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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-