Extraction and preconcentration of parabens in liquid pharmaceutical samples by Dispersive Liquid-Liquid Microextraction based on deep eutectic solvent

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Research Article

Keywords: Paraben, deep eutectic solvent, dispersive liquid liquid microextraction, pharmaceutical samples, high-performance liquid chromatography

Posted Date: February 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1337548/v1

Abstract

In this paper dispersive liquid-liquid microextraction using deep eutectic solvent, as one of extraction solvent was considered for preconcentration and determination of parabens in liquid pharmaceutical samples. Deep eutectic solvent (DES) composed of choline chloride (ChCl) hydrogen bond acceptor) and hydrogen bond donor (glucose) was provided the highest extraction efficiency. Thermogravimetry analysis (TGA), X-ray diffraction and Fourier-transform infrared spectroscopy (FT-IR) were selected to prove that the synthesis of DES was done successfully. The Liquid chromatography with photodiode array detection (HPLC-DAD) was used for analysis of paraben species. Parameters affecting the extraction efficiency were studied and optimized through univariate analysis and experimental design. Under the optimal conditions (pH of aqueous solution: 4.5, ethanol as the disperser solvent, glucose DES as the extraction solvent) the linearity range of 0.1-5000 ng mL^{-1} was obtained with the coefficient of determination (R^2) ranging in 0.993-0.9962. Limits of detection (LOD) ranged from 0.04 – 0.15 ng mL^{-1} with the relative standard deviations from 1.78–6.85%. The developed method was applied to determination of parabens in liquid pharmaceuticals such as ampule, syrups and nose drop samples. The relative recoveries in these real samples were in the range of 80.95-103.12%.

1. Introduction

Like other products, such as food, detergents, cosmetics, and sanitary products, pharmaceuticals especially those based on water need preservatives to prevent microbial contamination [1]. Parabens are one of the most commonly used preservatives in these compounds [2]. Parabens are esters of *p*-hydroxybenzoic acid. The most common parabens are methylparaben, ethylparaben, propylparaben, butylparaben. By adding parabens will help to extend product's shelf life and protect us from germs [3]. These preservative compounds (parabens) with low toxicity, good stability, non-volatility and non-irritability properties are efficient for such applications [4]. However, we cannot ignore the destructive effects of these materials. Parabens could change endogenous hormone action or synthesis, they may also affect the reproductive system or nervous system [5]. Parabens specious have high octanol water coefficients which lead to dissolve them in fatty tissues (bioaccumulate) [6] and they have been proven to be one of the cause of breast cancer. As, the use of certain types of parabens has been prohibited [7]

In order to find out the amount of parabens in different samples, the preconcentration and extraction of analytes usually performed before instrumental analysis.

Liquid phase extraction and solid phase extraction techniques are two main categories of extraction methods that are carried out in variety of ways. For example, liquid phase microextraction (LPME) [8], single-drop microextraction (SDME) [9], hollow-fiber liquid-phase microextraction (HF-LPME) [10] and dispersive liquid-liquid microextraction (DLLME) are modified forms of liquid phase extraction technique. They offer higher enrichment factor and/or extraction efficiency and lower organic solvent consumption rather than classic techniques [11, 12]. Among these techniques, dispersive liquid-liquid microextraction has significant attention by providing high enrichment factor which is dispersion of extraction solvent throughout the sample solution [13, 14].

Deep eutectic solvents were recently introduced to solvents, they are referred to as green solvents. They are mixture of Lewis or bronsted acids and bases. In these solvents one or more compound mixed and produced a eutectic solvent with a melting point significantly lower than either of the individual components, they are mostly liquid at room temperature. The components should combine in particular molar ratio to observe this state [15, 16].

Deep eutectic solvents provide valuable benefits such as easy preparation with high purity, environmental compatible, safe, cheap, and low toxicity [17, 18].

In DLLME for extraction of analytes, the extracting solvent which is microliters of a water-immiscible high density organic solvent in the form of a cloudy solution (fine droplets) dispersed throughout the aqueous phase by means of dispersive solvent (miscible in both extracting and aqueous phases) [19, 20]. Since the extraction solvent dispersed in all part of the sample solution, this technique has the great extraction efficiency. The use of deep eutectic solvents as extraction solvent in DLLME technique will also help improve the enrichment factor. Choosing the DES in this technique depend on some physical features

like viscosity, density, solubility, freezing point and polarity [21, 22]. Usually, the DES with higher density than water will be used overall, these solvents are extensively used as an extraction solvent in dispersive liquid liquid microextraction for extraction of various analytes.

In the present study we develop application of DES based glucose for DLLME extraction of parabens in liquid pharmaceutical samples.

2. Experimental

*2.1. Materia*ls

The analytical purity methyl paraben (MP) (logP 1.91, pKa 8.87), ethyl paraben (EP) (logP 2.34, pKa 8.90), propyl paraben (PP) (logP 2.94, pKa 8.87), butylparaben (BP) (logP 3.50, pKa 8.79) were obtained from Sigma-Aldrich (St. Louis, USA).

The HPLC-grade methanol and acetonitrile, ethanol, acetone, hydrochloric acid (37%), dichloromethane, and ethylene glycol were obtained from Merck (Darmstadt, Germany).

glucose, choline chloride, phenol, menthol, glycerol, sodium hydroxide and sodium chloride all of these reagents and other chemicals were of analytical grade. Doubly distilled water was used for preparation of aqueous solution prepared in the lab using a Water Purification System (HUMAN POWER 1, Korea).

Pharmaceutical samples such as nasal drop, syrup and ampoule were purchased from neighborhood pharmacies.

2.2. Preparation of standard solutions

The mixed stock solution of MP, EP, PP, BP was prepared in methanol at a concentration of 1000 $\mu g mL^{-1}$ and stored at 4 ^{0}C. Also, the standard working solutions were daily prepared by dilution of stock standard solution with distilled water to the required concentrations. The pH of aqueous solutions was adjusted by hydrochloric acid or sodium hydroxide. After finding optimized conditions, different samples prepared in acetic acid- sodium acetate buffer.

2.3. Instrumentation

Separation and determination of parabens were performed by HPLC-DAD. The HPLC system consisted of Agilent 1260 (Santa Clara, CA 95051. *United States)*, Agilent 1260 Infinity Bio-inert Quaternary Pump, Agilent 1260 Infinity Bio-inert Manual Injector valve equipped with a 20 µL sample loop, a vacuum degasser, and a column compartment, coupled to a DAD, A Zorbax Eclipse XDB C18 column, 150 mm × 4.6 mm, 5 µm, at an oven temperature of 25°C was used for separation, Agilent 1260 Infinity Diode Array Detectors (DAD) and Agilent ChemStation software. The degassed mobile phase was a mixture of methanol-pure HPLC grade water (60:40%, v/v) and the flow rate was 1 mL min^{-1}. The analytes were detected by DAD at wavelength, 254 nm. Centrifugation of solutions were carried out by Iranian Behdad Digital centrifuge. FT-IR spectroscopy analysis was applied to analyze the presence of molecular interactions between choline chloride and glucose. This investigation was performed by Thermo-Nicolet AVATAR 370 FT-IRw/SMART Endurance ATR (Canada) in the range of 400-4000 cm^{-1} in spectral-grade KBr pellets. Aliquots of 1.0 mL liquid sample (DES) was scanned in the wavelength range of 4000-400 cm^{-1}. In order to investigate the thermal stability of DES, glucose and choline chloride a TGA-50, shimadzu was used from RT up to 823 K at heating rates of 10 K/min.

2.4. Preparation of real samples before extraction

0.5 milliliter of each liquid pharmaceutical samples was diluted to 10 mL by distilled water and then centrifuged. 1.0 mL of diluted sample was taken to perform extraction.

2.5. Dispersive liquid-liquid microextraction procedure

A 5.0 mL of aqueous buffering solution of parabens was placed in a 10.0 mL screw cap glass test tube with conic bottom. 798.0 µL of ethanol (as a disperser solvent) and the 136.0 µL of glucose DES (as the extraction solvent) were mixed and injected

rapidly into the aqueous sample. The mixture was shaken. A cloudy solution (water/disperser solvent/extraction solvents) was formed in the test tube. In this step, the parabens in water sample were extracted into the fine droplets of extraction solvents. The mixture was then centrifuged for 5 min at 6000 rpm to accelerate phase separation. Finally, after decanting the upper phase, 10 µL of the sedimented phase was injected directly into the HPLC system for further analysis.

The DES peak should have no interferences with analyte peaks in the HPLC chromatogram (during separation).

3. Results And Discussion

3.1. Optimization of extraction conditions

Kind of solvents were considered and optimized in univarient way. Then, during one step, the other factors influencing the DLLME optimized simultaneously by applying experimental design.

3.1.1. Selection kind of DES

The extraction solvents in liquid extraction must have high affinity toward analytes in sample, appropriate chromatographic behavior, easy dispersion in aqueous phase and then separation from it in order to analyzing by instrument [23, 24]. Thus, choosing the most suitable extraction solvent is of primary importance for achieving good selectivity of the target compounds.

At first, several kind of DES solvents were synthesized and extraction procedure was carried out by help of them. The composition and preparation procedure of different kinds of DES were described in Table 1.

Table 1
Different DES composition

DES Name	DES constitute	Salt/HBD (mol/mol)	DES syntheses
MC	Menthol: ChCl	2:1	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed
PC	Phenol: ChCl	2:1	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed
GCW	Glucose: ChCl: Water	1:2:2	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed
GLYC	Glycerol: ChCl	2:1	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed
EGC	Ethylene glycol: ChCl	4:1	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed
MEGCFe	Ethylene glycol: ChCl: $FeCl_3$	4:1:1	Heating the mixture at 80 °C until a clear and homogeneous liquid formed
MD	DL menthol :Dodecanoic acid	2:1	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed
MA	DL menthol :Acetic acid	1:1	Heating mixture at 85ºC with constant stirring until a homogeneous liquid formed

The preparation process for all of these DES is almost the same and easy. They were usually synthesized by heating different hydrogen bond donor (HBDs) and ammonium salt (eg. ChCl) to 80~85 ^{O}C along with constant stirring until the emergence clear liquid. To keep the temperature constant, the process was performed in an oil bath.

To consider the performance of each DES on extraction efficiency, 5.0 mL aqueous sample of parabens (1 ppm) was selected, 1 mL methanol as dispersing solvent plus the 200 µL of each kind of DES as the extraction solvents were injected into the aqueous solution after shaking, the sample solution was centrifuged for 5 min at 6000 rpm, 10 µL of the sedimented phase used for quantification analysis by HPLC.

Extraction efficiency (peak area) was evaluated based on the type of DES and shown in Fig. 1.

Magnetic DES also can be seen in this consideration, by entering $FeCl_3$ in DES constitute, the

$FeCl^-_4$ anion formed and the solvent gets magnetic, however, extraction with this solvent was not very efficient rather than others.

Based on this diagram, the highest extraction efficiency (peak area) was obtained by using DES of glucose (GCW) as an extraction solvent. In general, density values of DES are higher than water which is favor of DLLME technique by reducing the requirement time for separation of the phases [19] .

Carbon tetrachloride (usual dispersive extraction solvent) was used without DES to extract paraben species, which did not have a satisfactory result.

3.1.2. Characterization of glucose DES

The FT-IR spectra of pure compounds as well as DES of glucose were obtained and analyzed.

Figure 2 depicted the IR spectrum of ChCl, the strong and almost broad peak in 3235 cm^{-1} related to stretching vibration of hydroxyl group (OH), this OH group can form inter and intramolecular hydrogen bond, bands at 1085 cm^{-1} and 1012 cm^{-1} are appeared for C–N stretching vibration, 1482 cm^{-1} refer to the presence of an alkyl groups. On the other hand, 3200 to 3400 cm^{-1} band assigned to OH groups (vibrational stretching) in glucose, 1026 cm^{-1}, C-O stretching vibration, 1376 cm^{-1} C-OH vibration, 772 cm^{-1}, C–H out-of-plane bending.

When glucose and choline chloride are hydrogen bonded together to form liquid deep eutectic, DES, FTIR spectrum pattern changed, the most important changes seen in hydroxyl band that participated in the formation of the hydrogen bond, as can be seen this peak gets wider, Fig. 3.

Band of vibration C-H group shift to 1479 cm^{-1} with change in intensity, on the other hand, the C-O stretching vibrations also appears in 1080 cm^{-1}[25–28].

Figure 4 presents the variation of weight percentage and derivative of the weight percentage (TGA-DTG) curves of glucose, choline chloride and DES samples in the temperature range from room temperature to 823 K. According to figure 4a and 4b, the thermal degradation of choline chloride consists of two distinct stages: first step related to water loss which was proved by OH band of FT.IR spectra pattern. The main thermal degradation step occurs in temperature range 562-618.8 K, leaving only 4.6%wt solid residue.

As seen Fig. 4, a rapid weight loss (5.79%wt) in the temperature interval of 337-357.5 K as moisture content of glucose. Glucose begins to decompose at around 456 K and with mass loss of 99.8%wt at 803K. Besides, figure 4 presents the onset thermal degradation of DES is lower than glucose and choline chloride. Based on FTIR spectrum pattern of DES, there is more mass loss in temperature range 303-433K (13.6%wt) as moisture content compared to glucose and choline chloride (Fig. 4). DES begins to decompose at around 448K. Besides, there is 22.5%wt residue mass at 803K.

Figure (5) shows the XRD pattern of the DES (GCW) and mixture of glucose and choline chloride. As it can be seen, the peaks are related to the crystal phases of glucose (JCPDS no.00-001-0374) and choline chloride (JDPDS no. 00-033-1581). Besides, the synthesized DES has amorphous structure as no significant sharp peak and completely peaks of glucose and choline chloride disappear.

3.1.3. Selection the disperser solvent

Disperser solvent is applied in DLLME to enhance the dispersion of extraction solvent throughout the aqueous phase by decreasing the interfacial tension. Therefore, this solvent must be able miscible in both the aqueous phase and the organic phase [29, 30]. By employing the disperser solvent, extraction efficiency will be improved. For this purpose, four usual disperser solvents, methanol, ethanol, acetonitrile, acetone, were examined. Extractions were performed by using 5.0 mL aqueous solution, 200 µL of DES (glucose) as the extraction solvent, 1.0 mL of different each of disperser solvent

Figure 6 depicts the most peak areas were obtained when the ethanol was the disperser solvent. Ethanol was chosen the disperser solvent in subsequent experiments. By means of ethanol as a disperser, acceptable repeatability obtained while the peak areas were lower than other solvents. Ethanol is the main parabens solvent that can dissolve them and of course completely miscible with water, therefore, the extraction efficiency will be improved [31].

With the use of acetonitrile as a disperser solvent, cloudy mode was sometimes not formed. That was the reason for reduced repeatability.

3.1.4. Optimization of dispersive liquid liquid microextraction conditions using central composite design

After selecting the extraction and disperser solvents individually, the other four important factors affecting the dispersive liquid-liquid extraction (pH of the aqueous sample, salt addition and volume of DES and ethanol) were simultaneously investigated and optimized in one step.

Optimization procedure was carried out by employing the response surface method (RSM) with a central composite design (CCD) technique. This design contains four main parameters, by applying the CCD method including 31 experiments (runs) with 7 Center points the relation between parameters and their response were obtained.

The low and high levels of these factors in two-level factorial (Full fraction) design were as follow: pH of sample solution (3-9), salt % (0-10%), volume of DES (50-150 µL) and volume of dispersion solvent (300-1000 µL). By establishing one block (1 day) 31 experiments were carried out. The peak area of each paraben species was considered as the response of each experiment.

To minimize the effect of uncontrolled factors on the response, all tests were done randomly. Table 1s. summarizes the design of experiments as uncoded and real values and shows the response value for extraction of each analyte. Practical response obtained in each experiment (Table 1s) was used to calculate the response descriptor model for each of the factors and the equation was obtained and applied (Table. 2s).

3.1.5. Analysis of variance (ANOVA)

The result and equations of the model was statistically analyzed by ANOVA method (Table 3s). The ANOVA method predicts one-way effects, interactions, and the second-order factors on the response.

The probability value defined as p value, the parameters having p value lower than 0.05 in the ANOVA indicated to be significant effect on the response at a confidence level of 95%.

On the other hand the F-value is the ratio of mean square for the individual term to the mean square for the residual.

In order to test the null hypothesis, F value and P value are compared. In this way, the statistical significance of effects can be estimated. The results of ANOVA method for the methyl paraben was considered as an example in Table 3s. According to the Table 3s, it can be found that all the variables had a significant effect on the response and had a $p \leq 0.05$. In this model, F-value is great that implies the model is significant [32, 33].

The evaluation the significance of the model was performed by the lack-of-fit test, Lack of Fit (LOF) is a symbol of the variation of data around the obtained model used for criterion judging the suitability of a model for fitting experimental data. If the LOF of

the model was significant indicates that model would be inappropriate for embedding empirical data, the resulting p value for the LOF is 0.915 (Table 3s) indicates the ability of the model to describe the experimental data and the obtained optimum points. The R^2 coefficient consideration will also help to confirm the result, R^2 coefficients compare experimental data and predict values by the model. For PP, R-sq was obtained 94.77% that means the data were fitted well and only 5.23% of the total variance was not explained by the model. Furthermore, the adjusted R-squared is a modified version of R^2 for the number of predictors in a model, while an R-squared value between 0 and 100 and shows the linear relationship in the sample of data even when there is no basic relationship, the adjusted R-squared gives the best estimate of the degree of relationship in the basic population. Methyl paraben had the adjusted R^2 value (R-sq(adj) (90.19%) that reveals the satisfactory correlation between the experimental data and the obtained model.

Finally, the experimental data were analyzed by constructing a polynomial equation, in fact, the mathematical equation between the detector response for each analyte and each of the factor. Desirability function (DF) condition also was applied to get the optimum conditions where the maximum peak area find out for each factor. DF values are between 0 to 1, indicating a minimum and maximum value of optimum conditions, respectively [34, 35].

In this research, Minitab 17 software was applied for prediction the optimum values for each of the studied parameters, obtaining the DF value and finding the desirable conditions profile.

The optimum conditions where the peak areas (responses) of each analyte meet its maximum value should be found. Peak areas are the symbol of the efficiency of the method. The graphs of maximizing the desirable conditions to attain optimum conditions could be seen in Fig. 1s.

According to Fig. 1s, it is clear that the best efficiency was obtained by setting the pH of aqueous solution 4.45, the amount of salt 5% and the volumes of glucose and ethanol 127.0 µL and 774.0 µL respectively.

Parabens are parahydroxybenzoic acid, they are hydrolyzed in acidic environment, in ionized formed disperse better in ethanol and then it is better absorb to extraction solvents, followed by preconcentration and extraction. This trend is similar to previous researches [36, 37].

DES of glucose has the hydroxyl groups (from glucose and choline chloride) that can participate in the formation of hydrogen bond, carboxy groups of parabens and hydroxyl groups of DES form hydrogen bond. Therefore medium acidic pH would be favor to this phenomena [38].

As the pH further increased, the peak areas decreased dramatically. Parabens are in ionic forms at pH higher than the pKa values of the analytes. It is difficult to absorb into the organic solvents. Therefore, pH adjustments were performed by using buffer of acetate at 4.5.

The influence of ionic strength of aqueous solution on the performance of extraction was investigated. It is performed by addition different amount of NaCl (0–10%). Increasing the salt up to 5% causes a significant increase in the extraction efficiency, however, further addition of NaCl due to the high viscosity of the solution and difficulty diffusion of analyte towards extraction solvent leads to a decrease in the yield.

As the amount of glucose was increased to 150.0 µL, the responses were also rise at the same time for all of the analytes. 774 µL ethanol as the disperser solvent would be sufficient for dispersing throughout the solution and helping the extraction and preconcentration of analytes.

3.2. Analytical performance

The method was evaluated under optimum condition, the linear range, limit of detections (LODs), limit of quantifications (LOQs), repeatability, enrichment factor (EF), and extraction recovery (ER) were obtained for this purpose. At first, several parabens solutions with known concentration in buffer solution were prepared, the DLLME were performed on them, then the analytical figures were calculated through these solutions.

Detection limit is defined as three times signal to noise, in other word, the minimum concentration of analyte that produces the chromatogram peak area equal to 3 times the peak area of the noise[39]. LOQ or limit of quantification is also calculated in same way only the signal-to-noise ratio will be 10 [39, 40]. Table 1

The reproducibility of the extraction procedure over one day and the repeatability of the method between three days at three levels of concentration (low, medium, high) were considered. The standard solutions of parabens prepared after performing the extraction procedure on them, the relative standard deviation between responses calculated and introduce as the precision of the method [41]. Table 2 shows these results.

The comparison between the results of extraction parabens from the standard samples with the known concentration and real sample under optimal conditions lead to find the recovery of the method and also matrix effect. The relative standard deviation (RSDs) was calculated to check the accuracy of the method.

The calibration curves were constructed using 10 concentration levels. By linear regression of the peak area versus standard concentrations of parabens.

The standard mixtures of 4 parabens in a concentration range of 0.01-5000 ng mL^{-1} were prepared for calibration curves, then linear regression of peak area against standard concentrations plotted individually for each analyte. The extraction procedure repeated 3 times for each concentration level.

Preconcentration factor expressed the ratio between the paraben concentration after extraction in sedimented phase to initial concentration [42, 43] .

Eqs. (1)(2) were used for calculation of enrichment factor and recovery, respectively.

$$EF = C_{sed} / C_a \quad (1)$$

$$ER\% = \frac{C_{sed} \cdot V_{sed}}{C_a \cdot V_a} \times 100 = \left(\frac{V_{sed}}{V_a} \right) \times EF \times 100 \quad (2)$$

C_{sed} was concentration of analyte in sedimented phase, C_{aq}, concentration of analyte in aqueous phase, V_{sed} volume of sedimented phase and V_a volume of aqueous phase.

Final volume of sedimented phase was 50.0 µL and initial aqueous volume was 5.0 mL

Table 2
The characteristics of the method

Analyte	Linear range	LOD	LOQ	R^2	EF	ER%
	ng mL^{-1}	*ng mL^{-1}*	*ng mL^{-1}*		(C_{sed}/C_{aq})	C_{sed}/C_{aq}×100
MP	0.1-5000	0.03	0.1	0.9962	47.73	47.73%
EP	0.5-5000	0.15	0.5	0.9955	55.39	55.39%
PP	0.1-5000	0.04	0.1	0.9959	67.65	67.65%
BP	0.1-5000	0.04	0.1	0.9934	75.05	75.05%

C_{sed}: concentration of analyte in sedimented phase, C_{aq}: concentration of analyte in aqueous phase.

Table 3
The method precision parameters

Precision n=5	Concentration *μg mL^{-1}*	MP	EP	PP	BP
	0.001	**4.41**	**3.68**	**6.85**	**5.95**
	0.1	**2.05**	**3.21**	**5.12**	**4.79**
	1	**1.78**	**2.21**	**4.94**	**3.48**
Reproducibility (Three days) Conc.0.5 *μg mL^{-1}*		4.57	4.31	7.08	8.25

3.3. Real sample analysis

The results of this study were evaluated by analyzing the parabens in real samples. Liquid pharmaceutical samples were chosen for investigation the capability of the method for extraction of parabens in real samples. Samples were prepared initially according to section 2.4.

Because the complete compositions (matrix) of these pharmaceuticals were unknown, the standard addition method was used to find the amount of parabens added to these samples.

2.0 mL of prepared pharmaceutical samples and different amounts of paraben standard solution at a concentration of 0.3 μg mL^{-1} and 0.5 g NaCl were added to 10 mL volumetric flasks, the solutions were diluted by buffer solution to the mark.
5.0 mL of each sample was taken to carried out the dispersive liquid liquid microextraction procedure under the optimum conditions on samples.
After extraction of the analytes by DLLME, the extracts were injected into HPLC for separation and determination of analytes. Parabens amounts were determined in different samples by means of the standard addition curves. The results were collected in Table 4.

According to Table 4, in different kind of pharmaceutical samples, paraben have been found. Therefore, these types of preservative are usually added to these pharmaceuticals. Among the parabens, propyl paraben and butyl paraben are more commonly used species.

Table 4
Parabens amounts in liquid pharmaceutical samples

Sample	MP ($\mu g\ mL^{-1}$) (n=3)	*R.R%	EP ($\mu g\ mL^{-1}$) (n=3)	R.R %	PP ($\mu g\ mL^{-1}$) (n=3)	R.R %	BP ($\mu g\ mL^{-1}$) (n=3)	R.R %
Nasal drop	**ND	-	ND	-	340±2.31	90.23	179± 3.23	103.12
Syrup no.1	830 ±5.12	80.95	ND	-	158±4.19	82.31	148± 3.18	93.98
Syrup no.2	ND		240±2.38	95.44	ND	-	209± 2.78	99.45
Syrup no.3	ND	-	ND	--	322± 4.70	101.25	ND	-
Ampoule no.1	ND	-	ND	-	713± 3.14	99.67	232.31± 3.15	
Ampoule no.2	ND	-	ND	-	243 ± 4.34	83.24	ND	-

* R.R %: Relative recovery, ** ND: Not defined

The accuracy of method was considered by comparison between the extraction of analytes in distilled water and the extraction of parabens in the real sample matrix. The accuracy of the method was confirmed by calculating the relative recovery. Relative recoveries were determined as the percent ratio of the concentrations found in real sample minus the concentration of analyte in the real sample without adding the standard to the spiked distilled water samples. This research was carried out at different concentration levels in the pharmaceutical samples and the results are presented in Table 4.

The sample chromatogram (syrup sample) shown in Fig. 8a related to the DLLME extraction of pharmaceutical sample without adding standard solution and Fig. 8b chromatogram after addition of standard solution.

The response of parabens significantly increased after extraction with the DLLME which demonstrated that the extraction by DES successfully pre-concentrated the parabens.

3.4. Comparison of the results of this study with similar pervious researches

Table 5 was provided for comparison the results of the present study with the other extraction methods by emphasizing dispersive liquid liquid extraction of parabens in different samples. Parabens can be found in almost all cosmetic and food samples and in all publications, four common parabens, BP MP, EP, PP, have been investigated. The optimized method exhibited comparable or lower limit of detection than earlier methods. The same trend obtained for linear range. It is quite clear that when there is a change in the conditions of the usual DLLME method such as using low-density organic solvent, solidification of floating organic drops or using DES solvent and so on, more satisfactory results are obtained. The lower LODs in our results can be attributed to the use of DES in extraction procedure, which has created better sensitivity. Extraction of parabens from pharmaceutical samples by DLLME was not considered in the past.

Table 5
Comparison table

Analyte	Analytical instrument	Extraction method	Real sample	Detection limit ($\mu g\ mL^{-1}$)	linearity range ($\mu g\ mL^{-1}$)	RSD (%)	Ref.
MP EP PP BP	Gas chromatography(GC) Flame ionization detector (FID)	DLLME	lake water	0.003 for MP and EP; 0.002 for PP and BP	0.05 - 1	2.0–10.0%	[44]
MP EP PP BP	HPLC-UV	DLLME low-density organic solvent	Tap water and Fruit juice samples	2.1×10^{-5} -4.6×10^{-5}	0.001–0.5	4.1-9.3%	[36]
MP,EP PP,BP isopropyl paraben (iPrP) isobutyl paraben (iBuP) heptyl paraben (HepP) octyl paraben (OctP)	HPLC-UV	DLLME solidification of floating organic drops	plasma samples and urine samples	Plasma, 0.0002–0.0004 Urine, 0.0001–0.0004	0.001–1.0	less than 5.4%	[45]
MP EP PP BP iBuP isoamylparaben,	GC-FID	DLLME	Aqueous cosmetic products	0.0048 – 0.025	MP,0.05—10 and 0.025—5.0 for the other five parabens	lower than 8.2%	[46]
MP EP PP BP	HPLC-DAD	In situ DES-LLME	Environmental water samples	0.0006-0.0008	0.003-1.0	less than 7.2%	[47]
MP EP PP BP	capillary electrophoresis (CE)	DLLME with back-extraction	Human milk and other food samples (tomato paste, pickle, mixed fruit juice and ice cream)	0.1 to 0.2	4.3–10.7	lower than 3.5%	[48]

Analyte	Analytical instrument	Extraction method	Real sample	Detection limit ($\mu g\ mL^{-1}$)	linearity range ($\mu g\ mL^{-1}$)	RSD (%)	Ref.
MP EP PP BP	HPLC-DAD	DLLME With DES	Liquid pharmaceutical samples	0.00003-0.00015	EP, 0.0005-5 and MP, PP, BP 0.0001-5.0	1.78-6.85	This study

4. Conclusion

The present study considered the dispersive liquid-liquid microextraction of parabens from liquid pharmaceutical samples. By applying deep eutectic solvent in extraction procedure, the new extraction solvent enters to reaction, which helps to better extract the analytes.

The results of TGA, XRD, and FT-IR instruments indicate that synthesis of DES from glucose and choline chloride was done successfully.

The proposed method provides satisfactory analytical merits, it is compatible with HPLC, it reduces the danger of exposure to toxic solvents used for extraction in conventional extraction procedures and requires short extraction time and low solvent. The method showed the low detection limits and relatively broad linear concentration range.

To the best of our knowledge, this was the first time that the DLLME with DES was applied for the determination of MP, EP, PP and BP in liquid pharmaceutical samples with acceptable relative recoveries.

This paper once again demonstrated the great potential of DLLME in preconcentration and analysis of analytes in a short period of time.

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Figures

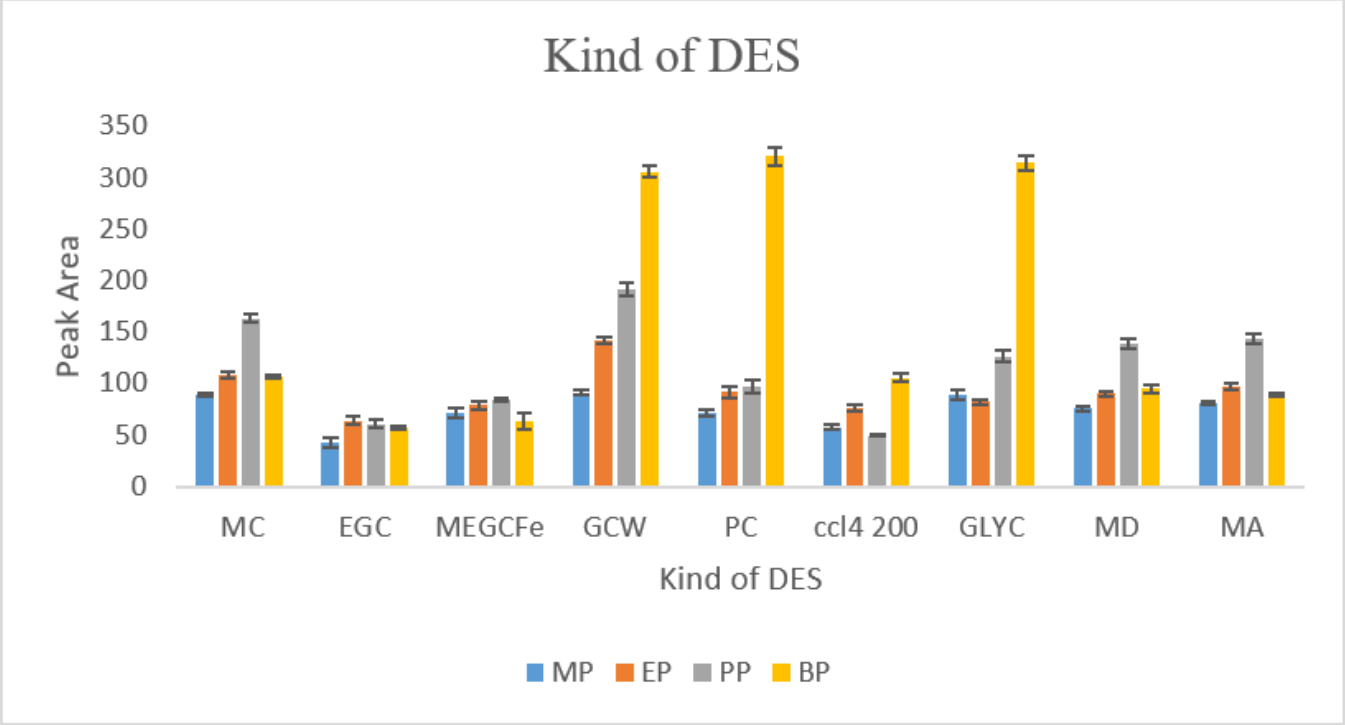

Figure 1

Kind of DES selection.

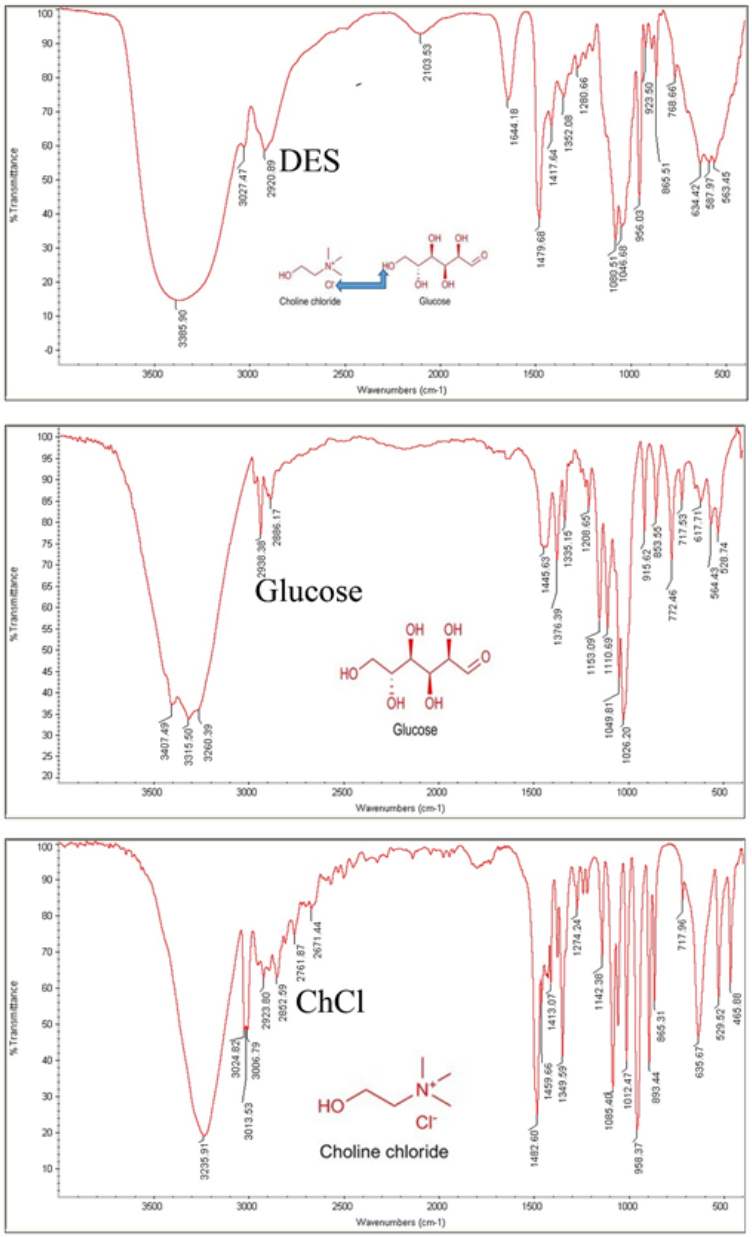

Figure 2

FT.IR spectra of DES, glucose, and cholin chlorid, ChCl.

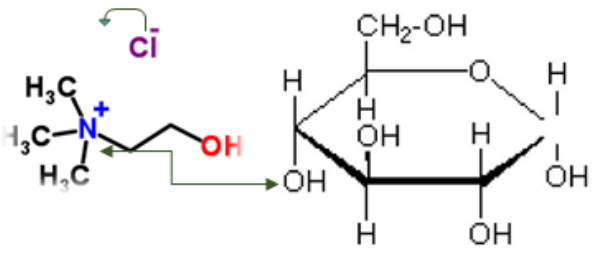

Figure 3

Hydrogen bonded between choline chloride and glucose.

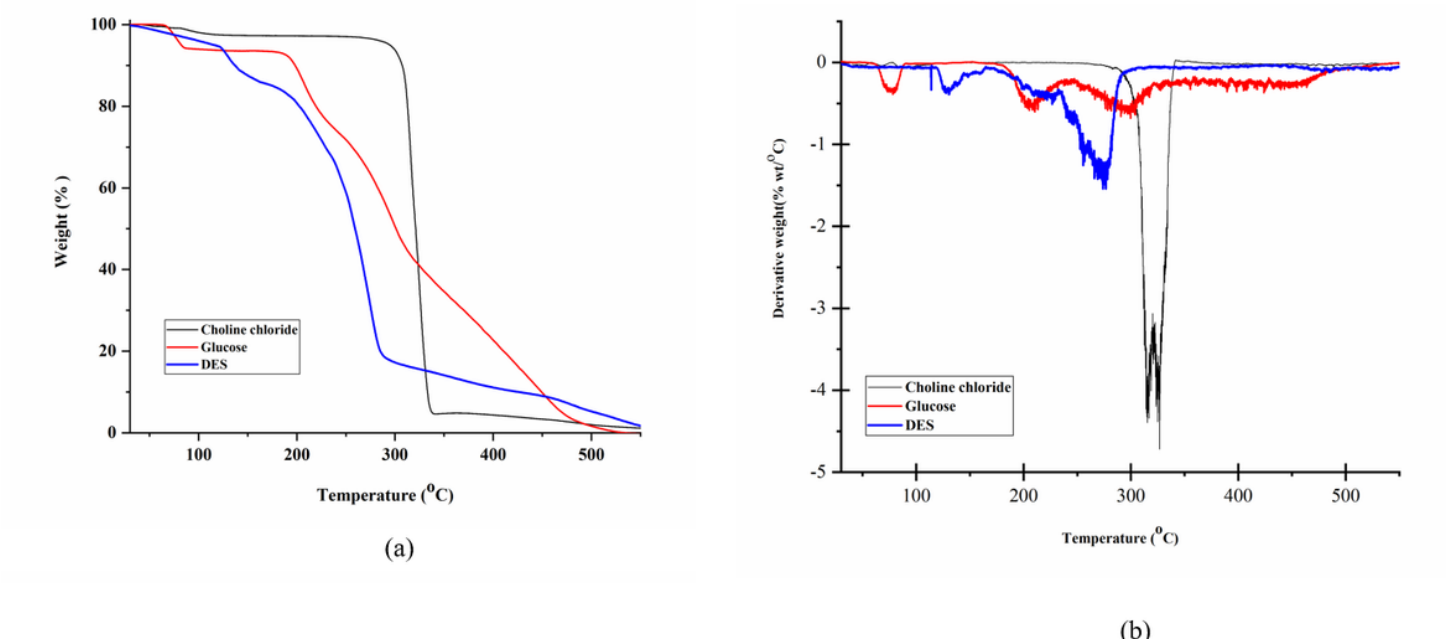

Figure 4

The variation of a) weight percentage (TGA) and b) the derivative of the weight percentage (DTG) curves of choline chloride, Glucose, DES as functions of temperature at 10 K/min.

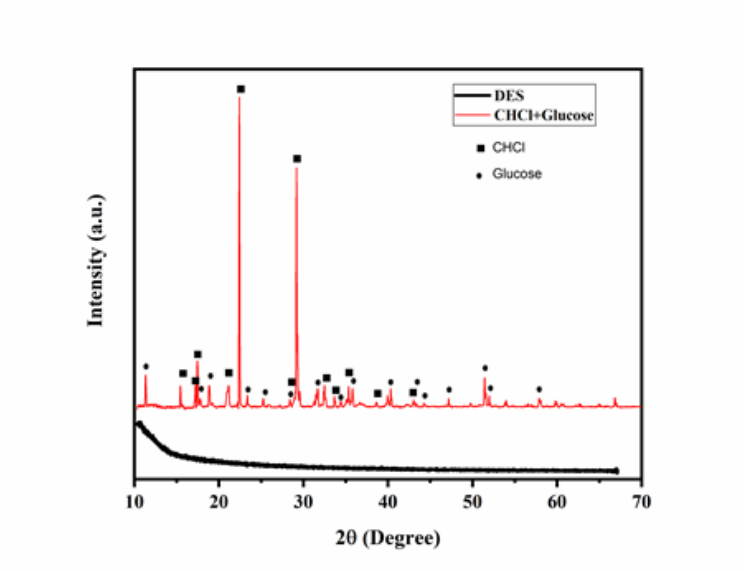

Figure 5

XRD graphs of DES and mixture of glucose and choline chloride.

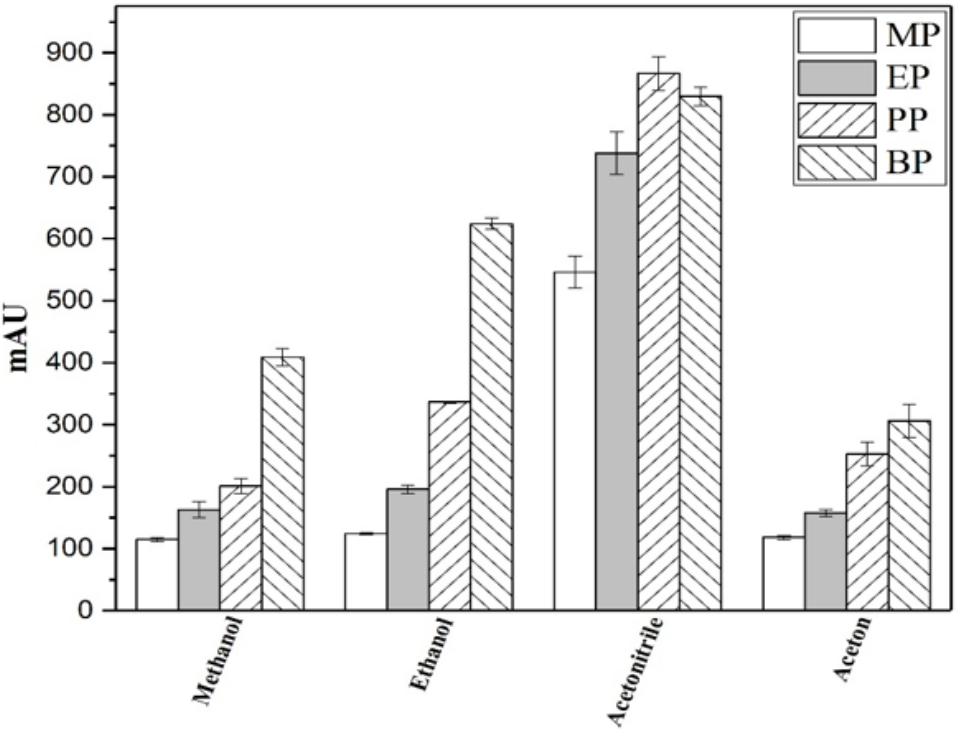

Figure 6

Kind of disperser solvent selection.

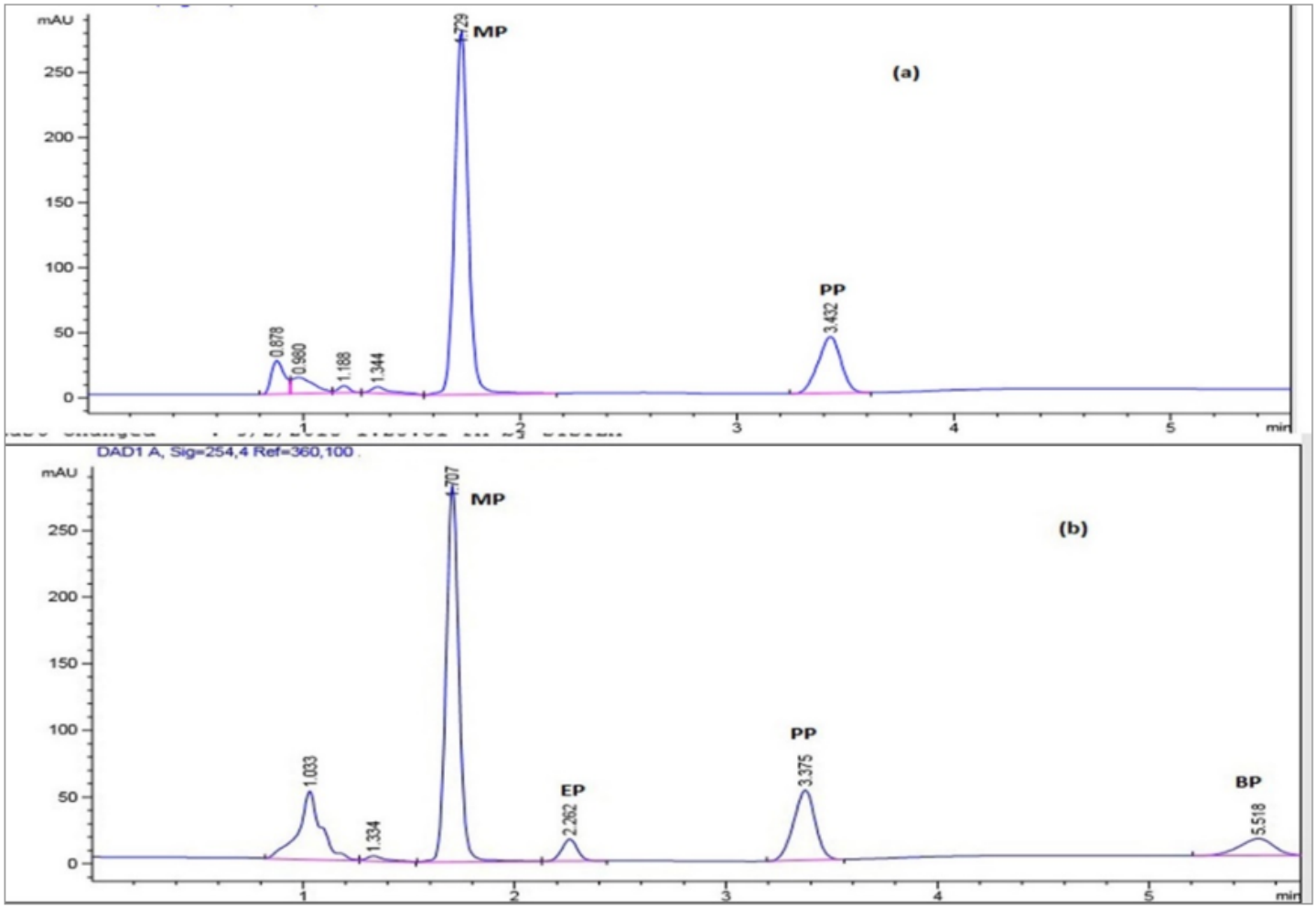

Figure 7

The sample chromatogram of extraction real sample (a) before and (b) after addition of standard solution

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NEWFIG.xlsx
- S2.docx