Application Note
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EU Priority PAH Analysis in Pumpkin Seed Oil Using Bond Elut EMR—Lipid Cleanup by GC/MS/MS

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Abstract

European Union (EU) priority polycyclic aromatic hydrocarbons (PAHs) were analyzed in pumpkin seed oil using a liquid-liquid extraction, followed by Bond Elut EMR—Lipid (EMR—Lipid) and PSA/C18/$MgSO_4$ dSPE (PSA/C18/$MgSO_4$) cleanup. PAHs were quantified using a gas chromatography triple quadrupole mass spectrometer (GC/MS/MS) equipped with an Agilent JetClean self-cleaning ion source (JetClean) and backflush (BF). The JetClean prevents PAH deposition in the source by introducing a low flow of hydrogen during analysis. Column lifetime is improved with the use of postrun midcolumn BF. High collision energy (50 eV) was used to remove matrix interference during multiple reaction monitoring (MRM) analysis of PAHs. PAH recoveries were within the EU regulation limits of 50 to 120 % for the mideluting PAHs at prespiked levels of 1, 10, and 50 ng/g. Heavy PAHs met the EU Regulation limits only at the 50 ng/g prespiked level. RSD recoveries were within EU regulation limits of below 20 % for all PAH investigated except for benzo(a)pyrene prespiked at 1 ng/g with an RSD of 23 %. Precision and accuracy analysis were used to verify method quantitation with accuracy of 100 ±20 % and RSD <20 % at all three prespiked levels. Limit of quantitation (LOQ) was 1 ng/g for all PAHs investigated except for cyclopenta(cd)pyrene and 5-methylchrysene at 10 ng/g. Linear calibration was observed with a R^2 >0.99.

Introduction

PAHs are a group of organic compounds composed of two or more aromatic rings that contain only carbon and hydrogen. The formation of PAHs occurs during industrial food processing (roasting, drying, and so on), high-temperature cooking (frying, grilling, and so on), or environmental exposure (incomplete combustion of fossil fuels or wood, and so on). In edible oils, the seed and kernel drying process is thought to be the most prominent source of PAHs with the use of direct firing[1]. High temperatures used in the seed roasting process is another possibility for contamination in edible oils. Environmental exposures, such as plants exposed to industrial and vehicle emissions, could also contribute to PAHs found in edible oils. Due to the lipophilic nature of PAHs, fats and lipids present the main source of PAHs in the diet[2].

Since 2005, the European Commission (EC) Regulation established maximum levels of benzo(a)pyrene in different food groups along with 15 other PAH analytes as carcinogenic by the Scientific Committee on Food (SCF). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) identified benzo(c)fluorene as a PAH that should be monitored as well. EC Regulations 1881/2006 and 835/2011 set the maximum permitted levels of PAH residues in oils and fats intended for human consumption or use as an ingredient in food to 2.0 µg/kg for benzo(a)pyrene and 10 µg/kg for the sum of benzo(a)pyrene, benzo(a) anthracene, benzo(b)fluoranthene, and chrysene[3,4].

Agilent Enhanced Matrix Removal—Lipid (EMR—Lipid) dSPE cleanup has gained a lot of attention since its introduction in 2015. The EMR—Lipid dSPE sorbent selectively interacts with the unbranched hydrocarbon chains of lipids, and leaves the bulky target analytes in solution for subsequent analysis. This makes EMR—Lipid ideal for multiclass and multiresidue analysis. The EMR—Lipid sorbent is convenient and effective in removing matrix interference, particularly lipids, to allow for low-level detection of the analyte of interest[5,6]. After EMR—Lipid dSPE cleanup, residual water is removed using an EMR–Polish dispersive kit containing $MgSO_4$ and NaCl, which is crucial for GC/MS/MS analysis. Then, a PSA/C18/$MgSO_4$ dSPE cleanup was used for further matrix cleanup and water removal.

The challenge of analyzing PAHs is due to their chemical properties, therefore, GC/MS/MS was modified for analysis[7]. PAHs tend to adhere to surfaces, are subject to desublimation, and are difficult to vaporize. Thus, the GC inlet, MSD transfer line, and MSD source were kept at high temperatures to minimize surface contact and improve vaporization. JetClean keeps the source clean by introducing a low flow of hydrogen during data acquisition[8]. The inlet liner contains glass wool to transfer heat and prevent PAHs from depositing at the bottom of the liner. Backflushing maintains column lifetime by removing heavy eluting matrix at the end of each analysis[9]. Since PAHs are resistant to change, a high collision energy of 50 eV was used in all of PAH MRM acquisitions to remove matrix interference while PAHs remained unaffected.

This Application Note investigated pumpkin seed oil because the matrix is fatty and complex with a high probability for PAH contamination. The fatty acid compositions in pumpkin seed oil include mainly palmitic acid (9.5 to 14.5 %), stearic acid (3.1 to 7.4 %), oleic acid (21 to 46.9 %), and linoleic acid (35 to 60.8 %)[10]. Pumpkin seed oil is produced by toasting pumpkin seeds above 100 °C, and pressing the seeds, using a hydraulic press, into a dark green oil. In addition to the roasting process, the other possibility for PAH contamination in pumpkin seed oil is that there is no refining step, which can drastically decrease the amount of PAHs[1].

Experimental

Solvents and sample preparation products

Solvents used in this study were HPLC- or GC-grade. Acetonitrile (ACN) (271004) and isooctane (650439) were bought from Sigma-Aldrich.

Sample preparation products used for sample cleanup consisted of the following:

- Bond Elut EMR—Lipid dispersive SPE in 15 mL tube (EMR—Lipid) (p/n 5982-1010)
- Bond Elut EMR—Lipid Polish in 15 mL tube containing NaCl and anhydrous $MgSO_4$ (EMR–Polish) (p/n 5982-0101)
- QuEChERS dispersive SPE, EN method, in 15 mL tube containing 150 mg PSA, 150 mg C18EC, and 900 mg of $MgSO_4$ (PSA/C18/$MgSO_4$) (p/n 5982-5156)

Standards and solutions

The PAH standard mix (STD) consisted of PAH standard mix (p/n 5191-4508). Isotopically labeled internal standard mix (IS) consisted of deuterated PAHs (p/n 5191-4509). For prespiking quality control (QC) samples, neat STD and IS spiking solutions were prepared in isooctane at desired concentrations, and spiked directly into pumpkin seed oil. For postspiking matrix-matched calibration samples, neat STD and IS spiking solution were prepared in isooctane at desired concentrations, and used to reconstitute dried matrix blank (MB) samples. Prespiking and postspiking levels were 1, 10, and 50 ng/g STD with 50 ng/g IS. Matrix-matched calibration levels were 1, 2, 5, 10, 25, 50, and 100 ng/g STD with 50 ng/g IS.

Sample preparation

Store-bought toasted pumpkin seed oil was used in this study. The sample was extracted with ACN followed by sample cleanup using EMR—Lipid, with residual water removed using EMR–Polish. The PSA/C18/$MgSO_4$ cleanup was used for further matrix cleanup and water removal. Figure 1 shows the detailed procedure for sample preparation.

GC/MS/MS analysis

The GC/MS/MS used in this study is an Agilent 7890B GC and an Agilent 7010 triple quadrupole GC/MS equipped with JetClean self-cleaning ion source and an Agilent 7693A Automatic Liquid Sampler (ALS). Tables 1 to 3 and Figure 2 list detailed GC and MSD parameters. Data were acquired using MassHunter GC/MS Acquisition B.07.06, and analyzed using MassHunter Qualitative Analysis B.07.00 and MassHunter Quantitative Analysis B.09.00.

1. Extraction

- Weigh 1 g of pumpkin seed oil into a 15 mL centrifuge tube.
  - QC samples: prespike PAH STD and IS spiking solution into the oil.
  - Create MB samples that do not contain STD and IS for matrix-matched calibration
- Vortex for two minutes at 5,000 rpm.
- Add 10 mL of ACN, vortex for 30 minutes at 2,000 rpm, and centrifuge for 10 minutes at 5,000 rpm.

2. EMR—Lipid cleanup

- Add 2.5 mL of water to EMR—Lipid, and vortex to mix.
- Transfer 5 mL of supernatant (from the extraction step) to the EMR—Lipid tube.
- Vortex for two minutes at 2,000 rpm and centrifuge for five minutes at 5,000 rpm.

3. EMR—Polish

- Decant supernatant to EMR—Polish.
- Vortex and shake vigorously.
- Centrifuge for five minutes at 5,000 rpm.

4. PSA/C18/$MgSO_4$ cleanup

- Transfer supernatant to PSA/C18/$MgSO_4$.
- Vortex for two minutes at 2,000 rpm and centrifuge for five minutes at 5,000 rpm.

5. Concentration

- Transfer 2 mL of supernatant to a glass centrifuge tube.
- Concentrate the sample to dryness (N_2 flow at 40 °C water bath).
- Reconstitute dried samples (10x)
  - Matrix-matched calibration: postspike MB with 200 µL postspiking solution.
  - QC samples: Add 200 µL of isooctane.
- Vortex for two minutes at 1,000 rpm, sonicate for 30 seconds in a water bath, and centrifuge for 30 seconds.
- Transfer to an autosampler vial with a 250 µL glass insert for GC/MS/MS analysis.

Figure 1. Detailed procedure to prepare pumpkin seed oil sample for GC/MS/MS analysis.

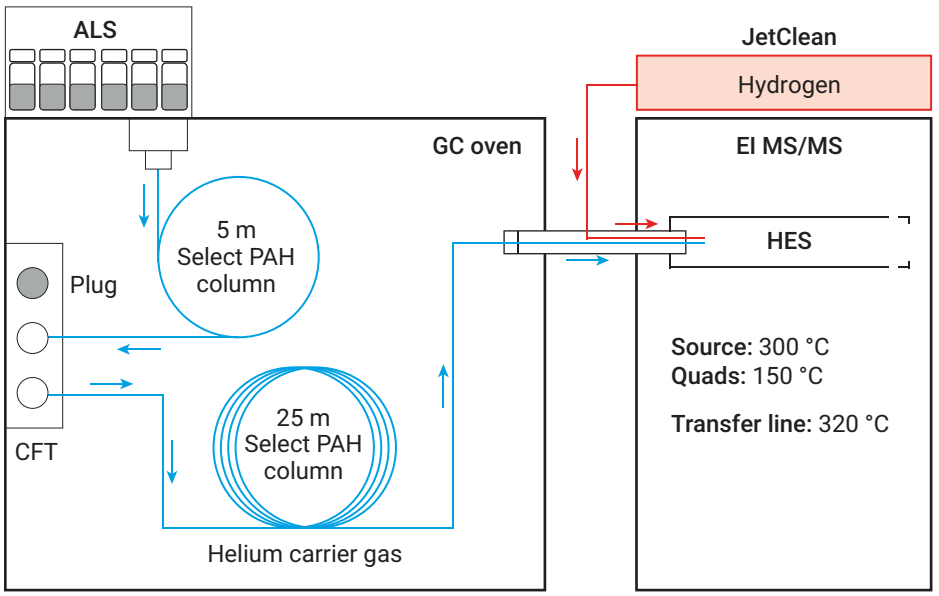

Figure 2. GC/MS/MS in electron ionization (EI) mode configured with BF and JetClean. Arrows indicate direction of helium carrier gas flow. CFT purged 3-way splitter is used to configure the two columns for BF. The JetClean self-cleaning ion source is configured to the MSD in Acquire and Clean mode to allow 0.33 mL/min of hydrogen flow to the source. ALS = automatic liquid sampler. HES = high efficiency source. CFT = Capillary Flow Technology.

The following GC/MS/MS modifications are crucial for low level PAH analysis:

- BF setup (Figure 2)
  - Cut 5 m off the Select PAH column (30 m length × 250 µm diameter × 0.15 µm film thickness).
  - Connect the 5 m section from the split/splitless inlet to the capillary flow technology (CFT).
  - Connect the rest of the 25 m from the CFT to the mass spectrometer detector (MSD).
  - Use the BF Wizard in the acquisition software for the following postrun procedures:
    - Decrease inlet pressure to 2 psi.
    - Increase CFT pressure to 70 psi.
    - Maintain an oven temperature of 320 °C.
    - Use 20 void volumes, giving a run time of 0.38 minutes.
- Inlet, transfer line, and source temperatures are at 320, 320, and 300 °C, respectively.
- The inlet liner must be an Ultra Inert 4 mm, single tapered with glass wool (to transfer heat to the PAHs).
- Operate the JetClean self-cleaning ion source in Acquire and Clean mode at 0.33 mL/min of hydrogen flow to the source.
- RT Lock to chrysene at 25.89 minutes to prevent shifting in retention time, and enable easy maintenance.

Table 1. GC parameters.

GC Conditions	
GC	7890B GC system
Inlet	Split/splitless
Mode	Splitless
Heater	320 °C
Pressure	14.4 psi
Total Flow	54.2 mL/min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Purge Flow to Split Vent	50 mL/min at 1 minute
Inlet Liner	Agilent 4 mm Ultra Inert inlet liner, single tapered with glass wool, 900 µL (p/n 5190-2293)
Oven	
Oven Ramp	Initial: 80 °C (Hold 0.5 minutes) Ramp 1: Rate at 120 °C/min to 120 °C Ramp 2: Rate at 40 °C/min to 180 °C Ramp 3: Rate at 3 °C/min to 280 °C Ramp 4: Rate at 120 °C/min to 330 °C (Hold 14 minutes)
Total Run Time*	50.08 minutes
Column Setup For Backflush	
Column	Agilent Select PAH (p/n CP7462)
Dimension	30 m length × 250 µm diameter, 0.15 µm film thickness
Column 1	
Dimensions	5 m × 250 µm, 0.15 µm (5 m cut from 30 m column)
In	Split/splitless inlet
Out	BF EPC
Pressure	14.4 psi
Flow	1.2 mL/min
Mode	Constant flow
Column 2	
Dimensions	25 m × 250 µm, 0.25 µm (Remaining after 5 m removed to make column 1)
Main Segment	24.83 m heated by oven
Segment 2	0.17 m heated by Thermal Aux 1
In	BF EPC
Out	MSD
Pressure	12.3 psi
Flow	1.5 mL/min
Mode	Constant flow
Carrier Gas	Helium
RT Locking	Locked to chrysene at 25.89 minutes*

* Instrument dependent

BF Operating Conditions	
Oven Temperature	330 °C
BF Pressure	70 psi
Inlet Pressure During BF	2 psi
Void Volumes	20
BF Time	0.38 minutes
BF Flow to Inlet	29.124 mL/min
Injector	7693A Automatic Liquid Sampler
Injection Volume	2 µL
Syringe Size	10 µL
Syringe	G4513-80203
Viscosity Delay	2 seconds
Thermal Aux 2 (MSD Transfer Line)	
Heater	320 °C

Table 2. MS parameters.

MSD Conditions	
MSD	7010 triple quadrupole LC/MS
Ion source	Electron ionization
Scan type	MRM
Electron energy	70 eV
Solvent delay	14 minutes
MS source	300 °C
MS quads	150 °C
Gain	10
Collision Cell	
He quench gas	4 mL/min
N_2 collision gas	1.5 mL/min
JetClean	
Operation	Acquire and Clean
Hydrogen flow	0.33 mL/min

Table 3. MRM transitions and scan segment.

Analyte[a]	RT (min)[b]	Quantifier[c]	Qualifier[c]	Dwell (ms)	Cycles per s	ms per Cycle
benzo(c)fluorene	21.2	216.0 → 215.0	216.0 → 216.0	350	1.4	702
benzo(a)anthracene-d_{12}	27.3	240.0 → 240.0	240.0 → 238.0	100	1.4	705.8
benzo(a)anthracene	27.5	228.0 → 228.0	228.0 → 226.0	100	1.4	705.8
chrysene-d_{12}	27.8	240.0 → 240.0	240.0 → 238.0	100	1.4	705.8
cyclopenta(cd)pyrene	27.9	226.0 → 226.0	226.0 → 225.0	100	1.4	705.8
chrysene	28.1	228.0 → 228.0	228.0 → 226.0	100	1.4	705.8
5-methylchrysene	31.3	242.0 → 240.0	242.0 → 242.0	230	1.4	692.7
benzo(b)fluoranthene-d_{12}	35.6	264.0 → 264.0	264.0 → 262.0	125	1.3	755.3
benzo(b)fluoranthene	35.8	252.0 → 252.0	252.0 → 250.0	125	1.3	755.3
benzo(k)fluoranthene-d_{12}	35.8	264.0 → 264.0	264.0 → 262.0	125	1.3	755.3
benzo(k)fluoranthene	35.9	252.0 → 252.0	252.0 → 250.0	125	1.3	755.3
benzo(j)fluoranthene	36.0	252.0 → 252.0	252.0 → 250.0	125	1.3	755.3
benzo(e)pyrene	37.1	252.0 → 252.0	252.0 → 250.0	170	1.5	683.6
benzo(a)pyrene-d_{12}	37.2	264.0 → 264.0	264.0 → 262.0	170	1.5	683.6
benzo(a)pyrene	37.2	252.0 → 252.0	252.0 → 250.0	170	1.5	683.6
dibenzo(ah)anthracene-d_{14}	40.7	292.0 → 292.0	292.0 → 290.0	60	1.4	730.2
indeno(1,2,3-cd)pyrene-d_{12}	40.8	288.0 → 288.0	288.0 → 286.0	60	1.4	730.2
dibenzo(ah)anthracene	40.8	278.0 → 276.0	278.0 → 278.0	60	1.4	730.2
indeno(1,2,3-cd)pyrene	40.8	276.0 → 274.0	276.0 → 276.0	60	1.4	730.2
benzo(ghi)perylene-d_{12}	42.1	288.0 → 288.0	288.0 → 286.0	150	1.1	905.4
benzo(ghi)perylene	42.2	276.0 → 276.0	276.0 → 274.0	150	1.1	905.4

[a] Isotopically labeled compounds (-d_{12} and -d_{14}) are used as internal standards (p/n 5191-4509). All other analytes are from a PAH standard mix (p/n 5191-4508). Gain = 10.

[b] Retention times are system-dependent

[c] Collision energy is 50 eV for quantifier and qualifier MRM transitions for all analytes and internal standards. Quantifier ions were chosen based on abundance and least interference. Unit resolution (0.7 amu) was used for all precursor and product ions.

Results and discussion

EMR—Lipid sample preparation

EMR—Lipid dSPE cleanup was modified specifically for PAH analysis in oil matrices. Pumpkin seed oil is a fatty and hydrophobic matrix that binds strongly to the highly hydrophobic PAH analytes. EMR—Lipid dSPE cleanup usually requires the use of 50 % water in the sample mixture to achieve efficient lipid removal. However, the addition of 50 % water reduces PAH solubility and negatively impacts analyte recoveries; therefore, the amount of water for EMR—Lipid sorbent activation was reduced to 2.5 mL (conventionally 5 mL). Pumpkin seed oil extract was then added immediately to the EMR—Lipid sorbent to ensure maximum interaction with the sorbent. EMR–Polish dSPE is normally recommended for residual water removal, but for GC/MS/MS analysis, this one-step water removal is not adequate, and an additional drying step is necessary. Therefore, the PSA/C18/$MgSO_4$ dSPE cleanup was used for further matrix and complete water removal. To achieve the desired quantitation limit of PAHs for GC/MS/MS analysis, the sample was concentrated and reconstituted 10 times with isooctane.

MS full scans were used to evaluate the matrix cleanup efficiency using EMR—Lipid dSPE cleanup. Without sample cleanup, pumpkin seed oil showed matrix and lipid interferences with raised baselines and column overload (Figure 3). Three methods of sample cleanup were compared:

- PSA/C18/$MgSO_4$ dSPE
- EMR—Lipid dSPE
- EMR—Lipid dSPE and PSA/C18/$MgSO_4$ dSPE

EMR—Lipid with additional PSA/C18/$MgSO_4$ cleanup was the most efficient cleanup for lipid and other matrix interference.

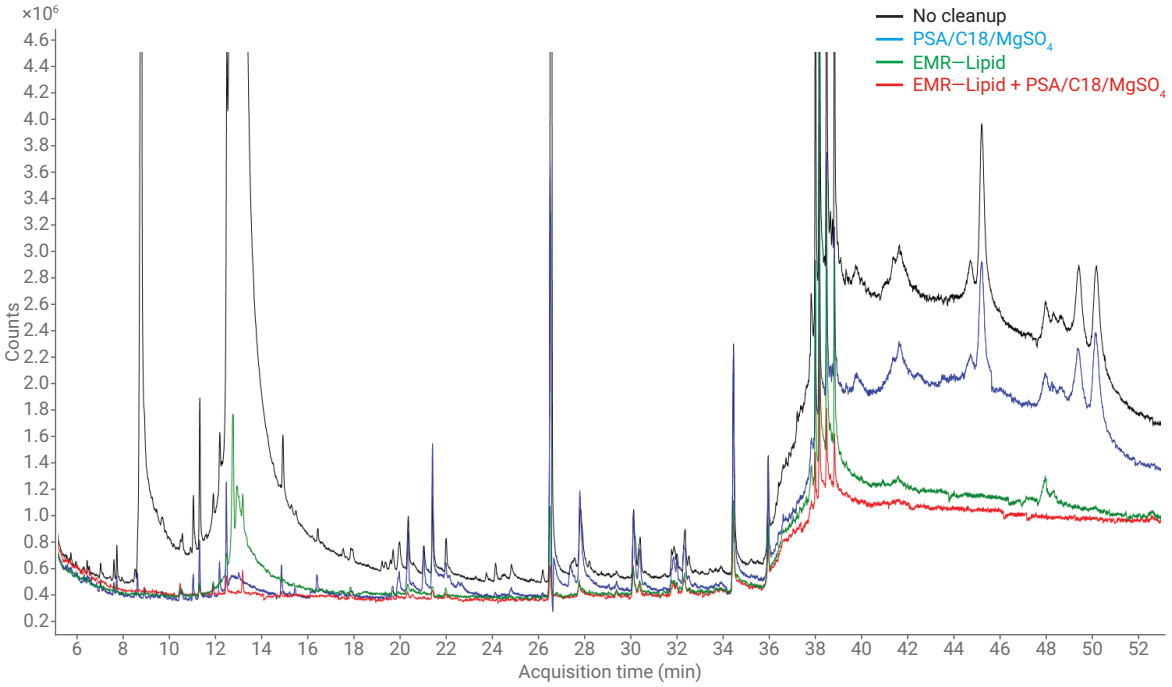

Figure 3. Pumpkin seed oil extracts with different cleanup procedures. MS scan range *m/z* 40 to 1,050. Black: no cleanup. Blue: PSA/C18/$MgSO_4$ cleanup. Green: EMR–Lipid cleanup. Red: EMR–Lipid with an additional PSA/C18/$MgSO_4$ cleanup.

GC/MS/MS analysis

Low-level PAH quantification was feasible in pumpkin seed oil by GC/MS/MS modified with JetClean and BF. The JetClean self-cleaning ion source is a module that introduces a low flow of hydrogen directly into the source during data acquisition to eliminate matrix deposition. BF reduces sample carryover by reversing the flow of the first column to flush out high-boiling matrix contaminants through the split vent at the end of analysis. The advantage of BF is that contaminants are not deposited onto the source. Without the need for column bake-out, the lifetime of the column is maintained, which also prevents column bleed from depositing onto the source.

MRM and a high collision energy (CE) were used for PAH analysis in pumpkin seed oil. Quantifier and qualifier ions were chosen based on ion abundance and significance. Since PAHs do not fragment readily, the precursor and product ion for the quantifier transitions is the molecular mass to the molecular mass ($[M]^+ \rightarrow [M]^+$). The qualifier transition is analyzed at $[M]^+ \rightarrow [M\text{-}2]^+$. The high CE of 50 eV helped eliminate matrix interference, while PAH remained unaffected (Figure 4). Table 4 lists the MRM transitions.

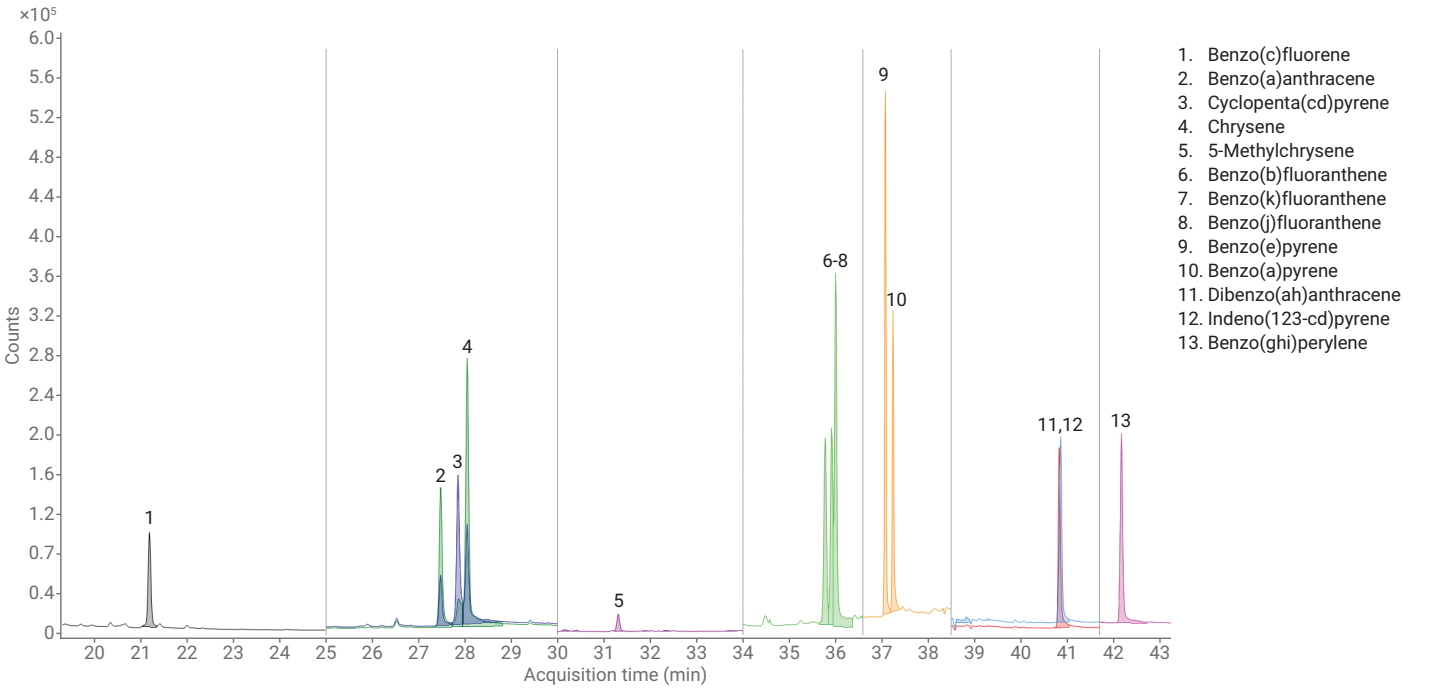

Figure 4. MRM TIC of quantifier ion from matrix-matched calibration of pumpkin seed oil spiked with 50 ng/g PAH. Collision energy of 50 eV. Quantifier transitions $[M]^+ \rightarrow [M]^+$ are plotted, where M represents molecular mass.

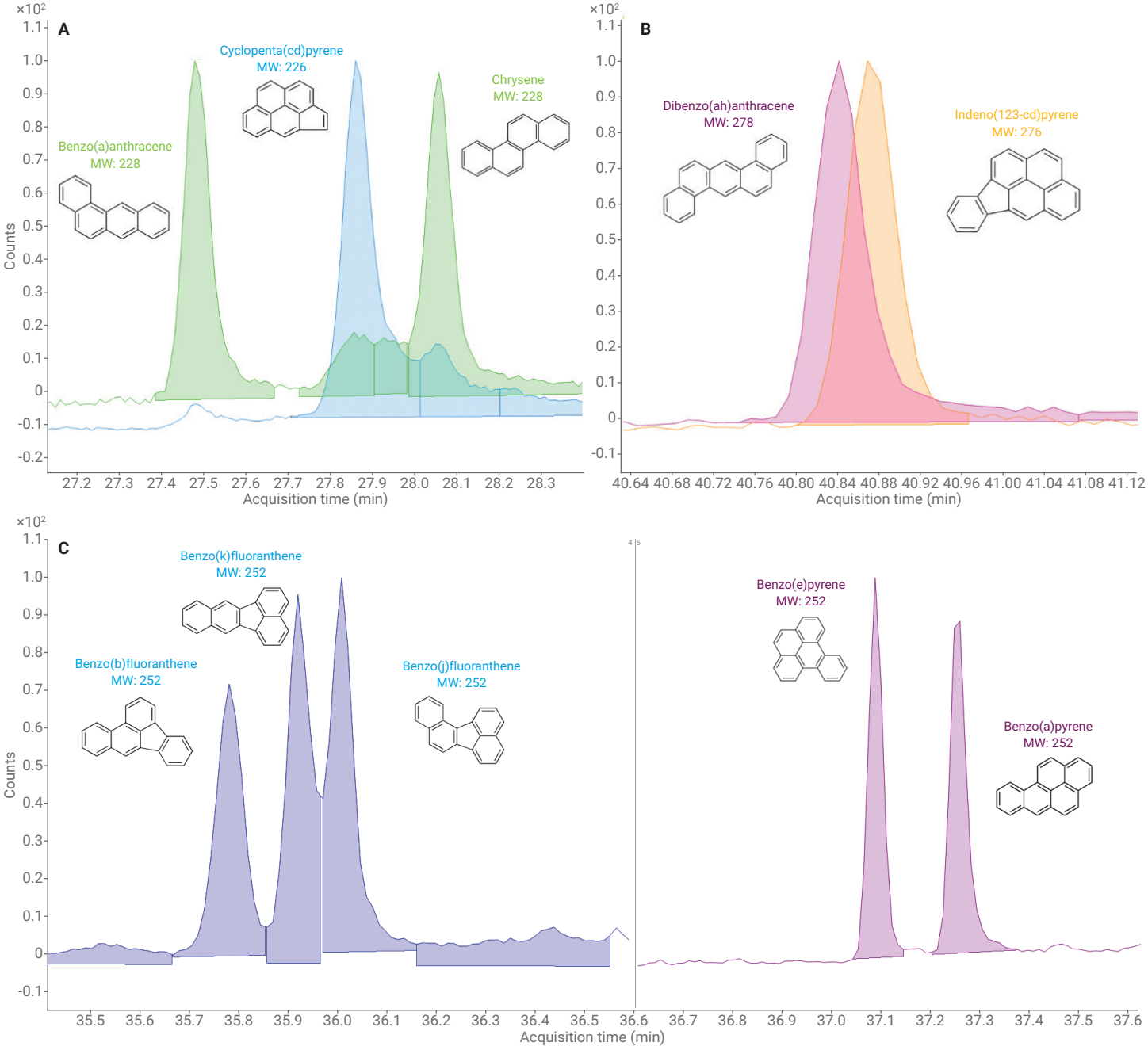

Figure 5. GC/MS/MS MRM chromatograms of pumpkin seed oil spiked with 5 ng/g PAHs (A-E). Collision energy of 50 eV. Quantifier transitions $[M]^+ \rightarrow [M]^+$ are plotted, where M represents molecular mass.

The Select PAH column provided excellent separation, good peak shape, and sensitivity for the pairs of PAH compounds that are typically difficult to separate on most GC columns because of their identical mass fragmentations[11]. The column aided in separation of the following PAH compound pairs that were investigated in this study (Figures 6A-C).

- benz(a)anthracene, cyclopenta(c,d)pyrene, and chrysene (molecular mass 226, 228 Da)
- Indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene (molecular mass 276, 278 Da)
- benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(j)fluoranthene (molecular mass 252 Da)
- benzo(e)pyrene and benzo(a)pyrene (molecular mass 252 Da)

Sensitivity, peak shape, and separation were also observed at the prespike level of 1 ng/g of the four PAHs monitored by the EU Commission Regulation (Figure 6).

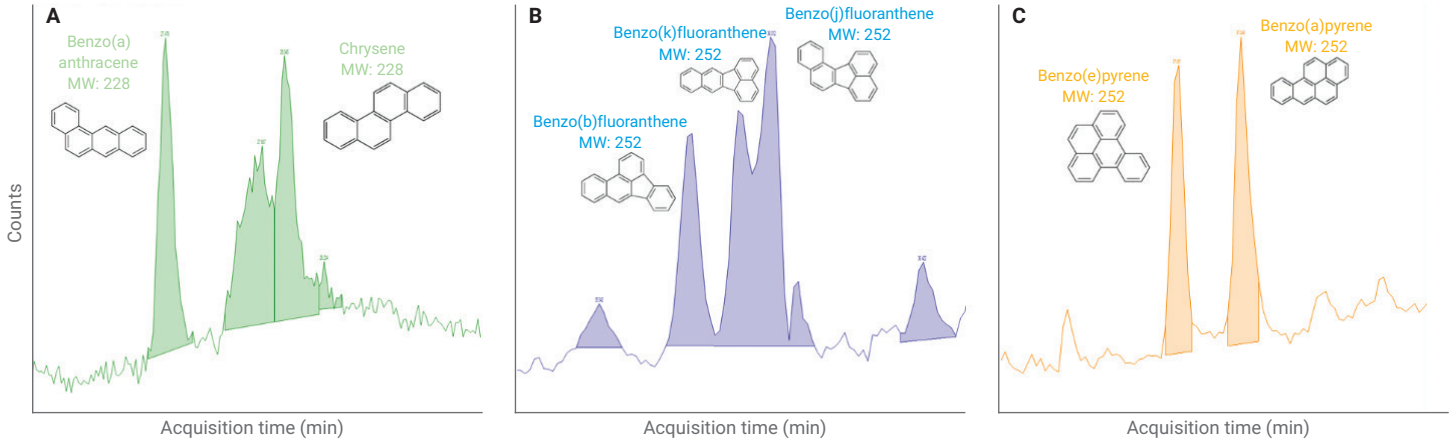

Figure 6. MRM TIC of pumpkin seed oil was prespiked with 1 ng/g PAH.

Accuracy and precision

Excellent accuracy and precision were achieved at 1, 10, and 50 ng/g in pumpkin seed oil using the developed sample preparation method. The accuracy ranged between 79 and 108 % for all spiking levels of analytes analyzed using IS correction (Figure 7A). All compounds at all spiked levels fell in the range of 80 to 120 % accuracy, except for benzo(k)fluoranthene, which was 79 % at the 1 ng/g prespike level. The RSD ranged between 2 and 17 % (Figure 7B). Cyclopenta(cd)pyrene and 5-methylchrysene at 1 ng/g were not detected.

LOQs and calibration linearity

A seven-point matrix-matched calibration was used for method quantitation. Matrix-matched calibration curves were generated at 1, 2, 5, 10, 25, 50, and 100 ng/g of PAHs with 50 ng/g of ISTD. Linear calibration was observed with an R^2 >0.99 using linear regression with weight of $1/x^2$ (Table 5). LOQs were 1 ng/g except for cyclopenta(cd)pyrene and 5-methylchrysene at 10 ng/g.

Table 5. Pumpkin seed oil seven-point matrix-matched calibration.

Analyte	LOQ	R^2
Bbenzo(c)fluorene	1	0.9991
Benzo(a)anthracene	1	0.9956
Cyclopenta(cd)pyrene	10	0.9949
Chrysene	1	0.9932
5-Methylchrysene	10	0.9958
Benzo(b)fluoranthene	1	0.9982
Benzo(k)fluoranthene	1	0.9981
Benzo(j)fluoranthene	1	0.9925
Benzo(e)pyrene	1	0.9975
Benzo(a)pyrene	1	0.9925
Dibenzo(ah)anthracene	1	0.9994
Indeno(123-cd)pyrene	1	0.9987
Benzo(ghi)perylene	1	0.9988

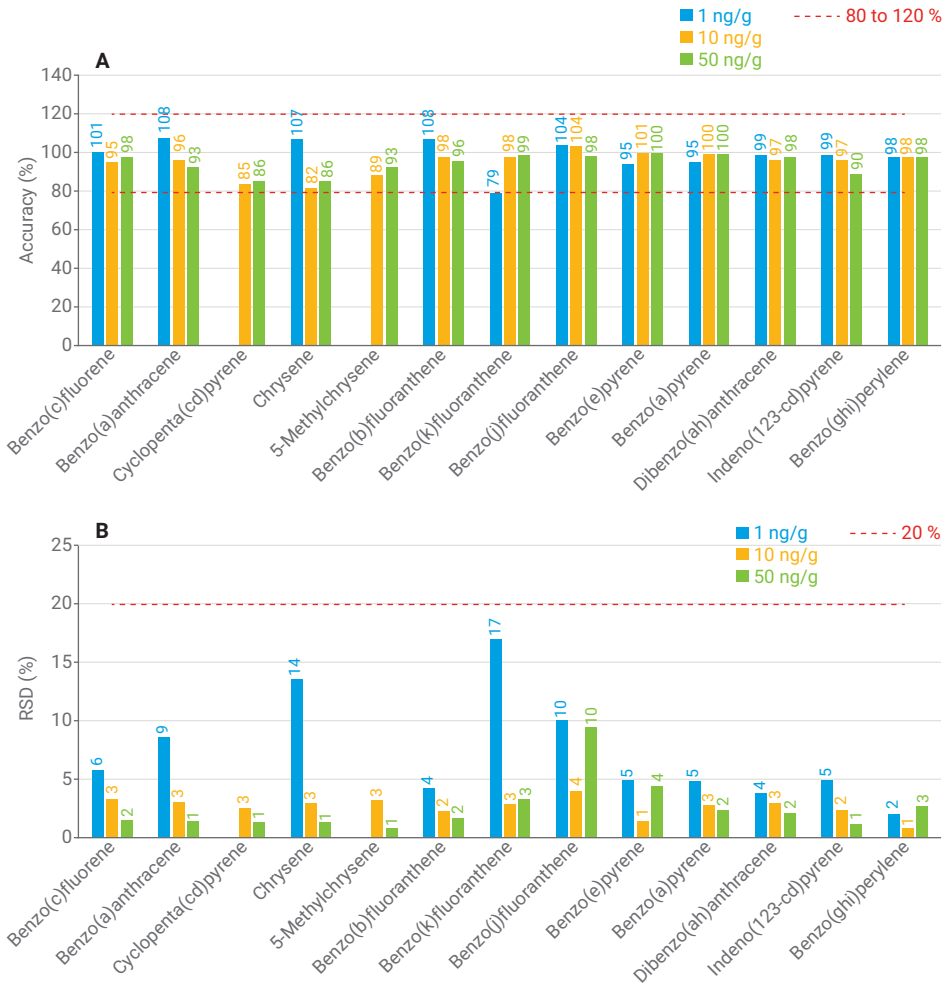

Figure 7. Accuracy (A) and Precision (B) of pumpkin seed oil spiked with 1, 10, and 50 ng/g PAH standard mix. IS was spiked at 50 ng/g. Analytes are plotted in order of increasing retention time. n = 6.

Recovery

Absolute recovery ranged from 27 to 94 % without the use of IS (Figure 6A). RSD of recovery ranged from 5 to 23 % (Figure 6B). The PAH absolute recoveries decrease with increasing molecular weight due to the decreasing PAH solubility in acetonitrile during the extraction step. Overall, the recoveries were within the limits set by the EU Commission Regulation of 50 to 120 % except for benzo(e)pyrene, benzo(a)pyrene, dibenzo(ah)anthracene, indeno(123-cd)pyrene, and benzo(ghi)perylene at some spiked levels. Benzo(e)pyrene and benzo(a)pyrene recoveries were 45 and 44 % at 10 ng/g spiked level, respectively. Dibenzo(ah)anthracene recoveries were 37 and 39 % at the 1 and 10 ng/g spiked levels, respectively. Indeno(123-cd)pyrene recoveries were 38 and 33 % at the 1 and 10 ng/g spiked levels, respectively. Benzo(ghi)perylene recoveries ranged from 34, 27, and 42 % at 1, 10, and 50 ng/g spiked levels. RSD% values were below 20 % for all analytes except benzo(a)pyrene with 23 %. However, the low recoveries can be corrected using IS for quantitation. Cyclopenta(cd)pyrene and 5-methylchrysene at 1 ng/g were not detected.

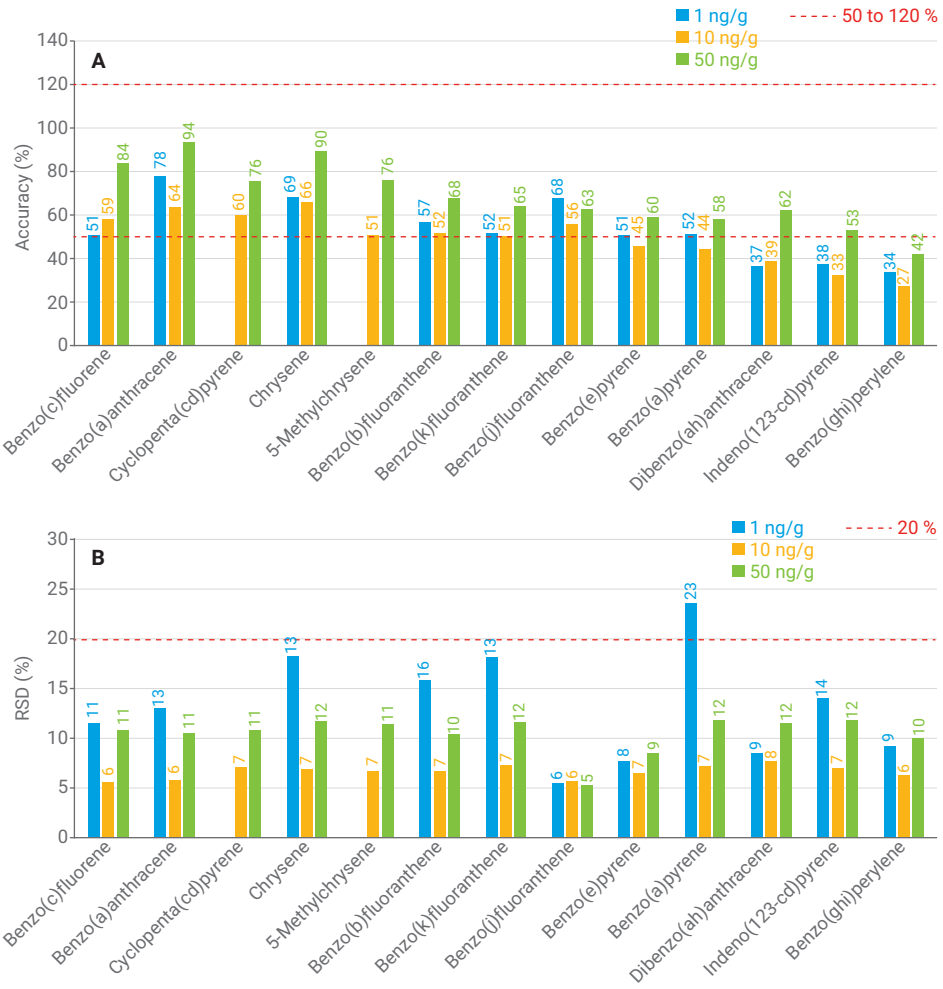

Figure 8. Recovery (A) and RSD (B) of pumpkin seed oil spiked with 1 ng/g (blue), 10 ng/g (yellow), and 50 ng/g (green) PAH standards. n=6. Analytes are plotted in order of increasing retention time.

Conclusions

A method was developed and validated for PAH analysis in pumpkin seed oil using liquid-liquid extraction followed by Bond Elut EMR—Lipid dSPE and PSA/C18/$MgSO_4$ cleanup by GC/MS/MS. EMR—Lipid dSPE cleanup was modified using less water for sorbent activation to improve the PAH recoveries during cleanup. GC/MS/MS was modified with JetClean and BF. These modifications allowed for recoveries that are within the limits of 50 to 120 % for most of the EU priority PAHs. Calibration linearity was achieved with R^2 >0.99. Accuracy was within 100 ±20 % except for benzo(k)fluoranthene, which was 79 % at the prespiked level of 1 ng/g. Precision was below 20 % for all analytes. Recoveries were within the EU Commission Regulation limit of 50 to 120 % for most of the mideluters, but present challenges for the heavier PAHs. This could be a result of PAH solubility to acetonitrile during extraction.

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