DONOR-ACCEPTOR COMPLEX CHROMATOGRAPHY-HPLC FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN EDIBLE OILS

A number of polycyclic aromatic hydrocarbons (PAHs) have been identified as carcinogens. For non-smokers who do not work in industries that produce PAHs, the main risk of PAH exposure comes from dietary intake. Edible oils used in food preparation have been identified as a potential source of dietary PAH exposure. HPLC is commonly used for determinations of PAHs, but separation of these samples requires removal of the oily matrix. Several solvent extraction and cleanup techniques have been employed, but are labor intensive and difficult to automate.

This study describes an improved automated procedure for the on-line matrix elimination and analysis of PAHs in edible oils. Donor-acceptor complex chromatography is used to retain PAHs as the oil matrix is flushed to waste. Software-controlled valve switching allows walk-away automation of the cleanup and analysis procedures, and reduces total time from 8–10 h down to approximately 80 min.

Chromatography Focus

INTRODUCTION

In 2004, the European Commission's Scientific Committee on Food concluded that dietary intake of 15 polycyclic aromatic hydrocarbons (PAHs) poses genotoxic and carcinogenic risks [1]. Vegetable oils were found to be one of the main sources of PAHs in European diets. Based on the committee's recommendations, and studies that have shown benzo[a]pyrene (BaP) to be a good indicator of overall PAH contamination in foods, the European Commission has imposed a limit of 2.0 µg/kg for BaP in edible oils [2]. However, the Scientific Committee on Food also recommended continued collection of data on the whole PAH profile in foods in order to monitor future changes in the PAH contamination of food commodities [1].

PAHs may appear in edible oils through the incomplete combustion of organic substances, or environmental contamination of the plants used to make the oils. PAHs are usually determined by HPLC combined with one or more detection methods, including UV, [3] fluorescence, [4] electrochemical, [5] and mass spectrometry (using atmospheric-pressure photoionisation) [6]. However, traditional HPLC methods of determining PAHs in edible oils are laborious and time consuming, requiring solvent extraction followed by either cleanup with a silica column, [7] or solid-phase extraction [8]. These manual sample preparation steps consume solvent, resources, and time.

Donor-acceptor complex chromatography (DACC) is a good alternative for matrix elimination for samples such as edible oils. PAHs strongly interact with DACC stationary phases, while the matrix is not retained and can be washed to waste. Compared to traditional methods, this cleanup technique uses less solvent, is less labour intensive, and saves considerable time [9]. However, methods using this approach often require several manual sample-handling and solvent exchange steps to prepare the sample for HPLC analysis, and therefore still require labour and are prone to errors.

To further reduce labour and errors, Van Stijn et al. [10] developed an automated process consisting of an LC-LC coupling of a cleanup DACC column with an analytical column, eliminating any manual cleanup steps. Although this solution solves the previously described challenges, the method is difficult to optimise and does not address users' requirements for ease of operation, process monitoring and documentation, validation, reporting, and automated diagnosis.

Based on the work of Van Stijn, Miao et al. [11] developed an automated on-line solution for determination of PAHs in edible oils, using two pumps and two switching valves. The method described in the present article further automates and optimises the method, using an HPLC system equipped with a dual-gradient pump and two switching valves, allowing on-line sample enrichment on a DACC column followed by HPLC analysis. On-line coupling of sample preparation and analysis eliminates the complex manual pretreatment required by traditional methods, reducing potential errors and increasing reproducibility. Analysis time per sample is reduced by a factor of 5 to 10, compared to traditional methods. Moreover, this automated system can run 24 h a day, significantly increasing sample throughput.

EXPERIMENTAL

Equipment: Experiments were performed using an UltiMate® 3000 HPLC system (Dionex Corporation, Sunnyvale, CA, USA) consisting of a DPG-3600A dual gradient pump with SRD-3600 air solvent rack, WPS-3000TSL autosampler, TCC-3200 thermostatted column compartment with two 2p-6p valves, and RF2000 fluorescence detector. Chromeleon® 6.80 Chromatography Management Software (Dionex) was used for system control and data collection and analysis.

Conditions:

DACC On-Line SPE Column: ChromSpher Pi, 3 × 80 mm (Varian, Inc, Palo Alto, CA, USA)

Analytical Columns: two Supelco® PAH columns, 4.6 × 250 mm (Sigma-Aldrich Co., St. Louis, MO, USA).

Mobile Phases: A) Water
B) Acetonitrile (loading and analysis)
C) Isopropanol (loading)

Loading Gradient: *Table 1*

Analytical Gradient: *Table 2*

Valve Switching Timing: *Table 3*

Flow Rate: 1 mL/min

Injection Volume: 80 µL (100 µL injection loop)

Column Temperature: 30 °C

Autosampler Temperature: 40 °C

Detection: Fluorescence (*Table 4*)

Table 1. Gradient Program for On-Line SPE (Right Pump)

Time	Flow rate (mL/min)	Solvent A (% vol.)	Solvent B (% vol.)	Solvent C (% vol.)	Curve (%)
0.00	0.35	0	0	100	--
12	0.35	0	0	100	5
12.1	0.35	20	80	0	5
20.9	0.35	20	80	0	5
20.91	0.35	0	100	0	5
50.9	0.35	0	100	0	5
51.5	0.35	0	0	100	5
66.5	0.35	0	0	100	5

Table 2. Gradient Program for Separation (Left Pump)

Time	Flow rate (mL/min)	Solvent A (% vol.)	Solvent B (% vol.)	Curve (%)
0.00	0.4	20	80	--
14.6	0.4	20	80	5
16	1	20	80	5
30	1	0	100	6
58	1	0	100	5
58.1	1	20	80	5
65	1	20	80	5
65.5	0.4	20	80	5
70	0.4	20	80	5

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Table 3. Valve Switching Programs for Valves 1 and 2

Time (min)	Valve 1	Valve 2
0.00	1-2	6-1
12.1	6-1	No movement
14.5	No movement	1-2
17	No movement	6-1
61.5	1-2	No movement

Table 4. Wavelength Changes for RF2000 Fluorescence Detector

Time (min)	Excitation Wavelength (nm)	Emission Wavelength (nm)
0.00	256	370
27.05	256	390
29.5	240	420
33.5	270	385
37.5	290	430
51.5	305	480
53.5	290	430

REAGENTS

Deionised water was generated using a Milli-Q® Gradient A10 system (Millipore Corporation, Billerica, MA, USA). HPLC grade acetonitrile (CH_3CN) and isopropanol were from ThermoFisher Scientific (Waltham, MA, USA). Activated granular charcoal (activated carbon), chemical pure grade, was from Shanghai Chemical Reagent Company (Shanghai, China). Standards were prepared from the Mix of PAHs, EPA Sample for Method 610, from Restek Corporation (Bellefonte, PA, USA), consisting of 200 µg/mL of each component, including phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene. Benzo[b]chrysene, 50 µg/mL, used as an internal standard (I.S.) was from AccuStandard, Inc. (New Haven, CT, USA).

SAMPLES

Three types of oil were tested, two brands of olive oil (olive oil 1 and 2 from Italy and Spain, respectively), and one brand of sesame oil (from China). Samples were filtered through a 0.45-µm PTFE membrane.

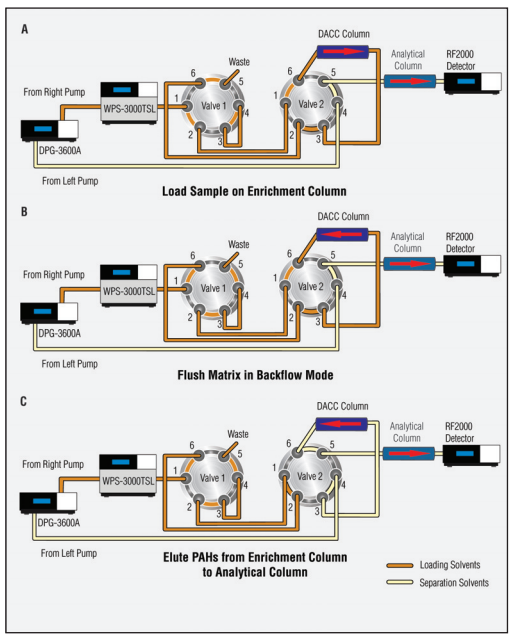

Figure 1. Flow diagram for on-line sample preparation and analysis of PAHs in edible oils. a) Valves positioned for sample injection onto the DACC enrichment column. b) Valves positioned for flushing oil matrix and isopropanol from the enrichment column in backflow mode. c) Valves positioned for elution of PAHs onto analytical column.

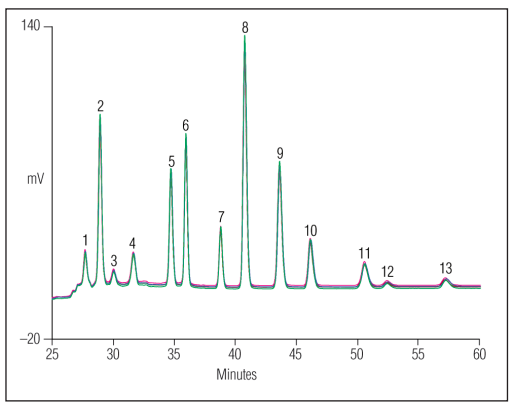

Figure 2. Overlay of seven injections of the 18.9 µg/kg PAH standard prepared in purified olive oil. Peaks: 1) phenanthrene, 2) anthracene, 3) fluoranthene, 4) pyrene, 5) benzo[a]anthracene, 6) chrysene, 7) benzo[b]fluoranthene, 8) benzo[k]fluoranthene, 9) benzo[a]pyrene, 10) dibenzo[a,h]anthracene, 11) benzo[g,h,i]perylene, 12) indeno[1,2,3-cd]pyrene, 13) benzo[b]chrysene (I.S.).

Olive oil 1 was purified for use as a blank and as the matrix for the standards. The oil was purified by heating with activated carbon for 2 h at 60°C with stirring, and was filtered through first a pleated filter, then a 0.45-µm PTFE membrane filter. The 200 µg/mL standard mix was first diluted to 1 µg/mL with isopropanol to make a stock standard. The stock standard was then diluted with isopropanol to make 0.1 and 0.2 µg/mL intermediate standards. The stock and intermediate standards were mixed with the purified matrix oil to make four working standards containing 1.0, 1.9, 9.5, and 18.9 µg/kg of each PAH, respectively. Each working standard was spiked with approximately 1 µg/kg of the I.S.

Description of the On-Line DACC-HPLC Method

The flow scheme, shown in *Figure 1*, couples the DACC cleanup directly with the analytical HPLC run, using two gradient pumps, contained in a single housing, and two column-switching valves. *Figure 1a* shows the valve positions as the filtered and undiluted oil is injected directly, using isopropanol (IPA) to transfer the sample onto the enrichment column (DACC column). The analytical separation column is simultaneously equilibrated using the second pump. After the PAHs have been retained on the DACC column, Valve 1 switches to flush out the oils and IPA, in a backflow mode, with acetonitrile/water *(Figure 1b)*. When all IPA and oils have been flushed to waste, the system switches the enrichment column into the analytical flow path *(Figure 1c)*.

RESULTS

Reproducibility, Detection Limits, and Linearity

Method reproducibility was estimated by making seven replicate injections of the 18.9 µg/kg working standard (*Figure 2*). Calibration linearity for the determination of PAHs was investigated by making five replicate injections of a mixed standard of PAHs prepared at four different concentrations. The internal standard method was used to calculate the calibration curve and for real sample analysis. *Table 5* summarises the retention time and peak area precision data and PAH method detection limits (MDLs). For more details on method ruggedness, please refer to Dionex Application Note 196 [12].

[a]Seven injections of olive oil sample 1 spiked with 18.9 µg/kg mixed PAH standards.

[b]The single-sided Student's test method (at the 99% confidence limit) was used for estimating MDL, where the standard deviation (SD) of the peak area of seven injections of olive oil sample 1 spiked with 1.9 µg/kg mixed PAHs standard is multiplied by 3.14 (at n = 7) to yield the MDL.

Table 5. Reproducibilitya and Method Detection Limitsb for PAH analysis

PAH	RT RSD	Area RSD	MDL (µg/kg)
Phenanthrene	0.064	6.733	0.42
Anthracene	0.055	4.350	0.26
Fluoranthene	0.072	4.491	1.19
Pyrene	0.044	4.965	0.69
Benzo[a]anthracene	0.031	4.628	0.68
Chrysene	0.026	4.469	0.34
Benzo[b]fluoranthene	0.027	4.325	0.21
Benzo[k]fluoranthene	0.027	4.173	0.39
Benzo[a]pyrene	0.031	4.399	0.75
Dibenzo[a,h]anthracene	0.041	4.383	0.41
Benzo[g,h,i]perylene	0.042	5.038	0.58
Indeno[1,2,3-cd]pyrene	0.048	4.484	0.59

SAMPLE ANALYSIS

Purification of olive oil 1 for use as a blank eliminated many ingredients from the original olive oil. However, there were still impurities left that could affect determination of some PAHs. To overcome this effect, the baseline of the purified olive oil blank was subtracted during data processing with Chromeleon software. Two olive oil samples and one sesame oil sample were analysed. The results are summarised in *Table 6. Figure 3* shows chromatograms of the oil samples. Spike recoveries for these PAHs ranged from 70 to 131%, and most fell within the range of 80 to 120%. Some PAHs were found in the edible oil

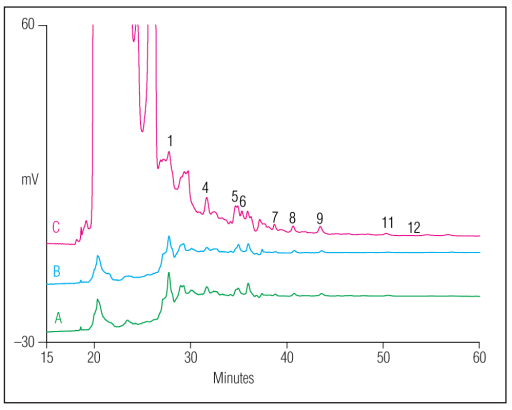

Figure 3. Overlay of chromatograms of a) olive oil 1, b) olive oil 2, and c) sesame oil. Peaks: 1) phenanthrene, 4) pyrene, 5) benzo[a]anthracene, 6) chrysene, 7) benzo[b]fluoranthene, 8) benzo[k]fluoranthene, 9) benzo[a]pyrene, 11) benzo[g,h,i]perylene, 12) indeno[1,2,3-cd]pyrene.

samples. Five PAHs, phenanthrene, anthracene, benzo[a]anthracene, chrysene and benzo[a]pyrene (BaP), existed in all three samples, and phenanthrene was by far the most abundant PAH. All three oils had BaP levels above the 2.0 µg/kg limit set by the European Commission.

DISCUSSION

The PAH levels discovered in the edible oils tested demonstrates the need for vigilance, if the safety of our food supply is to be assured. As researchers identify hazards in our food supplies, water, or environment, new methods have to be developed that allow these hazards to be detected and prevented. Automation and optimisation of these methods is critical, to give regulatory agencies and manufacturers the ability to detect the levels deemed hazardous in a timely manner. This optimised method for determining PAHs in edible oils provides the low detection limits required by EU regulation, while increasing automation, simplifying the system setup, and decreasing analysis time to make the method more practical for real-world application.

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Table 6. Analytical Results for Olive Oil 1, Olive Oil 2, and Sesame Oil

PAH	Olive Oil 1			Olive Oil 2	Sesame Oil
	Detected (µg/kg)	Added (µg/kg)	Recovery (%)	Detected (µg/kg)	Detected (µg/kg)
Phenanthrene	37	5	120	13.2	52
Anthracene	4.5	5	109	3.2	6.1
Fluoranthene	1.0	5	112	ND	ND
Pyrene	2.2	5	131	1.3	ND
Benzo[a]anthracene	2.8	5	108	2.1	18
Chrysene	4.4	5	110	3.2	5.3
Benzo[b]fluoranthene	ND	5	90	ND	ND
Benzo[k]fluoranthene	ND	5	84	ND	ND
Benzo[a]pyrene (BaP)	2.7	5	106	2.5	3.9
Dibenzo[a,h]anthracene	ND	5	84	ND	ND
Benzo[g,h,i]perylene	ND	5	70	ND	1.2
Indeno[1,2,3-cd]pyrene	ND	5	82	ND	ND