Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1062 (2005) 247–254
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Isotope dilution determination of polycyclic aromatic hydrocarbons in olive pomace oil by gas chromatography–mass spectrometry

Gianfranco Diletti, Giampiero Scortichini*, Rossana Scarpone, Giuseppe Gatti, Luigi Torreti, Giacomo Migliorati

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy

Received 10 May 2004; received in revised form 29 October 2004; accepted 3 November 2004
Available online 2 December 2004

Abstract

A gas chromatographic (GC) method with mass spectrometry detection (MS) for the determination of eight polycyclic aromatic hydrocarbons (PAHs) in olive pomace oil has been developed. The oil was diluted with *n*-pentane and extracted by liquid–liquid partition with dimethyl sulphoxide (DMSO). After water addition and back-extraction with cyclohexane, a thin-layer chromatography on silica gel was performed as a further purification step. The PAHs spot was scraped off from the plate and the final extract was concentrated and analysed by GC–MS in full scan mode. The eight PAHs under investigation were determined in the presence of the corresponding labelled compounds added as internal standards to the sample at the beginning of the analytical process. The identified PAHs were then quantified by the isotope dilution methodology assuring the compensation of the concentration of each analyte for any variation in the sample preparation. The method precision was satisfactory with relative standard deviation (R.S.D.) values in the range 3.6–12.7% for all PAHs. The average recovery rates ranged from 69.0 to 97.5%. Accuracy was also calculated for benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene by analysing a certified reference material (CRM 458, coconut oil) with adequate results. All response curves exhibited a linear fit from 0.1 to 10 $\mu g\,ml^{-1}$ and the determination coefficients R^2 were better than 0.9942. The limits of detection (0.1–0.4 $\mu g\,kg^{-1}$) were acceptable when compared with the maximum permitted limit of 2 $\mu g\,kg^{-1}$ for each of the eight considered PAHs and 5 $\mu g\,kg^{-1}$ for the sum of the eight PAHs established by the Italian legislation. Measurement uncertainty was finally calculated identifying and quantifying the uncertainty components of the analytical process. The relative expanded uncertainties (U_c), expressed as percent values were in the range 8.5–11.4% thus appropriate for residues quantification in the range of concentrations considered in the present study.

Keywords: Polycyclic aromatic hydrocarbons; Isotope dilution; Olive oil

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds containing two or more fused aromatic rings constituted of carbon and hydrogen atoms. They are widely spread in the environment and mainly arise from incomplete combustion or pyrolysis of organic matter, from industrial processes and other human activities. Some members of this class of chemicals have been demonstrated to be genotoxic and mutagenic in experimental animals. Although it is difficult to extrapolate toxicity data from animals to humans, fifteen PAHs have been regarded as potentially genotoxic and carcinogenic to humans by the Scientific Committee on Food (SCF) of the European Union [1]. The main source of exposure to PAHs for non-smoking humans is food that can be contaminated by environmental PAHs from air, soil and water, and during processing and cooking. Due to their high lipophilic characteristics PAHs can heavily contaminate oils and fats. As a consequence these food commodities are among the major contributors to PAHs dietary intake. In particular PAHs have been observed in a range of vegetable oils such as olive, sunflower and grapeseed oils [2,3]. High PAH levels have been recently found in pomace oil due to the technological process applied to

* Corresponding author. Tel.: +39 0861 332242; fax: +39 0861 332251.

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.11.019

dry the olive oil pomace prior to the solvent extraction of the residual oil. The dry residue has been used in the heating process to dry subsequent batches with PAHs formation and oil contamination [4]. In relation to the excessive PAHs levels found in olive pomace oil, the Italian Ministry of Health has established maximum residue limits (MRL) for a list of eight PAHs in such food matrix (2 $\mu g\,kg^{-1}$ for each PAH and 5 $\mu g\,kg^{-1}$ for their sum) [5]. Several methods for PAHs determination in oils and fats have been described [2,6]. PAHs are usually extracted by liquid–liquid partition, in some case preceded by a saponification step or even by caffein complexation. Purification is performed through one or more procedures as column chromatography, thin-layer chromatography (TLC) and solid-phase extraction (SPE). The determination of PAHs is carried out by high-performance liquid chromatography (HPLC) with spectrofluorometric detection or by high-resolution gas chromatography (HRGC) coupled to flame ionisation detection (FID) or mass spectrometry (MS). The best techniques in terms of sensitivity and selectivity are represented by HPLC with fluorescence detection and HRGC–MS, capable to reach detection limits below 1 $\mu g\,kg^{-1}$. In order to check the PAHs content in pomace oil in respect to the lately introduced legal limits in Italy a suitable method was required. The aim of this work was to develop an analytical technique to reliably quantify the PAHs content in vegetable oils at levels below 1 ppb. On the basis of a preliminary evaluation of sample extraction and clean-up techniques the method described by Corradetti et al. [7] afterwards validated by Menichini et al. [8] was adopted. The instrumental analysis has been performed by HRGC coupled to MS detection introducing the isotope dilution technique for the identification and quantification of the eight PAHs taken into account. The required high recovery rates and precision and low detection limits were thus obtained as demonstrated by the validation data showed in this paper.

2. Experimental

2.1. Reference materials

The reference standards were supplied by Labor Dr. Ehrenstorfer-Schafers (Augsburg, Germany): indeno[1,2,3-*cd*]pyrene (IP), benzo[*ghi*]perylene (BghiP), benzo[*e*]pyrene (BeP), benzo[*b*]fluoranthene (BbF), benzo[*a*]anthracene (BaA), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenzo[*a*,*h*]anthracene (DBahA). The internal standards were from Cambridge Isotope Labs. (Andover, MA, USA) as solutions in isooctane containing 200 $\mu g\,ml^{-1}$ of each of the following compounds: [$^2H_{12}$]indeno[1,2,3,-*cd*]pyrene ([$^2H_{12}$]-IP), [$^2H_{12}$]benzo[*ghi*]perylene ([$^2H_{12}$]-BghiP), [$^2H_{12}$]benzo[*e*]pyrene ([$^2H_{12}$]-BeP), [$^2H_{12}$]benzo[*b*]fluoranthene ([$^2H_{12}$]-BbF), [$^2H_{12}$]benzo[*a*]anthracene ([$^2H_{12}$]-BaA), [$^2H_{12}$]benzo[*k*]fluoranthene ([$^2H_{12}$]-BkF), [$^2H_{12}$]benzo[*a*]pyrene ([$^2H_{12}$]-BaP) and [$^2H_{14}$]dibenzo[*a*,*h*]anthracene ([$^2H_{14}$]-DBahA). The compound [$^2H_{10}$]fluoranthene ([$^2H_{10}$]-F) at 100 $\mu g\,ml^{-1}$ in acetonitrile (Labor Dr. Ehrenstorfer-Schafers) was used as recovery standard in order to calculate the internal standards recovery rates. Two certified reference materials were also used: PAHs in coconut oil CRM 458 and CRM 459 were purchased from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). CRM 458 contained BkF ($1.87 \pm 0.18\,\mu g\,kg^{-1}$), BaP ($0.93 \pm 0.09\,\mu g\,kg^{-1}$), BghiP ($0.97 \pm 0.07\,\mu g\,kg^{-1}$) and IP ($1.00 \pm 0.07\,\mu g\,kg^{-1}$). CRM 459 was negative for the same four PAHs (BkF < 0.2 $\mu g\,kg^{-1}$, BaP < 0.3 $\mu g\,kg^{-1}$, BghiP < 0.2 $\mu g\,kg^{-1}$ and IP < 0.2 $\mu g\,kg^{-1}$). Perfluorobutylamine (FC43) was obtained from Fluka (Buchs, Switzerland) and used for the mass scale calibration of the detector.

2.2. Solvents, reagents and other materials

Solvents used for the analytical procedure including cyclohexane, hexane, toluene, dichloromethane were for organic residue analysis quality, while *n*-pentane was HPLC grade and dimethyl sulphoxide (DMSO) was UV-spectroscopy grade. All solvents were supplied from J.T. Baker (Deventer, The Netherlands). Anhydrous sodium sulphate was from Carlo Erba (Milan, Italy). TLC silica gel plates (20 cm × 20 cm, thickness 1 mm) were from Merck (Darmstadt, Germany). GFA filters were purchased from Whatman (Clifton, NJ, USA).

2.3. Standards preparation

Labelled PAHs internal standards: from primary standard solutions at 200 $\mu g\,ml^{-1}$ of single PAHs in isooctane, two mixture solutions were prepared containing 20 and 4 $\mu g\,ml^{-1}$ in toluene of each PAHs, respectively. Labelled recovery standard: [$^2H_{10}$]-F solution at 100 $\mu g\,ml^{-1}$ in acetonitrile was diluted to 1 $\mu g\,ml^{-1}$ in toluene. Native PAHs: stock standard solutions at 1000 $\mu g\,ml^{-1}$ in toluene were prepared and then a mixture containing the eight PAHs with a concentration of 1 $\mu g\,ml^{-1}$ in toluene was obtained. Seven GC–MS calibration solutions were prepared from 20 $\mu g\,ml^{-1}$ labelled PAHs standard solutions and 1 $\mu g\,ml^{-1}$ native PAHs standard solution and their concentrations were as follows: 0.1–0.2–0.4–0.8–1.2–2.0–10 $\mu g\,ml^{-1}$ for native PAHs, 1 $\mu g\,ml^{-1}$ for labelled PAHs in all solutions.

2.4. Sample preparation

The oil specimens were treated according to Corradetti et al. [7] with some minor changes. The oil was homogenised by manual shaking. A 10 g aliquot of sample was weighed in a glass vial and spiked with 5 μl of the labelled 4 $\mu g\,ml^{-1}$ PAHs solution. The sample was diluted in 10 ml of *n*-pentane and quantitatively transferred to a 100 ml separatory funnel washing the glass vial with 10 + 5 ml of *n*-pentane. Then 15 ml of DMSO were added and the separatory funnel was

shaken for 1 min. The lower DMSO phase was decanted and transferred to a 250 ml separatory funnel. The extraction was repeated two more times with 10 ml of DMSO. The combined DMSO extracts were added of 70 ml of water and PAHs were back-extracted three times with 50 ml of cyclohexane. The combined cyclohexane extracts were washed with 100 ml of water, drained through a filter funnel containing 5 g of anhydrous sodium sulphate and concentrated using a rotary evaporator with a water bath temperature of 40 °C to approximately 50 μl and subjected to a TLC clean up. The TLC separation was performed onto a silica gel plate 20 cm × 20 cm, 1 mm thickness. The TLC plate was developed with toluene–hexane (1:1, v/v) to a height of 11 cm. After solvent evaporation under fume hood the plate was observed with 254 nm UV light in order to detect the PAHs spot (R_F about 0.8). The silica gel spot was then scraped off and transferred onto a funnel containing a GFA filter. PAHs were eluted from silica gel by washing with 4 × 4 ml of methylene chloride and concentrated to about 100 μl at 40 °C under nitrogen stream. Finally the extract was transferred to a 2 ml conical vial, evaporated to dryness under nitrogen stream and immediately dissolved in 20 μl of [$^2H_{10}$]-F standard solution at 1 μg ml^{-1} in toluene.

2.5. GC–MS analysis

PAHs identification and quantification were performed by using a GC–MS system consisted of a gas chromatograph Trace GC series 2000 (ThermoQuest, Milan, Italy), equipped with a split–splitless injector and an autosampler AS2000. The GC was coupled to an ion trap mass detector Polaris Q (Finnigan, Bremen, Germany). The GC–MS analyses were performed on a capillary column DB-5MS, 30 m × 0.25 mm × 0.2 μm (J&W Scientific, Folsom, CA, USA) with the following oven temperature programme: 98 °C for 1 min, 20 °C min^{-1} to 265 °C, 265 °C for 0.1 min, 1 °C min^{-1} to 310 °C, 310 °C for 1 min, 1 °C min^{-1} to 320 °C, 320 °C for 5 min. Helium was used as carrier gas at 1 ml min^{-1} flow rate. Injections were made in the splitless mode (injection volume 1 μl, splitless time 0.6 min) and the injector temperature was set at 260 °C. The mass spectrometer was operated in the full scan mode by monitoring signals in the mass range 50–450 *m/z*. The ion source temperature was 220 °C, while the FC43 was used for spectrometer mass calibration.

2.6. Validation tests

Ten blank samples consisted of olive pomace oil were assayed and chromatograms inspected for peaks that might correspond to one or more of the PAHs under investigation. Eighteen olive pomace oil samples were spiked with 2, 5 and 20 μg kg^{-1} of native PAHs (six replicates at each spiking level) and 20 ng of each of the corresponding labelled compounds (internal standards). Moreover, two certified reference materials were analysed: CRM 458 contained BkF, BaP, BghiP and IP, while CRM 459 was negative for the same four PAHs. Standard solutions containing from 0.1 to 10 μg ml^{-1} of each PAHs, 1 μg ml^{-1} of the corresponding labelled standards and 1 μg ml^{-1} of [$^2H_{10}$]-F (recovery standard) were injected to calibrate the GC–MS system. The following parameters were studied on the basis of the obtained results: the detection limit (LOD), the linearity of response, the intra-day precision (repeatability) and the accuracy. The concentrations of the native PAHs added to the blanks prior to extraction were calculated from the native PAHs response factors relative to their appropriate labelled standards, starting from the assumption that the isotopic compound resembles the analytical behaviour of the corresponding native compound. The labelled PAHs recovery rates were calculated from their response factors relative to the recovery standard added prior to the GC injection, in order to compensate the recoveries for an eventual injection error and matrix effects.

In order to meet the ISO/IEC 17025 requirements the measurement uncertainty was also estimated according to the EURACHEM/CITAC guidelines [9,10].

3. Results and discussion

The aim of this work was to develop a reliable method for quantifying and confirming eight PAH (IP, BghiP, BeP, BbF, BaA, BkF, BaP and DBahA) in olive pomace oil since legal limits have been fixed in Italy for these compounds in such vegetable oil. The analytical method was thus validated at concentrations corresponding to the MRL (2 μg kg^{-1} for each PAHs) and above the MRL (2.5 and 10 times the MRL), considering the high PAH concentrations found in olive pomace oil produced in Italy and other European countries in recent years [4].

3.1. Extraction and clean up

PAHs are known to be light-sensitive. Consequently all operation in samples and standard preparation were conducted minimising the PAHs light exposure. The sample pretreatment method was chosen on the basis of the required LODs for the investigated PAHs in pomace oil, in relationship to the selected instrumental analysis. With this aim, it was necessary to start the analysis from at least 10 g of sample and to dissolve the final extract in 20 μl of solvent because of the injection volume in GC–MS is limited to 1–2 μl, by using the splitless injection mode. Alternative SPE clean up procedures were taken into account, considering their advantages in terms of speed of analysis and reduced solvent consumption but they were not compatible with our analytical scheme, because of the limitation in the amount of fat that can be loaded onto a SPE cartridge [11]. The liquid–liquid partitioning process was then chosen among the PAHs extraction methods from oils and fats described in the literature since it was recognised as the best procedure in comparison to caffeine complexation and saponification when followed by SPE

Table 1
Recovery data for the labelled PAHs added to samples prior to the analyses (spiking level 2 μg kg^{-1}, $n = 18$)

Labelled PAH	Average recovery (%)	Standard deviation	Relative standard deviation (%)
[$^2H_{12}$]Benzo[*a*]anthracene	77.3	8.5	11.0
[$^2H_{12}$]Benzo[*b*]fluoranthene	77.3	12.3	15.9
[$^2H_{12}$]Benzo[*k*]fluoranthene	79.0	10.5	13.3
[$^2H_{12}$]Benzo[*e*]pyrene	72.3	11.5	16.0
[$^2H_{12}$]Benzo[*a*]pyrene	76.4	12.4	16.2
[$^2H_{12}$]Indeno[1,2,3-*cd*]pyrene	64.8	13.1	20.2
[$^2H_{12}$]Benzo[*ghi*]perylene	67.8	14.2	21.0
[$^2H_{14}$]Dibenzo[*a*,*h*]anthracene	65.5	11.5	17.5

or column chromatography [12,13]. Oil samples were diluted with an organic solvent and partitioned in DMSO in order to selectively extract PAHs from most of the lipidic matter that mainly consists of triglycerides. The DMSO extract was then diluted with water and the subsequent variation of the PAHs coefficients of partition allowed the back-extraction with cyclohexane. Owing to the cyclohexane low boiling point, it was possible to achieve a quick concentration of the extract in rotary evaporator at 40 °C. This procedure allows reducing the mass of residue to a 10% of the initial value [6]. The following TLC purification step was an efficient tool in order to separate the PAHs fraction from the co-extracted substances. The PAHs spot was easily identified onto the TLC plate under 254 nm UV light for the intense fluorescence due to the presence of the labelled PAHs.

With regard of sample preparation complexity the use of isotopically labelled standards permitted the compensation of the analytes concentrations for any variation in the sample preparation that was closely reflected by their reference compounds. This choice was appropriate as demonstrated by the variability in the recovery percentages of the PAHs labelled standards added to pomace oil at 2 μg kg^{-1} (Table 1).

3.2. Instrumental analysis

The identification of PAHs by GC–MS was obtained by recording the mass spectra in full scan mode with computer-aided library searching and on the chromatographic retention time of the PAHs. Single ion monitoring (SIM) acquisition mode was also performed without a significant sensitivity improvement in comparison with the full scan mode, while the evaluation of MS–MS capabilities of the ion-trap were not satisfactory in term of repeatability of quantitative response.

The ratio between the peak height of the molecular ion (base peak) and of a characteristic fragment (confirmatory ion) was measured: the ion abundance ratio calculated in negative samples spiked at 2 μg kg^{-1} and in the calibration solutions are reported in Table 2. The obtained results demonstrate the good reproducibility of the ion ratio measurements and the agreement between the ion ratio values in the cali-

Table 2
PAHs selected diagnostic ions and reproducibility of ion ratio measurements

PAH	Diagnostic ions (*m*/*z*)		Ions relative abundances[a]	
	Quantification ion	Confirmation ion	Samples[b], mean ± R.S.D. (%)	Standards[c], mean ± R.S.D. (%)
Benzo[*a*]anthracene	228	226	35 ± 2.7	31 ± 2.4
Benzo[*b*]fluoranthene	252	250	29 ± 6.2	27 ± 7.6
Benzo[*k*]fluoranthene	252	250	27 ± 4.2	28 ± 8.9
Benzo[*e*]pyrene	252	250	35 ± 9.9	36 ± 13.1
Benzo[*a*]pyrene	252	250	29 ± 8.2	29 ± 8.9
Indeno[1,2,3-*cd*]pyrene	276	277	26 ± 3.8	25 ± 2.6
Benzo[*ghi*]perylene	276	277	25 ± 5.1	27 ± 12.4
Dibenzo[*a*,*h*]anthracene	278	279	26 ± 8.1	25 ± 3.8
[$^2H_{12}$]Benzo[*a*]anthracene	240	–	–	–
[$^2H_{12}$]Benzo[*b*]fluoranthene	264	–	–	–
[$^2H_{12}$]Benzo[*k*]fluoranthene	264	–	–	–
[$^2H_{12}$]Benzo[*e*]pyrene	264	–	–	–
[$^2H_{12}$]Benzo[*a*]pyrene	264	–	–	–
[$^2H_{12}$]Indeno[1,2,3-*cd*]pyrene	288	–	–	–
[$^2H_{12}$]Benzo[*ghi*]perylene	288	–	–	–
[$^2H_{14}$]Dibenzo[*a*,*h*]anthracene	292	–	–	–
[$^2H_{10}$]Fluoranthene	212	–	–	–

[a] 100 × (peak height confirmation ion/peak height quantification ion).
[b] Blank samples spiked at 2 μg kg^{-1}, $n = 6$.
[c] Standards from calibration curve (0.1–10 μg ml^{-1}, $n = 7$).

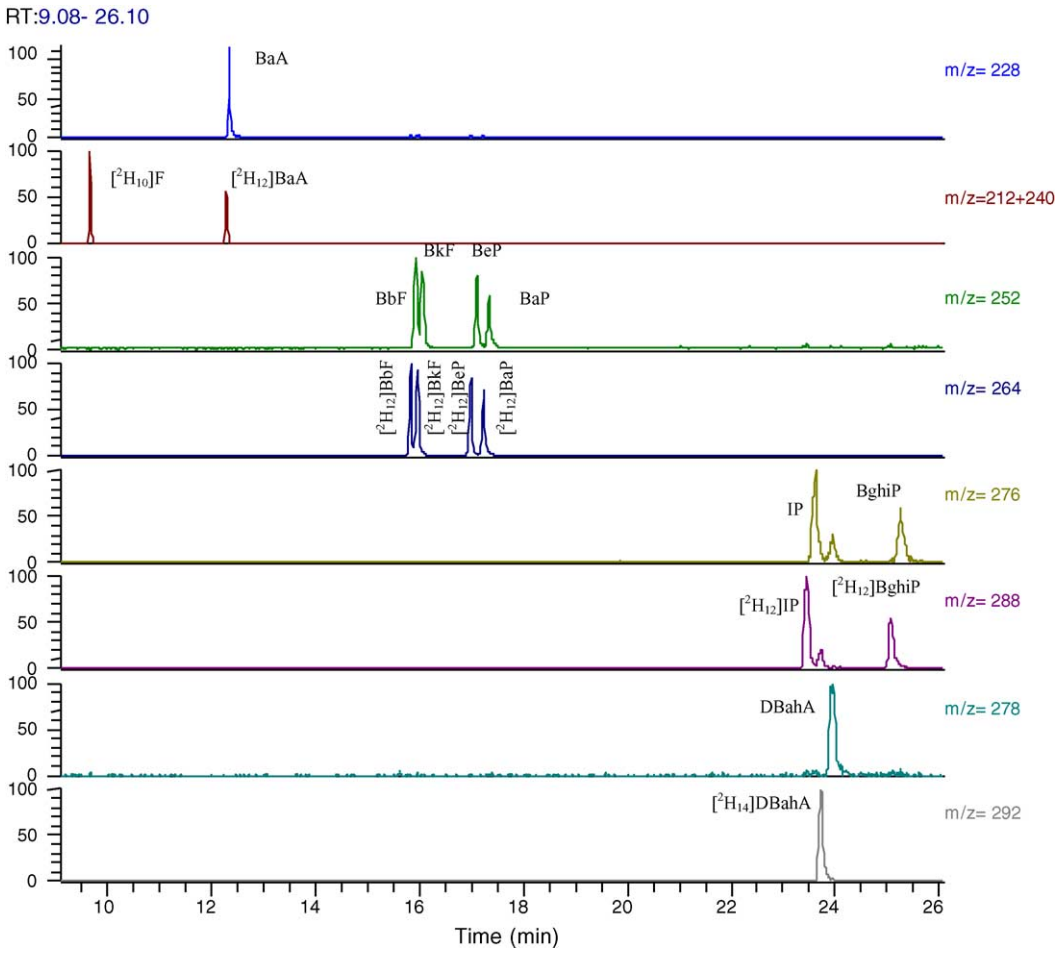

Fig. 1. Standard solution containing 0.2 μg ml^{-1} of native PAHs and 1 μg ml^{-1} of labelled PAHs (RT: retention time in minutes).

bration solutions and in the samples at the lowest fortification level. PAHs quantification was accomplished by measuring the peak height of the molecular ions since they are the most abundant ions due to the weak fragmentation of PAHs and this enhanced the sensitivity of the method. The calibration data were response factors (calculated dividing the target analyte peak height by the corresponding isotopic standard peak height) versus the concentration expressed in μg ml^{-1} of the PAH. The peak areas were also calculated but, in general terms, the repeatability was worse than that obtained from peak height measurement. This could be due to the fact that peak height measurement is much less influenced by the integration parameters, in particular at concentrations near the LODs. Typical chromatograms for a standard solution, a blank sample and a spiked sample at 2 μg kg^{-1} are presented in Figs. 1–3.

3.3. Validation

The specificity study performed on olive pomace oil blank samples showed no significant peaks interfering with the target analytes, even though the presence of other peaks was noted, in particular near the peak of BaA. The most abundant among these peaks was identified as chrysene. The proposed method demonstrated its suitability for quantitative analysis of chrysene in olive oil in several FAPAS proficiency tests. In this regard, chrysene deuterated reference standard was used in the isotope dilution analysis. No further investigations were undertaken about other peaks observed in the chromatograms of samples, since the aim of this work was to determine the eight PAHs for which a tolerance limit was established.

The analysis of a blank certified reference material gave results in compliance with the certified values even though it

Table 3
PAHs precision and recovery data obtained from the analysis of pomace oil blank samples spiked at 2–5–20 μg kg^{-1} (n = 6 for each spiking level)[a]

PAHs (μg kg^{-1})	BaA	BbF	BkF	BeP	BaP	IP	BghiP	DBahA
2	94.1 ± 4.5	87.5 ± 7.8	78.3 ± 4.8	95.5 ± 4.2	78.8 ± 5.8	94.4 ± 7.9	97.5 ± 11.2	97.0 ± 6.2
5	74.5 ± 10.1	77.5 ± 3.6	73.3 ± 4.2	87.7 ± 3.9	74.0 ± 11.9	88.2 ± 8.5	91.0 ± 15.5	88.9 ± 13.0
20	69.0 ± 9.4	72.1 ± 10.4	71.8 ± 9.8	80.9 ± 8.3	78.1 ± 9.4	88.7 ± 11.0	86.4 ± 7.4	91.8 ± 12.7

[a] Each value is mean recovery (%) ± relative standard deviation (%).

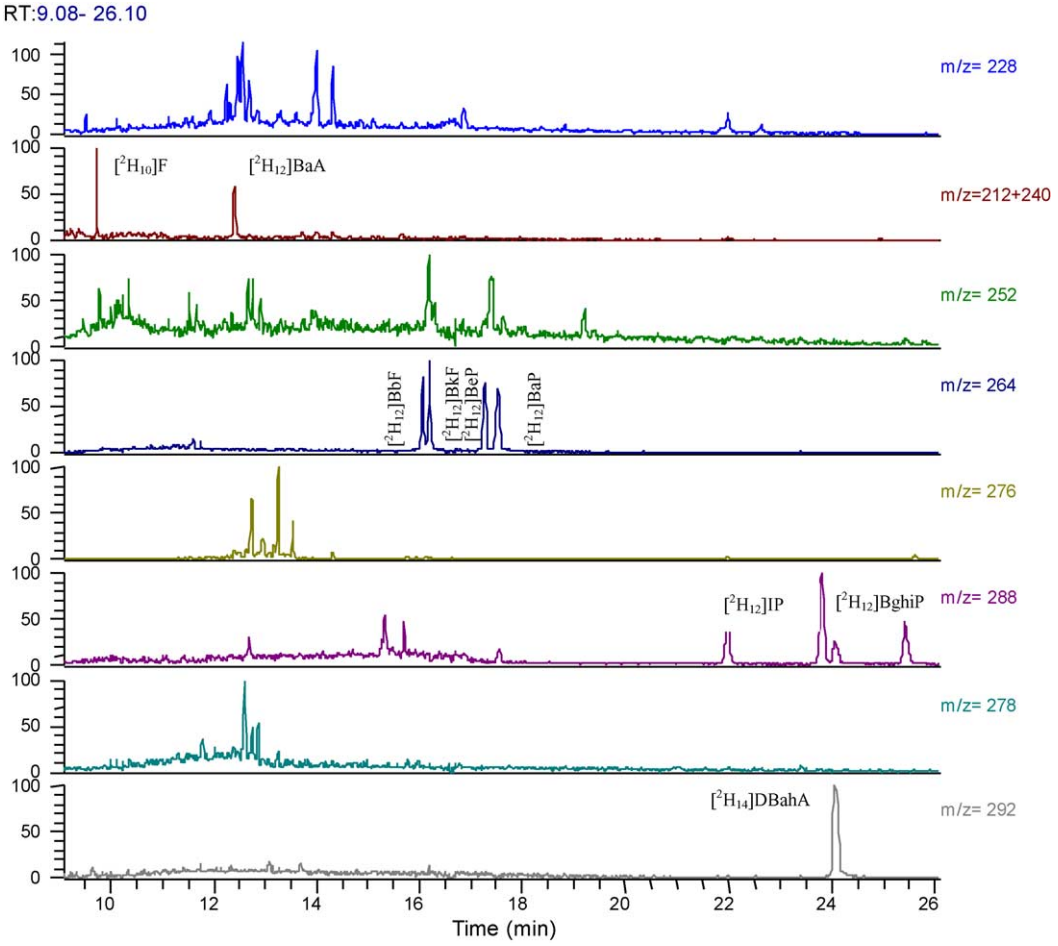

Fig. 2. Pomace oil blank sample.

was constituted of a different vegetable oil (coconut oil) from the one under examination. Precision and accuracy data are shown in Table 3. The precision of the method was evaluated performing a repeatability experiment and it is expressed as the relative standard deviation (R.S.D.) obtained for each analyte. The R.S.D. values are to be considered acceptable being in the range 3.6–12.7%. The mean recovery values of the substances under investigation achieved from the repeatabil-

Table 4
PAHs accuracy data obtained from analysis (five replicates) of a certified reference material (CRM 458, coconut oil)

PAH	Concentration found (μg kg^{-1})						Certified value (μ)g kg^{-1})	Accuracy (%)
	First	Second	Third	Fourth	Fifth	Average		
Benzo[*k*]fluoranthene	1.72	1.90	2.03	1.95	1.88	1.90	1.87 ± 0.18	101.6
Benzo[*a*]pyrene	0.88	0.90	0.94	1.05	0.78	0.91	0.93 ± 0.09	97.8
Indeno[1,2,3-*cd*]pyrene	0.90	1.00	1.00	1.02	0.91	0.97	1.00 ± 0.07	97.0
Benzo[*ghi*]perylene	1.00	1.09	1.15	1.23	0.87	1.07	0.97 ± 0.07	110.3

Table 5
Results of the regression analysis of the data of the calibration graphs ($n = 8$)

PAH	Slope	Standard deviation slope	Intercept	Standard deviation intercept	R^2
Benzo[*a*]anthracene	1.922	0.014	61.9	51.4	0.9997
Benzo[*b*]fluoranthene	0.979	0.031	206.7	11.7	0.9942
Benzo[*k*]fluoranthene	0.982	0.020	134.3	72.9	0.9975
Benzo[*e*]pyrene	1.181	0.004	1.2	15.9	0.9999
Benzo[*a*]pyrene	1.096	0.012	54.7	42.3	0.9993
Indeno[1,2,3-*cd*]pyrene	1.148	0.010	14.9	37.8	0.9995
Benzo[*ghi*]perylene	1.571	0.012	−73.3	43.3	0.9997
Dibenzo[*a*,*h*]anthracene	1.012	0.015	90.2	55.2	0.9987

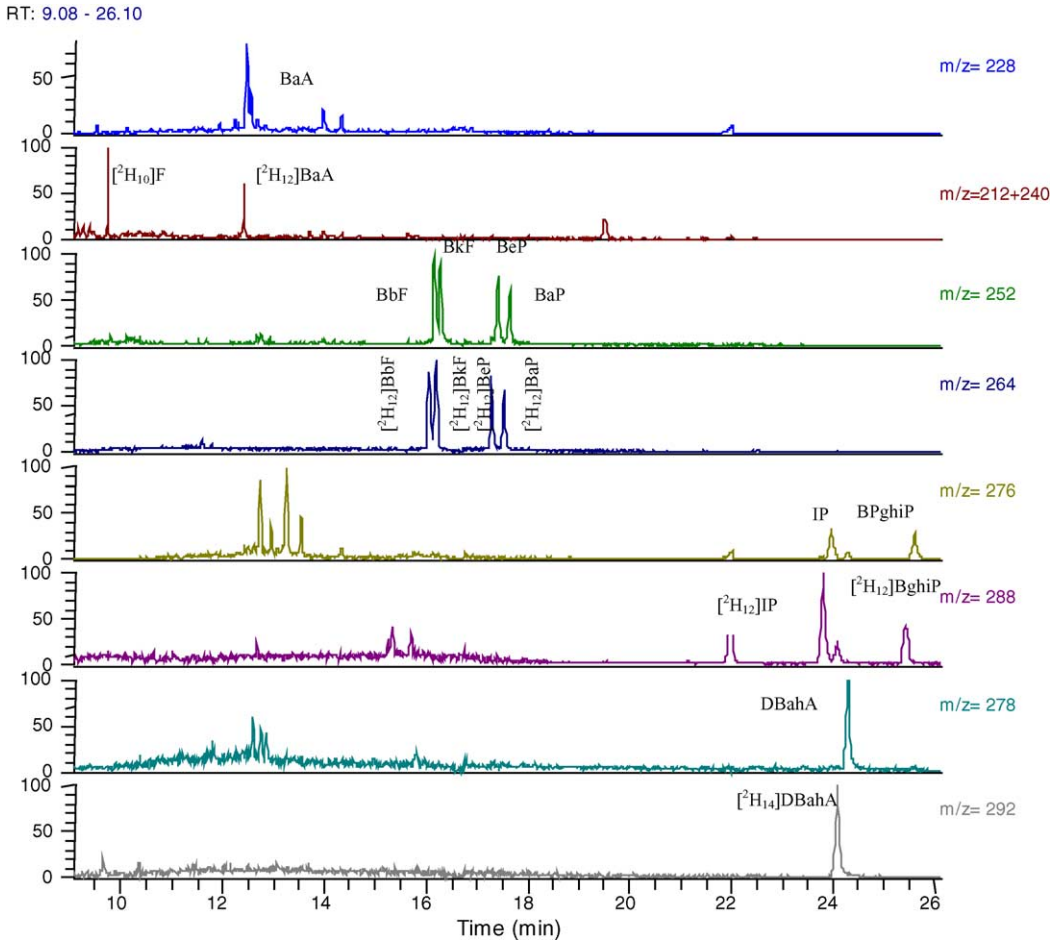

Fig. 3. Pomace oil blank sample spiked with 2 μg kg^{-1} of PAHs mixture.

ity test permitted to estimate the accuracy of the method that was satisfactory (recovery rates ranging from 69.0 to 97.5%). Accuracy was also calculated analysing a certified reference material (CRM 458, coconut oil). The accuracy evaluation was limited to BkF, BaP, IP and BghiP while the other four target PAHs were not present in the CRM 458. The results presented in Table 4 were fully satisfactory for BkF, BaP and IP while an overestimation was obtained for BghiP (110.3% in comparison to the certified value). The BghiP bias has been likely due to a chromatographic separation problem. BghiP is the last eluted PAH and the observed peak tailing may be responsible for an inaccuracy in the peak integration. Nevertheless, the trueness of measurement for BghiP was within the guideline ranges defined by the European Commission Decision 2002/657/EC [14]. Furthermore method performance was checked by participation in proficiency tests (FAPAS® UK Series 6-Round 15-17/2003) whose results have been found within ±1 *z*-score from the reference consensus values for all the considered PAH. All response curves exhibited a linear fit from 0.1 to 10 μg ml^{-1} and the determination coef-

Table 6
PAHs measurement uncertainty quantification[a] (probability level $p = 0.05$; coverage factor $k = 2$)

Parameter	BaA	BbF	BkF	BeP	BaP	IP	BghiP	DBahA
Rep[b]	0.0448	0.0322	0.0217	0.0279	0.0239	0.0251	0.0375	0.0265
Cal[c]	0.0051	0.0220	0.0143	0.0023	0.0070	0.0053	0.0043	0.0088
u_c[d]	0.0569	0.0522	0.0433	0.0446	0.0427	0.0431	0.0515	0.0446
U_c (%)[e]	11.4	10.4	8.7	8.9	8.5	8.6	10.3	8.9

[a] Relative standard uncertainty values for mass (0.0244), volume (0.0244) and reference standards purity (0.0029) were included in the combined standard uncertainty calculation but they are not reported in the table since they have the same values for all the target PAHs.
[b] Repeatability relative standard uncertainty.
[c] Calibration curve relative standard uncertainty.
[d] Relative combined standard uncertainty.
[e] Percent relative expanded uncertainty.

ficients R^2 were better than 0.9942. The results of the regression analysis of the data are summarised in Table 5. The LODs were calculated at a signal-to-noise ratio ≥3: 0.1 μg kg^{-1} for DBahA, 0.2 μg kg^{-1} for BaA, BbF, BkF, BeP, BaP and IP, 0.4 μg kg^{-1} for BghiP. The obtained LOD values were fit for the purpose taking into account the maximum permitted limit of 2 μg kg^{-1} for each considered PAHs and 5 μg kg^{-1} for the sum of the eight PAHs established by the Italian legislation. Measurement uncertainty quantification was accomplished following the EURACHEM/CITAC guidelines [10]. The uncertainty components associated with the potential sources of uncertainty identified in the analytical process were quantified and then combined to calculate the relative expanded uncertainty (U_c), expressed as percent value. The %U_c values ($p = 0.05$; $k = 2$) for PAHs were in the range 8.5–11.4% (Table 6) and they are appropriate for residues quantification in the range of concentrations considered in the present study.

References

[1] European Union Health and Consumer Protection Directorate General, Opinion of the Scientific Committee on Food on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food, 2002; http://europa.eu.int/comm/food/fs/sc/scf/index_en.html.
[2] W. Moreda, M.C. Perez-Camino, A. Cert, J. Chromatogr. A 936 (2001) 159.
[3] S. Moret, A. Dudine, L.S. Conte, J. Am. Oil Chem. Soc. 77 (2000) 1289.
[4] European Union Health and Consumer Protection Directorate General, Final report on a mission carried out in Italy from 27 to 31 May 2002 in order to assess the control measures in place for vegetable oil production and in particular for the assessment of controls on PAH (polycyclic aromatic hydrocarbons) contamination of such oils, DG (SANCO)/8602/2002, MR final.
[5] Italian Regulation, O.M. 18 September 2001, Off. J. No. 225 (2001) 17.
[6] S. Moret, L.S. Conte, J. Chromatogr. A 882 (2000) 245.
[7] E. Corradetti, C. Abbondanza, L. Mazzanti, G. Poli, Boll. Chim. Igien. 39 (1988) 297.
[8] E. Menichini, A. Di Domenico, L. Bonanni, E. Corradetti, L. Mazzanti, G. Zucchetti, J. Chromatogr. 555 (1991) 211.
[9] ISO/IEC 17025, General Requirements for the Competence of Calibration and Testing Laboratories, 1999.
[10] EURACHEM/CITAC, Quantifying Uncertainty in Analytical Measurement, second ed., 2000.
[11] S. Moret, L.S. Conte, J. Sep. Sci. 25 (2002) 96.
[12] S. Moret, R. Bortolomeazzi, S. Rebecca, L.S. Conte, Riv. Ital. Sost. Grasse. 73 (1996) 141.
[13] P. Welling, B. Kaandorp, Z. Lebensm, Unters. Forsch 183 (1986) 111.
[14] European Union Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002/657/EC, Off. J. Commun. No. L 221 (2002) 8.