

A Vortex-Assisted Deep Eutectic Solvent for Parabens Extraction in Cosmetic Samples Coupled with High Performance Liquid Chromatography

(Pelarut Eutektik Dalam Berbantu Vortex untuk Pengekstrakan Paraben dalam Sampel Kosmetik Digandingkan dengan Kromatografi Cecair Berprestasi Tinggi)

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ABSTRACT

Parabens, commonly used preservatives in food, cosmetics, and pharmaceutical items, inhibit harmful germ growth. Deep Eutectic Solvents (DES) are intriguing for analyte extraction due to superior properties formed through hydrogen bond acceptors and donor interactions. However, the studies on the optimal DES-based extraction conditions for paraben-containing cosmetics and the accuracy of 'paraben-free' labels are limited. The most effective DES (DL-Menthol and decanoic acid, 1:2 molar ratio) was applied to cosmetic samples after heating at 80 °C following optimization parameters. FTIR study confirmed hydrogen bonds in DES samples. Both paraben-containing and paraben-free samples showed methyl, ethyl, and propyl paraben peaks from the HPLC analysis. Cosmetic sample paraben concentrations ranged from 0.53 mg/L to 26.08 mg/L (non-spiked). LOD and LOQ were 0.24 mg/L-1.62 mg/L, 0.73 mg/L, and 4.8 mg/L, respectively. Goals were met by establishing a paraben extraction method using DES and investigating paraben quantities in cosmetics.

Keywords: Cosmetic; deep eutectic solvent; high performance liquid chromatography; paraben; vortex

ABSTRAK

Paraben, bahan pengawet yang biasa digunakan dalam makanan, kosmetik dan bahan farmaseutikal, merencat pertumbuhan kuman berbahaya. Pelarut Eutektik (DES) menarik minat untuk pengekstrakan analit kerana sifat unggul yang terbentuk melalui interaksi penerima dan penderma ikatan hidrogen. Namun, kajian pengekstrakan menggunakan DES yang optimum untuk kosmetik mengandungi paraben dan ketepatan label 'bebas paraben' masih terhad. DES yang paling berkesan (DL-Menthol dan asid dekanolik, nisbah molar 1:2) digunakan pada sampel kosmetik selepas dipanaskan pada 80 °C mengikut pengoptimuman parameter. Kajian FTIR mengesahkan kewujudan ikatan hidrogen dalam sampel DES. Analisis HPLC menunjukkan puncak metil, etil dan propil paraben sama ada sampel mengandungi paraben atau bebas paraben. Kepekatan paraben dalam sampel kosmetik adalah dalam julat 0.53 mg/L hingga 26.08 mg/L (tidak disuntik dengan paraben). LOD dan LOQ masing-masing adalah 0.24 mg/L-1.62 mg/L, 0.73 mg/L, dan 4.8 mg/L. Matlamat telah tercapai dengan membentuk kaedah pengekstrakan paraben yang berkesan menggunakan DES dan mengkaji kuantiti paraben dalam kosmetik.

Kata kunci: Kosmetik; kromatografi cecair berprestasi tinggi; paraben; pelarut eutektik; vorteks

INTRODUCTION

Numerous preservatives and additives are frequently used in food, medication, and cosmetic products to delay aging and deterioration and increase shelf life (Antakli,

Kabani & Shawa 2013). Through the consumption of processed foods, pharmaceuticals, or the use of cosmetics and personal care products, consumers were exposed to a variety of chemicals. However, these protectors'

propensity for allergic stimulation could harm users. The parabens are frequently utilised as preservatives to prevent the growth of unfavourable bacteria in food, cosmetics, personal care, and pharmaceutical items. However, according to several studies, parabens possess the potential to harm human health due to their link to estrogenic and carcinogenic risks (Ge et al. 2020).

Parabens are less likely to trigger an adverse reaction in babies than commercial preservatives. In the human body, parabens mimic the effects of the estrogen hormone in women. Numerous worries exist over how this can impact people's health (Gosens et al. 2013). Along with their antibacterial properties, parabens also have an estrogenic action that has the potential to either benefit or harm the endocrine system. Additionally, it has been discovered that parabens promote the growth of breast cancer cells in culture (Özak 2020). Due to rising consumer awareness regarding the safety of cosmetic products, many paraben-free cosmetics have been offered in recent years. However, because of the rising demand, cosmetic manufacturers falsify the 'paraben-free' designation on cosmetic samples (Yıldız & Çabuk 2018).

Hence, it is important in developing a precise and dependable approach to identifying parabens in cosmetic products is crucial. Thus, making the appropriate parabens determination is vital. Gas chromatography, capillary electrophoresis, high-performance liquid chromatography, liquid chromatography-mass spectrometry and immunological methods are examples of analytical methods developed for the detection and quantification of parabens in various matrices, including cosmetics (Beh et al. 2021; Hikmawanti et al. 2021). However, high-performance liquid chromatography (HPLC) is chosen in this study because it offers high sensitivity and selectivity (Hikmawanti et al. 2021).

Typically, a paraben can be identified by name, such as methylparaben, propylparaben, butylparaben, or ethyl paraben (FDA 2022). The FDA has no specific requirements that apply only to preservatives used in cosmetics. The law treats cosmetic preservatives the same as other cosmetic components. Under the Federal Food, Drug, and Cosmetic Act, cosmetic goods and ingredients (apart from colour additives) are exempt from FDA examination (FD&C Act). The maximum residue limit (MRL) of parabens in cosmetics can vary depending on the specific paraben compound and the regulatory agency overseeing cosmetics. In the European Union (EU), the Scientific Committee on Consumer Safety (SCCS) has established specific concentration limits

for different parabens in cosmetic products (Yih et al. 2019). For example, methyl paraben and ethyl paraben individually or in combination should not exceed 0.8% of the total content of the finished product (Yih et al. 2019). However, it is unlawful to advertise a cosmetic in interstate commerce if it is tainted or mislabelled (FDA 2022).

The Greek word 'eutectic', which means low melting, refers to substances with a lower melting point than their constituents (El-Deen & Shimizu 2021). As opposed to other solvents, they also have the benefits of a low eutectic point, specific polarity, surface tension, and thermal stability, as well as low toxicity and minimal environmental impact. DESs are frequently made by combining a hydrogen bond acceptor (HBA) like choline chloride with a hydrogen bond donor (HBD) such as phenols, carboxylic acids, sugars, urea, or polyalcohols in a specific molar ratio (Florindo, Branco & Marrucho 2019). They are viewed as a unique family of ionic liquids because of their similar physical features (ILs). Some ionic liquids have some downsides, including toxicity, preparation requirements, and expensive costs (Shamsuri & Kuang 2011). DESs differ from ILs in that they are more affordable, environmentally friendly, simple to prepare with high purity, and biodegradable (Ge et al. 2019). Deep eutectic solvents, commonly called 'green solvents', were introduced to the solvent community as potential sustainable substitute extraction solvents.

The usage of the terms 'non-toxicity' and 'biodegradability' must be taken into consideration. However, the high density and viscosity of DESs make them more challenging to be utilized than conventional solvents (Li et al. 2020). Because of this, higher temperatures or other parameters are frequently required. In addition, the physicochemical properties of the DESs can be changed by selecting the right HBD and HBA and their molar ratios, as well as by including water (Bazmandegan-Shamili et al. 2018).

In this study, parameter studies were conducted on molar ratio, pH, and vortex time to achieve the optimum conditions for extracting paraben from actual cosmetic samples by the synthesized DES. This research applied DL-Menthol and decanoic acid as HBA and HBD, respectively, in a molar ratio of 1:2 to investigate the concentration of parabens in actual cosmetics. These chemicals are chosen because these compounds are biodegradable, have low toxicity level and easily available (Li et al. 2020). The benefit of this study is to give awareness to consumers about the paraben levels in their daily personal care products.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

TABLE 1. List of chemicals

Chemicals	Purity	Brand	Exporter
DL-Menthol	>= 95%	Sigma-Aldrich	German
Polyethylene glycol	-	Aldrich Chemistry	German
Decanoic acid	>= 98%	Sigma-Aldrich	German
Choline chloride	98%	Sigma-Aldrich	German
Ethylene glycol	99.5%	Qrec (Asia) Sdn Bhd	Malaysia
Glucose	-	John Kollin Chemicals	United States
Hydrochloric acid	37%	R&M Chemicals	Malaysia
Sodium hydroxide	>= 98%	Sigma-Aldrich	German
Sodium chloride	99.5%	Bendosen	Malaysia
Methanol	99.99%	Fisher Chemicals	United Kingdom
Methyl p-hydroxybenzoate	99%	Aldrich Chemistry	German
Ethyl p-hydroxybenzoate	>= 99%	Sigma-Aldrich	German
Propyl p-hydroxybenzoate	99%	Aldrich Chemistry	German
Benzyl p-hydroxybenzoate	-	Sigma-Aldrich	German

INSTRUMENTATION

The HPLC model used was an Agilent 1100s HPLC manufactured by Spectralab Scientific Inc, Canada. Separation was performed on a Zorbax SB-C18 column (150 × 4.6 mm² ID, particle size 5 μm). A gradient elution mode was used with a flow rate of 1.0 mL min⁻¹. A mixture of water (A) and methanol (B) was used as the mobile phase. The elution profile was as follows: starting with 40% A and 60% B. The injection volume was 10 μL. The detection wavelength was 254 nm (Ge et al. 2019).

PREPARATION OF DES

The preparation of DES was adapted from the study by Ge et al. (2019). HBA and HBD were combined in the proper molar ratios to create DESSs, which were then stirred with a magnetic stirrer at a temperature between 80 °C and 100 °C to create a clear, colourless liquid (Figure 2). The acquired DESSs were stored in bottles till usage and chilled to room temperature. The molar ratios of HBA and HBD for DES synthesis are shown in Table 2. Figure 1 shows the pairs of HBA and HBD for DES

preparation and the molar ratio can be referred to Table 2. Figure 2 shows the procedure of making DES while Figure 3 shows the method of extracting parabens by using actual samples.

PREPARATION OF STANDARD CURVE

For the preparation of the stock solution, 40 mg of methyl paraben was measured and placed in a 100 mL volumetric flask followed by the addition of 70% ethanol to the volume. The procedure was repeated for the stock solutions preparation of ethyl, propyl, and benzyl parabens. The prepared stock solutions were stored at the temperature of 4 °C until usage. For the standard curve calibration, 0.25, 0.50, 0.75, 1.0 and 1.25 mL of each stock standard were added, respectively, in 50 mL volumetric flasks followed by dilution with 70% v/v ethanol to volume. These dilutions created the following concentrations of standard solutions: 2, 4, 6, 8, and 10 ppm, respectively. A syringe with a filter was used to collect, filter and place 1.25 mL from each concentration inside the HPLC vial for RP-HPLC analysis.

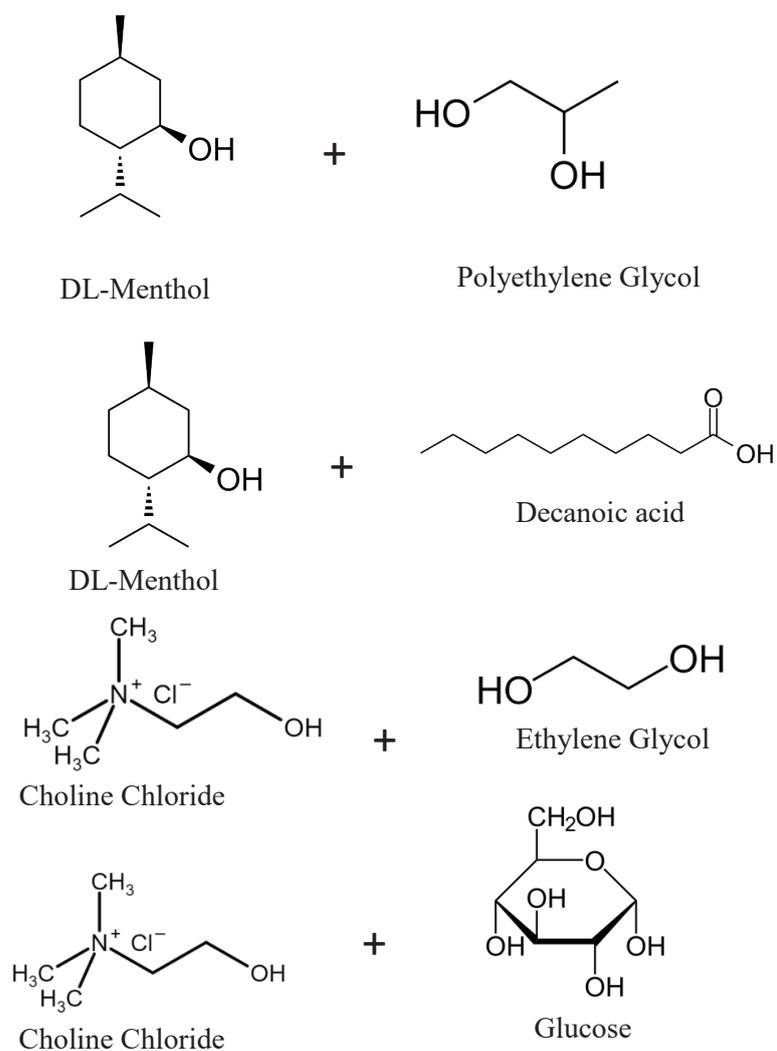


FIGURE 1. Molecular Structure of HBD and HBD for DES preparation

TABLE 2. DES selection type of parameter optimizer (Ariffin et al. 2019)

Abbreviation	Hydrogen bond acceptor	Hydrogen bond donor	Molar ratio
DES-1			1:1
DES-2		Polyethylene glycol (PG)	1:2
DES-2	DL-Menthol (DL)		2:1
DES-4			1:1
DES-5		Decanoic acid (DA)	1:2
DES-6			2:1
DES-7			1:1
DES-8		Ethylene glycol (EG)	1:2
DES-9	Choline chloride (CC)		2:1
DES-10			1:1
DES-11		Glucose (G)	1:2
DES-12			2:1

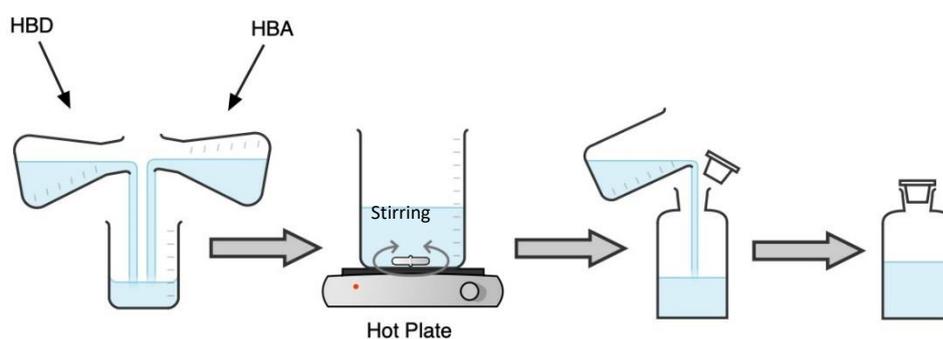


FIGURE 2. Procedure of preparing deep eutectic solvent

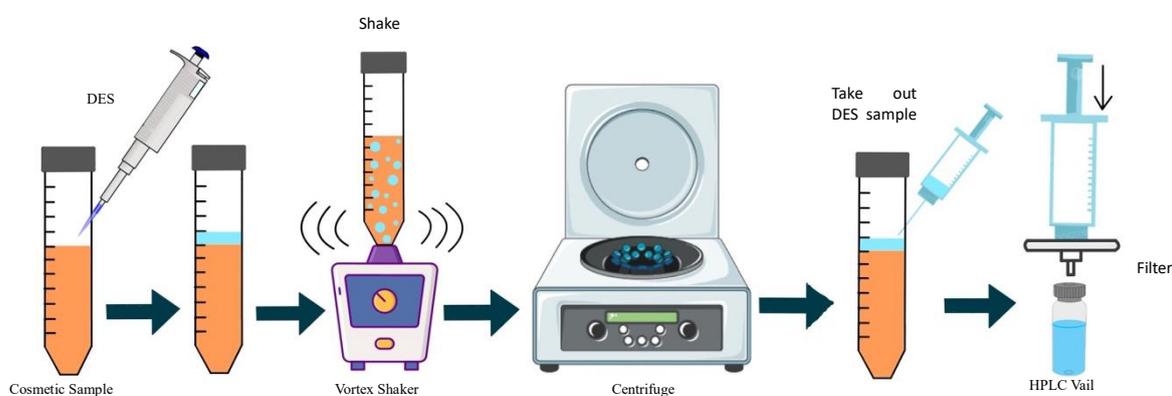


FIGURE 3. The procedure of extracting parabens by using actual samples

FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

The process for synthesizing DES involved combining the hydrogen bond acceptor (HBA) and hydrogen donor (HBD), following the molar ratio stated in Table 2, in Scott's bottles at the temperature of 60 °C to 80 °C with continuous magnetic stirring until a clear liquid form. The prepared DES was cooled to ambient temperature and kept in a desiccator until needed. The resulting DES samples were analyzed using FTIR to confirm the hydrogen bond formation between HBA and HBD.

DETERMINATION OF OPTIMUM DES SYSTEMS FOR PARABEN EXTRACTION

DES solvents with the four best combinations of hydrogen bond donors (HBDs) and acceptors (HBAs) were chosen. Choline chloride (HBA) and glucose (HBD), DL-

Menthol (HBA) and decanoic acid (HBD), and choline chloride (HBA) and ethylene glycol (HBD) are among the four forms of DES (HBD).

MOLAR RATIO

The DES has been prepared according to Table 2. 2 mL of the prepared DES was mixed with 8 mL of the sample into a centrifuge tube. The 8 mL sample constituted of standard 2 mL methyl p-hydroxybenzoate (MP), 2 mL ethyl p-hydroxybenzoate (EP), 2 mL propyl p-hydroxybenzoate (PP) and 2 mL of benzyl p-hydroxybenzoate (BP). The mixture was then vortexed for 1 min. When a cloudy solution containing fine DES droplets appeared, the solution was centrifuged (5000 rpm, 4 min). The DES phase that settled at the top of the centrifuge tube was collected and analysed using RP-HPLC (Figure 3).

SOLUBILITY OF DES IN WATER

In the previous experiment, the DES with the highest concentration of alkyl paraben detection was combined with ultrapure water at a volume ratio of 1:3 (Razavi et al. 2022). The mixture was centrifuged at 5000 rpm for 4 min after being vortexed for 30 s. Observations on the miscibility of the mixture were done once water was added to confirm the solubility of DES.

EFFECT OF VORTEX TIME

The DES with the highest concentration of alkyl paraben detection in the previous experiment was mixed with 8 mL of samples in the centrifuge tube. The 8 mL sample constituted of standard 2 mL methyl p-hydroxybenzoate (MP), 2 mL ethyl p-hydroxybenzoate (EP), 2 mL propyl p-hydroxybenzoate (PP) and 2 mL of benzyl p-hydroxybenzoate (BP). The mixture was then vortexed for different times of 1, 2, and 5 min, respectively. When a cloudy solution containing fine DES droplets appeared, the solution was centrifuged (5000 rpm, 4 min). The DES phase that settled at the top of the centrifuge tube was collected and analysed using RP-HPLC.

EFFECT OF AQUEOUS PHASE pH

The DES with the highest concentration of alkyl paraben detection in the previous experiment was mixed with 8 mL of samples in the centrifuge tube. The 8 mL sample constituted of standard 2 mL methyl p-hydroxybenzoate (MP), 2 mL ethyl p-hydroxybenzoate (EP), 2 mL propyl p-hydroxybenzoate (PP) and 2 mL of benzyl p-hydroxybenzoate (BP). The pH value of the aqueous solution was adjusted between 2, 5, 7, and 12 using 0.1 M HCl or 0.1 M NaOH. The mixture was then vortexed for 1 min because it gave the highest concentration of alkyl paraben detection. When a cloudy solution containing fine DES droplets appeared, the solution was centrifuged (5000 rpm, 4 min). The DES phase that settled at the top of the centrifuge tube was collected and analysed using RP-HPLC.

DES-BASED EXTRACTION WITH REAL COSMETIC PRODUCTS

Ten types of cosmetics were used (5 with parabens (spiked samples) and 5 without parabens). The DES with the highest concentration of alkyl paraben detection through the experiment was mixed with a cosmetic sample (0.05 g diluted) into a centrifuge tube. The

mixture was then vortexed for 1 min. When a cloudy solution containing fine DES droplets appeared, the solution was centrifuged (5000 rpm, 4 min). The DES phase that settled at the top of the centrifuge tube was collected and analysed using RP-HPLC. The test was repeated by adding 2 mL of methyl p-hydroxybenzoate (MP), 2 mL of ethyl p-hydroxybenzoate (EP), 2 mL of propyl p-hydroxybenzoate (PP), 2 mL of butyl p-hydroxybenzoate (BP) into the cosmetic samples.

RESULTS AND DISCUSSION

STANDARD CURVES

Table 3 presented the findings from evaluating the calibration graphs' linear range, precision, limits of detection (LODs), quantification (LOQs), enrichment factor (EF), and relative standard deviation (RSD). From the table, it was shown that MP has the higher LOD while PP had the lowest LOD. This is because of the less interaction of methyl group in methyl paraben towards the adsorption of DES chemicals compared with the long chain of propyl group in the propylparaben which has the good interaction in the adsorption with the DES chemicals. These structural variations may alter how the chemicals interact with the analytical technique, which may alter how detectable they are in adsorption process (Özak 2020).

A compound's detectability may also depend on how easily it dissolves in the solvent used. According to research by Mishra et al. (2023), the solubility of parabens in different solvents can vary significantly due to differences in molecular interactions. For instance, propylparaben demonstrates greater solubility in polar solvents like methanol or ethanol compared to less polar solvents like hexane. This increased solubility enhances its detectability in analyses conducted using polar solvent systems, potentially resulting in a lower LOD.

This statement is aligned with the study by Jala et al. (2023) which highlights the importance of solvent selection in achieving optimal sensitivity in paraben detection assays. By utilizing solvents with higher affinity for specific parabens, researchers can improve LODs and enhance the precision and accuracy of analytical methods. In summary, the solubility of parabens in chosen solvents significantly influences their LODs in analytical procedures. Understanding these solubility dynamics is crucial for selecting appropriate solvent systems to achieve the desired sensitivity and accuracy in paraben analysis.

According to the research of Ge et al. (2020), decanoic acid's O-H vibration is associated with broadband at 3476 cm^{-1} . According to Figure 4, the DES solvent produces an FT-IR wavenumber of 3648 cm^{-1} for the O-H vibration peak when combined with DL-Menthol and decanoic acid at a molar ratio of 2:1. One explanation is that the hydrogen bonding weakens the bonds and reduces the force constants, resulting in a decrease in stretching frequencies (Ge et al.

2020). Additionally, hydrogen bonding can affect the intensity and broadening of certain bands in the FTIR spectrum. The presence of hydrogen bonding may cause broadening and splitting of peaks, particularly in the regions corresponding to O-H. Therefore, the shifting in the O-H group's wavelength number between DES and ethylene glycol indicates DES's creation and the presence of hydrogen bonds. The O-H bond's electron density may alter because of this interaction, changing the absorption frequency (Kolesov 2021).

TABLE 3. Data summary for standard curve

Analyte	Retention time (min)	Linearity (ppm)	R	LOD (mg/L)	LOQ (mg/L)	RSD%	EF%
MP	3.925	2-10	0.9914	1.6	4.8	2.0	49.6
EP	4.966	2-10	0.9973	0.9	2.7	1.3	49.9
PP	6.632	2-10	0.9997	0.2	0.7	1.0	50.0
BP	8.255	2-10	0.9973	0.9	2.7	1.0	49.9

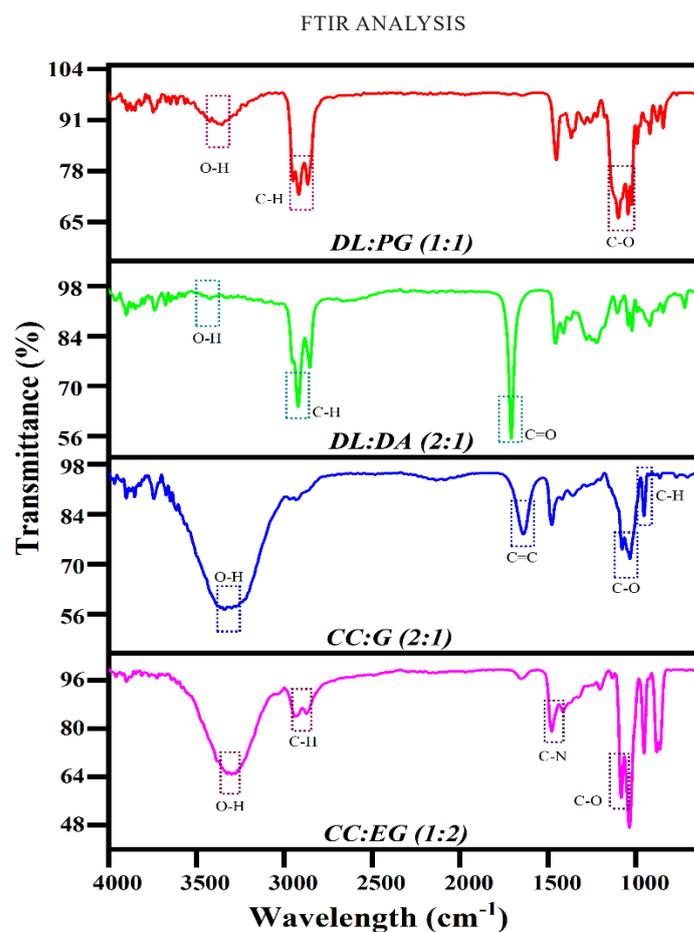


FIGURE 4. FTIR spectrum results from 4 types of DES. DL:PG = DL-Menthol:Polyethylene Glycol; DL:DA = DL-Menthol:Decanoic Acid; CC:G = Choline Chloride:Glucose; CC:EG = Choline Chloride:Ethylene Glycol

DETERMINATION OF OPTIMUM DES SYSTEMS FOR
PARABEN EXTRACTION
MOLAR RATIO

It was discovered that mixture of choline chloride and ethylene glycol at a ratio of 2:1 and 1:1 produced a viscous DES solution. Since DES is made from ethylene glycol; thus, DES has a high freezing point and appears foggy when it includes too many choline chloride halide salt anions (Ge et al. 2020). They stated that the density of the molar ratio of choline chloride to ethylene glycol (1:1) is greater than the molar ratio of choline chloride to ethylene glycol (1:2).

Consequently, it was discovered through the trials that the molar ratio of choline chloride to ethylene glycol (1:2) is the best when compared to the molar ratio of the same HBD and HBA. The synthesized DES is used immediately as it was found that the viscosity of the DES solution increased when the temperature was decreased thus leading the DES to be unsuitable for usage (Li et al. 2020).

It was also discovered that the DES of a water mixture at various molar ratios with HBD and HBA of choline chloride and glucose has no two layers formed when the DES was combined with a sample containing parabens. This occurred because, during the synthesis of DES, the molar ratio of water to choline chloride was unable to dilute the physical state of both HBA and HBA, leading to an increase in the molar ratio of water to choline chloride increased by 10 times to obtain DES in liquid form (Razavi et al. 2022). Water, however, can interfere with the hydrophobic interactions in DES

and lessen the substance's total hydrophobicity. The ability of DES to dissolve hydrophobic substances or carry out specific hydrophobic-based operations may be impacted by this (Dwamena 2019). As a result, hydrophobic DES' applicability for several applications has been jeopardized (Shah & Mjalli 2014). Therefore, none of the three DES choline chloride: glucose molar ratios are selected.

It can be observed from the bar chart in Figure 5 that the peak area of DES DL-Menthol: decanoic acid at a molar ratio (1:2) has the highest peak area of 300.62 mAU compared with other DES. According to the writing of Ge et al. (2019), it was found that DES DL-Menthol: decanoic acid at a molar ratio (2:1) provided the highest detection of paraben of parabens in water samples. The results obtained differ from literature studies, possibly due to several factors.

Solubility and partitioning are some of them. The solubility and partitioning of the desired molecule can occasionally be improved by using a specific molar ratio of the extraction agent (Li et al. 2020). Maximizing the extraction effectiveness and raising the target product's solubility is possible using a 1:2 molar menthol to decanoic acid ratio. However, parabens are typically less soluble in menthol. Although paraben solubility in menthol may be somewhat limited, it is commonly regarded as poor. Due to ester functional groups, parabens are polar chemicals, whereas menthol is generally nonpolar. Compounds that are polar or nonpolar typically have less solubility than the opposite.

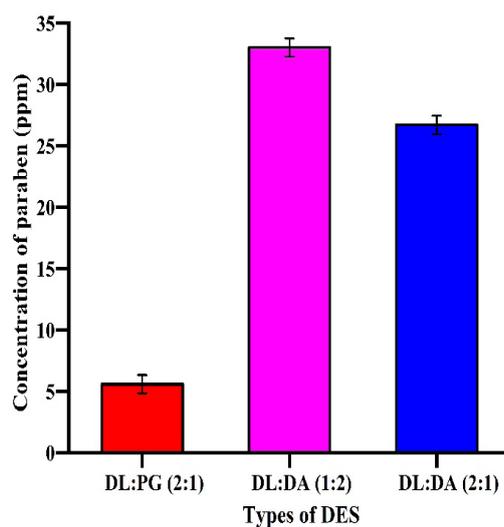


FIGURE 5. Bar chart of concentration of paraben vs. type DES (methylparaben)

The peak area graph for the two parabens, methyl and ethyl, from the HPLC analysis, is shown in Figure 6. Using the graph as a guide, it was discovered that the vortex time at 1 min generated the most significant peak area for methyl paraben, followed by 3 and 5 min. Regarding the peak area for ethyl paraben, the 2nd min has the highest value, followed by the 1st min and the 5th min.

According to the literature review, the most optimal vortex time obtained was in the first minute for Ge et al. (2020), whereas Sivrikaya (2019) indicated that it was in the third minute. Long-term vortexing may increase the solvent's air-exposed surface area, thus resulting

in more evaporation loss. This may lower the solvent concentration or alter the eutectic mixture's chemical composition. Therefore, it can be said that a vortex lasting one minute is the best for extracting parabens from cosmetic samples. Due to the high vortex speed factor experienced throughout the experiment, a shorter vortex period may be adequate to get acceptable findings in some circumstances.

Through the preceding figure, it can also be deduced that for methyl paraben, there is a substantial difference between the peak area in the first and third minutes, but that the difference is not significant for ethyl paraben at either time. The best scenario for a 1-minute vortex was chosen for further parameter optimization.

EFFECT OF VORTEX TIME

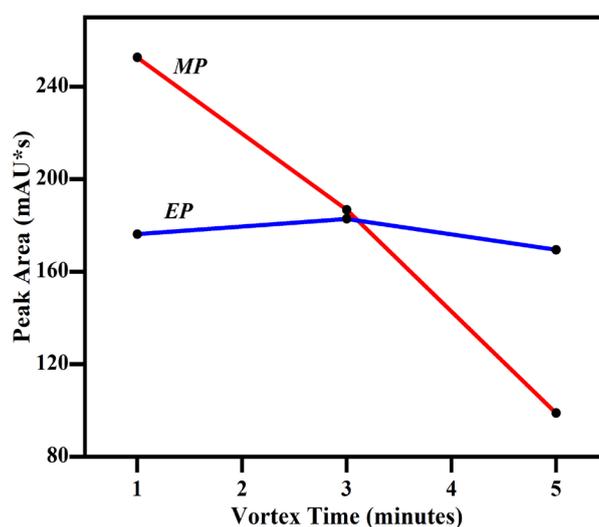


FIGURE 6. Graph of peak area against vortex time

EFFECT OF AQUEOUS PHASE pH

The most advantageous peak area for methyl paraben, according to Figure 7, is at pH 7, followed by pH 12, pH 4, and then pH 2. Methyl paraben can function effectively in the pH range of 4.5-7.5 and is resistant to hydrolysis (Polaka et al. 2022). The most stable form of methyl paraben is still present in this pH range. Referring to Figure 7, it was discovered that the peak area for ethyl paraben is highest at pH 2, followed by pH 4, pH

12, and then pH 7. Ethyl paraben is considered stable throughout a wide pH range of 4–8.

According to Temova et al. (2021), a formulation's activity increases with decreasing pH. In many cosmetic and personal care products, ethyl paraben, a preservative, is typically in the acidic range. This could account for the findings for the most prominent peak area at pH 2. The results of the peak area for pH 12 do not correspond to the range found in the literature study.

This is because of the systematic error, which occurs during the experiment and makes it such that the pH meter used, despite being calibrated, is not remarkably accurate. According to the works of Ge et al. (2019), pH 7 was discovered to be the pH that produced the best results. Based on the findings, pH 7 was selected since it is stable for both methyl and ethyl parabens.

APPLICATION OF THE DEVELOPED METHOD TO REAL SAMPLE

For each sample that has been examined, the concentration value has been determined based on Figure 8. According to the observation, the chosen DES was successful in extracting MP, MP (spiked), EP, EP (spiked), and PP from Product F. When considering the makeup of product F, it turns out that MP, EP, and PP are present in the ingredient list. There is an increase in the section of the graph where PP spiked has the highest concentration at 38.00 mg/L. Regarding product G, DES successfully extracted spiked MP, EP, EP, and PP. Like Product F, Product G likewise includes MP, EP, and PP with their content. The spiked PP also shows the highest concentration for this Product G which is at 41.84 mg/L.

The chosen DES was then found to successfully extract both MP spiked, and EP spiked for Product H. When comparing with other cosmetic products, Product H's concentration is one of the smallest which is 3.61 mg/L for MP spiked and 1.84 mg/L for EP spiked. Product I was likewise successfully extracted using the

chosen DES solvent with MP and EP spiked. When MP was spiked (39.66 mg/L), Product I displayed the most significant concentration. DES has finally recovered MP spiked, and EP spiked data from Product J successfully, precisely like it did for Product H and Product I. Spiked MP, followed by spiked EP, produces the Product's most significant concentration.

Product A provides the highest concentration (5.11 mg/L) compared with other cosmetic goods, as shown in Figure 9. It was discovered that spiked MP produced a high concentration which is at 6.87 mg/L for Product A according to Figure 9. No methyl paraben is listed as an ingredient in Product A's cosmetic composition. Therefore, hypothesis 2, where this paraben-free cosmetic product contains a variety of parabens, has been successfully achieved. Following Product A, Product B also contains methylparaben, and the concentration for spiked MP has the most prominent peak area (3.38 mg/L).

The graph also shows that Product C offers MP and EP extraction values in addition to its spike. It turns out that the information on the sample box does not mention their inclusion in the sample in any way. The presence of EP in this cosmetic sample can also be noticed in the graphic for Product D. The highest concentration came from spiked EP (1.05 mg/L). Finally, it can be demonstrated that Product E's cosmetic sample is parabens-free. This indicates that the information stated for Product E, where parabens are not added to

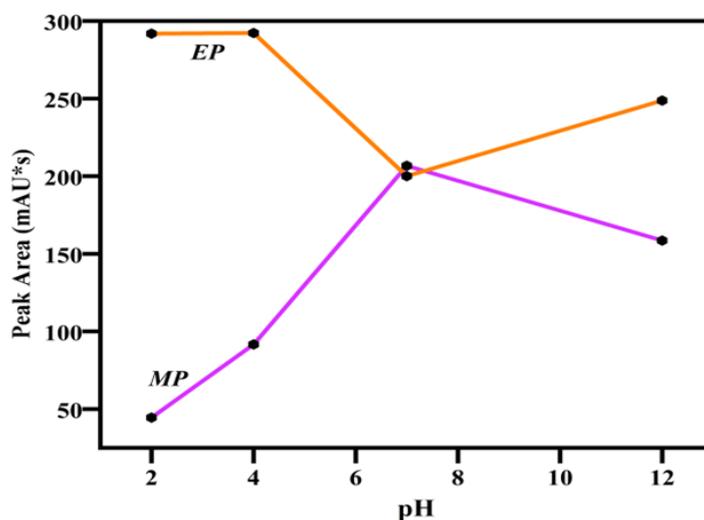


FIGURE 7. Graph of peak area against solvent pH

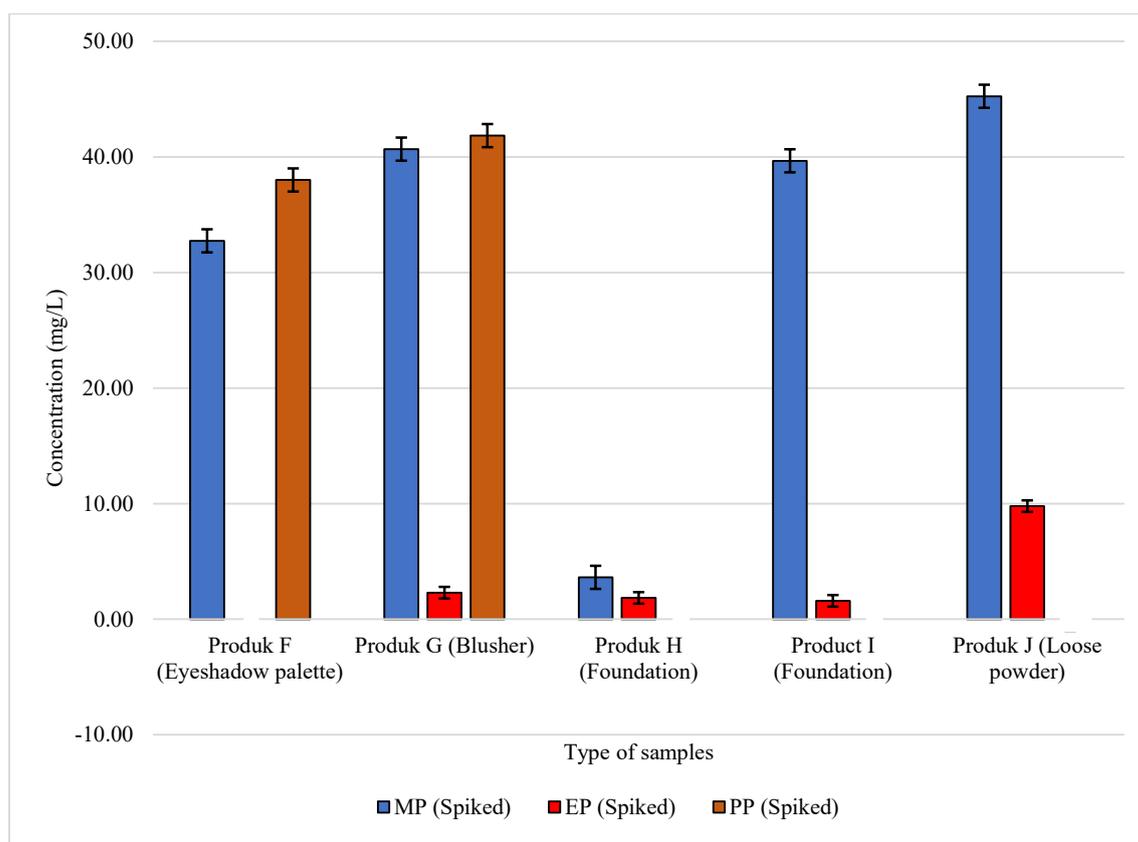


FIGURE 8. Bar chart of concentration against sample type (contains parabens)

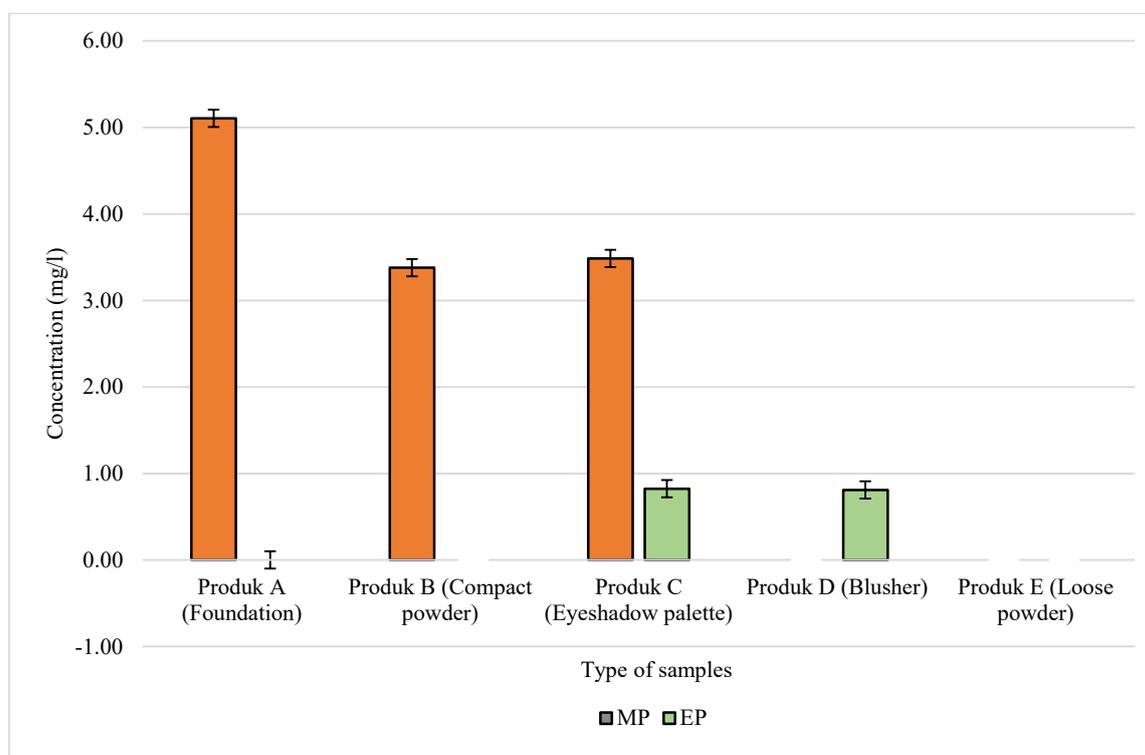


FIGURE 9. Bar chart of concentration against sample type (Free parabens)

cosmetics, is accurate. Figure 14 shows the HPLC peak for Product G (Spiked), where the methyl paraben was extracted at a retention time of 3.6 min, the ethyl paraben was extracted at 4.7 min, and the propyl paraben was extracted at 6.0 min.

The amount of methyl and ethyl parabens in cosmetics does not go above the maximum allowed paraben concentration in a single product, which is 0.8% (Polaka et al. 2020). It was discovered that certain paraben-free cosmetics contain trace amounts of methyl and ethyl paraben. This raises some concerns because

there is no mention of the usage of parabens in the Product's components. However, the overall content is below the 0.8% maximum paraben concentration. However, given that most parabens are present in conventional cosmetic products, everyday usage of these products over time might cause parabens to accumulate and do more harm than good, even if the Product's paraben content is safe. This occurs when paraben-containing cosmetics enter the body through the skin. Therefore, as prevention is better than cure, we should avoid using any product that contains paraben components.

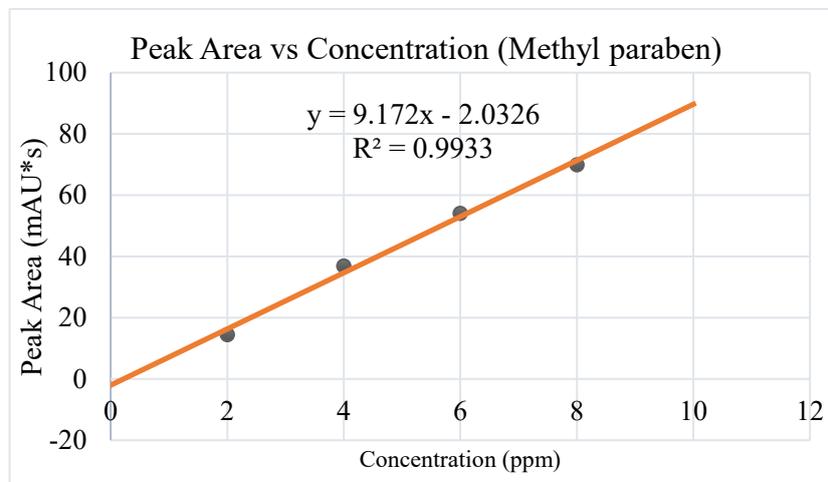


FIGURE 10. Standard calibration curve of methyl paraben

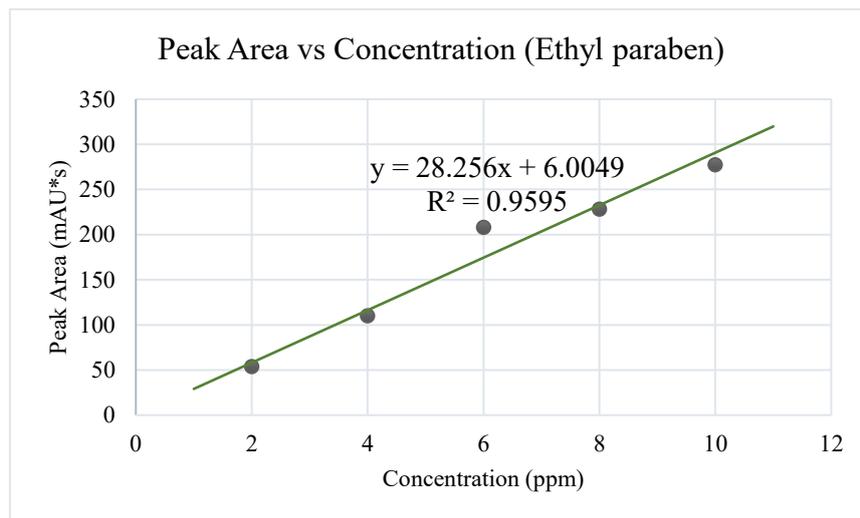


FIGURE 11. Standard calibration curve of ethyl paraben

MECHANISM OF INTERACTION IN DEEP EUTECTIC
SOLVENT WITH PARABEN

DESS are mixture of two or more substances with melting points that are lower than those of the constituent substances alone. The decrease in melting point is caused by these solutions' nonideality, which results from strong hydrogen bonds, favourable van der Waals

interactions, and positive mixing entropy (Smith, Abbott & Ryder 2014). Due to their hydrophilicity, DESs utilized in samples that contain water have limitations. As a result, the creation and application of hydrophobic DESs in sample preparation techniques have garnered considerable interest (Beh et al. 2021; Ge et al. 2019).

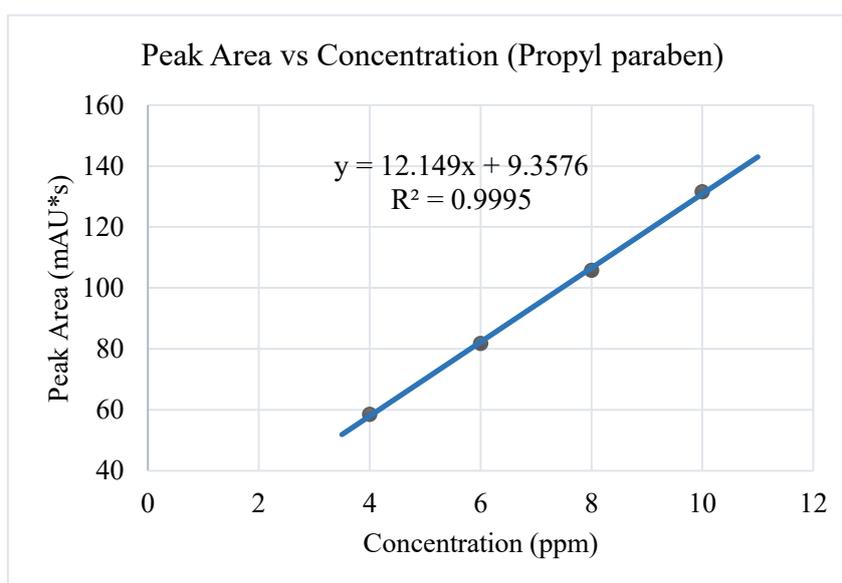


FIGURE 12. Standard calibration curve of propyl paraben

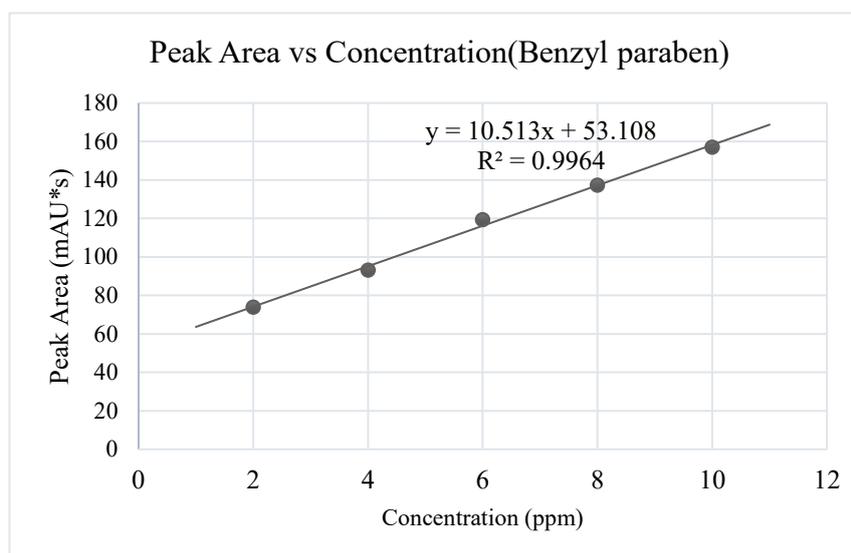


FIGURE 13. Standard calibration curve of benzyl paraben

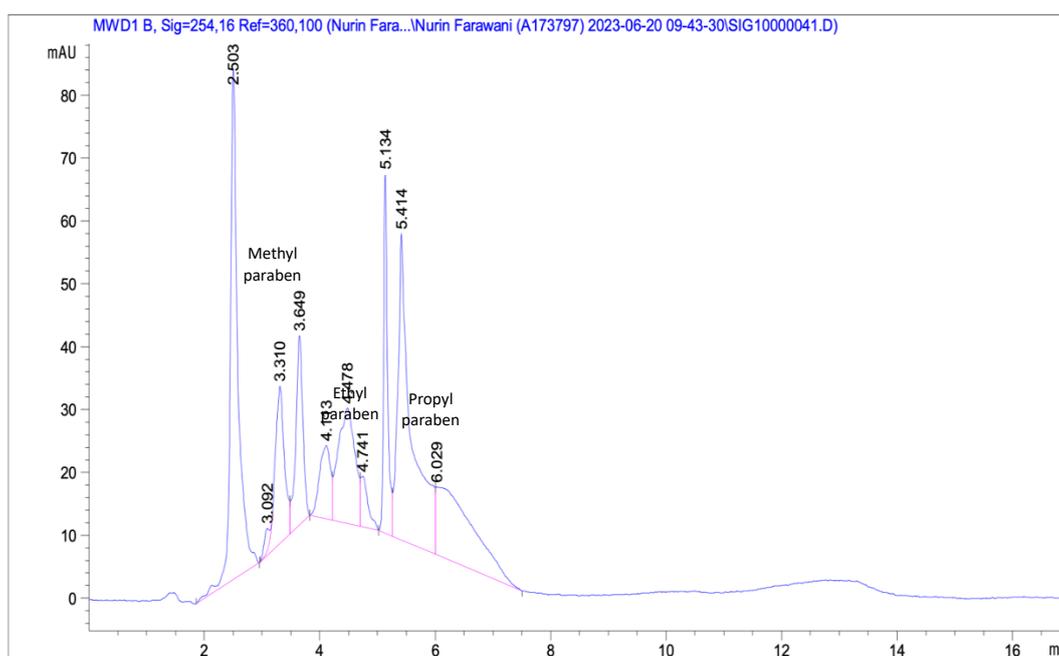


FIGURE 14. HPLC results of Product G (Spiked)

A form of hydrophobic DESs based on DL-menthol was created by Florindo, Branco and Marrucho (2017) and used to LLE of pesticides from aqueous samples. To extract parabens from environmental water samples, an *in situ* DES-LLME method using high-performance liquid chromatography (HPLC) was developed (Ge et al. 2019). A chemical is extracted from a mixture using a solvent in a process known as liquid-liquid extraction (LLE). Partitioning with solvents is more precise. It denotes that compounds have a ‘choice’ between two solvents in which to dissolve. A single solvent can dissolve some substances. In the opposite solvent, some substances dissolve. In this manner, the mixture’s components are divided into two categories (Ge et al. 2020) (Figure 15).

Two solvents that are not miscible with one another are necessary for solvent partitioning. Water is frequently one of the solvents. The alternative solvent is a substance that does not mix well with water, such as diethyl ether (which is also known as just ‘ether’ because it is the most prevalent form of ether). A mixture of ether and water will appear to have two layers if you look at it closely since the two substances do not mix well together. Same goes for the hydrophobic DES. It is immiscible in the cosmetic sample causing it to form two layers.

Based on how polar the paraben is with the DES, it will be separated from the cosmetic sample. Due to their lower density than water, most organic solvents

(DES) float to the surface. As a result, a syringe will be used to extract the top layer of this separation, which comprises DES and paraben. Both hydrophobic (nonpolar) and hydrophilic (polar) areas can be found in the chemical structure of parabens. Parabens typically consist of an ester group (hydrophilic) and an aromatic ring (hydrophobic). The polarity of the molecule is caused by the ester group, and the nonpolarity is caused by the aromatic ring.

According to Figure 16, in a formulation, DL-menthol and parabens can interact by a variety of intermolecular forces, including hydrogen bonds, van der Waals forces, and dipole-dipole interactions. The hydroxyl group (-OH) in DL-menthol can form hydrogen bonds with the carbonyl oxygen (-COO-) in methylparaben. The carbon chain of decanoic acid has a carboxylic acid group (-COOH) at one end. Due to oxygen’s greater electronegative charge than hydrogen’s, the hydrogen atom in this -COOH group is partially positively charged (+), forming a polar bond. On the other hand, methylparaben has a hydroxyl group (-OH), which is another polar group since the oxygen atom is likewise somewhat negatively charged (-). Decanoic acid’s -COOH group’s partial positive charge on the hydrogen might draw methylparaben’s -OH group’s partial negative charge on the oxygen. Between the two molecules, a hydrogen bond is created by the electrostatic attraction (Jala et al. 2023).

Table 4 shows the comparison study for application of deep eutectic solvents using various of samples. It shows that the trend of application for deep eutectic solvents are increasing in application for pharmaceuticals and cosmetic products. This is because

the DES method is simple procedures, without using the complex apparatus, consume small amount of chemicals and finish in short time. The LOD obtained for current study are acceptable compared with the previous study which is 0.2 -1.6 mg/L.

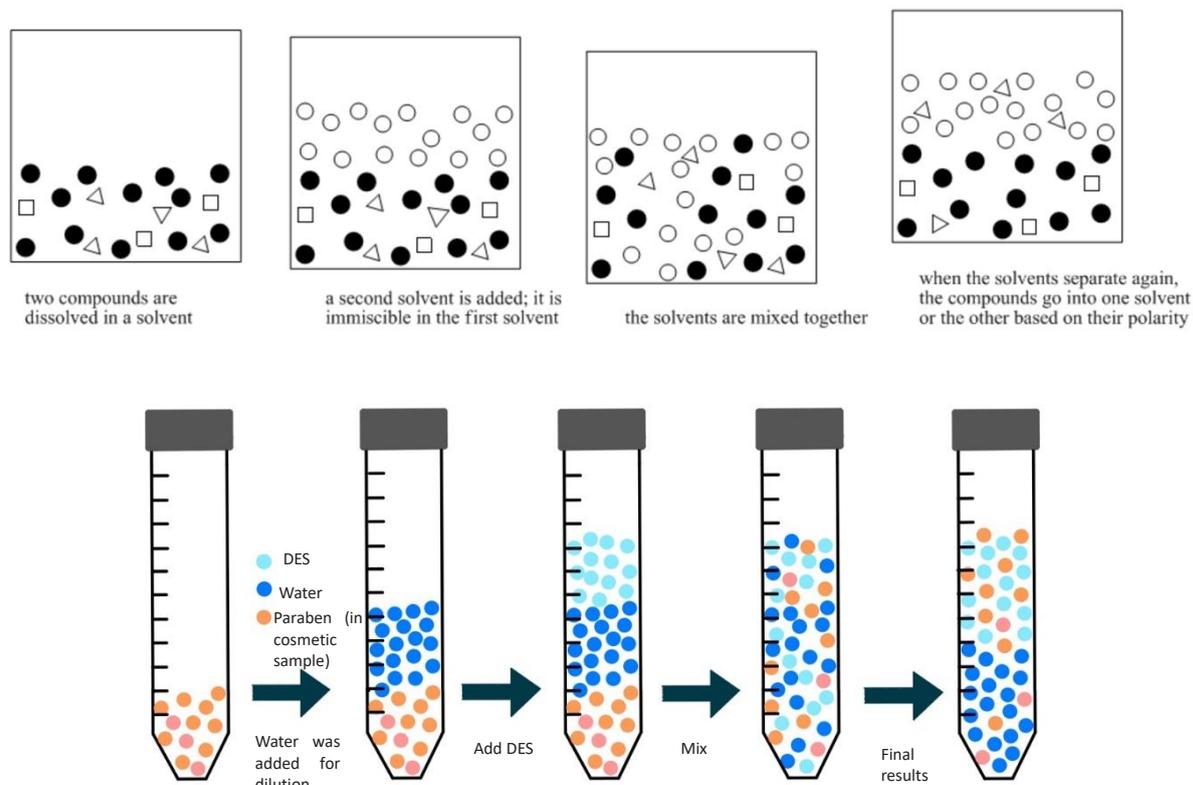


FIGURE 15. Solvent partitioning (separation of LLME) (Ge et al. 2020)

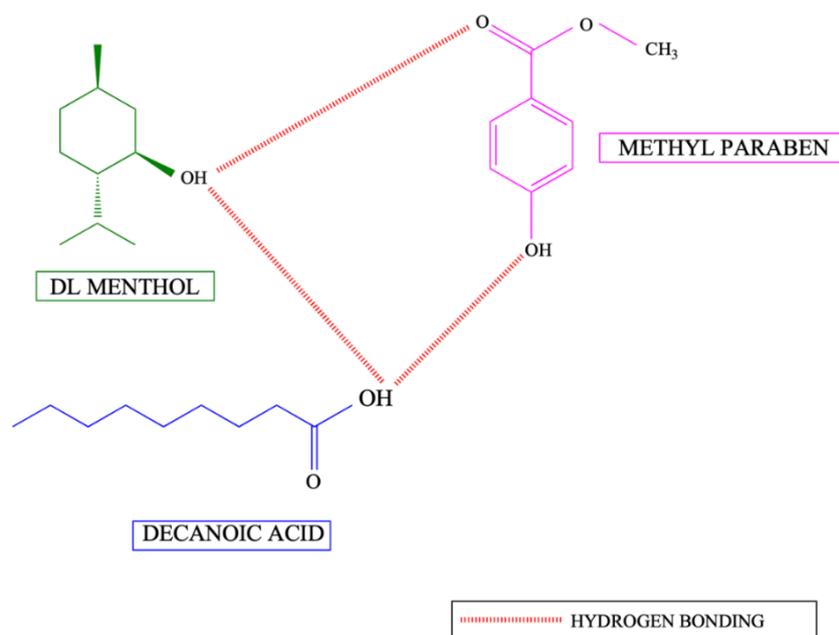


FIGURE 16. Mechanism of interaction between methylparaben and DL-Menthol as HBA, Decanoic acid as HBD (Dalmaz & Özak 2022)

TABLE 4. Comparison with previous studies

Study	Concentration of paraben extracted (mg/L)	HBA and HBD	Molar ratio	LOD (mg/L)	LOQ (mg/L)	Reference
Extraction of parabens in cosmetic oil products	Eyeshash oil	Choline chloride (HBA) and ethylene glycol (HBD)	1:2	MP: 0.05	MP: 0.18	(Sivrikaya 2019)
	MP: 3.81			EP: 0.06	EP: 0.21	
	EP: -			PP: 0.05	PP: 0.16	
	PP: 3.15			BP: 0.05	BP: 0.17	
Extraction of Parabens in Food, Cosmetics and Pharmaceutical Products	Hand Cream	DL-Menthol (HBA) and polyethylene glycol (HBD)	1:1	MP: 2E-3	MP: 5E-3	(Ge et al. 2020)
	MP: 680			EP: 1E-3	EP: 3E-3	
	EP: 1400			PP: 1E-3	PP: 3E-3	
	PP: -			BP: 3E-4	BP: 1E-3	
Extraction and preconcentration of parabens in liquid pharmaceutical samples	Syrup no.1	Choline chloride (HBA) and glucose (HBD)	2:1	MP: 3E-5	MP: 1E-4	(Razavi et al. 2022)
	MP: 830			EP: 1.5E-4	EP: 5E-4	
	EP: -			PP: 4E-5	PP: 1E-4	
	PP: 158			BP: 4E-5	BP: 1E-4	
Paraben extraction in water samples	Domestic waste-water	DL-Menthol (HBA) and Decanoic acid (HBD)	2:1	MP: 8E-4	MP: 3.1E-3	(Ge et al. 2019)
	MP: 3.5E-3			EP: 6E-4	EP: 2.5E-3	
	EP: -			PP: 6E-4	PP: 2.5E-3	
	PP: -			BP: 8E-4	BP: 3.1E-3	
Eutectic solvent extraction method for the extraction of parabens in cosmetics	Product G	DL-Menthol (HBA) and decanoic acid (HBD)	1:2	MP: 1.6	MP: 4.8	current study
	MP: 24.26			EP: 0.9	EP: 2.7	
	EP: 0.57			PP: 0.2	PP: 0.7	
	PP: -			BP: 0.9	BP: 2.7	
	BP: -					

CONCLUSION

Due to the high methyl paraben extraction using RP-HPLC results from the chosen samples, DES consisting of DL-Menthol: decanoic acid with a molar ratio (1:2) was selected as the most optimal DES for the first objective. Next, it was discovered that the 1-min vortex duration offered the best circumstances for the DES DL-Menthol: decanoic acid ratio (1:2) while optimizing the vortex time parameters. Additionally, it was discovered that when pH optimization factors were used, pH 7 produced the highest stable peak areas for both methyl and ethyl parabens. For the second aim, analysis was done on actual samples, five of which contained parabens and five of which did not. Through testing, it was discovered that the DES solvent chosen from the first aim successfully

extracted methyl, ethyl, and propyl parabens from cosmetic samples containing parabens. The peak areas of samples that have received injections of paraben standards are also higher than the initial peak areas. In contrast, it was discovered that 4 out of 5 cosmetic sample packets that claimed to be paraben-free included trace amounts of methyl and ethyl paraben. Therefore, the hypothesis that parabens will be in both samples containing parabens and paraben-free has been accepted.

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