Turk J Pharm Sci 2023;20(5):328-334
DOI: 10.4274/tjps.galenos.2022.42387

ORIGINAL ARTICLE

Ultrasound- and Vortex-Assisted Dispersive Liquid-Liquid Microextraction of Parabens from Personal Care Products and Urine, Followed by High-Performance Liquid Chromatography

Pelin KÖSEOĞLU YILMAZ*, Ufuk KOLAK

İstanbul University Faculty of Pharmacy, Department of Analytical Chemistry, İstanbul, Türkiye

ABSTRACT

Objectives: Parabens, which are *p*-hydroxybenzoic acid esters, are used as preservatives in personal care products, pharmaceuticals, and food because of their antimicrobial activity. However, they are also classified as suspected endocrine disruptors and carcinogens. In the present study, we aimed to optimize an ultrasound and vortex-assisted dispersive liquid-liquid microextraction (DLLME) procedure for the simultaneous extraction of methyl, ethyl, isopropyl, propyl, isobutyl, and butyl parabens from personal care products and urine.

Materials and Methods: The extraction solvent type, extraction solvent volume, disperser solvent volume, sodium chloride concentration, ultrasonication time, and vortex application time were evaluated to obtain optimum recoveries by ultrasound and vortex-assisted DLLME. Parabens were detected using a validated high performanc-liquid chromatography (HPLC) method with fluorescence detection. Method validation was performed by examining linearity, the limit of detection, limit of quantification, accuracy, and precision.

Results: The limits of detection and quantification of the HPLC method were between 0.09-0.18 µg/mL and 0.28-0.54 µg/mL, respectively. Precision was examined as the relative standard deviation, which was 0.22-1.81% and 1.12-2.03% for intra- and interday studies. Recovery percentages were higher than 96.00%. Samples of two paraben-free personal care products and synthetic urine were spiked with the analyses at 0.02 µg/mL and were successfully analyzed using the developed procedure with recovery values higher than 82.00%.

Conclusion: The proposed procedure provided quantification of selected parabens at 20 ng/mL in analyzed personal care products and urine matrices with good precision and accuracy.

Key words: High-performance liquid chromatography, liquid-liquid microextraction, paraben, personal care product, urine

INTRODUCTION

Parabens, which are *p*-hydroxybenzoic acid esters, are widely used in various types of food, pharmaceuticals, and personal care products as preservatives because of their antimicrobial activity within a wide pH range, high stability, water solubility, and low cost. Among them, methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP) are mostly used individually or as mixtures.[1,2] However, recent studies have shown the affinity of parabens for binding to estrogen.[3,4] Their estrogenic effect was assumed to be able to cause breast cancer.[5,6] In addition, some negative impacts on the male reproductive system were reported.[7] Regarding the related research, parabens are classified as suspected endocrine disruptors and carcinogens.

One of the ways of exposure to parabens is through personal care products containing various types of parabens as preservatives because parabens are absorbed through the skin.[8] The maximum permitted level for PP and BP is 0.14%, when

*Correspondence: pelink@istanbul.edu.tr, Phone: +90 212 440 00 00 - 13504, ORCID-ID: orcid.org/0000-0002-9871-1710
Received: 10.08.2022, Accepted: 23.12.2022

used individually or together with other esters in cosmetics by the European Commission. The use of isopropylparaben (IPP), isobutylparaben (IBP), phenylparaben, benzylparaben, and pentylparaben has been restricted.[9]

The reliable analysis of parabens has become an issue of great scientific interest because of their suspected damage to human health. Various types of pre-treatment techniques have been developed for the pre-concentration or extraction of parabens, considering low concentrations and complex sample matrices.[8,10,11] Among them, dispersive liquid-liquid microextraction (DLLME) is a common technique with advantages, including the requirement of a lower extraction solvent volume, lower sample amount, and less time-consumption compared with traditional procedures. The extraction solvent is immiscible with the sample solution, and the disperser solvent is used to obtain better contact between them.[12] In a previous study, MP, EP, PP, and BP were successfully extracted by DLLME from breast milk.[8] de Oliveira et al.[13] also determined 17 potential endocrine-disrupting chemicals, including MP, EP, PP, BP, and benzylparaben, by DLLME coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) in human saliva. In another study, DLLME of MP, EP, PP, and BP from pharmaceuticals and personal care products was performed.[14]

Parabens are generally extracted from various matrices using chlorinated solvents, which may negatively affect human health and are not environmentally friendly.[13,14] A special technique of DLLME, called ultrasound and vortex-assisted DLLME (USVADLLME), was developed by which the required volume of hazardous extraction solvents was reduced. In the USVADLLME procedure, ultrasonication provides better dispersion, and vortexing prevents biphasic system formation.[15]

High-performance liquid chromatography (HPLC)[16,17] and gas chromatography (GC)[18,19] are two common methods for the detection of parabens in different types of sample matrices such as food products,[20] biological fluids,[16,17,21] environmental samples,[22,23] pharmaceuticals,[24,25] and personal care products.[26,27] Among them, GC methods may require steps of derivatization or preconcentration. HPLC with ultraviolet (UV) or diode array detection has disadvantages, such as interference of other ingredients and high detection limits. LC-MS or LC-MS/MS may avoid all of these drawbacks, but these systems are unavailable in many laboratories because of their high costs. On the other hand, HPLC with fluorescence detection (FD) may also be used because it has higher selectivity than UV detection and is more available than MS systems. An HPLC-FD method was developed, validated, and applied successfully for four types of parabens, namely MP, EP, PP, and BP, in cosmetic products in a recent work.[28] In addition, Yılmaz and Tokat[29] developed a method for MP, EP, PP, IBP, and benzyl paraben (BzP) using HPLC-FD in cosmetics.

In the present study, we aimed to optimize a USVADLLME procedure for extracting six parabens (MP, EP, IPP, PP, IBP, and BP) (Figure 1) from personal care products and synthetic urine. For the quantification of the extracted parabens, an HPLC-FD method was developed and validated according to the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and the advantages of FD mentioned above. The proposed USVADLLME technique has never been used for extracting selected analysts. To the best of our knowledge, the proposed work is the first USVADLLME coupled with the HPLC-FD method for the detection of parabens in personal care products and urine.

MATERIALS AND METHODS

Reagents and chemicals

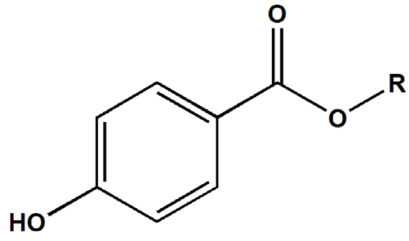
Standard materials of MP, EP, IPP, PP, IBP, BP, and synthetic urine (Surine™ Negative Urine Control) were purchased from Sigma (Darmstadt, Germany). HPLC grade methanol (MeOH), *o*-phosphoric acid, sodium chloride (NaCl), dichloromethane (CH_2Cl_2), and chloroform ($CHCl_3$) were obtained from Merck (Darmstadt, Germany). The chemicals were of analytical grade. A stock solution at 100.00 µg/mL for each analyte was prepared with MeOH (HPLC grade) and stored at 4 °C. The stock solution was diluted daily with the mobile phase to obtain standard paraben solutions at the desired concentrations. The paraben-free tonic sample (T) and the paraben-free micellar water sample (MW) were obtained from a commercial supplier in İstanbul, Türkiye (2018).

HPLC conditions

The analysis of the parabens was performed by an HPLC system (LC20AT, Shimadzu, Kyoto, Japan) with FD (RF20A). Analytes were separated using a C18 analytical column (4.6 x 250 mm, 5.0 µm) (Intersil ODS-3, GL Sciences Inc., Tokyo, Japan). The mobile phase system consisted of 50% phosphate buffer (0.1 M, pH 7) and 50% MeOH. Isocratic elution was applied at 1.0 mL/min. The column temperature was 40 °C. The excitation and emission wavelengths were 254 and 310 nm, respectively. The injection volume was set to 20 µL. Data analyses were performed using LabSolutions software (version 1.25).

USVADLLME procedure

A total of 150 µL of $CHCl_3$ and 50 µL of MeOH were transferred into a conical-bottom glass test tube with a screw cap containing 5 mL of sample solution. NaCl was then added (2.0 g/L). The



R: CH_3, methyl paraben
R: C_2H_5, ethyl paraben
R: C_3H_7, propyl and isopropyl paraben
R: C_4H_9, butyl and isobutyl paraben

Figure 1. Chemical structures of the analyzed parabens

solution was vortexed (VTX-3000L, Harmony, Tokyo, Japan) for 4 min and ultrasonicated for 90 s (Elma Hans Schmidbauer GmbH & Co. KG, Siegen, Germany). Following ultrasonication, the solution was centrifuged for 3 min at 4,000 rpm (VWR Compactstar CS4, VWR International Ltd, Leicestershire, UK). A microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) was used to separate the $CHCl_3$ phase, which was then evaporated under N_2 flow. The residue was dissolved in 100 µL of the mobile phase before HPLC analysis. The extraction procedure was performed in triplicate for all analyzed samples.

RESULTS AND DISCUSSION

The selection of HPLC conditions

Various mobile phase types were examined using MeOH, acetonitrile, acetic acid, formic acid, and phosphate buffers for the suitable separation of the parabens. A mobile phase system consisting of 50% phosphate buffer (0.1 M, pH 7) and 50% MeOH with isocratic elution was selected, considering the parameters of baseline drift, retention time, and resolution. An excitation wavelength of 254 nm and an emission wavelength of 310 nm were selected to obtain optimum signals for all analytes.

Method validation

Validation of the proposed HPLC-FD method was evaluated using the parameters of linearity, LOD, LOQ, precision, and accuracy. A representative chromatogram of the analytes (2.50 µg/mL) is shown in Figure 2.

A 6-point calibration curve was prepared for each paraben (0.50-10.00 µg/mL). The linearity was examined using regression results. Suitable linearities were obtained for all analytes ($r > 0.99$) (Table 1).

LODs were calculated as 3.3 times, whereas LOQs were determined as 10 times the standard deviation/slope ratio of the calibration curve. The LODs were in the range of 0.09-0.18 µg/mL, and LOQs were between 0.28-0.54 µg/mL. The analytical figures of merit for parabens are given in Table 1.

Precision was examined by intra- and interday studies at 0.50, 2.50, and 10.00 µg/mL. The standard solutions at three concentration levels were analyzed in triplicate consecutively on first day and in triplicate on three different days (Table 2). The results were calculated as the percentage of relative standard deviation (RSD %). The accuracy was determined as the recovery percentage (%) (Table 2). All %RSD values were lower than ≤ 2.03, and the recoveries were higher than 96.00.

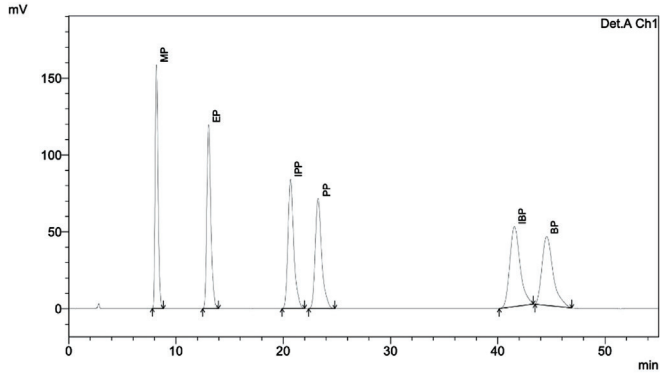


Figure 2. Representative HPLC-FD chromatogram of the analyzed parabens (at 2.50 µg/mL)

HPLC: High performance liquid chromatography, FD: Fluorescence detection

Optimization of the USVADLLME procedure

To optimize the USVADLLME procedure, extraction solvent type, extraction solvent volume, disperser solvent volume, NaCl concentration, ultrasonication, and vortex times were examined. All trials were performed in triplicate. The extraction recovery values were evaluated to determine the optimal extraction conditions.

Optimization of extraction solvent type and volume

A literature survey revealed that chlorinated organic solvents were effective for the extraction of parabens from various sample matrices.[13,14] The extraction capabilities of CH_2Cl_2 and $CHCl_3$ were examined. The selected solvents match the criteria for liquid-liquid extraction because they have higher density than the sample solutions, are poorly soluble in the sample solutions, and are volatile enough to be easily separated. The extraction trials were performed with the standard solution containing each analyte at a concentration 0.02 µg/mL. $CHCl_3$ provided better extraction recoveries for all the analytes (Figure 3). Different volumes of $CHCl_3$ as 100, 150, and 200 µL were used to determine the optimum extraction solvent volume and 150 µL provided almost the same extraction performance with 200 µL (Figure 4).

Optimization of the disperser solvent volume

A cloudy solution of the sample and the extraction solvent is formed using the disperser solvent, which determines the degree of dispersion. The disperser solvent was MeOH because of its good dispersing ability in mixtures of water and $CHCl_3$. The extraction trials were performed with 25, 50, and 100 µL of MeOH, and 50 µL of MeOH was suitable for complete dispersion with similar recovery results obtained with higher volumes (Figure 5).

Optimization of NaCl concentration

The presence of NaCl lowers the solubility of the parabens in the aqueous phase by the salting-out effect. Trials were performed without the addition of NaCl and with the addition of NaCl at concentrations of 2 g NaCl/L and 3 g NaCl/L. The optimum concentration was 2 g NaCl/L (Figure 6).

Optimization of ultrasonication and vortex times

The time ranges of 30, 60, and 90 seconds (sec) were examined to determine the optimum ultrasonication time for high recovery of the parabens, whereas the effect vortex time was examined at 2, 4, and 6 min. Recoveries higher than 80.00% were achieved with an ultrasonication time of 90 sec and vortex time of 4 min (Figures 7, 8).

Real sample analysis

The developed procedure was used for the extraction and determination of six parabens simultaneously in a cosmetic T,

Table 1. Analytical figures of merit for parabens

Analyte	t_R (min)[a]	Calibration range (µg/mL)	Linear equation	r	LOD (µg/mL)	LOQ (µg/mL)	Tailing factor (t)	Resolution (R_s)
MP	8.168 ± 0.004	0.50-10.00	y: 1114911 x 105530	0.9972	0.10 ± 0.01	0.30 ± 0.02	1.250 ± 0.009	-
EP	13.031 ± 0.010	0.50-10.00	y: 1209791 x 320800	0.9956	0.10 ± 0.02	0.30 ± 0.03	1.293 ± 0.010	8.459 ± 0.013
IPP	20.660 ± 0.013	0.50-10.00	y: 1205857 x 129613	0.9973	0.10 ± 0.02	0.30 ± 0.02	1.325 ± 0.017	9.506 ± 0.032
PP	23.229 ± 0.017	0.50-10.00	y: 1134769 x 123760	0.9973	0.09 ± 0.01	0.28 ± 0.01	1.356 ± 0.012	2.595 ± 0.011
IBP	41.523 ± 0.037	0.50-10.00	y: 1329085 x 158082	0.9970	0.12 ± 0.03	0.36 ± 0.03	1.273 ± 0.017	13.343 ± 0.099
BP	44.521 ± 0.042	0.50-10.00	y: 1248312 x 164808	0.9976	0.18 ± 0.03	0.54 ± 0.03	1.447 ± 0.024	1.709 ± 0.007

[a]Mean ± SD, n: 6, SD: Standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben, LOD: Limit of detection, LOQ: Limit of quantification

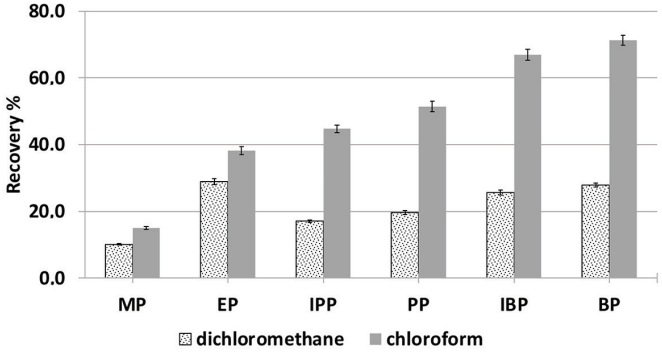


Figure 3. Optimization of the extraction solvent type. Disperser solvent (MeOH) volume, 50 µL; extraction solvent volume, 100 µL; ultrasonication time, 30 sec; vortex time: 4 min. n: 3, RSD values were in the range of 2.12-3.05%

MeOH: Methanol, RSD: Relative standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben

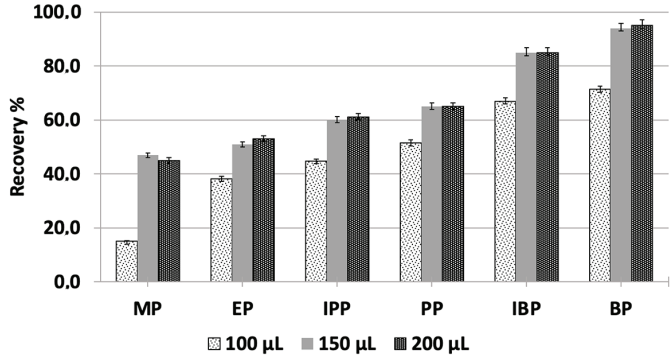


Figure 4. Optimization of the extraction solvent volume. Extraction solvent, $CHCl_3$; disperser solvent (MeOH) volume, 50 µL; ultrasonication time, 30 sec; vortex time: 4 min. n: 3, RSD values were in the range of 1.59-2.25%

$CHCl_3$: Chloroform, MeOH: Methanol, RSD: Relative standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben

MW, and synthetic urine sample. The conditions were optimized as: extraction solvent volume, 150 µL; dispersing solvent volume, 50 µL, NaCl concentration, 2 g/L; ultrasonication time, 90 sec; vortex time, 4 min. Because the samples were paraben-free, they were spiked at 0.02 µg/mL before the extraction procedure. The extraction procedure and HPLC-FD analyses

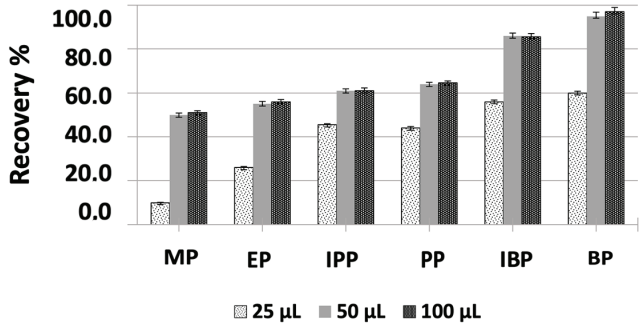


Figure 5. Optimization of the disperser solvent volume. Extraction solvent ($CHCl_3$) volume, 150 µL; disperser solvent, MeOH; ultrasonication time, 30 sec; vortex time: 4 min. n: 3, RSD values were in the range of 1.00-2.10%

$CHCl_3$: Chloroform, MeOH: Methanol, RSD: Relative standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben

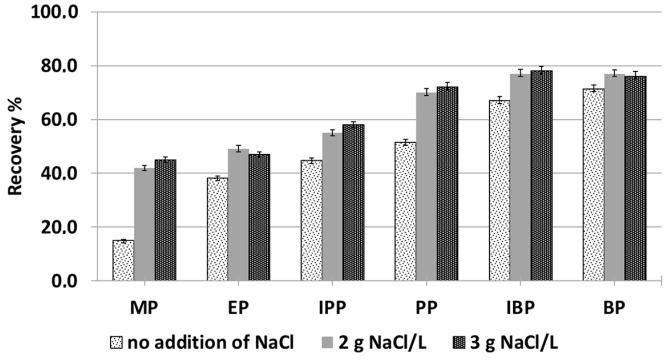


Figure 6. Optimization of the NaCl concentration. Extraction solvent ($CHCl_3$) volume, 100 µL; disperser solvent (MeOH) volume, 50 µL; ultrasonication time, 30 sec; vortex time: 4 min. n: 3, RSD values were in the range of 2.08-2.79%

NaCl: Sodium chloride, $CHCl_3$: Chloroform, MeOH: Methanol, RSD: Relative standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben

were performed in triplicate. Recoveries were higher than 82.00, and the enrichment factors were in the range of 41.07-49.05 (Table 3).

The optimized USVADLLME procedure provided the determination of parabens in different matrices at 20 ng/mL.

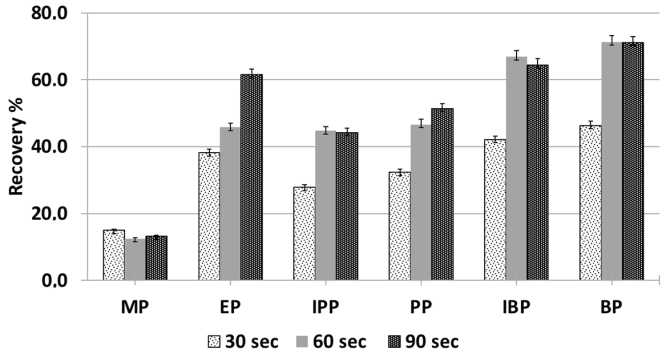


Figure 7. Optimization of ultrasonication time. Extraction solvent ($CHCl_3$) volume, 100 µL; disperser solvent (MeOH) volume, 50 µL; vortex time: 4 min. n: 3, RSD values were in the range of 2.51-3.12%

$CHCl_3$: Chloroform, MeOH: Methanol, RSD: Relative standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben

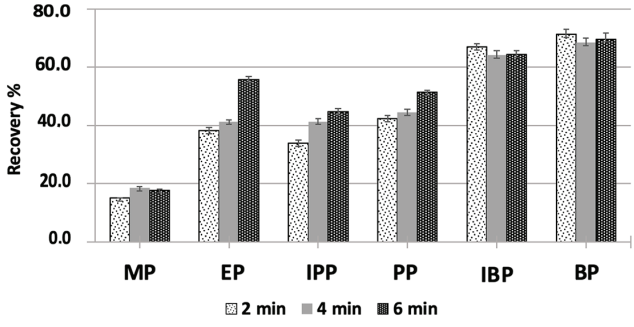


Figure 8. Optimization of the vortex time. Extraction solvent ($CHCl_3$) volume, 100 µL; disperser solvent (MeOH) volume, 50 µL; ultrasonication time, 30 sec. n: 3, RSD values were in the range of 1.15-2.93%

$CHCl_3$: Chloroform, MeOH: Methanol, RSD: Relative standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben

Yılmaz and Tokat[29] also developed an HPLC-FD method for the determination of different parabens (MP, EP, PP, IBP, and BzP) in cosmetics. Distinctly, in that study, a preconcentration method was not applied. The LOQs were in the range of

Table 3. Analysis results of spiked (0.02 µg/mL) real samples[a]

Sample	Analyte	Recovery (%)[b]	RSD (%)	Enrichment factor
T	MP	82.13 ± 2.01	2.45	41.07
	EP	84.53 ± 2.44	2.89	42.27
	IPP	88.53 ± 3.03	3.42	44.27
	PP	92.80 ± 2.88	3.11	46.40
	IBP	94.93 ± 1.22	1.29	47.47
	BP	95.47 ± 2.44	2.56	47.73
MW	MP	83.85 ± 1.41	1.68	41.93
	EP	88.90 ± 1.64	1.84	44.45
	IPP	90.52 ± 0.55	0.61	45.26
	PP	94.99 ± 1.37	1.44	47.50
	IBP	95.53 ± 1.05	1.09	47.77
	BP	94.89 ± 1.82	1.91	47.45
Urine	MP	95.03 ± 2.01	2.45	47.52
	EP	94.51 ± 2.44	1.89	47.26
	IPP	98.09 ± 3.03	2.03	49.05
	PP	92.86 ± 2.88	2.10	46.43
	IBP	94.96 ± 1.22	1.85	47.48
	BP	95.45 ± 2.44	2.01	47.73

[b]Mean recovery % ± standard deviation SD: Standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben, MW: Micellar water, RSD: Relative standard deviation, T: Cosmetic tonic

Table 2. Precision and accuracy of the developed HPLC-FD method

		MP	BP	IPP	PP	IBP	BP
Intraday (n: 3)[a]	C[c] (µg/mL)						
	0.50	1.43	1.30	1.58	1.81	1.26	1.61
	2.50	0.61	0.52	0.56	0.50	0.62	0.74
	10.00	0.60	0.29	0.25	0.23	0.22	0.27
Interday (n: 3)	C (µg/mL)						
	0.50	1.59	1.77	1.32	1.12	1.28	1.42
	2.50	1.82	1.65	1.66	1.46	1.42	1.45
	10.00	1.80	1.94	2.01	2.03	1.54	1.58
Recovery (%) (n: 3)[b]	C (µg/mL)						
	0.50	98.41 ± 1.77	96.71 ± 1.18	101.34 ± 1.15	101.11 ± 1.26	100.74 ± 1.40	99.67 ± 1.67
	2.50	98.46 ± 0.61	98.50 ± 0.52	98.16 ± 0.55	98.28 ± 0.48	99.07 ± 0.61	98.75 ± 0.73
	10.00	98.55 ± 0.59	98.61 ± 0.29	98.68 ± 0.25	98.76 ± 0.22	99.78 ± 0.22	99.94 ± 0.27

[a]Relative standard deviation (%), Mean recovery % ± SD, [c]Concentration (µg/mL), SD: Standard deviation, MP: Methylparaben, EP: Ethylparaben, IPP: Isopropylparaben, PP: Propylparaben, IBP: Isobutylparaben, BP: Butylparaben, HPLC: High-performance liquid chromatography, FD: Fluorescence detection

0.88-0.97 µg/mL, and it was not possible to quantify the analytes at lower concentrations. This procedure can be used for much lower concentrations with good precision and accuracy, which is an important advantage, especially for biological samples. In addition, USVADLLME may be effective for separating various interferences in complex matrices. On the other hand, the sample preparation time is longer and a chlorinated solvent such as $CHCl_3$ is used for the extraction. However, LOQs are lower without requiring a more sophisticated instrument such as LC-MS or GC-MS.

CONCLUSION

To the best of our knowledge, the present report could be considered as the first research on the determination of the selected parabens simultaneously by USVADLLME-HPLC-FD. Reliable paraben analysis could be achieved by the developed and validated HPLC-FD method. The proposed extraction procedure provided quantification of parabens at 20 ng/mL level without using a more sophisticated instrument such as LC-MS or GC-MS, was easy to perform and could be used for different aqueous personal care products and urine matrices. In addition, the use of low volumes of the extraction and dispersing solvents lower the cost.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: P.K.Y., U.K., Design: P.K.Y., U.K., Data Collection or Processing: P.K.Y., Analysis or Interpretation: P.K.Y., Literature Search: P.K.Y., Writing: P.K.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by the Scientific Research Projects Coordination Unit of İstanbul University (project number: FBA-2018-27374).

REFERENCES

1. Tavares RS, Martins FC, Oliveira PJ, Ramalho-Santos J, Peixoto FP. Parabens in male infertility-is there a mitochondrial connection? Reprod Toxicol. 2009;27:1-7.
2. Shen HY, Jiang HL, Mao HL, Pan G, Zhou L, Cao YF. Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. J Sep Sci. 2007;30:48-54.
3. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicol Appl Pharmacol. 1998;153:12-19.
4. Harvey PW, Darbre P. Endocrine disrupters and human health: could oestrogenic chemicals in body care cosmetics adversely affect breast cancer incidence in women? J Appl Toxicol. 2004;24:167-176.
5. Barr L, Metaxas G, Harbach CA, Savoy LA, Darbre PD. Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum. J Appl Toxicol. 2012;32:219-232.
6. Golden R, Gandy J, Vollmer G. A review of the endocrine activity of parabens and implications for potential risks to human health. Crit Rev Toxicol. 2005;35:435-458.
7. Kang KS, Che JH, Ryu DY, Kim TW, Li GX, Lee YS. Decreased sperm number and motile activity on the F1 offspring maternally exposed to butyl *p*-hydroxybenzoic acid (butyl paraben). J Vet Med Sci. 2002;64:227-235.
8. Grecco CF, Souza ID, Acquaro Juniora VR, Queiroz MEC. Determination of parabens in breast milk samples by dispersive liquid-liquid microextraction (DLLME) and ultra-high-performance liquid chromatography tandem mass spectrometry. J. Braz. Chem. Soc. 2019;30:48-59.
9. Consumers: Commission improves the safety of cosmetics, European Commission. 2020. Available from: https://ec.europa.eu/commission/presscorner/detail/en/IP_14_1051.
10. Canosa P, Rodríguez I, Rubí E, Bollaín MH, Cela R. Optimisation of a solid-phase microextraction method for the determination of parabens in water samples at the low ng *per* litre level. J Chromatogr A. 2006;1124:3-10.
11. Chávez-Moreno CA, Hinojosa-Reyes L, Ruiz-Ruiz EJ, Hernández-Ramírez A, Guzmán-Mar JL. Optimization of solid-phase extraction of parabens and benzophenones in water samples using a combination of Plakett-Burman and Box-Behnken designs. J Sep Sci. 2018;41:4488-4497.
12. Rezaee M, Assadi Y, Milani Hosseini MR, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid-liquid microextraction. J Chromatogr A. 2006;1116:1-9.
13. de Oliveira ML, Rocha BA, Souza VCO, Barbosa F Jr. Determination of 17 potential endocrine-disrupting chemicals in human saliva by dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry. Talanta. 2019;196:271-276.
14. Hassan M, Alshana U, Ertaş N. Dispersive liquid-liquid microextraction of parabens from pharmaceuticals and personal care products prior to their determination using HPLC-DAD. Turk J Chem. 2019;43:1634-1645.
15. Cinelli G, Avino P, Notardonato I, Centola A, Russo MV. Rapid analysis of six phthalate esters in wine by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography-ion trap mass spectrometry. Anal Chim Acta. 2013;769:72-78.
16. Rodríguez-Gómez R, Zafra-Gómez A, Camino-Sánchez FJ, Ballesteros O, Navalón A. Gas chromatography and ultra high performance liquid chromatography tandem mass spectrometry methods for the determination of selected endocrine disrupting chemicals in human breast milk after stir-bar sorptive extraction. J Chromatogr A. 2014;1349:69-79.
17. Tahan GP, Santos NKS, Albuquerque AC, Martins I. Determination of parabens in serum by liquid chromatography-tandem mass spectrometry: correlation with lipstick use. Regul Toxicol Pharmacol. 2016;79:42-48.
18. Lu D, Feng C, Wang D, Lin Y, Ip HS, She J, Xu Q, Wu C, Wang G, Zhou Z. Analysis of twenty phenolic compounds in human urine: hydrochloric acid hydrolysis, solid-phase extraction based on K_2CO_3-treated silica, and gas chromatography tandem mass spectrometry. Anal Bioanal Chem. 2015;407:4131-4141.

19. Azzouz A, Rascón AJ, Ballesteros E. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2016;119:16-26.
20. Han Y, Jia X, Liu X, Duan T, Chen H. DLLME Combined with GC-MS for the determination of methylparaben, ethylparaben, propylparaben and butylparaben in beverage samples. Chromatographia. 2010;72:351-355.
21. Ren L, Fang J, Liu G, Zhang J, Zhu Z, Liu H, Lin K, Zhang H, Lu S. Simultaneous determination of urinary parabens, bisphenol A, triclosan, and 8-hydroxy-2'-deoxyguanosine by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Anal Bioanal Chem. 2016;408:2621-2629.
22. Lee JW, Lee HK, Moon HB. Contamination and spatial distribution of parabens, their metabolites and antimicrobials in sediment from Korean coastal waters. Ecotoxicol Environ Saf. 2019;180:185-191.
23. Liao C, Shi J, Wang X, Zhu Q, Kannan K. Occurrence and distribution of parabens and bisphenols in sediment from northern Chinese coastal areas. Environ Pollut. 2019;253:759-767.
24. Moreta C, Tena MT, Kannan K. Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals. Environ Res. 2015;142:452-460.
25. Ma WL, Zhao X, Lin ZY, Mohammed MO, Zhang ZF, Liu LY, Song WW, Li YF. A survey of parabens in commercial pharmaceuticals from China and its implications for human exposure. Environ Int. 2016;95:30-35.
26. Guo Y, Wang L, Kannan K. Phthalates and parabens in personal care products from China: concentrations and human exposure. Arch Environ Contam Toxicol. 2014;66:113-119.
27. Eriksson E, Andersen HR, Ledin A. Substance flow analysis of parabens in Denmark complemented with a survey of presence and frequency in various commodities. J Hazard Mater. 2008;156:240-259.
28. Zgoła-Grzeskowiak A, Werner J, Jeszka-Skowrona M, Czarczynska-Goslinska B. Determination of parabens in cosmetic products using high performance liquid chromatography with fluorescence detection. Anal Methods. 2016;8:3903-3909.
29. Yılmaz PK, Tokat MA. Simultaneous determination of six parabens in cosmetics by a new high performance liquid chromatography method with fluorescence detection. Methods Objects Chem Anal. 2020;15:27-32.