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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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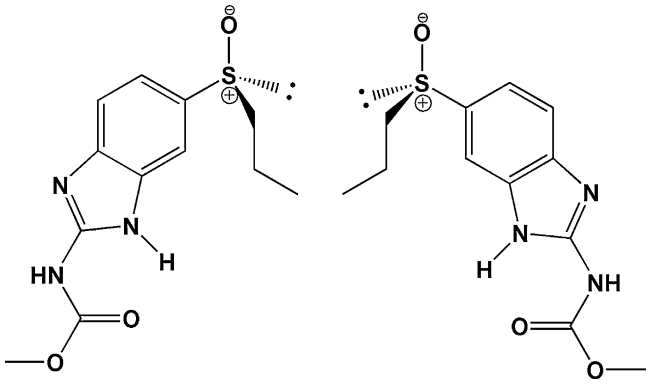

Fig. 1. Chemical structures of albendazole sulfoxide enantiomers.

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.