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Comparative study of the enantioselective separation of several antiulcer drugs by high-performance liquid chromatography and supercritical fluid chromatography☆

L. Toribio*, M.J. del Nozal, J.L. Bernal, C. Alonso, J.J. Jiménez

Department of Analytical Chemistry, Faculty of Science, University of Valladolid, Prado de la Magdalena s/n, 47005 Valladolid, Spain

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Abstract

A comparative study of the enantiomeric separation of several antiulcer drugs such as omeprazole, lansoprazole, rabeprazole and pantoprazole using HPLC and supercritical fluid chromatography (SFC) on the Chrialpak AD column is presented in this work. The results show that employing the above mentioned column only two compounds (omeprazole and pantoprazole) could be enantiomerically resolved using HPLC, on the contrary SFC allowed the enantiomeric separation of all the compounds studied with higher resolutions and lower analysis times.

Keywords: Enantiomeric separation; Supercritical fluid chromatography; Liquid chromatography; Antiulcer drugs

1. Introduction

Since it is well known that the enantiomers of a compound can display quite different activity and toxicity profiles, the number of chiral pharmaceutical candidates has been increasing in the last few years and many of them are moving to the enantiopure formulations [1,2]. Therefore, the pharmacological evaluation of each enantiomer and the enantiomeric purity of a drug are important tasks in drug development. As a consequence, the separation of enantiomers is a subject of growing interest not only in the pharmaceutical industry but also in the analytical chemistry area.

HPLC is one of the most widely used separation techniques in this field. Although the separation is slower and shows less efficiency with regards to GC, it can be used over a vast range of compounds including those which are thermally labiles or have high molecular weights. For this reason, and because of the fact that a broad range of columns has been developed for this technique, HPLC has been the most used for the separation of chiral drugs [3–7]. However, more recently, supercritical fluid chromatography (SFC) has emerged as a powerful alternative and in some cases as a complementary technique in the area of chiral separations [8,9]. The singular properties of supercritical fluids provide several advantages such as: higher efficiencies, higher resolutions in shorter analysis time and faster column equilibration [10].

Among the different chiral stationary phases (CSPs), the polysaccharide based ones have shown a very broad applicability to different compounds, being the phenyl carbamate derivatives one of the most successful CSPs [11–15]. Concretely the Chiralpak AD and Chiralcel OD columns have demonstrated to be highly effective not only in HPLC but also in SFC [16].

In this work, a study of the enantiomeric separation of several chiral antiulcer drugs including omeprazole, lansoprazole, pantoprazole and rabeprazole, using HPLC on the Chiralpak AD column is presented. The results are compared with those obtained using SFC [17].

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* Corresponding author. Tel.: +34 9 8342 3262; fax: +34 9 8342 3013.
E-mail address: ltoribio@qa.uva.es (L. Toribio).

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2. Experimental

2.1. Reagents

The organic solvents methanol, absolute ethanol, acetonitrile and hexane were purchased from Scharlau (Madrid, Spain) and 2-propanol from Lab-Scan (Deslian, Ireland). All the solvents were HPLC grade. Triethylamine (TEA) and trifluoroacetic acid (TFA) were of analytical grade from Sigma–Aldrich (Madrid, Spain).

Carbon dioxide was SFC grade and purchased from Carburos Metálicos (Barcelona, Spain).

2.2. Compounds

The compounds studied (Fig. 1) were purchased from Sigma–Aldrich (Madrid, Spain), and all of them were in their racemic form. The stock solutions of the individual drugs were prepared in ethanol at the 100 mg/L level.

2.3. Instrumentation

The supercritical fluid chromatograph used was a HP 1205 A model from Hewlett Packard (Wilmington, DE, USA) equipped with a diode array detection (DAD) system and a Rheodyne 7410 injector of 20 μL loop volume (Cotati, CA, USA), and operated in downstream mode.

The liquid chromatograph consisted of a Constantmetric II pump from Milton Roy (Madrid, Spain), a 4100 variable wavelength UV–vis detector from LDC Analytical (Madrid, Spain), a manual Rheodyne 7125 (Cotati) and a JLC 6000 software from Jones Chromatography (Littleton, USA). The detection wavelength was set at 285 nm.

A Chiralpak AD column, 250 nm × 4.6 mm, packed with the 3,5-dimethylphenylcarbamate derivative of amylose, coated on 10 μm silica-gel support, was obtained from J.T. Baker (Deventer, The Netherlands).

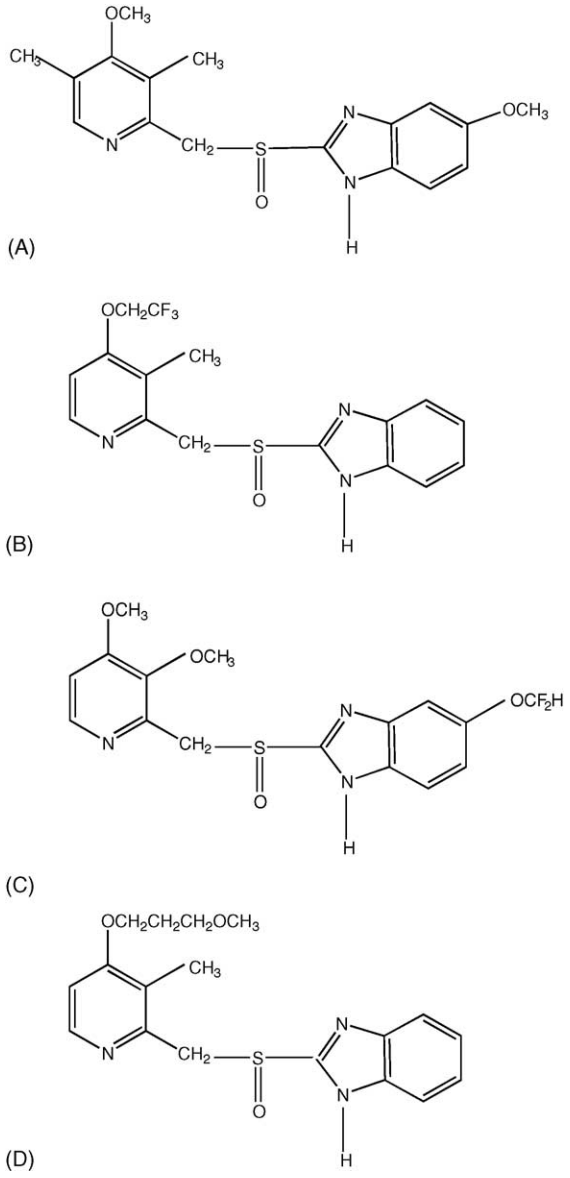

Fig. 1. Structure of the compounds: (A) omeprazole, (B) lansoprazole, (C) pantoprazole, and (D) rabeprazole.

3. Results and discussion

As the Chiralpak AD column used is design for working in normal-phase mode, the HPLC study was started using binary mixtures of hexane–ethanol or hexane/2-propanol. The percentages of organic modifier ranged from 0% to 25% in the case of 2-propanol, from 0% to 15% with ethanol, and a 60% of ethanol as a polar modifier was also used, taking into account the miscibility range of the solvents. Because most of the compounds could not be baseline resolved, the study was improved using polar organic mobile phases, which recently have attracted a lot of interest for chiral separations [18,19]. One hundred percent of methanol, ethanol and acetonitrile were the mobile phases employed. Due to the high pressure drop, a 100% of 2-propanol could not be used. Although a flow-rate of 0.2 mL/min could be possible, the compounds were highly retained.

The flow-rate was fixed at 1 mL/min, except when the pressure drop obligated to work at lower flow-rate to avoid damaging the column, as in the case of 60 and 100% of ethanol, where the flow-rate were 0.75 and 0.5 mL/min, respectively.

Working in normal-phase mode with 2-propanol as a polar modifier, the omeprazole enantiomers could not be baseline resolved in isocratic conditions. Although the effect of the temperature was studied to improve the separation, similar resolution was obtained, but the retention decreased with increasing temperature. However, the resolutions were over 1.5 when a gradient elution was used (Fig. 2). Although a baseline resolution is possible in HPLC in these conditions, the enantioresolutions were not so high as in SFC.

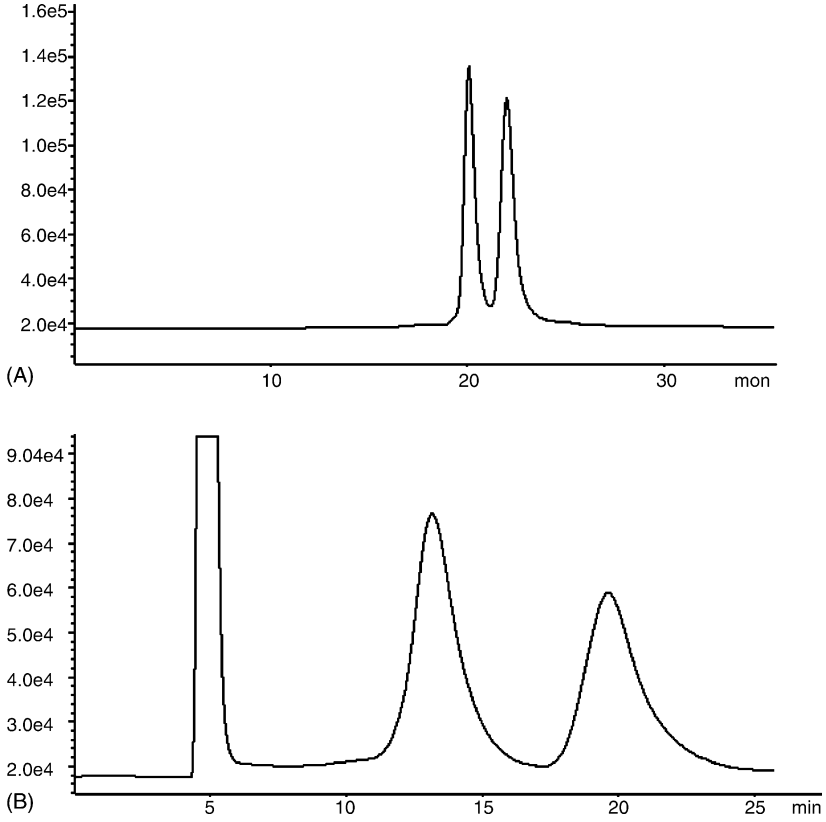

Fig. 2. HPLC enantiomeric separation of omeprazole. Chromatographic conditions: (A) gradient elution of 2-propanol: from 5% (5 min) to 30% at 2.5% per minute, flow-rate of 1 mL/min, (B) 60% of ethanol, flow-rate of 0.75 mL/min. The temperature was 25 °C in both cases.

The omeprazole enantiomers were overlapped when the percentage of ethanol was varied from 0% to 15%. On the other hand, the resolution was 1.9 with a 60% of ethanol (Fig. 2).

Working with 100% of methanol as polar mobile phase at 25 °C, higher resolutions than in normal-phase mode were obtained. TEA and TFA, as modifier additives, were added to enhance solute peak shape, getting also a decrease on the retention times. The influence of the modifier additives is shown in Table 1. As it can be seen, the best result was achieved when 0.1% of TEA and 0.1% of TFA where added simultaneously. The resolution was also good when a 0.05% of TEA was used instead of working with both additives, but the enantiomers were more retained.

The enantiomers could not be baseline resolved using ethanol and acetonitrile as polar mobile phases.

However, the enantiomers of omeprazole could be separated by SFC with a resolution higher than 2, being the analysis times lower than 10 min (Fig. 3). Comparing with the results obtained by HPLC, similar analysis times could only be achieved working with methanol and additives as polar mobile phase. However, the enantioresolutions in SFC were higher and consumption of organic solvents was reduced, which is also advantageous.

The baseline separation of pantoprazole enantiomers by HPLC was only achieved with binary mixtures of hexane-2-propanol (Fig. 4). The results are shown in Table 2. The effect of the temperature was also studied, and as it happened for omeprazole, similar enantioresolutions are obtained, getting only a retention decrease when the temperature increased. When the ethanol percentage was varied from 0% to 15%, the solutes were retained over 60 minutes, and with a higher percentage (60%) the enantioresolution was very low. The

Table 1
Influence of the additives in the enantiomeric separation by HPLC of omeprazole using methanol as polar mobile phase

Modifier additives	t_1 (min)	t_2 (min)	$\alpha_{2/1}$	R_s
	9.15	16.16	2.14	2.19
0.1% TEA + 0.1% TFA	5.03	8.39	2.66	4.35
0.05 % TEA + 0.05% TFA	5.23	9.03	2.71	2.24
0.05% TEA	7.35	16.11	3.02	3.44

Chromatographic conditions: 25 °C, flow-rate of 1 mL/min.

Table 2
Effect of the percentage of 2-propanol in the chiral separation by HPLC of pantoprazole

	t_1 (min)	t_2 (min)	$\alpha_{2/1}$	R_s
15%	34.50	44.87	1.33	1.92
20%	19.62	24.92	1.32	1.71
25%	14.35	18.12	1.33	1.74

Chromatographic conditions: 35 °C, flow-rate of 1 mL/min.

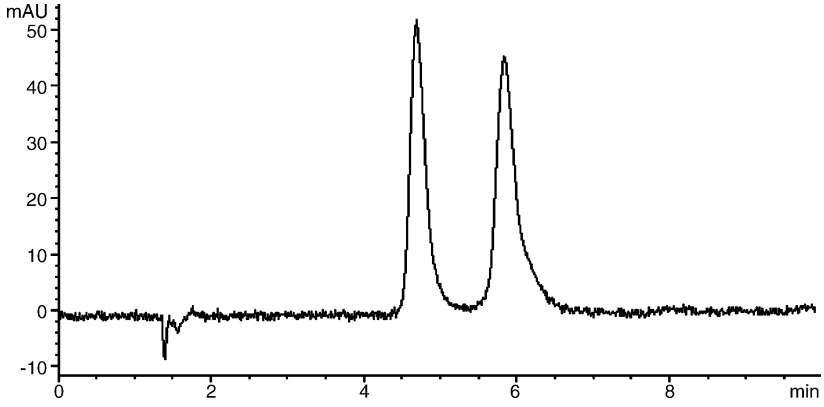

Fig. 3. Enantiomeric separation of omeprazole by SFC. Chromatographic conditions: 20 MPa, 35 °C, 30% 2-propanol, flow-rate of 2 mL/min. See ref. [17].

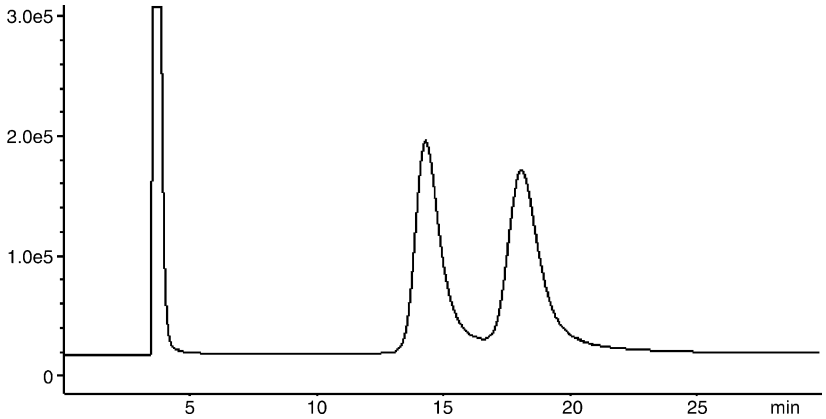

Fig. 4. HPLC enantiomeric separation of pantoprazole. Chromatographic conditions: 35 °C, 75:25 hexane/2-propanol, flow-rate of 1 mL/min.

pantoprazole enantiomers were overlapped working in polar-phase mode.

Comparing the results obtained by HPLC with SFC, the last technique could achieved resolutions higher than 2 (Fig. 5), what was no possible by HPLC. Moreover, retention times were more than twice longer by HPLC. So the enantiomeric separation of pantoprazole using SFC was better than in HPLC.

Lansoprazole and rabeprazole enantiomers could not be baseline resolved neither in normal-phase mode nor polar mode. In the case of lansoprazole, the enantiomers were retained more than 20 min in normal-mode with 2-propanol,

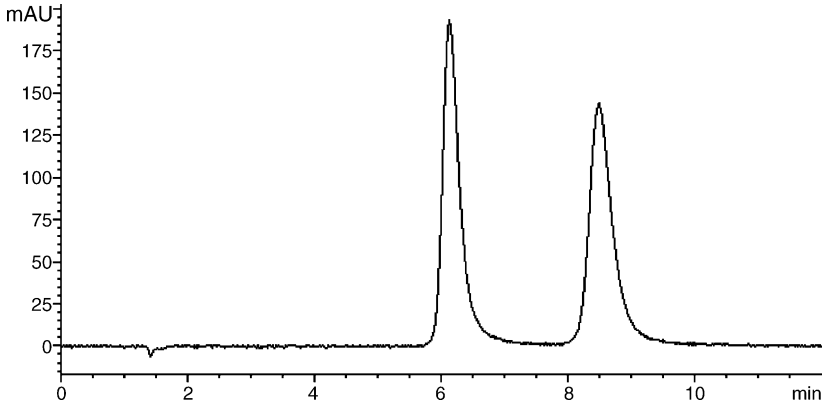

Fig. 5. Enantiomeric separation of pantoprazole by SFC. Chromatographic conditions: 20 MPa, 35 °C, 25% 2-propanol, flow-rate of 2 mL/min. See ref. [17].

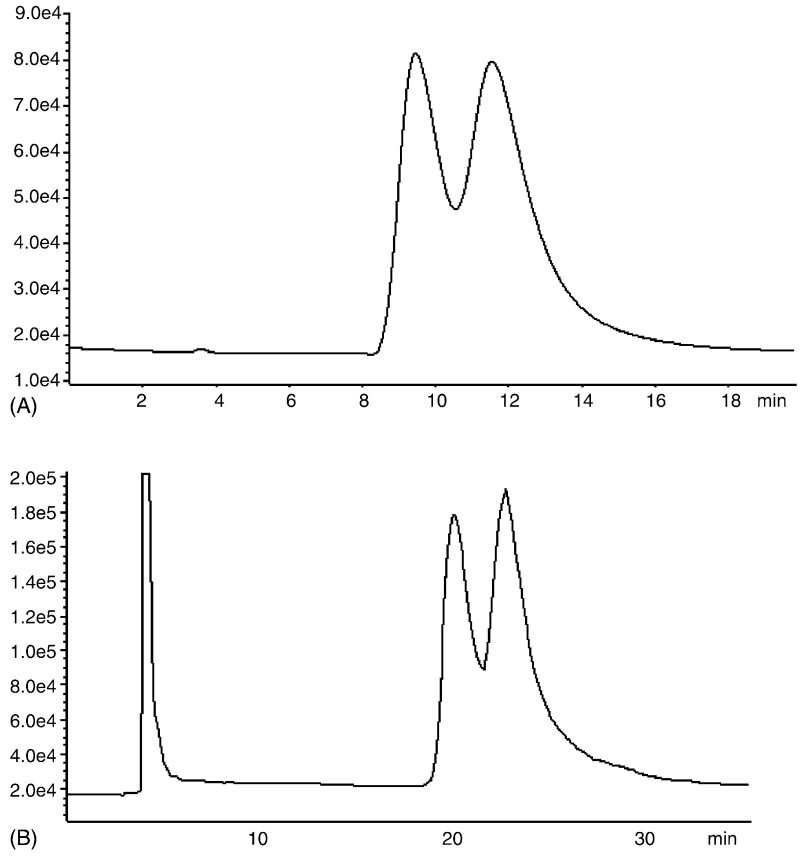

Fig. 6. Enantiomeric separation by HPLC: (A) lansoprazole. Chromatographic conditions: 25 °C, 100% acetonitrile, flow-rate of 1 mL/min. (B) rabeprazole. Chromatographic conditions: 25 °C, 85:15 hexane/2-propanol, flow-rate of 1 mL/min.

being the resolution very low. Changes in the temperature and gradient elution were tried without improving the results. The best result was obtained using 100% of acetonitrile as polar mobile phase, being the enantioresolution 0.72 (Fig. 6). Using ethanol, in normal and also in polar mode, the lansoprazole enantiomers could not be separated, being always overlapped.

The best resolution of rabeprazole was achieved working in normal-phase mode, with a percentage of 15% in 2-propanol, where the resolution was 0.84 (Fig. 6). As in the cases of previous compounds, the temperature was varied to improve the separation. The results were not better because a decrease in the resolution with increasing temperature could be seen; a decrease in the retention times was also observed. Using ethanol in normal-phase mode and also methanol in polar mode, the enantiomers were overlapped, and very low resolutions were obtained with 100% of ethanol and 100% of acetonitrile.

However, the chiral separation of lansoprazole and rabeprazole by SFC, which has already been reported in our previous work, achieved resolutions that were in most cases higher than 2 [18]. Therefore, SFC is a better technique than HPLC for the enantiomeric separation of these compounds by the assayed column.

4. Conclusion

The chiral separation by HPLC could only be reached for omeprazole and pantoprazole, but not for lansoprazole and rabeprazole. However, SFC allows the enantiomeric separation of all the compounds studied with resolutions that were in most of the cases higher than 2 and analysis times lower than 10 min. When the separation by HPLC was possible, the peaks were broadened, lower selectivity factors and resolutions were provided, and the retention times were longer. Moreover the consumption of organic solvents in HPLC was quite higher and, due to problems of miscibility with hexane in normal-phase mode, only ethanol and 2-propanol could be used as polar modifiers.

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