CHEMIJA. 2010. Vol. 21. No. 2–4. P. 112–117

Dispersive liquid–liquid microextraction of parabens

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Dispersive liquid–liquid microextraction is suggested for sampling and preconcentration of parabens. Effects of extraction solvent type, extraction and disperser solvent volume, extraction time and the ionic strength of the solution on the extraction efficiency were investigated. Chlorobenzene containing *n*-nonadecane as an internal standard was used as an extracting solvent, and acetone was used as a disperser solvent. The calibration graphs were linear up to 10 mg mL^{-1}, the correlation coefficients were 0.997–0.999, the enrichment factors varied from 20 for methylparaben to 190 for butylparaben, and the detection limits were 210, 23, 15 and 8 μg L^{-1} for methylparaben, ethylparaben, propylparaben and butylparaben, respectively. The repeatability of the results was acceptable (relative standard deviations up to 11.2%). A possibility to apply the proposed method for paraben determination in water samples was demonstrated.

Key words: dispersive liquid–liquid microextraction, gas chromatography, parabens, water samples

INTRODUCTION

Parabens are *p*-hydroxybenzoic acid esters. They are effective antibacterial and anti-fungal agents and are widely used as preservatives in cosmetics and pharmaceuticals and even in foods and beverages [1].

Until recently parabens had been considered to be absorbed through the skin from body care products, but then rapidly metabolised and excreted. They were considered safe, at the most causing skin irritation and contact dermatitis in persons with paraben allergies [1]. However, some years ago it has been demonstrated that paraben hydrolysis by skin esterases is incomplete [2], and paraben preservatives are oestrogenic, affect the human endocrine system and probably cause breast cancer [3] and male reproductive disorders [4]; high concentrations of propylparaben and butylparaben show genotoxicity [5]. A higher rate of melanoma in younger people correlates with the greater use of paraben-containing skincare / suncare products [6], Moreover, parabens may undergo different transformation reactions rendering even more toxic pollutants. For example, tap water and swimming pool water are amended with free chlorine to ensure its bacteriological quality. In the presence of chlorine, parabens can be converted into more toxic and persistent chlorinated by-products [7, 8].

Because of their high use, parabens are continuously released in the environment and are present in natural water [7, 9]. Awareness of parabens in the environment and their negative effects on human health led to increasing interest in their trace analysis. Gas chromatography is one of the most common methods of paraben analysis [10]. However, since environmental concentrations of parabens are low, it is necessary to perform preconcentration prior to the chromatographic analysis.

There are a few published methods for paraben extraction from aqueous matrices. Solid phase extraction is the most common [7, 9–12], but it requires large amounts of toxic organic solvents. Thus, microextraction techniques are gaining interest. A few articles have been published on paraben preconcentration using solid phase microextraction [1, 13, 14]. Liquid phase microextraction (LPME) has been developed as a miniaturised version of liquid–liquid extraction. Parabens have been extracted from water samples, using LPME techniques such as single drop microextraction [15] and hollow fibre LPME [16].

The recently introduced LPME technique of dispersive liquid–liquid microextraction (DLLME) [17] is based on a ternary solvent system. A mixture of water-immiscible extraction solvent (as a rule, with a higher density than water), dissolved in a water-miscible disperser solvent, is injected rapidly into the aqueous phase. A cloudy solution is formed. It consists of fine droplets of extraction solvent, which are dis-

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persed into aqueous phase. Due to the relatively large surface area of the finely dispersed extraction solvent, the extraction of the analytes is achieved rapidly. Following centrifugation, the extraction solvent containing the analytes is sedimented and analysed by an appropriate method.

DLLME is a simple to operate, rapid and inexpensive method of extraction with high preconcentration factors and low sample volume requirements. At present, DLLME, with the subsequent gas chromatographic analysis, was applied for the extraction of polyaromatic hydrocarbons [17, 18], organophosphorus pesticides [19], phenols [20], halogenated organic compounds [21, 22], phthalate esters [23, 24], triazine herbicides [25], anilines [26], aromatic hydrocarbons [27] and fatty acids [28]. While we progressed with our work, Farajzadeh et al. published an article concerning the DLLME of parabens [29]. However, a modified DLLME version with a lighter than water extraction solvent was applied in the latter work.

In this work, a method based on traditional DLLME followed by GC detection was developed for the determination of parabens in aqueous matrices.

EXPERIMENTAL

Reagents

Methyl-4-hydroxybenzoate (methylparaben) (99%), ethyl-4-hydroxybenzoate (ethylparaben) (99%), propyl-4-hydroxybenzoate (propylparaben) (99%), butyl-4-hydroxybenzoate (butylparaben) (99%), chloroform ($CHCl_3$) (≥99%), carbon tetrachloride (CCl_4) (≥99.5%), chlorobenzene (C_6H_5Cl) (≥99%), bromobenzene (C_6H_5Br) (≥99%), 1,2-dichlorobenzene ($C_6H_4Cl_2$) (≥99%) and *n*-nonadecane (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and NaCl (analytical grade) was purchased from "Reachim" (Ukraine).

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

DLLME procedure

Eight millilitres of aqueous solution of methylparaben, ethylparaben, propylparaben and butylparaben was placed in a 12 mL centrifuge tube with a conic bottom; 0.5 mL of the solution containing 0.48 mL of acetone (as disperser solvent), and 20 µL of chlorobenzene (as extraction solvent) containing *n*-nonadecane as internal standard (5 µg mL^{-1}) were rapidly injected with a 1 mL syringe. The cloudy solution formed was centrifuged for 2 min at 5000 rpm. Chlorobenzene phase with the analytes was sedimented on the bottom of the tube. One millilitre of the extraction phase was taken using a 10 µL microsyringe (Hamilton, Reno, NV, USA) and injected into a gas chromatograph.

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 an Equity™-5 fused silica capillary column (30 m × 0.53 mm, 1.5 µm film thickness) supplied by Supelco (Bellefonte, PA, USA). The injector temperature was 280 °C and the detector temperature 260 °C. The oven temperature was programmed, i. e. initially set at 100 °C for 2 min, then gradually ramped to 120 °C (2 °C min^{-1}), 230 °C (5 °C min^{-1}) and held 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

Method development

Extraction solvent

An extraction solvent for DLLME should fulfil some requirements: generally, it should have a higher density than water, should demonstrate a good extraction capability of the compounds of interest, and its solubility in water should be low. Most of halogenated solvents answer those requirements, thus chloroform, carbon tetrachloride, chlorobenzene, bromobenzene and 1,2-dichlorobenzene were compared in the extraction of parabens. The physical properties of the selected organic solvents are presented in Table 1.

In order to select a proper extraction solvent, a solution containing 0.5 mL of acetone and 40 µL of extraction solvent was rapidly injected into 8 mL of aqueous solution containing 10 mg L^{-1} of parabens. The cloudy solution formed was centrifuged, and 1 µL of the organic phase was taken for analysis. Chloroform was not suitable for paraben extraction as it was too soluble in the aqueous phase (8 g L^{-1}), so a cloudy solution and consequently a separate organic phase did not form. CCl_4 presented a significantly lower extraction efficiency than halogenated aromatic solvents (C_6H_5Cl, C_6H_5Br and $C_6H_4Cl_2$) (Fig. 1). This fact corresponds rather well to the principle "like dissolves like", as parabens also contain a benzene ring. As the extraction efficiency of C_6H_5Cl, C_6H_5Br and $C_6H_4Cl_2$ was similar, in order to achieve an easier chromatographic separation of the analytes from the solvent peak, a solvent with the lowest boiling point (C_6H_5Cl) was chosen for extraction.

To investigate the effect of the extraction solvent volume, a solution containing 0.5 mL of acetone and different volumes of extraction solvent was rapidly injected into 8 mL

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

Solvent	Boiling point, °C	Density, g mL^{-1}	Water solubility, g L^{-1}
$CHCl_3$	62	1.48	8
CCl_4	76.5	1.59	0.8
C_6H_5Cl	132	1.11	0.5
C_6H_5Br	153	1.50	0.4
$C_6H_4Cl_2$	180	1.30	0.15

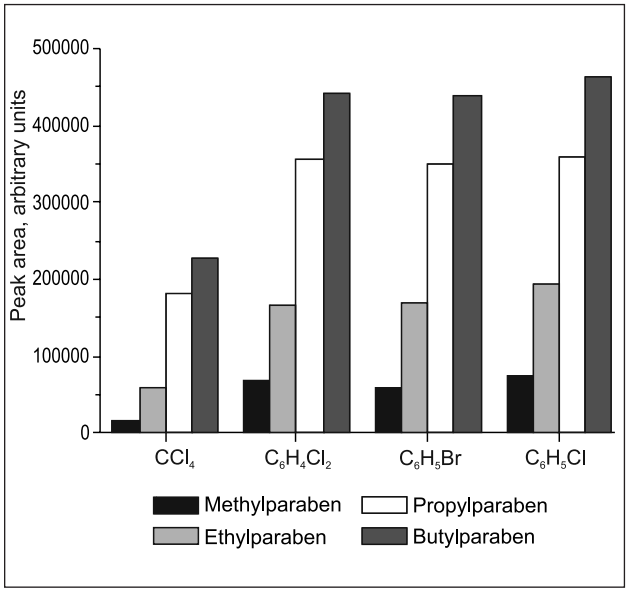

Fig. 1. Effect of extraction solvent on extraction efficiency. Extraction conditions: sample volume 8 mL, concentration of parabens 10 mg L^{-1}, acetone volume 0.5 mL, extraction solvent volume 40 µL

of aqueous solution containing 10 mg L^{-1} of parabens, and centrifugation was carried out. With increasing the extraction solvent volume, the peak areas initially increased and reached the maximum at 20 µL. Probably because of a partial sedimentation of chlorobenzene on the centrifuge tube walls, in the case of 15 µL chlorobenzene, its volume in the bottom of the centrifuge tube was too small, and together with the extraction phase some water phase was withdrawn into a microsyringe. Thus, the real amount of the injected extraction phase was less than 1 µL, resulting in decreased peak areas of the analytes.

On the other hand, when the extraction solvent volume exceeded 20 µL, because of the more intensive dilution of the analytes, the peak areas of the analytes decreased (Fig. 2). To achieve low detection limits, 20 µL of extracting solvent was selected.

Disperser solvent

The main requirement for disperser solvent is its miscibility with extraction solvent and aqueous phase [17]. Only a few solvents fulfil this requirement. In most of the publications concerning DLLME, acetone, acetonitrile and methanol were examined as disperser solvents [30–34]. According to the results presented in the publications, the recovery variations using different disperser solvents were not remarkable. Referring to the data and considering its low toxicity and cost, acetone was selected as a disperser solvent in our work.

To investigate the effect of the disperser solvent volume, different acetone volumes (0.2–1.5 mL) and 20 µL of extracting solvent were used. With increasing the acetone volume, the peak areas initially increased (Fig. 3). At a low acetone volume, the cloudy state was not stable, and probably this caused incomplete extraction. On the other hand, when the acetone volume exceeded 1.0 mL, the solubility of the parabens in the water–acetone mixture increased and their concentration in the sedimented phase decreased. According to the results, 0.4–1.0 mL acetone volume is the optimum. In order to have a convenient 0.5 mL acetone–chlorobenzene mixture volume for injection and considering that the optimum chlorobenzene volume is 20 µL, 0.48 mL of acetone volume was selected for the further work.

Extraction time

The extraction time was defined as the time interval between the injection of the mixture of disperser and extraction sol-

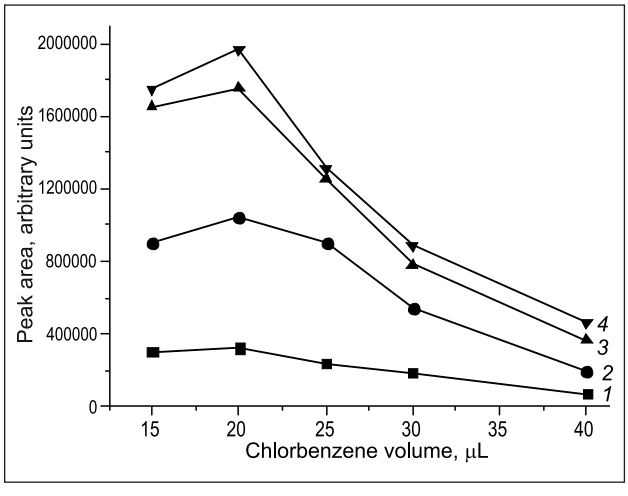

Fig. 2. Effect of extraction solvent (chlorobenzene) volume on the extraction efficiency of methylparaben (*1*), ethylparaben (*2*), propylparaben (*3*) and butylparaben (*4*). Extraction conditions: sample volume 8 mL, concentration of parabens 10 mg L^{-1}, acetone volume 0.5 mL

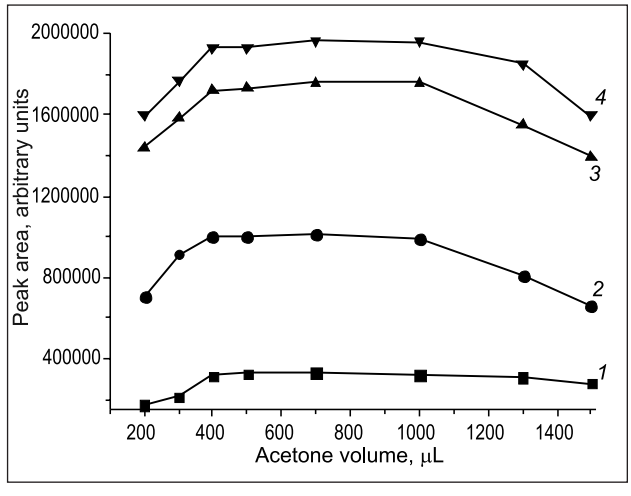

Fig. 3. Effect of disperser solvent (acetone) volume on the extraction efficiency of methylparaben (*1*), ethylparaben (*2*), propylparaben (*3*) and butylparaben (*4*). Extraction conditions: sample volume 8 mL, concentration of parabens 10 mg L^{-1}, chlorobenzene volume 20 µL

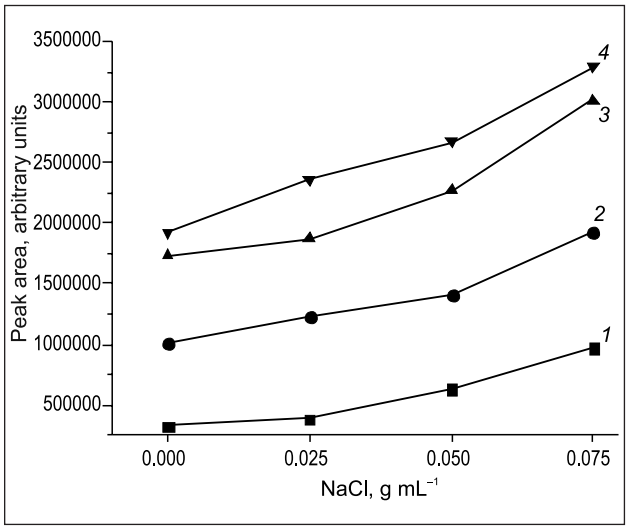

Fig. 4. Effect of NaCl content on the extraction efficiency of methylparaben (*1*), ethylparaben (*2*), propylparaben (*3*) and butylparaben (*4*). Extraction conditions: sample volume 8 mL, concentration of parabens 10 mg L^{-1}, chlorobenzene volume 20 µL, acetone volume 0.48 mL

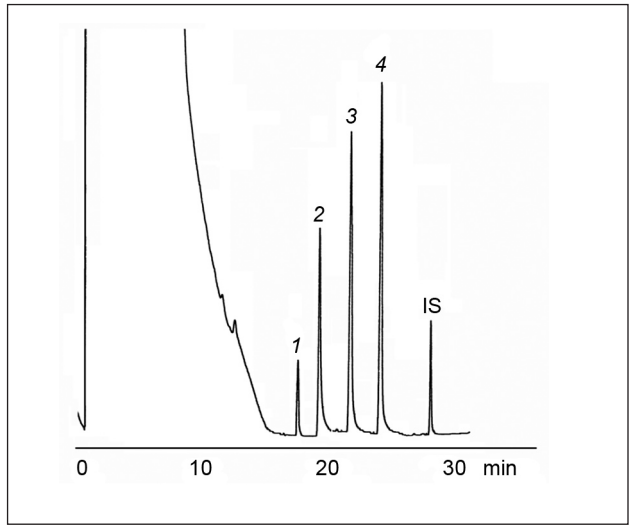

Fig. 5. A chromatogram of a standard solution of parabens (10 mg L^{-1}). *1* – methylparaben, *2* – ethylparaben, *3* – propylparaben, *4* – butylparaben, IS – *n*-nonadecane. Extraction conditions: sample volume 8 mL, chlorobenzene volume 20 µL, acetone volume 0.48 mL. For GC conditions, see Experimental

vents and the centrifugation. DLLME extraction time up to 20 min was investigated. Peak area variations at different extraction times were not significant. Evidently, as shown in [19], the surface area between the aqueous and organic phases is large, and 20–30 seconds (the time between the injection and the beginning of centrifugation) are sufficient for extraction.

Effect of ionic strength

The ionic strength of the solution was modified by adding NaCl which is commonly used for this purpose. When chlorobenzene was used as an extracting solvent, small quantities of NaCl (up to 0.075 g mL^{-1} of NaCl) promoted the transport of the analytes to the extracting drop (Fig. 4). However, with the further increase of NaCl concentration, the sedimented phase did not form any more, possibly because of the water phase density which increased with NaCl addition. Thus, the density of the organic phase was lower than that of the aqueous phase. Therefore, the organic phase formed the upper phase in the two-phase system. To avoid this, in further experiments no NaCl was added to the samples.

Quality parameters of the method

The quality parameters of the suggested method, such as linearity detection limits, enrichment factors and repeatability, were calculated under the optimized extraction conditions. However, before that, in order to improve the repeatability, *n*-nonadecane (5 µg mL^{-1}) had been added to the extraction solvent as an internal standard. A chromatogram of a standard solution of parabens with the internal standard is presented in Fig. 5.

To calculate the enrichment factor, three replicate extractions were performed in optimal conditions from the aqueous solution containing 10 µg mL^{-1} of each analyte. The enrichment factor was calculated as the ratio of the final analyte concentration in the extraction solution and its concentration in the original solution. The actual concentration of each extracted analyte was calculated from the calibration curves. The enrichment factors are presented in Table 2.

The calibration curves were drawn with three replicate injections of the extracts obtained after applying the DLLME procedure with 7 calibration points. The linear ranges were from 350, 35, 25 and 14 µg L^{-1} up to 10 µg mL^{-1} for methylparaben, ethylparaben, propylparaben and butylparaben, respectively. The correlation coefficients were 0.997–0.999. To calculate the detection limits, three replicate extractions were performed. The detection limits, defined as a triple base-line noise, are presented in Table 2.

Table 2. **Enrichment factors, detection limits and repeatabilities**

Analyte	Enrichment factor	Detection limit, µg L^{-1}	RSD, % (n = 5)	
			1 µg mL^{-1}	10 µg mL^{-1}
Methylparaben	20	210	11.2	10.6
Ethylparaben	115	23	10.3	9.8
Propylparaben	160	15	9.7	6.8
Butylparaben	190	8	7.8	6.5

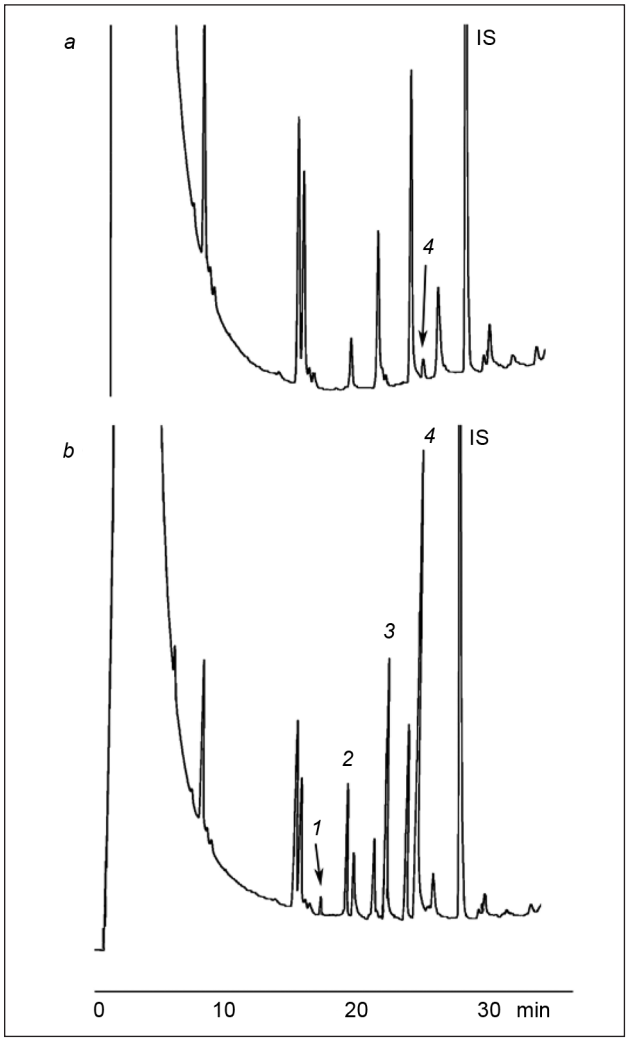

Fig. 6. Chromatograms of swimming pool water: *a* – non spiked, *b* – spiked with a standard solution of parabens (1 mg L^{-1}). *1* – methylparaben, *2* – ethylparaben, *3* – propylparaben, *4* – butylparaben, IS – *n*-nonadecane. Extraction conditions: sample volume 8 mL, chlorobenzene volume 20 µL, acetone volume 0.48 mL. For GC conditions, see Experimental

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

Application

The proposed method is particularly useful when low concentrations of parabens in simple matrices have to be determined. Tap water (from the laboratory) and swimming pool water were extracted by the optimized DLLME method. The tap water sample was analysed immediately, and swimming pool water was analysed 4 hours after sampling without any pretreatment. The results showed that the tap water was free of parabens or their concentrations were below detection limits. Butylparaben (28 µg L^{-1}; RSD = 9.8, n = 3) was determined in the swimming pool water (Fig. 6). Its main source might be body care cosmetics used by the swimming pool visitors.

To assess the matrix effect, 8 mL of tap water and of swimming pool water were spiked with 1 and 10 µg mL^{-1} of parabens. The concentration of parabens was calculated using the 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. Data of the analysis demonstrated a low matrix effect on DLLME with the recoveries close to 100%.

CONCLUSIONS

The paper describes the use of dispersive liquid–liquid microextraction technique of the sampling and preconcentration of parabens. The proposed method provides high enrichment factors, it is compatible with GC, precise, reproducible and linear over a broad concentration range, environmentally friendly. Only 20 microlitres of the extracting solvent are used for the extraction. Moreover, DLLME is a particularly time-saving technique as the extraction occurs instantaneously. The technique was successfully applied for determination of parabens in real water samples.

ACKNOWLEDGEMENTS

This work was supported by the Lithuanian State Studies Foundation.

Received 28 May 2010
Accepted 16 June 2010

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PARABENŲ DISPERSINĖ SKYSČIŲ–SKYSČIŲ MIKROEKSTRAKCIJA

S a n t r a u k a

Lakiesiems aromatiniams angliavandeniliams sukoncentruoti iš vandens mėginių pasiūlytas dispersinės skysčių–skysčių mikroekstrakcijos metodas. Ištirta ekstrahento prigimties ir tūrio, disperguojančiojo tirpiklio tūrio, ekstrakcijos trukmės ir tirpalo joninės jėgos įtaka ekstrakcijos efektyvumui. Ekstrahentu pasirinktas chlorbenzenas, disperguojančiuoju tirpikliu – acetonas, vidiniu standartu – *n*-nonadekanas. Kalibracinės kreivės tiesinės iki 10 mg mL^{-1} analičių koncentracijos, koreliacijos koeficientai 0,997–0,999, sukoncentravimo laipsnis nuo 20 (metilparabeno) iki 190 (butilparabeno), aptikimo ribos 210 μg L^{-1} (metilparabeno), 23 μg L^{-1} (etilparabeno), 15 μg L^{-1} (propilparabeno) ir 8 μg L^{-1} (butilparabeno). Santykiniai standartiniai nuokrypiai ne didesni kaip 11,2 %. Parodyta šio metodo taikymo parabenams vandens mėginiuose nustatyti galimybė.