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Original Paper

Semipreparative chiral supercritical fluid chromatography in the fractionation of lansoprazole and two related antiulcer drugs enantiomers

The semipreparative chiral separation of lansoprazole and two related compounds (pantoprazole and rabeprazole) using supercritical fluid chromatography (SFC) is presented in this work. Different loads were evaluated in order to obtain high enantiomeric purities and production rates. The volumes injected were 1, 2 and 4 mL. The concentrations of the racemic mixtures were 3 and 6 g/L for lansoprazole and 1.5 g/L for pantoprazole and rabeprazole. In all the cases, the recoveries, for a purity higher than 99.9%, were better for the second eluted enantiomer than for the first one. This fact conditioned the production rate of the first eluted enantiomer that, considering a fixed purity, was always lower than that obtained for the other one. In the case of lansoprazole it was possible to obtain 0.025 and 0.090 mg/min of the first and second eluted enantiomer, respectively, with an enantiomeric purity of 99.9%. For rabeprazole enantiomers 0.037 and 0.062 mg/min, and in the case of pantoprazole the results were better (0.062 and 0.122 mg/min) due to the higher resolution.

Keywords: Enantiomeric resolution / Lansoprazole / Pantoprazole / Rabeprazole / Semipreparative chromatography

Received: September 20, 2007; revised: November 28, 2007; accepted: December 2, 2007

DOI 10.1002/jssc.200700457

1 Introduction

Lansoprazole, pantoprazole and rabeprazole are substituted benzimidazole sulfoxides with a selective and long-acting proton pump inhibitor activity (PPIs). They are some of the most potent therapeutic agents used in the treatment of acid–peptic disorders such as gastroesophageal reflux disease, peptic ulcer disease, gastric ulcer and pathologic gastrointestinal hypersecretory conditions (Zollinger-Ellison syndrome) [1].

These drugs present an asymmetric sulfoxide centre in their molecules and are used clinically as racemic mixtures, *i.e.* 50:50 mixture of (+)-(*R*)- and (−)-(*S*)-enantiomers.

However, despite the fact that identical pharmacological effects of the (*R*)- and (*S*)-enantiomers of lansoprazole, for the inhibition of acid secretion have been considered until now, there are studies which reflect that (−)-(*S*)-lansoprazole is more effectively metabolised to pharmacologically inactive 5-hydroxy and sulphone metabolites [2, 3]. Pantoprazole and rabeprazole have also been found to have enantioselective pharmacokinetics since they present differences in the kinetic parameters and effects of its enantiomers: slightly higher serum concentrations of the (−)-(*S*)-enantiomer compared with those of the (+)-(*R*)-form [4, 5].

The interest in generating individual enantiomers has become a priority in the last few years for the pharmaceutical industry, with many of the top-selling drugs in the world now being sold in the enantiopure form [6]. Owing to the existence of pharmacological and toxicological differences between a pair of enantiomers, the study of its biological activities and effects is an important step in any chiral drug development process, and therefore small quantities of them are required for the comparative tests (www.fda.gov/cder/guidance/stereo.htm). Chiral chromatography has been proven to be an important tool for the generation of small quantities of enantiomers during early pharmaceutical research and development [7–10]. Although preparative HPLC on chiral stationary phases (CSPs) has usually been the choice for many separations, preparative chiral supercritical fluid chromatography (SFC) has emerged as a competitive technique in the last few years [10–16]. This

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Abbreviations: CSP, chiral stationary phase; **SFC,** supercritical fluid chromatography

technique offers a green advantage, reducing the solvent use and waste generation. Moreover, due to the low viscosity of supercritical fluids, separations can be performed at high flow rates, which contribute to a higher productivity.

Among the wide variety of CSPs available for the direct separation of enantiomers, the commercial polysaccharide based CSPs, are some of the most popular. They have shown good chiral recognition ability towards a wide number of different compounds [7, 17 – 24].

The aim of this work was to study the semipreparative SFC enantiomeric separation of lansoprazole, pantoprazole and rabeprazole, trying to obtain small quantities of each enantiomer with a high optical purity. For this purpose the Chiralpak AD column was used and different injection volumes and concentrations of the racemic mixtures were evaluated.

2 Experimental

2.1 Reagents

Lansoprazole, pantoprazole and rabeprazole (Fig. 1) were purchased in their racemic form from Sigma – Aldrich (Madrid, Spain).

The organic solvents methanol and absolute ethanol were obtained from Scharlau (Madrid, Spain) and 2-propanol from Lab-Scan (Deslian, Ireland). All the solvents were of HPLC grade. Carbon dioxide was of SFC grade and purchased from Carburos Metálicos (Barcelona, Spain).

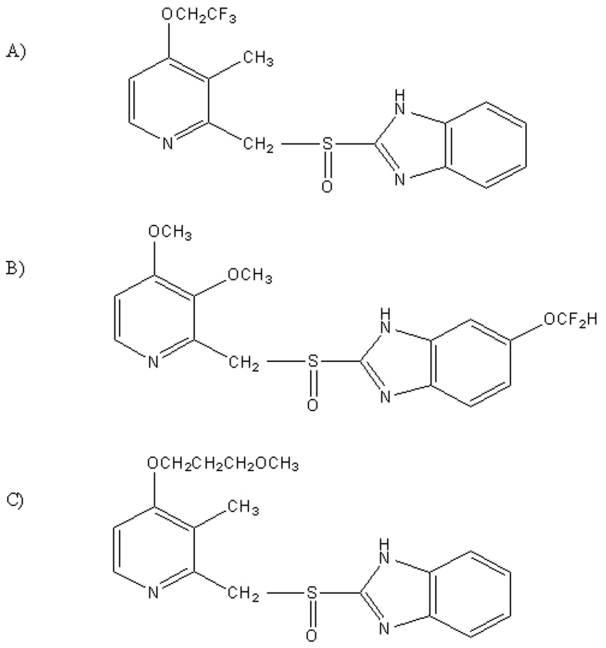

Figure 1. Structure of the compounds: (A) lansoprazole, (B) pantoprazole, (C) rabeprazole.

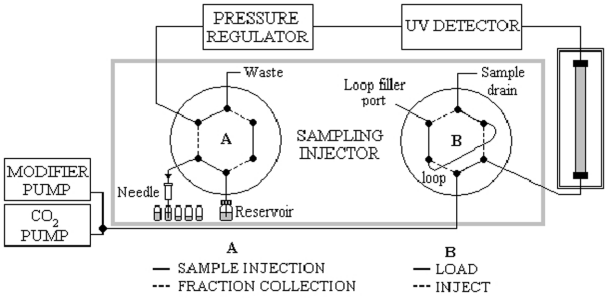

Figure 2. 233 XL valve-switching system.

2.2 Instrumentation

A modular supercritical fluid chromatograph was adapted to work at a semipreparative scale. It was equipped with two intelligent preparative pumps model PU-1586 from Jasco (Tokyo, Japan) used to supply the CO_2 and the organic modifier. The head of the CO_2 pump was cooled at 0°C using a thermostatic bath model Frigomix U from B. Braun (Melsungen, Germany). The injector was a Gilson 233XL sampling-injector (Villiers-le-Bel, France). This system was also employed to collect the fractions by means of a valve-switching device (Fig. 2). The left valve is used for aspirating the sample and collecting the fractions, and the right one for sample injection. Loop volumes of 1, 2 and 4 mL were used, and the collections were performed into 22 mL glass vials equipped with a pierced-cap and previously filled with 2 mL of the organic modifier employed for the chromatographic separation.

Pressure was controlled using a backpressure regulator model BP-1580-81 from Jasco. The detector employed was an UV – Vis detector model HP series 1050 from Hewlett – Packard (Palo Alto, CA, USA) equipped with a high-pressure flow cell. The detection wavelength was set at 285 nm. The column was placed into a column thermostat Jet-Stream Plus model from Thermotechnic Products (Langenzersdorf, Austria).

The supercritical fluid chromatograph used to analyse the collected fractions was an HP 1205 A model from Hewlett – Packard (Wilmington, DE, USA), equipped with a diode array detector (DAD) and a Rheodyne 7410 injector of 20 μL loop volume (Cotati, CA, USA).

The chiral columns employed were Chiralpak AD column, 250 × 4.6 mm and 250 × 10 mm, packed with the 3,5-dimethylphenylcarbamate derivative of amylose, coated on 10 μm silica-gel support. They were obtained from Chiral Technologies (Cedex, France).

3 Results and discussion

The enantiomeric separation of the compounds at analytical scale was previously studied by our research group

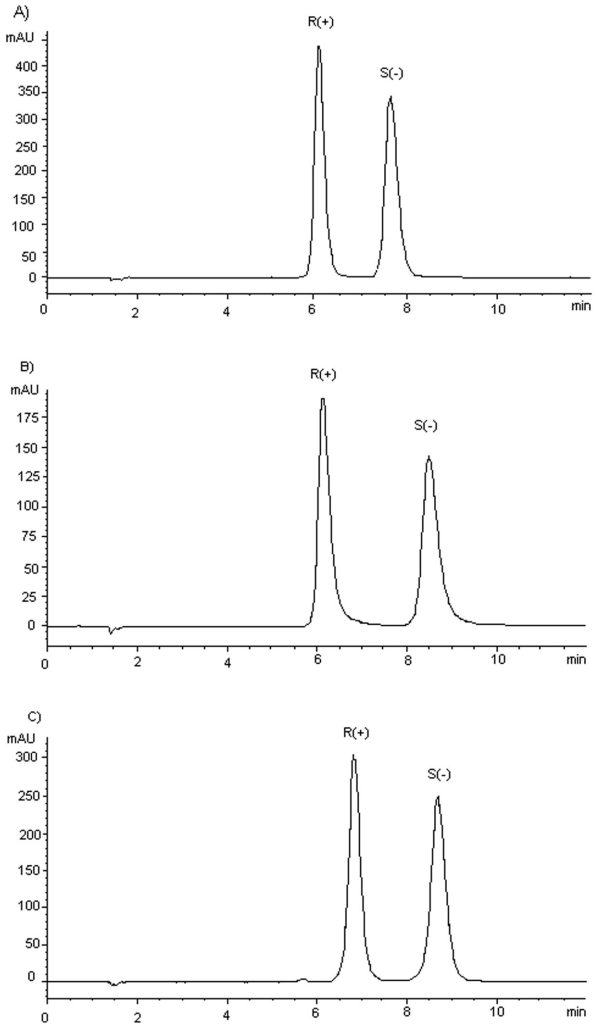

Figure 3. Chromatograms obtained with the column Chiralpak AD 4.6 × 250 mm, 20 MPa, 2 mL/min, 35°C and (A) lansoprazole, 20% methanol; (B) pantoprazole, 25% 2-propanol; (C) rabeprazole, 25% methanol.

[21]. The conditions which provided analysis times shorter than 10 min and resolutions higher than 3 were taken into account for the preparative study. Accordingly, a pressure of 20 MPa and a temperature of 35°C were selected for all the compounds, the organic modifier was 20% methanol for lansoprazole, 25% 2-propanol for pantoprazole and 25% methanol for rabeprazole. Under these conditions, the analytical resolutions were 3.45, 4.33 and 3.56, respectively (Fig. 3), being the *R*-(+)-enantiomer the first eluted isomer.

The flow rate, at semipreparative scale, was 8 mL/min in order to get similar retention times than those obtained in the analytical separation, and the injection volumes used in this work were 1, 2 and 4 mL.

The addition of solvent before the backpressure regulator, to prevent the analyte deposition on the tubing walls, was needless due to the high percentage of modifier used during the separation.

The fraction collection was performed at atmospheric pressure, filling the sealed vials with 2 mL of the modifier used in the separation. This volume was necessary to avoid the loss of the analyte with the aerosol formed during the expansion of the mobile phase. In all the cases, fractions were collected at regular intervals of time, with a slice time of 1 min, from the beginning to the end of each peak. The fractions were injected in the analytical supercritical fluid chromatograph in order to quantify the amount of each enantiomer and its enantiomeric purity. In Fig. 4, some of the chromatograms obtained for the fractions of lansoprazole are shown. Results from fractions and combinations of them were used to obtain the plots of enantiomeric purity *versus* recovery or production rate.

3.1 Separation of lansoprazole

Two solutions of racemic lansoprazole were prepared in ethanol at concentration levels of 3 and 6 g/L. Column overloading was performed by increasing the solution concentration or the volume injected (concentration or volume overloading). It should be noted that if the same load is considered, the peakwidth was always higher using volume overloading than concentration overloading (Fig. 5).

In general, when an enantiomeric purity higher than 99.9% was required, the recoveries obtained for the first eluted enantiomer were low. Even using a small load (injecting 1 mL of the solution of 6 g/L) the recovery was close to 20%, nevertheless decreasing the requirements of purity to 95%, the recovery increased to 85% (Fig. 6). The lowest purities were obtained injecting 4 mL, therefore 100% of the enantiomer was almost obtained with enantiomeric purities between 70 and 60%. The second enantiomer could be recovered in a higher extent with an enantiomeric purity higher than 99.9%. The highest recovery (50%) was achieved working with loads of 1 mL of a solution of 6 g/L. In both cases, and for a particular level of purity, the recovery decreased when the volume injected increased, obtaining the highest recoveries, with the highest purities, by injecting the lowest volume.

The production rates obtained depended heavily on the injected load, as can be seen in Fig. 7 for the second eluted enantiomer. The production rates, for an enantiomeric purity higher than 99.9%, were superior using a solution of 6 g/L than 3 g/L, and rose with the volume for both concentrations.

The production rates of the first enantiomer, at high enantiomeric purity levels, were highly influenced by the low recoveries obtained, and thus elevated production rates for the biggest loads could only be achieved

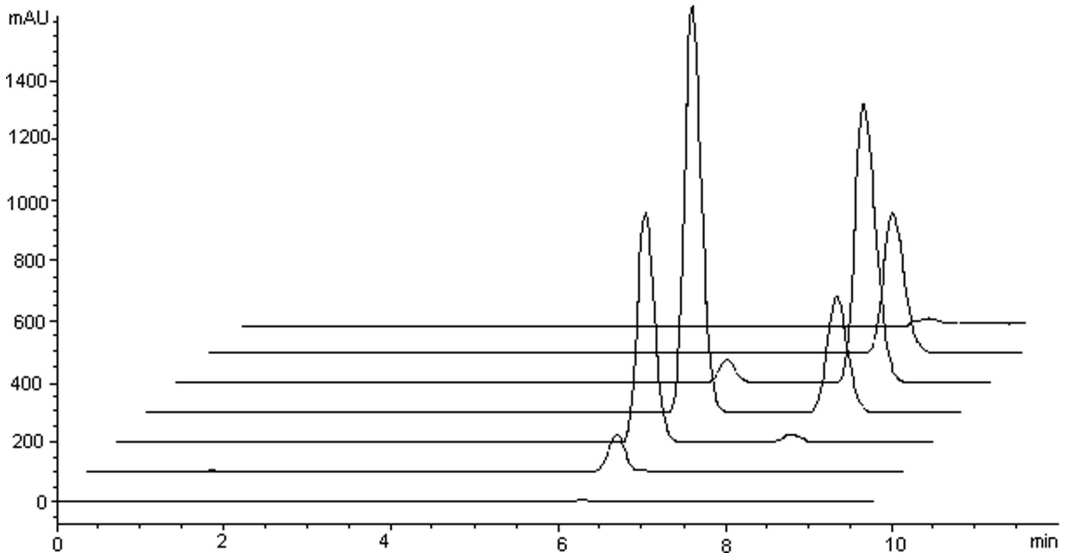

Figure 4. Chromatograms of the fractions collected in the semipreparative separation of racemic lansoprazole (6 g/L, 1 mL injected). Chromatographic conditions: column Chiralpak AD 4.6 × 250 mm, 20 MPa, 2 mL/min, 35°C and 20% methanol.

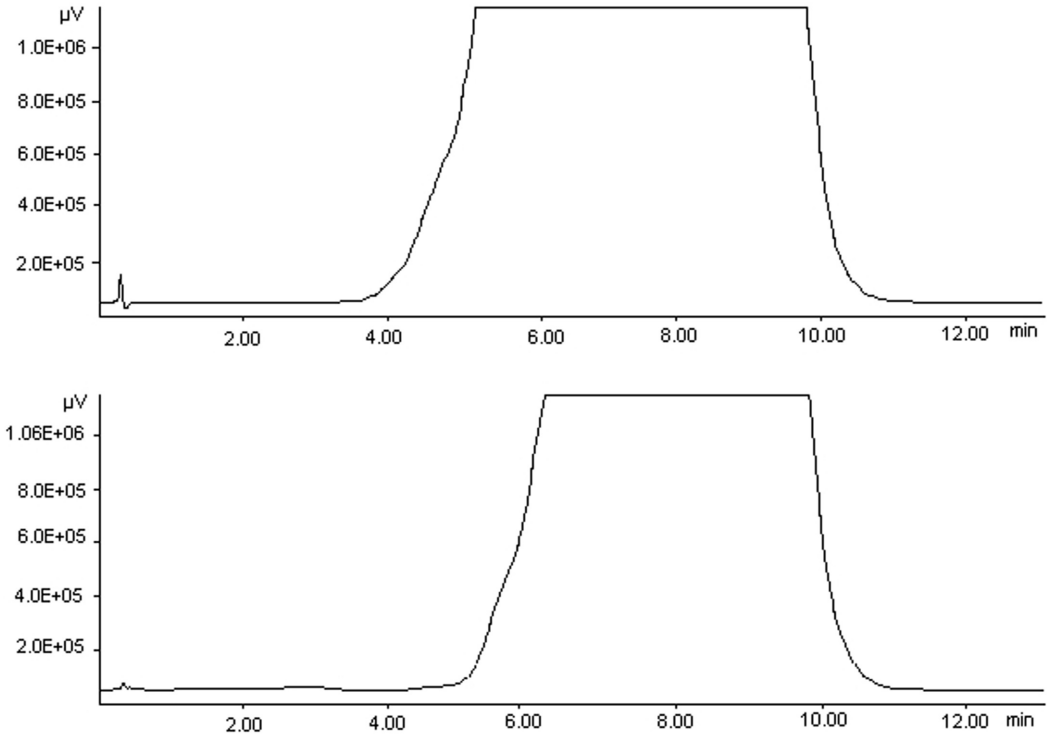

Figure 5. Chromatograms obtained for lansoprazole with the column Chiralpak AD 10 × 250 mm. Chromatographic conditions: 20 MPa, 8 mL/min, 35°C, 20% of methanol and (A) 2 mL, 3 g/L; (B) 1 mL, 6 g/L.

with purities below 70% (Fig. 8). Moreover, 0.025 mg/min of the first enantiomer and 0.090 mg/min of the second one could be obtained with an enantiomeric purity higher than 99.9% (working with loads of 1 mL of 3 g/L and 4 mL of 6 g/L, respectively). Decreasing the requirements of purity it is possible to improve the production rate to 0.097 mg/min (97% of enantiomeric purity) for the first eluted enantiomer or to 0.286 mg/min (98% of enantiomeric purity) for the second one.

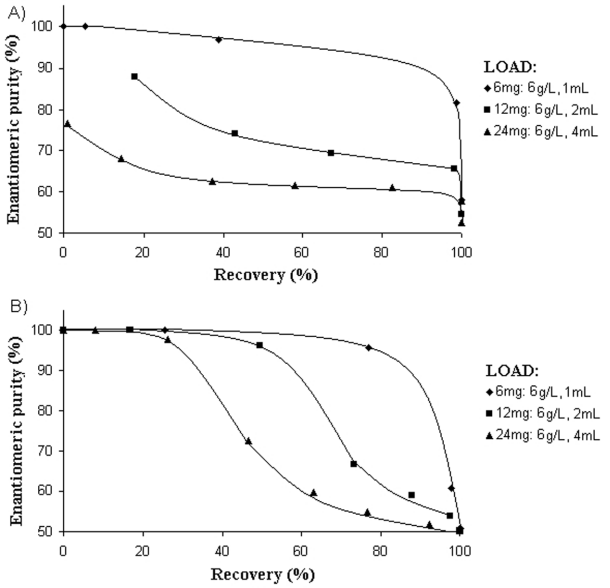

Figure 6. Enantiomeric purity *versus* recovery. Chromatographic conditions: 20 MPa, 8 mL/min, 35°C, 20% of methanol. (A) *R*-(+)-Lansoprazole; (B) *S*-(−)-lansoprazole.

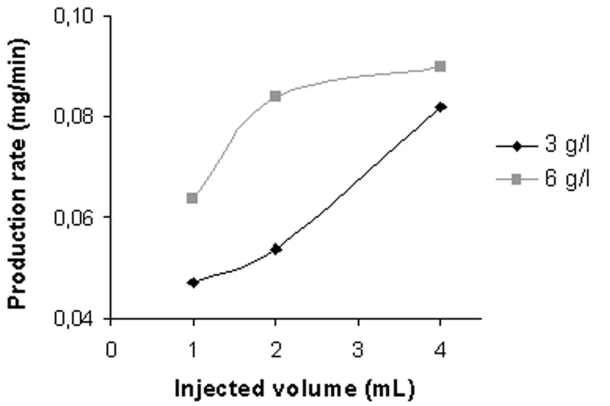

Figure 7. Production rate of *S*-(−)-lansoprazole for an enantiomeric purity higher than 99.9%. Chromatographic conditions: 20 MPa, 8 mL/min, 35°C, 20% of methanol.

3.2 Separation of pantoprazole and rabeprazole

In this case, and due to solubility reasons, the maximum concentration of pantoprazole or rabeprazole racemic mixtures that could be reached was 1.5 g/L in ethanol. This fact limited the column overloading that was only possible by increasing the injected volume, and so volume overloading was performed.

Plots of enantiomeric purity *versus* recovery and production rate were also drawn for pantoprazole and rabeprazole. Similarly to the case of lansoprazole, the best recoveries, with an enantiomeric purity higher than 99.9%, were obtained for the second eluted enantiomer (Fig. 9), except for the load of 1 mL where the results were

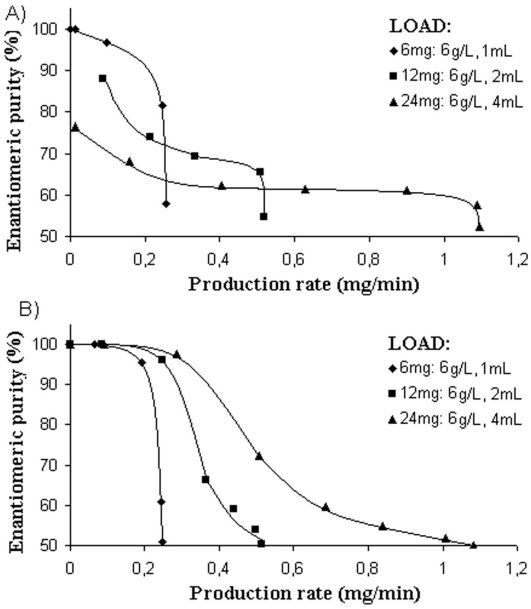

Figure 8. Enantiomeric purity *versus* production rate. Chromatographic conditions: 20 MPa, 8 mL/min, 35°C, 20% of methanol. (A) *R*-(+)-Lansoprazole; (B) *S*-(−)-lansoprazole.

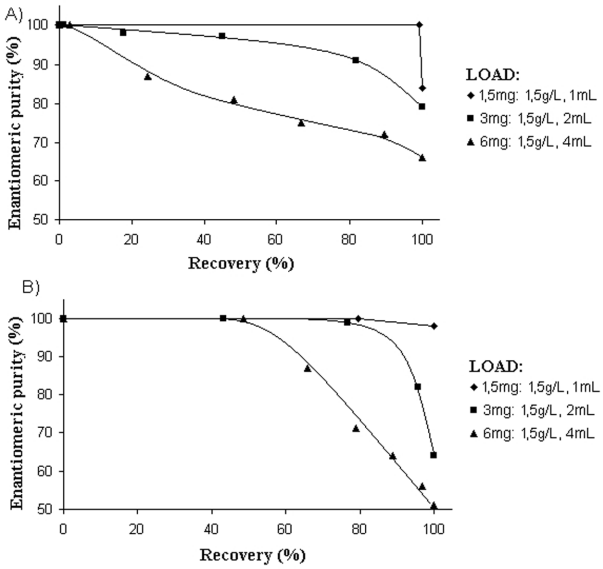

Figure 9. Enantiomeric purity *versus* recovery. Chromatographic conditions: 20 MPa, 8 mL/min, 35°C, 25% of 2-propanolol. (A) *R*-(+)-Pantoprazole; (B) *S*-(−)-pantoprazole.

similar for both enantiomers. Moreover the recoveries decreased when the injection volume increased, especially considering the greatest enantiomeric purities. It should be noted that due to the higher analytical enantioresolution achieved for pantoprazole (4.33 *vs.* 3.56 for rabeprazole), better recoveries of both enantiomers were achieved independently of the load.

The results obtained for the production rate of rabeprazole enantiomers are presented in Fig. 10. As it can be

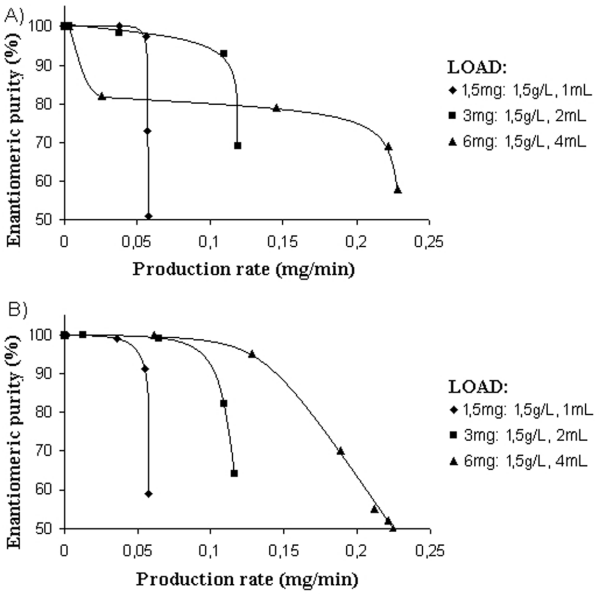

Figure 10. Enantiomeric purity *versus* production rate. Chromatographic conditions: 20 MPa, 8 mL/min, 35°C, 25% of methanol. (A) *R*-(+)-Rabeprazole; (B) *S*-(−)-rabeprazole.

seen, in the case of the second eluted enantiomer the production rate for the highest purity (>99.9%) increased with the load, obtaining the greatest values for a volume of 4 mL. In contrast, the opposite effect was observed for the first eluted enantiomer. In this case, and considering purities higher than 80%, the highest production rates were obtained for the lowest load. This way 0.037 mg/min of the first enantiomer of rabeprazole could be achieved injecting 1 mL and 0.062 mg/min of the second one injecting 4 mL, always with an enantiomeric purity higher than 99.9%. Nevertheless, these results can be improved if the requirements of enantiomeric purity decrease. For example, 0.128 mg/min of the second enantiomer can be obtained in the same conditions but with an enantiomeric purity of 95%.

For pantoprazole the graphics were similar to those obtained for rabeprazole, but the results were better and specially influenced by the higher analytical resolution and higher recoveries. In this case the production rates were 0.062 (1 mL) and 0.122 mg/min (4 mL) of the first and second enantiomers, respectively, and with a purity higher than 99.9%.

4 Conclusions

The results obtained in this work show that chiral SFC at semipreparative scale is useful to obtain individually the enantiomers of a racemic mixture. Moreover the semipreparative separation of lansoprazole, pantoprazole and rabeprazole can be performed using SFC on the Chiralpak AD column.

In all the cases, the recoveries at high enantiomeric purity levels decreased when the load increased, therefore the greatest values were obtained using an injection volume of 1 mL.

The opposite effect was observed for the production rate, in this case the highest load (using 4 mL) supplied the largest production rates, especially for the second eluted enantiomer. It should be noted that the production rates of the first eluted enantiomer, at high enantiomeric purity levels, were deeply influenced by the low recoveries obtained using the injection volume of 4 mL. This fact made that the highest values, for this enantiomer, were obtained with the lowest loads.

It should be noted that, in all the cases, the recoveries and production rates obtained for the first eluted enantiomer, were lower than those obtained for the second one, specially if high purity levels are considered. This made us to think that, in overloading conditions, the peaks were distorted at the front. This fact could be the cause that several fractions of the first enantiomer were contaminated with the second one, lowering the recoveries and production rates.

Authors acknowledge the Spanish Junta de Castilla y León (project VA037A05) for financial support.

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