Application Note

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Enhanced PAH Analysis in Crude Palm Oil, Crude Palm Kernel Oil, and Coconut Oil

With Agilent Bond Elut EMR–Lipid

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Abstract

This application note addresses the challenges of analyzing polycyclic aromatic hydrocarbons (PAHs) in crude matrices and high-fat coconut oil, using a donor-acceptor complex chromatography (DACC) enrichment column with direct injection. A simple and quick cleanup with Agilent Bond Elut Enhanced Matrix Removal-Lipid (EMR–Lipid) reduces interfering compounds and provides a cleaner baseline for PAH identification and quantification. Additionally, the method is optimized to monitor PAH levels, especially chrysene, using an emission wavelength of 380 nm in the tested matrices.

Introduction

Detecting PAHs in palm oil and coconut oil is crucial due to their potential carcinogenicity.[1] With their high lipophilicity, PAHs tend to bioaccumulate in oils, posing a risk to human health.[2] PAHs form during the process of drying the seeds and kernels at high temperatures or through direct heating.[3] The European Union (EU) imposed a maximum limit of 2 µg/kg of benzo(a)pyrene in oils, fats, and coconut oil that are ready for consumption.[4] To optimize the removal of PAHs during the refining process, refiners need to monitor PAH levels in these crude matrices.

Previous studies demonstrated a technique of directly injecting edible plant oils, such as sunflower oil, into a high-performance liquid chromatography (HPLC) system without the need for sample preparation.[5] A DACC enrichment column helps enrich the sample prior to separation in an analytical column. This technique is applicable to matrices that have been processed and are ready for consumption.

However, there were challenges for palm oil refiners when adopting these methods. Their crude oil may still contain unwanted compounds such as gums, phospholipids, and other impurities[6], which interfered with the baselines during PAH separation. Additionally, coconut oil contains high levels of fats and, without any sample treatment, would rapidly solidify and clog the HPLC flow path.

This application note enhances settings from previous research[5] to accommodate PAH analysis in crude oils as well as in coconut oil. It introduces a simple and quick cleanup step using Bond Elut EMR–Lipid and EMR–Lipid Polish to remove interferences effectively. This also helps to shorten the sample loading time in the DACC by 50%.

Experimental

Instruments

The system comprised the following modules:

- Agilent 1260 Infinity II flexible pump (G7104C)
- Agilent 1260 Infinity II fluorescence detector (G7121B)
- Agilent 1260 Infinity II multisampler (G7167C)
- Agilent 1290 multicolumn thermostat (G7116B) with Agilent InfinityLab Quick-Change 2-position/6-port switching valve (part number 5067-4241)
- Agilent 1290 Infinity II binary pump (G7120A)
- Agilent 1290 Infinity II valve drive (G1170A) with InfinityLab Quick-Change 2-position/6-port switching valve (part number 5067-4241)

Chemicals and standards

HPLC grade isopropanol (IPA) was obtained from Merck Malaysia. Agilent InfinityLab Ultrapure LC/MS grade acetonitrile (ACN, part number 5191-4496) and Agilent InfinityLab LC/MS grade water (part number 5190-6897) were also used.

The Agilent EPA 600 Series PAH standard (part number PAH-600-1) contains 16 PAHs at 100 µg/mL for each analyte. The compounds in the mixture are listed according to their elution order in an Agilent Pursuit PAH column (part number A7000250X046).

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz(a)anthracene
10. Chrysene
11. Benzo(b)fluoranthene
12. Benzo(k)fluoranthene
13. Benzo(a)pyrene
14. Dibenz(a,h)anthracene
15. Benzo(ghi)perylene
16. Indeno(1,2,3-cd)pyrene

Cleanup kit

The cleanup kit consisted of Agilent Bond Elut QuEChERS dSPE EMR–Lipid (part number 5982-1010) and Bond Elut EMR–Lipid Polish 15 mL tube containing NaCl/$MgSO_4$ (part number 5982-0101).

Samples

The samples were crude palm oil (CPO), crude palm kernel oil (CPKO), and coconut oil (CO). All were sourced from a local refinery. The coconut oil had been neutralized, bleached, and deodorized.

Columns

The DACC enrichment column used was Agilent ChromSphere Pi 3.0 × 80 mm (part number CP28159). The analytical column used was Agilent Pursuit 200Å PAH, 4.6 × 250 mm, 5 µm (part number A7000250X046).

Software

Agilent OpenLab CDS version 2.7 was used for data acquisition and interpretation.

Sample cleanup procedure

EMR–Lipid has been demonstrated to remove matrix interferences effectively, especially lipids, in other types of oil to allow low-level detection of PAHs. EMR–Polish is used as an additional cleanup step to remove residual water after EMR–Lipid extraction. The samples are solid at room temperature, so they were melted at 60 °C before extraction. See the detailed procedure in Figure 1.

To increase the miscibility of PAHs with the testing matrices, PAH standards were dissolved in isopropanol and added to the matrices at concentration levels of 0.1, 0.5, 1.0, 5.0, and 10.0 ppb to create matrix-matched calibration curves.

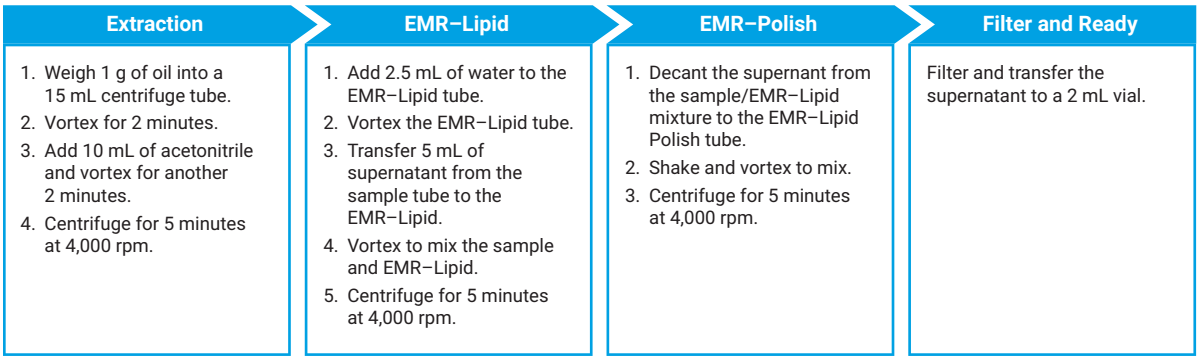

Figure 1. Procedure to clean up oil samples for HPLC analysis.

Method description

The method used, illustrated in Figure 2, was the same as that described in a previous application note on the analysis of PAHs in edible oils.[5] Briefly, a sample was loaded into the DACC column through the loading pump (Figure 2A). The sample was enriched in the DACC column for 5 minutes, followed by backflushing with water and acetonitrile to remove the IPA (Figure 2B). Finally, the sample was transferred from the DACC column to the analytical column (Figure 2C). The gradients for the analytical and loading pumps were adjusted according to the chromatographic condition.

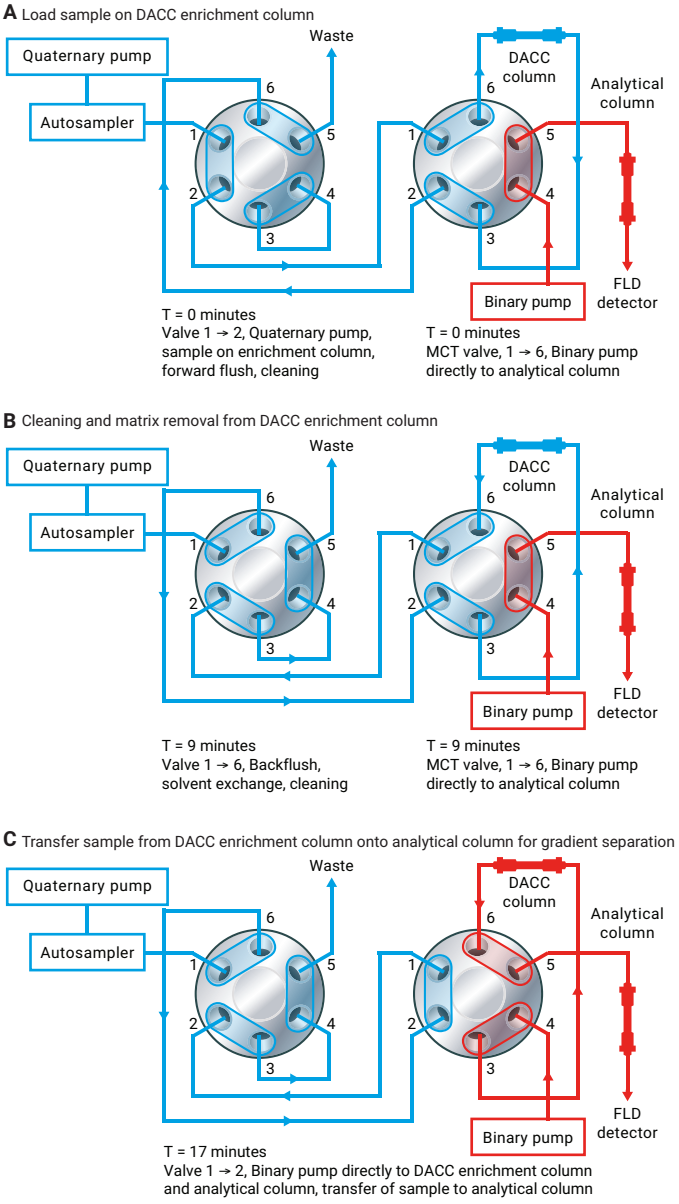

Figure 2. Valve diagrams of (A) sample loading, (B) matrix removal, and (C) sample transfer to the analytical column from the DACC column after cleanup. Figure adapted from application note 5991-2772EN.[5]

Chromatographic conditions

The chromatographic conditions used for this method are detailed in Table 1.

Table 1. Chromatographic conditions used in this analysis.

Parameter	Value
Flexible Pump (Loading Pump)	
Solvent A	Water
Solvent B	ACN
Solvent C	IPA
Initial Flow Rate	0.4 mL/min
Gradient	Time (min) %A %B %C Flow rate (mL/min) 0.00 0 0 100 0.4 5.00 0 0 100 0.4 5.10 40 60 0 1.0 8.00 40 60 0 1.0 8.10 30 70 0 1.0 17.00 30 70 0 1.0 18.00 1 99 0 1.0 28.00 1 99 0 1.0 29.00 0 0 100 0.4
Stop Time	65 min
Binary Pump (Analytical Pump)	
Solvent A	Water
Solvent B	ACN
Initial Flow Rate	0.4 mL/min
Gradient	Time (min) %A %B Flow rate (mL/min) 0.00 30 70 0.4 5.00 30 70 0.4 6.00 30 70 1.0 17.00 30 70 1.0 47.00 1 99 1.0 60.00 1 99 1.0 60.1 30 70 1.0
Stop Time	65 min
Autosampler	
Injection Volume	100 µL
Sampler Temperature	40 °C
Multicolumn Thermostat (MCT)	
Temperature	25 °C
Valve Position	Time (min) Valve position 0.00 1 → 6 9.00 1 → 2 17.00 1 → 6
External Valve	Time (min) Valve position 0.00 1 → 2 8.00 1 → 6 29.00 1 → 2
Fluorescence Detector (FLD)	
Peak Width	4.63 Hz
Excitation Wavelength	260 nm
Emission Wavelength	A: 380 nm B: 440 nm C: 500 nm
Photomultiplier (PMT) Gain	13

Results and discussion

Using this optimized method, excellent separation is demonstrated across three matrices for 12 out of 16 PAHs in the PAH standard mixtures (Figure 3). With well-resolved peaks, compounds can easily be identified. Naphthalene, acenaphthylene, acenaphthene, and fluorene were unable to be retained in the DACC column and thus were flushed away during sample cleaning.[5] Emission wavelength was optimized from 350 to 380 nm, as phenanthrene, anthracene, pyrene, and chrysene were found to show the best sensitivity and selectivity at these wavelengths within the tested matrices.

Chrysene, benz(a)anthracene, benzo(b)fluoranthene, and benzo(a)pyrene are four critical PAHs often monitored by refiners due to EU regulations.[4] Very low amounts (below 0.1 ppb) of PAHs are present in both CPO and CPKO (Figures 4 and 5). An ultra-low amount of chrysene is present in CO (Figure 6A). Therefore, these matrices were deemed suitable to be used as blank matrices for examination of matrix effects in this study. Phenanthrene was removed from the analysis due to low amounts of contaminant in the matrices that affected the accuracy of measurement at 0.1 and 0.5 ppb levels.

The cleanup step reduced interfering compounds and gave a cleaner baseline. The chrysene peak in coconut oil was separated well between two adjacent peaks, helping to identify the contaminant peak with confidence (Figure 6A).

Excellent linearity is demonstrated not only for the four critical PAHs, but also for other PAHs from 0.1 ppb to 10 ppb. At a concentration of 0.1 ppb, dibenz(a,h)anthracene was not detected in CPO and CPKO, while neither dibenz(a,h) anthracene nor benzo(g,h,i)perylene were detected in CO (Table 2).

For each matrix, at a concentration of 1 ppb, the average standard deviation (SD), and the percent residual standard deviation (%RSD) of retention time, area, and concentration were calculated (Tables 3 to 5). Most calculated %RSD values were well below 1% for all the matrices. The recoveries for all compounds in all matrices were between 90 and 120%.

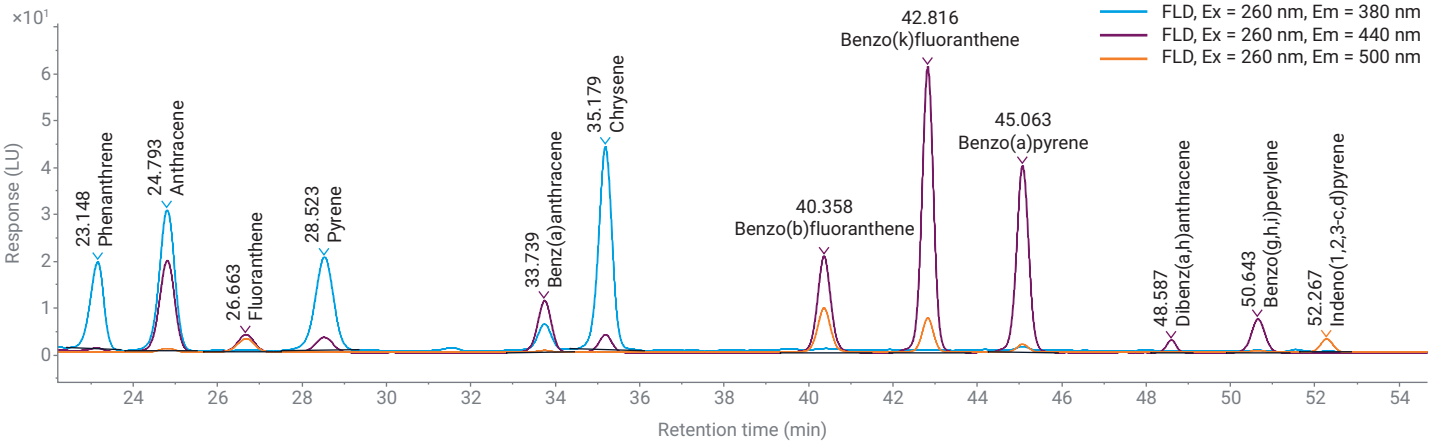

Figure 3. Separation of phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-c,d)pyrene in CPO matrix with the Agilent Pursuit 200Å PAH column.

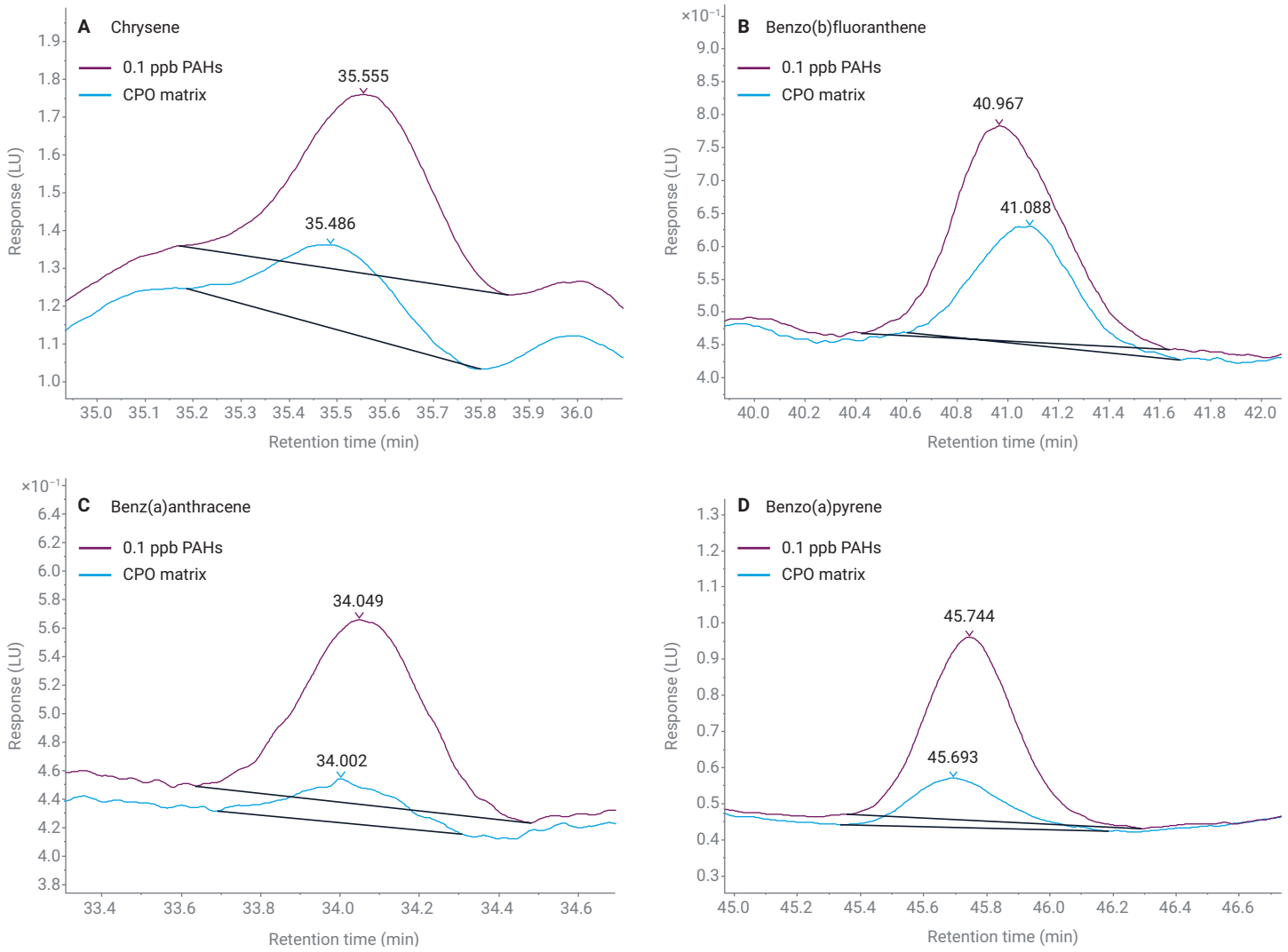

Figure 4. Comparison of 0.1 ppb PAHs (purple) in CPO matrix (cyan). (A) Chrysene, (B) benzo(b)fluoranthene, (C) benz(a)anthracene, and (D) benzo(a)pyrene.

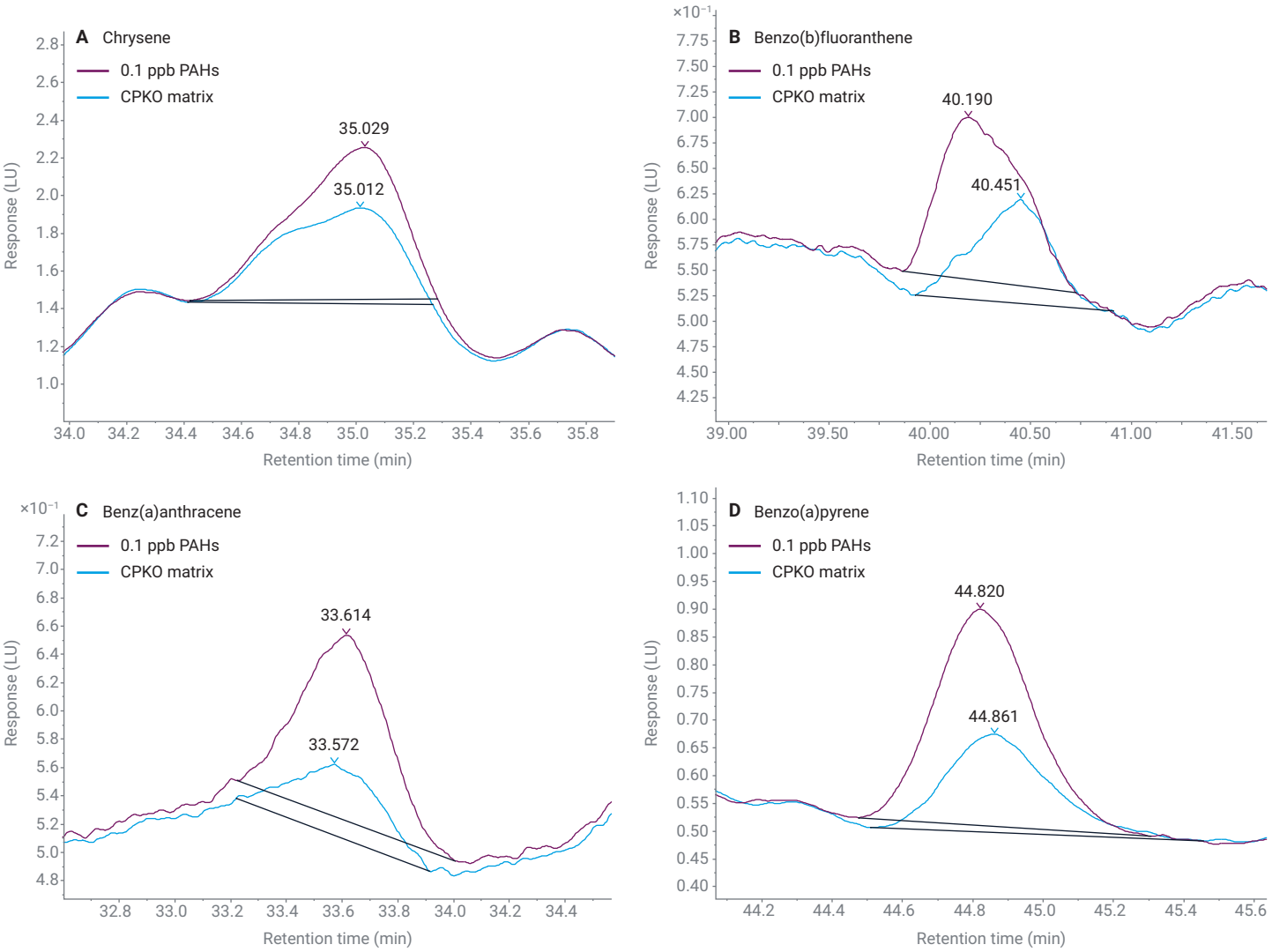

Figure 5. Comparison of 0.1 ppb PAHs (purple) in CPKO matrix (cyan). (A) Chrysene, (B) benzo(b)fluoranthene, (C) benz(a)anthracene, and (D) benzo(a)pyrene.

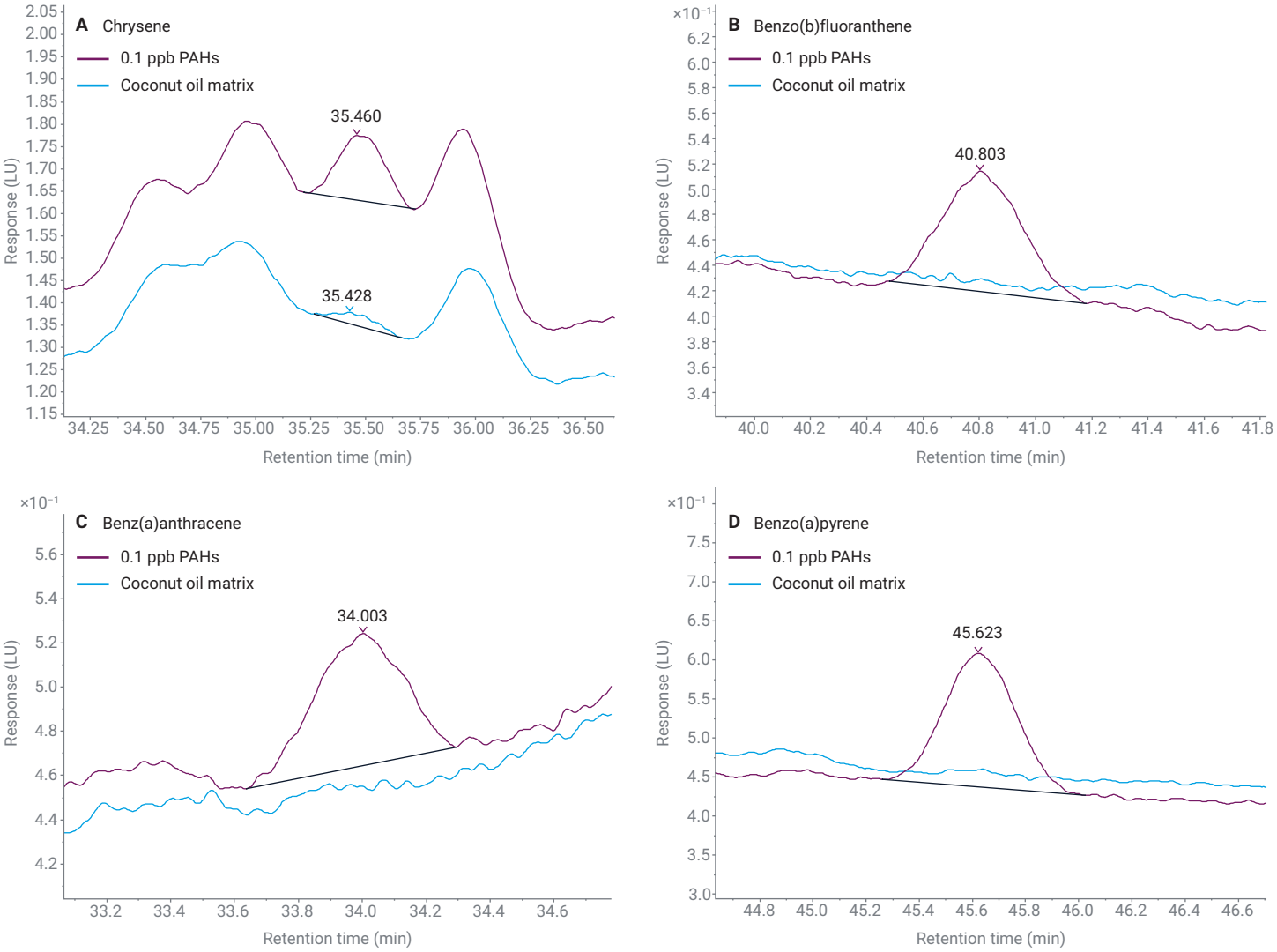

Figure 6. Comparison of 0.1 ppb PAHs (purple) in coconut oil matrix (cyan). (A) Chrysene, (B) benzo(b)fluoranthene, (C) benz(a)anthracene, and (D) benzo(a)pyrene.

Table 2. Linearity and signal-to-noise ratio (S/N) for PAHs at 0.1 ppb and 0.5 ppb levels. Values are shown for crude palm oil (CPO), crude palm kernel oil (CPKO), and coconut oil (CO).

Compound Name	CPO			CPKO			CO		
	Linearity (R^2)	S/N (0.1 ppb)	S/N (0.5 ppb)	Linearity (R^2)	S/N (0.1 ppb)	S/N (0.5 ppb)	Linearity (R^2)	S/N (0.1 ppb)	S/N (0.5 ppb)
Anthracene	0.9995	16.5	36.5	0.9992	6.7	19.0	0.9993	15.6	44.5
Fluoranthene	0.9997	5.8	12.3	0.9991	2.0	5.4	0.9995	4.5	13.2
Pyrene	0.9995	8.8	19.3	0.9996	5.2	13.1	0.9996	11.3	26.2
Benz(a)anthracene	0.9997	6.0	28.4	0.9991	3.3	11.5	0.9998	3.9	30.7
Chrysene	0.9992	8.0	42.4	0.9996	8.0	20.2	0.9996	2.1	41.8
Benzo(b)fluoranthene	0.9996	15.2	54.8	0.9992	4.0	17.9	0.9998	6.2	49.5
Benzo(k)fluoranthene	0.9997	29.9	148.8	0.9990	8.4	52.3	0.9995	19.9	147.4
Benzo(a)pyrene	0.9997	23.7	102.6	0.9991	9.9	34.6	0.9993	11.3	86.5
Dibenz(a,h)anthracene	0.9999	N.D	6.3	0.9995	N.D	1.7	0.9993	N.D.	5.4
Benzo(ghi)perylene	0.9997	3.8	18.0	0.9993	1.6	4.0	0.9996	N.D.	10.6
Indeno(1,2,3-cd)pyrene	0.9997	4.9	25.4	0.9990	3.8	13.7	0.9998	2.7	13.1

N.D.: Not determined

Table 3. Average, standard deviation (SD), and percent residual standard deviation (%RSD) calculated for retention time, area, concentration, and accuracy for crude palm kernel oil (CPKO). Data are based on three injections of CPKO at a concentration of 1 ppb.

	CPKO (n = 3)									
	Retention Time (min)			Area			Concentration (ppb)			
Compound Name	Average	SD	%RSD	Average	SD	%RSD	Concentration	SD	%RSD	Recovery %
Anthracene	24.800	0.007	0.003	84.340	0.453	0.537	0.934	0.006	0.625	93.41
Fluoranthene	26.662	0.014	0.052	11.402	0.092	0.803	1.019	0.009	0.845	101.94
Pyrene	28.499	0.016	0.056	65.938	0.226	0.342	1.003	0.004	0.381	100.34
Benz(a)anthracene	33.631	0.015	0.043	19.078	0.075	0.393	0.933	0.004	0.381	93.30
Chrysene	35.049	0.011	0.031	86.320	0.414	0.480	1.027	0.005	0.518	102.70
Benzo(b)fluoranthene	40.189	0.005	0.013	29.061	0.159	0.547	0.927	0.005	0.535	92.74
Benzo(k)fluoranthene	42.607	0.007	0.017	76.476	0.217	0.284	0.969	0.003	0.275	96.91
Benzo(a)pyrene	44.855	0.013	0.030	49.396	0.112	0.226	0.944	0.002	0.221	94.44
Dibenz(a,h)anthracene	48.339	0.031	0.064	1.931	0.025	1.313	0.902	0.010	1.133	90.21
Benzo(ghi)perylene	50.390	0.018	0.035	9.613	0.080	0.827	1.062	0.009	0.814	106.15
Indeno(1,2,3-cd)pyrene	51.959	0.044	0.085	3.110	0.037	1.186	0.965	0.011	1.176	96.48

Table 4. Average, standard deviation (SD), and percent residual standard deviation (%RSD) calculated for retention time, area, concentration, and accuracy for coconut oil (CO). Data are based on three injections of CO at a concentration of 1 ppb.

	CO (n = 3)									
	Retention Time (min)			Area			Concentration (ppb)			
Compound Name	Average	SD	%RSD	Area	SD	%RSD	Concentration	SD	%RSD	Recovery %
Anthracene	24.811	0.014	0.056	69.134	0.154	0.223	1.103	0.003	0.223	110.30
Fluoranthene	26.668	0.008	0.029	10.152	0.040	0.395	1.161	0.005	0.395	116.12
Pyrene	28.505	0.011	0.038	52.272	0.234	0.448	1.170	0.005	0.448	116.98
Benz(a)anthracene	33.675	0.023	0.069	18.842	0.041	0.218	1.094	0.002	0.218	109.36
Chrysene	35.105	0.027	0.078	76.314	0.241	0.316	1.199	0.004	0.316	119.91
Benzo(b)fluoranthene	40.252	0.029	0.072	26.952	0.064	0.237	1.026	0.002	0.237	102.57
Benzo(k)fluoranthene	42.684	0.035	0.083	74.201	0.346	0.466	1.159	0.005	0.466	115.85
Benzo(a)pyrene	44.928	0.037	0.082	47.380	0.280	0.591	1.125	0.007	0.591	112.45
Dibenz(a,h)anthracene	48.418	0.045	0.094	2.158	0.010	0.472	1.069	0.005	0.472	106.85
Benzo(ghi)perylene	50.472	0.043	0.085	7.682	0.016	0.214	1.179	0.003	0.215	117.91
Indeno(1,2,3-cd)pyrene	52.047	0.057	0.110	2.937	0.027	0.923	1.197	0.011	0.923	119.65

Table 5. Average, standard deviation (SD), and percent residual standard deviation (%RSD) calculated for retention time, area, concentration, and accuracy for crude palm oil (CPO). Data are based on three injections of CPO at a concentration of 1 ppb.

	CPO (n = 3)									
	Retention Time (min)			Area			Concentration (ppb)			
Compound Name	Average	SD	%RSD	Average	SD	%RSD	Average	SD	%RSD	Recovery %
Anthracene	24.799	0.002	0.007	80.685	0.162	0.200	0.900	0.002	0.232	90.03
Fluoranthene	26.679	0.010	0.037	12.184	0.112	0.919	1.003	0.010	1.021	100.26
Pyrene	28.532	0.019	0.067	79.296	0.102	0.129	1.178	0.002	0.141	117.76
Benz(a)anthracene	33.777	0.048	0.141	27.631	0.251	0.907	1.014	0.010	0.948	101.44
Chrysene	35.234	0.061	0.174	109.581	0.395	0.360	1.020	0.004	0.393	102.04
Benzo(b)fluoranthene	40.435	0.081	0.200	52.085	0.271	0.520	1.008	0.006	0.576	100.84
Benzo(k)fluoranthene	42.907	0.099	0.231	125.150	0.738	0.590	1.037	0.006	0.595	103.66
Benzo(a)pyrene	45.152	0.096	0.213	89.901	0.525	0.583	1.051	0.006	0.599	105.13
Dibenz(a,h)anthracene	48.698	0.114	0.233	4.663	0.008	0.165	1.014	0.002	0.167	101.37
Benzo(ghi)perylene	50.746	0.117	0.231	19.327	0.012	0.064	1.081	0.001	0.068	108.07
Indeno(1,2,3-cd)pyrene	52.409	0.141	0.269	6.440	0.006	0.086	0.999	0.001	0.092	99.86

Conclusion

The method presented in this application note has been optimized for the analysis of polycyclic aromatic hydrocarbons (PAHs) in crude palm matrices as well as in fat-rich coconut oil with simple sample preparation. Two enhancements were introduced in this method. The fluorescence detector (FLD) emission wavelength was optimized to 380 nm to achieve a lower detection limit in the tested matrices, and the EMR–Lipid cleanup removed interfering compounds that could cause a high chromatography baseline and clog the HPLC flow path. The high linearity, reproducibility, accuracy, and sensitivity demonstrate that this method is well-suited for monitoring PAH levels during refining, as well as for detecting PAHs in refined edible oils.

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