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Molecularly imprinted solid phase extraction of urinary diethyl thiophosphate and diethyl dithiophosphate and their analysis by gas chromatography–mass spectrometry

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ABSTRACT

An analytical method involving molecularly imprinted solid phase extraction (MISPE) and gas chromatography–mass spectrometry (GC–MS) was developed for the analysis of organophosphates metabolites (diethyl thiophosphate – DETP and diethyl dithiophosphate – DEDTP) in human urine samples. A DETP molecularly imprinted polymer (MIP) was synthesized using 4-vinylpiridine as the functional monomer and ethylene glycol dimethacrylate as the cross-linker. The conditioning step of the MISPE was conducted by running 3 mL of acetonitrile, 3 mL of 0.1 mol L^{-1} dibasic phosphate buffer at pH 11 and 2 mL of water through the molecularly imprinted polymer (MIP) cartridge. The extraction step was executed using 1.0 mL of a urine sample, with the pH previously adjusted to 3.0. Finally, the analytes were eluted with 3 mL of acetonitrile and derivatized with 3% 2,3,4,5,6-pentafluorobenzyl bromide solution at room temperature for 1 h. The sample was analyzed by GC–MS in the SIM (selected ion monitoring) mode. Analytical calibration curves for DETP and DEDTP were constructed using a pool of urine samples and six levels of concentration. The method was found to be linear from 10 to 500 μg L^{-1} ($r > 0.99$) with limits of quantification of 10 μg L^{-1} for both analytes. The within-day and between-day precisions were evaluated (as %RSD) and all the results were <15% for both analytes. The method was accurate (relative error < ±15%), with good robustness.

1. Introduction

Organophosphate pesticides (OPs) have been widely and effectively used as insecticides, with many applications in agricultural and residential settings [1]. In humans, these compounds are metabolized to dialkyl phosphates (DAPs) and excreted in the urine (80–90% of the total dose within 48 h) [2,3]. Urinary DAPs, such as dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP) and diethyl dithiophosphate (DEDTP), are commonly used as biomarkers of organophosphate exposure [4,5]. Fig. 1 shows the chemical structure of DETP and DEDTP, the main urinary metabolites of the dissulfoton.

The determination of DAPs by liquid chromatography has not been a common strategy due the difficulty in separating these metabolites by this technique [5]. Several methods based on the gas chromatographic determination of the derivatized DAP metabolites can be easily found in the literature [3–5]. However, due to the presence of several concomitants in the urine samples, the use of efficient extraction techniques is necessary to avoid interference problems during the derivatization and chromatographic analyses. In this way, some specific techniques can be pointed out, such as liquid–liquid extraction [6–9], solid phase extraction [10,11], ionic exchange extraction [12], extractive derivatization [13], azeotropic distillation [14–16] and lyophilization [17–20], among others. However, when unspecific methods are used, some concomitants may remain [14]. An efficient alternative for circumventing this problem is the use of selective sorbents such as molecularly imprinted polymers (MIPs) [21–23]. MIPs are cross-linked synthetic polymers obtained by the copolymerization of a functional monomer with a cross-linker in the presence of a template molecule. After polymerization, the polymer is washed. Removal of the template leaves specific recognition sites in the polymer that are complementary to the template in terms of size, shape and chemical functionality. The imprinted polymer is able to selectively rebind the template molecule (analyte) and

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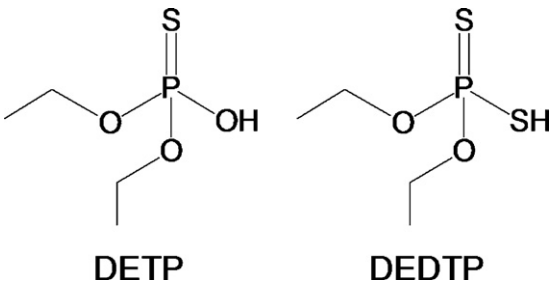

Fig. 1. Dialkyl phosphate metabolites.

other molecules with analogous structures [24,25]. MIPs have been extensively used in the solid phase extraction of several analytes in different matrices [26–30].

Based on the relevant characteristics of MIP compared to other adsorbents, such as selectivity, sensitivity, high stability, high lifetime, and low cost, we perceived, for the first time, the possibility of synthesizing a MIP for selective extraction of DETP and DEDTP in urine samples, followed by the separation and quantification of these analytes using gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Chemicals and solutions

All the HPLC grade (99.99%) organic solvents, such as acetonitrile, tetrahydrofuran, hexane and methanol were obtained from Vetec (Rio de Janeiro, Brazil). The solutions were prepared with deionized water (18.2 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, USA). For the MIP synthesis, DETP, 4-vinylpyridine, ethylene glycol dimethacrylate (EGDMA), 2,2′-azobisisobutyronitrile (AIBN) (all from Sigma–Aldrich, Steinheim, Germany) were used as template, functional monomer, crosslinking reagent and initiator, respectively. Acetonitrile was used as the solvent. A solution of methanol:acetic acid (Merck, Darmstand, Germany) at a ratio of 9:1 (v/v) was used during the washing of the MIP to remove the template. Stock solutions of DETP and DEDTP (Sigma–Aldrich, Steinheim, Germany) were prepared at 1.0 mg L^{-1} in HPLC grade acetonitrile, placed in an amber flask and kept at −20 °C for up to 30 days. Working solutions of 0.1–500 μg L^{-1} were prepared daily by diluting the standard solution in acetonitrile. A solution of 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) (Sigma–Aldrich, Steinheim, Germany) in acetonitrile was used as the derivatization reagent.

2.2. Gas chromatography and mass spectrometry conditions

The electron ionization (70 eV) mass spectrometric analysis was performed using a GC–MS QP-2010 from the Shimadzu® Corporation (Kyoto, Japan) equipped with a RTx®-5MS (30 m × 0.25 mm i.d. × 0.25 μm) capillary column (RESTEC, Bellefonte, USA). Pure helium (99.999%) with a column flow of 1.53 mL min^{-1} was used as the carrier gas. A 2 μL aliquot of the standard/sample was injected in the splitless mode and analyzed under the following conditions. The initial temperature of the column was maintained at 140 °C for 1 min, raised to 230 °C at 40 °C min^{-1} and maintained at 230 °C for 1 min. The column temperature was then raised to 260 °C at 20 °C min^{-1}, raised to 300 °C at 40 °C $min^{-1}$and maintained at 300 °C for 0.5 min. The injector, interface and ion source temperatures were 270, 300 and 230 °C, respectively. The quantitative analysis was achieved in selected ion monitoring mode (SIM) with an event time of 0.2 s for each analyte. The ions at *m*/*z* 350 and *m*/*z* 366 were used to quantify DETP and DEDTP, respectively. The ions at *m*/*z* 350, 274 and 213 as well as the ions at *m*/*z* 366, 185 and 157 were employed to confirm the identity of DETP and DEDTP, respectively. The data files were acquired with the GCMS-Solution software®.

2.3. MIP synthesis

The synthesis of the DETP-imprinted polymer was based on non-covalent interactions between the template and the functional monomer. In a 25 mL glass flask, 1 mmol of DETP and 4 mmol of 4-vinylpyridine were dissolved in 6 mL of acetonitrile and this solution was sonicated at room temperature. After 5 min, 16 mmol of EGDMA and 0.75 mmol of AIBN were added, and the mixture was purged with nitrogen in the ultrasonic bath for 10 min. The flask was sealed and immersed into a water bath at 65 °C for 24 h. After polymerization, the monolith obtained was mechanically ground, and the particle size was selected using a steel sieve (75–106 μm). The particles were transferred to a glass flask and washed in an ultrasonic bath with 4:1 (v/v) methanol/acetic acid for 1.5 h. The washing procedure was repeated 10 times, and the washing solution was renewed for each repetition. The efficacy of template removal was checked analyzing the eluates of the washing solutions by GC–MS until nothing could be detected. The polymer particles were dried at 70 °C, and 50 mg were packed in polypropylene cartridges. The non-imprinted polymer (NIP) was synthesized in the same way as the MIP, in the absence of the template.

2.4. Sample preparation and MISPE procedure

The urine sample handling was approved by the Ethics Committee of the Federal University of Alfenas (no. 296/2010).

Initially, 3 mL of acetonitrile, 3 mL of 0.1 mol L^{-1} dibasic phosphate buffer at pH 11.0 and 2 mL of water were flowed through the MIP cartridge at 1 mL min^{-1} during the conditioning step. Then, 1.0 mL of human urine sample at pH 3.0 (adjustment with 0.1 mol L^{-1} nitric acid aqueous solution) was percolated through the cartridge at 1 mL min^{-1}, and DETP and DEDTP were selectively extracted. Finally, the analytes were eluted with 3 mL of acetonitrile, and the extract was evaporated to dryness under a nitrogen stream. The derivatization reaction was performed according to the procedure described by De Alwis et al. [5], with some modifications. K_2CO_3 (15.0 mg) and 250 μL of 3% PFBBr solution (v/v in acetonitrile) were added to the tube containing the residue from the MISPE. The extract was allowed to stand for 1 h at room temperature for the derivatization reaction. Then, the derivatized extract was evaporated to dryness under a nitrogen stream and reconstituted in 100 μL of tetrahydrofuran before the GC–MS analysis.

2.5. Validation study

The following validation parameters were evaluated: linearity, sensitivity, precision, accuracy, recovery, detection and quantification limits and robustness. This study was performed using a pool of blank human urine samples spiked with DETP and DEDTP. The linearity and sensitivity were established through the calibration curve obtained by a sextuplicate analysis of DETP and DEDTP at six concentration levels (10, 50, 100, 200, 350 and 500 μg L^{-1} for DETP and DEDTP). Linearity and sensitivity were expressed as the correlation coefficient (*r*) and the slope of the calibration curve, respectively. Intra-assay precision and accuracy were assessed with five replicates from each concentration level (10, 50, 200 and 500 μg L^{-1} for DETP and DEDTP) on the same day. Inter-assay precision and accuracy were evaluated by three replicates analyzed at each concentration level (10, 50, 200 and 500 μg L^{-1} for both analytes) on separate days. The results were expressed as percent relative standard deviations and percent relative errors for