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Dispersive liquid-liquid microextraction of parabens from pharmaceuticals and personal care products prior to their determination using HPLC-DAD

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Abstract: Dispersive liquid–liquid microextraction followed by a back-extraction step was combined with HPLC-DAD for the determination of four parabens (i.e. methyl-, ethyl-, propyl-, and butylparaben). Optimum extraction conditions were found as follows: 225 μL of chloroform, 0.75 mL of ethanol, 7.5 mL of aqueous solution and within an extraction time of 15 s. Back-extraction into 100 μL of 50 mM sodium hydroxide solution within 20 s resulted in a reversed-phase HPLC-compatible extract. The analytes were separated at 20 °C using methanol (A) and water (B), 40:60 (A:B, v/v) as the mobile phase, a flow rate of 1.0 mL min^{-1} and an injection volume of 20 μL. DAD was set at 258 nm to monitor the analytes. Limits of detection and quantitation were as low as 0.1 and 0.3 μg mL^{-1}, respectively. Coefficients of determination (R^2) were higher than 0.9950 and percentage relative recoveries were found in the range of 86.5–114.5% for the four parabens from pharmaceuticals and personal care products.

Key words: Dispersive liquid–liquid microextraction, high-performance liquid chromatography, parabens, personal care products, pharmaceuticals

1. Introduction

Esters of *p*-hydroxybenzoic acid, or parabens, are extensively employed as preserving agents in a large number of processed food, pharmaceuticals and personal care products (PCPs) including creams, body lotions, mouth rinses, toothpastes, and shampoos. Owing to their neutrality, lack of odor, thermal stability antimicrobial properties, and low toxicity, they are ideal preservatives [1]. Methyl- (MP), ethyl- (EP), propyl- (PP), and butylparaben (BP) are the most widely used parabens. They are generally applied individually or in combination to achieve maximum activity since they show synergistic effect [2]. Some parabens have been shown to possess low estrogenic activity [3] and to affect the male reproductive system of rats and mice [4]. Thus, recently, their use as preservatives has been criticized. In Europe, the concentration of each paraben in PCPs should not exceed 0.4% and the total concentration should be less than 0.8% (EU Cosmetics Directive 76/768/EEC). Nevertheless, a combined maximum concentration of PP and BP up to 0.19% per weight has been recently recommended [5].

Analysis of pharmaceuticals and PCPs for parabens has been performed using flow-injection ultraviolet spectrophotometry (FI-UV) [6], gas chromatography-mass spectrometry (GC-MS) [7] and flame-ionization detection (GC-FID) [8], capillary electrophoresis-UV (CE-UV) [9], and ultra-performance liquid chromatography

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(UPLC) [10]. High-performance liquid chromatography-ultraviolet (HPLC-UV) remains the most common analytical technique for this purpose. However, due to the complexity of these matrices, applying an extraction step like liquid–liquid extraction (LLE) or solid-phase extraction (SPE) was necessary prior to separation and/or detection [11,12]. Nevertheless, these traditional extraction methods require significant amounts of the sample and toxic organic solvents, and the applied procedures are rather long, making them labor-intensive, cost-ineffective, and environmentally unfriendly [13]. Over the past decade, the focus on miniaturization of sample preparation techniques has been increasing in order to overcome these inherent problems. Solid-phase microextraction (SPME) [14] and dispersive liquid-liquid microextraction (DLLME) [15] have been applied to preconcentrate parabens from different matrices. Although SPME is considered a solvent-free technique, most commercially available fibers used in SPME are still expensive and may suffer from fragility, limited lifetime, and potential for sample carry-over [16].

DLLME has drawn much attention as an outstanding technique that offers simple and cost-effective extraction of analytes within a short time [17]. The novelty of this method relies on the rapid injection of a water-miscible disperser solvent into the donor phase to disperse the water-immiscible organic acceptor phase. Efficient extraction is accomplished rapidly due to emulsion formation. Upon centrifugation, the analyte-rich organic phase is collected for analysis. Due to compatibility of the extraction solvent with GC, it was the first analytical technique combined with DLLME [17]. Nonetheless, for polar analytes like parabens, a tedious derivatization step prior to GC is generally required [18,19].

In this study, DLLME-HPLC-DAD is proposed for quantitation of the four parabens in pharmaceuticals (antacid suspension, antiinflammatory solution, and anticough syrup) and PCPs (aftershave cream, baby cream, mouth rinse, and wet wipes) with a main focus on minimizing the extraction time and organic solvents without derivatization. Influential extraction and back-extraction parameters were examined and optimized.

2. Experimental

2.1. Chemicals and reagents

MP ($\log P$ 1.67, $\mathrm{p}K_a$ 8.50), EP ($\log P$ 2.03, $\mathrm{p}K_a$ 8.50), PP ($\log P$ 2.55, $\mathrm{p}K_a$ 8.50), BP ($\log P$ 3.00, $\mathrm{p}K_a$ 8.50), HPLC-grade acetonitrile (ACN), ethanol (EtOH), and methanol (MeOH) were obtained from Sigma-Aldrich (USA). Chloroform (CF, $\log P$ 1.83), diphenyl ether (DPE, $\log P$ 3.47), sodium hydroxide, and acetic acid were purchased from Merck (Germany). 1-Undecanol (1-UN, $\log P$ 3.92) and 1-dodecanol (1-DO, $\log P$ 4.36) were acquired from Sigma-Aldrich (Germany). Deionized (DI) water (18.2 MΩ.cm), treated with Purelab Ultra Analytic (ELGA LabWater, UK), was used. MarvinSketch (Version 5.3.8, ChemAxon, USA) was used to calculate $\log P$ and $\mathrm{p}K_a$ values.

2.2. Standard solutions of parabens

Appropriate amounts of parabens were used to prepare individual stock solutions at concentrations of 2000 μg mL^{-1} in ACN. Working standard solutions were freshly prepared in DI water from the stock solutions. Mobile phases and aqueous solutions were filtered using a vacuum filtration system through 0.20 μm regenerated cellulose-membrane filters (Whatman, Germany), whereas samples were filtered through 0.22 μm sterile nylon syringe filters (Chromfil, China). Degassing of the solutions was carried out using a digital ultrasonic bath (Isolab, Germany).

2.3. Instrumentation

An HPLC instrument (Agilent Technologies 1200 series, USA) having an online vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, and a diode-array detector (DAD) was used throughout the experiments. ChemStation software (Rev. B.03.01, Agilent Technologies, USA) was used to obtain, store, and evaluate the chromatograms. An optimum wavelength of 258 nm for the four parabens was found from the instrument's Data Analysis software. The analytes were separated on a reversed-phase column, i.e. ACE-C18 (125 $\times$ 3 mm $\times$ 5 μm). The mobile phase consisted of DI water and MeOH at a composition of 60:40 (v/v). The flow rate was set at 1.0 mL min^{-1}, column temperature at 20 °C, and injection volume at 20 μL. Back-pressure was typically 220 bar.

2.4. Sample preparation

Pharmaceuticals (i.e., antacid suspension, antiinflammatory solution, and anticough syrup) were obtained from local pharmacies (Nicosia, TRNC). Dilution of the samples (e.g., 1000 times with DI water) was adopted for antacid suspension, antiinflammatory solution, and the anticough syrup.

PCPs (aftershave cream, baby cream, mouth rinse, and wet wipes) were obtained from local markets (Nicosia, TRNC). Dilution of the samples was adopted for the aftershave cream, mouth rinse and baby cream, whereas solid-liquid extraction (SLE) into EtOH was applied for wipes.

0.25 ($\pm$0.01)-g samples of aftershave cream and baby cream were accurately weighed in a beaker before being mixed with DI water and ultrasonicated for 10 min at 60 °C. The mixture was transferred into a 25-mL volumetric flask and the volume was made up to the mark with DI water after being cooled down to room temperature. The solution was diluted 30 times prior to application of DLLME. SLE was adopted for the wet wipes; a 0.6 $\pm$(0.01)-g piece of the wipe was cut and accurately weighed, transferred into a graduated polypropylene centrifuge tube with a screw-cap and immersed into 2.0 mL of EtOH before being sonicated for 5 min. Next, the wipe was hanged by the cap and centrifuged for 1 min at 6000 rpm to obtain the solution from the wipe. The resulting solution was diluted 100 times prior to DLLME. Dilution factors for the samples were chosen in order to fit the final concentration of the analytes into the linear dynamic range (LDR).

2.5. DLLME procedure

To an aliquot of the sample solution (1.0 mL), 750 μL of EtOH was added and the volume was made up to 7.5 mL with DI water in a 15-mL centrifuge tube. The solution was acidified with 100 μL of acetic acid to ensure that the analytes were in their unprotonated forms before 225 μL of CF was added. Vortexing the mixture for 15 s caused the solution to turn cloudy. Upon centrifugation (6000 rpm, 1 min), CF subsided at the bottom of the test tube, which was completely transferred into a 1.5-mL snaplock microcentrifuge tube using a 100-μL HPLC syringe (Hamilton, USA). Finally, a back-extraction step was applied using 100 μL of the back-extraction solution (BES, 50 mM NaOH) to extract the analytes into an aqueous solution. Following vortexing for 20 s and centrifugation (6000 rpm, 1 min), a portion of the supernatant aqueous solution was transferred into a 1.5-mL capped glass vial (Agilent Technologies, USA) for analysis and a portion of 20 μL was directly injected into HPLC.

3. Results and discussion

3.1. Optimization of DLLME parameters

Parameters influencing DLLME, which included the type and volume of the extraction solvent, the type and volume of the disperser solvent, volume of the aqueous phase, extraction time, as well as BES volume and back-extraction time, were optimized. Peak areas were used to investigate the effect of each parameter on the extraction efficiency using the one-variable-at-a-time approach.

3.1.1. Type and volume of the extraction solvent

Favorable extraction solvents for DLLME have higher density than water for easy collection. However, the use of low-density solvents with low melting point has also been suggested [20]. Such solvents should have high extractability of the analytes, low water solubility and negligible volatility. Based on these requirements, four extraction solvents, namely, CF (density, d: 1.48 g mL^{-1}), DPE (d: 1.08 g mL^{-1}), 1-UN (d: 0.83 g mL^{-1}; melting point (m. p.): 11 °C) and 1-DO (d: 0.83 g mL^{-1}; m. p.: 22–26 °C) were studied. Low-density solvents were collected through freezing the floating organic drop [21]. CF was found to provide the highest peak areas (Figure 1a). Hence, CF was selected as the optimum extraction solvent for later experiments.

Different volumes of CF (i.e. 125, 150, 200, 225, and 250 μL) were tested under the same DLLME conditions. Increasing the volume of CF from 125 to 225 μL, increased peak areas due to increase of extraction efficiency but decreased afterwards due to dilution (Figure 1b). Thus, a volume of 225 μL of CF was considered optimum.

3.1.2. Type and volume of the disperser solvent

A suitable disperser solvent for DLLME should be miscible with the donor aqueous solution and the acceptor organic solvent in order to increase the surface area of contact for the analyte. This experiment was done using 1.0 mL of ACN, MeOH, and EtOH as the disperser solvents. Although the recoveries obtained with MeOH were higher for PP and BP, they were higher for MP and EP with EtOH (Figure 1c). Due to the higher toxicity of MeOH than that of EtOH and the wider use of MP and EP in the studied formulations, EtOH was selected as optimum.

Five volumes of EtOH (i.e. 0.50, 0.75, 0.90, 1.00, and 1.25 mL) were tested. Peak areas increased upon increasing the disperser solvent volume up to 0.75 mL (Figure 1d) and decreased thenceforth. This observation was linked to the increase of the solubility of the analytes in the aqueous sample solution with higher concentrations of EtOH. As a result, 0.75 mL of EtOH was chosen as optimum.

3.1.3. Volume of the aqueous phase

For evaluating the effect of volume of the aqueous phase, the solution was completed with DI water to a final volume in the range of 6.5 to 9.0 mL with constant volumes of EtOH (0.75 mL) and CF (225 μL) prior to DLLME. It was noticed that increasing the volume from 6.5 to 7.5 mL, rapidly increased peak areas but slowly decreased them afterwards (Figure 1e), which was also related to the solubility of the studied parabens in the aqueous solution containing EtOH. Hence, the volume of the aqueous phase was completed to 7.5 mL with DI water as an optimum volume in subsequent experiments.

3.1.4. Extraction time

The effect of vortex, or extraction, time on peak areas was investigated by vortexing the mixture for different time intervals (i.e. 0, 15, 30, 45, 60, and 75 s). Through increasing the extraction time from 0 to 15 s, peak areas increased and remained almost constant afterwards indicating that equilibrium has been reached rapidly within 15 s (Figure 1f). Accordingly, an extraction time of 15 s was decided on as optimum.

3.2. Optimization of back-extraction parameters

Influence of BES (50 mM NaOH) volume on peak areas was inspected within 50–175 μL. Peak areas increased rapidly upon the addition of 100 μL of BES, beyond which, it started to decrease gradually with further increase of BES volume because of dilution (Figure 1g). The impact of back-extraction time was also examined within the range of 0–30 s. Peak areas remained almost constant. Vortexing for 10 s was selected as optimum, however, for better reproducibility (Figure 1h).

3.3. Analytical performance

Evaluating the efficiency of the proposed method was done through constructing standard-addition calibration graphs as peak area versus concentration via spiking the samples with the analytes at concentration levels of 1.0 to 5.0 μg mL^{1} (N=3 at each level). Under optimized conditions, limits of detection (LOD, calculated based on 3Sb/m, where Sb is the standard deviation of the intercept and m is the slope of the regression equation), varied between 0.1 and 0.6 μg mL^{1}, and limits of quantitation (LOQ, based on 10Sb/m) from 0.3 to 2.0 μg mL^{1}. Linear relationship was obtained from LOQ up to 5.0 μg mL^{1} for all analytes, with R^{2} being larger than 0.9950. Reproducibility was assessed using intra- and interday precision through calibration in the same day and within three consecutive days, respectively. Reproducibility was studied at each concentration level used in the calibration graphs and the average value was reported. The average percentage relative standard deviation (% RSD) of peak areas were in the ranges of 1.8–5.4% and 2.7–6.7% for intra- and inter-day, respectively, despite the complexity of the samples. Analytical performance parameters of the developed method are outlined in Table 1.

3.4. Recovery studies and matrix effect

Under optimized conditions, the developed method was implemented to analyze three types of pharmaceuticals (i.e. antacid suspension, anticough syrup, and antiinflammatory solution) and four types of PCPs (i.e. aftershave cream, baby cream, mouth rinse, and wet wipes). Representative chromatograms of the analyzed samples are given in Figure 2. Good selectivity of the method was revealed from void of interfering peaks from the samples at the retention times of parabens. Percentage relative recoveries (%RR), calculated for samples spiked at three concentration levels (2.0, 3.0, and 4.0 μg mL^{-1} of each analyte), ranged from 86.5% to 114.5% (Table 2). Potential matrix effect was judged through a comparison of the slopes of the calibration graphs from the regression equations (Table 1) via calculating the P value using single-factor ANOVA. It was found that the difference was statistically significant ($P < 0.05$), which pointed out the presence of matrix effect. Therefore, the standard-addition method was necessary to eliminate this effect.

Table 1. Analytical figures of merit of DLLME-HPLC.

Sample	Paraben	Regression equation[a]	R^{2b}	LOD[c] (μg mL^{-1})	LOQ[d] (μg mL^{-1})	LDR[e] (μg mL^{-1})	%RSD[f]	
							Intraday	Interday
Aftershave cream	MP	$Y = 146.5\ (\pm 2.6)\ X + 47.0\ (\pm 5.2)$	0.9952	0.3	1.0	1.0–5.0	3.5	3.9
	EP	$Y = 472.1\ (\pm 7.2)\ X + 27.8\ (\pm 14.3)$	0.9958	0.2	0.7	0.7–5.0	2.7	4.5
	PP	$Y = 581.7\ (\pm 7.0)\ X - 15.6\ (\pm 14.5)$	0.9977	0.2	0.7	0.7–5.0	3.2	5.7
	BP	$Y = 697.8\ (\pm 8.7)\ X - 29.7\ (\pm 18.0)$	0.9975	0.2	0.7	0.7–5.0	3.8	5.2
Antacid Êsuspension	MP	$Y = 144.7\ (\pm 2.6)\ X + 130.8\ (\pm 5.3)$	0.9974	0.3	1.0	1.0–5.0	3.1	4.2
	EP	$Y = 500.5\ (\pm 6.3)\ X - 36.3\ (\pm 12.0)$	0.9984	0.2	0.7	0.7–5.0	3.3	4.5
	PP	$Y = 591.8\ (\pm 7.0)\ X + 52.5\ (\pm 12.6)$	0.9986	0.2	0.7	0.7–5.0	3.1	3.9
	BP	$Y = 751.9\ (\pm 19.3)\ X - 156.2\ (\pm 36.9)$	0.9954	0.3	1.0	1.0–5.0	4.1	4.5
Anticough syrup	MP	$Y = 153.5\ (\pm 3.3)\ X + 202.3\ (\pm 8.9)$	0.9955	0.4	1.3	1.3–5.0	2.4	3.9
	EP	$Y = 564.6\ (\pm 6.1)\ X + 4.7\ (\pm 15.5)$	0.9984	0.2	0.7	0.7–5.0	4.7	5.8
	PP	$Y = 631.0\ (\pm 5.1)\ X - 1.1\ (\pm 14.0)$	0.9992	0.2	0.7	0.7–5.0	3.7	4.9
	BP	$Y = 689.2\ (\pm 10.0)\ X - 2.8\ (\pm 27.3)$	0.9975	0.3	1.0	1.0–5.0	4.2	6.7
Anti-inflammatory solution	MP	$Y = 162.4\ (\pm 5.0)\ X + 190.3\ (\pm 11.1)$	0.9958	0.4	1.3	1.3–5.0	5.3	5.4
	EP	$Y = 509.7\ (\pm 8.7)\ X - 41.8\ (\pm 17.0)$	0.9960	0.2	0.7	0.7–5.0	3.8	5.9
	PP	$Y = 551.4\ (\pm 8.8)\ X - 19.1\ (\pm 8.4)$	0.9970	0.2	0.7	0.7–5.0	2.5	3.6
	BP	$Y = 635.6\ (\pm 10.4)\ X + 5.3 (\pm 5.3)$	0.9973	0.2	0.7	0.7–5.0	2.3	3.4
Baby cream	MP	$Y = 164.0\ (\pm 2.3)\ X + 28.6\ (\pm 5.1)$	0.9973	0.2	0.7	0.7–5.0	4.2	4.8
	EP	$Y = 545.9\ (\pm 6.7)\ X + 3.6 (\pm 16.6)$	0.9975	0.2	0.7	0.7–5.0	4.1	6.5
	PP	$Y = 608.8\ (\pm 5.6)\ X + 80.4\ (\pm 11.8)$	0.9974	0.2	0.7	0.7–5.0	2.6	4.7
	BP	$Y = 761.9\ (\pm 5.5) X - 2.7 (\pm 7.5)$	0.9992	0.1	0.3	0.3–5.0	2.7	4.8
Mouth rinse	MP	$Y = 166.1\ (\pm 1.5) X + 27.4 (\pm 3.3)$	0.9988	0.2	0.7	0.7–5.0	3.8	4.4
	EP	$Y = 549.8\ (\pm 2.7)\ X + 1.8\ (\pm 5.6)$	0.9996	0.1	0.3	0.3–5.0	2.8	4.8
	PP	$Y = 608.8\ (\pm 5.6) X + 80.4 (\pm 11.8)$	0.9986	0.2	0.7	0.7–5.0	3.3	5.6
	BP	$Y = 769.3\ (\pm 5.4)\ X - 5.1\ (\pm 7.5)$	0.9994	0.1	0.3	0.3–5.0	4.2	5.3
Wet wipes	MP	$Y = 164.8\ (\pm 2.3)\ X + 71.1\ (\pm 6.0)$	0.9979	0.2	0.7	0.7–5.0	5.4	6.2
	EP	$Y = 543.6\ (\pm 8.7)\ X + 40.9\ (\pm 22.2)$	0.9974	0.3	1.0	1.0–5.0	1.8	2.7
	PP	$Y = 578.8\ (\pm 5.3)\ X + 129.1\ (\pm 11.0)$	0.9990	0.2	0.7	0.7–5.0	2.4	3.9
	BP	$Y = 709.0\ (\pm 22.5)\ X - 25.0\ (\pm 54.6)$	0.9950	0.6	2.0	2.0–5.0	5.1	6.3

[a] $\times Peak\ area = slope\ (\pm SD)\ [paraben\ concentration\ (\mu g\ mL^{-1})] + intercept\ (\pm SD)$.

[b] Coefficient of determination.

[c] Limit of detection.

[d] Limit of quantitation.

[e] Linear dynamic range.

[f] Percentage relative standard deviation ($n = 3$).

3.5. Comparison with other methods

Competence of the proposed DLLME-HPLC-DAD method was evaluated through a comparison with other methods for the determination of parabens in terms of extraction time, total consumed volume of organic solvents per sample, LOD, LOQ, LDR, R^2, %RR, and %RSD (Table 3). Rapidness, simplicity, and necessity for the least volume of organic solvents were among the main features of this method. The extraction time was only 0.6 min due to emulsion formation and the infinitely large surface for extraction. Equilibrium was established after a longer time in the other methods applying SPME, LLE, and SPE. When applied by Jain et al. [8], DLLME required 1 min only but the volume of organic solvent was 250 mL, whereas only 0.98 mL were required in our study. Sensitivity, as revealed by LODs and LOQs, was similar to that obtained in the other studies but lower than that obtained with SPME-GC-MS (Table 3). Nonetheless, this was due to the use

Table 2. Percentage relative recoveries from pharmaceuticals and PCPs.

Sample	Added, μg mL^{-1}	Found, μg mL^{-1} (μg g^{-1})				%RR[a]			
		MP	EP	PP	BP	MP	EP	PP	BP
Aftershave cream	-	4.6 (912.6)	1.9 (380.8)	< LOD	< LOD	-	-	-	-
	2.00	1.99	1.85	1.74	1.67	99.3	93.3	94.2	95.8
	3.00	3.09	2.99	2.95	2.96	103.0	96.9	98.6	100.3
	4.00	4.27	4.29	4.42	4.63	106.7	100.5	103.1	104.8
Antacid suspension	-	3.8 (761.9)	< LOD	1.4 (276.3)	< LOD	-	-	-	-
	2.00	1.73	1.66	1.56	1.47	86.5	96.0	94.1	94.0
	3.00	2.71	2.70	2.64	2.59	90.2	99.9	97.6	98.3
	4.00	3.76	3.90	3.94	4.04	93.9	103.8	101.1	102.6
Anticough syrup	-	196.4 (196.4)	< LOD	< LOD	< LOD	-	-	-	-
	2.00	2.06	1.98	1.87	1.73	102.9	100.8	94.4	92.9
	3.00	3.18	3.22	3.18	3.13	106.0	106.0	98.7	98.3
	4.00	4.37	4.65	4.79	4.97	109.2	111.3	103.0	103.8
Anti-inflammatory solution	-	184.8 (184.8)	< L OD	< LOD	< LOD	-	-	-	-
	2.00	1.79	1.59	1.48	1.45	89.6	88.8	93.2	98.2
	3.00	2.85	2.66	2.56	2.59	94.9	93.6	96.2	101.0
	4.00	4.01	3.95	3.92	4.07	100.3	98.5	99.3	103.9
Baby cream	-	2.8 (555.3)	< LOD	2.4 (473.7)	< LOD	-	-	-	-
	2.00	1.90	1.84	1.82	1.95	94.8	97.0	99.2	107.0
	3.00	2.98	3.05	3.13	3.47	99.3	102.3	102.8	110.7
	4.00	4.15	4.47	4.76	5.44	103.8	107.6	106.5	114.5
Mouth rinse	-	266.0 (266.0)	< LOD	705.3 (705.3)	< LOD	-	-	-	-
	2.00	1.96	1.90	1.84	1.72	97.8	97.2	96.6	94.0
	3.00	3.06	3.09	3.12	3.08	101.9	101.0	101.0	98.7
	4.00	4.24	4.44	4.69	4.85	106.0	104.8	105.5	103.5
Wet wipes	-	6.9 (1092.2)	< LOQ	11.3 (1791.9)	< LOQ	-	-	-	-
	2.00	2.01	2.06	1.96	1.76	100.5	102.3	95.4	89.6
	3.00	3.19	3.33	3.28	3.13	106.3	104.5	98.5	95.3
	4.00	4.48	4.79	4.87	4.91	112.1	106.8	101.7	101.0

[a] Percentage relative recovery, a value obtained considering extraction yields from standard-addition calibrations.

of MS detectors, which are more sensitive, yet more expensive, than UV. In addition, high cost, fragility, short lifetime, and possible sample carry-over are some shortcomings of SPME fibers [16]. Precision was also as good as that of the other methods but better than that of SPME.

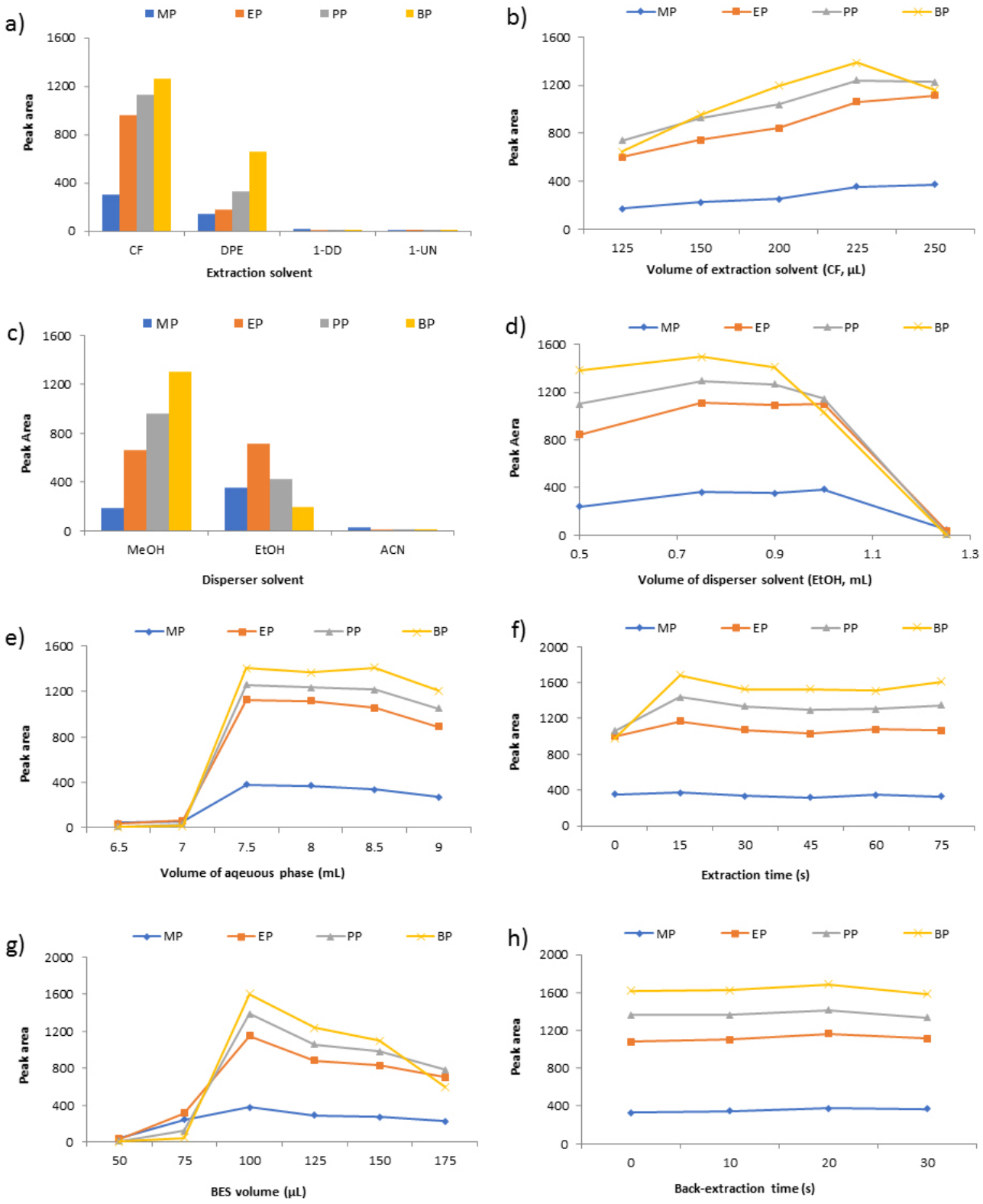

Figure 1. Effect of experimental parameters on extraction efficiency of DLLME: (a) Extraction solvent, (b) Volume of extraction solvent, (c) Dispeser solvent, (d) Volume of disperser solvent, (e) Volume of aqueous phase, (f) Extraction time, (g) BES volume and (h) Back-extraction time.

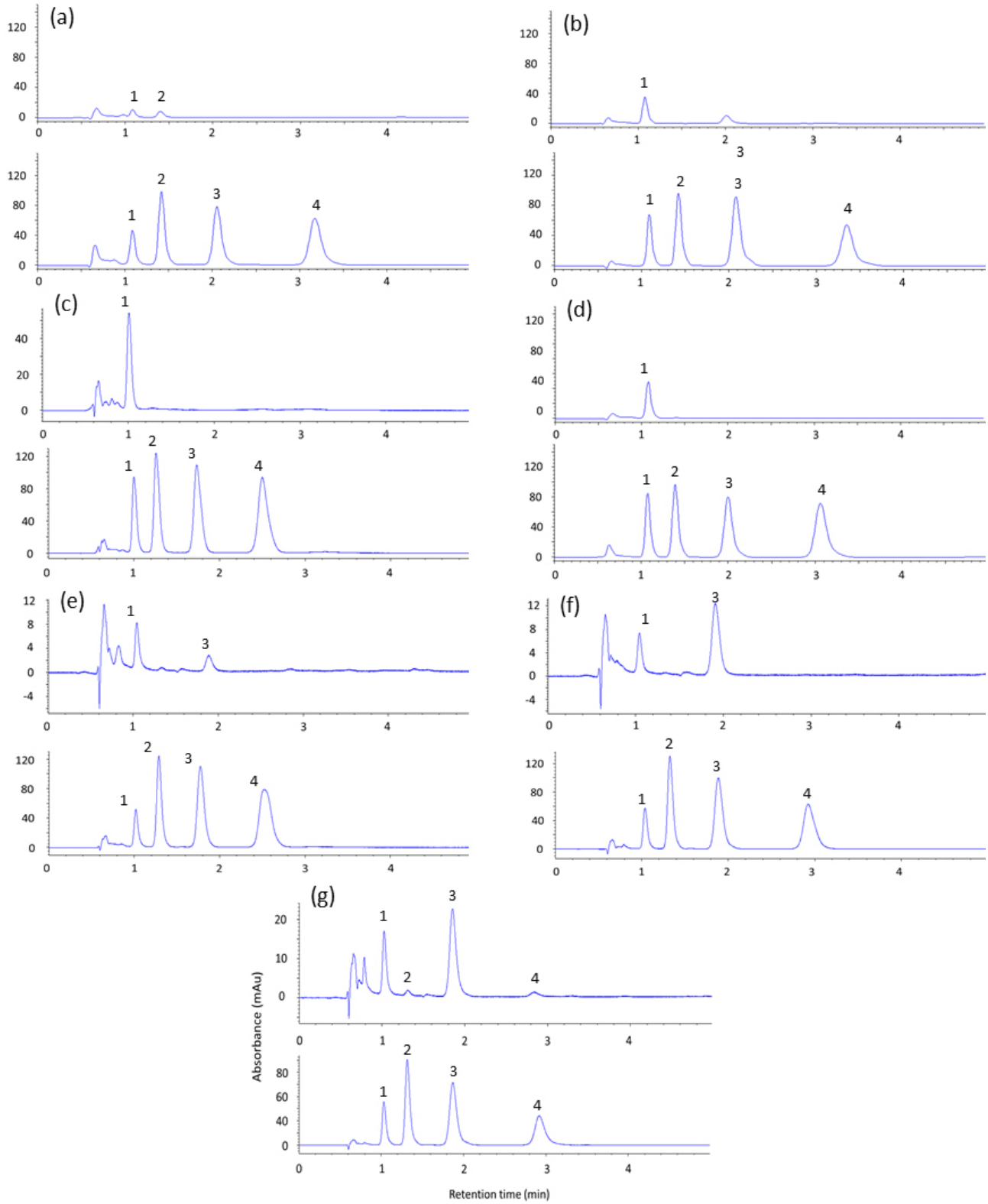

Figure 2. Representative chromatograms after DLLME. Top: unskiped sample; bottom: spiked sample at 3.0 μg mL^{-1} of each paraben. (a) Aftershave cream, (b) Antacid Suspension, (c) Anti-cough syrup, (d) Anti-inflammatory solution, (e) Baby cream, (f) Mouth rinse, and (g) Wet wipes. Peaks: 1, MP; 2, EP; 3, PP; 4, BP.

Table 3. Comparison of DLLME-HPLC with other reported methods for the exraction and determination of parabens.

Paraben	Sample	Extraction method/ Technique[a]	Extraction time (min)	$V^{b}_{org.}$ (mL)	LOD^{c} (μg mL^{-1})	LOQ^{d} (μg mL^{-1})	LDR^{e} (μg mL^{-1})	R^2	$\%RR^{f}$	$\%RSD^{g}$	Ref.
MP, EP, PP	PCPs, food, pharmaceutical	DLLME-GC-FID	10	~ 6	0.005–0.015	0.02-0.05	0.02–30	> 0.992	25–72	< 3	[2]
MP, EP, PP, BP	PCPs, food, waters	DLLME-GC-FID	1	250	0.029–0.102	0.095-0.336	0.1–10	> 0.9913	81.56–101.4	< 6.86	[8]
MP, EP, PP, BP	PCPs	SPE-CE-UV	~ 15	7.1	0.1–0.15	-	0.50–500	> 0.995	62.6–100.4	< 6.92	[9]
MP, EP, PP, BP	Cream	LLE-HPLC-UV	30	3	0.2	-	0.5–200	> 0.9997	91.3–116.3	< 4.68	[11]
MP, EP, PP, BP	Toothpaste, saliva	SPE-HPLC-UV	-	$\sim$ 10	0.1–0.3	0.3-1.0	0.3–50	> 0.9984	86–113	< 6.8	[12]
MP, EP, PP, BP	PCPs	SPME-GC-MS	40	2	0.4–8.5 (ng g^{-1})	-	1–2000 (ng g^{-1})	> 0.9922	83–98	< 10.3	[14]
MP, EP, PP, BP	Pharmaceuticals and PCPs.	DLLME-HPLC	0.6	1.0	0.1–0.6	0.3–2.0	0.1–5.0	> 0.9950	86.5–114.5	< 6.7	This study

[a] Solid-phase microextraction (SPME), Liquid-liquid extraction (LLE), Solid-phase extraction (SPE).

[b] Total volume of organic solvents consumed per sample.

[c] Limit of detection.

[d] Limit of quantitation.

[e] Linear dynamic range.

[f] Percentage relative recovery.

[g] Percentage relative standard deviation.

4. Conclusion

In this report, DLLME combined with a back-extraction step was illustrated to be a superior sample clean-up and preconcentration technique for parabens from pharmaceuticals and personal care products prior to HPLC-DAD determination. Rapidness, use of the least volume of organic solvents, low amounts of organic waste, straightforwardness, cost-effectiveness, and good selectivity were among the main advantages of this method. Regardless of the complexity of the studied samples, high recoveries, good reproducibility and interference-free chromatograms were attained in all cases. Applicability of DLLME-HPLC-DAD to different matrices with minimum modification of the pretreatment and extraction procedure encourages its use for the quantitation of parabens in pharmaceuticals and personal care products in routine analysis.

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