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Hollow fibre supported liquid membrane extraction for BTEX metabolites analysis in human teeth as biomarkers

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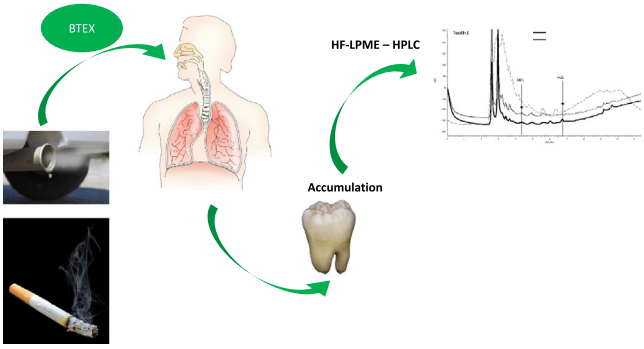
HIGHLIGHTS

- A simple method for BTEX and their metabolites in dental tissue is presented.
- The optimised HF-LPME method minimizes the use of solvents in comparison to other procedures.
- Extraction efficiencies depend on organic solvent, extraction time, stirring.
- Salt concentration in donor solution has a significant effect in extraction yield.
- The new method was applied to 8 real human samples.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of human teeth as biomarkers has been previously applied to characterize environmental exposure mainly to metal contamination. Difficulties arise when the contaminants are volatile or its concentration level is very low. This study presents the development of a methodology based on the transport through hollow fibre membrane liquid-phase microextraction (HF-LPME), followed by HPLC-UV measurement, to determine three different metabolites of BTEX contaminants, mandelic acid (MA), hyppuric acid (HA), and methylhippuric acid (4mHA). The driving force for the liquid membrane has been studied by using both non-facilitated (pH gradient 2–12) and facilitated transport (ionic and non-ionic carriers). Enrichment factors of several hundreds were accomplished. Different ionic and non-ionic water insoluble compounds were used as metabolite carriers for the facilitated transport at HF-LPME. Three organic solvents were used to constitute the liquid membrane, dodecane, dihexyl ether and *n*-decanol. Other parameters affecting the extraction process, such as extraction time, stirring speed, acceptor buffer and salt content were optimised in spiked solutions and selected those that presented the best enrichment factors for all analytes. Final conditions were established for donor solution as 20 mL, pH 2 of 0.5 M NaCl, the OLM (Organic Liquid Membrane) as *n*-decanol and the acceptor solution as 40 μL of 1 M NaOH. The selected extraction time was 20 h with stirring speed of 500 rpm. Validation of the optimised method included the determination of individual linearity range (MA: 0.002–5.7 μg; HA: 0.01–7.9 μg; 4mHA 0.002–5.3 μg), limits of detection (MA: 1.6 ng; HA: 0.2 ng; 4mHA 0.2 ng), repeatability (RSD 7–10%) and reproducibility (5–8%). The developed method was applied to the analysis of MA, HA and 4mHA in teeth samples of 8 workers exposed to BTEX.

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1. Introduction

Benzene, toluene, ethylbenzene and xylenes (BTEX) and their corresponding metabolites, such as mandelic acid (MA), hippuric acid (HA) and 4-methyl hippuric acid (4mHA) have been widely investigated, outstanding to the health risks associated with the occupationally BTEX exposure (Moon et al., 1997; Shen, 1998; Andreoli et al., 1999; Tolnai et al., 2000; Wang et al., 2003; Yimrungruang et al., 2008; Moolla et al., 2015), due to the high toxicity of these volatile organic compounds, which are easily absorbed via the lungs and the skin (Reese and Kimbrough, 1993). Mandelic and hippuric acids are aromatic carboxylic acids obtained by oxidation of the alkyl side chain of ethylbenzene, xylenes and toluene (Angerer and Hörsch, 1992). Their concentrations on biological samples have been used to know the degree of occupational or environmental BTEX exposure (Bolden et al., 2015).

Some analytical procedures consisting in the combination of a previous pre-concentration step followed by HPLC or capillary zone electrophoresis (ZCE) are used to monitor BTEX metabolites content in biological fluids, such as urine and blood of workers exposed to BTEX levels (Moon et al., 1997; Wang et al., 2003; Angerer and Schaller, 1988; Astier, 1992; Aranda-Rodriguez et al., 2015; Fustinoni et al., 2010; Hrivňák and Kráľovičová, 2009; Moro et al., 2010; Yang and Liu, 2010). Hollow fibre membrane liquid-phase microextraction (HF-LPME) in combination with HPLC or CZE is one of the most interesting pre-treatment method of complex samples in the environmental and bioanalytical applications (Lee et al., 2008; Dong et al., 2015; Goh and Lee, 2017), due to high enrichment, efficient sample clean-up, and the low consumption of organic solvent. This method consists in the extraction of target analytes from aqueous samples to an organic liquid membrane (OLM) supported in the wall of a small porous hollow fibre and later to an acceptor phase presents inside the lumen of hollow fibre. Several alternatives in membrane-based solvent extraction such as HF-LPME are widely applied for recovery and concentration of carboxylic acids from different matrixes (Schlosser et al., 2005), BTEX from water samples (Ma et al., 2011) and hippuric acid from urine (Bahrami et al., 2017).

In the literature, a recent method has been established for BTEX metabolites analysis in human teeth as biomarkers (González et al., 2017), which reported a comparison of different methodologies in the literature using SMPE for the BTEX analyses. Other applications found in the literature are approaches for the analysis of cumulative organic compounds (Murtomaa et al., 2007; Pascual et al., 2003; Marchei et al., 2008) and fluorine (Mehta, 2013).

The aim of the present study is to develop a method for simultaneous determination of MA, HA and 4mHA as BTEX metabolites in human teeth, to establish a capable and future tool for categorizing cumulative exposure to BTEX, and its relationship with dental health. For the first time, a HF-LPME extraction method followed by HPLC analysis was investigated in spiked solutions to obtain a methodology for determining the level of environmental exposure to BTEX in dental samples as new biomarkers. The investigation consisted in the optimisation of different parameters involved in the three-phase extraction procedure: type of organic solvent used as OLM, type of transport (non-facilitated and facilitated with carriers), extraction time, stirring speed, salinity and type of buffers as acceptor solutions. Finally, the validation of the HF-LPME-HPLC method was established and used to determine BTEX metabolites content (MA, HA, and 4mHA) in human teeth.

2. Experimental

2.1. Chemicals and samples

Standards and reagents: 98% mandelic acid (MA), >97% hippuric acid (HA), 98% 4-methylhippuric acid (4mHA), methyltrioctylammonium chloride (Aliquat 336), and organic solvents: HPLC grade methanol, >98% dodecane, >97% dihexyl ether, 97% *n*-decanol, 99% tributyl phosphate (TBP), 99% trioctylphosphine oxide (TOPO), 98% trioctylamine (TOA), were purchased from Sigma-Aldrich (Madrid, Spain). The salts and acids: 99.5% NaCl, 98% NaOH and 98% $Na_3PO_4.12H_2O$, 37% HCl and 85% H_3PO_4, were obtained from Panreac (Barcelona, Spain). Water was purified by a Milli-Q Plus system from Millipore (Milford, MA, USA).

A standard stock solution of each acid analytes (2000 mg L^{-1}) was prepared by dissolving 20 mg of standard in 10 mL of HPLC-grade methanol-water (1:1). A working solution (20 mg L^{-1}) containing the three analytes was prepared by mixing appropriate amount of the stock solution and diluting with water to a given volume. The standard stock solutions and working solution were stored at 4 °C. Twenty millilitres of donor sample solutions were prepared by taking appropriate amount of the working solution and diluting to the required volume of 0.5 M or 0.01 M NaOH to obtain 100 μg L^{-1} and then adjusting to expected pH value by drop wise adding concentrated hydrochloric acid. Acceptor solutions (1 M NaOH at pH 12, 1 M Na_3PO_4 at pH 11.7 or 2 M NaCl at pH 6.0) were prepared by dissolving appropriate amount in 1000 mL of water and adjusted to aforesaid pH.

Human teeth were collected, with patient's consent, at dental clinic of Drassanes in Barcelona between March and April 2009. Age, profession and smoking characteristic were registered for each donor. Individual human tooth was washed with water by the dentist after extraction, and placed in 20 mL glass vial containing 10 mL of 1 M NaOH solution. Each vial was sealed and ultrasonicated for 4 h as optimised extraction time of analytes from teeth (González et al., 2017). Then, 9 mL of water and 1 mL of concentrated hydrochloric acid were added to obtain the 20 mL samples at pH 2 for HF-LPME extraction procedure.

2.2. General extraction procedure

The extraction procedure followed was a slight modification from the described by Liu et al. (Liu et al., 2005), Berhanu et al. (Berhanu et al., 2006) and in our previous work (Chaieb et al., 2015). A hollow fibre (HF) Accurel® PP polypropylene Q3-2 (200-μm wall thickness, 600-μm inner diameter, 0.2 μm pore size; Membrana GmbH, Wuppertal, Germany), was cut manually into approximately 15 cm length (40-μL lumen) leaving both ends free for subsequent use. The impregnation of the fibre to form the OLM was established as follows: first, the lumen of a single hollow fibre was flushed and filled with the organic solvent using a syringe and the two ends were sealed with a small piece of silicone tubing. Next, the fibre was immersed in a vial with 20 mL of organic solvent and it was ultrasonicated for 5 min. After that, the two ends were open and the lumen was slowly flushed with 200 μL of distilled water to remove any organic solvent inside it. Then, the acceptor solution (300 μL) was flushed into the lumen to guaranty the complete filling of it, as well as the removal of any air bubbles. The two ends of the fibre were sealed again, soaked in distilled water for 1 min and this ready HF-LPME device was transferred to the 20 mL vial of donor sample solution. After the extraction, the acceptor solution, containing the analytes, was collected from the lumen into 2 mL vials with inserts of 250 μL. The sample was collected by opening one of the ends of the sealed fibre and connecting it to a retracted syringe needle. The other end was then opened and put into the vial. Next, the syringe plunger was pushed in, to dispense the acceptor solution containing the analyte into the vial to recover the 40 μL solution. Prior to HPLC analysis, 2–3 drops of 0.1 M HCl were added to adjust pH to 2. Finally, drops of water were added to reach a final volume of 100 μL in calibrated inserts vials. After capping, the vial was put on the autosampler of the HPLC system for the injection of 20 μL of each sample by duplicate. All HF-LMPE procedures were performed by triplicate.

2.3. Chromatographic conditions

A high-pressure liquid chromatograph equipped with an autosampler AS3000, gradient pumps P4000 and UV detector