

Comparison of different modifications on QuEChERS sample preparation method for PAHs determination in black, green, red and white tea

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Abstract The aim of this work was the evaluation of QuEChERS extraction method for polycyclic aromatic hydrocarbon (PAH) determination in various types of tea. In the experiment, different kinds of extraction solvents, sorbents and a final method of sample preparation were compared. The final extracts were analysed by gas chromatography-selected ion monitoring-mass spectrometry. The results suggest that acetonitrile extraction, clean up with SAX and final liquid–liquid extraction was the best combination giving the most purified extracts and acceptable compound recoveries for different types of teas. In the study of real samples, compounds belonging to light PAHs were mostly detected, and heavy polycyclic aromatic hydrocarbons, including benzo[a]pyrene, were not identified in any of samples.

Keywords GC-MS · Extraction · PAHs · Tea · QuEChERS · SPE sorbents

Introduction

Tea, because of its aroma, flavour and potential health-promoting properties, is one of the most known and consumed beverage in the world. Each type of tea has its own characteristics including a different taste, health benefits and even different levels of caffeine. White tea is the purest and least processed of all teas; green and red teas contain healthy antioxidants; black tea is fully fermented, and it has a high

level of caffeine. Despite the taste and healthy properties of tea, some of chemical contaminants present in it may pose a health threat to tea drinkers. Tea leaves, due to their large surface area, are particularly vulnerable to the deposition of harmful chemicals from polluted air. Among them, polycyclic aromatic hydrocarbons (PAHs) are one of the most dangerous. The presence of PAHs in tea leaves is also caused by fresh leaf treatment process—drying or roasting during which wood, oil or coal are used for burning (Lin et al. 2005). Among PAHs, 15 of these compounds were recognised as clearly mutagenic and carcinogenic by the Scientific Committee on Food, and benzo[a]pyrene and dibenzo[a,h]anthracene were reported to be the most carcinogenic (Scientific Committee on Food 2002). However, the European Food Safety Authority concluded that benzo[a]pyrene is not a suitable marker for the occurrence of PAHs in food and that a system of four or eight specific substances would be the most suitable markers of PAH in food (Commission Regulation (EU) No 836/2011). As a consequence, Commission Regulation No 835/2011 amended Regulation (EC) No 1881/2006 in order to set maximum levels for the sum of four polycyclic aromatic hydrocarbons (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene).

Analysis of PAHs in tea encounters certain difficulties because tea contains a lot of co-extractives such as caffeine, polyphenols, sugars, organic acids and pigments, especially high levels of chlorophyll. The content of these substances strongly depends on type of tea, and black tea is considered to be the most difficult matrix due to high content of interfering substances. Moreover, non-volatile matrix components might be deposited in the gas chromatography (GC) inlet and in the GC column giving rise to the formation of new active sites, which results in GC signal diminishment (Cajka et al. 2012). For this reason, it is necessary to utilise an effective method of extraction and clean-up procedures of tea samples. As yet,

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conventional procedures of sample preparation for PAHs determination in teas have included extraction using various solvents (acetone, ethyl acetate, dichloromethane, hexane) and clean-up step, usually with the use of solid-phase extraction (SPE) or gel permeation chromatography (Lin et al. 2005; Singh et al. 2011). Apart from these approaches, accelerated solvent extraction and stir bar sorptive extraction were successfully applied to PAH analysis (Ziegenhals et al. 2008; Zuin et al. 2005). The purified extract is then analysed by gas chromatography or liquid chromatography in combination with different types of detectors. Unfortunately, these classical methods mentioned above are labourious, time-consuming, require advanced analytical equipment and use large volumes of toxic and hazardous solvents, incurring consequent costs to the customer. Recently, modern analytical chemistry seeks to develop the so-called procedures green chemistry, in which a small amount of samples and reagents are utilized; only an easy operation and simple analytical laboratory equipment are employed; and the reagents and chemicals used for analysis are harmless to health and environment. One of them is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) developed originally for pesticide residues determination in food of plant origin (Anastassiades et al. 2003). Since that time, a lot of modifications of QuEChERS method have been done mainly including application of different solvent and sorbents. In recent research, Cajka et al. (2012) proposed a new approach with use of liquid–liquid extraction (LLE) of the final extracts. The effectiveness of the method made it commonly used for the determination of other organic contaminants such as mycotoxins, drugs and, finally, PAHs in food (in fish and seafood). In these approaches, PAHs were extracted with acetonitrile, ethyl acetate or with a mixture of acetone, ethyl acetate and isooctane and cleaned with MgSO_4 , silica, primary secondary amine or C_{18} (Cai et al. 2012; Forsberg et al. 2011; Gratz et al. 2011; Ramalhosa et al. 2009; Kalachova et al. 2011; Smoker et al. 2010). Recently, the application of QuEChERS method for PAH determination in environmental samples has been also reported (Albinet et al. 2013; Pule et al. 2012). In our previous study, the QuEChERS method was adapted for simultaneously determination of pesticide and PAHs in fresh herbs (Sadowska-Rociek et al. 2013). However, except for research performed by Drabova et al. (2012), there has been no attempt to adapt QuEChERS method for the determination of PAHs in tea samples. For this reason, we decided to evaluate the application of QuEChERS extraction method for PAH determination in infusions of various types of tea. In the experiment, we assessed the suitability of different kinds of extraction solvents (acetonitrile and ethyl acetate), sorbents (PSA, GCB, SAX, NH_2) and a final method of sample preparation (by solvent evaporation to dryness and dissolving the residues in hexane) or by LLE with the solvent exchange. The usefulness of the each variant of the analytical procedure was verified

basing on the recovery ratio of analysed compounds. The study was performed for black, green, red and white teas.

Materials and methods

Chemicals and reagents

Acetonitrile (MeCN), HPLC-grade, and ethyl acetate (EtAc), for liquid chromatography LiChrosolv[®] and formic acid, 98 %, p.a., were purchased from Merck KGaA, Germany. Magnesium sulphate anhydrous p.a. and sodium chloride p.a. were purchased from Chempur SA, Poland. PSA, GCB, C_{18} , SAX and NH_2 SPE Bulk Sorbent derived from Agilent Technologies, USA. EPA 525 PAH Mix-B (containing 12 compounds: acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, fluorene, indeno[1,2,3-cd]pyrene, phenanthrene and pyrene) and anthracene- d_{10} were obtained from Supelco, USA. Stock, intermediate and working standard solutions of PAHs at concentration $1 \mu\text{g mL}^{-1}$ and anthracene- d_{10} (syringe standard) at concentration $1 \mu\text{g mL}^{-1}$ were prepared in hexane. Deionised water (18 M Ω) was produced by a Milli-Q system (Millipore; USA).

Instrumentation

Varian 4000 gas chromatography-mass spectrometry (GC-MS) (Varian, Inc., USA) system consisted of 3800 GC with CP-8410 autoinjector (Bruker, USA), and a 4,000 Ion Trap MS detector were used to perform the GC-MS analyses. The injector was CP-1177 split/splitless capillary injector, with a temperature of 270 °C and an injection volume of 1.0 μL . Each injection (splitless mode; Agilent focus liner with deactivated glass wool) was performed in triplicate. Chromatographic separations were conducted using a Zebron MultiResidue-1 column (30 m \times 0.25 mm \times 0.25 μm ; Phenomenex Inc., USA). The GC oven was operated with the following temperature program: initial temperature 50 °C (1.0 min)–15 °C min^{-1} –320 °C (6.0 min). Helium 5.0 (Linde Gas, Poland) was used as the GC carrier gas at a flow rate of 1.0 mL min^{-1} . The ion trap mass spectrometer was operated in the internal ionisation mode, scan from m/z 45 to 500. The emission current of the ionisation filament was set at 15 μA and dwell time at 65 ms. Analyses were conducted in the selected ion monitoring mode (SIM) based on the use of one quantitative ions. Analysed compounds were identified according to their qualitative ions and retention times (Table 1). The trap and the transfer line temperatures were set at 180 and 220 °C, respectively. The analyses were carried out with a solvent delay of 8.0 min. Acquisition and processing data were performed using Varian Start Workstation software and NIST 2.0 library.

Table 1 Data of the identification and quantification of analysed compounds

Rt [min]	Compound	Quantification ion	Confirmation ions	Calibration slope	Correlation coefficient, <i>r</i>	LOD [$\mu\text{g kg}^{-1}$]	LOQ [$\mu\text{g kg}^{-1}$]
10.53	Acp	152.1	151.1, 153.1	0.008	0.9972	0.3	0.9
11.68	Flu	166.1	164.1, 165.1	0.005	0.9978	0.3	0.9
13.33	Ant	178.1	188.1, 179.1	0.008	0.9992	0.3	0.9
13.40	Ant-d ₁₀	188.0	188.1, 177.9	–	–	–	–
13.43	Phen	178.1	178.1, 179.1	0.009	0.9987	0.3	0.9
15.75	Pyr	202.1	200.1, 203.1	0.010	0.9975	0.2	0.6
17.82	B[a]a	228.1	226.1, 229.1	0.010	0.9991	0.3	0.9
17.88	Chr	228.1	226.1, 229.1	0.012	0.9981	0.2	0.6
19.62	B[b]f	252.1	250.1, 253.1	0.011	0.9987	0.3	0.9
19.67	B[k]f	252.1	250.1, 253.1	0.014	0.9968	0.3	0.9
20.27	B[a]p	252.1	250.1, 253.2	0.010	0.9961	0.3	0.9
23.00	I[cd]p	278.2	276.1, 279.1	0.010	0.9976	0.4	1.2
23.75	D[ah]a	276.0	277.1, 278.0	0.011	0.9993	0.4	1.2

Acp acenaphthylene, *Flu* fluorene, *Ant* anthracene, *Phen* phenanthrene, *Pyr* pyrene, *B[a]a* benzo[a]anthracene, *Chr* chrysene, *B[b]f* benzo[b]fluoranthrene, *B[k]f* benzo[k]fluoranthrene, *B[a]p* benzo[a]pyrene, *I[cd]p* indeno[1,2,3-cd]pyrene, *D[ah]a* dibenzo[a,h]anthracene, *R_t*, retention time, *LOD* limit of detection, *LOQ* limit of quantification

T25 digital ultra-turrax disperser (IKA, Germany), MS1 Minishaker (IKA, Germany) and MPW 350 R Centrifuge (MPW Med. Instruments, Poland) were used during sample preparation. Accublock™ (Labnet, USA) with nitrogen 5.0 (Linde Gas, Poland) was used to evaporate the solvent and concentrate the extracts.

Sample preparation

The whole experiment was accomplished for black tea, and the best variant of the method was then also tested separately for samples of black, green, red and white teas. The tea samples were used for preparation of blank and fortified samples and were purchased from the local market. As yet, tea and related food

matrices with certified concentrations of PAHs are not commonly available. Thus, the usefulness of the method was verified on the basis of the recovery ratio of analysed compounds (analysis of spiked samples). Recovery studies involved three samples of tea being fortified with the standard solution. The extraction process

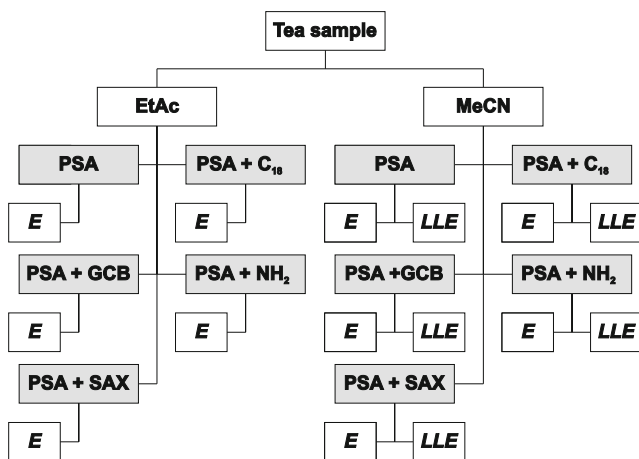


Fig. 1 Scheme of the experiment; *EtAc* ethyl acetate, *MeCN* acetonitrile; *E* evaporation; *LLE* liquid–liquid extraction; *PSA*, *NH₂*, *C₁₈*, *GCB*, *SAX*: sorbents

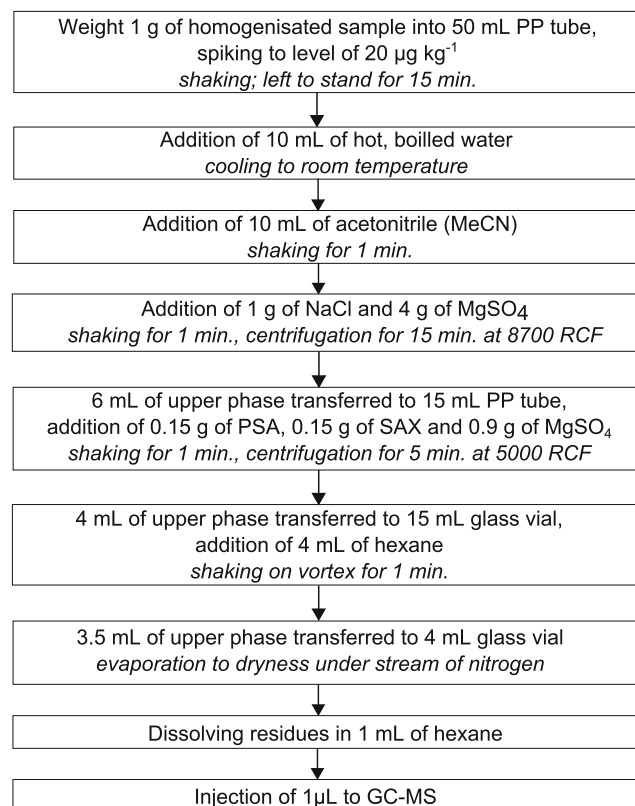


Fig. 2 Scheme of final analytical procedure; *PSA*, *SAX*: sorbents

was conducted as follows: A representative portion of each tea was cut, macerated and homogenized in a blender. One gram of tea was weighted into a 50-mL centrifuge tube, spiked with mixture of standards to the level of 20 $\mu\text{g kg}^{-1}$, mixed and left to stand for 15 min at room temperature prior to extraction. Then, 10 mL of hot, boiled water was added to the sample. Addition of water to the tea sample is a crucial step, because firstly, it imitates the real tea infusion; secondly, it helps improve the extraction yield (Cajka et al. 2012; Drabova et al. 2012). After cooling to a room temperature, fifteen different procedures were applied to further preparation of the tea extracts (Fig. 1).

Ten millilitres of acetonitrile or ethyl acetate was added, and the mixture was shaken vigorously for 1 min. Next, 1 g NaCl and 4 g MgSO_4 were added, with the tube being shaken immediately after addition of the salt. Then each sample was shaken vigorously for 1 min and centrifuged for 15 min at 8,700 RCF. Six millilitres of the supernatant was transferred into a PP 15-mL tube containing an appropriate amount of tested sorbent (according to the scheme at Fig. 1) and 0.9 g MgSO_4 . The tube was shaken for 2 min and centrifuged for 5 min at 5,000 RCF. A 4-mL amount from the extract was

transferred into a screw cup vial, and then 100 μL of the anthracene- d_{10} solution was added. The extract was: (1) evaporated under a stream of N_2 at a temperature of 40 $^\circ\text{C}$ to dryness, and residues were dissolved in 1 mL of hexane (evaporation, E), or (2) a 4 mL of hexane was added; the mixture was thoroughly shaken in a vortex for 1 min, and 3.5 mL of upper hexane layer was transferred into a screw cup vial (LLE). The extracts were evaporated under a stream of N_2 as previously described. The extracts were then analysed by GC-MS. The schema of the analytical procedure is shown in Fig. 2. Blank samples and reagent blanks were prepared similarly but were not fortified.

Standards preparation

For all procedures, a series of standard solutions in pure solvent were prepared by dilution of the standard mixture solution in hexane at the ranges 0.1, 1, 5, 10, 40, 70 and 100 ng mL^{-1} . Each standard solution contained 100 μL of the anthracene- d_{10} solution and was prepared in triplicate. GC-MS chromatograms of standard solution and fortified sample are presented in Fig. 3.

Fig. 3 GC-MS chromatograms of **a** standard solution at 40 ng mL^{-1} and **b** fortified sample (variant MeCN+PSA+SAX+LLE); 1 acenaphthylene; 2 fluorene; 3 anthracene; 4 anthracene- d_{10} ; 5 phenanthrene; 6 pyrene; 7 benzo[a]anthracene; 8 chrysene; 9 benzo[b]fluoranthrene; 10 benzo[k]fluoranthrene; 11 benzo[a]pyrene; 12 indeno[1,2,3-cd]pyrene; 13 dibenzo[a,h]anthracene

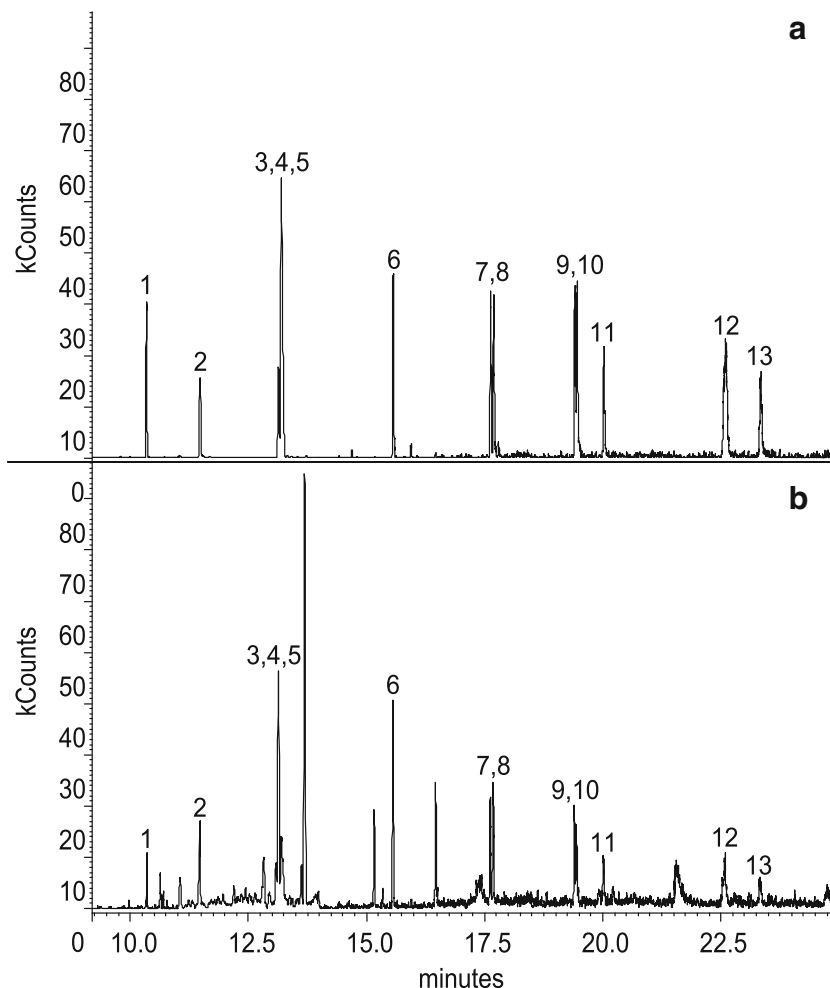


Fig. 4 Comparison of GC-MS chromatograms (SIM mode) for tested solvents; *EtAc* ethyl acetate; *MeCN* acetonitrile; *E* evaporation; *LLE* liquid–liquid extraction

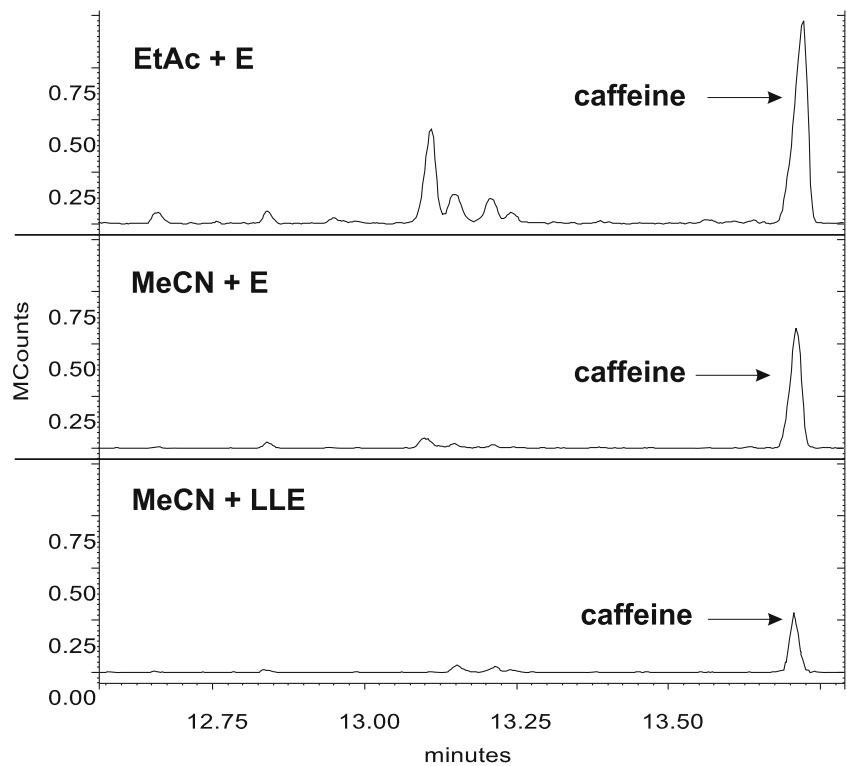
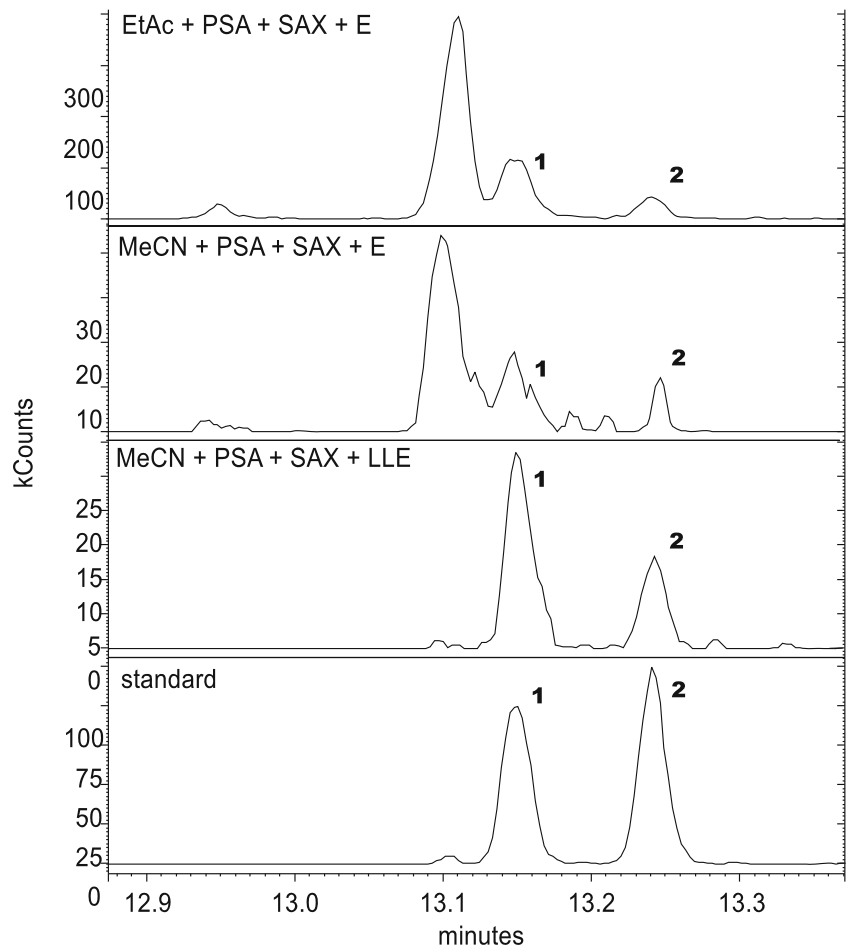


Fig. 5 GC-MS chromatograms of various combinations of QuEChERS method; *1* anthracene, *2* phenanthrene; *EtAc* ethyl acetate, *MeCN* acetonitrile; *E* evaporation; *LLE* liquid–liquid extraction; PSA, SAX: sorbents



Results and discussion

Calibration curves were constructed by plotting integrated peak areas, divided by the area of anthracene-d₁₀, against concentrations of compounds. Peak areas have been reduced by the area of the peaks of compounds derived from blank (hexane). Therefore, calibration curves were calculated without y-intercept, which the high value could significantly affect the calculation of the results making them inaccurate. Limit of detection (LOD) and limit of quantification (LOQ) were estimated based on the signal of the background noise measured from the chromatograms of standard at the lowest calibration level. The limit of detection was calculated as three times higher than the level of noise, and the limit of quantification were equal to ten times of the noise level. Table 1 summarizes the obtained results. In all cases, the correlation coefficient was higher than 0.99, and LOQs for almost all analysed PAHs were below 0.9 µg kg⁻¹, with the exception for the heaviest compounds: indeno[1,2,3-c,d]pyrene and dibenzo[a,h]anthracene (1.2 µg kg⁻¹).

Determination of organic compounds in different types of tea involves specific problems with extraction,

clean-up and analysis by GC-MS due to the complexity of the tea matrix (presence of phenolic compounds and caffeine) that can interfere with the analytes resulting in enhance or suppression of chromatographic peaks and ambiguity of identification.

Phenolic compounds make up 25–35 % of the dry matter content of young, fresh tea leaves. They are responsible for the colour of tea. Flavanol compounds are 80 % of the phenols, while the remainder is proanthocyanidins, phenolic acids, flavonols and flavones. During fermentation, the flavanols are oxidised enzymatically to compounds that are responsible for the colour and flavour of black tea. The reddish-yellow colour of black tea extract is largely due to theaflavins and thearubigins. The greenish or yellowish colour of green tea results from the presence of flavonols and flavones. Tea leaves contain also pigments, chlorophyll and carotenoids. Chlorophyll is degraded during tea processing to chlorophyllides and pheophorbides that are brownish in colour. Fourteen carotenoids (i.e. xanthophylls, neoxanthin, violaxanthin and β-carotene) have been identified in tea leaves. However, their content decreases during the processing of black tea (Belitz et al. 2009).

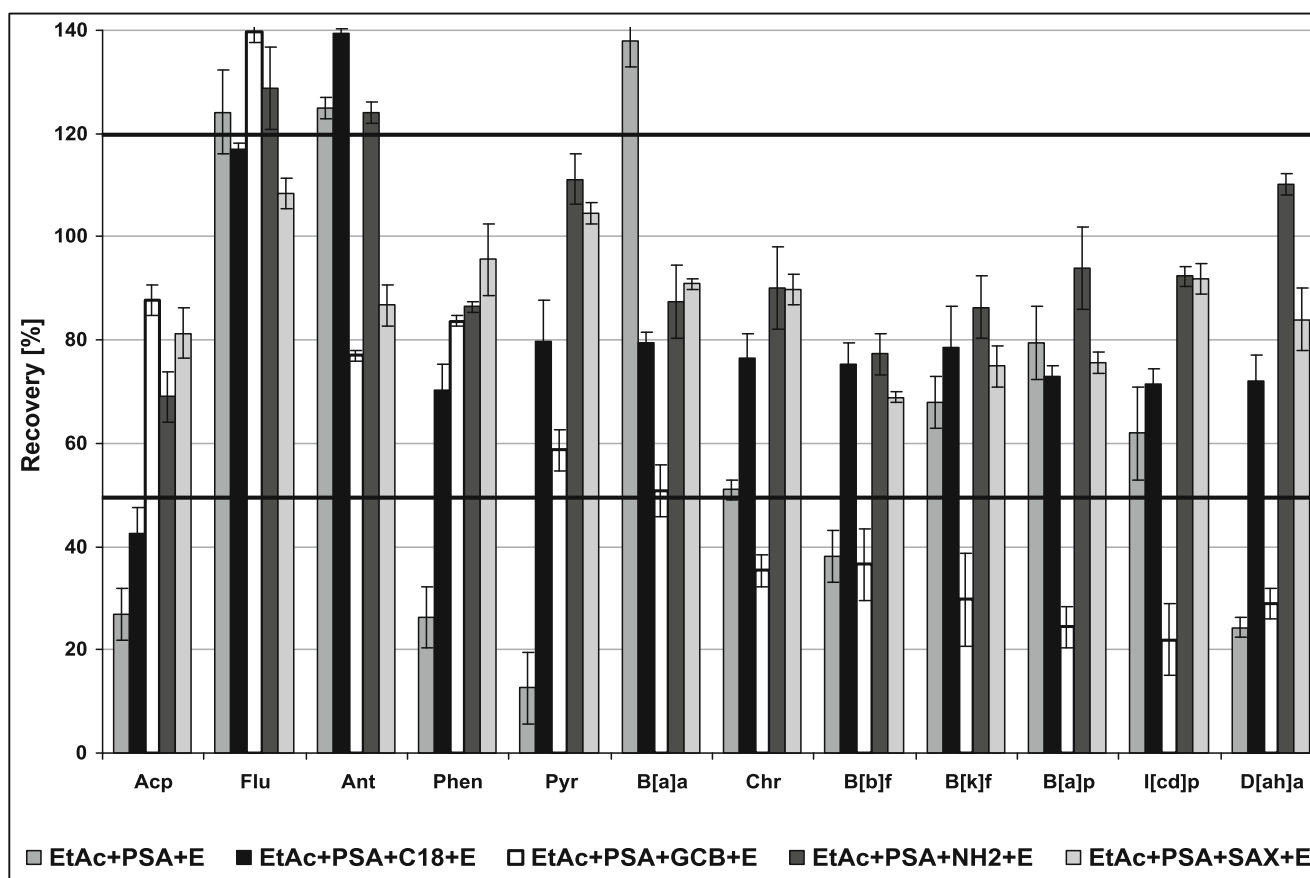


Fig. 6 Recovery values for variants with ethyl acetate (EtAc) and evaporation (E); PSA, NH₂, C₁₈, GCB, SAX: sorbents; Acp acenaphthylene; Flu fluorene; Ant anthracene; Phen phenanthrene; Pyr pyrene; B[a]a

benzo[a]anthracene; Chr chrysene; B[b]f benzo[b]fluoranthrene; B[k]f benzo[k]fluoranthrene; B[a]p benzo[a]pyrene; I[cd]p indeno[1,2,3-cd]pyrene; D[ah]a dibenzo[a,h]anthracene

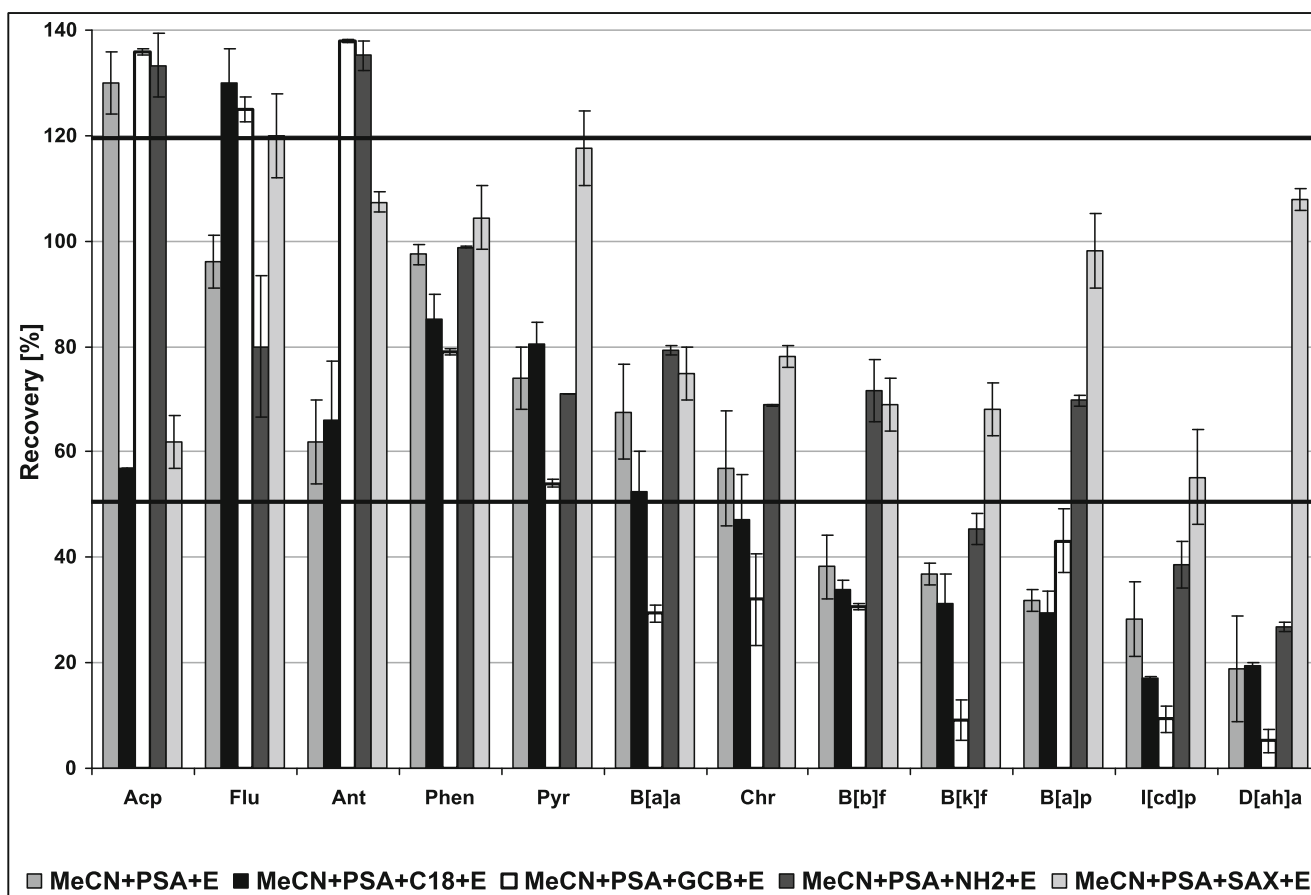


Fig. 7 Recovery values for variants with acetonitrile (MeCN) and evaporation (E); PSA, NH₂, C₁₈, GCB, SAX: sorbents; *Acp* acenaphthylene; *Flu* fluorene; *Ant* anthracene; *Phen* phenanthrene; *Pyr* pyrene; *B[a]a*

benzo[*a*]anthracene; *Chr* chrysene; *B[b]f* benzo[*b*]fluoranthrene; *B[k]f* benzo[*k*]fluoranthrene; *B[a]p* benzo[*a*]pyrene; *I[cd]p* indeno[1,2,3-*cd*]pyrene; *D[ah]a* dibenzo[*a,h*]anthracene

Therefore, we decided to evaluate different modifications of QuEChERS method, and we tested their capabilities of simultaneous good analyte extraction from a sample and the least possible extraction of undesirable interfering components, such as phenolic compounds responsible for tea colour and caffeine. Acetonitrile was the first solvent, used typically in QuEChERS method for pesticide extraction, and ethyl acetate was the second tested solvent, chosen for the fact that it is often applied for the extraction of PAHs. Additionally, an investigation was also made to find out if the use of certain sorbents would improve the purification step. Primary secondary amine (PSA) removes sugars, fatty and other acids; graphitized carbon black (GCB) is used for removal of pigments, such as chlorophyll. C₁₈ is the most hydrophobic, because of its extreme retentive nature for non-polar compounds, such as fat. NH₂ sorbent, a weaker anion exchanger, extracts strong acids that have one or more negative charges. SAX is a strong anion exchange sorbent ideally suited for the extraction of compounds such as carboxylic acids (Agilent Technologies 2013).

Finally, we compared yield of evaporation and liquid-liquid exchange step. The choice of the most suitable solvent,

sorbent and final preparation of the extract was based on a quantitative (PAH recovery ratio values) and a qualitative approach (sample colour, its transparency and the content of impurities) and the quality of the chromatograms obtained.

Appearance of the extracts

The experiment showed that the cleanest extracts were obtained after MeCN extraction and clean-up with GCB, SAX or NH₂. The efficiency in removal colour by GCB can be explained by its properties associated with the pigments removing from the samples. On the other hand, it was supposed that the use of SAX or NH₂ enabled to eliminate some phenolic compounds, taking into account the properties of these sorbents (extraction of certain acids).

In case of extraction with ethyl acetate, the tea extracts were more dark-coloured and contaminated than after the use of acetonitrile. As can be seen from Fig. 4, use of ethyl acetate, in contrast to acetonitrile, also resulted in the extraction of interferences such as caffeine, from the sample. The peak of caffeine was usually higher in samples extracted with ethyl acetate comparing with samples extracted with acetonitrile,

regardless of type of sorbent used. Caffeine was partially removed from the sample where acetonitrile was used for the extraction.

However, a substantial improvement of extract purification was achieved using liquid–liquid extraction (exchange acetonitrile to hexane). This phenomenon, clarified by Cajka et al. (2012), is based on a worse solubility of usually polar matrix co-extractives in non-polar hexane than in more polar acetonitrile. On the other hand, the application of final evaporation of the extracts to dryness and then dissolving the residues in hexane resulted in incomplete separation of some peaks and signal enhance. This situation was noticed, e.g. for anthracene (Fig. 5).

Recovery studies

Recovery studies were conducted after fortification to the levels of 20 µg kg⁻¹. The percent recoveries were calculated by subtracting values in an unspiked blank sample from values in the spiked sample. The received values of the recovery varied and strongly depended on type of the

extraction solvent and compound formula, and finally, they were also influenced by the matrix components.

The recoveries within 50–120 % (according to Regulation 836/2011) for all 12 compounds were provided by the combinations where SAX sorbent was used for the clean-up the extract (EtAc+PSA+SAX+E, MeCN+PSA+SAX+E, MeCN+PSA+SAX+LLE). Similar results were achieved for the combinations EtAc+PSA+NH₂+E and EtAc+PSA+C₁₈+E. However, in these variants, the recoveries for anthracene were too high. The recovery values for all combinations of the method are presented in Figs. 6, 7 and 8.

Overall, some characteristic patterns were observed. The recoveries for GCB and C₁₈ were usually too low. It was probably due to the properties of these sorbents that retain some non-polar compounds, especially those with planar structure. Surprisingly, recovery values in combination EtAc+PSA+C₁₈+E were relatively good, despite the use of C₁₈. This fact can be explained as the result of cross-compensation of ethyl acetate, which increased the recovery of certain compounds, and C₁₈, which decreased the recovery values. The application of SAX and, in some cases, NH₂ gave the most appropriate

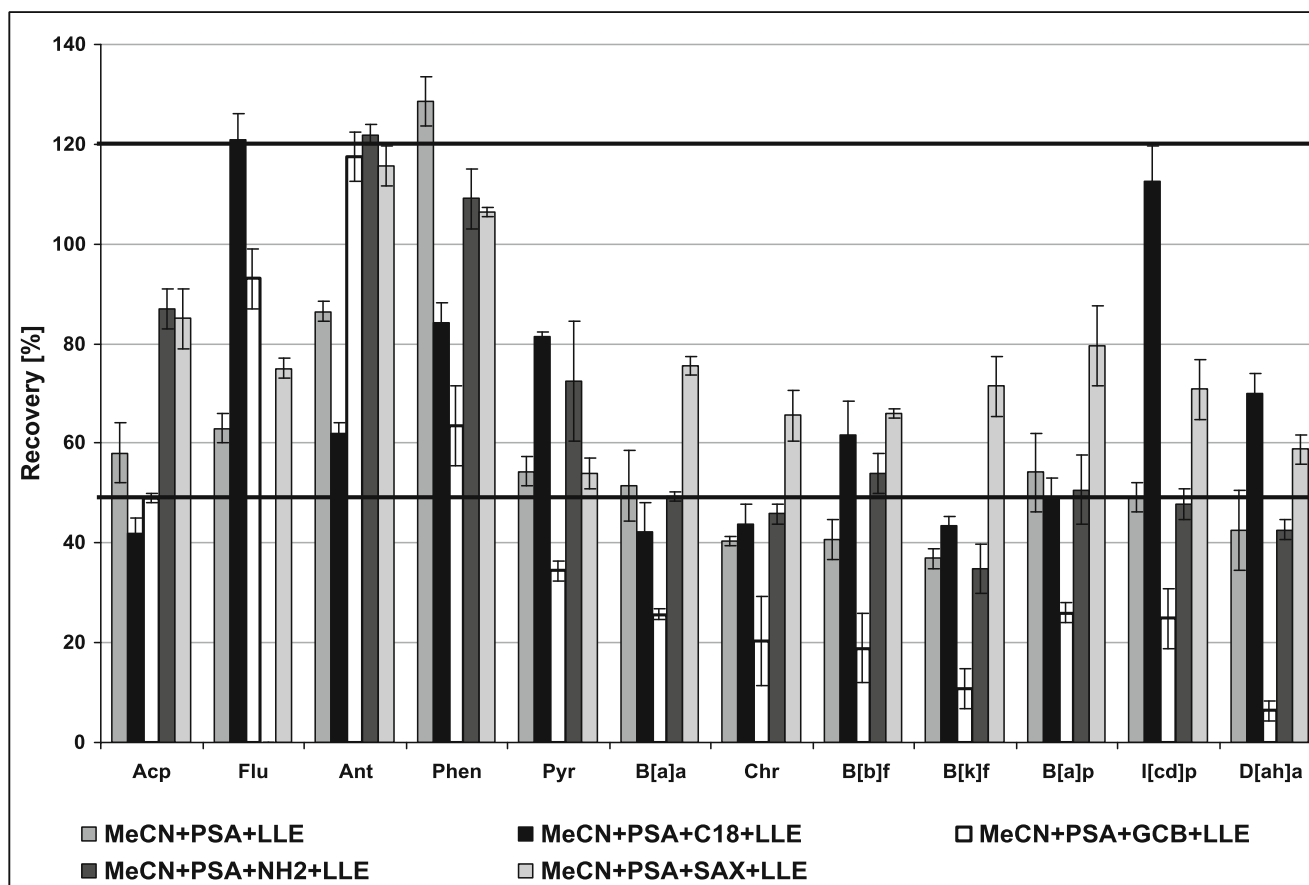


Fig. 8 Recovery values for variants with acetonitrile (MeCN) and liquid–liquid extraction (LLE); PSA, NH₂, C₁₈, GCB, SAX: sorbents; Acp acenaphthylene; Flu fluorene; Ant anthracene; Phen phenanthrene; Pyr pyrene;

B[a]a benzo[a]anthracene; Chr chrysene; B[b]f benzo[b]fluoranthrene; B[k]f benzo[k]fluoranthrene; B[a]p benzo[a]pyrene; I[cd]p indeno[1,2,3-cd]pyrene; D[ah]a dibenzo[a,h]anthracene

values of the recovery, while the application of only PSA sorbent resulted in diversified values of recoveries.

Considering the recoveries for each compound, some interesting observations can be made. For almost all combinations of the tested methods, the recoveries of fluorene and anthracene were significantly too high. For these compounds, the chromatographic signal and thus its mean recovery values were several times higher than expected. The results can be explained by the presence of co-extractive components from the matrix, having the same qualifier ions as analytes and interacting with them.

RSD values

The repeatability of recovery values, expressed as the relative standard deviation (RSD) of the spiked sample concentrations, was lower than 20 % for almost all samples, only in some cases exceeding this level. Although the differences between RSD values for each combination of the method were discovered, there was no explicit relation between these values.

Selection of the best variant of the method

Considering: (1) the appearance of extracts and the content of interacting matrix co-extractive components and (2) the

recovery ratio values, we concluded that variant MeCN+PSA+SAX+LLE is the best combination. Acetonitrile did not extract a lot of the matrix, and additionally, it was possible to separate it from the hexane. PSA and SAX efficiently cleaned up the extracts, and, finally, our proposed technique of LLE from acetonitrile to hexane proved to be the most efficient in cleaning up the extracts (removal peaks of matrix, such as caffeine) and significantly reduced colour of the extracts. The selected combination of the QuEChERS method was tested also for other types of tea: green tea, red tea and white tea at the fortification level of $20 \mu\text{g kg}^{-1}$ (Fig. 9) and also additionally at $2 \mu\text{g kg}^{-1}$ (Fig. 10). The results of the recoveries showed that this variant was also acceptable for other types of tea. Recoveries of all compounds were within established limits. At the fortification level of $20 \mu\text{g kg}^{-1}$, the lowest results were for the samples of red tea while, for the samples of green and white tea, the highest. At the second spiking level, $2 \mu\text{g kg}^{-1}$, the results were also acceptable, but they were slightly lower.

Overall, the combination MeCN+PSA+SAX+LLE offered the cleanest extracts, giving also acceptable compound recoveries for different tea types, as well as the percentage of compounds for which the recovery was in the accepted limits. The advantage of LLE applied at the end of the analytical procedure

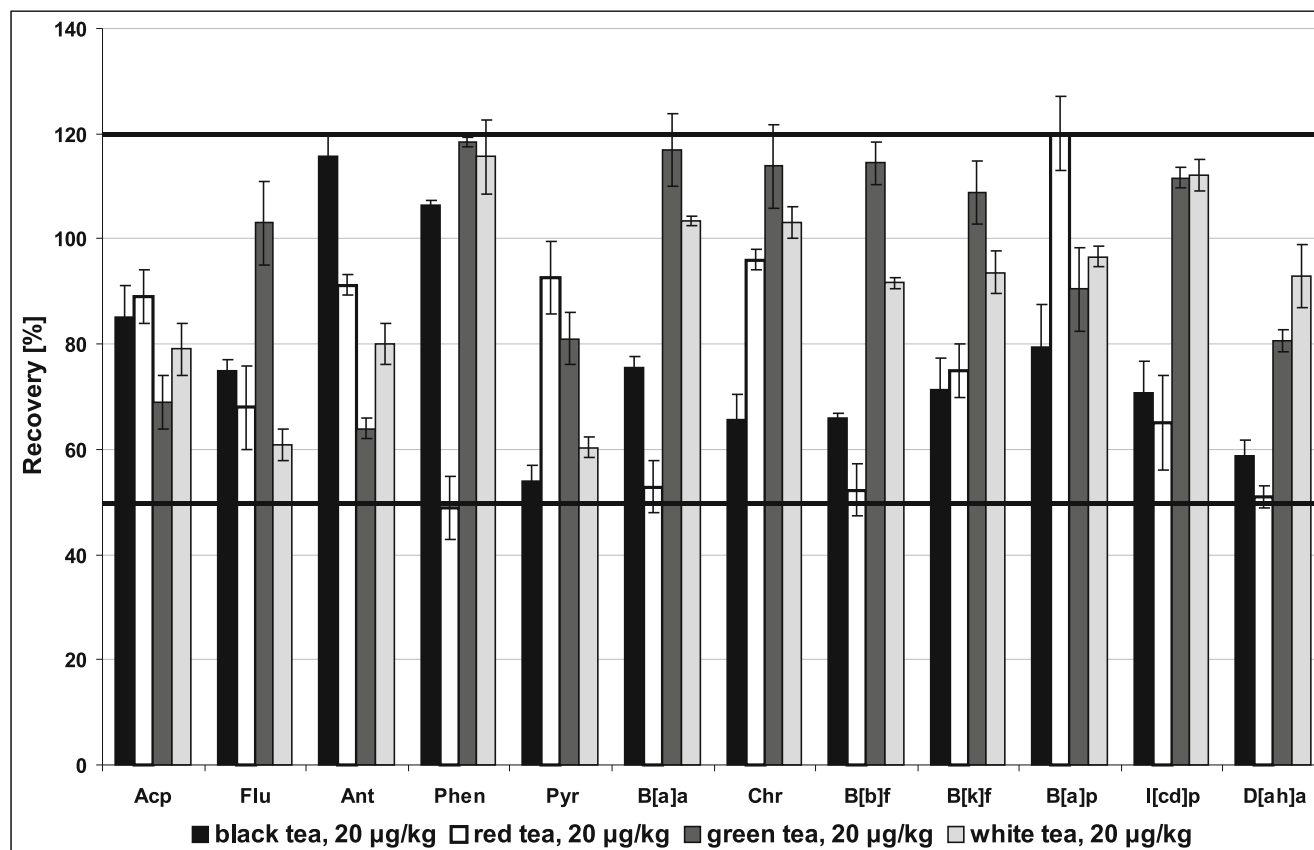


Fig. 9 Comparison of recovery values at the fortification level of $20 \mu\text{g kg}^{-1}$ for various types of tea; *Acp* acenaphthylene; *Flu* fluorene; *Ant* anthracene; *Phen* phenanthrene; *Pyr* pyrene; *B[a]a* benzo[a]anthracene; *Chr* chrysene;

B[b]f benzo[b]fluoranthrene; *B[k]f* benzo[k]fluoranthrene; *B[a]p* benzo[a]pyrene; *I[cd]p* indeno[1,2,3-cd]pyrene; *D[ah]a* dibenzo[a,h]anthracene

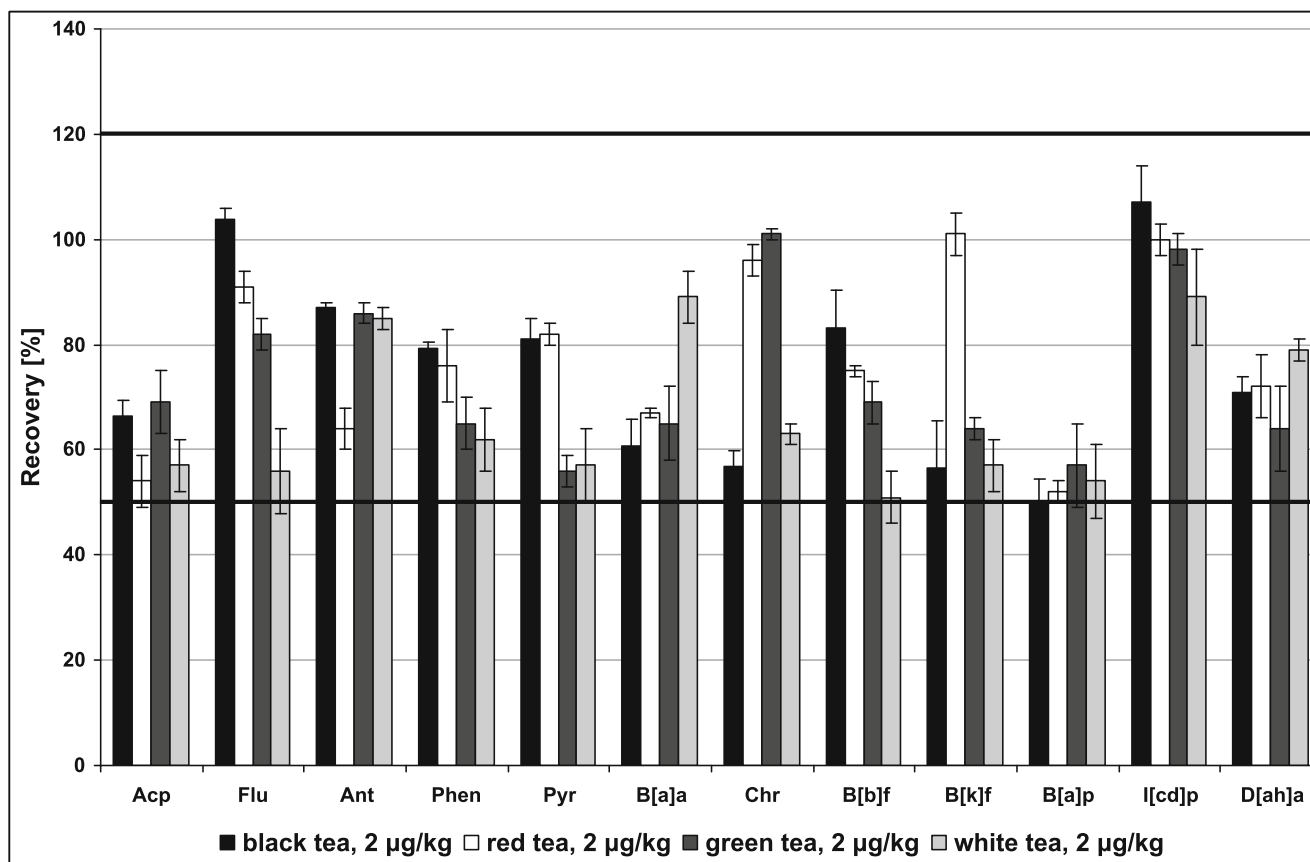


Fig. 10 Comparison of recovery values at the fortification level of $2 \mu\text{g kg}^{-1}$ for various types of tea; *Acp* acenaphthylene; *Flu* fluorene; *Ant* anthracene; *Phen* phenanthrene; *Pyr* pyrene; *B[a]a* benzo[a]anthracene; *Chr* chrysene;

B[b]f benzo[b]fluoranthrene; *B[k]f* benzo[k]fluoranthrene; *B[a]p* benzo[a]pyrene; *I[cd]p* indeno[1,2,3-cd]pyrene; *D[ah]a* dibenzo[a,h]anthracene

is the use of a small volume of solvents (4 mL) compared with traditional LLE, where large solvent quantities are required. Furthermore, the reagents used in this study (acetonitrile and hexane) are less toxic and odorous than dichloromethane or acetone that are commonly used in traditional LLE.

Monitoring study

In the study, 22 samples of various types of tea from seven manufacturers were analysed including five samples of white tea, seven samples of green tea, seven samples of black tea and three samples of red tea. All samples were delivered from Polish market in 2012.

Preparation of the samples was carried out according to the procedure described above with the application of variant MeCN+PSA+SAX+LLE.

In all analysed samples of tea, eight compounds belonging to the group of PAHs were detected: fluorene, phenanthrene, anthracene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthrene and benzo[k]fluoranthrene (Table 2). The highest value was observed for fluorene ($22.0 \mu\text{g kg}^{-1}$) and

lowest for benzo[b]fluoranthrene and benzo[k]fluorentrene (3.7 and $2.2 \mu\text{g kg}^{-1}$). Dibenzo[ah]anthracene and benzo[a]pyrene (PAH marker) were not detected in any of the analysed sample. The other PAH markers, benzo[a]anthracene, chrysene and benzo[b]fluoranthrene were found in 16, 13 and 7 samples, respectively. The levels of benzo[b]fluoranthrene were relatively low and ranged from 1.8 to $6.4 \mu\text{g kg}^{-1}$. The last two markers characterised by a higher content, 1.6 – $19.4 \mu\text{g kg}^{-1}$ for chrysene and 2.4 – $46.5 \mu\text{g kg}^{-1}$ for benzo[a]anthracene. The total contents of PAHs ranged from 30.1 to $147.1 \mu\text{g kg}^{-1}$. White and green teas were characterised by a lower content of PAHs (respectively 11.8 and $10.6 \mu\text{g kg}^{-1}$) than black and red tea (16.9 and $17.0 \mu\text{g kg}^{-1}$). The highest content of PAHs have been identified in samples of teas coming from manufacturers E and F ($16.5 \mu\text{g kg}^{-1}$), the lowest one – in samples of manufacturer B ($9.1 \mu\text{g kg}^{-1}$).

So far, any maximum residue levels of PAH in samples of teas have not established. Existing regulations (Commission Regulation (EU) No 835/2011) apply to various samples of foodstuffs except of tea. However, in the recent literature, some studies concerning the analyses of PAHs in tea have been made as yet. In the study of Grover et al. (2013), the

Table 2 PAHs content in analysed samples of tea

	PAHs content [$\mu\text{g kg}^{-1}$]												ΣPAHs
	Acp	Flu	Ant	Phe	Pyr	B[a]a	Chr	B[b]f	B[k]f	B[a]p	I[cd]p	D[ah]a	
White A	n.d.	14.8±1.2	46.9±0.5	11.9±1.6	5.6±0.4	14.8±0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	94.0
Red A	n.d.	12.7±0.8	n.d.	9.7±0.6	n.d.	32.9±2.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	55.3
Green A	n.d.	12.4±0.9	32.6±0.4	n.d.	6.2±0.4	12±2.1	2.8±0.2	n.d.	n.d.	n.d.	n.d.	n.d.	66.0
Black A	n.d.	n.d.	12.4±0.6	29.8±0.4	13.5±1	2.4±0.5	1.6±0.1	n.d.	1.5±0.1	n.d.	n.d.	n.d.	61.2
Green B	n.d.	n.d.	11.4±0.4	16.7±1.2	3.7±0.3	14.1±1	3.7±0.1	2±0.1	4±0.2	n.d.	n.d.	n.d.	55.6
Black B	n.d.	21.6±0.3	2.7±0.4	21.4±1.5	n.d.	3.6±0.9	n.d.	3.4±0.1	n.d.	n.d.	n.d.	n.d.	52.7
White C	n.d.	n.d.	n.d.	6.8±0.5	6.7±0.8	16.6±1.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	30.1
Black C	n.d.	26.4±0.3	6±0.3	9.8±0.5	24.7±2.1	3.1±0.2	13.1±0.4	n.d.	1.6±0.3	n.d.	n.d.	n.d.	84.7
Green C	n.d.	13.2±0.1	2.9±0.1	26.8±0.2	12.8±1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	55.7
White D	n.d.	1.2±0.1	6.8±0.1	6.7±0.4	n.d.	13.4±0.8	13.5±0.4	n.d.	1.7±0.1	n.d.	n.d.	n.d.	43.3
Red D	n.d.	n.d.	26.7±0.3	n.d.	5±0.1	n.d.	12.3±0.6	n.d.	n.d.	n.d.	n.d.	n.d.	44.0
Green D	n.d.	16.7±0.1	n.d.	3.2±0.6	n.d.	19.2±0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	39.1
Black D	n.d.	49.7±0.2	n.d.	16.6±0.8	32.7±1.9	n.d.	13.4±0.4	8.1±0.5	1.7±0.1	n.d.	n.d.	n.d.	122.2
White E	n.d.	5.6±0.3	23.7±1.3	23.4±2	6.7±0.4	2.4±0.8	12.2±0.3	n.d.	n.d.	n.d.	n.d.	n.d.	74.0
Green E	n.d.	n.d.	3.8±0.1	1.9±0.1	16±1.5	19.4±1.4	n.d.	1.8±0.2	n.d.	n.d.	n.d.	n.d.	42.9
Black E	n.d.	n.d.	24.8±1.6	6±0.4	63.4±0.3	46.5±0.6	n.d.	6.4±0.2	n.d.	n.d.	n.d.	n.d.	147.1
Red F	n.d.	46.7±1.2	n.d.	6.8±0.5	13.7±0.1	n.d.	3.2±0.5	n.d.	n.d.	n.d.	n.d.	n.d.	70.4
Green F	n.d.	36.7±1.3	n.d.	n.d.	n.d.	11.2±0.4	n.d.	n.d.	2.6±0.2	n.d.	n.d.	n.d.	50.5
Black F	n.d.	n.d.	30.9±1.4	n.d.	n.d.	6.9±0.6	6.3±0.1	n.d.	n.d.	n.d.	n.d.	n.d.	44.1
White G	n.d.	13.6±0.2	n.d.	n.d.	6.4±0.4	n.d.	19.4±0.1	2.2±0.1	n.d.	n.d.	n.d.	n.d.	41.6
Green G	n.d.	12.6±0.9	6.9±0.5	2.8±0.2	13.9±1.6	n.d.	1.9±0.1	n.d.	1.6±0.1	n.d.	n.d.	n.d.	39.7
Black G	n.d.	46.4±0.8	9±0.4	n.d.	45.7±0.9	23.5±2	18.2±0.4	1.9±0.1	2.5±0.1	n.d.	n.d.	n.d.	147.2

Acp acenaphthylene, Flu fluorene, Ant anthracene, Phen phenanthrene, Pyr pyrene, B[a]a benzo[a]anthracene, Chr chrysene, B[b]f benzo[b]fluoranthrene, B[k]f benzo[k]fluoranthrene, B[a]p benzo[a]pyrene, I[cd]p indeno[1,2,3-cd]pyrene, D[ah]a dibenzo[a,h]anthracene, A–G tea manufacturer, n.d. not detected

presence of 16 priority PAHs was studied in fresh leaves and at various stages of manufacturing. Benzo[a]pyrene was found in dried tea leaves only whereas dry tea leaves showed higher content of total 16 PAHs about 3 and 211 times than present in withered and dried leaves, respectively. Chrysene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]pyrene and benzo[a]anthracene were not found at all. In another research conducted in India (Singh et al. 2011), PAHs in tea have been studied for five locally available brands. Benzo[a]pyrene was found in two samples at levels of 14 and 16.6 $\mu\text{g kg}^{-1}$, and naphthalene was found in all the tea samples in the range of 33.3 to 268.8 $\mu\text{g kg}^{-1}$. In other study, performed in India, concentration of total PAHs in different brands of tea infusions varied from 18.79 to 31.37 $\mu\text{g L}^{-1}$. Mean concentration of total PAHs was 27.56 $\mu\text{g L}^{-1}$ (Bishnoi 2005). Drabova et al. (2012) determined the levels of sum of benzo[a]anthracene, chrysene, benzo[b]fluoranthrene and benzo[a]pyrene in black and green tea leaves in the range from 7.4 to 700 and from 4.5 to 102 $\mu\text{g kg}^{-1}$, respectively. Contamination of tested tea samples by benzo[a]pyrene was in the range of 0.2–152 $\mu\text{g kg}^{-1}$. Li et al. (2011) analysed PAHs in 15 tea samples; the detected

concentration of benzo[a]pyrene and benzo[b]fluoranthrene was from 0 to 348 $\mu\text{g kg}^{-1}$. However, there were no obvious relations between various areas in China or between different kinds of tea. In other Chinese work, the total contents of the 16 PAHs in the tea samples ranged from 323 to 8800 $\mu\text{g kg}^{-1}$ with the highest PAHs found in a black tea. Three- to four-ring PAHs were dominant in all tea samples (Lin et al. 2005). In German study (Ziegenhals et al. 2008), it was observed that the total contents of the 16 PAH in tea samples ranged from 1.4 to 2.662 $\mu\text{g kg}^{-1}$. The analysed tea samples showed an increasing presence of PAH in the following order: herbal and fruit tea, black tea, green tea, white tea and mate-tea. Finally, in Poland, Ciecierska and Obiedziński (2009) found a high level of four light PAHs (from 63 to 92 %) in the total content of 19 PAHs for all tested leaf tea brands. Benzo[a]pyrene was not detected in any of the analysed samples. In other Polish work, conducted by Ciemiński and Mocek (2010), the total content of 23 PAHs varied between ranges 22.9 to 2945.5 $\mu\text{g kg}^{-1}$ and 2.7 to 63.1 $\mu\text{g kg}^{-1}$ for benzo[a]pyrene. The analysed tea samples showed an increasing presence of PAH in the following order: black tea < red tea < green tea < white tea. The dominant PAHs in tea infusion were

two-, three- and four-ring PAHs, while the most toxic compounds were found at trace amounts. The concentrations of total 23 PAHs and benzo[a]pyrene in tea infusions ranged from 332.5 to 2245.9 ng L⁻¹ and 0.35 to 18.7 ng L⁻¹, respectively.

Overall, our outcomes are in good agreement with the results obtained by other authors. The comparison of the levels of selected compounds belonging to PAH markers and the sums of total PAHs provided by us and other researchers were presented in Table 3. In the samples of black tea, the contents of PAH markers obtained in this study were comparable to the results achieved in Poland by Ciecierska et al. (2007) and also in Germany, by Ziegenhals et al. (2008). The highest levels of these compounds were determined by Lin et al. (2005) in China and Drabova et al. (2012). In the samples of green and white tea, the PAH levels determined in our research were significantly lower than the other results, especially those provided by Ziegenhals et al. (2008). Ultimately, in the red tea samples, all results were diversified and difficult to compare.

In case of the sum of total PAHs, the results presented in this study were lower than those submitted by other authors. However, the sum of total PAHs, reported by various authors, included the sum of different number of compounds and, therefore, comparing them may be not reliable.

Conclusion

In general, a new approach for analysis of PAHs in various type of tea has been proposed, using the different combination of QuEChERS method. Our preliminary experiments showed that the QuEChERS-based extraction method with some modifications (use LLE at the end of the analytical procedure) could be successfully applied for the determination of selected PAHs in teas. However, the experiments indicate that teas are the matrices that require careful sample prep to ensure valid results.

In the study of real samples, compounds belonging to light PAHs were mostly detected while the most dangerous heavy

Table 3 Comparison of PAH contents in tea determined by different authors

Sampling place	PAHs content (µg kg ⁻¹)				
	B[a]a	Chr	B[b]f	B[a]p	ΣPAHs
<i>Black tea</i>					
Poland (this study)	2.4–46.5	1.6–18.2	1.9–8.1	n.d.	44.1–147.2
Poland (Ciemniak and Mocek 2010)	n.a.	n.a.	n.a.	2.9–63.1	26.2–440.6
Poland (Ciecierska et al. 2007)	1.7–15.4	0.9–18.9	0.5–2.5	n.d.	18.8–285.8
India (Grover et al. 2013)	n.a.	n.a.	210.4	1,574.1	6329.3
India (Singh et al. 2011)	n.a.	n.a.	n.a.	14.0–16.6	101.9–464.0
Czech Republic (Drabova et al. 2012)	1.4–196.1	3.9–229.0	0.7–59.2	0.2–151.7	12.7–988.3
China (Li et al. 2011)	n.a.	n.a.	n.a.	9.4	n.a.
China (Lin et al. 2005)	30.4–175.0	45.4–24.1	22.0–37.6	5.9–39.7	775.7–8,800
Germany (Ziegenhals et al. 2008)	1.3–13.1	3.4–18.1	1.5–8.1	1.3–14.1	14.0–80.8
<i>Green tea</i>					
Poland (this study)	11.2–19.4	2.8–3.7	1.8–2	n.d.	39.1–66.0
Poland (Ciemniak and Mocek 2010)	n.a.	n.a.	n.a.	5.6–31.0	46.9–1,468.8
Poland (Ciecierska et al. 2007)	1.47–22.9	2.4–19.9	0.8–13.5	n.d.	62.0–231.0
Czech Republic (Drabova et al. 2012)	0.7–28.3	2.9–42.4	0.7–23.9	0.2–17.9	10.3–159.7
China (Li et al. 2011)	n.a.	n.a.	n.a.	4.5–23.0	n.a.
China (Lin et al. 2005)	7.0–25.3	18.2–29.6	10.8–25.3	3.1–7.4	323.0–550.0
Germany (Ziegenhals et al. 2008)	1.8–40.4	6.7–61.5	1.2–17.1	1.6–32.6	19.6–286.1
<i>Red tea</i>					
Poland (this study)	32.9	3.2–12.3	n.d.	n.d.	44.0–70.4
Poland (Ciemniak and Mocek 2010)	n.a.	n.a.	n.a.	9.7–14.8	73.6–92.4
Poland (Ciecierska et al. 2007)	3.4–5.9	5.2–7.2	0.74	n.d.	69.9–243.3
<i>White tea</i>					
Poland (this study)	2.4–16.6	12.2–19.4	2.2	n.d.	30.1–94.0
Poland (Ciemniak and Mocek 2010)	n.a.	n.a.	n.a.	7.6–48.7	92.0–396.6
Germany (Ziegenhals et al. 2008)	14.1–80.1	19.0–95.1	14.5–44.6	8.2–22.1	107.9–349.7

B[a]a benzo[a]anthracene, Chr chrysene, B[b]f benzo[b]fluoranthrene, B[a]p benzo[a]pyrene, n.a. not analysed (data not available in a study), n.d. not detected

polycyclic aromatic hydrocarbons, including benzo[a]pyrene, were not identified in any of samples.

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