Application Note

Food Testing and Agriculture

Determination of 14 Polycyclic Aromatic Hydrocarbon Compounds in Edible Oil

Using Captiva EMR—Lipid Cleanup by GC/MS/MS

Author

Limian Zhao
Agilent Technologies, Inc.

Abstract

This Application Note presents the development and validation of a multiresidue method for the analysis of heavy polycyclic aromatic hydrocarbon (PAH) (more than four rings) residues in five edible oils. The oils were: pumpkin seed oil, olive oil, avocado oil, almond oil, and grape seed oil. Oil samples were extracted by liquid/liquid extraction (LLE) using 20:80 ethyl acetate/acetonitrile as the extraction solvent, followed by Captiva EMR—Lipid hyphenated with Bond Elut Jr PSA pass-through cleanup. The cleaned sample eluent was then back-extracted using isooctane to remove water before GC/MS/MS analysis. The combined use of EMR—Lipid and PSA pass-through cleanup provided efficient and selective cleanup of oil matrix, resulting in above 95% oil co-extractives residue removal. The extra clean sample background noise allows the use of a large volume injection method on GC/MS/MS method, and provides the desired limit of quantitation (LOQ) (0.9 to 2 ng/g) required by the European Commission regulation, with acceptable quantitation accuracy and precision results.

Introduction

PAHs are a large class of ubiquitous and toxic compounds characterized by a thermodynamically stable fused aromatic ring structure. PAH compounds can be classified according to the number of condensed aromatic rings, as light (two to three rings) or heavy (four to six rings) PAHs. The heavy PAHs are more stable and toxic than the lighter ones. 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. The use of high temperatures in the seed roasting process is another possibility for contamination. Additionally, with the high lipophilicity, PAHs also tend to bio-accumulate in oil. Due to their suspected or proven mutagenic and carcinogenic activity, these compounds have been widely investigated and regulated. The U.S. Food and Drug Administration (FDA) requires PAH analysis at low-ppb levels in seafood.[1] The European Commission (EC) specified the criteria for the methods of analysis of four heavy PAH compounds, benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, and chrysene, down to an LOQ of 0.9 µg/kg, and limit of detection (LOD) of 0.3 µg/kg for each of the four PAHs.[2]

The main challenges for the analysis of PAHs, especially heavy PAHs, in oil include:

- Extraction of PAH analytes from the oil matrix with minimal oil co-extractives
- Selective sample extract cleanup to remove unwanted oil co-extractives and retain the target PAH compounds

To increase the extraction efficiency and cleaning the oil matrix, the method normally involves using a large volume of solvent with multiple extractions, longer extraction time, SPE, or freezing or GPC cleanup, and repeated drying with a large volume to be concentrated.[3–7]

Agilent Enhanced Matrix Removal—Lipid (EMR—Lipid) dSPE cleanup has gained considerable attention since its introduction in 2015. The EMR—Lipid dSPE sorbent selectively interacts with the unbranched hydrocarbon chains of lipids, leaving the bulky target analytes in solution for analysis. This selective interaction makes it ideal for multiclass, multiresidue analysis in fatty food matrices. Captiva EMR—Lipid cartridges require less water for sorbent activation (20%) compared to the traditional Bond Elut EMR—Lipid (50%). This helps simplify the workflow and improve the recoveries of hydrophobic compounds during cleanup.[8] The primary secondary amine (PSA) sorbent interacts with fatty acids efficiently, providing additional cleanup after Captiva EMR—Lipid, but not impacting neutral PAH recovery. The Bond Elut Jr PSA can be attached easily to the EMR—Lipid cartridges. With the use of pressure or vacuum, sample flows through two kinds of sorbent sequentially, achieving the optimal oil matrix cleanup.

This study investigates sample preparation using Captiva EMR—Lipid cartridge hyphenated with Bond Elut Jr PSA pass-through cleanup for the analysis of 14 PAH compounds in oil by GC/MS/MS. This method was developed to improve the limitations of the previous method for using Bond Elut EMR—Lipid dSPE cleanup on PAH determination in food.[9,10] Figure 1 shows the structure and LogP value of the heavy PAHs investigated in this study.

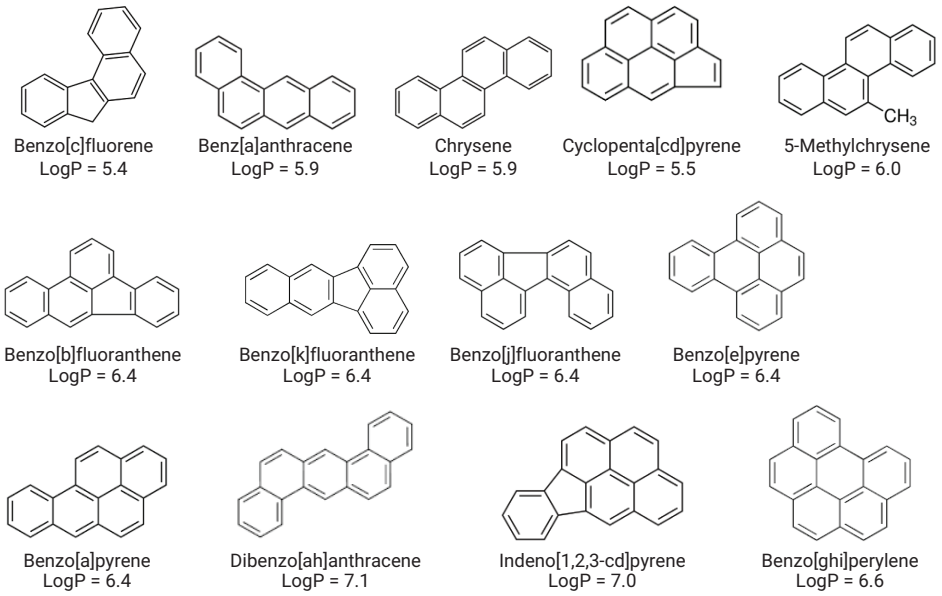

Figure 1. Heavy PAH analytes structure and LogP.

Experimental

Chemicals and reagents

The PAH standard mix (part number 5191-4508) and deuterated PAH internal standard (IS) mix (part number 5191-4509) were acquired from Agilent Technologies, Inc. HPLC grade acetonitrile (ACN), acetone, and ethyl acetate (EtOAc) were from Honeywell (Muskegon, MI, USA). Reagent grade isooctane was from Sigma-Aldrich (St. Louis, MO, USA).

Solutions and standards

Two working solutions were prepared from the stock solutions at 4 µg/mL and 250 ng/mL in acetone. An IS working solution was prepared at 10 µg/mL in acetone. Both working solutions were stored in amber glass vials in a refrigerator at 4 °C.

The 20:80 EtOAc/ACN extraction solvent was prepared by mixing 100 mL of EtOAc with 400 mL of ACN, and storing at room temperature. The 16:64:20 ACN/EtOAc/water elution solution was prepared by mixing 200 mL of extraction solvent and 50 mL of water, and storing at room temperature.

Equipment and material

The study was performed using an Agilent 7890B GC coupled with an Agilent 7000D triple quadrupole GC/MS. The GC system was equipped with an electronic pneumatic control (EPC), a multimode inlet (MMI) with air cooling, an Agilent 7693A automatic liquid sampler (ALS), and a backflush system based on a purged Ultimate union controlled by an AUX EPC module. Agilent MassHunter workstation software was used for data acquisition and analysis.

Sample preparation equipment included: a Centra CL3R centrifuge (Thermo IEC, MA, USA), Multi Reax Test Tube Shaker (Heidolph, Schwabach, Germany), pipettes and repeater (Eppendorf, NY, USA), Agilent positive pressure manifold 48 processor (PPM-48) (part number 5191-4101), Captiva EMR—Lipid cartridge, 6 mL, 600 mg (part number 5190-1004), and Bond Elut Jr PSA, 500 mg (part number 12162042B).

Instrument conditions

The GC/MS/MS instrument conditions were established based on a previously published method.[11] Table 1 lists the conditions of GC/MS/MS operation, and Table 2 lists the PAHs dMRM method parameters.

Table 1. Agilent 7890B GC and Agilent 7000D GC/MS/MS conditions.

Parameter	Value
Column 1	Agilent J&W DB-EUPAH UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-9632 UI), front MM inlet to AUX EPC 4
Column 2	Agilent J&W Silcotek deactivated tubing, 1.36 m × 0.15 mm, 0 µm (p/n 160-7625-5), AUX EPC 4 to MSD
Carrier Gas	Helium
Mode	Constant flow
Column 1 Flow	1.106 mL/min
Column 2 Flow	1.942 mL/min
Inlet	MMI inlet
Injection Mode	Large volume injection (solvent vent)
Injection Volume	5 µL
Inlet Temperature Gradient	85 °C hold for 0.03 minutes, ramp to 325 °C by 600 °C/min, hold for 5 minutes
Solvent Elimination	Inlet temp: 85 °C; vent pressure: 5 psi; vent flow: 100 mL/min; vent for 0.03 minutes
Inlet Liner	Ultra Inert liner, 4 mm id, single taper w/ wool, p/n 5190-2293
Oven Temperature Program	80 °C hold for 1 minute, ramp to 200 °C by 25 °C/min, then to 335 °C by 8 °C/min, hold for 9.325 minutes
Max Oven Temperature	340 °C
Run Time	32 minutes
Backflush Conditions	2 minutes post run 335 °C oven temperature 50 psi AUX EPC pressure, and 2 psi inlet pressure
Transfer Line Temperature	320 °C
Source Temperature	Xtr 350 EI source, 320 °C
Quadrupole Temperature	150 °C
Data Monitoring	Dynamic MRM mode
Solvent Delay	3 minutes
Gain Factor	20

Sample preparation

The edible oil was weighed (2.5 g) into 50 mL centrifuge tubes and spiked as necessary with standard and IS solutions. The sample was then vortexed thoroughly for one minute and equilibrated for 15 minutes. Oil samples were then prepared using the procedure shown in Figure 2, featuring three major parts:

1. Sample extraction by a two-step liquid-liquid extraction (LLE)
2. Sample extract pass-through cleanup using Captiva EMR—Lipid, hyphenated with Bond Elut Jr PSA cartridges
3. Post treatment for water removal using isooctane back-extraction (BE)

The entire workflow introduced a four-fold dilution of the original sample concentration.

Evaluation of matrix co-extractives removal

The matrix removal was investigated by gravimetric determination of sample co-extractive residue. The co-extractive residue weight was collected based on 1 mL of final sample extract with correction for the dilution factor when applicable.

The cleanup efficiency of Captiva–EMR can be seen based on the amount of residue left over after drying 1 mL of sample extract. "No cleanup" refers to sample extract that was collected after extraction followed by isooctane BE (no cleanup was performed). "EMR—Lipid + PSA cleanup" refers to sample extract with Captiva EMR—Lipid hyphenated with Bond Elut Jr PSA cleanup and back-extracted with isooctane. Samples were collected in replicates of two (n = 2), and the average weight was used to determine the percent matrix removal.

Table 2. List of PAHs for analysis, retention time (RT), and MS/MS conditions.

PAH compound	RT (min)	First MS/MS (*m/z*)	CE (V)	Second MS/MS (*m/z*)	CE (V)
Benzo[c]fluorine	16.49	215.8 → 214.8	50	215.8 → 212.8	50
Benzo[a]anthracene-D_{12}	18.96	240 → 240	50	240 → 240	50
Benz[a]anthracene	19.05	228.1 → 226.1	30	228.1 → 224.1	35
Chrysene-D_{12}	19.22	240.1 → 236.1	35	240.1 →238.1	50
Chrysene	19.32	228.1 → 226.1	30	226.1 → 224.1	40
Cyclopenta[cd]pyrene	19.33	226 → 226	50	226 → 225	50
5-Methylchrysene	20.59	241.8 → 240.8	50	241.8 → 238.8	50
Benzo[b]fluoranthene-D_{12}	22.3	264 → 264	50	264 → 262	50
Benzo[b]fluoranthene	22.38	252.1 → 250.1	30	252.1 → 252.1	50
Benzo[k]fluoranthene-D_{12}	22.38	264.1 → 264.1	50	264.1 → 262.1	50
Benzo[k]fluoranthene	22.45	252.1 → 252.1	50	252.1 → 250.1	50
Benzo[j]fluoranthene	22.55	251.8 → 251.8	50	251.8 → 249.8	50
Benzo[e]pyrene	23.5	251.8 → 251.8	50	251.8 → 249.8	50
Benzo[a]pyrene-D_{12}	23.57	264 → 264	50	264 → 262	50
Benzo[a]pyrene	23.66	252 → 250	50	125.1 → 124.1	10
Dibenzo[a,h]anthracene-D_{14}	27.28	292 → 292	50	292 → 290	50
Dibenzo[a,h]anthracene	27.44	277.8 → 277.8	50	277.8 → 275.8	50
Indo[1,2,3-cd]pyrene-D_{12}	27.41	288 → 288	50	288 → 286	50
Indo[1,2,3-cd]pyrene	27.54	277 → 277	50	276 → 274	50
Benzo[g,h,l]perylene-D_{12}	28.97	287.8 → 287.8	50	287.8 → 285.8	50
Benzo[g,h,l]perylene	29.12	275.8 → 275.8	50	275.8 → 273.8	10

Method validation

The optimized sample preparation method was validated in terms of analyte recoveries, quantitation accuracy and precision, LOQ, and calibration curve linearity in pumpkin seed oil. The method was then cross-verified in olive oil, avocado oil, grape seed oil, and almond oil for the recoveries and reproducibility at the LOQ level. The calibration standards included 1, 2, 5, 10, 20, 50, 100, 250, 400, and 500 ng/g in pumpkin seed oil. Four concentrations of QC samples were quantified against calibration curves at n = 6 for LOQ level (0.9 ng/g), low level (2 ng/g), mid level (10 ng/g), and high level 100 ng/g in pumpkin seed oil. Two concentrations of QC samples, n = 6 at LOQ level (0.9 ng/g) and low level (2 ng/g), were assessed with recoveries and reproducibility in four other oils for method cross-verification. Analyte identification and quantitation were determined from retention times and MRM transitions.

Results and discussion

EMR—Lipid and PSA sorbent

EMR—Lipid sorbent uses a novel chemistry that combines size exclusion and hydrophobic interactions providing high lipid removal selectivity and efficiency. Only the unbranched hydrocarbon chains of lipid-like molecules can enter the pores of the EMR—Lipid sorbent and be retained by hydrophobic interactions. Target analytes that do not have lipid-like structures are unable to enter the sorbent pores, and remain in solution for subsequent analysis. As a result, EMR—Lipid sorbent can deliver high analyte recovery, and efficiently remove lipids for most lipid classes. However, EMR—Lipid sorbent is limited for fatty acid removal due to unsteady interaction with fatty acid molecules, especially for short chain fatty acids.

PSA is a sorbent that interacts efficiently with acidic compounds to remove fatty acids. PSA sorbent has been widely used for acid cleanup in fruits and vegetables, but often has a negative impact on the recoveries of acidic analytes. However, for neutral target analytes, such as PAHs, the use of PSA sorbent provides further cleanup without impacting the target analyte recoveries.

Optimization of sample preparation

The method was developed based on the method for PAH analysis in salmon and beef.[12] The method worked well for salmon and beef matrices, but was not as successful when applied to a complex oil matrix such as pumpkin seed oil. The unremoved matrix interferences caused a raised baseline, which reduced the method sensitivity in oil. Further cleanup was needed to achieve the desired detection/quantitation limit.

PSA sorbent could make up EMR—Lipid cleanup without negatively affecting neutral PAH compounds. The Bond Elut Jr PSA cartridge can be hyphenated easily with a Captiva EMR—Lipid cartridge to provide sequential cleanup in one step (Figure 3). The convenient use of Jr PSA easily provided additional cleanup without an extra sample preparation step. However, the attached Jr PSA cartridges made the gravity elution difficult, and required an external force such as positive pressure or vacuum to initialize and maintain a steady elution flow.

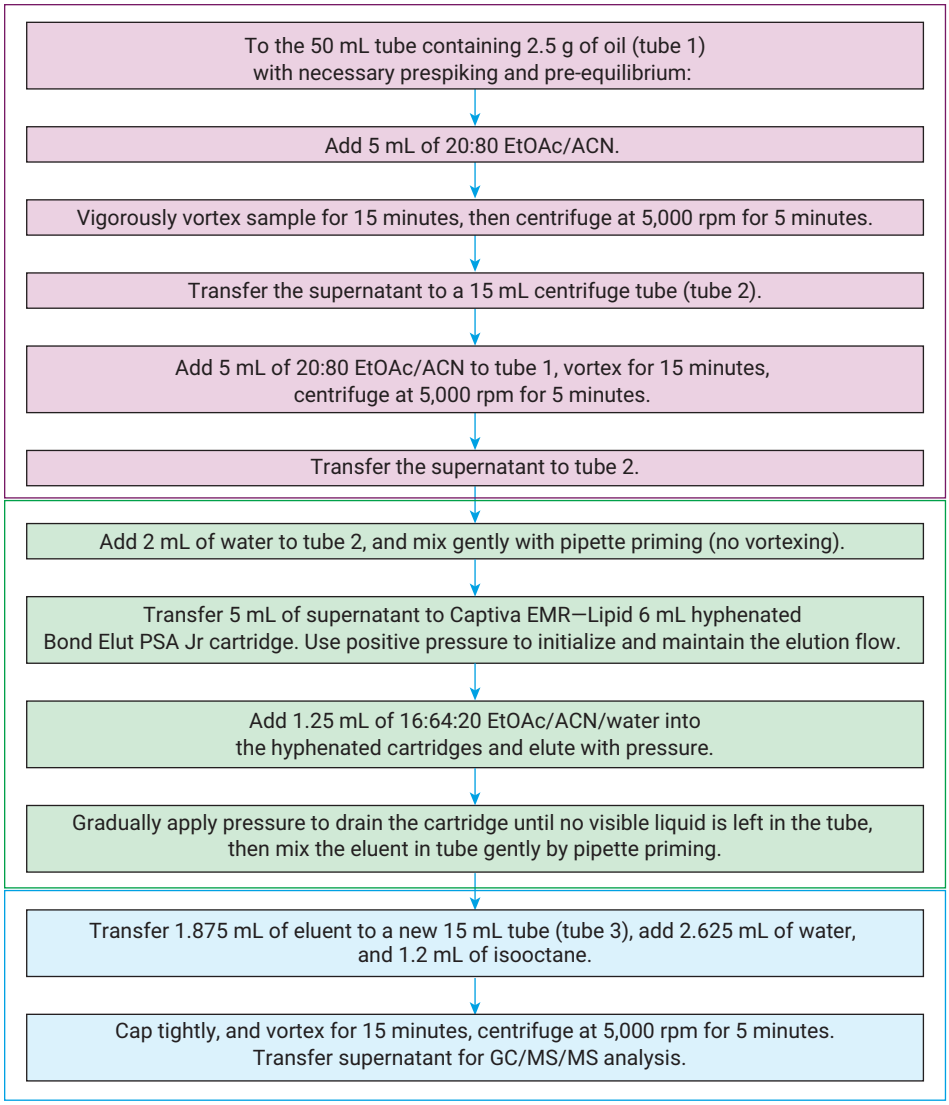

Figure 2. Flow diagram for the edible oil preparation procedure using liquid/liquid extraction followed with Agilent Captiva EMR—Lipid hyphenated with Bond Elut Jr PSA cleanup.

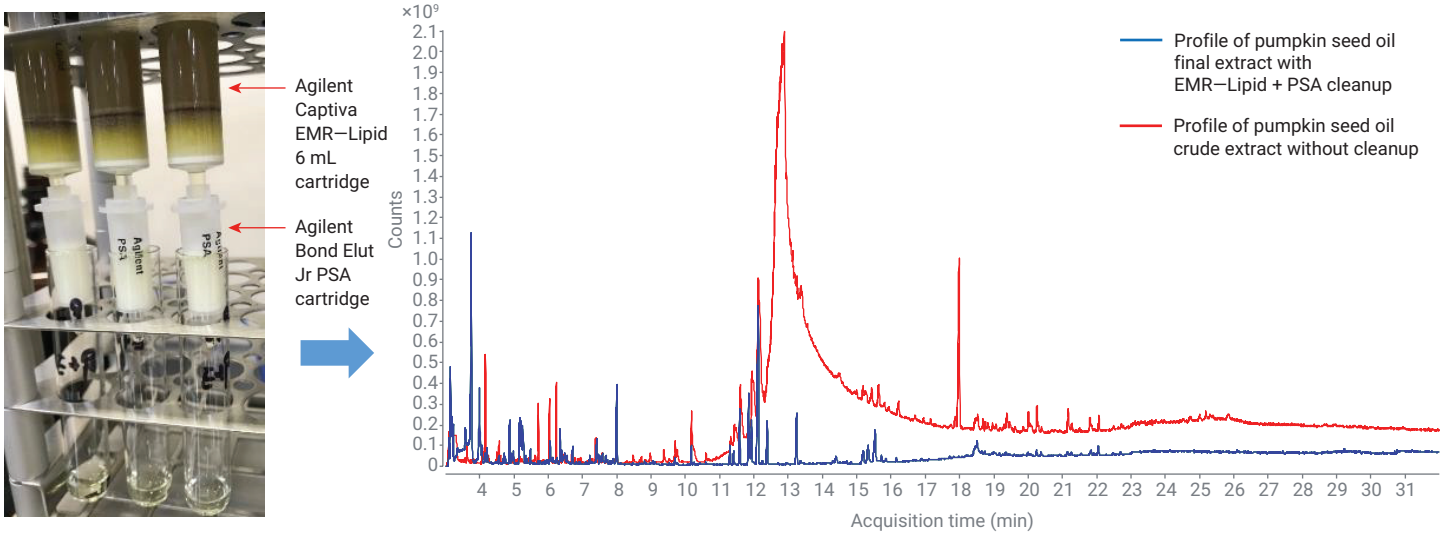

Figure 3. Agilent Captiva EMR–Lipid hyphenated with Bond Elut Jr PSA provides efficient cleanup for pumpkin seed oil matrix, with demonstration of attached cartridges (left) and a GC/MS full scan for cleanup efficiency (right).

Method validation

The quantitation method validation includes LOQ, calibration curve linearity, analyte accuracy, and precision at three spiking levels. Eight IS compounds were used for analyte quantitation: benzo[a]anthrancene-D_{12}, chrysene-d_{12}, benzo[b]fluoranthene-D_{12}, benzo[k] fluoranthene-D_{12}, benzo[a]pyrene-D_{12}, dibenzo[a,h]anthracene-D_{14}, indo[1,2,3-cd] pyrene-D_{12}, and benzo[g,h,l]perylene-D_{12}.

Figure 4 shows the matrix blank and critical PAH compound chromatograms at LOQ level (0.9 ng/g) in pumpkin seed oil.

Table 3 summarizes the method quantitation results in pumpkin seed oil. Figure 5A shows the recovery data of the PAH compounds from pumpkin seed oil at four spiking levels using the optimized method. Figure 5B shows the recovery data of four edible oils at the low spiking levels of 0.9 and 2 ng/g. The oils were olive oil, avocado oil, grape seed oil, and almond oil.

Heavy PAH compounds are highly hydrophobic, with logP >5. This feature makes them extremely difficult to extract from oil matrix. From the data shown in Figure 5, lower recoveries were observed for high spiking concentrations and more hydrophobic PAHs.

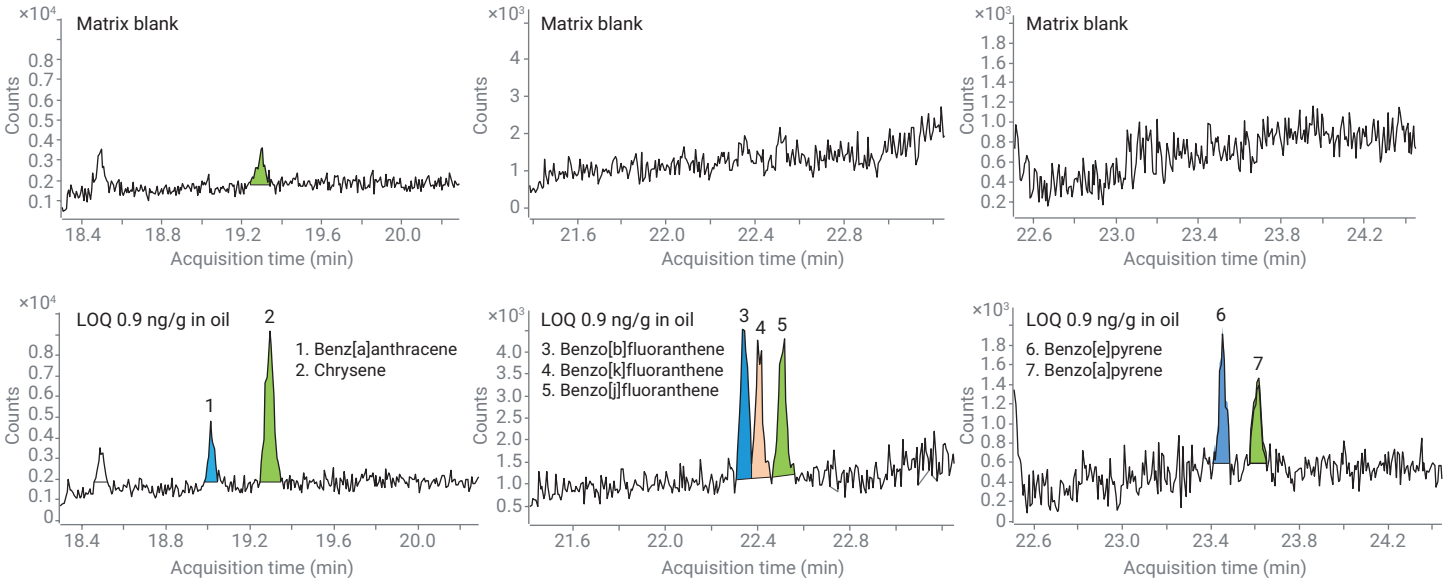

Figure 4. Separation observed for EU Commission-monitored PAH. MRM chromatograms are at LOQ of 0.9 ng/g in pumpkin seed oil (bottom row) with the matrix blank (top row).

Table 3. Quantitative validation results for the analysis of PAHs in pumpkin seed oil using the optimized method.

Target PAH	IS Used for Quantitation	Calibration Curve			Mean Accuracy and RSD%, n = 6							
					LOQ (0.9 ng/g)		Low QC (2 ng/g)		Mid QC (10 ng/g)		High QC (100 ng/g)	
		LOQ (ng/g)	HOQ (ng/g)	R^2	Accuracy (%)	RSD	Accuracy (%)	RSD	Accuracy (%)	RSD	Accuracy (%)	RSD
Benzo[a]fluorene	Benzo[a]anthrancene-D_{12}	0.9	200	0.9876	104	11.3	94	11.4	107	11.5	109	3.4
Benz[a]anthracene		0.9	200	0.9935	109	7.0	97	7.0	90	4.8	95	3.2
Chrysene	Chrysene-D_{12}	0.9	200	0.9961	112	5.3	93	6.2	88	3.1	87	3.0
Clyclopenta[cd]pyrene		0.9	200	0.9940	105	7.0	101	7.6	85	4.7	84	2.9
5-Methylchrysene	Benzo[b]fluoranthene-D_{12}	0.9	200	0.9885	106	3.5	96	4.5	98	4.9	105	5.1
Benzo[b]fluoranthene		0.9	200	0.9944	114	7.8	101	6.8	93	3.8	96	4.3
Benzo[k]fluoranthene	Benzo[k]fluoranthene-D_{12}	0.9	200	0.9954	100	8.6	94	4.3	90	6.5	91	5.6
Benzo[j]fluoranthene		0.9	200	0.9942	108	10.0	108	5.2	101	6.1	105	6.9
Benzo[e]pyrene	Benzo[a]pyrene-D_{12}	0.9	200	0.9953	113	4.7	104	5.6	101	3.1	109	4.6
Benzo[a]pyrene		0.9	200	0.9917	110	7.2	96	6.5	90	1.4	94	3.7
Dibenzo[ah]anthracene	Dibenzo[ah]anthracene-D_{14}	0.9	200	0.9944	104	9.4	87	15.4	87	4.3	90	2.7
Indo[1,2,3-cd]pyrene	Indo[1,2,3-cd]pyrene-D_{12}	0.9	200	0.9967	105	8.1	103	6.7	88	4.3	89	3.4
Benzo[ghl]perylene	Benzo[ghl]perylene-D_{12}	0.9	200	0.9963	103	5.6	94	7.1	88	2.7	89	6.0

IS = internal standard; LOQ = limit of quantification (low end); HOQ = high limit of quantification; QC = quality control

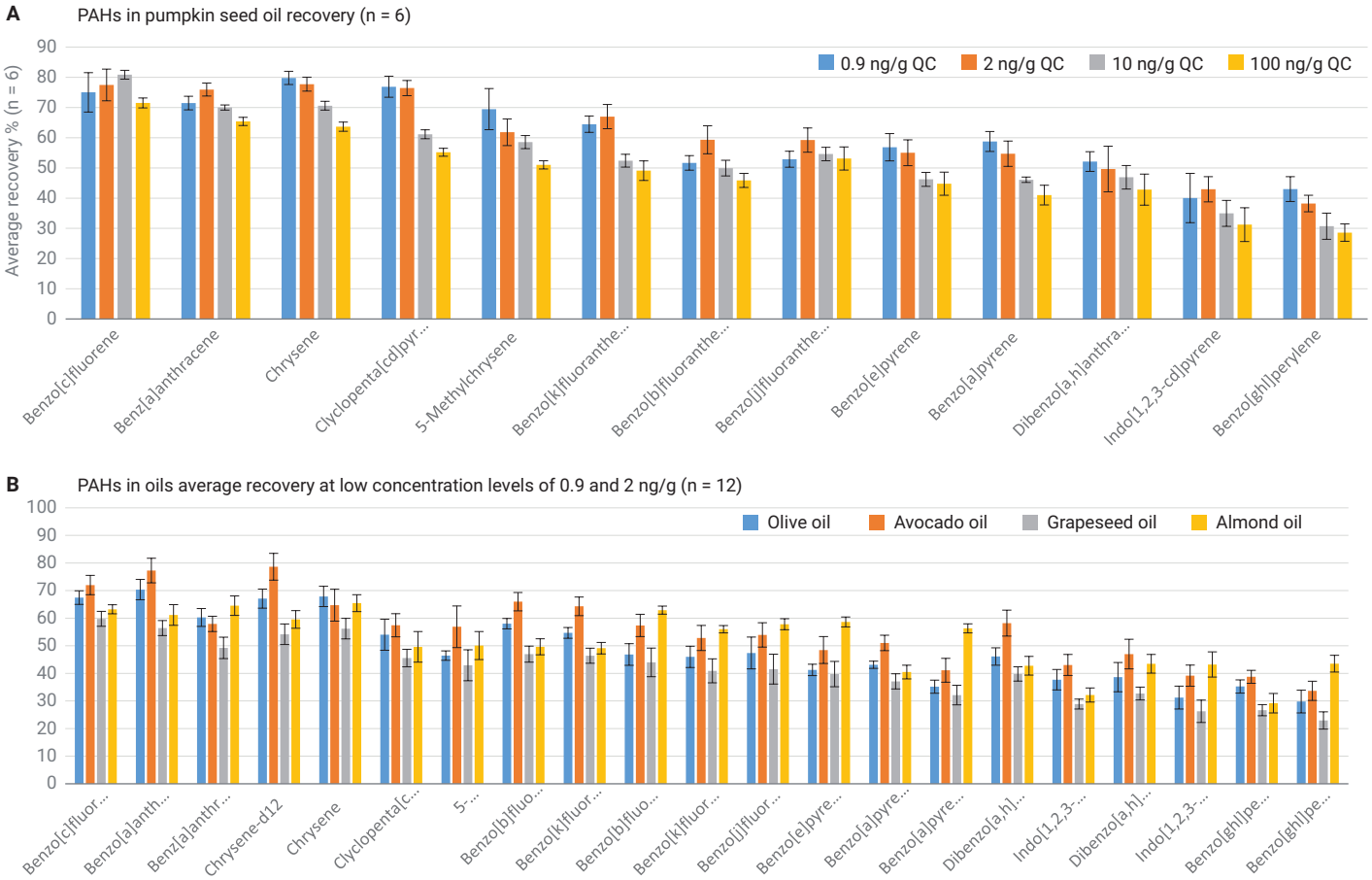

Figure 5. Heavy PAH compounds recoveries from edible oils. A) Recovery data in pumpkin seed oil at four different spiking levels; B) average recovery data in other four oils at two low spiking levels.

Recovery investigation indicated that the major loss of heavy PAHs happened during the extraction step. Therefore, ways to improve extraction efficiency will be investigated, including extracting with a more hydrophobic yet still water-miscible solvent mixture, and using sonication to aid analyte partition.

The low recoveries can be corrected using an appropriate, stable labeled internal standard. With a significantly clean sample matrix, the large volume injection method improved the sensitivity with excellent reproducibility. As a result, this simple method provides excellent quantitation results to meet regulatory requirements, which is demonstrated in Table 3 for validation results in pumpkin seed oil, and Figure 6 for relative recovery results cross-validated in four other edible oils.

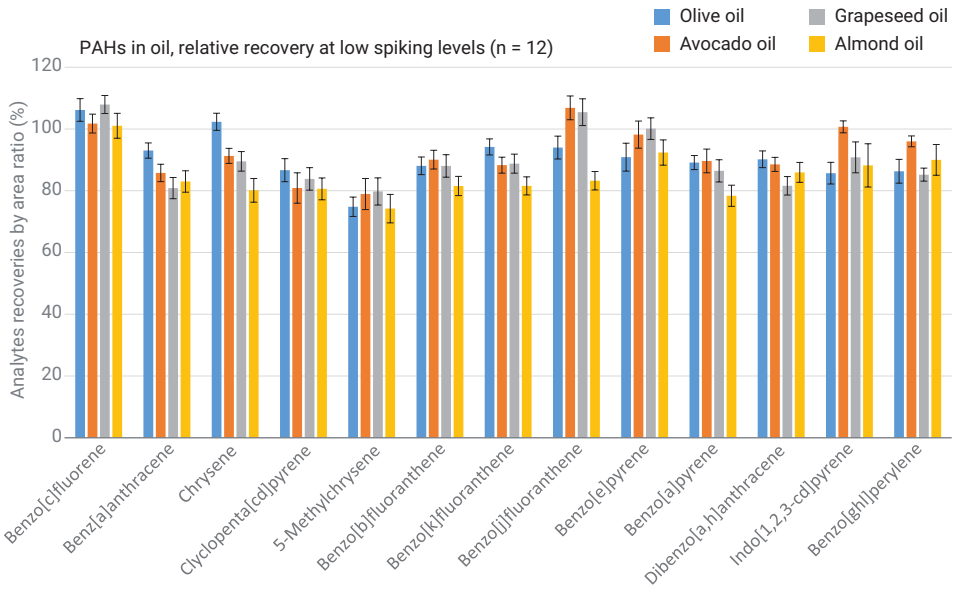

Figure 6. Heavy PAH recoveries by area ratio in four edible oils at low spiking levels (0.9 and 2 ng/g in oil).

Assessment of matrix cleanliness

The sample matrix residue in the final extract and matrix residue removal by cleanup was investigated in each oil. Figure 7 shows the visual appearance of sample dried residue for pumpkin seed oil and olive oil, with the actual residue weight in the table. Based on the difference in dried residue weight between the sample without cleanup and with EMR—Lipid plus PSA cleanup, Captiva EMR—Lipid hyphenated with Bond Elut Jr PSA cleanup provided more than 95% matrix removal for the five edible oils.

The overlapped GC/MS full scan chromatograms shown in Figure 3 demonstrate excellent sample background cleanup from the optimized method for pumpkin seed oil. Similar chromatograms were obtained for comparison against four other edible oils. These results proved that the efficient matrix cleanup can provide a significantly cleaner chromatographic background for reliable analysis.

Edible Oil		Pumpkin Seed Oil	Olive Oil	Avocado Oil	Grape Seed Oil	Almond Oil
Oil Extract no Cleanup (mg/mL Crude Extract, n = 2)		15.85	14.11	19.00	19.10	11.51
EMR–Lipid + PSA Cleanup	Residue (mg/mL Final Extract, n = 2)	0.82	0.46	0.68	0.37	0.09
	Matrix Residue Removal (%)	95%	97%	96%	98%	99%

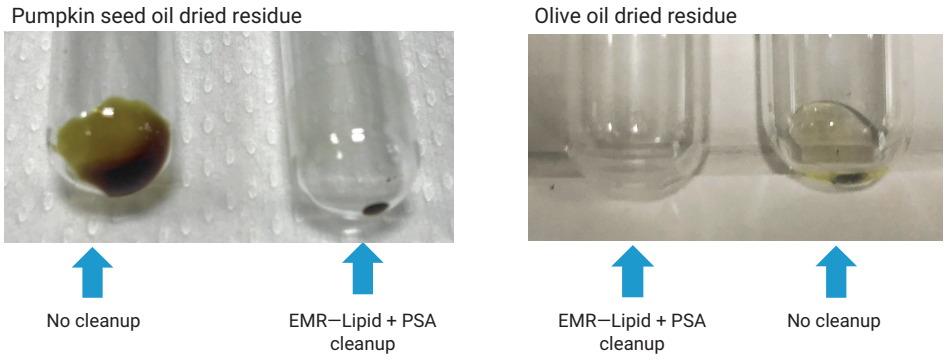

Figure 7. Matrix residue removal assessment by residue weight and appearance.

Conclusion

A simple, rugged, and reliable method using liquid-liquid extraction followed by Agilent Captiva EMR—Lipid hyphenated with Bond Elut Jr PSA cartridge cleanup was developed and validated for the analysis of heavy PAHs in edible oil. The convenient, hyphenated cartridges provide the sequential oil matrix cleanup, without adding an additional sample preparation step. The oil matrix co-extractive residue provided >95% removal with significantly cleaner chromatographic backgrounds. The quantitative analysis showed excellent accuracy (100 ±15%) and reproducibility (RSD <15%) with an LOQ of 0.9 ng/g in oil. Improvements to efficiencies in the PAH extraction step will be further investigated in edible oil. These results demonstrate that the optimized method provides high matrix cleanup and reliable quantitation results for the analysis of heavy PAHs in edible oils.

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Printed in the USA, October 17, 2019
5994-1483EN

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