Food Analytical Methods
https://doi.org/10.1007/s12161-018-1240-z

Multi-Walled Carbon Nanotubes as Effective Sorbents for Rapid Analysis of Polycyclic Aromatic Hydrocarbons in Edible Oils Using Dispersive Solid-Phase Extraction (d-SPE) and Gas Chromatography—Tandem Mass Spectrometry (GC-MS/MS)

D. Zacs[1] · I. Rozentale[1] · I. Reinholds[1] · V. Bartkevics[1]

Received: 22 August 2017 / Accepted: 16 March 2018

Abstract
A method was developed for the analysis of four polycyclic aromatic hydrocarbons regulated in the European Union, namely, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene in edible oils. Multi-walled carbon nanotubes were applied for dispersive solid-phase extraction of analytes from the oils after dilution with *n*-hexane followed by elution of analytes with toluene under reflux conditions. Gas chromatography coupled to tandem mass spectrometry was used for quantitative determination of the analytes. The efficiency of four nanotube-based sorbents was evaluated. The performance characteristics of the method permitted reliable measurement of four regulated polycyclic aromatic hydrocarbons in oil samples according to the criteria stated in the Commission Regulation No. 836/2011 and considering the maximum levels (MLs) stated in the Commission Regulation (EU) No. 835/2011. The concentrations of the selected analytes in real-world samples determined by our developed method and by previously validated gel permeation chromatography method were found to be in a good agreement.

Keywords Multi-walled carbon nanotubes · Polycyclic aromatic hydrocarbons · Dispersive solid-phase extraction · Edible oils · GC-MS/MS

Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise a large group of ubiquitous compounds, many of which are toxic. PAHs are formed during the combustion and high-temperature pyrolysis of organic materials and have been found in various matrices, including many types of food products. Despite the structural diversity of PAHs, only selected representatives are considered to be toxicologically significant, among which benzo[*a*]anthracene (B[a]A), chrysene (Chr), benzo[*b*]fluoranthene (B[b]F), and benzo[*a*]pyrene (B[a]P) are recognized as a subgroup of four priority PAHs (PAH4) that are regulated in food products in EU member states according to Commission Regulation (EU) No. 836/2011 (Commission Regulation (EU) No. 836/2011b). A number of studies have identified food as one of the most important pathways for the exposure of humans to PAHs, and taking into account their lipophilic nature and sources of origin, edible oils can be highly susceptible to occasional contamination with PAHs (Moret and Conte 2000). Edible oils are consumed in high volumes and used as indispensable ingredients in food formulations (Dennis et al. 1991), with thousands of food industry facilities worldwide producing millions of metric tons of edible oils annually. Thus, in order to protect consumers from the hazards of PAHs, the control of these contaminants in oils is of major importance and food testing laboratories must ensure high sample throughput and testing speed in order to provide a timely warning to the producers and relevant authorities in the case of a contamination crisis. For this reason, laboratories should be provided with rapid, sensitive, reliable, and cost effective analytical methods for the determination of PAHs.

A large number of analytical methods for the determination of PAHs in edible oils have been proposed over the years,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12161-018-1240-z) contains supplementary material, which is available to authorized users.

✉ D. Zacs
dzintars.zachs@gmail.com

[1] Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes iela 3, Riga LV-1076, Latvia

Published online: 27 March 2018

relying on different pre-concentration and purification procedures. The main obstacle in the analysis of PAHs in oils is that the major constituents of these matrices are high molecular weight compounds (e.g., triglycerides and fatty acids); thus, laborious procedures are needed in order to separate analyte fractions from the matrix and to achieve the desired purity of the final extract. Gel permeation chromatography (GPC) as well as fat saponification have been recognized as the most efficient cleanup methods in the analysis of foods with high fat content (Plaza-Bolanos et al. 2010). However, these methods are time-consuming and additional cleanup steps (e.g., solid phase extraction (SPE) or liquid - liquid extraction (LLE)) are typically needed. Therefore, the search for alternative analytical protocols is still of great relevance.

The application of multi-walled carbon nanotubes (MWCNTs) as sorbents for SPE in modern analytical methods provides new opportunities for design of rapid processing and selective isolation of analytes (Ravelo-Pérez et al. 2010; Song et al. 2017). Considering the highly hydrophobic properties of MWCNTs and their unique tubular structure with the ability for π-stacking (Pan et al. 2005), these nanomaterials provide superior potential for sorption of aromatics, with a special emphasis on planar compounds. Thus, affirming the effective sorption of planar aromatics, MWCNTs were found to be much more effective sorbents of polychlorinated dibenzo-*p*-dioxins in comparison to activated carbon (Yu et al. 2000). Despite the great potential of MWCNTs for selective sorption of planar aromatic contaminants, only isolated references report the application of these nanomaterials as sorbents for such planar molecules as PAHs, and only handful among them have been devoted to the extraction of fatty matrices (Zhao et al. 2011; Herrera-Herrera et al. 2012; Ma et al. 2010; Menezes et al. 2015; Moazzen et al. 2013; Paszkiewicz et al. 2017; Wu et al. 2010). Therefore, this application of MWCNTs in analytical chemistry remains insufficiently investigated. The principal aim of the current study was to elaborate an analytical method for the determination of PAHs in edible oils by applying dispersive SPE (d-SPE) with MWCNTs as sorbents for selective extraction of analytes, thus eliminating the most common time-consuming techniques (e.g., GPC, saponification), followed by detection with gas chromatography—tandem quadrupole mass spectrometry (GC-MS/MS). An additional advantage of the developed method is the application of semiautomatic instrumentation, which facilitates the desorption procedure and provides the capability of parallel sample processing. The whole procedure was optimized, including the type of MWCNTs, as well as the extraction and desorption conditions. The described method met the sensitivity criteria stated in Commission Regulation (EU) No 836/2011 (Commission Regulation (EU) No. 836/2011b) and confidence in the methodology was also provided by the analysis of reference material (RM). The method was applied for the analysis of a number of real samples and the results were in good agreement with those obtained by using the established method based on a GPC cleanup protocol (Rozentale et al. 2015). It is anticipated that the developed method may be applicable not only to the edible oils, but also to other fatty matrices and to different analytes of planar chemical structure.

Experimental

Chemicals and Materials

Four individual PAH standards (B[*a*]A, Chr, B[*b*]F, and B[*a*]P) (50 ng μL^{-1} of each in acetonitrile) and isotopically labeled deuterated surrogates serving as internal standards (B[*a*]A-d_{12}, Chr-d_{12}, B[*b*]F-d_{12}, and B[*a*]P-d_{12}) (1 ng μL^{-1} of each in cyclohexane) were obtained from LGC Standards (Bury, UK). Stock solutions were prepared in toluene and were stored at − 18 °C in amber colored glassware. Pesticide grade solvents (cyclohexane, *n*-hexane, toluene, *m*-xylene, acetone, and ethyl acetate (EtOAc)), as well as Celite-545 were purchased from Sigma-Aldrich (Steinheim, Germany). Calibration solutions were prepared by serial dilution of stock solutions in toluene. Four different types of MWCNTs were employed as potential sorbents for d-SPE. Baytubes® C150P MWCNTs (further denoted as CNT-1) were obtained from Covestro AG (Leverkusen, Germany) with the purity greater than 95%, outer diameter (O.D.) of 13 nm, internal diameter (I.D.) of 4 nm, length > 1 μm, and the density range of 1.3–1.5 g/cm^3 (according to manufacturer's specifications). Other three MWCNTs—TNIM4 (abbreviated as CNT-2), TNIMH4 (CNT-OH; hydroxy group content 2.48 wt.%), and TNIMC4 (CNT-COOH; carboxyl group content 1.55 wt.%) were purchased from Chengdu Organic Chemicals Company (Sichuan Sheng, China). The latter three MWCNTs were of > 95% purity, with 5–10 nm I.D., 10–30 nm O.D., 10–30 μm length, and the average density of 2.1 g/cm^3.

Samples

Fifteen edible oil samples were analyzed by using the elaborated method. All samples were collected during the period from February to May 2017 within the framework of a monitoring program for the control of PAHs in food products. The samples were transported to the laboratory while protected from ambient UV radiation. To avoid potential chemical composition changes, the samples were immediately processed upon arrival and the final extracts were stored at − 20 °C prior to the instrumental analysis.

Sample Extraction and Cleanup

Preparation of MWCNT-Based Sorbents for d-SPE and Control of Background Contamination

Taking into account that relatively small amounts of MWCNTs are required for sufficient recovery of PAHs from the matrix aliquot, and in order to facilitate handling operations, MWCNTs were dispersed in Celite-545 at the ratio of 5/95 (*w*/*w*). Considering the affinity of MWCNTs towards planar aromatic compounds and the ubiquity of PAHs, there was a need to control the probable background contamination of SPE sorbents based on MWCNTs. Procedural blanks consisting of the materials and chemicals included in the analytical protocol were treated in the same manner as real samples. Procedural blanks showed the background presence of significant quantities of PAHs in commercially available MWCNTs selected for the current study. For example, applying 50 mg of untreated MWCNTs for the extraction of 1 g of oil resulted in elevated B[*a*]A concentrations by up to 0.6 ng g^{-1} for CNT-1, 0.8 ng g^{-1} for CNT-2, 1.7 ng g^{-1} for CNT-COOH, and 2.5 ng g^{-1} for CNT-OH. The source of this contamination is unknown; however, this fact should be considered during the application of MWCNTs as sorbents in PAH analysis. In order to avoid background contamination, the prepared MWCNT-based sorbent mixtures were washed with hot toluene under reflux conditions for 48 h, dried overnight at room temperature under aluminum foil and heated at 115 °C for 4 h. The sorbents pre-treated according to this procedure did not show signals of the selected PAHs and were applied for further analyses.

Sample Extraction and Clean-up

A 1.0 g aliquot of oil sample was placed in a 15 mL glass tube, spiked with 100 μL of isotopically labeled PAH surrogates in toluene (100 pg μL^{-1} of each surrogate) and *n*-hexane (10 mL) was added. The sample was vortexed and pre-treated d-SPE sorbent (1.0 g) consisting of 50 mg of MWCNTs and 950 mg of Celite-545 was added. After performing the d-SPE procedure by vigorous shaking of the glass tube for 25 min, the sample was centrifuged at 3000 rpm for 5 min and the upper *n*-hexane phase was eliminated. Subsequently, a 10 mL portion of washing solvent (cyclohexane, *n*-hexane, acetone, and EtOAc were tested) was added, and after vigorous shaking for 5 min, the centrifugation was repeated and the washing solvent was removed. The resultant d-SPE sorbent was dispersed in 5 mL of toluene and the obtained slurry was quantitatively transferred into an extraction thimble for the semi-automatic Soxtec™ 2055 Extraction System (Hillerød, Denmark), in which up to six samples could be loaded in parallel. PAHs were eluted from the d-SPE sorbent into a glass vessel with toluene (35 mL) according to the following program: immersion of the sample in refluxing solvent for 15 min with further rinsing of the thimble for 60 min, followed by careful elimination of the bulk of the solvent for 15 min. The residue was quantitatively transferred with *n*-hexane (5 mL) to a 10 mL glass tube and complete solvent evaporation was performed under a gentle stream of nitrogen. The resulting sample was reconstituted in toluene (50 μL) containing benzo[ghi]perylene-d_{12} (200 pg μL^{-1}) as recovery standard and analyzed by the means of GC-MS/MS.

GC-MS/MS Analysis

Instrumental analysis of the target analytes was carried out on a Thermo Scientific Trace GC Ultra gas chromatograph coupled to a Thermo Scientific TSQ Quantum XLS Ultra mass selective detector. The system was equipped with a Select PAH (Agilent Technologies, Santa Clara, CA, USA) capillary column (30 m × 0.25 mm I. D., 0.15 mm film thickness) and operated in splitless mode. The GC operating conditions were as follows: helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}, inlet temperature 260 °C, MS transfer line temperature 280 °C. The oven temperature was set initially at 80 °C (2 min hold) and then increased to 265 °C at 15 °C min^{-1}. At 265 °C the temperature was increased at a rate of 5 °C min^{-1} to 290 °C and then to 320 °C at a rate of 20 °C min^{-1} (20 min hold). The injection volume was 1 μL. The tandem quadrupole mass spectrometer was operated in positive ion mode with electron impact (EI) ionization at electron energy of 70 eV. The emission current was 50 mA and the source temperature was 250 °C. The collision gas was argon, supplied at 1.0 mTorr pressure in the collision chamber. The system was controlled by Xcalibur software (ThermoScientific). The data were acquired by operating the mass spectrometer in selective reaction monitoring (SRM) mode, while using $[M]^+ \rightarrow [M–2H]^+$ transitions for quantification and $[M]^+ \rightarrow [M–2CH]^+$ transitions for confirmation of the selected PAHs. Collision energy of 30 eV was found to be optimal for all selected ion transitions.

Quality Assurance/Quality Control

Identification criteria for the analytes were based on the retention times of native PAHs and deuterated PAH surrogates and the isotopic peak ratios of the SRM transitions. The acceptable deviation for the isotopic peak ratio of two monitored ions or SRM transitions (target/confirmation) was within 15% of the value obtained for the medium calibration point. A five-point calibration curve was checked with relative response factors (RRFs) over the sample concentration range of 0.10–10.0 ng g^{-1} and was used for quantifying the analytes of interest in each sample run. Procedural blanks were taken through all steps of the analytical procedure and analyzed in each sample sequence. The quantification of analytes of

interest was based on stable isotope dilution with the deuterated PAH surrogates and on internal standardization.

Validation of the Analytical Method

The proposed method for determination of PAHs was validated while taking into account the Commission Regulations (EU) No. 836/2011, 2015/1933, and 2016/582 (Commission Regulation (EU) No. 836/2011b; Commission Regulation (EU) No. 2015/1933; Commission Regulation (EU) No. 2016/582). On the basis of these regulations, the limits of detection and quantification (LOD and LOQ), as well as the recovery and precision of the analytical procedure, were established. The method LODs and LOQs were calculated according to the Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food (Wenzl et al. 2016).

Recovery experiments were performed by spiking corn oil with PAHs at two different concentrations 1.0 and 2.0 μg kg^{-1}. Each spiking level was repeated ($n = 5$), resulting in 2 × 5 individual sample analyses. A linear calibration function including equal weighting index was applied. The correlation coefficient (R^2) values were calculated for seven spiking levels (0.10, 0.20, 0.40, 0.60, 1.0, 5.0, and 10.0 μg kg^{-1}). Recoveries were calculated as the differences in PAH content in spiked and non-spiked samples relative to the spiking level. For these recovery analyses, the repeatability of the method was expressed as the relative standard deviation (RSD) in %. In accordance with Commission Regulation (EU) No. 836/2011 (Commission Regulation (EU) No. 836/2011b), the $HORRAT_r$ values are regarded as a measurement of the method precision. The $HORRAT_r$ values were calculated for all four selected PAHs analyzed at each of the two different concentrations.

Results and Discussion

Experiment design for the optimization of extraction and clean-up procedures included the following steps: (1) selection of organic solvent for the dilution of oil samples to enhance the analyte sorption efficiency from the matrix (the parameter was assessed as a decrease of analyte concentration in the solution that was treated with MWCNT sorbent and was expressed as sorption efficiency (%)), (2) selection of an appropriate solvent for analyte desorption (the solvent from which analytes were poorly adsorbed and maximally remained in the solution was regarded as optimal), (3) selection of the MWCNT sorbent type, considering (a) the sorption capacity of each MWCNT type towards PAHs in the presence of matrix; (b) the effectiveness of PAH desorption from the MWCNTs (expressed as recovery of analytes after desorption step), (c) the affinity of MWCNTs towards matrix co-extractives (expressed as the amount of matrix co-extractive residue observed after treatment of the matrix with MWCNT sorbent) and selection of solvent for the washing step in order to reduce the possible influence of matrix co-extractives (expressed as recovery of analytes after desorption step if washing step was included). All experiments devoted to the optimization of extraction procedure were carried out using deuterated PAH surrogates as target compounds in order to eliminate the influence of the possible native PAH background in the laboratory environment and considering that isotopically labeled surrogates behaved analogously to the analytes in the presence of matrix components.

Selection of Solvents for Sample Dilution and Analyte Desorption

One of the most convenient ways for providing better transfer of analytes from the oil matrix to d-SPE sorbent is decreasing the viscosity of the sample by dilution with an appropriate organic solvent (dilution solvent). Selection of such solvent should be based on the following aspects: (a) miscibility of the sample matrix in the solvent, (b) complete solubility of analytes in the solvent, (c) the affinity of sorbent for the solvent molecules should be as low as possible in comparison to the affinity for analyte molecules, in order to ensure quantitative sorption of the analytes from the sample solution. Contrary to the case of sample dilution solvent, the affinity of sorbent for the desorption solvent molecules should be at least comparable to the affinity for analyte molecules, providing for competitive desorption of the analyte. Six organic solvents were tested as possible sample dilution and analyte desorption media and the results of sorption experiments expressed as analyte sorption efficiency are illustrated in Fig. 1. The best results among the tested aliphatic compounds as sample dilution solvents were obtained with *n*-hexane, from which the selected PAHs were almost completely adsorbed on the MWCNTs. Contrary to the previous study on the application of MWCNTs as sorbents in the analysis of PAHs in food products (Moazzen et al. 2013), in which the authors stated that PAHs could be desorbed from the MWCNTs even with polar solvents (e.g., methanol), the results of our experiments showed acceptable desorption of PAHs only by applying solvents of aromatic nature. Among the two tested aromatic desorption solvents, toluene was found to be more effective in comparison to *m*-xylene and provided better recovery of analytes from the MWCNT sorbents. A probable explanation for this effect is the stronger π-π interaction between toluene molecules and MWCNTs due to the smaller size of toluene molecules in comparison to *m*-xylene.

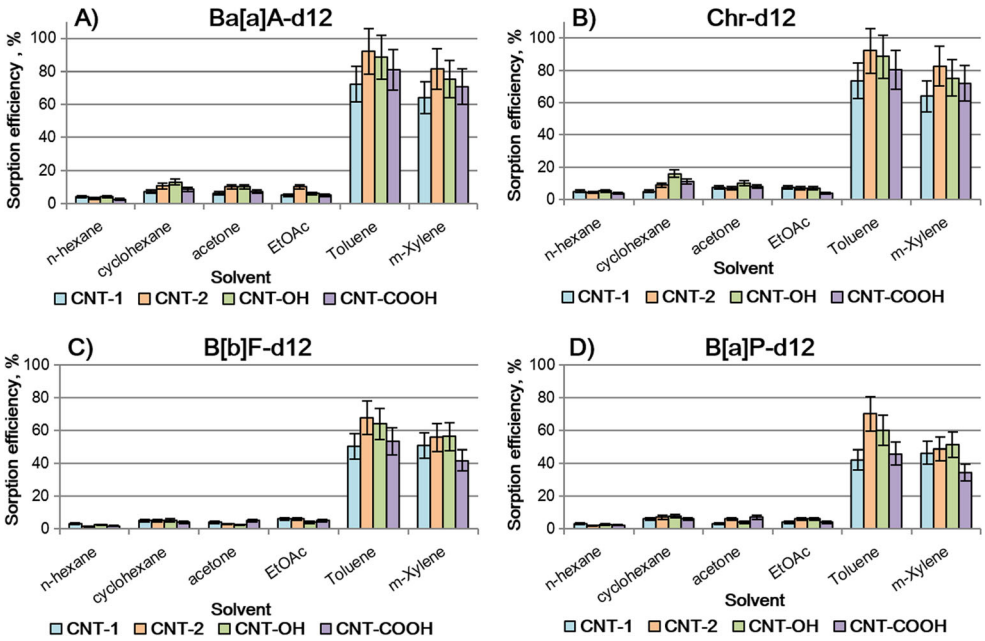

Fig. 1 Sorption of the analytes on different MWCNTs from standard solutions in selected organic solvents

Selection of MWCNT Sorbent

Adsorption of PAHs on Different MWCNTs

In order to assess the sorption potential of MWCNTs towards PAH analytes in the presence of matrix components, the capacity of each MWCNT type was evaluated in terms of recovery of PAHs-d_{12} from aliquots of oil treated according to the "Sample extraction and clean-up" section ($n = 3$ for each MWCNT type). As shown in Fig. 2, generally the lowest affinity towards the selected PAHs was observed in the case of CNT-OH nanomaterial. The other three sorbents were found to provide comparable extraction efficiency under the fully optimized conditions, with the exception of B[*a*]P-d_{12}, for which CNT-1 was less effective than CNT-2 and CNT-COOH.

Desorption Conditions and Recovery of Analytes from MWCNT Sorbents

Considering the planar structure of PAHs, the dominant sorption mechanism likely involved strong π-π electron interactions between the aromatic structure of analytes and the active surface of MWCNT sorbent (Wang et al. 2009). Despite the known benefits of π-π electron interactions for quantitative and selective sorption of aromatics, the strong affinity of MWCNT sorbents for PAH analytes resulted in poor absolute recoveries of the selected PAHs-d_{12} during the initial desorption attempts. Thus, it was found that extraction with toluene under sonication conditions was not effective for the desorption of analytes from the MWCNT sorbent, while acceptable recoveries of the selected analytes could be obtained by direct elution with toluene. It should be noted that in the case of applying direct elution of the analytes from the MWCNT sorbent the necessary volume of toluene was very high (up to 200 mL per sample) and was similar to the volume typically applied to elute the fraction of planar molecules from activated carbon in the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans, where analyte sorption is based on a similar π-π electron interaction mechanism (Fernandes et al. 2011; Zacs et al. 2015). In order to overcome this drawback and to reduce the necessary volume of elution solvent, washing with hot toluene under reflux conditions was applied for analyte elution from the MWCNT sorbent. Although the reflux procedure required only a few mL of solvent to be in contact with the treated sorbent, the design features of the extraction system required 35 mL of toluene to be used for the desorption procedure. It was found that the effectiveness of PAH elution from the MWCNTs increased with decreasing molecular weights of the analytes, showing absolute recoveries in the following order: B[*a*]A-d_{12} ~ Chr-d_{12} > B[*b*]F-d_{12} ~ B[*a*]P-d_{12}. The degree of analyte desorption was found to be highly dependent on the duration of refluxing, with 60 min extraction with toluene under reflux conditions selected as a reasonable compromise between the desorption efficiency of target PAHs and the labor and time consumption of the procedure. The best analyte recovery rates were in the range from 75 to 90%, observed for sorbents based on CNT-2 and CNT-COOH, (Fig. 2a, b), while sorbents prepared from CNT-1 and CNT-OH

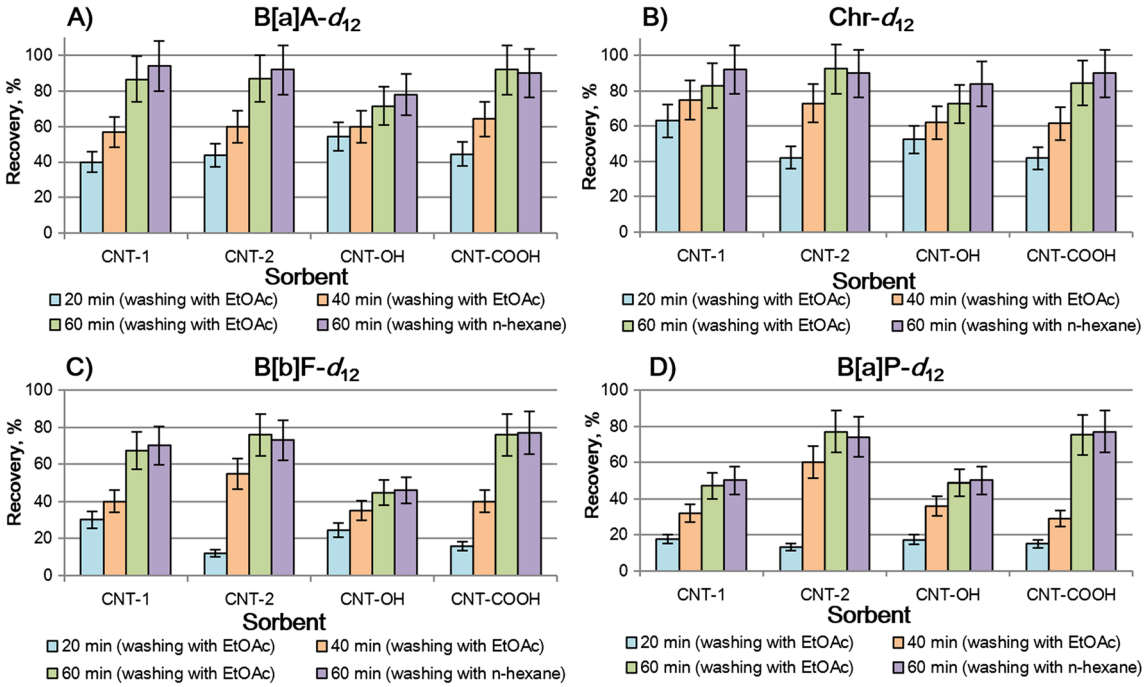

Fig. 2 Recovery rates for deuterated PAH surrogates observed with different MWCNTs, reflux times, and desorption solvents

were less suitable, providing absolute recoveries for B[*a*]P-d_{12} equal to only about 50% (Fig. 2d).

Evaluation of Coextractive Propensity of MWCNTs and the Selection of Washing Solvent

Despite the selective sorbent properties of MWCNTs, the adsorption of some matrix components of aromatic nature (e.g., dyes) could be also expected. A greater role of sorption mechanisms other than π-π electron interaction could be expected in the case of CNT-OH and CNT-COOH sorbents due to the presence of hydroxy and carboxyl groups in their structure. The affinity of the tested MWCNTs towards matrix co-extractives was evaluated by treating aliquots of oil diluted with *n*-hexane according to the procedure described in "Sample extraction and clean-up" section and further gravimetric measurement of the residual matrix remaining after the evaporation of *n*-hexane. As it was expected, the chemically modified CNT-OH and CNT-COOH sorbents were found to be more susceptible to retaining the matrix in comparison to CNT-1 and CNT-2, pointing to different sorption mechanisms. The initial extraction experiments showed that post-extraction washing of the sorbent is clearly required. As shown in Fig. 3, *n*-hexane was not sufficiently effective as a post-extraction washing solvent, while the more polar EtOAc efficiently removed the remaining matrix components from the MWCNT sorbents without significant loss of analytes (Fig. 2).

From the results reviewed in "Selection of Solvents for Sample Dilution and Analyte Desorption" and "Selection of MWCNT Sorbent" sections, it can be concluded that all of the tested MWCNTs have a great potential for the sorption of PAHs from oil samples, providing good recoveries of analytes and acceptable purity of the final extracts. Based on the superior analyte recovery rates and the lower retention of matrix components, CNT-2 was used in the final method in combination with EtOAc as a post-extraction washing solvent.

Validation

The method was validated using spiked corn oil and the results of spiking experiments were corrected by taking into account the concentrations of pseudo-blank samples. The mean concentrations of PAHs in the pseudo-blank samples were: B[*a*]A – 0.19 μg kg^{-1}, Chr – 0.41 μg kg^{-1}, B[*b*]F – 0.24 μg kg^{-1}, B[*a*]P – 0.12 μg kg^{-1}. Essential parameters such as sensitivity, selectivity, linearity, accuracy, and precision were investigated. The overview of validation parameters for the elaborated

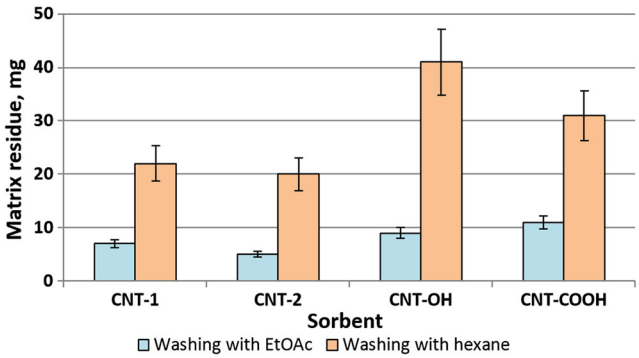

Fig. 3 Matrix residue on the tested MWCNTs after post-extraction washing with different solvents, determined by extracting 1 g of oil

method is given in Table 1. Since isotope dilution and internal standardization were used, solvent-matched calibration standards were chosen for quantitative purposes. Seven calibration levels covered the concentration range between 0.10 and 10.0 μg kg^{-1} and, in order to avoid heteroscedasticity, the calibration range was split into two parts. The lower part was applicable for LOD calculation and covered the range from 0.10 to 0.60 μg kg^{-1}, whereas the second calibration curve was set up for the whole calibration range between 0.1 and 10 μg kg^{-1}. The obtained correlation coefficients of the linear regression curves were greater than 0.998 with the residual values of less than 15% for all selected PAHs.

The calculation of method LOD was based on a single analysis of test sample, ten independent analyses of the pseudo-blank, and equal probabilities ($\alpha = ß = 0.05$) for false positive and false negative detections. The pseudo-blank samples were processed by applying the whole analytical procedure. Homoscedasticity was assumed for the analyte concentration range between LOD and the spiking level, and the probabilities of type I and type II errors (α and ß errors) were set to 0.05. The method LOD and LOQ for each analyte were calculated based on the Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food (Wenzl et al. 2016). The obtained method LODs and LOQs for single compounds were far below the levels set in Commission Regulations (EU) No. 836/2011 and 2015/1933 (Commission Regulation (EU) No. 836/2011b; Commission Regulation (EU) No. 2015/1933), with the actual values ranging from 0.06 to 0.21 μg kg^{-1} and from 0.19 to 0.71 μg kg^{-1} (Table 1), respectively.

The results of spiking experiments show that the mean recovery values for target analytes ranged from 96 to 107% and all of the obtained $HORRAT_r$ values were far below 2, thus completely meeting the criteria stated in the Commission Regulation No 836/2011 (Commission Regulation No 836/2011b), while the intra-day and inter-day precision in terms of RSDs were in the range of 2–5% and 4–6%, respectively. The expanded uncertainties for individual PAH compounds and for the combined PAH4 group were calculated for the lowest validation level (1 μg kg^{-1}) with the refined level of confidence of 95% ($k = 2$). The expanded uncertainty values for B[*a*]P and ∑PAH4 were 10% and 11%, respectively.

The accuracy of measurements or "trueness" of the method was demonstrated by the analysis of a real sample that was inter-laboratory tested for the content of selected PAHs within the framework of the "14th inter-laboratory comparison organized by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons - Four marker PAHs in food supplements". The accuracy data derived from the analyses of this material represent a good agreement of the determined concentrations with the provided consensus values (Table 1). The calculated accuracy expressed as a percentage of the measured concentration vs. the consensus value was

Table 1 Performance characteristics of the developed analytical method

Compound	Linearity, μg kg^{-1}	LOD, μg kg^{-1}	LOQ, μg kg^{-1}	1st spiking level				2nd spiking level				Analysis of fish oil CRM[d] ($n = 6$)			$HORRAT_r$	Uncertainty, %
				Spiking level, μg kg^{-1}	Recovery ($n = 5$)[a], %	Intra-day RSD_r, ($n = 5$)[b], %	Inter-day RSD_r, ($n = 5$)[c], %	Spiking level, μg kg^{-1}	Recovery ($n = 5$)[a], %	Intra-day RSD_r, ($n = 5$)[b], %	Inter-day RSD_r, ($n = 5$)[c], %	Obtained value ± SD, μg kg^{-1}	Consensus value, μg kg^{-1}	Accuracy ±RSD_r, %		
B[a]A	0.1–10	0.09	0.30	1.0	108	5	6	2.0	107	2	4	3.60 ± 0.33	3.33 ± 0.28	108 ± 9	0.34	10
Chr	0.1–10	0.21	0.71	1.0	104	3	5	2.0	105	2	4	3.45 ± 0.20	3.57 ± 0.39	97 ± 6	0.31	12
B[b]F	0.1–10	0.08	0.27	1.0	107	3	5	2.0	101	4	6	4.78 ± 0.42	4.34 ± 0.26	110 ± 9	0.38	10
B[a]P	0.1–10	0.06	0.19	1.0	98	2	4	2.0	96	4	5	3.53 ± 0.20	3.29 ± 0.19	107 ± 6	0.31	10
∑ PAH4				4.0	104	2	4	8.0	102	2	4	15.4 ± 1.15	14.5 ± 0.58	106 ± 8	0.28	11

SD standard deviation, *RSD*, relative standard deviation

[a] The average recovery (%) for selected PAHs at the corresponding spiking level, calculated from the data obtained on two different days

[b] The average intra-day precision (%) for selected PAHs at the corresponding spiking level, calculated from the data obtained on two different days

[c] The average inter-day precision (%) for selected PAHs at the corresponding spiking level, calculated from the data obtained on two different days

[d] Fish oil test material provided within the framework of the "14th inter-laboratory comparison organized by the European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons—Four marker PAHs in food supplements" (EC-JRC-IRMM, Geel, Belgium)

within the range of 97–110%, with the RSDs from 6 to 9% and the retrospectively calculated *z*-scores of 0.40 for B[*a*]A, 0.31 for Chr, 1.69 for B[*b*]F, 1.26 for B[*a*]P, and 1.52 for the sum of PAH4, respectively. Generally, considering a number of references published over the past decade (Plaza-Bolanos et al. 2010; Purcaro et al. 2013; Zelinkova and Wenzl 2016) which were devoted to the analytical methods for the analysis of PAHs in oils and fats, it can be concluded that the developed method has similar or superior performance characteristics than the majority of proposed protocols, indicating good reliability of the elaborated method for the analysis of 4 EU-regulated PAHs in edible oils.

Application to Real Samples and Comparison with the GPC Method

The applicability of the elaborated method was assessed through the analysis of selected PAHs in fifteen edible oil samples. The obtained analyte concentrations are presented in Table S1. The concentrations of PAHs observed with the MWCNT sample preparation protocol varied from 0.19 to 6.99 μg kg^{-1} for B[*a*]P and from 1.60 to 31.2 μg kg^{-1} for ∑PAH4. The concentrations of PAHs in five of the samples were above the MLs of 2.0 μg kg^{-1} for B[*a*]P and 10.0 μg kg^{-1} for ∑PAH4 stated in Commission Regulation (EU) No 835/2011 (Commission Regulation (EU) No. 835/2011a), thus the efficiency of the method was evaluated over a broad range of analyte concentrations (from 0.1× to 3.5× of the ML for B[*a*]P and from 0.15× to 3× of the ML for the ∑PAH4). A functional relationship between the data obtained by two different methods could serve as one of the approaches for the evaluation of comparability of the obtained results. In order to assess the effectiveness of the elaborated d-SPE method, all of the tested oil samples that were prepared and analyzed according to the procedure described in "Sample Extraction and Cleanup" and "GC-MS/MS Analysis" sections were additionally reanalyzed using a well-established and validated GC-MS/MS method (Rozentale et al. 2015), in which a GPC-based sample preparation procedure was applied. The functional relationship between the d-SPE and GPC methods for selected PAHs is shown in Fig. 4. Two regression curves were plotted: the bisecting line and the

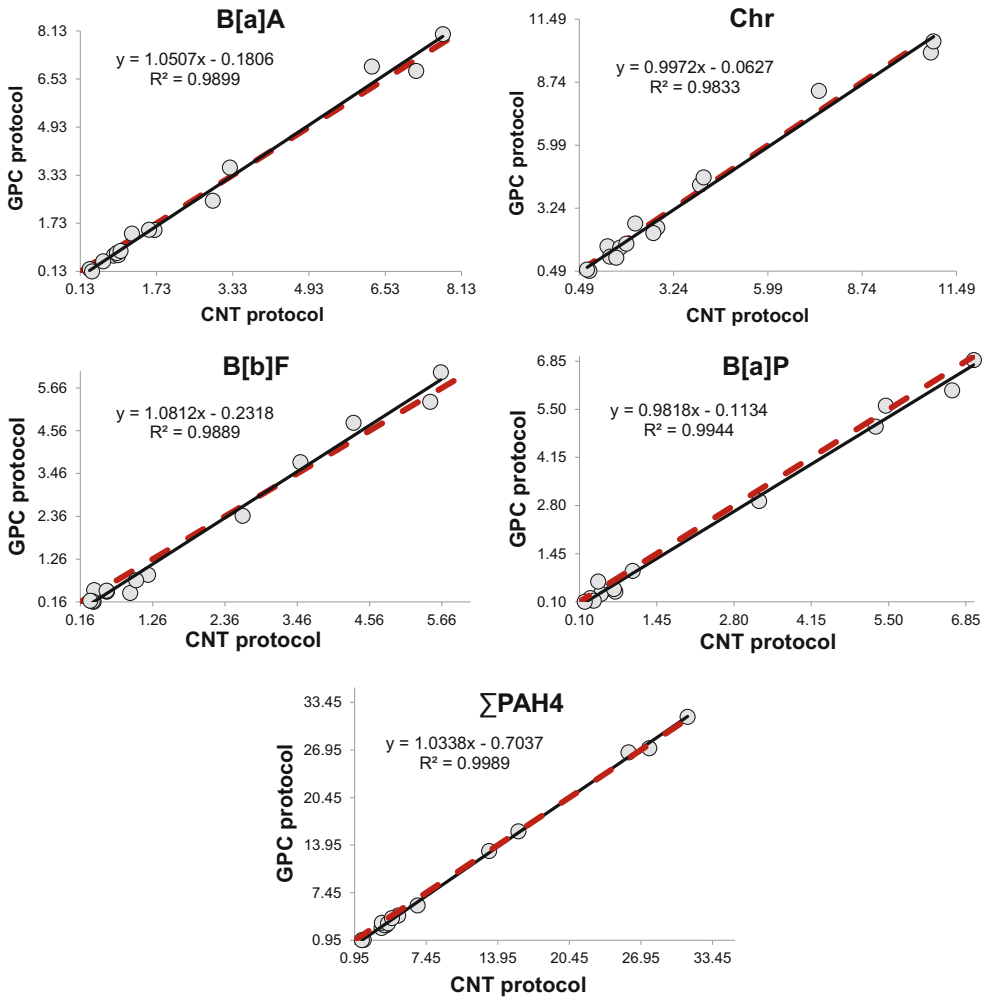

Fig. 4 Representation of the functional relationships between results obtained by the elaborated d-SPE method and GPC method for selected PAHs

functional relationship between methods calculated by the regression method. As it has been shown, all of the selected PAHs practically merged with the bisecting line and the observed bias could be acknowledged to be in the range of method uncertainty, affirming the equivalency of the developed method in terms of providing reliable results. Taking into account all of the aforementioned considerations, it can be seen that the developed method is convenient and rapid, while the whole proposed procedure using d-SPE for six parallel samples can be completed in less than 3 h.

Conclusions

A novel MWCNT-based d-SPE method has been elaborated in this study for selective extraction of four EU-regulated PAHs from edible oils. The presented methodology is selective, rapid, and met the analytical requirements stated in the Commission Regulation No 836/2011. The sorption efficiency on four types of MWCNTs was evaluated. Pre-concentration and clean-up procedures were optimized. The method was extensively validated and further confidence was provided by the analysis of reference material. To our knowledge, this is the first study on the application of MWCNTs as d-SPE sorbents for the analysis of PAHs in oil samples, which provides almost quantitative recovery of analytes in combination with minimum presence of co-extractives in the final extract. A good agreement between the results obtained for PAHs with the elaborated MWCNT method and a previously published GPC method emphasized the great potential of MWCNT-based sorbents in the analysis of these contaminants in oil samples and provides a respectable alternative to well-established sample preparation protocols.

Funding This research was co-financed by ERDF (85%) and state budget (7.5%) under the project No.1.1.1.1/16/A/258.

Compliance with Ethical Standards

Conflict of Interest Dzintars Zacs declares that he has no conflicts of interest. Irina Rozentale declares that she has no conflicts of interest. Ingars Reinholds declares that he has no conflicts of interest. Vadims Bartkevics declares that he has no conflicts of interest.

Ethical Approval This study does not contain any data obtained from human participants or animals by any of the authors.

Informed Consent Not applicable

References

Commission of the European Communities (2011a) Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. OJEU L 215:4–8

Commission of the European Communities (2011b) Commission Regulation (EU) No. 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. OJEU L 215:9–16

Commission of the European Communities (2015) Commission Regulation (EU) No 2015/1933 of 27 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in cocoa fibre, banana chips, food supplements, dried herbs and dried spices. OJEU L 282:11–13

Commission of the European Communities (2016) Commission Regulation (EU) No 2016/582 of 15 April 2016 amending Regulation (EC) No 333/2007 as regards the analysis of inorganic arsenic, lead and polycyclic aromatic hydrocarbons and certain performance criteria for analysis. OJEU L 101:3–6

Dennis MJ, Massey RC, Cripps G, Venn I, Howarth N, Lee G (1991) Factors affecting the polycyclic aromatic hydrocarbon content of cereals, fats and other food products. Food Addit Contam 8:517–530. https://doi.org/10.1080/02652039109374004

Fernandes AR, Rose M, Mortimer D, Carr M, Panton S, Smith F (2011) Mixed brominated/chlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls: simultaneous congener-selective determination in food. J Chromatogr A 1218:9279–9287. https://doi.org/10.1016/j.chroma.2011.10.058

Herrera-Herrera AV, González-Curbelo MA, Hernández-Borges J, Rodríguez-Delgado MA (2012) Carbon nanotubes applications in separation science: a review. Anal Chim Acta 734:1–30. https://doi.org/10.1016/j.aca.2012.04.035

Ma J, Xiao R, Li J, Yu J, Zhang Y, Chen L (2010) Determination of 16 polycyclic aromatic hydrocarbons in environmental water samples by solid-phase extraction using multi-walled carbon nanotubes as adsorbent coupled with gas chromatography-mass spectrometry. J Chromatogr A 1217:5462–5469. https://doi.org/10.1016/j.chroma.2010.06.060

Menezes HC, de Barcelos SM, Macedo DF, Purceno AD, Machado BF, Teixeira AP, Lago RM, Serp P, Cardeal ZL (2015) Magnetic N-doped carbon nanotubes: a versatile and efficient material for the determination of polycyclic aromatic hydrocarbons in environmental water samples. Anal Chim Acta 873:51–56. https://doi.org/10.1016/j.aca.2015.02.063

Moazzen M, Ahmadkhaniha R, Eshaghi Gorji M, Yunesian M, Rastkari N (2013) Magnetic solid-phase extraction based on magnetic multi-walled carbon nanotubes for the determination of polycyclic aromatic hydrocarbons in grilled meat samples. Talanta 115:957–965. https://doi.org/10.1016/j.talanta.2013.07.005

Moret S, Conte LS (2000) Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. J Chromatogr A 882:245–253. https://doi.org/10.1016/S0021-9673(00)00079-0

Pan CS, Xu SY, Zou HF, Guo Z, Zhang Y, Guo BC (2005) Carbon nanotubes as adsorbent of solid-phase extraction and matrix for laser desorption/ionization mass spectrometry. J Am Soc Mass Spectrom 16:263–270. https://doi.org/10.1016/j.jasms.2004.11.005

Paszkiewicz M, Caban M, Bielicka-Gieldon A, Stepnowski P (2017) Optimization of a procedure for the simultaneous extraction of polycyclic aromatic hydrocarbons and metal ions by functionalized and non-functionalized carbon nanotubes as effective sorbents. Talanta 165:405–411. https://doi.org/10.1016/j.talanta.2016.10.049

Plaza-Bolanos P, Garrido Frenicha A, Martínez Vidal JL (2010) Polycyclic aromatic hydrocarbons in food and beverages. Analytical methods and trends. J Chromatogr A 1217:6303–6326. https://doi.org/10.1016/j.chroma.2010.07.079

Purcaro G, Moret S, Conte LS (2013) Overview on polycyclic aromatic hydrocarbons: occurrence, legislation and innovative determination in foods. Talanta 105:292–305. https://doi.org/10.1016/j.talanta.2012.10.041

Ravelo-Pérez LM, Herrera-Herrera AV, Hernández-Borges J, Rodríguez-Delgado MA (2010) Carbon nanotubes: solid-phase extraction. J Chromatogr A 1217:2618–2641. https://doi.org/10.1016/j.chroma.2009.10.083

Rozentale I, Stumpe-Viksna I, Zacs D, Siksna I, Melngaile A, Bartkevics V (2015) Assessment of dietary exposure to polycyclic aromatic hydrocarbons from smoked meat products produced in Latvia. Food Control 54:16–22. https://doi.org/10.1016/j.foodcont.2015.01.017

Song X-Y, Chen J, Shi Y-P (2017) Different configurations of carbon nanotubes reinforced solid-phase microextraction techniques and their applications in the environmental analysis. TrAC Trends Anal Chem 86:263–275. https://doi.org/10.1016/j.trac.2016.11.006

Wang X, Tao S, Xing B (2009) Sorption and competition of aromatic compounds and humic acid on multiwalled carbon nanotubes. Environ Sci Technol 43:6214–6219. https://doi.org/10.1021/es901062t

Wenzl T, Haedrich J, Schaechtele A, Robouch P, Stroka J (2016) Guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in feed and food; EUR 28099. Publications Office of the European Union, Luxembourg ISBN 978-92-79-61768-3

Wu H, Wang X, Liu B, Lu J, Du B, Zhang L, Ji J, Yue Q, Han B (2010) Flow injection solid-phase extraction using multi-walled carbon nanotubes packed micro-column for the determination of polycyclic aromatic hydrocarbons in water by gas chromatography-mass spectrometry. J Chromatogr A 1217:2911–2917. https://doi.org/10.1016/j.chroma.2010.02.040

Yu MF, Lourie O, Dyer MJ, Moloni K, Kelly TF, Ruoff RS (2000) Strength and breaking mechanism of multiwalled carbon nanotubes under tensile load. Science 287:637–640. https://doi.org/10.1126/science.287.5453.637

Zacs D, Rjabova J, Viksna A, Bartkevics V (2015) Method development for the simultaneous determination of polybrominated, polychlorinated, mixed polybrominated/chlorinated dibenzo-p-dioxins and dibenzofurans, polychlorinated biphenyls and polybrominated diphenyl ethers in fish. Chemosphere 118:72–80. https://doi.org/10.1016/j.chemosphere.2014.06.032

Zelinkova Z, Wenzl T (2016) The occurrence of 16 EPA PAHs in food—a review. Polycycl Aromat Compd 35:248–284. https://doi.org/10.1080/10406638.2014.918550

Zhao Q, Wei F, Luo Y-B, Ding J, Xiao N, Feng Y-Q (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 59:12794–12800. https://doi.org/10.1021/jf203973s