Analytical Methods

The potential of solvent-minimized extraction methods in the determination of polycyclic aromatic hydrocarbons in fish oils

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Abstract

Fish oil has been identified as one of the most important contributors to the level of Persistent Organic Pollutants (POPs) in feed products. The determination of polycyclic aromatic hydrocarbons (PAHs) in fish oils is complicated due to the fat matrix, which affects both extraction efficiency and analytical quality. This article reviews and addresses two of the most relevant analytical methods for determining 11 mutagenic and carcinogenic PAHs, as well as two EPA indicator PAHs in fish oils. We discuss and critically evaluate two different extraction procedures, such as ultrasound-assisted solvent extraction (USAE) and ultrasound-assisted emulsification–microextraction (USAEME). Clean-up of extracts was performed by solid-phase extraction using C18 and glass columns containing silica gel and florisil for USAE or only C18 for USAEME. Detection of the selected PAHs was carried out by high-performance liquid chromatography coupled with fluorescence detection for determination. Optimization of the variables affecting extraction by the selected extraction techniques was conducted and recoveries ranged from 70% to 100% by USAE and from 70% to 100% by USAEME with estimated quantification limits between 0.020 and 2.6 µg/kg were achieved. Moreover, the applicability of the selected methods was evaluated by the analysis of real samples. To our knowledge, this is the first time that USAEME has been applied to the determination of PAHs in food matrices, such as oil fish samples. The methods proposed were applied to the determination of the target PAHs in fish samples from different countries, and it was found that the low PAH contamination of the selected fish oils could mainly occur by atmospheric sources.

1. Introduction

The term polycyclic aromatic hydrocarbons (PAHs) refers to the compounds made up of carbon and hydrogen atoms grouped into rings containing five or six carbon atoms. They are formed during the incomplete combustion or pyrolysis of organic matter by a series of complex chemical reactions (Dhammapala, Claiborn, Simpson, & Jimenez, 2007; Ergut et al., 2006; Voutsa, Terzi, Muller, Samara, & Kouimitzis, 2004).

According to the Commission Regulation No. 1881/2006, benzo[a]pyrene (B[a]P) could be used as a marker for the occurrence of these carcinogenic PAHs in food and maximum levels of B[a]P were determined. Nevertheless numerous studies concerning of these compounds in different types of food show that B[a]P is not a suitable indicator of the occurrence of PAHs in food (EFSA, 2008). Commission Regulation No 835/2011 (Commission Regulation, 2011a) amends Regulation No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs and establishes the four PAHs (PAH4): B[a]P, chrysene (Chr), benzo[b]fluoranthene (B[b]F) and benzo[a]anthracene (B[a]A) as the most suitable indicators of PAH in food.

The Scientific Committee on Food (SCF) has assessed the health risk to consumers associated with exposure to PAHs in foodstuffs (EFSA, 2008). The report of the SCF shows that presently, there are no reliable data for the content of PAH in several products and those intensive studies showed that feed ingredients are one of the major sources of contamination. Ingredients/additives are incorporated into animal feed to add colour, ensure stability for nutrients, provide flavour, and prevent mold growth. Specifically, PAHs in fish are a result of contamination of fresh and coastal waters. The residue levels of PAHs in aquatic organisms depend on contamination of their habitat and ability of these organisms to metabolise the contaminants. Fish oils are an excellent source of n-3 fatty acids such as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) having important growth- and health-related functions. However, due to the low metabolization capability of fish, fish oils could contain elevated levels of lipophilic pollutants, such as PAHs. Currently, Galicia (NW Spain) imports most of their animal feed ingredients. A survey is needed for systematic monitoring in the future. Maximum levels of B[a]P and PAH4, in a range of foodstuffs are now specified in Commission Regulation No 835/2011 (Commission Regulation, 2011a).
Nevertheless, no EU maxima levels for PAHs are regulated for feed ingredients such as fish oils.

Sample preparation is an important stage in the analytical process, especially for analysis at low concentrations in complex matrices, such as fat samples. Most of the methods applied to the determination of PAHs in fat liquid matrices involve a three-stage methodology including saponification step with KOH–methanolic solution, liquid–liquid extraction (LLE) with hexane, cyclohexane or isooctane. Then, the extract is cleaned up on a packed column (silica, alumina, florisoril…) (Barranco et al., 2003; Plaza-Bolaños, Garrido Frenich, & Martínez Vidal, 2010). Some authors determined PAHs in vegetable oils by liquid–liquid partition between dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF) and an organic solvent (hexane, pentane, cyclohexane), followed by clean up on silica gel (Vázquez Troche, García Falcón, González Amigo, Lage-Yusté, & Simal Lozano, 2000). Recently, a new liquid–liquid microextraction method termed dispersive liquid–liquid microextraction (DLLME) was developed by Rezaee et al. (2006). DLLME method consists in the rapid injection of an appropriate mixture of an extraction solvent and a disperser solvent into an aqueous sample, thus forming a cloudy solution in which the extraction solvent is dispersed throughout the sample. Since its introduction, DLLME has been used for determination of polycyclic aromatic hydrocarbons (PAHs) in water (Guo & Lee, 2011; Leong, Chang, Fuh, & Huang, 2010), smoked fish (Ghasemzadeh-Mohammadi, Mohammadi, Hashemi, Khaksar, & Haratian, 2012) or solid samples (Rezaee et al., 2010).

Ultrasound energy has also been used to accelerate the extraction of PAHs from environmental solid samples (Luque-Garcia & Luque de Castro, 2003). Ultrasound baths provide a simple and cheap way to deliver ultrasound energy, as well as, they allow the use of a large variety of solvents as extractants. The analytical use of ultrasound-generated emulsions has recently found a growing interest to improve efficiency in liquid–liquid extraction since they increase the speed of the mass transfer between the two immiscible phases implied. This approach was successfully applied by Luque de Castro’s research group (2007) for the simultaneous isolation of polar and nonpolar compounds in solid plant samples. They used methanol/water (dispersed-phase)–hexane (continuous phase) emulsions formed in the presence of ultrasound and a solid sample allows polar and nonpolar compounds to be transferred to the dispersed and continuous phase, respectively. The application of a miniaturised approach to this technique by using a microvolume of extracting organic phase was firstly used by Regueiro, Llompart, García-Jares, Garcia-Monteagudo, and Cela (2008) for determining emergent contaminants and pesticides in environmental waters. In this way, they proved that ultrasound-assisted emulsification–microextraction (USAEME) is a very efficient and fast analyte extraction.

To the best of our knowledge, only limited publications could be found for its application in food samples but none of them for the determination of PAHs (Cheng, Xia, Zhou, Guo, & Chen, 2011; Pizarro, Sáenz-González, Pérez-del-Notario, & González-Sáiz, 2011). These organic compounds were only extracted with this technique in water samples (Cheng, Matsadqiq, Liu, Zhou, & Chen, 2011; Ozcan, Tor, & Aydin, 2010; Saleh, Yamini, Faraji, Rezaee, & Ghambarian, 2009). Therefore, this paper describes the first application of ultrasound-assisted emulsification microextraction for the determination of PAHs in food matrices, such as oil samples.

The aim of this study was to propose and compare different analytical procedures, such as ultrasound-assisted solvent extraction and ultrasound-assisted emulsification–microextraction for determining 11 mutagenic and carcinogenic PAHs listed on Commission Regulation 1881/2006 (Commission Regulation, 2006) and Commission Regulation No 835/2011 (Commission Regulation, 2011a), as well as two EPA indicator PAHs in fish oils. Liquid chromatography (LC) coupled to fluorescence detection (FD) was selected as detection technique. Fluoranthene was selected as representative of three benzenic ring-PAHs. Pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[k]fluoranthene were selected as markers for four benzenic ring-PAHs. Benzo[a]pyrene, dibenzo[a]anthracene, and indeno[1,2,3-cd]P were selected as being representative of the five benzenic ring group of PAHs, and benzo[ghi]perylene as marker of six benzenic ring-PAHs.

2. Materials and methods

2.1. Chemicals, solutions and materials

The thirteen PAHs studied (benzo[b]fluoranthene (B[b]F, 98%), benzo[k]fluoranthene (B[k]F, 98%), benzo[a]pyrene (B[a]P, 97%), benzo[ghi]perylene (B[ghi]P, 98%), benzo[a]anthracene (B[a]A, 98%), chrysene (Chr, 99%), Fluoranthene (F, 99%), indeno[1,2,3-cd]pyrene (I[1,2,3-cd]P), 5-methylchrysene (5-MChr), Pyrene (P, 98%), dibenzo[a]anthracene (DB[a]A, 97%), dibenzo[a]pyrene (DB[a]P, 99%) and benzo[fluoranthene (B]F, 100%), were purchased from Sigma Aldrich (Madrid, Spain). These PAHs were used as markers for the fifteen PAHs of toxicological significance. F was selected as representative of three benzenic ring-PAHs. P, Chr, B[a]A, B[b]F and B[k]F were selected as markers of four benzenic ring-PAHs. B[a]P, DB[a]A and I[1,2,3-cd]P were selected as being representative of the five benzenic ring group of PAHs. B[ghi]P and DB[a]P were selected as marker of other six benzenic ring-PAHs. HPLC grade acetonitrile, DMSO and water, and analytical grade n-hexane were all supplied by Panreac (Madrid, Spain). Individual 100 mg/L stock solutions of PAHs were prepared by dissolving about 0.010 g of product in a small amount of acetonitrile or n-hexane and diluting to 100 mL with the same solvent, which was selected depending on the solubility of the PAHs. From these solutions, solutions containing 10 and 0.10 mg/L concentrations of the different PAHs in n-hexane were prepared separately. From these diluted individual solutions, mixed solutions with PAHs ranging from 10 to 700 µg/L were prepared in acetonitrile following evaporation of the hexane. Working standard solutions used to construct the calibration line were prepared in acetonitrile by dilution to reach concentrations between 0.020 and 40 µg/L.

The solid-phase extraction was performed with Strata C18-E cartridges (55 µm, 70A, 2 g, 12 mL). Glass columns, Florisil and Silica supplied from Panreac (Barcelona, Spain) were used as solid-phase extraction (SPE) for purification.

The extracts were evaporated using a TurvoVap evaporator (Caliper Life Sciences, Barcelona, Spain) provided with nitrogen. Analytical grade C-45 nitrogen was supplied by Carburos Metálicos (Vigo, Spain).

Additional equipment included an ultrasonic bath (P-Selecta, Barcelona, Spain), an oven (P-Selecta, Barcelona, Spain) a ProBlot hybridization oven (Labnet, USA), a Rotina 35R centrifuge (Hettich, Germany), an Avanti J-26 XP centrifuge with a Rotina JA-25.50 (Beckman Coulter, U.S.A.), an analytical precision scale (Sartorius, Madrid, Spain) and a vortex shaker (Heidelberg, Barcelona, Spain).

Disposables used were nylon filters (0.45 µm), micropipettes (200–1000 µL), polypropylene centrifuge tubes and injection vials (2.0 mL) furnished with screw caps and PTFE-lined butyl rubber septa and inserts (200 µL).

2.2. Extraction of PAHs

2.2.1. Ultrasound-assisted solvent extraction (USAE)

About 1.0 g of purified fish oil was extracted three times with 5 mL acetonitrile by shaking with vortex for 3.0 min and then by USAE for 5.0 min. Then, the samples were centrifuged (4000 rpm)
In the present work PAH recoveries were determined by spiking blank sample (n = 6) a PAH standard in n-hexane in a concentration ranged as Table 1 shown, which were stored in the dark for 24 h to facilitate equilibration with the sample matrix. Spiking levels were shown in Table 1, as well as the recoveries obtained and the high sensitivity of these methods, illustrated by the quantification limits (LOQs). LOQs were evaluated based on the noise obtained with the analysis of unfortified oil samples (n = 4). LOQ was defined as the concentration of the analyte the produced a signal-to-noise ratio of 10 (ACS, 1980) and was then tested experimentally by spiking blank samples at such levels.

Once a positive sample is encountered, additional confirmation is necessary in order to avoid false positives. Coeluting undetected matrix components may reduce or enhance the instrument signal of the analytes. Thus, response factors obtained from standard solution and in matrix-loaded sample may differ significantly and matrix effects must be eliminated and compensated to obtain quantitatively accurate results. In this way, and although quantitative recoveries were obtained and they evidence that no significant matrix effect take place, a comprehensive evaluation of signal suppression was performed for each analyte in order to assess its effect on the quantification. Two different types of calibration curves were studied: calibration curves prepared using n-hexane and calibration-set solutions prepared in sample matrix. The data obtained from the analysis of each calibration set were fitted to straight lines by the least squares method and slopes of each calibration curve were compared calculating an F statistic. In this case the slopes were not statistically different (p < 0.05) and therefore, it could be confirmed that the matrix content does not introduce a systematic bias in the analytical signals.

3.2. Performance of extraction procedures

Optimization of extraction parameters was addressed in fortified fish oil samples at PAH range as Table 1 shows. The fortification was carried out at least 24 h before to allow time for the analyte to interact with the matrix of the sample and thus, real conditions could be simulated.

3.2.1. Ultrasound-assisted solvent extraction (USAES)

The method presented in this paper was based on the developed by Vázquez Troche et al. (1996) and Barranco et al. (2003) and therefore, no experimental design was applied. When we use a liquid–liquid partition using only acetonitrile, some impurities from oil samples are eliminated, avoiding the use of n-hexane to previous dissolution of the sample. In this way 1.0 g oil sample were extracted (n = 3) three times by ultrasound-assisted solvent extractions with 15 mL of the selected solvents during 20 min each. The extract obtained was cleaned up, evaporated till dryness and re-dissolved to a final volume of 1.0 mL with acetonitrile for HPLC analysis. A lower extraction volume (10 and 5.0 mL) and time (10 and 5.0 min) were also tested. Since no differences were found by Sep-Pack Silica Plus cartridges was not enough for the selected samples. Barranco et al. (2003) after dilution of oil samples with n-hexane used C18 SPE with reversed phase sorbents such as N,N-Dimethylformamide (DMF):H2O (9:1).

In the present work we developed a LLE extraction using ACN following by a C18 SPE clean up. In this case, we optimised the solvent and the breakthrough volume to retain the target PAHs on the SPE cartridge. Teixeira, Casal, and Oliveira (2007) assessed an extraction analytical method involved a LLE with ACN:acetonite (6:4) and solid-phase clean up (C18 and Florisil). The main
drawback of using ACN directly on the SPE cartridge is the PAH retention on the cartridge. Although Teixeira et al. (2007) transferred 2.0 mL ACN:acetone (6:4) to the C_{18} Cartridge (12 cc, 2 g), in our case no retention of PAHs was observed and quantitative losses were detected. The LLE extract was evaporated till 0.50 mL ACN to avoid losses and the sides of tube were washed with 1.0 mL additional acetonitrile. Afterwards, the cartridge was dried under a flow of nitrogen in a TurvoVap for 15 min. The C_{18} cartridge was activated with 10 mL ACN and the elution step was also optimised. When only n-hexane was used as elution solvent, no quantitative recoveries were reached. Mixtures between n-hexane and dichloromethane (1:1, 1:2 and 1:3) were assayed and better results were observed with higher amounts of dichloromethane. Therefore, elution only with 60 mL dichloromethane was developed and the best recoveries were reached.

In the literature, the extraction methods of PAHs in oils using C_{18} Cartridges (Bogusz, El Hajj, Ehaideb, Hassa, & Al-Tufail, 2004; Teixeira et al., 2007) included another clean up step with Florisil bonded phase cartridge, unsatisfactory for the selected samples.

3.2.2. Ultrasound-assisted emulsification–microextraction (USAEME)

3.2.2.1. Preliminary studies. Regarding the USAEME process, the selection of a suitable extractant for determining PAHs in oils is limited by several characteristics that are necessary for emulsification in the presence of ultrasonic radiation. Some of these characteristics are the density of oil and its low solubility.

Firstly, we selected an emulsifier of oil samples to facilitate the emulsion formation and leaching of the target compounds. This solvent was used previously as a dilution solvent in order to modify the partition coefficients (Moret & Conte, 2000). In this way, n-hexane was selected due to its miscibility with fat matrices such as oils.

Later, we studied heavy solvents to extract PAHs so the organic phase was sedimented at the bottom of the conical tube. Most of the heavy solvents are halogenated compounds; several of them were initially tested in order to evaluate their emulsification capacity and the possible presence of interferences during mass spectrometry detection. Dichloromethane (CH_{2}Cl_{2}), carbon tetrachloride (CCl_{4}), chloroform (CHCl_{3}), DMSO (C_{2}H_{6}OS) and 1,2 dichloroethane (C_{2}H_{4}Cl_{2}) were initially considered as possible extracting solvents. About 1.0 g oil, 2.0 mL n-hexane and 200 mL of every solvent were ultrasound irradiated for 10 min but no emulsification was observed in all cases with the exception of DMSO. The higher solubility of the dichloromethane, carbon tetrachloride, chloroform and 1,2 dichloroethane is supposed to be the cause of no emulsion formation, so these solvents were ruled out for further optimization.

The use of DMSO could also prevent a change in solvent for cleaning up. In this way, the present authors extracted PAHs from fat matrices, using DMSO following by a cleanup step using C_{18} cartridges (García-Falcón & Simal-Gándara, 2005a). For such purpose, fish oil extracts from samples without PAHs were employed to select conditions providing high recovery and sensitivity only in the C_{18} SPE. Based on our experience, the use of DMSO on reverse phase solvent extraction needs water to provide PAH retention on SPE cartridge. Ratios DMSO:H_{2}O (1:2) showed the best retention. To ensure the complete PAH retention on C_{18} SPE cartridge, 5 mL acetonitrile and 10 mL water were selected to activate the column. After that, the cartridge was dried under a flow of nitrogen in a TurvoVap for 20 min. Elution was quantitative only with n-hexane and 20 mL were enough to recover the selected PAHs. The extracts obtained were redissolved in 1.0 mL acetonitrile and filtered for LC-FD analysis.

3.2.2.2. Optimization of USAEME. The influence of the variables potentially affecting the efficiency of USAEME was evaluated by using a response surface design. The three selected variables were: volume of DMSO and n-hexane, and the extraction time. Firstly, the volume of emulsifier of oil samples to facilitate the emulsion formation and leaching of the target compounds could be important. Sample weight (1.0 g oil) was kept constant and in this way, the volume ratio between emulsifier and sample was studied at values of 1.0 g/l, spike added in µg/L and recoveries (%RSD) (n = 6) for PAHs.

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Range of RPFs</th>
<th>LOQ (µg/kg)</th>
<th>Instrument linearity</th>
<th>Spike added (µg/L n-hexane)</th>
<th>Recovery (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USAE</td>
<td>USAEME</td>
<td>Standards concentration range (µg/L acetonitrile)</td>
<td>r²</td>
<td>USAE</td>
</tr>
<tr>
<td>F</td>
<td>0.0090–0.20</td>
<td>1.1</td>
<td>1.1</td>
<td>0.50–14</td>
<td>0.9991</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>2.6</td>
<td>2.4</td>
<td>2.0–20</td>
<td>0.9985</td>
</tr>
<tr>
<td>B[α]A</td>
<td>0.020–0.40</td>
<td>0.64</td>
<td>0.65</td>
<td>0.020–3.4</td>
<td>0.9990</td>
</tr>
<tr>
<td>Chr</td>
<td>0.040–0.20</td>
<td>0.23</td>
<td>0.25</td>
<td>0.12–20</td>
<td>0.9987</td>
</tr>
<tr>
<td>5-Mch</td>
<td>0.12–0.15</td>
<td>0.12–0.20</td>
<td>0.9988</td>
<td>12</td>
<td>84 (6.0)</td>
</tr>
<tr>
<td>B[j]F</td>
<td>0.010–1.00</td>
<td>0.55</td>
<td>0.60</td>
<td>0.30–60</td>
<td>0.9995</td>
</tr>
<tr>
<td>B[k]F</td>
<td>0.10–2.00</td>
<td>0.13</td>
<td>0.20</td>
<td>0.040–6.0</td>
<td>0.9999</td>
</tr>
<tr>
<td>B(lj)F</td>
<td>0.030–0.030</td>
<td>0.020</td>
<td>0.030</td>
<td>0.010–1.0</td>
<td>0.9999</td>
</tr>
<tr>
<td>B[α]P</td>
<td>1.0</td>
<td>0.21</td>
<td>0.25</td>
<td>0.010–2.0</td>
<td>0.9987</td>
</tr>
<tr>
<td>D[a,j]P</td>
<td>10–40</td>
<td>0.25</td>
<td>0.26</td>
<td>0.15–25</td>
<td>0.9999</td>
</tr>
<tr>
<td>D[a]Ja</td>
<td>1.0–10</td>
<td>0.28</td>
<td>0.30</td>
<td>0.040–7.0</td>
<td>0.9979</td>
</tr>
<tr>
<td>B[ghi]P</td>
<td>0.0090–0.0090</td>
<td>1.5</td>
<td>1.6</td>
<td>0.30–60</td>
<td>0.9989</td>
</tr>
<tr>
<td>l[1,2-3-c]d</td>
<td>0.070–0.070</td>
<td>2.0</td>
<td>2.5</td>
<td>0.20–30</td>
<td>0.9968</td>
</tr>
</tbody>
</table>
the graphs represents the statistically significant bound at the 95% confidence level.

The analysis of these results showed that not all the initially selected variables produced a significant effect. As it can be seen, volume extraction and, in some cases, time were the factors with greatest effect (in fact, the only statistically significant factors for some of the target PAHs). As it could be expected, the PAH peak areas increase when extraction volume of DMSO increase. This influence is also manifested by the existence of a significant AA interaction. This second order factor represents the quadratic effect of this factor.

Instead, the highest PAH peak areas for some of the target PAHs were obtained with 5.0 min extraction time, such as B[a]P, D[a,h]A, B[ghi]P and I[1,2,3-c,d]P, in the lower level of this factor. Fast rates of mass transport are supposed during liquid–liquid processes in acoustically emulsified media. Therefore, very short times should be required in comparison with conventional liquid–liquid extraction techniques.

In view of the results of the experimental design, the selected conditions for the ultrasound-assisted emulsification–microextraction of the target compounds from oil samples were as follows: an extraction time of 5.0 min, volume ratio between emulsifier and sample 1 (1.0 mL n-hexane) and 400 μL DMSO. Consecutive extraction cycles were also assayed and the best results were obtained with 3 cycles.

### 3.3. Comparison of extraction techniques

A comparison of general parameters for the different extraction methods is shown in Table 2. The extraction solvent and time for USAEME were lower than for USAE, using both no sophisticated equipment.

#### 3.3.1. Validation of the analytical methods

The validation procedure of the optimised USAE and USAEME methods comprised the determination of linearity, limits of quantification (LOQs), and the study of matrix effects.

External standard calibration was chosen to quantify analyte values for LC/FD techniques using multicomponent standards, using eight calibration standards (0.010 to 60 μg/L).

In Table 1, a complete description of standard linearity supported by regression data that include slope, intercept, standard deviations of both and precisions of the curves (RSD%) is shown. To verify the linearity range, a Mandel fitting test (P = 99%) was additionally performed. Linear calibration curves fit reasonably in a concentration scale of two or three orders of magnitude, depending on the compound.

The high sensitivity of these methods is illustrated by the detection (LODs) and (LOQs). They were evaluated on the basis of the noise obtained with the analysis of unfortified fish oil samples (n = 4). LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively (ACS, 1980) and were then tested experimentally by spiking blank samples of fish oils at such levels.

#### 3.3.2. Comparison of USAE and USAEME recoveries

The aim of the present work is developing a methodology suitable for the analysis of the target compounds in three different kinds of oil samples including raw or refined fish oils. Samples were spiked with the target compounds at PAH range as Table 1 shows. As can be seen, recoveries ranged from 70% to 100% (RSD = 2.0–9.0%) for USAE and from 70% to 108% (RSD = 3.0–10%) for USAEME, so no significant matrix effects were found.
3.4. Application to the determination of target PAHs fish oil samples from different fishing areas

The proposed method was then applied to the analysis of fish oil samples from different fishing areas (Spain, Africa and North Europe). They were raw and refined fish oils, intended for feeds. Analyses were performed at least in triplicate and results of the target compounds found are shown in Fig. 2. F, P and B[ jel]F were the compounds present to a greater extent. Among them, B[a]A, B[k]F and B[a]P were also found in higher concentrations in national fish oil. B[k]F and B[a]P were detected in lower concentrations in the rest of the selected fish oils. The rest of PAHs were not found in any of the samples. The present and other authors found similar results in fish oils (Fernández-González, Yebra-Pimentel, Martínez-Carballo, & Simal-Gándara, 2012; Nácher-Mestre et al., 2010; Yebra-Pimentel, Fernández-González, Martínez-Carballo, & Simal-Gándara, 2012a; Yebra-Pimentel, Fernández-González, Martínez-Carballo, & Simal-Gándara, 2012b). No EU maxima levels for PAHs are regulated for feed and feed ingredients. Commission Regulation No. 1881/2006 (Commission Regulation, 2006) and No. 835/2011 (Commission Regulation., 2011a; Commission Regulation., 2011b) specifies a maximum level of 2.0 μg/kg for B[a]P and 10 μg/kg for PAH4 in oils and fats excluding cocoa butter, 5.0 μg/kg for B[a]P in smoked meat and smoked meat products and muscle meat of smoked fish and smoked fishery products and 1.0 μg/kg for B[a]P and for PAH4 in processed cereal-based foods, intended for direct human consumption or use as an ingredient in foods. Therefore, the results shown in Fig. 2 pointed out that none of the selected fish oils contain important PAH content.

Usually, in environmental and food samples, the molecular patterns of PAHs are like fingerprints, what make possible to hypothesize about which processes generate them by studying their distribution in samples (Orecchio & Papuzza, 2009). Two of the ratios commonly used in the literature are F/P and F/F + P (F/202), B[a]A/Chr and B[a]A/B[a]A + Chr (B[a]A/228) (Yunker et al., 2002). Generally, higher F/202 ratios than 0.40 belong to pyrolytic sources. The same consideration could be applied to the F/P ratio, where values greater than 1.0 are classically related to pyrolytic origins, namely to coal combustion (Sicre et al., 1987). The highest ratios were reached for oil fish from Spain but ratios are lower than 0.40 for F/202 and than 1.0 for F/P. Therefore, we could postulate that PAH contamination of the selected fish oils would occur by atmospheric sources (Pontevedra-Pombal et al., 2012).

4. Conclusions

Comparisons between two different extraction procedures, such as USAE and USAEME have been carried out. They do not demand sophisticated equipment but, on the contrary, USAE demands time and solvent. In this way, simple, fast and robust methods have been developed and validated for determining 11 mutagenic and carcinogenic PAHs, as well as two EPA indicator PAHs in fish oils. LC coupled to FD was selected as detection technique. Recoveries, LOQ and RSD% of the developed procedures demonstrate their suitability for routine monitoring of PAHs in raw or refined fish oils.

Table 2
Comparison of general parameters for the different extraction techniques: USAE and USAEME.

<table>
<thead>
<tr>
<th>General parameter</th>
<th>USAE</th>
<th>USAEME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction time (h)</td>
<td>3 h</td>
<td>1.5 h</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>150 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Fig. 2. Results of the analysis of fish oil samples from different fishing areas (Spain, Africa and North Europe).
The proposed methods were then applied to the analysis of three fish oil samples from different sites (Spain, North Europe and South America). Since no matrix effects were observed even in the most complex samples, quantification could be easily performed by external calibration using standards in acetonitrile. F, P and B[b]F were the compounds present to a greater extent but the PAH content were lower than the EU maxima levels. F/P and F/202 ratios were also studied to determine the origin of the PAHs detected.

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References


