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Direct Separation of Albendazole Sulfoxide Enantiomers by Liquid Chromatography on a Chiral Column Deriving From (S)-*N*-(3,5-Dinitrobenzoyl)tyrosine: Application to Enantiomeric Assays on Plasma Samples

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ABSTRACT The direct enantiomeric resolution of albendazole sulfoxide (SOABZ), an anthelmintic drug belonging to the benzimidazole class, is reported on a chiral stationary phase (CSP) synthesized by covalent binding of (S)-*N*-(3,5-dinitrobenzoyl)tyrosine-*O*-(2-propen-1-yl) methyl ester on a γ-mercaptopropyl-silanized silica gel. A comparison with the resolution achieved on commercially available Pirkle-type CSPs obtained from *N*-(3,5-dinitrobenzoyl) derivatives of (R)-phenyglycine or (S)-phenylalanine is described. Some structurally related chiral sulfoxides including oxfendazole (SOFBZ) are also studied. Optimization of the mobile phase nature and composition is investigated showing that a hexane–dioxane–ethanol ternary mixture affords an almost baseline resolution (R_s = 1.25); however, in this case, albendazole sulfone (SO_2ABZ) is eluted between the two sulfoxide enantiomers; accordingly, a hexane–ethanol mobile phase would be preferred for biological samples containing both metabolites. The influence of temperature on the resolution is depicted with a hexane–ethanol mobile phase. Finally, application to the enantiomeric assays of SOABZ in plasmatic extracts of rat, sheep, bovin, and man after oral administration of albendazole (sulfoxidized to SOABZ and SO_2ABZ) is reported. Some distortions in the enantiomeric ratios are evidenced depending on the species.

KEY WORDS: enantiomeric separations, chiral stationary phase, Pirkle-type phases, HPLC, anthelmintics, benzimidazole sulfoxides, albendazole, fenbendazole, oxfendazole

INTRODUCTION

Albendazole (ABZ sulfide) is a broad-spectrum anthelmintic belonging to the important benzimidazole chemical class and marketed as Zental® and Valbazen®. This drug is active in animals[1] and man[2] against liver flukes, tapeworms, lungworms, and gastrointestinal roundworms. After oral administration of albendazole the major metabolite present in plasma fluid is the chiral sulfoxide of albendazole (SOABZ; Fig. 1) which is responsible for the anthelmintic activity.[3,4] Substantial amounts of albendazole sulfone (SO_2ABZ) are also detectable in plasma[4] (Scheme 1) while the parent compound is generally completely metabolized. No study of the enantiomeric composition of the sulfoxide metabolite in vivo has been published as yet and hitherto the two enantiomers were expected rather to exist as a racemic mixture. Besides the anthelmintic properties, SOABZ exhibits also some teratogenic effects (antimicrotubular properties) evidenced in rats.[5,6] The liquid chromatographic resolution of SOABZ enantiomers on chiral stationary phases (CSPs) provided us with a direct, easy, and reproducible means to achieve accurate enantiomeric assays of these metabolites: this can then be applied to the study of stereoselective disposition pharmacokinetics in animals and man.

In this paper the resolution of SOABZ enantiomers on a CSP deriving from (S)-*N*-(3,5-dinitrobenzoyl)tyrosine (CSP 1; Fig. 2) and the application to enantiomeric assays on plasma or urinary samples are described. This

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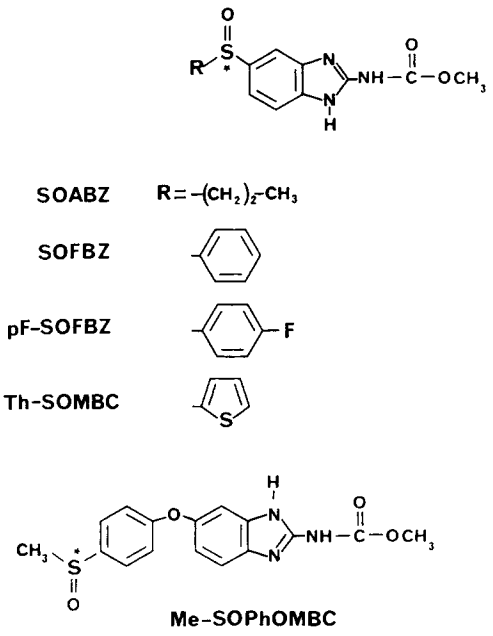
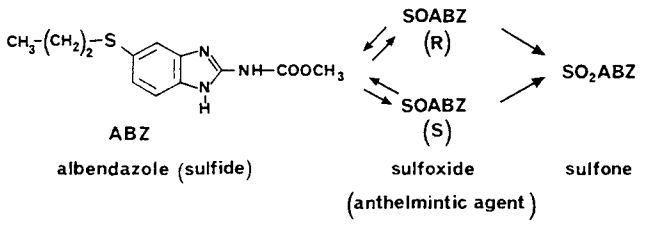


Fig. 1. Chemical structure of chiral sulfoxides studied in the present paper.



Scheme 1. Schematic metabolic pathway after oral administration of ABZ.

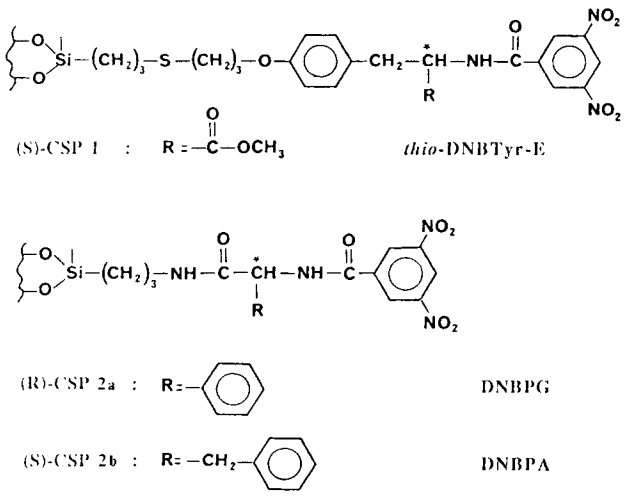


Fig. 2. Chemical structure of the investigated CSPs.

laboratory-made CSP is compared to two other commercially available Pirkle-type CSPs displaying a related chemical structure. Results concerning some structurally related sulfoxides (Fig. 1) including oxfendazole (fenbendazole sulfoxide SOFBZ; Panacur®, Synanthic®), another anthelmintic drug, are also presented.

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. UV absorption maxima of SOABZ are at 220 and 290 nm. The column and solvent were thermostated with a Haake Model D8-V circulator bath (−5°−150°C) (Roucaire, Vélizy-Villacoublay, France) and a water cooling-jacket. All tubing connections were heat insulated.

Chiral Stationary Phases

All the CSPs structures are gathered in Figure 2. General procedures for the synthesis of the stationary phase deriving from (S)-tyrosine (CSP 1: *thio*-DNBTyr-E) were depicted previously.[7] CSPs 2a and 2b were obtained starting from (R)-phenylglycine and (S)-phenylalanine, respectively.[8] These two Pirkle-type CSPs are also commercially available: CSP 2a as DNBPG column from J.T. Baker (Sochibo, Vélizy-Villacoublay, France) and CSP 2b (DNBPA) as Chiraline LO151 from S.F.C.C. (Neuilly-Plaisance, France); when tested, the commercialized CSP gave the same results as the laboratory-made CSP 2a and 2b. The chiral selectors were covalently bound to either LiChrosorb Si-60 (5 or 7 μm) modified with γ-mercaptopropyltrimethoxysilane (CSP 1) or γ-aminopropyl silica gel (LiChrosorb-NH_2 Si-60, dp = 5 μm, CSP 2a and 2b). Silica gels were purchased from Merck (Darmstadt, FRG).

Mobile Phase and Solutes

Ethanol, 2-propanol, dioxane, and *n*-hexane were of LiChrosolv grade, purchased from Merck (Darmstadt, FRG). This quality of solvent allowed UV detection at 220 nm. Chloroform of analytical grade (stabilized with 0.6% w/w of ethanol) was purchased from Prolabo (Paris, France).

Synthetic albendazole ABZ, fenbendazole FBZ, and their respective racemic sulfoxide and sulfone derivatives were purchased from SmithKline Beckman (U.S.A.), Synthex (U.S.A.), or Hoechst (FRG). After oral administration of albendazole, blood samples were taken at the postdose rate date corresponding to the plasma optimum of SOABZ (Table 1). Dose rates administered were consistent with the metabolic ability of each species to form SOABZ metabolites.

Extraction procedures and purification

Plasma and urine samples were extracted by diethylether according to a method previously described.[9] For

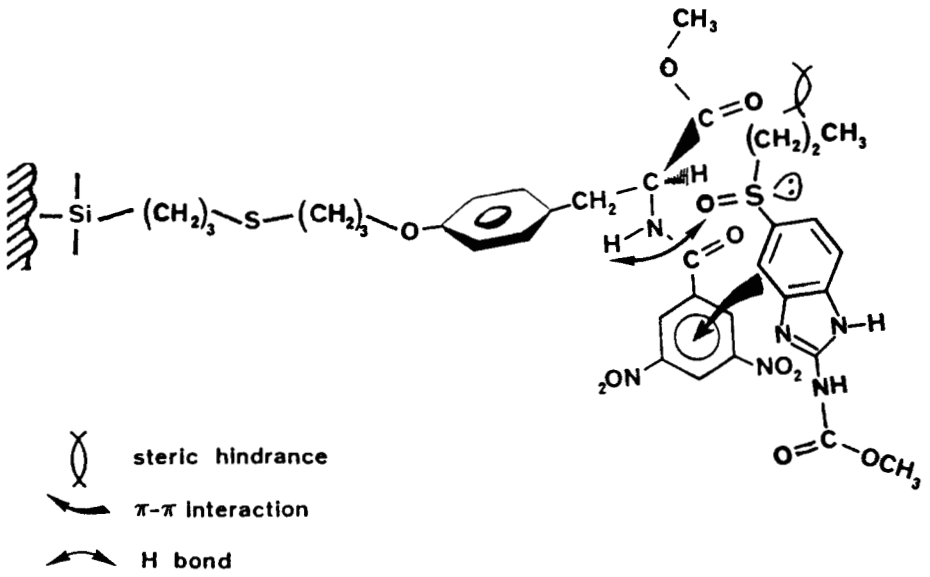


Fig. 3. Proposed chiral recognition mechanism for the resolution of SOABZ enantiomers on CSP 1.

TABLE 1. Pharmacokinetic parameters for albendazole sulfoxide (SOABZ) after oral administration of albendazole (ABZ)[a]

Species	Dose rate (mg kg^{-1})	t_{max} (h)	C_{max} (μg ml^{-1})
Rat	20	3	5.6
Man	10	4	0.4
Sheep	15	10	3.3
Bovin	20	16	0.5

[a] t_{max} is the time at which the maximum concentration (C_{max}) of SOABZ is reached.

TABLE 2. Compared resolution of SOABZ enantiomers on CSP 1, 2a, and 2b[a]

CSP	k'_2[b]	α[c]	R_s[d]
CSP 1	12.3	1.11	1.1
CSP 2a	14.0	1.06	0.5–0.6
CSP 2b	18.9	1.07	0.7

[a] Columns: 150 × 4.6 mm i.d. Mobile phase: hexane–ethanol, 88:12 (v/v); flow rate: 2 ml min^{-1}. UV detection at 220 nm. Temperature: 25°C.
[b] Capacity factors k'_1 and k'_2 (respectively of the first and second eluted enantiomers) were calculated from dead retention time t_0 (t_0 = 0.95 min) as follows: $k' = (t_r - t_0)/t_0$.
[c] Selectivity $\alpha = k'_2/k'_1$.
[d] R_s (resolution factor) = 2 (distance of the two enantiomer peak positions/sum of the bandwidths of the two peaks at their bases) = $2\,(t_{r2} - t_{r1})/(w_1 + w_2)$ or estimated when poor.[21]

species where SO_2ABZ was more abundant than SOABZ, a TLC isolation of SOABZ[3] was performed.

Dried extracts were dissolved in chloroform of analytical grade (stabilized with 0.6% w/w of ethanol) prior to their injection on the chiral column. Each sample was injected at least 5-fold to determine the enantiomeric ratios.

Optimization of the CSP structure. A review of literature data shows that the resolution of chiral sulfoxides has been successfully investigated on several CSPs including commercially available CSPs. Pirkle et al.[10,11] described the resolution of chiral sulfoxides containing a π-acid moiety on a π-basic suitably designed CSP synthesized from (R)-2,2,2-trifluoro-1-(9-anthryl)ethanol. These authors reported the further analytical[12] and preparative[13] scale separation of arylalkyl-, diaryl-, cyclic sulfoxides on the first reciprocal (R)-*N*-(3,5-dinitrobenzoyl)phenylglycine derived CSP [(R)-DNBPG, CSP 2a, Fig. 2]. Various chiral sulfoxides were also easily resolved on a so-called DACH-DNB CSP, designed by Gargaro et al.,[14] containing the 3,5-DNB derivative of (R,R)-1,2-diaminocyclohexane, when operating with a hexane–dioxane mobile phase. Preparative chromatography was then allowed on this CSP. Cellulose derivatives coated on silica gel (Chiracel OB[15] and OC[15,16]) also display a good ability to resolve chiral sulfoxides and other classes of chiral sulfur compounds (sulfoximines, sulfinamides, and sulfilimines). Finally, bovine serum albumin (BSA) bonded phases proved to be effective in the resolution of sulfoxides enantiomers as demonstrated by Allenmark et al.[17,18] for a series of pharmacologically active sulfoxides containing a benzimidazole ring such as Omeprazole®.

Considering the above-mentioned results we first studied the direct resolution of SOABZ enantiomers on various CSPs including commercially available ones.[19] No

resolution was observed on DACH-DNB, BSA, and Chiralcel OC CSPs[19] in standard conditions. Chiralcel OB,[20] DNBPG-, and DNBPA-CSPs gave a poor resolution inappropriate to enantiomeric assays ($R_s \leq 0.8$) (Table 2). Laboratory-made CSP 1 happened to be the most suitable (Table 2). The broad applicability of this CSP has been previously described[7] as well as its specific chromatographic behaviour.[22,23] Considering selectivity value, variations observed on CSPs 1, 2a, and 2b probably arise from particular conformation and steric hindrance features of these CSPs. First the binding mode of the chiral graft is involved: on CSP 1 the chiral carbon centre is remote, away from the silica matrix due to a longer "spacer arm" compared to CSPs 2a and 2b. An

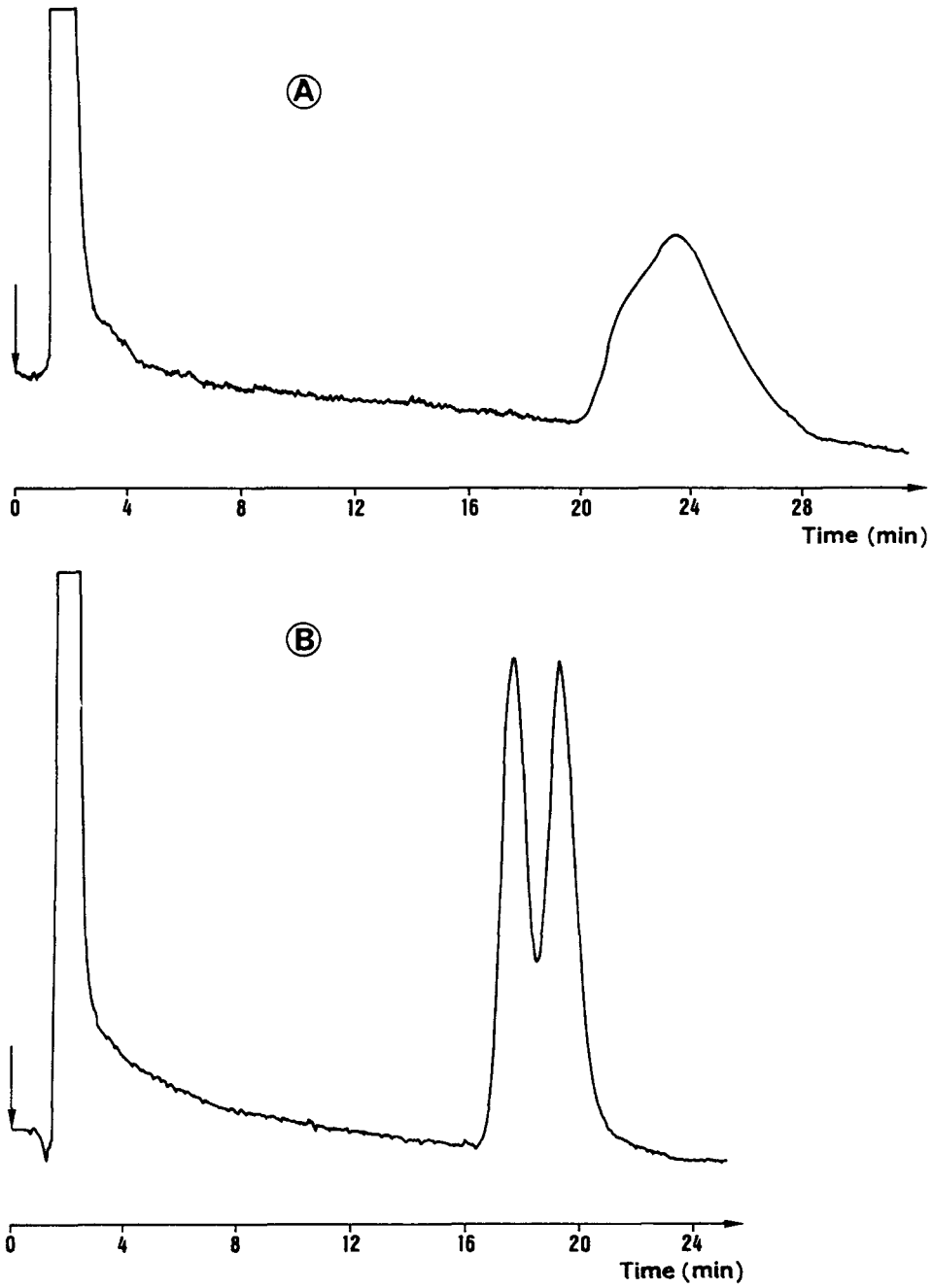


Fig. 4. Influence of the addition of water to a hexane–2-propanol mobile phase on the resolution of SOABZ enantiomers. **A**: Mobile phase: hexane–2-propanol, 80 : 20 (v/v); **B**: mobile phase: hexane–2-propanol [containing 2% of water (v/v)] 80 : 20 (v/v). Same other operating conditions as in Table 4; 0.4 μg injected.

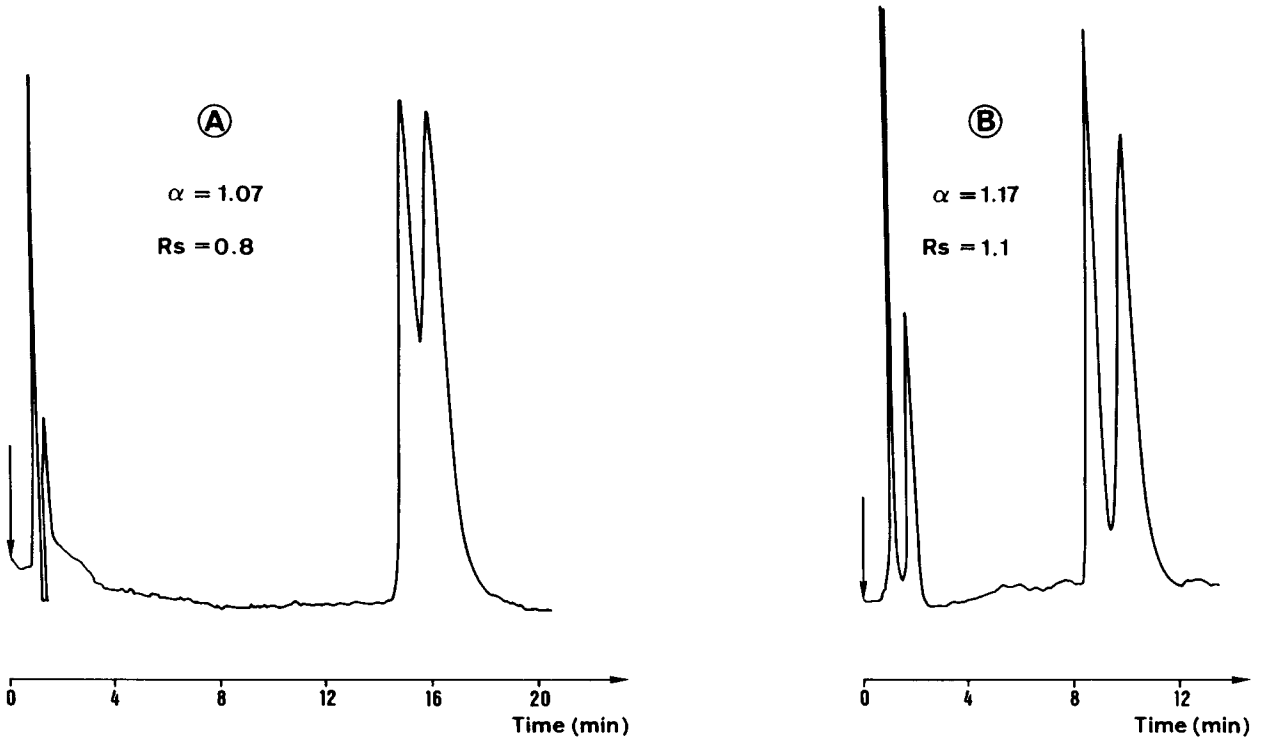


Fig. 5. Influence of chloroform in mobile phase on the resolution of SOABZ enantiomers on CSP 2b. Mobile phases: **A**: hexane–ethanol, 88 : 12 (v/v); **B**: hexane–chloroform–ethanol, 60 : 35 : 5 (v/v). Same other operating conditions as in Table 5.

additional amide dipole is created on CSPs 2a and 2b owing to the linkage on aminopropylsilanized silica gel; intramolecular hydrogen bonding can be then expected between these two amide moieties. Second, changing a phenyl substituent (CSP 2a) to a benzyl one (CSPs 1 and 2b) on the asymmetric carbon induces a higher flexibility and less steric hindrance[22] around this chiroselective site: CSP 2b displays an intermediate behaviour between CSP 1 and CSP 2a (Table 2). Since CSP 1 affords a higher selectivity value, the sulfoxide is expected to better match the conformation of CSP 1.

Two main attractive interactions can be advocated in the formation of transient diastereomeric solute–CSP complexes[24]: a hydrogen bonding interaction between the dinitrobenzamide NH moiety and S=O group in addition to a π–π stacking of the DNB and benzimidazole groups (Fig. 3). On CSP 2a and 2b a competing hydrogen bonding interaction can, however, take place with the linkage amide group. CSP 1 was evaluated for the resolution of some other closely related sulfoxides; chromatographic data listed in Table 3 show low and homogeneous selectivity values.

TABLE 3. Resolution of chiral sulfoxides structurally related to SOABZ on CSP 1[a]

Solute	k'_2	α	R_s
SOABZ	8.25	1.09	1.1
SOFBZ	11.65	1.05	0.7
pF-SOFBZ	10.84	1.06	0.9
Th-SOMBC	14.40	1.05	0.7
Me-SOPhOMBC	24.21	1.05	0.6

[a]Column: 250 × 4.6 mm i.d. Mobile phase: hexane–ethanol, 86 : 14 (v/v); flow rate: 2 ml min^{-1}. UV detection at 220 nm. Temperature: 40°C.

Retention times on CSP 1 are lower than on CSP 2a and 2b (Table 2; the coverage rate of the three CSPs calculated from microanalyses was about 0.3 mmol of chiral graft per gram of CSP[22]). The binding way of the chiral selector can, once more, account for this observation. Nonstereoselective adsorption of the sulfoxide on unbound aminopropyl "arms" and on the remaining silanol groups of CSP 2a and 2b can indeed be considered. Avoiding nonstereoselective interactions generally leads to lower retention times and better enantiorecognition (expressed as selectivity value) as observed on CSP 1: the above-mentioned adsorption may be limited to a certain extent on mercaptopropylsilanized silica gel owing partly to the longer "spacer arm" of CSP 1. Irreproducible random peak shape distortions occurred especially on CSP 2a and 2b, depending on the mobile phase composition and above all on the solvent used for solubilizing the sulfoxide: peak broadening, tailing, and sometimes splitting. Methanol, chloroform, tetrahydrofuran, acetonitrile, and various hexane–alcohol mixtures were tested. Chloroform gave the best results. For low concen-

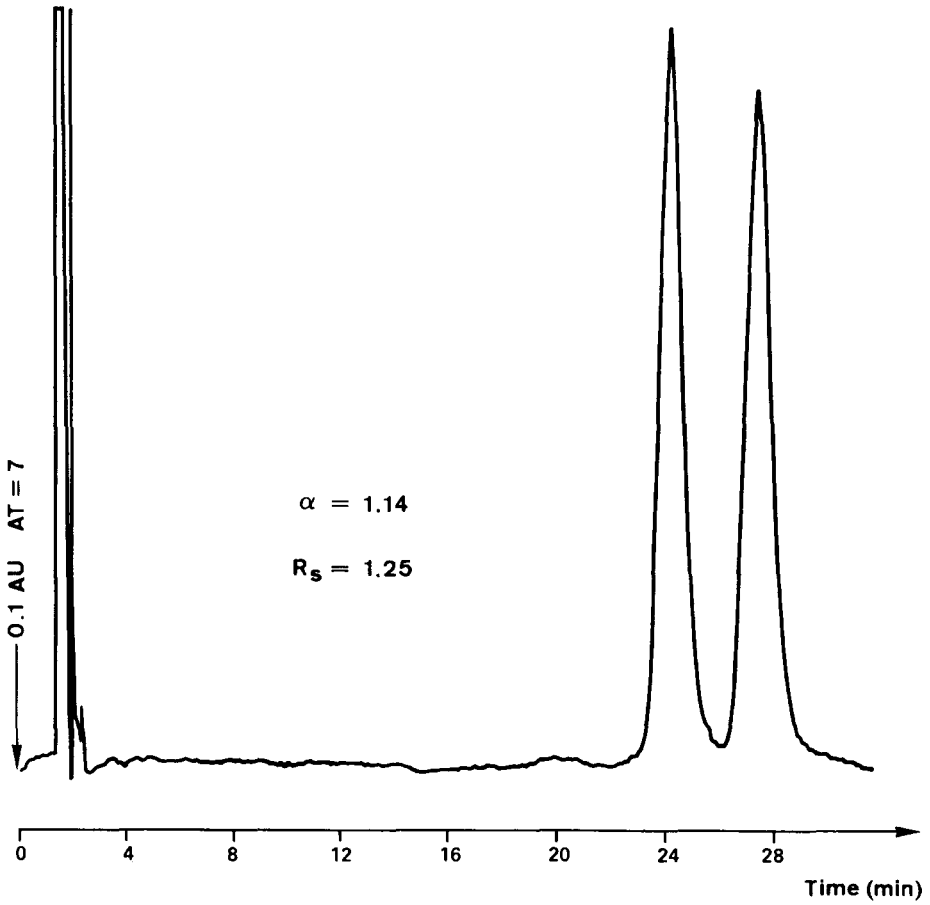


Fig. 6. Resolution of SOABZ enantiomers on CSP 1. Column: 25 cm × 4.6 mm i.d. Operating conditions: mobile phase, hexane–dioxane–ethanol 80:16:4 (v/v/v); flow rate, 2 ml min^{-1}; temperature, 25°C, UV detection at 230 nm. Injection of 8 μg of racemic SOABZ in 20 μl of chloroform.

trated samples 2-propanol (or ethanol)–hexane [30:70; (v/v)] mixtures can be also chosen. These chromatographic deviations can be attributed to specific intermolecular associations of the sulfoxide molecules favoured by the injection solvent and/or preferential solvation by the polar eluent.

Accurate measurement of enantiomeric ratios for stereochemical studies requires baseline resolution; this prompted us to improve the resolution by means of optimization of the mobile phase nature and composition.

Optimization of the mobile phase nature and composition. We attempted to improve both selectivity and efficiency. Ethanol, 2-propanol, chloroform, dioxane, and acetonitrile were evaluated as polar component in hexane; these solvents belong to different selectivity groups according to the Snyder classification.[25] Alcohols act typically as proton acceptors (with a minor proton donor character), chloroform as a proton donor (which may explain the better solubility of SOABZ in this solvent), and dioxane and acetonitrile as strong dipoles and moderate proton acceptors.

The addition of small amounts of water was investigated on CSP 1 with binary hexane–alcohol mobile phases (Table 4). With a hexane–2-propanol eluent it

TABLE 4. Influence of the addition of water to a hexane–alcohol mobile phase on the resolution of SOABZ enantiomers[a]

Alcohol [% (v/v) in hexane]	Water [% (v/v) in alcohol]	k'_2	α
Ethanol (10)	0	17.9	1.11
	1	18.6	1.10
	2	20.0	1.08
2-Propanol (20)	0	15.0	≥ 1.00[b]
	1	13.5	1.10
	2	12.2	1.10

[a] Column: CSP 1, 250 × 4.6 mm i.d. Flow rate: 2 ml min^{-1}. UV detection at 220 nm. Temperature: 25°C.
[b] Large peak with beginning of resolution (see Fig. 4).

TABLE 5. Influence of the addition of chloroform to the mobile phase on the resolution of SOABZ enantiomers on CSP 1, 2a, and 2b[a]

CSP	Mobile phase composition [% (v/v)]	k'_2	α	R_S
CSP 1	Hexane–ethanol, 90 : 10	12.17	1.12	1.1
	Hexane–chloroform–ethanol, 78 : 17 : 5	7.66	1.16	1.1
CSP 2a	Hexane–ethanol, 88 : 12	10.90	1.06	0.6
	Hexane–chloroform–ethanol, 60 : 35 : 5	5.42	1.09	0.8
CSP 2b	Hexane–ethanol, 88 : 12	15.35	1.07	0.7
	Hexane–chloroform–ethanol, 60 : 35 : 5	9.66	1.17	1.0

[a]Columns: 250 × 4.6 mm i.d. for CSP 1 and 2a, 150 × 4.6 mm i.d. for CSP 2b. Flow rate: 2 ml min^{-1}. UV detection at 290 nm. Temperature: 40°C.

entailed no significant increase in selectivity but a noticeable improvement of efficiency was evidenced (Fig. 4) resulting in a better resolution. We can assume that the molecules of water are preferentially adsorbed on the remaining silanol groups and unbound mercaptopropyl groups canceling nonstereoselective interaction with the solute and leading to a better kinetics of exchange (sorption/desorption) of the solute. Compared to 2-propanol, ethanol is more easily adsorbed on residual silanol groups[26]; indeed, with hexane–ethanol mobile phase addition of water was not prominent though a surprising increase of retention times was noted while selectivity slightly decreased: decreased solubility of SOABZ in water added eluent may account for this stronger retention on the CSP; moreover the efficiency was not significantly affected.

Chromatographic behaviour evidenced with ternary hexane–chloroform–ethanol is similar to the one previously reported with regard to the resolution of chiral phosphine oxides on Pirkle-type CSPs.[27] A concomitant noticeable decrease of the retention times and increase in the selectivity are focused when mixing two isoeluotropic hexane–ethanol and hexane–chloroform mixtures as a new ternary eluent. This phenomenon was above all observed on CSP 2b (Fig. 5) on which a significant enhancement of the resolution is evidenced (Table 5). Improvement of SOABZ solubility in mobile phases containing chloroform favours solute/mobile phase interactions and retention is accordingly reduced.[26] Nevertheless chloroform does not fit for low UV detection at the absorption maximum of SOABZ (220 nm) owing to its high cut-off wavelength (245 nm).

Dioxane appeared to be a good polar additive, as already reported for the resolution of other sulfoxides,[14] affording an almost baseline resolution (Fig. 6); unlike chloroform it can be used at low UV detection (cut-off at 215–220 nm). Table 6 gathers the chromatographic data acquired on CSPs 1, 2a, and 2b with optimized ternary hexane–dioxane–ethanol mobile phases. Finally, investigations with hexane–acetonitrile–ethanol mobile phases did not afford promising results: both efficiency and selectivity were poor.

The resolution of SOFBZ enantiomers with a hexane–dioxane–ethanol ternary mobile phase on CSP 1 was still low (Table 7).

TABLE 6. Comparison of the resolution of SOABZ enantiomers on CSP 1, 2a, and 2b using a ternary hexane–dioxane–ethanol mobile phase[a]

B in hexane (v/v) (%)	CSP 1 15	CSP 2a 20	CSP 2b 20
k'_2	26.8	23.0	29.5
α	1.17	1.07	1.07
R_S	1.2	0.7	0.7

[a]Columns: 150 × 4.6 mm i.d. Flow rate: 2 ml min^{-1}. UV detection at 220 nm. Temperature: 25°C.

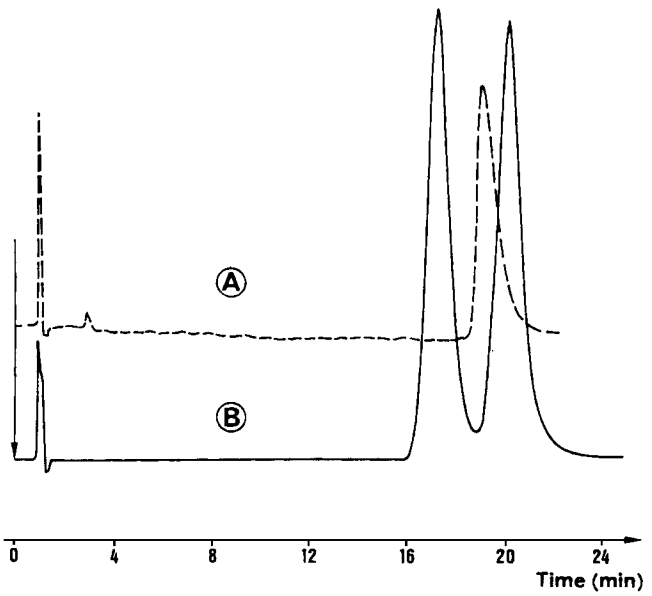


Fig. 7. Peaks overlapping occurring during the resolution of SOABZ(B) enantiomers and elution of SO_2ABZ(A) with a ternary hexane–dioxane–ethanol, 80 : 16 : 4 (v/v/v) mobile phase. Column: 150 × 4.6 mm i.d. Same other operating conditions as in Table 7.

TABLE 7. Optimization of the mobile phase nature and composition for the enantiomeric resolution of SOABZ and SOFBZ on CSP 1[a]

Solute	Mobile phase nature	Composition optimized	k'_2	α	R_S
SOABZ	Hexane–chloroform–ethanol	78 : 17 : 5	8.2	1.20	1.1
	Hexane–acetonitrile–ethanol	85 : 12 : 3	9.7	1.09	0.8
	Hexane–dioxane–ethanol	75 : 20 : 5	10.5	1.13	1.1
		80 : 16 : 4	16.6	1.14	1.25
SOFBZ	Hexane–chloroform–ethanol	78 : 17 : 5	10.7	1.10	0.8
	Hexane–acetonitrile–ethanol	90 : 7.5 : 2.5	13.0	1.04	0.5
	Hexane–dioxane–ethanol	80 : 16 : 4	21.0	1.06	0.7

[a]Column: 250 × 4.6 mm i.d. Flow rate: 2 ml min^{-1}; temperature: 25°C; UV detection at 220 nm.

On CSP 1, optimization of the mobile phase nature and composition (Table 7) shows that the highest resolution factor is achieved for SOABZ with a hexane–dioxane–ethanol ternary mixture. Unfortunately in this case SO_2ABZ is eluted exactly between the two enantiomers of SOABZ (Fig. 7). For samples containing amounts of SO_2ABZ, a hexane–ethanol mobile phase must then be preferred for accurate assays of SOABZ enantiomers. For further chiral preparative purposes, ternary hexane–dioxane–ethanol mobile phases will be recommended to afford higher optical purities. The reproducibility of selectivity value was checked on six columns (length: 250 or 150 mm) packed with different CSP 1 synthesis lots: $\alpha = 1.11 \pm 0.01$ with a hexane–ethanol 88 : 12 (% v/v) mobile phase. CSP 1 displayed good stability during time.

Influence of temperature. Experiments were carried out on CSP 1 with a hexane–ethanol, 86 : 14 (v/v) mobile phase (Fig. 8). The selectivity slightly increased with decreasing temperature but the concomitant loss in ef-

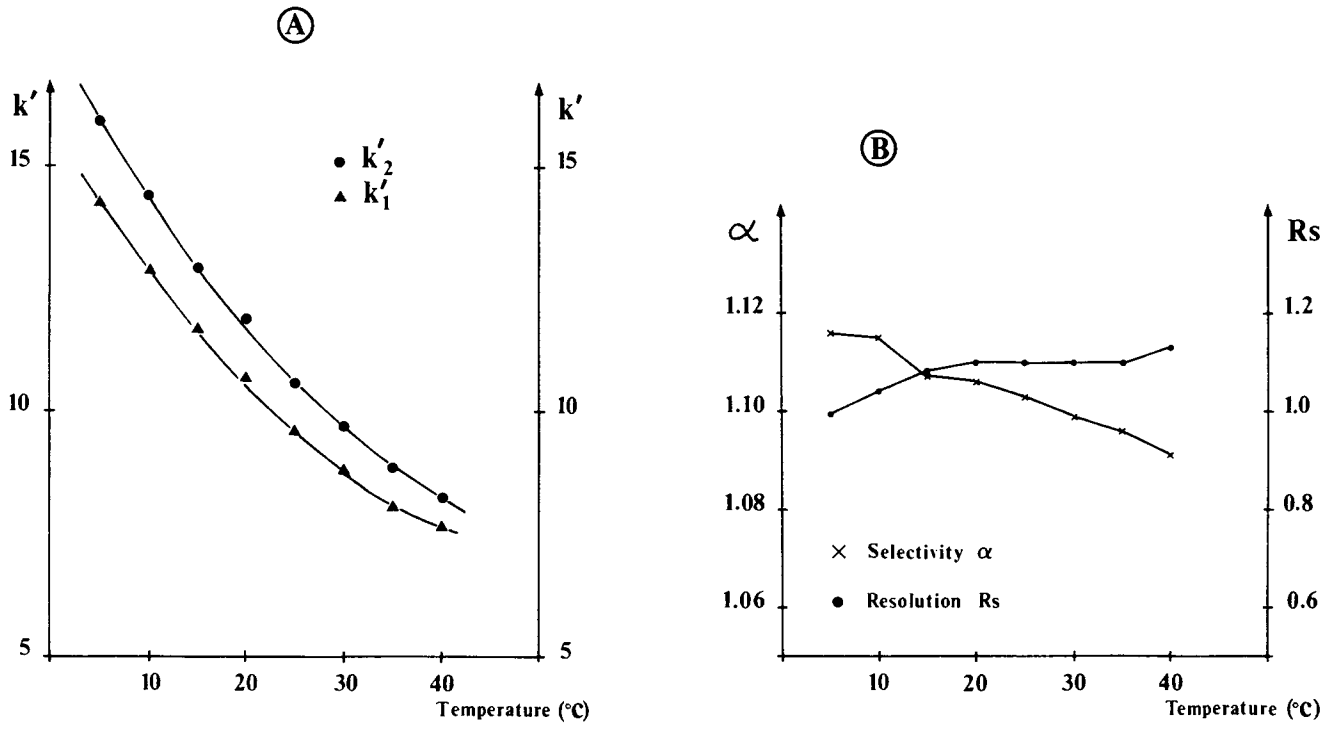


Fig. 8. Influence of temperature on the resolution of SOABZ enantiomers on CSP 1. **A**: Capacity factors k' versus temperature; **B**: selectivity α and resolution R_S versus temperature. Column: 250 × 4.6 mm i.d., dp 7 μm. Mobile phase: hexane–ethanol, 86 : 14 (v/v); flow rate: 2 ml min^{-1}. UV detection at 220 nm.

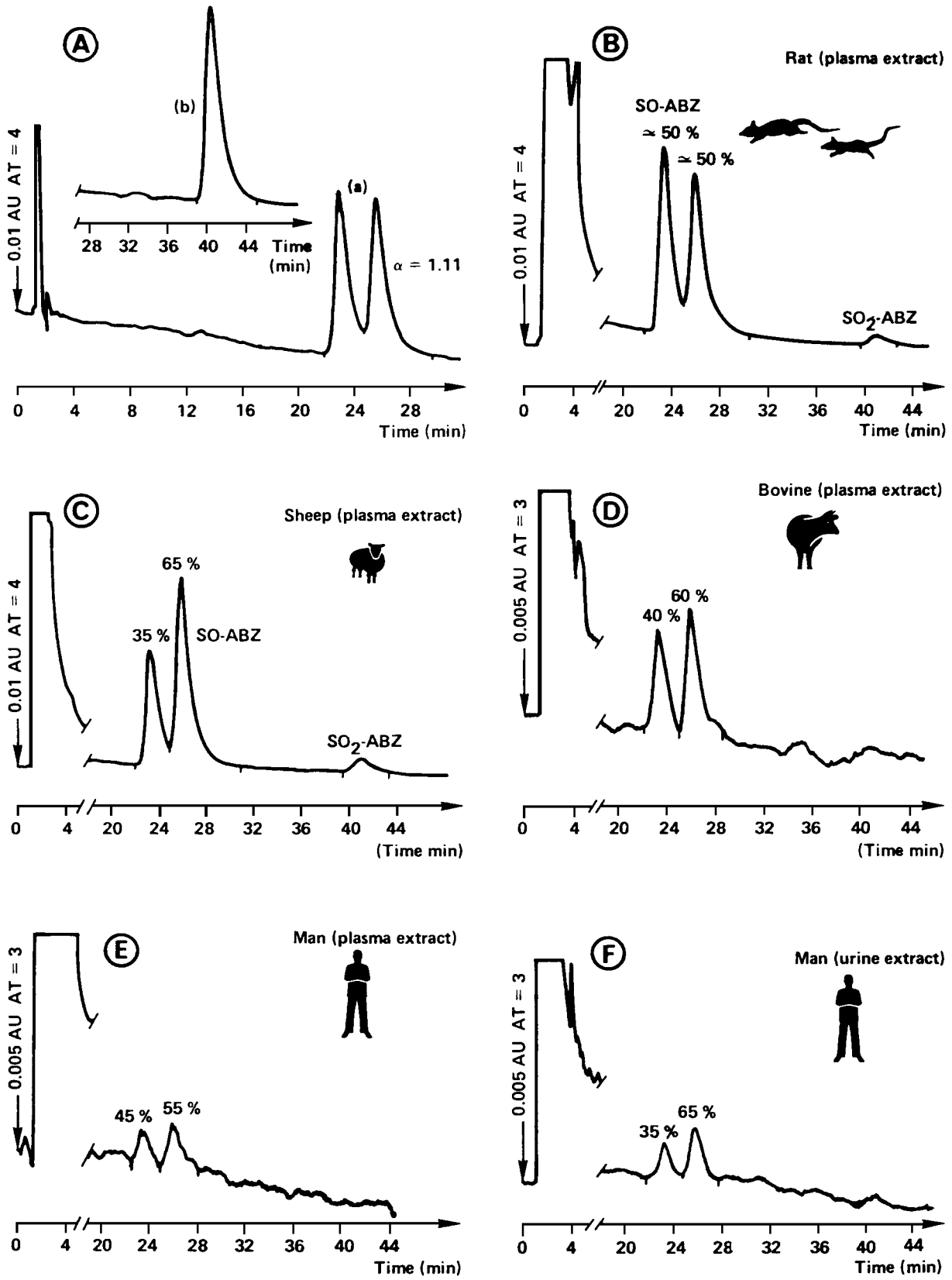


Fig. 9. Assays of SOABZ enantiomers in various plasma or urine samples on CSP 1. **A:** (a) Synthetic racemic SOABZ; 200 ng injected; first peak, 48%; second peak, 52% (from integrator data). (b) Synthetic SO_2ABZ. Retention time of ABZ in the same operating conditions: t_r; 5.6 min. Average amount of SOABZ determined: **B**: 200 ng; **C**: 200 ng; **D**: 60 ng; **E**: 20 ng; and **F**: 20 ng. Amount of SO_2ABZ: B: SOABZ 98%, SO_2ABZ 2%; C: SOABZ 95%, SO_2ABZ 5%. Column: 250 × 4.6 mm i.d. Mobile phase: hexane–ethanol, 90 : 10 (v/v); flow rate, 2 ml min^{-1}. UV detection at 220 nm. Temperature: 25°C.

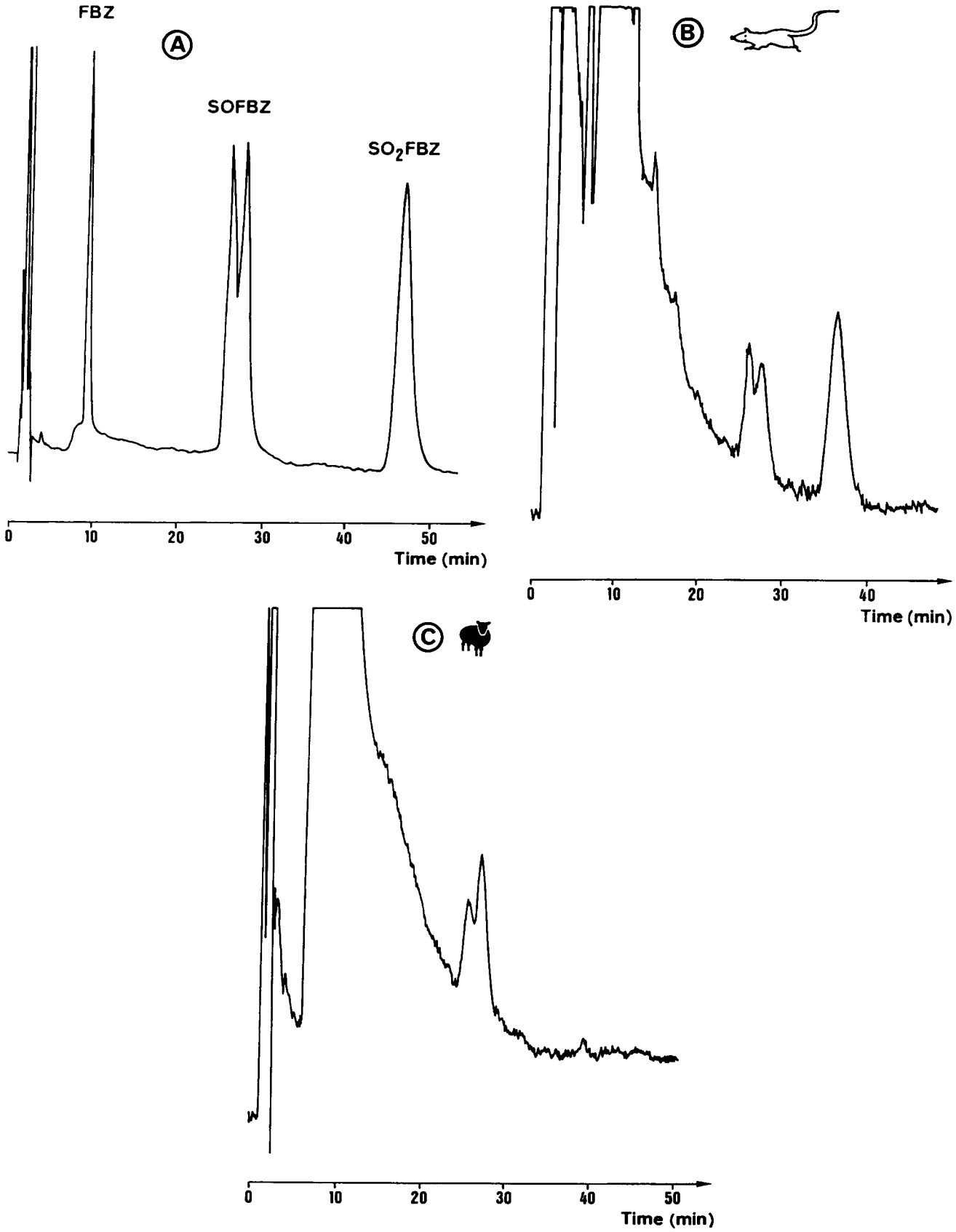


Fig. 10. Resolution of SOFBZ in plasmatic extracts on CSP 1. **A**: Coinjection of FBZ, SOFBZ, and SO_2FBZ. **B**: Rat sample ($\simeq$ 8 ng); the impurity at t_r = 35 min does not correspond to SO_2FBZ and was not identified. **C**: Sheep sample ($\simeq$ 8 ng). Mobile phase: hexane–ethanol, 86 : 14 (v/v). Same other operating conditions as in Figure 9.

TABLE 8. Enantomeric assays of SOABZ in plasma samples on CSP 1[a]

Species	First peak (%)	Second peak (%)
Rat	48.5 ± 1	51.5 ± 1
Sheep	33.5 ± 1	66.5 ± 1
Bovin	39.0 ± 2	61.0 ± 2
Man (urine)	37 ± 3	63 ± 3
Man (plasma)	47 ± 3	53 ± 3

[a]Same operating conditions as in Figure 9.

ficiency resulted in a poorer resolution. The temperature had a minor influence on the resolution and room temperature will be chosen for routine analyses.

Pharmacological application: assays of albendazole sulfoxide enantiomers in biological samples. Chromatographic assays of SOABZ enantiomers in various plasma or urine extracts are presented in Figure 9. When no trace of SO_2ABZ (separated by TLC) was detected (bovine and man), relative percentages of SOABZ enantiomers were checked using the ternary mixture. Some distortions in enantiomeric ratios were evidenced in the sheep and bovine extracts (Table 8). For man, more concentrated samples will be examined to confirm the preliminary results. In these samples no traces of the parent sulfide ABZ have been detected; traces of albendazole sulfone were present in the sheep and rat plasma extracts at low levels.

Figure 10A displays the elution of a standard mixture of FBZ, SOFBZ enantiomers and SO_2FBZ. Plasma extracts containing SOFBZ were also studied (Fig. 10B and 10C) and here too the sheep extract seemed to exhibit distortion in enantiomeric ratio.

A more comprehensive pharmacological study is needed to check (below 10 ng injected the determination of enantiomeric purity is no more accurate) and explain these preliminary results; it is now in progress.

CONCLUSION

The findings reported in this paper outline, once again,[22] that small changes in the chemical structure of the CSP chiral selector can induce significant enhancement in the enantiorecognition ability (CSP 2a and 2b). The binding way of the chiral selector affects both selectivity and efficiency. CSP 1 deriving from (S)-tyrosine (*thio*-DNBtyrE) afforded reproducible and accurate assays of SOABZ enantiomers that makes this CSP suitable for routine analysis. CSPs with closely related structures as CSP 1[22] are now under consideration. In addition to enantiomeric assays, the question was to possess pure SOABZ enantiomers to give a better insight into their respective biological behaviours (especially for embryotoxicity studies). However the enantioselective sulfoxidation of ABZ sulfide still remains a challenge. Some recent trials according to a procedure described by Zhao et al.[28] failed partly because of poor ABZ solubility in dichloromethane.[29] An alternative way will consist in the chromatographic preparation of SOABZ enantiomers on CSP 1 and related CSPs and is now under investigation.

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