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Note

Direct resolution of anthelmintic drug enantiomers on Chiral-AGP protein-bonded chiral stationary phase

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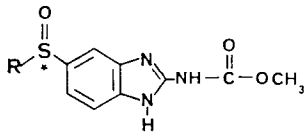
Albendazole (ABZ, Zental® or Valbazen®) and fenbendazole (FBZ, Panacur®) are benzimidazole anthelmintic drugs, used in the treatment of helminthiases in animals[1] and man[2]. After oral administration, the sulphide (ABZ or FBZ) is oxidized to the corresponding sulphoxide SOABZ (or SOFBZ)[3,4], which bears an asymmetric sulphur centre (Fig. 1). The sulphone compound SO_2ABZ (or SO_2FBZ) is also present as a metabolite. The sulphoxide is considered to be responsible for the anthelmintic activity; however, apart from this therapeutic property, embryotoxicity has been found in rats[5,6].

The liquid chromatographic (LC) separation of SOABZ and SOFBZ enantiomers on chiral stationary phases (CSPs) will provide a useful means of studying stereoselectivity in the pharmacokinetic disposition of these metabolites in animals and man. The resolution of SOABZ and SOFBZ enantiomers has been described previously on a CSP synthesized from (*S*)-N-(3,5-dinitrobenzoyl)tyrosine as chiral selector[7]. A baseline resolution of SOABZ enantiomers was achieved allowing enantiomeric assays of these metabolites in human, bovine, sheep and rat plasma samples[7]. Differences in the enantiomeric ratios were found, depending on the species,



SOABZ R = $-(CH_2)_2-CH_3$

SOFBZ 

Fig. 1. Structures of albendazole sulphoxide (SOABZ) and fenbendazole sulphoxide (SOFBZ).

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which could be attributed to different enzymatic processes. However, the resolution of SOFBZ enantiomers was poor (resolution factor $R_s \leqslant 0.8$) and still remained a challenge.

The use of bovine serum albumin (BSA) bonded phase (Resolvosil-BSA-7 column, available from Macherey, Nagel & Co., Düren, F.R.G.) has been described by Allenmark *et al.*[9] for the resolution of pharmacologically active chiral sulphoxides containing a benzimidazole moiety. This paper reports the direct LC resolution of SOABZ and SOFBZ enantiomers on a commercially available CSP, Chiral-AGP (ChromTech, Stockholm, Sweden) developed by Hermansson[10] and Enquist and Hermansson[11], and for which plasma α_1-acid glycoprotein is immobilized according to a novel technique on spherical porous silica particles ($d_p = 5$ μm).

EXPERIMENTAL

Apparatus

Analytical chromatography was performed with a modular liquid chromatograph (Gilson, Villiers-le Bel, France) equipped with a Model 802C manometric module, a Gilson 811 (1.5-ml) dynamic mixer and a Model 116 variable-wavelength UV detector. The column and solvent were thermostated with a Haake Model D8-V circulator bath (−5 to 150°C) (Roucaire, Vélizy-Villacoublay, France) and a water cooling-jacket. All tubing connections were heat-insulated.

Chiral stationary phases

A 100 × 4.0 mm I.D. Enantiopac column was purchased from LKB (Les Ulis, France). The plasma protein α_1-AGP was immobilized on a 10-μm diethylaminoethylsilica gel by ionic bonding followed by cross-linking. A Chiral-AGP (100 × 4.6 mm I.D.) column was purchased from ChromTech. In both instances t_0 was measured by injection of methanol.

Solutes and solvents

Samples of ABZ and FBZ sulphides, the corresponding SOABZ and SOFBZ sulphoxides (as racemates) and SO_2ABZ and SO_2FBZ sulphones were donated by Professor P. Delatour (Laboratoire de Biochimie, I.N.R.A. 54189, Ecole Nationale Vétérinaire de Lyon, France).

Aqueous buffer solutions were prepared from sodium phosphate buffer (25 m*M*, pH 6.88) purchased from Merck (Darmstadt, F.R.G.). Deionized water was doubly distilled on a Büchi-Fontavapor 285 apparatus (Roucaire). The pH of the aqueous buffer eluent was controlled with a Model Minisis 8000 pH/millivoltmeter (Tacussel, Villeurbanne, France) and Tacussel glass TB/HS and Tacussel C8 calomel reference electrodes. Aqueous solvents were filtered through 0.65-μm Type DAWP Millipore membrane filters (Touzart et Matignon, Paris, France) and then degassed with helium. 2-Propanol was of LiChrosolv grade purchased from Merck.

Solutes were first dissolved in 2-propanol in an ultrasonic bath; the solution was then diluted 5-fold with 8 m*M* sodium phosphate buffer (pH 7.0). The concentrations of the solutes were chosen around $3 \cdot 10^{-2}$ mg ml^{-1}, corresponding to an amount injected of about 2 nmol (20 μl)

RESULTS AND DISCUSSION

As shown in Fig. 2 for the resolution of SOFBZ enantiomers, the Chiral-AGP column displays a higher efficiency than the Enantiopac column. Moreover, shorter analysis times are afforded, as the Chiral-AGP column can be used without flow restrictions.

The retention and enantioselectivity (α) are highly affected by the concentration of 2-propanol in the mobile phase, as demonstrated in Table I. A decrease in the 2-propanol content from 2% to 0% resulted in a strong improvement in the enantioselectivity and an increase in retention. This is in accordance with previous findings for other classes of compounds[10–16]. The enantiomers of SOFBZ are, therefore, completely resolved using a mobile phase without 2-propanol (Fig. 3). The organic modifier probably competes with the uncharged solute for binding to the protein, as previously suggested by Hermansson and co-workers[11,12,16], through hydrophobic and/or hydrogen bonding interactions. The stereospecific binding of the chiral drug to the binding sites of α_1-acid glycoprotein may be altered in the presence of 2-propanol owing to conformational changes in the flexible protein structure.

From the chromatogram in Fig. 2B, it is obvious that a separation factor of *ca.* 1.25 is insufficient to obtain a complete baseline resolution of SOFBZ. However, a complete resolution ($R_s \geqslant 1.5$) of SOFBZ is achieved using a mobile phase without 2-propanol (Fig. 3). Under the same mobile phase conditions, SOABZ enantiomers

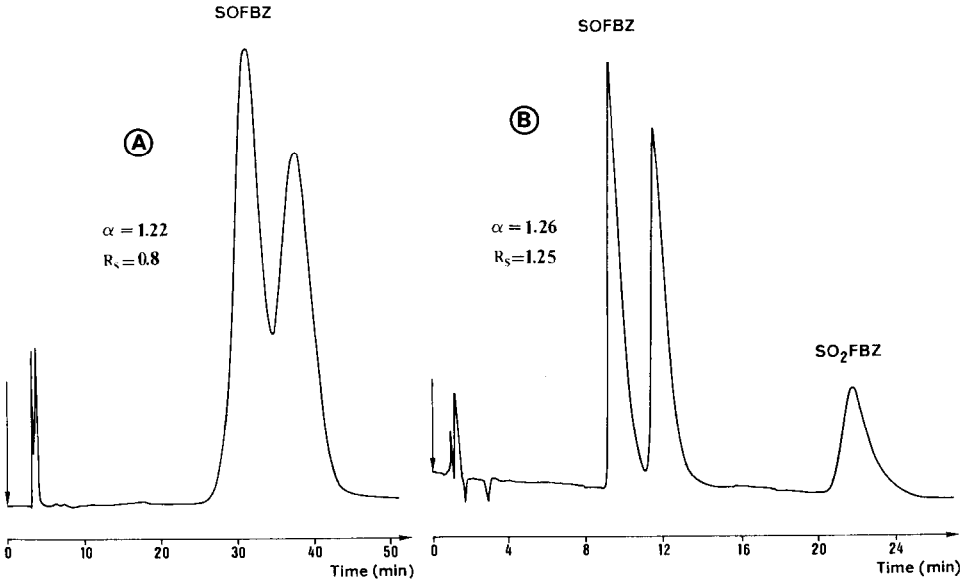

Fig. 2. Comparison of resolution of oxfendazole enantiomers (SOFBZ) on two acid α_1-glycoprotein bonded columns. (A) Enantiopac column (d_p = 10 μm); mobile phase, 8 m*M* sodium phosphate buffer (pH 7.0) + 0.1 m*M* NaCl + 2% (v/v) 2-propanol; flow-rate, 0.3 ml min^{-1}. (B) Co-injection of SOFBZ and SO_2FBZ on a Chiral-AGP column (d_p = 5 μm); mobile phase, 8 m*M* sodium phosphate buffer (pH 7.0) + 2% (v/v) 2-propanol; flow-rate, 0.9 ml min^{-1}; temperature, 25°C; UV detection at 220 nm. Efficiencies measured by means of reduced plate height, $h = L/Nd_p$: (A) $h \approx 40$, $k'_2 = 10.0$; (B) $h \approx 20$, $k'_2 = 10.8$.

TABLE I

INFLUENCE OF THE ADDITION OF 2-PROPANOL TO THE AQUEOUS MOBILE PHASE ON THE RESOLUTION OF SOABZ AND SOFBZ ENANTIOMERS AND ON THE RETENTION OF SO_2ABZ AND SO_2FBZ SULPHONES

Column, Chiral-AGP; mobile phase, 8 m*M* sodium phosphate buffer (pH 7) with 2-propanol added; flow-rate, 0.9 ml min^{-1}; temperature, 25°C; UV detection at 220 nm.

2-Propanol (%,v/v)	*SOABZ*			*SO_2ABZ, k'*	*SOFBZ*			*SO_2FBZ, k'*
	k'_2[a]	α[b]	R_s[c]		k'_2	α	R_s	
2	2.70	1.35	1.1	2.69	10.80	1.26	1.1	20.6
1	3.97	1.51	1.25	3.70	14.23	1.28	1.25	
0	7.33	1.68	1.4	6.37	18.94	1.54	1.6	

[a] The capacity factor k'_2 (of the second eluted enantiomer) was calculated as follows: $k'_2 = (t_{R2} - t_0)/t_0$.

[b] Selectivity $\alpha = k'_2/k'_1$.

[c] R_s (resolution factor) = 2 (distance of the two enantiomer peak positions/sum of the band widths of the two peaks at their bases); $R_s = 2(t_{R2} - t_{R1})/(w_1 + w_2)$.

are almost baseline resolved, despite the peak asymmetry of the first eluted enantiomer (Fig. 4).

SOFBZ enantiomers are more retained than the SOABZ enantiomers; enhancement of hydrophobic interactions with the protein caused by the phenyl group of SOFBZ may be responsible.

The capacity factors of the sulphone compounds are also given in Table I and demonstrate that SO_2ABZ is eluted between the two enantiomers of SOABZ (Fig. 4). This constitutes a drawback for accurate assays of SOABZ enantiomers in biological samples containing the sulphone metabolite. Fortunately, such peak overlapping does not occur with SOFBZ and SO_2FBZ (Fig. 2B).

The above findings demonstrate the chiral recognition ability of Chiral-AGP towards a new family of anthelmintic drugs. However, further investigations will be

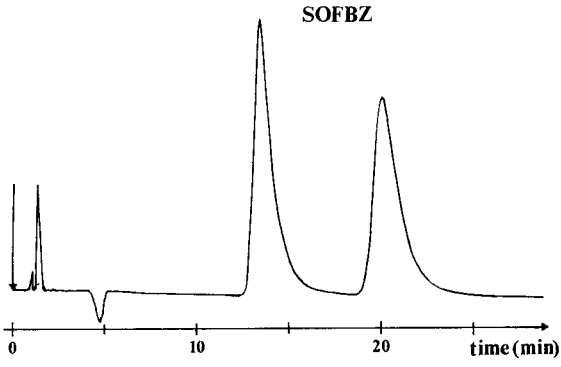

Fig. 3. Resolution of SOFBZ enantiomers on Chiral-AGP column. Reduced plate height, $h_{SOFBZ} \approx 25$. Mobile phase, 8 m*M* sodium phosphate buffer (pH 7.0); flow-rate, 0.9 ml min^{-1}; temperature, 25°C; UV detection at 220 nm.

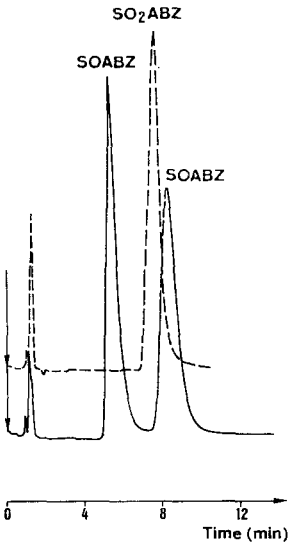

Fig. 4. Resolution of SOABZ enantiomers and comparison of elution of SO_2ABZ on Chiral-AGP column. Reduced plate height, $h_{SOABZ} \approx 40$. Mobile phase, 8 m*M* sodium phosphate buffer (pH 7.0); flow-rate, 0.9 ml min^{-1}; temperature, 25°C; UV detection at 220 nm.

undertaken to improve the resolution (especially in terms of efficiency) and prevent peak overlapping of SOABZ and SO_2ABZ. Other organic modifiers (various alcohols, aprotic polar solvents, etc.[11]) are under study, and also the influence of temperature.

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REFERENCES

1 V. J. Theodorides, R. J. Gyurik, W. D. Kingsbury and J. C. Parish, *Experientia*, 32 (1976) 702.
2 A. G. Saimot, A. C. Crémieux, J. M. Hay, A. Meulemans, M. D. Giovanangeli, B. Delaitre and J. P. Coulaud, *Lancet*, 17 (1983) 652.
3 R. J. Gyurik, A. W. Chow, B. Zaber, E. L. Brunner, J. A. Miller, A. J. Villani, L. A. Petka and R. C. Parish, *Drug Metab. Dispos.*, 9 (1981) 503.
4 S. E. Marriner, D. L. Morris, B. Dickson and J. A. Bogan, *Eur. J. Clin. Pharmacol.*, 30 (1986) 705.
5 P. Delatour, R. C. Paris and R. J. Gyurik, *Ann. Rech. Vét.*, 12 (1981) 159.
6 P. Delatour, F. Garnier, E. Benoit and Ch. Longin, *J. Vet. Pharmacol. Ther.*, 7 (1984) 139.
7 A. Tambuté, A. Begos, M. Lienne, P. Macaudière, M. Caude and R. Rosset, *New J. Chem.*, in press.
8 M. Lienne, M. Caude, R. Rosset, A. Tambuté and P. Delatour, *Chirality*, 1 (1989) 45.
9 S. Allenmark, B. Bomgren, H. Boren and P. O. Lagerström, *Anal. Biochem.*, 136 (1984) 293.
10 J. Hermansson, paper presented at the *1st International Symposium on Separation of Chiral Molecules, Paris, May 31st–June 2nd, 1988.*
11 M. Enquist (Eriksson) and J. Hermansson, paper presented at the *12th International Symposium on Column Liquid Chromatography, Washington, DC, June 19–24th, 1988.*
12 J. Hermansson, *J. Chromatogr.*, 269 (1983) 71.

13 J. Hermansson, *J. Chromatogr.*, 325 (1985) 379.
14 J. Hermansson, *J. Chromatogr.*, 298 (1984) 67.
15 G. Schill, I. W. Wainer and S. A. Barkan, *J. Liq. Chromatogr.*, 9 (1986) 641.
16 J. Hermansson and G. Schill, in M. Zief and L. Crane (Editors), *Chromatographic Chiral Separations*, Vol. 40, Marcel Dekker, New York, 1987, p. 245; and references cited therein.