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Analytical Methods

Determination of parabens in human milk and other food samples by capillary electrophoresis after dispersive liquid–liquid microextraction with back-extraction

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ABSTRACT

Dispersive liquid–liquid microextraction (DLLME) with back-extraction was used prior to capillary electrophoresis (CE) for the extraction of four parabens. Optimum extraction conditions were: 200 μL chloroform (extraction solvent), 1.0 mL acetonitrile (disperser solvent) and 1 min extraction time. Back-extraction of parabens from chloroform into a 50 mM sodium hydroxide solution within 10 s facilitated their direct injection into CE. The analytes were separated at 12 °C and 25 kV with a background electrolyte of 25 mM borate buffer containing 5.0% (v/v) acetonitrile. Enrichment factors were in the range of 4.3–10.7 and limits of detection ranged from 0.1 to 0.2 μg mL^{-1}. Calibration graphs showed good linearity with coefficients of determination (R^2) higher than 0.9957 and relative standard deviations (%RSDs) lower than 3.5%. DLLME–CE was demonstrated to be a simple and rapid method for the determination of parabens in human milk and food with relative recoveries in the range of 86.7–103.3%.

1. Introduction

Parabens or esters of *p*-hydroxybenzoic acid are used individually or in combination as antimicrobial preservatives in over 13,200 kinds of food, personal care products (PCP) and pharmaceuticals due to their broad spectrum of action against numerous microorganisms, efficacy, lack of perceptible odour, taste, discoloration or hardening effect and for their stability over a wide pH range (Canosa, Rodriguez, Rubi, Bollain, & Cela, 2006). Despite their benefits, a controversy surrounding their use has been mounting since 2004 when intact esters of the five commonly used parabens, methyl- (MP), ethyl- (EP), propyl- (PP), butyl- (BP) and isobutyl-paraben (iso-BP) were found in human breast cancer tissues at a mean concentration of 20.6 ng g^{-1}. Although the source of parabens could not be identified, it was suggested that dermal absorption from PCPs applied to the breast region over the long term might have contributed (Darbre et al., 2004). Comparison to the concentrations of each of the parabens measured in human breast tissue (Barr, Metaxas, Harbach, Savoy, & Darbre, 2012) as converted to oestrogen equivalents, it has been seen that even the highest concentrations measured in human breast tissue could be achieved by very few such applications of lotion and this should be considered in the context of exposure of a large global population where on average each consumer would use not one but multiple personal care products on a daily basis (Darbre & Harvey, 2014).

Since breast milk is the main route of exposure to such chemicals for breastfed infants, the analysis of breast milk for parabens would be of scientific interest. In a recent study, MP and PP were detected in human milk at concentrations ranging from 0.53 to 3.00 and at 0.33 ng mL^{-1}, respectively (Ye, Bishop, Needham, & Calafat, 2008). Even though they are still not regulated in food, their total maximum concentration does not generally exceed 0.1% w/w (Soni, Burdock, Taylor, & Greenberg, 2001). To ensure safety of the food chain, consumer demand requires that such chemicals be monitored in foodstuff, especially those commonly included in the daily diet. Consequently, the development of simple, rapid and accurate analytical methods for the determination of parabens is highly desirable to monitor them and to set legislations.

The reported methods for the determination of parabens in human milk and food are mainly based on high-performance liquid chromatography (HPLC) (Moradi & Yamini, 2012; Ye et al., 2008) and gas chromatography (GC) (Ochiai et al., 2002; Tsai & Lee, 2008). HPLC methods developed for parabens generally require

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large volumes of organic solvents for the mobile phase and that the extract be evaporated and replaced with a compatible solvent composition (Cabuk, Akyuz, & Ata, 2012; Zotou, Sakla, & Tzanavaras, 2010). Due to their polar nature, analysis of parabens by GC requires a tedious derivatisation step. Although instantaneous derivatisation has also been reported for parabens in food (Jain et al., 2013), large volumes of organic solvents were required in the sample preparation step. Recently, there has been an increasing interest in applying capillary electrophoresis (CE) for the determination of a wide range of analytes including parabens (Blanco, Coello, Iturriaga, Maspoch, & Romero, 2001; Cheng, Wang, Chen, & Wu, 2012; Maijo, Borrull, Aguilar, & Calull, 2013) since it is considered as a green analytical technique with low consumption of samples and reagents, extremely high separation efficiency, high versatility in terms of multiple separation modes and excellent biocompatibility.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have been widely applied to extract and/or preconcentrate parabens (Gao & Legido-Quigley, 2011; Ye, Shi, Li, & Wang, 2013; Zotou et al., 2010) prior to their determination. However, these traditional techniques consume large sample volumes and toxic organic solvents and require prolonged steps which make them labour-intensive, expensive and environmentally-unfriendly (Rezaee, Yamini, & Faraji, 2010). Thus, miniaturised sample preparation techniques have been proposed for extraction of parabens which include stir-bar sportive extraction (SBE) (Ochiai et al., 2002), solid phase microextraction (SPME) (Tsai & Lee, 2008), solidified floating vesicular coacervative drop microextraction (SFVCDME) (Moradi & Yamini, 2012) and dispersive liquid–liquid microextraction (DLLME) (Farajzadeh, Djozan, & Bakhtiyari, 2010; Jain et al., 2013).

DLLME has found wide acceptance as an outstanding technique for its simplicity, cost effectiveness and ability to provide high extraction efficiencies within a very short time due to the extensive surface contact between the droplets of the extraction solvent and the sample (Rezaee et al., 2006). In this method, a water-miscible disperser solvent is injected into an aqueous sample to help the dispersion of the organic water-immiscible extraction solvent. Extraction equilibrium is achieved in a short time due to the extensive surface contact between the droplets of the extraction solvent and the sample. Upon centrifugation, an extraction phase which is abundant with the analytes is obtained. Despite its successful combination with many atomic and chromatographic techniques, there are still very few reports on the application of DLLME prior to CE (Herrera-Herrera, Hernandez-Borges, Borges-Miquel, & Rodriguez-Delgado, 2010; Wen, Li, Zhang, & Chen, 2011) which might be linked to incompatibility of the final organic extract with the electrophoretic system. For ionisable analytes such as parabens, a simple back-extraction step into an aqueous solution having a suitable pH not only fulfils the instrument compatibility requirement but would also give a good control of the ionic strength in the extract which would minimise matrix effect and improve reproducibility. DLLME coupled with CE was recently used for the determination of parabens in other matrices, such as cosmetics (Xue, Chen, Luo, Wang, & Sun, 2013). To the best of our knowledge, this is the first report on applying DLLME with a back-extraction step prior to CE for the determination of parabens in food samples.

Recently, we have published a simple efficient method based on DLLME-back extraction prior to CE for the determination of non-steroidal anti-inflammatory drugs (NSAIDs) in bovine milk and dairy products (Alshana, Goger, & Ertas, 2013). The aim of this study is to extend the applicability of this method to a wider range of food samples such as, human breast milk, tomato paste, mixed fruit juice, pickle and ice cream with a focus on minimum consumption of organic solvents. Effective experimental parameters on extraction efficiency which include the type and volume of extraction and disperser solvents, salt concentration, extraction time and volume of back-extraction solution (BES) were investigated and optimised.

2. Experimental

2.1. Chemicals and reagents

MP (log$P$ 1.91, pK_a 8.87), EP (log$P$ 2.34, pK_a 8.90) PP (log$P$ 2.94, pK_a 8.87), BP (log$P$ 3.50, pK_a 8.79) (Angelov, Vlasenko, & Tashkov, 2008), HPLC-grade acetonitrile (ACN), ethanol (EtOH) and methanol (MeOH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$) was obtained from Sigma–Aldrich (Munich, Germany). Sodium chloride, chloroform (CF, log$P$ 1.8), carbon tetrachloride (CTC, log$P$ 3.0), sodium hydroxide and phosphoric acid were acquired from Merck (Darmstadt, Germany). 1-undecanol (1-UN, 99.0%, log$P$ 3.9) and 1-dodecanol (1-DO, 98.0%, log$P$ 4.4) were from Sigma–Aldrich (Steinheim, Germany). All reagents were of analytical grade. Deionised (DI) water (18.2 MΩ cm) treated with Millipore (Simplicity, 185 water purification system) Milli-Q water purification apparatus was used for all aqueous solutions.

2.2. Paraben standard solutions

Individual stock solutions of parabens at a concentration of 2000 μg mL^{-1} were prepared in ACN and stored at −20 °C. Mixed standard solutions were freshly prepared at each working session from the stock solutions by proper dilutions with DI water. All solutions were degassed using a sonicator (J.P. Selecta, s.a., Barcelona, Spain) and filtered through 0.20 μm filters (Econofilters, Agilent Technologies, Darmstadt, Germany) before use.

2.3. CE apparatus and conditions

The experiments were performed using an HP^{3D} CE (Agilent Technologies, Waldbronn, Germany) equipped with an online diode-array UV detector (DAD) which was operated at a wavelength of 298 nm, an optimum wavelength for the target analytes, as determined using 'Isoabsorbance' and '3D' plots in the instrument's 'Data Analysis' software (Agilent Technologies, Waldbronn, Germany). Separations were achieved using uncoated fused-silica capillaries (Agilent Technologies, USA) of 75 μm i.d. and 48.5 cm total length with effective length to the detector of 40 cm. Injections were done at the anodic while detection was performed at the cathodic end of the capillary. For optimum resolution and efficiency, capillary temperature was maintained at 12 °C, separation voltage at 25 kV and a background electrolyte (BGE, 25 mM borate buffer at pH 9.2 containing 5.0% ACN, v/v) were used. The analytes, back-extracted into BES (50 mM sodium hydroxide solution, pH 12.7), were injected for 5 s at 50 mbar. With this BGE composition, a 40-cm effective capillary length was sufficient to obtain a baseline resolution of all peaks within acceptable analysis time. Under these conditions, the current was typically 110 μA.

New capillaries were successively flushed with DI water (10 min), 1.0 M sodium hydroxide (20 min), DI water (15 min) and finally with the BGE for 20 min. To ensure reproducibility, the capillary was flushed with the BGE (2 min) at the end of each run. The capillary was flushed for 10 min with DI water at the end of each working session and the capillary tips were kept inside DI water vials till the next working session.

Standard calibration graphs for capillary zone electrophoresis (CZE) without extraction were obtained by plotting peak areas ver-

sus concentrations of the analytes in working standard solutions prepared by diluting the mixed standard solutions in the BES.

2.4. Sample preparation

Breast milk samples were obtained from a healthy volunteer (28 years old, Ankara, Turkey) and food samples (tomato paste, pickle, mixed fruit juice and ice cream) were obtained from local markets (Ankara, Turkey). Dilution of the sample was adopted for tomato paste and pickle, whereas salting-out extraction (SOE) with ACN was applied for mixed fruit juice, ice cream and breast milk as described below.

2.4.1. Dilution

(a) Tomato paste: 0.5 (±0.01) g was accurately weighed and transferred into a glass test tube and mixed with DI water before being sonicated for 10 min at 60 °C and the volume was made up to 25 mL with DI water. A portion of 1.0 mL of this solution was used for DLLME.

(b) Pickle: a sample of 75.0 (±0.4) g was thoroughly blended using a kitchen blender with stainless steel blades and the volume was made up to 250 mL with DI water. The mixture was centrifuged for 10 min at 4000 rpm, filtered and 1.0 mL of the supernatant was directly subjected to DLLME.

2.4.2. Salting-out extraction (SOE)

In SOE, 1.0 mL of the sample was vortexed with 100 μL concentrated phosphoric acid for 10 s. Then, 1.5 mL ACN and 0.5 mL of saturated sodium chloride solution were added and the mixture was vortexed for 1 min and centrifuged for 3 min at 4000 rpm. An aliquot of 1.0 mL of the supernatant solution (i.e., ACN) was subjected to DLLME.

(a) Breast milk samples were frozen at −20 °C, thawed at room temperature, shaken and centrifuged for 10 min at 4000 rpm. An aliquot of 1.0 mL of the supernatant solution was pre-treated with SOE.

(b) Mixed fruit juice: the samples were shaken and centrifuged for 10 min at 4000 rpm. A portion of 1.0 mL of the supernatant solution was pre-treated with SOE.

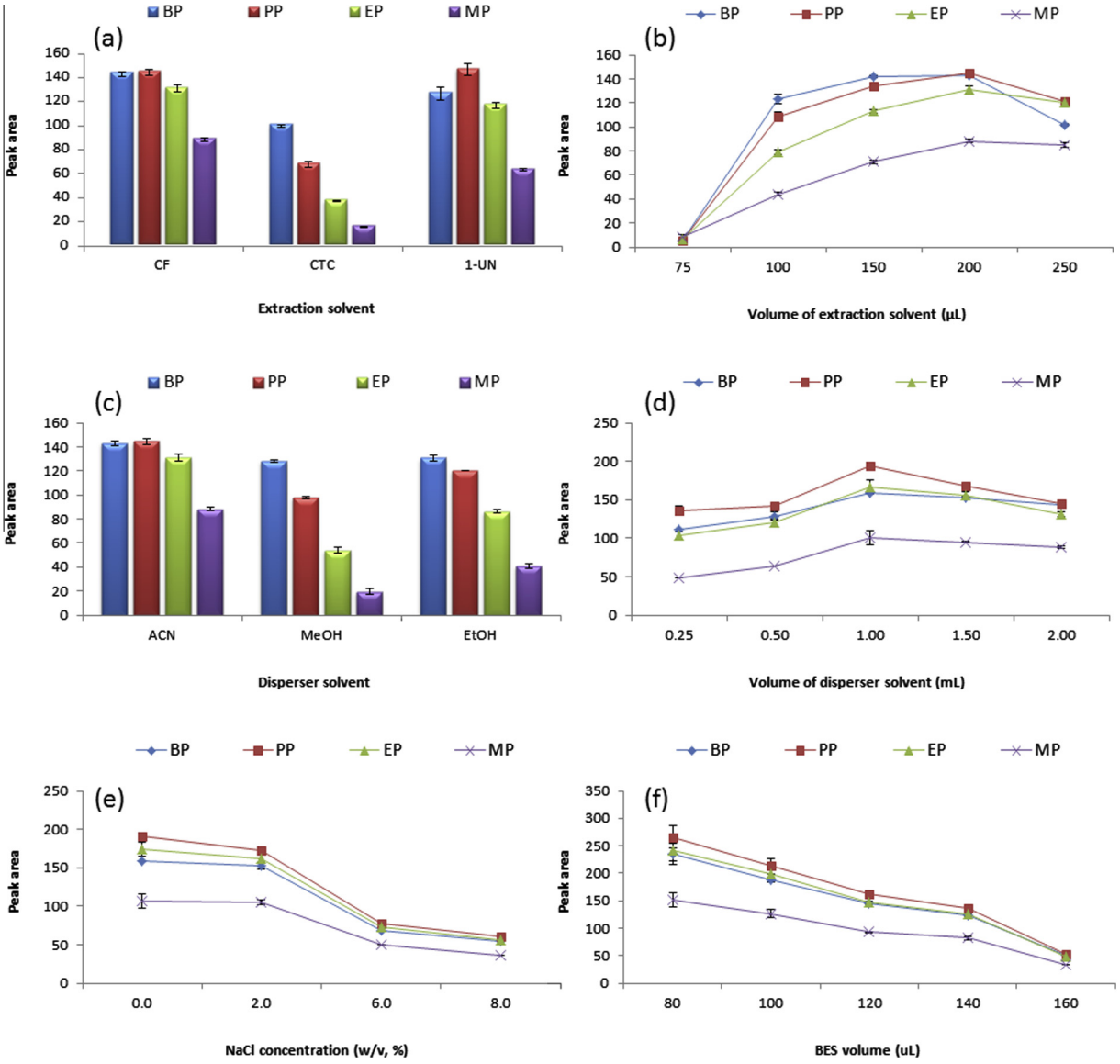

Fig. 1. Effect of experimental parameters on extraction efficiency of DLLME: (a) extraction solvent, (b) volume of extraction solvent, (c) disperser solvent, (d) volume of disperser solvent, (e) NaCl concentration, and (f) BES volume.

(c) Ice cream: 5.0 (±0.1) g were accurately weighed and homogenised in 5 mL DI water by vortexing for 1 min. This mixture was then centrifuged for 10 min at 4000 rpm and 1.0 mL of the supernatant solution was pre-treated with SOE.

Tomato paste, pickle, mixed fruit juice and ice cream samples were stored at 4 °C and analysed within one week of the time they were received. Breast milk was frozen, thawed and analysed within the same day of collection. For recovery studies, all samples were allowed to stand for 15 min after being spiked with the analytes for equilibration.

2.5. DLLME procedure

The DLLME procedure involved transferring 1.0 mL of the sample solution (tomato paste and pickle) or 1.0 mL ACN resulting from SOE (for breast milk, mixed fruit juice and ice cream) into a screw-cap 15-mL conical centrifuge graduated polypropylene test tube. Next, 100 μL concentrated phosphoric acid, 200 μL CF and 1.0 mL ACN (when not already present) were added. The volume was then made up to 8.0 mL with DI water and vortexed for 1 min, which resulted into the formation of a cloudy solution. The dispersed fine droplets of CF sedimented at the bottom of the test tube upon centrifugation (4000 rpm, 3 min) and were quantitatively transferred into a 1.0-mL snaplock microtube using a 100-μL HPLC syringe (Hamilton, USA). Finally, parabens were back-extracted into 80 μL of BES upon vortexing for 10 s and centrifugation (4000 rpm, 1 min) for direct injection into CE.

3. Results and discussion

3.1. Optimisation of CE conditions

Optimisation of CE conditions was performed with standard solutions prepared in BES (50 mM sodium hydroxide solution, pH 12.7). The evaluation was based on peak area, migration time, and resolution of the electrophoretic peaks. Although acetate (pH 4.2), phosphate (pH 7.0) and borate (8.3–9.8) were taken into consideration as the BGE, only with borate buffer was a baseline resolution of the four parabens achieved.

The acidity of the running buffer affects the zeta-potential, the electroosmotic flow (EOF) as well as the overall charge of the analytes, which determines the migration time and separation. The effect of the running buffer pH on the migration time of the analytes was investigated in the pH range of 8.3–9.8. Although peak area was not significantly affected, resolution and migration time increased as pH increased within this range. Therefore, as a compromise between resolution and migration time pH 9.3 was chosen.

The effect of borate buffer (pH 9.3) at different concentration levels (10–50 mM) was studied. Although it had a little effect on peak areas, increasing buffer concentration within this range improved resolution. Further increase in concentration above 25 mM increased the current above 130 μA and was thus avoided. Moreover, adding ACN (as an organic modifier) at different concentrations (2.5–10.0%, v/v) to the BGE was tested. Resolution improved upon the addition of up to 5.0% ACN where it started to decrease. Consequently, a 25 mM borate buffer of containing 5.0% ACN was selected, as this BGE provided the best separation and highest sensitivity within optimum migration time. A separation voltage of 25 kV and a temperature of 12 °C were found to give the lowest migration time while peak resolutions were higher than 2.

3.2. Sample pre-treatment

Due to the wide range of matrices investigated, two procedures were evaluated before applying DLLME. Dilution of the sample was used for tomato paste and pickle, whereas SOE with ACN was adopted for mixed fruit juice, ice cream and breast milk. From our previous experience with milk and dairy products (Alshana et al., 2013) and preliminary experiments of this study, SOE was considered as a better alternative of such samples in terms of protein precipitation, "clean" extracts and high extraction efficiency.

In SOE, different volumes of ACN (1.0–4.0 mL) were investigated for 1.0–3.0 mL sample. The use of 1.5 mL ACN for 1.0 mL sample resulted in the salt-induced phase separation of ca. 1.2 mL ACN, 1.0 mL of which was used for further experiments and gave the highest extraction efficiency among the other volumes investigated (data not shown). The amounts of salts (sodium chloride, magnesium sulphate and calcium chloride) and the corresponding signals obtained after extraction were compared. The largest peak areas for the four parabens were obtained with 0.5 mL of saturated solution of sodium chloride, such that this value was selected. Finally, extraction time of 1 min was chosen since no significant increase in the analytical signals occurred upon increasing the vortex-mixing time.

Table 1
Analytical performance parameters of CZE and DLLME–CE in aqueous standards.

Method	Paraben	Regression equation[a]	R^2	RSD (%, n = 3)		LOD[b] (μg mL^{-1})	LOQ[c] (μg mL^{-1})	LDR[d] (μg mL^{-1})	EF[e]
				Intraday	Interday				
CZE	MP	y = 1.77(±0.02) x + 2.45(±1.24)	0.9987	1.1	1.6	2.1	7.0	7.0–100	–
	EP	y = 2.03(±0.01) x + 1.39(±0.58)	0.9998	0.5	0.8	0.9	3.0	3.0–100	–
	PP	y = 1.79(±0.02) x + 2.35(±1.20)	0.9989	1.1	1.8	2.0	6.7	6.7–100	–
	BP	y = 1.68(±0.01) x + 0.23(±0.67)	0.9996	0.6	1.0	1.2	4.0	4.0–100	–
DLLME-CE after dilution	MP	y = 11.84(±0.24) x − 1.18(±0.82)	0.9959	2.0	3.3	0.2	0.7	0.7–6.0	6.7
	EP	y = 19.41(±0.21) x − 0.64(±0.71)	0.9988	1.1	1.7	0.1	0.3	0.3–6.0	9.6
	PP	y = 20.52(±0.14) x + 0.28(±0.45)	0.9995	0.7	1.0	0.1	0.3	0.3–6.0	11.5
	BP	y = 18.51(±0.12) x + 0.25(±0.39)	0.9996	0.6	1.0	0.1	0.3	0.3–6.0	11.0
DLLME-CE after SOE	MP	y = 8.23(±0.23) x − 0.21(±0.77)	0.9920	2.8	4.5	0.3	1.0	1.0–6.0	4.6
	EP	y = 14.89(±0.42) x − 0.01(±1.41)	0.9920	2.8	4.5	0.3	1.0	1.0–6.0	7.3
	PP	y = 16.38(±0.44) x + 0.17(±1.48)	0.9924	2.7	4.3	0.3	1.0	1.0–6.0	9.2
	BP	y = 14.74(±0.39) x + 0.46(±1.31)	0.9926	2.6	4.2	0.3	1.0	1.0–6.0	8.8

[a] Peak area = slope(±SD) × [paraben concentration (μg mL^{-1})] + intercept(±SD).
[b] Limit of detection.
[c] Limit of quantitation.
[d] Linear dynamic range.
[e] Enrichment factor: ratio of calibration slope with DLLME–CE to that with CZE.

Table 2
Analytical performance parameters of DLLME–CE in food samples.

Matrix	Paraben	Regression equation[a]	R^2	RSD (%, n = 3)		LOD[b] (μg mL^{-1})	LOQ[c] (μg mL^{-1})	LDR[d] (μg mL^{-1})	EF[e]
				Intraday	Interday				
Tomato paste	MP	y = 12.38(±0.09) x − 0.09(±0.29)	0.9995	0.7	1.1	0.1	0.3	0.3–6.0	7.0
	EP	y = 17.94(±0.11) x − 0.51(±0.33)	0.9997	0.6	0.9	0.1	0.3	0.3–6.0	8.8
	PP	y = 19.09(±0.22) x − 0.93(±0.67)	0.9990	1.2	1.7	0.1	0.3	0.3–6.0	10.7
	BP	y = 17.51 (±0.41)x − 1.43(±1.25)	0.9958	2.3	3.4	0.2	0.7	0.7–6.0	10.4
Pickle	MP	y = 10.88(±0.20) x − 0.93(±0.67)	0.9966	1.8	2.9	0.2	0.7	0.7–6.0	6.1
	EP	y = 17.47(±0.23) x − 1.08(±0.76)	0.9984	1.3	2.1	0.1	0.3	0.3–6.0	8.6
	PP	y = 18.04(±0.29) x − 1.49(±0.97)	0.9975	1.6	2.6	0.2	0.7	0.7–6.0	10.1
	BP	y = 15.40(±0.09) x − 0.11(±0.34)	0.9997	0.6	1.1	0.1	0.3	0.3–6.0	9.2
Breast milk	MP	y = 8.13(±0.06) x − 0.27(±0.21)	0.9996	0.7	1.2	0.1	0.3	0.3–6.0	4.6
	EP	y = 14.31(±0.31) x − 0.37(±0.44)	0.9993	1.5	2.2	0.1	0.3	0.3–6.0	7.0
	PP	y = 15.66(±0.08) x − 0.22(±0.27)	0.9998	0.5	0.8	0.1	0.3	0.3–6.0	8.7
	BP	y = 14.13(±0.04) x − 0.09(±0.13)	0.9999	0.3	0.4	0.1	0.3	0.3–6.0	8.4
Mixed fruit juice	MP	y = 9.08(±0.19) x − 1.08(±0.65)	0.9957	2.1	3.4	0.2	0.7	0.7–6.0	5.1
	EP	y = 16.50(±0.33) x − 1.52(±1.09)	0.9962	2.0	3.1	0.2	0.7	0.7–6.0	8.1
	PP	y = 18.57(±0.32) x − 1.23(±1.09)	0.9977	1.7	2.8	0.2	0.7	0.7–6.0	10.4
	BP	y = 17.18(±0.34) x − 1.16(±1.25)	0.9969	2.0	3.5	0.2	0.7	0.7–6.0	10.2
Ice cream	MP	y = 7.67(±0.13) x − 0.73(±0.43)	0.9974	1.7	2.7	0.2	0.7	0.7–6.0	4.3
	EP	y = 13.73(±0.25) x − 1.26(±0.83)	0.9969	1.8	2.9	0.2	0.7	0.7–6.0	6.8
	PP	y = 14.94(±0.25) x − 1.29(±0.83)	0.9974	1.7	2.6	0.2	0.7	0.7–6.0	8.3
	BP	y = 13.39(±0.25) x − 1.24(±0.83)	0.9967	1.9	3.0	0.2	0.7	0.7–6.0	8.0

[a] Peak area = slope(±SD) × [paraben concentration (μg mL^{-1})] + intercept(±SD).
[b] LOD: limit of detection.
[c] LOQ: limit of quantitation.
[d] Linear dynamic range.
[e] Enrichment factor: ratio of calibration slope with DLLME–CE to that with CZE.

3.3. Optimisation of DLLME conditions

DLLME parameters such as the type and volume of extraction and disperser solvents, ionic strength and extraction time were optimised. Peak areas, giving better precision than peak heights, were used to evaluate the impact of each parameter on extraction efficiency using the one-factor-at-a-time approach. All experiments were performed in triplicate and average values were considered for evaluation.

3.3.1. Type and volume of the extraction solvent

CF (density, d: 1.483 g mL^{-1}), CTC (d: 1.587 g mL^{-1}), 1-UN (d: 0.830 g mL^{-1}; melting point, m. p.: 13–20 °C) and 1-DO (d: 0.833 g mL^{-1}; m. p.: 24–27 °C) were investigated for selecting the extraction solvent. Initial experiments were performed using 2.0 mL of ACN as the disperser solvent and 200 μL of the extraction solvent in the absence of sodium chloride. With all of these extraction solvents, a stable cloudy solution formed. However, the use of 1-DO as the extraction solvent caused current-drop during electrophoretic separation. CF, giving the highest extraction efficiency among the other investigated solvents for the four parabens (Fig. 1a), was selected as the extraction solvent.

To evaluate the effect of extraction solvent volume, different volumes of CF (75, 100, 150, 200 and 250 μL) used with a constant volume of ACN (2.0 mL) were subjected to the same DLLME procedure. Fig. 1b shows the graphs of peak areas versus volume of extraction solvent. By increasing the CF volume from 75 to 200 μL, peak areas increased and then decreased afterwards. Therefore, 200 μL of CF was selected as the optimal solvent extraction volume. It is worthy to note that the collected volume of CF after extraction was 200 ± 10 μL (n = 15).

3.3.2. Type and volume of the disperser solvent

Experiments for choosing the disperser solvent were performed using 2.0 mL of ACN, MeOH and EtOH. Among these, ACN was found to provide the highest extraction efficiency (Fig. 1c) owing to the synergic effect of good compatibility of ACN with aqueous solutions and the low distributive ratio of analytes in mixtures of ACN and water (Xiong, Ruan, Cai, & Tang, 2009). Hence, ACN was chosen as the disperser solvent in the following experiments.

Different volumes of ACN (0.25, 0.50, 1.00, 1.50 and 2.00 mL) in the presence of a constant volume of 200 μL CF were tested. It was observed that with 0.25 mL of ACN, the cloudy state was not formed well and extraction was disturbed. The results showed that extraction efficiency increased up to 1.00 mL of the disperser volume (Fig. 1d) and decreased thereafter which was thought to be due to higher solubility of parabens in the aqueous phase in the presence of high volumes of ACN. As a result, 1.00 mL of ACN was selected as the optimum disperser volume.

3.3.3. Ionic strength and extraction time

The experimental results shown in Fig. 1e, indicated that extraction efficiency decreased with increasing sodium chloride concentration from 0% to 8.0% (w/v). It was also noted that the cloudy state did not form well when 8.0% and above were added to the sample solution due to the decrease of ACN miscibility with water. Consequently, no salt was added in subsequent experiments.

The effect of extraction time was examined in the range of 0–3 min with the other experimental conditions kept constant. Calculations indicated that extraction time did not have any significant influence on the signals of the analytes (data not shown). An extraction time of 1 min was used, however, to ensure good reproducibility in further experiments.

3.4. Optimisation of back-extraction conditions

The effect of BES volume on the extraction efficiency was studied over the range of 80–160 μL. Extraction efficiency decreased gradually upon increasing BES volume due to dilution of the analytes (Fig. 1f). Nevertheless, lower volumes than 80 μL could not be used due to loss of phase separation. Hence, subsequent experiments were performed using 80 μL as the BES volume. The effect of

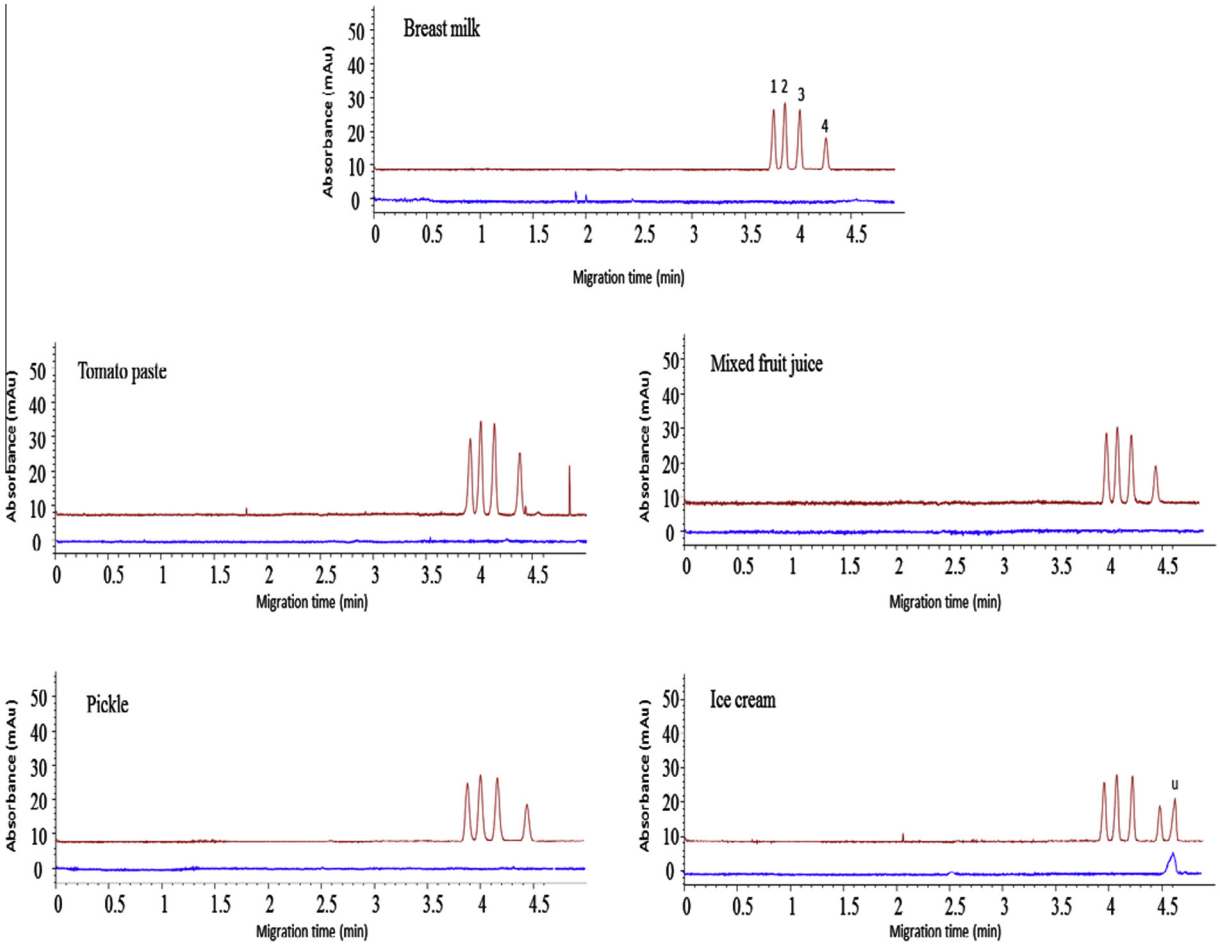

Fig. 2. Representative electropherograms with DLLME–CE. Top: sample spiked at 3.0 μg mL^{-1} of each paraben; bottom: unspiked sample. Peaks: 1, BP; 2, PP; 3, EP; 4, MP; and u, unknown peak.

back-extraction time on efficiency was also investigated and was found to have no effect. A back-extraction time of 10 s was chosen in further experiments and gave good reproducibility.

3.5. Analytical performance

Standard calibration graphs for CZE without DLLME were obtained by plotting peak areas versus concentrations of the analytes in working standard solutions (Section 2.3). Under optimised CZE conditions, LODs (calculated based on $3S_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the regression equations), ranged from 0.9 to 2.1 μg mL^{-1} (Table 1) and limits of quantitation (LOQ, based on $10S_b/m$) ranged from 3.0 to 7.0 μg mL^{-1}.

To evaluate the efficiency of the two sample pre-treatment steps used before DLLME, 1.0 mL of standard aqueous solutions at concentrations up to 6.0 μg mL^{-1} was treated by dilution to 8.0 mL with DI water or by SOE as described in Section 2.4 for the other samples. As shown in Table 1, DLLME after dilution resulted in enrichment factors (EF, calculated as the ratio of calibration slope with DLLME to that with CZE) of 6.7–11.5 while with SOE, they were 4.6–9.2. Expectedly, lower EFs were obtained with the latter method due to the loss of analytes in the ACN portion that remained in the aqueous solution. Nonetheless, attempts to completely collect ACN after SOE resulted in "unclean" extracts.

To examine the performance of the proposed DLLME–CE method with real food samples, matrix-matched calibration graphs were constructed by spiking sample solutions with appropriate amounts of a mixed standard solution of the target analytes. A series of samples containing a mixture of the four parabens at five concentration levels of 0.0, 1.5, 3.0, 4.5 and 6.0 μg mL^{-1} was used. The samples were then subjected to the DLLME procedure optimised above. For each level, triplicate extractions were performed and average peak areas were used for quantification. Regression equations, coefficients of determination (R^2), precision in terms of intraday and interday percentage relative standard deviation (%RSD), LODs, LOQs, linear dynamic ranges (LDR) and EFs as compared to CZE are summarised in Table 2.

The response was linear over the concentration range from their corresponding LOQs up to 6.0 μg mL^{-1} for all analytes, with R^2 ranging from 0.9957 to 0.9999. LODs ranged between 0.1 and 0.2 μg mL^{-1} and LOQs between 0.3 and 0.7 μg mL^{-1}. Reproducibility of the proposed method was evaluated in terms of intraday and interday precision, by extracting the spiked samples at the five concentration levels of the calibration graphs for each paraben in the same day and in three consecutive days, respectively. An acceptable precision was obtained in all cases with %RSD values below 2.3% for intraday and 3.5% for interday experiments. EFs with DLLME–CE as compared to CZE (Table 1) were in the range of 4.3–10.7 (Table 2).

3.6. Matrix effect and recovery studies

For evaluating the applicability, recovery and possible matrix effect of the proposed DLLME–CE method, four types of foodstuff (tomato paste, mixed fruit juice, pickle and ice cream) and human

Table 3
Relative recoveries of parabens from milk and food samples.

Matrix	Added (μg mL^{-1})	Found (μg mL^{-1}, ±SD, n = 3)				%RR[a]			
		MP	EP	PP	BP	MP	EP	PP	BP
Tomato paste	–	<LOD	<LOD	<LOD	<LOD	–	–	–	–
	3.00	3.00 (±0.02)	2.90 (±0.02)	2.90 (±0.03)	2.80 (±0.06)	100.0	96.7	96.7	93.3
	6.00	5.90 (±0.04)	6.00 (±0.04)	6.00 (±0.07)	6.10 (±0.14)	98.3	100.0	100.0	101.7
Pickle	–	<LOD	<LOD	<LOD	<LOD	–	–	–	–
	3.00	2.90 (±0.05)	2.90 (±0.04)	3.00 (±0.05)	3.00 (±0.02)	96.7	96.7	100.0	100.0
	6.00	6.10 (±0.11)	6.10 (±0.08)	6.10 (±0.10)	6.00 (±0.04)	101.7	101.7	101.7	100.0
Breast milk	–	<LOD	<LOD	<LOD	<LOD	–	–	–	–
	3.00	2.70 (±0.02)	2.80 (±0.04)	2.80 (±0.01)	2.90 (±0.01)	90.0	93.3	93.3	96.7
	6.00	6.00 (±0.04)	6.10 (±0.09)	6.00 (±0.03)	6.00 (±0.02)	100.0	101.7	100.0	100.0
Mixed fruit juice	–	<LOD	<LOD	<LOD	<LOD	–	–	–	–
	3.00	2.70 (±0.06)	2.60 (±0.05)	2.60 (±0.04)	2.60 (±0.05)	90.0	86.7	86.7	86.7
	6.00	6.00 (±0.13)	6.00 (±0.12)	6.00 (±0.10)	6.10 (±0.12)	100.0	100.0	100.0	101.7
Ice cream	–	<LOD	<LOD	<LOD	<LOD	–	–	–	–
	3.00	2.90 (±0.05)	2.90 (±0.05)	2.90 (±0.05)	2.80 (±0.05)	96.7	96.7	96.7	93.3
	6.00	6.10 (±0.10)	6.20 (±0.11)	6.10 (±0.10)	6.20 (±0.12)	101.7	103.3	101.7	103.3

[a] % Relative recovery, percentage value obtained considering extraction yields from matrix-matched calibrations.

Table 4
Comparison of DLLME–CE with other methods for the determination of parabens in food samples.

Analytes	Extraction method/technique	Extraction time (min)	V_{org}[a] (mL)	LOD[b] (μg mL^{-1})	LOQ[c] (μg mL^{-1})	LDR[d] (μg mL^{-1})	R^2	EF	%RR[e]	%RSD[f]	Refs.
EP, PP, BP	SBSE–GC–MS[g]	120	–	0.015–0.020	–	0.1–100	>0.9984	–	95–105	<4.2	Ochiai et al. (2002)
MP, EP, PP, BP	USE-UHPLC–ESI-MS/MS[h]	20	~50	0.01–0.08 (ng g^{-1})	0.03–0.20 (ng g^{-1})	0.10–20.00 (ng mL^{-1})	>0.9990	–	89.41–99.30	<3.16	Lv et al. (2012)
MP, EP, PP	DLLME–GC–FID[i]	10	~6	0.005–0.015	0.02–0.05	0.02–30	>0.992	100–276	25–72	<3	Farajzadeh et al. (2010)
MP, EP, PP, BP	DLLME–GC–FID	1	250	0.029–0.102	0.095–0.336	0.1–10	>0.9913	–	81.56–101.4	<6.86	Jain et al. (2013)
MP, EP, PP, BP	DLLME–CE	1–2	1.2–1.7	0.1–0.2	0.3–0.7	0.3–6.0	>0.9957	4.3–10.7	86.7–103.3	<3.5	This study

[a] Total volume of organic solvents consumed per sample.
[b] Limit of detection.
[c] Limit of quantitation.
[d] Linear dynamic range.
[e] % Relative recovery.
[f] % Relative standard deviation.
[g] Stir-bar sorptive extraction–gas chromatography–mass spectrometry.
[h] Ultrasound-assisted extraction-ultra-high-performance liquid chromatography with electrospray ionisation tandem mass spectrometry.
[i] Dispersive liquid–liquid microextraction–gas chromatography–flame ionisation detector.

breast milk were examined. Typical electropherograms of unspiked and spiked samples are shown in Fig. 2. Absence of interfering peaks at the migration times of parabens indicated good selectivity of the method. Furthermore, the four analytes were baseline separated in less than 4.5 min. The recoveries obtained for unspiked and spiked samples at two concentration levels (3.0 and 6.0 μg mL^{-1} of each paraben) are listed in Table 3. Accordingly, the recoveries obtained from matrix-matched calibrations were in the ranges of 86.7–103.3. Matrix effect was checked by comparing the slopes of calibration graphs (Table 2). Different slopes indicated the presence of matrix effect which was linked to the large difference in the contents of the samples. However, this matrix effect was eliminated by using matrix-matched calibrations as can be inferred from good % relative recovery (RR) values (Table 3).

3.7. Comparison with other preconcentration methods

Efficiency of the presented DLLME–CE method for the selected parabens was compared with other reported methods for parabens in food considering aspects such as extraction time, total volume of organic solvents consumed per sample, LOD, LOQ, LDR, R^2, EF, %RR and precision. In comparison with other methods, the main advantages of the proposed method were rapidness, simplicity and requirement of the least amount of organic solvents for analysis. As listed in Table 4, the extraction time was only 1–2 min in this study (including SOE and back-extraction), which was due to the infinitely large surface area of contact between the extraction solvent and the sample solution during emulsion formation. Other methods such as stir-bar sorptive extraction (SBSE) (Ochiai et al., 2002) and ultrasound-assisted extraction (USE) (Lv, Wang, Hu, Tai, & Yang, 2012) required much longer time for equilibrium to be established. The DLLME method proposed by Jain et al. (2013) was also fast but the volume of organic solvent was high (i.e., 250 mL), whereas in the proposed method, 1.2–1.7 mL (including SOE) were only required. LODs and LOQs were comparable with those obtained with the others but higher than USE-UHPLC–ESI-MS/MS. A narrower LDR when compared with FID and MS detectors was obtained as it is well known that these detectors have wider linear range with respect to absorption-type detectors. MS–MS detector is inherently more sensitive than UV but it is much more expensive, complicated and still not affordable by many analytical laboratories. Precision of the proposed method was also comparable with the others.

4. Conclusion

In this study, the applicability of DLLME followed by a back-extraction step as an efficient sample clean-up and preconcentration technique of parabens in human milk and other complex foodstuff samples prior to CE was successfully illustrated. A combination of DLLME and CE for the determination of the most widely used four parabens in these matrices was demonstrated to enhance the sensitivity of CE by 4.3–10.7 times resulting in LODs as low as 0.1 μg mL^{-1}. As compared with other sample preparation techniques, the proposed method offers numerous advantages, such as rapidity, use of minimum amounts of organic solvents, consequently less organic waste, simplicity, low cost, ease of operation, and high selectivity. Despite the complexity of the matrices studied, good recoveries, high reproducibility and interference-free electropherograms were achieved in all cases. Applicability of DLLME–CE to a wide range of food matrices including human milk without a considerable variation in the pre-treatment and extraction procedures indicates that this method could be of great interest for the determination of parabens in foodstuff and human breast milk in routine analytical laboratories.

Conflict of Interest

The authors have declared no conflict of interest.

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References

Alshana, U., Goger, N. G., & Ertas, N. (2013). Dispersive liquid–liquid microextraction combined with field-amplified sample stacking in capillary electrophoresis for the determination of non-steroidal anti-inflammatory drugs in milk and dairy products. *Food Chemistry, 138*(2–3), 890–897.

Angelov, T., Vlasenko, A., & Tashkov, W. (2008). HPLC determination of pKa of parabens and investigation on their lipophilicity parameters. *Journal of Liquid Chromatography & Related Technologies, 31*(2), 188–197.

Barr, L., Metaxas, G., Harbach, C. A. J., Savoy, L. A., & Darbre, P. D. (2012). Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum. *Journal of Applied Toxicology, 32*(3), 219–232.

Blanco, M., Coello, J., Iturriaga, H., Maspoch, S., & Romero, M. A. (2001). Resolution of isomers of sorbitolparaben esters by chromatographic and electrophoretic techniques. *Journal of Chromatography B, 752*(1), 99–105.

Cabuk, H., Akyuz, M., & Ata, S. (2012). A simple solvent collection technique for a dispersive liquid–liquid microextraction of parabens from aqueous samples using low-density organic solvent. *Journal of Separation Science, 35*(19), 2645–2652.

Canosa, P., Rodriguez, I., Rubi, E., Bollain, M. H., & Cela, R. (2006). Optimisation of a solid-phase microextraction method for the determination of parabens in water samples at the low ng per litre level. *Journal of Chromatography A, 1124*(1–2), 3–10.

Cheng, Y.-C., Wang, C.-C., Chen, Y.-L., & Wu, S.-M. (2012). Large volume sample stacking with EOF and sweeping in CE for determination of common preservatives in cosmetic products by chemometric experimental design. *Electrophoresis, 33*(9–10), 1443–1448.

Darbre, P. D., Aljarrah, A., Miller, W. R., Coldham, N. G., Sauer, M. J., & Pope, G. S. (2004). Concentrations of parabens in human breast tumours. *Journal of Applied Toxicology, 24*(1), 5–13.

Darbre, P. D., & Harvey, P. W. (2014). Parabens can enable hallmarks and characteristics of cancer in human breast epithelial cells: A review of the literature with reference to new exposure data and regulatory status. *Journal of Applied Toxicology, 34*(9), 925–938.

Farajzadeh, M. A., Djozan, D., & Bakhtiyari, R. F. (2010). Use of a capillary tube for collecting an extraction solvent lighter than water after dispersive liquid–liquid microextraction and its application in the determination of parabens in different samples by gas chromatography-flame ionization detection. *Talanta, 81*(4–5), 1360–1367.

Gao, W., & Legido-Quigley, C. (2011). Fast and sensitive high performance liquid chromatography analysis of cosmetic creams for hydroquinone, phenol and six preservatives. *Journal of Chromatography A, 1218*(28), 4307–4311.

Herrera-Herrera, A. V., Hernandez-Borges, J., Borges-Miquel, T. M., & Rodriguez-Delgado, M. A. (2010). Dispersive liquid–liquid microextraction combined with nonaqueous capillary electrophoresis for the determination of fluoroquinolone antibiotics in waters. *Electrophoresis, 31*(20), 3457–3465.

Jain, R., Mudiam, M. K. R., Chauhan, A., Ch, R., Murthy, R. C., & Khan, H. A. (2013). Simultaneous derivatisation and preconcentration of parabens in food and other matrices by isobutyl chloroformate and dispersive liquid–liquid microextraction followed by gas chromatographic analysis. *Food Chemistry, 141*(1), 436–443.

Lv, J., Wang, L., Hu, X., Tai, Z., & Yang, Y. (2012). Rapid determination of 10 parabens in spices by high performance liquid chromatography–mass spectrometry. *Analytical Letters, 45*(14), 1960–1970.

Maijo, I., Borrull, F., Aguilar, C., & Calull, M. (2013). Different strategies for the preconcentration and separation of parabens by capillary electrophoresis. *Electrophoresis, 34*(3), 363–373.

Moradi, M., & Yamini, Y. (2012). Application of vesicular coacervate phase for microextraction based on solidification of floating drop. *Journal of Chromatography A, 1229*, 30–37.

Ochiai, N., Sasamoto, K., Takino, M., Yamashita, S., Daishima, S., Heiden, A. C., et al. (2002). Simultaneous determination of preservatives in beverages, vinegar, aqueous sauces, and quasi-drug drinks by stir-bar sorptive extraction (SBSE) and thermal desorption GC–MS. *Analytical and Bioanalytical Chemistry, 373*(1–2), 56–63.

Rezaee, M., Assadi, Y., Hosseinia, M. R. M., Aghaee, E., Ahmadi, F., & Berijani, S. (2006). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A, 1116*(1–2), 1–9.

Rezaee, M., Yamini, Y., & Faraji, M. (2010). Evolution of dispersive liquid–liquid microextraction method. *Journal of Chromatography A, 1217*(16), 2342–2357.

Soni, M. G., Burdock, G. A., Taylor, S. L., & Greenberg, N. A. (2001). Safety assessment of propyl paraben: A review of the published literature. *Food and Chemical Toxicology, 39*(6), 513–532.

Tsai, T. F., & Lee, M. R. (2008). Determination of antioxidants and preservatives in cosmetics by SPME combined with GC–MS. *Chromatographia, 67*(5–6), 425–431.

Wen, Y. Y., Li, J. H., Zhang, W. W., & Chen, L. X. (2011). Dispersive liquid–liquid microextraction coupled with capillary electrophoresis for simultaneous determination of sulfonamides with the aid of experimental design. *Electrophoresis, 32*(16), 2131–2138.

Xiong, C., Ruan, J., Cai, Y., & Tang, Y. (2009). Extraction and determination of some psychotropic drugs in urine samples using dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 49*(2), 572–578.

Xue, Y., Chen, N., Luo, C., Wang, X., & Sun, C. (2013). Simultaneous determination of seven preservatives in cosmetics by dispersive liquid–liquid microextraction coupled with high performance capillary electrophoresis. *Analytical Methods, 5*(9), 2391–2397.

Ye, X. Y., Bishop, A. M., Needham, L. L., & Calafat, A. M. (2008). Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk. *Analytica Chimica Acta, 622*(1–2), 150–156.

Ye, N., Shi, P., Li, J., & Wang, Q. (2013). Application of graphene as solid phase extraction absorbent for the determination of parabens in cosmetic products by capillary electrophoresis. *Analytical Letters, 46*(13), 1991–2000.

Zotou, A., Sakla, I., & Tzanavaras, P. D. (2010). LC-determination of five paraben preservatives in saliva and toothpaste samples using UV detection and a short monolithic column. *Journal of Pharmaceutical and Biomedical Analysis, 53*(3), 785–789.