Journal of Chromatography A, 1218 (2011) 2865–2870

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

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

Juliana Cristina Barreiro, Kenia Lourenço Vanzolini, Quezia Bezerra Cass*

Chemistry Department, Federal University of São Carlos, P.O. Box 676, São Carlos 13565-905, SP, Brazil

ARTICLE INFO

Article history:
Received 26 December 2010
Received in revised form 22 February 2011
Accepted 23 February 2011
Available online 2 March 2011

Keywords:
Enantiomers
Lansoprazole
Matrix effects
Native samples
Pantoprazole
Tandem mass-spectrometry

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].

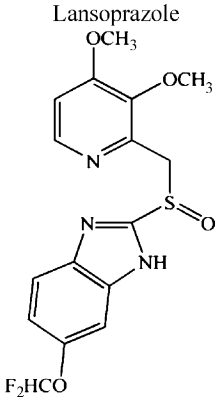
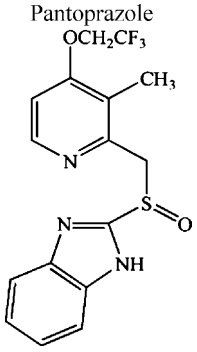
Trying to overcome this important drawback, sample clean-up procedures aiming to reduce introduction of matrix component into the API interface have been examined by a number of researchers [7,10,23]. Within this context, a large number of different restricted-access media (RAM) supports phases have 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

* Corresponding author. Tel.: +55 1633518087; fax: +55 16 3351 8350.
E-mail address: quezia@pq.cnpq.br (Q.B. Cass).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.02.064

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

Table 2
Time events for the column-switching procedure.

Event	Valve position	Pump	Time (min)
Humic substances are excluded by RAM column	1	Pump 1 (eluent A)	0.00–3.00
Conditioning of the chiral column	1	Pump 2 (eluent D)	0.00–7.20
Elution of retained components on the RAM	1	Pump 1 (eluent B)	3.01–9.60
Analytes are transferred to the chiral column	2	Pump 1 (eluent B)	7.20–9.60
Analysis of the enantiomers	1	Pump 2 (eluent D)	9.61–40.0
Washing of RAM column	1	Pump 1 (eluent C)	9.61–15.0
Conditioning of RAM column	1	Pump 1 (eluent A)	15.01–20.0

Pump 1: eluents (A) H_2O; (B) CH_3CN:H_2O (35:65 v/v); (C) CH_3CN:H_2O (80:20 v/v), flow rate: 1.0 mL min^{-1}. Pump 2: eluent (D) CH_3CN:H_2O (35:65 v/v), flow rate: 1.0 mL min^{-1}. λ_{max}: 285 nm.

degradation. No evidence of degradation was observed. Through an appropriated combination of stock solution, standard working solutions for calibration and quality controls (QC) were prepared, respectively, for (±) LAN: 1.20, 2.40, 4.80, 6.40, 9.60, 19.2, 38.4 and 76.8 μg L^{-1}; 1.44, 40.0 and 70.0 μg L^{-1}, and for (±) PAN: 0.800, 1.60, 3.20, 6.40, 12.8, 25.6 and 51.2 μg L^{-1}; 0.960, 14.0 and 20.0 μg L^{-1}.

To prepare the calibration standards and quality control samples, aliquots (200 μL) of the appropriated 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 2.00 mL of springwater from Monjolinho River (site 1) and the solutions were vortex-mixed during 20 s. Aliquots of 1500 μL were transferred to autosampler vials, from which 1000 μL were injected into the column-switching LC system. The samples were prepared in triplicate. The calibration curves were constructed from a least-squares linear regression by plotting the peak area against the concentration of each enantiomer.

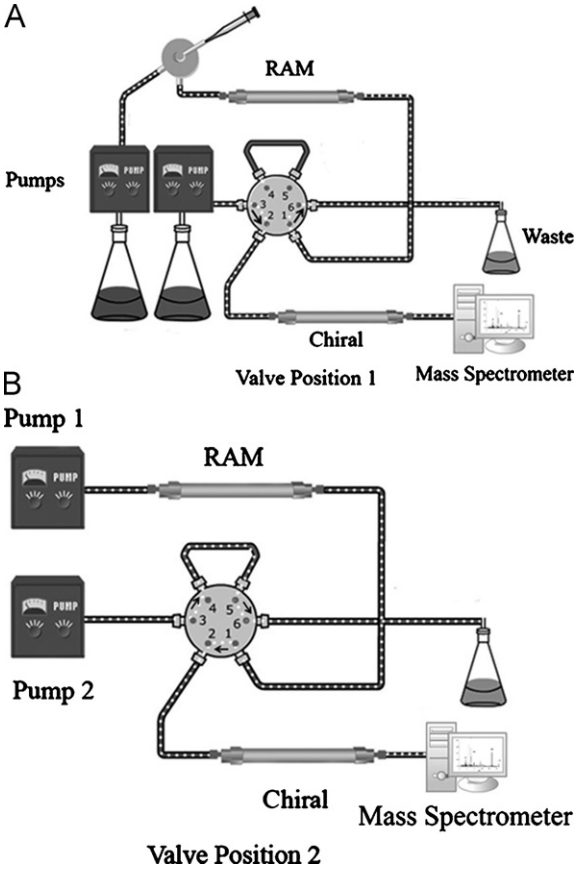


Fig. 1. Schematic diagram of the column-switching system (A) valve position 1 and (B) valve position 2.

2.7. Matrix effect

The matrix effect was evaluated by on-line extraction and post-column infusion on column-switching configuration. In the on-line extraction procedure, the percentage of the matrix effect (ME) is obtained by the ratio between the peak area obtained from solutions of the compounds (100 ng mL^{-1}) in ultrapure water and spring water (site 1) [19].

2.8. Method validation

The method validation was carried out in accordance with internationally accepted criteria [35]. The linearity was evaluated using external calibration curves with seven calibration levels for each enantiomer prepared in triplicate.

Intra and inter-day precision of the method were determined by the analysis of three QC samples of [(±) LAN 0.720, 20.0 and 35.0 μg L^{-1} and (±) PAN 0.480, 7.00 and 10.0 μg L^{-1}]. Five samples of each concentration were prepared in spring water. The accuracy was evaluated by back-calculation and expressed as the percentage of deviation between the amount found and the amount added at the three concentrations examined.

The extraction transfer efficiency was measured using the three QC samples. The percentage of recovery was obtained comparing the peak-area ratios of QC samples to the ones prepared at the same concentration in ultrapure water.

The LOD (as signal to noise ratio of 3) and LOQ were determined from spiked springwater samples. The LOQ was the lowest calibration level, and the accepted criteria were that both the precision and the accuracy for the three samples were ≤20% variability.

The stability of the enantiomers of LAN and PAN was evaluated by comparing the assay results of three spiked QC samples in different days, at room temperature, as freshly prepared (time 0), 24 and 48 h (autosampler stability) samples. RSD of less than 15% was the criterion for the stability evaluation [35].

3. Results and discussion

3.1. Method development

The use of RAM-BSA coupled to polysaccharide-based chiral columns has been successfully employed in methods for quantification of PPIs enantiomers in human plasma [36–38] and, more recently, in wastewater samples [19].

It is important to stress that chiral columns are easily deteriorated by clogging with macromolecules. Thus, when it is used in the second dimension, for maintaining the chiral resolution, the first dimension has to be efficient in clean-up justifying the selection of the RAM-BSA column for exclusion of humic substances [19].

Previously, complete studies on the enantioresolution of lansoprazole, and pantoprazole using cellulose and amylose phases were carried out [39]. We have already demonstrated the use of the non-commercial chiral column of amylose tris-(3,5-

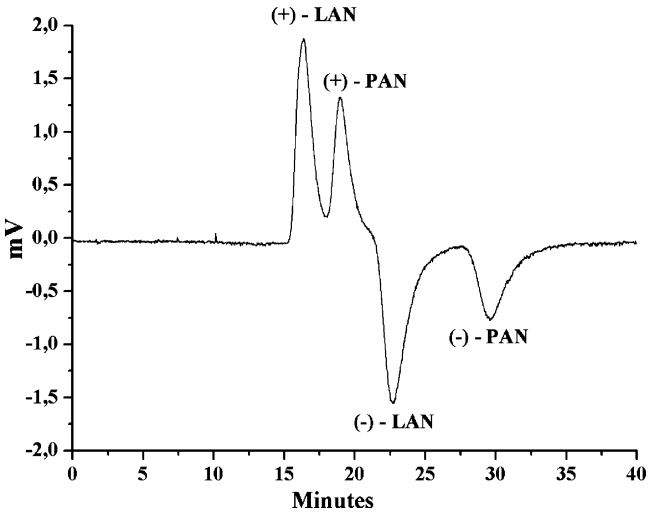


Fig. 2. Representative chromatograms of the elution order for the (±) LAN and (±) PAN enantiomers prepared in mobile phase (200 μg mL^{-1}) in the chromatographic conditions illustrated in Table 2.

dimethoxyphenylcarbamate), under reverse elution mode, for quantification of these PPIs [37,38], providing, thus, enough reasoning for selecting this chiral column.

The column-switching system used is schematically illustrated in Fig. 1. First, the chromatographic exclusion profile of humic substances for the blank matrix (spring water) was evaluated using the selected RAM-BSA C_8 column connected directly to a UV detector. The chromatographic conditions were optimized with: the macromolecules' exclusion time of 3 min, at the flow rate of 1 mL min^{-1}, and a sample injection of 1000 μL using 100% of ultrapure water as mobile phase (Fig. 1 and Table 2 – eluent A). Under this condition, LAN and PAN were retained as racemic mixtures by the RAM column. To estimate the transfer time, the spring water was spiked with high concentrations of (±) PAN and (±) LAN (50 μg mL^{-1}). The analytes were transferred to the chiral column using CH_3CN:H_2O (35:65, v/v). In order to avoid undesired adsorption of humic substances and/or other substances into the column, CH_3CN:H_2O (80:20, v/v) was used as solvent for the clean-up of the RAM column (Table 2).

The elution order of enantiomers was determined, under the developed chromatographic conditions, using a circular dichroism detector coupled to the 2D chromatographic system. The first enantiomer to elute was (+)-LAN followed by (+)-PAN, (−)-LAN and (−)-PAN, respectively (Fig. 2).

3.2. Validation study

3.2.1. Matrix effects

The matrix effects were investigated by post-column infusion and by on-line sample extraction, as described in Section 2. During the post-column infusion assay, ion suppression was observed only in the beginning of the chromatogram. However, on the on-line sample extraction the PAN enantiomers showed much higher ion suppression: (+)-LAN 13% and (−)-LAN 3% and (+)-PAN 27% and (−)-PAN 32%. As Trufelli et al. [26] point out, information regarding the signal enhancement is still lacking. The importance of chemical structure on the matrix effect is here emphasized since PAN is analogous to LAN. Recently, van Nuijs et al. reported [40] 90% signal suppression for racemic PAN when using Oasis MCX cartridges as sample preparation. This demonstrates the high efficiency of the RAM BSA column for reducing matrix effects of environmental samples.

Table 3
Linearity parameters, detection and quantification limits for the method.

Compounds	Range (μg L^{-1})	r	LOD (μg L^{-1})	LOQ (μg L^{-1})
(+)-LAN	0.600–38.4	0.999	0.150	0.600
(−)-LAN		0.999		
(+)-PAN	0.400–25.6	0.999	0.200	0.400
(−)-PAN		0.997		

3.2.2. Detection

The LAN and PAN enantiomers were analyzed in positive ion mode (ESI+), while multiple reaction-monitoring (MRM) mode was carried out for the acquisition. The protonated molecular ion $[M+H]^+$ of LAN (m/z = 370) and PAN (m/z = 384) was used as precursor ion. The isolation width was set at 2.0 m/z. Two MRM transitions were monitored and based on the EU Commission Decision 2002/657/EC the MRM ratio and the deviation of the retention time were used to confirm the presence of LAN and PAN in the samples [41]. The first transitions for both pair of the enantiomers were used for quantification and the second ones for confirmatory purposes. The first transition for the LAN corresponds to the loss of $[M-C_7H_5N_2]^+$ (m/z = 252) and the second one to the loss of $[SO]^+$ (m/z = 205). For the PAN, the first transition corresponds to the loss of $[M-C_8H_5N_2F_2O]^+$ (m/z = 200) and the second one to the loss of $[CSOH]^+$ (m/z = 138) (Fig. 3). The MS/MS fragmentation pattern is in accordance with the data previously reported for this compound [42,43].

3.2.3. Linearity, LOQs, LODs, extraction efficiency, accuracy and precision

The calibration curves were linear in the range studied for each enantiomer of LAN and PAN, respectively, with mean correlation coefficients (n = 3) of 0.99 or higher. The LOQ and LOD obtained are given in Table 3. Accuracy values between 86 and 108% and inter and intra-day precision with RSD in the range of 0.2–7.4% were obtained with extraction efficiency between 99 and 110%. No any tendencies in the change of the values between days or compounds could be observed. All values were in the range of accepted criteria, especially considering that QCs were prepared as replicates (n = 5) and not just a consecutive sequence of injections of a same sample [35]. Although the spiked samples showed stability for autosampler time (16 h), they were unstable in 24 h period, with results of accuracies out of the established criteria [35].

3.3. Application of the method to wastewater samples

The developed method was applied to wastewater samples collected directly from the discharge along Monjolinho River. For the analyses, new calibration curves were made for each sample batch. Three QC were injected between samples. LAN and PAN enantiomers were not found at the sampling sites.

To our knowledge, there are no methods in the literature for quantification of LAN and PAN enantiomers. However, methods using off-line SPE for quantification of either LAN or PAN as racemates in aqueous matrices (such as rivers, influents and effluents of WWTP) have been recently reported [40,42–44]. The LAN was not detected in these different matrices studied, whereas PAN was found in levels of 0.15 μg L^{-1} and 0.18 μg L^{-1} in treated effluents [43,44]. In river water, it was found at 0.013 μg L^{-1} [44].

The concentrations reported for racemic PAN are in the range (around 5×) of the LOQs here described for each enantiomer of PAN. Bearing in mind that the published methods used pre-concentrated samples and also the low stability of these PPIs in sample preparation [40,45], a direct injection of native aqueous matrix for tracking the enantiomers of LAN and PAN is innovative and should be further explored for evaluating enantiomeric fraction of these PPIs.

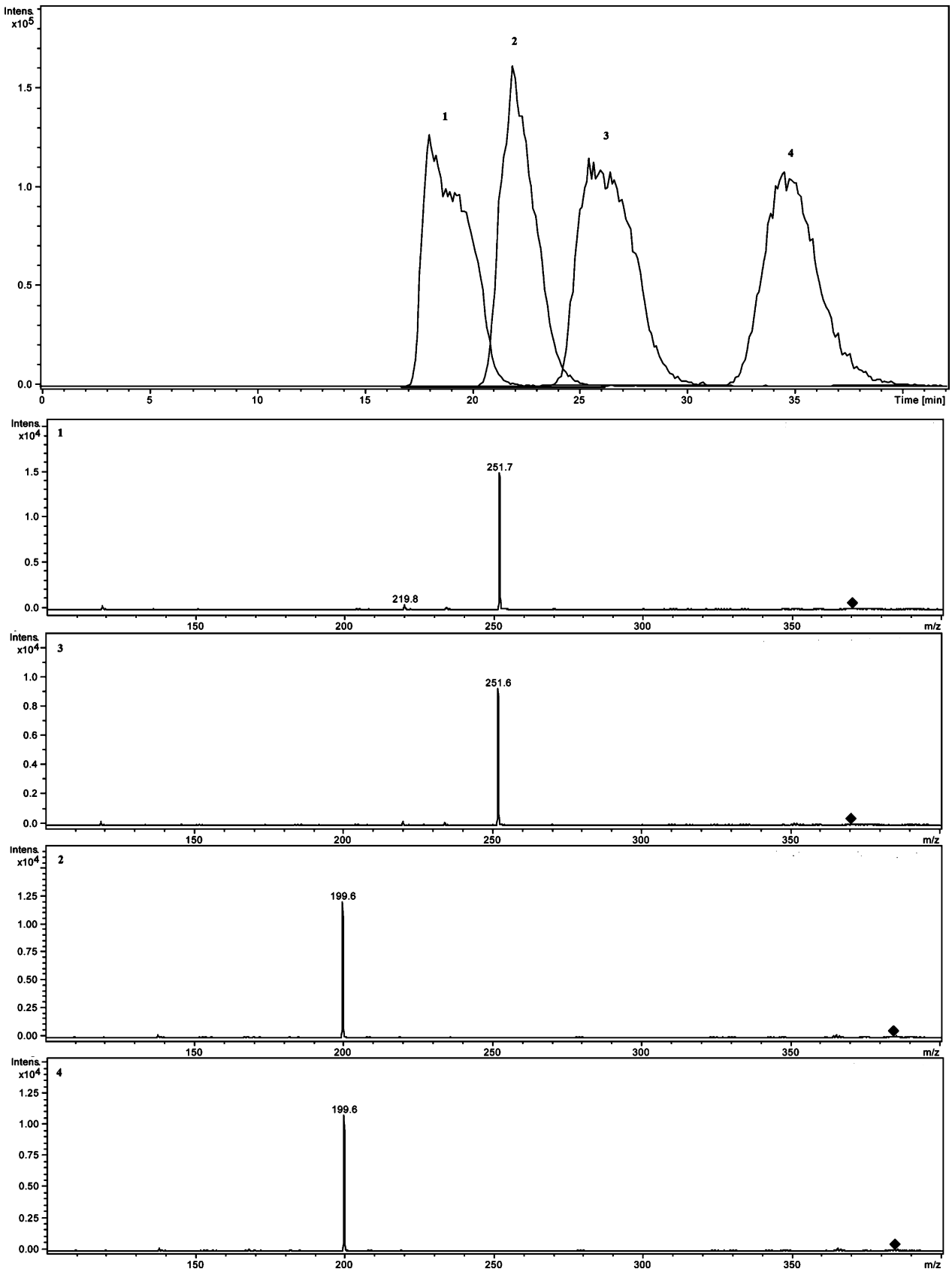


Fig. 3. Extracted ion chromatograms and mass spectra for the medium QC sample.

4. Conclusion

The use of a coupled achiral–chiral method was successfully achieved for simultaneous quantification of LAN and PAN enantiomers in native wasterwater samples. The analysis time is of only 40 min without any sample treatment and it is expected to be a new tool for environmental purpose such as: monitoring work, studies on biotic and abiotic enantioselective degradation and on temporal changes of the enantiomeric fractions.

The method provided good sensitivity and selectivity of detection according with European Commission Decision [41].

Acknowledgements

The authors acknowledge financial support from Fundação de Amparo à Pesquisa e Desenvolvimento do Estado de São Paulo (FAPESP, Grants 2006/05126-9 and 2008/05778-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant 471775/2008-8).

References

[1] S.D. Richardson, Anal. Chem 82 (2010) 4742.
[2] C. Lacey, G. McMahon, J. Bones, L. Barron, A. Morrissey, J.M. Tobin, Talanta 75 (2008) 1089.
[3] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, in: 14th European Conference on Analytical Chemistry, Anal. Bioanal. Chem. (2008) 1293.
[4] E. Botitsi, C. Frosyni, D. Tsipi, Anal. Bioanal. Chem. 387 (2007) 1317.
[5] M.D. Hernando, E. Heath, M. Petrovic, D. Barcelo, in: Workshop on Analysis and Removal of Contaminants from Wastewaters for the Implementation of the Water Framework Directive, Anal. Bioanal. Chem. (2006) 985.
[6] N. Lindqvist, T. Tuhkanen, L. Kronberg, Water Res. 39 (2005) 2219.
[7] T.V. Madureira, J.C. Barreiro, M.J. Rocha, Q.B. Cass, M.E. Tiritan, J. Chromatogr. A 1216 (2009) 7033.
[8] A.Y.C. Lin, T.H. Yu, C.F. Lin, Chemosphere 74 (2008) 131.
[9] S. Terzic, I. Senta, M. Ahel, M. Gros, M. Petrovic, D. Barcelo, J. Muller, T. Knepper, I. Marti, F. Ventura, P. Jovancic, D. Jabucar, Sci. Total Environ. 399 (2008) 66.
[10] M. Kostopoulou, A. Nikolaou, Trends Anal. Chem 27 (2008) 1023.
[11] D. Fatta, A. Nikolaou, A. Achilleos, S. Meric, Trends Anal. Chem. 26 (2007) 515.
[12] T. Harner, K. Wiberg, R. Norstrom, Environ. Sci. Technol. 34 (2000) 218.
[13] N.H. Hashim, S. Shafie, S.J. Khan, Environ. Technol 31 (2010) 1349.
[14] L.N. Nikolai, E.L. McClure, S.L. MacLeod, C.S. Wong, J. Chromatogr. A 1131 (2006) 103.
[15] L.J. Fono, D.L. Sedlak, Environ. Sci. Technol. 39 (2005) 9244.
[16] I. Ali, P. Singh, H.Y. Aboul-Enein, B. Sharma, Anal. Lett. 42 (2009) 1747.
[17] S.L. MacLeod, P. Sudhir, C.S. Wong, J. Chromatogr. A 1170 (2007) 23.
[18] S.L. MacLeod, C.S. Wong, Water Res 44 (2010) 533.
[19] J.C. Barreiro, K.L. Vanzolini, T.V. Madureira, M.E. Tiritan, Q.B. Cass, Talanta 82 (2010) 384.
[20] B. Kasprzyk-Hordern, V.V.R. Kondakal, D.R. Baker, J. Chromatogr. A 1217 (2010) 4575.
[21] H. Huhnerfuss, M.R. Shah, J. Chromatogr. A 1216 (2009) 481.
[22] S. Perez, D. Barcelo, Trends Anal. Chem. 27 (2008) 836.
[23] M. Petrovic, M. Farre, M.L. de Alda, S. Perez, C. Postigo, M. Kock, J. Radjenovic, M. Gros, D. Barcelo, J. Chromatogr. A 1217 (2010) 4004.
[24] G.W. Lien, C.Y. Chen, G.S. Wang, J. Chromatogr. A 1216 (2009) 956.
[25] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, J. Chromatogr. A 1217 (2010) 3929.
[26] H. Trufelli, P. Palma, G. Famiglini, A. Cappielo, Mass Spectr. Rev. (2010), doi:10.1002/mas.20298.
[27] N.M. Cassiano, V.V. Lima, R.V. Oliveira, A.C. de Pietro, Q.B. Cass, Anal. Bioanal. Chem. 384 (2006) 1462.
[28] N.M. Cassiano, J.C. Barreiro, M.C. Moraes, R.V. Oliveira, Q.B. Cass, Bioanalysis 1 (2009) 577.
[29] J. Chico, S. Meca, R. Companyo, M.D. Prat, M. Granados, J. Chromatogr. A 1181 (2008) 1.
[30] K. Hoshina, S. Horiyama, H. Matsunaga, J. Haginaka, J. Chromatogr. A 1216 (2009) 4957.
[31] J. Ding, N.Q. Ren, L.G. Chen, L. Ding, Anal. Chim. Acta 634 (2009) 215.
[32] S.A. Matlin, M.E. Tiritan, A.J. Crawford, Q.B. Cass, D.R. Boyd, Chirality 6 (1994) 135.
[33] N.M. Cassiano, Q.B. Cass, A.L.G. Degani, I.W. Wainer, Chirality 14 (2002) 731.
[34] M.L. Menezes, G. Felix, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 3221.
[35] U.S. Food and Drug Administration, Bioanalytical method validation, in: Guidance for Industry, U.S. Food and Drug Administration, 2001, p. 1.
[36] Q.B. Cass, V.V. Lima, R.V. Oliveira, N.M. Cassiano, A.L.G. Degani, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 798 (2003) 275.
[37] R.F. Gomes, N.M. Cassiano, J. Pedrazzoli, Q.B. Cass, Chirality 22 (2010) 35.
[38] Q.B. Cass, A.L.G. Degani, N.M. Cassiano, J. Pedrazolli, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 766 (2002) 153.
[39] Q.B. Cass, A.L.G. Degani, N.M. Cassiano, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 2083.
[40] A.L.N. van Nuijs, I. Tarcomnicu, W. Simons, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Anal. Bioanal. Chem. 398 (2010) 2211.
[41] Commission Decision (2002/657/EC) of 12 August 2002, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities L221, Brussels, Belgium, p. 8.
[42] M. Gros, M. Petrovic, D. Barcelo, Talanta 70 (2006) 678.
[43] E. Gracia-Lor, J.V. Sancho, F. Hernandez, J. Chromatogr. A 1217 (2010) 622.
[44] K. Nodler, T. Licha, K. Bester, M. Sauter, J. Chromatogr. A 1217 (2010) 6511.
[45] M. DellaGreca, M.R. Iesce, L. Previtera, M. Rubino, F. Temussi, M. Brigante, Chemosphere 63 (2006) 1087.