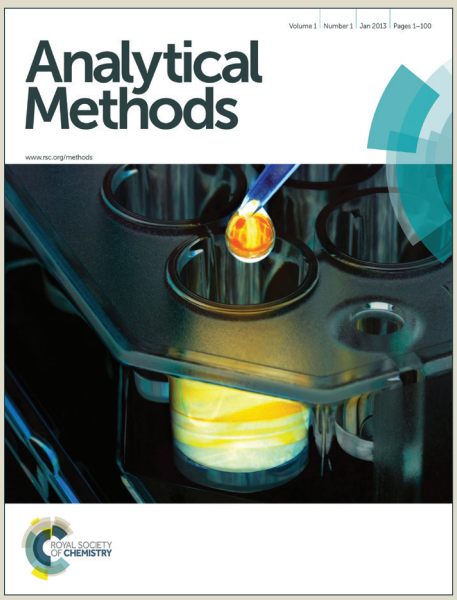
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Single laboratory validation of an environmental friendly single extraction and cleanup method for quantitative determination of four priority polycyclic aromatic hydrocarbons in edible oils and fats

Stephen W.C. Chung[1], Jason S.Y. Lau

Food Research Laboratory, Centre for Food Safety, Food and Environmental Hygiene Department, 4/F Public Health Laboratory Centre, 382 Nam Cheong Street, Hong Kong

1 Author to whom correspondence should be addressed.
e-mail: swcchung@fehd.gov.hk

Abstract

This paper reports a simple, rapid, reliable and environmental friendly gas chromatography-mass spectrometry (GC-MS) method for analyzing polycyclic aromatic hydrocarbons (PAHs) in edible oils and fats. The fat sample was firstly dissolved in acetonitrile while oils loaded directly onto a solid phase extraction cartridge. PAHs were eluted with acetonitrile. Owing to the background interference, GC-MS was found applicable for 4 European Union (EU) priority PAHs (benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) in various types of edible oils and fats, but not for 15+1 EU-priority PAHs. An adequate linear relationship was obtained in the studied concentration range (0.1 – 60.0 μg kg^{-1}) in sample; analytical limits of detection and quantification were 0.1 and 0.25 μg kg^{-1} respectively. Suffered from lack of certified reference material, spiked recovery and a FAPAS quality control material were employed to assess the accuracy. The mean spiked recoveries for 4 PAHs, studied at concentration levels of 0.25 (method limit of quantification (MLOQ)), 1.0 and 2.0 μg kg^{-1}, were ranged from 86 to 114%. Precision values, expressed as relative standard deviation, were below 10% at aforementioned spiking levels. Extraction and cleanup of a batch of 20 samples can be completed within an hour by one worker. The developed method was successfully applied for the PAHs determination in real commercial samples, including lard, olive, corn, peanut, sunflower seed, rapeseed, sesame and vegetable oil.

1. Introduction

Polycyclic aromatic hydrocabons (PAHs) refers to a large group of over 100 organic chemicals containing two or more fused aromatic rings made up of carbon and hydrogen atoms. PAHs are formed during the thermal decomposition of organic mass such as coal, crude oil and natural gas, and incomplete burning of coal, oil, gas, garbage, especially at limited access of oxygen in the range of 500 – 900 °C [1]. They are ubiquitous in the environment, being present in air, soil and water. PAHs in foods can result from the transfer from contaminated air, water and soil, depositing PAHs directly on food. The other significant source is the formation and deposition of PAHs during heat processing using methods such as barbecuing, smoking, drying, roasting, baking, frying or grilling [1]. Except for smokers, the main source of exposure to PAHs for the adult is food, which contributed to more than 90% of total exposure [2]. Other minor routes of exposure to PAHs are inhalation of polluted ambient and indoor air, ingestion of house dust, and dermal absorption from contaminated soil and water [3].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed a risk assessment on PAHs in 2005 and mentioned the major contributors to human intakes of PAHs were cereals and cereal products (owing to high consumption in the diets of many countries) and vegetable fats and oils (owing to higher concentrations of PAHs in this food group). For fats and oils, drying of cereals and plants used for production of crude vegetable oils using direct application of combustion gases can result in contamination of the product with PAHs [1]. Direct fire-drying and heating processes used during the production of some oils of plant origin and in particular residue oil can result in high levels of PAHs. According to information provided by local trade members, nutty oil such as peanut oil and sesame oil may contain higher levels of benzo[*a*]pyrene (BaP) because deodorization was not introduced [4] or pyrazine (the chemical which produced typical nutty flavours) in the nut may convert to BaP [5] during oil processing. Nevertheless, the level of BaP in oil would be much reduced after oil refining processes (based on the deodorization step) and the final level depending on the refining conditions adopted [6]. Hence, effective refining of crude oils can remove PAHs and is crucial to ensure that the products are safe.

Maximum levels (MLs) have been set for PAHs in key foodstuffs, e.g. meat and meat products, fish and fishery products, milk and milk products, oils and fats, via European Commission Regulation No 835/2011, the framework European Union (EU) legislation which sets maximum levels for chemical contaminants in foodstuffs. Besides, the Korean [7] and Chinese [8] government recommended a value of 2 and 10 μg kg^{-1} as the maximum tolerance level of BaP in edible oils and fats respectively. These MLs are set at a very low

level (as low as reasonably achievable for the particular foodstuff in question), in order to ensure that the health of consumers is not affected by consuming these products. For ensuring that these MLs are not being exceeded, routine surveillance of food must be carried out, involving the taking of samples of potentially contaminated produce, followed by laboratory analysis to determine the levels of PAHs in the product.

In 2000, Moret et al. [9] reviewed the analytical methods for testing PAHs in edible oils and mentioned sample preparation relied on tedious and time-consuming procedures. In 2003, Barranco et al. [10] studied different solid phase extractions (SPEs) for extracting PAHs from edible oils in 2003. Subsequently, single extraction and cleanup step has been reported [11-14] for extracting PAHs from oils and fats. Moret and Conte [11] employed a silica cartridge to retain triglycerides, then elute PAHs with a mixture of n-hexane and dichloromethane. Bogusz et al. [12] used a self-packed SPE, filled with Florisil and Nucleoprep C18, to retain triglycerides and BaP was eluted with acetonitrile. In the contrast, Veyrand et al. [15] and Cortesi and Fusari [13] used PS/DVB cartridge to retain PAHs. Triglycerides were firstly washed away with a mixture of isooctane and cyclohexane, then PAHs were eluted with dichloromethane. Recently, Zhao et al. [16] employed magnetic multi-walled carbon nanotubes as magnetic SPE for determination of 8 PAHs in edible oils. These publications were either employed chlorinated solvent or analyzed limited number of PAHs in olive oil only.

The aim of this work was to optimize and validate a rapid and environmental friendly method for determination of PAHs in edible oils and fats with a commercial available SPE while its application is for regulatory monitoring of PAHs in various types of edible oils. The developed method involves a single SPE extraction and cleanup step. The resulting extract was then applied to the gas chromatography-mass spectrometry (GC-MS) for quantitative and qualitative analysis of 4 EU priority PAHs with one quantifier and one qualifier per each compound. The target analytes had been spiked to a variety of in real commercial samples, including lard, olive, corn, peanut, sunflower seed, rapeseed, sesame and vegetable oil. Satisfactory spike recovery result was obtained and no significant interference was encountered in these matrices when spiked at the method limit of quantitation (MLOQ) of 0.25 $\mu g\ kg^{-1}$.

2. Experimental

2.1 Chemicals and standards

All solvents used were of pesticide grade and all reagents were of analytical grade. Supelclean™ EZ-POP NP was obtained from Supelco (Sigma-Aldrich Corp., St. Louis, USA). All edible oil and fat samples were collected from local retailers and restaurants.

Native PAH mixed standards, including BaP, benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF) and chrysene (CHR), were purchased from Wellington Laboratories Inc. (Ontario, Canada) and Dr. Ehrenstorfer (Augsburg, Germany). The purities of the PAH standards were of 98% or above. Working standard solutions for calibration were prepared by appropriate dilution of PAH mixed standard with isooctane. Isotopically labelled mixed standards, including d_{12}–BaP, d_{12}–BaA, d_{12}–BbF, d_{12}–CHR, were purchased from Wellington Laboratories Inc. (Ontario, Canada). A working internal standard solution mix used for spiking containing all internal standards of 20 ng mL^{-1} was prepared by appropriate dilution with cyclohexane. All solutions were stored at −20°C.

2.2 Sample preparation procedure

Conditioned the EZ-POP NP cartridge with 10 mL of acetone and dried by passing air with vacuum for 10 min. For liquid samples, shake vigorously or inverting up and down the liquid content within the container. Weighed accurately 0.4 g of oil and placed onto the cartridge. For solid samples, the entire sample was blended with a high speed blender. Weighed accurately 0.4 g sample into a glass tube and then melt it in a warm water bath. After then, added 1 mL of acetonitrile and vortexed for 30 sec and loaded onto the catridge. 0.1 mL of internal standard spiking solution was added onto the cartridge. Let the sample penetrate into the cartridge with gravity. 15 mL of acetonitrile was then added to elute out the target analytes at a rate of about 1 drop per sec. 0.1 mL of toluene was added to the eluate as trapping agent [17] before the resulting extract was evaporated to almost dryness by a nitrogen stream at room temperature. The residue was then dissolved in 0.1 mL isooctane for GC-MS determination of PAHs with internal standardization.

2.3 Gas chromatograph – mass spectrometry (GC-MS)

The analysis of the residues was carried out on an Agilent 6890 gas chromatograph equipped with a Series 5973 Network mass selective detector, a Series 7683A automatic sampler and a data processing system with ChemStation software (Version B.03.02) (Agilent, Avondale,

USA). GC separation was performed on a DB-EUPAH fused-silica capillary column (20 m, 0.18 mm I.D., 0.14 μm film thickness, Agilent). Ultra-high-purity helium (99.999%) was used as the carrier gas.

A split-splitless injection system operated in pulsed splitless mode with quartz Gooseneck splitless injector liner (4 mm id, 6.5 mm od and 78.5 mm length) was employed. Injection volume was 1 μL. One min after the injection, the split valve was activated to a total flow-rate of 60 mL min^{-1} for 1 min. Afterwards, the total flow was set to 20 mL min^{-1}. For the column carrier gas, it was operated in ramp flow mode. The gas flow was initially set at 1.0 mL min^{-1}. After 0.2 min, it was increased to 1.7 mL min^{-1} (ramp rate of 5.0 mL min^{-2}). The initial oven temperature was set at 45 °C. After the sample was injected for 0.8 min, it was increased to 200 °C (ramp rate of 45 °C min^{-1}), 225 °C (ramp rate of 2.5 °C min^{-1}), 266 °C (ramp rate of 3 °C min^{-1}), 300 °C (ramp rate of 5 °C min^{-1}) and finally to 320 °C (ramp rate of 10 °C min^{-1}). The temperature of the injector was set at 325 °C.

The mass spectrometer was operated in electron ionization mode at 70 eV. The temperatures of the ion source, the quadrupole and the transfer line were set at 300, 150 and 320 °C respectively. Qualitative and quantitative analysis was carried out by selectively monitored the detector response of characteristic ions in 2 time segments with one and three scan events designated for each internal standard and target analyte respectively. The quantitative ion and secondary (identification) ions measured for each analyte are listed in Table 1. The extracted ion chromatograms of 4 EU priority PAHs of a standard solution are illustrated in Figure 1.

2.4 Quantitation and identification

Calibration curves were constructed for all target analytes by injecting 6 calibration standard solutions directly into the GC, at the concentrations 0.4, 1, 2, 10, 50 and 250 μg L^{-1}, for PAHs with a method limit of quantitation (MLOQ) of 0.25 μg kg^{-1}. Calibration curve was constructed each time a new sample set was analyzed in order to accurately compensate for the day-to-day variation of the control standards. For simultaneous quantitation and identification purposes, one secondary ion was used in order to avoid false positives at trace PAH levels. According to the European Commission Regulation 2002/657/EC, identification of an analyte above the MLOQ in the sample is made when the following interpretation criteria are fulfilled:

1. the tolerance criteria for relative retention time should be within ±0.5% when comparing the unknown peak (in the test sample) with that of the corresponding analyte peak in the calibration standard;
2. a minimum of at least one ion ratio shall be measured;

3. the quantitative ion and the one identification ion should be present with signal-to-noise (S/N) ratio greater than 3; and
4. the identification ion/ quantification ion ratio in the sample and the previously injected standard should not differ by more than the maximum tolerances as stipulated in the 2002/657/EC.

If the above criteria were met, then identification of the analyte in the sample was reported.

2.5 Matrix effect

The proposed method is meant to be a versatile method for common PAHs in different variety of edible oils and fats. Matrix-matched calibration curves were prepared at three concentration levels (MLOQ, 4 x MLOQ and 8 x MLOQ (ML)) three times in four edible oils and fats. Matrix effect, expressed as the percent deviation of slope of relative response ratio against relative concentration of the PAH in matrix from the corresponding slope in isooctane, was also evaluated in the selected matrices (Table 2).

2.6 Validation study

The validation study was performed on the basis of the above-mentioned criteria. Analytical characteristics evaluated were sensitivity, mean spiked recovery, accuracy (as a measure of trueness), precision (expressed as repeatability and reproducibility), and selectivity. With this objective, spike recovery tests were conducted three times at MLOQ, 4 x MLOQ and 8 x MLOQ (ML) levels, respectively, with four typical edible oils and fats. The recovery results were summarized in Table 3. Besides, recovery experiments with spiked blank samples were performed at 8 x MLOQ (ML) during the real sample analyses. At least one pair of replicate was used for each sample matrix and at each spiking level. Linearity was studied using standards, not matrix-matched, across the six concentrations between 0.4 and 250 μg L^{-1} (corresponding to 0.1 to 62.5 μg kg^{-1} in sample).

The MLOQ was established as the lowest quantifiable concentration tested amongst the targeted PAHs, for which recovery and precision were assessed in accordance with the criteria established for analysis of PAH residues in foods. The trueness cannot be assessed with appropriate certified reference material as BCR 458 (coconut oil) was already out of stock. Instead, a Food Analysis Performance Assessment Scheme (FAPAS) quality control material, T0657QC (palm oil), was used for validation of trueness.

3. Results and discussion

3.1 Extraction cum cleanup of PAHs

As PAHs are fat soluble compounds, other fatty substances would be co-extracted from the sample at the same time. These fatty substances are highly soluble in organic solvent and tend to adsorb in the GC system resulting in poor chromatographic performance and shorten the lifetime of the GC column. Besides, co-extracted substances might also induce matrix enhancement / suppression effect. Furthermore, the remaining lipids would also affect the efficiency of solid phase extraction (SPE) cleanup if applied. Therefore, the success of the analysis of PAHs critically relies on the efficiency of lipids removal.

For extracting 15+1 EU-priority PAHs from edible oils, three different ready-to-use solid phase extraction (SPE) cartridges are commercially available, via. styrene-divinylbenzene copolymer (SDB), SupelMIP™ and EZ-POP NP cartridge, were considered initially. Firstly, Jung et al. [5] showed SDB cartridge provided good recoveries of > 70% for 12 PAHs and < 70% for cyclopenta[*c,d*]pyrene, 5-methylchrysene, dibenzo[*a,l*]pyrene and CHR. Besides, a late eluting broad background was found during the initial stage of method development. Secondly, SupelMIP™ composed of highly cross linked polymer-based molecular recognition elements engineered to bind certain PAHs with high selectivity. The recoveries of BaA and CHR from olive oil were shown to be less than 80% [18]. Thus, both cartridges were not considered for further study.

The Supelclean EZ-POP NP, a dual-layer SPE cartridge containing Florisil® and Z-Sep/C18, was designed for the extraction of nonpolar analytes from oil matrices. It was based on Lewis acid/base and hydrophobic interactions so that fatty matrix interferences are preferentially retained by the cartridge while non-polar analytes are eluted out. By this way, lipophilic substances can be retained in the SPE. For optimization of elution volume of acetonitrile, portions of 3 mL were consecutively collected for GC-MS analysis. Figure 2 showed the elution profile of 4 target analytes. Although all target PAHs eluted out within first 4 fractions in blank spike, only over 90% was eluted out for the first 5 fractions when target PAHs was spiked in a blank olive oil. Around 7% of PAHs was eluted in the sixth fraction. However, the full scan MS study showed that matrix started to elute out from the sixth fraction. Therefore, only 15 mL of acetonitrile was collected during elution. As such, the use of labelled internal standard is necessary for obtaining better recoveries. To our understanding, this is the first reported case that use EZ-POP NP cartridge for removing various edible fats so as to analyze PAHs. By using such cartridge, 4 EU priority PAHs in tested matrices could be extracted and purified in single step with high sample throughput of

20 samples per hour per person.

For full scan MS analysis of various edibel oils and fats after extraction cum clean-up by EZ-POP NP, sesame oil gave cleanest background except for two late eluting compounds. By matching the mass spectra of these compounds with MS library, they were found to be polyphenols, viz. sesamin and sesaminol/sesamolin (Figure S1). Owing to these polyphenols get minor mass fragments as dibenzo[*a,h*]anthracene and benzo[*g,h,i*]perylene, the extract cannot be employed for 15+1 EU-priority PAHs analysis but found suitable for PAH4. Amongst other edible oils after clean-up with EZ-POP NP cartridge, sunflower seed oil and peanut oil showed to have much dirtier background (Figure S2) when compared to other oils. For edible fat, lard, the background after the clean-up is quite similar to sunflower seed oil. Thus, the validation study was focused in lard and these three oils. Owing to the dirty background of these edible oils and fats after single extraction cum clean-up by EZ-POP NP, they posed difficulty to quantify 15+1 EU-priority PAHs or PAH8 with GC-MS. For better understanding the cleaniness of background on PAH4 analyses, the extracted ion chromatograms were also incorporated in Figure S2. However, peanut oil without detectable amount of PAH4 could not be found, their corresponding peaks were marked for easy reference.

3.2 GC analysis of PAHs

Overlapping of peaks is commonly occurred for the 15+1 EU-priority PAHs, especially when cyclopenta[*c,d*]pyrene, BaA and CHR are involved. In our first attempt to separate 15+1 EU-priority PAHs, Rxi-PAH column (by Restek) was used and could not completely separate indeno[*1,2,3-cd*]pyrene and dibenz[*a,h*]anthracene after trying many different GC running conditions. Although they have a mass difference of 2 amu, both of them gets the ion of *m/z* 276 and difficult to quantify them if they were included in the scope of analysis. The DB-EUPAH column (by Agilent) was found to have better separation of PAHs and could provide almost baseline separation for all 15+1 EU-priority PAHs in a run of around 40 min. (Figure 3). Afterwards, it was found that another column, Trace TR-50ms (50% phenyl), can provide same elution profile and complete resolution of the 15+1 EU-priority PAHs [19].

The GC-MS methods have become popular methods for analyzing PAHs in foods. This is due to the selectivity of the MS-detector, the use of mass spectrum data for reliable confirmation of PAHs, especially for more than 100 PAHs can be found in the environment with similar physical properties. Single quadrupole MS is normally running in electron ionization (EI) mode with target analytes monitored by selective ion monitoring (SIM). However, PAHs were difficult to breakdown under EI mode and led to have lower signal

responses for identification ions. Thus, one single identification ion was normally employed for confirmation.

3.3 Matrix effect

In this work, four matrices were selected for the evaluation of matrix effect at MLOQ, 4 x MLOQ and 8 x MLOQ (ML) levels. The slopes obtained in the calibration with matrix matched-standards were compared with those obtained with standard solutions. Evaluation data of matrix effect are presented in Table 2. Mild matrix effect (suppression or enhancement of less than 10%) were found for all of the analyte-matrix pairs. Therefore, we did not perform quantitation using calibration with matrix-matched standards. This eliminated the trouble of finding representative blank matrices similar to various types of food samples.

3.4 Analytical performance

The accuracy and repeatability of the method were studied by means of recovery experiments at three spiking levels, MLOQ, 4 x MLOQ and 8 x MLOQ (ML) (i.e. 0.25 μg kg^{-1}, 1.0 μg kg^{-1} and 2.0 μg kg^{-1}). Lard, peanut, sunflower seed and sesame oil were selected as the representative matrices in the validation. All recovery experiments were performed three times at each level as suggested by the EC No 333/2007. The overall performance of initial validation was summarized into 4 edible oil and fat matrices in Table 3. The average recoveries were ranged from 86 to 114% and average coefficients of variation were (CV) below 10%, which fulfilled the EC No 333/2007 recommendations. Linearity was verified through calibration curves of six concentration levels from 0.4 and 250 μg L^{-1} (corresponding to 0.1 to 62.5 μg kg^{-1} in sample). The coefficients of determination (R^2) were found to be >0.995.

The trueness of the method was demonstrated by analyzing a FAPAS quality control material, palm oil. The results of analyses of each PAHs are given in Table 4 and demonstrated they comply well with the assigned range as specified by the producer.

On-going performance of the method was monitored by recovery experiments of real sample spikes at ML during the real sample studies. Within-laboratory reproducibility, expressed as standard deviation on on-going performance of the method, was found to be less than 10% for 4 analytes. Hence, the robustness of the method was also demonstrated.

3.5 Codex Alimentarius Commission's requirements

With reference to Codex's procedure manual that established working instruction for the implementation of the criteria approach, the general requirements for analyte level lower than 0.1 mg kg^{-1} were verified against the method performance. As specified in the procedure manual, the limit of detection (LOD) and limit of quantitation (LOQ) should be equal to or lower than 1/5 and 2/5 of the ML (2.0 μg kg^{-1}) respectively. Hence, the LOD and LOQ should be lower than 0.4 and 0.8 μg kg^{-1} respectively for edible oils and fats on whole weight basis. Therefore, the established LOD and LOQ (i.e. 0.1 and 0.25 μg kg^{-1}) of the method achieved and fulfilled Codex's requirements for regulatory enforcement. For the Codex's requirements on precision and recovery, the method performance as mentioned above fulfilled the theoretical relative standard deviation and the expected recovery of 22 % and 60 to 115 % respectively.

4. Application of the method to real samples

The developed method was applied to the analysis of 88 edible oils and fats. In order to assure the quality of the results, reagent blank (obtained by performing the whole procedure without sample) was used to remove any possibility of false positive due to contamination in the instruments or reagents employed. Replicate analysis of spiked samples at ML was also performed to assess the extraction efficiency, spike recovery as well as precision. Despite the wide variety of sample matrices spiked (n=22) in this study, the worst individual single recoveries fell within the range of 84 – 118% for BaA in a soybean oil and BaP in a vegetable oil respectively, which matched the generalized acceptable range for routine PAHs analysis as stipulated in the EC No 333/2007.

The BaP and PAH4 content in 88 samples against EU regulatory limits were summarized in Table 5. For PAH4, most of them were found to contain trace amount of at least one of them, except for 15 analyzed samples. Only 45 out of 88 samples were found to contain detectable amount of BaP but less than 10 μg kg^{-1}, seven of them were found to greater than 2 μg kg^{-1}. Hence, all the samples complied with Chinese standard, but not EU regulatory limit on BaP. For PAH4, fifteen of them contained the total content greater than 10 μg kg^{-1}. Only five of them were exceeded the ML as set by the EU on BaP and PAH4 too. They were three peanut oils, one sesame oil and one soybean oil. Based on the above-mentioned results, peanut oil, sesame oil and soybean oil could contain higher level of BaP for maintaining typical nutty flavours during oil processing.

Amongst the 12 lard samples, none was found to contain detectable BaP, i.e. below LOD. Similarly, only one out of 13 olive oil got trace amount of BaP. For rapeseed (canola) oil, their BaP contents fully complied with EU's regulated levels of 2 μg BaP kg^{-1} and 10 μg PAH4 kg^{-1}. A total of 27 soybean and 5 vegetable oil samples were also analyzed and their BaP contents still complied with EU's regulated level after accounted for measurement uncertainty. Sunflower seed oil seems to be another problematic oil, 2 out of 3 samples got high level of BaP content of greater than 2 μg kg^{-1}. Nevertheless, the level of BaP in oil should be much reduced after oil refining processes (based on the deodorization step) and the final level depending on the refining conditions adopted. In general, the finding is similar to that of EFSA published in 2008. Amongst others, 7.3% of edible oils and fats was found to have BaP content exceeded the maximum level of 2 μg kg^{-1}.

5. Conclusions

A simple, rapid and environmental friendly analytical method employing single SPE extraction cum clean-up that allows efficient and matrix effect free extraction and enrichment of 4 EU regulated PAHs from various edible oils and fats has been developed and validated. Combined with GC-MS, the method achieves MLODs in the sub μg kg^{-1} concentration range for 4 target analytes in a wide range of edible oils and fats. Compared to published methods for PAHs analysis, the method presented here represents a significant step forward with respect to:

- Applicability. The rigorous extraction cum clean-up approach exploiting the lipophilic properties of PAHs makes the method applicable to a wide range of edible oils and fats.
- Sensitivity. MLOQ is sufficient low for regulatory enforcement.
- Reliability of results. This was demonstrated with results of a FAPAS quality control sample.
- Environmental friendliness. Only small volume of acetonitrile, acetone and negligible amount of isooctane were used for each sample.

Besides, the method has successfully determined PAHs in the various edible oils and fats. Furthermore, the method performance of this method also satisfied with the criteria of EC No 333/2007. In conclusion, up to now, the developed method is one of the few reported rapid, simple and environmental friendly methods that can determine PAH4 in edible oils and fats and fulfil the required method performance criteria as set by the Codex.

6. References

1. World Health Organization (WHO) (1998). Environmental Health Criteria 202, Selected Non-heterocyclic PAHs Geneva

2. European Food Safety Authority (EFSA) (2008). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food Question N° EFSA-Q-2007-136 The EFSA Journal 724:1-114

3. International Agency for Research on Cancer (IARC) (2012). Benzo[a]pyrene In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100F A Review of Human Carcinogens: Chemical Agents and Related Occupations

4. Wu XM, Wu WJ (2012) Liquid–liquid extraction of polycyclic aromatic hydrocarbons in four different edible oils from China. Food Chemistry 134 (1):597-601

5. Jung SY, Park JS, Chang MS, Kim MS, Lee SM, Kim JH, Chae YZ (2013) A Simple Method for the Determination of Polycyclic Aromatic Hydrocarbons (PAH) in Edible Oil Employing Solid Phase Extraction (SPE) Cartridge Purification. Food Sci Biotechnol 22 ((S)):241-248

6. European Commission. Background document to the opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food. Polycyclic Aromatic Hydrocarbons – Occurrence in foods, dietary exposure and health effects. European Commission; December 2002

7. Korea Food and Drug Administration. Food Code Article 2. Common Standards & Specifications for General Foods.

8. Chinese National Food Safety Standard GB2762-2012 《General Standard of Contaminants in Foods 》 (2012).

9. Moret S, Conte LS (2000) Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. J Chromatogr A 882 (1-2):245-253

10. Barranco A, Alonso-Salces RM, Bakkali A, Berrueta LA, Gallo B, Vicente F, Sarobe M (2003) Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. J Chromatogr A 988 (1):33-40

11. Moret S, Conte LS (2002) A rapid method for polycyclic aromatic hydrocarbon determination in vegetable oils. Journal of Separation Science 25 (1-2):96-100

12. Bogusz MJ, El Hajj SA, Ehaideb Z, Hassan H, Al-Tufail M (2004) Rapid determination of benzo(a)pyrene in olive oil samples with solid-phase extraction and low-pressure, wide-bore gas chromatography-mass spectrometry and fast liquid chromatography with fluorescence detection. J Chromatogr A 1026 (1-2):1-7

13. Cortesi N, Fusari P (2005) Developments in the determination of polycyclic aromatic hydrocarbons in vegetable oils. Rivista Italiana delle Sostanze Grasse 82 (5):167-172

14. Purcaro G, Moret S, Conte LS (2008) Rapid SPE-HPLC determination of the 16 European priority polycyclic aromatic hydrocarbons in olive oils. J Sep Sci 31 (22):3936-3944

15. Veyrand B, Brosseaud A, Sarcher L, Varlet V, Monteau F, Marchand P, Andre F, Le Bizec B (2007) 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. J Chromatogr A 1149 (2):333-344

16. Zhao Q, Wei F, Luo YB, Ding J, Xiao N, Feng YQ (2011) Rapid magnetic solid-phase extraction based on magnetic multiwalled carbon nanotubes for the determination of polycyclic aromatic hydrocarbons in edible oils. J Agric Food Chem 59 (24):12794-12800

17. Gomez-Ruiz JA, Cordeiro F, Lopez P, Wenzl T (2009) Optimisation and validation of programmed temperature vaporization (PTV) injection in solvent vent mode for the analysis of the 15+1 EU-priority PAHs by GC-MS. Talanta 80 (2):643-650

18. Supleco Extraction & Analysis of PAHs in Olive Oil Using SupelMIP™ SPE – PAHs and GC-MS. Application Note 192

19. Ziegenhals K, Hubschmann HJ, Speer K, Jira W (2008) Fast-GC/HRMS to quantify the EU priority PAH. J Sep Sci 31 (10):1779-1786

Figure 1. Extracted ion chromatogram of 4 EU-priority PAHs of a standard solution at 2 µg L^{-1} (corresponding to 0.5 µg kg^{-1} in sample).

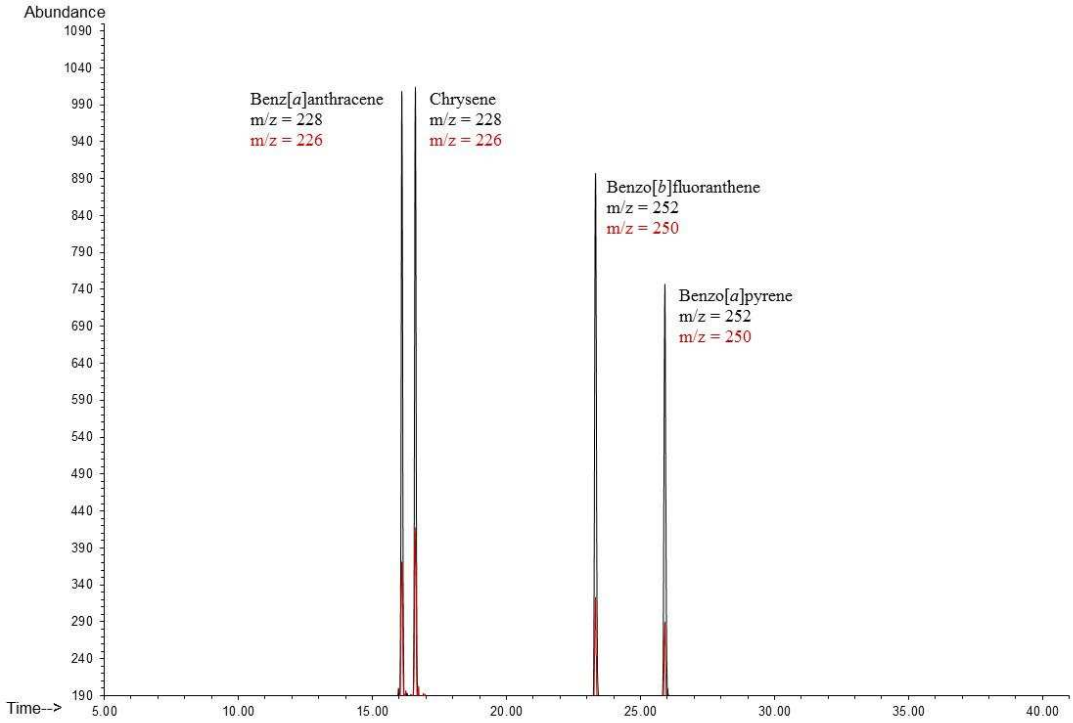


Figure 2. Elution profile of 4 EU priority PAHs with EZ-POP NP.

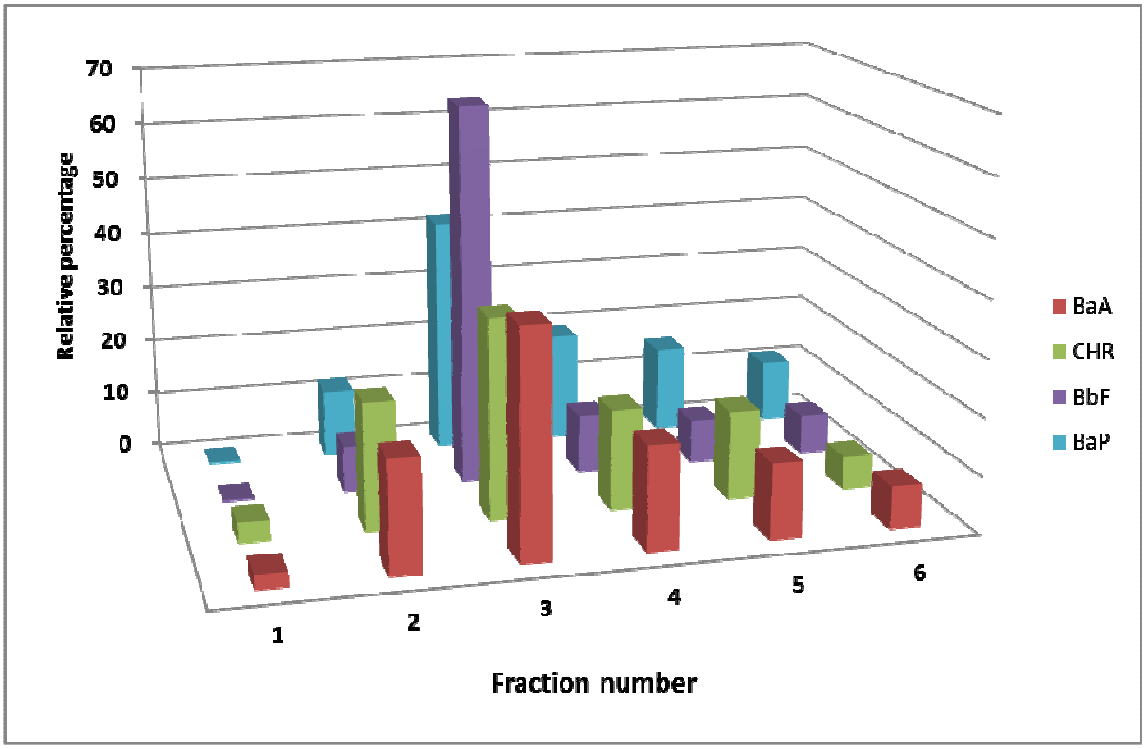
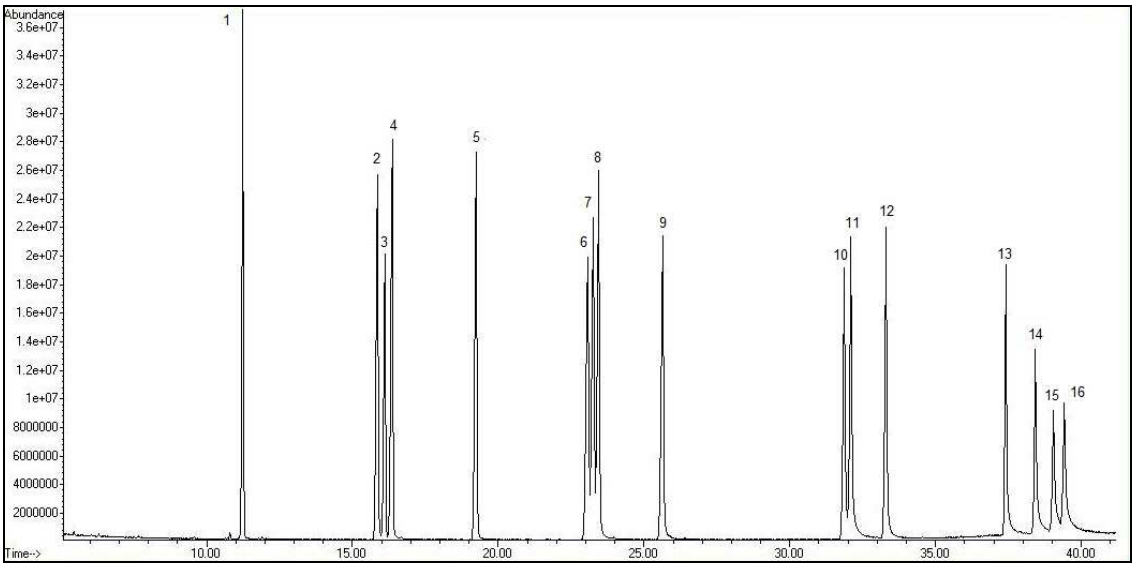


Figure 3. Total ion chromatogram of 15+1 EU-priority PAHs at 250 μg L^{-1}.



Note: 1: Benzo[*c*]fluorene; 2: Benz[*a*]anthracene; 3: Cyclopenta[*c,d*]pyrene; 4: Chrysene; 5: 5-Methylchrysene; 6: Benzo[*b*]fluoranthene; 7: Benzo[*k*]fluoranthene; 8: Benzo[*j*]fluoranthene; 9: Benz[*a*]pyrene; 10: Indeno[*1,2,3-cd*]pyrene; 11: Dibenzo[*a,h*]anthracene; 12: Benzo[*g,h,i*]perylene; 13: Dibenzo[*a,l*]pyrene; 14: Dibenzo[*a,e*]pyrene; 15: Dibenzo[*a,i*]pyrene; 16: Dibenzo[*a,h*]pyrene.

Table 1. Gas Chromatography Mass Spectrometry SIM Table.

Time window	Start time (min)	Analyte (native or IS)	Retention time (min)	Dwell time (ms)	Q0	Q1	Response ratio
1	14.0	d_{12}-BaA (IS)	15.96	100	240		
		BaA	16.10	100	228	226	0.27
		d_{12}-CHR (IS)	16.44	100	240		
		CHR	16.60	100	228	226	0.31
2	22.5	d_{12}-BbF (IS)	23.18	100	264		
		BbF	23.34	100	252	250	0.24
		d_{12}-BaP (IS)	25.76	100	264		
		BaP	25.93	100	252	250	0.24

Table 2. Matrix effects ($Slope_{matrix}/Slope_{solvent}$) in different edible oils and fats.

PAH	Lard	Peanut oil	Sunflower seed oil	Sesame oil
BaA	0.99	1.03	1.00	1.00
CHR	0.97	0.98	1.03	1.06
BbF	0.98	1.01	1.00	0.97
BaP	1.00	0.97	0.98	1.05

Table 3. Spiked recoveries (n=3) results in different edible oils and fats conducted at MLOQ, 4 x MLOQ and 8 x MLOQ (ML) levels, i.e. 0.25, 1.0 and 2.0 μg kg^{-1}, respectively.

Matrix	Spike level (μg kg^{-1})	Mean Recovery			
		BaA	CHR	BbF	BaP
Lard	0.25	101	110	89	95
	1	91	104	86	90
	2	100	101	88	93
Peanut oil	0.25	111	98	90	100
	1	103	100	102	96
	2	105	99	91	91
Sunflower seed oil	0.25	107	108	98	110
	1	99	104	87	95
	2	104	103	89	93
Sesame oil	0.25	102	114	107	106
	1	94	100	86	106
	2	99	109	89	100
Mean		**101**	**104**	**92**	**98**
RSD (%)		**5.5**	**5.2**	**7.7**	**7.0**

Table 4. Validation data obtained from FAPAS T0657QC, palm oil, (n=2).

PAH	Run 1	Run 2	Mean value ($\mu g\ kg^{-1}$)	Assigned value ($\mu g\ kg^{-1}$)
BaA	1.4	1.5	1.45	1.51 ± 0.67
CHR	1.9	2.0	1.95	1.91 ± 0.83
BbF	1.5	1.6	1.55	1.48 ± 0.65
BaP	1.2	1.0	1.15	1.10 ± 0.49

Table 5. Summary of compliance of 88 real samples to EU regulatory limits.

	Filled EU requirements		Exceeded EU requirements	
	BaP $\leqq$ 2 μg kg^{-1}	PAH4 $\leqq$ 10 μg kg^{-1}	BaP > 2 μg kg^{-1}	PAH4 > 10 μg kg^{-1}
Coil oil	2	2	---	---
Grapeseed oil	1	1	---	---
Olive oil	13	13	---	---
Peanut oil	3	1	3	5
Rapeseed oil	12	12	---	---
Sesame oil	6	5	1	2
Soybean oil	26	20	1	7
Sunflower seed oil	1	3	2	---
Vegetable oil	5	4	---	1
Lard	12	12	---	---