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ORIGINAL PAPER

A fast and green preconcentration method based on surfactant ion pair-switchable solvent dispersive liquid–liquid microextraction for determination of phenazopyridine in pharmaceutical and biological samples

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Abstract
Herein, a novel, fast, green and sensitive surfactant ion pair-switchable solvent dispersive liquid–liquid microextraction (SIP-SS-DLLME) method was developed for the preconcentration of phenazopyridine. Protonated triethylamine bicarbonate is synthesized by the reaction of triethylamine and CO_2 in the presence of water. This protonated switchable solvent (soluble in water) easily converted to triethylamine which is insoluble in water. Aliquat 336 was used as an ion-pair agent in this method, which results in the increase of the phenazopyridine extraction into the switchable solvent. Variables affecting the performance of extraction were studied and optimized. The relative standard deviation (RSD) was 3.1% for five repeated determinations containing 20 µg/L of phenazopyridine. The linear range of the method for microextraction and determination of phenazopyridine was found to be 5–180 µg/L with a detection limit of 0.88 µg/L. The presented method was applied successfully for the determination of phenazopyridine in pharmaceutical and biological samples.

Keywords Phenazopyridine · Aliquat 336 · Surfactant ion pair-switchable solvent dispersive liquid–liquid microextraction · Pharmaceutical and biological samples

Introduction

Drugs are biologically active organic compounds with various chemical structures. Phenazopyridine hydrochloride with the structure of azo dye, is widely used as an analgesic and for the treatment of urinary tract infections [1]. In patients with kidney disease, overdose of this drug causes kidney failure [2]. Although, azo dyes are known to be associated with cancer and evidence has been shown in animal model, till date, human cancer has not been reported in relation to the use of phenazopyridine [3]. For this reason, the determination of phenazopyridine is of great interest to researchers.

Reports have shown that several analytical techniques such as UV–visible spectrophotometry [4], electrochemical methods [3, 5] and chromatographic methods [6, 7] have been used for the determination of phenazopyridine drug in pharmaceutical and biological samples. However, chromatographic technique suffers some drawbacks such as complexity of the instrumental and time-consuming process, high cost and high consumption of organic solvents [8]. Therefore, spectrophotometric techniques were considered. UV–Vis spectrophotometry is an easy, available, cheap, and very attractive technique for determination of phenazopyridine, which the United States Pharmacopeia has recommended [9]. Due to trace amounts of Phenazopyridine in biological samples, and the possibility of interference caused by sample matrix, a separation step is indispensable, especially if a spectrometric technique is employed.This technique can be easily applied after the preconcentration/extraction step, with new microextraction methods such as, dispersive liquid–liquid microextraction (DLLME), cold-induced aggregation microextraction, static liquid-phase microextraction and cloud point microextraction [10, 11]. Among the above, the DLLME technique has been of great interest to researchers because of its simplicity, fast operation and low cost. This procedure is based on the addition

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of a microliter solvent in the presence of a dispersant agent [12]. The introduction of surfactants/dispersant agents in the DLLME can reduce consumption of organic solvent, increase the interaction of analyte-extraction phase and increase the stability of solvent [11, 13].

Recently, there has been a great interest in the use of green solvents in extraction technique. Many green solvents are found to be properly used as extraction phase because they frequently exhibit remarkably improved properties when compared with the conventional organic solvents [11]. The intriguing properties of green solvents, including good extraction efficiency for pesticides, drug and heavy metal ions, excellent chemical and thermal stability, adjustable viscosity and eco-friendly, have expanded its applications as an extraction phase for DLLME [14, 15].

Switchable solvent (SS), a kind of green and smart solvent, is used in catalytic processes, purification, extraction and separation of chemicals [16]. The feature of this solvent is based on the polarity conversion behavior by carbon dioxide. CO_2 reacts with water–amine mixture (e.g., water–triethylamine) to form an ammonium carbonate (e.g., protonated triethylamine bicarbonate), which is soluble in water [17]. Furthermore, it is cheap, readily available, safe and quickly removed by purging with inert gas or addition of NaOH. The most important properties of switchable solvent include simple and rapid separation, safe and eco-friendly [18]. During the last few years, there was an increased interest in switchable solvents such as extraction reagent in the analytical separation for the preconcentration and extraction of metal complexes and pesticides [18–20].

Despite the considerable features of this solvent, no studies have been conducted on extraction in the presence of cationic surfactant. Due to the widespread use of this new and green solvent in the microextraction of various analytes, Aliquat 336 is considered as a carrier agent [21]. Aliquat 336 as an ionic surfactant, has been used extensively as phase transfer catalyst [22] and extraction phase of separation [23] and modifier of adsorbents [21]. As an ion-pair or carrier agent in DLLME, it has been proven to possess great ability for the separation/preconcentration of many kinds of heavy metals and organic pollutants.[24, 25] The combination of Aliquat 336 ionic liquid and switchable solvent will have the combined advantages of ionic surfactants and switchable solvents. Aliquat 336 as an excellent cationic surfactant is a significant carrier agent in the extraction and preparation of green sorbents [26]. In the reaction medium, it leads to stability by decreasing volatility and increasing viscosity and hydrophobicity.

Research on the use of switchable solvents as extraction phase in separation is still few, it will be of great value to develop the application of switchable solvents so as to increase the ability of extraction, minimize consumption of organic solvents and shorten extraction time [27]. From the literature, there is no paper on the application of surfactant ion pair-switchable solvent dispersive liquid–liquid microextraction (SIP-A-SS-DLLME) in phenazopyridine analysis by UV–Vis spectrophotometry. Following this technique, Aliquat 336 (as surfactant ion pair) was dispersed in aqueous solution containing the sample and switchable solvent (protonated triethylamine bicarbonate) with the assistance of a vortex mixer. Then, protonated triethylamine bicarbonate was easily changed into triethylamine (insoluble in water) by adding NaOH.

In this paper, a study on the coapplication of ionic surfactant and switchable solvent in the present vortex agent was presented and their capabilities in the preconcentration of phenazopyridine was explored. It is shown that the sensitivity increased due to the combination of ionic surfactant and switchable solvent. The linear range, limit of detection and relative standard deviation (RSD) were investigated. The study results showed that the presented method could be used to determine phenazopyridine in pharmaceutical and biological samples.

Experimental

Instruments

Cintra 101 UV–Vis spectrophotometer with glass cell was used for absorbance measurements (GBC Scientific Equipment, Australia). Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were recorded on a Thermo Nicolet IR 100 spectrometer with a diamond ATR crystal, (USA), (Germany). A Benchtop pH/mV Meter-860,031 pH meter (India) was utilized for pH measurements.

Materials and chemicals

All materials used in this study are of analytical purity. Phenazopyridine hydrochloride was obtained from the Iranian Pharmaceutical Company, Shahredaru (Tehran, Iran). Triethylamine, NaOH and HCl were purchased from Merck (Darmstadt, Germany). Tricaprylylmethylammonium chloride (Aliquat 336) was purchased from Sigma-Aldrich Company (Darmstadt, Germany). To prepare Aliquat 336 (3%w/v) solution, 3 g of Aliquat 336 was added to ethanol and diluted to mark in a 10 mL volumetric flask. Using hydrochloric acid and sodium hydroxide solutions, desired pH was obtained.

The synthesis of hydrophilic form of switchable solvent

In the preparation of switchable solvent, exposure to a 1:1 mixture of the two immiscible liquids, namely triethylamine

(TEA) and water, to gaseous CO_2 at ambient temperature and pressure caused the alteration of the two-phase liquids mixture, resulting in a single-phase liquid, which is monophasic and soluble in water. About 20 g of dry ice ($CO_{2(s)}$) was gradually added into a beaker containing 200 mL of ultrapure water and 200 mL trimethylamine (TEA) at room temperature under magnetic stirring. The solution was stirred at room temperature for 2 h to ensure that the triethylamine bicarbonate (p-TEA-CO_3, as switchable solvent) product was obtained.

Real samples preparation

Tablet samples were obtained from Shahre Daru Pharmaceutical Company (Tehran, Iran). Five tablets of phenazopyridine were powdered accurately and weighed, and equivalent of 25 mg was dissolved in water (under ultrasonic radiation for 5 min) and transferred into a 250 mL calibrated flask and made up to volume with distilled water. A suitable volume of this sample solution was diluted further with water so that the concentration of phenazopyridine in the final solution is within the linear range.

Urine sample (drug-free) of a healthy volunteer was placed in a polypropylene centrifuge tube and stored before analysis in the freezer. The spiked urine sample with phenazopyridine was prepared by adding the specified volume of a standard solution of phenazopyridine to a 10 mL calibration flask and then diluted to the mark with urine. This urine sample was centrifuged at 5000 rpm for 10 min and in the following, supernatant was transferred to a clean tube. Finally, the supernatant was filtered (with 0.45 µm filter) and diluted with distilled water (to reduce matrix effects).

Drug-free plasma (blank) was provided by the Blood Transfusion Organization of Khuzestan and stored before analysis in the freezer. The spiked plasma sample with phenazopyridine was prepared by adding the appropriate mixed standard solution of phenazopyridine with drug-free plasma. One milliliter of plasma was transferred into a centrifuge tube containing 0.5 mL zinc sulfate (0.7 M) and 0.1 mL 1 M sodium hydroxide solution. Then, by centrifugation (5000 rpm at 4 min), the supernatant was separated from the precipitate protein. The solution obtained was transferred into a 10 mL calibrated flask and then diluted to mark with distilled water [28].

Recommended procedure

An aliquot 400 µL of Aliquat 336 (3%w/v) was transferred into a 15 mL conical-bottom glass centrifuge tube containing 10 mL solution of phenazopyridine and 750 µL p-TEA-CO_3 solvent (as switchable solvent) at pH 11, was prepared. Then, 2 mL of NaOH (10 M) was added to the mixture. The resulting solution was vigorously shaken with a vortex mixer for 1 min at 2800 rpm. Separation of extraction phase did not need centrifugation. Finally, a volume of 200 µL of supernatant was transferred into a glass cell to measure the increase in the absorbance at 416 nm against a blank solution which was prepared with the same extraction technique except that distilled water was used instead of phenazopyridine.

Results and discussion

SIP-SS-DLLME for extraction of phenazopyridine

The obtained protonated triethylamine bicarbonate (p-TEA-CO_3) was characterized by ATR-FTIR(to study the functional groups) and used in the following experiments [16] (shown in Fig. 1). The FTIR spectra of the protonated triethylammonium carbonate (P-TEA-C) to triethylamine (TEA) solvents obtained by Attenuated Total Reflection (ATR)-FT-IR spectroscopy are shown in Fig. 1b. The three bands achieved at 2967, 2937, and 2873 cm can be attributed to the stretching vibrations of CH2-symmetric and CH2-asymmetric stretch modes. The absorption band at 3438 cm signifies the stretching vibration of C–N bond in the FT-IR spectra of TEA. The FTIR spectra of P-TEA-C show several new spectral bands, such as those of N–H stretching and bending vibrations and C–N stretching vibration originally occurring at 1540, 1451, 1300, 1166, and 894 cm, respectively.

In this study, SIP-SS-DLLME combined with UV–Visible spectrophotometry was used for the analysis of phenazopyridine in pharmaceutical and biological samples. The interaction mechanism (Fig. 2) of the presented technique can be expressed by three types of analyte transfer routes into the switchable solvent during extraction process: (1) hydrophobic interaction between the hydrophobic nonpolar regions of phenazopyridine, Aliquat 336 and trimethylamine (switchable solvent); (2) π-cation interactions between the aromatic rings of phenazopyridine and the quaternary nitrogen of Aliquat 336; (3) hydrogen bonds between the amine groups of phenazopyridine and the amine group of switchable solvent.

When Aliquat 336 (ionic surfactant) was added to the aqueous solution containing analyte and switchable solvent, it was suspended on the sample surface due to its immiscibility with the aqueous solution, which is dispersed by vortex mixer. Vortex mixer caused the fast creation of tiny droplets of the Aliquat 336 in the aqueous solution containing sample and switchable solvent (ionic form), and the collision surface between ionic liquid, switchable solvent and phenazopyridine was enlarged. The presence of Aliquat 336 in the extraction phase, switchable solvent, has several advantages: increase the stability of the extraction phase and decrease the volatility of the switchable solvent. As an extraction phase, together with the alternating solvent, there is a cooperative

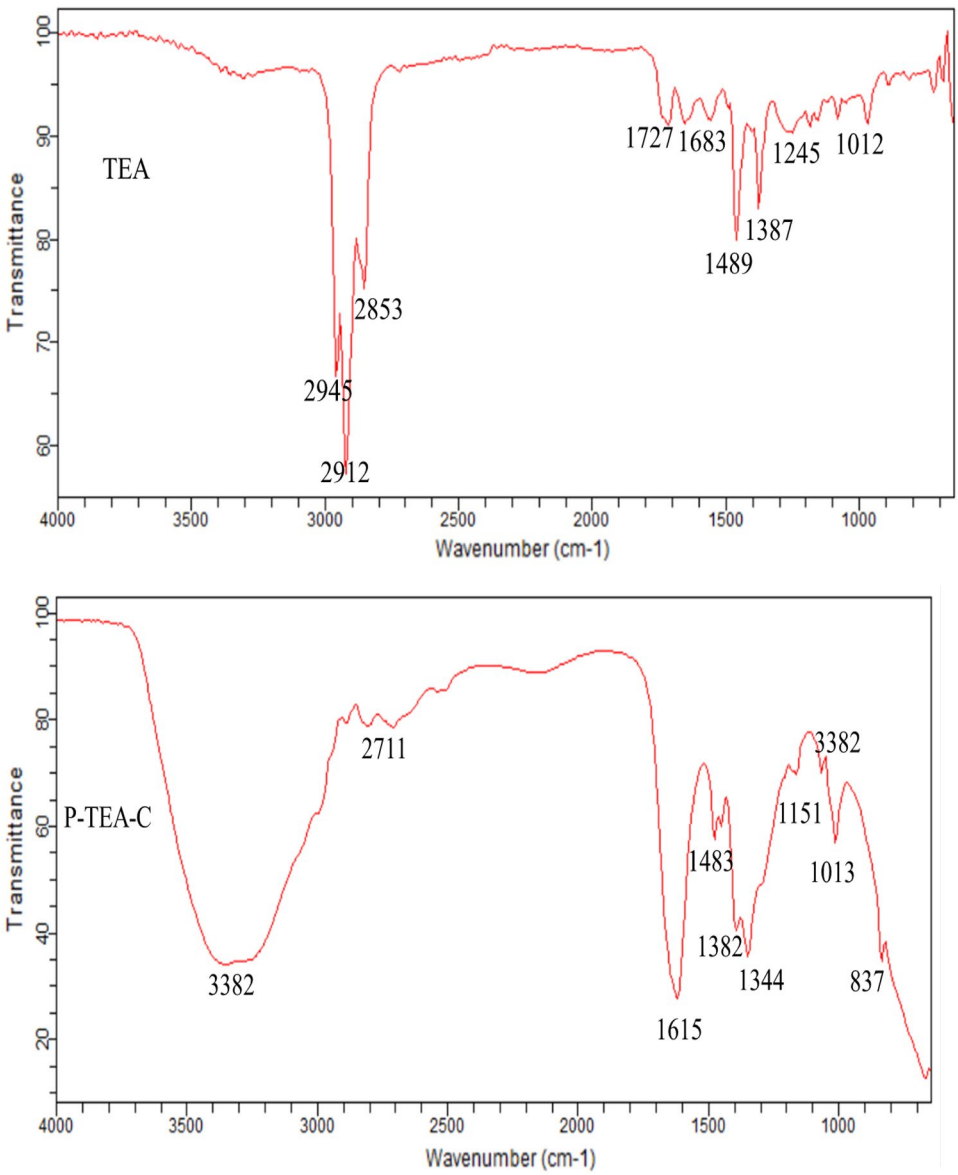

Fig. 1 ATR-FT-IR spectra of trimethylamine (TEA) and protonated triethylammonium carbonate (p-TEA-CO3)

effect that, with increase in preconcentration, the sensitivity of the measurement increases.

In this work, the adsorption peak of phenazopyridine at 416 nm was obtained and the difference in peak absorption relative to the blank was used as an analytical signal. The experimental parameters, including pH value of the solution, the amount of Aliquat 336 and the volume of extraction were studied in detail. The effects of optimization parameters were evaluated using one variable at a time method.

The optimization of pH value of solution

The pH of the sample solution was introduced as the main parameter in the extraction phase-analyte interaction. To determine the most suitable pH of the sample solution, extraction was evaluated in the pH range of 5–11 (Fig. 3). It was found that the extraction efficiency increased with increase in the pH of the sample solution up to 9, and the higher pH had neither enhancement nor negative effect on the extraction. As phenazopyridine is a weak base, it could be extracted well at relatively high pH, because the molecule cannot be protonated under basic conditions (pka of phenazopyridine is 5.10). Accordingly, with a decrease in pH of the sample solution, the electrostatic repulsion between the Aliquat 336 and the protonated phenazopyridine occurred, which led to decrease in the extraction efficiency. Therefore, pH 9 was used for further experiments.

Effect of the Aliquat 336

The addition of Aliquat 336 as a cationic surfactant along with a switchable solvent into the sample solution was the

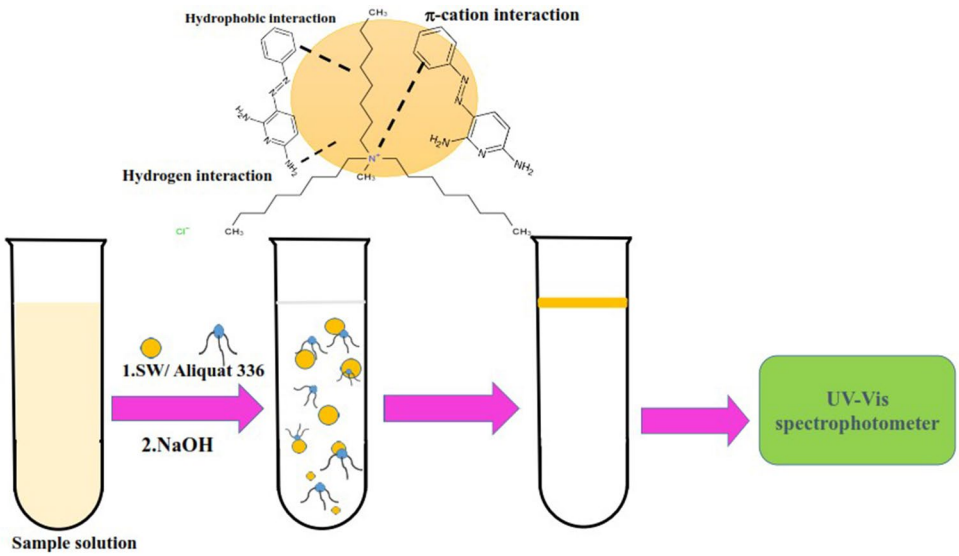

Fig. 2 Schematic of the surfactant ion pair-switchable solvent dispersive liquid–liquid microextraction for determination for phenazopyridine in pharmaceutical and biological samples

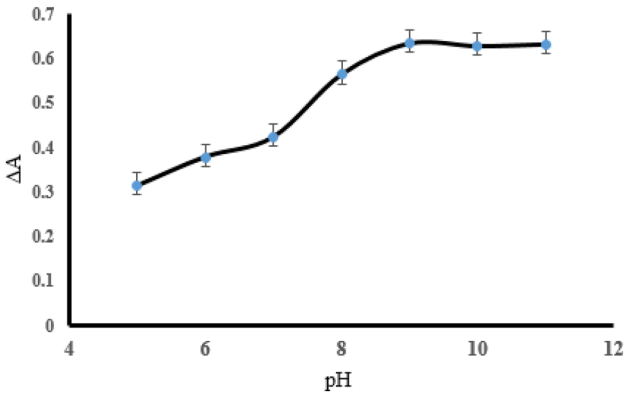

Fig. 3 The effect of the pH valve of sample solution on absorption

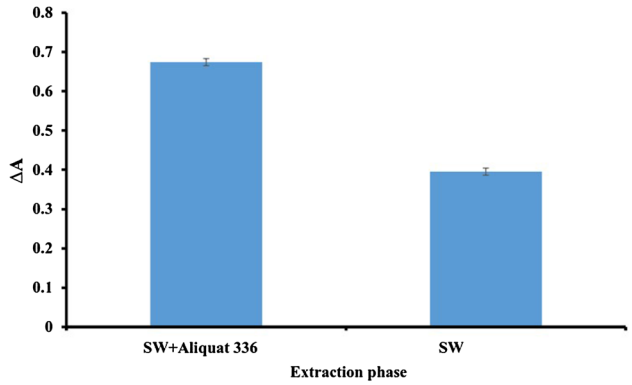

Fig. 4 The effect of the extraction phase used for the SIP-SS-DLLME of phenazopyridine: switchable solvent (750 µL) + Aliquat 336 (400 µL of 3% w/v) and switchable solvent (750 µL)

significant benefit of this study: the ions of Aliquat 336 and triethylamine (switchable solvent) are hydrophobic, which lead to π-cation interaction with phenazopyridine and enhanced extraction of the switchable solvent. The effects of Aliquat 336 on the switchable solvent in the extraction phase were investigated under similar experimental conditions (Fig. 4).

Different volumes of Aliquat 336 (as ionic surfactant) (3% w/v) in a range of 100–500 µL were used to extract phenazopyridine from the sample solution. The results indicated (Fig. 5) that 400 µL of Aliquat 336 (3% w/v) was enough for the extraction, and further increase in the volume of Aliquat 336 reduced the extraction percentage of phenazopyridine, which is probably due to increase in the dilation of the extracted analyte. Therefore, 400 µL was selected as the volume of the Aliquat 336.

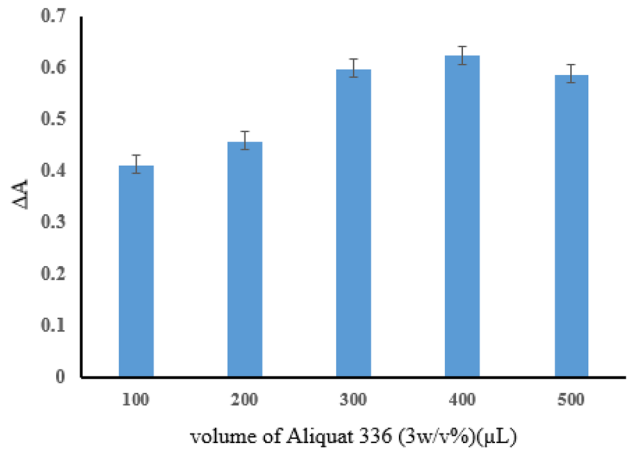

Fig. 5 The amount of Aliquat 336 (3% w/v) used for the SIP-SS-DLLME

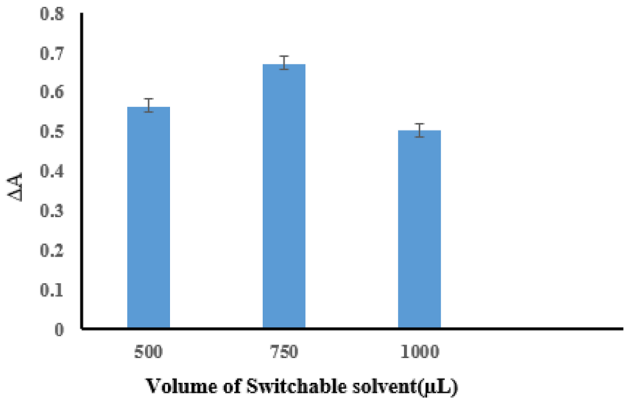

Fig. 6 Effect of volume of ionic-state of switchable solvent in SIP-SS-DLLME method

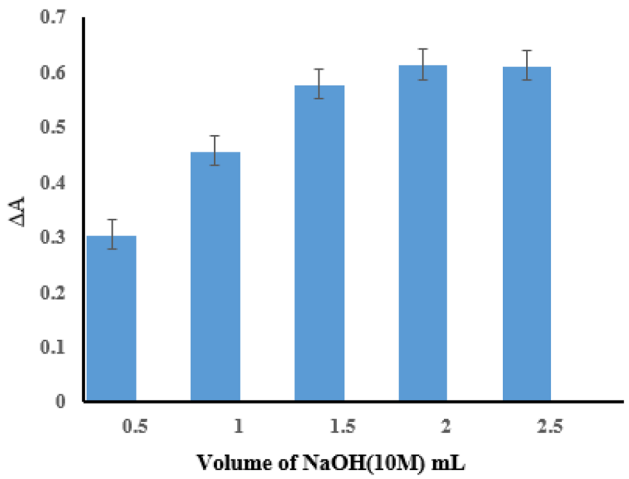

Fig. 7 Effect of NaOH (10 M) in SIP-SS-DLLME method

Effect of the extraction volume

In this extraction method, 10 M sodium hydroxide was added to a solution containing the sample, p-TEA-CO3 and Aliquat 336 as ionic liquid to form the extraction phase. In this study, 10 mL sample solution of phenazopyridine was used. The volume of the switchable solvent is a very important parameter in the microextraction. For this reason, the effect of the volume of protonated solvent (500, 750 and 1000 µL) was studied. It was found that extraction efficiency increased with increase in the concentration of solvent (protonated) up to 750 µL, and decreased when higher volumes were used (Fig. 6). It should be noted that in the conversion of the protonated triethylamine bicarbonate (water-soluble) to triethylamine (water insoluble), the volume of solvent (non-protonated) obtained as the extraction phase was half of the volume of solvent (protonated) added to the sample solution.

The volume of NaOH (10 M) (as trigger) in the range of 0.5–2.5 mL was studied. Based on the results, the suitable volume of NaOH required for phase separation was 2 mL. Complete reaction of non-ionic solvent formation (TEA) was attained after adding 2 mL of phenazopyridine drug (Fig. 7).

Analytical figures of merit

Under optimal conditions, the presented method was investigated for linearity, limit of detection and relative standard deviation. The method showed linearity over the calibration range of 5–180 µg/L. Table 1 shows the results of the statistical analysis of the experimental data. Detection limit (LOD) for phenazopyridine was calculated as follows: $\mathrm{LOD} = 3S_b/m$, where S_b is the standard deviation of intercept and m is the slope. The mean of the absorbance of five isolate samples solution extract of the studied phenazopyridine had a relative standard deviation of 3.1%. The inter-day precision was investigated through replicate analysis of standard solution of 50 µg/L phenazopyridine on three successive days. Intra-day and inter-day precisions equal to 2.3 and 3.9%, respectively, were obtained.

Table 1 Analytical figures of merit for the SIP-SS-DLLME method for determination of phenazopyridine

Linear range (µg/L)	R^2	LOD (µg/L)		RSD (%) ($n=3$)
		Intraday	Interday	
5–180 ($y=0.007x+0.0971$)	0.9981	0.88	2.3	3.9

Table 2 Tolerance Limits of species in presence of 100 µg/L phenazopyridine

Added species	Tolerance limit (mg/L)
Na^+, K^+, Cl^-, SO_4^{2-}, NO_3^-	1000
Mg^{2+}, Zn^{2+}	500
Ca^{2+}, Fe^{2+}	50
Fe^{3+}	25

Interference studies

To achieve the analytical uses of the introduced method, the effects of some probable species in the actual sample on the extraction results were investigated. For this purpose, 10 ml containing 100 µg/L of phenazopyridine and the probable species studied with a specific concentration were used. Extraction of phenazopyridine analyte using the highest concentration of possible interferent species with 5% error was investigated. Results of the effect of probable interferent species on extraction of phenazopyridine are shown in Table 2.

Table 3 Analysis of the pharmaceutical and biological samples

Sample	Added (µg/L)	Founded ($n=3$; µg/L)	Recovery%
Tablet	20	20.3 ± 0.7	101.6
	50	50.9 ± 1.9	101.8
Urine	–	N.D*	–
	30	30.1 ± 1.2	100.3
	70	71.2 ± 2.84	101.7
Plasma	–	N.D	–
	30	30.8 ± 1.3	102.8
	70	69.2 ± 3.3	98.8

*Not detected

Application for the analysis of actual samples

Applicability of this extraction method to actual samples was evaluated by separation/preconcentration and analysis of phenazopyridine in the tablet, plasma and urine samples. The results are given in Table 3. The recoveries are 98.8–102.8% which shows that there is no serious interference in the extraction and determination of phenazopyridine in the tablet, plasma and urine samples.

Comparison of the SIP-SS-DLLME and other methods

The extraction and determination methods of phenazopyridine in the actual samples are compared in Table 3. In the investigation of the methods, the spectrophotometric method was simple, fast and available as compared to the chromatographic methods that are expensive, complicated and use organic solvents. The method presented in this study is a green, simple and fast methodology in which extraction was done without specific tools and with minimal consumption of chemicals. This extraction method has good sensitivity to the determination of phenazopyridine in pharmaceutical and biological samples (Table 4).

Table 4 comparison of the presented method(SIP-SS-DLLME) with the other extraction methods for determination of phenazopyridine

Method	LOD[e] (µg/L)	Linear range (µg/L)	References
HF-LLLME-FIA[a]	0.5	5–200	[29]
LLE-LC/UV[b]	10	50–10,000	[30]
EME-HPLC-UV[c]	0.5-1	0.5–1000,1–1000	[6]
CCT-CPE[d]-spectrophotometric	500	500–25000	[4]
SIP-SS-DLLME	0.88	5–180	This method

[a]Hollow fiber-based liquid–liquid–liquid microextraction followed by flow injection

[b]Liquid–liquid extraction-liquid chromatography-UV

[c]Electromembrane extraction-HPLC-UV

[d]Cold column trapping-cloud point extraction

[e]Limit of detection

Conclusions

A novel green, simple, rapid and sensitive preconcentration method (SIP-SS-DLLME) was developed based on the cooperative effect of ionic surfactant and switchable solvent on the UV–Vis spectrophotometry of phenazopyridine in pharmaceutical and biological samples. Separation/preconcentration through the polarity conversion of P-TEA-BC/TEA solvent was introduced as a safe and green extraction method without the need for a dispersive solvent or special equipment. The switchable solvent release in a water-insoluble form at the extraction site resulted in the surfactant dispersion and formation of a cloudy solution in the samples associated with the rapid accumulation of the extraction phase. It clearly showed that the presence of Aliquat 336 as an ionic surfactant besides switchable hydrophilic solvent in the extraction phase provides an excellent method to improve the preconcentration performance. Aliquat 336 as a cationic surfactant in the switchable solvent medium led to stability by decreasing volatility and increasing viscosity and hydrophobicity. The proposed approach had the significant advantages of ease of operation using green solvents without the need for a specific device, enhanced stability and capability of the extraction phase, and reduced extraction time compared to any other sensitive methods, such as cloud point extraction (CPE) and electro-membrane extraction (EME).

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