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Determination of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor–acceptor complex chromatography and high-performance liquid chromatography with fluorescence detection

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Abstract

Various off-line methods for clean-up and sample enrichment are available for the analysis of polycyclic aromatic hydrocarbons (PAHs) in edible oils and fats. These methods consist of laborious and time consuming procedures. This study reports an on-line method using LC–LC coupling. After clean-up of the sample on a donor–acceptor complex chromatography (DACC) column the PAHs are transferred to and separated on an analytical HPLC column. Quantification is carried out with fluorescence detection. The DACC column clean-up is fast and is carried out during the HPLC run of the previous sample. Compared to the traditional methods this automated on-line method saves considerable time and significantly reduces the amount of solvent waste. The method uses common HPLC equipment and its performance has been evaluated.

Keywords: Oils; Food analysis; Column switching; Donor–acceptor complex chromatography; Polynuclear aromatic hydrocarbons

1. Introduction

The presence of polycyclic aromatic hydrocarbons (PAHs) in edible oils and fats has been reported by numerous investigators [1–15]. The PAHs are formed during pyrolytic processes like incomplete combustion of organic substances or have a petrogenic origin (mineral oils). Edible oils and fats may be contaminated by environmental pollution and/or processing steps prior to refining. Their presence in oils is a health concern due to the carcinogenicity of the PAHs.

Different levels of PAHs have been observed in crude edible oils. Refining of the oils (deodorization, bleaching, charcoal treatment) under the appropriate conditions, which depend on the initial level of the PAHs, reduces the content of the individual PAHs to a μg/kg level.

The analysis of PAHs in edible oils has been described by many authors [9–16]. These methods include complex and laborious extraction and clean-up procedures to isolate the low levels of PAHs present. It is obvious that a fast (automated) method would greatly facilitate the analysis of PAHs.

Recently a number of studies on donor–acceptor complex chromatography (DACC) has been published [17–20]. With this technique PAHs can be extracted from different matrices. PAHs are electron donors (π-electrons) and the strong interaction of the PAHs with an electron acceptor stationary phase results in retention of the PAHs and elution of (the bulk of) the other components of the oil.

In this study an automated on-line method for the determination of PAHs in edible oils and fats is

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described, which can easily be applied as a routine analysis. The method consists of an LC–LC coupling of a clean-up DACC column to an analytical column for the separation. PAHs are quantified with fluorescence detection.

Compared to the traditional methods this automated on-line method significantly reduces the amount of solvent used and saves considerable time. The DACC column clean-up is fast and is carried out during the HPLC run of the previous sample. The total analysis time of one sample is approximately 80 min, with the traditional methods 8–10 h. Moreover the system can run 24 h a day. The quantification limits of 0.1 μg/kg of the individual PAHs have been retained with the DACC method and the method automatically corrects for possibly incomplete recoveries because the calibration samples are subjected to the same treatment as the samples to be analyzed. The system uses regular HPLC instrumentation.

2. Experimental

2.1. Equipment

The DACC–HPLC system consists of a Triathlon autosampler, a model 480 ternary HPLC pump, a Mistral column thermostat, two on-line degassers GT-103 from Separations (Hendrik Ido Ambacht, Netherlands), a solid-phase extraction (SPE) unit from Chrompack (Bergen op Zoom, Netherlands) and an LC-240 fluorescence detector from Perkin-Elmer (Gouda, Netherlands). A Jasco FP-920 fluorescence detector from Separations was used in a part of the method performance evaluation. The TC4 data acquisition software was from Perkin-Elmer. The DACC column (80×3 mm Chromspher PI) and the analytical columns (250×4.6 mm Chromspher 5 PAH) were from Chrompack.

2.2. Chemicals

Fluoranthene, pyrene, chrysene, 1,2-benzanthracene, benz[*e*]pyrene, benz[*a*]pyrene, 1,12-benzperylene, anthanthrene, 1,2,5,6-dibenzanthracene, coronene, indeno[123-*cd*]pyrene, benz[*a*]fluoranthene, benz[*b*]fluoranthene, benz[*j*]fluoranthene and benz[*k*]fluoranthene were obtained from BCR (Geel, Belgium). Anthracene, phenanthrene and perylene were obtained from Promochem (Augsburg, Germany). Acetonitrile, ethylacetate and isopropanol were of analytical grade purchased from Merck (Darmstadt, Germany).

2.3. Preparation of the calibration samples and the oil samples

The calibration samples were prepared by spiking PAHs at different concentrations (0.1–3.5 μg/kg of each PAH) into blank oil. Blank oils were prepared from (refined) oils by treatment with 4% charcoal at 60°C for 15 min under vacuum. The treated oils were analyzed for residual PAHs.

Before injection 12.5% (v/v) isopropanol is added to all samples and the samples are filtered over a 0.45 μm filter.

Oil samples with individual PAH levels >3.5 μg/kg are diluted with (the same type of) blank oil and analyzed again. If many samples with expected high PAH levels have to be analyzed, it is also possible to adjust the range of the calibration curve.

2.4. Experimental conditions

The connection scheme of the DACC–HPLC system is given in Fig. 1.

Both the DACC column and the analytical columns were placed in the column thermostat which was kept at 20.0°C. A user program of the autosampler controlled the three switching valves of the system and the solvent select valve of the SPE unit. The program also started the HPLC gradient.

A volume of 250 μl of sample is injected onto the DACC column and the clean up is performed with 0.35 ml/min isopropanol as mobile phase. After 10–12 min, depending on the type of oil, the backflush mode and the HPLC gradient are initiated. During the first 145 s the backflush eluent is sent to waste, whereafter the eluent is directed to the analytical column. The HPLC gradient, the timed events and the wavelength program of the fluorescence detector are listed in Table 1.

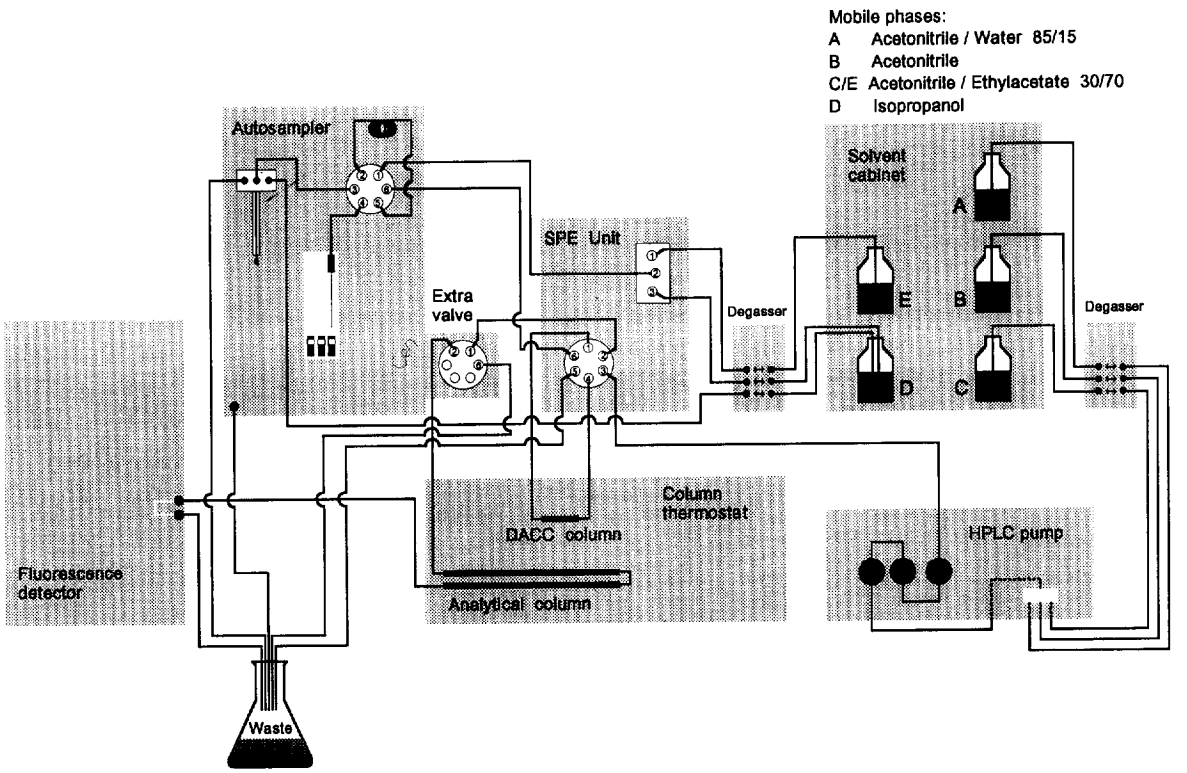

Fig. 1. Overview of the DACC–HPLC system with fluid connections.

2.5. Column switching mechanism

The column switching scheme is illustrated in Fig. 2 and consists of the following steps:

2.5.1. Step 1

Length of time ±4 min. The syringe of the autosampler fills the sample loop with sample from the autosampler vial. The DACC column is conditioned with isopropanol and the analytical columns are conditioned with the initial mobile phase composition of the gradient.

2.5.2. Step 2

Length of time 10–12 min depending on the type of oil. The injector valve is switched and the clean-up of the sample is performed on the DACC column. The syringe and needle of the autosampler are rinsed.

2.5.3. Step 3

Length of time 145 s. The SPE valve, the extra valve and the solvent select valve of the SPE unit are switched. The SPE valve sets the DACC column to the backflush mode. The extra valve sends the redundant isopropanol to waste. The injection system is rinsed.

2.5.4. Step 4

Length of time 5 min. The extra valve is switched. The backflush eluent is sent to the analytical column.

2.5.5. Step 5

Length of time 57 min. The SPE valve is switched. The DACC column is set to the normal mode and is rinsed. The separation on the analytical column is continued. After 20 min the solvent select valve is switched and the DACC column is re-conditioned with isopropanol.

While the sample is analyzed, followed by re-

Table 1
HPLC conditions

DACC column: 80×3 mm Chromspher PI
Mobile phase: 2-Propanol, flow-rate 0.35 ml/min
Analytical column: two 250×4.6 mm Chromspher 5 PAH

Mobile phase	Time (min)	Flow (ml/min)	Water (%)	Acetonitrile (%)	Ethyl acetate (%)
	0	0.4	15	85	0
	2.5	0.4	15	85	0
	3.0	1	15	85	0
	12	1	6	94	0
	20	1	4.5	95.5	0
	26	1	4	89	7
	39	1	0	30	70
	50	1	0	30	70

Detection — Fluorescence with timed wavelength program

Time (min)	Excitation (nm)	Emission (nm)	PAH detected
0	244	375	(1) Phenanthrene
13.5	244	420	(2) Anthracene
15.1	280	460	(3) Fluoranthene
16.7	330	388	(4) Pyrene
20.7	280	420	(5) 1,2-Benzanthracene
24.2	261	400	(6) Chrysene
27.4	250	510	(7) Benz[*a*]fluoranthene
30.4	324	392	(8) Benz[*e*]pyrene
32.3	430	465	(9) Perylene
34.6	396	430	(10) Benz[*k*]fluoranthene
36.4	378	403	(11) Benz[*a*]pyrene
38.5	290	440	(12) 1,2,5,6-Dibenzanthracene
			(13) 1,12-Benzperylene
41.6	296	500	(14) Indeno[123-*cd*]pyrene
43.7	298	438	(15) Anthanthrene
			(16) Coronene

Note: If benz[*b*]fluoranthene and benz[*j*]fluoranthene have to be determined, the program lines concerning benz[*a*]fluoranthene, benz[*e*]pyrene and perylene should be replaced by:

Time (min)	Excitation (nm)	Emission (nm)	PAH detected
27.4	310	508	Benz[*j*]fluoranthene
31.4	346	443	Benz[*b*]fluoranthene

conditioning of the HPLC column, the system is started to process step 1 and 2 of the next sample.

3. Results

Several parameters had to be examined to establish the optimal conditions (Sections 2.3–2.5) and define the necessary equipment (Section 2.1) to automate the system.

3.1. Optimization of the clean-up

A number of polar and apolar mobile phases were tried for the separation of PAHs from other components of edible oils on the DACC column. The best separation was obtained with isopropanol (IPA), independent of the type of oil. The examined oils were sunflower, olive, coconut, bean, fish, sesame and palm oil.

The IPA/oil ratio of oil samples to be injected influences the separation on the DACC column. It

appeared that oil/IPA mixtures demix in the range of 60–400% IPA, depending on the type of oil. To be able to inject as much oil as possible, mixtures in the range of 0–60% IPA were analyzed. The best separation for all oils was obtained with an oil/IPA ratio of 100:12.5 (v/v), with exception of palm oil which needs a larger percentage of IPA to avoid precipitation in the system.

The dimensions of the DACC column that can be used are limited. Since IPA has a negative influence on the analytical separation of the PAHs, in backflush most of the IPA is sent to waste as described in Section 3.2. However, a certain amount of IPA mixes with the front of the backflush eluent, which contains the PAHs, and reaches the analytical column. The larger the dimensions of the DACC column, the more IPA will be directed to the analytical column.

Several columns with different lengths and internal diameters were tested. The best results were obtained with a DACC column of 80×3 mm. After transfer of the PAHs the resulting separation on the analytical column was sufficient to be able to switch the excitation and emission wavelengths between the eluting PAHs. The column allowed a (maximum) injection volume of 250 μl, which gave the desired quantification limits of 0.1 μg/kg.

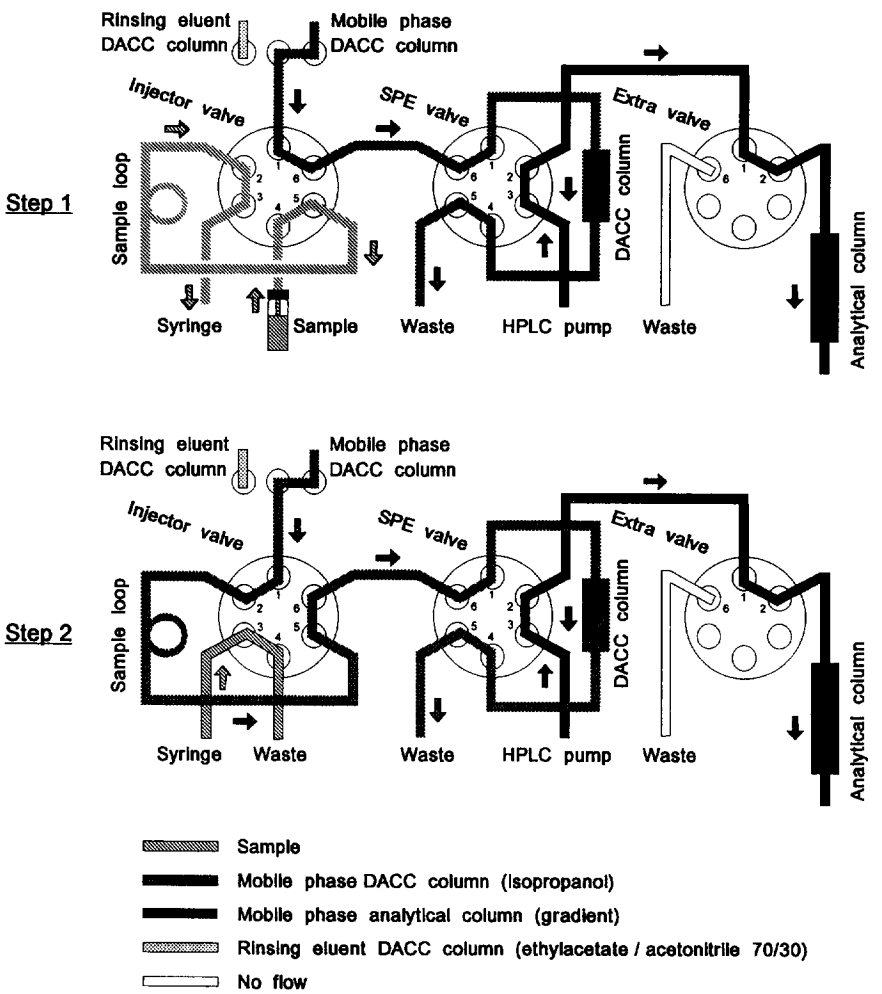

Fig. 2 (*continued on p. 268*).

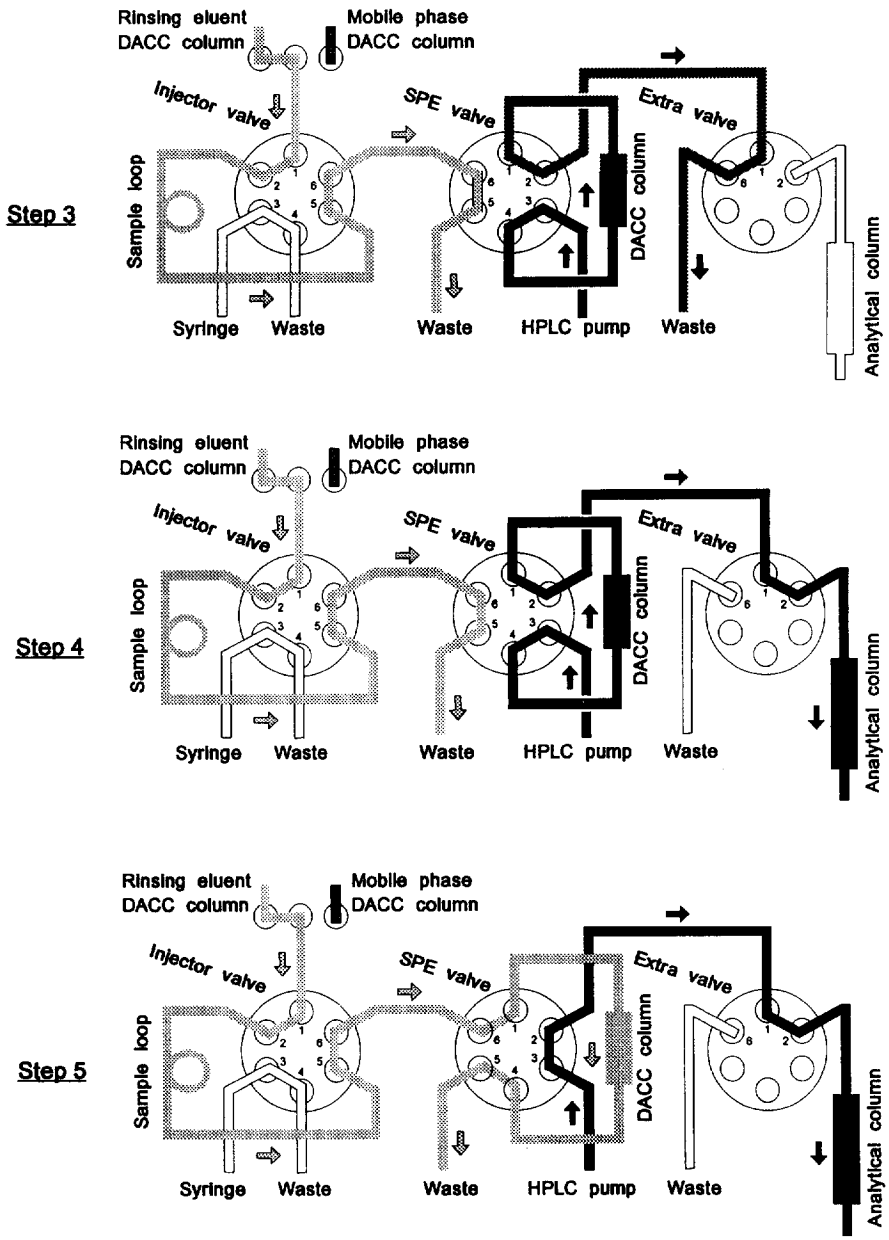

Fig. 2. Different steps of the column switching mechanism described in Section 2.5.

All columns were placed in a column thermostat which was kept at 20°C. Higher temperatures (30°C) resulted in a worse separation on the DACC column, lower temperatures (10°C) caused precipitation of the oil in the system.

The flow of the IPA was set at 0.35 ml/min, higher flows caused too much pressure in the system after injection of the sample. Under these conditions the clean up time was 10–12 min, depending on the type of oil.

3.2. Optimization of the backflush

To minimize the amount of IPA reaching the analytical column an extra valve has been inserted between the valve which initiates the backflush mode and the analytical column. The extra valve is switched to waste during the first 145 s of the backflush mode, using the system and experimental conditions described in Sections 2.1 and 2.4 respectively.

The initial composition of the mobile phase for the analytical separation, a mixture of acetonitrile–water, is used as backflush eluent. A higher percentage of acetonitrile results on the one hand in a sharper peak shape of the eluting PAHs, but on the other hand it decreases the separation on the analytical column. The optimal results were obtained with a acetonitrile–water (85:15, v/v) mixture.

3.3. Analytical separation

The HPLC conditions are listed in Table 1. In order to meet the required quantification limits the fluorescence detector has to switch to specific wavelengths for each PAH. Therefore it is necessary to have a baseline of a certain length between each eluting PAH. This was achieved by the use of two coupled 25 cm columns. The total column length of 50 cm made it necessary to introduce ethylacetate in the last part of the gradient to get reasonable retention times of the last eluting PAHs. In a single analysis 16 different PAHs are quantified as stated in Table 1.

Although for some PAHs the optimal excitation and emission wavelengths could not be used due to interfering peaks in the chromatogram, the desired quantification limits were achieved.

With the fluorescence conditions given in Table 1 the system is blind to two other PAHs, benz[*j*]fluoranthene and benz[*b*]fluoranthene. Alternatively, quantification of benz[*j*]fluoranthene and benz[*b*]fluoranthene is possible by selecting other wavelengths and ignoring the detection of benz[*e*]pyrene, perylene and benz[*a*]fluoranthene. The differences in the detector program are listed in Table 1.

Chromatograms of a blank olive oil spiked with 1.0 μg/kg of each PAH and of an olive oil are presented in Figs. 3 and 4 respectively.

3.4. Comparison with the caffeine complexation method

Ten olive oils have been analyzed with both the caffeine complexation method and the DACC method. The results of this comparison are listed in Table 2. The levels found for the 5- and 6-ring PAHs are in good agreement. With the DACC method higher levels of the 3- and 4-ring PAHs are found. Since detection of these PAHs at other wavelengths gave the same results, it should be concluded that the caffeine method gives systematically too low values, which can be explained by the loss of PAHs during the evaporation steps.

3.5. Method performance

The performance of the method has been evaluated for coconut, sunflower, olive and bean oil. The four oils were spiked with the 16 PAHs at ten different levels in the range of 0.1–3.5 μg/kg. The lowest and highest level were analyzed in tenfold, the middle level in sixfold and the other levels in duplicate. The samples were analyzed in one series, together with blanks. Afterwards, without interrupting the system, another detector was installed and the appropriate coconut samples were analyzed again to establish the quantification limits of the method using a second detector.

3.5.1. Confidence limits

All calibration curves showed increasing variances at increasing concentration levels (heteroscedasticity). The individual PAHs showed similar confidence intervals (95%) in the four investigated oils. These intervals are in the same range for most of the PAHs. The only exception was anthanthrene which gave a good performance in coconut oil but gave sometimes irreproducible results in the other oils. The (clustered) confidence intervals at two different levels are listed in Table 3.

3.5.2. Quantification limits

The quantification limits of the individual PAHs have been calculated in two different ways, from

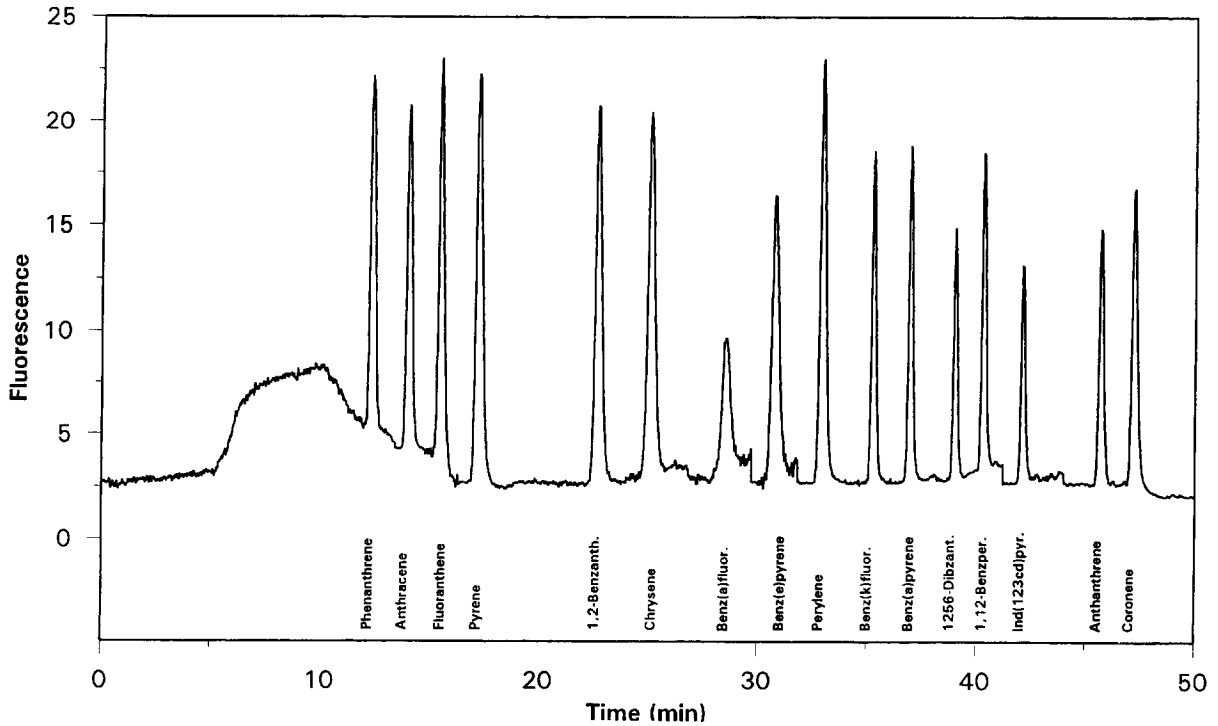

Fig. 3. Analysis of an olive oil spiked with 1.0 μg/kg of each PAH.

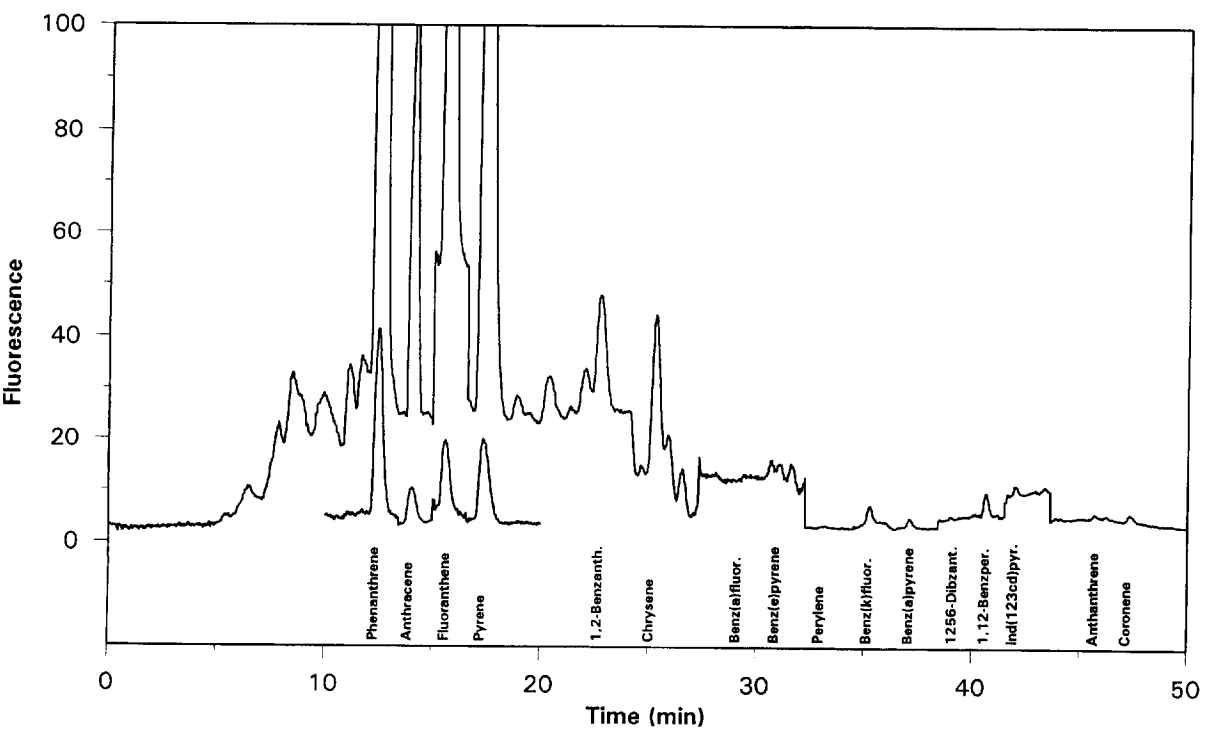

Fig. 4. Analysis of an olive oil, including a part of a second analysis 15× diluted.

Table 2
Comparison of the DACC method (A) with the caffeine complexation method (B)

PAH	Oil																			
	1		2		3		4		5		6		7		8		9		10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
(1) Phenanthrene	40.5	22.0	31.5	22.5	15.0	9.7	30.0	13.5	81.0	32.9	16.5	14.5	19.5	17.2	15.0	12.2	27.0	21.0	48.0	32.1
(2) Anthracene	2.1	0.6	2.2	0.9	0.6	0.3	1.7	0.6	7.5	1.9	1.3	0.7	1.3	0.6	0.6	0.4	1.7	0.8	2.9	1.1
(3) Fluoranthene	12.0	7.6	9.0	7.5	2.9	2.8	9.0	6.5	22.5	15.4	4.5	4.1	4.5	3.5	4.5	2.7	7.5	6.1	13.5	9.2
(4) Pyrene	9.0	5.1	9.0	6.1	3.2	2.1	7.5	4.5	21.0	11.8	6.0	3.6	6.0	2.7	7.5	3.9	7.5	5.1	10.5	6.9
(5) 1,2-Benzanthracene	1.0	0.8	0.9	1.2	0.2	0.3	0.8	0.7	3.0	2.3	0.5	0.8	0.6	0.6	0.5	0.7	0.8	1.7	1.2	<
(6) Chrysene	2.4	1.5	1.8	2.0	0.7	0.9	1.6	1.4	6.0	3.4	1.0	1.2	1.2	0.7	0.8	0.8	2.5	1.8	2.3	1.9
(7) Benz[*a*]fluoranthene	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<
(8) Benz[*e*]pyrene	<	0.1	<	0.4	<	0.1	<	0.3	<	0.4	<	0.3	<	0.6	<	0.6	<	0.4	<	<
(9) Perylene	0.1	<	0.1	<	0.1	<	0.2	<	0.1	<	0.1	<	0.1	<	0.1	<	0.1	<	0.1	<
(10) Benz[*k*]fluoranthene	0.2	0.1	0.2	0.2	0.1	<	0.3	0.2	0.3	0.2	0.1	<	0.2	0.1	0.1	<	0.2	0.1	0.3	0.2
(11) Benz[*a*]pyrene	0.2	0.1	0.3	0.2	0.1	<	0.4	0.2	0.4	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.3	0.2
(12) 1,2,5,6-Dibenzanthracene	<	<	0.1	<	<	<	0.1	<	0.1	<	0.1	<	<	<	0.2	<	<	<	<	<
(13) 1,12-Benzperylene	0.2	0.1	0.2	0.2	0.1	<	0.4	0.3	0.2	0.2	0.4	0.3	0.2	0.1	0.7	0.4	0.2	<	0.3	0.2
(14) Indeno[123-*cd*]pyrene	0.1	0.1	0.1	0.2	<	<	0.2	0.3	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.1
(15) Anthanthrene	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	0.1	<	<
(16) Coronene	0.2	<	0.2	0.2	0.2	<	0.2	0.2	0.2	<	0.3	0.3	0.2	<	0.4	0.3	0.1	0.1	0.2	0.1

Results (μg/kg) of the analyses of ten different oils.
< Signifies value below 0.1 μg/kg.

Table 3
95% Confidence intervals (clustered) at two different levels of quantitation

PAH	Level	
	0.5 μg/kg ±	3.0 μg/kg ±
(2) Anthracene	0.02–0.04	0.04–0.10
(4) Pyrene		
(5) 1,2-Benzanthracene		
(6) Chrysene		
(8) Benz[*e*]pyrene		
(10) Benz[*k*]fluoranthene		
(11) Benz[*a*]pyrene		
(12) 1,2,5,6-Dibenzanthracene		
(13) 1,12-Benzperylene		
(14) Indeno[123-*cd*]pyrene		
(16) Coronene		
(3) Fluoranthene	0.04–0.07	0.10–0.16
(7) Benz[*a*]fluoranthene		
(9) Perylene		
(1) Phenanthrene	0.04–0.09	0.11–0.21
(15) Anthanthrene (Coconut oil)	0.04	0.10

The individual PAHs showed similar confidence intervals (95%) in the four investigated oils. PAHs with intervals in the same range are clustered.

lowest concentration level (A) and from the noise of the baseline (B).

(A) The limit of quantification was calculated as 10 times the standard deviation of ten analyses of the lowest concentration level. The variance of these analyses was considered to be equal to the variance of real blank samples.

(B) The limit of quantification was calculated as 10 times the standard deviation of the noise of the baseline of blank samples.

The quantification limits of both methods were in good agreement. For all oils the limits were in the range of 0.02–0.1 μg/kg, which was equal to or lower than the desired level of 0.1 μg/kg.

The method performance has been evaluated using the Perkin-Elmer LC-240 fluorescence detector. Afterwards the determination of the quantification limits, according to method B, was repeated with the Jasco FP-920 detector (Separations). The signal/noise ratio of this detector resulted in an improvement of the quantification limits by a factor from 2 to 10.

4. Conclusions

A new automated on-line method for the determination of PAHs in edible oils and fats has been developed using an LC–LC coupling of a clean-up DACC column to an analytical column for the separation. PAHs are quantified with fluorescence detection.

Compared to the traditional methods this method significantly reduces the amount of solvent waste and considerably saves time. The total analysis time of one sample is approximately 80 min, with the traditional methods 8–10 h.

The method showed excellent confidence limits at μg/kg level of quantification.

The quantification limits of the individual PAHs are <0.1 μg/kg and the method automatically corrects for possible incomplete recoveries because the calibration samples are subjected to the same treatment as the samples to be analyzed.

The system uses regular HPLC instrumentation.

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