Talanta 81 (2010) 1360–1367

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Use of a capillary tube for collecting an extraction solvent lighter than water after dispersive liquid–liquid microextraction and its application in the determination of parabens in different samples by gas chromatography—Flame ionization detection

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ARTICLE INFO

Article history:
Received 30 December 2009
Received in revised form 5 February 2010
Accepted 8 February 2010
Available online 13 February 2010

Keywords:
Dispersive liquid–liquid microextraction
Sample preparation
Preservatives
Parabens
Gas chromatography

ABSTRACT

In this study a new dispersive liquid–liquid microextraction (DLLME) method is presented on the basis of a safe organic solvent, octanol, which is lighter than water. The proposed method is used for the extraction and pre-concentration of some preservatives including methyl paraben (Mep), ethyl paraben (Etp) and propyl paraben (Prp) from different matrices. The extracted compounds are monitored by gas chromatography-flame ionization detector (GC-FID). A mixture of suitable extraction and dispersive solvents including 20 μL octanol and 0.5 mL acetone is quickly injected into the aqueous sample. The mixture is centrifuged for 10 min at 6000 rpm, so a small drop of extraction solvent collects on the water surface. A portion of the collected solvent is removed by a capillary tube through simple dipping the tube into organic solvent drop. 0.4 μL of extract into the tube is removed by a microsyringe and injected into GC. Some effective parameters such as kinds and volumes of extraction and dispersive solvents as well as extraction time have to be investigated. Under optimum conditions, enrichment factors and recoveries of the studied compounds were obtained in the range of 100–276 and 25–72%, respectively. Linear ranges of the calibration curves were between 0.05 and 30 for methyl- and 0.02 and 30 $\mu g\,mL^{-1}$ for ethyl- and propyl parabens, respectively. Limit of detection for methyl paraben was 0.015 $\mu g\,mL^{-1}$ and those of ethyl- and propyl parabens were 0.005 $\mu g\,mL^{-1}$. Relative standard deviations (RSDs %) for six repeated measurements ($C = 2\ \mu g\,mL^{-1}$) were 2% for methyl-, and ethyl parabens and 3% for propyl parabens, respectively.

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1. Introduction

Dispersive liquid–liquid microextraction (DLLME) was first introduced by Assadi and co-workers which it offers an outstanding method to solve many sampling problems [1]. It is based on a ternary component solvents system similar to homogeneous liquid–liquid extraction method (HLLE) [2,3] and cloud point extraction (CPE) [4]. In this method, an appropriate mixture of an extraction solvent and a disperser is rapidly injected into aqueous sample by a syringe, so a cloudy solution is formed. The advantages of DLLME method are operation simplicity, rapidity, low cost, high recoveries and enrichment factors. So far, DLLME has been successfully used for the extraction and pre-concentration of triclosan and methyl triclosan [5], antioxidants [6], organic compounds [1], chlorophenols [7], and polychlorinated biphenyls [8] from aqueous samples. In a common DLLME method, an especially designed extraction vessel and a chlorinated solvent such as chloroform, carbon tetrachloride, chlorobenzene, etc (extraction solvent) which is heavier than water are used. The mentioned solvents are highly toxic and their use is not recommended. In the previous study [9] in order to use more safe solvents such as alcohols and alkanes, which are lighter than water, a new extraction vessel was designed and successfully used for the extraction and pre-concentration of trace amounts of some organophosphorus pesticides from aqueous solutions. This vessel is relatively sophisticated and not easy to use in different sample sizes. Therefore development of a new DLLME method, free of toxic solvents and using very simple extraction vessel is highly taken into consideration.

p-Hydroxy benzoic esters (parabens) have been used as preservatives for over seven decades [10]. Methyl paraben (Mep) and propyl paraben (Prp) are effective antibacterial and anti-fungal agents, which are commonly used as preservatives in foods, beverages, cosmetics and pharmaceuticals [11]. Antimicrobial activity

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doi:10.1016/j.talanta.2010.02.035

of parabens increases as the chain length of the ester group increases, but at the same time their solubility in water decreases. To achieve maximum activity, parabens are used as mixtures of esters since they show synergistic effects. Microbial replication generally occurs in the aqueous phase of oil/water mixtures and amount of parabens dissolved in the aqueous phase generally determines the preservative efficiency. The parabens meet several criteria of an ideal preservative; they have a broad spectrum of antimicrobial activity, are safe to use (i.e., relatively non-irritating, non-sensitizing and of low toxicity), are stable over a wide pH range, and are sufficiently soluble in water to produce the desired effective concentration in the aqueous phase [12,13]. Recently, preservatives in costumer products have received criticism because of their possible side-effects on human health. As a result, a fast, simple and accurate method of analysis is necessary [14]. The reported methods for the determination of parabens in foods, cosmetics and pharmaceuticals are based on high-performance liquid chromatography (HPLC) [15–20], capillary electrophoresis (CE) [21] and gas chromatography (GC) [22,23]. HPLC is the most common method used for detecting these compounds, which is often combined with a pretreatment procedure to remove non-polar matrices. GC with or without derivatization is employed for analysis of parabens [24]. In order to determine the parabens in different samples, an extraction or pre-concentration step is often required prior to their analysis. Different sample preparation methods have been adopted for this purpose. They include solid phase extraction (SPE) [25–27], solid phase microextraction (SPME) [13,28], and shaking and sonication extraction [29]

In this study, a very simple and toxic solvent-free DLLME method has been developed for the extraction and pre-concentration of parabens in complex matrices for their subsequent GC analysis.

2. Experimental

2.1. Reagents and standards

Methyl-, ethyl-, and propyl esters of 4-hydroxy benzoic acid were purchased from Sigma (St. Louis, MO, USA). *n*-Octanol, *n*-pentanol and toluene as extraction solvents and other chemicals such as methanol, acetone, acetonitrile, tetrahydrofuran (THF), undecanol, sodium chloride, hydrochloric acid, and sodium hydroxide were from Merck Company (Darmstadt, Germany). Ethanol and *n*-hexanol were from Fluka (Switzerland). De-ionized water (Ghazi Company, Tabriz, Iran) was used in preparation of aqueous solutions.

2.2. Standard solutions and real samples

Due to the limited solubility of parabens in water, 0.0100 g of each parabens was dissolved in 10 mL methanol to obtain a stock solution with a concentration of 1000 mg L^{-1}. Working standard solutions (10 mg L^{-1}) were prepared by appropriate dilutions of the stock solutions with de-ionized water. The undecanol solution was prepared at a concentration of 500 mg L^{-1} in octanol (extraction solvent). In order to prepare the model solution, 0.0100 g of each paraben was dissolved in 10 mL octanol (each 1000 mg L^{-1}). This mixture was injected to the separation system each day (three times) for quality control. The obtained peaks areas were used for the calculation of enrichment factors and recoveries of the studied compounds. Real samples such as mouth rinse solution (hygienic product), diclofenac gel and lidocain hydrochloride solution (pharmaceuticals), and sauces and tomato paste (food samples) were purchased from local pharmacies and supermarkets to analyse their parabens contents.

2.3. Instrumentation

A gas chromatograph (GC-15A, Shimadzu, Japan) with a split/splitless injector system, and a flame ionization detector was used for separation and determination of parabens. Ultra pure helium (99.9999%, Air products, UK) was used as the carrier gas at a constant linear velocity of 30 cm s^{-1}. The injection port was held at 270 °C in the splitless mode with a purge time of 45 s. Separation was carried out on an SPB-50 capillary column (30 m × 0.22 mm i.d., 0.25 μm film thickness). The oven temperature was programmed as follows: initial temperature 130 °C (held 1 min), from 130 °C to 230 °C at a rate of 10 °C min^{-1}, and finally held at 230 °C for 2 min. The total time for one GC run was 13 min. The FID temperature was maintained at 270 °C. Hydrogen gas for FID was generated with a hydrogen generator (OPGU-1500S, Shimadzu, Japan) at a flow rate of 40 mL min^{-1}. The flow rate of air for FID was 300 mL min^{-1}. The Centurion scientific centrifuge (model D-7200, Hettich, Germany) was used for centrifuging.

2.4. Preparation of real samples

(a) Mouth rinse solution (I) and lidocain hydrochloride (injection solution) (II): 1 mL of (I) or (II) were diluted to 100 mL with de-ionized water, separately. 10 mL of these solutions were subjected to DLLME method before and after spiking with parabens at different concentrations.
(b) Diclofenac gel: 1 g of the diclofenac gel was accurately weighed and transferred into a centrifuge tube. Six milliliters ethanol was added and the mixture was sonicated for 10 min and then centrifuged at 6000 rpm for 10 min. The supernatant solution was transferred into a 50-mL volumetric flask and diluted with de-ionized water.
(c) Food samples: 1 g of sauces or tomato paste was accurately weighed, mixed with 100 mL de-ionized water and homogenized by a magnetic stirrer for 5 min at 300 rpm. The mixture was filtered through a filter paper (DP 135 125, Filalbet, Barcelona, Spain). pH of all real samples after dilution with de-ionized water and before performing extraction procedure was in the range of 4–6. Samples were analyzed without pH adjustment.

2.5. Dispersive liquid–liquid microextraction procedure

10.0 mL of standard or sample solution was transferred into a 12-mL glass test tube. 2 g NaCl was added and the tube was shaken to dissolve NaCl. 0.5 mL acetone (as disperser) containing 20 μL octanol (as extraction solvent) was rapidly injected into solution using a 5-mL syringe. A cloudy solution (water, acetone and octanol) was formed which was stable for a long time. The mixture was centrifuged for 10 min at 6000 rpm. Organic solvent (octanol) was gathered on the surface of aqueous phase as a small drop. A portion of the collected organic phase is removed by a capillary tube (glass capillary tube, 100 mm length and 1.5 mm o.d., Electrothermal, Denmark) through simple dipping the tube into organic solvent drop. An amount of 0.4 μL of organic phase was removed using a 1-μL zero dead volume GC microsyringe (Hamilton, Switzerland) and injected into the GC injector port for analysis. All experiments were performed in triplicates and mean of the results was used in plotting of graphs and preparation of tables.

2.6. Calculation of enrichment factor and extraction recovery

Enrichment factor (EF) is defined as the ratio of analyte concentration in the collected phase (C_{coll}) to the initial concentration of

analyte (C_0) within the sample.

$$EF = \frac{C_{coll}}{C_0} \quad (1)$$

C_{coll} was calculated from calibration graphs plotted by direct injection of standard solutions of parabens in octanol.

The extraction recovery (ER) is defined as the percentage of the total analyte amount (n_0) extracted into the collected phase (n_{coll}).

$$ER = \frac{n_{coll}}{n_0} \times 100 = \left[\frac{C_{coll} \times V_{coll}}{C_0 \times V_{aq}}\right] \times 100$$

$$ER = \left(\frac{V_{coll}}{V_{aq}}\right) \times EF \times 100 \quad (2)$$

where V_{coll} and V_{aq} are the volumes of the collected phase and aqueous solution, respectively.

3. Results and discussion

3.1. Designing of extraction

Preliminary experiments showed that, after centrifuging the cloudy mixture consisting of water, octanol (extraction solvent), and acetone (disperser), a small drop of extraction solvent is collected on the surface of water. In the second stage, a commercially available capillary tube was used to collect a portion of the drop of extract. It was done by simply dipping of the tip of capillary tube into organic solvent drop. Thereafter, capillary tube is withdrawn from test tube. Finally a portion of the extract (0.4 μL) is removed from capillary tube by a microsyringe and injected into GC. This system is very simple and does not require any expensive materials or equipments. As mentioned above only a part of the organic phase is introduced into the capillary tube. Therefore, an easy method for calculation of volume of the collected phase was used. For this purpose, the extraction was performed using an extraction solvent containing a non-soluble compound in water, i.e., undecanol in octanol (500 mg L^{-1}). To determine the volume of organic phase collected on the surface of aqueous sample after centrifuging, undecanol peak area was compared with its peak area after direct injection of undecanol in the extraction solvent at equivalent concentration. The following equation was used for this purpose.

$$V_{coll} = \left(\frac{A_{standard}}{A_{sample}}\right) \times V_{added} \quad (3)$$

where A_{sample} and $A_{standard}$ are peak areas of undecanol after extraction and direct injection of 500 mg L^{-1} undecanol in extraction solvent, respectively. V_{added} is the extraction solvent volume containing 500 mg L^{-1} of undecanol which is injected into aqueous solution along with a disperser. It is noted that $K_{o/w}$ for undecanol is $10^{4.2}$. Therefore back extraction of undecanol into aqueous phase is negligible. However the difference in peak areas of undecanol in two cases (direct injection of standard solution and injection of the collected phase) can be attributed to decrease of extraction solvent volume during extraction due to its solubility in aqueous phase or evaporation.

3.2. Selection of the extraction solvent

Extraction solvent is selected on the basis of its extraction capability, good gas chromatographic behaviour and formation of the cloudy state in the presence of a disperser when it is injected into an aqueous solution. As described previously, the aim of this work is using lighter extraction solvents than water. Due to the vaporization during extraction and centrifuging, low boiling point solvents are not satisfactory. On the other hand, solvent boiling point must not be so high because its chromatographic peak can overlapped with analytes peaks or appeared after them, which leads to low chromatographic resolution. Therefore we have investigated the applicability of *n*-octanol, *n*-hexanol, *n*-pentanol and toluene for the extraction of parabens. Density of the selected organic solvents is 0.82, 0.81, 0.81, and 0.87 g mL^{-1}, respectively. A series of experiments were performed by using 100 μL of the selected extraction solvents and 0.5 mL acetone as a disperser. The obtained results revealed that by using pentanol, toluene and hexanol, no organic phase is collected on the surface of aqueous phase. By using octanol, 86% of the solvent is collected as a drop. Therefore, octanol was selected as the extraction solvent.

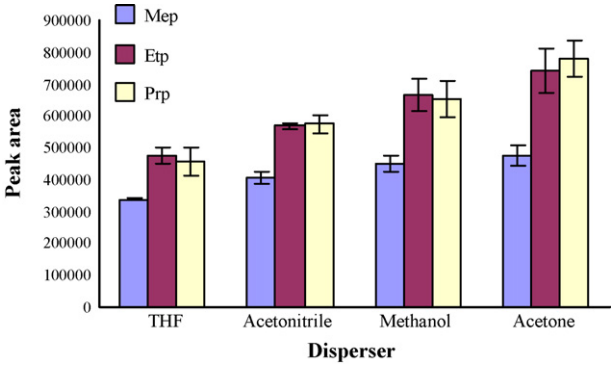

Fig. 1. Effect of disperser solvent kind on the extraction efficiency. Extraction conditions: extraction solvent, 100 μL octanol; disperser volume, 0.5 mL; sample volume, 10 mL; analytes concentrations, 10 mg L^{-1} of each paraben; extraction time, 0 min; centrifuging time, 5 min and centrifuging speed, 6000 rpm. The bars indicate the maximum and minimum of three determinations.

3.3. Selection of the disperser

The role of a disperser is dispersion of an extraction solvent into aqueous sample to make extensive contact area between them and facilitating the mass transfer of analyte from water to organic solvent which causes considerable acceleration in the extraction of analytes. Miscibility of dispersion solvent either in extraction phase or aqueous phase (sample) is the main point for its selection. Therefore in this section the ability of acetone, acetonitrile, THF and methanol was investigated. For this purpose, extraction of analytes was carried out by using 0.5 mL of each disperser containing 100 μL octanol (extraction solvent). 0.4 μL of extract was analysed by GC. Chromatographic peaks areas as a function of disperser kind are illustrated in Fig. 1. According to these results, and owing to less toxicity, low cost and high recoveries of analytes, acetone is selected for further experiments.

3.4. Optimization of disperser volume

Different volumes of acetone (0.25, 0.50, 1.0, 1.5, and 2.0 mL) containing a constant volume of octanol were tested. The experimental observations showed that with low volume of acetone (0.25 mL), the cloudy state is not formed well so extraction is disturbed. The obtained results showed that extraction efficiency is high in the range of 0.5–1.5 mL of disperser volume and decreases thereafter. It is most possible that at high volume of acetone (2 mL), the solubility of parabens in water increases and therefore enrichment factor decreases. As a result, 0.5 mL was selected as the optimum disperser volume in the further experiments.

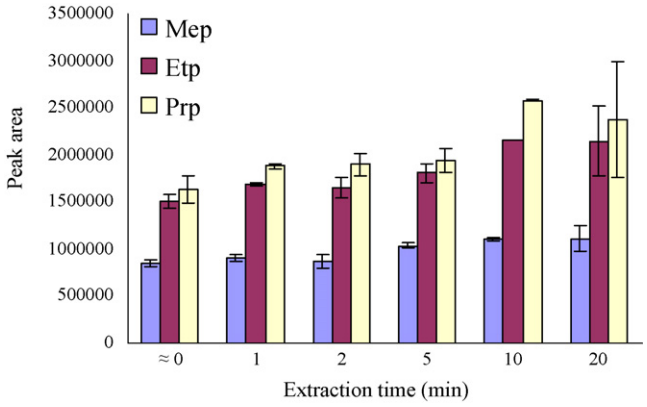

Fig. 2. Effect of extraction time on the extraction efficiency. Extraction conditions: extraction solvent, octanol (40 μL); disperser, acetone (0.5 mL); sample volume, 10 mL; analytes concentrations, 10 mg L^{-1} of each paraben; centrifuging time, 5 min and centrifuging speed, 6000 rpm. The bars indicate the maximum and minimum of three determinations. In the case of ≈0, centrifuging is performed immediately after adding acetone and octanol mixture.

3.5. Effect of extraction time

In a DLLME procedure, extraction time is defined as an interval time between injection of mixture of disperser and extraction solvent, and before centrifugation. The effect of time was examined in the range of 1–20 min as well as immediately after adding octanol + acetone mixture while the other experimental conditions are maintained constant. The obtained results show that transferring of analytes from aqueous phase to extraction solvent is very fast (Fig. 2). From these data it can be concluded that extraction time is not an important factor in this study. However considering smallest error bars (high repeatability), 10 min was selected as optimum extraction time.

3.6. Effect of salt addition on the extraction efficiency and extract drop volume

To evaluate this parameter, two series of experiments were performed: in the first series of experiments, 20, 30, 40, 50, 75, and 100 μL of octanol were dissolved in 0.5 mL portions of acetone. The mixtures were used separately for the extraction of analytes from 10 mL of aqueous standards solution containing 10 mg L^{-1} of each paraben and 2 g NaCl. In the second series, 0.5 mL portions of acetone containing 20 μL of octanol were used for the extraction of analytes from 10 mL aqueous standards solution containing 10 mg L^{-1} of each paraben with various percents of NaCl (5, 10, 20, and 30%, w/v). The extracted amounts of analytes based on the chromatographic peaks areas for each analyte in the first series of experiments are illustrated in Fig. 3. From these results, it can be seen that the drop volume (volume of the collected phase) increases in the presence of 20% NaCl compared to the cases in which NaCl was not added. This is certainly due to the salting out effect and decreasing of organic solvent miscibility with water. This phenomenon allows us to decrease the volume of extraction solvent (20 μL) and increase enrichment factor without losing extract drop volume. The results obtained in second series of experiments revealed that for the low homologues, especially methyl paraben, a relatively large analytical signal is obtained by increasing the percentage of salt from 5% to 20% and then it remains constant up to 30%. This confirms the significant effect of salt on the extraction efficiency of methyl paraben, while in the case of heavier homologue, i.e., propyl paraben, salting out effect is negligible. Therefore, the further studies were performed in the presence of 20% (w/v) NaCl and 20 μL extraction solvent.

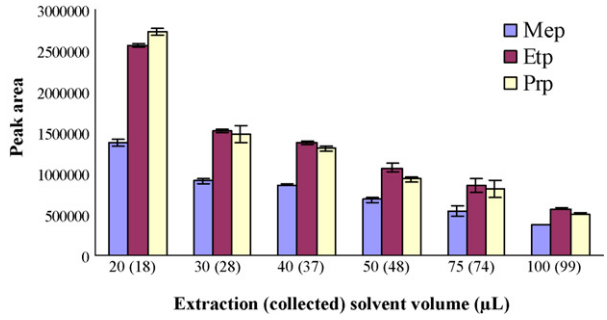

Fig. 3. Effect of extraction (collected) solvent volume on the analytical signals. Extraction conditions: disperser, acetone (0.5 mL); sample volume, 10 mL; analytes concentrations, 10 mg L^{-1} of each paraben; NaCl amount, 2 g; centrifuging time, 5 min and centrifuging speed, 6000 rpm. The bars indicate the maximum and minimum of three determinations. Volume of the collected phase is given into parentheses (*X*-axis).

3.7. Effect of pH

The pH of aqueous solution is a significant factor in the extraction of an acidic or alkaline analyte. The effect of sample pH on the extraction efficiency of the studied compounds from aqueous samples was investigated in the range of 2–12. The obtained results presented in Fig. 4, indicate that the peaks areas increase with increasing pH, reach a maximum at pH 4 and remain constant up to pH 6, and then decrease thereafter. Decreasing extraction efficiency at higher pHs has two different possible origins. Firstly, considering acidic constants of parabens (methyl paraben, $pK_a = 8.2$; ethyl paraben, $pK_a = 8.2$; and propyl paraben, $pK_a = 8.4$), they are in ionic forms at higher pHs and cannot be extracted into organic solvents. Secondly, hydrolysis of parabens will occur at elevated pH values [12]. Therefore they can considerably be extracted at pH values below pK_a values (<8). On the other hand, decreasing of extraction efficiency at more acidic pH (below pH 4) is due to the hydrolysing of ester group of the parabens. The pH of original working solution was 6.1. Therefore in the further experiments there is no need for pH adjustment.

3.8. Optimization of centrifuging time and speed

In this method, time and speed of centrifugation are the main effective parameters and must be optimized.

3.8.1. Optimization of centrifuging time duration

To achieve the optimum value of this parameter, some experiments were carried out in the range of 1–15 min and the obtained results are illustrated in Fig. 5. From these results, it is concluded

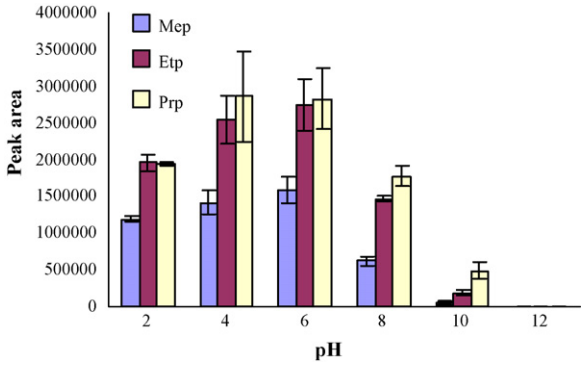

Fig. 4. Effect of sample pH on the extraction efficiency of parabens. Extraction conditions (except pH) are the same as in Fig. 3. The bars indicate the maximum and minimum of three determinations.

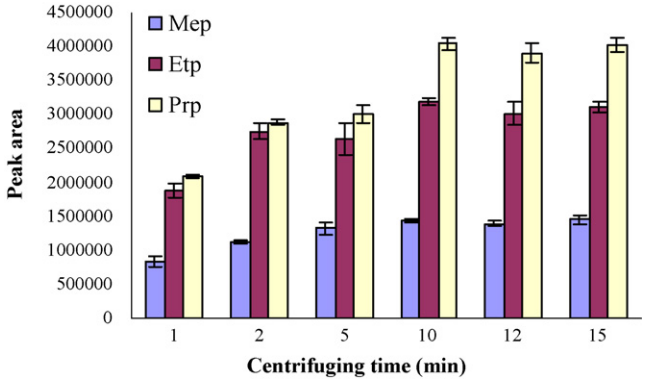

Fig. 5. Effect of centrifuging time on the extraction efficiency of parabens. Extraction conditions: extraction solvent, octanol (20 μL); disperser, acetone (0.5 mL); sample volume, 10 mL; analytes concentrations, 10 mg L^{-1} of each paraben; extraction time, 10 min; centrifuging speed, 6000 rpm and NaCl amount, 2 g. The bars indicate the maximum and minimum of three determinations.

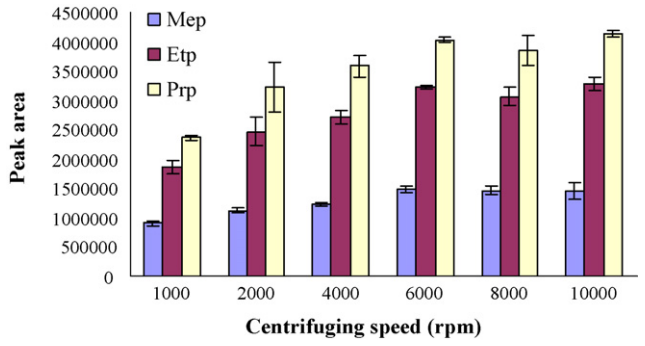

Fig. 6. Effect of centrifugation speed on the extraction efficiency of parabens. Extraction conditions: extraction solvent, octanol (20 μL); disperser, acetone (0.5 mL); sample volume, 10 mL; analytes concentrations, 10 mg L^{-1} of each paraben; extraction time, 10 min; centrifuging time,10 min and NaCl amount, 2 g. The bars indicate the maximum and minimum of three determinations.

that the extraction efficiency increases with increasing centrifugation time and remains constant after 10 min. These are normal and logical results, because after complete separation of organic phase from sample solution, longer centrifugation cannot play any effective role. Therefore 10 min centrifuging time duration was selected in the further experiments.

3.8.2. Optimization of centrifuging speed

For this purpose, some experiments were carried out at centrifugation speed range of 1000–10,000 rpm with 1000 rpm intervals for 10 min. The obtained extraction efficiencies according to the chromatographic peaks areas are plotted as a function of centrifugation speed in Fig. 6. These results reveal that the extraction efficiencies increase with increasing of speed and reach plateaus after 6000 rpm. 6000 rpm was therefore selected as the optimum centrifugation speed.

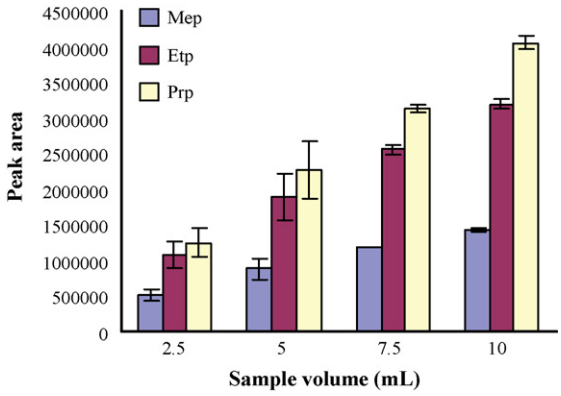

Fig. 7. Effect of sample volume on the extraction efficiencies of parabens. Extraction conditions: extraction solvent, octanol (20 μL); disperser, acetone (0.5 mL); analytes concentrations, 10 mg L^{-1} of each paraben; NaCl concentration, 20% (w/v) and sample pH, not adjusted. The bars indicate the maximum and minimum of three determinations.

3.9. Optimization of sample volume

DLLME procedure was performed for extraction of the studied compounds from 2.5, 5.0, 7.5, and 10.0 mL standard solution (containing 10 mg L^{-1} of each analyte). The obtained results are shown in Fig. 7. These results prove that analytical signals increase by increasing the sample volume. This is due to the increase of enrichment factors of the analytes in extract related to the additional amounts of analytes in the system. However, more increasing of sample size is not reasonable, because it prevents the formation of organic phase drop. Furthermore, due to decreasing the volume ratio of organic phase/aqueous phase, the extraction efficiency can be also reduced. Therefore, in this study 10 mL was selected as the optimum sample size.

3.10. Quantitative features of the method

Quantitative characteristics of the proposed method are obtained under the optimized conditions and shown in Table 1. Linearity ranges of the calibration curves are 0.05–30 for methyl paraben and 0.02–30 μg mL^{-1} for ethyl- and propyl parabens. Square of correlation coefficients (R^2) ranged from 0.992 to 0.999. The enrichment factors of the selected parabens were relatively high (100–276). Repeatability of the method was investigated by six repeated experiments performed under the optimized conditions. The relative standard deviations (RSDs %) for the studied parabens ranged from 4% to 8% without internal standard and 2–3% with internal standard (undecanol), respectively. The limits of detection (LODs) based on signal-to-noise ratio (S/N) of 3 ranged from 5 to 15 ng mL^{-1}, which are very low for GC-FID.

Table 1
Quantitative features of the proposed DLLME-GC-FID method.

Analyte	LR[a] (μg mL^{-1})	R^2[b]	LOD[c] (ng mL^{-1})	LOQ[d] (ng mL^{-1})	RSD[e] (%)	RSD[f] (%)	EF ± SD[g]	R% ± SD[h]
Methyl paraben	0.05–30	0.999	15	50	2	8	100 ± 7	25 ± 0.2
Ethyl paraben	0.02–30	0.999	5	20	2	4	202 ± 4	52 ± 1
Propyl paraben	0.02–30	0.992	5	20	3	6	276 ± 7	72 ± 2

[a] Linear range.
[b] Square of correlation coefficient.
[c] Limit of detection (S/N = 3).
[d] Limit of quantification (S/N = 10).
[e] Relative standard deviation (C = 2 μg mL^{-1}, n = 6) with internal standard (undecanol).
[f] Relative standard deviation (C = 2 μg mL^{-1}, n = 6) without internal standard.
[g] Mean enrichment factor ± standard deviation, EF = C_{coll}/C_0 (n = 3).
[h] Mean extraction recovery ± standard deviation (n = 3).

Table 2
Comparison of the proposed DLLME method with other methods used in pre-concentration and determination of the target analytes.

Method	Compound	LR (μg mL^{-1})	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	RSD (%)	Extraction time (min)	Sample volume (mL)	Ref.
SPME-IMS[a]	Mep	0.05–1.0	0.988	10	30	7.1	15	3	[30]
	Etp	0.05–1.0	0.990	10	30	6.0			
	Prp	0.05–1.0	0.991	5	15	4.3			
SPME-GC/MS[b]	Mep	0.01–2	0.999	6.0	–	10.0	40	30	[31]
	Etp	0.01–0.4	0.999	8.5	–	10.3			
	Prp	0.001–1	0.999	0.4	–	8.2			
SFE-CZE-UV[c]	Mep	0.35–60	0.999	1.0	–	1.70	15 (static) 20 (dynamic)	20	[24]
	Etp	0.35–60	0.999	1.1	–	0.63			
	Prp	0.35–60	0.999	1.2	–	0.60			
SPME-GC/-MS	Mep	–	0.999	–	0.025	8.3	40	10	[13]
	Etp	–	0.997	–	0.005	6.3			
	Prp	–	0.998	–	0.002	2.4			
SFE-LC/MS[d]	Mep	0.01–1	0.996	4.7	–	18.6	5 (static) 20 (dynamic)	–	[32]
	Etp	0.02–2	0.997	13.5	–	4.7			
	Prp	0.02–2	0.999	13.4	–	5.7			
SBSE-GC/MS[e]	Mep	–	–	–	–	–	120	10	[33]
	Etp	0.1–100	0.999	15	–	–			
	Prp	0.1–100	0.998	17	–	–			
DLLME-GC-FID	Mep	0.05–30	0.999	15	50	2	10	10	This study
	Etp	0.02–30	0.999	5	20	2			
	Prp	0.02–30	0.992	5	20	3			

[a] Solid phase microextraction-ion mobility spectrometry.
[b] Solid phase microextraction-gas chromatography–mass spectrometry.
[c] Supercritical fluid extraction-capillary zone electrophoresis-ultraviolet visible detection.
[d] Supercritical fluid extraction-liquid chromatography–mass spectrometry.
[e] Stir-bar sportive extraction-gas chromatography–mass spectrometry.

3.11. Comparison of the proposed DLLME method with other methods

Some representative analytical characteristics of the proposed method were collected in Table 2 and compared with literature data obtained from other methods. This comparison proves that, the proposed extraction method is very simple and does not need any complex and expensive instrument and it is promising as a sensitive, rapid and repeatable method for the extraction and pre-concentration of parabens from aqueous samples.

3.12. Application

To evaluate the reliability of the proposed method for the extraction and pre-concentration of parabens from real samples, different samples such as mouth rinse solution (hygienic product), diclofenac

Table 3
Recovery percent of the selected parabens from different hygienic product, pharmaceuticals, and food samples.

Sample	Added (mg)			Found (mg)			Relative recovery (%)		
	Mep	Etp	Prp	Mep	Etp	Prp	Mep	Etp	Prp
Mouth rinse solution	–	–	–	12.0 (1200)[a]	n.d.[b]	2.0 (200)	–	–	–
	2.5	5.0	2.5	14.1 ± 0.3[c]	5.6 ± 0.1	4.6 ± 0.4	84 ± 11[d]	113 ± 2	104 ± 16
	5.0	5.0	5.0	16.6 ± 1.0	5.0 ± 0.4	7.3 ± 0.03	92 ± 1	99 ± 7	106 ± 1
	10	5.0	10	22.2 ± 0.2	5.5 ± 0.1	13.7 ± 0.2	102 ± 2	110 ± 1	117 ± 2
Lidocain hydrochlorie	–	–	–	n.d.	n.d.	n.d.	–	–	–
	2.5	2.5	2.5	2.2 ± 0.4	2.7 ± 0.3	2.1 ± 0.1	86 ± 14	108 ± 12	84 ± 4
	5.0	5.0	5.0	5.1 ± 0.5	5.1 ± 0.2	5.1 ± 0.3	103 ± 9	102 ± 4	102 ± 6
	10	10	10	9.4 ± 0.4	9.0 ± 0.5	8.6 ± 0.9	94 ± 4	88 ± 5	86 ± 8
Diclofenac gel	–	–	–	n.d.	n.d.	n.d	–	–	–
	2.5	2.5	2.5	2.1 ± 0.2	2.2 ± 0.2	2.6 ± 0.2	84 ± 8	88 ± 9	104 ± 6
	5.0	5.0	5.0	5.0 ± 0.4	4.6 ± 0.2	5.4 ± 0.2	96 ± 8	91 ± 5	109 ± 5
	10	10	10	9.3 ± 0.7	10.5 ± 0.8	11.3 ± 0.6	93 ± 6	105 ± 8	113 ± 6
Tomato paste	–	–	–	n.d.	n.d.	n.d	–	–	–
	2.5	2.5	2.5	2.2 ± 0.8	2.4 ± 0.1	3.3 ± 0.5	88 ± 4	96 ± 4	131 ± 23
	5.0	5.0	5.0	5.0 ± 0.4	4.6 ± 1.0	5.3 ± 0.4	99 ± 7	92 ± 18	106 ± 8
	10	10	10	8.2 ± 0.6	8.3 ± 0.6	10.3 ± 0.5	82 ± 6	83 ± 6	103 ± 5
Sauces	–	–	–	n.d.	n.d.	n.d.	–	–	–
	2.5	2.5	2.5	1.5 ± 0.3	1.4 ± 0.2	1.1 ± 0.1	58 ± 11	58 ± 6	42 ± 6
	5.0	5.0	5.0	4.2 ± 0.3	4.15 ± 1.0	4.0 ± 0.6	83 ± 6	83 ± 19	81 ± 11
	10	10	10	8.6 ± 0.4	7.9 ± 0.1	8.1 ± 0.5	86 ± 3	79 ± 1	81 ± 6

[a] Data into the parentheses are the concentrations (mg L^{-1}) of analytes in samples obtained by the proposed method.
[b] Not detected.
[c] Mean found amount ± standard deviation (n = 3).
[d] Mean recovery ± standard deviation (n = 3).

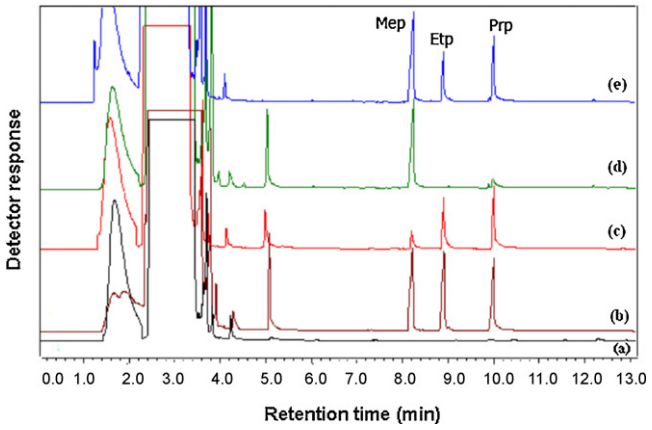

Fig. 8. Chromatograms of (a) blank, (b) direct injection (0.4 μL) of standard solution of parabens (each 1000 mg L^{-1}), (c) standard solution of parabens (each 5 mg L^{-1}), (d) mouth rinse solution, and (e) mouth rinse solution spiked with analytes (each 5 mg L^{-1}). In all cases (except chromatogram b) DLLME method was performed on them and 0.4 μL of the collected phase was injected into GC. For chromatographic conditions see the text.

gel and lidocain hydrochloride solution (pharmaceuticals), sauces and tomato paste (food samples) were selected, spiked and subjected to the DLLME-GC-FID analysis. Results are presented in Table 3. Also Figs. 8 and 9 show typical chromatograms obtained from different samples. Methyl- and propyl parabens in the studied mouth rinse solution were detected. GC quantitative analysis reveals that in the optimum conditions, more than 82% of the added parabens can be recovered and analysed (except in sauces sample).

4. Conclusions

This paper describes the development of a DLLME-GC-FID technique for the determination of methyl-, ethyl-, and propyl parabens in foods, pharmaceuticals and aqueous samples. The procedure does not require derivatization step and provides a fast, sensitive, and reliable method for detecting and quantization of the selected parabens in relatively complex matrices. The main advantages of this method include the reduction of labour-intensive and time-consuming extraction steps, very little solvent consumption in extraction, small sample volume and short analysis time. Using a safe organic solvent (octanol) as extraction solvent is another important feature of the proposed DLLME method in this study. A simple procedure with use of a capillary tube has been utilized for collection of the organic phase after centrifuging.

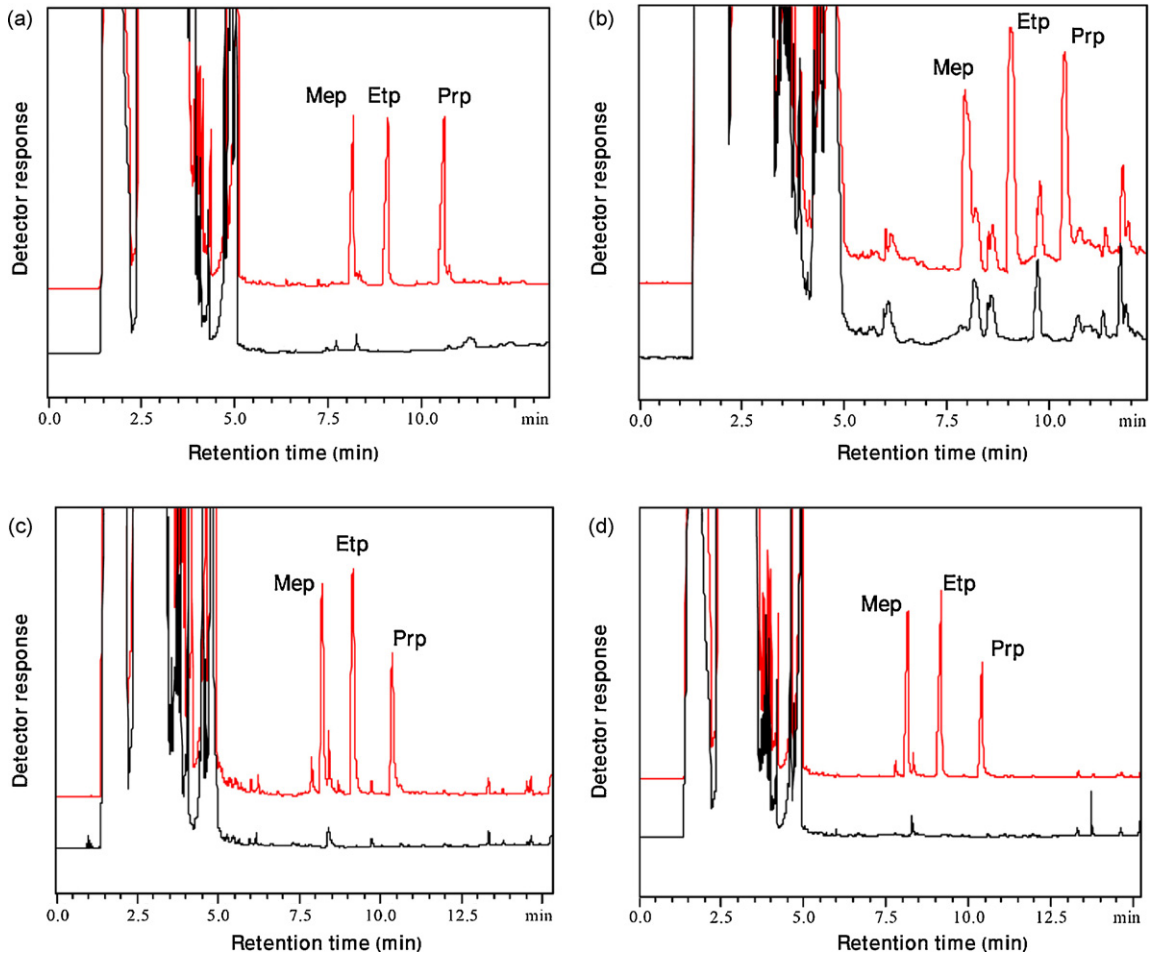

Fig. 9. Typical chromatograms of (a) lidocain hydrochloride, (b) diclofenac gel, (c) sauces, and (d) tomato paste. In all cases lower chromatograms belong to unspiked samples and upper chromatograms belong to spiked samples with parabens (each 5 mg L^{-1}). DLLME technique was performed on them and 0.4 μL of the collected phase was injected into GC. For chromatographic conditions see the experimental section.

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