Cent. Eur. J. Chem. • 7(3) • 2009 • 285-290
DOI: 10.2478/s11532-009-0047-6

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Central European Journal of **Chemistry**

Hollow fibre liquid phase microextraction of parabens

Invited Paper

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Received 30 December 2008; Accepted 09 March 2009

Abstract: A hollow fibre liquid phase microextraction for gas chromatographic determination of some p-hydroxybenzoic acid esters has been developed. Chlorobenzene containing tetradecane as internal standard was used for the extraction. Optimized extraction was carried out at room temperature for 40 min in the presence of 0.4 g mL^{-1} NaCl in the sample solution. Calibration was linear up to 30 mg L^{-1}. Correlation coefficients were 0.996 - 0.998. Enrichment factors were 21, 95 and 154, and detection limits were 0.20, 0.03 and 0.01 μg mL^{-1} for methylparaben, ethylparaben and propylparaben, respectively. Reproducibility was acceptable with relative standard deviations up to 11.7%. The technique was tested for water and urine analysis.

Keywords: *Hollow fibre liquid phase microextraction • Gas chromatography • Parabens*

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1. Introduction

Parabens are p-hydroxybenzoic acid esters. Due to their bactericidal and fungicidal properties they are used extensively as preservatives in pharmaceutical preparations, cosmetics and skin care products [1]. For example, in 1984 parabens were used in 13200 different cosmetic formulations. In 1995 a survey of 215 cosmetic products found parabens in 99% of leave-on products and 77% of rinse-off products [2]. The European Union Cosmetics Directive restricts cosmetic products to a maximum concentration of each paraben of 0.4% and a total maximum concentration of 0.8% [2]. Parabens are also registered for use in foods; however this use is more strictly regulated and there have been recommendations that it be withdrawn.

For many years parabens were considered to have low toxicity, primarily causing allergic reactions. However, some years ago it was demonstrated that parabens are readily absorbed through the skin from body care products and their hydrolysis by skin esterases is incomplete [3]. Parabens are oestrogenic, affect the human endocrine system and probably cause breast cancer [4,5] and male reproductive disorders [2]. Moreover, a higher rate of melanoma in younger people correlates with greater use of paraben-containing skincare/sun protection products [6], and high concentrations of propylparaben and butylparaben show genotoxicity [7].

Because of their common use, parabens are continuously released into the environment *via* sewage [8,9]. Although removed to considerable extent during sewage treatment [9], their presence has been detected in natural water [10]. Awareness of parabens in the environment and their negative effects on human health and wildlife has led to increased interest in their trace analysis.

One of the most common methods of parabens analysis is gas chromatography [5]. Since their environmental concentrations are low and cosmetics present complex matrices it is necessary to perform preconcentration or isolation prior to the chromatographic analysis.

There are few published methods for parabens extraction from aqueous matrices. Solid phase extraction is the most common [5,9-12], but because it requires large amounts of toxic organic solvents microextraction techniques are gaining interest. Solid phase microextraction is a miniaturised version of

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solid phase extraction. It is its simple, solventless and environmentally friendly. Nevertheless, to our knowledge there have been few reports on paraben SPME [1,8,13].

In recent years liquid phase microextraction has been developed as a miniaturised version of liquid-liquid extraction. Among different versions, hollow fibre liquid phase microextraction (HFLPME), proposed by Norwegian scientists in 1999 [14], is gaining interest. The technique utilizes porous hydrophobic polypropylene hollow fibre as a membrane. The fibre is impregnated with an organic phase and inside the hollow fibre is a receiving phase (the same or different from that used for impregnation). This new microextraction methodology is an attractive alternative to single drop microextraction because apart from being simple and fast it also enables clean extract formation. The low fibre cost enables its disposal after a single extraction, excluding cross-contamination and avoiding regeneration.

HFLPME has been successfully applied to the determination of drugs [15,16], aromatic amines [17,18], pesticides [19,20], and phthalates [21] in water and in complex matrices such as urine and plasma. However, to our knowledge, HFLPME has not been applied to parabens preconcentration.

In the present work, HFLPME has been developed for gas chromatographic analysis of parabens in water and urine.

2. Experimental Procedures

2.1 Reagents

Methyl-4-hydroxybenzoate (methylparaben) (99%), ethyl-4-hydroxybenzoate (ethylparaben) (99%), propyl-4-hydroxybenzoate (propylparaben) (99%), n-octane (C_8H_{18}) (98%), tetradecane (99%), toluene (C_7H_8) (99%), amyl acetate ($C_7H_{14}O_2$) (98%), carbon tetrachloride (CCl_4) (≥99.5%), chlorobenzene (C_6H_5Cl) (≥99%) and acetone (≥99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaCl (analytical grade) was purchased from Reachim (Doneck, Ukraine).

A standard stock solution containing methylparaben, ethylparaben and propylparaben (each 1mg mL^{-1}) was prepared in acetone and stored at 4°C. Working standards were prepared daily by diluting this solution with distilled water.

Tap water was collected in the laboratory immediately before analysis. Human urine samples were supplied by volunteers. Samples were analysed without pretreatment.

2.2 Hollow-fibre liquid phase microextraction procedure

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 length pieces. One end of each piece was heat-sealed using a soldering iron. The effective internal volume was approximately 5 µL. Before use, the hollow fibres were sonicated in acetone for 10 min, then removed and allowed to dry at room temperature. Each piece was used only once.

The unsealed end of the fibre was connected to a 0.7 mm diameter syringe needle inserted through the silicone rubber septum in an extraction vial cap. For several minutes the hollow fibre was immersed in the receiving phase which impregnated its walls and filled it. Then the fibre was withdrawn, washed with distilled water and immersed into a 12 mL sample *via*l containing 8 mL sample. This was then placed on a magnetic stirrer. After extraction, the cap together with needle and fibre was removed from the vial. 1 µL of the extract was withdrawn with a 10 µL microsyringe and injected into the GC.

2.3 GC analysis

Gas chromatography was carried out on 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) using an Equity™-5 fused silica capillary column (30 m × 0.53 mm, 1.5 µm film thickness) supplied by Supelco (Bellefonte, PA, USA). Splitless injection mode was used. Injector temperature was 280°C; detector temperature was 260°C. The oven temperature was programmed: initially 90°C (5 min), ramped to 120°C (2°C min^{-1}), then to 200°C (6°C min^{-1}) and held for 5 min. The gas flow rates were: carrier (nitrogen) 10, make-up gas (nitrogen) 20, hydrogen 30, and air 300 mL min^{-1}.

3. Results And Discussion

3.1 Method development

Three replicate extractions were performed for the investigation of each extraction parameter.

The first step was to select the HFLPME extractant. This must extract the analytes quite well, be practically insoluble in water, and be separated from the analyte peaks in the chromatogram. In addition, it must penetrate the polypropylene fibre pores. Moreover, for convenience, the solvent should be visible in the hollow fibre to determine whether it is well-filled.

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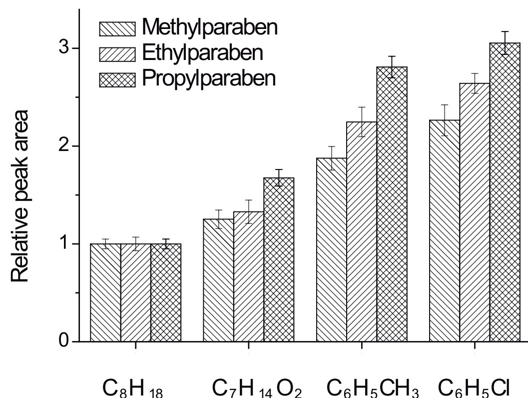

Figure 1. Effect of HFLPME solvent on methylparaben, ethylparaben and propylparaben peak areas. Concentration of each analyte is 5 μg mL^{-1}. Extraction time is 15 min. Solution stirring rate is 1500 rpm. Peak areas normalised to the corresponding peak areas of $C_{14}H_{30}$.

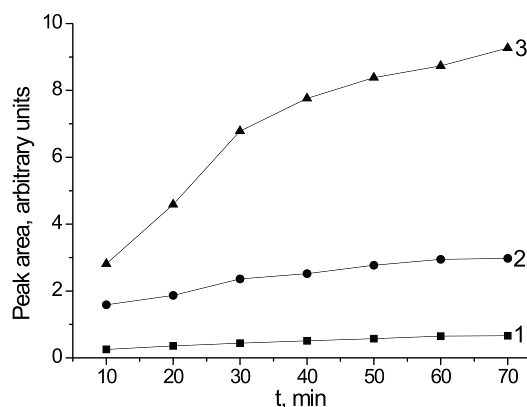

Figure 2. Effect of HFLPME time on the peak areas of (1) methylparaben, (2) ethylparaben and (3) propylparaben. Concentration of each analyte is 5 μg mL^{-1}. Solution stirring rate is 1500 rpm.

Five different solvents were tested: carbon tetrachloride, n-octane, toluene, chlorobenzene and amyl acetate. For preliminary studies the extraction time was 15 min.

Carbon tetrachloride was too volatile (boiling point 76.7°C). After 15 min extraction less than 1 µL solvent remained in the capillary. The extraction efficiencies of the other four solvents are shown in Fig. 1. Octane and amyl acetate showed lower extraction efficiencies. In addition, those two solvents were practically invisible in the hollow fibre so it was difficult to observe the receiving phase. In contrast, immediately after immersion into toluene or chlorobenzene, the fibre walls became transparent and the solvent level in the capillary could be easily seen. Within a few minutes the solvent filled the fibre. Thus, impregnation of the fibre walls and fibre filling were accomplished in a single step and the delicate procedure of filling the hollow fibre with a microsyringe was eliminated. As chlorobenzene showed higher extraction efficiency (Fig. 1), it was chosen as the parabens extractant.

HFLPME allows high stirring rates (since the receiving phase is protected by the hollow fibre) to reduce the equilibration time. In this work we used the magnetic stirrer's maximum rate (1500 rpm).

Maximum extraction efficiency would be achieved when equilibrium is established. Extraction times between 10 and 70 min were evaluated. According to the curves in Fig. 2, equilibrium was not reached even after a 70 min extraction. However, it is possible to work at a non-equilibrium state if constant extraction conditions are maintained. For further work a 40 min extraction was chosen as it was sufficiently long to reach high extraction efficiency yet dissolution or evaporation of the receiving phase was not observed.

The addition of salt generally causes a decrease in organic compounds' water solubility due to its engagement in the ions' hydration spheres and reduced water activity. Consequently, analyte movement to the receiving phase is favoured. This has been widely used to enhance analyte extraction.

The extraction was performed in the presence of different concentrations of NaCl (Fig. 3). Extraction efficiency gradually increases with increasing NaCl concentration and the maximum signal was achieved when the solution was saturated with NaCl. In further experiments, 0.4 g mL^{-1} of NaCl was added to the samples. A chromatogram of the standard parabens solution extract obtained under optimized conditions is presented in Fig. 4.

3.2 Validation of the method

The quality parameters of the method such as linearity, limits of detection, enrichment factors and reproducibility were determined under the optimized extraction conditions. However, tetradecane (50 µg mL^{-1}) was added to the extraction solvent as an internal standard to improve reproducibility. Calibration curves were determined from three replicate direct injections at 7 calibration points. The regression equations and the enrichment factors are presented in Table 1. The linear ranges for all the parabens investigated were up to 30 mg L^{-1}. Correlation coefficients were 0.996-0.998.

Detection limits were defined as three times base-line noise (Table 1). The reproducibility was determined by five replicate analyses of two parabens concentrations.

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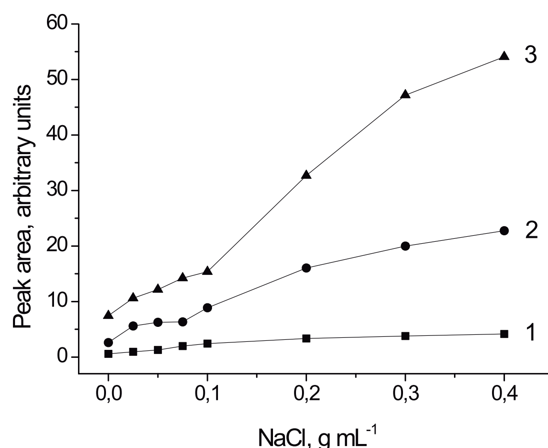

Figure 3. Effect of NaCl content on the peak areas of (1) methylparaben, (2) ethylparaben and (3) propylparaben. Concentration of each analyte is 5 μg mL^{-1}. Extraction time is 40 min. Solution stirring rate is 1500 rpm.

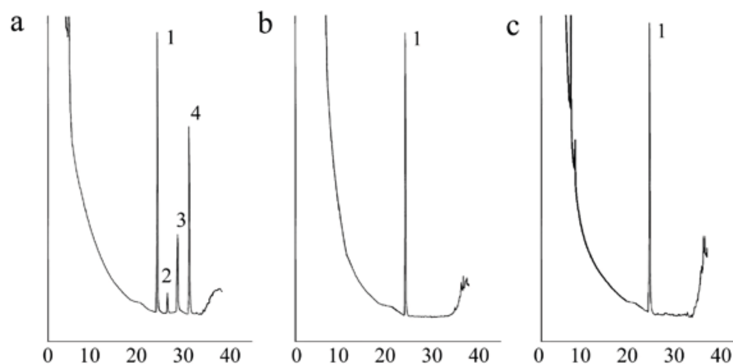

Figure 4. Chromatograms of (a) standard solution of parabens, (b) tap water, and (c) urine obtained after HFLPME. Peak identification: (1) internal standard tetradecane (50 μg mL^{-1}), (2) methylparaben, (3) ethylparaben and (4) propylparaben. Concentration of each analyte in the standard solution is 1 μg mL^{-1}. NaCl concentration is 0.4 g mL^{-1}. Extraction time is 40 min. Solution stirring rate is 1500 rpm. For GC conditions see Section 2.

Table 1. Regression equations, enrichment factors, detection limits and reproducibilities of the method.

Analyte	Equation	Enrichment factor	Detection limit, μg mL^{-1}	RSD, % (n = 5) Concentration, μg mL^{-1} 1	10
Methylparaben	Y = 0.3864X - 0.4470	21	0.20	11.7	8.1
Ethylparaben	Y = 0.8122X - 0.7572	95	0.03	9.0	6.5
Propylparaben	Y = 0.8883X - 0.1967	154	0.01	7.6	9.4

Table 2. Recoveries of spiked parabens in tap water and urine (n=5).

Analyte	Spiked Level, μg mL^{-1}	Tap water Calibration curve method		Urine Calibration curve method		Urine Standard addition method	
		Recovery, %	RSD, %	Recovery, %	RSD, %	Recovery, %	RSD, %
Methylparaben	1	95.9	9.1	55.9	9.1	101.8	13.0
	2	98.3	10.0	58.3	10.0	97.0	12.2
Ethylparaben	0.1	96.2	13.1	57.5	11.2	96.9	12.5
	1	100.8	8.4	60.8	8.4	96.6	10.2
	2	104.1	6.8	64.1	6.8	100.5	8.8
Propylparaben	0.1	96.3	11.1	58.6	9.8	102.9	9.5
	1	96.6	8.1	65.6	8.1	104.1	8.2
	2	102.7	6.9	67.7	6.9	99.2	9.6

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Relative standard deviations (RSDs) are summarized in Table 1. These data show satisfactory reproducibility.

For the enrichment factor determination, three replicate extractions from the aqueous standard containing 1.0 mg L^{-1} of each analyte were performed under optimal conditions. The actual concentration of each extracted analyte was calculated from the calibration curves. The enrichment factor was calculated as the ratio of the final analyte concentration in the hollow fibre to its concentration in the original solution.

3.3 Application

Laboratory tap water was extracted using the optimized HFLPME method and analysed immediately without pretreatment. The water was free of parabens, or their concentrations were below the detection limits (Fig. 4). To assess the matrix effect, 8 mL of tap water was spiked with 0.1, 1 and 2 µg mL^{-1} of parabens and analyzed. Parabens concentrations were determined from calibration curves obtained in distilled water. Relative recoveries were determined as the ratio of the concentrations found in real and distilled water samples spiked at the same analyte concentrations. The data (Table 2) demonstrate little natural water matrix effect on HFLPME.

Parabens tend to absorb from body care products into the body and are found in human urine [2,22,23]. In order to evaluate parabens intake a fast and simple method of their determination in urine is required. Urine is a more complex matrix than tap water. However, as HFLPME enables clean extraction, we expected HFLPME to extract parabens from urine without pretreatment.

Urine HFLPME extracts were colourless and transparent and no paraben peaks were observed (Fig. 4). Matrix effects on extraction efficiency were studied by analysis of urine spiked with 0.1, 1 and 2 µg mL^{-1} of each analyte. Concentrations were determined from calibration curves obtained in distilled water. Relative recoveries were 55.9–67.7% (Table 2) indicating a significant matrix effect. To eliminate this, parabens concentrations were determined by the method of standard additions, after adding 0.1, 1 and 2 µg mL^{-1} of each analyte to the sample. Relative recoveries were 96.6–104.1% (Table 2). Reproducibility determined by five replicate analysis was comparable with those for aqueous solutions (Table 2).

4. Conclusions

The paper describes the use of hollow fibre liquid phase microextraction for parabens sampling and preconcentration. The method is precise, reproducible and linear over a wide concentration range. It provides high enrichment factors for parabens with higher molecular weight. Only few microlitres of extracting solvent are used. The technique is compatible with GC. The hollow fibre prevents co-extraction of large molecules; the method can thus be applied to parabens extraction from complex matrices such as urine. The extract obtained does not need additional purification. Due to its simplicity, speed and low cost the method is a promising technique for paraben analysis.

Acknowledgements

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

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