Analysis of PAHs in Edible Oils by Online Enrichment, Matrix Removal and Fluorescence Detection

Application Note

Food Testing and Agriculture – Pesticides, Mycotoxins, and Other Contaminants

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Abstract

This Application Note demonstrates the use of the Agilent 1260 Infinity LC System for direct analysis of polycyclic aromatic hydrocarbons (PAH) from edible plant oils without sample preparation. A column switching method that allows the enrichment of PAHs and matrix removal directly from edible plant oils with subsequent separation and sensitive fluorescence detection (FLD) is shown. Calibration curves and performance data for the major PAHs required by European authorities are discussed.

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Introduction

PAHs originate from incomplete combustion of petrochemical products and are, therefore, a wide spread pollutant and contaminate. Due to their carcinogenic potential, they have to be monitored in the environment and foodstuff. The European Union 835/2011 commission directive defines the maximum content of benzo(a)pyrene in different foodstuffs[1]. Benzo(a)pyrene is considered to be a primary marker for the presence of PAHs and, as an example, must not exceed 2 ppb in edible oils. To improve the profiling of PAHs in food products, benz(a)anthracene, chrysene, and benzo(b)fluoranthrene are defined as additional markers. The maximum contamination for those four PAHs together must not exceed 10 ppb. The commission directive of the European union 2005/10EC[2] discusses sampling methods and performance criteria for the determination of benzo(a)pyrene.

Sample preparation, in the analysis of foodstuff for PAHs, is laborious and time consuming. For edible oils, the sample typically has to be saponified, extracted, and cleaned up over several steps for HPLC analysis[3]. To overcome this, an online approach for sample preparation and analysis was developed by means of LC/LC column coupling. This approach is based on donor-acceptor complex chromatography (DACC) where the PAHs are selectively retained on a DACC column and, after removal of the matrix, analyzed on a PAH column[4,5].

This Application Note demonstrates the use of the Agilent Infinity 1260 HPLC for direct analysis of poly aromatic hydrocarbons (PAH) from edible oils without sample preparation. A column switching method that allows the enrichment of PAHs directly from edible oils on a DACC column with subsequent separation and sensitive fluorescence detection (FLD) is shown. Calibration curves and performance data for the major PAHs required by European authorities are also discussed.

Experimental

Equipment

- Agilent 1260 Infinity Binary Pump (G1312B) with external degasser (G1322A)
- Agilent 1260 Infinity Quaternary Pump (G1311B) with internal degasser
- Agilent 1260 Infinity Standard Autosampler (G1329B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with 6-port/2-position Agilent Quick-Change valve head (p/n 5067-4137)
- Agilent 1290 Infinity Valve Drive (G1170A) with 6-port/2-position Agilent Quick-Change valve head (p/n 5067-4137)
- Agilent 1290 Infinity FLD (G1321A)

Software

- Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.04

Columns

- Enrichment column: Agilent ChromSpher Pi, 3.0 × 80 mm (p/n CP28159)
- Analytical column: Agilent ZORBAX Eclipse PAH, 3.0 × 250 mm, 5 µm (p/n 959990-318)

HPLC Method

Quaternary pump

Solvent A	Water
Solvent B	ACN
Solvent C	iPrOH
Initial flow rate	0.4 mL/min
Initial solvent composition	100 % C
Gradient	See Table 1 and Figure 1
Stop time	60 minutes

Binary pump

Solvent A	Water
Solvent B	ACN
Initial flow rate	0.4 mL/min
Initial solvent composition	70 % B
Gradient	See Table 2 and Figure 2
Stop time	60 minutes

Standard Autosampler

Injection volume	100 µL
Sample temperature	RT
Draw and eject speed	100 µL/min
Needle wash in vial with ACN	
Injector program	Draw default volume from sample, wash needle from vial, Inject, wait 10 minutes, valve to bypass

TCC

Temperature	25 °C
Initial valve position	0 minutes 1 → 6, See Table 3 and Figure 3

External valve

Initial position	0 minutes 1 → 2, See Table 3 and Figure 3

FLD

Peak width	9.26 Hz
Excitation wave length	260 nm
Emission wave length	A: 350 nm B: 440 nm C: 500 nm
PTM gain	0 minutes – 13, 26.9 minutes – 13, 27.0 minutes – 14, 37.3 minutes – 14, 37.4 minutes – 13

Method description

After the injection of the oil sample onto the enrichment column, the autosampler and the enrichment column was flushed with isopropanol (iPrOH) from the quaternary pump for 10 minutes at 0.4 mL/min before the sampler was switched to bypass (Figure 1 and Table 1 show the quaternary pump programming, and Figure 3A and Table 3 show the valve positions).

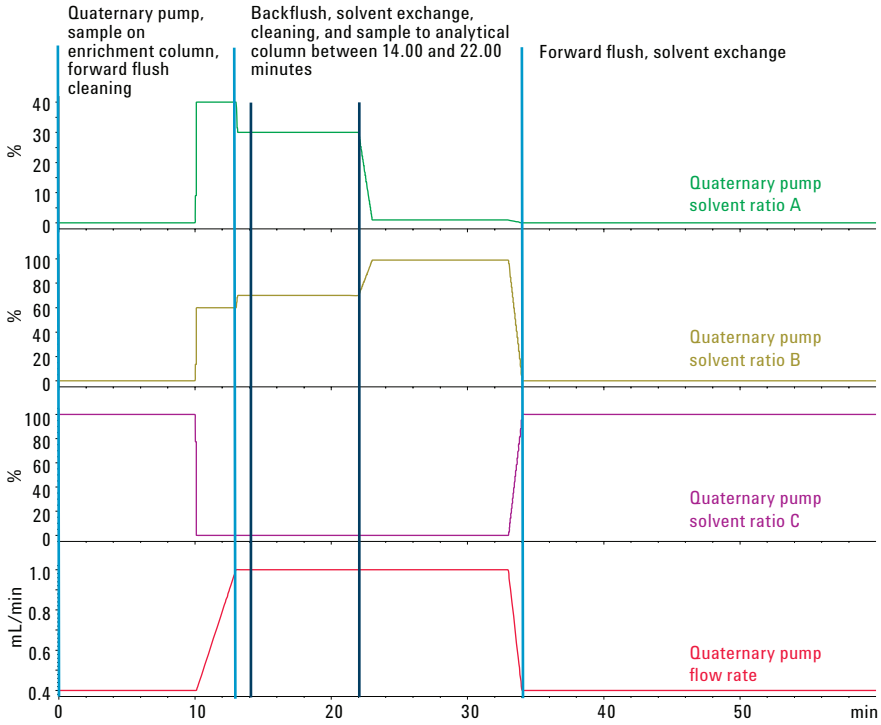

Figure 1. Quaternary pump gradient (Light green: Solvent A, Water; Green: Solvent B, ACN, Purple: Solvent C, iPrOH), flow rate profile (Red) and valve switching times (vertical blue and black lines, see Table 3 and Figure 3).

Table 1. Gradient table and flow rates for quaternary pump.

Time	Solvent composition (%)			Flow rate (mL/min)
0.00	A: 0	B: 0	C: 100	0.4
10.00	A: 0	B: 0	C: 100	0.4
10.10	A: 40	B: 60	C: 0	1
13.00	A: 40	B: 60	C: 0	1
13.10	A: 30	B: 70	C: 0	1
22.00	A: 30	B: 70	C: 0	1
23.00	A: 1	B: 99	C: 0	1
33.00	A: 1	B: 99	C: 0	1
34.00	A: 0	B: 0	C: 100	0.4

After 10 minutes, the iPrOH flow was stopped and acetonitrile (ACN)/water started to flush the iPrOH out of the enrichment column with 1 mL/min. At 13 minutes, the external valve was switched to flush the enrichment column backwards for final matrix removal (Figure 3B). At 14 minutes, the valve in the TCC was switched to connect the enrichment column directly to the analytical column (Figure 2 and Table 2 show the binary pump programming, and Figure 3C and Table 3 show the valve positions). Before both columns were switched in line, the flow on the analytical column side was also brought up to 1 mL/min (Figure 2, Table 2). At 22 minutes, the valve in the TCC was switched back, and the separation of the PAHs by the ACN/water gradient from the binary pump started (Figure 2, Table 2, Figure 3B, and Table 3).

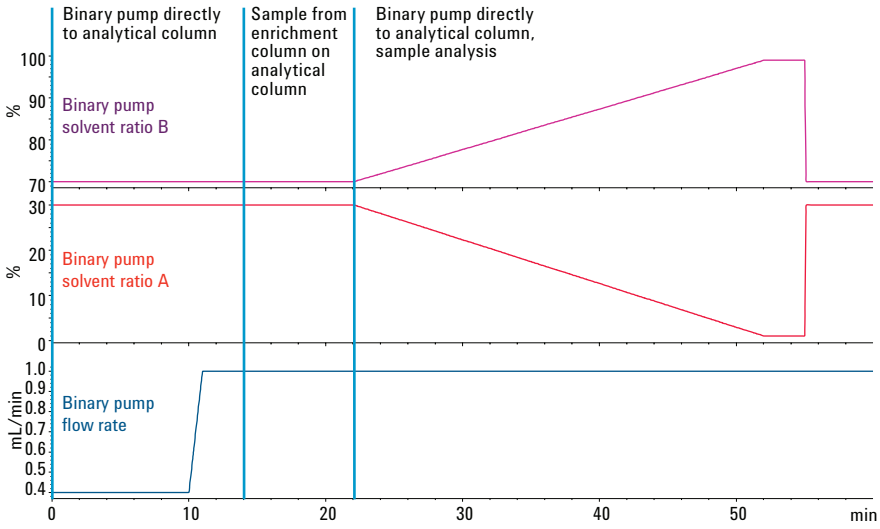

Figure 2. Binary pump gradient (Red: Solvent A, Water; Purple: Solvent B, AcN), flow rate profile (Blue) and valve switching times (vertical blue lines, see Table 3 and Figure 3).

Table 2. Gradient table and flow rates for binary pump.

Time	Solvent composition (%)		Flow rate (mL/min)
0.00	A: 30	B: 70	0.4
10.00	A: 30	B: 70	0.4
11.00	A: 30	B: 70	1
22.00	A: 30	B: 70	1
52.00	A: 1	B: 99	1
55.00	A: 1	B: 99	1
55.10	A: 30	B: 70	1

Table 3. Switching times and functions of the valves in the TCC and external valve box.

Time	TCC valve	Function	Valve	Function
0.00	1 → 6	Binary pump directly to analytical column	1 → 2	Quaternary pump, sample on enrichment column, forward flush cleaning
13.00			1 → 6	Back flush, solvent exchange, cleaning
14.00	1 → 2	Sample from enrichment column on analytical column		
22.00	1 → 6	Binary pump directly to analytical column, sample analysis		
34.00			1 → 2	Forward flush, solvent exchange

While the gradient from the binary pump separates the PAHs, the quaternary pump was used to clean the enrichment column with high ACN content (Figure 1, Table1, Figure 3B, and Table 3). At 34 minutes, the external valve was switched back and the quaternary pump started to deliver iPrOH to bring the enrichment column back to initial conditions for the next sample injection (Figure 1, Table 1, Figure 3A, and Table 3).

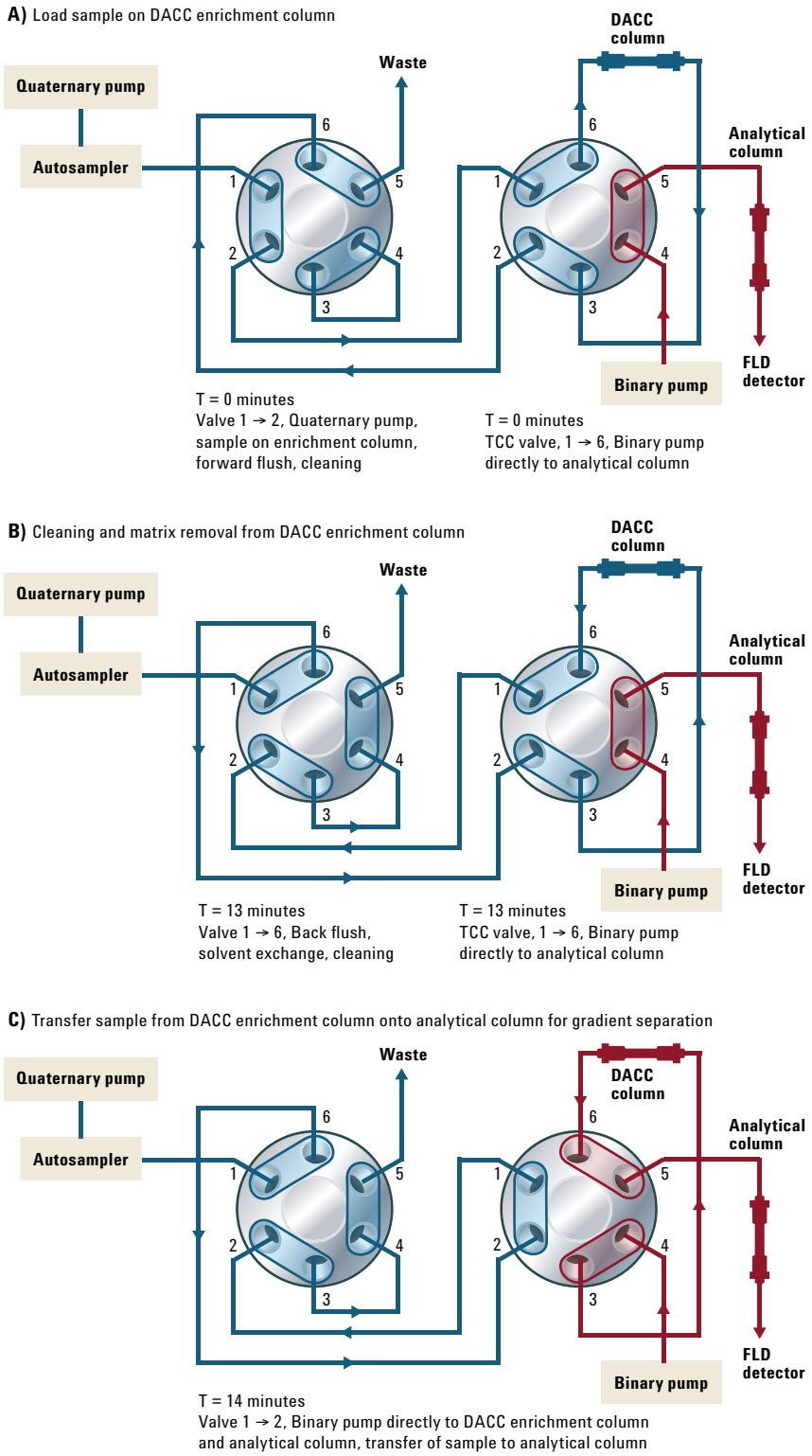

Figure 3. Valve positions and their functions.
A) Load sample on DACC enrichment column
B) Cleaning and matrix removal from DACC enrichment column
C) Transfer sample from DACC enrichment column onto analytical column for gradient separation

Standard

Supelco PAH Calibration Mix, obtained from Sigma-Aldrich, Germany, (p/n 47940-U).

The PAH Calibration Mix contained the following compounds at a concentration of 10 µg/mL (10 ppm), and was used as a stock solution to generate the dilution series in i-PrOH and sunflower oil with concentration levels at 10, 5, 2, 1, 0.5, 0.2, and 0.1 ppb to create calibration curves, limit of quantification (LOQ), limit of detection (LOD), statistical evaluation data, precision, and accuracy data:

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

Sample

A bottle of sunflower oil was bought from a local super market. The oil was spiked with the standard PAH mixture at the same concentration levels as used for the calibration. The samples were injected directly without any further purification.

Results and Discussion

The developed method showed a separation and detection capability for Compound 5 to Compound 16 inherent in the calibration standard mixture. Compounds 1 to 4 were flushed away from the trapping column during cleaning with iPrOH and exchange of the solvent to acetonitrile/water due to their weak retention on the enrichment column (Figure 4). An excitation wavelength of 260 nm was used for the fluorescence detection of the four PAHs mentioned in the European Commission Report. Chrysene showed its best sensitivity and selectivity for the emission wavelength at 350 nm and the other three PAHs, benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene at an emission wavelength of 440 nm (the emission wavelength at 500 nm was used for the detection of indeno(1,2,3-cd)pyrene).

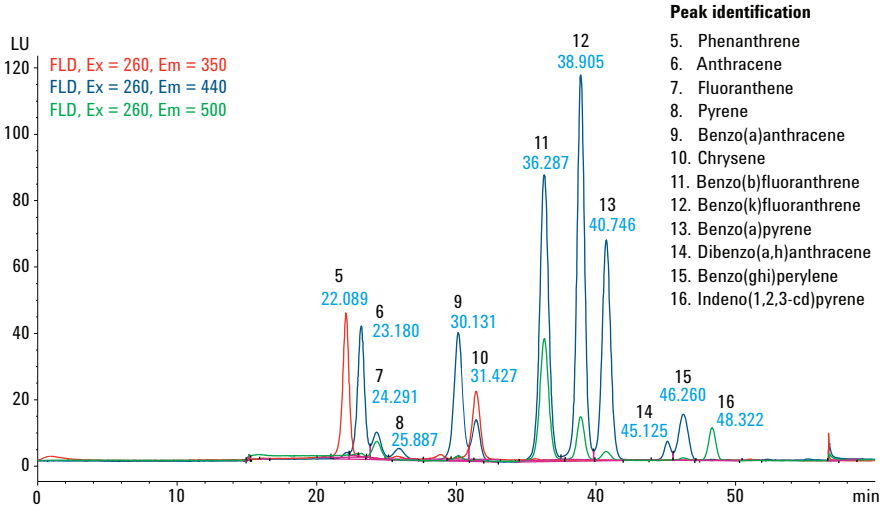

Figure 4. All PAHs analyzable with the described method at a concentration level of 10 ppb.

A concentration range of 0.5 ppb up to 10 ppb was used to create the calibration curves, which show excellent linearity for all four compounds (Figure 5). The concentration level at 0.5 ppb was used as the lowest level for all calibration curves because chrysene reached its LOQ at that point with a signal-to-noise (S/N) ratio of 10. The LOD was found at 0.2 ppb at S/N = 2.5 (Figure 6). The other compounds of interest showed a much lower LOQ and LOD. These values for LOQ and LOD were calculated from the S/N ratios measured at 0.5 ppb and 0.2 ppb, respectively (Table 4). It can be seen that the S/N ratios for benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene are much lower than for chrysene. In the EU commission directive[1], only benzo(a)pyrene shows a direct requirement for the LOQ and LOD. The required LOQ is 0.9 ppb and the required LOD is 0.3 ppb. It can be seen that these values are exceeded with the actual LOQ below 0.2 ppb and the actual LOD below 0.1 ppb.

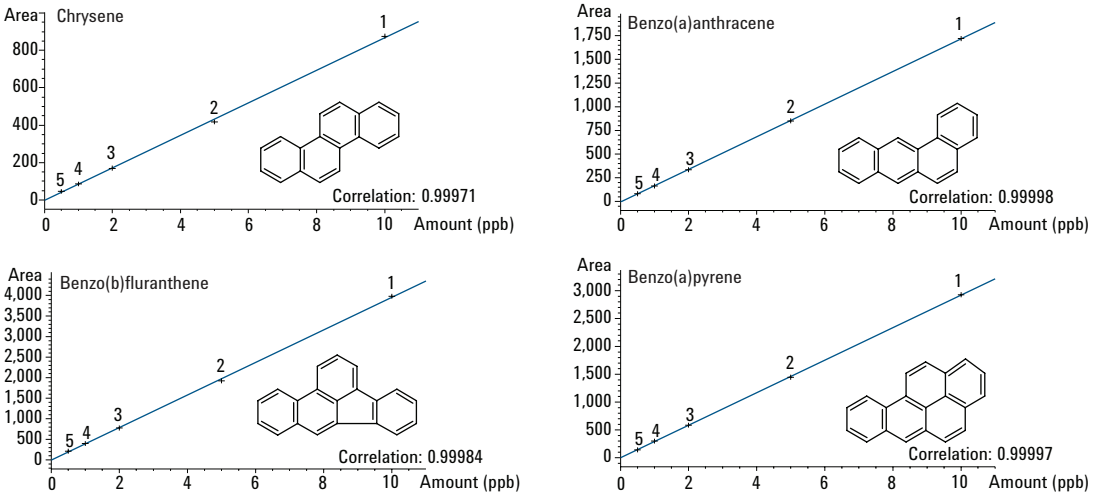

Figure 5. Calibration curves for chrysene, benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene from 0.5 ppb to 10 ppb.

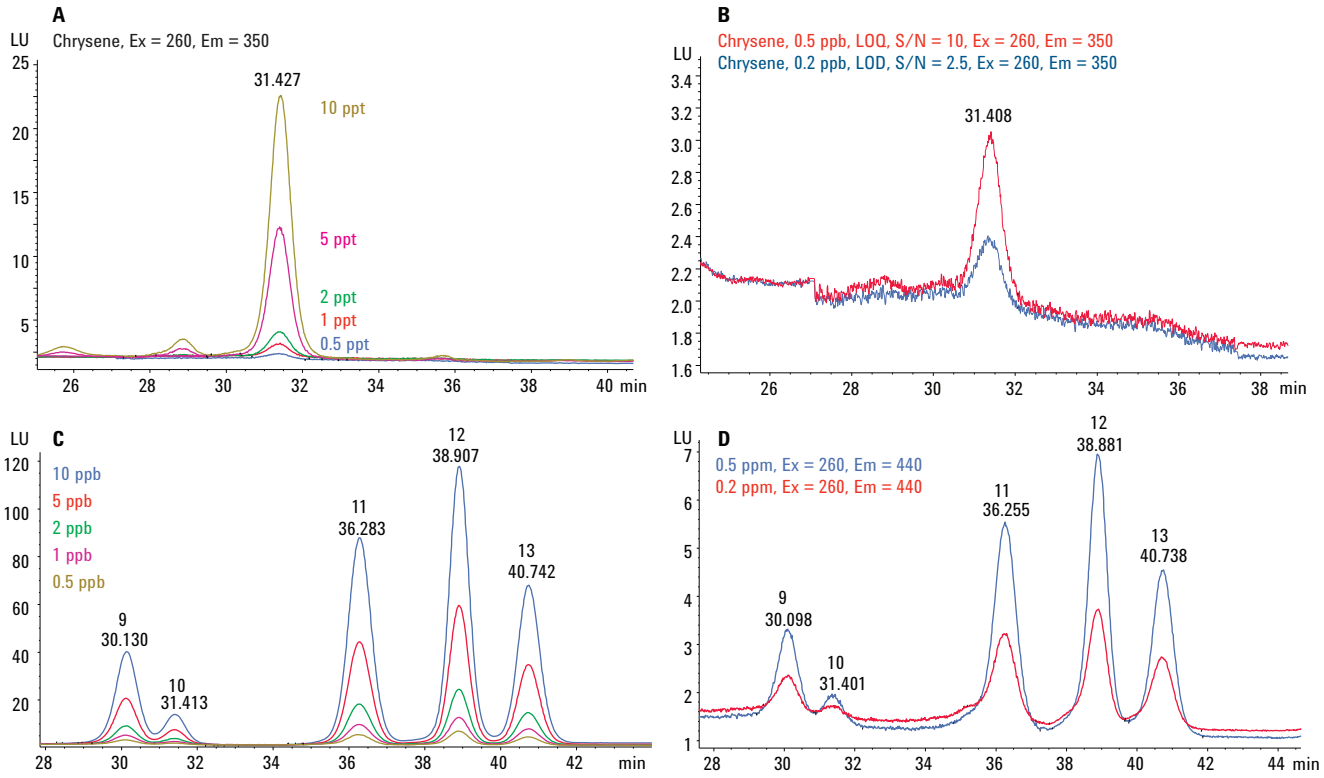

Figure 6. LOD and LOQ of chrysene as well as signals of benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene at 0.5 ppb and 0.2 ppb. A) Chrysene calibration points 10 ppb–0.5 ppb, LOQ at 0.5 ppb. B) Chrysene, LOQ 0.5 ppb at S/N = 10 and LOD of 0.2 ppb at S/N = 2.5. C) Benzo(a)anthracene (9), benzo(b)fluoranthrene (11), and benzo(a)pyrene (13), calibration points 10 ppb–0.5 ppb. D) Benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene at 0.5 ppb and 0.2 ppb, LOQs are calculated from the individual S/N at 0.5 ppb and LODs are calculated from individual S/N at 0.2 ppb (Table 4).

Table 4. LOQs and LODs, measured for chrysene. Others are calculated from S/N ratios measured at 0.5 ppb and 0.2 ppb.

	Benzo(a)anthracene	Chrysene	Benzo(b)fluranthrene	Benzo(a)pyrene
S/N at 0.5 ppb	16	10	38	29
LOQ (ppb)	0.31	0.50	0.13	0.17
S/N at 0.2 ppb	6.5	2.5	16	13
LOD (ppb)	0.01	0.20	0.04	0.05

The carryover was determined by injecting the standard at 10 ppb followed by a blank injection. There was no carry over detectable for Chrysene. For Benzo(a)anthracene, Benzo(b)fluoranthrene, and Benzo(a)pyrene there was approximately 0.2 % carry over detectable (Figure 7).

As a statistical evaluation, the relative standard deviation (RSD) for retention time and area were measured for all four compounds by multiple injections of the 10 ppb sample. The retention time RSDs were typically below 0.5 % and the area RSDs were typically below 1 % (Table 5).

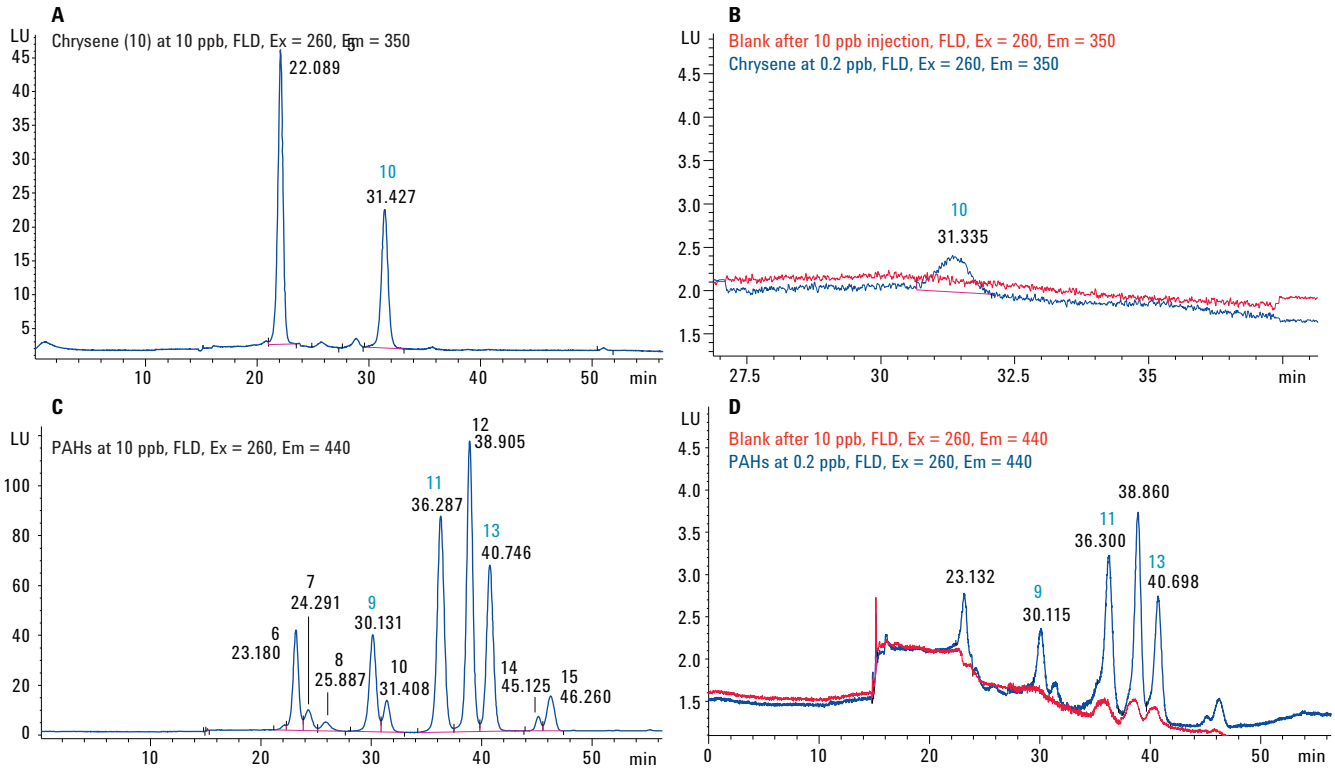

Figure 7. Determination of carryover from chrysene (A and B) and benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene (C and D). A) 10 ppb of chrysene followed by B) a blank showed no carryover (red trace, blue trace for comparison at 0.2 ppb). C) 10 ppb of benzo(a)anthracene, benzo(b)fluoranthrene, and benzo(a)pyrene, followed by a blank D) showed approximately 0.2 % carryover (red trace, blue trace for comparison at 0.2 ppb).

Table 5. Retention time and area precision, 10 ppt, n = 10.

	Retention time (min)			
	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthrene	Benzo(a)pyrene
AV	30.18	31.469	36.405	40.888
SD	0.0115	0.0175	0.0106	0.0088
RSD (%)	0.038	0.056	0.029	0.022
	Area			
AV	1664.07	863.88	3903.01	2734.27
SD	7.2324	4.8564	8.0112	29.2663
RSD (%)	0.435	0.562	0.205	1.07

Finally, a dilution of the PAHs sample in sunflower oils at the concentration levels 10, 5, and 2 ppb was measured to determine the area precision and concentration accuracy under the influence of matrix (Table 6). The area precision was typically below an RSD of 1 % and the accuracy is, due to the under laying matrix, typically in the range of 100 % to 120 % of the spiked amount. This is in accordance with the range of 80 % to 120 % requested for benzo(a)pyrene[1].

Table 6. Repeated (n = 5) injections of 10, 5, and 2 ppb of all four relevant PAHs in sunflower oil matrix for the determination of are precision and concentration accuracy.

	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthrene	Benzo(a)pyrene
Concentration	10 ppt	10 ppt	10 ppt	10 ppt
average	10.13	10.52	10.14	10.18
s.d.	0.0849	0.0377	0.0616	0.1344
RSD (%)	0.84	0.36	0.61	1.32
accuracy (%)	101.3	105.2	101.4	101.8
Concentration	5 ppt	5 ppt	5 ppt	5 ppt
average	5.25	6.00	5.37	5.62
s.d.	0.0169	0.0524	0.0094	0.0205
RSD (%)	0.32	0.87	0.18	0.36
accuracy (%)	105.0	120.0	107.4	112.4
Concentration	2 ppt	2 ppt	2 ppt	2 ppt
average	2.40	3.11	2.62	2.41
s.d.	0.0216	0.0623	0.0082	0.0081
RSD (%)	0.90	2.00	0.31	0.34
accuracy (%)	120.0	155.5	131.0	120.5

Conclusion

This Application Note demonstrates the capability of the Agilent 1260 Infinity LC System to analyze PAHs directly from edible oils without any sample preparation. The analysis was performed by a method comprising enrichment and matrix removal on an enrichment column prior to separation and FLD detection.

The achieved LOQ (0.2 ppb) and LOD (0.1 ppb) for benzo(a)pyrene exceed the values required by the commission directive of the European Union (LOQ 0.9 ppb and LOD 0.3 ppb).

References

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