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Review

Polycyclic aromatic hydrocarbons in food and beverages. Analytical methods and trends

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are compounds widespread in the environment, many of them showing carcinogenic effects. These compounds can reach the food chain by different ways and, therefore, the analysis of PAHs in food is a matter of concern. This article reviews the extraction methodologies together with the separation and detection techniques which are currently applied in the determination of PAHs in food and beverages. Specific extraction conditions, performance characteristics, chromatographic and detection parameters are discussed. A review of the occurrence of these compounds in the matrixes under study is also provided.

Contents

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) or polyarenes constitute a large class of organic compounds (about 10,000 substances) characterized by a structure made up of carbon and hydrogen atoms forming two or more fused aromatic rings without any heteroatom or substituent (the most important compounds are shown

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in Table 1). The compounds containing five or more aromatic rings are known as "heavy" PAHs, whereas those containing less than five rings are named "light" PAHs. Both kinds of PAHs are non-polar compounds showing high lipophilic nature, although heavy PAHs are more stable and toxic than the other group.

PAHs are ubiquitous environmental contaminants which are widespread in the air bonded to particulate matter. In spite of PAHs show hydrophobic properties (especially heavy PAHs), they are also found in water. These compounds are produced during a variety of combustion and pyrolysis processes from anthropogenic and natural sources. A high amount of PAHs are emitted from processing coal, during incomplete combustion of organic matter (e.g. wood and fossil fuels), from motor vehicle exhaust and cigarettes [1,2]. Forest fires, volcanoes or hydrothermal processes are natural emission sources of PAHs [3].

A number of PAHs are considered as genotoxic carcinogens, and biological and mutagenic effects have also been reported [3]. Other PAHs not defined as carcinogens may act as synergists [2]. The occurrence of PAHs in the environment is therefore a cause of concern since humans are exposed to these compounds, for instance, by the air. However, one of the major routes of human exposure to PAHs in non-smoking people is food; for smokers, the contribution of cigarette smoke may be similar [4]. Food can be contaminated by PAHs present in the environment, i.e. PAHs can accumulate on the waxy surface of many vegetables and fruits [4]. Indeed, the presence of PAHs in uncooked food, such as vegetables, seeds and grains, has been demonstrated [5]. These products do not take up significant amounts of PAHs from the soil, but from air particles [2] through deposition of contaminated matter. Nevertheless, other studies show contradictory results about the possibility of vegetables to take PAHs from soil and water and metabolize them [6]. Another example of possible PAH contamination in food is due to traffic, i.e. crops or livestock close to urban roads could be exposed to PAHs and nitro-PAHs (derivates from PAHs) [5]. Other food products, such as seafood and fish, can be exposed to PAHs present in water and sediments and the PAH content greatly depends on the ability of the aquatic organisms to metabolize them (e.g. bivalve mollusks accumulate more PAHs than vertebrate fish, which metabolize these compounds very rapidly).

On the other hand, PAHs can be found in food products as a consequence of certain industrial processing methods, such as smoking, heating (grilling, roasting) and drying, which permit the direct contact between food and combustion products; these are important sources of PAH contamination for seeds, edible oils, and meat and dairy products [4]. In edible oils, the oilseed drying processes by direct combustion can be an important source of contamination in a variety of vegetable oils [7].

Furthermore, the use of smoke flavoring products (SFP), which are utilized to improve organoleptic characteristics, has increased in food industry. Since SFP are produced from smoke condensates, they are another significant source of PAHs in food. In food industry, materials as polyethylene are normally used. This material is effective in lowering PAH load from a contaminated food, but an opposite effect can be observed when using recycled polyethylene film in oil packaging since it could contaminate vegetable oils with PAHs by rediffusion [8].

In general, PAHs are not present individually but in mixtures. PAHs that have been extensively monitored are the compounds included in the United States Environmental Protection Agency (US-EPA) list of priority organic pollutants (the so-called 16 EPA PAHs) [9]. Since 2005, the European Union (EU) list of PAHs [10] (15 compounds) has also been included in the monitoring studies [2] (Table 1). Benzo[*a*]pyrene (BaP) is probably the most studied PAH. The International Agency for Research on Cancer (IARC) described BaP as probable human carcinogen in 1987 [11]. Thus, the determination of BaP has been widely used in environmental analysis as marker for the entire PAH content. Maximum permitted concentrations in foodstuffs for BaP have been established by the EU [12], as well as methods of sampling and analysis in order to perform official controls of this compound [13]. However, BaP contributes only with 1–20% of the total content of PAH, and other aromatic compounds can be present as well [1]. In this sense, in 2002, the Scientific Committee on Food (SCF) of the European Commission considered that despite the use of BaP as a marker of occurrence and carcinogenic effect of the PAH content in food, it suggested that this evaluation should be accompanied by additional analysis of other PAHs in order to establish a PAH contamination profile in food commodities [14]. In 2007, the European Food Safety Authority (EFSA) pointed out that the supposition that BaP was a good indicator of any PAH contamination was uncertain [4]. The SCF noticed that a number of derivatives of PAHs, such as nitro-PAHs and oxygenated PAHs, as well as heterocyclic aromatic compounds (e.g. acridine, carbazole) can be generated by incomplete combustion or reactions in air [14]. Consequently, the determination of all these compounds in food is also an issue of concern.

The monitoring of other PAHs has been strongly recommended by the EU [10]. The EFSA also remarked that food categories such as herbs and spices, food supplements, coffee, tea and herbal infusions and other cereal and grain beverages are not covered by existing legislation [1,2,4,12]. Later, in 2008, the EFSA established that BaP is not a suitable indicator for the occurrence of PAHs in food and that occurrence data for benzo[*c*]fluorene (BcF) are needed [15]. It is important to notice that this compound is not included in either the EPA or the EU list of PAHs.

Therefore, the need for reliable data about the concentration of PAHs in food is increasing in order to establish new maximum permitted levels. In this sense, analytical laboratories play an important role since they must have adequate methods for the analysis of PAHs and their derivatives in food.

This review shows an overview of the analytical methodologies applied in the determination of PAHs in food and beverages (e.g. edible oils, smoked foodstuffs, milk or infusions), including recent approaches. The main techniques applied in the extraction and clean-up of the extracts and in the detection and quantification of the analytes have been reviewed, focusing on liquid chromatography (LC) coupled to fluorescence (FLD) and ultraviolet–visible (UV) detection and gas chromatography (GC) coupled to mass spectrometry (MS). New trends based on instrumental analysis and recent extraction techniques, some of them applied in other fields of food safety and environmental analysis, have been pointed out.

The review covers the relevant literature published since the year 2000 and certain previous references highly cited. The previous articles have already been revised and discussed elsewhere [7,8,16].

2. Sample preparation: extraction and clean-up

As general precautions to be considered when determining PAHs, it is important to protect the solutions against light since these compounds are light sensitive and they can decompose by photoirradiation and oxidation [17]. Thus, light exposure during the sample pre-treatment has to be carefully controlled [17,18]. Besides, concentration to dryness should be avoided in order to diminish possible losses due to evaporation of the lower molecular weight compounds [17].

2.1. Liquid matrices

2.1.1. Fatty matrices

It is well known that one of the main difficulties in the analysis of fatty matrices is due to their high fat content (e.g. lipids, triglycerides, fatty acids) [19]. For this reason, the extraction of PAHs from

Table 1
Summary of the most important PAHs analyzed in food.

Compound	Abbreviation	Structure	Molecular weight (amu)	Boiling point (°C)	CAS No.
Acenaphthene[a]	ACP		154	279	83-32-9
Acenaphthylene[a]	ACY		152	280	208-96-8
Anthracene[a]	ANT		178	340	120-12-7
Benz[*a*]anthracene[a,b]	BaA		228	438	56-55-3
Benzo[*b*]fluoranthene[a,b]	BbFA		252	N.A.	205-99-2
Benzo[*k*]fluoranthene[a,b]	BkFA		252	N.A.	207-08-9
Benzo[*ghi*]perylene[a,b]	BghiP		276	>500	191-24-2
Benzo[*a*]pyrene[a,b]	BaP		252	495	50-32-8
Chrysene[a,b]	CHR		228	448	218-01-9
Dibenzo[*a,h*]anthracene[a,b]	DBahA		278	524	53-70-3
Fluoranthene[a]	FA		202	384	206-44-0
Fluorene[a]	FLR		166	298	86-73-7
Indeno[1,2,3-*cd*]pyrene[a,b]	IP		276	N.A.	193-39-5
Naphthalene[a]	NPH		128	218	91-20-3
Phenanthrene[a]	PHE		178	340	85-01-8
Pyrene[a]	PYR		202	404	129-00-0
Benzo[*j*]fluoranthene[b]	BjFA		252	N.A.	205-82-3
Cyclopenta[*cd*]pyrene[b]	CPcdP		226	N.A.	27208-37-3
Dibenzo[*a,e*]pyrene[b]	DBaeP		302	N.A.	192-65-4
Dibenzo[*a,h*]pyrene[b]	DBahP		302	N.A.	189-64-0
Dibenzo[*a,i*]pyrene[b]	DBaiP		302	N.A.	189-55-9
Dibenzo[*a,l*]pyrene[b]	DBalP		302	N.A.	191-30-0
5-Methylchrysene[b]	MCH		242	N.A.	3697-24-3

N.A.: Data not available.
[a] 16 EPA priority PAHs [9].
[b] UE PAHs of concern in food [10].

these complex matrices is usually a laborious and time-consuming stage (Fig. 1). The removal of lipidic material is important not only to minimize the maintenance of the chromatographic system (especially when using GC) but also to reach low detection limits (LODs). The need for high sensitivity is justified by the low concentrations of PAHs fixed as maximum levels permitted in current legislation [1,2,8,12,20].

Extraction of PAHs from foodstuffs has traditionally relied on a three-stage methodology including saponification, liquid–liquid extraction (LLE) and clean-up by column chromatography or, more recently, solid-phase extraction (SPE).

One of the most studied fatty commodities is edible oils since they can be exposed to PAHs by the use of heating processes (e.g. during solvent evaporation) [21] or solvent extraction dur-

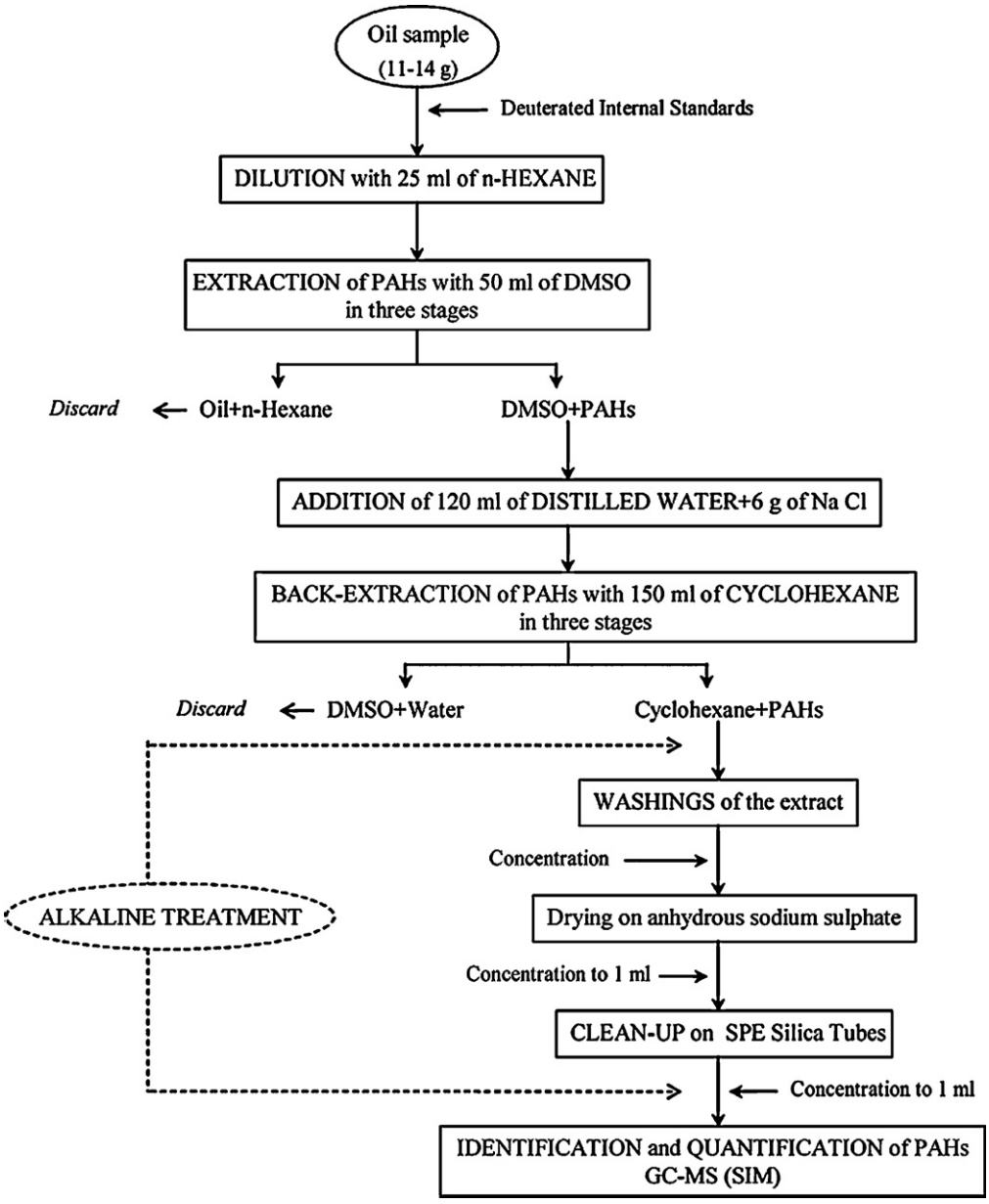

Fig. 1. Example of a scheme of a methodology employed for the extraction of PAHs in olive pomace oil samples, an example of fatty matrix. Reprinted from [22] copyright 2004, with permission from American Chemical Society.

ing their production. Besides, the drying of the raw material (e.g. seeds) with combustion gases before oil extraction generates high amounts of PAHs [22]. The reported methods in bibliography described the use of two general strategies for the sample extraction and clean-up. The first strategy involves the dilution of the sample, LLE and a subsequent clean-up by SPE [17,23,24]; the other general methodology carries out a single SPE-stage after the sample dilution [22,23,25,26]. The dilution step is normally performed with *n*-hexane in order to modify the partition coefficients [8]. A variety of solvents are used in LLE, but the most common are dimethylsulfoxide (DMSO) and cyclohexane (Table 2). For the clean-up by SPE, very different cartridges have been utilized, such as C_{18}/C_8, aminopropyl, silica and polystyrene/divinylbenzene (PS-DVB) sorbents. For SPE (as extraction method), C_{18}/Florisil mixtures [21] and PS-DVB [26] have been used. Some authors have described PS-DVB sorbents as extremely selective to PAHs, considering this material as suitable to most of food matrices [27].

Recently, humic acid-bonded silica has been proposed as a new sorbent for the extraction of PAHs by SPE using BaP as example [25]. The retention of PAHs in this sorbent is based on charge-transfer and hydrophobic interactions. Nevertheless, additional applications of this SPE material have not been found.

The performance of a saponification step prior to the LLE is also described in order to reduce the lipidic content (e.g. triacylglicerols), for instance using mixtures of KOH or NaOH solutions containing ethanol or methanol [1,7,8,17]. However, losses of BaP have been reported by partial portioning to the alcoholic phase when using this procedure [17], and other authors suggested that saponification could negatively affect the most labile compounds [8].

The formation of caffeine complexes with PAHs prior to LLE has been also reported by mixing the sample with a caffeine:formic acid solution, although it is not currently applied. The complexes are then decomposed by extracting with an aqueous sodium chloride solution [7,8].

Table 2
Summary of extraction and clean-up methods in the discussed matrices.

Matrix	Extraction	Clean-up	Separation/ detection	Recovery (%)	RSD (%)	Ref.
Liquid fatty matrices						
Edible oils	Dilution (*n*-hexane); LLE (2 × DMF/water, 9:1, v/v)	SPE (C_{18}/C_8)	LC–FLD	50–103	Intra-day: 3–6 Inter-day: 5–2	[23]
Edible oils	Dilution (*n*-hexane)	(A) DACC column (Varian ChromSpher 5π, 80 × 3 mm i.d., 5 μm) (B) Column chromatography (alumina)	LC–FLD	(A) 88–105 (B) 67–103	(A) 3–8 (B) 3–8	[28]
Edible oils	Dilution (*n*-hexane); SPE (silica)	–	LC–FLD	32–151	1–17	[22]
Edible oils	Dilution *n*-hexane; SPE (Humic acid-bonded silica)	–	LC–FLD	79–103	Intra-day: 1–9 Inter-day: 3–9	[25]
Edible oils	Dilution (*n*-heptane); LLE (4 × 20 mL DMSO); LLE (3 × 50 mL cyclohexane); LLE (2 × 100 mL water)	Column chromatography (silica gel + Na_2SO_4, 200 × 22 mm)	LC–FLD	58–99	N.A.[a]	[29]
Edible oils, fat	Dilution (isohexane:butyldimethylether,)%:5, v/v); SPE (PS-DVB)	–	LC–FLD	60–95	N.A.	[26]
Edible oil, smoked meat	(A) Smoked meat: Saponification (10 mL KOH 2 M in EtOH:water, 9:1, v/v, 1 h); LLE (2 × 2 mL cyclohexane) (B) Oil: Addition 15 mL cyclohexane; LLE (15 mL DMF:water, 9:1, v/v); LLE (15 mL water); LLE (2 × 15 mL cyclohexane)	SPE (aminopropyl, C_{18})	GC–MS	(A) 60–134	(A) N.A.	[17]
Olive oil	(A) SPE (C_{18} Nucleoprep+Florisil) (B) MSPD (C_{18}+Florisil)	–	GC–MS, LP-GC–MS, LC–FLD	(A) 77–79 (B) 55–66	(A) 4–6 (B) 8–11	[21]
Olive oil	Dilution (25 mL *n*-hexane); LLE (50 mL DMSO); addition 120 mL water + 6 g NaCl; LLE (3 × 150 mL cyclohexane); LLE (100 mL water)	SPE (silica) Optional: Soxhlet (100 mL MeOH:water, 8:2, v/v + KOH, 4 h); LLE (3 × 150 mL cyclohexane)	GC–MS	52–80[b]	N.A.	[24]
Olive oil	HS-SPME (DVB/Car/PDMS 50/30 μm)	–	GC–MS	74–28	Intra-day: 3–16 Inter-day: 1–14	[33]
Olive oil	HS	–	GC–MS(-MS)	96–99	3–9	[32]
Oil, food mixture	PLE (celite + Florisil, *n*-hexane:acetone, 1:1, v/v)	SPE (PS-DVB)	GC–MS/MS	12–70 (in food by isotopic dilution)	3–21 (in food)	[27]
Olive, olive-pomace oil	SLE or LLE (ACN/*n*-hexane, 83:17, v/v)	GPC (styrene-divinylbenzene copolymer, 5 mL min^{-1} CH_2Cl_2)	GC–MS/MS	84–110	3–8	[31]
Olive pomace oil	Dilution (25 mL *n*-pentane); LLE (15 mL, 2 × 10 mL DMSO); addition 70 mL water; LLE (3 × 50 mL cyclohexane); LLE (100 mL water)	TLC (silica gel)	GC–MS	69–98	4–13	[18]
Vegetable oils	Dilution (*n*-hexane); SPME (Carbopack Z/PDMS, 15 μm)	–	GC–MS	N.A.	Intra-day: 2–5 Inter-day: 2–6	[34]
Vegetable oils	Dilution (*n*-hexane); SPME (Carbopack Z/PDMS, 15 μm)	–	GC × GC–MS	N.A.	3–35	[35]
Fish oil, fish	Homogenization (Na_2SO_4); saponification (10 mL methanolic KOH 1 M, 3 h); LLE (2 × 8 mL *n*-hexane)	SPE (Florisil)	GC–MS(/MS)	64–124	1–37	[95]
Fish oil, dried plants	(A) Fish oil: SLE with rotary agitator (3 × CH_2Cl_2/cyclohexane, 1:1, v/v); centrifugation (B) Dried plants: dilution (cyclohexane)	(A), (B) DACC column (Varian Chromspher π, 80 × 3 mm i.d., 5 μm)	LC–FLD	74–120	Intra-day: 2–4 Inter-day: 4–11	[30]
Milk	HS-SPME (PDMS-DVB)	–	GC–MS	90–113	5–15	[39]
Milk	Dilution (water), SPME (PDMS-DVB)	–	GC–MS	88–112	<20	[40]
Milk	Addition sodium oxalate; LLE (250 mL MeOH); LLE (250 mL diethyl ether); LLE (250 mL petroleum ether)	Column chromatography (silica gel)	GC–MS	40–125	N.A.	[36]
Milk	LLE (20 mL cyclohexane:ethyl acetate, 1:1, v/v); centrifugation	SPE (styrene-divinylbenzene copolymer Envi Chrom); addition 2 mL cyclohexane + 2 mL MeOH:water (80:20, v/v); centrifugation; LLE (2 mL cyclohexane); centrifugation PAHs: Cyclohexane fraction; saponification (5 mL KOH 10%, 90 °C, 80 min); addition 3 mL water + 5 mL cyclohexane; centrifugation Hydroxi-PAHs: MeOH layer; LLE (4 mL water:ethyl acetate, 1:1, v/v); centrifugation	GC–MS (Derivatization)	N.A.	N.A.	[37]

Table 2 (*Continued*)

Matrix	Extraction	Clean-up	Separation/ detection	Recovery (%)	RSD (%)	Ref.
Milk	Saponification (4 mL NaOH 0.4M in EtOH:water, 9:1, v/v, 60 °C, 30 min); LLE (2 × 2 mL *n*-hexane)	–	LC–FLD	90–105	Intra-day: 4–10 Inter-day: 7–10	[38]
Liquid non-fatty matrices						
Coffee	LLE (*n*-hexane)	SPE (silica)	LC–FLD	87–103	5–8	[41]
Coffee brew	SPE (BondElut PPL polystyrene-divinylbenzene)	–	LC–FLD	84–89	1–6	[44]
Coffee	MIP-SPE	–	LC–FLD	Coffee: 73	Coffee: 5	[47]
Tea	SPE (C_{18})	–	LC–FLD	44–103	3–17	[45]
Tea infusion	(A) HS-SPME (PDMS-DVB 60 μm) (B) SPME (Confirmation GC–MS, PDMS-DVB 65 μm)	–	LC–FLD, GC–MS (Confirmation)	N.A.	4–16	[48]
Mate tea	SBSE: 10-mm bars coated with PDMS (0.5 mm), room temperature, 2 h, 160 μL ACN:water, 4:1, v/v (desorption)	–	LC–FLD	24–87	1–11	[49]
Beverages	Addition 10% MeOH; MASE (polypropylene, ethyl acetate)	–	GC–MS	65–92	Intra-day: 6–18 Inter-day: 10–18	[51]
Sugarcane juice	(A) SBSE-TD: Addition of NaCl; 10-mm bars coated with PDMS (0.5 mm), room temperature, 3 h (B) MASE: polypropylene, 800 μL cyclohexane	-	GC–MS	(A) 2 (B) 14	(A) 19 (B) 4	[50]
Cachaça (spirit)	LLE (50 mL, 2 × 25 mL DMF:water, 9:1, v/v); addition 100 mL Na_2SO_4 1%; LLE (50 mL, 2 × 35 mL cyclohexane)	Column chromatography (silica gel-15% water + Na_2SO_4, 200 × 10 mm)	LC–FLD	70–97	12–21[c]	[42]
Spirits	SPE (C_{18})	–	LC–FLD	82–113	1–9	[46]
Solid fatty matrices						
Meat	Freeze-drying; Soxhlet (25 mL KOH 25% + MeOH 200 mL, 3 h); addition *n*-hexane (150 mL); LLE (100 mL water), LLE (150 + 100 mL *n*-hexane); LLE (3 × 100 mL water)	SPE (Florisil)	GC–MS	62–91	4–16	[53]
Meat	(A) Freeze-drying; USE (*n*-hexane) (B) Soxhlet (25 mL KOH 50% + MeOH 200 mL, 3 h); addition *n*-hexane (150 mL); LLE (100 mL water), LLE (150 + 100 mL *n*-hexane)	(A), (B) SPE (Florisil)	LC–UV, LC–FLD	(A) 74–111 (B) 72–102	N.A.	[52]
Smoked meat	PLE (*n*-hexane, 100 °C, 10 MPa)	GPC (Bio-Beads S-X3, Cyclohexane:ethyl acetate, 1:1, v/v) SPE (silica)	GC–EI-MS	58–75	<20	[60]
Smoked meat	PLE (*n*-hexane, 100 °C, 10 MPa)	GPC (Bio-Beads S-X3, Cyclohexane:ethyl acetate, 1:1, v/v) SPE (silica)	GC–EI-MS	N.A.	N.A.	[61]
Smoked meat	Saponification (MeOH + KOH)	SPE (Florisil)	LC–UV, LC–FLD, GC–EI-MS	68–99	N.A.	[54]
Smoked meat	SPME-DED (PDMS 100 μm)	–	GC–MS	N.A.	5–18	[62]
Smoked meat	PLE (*n*-hexane, 100 °C, 100 bar)	GPC (Bio-Beads S-X3, 420 mm × 25 mm; cyclohexane:ethyl acetate, 1:1, v/v) Column chromatography (silica)	GC–MS	75–110	3–12	[59]
Smoked meat	MAE (*n*-hexane, 115 °C)	SPE (silica)	LC–FLD	77–103	1–10	[71]
Fish, smoked meat	(A) Pork: PLE (Supelclean LC-18 + Na_2SO_4, CH_2Cl_2:ACN, 90:10, v/v, 1500 psi, 100 °C) (B) Smoked meat and fish: PLE (C_{18} + Na_2SO_4, CH_2Cl_2:ACN, 90:10, v/v, 1500 psi, 100 °C)	LLE (2 × 1 mL H_2SO_4 9 M); LLE (water); column chromatography (Florisil, 6 g, 1 cm i.d.)	GC–MS	(A) 54–102 (B) 35–93	(A) 4–12 (B) 2–18	[58]
Fish	Homogenization (Na_2SO_4), Soxhlet (CH_2Cl_2:*n*-hexane, 1:1, v/v, 16 h)	Addition water + K_2CO_3 + acetic anhydride; dilution water; LLE (3 × 100 mL CH_2Cl_2); LLE (2 × 2 mL K_2CO_3); column chromatography (silica gel + Na_2SO_4); GPC (2 mL min^{-1} CH_2Cl_2)	GC–MS	N.A.	2.2–20.0	[67]
Fish	(A) Soxhlet (170 mL *n*-hexane:acetone, 1:1, v/v, 6 h) (B) PLE (*n*-hexane:acetone, 1:1, v/v, 100 °C, 10 MPa)	GPC (Bio-Beads S-X3, 500 mm × 8 mm; $CHCl_3$)	LC–FLD	N.A.	N.A.	[65]
Fish	HS-SPME (polyacrilate)	–	GC–MS	N.A.	N.A.	[74]
Fish	MAE (4 mL saturated KOH in MeOH + 10 mL *n*-hexane, 129 °C); centrifugation	SPE (Silica)	LC–FLD	86–98	1–5	[70]
Fish	Lyophilization; MSPD (C_{18} + Na_2SO_4)	Simultaneous SPE (Florisil + C_{18})	LC–FLD	80–105	2–6	[76]
Fish	Homogenization (Na_2SO_4), Soxhlet (150 mL, CH_2Cl_2, 16 h)	Column chromatography (silica gel + Na_2SO_4, 1 cm i.d.)	GC–MS	Fish: 70–118	<10	[64]

Table 2 (*Continued*)

Matrix	Extraction	Clean-up	Separation/ detection	Recovery (%)	RSD (%)	Ref.
Fish	QuEChERS method: SLE (ACN); vortex; induced partition ($MgSO_4$ + sodium acetate); centrifugation	–	LC–FLD	64–110	<8	[77]
Fish, seafood	Saponification (10 mL ethanolic KOH 1M, 3 h, 80 °C); addition 10 mL water; LLE (2 × 20 mL cyclohexane)	–	LC–FLD	41–67	3–18	[68]
Fish, seafood	MAE (15 mL acetone, 21 psi, 80% microwave power)	(A) SPE (Florisil) (B) GPC (Envirosep ABC, 350 mm × 21.2 mm, 5 mL min^{-1} CH_2Cl_2)	GC–MS	N.A.	N.A.	[73]
Fish, mussel	Dilution (NaCl solution 24%), HS-SPME (PDMS-DVB)	–	GC–MS	8–111	7–15	[75]
Fish, palm dates	Soxhlet (150 mL, *n*-hexane, 8 h)	Column chromatography (silica gel + Florisil + Na_2SO_4)	GC–MS	59–112	1–24	[66]
Shellfish	Freeze-drying; Soxhlet (CH_2Cl_2, 24 h)	GPC (Bio-Beads S-X3; $CHCl_3$) Column chromatography (aluminosilicate)	GC–MS	62–123	9–21	[63]
Mussel	Lyophilization; PLE (*n*-hexane:CH_2Cl_2, 1:1, v/v, 150 °C, 150 psi)	Saponification (25 mL KOH 6 M, ambient temperature, 24 h)	GC–MS	64–121	3–30	[69]
Cheese	Saponification (10 mL KOH ethanolic solution); addition water (10 mL); LLE (2 × 20 mL cyclohexane)	SPE (Isolute silica 500 mg)	LC–FLD	84–89	N.A.	[80]
Cheese	Soxhlet (Na_2SO_4 + 170 mL *n*-hexane: CH_2Cl_2, 1:1, v/v, 7 h)	GPC (Bio-Beads S-X3, CH_3Cl)	LC–FLD	52–94	9–34	[78]
Cheese	(A) Cheese: lipid extraction (N.A.); addition 30 mL cyclohexane; LLE (DMF:water, 9:1, v/v); LLE 30 mL cyclohexane	(A), (B) SPE (Silica)	LC–FLD	75–96	N.A.	[79]
Infant milk, infant cereals	(A) Parent PAHs: USE (3 × 10 mL *n*-hexane); centrifugation (B) Hydroxy-PAHs: USE (3 × 9/6/5 mL ACN:ethyl acetate, 70:30, v/v, containing 0.8 g/L tert-butyl hydroquinone); centrifugation; hydrolysis of conjugated PAHs (β-glucuronidase/aryl sulphatase)	(A) SPE (Silica) (B) SPE (C_{18})	(A) LC–FLD (B) LC–MS	Infant milk: 70–116 Infant cereals: 82–103	Infant milk: 4–9 Infant cereals: 2–7	[94]
Solid non-fatty matrices						
Bread, potato	(A) Bread: Addition 1 mL water; USE (3 mL ethyl ether:CH_2Cl_2, 1:1, v/v) (B) Potato: USE (as explained in (A))	–	LC–FLD, GC–MS (Confirmation)	70–86	4–11	[85]
Cane sugar	SLE (100 mL cyclohexane); LLE (50 mL, 2 × 25 mL DMF:water, 9:1, v/v); addition 100 mL Na_2SO_4 1%; LLE (50 mL, 2 × 35 mL cyclohexane)	Column chromatography (silica gel-15% water + Na_2SO_4, 200 × 10 mm)	LC–FLD	74–86	3–22[c]	[86]
Foodstuffs	Soxhlet (150 mL, CH_2Cl_2, 8 h)	Column chromatography (silica gel)	LC–FLD	70–10	Repeatability: <7 Reproducibility: <6	[88]
Food supplements	SLE with rotary agitator (3 × cyclohexane/CH_2Cl_2, 1:1, v/v + HF-M + alumina); centrifugation	Column chromatography (silica gel)	LC–FLD	63–116	N.A.	[100]
Fruits, vegetables	Saponification (100 mL KOH methanolic, 5 h); addition MeOH:water (100 mL, 9:1, v/v); LLE (2 × 150 mL cyclohexane); LLE (100 mL MeOH:water, 9:1, v/v; 100 water); LLE (N,N-dimethylformamide:water, 9:1, v/v)	Column chromatography (silica gel-15% water + Na_2SO_4, 200 × 10 mm)	LC–FLD, GC–MS	74–99	3–21	[84]
Ground coffee	PLE (*n*-hexane:acetone, 1:1, v/v; 150 °C)	Saponification (EtOH+KOH, 30 min) -LLE (100 mL cyclohexane); LLE (3 × 100 mL water) SPE (silica)	LC–FLD, GC–MS/MS, LC–UV	64–106	1–12	[87]
Tea leaves	USE (3 × 20 mL CH_2Cl_2:acetone, 1:1, v/v)	Column chromatography (silica)	LC–UV	>70	>20	[81]
Tea leaves	Soxhlet (CH_2Cl_2:acetone, 1:1, v/v, 18 h)	SPE (Florisil)	GC–EI-MS	N.A.	N.A.	[82]
Vegetables	Soxhlet (300 mL *n*-hexane:acetone, 1:1, v/v, 24 h)	SPE (Acid treated silica, aromatic sulfonic acid)	GC–MS	69–111	3–12	[83]

Abbreviations: ACN: acetonitrile; DACC: donor–acceptor complex chromatography; DMF: dimethylformamide; DMSO: dimethylsulfoxide; DVB: divinylbenzene; EtOH: ethanol; GC–MS: gas chromatography coupled to mass spectrometry; GC × GC–MS: multidimensional GC–MS; HF-M: modified diatomaceous earth; HS-SPME: headspace solid-phase microextraction; GPC: gel permeation chromatography; i.d.: internal diameter; LC–FLD: liquid chromatography coupled to fluorescence detection; LC–UV: LC coupled to ultraviolet–vis detection; LLE: liquid–liquid extraction; LP-GC–MS: low pressure GC–MS; MAE: microwave-assisted extraction; MASE: membrane-assisted solvent extraction; MeOH: methanol; MIP-SPE: molecularly imprinted polymers solid-phase extraction; MSPD: matrix solid-phase dispersion; PDMS: polydimethylsiloxane; PLE: pressurized-liquid extraction; PS-DVB: polystyrene/divinylbenzene; SBSE: stir bar sorptive extraction; SBSE-TD: SBSE-thermal desorption; SFE: supercritical fluid extraction; SLE solid–liquid extraction; SPE: solid-phase extraction; SPME: solid-phase microextraction; SPME-DED: SPME coupled to a direct extraction device; TLC: thin layer chromatography; USE: ultrasound extraction.

[a] N.A.: Data not available.

[b] Values corresponding to isotope labeled compounds.

[c] Coefficient of variation.

Column chromatography has also been applied as clean-up using alumina [28] and silica gel [29]. The utilization of donor–acceptor complex chromatography (DACC) for the clean-up of diluted oil samples is also described [28,30]. DACC is based on a strong π–π interaction produced between the sorbent and the PAHs; then, certain matrix components, such as neutral lipids and tocopherol, can be eluted by using a non-π-electron containing solvents. After that, the PAHs are eluted with an appropriate organic solvent that removes the interaction (e.g. *n*-hexane:tetrahydrofuran mixtures, acetonitrile). However, some problems can be found. The lightest PAHs (namely naphthalene (NPH), acenaphthylene (ACY), acenaphthene (ACP) and fluorene (FLR)) co-eluted with the fatty fraction and the use of more than 5% of tetrahydrofuran caused additional co-elution problems.

In general, the reported recoveries applying the methodologies commented above are quite similar, showing good relative standard deviation (RSD) values (<10%). However, the application of the saponification stage prior to an LLE and SPE clean-up can provide very high recovery (>120%) values for some compounds [17]. Despite the problems reported for the application of DACC, the recoveries reported for edible oils are slightly higher than the recoveries obtained using LLE and/or SPE (Table 2).

Gel permeation chromatography (GPC, also size exclusion chromatography, SEC) has been utilized after LLE operating in the normal phase mode (e.g. mobile phase: dichloromethane; stationary phase: styrene-divinylbenzene copolymer) [31]. This kind of chromatography has been extensively used for the purification of fatty extracts separating lipids from the analytes. Despite GPC is broadly applied in food analysis, its application in the reported methods for edible oils is scarce (Table 2). The reported recoveries are higher than those using other methodologies, although this procedure (LLE + GPC) was only carried out for the determination of medium molecular weight PAHs. GPC is a semi-automatic clean-up, which is an obvious advantage, but the solvent consumption is moderated, especially considering a typical flow of 5 mL min^{-1} and 30–40-min running times. Thus, about 150–200 mL of solvent per sample can be required, which could explain its low use.

Bogusz et al. [21] carried out a comparison between the performance of SPE and matrix-solid phase dispersion (MSPD) for the extraction of PAHs from olive oil. In the MSPD technique, a small amount of sample (typically 0.5 g) is mixed with a solid support (e.g. C_{18} material) in a mortar. Then, the mixture is transferred to an SPE reservoir and eluted in the same way as in SPE. The main advantage of MSPD is the low amount of solvent needed. Nevertheless, reproducibility problems are often observed, with medium-high RSD values. The extraction of PAHs with this technique is not an exception: MSPD provided lower recoveries and worse repeatability than the SPE procedure used. However, MSPD is simpler and faster than the LLE-based methods.

Due to the volatile character of certain PAHs, they have been also determined by head-space (HS) and solid-phase microextraction (SPME) techniques, namely, HS [32], HS-SPME [33] and SPME [34,35], with or without a previous dilution of the oil. HS-SPME has been applied for the analysis of PAHs showing a molecular weight ≤202 using a DVB/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber, which was chosen on the basis of its affinity for low/medium molecular weight compounds [33]. The recovery values were adequate, even for NPH, which is a problematic compound because of well-known losses during the evaporation stages in the extraction. This can be explained since the HS-SPME does not involve any evaporation or pre-concentration stage. However, the RSD values are higher in comparison to other extraction techniques. The application of SPME with direct immersion of the fiber in the oil has also been reported [34,35], using Carbopack/PDMS fibers and dilution of the oil. In the analysis of fatty commodities, one of the main problems when using SPME is matrix effect, causing a decrease in the fiber efficiency. In order to decrease the possible matrix effect, the fiber needs to be rinsed with *n*-hexane prior to desorption and injection in order to remove triglycerides residues on the surface of the fiber [34,35]. Purcaro et al. [35] described the use of an SPME method for the determination of the EU list of PAHs with low RSD values (<11%, except for BcF (16%) and cyclopenta[*cd*]pyrene (CPcdP) (35%)). It was observed that at high extraction times (>30 min), the extraction efficiency decreased for some compounds, due to a rising effect by the organic solvent used in the dilution. Desorption time was limited to 10 min to avoid broadening of the peaks of the later eluted compounds (heavy PAHs). As commented above, the SPME methodologies are limited with respect to precision. This is one of its drawbacks, the lack of reproducibility, together with a short lifetime of the fibers and possible carry-over problems. On the contrary, the use of HS/SPME technique reduces sample handling and minimizes solvent consumption.

Another fatty liquid matrix that has been monitored for PAHs is milk, but to a lesser extent. Although the fat content of this matrix is much lower than the fat percentage of edible oils, the methodologies used in the revised literature are quite similar (Table 2). LLE-based methods are therefore applied, but the number of LLE stages is more reduced [36–38]. Lutz et al. [37] described the analysis of PAHs and hydroxy-PAHs using the same extraction procedure involving LLE, SPE as clean-up and subsequent LLE steps for clean-up (different for each group). It is important to notice that any SPE procedure has been found for the extraction of PAHs from milk (Table 2). The use of saponification is also reported; the alcohol percentage was found to influence the extractability of the compounds, increasing the extractability but also the intensity of interfering peaks when working at 100% ethanol. HS-SPME [39] and SPME have been also applied [40]. In both studies, PDMS-DVB fibers were used; in the case of SPME, the sample was diluted with water prior to the extraction. The HS-SPME modality was also evaluated but it was not able to extract the high molecular weight PAHs [40]. In a later study, the HS-SPME mode was used for the extraction of PAHs containing up to four aromatic rings [39]. Similar recoveries were obtained, although better RSD values were found in the HS-SPME procedure. In comparison to the LLE-based methodologies, these two microextraction techniques provided an improvement in the recovery values.

More detailed information about extraction methods is shown in (Table 2).

2.1.2. Non-fatty matrices

The monitoring of PAHs has been carried out in a number of non-fatty liquid matrices, namely coffee, tea, alcoholic beverages and juice. In general, the extraction of PAHs from these commodities is performed by procedures less laborious than the protocols used for fatty matrices since the amount of lipidic material, and thus the possible matrix interferences, is much lower than in that group of matrices (Table 2).

The use of LLE with subsequent SPE clean-up (silica sorbent) has been reported in coffee brew [41]. The application of a single SPE stage was discarded because of clogging problems when passing instant coffee solutions through the SPE cartridge (C_{18}); the application of microwave-assisted extraction (MAE) was also ruled out due to stability problems observed for some PAHs. LLE and subsequent clean-up using column chromatography with silica gel has been recently applied for cachaça (Brazilian spirit) [42].

In the extraction of PAHs by SPE using reversed-phase or polymeric sorbents, some questions must be taken into account. Depending on the solvent used, adsorption problems related to PAH solubility can be found (e.g. adsorption onto the glassware walls). The addition of a small percentage (e.g. 10%) of an organic solvent

(i.e. methanol, acetonitrile or 2-propanol) to the sample can be used to increase solubility and minimize this adsorption. However, the optimization of the solvent percentage is a critical point since low percentages cannot improve the solubilization of the heavy PAHs, whereas a high percentage can reduce the breakthrough volume for the light PAHs [43].

SPE-based procedures have been used for the analysis of PAHs in coffee [44], but also in tea [45] and spirits [46] using different cartridges, such as PS-DVB and C_{18}. However, Houessou et al. [44] consider PS-DVB sorbents as the most suitable material for the extraction of PAHs from liquid samples due to π–π interactions that can increase the retention in comparison to C_{18} or silica sorbents, which do not show this type of interaction. Moreover, slightly better reproducibility was found when using PS-DVB cartridges instead of C_{18} sorbents for the analysis of coffee. The addition of methanol or acetonitrile to the sample has been described in order to minimize the adsorption of PAHs onto the glass and/or cartridge surfaces [44–46]. However, contradictory results concerning the methanol percentage have been reported: about 1% (v/v) of methanol has been described as the optimal amount in order to avoid competitive effects between PAHs and the solvent on the stationary phase [44] for tea samples, whereas higher percentages (10–20%, v/v) have been used in coffee samples and spirits [45,46]. Alternatively, SPE has been performed using molecularly imprinted polymers (MIP-SPE) for the extraction of BaP in coffee with adequate performance characteristics; the MIP-SPE sorbent was compared to C_{18}, obtaining better recovery values for the first approach [47].

LLE [42] and SPE [46] have been utilized for the analysis of spirits, obtaining similar recovery values but better RSDs when using SPE, probably due to the high number of LLE steps included in the first method. Moreover, the SPE-based methodology was applied for the analysis of a higher number of PAHs (15) in comparison with the LLE method (5).

HS-SPME has been applied for the analysis of PAHs in tea infusions; a variety of fibers were evaluated, including polyacrilate (polar), PDMS (non-polar), and PDMS-DVB (medium polarity). The optimal results were obtained using PDMS-DVB fibers [48]. Nevertheless, some drawbacks have been reported, such as overloading problems due to insufficient coating film of the fibers [49]. In this sense, two recent approaches described the application of stir bar sorptive extraction (SBSE) for the analysis of mate tea [49] and sugarcane juice [50]. In SBSE, the adsorption process occurs on bars and so the amount of coating film is higher, increasing the adsorption capacity in comparison with SPME and also minimizing the amount of co-extracted matrix material (Fig. 2). SBSE is currently considered as an environmentally friendly technique since it permits the reduction of solvent consumption to the minimum (no solvent required). SBSE with thermal desorption (SBSE-TD) is the solvent-free mode but it requires a special device in the chromatographic system to carry out the desorption of the analytes. As an alternative, the desorption process can be performed by using an appropriate solvent; in this case, the volume needed is still reduced (from a few microliters up to 1–2 mL). This modality has been used in mate tea samples with results comparable to LLE [49]; this study described the negative effect of the addition of NaCl to increase the ionic strength since the presence of salt provokes the transfer of the PAHs to the surface of the solution, minimizing the interaction with the sorbent on the bar: it is the "oil effect". The addition of methanol or acetonitrile to reduce adsorption onto the walls was discarded since any improvement was found. SBSE-TD was applied satisfactorily for the determination of only BaP in sugarcane juice [50]. Bearing in mind that the matrices were different, worse RSD values were obtained for BaP with this modality (19% by SBSE-TD and 4–6% by SBSE), which is an unexpected data since the SBSE-TD involves an automated desorption that should improve this parameter. The performance of SBSE-TD was compared to another environmentally friendly technique, membrane-assisted solvent extraction (MASE) [50], concluding that MASE provided better recovery and RSD values. MASE was also applied for the determination of PAHs in several beverages [51]; in this case, a higher number of compounds were monitored (16) and the addition of methanol was found adequate since it improved the enrichment of the compounds in the membrane bag. It is important to notice that the recovery of the more volatile PAHs (e.g. NPH) was more than acceptable, probably due to the fact that any evaporation stage was needed in this procedure.

More detailed information about extraction methods is shown in (Table 2).

2.2. Solid matrices

2.2.1. Fatty matrices

There are two food commodities that have been extensively monitored for PAHs, meat and fish (Table 2). The analysis of PAHs in meat, especially smoked meat, is due to the occurrence of these compounds after traditional or industrial smoking processes. Despite there are controversial results, many authors agree on the facts that vertebrate fish did not accumulate PAHs in their tissue as they rapidly metabolize them. However, PAHs can bioaccumulate in fatty tissues and fish is not free from the exposure to these contaminants from the environment.

Despite this review aims to cover the most relevant literature after 2000, there are previous studies widely referred which are related to the analysis of meat using solid–liquid extraction (SLE), and SPE for clean-up [52–54]. Chen et al. [52,53] proposed the extraction of the sample, which was previously lyophilized, by SLE using ultrasounds or ultrasound extraction (USE), and subsequent SPE clean-up with Florisil. This method was then compared to a more laborious procedure involving saponification using a Soxhlet extractor, and a number of LLE steps prior to a final SPE clean-up (Florisil). Despite the recovery values were quite similar and USE reduced the extraction time and solvent consumption, the Soxhlet method was eventually selected due to the saponification stage was described as necessary for a successful determination of the selected PAHs. Chiu et al. [54] also described the performance of a saponification stage in a similar way as described in [52].

The utilization of supercritical fluid extraction (SFE) together with C_{18} sorbent inside the SFE chamber to perform a simultaneous extraction and clean-up of the samples has been described. Although promising results were obtained, the application of SFE has not succeeded, as in other analytical fields [55–57].

Wang et al. [58] first described the use of pressurized liquid extraction (PLE) for the analysis of PAHs in meat samples. A dichloromethane/acetonitrile mixture was used and C_{18} or C_8 bulk sorbent and sodium sulphate were used to prepare the extraction cell. Although the performance of the SLE process by using PLE allowed the semi-automation of the extraction stage, a laborious clean-up procedure was still applied since partitioning with sulphuric acid and column chromatography (Florisil) were also performed. More recent PLE-based methodologies have been published for this aim, but using in the clean-up stage GPC and column chromatography [59] or GPC and SPE [60]. These studies utilized *n*-hexane as extraction solvent and polymeric-based columns (styrene DVB) for the GPC process (normal phase). This stage permitted the removal of a high percentage of lipids from the matrix; however, this was insufficient and an additional clean-up step was needed, as described in both studies. Jira et al. [59] pointed out the use of GPC as an effective way of removing lipidic material instead of saponification; for the remaining lipids and polar compounds, silica gel column chromatography was chosen. The use of sea sand and/or drying material to homogenize the sample was discarded

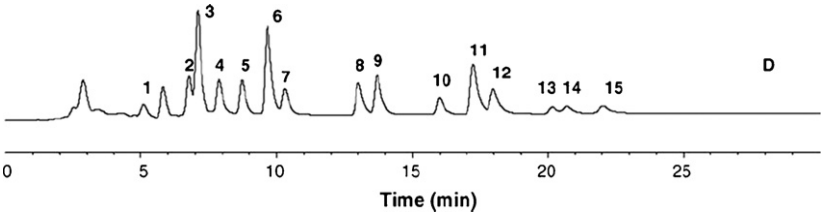

Fig. 2. HPLC–FLD chromatogram obtained by SBSE from Mate tea spiked with 500 ng L^{-1} (extraction time: 240 min). Peak identities are (1) naphthalene (NPH); (2) acenaphthene (ACP); (3) fluorene (FLR); (4) phenanthrene (PHE); (5) anthracene (ANT); (6) fluoranthene (FA); (7) pyrene (PYR); (8) benz[*a*]anthracene (BaA); (9) chrysene (CHR); (10) benzo[*b*]fluoranthene (BbFA); (11) benzo[*k*]fluoranthene (BkFA); (12) benzo[*a*]pyrene (BaP); (13) dibenzo[*a,h*]anthracene (DBahA); (14) benzo[*ghi*]perylene (BghiP) and (15) indeno[1,2,3-*cd*]pyrene (IP). Reprinted from [49] copyright 2005, with permission from Elsevier.

because of certain PAHs (pyrene (PYR), benz[*a*]anthracene (BaA), BaP, indeno[1,2,3-*cd*]-pyrene (IP), dibenzo[*a,h*]anthracene (DBahA) and benzo[*ghi*]perylene (BghiP)) could be adsorbed on these materials. The use of GPC and subsequent SPE by silica gel is a similar procedure also reported [60,61]. The use of GPC and column chromatography offered higher recoveries and lower RSD values in comparison with GPC plus SPE, although the number of monitored PAHs was superior in this last study.

In relation to the recovery rates, in general, the results obtained by LLE-based and PLE-based procedures are very similar; although it is obvious that PLE shows certain advantages, such as automation of the process and less solvent and time consumption.

Although SPME is normally used with liquid samples, Martin et al. [62] described the application of this extraction technique to the analysis of PAHs in meat by using a direct extraction device (SPME-DED). This device contains a gelatine support for the SPME fiber that permits the interaction between the fiber and the solid sample (Fig. 3). Although recovery values were not provided, the RSD values obtained were consistent.

Fish (and seafood) is the second most studied fatty matrix in PAH analysis. Despite the disadvantages of Soxhlet have been largely discussed (e.g. solvent consumption, time-consuming, non-selective), its efficiency still makes it used and selected in PAH analysis. In this type of commodity, the use of Soxhlet extraction (e.g. dichloromethane, *n*-hexane) is widely reported [63–67]; lyophilization [63] and homogenization with sodium sulphate [64,67] have been applied prior to the SLE process. As in previous matrices, a clean-up stage is mandatory after the Soxhlet extraction due to the high amount of co-extracted material. The use of GPC is preferred for this aim [63,65], although saponification plus subsequent LLE stages and column chromatography [66,67] have also been described. The main disadvantage of the application of GPC in this type of matrices is not only the moderate solvent consumption but also the widespread utilization of chlorinated solvents such as dichloromethane or chloroform as mobile phases (Table 2). In relation to the performance characteristics, the procedures using Soxhlet provided adequate recoveries but the reported RSD were quite high for certain compounds (e.g. 2–20%); these high RSD values could be due to the application of such time-consuming protocols involving numerous steps.

Although the performance of a saponification and subsequent LLE stages is less time-consuming than Soxhlet plus GPC or LLE, it provided low recoveries and similar RSD values [68].

PLE is applied as a suitable option to the aforementioned procedures [58,65,69]. The decrease in the extraction time against the Soxhlet methods is an obvious advantage. However, PLE is also a non-selective extraction and further clean-up is normally performed. Wang et al. [58] described that the removal of the fatty material in the PLE extracts was incomplete when applying saponification and an acid treatment with sulphuric acid (18 M) was found to cause decomposition of several PAHs (namely ACY, anthracene (ANT), and BaP) and losses of signal (e.g. ACP, IP, DBahA and BghiP). Nevertheless, this effect was not observed when a less concentrated solution was used (9 M). On the contrary, Mar-

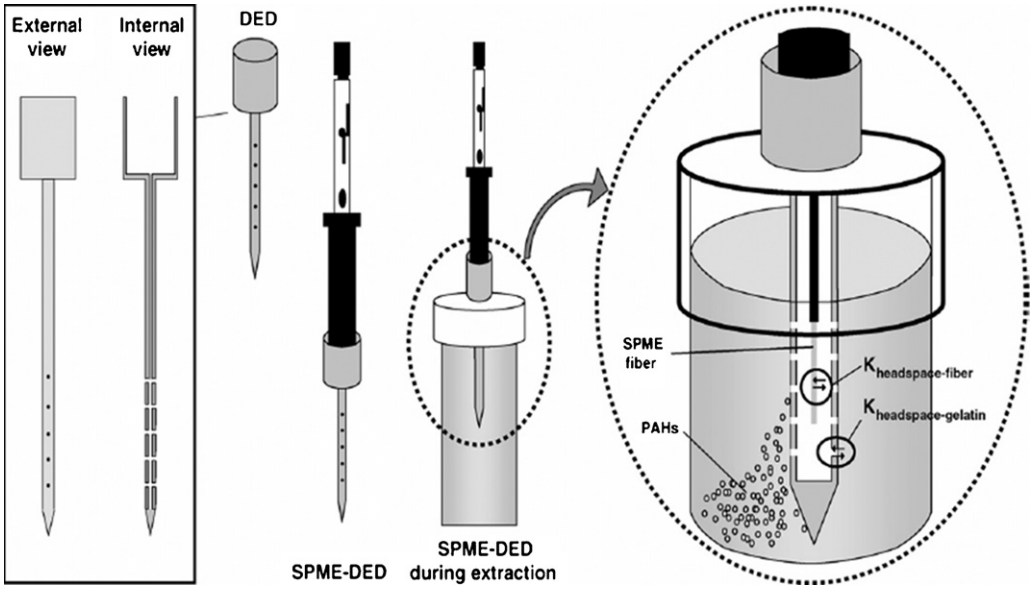

Fig. 3. Scheme of the use of SPME-DED in model systems of gelatine for the determination of the 16-EPA PAHs in smoked meat by GC–MS. The diffusion process of the analytes from the matrix to the headspace of the DED and the equilibriums implied in the process are shown. Reprinted from [59] copyright 2007, with permission from Elsevier.

tinez et al. [69] utilized a saponification stage for the clean-up of the raw PLE extracts with adequate recoveries, although a different extraction solvent was used (*n*-hexane:dichloromethane (1:1) instead of dichloromethane:acetonitrile (9:1)). In this study, Soxhlet extraction, USE and PLE were compared; similar results were found when using PLE and USE, although this last methodology was less repetitive. Surprisingly, the classical Soxhlet extraction yielded worse recovery and RSD values than PLE and USE. In a similar study, Janska et al. [65] established that the results provided by PLE and USE were not significantly different from those found by using the Soxhlet extraction, using GPC for the clean-up of the extracts in all cases, but higher repeatability was observed with PLE. The utilization of extraction mixtures containing a water-miscible solvent was strongly recommended in PLE to enhance the penetration into wet fatty matrices, such as fish [65].

The saponification stage has also been performed together with the extraction step by MAE [70], reducing the whole extraction time. However, further purification by SPE (silica) was needed and the number of analyzed PAHs was reduced (7 compounds). Additionally, direct SPE or GPC clean-up of MAE extracts has been applied; despite any recovery or precision rates were provided, the results of the analysis of a certified reference material were adequate [71–73].

The use of HS-SPME has been described for the analysis of PAHs showing up to 4 rings in fish and seafood using polyacrylate [74] and PDMS-DVB fibers [75]. Two approaches can be used considering that these matrices are solid samples: first, the sample can be put directly into the HS vial [74]; a second option involves the homogenization of the sample with a liquid solution [75]. Any comparison could be established between both procedures (with and without homogenization of the sample with solvent) since performance characteristics were not provided in the method using the raw sample.

MSPD was also evaluated for the determination of 6 PAHs in fish and seafood [76]. For clean-up purposes, an acid treatment with sulphuric acid impregnated silica gel was evaluated thanks to its compatibility with MSPD. However, the compounds were retained in the sorbent, although the lipidic removal was effective. The recovery rates were adequate and the RSD values were significantly low, which is remarkable since one of the main drawbacks of MSPD is its moderately low repeatability/reproducibility. As previously discussed, sulfuric acid can be used for the removal of lipids.

Recently, Ramalhosa et al. [77] evaluated the so-called QuEChERS method (acronymic name from quick, easy, cheap, effective, rugged and safe) in fish. This method is a procedure extensively applied and originally developed for the analysis of pesticide residues in food samples. The described approach results very appropriate for the analysis of volatile PAHs, such as NPH, ACP or FLR, often lost during pre-concentration stages, since it does not involve any evaporation step. The results obtained for heavy PAHs were also consistent, as verified by the analysis of a certified reference material. Beside this, the QuEChERS method is much easier than the typical procedures described for the analysis of PAHs (e.g. Soxhlet, LLE, etc.), showing adequate performance characteristics.

Smoked cheese is another fatty matrix of interest for the monitoring of PAHs, although the number of related studies is very scarce. As in other fatty matrices, Soxhlet plus GPC [78] and LLE-based methods [79], sometimes including saponification [80], are described in literature. SPE has been applied for clean-up purposes using silica sorbents. The recovery values for the revised references are adequate and comprise between 52 and 96%. For the most volatile compounds, Suchanova et al. [78] remarked the poor recoveries obtained (namely, NPH, ACP and FLR). This is a well-known fact that has been confirmed in many other studies; however, these authors do not consider important to improve the efforts in increasing the recovery rates for these PAHs since they are not health concerns in terms of carcinogenicity.

More detailed information about extraction methods is shown in (Table 2).

2.2.2. Non-fatty matrices

Besides the matrices discussed before, PAHs have been monitored in a variety of solid foodstuffs, i.e. tea leaves [81,82], vegetables [83–85], fruits [84], bread [85], cane sugar [86], fatty food mixtures [27], ground coffee [87] and palm dates [66] (Table 2). The methodologies applied are not different from those applied in the main food groups. Soxhlet, LLE, PLE or USE have been utilized in the extraction step (involving in some cases a saponification stage), whereas GPC or SPE have been applied in the clean-up step. Borjadandi et al. [88] reported the analysis of PAHs in a great variety of food samples, such as fish, seafood, meat products, vegetable oils, breads and pastries. For this aim, a generic methodology based on Soxhlet extraction was applied, concluding that this extraction technique is the most suitable for the determination of very different food commodities with adequate performance.

More detailed information about extraction methods is shown in (Table 2).

3. Chromatographic and detection techniques

In general, the determination of PAHs is carried out by liquid chromatography coupled to fluorescence (LC–FLD) or ultraviolet–visible detection (LC–UV), or gas chromatography coupled to mass spectrometry (GC–MS) detection, techniques which are discussed below.

3.1. Liquid chromatography coupled to UV and FLD

In the past, the determination of PAHs by LC was carried out by using UV detection [52,54]. However, it is well known that UV detection shows a number of disadvantages, such as selectivity problems and sensitivity limitations, and it cannot discriminate matrix interferences, especially in complex matrices. On the contrary, FLD is more selective and sensitive than UV detection, and it is currently the detection system of choice in LC, normally with variable excitation and emission wavelengths. LC–FLD has been extensively applied for the determination of PAHs in very different matrices, including foodstuffs and beverages, since it is cheap and simple, in comparison to other detection systems. Indeed, LC–FLD has been the basis of different official methods for the analysis of PAHs in food [2,89] (Table 3).

In this sense, LC–FLD has been largely used for the determination of the EPA priority list of PAHs [22,23,46,49,52,65,77]. It has been reported that ANT and perylene (PER) are best measured by FLD due to their selective and sensitive fluorescence characteristics [3]. CPcdP does not give rise to fluorescence and can only be quantified by UV detection [1]. Despite there is an improvement in comparison to UV detection, FLD can still show a lack of selectivity, and then GC–MS is applied in order to confirm the positive results [17,54,84,87].

Moreover, some authors describe certain selectivity problems due to the presence of alkylated PAHs [16], which are considered the main impurities of PAH fractions. These compounds show similar fluorescence responses to the unsubstituted PAHs. Another disadvantage is the impossibility of using certain isotopically labeled compounds because of FLD cannot distinguish these ones from the native PAHs. As an alternative, benzo[*b*]chrysene or deuterated compounds, which can be chromatographically separated, have been used [1].

Table 3
Summary of separation and detection techniques in the discussed matrices.

Matrix	Separation/detection technique	Separation remarks	Detection remarks	LOD (units)	LOQ (units)	Ref.
Liquid fatty matrices						
Edible oils	LC–FLD	Vydac C_{18} (250 × 4.6 mm i.d., 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 250–290, λ_{em} = 330–500 nm	N.A.[a]	0.3–6.0 ng g^{-1}	[23]
Edible oils	LC–FLD	Vydac C_{18} (250 × 4.6 mm i.d., 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 250–300, λ_{em} = 330–500 nm	0.03–0.2 ng g^{-1}	0.1–8.0 ng g^{-1}	[28]
Edible oils	LC–FLD	Supelcosil LC-PAH (250 mm × 3 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 240–290, λ_{em} = 330–484 nm	N.A.	N.A.	[22]
Edible oils	LC–FLD	Thermo Hypersil ODS (200 mm × 4.6 mm × 5 μm) Isocratic elution: MeOH:water, 9:1, v/v	λ_{ex} = 255, λ_{em} = 420 nm	0.06 μg kg^{-1}	0.2 μg kg^{-1}	[25]
Edible oils	LC–FLD	C-18 Lichrocart (125 mm × 4 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 242–350, λ_{em} = 380–443 nm	0.1–4.0 ng	N.A.	[29]
Edible oils, fat	LC–FLD	(250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 245–300, λ_{em} = 376–418 nm	0.2–0.8 μg kg^{-1}	N.A.	[26]
Edible oil, smoked meat	GC–EI-MS	Injection: Pulsed splitless Supelco SPB-5 (25 m × 0.20 mm × 0.33 μm)	Q, SIM	0.06 μg kg^{-1}	0.20 μg kg^{-1}	[17]
Olive oil	(A) GC–EI-MS	(A) Injection: N.A.; DB-5ms (30 m × 0.25 mm, 0.25 μm)	(A),(B) SIM	(A) 1 ng g^{-1}	(A) 3.4 ng g^{-1}	[21]
	(B) LP-GC–EI-MS	(B) Injection: N.A.; Rapid MS FS CP-Sil 8 (10 m × 0.53 mm, 0.50 μm) + restrictor (0.6 m × 0.25 mm)	(C), (D) λ_{ex} = 370, λ_{em} = 470 nm	(B) 1.6 ng g^{-1}	(B) 5.5 ng g^{-1}	
	(C) LC–FLD	(C) CP EcoSpher 4 PAH (150 mm × 3 mm); isocratic elution: ACN:water (85:15, v/v)		(C) 0.5 ng g^{-1}	(C) 1.7 ng g^{-1}	
	(D) DACC	(D) CP ChromSpher π (20 mm × 3 mm); isocratic elution: ACN:water (85:15, v/v)		(D) 0.3 ng g^{-1}	(D) 1.1 ng g^{-1}	
Olive oil	GC–EI-MS	Injection: Pulsed splitless HP-5ms, (60 m × 0.25 mm × 0.25 μm)	Q, SIM	N.A.	N.A.	[24]
Olive oil	GC–EI-MS	Injection: Splitless Supelcowax-10 and HP-5ms, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	0.05–1.60 μg kg^{-1}	0.20–5.20 μg kg^{-1}	[33]
Olive oil	GC–EI-MS(/MS)	Injection: Splitless VF-5ms, 5% phenyl-95% methylpolysiloxane, (30 m × 0.25 mm × 0.25 μm)	QqQ, SIM, SRM	0.02–0.06 μg kg^{-1}	0.07–0.26 μg kg^{-1}	[32]
Oil, food mixture	GC–EI-MS/MS	Injection: N.A. Zebron ZB-5ms, (30 m × 0.25 mm × 0.25 μm)	QqQ, SRM	0.008–0.150 μg kg^{-1} (dry weight)	0.024–0.920 μg kg^{-1} (dry weight)	[27]
Olive, olive-pomace oil	GC–EI-MS/MS	Injection: LVI + PTV HP-5, crosslinked 5% phenyl-95% methylpolysiloxane, (30 m × 0.25 mm × 0.25 μm)	IT, Product-ion scan, Resonant mode	0.05–0.07 μg kg^{-1}	0.1–0.2 μg kg^{-1}	[31]
Olive pomace oil	GC–EI-MS	Injection: splitless DB-5ms (30 m × 0.25 mm × 0.20 μm)	IT, Full scan	0.1–0.4 μg kg^{-1}	N.A.	[18]
Vegetable oils	GC–EI-MS	Injection: Splitless SPB-5, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	0.2 μg kg^{-1}	0.5 μg kg^{-1}	[34]
Vegetable oils	GC × GC–EI-MS	Injection: Splitless First dimension: SGE BPX5 (30 m × 0.25 mm × 0.25 μm) Second dimension: SGE BPX50, 50% phenyl polysilphenylenesiloxane (1 m × 0.1 mm × 0.1 μm)	TOF, Full scan	0.1–1.4 μg kg^{-1}	0.4–4.6 μg kg^{-1}	[35]
Fish oil, fish	(A) GC–EI-MS/MS	(A), (B) Injection: Splitless; HP-5ms, 5% diphenyl-95% dimethyl polysiloxane, (30 m × 0.25 mm × 0.25 μm)	(A) QqQ, SRM	0.02–1.25 μg kg^{-1}	0.125–1.250 μg kg^{-1}	[95]
	(B) GC–EI-MS (Confirmation)		(B) TOF, Full scan			
Fish oil, dried plants	LC–FLD	Varian Pursuit 3 PAH (100 mm × 4.6 mm × 1/4″) Gradient elution: A: ACN; B: MeOH; C: water	λ program: λ_{ex} = 222–380, λ_{em} = 353–499 nm	0.07–7.80 μg kg^{-1}	0.13–16 μg kg^{-1}	[30]
Milk	GC–EI-MS	N.A.	Q, SIM	0.2–5.0 ng L^{-1}	0.7–16.6 ng L^{-1}	[39]
Milk	GC–EI-MS	Injection: Splitless HP-5ms, 5% diphenyl-95% dimethyl polysiloxane, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	0.003–1.56 μg L^{-1}	N.A.	[40]
Milk	GC–EI-MS	Injection: N.A.	Q, SIM	N.A.	N.A.	[36]

Table 3 (*Continued*)

Matrix	Separation/detection technique	Separation remarks	Detection remarks	LOD (units)	LOQ (units)	Ref.
		DB-XLB, proprietary phase, (60 m × 0.25 mm × 0.25 μm)				
Milk	GC–EI-MS	Injection: Splitless OV-1 (30 m × 0.25 mm × 0.25 μm)	Q, SIM -Derivatization: (MSTFA)	0.04–0.39 ng mL^{-1}	N.A.	[37]
Milk	LC–FLD	Wakosil-PAHs (250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: MeOH:water, 8:2, v/v	λ program: λ_{ex} = 248–364, λ_{em} = 360–500 nm	1.3–76.0 ng kg^{-1}	N.A.	[38]
Liquid non-fatty matrices						
Coffee	LC–FLD	C18 Supelcosil LC-PAH (250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 274–300, λ_{em} = 406–470 nm	0.01–0.05 μg L^{-1}	0.04–0.20 μg L^{-1}	[41]
Coffee	LC–FLD	C18 Supelcosil LC-PAH (250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 230–250, λ_{em} = 410–420 nm	0.8–10.0 ng L^{-1} [b]	2.5–33.2 ng L^{-1} [c]	[44]
Coffee	LC–FLD	Isocratic elution: ACN:water, 4:6, v/v Phenomenex Envirosep PP (125 mm × 3.2 mm)	λ program: λ_{ex} = 252–300, λ_{em} = 322–500 nm	N.A.	N.A.	[47]
Tea	LC–FLD	Nova-Pak C_{18} (150 mm × 3.9 mm × 4 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 250–295, λ_{em} = 365–465 nm	0.016–0.140 ng mL^{-1}	N.A.	[45]
Tea infusion	(A) LC–FLD	(A) LiChrospher PAH, modified RP-18 silica gel (250 mm × 4.6 mm × 5 μm); gradient elution: A: ACN; B: water	(A) λ program:	5–145 ng L^{-1}	N.A.	[48]
	(B) GC–EI-MS (Confirmation)	(B) Injection: Splitless; HP-5ms, (30 m × 0.25 mm × 0.25 μm)	λ_{ex}=250, λ_{em} = 330–500 nm (B) Q, SIM			
Mate tea	LC–FLD	Vydac 201TP52 (250 × 2.1 mm i.d., 5 μm) Gradient elution: A: ACN; B: water		0.1–8.9 ng L^{-1}	0.3–30 ng L^{-1}	[49]
Beverages	GC–EI-MS	Injection: LVI + PTV HP-5ms, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	3–27 ng L^{-1}	30–133 ng L^{-1}	[51]
Sugarcane juice	GC–EI-MS	Injection: (A) SBSE: Splitless; (B) MASE: LVI + PTV HP-5ms, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	(A) 0.04 μg L^{-1} (B) 0.06 μg L^{-1}	N.A.	[50]
Cachaça (spirit)	(A) LC–FLD	(A) Vydac 201TP54 (250 mm × 4.6 mm × 5 μm); isocratic elution: ACN/water (75:25, v/v)	(A) λ_{ex} = 290, λ_{em} = 430	0.006–0.090 μg L^{-1}	N.A.	[42]
	(B) GC–EI-MS (Confirmation)	(B) Injection: Splitless + PTV; HP-5ms, (30 m × 0.25 mm × 0.25 μm)	(B) Q, SIM			
Spirits	LC–FLD	C18 Supelcosil LC-PAH (250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 270–356, λ_{em} = 330–500 nm	1.08×10^{-3}–1.28×10^{-2} μg L^{-1}	0.11–10.93 μg L^{-1}	[46]
Solid fatty matrices						
Meat	GC–EI-MS	Injection: splitless DB-5ms (30 m × 0.32 mm × 0.25 μm)	IT, Full scan	5–50 pg	N.A.	[53]
Meat	(A) LC–UV	ED Envirosep-pp C18 column (125 mm × 4.6 mm × 5 μm)	(A) λ = 254 nm	(A) 0.03–1.54 ng	N.A.	[52]
	(B) LC–FLD	Gradient elution: A: ACN; B: water	(B) λ program: λ_{ex} = 254–270, λ_{em} = 340–420 nm	(B) Not detected-6 pg		
Smoked meat	GC–EI-MS	Injection: splitless TR-50MS, 50% phenyl polysilphenylene-siloxane (10 m × 0.1 mm × 0.1 μm)	Magnetic sector, Full scan	0.001–0.049 μg kg^{-1}	N.A.	[60]
Smoked meat	GC–EI-MS	Injection: splitless TR-50MS, 50% phenyl polysilphenylene-siloxane (10 m × 0.1 mm × 0.1 μm)	Magnetic sector, Full scan	0.001–0.045 μg kg^{-1}	N.A.	[61]
Smoked meat	(A) LC–UV (B) LC–FLD	(A), (B) ED Envirosep-pp C18 column (125 mm × 4.6 mm × 5 μm); gradient elution: A: ACN; B: water	(A) λ = 254 nm (B) λ program: λ_{ex} = 254–320, λ_{em} = 340–533 nm	(A) 0.03–1.54 ng (B) 2×10^{7}–6 pg	N.A.	[54]
	(C) GC–EI-MS (Confirmation)	(C) Injection: splitless; DB-5ms (30 m × 0.32 mm × 0.25 μm)	(C) IT, Full scan	(C) 5–50 pg		
Smoked meat	GC–EI-MS	Injection: Splitless HP-5, (50 m × 0.32 mm × 1.05 μm)	Q, SIM	0.008–0.102 ng mL^{-1}	N.A.	[62]
Smoked meat	GC–EI-MS	Injection: Splitless	Magnetic sector, SIR	N.A.	N.A.	[59]

Table 3 (*Continued*)

Matrix	Separation/detection technique	Separation remarks	Detection remarks	LOD (units)	LOQ (units)	Ref.
		DB-5ms (60 m × 0.25 mm × 0.25 μm)				
Smoked meat	LC–FLD	Supelcosil LC-PAH (250 mm × 3 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program (detector A): λ_{ex} = 250–290, λ_{em} = 350–470 nm λ program (detector B): λ_{ex} = 240–290, λ_{em} = 330–484 nm	N.A.	<0.2–0.6 μg kg^{-1}	[71]
Fish, smoked meat	GC–EI-MS	Injection: Splitless HP-5ms, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	0.002–0.100 μg mL^{-1}	N.A.	[58]
Fish	GC–EI-MS	Injection: Pulsed splitless DB-5ms, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	2–7 ng g^{-1} [b]	N.A.	[67]
Fish	LC–FLD	LiChroCART (250 mm × 4.0) with LiChrospher PAHs sorbent Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 217–295, λ_{em} = 341–484 nm	N.A.	N.A.	[65]
Fish	GC–EI-MS	Injection: Splitless HP-5ms, (60 m × 0.25 mm × 0.25 μm)	Q	N.A.	N.A.	[74]
Fish	LC–FLD	Vydac 201TP52 (250 mm × 2.1 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 245–294, λ_{em} = 410–500 nm	0.1–0.5 ng g^{-1} (dry weight)	0.2–1.8 ng g^{-1} (dry weight)	[70]
Fish	LC–FLD	Vydac 201TP52 (250 mm × 2.1 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 245–294, λ_{em} = 410–500 nm	0.04–0.32 ng g^{-1}	0.13–1.07 ng g^{-1}	[76]
Fish	GC–EI-MS	Injection: Splitless DB-5ms, 5% phenyl 95% dimethyl arylene siloxane(30 m × 0.25 mm × 0.25 μm)	IT, Full scan	0.02–1.70 μg mL^{-1}	0.06–5.00 μg mL^{-1}	[64]
Fish	LC–FLD	CC 150/4 Nucleosil 100-5 C18 PAH (150 mm × 4.0 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 315–590, λ_{em} = 260–290 nm	0.04–0.56 ng g^{-1}	0.12–1.90 ng g^{-1}	[77]
Fish, seafood	LC–FLD	Phenomenex C18 Envirosep (125 mm × 4.6 mm × 3 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 250–290, λ_{em} = 380–450 nm	0.01–0.49 ng g^{-1}	0.02–0.62 ng g^{-1}	[68]
Fish, seafood	GC–EI-MS	Injection: Splitless HP-5, (30 m × 0.25 mm × 0.25 μm)	Q, Full scan	N.A.	N.A.	[73]
Fish, mussel	GC–EI-MS	Injection: Splitless HP-5ms, 5% diphenyl-95% dimethyl polysiloxane, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	8–450 pg g^{-1}	50–1500 pg g^{-1}	[75]
Fish, palm dates	GC–EI-MS	Injection: Splitless CP-SIL 8CB-MS arylene-modified 5% phenyl-95% methyl polydimethylsiloxane (30 m × 0.25 mm × 0.25 μm)	IT, SIS	0.13–4.29 μg L^{-1}	0.43–14.29 μg L^{-1}	[66]
Shellfish	GC–EI-MS	Injection: Splitless VF-5ms, (30 m × 0.25 mm × 0.25 μm)	IT, SIS	0.52–0.81 ng g^{-1} [b]	N.A.	[63]
Mussel	GC–EI-MS	Injection: Splitless DB-5, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	0.5–8.0 μg kg^{-1} (dry mass)	N.A.	[69]
Cheese	LC–FLD	Envirosep-PP (125 × 4.6 mm i.d., 4.6 μm) Isocratic elution: ACN/water (88:12, v/v)	λ_{ex} = 295, λ_{em} = 404 nm	0.006 μg kg^{-1}	0.021 μg kg^{-1}	[80]
Cheese	LC–FLD	Supelcosil LC-PAH (250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 216–295, λ_{em} = 320–484 nm	0.01–0.25 μg kg^{-1}	N.A.	[78]
Cheese	LC–FLD	Supelcosil LC-PAH (250 mm × 4.6 mm × 5 μm) Gradient elution: A: ACN; B: water	λ program: λ_{ex} = 224–268, λ_{em} = 320–400 nm	N.A.	0.01–0.90 μg kg^{-1}	[79]
Infant milk, infant cereals	(A) Parent and hydroxy-PAHs: LC–FLD (B) Hydroxy-PAHs: LC–MS (Confirmation)	(A) Luna C_8 Supelcosil (120 mm × 2.0 mm × 5 μm); gradient elution: A: ACN; B: water (B) Hypersil Green PAH (100 mm × 4.6 mm × 5 μm); gradient elution: A: ACN; B: water	(A) λ program: λ_{ex} = 274–393, λ_{em} = 406–470 nm (B) QqQ, SRM, ESI (−)	(A) 0.01–0.70 μg kg^{-1} (B) 0.10–0.15 μg kg^{-1}	(A) 0.03–1.70 μg kg^{-1} (B) 0.25–0.50 μg kg^{-1}	[94]
Solid non-fatty matrices						
Bread, potato	(A) LC–FLD (B) GC–MS/MS (Confirmation)	(A) Hypersil Green PAH (100 mm × 4.6 mm × 5 μm); gradient elution: A: ACN; B: water (B) Injection: Splitless; VA-5, (30 m × 0.25 mm × 0.25 μm)	(A) λ program: λ_{ex} = 250–300, λ_{em} = 325–465 nm (B) Q, full scan	0.007–6.400 μg L^{-1}	0.023–21.300 μg L^{-1}	[85]

Table 3 (*Continued*)

Matrix	Separation/detection technique	Separation remarks	Detection remarks	LOD (units)	LOQ (units)	Ref.
Cane sugar	LC–FLD	Vydac 201TP54 (250 mm × 4.6 mm × 5 μm) Isocratic elution: ACN/water (75:25, v/v)	λ_{ex} = 290, λ_{em} = 430	0.01–0.17 μg kg^{-1}	N.A.	[86]
Foodstuffs	LC–FLD	Spherisorb ODS2-C_{18} (250 mm × 4.6 mm i.d., 5 μm) Gradient elution: A: ACN; B: water	λ_{ex} = 250–300, λ_{em} = 330–500 nm	0.0007–0.013 ng (L^{-1} [b]	N.A.	[88]
Food supplements	LC–FLD	Varian C_{18} Pursuit 3 PAH (100 mm × 4.6 mm i.d., 3 μm) Gradient elution: A: ACN; B: MeOH; C: water	N.A.	0.1–29.8 μg kg^{-1}	0.2–59.7 μg kg^{-1}	[100]
Fruits, vegetables	(A) LC–FLD	(A) C18 Vydac 201 TP (250 mm × 4.6 mm i.d., 5 μm); isocratic elution: ACN:water (75:25, v/v)	(A) λ_{ex} = 290, λ_{em} = 430 nm	(A) 0.07–1.29 μg kg^{-1} [b]	N.A.	[84]
	(B) GC–EI-MS (Confirmation)	(B) Injection: Splitless; Supelco 5% diphenyl-95% dimethylpolysiloxane, (30 m × 0.25 mm × 0.25 μm)	(B) Q, SIM			
Ground coffee	(A) LC–FLD	(A) Supelcosil LC-PAH (250 mm × 4.6 mm × 5 μm); gradient elution: A: ACN; B: water	(A) λ program:	0.11–0.18 μg kg^{-1} [b]	N.A.	[87]
	(B) GC–MS/MS (Confirmation)	(B) Injection: programmed temperature vaporization; Rtx-5MS (30 m × 0.25 mm × 0.25 μm)	λ_{ex} = 220–286, λ_{em} = 340–420 nm			
	(C) LC–UV (Confirmation)	(C) C18 Supelcosil LC-PAH (150 mm × 3.0 mm × 5 μm); gradient elution: A: ACN; B: water	(B) IT, Product ion scan			
Tea leaves	LC–UV	Elution: N.A. Agilent C-18 (250 mm × 4.6 mm)	N.A.	0.16–1.27 μg kg^{-1}	N.A.	[81]
Tea leaves	GC–EI-MS	Injection: N.A. HP-5ms (30 m × 0.25 mm × N.A.)	Q	N.A.	N.A.	[82]
Vegetables	GC–EI-MS	Injection: Splitless HP-5ms, (30 m × 0.25 mm × 0.25 μm)	Q, SIM	N.A.	N.A.	[83]

Abbreviations: ACN: acetonitrile; DACC: donor–acceptor complex chromatography; ESI (−): electrospray ionization in negative mode; GC–EI-MS: gas chromatography coupled to mass spectrometry operating in electronic ionization; GC–EI-MS/MS: GC coupled to tandem MS; GC × GC–MS: multidimensional GC–EI-MS; IT: ion trap analyzer; LC–FLD: liquid chromatography coupled to fluorescence detection; LC–UV: LC coupled to ultraviolet–vis detection; LP-GC–EI-MS low pressure GC–MS in electronic ionization; LVI: large-volume injection; MASE: membrane-assisted solvent extraction; MeOH: methanol; MSTFA: N-methyl-N-(trimethylsilyl)trifluoroacetamide; PTV: programmed-temperature vaporization; Q: single quadrupole analyzer; QqQ: triple quadrupole analyzer; SBSE: stir bar sorptive extraction; SIM: single-ion monitoring; SIR: selected ion recording; SIS: selected-ion storage; SRM: selected-reaction monitoring; TOF: time of flight analyzer; λ_{ex}: excitation wavelength; λ_{em}: emission wavelength.

[a] N.A.: Data not available.

[b] Method detection limit (MDL).

[c] Method quantification limit (MQL).

In relation to sensitivity, the reported limits of detection (LODs) are frequently found at the sub-ppb level (e.g. 0.01–1 μg L^{-1} or μg kg^{-1}), and in some applications in beverages, LODs at the ppt level (e.g. 0.01–1 μg L^{-1} or μg kg^{-1}) have been achieved [44,48,49]. This fact can be justified since in this type of samples, pre-concentration techniques such as SPE or SBSE have been applied.

Despite the widespread use of columns with particle size ≤2 μm in trace analysis (i.e. pesticide or veterinary drug residue analysis [90]), the utilization of ultra-high performance liquid chromatography (UHPLC) has not been reported in PAH analysis, up to our knowledge. The main advantages of UHPLC are well known (e.g. reduction of running time or narrower peaks than conventional LC) and its application has rapidly increased. Thus, the coupling of UHPLC to FLD would provide an increase in chromatographic resolution that could improve the discrimination of co-eluted interferences, especially in complex matrices such as foodstuffs.

Although FLD is the most utilized detection system for the analysis of PAHs in food and beverages by LC, MS has also been applied in other matrices such as environmental matrices [91,92] or biological matrices [93]. Up to our knowledge, only a study related to the determination of hydroxy-PAHs describes the application of LC–MS using electrospray ionization (ESI) in negative mode [94]. Due to their non-polar character, atmospheric-pressure chemical ionization source (APCI) [91] and atmospheric-pressure photoionization (APPI) [92,95] have been applied as ionization techniques. However, the application of LC–MS using APCI or APPI as ionization modes for the determination of PAHs in food commodities has not been described yet.

More detailed information about determination methods and conditions is shown in (Table 3).

3.2. Gas chromatography coupled to MS

GC–MS is the main alternative to LC–FLD and it is applied in all kind of food samples. Besides, GC–MS-based methods are more frequently found in the more recent bibliography [27,34,35,39,51,60,62,75,96,97]. As in the case of LC–FLD, there are official methods for the analysis of PAHs by GC–MS, such as the EPA method 8100 regarding the analysis of PAHs by GC [3,98] (Table 3).

The utilization of GC–MS shows several advantages in comparison to LC–FLD, mainly in their resolution capability. GC offers high chromatographic resolving power and MS provides high mass selectivity and structural information. GC–MS permits the determination of non-fluorescence PAHs, such as CPcdP, or PAHs showing poor fluorescence, such as NPH, ACY, ACP and FLR [99].

For the separation stage, columns with a stationary phase based on 5% phenyl-95% methylpolysiloxane substitution are widely applied in the revised literature (e.g. typical HP-5 or HP-5ms). This type of column is also commonly utilized for the analysis of other organic contaminants and residues at trace level. However, obtaining an adequate resolution can become a critical point depending on the target PAHs since there are several groups of compounds which can co-elute (Fig. 4). This issue is particularly important when these PAHs cannot be resolved mass spectrometrically by extracting their corresponding traces (e.g. isomers). If the overlapped compounds show isobaric ions, an accurate quantification is therefore difficult or impossible. Five groups of PAHs present this type of resolution problems: (i) chrysene (CHR) and triphenylene; (ii) CPcdP, BaA and CHR; (iii) benzo[*b*]fluoranthene (BbFA), benzo[*j*]fluoranthene (BjFA) and benzo[*k*]fluoranthene BkFA; (iv) dibenzo[*a,c*]anthracene and DBahA; and (v) DBahA and IP [3,97].

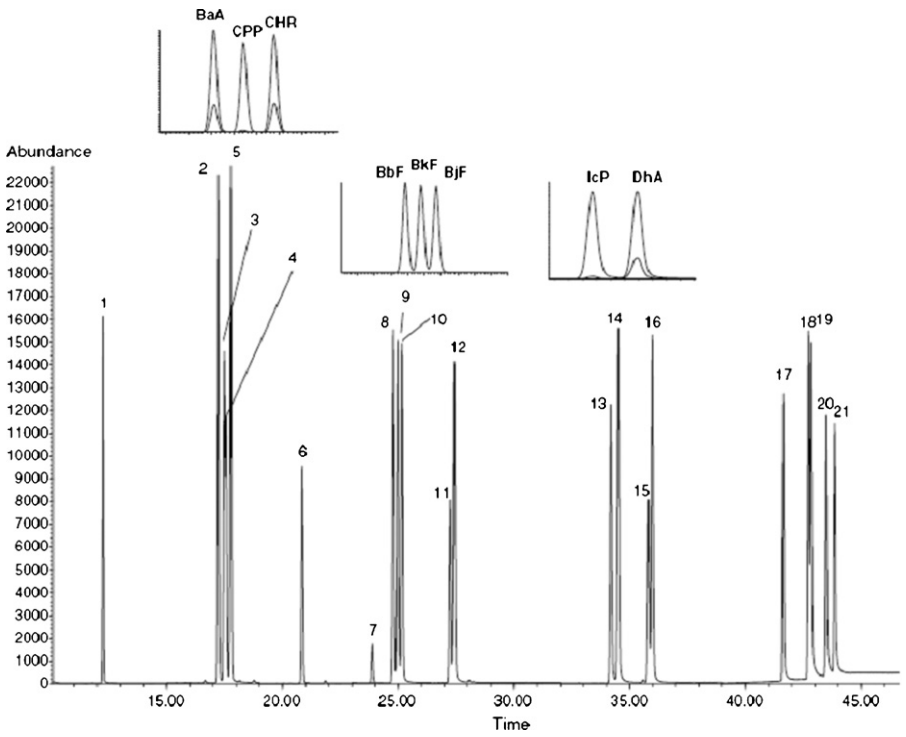

Fig. 4. GC–MS chromatogram of the 15 + 1 EU PAHs on a DB-17MS column 20 m length, 0.18 mm i.d. and 0.14 μm film thickness. Peak identities are (1) benzo[*c*]fluorene (BbF); (2) benz[*a*]anthracene (BaA); (3) cyclopenta[*cd*]pyrene (CPcdP); (4) d12-chrysene (d12-CHR); (5) chrysene (CHR); (6) 5-methylchrysene (MCH); (7) 9-fluorobenzo[*k*]fluoranthene (FBkF); (8) benzo[*b*]fluoranthene (BbF); (9) benzo[*k*]fluoranthene (BkF); (10) benzo[*j*]fluoranthene (BjF); (11) d12-benzo[*a*]pyrene (d12-BaP); (12) benzo[*a*]pyrene (BaP); (13) indeno[1,2,3-*cd*]pyrene (IP); (14) dibenzo[*a,h*]anthracene (DBahA); (15) d12-benzo[*ghi*]perylene (d12-BghiP); (16) benzo[*ghi*]perylene (BghiP); (17) dibenzo[*a,l*]pyrene (DBalP); (18) d12-coronene (d12-COR); (19) dibenzo[*a,e*]pyrene (DBaeP); (20) dibenzo[*a,i*]pyrene (DBaiP) and (21) dibenzo[*a,h*]pyrene (DBahP). Reprinted from [90] copyright 2009, with permission from Springer.

The determination of heavy PAHs, such as dibenzopyrenes, by using typical 5ms columns is also problematic as they show a strong interaction with the stationary phase provoking broadening peak and sensitivity problems. Some dibenzopyrenes are included in the EU-list (dibenzo[*a,e*]pyrene (DBaeP), dibenzo[*a,h*]pyrene (DBahP), dibenzo[*a,i*]pyrene (DBaiP), dibenzo[*a,l*]pyrene (DBalP)) and consequently, adequate analytical methods are needed for their monitoring. However, most of the revised literature is focused on the EPA list, and thus, DBahA (278 amu) is the heaviest compound which is normally determined, regardless the most heavy PAHs.

Although columns showing a more polar stationary phase have been pointed out as adequate for the determination of dibenzopyrenes (302 amu), their application in food analysis is not widespread [61]. In relation to this, Gómez-Ruiz et al. [97] evaluated thoroughly the performance of different stationary phases (Fig. 5) for the analysis of the EU priority PAHs, including the typical 5%-phenyl columns and other more polar columns, such as 50% phenyl-50% methylpolysiloxane columns (e.g. DB-17ms) and a recently commercialized mid-polar to polar phase (Optima® δ-6) (Fig. 5). The utilization of a 50% phenyl-50% methylpolysiloxane column (mid-polar phase) solved the resolution problems of three groups of co-eluted PAHs: DBahA-IP, BbFA-BjFA-BkFA and CPcdP-BaA-CHR, whereas a tailor-made DB-17ms column (20 m) showed the best results for the suitable determination of the four aforementioned dibenzopyrenes, improving their peak shape and signal-to-noise (S/N) ratios.

Veyrand et al. [27] proposed the utilization of several mathematical formulae, which are based on full scan spectra and relative abundances, in order to quantify separately BaA and CDcdP. In relation to the quantification issue, Wolska et al. [100] described the different problems when using isotope-labeled standards, as recovery standards, in PAH analysis. In this study, this strategy permitted the improvement of the accuracy and precision in the determinations.

A few applications utilize fast chromatography modes, such as shorter columns (e.g. 10 m × 0.1 mm) [60,61]; or comprehensive or multidimensional GC (GC × GC) [35]. In this last modality, a first separation is performed with a non-polar column (e.g. 5% phenyl polysilphenylene-siloxane, 30-m length, typical separation), and, then, a second separation is carried out with a polar column (e.g. 50% phenyl polysilphenylene-siloxane, 1-m length, separation based on polarity). The resolution power is increased but the raw data and chromatograms are considerably complex and powerful software tools are requested (Fig. 6). Additionally, the use of columns with >30-m length has been reported in specific applications in order to improve the resolution of certain groups of isomers and some methyl derivatives [16].

In relation to the sample injection, the applied technique can be a key factor since certain PAHs show very high boiling points ("heavy" PAHs). In literature, splitless injection is preferred (e.g. [18,31,34]). Other techniques utilized are programmed-temperature vaporization (PTV) (e.g. [31,87]), cold on-column injection, which is also used since it improves peak shape in the early eluting PAHs (those with low molecular mass) [3], and large-volume injection (LVI), which is rarely applied [27,49,51] despite the fact that it can increase sensitivity. LVI and on-column injection have been considered as a way of diminishing the discrimination of dibenzopyrenes [97]. In this sense, the combination of PTV and LVI has been successfully applied for the analysis of both light and heavy PAHs in environmental analysis [101], and more recently in food [102]. In this study, the optimized PTV in solvent mode (combined with LVI) always provided higher sensitivity than the PTV process used in splitless mode, and improved S/N ratios for the more heavy PAHs (especially important for DBaeP, DBaiP and DBahP).

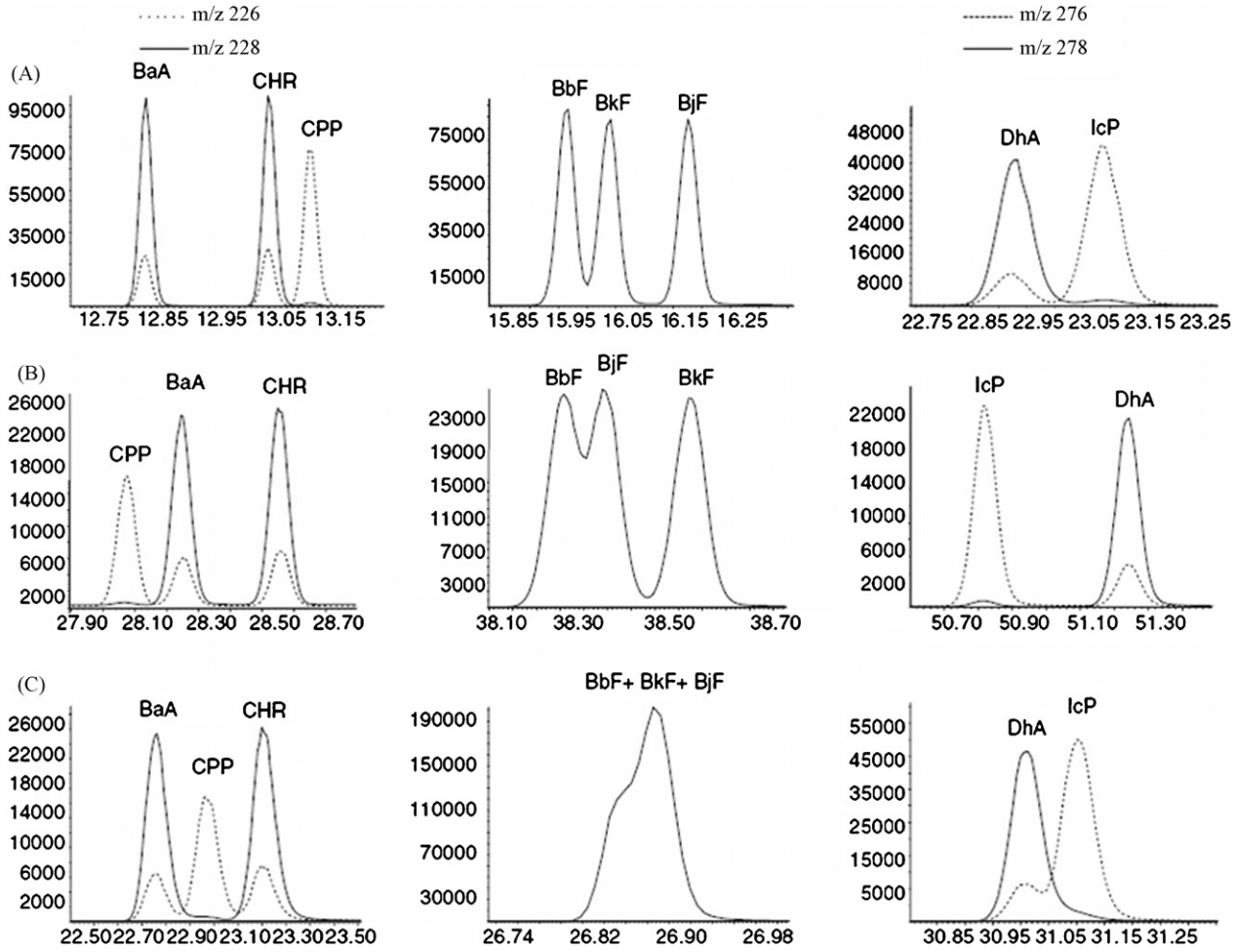

Fig. 5. Chromatographic separation of critical pairs/triplets by GC–MS obtained on three different stationary phases: (a) DB-17MS column, 60 m length, 0.25 mm i.d., 0.25 μm film thickness; (b) DB-5MS column, 60 m length, 0.25 mm i.d., 0.25 μm; (c) Optima® δ-6 column, 30 m length, 0.25 mm i.d., 0.25 μm. Reprinted from [90] copyright 2009, with permission from Springer.

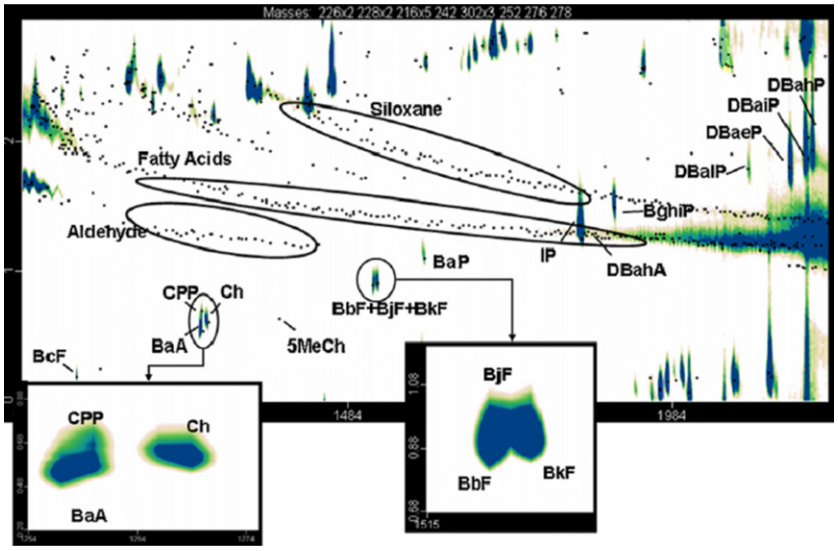

Fig. 6. Example of SPME-GC × GC-TOF-MS analysis showing a contour plot of a vegetable oil sample spiked with a PAHs solution (others groups of compounds are also shown). Abbreviations: BaA, benz[*a*]anthracene; BbF, benzo[*b*]fluoranthene; BghiP, benzo[*ghi*]perylene; BjF, benzo[*j*]fluoranthene; BkF, benzo[*k*]fluoranthene; BcF, benzo[c]fluorene; BaP, benzo[*a*]pyrene; CCP, cyclopenta[*cd*]pyrene; Ch, chrysene; DBahA, dibenzo[*a,h*]anthracene; IP, indeno[1,2,3-*cd*]pyrene; 5MeCh, 5-methylchrysene; DBaeP, dibenzo[*a,e*]pyrene; DBahP, dibenzo[*a,h*]pyrene; DBaiP, dibenzo[*a,i*]pyrene; DBalP, dibenzo[*a,l*]pyrene. Reprinted from [36] copyright 2007, with permission from Elsevier.

It is well known that MS has become the most popular detection system in trace analysis due to its intrinsic characteristics such as selectivity, sensitivity, different available monitoring modes, etc. In the determination of PAHs in food and beverages, GC–MS can offer an improvement in selectivity in comparison to LC–FLD; besides, identification and confirmation can be carried out in a single step. Indeed, the re-injection of samples by GC–MS for confirmation purposes when using LC–FLD is often reported (Table 3).

Most of the studies use single quadrupole analyzers (Q) working in the single ion monitoring mode (SIM) (Table 3), whereas other analyzers such as ion trap (IT) [18,31,63,64,66] and triple quadrupole (QqQ) [32,96] are rarely used. For these instruments, selected-ion storage or product ion scan mode (for IT), and selected-reaction monitoring (for QqQ) are normally applied. High-resolution mass spectrometry (HRMS) analyzers (e.g. time-of-flight [34,35,96], magnetic sectors [59–61]) have been also used (Fig. 6), but because of its high cost, its application is not common.

The application of tandem MS and other advanced analyzers would provide an increase in selectivity. However, the use of single-stage MS and Q analyzers is widespread, which could be explained as a consequence of the highly stable structure of PAHs. Despite the use of a high-energy ionization mode, such as electronic ionization (EI), the number of fragments produced is extremely low, mainly the $[M-H]^+$ or $[M-2H]^+$ [27]. These ions are at the same time very stable and complicated to fragment by MS/MS, providing product ions with a few *m/z* units less than the precursor ion. Besides, the application of higher energy values does not change this pattern significantly (e.g. 150 eV).

Finally, in relation to sensitivity, the majority of LODs reported are at the (sub)-/ppb level (Table 3). Significant differences between the performance of Q and other analyzers have not been found. The studies using Q or QqQ and IT analyzers reported LODs (and LOQs when determined) at the sub-ppb level (e.g. [27,31,96]). It must be noticed that similar results for lower limits have also been found when using LC–FLD and GC–MS(/MS). However, the comparison between the LOQs obtained by GC–MS(/MS) and LC–FLD was not possible due to the lack of reported LOQs in many of the revised references.

More detailed information about determination methods and conditions is shown in (Table 3).

4. Determination of PAHs in real samples

A summary of the reported concentrations of PAHs found in real food and beverage samples is shown in Table 4.

A comparison between refined and unrefined oil showed that the levels of BaP in most of refined oils were <1.5 $\mu g\,kg^{-1}$, while for oil of unrefined or oils used for frying, BaP concentrations were found to be >2.0 $\mu g\,kg^{-1}$ (above the maximum permitted level in the Spanish legislation) [25]. Besides, the use of activated carbon in the refinement process, which produces an efficient removal of BaP, explained the lower levels of this compound found in refined oil. In fried oil, BaP levels were higher than in fresh oil; the authors justified this difference because of the high temperatures applied. However, other authors state that the maximum temperature reached during oil frying is not high enough as to generate PAHs [103].

Another comprehensive study (296 samples) [29] revealed that 66.4% of the analyzed edible oil samples exceeded the German Society of Fat Sciences limit (25 $\mu g\,kg^{-1}$). Olive oil showed the maximum concentration (265 $\mu g\,kg^{-1}$) of heavy PAHs, whereas rice brand oil showed the minimum values (4.6 $\mu g\,kg^{-1}$). Phenanthrene (PHE) and ANT were found in more than half of the samples (58.3% and 53%, respectively), whereas BaP was found only in 25.5% of them. One of its isomers, benzo[*e*]pyrene, which is not normally monitored, was found in 31.2% of the studied oils. In total, 88.5% of samples showed PAH contamination, and only 11.5% of them were devoid of any PAH.

Olive pomace oil has also been under study and a high number of PAHs (both light and heavy) have been found in most of the analyzed samples [24]. Besides, it is important to point out that alkyl derivatives, which are rarely determined, were also found and, in many cases, at higher concentrations than the parent PAHs. The high PAH concentration determined in refined olive and olive pomace oil in comparison to virgin olive oil was explained as a consequence of the refining process, which can partially remove these contaminants [31].

Another study focused on the analysis of a variety of edible oils reported that CHR was the most abundant PAH. This result can be related to the high concentrations of 5-methylchrysene reported by the aforementioned study [24]. In relation to olive pomace oil, the authors remarked that the amount of BaP increased from 0.5 $\mu g\,kg^{-1}$ in olive pomace oil samples to 16.1 $\mu g\,kg^{-1}$ in dried oil. Thus, drying stages in the presence of combustion gases can increase PAH contamination [35].

The concentrations of PAHs found in milk samples are, in general, lower than those reported in edible oils (<20 $\mu g\,kg^{-1}$) [36,38,39], which can be due to different reasons: there is not an evident carryover of PAHs along the food chain; milk is less exposed to environmental contamination; and different food processes that are applied in each commodity (Table 4).

Grova et al. [36] described the monitoring of milk samples obtained under different possible sources of contamination, such as cement factories or motorways (Fig. 7). Not surprisingly, milk from farms nearby these sources showed maximum concentrations higher than the concentrations determined in milk from control farms. PAHs with more than four aromatic cycles were not detected and BaP (considered as marker of exposure) was not detected either.

The monitoring of PAHs in infant formula revealed higher PAH concentrations than in commercial and human milk [38]. This important result was explained as a consequence of drying processes which can provoke the formation of PAHs.

Since the manufacturing process in coffee industry also includes roasting stages, coffee samples have been also analyzed in several studies. The results reported by García Falcón et al. [41] showed that PAHs were not found in instant coffee samples but in highly roasted coffee without caffeine. In these samples, BbF, BkF and BaP were found at very low levels: 0.03–0.1 $\mu g\,kg^{-1}$ for BbF and 0.01–0.04 $\mu g\,kg^{-1}$ for BkF and BaP. Houessou et al. found significant differences in the PAH content of lots of coffee from the same origin [44]. These results were potentially attributed to variations in the roasting conditions, and the need for systematic analysis of coffee brews was pointed out. Lai et al. [47] also observed variations in the BaP concentration found in different coffee samples due to the same roasting process. However, Houessou et al. [87] determined in another study that PHE and PYR were mainly found regardless the coffee lot considered. The absence of the highly toxic DBahA in the analyzed samples was also remarked.

In mate infusions, BaP was found in the majority of samples. Considering the European legislation for drinking water as reference, concentrations 5–11.2 times higher than the maximum limit for BaP (0.01 $\mu g\,L^{-1}$) were found [49].

The determination of PAHs in tea leaves demonstrated that the PAH content in the crude black tea and black tea were much higher than the levels found in tea leaves not submitted to the drying stage, which is one of the manufacturing processes in black tea industry [81].

The monitoring of PAHs in spirits revealed that BaA and BbFA were detected in 96% of the analyzed cachaça samples and only one

Table 4
Summary of analyzed PAHs and concentrations found in real samples.

Analytes	Type of sample	Concentration[a]	Observations	Reference
Liquid fatty matrices				
16 EPA PAHs[b]	Edible oils	0.3 (BaA, IP)[c]–1145 (PHE)[d] ng g^{-1}	47 samples	[23]
BaP	Edible oils	Refined oil: <1.5 μg kg^{-1} Unrefined oil: >2 μg kg^{-1}	8 samples (refined, unrefined oils)	[25]
ACP, ANT, BaP, BeP, BghiP, CHR, COR, CPdefPHE, PHE, PYR	Edible oils	Refined vegetable oil: 40.2 μg kg^{-1} (total PAH content) Olive oil: 624 μg kg^{-1} (total PAH content)	296 samples	[29]
BaP	Olive oil	84–89 ng g^{-1}	48 samples	[21]
16 EPA PAHs + 4 EU PAHs[b] + (>35)	Olive oil	0.30–320 (3-methylCHR) μg kg^{-1}	5 samples (olive pomace oil)	[24]
ACP, ACY, ANT, FA, FLR, NPH, PHE, PYR + 2 alkyl derivatives	Olive oil	0.4 (ACP, ACY)–26 (PHE) μg kg^{-1}	10 samples (extra virgin olive oil)	[33]
BkFA, BghiP, BeP, BaP	Olive, olive-pomace oil	0.3 (BghiP, BeP, BaP)–88.7 (BkFA) μg kg^{-1}	25 samples (virgin olive oil, olive pomace oil)	[31]
ACP, ACY, ANT, BaA, BbFA, BjFA, BkFA, BcF, BghiP, BaP, CHR, CPcdP, DBahA, DBaeP, DBahP, DBaiP, DBalP, FA, FLR, IP, MCH, NPH, PHE, PYR,	Vegetable oils	0.5 (BaP)–133.2 (CHR) μg kg^{-1}	14 samples (olive oil, extra virgin olive oil, pomace olive oil, sunflower oil)	[35]
16 EPA PAHs	Fish oil, fish	Fish: 0.06 (BaA)–11.4 (PYR) μg kg^{-1} Fish feed: 0.2 (ACP, ACY)–242 (NPH) μg kg^{-1} Fish oil: 0.3 (ACP, BbFA, BkFA)–38.2 (PHE) μg kg^{-1} Linseed oil: 0.3 (BaP)–16.7 (FA) μg kg^{-1} Palm oil: 0.2 (ACY)–1.4 (BaP) μg kg^{-1} Rapeseed oil: 0.2 (ACY)–1.9 (NPH) μg kg^{-1}	31 samples (fish, fish feed, fish oil, linseed oil, palm oil, rapeseed oils) Fish exposed to long-term feed trials	[96]
ACP, ACY, ANT, BaA, CHR, FA, FLR, NPH, PHE, PYR	Milk	31.9 (PYR)–160.5 (PHE) μg L^{-1}	10 samples	[39]
16 EPA PAHs	Milk	Only 8 PAHs found: ACP, ACY, ANT, BaA, FA, FLR, NPH, PYR Control: 0.3 (ACP)–6.4 (NPH) ng g^{-1} (milk fat) Cementwork: 0.2 (ACP)–16.2 (FLR) ng g^{-1} (milk fat) Motorway: 0.5 (ACP)–10.7 (FLR) ng g^{-1} (milk fat) Combined sources: 0.8 (ACP)–15.2 (NPH) ng g^{-1} (milk fat)	14 samples (control, cementwork, motorway, combined sources) Average values	[36]
ANT, BaP, BaA, BbFA, BkFA, BghiP, CHR, DBahA, FA, IP, PHE, PYR	Milk	Milk: 0.01 (ANT, IP)–0.35 (BbFA) μg kg^{-1} Infant formula: 0.02 (ANT)–0.40 (PHE) μg kg^{-1}	17 samples (commercial milk and infant formula)	[38]
Liquid non-fatty matrices				
BaA, BbFA, BkFA, BaP, BghiP, DBahA, IP	Coffee	0.01 (all except BbF)–0.1 (BbFA) μg kg^{-1}	12 samples (with and without caffeine, natural roasting)	[41]
BaP	Coffee	1.1 ng g^{-1}	1 sample (standard addition method)	[47]
ANT, BaA, BbFA, BkFA, BaP, CHR, FA, FLR, NPH, PHE, PYR	Tea infusion	4 PAHs found: 6.6 (PHE)–82 (FLR) ng mL^{-1}	6 samples	[48]
16 EPA PAHs (except ACY)	Mate tea	1.4 (BaA)–1156 (ACP) ng L^{-1}	11 samples	[49]
BaP	Sugarcane juice	0.05–0.11 μg L^{-1}		[50]
BaA, BbFA, BkFA, BaP, DBahA	Cachaça (spirit)	0.01 (BkFA)–0.83 (BbFA) μg L^{-1}	25 samples	[42]
16 EPA PAHs (except ACY)	Spirits	From burned sugar cane: 0.003 (BghiP)–138 (BaA) μg L^{-1} From non-burned sugar cane: 0.002 (BaP)–3.13 (PHE) μg L^{-1}	131 samples (from burned and non-burned sugar cane crops)	[46]
Solid fatty matrices				
16 EPA PAHs	Smoked meat	<0.20 (BaA, BbFA, BkFA, BaP, BghiP, CHR, FA, PY)–38.59 (PHE) μg kg^{-1}	7 samples (smoked meat)	[17]
15 EU PAHs + BcF	Smoked meat	Before processing: 0.003 (DBalP)–0.101 (BcF) μg kg^{-1} Traditional smokehouse: 0.002 (DBahP, DBalP)–2.134 (BcF) μg kg^{-1} Industrial smokehouse: 0.003 (DBahA, DBalP)–1.539 (BcF) μg kg^{-1}	22 samples (before processing and traditional and industrial smokehouse)	[60]
15 EU PAHs + BcF	Smoked meat	0.001 (DBaHP, DBalP)–10.6 (BcF) μg kg^{-1}	32 samples	[61]
BaA, BbFA, BkFA, BghiP, BaP, CHR, DBahA, FA, IP, PYR	Smoked meat	0.1 (DBahA, BbFA)–26.22 (FA) μg kg^{-1}	18 samples	[59]
BaA, BbFA, BkFA, BaP, DBahA, IP	Fish	14.2 (BaA)–51.4 (BbFA) ng g^{-1}	10 samples	[70]
BaA, BbFA, BkFA, BaP, DBahA, IP	Fish	14.37 (BaP)–42.49 (BbFA) ng g^{-1}	10 samples	[76]
16 EPA PAHs	Fish	0.42 (ACY)–34.48 (BghiP) μg g^{-1}	Number of samples not defined	[64]
16 EPA PAHs (except ACY) + DBalP	Fish	0.12 (PHE)–4.99 (NPH) ng g^{-1}	27 samples	[77]
ANT, BaA, BbFA, BkFA, BaP, BghiP, CHR, DBahA, FA, IP, PYR	Fish, seafood	Summer: 0.12 (ANT)–23.23 (PYR) ng g^{-1} (average values) Winter: 0.35 (FA)–46.01 (CHR) ng g^{-1}(average values)	Number of samples not defined	[68]
ACP, ACY, ANT, FA, FLR, NPH, PHE, PYR	Fish, mussel	0.52 (NPH)–8.00 (PHE) ng g^{-1}	8 samples PAHs showing up to 4 rings	[75]
16 EPA PAHs	Shellfish	24.4–140.0 ng g^{-1} (total PAH content)	10 samples	[63]

Table 4 (*Continued*)

Analytes	Type of sample	Concentration[a]	Observations	Reference
BaP	Cheese	Samples smoked with straw/cardboard: 0.38–2.40 μg kg^{-1} Samples smoked with wood shavings/liquid smoke flavorings: 0.18–0.80 μg kg^{-1}	96 samples	[80]
16 EPA PAHs	Cheese	0.01 (BkFA, BaP, DBahA)–60.0 (NPH, PHE) μg kg^{-1}	36 samples	[78]
16 EPA PAHs	Cheese	0.12–6.21 μg kg^{-1} (total PAH content)	16 samples Analysis before and after smoking	[79]
Solid non-fatty matrices				
16 EPA PAHs (except ACY) + BeP	Bread, potato	Mashed potato: 9.35–17.10 μg kg^{-1} (total PAH content) Potato: 8.47–17.20 μg kg^{-1} (total PAH content) Toasted bread: 7.38–18.00 μg kg^{-1} (total PAH content)	5 samples	[85]
BaA, BbFA, BkFA, BaP, DBahA	Cane sugar	Typical sugar: 0.015 (BaP)–0.300 (BaA) μg kg^{-1}(average values) Organic sugar: 0.002 (BkFA)–0.104 (BaA) μg kg^{-1}(average values)	57 samples (18 organic samples)	[86]
16 EPA PAHs (except ACY)	Foodstuffs	0.08 (ANT)–61.4 (PYR) ng g^{-1}	Number of samples not defined	[88]
15 EU PAHs + BcF	Food supplements	0.02 (BaA, BkFA, BghiP, DAaeP)–32.50 (BcF) μg kg^{-1}	20 samples	[104]
BaA, BbFA, BkFA, BaP, BeP, BghiP, CHR, DBahA, FA, PYR	Fruits, vegetables	Lettuce: 0.08 (BaP)–8.68 (FA) μg kg^{-1} (average values) Tomato: 0.08 (BaP)–6.19 (FA) μg kg^{-1}(average values) Cabbage: 0.06 (BkFA)–5.53 (BkFA) μg kg^{-1}(average values) Fruits: 0.08 (BaP)–6.22 (BghiP) μg kg^{-1}(average values)	Number of samples not defined (combined samples of lettuce, tomato, cabbage, apple, grape and pear)	[84]
16 EPA PAHs	Tea leaves	Leaves: 0.42 (ANT)–83.40 (PYR) μg kg^{-1}(dry mass) Crude tea: 2.35 (DBahA)–1120.00 (PHE) μg kg^{-1}(dry mass) Tea: 8.42 (DBahA)–3930.00 (PHE) μg kg^{-1}(dry mass)	6 samples Leaves analyzed during the whole tea manufacturing process	[81]
ACP, ACY, ANT, BaA, BbFA, BkFA, BaP, CHR, FA, FLR, IP, NPH, PHE, PYR	Vegetables	Potato: 0.23 (ACY)–459 (IP) μg kg^{-1}(average values) Carrot: 0.40 (NPH)–291 (IP) μg kg^{-1}(average values)	21 samples (organic agriculture)	[83]

Abbreviations: COR: Coronene; BeP: Benzo[*e*]pyrene; CPdefPHE: Cyclopenta[*d,e,f*]phenanthrene; 3-methylCHR: 3-methylchrysene; BcF: Benzo[*c*]fluorene; BeP: Benzo[*e*]pyrene (For other abbreviations see Table 1).

[a] Compounds showing the minimum and maximum values of the range are shown in parentheses.

[b] For more details see Table 1.

[c] Compound(s) showing the minimum concentration found.

[d] Compound(s) showing the maximum concentration found.

sample did not contain any of the 5 target PAHs [42]. Different PAH profiles were found in different types of this spirit: BaP showed higher concentrations when burned sugar cane was used in the production, although always below the limit established by the EU for food products (2 μg L^{-1}) [46].

Meat samples have been extensively monitored for PAHs, especially those meat products based on smoked meat. The concentrations found in these products are quite lower than the concentrations found in other important food matrices, such as edible oils. Mottier et al. [17] described that the levels of carcinogenic PAHs were below the LOQ in almost all samples (Fig. 8) and the compounds listed as carcinogenic by the IARC (BaA, BbFA, BkFA, BaP, IP and DBahA were not the major PAHs present in the samples). Beside this, samples containing higher fat content showed a higher PAH total content, which was in accordance with previous studies describing the ratio between PAH formation (during grilling) and fat content.

The smoking technology utilized in the production of smoked products was also found a key factor in the PAH content of the final foodstuff [60]. In a similar study [61], BcF showed the highest

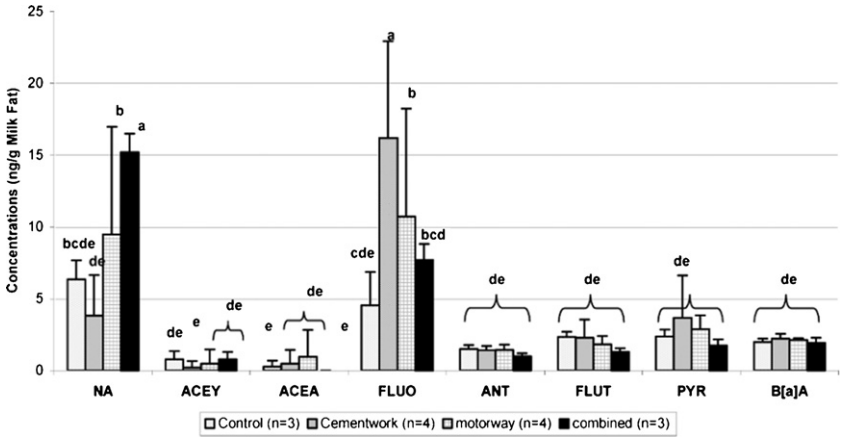

Fig. 7. Concentration of PAHs in milk samples collected close to various potential contamination sources. Abbreviations: NA, naphthalene; ACEY, acenaphthylene; ACEA, acenaphthene; FLUO, fluorene; ANT, anthracene; FLUT, fluoranthene; PYR, pyrene; B[a]A, benz[*a*]anthracene. Reprinted from [37] copyright 2002, with permission from American Chemical Society.

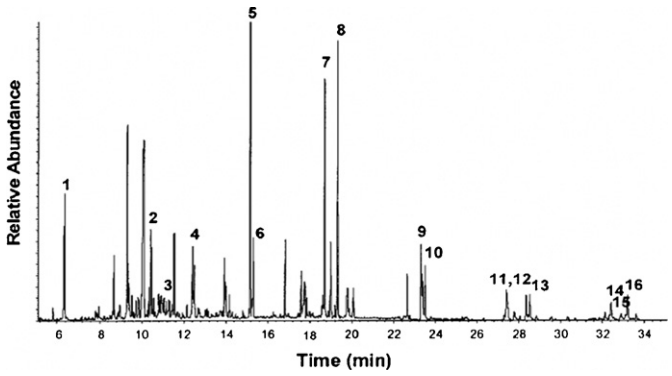

Fig. 8. GC–MS/MS chromatogram of a meat sausage sample containing endogenous PAHs and internal standards (1 μg kg^{-1}). Peak identities are (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) benz[*a*]anthracene; (10) chrysene; (11) benzo[*b*]fluoranthene; (12) benzo[*k*]fluoranthene; (13) benzo[*a*]pyrene; (14) indeno[1,2,3-*cd*]pyrene; (15) dibenzo[*a,h*]anthracene; (16) benzo[*ghi*]perylene. Reprinted from [17] copyright 2000, with permission from American Chemical Society.

concentration in all types of samples. Besides, the total PAH content increased during the different smoking steps.

In general, PAH content in fish (Fig. 9) and seafood samples are considerably higher than those found in meat and similar to the levels determined in edible oils (Table 4). The use of BaP as a marker of the total PAH content was again discussed since it was not detected in the analysis of 27 fish samples when other PAHs were found [77]. This fact was also confirmed in another study [68] focused on the analysis of fish and seafood. Despite several PAHs were found in all samples (ANT, fluoranthene (FA), PYR, BaA, CHR, BbFA and BkFA), BaP was only detected in Mediterranean mussels. In this kind of samples, a seasonal variation was found: 72% of mussels collected in winter exceeded the EU MRL set for BaP, whereas the values shown by mussels collected in summer were below this limit. Moreover, comparing the PAH levels of the different organisms evaluated, mussels showed the maximum amounts.

The evaluation of the PAH profiles in food from marine origin revealed that PYR was the major PAH, representing more than 80% of the total content in all samples, except in prawns, where NPH showed the maximum contribution (49%) [88]. Another compound often found was PHE, which is one of the main components of crude oil. In this sense, the authors remarked that profiles showing high percentages of light PAHs are typical of oil-polluted areas and intense oil activity.

A thorough study of the levels of BaP in cheese (96 samples) demonstrated that the concentrations in cheese smoked with straw and cardboard were statistically higher than the concentrations found in samples smoked and aromatized with wood shavings and SFPs [80]. However, samples treated with SFPs showed BaP concentrations exceeding the limits set by the EU. BaP levels were dependent on the smoking process (temperature, time, etc.), which was also observed in other smoked products. In another study [78], the PAH content found in home-made smoked cheese was up to 10 times higher than in cheese smoked under industrial conditions (Fig. 10). This trend was also observed for BaP. Besides, significant differences were observed in the PAH content when comparing the cheese surface and internal parts of the product.

The analysis of cane sugar showed PAH contamination in 57% of the samples; BaA was found in 51% of the samples [86]. The obtained results suggested that the PAH content relied on the amount of burnt sugar cane utilized (if so), and in the number of steps involving the refining process. Furthermore, PAHs were also detected in 33% of the organic samples analyzed. The deposition of airborne particles containing PAHs on the plant surface was proposed as a possible explanation, probably because of the environmental pollution.

Danyi et al. [104] determined that 50% of the food dietary supplement samples submitted to analysis showed PAH concentrations above the limit established by the EU (2 μg kg^{-1}) for one to seven PAHs. In general, light PAHs were mostly found and several genotoxic PAHs were found at relatively high concentrations in certain food supplements from plant origin.

A recent study involving the monitoring of parent PAHs and hydroxy-PAHs in infant milk and cereals demonstrated the absence

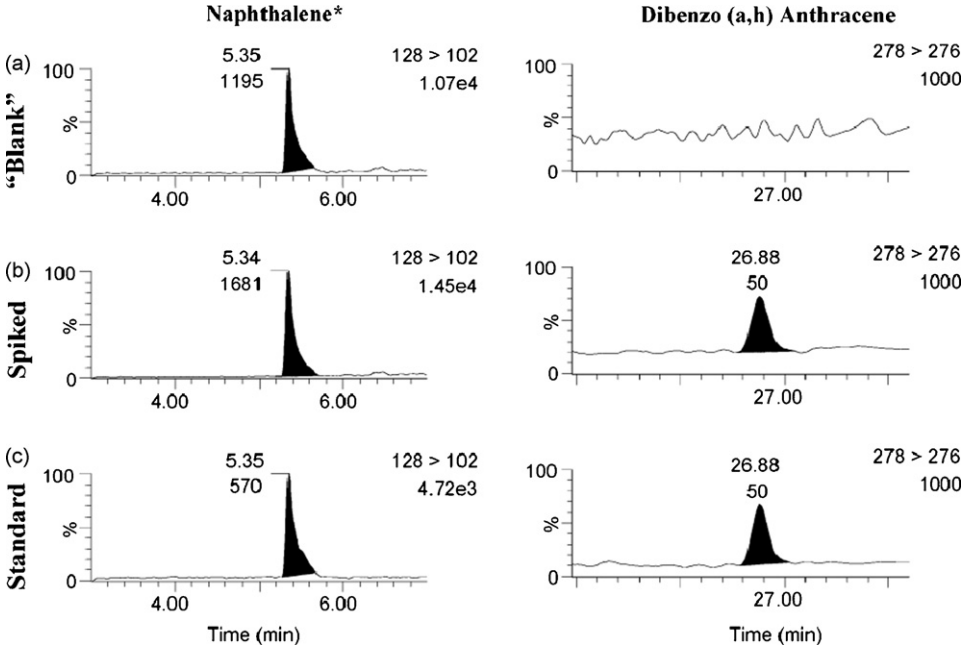

Fig. 9. GC–MS/MS chromatograms in the selected-reaction monitoring (SRM) mode of different fish samples: (a) non-spiked sample; (b) spiked sample (1.25 μg kg^{-1} for naphthalene and 0.125 μg kg^{-1} for dibenzo[*a,h*]anthracene); and (c) matrix-matched standard (10 ng mL^{-1}, equivalent to 1.25 μg kg^{-1} for naphthalene; and 1 ng mL^{-1}, equivalent to 0.125 μg kg^{-1} for dibenzo[*a,h*]anthracene). Reprinted from [89] copyright 2009, with permission from John Wiley and Sons.

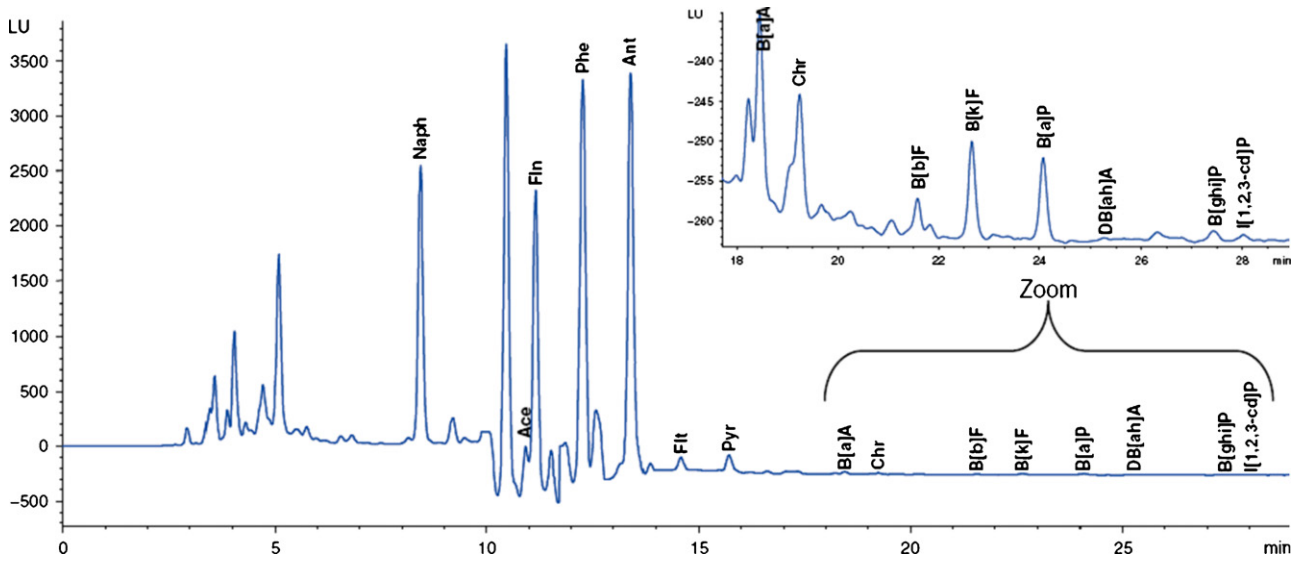

Fig. 10. HPLC–FLD chromatogram of a real smoked cheese sample with PAH concentrations in the range 0.03–60 μg kg^{-1}. Abbreviations: Naph, naphthalene; Ace, acenaphthene; Fln, fluorene; Phe, phenanthrene; Ant, anthracene; Flt, fluoranthene; Pyr, pyrene; B[a]A, benz[*a*]anthracene; Chr, chrysene; B[b]F, benzo[*b*]fluoranthene; B[k]F, benzo[*k*]fluoranthene; B[a]P, benzo[*a*]pyrene; DB[ah]A, dibenzo[*a,h*]anthracene; B[ghi]P, benzo[*ghi*]perylene; I[1,2,3-cd]P, indeno[1,2,3-*cd*]pyrene. Reprinted from [73] copyright 2008, with permission from John Wiley and Sons.

of PAH metabolites. However, parent PAHs were found in two samples (total number of samples: 36), namely B[*k*]F at 0.1 and 0.3 μg kg^{-1} [94].

The study carried out by Rojo-Camargo et al. [84], which was focused on the analysis of a variety of vegetable and fruit samples, showed that BaA was detected in 89% of the samples. Vegetables, which are rarely monitored, also showed PAH contamination at levels higher than those found in fruits (4.38–17.93 μg kg^{-1}, and 3.77–4.05 μg kg^{-1}, respectively). Lettuce and grape were the matrixes showing higher PAH total content. Moreover, vegetable samples collected close to road traffic revealed higher PAH levels than in samples grown in rural areas. It is important to notice that organic samples also shown PAH contamination, probably due to the exposure to environmental pollution, as aforementioned in sugar cane samples.

5. Conclusions

The determination of PAHs in foodstuffs and beverages has focused attention for a long time, as it is demonstrated by existing publications in the early 1990s. However, a high percentage of this literature has been devoted to the analysis of the PAHs included in the well-known EPA list, and especially BaP. It seems necessary to increase and improve the available information and data about the occurrence of other PAHs, such as those included in the EU list (e.g. dibenzopyrenes) or transformation products (e.g. alkyl derivatives or hydroxy-PAHs), in order to achieve a better knowledge about PAH levels in foodstuffs. On the other hand, the revised literature shows that edible oils and animal products are the most analyzed matrices for PAHs, which was expected as many of the current legislation is established for them. Additional data about the levels of PAHs found in other food matrices, such as vegetables or livestock, which can be exposed to PAH contamination when roads or traffic are nearby, could be of interest.

In relation to the extraction techniques, conventional techniques such as Soxhlet (solid matrices), LLE and SPE (liquid matrices) are still widely used, although the application of less-solvent-consuming techniques, such as MSPD, and microextraction techniques, such as LPME, has been recently reported. The performance of clean-up stages is also requested for most of applications, but the utilization of techniques such as SPME or HS-SPME has permitted the reduction of the pre-treatment stage. The clean-up stage is still a time-consuming step, especially in fatty matrices. SPE and GPC are the most applied techniques for this aim.

It is important to notice, that the implementation of the most recent extraction techniques, such as LPME, MSPD or (HS)-SPME is still reduced in routine analysis, but they must be considered as new approaches that can be useful in the near future for this aim in terms of solvent consumption or sample throughput.

With respect to separation and detection issues, LC–FLD and GC–MS(/MS) are the outstanding techniques, although any of them is particularly preferred. The revised literature shows the utilization of both techniques without regarding the type of sample or extraction methodology. Nevertheless, it must be noticed that GC–MS(/MS) instruments have been used in the most recent studies. This trend is contrary to the so-called GC-to-LC movement observed in the analysis of other organic compounds at trace levels (e.g. veterinary drugs). This fact can be justified due to the need for a more accurate confirmation of the identity of the compound, which is not possible only with determinations by LC–FLD. Besides, the lack of native fluorescence of certain PAHs does not permit the monitoring of such compounds, which is an obvious drawback in comparison to GC–MS(/MS).

6. Trends

Bearing in mind the aforementioned considerations and conclusions and the current challenges, more research and efforts should be focused on specific issues such as:

- A more thorough evaluation of recent API sources for LC instruments, such as APPI, which are designed for more non-polar compounds, in order to establish their possible utilization for the determination of PAHs in LC–MS systems. The comparison of these instruments with conventional LC–FLD would be also of interest since simultaneous identification and confirmation would be possible.
- The feasibility of the APPI sources for the determination of PAHs, but especially compounds not included in the EPA list (e.g. heavy PAHs such as dibenzopyrenes) should be evaluated.
- UHPLC instruments, which are widely applied for the analysis of other organic compounds at trace levels, have not been used for

PAH analysis yet. The increase in resolution provided by these systems may also allow an increase in selectivity, especially in very complex matrices and when using FLD as detection technique. Besides, the study of the suitability of UHPLC coupled to MS and the aforementioned ionization sources in this field would be also desirable.

- More data about the utilization of specific columns for PAH analysis in food by GC should be generated. Although the typical 5%-phenyl columns are widely used, the separation of certain groups is not achievable and heavy PAHs, such as dibenzopyrenes, do not show adequate peak shape and/or sensitivity. These problems can be overcome employing other stationary phases, but their use is very scarce.
- Extra efforts should be made in order to improve the data about PAH concentrations in food products exposed to possible contamination, such as agricultural areas near to road traffic.
- Data about PAHs out of the EPA and EU lists should be increased in order to propose new maximum concentrations as well as extended lists of priority PAHs.

Acknowledgments

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