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RESEARCH ARTICLE

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Extraction and pre-concentration of parabens in liquid pharmaceutical samples by dispersive liquid–liquid microextraction based on deep eutectic solvents

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Abstract

In this paper dispersive liquid–liquid microextraction using deep eutectic solvent (DES), as an extraction solvent, was applied for the pre-concentration and determination of parabens in liquid pharmaceutical samples. A DES composed of a hydrogen bond acceptor [choline chloride (ChCl)] and a hydrogen bond donor (glucose) achieved the highest extraction efficiency. Therefore, this solvent was selected as the extraction solvent. After the synthesis of this solvent, its various properties were investigated. Thermogravimetric analysis, X-ray diffraction, and Fourier transform infrared spectroscopy were used for this purpose and the successful synthesis of the solvent was confirmed. HPLC with photodiode array detection was used for the analysis of paraben species. Parameters affecting the extraction efficiency were monitored and optimized through univariate analysis and experimental design. Under the optimal conditions (pH of aqueous solution 4.5, ethanol as the disperser solvent, and 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) between 0.993 and 0.9962. Limits of detections ranged from 0.04 to 0.15 ng mL^{-1}, with relative standard deviations from 1.8% to 6.8%. The developed method was applied to the determination of parabens in liquid pharmaceuticals such as ampule, syrups, and nose drop samples. A certain amount of paraben was added to the tested real samples to increase their shelf life. The relative recoveries in these real samples ranged between 80.9% and 103.1%.

KEYWORDS
deep eutectic solvent, dispersive liquid–liquid microextraction, high-performance liquid chromatography, paraben, pharmaceutical samples

1 | INTRODUCTION

As is the case with products such as food, detergents, cosmetics, and sanitary products, pharmaceuticals, especially those water based ones, also need preservatives to prevent them from microbial contamination (Kashid et al., 2011). Parabens are one of the most commonly used preservatives in these compounds (Dodge et al., 2015). Parabens are esters of *p*-hydroxybenzoic acid. The most common parabens are methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP). Adding parabens will help extend product's shelf life and improve germ protection (Croitoru et al., 2016). Parabens are preservative compounds with low toxicity, good stability, non-volatility, and non-irritability properties and as such are efficient for preserving various products (Castelain & Castelain, 2012). However, we cannot ignore the destructive effects of these materials. Parabens could change endogenous hormone action or synthesis; besides, they may

affect the reproductive system or nervous system (Błędzka, Gromadzińska, & Wąsowicz, 2014). Parabens have high octanol water coefficients, which allows them to dissolve in fatty tissues (bioaccumulate; Darbre et al., 2004). Importantly, they have been proven to be one of the causes of breast cancer. As a result, the use of certain types of parabens has been prohibited (Qian, 2019).

To understand the amount of parabens in different samples, preconcentration and extraction of analytes are usually performed before instrumental analysis.

Liquid-phase extraction and solid-phase extraction are two main categories of extraction methods that are performed in a variety of ways. For example, liquid-phase microextraction (Ali Sarafraz-Yazdi & Amiri, 2010), single-drop microextraction (Gioti et al., 2005), hollow-fiber liquid-phase microextraction (Sarafraz-Yazdi & Es' Haghi, 2006), and dispersive liquid–liquid microextraction (DLLME) are modified forms of liquid-phase extraction. They offer a higher enrichment factor and/or extraction efficiency and lower organic solvent consumption rather than the classic techniques (Pengionan, 2017; Yazdi et al., 2008). Among the aforesaid techniques, DLLME has received significant attention, as it provides a high enrichment factor, that is, dispersion of extraction solvent throughout the sample solution (C.-W. Chen et al., 2018; Grecco et al., 2019).

Most solvents used in liquid-phase extraction are toxic organic solvents. Therefore, it is worth searching for an eco-friendly solvent that is effective in extraction.

Deep eutectic solvents (DESs) are recently identified green solvents that are a mixture of Lewis or Brønsted acids and bases. In these solvents two or more compounds are mixed to produce an 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 achieve this state (Aroso et al., 2016; Zhang et al., 2019).

DESs are a new type of ionic liquids, with the advantages of low vapor pressure, high thermal and chemical stability, low melting temperature, wide liquid range, and non-flammable properties; DES is an improved form of ionic liquids. They are also biodegradable (environmentally friendly) and safe, so their use is expanding (Dil et al., 2020; Faraji, 2019; Lan et al., 2017). However, only a few studies have focused on predicting the properties of DES and investigating their toxicity (Hansen et al., 2020; Lomba et al., 2021).

Many review articles have comprehensively investigated the properties of these solvents (El Achkar et al., 2021; Fuad et al., 2021). These articles have considered the necessary conditions for the production of these solvents, the required molar percentage of each component, their physicochemical properties (density, viscosity, ionic conductivity, surface tension and polarity), how to change these properties according to the intended application, the effect of these factors on the structure of these solvents, and the possibility of creating new compounds. Sometimes these articles have been paid attention in a specialized and special or partial way. For example, investigating the use of DES in the food industry (J. Chen et al., 2019) or pharmaceutical industry (Mbous et al., 2017). In addition to the outstanding advantages of DESs, natural-type DESs are even more eco-friendly due to their natural origin (Liu et al., 2018). The use of these solvents in analytical chemistry has been investigated in different areas, such as in the microextraction of target before analytical studies, modification of particles, application in solid samples, and using DES as an eluent in dispersive extraction or chromatography (Shishov et al., 2017).

In DLLME for the extraction of analytes, the extracting solvent, which is usually a water-immiscible high-density organic solvent (volume in microliters) in the form of a cloudy solution (fine droplets), is dispersed throughout the aqueous phase using a dispersive solvent (miscible in both extracting and aqueous phases; Cunha & Fernandes, 2018; S. Ma et al., 2019). As the extraction solvent disperses to all parts of the sample solution, this technique presents significant extraction efficiency. Using DESs as an extraction solvent in DLLME will also help improve the enrichment factor. Choosing the DES in this technique will depend on some physical features such as viscosity, density, solubility, freezing point, and polarity (El-Deen & Shimizu, 2020; W. Ma et al., 2017). Usually, a DES with higher density than water will be used. These solvents are also extensively used as an extraction solvent in DLLME for the extraction of various analytes.

This study considered the DLLME of parabens from liquid pharmaceutical samples. The innovation of this work was the preparation and synthesis of the DES and the use of this solvent in dispersive extraction instead of common extraction solvents. Few studies have used DESs for extraction. These solvents are eco-friendly and the extraction efficiency was acceptable. Characterization tests were performed on the solvent and the correct synthesis of the solvent was proved.

To the best of our knowledge, this is 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 the pre-concentration and analysis of analytes in a short period.

2 | EXPERIMENTAL

2.1 | Materials

Analytical purity–grade MP (logP 1.91, pK_a 8.87), EP (logP 2.34, pK_a 8.90), PP (logP 2.94, pK_a 8.87), BP (logP 3.50, pK_a 8.79), and choline chloride (ChCl) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

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

Glucose, glycerol and sodium hydroxide, were from Merck with analytical grade. Double-distilled water was used for the preparation of aqueous solution in the laboratory using a Water Purification System (HUMAN POWER 1, Korea). Sodium chloride was supplied by reidel-deHaen.

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, and BP was prepared in methanol at a concentration of 1000 μg mL^{-1} and stored at 4°C. The standard working solutions were daily prepared by diluting a stock standard solution with distilled water to the required concentrations. The pH of aqueous solutions was adjusted by adding hydrochloric acid or sodium hydroxide, as required. After finding optimized conditions, different samples were prepared in acetic acid–sodium acetate buffer.

2.3 | Instrumentation

Parabens were separated and determined by HPLC–DAD (diode array detection). The HPLC system comprised the following systems: Agilent 1260 (Santa Clara, CA, USA), 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 C_{18} column, 150 mm × 4.6 mm, 5 μm, with an oven temperature of 25°C used for separation, Agilent 1260 Infinity Diode Array Detectors, 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 254 nm (wavelength). Solutions were centrifuged on an Iranian Behdad digital centrifuge. Fourier transform infrared (FT-IR) spectroscopy analysis (Thermo-Nicolet AVATAR 370 FT-IR/SMART Endurance ATR, Canada) was performed in the range of 400–4000 cm^{-1} using spectral-grade KBr pellets to identify molecular interactions between ChCl and glucose. Aliquots of 1.0 mL liquid sample (DES) was scanned in the wavelength range of 4000–400 cm^{-1}. To investigate the thermal stability of DES, glucose, and ChCl, a thermogravimetric analyzer (TGA-50; Shimadzu) was used from room temperature up to 823 K at a heating rate of 10 K min^{-1}.

2.4 | Preparation of real samples before extraction

As all samples (drugs) were water based, removing only their interface materials before the pre-concentration step was sufficient. Then, 0.5 mL of each liquid pharmaceutical sample was diluted to 10.0 mL by adding distilled water and then the mixture centrifuged; 1.0 mL of diluted sample was taken to perform extraction.

2.5 | Dispersive liquid–liquid microextraction procedure

A 5 mL aqueous buffering solution of parabens was placed in a 10-mL screw cap glass test tube with conic bottom. About 798 μL ethanol (disperser solvent) and 136 μL glucose DES (extraction solvent) were mixed and injected rapidly into the aqueous sample. The mixture was shaken until a cloudy solution (water/disperser solvent/extraction solvents) was formed in the test tube. In this step, the parabens in the water sample were extracted into the fine droplets of the extraction solvent. The mixture was then centrifuged for 5 min at 6000 rpm (RFC 1900 g) to accelerate phase separation. Finally, after decanting the upper phase, 10 μL of the sedimented phase was directly injected 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

Different solvents were considered and optimized in a univariant way. Then, the other factors influencing the DLLME were simultaneously optimized by applying the experimental design.

3.1.1 | Selection of the appropriate DES

The extraction solvents used in liquid extraction must have high affinity toward analytes in the sample, appropriate chromatographic behavior, easy dispersion in the aqueous phase, so it can be separated from the mixture easily prior to the analysis (Ge et al., 2018; Saraji & Boroujeni, 2014). Thus, choosing the most suitable extraction solvent is of primary importance for achieving good selectivity of the target compounds.

First, several kinds of DESs were synthesized and extraction was performed using each. The composition and preparation procedure of different kinds of DES are described in Table 1.

The method for the preparation of all these DESs is almost the same and easy. They were usually synthesized by heating different hydrogen bond donors and ammonium salt (e.g. ChCl) to 80–85°C along with constant stirring until a clear liquid emerges. To keep the temperature constant, the process was performed in an oil bath.

To consider the impact of each DES on extraction efficiency, a 5 mL aqueous sample of parabens (1 ppm) was selected; then, 1 mL methanol as the dispersing solvent and 200 μL of each type of DES as the extraction solvents were injected into the aqueous solution after shaking the sample of parabens. The sample solution mixture was centrifuged for 5 min at 6000 rpm (RFC 1900 g), and 10 μL of the sedimented phase was used for quantification analysis by HPLC.

Extraction efficiency (peak area) was evaluated based on the type of DES (Figure 1).

Magnetic DES also can be seen in this consideration, by entering $FeCl_4$ 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 Figure 1, the highest extraction efficiency (peak area) was obtained using DES of glucose (glucose choline chloride and water [GCW]) as an extraction solvent. In general, density values of DESs are higher than that of water, which is in favor of the DLLME technique, as it reduces the requirement time for separation of the phases (Cunha & Fernandes, 2018).

TABLE 1 Different DES composition (molar ratio and syntheses procedure)

DES name	DES composition	Salt/hydrogen bond donor (mol/mol)	DES syntheses
MC	Menthol:ChCl	2:1	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed
PC	Phenol:ChCl	2:1	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed
GCW	Glucose:ChCl:water	1:2:2	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed
GLYC	Glycerol:ChCl	2:1	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed
EGC	Ethylene glycol:ChCl	4:1	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed
MEGCFe	Ethylene glycol:ChCl:$FeCl_3$	4:1:1	Heating the mixture at 80°C until a clear and homogeneous liquid is formed
MD	DL-Menthol:dodecanoic acid	2:1	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed
MA	DL-Menthol:acetic acid	1:1	Heating the mixture at 85°C with constant stirring until a homogeneous liquid is formed

Abbreviations: ChCl, choline chloride; EGC, ethylene, glycol, choline chloride; GCW, glucose, choline chloride, water; GLYC, glycerol, choline chloride; MA, menthol, acetic acid; MC, menthol, choline chloride; MD, menthol, dodecanoic acid; MEGCFe, magnetic ethylene glycol, $FeCl_3$; PC, phenol, choline chloride.

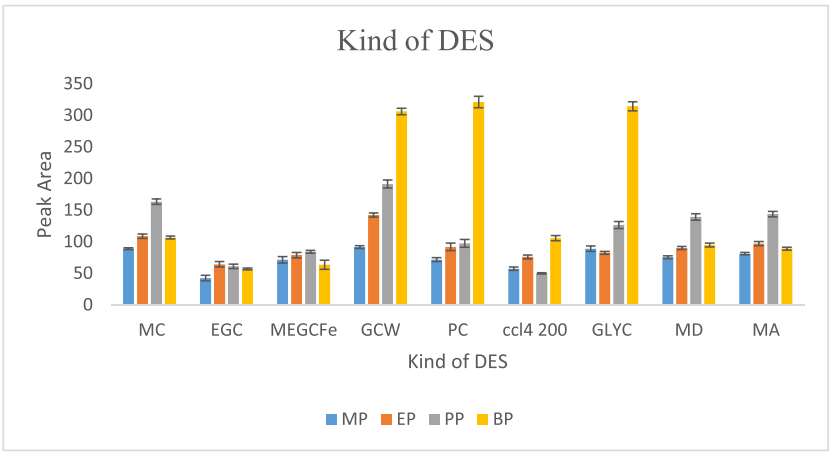

FIGURE 1 Deep eutectic solvent (DES) selection. Other conditions: 5.0 mL aqueous sample, 1 mL methanol (dispersing solvent), and 200 µL of each kind of DES as the extraction solvents. BP, butylparaben; EP, ethylparaben; MP, methylparaben; PP, propylparaben.

By contrast, according to previous studies, glucose can participate in the transesterification reaction with parabens and absorb parabens. In this reaction, the hydroxyl group of sugar reacts with the ester group of paraben and produces a new ester (M. Ma et al., 2002).

Carbon tetrachloride (usual dispersive extraction solvent) was used without DES to extract paraben species; however, this did not present a satisfactory result.

3.1.2 | Characterization of glucose DES

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

Figure 2 depicts the FT-IR spectrum of ChCl. A strong and almost broad peak at 3235 cm^{-1} related to stretching vibration of the hydroxyl group (OH) can be noted. This OH group can form inter- and intramolecular hydrogen bond. The bands at 1085 and 1012 cm^{-1} are related to the C–N stretching vibration, and the band at 1482 cm^{-1} refers to the presence of an alkyl group. Bands at 3200–3400 cm^{-1} are assigned to OH groups (vibrational stretching) in glucose, 1026 cm^{-1} to the C–O stretching vibration, 1376 cm^{-1} to the C–OH vibration, and 772 cm^{-1} to the C–H out-of-plane bending.

When glucose and ChCl are hydrogen bonded together to form a liquid deep eutectic (DES), the FT-IR spectral pattern changed. The most important changes in the hydroxyl band that participated in the formation of the hydrogen bond are shown in Figure 3.

The vibration band of the C–H group shifts to 1479 cm^{-1} with a change in intensity. Meanwhile, the C–O stretching vibrations also appear at 1080 cm^{-1} (Ibrahim et al., 2006; Kędzierska-Matysek et al., 2018; Mulia et al., 2015; Troter et al., 2016).

The electron pair of the glucose hydroxyl participates in the nucleophilic reaction and enters the nitrogen vacancy of the amine group.

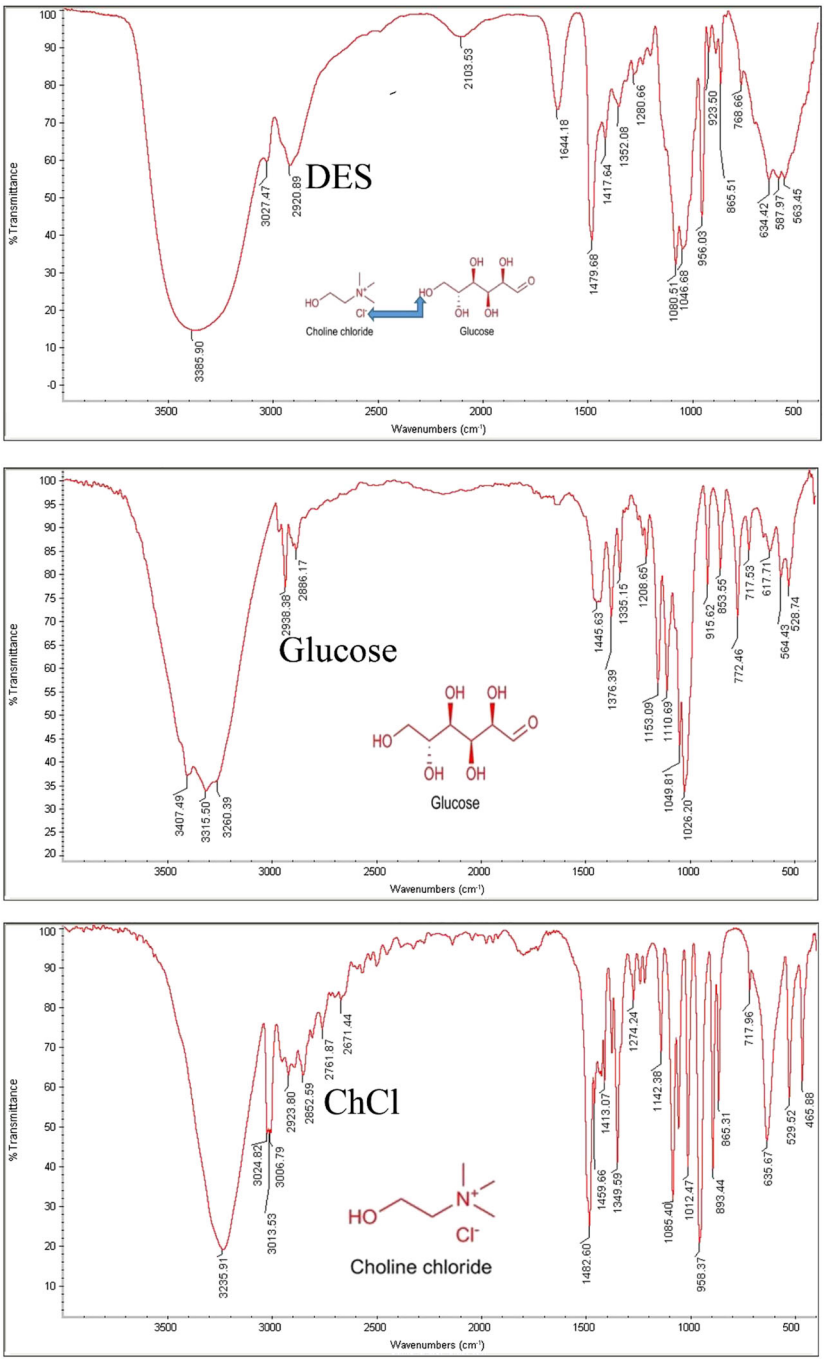

FIGURE 2 Fourier transform infrared spectra of deep eutectic solvent (DES), glucose, and choline chloride (ChCl).

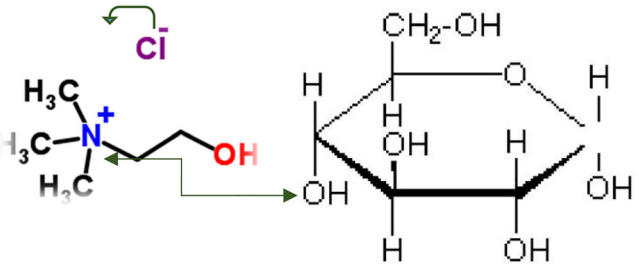

FIGURE 3 Hydrogen bond between choline chloride and glucose.

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

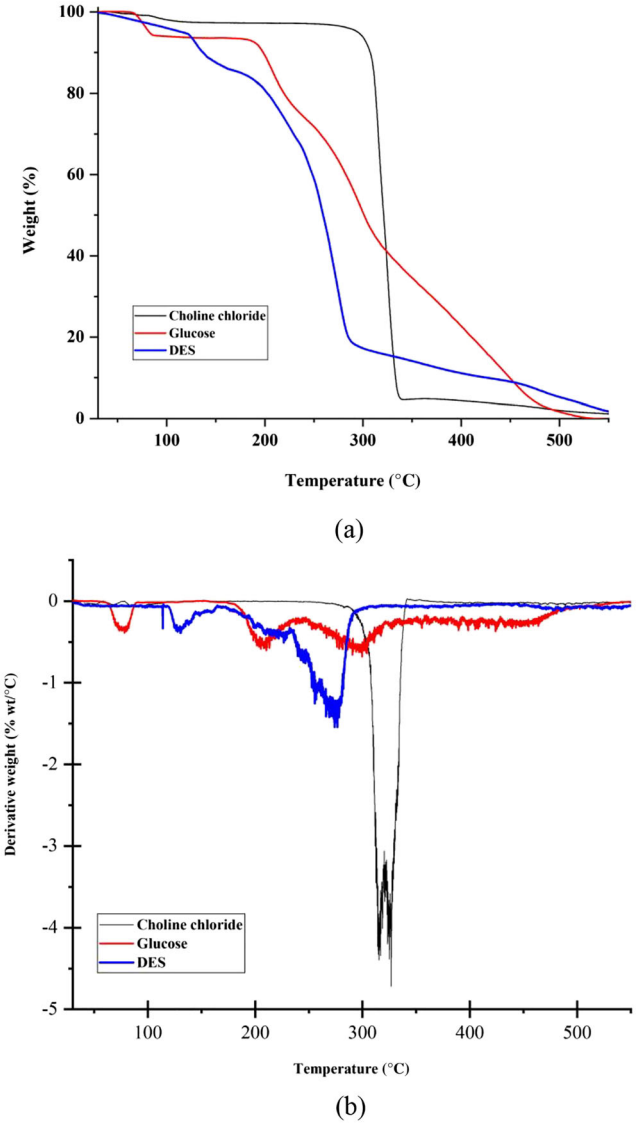

FIGURE 4 The variation of (a) weight percentage and (b) the rate of material weight changes upon heating is plotted against temperature

As seen Figure 4, a rapid weight loss (5.79%wt) occurs in the temperature range of 337–357.5 K, which is mainly due to the loss of moisture content of glucose. Glucose begins to decompose at around 456 K and with mass loss of 99.8%wt at 803 K. Besides, Figure 4 indicates that the thermal degradation onset of DES is lower than that of glucose and ChCl. Based on the FT-IR spectral pattern of DES, there is more mass loss in the temperature range from 303 to 433 K (13.6%wt), due to the loss of moisture content, compared with the mass loss of glucose and ChCl (Figure 4). The DES begins to decompose at around 448 K. Besides, there is a 22.5%wt residue mass at 803 K.

Figure 5 shows the X-ray diffraction pattern of the DES (GCW) (Glucose Choline chloride, Water) and mixture of glucose and ChCl. As can be seen, the peaks are related to the crystal phases of glucose (JCPDS no. 00–001-0374) and ChCl (JDPDS no. 00–033-1581). Besides, the synthesized DES has an amorphous structure, as there is no significant sharp peak and peaks of both glucose and ChCl disappear completely.

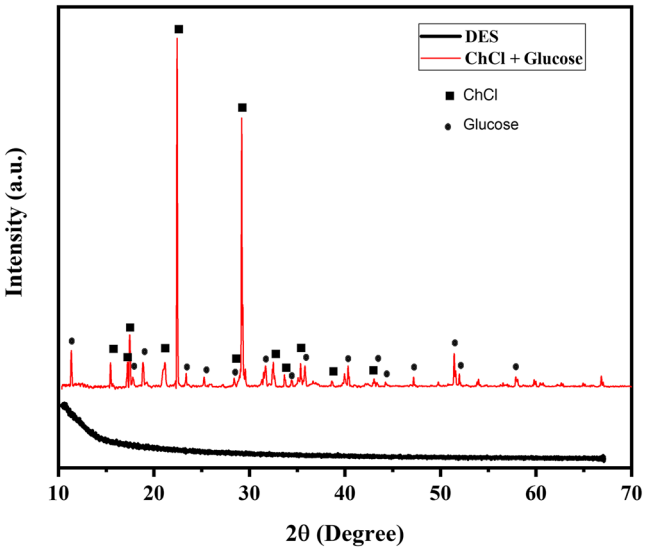

FIGURE 5 X-ray diffraction graphs of the DES and mixture of glucose and choline chloride.

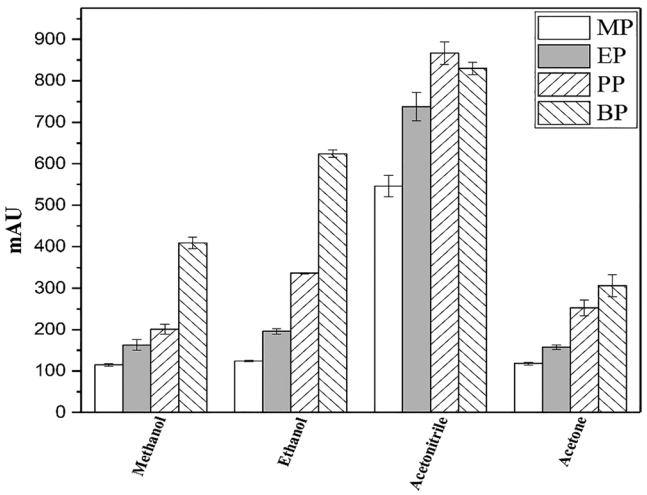

FIGURE 6 Disperser solvent selection. Reaction conditions: 5 mL aqueous solution, 200 µL deep eutectic solvent (DES; glucose) as the extraction solvent, and 1 mL of each disperser solvent. BP, butylparaben; EP, ethylparaben; MP, methylparaben; PP, propylparaben

3.1.3 | Selection of the disperser solvent

A 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 (Berijani, et al. 2006; Tabrizi & Rezazadeh, 2012). By using the disperser solvent, the extraction efficiency will be improved. For this purpose, four usual disperser solvents, namely, methanol, ethanol, acetonitrile, and acetone, were examined. Extractions were performed using 5 mL aqueous solution, 200 µL DES (glucose) as the extraction solvent, and 1 mL of each disperser solvent.

Most peak areas were obtained when ethanol was the disperser solvent (Figure 6). Thus, ethanol was chosen the disperser solvent in

subsequent experiments. Using ethanol as a disperser, acceptable repeatability was achieved while the peak areas were higher than those of other solvents. Ethanol is the main parabens solvent that can dissolve them. Besides, it is completely miscible with water, thus also improving the extraction efficiency (Yang & Rasmuson, 2010).

As can be seen in this figure, peak areas of analytes with the use of acetonitrile as a disperser solvent were much higher than those of ethanol, although the cloudy mode was sometimes not formed with this solvent, which explains the reduced repeatability.

3.1.4 | Optimization of dispersive liquid–liquid microextraction conditions using a central composite design

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

An optimization procedure was carried out by employing the response surface method with a central composite design technique. This design includes four main parameters. By applying the central composite design method, including 31 experiments (runs) with 7 center points, the relation between various parameters and their response were obtained.

The low and high levels of these factors in the two-level factorial (full fraction) design were as follows: 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 performed. 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 randomly performed. Table S1 summarizes the design of experiments as uncoded and real values and shows the response value for the extraction of each analyte. Practical response obtained in each experiment (Table S1) was used to calculate the response descriptor model for each factor and the equation was obtained and applied (Table S2).

3.1.5 | Analysis of variance

The result and equations of the model were statistically analyzed by analysis of variance (ANOVA; Table S3). The ANOVA method predicts one-way effects, interactions, and the second-order factors on the response.

The probability value is defined as the *P* value. Parameters with *P* values lower than 0.05 in the ANOVA were indicated to have a significant effect on the response at a confidence level of 95%.

The *F*-value is the ratio of mean square for the individual term to the mean square for the residual.

To test the null hypothesis, both *F*-value and P-value are compared. In this way, the statistical significance of effects can be estimated. The ANOVA results for MP are considered as an example in Table S3. According to Table S3, all the variables had a significant effect on the response and had a $P \leq 0.05$. In this model, the *F*-value is high enough, which implies the model is significant (Fratoddi et al., 2018; Sadhukhan et al., 2016).

The significance of the model was evaluated by the lack-of-fit (LOF) test. 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 is significant, the model would be inappropriate for embedding empirical data. The *P*-value for the LOF in this study was 0.915 (Table S3), which 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, as R^2 coefficients compare experimental data and predict values by the model. For PP, the R^2 value was 94.77%, indicating that the data fitted well and only 5.23% of the total variance was not explained by the model. Furthermore, the adjusted R^2 is a modified version of R^2 for the number of predictors in a model, with an R^2 value between 0 and 100 indicating the linear relationship in the sample of data even when there is no basic relationship. The adjusted R^2 value gives the best estimate of the degree of relationship in the basic population. The adjusted R^2 value for MP is 90.19%, which reveals the satisfactory correlation between the experimental data and the obtained model.

Finally, the experimental data were analyzed by constructing a polynomial equation, which is a mathematical equation between the detector response for each analyte and each factor. The desirability function (DF) condition also was applied to get the optimal conditions, in which the maximal peak area was identified for each factor. The DF values are between 0 and 1, indicating a minimum and maximum value of optimal conditions, respectively (Azharul Islam et al., 2010; Trinh & Kang, 2010).

In this work, Minitab 17 was applied for the prediction of optimal values for each studied parameter, obtaining the DF value, and finding the desirable conditions profile.

The optimal conditions where the peak areas (responses) of each analyte meet its maximum value should be identified. Peak areas are the symbol of the efficiency of the method. The graphs of maximizing the desirable conditions to attain optimal conditions are presented in Figure S1.

The best efficiency was obtained by setting the pH of aqueous solution to 4.4, the amount of salt to 5.0%, and the volumes of glucose and ethanol to 127.0 and 774.0 μL, respectively (Figure S1).

Parabens are *para*-hydroxybenzoic acid which are hydrolyzed in the acidic environment. When in an ionized form, they disperse better in ethanol and can better absorb extraction solvents. This trend is similar to previous studies (Çabuk et al., 2012; Razavi & Es' haghi, 2019).

The glucose DES has hydroxyl groups (from glucose and ChCl) that can participate in the formation of a hydrogen bond, whereas the carboxy groups of parabens and the hydroxyl groups of DES form a hydrogen bond. Therefore, medium acidic pH would favor this phenomenon (Dai et al., 2014).

As the pH increased further, the peak areas decreased dramatically. Parabens are in ionic forms at pH higher than the pK_a values of

the analytes. It is difficult to absorb ionic form of analyte into the hydrophobe organic solvents. Therefore, pH adjustments were performed using an acetate buffer at 4.5.

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

As the amount of glucose was increased to 150 μL, the responses also rose at the same time for all analytes. Ethanol (774 μL) as the disperser solvent would be sufficient for dispersing throughout the solution and help in the extraction and preconcentration of analytes.

3.2 | Analytical performance

The proposed method was evaluated under optimal conditions, and the linear range, limit of detections, limit of quantifications, repeatability, enrichment factor, and extraction recovery were obtained for this purpose. At first, several paraben solutions with known concentration in buffer solution were prepared, which were subjected to DLLME, and then the analytical figures were calculated through these solutions.

Detection limit is defined as three times the signal-to-noise ratio. In other words, this corresponds to the minimum concentration of the analyte that produces the chromatogram peak area equal to three times the peak area of the noise (Prichodko et al., 2009). Limit of quantification is also calculated in same way, but the signal-to-noise ratio will be 10 (Table 1; Prichodko et al., 2009; Shrivastava & Gupta, 2011).

The reproducibility of the extraction procedure over 1 day and the repeatability of the method between 3 days at three levels of concentration (low, medium, and high) were considered. Details on the standard solutions of parabens prepared after performing the extraction procedure on them, the relative standard deviation between responses calculated, and the precision of the method (Prichodko, Mockunaite, et al., 2011) are presented in Table 2.

A comparison between the results of parabens extraction from the standard samples with the known concentration and the real sample under optimal conditions lead to find the recovery of the method and also the matrix effect. The relative standard deviation was calculated to check the accuracy of the method (Table 3).

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 four parabens in a concentration range of 0.01–5000 ng mL^{-1} were prepared for calibration curves, and the linear regression of peak area against standard concentrations was plotted individually for each analyte. The extraction procedure was repeated three times for each concentration level.

The pre-concentration factor expressed the ratio of the paraben concentration after extraction in the sedimented phase to its initial concentration (Lin et al., 2015; Xu et al., 2014).

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

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

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

where C_{sed} is the concentration of the analyte in the sedimented phase; C_{aq} is the concentration of the analyte in the aqueous phase; V_{sed} is the volume of the sedimented phase and V_a is the volume of the aqueous phase.

The final volume of the sedimented phase was 50 μL and the initial aqueous volume was 5 mL.

TABLE 2 The characteristics of the method (obtained analytical figures in optimal condition)

Analyte	Linear range, ng mL^{-1}	LOD, ng mL^{-1}	LOQ, ng mL^{-1}	R^2	EF (C_{sed}/C_{aq})	ER% (V_{sed}/V_{aq}) × EF × 100
MP	0.1–5000	0.03	0.1	0.9962	48	47.7
EP	0.5–5000	0.15	0.5	0.9955	55	55.4
PP	0.1–5000	0.04	0.1	0.9959	68	67.6
BP	0.1–5000	0.04	0.1	0.9934	75	75.1

Abbreviations: BP, butylparaben; C_{aq}, concentration of the analyte in the aqueous phase; C_{sed}, concentration of the analyte in the sedimented phase; EF, enrichment factor; EP, ethylparaben; ER, extraction recovery; LOD, limit of detection; LOQ, limit of quantification; MP, methylparaben; PP, propylparaben.

TABLE 3 The method precision parameters (inter- and intra-days precisions)

Parameter	Concentration, μg mL^{-1}	Methylparaben	Ethylparaben	Propylparaben	Butylparaben
Precision (n = 5)	0.001	4.4	3.7	6.8	5.9
	0.1	2.1	3.2	5.1	4.8
	1	1.8	2.2	4.9	3.5
Reproducibility (3 days) Concentration: 0.5 μg mL^{-1}		4.6	4.3	7.1	8.2

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 investigating the capability of the method for the extraction of parabens in real samples. Samples were prepared initially according to the "2.4. Preparation of real samples before extraction" section.

As 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.

The prepared pharmaceutical samples (2 mL) 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, and the solutions diluted by buffer solution to the mark.

Each sample (5 mL) was taken to perform DLLME under optimal conditions.

After extraction of the analytes by DLLME, the extracts were injected into an HPLC system for separation and determination of analytes. Amounts of parabens were determined in different samples using the standard addition curves. The results are presented in Table 4.

According to Table 4, paraben has been found in in different kinds of pharmaceutical samples. Therefore, it can be deduced that parabens as preservatives are usually added to these pharmaceuticals.

TABLE 4 Amounts of parabens in liquid pharmaceutical samples (dispersive extraction on real samples under optimal conditions)

Sample	MP (μg mL^{-1}) (n = 3)	RR%	EP (μg mL^{-1}) (n = 3)	RR%	PP (μg mL^{-1}) (n = 3)	RR%	BP (μg mL^{-1}) (n = 3)	RR%
Nasal drop	ND	–	ND	–	340 ± 2.3	90.2	179 ± 3.2	103.1
Syrup number 1	830 ± 5.1	80.9	ND	–	158 ± 4.2	82.3	148 ± 3.2	93.9
Syrup number 2	ND		240 ± 2.4	95.4	ND	–	209 ± 2.8	99.4
Syrup number 3	ND	–	ND	–	322 ± 4.7	101.2	ND	–
Ampoule number 1	ND	–	ND	–	713 ± 3.1	99.7	232.31 ± 3.1	98.2
Ampoule number 2	ND	–	ND	–	243 ± 4.3	83.2	ND	–

Abbreviations: BP, butylparaben; EP, ethylparaben; MP, methylparaben; ND, not defined; PP, propylparaben; RR, relative recovery.

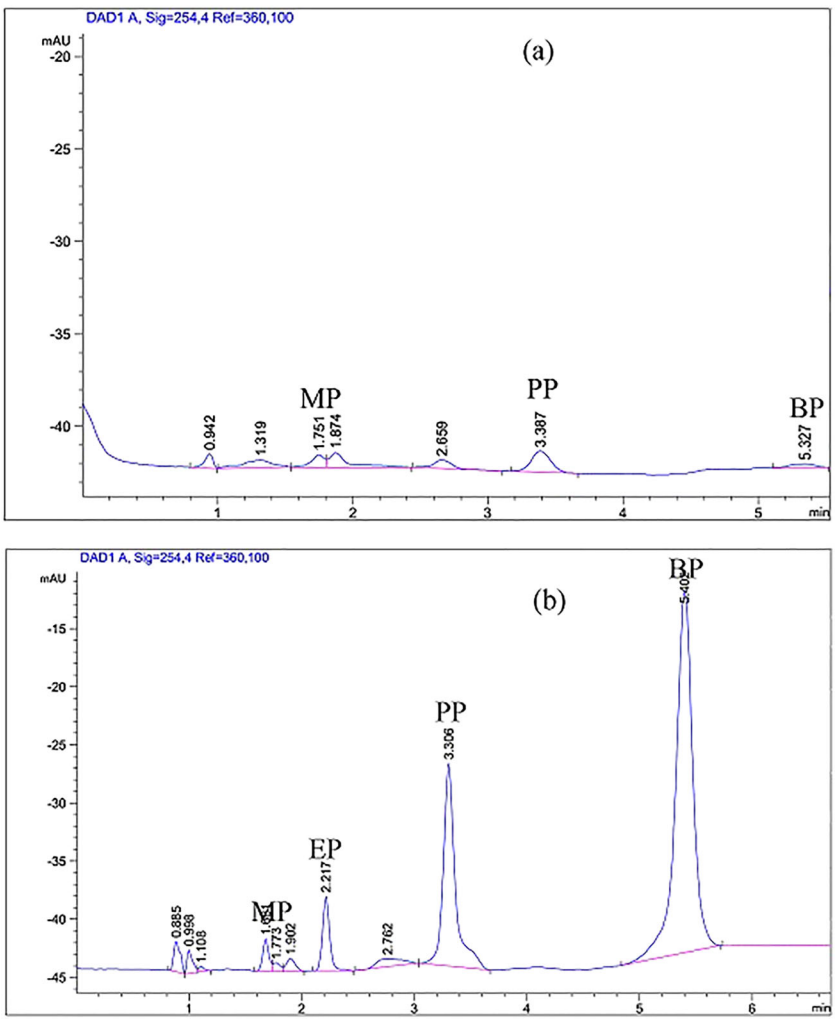

FIGURE 7 A sample chromatogram of the extraction of real sample (a) before and (b) after the addition of a standard solution. Extraction conditions: pH of aqueous solution, 4.4; amount of salt, 5%; and volumes of glucose and ethanol, 127 and 774 μL, respectively. BP, butylparaben; EP, ethylparaben; MP, methylparaben; PP, propylparaben.

Among the different parabens detected, PP and BP were the more commonly used species.

The accuracy of the method was evaluated by performing a 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 performed at different concentration levels in the pharmaceutical samples and the results are presented in Table 4.

The sample chromatogram (syrup sample) shown in Figure 7a is related to DLLME of pharmaceutical sample without adding a standard solution, whereas that in Figure 7b relates to the extraction after the addition of a standard solution.

The response of parabens significantly increased after DLLME, which indicated that the extraction using DES successfully preconcentrated the parabens.

3.4 | Comparison of the results of this study with similar previous studies

Table 5 provides a comparison of the results of this study with other extraction methods by emphasizing dispersive liquid–liquid extraction of parabens in different samples. Parabens can be found in almost all

TABLE 5 Comparison table (results of similar studies were compared)

Analyte	Analytical instrument	Extraction method	Real sample	Detection limit (μg mL^{-1})	Linearity range (μg mL^{-1})	Relative standard deviation (%)	Reference
MP EP PP BP	GC Flame ionization detector	DLLME	Lake water	0.003 for MP and EP; 0.002 for PP and BP	0.05–1	2.0–10.0%	Levchyk and Zui (2015)
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%	Çabuk et al. (2012)
MP EP PP BP Isopropyl paraben Isobutyl paraben Heptyl paraben Octyl paraben	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%	Shen et al. (2017)
MP EP PP BP Isobutyl paraben isoamylparaben	GC–flame ionization detector	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%	Wei et al. (2014)
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%	Ge et al. (2019)
MP EP PP BP	Capillary electrophoresis	DLLME with back-extraction	Human milk and other food samples (tomato paste, pickle, mixed fruit juice, and ice cream)	0.1–0.2	4.3–10.7	Lower than 3.5%	Alshana et al. (2015)
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	1.8–6.8	This study

Abbreviations: BP, butylparaben; DAD, diode array detector; DES, deep eutectic solvent; DLLME, dispersive liquid–liquid microextraction; EP, ethylparaben; MP, methylparaben; PP, propylparaben.

cosmetic and food samples and in all publications, four common parabens (BP, MP, EP, and PP) have been investigated. The optimized method exhibited comparable or lower limit of detection than previous methods. The same trend was seen for the linear range. It is thus quite clear that when there is a change in the conditions of the usual DLLME method, such as using a low-density organic solvent, solidification of floating organic drops, or using a DES, more satisfactory results are obtained. The lower limit of detections in our results can be attributed to the use of DES in the extraction procedure, which created better sensitivity. Extraction of parabens from pharmaceutical samples by DLLME was not considered in the past.

4 | CONCLUSION

In this study, we investigated DLLME of parabens from liquid pharmaceutical samples. Using DES and dispersing solvents, we extracted the spiked samples of paraben with a known concentration and the best extraction conditions (optimal) were obtained. Under the optimal conditions, analytical figures with a suitable linear range (0.1–5000) was obtained. The detection limit of the method was 0.04–0.15, indicating an acceptable level. The pre-concentration factor was in the average range for the dispersive method, although in some other studies, a much higher enrichment factor was obtained by using this extraction technique. The extraction and pre-concentration steps on pharmaceutical samples showed that one or more species of parabens are present in each medicinal sample tested. Parabens are widely used to increase the shelf life of drugs. Upon application in the extraction procedure, the DES enters into the reaction pathway, which helps to better extract the analytes. The thermogravimetric analysis, X-ray diffraction, and FT-IR results indicate that the synthesis of DES from glucose and ChCl was successful. The proposed method provides satisfactory analytical merits, is compatible with HPLC, reduces the danger of exposure to toxic solvents used for extraction in conventional extraction procedures, and requires a short extraction time and a low volume of the solvent. The method also showed low detection limits and a relatively broad linear concentration range.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

Alshana, U., Ertaş, N., & Göğer, N. G. (2015). Determination of parabens in human milk and other food samples by capillary electrophoresis after dispersive liquid–liquid microextraction with back-extraction. *Food Chemistry*, *181*, 1–8. https://doi.org/10.1016/j.foodchem.2015.02.074

Aroso, I. M., Silva, J. C., Mano, F., Ferreira, A. S., Dionísio, M., Sá-Nogueira, I., Barreiros, S., Reis, R. L., Paiva, A., & Duarte, A. R. C. (2016). Dissolution enhancement of active pharmaceutical ingredients by therapeutic deep eutectic systems. *European Journal of Pharmaceutics and Biopharmaceutics*, *98*, 57–66. https://doi.org/10.1016/j.ejpb.2015.11.002

Azharul Islam, M., Nikoloutsou, Z., Sakkas, V., Papatheodorou, M., & Albanis, T. (2010). Statistical optimisation by combination of response surface methodology and desirability function for removal of azo dye from aqueous solution. *International Journal of Environmental and Analytical Chemistry*, *90*(3–6), 497–509. https://doi.org/10.1080/03067310903094503

Berijani, S., Assadi, Y., Anbia, M., Milani Hosseini, M. R., & Aghaee, E. (2006). Dispersive liquid–liquid microextraction combined with gas chromatography-flame photometric detection: Very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water. *Journal of Chromatography A*, *1123*(1), 1–9. https://doi.org/10.1016/j.chroma.2006.05.010

Błędzka, D., Gromadzińska, J., & Wąsowicz, W. (2014). Parabens. From environmental studies to human health. *Environment International*, *67*, 27–42. https://doi.org/10.1016/j.envint.2014.02.007

Çabuk, H., Akyüz, M., & Ata, Ş. (2012). A simple solvent collection technique for a dispersive liquid–liquid microextraction of parabens from aqueous samples using low-density organic solvent. *Journal of Separation Science*, *35*(19), 2645–2652. https://doi.org/10.1002/jssc.201200317

Castelain, F., & Castelain, M. (2012). Parabens: A real hazard or a scare story? *European Journal of Dermatology*, *22*(6), 723–727. https://doi.org/10.1684/ejd.2012.1835

Chen, C.-W., Hsu, W. C., Lu, Y. C., Weng, J. R., & Feng, C. H. (2018). Determination of parabens using two microextraction methods coupled with capillary liquid chromatography-UV detection. *Food Chemistry*, *241*, 411–418. https://doi.org/10.1016/j.foodchem.2017.09.031

Chen, J., Li, Y., Wang, X., & Liu, W. (2019). Application of deep eutectic solvents in food analysis: A review. *Molecules*, *24*(24), 4594. https://doi.org/10.3390/molecules24244594

Croitoru, M. D., Miklos, A., Fogarasi, E., Coman, M., & Fülöp, I. (2016). Evaluation of the ingestion of Parabens during the use of dental care products by healthy human volunteers. *Life*, *9*(13), 14.

Cunha, S. C., & Fernandes, J. O. (2018). Extraction techniques with deep eutectic solvents. *TrAC Trends in Analytical Chemistry*, *105*, 225–239. https://doi.org/10.1016/j.trac.2018.05.001

Dai, Y., Verpoorte, R., & Choi, Y. H. (2014). Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (Carthamus tinctorius). *Food Chemistry*, *159*, 116–121. https://doi.org/10.1016/j.foodchem.2014.02.155

Darbre, P. D., Aljarrah, A., Miller, W. R., Coldham, N. G., Sauer, M. J., & Pope, G. (2004). Concentrations of parabens in human breast tumours. *Journal of Applied Toxicology: An International Journal*, *24*(1), 5–13. https://doi.org/10.1002/jat.958

Dil, E. A., Ghaedi, M., Asfaram, A., Tayebi, L., & Mehrabi, F. (2020). A ferrofluidic hydrophobic deep eutectic solvent for the extraction of doxycycline from urine, blood plasma and milk samples prior to its determination by high-performance liquid chromatography-ultraviolet. *Journal of Chromatography A*, *1613*, 460695.

Dodge, L. E., Kelley, K. E., Williams, P. L., Williams, M. A., Hernández-Díaz, S., Missmer, S. A., & Hauser, R. (2015). Medications as a source of paraben exposure. *Reproductive Toxicology*, *52*, 93–100. https://doi.org/10.1016/j.reprotox.2015.02.002

El Achkar, T., Greige-Gerges, H., & Fourmentin, S. (2021). Basics and properties of deep eutectic solvents: A review. *Environmental Chemistry Letters*, *19*(4), 3397–3408. https://doi.org/10.1007/s10311-021-01225-8

El-Deen, A. K., & Shimizu, K. (2020). A green air assisted-dispersive liquid-liquid microextraction based on solidification of a novel low viscous ternary deep eutectic solvent for the enrichment of endocrine disrupting compounds from water. *Journal of Chromatography A*, *1629*, 461498. https://doi.org/10.1016/j.chroma.2020.461498

Faraji, M. (2019). Determination of some red dyes in food samples using a hydrophobic deep eutectic solvent-based vortex assisted dispersive liquid-liquid microextraction coupled with high performance liquid chromatography. *Journal of Chromatography A*, *1591*, 15–23. https://doi.org/10.1016/j.chroma.2019.01.022

Fratoddi, I., Rapa, M., Testa, G., Venditti, I., Scaramuzzo, F. A., & Vinci, G. (2018). Response surface methodology for the optimization of phenolic compounds extraction from extra virgin olive oil with functionalized gold nanoparticles. *Microchemical Journal*, *138*, 430–437. https://doi.org/10.1016/j.microc.2018.01.043

Fuad, F. M., Nadzir, M. M., & Harun, A. (2021). Hydrophilic natural deep eutectic solvent: A review on physicochemical properties and extractability of bioactive compounds. *Journal of Molecular Liquids*, *339*, 116923. https://doi.org/10.1016/j.molliq.2021.116923

Ge, D., Wang, Y., Jiang, Q., & Dai, E. (2019). A deep eutectic solvent as an extraction solvent to separate and Preconcentrate Parabens in water samples using in situ liquid-liquid microextraction. *Journal of the Brazilian Chemical Society*, *30*(6), 1203–1210. https://doi.org/10.21577/0103-5053.20190014

Ge, D., Zhang, Y., Dai, Y., & Yang, S. (2018). Air-assisted dispersive liquid–liquid microextraction based on a new hydrophobic deep eutectic solvent for the preconcentration of benzophenone-type UV filters from aqueous samples. *Journal of Separation Science*, *41*(7), 1635–1643. https://doi.org/10.1002/jssc.201701282

Gioti, E. M., Skalkos, D. C., Fiamegos, Y. C., & Stalikas, C. D. (2005). Single-drop liquid-phase microextraction for the determination of hypericin, pseudohypericin and hyperforin in biological fluids by high performance liquid chromatography. *Journal of Chromatography a*, *1093*(1–2), 1–10. https://doi.org/10.1016/j.chroma.2005.07.038

Grecco, C. F., Souza, I. D., Acquaro, V. R., & Queiroz, M. E. (2019). Determination of parabens in breast milk samples by dispersive liquid-liquid microextraction (DLLME) and ultra-high-performance liquid chromatography tandem mass spectrometry. *Journal of the Brazilian Chemical Society*, *30*, 48–59.

Hansen, B. B., Spittle, S., Chen, B., Poe, D., Zhang, Y., Klein, J. M., Horton, A., Adhikari, L., Zelovich, T., Doherty, B. W., Gurkan, B., Maginn, E. J., Ragauskas, A., Dadmun, M., Zawodzinski, T. A., Baker, G. A., Tuckerman, M. E., Savinell, R. F., & Sangoro, J. R. (2020). Deep eutectic solvents: A review of fundamentals and applications. *Chemical Reviews*, *121*(3), 1232–1285. https://doi.org/10.1021/acs.chemrev.0c00385

Ibrahim, M., Alaam, M., el-Haes, H., Jalbout, A. F., & Leon, A. (2006). Analysis of the structure and vibrational spectra of glucose and fructose. *Ecletica Quimica*, *31*(3), 15–21. https://doi.org/10.1590/S0100-46702006000300002

Kashid, R. M., Singh, S. G., & Singh, S. (2011). Simultaneous determination of preservatives (methyl paraben and propyl paraben) in sucralfate suspension using high performance liquid chromatography. *Journal of Chemistry*, *8*(1), 340–346.

Kędzierska-Matysek, M., Arkadiusz, M., Mariusz, F., Joanna, B., Anna, W., Alicja, M., Edyta, C., Radosław, W., Dariusz, K., & Bożena, G. (2018). Application of FTIR spectroscopy for analysis of the quality of honey. In BIO web of conferences. EDP Sciences.

Lan, D., Wang, X., Zhou, P., Hollmann, F., & Wang, Y. (2017). Deep eutectic solvents as performance additives in biphasic reactions. *RSC Advances*, *7*(64), 40367–40370. https://doi.org/10.1039/C7RA06755K

Levchyk, V. M., & Zui, M. F. (2015). Gas chromatographic determination of parabens after derivatization and dispersive microextraction. *French-Ukrainian Journal of Chemistry*, *3*(2), 72–79. https://doi.org/10.17721/fujcV3I2P72-79

Lin, J., Chen, W., Zhu, H., & Wang, C. (2015). Determination of free and total phthalates in commercial whole milk products in different packaging materials by gas chromatography-mass spectrometry. *Journal of Dairy Science*, *98*(12), 8278–8284. https://doi.org/10.3168/jds.2015-10066

Liu, Y., Friesen, J. B., McAlpine, J. B., Lankin, D. C., Chen, S.-N., & Pauli, G. F. (2018). Natural deep eutectic solvents: Properties, applications, and perspectives. *Journal of Natural Products*, *81*(3), 679–690. https://doi.org/10.1021/acs.jnatprod.7b00945

Lomba, L., Ribate, M. P., Sangüesa, E., Concha, J., Garralaga, M. P., Errazquin, D., García, C. B., & Giner, B. (2021). Deep eutectic solvents: Are they safe? *Applied Sciences*, *11*(21), 10061. https://doi.org/10.3390/app112110061

Ma, M., Lee, T., & Kwong, E. (2002). Interaction of methylparaben preservative with selected sugars and sugar alcohols. *Journal of Pharmaceutical Sciences*, *91*(7), 1715–1723. https://doi.org/10.1002/jps.10167

Ma, S., Gao, F., Lu, W., Zhou, N., You, H., Li, J., & Chen, L. (2019). Dispersive liquid-liquid microextraction coupled with pressure-assisted electrokinetic injection for simultaneous enrichment of seven phenolic compounds in water samples followed by determination using capillary electrophoresis. *Journal of Separation Science*, *42*(13), 2263–2271. https://doi.org/10.1002/jssc.201900106

Ma, W., Tang, W., & Row, K. H. (2017). Characterization of deep eutectic solvents for dispersive liquid–liquid microextraction for phenolics. *Analytical Letters*, *50*(14), 2177–2188. https://doi.org/10.1080/00032719.2016.1277533

Mbous, Y. P., Hayyan, M., Hayyan, A., Wong, W. F., Hashim, M. A., & Looi, C. Y. (2017). Applications of deep eutectic solvents in biotechnology and bioengineering—Promises and challenges. *Biotechnology Advances*, *35*(2), 105–134. https://doi.org/10.1016/j.biotechadv.2016.11.006

Mulia, K., Krisanti, E., Terahadi, F., & Putri, S. (2015). Selected natural deep eutectic solvents for the extraction of α-mangostin from mangosteen (Garcinia mangostana L.) pericarp. *International Journal of Technology*, *6*(7), 1211–1220. https://doi.org/10.14716/ijtech.v6i7.1984

Pengionan, K. G. P. N. (2017). Extraction of methylparaben in cosmetics using dispersive liquid-liquid microextraction based on solidification of floating organic drop coupled with gas chromatography flame ionization detector. *Malaysian Journal of Analytical Sciences*, *21*(6), 1289–1298.

Prichodko, A., Jonusaite, K., & Vickackaite, V. (2009). Hollow fibre liquid phase microextraction of parabens. *Open Chemistry*, *7*(3), 285–290. https://doi.org/10.2478/s11532-009-0047-6

Prichodko, A., Mockūnaitė, M., Šmitienė, V., & Vičkačkaitė, V. (2011). Hollow fiber liquid phase microextraction of derivatized parabens. *Chemija*, *22*(3), 155–161.

Qian, F. (2019). Manufacturing method of preservative-free facial mask and process equipment thereof. Google Patents.

Razavi, N., & Es' haghi, Z. (2019). Curcumin loaded magnetic graphene oxide solid-phase extraction for the determination of parabens in toothpaste and mouthwash coupled with high performance liquid chromatography. *Microchemical Journal*, *148*, 616–625. https://doi.org/10.1016/j.microc.2019.04.057

Sadhukhan, B., Mondal, N. K., & Chattoraj, S. (2016). Optimisation using central composite design (CCD) and the desirability function for sorption of methylene blue from aqueous solution onto Lemna major. *Karbala International Journal of Modern Science*, *2*(3), 145–155. https://doi.org/10.1016/j.kijoms.2016.03.005

Sarafraz-Yazdi, A., & Amiri, A. (2010). Liquid-phase microextraction. *TrAC Trends in Analytical Chemistry*, *29*(1), 1–14. https://doi.org/10.1016/j.trac.2009.10.003

Sarafraz-Yazdi, A., & Es' Haghi, Z. (2006). Comparison of hollow fiber and single-drop liquid-phase microextraction techniques for HPLC determination of aniline derivatives in water. *Chromatographia*, *63*(11–12), 563–569. https://doi.org/10.1365/s10337-006-0801-2

Saraji, M., & Boroujeni, M. K. (2014). Recent developments in dispersive liquid–liquid microextraction. *Analytical and Bioanalytical Chemistry*, *406*(8), 2027–2066. https://doi.org/10.1007/s00216-013-7467-z

Shen, X., Liang, J., Zheng, L., Lv, Q., & Wang, H. (2017). Application of dispersive liquid–liquid microextraction for the preconcentration of eight

parabens in real samples and their determination by high-performance liquid chromatography. *Journal of Separation Science*, *40*(22), 4385–4393. https://doi.org/10.1002/jssc.201700722

Shishov, A., Bulatov, A., Locatelli, M., Carradori, S., & Andruch, V. (2017). Application of deep eutectic solvents in analytical chemistry. A review. *Microchemical Journal*, *135*, 33–38. https://doi.org/10.1016/j.microc.2017.07.015

Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*(1), 21. https://doi.org/10.4103/2229-5186.79345

Tabrizi, A. B., & Rezazadeh, A. (2012). Development of a dispersive liquid-liquid microextraction technique for the extraction and spectrofluorimetric determination of fluoxetine in pharmaceutical formulations and human urine. *Advanced Pharmaceutical Bulletin*, *2*(2), 157.

Trinh, T. K., & Kang, L.-S. (2010). Application of response surface method as an experimental design to optimize coagulation tests. *Environmental Engineering Research*, *15*(2), 63–70. https://doi.org/10.4491/eer.2010.15.2.063

Troter, D., Zlatković, M., Đorđević, B., Đokić-Stojanović, D., Konstantinović, S., Todorović, Z., & Veljković, V. (2016). Preparation and FTIR characterization of choline chloride–based deep eutectic solvents with thiourea and dimethylurea. Paper presented at the Physical Chemistry 2016 13th International Conference on Fundamental and Applied Aspects of Physical Chemistry.

Wei, H., Yang, J., Zhang, H., & Shi, Y. (2014). Dispersive liquid-liquid microextraction for simultaneous determination of six parabens in aqueous cosmetics. *Chemical Research in Chinese Universities*, *30*(3), 368–373. https://doi.org/10.1007/s40242-014-3566-4

Xu, D., Deng, X., Fang, E., Zheng, X., Zhou, Y., Lin, L., Chen, L., Wu, M., & Huang, Z. (2014). Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, *1324*, 49–56. https://doi.org/10.1016/j.chroma.2013.11.017

Yang, H., & Rasmuson, Å. C. (2010). Solubility of butyl paraben in methanol, ethanol, propanol, ethyl acetate, acetone, and acetonitrile. *Journal of Chemical & Engineering Data*, *55*(11), 5091–5093. https://doi.org/10.1021/je1006289

Yazdi, A. S., Razavi, N., & Yazdinejad, S. R. (2008). Separation and determination of amitriptyline and nortriptyline by dispersive liquid–liquid microextraction combined with gas chromatography flame ionization detection. *Talanta*, *75*(5), 1293–1299. https://doi.org/10.1016/j.talanta.2008.01.039

Zhang, K., Li, S., Liu, C., Wang, Q., Wang, Y., & Fan, J. (2019). A hydrophobic deep eutectic solvent-based vortex-assisted dispersive liquid–liquid microextraction combined with HPLC for the determination of nitrite in water and biological samples. *Journal of Separation Science*, *42*(2), 574–581. https://doi.org/10.1002/jssc.201800921

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