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A column-switching method for quantification of the enantiomers of omeprazole in native matrices of waste and estuarine water samples

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ABSTRACT

This work reports the use of a two-dimensional liquid chromatography (2D-LC) system for quantification of the enantiomers of omeprazole in distinct native aqueous matrices. An octyl restricted-access media bovine serum albumin column (RAM-BSA C_8) was used in the first dimension, while a polysaccharide-based chiral column was used in the second dimension with either ultraviolet (UV–vis) or ion-trap tandem mass spectrometry (IT-MS/MS) detection. An in-line configuration was employed to assess the exclusion capacity of the RAM-BSA columns to humic substances. The excluded macromolecules had a molecular mass in the order of 18 kDa. Good selectivity, extraction efficiency, accuracy, and precision were achieved employing a very small amount (500 μL or 1.00 mL) of native water sample per injection, with detection limits of 5.00 $\mu g\,L^{-1}$, using UV–vis, and 0.0250 $\mu g\,L^{-1}$, using IT-MS/MS. The total analysis time was only 35 min, with no time spent on sample preparation. The methods were successfully applied to analyze a series of waste and estuarine water samples. The enantiomers were detected in an estuarine water sample collected from the Douro River estuary (Portugal) and in an influent sample from the wastewater treatment plant (WWTP) of São Carlos (Brazil). As far as we are concerned, this is the first report of the occurrence of (+)-omeprazole and (−)-omeprazole in native aqueous matrices.

1. Introduction

Sample preparation previous to instrumental analysis is a crucial step for establishing a selective and sensitive chromatographic method for trace analysis in complex matrices. Different sample extraction methods and preparation techniques are often involved in the pre-treatment of complex matrices, such as biological and environmental samples [1–3]. In order to improve this type of analysis, faster analytical methods have been developed with concomitant higher sensitivity and selectivity. A large number of extraction techniques for enhancing sensitivity, selectivity, and sample cleanup have also been developed mainly in the field of solid-phase extraction (SPE), such as multifunctionalized sorbents [4,5]. However, SPE is generally employed in an off-line mode and presents some drawbacks, such as long analysis time, use of high amounts of organic solvents, and the generation of waste cartridges; furthermore, it leads to the use of large volumes of samples, especially in environmental analyses [6,7].

In order to achieve automatization, a large number of different restricted-access media (RAM) supports, such as alkyl-diol-silica (ADS), internal surface reversed phase (ISRP), semi-permeable surface (SPS), shielded hydrophobic phase (SHP), mixed-function phase (MFP), and protein-coated silica, have been developed to allow the direct injection of biological fluids and food samples into liquid chromatography systems (LC) [8–10]. However, only few works reported the use of RAM supports for environmental samples [11–13]. In a recent work, a RAM molecularly imprinted polymer (MIP) column with a large injection volume (50 mL) was used for the analysis of pharmaceuticals in river water [14], while in a previous work developed by Chico et al. [15] a pre-concentration step using SPE was employed before the injection into the RAM column. Ding et al. [16] achieved high sensitivity in a LC–MS method for the analysis of macrolide antibiotics using a RAM column in the backflush mode, leading to an injection volume of only 1 mL.

RAM supports allow the extraction/concentration of the analytes through a combination of size exclusion and conventional hydrophobic or ion-exchange interactions, promoting the exclusion of macromolecules while retaining micromolecules [2]. Thus, compounds of low molecular mass are extracted and enriched, into the pore phase, whereas the outer surface of the particles has a

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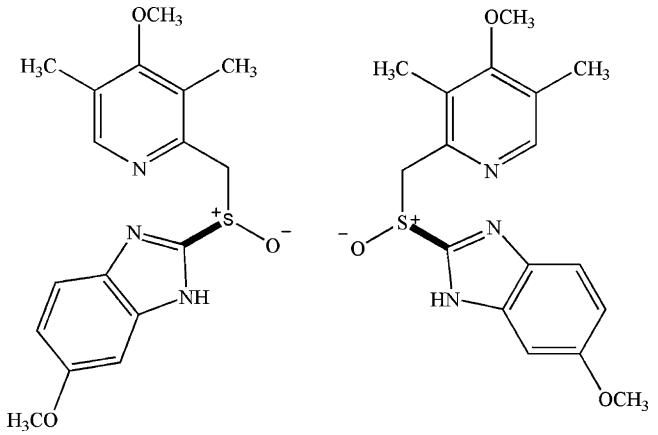

Fig. 1. Chemical structures of the enantiomers of omeprazole.

special topochemistry to prevent adsorption of large molecules, such as humic substances from environmental water matrices, thus allowing their exclusion in the void volume [15].

In this work we evaluated the exclusion capacity of RAM-BSA C_8 and C_{18} columns. For this, environmental water samples and certified standards of aquatic humic substances were analyzed using RAM columns; the results were compared to the ones obtained by the use of a high-performance size-exclusion chromatography column (HPSEC). The RAM-BSA C_8 column was used in the first dimension of a 2D-LC system for sample cleanup, while a polysaccharide-based chiral column was used in the second dimension for the enantioselective separation of omeprazole (OME) (Fig. 1).

The interest in developing methods for the determination of chiral pharmaceuticals in the environment is due to the fact that these compounds are now an important issue in the design, discovery, and development of new drugs. Although stereochemistry plays an important role in pharmacology, a large number of chiral drugs under clinical use are still racemic mixtures. The advances in environmental chiral analysis led to a new awareness of the importance of stereoselective behaviors and the fate of chiral drugs [17–20].

In this paper we report the development and validation of, as far as we know, the first procedure for the quantification of the enantiomers of omeprazole in environmental native water matrices by LC with an achiral–chiral column-switching approach, using either UV–vis or IT-MS/MS detection.

2. Experimental

2.1. Chemicals and equipments

All the organic solvents were LC grade from Mallinckrodt Baker (St. Louis, MO, USA). The water used for the mobile phase was purified through a Milli-Q system (Millipore, São Paulo, Brazil). Bovine serum albumin (fraction V powder, minimum 98%) was purchased from Sigma (St. Louis, MO, USA). Sodium polystyrene sulfonates (Polymer Laboratories, Amherst, MA, USA) were used as molecular-mass calibration standards (8, 18, 46 and 100 kDa). Nylon membranes (47 mm i.d. × 0.45 μm, Millipore, São Paulo, Brazil) were used to filter all the mobile phases and water samples. Glutaraldehyde, potassium dihydrogen phosphate, and sodium borohydride were from Merck (Darmstadt, Germany). Omeprazole was generously donated by LIBBS (São Paulo, SP, Brazil). All other reagents were of analytical grade. The mobile phases were prepared in a volume/volume ratio.

Two LC systems were used. The first equipment consisted of two Shimadzu LC-10 ATVP pumps (Kyoto, Japan), with one of the pumps having a FCV-10AL valve for selecting solvent, a SIL-10ADVP autosampler with a 500 μL loop, a DGU-14A degasser, a SPD-10A UV-vis detector, and a SCL-10AVP interface. A LC 7000 Nitronic EA (Sulpelco, St. Louis, MO, USA) six-port valve was used for the automated column-switching. Data acquisition was done using a Shimadzu CLASS-VP software. The second LC system had two Shimadzu LC-20AD pumps (Kyoto, Japan), a SIL-20A autosampler with a 2.0 mL loop, a DGU-20A5 degasser and a CBM-20A interface. The automated column-switching system was also a LC 7000 Nitronic EA six-port valve, and an Esquire 6000 IT mass spectrometer (Bruker Daltonics, Germany) equipped with an ESI source, operating in a positive mode. Data acquisition was carried out using the Data Analysis software (Bruker Daltonics, Germany).

A Total Organic Carbon Analyzer – TOC VCPH Shimadzu was used in the TOC analyses.

2.2. Chromatographic columns

The chiral phase tris-(3,5-dimethylphenylcarbamate) of amylose coated onto APS-Nucleosil (500 Å, 7 μm, 20%, w/w, 150 mm × 4.6 mm i.d.) (CSP) was prepared as described elsewhere [21,22]. The RAM-BSA columns (50 mm × 4.6 mm i.d.) using silica octyl and octadecyl Luna® (10 μm particle size and 100 Å pore size) were prepared as before [23], based on the protocol previously described by Menezes and Felix [24].

An analytical Tsk-Gel® column (Tosoh Bioscience, G3000PW$_{XL}$, 300 mm × 7.8 mm i.d., and 6.0 μm particle size) was used to evaluate the exclusion of aquatic humic substances from the collected water samples.

2.3. Standard solution and spiked sample preparation

A 200 mg L^{-1} stock solution of omeprazole (OME) (±)-(6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl)-1*H*-benzo[*d*]imidazole] (200 mg L^{-1}) was prepared and diluted to 20 μg mL^{-1} in methanol for the LC–UV–vis method; from which a stock solution of 1000 μg L^{-1} was also prepared for the LC–IT-MS/MS method. Using the appropriate stock solution, two sets of standard working solutions for calibration and two sets for quality controls (QC) were prepared with the following concentrations: 12,800, 6,400, 3,200, 1,600, 800, 400, 300 μg L^{-1}, and 360, 6,000, 10,000 μg L^{-1}, respectively (LC–UV–vis); 8.00, 6.00, 4.00, 3.00, 2.00, 1.50, 1.00 μg L^{-1}, and 1.20, 4.80, 6.40 μg L^{-1} (LC–IT-MS/MS). All stock and working solutions were stable during 2 months when stored at 4 °C in amber bottles; no evidence of degradation of the analytes was observed in the chromatograms.

To prepare the calibration standards and quality control samples, either 100 or 200 μL aliquots of the appropriate standard working solutions were placed in a series of test tubes and the solvent was evaporated to dryness under a nitrogen stream. The dried analytes were reconstituted using either 1.00 mL or 2.00 mL of spring water from the Monjolinho River (São Carlos, Brazil). The solutions were vortex-mixed during 20 s and aliquots of 700 μL or 1500 μL were transferred to autosampler vials from which 500 μL (LC–UV–vis) or 1,000 μL (LC–IT-MS/MS) were injected into the column-switching LC systems.

2.4. Evaluation of RAM-BSA C_8 and C_{18} columns for the exclusion of humic substances

The exclusion was evaluated using certified standards of aquatic humic (HAs) and fulvic (FAs) acids (IHSS, International Humic Substance Society, donated by Embrapa-CNPDIA – São Carlos). Ultrapure water was used to prepare 1.00 mg L^{-1} solutions of HAs and FAs; the pH was adjusted to 8.2 with NaOH or HNO_3. A phosphate buffer (pH 6.8, 0.1 M NaCl) was used as mobile phase at a