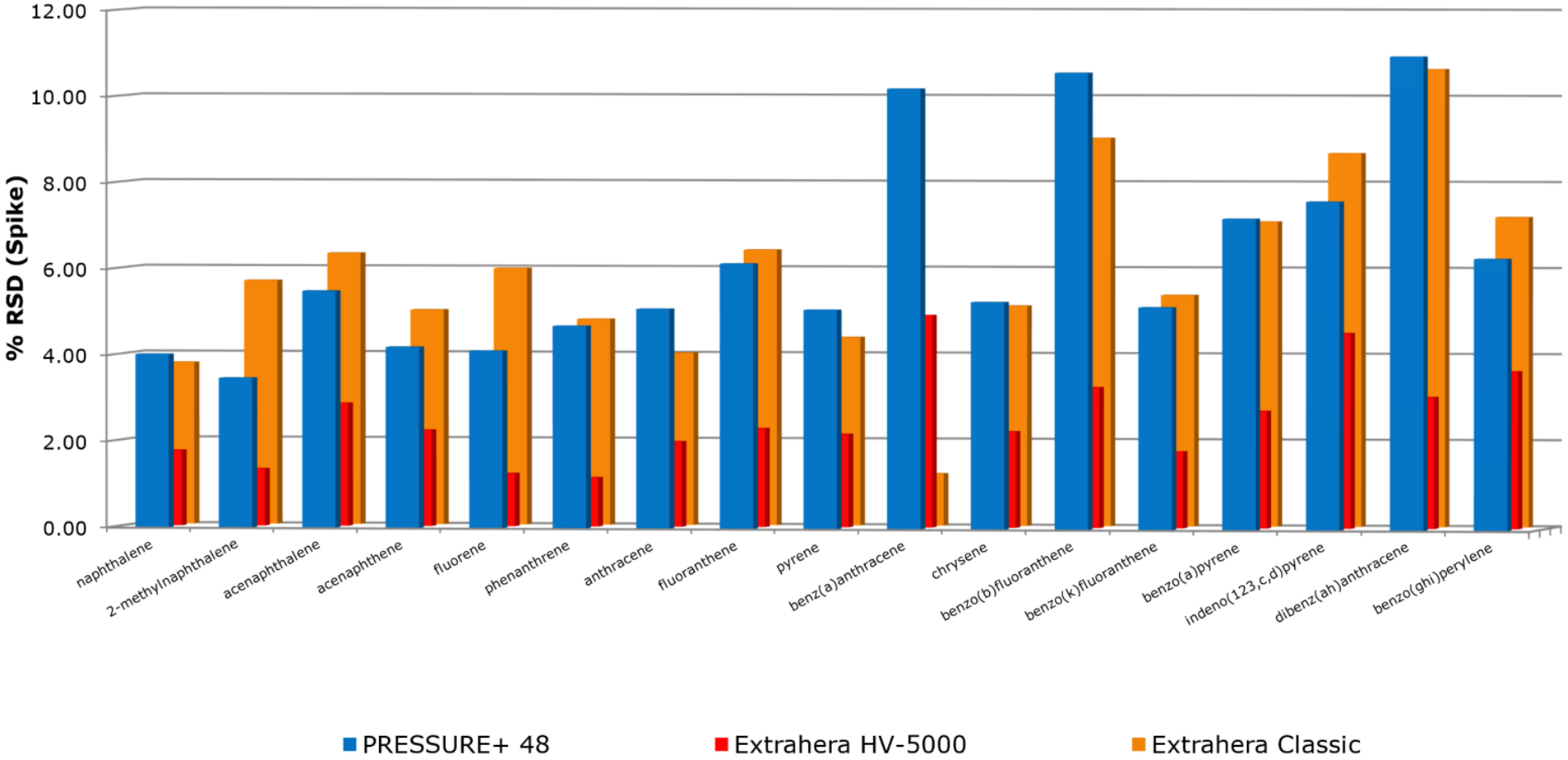
Extrahera HV-5000 Processing Aromatics



Aliphatic RSDs



Aromatic RSDs



Maximizing EPH Workflow- Aqueous

Sample

Extraction

Extract drying & solvent exchange

Fractionation

Concentration
Twice the extracts

Analysis by GC/FID

Benefits of the Biotage EPH Solution

- Isolute EPH sorbent has been optimised to ensure there is no breakthrough of the aromatic compounds into the aliphatic hydrocarbon fraction or high MW aliphatic hydrocarbons into the aromatic fraction.
- Cleanliness of Isolute EPH column components are fit for purpose
- QC of the Isolute EPH column has been tailored to cover these key features
- COA comes with fractionation check data

- **Can be integrated with the Biotage Extrahera for a streamlined solution that drastically improve productivity in the extraction lab**
- **Eliminates analyst variability due to removal of manual intervention**

Questions and Answers

Thank you for attending
For more information, please visit
Biotage.com

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