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Stir bar sorptive extraction and high-performance liquid chromatography–fluorescence detection for the determination of polycyclic aromatic hydrocarbons in Mate teas

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Abstract

A simple procedure based on stir bar sorptive extraction (SBSE) and high-performance liquid chromatography–fluorescence detection (HPLC–FLD) is presented for the determination of 15 polycyclic aromatic hydrocarbons (PAHs) in herbal tea prepared with Mate leaves (*Ilex paraguariensis* St. Hil.). The influence of methanol and salt addition to the samples, the extraction time, the desorption time and the number of desorption steps, as well as the matrix effect, were investigated. Once the SBSE method was optimised (10 mL of Mate tea, 2 h extraction at room temperature followed by 15 min desorption in 160 μL of an acetonitrile (ACN)–water mixture), analytical parameters such as repeatability (≤10.1%), linearity ($r^2 \geq 0.996$), limit of detection (LOD, 0.1–8.9 ng L^{-1}), limit of quantitation (LOQ, 0.3–29.7 ng L^{-1}) and absolute recovery (24.2–87.0%) were determined. For calibration purposes, a reference sample was firstly obtained by removing the analytes originally present in the lowest contaminated Mate tea studied (via SBSE procedure) and then spiked at 1–1200 ng L^{-1} range. The proposed methodology proved to be very convenient and effective, and was successfully applied to the analysis of 11 Mate tea samples commercialised in Brazil. The results of the commercial Mate tea samples found by the SBSE approach were compared with those obtained by liquid–liquid extraction (LLE), showing good agreement.

Keywords: Stir bar sorptive extraction; Polycyclic aromatic hydrocarbons; Mate tea; *Ilex paraguariensis* St. Hil

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds formed and released during the incomplete combustion of organic matter by natural (e.g. carbonisation) and anthropogenic processes [1]. Most PAHs are toxic but, on the basis of their occurrence and carcinogenicity, only 16 of them have been selected as priority contaminants by the European Union (EU) and by the US Environmental Protection Agency (EPA) [2]. Despite the wide distribution of PAHs and the serious health risks to all living organisms exposed to them, only a few reports have been published regarding PAHs in food samples and, more specifically, in tea [3–5].

Mate leaves (*Ilex paraguariensis* St. Hil.) are widely employed in Brazil, Argentina and Paraguay for the preparation of several types of beverages, such as the "chimarrão", "tererê", soft drinks, and teas, among others. These beverages, considered stimulants (owing to the presence of alkaloids such as caffeine) are also considered functional foods, since they may present antioxidant properties (due to compounds such as flavonoids and vitamins). During processing, the leaves and twigs of wild or cultivated origin are usually dried by direct exposure to flames (toasting), followed, in some cases, by roasting. It is well known that these processes may contribute significantly to the formation and increased concentration of PAHs in teas, being responsible for the

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presence of PAHs in herbal infusions at the 10–10^2 ng L^{-1} level [6–8].

In general, the methodologies employed to determine PAHs in liquid food matrices are intricate and time-consuming procedures involving liquid–liquid extraction (LLE) [9] and solid-phase extraction (SPE) [10], which require, especially in the case of LLE, large amounts of samples, large volumes of hazardous solvents and at least one clean-up stage prior to the identification and quantification step. A possible solution to offset some of these limitations would be the use of modern, more selective, simpler, faster and more environmentally friendly sample preparation techniques to simplify or even eliminate the subsequent extract purification processes. Some of the techniques established over recent years and successfully applied to determine PAHs in aqueous samples include liquid-phase microextraction (LPME) [11], membrane-assisted solvent extraction [12], solid-phase microextraction (SPME) [13] and stir bar sorptive extraction (SBSE) [14].

SPME, a sample preparation technique that drove the development of SBSE, was introduced by Arthur and Pawliszyn [15]. This technique is based on the equilibrium of target analytes between a fused silica fibre coated with a thin film of sorbent and the sample matrix. Although SPME, using the most common liquid polymeric coating polydimethylsiloxane (PDMS), is a simple and fast technique, its applicability is limited and low recoveries are obtained for analytes with an octanol–water partition constant (K_{ow}) of $<10^4$. In fact, according to Garcia-Falcón et al. [16], the use of SPME employing a 100 μm-PDMS fiber was not suitable for the determination of PAHs in drinking water at the levels established by European legislation. In that work, SPME yielded limits of detection (LOD) from 3 ng L^{-1} (benzo(a)anthracene) to 37 ng L^{-1} (indeno(1,2,3)pyrene), which were very close to the maximum level allowed by the EU.

The disadvantage of relative low recovery of SPME can be partially eliminated by the use of commercially available fibres containing adsorption-based coatings, such as carbowax-divinylbenzene, but these fibres have only a limited number of active surface sites where adsorptive processes occur. Hence, the linear concentration range for some analytes is limited and the extraction is more susceptible to the effects of the matrix [17].

In addition to commercial SPME fibers, some new stationary phases have been prepared in order to improve extraction capacity, selectivity, sensitivity, repeatability and durability. Thus, Djozan and Assadi [18] proposed a modified pencil lead as a new SPME fiber for direct extraction of PAHs from water, followed by capillary gas chromatography analysis. Despite its robustness and low cost, the selectivity and efficiency of the modified lead fiber were similar to those of commercial fibers. The LODs of the proposed modified fiber for the compounds studied were in the 10–70 ng L^{-1} range.

One possibility to overcome the relatively low extraction capacity of SPME (due to the small amount of coating film of around 0.5 μL or less) is the use of stir bar sorptive extraction. SBSE was proposed by Baltussen et al. [19], utilising 10 and 40 mm long glass stir bars coated with 55 and 219 μL of PDMS. In fact, because of the lower phase ratio between the aqueous and the PDMS phase compared with SPME, considerably higher recoveries for PAHs have been achieved by the SBSE procedure [20]. This advantage is particularly important because the amount of PAHs transferred from the leaves to the tea is usually low and depends on their solubility in hot water, requiring an extraction procedure with high pre-concentration capability for the determination of such contaminants in herbal infusions. On-site determination of 24 PAHs in seawater by SBSE and thermal desorption and gas chromatography coupled to mass spectrometry (SBSE/TD–HRGC–MS) was performed by Roy et al.[21]. It was showed that the coupling of SBSE and a transportable HRGC–MS allowed for the separation, identification and quantification at concentrations of around 0.1–1200 ng L^{-1} after 1 h of extraction. However, these levels still remain too high for monitoring seawater quality and the use of a cold trap focusing system is being studied to lower the LODs and improve repeatability.

As described by Popp et al. [22], the combination of SBSE and solvent desorption with subsequent high-performance liquid chromatography–fluorescence detection (SBSE/HPLC–FLD) was successfully employed to determine PAHs in water samples. Although the use of SBSE/TD–HRGC–MS allowed for unequivocal identification and for LODs similar to those obtained through SBSE/HPLC–FLD (ng L^{-1} range), the latter combination could avoid losses of the more volatile PAHs by vaporisation. Such losses occur because part of the enriched volatiles are vaporised from the stir bar when the thermodesorption glass tubes are transferred from the autosampler to the desorption unit [23]. Also, Popp et al. [24] studied the application of polysiloxane rods (1 mm diameter × 10 mm length) for the extraction of PAHs from natural water, followed by solvent desorption and HPLC–FLD determination. The results obtained with the alternative polysiloxane-based method were comparable to those found by SBSE. However, although silicone rods are much cheaper than stir bars, the latter requires a longer extraction time to reach the equilibrium condition (7 h). To date, the literature is devoid of studies on the analysis of food matrices employing sorptive sample preparation techniques to determine PAHs. This fact reinforces the need for modern methodologies to be developed to determine these contaminants in food, matrices of recognised complexity for which so far there are neither official analytical methods nor maximum levels established by regulatory agencies [25]. The aim of the present work was to develop and validate a very simple and effective analytical procedure based on SBSE/HPLC–FLD to determine PAHs in Mate tea. The method was applied to analyse 11 commercial samples of Brazilian Mate tea. The performance of the SBSE method was compared with the traditional liquid–liquid extraction, showing good agreement.

2. Experimental

2.1. Materials

Acetonitrile (ACN, HPLC Ultra Gradient Grade) and HPLC water were purchased from Baker (Deventer, The Netherlands), while anhydrous Na_2SO_4 (PA), NaCl (PA), methanol (LiChrosolv), methylene chloride (LiChrosolv) and *n*-hexane (Suprasolv) were obtained from Merck (Darmstadt, Germany). The PAH calibration mix (10 μg of each compound per mL ACN) was supplied by Supelco (Bellefont, USA). Standard solutions containing the 16 EPA–PAHs were prepared in ACN, in concentrations ranging from 1 to 1200 ng L^{-1}. The HPLC–FLD was optimized and validated with 15 of the EPA–PAHs, since acenaphthylene is undetectable by fluorescence detection.

The 10-mm-long stir bars coated with a 0.5 mm film thick layer of PDMS (Twister™) were obtained from Gerstel (Mülheim an der Ruhr, Germany). Ten milliliters Erlenmeyer flasks capped with teflon/silicone septa (Supelco, Bellefont, USA) were employed for all the extractions.

Samples of Mate leaves (*Ilex paraguariensis* St. Hil.) were obtained from different Brazilian herbal suppliers. The plant material was powdered, sieved (1–2 mm) and stored in sealed packs protected from humidity, heat and light. Mate teas were freshly prepared before use (simulating as closely as possible the conditions in which these infusions are prepared in everyday practice) by steeping 1.0 g of dried leaves in 100 mL of boiling water for 5 min, filtering and allowing it to cool down at room temperature.

2.2. Sample preparation

2.2.1. SBSE method

Prior to use, each stir bar was placed into a vial containing 1 mL of methylene chloride-methanol mixture (1:1) and magnetically stirred for 5 min. This procedure was repeated three times with fresh portions of the solvent mixture. After drying with a lint-free tissue, the stir bar was conditioned at 280 °C under a nitrogen flow (30 mL min^{-1}) for 2 h. Once conditioned, the stir bar was immediately employed to extract the PAHs from 10 mL of Mate tea at room temperature, using a stirring speed of 750 rpm. After 2 h of extraction, the stir bar was removed from the tea sample using magnetic tweezers, cleaned with a lint-free tissue and placed in a vial with a 250 μL glass insert, which was then filled with 160 μL of an ACN–water mixture (4:1). After 15 min for the solvent desorption of the analytes, the stir bar was removed and the vial containing the extract was put into the HPLC–FLD's autosampler.

2.2.2. LLE method

One hundred milliliters of Mate tea were placed in an Erlenmeyer flask and extracted three times with 3 mL of *n*-hexane, stirring at 750 rpm for 10 min. The combined organic extract was dried with Na_2SO_4, then filtered and concentrated at room temperature near to dryness under a mild argon flow. The residue was then redissolved in 1 mL of ACN and analysed by HPLC–FLD.

2.3. Chromatographic analysis

The chromatographic analyses were performed using a Hewlett-Packard Series 1100 HPLC system (Waldbronn, Germany), equipped with a programmable fluorescence detector (G1321A) and fitted with a Vydac™ 201 TP 52 column (5 μm particle size; 250 mm × 2.1 mm I.D.) and a Vydac™ safeguard column (W. R. Grace & Co., Hesperia, USA). ACN and water were employed as the mobile phase. The gradient elution started with 50% water and 50% ACN, after which the ACN content was increased to 60% (0–2 min), 90% (2–13.5 min) and 95% (13.5–19 min). This level was held constant until the end of the analysis. The column temperature was set at 22 °C. The excitation and emission wavelength programs used were similar to those cited in [22]. A volume of 16 μL of the Mate tea extract obtained by the SBSE procedure was injected into the HPLC–FLD system. The Mate tea extract obtained by LLE was filtered through a regenerated cellulose membrane filter with a 0.45 μm pore size (Agilent, Waldbronn, Germany) and then, a 4 μL aliquot was employed for the HPLC–FLD analysis. The chromatographic data were collected and processed using an HP Chemstation software.

3. Results and discussion

3.1. Development of the SBSE method

Optimal conditions for the SBSE method were studied using the least contaminated Mate tea spiked with 50 ng L^{-1} of each EPA–PAH. The parameters investigated were the addition of salt and methanol to the tea samples, the time of extraction, the solvent desorption time and the number of desorption steps. In the general procedure adopted throughout the experiments, 10 mL of tea sample were poured into an Erlenmeyer flask and magnetically stirred (750 rpm) at room temperature for a suitable length of time. The stir bar was then exposed to the previously investigated desorption solution [22] and the extracted compounds were analysed by HPLC–FLD.

3.1.1. Influence of the addition of methanol and NaCl

The study of the effect of ionic strength indicated that the addition of NaCl to the Mate teas up to saturation considerably reduced the extraction efficiency. When compared with an unsalted sample, the PAHs recoveries for the salted sample ranged from 11.7% (pyrene) to 64.5% (naphthalene). This phenomenon was also reported by other authors [14,26] and is likely attributable to the "oil effect", i.e., the presence of salt in the solution promotes the movement of the non-polar PAHs to the water surface, minimising their interaction

with the PDMS of the stir bar, thus reducing the recovery of analytes. Therefore, all the subsequent experiments were conducted using tea samples without NaCl.

The effect of rinsing the stir bars before the desorption step was also examined. After extraction, the stir bars were removed from the unsalted and salted teas and dipped for 30 s in clean water to remove any possible suspended organic matter and interfering compounds originating from the samples. A comparison of the procedure with and without stir bar rinsing indicated that the stir bar cleaning process did not affect the responses obtained for any of the PAHs nor did it provide cleaner chromatograms. Subsequent experiments were therefore conducted without the stir bar rinsing step.

An investigation was also made to find out if the addition of methanol to the teas would affect the extraction efficiency. The use of methanol (or other organic solvents such as ACN) [27] was found to minimise potential PAHs adsorption on glass materials (wall effect). However, when compared to the pure tea sample, the addition of 20% of methanol to the tea did not increase the recovery rates of the analytes. The recovery values of the less volatile compounds were similar, while the more volatile ones were slightly lower (e.g., naphthalene 32.0%, acenaphthene 63.5%, fluorene 54.6%, phenanthrene 68.1% and anthracene 75.2%). This result can be explained by the fact that the solubility of these more volatile PAHs increased slightly when 20% of methanol was added to the tea samples, reducing the PAHs' partition to the stir bar PDMS [10]. Further measurements were performed with pure teas, without methanol.

3.1.2. Extraction time and solvent desorption

To optimise the extraction time of the PAHs from tea, the SBSE time was varied from 15 to 180 min (15, 30, 40, 60, 90, 120 and 180 min). As Fig. 1 shows, a sampling time

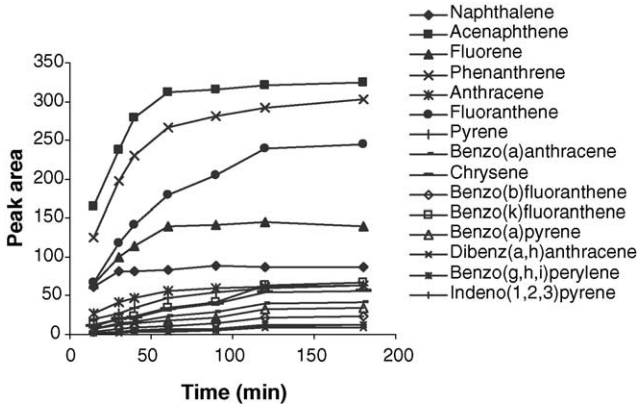

Fig. 1. Exposure time profiles of the PAHs studied (10 mL of tea sample containing 50 ng L^{-1} of each analyte; desorption time: 10 min).

of about 90 min was required for the lower-molecular mass analytes to reach the equilibrium condition. However, the other PAHs required extraction times of more than 120 min. Therefore, an extraction time of 120 min was adopted as the best compromise between extraction efficiency and overall sorption time for all the analytes.

According to Popp et al. [28], 160 μL of an ACN–water mixture (4:1) were adequate for the solvent desorption of the analytes from the stir bar, so the same amount was employed in this study. This was the smallest volume of solvent that allowed for complete immersion of the stir bar. PAH desorption profiles were obtained by investigating 5, 10, 15 and 20 min of desorption time. Fig. 2 shows that 15 min sufficed to ensure quantitative PAHs desorption. This time was therefore employed in all subsequent experiments. To verify the number of steps needed for complete analyte's desorption, the procedure was repeated twice using fresh solvent solution aliquots, taking 5 min for each additional desorption

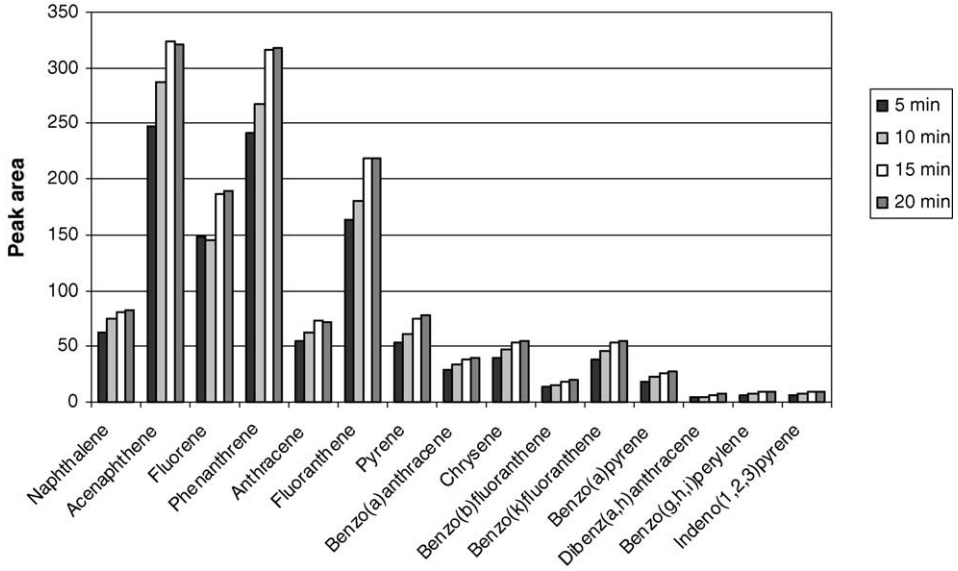

Fig. 2. Dependence of the PAHs peak areas on the desorption time of the stir bars (10 mL of tea sample containing 50 ng L^{-1} of each analyte; extraction time: 2 h).

process (i.e., 15 min for the first extract, 5 min for the second and 5 min for the third), making up a total of 25 min. The analysis of the last two extracts confirmed an insignificant carryover. Since 84.0–100% (benzo(k)fluoranthene and benzo(g,h,i)perylene, respectively) of the desorbed PAHs were found in the first extract, the subsequent measurements were taken using a single desorption step of 15 min.

3.2. SBSE method validation

After optimisation studies, the SBSE/HPLC–FLD method was validated based on quality criteria such as precision, linearity, LOD, limit of quantitation (LOQ) and absolute recovery.

The method's repeatability was investigated using 12 different stir bars to extract the EPA–PAHs (50 ng L^{-1}) from the tea sample. As Table 1 indicates, the relative standard deviation (RSD) obtained for the PAHs' peak area varied from 6.0% (naphtalene) to 10.1% (benzo(g,h,i)perylene). Similar RSD values have been described in literature [22] for the determination of PAHs in aqueous samples. These results could be considered satisfactory (since 12 different stirrers were employed), confirming that parallel extractions could be successfully performed, allowing for a reduction of the total analysis time.

Having confirmed the applicability of parallel SBSE procedures, the method's linearity was evaluated. In order to perform quantitative analyses using the external standard method, the calibration levels shall be close to the expected analyte concentrations and they must be prepared in a blank tea sample to compensate matrix effects. However, as reported by Sandra et al. [29], a blank sample to make up the matrix effects is not easily available. Therefore, after a preliminary investigation of the 11 commercial Mate tea samples with regard to their PAHs content, the lowest contaminated tea was used to construct the calibration curves (sample no. 7). Nevertheless, even this sample presented some of the PAHs under study, at concentrations ranging from 6.1 ng L^{-1} (chrysene) to 361.0 ng L^{-1} (acenaphthene). The possibility of quantifying the PAHs in the tea samples by the standard addition method at five or six calibration levels was considered, but this method is reportedly time-consuming and thus undesirable, especially when a considerable number of samples have to be quantified [29]. So, in order to obtain a blank of the Mate tea and be able to employ the external standard method, a cleaning step was proposed to remove the pre-existing PAHs from the Mate tea. The SBSE procedure described under Section 2.2.1 was performed twice for the same sample and, after which the 12 cleaned 10 mL-portions of tea were spiked at 12 calibration levels in the 1–1200 ng L^{-1} range. In general, the slopes of the calibration curves for water were up to 50% higher that the slopes for the tea sample; such matrix effects were already been observed and discussed in the literature for organic contaminants extracted from spiked water and tea samples [30]. However, the slopes for tea samples were very similar to the ones for the cleaned tea samples (in extreme cases, 11%), indicating that the matrix effects are almost identical in the two cases. In fact, the calibration curves were nearly identical for the analytes which were absent in the original tea sample (e.g., dibenz(a,h)anthracene and benzo(g,h,i)perylene). Although a few small endogenous compounds were extracted from the sample (Fig. 3), the SBSE procedure proved to be efficient and selective in removing PAHs from the tea without impairing the analytical quality parameters investigated. We also found that calibrating by spiking the cleaned Mate tea resulted in a better linearity ($r^2 \geq 0.996$ versus $r^2 \geq 0.989$) in the wide concentration range studied.

Thus, employing the cleaned tea sample, the LOD for the analytes were calculated from sample signals with known concentrations (for instance, 0.5 and 1.0 ng L^{-1}), taking into account a signal-to-noise ratio of 3:1, while a signal-to-noise ratio of 10:1 was used for the LOQ determination [31]. Table 1 presents the LOD and LOQ values obtained for the analytes by SBSE/HPLC–FLD at ng L^{-1} levels, which were considered adequate for the analytical purposes [1].

In addition, the absolute recovery of the analytes by the SBSE/HPLC–FLD method was evaluated by analysing three samples of cleaned Mate tea, each one spiked at two concentration levels. As can be seen in Table 2, the recovery for the PAHs varied from 87.0% (phenanthrene) to 24.2% (dibenz(a,h)anthracene), being lower for the more hydrophobic PAHs since the equilibrium for those compounds was not achieved within the 2 h extraction. Similar recoveries of PAHs from aqueous matrices using SBSE have been reported in the literature [14].

The application of other sorptive extraction system based on SPME for the determination of PAHs in liquid samples with analysis by HPLC have been described in the literature [32]. Precision and LODs for that SPME method were found to be between 5–20.8% and 1–5 μg L^{-1}, respectively. Thus, the overall results indicate that the proposed

Table 1
Repeatability (peak area), LOD and LOQ (ng L^{-1}) of the PAHs obtained by the optimised SBSE/HPLC–FLD method

Compound	RSD ($n = 12$)[a] (%)	LOD	LOQ
Naphthalene	6.0	0.1	0.3
Acenaphthene	8.1	3.7	12.3
Fluorene	9.5	0.2	0.8
Phenanthrene	7.8	8.9	29.7
Anthracene	9.5	1.3	4.3
Fluoranthene	8.8	0.1	0.3
Pyrene	8.7	0.5	1.7
Benzo(a)anthracene	9.2	0.9	3.0
Chrysene	9.1	2.8	9.3
Benzo(b)fluoranthene	10.1	2.4	8.0
Benzo(k)fluoranthene	9.5	0.7	2.3
Benzo(a)pyrene	9.8	1.2	4.0
Dibenz(a,h)anthracene	9.6	3.5	11.7
Benzo(g,h,i)perylene	10.1	2.5	8.9
Indeno(1,2,3)pyrene	8.9	2.3	7.7

[a] Mean values obtained using 12 different stir bars to extract the analytes from a 50 ng^{-1} spiked tea sample.

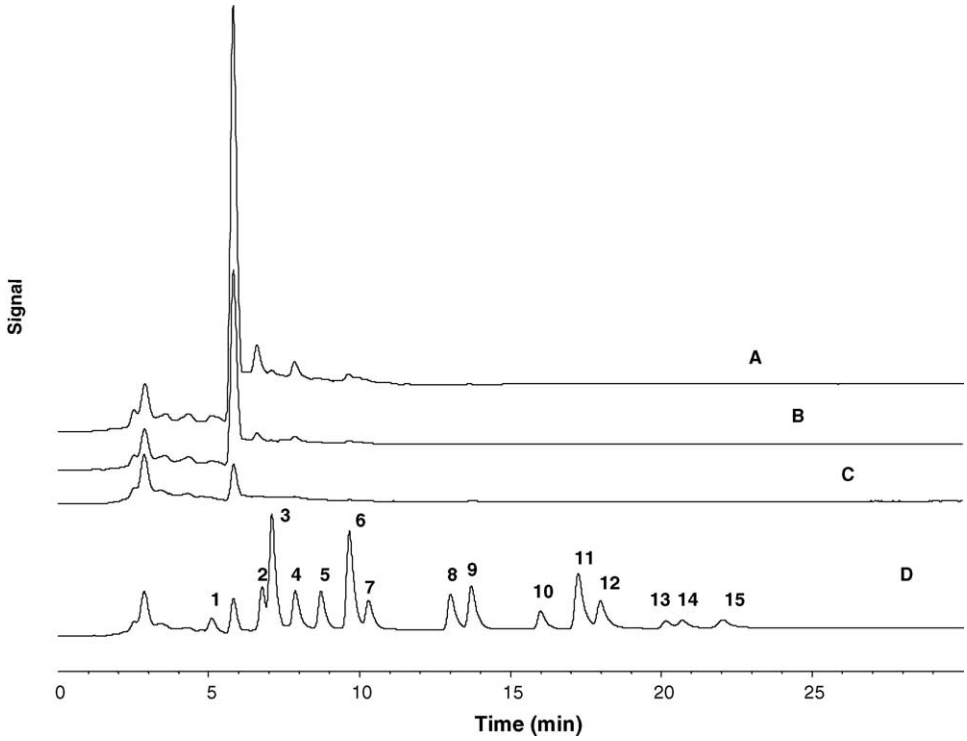

Fig. 3. HPLC–FLD chromatograms obtained: (A) for 120 min of SBSE from Mate tea; (B) for 240 min of SBSE from Mate tea (cleaned Mate tea); (C) for 120 min of SBSE from cleaned Mate tea and (D) for 120 min of SBSE from cleaned Mate tea spiked with 500 ng L^{-1}. Peak identification: 1, naphthalene; 2, acenaphthene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benzo(a)anthracene; 9, chrysene; 10, benzo(b)fluoranthene; 11, benzo(k)fluoranthene; 12, benzo(a)pyrene; 13, dibenz(a,h)anthracene; 14, benzo(g,h,i)perylene and 15, indeno(1,2,3)pyrene.

SBSE/HPLC–FLD method offers a satisfactory route for the extraction and determination of 15 EPA–PAHs in Mate tea samples.

3.3. Analysis of Brazilian Mate tea samples

Eleven samples of Mate leaves (dried and powdered plant material) were obtained from different Brazilian herbal drug suppliers and processed as described under Section 2.1. After the infusion process (1 g herbal material/100 mL boiling water), the cold teas were analysed using the proposed SBSE/HPLC–FLD method. Table 3 shows the results obtained for the samples containing the PAHs under study. Fig. 4 shows representative chromatograms of the Mate teas studied.

Table 2
Statistical data on PAHs absolute recovery of Mate tea sample by the optimised SBSE/HPLC–FLD method ($n = 3$)

Compound	100 ng L^{-1}		1000 ng L^{-1}	
	R (%)	RSD (%)	R (%)	RSD (%)
Naphthalene	70.3	6.0	66.8	3.1
Acenaphthene	85.8	8.9	76.5	2.7
Fluorene	84.0	3.8	81.8	1.4
Phenanthrene	87.0	6.7	86.1	1.0
Anthracene	85.3	2.5	84.7	0.8
Fluoranthene	83.5	5.8	82.2	1.7
Pyrene	78.8	9.5	80.2	3.0
Benzo(a)anthracene	69.3	4.4	65.2	3.0
Chrysene	70.6	4.0	67.6	3.1
Benzo(b)fluoranthene	46.8	6.7	44.9	5.9
Benzo(k)fluoranthene	45.8	6.9	44.2	3.1
Benzo(a)pyrene	45.0	6.0	44.8	3.8
Dibenz(a,h)anthracene	26.2	6.9	24.2	4.4
Benzo(g,h,i)perylene	29.0	5.7	26.4	5.5
Indeno(1,2,3)pyrene	25.2	11.3	27.4	7.0

It was possible to observe that all the commercial samples analysed here showed considerably high levels of PAHs. This fact can be confirmed when the EU criteria for drinking water quality [33] are taken as reference, since the maximum level allowed for one of the most harmful PAHs, benzo(a)pyrene, is 10 ng L^{-1} while 100 ng L^{-1} is the maximum limit for the sum of five of them (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)fluoranthene and indeno(1,2,3)pyrene). With respect to the toxicity of the samples, the TEQ (toxicity equivalent quantity) [34,35] was calculated (Table 3) as the sum of the TEQ_i value for the individual PAHs. The TEQ_i value was calculated for each PAH [36] considering its concentration in the sample (C_{PAHi}) and its toxic equivalency factor (TEF_{PAHi}) from the literature [37], so that:

$$TEQ = \sum(TEQ_i) = \sum(C_{PAH_i} \times TEF_{PAH_i})$$

The TEF values (Table 3) show that benzo(a)pyrene and dibenz(g,h,i)perylene are the most potent carcinogenic PAHs. Since the later was not present in any of the studied samples,

Table 3
Individual PAH's levels and TEF values [37], total PAHs concentration and TEQ values in commercial Brazilian Mate tea using the proposed SBSE/HPLC–FLD method

Compound	Mate teas[a] ($ng\,L^{-1}$)											
	TEF	1	2	3	4	5	6	7	8	9	10	11
Naphthalene	0.001	135.4 (8.5)	80.3 (15.4)	129.5 (12.4)	101.4 (10.8)	87.1 (19.3)	169.4 (17.9)	80.0 (7.6)	100.1 (7.4)	63.7 (14.7)	102.0 (6.5)	109.2 (8.3)
Acenaphthene	0.001	428.6 (2.0)	444.1 (2.9)	290.2 (1.8)	357.2 (1.7)	473.5 (1.0)	1156.2 (1.0)	361.0 (1.3)	452.2 (1.5)	408.0 (1.5)	320.4 (2.2)	424.0 (2.8)
Fluorene	0.001	39.9 (3.5)	23.1 (12.7)	15.7 (1.7)	22.3 (1.4)	23.9 (1.8)	38.7 (1.4)	7.6 (3.0)	66.0 (1.3)	50.2 (6.6)	48.9 (4.5)	50.2 (2.2)
Phenanthrene	0.001	397.3 (1.8)	373.8 (2.6)	202.6 (4.4)	358.4 (5.4)	624.0 (7.4)	535.0 (7.3)	135.0 (2.0)	479.7 (7.5)	357.2 (1.7)	357.1 (1.9)	350.1 (1.5)
Anthracene	0.01	52.2 (2.5)	–	–	–	74.1 (2.2)	78.5 (8.5)	–	60.6 (1.5)	–	39.8 (4.8)	–
Fluoranthene	0.001	67.8 (12.0)	60.1 (6.1)	34.7 (13.6)	78.2 (3.4)	154.1 (13.3)	151.0 (9.0)	24.9 (11.1)	78.9 (4.0)	18.5 (1.4)	47.2 (6.4)	21.1 (11.2)
Pyrene	0.001	63.7 (11.2)	46.9 (2.2)	31.5 (13.4)	51.9 (2.1)	129.1 (9.2)	118.3 (13.4)	20.0 (4.8)	69.7 (5.0)	–	59.5 (2.0)	–
Benzo(a)anthracene	0.1	3.7 (5.5)	6.5 (11.4)	6.7 (11.0)	15.5 (2.5)	50.9 (6.6)	15.6 (2.6)	7.2 (8.5)	4.2 (2.1)	1.4[b] (7.3)	3.3 (2.7)	1.4[b] (10.4)
Chrysene	0.01	6.6[b] (12.0)	–	4.2[b] (13.2)	14.4 (6.9)	40.5 (1.3)	17.5 (5.3)	6.1[b] (6.8)	9.5 (2.0)	4.7[b] (9.9)	7.1[b] (4.9)	4.7[b] (12.7)
Benzo(b)fluoranthene	0.1	12.2 (1.5)	10.2 (2.3)	10.5 (3.0)	12.9 (5.1)	21.9 (1.7)	13.1 (2.0)	–	11.2 (1.7)	–	10.8 (2.2)	–
Benzo(k)fluoranthene	0.1	4.3 (8.9)	3.0 (15.9)	3.1 (1.4)	4.0 (3.2)	7.2 (3.6)	3.8 (1.5)	–	3.2 (11.7)	–	3.3 (12.4)	–
Benzo(a)pyrene	1	13.8 (2.2)	11.5 (10.3)	11.3 (1.7)	14.1 (1.4)	22.6 (1.2)	11.6 (1.1)	–	11.8 (4.0)	–	12.2 (1.5)	–
Dibenz(a,h)anthracene	5	–	–	–	–	–	–	–	–	–	–	–
Benzo(g,h,i)perylene	0.001	7.2[b] (2.0)	–	–	–	16.2 (2.0)	–	–	–	–	7.2[b] (2.6)	–
Indeno(1,2,3)pyrene	0.1	–	9.2 (18.0)	–	6.8[b] (12.2)	25.2 (1.2)	10.0 (4.8)	–	7.1[b] (4.4)	–	8.3 (2.3)	–
Total		1232.7	1068.6	739.9	1037.0	1750.3	2318.8	641.8	1354.2	903.8	1027.0	961.1
TEQ	6.427	17.6127	15.4183	14.0762	19.1334	35.9197	18.9786	1.4095	16.3176	1.0846	18.7741	1.1416

In brackets, the RSD (%) from analysis of commercial mate tea samples ($n = 3$).

[a] $(-) \leq$ LOD.

[b] $\leq$LOQ.

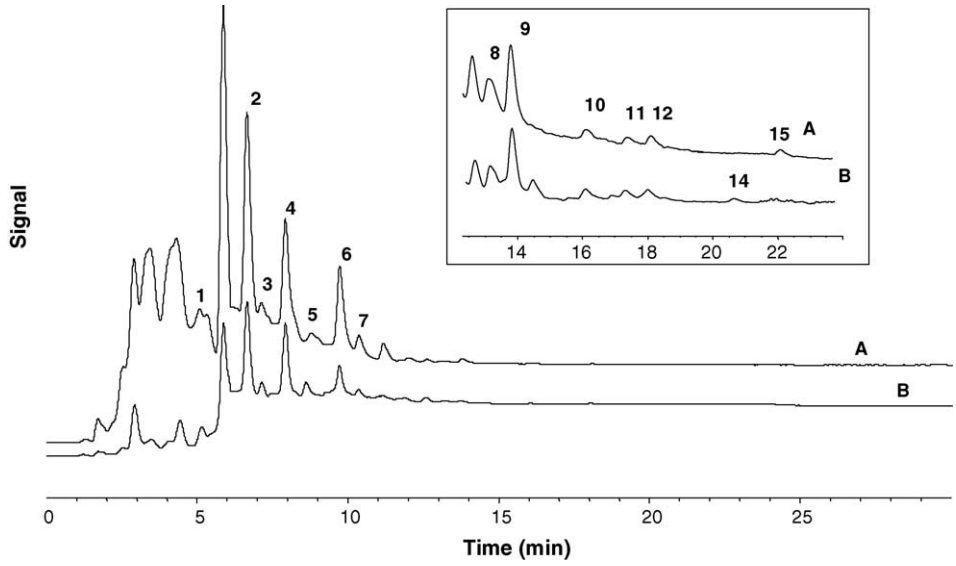

Fig. 4. Representative HPLC–FLD chromatograms of: (A) sample no. 1 and (B) sample no. 7 obtained by the SBSE method. See Fig. 3 for peak identification.

benzo(a)pyrene played a very important role in the toxicity of the samples; therefore, the TEQ values for samples 7, 9 and 11 were very low (no benzo(a)pyrene detected). This behaviour was also reflected in the good correlation between the TEQ value and the benzo(a)pyrene concentration ($r = 0.97$), compared with the poor correlation ($r = 0.56$) between the TEQ and the total PAHs concentration. Furthermore, although sample 6 showed the highest PAHs content, the toxicity of this sample was much lower than that of sample 5, which showed the highest benzo(a)pyrene content of all the samples.

To confirm the PAHs results found by SBSE/HPLC–FLD, samples 6 and 7 (the teas with the highest and lowest levels of total PAHs contamination, respectively) were subjected to a conventional LLE-based assay [38]. Fig. 5 indicates that most of the PAH levels determined by the SBSE and LLE methods are comparable; in fact, a reasonable good correlation ($r = 0.98$) between both of them has been found for such Mate samples. Although both procedures revealed traces of the EPA–PAHs, the latter presented several disadvantages such as the formation of emulsion and poor precision [39].

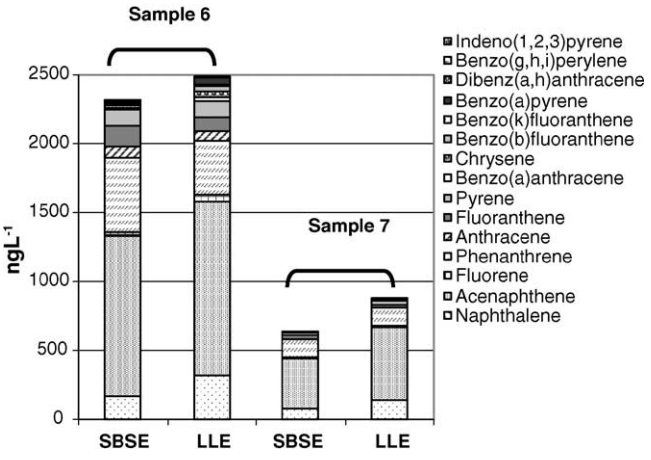

Fig. 5. Comparison of mean PAH concentration profiles of samples no. 6 and no. 7, obtained by the proposed SBSE/HPLC–FLD and LLE/HPLC–FLD methods.

4. Conclusions

The determination of PAHs from Mate tea was successfully performed by SBSE, followed by desorption in a small volume of solvent and subsequent HPLC–FLD analysis. The proposed method proved very simple, easy, precise and effective, and is an environmentally friendly alternative methodology to determine PAHs in complex food matrices. Moreover, the possibility of parallel SBSE accelerates the entire procedure, which is especially important when routine analyses are required.

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