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MODIFIED ANALYTICAL METHOD FOR POLYCYCLIC AROMATIC HYDROCARBONS, USING SEC FOR SAMPLE PREPARATION AND RP-HPLC WITH FLUORESCENCE DETECTION. APPLICATION TO DIFFERENT FOOD SAMPLES

E. Węgrzyn, S. Grześkiewicz, W. Popławska, and *B. K. Głód*

Meat and Fat Research Institute, ul. Jubilerska 4, 04-190 Warszawa, Poland

SUMMARY

An HPLC method with fluorescence detection has been developed for determination of eight polycyclic aromatic hydrocarbons (PAH) with four to six condensed aromatic carbon rings in edible oils and smoked products. The method employs preparative size-exclusion chromatography for efficient one-step lipid removal without saponification; benzo[*b*]chrysene is used as internal standard for quantification. Two other methods (liquid–liquid extraction and solid-phase extraction) were tested for one-step clean-up and sample enrichment but it was found that one-step procedures did not remove lipids completely. Linearity of calibration plots was good for all PAH in the concentration range from the detection limit (approx. 0.1 ppb) to 100 ppb. The repeatability (RSD, $n = 6$) for different PAH ranged from 0.5 to 5%. Analysis of standard reference materials from the National Institute of Standards and Technology (mussel tissue, SRM 2978), the Community Bureau of Reference (coconut oil, CRM 458), and the Central Science Laboratory (olive oils, FAPAS 0615, 0618, and 0621) resulted in a good agreement between measured and certified concentrations. The method described has been used for determination of the PAH content of twelve samples of edible oil, rape seed, milk powder, hens' egg white and yolk, smoked sausage, white cottage cheese, and sprats. The PAH were identified from their fluorescence spectra.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are organic compounds containing two or more fused aromatic rings made up of carbon and hy-

drogen atoms. They belong to a group of ubiquitous environmental contaminants formed and released during incomplete combustion or by industrial processes. They are characterized by high mutagenic [1] and carcinogenic [2] potential. PAH can arise both naturally and as a result of anthropogenic activity. The latter is a much more important contributor of environmentally hazardous compounds.

Exposure to PAH occurs mainly by inhalation of air and by ingestion of food and drinking water [3,4]. Although food can be contaminated by environmental (air, dust and soil) PAH, PAH in food are mainly formed during industrial processing and food preparation, for example smoking, roasting, baking, drying, frying, or grilling [5]. PAH formation during smoking depends on such conditions as type and composition of wood, type of generator, oxygen accessibility, temperature, and time. The occurrence of PAH in vegetable oils is mostly related to their thermal treatment and the process used to dry the seeds, in which combustion gases may make contact with the seeds [6]. The main contributors to PAH uptake from food are oil and fat (50%), because of the strongly lipophilic nature of PAH, cereals (30%), and vegetables (10%) [7]. Concentrations in refined oils are in the range of few $\mu g\ kg^{-1}$ whereas in crude oils levels may exceed 2000 $\mu g\ kg^{-1}$ [8]. Their presence in food is, therefore, a matter of concern and requires continuous monitoring.

Some PAH are expected to be human carcinogens [9]. Several organizations have proposed maximum values for PAH in food products. On the many hundreds of PAH, the most studied is benzo[*a*]pyrene, which is often used as a marker for PAH in ambient air and food [10,11]. The International Agency for Research on Cancer (IARC) has classified three PAH (benzo[*a*]anthracene, benzo[*a*]pyrene, and dibenzo[*a*,*h*]anthracene) as *probably carcinogenic to humans* and three (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and indeno[1,2,3-*c*,*d*]pyrene) as *possibly carcinogenic to humans* [12]. The US EPA suggests determination of 16 PAH in food [13]. Among these, eight (benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]perylene) have high carcinogenic potential. In the paper we describe analysis of these eight PAH.

The analytical methods most frequently used for determination of the carcinogenic PAH are HPLC with fluorescence detection [3,4] and GC –MS [14–16]. Traditionally, procedures such as Soxhlet, solid-phase, and liquid–liquid extraction, with previous saponification with KOH–methanol solution [17], have been described for sample clean-up. These methods are,

however, very difficult and time and solvent-consuming, and, because they involve long and complex procedures, are unsuitable for routine analysis [18,19]. The reason is the complicated nature of lipophilic matrices, the physicochemical properties of which (solubility, molecular weight, etc.) are similar to those of PAH [20]. It has, however, been found that no clean-up is necessary for non-fatty food [17]. One of the limitations of single-wavelength UV and fluorescence detection is the lack of peak-purity determination and qualitative analysis other than retention time. A second, additional, method of analysis, for example GC–MS is, therefore, usually recommended when the compound cannot be easily identified by HPLC [21]. Compounds can also be identified by use of a diode-array detector. This detector is not recommended for PAH determination, however, because of its lack of selectivity and high detection limit.

The objective of our work was to simplify conventional techniques for determination of PAH in fatty samples and to perform clean-up in one-step, thus avoiding losses of the pollutants and saving time. Three methods of sample preparation (removal of lipids and other high-molecular-mass compounds) have been tested: liquid–liquid extraction, solid-phase extraction [21], and preparative size-exclusion chromatography (SEC). SEC has already been found to be a suitable technique for improving previous clean-up methods for analysis of PAH in non-fatty solid food [17] and in plant matrices [22]. In this paper we report an HPLC method, with fluorescence detection, for analysis of PAH in edible oils and solid food products. To evaluate peak purity and for qualitative analysis the fluorescence excitation and emission spectra of each PAH in the samples were compared with those obtained from standard solutions. The method has been tested on a wide range of vegetable oils and solid food products. As far as we are aware it is first description in the literature of analysis of PAH in such wide range of different samples.

EXPERIMENTAL

Apparatus

The preparative chromatographic system consisted of an ASI-100 automatic sample injector, a P-580 degasser and pump, a UVD-340S diode-array detector, a UCI-100 universal chromatography interface (Dionex–Softron, Germering, Germany), a Foxy Jr. fraction collector (Isco, Lincoln, USA), and a size-exclusion column packed with PLgel, based on PS/DVB,

5 μm, 50 Å, 600 mm × 7.8 mm i.d., with guard column (Polymer Laboratories, Amherst, USA). The system was controlled by use of Chromeleon v. 6.20 (Dionex-Softron, Germering, Germany) software installed on an IBM-PC Pentium computer.

The analytical chromatographic system consisted of an Agilent 1100 series G-1322A vacuum degasser, a G-1313A automatic sample injector, a G-1311A quaternary pump, a G-1316A column thermostat, a G-1315B diode-array detector, a G-1321A scanning fluorescence detector (all Agilent Technologies, Palo Alto, USA), and a Hypersil Green PAH column with guard column, 5 μm, 250 mm × 3 mm i.d. (Thermo Electron Corporation, Runcorn, UK). The system was controlled by use of Chem Station for LC 3D software (Agilent Technologies) installed on an IBM PC-compatible Pentium computer.

Sample preparation was performed using a UM4EV2A homogenizer (Bosch, Germany), a 317b high-speed centrifuge with 310b controller (Mechanika Precyzyjna, Warsaw, Poland), a vortex mixer (JWE-electronic, Warsaw, Poland), and a BF5 water bath (Falc, Luvarno, Italy).

Materials

Acetonitrile, dichloromethane, chloroform, and methanol, all HPLC grade, were obtained from Labscan (Dublin, Eire). Florisil, 100–200 mesh size, was from Fluka (Buchs, Switzerland). LC-18 Supelclean solid-phase extraction tubes were from Supelco (Bellefonte, USA). Benzo[*a*]anthracene (B[*a*]A), chrysene (Ch), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), indeno[1,2,3-*c*,*d*]pyrene (IP), dibenzo[*a*,*h*]anthracene (dBA), benzo[*g*,*h*,*i*]perylene (BP), and benzo[*b*]chrysene (B[*b*]Ch), 10 ng μL^{-1} in acetonitrile, were from Ehrenstorfer (Augsburg, Germany). SRM-1647d (16 PAH listed by the US Environmental Protection Agency; EPA) and SRM-2978 (mussel tissue, organic contaminants; Raritan Bay, New Jersey, USA) were from the National Institute of Standards and Technology (NIST; Gaithersburg, USA), CRM-458 (PAH in coconut oil) was from the Community Bureau of References (Luxembourg, EU), and FAPAS 0615, 0618, and 0621 (environmental contaminants in olive oil test materials) were from the Central Science Laboratory (York, UK). Other chemicals were of analytical-reagent grade and were used without further purification. Milli-Q (Millipore, Bedford, USA) water was used to prepare all solutions. Mobile phases were filtered through a Millipore 0.22-μm membrane filter before use. Samples of edible oils (coconut crude and refined, rape-seed refined and cold pressed, sunflower,

olive, soya-bean, grapes, flax, pumpkin, peanut, sesame, and whale oil) and solid food products (rape-seed, smoked sausage, smoked white cottage cheese, smoked sprats, and egg and milk powder) were commercially available and obtained from the nearest supermarket.

Procedure

Standards

Stock standard solutions (B[*b*]Ch, individual PAH, and the 16 US EPA solutions) were prepared by dissolving PAH standards at a concentration of 50 μg L^{-1} (50 μg L^{-1} B[*a*]P in 16 US EPA standards) in acetonitrile. Solutions were stored at 4ºC in the dark and were stable for approximately three months. Calibration standard PAH solutions were prepared by suitable dilution of the stock solutions with acetonitrile. B[*b*]Ch was used as internal standard and was added to the samples, at a final concentration of 5 μg kg^{-1}, before any procedure was started. Two certified reference materials SRM-2978 (mussel tissue) and CRM-458 (coconut oil) and the three FAPAS samples 0615, 0618, and 0621 (olive oil) were used to validate the method.

Sample Preparation

To obtain repeatable results the laboratory temperature was kept below 22ºC, to reduce the solubility of short-chain fatty acids. All evaporation and clean-up procedures were conducted in a vented hood to minimize contamination with laboratory PAH and organic solvents.

Edible oil samples (1 g) were spiked with B[*b*]Ch (50 μg L^{-1}, 100 μL) and diluted with dichloromethane to a final volume of 5 mL before the clean-up procedure.

Solid samples (sausage, cheese, sprats, cooked egg, or SRM-2978) were cut into small pieces then mechanically homogenized in a stainless steel blender, in accordance with a procedure described elsewhere [23]. Sample (1 g) was spiked with B[*b*]Ch (50 μg L^{-1}, 100 μL), and 1.5 mL methanol was added. The sample was vortex mixed for 1 min, 3 mL chloroform was added, the sample was again vortex mixed for 1 min, 1.5 mL water was added, and the sample was finally vortex mixed for 3 min. The mixture obtained was centrifuged for 10 min at 10000 rpm, furnishing three layers – liquid (water–methanol solution), solid (scum containing proteins and other large-molecule compounds), and liquid (chloroform solution containing PAH). The chloroform phase was poured from the tube through a

folded filter paper. The sample was re-extracted, as previously described, with 3 mL chloroform, vortex mixed for 3 min, and centrifuged for 10 min at 10000 rpm. The combined extracts were evaporated on a water bath (40ºC) under a stream of nitrogen and diluted with 2 mL dichloromethane.

Sample Clean-Up Procedures

Liquid–liquid extraction was performed [24] with acetonitrile or acetonitrile–acetone, 60:40% (*v*/*v*). Equal volumes of sample and extractant were vortex mixed for 1 min and centrifuged for 10 min at 10000 rpm. The supernatant was evaporated on a water bath (40ºC) under a stream of nitrogen and diluted with 200 µL acetonitrile before chromatographic analysis.

The SPE column was prepared by filling a 1-cm i.d. RP-18 column with 2 g Florisil. After rinsing the system with 10 mL deionized water the column was conditioned with 10 mL methanol. The column was dried, the extract was loaded slowly, and the column was left to dry for 1 min in the air. PAH were eluted from the column with 10 mL dichloromethane. The collected eluent was evaporated on a water bath (40ºC) under a stream of nitrogen and diluted with 200 µL acetonitrile before chromatographic analysis.

Preparative SEC was performed under isocratic conditions (100% dichloromethane) at a flow rate of 1 mL min^{-1} and at ambient temperature. The column was stabilized for 1 h before chromatographic measurements. The injection volume was 400 µL. Chromatograms were monitored at 254 nm and fractions were collected between 18 and 24 min. Aliquots were evaporated to dryness on a water bath (40°C) under a stream of nitrogen and the residue was dissolved in 200 µL acetonitrile for chromatographic analysis (HPLC FLD).

HPLC Analysis

Analytical chromatography was performed with a flow rate of 0.8 mL min^{-1} at 25°C. The injection volume was 20 µL. The column was stabilized at 25°C for 1 h before chromatography. The mobile phase was a gradient prepared from water (component A) and acetonitrile (component B). Details of the gradient are given in Table I. The output signal from the fluorescence detector was displayed continuously on the computer. Excitation and emission wavelengths were programmed as reported in Table II. Optimum wavelengths were obtained from stop-flow three-dimensional

fluorescence spectra (the signal was plotted against excitation and emission wavelengths).

Table I

The mobile phase gradient

Time (min)	Water (%)	Acetonitrile (%)
0	50	50
20	0	100
35	0	100
40	50	50
45	50	50

Table II

Programmed excitation and emission wavelength pairs

Time (min)	PAH	$\lambda_{ex.}$ (nm)	$\lambda_{em.}$ (nm)
0.0	Small-molecule PAH	248	375
13.9	B[*a*]A, Ch	270	385
19.0	B[*b*]F	256	446
20.8	B[*k*]F, B[*a*]P, dBA, BP	295	410
25.7	IP	274	507
27.0	B[*b*]Ch	295	410

Data Analysis

Measurements were repeated three times for each sample and the results were averaged and expressed relative to the average result for the blank control, containing no PAH. Results were compared by use of Student's *t*-test for independent variables. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Sample Preparation and Clean-Up

Preparation and clean-up of food samples before PAH analysis are traditionally performed by very long and laborious procedures [17–19] which include saponification, extraction, and clean-up steps. For example, the ISO directive requires two liquid–liquid extractions and two solid–

phase extractions [24]. The objective of our work was to develop a simple, one-step cleanup procedure suitable for different food products, both liquids (oils) and solids. Fat/oil samples were prepared merely by dissolution in dichloromethane. Solid samples were homogenized and the fat was obtained from the solid residue by extraction.

In pilot experiments three clean-up procedures were tested – liquid–liquid extraction, solid-phase extraction, and preparative SEC. It was found that the first two procedures left fat residues in the sample – these were observed as small drops on the walls of test tubes. This oil was not removed even after a second liquid–liquid extraction. Injection of such samples resulted in generation of substantial backpressure in the analytical column and reduced retention reproducibility. The problem disappeared when SEC was used and it was also found the assay could be used on all the food products tested. It was of particular interest that both solid and liquid agriculture products could be analyzed by use of the proposed assay, as is shown in Fig. 1. Although the fat content of these samples differs substantially, the eluate fraction collected between 18 and 24 min includes all the PAH in the sample, as was confirmed by comparison of retention times with those of standards. As a precaution, solvents should be checked to ensure they are not contaminated with PAH. Although high-quality solvents were used some light PAH were detected. Contamination as a result of solvents was found to be more serious in extraction procedures that required more solvent than was required by SEC.

HPLC Analysis

The separation and detection of PAH have already been described in the literature. Separation is usually performed on a reversed-phase column with acetonitrile–water mobile phases and fluorescence detection [3, 4]. Under these conditions retention is proportional to sample molecular weight, i.e. hydrophobicity [25]. Gradient elution conditions are given in Table I. They are a compromise between short separation time and cleaning of the column after injection of the complex food sample matrix. Because excitation and emission fluorescence spectra measured with different instruments can vary slightly, the maximum excitation and emission wavelengths were obtained separately for all the compounds investigated (Table II). First, the maximum emission wavelength was determined by scanning emission between 300 and 550 nm at an excitation wavelength of 350 nm for the 16 PAH listed by the US EPA. Maximum excitation wavelengths were then determined by scanning excitation between 200 and 400 nm and recording

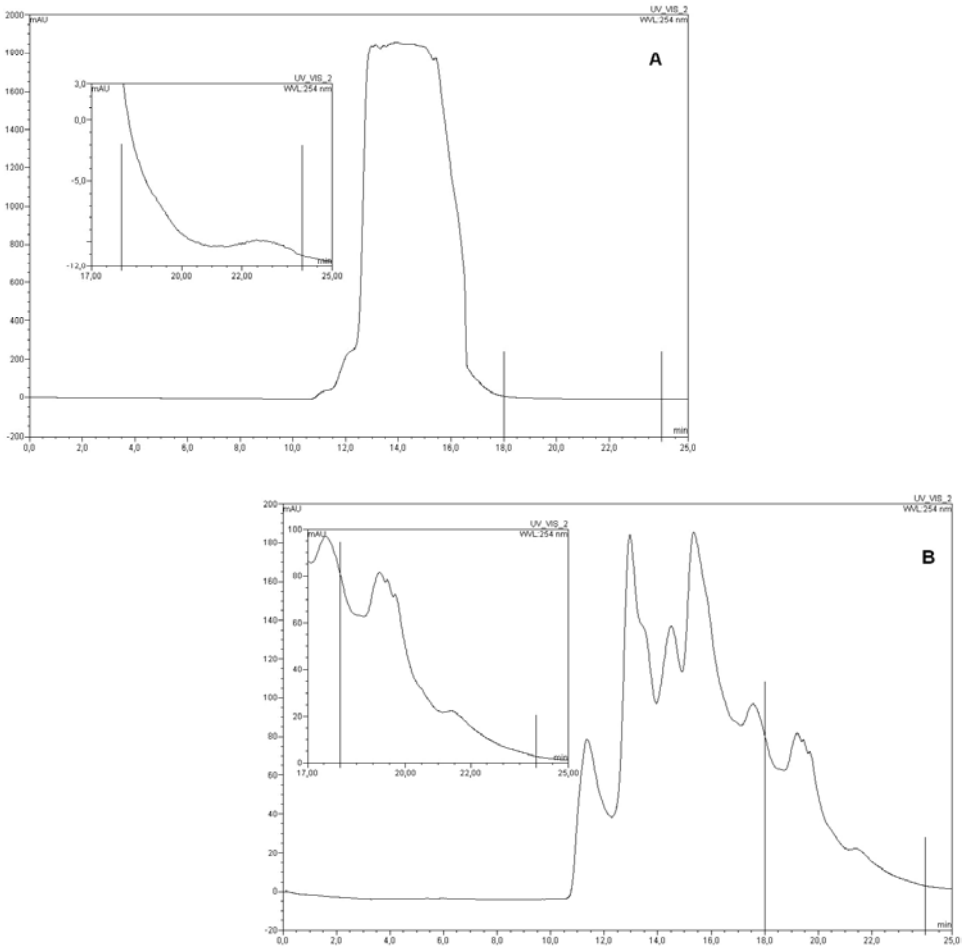

Fig. 1

SEC chromatograms obtained from rape seed oil (A) and smoked sprats (B). Chromatographic conditions: PLgel column, 5 μm, 50 Å, 600 mm × 7.8 mm i.d.; mobile phase dichloromethane, UV detection at 254 nm; ambient temperature, flow rate 1 mL min^{-1}, injection volume 400 μL

at the emission wavelength determined previously. Finally, the results obtained were confirmed by acquiring stop-flow three-dimensional fluorescence spectra (signal plotted against excitation and emission wavelengths) as presented in Fig. 2 (the huge peak on the diagonal arises as a result of a Rayleigh scattering and Raman effect). For each of the two groups of compounds (Table II) with very similar retention times, a set of excitation/emission wavelengths as near as possible to the maximum wave-

length for each compound was selected. The wavelengths chosen are listed in Table II.

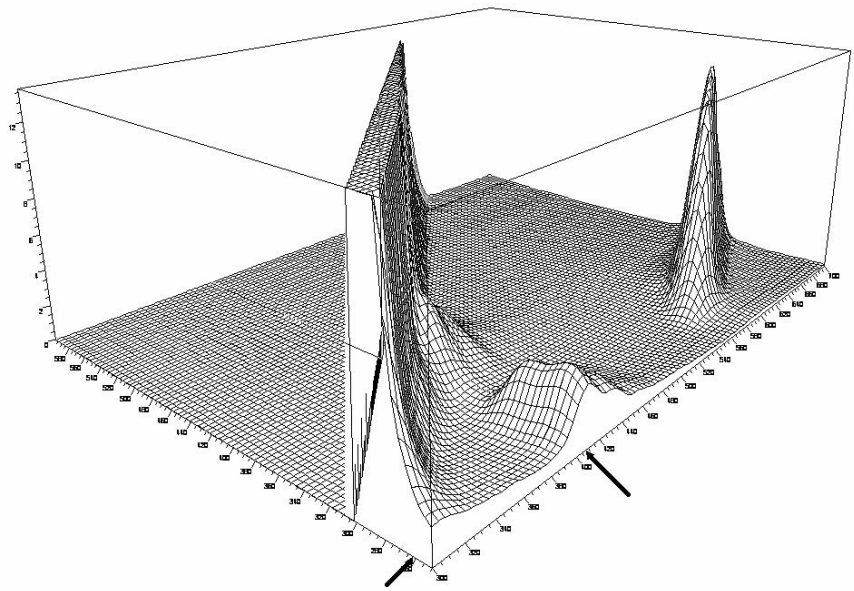

Fig. 2

Stop-flow three dimensional fluorescence spectra (plot of signal against excitation and emission wavelengths) for B[a]P. The arrows indicate the set of excitation/emission wavelengths selected

The chromatogram obtained for the 16 PAH listed by the US EPA is presented in Fig. 3. It is apparent that good separation was achieved for the eight carcinogenic PAH. Optimum chromatographic conditions were determined and the method was validated by the determination of linearity, precision, and detection limit (D_L). The D_L was defined as the lowest absolute concentration of analyte in a sample that could be detected but not necessarily quantified (the signal was three times the noise level). The limit of quantification, defined as the lowest concentration of analyte in a sample that could be determined with acceptable precision and accuracy (signal six times the noise level), was twice as high as the D_L. The precision of the method was determined by measurement of repeatability, which was determined by analysis of the same standard mixture six times, on the same day, under the same experimental conditions. The relative standard deviation (RSD) obtained for retention time was less than 1% for all the

PAH investigated and in the range 0.5–5% for the surface area of the peaks. Inter-day repeatability was always less than 6% (five between-day replicates for three different concentrations). The linearity of calibration plots was good (regression coefficients exceeded 0.995) for all the PAH in the concentration range 0.1–100 μg kg^{-1}. The retention times and detection limits obtained for all the PAH by use of the assay are listed in Table III. It should be noted that although IP is satisfactorily separated from the other PAH, its detection limit is much higher than those obtained for other PAH (Fig. 3, Table III), as has been observed in other work [17]. Because this detection limit was usually too high for real samples, IP was not determined.

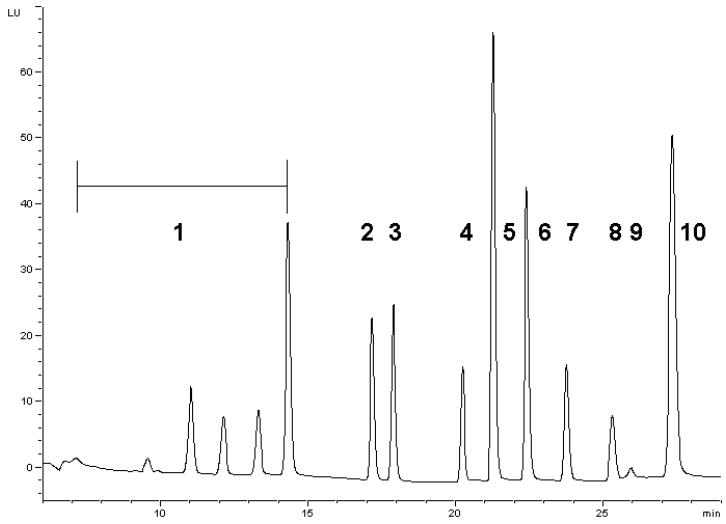

Fig. 3

HPLC chromatogram obtained from the 16 PAH listed by the US EPA (1, not analyzed small-molecule PAH; 2, B[*a*]A; 3, Ch; 4, B[*b*]F; 5, B[*k*]F; 6, B[*a*]P; 7, IP; 8, dBA; 9, BP; 10, B[*b*]Ch). Chromatographic conditions: 250 mm × 3 mm i.d., 5 μm particle, Hypersil Green PAH column with guard column; mobile phase, acetonitrile–water gradient (Table I). The fluorescence detector excitation and emission wavelengths are listed in Table II. The temperature was 25°C, the flow rate 0.8 mL min^{-1}, and the injection volume 20 μL

Validation of the Assay

Coconut oil (CRM 458), mussel tissue (SRM 2978), and olive oil (FAPAS 0615, 0618 and 0621) reference materials containing certified concentrations of some PAH were used to validate the method. The certi-

Table III

Analytical characteristics of the chromatographic method

PAH	t_R (min)	D_L (μg kg^{-1})
B[*a*]A	17.1	0.06
Ch	17.8	0.08
B[*b*]F	20.2	0.1
B[*k*]F	21.3	0.04
B[*a*]P	22.4	0.08
dBA	23.7	0.1
BP	25.2	0.2
IP	25.9	4.8

t_R is the retention time and D_L the detection limit

Table IV

The accuracy of the assay

Reference material		Amount (μg kg^{-1})					
		B[*a*]A	Ch	B[*b*]F	B[*k*]F	B[*a*]P	BP
CRM 458 Coconut oil	Certified value		4.9 ± 0.4		1.97 ± 0.18	0.93 ± 0.09	0.97 ± 0.07
	Measured value		5.83		2.16	1.03	0.96
SRM 2978 Mussel tissue	Certified value	25 ± 7	59 ± 10	58 ± 15	24.1 ± 3.4	7 ± 3	19.7 ± 4.4
	Measured value	26.96	57.65	52.71	24.01	7.85	23.15
FAPAS 0615 Olive oil	Certified value		22.3 ± 2.05	4.41 ± 0.58		2.36 ± 0.15	2.36 ± 0.2
	Measured value		22.05	3.79		2.23	2.14
FAPAS 0618 Olive oil	Certified value	38.75 ± 1.92		24.90 ± 1.86		18.66 ± 0.96	17.83 ± 96
	Measured value	35.21		21.84		20.73	16.36
FAPAS 0621 Olive oil	Certified value	43.2 ± 1.88		30.8 ± 1.38		28.2 ± 1.03	18.9 ± 0.91
	Measured value	40.07		27.47		25.59	16.87

fied and measured values are compared in Table IV. Good results were obtained for all the PAH and concentrations investigated. Differences between measured and certified values were below 12%. These differences (with the exception of chrysene in CRM 458) fulfilled the criterion for acceptance because they were smaller than twice the uncertainty for the

reference materials. For the FAPAS samples all our results were in a widely accepted z-score between +1.96 and −1.96 ($\alpha = 0.05$) with 50% precision [26,27].

The selectivity of the assay was estimated by evaluating the purity of the PAH peaks. The excitation and emission spectra of each PAH peak in the chromatograms obtained from the different samples assayed were compared with those obtained from standard solutions. Peak purity, expressed as the quality of matching, was always greater than 95%. As an example, Fig. 4 shows the excitation and emission spectra of B[*a*]P standard and of the chromatographic peak from rapeseed oil at the same retention time. The spectra are identical, confirming the peak is B[*a*]P.

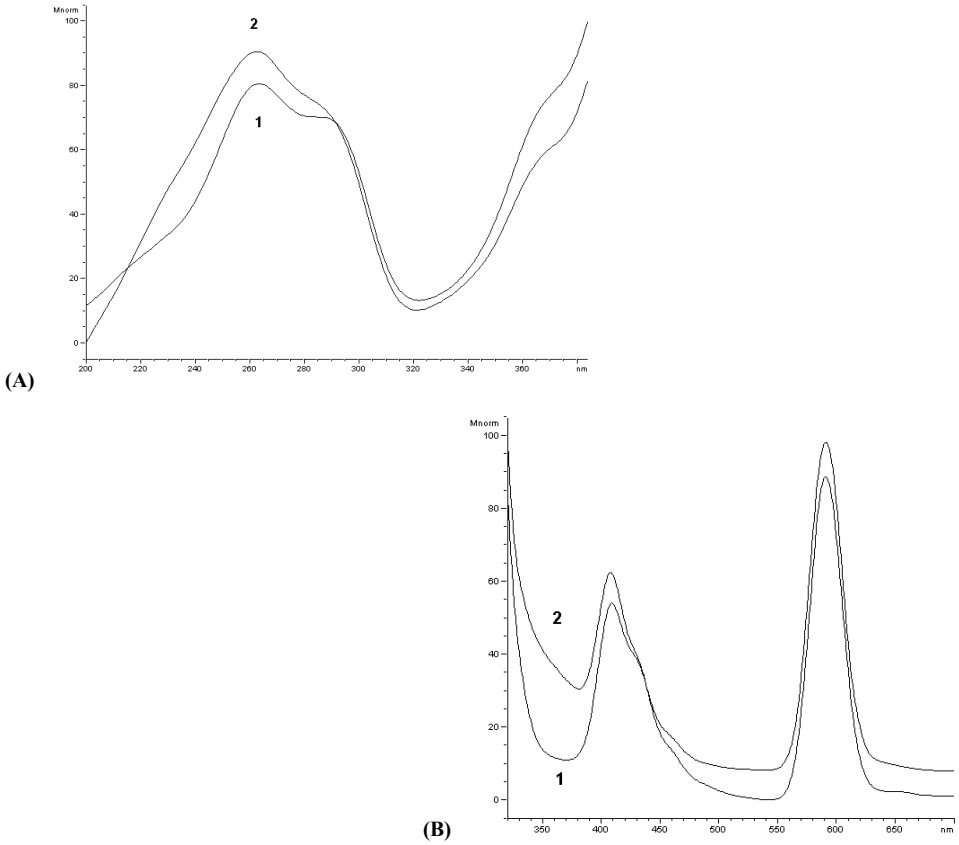

Fig. 4

Comparison of the excitation (A) and emission (B) spectra of B[*a*]P standard (1) with the same spectra obtained from the chromatographic peak, with the same retention time, from rape-seed oil (2). Chromatographic conditions as for Fig. 3

Determination of the PAH in Food Products

The method described above was used to determine PAH in 20 samples of different kinds of edible oil and solid food sample (Table V). The assay was found to be suitable for analysis of PAH in sample from different matrices. Some PAH were detected but not quantified, because their concentrations were below the limit of quantification. Concentrations of B[*a*]P (and of the other PAH) were below the maximum value for edible oils (2 μg kg^{-1}) suggested by the EU-SFC [11] except for rapeseed, pumpkin, and sesame oils. Smoking procedures increase B[*a*]P concentrations in food. In all the samples of smoked food investigated the concentration of B[*a*]P did not exceed the maximum amount permitted (European Regulation EC 208/2005, of 4th February 2005, set a limit of 2 μg kg^{-1} for oils and fats intended for direct human consumption and 5 μg kg^{-1} for

Table V

Concentrations (μg kg^{-1}) of PAH in different liquid and solid food samples

Sample	B[*a*]A	Ch	B[*b*]F	B[*k*]F	B[*a*]P	dBA	BP
Liquid samples – edible oils							
Coconut crude oil	62.6	105.3	55.1	12.1	40.6	nd	15.8
Coconut refined oil	nd	nd	nd	0.09	0.10	nd	nd
Rape-seed (cold pressed)	4.70	5.57	5.56	2.17	4.35	0.46	4.05
Universal (refined rape-seed)	0.16	0.61	0.48	0.22	0.24	0.15	1.35
Olive	0.49	1.97	0.97	0.40	0.69	0.08	1.42
Sunflower	nd	0.44	0.22	0.15	0.27	nd	0.60
Soya-bean	0.60	2.10	0.91	0.56	0.98	0.20	nd
Grape-seed	0.35	1.69	0.35	0.26	0.51	nd	1.09
Flax	0.62	0.87	0.56	0.20	0.45	nd	1.09
Pumpkin	0.11	0.82	0.57	0.16	0.46	nd	1.02
Peanut	5.05	5.20	3.97	1.59	3.11	0.30	2.46
Sesame	5.53	6.14	3.83	1.47	3.06	0.29	2.35
Whale oil	nd	nd	nd	nd	0.09	nd	nd
Solid food samples							
Rape-seed	5.28	4.47	3.62	1.66	2.73	0.21	4.25
Smoked sausage	nd	0.43	nd	0.17	0.29	nd	nd
Smoked white cottage cheese	1.31	1.16	0.71	0.23	0.77	0.10	0.60
Smoked sprats	21.5	18.4	11.6	5.20	12.0	nd	4.20
Milk powder	0.12	0.49	0.20	0.12	0.28	nd	0.62
Hens egg white	0.07	nd	nd	nd	nd	nd	nd
Egg yolk	0.12	nd	nd	nd	nd	nd	0.36

smoked food). Refining, in contrast, reduces the concentration of PAH, as is apparent from the results for the crude and refined coconut oils. Especially interesting was that the concentration of B[*a*]P in edible oils obtained by cold pressure, without further purification, exceeded the maximum value proposed by the EU directive [11]. Typical chromatograms obtained from two different samples, both liquid (whale oil) and solid (smoked sausage) are shown in Fig. 5. For both samples the assay performed well, enabling nearly baseline separation of PAH.

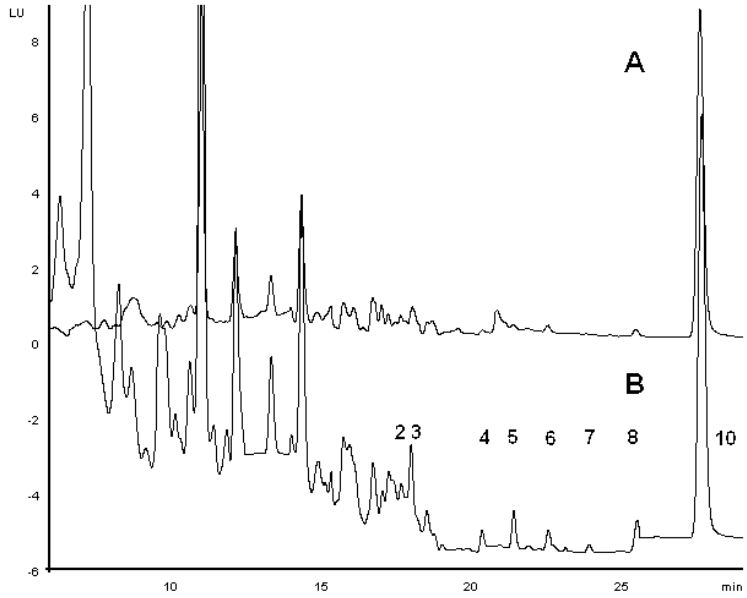

Fig. 5

HPLC chromatogram obtained from PAH in whale oil (A) and smoked sausage (B). PAH and chromatographic conditions as for Fig. 3

CONCLUSIONS

A rapid SEC method is proposed for isolation and purification of PAH from different foods. Of the three clean-up procedures investigated only SEC enables one-step clean-up. This clean-up procedure enables the assay to be applied to all types of edible oil and solid food product. The proposed method is selective and sufficiently sensitive for determination of PAH in different food matrices. The assay is, therefore, suitable for routine analysis.

This study emphasizes the use of fluorescence spectra to evaluate peak purity and for qualitative analysis.

It was found that the concentration of B[*a*]P in edible oils obtained by cold pressure, without further purification, exceeds maximum levels proposed by the EU directive [11].

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