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Direct injection of native aqueous matrices by achiral–chiral chromatography ion trap mass spectrometry for simultaneous quantification of pantoprazole and lansoprazole enantiomers fractions

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ABSTRACT

A two-dimensional liquid chromatography system coupled to ion-trap tandem mass spectrometer (2DLC-IT–MS/MS) was employed for the simultaneous quantification of pantoprazole and lansoprazole enantiomers fractions. A restricted access media of bovine serum albumin octyl column (RAM-BSA C_8) was used in the first dimension for the exclusion of the humic substances, while a polysaccharide-based chiral column was used in the second dimension for the enantioseparation of both pharmaceuticals. The results described here show good selectivity, extraction efficiency, accuracy, and precision with detection limits of 0.200 and 0.150 $\mu g\,L^{-1}$ for the enatiomers of pantoprazole and lansoprazole respectively, while employing a small amount (1.0 mL) of native water sample per injection. This work reports an innovative assay for monitoring work, studies of biotic and abiotic enantioselective degradation and temporal changes of enantiomeric fractions.

1. Introduction

During the last decade, pharmaceutically active compounds have become an important environmental pollutant, due to their presence in aquatic systems, such as: wastewater-treatment-plant (WWTP) effluents, receiving waters (rivers and lakes), drinking water, and groundwater [1–9]. These contaminants are considered as pseudo-persistent pollutants, since their outflow in the environment is continuously at very low concentrations. The concentrations usually found for these contaminants ($\mu g\,L^{-1}$ in wastewaters to $ng\,L^{-1}$ in surface waters) vary according to: frequency of use, excretion of non-metabolized form, and persistence to biodegradation [10,11]. Nonetheless, their toxic effects on the biota are not yet clarified. They still require new analytical tools for the improvement of the methods employed on their determination in environmental samples.

A large number of chiral drugs are consumed as a racemic mixture, whereas others as a single enantiomer. At environmental conditions, the enantiomers can undergo abiotic and biotic processes, which are responsible for their availability differences to the biota. Enantiomeric fraction has the potential to be a useful maker of temporal changes of chiral pharmaceuticals in the environment [12–14]. For that, enantioselective analyses are required. However, few reports are available on the analysis of chiral pharmaceuticals and illicit drugs in the environment [14–20]. This limitation is due mainly to the narrow number of enantioselective methods for drugs in environmental samples [20,21].

The use of liquid chromatography–tandem mass spectrometry (LC–MS/MS) has allowed the quantification and confirmation at trace levels of enantiomers in environmental samples [13,22,23]. Nevertheless, matrix-dependent signal suppression or enhancement is responsible for the main problem of atmospheric pressure ionization (API) mass spectrometry [24–26].

Trying to overcome this important drawback, sample clean-up procedures aiming to reduce introduction of matrix component into the API interface have been examined by a number of researchers [7,10,23]. Within this context, a large number of different restricted-access media (RAM) supports phases have been developed [27,28]. The versatility on the use of these RAM supports has been established for on-line clean-up of biological fluids, food and, more recently, aqueous environmental samples [27–31]. Recently, we have [19] reported the capacity of a restricted-access media bovine serum albumin (RAM BSA) column to exclude humic substances, which are the major source of matrix effects in environmental samples.

This article presents an analytical method for simultaneous quantification of lansoprazole (LAN) and pantoprazole (PAN) enantiomers (Table 1), by direct injection of native aqueous samples

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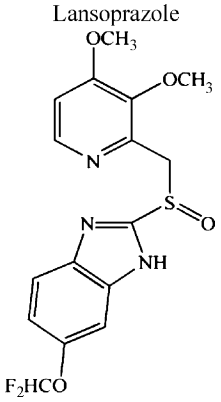
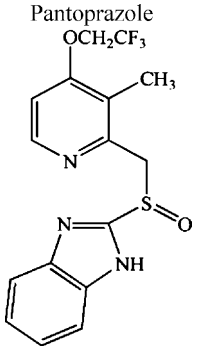
Table 1
Chemical structures of the lansoprazole and pantoprazole and their physicochemical properties [42,44].

Compounds	Molecular mass	Log K_{ow}	pK_a
Lansoprazole 	369.3	2.58	8.73
Pantoprazole 	383.1	1.69	7.7

using a 2DLC-IT–MS/MS. These pharmaceuticals are widely used as proton pump inhibitors (PPIs) for the treatment of acid related diseases [30].

This work brings an innovative mode for tracking enantiomers fractions in native aqueous matrices.

2. Experimental

2.1. Study area and sampling

Water samples were collected in January 2009, during the summer season, along Monjolinho River (São Carlos, SP, Brazil), from the outfalls of untreated wastewater discharges. The site (1), with latitude: 22°00′33″S and longitude: 47°50′07″W, refers to the springwater of the Monjolinho used as the blank matrix. The other sampling sites are located on areas considered to be susceptible to human and industrial contaminations: latitude: 22°01′19.5″S, longitude: 47°54′50.3″W (2); and agricultural run-off: latitude: 22°00′33″S, longitude: 47°50′07″ W (3) and latitude: 21°59′25.2″S, longitude: 47°53′29.4″W (4).

The wastewater samples were collected in amber glass bottles (100 mL) pre-rinsed with ultrapure water and kept on ice for transportation to the laboratory. The collected samples were vacuum filtered through 0.45 μm nylon membrane glass fiber filters, to remove suspended particles. Methanol LC grade (3 drops to each liter collected) was added to it and, then stored at 4 °C.

2.2. Reagents and chemicals

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, SP, Brazil). Bovine serum albumin was purchased from Sigma (fraction V powder minimum 98%, St. Louis, MO, USA). Nylon membranes (47 mm i.d. × 0.45 μm, Millipore, São Paulo, SP, Brazil) were used to filter the water samples. LAN – (±) 2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl]-1H-benzoimidazole and PAN – (±) 5-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-3H-benzoimidazole were generously supplied by Boehiringer Ingelhein (São Paulo, SP, Brazil) and Eurofarma (São Paulo, SP, Brazil), respectively. All other reagents were of analytical grade. The mobile phases were prepared in a volume/volume relation.

2.3. Chromatographic instrumentation

The LC system (Shimadzu, Kyoto, Japan) used consisted of two LC-20AD pumps, with one of the pumps having: an FCV-10AL valve for selecting solvent, a SIL 20A autosampler with a 2.00 mL loop, a DGU-20A5 degasser, and a CBM-20A interface. A LC 7000 Nitronic EA (Sulpelco, St. Louis, MO, USA) six-port valve was used for the automated column-switching. The LC system was coupled to an Esquire 6000 IT mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) equipped with an ESI source, operating in a positive mode. Data acquisition was carried out using a Data Analysis software (Bruker Daltonics GmbH, Bremen, Germany). All LC analyses were performed at room temperature (±25 °C). The analysis was completed in 40 min, enantiomeric elution order was determined at the established chromatographic conditions using a JASCO CD-2095 plus chiral detector at λ_{max} 285 nm. For that, a solution of 200 μg mL^{-1} of (±)-LAN and (±)-PAN prepared in mobile phase was injected (20 μL) into the chiral column.

2.4. Chromatographic columns

The columns were prepared as described elsewhere [32,33]. The chiral column used (150 × 4.60 mm i.d.) was non-commercial of tris-(3,5-dimethoxyphenylcarbamate) of amylose coated onto APS-Nucleosil (500 Å, 7 μm, 20% w/w). The RAM-BSA column (50.0 × 4.60 mm i.d.) was prepared using octyl silica (Luna®, 10 μm particle size and 100 Å pore size). The immobilization of BSA was done *in situ*, based on the protocol previously described by Menezes and Felix [34].

2.5. Column-switching procedure and analysis conditions

The column-switching systems used for coupling the RAM and the chiral columns are illustrated in Fig. 1. The position of the column-switching device alternates between positions 1 and 2. It was controlled by CLASS-VP software through the timed events. First, the switching-valve was set to position 1 and, then 1000 μL of water sample were injected into the RAM column. The time sequence used is listed in Table 2. The flow rate used was 1.0 mL min^{-1}. For the IT-MS/MS detection, the flow rate of the mobile phase was split into the source at 100 μL min^{-1} by means of a T-piece. The optimization of the ionization source, voltages on the lenses and trap conditions were all achieved with the expert tune mode of Bruker Daltonics Esquire control software. IT-MS/MS parameters for the analysis were the following: nebulizer pressure of 30 psi, drying gas flow of 8 L min^{-1}, temperature of 325 °C, capillary voltage of 4000 V and fragmentation amplitude of 0.42 V for (±)-LAN, and 0.38 V for (±)-PAN.

2.6. Standard solutions and spiked sample preparation

Stock solutions of 200 μg mL^{-1} were prepared separately by dissolving of 2.00 mg of each compound in methanol (10.0 mL). The solutions were stored in the dark at 20 °C in ambar bottles to avoid