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Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach

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Abstract

An efficient and selective analytical method for the determination and the quantification of 19 polycyclic aromatic hydrocarbons (PAHs) in food and oil has been developed. This method includes the monitoring of 15 PAHs stated as a priority by the EU in their 2005/108 recommendation. The samples were extracted according to a selective extraction step using pressurized liquid extraction followed by a purification with polystyrene-divinylbenzene SPE. Identification and quantification were performed using GC–MS/MS, with an isotope dilution approach using ^{13}C-labelled PAHs. The novel combination of selective extraction followed by purification provides highly purified analytes combined to a fast and automated method. The advantages of GC–MS/MS as compared to other detection methods are tremendous in terms of sensitivity, selectivity and interpretation facilities. Limits of detection varied between 0.008 and 0.15 μg kg^{-1}, limits of quantification between 0.025 and 0.915 μg kg^{-1} for PAHs in food. The calibration curves showed a good linearity for all PAHs ($R^2 > 0.99$) and precision and recovery were fit for purpose. Trueness of the method was carried out using the US National Institute of Standards and Technology SRM 2977 reference material.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); GC–MS/MS; Isotope dilution; 2002/657/EC

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds containing two or more aromatic rings and belonging to the Food and Environment Contaminants. They are produced by natural and anthropogenic processes. Among these sources, pyrogenic (incomplete burning of coal, oil, gas, wood, garbage or other organic substances) and petrogenic inputs are the two main sources of PAHs [1,2]. Non-smokers are mainly exposed to PAHs through food and air. PAHs are significantly present in food due to heat processes such as smoking, grilling and smoke-drying. Food can also be naturally contaminated by an accumulation of PAHs in the food chain, due to their lipophilic properties and their propensity to accumulate in adipose tissue. Exposure to PAHs is a major concern for human health, as PAHs are classified as carcinogenic, especially because they are metabolized to dihydrodiols by hydrocarbon hydroxylases present in the liver. Dihydrodiols and their epoxide derivatives bind to DNA and proteins, starting mutagenic processes in the cells [3,4]. Benzo[*a*]pyrene is identified as a potent carcinogenic species and has been chosen as indicator for total PAHs. In view of the presence of PAHs in food and their significant toxicity, control of these compounds in food is necessary. In 1984, the US Environmental Protecting Agency (EPA) proposed a list of 16 PAHs which are known as carcinogenic or mutagenic. In the European Union, a new legislation was adopted in 2005 [5] and provided a list of 15 PAHs (8 in common with US EPA and 7 new compounds) which were of major concern for human health due to their toxic properties (Table 1). Another legislation [6] setting new limits for benzo[*a*]pyrene has been put in force in several food categories (oils, baby food, smoked meat, crustaceans, bivalve molluscs). Consequently, analytical methods are necessary in order to control these 15 European priority polycyclic aromatic hydrocarbons in food. Several methods have been

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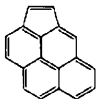
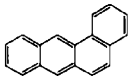
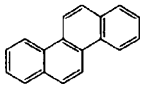
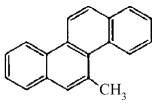
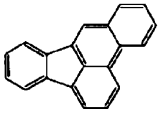
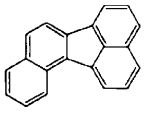
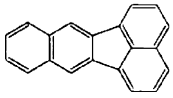
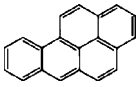
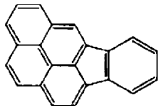
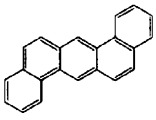
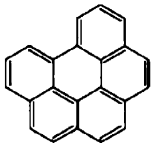
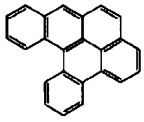
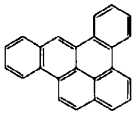
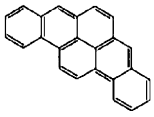
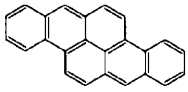
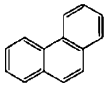
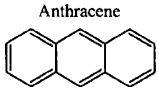
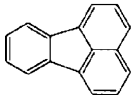
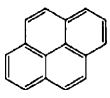
Table 1
List of the 19 studied PAHs, corresponding to the compounds of EU priority list and 4 other included in the US EPA list

	Chemical structure of the 19 studied PAHs		
15 EU priority PAHs	Cyclopenta[*c,d*]pyrene 	Benz[*a*]anthracene 	Chrysene 
	5-Methylchrysene 	Benzo[*b*]fluoranthene 	Benzo[*j*]fluoranthene 
	Benzo[*k*]fluoranthene 	Benzo[*a*]pyrene 	Indéno[1,2,3-*c,d*]pyrene 
	Dibenz[*a,h*]anthracene 	Benzo[*g,h,i*]perylene 	Dibenzo[*a,l*]pyrene 
	Dibenzo[*a,e*]pyrene 	Dibenzo[*a,i*]pyrene 	Dibenzo[*a,h*]pyrene 
Four other studied PAHs included in the list of US EPA	Phenanthrene 	Anthracene 	
	Fluoranthene 	Pyrene 	

described for the analysis of PAHs, with different techniques of extraction, purification and detection. The method commonly used as a reference for the extraction of lipophilic organic compounds was based on soxhlet [7–12]. Nowadays, this extraction method is replaced by other techniques which give advantages (automation, reduced extraction time and lower solvent used) such as pressurized liquid extraction (PLE) [7,8,10,12–21], supercritical fluid extraction (SFE) [7,8,12] or microwave-assisted extraction (MAE) [8,22]. Selective extraction may also be used by adding a purification phase in the PLE cell [15,18,23] which allows for a pre-purification step. As regards purification, the method used depends on the investigation matrix. Gel permeation chromatography [10,11,21,23–25], silica gel [11,26,27] or open-column liquid chromatography with florisil [28,29] are mainly used. Finally, PAHs are analyzed either by GC–MS [12,14,16,17–19,21,23,26,28–31], GC–MS/MS [24,25] or HPLC with fluorescence detection [7,10,22, 27,32–34].

This paper describes a GC–MS/MS method for the determination of 19 PAHs (including the 15 European priority PAHs) in food and oil, using selective PLE, purification with SPE with polystyrene-divinylbenzene phase and GC–MS/MS detection. For trueness assessment, the standard reference mussel tissue material (SRM 2977) of the National Institute of Standards and Technology was used.

2. Experimental

2.1. Reagents and material

All solvents used (e.g. dichloromethane, hexane, acetone, ethanol, cyclohexane, ethyl acetate and toluene) were obtained in picograde quality from Promochem (Wesel, Germany). Florisil (100–200 mesh) was provided by Promochem.

SPE Envi Chrom-P cartridges were provided by Sigma–Aldrich (St. Quentin Fallavier, France).

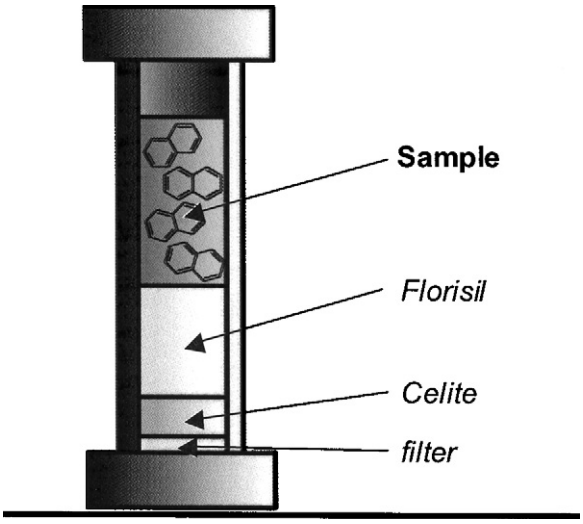


Fig. 1. ASE cell for the selective extraction step.

The isotopic labelled internal standard compounds ^{13}C PAHs and ^{12}C PAHs were purchased from Promochem. Fluorinate PAHs were purchased from Chiron (Norway).

2.2. Instruments and equipments

Samples were extracted by ASE 300® (Accelerated Solvent Extractor), from Dionex Corp.

For GC–MS/MS analysis, a gas chromatograph (Agilent, 6890 Series) with split/splitless injector and a programmable oven reaching up to 350 °C was coupled to a Quattro-micro GC triple quadrupole analyzer (Waters, Micromass) operating in electron ionization mode. Gas chromatography was performed on a non-polar column Zebron ZB-5MS (30 m × 0.25 mm × 0.25 μm) purchased from Phenomenex (Le Pecq, France).

2.3. Sample preparation

Ten grams of food sample were freeze-dried. The dry residue obtained was weighed in order to determine its water content. One gram of the dry residue was taken and spiked with a ^{13}C-labelled internal standard.

For the analysis of oil, 1 g was weighed and spiked with a ^{13}C-labelled internal standard. Three milliliters of cyclochexane were added, and the resulting mixture was shaked for homogenization.

2.4. Sample extraction and purification

A cellulose filter was placed at the bottom of the ASE cell (66 ml) and filled up with 1.0 g of celite and 15.0 g of florisil. The florisil was pre-washed in the ASE system with CH_2Cl_2. One gram of the dry residue sample was introduced into the cell (Fig. 1), and extraction was performed with a mixture hexane/acetone (50/50, v/v). The extract was purified onto a SPE cartridge (Envi Chrom-P): after conditioning, the sample was loaded. The cartridge was washed with a mixture of cyclohexane/ethanol (70/30, v/v) and compounds were eluted using 12 ml of cyclohexane/ethyl acetate (40/60, v/v) as mobile phase. Fluorinated PAHs were used as external standards. Two microliters of the final extract (in toluene) were analyzed by GC–MS/MS.

2.5. GC–MS/MS conditions

The sample solutions were injected in the splitless mode. The injector temperature was kept at 300 °C and the transfer line temperature was set at 320 °C. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The column temperature program was as follows: 110 °C (1 min), 20 °C/min to 240 °C (0 min), 5 °C/min to 320 °C (10 min). Electron ionization (EI) was operated at 70 eV in any case. Temperature of the source was kept at 230 °C. Argon was used as collision gas at a pressure of approximatively 3×10^{-4} mbar. PAHs were detected and quantified by monitoring two specific transitions. Table 2 gives retention time indications and the monitored transitions for each compound.

3. Results and discussion

3.1. Extraction

Pressurized liquid extraction has been used to extract PAHs from food. A stationary phase has been added in the cell to assure a preliminary purification of the extract. The nature of the stationary phase and extraction solvent was optimized, and the use of celite/florisil combined to hexane/acetone proved to be an efficient option even if florisil contains significant trace of PAHs and need consequently washing steps before any use. This precaution is easily performed programming a pre-wash cycle of the phase in the ASE cell before sample introduction. Dichloromethane was found to be efficient to decontaminate the stationary phase.

3.2. Clean-up

Several methods have described the purification of PAHs in various matrices. Among the many purification protocols, saponification [26,28], gel permeation chromatography [21,23,24], silica gel [23,26,30] and florisil [17,28,29] are the most popular.

The use of a polystyrene-divinylbenzene (PS-DVB) has been described for purification of PAHs in sediment [17] or steroids in various biological fluids or tissues [35]. This phase is extremely selective to PAHs and sufficient as such before GC–MS/MS injection for most food application.

For oil purification, gel permeation [24] or thin-layer chromatography on silica gel [31] have been described. Nevertheless, these approaches could not be considered for a high throughput approach. Operative conditions described in this text enable a rapid and efficient method, with only one purification step.

Different SPE PS-DVB manufacturers were tested. Best results were obtained with Supelclean Envi-Chrom-P (Supelco®). Conditions of the purification step were optimized

Table 2
Indicative retention times and PAHs monitored transitions

Compounds	Indicative RT (min)	Transition 1	Collision T1 (eV)	Transition 2	Collision T2 (eV)
Phenanthrene $^{13}C_6$ (IS)	7.59	184 > 182	25		
Phenanthrene	7.59	178 > 176	25	178 > 152	15
Anthracene $^{13}C_6$ (IS)	7.67	184 > 182	25		
Anthracene	7.67	178 > 176	25	178 > 152	15
3-Fluoro fluoranthene (ES)	9.25	220 > 218	30		
Fluoranthene $^{13}C_6$ (IS)	9.40	208 > 206	30		
Fluoranthene	9.40	202 > 200	30	202 > 152	30
Pyrene $^{13}C_3$ (IS)	9.81	205 > 203	30		
Pyrene	9.81	202 > 200	20	202 > 152	30
3-Fluoro chrysene (ES)	12.50	246 > 244	30		
Cyclopenta[*c*;*d*]pyrene	12.69	226 > 224	30	226 > 200	35
Benz[*a*]anthracene $^{13}C_6$ (IS)	12.74	234 > 232	30		
Benz[*a*]anthracene	12.74	228 > 226	30	228 > 202	20
Chrysene $^{13}C_6$ (IS)	12.84	234 > 232	30		
Chrysene	12.84	228 > 226	30	228 > 202	20
5-Methylchrysene	14.18	242 > 240	30	242 > 226	25
9-Fluoro benzo[*k*]fluoranthene (ES)	15.80	270 > 268	30		
Benzo[*b*]fluoranthene $^{13}C_6$ (IS)	16.09	258 > 256	30		
Benzo[*b*]fluoranthene	16.09	252 > 250	30	252 > 226	20
Benzo[*j*]fluoranthene	16.10	252 > 250	30	252 > 226	20
Benzo[*k*]fluoranthene $^{13}C_6$ (IS)	16.18	258 > 256	30		
Benzo[*k*]fluoranthene	16.18	252 > 250	30	252 > 226	20
Benzo[*a*]pyrene $^{13}C_4$ (IS)	17.12	256 > 254	30		
Benzo[*a*]pyrene	17.12	252 > 250	30	252 > 226	20
Indeno[1,2,3-*c*,*d*]pyrene $^{13}C_6$ (IS)	20.65	282 > 280	45		
Indeno[1,2,3-*c*,*d*]pyrene	20.65	276 > 274	45	276 > 272	50
Dibenz[*a*,*h*]anthracene $^{13}C_6$ (IS)	20.79	284 > 282	30		
Dibenz[*a*,*h*]anthracene	20.79	278 > 276	30	278 > 252	20
Benzo[*g*,*h*,*i*]perylene $^{13}C_{12}$ (IS)	21.41	288 > 286	45		
Benzo[*g*,*h*,*i*]perylene	21.41	276 > 274	45	276 > 272	50
Dibenzo[*a*,*l*]pyrene	24.83	302 > 300	40	302 > 298	50
Dibenzo[*a*,*e*]pyrene $^{13}C_6$ (IS)	25.79	308 > 306	40		
Dibenzo[*a*,*e*]pyrene	25.79	302 > 300	40	302 > 298	50
Dibenzo[*a*,*i*]pyrene $^{13}C_{12}$ (IS)	26.14	314 > 312	40		
Dibenzo[*a*,*i*]pyrene	26.14	302 > 300	40	302 > 298	50
Dibenzo[*a*,*h*]pyrene	26.32	302 > 300	40	302 > 298	50

IS, internal standard; ES, external standard.

for conditioning, washing and elution. First, conditioning with ethyl acetate was used with the purpose of eliminating all traces of PAHs contained in the cartridge. Indeed, some column batches showed a significant contamination by PAHs, as observed with florisil phase. Pyrene, fluoranthene and benzo[*g*,*h*,*i*]perylene were estimated at approximatively 1 ng in every cartridge. Contamination with lightest PAHs such as pyrene or fluoranthene was recurrent, but the presence of benzo[*g*,*h*,*i*]perylene was very surprising at this contamination level. For the washing and elution steps, different tests were performed by progressively increasing the solvent polarity and the eluotropic force.

A critical step for the lighter PAHs analysis was the evaporation of solvent, especially at the end of the purification step, when no interference with any matrix compounds could trap the PAHs. It has been demonstrated that evaporation may cause a 20–70% loss of 3-ring PAHs [30]. In order to limit the loss, the evaporation under a gentle stream of nitrogen at moderated temperature (40 °C) was preferred; precautions were taken to stop the nitrogen flow as soon as the solvent was evaporated.

3.3. Detection: compared mass spectrometry approaches

The high stability of PAHs makes them difficult to fragment, even under drastic electron ionization conditions. Fragmentation of PAHs after EI leads nearly exclusively to the observation of the molecular ion ($M^{+\bullet}$). Nevertheless, other ions are commonly formed such as $[M-2H]^{+\bullet}$ or double-charged analytes M^{2+} but to a lower extent.

The mass spectrum thus obtained was then poor in fragment ions so that the identification of each compound on single MS was limited. A typical example of observed ion chromatograms for $^{13}C_3$-pyrene (internal standard, *m*/*z* 205), $^{13}C_6$-fluoranthene (internal standard, *m*/*z* 208), fluoranthene and pyrene (*m*/*z* 202 and 101) in smoked tuna sample is shown in Fig. 2. For some traces such as the one shown for $^{13}C_6$-fluoranthene (*m*/*z* 208) and fluoranthene/pyrene (*m*/*z* 101), the signal is affected by co-extracted compounds. Moreover, the signal to noise ratio of $^{13}C_3$-pyrene is weak, even at 0.5 μg kg^{-1} level. The main advantage of the MS/MS technique is the possible fragmentation of PAHs in the collision cell, leading, under drastic conditions (high energy voltage applied to the collision cell:

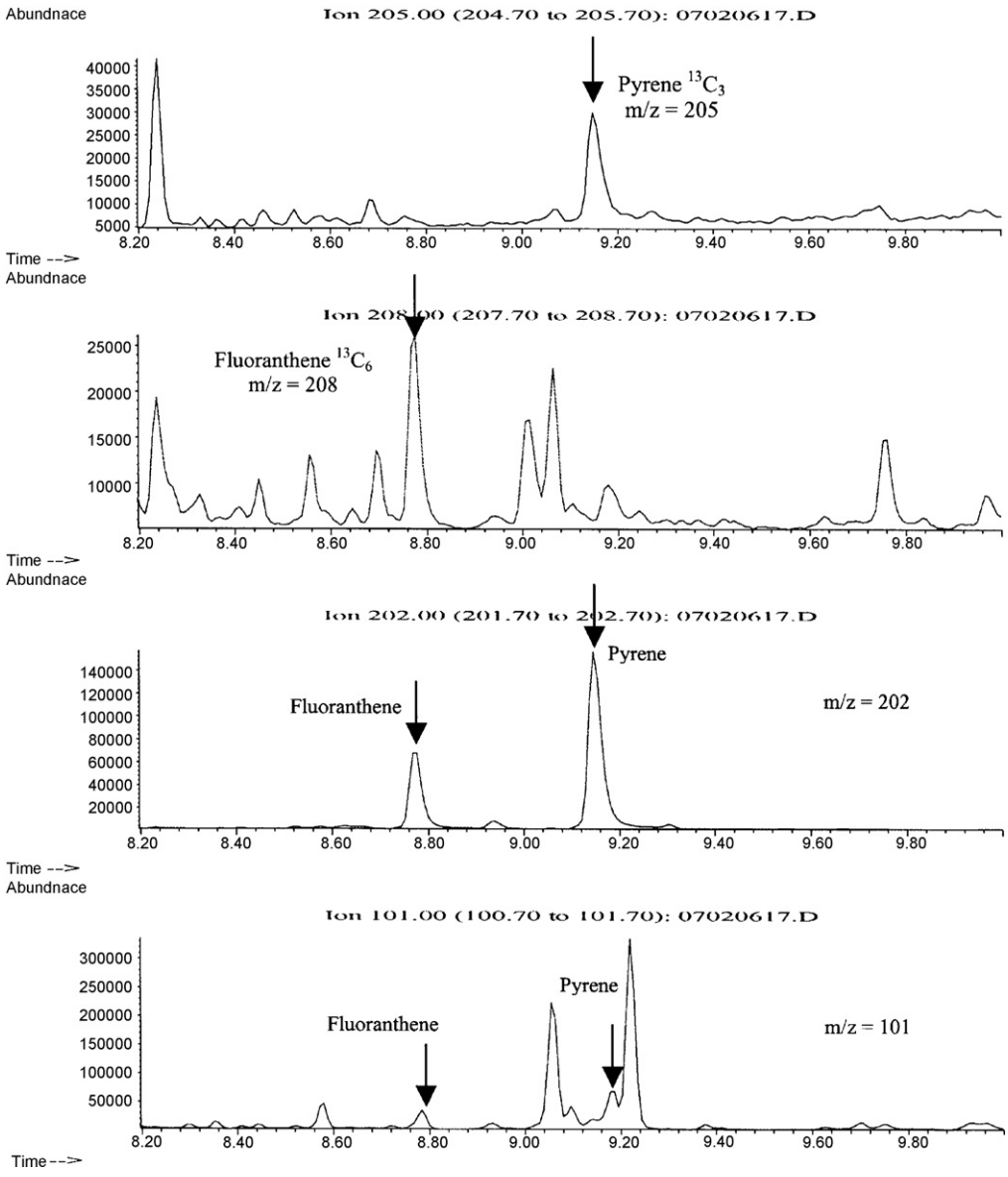


Fig. 2. Ion chromatograms (GC–MS, EI ionization, SIM acquisition) corresponding to $^{13}C_3$-pyrene (*m/z* 205; internal standard), $^{13}C_6$-fluoranthene (*m/z* 208; internal standard), fluoranthene and pyrene (both *m/z* 202 and 101) in a smoked tuna sample.

30–50 V), to the production of specific daughter ions, such as $[M-2H]^{+\bullet}$, $[M–C_2H_2]^{+\bullet}$, and in some cases $[M–C_4H_2]^{+\bullet}$ for fluoranthene and pyrene or $[M-2H–2H]^{+\bullet}$ ions for the heaviest PAHs. An example of fragmentation of benzo[*a*]pyrene under relatively low energy voltage of collision cell (30 V) is shown in Fig. 3. Daughter ions $[M-2H]^{+\bullet}$ (*m/z* 250), $[M–C_2H_2]^{+\bullet}$ (*m/z* 226) and $[M–C_4H_2]^{+\bullet}$ (*m/z* 202) can be observed, which allow to obtain very specific transitions under drastic conditions: the high energy voltage applied to the collision cell allows for an important fragmentation of interferences and enables an important sensitivity of the monitored transitions. The observed monitoring transitions are shown for the previous smoked tuna sample in Fig. 4. The results are tremendous in terms of S/N improvement: internal standards (fluoranthene $^{13}C_6$ and pyrene $^{13}C_3$) are intensively detected compared to the MS detection. The diagnostic absence/presence of fluoranthene and pyrene is highly facilitated, and the quantification does not suffer from the presence of any interference. The given example is transposable to all compounds. Moreover, the use of MS detection involves a difficulty to identify target analytes unambiguously, especially with regard to the criteria of the 2002/657/EC decision [36]; indeed four ions should be monitored and fit the identification criteria. When MS/MS techniques are used, two specific transitions are sufficient to guarantee reliable identification of the analytes. HRMS approach was evaluated and compared to MS/MS and single MS. For that, fish sample was purified and quantified using GC–MS, GC–MS/MS and GC–HRMS. Ions chromatograms corresponding to $^{13}C_4$-benzo[*a*]pyrene (internal standard) and benzo[*a*]pyrene in a fish sample at 0.07 $\mu g\,kg^{-1}$ of wet matter are reported in Fig. 5. At this concentration level, single MS detection was clearly insufficient to detect B*a*P: only molecular ion (*m/z* = 252)

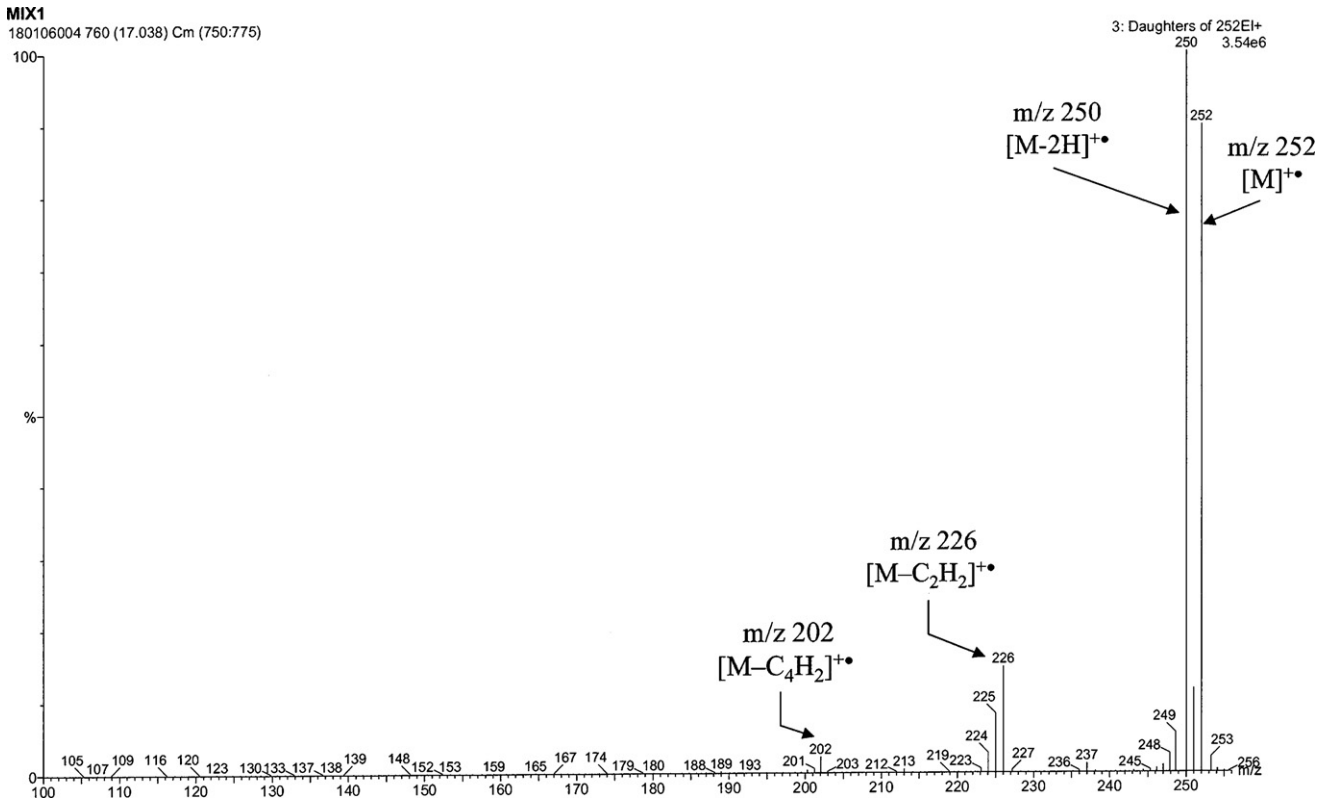


Fig. 3. Mass spectrum daughter scan [*m*/*z* 252] obtained for an injection of standard solution of benzo[*a*]pyrene.

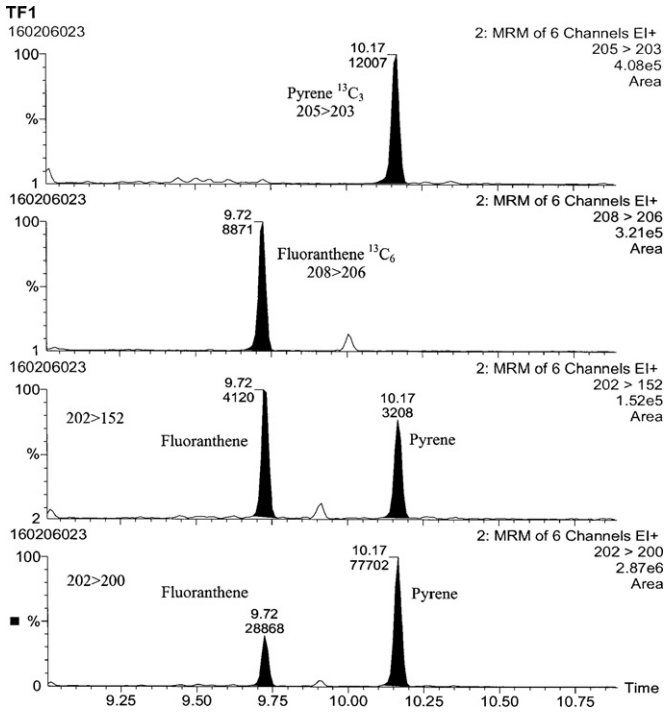


Fig. 4. Ion chromatograms (GC–MS/MS, EI ionization, SRM acquisition) corresponding to $^{13}C_3$-pyrene (*m*/*z* 205 > 203; internal standard), $^{13}C_6$-fluoranthene (208 > 206; internal standard), fluoranthene and pyrene (both 202 > 152 and 202 > 200) in a smoked tuna sample.

was detected with a signal-to-noise ratio below 3. MS/MS allowed for an unambiguous detection, as both transitions were detected with sufficient signal-to-noise ratio (respectively, 29 and 11). HRMS was comparable to MS/MS in term of chromatographic profile (S/N = 66 and 17). Indeed, PAHs do not show any mass deficiency, so that the increasing of resolution does not significantly improved the S/N. Because economically more realistic and technically more achievable, MS/MS on triple quadrupole was preferred to HRMS on a magnetic sector instrument.

3.4. Chromatographic aspects

One of the main challenges regarding PAHs analysis concerns the chromatographic separation especially because co-eluting analytes are most part of the time characterized by the same mass. Despite many attempt to separate these isomers (stationary phase, column characteristics, temperature program) some PAHs could not be separated and some critical pairs remained. This was the case of the 3 benzofluoranthenes. Separation between the three compounds was impossible by capillary GC.

Benzo[*b*]fluoranthene (B*b*F) and benzo[*j*]fluoranthene (B*j*F) were co-eluted whatever the type of column or gradient temperature used. Benzo[*k*]fluoranthene (B*k*F) could be separated in a standard mixture, but the resolution dropped in case of complex matrix analysis or when present in higher concentration. Despite the fact that it is possible to determine the concentration of B*k*F separately, it will be preferable to consider the three compounds as a single one and to give a global concentration. Moreover,

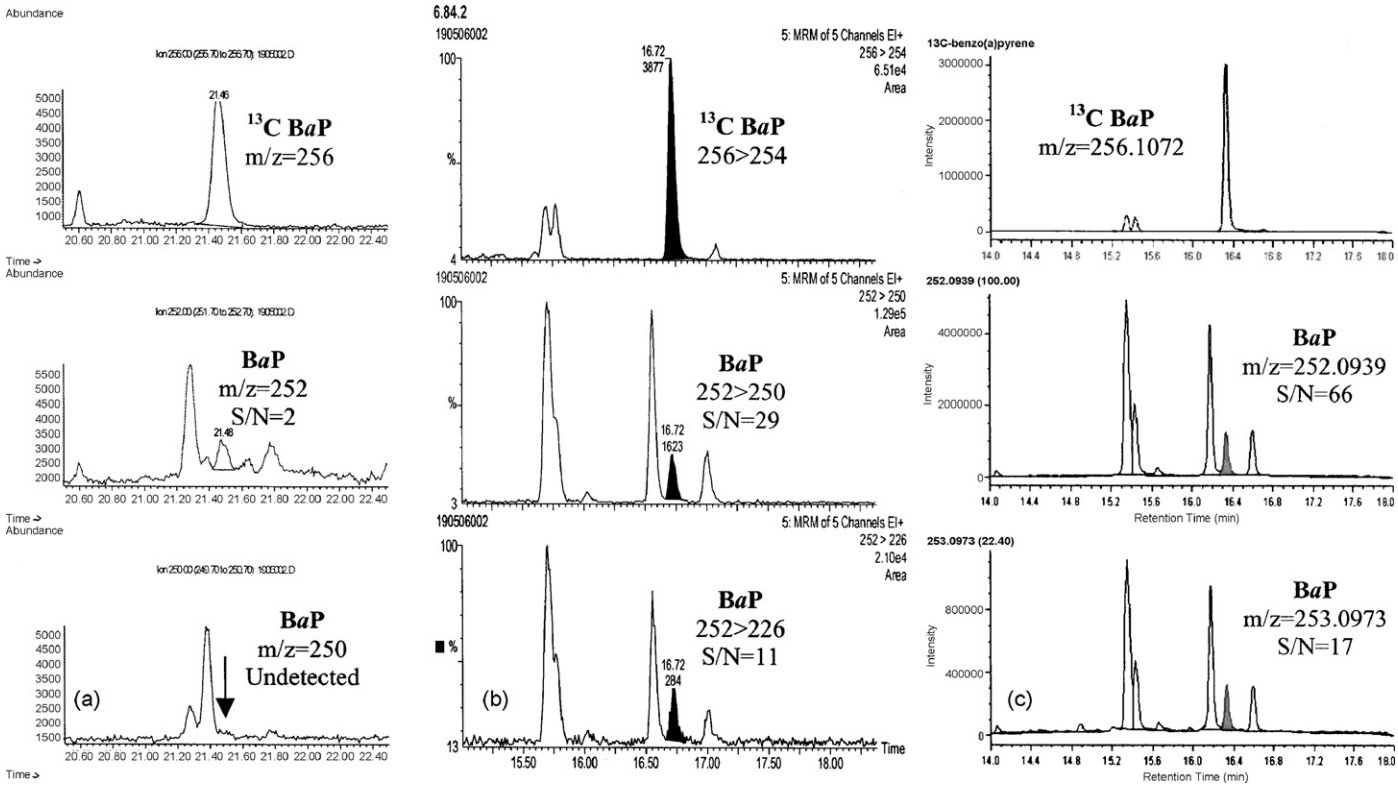


Fig. 5. Chromatograms corresponding to $^{13}C_4$-benzo[*a*]pyrene (internal standard) and benzo[*a*]pyrene in a fish sample at 0.07 μg/kg wet matter using: (a) GC–MS, (b) GC–MS/MS, (c) GC–HRMS.

these three compounds have the same toxic equivalent factor (TEF), and therefore contribute in the same proportion to the toxicological effect. It is finally relevant to quantify the sum of the three.

Another analytical challenge is the quantification of cyclopenta[*c*;*d*]pyrene (CPP). CPP could only be partially separated from benz[*a*]anthracene (BaA). Despite multiple tests, no efficient chromatographic separation was possible.

Unfortunately, after EI, CPP generates a signal at *m/z* 228 and *m/z* 226 which corresponds to the $[M]^{+\bullet}$ and $[M-2H]^{+\bullet}$ of BaA. The mass spectrum (full scan acquisition) of both compounds is shown in Fig. 6 and illustrates the presence of the two ions for the two compounds with their relative intensity.

Consequently, each area of the signal 228 > 226 and 226 > 224 corresponds to the sum of CPP and BaA. Fig. 7 illustrates this observation: co-elution was observed for the peak corresponding

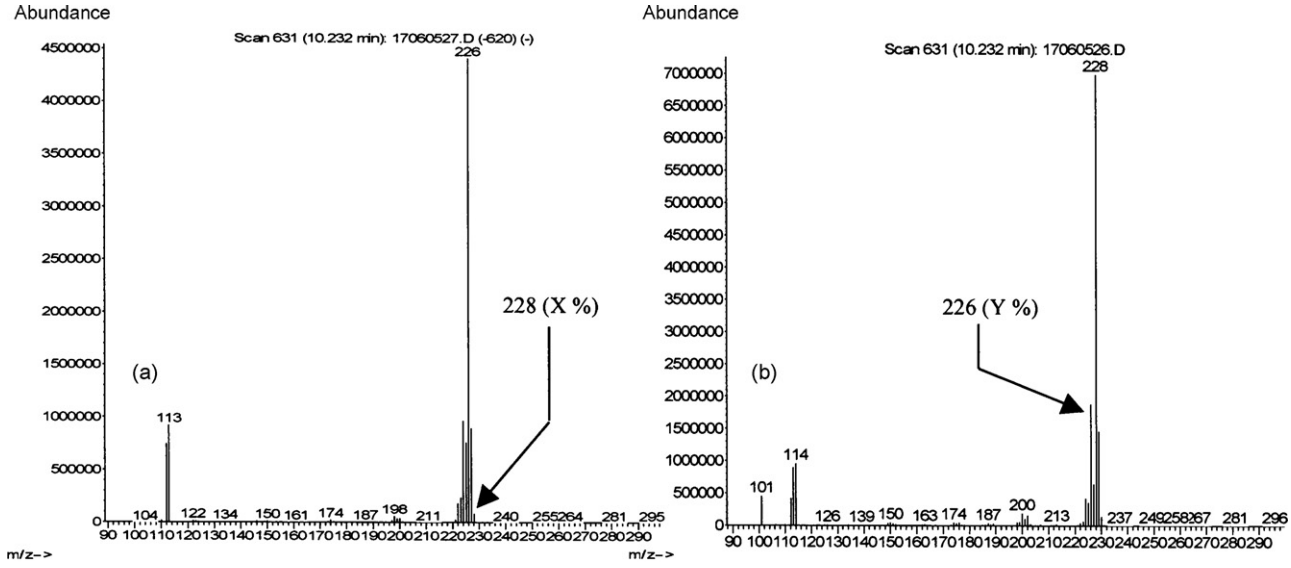


Fig. 6. Mass spectra full scan of: (a) cyclopenta[*c*,*d*]pyrene (CPP) and (b) benzo[*a*]anthracene (BaA) with relative intensity of monitoring ions.

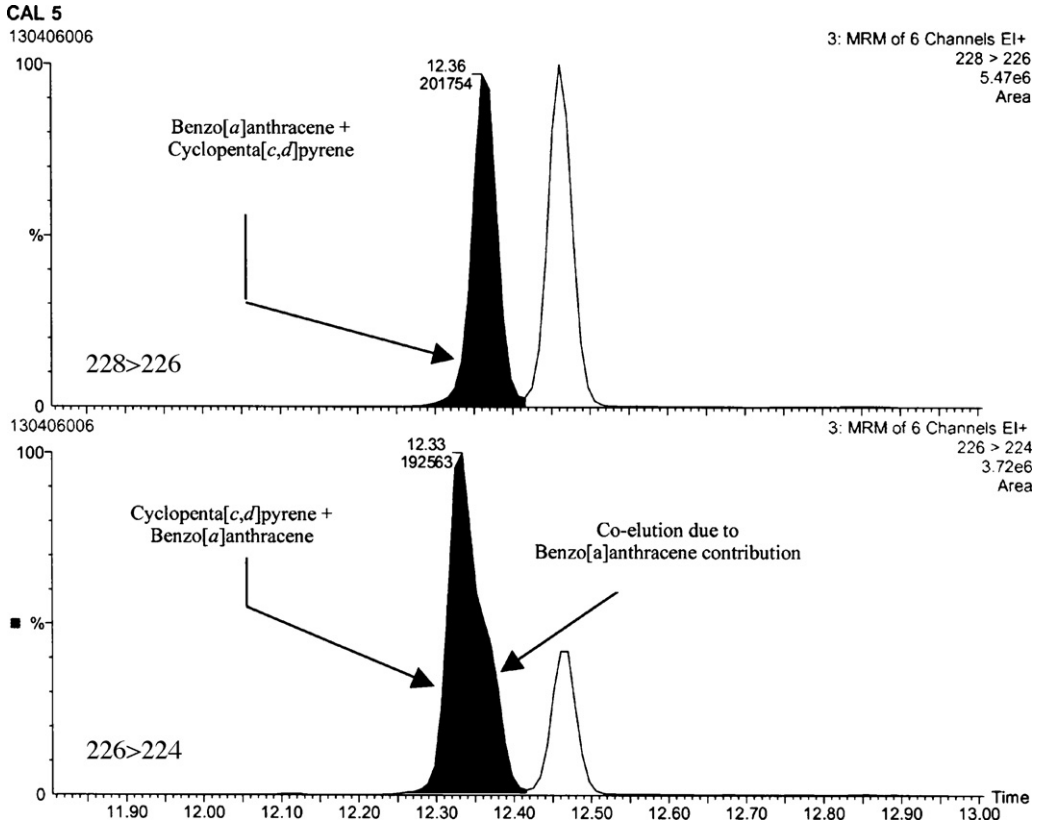


Fig. 7. GC–MS/MS chromatogram (SRM acquisition mode) of a mixture of different PAHs following 228 > 226 and 226 > 224 transitions.

to transition 226 > 224 due to the presence of both PAHs. For the transition 228 > 226, contribution of CPP is too small to be observed.

Regarding the different ratio between monitoring ions, concentrations could be estimated with this formula:

$$\text{Area}\,228 > 226 = \text{Area}\,\text{BaA}_{228>226} + \text{Area}\,\text{CPP}_{228>226}$$

$$\text{Area}\,226 > 224 = \text{Area}\,\text{BaA}_{226>224} + \text{Area}\,\text{CPP}_{226>224}$$

$$\text{Area}\,\text{CPP}_{228>226} = X\,\text{Area}\,\text{CPP}_{226>224}$$

$$\text{Area}\,\text{BaA}_{226>224} = Y\,\text{Area}\,\text{BaA}_{228>226}$$

where X and Y are the relative heights (Fig. 6).

So,

$$\text{Area}\,228 > 226 = \text{Area}\,\text{BaA}_{228>226} + X\,\text{Area}\,\text{CPP}_{226>224}$$

$$\text{Area}\,226 > 224 = Y\,\text{Area}\,\text{BaA}_{228>226} + \text{Area}\,\text{CPP}_{226>224}$$

We could deduce:

$$\text{Area}\,\text{BaA}_{228>226} = \frac{\text{Area}\,228 > 226 - X\,\text{Area}\,226 > 224}{1 - YX}$$

$$\text{Area}\,\text{CPP}_{226>224} = \frac{\text{Area}\,226 > 224 - Y\,\text{Area}\,228 > 226}{1 - YX}$$

The areas corresponding to each compound could be calculated accurately with these formulae. For the particular case of BaA and CPP, X and Y equal 2.5 and 30%, respectively. These values have obviously to be estimated on a daily basis by injection of a standard solution.

The chromatographic resolution in-between indeno[1,2,3-*c*,*d*]pyrene (IP) ($M = 276$ Da) and dibenz[*a*,*h*]anthracene (Db*ah*A) ($M = 278$ Da) is a critical point for the same reason. Nevertheless, we succeeded to separate both compounds, by ad hoc stationary phase (Zebron ZB-5MS) and optimized GC temperature program.

3.5. Optimization of the heaviest PAHs detection

Four dibenzopyrenes have to be monitored according to Commission Recommendation [5]; these four PAHs of high molecular mass do not show good chromatographic properties with most part of the time significant peak tailing and finally high limits of detection. We investigated injection pressure modes to solve this problem; constant flow selection greatly improved peak shape especially at high temperature (300 °C). Improvement in terms of S/N of heavy PAHs is shown in Fig. 8: high temperature of the insert causes a slight decrease in the lightest PAHs (retention time between 6 and 8 min) but increases the dibenzopyrenes signal significantly.

3.6. Quantitative determination

The quantitative determination was made by isotopic dilution using labelled compounds. ^{13}C PAHs have been

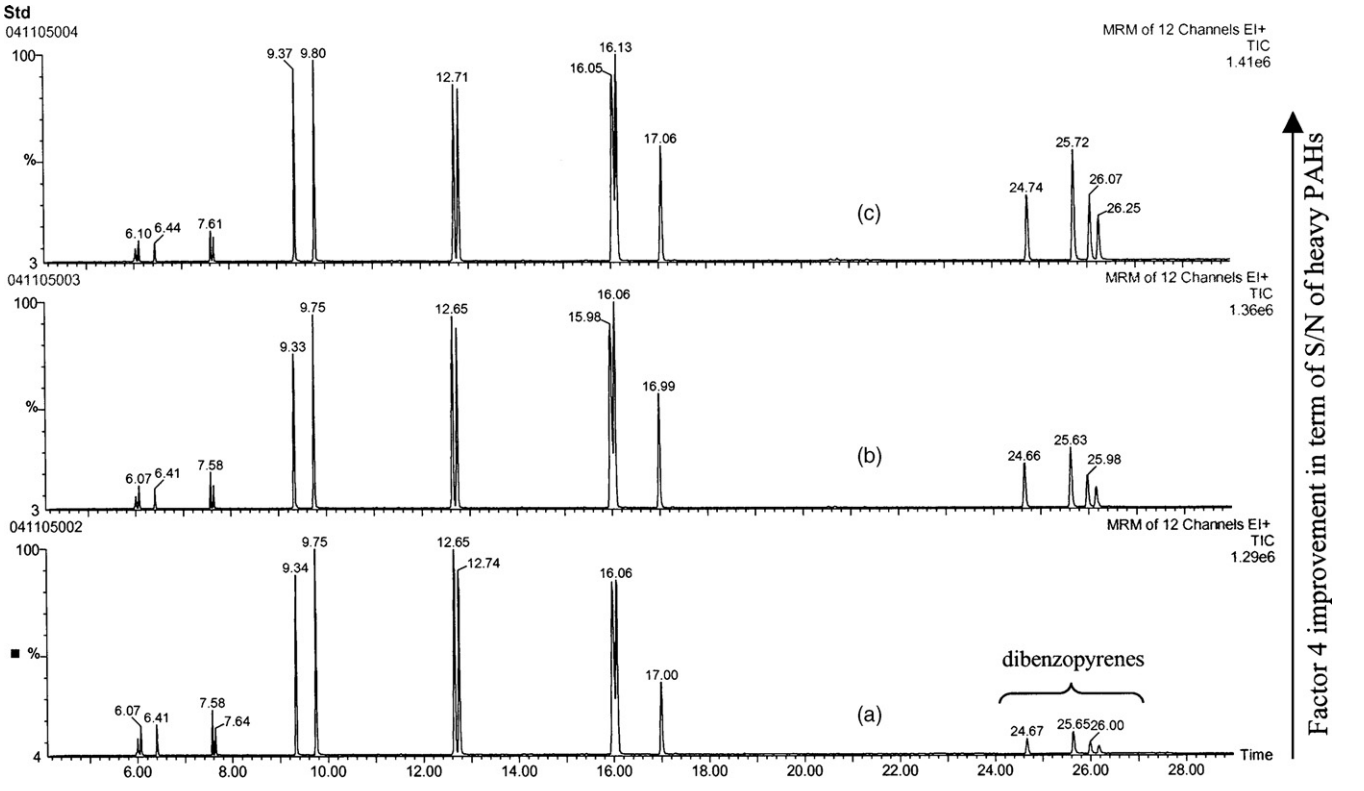


Fig. 8. GC–MS/MS chromatogram of a mixture of studied PAHs with different insert temperatures: (a) $T = 250\,^{\circ}C$, (b) $T = 280\,^{\circ}C$ and (c) $T = 300\,^{\circ}C$.

preferred to ^{2}H PAHs because they present higher stability with no risk of $^{2}H/^{1}H$ exchange. Fifteen ^{13}C-labelled PAHs are added to each sample prior the extraction. Recoveries are calculated for the labelled compounds using fluorinated PAHs as external standards. These compounds are added into the final extract just before GC–MS/MS injection.

3.7. Performances

Results of the validation are summarized in Table 3. Intermediate precision has been assessed on the basis of the quantification of the same sample 20 times by two operators. Relative standard deviations were in the range (2.9–8.4%) for most PAHs. Only anthracene has a high value due to its concentration being close to the limit of detection (LOD) of the method (and below the limit of quantification, LOQ). LODs and LOQs were estimated on the signal observed at the lowest point of the calibration curve: calculations were performed on the basis of an extrapolation at S/N = 3 for LODs and S/N = 10 for LOQs. These values were ranged from 0.01 to 0.15 $\mu g\,kg^{-1}$ and 0.02 to 0.92 $\mu g\,kg^{-1}$, respectively. These limits are fully compatible with the concentration range potentially met in food. The linearity was assessed on seven calibration levels for each analyte over the respective range of 0.01–4 $\mu g\,kg^{-1}$ of wet matter sample (equivalent to a range of approximatively 0.1–50 $\mu g\,kg^{-1}$ of dry matter, especially for food such as molluscs which contain lot of water). Coefficient correlation (R^2) was better than 0.99 for all analytes, the use of ^{13}C-PAHs greatly help the method in that sense. Recoveries were found generally in-between 30 and 70%, except for the heaviest one (dibenzopyrene). These values could appear very low for some compounds, but the use of ^{13}C-labelled for the majority of the PAHs guarantee an accurate calculated concentration inspite of the low levels of recuperation. Moreover, the sensitivity of the GC–MS/MS system used compensated the weak recovery. Robustness was tested by analyzing different types of food with the analytical procedure. Fig. 9 illustrates nine different matrices analyzed; the profile of benzo[*a*]pyrene is the same whatever the type of food (liquid, solid, oil). The trueness of the method was tested by analyzing one certified reference material (Mussel tissue SRM 2977). Results are shown in Table 4. Concentrations obtained for chrysene, benzofluoranthenes, dibenzo[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]perylene are in accordance with the certified values. Concentration found for benzo[*a*]pyrene was found significantly lower than the reference sample. The trueness in this case was 22%. The certified value could raise some questions with regard to the concentrations estimated by laboratories: benzo[*a*]pyrene is known to be light-sensitive and not fully stable in freeze-drying material. Measured concentrations reported on sample SRM 2977 in different studies published after 2005 were very similar: while we measured a concentration of 6.55 $\mu g\,kg^{-1}$, the other values were 6.70 $\mu g\,kg^{-1}$ [34], 6.50 $\mu g\,kg^{-1}$ [23] and 7.0 $\mu g\,kg^{-1}$ [32]. These values are very homogenous, even if the procedures are different for extraction (matrix solid-phase dispersion [34], PLE [23] or microwave-assisted extraction [32]), purification (permeation gel [23], silica cartridge [32] or PS-DVD cartridge) or detection (HPLC coupled to fluorescence

Table 3
Performance of the purification method—analysis of a food mixture (pool constituted with mussel, oyster, salmon, tuna, artichoke, sausages, crab, herring, prawn and winkle)

Compounds	Measured concentration ($\mu g\,kg^{-1}$)	Intermediate precision (RSD %)	Linearity (R^2)	Slope $(\mu g\,kg^{-1})^{-1}$	Intercept ($\mu g\,kg^{-1}$)	LOD ($\mu g\,kg^{-1}$ of dry matter)	LOQ ($\mu g\,kg^{-1}$ of dry matter)	Recovery (%)
Phenanthrene	1.26	4.4	0.9986	2.38	26.50	0.150	0.470	38
Anthracene	0.25	20.5	0.9999	2.64	3.10	0.150	0.920	31
Fluoranthene	0.97	3.1	1.0000	0.38	0.56	0.030	0.100	50
Pyrene	0.76	3.3	0.9972	0.08	0.38	0.046	0.155	70
Cyclopenta[*c*;*d*]pyrene	ND	–	0.9999	0.15	0.02	0.040	0.130	–
Benzo[*a*]anthracene	0.86	4.1	0.9997	0.35	0.03	0.008	0.026	67
Chrysene	0.74	8.4	0.9999	0.36	0.10	0.010	0.032	69
5-Methylchrysene	ND	–	0.9999	0.30	0.04	0.023	0.074	–
Sum of benzofluoranthenes	2.47	2.9	1.0000	0.47	0.02	0.017	0.055	68
Benzo[*a*]pyrene	0.58	8.0	0.9994	0.30	0.09	0.011	0.037	57
Indeno[1,2,3-*c*,*d*]pyrene	0.57	7.6	1.0000	0.69	0.03	0.016	0.052	41
Dibenzo[*a*,*h*]anthracene	0.18	7.3	0.9999	0.21	0.02	0.009	0.030	43
Benzo[*g*,*h*,*i*]perylene	0.57	4.5	1.0000	0.74	0.03	0.016	0.052	38
Dibenzo[*a*,*l*]pyrene	ND	–	0.9995	0.34	0.08	0.008	0.024	–
Dibenzo[*a*,*e*]pyrene	ND	–	1.0000	0.44	0.02	0.008	0.024	17
Dibenzo[*a*,*i*]pyrene	ND	–	0.9995	0.82	0.16	0.008	0.024	12
Dibenzo[*a*,*h*]pyrene	ND	–	0.9991	0.75	0.15	0.008	0.024	–

Intermediate precision observed for two operators ($n = 20$); linearity range, 0–4 $\mu g\,kg^{-1}$ of dry matter; ND, not detected.

Table 4
Measured concentrations vs. certified ones in NIST SRM 2977

Compounds	Certified reference values ($\mu g\,kg^{-1}$)	Values determined by the method ($\mu g\,kg^{-1}$)	Trueness (%)
Benzo[*a*]anthracene	20.34 ± 0.78	14.87	26.9
Chrysene	49 ± 2	51.59	3.9
Sum of the three benzofluoranthenes	19.61	20.75	5.5
Benzo[*a*]pyrene	8.35 ± 0.72	6.55	21.6
Indeno[1,2,3-*c*,*d*]pyrene	4.84 ± 0.81	3.53	27.1
Dibenzo[*a*,*h*]anthracene	1.41 ± 0.19	1.62	13.0
Benzo[*g*,*h*,*I*]perylene	9.53 ± 0.43	8.95	6.1

detection [32,34], GC–MS [23] and GC–MS/MS). These results questioned the current concentration of benzo[*a*]pyrene of the reference sample. Further trueness assessment has also been done to verify the analytical method through participation to Inter-Laboratory assay organised by the Community Reference Laboratory for PAHs in food (JRC-IRMM, Geel, Belgium) on oil samples. Results are presented in Table 5. Our values are very close to the median. To evaluate our results, we have calculated *z*-score values defined as the measured concentration minus the median divided by the standard deviation (values exceeding

Table 5
Measured concentrations, median concentration and *z*-score obtained for the inter-laboratory assay (52 laboratories) dedicated to analysis of PAH in an oil sample

Compounds	Median concentration ($\mu g\,kg^{-1}$)[a]	Measured concentration ($\mu g\,kg^{-1}$)[b]	*z*-Score
Cyclopenta[*c*;*d*]pyrene	1.1	1.4	−0.14
Benzo[*a*]anthracene	2.9	3.6	0.54
Chrysene	10.1	11.8	0.69
Sum of benzofluoranthenes	14.2	14.0	0.44
Benzo[*a*]pyrene	2.4	2.4	−0.10
Indeno[1,2,3-*c*,*d*]pyrene	6.9	6.9	0.02
Dibenzo[*a*,*h*]anthracene	2.9	2.5	−0.42
Benzo[*g*,*h*,*i*]perylene	1.6	1.8	−0.03
Dibenzo[*a*,*l*]pyrene	2.4	3.2	0.33
Dibenzo[*a*,*e*]pyrene	0.8	0.8	−0.30
Dibenzo[*a*,*i*]pyrene	6.9	8.5	0.37
Dibenzo[*a*,*h*]pyrene	0.8	1.0	−0.31

[a] Determination based on results from 51 laboratories.
[b] Determined using the described analytical method.

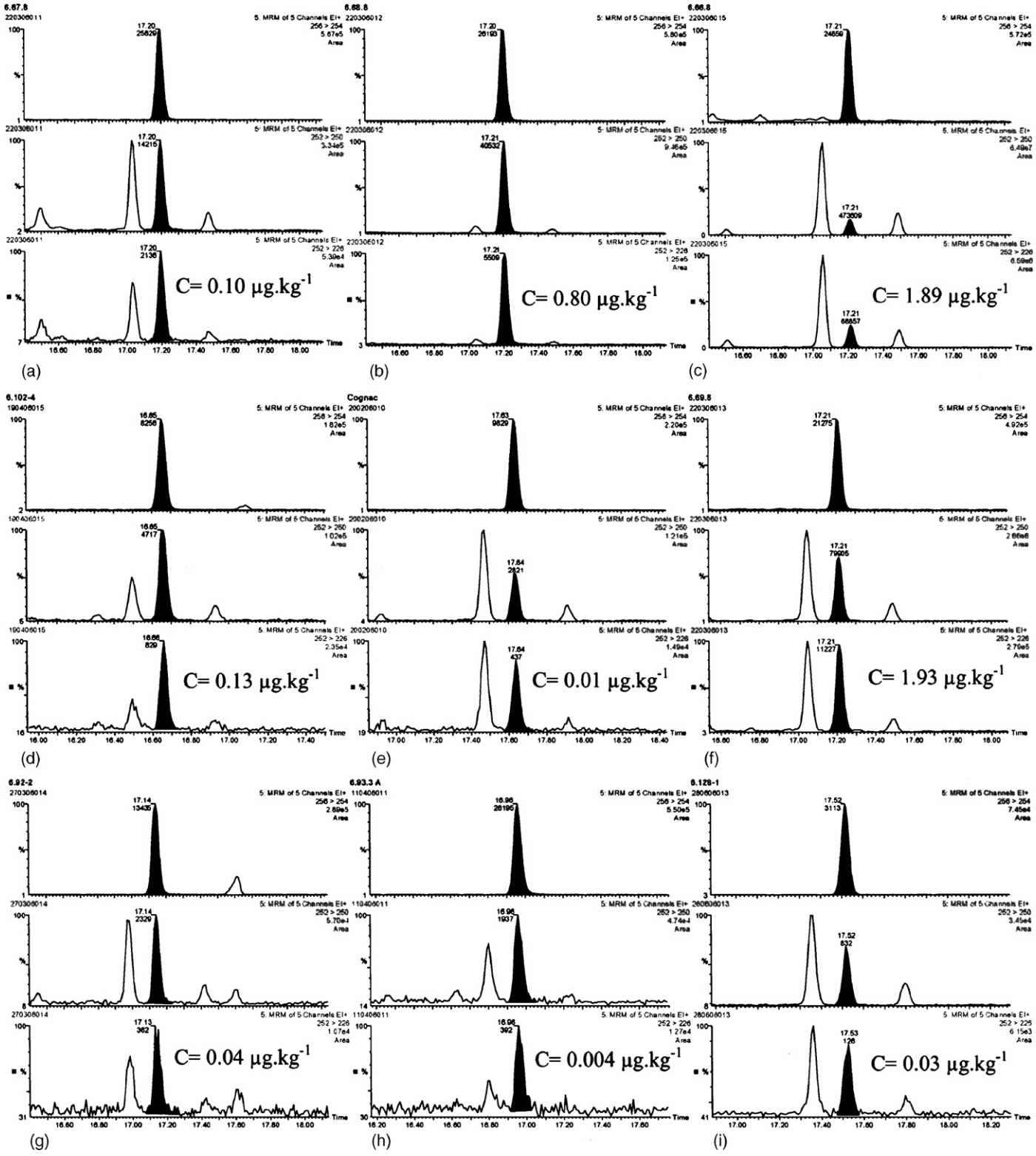


Fig. 9. Chromatographic profiles of $^{13}C_4$-benzo[*a*]pyrene (256 > 254, upper traces) and benzo[*a*]pyrene (252 > 250 and 252 > 226) with estimated concentrations (µg kg^{-1} of wet matter) in different types of food: (a) smoked salmon, (b) oil, (c) mussel, (d) duck breast, (e) cognac, (f) artichoke, (g) dried sausage, (h) wine and (i) fish.

twice the standard deviation were considered as outliers and were rejected for the median value calculation). All analytes lead to good *z*-score values. Thus, based on these results, this analytical method shows high precision for PAHs analysis.

4. Conclusions

A method for the determination of 19 PAHs (including the 15 EU priority PAHs) has been developed in food and oil sam-

ple. The analytical strategy consisted in a selective extraction using an ASE system followed by purification on polystyrene-divinylbenzene SPE. GC–MS/MS was used to measure at trace levels these analytes with a fit-for-purpose sensitivity and specificity, which authorise robust PAHs monitoring whatever the type of the tested food sample. Application on samples permitted to prove its suitability and to collect data on PAHs contamination profile in food.

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