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Multimilligram enantioresolution of sulfoxide proton pump inhibitors by liquid chromatography on polysaccharide-based chiral stationary phase

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Abstract

The enantiomers of sulfoxide proton pump inhibitors – omeprazole, lansoprazole, rabeprazole and Ro 18-5364 – were enantiomerically separated by liquid chromatography at multimilligram scale on a polysaccharide-based chiral stationary phase using normal and polar organic conditions as mobile phase. The values of the recovery and production rate were significant for each enantiomer; better results were achieved using a solid-phase injection system. However, this system was applied just for the enantiomeric separation of omeprazole to demonstrate the applicability of this injection mode at milligram scale. The chiroptical characterization of the compounds was performed using a polarimeter and a circular dichroism detector. The higher enantiomeric purity obtained for the isolated enantiomers suggests that the methods here described should be considered as a simple and rapid way to obtain enantiomeric pure standards for analytical purpose.
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Keywords: Omeprazole; Lansoprazole; Rabeprazole; Ro 18-5364; Semipreparative chromatography; Enantiomer separation

1. Introduction

Omeprazole ((±)-5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridin-2-yl) methylsulfinyl]-3H-benzoimidazole)—OME; lansoprazole ((±)2-[(3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl)methylsulfinyl]-1H-benzoimidazole)—LAN; rabeprazole ((±)-2-[[4-(3-methoxypropoxy)-3-methyl-pyridin-2-yl] methylsulfinyl]-1H-benzoimidazole)—RAB; and more recently, Ro 18-5364 ((±)-5,7-dihydro-2-(4-methoxy-3-methyl-2-pyridinyl)methylsulfinyl)-5, 5, 7, 7-tetramethylindeno-[5, 6-d]-imidazol-6-(1H)-one)—Ro (Fig. 1) have been widely used as anti-ulcer drugs – proton pump inhibitors (PPIs) – for the treatment of gastric acid hypersecretion disorders by covalently binding to the proton pump (H^+/K^+ ATPase) at the surface of gastric parietal cells inhibiting the final step in secretion of the H^+ ions into the gastric lumen [1–6].

All these PPIs have a stereogenic center at the sulfur atom and they are clinically administered as a racemic mixture [7–12]. Nevertheless, AstraZeneca has carried out the chiral switch of omeprazole to its (*S*)-(−)-enantiomer, as esomeprazol magnesium, and under the trade name of Nexium™ it was launched in Europe in 2000 and in the United States in 2001 [7,13,14]. The PPIs are extensively metabolized in the liver via cytochrome P450 enzyme system exhibiting polymorphic metabolism in humans justifying the great interest in enantiomeric separation methods for this class of pharmaceutical compounds [6,15–18].

A number of publications have reported the successful resolution of these PPIs, at analytical (*e.g.* human plasma determination) and preparative scale separation, on different types of chiral stationary phases including the derivatives of amylose and cellulose [1,3,5,8,17–25].

The polysaccharide-based phase has shown to be effective on multimodal elution (normal, reverse and organic polar mode), broadening the application for different compounds—being the phenyl carbamate derivatives the most successful CSPs

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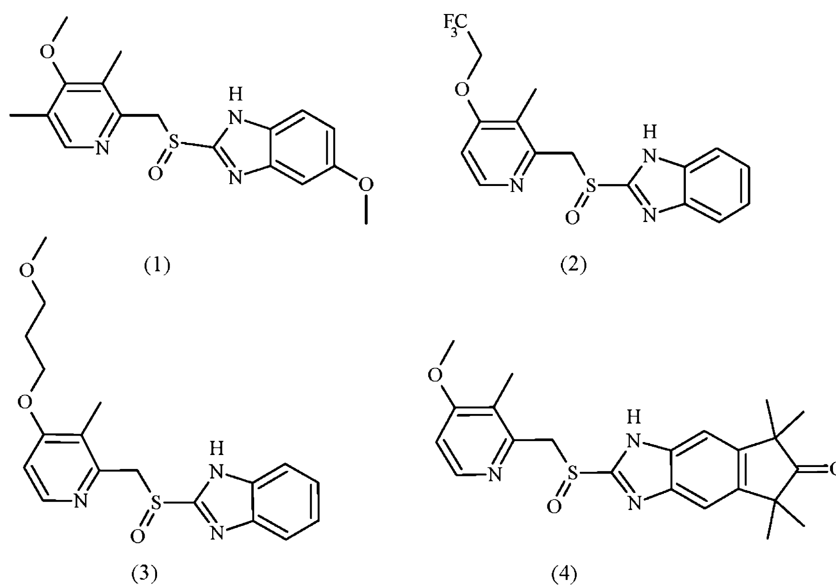

Fig. 1. Chemical structure of PPIs (1) OME, (2) LAN, (3) RAB and (4) Ro.

[26,27]. The use of these chiral phases has been previously demonstrated on many occasion [28–34], as in the direct separation of multimilligram quantities of gossypol enantiomers using tris-3,5-dimethylphenylcarbamate cellulose phase on the reversed mode of elution, with efficient recovery for the enantiomers (80%) [35]. Sousa et al. [36] also showed the versatility of these polysaccharide phases, under polar organic conditions, in the separation of the xanthonolignoids enantiomers in multimilligram scale.

A thorough literature search has revealed that only a few liquid chromatographic methods are described for preparative scale separations of the racemic benzimidazoles mixtures [24, 37,38]. Thus, the aim of the present work was to evaluate the enantiomeric separation of OME and related sulfoxides, LAN, RAB and Ro, in multimilligram scale by HPLC on polysaccharide-based phases under normal phase (hexane: ethanol) and polar organic (methanol) conditions. The conditions described in this paper develop an alternative method for achieving enantiomeric pure standards for analytical purpose. Moreover, OME was used as a model compound for comparing the productivity and recovery of the conventional injection method, classical loop injection, with the solid-phase injection mode. Also, the enantiomeric purities of the isolated PPIs enantiomers were verified by polarimeter and circular dichroism (CD) spectroscopy.

2. Experimental

2.1. Equipment

The preparative HPLC system consisted of a Shimadzu LC-6AD pump, a Rheodyne 7725 injector fitted with a 200 μL loop or a cylindrical stainless steel pre-column coated with Teflon for the injections of samples, and a 10 AVvp variable wavelength UV–vis detector with a CBM SCL-10 AVvp interface. Data acquisition was performed using CLASS-VP software. The solid-phase injector system was the same used and described by Sousa et al. [36].

The analytical system consisted of a two Shimadzu LC-10 ADvp pumps, an SIL-10 ADvp auto injector fitted with a 20 μL loop, and an SPD-M10 AVvp UV–vis detector with a SCL-10 AVvp interface. Data acquisition was performed using LAB SOLUTION software. A circular dichroism (CD) detector was used for the identification of enantiomers. The analysis was performed in the analytical system, described above, with a JASCO CD-2095 plus chiral detector. All HPLC analyses were performed at room temperature. The optical activity of the separated enantiomers was defined in ethanol, at room temperature, using a PerkinElmer Model 241 polarimeter with a sodium lamp.

2.2. Materials and reagents

All solvents were of HPLC grade, purchased from Merck (Darmstadt, Germany) and Mallinckrodt Baker (St. Louis, USA). Triethylamine 99% (TEA) and acetic acid glacial (Ac) were purchased from Sigma–Aldrich (Steinheim, Germany) and JT Baker (Mallinckrodt Baker, Mexico). The mobile phases were prepared in a volume/volume relation and degassed for 10 min in an ultrasonic bath prior to use. The OME was generously supplied by Fundação Oswaldo Cruz (FIOCRUZ), LAN by Boehiringer Ingelhein, RAB by Clinical Research Center, and Ro 18-5364 by F. Hoffmann–LaRoche Ltd.

2.3. Chiral stationary phases

The columns were prepared at the UFSCar laboratory as described elsewhere [30,33,39]. The tris-3,5-dimethylphenylcarbamate and tris[(*S*)-1-phenylethylcarbamate of amylose were coated on to APS-Nucleosil (500 Å, 7 μm, 20%, w/w) and packed into a stainless-steel 20 cm × 0.7 cm i.d. size column for semipreparative chromatography (CSP

Table 1
Semipreparative and analytical chromatographic conditions for the OME, LAN, RAB and Ro separations

Compounds	Semipreparative conditions				Analytical conditions		
	Column(CSP)	Mobile phase	Flow rate (mL min^{-1})	λ (nm)	Column (CSP)	Mobile phase	Flow rate (mL min^{-1})
OME	1	MeOH (100%)	3.0	302	3	MeOH (100%)	1.0
LAN	5	hexane:ethanol (70:30, v/v)	2.5	289	4	hexane:ethanol (70:30, v/v)	0.8
RAB	2	hexane:ethanol (80:20, v/v + 0.02% Ac + 0.02% TEA)	2.0	289	4	hexane:ethanol (80:20, v/v + 0.02% Ac + 0.02% TEA)	0.8
Ro	2	hexane:ethanol (80:20, v/v + 0.02% Ac + 0.02% TEA)	2.0	289	4	hexane:ethanol (80:20, v/v + 0.02% Ac + 0.02% TEA)	0.8

1 and 2) and into a 15 cm × 0.46 cm i.d. size column for analytical separation (CSP 3 and 4, respectively). Amylose tris[(*S*)-1-phenylcarbamate coated on to APS-Hypersil® (120 Å, 5 μm, 25%, w/w) packed into a stainless-steel 20 cm × 0.7 cm semipreparative column (CSP 5) was also used. A Shandon HPLC packing pump was employed for column packing.

2.4. Sample preparation

For the conventional sample injection method, at multi-milligram scale, the solutions were prepared as racemic mixture of each compound in an organic solvent (methanol or ethanol).

For OME, 16.0 mg was dissolved in 2 mL of methanol (8.0 mg mL^{-1} of each enantiomer); RAB, 35.2 mg was dissolved in 2 mL of ethanol (17.6 mg mL^{-1} of each enantiomer); Ro, 15.0 mg was dissolved in 1.6 mL of ethanol (9.37 mg mL^{-1} of each enantiomer); LAN, 15.0 mg was dissolved in 2.0 mL of ethanol (7.5 mg mL^{-1} of each enantiomer).

For solid-phase injection, 4.0 mg of OME was mixed with 500 mg of Merck silica gel 60 (0.50–0.20 mm). The mixture was moistened with methanol and packed in a stainless-steel pre-

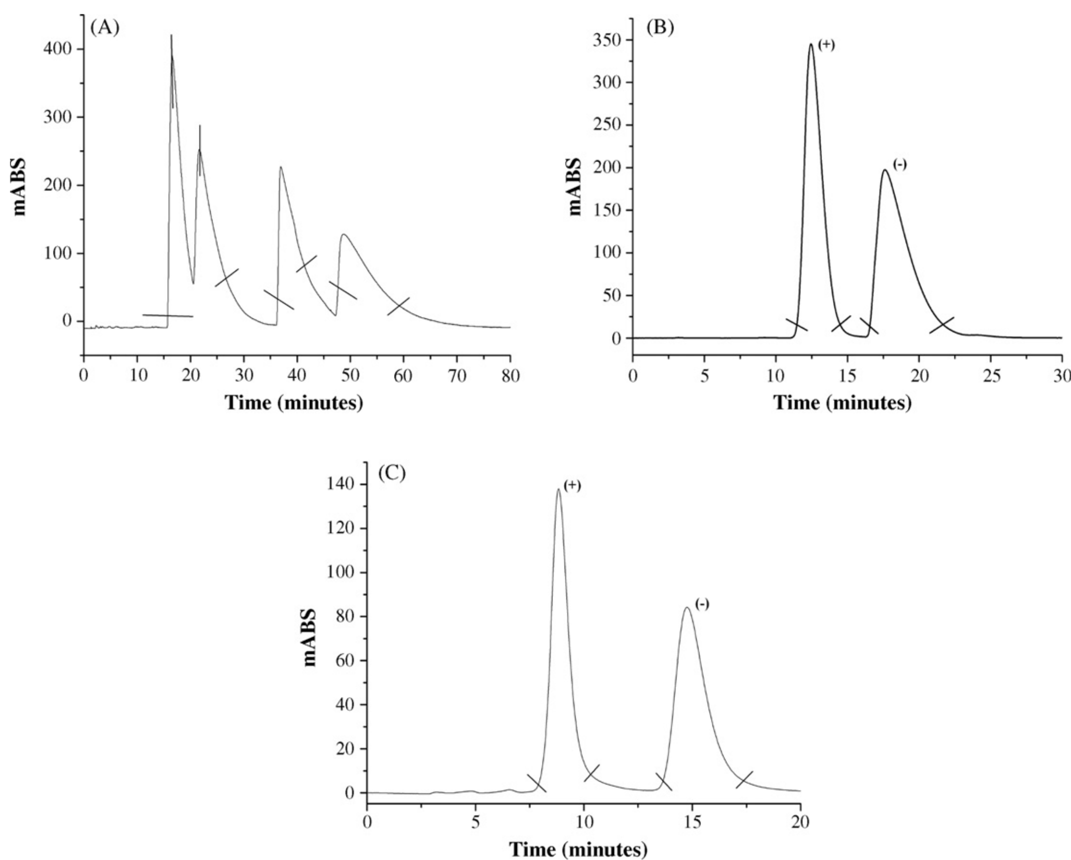

Fig. 2. Chromatograms of the enantiomeric semipreparative separations of (A) LAN, (B) RAB and (C) Ro. Chromatographic conditions—(A) column: CSP 5, mobile phase: hexane:ethanol (70:30, v/v), flow-rate 2.5 mL min^{-1}, λ = 289 nm; (B) and (C) column: CSP 2, mobile phase: hexane:ethanol (80:20, v/v + 0.02% Ac + 0.02% TEA), flow-rate: 2.0 mL min^{-1}, λ = 289 nm.

Table 2
Recovery, e.p., and specific rotation ($[\alpha]_D$) for OME, LAN, RAB and Ro

Enantiomer	Recovery (%)	e.p. (%)	$[\alpha]_{D\ ethanol\ (25\,^{\circ}C)}$	Yield (mg h^{-1})
(+) OME	88.7	97.5	+81.8	7.1
(−) OME	91.2	99.7	−101.0	7.3
(+) OME	96.2	99.2	+98.0	3.08
(−) OME	98.7	96.1	−83.7	3.16
(+) LAN	84.0	99.9	+142.4	1.51
(−) LAN	69.3	95.3	−124.4	0.97
(+) RAB	91.5	99.9	+74.7°	3.86
(−) RAB	89.2	99.6	−73.4°	3.77
(+) Ro	90.7	99.5	+76.4	2.55
(−) Ro	86.7	99.1	−74.6	2.44

column (15 mm × 8.0 mm i.d.). Each run with the solid-phase injector consisted in connecting the pre-column on the top of the semipreparative chiral column.

2.5. Chromatographic conditions

Semipreparative chromatographic separations were first achieved through multiple injections fitted with a 200 μL loop, under different flow rate and mobile phase conditions. The collected fractions of each enantiomer were rotoevaporated at room temperature and analyzed to determine their enantiomeric purity using the analytical column with a 20 μL loop. Mobile phase compositions and chromatographic parameters are summarized in Table 1.

3. Results and discussion

3.1. Multimilligram separation using the conventional injection method for LAN, RAB and Ro

A number of publications have described resolution of chiral sulfoxides, such as the benzimidazoles PPIs, on different types of chiral stationary phases including the polysaccharide-based column and thus, justifying their selection for carrying out this work [2,17,18,22,26,28,31,32,40–44].

To achieve better separation and good band shapes for the selected PPIs different mobile phases containing varying percentages of organic phase were systematically evaluated [17,18,45]. These evaluations confirmed the high chiral recognition ability of the amylose tris[(*S*)-1-phenylethylcarbamate] phase for this class of analytes at the normal elution mode, using ethanol as organic modifier, and a low resolution at reversed-phase and polar elution mode. The amylose tris-3,5-dimethylphenylcarbamate phase, on the other hand, showed a higher resolution power for these PPIs at reverse-phase elution mode [17,18,26,45], whereas the polar elution mode using methanol afforded base-line resolution of OME. Nevertheless, the different parameters, like separation factor (α), resolution (R_s) and retention factors (k), obtained [45] will be discussed in a later paper. So, the results reported in this paper have been based on the optimized conditions previously obtained during extensive chromatographic investigation.

The conventional mode of injection system was used to perform the semipreparative separation of LAN, RAB and Ro. Representative chromatograms are shown in Fig. 2. All three compounds were eluted at normal elution mode for the semipreparative and analytical separations using hexane:ethanol (70:30, v/v) for LAN and hexane:ethanol (80:20, v/v + 0.02% AcOH + 0.02% TEA) for RAB and Ro, as mobile phases (Table 1). TEA and AcOH were added as modifier additives to enhance solute band shape, getting also a decrease on the retention time [30]. For LAN the racemic mixture was injected in 10 applications of 200 μL each with recycling; whereas for RAB and Ro the racemic mixtures were injected in 10 and 8 applications, respectively, in the same amount. Recycling was used only for the LAN compound to improve the efficiency of the separation and thus achieving the desired purity [46]. We previously demonstrated the use of recycling for improving sepa ration at multimilligram scale [35]. Here, we used it again due to the lower resolution level observed for the semipreparative column used owing to the silica used as support. The Hypersil® silica has higher acidity when compared with Nucleosil® silica [47] and we previously demonstrated [26] that this can diminishes the enantioresolution of polar compounds due to secondary non-specific interactions and it might be responsible for the low recovery level observed for the LAN enantiomers (Table 2).

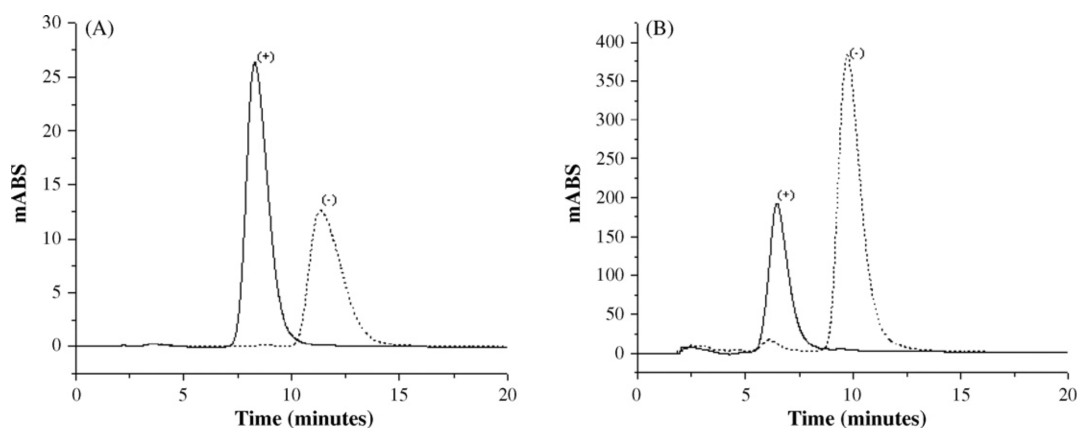

Fig. 3. Analytical evaluations of collected fractions from the semipreparative separations of (A) (+) RAB/(−) RAB and (B) (+) Ro/(−) Ro. Chromatographic conditions—column: CSP 4, mobile phase: hexane:ethanol (80:20, v/v + 0.02% Ac + 0.02% TEA), flow-rate 0.8 mL min^{-1}, injection volume: 20 μL, λ = 289 nm.

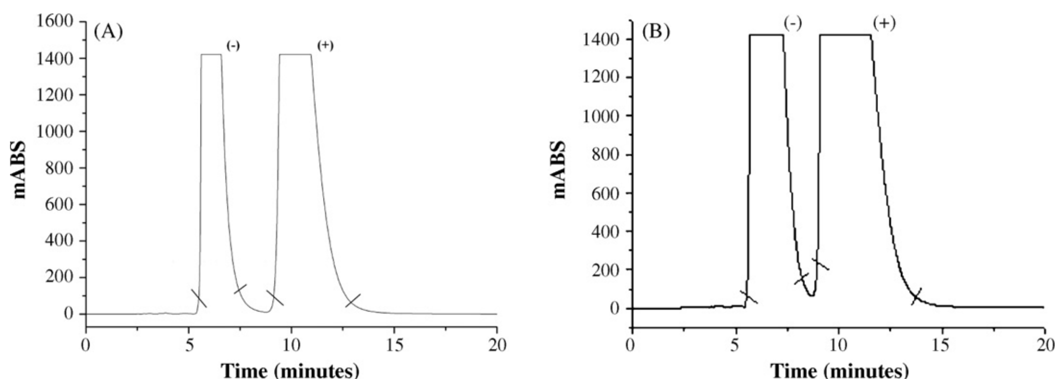

Fig. 4. Chromatograms of the enantiomeric semipreparative separations of OME: (A) conventional system and (B) solid-phase injection. Chromatographic conditions—column: CSP 1, mobile phase: 100% MeOH, flow-rate 3.0 mL min^{-1}, $\lambda = 302$ nm.

The fractions were collected at time intervals of 3 min (first enantiomer) and 5 min (second enantiomer), from the beginning to the end of each band of RAB; while for Ro the times used were 2 min (first enantiomer) and 5 min (second enantiomer).

The analytical chromatograms of the isolated enantiomers of RAB and Ro are given as selected examples in Fig. 3A and B, respectively. Table 2 gives also the calculated enantiomeric purity (e.p.) of all isolated enantiomers. The (−)-enantiomers eluted as the more retained chromatographic band for LAN, RAB and Ro. The second chromatographic band is always more difficult to isolate with high e.p., especially when the enantiomeric resolution is lower. This was observed for the (−)-LAN enantiomer.

It is worth emphasizing that the literature about enantiomeric separation of Ro is quite poor [38] and this paper is the first to report it in a multimilligram scale.

The enantiomeric purity reported here for all compounds was defined as the ratio between *R* (enantiomer) area divided by the sum of the *R* and *S* area multiplied by 100 [48,49].

3.2. Multimilligram separation of OME using the conventional and solid-phase injections systems

Solubility should be considered as an important factor at multimilligram/preparative scale chromatographic separation, as it can limit the injected quantity, damaging the productivity of the separation. Thus, solid-phase injection arises as alternative method as allows a larger amount of an enantiomeric mixture to be loaded in cases of poor solubility. This system was used with success for the multimilligram separation of compounds with low solubility, *e.g. rac-trans*-kielcorin C racemate [36]. OME is quite soluble, however great differences in the productivity of this method were observed compared to the conventional injection system (Table 2).

For the OME semipreparative separation, using the conventional injection system, 16 mg amount of OME racemic mixture was injected in 10 applications of 200 μL each, while in the solid-phase injection just four applications were necessary (Fig. 4). In both systems, the separation was performed in closed recycling loop in the polar elution mode with 100% of methanol.

The fractions were collected at 2 min time intervals, from the beginning to the end of each peak for the first enantiomer and at 3 min for the second. These collected fractions were analyzed in the analytical column, in order to evaluate the enantiomeric purity of each enantiomer, using the same mobile phase of the semipreparative conditions (Table 1). The (−)-enantiomer of OME, in this elution condition, eluted as the first enantiomer. The e.p. of the isolated enantiomers are given in Table 2.

The comparison of the two injection systems, solid-phase and traditional injector loop, for the OME multimilligram separation, demonstrated that the analysis was 2.5 times faster for

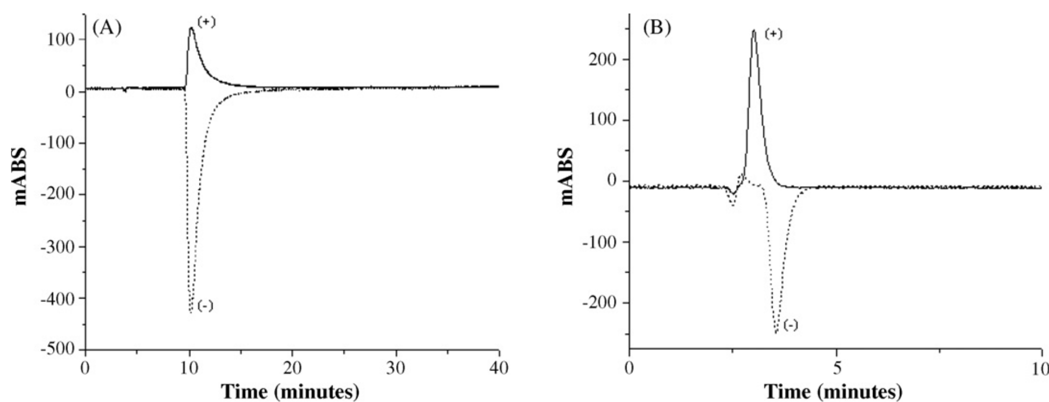

Fig. 5. The CD chromatograms of the isolated enantiomers – (A) OME and (B) LAN at the polarimeter conditions – 100% ethanol, injection volume: 20 μL, concentration 50 μg mL^{-1}, $\lambda_{OME} = 302$ nm and $\lambda_{LAN,\ RAB\ and\ Ro} = 289$ nm.

the first system. In addition, this system allows the use of larger amounts of racemic mixture, at each injection, decreasing the injection numbers, time of analysis and the solvent used. In spite of this, the recoveries or e.p. of the enantiomers isolated by both systems did not show significant differences; however, it was observed that the throughput was 2.3 times higher using the solid-phase system (Table 2).

The absolute configurations for the PPIs have been reported [38]. Thus, in order to assess the absolute configuration of each of the isolated enantiomers, the optical activities of the enantiomers of highest enantiomeric purity were measured using a polarimeter ($[\alpha]_{\mathrm{D\ ethanol,\ 25\,^{\circ}C}}$) [50]. The results of optical rotation were used for comparison with the published results [38]. Values are given in Table 2. The results show that the optical rotations of the isolated enantiomers are in agreement with the results observed using the CD detector. Since the CD detector responds only to chiral compounds, its use is of great value for complex samples, where only the chiral bands are of interest, making simple enantiomeric purity evaluation. Furthermore, this type of detection is stable for changes in temperature and mobiles phases [51]. Fig. 5A and B illustrates the chromatograms obtained using the CD detector for the enantiomers of OME and LAN, respectively.

4. Conclusions

The polysaccharide-based phases demonstrated excellent capacity for enantiomeric resolution in multimilligram scale for the benzimidazoles series of drugs. All enantiomers of each compound were obtained with a high degree of enantiomeric purity and high recoveries. For LAN enantiomers lower recoveries and productivities were observed; however, the first enantiomer (+)-(*R*)-lansoprazole showed high enantiomeric purity. For all the enantiomeric separations, with exception of OME, the first enantiomer to elute was (+)-*R*-enantiomer, with a lower retention time.

The highest production rates were achieved when the solid-phase injector system was compared with the conventional injection system; proving that the solid-phase should be taken as an alternative injection mode for multimilligram scale chiral separations. Therefore, both injection systems used have shown satisfactory recovery rate.

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