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Evaluation of surfactant assisted pressurized liquid extraction for the determination of glycyrrhizin and ephedrine in medicinal plants

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Abstract

Surfactant assisted pressurized liquid extraction (PLE) with a laboratory made system was applied for the extraction of glycyrrhizin in *Radix glycyrrhizae*/liquorice and ephedrine in *Ephedra sinica*. The proposed system set-up for this current work was simpler as no heating and back pressure regulator was required. Extraction with surfactant assisted PLE was carried out dynamically at a flow of 1.5 mL min^{-1}, at room temperature, under an applied pressure of 10–20 bar with an extraction time of 45–50 min. The extraction efficiencies of the proposed method using surfactants such as sodium dodecyl sulfate (SDS) and Triton X-100 were compared with sonication using organic solvent for different batches of medicinal plants materials. For the determination of glycyrrhizin in *R. glycyrrhizae*, the extraction efficiencies of surfactant assisted PLE with SDS and Triton X-100 was observed to be comparable with sonication. The method precision was found to vary from 1.6 to 2.6% (R.S.D., $n = 6$) on different days. For ephedrine in *E. sinica*, surfactant assisted PLE with SDS was found to give higher extraction efficiencies compared to Triton X-100. The overall method precision for surfactant assisted PLE with SDS for ephedrine in *E. sinica* was found to vary from 1.5 to 4.1% (R.S.D., $n = 6$) on different days. The marker compounds present in the various medicinal plant extracts were determined by gradient elution HPLC. Our data showed the possibility of PLE at room temperature and the advantages of eliminating the use of organic solvents in the extraction process.

Keywords: Surfactant assisted pressurized liquid extraction; Medicinal plants; Ephedrine; Glycyrrhizin; High performance liquid chromatography; Extraction methods

1. Introduction

Radix glycyrrhizae/liquorices and *Ephedra sinica*/Herba Ephedrae are medicinal plants found in the Chinese Pharmacopoeia [1], World Health Organization (WHO) monographs [2,3] and others [4,5]. For *R. glycyrrhizae*, the major constituents are glycyrrhizin (glycyrrhizic acid, glycyrrhizinic acid) as shown in Fig. 1A). It was known to contain 2–9% of glycyrrhizin. The chemical substance, glycyrrhizin present in *R. glycyrrhizae* is responsible for its sweetness, which is 50 times that of surcose. Other flavonoid constituents present in *R. glycyrrhizae* include liquiritigenin and isoliquiritigenin [2,4]. For Herba Ephedrae, the major active principle is (−)-ephedrine (Fig. 1B), it is found in the concentrations of 40–90% of the total alkaloid fraction, accompanied by (+)-pseudoephedrine. Other trace alkaloids in the alkaloid complex include (−)-norephedrine, (+)-norpseudoephedrine, (−)-methylephedrine and (+)-methylpseudoephedrine. The total alkaloid content can exceed 2%, depending on the species [2,4].

Currently, the various approaches for the preparation of samples and determination of bioactive or marker compounds in botanical extracts and herbal preparations are discussed in a few review papers [6–9]. In the monograph stated in the pharmacopeias, methods of extraction such as sonication, heating under reflux, Soxhlet extraction and others are commonly used. However, such methods can be time consuming, require the use of large amount of organic solvent and may have lower extraction efficiencies. Thus, this will lead to higher cost involved during the extraction process. In the move to reduce or eliminate the use of organic solvent and improve the extraction processes,

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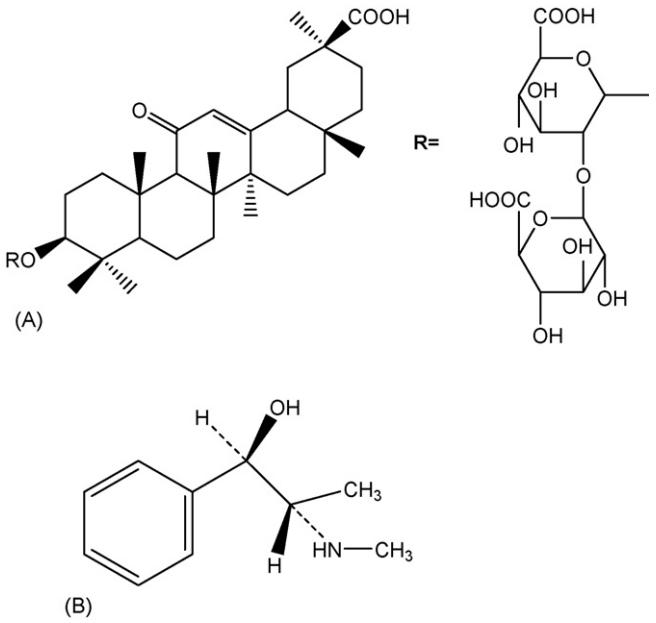

Fig. 1. Chemical structures of (A) glycyrrhizin present in *Radix glycyrrhizae* and (B) ephedrine present in *Ephedra sinica*.

pressurized hot water extraction (PHWE) extraction had been developed for the extraction of compounds present in medicinal plants [10].

Methods using a laboratory made PHWE system were applied for the extraction of thermally labile and non-polar to polar components in botanicals. These included berberine in *Coptidis rhizoma*, glycyrrhizin in *R. glycyrrhizae* (liquorice), baicalein in *Scutellariae radix*, hydrophobic marker compounds in *Radix codonopsis pilosula* and tanshinone I and IIA in *Salvia miltiorrhiza* [11–13]. Recently, studies have demonstrated the use of micellar media as an alternative to organic solvents for use as extractants of organic pollutants from liquid and solid environmental samples [14]. To improve the recoveries of marker compounds in *R. c. pilosula*, a method combining surfactants such as sodium dodecyl sulfate (SDS) or Triton X-100 and PHWE with an applied temperature at 95 °C was developed [12]. The feasibility of employing aqueous non-ionic surfactants solutions as an alternative solvent system in pressurized liquid extraction (PLE) was demonstrated by another research group using the root of American ginseng as model solid samples. When compared to ultrasonic extraction with an organic solvent, the presence of a common non-ionic surfactant (Triton X-100) in water at a concentration above its critical micelle concentration and higher applied temperature was shown to give comparable amount of pharmacological active compounds extracted from ginseng roots [15]. To our best knowledge, reports on the use of surfactant assisted PLE at room temperature with medicinal plant materials are rather limited.

The aim of the current work is to develop a simple method for the rapid determination of glycyrrhizin in *R. glycyrrhizae* and ephedrine in Herba Ephedrae using surfactant assisted PLE at room temperature. The instrumentation used was based on a laboratory made system. The extraction efficiencies of the proposed method using surfactants such as SDS and Triton X-100 were compared with sonication using organic solvent for different medicinal plants materials. The botanical extracts obtained were analyzed using reversed phase HPLC.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade. Glycyrrhizin, ephedrine hydrochloride and sand purified by acid (about 40–100 mesh) were purchased from Sigma (St. Louis, MO, USA). Methanol and ethanol were purchased from Hayman (Witham, Essex, England). Ultra pure water was obtained from Millipore Alpha-Q water system (Millipore, Bedford, MA, USA).

2.2. Preparation of references standards

Stock solutions of glycyrrhizin and ephedrine at 1000 mg L^{-1} were prepared in methanol. For all analysis, the working solutions of glycyrrhizin and ephedrine were prepared in the range of 0–100 mg L^{-1} in methanol. Linearity of glycyrrhizin and ephedrine were established between 0 and 100 mg L^{-1} with correlation coefficient $r^2 \geq 0.99$. For the quantatition of marker compounds in medicinal plants, a three-point calibration based on the linearity established was used. The system precision (R.S.D., $n = 6$) for glycyrrhizin and ephedrine were found to be less than 2.0% on different days.

2.3. Preparation of medicinal plants

To prepare a homogenous sample, different types of medicinal plants were ground using an IKA MF10 microfine grinder (Staufen, Germany) with sieve insert of hole size 0.5 mm. For pressurized liquid extraction, 0.1–0.5 g of the medicinal plant samples were weighed directly into a glass tube and mixed thoroughly with a high proportion of sand. The sand and plant material mixture were transferred into the extraction cell for pressurized liquid extraction as mentioned below. For all experiments, the sample powder was sandwiched between the sand. The extraction cells were finally filled with sand to avoid any voids.

2.4. Sonication

For glycyrrhizin, the procedures were adopted from our earlier reports [11,16]. Briefly, an accurately weighed 0.6 g of ground sample was extracted with 20 mL of methanol/water mixture (70:30) by sonication at room temperature for 10 min and centrifuged at 2000 rpm for 10 min. The procedures were repeated three times. The extracts were combined and transferred into a 100 mL volumetric flask. For ephedrine, the procedures were adopted from other reports [17–19]. Briefly, an accurately weighed 0.3 g of ground sample was extracted with 20 mL of methanol/water mixture (80:20) by sonication at room temperature for 10 min and centrifuged at 2000 rpm for 10 min. The procedures were repeated three times. The extracts were combined and transferred into a 100 mL volumetric flask.

2.5. Surfactant assisted pressurized liquid extraction (PLE) system

The instrumentation used was laboratory assembled. For surfactant assisted PLE, different amount of SDS (0.1–0.4%, w/v) or Triton X-100 (0.1–1.0%, v/v) in 1 L of water was used as the extraction liquid. The stainless steel tubings used were 1/16 in. o.d. and 0.18 mm i.d. The extraction cells were of stainless steel with 10 mm × 150 mm i.d. (approximately 10–11 mL). The pump used was an isocratic Shimadzu LC10 series pump (Kyoto, Japan) and the flow rate was set at 1.5 mL min^{-1}. The pressure in the system indicated by the HPLC pump was between 4 and 20 bar. The extraction cell was pre-filled with water to check for possible leakage before extraction. Extraction with water was carried out for a period of 40–50 min and 45–50 mL of liquid was collected in a 100 mL volumetric flask. In between runs, the system was washed with water for 5 min.

2.6. HPLC conditions

For all experiments, a Shimadzu LC 10 series HPLC system equipped with a binary gradient pump, autosampler, column oven and diode array detector was used. For the separation of glycyrrhizin, the gradient elution consists of mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The initial condition was set at 20% of B, gradient up to 100% B in 20 min before returning to initial condition for 10 min. For ephedrine, the gradient elution consists of mobile phase of (A) 5 mM SDS in water and (B) acetonitrile (ACN). The initial condition was set at 20% of B, gradient up to 100% B in 20 min before returning to initial condition for 10 min. Detection was at 254 nm for the analysis of glycyrrhizin and 210 nm for ephedrine. Oven temperature was set at 40 °C and flow rate was at 1.0 mL min^{-1}. For all experiments, 10 μL of standards and sample extracts were injected. The column used for separation was Hypersil Elite C18, 150 mm × 4.6 mm i.d., 5 μm (Runcorn, Cheshire, England).

3. Results and discussion

3.1. Optimization of surfactant assisted PLE for medicinal plants

The parameters that can affect the extraction efficiencies of PHWE included temperature, extraction time, addition of organic solvent or different amount of surfactants added [10–13]. For the extraction of bioactive and marker compounds in botanicals and medicinal plants, the temperature used in PHWE has a critical effect on the method accuracy and extraction efficiencies. Increasing the extraction temperature from 125 to 180 °C will result in higher recoveries for the extraction of certain compounds from plant materials. However, an increase in the extraction temperature to a certain point will cause the analytes present in the medicinal plants to degrade [10–13,15]. It has been observed that the geometry of the extraction cell or vessel and the flow direction of the water had only minor effect on the recoveries of the target analytes in the solid samples [20].

Surfactants are amphiphilic molecules, the head of which is polar or hydrophilic and the tail is hydrophobic. The tail is generally a hydrocarbon chain with different number of carbon atoms, may be linear or branched and contains aromatic rings. The surfactant molecules can associate in aqueous solution to form molecular aggregates called micelles. One of the most important properties of these organized structures is their good capacity to solubilize solutes of different character. This will allow sparingly soluble or non-water soluble to be solubilized in water because they tend to bind to the micelles in solution [14]. The improvement of subcritical water extraction with micelle formation had been applied for the removal of polycyclic aromatic hydrocarbons in soil and environmental samples. The environmental samples were subjected to static–dynamic extraction with SDS–water at a pressure of 50 bar and a temperature of 150–225 °C. Although SDS had been applied successfully in the extraction of non-polar components such as polyaromatic hydrocarbons in soil [21], non-ionic surfactant such as Triton X-100 gave higher extraction efficiencies for the hydrophobic components in *R. c. pilosula* [12]. Similarly, surfactant such as Triton X-100 added into the extraction cell prior to supercritical fluid extraction was reported to accelerate the quantitative extraction of cholesterol in solid food sample [22]. In ultrasonically assisted extraction of ginsenosides from American ginseng, the use of aqueous surfactant solution containing 10% Triton X-100 as the extraction fluid was found to result in faster extraction kinetics and higher recovery compared to methanol and water [15,23]. Using non-ionic surfactant such as Triton X-100 with PLE, it was observed that good extraction efficiencies were obtained when a lower applied temperature was used [12,15]. For the current work, the applied temperature was at room temperature, hence, parameters that required optimization include the different types of surfactant such as SDS and Triton X-100, amount of surfactant added into the extraction fluid and the extraction time. Water without the addition of any surfactants at room temperature was not used as it is observed to be too polar to efficiently dissolve most organics that are associated with botanicals [11,12].

The effects of two different types of surfactants such as SDS and Triton X-100 added into the extraction fluid were shown in Figs. 2 and 3, respectively. For the analysis of glycyrrhizin in *R. glycyrrhizae* (Fig. 2A and B), the amount of SDS and Triton X-100 added into the extraction fluid did not have a significant effect on the amount of glycyrrhizin extracted from the various batches of medicinal plants. At the same time, the amount of glycyrrhizin extracted was not affected by the two different types of surfactants used. For the determination of ephedrine in Herba Ephedrae (Fig. 3A), the amount of ephedrine extracted increased with the amount of SDS added in the extraction fluid. However, the amount of ephedrine extracted was not affected by the amount of Triton X-100 added, as shown in Fig. 3B. In this study, a higher concentration of SDS and Triton X-100 was not used as a gel like extract that was difficult for the system to handle was obtained. As reported in our earlier works, the variation of the data presented in Figs. 2 and 3 was due to the non-homogeneity of the medicinal plant samples used [11–13]. Based on the data obtained in Figs. 2 and 3, the amount of surfactants added into the extraction fluid (0.4%, w/v, of SDS and

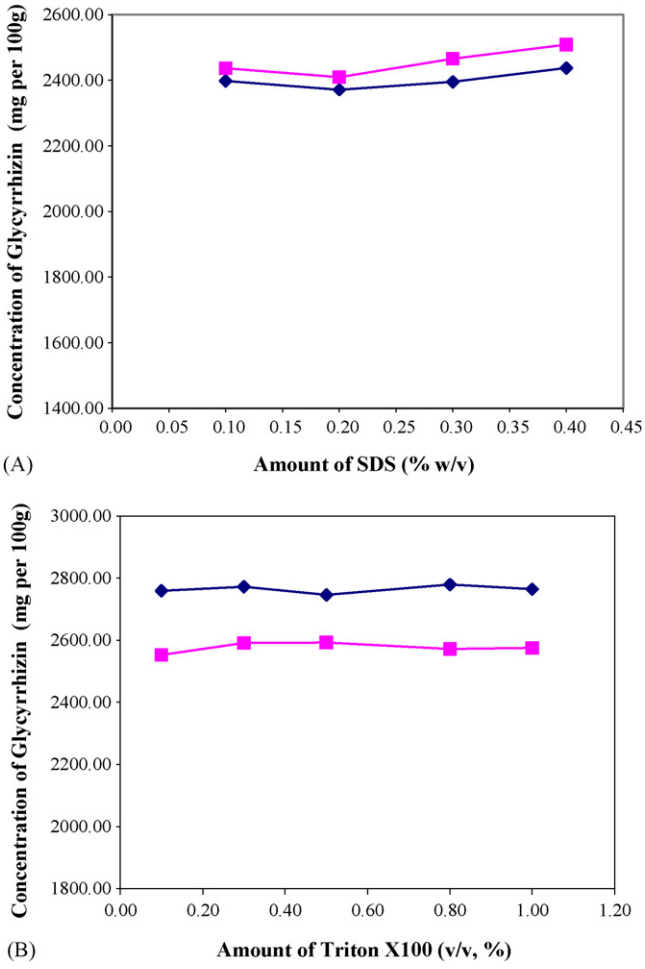

Fig. 2. (A) Effect of different amount of SDS in water on the extraction of glycyrrhizin by surfactant assisted PLE ($n = 3$, R.S.D., 1–3%) and (B) effect of different amount of Triton X-100 in water in the extraction of glycyrrhizin by surfactant assisted PLE ($n = 3$, 1–3%). The study was performed with two different batches of medicinal plant materials.

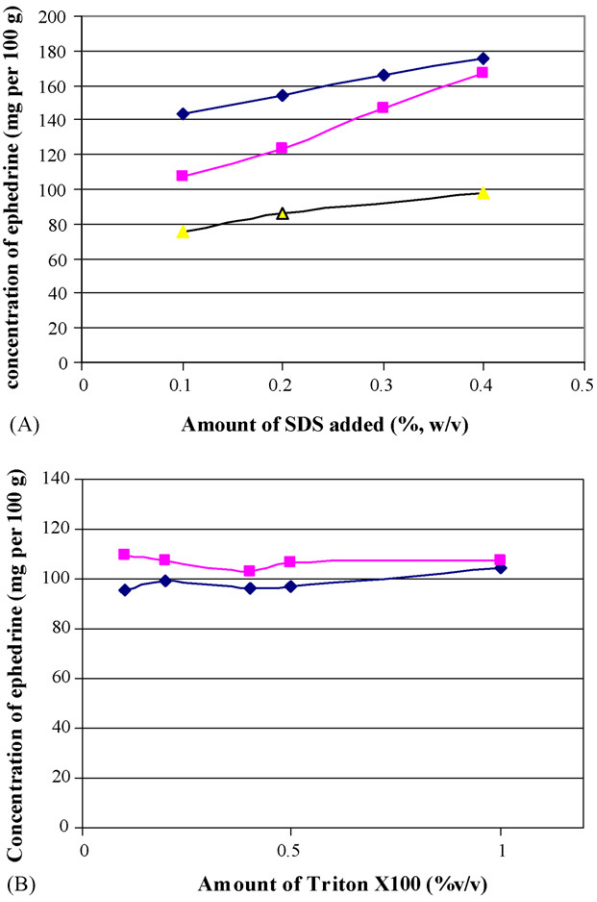

Fig. 3. (A) Effect of different amount of SDS in water on the extraction of ephedrine by surfactant assisted PLE ($n = 3$, 1–3%) and (B) effect of different amount of Triton X-100 in water on the extraction of ephedrine by surfactant assisted PLE ($n = 3$, 1–3%). The study was performed with two or three different batches of medicinal plant materials.

1.0%, v/v, of Triton X-100) were selected for further experiments.

The time for surfactant assisted PLE was set at 45–50 min. From Fig. 4A and B, it was found that a significant portion of the target analytes would be extracted within 45–50 min. Our data was consistent with other report where a significant portion of the target analytes would be extracted by PHWE within 40–60 min [24]. Hence, a model based solely on the thermodynamic distribution coefficient K_D, which assumes that analyte desorption from the matrix is rapid compared to elution is used to describe the extraction profiles obtained with hot water [24]. Lastly, the pressure was generated based on the packing materials in the extraction cell and was reported to have negligible effect on the extraction efficiency [10–13,15].

3.2. Applications of surfactant assisted PLE to medicinal plant samples

In the validation of analytical methods using PHWE, it is often required to compare the extraction efficiencies with reference methods such as Soxhlet extraction, sonication and others. For botanicals and herbal preparations, the bioactive or marker compounds are present naturally and significant analyte–matrix interaction will be present. Hence, spiking of the target compounds into the plant matrix will not mimic the real environment [11–13,16]. The high recovery obtained in the spiking experiments may not imply that the method has good accuracy.

Based on the results obtained from the optimization experiments, the performance of the proposed method was compared with sonication with organic solvent on the same day. It is important that all comparisons are done on the same day as loss of water and possible degradation may occur as a result of the storage conditions of the plant materials. Our earlier work had showed that the amount of glycyrrhizin in *R. glycyrrhizae* can vary from 6.9 to 8.1% (R.S.D., $n = 6$) [16]. At the same time, we have evaluated the homogeneity of plant samples used, the amount of glycyrrhizin present in *R. glycyrrhizae* can vary from 0.7 to 9.5% (R.S.D., $n = 5/6$). For the determination of glycyrrhizin in *R. glycyrrhizae*, taking into account the small sample size used and sample in-homogeneity, the extraction efficiencies of surfactant assisted PLE with SDS and Triton X-100 was observed to be comparable with sonication (Tables 1 and 2). The method

Table 1
Comparison of amount of glycyrrhizin extracted from different batches of in *Radix glycyrrhizae* using sonication with 70% aqueous methanol and surfactant assisted PLE with 0.4% (w/v) SDS/H_2O

	Sonication (mg g^{-1})	Surfactant assisted PLE with 0.4% (w/v) SDS/H_2O (mg g^{-1})
Glycyrrhizin in *R. glycyrrhizae* 1	24.1 ± 0.2 (R.S.D.: 1.1, $n=3$)	23.3 ± 0.4 (R.S.D.: 1.7, $n=6$)
Glycyrrhizin in *R. glycyrrhizae* 2	26.3 ± 0.3 (R.S.D.: 1.3, $n=3$)	25.0 ± 0.4 (R.S.D.: 1.7, $n=6$)
Glycyrrhizin in *R. glycyrrhizae* 2	32.7 ± 0.8 (R.S.D.: 2.4, $n=3$)	30.5 ± 0.8 (R.S.D.: 2.6, $n=6$)

Table 2
Comparison of amount of glycyrrhizin extracted from different batches of in *R. glycyrrhizae* using sonication with 70% aqueous methanol and surfactant assisted PLE with 1.0% (v/v) Triton X-100/H_2O

	Sonication (mg g^{-1})	Surfactant assisted PLE with 1.0% (v/v) Triton X-100/H_2O (mg g^{-1})
Glycyrrhizin in *R. glycyrrhizae* 1	26.2 ± 0.8 (R.S.D.: 0.3, $n=3$)	24.8 ± 0.1 (R.S.D.: 0.6, $n=6$)
Glycyrrhizin in *R. glycyrrhizae* 1	25.5 ± 0.1 (R.S.D.: 0.6, $n=3$)	24.1 ± 0.4 (R.S.D.: 1.6, $n=6$)
Glycyrrhizin in *R. glycyrrhizae* 2	28.5 ± 0.2 (R.S.D.: 0.5, $n=3$)	27.4 ± 0.4 (R.S.D.: 1.7, $n=5$)

Table 3
Comparison of amount of ephedrine extracted from different batches of *Ephedra sinica* using sonication with 80% aqueous methanol and surfactant assisted PLE with 0.4% (w/v) SDS/H_2O

	Sonication (mg 100 g^{-1})	Surfactant assisted PLE with 0.4% (w/v) SDS/H_2O (mg 100 g^{-1})
Ephedrine in *E. sinica* 1	104.8 ± 0.1 (R.S.D.: 0.1, $n=3$)	98.1 ± 3.3 (R.S.D.: 3.4, $n=6$)
Ephedrine in *E. sinica* 1	101.3 ± 0.9 (R.S.D.: 0.9, $n=3$)	96.4 ± 2.1 (R.S.D.: 2.2, $n=6$)
Ephedrine in *E. sinica* 2	173.3 ± 6.1 (R.S.D.: 3.5, $n=3$)	172.0 ± 7.1 (R.S.D.: 4.1, $n=6$)
Ephedrine in *E. sinica* 2	179.3 ± 4.3 (R.S.D.: 2.4, $n=3$)	183.0 ± 2.8 (R.S.D.: 1.5, $n=6$)
Ephedrine in *E. sinica* 2	183.2 ± 1.2 (R.S.D.: 0.7, $n=3$)	178.4 ± 3.5 (R.S.D.: 2.0, $n=6$)

Table 4
Comparison of amount of ephedrine extracted from different batches of *E. sinica* using sonication with 80% aqueous methanol and surfactant assisted PLE with 1.0% (v/v) Triton X-100/H_2O

	Sonication (mg 100 g^{-1})	Surfactant assisted PLE with 1.0% (v/v) Triton X-100/H_2O (mg 100 g^{-1})
Ephedrine in *E. sinica* 1	136.0 ± 13.2 (R.S.D.: 9.7, $n=3$)	123.1 ± 3.0 (R.S.D.: 2.4, $n=6$)
Ephedrine in *E. sinica* 1	113.3 ± 2.4 (R.S.D.: 2.1, $n=3$)	102.8 ± 2.2 (R.S.D.: 2.1, $n=6$)
Ephedrine in *E. sinica* 2	128.1 ± 1.0 (R.S.D.: 0.8, $n=3$)	113.3 ± 1.7 (R.S.D.: 1.50, $n=6$)
Ephedrine in *E. sinica* 2	132.4 ± 7.1 (R.S.D.: 5.4, $n=3$)	111.2 ± 1.8 (R.S.D.: 1.7, $n=6$)

precision was found to vary from 1.6 to 2.6% (R.S.D., $n=6$) on different days.

For ephedrine in *E. sinica*, surfactant assisted PLE with SDS was found to give higher extraction efficiencies compared to Triton X-100 (Tables 3 and 4). The overall method precision for surfactant assisted PLE with SDS for ephedrine in *E. sinica* was found to vary from 1.5 to 4.1% (R.S.D., $n=6$) on different days. The method recovery ranging from 93.6 to 102.0% was obtained for SDS and 84.0 to 90.7% was obtained for Triton X-100. For the extraction of ephedrine in *E. sinica*, compared to Triton X-100, the presence of anionic surfactant such as SDS in the extraction fluid further enhanced the solubility of the target analyte from the sample matrix into the mobile phase and resulted in higher extraction efficiencies.

For surfactant assisted PLE in a dynamic mode, the presence of surfactant such as SDS and Triton X-100 in the water enhanced the solubility of the target compounds and pushed the target analytes in the sample matrix in the mobile phase to completeness with the fresh liquid pumped through the sample continuously. In this model of extraction, a high dilution of the aqueous extract was produced as higher extractant volume was used. As the target compounds were naturally occurring in *R. glycyrrhizae* and *E. sinica*, strong analyte–matrix interaction will be present. Even at room temperature, the addition of surfactants in the extractant liquid was proposed to disrupt the strong analyte–matrix interaction present naturally in the plant materials and improve the extraction efficiencies.

With the additional step of dispensing the plant samples with sand for surfactant assisted PLE and account of sample non-homogeneity, good method precision was observed for the plant materials studied. Representative chromatograms of surfactant assisted PLE and sonication with organic solvent for

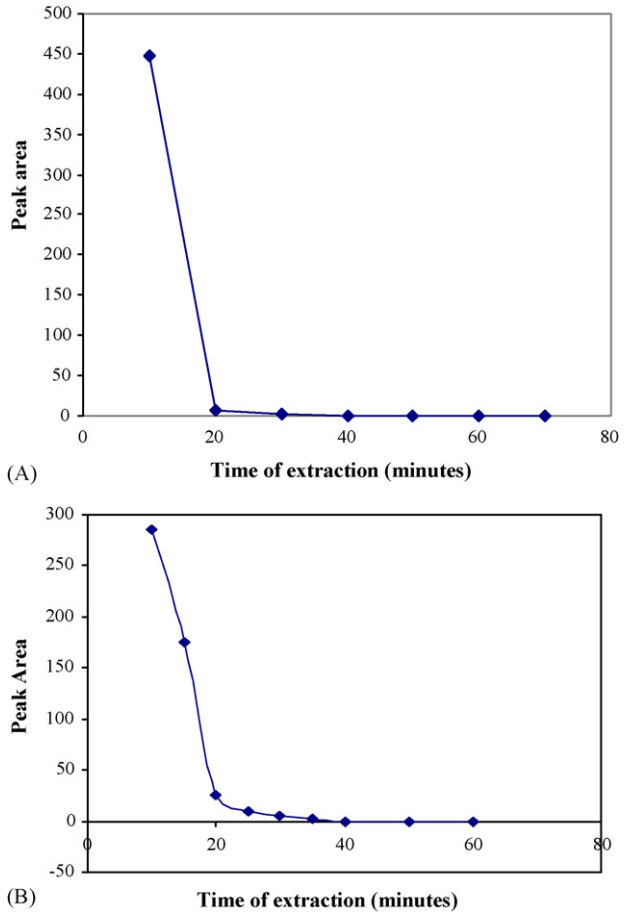

Fig. 4. Extraction profiles of surfactant assisted PLE (0.4%, w/v, of SDS in water) for the extraction of (A) glycyrrhizin in *R. glycyrrhizae* and (B) ephedrine in *E. sinica*. A significant portion of the target analytes would be extracted within 30–50 min.

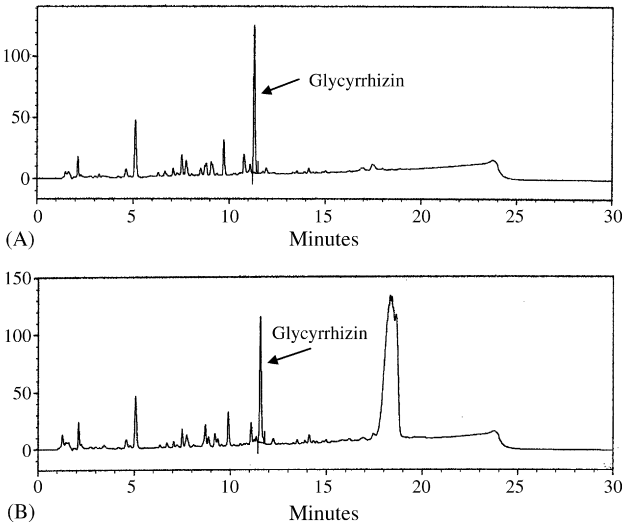

Fig. 5. Chromatogram obtained for (A) glycyrrhizin in Licorice by surfactant assisted PLE with 0.4% SDS/water, (B) glycyrrhizin in Licorice by surfactant assisted PLE with 1.0% Triton X/water. HPLC condition: 0.1% formic acid in water (pump A) and 0.1% formic acid in ACN (pump B) as mobile phase. At initial condition, gradient of pump B is set at 20% and increases to 100% in 20 min and then return to initial condition for 10 min. Detection was at 254 nm. Oven temperature was at 40 °C and flow rate was set at 1.0 mL min^{-1}.

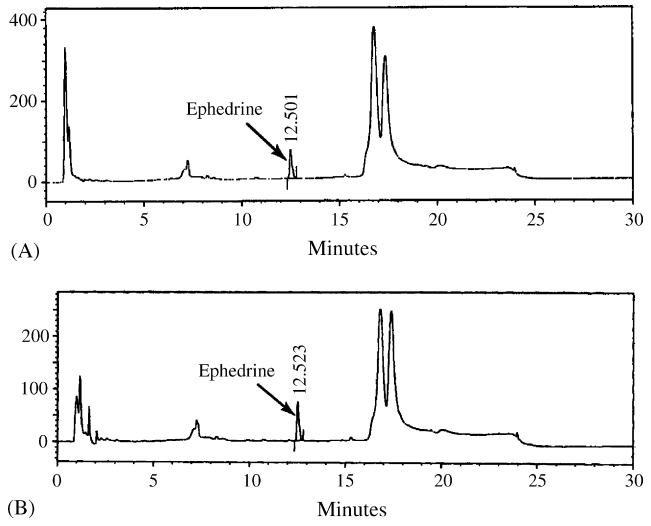

Fig. 6. Chromatogram obtained for (A) ephedrine in *E. sinica* by surfactant assisted PLE with 0.4% SDS/water, (B) ephedrine in *E. sinica* by sonication with 80% methanol. HPLC condition: 5 mM SDS in water (pump A) and ACN (pump B) as mobile phase. At initial condition, gradient of pump B is set at 20% and increases to 100% in 20 min and then return to initial condition for 10 min. Detection was at 210 nm. Oven temperature was at 40 °C and flow-rate was set at 1.0 mL min^{-1}.

glycyrrhizin in *R. glycyrrhizae* and ephedrine in *E. sinica* were shown in Figs. 5 and 6, respectively. The presence of glycyrrhizin and ephedrine in the botanical extracts were confirmed by comparison of the retention time and UV spectra with the standard compounds.

4. Conclusions

The current study showed that glycyrrhizin and ephedrine present in medicinal plants could be extracted using surfactant assisted PLE at room temperature. Compared to other reports, the proposed system set-up for surfactant assisted PLE in this work was simpler as no heating and back pressure regulator was required. With the additional step of dispensing the plant samples with sand for PLE and account of sample non-homogeneity, good method precision and accuracy were observed for the plant materials studied. In brief, surfactant assisted PLE with SDS and Triton X-100 proved to be at least equivalent or better compared to sonication with organic solvent in terms of quantitative analysis of marker compounds in *R. glycyrrhizae* and *E. sinica*. The proposed method showed that it was possible to eliminate the use of organic solvents in the extraction process. It will provide the increasingly needed alternative approaches for assuring the quality of botanicals and herbal preparations and propel the move towards evidenced based medicine.

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