

PAH Analysis in Fatty and Complex Matrix using GC/MS/MS

Pumpkin Seed Oil

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Overview

Project Outline

Goal

- Investigate GC/MS/MS for analysis of EU Priority PAHs in Pumpkin Seed Oil

European Commission PAH Regulation

- Maximum Level
- Recovery and Deviation

Sample Preparation

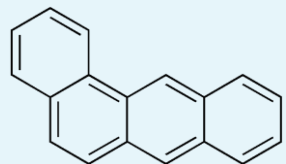
- BondElut Enhanced Matrix Removal – Lipid (EMR-Lipid)

GC/MS/MS Modifications

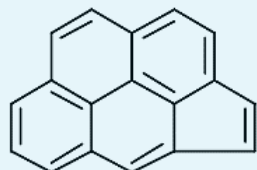
- Backflush (BF)
- Jetclean
- Retention time locking (RTL)
- Consumables
- Parameters

Polycyclic Aromatic Hydrocarbons (PAHs) Evaluated

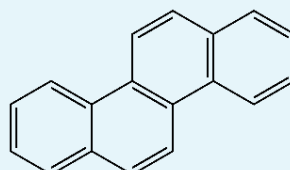
16 EU Priority PAHs



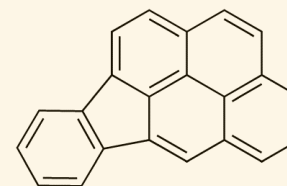
Benz[a]anthracene
MW: 228



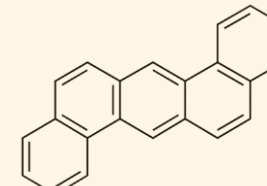
Cyclopenta[cd]pyrene
MW: 226



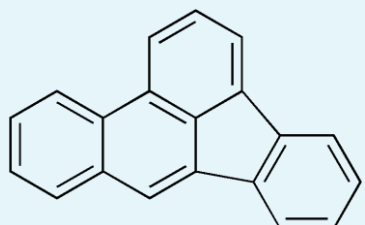
Chrysene
MW 228



Indeno[1,2,3-cd]pyrene
MW 276

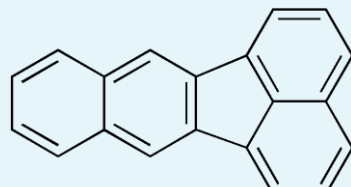


Dibenzo[a,h]anthracene
MW 278

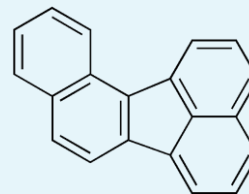


Benzo[b]fluoranthene
MW: 252

Isomeric PAHs

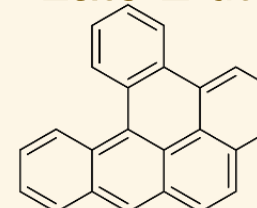


Benzo[k]fluoranthene
MW: 252

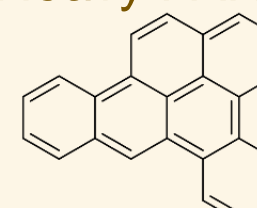


Benzo[j]fluoranthene
MW: 252

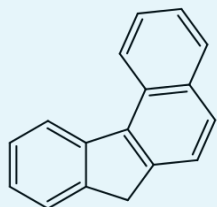
Late Eluters/Heavy PAHs



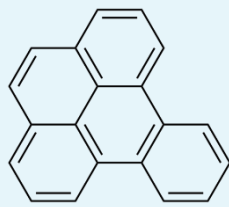
Dibenzo[a,l]pyrene
MW 302



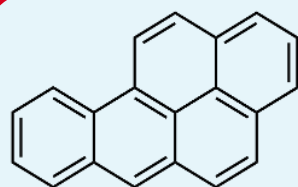
Dibenzo[a,e]pyrene
MW 302



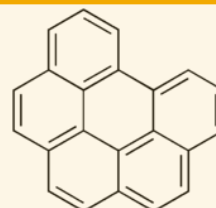
Benzo[c]fluorene
MW: 216



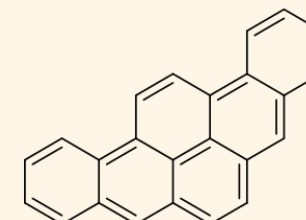
Benzo[e]pyrene
MW 252



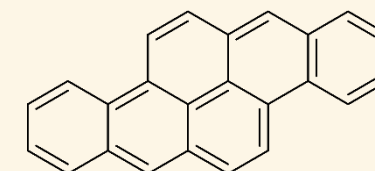
Benzo[a]pyrene
MW 252



Benzo[ghi]perylene
MW 276



Dibenzo[a,i]pyrene
MW 302



Dibenzo[a,h]pyrene
MW 302

EU Commission Regulations on PAHs

Foodstuff		Maximum Levels (ug/kg)	
Points	Foodstuff	benzo[a]pyrene	Sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, and chrysene
6.1.1	Oils and fats	2	10
6.1.2	Cocoa bean	5	30
6.1.4	Smoked meat	2	12
6.1.5	Smoked fish	2	12
6.1.7	Smoked bivalve mollusk	6	35

EU Commission No 1881/2006 Section 6

Pumpkin Seed Oil

PAH contamination occur during industrial preparation

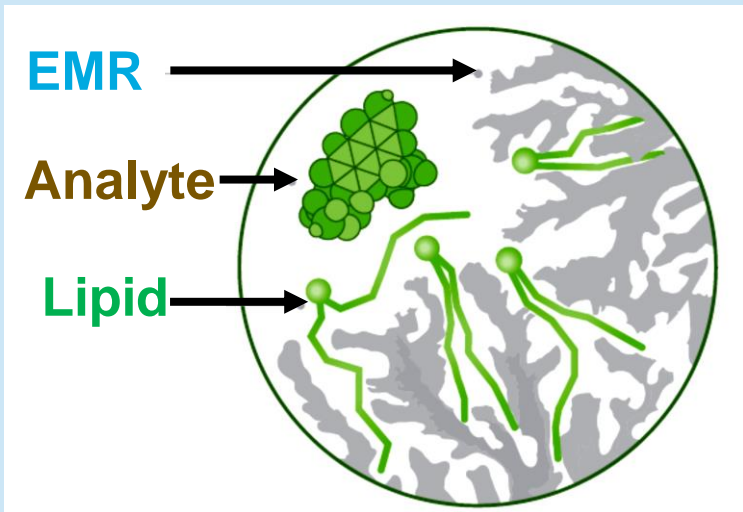


Pumpkin seed oil

- Produced by **roasting** pumpkin seeds and pressed into a dark green oil
- **Fatty matrix**: 10-14% Steric acid, 3-7% oleic acid, and 21-47% linoleic acid
- **No refining** step (which can decrease PAHs)

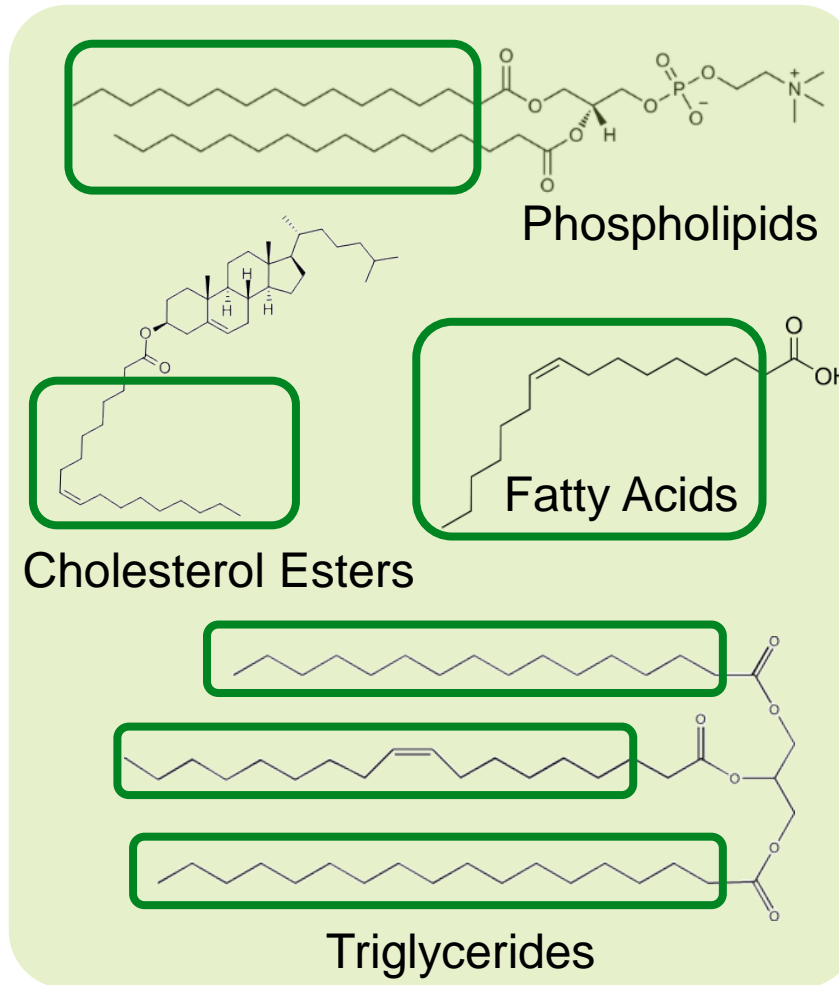
Enhanced Matrix Removal – Lipid (EMR-Lipid)

What is EMR-Lipid?
Sorbent Technology!



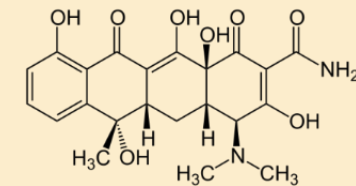
Traps Lipids by
Size exclusion
Hydrophobic interaction

What does EMR Remove?
Lipids!

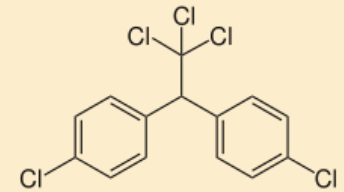


What is **not** removed?
Target Analyte

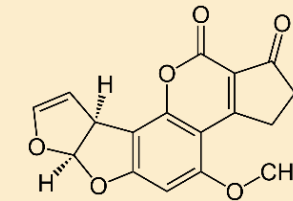
Veterinary drugs



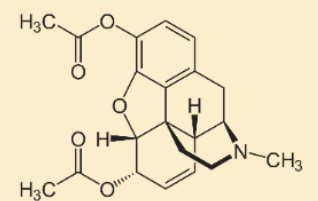
Pesticides



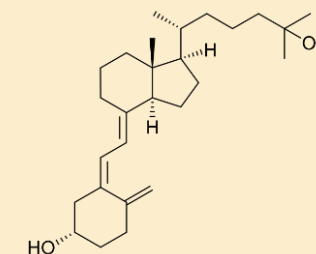
Mycotoxins



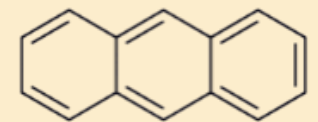
Drugs of Abuse



Metabolites

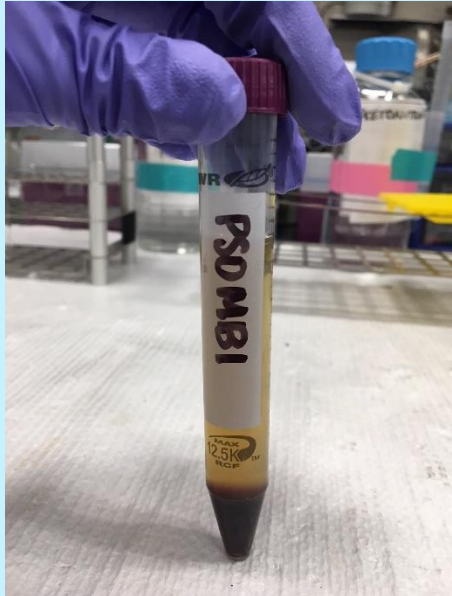


PAHs



EMR-Lipid Sample Preparation

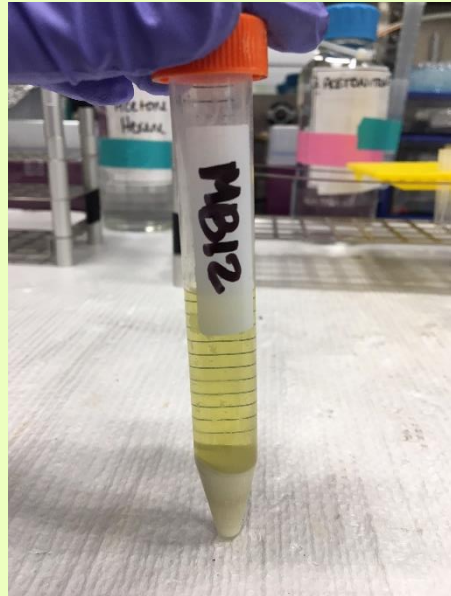
Extraction



Add sample
PreSpike QC Samples

Add **Acetonitrile**
Vortex and Centrifuge

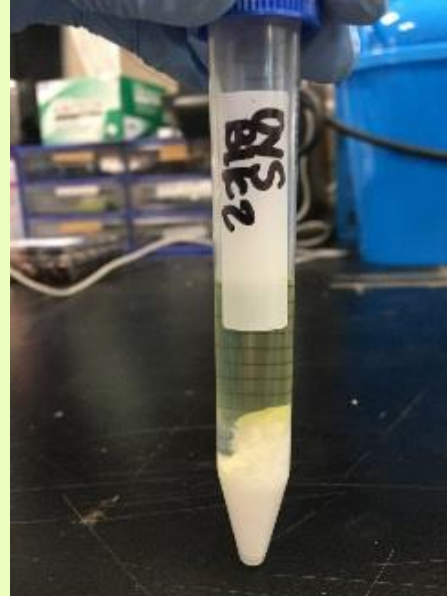
EMR-Lipid



Add **water** to **EMR-Lipid**
Vortex

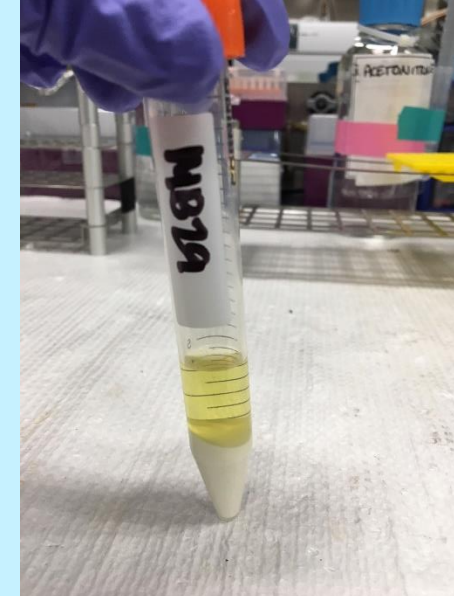
Add **supernatant**
Vortex and Centrifuge

EMR-Polish



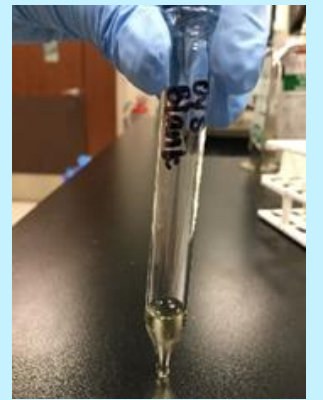
Decant to Polish
Vortex and Centrifuge

PSA/C18

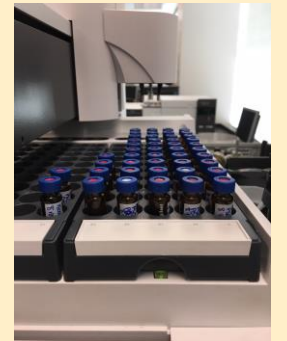


Transfer to PSA/C18 EC
Vortex and Centrifuge

Concentration

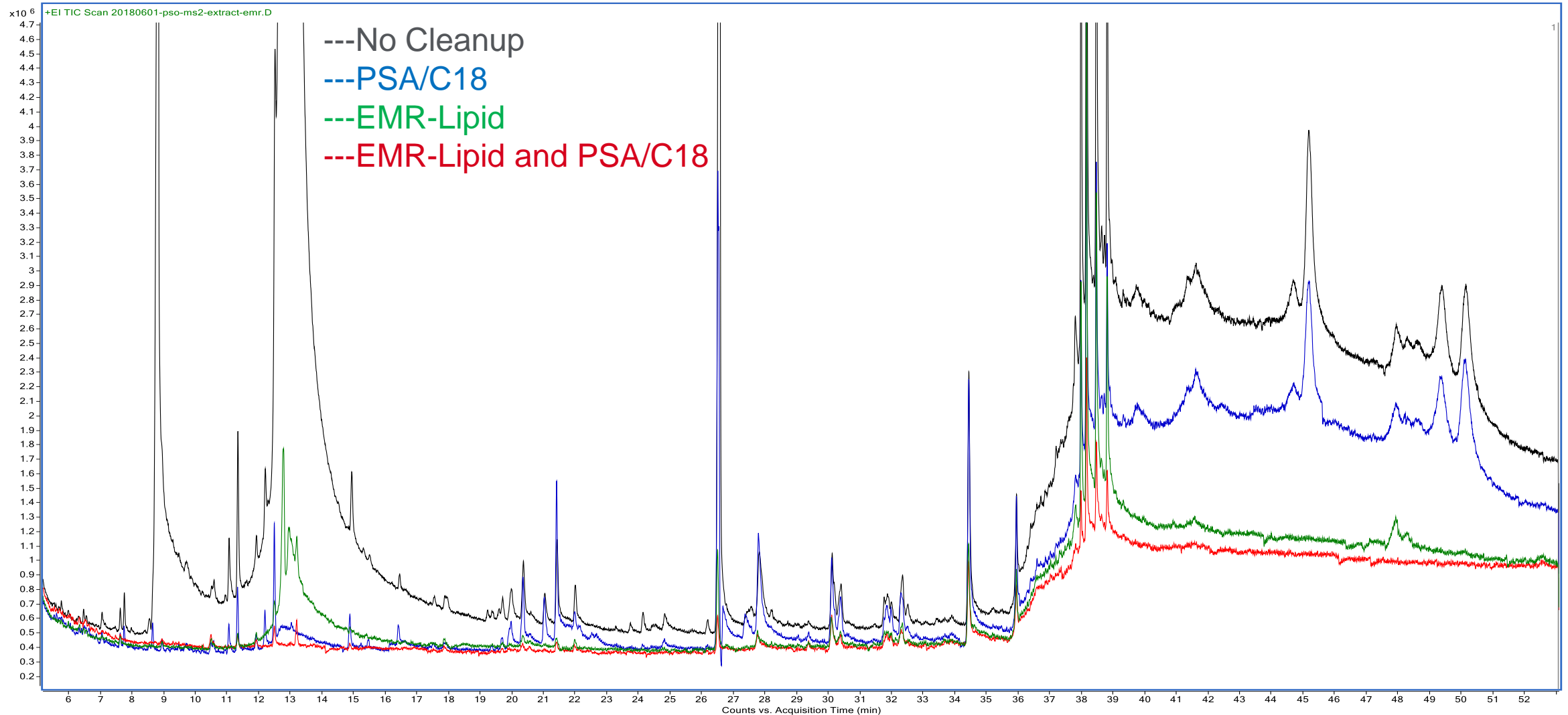


GC/MS/MS



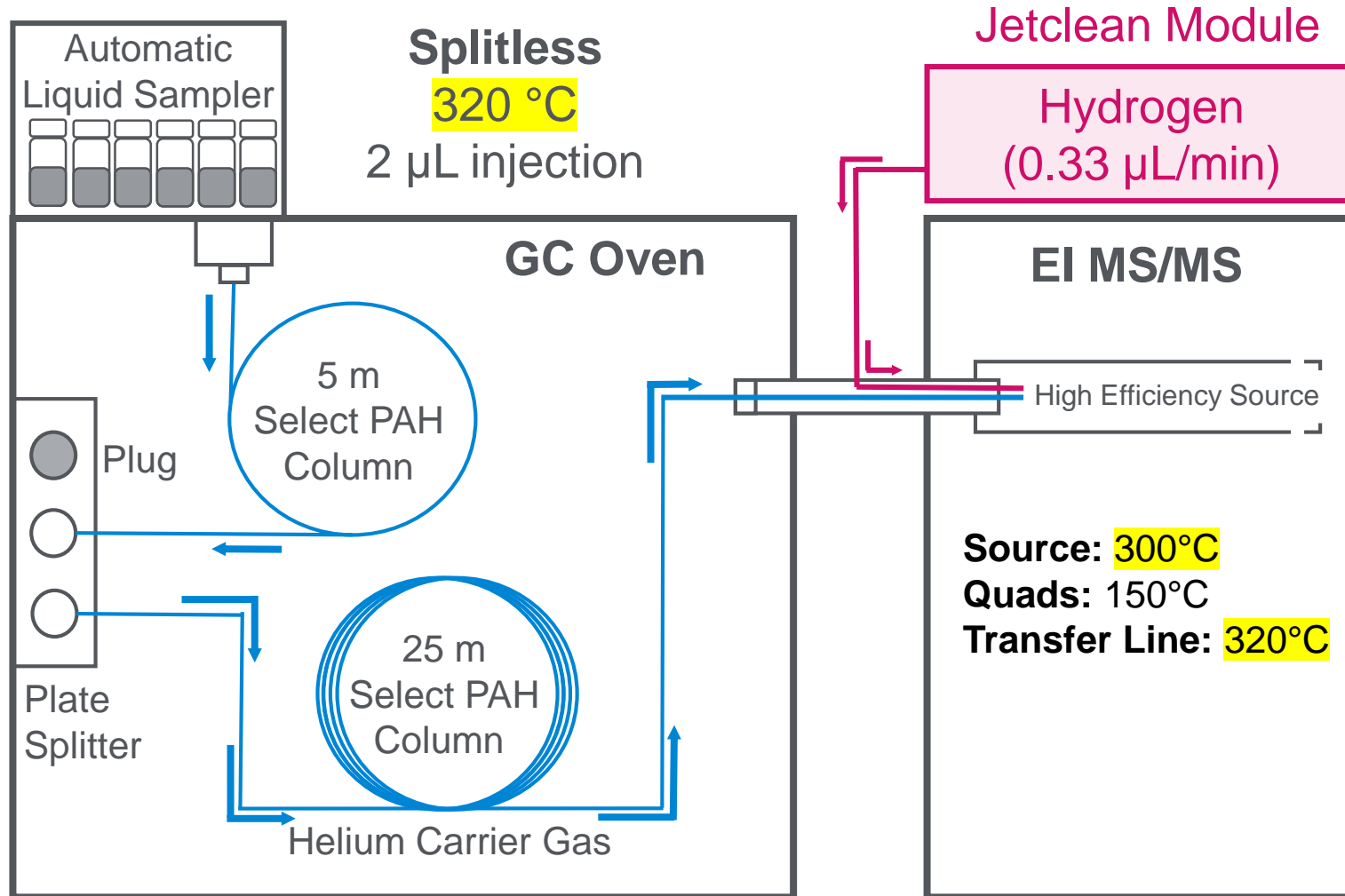
Pumpkin Seed Oil – Lipid cleanup using EMR

EMR-Lipid with PSA/C18 is the preferred cleanup procedure



GC/MS/MS Configuration

Backflush, Jetclean and RT Locking



Liner

- 4-mm single-tapered liner with **glass wool**

Column

- Select PAH
- 30 m x 250 µm x 0.25 µm
- Column 1 Flow at 1.2 mL/min
- Column 2 Flow at 1.5 mL/min

Oven program

- Initial: 80 °C (0.5 min)
- Ramp at 120 °C/min to 120 °C
- Ramp at 40 °C/min to 180 °C
- Ramp at 3 °C/min to 280 °C
- Ramp at 120 °C/min to 325 (18 min)

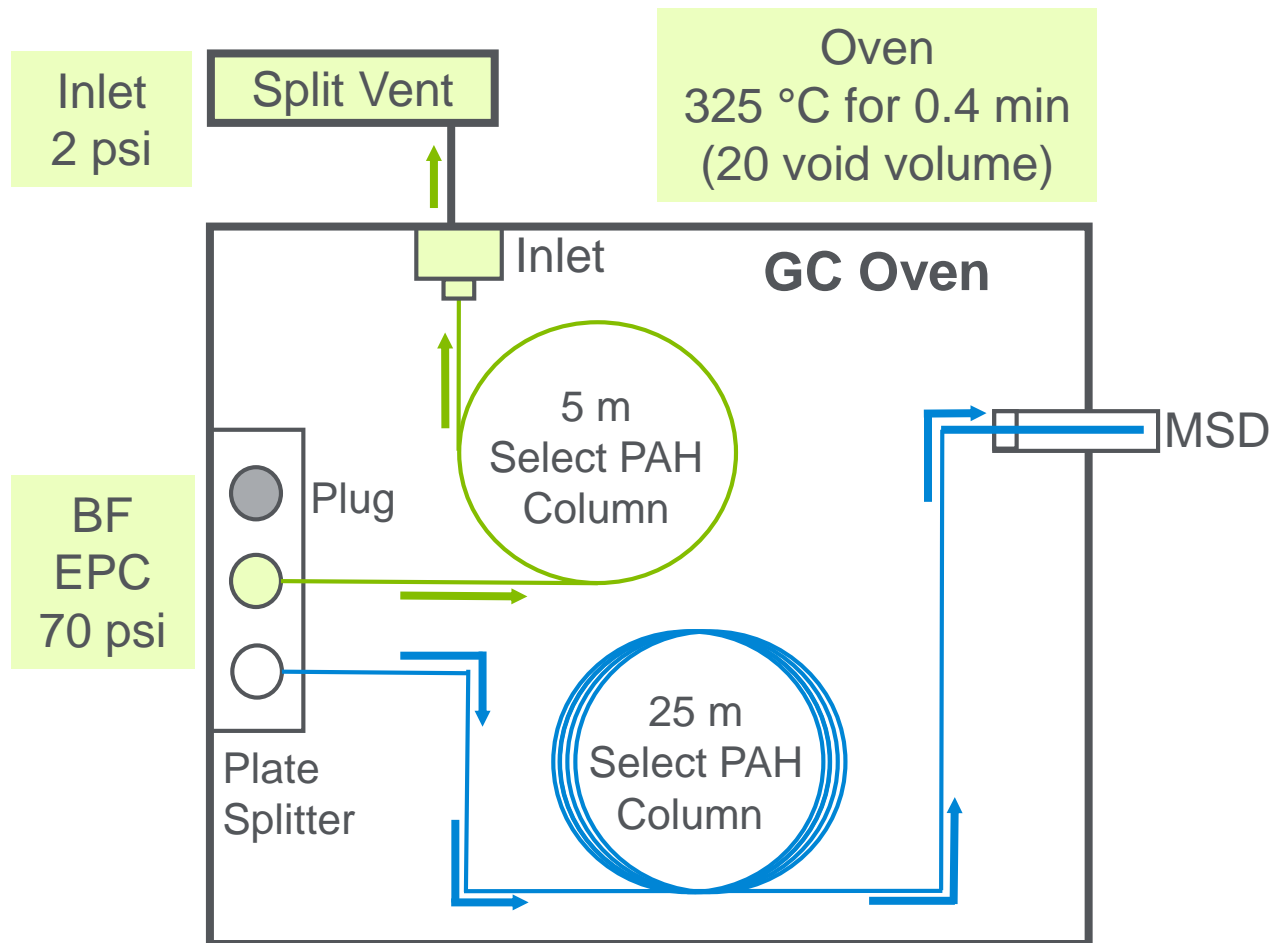
Modifications

- Backflush (BF)
- JetClean
- Retention Time Locking (RTL)

BackFlush

To maintain column lifetime

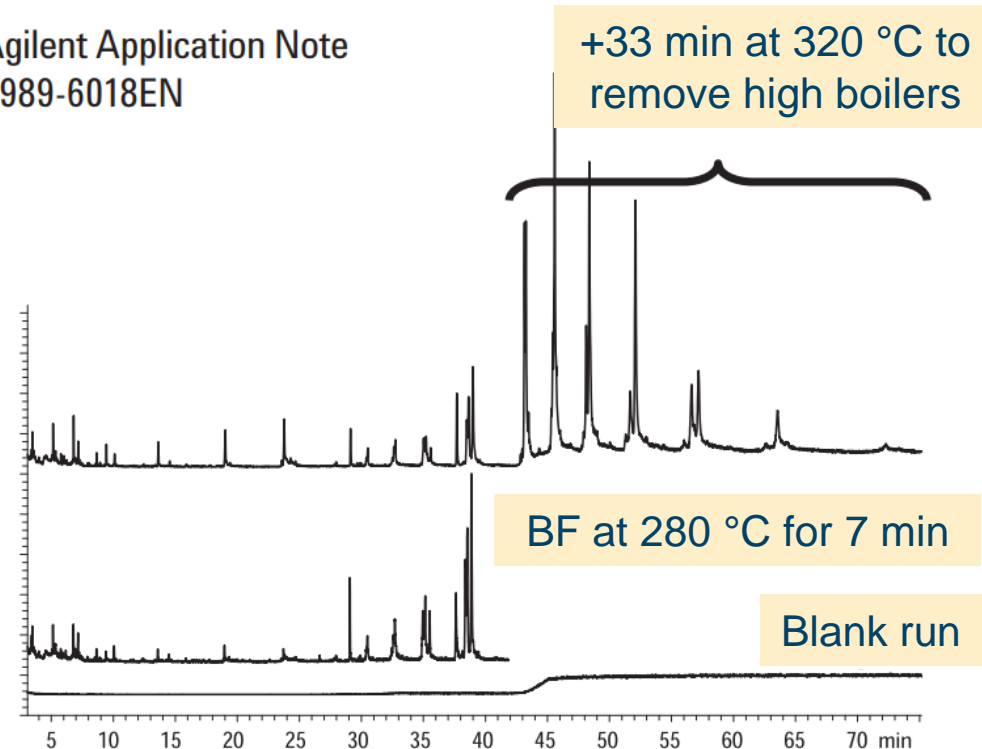
How it works? At the end of every run...



Reduce cycle time and eliminates carryover

Improving Productivity and Extending Column Life with Backflush

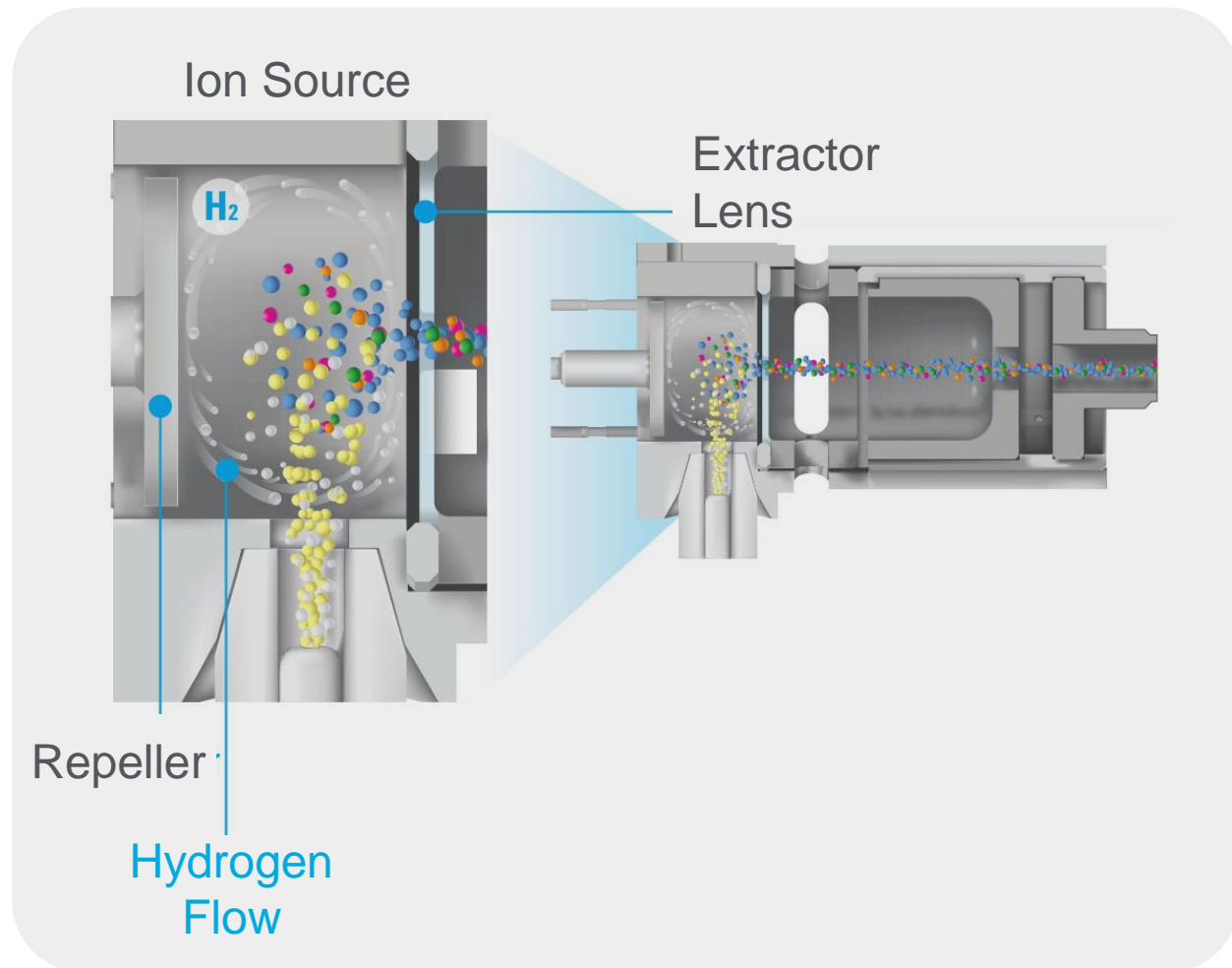
Agilent Application Note 5989-6018EN



JetClean Self-Cleaning Ion Source

Reduces the frequency of source cleaning

How does Jetclean work?

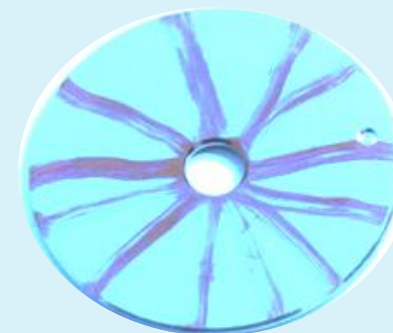


Jetclean Applications

Rhodamine 6 deposit
(red ink)



Artificial
Contamination



Helium
Bakeout



After Jetclean
(one cycle)

US Patent: In-situ conditioning in mass spectrometer systems US 8378293 B1, US 8513593 B2 *Japan* - Patent No. 6267856 Patent pending in Germany and China

Retention Time Locking (RTL)

Allows for close match of retention times from analysis to analysis

Choose a compound for RT Locking

Enter mass of Locking Compound

Acquire RT vs P Calibration Data

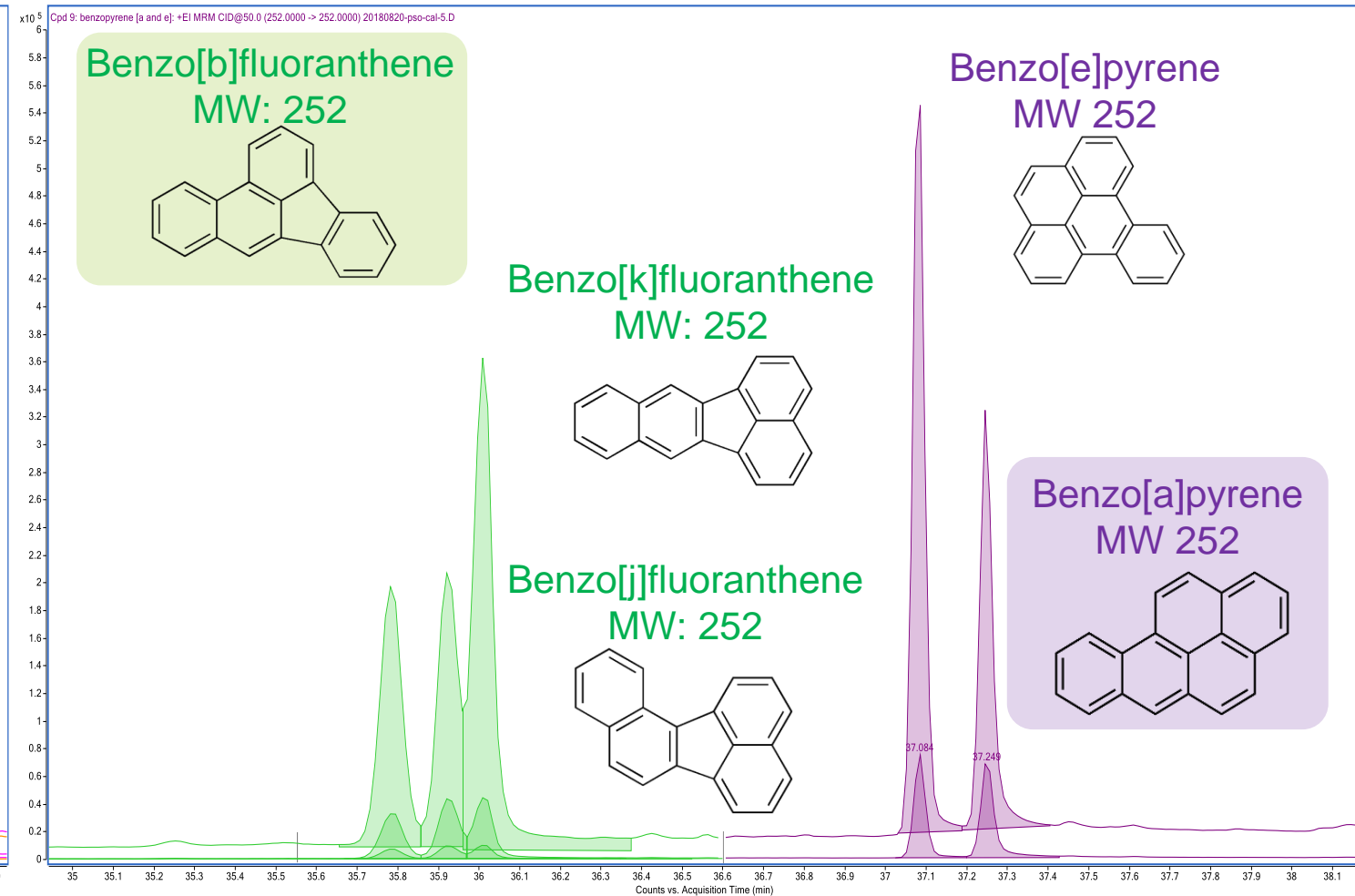
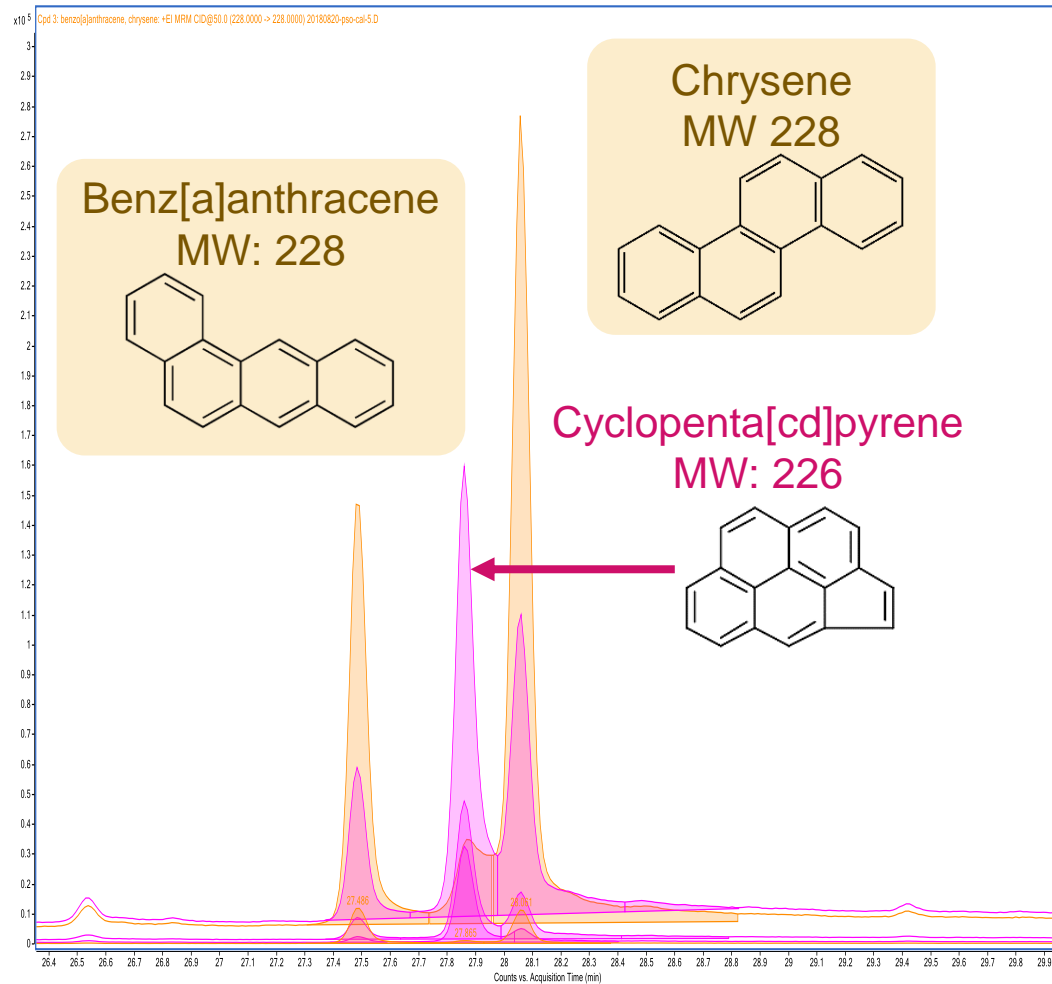
Calibration data is saved in the method

Inlet P is adjusted to achieved RT

Automated

Pumpkin Seed Oil

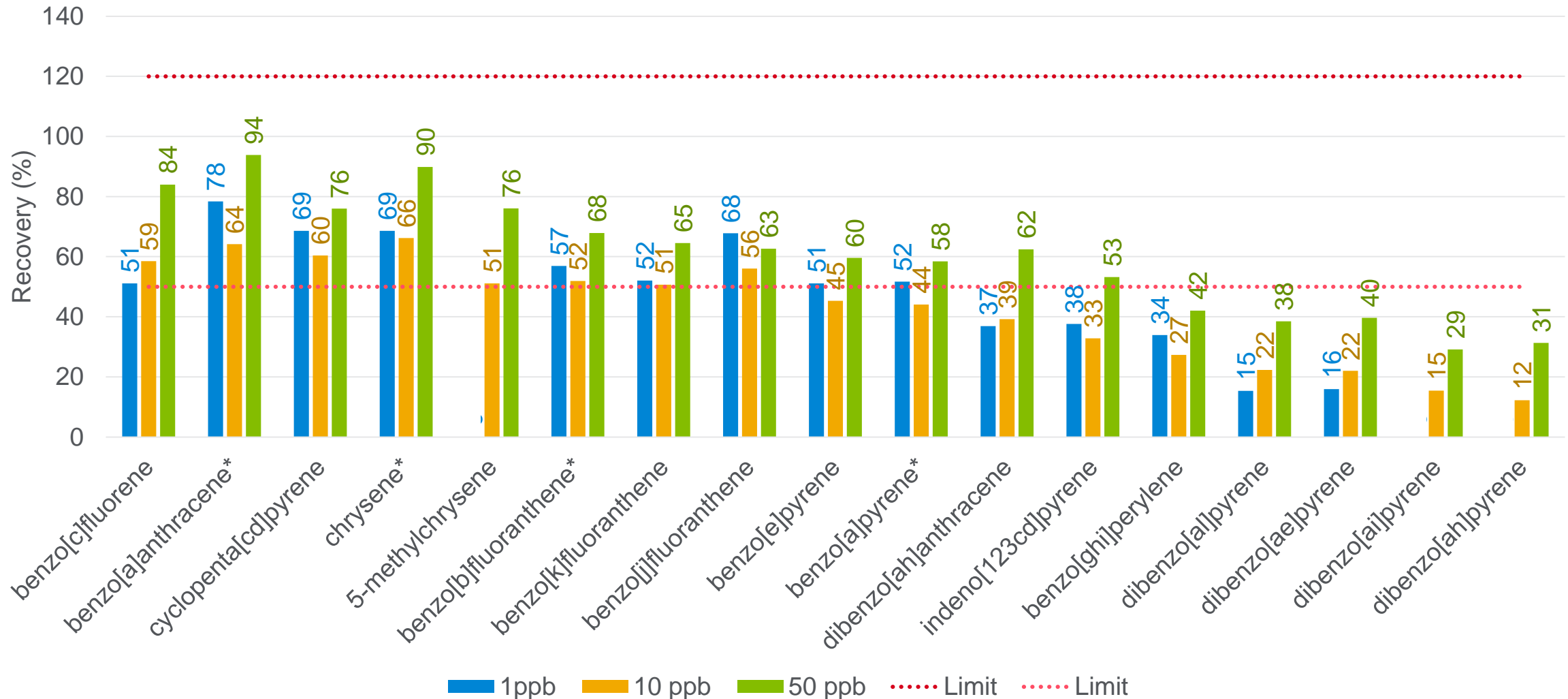
$[M]^+ \rightarrow [M]^+$ and $[M]^+ \rightarrow [M-2]^+$ at Collision Energy of 50 eV to destroy matrix interference



Matrix-Matched Calibration at 50 ppb

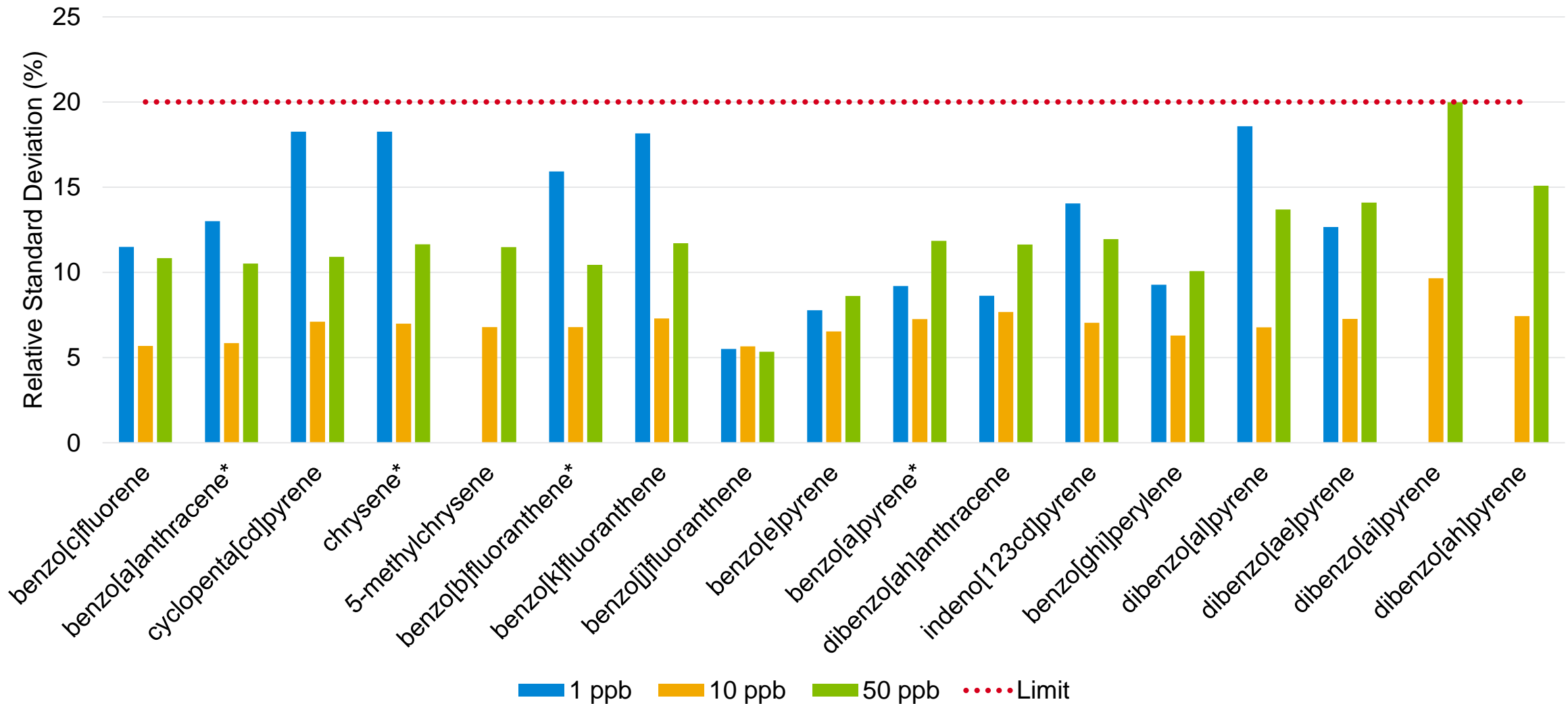
Pumpkin Seed Oil – Recovery

Recoveries are within range for the early and mid-eluters



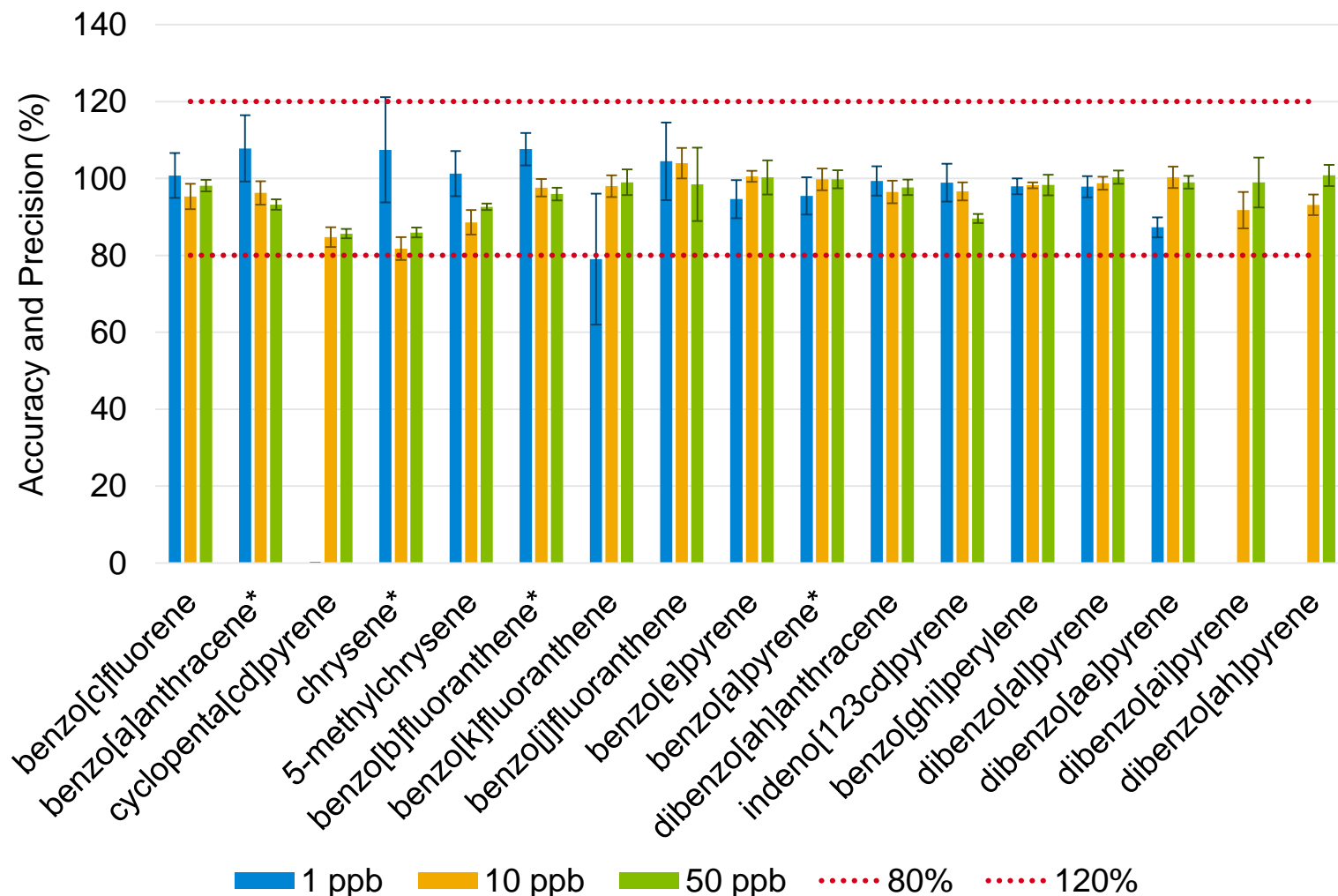
Pumpkin Seed Oil – Recovery RSD

Recovery RSD are within limits



Pumpkin Seed Oil – Accuracy and Precision

Accuracy and Precision are within limits. R2 linearity is >0.99



Analyte	R2
benzo[c]fluorene	0.9991
benzo[a]anthracene	0.9956
cyclopenta[cd]pyrene	0.9949
chrysene	0.9932
5-methylchrysene	0.9958
benzo[b]fluoranthene	0.9982
benzo[k]fluoranthene	0.9981
benzo[j]fluoranthene	0.9925
benzo[e]pyrene	0.9975
benzo[a]pyrene	0.9925
dibenzo[ah]anthracene	0.9994
indeno[123cd]pyrene	0.9987
benzo[ghi]perylene	0.9988
dibenzo[a]pyrene	0.9971
dibenzo[ae]pyrene	0.9953
dibenzo[ai]pyrene	0.9954
dibenzo[ah]pyrene	0.9977

Calibration 1, 2, 5, 10, 50, 80, 100 ppb

PAH References

Application Note 5991-3003EN



Optimized PAH Analysis Using the Agilent Self-Cleaning Ion Source and the Enhanced PAH Analyzer

Application Note

PAH, environmental, gas chromatography, mass spectrometry, semivolatiles

Author

Mike Szelewski and
Bruce D. Quimby
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808


Abstract

The Agilent Enhanced PAH Analyzer was used for the analysis of polycyclic aromatic hydrocarbons (PAHs) with the Self-Cleaning Ion Source in Continuous Cleaning Mode. Both the Agilent 5977A Series GC/MSD System and Agilent 7000B Triple Quadrupole GC/MS versions of the analyzer were used. All instrument parameters including inlet, column, and MS were investigated and optimized. Linearity and ISTD reproducibility, across a calibration range of 1–1,000 pg, were improved while maintaining sensitivity.

Peer Review Journal


Journal of Chromatography A, 1419 (2015) 89–98

Contents lists available at ScienceDirect



Journal of Chromatography A


journal homepage: www.elsevier.com/locate/chroma



Modified ion source triple quadrupole mass spectrometer gas chromatograph for polycyclic aromatic hydrocarbon analyses

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^b Agilent Technologies, Wilmington, DE 19808, United States



Journal of Chromatography A 1419 (2015) 89–98

Collaboration with **Oregon State University**
Dept. of Environmental and Molecular Toxicology

Future/Current work

Investigate other matrices

Foodstuff		Maximum Levels (ug/kg)	
Points	Foodstuff	benzo[a]pyrene	Sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, and chrysene
6.1.1	Oils and fats	2	10
6.1.2	Cocoa bean	5	30
6.1.4	Smoked meat	2	12
6.1.5	Smoked fish	2	12
6.1.7	Smoked bivalve mollusk	6	35

EU Commission No 1881/2006 Section 6

Summary

EMR-Lipid with PSA/C18

- To clean up pumpkin seed oil

GC/MS/MS

- **Jetclean** to keep the source clean
- **Backflush (BF)** to maintain column lifetime
- **Retention Time Locking (RTL)** to prevent RT shift
- **High temperatures** to prevent PAHs from sticking to surfaces
- Single-tapered **liner with glass wool** to prevent PAH condensation in inlet base

Pumpkin Seed Oil

- Recoveries and RSD are within limits for the early and mid-eluting PAHs
- Precision and Accuracy are within limits
- Calibration linearity is achieved

Thank you!

CVUA-MEL (Germany)

Thorsten Bernsmann, Ph.D.

Agilent Technologies, Inc.

John Lee

Limian Zhao

Bruce Quimby, Ph.D.

Mike Szelewski, Ph.D.

Joerg Riener, Ph.D.

AOAC

New Blood Session Chairs!

BondElut EMR-Lipid



Drying Pouches

Extraction

EMR-Lipid dSPE

EMR polish

GC/MS/MS



7890 Gas Chromatograph
7010 Triple Quadrupole
Mass Spectrometer