ELSEVIER

Journal of Chromatography B, 770 (2002) 3–18

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives

Peter Šimko*

Food Research Institute, Priemyselná 4, P.O. Box 25, 824 75 Bratislava, Slovak Republic

Abstract

Formation, factors affecting concentrations, legal limits and occurrence of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavour additives are briefly reviewed. The most used techniques such as thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC) are evaluated. Also, sample preparation, pre-separation procedures, separation and detection systems being used for determination are discussed with emphasis to latest development in applied food analysis and the chosen data regarding the concentration of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavour additives are summarised.

Keywords: Reviews; Food analysis; Polycyclic aromatic hydrocarbons

Contents

*Fax: +421-2-5557-1417.
E-mail address: peter.simko@vup.sk (P. Šimko).

1570-0232/02/$ – see front matter
PII: S0378-4347(01)00438-8

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of chemical compounds known to be cancer causing agents. Some, while not carcinogenic, may act as synergists. PAHs are being found in water, air, soil, and, therefore also in food. They originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal — derived products, with combustion sources predominating [1]. So, food contamination is realistic in every stage of food 'live' with regard to permanent formation and presence of these compounds in the environment. But, PAHs may also form directly in food as a result of some heat processes (charcoal grilling [2–4], roasting [5], smoke drying [6], smoking — discussed in Section 1.4). Important ways of contaminating food are contaminated additives [7] and migration from contaminated package [8,9]. Formation, determination and occurrence of PAHs in foodstuffs have been reviewed [1,10–12,25] in general, but particular attention to smoked meat products as well as smoke flavouring additives (SFAs) has not been paid. This review brings not only history but also the latest information about analytical aspects of PAHs; determination of the products and a mention on factors which affect PAHs concentrations themselves in samples during storage and analytical determination.

1.1. History

The fact that chemicals could cause cancer arose from the observations of Percival Pott of St Bartholomew's Hospital in London in 1775 when he had noted high incidence of cancer of the scrotum among chimney sweeps who often had to climb up inside chimneys to sweep the soot down. Although he deduced correctly that the soot was responsible for the cancer, at this time it was not possible to determine the compounds responsible for such serious tissue damage. In 1920, Japanese workers discovered that painting extracts of soot onto the skin of mice caused tumours of the skin. In 1929, the first pure chemical carcinogen DahA was isolated from soot extract at the Chester Beatty Research Institute by Kennaway. In 1953, Doll, on the basis of wide epidemiological and statistical analysis proved, that cigarette smoking was a prime cause of lung cancer. Careful analysis of the smoke and tar obtained from cigarettes showed, that it contained many carcinogenic PAHs, from which BaP was assessed as the most dangerous compound.

1.2. Characterisation of PAHs

PAHs are compounds consisting of two or more condensed aromatic rings, lineared together, either *cata*-annellated (linearly-, or angularly), or *peri*-condensed. *Cata*-condensed PAHs are alternant systems containing only six-membered rings and closed shell systems having all bonding orbitals occupied by two electrons. The entire group of *cata*-condensed PAHs can be further divided into branched and non-branched systems. Branched systems are thermodynamically more stable and chemically less reactive than non-branched of the same size. Conversely, *peri*-condensed PAHs are either closed shell systems or neutral free orbitals, in which at least one electron is in a non-bonding orbital. Free radicals of this type are stable only if the systems have an odd number of carbon atoms. In addition, *peri*-condensed PAHs can be further divided into alternant and non-alternant, depending on the presence of five- or six-membered rings in the molecule [10]. These variabilities, including the existence of alkylated derivatives, make for a large number of various isomers. Grimmer and Böhnke [27] have reported about 100 various PAHs compounds present in smoked fish.

1.3. Effect of PAHs to organisms

In recent years significant progress has been made in the understanding of the biological action of PAHs. These compounds enter the organism by inhalation, ingestion or penetration with a following distribution to the various organs, where they interact with aryl hydrocarbon hydroxylases (where the dominant role plays cytochrome P_{450}), which are most abundant in the liver, followed by hydrolysis to dihydrodiols. The products are the true active species, the 'ultimative carcinogens' the so-called 'bay region' dihydrodiol epoxides. These compounds form covalent adducts with proteins and nucleic acids. The DNA adducts are thought to initiate cell mutation and eventual malignancy [1,12]. Lately, the

direct mutagenic potential of 14 PAHs and PAHs containing fractions isolated from smoked and charcoal broiled samples was studied towards strains TA 98 and TA 100 using the Ames test. As found, the most potent mutagenicity was observed with PAHs fractions isolated from smoked fish [51].

1.4. Formation of PAHs during smoking

Food smoking belongs to one of the oldest food technologies which mankind has used for at least 10 000 years. Probably as a protection against canines man hung catches over the fire. From this time, smoking started to be widely used not only for special organoleptic profiles of smoked products, but also for the inactivating effect of smoke (and heat) on enzymes and microorganisms. Today, smoking technology uses mainly the special effects of various sensory active components (phenol derivates, carbonyls, organic acids and their esters, lactones, pyrazines, pyrols and furan derivates [13]) contained in smoke for aromatisation of meat products to make food with a specific organoleptic profile, widely demanded on the market. Smoke is generated by thermal pyrolysis of a certain kind of wood when there is limited access of oxygen. Temperature of smoke generally plays a very important role, because the amount of PAHs in smoke, formed during pyrolysis increases linearly with the smoking temperature within the interval 400–1000°C [14]. Direct exposition of meat products to smoke brings about higher concentrations of PAHs as compared to indirect methods, when PAHs are partially eliminated by condensation in tars [15]. Also, hot smoking used for treating, a main part of meat production, brings about higher concentrations of PAHs than cold smoking, used for fermented, thermally non-processed meat products [16,17]. Heavy or 'wild' smoking increases PAHs concentration to high levels [6,15,32,71,77]. In some types of products it is possible to decrease the concentration of PAHs by cooking [18]. The highest concentration of PAHs in smoked products is immediately after finishing the smoking, then it decreases due to light decomposition and interaction with present compounds [19,20,72]. However, PAHs also penetrate into smoked products, where they are protected from light and oxygen, and after some time, the concentration stabilizes at a certain constant level [21]. A relatively new alternative for traditional smoking is the use of SFA. It was in the late 1800s when the Kansas pharmacist Wright developed and patented the first liquid SFA prepared from smoke condensate. Nowadays, SFAs are being produced and applied widely in innumerable variations of taste and odour in solid and liquid state, applied in many ways, but the fundamental base for both modifications is identical — the use of decomposition products of wood pyrolysis.

1.5. Legal limits

Taking account of the situation regarding the presence of PAHs in smoked food, and problems to assess and interpret correctly the variable concentrations of individual carcinogens with different biological effect, BaP has been chosen as the general indicator of total PAHs presence in smoked foods in Germany, and a maximum acceptable concentration of 1 $\mu g\ kg^{-1}$ BaP in smoked foods has been set in force since 1973 even in spite of the fact that BaP constitutes only between 1 and 20% of the total carcinogenic PAHs [76]. Later, other countries, e.g. Austria, Czech Republic, Switzerland, Italy and the Slovak Republic, have also adopted the same limit. Regarding SFA [22] JECFA adopted a specification, which requires that the concentration of BaP should not exceed a limit of 10 $\mu g\ kg^{-1}$. For foods aromatised with SFA the EU set a maximum permissible limit for BaP in the EEC Directive 88/388 to a level of 0.03 $\mu g\ kg^{-1}$ [23].

2. Analysis of PAHs

Usually, PAHs are presented in food at $\mu g\ kg^{-1}$ levels. For this, algorithm of the analysis is as follows: extraction/hydrolysis, liquid/liquid partition, clean-up procedures, concentration, chromatographic separation and, of course, determination. Although all steps are very important, chromatographic separation is the most important for the correct evaluation of real risk assessment, because while BaP is a very strong carcinogenic agent, carcinogenic activity of its isomer BeP is quite low. Methodology of PAHs analysis was strongly affected

by levels of development of chromatographic methods. At the beginning, a separation of BaP isomers by PC and CC was practically impossible [24]. With regard to complex mixtures of PAHs, the presence of a variety of interfering substances and the need to assess correctly real concentrations of the most dangerous compounds at minimum, it was necessary to overcome problems regarding the resolution of so-called 'benzpyrene fraction' which consisted at this time of BaP and its isomers BeP, BkF, BbF and Per. In 1968, at a joint meeting of IUCC and IARC, the joint working group specified that any acceptable analytical method should be capable of separating at least BaA, BaP, BeP, BghiP, Py, BkF, and Cor [25]. Collaborative studies of a method specific for BaP and a general procedure for PAHs were conducted under the auspices of the AOAC and IUPAC. The procedure consisted of an initial saponification of the sample in ethanolic potassium hydroxide solution, followed by a partition step between DMSO and an aliphatic solvent and column chromatography on pre-treated Florisil. For determination of individual PAHs, a cellulose reversed-phase technique in conjunction with cellulose acetate multiphase technique was used. The method was adopted as an AOAC official first action method in 1973 and accepted as a recommended method by IUPAC. Statistical evaluation of the data obtained by interlaboratory tests, in which ham samples were fortified with BaP, BeP, BaA, and BghiP at a level of 10 $\mu g\ kg^{-1}$ and analysed by the above mentioned method showed a standard deviation between 7.4 and 12.7%. On this basis, the method has been adopted as the official method of AOAC [26].

2.1. Sample preparation

Smoked meats and SFA represent two different matrices, which have in common only the organoleptic profile and the compounds to be determined. For this, different procedures for sample pre-treatment are taken in order to reach the highest recoveries of analytes, as possible.

2.1.1. Sample treatment of processed meats

From the analytical point of view, meat and its products belong to problematic matrices with regard to the presence of various interfering compounds. Moreover, PAHs such as lipophilic compounds have a tendency to diffuse not only into the non-polar part of the sample but also into the inside of tissue cells due to the existing concentration gradient. For this reason a simple solvent extraction with non-polar solvent seems to be insufficient to reach high recovery. Grimmer and Böhnke [27] isolated PAHs from smoked fish and smoked-dried cobra with boiling methanol prior to sample hydrolysis with methanolic KOH. It was found that only about 30% BaP and other PAHs was extractable from the samples, whereas an additional alkaline hydrolysis of meat protein yielded another 60% of PAHs. It was concluded that PAHs were linked adsorptively to high molecular structures not destroyed with boiling methanol. Although more than 80% of the methanol used could be decanted, this contained only one-third of the PAHs contained in the sample. As postulated, alkaline hydrolysis with aqueous methanolic KOH is an absolute necessarity to isolate PAHs quantitatively from such types samples. Alkaline hydrolysis takes usually 2 to 4 h, depending on the character of the sample. Lean tissues take less time than adipose and collagen containing tissues. Under reflux alkaline hydrolysis sample treatment was adopted following the many experimental works [28–32]. On the other hand, by Vassilaros et al. [33], the use of an alcohol is superfluous and contributes to interference problems in the final analysis because of methyl esters formation from fatty acids and methanol which are then difficult to remove from the PAHs fraction. Takatsuki et al. [34] found that during alkaline hydrolysis BaP may be partially decomposed by the coexistence of alkaline conditions, light oxygen, and peroxides in aged ethyl ether. They proposed to use amber glass, the addition of Na_2S as antioxidant, distillation ethyl ether just before use and prevention of air from contact with adsorbents. To protect PAHs from light decomposition, Karl and Leinemann [35] used brown glassware carefully rinsed with acetone before using an alkalic hydrolysis. Even though, some authors also recommended direct extraction with organic solvents. Potthast and Eigner [36] proposed a procedure based on the mixing of preground samples with chloroform and anhydrous Na_2SO_4 to remove water from the extract. After adding Celite, the fat portion became uniformly distributed over the surface of the adsorbent. Al-

though some authors achieved a recovery 95–100% of BaP added at a level of 10 μg, there is a real assumption that they recovered only 'free' PAHs accessible with solvent. This procedure was used also in Alonge work [37]. Extraction of fat from smoked fish was also used by Afolabi et al. [6]. Cejpek et al. [38] tested the efficiency of some organic solvents to obtain fat portion of meat samples. The best efficient solvent was a mixture of chloroform–methanol (2:1), less effective was chloroform and the worst yields were achieved with methanol. This confirms observations of Grimmer and Böhnke [27] regarding insufficient capability of methanol to extract quantitatively PAHs from meat samples. Otherwise, the use of chloroform–methanol mixture, called also Folch agent, is widely used in food analysis for extraction of lipids and lipoproteins, when methanol makes possible extraction of lipids from inside of cells by denaturation of cell wall proteins [67]. Joe et al. [39] digested samples of smoked food with KOH, and PAHs extracted with Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane). Chen et al. [40] compared the efficiency of extraction from freeze-dried sample, when sonication and Soxhlet procedures were employed. Recovery studies showed that Soxhlet extraction was more suitable prior to the sonication method. Accelerated procedure of extraction was tested by Wang et al. [41]. Samples were extracted in a Dionex extractor as well as Soxhlet apparatus. ASE was found to be comparable with, and even better than, the reference Soxhlet method, when significant reductions in time of extraction (20 min) and solvent consumption (20–30 ml) were achieved. Garcia-Falcón et al. [23] accelerated the extraction of PAHs from freeze-dried samples into hexane with microwave treatment and hexane extract then saponified with ethanolic KOH.

2.1.2. Sample treatment of LSF

Sample treatment of the SFA matrix is different from the treatment of processed meats due to the easy solution of FSF in liquid state in organic solvent. For this, there is no reason to treat samples by time-consuming hydrolysis under reflux [27]. Different situations could arise, when SFA were analysed in solid state (e.g. applied on starch, gelatine, or encapsulated). In spite of this, some authors preferred alkaline hydrolysis of liquid SFA under reflux. However, adding KOH is strongly recommended to transform phenols to polar, non-extractable form of phenolates prior to the PAHs extraction with non polar solvent. White et al. [43] alkalized water soluble liquid SFA and resinous condensates which settled out of SFA after storage with KOH solution and extracted PAHs into iso-octane. Silvester [44] extracted PAHs from alkalised liquid SFA with hexane. Radecki [45] alkalised liquid SFA with ethanolic KOH solution and maintained it at 60°C for 30 min prior to extraction into cyclohexane. After alkalisation, a direct extraction of PAHs with cyclohexane was used by Šimko et al. [46]. Gomaa et al. [47] saponified liquid SFA with methanolic KOH for 3 h and then PAHs extracted into cyclohexane. Laffon Lage et al. [48] used the SPE technique on Sep Pak C_{18} for PAHs isolation and compared this to the SFE procedure, when the sample for SFE was mixed with alumina and the extracted PAHs were concentrated in an oktadecylsilane trap. In both cases, 91% recoveries of BaP spiked at 15 ng were found and statistically no serious differences were observed. Taking account of the expensive SFE extractor, they recommended for use the simple SPE procedure. Guillén et al. [49,50] alkalized liquid SFA with methanolic KOH and heated under reflux for 3 h, followed by the extraction of PAHs into DCM, or cyclohexane, respectively.

2.2. Pre-separation procedures

At this point in both procedures it could be said that the following steps are more or less the same for processed meats and SFA. However, sometimes mainly after adipose tissue hydrolysis, a presence of lipoproteins in non-polar solvent needs their removal prior to pre-separation with a one-step liquid–liquid partition between non polar and polar solvent (e.g. DMF–water hexane [27], methanol–water, or DMSO–water-cyclohexane [31,35]), or two-step liquid–liquid partition (e.g. NaCl–water and DMF–water [52]), or precipitation with Na_2WO_4 [17–19]. For preseparation, deactivated Florisil [17–19, 31,41,47,49] silica gel [30,34,3], alumina [44] and Celite [36,37] are used frequently. The only study [44] reported that elution of BaP from Florisil and silica gel with hexane was impossible and for this

reason an alumina was recommended for preseparation of concentrated PAHs extracts. Guillén et al. [50] preferred elution of silica with cyclohexane prior to Florisil dichlormetane elution in order to obtain higher recoveries with reduced amounts of interfering substances, which were eluted from Florisil with dichlormethane, as shown in Fig. 1. Another effective preseparation procedure is also GPC on Sephadex LH 20 [28] or BioBeads S-X3 [38], respectively. Motier et al. [3] cleaned concentrated cyclohexane extracts by SPE, using conditioned Isolute aminopropyl and C_{18} columns. Also, the use of two different techniques was used, when cyclohexane extract first cleaned with GPC on Sephadex LH 20, followed by silica gel [52]. Even, the last procedure is possible to carry out in reverse mode [6]. In all cases, removal of organic solvents by vacuum evaporation is a repeating operation. This may be a critical step, mainly if there is a presumption of presence of light PAHs (Fl, Ant, Phe), extracts should not be evaporated to dryness because these PAHs are volatile. This cautious manipulation is not necessary, if only PAHs with boiling points above 370°C are determined [27].

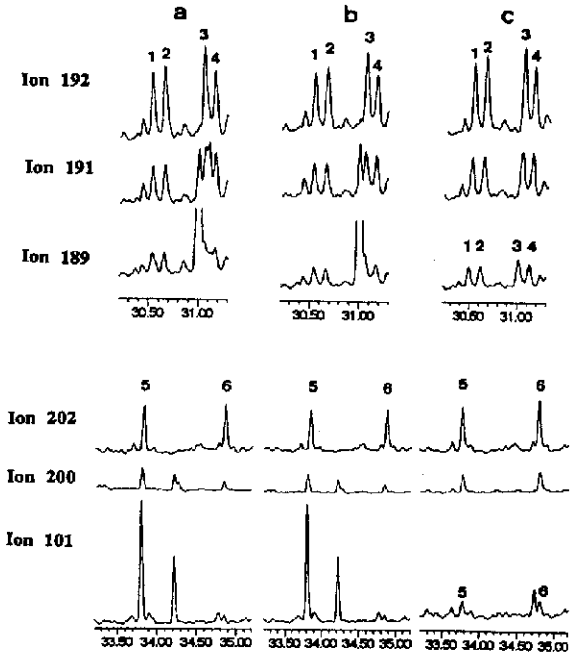

Fig. 1. Extracted chromatograms of ions 192, 191, 189, 202, 200, and 101 in (a) the eluate from the first Florisil tube used for the cleanup, (b) the second fraction from the second Florisil tube and (c) the second fraction from the silica tube. The peaks designated 1, 2, 3, 4, 5, and 6 correspond to 3-, 2-, 9-, and 1-methylphenanthrenes, Fu, and Py. Reprinted with permission from Ref. [49]. Copyright (2000) American Chemical Society.

2.3. TLC

TLC belongs to the older analytical methods used for determination of PAHs in various matrices. Haenni [54] discussed the development of analytical tools for control of PAHs in food additives and in food by the use of ultraviolet specification within specific wavelength ranges. To this, Schaad [24] reviewed various chromatographic separation procedures, including TLC. At this time analytical problems of PAHs separation are discussed in Section 2. Analysis of PAHs. White et al. [43] used two systems for PAHs separation. The first consisted of 20% *N*,*N*-dimethylformamide in ethyl ether as a stationary phase and isooctane as a mobile phase. Fluorescent spots were scraped from the cellulose layer and eluted with hot methanol. After concentration, the sample was developed in the second system, using ethanol–toluene–water (17:4:4) as developer. Fluorescent spots were eluted again from the cellulose acetate layer and the ultraviolet spectrum was recorded against isooctane in a reference cell. The observed maxima were compared with those in the spectra of known PAHs obtained under the same instrumental conditions. Estimation of quantity of the identified compounds was made by the baseline technique in conjunction with spectra of these PAHs and identification was confirmed by spectrophotofluorometry. This method has become the basis of AOAC Official Method 973.30, adopted in 1974 [26].

2.4. GC

Nowadays, GC is widely used for determination of PAHs in food analysis. The determination of the large number of PAHs in samples requires columns with high efficiency. To separate some critical pairs as well as isomers of methyl derivatives of certain PAHs, capillary columns (50 m×0.3–0.5 mm) which can achieve 50 000–70 000 HETP are especially convenient. Packed columns used for determination

of PAHs [27] had lower HETP ranging between 20 000 and 30 000. Two stationary phases, OV-17 and OV-101 were used for separation of BaP from BeP, DajA from DahA, and Phe from Ant. Successful separation of Chr from BaA was achieved using the OV-17 stationary phase. The separation of BbF, BjF, and BkF isomers on packed columns was not possible [27]. Radecki et al. [59] tested various stationary phases (GE SE 30; OV-1; SE-52; OV-7; OV-101; BMBT; BBBT) on Chromosorb W, Chromosorb W HP, Gas Chrom and Diatomite CQ supports in packed columns to develop a precise GC method for assaying BaP in SFA. Following from experiments, separation of BaP from BeP and Per was not possible to achieve using SE 30, OV-1, SE-52, OV-7, and OV-101 stationary phases. Nematic phases gave a good separation of BaP from its isomers, but they were not suitable for analysis with regard to their poor thermal stability. Detection of PAHs is not a serious problem, because an FID response is the same for all compounds and is linear over a large concentration range (about $1–10^6$), according to the carbon content. Typical GC–FID chromatogram of PAHs fraction from smoked fish is shown in Fig. 2. However, the use of FID is sometimes hampered by the need for very thorough clean-up procedures with the accompanying risk of severe losses and possible misidentification [55]. MSD has also successfully been used for PAHs analysis in many cases [56]. Especially, the use of MSD in the SIM mode makes it possible to simplify the time-consuming clean-up procedure [55], and it is recommended especially for quantitative analysis. GC–MS–SIM of PAHs isolated from smoked salmon is shown in Fig. 3. In Fig. 4 the GC–MS–SIM chromatogram of BaP isolated from LSF is displayed. ITD has many of the advantages prior to traditional MSD. The ITD utilizes electric fields to hold the ions within the ion storage regions. The ITD is then scanned through the mass range, causing the ions to be ejected from this region sequentially, from low to high mass. The ejected ions are detected by a conventional electron multiplier. Thus the characteristic of the ITD is that ionisation and mass analysis take place in the same space. This contrasts with a conventional MSD, which requires a separate ionisation source, focusing lenses and analyser, and associated low mechanical tolerances [57,58]. Some-

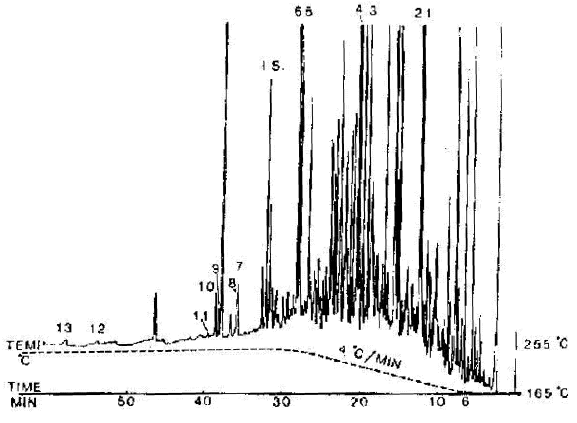

Fig. 2. GC–FID chromatogram of PAHs from hot smoked fish. 1-Phe, 2-Ant, 3-Fu, 4-Py, 5-BaA, 6-Chr and Tph, 7-BbF, 8-BjF and BkF, 9-BeP, 10-BaP, 11-Per, 12-IPy, 13- BghiP, I.S., internal standard (ββ-Binaphthyl). Reprinted with permission from Ref. [30]. Copyright (1982) Springer-Verlag GmbH and Co. KG.

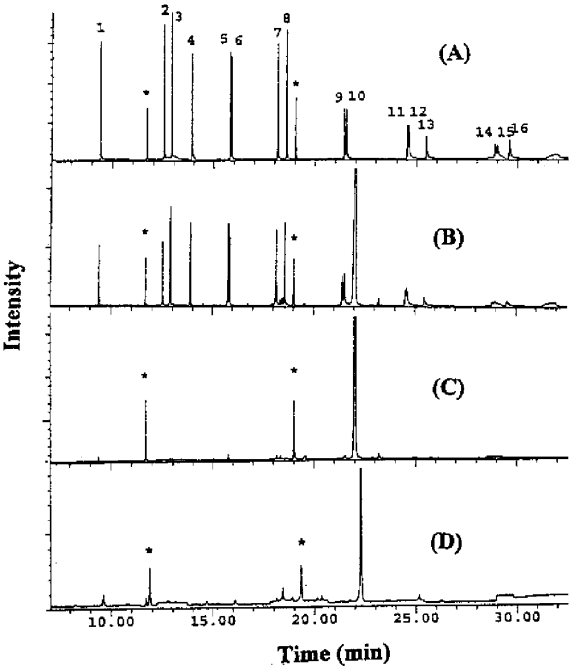

Fig. 3. SIM chromatograms: (A) standard solution of PAHs 1-Na, 2-Acy, 3-Ace, 4-Fl, 5-Phe, 6-Ant, 7-Fu, 8-Py, 9-BaA, 10-Chr, 11-BkF, 12-BjF, 13-BaP, 14-Ipy, 15-DahA, 16-BghiP. (B) Extracts from fish tissue spiked with PAHs. (C) Extracts from fish tissue without spiking. (D) Extract from smoked salmon tissue. * Internal standards. Reprinted with permission from Ref. [41]. Copyright (1999) American Chemical Society.

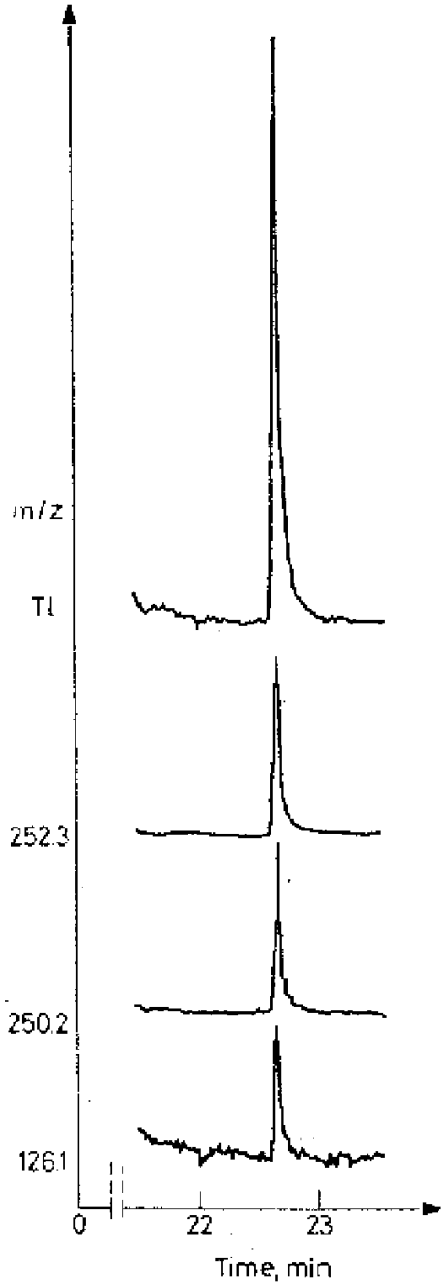

Fig. 4. GC–MS chromatogram of LSF obtained by SIM technique. Scanned masses of ions: 252.3, 250.2, and 126.1. TI, the sum of all three masses of scanned ions. Reprinted from Ref. [46]. Copyright (1992) Akadémiai Kiadó.

times, separation of isomers is quite a serious problem even when capillary columns are used. Dennis et al. [61] did not separate BjF from BkF. Speer et al. [60] were not able to separate Chr from Tph, BbF, BjF, and BkF to each other, and DahA from DacA. Problems associated with separation of Chr from Tph are also reported in works of Guillén et al. [49,50]. Wise et al. [68] reported on difficulties in separating isomers BbF and BkF. A review of pre-separation procedures as well as GC conditions to be used for determination of PAHs in smoked meat products and SFA are in Table 1.

2.5. HPLC

In recent years the HPLC method has been used extensively for determination of PAHs in food, as reported in review works [1,4,10–12]. Formerly used in stationary phases, such as alumina and silica gel they were later replaced with chemically bonded phases, particularly reverse phases such as ODS, widely used at the present time. For determination of PAHs in food, Hunt et al. [63] developed a PPS stationary phase and compared it with the ODS. As found, PPS column was able to separate BkF from Per, which was impossible using ODS column. HPLC has some advantages in PAHs analysis as follows [11]:

- separation of isomers shows very good resolution
- sufficient sensitivity and specificity of UVD and FLD
- molecular sizes of PAHs can be estimated on the basis of the retention time using RP column
- possibility to determine compounds with high molecular mass
- analysis are usually carried out at ambient temperature, there is no risk of thermal decomposition of analytes

In Fig. 5 there is shown a chromatogram of HPLC–FLD of smoked salami and in Fig. 6 is presented a chromatogram of HPLC–FLD of LSF, both obtained at isocratic elution. A HPLC–FLD chromatogram of a smoked fish sample obtained at programmed elution is shown in Fig. 7. HPLC equipped with MSD is an effective tool for charac-

Table 1
Pre-separation procedures as well as GC conditions to be used for determination of PAHs in smoked meat products and SFA

Sample	Sample treatment and preseparation	Column/stationary phase	Temperature program	Detection	Ref.
Barbecued sausages	Saponification with mixture of ethanol, water and KOH, extraction with cyclohexane, preseparation by SPE on Isolute aminopropyl and C_{18} columns	25 m×0.2 mm capillary column /SPB-5	80°C for 0.5 min →230°C at 8°C/min →300°C at 5°C/min	MSD	[3]
Smoked fish	Extraction with pentane, pre-cleaning on silica gel and Sephadex LH-20	25 m×0.2 mm quartz capillary column/SE-54	100→260°C, 3°C/min	MSD	[6]
Smoked fish	Saponification in methanolic KOH, liquid–liquid extraction (methanol–water–cyclohexane and DMF–water–cyclohexane) and GPC on Sephadex LH 20	10 m×2 mm packed columns/5% OV-101 and OV-17 on sorbent Gas Chrom	1. 120→250°C, 1°C/min 2. 250°C, isothermal	FID MSD	[27]
Smoked sausages	Saponification in methanolic KOH, liquid–liquid extraction (methanol–water–cyclohexane and DMF–water–cyclohexane) precleaning on silica gel and GPC on Sephadex LH 20	10 m×2 mm packed column/5% OV-101 on sorbent Gas Chrom	260°C isotermal	FID	[28]
Smoked meat products	Saponification with mixture of methanol, water and KOH, partition with DMF, precleaning on Kiesel gel 60	25 m×0.28 mm capillary column/ SE-54	240°C isothermal	MSD	[29]
Smoked fish and fish products	Saponification in methanolic KOH, liquid–liquid extraction (methanol–water–cyclohexane and DMF–water–cyclohexane) precleaning by CC on silica gel and GPC on Sephadex LH 20	55 m×0.3 mm glass capillary column/SE-54	165°C for 6 min, 165→255°C, at 4°C/min	FID	[30]
Smoked fish, smoked meat spreads	Saponification with mixture of methanol, water and KOH, extraction with cyclohexane, cleaning-up on Florisil, partitioning with DMSO/hexane	30 m×0.25 mm capillary column/DB-5	25°C, →180°C rapidly, →320°C, at 8°C/min	FID, MSD	[31]
Smoked fish	Saponification with methanol–water–KOH mixture under reflux, extraction into cyclohexane, extraction of PAHs with caffeine/formic acid, washing with NaCl solution, extraction into cyclohexane, preseparation on silica gel	30 m×0.25 mm capillary fused silica column/DB-5	110°C isotermal for 1.5 min → 210°C at 30°C/min →290°C at 3°C/min →300°C at 10°C/min	MSD	[35]
Smoked salmon, sausages, pork	Direct solvent extraction (ASE), clean-up on Florisil	30 m×0.25 mm capillary column/ cross-linked 5% phenyl methyl siloxane HP-5MS	40°C isothermal for 1 min → 250°C at 12°C/min →310°C at 5°C/min	MSD	[41]
SFA	Alkalisation with KOH solution, extraction with cyclohexane, clean-up on silica	25 m×0.2 mm fused-silica capillary column/HP-5 cross linked with 5% phenylmethylsilicone	50°C isothermal for 0.5 min → 180°C at 30°C/min →300°C at 7°C/min	MSD	[46]
SFA	Heating with methanolic KOH under reflux, extraction with cyclohexane, cleaning-up by SPE technique on Florisil	60 m×0.25 mm fused-silica capillary column/HP-5MS, 5% phenyl methyl siloxane	50°C isothermal for 0.5 min → 130°C at 8°C/min→290°C at 5°C/min	MSD	[49]
SFA	Heating with methanolic KOH under reflux, extraction with cyclohexane, cleaning-up by SPE technique on LC silica	60 m×0.25 mm fused-silica capillary column/HP-5MS, 5% phenyl methyl siloxane	50°C isothermal for 0.5 min → 130°C at 8°C/min →290°C at 5°C/min	MSD	[50]
Smoked meats	Saponification with methanolic KOH, extraction with cyclohexane, partition with DMF/water, clean up on silica gel and with GPC on Bio Beads S-X3	50 m capillary column/ DB-5	70°C, →280°C at 5°C/min	MSD	[60]
Smoked meats	Saponification with methanolic KOH, extraction with *n*-hexane, clean-up by SPE on Florisil	30 m×0.32 mm/DB-5	70°C isothermal for 1 min → 150°C at 10°C/min →280°C at 4°C/min	ITD	[62]
Smoked chicken	Extraction with methanol in Soxhlet app.,+KOH, extraction into *n*-hexane, clean-up on Pep-Pak Florisil	30 m×0.32 mm/DB-5	70°C isothermal for 1 min → 150°C at 10°C/min→280°C at 4°C/min hold for 14 min	ITD	[78]

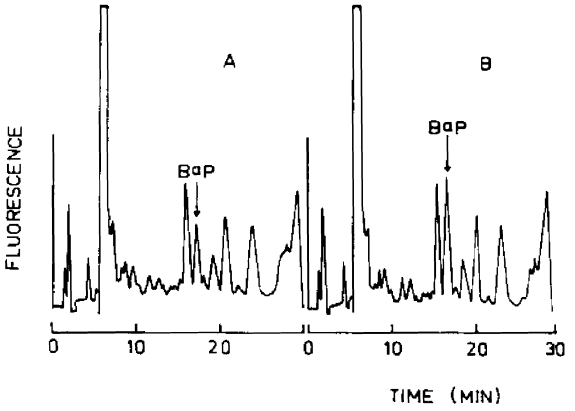

Fig. 5. HPLC–FLD chromatogram of smoked salami (A), and the chromatogram of the same sample with addition of BaP (B). Isocratic elutions. Reprinted from Ref. [20]. Copyright (1991) Springer-Verlag GmbH and Co. KG.

terization of high molecular, thermally unstable compounds, e.g. BaP metabolites were identified and determined by this method in microbore mode [69]. Due to high absorption of the light in the UV part of

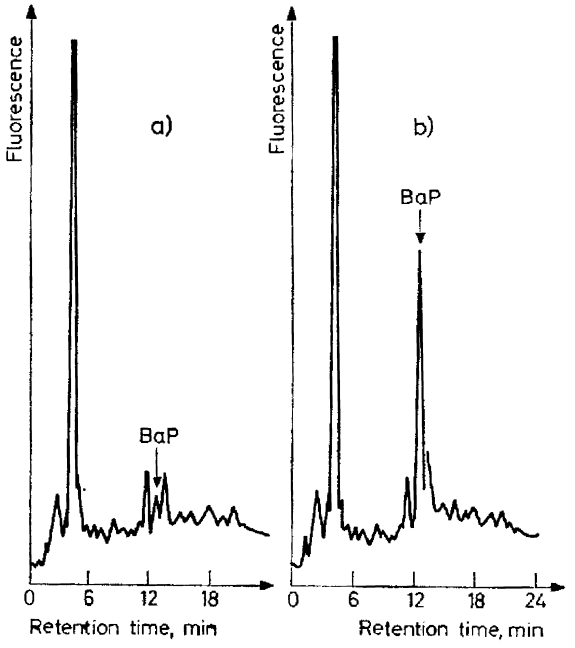

Fig. 6. HPLC–FLD chromatogram of LSF (a), the chromatogram of the same sample with addition of BaP (b). Isocratic elutions. Reprinted from Ref. [46]. Copyright (1992) Akadémiai Kiadó.

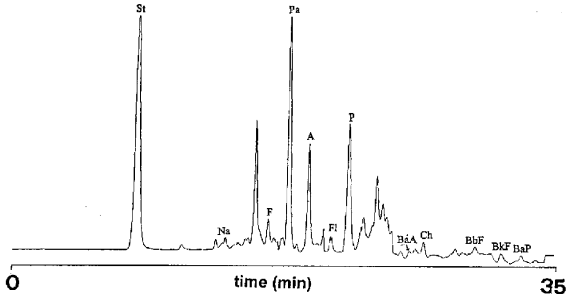

Fig. 7. HPLC–FLD chromatogram of a smoked fish sample. Programmed elution. Reprinted with permission from Ref. [53]. Copyright (1999) American Chemical Society.

the spectrum, and intensive fluorescence, both types of detectors are able to detect reliable concentrations at the $\mu g\ kg^{-1}$ levels. On the other hand, measurements by non-specific detection systems, particularly optical detectors, though often precise, can also be much less accurate due to possible chemical interferences not having been chromatographically resolved or otherwise avoided prior to the measurement. The major impurities in the PAHs fractions appear to be alkylated PAHs, which have very similar responses in optical detection systems to their unsubstituted analogues [70]. Regarding DAD, confirmation of peak purity and identification is possible, but due to the broad absorption bands in UV spectra it is highly probable that there will be some interference, if one particular wavelength is chosen for quantification. In any case, identification must be based on retention time. FLD provide very high selectivity and sensitivity, particularly those with excitation and emission wavelengths that can by varied throughout the analyses. However, fluorescence suffers from not being able to provide 'broad spectrum' analyses (i.e. a wide variety of compounds) because of the presence of alkylated PAHs compounds. A review of pre-separation procedures as well as HPLC conditions to be used for determination of PAHs in smoked meat products and SFA are shown in Table 2.

2.6. Comparison of GC and HPLC methods

In many works, also mentioned here, authors studied advantages and drawbacks of both methods,

Table 2
Pre-separation procedures as well as HPLC conditions to be used for determination of PAHs in smoked meat products and SFA

Sample	Sample treatment and preseparation	Column/stationary phase	Mobile phase	Detection	Ref.
SFA, smoked meats	Saponification with ethanolic KOH, extraction into cyclohexane, washing with saturate NaCl solution, clean-up on silica gel	25 cm×4 mm Lichrosorb RP 18	Acetonitrile–water 8:2, isocratic, 1.5 ml min^{-1}	FLD Ex: 305, 381 nm Em: 389, 430, 520 nm	[7]
Smoked meat products	Saponification with mixture of methanol, water and KOH, extraction with cyclohexane, washing with Na_2WO_4 solution, clean-up on Florisil	30 cm×3 mm, Separon SGX C_{18} RP, 5 μm,	Acetonitrile–water 3:1, isocratic, 1.5 ml min^{-1}	FLD Ex/Em 310/410 nm	[17–20]
Smoked fish, smoked meat spreads	Saponification with mixture of methanol, water and KOH, extraction with cyclohexane, cleaning-up on Florisil, partitioning with DMSO/hexane	25 cm×4.6 mm, RP-18, 5 μm,	Acetonitrile–water 7:3, isocratic, 3 ml min^{-1}	UVD 254 nm FLD Ex/Em 250/370 nm	[31]
Fish, shellfish	Saponification with methanol–water–KOH mixture under reflux, extraction into *n*-hexane, clean-up on silica gel	Radial-Pak PAH	Acetonitrile–water 8:2, isocratic, 1 ml min^{-1}	FLD Ex/Em 370/410 nm	[34]
Smoked fish	Saponification with methanol–water–KOH mixture under reflux, extraction into cyclohexane, extraction of PAHs with caffeine/formic acid, washing with NaCl solution, extraction into cyclohexane, preseparation on silica gel	ET 15 cm×4 mm, Nucleosil 5 C_{10} PAH	Acetonitrile–water 7:3 for 1 min then gradient linearly up to 9:1 in the 19th min, then to 100% acetonitrile from 20 to 40 min then isocratic till 55 min	UVD 240, 254, 260 nm FLD Ex/Em 300/408 and 280/395 nm	[35]
Smoked sausage, smoked meat	Extraction with chloroform–methanol mixture, preseparation by GPC on Bio Beads S-X3	15 cm×4.6 mm Supelcosil LC PAH, 5 μm	A: methanol–acetonitrile–water 50:25:25; B: acetonitrile; 1 min 100% A, 25th min 100% B.	FLD Variable Ex (240–293) Em (340–498) nm	[38]
Smoked frankfurters, smoked meats	Extraction with methanol in Soxhlet app.,+KOH, extraction into *n*-hexane, clean-up on Pep-Pak Florisil	12.5 cm×4.6 mm Envirosep-pp C_{18} 5 μm,	I. Acetonitrile–water 7:3, isocratic, 2 ml min^{-1} II. Acetonitrile–water 40:60, gradient to 100% acetonitrile within 25 min III. Acetonitrile–water 55:45, gradient to 100% acetonitrile within 23 min	UVD 230–360 nm FLD Variable Ex (232–302) Em (330–484) nm	[40]
SFA	Alkalisation with NaOH solution, extraction with hexane, clean-up on alumina	25 cm×4.6 mm Partisil 10 ODS	Methanol–acetonitrile–water 35:35:30, isocratic	FLD Ex/Em 280/390 nm	[44]
SFA	Alkalisation with ethanolic and aqueous NaOH, extraction into cyclohexane, partitioning with DMSO–water, extraction into cyclohexane	30 cm×4 mm, μBondapak C_{18}/Corasil	Methanol–water 7:3, 2 ml min^{-1}	UVD 280 nm	[45]
SFA, smoked food products	SFA: Saponification with methanolic KOH, extraction into cyclohexane, purification on Florisil Meat products: digestion with KOH solution, extraction with Freon 113, purification on Florisil	25 cm×4.6 mm, Supelcosil LC-PAH	Acetonitrile–water 60:40 for 5 min then 100% of acetonitrile in 15 min hold for 15 min then decrease to 60% over 10 min	FLD Ex/Em 254/375 nm	[47]
Smoked fish	Direct extraction with chloroform, preseparation on preparation silica column	Preparation column: 25 cm×4.6 mm, silica 5 μ, Analytical column: 15×4.6 mm 5 μ particle, Supelcosil LC-PAH	Preparation column: pentane/5% DCM, 0.8 ml min^{-1} Analytical column: Water/acetonitrile 6:4 for 5 min then to 100% acetonitrile over 40 min, 1.5 ml min^{-1}	FLD Variable nm	[53]
Smoked fish, ham	Saponification with mixture of methanol, water and KOH, extraction with cyclohexane, partitioning with DMSO/hexane	Spherisorb ODS 5 μm precolumn and 5 μm VydacODS analytical column	Acetonitrile–water 6:4, linearly to 9:1 over 35 min	FLD Ex/Em 290/430 nm	[61]
Smoked meat products	Saponification with methanolic KOH, extraction with *n*-hexane, preseparation by SPE on CN bonded silica	Nucleosil 100–5 C_{18} PAK	Acetonitrile–water 8:2, isocratic, 0.5 ml min^{-1}	FLD Ex/Em 290/430 nm	[64]
Smoked fish	Saponification with methanol–water–KOH mixture under reflux, extraction into *n*-hexane, clean-up on silica gel	15 cm×6 mm, ODS, 5 μ particle,	Acetonitrile–water 8:2, isocratic, 1 ml min^{-1}	FLD Ex/Em 370/410 nm	[65]
SFA, smoked foods	SFA: Saponification with methanolic KOH, extraction into cyclohexane, purification on Florisil Smoked products: digestion with KOH solution, extraction with Freon 113, purification on Florisil	12.5 cm×4 mm Lichrosphere 100 RP-18	A: water; B: methanol–acetonitrile 1:1; I. segment: 1:80 to 100% B in 20 min II. segment: 100% B for 5 min III. segment: 100 to 80 B in 5 min	FLD Ex/Em 365/418 nm	[66]
Smoked meat products	Saponification with methanolic KOH, extraction into cyclohexane, preseparation by SPE on Kiesel gel	12.5 cm×4 mm, Chrompack PAH-Säule	Acetonitrile–water 9:1, isocratic, 0.5 ml min^{-1}	FLD Ex/Em 290/430 nm	[77]
Smoked chicken	Extraction with methanol in Soxhlet app.,+KOH, extraction into *n*-hexane, clean-up on Pep-Pak Florisil	12.5 cm×4.6 mm Envirosep-pp 5 μm C_{18}	Acetonitrile–water 55:45, gradient to 100% acetonitrile within 23 min 1.2 ml min^{-1}	FLD Variable nm	[78]

when studies were aimed especially at recovery studies, quality of separation processes, time of analysis, price of equipments, etc. Dennis et al. [61] compared results of the analysis of some food (two smoked) obtained by GC and HPLC. Thirty-five pairs of analysis were tested using statistical procedure (Student's *t*-test). From this, 25 were not significantly different within the 95% confidence limits employed. But, data for BkF/benzofluorantenes and DahA/dibenzoanthracenes were not compared because different analytes were being measured. Standard deviations indicated that repeatability of both methods was very good, being usually within 10%. The methods appeared well able to compare data throughout the wide range (0.2–1000 $\mu g\ kg^{-1}$). In conclusion, it was stressed that capillary GC possessed a much greater resolving power, in terms of plate number, so that many more PAHs can be separated and determined. To the opposite, end, HPLC was able to separate individual isomers (BbF and BkF; Chr and Tph) i.e. it had a greater selectivity. Chiu et al. [78] compared separation and detection conditions of both methods analysing smoked chicken. As found, 16 priority PAHs polutants can be simultaneously separated by HPLC using a gradient solvent system and detected by FLD with seven settings of programmable wavelength. With GC, a temperature programming method can also resolve 16 PAHs. The presence of impurities in smoked meat products can interfere with the identification and quantification of PAHs by HPLC. With ITD, the PAHs can be identified even in the presence of fat — or PAHs — like impurities. The retention times by HPLC were shorter than those by GC when HPLC had a better separation for most compounds than GC. Sim et al. [70] compared GC and HPLC methods analysing 16 PAHs pollutants. As pointed out, the chromatographic resolution may be divided into a combination of column capacity, column efficiency and separation selectivity. GC has a higher column efficiency and thus has an advantage for complex mixture analysis, but HPLC can often have a higher column selectivity, which is more suitable for separation of isomeric compounds. Thus, both methods should be viewed as complementary in the analysis of PAHs and they are essential for precise and reliable analysis.

3. Occurrence of PAHs

Immediately after the information regarding carcinogenic effect, research workers started to find PAHs concentrations in smoked meat products. However, there were problems in assessing real toxicological risk with regard to the fact, that different numbers of compounds were determined from case to case. So, although roughly 60% of compounds have been found to be carcinogenic in mammals [11], and BaP concentration constitutes only between 1 and 20% of the total carcinogenic PAHs [76], it seems correct at this stage to simplify the problem of risk assessment by taking into account only BaP. Of course, if the situation is changed in the future, a new model of risk assessment, assessing also the presence of other PAHs, will be very realistic and needed. In Table 3 are shown data regarding BaP concentrations to be found in smoked meat products. These data approve that technologically correct smoking process contaminates products only with small concentrations of PAHs. Far more dangerous is the smoking process in uncontrolled conditions, typical for home 'wild' smoking in preparation of heavy smoked 'farm' products as well as smoking to be being done in developing countries without any technological and knowledge and hygienic control. These products may bring a real risk to the consumer of cancer, especially after a long period of consumption. In Table 4 some data about BaP in SFA are displayed. Although these concentrations are quite high, it is necessary to take into account that SFA are applied in small quantities and for this the final PAHs concentrations in aromatised products is lowered by some orders.

4. Conclusion

Determination of PAHs in smoked meat products is a permanent process. The real risk assessment realisation will need in the future simple, versatile and precise methods for measurement of PAHs in food. As mentioned by Tamakawa [11], the main route for human exposure to BaP is diet (80%). So, it will be necessary to continue developing and unifying the analytical methods applicable for on-line

Table 3
Occurrence of BaP in smoked meat products

Sample	No. of analysed positive samples	Concentration of BaP [μg kg^{-1}]		Ref.
		Min.	Max.	
Fish	5/5	11.1[a]	66.9[a]	[6]
Ham, bacon, fish, sausage	19/19	0.3	18	[7]
Frankfurters, meat, sausages	8/8	0.1	12.0	[15]
Ham, pork, meat products	74/69	0.2	56.5	[16]
Fish, sausages, spread, salami	17/17	0.1	9.5	[17]
Fermented products, frankfurters	17/7	0.05	0.15	[28]
Sausages, special products	386	0.6	100	[29]
Fish and fish products	70/57	0.1	11.3	[30]
Dark smoked meat products	5/5	17.1	39.9	[32]
Fish	62	0.1	4.1	[35]
Sausage, pork	2/2	0.3	5.2	[38]
Ham, bacon	3/3	0.2	0.4	[39]
Sausage, fish, pork tasso	5/0	–	–	[41]
Fish	Unknown	0.3	1.4	[42]
Sausages, poultry, bacon	5/3	0.1	0.4	[47]
Fish	11/4	0.1	0.3	[53]
Oysters	2/2	10.1	12.2	[60]
Fish	6/6	1.5	2.8	[65]
Ribbons, ham, sausages, bacon	6/5	0.2	1.3	[66]
Fish	20/20	20 850[b]	66 910[b]	[71]
Mutton meat	5/5	0.1	5.6	[72]
Bacon, frankfurters	Unknown	1.2	3.6	[73]
Salami, bacon	4/4	0.2	0.5	[74]
Products, fish	4/4	0.2	1.5	[75]
Heavy smoked ham, products	196/196	0.03	>10.0	[77]

[a] Concentration of BaP in dry matter.
[b] Probably the correct concentration is in the range 20.85–66.91 μg kg^{-1}.

Table 4
Occurrence of BaP in SFA

Sample	Number of analysed positive samples	Concentration of BaP [μg kg^{-1}]		Ref.
		Min.	Max.	
SFA	10/10	0.2	6.3	[23]
SFA liposoluble form	6/6	0.3	48	
Smoke concentrate aroma	Unknown	Less than 0.1	44	
Sediments from smoke aroma	Unknown	25	3800	[43]
SFA	3/3	0.3	0.8	[46]
SFA	11/10	0.1	3.4	[47]
SFA	5/3	1.1	2.9	[49]
STA	5/2	0.04	0.06	[50]
SFA	11/10	0.1	336.6	[66]

analysis, comparable throughout the world which makes it possible to determine real risk assessment resulting from permanent PAHs presence in the food chain.

5. Nomenclature

AOAC	Association of Official Analytical Methods
Ace	Acenaphthene
Acy	Acenaphthylene
Ant	Anthracene
ASE	Advanced solvent extraction
BaA	Benzo(a)anthracene
BaP	Benzo(a)pyrene
BeP	Benzo(e)pyrene
BghiP	Benzo(g,h,i)perylene
BbF	Benzo(b)fluoranthene
BjF	Benzo(j)fluoranthene
BkF	Benzo(k)fluoranthene
BMBT	*N*,*N*′-bis[*p*-methoxybenzilidene]-α, α′-bi-*p*-toluidine
BBBT	*N*,*N*′-bis[*p*-butoxybenzilidene]-α, α′-bi-*p*-toluidine
CC	Column chromatography
Cor	Coronene
DAD	Diode array detector
DCM	Dichlormethane
DacA	Dibenzo(a,c)anthracene
DahA	Dibenzo(a,h)anthracene
DajA	Dibenzo(a,j)anthracene
DMFA	Dimethylforamide
DMSO	Dimethylsulfoxide
EU	European union
FID	Flame ionisation detector
Fl	Fluorene
Fu	Fluoranthene
FLD	Fluorimetric detector
ITD	Ion trap detector
GC	Gas chromatography
GPC	Gel permeation chromatography
HETP	High equivalent theoretical plate
HPLC	High pressure liquid chromatography
Chr	Chrysene
IARC	International Agency for Research on Cancer
IPy	Indeno(1,2,3-c,d)pyrene
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LSF	Liquid smoke flavour
MSD	Mass spectrometry detector
Na	Naphthalene
ODS	Octadecylsilane
PAHs	Polycyclic aromatic hydrocarbons
PC	Paper chromatography
Per	Perylene
Phe	Phenanthrene
Py	Pyrene
PPS	Phtalimidopropylsilane
RP	Reverse phase
SIM	Selected ion monitoring
SFA	Smoke flavouring additive
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
SFA	Smoke flavouring additives
TIC	Total ion chromatogram
TLC	Thin layer chromatography
Tph	Triphenylene
UICC	International Union Against Cancer
UVD	Ultraviolet detector

References

[1] K.D. Bartle, in: C. Creaser, R. Purchase (Eds.), Food Contaminants, Sources and Surveillance, The Royal Society of Chemistry, Cambridge, 1991, p. 41.
[2] K. Fretheim, Food Chem. 10 (1983) 129.
[3] P. Mottier, V. Parisod, R.J. Turesky, J. Agric. Food Chem. 48 (2000) 1160.
[4] J. Pánek, J. Davídek, Z. Jehličková, in: J. Davídek (Ed.), Natural Toxic Compounds of Foods. Formation and Change During Food Processing and Storage, CRC Press, Boca Raton, FL, 1995, p. 195.
[5] N. Kruijf, T. Schouten, G.H.D. Stegen, J. Agric. Food Chem. 35 (1987) 545.
[6] A.O. Afolabi, E.A. Adesulu, O.L. Oke, J. Agric. Food Chem. 31 (1983) 1083.
[7] T. Stijve, C. Hischenhuber, Deutsch. Lebensm. Rundsch. 83 (1987) 276.
[8] K. Grob, M. Biedermann, A. Caramaschi, B. Pacciareli, J. High Resol. Chrom. 14 (1991) 33.
[9] P. Šimko, V. Khunová, P. Šimon, M. Hrubá, Int. J. Food Sci. Technol. 30 (1995) 807.

[10] M.D. Guillén, Food Addit. Contam. 11 (1994) 669.
[11] K. Tamakawa, T. Kato, M. Oba, in: L. Nollet (Ed.), Handbook of Food Analysis, Marcel Dekker, New York, 1996, p. 1641.
[12] W. Stahl, G. Eisenbrand, in: R. Macrae (Ed.), HPLC in Food Analysis, Academic Press, London, 1988, p. 377.
[13] J.A. Maga, Food Rev. Int. 3 (1987) 139.
[14] L. Tóth, W. Blaas, Fleischwirtsch 52 (1972) 1419.
[15] A. Roda, P. Simoni, E.N. Ferri, S. Girotti, A. Ius, P. Rauch, M. Poplstein, M. Pospisil, P. Pipek, I. Hochel, L. Fukal, J. Sci. Food Agric. 79 (1999) 58.
[16] K. Potthast, Fleischwirtsch 58 (1978) 371.
[17] P. Šimko, M. Gombita, J. Karovičová, Nahrung 35 (1991) 103.
[18] P. Šimko, Š. Gergely, J. Karovičová, M. Drdák, J. Knezo, Meat Sci. 34 (1993) 301.
[19] P. Šimko, Food Chem. 40 (1991) 293.
[20] P. Šimko, J. Karovičová, M. Kubincová, Z. Lebensm. Unters. Forsch. 192 (1991) 538.
[21] P. Šimko, J. Knežo, Nahrung 36 (1992) 208.
[22] Report of the Joint FAO/WHO Expert Commission on Food Additives, 31 (1987) s.759.
[23] M.S. García-Falcón, J. Simal-Gandara, S.T. Carril-Gonzalez-Barros, Food Addit. Contam. 17 (2000) 957.
[24] R. Schaad, Chromatogr. Rev. 13 (1970) 61.
[25] J.W. Howard, T. Fazio, J. Off. Anal. Chem. 63 (1980) 1077.
[26] AOAC Official Method 973.30. 16th ed., AOAC International, Arlington, 1995, p. 48.
[27] G. Grimmer, H. Böhnke, J. Assoc. Off. Anal. Chem. 58 (1975) 725.
[28] K. Fretheim, J. Agric. Food Chem. 24 (1976) 976.
[29] P.H. Binnemann, Z. Lebensm Unters. Forsch. 169 (1979) 447.
[30] B.K. Larsson, Z. Lebensm Unters. Forsch. 174 (1982) 101.
[31] J.F. Lawrence, D.F. Weber, J. Agric. Food Chem. 32 (1984) 795.
[32] P. Šimko, J. Dubravický, V. Smirnov, Potrav. Vedy 7 (1989) 59.
[33] D.L. Vassilaros, P.W. Stoker, G.M. Booth, M.L. Lee, Anal. Chem. 54 (1982) 106.
[34] K. Takatsuki, S. Suzuki, N. Sato, I. Ushizawa, J. Assoc. Off. Anal. Chem. 68 (1985) 945.
[35] H. Karl, M. Leinemann, Z. Lebensm. Unters. Forsch. 202 (1996) 458.
[36] K. Potthast, G. Eigner, J. Chromatogr. 103 (1975) 173.
[37] D.O. Alonge, Acta Aliment. 16 (1987) 263.
[38] K. Cejpek, J. Hajšlová, Z. Jehličková, J. Merhaut, Int. J. Environ. Anal. Chem. 61 (1995) 65.
[39] F.L. Joe, J. Salemme, T. Fazio, J. Assoc. Off. Anal. Chem. 67 (1984) 1076.
[40] B.H. Chen, C.Y. Wang, C.P. Chiu, J. Agric. Food Chem. 44 (1996) 2244.
[41] G. Wang, A.S. Lee, M. Lewis, B. Kamath, R.K. Archer, J. Agric. Food Chem. 47 (1999) 1062.
[42] Ch. Gertz, Z. Lebensm. Unters. Forsch. 173 (1981) 208.
[43] R.H. White, J.W. Howard, C.J. Barnes, J. Agric. Food Chem. 19 (1971) 143.
[44] D.S. Silvester, J. Food Technol. 15 (1980) 413.
[45] A. Radecki, H. Lamparczyk, J. Grzybowski, J. Halkiewicz, J. Chromatogr. 150 (1978) 527.
[46] P. Šimko, J. Petrík, J. Karovičová, Acta Aliment. 21 (1992) 107.
[47] E.A. Gomaa, I.J. Gray, S. Rabie, C. Lopez-Bote, A.M. Booren, Food Addit. Contam. 10 (1993) 503.
[48] B. Laffon Lage, S. García Falcón, M.S. Gonzalez Amigo, M.A. Lage Yusty, J. Simal Lozano, Food Addit. Contam. 14 (1997) 469.
[49] M.D. Guillén, P. Sopelana, M.A. Partearroyo, J. Agric. Food Chem. 48 (2000) 126.
[50] M.D. Guillén, P. Sopelana, A. Partearroyo, J. Agric. Food Chem. 48 (2000) 5083.
[51] K. Kangsadalampai, C. Butryee, K. Manoonphol, Food Chem. Toxicol. 35 (1997) 213.
[52] G.M.A.H. Vaessen, A.A. Jekel, M.M.A.A. Wilbers, Toxic. Environ. Chem. 16 (1988) 281.
[53] S. Moret, L. Conte, D.J. Dean, Agric. Food Chem. 47 (1999) 1367.
[54] E.O. Haenni, Residue Rev. 24 (1968) 42.
[55] J. Tuominen, K. Wickström, H. Pyysalo, J. High Resol. Chrom. Chrom. Comm. 9 (1986) 469.
[56] M.L. Lee, M.V. Novotny, K.D. Bartle, Analytical Chemistry of Polycyclic Aromatic Hydrocarbons, Academic Press, New York, 1981, p. 242.
[57] P.T. Williams, G.E. Andrews, K.D. Bartle, P. Bishop, P. Watkins, Biomed. Environ. Mass Spectrom. 15 (1988) 517.
[58] P.J. Nyman, G.A. Perfetti, F.L. Joe, G.W. Diachenko, Food Addit. Contam. 10 (1993) 489.
[59] A. Radecki, H. Lamparczyk, J. Grzybowski, J. Halkiewicz, J. Chromatogr. 150 (1978) 527.
[60] K. Speer, E. Steeg, P. Horstmann, T. Kuehn, A. Montag, J. High Resol. Chrom. Chrom. Commun. 13 (1990) 104.
[61] M.J. Dennis, R.C. Massey, D.J. McWeeny, B. Larsson, A. Eriksson, G. Sahlberg, J. Chromatogr. 285 (1984) 127.
[62] B.H. Chen, Y.S. Lin, J. Agric. Food Chem. 45 (1997) 1394.
[63] D. Hunt, P. Wild, N.T. Crosby, J. Chromatogr. 130 (1977) 320.
[64] K. Hartmann, Deutsch. Lebensm. Rundsch. 96 (2000) 163.
[65] G. Ova, S. Onaran, Adv. Food Sci. 20 (1998) 168.
[66] H.Y. Yabiku, M.S. Martins, M.Y. Takahashi, Food Addit. Contam. 10 (1993) 399.
[67] J. Folch, M. Lees, G.H. Sloane Stanley, J. Biol. Chem. 226 (1957) 497.
[68] A.S. Wise, L.C. Sander, W.E. May, J. Chromatogr. 642 (1993) 329.
[69] R.H. Bieri, J. Greaves, Biom. Environ. Mass Spectr. 14 (1987) 555.
[70] P.G. Sim, R.K. Boyd, R.M. Gershey, R. Guevremont, W.D. Jamieson, M.A. Quillian, R.J. Gergely, Biom. Environ. Mass Spectr. 14 (1987) 375.
[71] D.O. Alonge, Acta Aliment. 16 (1987) 263.
[72] M. J Dennis, G.S. Cripps, A.R. Tricker, R.C. Massey, D.J. McWeeny, Food Chem. Toxicol. 22 (1984) 305.
[73] K.S. Rhee, L.J. Bratzler, J. Food Sci. 35 (1970) 146.

[74] C. Lintas, M.C. de Matthaeis, Food Cosmet. Toxicol. 17 (1979) 325.
[75] T. Panalaks, J. Environ. Sci. Health Bull. 11 (1976) 299.
[76] J.B. Andelman, M.J. Suess, Bull. WHO 43 (1970) 479.
[77] W. Rauter, Ernährung 21 (1997) 447.
[78] C.P. Chiu, Y.S. Lin, B.H. Chen, Chromatographia 44 (1997) 497.