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Automated micro-solid-phase extraction clean-up of polycyclic aromatic hydrocarbons in food oils for analysis by gas chromatography – orbital ion trap mass spectrometry

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Abstract

Food can contain unwanted compounds and need to be analyzed for compounds like carcinogenic polycyclic aromatic hydrocarbons to ensure consumer safety. The analytes need to be extracted from the sample matrix and cleaned-up to enable detection. However,

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established methods for clean-up are labor-intensive and have a high expenditure on organic solvents. Here we show a newly developed micro solid-phase-extraction cartridge method, to automate the clean-up for analysis of polycyclic aromatic hydrocarbons in sunflower oil using GC-Quadrupole-Orbitrap-MS with a TriPlus autosampler. This automated micro solid-phase-extraction cartridge method needs only 4 μL of vegetable oil sample and requires only 360 μL acetonitrile for elution, and, therefore, it needs only small amounts of organic solvent. Two different micro solid-phase-extraction cartridge methods were developed, one using two commercially available cartridges with Florisil and Octadecylsilane /Z-Sep/CarbonX, and the other method using one commercially available cartridge with Florisil followed by one self-made cartridge with Octadecylsilane/Z-Sep. The latter method showed successful lipid removal and was further validated for 22 of 24 polycyclic aromatic hydrocarbon compounds in sunflower oil at a spiked level of 1090 $\mu\text{g}/\text{kg}$ with recoveries ranging from 53-118% and relative standard deviation below 22%. This method shows promising short-time clean-up with low expenditure of solvents.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of contaminants consisting of two or more fused aromatic rings that are known to contain carcinogenic and mutagenic compounds [1]. Sources of PAHs can be the formation during incomplete combustion of organic matter, air pollution, or as a result of processes like drying, roasting, and smoking [1]. Contamination of food can occur via several routes. Food consumption is an important source of exposure to PAHs and maximum limits (MLs) of PAHs are therefore set for certain

food commodities by the European Commission [2]. The European Commission recommended in 2005 to monitor 15 carcinogenic PAH compounds [3] and later established MLs for PAHs in oils and fats for benzo[*a*]pyrene (BaP) at 2 µg/kg and the sum of four specific PAHs, the so-called PAH4, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and BaP at 10 µg/kg [2].

Analysis of PAH in high lipid-containing food samples is challenging as compounds like triglycerides and other lipophilic matrix compounds need to be removed before analysis with instruments such as GC-MS[4–6].

PAH analysis of liquid food samples can be done by diluting the sample in an organic solvent, or by liquid-liquid extraction, and further clean-up by e.g. gel permeation chromatography (GPC), or solid-phase extraction (SPE) [7–10]. Another option is to directly clean-up the liquid sample by solid-phase extraction and leaving out the extraction part[11].

In general, clean-up methods, such as GPC, are laborious, time-consuming, and require significant training. Therefore, there is a need for a faster clean-up [12]. Among existing methods, SPE has an advantage as it uses less organic solvents than liquid-liquid extraction [13] and GPC [12] while holding the opportunity for automation [13].

Automation of clean-up methods for SPE[14] has been shown to increase efficiency, improve precision, yield higher sample throughput, and reduce the overall workload. Automated SPE methods using mini-columns, or so-called micro solid-phase-extraction cartridges (µSPE cartridges), have already been successfully implemented for clean-up of Quick Easy Cheap Effective Rugged Safe (QuEChERS) extracts used for the analysis of PAHs, pesticides, and other analyte classes in food samples [14–17]. The clean-up of extracts

containing PAHs with μ SPE cartridges was previously done for QuEChERS extracts, with previous compound extraction [17].

The objective of this work was to develop a one-step clean-up method to analyze food oil samples directly using automated cleanup with μ SPE Cartridges and a GC-Quadrupole-Orbitrap-MS (GC-Q-Orbitrap-MS). A new μ SPE cartridge was therefore developed, inspired by the procedure described for SPE cartridge EZ-Pop NP, which consists of a double layer construction with a layer of Florisil®, and a mix of Octadecylsilane (C18) and Z-Sep® [11]. Two different μ SPE cartridges columns were used to mimic this double-layer structure, as a two-layer μ SPE cartridge was not yet available. With this new approach we were able to develop a new fast automation method of the complex SPE process, which included conditioning, drying, and elution of two μ SPE cartridges in serial within 30 minutes.

2 Materials and Methods

2.1 Chemicals and analytical standards

Acetonitrile and heptane (HPLC grade) were purchased from Biosolve (Valkenswaard, The Netherlands), formic acid (>99%) and ammonium formate (>99%) were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). A PAH-mix of in total 24 of EPA+EU(15+1) PAHs in toluene at 100 μ g/mL was obtained from Chiron AS (Trondheim, Norway), containing acenaphthene, acenaphthylene, anthracene, fluoranthene, fluorene, naphthalene, phenanthrene, pyrene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, benzo[*k*]fluoranthene, chrysene, dibenz[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, benzo[*c*]fluorene,

benzo[*j*]fluoranthene, cyclopenta[*cd*]pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene and dibenzo[*a,l*]pyrene. A 16 PAH internal standard mix, containing ^{13}C labelled polycyclic aromatic hydrocarbon standards in nonane at 5 $\mu\text{g/mL}$ (acenaphthene ($^{13}\text{C}_6,99\%$), acenaphthylene ($^{13}\text{C}_6,99\%$), anthracene ($^{13}\text{C}_6,99\%$), benz[*a*]anthracene ($^{13}\text{C}_6,99\%$), benzo[*a*]pyrene ($^{13}\text{C}_4,99\%$), benzo[*b*]fluoranthene ($^{13}\text{C}_6,99\%$), benzo[*ghi*]perylene ($^{13}\text{C}_{12},99\%$), benzo[*k*]fluoranthene ($^{13}\text{C}_6,99\%$), chrysene ($^{13}\text{C}_6,99\%$), dibenz[*a,h*]anthracene ($^{13}\text{C}_6,99\%$), fluoranthene ($^{13}\text{C}_6,99\%$), fluorene ($^{13}\text{C}_6,99\%$), indeno[1,2,3-*cd*]pyrene ($^{13}\text{C}_6,99\%$), naphthalene ($^{13}\text{C}_6,99\%$), phenanthrene ($^{13}\text{C}_6,99\%$), pyrene ($^{13}\text{C}_6,99\%$)) was obtained from LGC standards (manufactured by Cambridge Isotope Laboratories (Tewksbury, USA)). Additional PAH labelled standards in isooctane (5 $\mu\text{g/mL}$) were 5-methylchrysene ($^{13}\text{C},99\%$), benzo[*c*]fluorene (7A,7B,8,9,10,11- $^{13}\text{C}_6,99\%$), cyclopenta[*cd*]pyrene (Ring- $^{13}\text{C}_6,99\%$), dibenzo[*a,l*]pyrene ($^{13}\text{C}_{12},99\%$), dibenzo[*a,i*]pyrene ($^{13}\text{C}_{12},99\%$) and dibenzo[*a,e*]pyrene ($^{13}\text{C}_{12},99\%$) obtained from dSP-Systems (Ede, The Netherlands). BCR-519, certified reference material for glycerides: Anhydrous butter fat was obtained from Merck (Darmstadt, Germany).

2.2 Sample and working solutions

Sunflower oil sample was obtained from the National Monitoring Program in the Netherlands collected in 2018. A PAH standard mix containing 24 compounds at 1 $\mu\text{g/mL}$ in heptane was made from the analytical standard PAH-mix. An internal standard mix containing 22 ^{13}C -labelled compounds was made from the 16 PAH internal standard mix and stock solutions of additional PAH labelled standards in isooctane (0.5 $\mu\text{g/kg}$); 5-methylchrysene ($^{13}\text{C}_6,99\%$), benzo[*c*]fluorene ($^{13}\text{C}_6,99\%$), cyclopenta[*cd*]pyrene ($^{13}\text{C}_6,99\%$),

dibenzo[*a,l*]pyrene ($^{13}\text{C}_{12}$, 99%), dibenzo[*a,i*]pyrene ($^{13}\text{C}_{12}$, 99%) and dibenzo[*a,e*]pyrene ($^{13}\text{C}_{12}$, 99%).

2.3 SPE

EZ-Pop NP purchased from Supelco, Sigma Aldrich (St. Louis, USA) was a dual-layer SPE containing both LC-Florisil® and a mix of Z-Sep® and C18.

2.4 Automated μ SPE cartridges

μ SPE cartridges were supplied by ITSP Solutions (Hartwell, GA, USA), with 30 mg of Florisil (30-UFLOR-T); or with 30 mg of C18/Z-Sep/CarbonX, (30-UC18HX-T). μ SPE cartridges with C18/Z-Sep sorbent taken from the lower layer of the EZ-Pop NP column (see 2.3) were prepared in the laboratory in the following manner: An EZ Pop NP column was opened with a knife and the upper Florisil layer and lower C18/Z-Sep mix layer were separated and stored in plastic tubes. A μ SPE cartridge was prepared by placing the empty media cup in a little holder and adding a bottom frit. Then, 30.0 mg of the sorbent (C18/Z-Sep mix from the lower layer) was added, and a top frit was placed on top of the sorbent. The needle guide device was pressed into the cartridge, without disturbing sorbent and the frit. The needle guide was closed by adding a crimp seal with a septum and the use of an electronic crimper (Agilent Technologies, Santa Clara, USA).

2.5 Method development

The method was developed based on the sample preparation procedure of the SPE cartridge EZ-Pop NP and an earlier publication in the field using μ SPE cartridges [11].

The μ SPE method workflow automation was developed on a PAL RTC Autosampler (CTC Analytics AG, Zwingen, Switzerland) equipped with tools to automate the handling of miniaturized SPE (μ SPE cartridges) in dimensions of 33 mm height x 8 mm diameter.

The PAL RTC is a modular robotic platform for automated sample preparation from simple liquid sample injection to complete sample preparation workflows. The PAL RTC can automatically switch between different automation tools, for example, a 10 μ L syringe for the accurate addition of small volumes followed by a sample dilution with a 1mL syringe.

All SPE steps (conditioning, drying, sample load, and elution) originated from the Supelclean™ EZ-POP NP procedure for oily samples [11] were scaled down to match the μ SPE cartridge packing volume. This involved a linear scale down of the volumes of solvent used for the individual workflow steps as well as for the ratio of elution volume to sample volume.

The scaled-down SPE method was then broken down to a number of workflow steps on the PAL RTC with optimization required of each of the individual workflow steps. This included all liquid handling steps, which had to be individually optimized. Specific challenges had to be addressed with the scale down of the aspiration and transfer steps of miniaturized volumes of the high viscous oil sample. Additionally, a new miniaturized μ SPE cartridge drying step in reference to the original SPE method [11] had to be developed by including an additional 1ml syringe to the μ SPE workflow for the purpose of dispensing air through the μ SPE cartridge.

Automation method development was realized based on a proprietary script interface (Script API) to control the PAL RTC available as part of a Software Developer Kit from CTC Analytics.

The final μ SPE method resulted in a fully automated workflow with a sample preparation time of 28 minutes. Sample preparation could be executed in parallel to a GC run, but this was not part of this study.

The lipid removal capacity of μ SPE cartridges was tested for sunflower oil using two different options. Option 1 used commercially available μ SPE cartridges Florisil (30-UFLOR-T) and C18/Z-Sep/CarbonX for the developed sequence (see Autosampler and sequence). Option 2 used μ SPE cartridges Florisil (30-UFLOR-T) and in-lab made C18/Z-Sep μ SPE cartridges as described in the sequence (see Autosampler and sequence). Therefore, the only difference was, that the second cartridge contained an in-lab made C18/Z-Sep sorbent (see automated μ SPE section) instead of the C18/Z-Sep/CarbonX in option 1. The resulting eluents were collected in a glass vial and analyzed by GC-FID using an in-house method [18], for the presence of diglycerides and triglycerides.

2.6 Validation

Method validation was performed using two spike levels with five replicates of each level, all analyzed within one day. The spike level of 1090 μ g/kg in oil was judged as adequate for validation experiments, based on the expected concentration in the sample after clean-up, which was very low due to the lack of a concentration step by evaporation. Calibration

standards were prepared in isooctane with concentrations of 5, 10, 25, 50, 100, and 250 ng/mL with 50 ng/mL concentration of internal standard. Spiked samples were prepared as follows: 100 μ L of the standard mix (1 μ g/mL) and 50 μ L of internal standard solution (0.5 μ g/mL) were added to 100 μ L (1090 μ g/kg) of sunflower oil. After spiking, the samples were evaporated with nitrogen, until only oil was left. The calculation of recovery was performed by dividing the concentration of replicates by the theoretical value. RSD was calculated based on repeatability, so the replicate experiments were done on the same day, in the same laboratory, and from the same person. For RSD calculation, the standard deviation of the replicates was divided by the average of their recovery values. Quantification of PAH used linear external calibration (see table 1). For identification, retention time, and accurate mass were used (see table 1). The calibration standards were prepared without using μ SPE.

2.7 Autosampler and sequence

The automated μ SPE method used a PAL autosampler from CTC Analytics, Zwingen, Switzerland. The autosampler consisted of a PAL RTC autosampler, a PAL RTC Tray Holder, a μ SPE cartridge tray to hold 54 cartridges, a μ SPE cartridge Elution Tray Cover to lock 2 mL vials in place, and a μ SPE cartridge Waste receptacle. All parts are included in the QuEChERS extracts clean-up application kit from CTC Analytics (PAL3-Kit-uSPE54). A sample preparation script was developed, with a duration of approximately 28 minutes, to perform the automated sample clean-up. The final μ SPE cartridge setting includes: 1.) Florisil cartridge (cartridge 1) conditioning with 240 μ L of acetone in the first tray followed by drying with 1 mL of air. 2.) Cartridge 1 was transported to the second tray. Below this tray, vials with 360 μ L glass inserts were placed. 3.) A volume of 4 μ L of the sample was loaded onto cartridge 1, followed by adding 360 μ L of acetonitrile, which was collected in a vial with

glass inserts below (vial 1). 4.) Conditioning the C18/Z-Sep cartridge (cartridge 2) with 240 μ L of acetone in the tray, followed by drying with 1 mL of air. 5.) Cartridge 2 was placed above the second tray. Below this tray, vials with 360 μ L glass inserts were stored. 6.) Then, 260 μ L eluate from vial 1 was loaded onto cartridge 2 and collected in the vial below (vial 2). See table 2 for details of the whole sequence.

The samples were prepared according to the μ SPE workflow (see table 2), locked with a crimp cap, and stored at -20°C. The samples and the calibration standards were analyzed together in a sequence at a later point, hence sample preparation and measurement of the samples were separated events.

After clean-up, 2 μ L of the final extract was injected into the GC-Q-Orbitrap-MS system for analysis.

2.8 Instrumentation

All samples were analyzed on a GC-Q-Orbitrap-MS system (Q-Exactive GC, Thermo Scientific, Bremen, Germany) using a TriPlus RSH autosampler, a TRACE 1310 gas chromatograph (Thermo Scientific, Bremen, Germany) with a programmed temperature vaporizer (PTV) inlet, an electron impact (EI) source, and connected to a hybrid quadrupole Orbitrap mass spectrometer. The system is described in more detail in a previous study [19]. GC-Q-Orbitrap-MS analysis: The maximum resolving power was 60000 full width at half maximum (FWHM) at m/z 200. Two μ L solvent vent injection of acetonitrile was performed using the following program: initial PTV temperature was 45°C, vent flow was 50 mL/min, injection time was 0.08 min, and vent time was 0.1min. After injection of 2 μ L, the temperature was

increased to 60°C at 14.5°C/s. As a next step, analytes were transferred to the column by closing the split valve (splitless time, 1.0 min) and by heating the PTV to 320°C at 10°C/s. The PTV was cleaned after the transfer by heating to 340°C for 5 minutes. GC separation occurred on a 30 m × 0.25 mm id, 0.25 µm TraceGOLD™ TG-OCP I column (Thermo Scientific, Bremen, Germany) coupled to a Siltek®-deactivated guard column (Restek, Bad Homburg, Germany). The temperature program started at 60°C for 1.5 min, and an increase in temperature occurred at a rate of 25°C/min until 135°C. The temperature was further increased with a rate of 10°C/minute to end at 325°C, and was held constant at the final temperature for 7 minutes. As carrier gas Helium 5.0 (99.999%; Linde Gas, Schiedam, The Netherlands) was used with a constant flow of 1.2 mL/min. The transfer line was maintained at 260°C and electron ionization was performed at 70 eV, having the source temperature at 230°C. Full scan mass spectrometry acquisition was performed in profile mode with an m/z range of 50–500. The nitrogen gas supply for the C-trap was 5.0 grade (99.999%; Linde Gas). The automatic gain control (AGC) target was implemented at 5e6 ions, and the maximum ion injection time was set to 25 ms [19].

Lipid measurements were performed similarly to a previous study [18]. The analysis was carried out by a Focus GC gas chromatograph (Thermo Scientific, Italy) with a flame ionization detector (GC–FID) and split– splitless injection port, set at 280 and 250 °C, respectively, and a split ratio of 1:30. Chromatographic separation was performed on a CP 7419 FAME capillary column (50 m × 0.25 mm inner diameter; Varian, Palo Alto, USA). Helium (1 ml min^{–1} flow) was used as a carrier gas. The oven was programmed in the following manner: initial temperature, 100 °C, increased at 5 °C/min to 230 °C, and held for 9 min. The sample volume injected was 1 µl. Glycerides were identified by comparing their

retention times with that of the available butter fat standard (anhydrous butter fat, BCR 519 certified reference material, Merck, Darmstadt, Germany).

3 Results and discussion

3.1 Method development

Method development of the μ SPE method was based on the μ -SPE clean-up workflow of QuEChERS extracts [15] and the original SPE workflow supplied with the SupelcleanTM EZ-POP NP cartridges [11]. Liquid handling steps were linearly scaled down to match the μ SPE cartridges. Furthermore, a new drying step of the cartridge was developed by loading 1ml of air on the cartridge after conditioning.

Besides the optimization of individual liquid-handling parameters, and the new step of drying of cartridges, the complete workflow of the PAL RTC needed quality control and validation. This was done utilizing different measures: First, checks were performed optically by monitoring complete liquid transfer steps and the lack of air bubble formation. Second, the complete workflow was reviewed in terms of pick up and displacement positions. Finally, the quality control and efficiency of the complete system was evaluated by fat retainment experiments with GC-FID, and later by using validation experiments.

Two different μ SPE cartridge options were evaluated based on the ability to remove lipids. Sunflower oil samples were analyzed by GC-FID without clean-up, after clean-up using the Florisil and C18/Z-Sep/CarbonX cartridge (option 1), and after a two-step-clean-up using a Florisil μ SPE cartridge followed by an in-lab made C18/Z-Sep cartridge, (option 2). In GC-FID

chromatograms from samples without clean-up pronounced peaks from diglycerides and triglyceride could be seen. Using the commercially available Florisil and C18/Z-Sep/CarbonX cartridge (option 1) diglycerides and triglycerides were still present, whereas using the proposed two-step clean-up (option 2), no di- and triglycerides were detected in the GC-FID chromatograms (see figure 1). Sunflower oil samples following the option 2 clean-up were also analyzed by GC-Q-Orbitrap-MS, showing singular background peaks with medium intensity. Based on the low occurrence of background ions and potential interfering ions, option 2 clean-up was considered “cleaner”, and it was decided to use this option for validation experiments.

3.2 Method validation

3.2.1 Sensitivity and linearity

The spike level of 1090 µg/kg is much higher than the ML of 10 µg/kg, needed for compliance testing for the sum of benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene [2,20,21]. However, as the purpose of this study was to provide “proof of principle” automation using µSPE cartridge clean-up in PAH analysis of oil samples, the method was not optimized for sensitivity. Linear, non-weighted external calibration curves were used for the six calibration standard levels analyzed in solvent in the range of 5- to 50 ng/mL showing $R^2 \geq 0.99$ for all 24 PAH compounds. These calibration standards were not processed using a µSPE cartridge.

3.2.2 Recoveries and reproducibility

Recoveries of all analytes were calculated using peak areas normalized to peak areas of internal standards (see table 3) and quantified based on an external, solvent-based

calibration curve made in solvent. The signal intensities of some internal standards (IS) were found to be too low to be useful, due to low sensitivity and overlapping peaks. Because of this, some analytes were normalized against other internal standards (see table 1).

Recoveries were ranging from 53 % (indeno[1,2,3-*cd*]pyrene) to 115 % (benzo[*b+j+k*]fluoranthene) with RSD values for repeatability below 22% for the five replicates (see table 3). Satisfactory results for recovery (50-120% [21]) were achieved for 22 of the 24 tested PAHs (see table 3). Naphthalene and fluorene were barely recovered at this spike level, which can be explained either by the solvent vent at GC injection or by the evaporation performed after spiking of the oil sample with the standard solutions.

3.2.3 Challenging analytes

Dibenzo[*a,i*]pyrene and dibenzo[*a,h*]pyrene had overlapping peaks (see figure 2) and the concentration of the corresponding internal standard was too low to be useful. They could therefore not be quantified. Other compounds that were eluting closely together but barely overlapping were anthracene with phenanthrene; benzo[*j*]fluoranthene with benzo[*k*]fluoranthene and benzo[*b*]fluoranthene; chrysene with benz[*a*]anthracene.

Dibenzo[*a,i*]pyrene showed a 93% recovery for the lower spiked level while showing 69% for the higher spike level. This difference can be explained due to a high RSD value at the high spiked level and overlapping interfering peaks of dibenzo[*a,i*]pyrene and dibenzo[*a,h*]pyrene.

3.2.4 Suggested Improvements

The automated two-step μ SPE cartridge clean-up has the potential for direct sample analysis and can be improved in several ways to increase sensitivity to reach lower

quantification limits. The first improvement could be to include an evaporation step of the eluent after μ SPE clean-up to concentrate the PAH compounds with improved detection levels. It will then need to include an investigation of whether the evaporation affects volatile, low molecular weight PAH (e.g. naphthalene), and whether it might be balanced using volatile internal standards. Alternatively, if evaporation is not feasible and samples are clean enough, another improvement could be the use of high volume injection in GC, hence injecting more of the sample into the system. To save time in the sample preparation process, the method could be tested without conditioning and drying together with a direct injection step on GC-Q-Orbitrap-MS. The GC-method could be further improved e.g. by application of a PAH selective GC column to improve peak separation and detection of PAH at lower concentration levels.

4 Concluding remarks

Significant advantages of the automated μ SPE proof of concept method are a fast workflow of 28 minutes, low usage of organic solvents, less manual labor, and therefore reduced costs. As μ SPE has mostly been used to automate QuEChERS procedures, our approach is in essence an extension of the scope of automated μ SPE to oil samples. As demonstrated, even challenging samples can be transferred to the concept of automated μ SPE.

Furthermore, our approach used a combination of sorbents with florisil and C18/Z-Sep that is new for automated μ SPE analysis and has, only been applied for SPE clean-up [11]. The μ SPE method combines elements known from traditional SPE, such as conditioning and drying, into a single automated workflow which have not been used before for μ SPE. Also, it showed how the concept of a multi-layered SPE column can be transferred to a μ SPE method by using two μ SPE cartridges.

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Conflict of interest statement

Thomi Preiswerk works for the company CTC, sells μ -SPE instrumentation, and advises in implementing μ -SPE workflow. There are no financial/commercial conflicts of interest for the other authors of this publication.

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Figure Captions

Figure 1: Overlay of GC-FID chromatograms of sunflower oil containing di- and triglycerides (around 17 and 26 minutes) using different clean-up procedures. Option 1: commercial available μ SPE cartridge clean-up; Option 2: proposed two-step μ SPE cartridge clean-up.

Figure 2: Chromatograms of polycyclic aromatic hydrocarbons in a spiked oil sample at 1090 μ g/kg. 1: naphthalene, 2: acenaphthylene, 3: acenaphthene, 4: fluorene, 5: anthracene, 6: phenanthrene, 7: fluoranthene, 8: pyrene, 9: benzo[*c*]fluorene, 10: benz[*a*]anthracene, 11: chrysene, 12: cyclopenta[*cd*]pyrene, 13: 5-methylchrysene, 14: benzo[*b*]fluoranthene, 15: benzo[*k*]fluoranthene, 16: benzo[*j*]fluoranthene, 17: benzo[*a*]pyrene, 18:

dibenz[*a,h*]anthracene, 19: indeno[1,2,3-*cd*]pyrene, 20: benzo[*ghi*]perylene, 21:

dibenzo[*a,l*]pyrene, 22: dibenzo[*a,e*]pyrene, 23: dibenzo[*a,i*]pyrene, 24: dibenzo[*a,h*]pyrene.

Table 1: Analyte parameters used for GC-Q-Orbitrap-MS experiments. t_r : Retention time, s: single peak, d: not separated peak.

Compound	Internal Standard (^{13}C , 99%)	t_r	Molecular formula	Accurate masses (m/z)	Peak
Naphthalene	Naphthalene	5.75	C ₁₀ H ₈	1.280.626	s
Acenaphthylene	Acenaphthylene	8.14	C ₁₂ H ₈	1.520.626	s
Acenaphthene	Acenaphthene	8.22	C ₁₂ H ₁₀	1.540.782	s
Fluorene	Fluorene	9.32	C ₁₃ H ₁₀	1.660.782	s
Anthracene	Anthracene	11.33	C ₁₄ H ₁₀	1.780.782	d
Phenanthrene	Phenanthrene	11.5	C ₁₄ H ₁₀	1.780.782	d
Fluoranthene	Fluoranthene	14	C ₁₆ H ₁₀	2.020.782	s
Pyrene	Pyrene	14.42	C ₁₆ H ₁₀	2.020.782	s
Benzo[<i>c</i>]fluorene	Benzo[<i>c</i>]fluorene	15.31	C ₁₇ H ₁₂	2.160.939	s
Benz[<i>a</i>]anthracene	Benz[<i>a</i>]anthracene	17.23	C ₁₈ H ₁₂	2.280.939	d
Chrysene	Chrysene	17.23	C ₁₈ H ₁₂	2.280.939	d
Cyclopenta[<i>cd</i>]pyrene	Cyclopenta[<i>cd</i>]pyrene	17.23	C ₁₈ H ₁₀	2.260.782	s
5-Methylchrysene	5-Methylchrysene	18.14	C ₁₉ H ₁₄	2.421.096	s
Benzo[<i>b</i>]fluoranthene	Benzo[<i>b</i>]fluoranthene	19.61	C ₂₀ H ₁₂	2.520.939	d
Benzo[<i>k</i>]fluoranthene	Benzo[<i>b</i>]fluoranthene	19.61	C ₂₀ H ₁₂	2.520.939	d
Benzo[<i>j</i>]fluoranthene	Benzo[<i>b</i>]fluoranthene	19.61	C ₂₀ H ₁₂	2.520.939	d
Benzo[<i>a</i>]pyrene	5-Methylchrysene	20.17	C ₂₀ H ₁₂	2.520.939	s
Dibenz[<i>a,h</i>]anthracene	5-Methylchrysene	22.28	C ₂₂ H ₁₄	2.781.096	s
Indeno[1,2,3- <i>cd</i>]pyrene	5-Methylchrysene	22.29	C ₂₂ H ₁₂	2.760.939	s
Benzo[<i>ghi</i>]perylene	Benzo[<i>ghi</i>]perylene	22.67	C ₂₂ H ₁₂	2.760.939	s
Dibenzo[<i>a,l</i>]pyrene	Benzo[<i>ghi</i>]perylene	24.36	C ₂₄ H ₁₄	3.021.096	s
Dibenzo[<i>a,e</i>]pyrene	Benzo[<i>ghi</i>]perylene	25.2	C ₂₄ H ₁₄	3.021.096	d
Dibenzo[<i>a,i</i>]pyrene	Benzo[<i>ghi</i>]perylene	25.5	C ₂₄ H ₁₄	3.021.096	d
Dibenzo[<i>a,h</i>]pyrene	Benzo[<i>ghi</i>]perylene	25.7	C ₂₄ H ₁₄	3.021.096	d

Table 2: Depiction of the μSPE workflow.

Sequence	Description	Tool
1	Change Tool to 1ml syringe	1ml syringe
2	Wash syringe two times with 1000 μL acetonitrile	1ml syringe
3	Load 240 μL of acetone to cartridge 1 (florisil) for conditioning	1ml syringe
4	Drying with air, 1000 μL , cartridge 1	1ml syringe

5	Change Tool to 10 μ L syringe (Injection Tool)	1ml syringe /Injection Tool
6	Wash syringe two times with 10 μ L acetonitrile	Injection Tool
7	Load 4 μ L of oil sample to the syringe (2 fill strokes prior to avoid bubbles)	Injection Tool
8	Load oil sample to cartridge 1	Injection Tool
9	Wash injection tool two times with 10 μ L acetonitrile	Injection Tool
10	Change Tool to 1ml syringe	1ml syringe
11	Wash syringe two times with 1000 μ L acetonitrile	1ml syringe
12	Elute cartridge to vial 1 with 360 μ L acetonitrile	1ml syringe
13	Wash syringe two times with 1000 μ L acetonitrile	1ml syringe
14	Load 240 μ L of Acetone Condition of cartridge 2 (C18/Z-Sep)	1ml syringe
15	Drying cartridge 2 with 1000 μ L air	1ml syringe
17	Load/Eluate 260 μ L of eluate 1 to cartridge 2 above vial 2	1ml syringe
18	Wash syringe two times with 500 μ L acetonitrile	1ml syringe

Table 3: Recovery and repeatability of automated micro solid-phase extraction proof of principle method of spiked sunflower oil samples.

Analyte	Recovery level	RSD
	1090 μ g/kg	(n=5)
Naphthalene	97	8
Acenaphthylene	101	4
Acenaphthene	107	8
Fluorene	102	5
Anthracene + Phenanthrene	99	5
Fluoranthene	107	5
Pyrene	97	5
Benzo[c]fluorene	93	5
Benz[a]anthracene + Chrysene	90	6
Cyclopenta[cd]pyrene	105	3
5-Methylchrysene	98	3
Benzo[b+j+k]fluoranthene	115	2
Benzo[a]pyrene	104	13
Dibenz[a,h]anthracene	96	8
Indeno[1,2,3-cd]pyrene	53	14
Benzo[g,h,i]perylene	73	22
Dibenzo[a,l]pyrene	69	17
Dibenzo[a,e]pyrene	72	19