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Albendazole sulfoxide enantiomers: Preparative chiral separation and absolute stereochemistry

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ABSTRACT

The enantiomeric separation of albendazole sulfoxide was carried out by simulated moving bed chromatography with variable zones (VARICOL). An overall recovery of 97% was achieved and enantiomeric ratios of 99.5% for raffinate and 99.0% for extract were attained. A total of 880 mg of (+)-albendazol sulfoxide and 930 mg of its antipode were collected after 55 cycles or 11 h of process, resulting in a mass rate of 2 g/day. Furthermore the absolute configuration of the enantiopure compounds was determined for the first time by vibrational circular dichroism (VCD) with the aid of theoretical calculations as (−)-(*S*) and (+)-(*R*)-albendazole sulfoxide.

1. Introduction

Albendazole is a broad-spectrum anthelmintic drug that when administered undergoes rapid hepatic oxidation, by the liver microsomal enzymes, producing the active metabolite albendazole sulfoxide which is then oxidized to the inactive metabolites albendazole sulfone and albendazole-2-amino sulfone [1].

Pharmacokinetic studies indicate that albendazole sulfoxide (Fig. 1) possesses both anthelmintic and toxicological effects. In addition, the sulfoxidation of albendazole is enantioselective and species dependent [1,2]. Enantioselective studies of the metabolism of albendazole on different organisms and biological matrices, or of the anthelmintic activity of albendazole sulfoxide, have been delayed by the lack of an efficient process for production of the enantiomers of this active metabolite in gram scale.

A variety of methods are available for large scale enantiomeric separation and one of the most efficient is chiral chromatography. Since the improvement of chiral stationary phases (CSPs) and chromatographic instrumentation, enantiomeric separation by preparative chromatography is an important option, particularly, because of method robustness, rapidity, simplicity and applicability [3].

In this approach, elution batch chromatography is perhaps the most famous technique used for the isolation of enantiopure compounds; stack injections and peak shaving recycling can provide efficient methods [3]. However, large-scale separations require large amounts of CSPs and mobile phase, which results in high costs, dilution conditions and difficulties associated with the evaporation/recycling of solvents [4].

In batch chromatography, the sample is injected on the top of the column and then collected at different times after the elution of the mobile phase. The stationary phase in this process is underused because a restricted section of the column bed contributes to the chiral discrimination. The simulated moving bed chromatography (SMB) technology appears to improve the packing utilization since it simulates the bed movement in the opposite direction of the mobile phase by a change of valves [5,6].

The VARICOL process, introduced by NOVASEP [7], is a nonconventional multicolumn system that operates with a non-synchronous shift of the inlet and outlet valves, which provide advantages; specially, in cases that a reduced number of columns is required.

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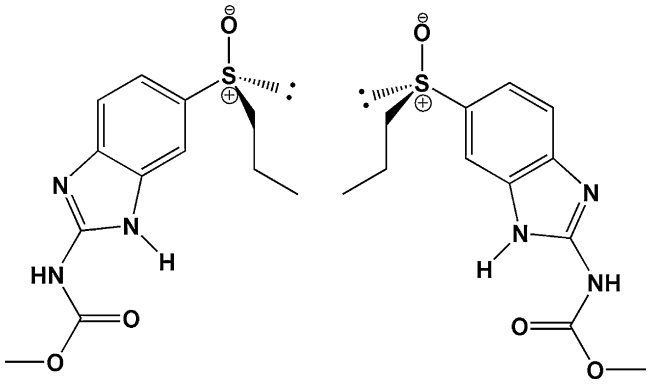

Fig. 1. Chemical structures of albendazole sulfoxide enantiomers.

Determination of the absolute handedness, known as absolute configuration (AC), of chiral molecules is an important step in any field related to chirality, especially in the pharmaceutical industry. Even though X-ray crystallography is a widely used approach to determine the AC of chiral sulfoxides, suitable quality crystals are required which may not be possible in many cases. As an alternative, VCD, associated with density functional theory (DFT) calculations, has been successfully used for this purpose in a variety of organic molecules [8–10], including sulfoxides [11–13].

Given the importance of albendazole sulfoxide [2,14] it is surprising that only two reports have appeared about the chiral preparative separation of the racemate [15,16] and, to our knowledge, the absolute configuration of those active metabolites has not yet been described. Accordingly, this work reports the results obtained by the use of the VARICOL process for the separation of albendazole sulfoxide enantiomers as well as the determination of their absolute configuration by vibrational circular dichroism and DFT calculations as (−)-(*S*)- and (+)-(*R*)-albendazole sulfoxide.

2. Experimental

2.1. Materials and methods

The analytical HPLC system consisted of a Shimadzu LC-10AD pump (Kyoto, Japan), a SPD-10A variable wavelength UV–vis detector, a rheodyne with a 250 μL loop or 2000 μL. This equipment is connected to a CBM-10A and for data acquisition Labsolutions software from Shimadzu was used. The SMB unit used was a MicroLAB-VARICOL and the softwares were HELP 10.3 and ACS, all obtained from NOVASEP (Pompey, France). The eluents used as mobile phase were HPLC grade; methanol was purchased from Tedia, while *n*-hexane and ethanol from J.T. Baker. The six CHIRALPAK AD columns (10 cm × 1.0 cm I.D., 20 μm) used in the VARICOL system are commercially available from Chiral Technologies (West Chester, USA). Racemic albendazole sulfoxide was gently donated by Ourofino Animal Health (Ribeirão Preto, Brazil). The specific rotations for albendazole sulfoxide enantiomers were determined with a polarimeter, using methanol as solvent and a concentration of 10 mg mL^{-1}.

2.2. Overload experiments and software simulation

A stock solution of albendazole sulfoxide (250 mg) was prepared by dissolving it in 25 mL of methanol. From this solution the following concentrations: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mg mL^{-1} were prepared. The overload experiments were carried out using the prepared series of standard solutions and the following chromatographic conditions: CHIRALPAK AD (10 cm × 1.0 cm I.D., 20 μm) column, MeOH (100%) as mobile phase with a flow rate of 3.0 mL min^{-1}, λ = 305 nm, and 2 mL of injection volume.

The values for the inflexion points of the chromatographic bands of the overload chromatograms were measure on the first derivative plot acquire with Origin software and then used to determinate the initial parameters process, with the software HELP 10.3. For feed concentration of 5.0 mg mL^{-1} the parameters were: $Q_{recycle}$ = 18.26, $Q_{extract}$ = 11.91, Q_{feed} = 1.42, $Q_{raffinate}$ = 2.12, period = 2.0, Zone I = 0.98, Zone II = 2.38, Zone III = 1.43, Zone IV = 1.21 and a period of change of 2.0. For the monitoring and the regulation of the separation process Advanced System Controlled (ACS) software was used.

2.3. Internal concentration profile

The internal concentration profile was obtained by the quantification of raffinate and extract samples on different positions of the VARICOL cycle. To prepare the calibrator standards a stock solution of 25 mg of albendazole sulfoxide in 10 mL of methanol was prepared. The standards calibration samples were then prepared on the following concentrations: 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mg mL^{-1}. Calibration curves were constructed by plotting the peak area against the concentration of each enantiomer. The raffinate and extract samples were collected on 0.94, 1.44, 1.94, 2.90, 3.40, 3.90, 5.32, 5.82, 7.25, 8.68, and 11.06 min during cycle 55 of the VARICOL system.

2.4. IR and VCD spectroscopy

IR and VCD spectra of the enantiomers of **1** in the mid-IR spectral region (950–1800 cm^{-1}) were recorded with a Dual-PEM FT-VCD spectrometer using a resolution of 4 cm^{-1} and a collection time of 14 h. The optimum retardation of the two ZnSe photoelastic modulators (PEMs) was set at 1400 cm^{-1}. VCD spectra were measured with the dual-PEM option by subtracting in real time the VCD spectra associated with each of the two PEMs as previously described [17]. Spectra were calibrated automatically, using the standard calibration files. VCD spectra were recorded in $CDCl_3$ solution (10 mg of each compound in 400 μL of $CDCl_3$ (0.089 M) in a BaF_2 cell with 100 μm path length). Minor instrumental baseline offsets were eliminated from the final VCD spectra by subtracting the VCD spectra of the enantiomer and its antipode and dividing by 2.

The details of the computational methods used can be found in Supplementary data.

3. Results and discussion

3.1. Albendazole sulfoxide multimilligram enantiomeric separation

The versatility of polysaccharide chiral phases for separation of chiral sulfoxides is well documented in the literature [18–21] and the enantiomeric resolution of albendazole sulfoxide by these stationary phases has been reported on normal [16] and polar organic [22] modes of elution. The CHIRALPAK AD (10 cm × 1.0 cm I.D., 20 μm) was efficient to discriminate albendazole sulfoxide enantiomers for both modes (Table 1).

Table 1
Chromatographic parameters for the separation of albendazole sulfoxide.

Mobile phase[a]	k_1	α	R_s
n-Hexane/ethanol (70:30, v/v)	7.25	2.33	3.10
Methanol (100%)	1.60	3.13	4.05

[a] CHIRALPAK AD (10 × 1.0 cm I.D., 20 μm), 2.5 mL min^{-1}, 290 nm.

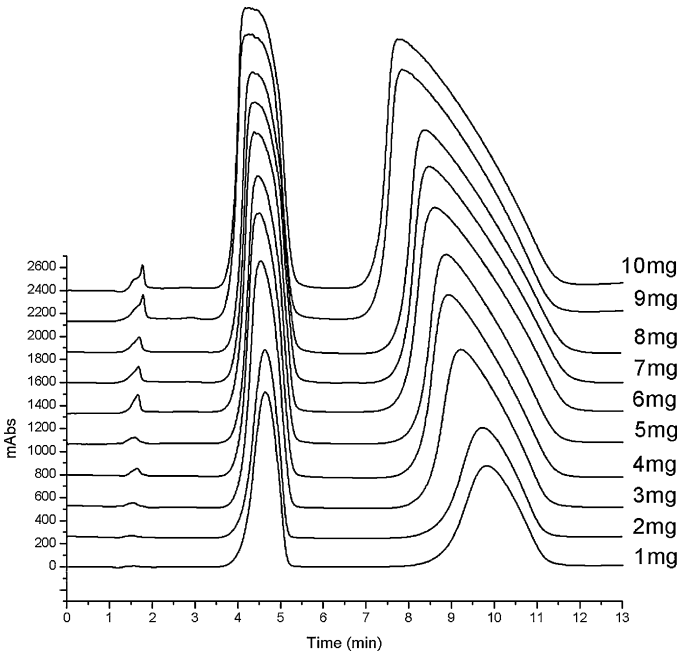

Fig. 2. Overload chromatograms obtained for the separation of albendazole sulfoxide by CHIRAPAK AD (10 cm × 1.0 cm I.D., 20 μm) using MeOH 100%, 3 mL min^{-1}, λ = 305 nm, injection volume of 2.0 mL.

For preparative separations the solubility is an important issue that should be investigated, since high solubility provides great productivities. In this way, polar organic solvents were preferentially selected, since albendazole sulfoxide is not soluble in hydrocarbon mobile phases.

The optimization of the enantiomeric resolution using VARICOL systems can be done after analytical chromatography experiments and with the application of modeling and simulation software.

The proper simulation of a chromatography separation requires reliable information about the equilibrium of the enantiomers between the mobile and stationary phase, specially, in cases of non-linear conditions. Different concentrations of racemic albendazole sulfoxide were systematically injected on one of the semipreparative CHIRALPAK AD columns used on the SMB system. The injection of diluted and concentrated solutions provided overload chromatograms (Fig. 2) that determined the interactions between the enantiomers and chiral stationary phase.

Based on these analytical experiments the different starting parameters (zones distributing, feed, mobile phase, raffinate, extract and recycling rates) were calculated using the HELP 10.3 software which is based on competitive Langmuir model adapted to include a correction term for solution concentration [23].

The feed concentration was set to 5.0 mg mL^{-1} which corresponds to half of the solubility of the racemate on the mobile phase at 25 °C. Applying the pre-determined starting parameters and operating at a cycle time of 12.10 min, the system achieved steady state – maximum concentration of binary mixture – after ten cycles. After that, the ACS software was turned on and the collection of the enantiomers was started.

During the separation process, in order to evaluate the enantiomeric purities of the extract and raffinate, samples were collected always at the beginning of cycles 11, 16, 21, 26, 40, 50 and 55. Launching the software ACS increased the purities of the two enantiomers collected. The enantiomeric ratio of raffinate and extract on cycle 11 was 64.0% and 40.0%, respectively, while after 5 cycles, the purities obtained were already 99.0% for the raffinate and 94.0% for the extract (the graphics can be found in Suppl. Fig. 1).

Table 2
Process parameters for the enantiomeric separation of albendazole sulfoxide.

Process Parameters	Values
Raffinate enantiomeric ratio (%)	99.5
Raffinate productivity (g/kg/day)	60.0
Raffinate rate of production (g/day)	1.90
Raffinate recovery (%)	95.0
Extract enantiomeric ratio (%)	99.0
Extract productivity (g/kg/day)	70.0
Extract rate of production (g/day)	1.99
Extract recovery (%)	99.0
Solvent consumption (L/g)	1.79

This software was also useful for monitoring and regulating the flow rates in the separation and cleaning zones throughout the cycles.

A total of 55 cycles was performed yielding 880 mg of raffinate (e.r. 99.5%) and 930 mg of extract (e.r. 99.0%). Recoveries of 95.0% for the first enantiomer and 99.0% for the second enantiomer were obtained. Table 2 presents the process parameters obtained for the enantiomeric resolution of albendazole sulfoxide.

The solubility of albendazole sulfoxide is very low in the organic solvents that can be used as mobile phase on adsorbed polysaccharide stationary phases. Among them, methanol is the one that best solubilizes this racemate, despite low concentration values.

Thus, although the enantiomeric separation had an impressive R_s, the low solubility resulted in productivity of only 10 g of each enantiopure compound per kilogram of stationary phase per day.

The enantiomeric rate of production for both enantiomers and high values of recoveries demonstrate the efficiency of the process, especially because the eluent used for the separation was recycled (97%) by simple distillation during the evaporation of the mobile phase.

Li and co-workers have reported a semi preparative method for the resolution of albendazole sulfoxide [16], but the simulated moving bed chromatography process developed here shows higher mass rate for both enantiomers and the same high values of enantiomeric purities.

In order to evaluate the zone distribution of raffinate and extract throughout one cycle, the internal concentration profile was calculated by the quantification of the enantiomers on cycle 55. Eleven samples were collected at specific times of the selected cycle, and then the enantiomeric purities were evaluated independently. After this, the areas of each enantiomer of the samples were interpolated by a calibration curve.

Ideally the internal concentration profile has to show no contamination in the collection zones. The extract is collected between zones I/II, while raffinate between zones III/IV. Besides that the

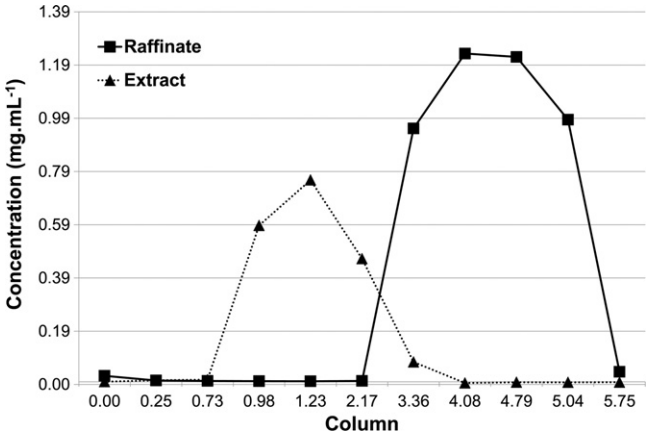

Fig. 3. Internal concentration profile obtained during the enantiomeric separation of albendazole sulfoxide on cycle 55.

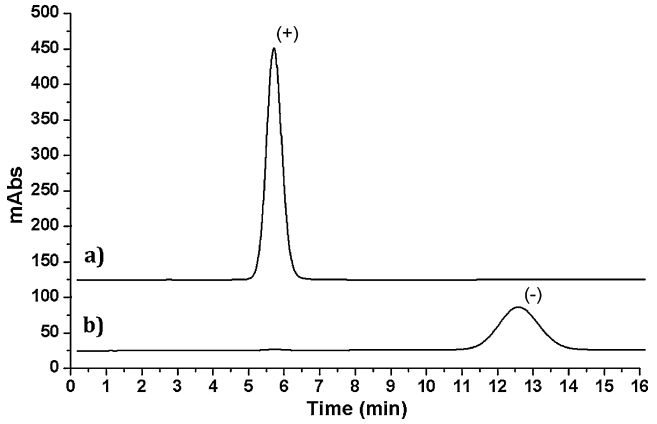

Fig. 4. Chromatograms obtained for (a) raffinate and (b) extract. Chromatographic conditions used: CHIRALPAK AD (10 cm × 1.0 cm I.D., 20 μm) column, MeOH (100%) as mobile phase at a flow rate of 3.0 mL min^{-1}, λ = 290 nm.

enantiomers should be collected at the highest concentrations; in this way, high purities and productivity will be attained for the separation process. Fig. 3 represents the internal concentration profile obtained during the resolution of albendazole sulfoxide enantiomers.

The internal concentration profile obtained demonstrates a small contamination of raffinate on the cleaning zone IV; however it did not affect the overall purities of the extract. In positions 0.98 and 4.79, where the extract and the raffinate were respectively collected, no evidence of impurities was detected, moreover a high concentration of the enantiomers was observed in these regions resulting in less dilute samples.

The $[\alpha]_D$ at 25 °C, was determined for both enantiomers and the values obtained were very similar to those previously reported [16]. Extract was identified as (−)-albendazole sulfoxide **(1)** $[\alpha]_D = -105$ and raffinate as (+)-albendazole sulfoxide **(2)** $[\alpha]_D = +108$ (Fig. 4).

3.2. Absolute configuration of albendazole sulfoxide enantiomers by VCD

In order to determine the absolute configuration of the enantiomers of albendazole sulfoxide, measurements and quantum chemistry calculations of IR and VCD spectra were carried out. By comparing IR and VCD measurements with the output of DFT calculations, the absolute configuration of each enantiomer of albendazole sulfoxide was unambiguously determined in solution as (−)-(*S*) and (+)-(*R*).

For the calculations, two combinations of hybrid functional and basis set were used, B3LYP/6-31G*, the most commonly used for VCD [24], and B3PW91/6-311G*, which uses a Gaussian basis set of triple-split valence. Even though B3LYP and B3PW91 generate qualitatively similar spectra [11], a larger basis set such as 6-311G* is supposed to promote better agreement with experimental data due to improved accuracy in the calculations.

The measurements were carried out at a concentration lower than 0.1 M in order to avoid aggregation, a previously observed occurrence in sulfoxides [11]. The experimental and calculated IR and VCD data for the enantiomers of albendazole sulfoxide are presented in Fig. 5. From that, it is possible to observe a very good agreement between the spectra of (−)-enantiomer and that calculated for the (*S*) configuration using both levels of theory. Such assignment was based on the presence of fundamentals 46, 49, 50, and, 52, which arise mainly from S—O stretching as well as aromatic C—H and non-aromatic C—H_2 bending vibrations of methylene groups directly linked to the stereogenic center. An interesting feature in the unpolarized absorption spectra (IR) is the difference in intensity between fundamentals 59 and 61 regarding calculated and experimental data. In the calculated spectra the fundamental 59, which comes from coupling of N-H bending and C—O stretching of the carbamate group (amide III band), is predicted to be substantially larger than that observed experimentally. This difference is probably due to an interchange in the calculated order of these two fundamental modes that occurs from the neglect of anharmonicity and intramolecular hydrogen bonding between N—H and C=O that

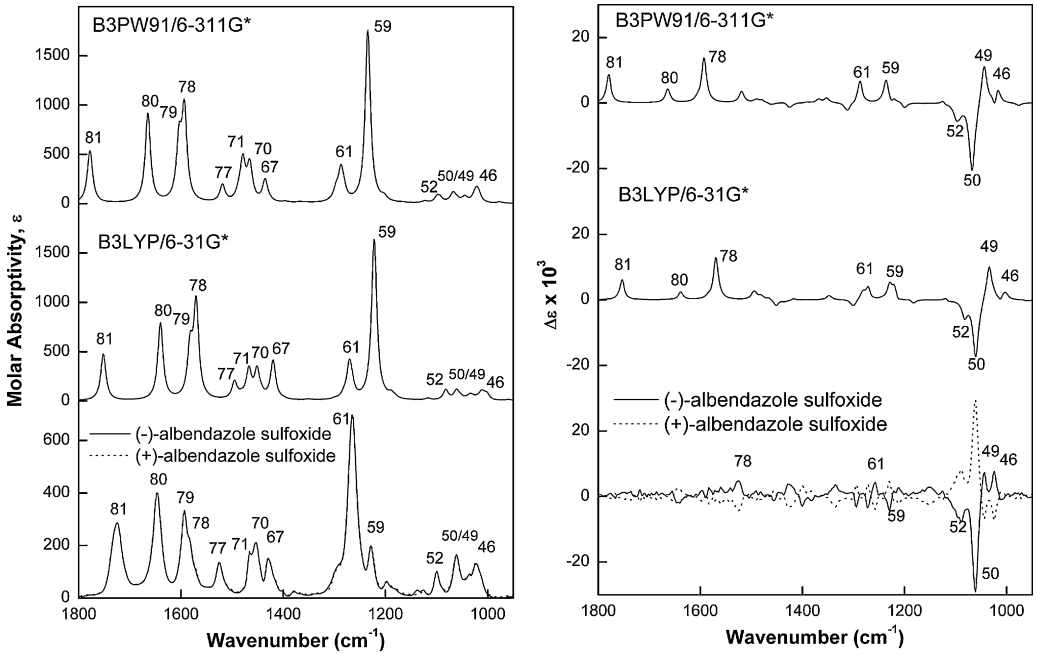

Fig. 5. Left: experimental IR for (−)-albendazole sulfoxide enantiomer (solid line) and (+)-albendazole sulfoxide enantiomer (dotted line) and calculated data for (*S*)-enantiomer at the B3LYP/6-31G* and B3PW91/6-311G* levels. On the right: experimental VCD for (−)-albendazole sulfoxide enantiomer (solid line) and (+)-albendazole sulfoxide enantiomer (dotted line) and calculated data for (*S*)-enantiomer at both levels of theory. The comparison of observed and calculated data establishes the absolute configuration of (−)-albendazole sulfoxide as (*S*). Numbers represent fundamentals.

forms a pseudo six-membered ring. With regard to the five lowest-energy conformers predicted to be significantly populated at room temperature, at both levels of theory, the main conformational changes were represented by 180° flipping about the S—CAr bond and different positioning of the flexible propyl substituent bound to the chiral sulfur atom (the structures can be found in Suppl. Fig. 2). It is also noteworthy that both levels of theory yielded basically the same lowest-lying conformers and almost identical VCD spectra, the only significant difference being the Boltzmann distribution. The latter aspect is expected when different basis set are employed [8] and slight changes in the Boltzmann distribution may be responsible for the fact that fundamentals 59, 61, 78, 80 and 81 were not cancelled out within the conformers identified, as observed in the experimental spectra. Finally, for the assignment of (*S*)-(−)-albendazole sulfoxide the output of the confidence level algorithm was as follows: ESI = 68.3 and Confidence Level of 100%.

4. Conclusion

The enantiomeric separation of albendazole sulfoxide by simulated moving bed chromatography with variable zones was successfully developed achieving enantiomeric ratios of 99.5% for the (+)-enantiomer and of 99.0% for (−)-enantiomer and thus, allowing the unambiguous determination of their absolute configuration, for the first time, as (−)-(*S*) and (+)-(*R*) by the application of VCD spectroscopy and DFT calculations. The overall recovery of 97% and the use of a mobile phase that was recycled by simple distillation are other positives facts of the process herein described. Furthermore, the mass rate of 2 g/day is a satisfactory figure for a variety of applications in pharmaceutical analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.01.070.

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