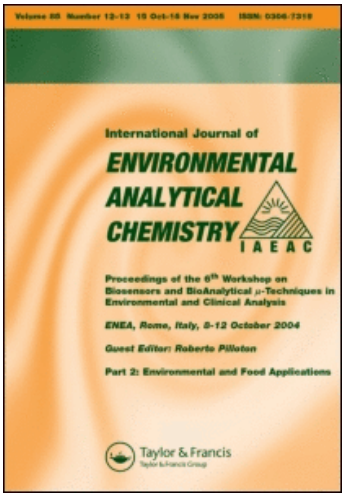


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Simplified Extraction and Cleanup Procedure for the Determination of PAHs in Fatty and Protein-Rich Matrices

K. Cejpek[a]; J. Hajšová[a]; Z. Jehllčková[a]; J. Merhaut[a]
[a] Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague 6, Czech Republic

To cite this Article Cejpek, K. , Hajšová, J. , Jehllčková, Z. and Merhaut, J.(1995) 'Simplified Extraction and Cleanup Procedure for the Determination of PAHs in Fatty and Protein-Rich Matrices', International Journal of Environmental Analytical Chemistry, 61: 1, 65 — 80
To link to this Article: DOI: 10.1080/03067319508026237
URL: http://dx.doi.org/10.1080/03067319508026237

Intern. J. Environ. Anal. Chem., Vol. 61, pp. 65–80
Reprints available directly from the publisher
Photocopying permitted by license only

SIMPLIFIED EXTRACTION AND CLEANUP PROCEDURE FOR THE DETERMINATION OF PAHs IN FATTY AND PROTEIN-RICH MATRICES

K. CEJPEK, J. HAJŠLOVÁ, Z. JEHLIČKOVÁ and J. MERHAUT

Institute of Chemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic

(Received, 7 February 1994; in final form, 16 January 1995)

A simplified analytical procedure for the determination of 12 priority polycyclic aromatic hydrocarbons (phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(ghi)perylene and indeno(1,2,3-cd) pyrene) in meat products and other biological materials has been developed. As a first step, ultrasonic extraction with chloroform for isolation of analytes was used. Gel permeation chromatography on Bio-Beads S-X3 utilising chloroform as mobile phase was applied to remove interferences (lipids, pigments etc.). HPLC with fluorescene detection was employed for quantitation of analytes. Recoveries at a μg/kg spiking level ranged from 53% (phenanthrene) to 112% (benzo(k)fluoranthene) with relative standard deviations in the range of 15% (benzo(k)fluoranthene) to 49% (anthracene).

KEY WORDS: Polycyclic aromatic hydrocarbons, smoked-meat products, fats, oils, gel permeation chromatography, HPLC, fluorescence detection.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants, a certain part of which represents the largest class of known environmental carcinogens. PAHs are mostly formed by air-deficient combustion of organic matter at temperatures in the range 500–900°C[1,2].

An exposure of humans (non-smokers) to PAHs occurs mainly via the intake of contaminated food. The levels of PAHs in ambient air and/or water are usually by one to two orders of magnitude lower.[3] Contamination of foods is mainly due to the environmental pollution. Thanks to some technological processes and cooking procedures, PAHs can also occur at higher levels in smoke- and heat-processed food.[4] Occasionally, some types of food additives or packaging materials contain PAHs as well.[5]

The determination of PAHs in such complex matrices as food samples is a difficult task because of the large number of PAHs and their occurrence at very low concentration levels. Moreover, the analysis is complicated by the need for protecting the analytes against photooxidative degradation. Attention should also be paid to the adequate sampling procedures[6,7] and to the storage of the samples[8–11].

The procedures used in a routine PAH analysis of biological matrices are time-consuming and cumbersome. Isolation and enrichment of PAHs commonly involve the following steps: alkaline digestion, several liquid/liquid extractions and one or two column cleanup procedures. An application of alcoholic saponification is considered mandatory as the first step of PAH analysis of insoluble fats and protein-rich foods (e.g., meat, fish, bacon and cheese) for the release of PAHs bound to lipid components of the matrix[12–14]. After the extraction of the hydrolysed sample, cleanup and enrichment are usually carried out by partitioning of PAHs between a polar aprotic solvent (e.g., DMF, DMSO) and a non-polar extraction solvent. The application of adsorption chromatography on silica gel and Florisil and/or size exclusion chromatography on Sephadex LH-20 gel is the most frequent way of further cleanup and PAH group separation. The concise survey of various isolation purification steps used for PAH determination in protein-rich foodstuffs is summarised in Table 1.

In principle, the requirements for the extent of purification depend on the chromatographic techniques used for identification and quantitation of the analytes. The use of HPLC for PAH analysis is advantageous especially in connection with selective fluorescence detection. In comparison with the HPLC-FLD method, the GC-MS determination has the same or slightly lower sensitivity but a higher confidence of the quanlitative results[24–26]. In this paper we present a new rapid method for the PAH determination in fatty and protein-rich food utilising direct extraction, simple cleanup of the extract, and HPLC-FLD method.

EXPERIMENTAL

Chemicals and experimental materials

Chloroform and acetone (p.a., Lachema Brno, Czech Republic) and water (ARTES, Aqua Artes Káraný, Czech Rep.) were purified by distillation in glass before use. Methanol (J. T. Baker, HPLC grade) and acetonitrile (J. T. Baker, HPLC 200) were used as supplied. Sodium sulphate—anhydrous, purum, was heated to 500°C for 5 h and stored in a tightly capped bottle (Lachema Brno, Czech Rep.). Potassium hydroxide (KOH) pellets were reagent grade (Lachema Brno, Czech Rep.). Glass wool was washed with chloroform just before use. All the glassware was washed with detergent and rinsed with distilled water and acetone before use.

The investigated samples, namely edible table (mixed) oil, rape oil, sunflower oil, lard, smoked mackerel, boiled pork meat (as a control blank) and smoked sausage (produced in a commercial plant), were purchased from a local food retailer. Smoked pork meat was produced in a private traditional smoke-house.

Standard compounds

A mixture of 16 US EPA priority pollutant PAHs—acenaphtene [Ace], acenaphtylene [Acy], anthracene [Ant], benz(a)anthracene [B(a)A], benzo(a)pyrene [B(a)P], benzo(b)fluoranthene [B(b)Fla], benzo(ghi)perylene [B(ghi)Per], benzo(k)fluoranthene [B(k)Fla], chrysene [Ch], dibenz(a,h)anthracene [DB(ah)A], fluoranthene [Fla], fluorene [Flu], indeno(1,2,3-cd)pyrene [I(cd)P], naphtalene [Nap], phenanthrene [Phe] and pyrene [Pyr] dissolved in methanol: dichloromethane (1:1, v/v) at concentrations of

Table 1 A survey of analytical procedures used for PAH determination in protein- and/or fat-rich food.

	Isolation and pre-concentration of analytes						
Samples	*Hydrolysis*	*Extraction*	*Cleanup I*	*Partition*	*Cleanup II*	*Identintification and quantitation**	*References*
food samples (non-specified)	alcoholic KOH	isooctane	Florisil/isooctane and benzene, add hexadecane and isooctane (benzene removal)	DMSO, water, isooctane	TLC - cellulose/isooctane-DMFA-diethyl ether and cellulose acetate/ethanol-toluene-water	UV spectrometry, spectrofluorometry	12
frankfurters cheese, fish	ethanolic KOH with sodium sulphate (in Soxhlet apparatus)	isooctane	Florisil/isooctane and benzene (hexadecane)	DMSO, water, isooctane	1.Florisil/benzene, 2.paper chromatogr./isooctane 3.TLC-cellulose acetate /ethanol-toluene-water	UV spectrometry, spectrofluorometry	13
meat, poultry, fish, yeast	methanolic KOH	cyclohexane	–	DMFA, water, cyclohexane	1.silica gel/cyclohexane 2.Sephadex LH20/isopropanol	GLC-FID (OV-101)	14
fish, shellfish	ethanolic KOH	n-hexane	–	–	silica gel/n-hexane	HPLC-FLD (Radial-Pak PAH column, eluent MeCN-water)	15
fish, shellfish, meat products	methanolic KOH	cyclohexane	Florisil/toluene	DMSO, water, hexane	–	HPLC-FLD (RP-18 Spheri 5, MeCN-water) GC-FID, GC-MSD (DB-5)	16
fish	aqueous KOH (acidification of the digestate)	cyclopentane--dichloromethane	–	–	1.potassium silicate and silica gel. 2.Bio Beads S-X3/all in cyclopentane-dichloromethane	GC-PID (DB-5)	17
food (market basket)	ethanolic KOH (in presence Na_2S)	n-hexane	–	–	Sep-Pak silica cartridge/ n-hexane	HPLC-FLD (Radial-Pak 5 PAH 10, MeCN-water)	18
smoked fish and sausage	methanolic KOH	cyclohexane	–	aqueous Na_2WO_4, cyclohexane	Florisil/cyclohexane	HPLC-FLD (Separon SGX C-18, MeCN-water)	19
shellfish	ethanolic KOH	isooctane	–	–	Bio-Beads S-X3/ cyclohexane-dichloromethane	HPLC-FLD (Vydac 201 TP54 C18, MeCN-water)	20
seafood	ethanolic KOH	1,1,2-trichlorotri fluoroethane	–	–	SPE on alumina, silica and C_{18}	HPLC-FLD (Vydac 201 TP54 C18, MeCN-water)	21
mussel, oyster	–	dichloromethane	silica-alumina/ dichloromethane	–	Phenogel 100-A/ dichloromethane	GC-FID (DB-5)	22
smoked meat, bacon and fish	ethanolic KOH	cyclohexane, water	–	–	silica gel/cyclohexane	HPLC-FLD (Lichrosorb RP18, MeCN-water)	23

* FID - flame-ionisation detector
PID - photo-ionisation detector
FLD - fluorescence detector
MSD - mass selective detector

100–2000 μg/ml was supplied by Supelco, USA. 3-methylcholanthrene [3-MeCh] (96%, Fluka, Switzerland) and coronene [Cor] (98%, Aldrich, Germany) were available as solids.

Apparatus

A kitchen meat grinder, a homogenizer (Ultra-Turrax TR–50, Germany), an ultrasonic bath (Tesla, Czech Rep.), and a rotary vacuum evaporator (Laboratorní přístroje, Czech Rep.) were used during the isolation steps.

The GPC (gel permeation chromatographic) system consisted of a HPLC pump HPP 4001 (Laboratorní přístroje, Czech Rep.), a six-port valve 7125 Rheodyne, CA, with 2 ml loop, and a stainless steel column, 50 cm × 0.8 cm i.d., (Tessek, Czech Rep.) filled with Bio-Beads S-X3, 200–400 mesh (Bio-Rad Laboratories, U.S.A.).

The liquid chromatograph was composed of a Hewlett-Packard 1050 Series Pumping System, a HP 1050 Series Autosampler, a HP 1046 A Fluorescence Detector, a HP 35900 A/D Interface and a HP ChemStation, version B.01.02.

Isolation

Five different isolation procedures for separation of PAHs from smoked-meat-product samples were investigated (Table 2):

Procedure A. Hydrolytic method according to Grimmer and Böhnke[14] (see Table 1 - hydrolysis and extraction).

Procedure B. Hydrolytic method according to Šimko[19] (see Table 1).

Procedure C. Extraction procedure with chloroform. 50–100 g of sample were ground in a meat grinder and 10 g of the representative sample were mixed and homogenised thoroughly in a beaker with 100 ml of chloroform. The homogenizer knives were rinsed with chloroform and the rinses were transferred to the beaker, covered with an

Table 2 Descriptions of tested procedures.

Procedure code	*Isolation*	*Cleanup*	*Determination*	*Reference*
A	MeOH/KOH hydrolysis, cyclohexane extraction	Bio-Beads S-X3 chloroform (GPC)	HPLC-FLD	14*
B	MeOH/KOH hydrolysis, cyclohexane extraction	Na_2WO_4 solution, Florisil/cyclohexane	HPLC-FLD	19
C	chloroform, sonication	Bio-Beads S-X3 chloroform (GPC)	HPLC-FLD	proposed procedure
D	chloroform:methanol (2:1, v/v), sonication	Bio-Beads S-X3 chloroform (GPC)	HPLC-FLD	28*
E	MeOH, sonication	Bio-Beads S-X3 chloroform (GPC)	HPLC-FLD	–

* for isolation step only.

aluminium foil, and put into an ultrasonic bath for 15 min at dark. The chloroform extract was carefully filtered through glass wool into a 250 ml round-bottom flask. 50 ml of chloroform were added to the extracted particles in the beaker and both sonication and filtration procedures were repeated twice. The combined extracts were evaporated on rotary vacuum evaporator (40°C) to about 3 ml and transferred quantitatively into a 10 ml graduated vessel.

Procedure D. Extraction with chloroform : methanol (2:1, v/v) (according to Folch[28]). The mixture of chloroform : methanol (2:1, v/v) instead of chloroform for ultrasonic extraction step was used. The combined extracts in a 250 ml round-bottom flask were transferred into 500 ml separating funnel. 80 ml water were added and the mixture was shaken for 2 min. After separation the lower layer was drained and concentrated as described in procedure *C*.

Procedure E. Extraction with methanol. Methanol instead of chloroform was used for ultrasonic extraction step. The extracts were filtered through the funnel with 5 g of anhydrous Na_2SO_4 and concentrated just to dryness. The solid residue was dissolved in 3 ml of chloroform and the solution was transferred quantitatively into a 10 ml graduated vessel.

Cleanup

The below cleanup step was performed for the extracts obtained by all investigated isolation procedures except for that of the procedure *B* - in this case the cleanup was performed according to the original procedure[19].

The GPC pump flow rate was set up on 0.7 ml/min and the column was let to equilibrate for 10 min. The extract was injected by all-glass syringe into 2 ml loop and eluted with chloroform at maximum pressure 0.6 MPa. The first 16.5 ml of eluate were discarded. The next 8.5 ml were collected and evaporated in 50 ml flask nearly to dryness. The chloroform residue was let to evaporate spontaneously. After immediate dissolving of dry residue in 0.2 ml acetonitrile, the solution was transferred into an amber vial and capped. The maximum fat (extract) amount that can be loaded without any risk of the column separation efficiency reduction is 350 mg.

HPLC determination

The HPLC-FLD equipment and analytical column Supelcosil LC PAH (150 mm × 4.6 mm, 5 µm) with pre-column Supelguard LC-18-DB (20 mm × 4.6 mm, 5 µm) were used under following conditions: gradient elution [A - MeOH : MeCN : H_2O (50:25:25, v/v/v), B - MeCN; 0 min – 100% A, 1 min – 100% A, 25 min – 100% B], injection volume 20 µl, column temperature 40°C. FLD timetable is shown in Table 3.

RESULTS AND DISCUSSION

Isolation

In our experiment we have applied an efficient direct extraction step utilising a suitable organic solvent to avoid the time-consuming procedure with a hydrolytic step. According

to earlier studies (see Table 1), alkaline alcoholic hydrolysis was claimed to be necessary for the release of PAHs from the protein-rich fatty matrices. Grimmer and Böhnke[14] documented this fact in their experiments: in contrast to methanolic saponification, low recovery (approx. 30%) was obtained when boiling methanol was used for extraction of analytes from smoked fish. These low recovery values were assumed to be caused by PAH affinity to the high molecular structures which were not destroyed and/or dissolved by boiling methanol.

The affinity of hydrophobic PAH molecules to non-polar and/or less polar components of biological material was assumed and the physical and chemical properties of both PAHs and analysed matrix were taken into consideration. The choice of a suitable extraction solvent for direct extraction of PAHs together with lipids came from our experience with the determination of lipid content in meat and related products. Several methods for total lipid determination are usually used. A rapid microwave - solvent extraction AOAC official method with dichloromethane is recommended for crude fat determination in meat products[27]. Another common procedure by Folch[28] utilises a mixture of chloroform : methanol (2:1, v/v) for more efficient extraction of lipids and lipoproteins.

Chloroform *(in procedure C)*, chloroform : methanol (2:1, v/v) *(in procedure D)*, and methanol *(in procedure E)* were used as tested extraction solvent systems. The penetration of solvents into the homogenised (disintegrated) sample was enhanced by sonication. The procedures *(C,D,E)* were tested on the extraction efficiencies of lipid compounds in meat samples (Table 4). Extraction efficiencies of chloroform *(procedure C)* for both lipids (Table 4) and PAHs (Table 6) in smoked meat were evidently higher than those achieved by methanol *(procedure E)* presumably due to the limited solubility of lipid structures in methanol. The extracted portions of lipids and PAHs obtained by a chloroform : methanol (2:1, v/v) mixture *(procedure D)* were higher and lower, respectively, than those achieved by pure chloroform *(procedure C)*. This could be caused by PAH losses during the liquid-liquid partition and/or by lower affinity of PAHs to extraction mixture. Thus chloroform was chosen as the most appropriate solvent for direct PAH extraction in meat and meat products as well as the solvent for simple dissolving of oils and fats prior to cleanup step.

The comparison of PAH contents' values achieved by aforementioned isolation procedures *(C,D,E)* with the data obtained from tested procedures including hydrolytic steps[14,19] *(A,B)*, is summarized in Tables 5a, 5b and 6. The chloroform extraction *(procedure C)* efficiency for PAHs was comparable with those values obtained by the standard hydrolytic method by Grimmer[14] *(procedure A)* - see Table 6. The purification step of the latter method was replaced by a simplified procedure with known parameters (GPC on Bio-Beads S-X3) due to the lack of detailed technical instructions in the

Table 4 Extraction efficiencies of investigated solvent systems for smoked sausage sample.

Extraction solvent	*Procedure code*	*Extracted "lipids" portion*	
		Average (% of sample weight)	*RSD (% of average)*
chloroform	C	26	8
chloroform:methanol (2:1, v/v)	D	37	5
methanol	E	6	14

Table 5a Characterisation of isolation procedure employing chloroform for extraction *(procedure C)*.

PAH	*Control blank* (boiled pork meat)*		*Recovery (control blank spiked on 2–4 μg/kg)*		*Sample – smoked sausage**	
	Average (μg/kg)	*RSD (%)*	*Average (%)*	*RSD (%)*	*Average (μg/kg)*	*RSD (%)*
Phe	(0.4)	58	53	36	21	29
Ant	n.d.	–	57	49	2.7	26
Fla	(1.0)	22	77	43	14	7
Pyr	(0.4)	40	88	26	17	6
B(a)A	n.d.	–	108	22	3.6	11
Ch	n.d.	–	104	25	2.0	10
B(b)Fla	n.d.	–	107	22	1.4	21
B(k)Fla	n.d.	–	112	15	0.57	26
B(a)P	n.d.	–	88	18	1.0	20
DB(ah)A	n.d.	–	91	22	n.d.	–
B(ghi)Per	n.d.	–	109	17	0.2	45
I(1,2,3-cd)P	n.d.	–	111	32	n.d.	–

n = 4, n.d. – not determined, *no correction for recovery.

Table 5b Characterisation of isolation procedure employing alkaline hydrolysis *(procedure B)*.

PAH	*Control blank* (boiled pork meat)*		*Recovery (control blank spiked on 2.6–5.2 μg/kg)*		*Sample – smoked sausage**	
	Average (μg/kg)	*RSD (%)*	*Average (%)*	*RSD (%)*	*Average (μg/kg)*	*RSD (%)*
Phe	4	25	57	44	12	33
Ant	0.4	70	70	34	1.6	–
Fla	0.8	37	72	24	10	10
Pyr	0.9	55	56	23	11	9
B(a)A	0.2	80	92	20	2.7	7
Ch	0.1	100	84	18	1.7	12
B(b)Fla	n.d.	–	43	16	1.0	20
B(k)Fla	0.02	60	46	17	0.25	33
B(a)P	n.d.	–	45	12	0.5	35
DB(ah)A	n.d.	–	44	18	n.d.	–
B(ghi)Per	n.d.	–	58	16	n.d.	–
I(1,2,3-cd)P	n.d.	–	56	26	n.d.	–

n = 4, n.d. – not determined, *no correction for recovery.

method description[14]. The comparison of our proposed procedure *(C)* with another hydrolytic one *(B)* was done in close co-operation with the author[19]. The efficiency of the direct extraction with chloroform was slightly higher than that of the procedure *B*. Blank samples of our procedure were better quality than those of the hydrolytic procedure *B* (Tables 5a and 5b).

Table 6 The comparison of PAH contents in smoked sausage and in smoked pork meat isolated by various extraction procedures.

PAH	*Content of PAHs determined by means of procedure:* A µg/kg	C µg/kg	C µg/kg	D µg/kg	E µg/kg
	Smoked pork meat		*Smoked sausage*		
Phe	140	133	21	26	12
Ant	48	57	2.7	3.1	0.8
Fla	216	263	15	14	5
Pyr	273	242	18	16	12
B(a)A	13.7	11.8	3.6	2.3	1.3
Ch	26.2	25.4	2.0	1.7	0.6
B(b)Fla	16.6	18.3	1.4	0.7	0.6
B(k)Fla	3.41	2.80	0.57	0.40	0.10
B(a)P	4.7	5.2	1.0	0.5	0.3
DB(ah)A	1.3	1.2	n.d.	n.d.	n.d.
B(ghi)Per	5.0	6.2	0.2	0.2	n.d.
I(1,2,3-cd)P	2.7	3.8	n.d.	n.d.	n.d.

n = 5, n.d. – not determined.

Cleanup

Direct extraction of both lipids and analytes (PAHs) is conditioned by the application of purifying step(s) which separate(s) analytes from lipids, pigments, and other undesired co-extracts, and therefore adsorption and/or gel permeation chromatography (GPC) are usually employed. The latter technique is a more advantageous alternative for the removal of interfering compounds with higher molecular weight.

Up to now most applications of GPC for PAH analyses in biological matrices had to resolve the purification of complex extract obtained after tissue hydrolysis. Sephadex LH-20 with mostly alcoholic eluent has been most frequently described[14,29–31]. We employed the system consisting of the widely used styrene-divinylbenzene copolymer Bio-Beads S-X3 with chloroform as a mobile phase (exclusion limit approx. 2000 Da). One of the reasons for applying this arrangement was good experience in its application for the separation of fat and PCBs in our laboratory[32]. More perfect conjugation of π-electrons in PAH molecules in comparison with PCBs gives better assumption for stronger retention of PAHs and consequently their better separation on styrene-divinylbenzene gel matrix with high conjugation of π-electrons.

The GPC elution curves for various lipids both of plant and animal origin are shown in Figure 1. The elution profiles were very similar and no tailing was recorded. Theoretically, a wide variety of products could be formed from the heat-treated oil (esp. unsaturated) as a result of polymerisation and oxidation, however, minimum changes in chromatographic behaviour occurred under the used GPC conditions (Figure 2). The elution curves of PAHs are illustrated in Figure 3. The maxima of elution bands for most of these compounds occurred between 19–21 ml. The earlier elution of 3-MeCh can be attributed to the lower extent of conjugation of π-electrons within its molecule and consequently to the weaker interaction with gel. The last eluted compound was coronene displaying the highest degree of π-electrons' conjugation of tested analytes. Generally, it can be assumed than not only pure "size exclusion mechanism" controls the elution of PAHs, but the adsorption phenomena (π-π interactions with gel) also significantly

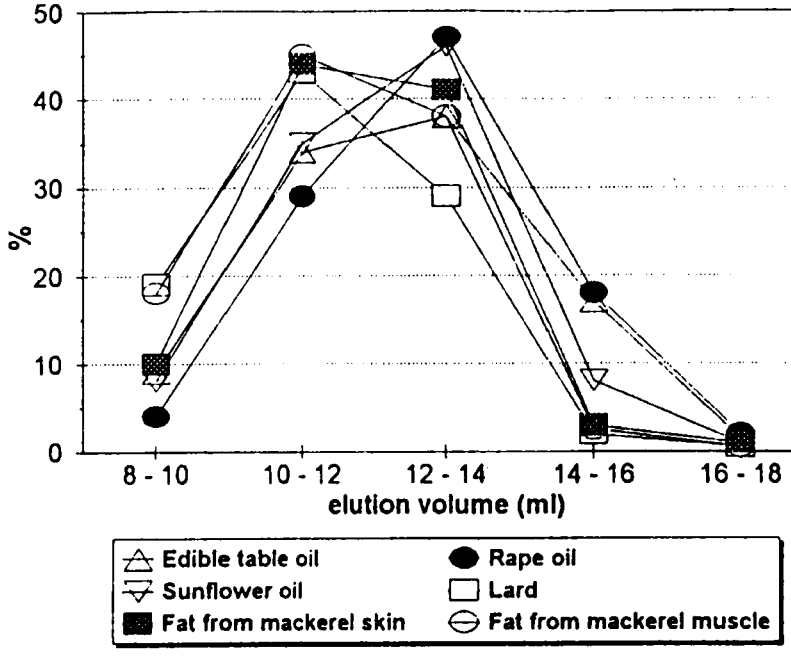


Figure 1 GPC elution profiles of various lipids.

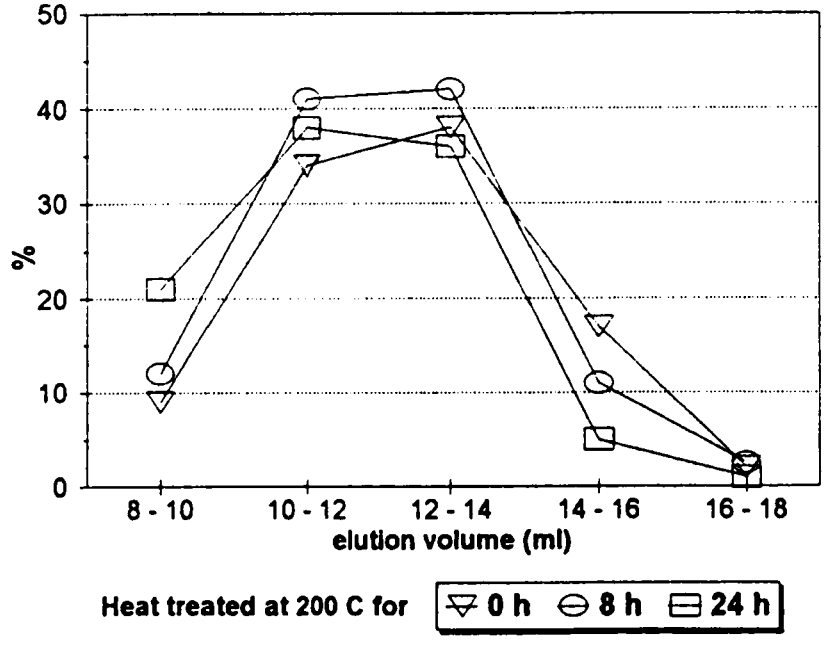
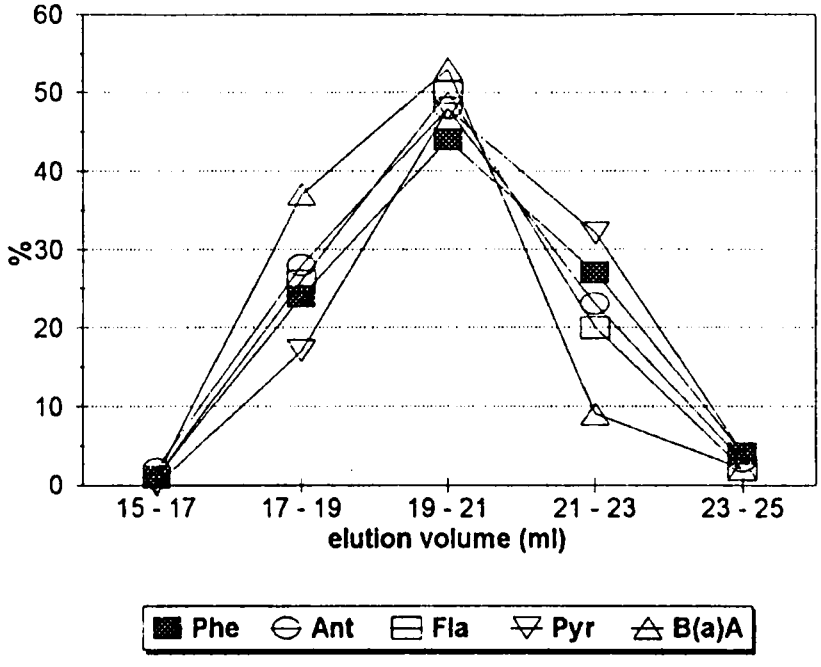


Figure 2 GPC elution profiles of heat-treated rape oil.



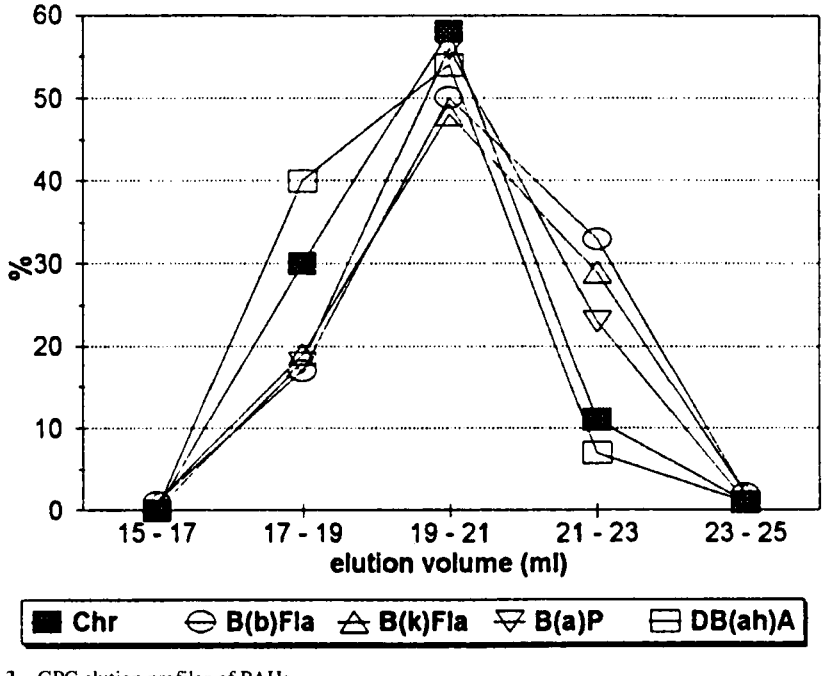


Figure 3 GPC elution profiles of PAHs.

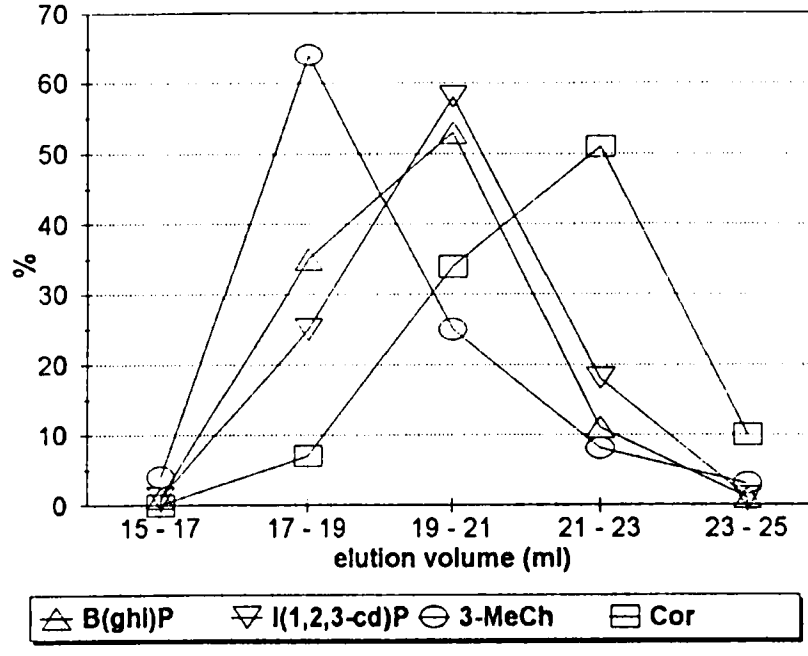


Figure 3 *(cont.)* GPC elution profiles of PAHs.

contributes to the increased retention of analytes. The use of a less polar mobile phase than chloroform would enhance this effect[33,34]. Fernandez *et al.*[33] studied the PAH behaviour on Bio-Beads S-X12 with THF as a mobile phase. In this system (size exclusion limit approx. 400 Da, relatively polar solvent) a strong size exclusion mechanism predominates for the cata-condensed PAHs while the peri-condensed ones are eluted according to adsorption principles. Klimisch *et al.*[34], who studied the application of GPC on Bio-Beads S-X8 (also with THF as a mobile phase) for the purification of extracts from environmental samples, drew a similar conclusion. Musial *et al.*[20] employed a less polar mobile phase, i.e. cyclohexane-dichloromethane (1:1, v/v) for the purification of shellfish-hydrolysate extract on Bio-Beads S-X3 after hydrolysis (see Table 1).

The recovery of PAHs in the course of GPC operation is shown in Table 7. The losses during substeps of the GPC step (viz. separation process on column and chloroform evaporation from collected fraction) were evaluated. It is evident that there are no losses during the actual separation process. Thus the source of the losses of more volatile and photooxidation sensitive PAHs during the GPC step is definitely the chloroform evaporation substep.

Identification and quantitation

HPLC analysis with fluorescence detection is one of the most appropriate methods for PAH determination in biological matrices. The optimisation of both separation and detection parameters of the method was performed. The application of column with polymeric C18 layer along with suitable mobile phase made possible separations of

Table 7 Recoveries of the GPC cleanup procedure (10–20 ng PAHs loaded on column).

PAH	*Molecular weight*	*Recovery of complete GPC step**		*Recovery of evaporating substep*	
	g/mol	*Average (%)*	*RSD (%)*	*Average (%)*	*RSD (%)*
Phe	178	75	22	69	32
Ant	178	66	31	68	36
Fla	202	92	28	92	26
Pyr	202	104	21	103	23
B(a)A	228	108	19	110	29
Ch	228	110	23	111	18
B(b)Fla	252	90	17	98	18
B(k)Fla	252	106	11	108	11
B(a)P	252	107	14	105	19
DB(ah)A	278	99	22	104	23
B(ghi)Per	276	109	12	111	11
I(1,2,3-cd)P	276	112	27	114	16

n = 5, *recoveries of both separation process on column and evaporating substep are included.

critical pairs (and/or groups) of analytes (mostly isomers), e.g. benz(a)anthracene - chrysene, benzo(a)fluorene - benzo(b)fluorene, benzo(k)fluoranthene - benzo(a)pyrene, benzo(e)pyrene - perylene - benzo(b)fluoranthene - benzo(j)fluoranthene and dibenz(a,h)anthracene - benzo(ghi)perylene[35,36]. Supelcosil LC PAH column and a MeCN : MeOH : H_2O gradient elution were employed to separate US EPA PAH priority pollutants (Figure 4). Other columns used by us, such as Lichrospher PAH 250–4 (Merck), display similar qualities of separation[35,36]. The column separation selectivity was sufficient for analysed PAHs as well as for co-extracts (Figure 5a and Figure 5b). Naphtalene, acenaphtene, acenaphtylene, and fluorene were not determined because low recoveries were achieved due to their high volatilities. The optimum fluorescence

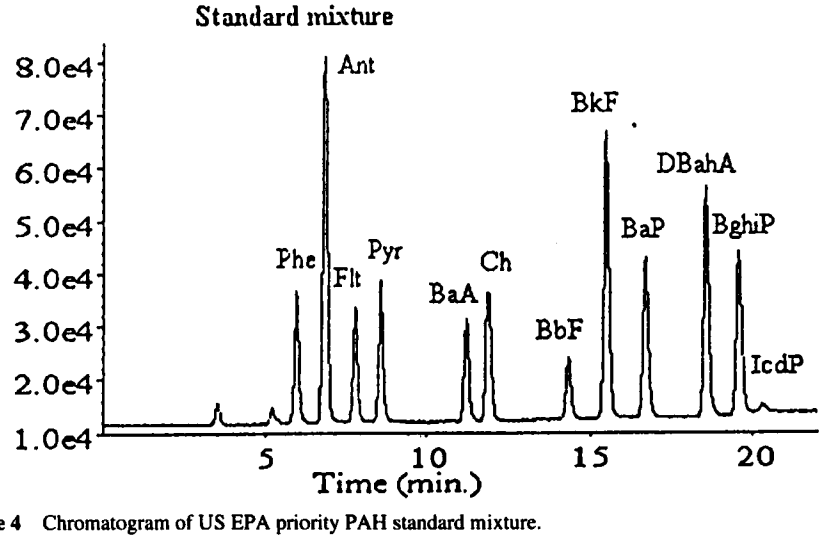


Figure 4 Chromatogram of US EPA priority PAH standard mixture.

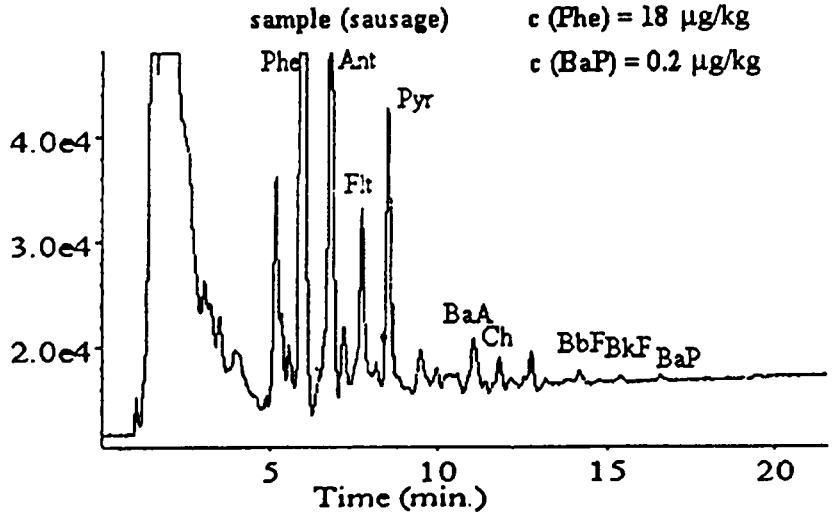


Figure 5a Chromatogram of purified extract of sausage with PAH content typical for meat products made by commercial technology with controlled smoking process.

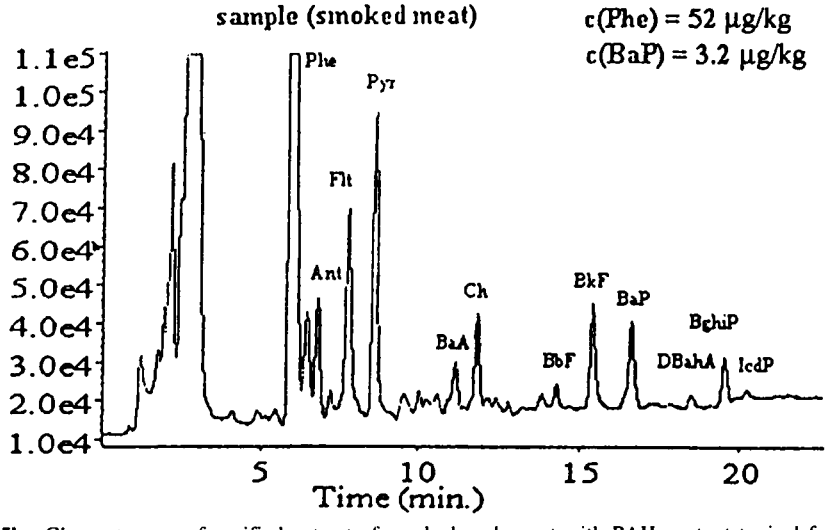


Figure 5b Chromatogram of purified extract of smoked pork meat with PAH content typical for meat products made in simple traditional smoke-house (without smoke cleaning).

parameters were determined and compromised for individual groups of analytes (Table 3).

Responses for analysed PAHs within the tested range 0.02–40 ng were linear. The linearity range corresponds to 0.1–200 µg/kg of individual PAH in sample when samples are processed by the proposed procedure *(C)*. One of the most important detection characteristics is the minimum detectable amount of PAH analysed under given

Table 3 Excitation and emission wavelengths used for PAH detection.

λ(exc)	*λ(em)*	*Detected analytes*
280 nm	370 nm	(Nap)
302 nm	330 nm	(Ace, Flu)
248 nm	374 nm	Phe, Ant
232 nm	420 nm	Fla, Pyr
264 nm	384 nm	B(a)A, Ch
300 nm	410 nm	B(b)Fla, B(k)Fla, B(a)P, DB(ah)A, B(ghi)Per
290 nm	484 nm	I(cd)P

conditions of the separation and detection systems. It is defined as the amount of separated standard material producing a response of mean gradient blank plus 3 standard deviations (Table 8). The limits of detection (L.O.D) for the analysed PAHs are also shown in Table 8. Those data are based on definition of L.O.D. as the analyte concentration the response of which is equivalent to the mean control blank response plus 3 standard deviations. Limits of quantitation (L.O.Q.) are given in Table 8. They are generally considered as 10 standard deviations above the average blank signal. A spiked control blank sample (boiled pork) was used for better evaluation of PAH recoveries. The obtained recovery data are acceptable at given levels of the analytes (Table 5a). Unfortunately, there are no available standard reference materials of the aforementioned foodstuffs' character with certified PAH contents. The only commercially prepared SRM of biological origin is the mussel tissue (e.g., NIST SRM 1974[11]).

The simplification and time-shortening of the sample processing procedure *(C)* prior to the HPLC analysis reduces the risk of PAH losses with respect to the photooxidative decomposition, volatility, and sorption capabilities of PAHs. The proposed procedure does not include any time-consuming hydrolysis and any adsorption chromatography with higher risk of PAH degradation[18]. The losses initiated by the light are of little importance, especially when the protection by aluminium foil during sonication, filtration and storage of sample is provided. The main losses can be attributed to PAH volatility and/or degradability occurred during solvent removing step.

Table 8 Analytical and statistical parameters of the developed procedure *(C)*.

PAH	*Minimum detectable amount**	*Limit of detection*	*Limit of quantitation*	*Method blank contents*	
				(μg/kg)	
	pg	*μg/kg*	*μg/kg*	*Average*	*RSD*
Phe	25	1	2	0.3	70
Ant	3	0.05	0.1	n.d.	–
Fla	30	1	3	0.8	37
Pyr	20	1	2	0.3	60
B(a)A	10	0.2	0.5	n.d.	–
Ch	10	0.2	0.5	n.d.	–
B(b)Fla	5	0.1	0.2	n.d.	–
B(k)Fla	1	0.02	0.05	n.d.	–
B(a)P	2	0.03	0.1	n.d.	–
DB(ah)A	5	0.05	0.2	n.d.	–
B(ghi)Per	3	0.03	0.1	n.d.	–
I(1,2,3-cd)P	10	0.1	0.2	n.d.	–

n.d. – not determined *in 20 μl aliquot injected into HPLC-FLD system.

CONCLUSIONS

A simplified and rapid analytical procedure for the determination of priority PAHs in protein-rich fatty products and oils using direct extraction with chloroform by sonication and GPC as purification step was developed. The HPLC-FLD system with a tailor-made PAH C18 column and programmable wavelengths' detector was employed.

Based on experimental results, the essential analytical and statistical parameters of the method were obtained. The accomplished comparative measurements confirmed that the extraction efficiency of standard procedures and the procedure proposed by us were comparable. The comparison with standard reference material was not carried out due to the lack of certified SRM related to the above food products. Our latest preliminary data indicate that our extraction and purification procedures will be also suitable for PAH determination in leafy plants, crops and vegetables.

Acknowledgements

This project was supported under a grant No. 509/93/2483 from the Grant Agency of Czech Republic. Special thanks also go to Dr. P. Šimko from Department of Food Chemistry and Analysis, Slovak Technical University, for his participation in the comparison study of various isolation procedures.

References

1. M. Zander, in: *Handbook of Polycyclic Aromatic Hydrocarbons*, (A. Bjorseth, ed., Marcel Dekker, New York, 1983), Vol. 1, Chapter 1., p. 1.
2. T. Vo-Dinh, in: *Chemical Analysis of Polycyclic Aromatic Compounds*, (T. Vo-Dinh, ed., John Wiley, New York, 1989), Chapter 1, p. 1.
3. J. Santodonato, Ch. A. Menzie and B. B. Potocki, *Environ. Sci. Technol.*, **26**, 1278–1284 (1992).
4. B. K. Larsson, G. P. Sahlberg, A. T. Eriksson and L. A. Busk, *J. Agric. Food. Chem.*, **31**, 867–873 (1983).
5. T. Fazio and J. W. Howard, in: *Handbook of Polycyclic Aromatics Hydrocarbons*, (A. Bjorseth, ed., Marcel Dekker, New York, 1983), Vol. 1, p. 461.
6. L. H. Keith (Ed.), *Principles of Environmental Sampling*, (ACS Professional Reference Book, Washington, DC, 1988), 458 pp.
7. L. H. Keith, *Environmental Sampling and Analysis: A Practical Guide*, (Lewis Publishers, Chelsea, Michigan, 1991), 144 pp.
8. W. Karcher, in: *Mobile Source Emissions Including Polycyclic Organic Species*, (D. Rondia *et al.*, eds., D. Reidel Publishing Company, 1983), pp. 127–149.
9. W. Karcher, *Reference Materials for the Analysis of PAHs*, in: *Handbook of PAHs*, (A. Bjorseth *et al.*, eds., Marcel Dekker, New York and Basel, 1985), Vol. 2, pp. 385–406.
10. Certificate of Analysis, *Standard Reference Material 1647, Priority Pollutant Polynuclear Aromatic Hydrocarbons (in Acetonitrile)*, (National Bureau of Standards, Gaithersburg, MD, 1990).
11. Certificate of Analysis, *SRM 1974 Organic in Mussel Tissue*, (National Bureau of Standards, Gaithersburg, MD, 1989).
12. *Official Methods of Analysis of the* AOAC, (Arlington, VA. 1990), 15th ed., pp. 1176–1178.
13. J. W. Howard, R. T. Teague, Jr., R. H. White and B. E. Fry, *J. Assoc. Off. Anal. Chem.*, **49**, 3, 595–611 (1966).
14. G. Grimmer and H. Böhnke, *J. Assoc. Off. Anal. Chem.*, **58**, 4, 725–733 (1975).
15. K. Takatsuki, S. Suzuki, N. Sato and I. Ushizawa, *J. Assoc. Off. Anal. Chem.*, **68**, 945–949 (1985).
16. J. E. Lawrence and D. F. Weber, *J. Agric. Food Chem.*, **32**, 789–797 (1984).
17. J. A. Lebo, J. L. Zajicek, T. R. Schwarz, L. M. Smith and M. P. Beasley, *J. Assoc. Off. Anal. Chem.*, **74**, 538–544 (1991).
18. K. Tamakawa, T. Seki and A. Tsunoda, *Food Sci. Technol.*, **52**, (Food Anal. HPLC), 697–711, (1992).
19. P. Šimko, *Food Chemistry*, **40**, 293–300 (1991).
20. Ch. J. Musial and J. F. Uthe, *J. Assoc. Off. Anal. Chem.*, **69**, 462–466 (1986).

21. G. A. Perfetti, P. J. Nyman, S. Fisher, F. L. Joe, Jr. and G. W. Diachenko, *J. Assoc. Off. Anal. Chem.*, **75**, 872–877 (1992).
22. M. M. Krahn, L. K. Moore, R. G. Bogar, C. A. Wigren, S.-L. Chan and D. W. Brown, *J. Chromatogr.*, **437**, 161–175 (1988).
23. T. Stijve and C. Hischenhuber, *Dtsch. Lebensm.-Rdsch.*, **83**, 276–282 (1987).
24. S. A. Wise, L. R. Hilpert, G. D. Byrd and W. E. May, *Polycyclic Aromatic Compounds*, **1** (1–2). 81–98 (1990).
25. M. J. Dennis, R. C. Massey, D. J. McWeeny, B. Larsson, A. Eriksson and G. Sahlberg. *J. Chromatogr.*, **285**, 127–133 (1984).
26. P. G. Sim, R. K. Boyd, R. M. Gershey, R. Guevremont, W. D. Jamieson, M. A. Quilliam and R. J. Gergely, *Biom. Environ. Mass Spectrometry*, **14**, 375–381 (1987).
27. *Official Methods of Analysis of the AOAC*, (AOAC, Arlington, VA, 1990), 15th ed., p. 932.
28. J. Folch, M. Lees and G. H. Sloane Stanley, *J. Biol. Chem.*, **226**, 497–509 (1957).
29. J. F. Lawrence, *Int. J. Environ. Anal. Chem.*, **24**, 113–131 (1986).
30. M. Novotny, M. L. Lee and K. D. Bartle, *J. Chromatogr. Sci.*, **12**, 606–612 (1974).
31. K. D. Bartle, in: *Food Contaminants Sources and Surveillance*, (C. Creaser and R. Purchase, eds., The Royal Society of Chemistry, Cambridge, 1988). p. 46.
32. J. Hajšlová and J. Poustka, *Application note A 16*, (Tessek, Praha, 1991).
33. P. Fernández, C. Porte, D. Barceló, J. M. Bayona and J. Albaigés, *J. Chromatogr.*, **456**, 155–164 (1988).
34. H. J. Klimisch and D. Reese, *J. Chromatogr.*, **67**, 299–307 (1972).
35. K. Cejpek, J. Hajšlová and Z. Jehličková, *Application note "Determination of PAHs in Environmental Samples" CZ Chrom 1/94*, (Merck Ltd., Praha, 1994).
36. K. Cejpek, J. Hajšlová and Z. Jehličková, *Application note "Determination of PAHs in Foods" CZ Chrom 2/94*, (Merck Ltd., Praha, 1994).