CHEMIJA. 2011. Vol. 22. No. 3. P. 155–161

Hollow fibre liquid phase microextraction of derivatized parabens

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Hollow fibre liquid phase microextraction in combination with an *in situ* derivatization followed by gas chromatographic determination is suggested for parabens sampling and preconcentration. The derivatization was carried out with acetic anhydride in alkaline conditions maintained with di-potassium hydrogen phosphate. The effects of the extraction solvent type, extraction time and the ionic strength of the solution on the extraction efficiency were investigated. Chlorobenzene containing *n*-hexadecane as the internal standard was used as an extracting solvent. The calibration graphs were linear up to 10 mg L^{-1}, correlation coefficients were 0.997–0.999, and detection limits were 18, 9.1, 6.4 and 5.1 μg L^{-1} for methylparaben, ethylparaben, propylparaben and butylparaben, respectively. Repeatabilities of the results were acceptable with relative standard deviations up to 9.2%. The possibility to apply the proposed method for paraben determination in facial tonics was demonstrated.

Key words: hollow fibre liquid phase microextraction, gas chromatography, parabens, derivatization, cosmetics

INTRODUCTION

Determination of esters of p-hydroxybenzoic acid (parabens) is of high interest because, due to their bactericidal and fungicidal properties, parabens are used extensively as preservatives in cosmetics, in personal care products applied on the skin, hair, scalp, lips, mucosae and nails, in pharmaceutical products and even in foods and beverages [1, 2]. The European Union Cosmetics Directive restricts the preservation of cosmetic products to a maximum concentration of each paraben of 0.4% and a total maximum concentration of 0.8% [3]. Parabens are also registered for use in foods; however, the latter use is more strictly regulated and even recommended to be withdrawn.

For many years, parabens were ranked among preservatives with a low toxicity. However, some years ago it has been demonstrated that paraben preservatives are genotoxic [4], oestrogenic, affect the human endocrine system and probably cause breast cancer [2, 5] and male reproductive disorders [3]. Also, a relationship was observed between the increasing melanoma rate and the greater use of paraben-containing skincare / suncare products [6]. Because of their high use, parabens are continuously released in the environment via sewage [7, 8]. Although they are removed to a considerable extent during sewage water treatment [8], their presence has been detected in natural water [9].

Because of the presence of parabens in the environment and their negative effects on human health, there is an increasing interest in their trace analysis. One of the most common methods for paraben analysis is gas chromatography [10]. However, due to their polar nature, prior to GC analysis parabens are often derivatized to reduce their adsorption in the chromatographic system, to improve sensitivity, peak separation and peak symmetry [11]. Post-extraction derivatization can be performed using alkylation with diazomethane [12] and silylation with bis(trimethylsilyl)trifluoroacetamide or *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide [7, 13]. However, this derivatization procedure involves additional steps which increase the time required for sample preparation.

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An alternative derivatization approach is *in situ* acetylation. The reaction can be performed in aqueous samples in a few minutes with high efficiency and using low-cost reagents. For *in situ* acetylation, derivatization reagents such as acetic anhydride or isobutyl chloroformate [14] are added into the aqueous sample.

Since the concentration of parabens in the environment is rather low and cosmetics present rather complex matrices for the analysis, it is necessary to apply a preconcentration or isolation step prior to the chromatographic analysis.

In recent years, microextraction techniques are gaining a growing interest. One of them is hollow fibre liquid phase microextraction (HFLPME) proposed in 1999 [15]. It is a miniaturized version of liquid–liquid extraction. The technique utilizes porous, hydrophobic polypropylene hollow fibre as a membrane. The fibre is impregnated with an organic phase, and inside the hollow fibre there is a receiving phase (the same or different as the organic phase used for hollow fibre impregnation). This new microextraction methodology, apart from being simple and fast, also enables clean extract formation. The low cost of the hollow fibre enables to dispose each extraction unit after a single extraction and thus to exclude cross-contamination problems from sample to sample and to avoid the need of regeneration of the extraction unit.

For paraben extraction, prior to GC analysis, some microextraction techniques have been applied. Underivatized parabens have been extracted using solid phase microextraction [1, 16–18], dispersive liquid phase microextraction [19–21], hollow fibre liquid phase microextraction [22]. For derivatized paraben extraction, solid phase microextraction [7, 23], stir bar sorptive extraction [24], single drop microextraction [25, 26] techniques have been applied.

However, to our knowledge, HFLPME has never been used for the preconcentration of derivatized parabens. In the present work, a method based on *in situ* acetylation of parabens, followed by HFLPME and GC determination, was developed.

EXPERIMENTAL

Reagents

Methylparaben (99%), ethylparaben (99%), propylparaben (99%), butylparaben (99%), o-xylene ($C_6H_4(CH_3)_2$ (99%), octanol-1 ($C_8H_{18}O$) (99%), chlorobenzene (C_6H_5Cl) (99%), bromobenzene (C_6H_5Br) (99%), acetic anhydride (99%), and acetone (99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaCl (analytical grade) was purchased from "Reachim" (Donetsk, Ukraine). Di-potassium hydrogen phosphate trihydrate ($K_2HPO_4{\cdot}3H_2O$) (99%) was purchased from Carl Roth GmbH+ Co. (Germany). The physical properties of the extraction solvents are shown in Table 1.

A standard stock solution containing 10 mg mL^{-1} of methylparaben, ethylparaben, propylparaben and butylparaben was prepared in acetone. The stock solution was stored refrigerated at +4 °C. Working standard solutions were prepared daily by diluting the stock standard solution with distilled water to the required concentrations.

Hollow-fibre liquid phase microextraction procedure

For HFLPME, 10 mL of sample was placed into a 12 mL vial containing a magnetic stirring bar; 0.2 g of $K_2HPO_4 \cdot 3H_2O$ was dissolved in the sample solution, and 10 µL of acetic anhydride was added. HPLPME was carried out using an Accurel Q 3/2 polypropylene hollow fibre membrane (Membrana, Wuppertal, Germany) with a 200 µm wall thickness, 0.2 µm pore size and 600 µm internal diameter. The hollow fibre was cut into 1.8 cm long pieces. One end of each piece was heat-sealed using soldering iron. The effective internal volume of a piece of the hollow fibre was approximately 5 µL. Each piece was used only once. Before use, the hollow fibres had been sonicated in acetone for 10 min, then removed from acetone and allowed to dry at room temperature.

The unsealed end of the fibre was connected to a 0.7 cm diameter syringe needle inserted into the silicone rubber septum placed in the extraction vial cap. For several minutes the hollow fibre was immersed into the receiving phase. The receiving phase impregnated its walls and penetrated inside the hollow fibre, filling it completely. Then the fibre was withdrawn from the receiving phase, washed with distilled water in order to eliminate the excess of the receiving phase, and immersed into the sample solution. The sample vial was placed on a magnetic stirrer. After the extraction, the vial cap together with the needle and the hollow fibre was removed from the vial, and 1 µL of the extract was withdrawn into a 10 µL microsyringe and injected into the GC system.

GC analysis

Gas chromatography was carried out in a Varian 3400 (Palo Alto, CA, USA) gas chromatograph equipped with a flame ionisation detector coupled with an SP4290 integrator (Spectra-Physics San Jose, CA, USA) and a Equity™-5 fused silica capillary column (30 m × 0.53 mm, 1.5 µm film thickness) supplied by Supelco (Bellefonte, PA, USA). The

Table 1. **Physical properties of extraction solvents**

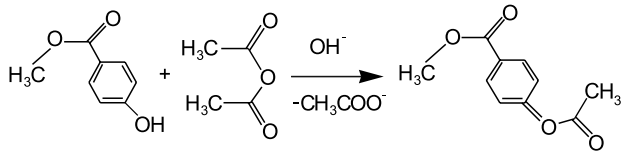
Solvent	Boiling point, °C	Refraction index	Water solubility, g L^{-1}
o-Xylene	144	1.505	0.18
Chlorobenzene	132	1.525	0.5
Bromobenzene	156	1.559	0.4
Octanol-1	195	1.428	0.0003

splittless injection mode was used. The injector and detector temperature was 280 °C. The oven temperature was programmed – initially set at 120 °C for 2 min, then gradually ramped to 176 °C (2 °C min^{-1}), 280 °C (50 °C min^{-1}) and kept for 1 min. The following gas flow rates were used: carrier (nitrogen) 10, make-up gas (nitrogen) 20, hydrogen 30 and air 300 mL min^{-1}.

RESULTS AND DISCUSSION

Derivatization conditions

Parabens can be derivatized before gas chromatographic analysis or analysed without a derivatization step. The purpose of derivatization is to convert the native form to a less polar and more volatile species. In addition, in-sample derivatization accomplished before the extraction step can increase the extractability of the analytes. According to the literature, *in-situ* acetylation of parabens with acetic anhydride is especially simple and fast [11], it was chosen in this work as a paraben derivatization procedure. The paraben acetylation reaction in the presence of an alkaline catalyst is outlined below:



The influence of derivatization on the paraben peak shape was examined. For this purpose, chromatograms of underivatizes and derivatized parabens were obtained in the same chromatographic conditions. For paraben derivatization, to 10 ml of 1 mg mL^{-1} paraben solution 0.5 g of $K_2HPO_4 \cdot 3H_2O$ and 100 µL of acetic anhydride were added. The chromatographic benefit of derivatization can be seen in Fig. 1. Underivatized parabens produced asymmetric, broad peaks with significant tailing due to the interaction of hydroxyl groups with the chromatographic system. The peaks of derivatized paraben were of improved shape, higher and narrower than the peaks of underivatized paraben.

Acetylation with acetic anhydride is normally performed in the presence of hydrogencarbonate or pyridine [14, 24]; however, according to [14], the use of hydrogenphosphate leads to a higher extraction efficiency than does hydrogencarbonate. Thus, in our work, di-potassium hydrogenphosphate was used as the basic catalyser.

The influence of pH on derivatization efficiency was examined, and it was determined that an increase in the paraben peak area took place at pH up to 7.5–8.0. At pH < 7.5, peaks of derivatized and underivatized parabens were observed in the chromatogram. At pH values higher than 8, derivatization efficiency did not change any more, and only derivatized paraben peaks were present. Thus, for the further work, the pH of the sample solution was adjusted to 9 by adding 0.02 g mL^{-1} of $K_2HPO_4 \cdot 3H_2O$.

Optimization of HFLPME conditions

The extracting solvent used in HFLPME has to meet some requirements: to extract the analytes quite well, to be practically insoluble in water and to be separated from the analyte

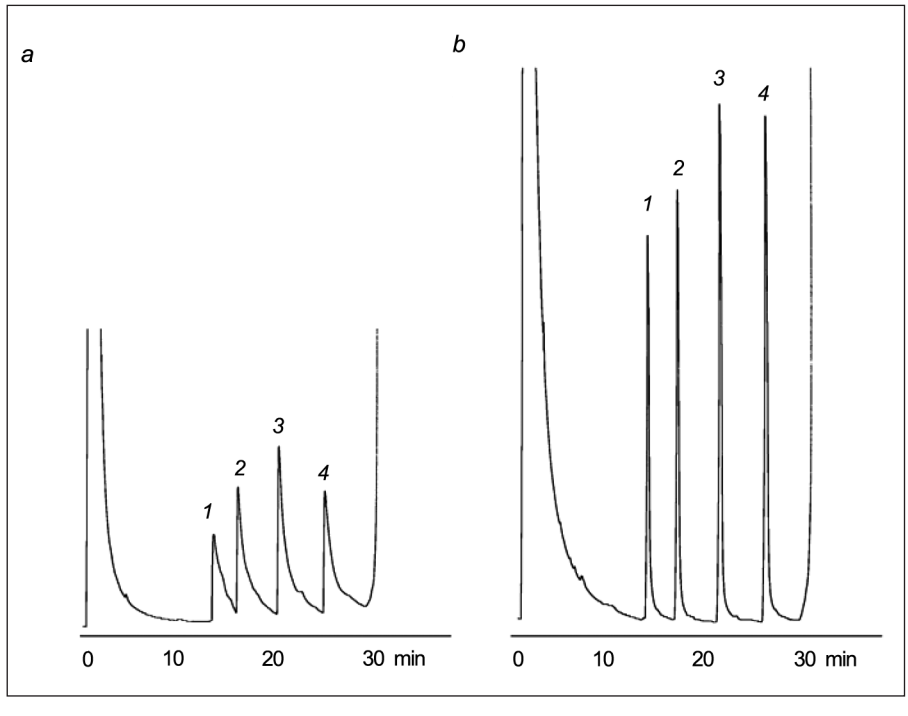


Fig. 1. Chromatograms of underivatized (*a*) and derivatized (*b*) paraben solution (1 mg mL^{-1}): *1* – methylparaben, *2* – ethylparaben, *3* – propylparaben, *4* – butylparaben. For GC conditions, see Experimental

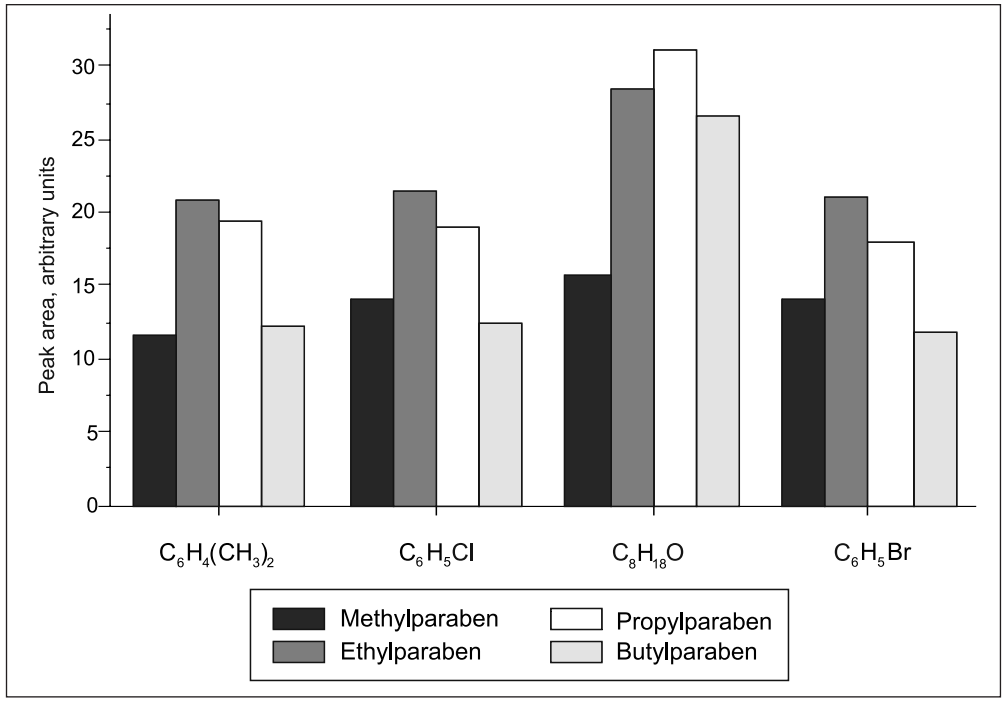


Fig. 2. Effect of HFLPME solvent on extraction efficiency. Extraction conditions: sample volume 10 mL, concentration of parabens 10 mg L^{-1}, 0.02 g mL^{-1} of $K_2HPO_4 \cdot 3H_2O$, acetic anhydride volume 10 µL, extraction time 20 min, solution stirring rate 800 rpm

peaks in the chromatogram. In addition, solvents for HFLPME must be able to penetrate into the pores of the polypropylene hollow fibre and, for easier work, the optical properties of the solvent should allow to see the solvent in the hollow fibre in order to control whether the hollow fibre is well-filled.

Octanol-1, chlorobenzene, bromobenzene and *o*-xylene were tested as extracting solvents. For preliminary studies, extraction was carried out for 20 min. As one can see in Fig. 2, the highest extraction efficiency was obtained using octanol-1. However, octanol-1 was practically invisible in the hollow fibre, so it was rather inconvenient for the work. Contrarily, the walls of the hollow fibre immersed into *o*-xylene, chlorobenzene or bromobenzene became transparent; thus, it was possible to observe how the solvent filled the space inside a hollow fibre. The extraction efficiencies of *o*-xylene, chlorobenzene and bromobenzene were quite similar. As chlorobenzene had the lowest boiling point, its retention time in the chromatogram was less than that of *o*-xylene and bromobenzene; chlorobenzene was better separated from the analytes and thus was chosen as an extracting solvent for parabens.

In order to alleviate injected extract volume error and to improve repeatability, *n*-hexadecane (10 µg mL^{-1}) was added to the extraction solvent as an internal standard.

Since the receiving phase is protected by the hollow fibre, there is a possibility to apply high stirring rates and thus to reduce the time required to reach the equilibrium of the analytes between the aqueous and the receiving phases. In this work, we applied the stirring rate of 800 rpm.

Extraction time was assessed to be 5 and 40 min. HFLPME is an equilibrium extraction technique, thus the amount of the parabens extracted depends on the equilibration time, which is reached when the further increase in the extraction time does not result in a significant increase in the amount of extracted parabens. As is seen in Fig. 3, the peak areas of extracted parabens leveled off after about 30 min. The opti-

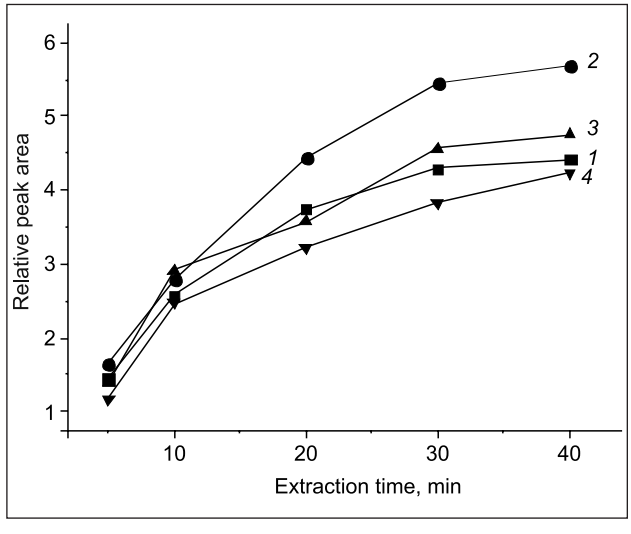


Fig. 3. Effect of HFLPME time on extraction efficiency: *1* – methylparaben, *2* – ethylparaben, *3* – propylparaben, *4* – butylparaben. Extraction conditions: sample volume 10 mL, concentration of parabens 10 mg L^{-1}, 0.02 g mL^{-1} of $K_2HPO_4 \cdot 3H_2O$, acetic anhydride volume 10 µL, extracting solvent chlorobenzene, solution stirring rate 800 rpm. Peak areas are normalised to *n*-hexadecane peak area

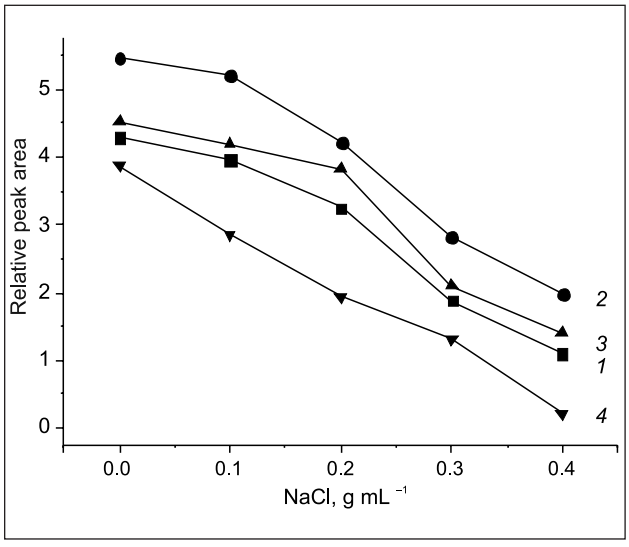


Fig. 4. Effect of NaCl content on extraction efficiency: *1* – methylparaben, *2* – ethylparaben, *3* – propylparaben, *4* – butylparaben. Extraction conditions: sample volume 10 mL, concentration of parabens 10 mg L^{-1}, 0.02 g mL^{-1} of $K_2HPO_4 \cdot 3H_2O$, acetic anhydride volume 10 µL, extracting solvent chlorobenzene, extraction time 30 min, solution stirring rate 800 rpm. Peak areas are normalised to *n*-hexadecane peak area

mum sample extraction time of 30 min was therefore chosen to achieve maximum sensitivity without extending the time of analysis.

Addition of salt to an aqueous sample solution generally causes a decrease in the solubility of organic compounds in water, and this feature has been widely used to enhance the extraction of analytes. The effect of salt addition prior to HFLPME had been investigated by different authors, and it was demonstrated that, depending on the target analytes, an increase in the ionic strength of aqueous solution may have various effects on extraction: it may enhance [27], not influence [28] or limit extraction [29–32]. To investigate the effect of salt on the HFLPME of derivatized parabens, extraction was performed in the presence of different concentrations of NaCl (from saltless up to saturation). The results presented in Fig. 4 demonstrate that the extraction efficiency gradually decreased with increasing the concentration of NaCl. In the literature, some explanations concerning the decrease of extraction efficiency are presented. In [29], it is supposed that the addition of salt might lead to an increased adsorption onto glassware, which may give rise to sample losses during extraction. According to [31], the presence of salt can cause an effect adverse for the extraction, whereby the physical properties of the extraction film are changed. We have assumed that in our case the dissolved NaCl probably changed the physical properties of the extraction film in the interface of the aqueous solution and organic phase, and thus the diffusion of the analytes into the organic phase was reduced. On the basis of the obtained results, NaCl was not added in the further experiments.

Validation of the method

The quality parameters of the suggested method, such as linearity, detection limits, and repeatabilities were calculated under optimized extraction conditions.

The calibration curves were drawn with three replicate direct injections. Linear ranges varied from 30, 15, 11 and 9 µg L^{-1} up to 10 mg L^{-1} for methylparaben, ethylparaben, propylparaben and butylparaben, respectively. Correlation coefficients for all the analytes were within 0.997–0.999.

The repeatabilities were determined by five-repetition analysis for two concentrations of parabens. Relative standard deviations (RSDs) were calculated and are summarized in Table 2. These data show that the repeatability of the method is satisfactory.

Detection limits defined as a triple base-line noise are presented in Table 2. The detection limits of the developed method were lower than those obtained in our laboratory using the same GC equipment and HFLPME of underivatized parabens [22] (Table 2).

Application

Parabens tend to absorb from body care cosmetics into the human body. Thus, the possibility to use the proposed technique for the determination of parabens in cosmetics has been demonstrated. The facial tonic "Matt Touch" (Lumene) was analysed. First of all, the tonic was analysed without preliminary dilution. In 10 mL of the tonic, 0.2 g of $K_2HPO_4 \cdot 3H_2O$ was dissolved, 10 µL of acetic anhydride was added, and HFLPME was carried out for 30 min. The GC analysis of the extract showed that the concentrations of parabens did not fall into the linear ranges of calibration curves. Thus, a 100-fold dilution of the tonic was needed. The chromatogram of the diluted tonic is presented in Fig. 5. The concentrations of the analytes were calculated using the standard addition method and were determined to be 168, 59, 33 and 53 mg L^{-1} for methylparaben, ethylparaben, propylparaben and butylparaben, respectively.

Table 2. **Repeatabilities and detection limits of parabens**

Analyte	RSD, % (n = 5)		Detection limit, µg L^{-1}	
	0.1 µg mL^{-1}	1 µg mL^{-1}	This work	[22]
Methylparaben	8.9	5.2	18	200
Ethylparaben	4.5	5.3	9.1	30
Propylparaben	7.7	5.6	6.4	10
Butylparaben	9.2	5.5	5.1	–

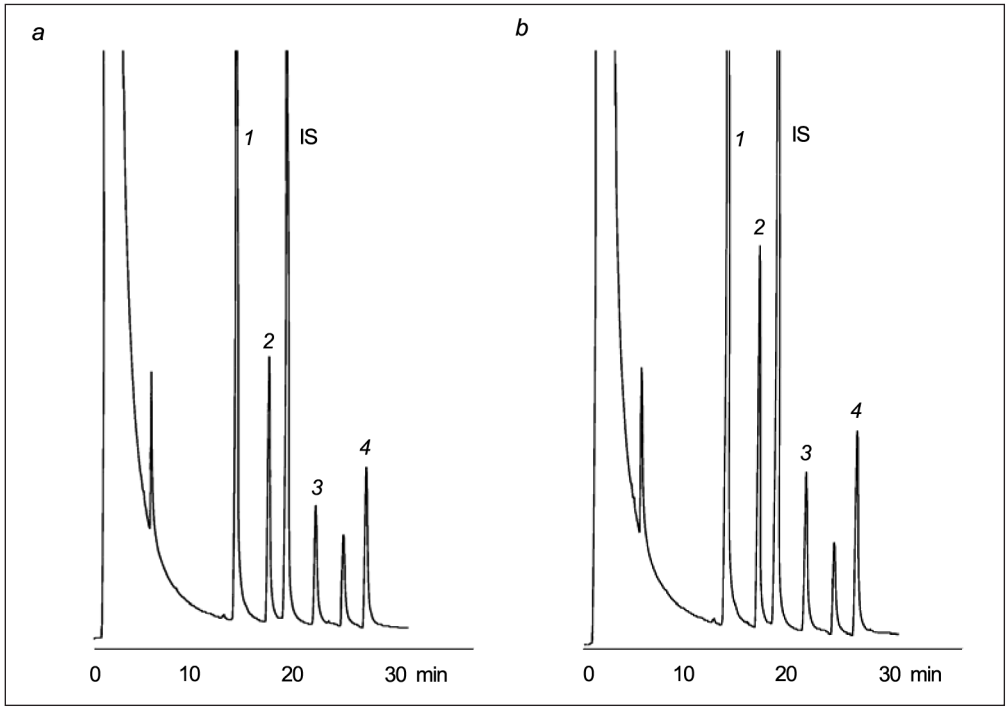


Fig. 5. Chromatograms of 100-fold diluted facial tonic: *a* – unspiked, *b* – spiked with a standard solution of parabens (0.5 mg L^{-1}). *1* – methylparaben, *2* – ethylparaben, *3* – propylparaben, *4* – butylparaben, IS – internal standard *n*-hexadecane. Extraction conditions: sample volume 10 mL, concentration of parabens 10 mg L^{-1}, 0.02 g mL^{-1} of $K_2HPO_4 \cdot 3H_2O$, acetic anhydride volume 10 µL, extracting solvent chlorobenzene, extraction time 30 min, solution stirring rate 800 rpm. For GC conditions, see Experimental

CONCLUSIONS

A method of hollow fibre liquid phase microextraction of derivatized parabens from water-based samples, followed by gas chromatographic determination, has been developed. For the derivatization of parabens, acetylation with acetic anhydride in the presence of K_2HPO_4 has been applied. The proposed method is compatible with GC, precise, reproducible and linear over a broad concentration range, environmentally friendly. The detection limits were lower than those obtained for underivatized parabens using the same GC equipment. Detection limits can be additionally reduced using mass spectrometric detection instead of flame ionisation detection.

ACKNOWLEDGEMENTS

This work has been supported by the Lithuanian State Studies Foundation.

Received 29 June 2011
Accepted 8 July 2011

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DERIVATIZUOTŲ PARABENŲ SKYSTAFAZĖ MIKROEKSTRAKCIJA KAPILIARE

S a n t r a u k a

Parabenų ekstrakcijai ir sukoncentravimui pasiūlytas skystafazės mikroekstrakcijos kapiliare metodas. Prieš ekstrakciją parabenai buvo derivatizuojami acto rūgšties anhidridu šarminėje terpėje, gautoje pridėjus di-kalio hidrofosfato. Išekstrahuoti parabenai analizuojami dujų chromatografijos metodu. Ištirta ekstrahento prigimties, ekstrakcijos trukmės ir tirpalo joninės jėgos įtaka ekstrakcijos efektyvumui. Ekstrahentu pasirinktas chlorbenzenas, vidiniu standartu – *n*-heksadekanas. Kalibracinės kreivės tiesinės iki 10 mg L^{-1} analičių koncentracijos, koreliacijos koeficientai 0,997–0,999, aptikimo ribos 18 μg L^{-1} (metilparabeno), 9,1 μg L^{-1} (etilparabeno), 6,4 μg L^{-1} (propilparabeno) ir 5,1 μg L^{-1} (butilparabeno). Santykiniai standartiniai nuokrypiai ne didesni kaip 9,2 %. Parodyta galimybė pritaikyti parengtą metodą parabenams nustatyti veido tonike.