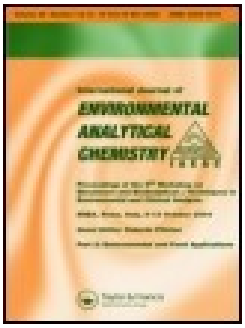

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ARTICLE

A novel vortex-assisted liquid phase microextraction method for parabens in cosmetic oil products using deep eutectic solvent

Sezen Sivrikaya

Polymer Engineering Department, Technology Faculty, Duzce University, Duzce, Turkey

ABSTRACT

The parabens, which are harmful to our bodies, are primarily utilized as preservatives in medicine, personal care products and cosmetics. A novel, more efficient, fast and cheap vortex-assisted liquid phase microextraction method based on deep eutectic solvents (DESs) was developed for the determination of parabens. The microextraction conditions were optimized using these solvents and the analytical parameters of the method were determined under optimal microextraction conditions. After extraction, the chromatographic separation of parabens was undertaken using high-performance liquid chromatography-UV detection. Experimental parameters, such as DES type, DES volume, dilution solvent volume and vortex extraction time were optimized. DES6 [ChCl-Ethylene glycol (1/2)] was the most suitable DES to work in this study. Detection limits for this method of 0.053 μg mL^{-1} for methylparaben, 0.061 μg mL^{-1} for ethylparaben, 0.049 μg mL^{-1} for propylparaben and 0.052 μg mL^{-1} for butylparaben were obtained. Correlation coefficients (R^2) for a concentration range of 0.1–100 μg mL^{-1} were higher than 0.9992 and relative standard deviation (RSD) values below 2.91% at parabens concentration of 2.5 μg mL^{-1} were obtained. The results of spike/recovery values of real samples were greater than 84%. When compared with other methods, the main advantages include lower LOD, short extraction time, rapidity, repeatability and simplicity.

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Parabens; deep eutectic solvents; vortex-assisted liquid phase microextraction; cosmetic oils

1. Introduction

In order to prevent aging and deterioration, many preservatives and additives are widely utilized in food, medicine and cosmetic products and thus extend shelf life [1,2]. Consumers are exposed to many chemicals through the ingestion of processed food and pharmaceutical products, or the application of personal care products and cosmetics. However, the tendency to allergic stimulation of these protectors may be harmful to consumers [3–5].

The parabens are p-hydroxybenzoates or alkyl esters of parahydroxybenzoic acid. This class of chemical includes methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BP), isobutylparaben, isopropylparaben and benzylparaben and their sodium salts [1,6]. Parabens are widely used as antifungal and antimicrobial agents in over 13,200 kinds of beverages, cosmetics and pharmaceuticals, because of their ability

CONTACT Sezen Sivrikaya sezensivrikaya@duzce.edu.tr

to stabilize, their broad antimicrobial spectrum and because they are non-volatile [1,3,6,7]. The most commonly used ones are the first four parabens, namely, methyl, ethyl, propyl and butyl paraben.

Studies on parabens have shown that parabens are genotoxic, may disrupt the endocrine system, possibly cause breast cancer and reduce sperm production [8–12]. In addition, different studies have also noticed the negative influences of parabens on reproductivity [13,14]. In order to deal with such problems, various regulatory bodies have introduced restrictions on the use of parabens in cosmetic and food products [15]. In addition, the European Union permits the use of parabens in cosmetic products with a maximum concentration of each paraben of 0.4% (w/w) and a total maximum concentration of 0.8% (w/w) expressed as p-hydroxybenzoic acid (EU Cosmetics Directive 76/768/EEC) [16–18].

From the standpoint of isolation and purification, it is difficult to remove impurities and identify parabens because of the complex sample matrices in which they are contained. A sample pre-treatment for the paraben determination in products is an important step to consider [19,20]. Many methods have been informed for the determination of the analyte from different media, such as solid phase microextraction (SPME), solid phase extraction (SPE) and molecular imprint polymers for stirring bar extraction. In addition, there are many advantages due to the fact that liquid phase micro extraction (LPME), shorter extraction time than SPME and mass transfer effect and a more environmentally friendly extraction process [15,21–26]. Many LPME techniques have been advanced for the extraction and determination of paraben groups from complex matrices. These include ultrasound-assisted dispersive liquid-liquid microextraction, single-drop microextraction, dispersive liquid-liquid microextraction (DLLME), stirring-assisted drop breakup microextraction, and solidified floating vesicular coacervative drop microextraction [14,15,21,27–29].

High-density chlorinated organic solvents (such as chlorobenzene, chloromethane, carbon tetrachloride and chloroform) were used in initial studies in applications involving liquid-phase microextraction. However, chlorinated solvents are extremely toxic and harmful to the environment. More recently, alternative eco-friendly solvent microextraction methods using long carbon chain alcohols, ionic liquids, and supramolecular solvents have been preferred [30,31]. Interest in microextraction methods using deep eutectic solvents (DESs) has increased because they are more environmentally friendly, economic and more useful than conventional solvents [32–35].

Deep eutectic solvents (DES) are a mixture of quaternary ammonium salts and hydrogen donors such as amines and carboxylic acids. The DESs form a two-layer eutectic mixture, forming a liquid with a much lower melting point than the constituent components. The melting point is caused by hydrogen bonds between quaternary ammonium salts and hydrogen donors [32,36,37]. They are preferred for extraction because of their many advantages, especially because of their high polarity and their ability to dissolve many organic and inorganic substances which are insoluble in conventional solvents. In addition, the DESs are classified as green solvents because they contain non-toxic components. They are also preferred because they are cheaper in industrial applications. DESs obtained from choline chloride have many advantages such as low cost, ease of preparation by mixing of components, do not need much preliminary preparation, are often biodegradable, have good biocompatibility and are non-toxic. They also have the advantage of being capable of mixing with water and certain organic solvents. In recent years they have also been used in microextraction-based analyses of organic materials in different environments [38–40].

The aim of this study is to develop a sensitive and specific method of analysis that can be used to identify and quantify parabens found in cosmetic oils for the protection of human health. The aim was to develop a method that is fast, easy to apply and economical. Recently, deep eutectic solvents have attracted considerable attention because they are economical and are more useful compared with classical solvents. In this study, a vortex-assisted liquid phase microextraction (V-LPME) method based on DES was developed. The developed method was compared with the existing methods in the literature and the advantages of the method were determined.

2. Experimental

2.1. Reagents and solutions

All chemicals used in this study were of analytical grade. Choline chloride (ChCl) (98%), urea (99%), glycerol (99%), ethylene glycol (>99%) and HPLC grade acetonitrile were bought from Sigma Aldrich (Steinheim, Germany). Methyl paraben, ethlyparaben, propylparaben and butlyparaben with purities higher than 98.5% were used as standards and were purchased from the Sigma Aldrich (Steinheim, Germany). Distilled water was obtained using a Milli-Q system (Millipore, Billerica, MA, USA).

A stock solution of parabens (methyl, ethyl, propyl and butyl) was initially prepared at 1000 µg mL^{-1} in acetonitrile, and was stored at 4°C. The working standard solutions were prepared by the suitable dilution of the stock solutions in acetonitrile. The cosmetic oil samples were purchased from commercial suppliers in Sakarya and Duzce, Turkey. The samples were first analyzed and those without parabens were selected for optimization and validation studies. These samples were then spiked with different concentration of paraben standards and followed by microextraction procedure.

2.2. Instrumentation

The chromatographic analysis was used an Agilent Technologies 1200 Series High Performance Liquid Chromatograph (HPLC) equipped with G1329A autosampler, a G1316A column oven, a G1314B UV-detector, a G1311A quaternary pump and G1322A degasser (Agilent, USA). Agilent ChemStation software v.B.04.02 was used for instrument control and data analysis. An ACE-C18 column (4.6 i.d.×250 mm, 5 µm, Mac-Mod, Chadds Ford, PA, USA) was used at chromatographic separation studies. A NF 200 centrifuge (Nüve, Ankara, Turkey), thermostatted hotplate stirrer (IKA C-MAG HS-7, Staufen, Germany) and a vortex mixer (VWR international model, Germany) were used for extraction of paraben in the sample preparation stage. A Fourier transform-infrared spectrometer Frontier Optica (PerkinElmer, Shelton, CT, USA) with ATR technique was used to define functional groups of DESs.

The chromatographic separation of parabens was obtained using a mobile phase composed of solvent A (water) and solvent B (acetonitrile) during a 35 min run by applying the following gradient program: 0 min – 35% solvent B; 0–25 min – 100% solvent B and 25–35 min–35% solvent B. Flow rate was adjusted as 1 mL min^{-1} and injection volume was adjusted as 20 µL, and the column temperature was adjusted as 30°C. The parabens were detected with a UV wavelength of 254 nm.

2.3. Preparation of DES

DESs were prepared using ChCl and hydrogen bond donors (urea, glycerol, ethylene glycol) at suitable molar ratios, followed by stirring with magnetic stirrer at 80–100°C until a clear, colorless liquid was composed. Table 1 indicates the molar ratios of hydrogen donors with ChCl and the obtained DES.

2.4. Vortex-assisted liquid phase microextraction procedure

Figure 1 shows a schematic diagram of vortex-assisted liquid phase microextraction procedure. In this procedure, 0.5 grams of cosmetic oil sample was put in a polypropylene tube. Then, 200 µL of DES was added and the mixture was rinsed for 10 s for the premixing. The obtained mixture was stirred by vortex for 3 min, and then centrifuged for 5 min at 4000 rpm. Two phases were separated. Finally, the lower phase was removed from the tube using a micropipette. The sample solution (20 µL) was injected in the HPLC-UV system for the determination of the parabens in cosmetic oil samples.

2.5. Optimization strategy

Effective analytical parameters, such as types and volume of DES, extraction time and volume of extraction solvent were studied. Optimization of these parameters was carried out using a 'one-factor at a time' experimental method with working solutions containing oil samples. All experiments were done in triplicate and the obtained average of the

Table 1. Created 6 DES types.

Abbreviation	Salt	HBDs	Ratio (salt: HBD)
DES1	ChCl	Urea	1:1
DES2		Urea	1:2
DES3		Glycerol	1:1
DES4		Glycerol	1:2
DES5		Ethylene glycol	1:1
DES6		Ethylene glycol	1:2

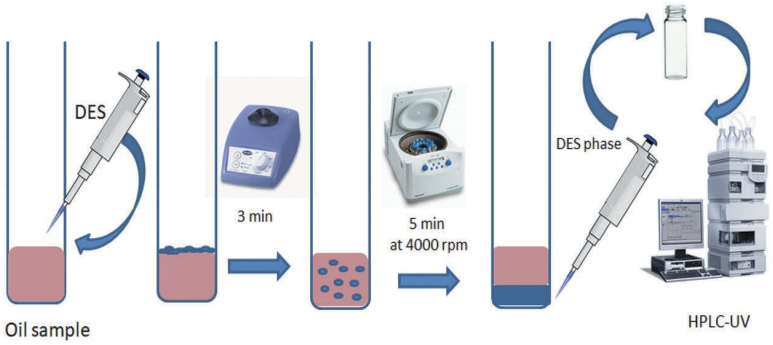

Figure 1. Schematic diagram of deep eutectic solvent based vortex-assisted liquid phase microextraction procedure.

results was used. The amount extracted under each of the conditions was determined and the extraction efficiency calculated accordingly.

3. Results and discussion

3.1. Characterization of DES

DES6 is formed by the hydrogen bond between choline chloride and ethylene glycol. FT-IR spectra of choline chloride, ethylene glycol and DES6 were made and are shown in Figure 2. When the FT-IR spectra are examined, the presence of an O-H vibration peak is seen at 3391 cm^{-1} for choline chloride and at 3374 cm^{-1} for ethylene glycol. The peak at 1086 cm^{-1} in the FTIR spectrum of choline chloride is indicative of the C-N vibration. While the O-H vibration in the spectrum of ethylene glycol is at 3374 cm^{-1}, the OH vibration of the DES6 is shifted to 3402 cm^{-1}. This change of O-H vibration shows the presence of hydrogen bonds between HBD and choline chloride when DES6 is obtained [34].

3.2. Effect of DES types

Six different DESs were prepared using ChCl and different HBDs. The extraction recoveries of four parabens (methyl, ethyl, propyl and butyl) for six different DESs are indicated in Figure 3. DES6 showed better extraction efficiency than the other DESs. The density of DES1 and DES5 was too high and therefore the study could not be performed using them. DES6 was found to have better extraction recovery than

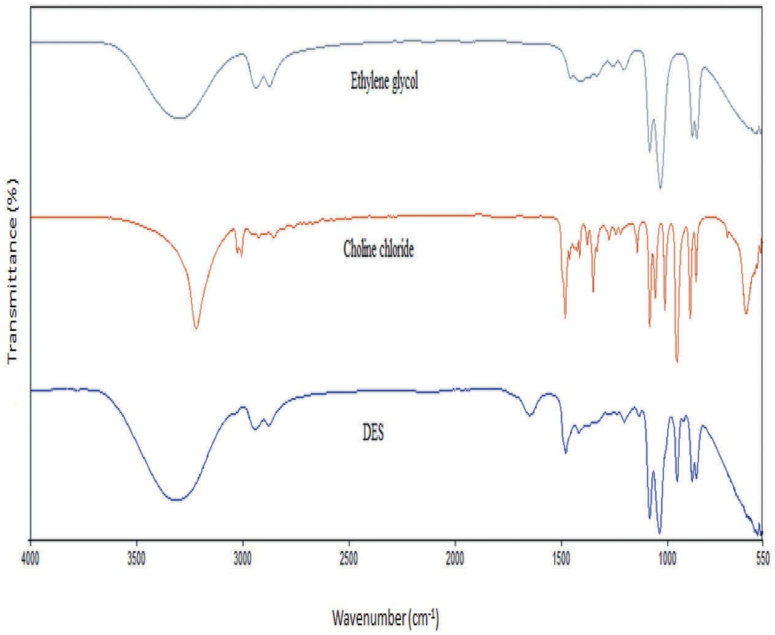

Figure 2. FT-IR spectra of choline chloride, ethylene glycol and DES6.

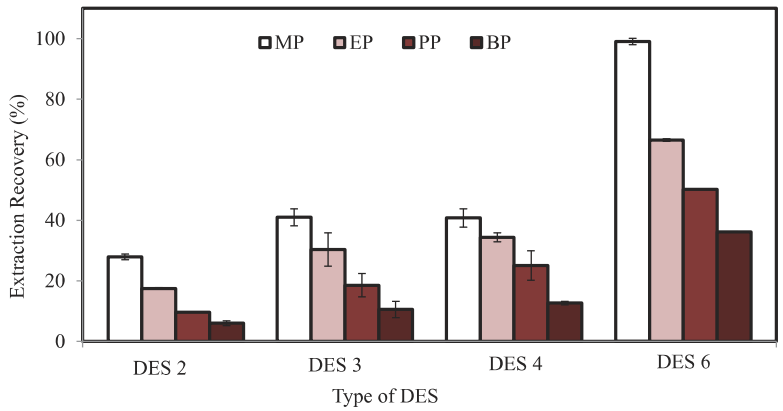

Figure 3. Effect of types of DESs (sample volume: 0.5 g; volume of DES: 200µL; extraction time: 3 min vortex extraction; volume of dilution solvent: 0.0 mL) N = 3.

other DESs. Due to the high recovery of extraction, the matrix may be bonded to viscosities which may prevent the solvent from dissipating.

3.3. Effect of DES volume

After determining the optimal DES, the effect of DES volume on extraction efficiency was then checked by using between 100 and 500 µL of DES6. When the examined of the results show that the maximum extraction recoveries for all parabens are quantitatively obtained at 200 µL. 200 µL DES was chosen as the optimum DES volume for subsequent studies.

3.4. Effect of dilution solvent volume

In this microextraction method, hexane was used as a dilution solvent for separating the parabens. The effect of hexane volume was changed within the range of 0.0 and 2.0 mL on the extraction recovery of parabens. The quantitative extraction was obtained when 0.0 mL of hexane was used. Therefore, since the extraction recovery was the same with and without the use of hexane, it was not used in further experiments. This was to avoid dilution of the analytes.

3.5. Effect of vortex time

The duration of contact between the type of mixing and the sample and solvent phases is important to ensure effective transfer of the target analyte in liquid phase microextraction studies. The vortex mixing apparatus was used to ensure DES6 interacted well with the parabens. Vortex mixing times of 0–10 min were investigated in this study. When the results are examined, it can be seen that duration of vortex mixing of over 3 minutes is required for the two phases to be completely separated from each other. A vortex time of 3 minutes is also suitable for obtaining a good recovery of parabens. A vortex time of 3 minutes was therefore chosen for the rest of the study.

3.6. Analytical performance

Calibration solutions of methyl, ethyl, propyl and butyl parabens were prepared over the concentration range 0.1–100 μg mL^{-1}. When analyzed under optimum working conditions, the regression equation, correlation coefficient, limit of detection (LOD), limit of quantification (LOQ) and relative standard deviation (RSD) were calculated from the calibration curves. The limit of detection (LOD) and the limit of quantification (LOQ) were defined as 3S/m and 10S/m, respectively; where S is the standard deviation of the eleven blank solutions and m is the slope of the calibration curves. These analytical performance values are shown in Table 2.

3.7. The application of developed method to real samples

The developed vortex-assisted liquid phase microextraction method was employed for the determination of parabens in cosmetic products such as massage, body, hair, nail, sun and eyelash and oils. The different brands of cosmetic oil samples were bought from cosmetic shopping markets. Parabens at various concentrations (5 and 50 μg mL^{-1}) were spiked into the selected cosmetic oils to determine the accuracy of the developed method. As can be seen in Table 3, the method of recovery of parabens in cosmetic oil samples was found to be highly reliable. The results clearly indicated that the spike/recovery results of real samples were greater than 84%, indicating that the method is accurate.

3.8. Comparison with other methods

The analytical performance of the vortex-assisted liquid phase microextraction method was compared with other extraction methods reported at literature. When compared with other methods, the main advantages include lower LOD, short extraction time, rapidity, repeatability and simplicity. As listed in Table 4, conventional methods have extraction times of between 5 and 90 minutes. This time period is often significantly greater than the 3–4 minutes required using this developed method. In addition, the fact that no organic solvent (hexane) is used in this study is another distinct advantage of this method. Finally, since the developed method requires low sample and organic solvent volumes, it can be regarded as being practical and environmentally friendly and an effective method for the separation of parabens from cosmetic oils has been achieved.

4. Conclusion

Six DESs were prepared with choline chloride and HBDs for the extraction of parabens from different cosmetic oils. It was determined that DES6, formed from choline chloride

Table 2. Analytical performance of the developed method.

Analyte	Linearity (μg mL^{-1})	Regression equation	Correlation coefficient (R^2)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	RSD (%) 2.5 μg mL^{-1}
Methylparaben	0.1–100	A = 208.25C + 25.69	0.9994	0.053	0.177	2.38
Ethylparaben	0.1–100	A = 148.76C + 106.35	0.9992	0.061	0.205	1.64
Propylparaben	0.1–100	A = 98.66C+ 50.76	0.9995	0.049	0.163	2.91
Butylparaben	0.1–100	A = 56.8C + 60.64	0.9992	0.052	0.174	2.16

Table 3. Application of developed method to real samples (N = 3).

		Massage oil		Body oil		Nail oil		Hair oil		Eyelash oil		Sun oil	
Parabens	Spiked level (μg mL^{-1})	Found (μg mL^{-1})	% R	Found (μg mL^{-1})	% R	Found (μg mL^{-1})	% R	Found (μg mL^{-1})	% R	Found (μg mL^{-1})	% R	Found (μg mL^{-1})	% R
MP	0	18.11	-	2.13	-	3.57	-	0.55	-	3.81	-	11.75	-
	5	22.94	99.26	7.18	100.7	8.04	93.84	4.81	86.69	8.28	94.00	14.88	88.82
	50	58.99	86.60	51.29	98.38	52.01	97.09	44.98	88.98	52.10	96.82	52.10	84.37
EP	0	10.21	-	-	-	-		0.49	-	-	-	-	
	5	14.65	96.34	4.98	99.61	5.02	100.3	4.67	84.97	4.63	92.64	4.73	94.61
	50	46.06	93.11	49.44	98.88	49.91	99.83	48.41	82.02	47.65	95.30	44.65	89.30
PP	0	-	-	-	-	1.25	-	0.30	-	3.15	-	-	-
	5	5.13	102.5	4.99	99.81	6.07	97.16	4.88	92.03	7.40	90.81	4.53	90.65
	50	45.15	90.30	49.41	98.82	51.68	100.8	47.75	84.99	52.57	98.91	45.57	91.15
BP	0	-	-	-	-	1.03	-	1.58	-	1.16	-	-	-
	5	4.94	98.86	5.02	100.4	6.13	101.7	5.59	84.99	5.52	89.64	4.29	85.82
	50	46.45	92.89	49.48	98.95	50.65	99.26	46.38	87.99	46.18	90.26	42.00	84.01

Table 4. Comparison of the developed method with other methods for the determination of parabens in cosmetics.

Sample Preparation methods	Instrument analysis methods	Sample matrix	Linearity range (μg L^{-1})	LOD (μg L^{-1})	RSD (%)	Extraction time (minute)	Ref.
VA-D-μ-SPE	HPLC-DAD	Waters, cosmetic creams, and human urine	10–600	1.5	<2.27	-	[41]
CL	HPLC	Wash-off cosmetics	4–7000	1.9–5.3	<3.1	-	[42]
SFE	LC–MS	Cosmetic products	10–2000	4.7–19.3	<18	5–20	[43]
SFVCDME	HPLC-UV	Cosmetic samples and water samples	0.5–100	0.2–0.5	<11.9	33	[44]
UA-DLLME-SFO	HPLC-MS /MS	Shampoo	0.5–100	0.062–0.343	<10	15	[45]
CZE	HPLC-UV	Foam shampoo	1000–40,000	20–50	<3.2	-	[46]
SAD–SPME	GC–PID	Mouth wash solution, hand creams, river water	0.2–100	0.05–0.3	<8	5	[47]
DLLME	GC–FID	Food, cosmetics and water	100–10,000	29–102	0.83–2.82	20 to 90	[48]
MSC	HPLC–DAD	cosmetics and personal care products	60–12,000	20–40	4.9	-	[49]
V-LPME	HPLC-UV	Cosmetic oil	100–100,000	48.7–63.5	1.64–4.48	3	This study

Sample Preparation methods: VA-D-μ-SPE: vortex-assisted dispersive micro-solid-phase extraction, CL: chemiluminescence, SFE: supercritical fluid extraction, SFVCDME: solidified floating vesicular coacervative drop microextraction, UA-DLLME-SFO: ultrasound assisted dispersive liquid–liquid microextraction based on the solidification of a floating organic drop, CZE: capillary zone electrophoresis, SAD-SPME: solvent assisted dispersive-solid phase microextraction, DLLME: dispersive liquid–liquid microextraction, MSC: multi-syringe chromatographic.

and ethylene glycol was the most effective for the extraction of parabens from cosmetic oils. The DES6 was synthesized and characterized, and then applied during the vortex-assisted liquid-phase microextraction of parabens from different cosmetic oil samples. The extraction of parabens with DES has never been studied before. The developed method for the extraction and detection of parabens in cosmetic oils is simple and environmentally friendly. The other advantages of this method are that it is very rapid, with an extraction time of as little as 3 minutes being required, it uses no organic solvents (hexane) and the LOD is very low compared with other methods. The results show that DES6 can quantitatively extract parabens from cosmetic oils when combined with vortex-assisted liquid phase microextraction (V-LPME). The methodology was validated using spike/recovery experiments with values greater than 84% for methyl, ethyl, propyl and butyl parabens being achieved.

Disclosure statement

No potential conflict of interest was reported by the author.

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