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Journal of Chromatography A, 1011 (2003) 155–161
JOURNAL OF CHROMATOGRAPHY A
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Use of semipreparative supercritical fluid chromatography to obtain small quantities of the albendazole sulfoxide enantiomers ☆

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Received 7 March 2003; received in revised form 24 June 2003; accepted 4 July 2003

Abstract

The semipreparative separation of the albendazole sulfoxide enantiomers using chiral supercritical fluid chromatography is presented in this work. For this purpose, a modular SFC chromatograph was adapted to work at semipreparative scale and a Chiralpak AD (250×10 mm) column was used. Different injection volumes were evaluated in order to obtain high purities and throughputs. Using the maximum load, it was possible to obtain 37 mg/h of the first eluted enantiomer with a purity of 99.9%, and 36.5 mg/h of the second eluted enantiomer with a purity of 95%.

Keywords: Enantiomer separation; Supercritical fluid chromatography; Preparative chromatography; Albendazole sulfoxide

1. Introduction

The interest in chiral compounds has increased more and more in the last few years, and the production of pure chiral drugs is one of the most important tasks in today's pharmaceutical industry. The need for obtaining the pure individual enantiomers at the first steps in the development of a chiral drug, has been promoted by the fact that a pair of enantiomers often show different pharmacological and toxicological profiles, and so, they must be tested and regulated separately.

There are two approaches to get enantiomerically pure compounds: stereoselective synthesis and preparation of the racemic mixture which is further resolved into its enantiomers. Stereoselective synthesis can be useful to prepare large quantities of enantiomers but, only one enantiomer is obtained, and the time it takes to develop can make it non-feasible when small quantities are needed. Using the racemic approach, both enantiomers are obtained, and the synthesis of the racemate has a lower degree of difficulty.

Among the different methods to separate enantiomers, preparative-scale chromatographic techniques

☆Presented at the 2nd Meeting of the Spanish Society of Chromatography and Related Techniques, Barcelona, 26–29 November 2002.

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)01179-8

on chiral stationary phases are a useful alternative, because they make possible the fast and relatively easy achievement of both enantiomers with a high degree of optical purity. Most of these separations have been performed using preparative HPLC [1–7], but the disadvantages of these methods are the sample dilution and the high consumption of organic solvents. Since patented by Perrut in 1984 [8] preparative supercritical fluid chromatography (prep-SFC) has been arousing great interest, and several papers reporting the use of prep-SFC have appeared in the last few years [9–16]. Prep-SFC offers several potential advantages over HPLC: it is faster, yielding at least a three-fold increase in the throughput, products can be recovered more easily than in HPLC because most of the mobile phase vaporises when depressurised, the consumption of organic solvent is low, and it has a wider range of applicability. Thus, prep-SFC is a valuable, and often a complementary tool to preparative HPLC, when the isolation of one or more compounds of a mixture is necessary.

One of the most important tasks in prep-SFC is sample collection. It has been performed by different ways: using packed beds and subsequent elution [17], at high pressure [10,18] using collection tubes contained in a pressurised vessel, or at atmospheric pressure [11–13,15] by bubbling the effluent through a trapping solvent. Employing bed traps, solute could be lost due to the possibility that modifier liquefy in the bed during collection and cause breakthrough. Collection at high pressure or at atmospheric pressure have yield high recoveries, and both of them are the most extensively used techniques.

Albendazole (ABZ) is a broad-spectrum anthelmintic whose activity is due to its principal metabolite: the albendazole sulfoxide (ABZSO), namely ricobendazole.

Recently, some works have showed enantioselectivity in the elimination of ABZSO in patients with neurocysticercosis [19] but, to our knowledge, all the pharmacological and toxicological studies have been done with racemic ABZSO.

The aim of this work has been to study the possibilities that semipreparative-scale supercritical fluid chromatography offers in the isolation of the enantiomers of albendazole sulfoxide to be used in pharmacological studies. For this purpose, a modular supercritical fluid chromatograph has been adapted to

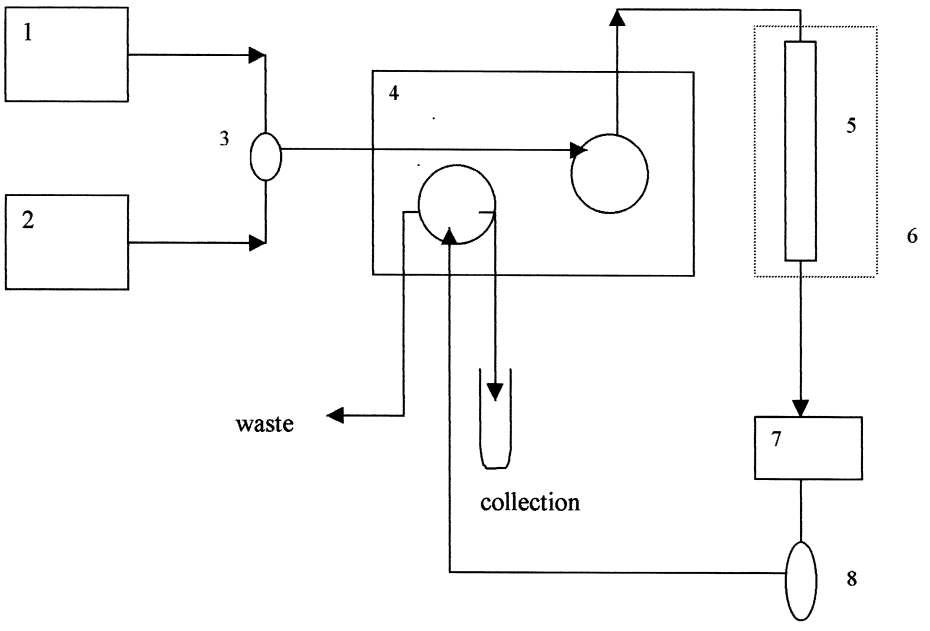

Fig. 1. Scheme of the semipreparative SFC system used. (1) Modifier pump, (2) CO_2 pump, (3) mixer, (4) sampling-injector, (5) column, (6) column thermostat, (7) UV detector, (8) pressure regulator.

work at semipreparative scale and several loads have been evaluated in order to obtain high throughputs and purities.

2. Experimental

2.1. Reagents

Albendazole sulfoxide (ABZSO) was purchased from Schering Plough. The standard solution was prepared in methanol at the 3 g/l level. Methanol and 2-propanol were HPLC grade and obtained from Lab-Scan (Dublin, Ireland). Carbon dioxide was SFC-grade and purchased from Carburos Metálicos (Barcelona, Spain).

2.2. Instrumentation

The semipreparative supercritical fluid chromatograph used, was a modular one adapted to work at semipreparative scale (Fig. 1). It was equipped with two intelligent preparative pumps model PU-1586 from Jasco Corporation (Tokyo, Japan), one of them was used to supply the organic modifier and the other to propel the CO_2. The pump-head of this last, was cooled at 0 °C using a thermostatic bath model Frigomix U from B. Braun (Melsugen, Germany). The injector was a Gilson 233XL sampling-injector (Villiers-le-Bel, France) and loop volumes of 0.5, 1, 2 and 5 ml were used. This system was also employed to collect the fractions. The collections were performed into 20-ml glass vials equipped with

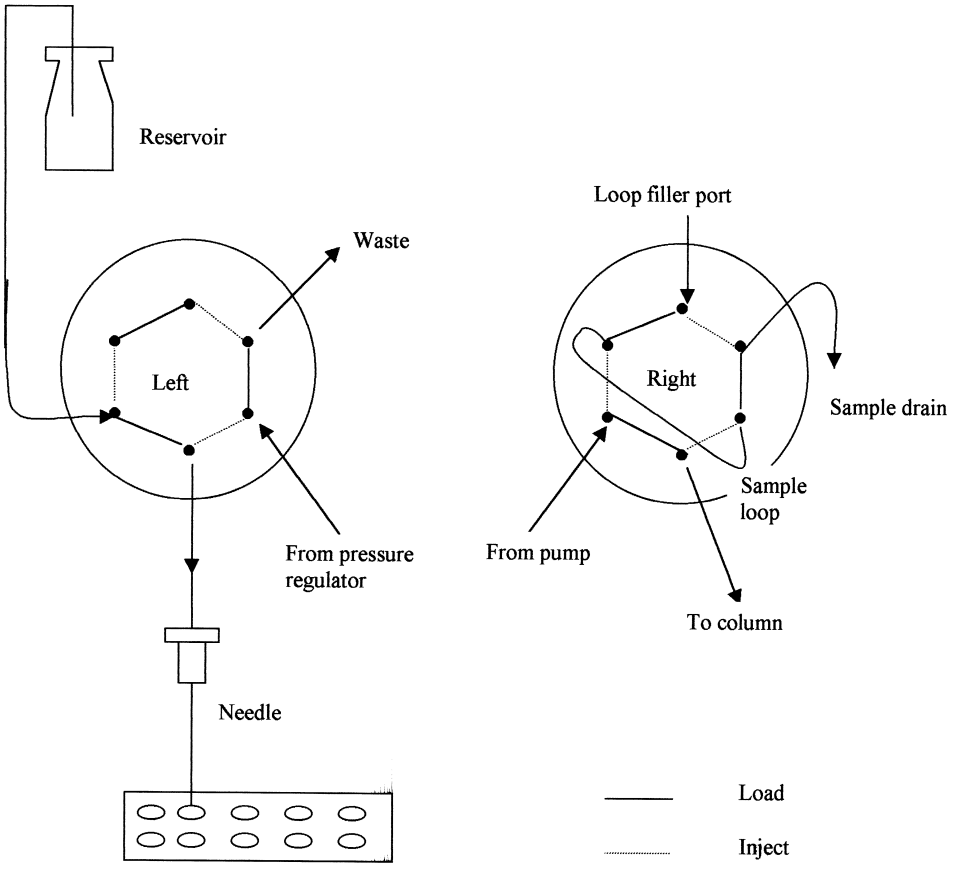

Fig. 2. 233 XL valve switching.

a pierced-cap and previously filled with 1 ml of 2-propanol. The 233XL device was equipped with a collection needle which moves down to the vial bottom as programmed. The 233XL valve switching system is shown in Fig. 2, the right valve is used for sample injection and the left one for collecting the fractions.

Pressure was controlled using a backpressure regulator model BP-1580.81 from Jasco (Tokyo, Japan). The detector employed was a 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 225 nm. The column was placed into a column thermostat Jet-Stream plus model from Thermotechnic Products (Langenzersdorf, Austria).

The supercritical fluid chromatograph used to analyze the fractions collected, was an HP G1205A model from Hewlett-Packard (Wilmington, DE, USA) equipped with a diode array detection (DAD) system and a 7410 Rheodyne (Cotati, CA, USA) valve of a 5-μl loop volume.

The chiral columns employed were Chiralpak AD 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 Daicel (Deventer, The Netherlands).

3. Results and discussion

Taking into account the results obtained in a previous work [20], the best conditions for the analytical separation of ABZSO on the Chiralpak AD column are 200 bar, 2 ml/min, 35 °C and 30% 2-propanol. Under these conditions, an analytical resolution of $R_s = 4$ was obtained in an analysis time of 10 min (Fig. 3).

The study of the semipreparative separation of ABZSO was carried out applying these conditions, but the flow-rate was increased to 8 ml/min, in order to get retention times similar to those obtained at analytical scale.

The fraction collector used in this work has been previously mentioned by other authors in their semipreparative SFC papers. Some of them [12] needed to add solvent (by means of another pump) before the pressure restrictor, in order to prevent the analyte deposition on the tubing walls. In our case,

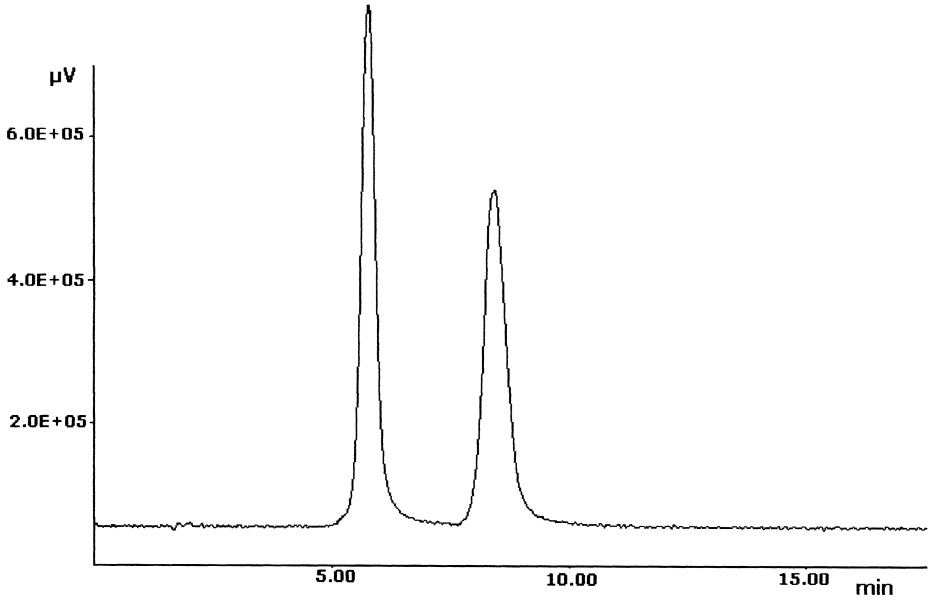

Fig. 3. Chromatogram obtained with the column Chiralpak AD 4.6×250 mm, 200 bar, 35 °C, 2 ml/min and 30% 2-propanol.

Table 1
Volume injected

Volume injected (ml)	Load (mg)
0.5	1.5
1	3
2	6
4	12
5	15

as the semipreparative separation was carried out using a high level of organic modifier (30% 2-propanol), this amount is enough to dissolve the analyte after the CO_2 depressurization and so, the addition of solvent was not necessary to obtain good recoveries.

The highest concentration of the ABZSO racemic mixture that was possible to reach, due to solubility reasons, was 3 g/l in methanol. This determined the column overloading which could only be achieved by increasing the volume injected always using a solution of 3 g/l. So the maximum column load used in this work was 15 mg (Table 1) that correspond to an injection volume of 5 ml. In Fig. 4, the chromatogram obtained using this load is showed.

Different loads (volumes) were assayed and fractions were always collected at regular intervals of time with a slice width of 1 min from the beginning to the end of each peak. The fractions were injected (5 μl) in the analytical supercritical fluid chromatograph in order to quantify the amount of analyte and its purity. Results from individual fractions and combinations of them, allowed to obtain the graphics shown in Figs. 5 and 6.

As it can be seen in Fig. 5, the lowest recoveries were obtained for the highest load and purity. It should be noted that even in the worst case, it was possible to recover more than 85% of the first eluted enantiomer with a purity higher than 99.9%. In the case of the last eluted enantiomer, it was more difficult to obtain high recoveries and high purities, specially working at the maximum load. This is due to the tailing of the first peak which contaminated the fractions collected. In this case, there is a marked decrease of the recovery with the increase of the load when the purity required is 98% or higher. For lower purities the decrease is similar to the obtained when collecting the first eluted enantiomer. Nevertheless, using the maximum load, it was possible to recover

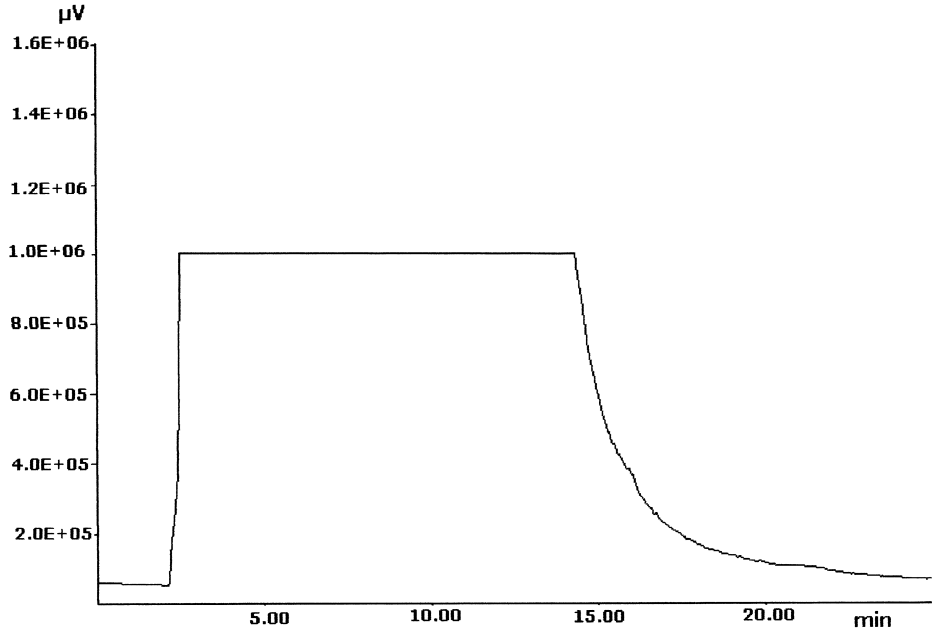

Fig. 4. Chromatogram obtained for a load of 15 mg with the column Chiralpak AD 10×250 mm, 200 bar, 35 °C, 8 ml/min and 30% 2-propanol. Volume injected 5 ml.

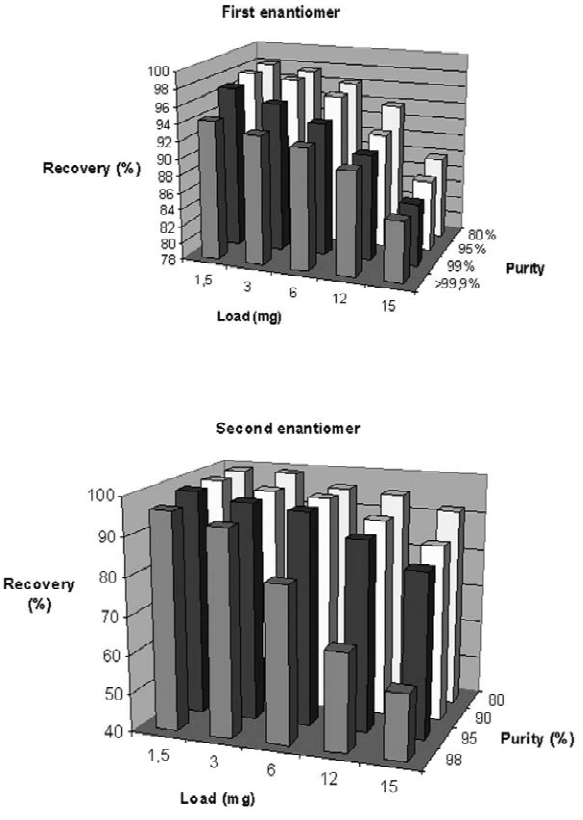

Fig. 5. Recoveries obtained for the different loads and purities.

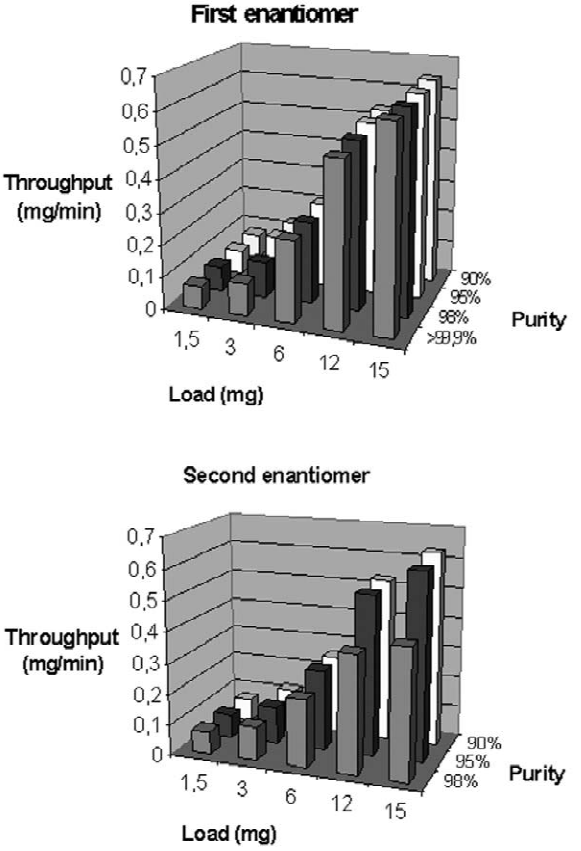

Fig. 6. Throughputs obtained for the different loads and purities.

more than 56% of the second eluted enantiomer with a purity of 98%.

As far as throughput is concerned (Fig. 6), it should be noted that it is more influenced by the load than by the purity desired. As it can be seen, for the first eluted enantiomer the throughput varied between 0.64 and 0.62 mg/min considering purities of 90% and higher than 99.9%, respectively. In the case of the second eluted enantiomer the throughput decreased markedly for a purity of 98%, in this case the value obtained was 0.42 mg/min against 0.61 mg/min obtained for purities of 95% or lower.

Using the maximum load assayed, the first eluted enantiomer could be obtained with a purity higher than 99.9% at a throughput of 37 mg/h, while the second eluted enantiomer could be obtained with a purity of 95% at a throughput of 36.5 mg/h.

4. Conclusions

The results presented show that chiral semipreparative SFC, on the Chiralpak AD column, can be useful to obtain the enantiomers of albendazole sulfoxide (ABZSO) with a high degree of purity and the quantities obtained can be enough to carry out pharmacological assays.

The low solubility of ABZSO in organic modifiers (3 g/l in methanol) determined the column overloading that could only be done by increasing the volume injected, and always using a solution of 3 g/l.

The recoveries decreased when the requirements of purity or the load increased, but it was always possible to recover more than 85% of the first eluted enantiomer with a purity higher than 99.9%. The second eluted enantiomer was more difficult to

obtain with a high degree of purity due to the tailing of the first peak, nevertheless purities of 98% with recoveries of 59% were obtained for the maximum load.

The load had a higher influence on the throughput than the purity. Using the maximum load assayed, the first eluted enantiomer could be obtained with a purity higher than 99.9% at a throughput of 37 mg/h, while the second eluted enantiomer could be obtained with a purity of 95% at a throughput of 36.5 mg/h.

Acknowledgements

The authors thank the Spanish Ministry of Science and Technology for financial support (project PPQ 2001-2072-C02-01). EMN thanks the Spanish Ministry of Education for a doctoral study grant (project No. 1FD97-0460, FEDER program)

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