

EXTRACTION OF METHYLPARABEN IN COSMETICS USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BASED ON SOLIDIFICATION OF FLOATING ORGANIC DROP COUPLED WITH GAS CHROMATOGRAPHY FLAME IONIZATION DETECTOR

(Pengekstrakan Metilparaben dalam Kosmetik Menggunakan Pengekstrakan Mikro
Cecair-Cecair Serakan Berdasarkan Pemejalan Organik Terampai Berganding dengan
Kromatografi Gas Pengesan Nyala Pengionan)

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Abstract

A simple and efficient method called dispersive liquid-liquid microextraction based on solidification of floating organic droplets (DLLME-SFO) method coupled with gas chromatography flame ionization detector (GC-FID) was developed for the extraction of methylparaben (MP) in cosmetics. In the optimized DLLME-SFO method, a mixture of n-hexadecane (70 μ L) as an extraction solvent and methanol (0.25 mL) as a disperser solvent was rapidly injected into a 5 mL sample solution (pH 6) containing 4 % (w/v) NaCl. After 4 minutes of centrifugation at 4000 rpm, the mixture was separated into two phases with the fine droplets of n-hexadecane floating at the top of the sample solution. Then, the test tube was placed in an ice bath for cooling and solidification purposes. The solidified extract was transferred into a small vial where it melted and injected into GC-FID system. The DLLME-SFO method gave a good linearity over the concentration range from 0.1 – 8 μ g/mL with coefficient of estimation (r^2) from 0.9996. The method also provides a low limit of detections (LODs) which is 0.0048 μ g/mL. Methylparaben was detected in both samples at the concentration level of 0.06 μ g/mL for sample 1 and 0.42 μ g/mL for sample 2 respectively.

Keywords: dispersive liquid-liquid microextraction, solidification of floating organic droplets, gas chromatography flame ionization detector, methylparaben, cosmetic

Abstrak

Kaedah yang senang dan berkesan iaitu pengekstrakan mikro cecair-cecair serakan berdasarkan pemejalan organik terampai (DLLME-SFO) berganding dengan kromatografi gas dengan dengan pengesan nyala pengionan (GC-FID) telah dihasilkan bagi penentuan metilparaben dalam alatan kosmetik. Dalam kaedah DLLME-SFO yang telah dioptimumkan, campuran n-heksadekana (70 μ L) sebagai pelarut pengekstrak dan metanol (0.25 mL) sebagai pelarut penyebar disuntik segera ke dalam 5 mL larutan sampel (pH 6) yang mengandungi 4% (w/v) NaCl. Selepas pengemparan selama 4 min pada 4000 rpm, campuran terpisah kepada dua fasa di mana titisan halus n-heksadekana terapung di atas larutan sampel. Kemudiannya, tabung uji diletakkan di dalam rendaman ais untuk penyejukan dan pemejalan. Ekstrak yang beku dimasukkan ke dalam vial di mana ia mencair dan disuntik ke dalam sistem GC-FID. Kaedah DLLME-SFO memberikan kelinearan yang baik untuk kepekatan diantara, 0.1 – 8 μ g/mL dengan pekali penentuan (r^2) 0.9996. Kaedah ini juga menunjukkan had pengesanan (LODs) rendah iaitu 0.0048 μ g/mL. Metilparaben telah dikesan dalam kedua-dua sampel pada tahap kepekatan 0.06 μ g/mL untuk sampel 1 dan 0.42 μ g/mL untuk sampel 2.

Katakunci: pengestrakan mikro cecair-cecair serakan, pemejalan organik terampai, gas kromatografi pengesan pengionan nyala, metilparaben, kosmetik

Introduction

A preservative is a natural or man-made chemical that is added to several types of products, for example foods, pharmaceuticals, paints, biological samples, wood, beverages and others. It is used to prevent decomposition or any undesirable chemical changes due to microbial activities; hence, products last longer and are safe for use [1, 2]. Hirose and coworkers [3] stated that these preservatives act as either antimicrobials or antioxidants which they will either inhibit the activity of or kill the bacteria, molds, insects and other microorganisms. Antimicrobials are to prevent the growth of molds, yeasts and bacteria while antioxidants keep the products from becoming rancid or developing black spots [4]. They suppress the reaction when the products come in contact with oxygen, heat, and some metals.

Parabens or ester of p-hydroxybenzoic acid are extensively used in formulations of personal care products due to having neutral pH, no perceptible odor or taste, and no discoloration or hardening effect [5]. Generally, parabens are stable in the air, and are resistant to hydrolysis in hot and cold water as well as in acidic solutions ($1 \leq \text{pH} < 7$). Despite their benefits, a controversy surrounds the discussion the effect of commonly used parabens, methylparabens (MP), which can cause side effects on consumers and organoleptic alterations in the cosmetics. The studies revealed that the use of parabens may cause cancer, genotoxicity and breast cancer [6, 7, 8]. Parabens are also reported to have side effects on males as it may decrease the reproduction potential, cause infertility and cause skin cancer diseases such as malignant, melanoma and contact eczema. Recently, the use of preservatives in consumer products has been the subject of criticism because of their possible side-effects on human health. According to European Union (EU) and USA legislation, 0.4% (w/w) of single paraben concentration and a maximum of 0.8% (w/w) for the mixture of parabens is allowed in a cosmetic product [9, 10].

There is no doubt that legislation lags behind the market. Numerous amounts of paraben-free cosmetic products have been marketed in recent years. Labels on these products usually claim 0% parabens or paraben-free cosmetics. However, the lack of specific regulation facilitates labeling fraud. Their widespread use makes parabens a group of chemicals to be controlled from an analytical point of view. Information about some analytical methods published for paraben determination can be found in the book edited by Salvador and Chisvert [11] and a review paper by Wang and Liu [12]. Liquid-liquid extraction (LLE), solid phase extraction (SPE), solid-phase microextraction (SPME), and matrix-solid phase dispersion (MSPD) coupled with either gas chromatography (GC) or high performance liquid chromatography (HPLC) have been critically examined for the identification of MP in cosmetics [13-15]. In a conventional LLE there are many well-known drawbacks and limitations, the most important of which are the large volumes of samples and extraction solvents required, and consequently the large amount of wastes generated, as well as the low preconcentration on samples.

Dispersive liquid-liquid microextraction (DLLME) has been introduced for the analysis of organic compounds, inorganic analytes and various types of analytes from different matrices such as water, tissue, biological fluids, and food matrices. DLLME is a miniaturized LLE using microliter volumes of extraction solvent, which is based on the equilibrium distribution process of the target analytes between sample solution and extraction solvent. This method offers simplicity of operation, rapidity, low cost, high recovery, high enrichment factor, and environmental benignity with wide application prospects in trace analysis [16, 17]. In conventional DLLME, the density of extraction solvent should be higher than water. The high density extraction solvents, being mostly halogenated, are generally hazardous to laboratory personnel and are not often compatible with reversed phase high performance liquid chromatography (HPLC).

Recently, a new microextraction method was developed, which is DLLME integrated with the solidification of a floating drop (DLLME-SFO) [18]. In DLLME-SFO, the extraction solvent was collected on the top of the test tube and was then cooled by inserting it into an ice bath for 5 minutes. The solidified extraction solvent was transferred into a suitable vial and immediately melted at room temperature; then it was finally injected into a suitable instrument.

In this work, we present a new application of DLLME-SFO in the extraction of MP in cosmetic creams and separation using GC-FID. In addition, the sample treatment step was employed for the extraction of target analyte in cosmetic creams before DLLME-SFO and thus reducing the matrix effect.

Materials and Methods

Chemicals and reagents

Methylparaben standard, 1-undecanol, 1-dodecanol, and *n*-hexadecane were supplied by Sigma-Aldrich (Buchs, Switzerland). HPLC grade of methanol, acetonitrile, and acetone were obtained from Fisher Scientific (UK). Sample pH was adjusted by the addition of diluted sodium chloride from Merck (Darmstadt, Germany). 100 mg mL⁻¹ solution was prepared by dissolving analyte in methanol and stored in the refrigerator at 4 °C before use. This stock solution was diluted with methanol to prepare a series of working solutions. Deionized water was prepared by the use of a Millipore Milli-Q (Milford, MA, USA) reagent water system. pH value was measured using a Cyberscan 510 pH meter; a Hettich (Oxford, UK) model EBA 20 centrifuge was used for phase separation. Two different local cosmetic sunblock's were used as real samples.

GC conditions

Gas chromatographic (GC-15A, Shimadzu, Japan) analysis was performed with a split or splitless injector system, and a flame ionization detector was used to determine methylparaben. A SPB-50 capillary column (30 m x 0.22 mm i.d., 0.25µm film thickness) was used for chromatographic separation and ultra-pure helium (99.99%, Air products, UK) was used as the carrier gas. The injection port was held at 270 °C in the splitless mode with a purge time of 45s. The oven temperature was programmed as follows: initial temperature 130 °C (held for 1 min), from 130 °C to 230 °C at the rate of 10 °C/min, and finally held at 230 °C for 2 minutes. The FID temperature was maintained at 270 °C. Hydrogen gas for FID was generated by a hydrogen generator (OPGU-1500S, Shimadzu, Japan) at a flow rate of 40 mL/min. The flow rate of air for FID was 300 mL/min.

Sample preparation

Sunblock cream samples (5g) were dissolved into 2:8 mL methanol and deionized water before 1 mL of concentrated HCl was added into the solution and exposed to sonication for 10 minutes. Then, the solution was diluted to 150 mL with deionized water, adjusted to pH 6.0, and then subjected to DLLME-SFO extraction.

DLLME-SFO procedure

Aqueous solution (5 mL) containing methylparaben (3.0 µg/mL) was placed in a 10-mL test tube. A mixture of *n*-hexadecane (70µL) and methanol (0.25 mL) was rapidly injected into the sample solution using a syringe. A cloudy suspension resulted from dispersion of fine droplets of *n*-hexadecane in the sample solution was then separated by centrifugation for 4 minutes at 4000 rpm. After centrifugation, the test tube was directly put into an ice bath for 5 minutes to solidify the floated *n*-hexadecane. After 5 minutes, the solidified solvent was collected using a small spatula and transferred into a conical vial, where it melted rapidly at room temperature. The floating extraction phase was collected using a microsyringe and 1µL was injected for GC-FID analysis.

Results and Discussion

Peak identification

In the GC-FID analysis, methylparaben was detected and eluted at 10.9 minutes (Figure 1). Generally, the area under the peak is directly proportional to its concentration.

Optimization of DLLME-SFO method

To determine the optimized extraction conditions, the peak area of MP was used to evaluate the extraction efficiency under different conditions. Six parameters were critically examined; types of extraction solvent and disperser solvent, volumes of extraction solvent and disperser solvent, effect of salt concentration and sample pH.

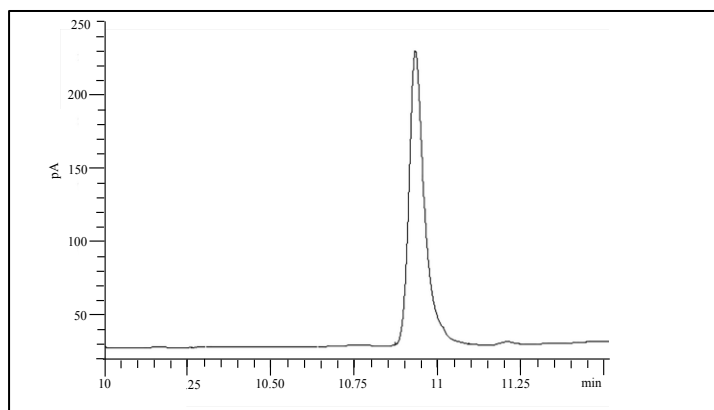


Figure 1. GC-FID chromatogram of MP at concentration 0.5 µg/mL

Selection of extraction solvents

An extraction solvent is crucial in the optimization of DLLME-SFO conditions. To achieve the optimum results, the extraction solvent must meet several criteria: high affinity for analytes, lower density than water, low solubility in aqueous solution, low melting point and volatility (Table 1), and perform a good chromatographic behavior [19]. In this study, 1-undecanol, 1-dodecanol, and *n*-hexadecane were tested in DLLME-SFO extraction (Figure 2). The results revealed that *n*-hexadecane showed better extraction efficiency compared to 1-undecanol and 1-dodecanol. Thus, it was selected as an extraction solvent and used in further experiments.

Table 1. Boiling point, melting point and density of extraction solvents [20]

Extraction Solvent	Boiling Point (°C)	Melting Point (°C)	Density (g/cm ³)
1-Undecanol	243	13-15	0.8298
1-Dodecanol	98	22-24	0.8309
<i>n</i> -hexadecane	287	18	0.7700

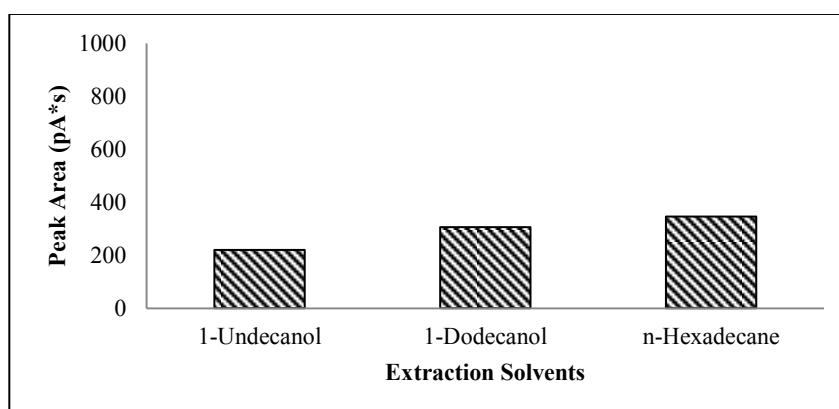


Figure 2. Effect of extraction solvent on extraction efficiency for DLLME-SFO of MP. Concentration of MP 3.00 µg/mL; volume of sample 5 mL (pH 6); volume extractant 70 µL; volume disperser solvent (methanol) 0.25 mL; extraction time 1 min; centrifugation 4000 rpm × 4 min; no salt added

Selection of disperser solvents

The miscibility of disperser solvent in diluted cosmetic solution and extraction solvent is one of the criteria that should be considered in order to enable the *n*-hexadecane to be dispersed as a fine particle in aqueous phase to form a cloudy solution (water/disperser solvent/extraction solvent). In such a case, the surface area between extraction solvent and aqueous phase can be definitely large, thus increasing the extraction efficiency [21]. Thereby, acetone, methanol, and acetonitrile were selected for this purpose of study. A series of experiments were conducted by using a mixture of 70 *n*-hexadecane and 0.25 mL of each disperser solvent (Figure 3). According to the obtained results, methanol was selected as the most suitable disperser because of the formation of a cloudy state with very fine droplets and high recovery of analyte.

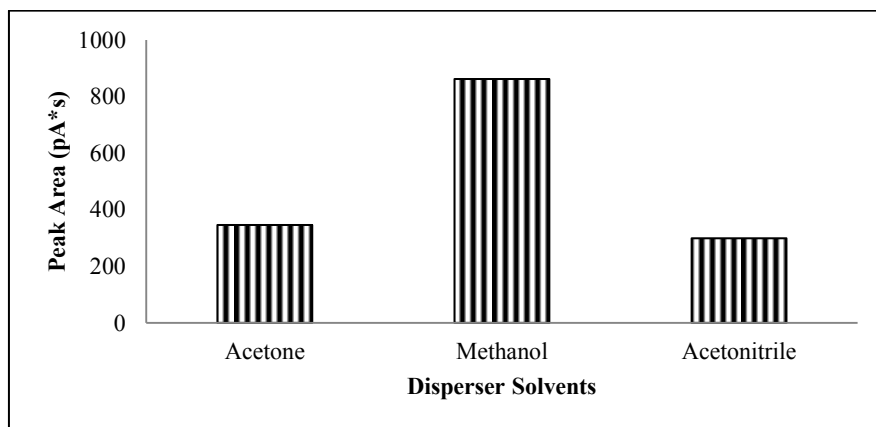


Figure 3. Effect of disperser solvent on extraction efficiency for DLLME-SFO of MP. Concentration of MP 3.00 $\mu\text{g/mL}$; volume of sample 5 mL (pH 6); volume extractant (*n*-hexadecane) 70 μL ; volume disperser solvent 0.25 mL; extraction time 1 min; centrifugation 4000 rpm $\times$ 4 min; no salt added

Effect of volume of extraction solvent

To study the effect of extraction volume on extraction efficiency in DLLME-SFO method, different volumes of *n*-hexadecane (60, 70, and 80 μL) containing 0.25 mL method were tested. Based on the results in Figure 4, the extraction recoveries decreased when the volume of *n*-hexadecane increased to 80 μL . This is due to the increasing volume of floating phase, and hence lessen the concentration of MP in *n*-hexadecane (dilution factor occurred). Therefore, 70 μL of *n*-hexadecane was chosen as the optimum volume for extraction solvent.

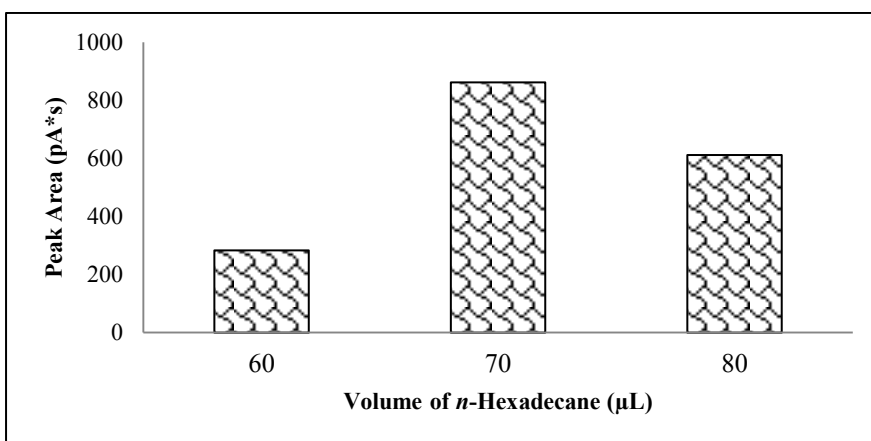


Figure 4. Effect of extraction solvent (*n*-hexadecane) volume for DLLME-SFO of MP. Concentration of MP 3.00 $\mu\text{g/mL}$; volume of sample 5 mL (pH 6); volume extractant (*n*-hexadecane) 60 - 80 μL ; volume disperser solvent (methanol) 0.25 mL; extraction time 1 min; centrifugation 4000 rpm $\times$ 4 min; no salt added

Effect of disperser solvent volume

The volume of disperser solvent directly affects the formation of cloudy solution (sample solution/disperser solvent/extraction solvent), volume of floated phase, and the degree of dispersion of extraction in aqueous phase and subsequently the overall extraction efficiency. To study the effect of disperser solvent volume on the extraction efficiency in DLLME-SFO method, different volumes of methanol (0.15, 0.25 and 0.50 mL) containing 70 μL of *n*-hexadecane were tested. According to the results in Figure 5, recovery of the analyte increased with the increasing volume of methanol up to 0.25 mL. This may be due to the increasing interaction of MP in water with the increasing volume of methanol. On the other hand, with a large volume of methanol, the solubility of analytes in aqueous phase increased; thus decreased the extraction efficiency. Finally, 0.25 mL of methanol was selected as the volume of disperser solvent in subsequent experiments.

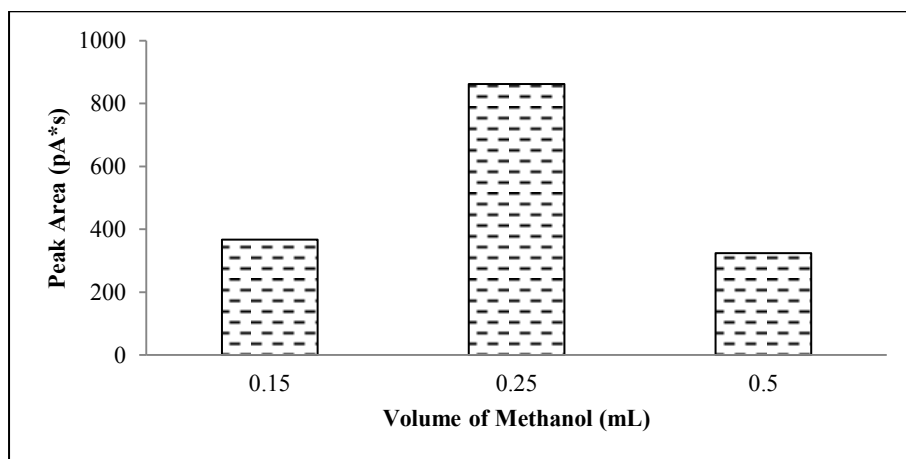


Figure 5. Effect of disperser solvent (methanol) volume for DLLME-SFO of MP. Concentration of MP 3.00 $\mu\text{g/mL}$; volume of sample 5 mL (pH 6); volume extractant (*n*-hexadecane) 70 μL ; volume disperser solvent (methanol) 0.15 - 0.25 mL; extraction time 1 min; centrifugation 4000 rpm $\times$ 4 min; no salt added

Effect of salt concentration

The function of adding a salt (NaCl) into the sample solution is to increase its ionic strength, decrease the solubility of the analytes in the sample solution and enhance the extraction efficiency [22, 23]. The concentration of salt used in this study was varied between 0 and 8% (w/v) and the effect on the extraction efficiency was investigated. According to the results in Figure 6, the peak areas of MP started to increase with the increasing amount of the NaCl concentration from 0 to 4%. However, with the addition of NaCl to 6%, it shows a dramatic decrease in extraction efficiency and a slight decrease when 8% NaCl was added. This phenomenon can be described regarding the salting-out effect happened in DLLME-SFO. Upon the addition of more than 6% of NaCl, the transportation of the analyte to the extraction solvent is dropped due to the increase of sample viscosity. By increasing the salt concentration, the diffusion of analyte towards organic solvent becomes more difficult; thus, 4% of NaCl was used in next experiments.

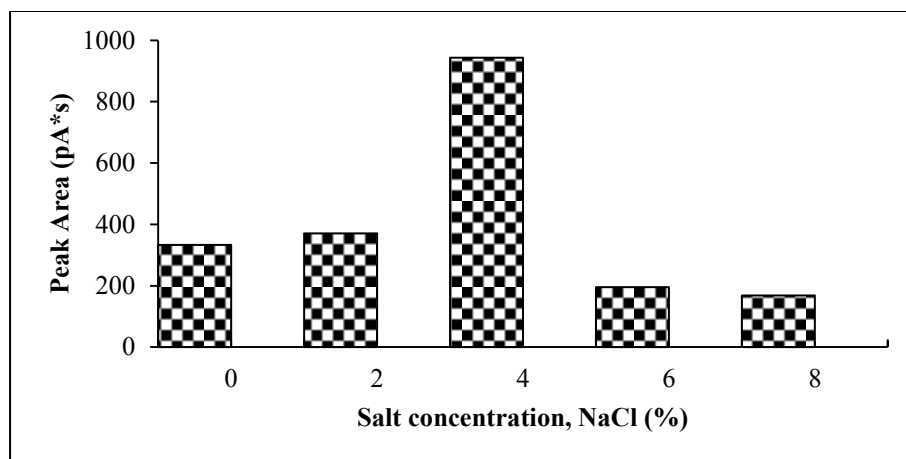


Figure 6. Effect of NaCl for DLLME-SFO of MP. Concentration of MP 3.00 $\mu\text{g/mL}$; volume of sample 5 mL (pH 6); volume extractant (*n*-hexadecane) 70 μL ; volume disperser solvent (methanol) 0.15 - 0.25 mL; extraction time 1 min; centrifugation 4000 rpm $\times$ 4 min

Effect of sample pH

The pH values of samples can affect the ratio of ionic form to molecular form of the analytes, especially for the analytes which are weak acid or weak alkali. According to Soni et. al. [4], at higher pH values ($\geq$ pH 8), methylparaben is in ionic form and the hydrolysis will occur and make it less to be extracted into organic solvent. Therefore, methylparaben should be extracted at pH values lower than its pKa value (methylparaben, pKa = 8.2) which is less than pH 8. Hence, the pH that was investigated in this study is in the range of 3–7 by adding appropriate sodium hydroxide or hydrochloric acid to the samples. The results illustrated in Figure 8 demonstrate that the optimum extraction efficiency of MP in DLLME-SFO was obtained at pH 6.0.

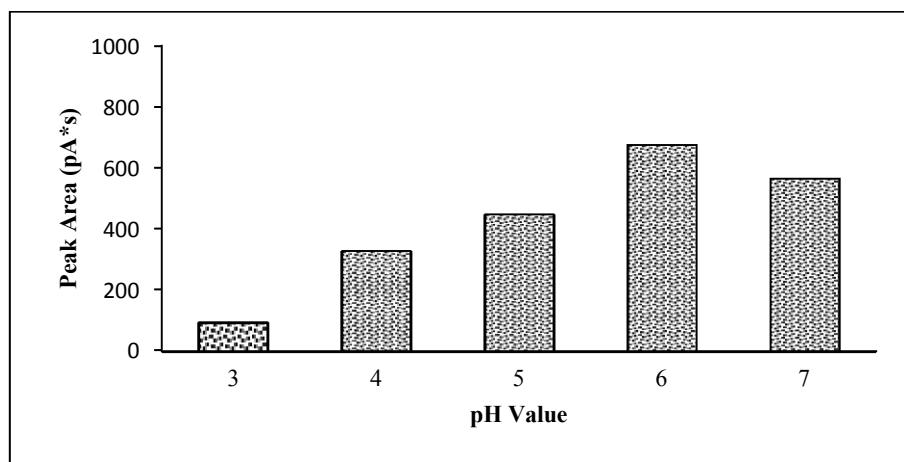


Figure 7. Effect of sample pH for DLLME-SFO of MP. Concentration of MP 3.00 $\mu\text{g/mL}$; volume of sample 5 mL (pH 6); volume extractant (*n*-hexadecane) 70 μL ; volume disperser solvent (methanol) 0.25 mL; NaCl (w/v) 4%; extraction time 1 min; centrifugation 4000 rpm $\times$ 4 min

Method validation and real sample analysis

The optimized DLLME-SFO conditions which is 70 μL of *n*-hexadecane as extraction solvent, 0.25 mL of methanol as disperser solvent, 4% of NaCl, and pH 6 of sample solution was carried out for the extraction validation in the range of 0.1 – 8 $\mu\text{g/mL}$. The results showed good linearity and acceptable LODs as summarized in Table 2.

Table 2. Correlation coefficients, LOD and LOQ of samples using GC-FID for DLLME-SFO

Paraben	Linear Range ($\mu\text{g/mL}$)	Correlation coefficients, R^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Methylparaben	0.1 – 8	0.9996	0.082	0.272

To investigate matrix effects and applicability of the technique on cosmetic samples, the final experiments were carried out on two different brands of sunblock labeled as Sample 1 and Sample 2. These samples were handled exactly according to the steps described in the sample preparation procedure and each sample was carried out with triplicate extraction. According to the chromatograms in Figure 8, MP was detected in both samples at the concentration of 0.06 $\mu\text{g/mL}$ (Sample 1) and 0.42 $\mu\text{g/mL}$ (Sample 2), respectively.

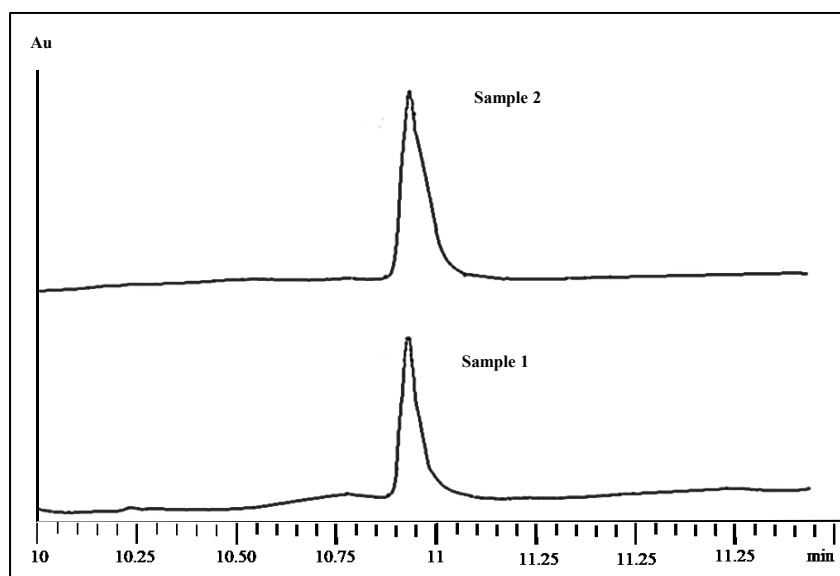


Figure 8. GC-FID chromatograms of DLLME-SFO extracts of Sample 1 and Sample 2

Conclusion

This study has demonstrated the successful application of DLLME-SFO technique coupled with GC-FID for the determination of methylparaben in cosmetic samples. This method proved to have a high precision and requires a shorter extraction time with a low volume of the lower toxicity of extraction solvent. It is convinced that DLLME-SFO possesses a great potential in the rapid preconcentration of analyte and provides good linearity over the investigated concentration range. Since this is the first time we reported the application of DLLME-SFO in an analysis of methylparaben, with satisfactory results, the system could readily be applied to determine other parabens from different and complex sample matrices, using different analytical instruments. Combination with other microextraction techniques is suggested to enhance the extraction efficiency and sample preconcentration. In addition, simplicity, ease of operation and less consumption of organic solvents are the additional advantages of the method.

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