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Matrix solid-phase dispersion combined to liquid chromatography–tandem mass spectrometry for the determination of paraben preservatives in mollusks

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ABSTRACT

A method for the extraction and determination of seven parabens, esters of 4-hydroxybenzoic acid, widely used as preservatives in personal care products, pharmaceuticals, etc., and two chlorinated derivatives (mono- and di-chloro methyl paraben) from mollusk samples was developed by combining matrix solid-phase dispersion (MSPD) and liquid chromatography–tandem mass spectrometry. MSPD parameters, such as solvent, solid support and clean-up sorbent, were optimized. Besides, since blank problems were observed for some parabens, these were investigated and blanks were tackled by precleaning all sorbents prior to use. Under final conditions, 0.5 g of freeze-dried mollusk were dispersed with 1.2 g of silica and packed into a cartridge containing 3 g of C18, as on-line clean-up sorbent. This cartridge was eluted with 10 mL of acetonitrile, evaporated and reconstituted in methanol for analysis. In the validation stage, successful linearity ($R^2 > 0.999$), recoveries (between 71 and 117% for most analytes), precision (RSD lower than 21%) and limits of detection and quantification (LOD and LOQ, lower than 0.4 and 1.4 ng g^{-1} dry weight respectively) levels were achieved. Finally, the new methodology was applied to mussel, clam and cockle samples. Methyl paraben was above the LOQ in five of the six samples (not found in one clam sample) at concentrations up to 7 ng g^{-1} dry weight. Ethyl paraben was found above the LOQ in mussel and cockle samples at a concentration level around 0.3 ng g^{-1}. *n*-Propyl paraben was only above the LOQ in one mussel sample.

1. Introduction

Parabens are among the main classes of personal care products (PCPs) [1]. They are esters of *p*-hydroxybenzoic acid used as antimicrobial preservatives in cosmetics, toiletries, pharmaceuticals and food. There are seven different parabens in use differing in the ester group, i.e. methyl- (MeP), ethyl- (EtP), *n*-propyl- (*n*-PrP), *iso*-propyl- (*i*-PrP), *n*-butyl- (*n*-BuP), *iso*-butyl (*i*-BuP) and benzyl- (BzP) paraben [1]. Among them, MeP and *n*-PrP are the most widely used in cosmetics, and are typically co-applied to increase preservative effects [2]. In European Union (EU) countries, the maximum allowable concentrations of parabens in cosmetic products is 0.4% for each single ester and 0.8% for mixtures of parabens [3]. However, the EU Scientific Committee on Consumer Safety (SCCS) has recommended lowering the limits of *n*-PrP and *n*-BuP to a maximum total concentration in cosmetics of 0.19% (as ester) [4]. Moreover, a draft of an actualization of the Regulation (EC) 1223/2009, based on those SCCS considerations established that some parabens such as *i*-PrP, *i*-BuP or BzP should not be used as preservatives in cosmetics [5]. In the United States of America (USA) there is no legislation regulating concentrations of parabens in cosmetics but the same threshold as the EU has been recommended by the Food and Drug Administration (FDA) [6]. On the other hand, parabens are classified as "generally regarded as safe" (GRAS) substances and approved for use in foods by USA FDA and the EU regulations [7].

The discussion on paraben safety is ongoing [8–11]. The main concern arises from their endocrine disrupting potential. In general, acute and chronic (eco)toxicity of parabens increases in the following order: MeP < EtP < PrP < BuP < BzP [12–14]. Moreover, at concentrations as low as those found in tap water, chlorine can react with parabens to produce chlorinated derivatives [15] which are considerably more toxic to aquatic organisms than the parent compounds [16].

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Parabens, as all PCPs, are products intended for external use on the human body and thus are not subjected to metabolic alterations. Therefore, large quantities of them enter the environment unaltered through regular usage [17]. Many of these compounds are used in large quantities, and recent studies have indicated that many are environmentally persistent, bioactive, and have the potential for bioaccumulation [2,18]. The main point sources of pollution are wastewater treatment plants (WWTPs). Thus, parabens were detected in several matrices throughout the world, such as surface [19–22] and drinking water [21,23,24], sediments and soils [25,26], and biota [27–29]. However, data concerning their presence in aquatic organism are scarce. Chlorinated derivatives of parabens have also been found in some environmental matrices, such as wastewater [30] and sludge [26], but data about the presence of these chlorinated transformation products in environmental samples is still scarce and the occurrence in biota samples has not been investigated yet.

Until today, no analytical methods have been developed for mollusks, while analytical procedures applied to the determination of parabens in fish samples are based on accelerated solvent extraction [31–33], high speed solvent extraction [28,29] or sonication [34]. Nevertheless, extracts from such complex fish matrices also require an additional clean-up step, since they produce high lipid content extracts which may interfere with chromatographic analysis. The most often applied purification step includes solid-phase extraction using a silica gel column [28,29,33] or gel permeation chromatography (GPC) [33,34]. Alternatively, matrix solid-phase dispersion (MSPD) has been successfully applied to the determination of parabens in sewage sludge [26] and dust [35] samples. MSPD requires less sample intake and solvent consumption and incorporates the extraction and clean-up in a single step with a notable simplification of the process, as shown by some previous applications to fish/mollusk analysis in the literature [36–39].

The aim of this study is the development of a new and simplified method for the extraction and determination of nine parabens (i.e. MeP, EtP, *n*-PrP, *i*-PrP, *n*-BuP, *i*-BuP, BzP and mono- and dichlorinated MeP) in mollusk samples (clams, mussels and cockles) using MSPD as sample preparation technique followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

2. Experimental

2.1. Chemicals

MeP, EtP, *n*-PrP, *n*-BuP, and BzP were purchased from Aldrich (Milwaukee, WI, USA). *i*-PrP and *i*-BuP were obtained from TCI Europe (Zwijndrecht, Belgium). Chlorinated derivatives of MeP: 3-chloro- and 3,5-dichloro-methyl paraben (ClMeP and Cl_2MeP, respectively) were obtained from ABCR GmbH (Karlsruhe, Germany). As surrogate internal standards (IS), the deuterium - labelled isotopic derivatives methyl 4-hydroxybenzoate-2,3,5,6-d_4 (MeP-d_4) and *n*-propyl 4-hydroxybenzoate-2,3,5,6-d_4 (*n*-PrP-d_4) were obtained from CDN Isotopes (Quebec, Canada). Stock solutions of each compound (~3000 μg mL^{-1}) and mixtures of all of them or their isotopically labelled analogues (20 μg mL^{-1}) were prepared in methanol and stored at −20 °C at the dark until use.

Methanol (MeOH), acetonitrile (ACN), ethyl acetate (AcOEt), diethylether and *n*-hexane (all of chromatographic analysis grade) were purchased from Merck (Darmstadt, Germany), and dichloromethane (DCM) from VWR Prolabo (Fontenay-sous-Bois, France). Alumina, florisil, diatomaceous earth, sea sand (50–70 mesh) and octadecyl-functionalized silica (C18) were provided by Sigma-Aldrich (Steinheim, Germany). Primary-secondary amine bonded silica (PSA) was acquired from Supelco (Bellefonte, PA, USA) and silica was obtained from Merck. Ultrapure water was obtained from a Milli-Q water purifier (18.2 MΩ cm^{-1}, Millipore, Billerica, MA, USA) in the laboratory. Silica and C18 were cleaned with 40 mL of ACN before use.

Empty polypropylene syringes (10 mL capacity) and 20 μm polyethylene frits were purchased from Biotage (Uppsala, Sweden).

2.2. Samples

Mussels (*Mytilus galloprovincialis*), manila clams (*Ruditapes philippinarum*) and cockles (*Cerastoderma edule*) were collected on the northern coast of Spain during 2014. All samples were collected, homogenized and freeze-dried by the Technological Institute for the Monitoring of the Marine Environment in Galicia (INTECMAR) before arriving into our laboratory and stored in amber glass bottles previously rinsed and wrapped with aluminum foil until chemical analysis. INTECMAR is the official organization controlling the quality of shellfish in the region and has all the legal permissions to sample these aquatic organisms.

2.3. Liquid chromatography–tandem mass spectrometry

The LC consisted of two ProStar 210 high-pressure mixing pumps (Varian, Walnut Creek, CA, USA), a Metachem Technologies vacuum membrane degasser (Bath, UK), an autosampler and a thermostatted column compartment ProStar 410 module (Varian).

A Luna C18 (2) column (100 mm × 2.0 mm, 3.2 μm particle diameter; 100 Å pore size) (Phenomenex, USA), maintained at 45 °C, was employed, with sample injection volume set to 10 μL. The flow rate employed to achieve a successful separation of target analytes was 0.2 mL min^{-1} using 5 mM of ammonium acetate in both Milli-Q water (A) and MeOH (B) as eluents. The applied binary gradient starts with a linear gradient from 40 to 65% B for 25 min, raised to 100% B in 0.5 min and kept constant until 28 min, followed by a last linear gradient for 2 min until 40% B kept constant until 33 min, for column back-conditioning.

The LC was coupled to a triple quadrupole mass spectrometer (Varian 340-MS) which incorporates an electrospray interface (ESI). Nitrogen, used as nebulizing and drying gas, was provided by a nitrogen generator (Domnick Hunter, Durham, UK). Argon (99.999%) was used as collision gas. Instrument control and data acquisition were performed with the Varian MS Workstation 6.9 software.

The MS was operated in the negative ESI mode. The ionization source working parameters were as follows: needle voltage −4000 V, ionization source temperature 50 °C, drying gas temperature (N_2) 200 °C, nebulizer gas pressure (N_2) 55 psi, drying gas pressure (N_2) 18 psi, collision gas pressure (Ar) 1.5 mTorr and centroid mode for acquisition.

Determination of all compounds was performed by recording two transitions for each analyte in the multiple reaction monitoring (MRM) mode. Specific ESI–MS/MS parameters for each analyte are listed in Table 1.

2.4. Matrix solid-phase dispersion

Under optimal conditions, 0.5 g of freeze-dried mollusk sample spiked with the IS (5 ng) was thoroughly homogenized in a glass mortar with 1.2 g of cleaned silica, used as solid support (dispersing agent). A 10 mL syringe barrel, containing a frit at the bottom, was filled with 3 g of C18 (as clean-up sorbent) followed by the homogenized sample and finally a second frit. Then, analytes were eluted with 10 mL of ACN. The eluate was concentrated to dryness under a nitrogen stream. Finally, the extract was reconstituted to a final volume of 100 μL in MeOH, being ready for LC–MS/MS analysis.