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Review

Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods

Giorgia Purcaro *, Sabrina Moret, Lanfranco S. Conte

Department of Food Science, University of Udine, via Sondrio 2A, Udine 33100, Italy

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ABSTRACT

Polycyclic aromatic hydrocarbons are ubiquitous compounds, well-known to be carcinogenic, which can reach the food in different ways. Thus the analysis of such compounds has always been of great importance. The aim of the present review, is not only to give an overview of the most recent sample preparation and analytical approaches (such as pressurized liquid extraction, solid-phase microextraction, supercritical fluid extraction, etc.), but also to introduce such a topic to researchers who want to approach it for the first time; therefore, the most significant references related to general aspects, such as formation, toxicity, risk assessment, occurrence in food, are reported and briefly discussed.

Contents

Abbreviations: A, anthracene; Ac, acenaphthene; ACN, acetonitrile; Ap, acenaphthylene; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; BaA, benz[*a*]anthracene; BaP, benzo[*a*]pyrene; BbF, benzo[*b*]fluoranthene; BcF, benzo[*c*]fluorene; BeP, benzo[*e*]pyrene; BghiP, benzo[*g,h,i*]perylene; BjF, benzo[*j*]fluoranthene; BkF, benzo[*k*]fluoranthene; Car, carbone; Ch, chrysene; CNTs, carbon nanotubes; CPP, cyclopenta[*c,d*]pyrene; DBaeP, dibenzo[*a,e*]pyrene; DBahA, dibenz[*a,h*]anthracene; DBahP, dibenzo[*a,h*]pyrene; DBaiP, dibenzo[*a,e*]pyrene; DBalP, dibenzo[*a,l*]pyrene; DVB, divinylbenzene; EC, European Commission; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; ESI, electronspray ionization; F, fluorene; FID, flame ionization detector; Fl, fluoranthene; FLD, fluorimetric detector; GC, gas chromatography; GC × GC, comprehensive GC; HPLC, high performance liquid chromatography; HS, head-space; IP, indeno[1,2,3-*cd*]pyrene; JECFA, Joint FAO/WHO Expert Committee on Food Additives; JRC, Joint Research Centre; LC × LC, comprehensive LC; LOD, limit of detection; LOQ, limit of quantification; LVI, large-volume injection; MAE, microwave assisted extraction; MeOH, methanol; MEPS, micro-extraction in packed syringe; MIPs, molecular imprinted polymers; mMWCNTs, magnetic multiwalled carbon nanotubes; MoE, Margin of Exposure; MS, mass spectrometer; MSPD, matrix solid-phase dispersion; MSPE, magnetic solid-phase extraction; Na, naphthalene; NaOAc, sodium acetate; NH_2, amino; P, pyrene; Pa, phenanthrene; PAHs, polycyclic aromatic hydrocarbons; PDMS, polydimethylsiloxane; PLE, pressurized liquid extraction; PS-DVB, styrene-divinylbenzene; PSA, primary secondary amine; PTV, programmed-temperature vaporization; SCF, Scientific Committee on Food; SPE, solid-phase extraction; SPME, solid-phase microextraction; TEF, toxic equivalent factor; UHPLC, ultra-high performance liquid chromatography; WHO, World Health Organization

* Corresponding author. Tel.: +39 432 558393; fax: +39 432 558130.
E-mail addresses: giorgia.purcaro@uniud.it, giopurcaro@gmail.com (G. Purcaro).

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of ubiquitous and toxic environmental and food processing contaminants. PAHs contain two or more fused aromatic rings, produced through incomplete combustion or pyrolysis of organic matter and geological processes [1]. They can be classified according to the number of condensed aromatic rings as light (2–3 rings) or heavy (4–6 rings) PAHs, the latter being more stable and toxic than the light ones.

Due to their proved carcinogenic activity, they have been largely investigated, both to fully understand the toxicity mechanism and to elucidate the main sources and occurrence in foods. A great effort has also been devoted to the improvement of the analytical method to determine such compounds in complex samples, such as food. Furthermore, legislation has tried to follow the new evidences by introducing, modifying and updating the related regulations, which have evolved significantly over the last decades.

Many reviews [2–9] related to PAH analysis in food and in environmental samples have been published over the years. The aim of the present review is to give a useful tool to scientists who want to approach the topic of PAHs contamination in foods. A particular attention has been devoted to a concise but complete overview of the general PAHs aspect, such as toxicity, sources and occurrence, giving the most important and complete references to deepen the specific topics. The development of the European legislation on the matter has been largely discussed. Finally, a review of the analytical methods for PAHs determination has been reported, with a focus on more innovative and less-solvent and time-consuming techniques developed over recent years.

2. Sources, toxicity and occurrence.

All compounds containing carbon and hydrogen can act as PAH precursors at high temperature (500–700 °C). In fact, they are partially cracked to smaller unstable fragments (pyrolysis), mostly radicals, which recombine to give relatively stable PAHs (pyrosynthesis). Formation of PAHs can occur also at lower temperatures (100–150 °C) but it requires a period on the geological time-scale and a larger amount of alkylated PAHs are originated, as in the case of natural fossil fuel formation [10].

PAHs are present in the environment due to natural sources (forest fires, volcanoes, hydrothermal processes, oil seepage and carbonisation) and anthropogenic ones (mainly, the combustion of fossil fuels and the direct release of oil and oil products) [1,11].

A number of PAHs have been proven genotoxic and mutagenic, while other PAHs not defined as carcinogenic may act as synergists [12]. It is interesting to highlight that PAHs themselves are not toxic, but that they are activated by the attempt of the organism to eliminate these xenobiotics by increasing the polarity through the addition of polar groups. The most important metabolites of benzo[*a*]pyrene (BaP) is BaP-7,8-diol-9,10-epoxide, which presents the highest tumour inducing activity, because it forms adducts with proteins or DNA. A deeper and detailed discussion of activation mechanism can be found in several documents published by the World Health Organization (WHO) and the European Scientific Committee on Food (SCF) [12–16]. Very little systematic information is available on the effect of alkylation on the biological activity of PAHs, nevertheless it seems strictly correlated with the position of substitution in the ring. In particular, methyl substitution might enhance the toxicity compared to the non-alkylated PAHs, while bulky substitutions would reduce the bio-activity of such compounds [17].

Humans can be exposed to PAHs through three main routes: inhalation, skin contact, and ingestion.

The rate of absorption by the lungs depends on the structure of the PAH, the size and the chemical nature of the particles where they are adsorbed [16–20]. Most of the toxicity studies on PAHs have been carried out on dermal, subcutaneous and inhalation exposure, mainly as an occupational issue. However, it has been demonstrated that ingestion through food and water is a major route of exposure [12,21]. The composition of the diet affects PAHs absorption in the intestinal mucosa. Indeed, absorption of ^{14}C-BaP in Wistar rats is increased by lipophilic foods and is inhibited by high-fibre foods [22].

The occurrence of PAHs in food is mainly due to environmental contamination, technological processing, and contact with non-food-grade quality mineral oil and contaminated packaging. Considering the numerous sources of PAHs that release them into the environment, the atmospheric fallout is an important cause of contamination in plant and vegetables directly in the field [23,24]. In particular, only light PAHs are present in the gaseous phase, while both the light and the heavy ones may be adsorbed on the particulate.

Among the technological processes, heating (i.e., grilling and smoking) involving direct contact with combustion gases is an important source of contamination [25,26]. The contribution to contamination levels depends on: the time and temperature of processing (higher temperature and longer time increase the amount of PAHs); the distance from the heat source (the higher the distance, lower the contamination level in foods); the kind of process (grilling, roasting, smoking, drying), in particular, if food is directly in contact with the combustion products; the type of fuel used (e.g., burning of carbon produce less PAHs than wood), and the amount of fat in the processed food (fat is the major precursor of PAHs). Finally, contamination with non-food-grade mineral oil may occur, mainly related to lubricant oil, jute bags, recycled paper packaging, printing inks [27–31].

The most important contributors to dietary intake are vegetable oils and fats, both as a seasoning and indirectly as an ingredient in food formulation [25]. Very high levels of PAHs can also be found in dried fruits (when combustion products are allowed to come directly in contact with food or due to contamination during transport) [32], and in smoked meat and fish products [26] (related to the smoking method) [33]. Among fishery products, blue fish species (i.e., tuna, mackerel and salmon) generally present lower contamination levels than molluscs, even if they originated from polluted areas. Indeed, fish, in contrast with bivalves, oxidize and metabolise PAHs to water-soluble compounds, which are eventually excreted. Several reports discuss in depth the occurrence of PAHs in food [12,34,35].

PAHs occurring in foods are always present as a complex mixture. This has to be considered during the selection of the most suitable risk assessment approach to evaluate the PAH toxicity. The risk assessment process consists of several steps including hazard identification, hazard characterisation, exposure assessment and risk characterization. Three of the most popular approaches are toxicity equivalent factor (TEF), comparative potency, and BaP as a surrogate (the detailed discussion of such topics are out of the aim of the

Table 1
Name, abbreviations, molecular weigth (mw), and formula of PAH reported by the Environmental Protection Agency (EPA) and the European Union (EU).

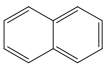
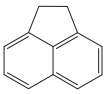
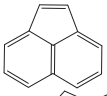
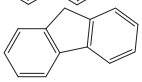
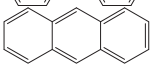
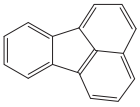
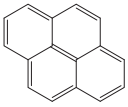
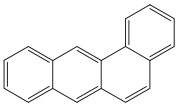
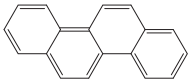
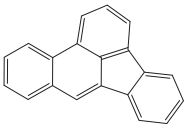
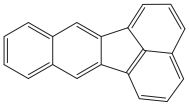
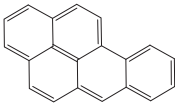
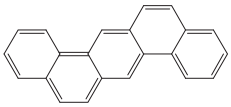
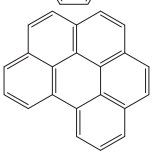
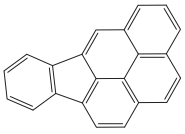
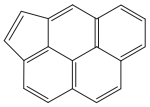
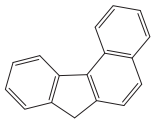
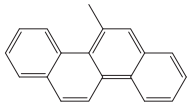
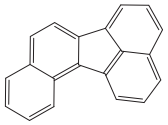
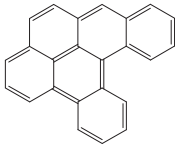
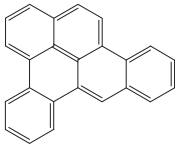
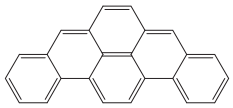
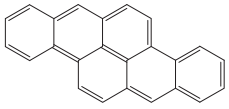
Compounds	Abbreviation	mw	Formula
EPA PRIORITY			
Naphthalene	Na	128	
Acenaphtene	Ac	154	
Acenaphthylene	Ap	152	
Fluorene	F	166	
Phenanthrene	Pa	178	
Anthracene	A	178	
Fluoranthene	Fl	202	
Pyrene	P	202	
EPA/EU PRIORITY			
Benz[*a*]anthracene	BaA	228	
Chrysene	Ch	228	
Benzo[*b*]fluoranthene	BbF	252	
Benzo[*k*]fluoranthene	BkF	252	
Benzo[*a*]pyrene	BaP	252	
Dibenz[*a*,*h*]anthracene	DBahA	278	
Benzo[*g*,*h*,*i*]perylene	BghiP	276	
Indeno[1,2,3-*cd*]pirene	IP	276	
Compounds ***EU PRIORITY***	**Abbreviation**	**mw**	**Formula**
Cyclopenta[*c*,*d*]pyrene	CPP	226	

Table 1 (*continued*)

Compounds *EU PRIORITY*	Abbreviation	mw	Formula
Benzo[*c*]fluorene	BcF	216	
5-methylchrysene	5MeCh	242	
Benzo[*j*]fluoranthene	BjF	252	
Dibenzo[*a*,*l*]pyrene	DBalP	302	
Dibenzo[*a*,*e*]pyrene	DBaeP	302	
Dibenzo[*a*,*i*]pyrene	DBaiP	302	
Dibenzo[*a*,*h*]pyrene	DBahP	302	

present paper, but to deepen such an issue readers are directed to specific literature [15,36,37]). The latter approach was considered by the European Commission until the European Food Safety Authority (EFSA) published an Opinion in 2007 highlighting some doubts in using such an approach [34].

When epidemiological data are not available, the risk assessment related to xenobiotic exposure are evaluated using animal assays. Animals are exposed to high levels of toxic compounds in order to compensate for the low number of experimental groups and to cause cancer in a reasonable time; therefore, the extrapolation of low levels of exposition to which human population may be exposed is needed. A wide range of models, giving very different conclusions, can be used to extrapolate these data [38]. In 2005, the SCF suggested the possibility to use the *Margin of Exposure* (*MoE*) approach [39], which is a rather complicated model. Since some knowledge of risk assessment is needed to understand such a model, readers interested in the topic are directed to the related reference [39]. Comparable approaches have been used by Health Canada for Priority Substances under the Canadian Environmental Protection Act [40], the National Health and Medical Research Council in Australia and New Zealand for the Toxicity Assessment for Carcinogenic Soil Contaminants [41], and the Joint FAO/WHO Expert Committee on Food Additives [42].

3. European legislation

The lack of a harmonized European legislation on PAH limits and determination methods became a priority issue after 2001, when a highly contaminated pomace olive oil was detected in the Czech Republic. A rapid alert procedure [43] (for a rapid information exchange among the members of EU to prevent the sale of hazardous products to humans) was immediately started. Following this finding, Spain, Italy, Greece and Sweden, fixed a limit of 5 μg/kg for the sum of 8 heavy PAHs (BaP, dibenz[*a*,*h*]anthracene-DBahA-, benz[*a*]anthracene- BaA-, benz[*e*]pyrene-BeP-, benzo [*b*]fluoranthene-BbF-, benzo[*k*]fluoranthene-BkF-, indeno[1,2,3-*cd*] pyrene-IP-, and benzo[*g*,*h*,*i*]perylene-BghiP-) and a limit of 2 μg/kg for each single PAH in olive pomace oil. Germany had a limit of 5 μg/kg for heavy PAHs and of 25 μg/kg for the sum of all the 16 PAHs highlighted by the US Environmental Protection Agency (EPA) in oil and fats. The Canadian legislation fixed a limit of 3 μg/kg for the sum of heavy PAHs (BaP, DBahA, BaA, BbF, BkF, IP, Ch and BghiP) calculated on the basis of the TEF [44]. These varieties of legal limits caused several commercial problems.

The 16 EPA environmental priority PAHs [45], or only BaP have been the main focus of the research community until the Opinion of the SCF in 2002 [12]. The SCF assessed 33 PAHs and identified 15 PAHs as both carcinogenic and genotoxic. Table 1 reports the structure of the EU and the EPA PAHs, highlighting the common ones.

The SCF opinion stated that BaP could be used as a marker since the profile of the measured PAHs in various foodstuffs was very similar. According to such an opinion, in 2005 the European Commission (EC) introduced Regulation No. 208/2005 [46], and the following Regulation No. 1881/2006 [47], which harmonised PAHs legislation among all the member states, and fixed a limit

Table 2
New limit fixed by the EC Regulation 835/2011 for benzo[*a*]pyrene and the sum of PAH4 (BaA, Ch, BbF and BaP) in foods. (Modified by regulation 835/2011).

Product	Maximum level µg/kg	
	BaP	Sum of PAH4
Processed cereal-based foods and baby foods for infants and young children Infant and follow-on formulae, including infant and follow-on milk Dietary foods for special medical purposes, intended specifically for infants	1	1
Oils and fats intended for direct human consumption or use as ingredient in food	2	10
Coconut oil for direct human consumption or use as ingredient in food	2	20
Cocoa beans and derived products	5[a]	35[b] 30[c]
Smoked sprats and canned smoked sprats Bivalve molluscs (fresh, chilled or frozen)	5	30
Heat treated meat and meat products sold to the final consumer (i.e., grilled and barbecued) Bivalve molluscs (smoked)	6	35
Muscle meat of smoked fish and smoked meat and meat products	5[d] 2[f]	30[e] 12[g]

[a] from 1/4/2013.
[b] from 1/4/2013 until 31/3/2015.
[c] from 1/4/2015.
[d] until 31/8/2014.
[e] from 1/9/2012 until 31/8/2014.
[f] from 1/9/2014.
[g] from 1/9/2014.

for the presence of BaP only in different classes of foods. Furthermore, all the Member States were recommended (Recommendation 2005/108/EC [48]) to investigate the levels of the 15 PAHs pointed out by the SCF and the one PAH (benzo[*c*]fluorene-BcF-) highlighted by the JECFA [41], in order to review the limits already set and to add new food classes by 1st April 2007. Finally, any specific official methods were dictated for the analysis of PAHs but the performance criteria to whom the method applied has to fit was fixed for BaP (Directive 2005/10/EC [49], then included in the Regulation No. 333/2007 [50]).

In April 2007 the legal limits were not changed, but in June the EFSA published a new report [34] about the data collected from the Member States raising doubts about the suitability of BaP as a marker. The high presence of Ch and BcF in most of the samples considered was also highlighted. In 2008, the EFSA CONTAM Panel [51] concluded that BaP is not a suitable marker for PAH occurrence in foods. Thus, the use of the sum of eight high molecular weight PAHs (PAH8) (BaA, Ch, BbF, BkF, BaP, DBahA, BghiP, IP), as well as the sum of a subgroup of four PAHs (PAH4), including BaA, Ch, BbF and BaP was suggested.

This latter EFSA Opinion was taken into account in August 2011 enacting a new Regulation (Reg. 835/2011 [52]), coming into force from 1st September 2012, and fixing new limits considering both BaP and the sum of four PAHs (PAH4). The food classes left suspended in the previous regulation were added in the latter one, namely cocoa beans and derivatives, and coconut oil. The new data collected have shown that the background levels of PAHs in some commodities are lower than previously reported, thus for such products the limits have been added, but giving more time to adapt the production technologies (as in the case of smoked products) and the Good Manufacturing Practices. The new limits fixed by the Regulation 835/2011 are shown in Table 2.

Furthermore, the EC, considering the Report 59046 [53] presented by the Joint Research Center (JRC) in 2010, extended the performance criteria fixed for BaP in the Regulation 333/2007 [50] to all the PAH4, with the Regulation 836/2011 [54] (Table 3), and gave specific guidelines for sampling. It is important to highlight that, if any of the 4 PAHs resulted below the limit of quantification (LOQ) it is considered as zero in the summing of the 4 PAHs.

Table 3
Performance criteria for the methods of analysis of BaP, BaA, BbF, Ch. (Modified from regulation 836/2011).

Parameter	Value
Precision (repeatability and reproducibility)	$HORRAT_r$ or $HORRAT_R < 2$ in ring test
Recovery	50–120%
LOD	≤ 0.3 µg/kg for each PAH
LOQ	≤ 0.9 µg/kg for each PAH

4. Sample preparation and analytical methods in food analysis

Natural products, thus foodstuffs processed to a different extent, are very complex samples, especially when trace compounds have to be determined. In fact, such analytes have to be separated from the bulk mass of different constituents, some of them very similar to the main constituents of the samples. In the specific case, PAHs are highly lipophilic, thus when extracted they are associated with the lipid constituents, which have to be removed by a cleaning step. Numerous approaches have been proposed over the years following the development of the lipid extraction methods (from solid to liquid extraction or liquid–liquid extraction to pressurized liquid extraction or supercritical fluid extraction), followed by different preparation steps (from partition methods to solid-phase extraction or solid-phase microextraction). Finally, the analytical determination can be carried out by both liquid chromatography (LC), including ultra-high performance LC (UHPLC), and gas chromatography (GC) with different detectors, or by comprehensive techniques ($GC \times GC$, and $LC \times LC$).

Since discussion on sample preparation may be correlated to the characteristics of the final analytical technique, the latter one will be discussed first, despite the natural chronological sequence. The discussion on the preparation methods (extraction and clean-up) in food analysis has been focused on innovative and more recently proposed methods, leading to a reduction of the solvents employed and time-consumed, since several reviews on general determination have already been published [2–6].

4.1. Analytical determination

4.1.1. Liquid chromatography

LC has been the most employed technique for long time, also in some official methods proposed by the International Standardization Organization [55–59] and of the US EPA [60]. In particular, the latter document discussed the choice of LC as a lack of resolution of GC in resolving some peak pairs of compounds. As mentioned above, most of the papers have dealt with the separation of the 16 EPA PAHs, with an interval of time during which few works focused on the 15+1 EU PAHs (between the Recommendation 2005/208 [48] and the EFSA Opinion in 2008 [51]).

The most employed columns are packed with specially designed C_{18} phase, with 100–250 mm length × 3.0–4.6 mm ID packed with 3–5 μm particles. The mobile phase used for the 16 EPA PAH separation is usually an acetonitrile (ACN) or methanol (MeOH) gradient in water, starting from 50 to 60% and linearly increasing to 100% of ACN/MeOH. The use of ACN enables an easier optimization of the elution parameters and a faster analysis, but good performance may be obtained also using MeOH, paying attention to a change in the elution order between DBahA and BghiP. The use of MeOH allows to reduce the analysis costs (since ACN has became very expensive in recent years). Instead, to separate the 15+1 EU PAHs a multi-step gradient is required, since the resolution of some peak pairs is difficult, such as P and BcF, BjF and BeP, DBahP and BghiP [61–63]. However, the mobile-phase gradient is not always sufficient; in fact the few works dealing with such a separation used more detectors in series. Specific wavelengths were employed in-series- spectrofluorometric detectors (FLD) to selectively detect one analyte or the other co-eluted compounds, while UV (a much less sensitive detector compared to FLD) was necessary to detect CPP, which does not give rise to fluorescence. Notwithstanding that the FLD, at present, is still the detector of choice for PAH analysis, thanks to its selectivity and sensitivity, in recent years the use of mass spectrometer (MS) has been increasing, giving sensitivity similar to FLD (in the order of 10 pg).

The MS has been more employed on environmental samples and biological metabolites, rather than food samples. The most exploited ion sources in LC–MS analysis are electrospray ionization (ESI) and atmospheric-pressure chemical ionization source (APCI) [64,65]. However, such interfaces give low efficiency in non-polar compound ionization. To come through this problem, several authors [66,67] used post-run chemical derivatization, by adding tropylium (TR^+) or Ag^+ (as silver nitrate) to the eluent as electron capture acceptors, to yield cationic PAH adducts. The addition of Ag^+ salt (as $AgNO_3$) gives better sensitivity than TR^+, but may deposit in the instrument, requiring more frequent maintenance.

The generally less employed atmospheric-pressure photoionization (APPI) ion source extends the range of ionisable compounds to many non-polar analytes. Furthermore, this type of interface shows less ion suppression than APCI and ESI. Usually, to further increase the ionization, a dopant (e.g., acetone or toluene) is used. The dopant effect depends on ionization energies and proton affinities of the analytes and eluents. For PAH analysis the use of acetone or toluene [68], or a mixture of toluene and anisole [69] have been proposed. APPI shows a lower sensitivity when 1 mL/min flow rate is employed, therefore the general trend in minimizing the solvent consumption in analytical chemistry (thus the LC flow rate) will probably lead to an increase in the use of APPI which generally performs better than APCI. UHPLC methods have been recently proposed [70–73] coupled with both FLD or MS. UHPLC compared to conventional HPLC, enables to improve the resolution, increase the throughput (total analysis time is about 3/4-fold less), and solvent consumption is drastically reduced (more than 70%), therefore, waste and costs are much lower. Cai and co-workers applied UHPLC coupled to APPI-MS/MS to analyse a standard mixture of PAHs [71]. All the 16 US EPA PAHs were successfully analysed in 3.5 min with picogram detection limits, using a chlorobenzene as a dopant. A longer gradient was needed when complex matrices, such as oyster samples, were analysed by APPI–MS, in order to separate isobaric mass matrix interferences [73]. Thus the entire analysis lasted 14 min. Even though toluene as a dopant may offer lower sensitivity compared to chlorobenzene, it was preferred by Smoker and co-workers [74] since less toxic. In 2011, Gosetti and co-workers [72] applied a UHPLC–(APCI–MS)/MS method for the simultaneous analysis of 13 PAHs and 12 aldehydes in different cooked foods. The chromatogram obtained after SPE purification of the extract is shown in Fig. 1.

4.1.2. Gas chromatography

Capillary GC technique is a valuable alternative, sometimes a necessity, for PAHs analysis considering that several PAHs cannot be easily detected in LC–FLD due to a too low fluorescence signal. In particular, GC has a higher peak capacity compared to LC and coupling to MS detectors is easy and relatively cheap, enabling high selectivity and sensitivity. GC–MS enables the determination of non-fluorescence PAHs, such as CPP and alkylated PAHs. Since PAHs are highly stable and produce mainly the molecular ion ($[M–H]^+$ or $[M–2H]^+$) along with few fragments, the single quadrupole analyzer is the most employed. However, some work reports the use of ion trap (IT) [75,76] and triple quadrupole (QqQ) [77,78]. Usually the single ion monitoring (SIM) acquisition mode is employed to enhance sensitivity (pg amount can be detected). Quantification is carried out using the isotope dilution determination, which involves the addition of deuterated or ^{13}C-labelled PAHs. GC-flame ionization detector (FID) is not suitable for such a determination since it is not sensitive enough and is subjected to background interferences. Very few papers report the use of a photo-ionisation detector (PID) [79]. In particular, since PID is a non-destructive detector, it was used coupled with FID, and the response ratios of the two detectors can be used for

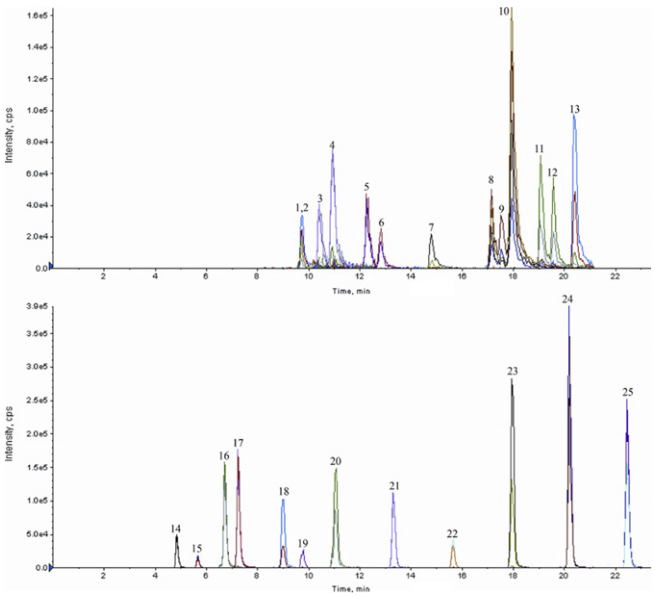


Fig. 1. A UHPLC–MS/MS chromatogram of a PAH standard mixture. (a) PAH separation in APCI PI mode; (b) DNPH-aldehyde separation in APCI NI mode. Peaks: (1) Ac, (2) F, (3) Pa, (4) A, (5) Fl, (6) P, (7) Ch, (8) BeP, (9) BkF, (10) BaP, (11) DBacA, (12) DBahA, (13) BghiP, (14) formaldehyde, (15) acetaldehyde, (16) acrolein, (17) propanal, (18) butanal, (19) benzadehyde, (20) pentanal, (21) hexanal, (22) heptanal, (23) octanal, (24) nonal, (25) decanal (reprinted with permission from Ref. 72).

identification purposes. However, much more reliable results and higher sensitivity are obtained using the MS.

As mentioned before, GC has been often considered by the US Official Authority [60] not reliable enough, mainly due to several co-elution problems. In fact, several PAHs pairs present critical resolution, namely (1) A and Pa, (2) Ch and triphenylene, (3) CPP, BaA and Ch, (4) BbF, BjF and BkF, (5) DBahP and DBacA, (6) DBahA and IP. Actually, these unresolved pairs depend on the stationary phase used, thus the selection of the suitable column is an important issue, which has been discussed in depth by Poster and co-workers [7] and Gómez-Ruiz and co-workers [80].

The complete resolution of the 15+1 EU PAHs was obtained with a careful choice of the suitable chromatography conditions, including the stationary phase, by carrying out a fast GC analysis on a 50% phenyl column (10 m × 0.1 mm × 0.1 μm), as shown in Fig. 2 [81].

In general, when performing GC analysis, the choice of a suitable injection mode is of great importance, since discrimination can occur, especially when less volatile compounds are involved. Usually the use of a multi-baffle liner or a packed liner can solve such a problem, but the type of packing material is important (e.g., glass wool, tenax®, PDMS, etc.). Indeed, in the case of PAHs, the presence of glass wool can cause analyte loss or peak shape distortion due to sorbent activation. Among the possible injection modes, the most employed for PAH analysis have been splitless injection, programmed-temperature vaporization (PTV), cold on-column injection, large-volume injection (LVI). In particular, the combination of PTV and LVI has been largely applied to reduce injection discrimination and increase sensitivity in environmental and food samples [80,82].

4.2. Sample preparation

Before approaching any PAH sample preparation methods, it is important to highlight several precautions that must always be applied. Since PAHs are ubiquitous, all the containers should be rinsed with high purity acetone or hexane before use. To avoid cross-contamination, the samples analyzed have to come in contact only with inert materials, such as aluminium, glass or stainless steel; plastic materials have to be completely avoided, especially polyethylene, since analytes adsorption may occur. Since PAHs are light sensitive, samples and extracts have to be protected from light by using amber or foil-wrapped vials. Finally, to avoid loss of the lighter compounds during drying, it is a good practice to avoid drastic evaporation processes and to leave the last drop to evaporate naturally or to use a keeper, such as toluene.

In general sample preparation involves an extraction and a purification step, which, according to the nature of the sample to be analyzed (liquid, solid, fatty or not-fatty), can be fused together in a unique and less time-consuming step. The latter approach is a general trend in the sample preparation field, therefore, the discussion will be focused on more innovative methods, which lead to minimising the solvents and manipulation, as well as the time-consumed.

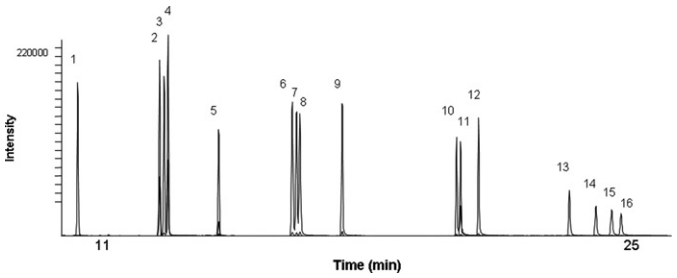


Fig. 2. GC–MS chromatogram of the 16 EU PAH on a 50% phenyl column (10 m × 0.1 mm × 0.1 μm). Peaks: (1) BcF, (2) BaA, (3) CPP, (4) Ch, (5) 5MeCh, (6) BbF, (7) BkF, (8) BjF, (9) BaP, (10) IP, (11) DBahA, (12) BghiP, (13) DBalP, (14) DBaeP, (15) DBaiP, (16) DahP. (Adapted with permission from Ref. 81).

The preparation techniques to be used can be roughly divided into two categories, according to the type of food analyzed. The first group is related to not-fatty foods, which are mainly water and beverages, thus usually the extraction and the purification step can be carried out in a single step. The second group is fatty foods, which includes both liquid (vegetable oils, milk, etc.) and solid (fish, meat, cheese, etc.) foods. In this case, extraction and purification can be carried out either separately or as a single step. The two distinct categories are discussed separately. Table 4 summarizes the applications discussed, considering the extraction and the analytical techniques, the range of detection limits (LODs) obtained and the PAHs analyzed.

4.2.1. Non-fatty foods

PAHs are organic compounds highly lipophilic, thus non-fatty foods are usually less contaminated. The contamination of the main product of this category (water) is related to PAHs bound to particulate matter, which is mainly removed during the water sanification process, namely flocculation, sedimentation and filtration, and to some extent during oxidation.

When liquid non-fatty samples are analysed, a loss of PAHs may occur due to solubility problems (e.g., adsorption onto the glassware or cartridge surfaces). Low amounts of organic solvent (e.g., MeOH, ACN, or 2-propanol) are added to the sample to minimize such a loss, however, the quantity to add is a critical point in the method optimization process (typically a 1–25% of the total volume). When analyzing such liquid samples, extraction and purification can easily be obtained by a unique solid-phase extraction (SPE) step, mainly retaining the analytes of interest, and then eluting them with an amount of solvent as low as possible [83]. Usually a mixture of solvent (polar and non-polar, such as hexane/dichloromethane, methanol/tetrahydrofurane, isooctane/cyclohexane) is employed as a compromise between the recovery of light and heavier PAHs. The most exploited SPE sorbent has been the C_{18}-bonded silica, but others have been tested as well (e.g., C_8, styrene-divinylbenzene – PS-DVB – cyano and phenyl sorbents). The choice of the sorbent is related to both the nature of the sample and, to some extent, to the final analytical method. For instance, it was shown that for PAH analysis in lake sediments, the use of a C_{18} and silica phase gave a suitable extract for HPLC-FLD analysis, but interference of aliphatic waxes with heavy PAHs was highlighted by the GC-MS analysis. Therefore, in such a case, a further clean-up step using activated silicic acid and neutral aluminium columns proved to be more effective [83].

The SPE-approach has been widely employed not only for drinking water, but also for beverage analysis, such as coffee [84], tea [85], alcoholic beverages (wine, beer and spirits) [86–88]. A review on SPE analysis of polycyclic aromatic compounds was published by Marcé and Borrul in 2000 [83].

Recently several novel phases have been tested as SPE sorbent, such as molecular imprinted polymers (MIPs) [89], sulfonated graphene sheets [90], and carbon nanotubes (CNTs) [91,92]. Lai and co-workers [93] synthesized a BaP-imprinted polymer and tested its performance in three kinds of sample, namely tap water, lake water and instant coffee. The recovery of BaP from tap water was significantly higher (96.5%) compared to the other two samples (84.7 and 72.5% for lake water and coffee, respectively). The recovery from the instant coffee was higher on the MIPs cartridge than on a C_{18} one (58.8%). The performance of CNTs was compared to a C_{18} SPE for PAHs analysis in

Table 4
Summary of the main approaches discussed in the text related to non-fatty and fatty foods.

Extraction technique		Sample	Analytical determination	PAHs analyzed	LOD (μg/L or μg/kg)[a]	References
Technique	**Sorbent material/ purification**					
Non-fatty food						
SPE	PS–DVB	Coffee brew	HPLC–FLD	F, BbF, BaP	0.19–2.49	84
	C_{18}	Tea infusion; spirits; sugar cane	HPLC–FLD	Na, Ac, Ap, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BeP, BaP, IP, DBahA, BghiP	0.001–0.14	85, 87, 88
	CNTs	Water	HPLC–UV, GC–MS	Na, Ac, F, Pa, A, Fl, P, BaA, BbF, BkF	0.009–0.058	91, 92
	MIPs	Water and coffee	HPLC–FLD	BaP	–	93
μ-SPE	Sulphonated graphene sheets	Water	GC–MS	Na, Ac, F, Pa, A, Fl, P	0.0008–0.004	90
MEPs	C_8	Water	GC–MS	A, F, Fl, P, Ch	0.001–0.005	94
SPME	PDMS	Water	GC–MS	Ac, Ap, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	0.003–0.12	95, 100–104
SBSE	PDMS	Water	GC–MS	Na, Ac, Ap, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP, 1-MeNa, 2-MeNa	0.0002–0.0020	96, 97, 99
MASE		Water and beverages	GC–MS	Na, Ac, Ap, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	0.003–0.004	105
Fatty food						
SPE	Si	Vegetable oil	HPLC–FLD	Na, Ac, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	<0.1	106
	C_{18}/Florisil	Olive oil	GC–MS; HPLC (DACC)–FLD	BaP	0.5–1.6	108
	NH_2	Vegetable oil	HPLC–FLD	BaA, Ch, BbF, BkF, BeP, BaP, IP, DBahA, BghiP	0.01–0.02	109
MSPD	Florisil; C_{18}	Fish sample	HPLC–FLD	DBalP	0.04–0.32	111, 136
MSPE	mMWCNT	Edible oil	GC–MS; HPLC (DACC)–FLD	BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	0.10–0.88	112
SPME	DVB/Car/PDMS	Olive oil	GC–MS	Na, Ac, Ap, F, Pa, A, Fl, P, 1-MeNa, 2-MeNa	0.05–1.6	114
	Carbopack Z/ PDMS	Vegetable oil	GC × GC–MS; GC–MS	BcF, CPP, BaA, Ch, BbF, BkF, BjF, BaP, IP, DBahA, BghiP, DBalP, DBaiP, DBaeP, DBahP, 5-MeCh	0.02–1.4	115, 116
	PDMS/DVB	Milk; fish sample	GC–MS	Na, Ac, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	0.003–1.56	117, 118, 142
	PDMS	Smoked food	GC–MS		0.008–0.1	143
PLE	SPE-Florisil	Smoked food; oil	GC–MS; GC–MS/MS	Na, Ac, Ap, F, Pa, A, Fl, P, BcF, CPP, BaA, Ch, BbF, BkF, BjF, BaP, IP, DBahA, BghiP, DBalP, DBaiP, DBaeP, DBahP, 5-MeCh	0.008–100	122, 124
	Alkaline digestion	Mussel	GC–MS	Na, Ac, Ap, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	0.5–8	123
	Fat retainer: polyacrylic acid in PLE cell	Smoked fish	GC–MS	Na, Ac, Ap, F, Pa, A, Fl, P, BcF, CPP, BaA, Ch, BbF, BkF, BjF, BaP, IP, DBahA, BghiP, DBalP, DBaiP, DBaeP, DBahP, 5-MeCh	0.2–4.4	128
MAE	μ-Droplet extraction with IL	Toasted cereals	HPLC–FLD	BcF, CPP, BaA, Ch, BbF, BkF, BjF, BaP, IP, DBahA, BghiP, DBalP, DBaiP, DBaeP, DBahP, 5-MeCh	0.007–22	144, 145
	SPE–Si	Fish; smoked meat; propolis	HPLC-FLD	F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP	0.1–0.5	132–134
QuEChERS	PSA, C_{18}	Fish; seafood	HPLC–FLD; UHPLC–APPI-MS; GC–MS	Na, Ac, Ap, F, Pa, A, Fl, P, BaA, Ca, BbF, BkF, BeP, BaP, IP, DBahA, BghiP, DBalP, plus several alkylated	0.005–8	71, 138–140

[a] The range include the lowest and the highest value reported in the related references.

environmental water, giving similar recoveries at low-spiked level, and significantly better at higher-spiked level in tap water. [91].

The same principles of SPE are applied in micro-extraction in the packed syringe (MEPS) approach, which was applied for the analysis of five PAHs (A, Ch, Fl, F, P) in water [94]. The water sample (50 μL) was extracted by drawing it 60 times through the syringe, and successively desorbed in a PTV injector at 40 °C by using methanol (30 μL) as the elution solvent. The performance of the method was compared to other published methods, namely SPME [95] and stir-bar sorptive extraction (SBSE) [96], giving similar results. The method is very promising, since it is faster and cheaper than SPE, but it would be interesting to assess the performance of such an approach considering the more toxic heavy PAHs.

In 2009, Barrek and co-workers [97] compared the performance of four commercial SPE cartridges (Oasis HLB, packed with *N*-vinylpyrrolidone-DVB; Strata-C_{18}, packed with a C_{18} sorbent; Strata-X, packed with *N*-vinylpyrrolidone-DVB; Envi-carb, packed with a graphitized carbon black), a stir-bar sorptive extraction (SBSE, coated with PDMS), a carbonaceous adsorbent (Ambersorb 572), and a C_{18} SPE disk (Envi-C_{18} DSK), for a multi-residue method. The analyzed compounds were 18 pesticides, 8 PAHs (A, Fl, Na, BaP, BbF, BghiP, BkF, IP), 5 endocrine-disruptors, and 4 organochlorine compounds. Both GC-MS and LC-FLD-MS/MS were employed for the final analytical determination. The best results were obtained using a Strata-X cartridge, with recoveries ranging from 60 to 105%, Envi-carb cartridge gave acceptable results for pesticides, but it was impossible to elute PAHs from such a support, since firmly adsorbed. SBSE and Ambersorb 572 gave recoveries sufficient for a multi-residue procedure, but not for ultra-trace analysis. SBSE is a very powerful, rapid and easy-to-use technique (the sorptive stirrer is placed directly into the water sample and then desorbed thermally or using low volume of solvent [98]), it gave better performance in terms of sensitivity and recovery compared to LLE with *n*-hexane [99] or SPME (using a PDMS fiber) [100].

The lower sensitivity and recovery values obtained using SPME, satisfactory for many purposes, are largely compensated by the ease of use of this technique, the availability of many different sorbent phase, and the good repeatability obtained when correctly handled; therefore, SPME has been largely applied as a solvent-free approach for PAH analysis in water [101], both in direct immersion (DI) and head-space (HS) extraction. However, since PAHs are a large class of compounds, the fiber selection is a critical point [102]. Indeed, the fiber phase largely affects the extraction yield. Doong and co-workers [103] showed that a Carbowax (CAR)/polydimethylsiloxane (PDMS) fiber (65 μm) has the best extraction efficiency for Na, but the worst for 4 or more ringed PAHs, while a polyacrilate (PA) fiber has similar extraction efficiency to a 100 μm PDMS fiber (Fig. 3).

In 2012, Bianchin and co-workers [104] proposed an interesting approach, which combined different extraction mode and temperature in a single extraction procedure. Using a PDMS/DVB fiber BTEX and PAHs were simultaneously extracted performing a previous extraction in DI mode at 80 °C for 48 min, followed by an HS extraction for 32 min at 10 °C. Results obtained compared to the single extraction modes are shown in Fig. 4.

Sensitivity problems are generally reported for SPME extraction, although several works have shown good LOD values in the 1–100 ng/L range.

Membrane-assisted solvent extraction (MASE) is a micro-extraction technique that exploits the diffusion of the analytes through a non-porous membrane into an organic solvent. Although competitive adsorption in some matrices may occur (especially fat-containing samples), it was successfully applied for PAHs analysis in different water-based beverages (e.g., water, wine and juice) without any matrix effect and obtaining results comparable to SPME and SBSE [105].

4.2.2. Fatty foods

In the fatty food category both edible fats and oils are included, and fatty extracts obtained from different foods, such as milk, fish, meat, etc. The main challenge in the analytical determination of trace compounds in such a matrix is to isolate the compounds of interest from the bulk presence of triglycerides and fatty acids. In the case of edible oils, the extraction and purification methods often coincide, and such a procedure may often be applied to the fatty extract deriving from different extraction procedure in solid matrices.

Among the most recently employed techniques, SPE is the approach of choice to meet sensitivity and rapidity requirements. Several applications have proposed SPE as a single extraction and clean-up step [106–108]. Both the purification mechanisms of SPE (retaining the bulk lipids and eluting the analytes, or vice-versa retention of the analytes while eluting lipids, then analytes elution with a small volume of solvent) have been exploited by using a different sorbent phase, namely C_{18}/Florisil, PS-DVB, silica, or a mixture of phases (C_{18} and Florisil). For instance, a silica cartridge retains the triglycerides, while PAH are eluted with a mixture of *n*-hexane and CH_2Cl_2 [106]; while using PS/DVB cartridges the opposite mechanism is exploited. In the latter approach, PAHs are retained in the sorbent phase, while triglycerides are washed away with a mixture of isooctane/cyclohexane, and then PAHs are eluted with CH_2Cl_2 [107]. In some complex samples, such as refined olive oil and olive pomace oil, the presence of a high amount of unsaturated hydrocarbons with cyclic moieties deriving from partial isomerisation of squalene and decomposition of steroidal alcohols during the refining process, requires the use of an additional purification step. Moreda and co-workers [109] proposed the use of an amino (NH_2) SPE cartridge after a previous silica one.

The same sorbent phases have been used for matrix-solid phase dispersion in both edible oils and solid-fatty foods [108,110,111]. Bogusz and co-workers [108] compared a laboratory-made SPE, obtained by packing two layers of sorbent (Florisil on the bottom and C_{18} on the top) into a plastic syringe, with a matrix-solid phase dispersion (MSPD) method. In the latter the oil sample was previously mixed with a C_{18} sorbent in a mortar and then added on the top of a Florisil-packed SPE cartridge. Despite the proven advantages obtained using MSPD for solid samples, SPE turned out to be simpler, more precise and with higher recoveries. A novel phase for MSPD was proposed for PAH analysis in 2011 by Zhao and co-workers [112]. More precisely, the proposed method consisted of a magnetic solid-phase extraction (MSPE) technique, based on the use of magnetic or magnetisable adsorbents, which are easily removed from the sample by the use of an external magnet. In such an application the carbon nano tubes (CNTs), hydrophobic nanomaterial with a large specific area and π–π electrostatic properties (largely employed as an adsorbent material, especially for pesticides analysis [113]), were employed in the form of magnetic multi-walled CNTs (mMWCNTs) by physically assembling magnetic nanoparticles (MNPs). The extraction procedure was very fast, consisting basically of mixing only the adsorbent with the matrix and subsequent washing before desorption of PAHs with 100 μL of toluene and GC–MS analysis. The main drawback was the time-consuming washing process of the adsorbent before use (about 3 days).

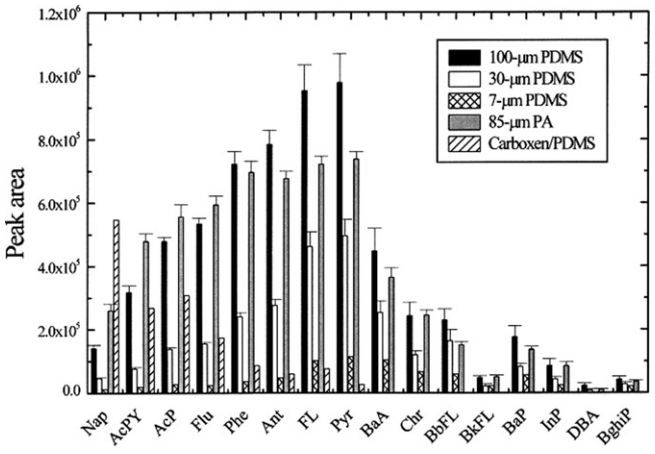


Fig. 3. PAH extraction efficiency obtained with five different SPME fiber from an aqueous solution. Extraction time 90 min at room temperature under magnetic stirring (reprinted with permission from Ref. 103).

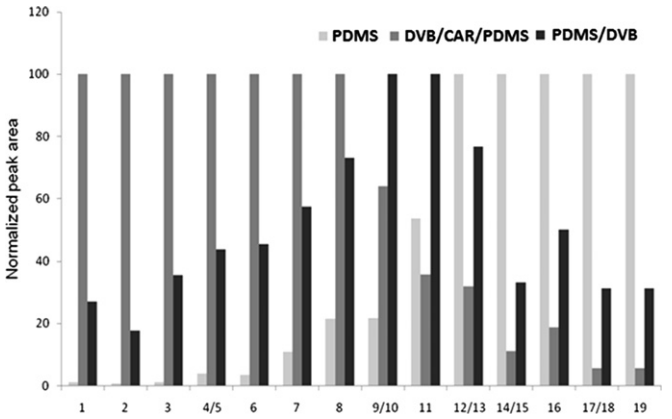


Fig. 4. Normalized peak area obtained with HS–DI–SPME, HS–SPME, DI–SPME. Compounds: (1) benzene, (2) toluene, (3) ethylbenzene, (4) *p*-xylene, (5) *m*-xylene, (6) *o*-xylene, (7) Ap, (8) F, (9) A, (10) Pa, (11) P, (12) BaA, (13) Ch, (14) BkF, (15) BaP, (16) BbF, (17) IP, (18) DBahA, (19) BghiP. (reprinted with permission from Ref. 104.).

Head-space (HS) SPME was used to analyze volatile aromatic hydrocarbons by using a PDMS/Car/DVB fiber exposed at 100 °C for 60 min, followed by GC–MS analysis [114]. LODs below 2 μg/kg were obtained for light PAHs with accuracy above 80%. Arrebola and co-workers [77] "forced" the volatility of PAHs by using higher extraction temperature (200 °C) and simply sampling 100 μL of the HS without any pre-concentration. Using a GC–MS/MS system for the final analysis, very low LODs were obtaining for 8 heavy PAHs (below 0.06 μg/kg) with recoveries higher than 96%.

Purcaro and co-workers used SPME by direct immersion (DI) of the fiber into a diluted oil sample for the analysis of the 16 EU PAHs [115] and later of the only BaP [116]. The fiber used was a Carbopack Z/PDMS, which primary extracts by π–π interaction between the carbon surface and the analytes, in particular, flat co-planar compounds. Such a mechanism is predominant in a non-polar solvent, where the effect of PDMS is greatly minimized. The fiber was dipped in a stirred 1.5 mL hexane solution containing 200 μL of oil sample. Before GC analysis, it was rinsed in pure hexane to remove any possible physically stacked triglycerides. The method was validated first for the analysis of the 16 EU PAHs by GC × GC-ToFMS obtaining both good accuracy values (above 80%, except for BcP, Ch, 5-MeCh and DBalP) and LODs (below 1.2 μg/kg). Later the same approach was used for BaP determination in a mono-dimensional GC–MS using a deuterated internal standard, obtaining a LOQ of 0.46 μg/kg and inter- and intra-day repeatability below 6%.

In 2007, SPME was applied for the first time for the analysis of the 16 EPA PAHs in milk and related products, followed by a GC–MS analysis [117]. The medium polar PDMS-DVB fiber was selected and HS and DI extraction procedure were compared. As expected, HS showed extraction only for light PAH (and only in milk), thus DI–SPME was chosen. Due to the high complexity of the matrices, the authors suggested the standard addition methods for quantification purposes. Later the same authors [118] presented an HS–SPME method for determination of PAHs with up to four aromatic rings in milk samples. Milk samples present a very strong matrix effect, in particular related to the fat content. Rodil and co-workers [105] attempted different approaches to reduce the strong lipid-binding of the analytes prior to the microwave-assisted solvent extraction (MASE). Neither the addition of methanol or detergent, nor the saponification with NaOH (which led to the formation of interferences that hindered a reliable determination of most of the compounds) succeeded in increasing the extraction yield. Extraction time was the only effective approach, but only when skimmed milk was analyzed (4 h extraction showed a recoveries ranging between 65 and 92%). However, good trueness results were obtained by using internal standard calibration (RSD% values below 25%).

All the techniques described above can be applied after a previous extraction, as a purification step to analyze solid samples. The general approach is to extract the lipid fraction, with which the PAHs are associated, thus further purification is needed. The traditional technique is Soxhlet extraction, followed by clean-up, performed by SPE, gel permeation chromatography (GPC), saponification or others. However, such a technique is often time- and solvent-consuming; while solid–liquid extraction (SLE) and ultrasound assisted extraction (USAE) are faster and less solvent-consuming techniques. The latter in particular, is widely diffused since it requires inexpensive equipment, it is easy to use, and it provides very good results if properly optimized [119]. More innovative extraction techniques have been exploited as well, like pressurized liquid extraction (PLE), microwave assisted extraction (MAE), supercritical fluid extraction (SFE), etc. The good performance of such techniques has been largely assessed by comparison with traditional extraction procedures, such as Soxhlet and USAE, in particular in environmental samples [120,121].

When approaching food samples, the main problem is the fat content of some products, which usually requires an additional purification step. Wang and co-workers [122] described the use of PLE for PAHs analysis in food samples (fish and pork tissues) for the first time. A further clean-up step was performed by eliminating the co-extracted lipids with concentrated sulphuric acid, and then purifying the extract in a Florisil cartridge. Martinez and co-workers [123] applied a further step using alkaline digestion and in some cases a further alumina SPE clean-up to remove co-extracted compounds from a mussel extract. Veyrand and co-workers applied a PS-DVB SPE cartridge for further purification of a mussel extract previous to GC–MS/MS analysis [124]. An interesting approach to perform a single extraction/clean-up step by using PLE for food samples was first proposed for PCB analysis by Björklund and co-workers [125,126]. The same approach devoted to PAHs analysis was first applied in sediment [127], and in 2009 in smoked fish [128]. Briefly, the PLE cells were packed with a cellulose filter at the bottom, follow by a fat retainer. The ground sample, mixed with polyacrylic acid (to avoid deactivation of the fat retainer by water) and Ottawa sand (to fill the void volume), was transferred to the top of the cell, and then extracted with a mixture of hexane/dichloromethane. Several fat retainers were compared, namely sulphuric-acid-impregnated silica, activated silica, Florisil®, and neutral, basic and acidic alumina. Silica gave the best compromise between fatty-free extract and recoveries among all the PAHs considered. For instance, sulphuric-acid-impregnated silica gave fatty-free extract but low recoveries for light PAHs [128]. A comparison between the extraction yields obtained using the main fat retainer is shown in Fig. 5.

The same approach to avoid further purification was used for SFE [129,130] analysis. C_{18} beads, used as the sorbent, were mixed with pureed crab tissue in the extraction thimble, in order to adsorb non-polar to slightly polar analytes during the CO_2 extraction. Fig. 6 shows the chromatograms obtained from the extraction using the conventional SFE technique with an inert sorbent and C_{18} beads.

Although no modifier was used, the proposed method gave recoveries over 99%, comparable to Soxhlet extraction. Methanol can be used as a modifier to increase the extraction of high molecular weight PAHs, but leading to the necessity of a further SPE purification [131]. SFE applications are mainly related to environmental samples, while only few applications are reported on food samples [120,131]. Instead, MAE has been largely applied in food samples. An interesting application, to reduce the extraction time and the losses of analytes, is related to simultaneous saponification and extraction in the same extraction vessel [24,132,133]. However, a further clean-up step on a SPE cartridge (Florisil or silica) was necessary to remove interferences when fish and meat samples were analyzed, while no further purification was needed for vegetable samples and others, such as propolis [134].

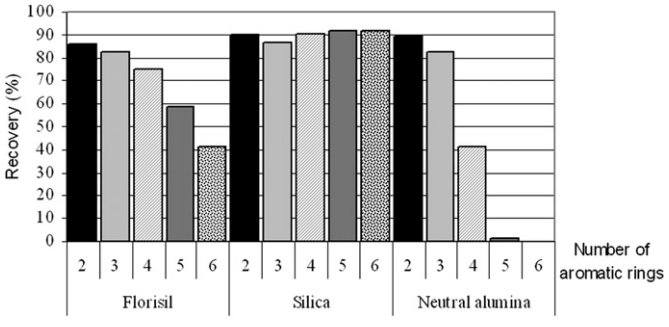


Fig. 5. PAH recoveries obtained from pork samples by PLE using a different fat retainer. PAH are grouped based on the number of aromatic rings. (reprinted with permission from Ref. 128).

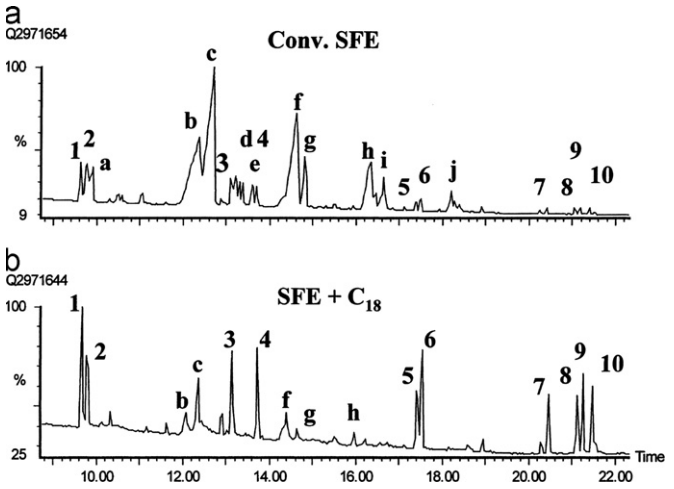


Fig. 6. GC–MS chromatograms obtained by SFE extraction of a spiked crab tissue. (A) conventional SFE using inert sorbent; (b) SFE plus C_{18} as sorbent. Peaks: (1) Pa. (2) A, (3) Fl, (4) P, (5) BaA, (6) Ch, (7) BbF, (8) BeP, (9) BaP, (10) Perylene, letters are natural lipids. (reprinted with permission from Ref. 129.).

Although applied to soil samples, an innovative application worthy of mentioning, was presented by Xu and Lee [135]. A graphite fiber, enveloped into a polypropylene membrane, was used as sorbent for a μ-SPE device placed in the MAE vessel during extraction. The device was then desorbed in a low volume (150 μL) of ACN and injected into a GC–MS, giving extremely low LOD (below 0.003 μg/kg).

Matrix solid-phase dispersion (MSPD) was proposed as an alternative to the above-described techniques for fish and seafood analysis. The reconstituted lyophilized sample was ground with C_{18} sorbent and then loaded in a syringe packed with a layer of Florisil and one of C_{18}, acting as co-column or clean-up phases; the analytes were eluted with acetonitrile. Recoveries above 90% were obtained from a spiked sample, while lower recoveries for BaA, Ch and A were obtained using a certified sample (mussel tissue) [136]. Such a phenomenon is common since spiked analytes are not structured in the matrix.

Over the last years great attention has been gained by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach, first developed in 2003 by Anastassiades and co-workers [137] for pesticides analysis.

In 2009 Ramalhosa and co-workers [138] proposed a modified QuEChERS approach for PAH extraction in fish samples. The PSA clean-up step was eliminated since no significant interferences were detected in the following LC–FLD analysis (most likely due to the selectivity of FLD) [138,139]. In fact, Smoker and co-workers [72] highlighted a slight ion suppression effect in the LC–(APPI–MS)/MS analysis without PSA clean-up.

Forsberg and co-workers [140] modified the QuEChERS method for high fat-containing samples by replacing ACN with a mixture of acetone, ethyl acetate and isooctane. Recoveries comparable to PLE and Soxhlet extraction were obtained. The method was applied to the analysis of 33 parent and substituted PAHs in salmon by GC–MS in SIM mode.

Due to the high matrix effect when analysing solid samples, only few applications dealt with SPME extraction and mainly with the extraction of light PAHs [141,142]. Nevertheless, the absence of any manipulation was interesting. Martin and Ruiz [143] presented an interesting tool, called direct extraction device (DED), which enabled to perform a HS-SPME into the solid matrix. The fiber was exposed into a small chamber, which presented several holes to allow the diffusion of analytes from the samples in the chamber HS. The performance was studied in a gelatine model, and then assessed in real smoked samples. Only light PAHs were extracted (up to P), but good repeatability and LOD values were obtained. Testing some other fiber phases (rather than PDMS) would be interesting to evaluate the possibility to extract higher molecular weight PAHs.

A very interesting application using the ionic liquid (IL) as an extraction surfactant, was presented by Germán-Hernández and co-workers [144,145]. ILs are a large group of non-molecular solvents characterized by low toxicity, negligible vapour pressure, high thermal stability, and many of them posses a low critical micelle concentration. The extraction of the 16 EU PAHs from toasted cereals was successfully carried out using aggregates of the IL 1-hexadecyl-3-butylimidazolium bromide (HDBIm-Br) dissolved in water. A MAE extraction was performed, and the surnatant was directly injected (possibly after pre-concentration) in HPLC–FLD without further clean-up. The pre-concentration was achieved by transforming the water soluble IL into a water insoluble IL through a reaction with $LiNTf_2$ (lithium bis[(trifluoromethane)sulfonyl]imide) salt [145]. Very good sensitivity (LODs ranging between 0.03 and 3.9 μg/kg, except for CPP and BjF) was achieved by simply diluting the microdroplet obtained in acetonitrile before HPLC injection. A low signal derived from the IL was detected in the first minutes of the LC-chromatogram, however, in a zone where no analytes of interest were eluted.

4.3. Hyphenated techniques

Hyphenated techniques have gained increasing interest over the years, in particular in trace analysis, since sample manipulation is highly reduced, and thus the risk of cross-contamination. Furthermore, off-line methods generally do not permit quantitative transfer of the fraction of interest. The present section will give a brief overview of some approaches proposed, coupling a preparation step with the analytical method (HPLC or GC). Multidimensional analytical techniques, such as GC × GC, can also be considered from a similar point of view. In fact, the increased separation power, obtained by coupling two columns with different selectivity, can enable the isolation of matrix interferences, thus in several cases a less intensive clean-up step can be performed [115].

Many LC–GC or LC–LC–GC techniques have been developed, but mainly to analyze alkylated PAHs derived from mineral oil contamination. Few works dealt with parent PAH determination in food. Vreuls and co-workers [146] developed an on-line HPLC–GC system for PAH analysis in edible oil. Using a silica column for sample pre-treatment, the triglycerides were retained by the stationary phase and eliminated by backflushing the column with MTBE. While the PAH fraction was eluted and transferred to the GC column via a loop-type interface. The loading limit of the HPLC column was 2 mg and the LODs of PAHs were about 20 μg/kg in the scan mode and 0.5 μg/kg in the SIM mode.

In 2002, Moret and co-workers [147] developed a method to analyze PAHs in oil and lipid extract using a normal phase (NP)–HPLC (silica column) coupled on-line with a reversed phase (RP)–HPLC (C_{18} column). The interface consisted of an on-line evaporator, which enabled the trapping of analytes while the solvent was easily evaporated through a solvent exit connected to a vacuum source. The analytes were removed from the packed chamber and injected onto the second column by using ACN, while the first column was rinsed in backflush to eliminate the triglycerides. An FLD detector was used and the recoveries obtained were above 80%

Donor-acceptor complex chromatography (DACC), previously used for off-line purification [148], was directly coupled to the following LC system [149,150]. The working principle of DACC is based π–π interactions between the sorbent and PAHs when a non-π-electron containing solvent is used as a mobile phase. Thus, the matrix components were eluted with isopropanol and

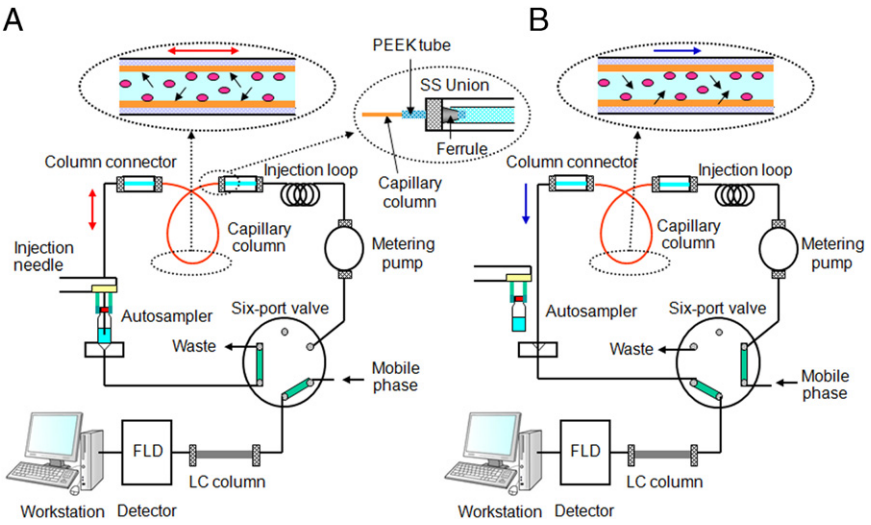


Fig. 7. Scheme of the in-tube SPME–HPLC–FLD system. (a) Load position (extraction) and (b) inject position (desorption) (reprinted with permission from Ref. [151]).

discarded, then PAHs were eluted in backflush onto the analytical column, exploiting a system of valves [149,150].

An interesting approach for analysis of dried tea leaves extracts was presented by Ishizaki and co-workers in 2010 [151]. A GC capillary column (CP-Sil 19CB, 60 cm × 0.32 mm id × 12 μm) was used as an in-tube SPME device and located on-line between the injection loop and the injection needle of an HPLC–FLD system. A scheme of the system is reported in Fig. 7. PAHs were trapped into the capillary column by repeated suction and ejection of the sample from the autosampler vial. Analytes were then desorbed by the mobile phase (water/ACN 45/55 v/v). Very low LODs were achieved for all the 16 EPA PAHs (below 5 pg/mL), and recovery yields were above 70%.

5. Conclusion

The analysis of PAHs in food, like other trace analysis in complex matrices, has been an interesting challenge for food chemistry researchers for a long time. The analytical methods proposed over the years have always followed the recent evolution in analytical instruments, sometimes being the pacesetter for new approaches. The general trend is a reduction of the solvent and time, trying to reduce the whole sample preparation into a single step, like for instance, in several PLE, MSPD and SPME applications. Such innovative techniques, along with hyphenated techniques, which also reduce sample handling, will be the most exploited approaches, suitable for routine analysis, as well.

Conflict of interest

The authors declare no conflict of interest

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