

# HC-0A-02: Analysis of Polycyclic Aromatic Hydrocarbons from Food

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**Abstract** Polycyclic aromatic hydrocarbons (PAHs) comprise a class of organic compounds of petrogenic origins or generated from the incomplete combustion of organic matter. Their presence in the environment can be a source of food contamination, which implies a potential risk to human health according to the International Agency for Research on Cancer. Additionally, the Food and Agriculture Organization of the United Nations (FAO/WHO) and the Scientific Committee on Food consider PAHs to be genotoxic and carcinogenic. The greatest concern has been raised regarding the possibility of cancer induction in humans exposed to PAHs from contaminated food. Therefore, many regulations and reports regarding strategies for and results of food monitoring have been published, including performance criteria for the sampling, chemical analysis and maximum permitted levels of these contaminants in food. A variety of techniques have been reported for the extraction, clean-up and determination steps of the analytical process. The details and advantages of each technique are discussed in this chapter. Additionally, because method validation is one measure that laboratories must implement to guarantee the reliability, traceability and comparability of their results, a review and critical analysis of the current practices in this area are also presented.

**Keywords** Polycyclic aromatic hydrocarbons • Analytical methods • Food • Method validation

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## 1 Introduction

Polycyclic or polynuclear aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings in linear, angular, or cluster arrangements. By definition, they contain only carbon and hydrogen (Baek et al. 1991; Albers 2002).

PAHs can be formed in any incomplete combustion or high temperature pyrolytic process involving fossil fuels, plastic products, cigarette smoke, waste incineration, forest fires, combustion engines and, more generally, in processes involving materials containing C and H (Codex Alimentarius 2005; Poster et al. 2006). Furthermore, a variety of PAHs are naturally present in crude oil (0.2–7 %) and coal, with configurations ranging from two to six rings and arising from the chemical conversion of natural precursors for PAHs, such as steroids and terpenes (Harvey 1998; Albers 2002; Feng et al. 2009; Pampanin and Sydnés 2013).

PAHs were one of the first atmospheric pollutants to be identified as being carcinogenic (ATSDR 1995; WHO 2006). Their presence in food implies a potential risk to human health according to the International Agency for Research on Cancer (IARC) (IARC 2012). Additionally, the Food and Agriculture Organization of the United Nations (FAO/WHO) (WHO 2006) and the Scientific Committee on Food (SCF) (EC 2002) consider PAHs to be genotoxic and carcinogenic and recommend monitoring the presence of PAHs in food.

The main techniques used for the detection and quantification of PAHs from food matrices include gas chromatography (GC) coupled to mass spectrometry (MS) and high performance liquid chromatography (HPLC) with fluorescence detection (FL). HPLC coupled to MS or GC tandem MS (MS/MS) and time of flight (TOF) are also used. Fat extraction and clean-up steps are often required for the determination of PAHs from complex matrices such as food. The applications of both conventional and recently developed extraction and clean-up techniques, such as pressurized liquid extraction (PLE) and QuEChERS (quick, easy, cheap, effective, rugged and safe), are presented. For the clean-up step, solid-phase extraction (SPE), including conventional SPE, dispersive SPE, solid-phase micro-extraction (SPME) and molecularly imprinted polymer (MIP) SPE, as well as gel permeation chromatography (GPC) and donor–acceptor complex chromatography (DACC) are shown. The details and advantages of each technique are discussed.

The importance of validation and a review of the approaches for the in-house validation of the proposed methods for PAHs determination from food are presented and confronted with the recommendations laid in harmonized international guidelines and the regulated performance requirements.

## 2 Physicochemical Properties of PAHs

Knowledge of the physico-chemical properties of the PAHs (Table 1) allows for an understanding of the distribution profile of these contaminants in food matrices, as well as their interactions and stability, which will guide the choice of strategies used for their extraction from food.

In the environment, PAHs occur as compounds containing between two and seven conjugated rings and have molar masses ranging from 128 to 302 g/mol (Ming-Ho 2005). According to the United States Environmental Protection Agency

**Table 1** Physico-chemical properties and classification by the International Agency for Research on Cancer (IARC) regarding the carcinogenicity of the sixteen polycyclic aromatic hydrocarbons (PAHs) prioritized by the United States Environmental Protection Agency (EPA 1986; IPCS 1998; IARC 2012)

PAHs	No of rings	Molecular weight (g/mol)	Solubility in water at 25 °C (mg/L)	Vapor pressure 25 °C (Pa)	Octanol-water partition coefficient Log KOW (-)	IARC group
Naphthalene	2	128	$3.1 \times 10^1$	$1 \times 10^1$	3.37	2B
Acenaphthylene	3	152	3.9	$9 \times 10^{-1}$	4.00	Not classified
Acenaphthene	3	154	3.8	$3 \times 10^{-1}$	3.92	3
Fluorene	3	166	1.9	$8 \times 10^{-2}$	4.18	3
Phenanthrene	3	178	1.1	$2 \times 10^{-2}$	4.57	3
Anthracene	3	178	$4.5 \times 10^{-2}$	$8 \times 10^{-4}$	4.54	3
Fluoranthene	4	202	$2.6 \times 10^{-1}$	$1.2 \times 10^{-3}$	5.22	3
Pyrene	4	202	$1.3 \times 10^{-1}$	$6 \times 10^{-4}$	5.18	3
Benzo(a)anthracene	4	228	$1.1 \times 10^{-2}$	$2.8 \times 10^{-5}$	5.61	2B
Chrysene	4	228	$2.0 \times 10^{-3}$	$8.4 \times 10^{-5}$	5.91	2B
Benzo(b)fluoranthene	5	252	$1.5 \times 10^{-3}$	$1.3 \times 10^{-7}$	5.80	2B
Benzo(k)fluoranthene	5	252	$7.6 \times 10^{-4}$	$1.3 \times 10^{-7}$	6.84	2B
Benzo(a)pyrene	5	252	$3.8 \times 10^{-3}$	$7.3 \times 10^{-7}$	6.50	1
Indeno(1,2,3-c,d)pyrene	6	276	$6.2 \times 10^{-2}$	$1.3 \times 10^{-8}$	6.58	2B
Dibenzo(a,h)anthracene	5	278	$6.0 \times 10^{-4}$	$1.3 \times 10^{-8}$	6.50	2A
Benzo(g,h,i)perylene	6	276	$2.6 \times 10^{-4}$	$1.4 \times 10^{-8}$	7.10	2B

*Group 1* sufficient evidence in humans or in animals and strong mechanistic data in humans, *Group 2A* limited evidence in humans and sufficient evidence in animals, *Group 2B* limited evidence in humans and less than sufficient evidence in animals, *Group 3* inadequate evidence in humans and inadequate or limited evidence in animals

(USEPA), sixteen PAHs are considered particularly important regarding the environmental monitoring of organic pollutants (EPA 1986).

The physico-chemical characteristics of these PAHs, such as solubility and vapor pressure, are important factors that drive the distribution of these contaminants in soluble and particulate phases throughout atmospheric, aqueous and biotic media. In general, the solubility of PAHs in water decreases with increasing molecular weight and ranges from highly insoluble, such as for benzo(g,h,i)perylene (solubility 0.0003 mg/L), to slightly soluble, such as for naphthalene (solubility 31 mg/L). PAHs can also be classified as moderately to highly soluble, with octanol-water partition coefficients (log KOW) ranging between 3.37 and 7.10 (IPCS 1998; Meire et al. 2007).

Most PAHs are of low volatility and have a high tendency to adsorb onto organic particulate matter. In the atmosphere, PAHs containing five or more aromatic rings are found predominantly in association with particulates, usually on small (<2.5  $\mu\text{m}$ ) particles, such as fly ash and soot. PAHs with two or three rings are almost entirely found in the vapor phase, whereas those with four rings exhibit an intermediate behavior (EC 2002). In aqueous environments, PAHs are generally found adsorbed on particulates and humic matter or dissolved in any oily contaminant that may be present in the water, sediment or soil (EFSA 2008b).

PAHs are chemically stable and resistant to degradation by hydrolysis. In the presence of light, they are susceptible to oxidation and photo-degradation. Depending on various parameters (such as the type of adsorption onto particles and molecular mass), the half-lives of PAHs in air range from a few hours to days. In soil, PAHs may also be degraded by microbial activity. The estimated half-lives of PAHs in soils vary from several months to several years (Harvey 1998; EC 2002).

### 3 Toxicity of PAHs

The toxicological properties of PAHs highlight the importance of determining these compounds in food, even at very low levels.

PAHs are the most extensively studied compounds with regard to their cancer-inducing properties in laboratory animals. Researchers have shown that certain PAHs can cause cancer when inhaled, ingested and even after skin contact. Immunosuppression, hepatic hypertrophy and changes in the growth of other tissues have also been reported in laboratory animals (ATSDR 1995). The evidence of carcinogenicity in humans comes from occupational studies in workers exposed to known sources of PAHs, such as oil refineries, charcoal ovens and chimneys (ATSDR 1995; WHO 2006).

Many PAHs are considered toxic even at low concentrations. Lower molecular weight compounds consisting of two or three rings (such as naphthalene, anthracene, and phenanthrene) have high acute toxicity but little or no carcinogenic potential. Conversely, compounds with higher molecular masses that consist of four, five or six rings (such as benzo(a)pyrene) have low acute toxicity but greater

carcinogenic potential (Meire et al. 2007). Acute effects affect mainly the liver and kidneys, leading to cutaneous inflammation, ulcerations and hyperkeratosis. Changes in the lymph nodes and immunosuppressive induction have also been reported (WHO 2006).

In 2012, the IARC updated the classification of benzo(a)pyrene to Group 1-Carcinogenic to humans (IARC 2012). This group includes 116 agents (or mixtures) with sufficient evidence of carcinogenicity in humans. In Table 1, the classification of the sixteen PAHs prioritized by the EPA is presented.

PAHs are considered to be pro-carcinogenic, i.e., they must be altered by metabolic activation to generate the active carcinogen capable of reacting with DNA and other molecules (Ming-Ho 2005). The carcinogenicity of PAHs is associated with the complexity of the molecule, i.e., the number of benzene rings present. The mechanism of toxicity involves the oxidation of the aromatic rings by the enzymes of the cytochrome P450 family, which generates epoxide intermediates that covalently bind to critical sites in the DNA, causing replication errors and allowing mutation to occur (Boström et al. 2002; EC 2002). The main sites are the amino groups of adenine and guanine (WHO 2006).

Some PAHs and their metabolites also bind the aryl hydrocarbon receptor, resulting in the up-regulation of several enzymes involved in the metabolism of PAHs and exhibiting complex and non-linear dose-response curves when mixtures of PAHs are present (WHO 2006). This phenomenon hinders the establishment of safe limits for the intake of these contaminants.

Thus, a minimum safe threshold for chronic exposure has not been established by international agencies and health authorities, such as the Agency for Toxic Substances and Disease Registry (ATSDR), the SCF and the EFSA. Instead, these agencies recommend that exposure to PAHs be as low as is reasonably achievable (Bulder et al. 2006).

Other international organizations have calculated a so-called “virtually safe dose” based on extrapolating the data obtained from experiments with laboratory animals. The EPA associates an intake of 0.14 ng/kg body weight/day of benzo(a)pyrene with a cancer risk of  $1 \times 10^{-6}$ . The Dutch National Institute of Public Health and Environment (RIVM) calculated the virtually safe dose for the same cancer risk to be 0.50 ng/kg body weight/day (Bulder et al. 2006).

## 4 PAH in Food

### 4.1 Contamination and Occurrence

The presence of PAHs in food has been extensively described in the literature (Diletti et al. 2005; Houessou et al. 2005; Ballesteros et al. 2006; Liguori et al. 2006; Ciecierska and Obiedzinsk 2007; Rose et al. 2007; Stanciu et al. 2008; Windal et al. 2008; Veyrand et al. 2007; Ziegenhals et al. 2009; Belo et al. 2012;

Pissinatti et al. 2014). There are several routes of food contamination by PAHs, and contamination can arise from both environmental pollution (fruits, vegetables and grains grown in industrial regions) and food processing (such as smoking and roasting) (Camargo and Toledo 2002; Tfouni et al. 2012). PAHs from environmental pollution have both natural sources (bitumens, coal, forest and prairie fires, oil seeps, plant debris) and anthropogenic sources (fossil fuels and combustion). Since the PAH compositions of the two sources overlap, the significance of anthropogenic PAH in the environment have been evaluated against a dynamic background of natural PAH (Yunker et al. 2002). Some authors have proposed the estimation of isomeric ratios to predict the source of the contamination (Budzinski et al. 1997; Yunker et al. 2002; Mannino and Orecchio 2008; Orecchio 2010).

The values of ratios between anthracene (An) and anthracene plus phenanthrene (An + Phe) when lower than 0.10 indicate low temperature sources (petroleum) while ratios bigger than 0.10 indicate a dominance of combustion (Yunker et al. 2002), because phenanthrene and anthracene are isomers and phenanthrene is more thermodynamically stable than anthracene (Budzinski et al. 1997). BaA/ (BaA + Chr) ratios lower than 0.20 involve petroleum, from 0.20 to 0.35 indicate either petroleum or combustion and upper than 0.35 combustion. Also, IP/ (IP + BghiP) ratios between 0.20 and 0.50 imply liquid fossil fuel combustion (Yunker et al. 2002). The ratio between fluoranthene (Fl) and fluoranthene plus pyrene (Fl + Py) have also been applied for PAHs source typing (Budzinski et al. 1997).

In vegetables and fruits, PAH contamination mainly occurs via the deposition of particles from air pollution on their surfaces. The concentrations depend on the location of cultivation and production, and samples cultivated in highly industrialized areas or near roads and express highways generally have higher levels of PAHs than those cultivated in rural areas. The association between PAHs with higher molecular masses and suspended particulate matter in the atmosphere is a major source of contamination (Nielsen et al. 1999). The concentrations of PAHs are generally higher at the surfaces of plants, such as fruits shells and edible leaves, than in the internal tissues. The higher levels of PAHs found in plants grown in areas with polluted air are especially evident in plants with a large exposed surface area, for example, lettuce, cabbage and spinach (EC 2002).

During the process of smoking, PAHs may be deposited on the surface and migrate into the food that is being smoked. Numerous factors in the smoking process influence the composition of the smoke and the absorption of PAHs by smoked food, with the combustion temperature during the generation of the smoke being a particularly critical parameter (Wretling et al. 2010).

In general, due to the lipophilic properties of PAHs, oils and fats are more susceptible to concentrated contamination from environmental sources or during the process of seed drying prior to oil extraction. Smoked foods, such as meat and fish, can also have high levels of PAH contamination. However, the contribution of these foods to the total ingested PAHs is not significant because the levels of these products in the population's diet are usually low (Codex Alimentarius 2009).

A survey published in 2008 by EFSA, which involved data from sixteen European countries, indicated that fish and seafood, vegetable oils, meat, coffee and tea, food supplements and spices as the main food groups susceptible to PAH contamination. Those types of food presented levels exceeding 10 µg/kg for the sum of the following eight PAHs: benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene ( $\Sigma$ PAH8). These results suggest that attention should be paid to coffee and tea, food supplements and spices because there are no current regulations for the PAHs levels in these food matrices (EFSA 2008a, b).

Studies conducted in Italy (Lodovici et al. 1995) highlighted the fact that exposure to PAHs from contaminated food intake is significantly higher than environmental exposure or exposure through inhalation or skin absorption. According to the EFSA Report, the diet is the largest non-occupational source of PAH exposure for non-smokers. In this European study, based on the dietary profiles of the 16 countries involved, an average dietary exposure for the whole population (including the non-consumers) would provide 235 ng/day of benzo(a)pyrene and 1729 ng/day of all eight PAHs, whereas a diet high in PAHs would provide 389 ng/day of benzo(a)pyrene and 3078 ng/day of all eight PAHs. The products with the largest contributions to PAH exposure, considering the median consumption estimated for consumers only, were seafood, cereals, vegetables, meat, oils and fats, fish and coffee in terms of both benzo(a)pyrene alone and all eight PAHs (Table 2). It must be emphasized that, in this study, the number of cereal and coffee samples was significantly lower than the other matrices studied (EFSA 2008a, b).

In this context, the use of benzo(a)pyrene as a marker for the presence of PAHs in food, as proposed by the SCF (EC 2002), is controversial. The survey data collected by the EFSA (EFSA 2008a, b) also show that the concentrations of benzo(a)pyrene and other PAHs, such as pyrene and benzo(a)anthracene, which are also considered toxic, presented a low correlation in products such as fish, crustaceans, tea and coffee. Among 9714 samples comprising 95 groups of Codex Alimentarius food matrices, 33 % had values exceeding the detection limit for one or more priority PAH, and benzo(a)pyrene was not detected.

According to the report published by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA 2004), in which PAHs occurrence data from more than eight thousand samples in 44 different food groups were compiled, the highest levels of benzo(a)pyrene were found in dried fruits (48.10 µg/kg wet weight), olive pomace oil (17.7 µg/kg), smoked fish (5.28 µg/kg), grape seed oil (4.2 µg/kg), smoked meat products (3.27 µg/kg), fresh mollusks (3.09 µg/kg), and spices/sauces and condiments (2.16 µg/kg).

Several studies have reported evidence of increased PAH concentrations in seafood after oil spills compared with baseline levels. Law et al. (2002) examined PAH levels based on datasets covering background levels and seafood after oil spills from 19 studies and found average total PAH concentrations between 20 and 1600 µg/kg in baseline monitoring studies and between 104 and 27,400 µg/kg after

**Table 2** Consumer exposure estimated by European Food Safety Authority (EFSA) to benzo(a)pyrene (BaP) alone,  $\sum$ PAH2 (BaP and chrysene),  $\sum$ PAH4 (PAH2, benzo(a)anthracene, and benzo(b)fluoranthene), and  $\sum$ PAH8 (PAH4, benzo(k)fluoranthene, benzo(ghi)perylene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene) for each food category for which occurrence data were available. (EFSA 2008a, b)

Food category	Consumption median (g/day)	Exposure (ng/day)			
		$\sum$ BaP	$\sum$ PAH2	$\sum$ PAH4	$\sum$ PAH8
Alcoholic beverages	413	4	12	25	74
Cereals and cereal products	257	67	129	257	393
Cheese	42	6	12	20	30
Coffee, tea and cocoa (expressed as liquid)	601	21	55	106	156
Fats (vegetal and animal)	38	26	112	177	239
Fish and fishery products	41	21	84	170	210
Fruits	153	5	40	75	87
Meat and meat products (including substitutes)	132	42	107	195	279
Seafood and seafood products	27	36	140	289	421
Sugar and sugar products (including chocolate)	43	5	13	25	39
Vegetables, nuts and pulses	194	50	124	221	378

Median consumption: estimated by the Member States for consumers only

different oil spills. Studies of oils spills suggest that several factors may play a role in determining the duration of PAH contamination, including the amount of sedimentation and the likelihood of subsequent resuspension of the oil, the composition of the oil, the rate of biodegradation (which tends to be higher in warmer climates), and the particular species of interest (Gohlke et al. 2011). Among fishery products, finfish species (i.e., tuna, mackerel and salmon) generally present lower contamination levels than shellfish (i.e., bivalve mollusks), even if they originated from polluted areas. Indeed, unlike bivalves, fish oxidize and metabolize PAHs to water-soluble compounds, which are eventually excreted (EU 2011b; Gohlke et al. 2011; Purcaro et al. 2013).

## 4.2 Regulation

Until 2011, the maximum permitted level described in European legislation was applied only for benzo(a)pyrene. The report published by the EFSA (2008a, b) concluded that benzo(a)pyrene was not a suitable marker for the occurrence of PAHs in food and that a system of four specific substances would be appropriate to



determine the carcinogenic potency of PAHs in food. Thus, the European regulation 1881/2006 was amended by the regulation 835/2011 (EU 2011b) with regard to the permitted levels for benzo(a)pyrene only, as well as for the sum of benzo(a)pyrene, chrysene, benz(a)anthracene, and benzo(b)fluoranthene ( $\sum$ PAH4).

The regulation EU/835/2011 (EU 2011b) also updated the matrices to be monitored based on new occurrence data. Therefore, it was concluded that maintaining a maximum level for PAHs in fresh fish (muscle) was no longer appropriate, as PAHs are quickly metabolized in fresh fish. Other limits were added, namely, for cocoa beans and derivatives and coconut oil (limits described in terms of fat). Other matrices affected by this legislation are as follows: oils and fats, smoked products, bivalve mollusks and baby food for infants and young children. For the latter, the maximum permitted levels were set at 1.0  $\mu\text{g/kg}$  (benzo(a)pyrene and  $\sum$ PAH4) and ranged up to 6  $\mu\text{g/kg}$  (benzo(a)pyrene) or 35  $\mu\text{g/kg}$  ( $\sum$ PAH4) for bivalve mollusks.

## 5 Analytical Methods for PAHs from Food

In Table 3, a review of the published analytical methods for the determination of PAHs from food is shown in chronological order. The matrices and measured parameters are listed, in addition to the analytical steps and parameters, such as sample size, extraction and clean-up techniques, and detection and quantification techniques, including details about chromatographic columns, detectors and the use of internal standards.

A discussion about the characteristics of the different techniques presented in Table 3, with notes concerning limitations and advantages of some of them, is made in the following Sect. 5.1–5.3.

### 5.1 *Extraction and Clean-up*

Traditional methods for the extraction and clean-up of PAHs from food matrices are laborious and require large amounts of solvent. Usually, sample extraction involves saponification with potassium hydroxide solution, followed by liquid–liquid partition with organic solvents and column purification using silica gel, alumina or Florisil as the stationary phase (Badolato et al. 2006; Stanciu et al. 2008). These methods have significant drawbacks, including the need for high purity solvents and the formation of emulsions that are difficult to break, which may affect the recovery of some measured parameters (Orecchio et al. 2009).

The need for more reliable analytical methods, as well as consideration of environmental and sustainability issues, has driven the development of new technologies for the extraction and purification of samples and the miniaturization of analytical systems.

**Table 3** Review of analytical approaches for the determination of PAHs in foods: food matrices, parameters measured, and extraction, clean-up and determination techniques

References	Food matrix	Parameter measured (PAHs and respective monitored ions)	Analytical method				Detection and quantification	Column	Detector details	Internal standard
			Sample size	Extraction	Clean-up					
Diletti et al. (2005)	Olive pomace oil	BaA (228 226), BbF (252 250), BkF (252 250), BaP (252 250), BeP (252 250), IP (276 277), DBaHA (278 279) and BghiP (276 277)	10 g	Liquid-liquid partition (10 mL n-pentane followed by 15 + 10 + 10 mL dimethyl sulfoxide and 50 mL cyclohexane three times)	TLC (silica gel)	GC-MS	DB-5MS (30 m × 0.25 mm i.d., 0.25 µm)	Ion Trap (Polaris Q, Thermo)	Isotope labeled ( <sup>2</sup> H)	
Houssou et al. (2005)	Coffee brew	Fl, BbF, BaP	50 mL	SPE (0.5 g polystyrene-divinylbenzene cartridge) Extraction (methanol:tetrahydrofuran 10:90, v/v)	No further clean-up	HPLC-FL	Supelcosil LC-PAH (150 mm × 3.0 mm i.d., 5 µm)	–	Not used	
Badolato et al. (2006)	Coffee	BaP	20 g	Soxhlet (250 mL acetone) Saponification (50 mL methanol: water 9:1, v/v and 1.49 g potassium hydroxide) Liquid-liquid partition (40 mL cyclohexane three times)	Column (5 g silica gel)	HPLC-FL	Lichrosphere 100 RP18 (150 mm × 4.6 mm i.d., 5 µm)	–	Not used	
Ballesteros et al. (2006)	Olive and olive-pomace oil	BkF (252 > 226 + 250 + 251), BeP (252 > 226 + 250 + 251), BaP (252 > 226 + 250 + 251) and BghiP (276 > 274 + 275)	2 g	Extraction (2 mL n-hexane, 10 mL acetonitrile and 3 mg of anhydrous sodium sulfate)	GPC (CL <sub>2</sub> CH <sub>2</sub> )	GC-MS/MS	HP-5 (30 m × 0.25 mm i.d., 0.25 µm)	Ion Trap (Saturn 2200, Varian)	p-terphenyl	
Liguori et al. (2006)	Blue mussel, salmon fillet, fish oil and fish feed	Na (128), 2-MeNa (142), 2,6-DiMeNa (156), Ap (152), Ac (154), 2,3,6-TriMeNa (170), F (166), Phe (178), A (178), 2-MePhe (192), 3,6-DiMePhe (206), Fl (202), Py (202), 1-MePy (216), Tph (228), Ch (228), BaA (228), BkF (252), BbF (252), BaP (252), 6-MeBaP (266), IP (276), DBaHA (278) and BghiP (276)	5 g (mussel), 1.3 g (salmon), 1.5 g (fish feed) and 0.25 g (fish oil)	PLE in cell clean-up (extraction solvent: dichloromethane and adsorbents: 22 g aluminum oxide, 16 g silica gel and 3 g magnesium sulfate)	GPC (CL <sub>2</sub> CH <sub>2</sub> )	GC-MS	DB-5MS (30 m × 0.25 mm i.d., 0.25 µm)	Single quadrupole (5972, HP)	Isotope labeled ( <sup>2</sup> H)	

(continued)

Table 3 (continued)

References	Food matrix	Parameter measured (PAHs and respective monitored ions)	Analytical method		Clean-up	Detection and quantification	Column	Detector details	Internal standard
			Sample size	Extraction					
Aguinaga et al. (2007)	Milk	Na (128, 127), Ap (152, 151), Ac (154, 153), F (166 165), Phe (178, 176), A (178, 176), Fl (202, 101), Py (202, 101), BaA (228, 114), Ch (228, 114), BbF (252, 126), BkF (252, 126), BaP (252, 126), IP (278, 276), DBaH (278 276), BghiP (276, 278)	5 mL (liquid sample) 5 g (solid sample)	Immersion SPME (100 µm polydimethylsiloxane, 65 µm olydimethylsiloxane-divinylbenzene and 85 µm polyacrylate)	No further clean-up	GC-MS	HP-5MS (30 m × 0.25 mm i.d., 0.25 µm)	Single quadrupole (5973, Agilent)	Not used
Purcuro et al. (2007b)	Vegetable oils	15 (+) EU priority PAHs	200 µL	Immersion SPME (15 µm Carboxpack Z/polydimethyl-siloxane)	No further clean-up	GC × GC-MS	BPX5 (30 m × 0.25 mm i.d., 0.25 µm) BPX50 (1 m × 0.1 mm i.d., 0.1 µm)	Pegasus III (LECO Corporation)	Not used
Purcuro et al. (2007a)	Vegetable oils	BaP (252)	200 µL	Immersion SPME (15 µm Carboxpack Z/polydimethyl-siloxane)	No further clean-up	GC-MS	SPB-5 (30 m × 0.25 mm i.d., 0.25 µm)	Quadrupole (QP 2010 Ultra, Shimadzu)	BaP-D12
Rose et al. (2007)	Olive pomace, sunflower and coconut oil	Ap (152 151), Ac (154 153), F (166 165), A (178 176), Phe (178 176), Fl (202 200), Py (202 200), BaA(226 224), BghiF (226 224), benzo (b)naphtha(2,1-d)thiophene (234 232), Ch (228 226), CPP (226 224), 5-MeCh (242 241), BbF (252 250), BbF (252 250), BkF (252 250), BeP (252 250), BaP (252 250), IP	5 g	Saponification (200 mL 2 mol/L methanolic potassium hydroxide) Liquid-liquid partition (100 mL cyclohexane and 50 mL dimethylformamide)	Column (2.5 g silica gel)	GC-MS	DB-5MS (60 m × 0.25 mm i.d., 0.25 µm)	Single quadrupole (TRACE, Thermo Finnigan)	Isotope labeled ( <sup>13</sup> C)

(continued)

Table 3 (continued)

References	Food matrix	Parameter measured (PAHs and respective monitored ions)	Analytical method		Clean-up	Detection and quantification	Column	Detector details	Internal standard
			Sample size	Extraction					
Veyrand et al. (2007)	Food and oil	(276 277), BghiP (276 277), An (276 277), DBaA (278 279), DBaP (302 303), DBaP (302 303), DBaP (302 303) and COR (300 301)							
		Phe (178 > 176, 152), A (178 > 176, 152), Fl (202 > 200, 152), Py (202 > 200, 152), CPP (226 > 224, 200), BaA (228 > 226, 202), Ch (228 > 226, 202), 5-MeCh (242 > 240, 226), BbF (252 > 250, 226), BbF (252 > 250, 226), BkF (252 > 250, 226), BaP (252 > 250, 226), IP (276 > 274, 272), DBaA (278 > 276, 252), BghiP (276 > 274, 272), DBaP (302 > 300, 298), DBaP (302 > 300, 298), DBaP (302 > 300, 298) and DBaP (302 > 300, 298)	10 g (food) and 1 g (oil)	Freeze-drying and PLE in cell clean-up extraction solvent: hexane: acetone 50:50, v/v and adsorbents: 15 g Florisil and 1 g Celite)	SPE (polystyrene divinylbenzene cartridge)	GC-MS/MS	Zebtron ZB-5MS (30 m × 0.25 mm i.d., 0.25 µm)	Triple quadrupole (Quattro micro, Waters, Micromass)	Isotope labeled ( <sup>13</sup> C)
Stanciu et al. (2008)	Coffee	BaA, BkF, BbF, BaP, BghiP, DBaA and IP	10 g	Soxhlet (hexane) Liquid-liquid partition (petroleum ether and acetonitrile)	Column (5 g aluminum oxide, 5 g silica-gel and 1 g anhydrous sodium sulfate)	HPLC-FL	Column SS (100 mm × 4.6 mm i.d.)	-	Not used

(continued)

Table 3 (continued)

References	Food matrix	Parameter measured (PAHs and respective monitored ions)	Analytical method				Detection and quantification	Column	Detector details	Internal standard
			Sample size	Extraction	Clean-up					
Windal et al. (2008)	Fish oil and dried plants	BcL, CPP, BaA, Ch, 5MeCh, BbF, BbF, BkF, BaP, DBaHP, DBaH, A, BghiP, IP, DBaEP, DBaHP and DBaHP	2 g	Dilution (10 mL dichloromethane:cyclohexane 50:50, v/v)	Automatic clean-up with DACC (oil) or SPE (0.5 g silica gel cartridges) followed by DACC (dirty matrices)	HPLC–UV/FL	Varian Pursuit 3 PAH (100 mm × 4.6 mm i.d., ¼")	Fluorescence (2475, Waters) UV (487, Waters)	Not used	
Danyi et al. (2009)	Food supplements (dried plants and plant extracts)	15 (+1) EU priority PAHs	1 g	Extraction (15 mL dichloromethane:cyclohexane 50:50, v/v, followed by centrifugation)	SPE (silica gel column)	HPLC/UV–FL	Cl8 Pursuit 3 PAH (100 mm × 4.6 mm i.d., 3 µm)	Fluorescence (996 PDA and 2475, Waters)	<sup>2</sup> H-DBaHP	
Lund et al. (2009)	Smoked fish	16 EPA priority PAH + BcL, DBaHP, DBaEP, DBaHP, Per, 5-MeCh and CPP	10 g	PLE in cell clean-up (extraction solvent: hexane:acetone 1:1, v/v and adsorbents: Ottawa sand and polyacrylic acid)	No further clean-up	GC–MS	ZB-5 (40 m × 0.18 mm i.d., 0.25 µm)	Single Quadropole (5975B, Agilent)	Ap-D8, BaP-D12, BaA-D12, IP-D12 and DBaHP-D14	
Orecchio et al. (2009)	Coffee (brew)	Ap (152, 151, 76), Ac (154, 152, 76), F (166, 165, 164), A (178, 188, 89), Phe (178 > 152, 176), Fl (202, 200, 101), Py (202, 200, 101), BaA (228, 114, 226), Ch (228, 114, 226), BaP (252, 126, 250), BbF (252, 126, 250), BkF (252, 126, 250), DBaH, A (278, 279, 139), BeP (252, 126, 250), Per (252, 126, 250), DBaHP (302, 151), DBaEP (302, 151), DBaHP (302, 151)	15 mL	Digestion under reflux (50 mL methanolic solution of potassium hydroxide 2 mol/L, 3 h) Liquid–liquid partition (10 mL hexane, three times)	No further clean-up	GC–MS	Equity-5 (30 m × 0.25 mm id, 0.5 µm)	Quadropole (QP5000, Shimadzu)	Ac-D10, Phe-D10, Ch-D12 and Per-D12	

(continued)

(continued)

**Table 3** (continued)

References	Food matrix	Parameter measured (PAHs and respective monitored ions)	Analytical method Sample size	Extraction	Clean-up	Detection and quantification	Column	Detector details	Internal standard
Smoker et al. (2010)	Shrimp	Na (128 > 102, 78), Ap (152 > 126, 102), Ac (154 > 152, 127), F (166 > 115, 139), A (178 > 152, 176), Phe (178 > 152, 176), Fl (202 > 200, 150), Py (202 > 200, 151), BaA (228 > 226, 200), Ch (228 > 226, 200), BaP (252 > 250, 226), BbF (252 > 224, 250), BkF (252 > 250, 226), BghiP (276 > 274, 250), IP (276 > 274, 250) and DBaA (278 > 276, 250)	10 g	QuEChERS (10 mL acetonitrile, 6 g magnesium sulfate, 1.5 g sodium chloride, followed by centrifugation)	Dispersive SPE (PSA and magnesium sulfate)	LC-MS/MS	PAH C18 (150 mm × 4.6 mm i.d., 5 µm)	Triple quadrupole (PhotoSpray APPI source and 4000 QTrap, Applied Biosystems)	Not used
Belo et al. (2012)	Soy, sunflower and olive oil	BaA (226, 228), BbF (250, 252), BkF (250, 252), IP (250, 252), Ch (226, 228), IP (277, 276), DBaA (279, 278) and BghiP (277, 276)	2 g	Low volume liquid-liquid partition (hexane and dimethylformamide)	SPE (octadecyl and silica gel cartridges)	GC-MS	DB-5MS (30 m × 0.25 mm i.d., 0.25 µm)	Single quadrupole (DSQ, Thermo)	Isotope labeled ( <sup>13</sup> C)
Cai et al. (2012)	Oysters	Na (128.1), Ap (152.1), Ac (154.1), F (166.1), Phe (178.1), A (178.1), Fl (202.1), Py (202.1), BaA (228.1), Ch (228.1), BbF (252.1), BkF (252.1), BaP (252.1), DBaA (278.1), BghiP (276.1) and IP (276.1)	10 g	QuEChERS (15 mL acetonitrile, 6 g magnesium sulfate, 1.5 g sodium chloride, followed by centrifugation)	Dispersive SPE (50 mg PSA and 150 mg magnesium sulfate)	UHPLC-MS	Zorbax Eclipse PAH column Rapid Resolution HT (50 mm × 2.1 mm i.d., 1.8 µm)	Single quadrupole (G6140A, with Syagen's PhotoMate® APPI® source, Agilent)	Not used

(continued)

Table 3 (continued)

References	Food matrix	Parameter measured (PAHs and respective monitored ions)	Analytical method		Clean-up	Detection and quantification	Column	Detector details	Internal standard
			Sample size	Extraction					
Drabova et al. (2012)	Vegetable oils and tea	15 (+1) EU priority PAHs	2 g	QuEChERS (10 mL ethyl acetate, 4 g magnesium sulfate, 2 g sodium chloride, followed by centrifugation)	MIP SPE-PAH (50 mg/3 mL)	GC × GC-TOFMS	BPX-50 (30 m × 0.25 mm i.d., 0.25 µm) BPX-5 (1 m × 0.1 mm i.d., 0.1 µm) SGE	Pegasus III (LECO Corporation)	Isotope labeled ( <sup>13</sup> C)
Londoño et al. (2013)	Milk	Ap, Ac, F, A, Phe, Fl, Py, BaA, Ch, BaP, BbF, BkF, BghiP, IP and DBaA	1 g	Low volume liquid-liquid partition (10 mL hexane three times)	SPE (silica gel cartridge, 690 mg, 55–105 µm particle size)	HPLC-UV/FL	PAH C18 (250 mm × 4.6 mm i.d., 5 µm)	UV-Vis (2698, Waters) Fluorescence (2475, Waters)	Not used
Payanan et al. (2013)	Edible oils	16 EPA priority PAHs	1 g	Low-temperature liquid-liquid partition (two extractions with 8 mL of acetonitrile: acetone 4:1, v/v and freezing for 24 h)	SPE (alumina cartridge)	HPLC-FL	PAH C18 (250 mm × 4.6 mm i.d., 5 µm)	–	Not used
So-Young et al. (2013)	Edible oils	15 EU priority PAHs	2 g	Extraction (8 mL isooctane:cyclohexane 1:1, v/v)	SPE (polystyrene divinylbenzene cartridges)	GC-MS/MS	VF-5 ms 5 % phenyl-95 % polydimethyl-siloxane (30 m × 0.25 mm i.d., 0.25 µm)	320-MS Bruker system (Bremen, Germany)	IP-D12 and Ch-D12
Taylor et al. (2013)	Fish and mussel	Na (127,128), Ap (151, 152), Ac (153, 154), F (165, 166), A (178, 176), Phe (178, 176), Fl (202, 201) Py (202, 201), BaA (228, 226), Ch (228, 226), BaP (252, 250), BbF (252, 250), BkF (252, 250), BghiP (276, 138), IP (276, 138), DBaA (278, 139) and 2-MeNa (142, 141)	10 g	QuEChERS (20 mL methylene chloride; 2 g magnesium sulfate)	No further clean-up	GC × GC-MS	BPX50 50 % phenyl polysilphenylene siloxane (30 m × 0.25 mm i.d., 0.50 µm). BPX1 100 % polydimethyl-siloxane (2 m × 0.1 mm i.d., 0.1 µm)	QP 2010 Ultra Shimadzu	F-D10 and F-D10

(continued)





Some authors have adopted GPC in the clean-up step to remove lipids and high molecular weight compounds from food extracts (Ballesteros et al. 2006; Liguori et al. 2006). However, the prevailing technique is SPE, in which the solvent volume and the time spent are considerably lower than those needed for liquid–liquid extraction and which is amenable to automation (Tobiszewski et al. 2009). Houessou et al. (2005) evaluated the extraction efficiency of octadecyl (C18, 0.5 g) and polystyrene-divinylbenzene copolymer (PS-DVB, 0.2–0.5 g) cartridges for the determination of PAHs from coffee brew samples. The PS-DVB phase was also used by Veyrand et al. (2007) and So-Young et al. (2013) for food and edible oil matrices, respectively. This phase is selective for PAHs due to its lipophilicity and ability to form  $\pi$ - $\pi$  bonds with the aromatic rings in PAHs. Although the results found by Houessou et al. (2005) indicated similar recovery values for both solid phases used, C18 and PS-DVB, better reproducibility was observed when the PS-DVB phase (0.5 g) was employed.

Silica gel have been the most widely used phase in the clean-up step by SPE to remove interfering molecules during the determination of PAHs from various food matrices, both in the form of packaged cartridges containing 0.5–2.0 g of silica gel (Windal et al. 2008; Belo et al. 2012; Londoño et al. 2013) and as columns prepared with up to 5 g of silica gel (Badolato et al. 2006; Rose et al. 2007; Stanciu et al. 2008; Danyi et al. 2009; Pissinatti et al. 2014). The alumina-N SPE cartridge showed satisfactory PAH recovery and highly efficient elimination of the lipid-interferences from the extracts of edible oils (Payanan et al. 2013).

The use of MIP SPE for the clean-up of PAH extracts from food samples has also been reported, including for vegetable oils and tea samples. SPE cartridges containing molecular recognition elements engineered to bind structurally related PAHs with high selectivity are commercially available. It is assumed that multiple interactions (such as hydrogen bonding, ionic, van der Waals and hydrophobic forces) take place between the measurands and MIP cavities and are responsible for their binding. Operatively, the MIP SPE technique is very similar to the traditional SPE performed on non-specific supports, and the usual steps of column conditioning, sample loading, column washing and measurands elution are carried out (Drabova et al. 2012).

Another variation of SPE, known as dispersive SPE (which is commonly used as part of QuEChERS extraction), has been reported for the determination of PAHs from matrices such as shrimp, oysters, fish and mussel (Smoker et al. 2010; Cai et al. 2012; Taylor et al. 2013). The procedure of dispersive SPE clean-up is simple: the sample extract and the sorbent are stirred together and centrifuged, and the supernatant is collected. This procedure results in better recoveries and more effective clean-up that removes the interferences and generates less waste due to the lower volume of organic solvent employed in the sample preparation when compared to traditional cartridge SPE (Oshita and Jardim 2015).

SPME is a rapid, simple and low solvent consuming technique widely used for environmental pollutants, including PAHs. Extraction is performed into a silica fiber coated with an appropriate stationary phase and can be carried out immersing the fiber in the liquid sample (direct immersion) or from the sample headspace

(Aguinaga et al. 2007; Purcaro et al. 2007a, b). For vegetable oils, Purcaro et al. (2007b) concluded that the use of GC  $\times$  GC was necessary in order to achieve a better resolution between interferents and PAHs of interest, while GC–MS, using the deuterated congerene as internal standard, provided enough selectivity when only benzo(a)pyrene was considered (Purcaro et al. 2007a). SPME followed by a GC–MS analysis was successfully applied for quantification of the 16 EPA priority PAHs in milk products (Aguinaga et al. 2007).

Donor–acceptor complex chromatography (DACC) was first developed for the analysis of PAHs in oil and fat samples (Windal et al. 2008). The samples are eluted using a column with a modified stationary phase that acts as an electron acceptor. This column retains electron donors, including PAHs, by  $\pi$ – $\pi$  interactions and is used for sample clean-up (ISO 2012). Commercial instruments have been designed for the on-line coupling of a DACC column to an HPLC reversed-phase column and fluorescence detector, allowing for the automated clean-up, separation and quantification of PAHs (Windal et al. 2008). This technique can be used for fats and oils without any other clean-up steps (ISO 2012) or may be applied to dirty matrices, such as dried plants, if an additional clean-up is performed (Windal et al. 2008).

PLE is a type of extraction that uses conventional organic solvents at high temperature (100–180°C) and pressure ( $6.9 \times 10^6$  to  $1.0 \times 10^7$  Pa) to extract organic components from solid or semi-solid samples. It was first used in 1995, and in 1996, this technique was approved by the EPA as a standard method for the analysis of organic contaminants, such as pesticides, chlorinated herbicides, polychlorinated biphenyls (PCBs) and dioxins, in environmental matrices (Mitra 2003). The elevated temperature and pressure affect the solvent, the sample and the interactions between them. For example, the boiling point of the solvent is increased by the high pressure, which enables the extraction to occur at higher temperatures, resulting in lower viscosity and lower surface tension. Additionally, high temperatures increase the solubility of the compounds by weakening the interactions, such as the Van der Waals forces and hydrogen bonds, thus allowing faster mass transfer. High pressure also allows the solvent to penetrate deeper into the matrix. Thus, using high pressure and temperature assures faster extraction and better recoveries (Mitra 2003). On average, one to three extraction cycles of up to 15 min each is sufficient. Using this technique, the solvent volume and extraction time are reduced compared to traditional extraction procedures.

Many authors have demonstrated the efficiency of the use of PLE for PAH analysis. Wang et al. (2007) compared PLE, Soxhlet and solvent extraction assisted by microwave (MAE) for PAHs extraction from soil samples. According to these authors, PLE showed higher extraction efficiency, in addition to the advantages described above.

A further advantage of PLE is that some instruments allow a simultaneous purification step to be performed by adding adsorbents to the extraction cell. The most commonly used adsorbents are fat retainers, such as alumina, silica or Florisil® (Liguori et al. 2006; Veyrand et al. 2007; Lund et al. 2009; Pissinatti et al. 2014). For low complexity matrices, it is possible to carry out a one-step

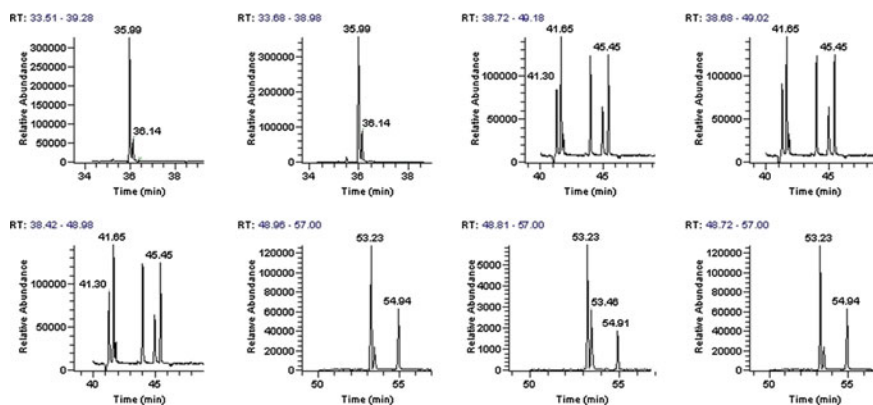
extraction/purification method (Kim et al. 2003), but because food matrices are complex, most authors have used pre-purification within the PLE cell to simplify later clean-up steps.

Recently, QuEChERS extraction, which has been widely applied for the analysis of pesticides, has been used to extract PAHs from food matrix (Smoker et al. 2010; Cai et al. 2012; Taylor et al. 2013; Pincemaille et al. 2014). Briefly, in this technique, samples are extracted with acetonitrile and inorganic salts (e.g., magnesium sulfate and sodium chloride). After centrifugation, an aliquot of the acetonitrile layer is filtered or cleaned up in dispersive mode. An acetonitrile QuEChERS extraction with PSA (clean-up sorbent that contains primary and secondary amines) and magnesium sulfate clean-up was used by Smoker et al. (2010) and Cai et al. (2012) for shrimp and seafood samples, respectively. The authors observed that spiked samples showed ion suppression and lower recovery rates without PSA clean-up. Pincemaille et al. (2014) used two different SPE purification steps after the initial QuEChERS extraction from tea samples: a first step on a C18-support to extract non-polar compounds and a second step on a polar support (Florisil®) to trap polar impurities. This technique represents an advance in determining PAHs mainly due to environmental friendly and high throughput characteristics.

## 5.2 *Determination and Quantification*

Many authors have reported the use of HPLC with a UV/FL detector as the detection and quantification technique for the analysis of PAHs from food (Houessou et al. 2005; Badolato et al. 2006; Stanciu et al. 2008; Windal et al. 2008; Danyi et al. 2009; Londoño et al. 2013; Payanan et al. 2013). Liquid chromatography coupled to MS has also been applied (Smoker et al. 2010; Cai et al. 2012). However, GC is the most widely used technique for these analytical scopes (Diletti et al. 2005; Ballesteros et al. 2006; Liguori et al. 2006; Aguinaga et al. 2007; Purcaro et al. 2007a, b; Rose et al. 2007; Veyrand et al. 2007; Lund et al. 2009; Orecchio et al. 2009; Belo et al. 2012; Drabova et al. 2012; So-Young et al. 2013; Taylor et al. 2013; Pincemaille et al. 2014; Pissinatti et al. 2014). According to Poster et al. (2006), for these compounds, GC has better selectivity, resolution and sensitivity compared to liquid chromatography. These authors also report the ease of coupling GC with MS, allowing for the confirmation and quantification of the compounds of interest and indicating that GC is preferable to LC. The thermal properties of PAHs are an important factor: PAHs are readily volatile but are not degraded by the higher temperatures used in GC. Typical chromatograms obtained for PAHs in food matrices by GC coupled to MS are illustrated in Fig. 1.

A study of the mass spectra of PAHs was carried out by Veyrand et al. (2007), who aimed to develop a method for the analysis of contaminants in food. According to these authors, the minimal fragmentation observed when using electron ionization under conventional conditions is attributable to the stability of PAH molecules, which results in a highly intense molecular ion signal and the presence of ions with



**Fig. 1** Typical chromatograms obtained for eight polycyclic aromatic hydrocarbons from food matrices (roasted coffee and fish samples) by gas chromatography coupled to mass spectrometry. Monitored ions ( $m/z$ ): BaA and Ch (226–228); BbF, BkF and BaP (250–252); IP and BghiP (276–277); and DBahA (278–279). RT: retention time. Roasted coffee: BaA 19.66 min; Ch 19.78 min; BbF 23.58 min; BkF 23.67 min; BaP 24.65 min; IP 28.29 min; DBahA 28.47 min; and BghiP 29.22 min. Fish: BaA 35.99 min; Ch 36.14 min; BbF 41.30 min; BkF 41.65 min; BaP 45.45 min; IP 53.23 min; DBahA 53.46 min and BghiP 54.94 min. BaA: benzo(a)anthracene; BaP: benzo(a)pyrene; BbF: benzo(b)fluoranthene; BghiP: benzo(g,h,i)perylene; BkF: benzo(k)fluoranthene; Ch: chrysene; DBahA: dibenzo(a,h)anthracene; and IP: indene(1,2,3-c,d)pyrene

the loss of two hydrogens. The same observation was reported by Poster et al. (2006), who concluded that this stability makes PAHs amenable to GC. However, this characteristic makes the use of tandem MS, in which the precursor ion is fragmented into specific product ions, problematic. As a result, very few works have described the use of MS/MS for PAH analysis (Ballesteros et al. 2006; Veyrand et al. 2007; Smoker et al. 2010).

HPLC coupled to MS is more commonly used for environmental samples than food samples. The higher detection limits needed for environmental samples can be easily achieved using this technique. The most commonly used ion sources in LC–MS analysis are electro spray ionization (ESI) and atmospheric-pressure chemical ionization (APCI). However, such sources are inefficient for non-polar compound ionization. Post-run chemical derivatization was proposed by several authors to overcome this problem, but this technique may result in deposits in the instrument and create the need for more frequent maintenance. The atmospheric-pressure photoionization (APPI) ion source extends the range of ionizable compounds to many non-polar substances. Furthermore, this type of interface shows less ion suppression than APCI or ESI. Additionally, to further increase the ionization efficiency, a dopant (e.g., acetone or toluene) can be used (Purcaro et al. 2013). Thus, the low sensitivity of HPLC–MS has only recently been overcome (Cai et al. 2012).

### 5.3 Use of Internal Standards

The isotope dilution mass spectrometry (IDMS) technique was first developed during the 1950s for analyzing inorganic elements. In 1970, it was extended to the field of organic chemistry, with applications in trace analysis to determine persistent organic pollutants and in medical tests (Sargent et al. 2002; Mechlinska et al. 2010). ID consists of modifying the natural isotopic composition of a target measurand present in the sample by adding a known amount of an isotopically labeled analog (internal standard). In MS, unlike spectrophotometric techniques, there is a fixed relationship between the quantity or concentration of a particular substance and the instrument response. The sensitivity for a given compound may vary over time or in accordance with the calibration of the equipment. These variations are added to variations caused by, for example, losses during extraction or the introduction of the analytical sample into the chromatographic system. Adding the internal standard at the beginning of the analytical procedure allows for compensation of losses and errors throughout the analytical process (Sargent et al. 2002).

The most important criterion for selecting a standard substance is that it mimics as closely as possible the physico-chemical properties of the target analyte. This is achieved by using similar molecules that isotopically labeled, especially with  $^{13}\text{C}$ ,  $^{37}\text{Cl}$  or  $^2\text{H}$ . Because the amount of internal standard added to the sample is known, the recovery percentage can be calculated and used as an indirect measurement of the target compound recovery (Mechlinska et al. 2010).

IDMS is often used for the analysis of PAHs to overcome systematic errors in analysis of PAH. In fact, almost all of the work published using MS have used this technique (Diletti et al. 2005; Liguori et al. 2006; Rose et al. 2007; Veyrand et al. 2007; Danyi et al. 2009; Lund et al. 2009; Orecchio et al. 2009; Belo et al. 2012; Drabova et al. 2012; So-Young et al. 2013; Taylor et al. 2013; Pincemaille et al. 2014; Pissinatti et al. 2014). Thus, because extraction methods are usually time consuming and laborious and because the volatility of the compounds can result in loss during the extraction procedure, the use of isotope dilution is almost a necessity for this type of analysis, allowing for acceptable accuracy, even at low concentrations ( $\mu\text{g/kg}$ ).

$^{13}\text{C}$ -labeled PAHs are preferred in MS methods due to their relatively high stability compared to deuterated species (Rose et al. 2007; Veyrand et al. 2007; Belo et al. 2012; Drabova et al. 2012; Pissinatti et al. 2014), but the high cost of these PAHS limits their use. Although a number of  $^{13}\text{C}$ -labeled PAH standards are commercially available, there is still a need for  $^{13}\text{C}$ -labeled analogues for various PAHs, such as benzo(j)fluoranthene, dibenzo(a,h)pyrene, dibenzo(a,l)pyrene, cyclopenta(c,d)pyrene, 5-methylchrysene and benzo(c)fluorine (EFSA 2008a, b). The lack of  $^{13}\text{C}$ -labeled standards may partly explain the high variation in the results reported for some PAHs in foods (Rose et al. 2007).

## 5.4 Validation Practices

Despite the significant number of works dedicated to the development and application of new technologies and equipment for the determination of PAHs in food samples, contemporary analytical techniques remain limited in their ability to obtain reliable information regarding the levels and forms of PAHs in the environment. This has caused researchers to alter their approaches to the determination of these compounds, which are present at low concentrations in highly variable and complex matrices, with regard to control issues and quality assurance of the results (Konieczka et al. 2010). In the context of laboratory quality management systems, specifically regarding the quality assurance of the analytical results, method validation is an extremely important tool.

Method validation is defined as the confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled (ISO 2005). Inherent in this definition is the need to evaluate the performance characteristics or parameters of the method, although judgement of the suitability of the method is also important (Magnusson and Ornemark 2014).

The performance parameters proposed by EURACHEM (Magnusson and Ornemark 2014) and harmonized by the Association of Official Analytical Chemists (AOAC International), International Organization for Standardization (ISO) and International Union of Pure and Applied Chemistry (IUPAC) (Thompson et al. 2002) in their respective guidelines for single-laboratory validation include the following: selectivity, linearity (tests for general matrix effects), trueness, precision (under repeatability and intermediate precision conditions), recovery, range, detection limit, limit of quantification, sensitivity, ruggedness and measurement uncertainty.

In this context, the European Commission established analytical performance criteria for the determination of the regulated PAHs (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) in food. Fully validated methods (i.e., methods validated by collaborative trials) were recommended when appropriate and available. Alternatively, in-house validated methods were indicated, provided that they fulfill some performance criteria: applicability (demonstrated using the regulated food matrices), selectivity (free from matrix or spectral interferences, verification of positive detection), recovery (between 50 and 120 %), relative standard deviation under repeatability and reproducibility conditions (HORRAT less than 2, HORRAT being the observed relative standard deviation divided by the value estimated from the modified Horwitz equation), limit of detection ( $\leq 0.30 \mu\text{g/kg}$  for each of the four substances) and limit of quantification ( $\leq 0.90 \mu\text{g/kg}$  for each of the four substances). Additionally, the in-house validated methods must produce results with a combined standard measurement uncertainty less than the established maximum standard measurement uncertainty (EU 2011a).

In Table 4, the validation procedures reported in the literature for methods used for the determination of PAHs in food are presented. This review showed that the current practices for validation do not address all of the parameters needed to assess

**Table 4** Review of current validation procedures practiced for the determination of PAHs in foods

References	Method validation		Selectivity/matrix effects	Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
Diletti et al. (2005)	Linearity/range/sensitivity		Ion relative abundances (spiked samples 2µg/kg and calibration solutions) No significant peaks interfering with the compound (blank samples and CRM blank of coconut oil, n = 5)	69–97.5 % (spiked samples at 2, 5 and 20 µg/kg, n = 6)	97.0–110.3 % (CRM BCR 458, coconut oil, for 4 PAHs, n = 5)	RSD repeatability from 3.6 to 15.5 % (spiked samples at 2, 5 and 20 µg/kg, n = 6) Intermediate precision not studied	0.2–0.4 µg/kg (signal/noise $\geq 3$ )	Not studied	8.5–11.4 %	Not studied
Houessou et al. (2005)	Estimation of regression parameters and $R^2$ from 0.9942–0.9999 (0.1–10 µg/mL, n = 8)		Analysis of blank samples Influence of coffee brew matrix in recovery by comparison with water	81.5–105.2 % (spiked samples at 2 µg/kg, n = 3)	Not studied	RSD repeatability from 5.1 to 17.3 % (spiked samples at 2 µg/kg, n = 3) Intermediate precision not studied	0.19–2.49 µg/kg (analytical) and from 0.76 to 9.96 µg/kg (method) (signal/noise = 3 based on the analysis of blanks, n = 7)	0.63–1.11 µg/kg (analytical) and from 2.52 to 33.2 µg/kg (method) (signal/noise = 3 based on the analysis of blanks, n = 7)	Not studied	Not studied
Badolato et al. (2006)	Estimation of regression parameters and $R^2$ of 0.9993 ( $0.1 \times 10^{-3}$ to 0.1 µg/mL)		Not studied	76–116 % (spiked samples at 1.01, 2.02 and 3.03 µg/kg, n = 3)	Not studied	RSD repeatability from 12 to 18 % (spiked samples at 1.01, 2.02 and 3.03 µg/kg, n = 3) Intermediate precision not studied	0.03 µg/kg (signal/noise = 3 based on the analysis of blanks, n = 6)	0.10 µg/kg (signal/noise = 10 based on the analysis of blanks, n = 6)	Not studied	Not studied
Ballesteros et al. (2006)	$R^2$ from 0.9922 to 0.9980 (0.3–200 µg/kg, matrix-matched calibration curve)		Ion analysis	84–110 % (spiked samples at 1, 2 and 10 µg/kg, n = 3)	Not studied	RSD repeatability from 5.11 to 7.76 % (1 µg/kg, n = 11)	0.05–0.07 µg/kg (signal/noise $\geq 3$ )	0.10–0.20 µg/kg (signal/noise $\geq 3$ )	Not studied	Not studied

(continued)

**Table 4** (continued)

References	Method validation		Selectivity/matrix effects	Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity									
Liguori et al. (2006)	R <sup>2</sup> of 0.999 (six concentration points between 10 and 600 ng/mL)	Compound signals in the presence of potential components of the sample were compared with the responses of standard solution	75–121 % (salmon fillet, fish feed and fish oil spiked at 60 and 100 ng, n = 3 and blue mussels spiked at 100 ng, n = 5)	CRM NIST SRM 2977 mussel tissue and T0621 olive oil, for four PAHs, n = 3	RSD repeatability from 0.2 to 19 % (salmon fillet, fish feed and fish oil and fish oil spiked at 60 and 100 ng, n = 3 and blue mussels spiked at 100 ng, n = 5) Intermediate precision not studied	0.42–3.4 pg/g (wet weight) for salmon fillet, 0.36–2.9 pg/g for fish feed, 2.2–18 pg/g for fish oil and 0.11–0.88 pg/g (dry mass) for blue mussels (signal/noise = 3)	1.4–11 pg/g (wet weight) for salmon fillet, 1.2–9.8 pg/g for fish feed, 7.2–30 pg/g for fish oil and 0.36–2.9 pg/g (dry mass) for blue mussels (signal/noise = 10)	Not studied	Not studied	
Aguinaga et al. (2007)	Estimation of regression parameters and R <sup>2</sup> from 0.9956 and 0.9999 (0.01–100 µg/L, 5 concentration levels, standard addition calibration curves)	Significant matrix effects (comparison of the slope of the calibration curve in solvent with the slope of the standard addition calibration curves by t test)	87.6–112 % (spiked samples of 4 matrices at 0.6–3.2 mg/L)	Not studied	RSD repeatability from 2 to 19.6 % (spiked sample at five times the quantitation limit of each compound, n = 8) Intermediate precision not studied	0.003–1.56 µg/L (signal/noise = 3)	Not studied	Not studied	Not studied	
										(continued)

(continued)



Table 4 (continued)

References	Method validation	Selectivity/matrix effects	Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
Purcaro et al. (2007a)	Estimation of regression parameters and $R^2$ of 0.999 Linearity tested by Mandel's fitting test and evaluation of regression assumptions, including homoscedasticity and lack of fit test (0.46–15.79 µg/kg, 7 concentration levels, n = 3)	Not studied	+6 to -2 % (spiked samples at 0.4 and 2.0 ng)	Not studied	RSD repeatability from 1.6 to 5.3 % RSD intermediary precision from 1.93 to 5.76 % (spiked samples at 0.4 and 2.0 ng)	0.03 ng corresponding to 0.17 µg/kg (average signal of the blank sample plus 2 times the blank standard deviation and the constant of the t-Student distribution, n = 8)	0.09 ng corresponding to 0.46 µg/kg (average signal of the blank sample plus 10 times the blank standard deviation and the constant of the t-Student distribution, n = 8)	Not studied	Not studied
Purcaro et al. (2007b)	Estimation of regression parameters and $R^2$ from 0.957 to 0.998 (1–36.8 µg/kg)	Not studied	+20.9 to -69.4 (spiked sample at 2 levels, n = 6)	Not studied	RSD repeatability from 12.8 to 34.5 % (one sample, n = 6)	0.1–1.4 µg/kg (signal/noise = 3)	0.4–4.6 µg/kg (signal/noise = 10)	Not studied	Not studied
Rose et al. (2007)	Weighted least-squares linear regression	Use of $^{13}\text{C}$ -isotopes in every sample aiming to correct for any variation due to matrix effects	53 a 128 % (spiked samples at 1, 2, 10 and 50 µg/kg, measured in four analytical runs)	CRM BCR 458, coconut oil, for 6 PAHs, n = 120	Repeatability not studied RSD intermediate precision from 0.02 to 0.48 % (unfortified, 1, 2, 10 and 50 µg/kg, measured in 4 analytical runs)	Less than 2 µg/kg (standard uncertainty at zero concentration multiplied by 3)	Lowest concentration that could be measured with a sufficiently low relative uncertainty	Less than 0.2 for 24 PAHs	Not studied
Veyrand et al. (2007)	Estimation of regression parameters $R^2$ from 0.9986 to 1 (0–4 µg/kg of dry matter)	Ion analysis Matrix effects not studied	12–70 % (same sample analyzed 20 times by two operators)	CRM NIST SRM 2977 mussel tissue for 4 PAHs Participation in an	Repeatability not studied RSD intermediate precision from 2.9 to 20.5 % (same sample)	0.08–0.15 µg/kg (signal observed at the lowest point of the calibration curve, extrapolation at signal/noise = 3)	0.25–0.915 µg/kg (signal observed at the lowest point of the calibration curve, extrapolation at signal/noise = 10)	Not studied	Analysis of different types of food

(continued)

Table 4 (continued)

References	Method validation		Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity	Selectivity/matrix effects							
Stanciu et al. (2008)	$R^2$ 0.9946-0.9993 (0.2–10 µg/L)	Not studied	83–105 % (spiked sample at 2 µg/kg)	inter-laboratory assay  Not studied	analyzed 20 times by two operators)  Not studied	0.02–0.04 ng/kg (signal/noise = 3, based on analysis of blank samples, n = 7)	0.2 ng/kg (signal/noise = 3, based on analysis of blank samples, n = 7)	Not studied	Not studied
Windal et al. (2008)	Linearity tested by Mandel's fitting test (5.2, 32, 66, 90 pg/L for the CPP and 0.2, 2, 6, 10 pg/L for the other PAH except for B <sub>1</sub> F for which the first level was 0.5 pg/L)	No significant matrix effects (comparison of the slope of the calibration curve in acetonitrile with the slope of the matrix-matched calibration curve by t test)	60 and 115 % (spiked samples at 2 µg/kg for all PAHs except B <sub>1</sub> F and I <sub>1</sub> C <sub>P</sub> 10 µg/kg and CPP 200 µg/kg, n = 6)	Participation in an inter-laboratory assay	RSD repeatability from 2.3 to 19 % (spiked samples at 2 µg/kg for all PAHs except B <sub>1</sub> F and I <sub>1</sub> C <sub>P</sub> 10 µg/kg and CPP 200 µg/kg, n = 6 on the same day) RSD intermediate precision from 1.3 to 13 % (spiked samples at 2 µg/kg for all PAHs except B <sub>1</sub> F and I <sub>1</sub> C <sub>P</sub> 10 µg/kg and CPP 200 µg/kg, n = 6, analysis of one sample on different days)	0.3–5.8 pg (54 pg for CPP) (instrumental—standard solutions in acetonitrile, signal/noise = 3) 0.07–0.75 µg/kg (7.8 pg for CPP) (method—spiked matrices	0.6–12 pg (104 pg for CPP) (instrumental—standard solutions in acetonitrile, signal/noise = 3) 0.13–1.5 µg/kg (16 pg for CPP) (method—spiked matrices	Not studied	Not studied

(continued)

Table 4 (continued)

References	Method validation		Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity	range/sensitivity							
Danyi et al. (2009)	R <sup>2</sup> from 0.98 to 1.00	Not studied	63–118 % (spiked samples at three levels, corresponding to the LOQ, 2 and 4 µg kg, except for BbF, IP and CPP)	Not studied	Repeatability HORRAT from 0 to 1.2 (spiked samples at 2 µg kg except for BbF, IP and CPP, n = 6, on the same day) Intermediate precision HORRAT from 0 to 0.4 (spiked samples at 2 µgkg except for BbF, IP and CPP, n = 6, one time during 6 independent days)	<0.1 µg/kg (extrapolation at signal/noise = 3 measured in the chromatogram of unspiked sample)	0.2–0.3 µg/kg except for CPP, IP, BbF and BcL (extrapolation at signal/noise = 6 measured in the chromatogram of unspiked sample)	Not studied	Not studied
Lund et al. (2009)	R <sup>2</sup> from 0.995 to 1.000	Not studied	53–108 %	Not studied	RSD repeatability from 3 to 87 % Intermediate precision not studied	0.2–4.4 ng/g	0.7–14.6 ng/g	Not studied	Fat-retention capacity (5 g fish homogenate n = 6, between 5 and 30 % fat, analyzed in random order in two runs during 2 days)

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**Table 4** (continued)

References	Method validation		Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity	Selectivity/matrix effects							
Orecchio et al. <a href="#">2009</a>	Five standard solutions	Not studied	≥ 85 %	Not studied	RSD repeatability from 4 to 16 % (3 replicates of 13 sample) Intermediate precision not studied	0.67–18 ng/L	<1 µg/kg	Not studied	Not studied
Smoker et al. <a href="#">(2010)</a>	R <sup>2</sup> from 0.996 to 1.000 (0.2, 0.5, 1, 2, and 10 µg/mL)	Ion analysis Matrix effects not studied	49–129 % without PSA and 74–122 % with PSA (spiked samples at 0.2 and 1.0 µg/g)	Not studied	Not studied	0.02–0.42 µg/g without PSA and from 0.02 to 0.51 µg/g with PSA (signal/noise = script of Analyst software)	Not studied	Not studied	Not studied
Belo et al. <a href="#">(2012)</a>	Weighted least-squares linear regression and R <sup>2</sup> from 0.9845 to 0.9896 (0, 0.75, 1, 2, 3 and 4 µg kg <sup>-1</sup> matrix matched calibration curve, n = 6)	Ion analysis Significant matrix effects (slopes of matrix-matched calibration curves were compared with slopes of pure solvent curves by t test)	54.01–114.69 % (spiked samples at 0.75, 2.00 and 4.0 µg/kg, n = 18)	Participation in inter-laboratory assay	RSD repeatability from 4.58 to 54.60 % and HORRAT from 0.31 to 3.72 RSD intermediate precision from 4.04 to 44.48 % and HORRAT from 0.18 to 2.02 (spiked samples at 0.75, 2.00 and 4.0 µg/kg, n = 18, analyzed	0.04–0.48 µg/kg (three times the standard deviation of the mean of blank oil sample, n > 20)	0.12–1.34 µg/kg (theoretical—ten times the standard deviation of the mean of blank oil sample, n > 20) The lowest concentration level of the calibration curve (practical)	14.5–92.3 % (top-down approach, considering the uncertainty of the calibration curve and intermediate precision)	Not studied

(continued)

**Table 4** (continued)

References	Method validation		Selectivity/matrix effects	Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity									
Cai et al. (2012)	Estimation of regression parameters and $R^2$ from 0.995 to 1.000 (1.5–400 pg, 18 concentration levels, $n = 3$ )		Ion analysis Definition of a gradient elution program in order to separate isobaric mass matrix interference peak responses from target measurands	77–110 % (spiked samples at 0.1–2 ppm level 1 and at 0.5–10 ppm level 2, $n = 3$ )	Not studied	RSD repeatability from 0.3 to 6.7 % (spiked samples at 0.1–2 ppm level 1 and at 0.5–10 ppm level 2, $n = 3$ ) Intermediate precision not studied	8–105.6 pg (instrument) and from 0.013 to 0.129 ppm (method) (six times the standard deviation of manually selected background signal/noise = 3 based on the analysis of low level calibration standards, $n = 3$ )	Not studied	Not studied	Not studied
Drabova et al. (2012)	Estimation of regression parameters and $R^2$ from 0.9963 to 0.9997 (0.05–100 ng/mL)		Ion analysis (use of GC $\times$ GC for separation of otherwise well-known critical groups of PAHs) Matrix effects not studied	73–103 % (spiked samples at 0.5, 2.5 and 5 $\mu\text{g/kg}$ , $n = 6$ )	Comparison with the routinely used method on the naturally contaminated sample	RSD repeatability from 2 to 12 % (spiked samples at 0.5, 2.5 and 5 $\mu\text{g/kg}$ , $n = 6$ ) Intermediate precision not studied	Not studied	0.05–0.2 $\mu\text{g/kg}$	Not studied	Not studied
Londoño et al. (2013)	A least three orders of magnitude had $R^2 > 0.991$		No significant peaks interfering with the measurands (blank samples)	79–105 % (spiked samples at 0.1–53 $\mu\text{g/kg}$ , $n = 3$ )	Verified through a reference solution	RSD repeatability from 1.2 to 9.4 % (spiked samples at 0.1–53 $\mu\text{g/kg}$ , $n = 3$ )	0.0004–4.9 $\mu\text{g/kg}$ (signal/noise = 3 based on the analysis of blanks, $n = 6$ )	0.0014–16.4 $\mu\text{g/kg}$ (signal/noise = 10 based on the analysis of blanks, $n = 6$ )	Not studied	Not studied

(continued)

**Table 4** (continued)

References	Method validation		Selectivity/matrix effects	Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity									
Payanan et al. (2013)	$R^2$ from 0.9739 to 0.9989 (spiked samples at 0.25–150.0 $\mu\text{g/kg}$ )	Not studied	45.9–118.5 % (spiked samples at 0.5, 1.0, 2.0 and 6.0 $\mu\text{g/kg}$ , $n = 10$ )	RM FAPAS T0631, olive oil, for 5 PAHs	Intermediate precision not studied	RSD repeatability from 0.1 to 6.2 % (spiked samples at 0.5, 1.0, 2.0 and 6.0 $\mu\text{g/kg}$ , $n = 10$ )	0.13–3.13 $\mu\text{g/kg}$ ( $n = 10$ )	0.25–6.25 $\mu\text{g/kg}$ ( $n = 10$ )	Not studied	Not studied
So-Young et al. (2013)	$R^2$ from 0.9763 to 0.9999 (0.5, 1, 5, 10, and 20 $\mu\text{g/kg}$ )	Ion analysis Matrix effects not studied	55.1–105 % (spiked samples at 100 $\mu\text{g/kg}$ , $n = 3$ )	96–97.4 % (CRM BCR 458, coconut oil, for 4 PAHs, $n = 5$ )	RSD repeatability from 0.8 to 7.5 % (spiked samples at 100 $\mu\text{g/kg}$ , $n = 3$ )	Intermediate precision not studied	0.01–0.06 $\mu\text{g/kg}$ (ratio between the standard deviation of the response and the slope of the calibration curve, multiplied by 3)	0.03–0.17 $\mu\text{g/kg}$ (ratio between the standard deviation of the response and the slope of the calibration curve, multiplied by 10)	Not studied	Not studied
Taylor et al. (2013)	Not studied	Not studied	Not studied	CRM NIST SRM 2947, mussel tissue, for 8 PAHs Comparison with a standard	Not studied	Not studied	Not studied	Not studied	Not studied	Not studied

(continued)

**Table 4** (continued)

References	Method validation		Recovery	Trueness	Precision	Detection limit	Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity	Selectivity/matrix effects							
Pincemaille et al. (2014)	R <sup>2</sup> from 0.9811 to 0.9991 (0, 0.1, 0.5, 1, 5 and 10 µg/kg for tea leaves and µg/L for infusions, matrix-matched calibration curve)	No significant peaks interfering with the measurands (blank samples, n = 5) Significant matrix effects (slopes of matrix-matched calibration curves were compared with slopes of non-matrix-matched calibration curves)	53–88 % (spiked samples with 1 µg/kg, n = 5)	HPLC–FLD method Not studied	Repeatability HORRAT from 0.2 to 1.0 Reproducibility HORRAT from 0.2 to 0.6	0.1–0.3 µg/kg or L (lowest concentration where both the quantifying and the qualifying transition presented a signal/noise = 3)	0.2–0.6 µg/kg or L (lowest concentration where both the quantifying and the qualifying transition presented a signal/noise = 10)	Not studied	Not studied
Pissinatti et al. (2014)	Estimation of regression parameters, the respective deviations, R <sup>2</sup> from 0.996 to 1.000 and evaluation of regression assumptions, including lack of fit test (50–800 pg/µL corresponding to 0.25–4.00 µg/kg, 6 concentration levels, n = 3)	Ions analysis No significant peaks interfering with the measurands (blank samples and CRM blank of coconut oil, n = 18) No significant matrix effects (comparison of the slope of the calibration curve in solvent with the slope of the matrix-matched calibration curve by t test)	87.08–111.28 % (samples spiked at 0.25, 1.00, and 3.00 µg/kg, n = 18)	Not studied	RSD repeatability from 3.26 to 23.75 % and HORRAT 0.22–1.62 RSD intermediate precision 3.29–33.34 % and HORRAT 0.15–1.52 (samples spiked at 0.25, 1.00, and 3.00 µg/kg, n = 18, analyzed on 3 different days, by	0.03–0.18 µg/kg (instrumental) (signal/noise = 3 based on the analysis of reagent blanks, n = 21)	0.11–0.59 µg/kg (instrumental) (signal/noise = 10 based on the analysis of reagent blanks, n = 21) (theoretical) (10 times the standard deviation of the response obtained for unspiked samples, n = 18) 0.26–3.09 (method) (lower concentration levels for which acceptable	11.85–66.06 % (top-down approach, considering the uncertainty of the calibration curve and the intermediate precision)	Not studied

(continued)

Table 4 (continued)

References	Method validation										Limit of quantification	Measurement uncertainty	Ruggedness
	Linearity/range/sensitivity	Selectivity/matrix effects	Recovery	Trueness	Precision	Detection limit							
					different analysts, employing different equipment and brands/batches of reagents)			recovery and precision were observed, n = 18)					

*Bz-L* benzo(e)fluorine; *BzF* benzo(f)fluoranthene; *CPP* cyclopenta(c,d)pyrene; *CRM* certified reference material; *GC*  $\times$  *GC* two-dimensional gas chromatography; *HORAT* the observed relative standard deviation divided by the value estimated from the modified Horwitz equation; *HPLC-FLD* high-performance liquid chromatography coupled to fluorescence detector; *IP* indene(1,2,3-c,d)pyrene; *n* number of observations; *PSA* cleanup sorbent that contains primary and secondary amines, used here in dispersive mode; *RM* reference material; *RSD* relative standard deviation, *R<sup>c</sup>* coefficient of determination



the fitness of the method for the stated purpose. Even for the evaluated parameters, experimental design and data analysis often did not follow the guidelines recommendations, as well as the regulated minimum performance criteria.

Although the use of the coefficient of determination as a test for linearity has been strongly discouraged (Thompson et al. 2002), this approach prevails in validation methods for PAHs in food (Diletti et al. 2005; Houessou et al. 2005; Badolato et al. 2006; Ballesteros et al. 2006; Liguori et al. 2006; Aguinaga et al. 2007; Purcaro et al. 2007b; Veyrand et al. 2007; Stanciu et al. 2008; Danyi et al. 2009; Lund et al. 2009; Orecchio et al. 2009; Cai et al. 2012; Drabova et al. 2012; Londoño et al. 2013; Payanan et al. 2013; So-Young et al. 2013; Pincemaille et al. 2014). A proper assessment of lack of fit and the verification of the regression assumptions (Souza and Junqueira 2005) have not been performed by most authors. Information about the experimental design revealed that several important aspects, such as the number of concentrations levels, evenly spaced distributions over the concentration range of interest and truly random replicates, have not been considered.

One relevant parameter that is frequently neglected is the matrix effects (Badolato et al. 2006; Ballesteros et al. 2006; Purcaro et al. 2007a, b; Veyrand et al. 2007; Stanciu et al. 2008; Danyi et al. 2009; Lund et al. 2009; Orecchio et al. 2009; Drabova et al. 2012; Payanan et al. 2013; So-Young et al. 2013; Taylor et al. 2013). According to the harmonized guideline (Thompson et al. 2002), the effects of a possible general matrix mismatch must be assessed during validation if the calibration standards were prepared as simple solutions of the measurands. Some strategies that were used to compensate for matrix effects were the matrix-matched calibration curves (Ballesteros et al. 2006; Belo et al. 2012; Pincemaille et al. 2014) and the calibration by standard addition (Aguinaga et al. 2007). Rose et al. (2007) argued that the use of  $^{13}\text{C}$ -isotopes corrected for any variation due to matrix effects. In fact, IDMS is discussed as an approach to overcome matrix effects. The use of matrix-matched curves is regarded as unnecessary to compensate matrix effects in this case, considering that the relative responses between the target measurand and the labeled analogue remain constant (Sargent et al. 2002; Hewavitharana 2011).

Trueness was not frequently investigated in the current practices for method validation because commonly certified reference materials were not used in the studies (Houessou et al. 2005; Badolato et al. 2006; Ballesteros et al. 2006; Aguinaga et al. 2007; Purcaro et al. 2007a, b; Stanciu et al. 2008; Danyi et al. 2009; Lund et al. 2009; Orecchio et al. 2009; Cai et al. 2012; Pincemaille et al. 2014; Pissinatti et al. 2014). Often, when these materials were employed, they did not represent the matrices defined in the scopes of the validation processes. This fact reflected another problem in this specific area that is the lack of materials for a significant range of food matrices. Comparison with a reference method (Drabova et al. 2012; Taylor et al. 2013) and participation in interlaboratorial assays (such as proficiency testing schemes) (Veyrand et al. 2007; Windal et al. 2008; Belo et al. 2012) were adopted by the researchers as alternatives in the absence of certified reference materials.

The validation processes were restricted to the evaluation of precision under repeatability conditions in most cases (Diletti et al. 2005; Houessou et al. 2005; Badolato et al. 2006; Ballesteros et al. 2006; Liguori et al. 2006; Aguinaga et al. 2007; Purcaro et al. 2007b; Lund et al. 2009; Orecchio et al. 2009; Cai et al. 2012; Drabova et al. 2012; Londoño et al. 2013; Payanan et al. 2013; So-Young et al. 2013). For precision assessment in a single-laboratory validation, two sets of conditions are important: repeatability conditions, related to the variations observed during a single run, and precision under run-to-run or intermediate precision conditions, related to variations in run bias. The variation in conditions among the runs must represent what happen in the routine use of the method, including representative variations in reagent batches, analysts, and instruments (Thompson et al. 2002). These findings indicated that an important aspect of precision had not been considered in the literature related to validation of methods for PAHs from food.

This fact may explain the small number of studies that considered the measure of uncertainty in the validation process (Diletti et al. 2005; Rose et al. 2007; Belo et al. 2012; Pissinatti et al. 2014), since the estimates of standard deviations obtained from the precision experiments represents the uncertainties associated with the random error (repeatability) and run (intermediate precision) terms, which are combined to estimate measurement uncertainty (Thompson et al. 2002).

Ruggedness is highlighted as one of the least studied parameters. In any method there are certain stages which, if not carried out sufficiently carefully, will produce significant effect on method performance. These stages should be identified by ruggedness tests. However, usually, the ruggedness is understood by the authors as part of the method development instead of the method validation (Magnusson and Ornemark 2014).

## 6 Conclusions

Techniques for the determination of PAHs in food have advanced, especially with regard to automation and miniaturization, as well as increased sensitivity and selectivity. However, the reported validation practices show that, in general, the performance evaluation of the methods is not conducted properly, which may compromise the reliability and comparability of the reported measurements and, consequently, the correctness of the actions taken.

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