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Short Communication

Direct enantiomeric separation of terfenadine and its major acid metabolite by high-performance liquid chromatography, and the lack of stereoselective terfenadine enantiomer biotransformation in man

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ABSTRACT

Direct enantiomeric separation of terfenadine and its major acid metabolite was achieved by using two different chiral stationary phase columns with two different mobile phase systems. Further, the enantiomeric composition of the human urinary acid metabolite has been determined, indicating a non-stereoselective biotransformation in man.

INTRODUCTION

Terfenadine, the first commercially available non-sedating anti-histamine, is a specific H_1-receptor antagonist that is also devoid of any anticholinergic, anti-serotoninergic and anti-andrenergic effects both *in vitro* and *in vivo* [1–4]. Terfenadine has a single asymmetric center in the molecule; hence, it exists as two enantiomers. The drug is being marketed as a racemate. *In vitro* pharmacological study has shown that both terfenadine enantiomers are equally potent in antagonizing the contractile effect of histamine in isolated guinea pig ileum. Animal studies have also demonstrated that both terfenadine isomers have similiar toxicity profiles [5].

In animal and human metabolic studies, terfenadine was shown to undergo high first-past effect, resulting in extremely low plasma concentrations and non-detectable urinary levels of unchanged compound [6,7]. This first-pass effect results in readily measurable plasma concentrations of the major metabolite (MDL 16,455, M-I, a carboxylic acid analogue of terfenadine which possesses anti-histaminic activity in animal models and presumably in man as well) (Fig. 1). M-I is also the major biotransformation product found in urine and feces of animal species and man.

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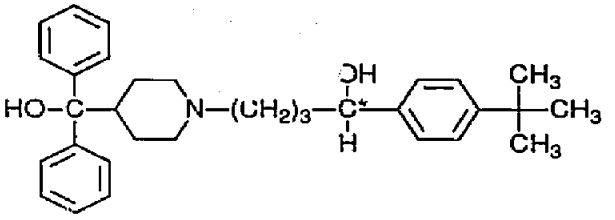
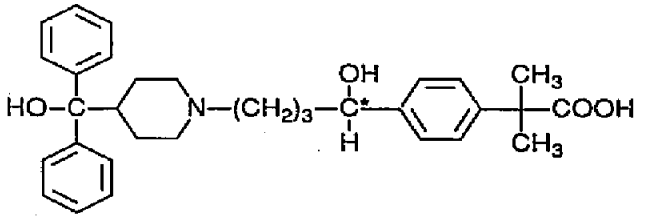


TERFENADINE



MDL 16,455
(ACID Metabolite MI)

Fig. 1. Structures of terfenadine and M-I. The asterisk denotes the asymmetric center.

This communication describes the direct enantiomeric separation of terfenadine and M-I by high-performance liquid chromatography (HPLC) and the analyses of the urinary enantiomeric contents of M-I from human subjects receiving therapeutic doses of terfenadine.

EXPERIMENTAL

Chemicals

Racemic terfenadine, (−)-(*S*)-terfenadine, (+)-(*R*)-terfenadine and racemic MDL 16,455 (M-I or 4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α,α-dimethylbenzene acetic acid hydrochloride) were obtained from Marion Merrell Dow Research Institute (Cincinnati, OH, USA). HPLC-grade methanol and 2-propanol were purchased from Burdick & Jackson (Muskegon, MI, USA). Other reagents and chemical utilized were of the highest purity commercially available.

Instrumentation

Chiral separation of terfenadine and M-I was performed with a component HPLC system (Waters Assoc., Milford, MA, USA) which consisted of a Model 6000A pump, a WISP Model 712 auto-injector and a Model 484 ultraviolet absorption detector set at either 210 or 254 nm. The chromatographic column for terfenadine separation was a prepacked 250 mm × 4.6 mm I.D. Cyclobond I (5 μm particle size, Astec, Whippany, NJ, USA) operated with a methanol–0.014 *M* sodium perchlorate (75:25, v/v) mobile phase, flowing at 0.2 ml/min.

For chiral separation of M-I, a pre-packed 150 mm × 4 mm I.D. Resolvosil

BSA-7 column (5-μm packing Anspec, Ann Arbor, MI, USA) was operated with a mobile phase consisting of 2-propanol–0.08 *M* sodium phosphate (pH adjusted to 8.0 with 1 *M* sodium hydroxide, 1.5:98.5, v/v). The flow-rate was set at 0.4 ml/min.

A urinary metabolite extract was also analyzed by thermospray liquid chromatography–mass spectrometry (TSP-LC–MS) for structural confirmation. The same LC column as described above for the chiral separation of the acid metabolite was connected to a Hewlett Packard 5970 MSD mass spectrometer (Palo Alto, CA, USA) via a Vestec 101 TSP interface (Houston, TX, USA). The vaporizer and ion source block temperatures were set at 145 and 270°C, respectively. The phosphate buffer in the mobile phase preparation was replaced with a more volatile ammonium formate buffer (0.08 *M*, pH 8.0) for the TSP operation. The mass spectrometer was operated at both scan and selected-ion modes (SIM).

Human urine collection

Periodic urine samples were collected from two male volunteers who were undergoing oral terfenadine therapy. Subject 1 had been undergoing chronic dosing, thus, he was not able to supply a pre-dose drug-free urine sample. Subject 2 just initiated therapy and was able to provide a pre-dose urine sample. Urine samples were stored frozen at −20°C until analyses.

Extraction of urine samples

A 3-ml aliquot of urine was adjusted to basic pH by the addition of 3 ml of a 0.1 *M* sodium bicarbonate solution and then extracted twice with 5 ml of ethyl acetate. The organic layers were combined and then dried in a heating block (55°C) under a stream of nitrogen. The dried residue was redissolved in 100 μl of a methanol–water (20:80, v/v) solution and 10–30 μl were injected for LC analysis.

RESULTS AND DISCUSSION

Chiral separation of terfenadine

The chromatogram for the enantiomeric separation of terfenadine is shown in Fig. 2. The chiral separation of terfenadine results from the ability of the lipophilic *tert.*-butylphenyl moiety of terfenadine to interact with the lipophilic cavity of the β-cyclodextrin. The mobile phase was chosen to be simple. The perchlorate in the mobile phase, however, was required to ion-pair with terfenadine and allow terfenadine to elute in a reasonable time with good peak shape. The flow-rate of 0.2 ml/min was a compromise as higher flow-rates resulted in less resolution between the enantiomers. The synthetic racemic terfenadine shows a typical 50:50 area ratio. Analyses of the individual (+)-(*R*)- and (−)-(*S*)-terfenadine enantiomers are also shown in the same figure. Analytical results indicated a chiral excess of 97.8 and 97.2%, respectively, for the two optical isomers. These results showed good agreement with the purity test done by using the technique of

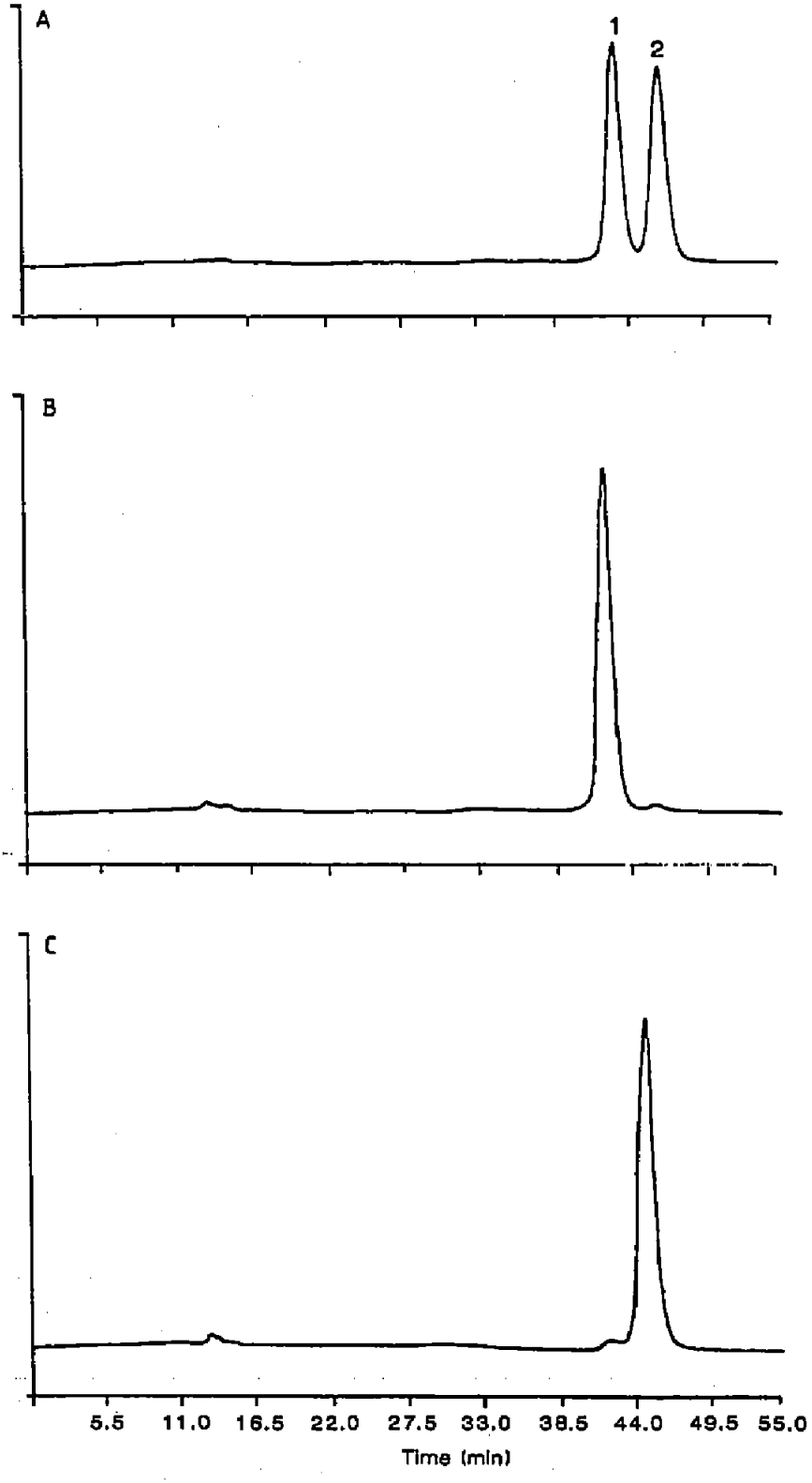


Fig. 2. Chiral separation of terfenadine. (A) Racemic terfenadine; (B) (+)-(*R*)-terfenadine; (C) (−)-(*S*)-terfenadine. Peaks: 1 = (+)-(*R*)-terfenadine; 2 = (−)-(*S*)-terfenadine- Amount injected: 1.2 μg of each compound in 5 μl of mobile solvent.

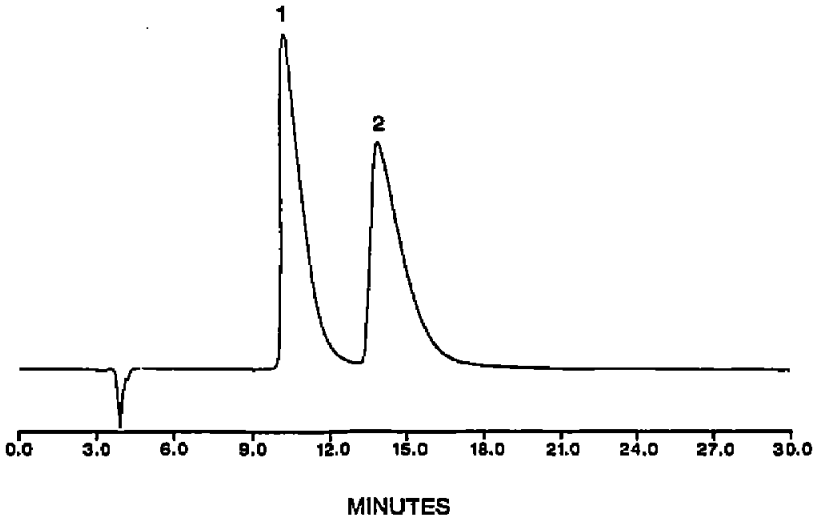


Fig. 3. Chiral separation of racemic M-I. Peaks: 1 = (−)-(*S*)-M-I; 2 = (+)-(*R*)-M-I. Amount injected: 1.2 μg in 3 μl of mobile solvent.

nuclear magnetic resonance with a chiral shift reagent [98 and 97% for (+)-(*R*)- and (−)-(*S*)-isomers, respectively].

Chiral separation of M-I

Fig. 3 shows a chromatogram for the chiral separation of M-I. Baseline separation was obtained for the two isomeric peaks of M-I. Retention times of about 10.2 and 14.4 min were obtained for the (*S*)- and (*R*)-enantiomers, respectively. The resolution of the enantiomers of M-I was found to increase as the mobile phase pH was raised. A pH of 8.0 was chosen as a compromise between resolution of the enantiomers and exposing the column packing to an undesirable pH. Earlier attempts at separating the enantiomers of M-I using a β-cyclodextrin bonded-phase column were unsuccessful. This was most likely because of the

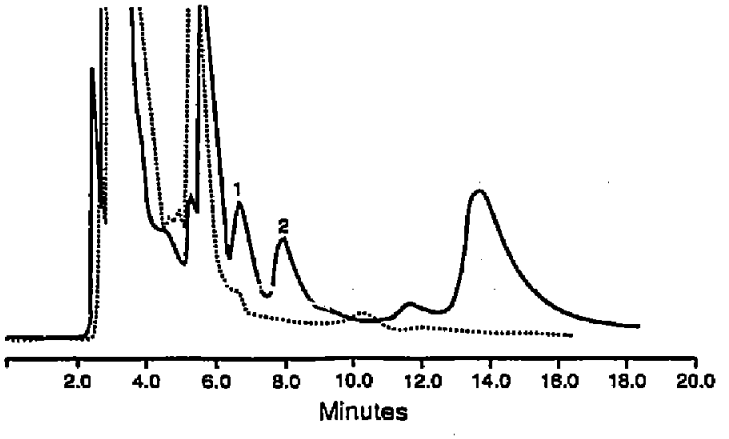


Fig. 4. LC chiral analysis (UV at 210 nm) of human urine sample. (· · · ·) Control urine; (———) 2 h post-seldane. Peaks: 1 = (−)-(*S*)-M-I: 2 = (+)-(*R*)-M-I. Amount injected: 10 μl.

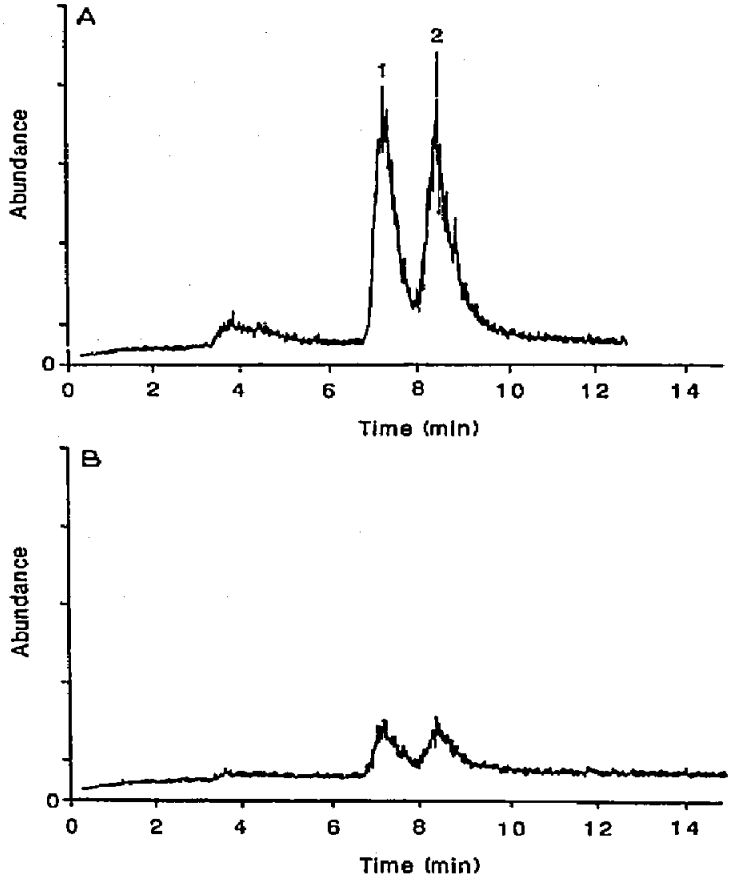


Fig. 5. TSP-LC–MS (selective-ion detection mode at 502 *m/z*, M + H ion of M-I). (A) 0–2 h sample; (B) 2–3 h sample. Peaks: 1 = (−)-(*S*)-M-I; 2 = (+)-(*R*)-M-I. Amount injected: 10 μl.

polar acid group of M-I rendered it unable to interact with the lipophilic β-cyclodextrin cavity unlike terfenadine.

Analyses of human urine

The chromatogram of the human volunteer who underwent terfenadine therapy was shown in Fig. 4. This urine sample contained the two enantiomeric peaks for the acid metabolite (M-I). The identities of these two peaks were confirmed by TSP-LC–MS. The TSP mass spectra (in SIM mode) are showed in Fig. 5. Both retention times and mass spectral data agreed with those of synthetic reference material. Analyses of the two selected time points indicated that the enantiomeric content exists in about a 50:50 area ratio, and this ratio does not change with time. The retention times in Fig. 3 are different from those in Fig. 4. This resulted from changing the phosphate buffer to the more volatile ammonium formate buffer for the TSP operation.

DISCUSSIONS AND CONCLUSION

The results of these experiments showed that direct chiral separation of terfenadine and its major acid metabolite can be achieved by using two different HPLC columns. The chiral separation of terfenadine enantiomers was easily accomplished using the Cyclobond I chiral β-cyclodextrin bonded phase. The structure of terfenadine with the *p-tert.*-butylphenyl group apparently has the right size to properly interact with the β-cyclodextrin cavity. On the BSA bonded-phase column no separation of terfenadine could be obtained. Terfenadine was strongly retained and eluted as a single broad peak using a mobile phase containing 8% isopropanol and buffer. This would suggest that terfenadine is tightly bound to BSA in a non-stereospecific way.

The acid metabolite of terfenadine, M-I, no longer has the *p-tert.*-butylphenyl group of terfenadine and its enantiomers cannot be separated using the β-cyclodextrin column. The BSA bonded-phase column, Resolvosil BSA-7, gave a good separation of the enantiomers of M-I. This is probably due to the ability of the M-I enantiomers to selectively bind to BSA in different ways.

Examination of the urinary M-I enantiomeric content seemed to indicate that terfenadine does not undergo any stereoselective isomeric interconversion in man. This conclusion was supported by the fact that after terfenadine therapy, the major acid metabolite (M-I) produced in urine showed a 50:50 enantiomeric composition.

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