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In-syringe dispersive micro-solid phase extraction using carbon fibres for the determination of chlorophenols in human urine by gas chromatography/mass spectrometry

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ABSTRACT

In this article, carbon fibres (CFs) are presented as sorbent material for the dispersive micro-solid phase extraction of twelve chlorophenols from urine samples. CFs are synthesized by a reagentless and green procedure consisting of heating raw cotton, a natural precursor, at high temperature (400 °C) in an inert atmosphere (Ar) during 2 h. The resulting fibres, which present good water dispersibility, are finally loaded on an in-syringe device. This device, which integrates the extraction and final elution of the analytes, is disposable and it is adapted to process low sample volumes. Working at the optimum conditions, the extraction procedure in combination with gas chromatography/mass spectrometry allows the determination of the analytes in urine at the low μg/L range. In fact, the limits of quantification (LOQs) of the analytes were in the interval from 1 μg/L to 2.5 μg/L with precision values, expressed as relative standard deviations (RSD), better than 13%. Relative recovery values, ranging from 74.5% to 113%, demonstrate the applicability of the proposed method.

1. Introduction

Chlorophenols (CPs) are organic compounds commonly used as pesticides, herbicides, disinfectants and precursors in the synthesis of other substances [1]. From the chemical point of view, CPs are phenols substituted in the range from 1 to 5 by chlorine atoms. The substitution degree is usually employed for their classification into mono, di-, tri-, tetra- and penta-chlorophenols. As any pesticide, CPs may be toxic also for humans. The toxicity of CPs is related to the number of chlorine atoms, being pentachlorophenol (PCP) the most harmful compound [2]. Indeed, CPs and derivatives may lead to acute toxicity, histopathological changes, mutagenicity and cancer [3].

Their intensive use and persistence make them common pollutants in some environmental compartments such as air (specially the more volatile) and water. In fact, environmental and workplace exposures are the main sources of toxicological concern [4]. CPs can be found in biofluids like urine or plasma, as a consequence of a direct exposure to these compounds or as a result of the metabolism of other structure-related substances [5].

The analysis of CPs in biofluids usually involves an extraction technique followed by gas chromatography (GC) or liquid chromatography (LC). GC methods use electron capture [6,7], flame ionization [8] and mass spectrometric (MS) [9,10] detection and require a previous derivatization of the analytes to boost their volatility, thus improving the sensitivity. On the other side, LC combined with UV [11], fluorescence, electrochemical [3,12] and MS [13–15] detection has been also successfully employed in this determination.

The isolation and preconcentration of CPs is a critical step for their determination in biological samples. The complexity of biofluids, due to the large number of exogenous and mainly endogenous compounds, may hinder the method selectivity. In addition, the low concentration of the analytes, either by a short exposure or a normal metabolism of the primary compounds, challenges the method sensitivity. Besides, the analytes may coexist in biofluids in the free form or bonded to other molecules like proteins (in blood) or glucuronic acid and sulfate (in urine). Therefore, the typical sample treatment workflow involves a hydrolysis (chemical or enzymatic) followed by an extraction technique. Liquid–liquid extraction (LLE) [9], solid phase extraction (SPE) [9], solid phase microextraction (SPME) [8], stir bar sorptive extraction (SBSE) [16] and hollow fiber-liquid phase microextraction (HF-LPME) [17] have been used for the isolation of CPs from urine samples [3,5].

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The development of new sorptive materials is a consolidated trend in solid phase (micro)extraction. In this context carbon-based materials, especially those that present a nanometric size, have been successfully applied for the extraction of diverse analytes [18,19]. In most of the cases, these outstanding materials are difficult to be synthesized in a simple procedure. The use of natural products as precursors as well as the design of easy and cleaner (low reagents consumption) synthesis is therefore particularly welcome [20]. In addition, these characteristics are in line with the Green Chemistry principles [21]. Carbon fibres (CFs) are good exponents of these materials since they can be obtained from natural products like cotton or linen, and cloths or felts. Their use for extraction purposes has been described elsewhere [20,22,23].

In this article, CFs synthesized after a thermal treatment of cotton under inert atmosphere, have been evaluated as sorbent for the extraction of twelve CPs from urine samples. A dispersive micro-solid phase extraction (d-μSPE) technique, developed in a lab-made in-syringe device is proposed for the extraction of the target analytes, which are finally determined by GC–MS.

2. Experimental section

2.1. Reagents

All the reagents were of analytical grade or better. Unless otherwise specified, they were purchased from Sigma Aldrich (Madrid, Spain). The analytes, 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,3-dichlorophenol (2,3-DCP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP), 3,4-dichlorophenol (3,4-DCP), 3,5-dichlorophenol (3,5-DCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) and pentachlorophenol (PCP) were dissolved in acetone (Panreac, Barcelona, Spain) to prepare stock standards at a concentration of 0.5 g/L which were stored at 4 °C in the dark. 4-bromophenol was used as internal standard (IS). Working standards were daily prepared by dilution of the stock solutions in ultrapure water (Millipore Corp., Madrid, Spain).

Synthetic urine (as blank matrix) was obtained from Cerilliant (Round Rock, TX, USA). β-glucuronidase (EC 3.2.1.31) from Helix pomatia (type H-1 ≥300,000 units/g solid) was used in the sample pre-treatment. β-glucuronidase (20000 U/mL) was prepared in 1 M ammonium acetate buffer (pH 5) and it was stored at −20 °C.

Potassium carbonate and acetic anhydride, both purchased from Merck (Hohenbrunn, Germany), were used for the derivatization of the analytes.

2.2. Collection and pre-treatment of samples

Blank urine samples from healthy individuals were collected and stored in PTFE flasks at −20 °C until their analysis. Before its extraction, the sample was thawed and decanted. The supernatant was taken and diluted 1:2 (v/v) with ultrapure water.

CPs are excreted in urine not only as free molecules but also as glucuronide and sulfate conjugates. For this reason, enzymatic hydrolysis is necessary to ensure the total quantification of CPs excreted in urine. For this reason, 25 μL of the enzyme solution was added to 10 mL of urine, samples being incubated overnight at 37 °C.

2.3. Derivatization of the analytes

The target analytes were derivatized prior to their extraction. This derivatization improves the extraction of the analytes making them less polar and enhances their volatility for GC analysis. For this purpose, for each mL of urine sample or aqueous standard, containing the analytes and the internal standard at 100 μg/L and 50 μg/L, respectively, 200 μL of 5 M K_2CO_3 solution and 200 μL of acetic anhydride were added to an amber-glass bottle. The solution was shaken during 5 min, opening the bottle regularly to release the carbon dioxide generated when CPs are acetylated [24].

Finally, the conductivity of the samples and standards was adjusted to 78.8 mΩ cm^{-1} before the extraction using sodium chloride as electrolyte. To avoid analyte dilution, sodium chloride was directly added to the samples/standards under continuous stirring until the taget conductivity, measured in a conductance meter (YSI Model 35, Yellow Springs Instrument Co. Ohio, USA), was achieved.

2.4. Synthesis and characterization of carbon fibres

Carbon fibres were synthetized in a simple, quick and cheap process. The procedure was as follows: 4 g of raw cotton were introduced in a stainless steel tube with a hermetic seal. In order to remove oxygen, the tube was purged with a stream of Ar during 20 min. After this time, the tube was sealed and heated at 400 °C for 2 h yielding a black solid. Fig. 1A shows pictures of raw cotton and CFs where a clear colour change (from white to black) is observed.

Scanning electron microscopy (SEM) images were obtained using a JEOL JSM 6300 microscope. Carbon content of the fibres was determined in a EuroVector Elemental Analyser EA3000 (EuroVector SpA, Milan, Italy). Micrographs and elemental analysis were obtained in the Central Service for Research Support (SCAI) of the University of Córdoba.

2.5. In-syringe μSPE

The in-syringe system, which has been published elsewhere [25], consists of a 10 mL syringe attached to a micropipette tip section with a cotton bead used as frit. 100 mg of carbon fibres, accurately weighted, are located (see Fig. 1B) inside the barrel of the syringe. The extraction procedure involves several sequential steps. Firstly, carbon fibres are conditioned by 2 mL of methanol followed by 2 mL of water. Secondly, 10 mL of treated sample (as indicated in Sections 2.2 and 2.3) is aspirated into the syringe making possible the interaction of the analytes with the sorbent, which is dispersed into the sample. This step is done sequentially in two different stages (5 mL × 2) to enhance that interaction. Thirdly, the material is cleaned up with 2 mL of water. After the water removal, which is done by several plunger movements, the analytes are eluted with 300 μL of chloroform. 2 μL of the extract is injected in GC/MS.

2.6. Instrument

GC/MS analyses were performed in an Agilent Technologies (Palo Alto, CA, USA) HP6890 gas chromatograph equipped with a HP5973 mass spectrometric detector based on a quadrupole analyzer and an electron multiplier detector. System control and data acquisition were achieved with a HP1701CA MS ChemStation software.

A column split ratio of 1:10 was selected for injection, using Helium (6.0 grade, Air liquid, Seville, Spain) at a flow rate of 1 mL min^{-1} as carrier gas. Chromatographic separations were performed on a fused silica HP-5MS capillary column, 30 m × 0.25 mm i.d. coated with 5% diphenyl, 95% dimethyl polysiloxane (film thickness 0.25 μm) from Agilent. The column temperature program was as follows: 2.5 min at 40 °C, raised up to 100 °C at 25 °C min^{-1}, then immediately ramped at 4 °C min^{-1} up to 180 °C and raised up to 300 °C at 40 °C min^{-1} and kept finally at this temperature for 5 min. The quadrupole mass spectrometer detector was operated in selected ion monitoring mode, recording the following fragmentions: *m/z* 128 (from 8.00 to 11.50 min), *m/z* 162 and *m/z* 172 (from 11.50 to 14.50 min), *m/z* 196 (from 14.50 to 19.00 min), *m/z* 232