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Ultrasound-assisted surfactant-enhanced emulsification microextraction and determination of caffeine and theophylline in human plasma and cocoa powder

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Abstract

A rapid and simple ultrasound-assisted surfactant-enhanced emulsification microextraction procedure with chloroform-decanoic acid (87:13 w/w) as the extractant mixture has been improved for the simultaneous preconcentration of caffeine and theophylline before their determination using high-performance liquid chromatography. The use of ultrasonic radiation and surfactant can enhance the dispersion of extraction solvent into an aqueous phase and simplify the emulsification process. The proposed method is an environmentally friendly pretreatment method because of its low solvent consumption. Various parameters influencing the efficiency of the method including pH, buffer, extraction solvent, extraction time, and interfering ions were studied. The linear calibration curves were obtained in the ranges of 0.5–150 and 2–150 ng mL^{-1} with limits of detection of 0.22 and 0.15 ng mL^{-1} for caffeine and theophylline, respectively. The proposed procedure was successfully utilized for the determination of caffeine and theophylline in cocoa powder and human plasma samples. Based on the obtained results, it can be concluded that the proposed method is potentially applicable as an efficient method for preconcentration and determination of caffeine and theophylline in real samples such as food and biological media.

Keywords Microextraction · HPLC · Caffeine · Theophylline · Human plasma · Cocoa powder

Introduction

A major step in the drug analysis is sample preparation, impressing the accuracy and reliability of the analysis where it is necessary to extract the analytes from the matrices for further analysis. So, sample preparation techniques for the extraction of drugs play a fundamental role in analytical procedures (Moein et al. 2014). Conventional sample preparation methods like liquid–liquid extraction (LLE) are time-consuming, multistep procedures and need significant amounts of organic solvents. The modern sample preparation techniques are mainly aimed to simplify and miniaturize the sample preparation procedure and decrease the organic solvents consumption (Sarafraz-Yazdi and Amiri 2010).

LLE can be miniaturized in several ways which have been led to different liquid-phase microextraction (LPME) techniques. The most popular of these techniques are hollow-fiber liquid-phase microextraction (HF-LPME) (Makahleh et al. 2019), single-drop microextraction (SDME) (Neri et al. 2019), and dispersive liquid–liquid microextraction (DLLME) (Moreira et al. 2020). DLLME is a fast and powerful extraction method that has been presented by Rezaee et al. 2006. In this technique, a mixture of water-miscible disperser solvent and microliter volumes of water-immiscible extraction solvent is used. The mixture is rapidly introduced into the sample solution via injection, and then a cloudy suspension appears. Formation of fine organic droplets in the sample solution as a result of high dispersion of extraction solvent can accelerate the analyte extraction. After extraction, a centrifugation step is necessary to collect the organic phase (Xue et al. 2014; Rosa et al. 2015). Recently, DLLME has been interested greatly because of its rapidity, simple operation, low volume of solvent consumption, cost-effectiveness, high recovery, and enrichment factor (EF) (Rezaee et al. 2006).

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Despite the advantages of DLLME, the major disadvantage of this method is the necessity of using a disperser solvent that is harmful and declines the efficiency of extraction. To overcome this drawback, a new microextraction technique based on ultrasonic dispersion of the extraction solvent termed ultrasound-assisted microextraction has been introduced by Regueiro et al. in (2008).

A new DLLME method named ultrasound-assisted surfactant-enhanced emulsification microextraction (UA-SEME) was introduced by Wu et al. in (2010b). In the UA-SEME method, surfactant emulsification was employed to the dispersion of the organic extractant into the aqueous phase under ultrasonication. Surfactants are amphiphilic molecules that are soluble in both organic and aqueous phases. They can reduce the interfacial tension between the two phases and serve as an emulsifier to increase the emulsification of the organic phase into an aqueous solution. This method does not need a disperser solvent, which decreases the partition coefficient of analytes by increasing the solubility of the analytes in the aqueous solution. Application of surfactant and ultrasonic radiation can simplify the emulsification process and accelerate the analyte mass transfer to the organic phase which leads to enhancement in the extraction efficiency in a very short time (Wu et al. 2010a; Rosa et al. 2015). UA-SEME is an eco-friendly technique that combines the benefits of both ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) (Ghoraba et al. 2018) and surfactant-assisted dispersive liquid–liquid microextraction (SA-DLLME) (Sobhi et al. 2018).

Caffeine (CF) and theophylline (TP) belong to methylxanthines drugs. Human beings daily consume methylxanthines because many beverages and food products such as tea leaves, coffee, and cocoa naturally contain CF and TP.

TP is similar to CF in terms of structure and pharmacological effects and it is one of the primary metabolites produced by metabolization of CF in the liver (Heckman et al. 2010). Same as CF, it can be found in natural sources such as cocoa beans and tea (Barnes 2013).

Measurement of drug concentrations in biologic fluids termed therapeutic drug monitoring (TDM) is needed for the avoidance of poisoning or side effects of drugs (Messer and Schmauss 2006), especially drugs with a narrow therapeutic index such as TP (Tamargo et al. 2015). Blood TP concentration must be kept between 10 and 20 µg mL^{-1} for the best bronchodilator effect; otherwise, its adverse effects occur over 20 µg mL^{-1} (Charehsaz et al. 2014). Blood caffeine level below 10 µg mL^{-1} was considered harmless. Caffeine concentration between 15 and 20 µg mL^{-1} in blood was considered elevated, but still not toxic or a danger to health, whereas levels between 80 and 180 µg mL^{-1} were associated with caffeine-related fatalities (Jones 2017). Therefore simple, sensitive, and reliable methods are necessary for preconcentration and extraction of CF and TP from pharmaceutical preparations, biological samples, and food products. Thus, a number of pretreatment methods have been applied before the determination of CF and TP like online in-tube solid-phase microextraction (IT-SPME) coupled to capillary liquid chromatography-diode array detection (Ponce-Rodríguez et al. 2020), accelerated solvent extraction (ASE) coupled with ultra-high-pressure liquid chromatography (UHPLC-PDA) (Ahmad et al. 2020), solid-phase extraction (Tian et al. 2009), ultrasonic-assisted dispersive micro solid-phase extraction combined with HPLC–UV (Sereshti et al. 2014).

In this paper, a fast and effective UA-SEME method using a chloroform-decanoic acid mixture was described for determining CF and TP in the food products and biological samples. The proposed method is an eco-friendly technique compared with conventional liquid–liquid extraction and DLLME because of very low organic solvent consumption and no need to a disperser solvent. HPLC–UV is one of the best choices for the separation and detection of these two alkaloids in terms of similar molecular structures. To the best of the authors' knowledge, excepting a limited number of studies concerning the use of LPME for the determination of CF (Amini and Hashemi 2018; Shishov et al. 2019; Elik et al. 2019; Sivrikaya 2020), no LPME procedure for simultaneous determination of CF and TP has been reported so far.

Material and methods

Reagents and solutions

CF, HPLC grade acetonitrile, analytical grade hydrochloric acid, sodium hydroxide, acetic acid, chloroform, dichloromethane, 1,2-dichloroethane, carbon tetrachloride, n-hexane, 1-decanol, 1-octanol, decanoic acid, sodium dodecyl sulfate, sodium chloride, cetyltrimethylammonium bromide, tetrapropylammonium bromide, Triton X-100 and acetonitrile were prepared from Merck (Darmstadt, Germany). TP, dioctyl sulfosuccinate sodium, and Aliquat 336 were prepared from Sigma-Aldrich (Steinheim, Germany). Triton X-114 was purchased from Acros Organics (Geel, Belgium). Cocoa powder was purchased from Parand Chocolate Company (Savojbolagh, Iran). Human plasma sample was prepared from the Iranian Blood Transfusion Organization (Damghan, Iran) and kept at −20 °C. Doubly distilled water was utilized for all experiments.

The stock solutions (100 µg mL^{-1}) of CF and TP were prepared in double-distilled water. Serial dilutions of the stock solutions were used to prepare suitable working standard solutions. Acetate buffer solution was prepared by mixing suitable volumes of 0.1 mol L^{-1} sodium hydroxide and 0.1 mol L^{-1} acetic acid to achieve pH=5.5. The acidic water solution (pH=2.8; 0.34 mol L^{-1} acetic acid) was prepared

Fig. 1 Chromatograms of the sample and blank solutions after UA-SEME procedure. Conditions: acetonitrile-acidic water (pH 2.8; 0.34 mol L^{-1} acetic acid) (10:90, v/v) as mobile phase, flow rate = 1.5 mL min^{-1} and λ = 273 nm
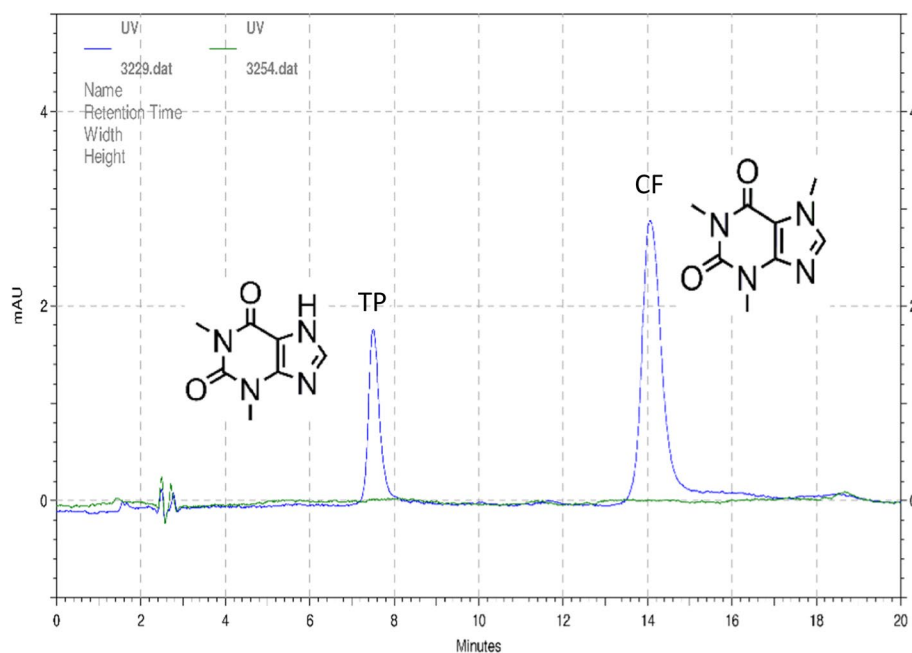
by adding a proper amount of glacial acetic acid to 100 mL HPLC grade water.

Apparatus

The HPLC system consisted of a Smartline HPLC pump (Knauer, Germany), a six-port injection valve with a 20 μL sample loop (Rheodyne, USA) and a UV absorption detector (Knauer) set at λ = 273 nm. A reversed-phase C18 column, (25 cm × 4.6 mm i.d.) with 5 μm particle size, Perfectsil ODS-3 (MZ-Analytical, Germany) protected by a guard column was employed as the separation column. The column was isocratically eluted (1.5 mL min^{-1}) with the mobile phase consisted of acidic water (pH = 2.8; acetic acid)-acetonitrile (90:10, v/v). All pH measurements were performed by a digital pH meter (Metrohm 780) calibrated with standard buffer solutions. An ultrasonic water bath with temperature control (Parsonic 2600 S, Pars Nahand, Iran) was used. A Hermle Z-300 centrifuge (Germany) was used for phase separation. The injections of organic phase into the HPLC instrument were performed using a 100 μL microsyringe (Hamilton, Switzerland).

US-SEME procedure

5 mL of a solution comprising 0.5–150 ng mL^{-1} of CF, 2–150 ng mL^{-1} of TP, and 1.25 mL of acetate buffer with pH = 5.5 was poured into a 10 mL conical centrifuge tube, and it was located in the ultrasonic bath. Then 170 μL of a homogenous mixture of chloroform-decanoic acid (87:13 w/w) was immediately introduced into the mentioned solution via injection. Thereafter the sonication was applied

to the tube for 3 min at ambient conditions. To achieve phase separation of the resulted emulsion, it was centrifuged for 4 min at 4000 rpm. Finally, 20 μL of the sedimented organic phase was withdrawn by a micro-syringe and injected into the HPLC system.

Statistical analysis

Relative standard deviation (RSD) was used to assess the precision of the suggested method via studying eight replicate solutions of CF and TP at two various concentrations.

Standard deviation and mean value of triplicate measurements of real specimens were calculated. Statistical analysis of the archived results was carried out by employing t-test and comparison between calculated t-values and the critical value (95% confidence level).

Results and discussion

Optimization of UA-SEME procedure

To achieve the optimal conditions for preconcentration and determination of CF and TP, different experimental factors were optimized. For this purpose, the peak areas of CF and TP were used as analytical signals. The typical chromatograms for the blank and extracted analytes solutions are presented in Fig. 1. The retention times of analytes were 7.6 and 14.08 min for TP and CF, respectively.
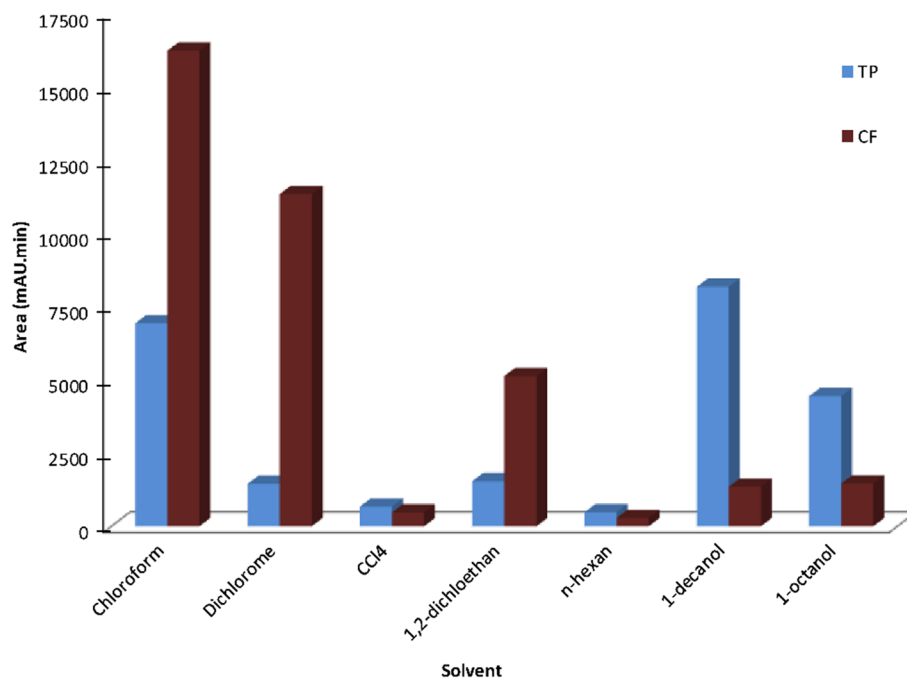
Selection of extraction solvent

Choosing a proper extraction solvent is a key parameter to
improve the performance of the UA-SEME procedure. The
favorable characteristics of extraction solvent are low water
solubility, excellent extraction ability for the analytes of
interest, and low surface tension to facilitate emulsion for-
mation. For this purpose, different organic solvents includ-
ing chloroform, 1-octanol, 1,2-dichloroethane, 1-decanol,
carbon tetrachloride (CCl$_4$), n-hexane, and dichloromethane
were studied. The tests were carried out with 200 μL of each
solvent. Based on the results shown in Fig. 2, the chloroform
was chosen as the best solvent for the UA-SEME procedure.

Selection of surfactant type and concentration

Selecting an appropriate surfactant is essential to attaining
high efficiency of the UA-SEME method because the tiny
droplet formation of the water-immiscible extraction sol-
vent is accelerated by surfactant as emulsifier and ultrasonic
radiation. The effect of several surfactants such as sodium
dodecyl sulfate (SDS), decanoic acid, tetrapropylammo-
nium bromide (TPAB), cetyltrimethylammonium bromide
(CTAB), tetrapropylammonium bromide (TPAB), Triton
X-100, dioctyl sulfosuccinate (DOS), Triton X-114, and Ali-
quat 336 on the analytical signal of the analytes was inves-
tigated. As shown in Fig. 3, among the surfactants studied,
decanoic acid gave the best result.

Moreover, surfactant concentration plays a key role in
the UA-SEME procedure. To investigate the influence of
surfactant concentration on extraction performance, a series

of mixtures containing various percentages of decanoic acid
in chloroform (0–37%, w/w) were investigated. According
to the obtained results that are represented in Fig. 3, 13%
(w/w) of decanoic acid in chloroform was selected as an
optimal concentration.

Effect of pH

The pH has a critical role in this procedure because the for-
mation of surfactant-analytes ion pairs in the sample solu-
tion is dependent on the pH. Hence, it must be controlled
to increase the EF and method sensitivity. The effect of pH
on the analytical signal of the CF and TP was investigated
in the pH range between 2.0 and 9.0. The HCl and NaOH
solutions were used for pH adjustment. The results (Fig. 4a)
demonstrated that the optimal pH value was equal to 5.5.

At the optimum pH value, CF (pKa = 8.3 (Hackett et al.
2008)) and TP (pKa = 8.52 (Romand et al. 2014)) are proto-
nated and comprise a positive charge (Scheme 1). In addi-
tion, the surfactant is deprotonated (pK$_a$ = 4.8) to produce
decanoate ions with a negative charge (Jafarvand and Shemi-
rani 2011). As a consequence of the electrostatic attraction,
the decanoate-protonated analytes ion pairs are formed. At
high pH values, the analytes are deprotonated and at low pH
values, the surfactant is protonated, which ion pair formation
is disturbed and so analytical signal is decreased.

Selection of buffer type and concentration

Based on Fig. 4a, the analytical signal of analytes was found
to be affected by the pH variation. Different real samples

Fig. 3 Effect of surfactant type and concentration of decanoic acid (inset Fig.) on the extraction of caffeine and theophylline. Conditions: 5 mL solution of caffeine and theophylline 50 ng mL^{-1}, extraction time = 6 min and 200 μL of extractant mixture (5% w/w surfactant in chloroform)
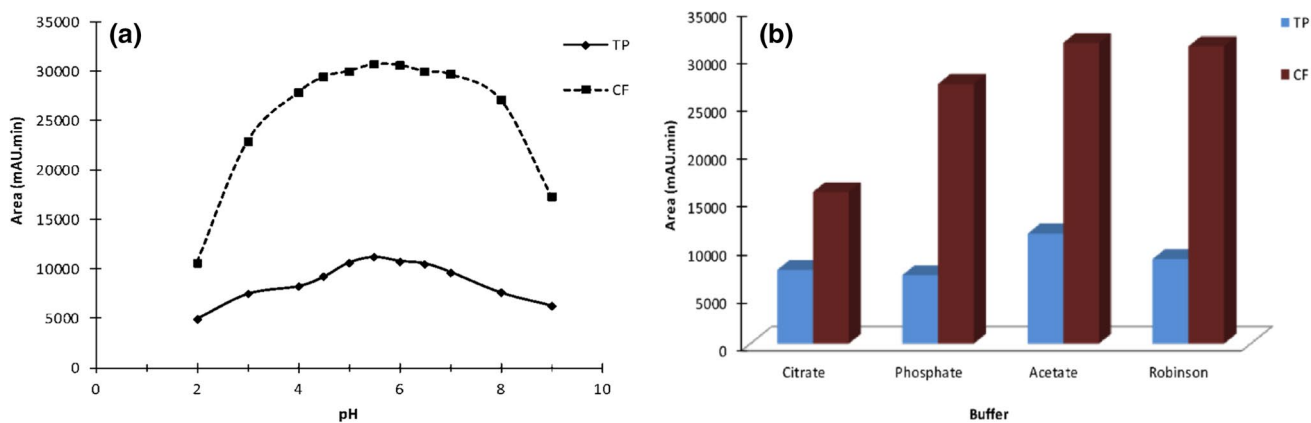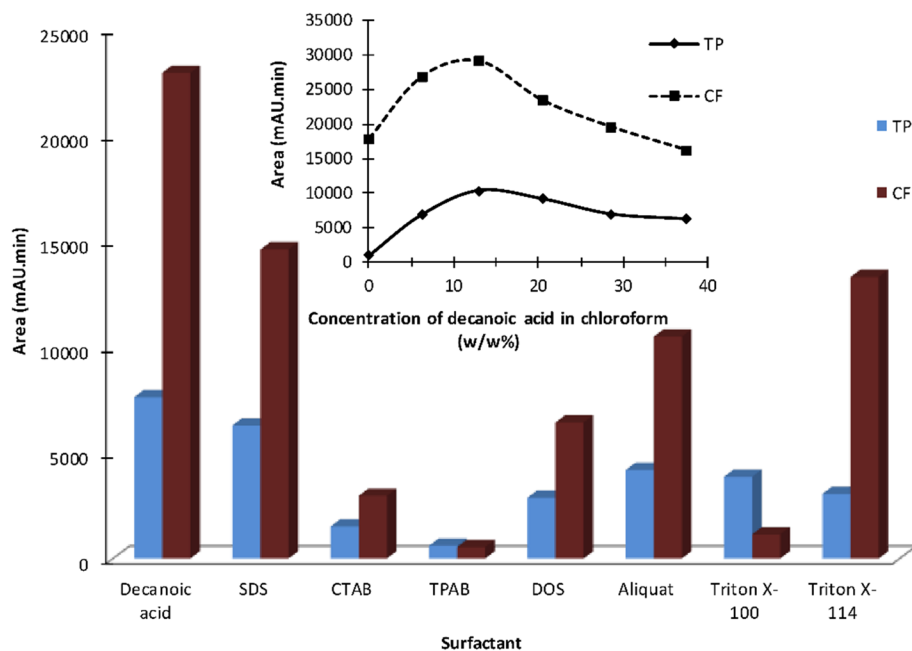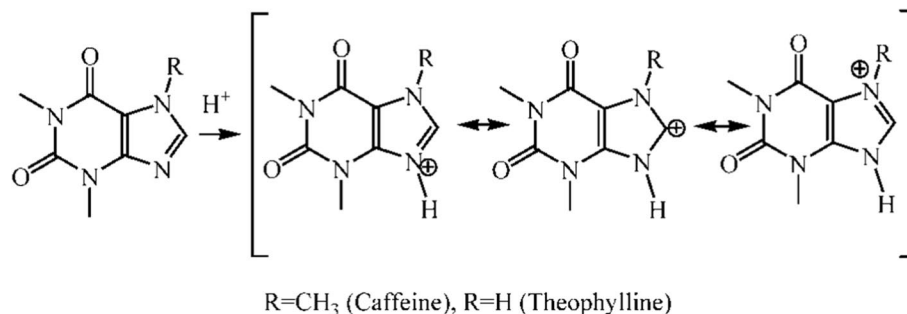

Fig. 4 **a** Effect of pH and **b** Effect of buffer type on the extraction of caffeine and theophylline. Conditions: 5 mL solution of caffeine and theophylline (50 ng mL^{-1}), extraction time = 6 min and 200 μL of chloroform-decanoic acid (87:13 w/w) as extractant mixture

Scheme 1 Protonation of caffeine and theophylline

R=CH$_3$ (Caffeine), R=H (Theophylline)

can change the pH of the solution, so the extraction procedure must be performed in controlled pH values. Therefore, the buffer solution should be used for the pH adjustment. The influence of buffer type on the performance of

the extraction procedure was studied by the use of various kinds of buffer (pH = 5.5). For this purpose, the same concentration (0.006 mol L^{-1}) of different buffers such as citrate, acetate, phosphate, and Britton-Robinson buffer were

used in the extraction procedure. According to the obtained results which are represented in Fig. 4b, the acetate buffer was selected as the best to achieve the highest effectiveness. Further tests were carried out on the concentration of the suitable buffer in the range of 0–0.027 mol L^{-1}, which indicated that 0.014 mol L^{-1} of acetate buffer with pH = 5.5 was sufficient to attain the maximum analytical signal (Fig. S1). Accordingly, to achieve this concentration, 1.25 mL of acetate buffer must be added to sample solution in further experiments.

Effect of the extractant mixture volume

The influence of the extractant mixture volume on the analytical signal was also studied. The chloroform-decanoic acid (87:13 w/w) at different volumes in the range of 170.0–300.0 μL was introduced to the UA-SEME method (Fig. S2). Based on the obtained results, 170 μL of the extractant mixture was selected as the optimum.

It was observed that the collected organic phase has a lower volume compared with the injected extractant mixture due to its solubility in the aqueous phase. Therefore, in the case of volumes lower than 170 μL, the collected phase was not sufficient for HPLC injection. Furthermore, at higher volumes of the extractant mixture, the analytical signal is reduced due to the dilution of analytes by increasing the sedimented phase volume.

Influence of extraction time

In the UA-SEME, the sonication time duration that was applied to the sample/extractant mixture solution was considered as the extraction time. The influence of sonication time was evaluated in the range of 1 to 15 min. Based on the achieved results (Fig. S3), the extraction time had no remarkable influence on the extraction; because the equilibrium state is achieved quickly as a result of the large contact surface between the aqueous phase and tiny droplets of extractant mixture in the emulsion. Therefore, ultrasound treatment for 3 min was chosen as the optimal extraction time to ensure the complete formation of a cloudy solution.

Influence of salt concentration

The influence of salt concentration on the extraction performance was studied by adding various NaCl concentrations in the range of 0–1 mol L^{-1} to the sample solutions. The experimental results (Fig. S4) demonstrated that the extraction performance was slightly diminished by enhancing the NaCl concentration.

The density and viscosity of the solution were increased by enhancing the amount of NaCl. A reduction in the emulsification efficiency was observed due to the absorption of ultrasound energy by the viscose solutions and its conversion to calorific energy. Consequently, the next experiments were performed without adding salt.

Effect of the extraction temperature

Temperature is also a key factor that may influence the analytical signal by affecting the mass transfer efficiency, emulsification process, and distribution coefficient. To study the influence of temperature, the method was performed at various temperatures ranging between 4 and 37 °C. Based on the obtained results (Fig. S5), the temperature had no noticeable influence on the analytical signal because the extraction was not limited by the mass transfer phenomenon. It can be explained by the fast equilibrium state obtained via the large contact surface formed between the extractant droplets and the aqueous phase as a result of simultaneous application of surfactant and ultrasound radiation. Hence, for simplicity further experiments were performed at room temperature.

Analytical features

Under the optimal conditions, the analytical features of the suggested procedure were achieved. The calibration graphs were achieved by plotting the analytes' peak areas versus the relevant concentrations. The linearity was obtained within the concentration range of 0.5–150 and 2–150 ng mL^{-1}, with correlation coefficients (r) 0.9998 and 0.9994 for CF and TP, respectively; where demonstrated excellent linearity for the suggested method. Linear regression equations were obtained as $A = 657.2 \times C_{CF} + 1976.44$ and $A = 265.42 \times C_{TP} + 215.58$; which A refers to the corresponding peak area (mAU.min),[1] C_{CF} and C_{TP} represent the concentration of CF and TP (ng mL^{-1}), respectively. Limits of detection (LODs) of the method were calculated as three times of standard deviation of the blank divided by the slope of the calibration graph ($3S_b/m$) for eight blank replications (Rahmani and Aibaghi 2022). According to the results, the LODs were attained 0.22 and 0.15 ng mL^{-1} for CF and TP, respectively.

Method repeatability was expressed in terms of RSD. Intraday precision was estimated via studying eight replicate solutions of CF and TP at two concentration levels (10 and 100 ng mL^{-1}). RSD were obtained 4.3%, 2.9% for 10 and 100 ng mL^{-1} of CF and 3.2%, 2.5% for 10 and 100 ng mL^{-1} of TP, respectively.

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[1] milli-Absorbance Unit × minute.

Table 1 Effect of the interfering ions and compounds on the UA-SEME of caffeine and theophylline

Foreign species	Tolerance ratio***
K+, Mg2+, Ca2+, Fe3+, Cr3+, Ag+*, Al3+, F-, Br-*, Cl-, SO42-, S2-, Citrate, Glucose, Fluvoxamine maleate	1000
Sn2+, Na+, Cd2+, Zn2+, Fe2+, Cu2+, Ag+**, H2PO4-, CO32-, I-, Br-**, EDTA, Glycine, Bromhexine hydrochloride	750
Pb2+, Ba2+, C2O42-	500
Urea, Chlorpropamide	250
Ni2+	100

*For theophylline, **For caffeine, ***Concentration of interfering species divided to the analyte concentration

Enrichment factor and consumptive index

The EF of the method was assessed by $EF = m_p/m$, where m_p and m are the slopes of the calibration graphs with and without pre-concentration step, respectively. The EFs of the method were obtained 51 for CF and 27 for TP.

The analytical performance of a preconcentration system can be characterized by evaluating the consumptive index (CI). The sample volume (V_s), in milliliter, which is needed to achieve a unit of EF is considered as the CI and calculated via $CI = V_s/EF$. According to this, the CIs of 0.098 and 0.185 mL were achieved for the CF and TP, respectively.

Interference studies

To apply the suggested UA-SEME method to real specimens, the influence of foreign species on the procedure was examined. Therefore, 5 mL solution comprising 50 ng mL^{-1} of each analyte, 1.25 mL of acetate buffer (pH = 5.5), and diverse species at different concentration levels were prepared and the optimal UA-SEME method was applied. The relative error of less than 5% in the analytical signal of CF

and TP was considered tolerable. The results are shown in Table 1. As can be seen, most species show low interference effect on the determination of the analytes.

Real samples analysis

To indicate the efficiency of the UA-SEME method in real specimens, the suggested procedure was used to the simultaneous determination of CF and TP in cocoa powder and human plasma samples.

Cocoa powder

For the extraction of CF and TP from cocoa, 0.020 g of cocoa powder and 20.0 mL of hot water (80 °C) were transferred to a 50 mL beaker and was placed in the 80 °C water bath for 20 min. The mixture was then cooled down to ambient temperature, and the liquid phase was separated by centrifugation at 4000 rpm (Brunetto et al. 2007). For CF determination 0.05 mL and for TP determination 0.5 mL of the mentioned liquid phase along with the buffer solution were transferred into a 5 mL volumetric flask and spiked with various concentrations of CF and TP and analyzed using the optimum procedure. The results are shown in Table 2. According to the results, CF and TP contents of cocoa powder were obtained 0.314 and 0.004 (% w/w), respectively.

Human plasma

In order to prepare plasma sample solutions comprising various concentrations of CF and TP, 0.5 mL of standard solutions of each analyte with different concentrations (0, 2, and 10 µg mL^{-1}) was poured into a volumetric flask and filled up to 10 mL with human plasma. For deproteinization of the plasma, 0.5 mL of these drugs-supplemented plasma solutions was transferred to the vial and placed in a boiling water bath for 10 min, then the solution was cooled to ambient temperature and 0.5 mL

Table 2 Determination of caffeine and theophylline in real samples

Real sample	Caffeine (ng mL^{-1})				Theophylline (ng mL^{-1})			
	Added	Found[a]	Recovery (%)	t-test[b]	Added	Found	Recovery (%)	t-test
Cocoa powder	0	15.7 ± 0.9	–	–	0	4.1 ± 0.4	–	–
	30	44.6 ± 2.0	96.3	0.9	30	34.2 ± 1.3	100.3	0.1
	50	66.0 ± 2.4	100.6	0.2	50	53.9 ± 1.7	99.6	0.2
	70	84.6 ± 2.5	98.4	0.8	70	73.3 ± 2.2	98.8	0.6
Human plasma[c]	0	29.6 ± 1.9	–	–	0	N.D[d]	–	–
	10	40.0 ± 1.5	104.0	0.5	10	10.2 ± 0.5	102.0	0.7
	50	79.7 ± 2.2	100.2	0.1	50	49.7 ± 0.8	99.4	0.6

[a]Average of three measurements ± Standard deviation. [b]Tabulated t-value for two degrees of freedom at 95% confidence level is 4.3. [c]Analyzed using standard addition method. [d]Not Detected

Table 3 Comparison of UA-SEME method and previously published LLE and LPME methods for the determination of caffeine and theophylline

Method	Analyte(s)	Detection	Real samples	LOD (ng mL^{-1})	RSD (%)	Linear range (ng mL^{-1})	Ref
LLE	Caffeine Theophylline Theobromine Paraxanthine	HPLC–DAD	Urine	80 (CF) 40 (TP)	2.9–6.9 (CF) 2.3–7.2 (TP)	1000–20,000	(Büyüktuncel 2010)
LLE	Theophylline	HPLC–UV	Saliva, Plasma	600	0.45–2.33	1000–50,000	(Charehsaz et al. 2014)
DLLME	Caffeine	GC-NPD	Teas, Coffees, Beverages	20	3.2	50–500,000	(Sereshti and Samadi 2014)
DLLME	Caffeine	Spectrophotometry	Coffee, Beverages	460	2.1	2000–75,000	(Frizzarin et al. 2016)
UAE-DLLME[b]	Caffeine	GC–MS	Teas	300	3.10	1000–5,000,000	(Sereshti et al. 2013)
SDLME[c]	Caffeine	Potentiometry	Saliva	11,651	2.0–2.6	1942–1,941,900	(Timofeeva et al. 2016)
CA-DES-ME[d]	Caffeine	UV–Vis	Foods, Beverages	7.5	1.2–1.6	25–700	(Elik et al. 2019)
DES-LPME[e]	Caffeine	HPLC–UV	Coffee	120	2.20	500–100,000	(Sivrikaya 2020)
DLLME	Caffeine	HPLC–UV	Teas, Energy drinks	0.9	3.8	1–200	(Yousefi et al. 2017)
A-HLLME[f]	Caffeine	HPLC–UV	Teas, Cola, Energy drinks	30	5	100–200,000	(Shishov et al. 2019)
HLLME[g]	Caffeine	GC–MS	Teas, Coffee	50	4.8	160–50,000	(Amini and Hashemi 2018)
UA-SEME	Caffeine Theophylline	HPLC- UV	Plasma, Cocoa powder	0.22 (CF) 0.15 (TP)	2.9–4.3 (CF) 2.5–3.2 (TP)	0.5–150 (CF) 2–150 (TP)	*This work*

[a]Not Reported, [b]Ultrasound-assisted extraction-dispersive liquid–liquid microextraction, [c]Single-drop liquid microextraction, [d]Chemometric-assisted deep eutectic solvent-based microextraction, [e]Deep eutectic solvent-based liquid-phase microextraction, [f]Automated homogeneous liquid–liquid microextraction, [g]Homogeneous liquid–liquid microextraction

of acetonitrile was added to the vial. After centrifugation, the supernatant was separated and evaporated by a gentle nitrogen stream to one-third of the volume. Then, it was transferred to a 5 mL volumetric flask and made up to volume with 1.25 mL acetate buffer solution (pH = 5.5) and double-distilled water and analyzed using the optimized UA-SEME method with the standard addition technique. The obtained results are demonstrated in Table 2.

Comparison with other methods

The analytical efficiency of the suggested UA-SEME procedure was compared with a number of previously reported LLE and LPME techniques for the determination of CF and TP and summary results are presented in Table 3. It was demonstrated that the proposed UA-SEME is better or comparable to most of the tabulated techniques (Table 3) by considering the LOD, linear range, or RSD.

Conclusion

An easy, efficient, and sensitive procedure on the basis of the UA-SEME technique combined with HPLC–UV was described for simultaneous determination of CF and TP. The proposed method is eco-friendly due to the absence of disperser solvent and low organic solvent consumption. The calibration curves and LOD of the procedure showed high levels of linearity and sensitivity to the analytes. The calibration ranges, LOD, and RSD values of the proposed method for the determination of the CF and TP are better than many reported techniques. The suggested procedure gave satisfactory results for extraction and quantitative determination of CF and TP in cocoa powder and plasma specimens. In conclusion, the suggested UA-SEME procedure is potentially applicable as an effective method for preconcentration and determination of CF and TP in real specimens such as food and biological media.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Ethics approval The authors declare that all experiments were followed according to the Damghan University ethical regulations.

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