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Determination of the metabolites of terfenadine in human urine by thermospray liquid chromatography–mass spectrometry*

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Abstract: Thermospray liquid chromatography–mass spectrometry (TSP LC–MS) was used to determine human urinary metabolites of terfenadine after oral administration of terfenadine tablets. In addition to the two previously identified major metabolites, azacyclonol (MDL 4829) and the 'acid' metabolite (MDL 16,455), three additional metabolites were also detected. One of the additional metabolites was identified as the 'alcohol' metabolite (MDL 17,523) and the other two were proposed to be an 'aldehyde' and a 'ketone-acid' metabolites from their TSP mass spectra. The results of this study demonstrated that TSP LC–MS is a useful technique for the study of terfenadine biotransformation.

Keywords: *Terfenadine; thermospray mass spectrometry; HPLC.*

Introduction

Terfenadine is a non-sedating antihistamine [1, 2] which undergoes extensive biotransformation on its first pass through the liver, i.e. high first pass effect, resulting in very low concentrations (often unmeasurable) in the blood plasma [3]. To date only two metabolites of terfenaldine have been identified in human samples, i.e. azacyclonol (MDL 4829) and the 'acid' metabolite (MDL 16,455) which were identified by gas chromatography–mass spectrometry (GC–MS) after extensive sample preparation that included multiple chemical derivatization steps [4]. Recently, thermospray liquid chromatography–mass spectrometry (TSP LC–MS) has been used to analyse human urine samples after oral administration of terfenadine tablets (Seldane® 60 mg). Three additional terfenadine-related metabolites were detected. This paper describes a useful TSP LC–MS procedure for the determination of the metabolites of terfenadine in human urine and proposes structures for the three unidentified metabolites of terfenadine.

Experimental

Materials

Terfenadine tablets (Seldane® 60 mg) and the authentic compounds of MDL 4829, MDL 16,455 and MDL 17,523 were propared by the Marion Merrell Dow Research Institute. Acetonitrile was HPLC-grade purchased from Burdick & Jackson Laboratories (Muskegon, MI, USA). Water was purified with a Barnstead NONOpure/ORGANICpure system (Boston, MA, USA). All other chemicals used were analytical-reagent grade.

TSP LC–MS analysis

The TSP LC–MS apparatus consisted of a Waters 600-MS multisolvent delivery HPLC system (Milford, MA, USA) and a Hewlett–Packard 5970 mass-selective detector (Palo Alto, CA, USA) that was mounted in a Vestec 101 TSP interface (Houston, TX, USA) [5, 6]. The vapourizer and ion source block temperatures were set at 152 and 265°C, respectively. Data were acquired and the instrument was controlled by a Hewlett–Packard 59970 ChemStation.

LC separations were performed on a Spherisorb, 5 μm, CN column (25 cm × 4.6 mm i.d.) purchased from Alltech Associates, Inc. (Deerfield, IL, USA). The mobile phase was acetonitrile–0.05 M ammonium acetate, pH 4.5 (40:60, v/v) at a flow rate of 1.2 ml min^{-1}. The column effluent was mixed, by use of a mixing T-piece located between a Waters 490-

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MS multiwavelength detector and the TSP interface, with 0.5 M ammonium acetate at a flow rate of 0.1 ml min^{-1}.

Preparation of urine samples

Two terfenadine tablets (Seldane® 60 mg) were ingested by a human subject and periodic urine samples were collected. A 5 ml volume of the 2–4 h samples were basified by adding 40 μl of 5 M sodium hydroxide solution and extracted twice with 8 ml of ethyl acetate. The organic layers were combined and then dried in a heating block set at 55°C under a stream of nitrogen. The residue obtained was redissolved in 200 μl of methanol–water (20:80, v/v) and 50 μl were used for TSP LC–MS analysis.

Results and Discussion

Figure 1 shows the chromatogram with spectrophotometric detection of a human urine sample after the oral administration of terfenadine tablets. The corresponding chromatograms obtained using mass detection are shown in Fig. 2. The chromatograms of the control (drug-free) urine sample also obtained by TSP LC–MS with mass detection are shown in Fig. 3 for comparison. As expected, no terfenadine peak (retention time at 16.5 min) was detected using either spectrophotometric or mass detection, which confirms its extensive biotransformation on the first pass through the liver [3]. Five terfenadine-related metabolites (peaks I–V) were detected in 6–11 min; their protonated molecular ions, MH^+, are specified in Fig. 2. The peak labelled E (Fig. 1) is an endogenous peak which was also observed in the control urine sample.

Metabolites I, II and IV were confirmed to be azacyclonol (MDL 4829), the 'acid' metabolite (MDL 16,455) and the 'alcohol' metabolite (MDL 17,523), respectively, based on comparison of the LC retention times and the MH^+ ions with the authentic samples as shown in Fig. 4. Their TSP mass spectra were also similar to those of the authentic samples. MDL 4829 and MDL 16,455 have been identified previously by GC–MS and reported to be the major metabolites of terfenadine [4]. However, MDL 17,523 has not been reported previously as a human metabolite. It, apparently, is the primary metabolic product of terfenadine when the biotransformation takes place at the *t*-butyl group as shown in Fig. 5.

The TSP mass spectrum of metabolite III exhibited an MH^+ ion at *m/z* 500, i.e. 2 mass units less than MDL 16,455. The spectrum also showed two fragment ions at *m/z* 482 and 456, which correspond to $[MH\text{-}H_2O]^+$ and $[MH\text{-}CO_2]^+$ ions, respectively. Metabolite III may be formed by further oxidation of MDL 16,455 metabolite by converting its secondary hydroxyl group to a ketone form and thereby forming a 'ketone-acid' structure. This tentatively proposed structure is strongly supported by its longer retention time and

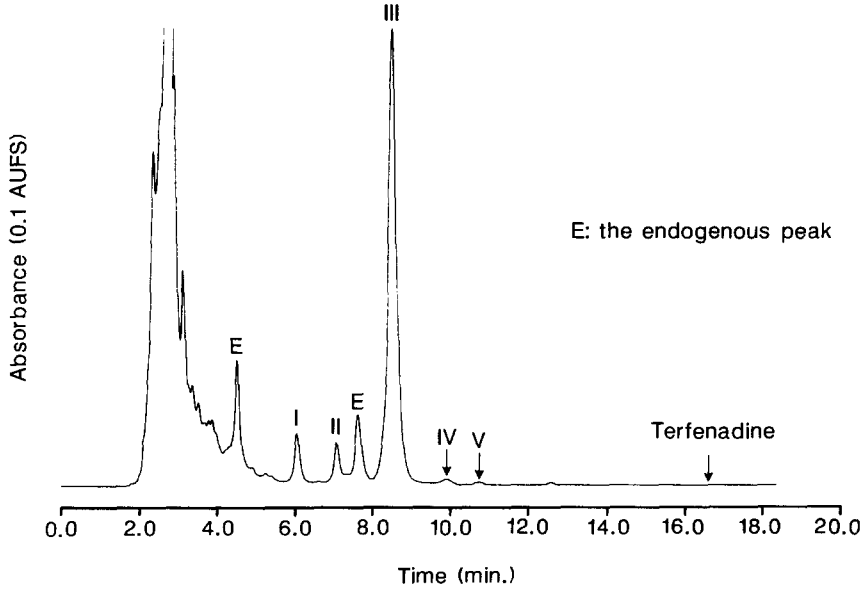

Figure 1
UV (254 nm) chromatogram of a human urine sample after oral administration of terfenadine tablets.

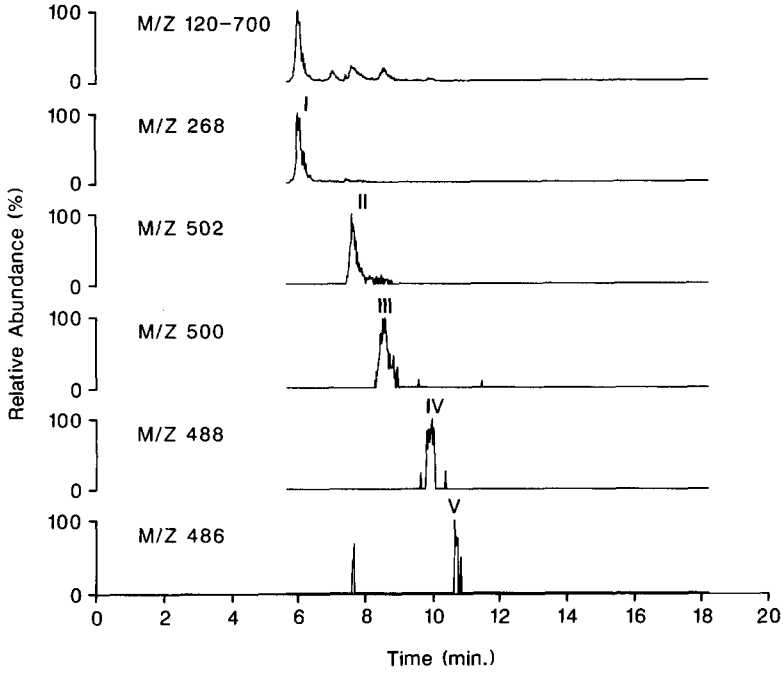

Figure 2
Selected ion chromatograms of a human urine sample after oral administration of terfenadine tablets.

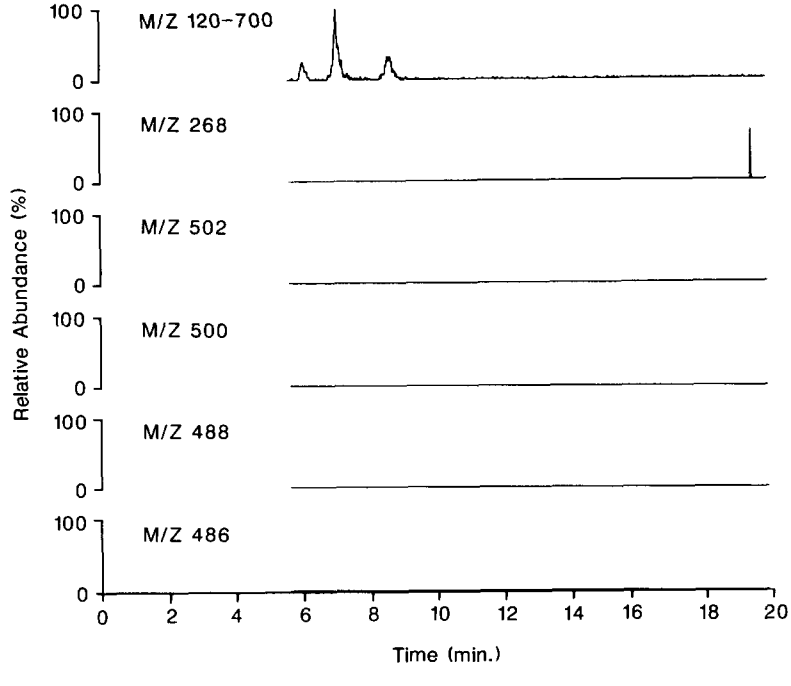

Figure 3
Selected ion chromatograms of a control human urine sample.

higher UV response than those of MDL 16,455. Although no authentic sample is available for confirmation, its high UV response is evident by its relatively large peak area with UV detection and low peak area with mass detection. It has been reported elsewhere that the 'ketone' analogue of terfenadine, MDL 9917, is retained about 1.2 times longer than terfenadine under similar reversed-phase LC conditions, and its response factor at 260 nm

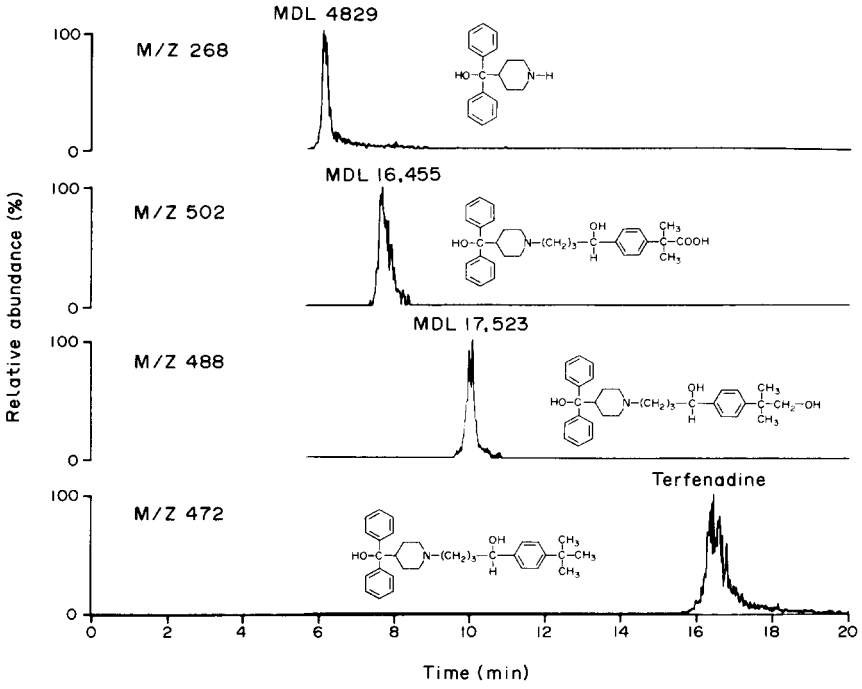

Figure 4
Selected ion chromatograms of authentic samples. Key: MDL 4829 (I); MDL 16,455 (II); and MDL 17,523 (IV).

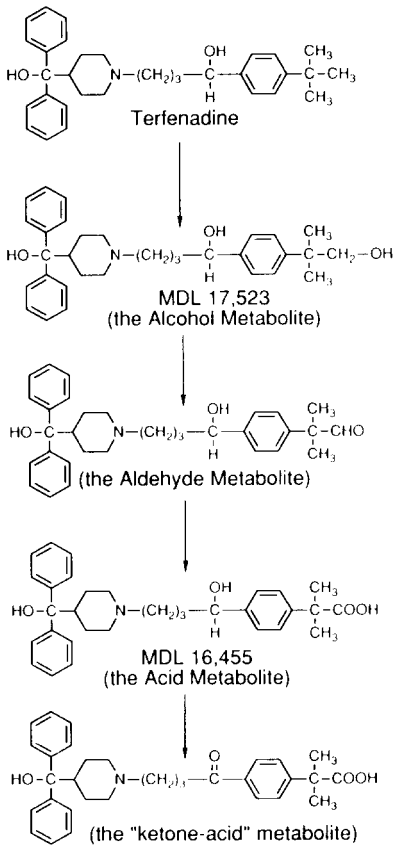

Figure 5
The proposed metabolic reaction at the *t*-butyl group of terfenadine.

was about 25 times that of terfenadine [7]. If the 'ketone acid' metabolite has a similar UV response factor it is present only as a small percentage of the total concentrations of metabolites.

The $[MH\text{-}H_2O]^+$ ion is a common fragment ion that was observed in the spectra of terfenadine, MDL 16,455, MDL 17,523 and metabolite III. However, the $[MH\text{-}CO_2]^+$ ion observed in the spectrum of metabolite III was not observed in the spectrum of MDL 16,455, which showed only two intense fragment ions of $[MH\text{-}H_2O]^+$ and $[MH\text{-}2H_2O]^+$. This indicates that the formation of the $[MH\text{-}CO_2]^+$ ion by this tentatively proposed 'ketone-acid' structure is unlikely. The ion at *m/z* 456 observed in the TSP mass spectrum of metabolite III could arise from a compound that coincidently co-eluted with metabolite III. It is possible that a compound that has an MH^+ ion at *m/z* 456 results from the decarboxylation of metabolite III: decarboxylation can occur through a non-enzymatic degradation process [8].

Although the amount of metabolite V present in the urine was very small, its MH^+ ion at *m/z* 486 was detectable by mass detection. However, no fragment ions were detected. The structure for metabolite V is proposed to be an 'aldehyde' derived from

further oxidation of the 'alcohol' metabolite, MDL 17,523. This postulated structure is based mainly on the presence of the ion at *m/z* 486 and the formation of MDL 17,523 and MDL 16,455 metabolites in the urine. It is a logical intermediate in the biotransformation of MDL 17,523 to MDL 16,455, though the 'aldehyde' metabolite is normally not stable in biological systems.

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