Microchemical Journal 167 (2021) 106278

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Polycyclic aromatic hydrocarbons analysis in tea infusions and tea beverages using membrane assisted solvent extraction

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ARTICLE INFO

Keywords:
Tea infusions
Tea beverages
Polycyclic aromatic hydrocarbons
Membrane assisted solvent extraction

ABSTRACT

Membrane Assisted Solvent Extraction (MASE) was applied as an extraction and enrichment technique of polycyclic aromatic hydrocarbons (PAHs) from tea infusions and fruit/herbal-tea beverages. PAHs have been separated and detected by high performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD). Variables affecting MASE comprising extraction temperature and time, stirring rate, acceptor solvent (hexane) volume, organic modifier in the donor phase (methanol) volume, aqueous donor phase pH and ionic strength were simultaneously studied by applying a Plackett–Burman design (PBD) as screening method. Results showed statistical significance for acceptor solvent volume, extraction time and stirring rate, which were optimised by an orthogonal 2^3 + star central composite design (CCD). Quantitative recoveries for all PAHs (within 78–116%) were obtained by using the optimized extraction conditions: 350 μL of hexane, extraction time of 70 min and stirring rate of 175 rpm. Extraction temperature, ionic strength and donor phase pH were statistically non-significant, which simplify the procedure. The MASE method has been found sensitive (LOQs < 43 ng L^{-1}) and precise (RSDs of < 13%). Finally, the method has been applied to assess PAHs levels in several tea infusions and fruit/herbal-tea beverages in the presence of surrogate standards. The total mean Σ_{16}PAHs in tea infusions were from 1.2 ng L^{-1} (white tea) to 151.7 ng L^{-1} (black tea), while total mean Σ_{16}PAHs was lower than 11.5 ng L^{-1} regarding tea fruit/herbal beverages. Furthermore, benzo(a)pyrene (BaP) concentrations were from < 1.5 ng L^{-1} (white tea) to 4.6 ng L^{-1} (green tea). Nevertheless, BaP concentrations obtained as well as the summation of BaA, Chry, BbF and BaP concentrations (4.6 ng L^{-1} for tea beverages to 7.5 ng L^{-1} for green tea infusions) did not exceeded the maximum levels according with European Union (EU) standards. Finally, BaP carcinogenic equivalent concentration (BaP_{eq}) and BaP mutagenic equivalent concentration (BaP_{Meq}) were estimated, ranging from 0.01 ng L^{-1} to 19.8 ng L^{-1} and 0.23 ng L^{-1} to 6.9 ng L^{-1}, respectively.

1. Introduction

Tea infusions and tea beverages, aromatic drinks prepared by brewing dried leaves, flowers, twigs or buds of *Camellia sinensis*, are popular dietary beverages widely consumed by millions of people worldwide [1]. Leaves of *Camellia sinensis* harvesting represents a global market of great economic importance. The presence of theanine and flavonoids and catechin compounds in *Camellia sinensis* leaves gives significant health benefits such as anti-oxidative, antibacterial, antiviral, chemo-preventive, anti-carcinogenic, and anti-mutagenic activities and type 2 diabetes risk and cholesterol reduction [2–4].

However, tea leaves could also contain several hazardous compounds (such as pesticides, heavy metals, or polycyclic aromatic hydrocarbons (PAHs)) as consequence of environmental pollution (atmospheric deposition and soil uptake), tea leaves harvesting (fertilizers and sewage irrigation) and manufacturing process [5].

PAHs (group of chemicals made up of more than one condensed aromatic rings) are well-known persistent organic pollutants formed as result of incomplete combustion of organic materials, whose adverse (carcinogenic, mutagenic, teratogenic and immunosuppressant) effects on human beings have been widely proved [6–8].

Some oxidizing and fermenting processes, as well as the use of the smoke for drying tea leaves and some fruits and roots, seeds or flowers of herbaceous plants by using open flames (from burning of organic natural

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https://doi.org/10.1016/j.microc.2021.106278
Received 10 February 2021; Received in revised form 30 March 2021; Accepted 8 April 2021
Available online 16 April 2021

resources), are usually part of tea processing, that may result in the generation and accumulation of high PAHs concentrations in tea leaves (mainly in black teas) [9,10]. Although PAHs are hydrophobic compounds, some studies suggest that 8% of total PAHs content (depending to the kind of tea and brewing conditions) could be transferred from dried tea leaves into hot water during brewing [11,12].

Due to PAHs harmful effects on human beings, several regulations regarding the analysis and maximum levels of PAHs in foodstuffs [13–15], as well as studies concerning PAHs levels in foodstuffs, have been published in the last years [16]. Benzo(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF) and benzo(a)pyrene (BaP) have been chosen as indicators for the occurrence of PAHs in food by the European Food Safety Agency [13]. Furthermore, a maximum level of BaP and the sum of BaA, Chry, BbF and BaP in foodstuffs (oils, cocoa beans, smoked meat and fishery products, bivalves, cereal and baby and dietary foods) were set according to European Union standards [14].

Several suitable methods for the quantification of PAHs in foodstuff (mainly based on fluorescence spectrophotometry coupled to multivariate calibration, high performance liquid chromatography with fluorescence detector (HPLC-FLD) and gas chromatography-mass spectrometry (GC–MS)) have been reported. However, due to complexity of tea matrix, extremely low concentrations of PAHs in tea infusions and the low detection levels required by current regulations [15], steps such as PAHs extraction, enrichment and isolation are critical prior to PAHs analysis in tea infusions and beverages. Thus, the development of fast, flexible, more cost-effective and environmental-friendly sample pre-treatment procedures, which guaranties the efficiency, selectivity, sensitivity and quantitative PAHs recoveries remains on interest. The more published sample pre-treatment to assess PAHs in tea infusions are those based on classical liquid–liquid extraction (LLE) [1,5,11,12,17–20] and solid phase extraction SPE [21–24], which commonly are time-consuming, require large amount of organic solvents and generate large volumes of wastes. New sample pre-treatment methods such as Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) [25–28] and micro-extraction approaches which minimised solvents amounts and offers automation facilities (dispersive micro-solid phase extraction (d-μSPE) [29–34], dispersive liquid–liquid micro-extraction (DLLME) [35–37], on line-SPE [38], stir bar sorptive extraction (SBSE) [39,40], solid phase micro-extraction (SPME) [41,42] and in-tube SPME [43]), have been recently used for PAHs enrichment from tea infusions.

Nevertheless, membrane micro-extraction techniques such as membrane assisted solvent extraction (MASE) were not still reported in the literature for PAHs analysis in tea infusions and tea beverages, despite meeting the Green Chemistry principles due to the reduction of organic solvents and the prevention of waste generation. MASE, firstly proposed by Hauser and Popp [44], is a non-exhaustive procedure based on the use of hydrophobic and size-exclusive polymeric micro-porous membranes which allow the separation of the sample from the solvent (acceptor phase). These membranes are filled with an organic solvent (400–1000 μL) and analytes diffuse through the membrane micro-porous from the donor phase (usually an aqueous sample) to the acceptor phase into membrane because of concentration gradient. MASE remove hydrophilic compounds and higher molecular weight hydrophobic matrix compounds allowing the handling of very complex matrices and the enrichment factors and detection limits enhancement. This approach has been successfully applied for PAHs extraction from wastewater [45,46], natural waters [47–49] and beverages (apple juice, red wine and milk) [49,50] and in air particulate matter after subcritical water extraction [51].

Most methodologies are focused on the extraction of PAHs in tea leaves despite tea infusion is mainly the way in which tea is consumed. Therefore, the knowledge of PAHs levels in tea infusion could allow a more realistic health risk assessment of PAHs associated with dietary intake. The aim of the present study was the optimisation of a simple, fast and "green" MASE procedure for the extraction and isolation of 16 EPA priority PAHs and benzo(e)pyrene (BeP) from tea infusion (green, red, black and white tea) and fruit/herbal tea beverages. Because many variables can affect MASE efficiency, experimental design approaches (Plackett–Burman and central composite designs) were used to perform the optimisation.

2. Materials and methods

2.1. Instrumentation

Chromatographic analysis was carried out by using a Waters® 2695 Alliance High Performance Liquid Chromatography (Waters, Milford, MA, USA) system equipped with an auto-sampler and coupled to a fluorescence detector (Waters® 2475) and a Waters® PAH C18 column (250 × 4.6 mm i.d., 5 μm particle size).

Membrane Assisted Solvent Extraction device (a 20 mL glass vial, a membrane insert made of dense PP (4 cm × 6 mm i.d. with a wall thickness of 0.03 mm), and metal funnel with PTFE ring) were purchased from Gerstel (Mülheim, Germany). Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (Selecta, Barcelona, Spain) was used to fixed temperature during the extraction step. CRISON GLP21 pH-meter with a glass–calomel electrode was from Crison (Barcelona, Spain).

2.2. Chemicals and reagents

PAH Calibration Mix CRM (2000 μg mL^{-1} in acetonitrile) including naphthalene (Naph), acenaphtylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Ft), pyrene (Pyr), chrysene (Chry), benzo(a)anthracene (BaA), benzo(k)fluoranthene (BkF), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DBahA), indeno(1,2,3-cd)pyrene (IP) and benzo (g,h,i)perylene (BghiP) was purchased from Supelco (Steinheim, Germany). BeP (10 μg mL^{-1} in acetonitrile) and isotopically labelled standards comprising anthracene-d10 (Ant-d10) (100 μg mL^{-1} in cyclohexane), benzo(e)pyrene-d12 (BeP-d12) (100 μg mL^{-1} in cyclohexane) and dibenzo(a,h)anthracene-d14 (DBahA-d14) (10 μg mL^{-1} in cyclohexane) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). In addition, 6-metilchrysene (6-m-Chry) (100 μg mL^{-1} in methylene chloride) was purchased from ChemService (West Cherster, PA, USA). Stock standard solutions were prepared in acetonitrile and stored in amber glass vials at − 18 °C for a maximum of 4 weeks. Salts were analytical grade purchased by Sigma-Aldrich (St. Louis, MO, USA) and Merck-Millipore (Darmstadt, Germany). Acetonitrile LiChrosolv®, ethyl acetate LiChrosolv® and hexane Suprasolv® were purchased from Merck-Millipore (Darmstadt, Germany). Methanol gradient quality and dimethyl sulfoxide (DMSO) for headspace GC were from Romil (Cambridge UK) and Panreac (Barcelona, Spain). Ultrapure quality water of 18 MΩ cm resistance filtered through 0.22 μm was from Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Selected teas and tea beverages

The analysis of PAHs in green (n = 3), red (n = 3), black (n = 3) and white (n = 3) tea bag samples and fruit/herbal-tea beverages (raspberry and basil black tea, peach and rosemary white tea, peach black tea and mint green tea) (n = 4) were purchased commercially on the Spanish markets and specialised establishments. Until the analysis, the tea bags were stored at in its original unopened packaging in a dry and dark compartment at room temperature, not longer than month, within the expiration date.

2.4. Preparation of tea infusion and tea beverages

Tea infusions were prepared by submerging a commercial packet bag of tea sample (about 2 g) in 100 mL of boiling ultrapure water for 6 min

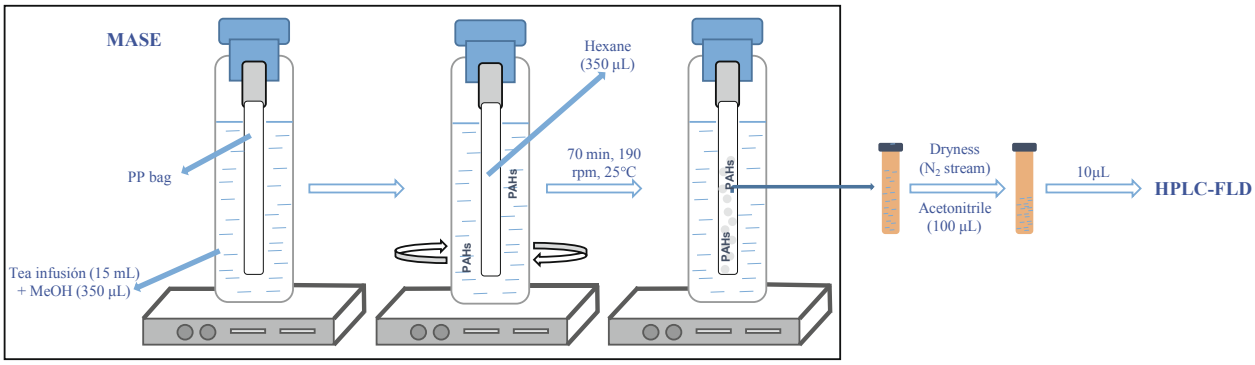

Fig. 1. Schematic diagram of the MASE-HPLC-FLD procedure for HAPs extraction and quantification from tea infusions.

as recommended by ISO 3103:2019 norm [52]. Afterwards, the infusion was filtered through 0.2 µm and kept in the dark at 4 °C for no>48 h before being analysed. Fruit/herbal-tea beverages were subjected to extraction without any pre-treatment.

2.5. Pahs extraction and pre-concentration procedures

A conventional 20 mL headspace vial was filled with 15 mL tea infusion/tea beverage sample and methanol (750 µL), and spiked with 10 µL of Ant-d10, BeP-d12 and DBahA-d14 solution used as surrogate standards (500 µg L^{-1} in acetonitrile). The PP membrane bag (attached to the metal funnel and fixed with a PTFE ring) was introduced into the vial and filled with 350 µL of hexane; then the vial was closed with a metallic crimp cap. MASE device was transferred into an orbital–horizontal shaker. After 70 min of shaking at 190 rpm, the hexane extract was collected from the membrane bag with a glass Pasteur pipette and poured into a 2 mL amber vial. Finally, the hexane was evaporated to dryness by gentle N_2 stream and reconstituted with 100 µL of 6-m-Chry (80 µg L^{-1}) in acetonitrile, used as internal standard (Fig. 1).

2.6. Pahs quantification by HPLC-FLD

The PAHs separation and quantification conditions were based on previous papers [53]. A gradient elution by using acetonitrile and water as mobile phases was carried out. The gradient started with 50% water and 50% acetonitrile, after which the acetonitrile content was increased to 100% (0–34 min) and finally a return to 50% acetonitrile content (34–40 min)). The flow rate and column temperature were set at 1.0 mL min^{-1} and 32 °C, respectively. Fluorescence excitation and emission wavelength programs used were similar to those cited in [53]. A volume of 10 µL of tea extract obtained after the extraction and pre-concentration procedure was injected into the HPLC–FLD system. In STable 1 are summarised the separation, condensed ring number and fluorescence detection settings for each PAH, while Fig. 2 shows typical HPLC-FLD chromatograms for a mixture of target PAH standards (10 µg L^{-1}) (a), and for a MASE extracts from black tea (b) and white tea (c) infusions obtained by using above conditions. Acy were not considered in the present study because of not being fluorescent.

2.7. Statistical treatment of data

A Plackett-Burman (PBD) (to screen variables) and central composite designs (CCD) (to optimise significant variables) were performed with Statgraphics version 7.0 routine (Statgraphics Graphics Corporation, ST. SC., USA).

3. Results and discussion

3.1. MASE optimization

3.1.1. Preliminary studies

The suitability of two acceptor solvents (hexane and ethyl acetate) and three solvent modifiers in the donor phase (acetonitrile, methanol and dimethyl sulfoxide) for PAHs extraction by MASE has been tested. Organic solvent should have a low volatility, which will restrict solvent evaporation during extraction in the shaker during the incubation of samples; and it should provide an appropriate extraction selectivity to provide high extraction PAHs recoveries. Additionally, miscible organic solvents in donor phase is required to avoid loss of PAHs by adsorption on glass flasks in which the MASE is performed in order to improve the extraction efficiency. However, a large amount of organic solvent could increase the solubility of the analytes in the donor solution, which might reduce the extraction efficiency of less non-polar PAHs [47]. As a preliminary experiment, 15 mL of NaCl saturated tea infusion/tea beverage sample spiked with target PAHs (25 µg L^{-1}) and Ant-d10 and DBahA-d14 (500 µg L^{-1}) at pH 5, 500 µL of acceptor phase and an organic modifier concentration in the donor phase of 5% (v/v) were used. Common MASE extraction conditions were used: extraction temperature of 25 °C, an extraction time of 70 min and orbital shaking of 150 rpm. Fig. 3 shows high mean PAHs recoveries (46.9%) when hexane and methanol were used as acceptor phase and organic modifier, respectively; while for ethyl acetate, mean PAHs recoveries were low (23.4–34.4%). High recoveries were also achieved for 2–3 rings (61.7%), 4 ring (45.3%) and 5–6 rings (33.8%) PAHs. However, analytical recoveries decreased when increasing the ring number (Fig. 3), while for 2–3 rings PAHs high analytical recoveries were achieved. Finally, low mean PAHs recoveries were achieved for acetonitrile (31.3%) and dimethyl sulfoxide (21.0%) when hexane is used as acceptor phase. The addition of methanol decreases the PAHs sorption on glass, allowing the analytes stay available in aqueous phase to diffuse through the membrane [54]. For the next stage in the optimisation process, hexane as acceptor phase and methanol as organic modifier were selected.

3.1.2. Optimization of procedure

Optimisation of PAHs extraction/pre-concentration by MASE involves study of many factors, such as extraction temperature and extraction time, hexane volume, NaCl concentration (ionic strength), stirring rate, pH sample and methanol concentration. Extraction efficiency of high non-polar PAHs could be increased by using high extraction temperatures, due to the adsorption on glass of the high non-polar PAHs is minimized [51]. High PAHs extraction efficiency is achieved after longer extraction times; however, large extraction times are not always a practical approach. Large hexane volume (acceptor phase) improves PAHs extraction; on contrary, enrichment factor and LODs of the method can be improved by decreasing the volume hexane. Ionic

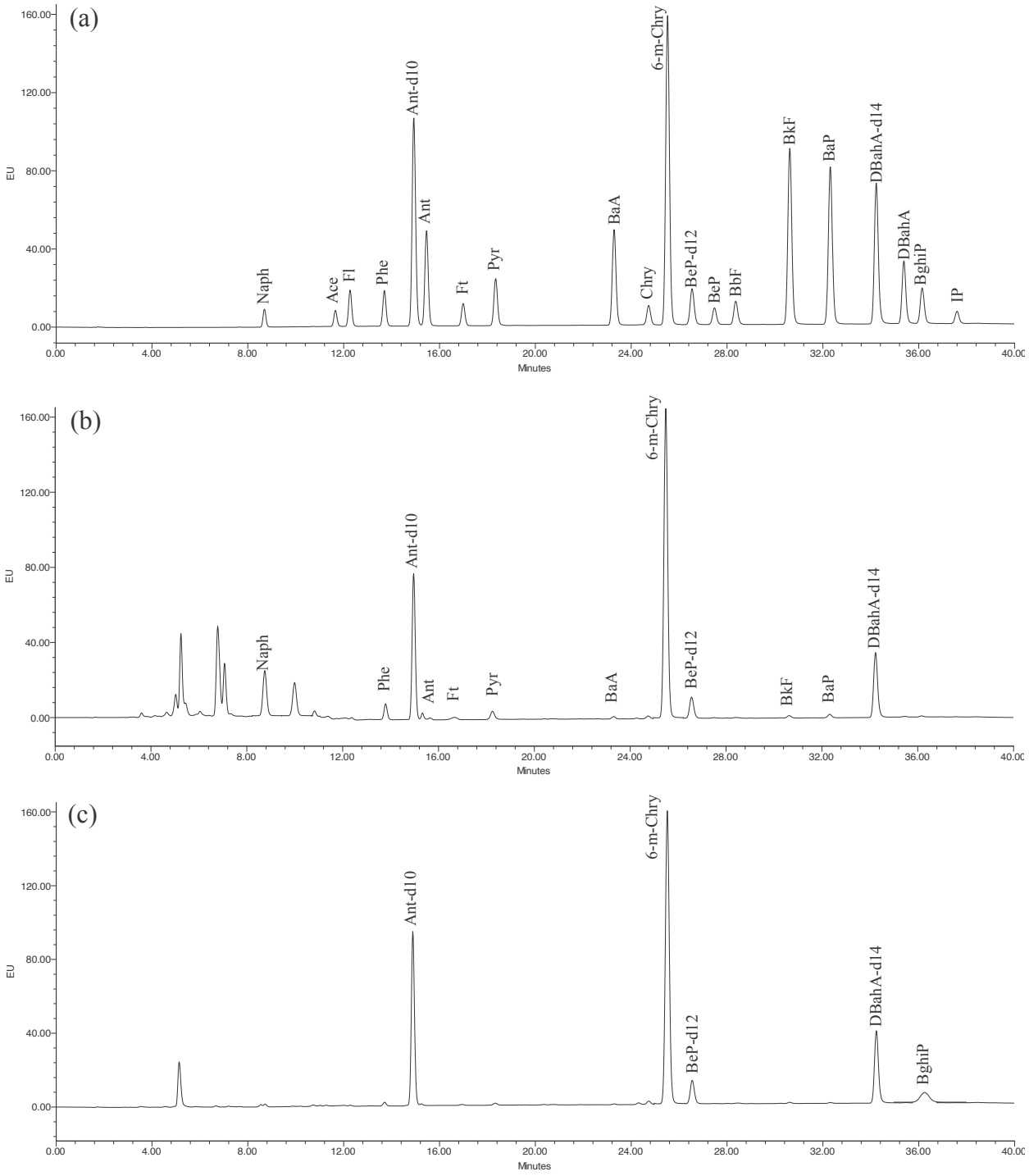

Fig. 2. HPLC-FLD chromatograms obtained for a mixture of target PAHs: 10 μg L^{-1} (a), and for a MASE extracts from black tea (b) and white tea (c) infusions using optimized conditions (Section 2.5 and 2.6). Naphthalene (Naph), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene-d10 (Ant-d_{10}), Anthracene (Ant), fluoranthene (Ft), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), 6-methylchrysene (6-m-Chry), benzo(e)pyrene-d12 (BeP-d_{12}), benzo(e)pyrene (BeP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene-d14 (DBahA-d_{14}), dibenz(a,h)anthracene (DBahA), benzo(g,h,i)perylene (BghiP) and indeno(1,2,3-cd)pyrene (IP).

strength of tea infusion increasing (salt addition) decrease the solubility in donor phase (tea infusion) of neutral-polar PAHs and therefore enhance extraction because of the salting-out effect. Also, a change in pH of tea infusion could have influence on the PAHs extraction [49]. Extraction efficiency of less polar PAHs could be improved by using high solvent modifier (methanol) concentration in the donor phase [47]. Finally, stirring rate affects the PAHs mass transfer from donor phase to the donor phase membrane interface, accelerating the extraction kinetics and reducing the time required to reach the thermodynamic equilibrium. In general, a stirring rate increasing enhances extraction efficiency.

The interdependence of some conditions (e.g. extraction temperature and extraction time or extraction time and stirring rate) must also be taken into account. Therefore, the classical univariate approach has

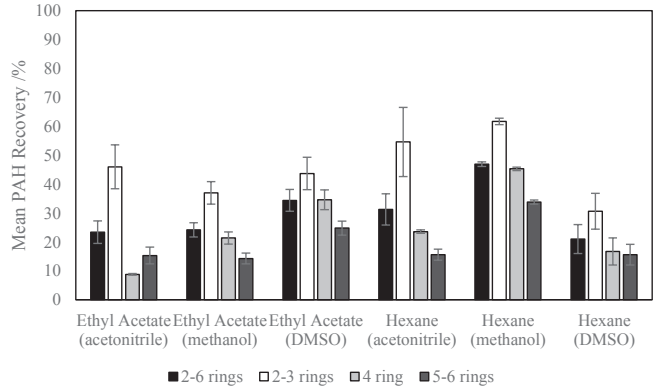

Fig. 3. Combine effect of nature of acceptor solution and organic modifier (5% (v/v)) on the mean 2–6 ring PAHs, 2–3 ring PAHs, 4 ring PAHs and 5–6 ring PAHs analytical recovery. Volume of the donor phase: 15 mL of NaCl saturated tea infusion sample at pH 5; target PAHs concentration: 25 μg L^{-1}; Ant-d10 and DBahA-d14 concentrations: 500 μg L^{-1}; volume of the acceptor phase: 500 μL; extraction time 73 min.; extraction temperature: 25 °C; shaking rate: 150 rpm; N = 3.

Table 1
Experimental field definition for the Plackett–Burman (PBD) and central composite (CCD) designs.

Variable	Symbol	Low level (–)	High level (+)	Unit
Extraction temperature	T	25	40	°C
Extraction time	t	45	90	min
Acceptor (hexane) volume	V	200	400	μL
Ionic strength [NaCl]	I	0	5	%
Stirring rate	S	75	200	rpm
pH	P	3	9	
Modifier [MeOH]	M	0	5	%
Dummy factor	D	− 1	+ 1	

several limitations compared with multivariate methods (e.g. factorial design). Multivariate methods involve simultaneous combinations of a number of parameters according to a predefined regime, the number of experiments could be 2^n for a system with '*n*' factors. For systems with a great number of variables, such as MASE procedures, the workload involves an impractical number of experiments. In order to reduce the number of experiments, a reduced fractional factorial design i.e. Plackett–Burman design (PBD) was selected for screening purposes. PBD allow us the evaluation of either system with only few experiments. These are two-level design for the study of K = n − 1 factors in *n* experiment runs where *n* is a multiple of four. Subsequently, the most significant factors from PBD were optimized by applying an orthogonal 2^n + star central composite design (CCD). In screening/optimization experiments tea infusions and beverage samples were spiked with target PAHs (25 μg L^{-1}).

3.1.2.1. Statistically significant variables affecting PAHs extraction by MASE. The statistical significance of the factors commented on above was evaluated by applying a $2^8 \times 3/64$ type III resolution PBD, for eight factors and three degrees of freedom. A series of 24 non-randomized experiments (12 runs and two replicates) was performed. Table 1 shows the experimental field definition for PBD design. The experiments were performed at two levels for each factor investigated, coded as "−1" (low) and "+1" (high), usually called boundaries of the experimental domain. These values were chosen basing on our experience in accordance with literature data, and considering the instrumental limitations (maximum stirring rate allowed by the platform shaker is 230 rpm). Table 1 also shows an eight factor, called a dummy factor, which was considered in the study. Dummy factors are imaginary variables for which the change from one level to another is not supposed to cause any physical change. This variable is commonly used to evaluate possible systematic error and/or the presence of an important factor that was not considered.

The response variables were the analytical recovery R (%) of the PAHs extracted in accordance with the equation:

$$R(\%) = \frac{\Sigma CPAH_{MASE}}{\Sigma CPAH_{add}} x100$$

where $\Sigma CPAH_{MASE}$ is the total PAHs concentration obtained after MASE procedure and $\Sigma CPAH_{add}$ is the total PAHs concentration added.

STable 2 lists the effect of factors on mean R (%) of 2–6 ring PAHs, 2-3ring PAH, 4ring PAH and 5-6ring PAH obtained in each experiment provided by the Statgraphics plus 7.0 routine. The statistical evaluation of the results was performed at a 95.0% confidence interval, obtaining a minimum t-value of 2.15, that was calculated using an iterative process (Statgraphics routine program). Then, variables with t-values higher than 2.15 were considered as statistically significant factors (Fig. 4). It can be seen that the variables extraction time (t), hexane volume (V) and stirring rate (S) are statistically significant for 2–6 ring PAHs, 2–3 ring PAHs, 4 ring PAHs and 5–6 ring PAHs. Concerning these variables, the

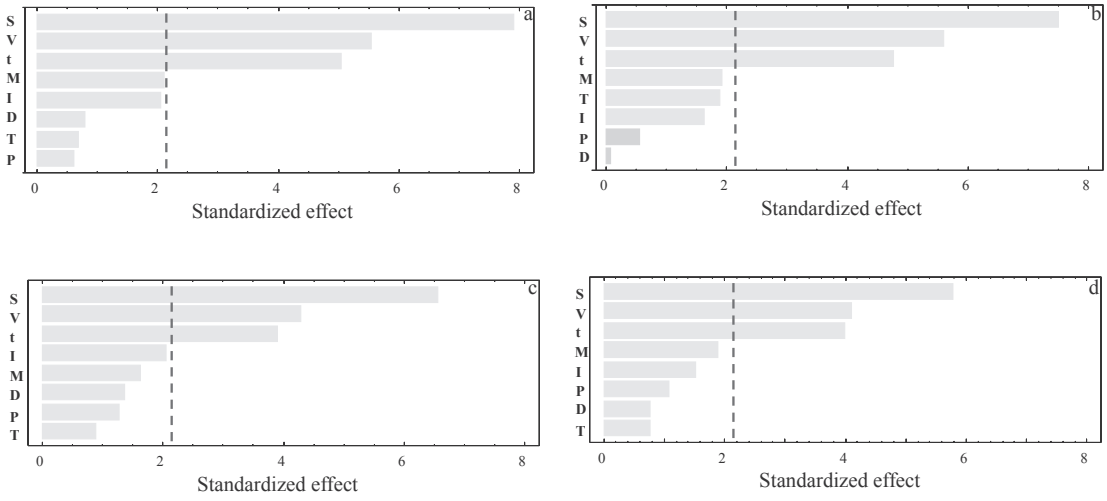

Fig. 4. Standardized (P = 95%) main effect Pareto charts for the PBD for PAHs pre-concentration by MASE using as response variables the mean 2–6 ring PAH analytical recovery (a), mean 2–3 ring PAH analytical recovery (b), mean 4 ring PAH analytical recovery (c), and mean 5–6 ring PAH analytical recovery (d). (T) extraction temperature, (t) static time, (V) hexane volume, (I) NaCl concentration, (S) stirring rate, (P) pH, (M) methanol volume and (D) dummy.

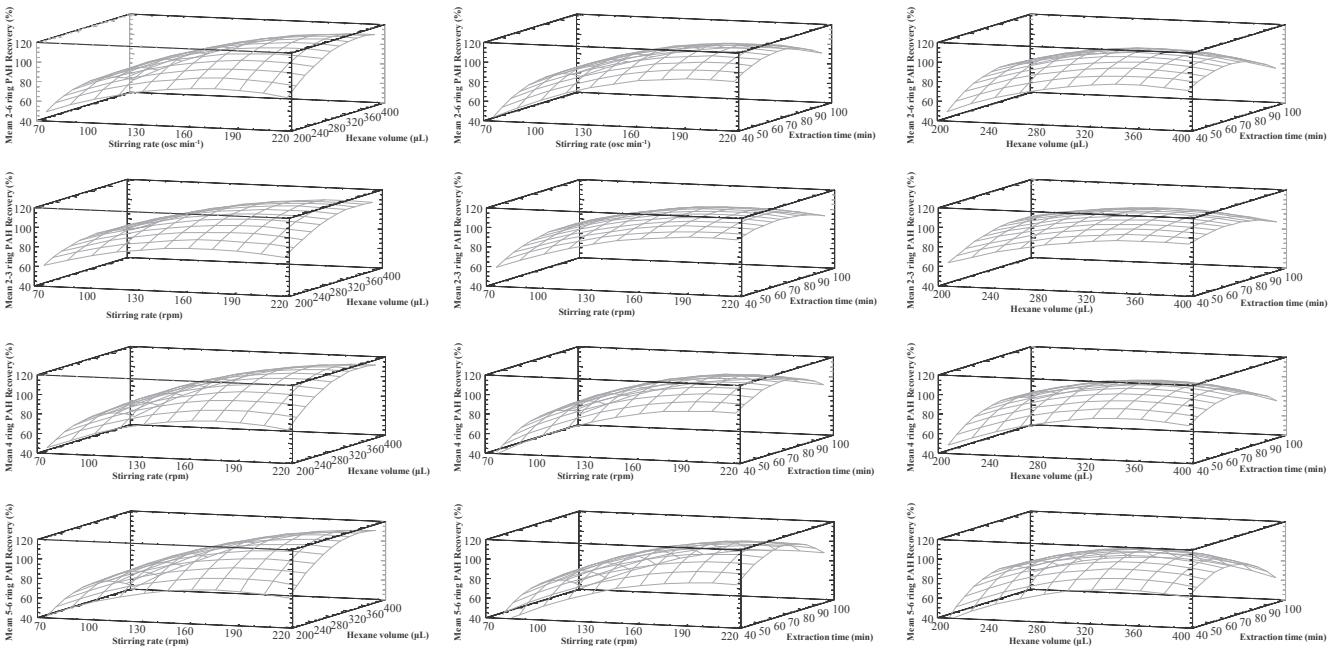

Fig. 5. Estimated response surface from the central composite design (CCD) using the mean 2–6 ring PAHs, mean 2–3 ring PAHs, mean 4 ring PAHs and mean 5–6 ring PAHs analytical recoveries as response variables.

effects were positives. This means an increase in the PAHs extraction efficiency when extraction time, stirring rate, and hexane volume is increased. Sample stirring facilitated the diffusion of PAHs through the interfacial layer, accelerate the PAHs extraction kinetics and reduces the time required to reach the thermodynamic equilibrium; which enhances extraction efficiency and improves the repeatability of the extraction method. This equilibrium is attained only after exposing the acceptor solution to the sample for a long period of time (extraction time). The sensitivity can be increased by decreasing the volume of the acceptor phase (hexane volume), the use of high volumes enhanced the PAHs recovery. The variables extraction temperature (T), NaCl and methanol concentrations and sample pH have not been statistically significant factors. This fact offers several advantages since the addition of NaCl (to set the ionic strength) and tea infusion pH adjustment were not necessary, which reduce the total pre-treatment time. Finally, the dummy factor was not statistically significant, thus it can be concluded that there is neither a systematic error nor unknown variables affecting the system under study.

3.1.2.2. Optimization of significant variables by central composite designs. Attending to the results commented above, extraction time (t), hexane volume (V) and stirring rate (S) were the most significant factors, and therefore, they were the experimental variables considered for a further optimization process. Concerning the remaining variables, extraction temperature and pH were set at 25 °C and 5.0 (tea infusion pH), respectively; with no addition of NaCl. Although the lack of statistical significance of methanol, the high value (5%) was set for this variable, in accordance with published results, to avoid the loss of PAHs by adsorption on glass flasks. The extraction time, hexane volume and stirring rate was optimized by applying an orthogonal 2^3 + star CCD with six error degrees of freedom, two centres, two replicates and sixteen runs. Table 1 shows the experimental field definition for the variables, STable 3 gives the effect of stirring time, hexane volume and extraction time on mean R (%) of PAHs, obtained in each experiment from CCD matrix (provided by the Statgraphics plus 7.0 routine) while SFigure 1–3 show HPLC-FLD chromatograms achieved for some experiments from CCD matrix. After performing the CCD experiments, the statistical evaluation of quadratic terms was significant for many cases, when the response variable was the mean of 2–6 ring PAHs, 2–3 ring PAHs, 4 ring PAHs and 5–6 ring PAH analytical recoveries (Fig. 5). The careful study of the results leads to the best-compromise conditions: 190 rpm, 350 µL of hexane and 70 min for stirring rate, hexane volume and extraction time, respectively. As can be seen, optimum stirring rate and extraction time are not the highest values in the design (Table 1). Bubbles formation at high stirring rate (>190 rpm) can decrease PAHs transfer to hexane phase. By the same way, the increase of bubbles formation could also explain the slightly decrease of PAHs recovery for high extraction times (>70 min) when high stirring rates are used.

Table 2
Calibration graphs (y = bx + a), correlation coefficients (R^2), relative response factors with respect to the corresponding surrogate (RRF) and RRF relative standard deviation (RSD_{RRF}, %) used for PAHs quantification by HPLC-FLD.

PAH	y = bx + a	R^2	RRF[a]	RSD_{RRF}
Naph	y = 74711x + 63898	0.9996	0.18[b]	6.0
Ace	y = 71779x + 69333	0.9995	0.17[b]	5.2
Fl	y = 165905x + 162840	0.9995	0.40[b]	4.8
Phe	y = 166594x + 149613	0.9996	0.41[b]	5.3
Ant	y = 458939x + 456564	0.9996	1.10[b]	5.4
Ft	y = 110464x + 96875	0.9996	0.27[b]	4.9
Pyr	y = 252351x + 207899	0.9995	0.60[b]	5.9
BaA	y = 518539x + 492390	0.9995	6.04[c]	4.0
Chry	y = 101314x + 9424	0.9997	1.26[c]	5.4
BeP	y = 97604x + 93724	0.9995	1.13[c]	4.3
BbF	y = 130536x + 67338	0.9995	1.56[c]	2.3
BkF	y = 1001145x + 877363	0.9994	11.8[c]	4.5
BaP	y = 937664x + 1154501	0.9995	2.90[d]	5.0
DBahA	y = 349690x + 345648	0.9994	1.09[d]	5.1
BghiP	y = 216605x + 326276	0.9995	0.66[d]	6.0
IP	y = 71607x + 76958	0.9995	0.22[d]	6.1

$$RRF = \frac{Area_{PAH} x Concentration_{surrogate}}{Area_{surrogate} x Concentration_{PAH}};$$

[a] Relative response factors (RRF) were calculated as follows:
[b] RRF calculated using Ant-d10.
[c] RRF calculated using BeP-d12.
[d] RRF calculated using DBahA-d14.

Table 3
LOD and LOQ (n = 5), intra-day (n = 3) and inter-day (n = 7) precision.

PAH	LOD		LOQ		Precision	
					RSD (%)	
	(ng L^{-1})	(ng g^{-1})[a]	(ng L^{-1})	(ng g^{-1})[a]	Intra-day	Inter-day
Naph	26.0	1.3	43.8	2.2	4.2	13.2
Ace	4.7	0.24	10.4	0.52	0.42	7.1
Fl	5.2	0.26	6.6	0.33	0.41	5.2
Phe	28.2	1.4	39.2	2.0	0.36	5.6
Ant	1.9	0.10	2.1	0.11	6.3	8.8
Ft	7.7	0.39	11.2	0.56	0.81	3.1
Pyr	5.4	0.27	8.8	0.44	0.50	6.9
BaA	0.6	0.03	1.0	0.05	0.49	7.6
Chry	5.7	0.29	9.5	0.48	2.3	5.9
BeP	2.8	0.14	5.8	0.29	0.54	8.3
BbF	3.0	0.15	5.1	0.26	0.46	6.7
BkF	0.76	0.04	0.82	0.04	0.47	4.9
BaP	0.81	0.04	1.5	0.08	0.55	8.6
DBahA	0.74	0.04	1.9	0.10	0.54	9.1
BghiP	1.0	0.05	1.1	0.06	0.47	6.1
IP	2.1	0.11	5.4	0.27	4.0	8.7

[a]Results calculated on the mass basis of leaves used for infusion preparation

3.2. Analytical performances and validation

Nine-point (0–200 μg L^{-1}) calibration curves were carried out with correlation coefficients $R^2 > 0.9976$ for all the PAHs tested (Table 2). Moreover, relative response factors (RRF) with regards to the corresponding surrogate standard (Ant-d10, BeP-d12 and DBahA-d14) were used to PAHs quantification from tea infusion and tea beverage extracts. Relative response factors (RRF) of each PAH were calculated (average of all RRFs considering all the calibration range) [55], showing satisfactory relative standard deviations RSD < 6.1% (Table 2). Furthermore, the internal standard (6-m-Chry) were used to evaluate the surrogate's recoveries for all the samples during the procedure, with recoveries between 74 and 113 %, 69–97% and 57–80% for Ant-d10, BeP-12 and DBahA-14 respectively.

The limits of detection (LODs) (mean blank + 3 SD criterion) and limits of quantification (LOQs) (mean blank + 10 SD criterion) and were estimated by analysing 5 procedural blanks. The LOD values were between 0.60 and 26.0 ng L^{-1} for BaA and Naph, respectively. Moreover, LOQ values ranged from 0.82 (BkF) to 43.3 ng L^{-1} (Naph) (Table 3). Procedural blanks were performed using ultrapure water to control possible contamination, and concentrations obtained were subtracted from the concentrations obtained in the samples. The precision of method was proved by analysing a tea infusion sample at detectable levels of PAHs. Good intra-day (n = 3) and inter-day (n = 7) precision was observed for the target compounds with relative standard deviations between 0.36 (Phe) and 4.2% (Naph) and 3.1 (Ft) and 13.2 (Naph), respectively (Table 3).

The trueness [56,57] of MASE-HPLC-FLD method was demonstrated by analysing four tea infusions samples and four fruit/herbal-tea beverages at two spiking levels (12.5 and 25 μg L^{-1} for all target PAHs) in triplicates, obtaining analytical recoveries that ranged between 76 and 117% (Table 4).

A comparison between analytical figures of merit (LODs, analytical recoveries, enrichment factors and precision) were performed by considering the methodology proposed in the present study and other procedures reported in the literature concerning PAHs analysis in tea infusions (Table 5). Organic solvent volumes used in the extraction/pre-concentration of PAHs procedure were also enclosed. The classic extraction/pre-concentration methods (LLE, SPE and QuEChERS, Table 5) require more sample treatment steps and high organic solvent consumption compared to micro-extraction procedures including the approach proposed in this work. LODs, analytical recoveries and repeatability of this work are similar than those achieved by other previous micro-extraction procedures (Table 5). However, the method that we propose in this study is more simple and rapid with previously reported methods. Complex extracting material preparation or synthesis of cotton based carbon fiber [29], agarose-chitosan-immobilized octadecylsilyl-silica (C18) [31], phenyl-functionalized magnetic sorbent [34], multi-walled carbon nanotubes-poly(vinyl alcohol) cryogel [38], fullerene functionalized Fe_3O_4@SiO_2@C_{60} magnetic nanoparticles [30], metal–organic framework (MOF) HKUST-1/ Fe_3O_4 magnetic nanoparticles [32], or ionic liquid of immobilized Fe_3O_4@3-(Trimethoxysilyl)propyl methacrylate@ionic liquid magnetic nanoparticles [33] are required for d-μSPE procedures. Although in IL-DLLME methods [35–37] organic solvents volumes are partial substituted by the use of ionic liquids, the synthesis of those commercialised liquids could not be "green" enough. Moreover, MASE procedure and HPLC-FLD quantification is more cost–effective when comparing with SPME or SBSE coupled to GC–MS. Then, our proposed MASE method offers high analytical potential and practical advantages for routine extraction and pre-concentration of PAHs in tea infusions. Finally, the analytical method developed meets the performance criteria for the LOD (<0.3 ng g^{-1}), LOQ (<0.9 ng g^{-1}) and analytical recovery (between 50 and 120%) described in the European Commission Regulation 836/2011 for BaA, Chry, BbF and BaP in foodstuffs (Table 5) [15].

3.3. Application

PAHs content distribution in several tea infusions (three green tea, three red tea, three black tea and three white tea) and four tea beverages

Table 4
Analytical recovery (n = 3) for several tea infusions fruit/herbal-tea beverages at two spiking levels (12.5 and 25 ng L^{-1}).

PAH	Green tea		Red tea		Black tea		White tea		Tea beverages	
	12.5 ng L^{-1}	25 ng L^{-1}	12.5 ng L^{-1}	25 ng L^{-1}	12.5 ng L^{-1}	25 ng L^{-1}	12.5 ng L^{-1}	25 ng L^{-1}	12.5 ng L^{-1}	25 ng L^{-1}
Naph	90 ± 12	92 ± 14	88 ± 6	87 ± 5	98 ± 18	79 ± 3	78 ± 11	82 ± 7	87 ± 12	78 ± 8
Ace	89 ± 6	99 ± 18	91 ± 3	84 ± 6	87 ± 14	88 ± 2	84 ± 1	91 ± 6	106 ± 15	101 ± 6
Fl	96 ± 4	99 ± 15	102 ± 3	89 ± 7	93 ± 12	84 ± 2	87 ± 4	100 ± 9	104 ± 1	93 ± 9
Phe	113 ± 7	104 ± 5	121 ± 1	96 ± 10	113 ± 12	94 ± 3	96 ± 5	92 ± 6	103 ± 3	92 ± 3
Ant	97 ± 1	87 ± 8	89 ± 10	93 ± 14	97 ± 10	86 ± 4	89 ± 8	81 ± 7	100 ± 4	87 ± 5
Ft	98 ± 11	92 ± 7	100 ± 9	88 ± 2	101 ± 9	86 ± 9	90 ± 2	102 ± 6	91 ± 1	94 ± 9
Pyr	100 ± 4	98 ± 2	99 ± 9	85 ± 5	102 ± 6	88 ± 10	89 ± 2	87 ± 5	93 ± 1	87 ± 3
BaA	98 ± 6	102 ± 5	95 ± 10	101 ± 11	95 ± 12	82 ± 4	91 ± 2	94 ± 8	112 ± 3	96 ± 7
Chry	98 ± 4	92 ± 5	102 ± 5	104 ± 10	92 ± 5	90 ± 3	92 ± 3	101 ± 5	96 ± 2	107 ± 14
BeP	88 ± 2	89 ± 1	92 ± 6	85 ± 6	87 ± 7	88 ± 8	79 ± 3	86 ± 7	91 ± 2	81 ± 10
BbF	80 ± 3	91 ± 3	86 ± 10	80 ± 5	86 ± 8	80 ± 9	78 ± 4	96 ± 3	95 ± 1	86 ± 6
BkF	80 ± 4	87 ± 5	80 ± 11	78 ± 8	84 ± 10	84 ± 11	76 ± 8	85 ± 9	95 ± 1	94 ± 7
BaP	117 ± 2	107 ± 3	116 ± 11	83 ± 3	109 ± 5	89 ± 10	89 ± 12	102 ± 10	107 ± 1	91 ± 6
DBahA	83 ± 1	84 ± 5	79 ± 14	84 ± 8	80 ± 4	86 ± 12	80 ± 8	87 ± 8	81 ± 2	96 ± 7
BghiP	114 ± 1	104 ± 4	113 ± 9	80 ± 2	108 ± 4	83 ± 11	81 ± 13	95 ± 3	100 ± 1	94 ± 5
IP	95 ± 1	78 ± 12	102 ± 14	88 ± 6	98 ± 3	84 ± 11	79 ± 6	83 ± 7	91 ± 2	79 ± 8

Table 5
A brief comparative of analytical parameters for analytical methods commonly used for PAH determination in tea infusions.

Pre-concentration technique	Target PAH	Solvents (Solvent volumes)	Determination technique	LOD (ng L^{-1})	Recovery (%)	EF	Precision RSD (%)	Ref.
LLE	Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	DCM (150 mL)	GC–MS	1.1–2.7	53–132	200	–[a]	[1]
LLE	Naph, Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Cyclohexane (6 mL), ACN (100 μL)	HPLC-FLD	0.2–10	40–120	–[a]	5–25	[5]
LLE	Naph, Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	DCM (160 mL), hexane (12 mL), ACN (2 mL)	HPLC-FLD	0.66–3.67[b]	72–103	–[a]	<20	[11]
LLE	Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Hexane (105 mL), RtOH (5 mL)	GC–MS-MS	23.1–97.1	72–108	–[a]	5.0–8.7	[12]
LLE	Naph, Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Cyclohexane (115 mL)	GC–MS	0.10–0.28	36–105	–[a]	–[a]	[17]
LLE	Naph, Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP, BcF, BcPhe, CPcdPyr, BjF, 7,12 dmaAnt, BeP, 3mCho, DBaiPyr, DBaePyr, DBahPyr, 5mChry, DBalPyr	Hexane (75 mL), formic acid (5 mL)	GC–MS	70–90[c]	62–108	–[a]	–[a]	[18]
LLE	BaA, Chry, BbF, BaP	Hexane (100 mL)	GC–MS	10[c]	72–110	–[a]	0.7–3	[19]
LLE	Naph, Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	DCM (161 mL), hexane (50 mL)	GC-FID	30–240[c]	64–98	–[a]	1.0–9.6	[20]
SPE	BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Formic acid 0.1% v/v (10 mL), formic acid 0.1% v/v with 40% MeOH (10 mL), and formic acid 0.1% v/v with 80% MeOH (10 mL), DCM with 10% hexane (8 mL), ACN (2 mL)	EEMF and RTEFM	0.05–0.22[d] for EEMF and 1.5–12.1[d] for RTEFM	28–104	142–520	–[a]	[21]
SPE	Naph, Acy, Ace Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	ACN (350 μL)	GC–MS	0.02–0.6	90–103	300	3.5–6.5	[22]
SPE	Phe, Ft, Pyr, BaA, BeP, BaP, DBahA, BghiP		HPLC-FLD	16–140	>54	–[a]	5.3–11	[24]
QuEChERS	BaA, Chry, BbF, BaP	ACN (10 mL)	HPLC-FLD	150–250[c]	51–93	–[a]	2.2–8.8	[25]
QuEChERS	BaA, Chry, BbF, BaP	EtOAc (10 mL), ACN (1 mL)	HPLC-FLD	30–50[c]	54–99	–[a]	4–7	[26]
QuEChERS	BaA, Chry, BbF, BaP	ACN (33 mL), acetone (6 mL), MeOH (24 mL), hexane (8 mL), DCM (6 mL)	GC–MS-MS	0.1[d]	67–88	–[a]	0.2–0.6	[27]
QuEChERS	Acy, Fl, Phe, Ant, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, IP	ACN (10 mL), hexane (5 mL)	GC–MS	0.2–0.4[e]	~50–~120	–[a]	<20	[28]
d-μSPE	Ace, Phe, Ant	Toluene (2 mL)	GC–MS	12–14	76–126	4.1–4.7	<7.8	[29]
d-μSPE	Naph, Acy, Ace Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Hexane (500 μL), acetone (500 μL)	GC–MS	0.8–14.3	92–107	–[a]	<10.6	[30]
d-μSPE	Phe, Pyr	MeOH (–[b])	HPLC-UV	0.55–0.67[d]	101–106	4–72	<13.5	[31]
d-μSPE	BaA, Chy, BbF, BkF, BaP, DBahA, BghiP, IP	ACN (1.5 mL)	UHPLC-FLD	6.1–21	70–75	–[a]	<19	[32]
d-μSPE	Phe, BaA, Chry, BbF, BkF, BaP, DBahA	Acetone (400 μL)	HPLC-FLD	0.1–10	88–105	106–125	2–3	[33]
d-μSPE	Fl, Ant, Ft, Pyr	Octanol (20 μL), acetone (200 μL)	GC–MS	3–16	86–109	61–239	1–3.8	[34]
IL-DLLME	BaA, Chry, BbF, BkF, BaP, DBahA, BghiP	ACN (200 μL)	HPLC-FLD	2–30.8	56–94	61–94	2–5	[35]
IL-DLLME	BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP, BcF, CPcdPyr, BjF, DBaiPyr, DBaePyr, DBahPyr, 5mChry, DBalPyr	Solvent free	HPLC-FLD	10–600	94–114	8.6–50	4.2–19	[36]
IL-DLLME	BaA, Chry, BbF, BkF, BaP	Acetone (500 μL), hexane (1 mL), ACN (300 μL)	HPLC-FLD	5–20	92–120	9–17	1–13	[37]
On line-SPE	BaA, Chry, BaP, BbF	ACN (25 μL)	HPLC-UV	50–200	83–98	–[a]	0.5–12	[38]
SBSE	Naph, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP	Solvent free	GC–MS	11–26	–[a]		3.5–7	[39]
SBSE	Naph, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	ACN (128 μL)	HPLC-FLD	0.1–8.9	24–87	–[a]	0.8–11.3	[40]
SPME		Solvent free	HPLC-FLD	4–145	–[a]		5–17	[41]

(continued on next page)

Table 5 (*continued*)

Pre-concentration technique	Target PAH	Solvents (Solvent volumes)	Determination technique	LOD (ng L^{-1})	Recovery (%)	EF	Precision RSD (%)	Ref.
	Naph, Acy, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry							
SPME	Phe, Ft, BaP	THF (100 μL)	HPLC-UV	0.1–50	91–107	–[a]	0.8–4.5	[42]
In-tube SPME	Naph, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Solvent free	HPLC-FLD	0.32–4.6	70–101	–[a]	1.9–19.6	[43]
MASE	Naph, Ace, Fl, Phe, Ant, Ft, Pyr, BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, IP	Hexane (350 μL)	HPLC-FLD	0.6–26	43	42.8	0.4–4.2	[This work]

Ace, acenaphthene; Acy, acenaphthylene; ACN, acetonitrile; Ant, anthracene; BaA, benzo(a)anthracene; BaP, benzo(a)pyrene; BbF, benzo(b)fluoranthene; BcF, benzo(c)fluorene; BcPhe, benzo(c)phenanthrene; BeP, benzo(e)pyrene; BghiP, benzo(g,h,i)perylene; BkF, benzo(k)fluoranthene; BjF, benzo(j)fluoranthrene; CFs, carbon fibers; Chry, chrysene; CPcdPyr, cyclopenta(c,d)pyrene; DBaePyr, dibenzo(a,e)pyrene; DBahA, dibenz(a,h)anthracene; DBahPyr, dibenzo(a,h)pyrene; DBaiPyr, dibenzo(a,i)pyrene; DBalPyr, dibenzo(a,l)pyrene; DCM, dichloromethane; DIW, deionised water; DSPE, dispersive solid-phase extraction; d-μSPE. dispersive micro-solid phase extraction; EEMF, excitation-emission matrix fluorescence; EtOAc, ethyl acetate; FLD, fluorescence detection; FID, flame ionization detector; Fl, fluorene; Ft, fluoranthene; GC–MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; IL, ionic liquid;[MOEDEA][FAP], ethyl-dimethyl-(2-methoxyethyl)ammonium tris (pentafluoroethyl)trifluorophosphate; IP, indeno(1,2,3-cd)pyrene; MASE, membrane assisted solvent extraction; MS-MS, tandem mass spectrometry; Naph, naphthalene; Phe, phenanthrene; Pyr, pyrene; QuEChERS, quick, easy, cheap, effective, rugged and safe; RTEFM, retention time-emission spectra matrices; RT, room temperature; SPME, solid phase microextraction; THF, tetrahydrofuran; UHPLC, ultra-high performance liquid chromatography; UV, ultraviolet; 3mCho, 3-methylcholanthrene; 5mChry, 5-methylchryzene; 7,12 dmaAnt, 7,12-dimethylbenz(a)anthracene.

[a] Not given.
[b] LOQ as ng L^{-1}.
[c] Expressed as mg kg^{-1}.
[d] Data expressed as μg L^{-1}.
[e] Expressed as μg kg^{-1}.

Table 6
PAHs concentration (mean value ± SD) in several commercial tea infusions.

PAH	Green tea		Red tea		Black tea		White tea		Fruit/herbal tea beverage
	ng L^{-1}	ng Kg^{-1}[a]	ng L^{-1}	ng Kg^{-1}[a]	ng L^{-1}	ng Kg^{-1}[a]	ng L^{-1}	ng Kg^{-1}[a]	ng L^{-1}
Naph	–[c]	–[c]	–[c]	–[c]	54.8 ± 0.57	2.7 ± 0.03	–[c]	–[c]	–[c]
Ace	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
Fl	–[c]	–[c]	6.9 ± 0.40	0.35 ± 0.02	–[c]	–[c]	–[c]	–[c]	–[c]
Phe	–[c]	–[c]	46.8 ± 3.4	2.3 ± 0.17	39.8 ± 2.3	2.1 ± 0.12	–[c]	–[c]	–[c]
Ant	2.9 ± 0.2	0.15 ± 0.01	5.5 ± 0.40	0.28 ± 0.02	4.8 ± 0.27	0.24 ± 0.10	–[c]	–[c]	–[c]
Ft	16.1 ± 1.3	0.81 ± 0.07	20.9 ± 1.2	1.0 ± 0.06	15.7 ± 1.1	0.79 ± 0.06	–[c]	–[c]	–[c]
Pyr	18.0 ± 2.4	0.90 ± 0.12	22.2 ± 2.1	1.1 ± 0.11	22.5 ± 2.4	1.1 ± 0.12	–[c]	–[c]	–[c]
BaA	2.9 ± 0.15	0.15 ± 0.008	3.0 ± 0.47	0.15 ± 0.02	3.5 ± 0.22	0.18 ± 0.01	–[c]	–[c]	1.8 ± 0.16
Chry	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
BeP	6.0 ± 0.46	0.30 ± 0.02	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
BbF	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
BkF	2.6 ± 0.17	0.13 ± 0.009	1.0 ± 0.17	0.05 ± 0.009	1.1 ± 0.21	0.06 ± 0.01	–[c]	–[c]	2.3 ± 0.18
BaP	4.6 ± 0.55	0.23 ± 0.03	2.6 ± 0.18	0.13 ± 0.009	3.6 ± 0.14	0.18 ± 0.007	–[c]	–[c]	2.8 ± 0.12
DBahA	1.7 ± 0.70	0.09 ± 0.04	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	2.0 ± 0.13
BghiP	6.6 ± 0.28	0,33 ± 0.01	3.0 ± 0.05	0.15 ± 0.003	5.9 ± 1.1	0.30 ± 0.06	1.2 ± 0.20	0.06 ± 0.01	2.6 ± 0.04
IP	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
$\Sigma_{16}C_{PAHs}$	61.4 ± 2.9	3.1 ± 0.15	111.9 ± 4.2	5.6 ± 0.21	151.7 ± 3.7	7.6 ± 0.19	1.2 ± 0.20	0.06 ± 0.01	11.5 ± 0.30
Σ_4C_{PAHs}[b]	7.5 ± 0.57	0.38 ± 0.03	5.6 ± 0.50	0.28 ± 0.03	7.1 ± 0.26	0.36 ± 0.01	–[c]	–[c]	4.6 ± 0.20
$\Sigma_{16}C_{BaPeq}$	19.8 ± 3.6	0.99 ± 0.18	3.2 ± 0.19	0.16 ± 0.009	4.3 ± 0.14	0.21 ± 0.007	0.01 ± 0.002	0.0005 ± 0.0001	13.2 ± 0.66
Σ_8C_{BaPMeq}	6.9 ± 0.60	0.34 ± 0.03	3.5 ± 0.20	0.18 ± 0.009	5.1 ± 0.30	0.26 ± 0.01	0.23 ± 0.04	0.01 ± 0.02	4.3 ± 0.10

[a] Results calculated on the mass basis of leaves used for infusion preparation.
[b] $\Sigma(C_{BaA} + C_{Chry} + C_{BbF} + C_{BaP})$.
[c] Concentration lower than LOQs.

expressed as ng L^{-1} is shown in Table 6. PAHs concentrations, expressed as ng g^{-1}, were calculated on the mass basis of leaves used for infusion preparation, are also shown in Table 6. As can be seen, Pyr and Ft were the most abundant PAHs in the green, red and black tea infusions; Phe is also abundant in red and black tea infusions; and Naph in black tea infusions. In addition, all PAHs, except for BghiP for white tea infusion and BaP, BghiP, BkF, DBahA abd BaA for tea beverages, were below LOQs. The total mean $\Sigma_{16}C_{PAHs}$ in samples were from 1.2 ng L^{-1} to 151.7 ng L^{-1}, showing high PAHs concentrations in black tea infusions, followed by red and green tea infusions, tea beverages and white tea infusions. The mean Σ_4C_{PAH} (sum of BaA, Chry, BbF and BaP concentrations) were 7.5 ± 0.57 ng L^{-1}, 5.6 ± 0.50 ng $L^{-1}$and 7.1 ± 0.26 ng L^{-1} for green, red and black tea infusions, respectively; and 4.6 ± 0.20 ng L^{-1} for tea beverages. BaP concentrations were from < 1.5 ng L^{-1} (white tea infusions) to 4.6 ng L^{-1} (green tea infusions). The wide PAHs content variations found in studied samples might be attributed to the different production and manufacturing processes of tea leaves [5–9]. As can be seen in Table 6, the maximum values of BaP (10 ng g^{-1}) and the sum of BaA, Chry, BbF and BaP (50 ng g^{-1}) for foodstuffs (food supplements containing botanicals, dried herbs and their preparations) set by the European Food Safety Agency [13] were not exceeded.

Finally, although the carcinogenicity of PAHs relatively to BaP equivalent (BaP_{eq}) concentration of 16 PAHs ($\Sigma_{16}C_{BaPeq}$) and the total mutagenicity of PAHs relatively to BaP mutagenic equivalent (BaP_{Meq})

concentration of 8 USEPA priority PAHs (Σ_8C_{BaPMeq}) regulatory standards have not been established yet, those concentrations were achieved (Table 6). $\Sigma_{16}C_{BaPeq}$ and Σ_8C_{BaPMeq} were calculated using the following formulas [58,59]:

$$\sum_{16} C_{BaP_{eq}} = \sum_{i=1}^{i=16} (C_{PAH_i} x TEF_i)$$

$$\sum_{8} C_{BaP_{Meq}} = \sum_{i=1}^{i=8} (C_{PAH_i} x MEF_i)$$

where C_{PAHi} represents the concentration of each PAH in tea infusions (expressed as ng L^{-1}), TEF_i is the toxic equivalence factor of the PAH_i relative to BaP and MEF_i is the mutagenic equivalence factor (MEF) value of the PAH_i. TEFs used in this study were: 0.001 for Naph, Ace, Fl, Phe, Ft and Pyr; 0.01 for Ant, Chry and BghiP; 0.1 for BaA, BbF, BkF and IP; 1.0 for BeP and BaP; and 5.0 for DBahA [60]. MEFs used in this study were: 0.082, 0.017, 0.25, 0.11, 1, 0.29, 0.19 and 0.31, for BaA, Chry, BbF, BkF, BaP, DBahA, BghiP, and IP, respectively [59].

Attending to the results obtained (Table 6), $\Sigma_{16}C_{BaPeq}$ values followed the order green tea infusions (19.8 ± 3.6 ng L^{-1}) > tea beverages (13.2 ± 0.66 ng L^{-1}) > black tea infusions (4.3 ± 0.14 ng L^{-1}) > red tea infusions (3.2 ± 0.19 ng L^{-1}) > white tea infusions (0.01 ± 0.02 ng L^{-1}). Finally, Σ_8C_{BaPMeq} values followed the order green tea infusions (6.9 ± 0.60 ng L^{-1}) > black tea infusions (5.1 ± 0.30 ng L^{-1}) > tea beverages (4.3 ± 0.10 ng L^{-1}) > red tea infusions (3.5 ± 0.20 ng L^{-1}) > white tea infusions (0.23 ± 0.04 ng L^{-1}).

4. Conclusions

An environmentally friendly, simple, highly efficient and cost-effective method for PAHs extraction and pre-concentration in tea infusions and tea beverages base on a MASE procedure has been proposed, at few ng L^{-1}. PAHs extracted were quantified by HPLC-FLD using Ant-d10, BeP-12 and DBahA-d14 as surrogate standards that compensate analyte losses and minimize possible matrix effects. Furthermore, MASE procedure was optimised by using a central composite design after the application of Plackett–Burman designs as factor screening. Plackett–Burman designs showed that extraction time, acceptor solvent (hexane) volume and stirring time were the most significant variables involved on the extraction and pre-concentration of PAHs in tea infusions and tea beverages by MASE. In addition, PAHs were efficiently extracted from tea infusions stirring at 190 rpm during 70 min and using 350 μL of hexane as acceptor phase, achieving the very restrictive criteria of LODs, LOQs and analytical recoveries for BaA, Chry, BbF and BaP determination in foodstuffs set by the European Commission Regulation 836/2011. Finally, the proposed method was successfully applied to several tea infusions and tea beverages, and carcinogenic and mutagenic BaP equivalent concentrations were calculated.

CRediT authorship contribution statement

Adriana Mañana-López: Investigation, Methodology. **Joel Sánchez-Piñero:** Data curation, Investigation, Methodology, Writing - review & editing. **Jorge Moreda-Piñeiro:** Conceptualization, Formal analysis, Software, Supervision, Writing - original draft, Writing - review & editing, Validation. **Isabel Turnes-Carou:** Conceptualization, Formal analysis, Supervision, Validation. **Soledad Muniategui-Lorenzo:** Funding acquisition, Project administration, Resources, Visualization. **Purificación López-Mahía:** . **:** Funding acquisition, Project administration, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by Xunta de Galicia (Programa de Consolidación y Estructuración de Unidades de Investigación Competitivas ref: ED431C 2017/28-2017-2020) and FEDER-MINECO (UNLC15-DE-3097, financed together (80/20%) with Xunta de Galicia. Joel Sánchez-Piñero acknowledges the Xunta de Galicia and the European Union (European Social Fund - ESF) for a predoctoral grant (ED481A-2018/164).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2021.106278.

References:

[1] M. Rameshrad, B.M. Razavi, H. Hosseinzadeh, Protective effects of green tea and its main constituents against natural and chemical toxins: A comprehensive review, Food Chem. Toxicol. 100 (2017) 115–137, https://doi.org/10.1016/j.fct.2016.11.035.
[2] M. da Silva Pinto, Tea,, a new perspective on health benefits, Food Res. Int. 53 (2013) 558–567, https://doi.org/10.1016/j.foodres.2013.01.038.
[3] H. Banerjee, P. Ganguly, S. Roy, D. Banerjee, M. Paramasivam, T. Banerjee, K. K. Sharma, Persistence and safety risk assessment of propineb in Indian tea, Environ. Monit. Assess. 170 (2010) 311–314, https://doi.org/10.1007/s10661-009-1234-9.
[4] A.M. Girelli, A. Apriceno, A.M. Tarola, F. Tortora, Determination of polycyclic aromatic hydrocarbons in tea infusions samples by high performance liquid chromatography with fluorimetric detection, J Food Qual. (2017 (2017)) 1076876, https://doi.org/10.1155/2017/1076876.
[5] International Agency for Research on Cancer – IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Overall evaluation of carcinogenicity. Polynuclear aromatic compounds Vol. 35, IARC, Lyon (2010) http://monographs.iarc.fr/ENG/ Monographs/vol1-42/mono35.pdf/ Accessed 15 December 2020.
[6] P. Karimi, K.O. Peters, K. Bidad, P.T. Strickland, Polycyclic aromatic hydrocarbons and childhood asthma, Eur. J. Epidemiol. 30 (2015) 91–101, https://doi.org/10.1007/s10654-015-9988-6.
[7] A. Ramesh, S.A. Walker, D.B. Hood, M.D. Guillén, K. Schneider, E.H. Weyand, Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons, Int. J. Toxicol. 23 (2004) 301–333, https://doi.org/10.1080/10915810490517063.
[8] O.H. Fred-Ahmadu, N.U. Benson, Polycyclic aromatic hydrocarbons (PAHs). Occurrence and toxicity in *Camellia sinensis* and herbal tea, Polycycl. Aromat. Compd. 39 (2019) 383–393, https://doi.org/10.1080/10406638.2017.1335216.
[9] L. Duedahl-Olesen, M.A. Navaratnam, J. Jewula, A.H. Jensen, PAH in some brands of tea and coffee, Polycycl. Aromat. Compd. 35 (2015) 74–90, https://doi.org/10.1080/10406638.2014.918554.
[10] D. Lin, Y. Tu, L. Zhu, Concentrations and health risk of polycyclic aromatic hydrocarbons in tea, Food Chem.l Toxicol. 43 (2005) 41–48, https://doi.org/10.1016/j.fct.2004.08.010.
[11] G. Gao, H. Chen, H., P. Liu, Z. Hao, G. Ma, Y. Chai, C. Wang, C. Lu, Residue pattern of polycyclic aromatic hydrocarbons during green tea manufacturing and their transfer rates during tea brewing, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 34 (2017) 990–999. https://doi.org/10.1080/19440049.2017.1316873.
[12] European Food Safety Authority EFSA Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food European Food Safety Authority Journal, 724 (2008) 1-114.
[13] Commission of the European Communities Commission Regulation (EC) No 835/2011, of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs, Off. J. Eur. Commun., L215 (2011) 4-8.
[14] Commission of the European Communities Commission Regulation (EC) 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, Off. J. Eur. Commun. (2011) 9-16.
[15] Y. Sun, S. Wu, G. Gong, Trends of research on polycyclic aromatic hydrocarbons in food: A 20-year perspective from 1997 to 2017, Trends Food Sci. Technol. 83 (2019) (1997) 86–98, https://doi.org/10.1016/j.tifs.2018.11.015.
[16] A. Ciemniak, K. Kuźmicz, M. Rajkowska-Myśliwiec, M.F. Cadena, Assessing the contamination levels of dried teas and their infusions by polycyclic aromatic hydrocarbons (PAHs), J. Consum. Prot. Food S. 14 (2019) 263–274, https://doi.org/10.1007/s00003-019-01229-1.

[17] M. Roszko, M. Kamińska, K. Szymczyk, R. Jędrzejczak, Dietary risk evaluation for 28 polycyclic aromatic hydrocarbons (PAHs) in tea preparations made of teas available on the Polish retail market, J. Environ. Sci. Health, Part B 53 (2018) 25–34, https://doi.org/10.1080/03601234.2017.1369323.
[18] J.-G. Lee, T. Lim, S.-H. Kim, D.-H. Kang, H.-J. Yoon, Determination and risk characterization of polycyclic aromatic hydrocarbons of tea by using the Margin of Exposure (MOE) approach, Food Sci. Biotechnol. 27 (2018) 1843–1856, https://doi.org/10.1007/s10068-018-0400-7.
[19] C.M.A. Iwegbue, G.O. Tesi, F.I. Bassey, B.S. Martincigh, G.E. Nwajei, C. Ucheaga, Determination of polycyclic aromatic hydrocarbons in water- and gin-based tea infusions of selected tea brands in Nigeria, Polycycl. Aromat. Compd. 36 (2016) 564–586, https://doi.org/10.1080/10406638.2015.1033433.
[20] M.A. Bravo, A. Gonzalez L., B. Valverde O., W. Quiroz V., C. Toledo-Neira, Evaluation of three-way fluorescence data-based for simultaneous determination of polycyclic aromatic hydrocarbons in tea infusion samples at sub-ppb levels by second-order multivariate calibration. Microchem. J. 151 (2019) 104208. https://doi.org/10.1016/j.microc.2019.104208.
[21] A.J. Rascón, A. Azzouz, E. Ballesteros, Use of semi-automated continuous solid-phase extraction and gas chromatography–mass spectrometry for the determination of polycyclic aromatic hydrocarbons in alcoholic and non-alcoholic drinks from Andalucía (Spain), J. Sci. Food Agric. 99 (2019) 1117–1125, https://doi.org/10.1002/jsfa.9279.
[22] D. Lin, L. Zhu, L. Luo, Factors affecting transfer of polycyclic aromatic hydrocarbons from made tea to tea infusion, J. Agric. Food Chem. 54 (2006) 4350–4354, https://doi.org/10.1021/jf060189j.
[23] M.N. Kayali-Sayadi, S. Rubio-Barroso, M.P. Cuesta-Jimenez, L.M. Polo-Díez, Rapid determination of polycyclic aromatic hydrocarbons in tea infusion samples by high-performance liquid chromatography and fluorimetric detection based on solid-phase extraction, Analyst 123 (1998) 2145–2148.
[24] A. Zachara, D. Gałkowska, L. Juszczak, Contamination of tea and tea infusion with polycyclic aromatic hydrocarbons, Int. J. Environ. Res. Public Health 15 (2018) 45, https://doi.org/10.3390/ijerph15010045.
[25] S.A.V. Tfouni, R.M. Reis, K. Kamikata, F.M.L. Gomes, M.A. Morgano, R.P. Z. Furlani, Polycyclic aromatic hydrocarbons in teas using QuEChERS and HPLC-FLD, Food Addit. Contam. Part B 11 (2018) 146–152, https://doi.org/10.1080/19393210.2018.1440638.
[26] J. Pincemaille, C. Schummer, E. Heinen, G. Moris, Determination of polycyclic aromatic hydrocarbons in smoked and non-smoked black teas and tea infusions, Food Chem. 145 (2014) 807–813, https://doi.org/10.1016/j.foodchem.2013.08.121.
[27] A. Sadowska-Rociek, M. Surma, E. Cieślik, Comparison of different modifications on QuEChERS sample preparation method for PAHs determination in black, green, red and white tea, Environ. Sci. Pollut. Res. 21 (2014) 1326–1338, https://doi.org/10.1007/s11356-013-2022-1.
[28] Z. Shi, J. Jiang, W. Pang, H. Ma, X. Chu, C. Zhou, H. Zhang, Dispersive micro-solid phase extraction using cotton based carbon fiber sorbent for the determination of three polycyclic aromatic hydrocarbons in tea infusion by gas chromatography-quadrupole mass spectrometry, Microchem. J. 151 (2019), 104209, https://doi.org/10.1016/j.microc.2019.104209.
[29] D.-B. Zhou, X. Sheng, F. Han, Y.-Y. Hu, L. Ding, Y.-L. Lv, W. Song, P. Zheng, Magnetic solid-phase extraction based on [60fullerene functionalization of magnetic nanoparticles for the determination of sixteen polycyclic aromatic hydrocarbons in tea samples, J. Chromatogr. A 1578 (2018) 53–60, https://doi.org/10.1016/j.chroma.2018.10.010.
[30] N.T. Ng, M.M. Sanagi, W.N.W. Ibrahim, W.A.W. Ibrahim, Agarose-chitosan-C18 film micro-solid phase extraction combined with high performance liquid chromatography for the determination of phenanthrene and pyrene in chrysanthemum tea samples, Food Chem. 222 (2017) 28–34, https://doi.org/10.1016/j.foodchem.2016.11.147.
[31] P. Rocío-Bautista, V. Pino, J.H. Ayala, J. Pasán, C. Ruiz-Pérez, A.M. Afonso, A magnetic-based dispersive micro-solid-phase extraction method using the metal-organic framework HKUST-1 and ultra-high-performance liquid chromatography with fluorescence detection for determining polycyclic aromatic hydrocarbons in waters and fruit tea infusions, J. Chromatogr. A 1436 (2016) 42–50, https://doi.org/10.1016/j.chroma.2016.01.067.
[32] Y. Shi, H. Wu, C. Wang, X. Guo, J. Du, L. Du, Determination of polycyclic aromatic hydrocarbons in coffee and tea samples by magnetic solid-phase extraction coupled with HPLC–FLD, Food Chem. 199 (2016) 75–80, https://doi.org/10.1016/j.foodchem.2015.11.137.
[33] F.W.M. Hassan, M. Raoov, S. Kamaruzaman, M.M. Sanagi, N. Yoshida, Y. Hirota, N. Nishiyama, N. Yahaya, Dispersive liquid–liquid microextraction combined with dispersive solid-phase extraction for gas chromatography with mass spectrometry determination of polycyclic aromatic hydrocarbons in aqueous matrices, J Sep Sci 41 (2018) 3751–3763, https://doi.org/10.1002/jssc.201800326.
[34] C. Rivera-Vera, G. Lasarte-Aragonés, M.A. Bravo, D. Muñoz-Lira, R. Salazar, C. Toledo-Neira, Ionic liquids-based dispersive liquid-liquid microextraction for determination of carcinogenic polycyclic aromatic hydrocarbons in tea beverages: Evaluation of infusion preparation on pollutants release, Food Control 106 (2019), 106685, https://doi.org/10.1016/j.foodcont.2019.06.011.
[35] M. Germán-Hernández, P. Crespo-Llabrés, V. Pino, J.H. Ayala, A.M. Afonso, Utilization of an ionic liquid in situ preconcentration method for the determination of the 15 + 1 European Union polycyclic aromatic hydrocarbons in drinking water and fruit-tea infusions, J. Sep. Sci. 36 (2013) 2496–2506.
[36] M.J. Trujillo-Rodríguez, O. Nacham, K.D. Clark, V. Pino, J.L. Anderson, J.H. Ayala, A.M. Afonso, Magnetic ionic liquids as non-conventional extraction solvents for the determination of polycyclic aromatic hydrocarbons, Anal. Chim. Acta 934 (2016) 106–113, https://doi.org/10.1016/j.aca.2016.06.014.
[37] C. Siritham, C. Thammakhet-Buranachai, P. Thavarungkul, P. Kanatharana, A preconcentrator-separator two-in-one online system for polycyclic aromatic hydrocarbons analysis, Talanta 167 (2017) 573–582, https://doi.org/10.1016/j.talanta.2017.02.035.
[38] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, Use of headspace sorptive extraction coupled to gas chromatography–mass spectrometry for the analysis of volatile polycyclic aromatic hydrocarbons in herbal infusions, J. Chromatog. A 1356 (2014) 38–44, https://doi.org/10.1016/j.chroma.2014.06.060.
[39] V.G. Zuin, L. Montero, C. Bauer, P. Popp, Stir bar sorptive extraction and high-performance liquid chromatography–fluorescence detection for the determination of polycyclic aromatic hydrocarbons in Mate teas, J. Chromatog. A 1091 (2005) 2–10, https://doi.org/10.1016/j.chroma.2005.07.057.
[40] P. Viñas, N. Campillo, N. Aguinaga, E. Pérez-Cánovas, M. Hernández-Córdoba, Use of headspace solid-phase microextraction coupled to liquid chromatography for the analysis of polycyclic aromatic hydrocarbons in tea infusions, J. Chromatog. A 1164 (2007) 10–17, https://doi.org/10.1016/j.chroma.2007.06.056.
[41] S.H. Loh, M.M. Sanagi, W.A.W. Ibrahim, M.N. Hasan, Multi-walled carbon nanotube-impregnated agarose film microextraction of polycyclic aromatic hydrocarbons in green tea beverage, Talanta 106 (2013) 200–205, https://doi.org/10.1016/j.talanta.2012.12.032.
[42] A. Ishizaki, K. Saito, N. Hanioka, S. Narimatsu, H. Kataoka, Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection, J. Chromatog. A 1217 (2010) 5555–5563, https://doi.org/10.1016/j.chroma.2010.06.068.
[43] B. Hauser, P. Popp, Membrane-assisted solvent extraction of organochlorine compounds in combination with large-volume injection/gas chromatography–electron capture detection, J. Sep. Sci. 24 (2001) 551–560, https://doi.org/10.1002/1615-9314.
[44] S. Ncube, G. Lekoto, E. Cukrowska, L. Chimuka, Development and optimisation of a novel three-way extraction technique based on a combination of Soxhlet extraction, membrane assisted solvent extraction and a molecularly imprinted polymer using sludge polycyclic aromatic hydrocarbons as model compounds, J. Sep. Sci. 41 (2018) 918–928, https://doi.org/10.1002/jssc.201701216.
[45] S. Ncube, N. Tavengwa, A. Soqaka, E. Cukrowska, L. Chimuka, Development of a single format membrane assisted solvent extraction-molecularly imprinted polymer technique for extraction of polycyclic aromatic hydrocarbons in wastewater followed by gas chromatography mass spectrometry determination, J. Chromatogr. A (1569 (2018)) 36–43, https://doi.org/10.1016/j.chroma.2018.07.061.
[46] J.G. March, F. Moukhchan, V. Cerdà, Application of in-vial membrane assisted solvent extraction to the determination of polycyclic aromatic hydrocarbons in seawater by gas chromatography–mass spectrometry, Anal. Chim. Acta 685 (2011) 132–137, https://doi.org/10.1016/j.aca.2010.11.028.
[47] A. Prieto, O. Telleria, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, Simultaneous preconcentration of a wide variety of organic pollutants in water samples Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction, J. Chromatogr. A 1214 (2008) 1–10, https://doi.org/10.1016/j.chroma.2008.10.060.
[48] R. Rodil, M. Schellin, P. Popp, Analysis of polycyclic aromatic hydrocarbons in water and beverages using membrane-assisted solvent extraction in combination with large volume injection–gas chromatography–mass spectrometric detection, J. Chromatogr. A 1163 (2007) 288–297, https://doi.org/10.1016/j.chroma.2007.06.031.
[49] V.G. Zuin, M. Schellin, L. Montero, J.H. Yariwake, F. Augusto, P. Popp, Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction as enrichment techniques for the determination of pesticide and benzo[a]pyrene residues in Brazilian sugarcane juice, J. Chromatogr. A 1114 (2006) 180–187, https://doi.org/10.1016/j.chroma.2006.03.035.
[50] C. Ramos-Contreras, E. Concha-Graña, P. López-Mahía, F. Molina-Pérez, S. Muniategui-Lorenzo, Determination of atmospheric particle-bound polycyclic aromatic hydrocarbons using subcritical water extraction coupled with membrane microextraction, J. Chromatogr. A 1606 (2019), 460381, https://doi.org/10.1016/j.chroma.2019.460381.
[51] ISO 3103:2019. Tea - Preparation of liquor for use in sensory tests.
[52] J. Sánchez-Piñero, J. Moreda-Piñeiro, E. Concha-Graña, M. Fernández-Amado, S. Muategui-Lorenzo, P. López-Mahía, Inhalation bioaccessibility estimation of polycyclic aromatic hydrocarbons from atmospheric particulate matter (PM10): Influence of PM10 composition and health risk assessment, Chemosphere 263 (2021), 127847, https://doi.org/10.1016/j.chemosphere.2020.127847.
[53] Y. Qian, T. Posch, T.C. Schmidt, Sorption of polycyclic aromatic hydrocarbons (PAHs) on glass surfaces, Chemosphere 82 (2011) 859–865, https://doi.org/10.1016/j.chemosphere.2010.11.002.
[54] USEPA, Method 800d - Determinative chromatographic separations (5th revision), (2018). https://www.epa.gov/sites/production/files/2015-12/documents/8000d.pdf (accessed March 15, 2021).
[55] Commission of the European Communities, Commission Decision 2002/657/EC: implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Off. J. Eur. Commun., L221/8.
[56] EN 15549:2008 - Air quality - Standard method for the measurement of the concentration of benzo[a]pyrene in ambient air, (2008). https://standards.iteh.ai/catalog/standards/cen/b3e83792-1321-4a61-bfc7-0b10b50ae9cc/en-15549-2008.

[57] P. Gao, D. Liu, L. Guo, C. He, N. Lin, Y. Xing, C. Yao, B. Wu, Z. Zheng, Y. Wang, J. Hang, Ingestion bioaccessibility of indoor dust-bound PAHs: Inclusion of a sorption sink to simulate passive transfer across the small intestine, Sci. Total Environ. 659 (2019) 1546–1554, https://doi.org/10.1016/J.SCITOTENV.2018.12.459.

[58] J.L. Durant, W.F. Busby, A.L. Lafleur, B.W. Penman, C.L. Crespi, Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols, Mutat. Res. Genet. Toxicol. 371 (1996) 123–157, https://doi.org/10.1016/S0165-1218(96)90103-2.

[59] V. Samburova, B. Zielinska, A. Khlystov, Do 16 polycyclic aromatic hydrocarbons represent PAH air toxicity? Toxics 5 (2017) 17, https://doi.org/10.3390/toxics5030017.