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Investigation of the stereoselective metabolism of the chiral H_1-antihistaminic drug terfenadine by high-performance liquid chromatography☆

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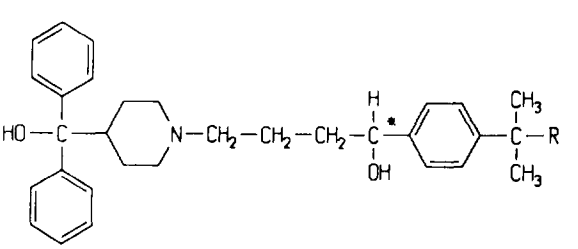
Abstract

The enantiomers of the racemic H_1-antihistaminic drug terfenadine (**1**) have been resolved by fractional crystallization of the diastereomeric salts with optically active 2-chlorotartranilic acid. The enantiomeric excess of both terfenadine enantiomers was determined using an achiral and a chiral HPLC system after formation of diastereomers with *S*-(+)-naphthylethylisocyanate. To investigate the metabolism of terfenadine after oral administration, an achiral HPLC system, equipped with a conventional reversed-phase column, was used to quantify the main metabolite MDL 16.455 (**2**) in human serum and urine. The determination of the enantiomeric composition of **2** was achieved using an Ultron ES-OVM® column as chiral stationary phase. Metabolite **2**, extracted from human blood plasma, was found to be enriched in the *R*-enantiomer, but was excreted in urine as racemate. The results of a study including six volunteers are presented.

1. Introduction

Terfenadine, Teldane®, α-[4-(1,1-dimethylethyl)phenyl] - 4 - (hydroxydiphenylmethyl) - 1 - piperidinebutanol, structure **1** in Fig. 1 is a H_1-antihistaminic drug. It is successfully used in the therapy of allergic diseases as allergic rhinitis and chronic urticaria [1]. The most important advantage over the H_1-antihistamic drugs of the first generation is its lack of effects on the central nervous system.

Terfenadine, a chiral drug, is used in therapy as the racemate. After oral application terfenadine is almost completely absorbed, undergoing a strong first-pass-metabolism [2], in which ca. 99% of the drug is metabolized to its carboxylic acid analogue MDL 16.455 (**2**) (Fig. 1), resulting in extremely low plasma concentrations and almost non-detectable urinary levels of the



1: R = CH_3 Terfenadine
2: R = COOH MDL 16.455

Fig. 1. Structures of terfenadine (**1**) and its major acid metabolite MDL 16.455 (**2**).

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☆ Dedicated to Professor Dr. H.J. Roth on the occasion of his 65th birthday.

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unmetabolized drug. The carboxylic acid metabolite **2** possesses about 30% of the H_1-antihistaminic activity of terfenadine and it was stated that this metabolite is exclusively responsible for the pharmacological effects of the drug [3].

Many chiral drugs show an enantioselective metabolism. However, in case of terfenadine it is reported [4] that metabolite **2** is excreted in human urine as a racemate indicating no enantioselectivity in the formation of **2**. In contrast an animal metabolic study with rats after oral administration of a high dose of terfenadine showed [5] that the *S*-enantiomer of terfenadine and the *R*-enantiomer of metabolite **2** was enriched in blood plasma. The absolute configuration of terfenadine had been determined previously by synthesis [6] and for terfenadine and its metabolite 2 by circular dichroism (CD) spectra [5]: the laevorotatory enantiomers are *S*-, the dextrorotatary enantiomers *R*-configurated.

This paper describes an economic method for the preparative separation of both enantiomers of terfenadine via diastereomeric salt formation with optically active 2-chlorotartranilic acid. The enantiomeric excess of both terfenadine enantiomers was determined using an achiral HPLC method after derivatisation of terfenadine (**1**) with *S*-(+)-naphthylethylisocyanate [*S*-(+)-NEIC] as well as by chiral HPLC columns. Furthermore, a chiral HPLC assay was used to determine the enantiomeric ratios of metabolite **2** in human serum and urine after oral application of the racemic drug.

2. Experimental

2.1. Chemicals

Terfenadine and the metabolite MDL 16.455 (**2**) were gifts from Marion Merrell Dow (Rüsselsheim, Germany). *n*-Hexane, 2-propanol, ethyl acetate and acetonitrile were Lichrosolv reagents (Merck, Darmstadt, Germany). The other chemicals were of analytical grade. Buffer solutions were prepared in double-distilled, deionized water and filtered (0.22 μm).

2.2. Apparatus

The HPLC system consisted of a Merck-Hitachi L 6200 pump (Darmstadt, Germany), a Merck-Hitachi variable-wavelength UV monitor 655A or a fluorescence detector Merck-Hitachi F-1250 and a Merck-Hitachi D-2500 chromato-intergrator.

2.3. Preparative separation

A mixture of racemic terfenadine (5.0 g, 10.6 mmol) and either (+)- or (−)-2-chlorotartranilic acid [7] (1.38 g, 5.32 mmol) were dissolved in 125 ml of hot ethanol (96%) and allowed to crystallize for 24 h at room temperature. Five recrystallization steps afforded the pure diastereomeric salts. After decomposition of the salts with 1 *M* NaOH and extraction with dichloromethane, the optically pure enantiomers [determination by HPLC on an achiral column after derivatization with *S*-(+)-NEIC and thereafter directly determined on chiral stationary phases] of terfenadine were obtained with a yield of >50%, m.p. 144–145°C.

The enantiomers were characterized by mass spectrometry, NMR and elemental analysis.

2.4. Achiral chromatography

The separation of the main metabolite MDL 16.455 (**2**) and the internal standard diphenylpyraline was achieved on a RP-Select B column (Merck, 125 × 4.6 mm I.D., particle size 5 μm) and a LiChrosorb NH_2 guard column. The mobile phase was a mixture of 0.01 *M* phosphate buffer pH 2.5–methanol–acetonitrile (52:48:1.5, v/v/v) with a flow-rate of 1.0 ml/min.

2.5. Indirect chromatographic resolution of terfenadine enantiomers

The enantiomeric purity of the enantiomers of terfenadine was determined by HPLC resolution on a RP-Select B column (Merck, 250 × 4.6 mm I.D., particle size 5 μm) after formation of the diastereomers with *S*-(+)-NEIC. The mobile

phase was methanol–0.1 *M* ammonium acetate–acetonitrile (75:25:1, v/v/v) and the flow-rate 0.5 ml/min. To form the diastereomers 12 mg of terfenadine were dissolved in 2 ml of dichloromethane and were allowed to react at room temperature for 20 h after addition of 40 μl *S*-(+)-NEIC (Sigma).

2.6. Chiral chromatography

Direct chromatographic resolution of terfenadine enantiomers was achieved by HPLC on a Ultron ES-OVM column (Grom, Germany, 150 × 4.6 mm, I.D., particle size 5 μm). The same column was used for the separation of the enantiomers of the metabolite MDL 16.455 (**2**). The use of a 0.01 *M* phosphate buffer pH 4.0–7.0 in combination with 2-propanol as organic modifier yields enantioseparations of **2**. The flow-rate was 0.5 ml/min and a fluorescence detector was used (λ_{ex} = 230 nm and λ_{em} = 280 nm).

Starting with a 0.01 *M* phosphate buffer pH 5.5–2-propanol (96:4, v/v) the simultaneous enantioseparation of **1** and **2** was achieved on a Ultron ES-OVM column. For the elution of terfenadine the concentration of the organic modifier 2-propanol was gradually increased up to 12% (Fig. 2).

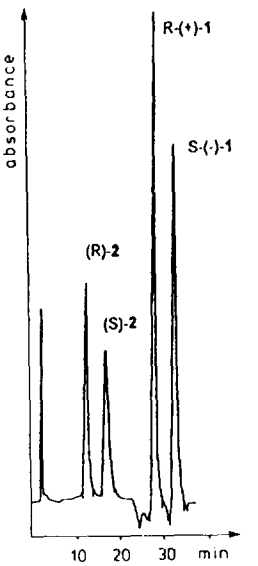

Fig. 2. Simultaneous resolution of terfenadine (**1**) and MDL 16.455 (**2**) on a Ultron ES-OVM column using a gradient HPLC system. Mobile phase composition: 0–19 min: 0.1 *M* phosphate buffer pH 5.5–2-propanol (94:4); 20–21 min: 4% 2-propanol up to 12%; 21–40 min: 0.1 *M* phosphate buffer pH 5.5–2-propanol (88:12); flow-rate, 1.0 ml/min; detection UV at 224 nm.

2.7. Quantification of MDL 16.455 in serum and urine (achiral assay)

For the quantification of **2** 1.0 ml of serum or 100 μl of urine, both adjusted with 1 ml 0.1 *M* sodium acetate buffer to pH 4.0, were extracted using Bakerbond octadecyl extraction columns 7020-3. After conditioning of the columns with 2 × 3 ml methanol, followed by 1 ml of double-distilled water and 2 ml 0.1 *M* sodium acetate buffer (pH 4.0), the sample was applied and soaked through the column by vacuum. The columns were washed with 2 ml double-distilled water, twice with 1 ml acetonitrile:water (1:2, v/v) and once with 0.7 ml acetonitrile. After drying the column for 10 min by suction the adsorbent was extracted with 1.5 ml of a 0.1 *M* solution of triethylamine in methanol. To quantify **2** in serum and urine on an achiral RP-Select B column the first aliquot was taken up in 50 μl of the mobile phase containing the internal standard diphenylpyraline. The second aliquot was used to determine the enantiomeric ratios of **2** in serum and urine.

2.8. Determination of the enantiomeric ratio of MDL 16.455 (2) in serum and urine by chiral chromatography (chiral assay)

After oral administration of racemic terfenadine the enantiomeric ratios of **2** were determined in the serum and urine samples by dissolving the second aliquot (see above) in 60 μl of the mobile phase without internal standard and HPLC analysis on an Ultron ES-OVM column. For these determinations a fluorescence detector (λ_{ex} = 230 nm, λ_{em} = 280 nm) was used.

2.9. In vivo study

Six healthy volunteers received once 120 mg terfenadine (Teldane forte, Merrell Dow) p.o. on an empty stomach. Blood was collected before and 0.5, 1.5, 2.5, 5, 8, 12, and 24 h after application. Serum was obtained by centrifugation at 1000 *g* for 25 min and stored frozen (−20°C) until analysis. Additionally the urine samples of three volunteers were collected after 2, 4, 6, 8, 10, 12 and 24 h, and were stored accordingly.

3. Results and discussion

3.1. Preparative separation and determination of the enantiomeric excess

Both enantiomers of terfenadine were obtained on a preparative scale by fractional crystallisation of the salts of (+)- and (−)-2-chlorotartranilic acid, respectively. They were used in this study as reference substances to determine the elution order of terfenadine enantiomers. To determine the enantiomeric excess (ee) of terfenadine in the beginning of our work an achiral HPLC system was used after derivatisation with *S*-(+)-NEIC. Later the enantiomeric purity of **1** was determined directly on a commercially available Ultron ES-OVM column. In both cases the enantiomeric excess (ee) was found to be higher than 99%.

With several chiral stationary phases (CSPs) such as Chiracel OD and Chiralpak AD we obtained good enantioseparations for **1** and **2** with an Ultron ES-OVM in a wide pH range of 5–7. During these experiments similar resolutions on the same column were published [5]. The enantioseparation of the main metabolite MDL 16.455 (**2**) was previously achieved on a Resolvosil BSA-7 protein column using a phosphate buffer pH 8.0 and 2-propanol as organic modifier [4]. Due to the high enantioselectivity of the Ultron ES-OVM column a simultaneous separation of **1** and **2** (Fig. 2) was achieved. However, because of the extensive first-pass effect of terfenadine it was not possible to analyze the very low serum and urine concentrations of terfenadine (**1**) using the Ultron column.

3.2. Quantitative determination of metabolite 2 in serum and urine (achiral assay)

Precision and accuracy of the assay were determined in blank serum samples spiked with known amounts of **2** in the range of 17.8–284.8 ng/ml (Table 1). Each concentration was analyzed three times. A linear correlation was found ($y = 0.0055x + 0.04004$, $r = 0.9876$). Recovery values were evaluated by comparing the peak areas from serum samples spiked with different amounts of **2**, and those of unextracted standard solutions. The recovery (± S.D.) of **2** was 80.2 ± 4.8% (Table 2). Fig. 3 shows typical chromatograms of (a) blank serum and (b) a serum sample 2.5 h after oral administration of 120 mg racemic terfenadine. Unmetabolized terfenadine was not detected in the serum samples.

Precision, accuracy and recovery of the assay in urine were determined accordingly in a concentration range of 71.2–569.7 ng/ml. A linear correlation was found ($y = 0.00479x + 0.00479$, $r = 0.9798$). The recovery (± S.D.) of **2** was

Table 1
Reproducibility (intra-day) and accuracy after extraction of MDL 16.455 (**2**) from serum and urine

	Concentration (ng/ml)	Average peak area 2/I.S.	S.E.M.
Serum:	17.8	0.1223	0.0038
	35.6	0.2280	0.0151
	71.2	0.4626	0.0059
	142.4	0.8190	0.0558
	284.8	1.6020	0.1181
Urine:	71.2	0.3432	0.0437
	142.4	0.6797	0.1335
	284.8	1.3186	0.1317
	569.7	2.6748	0.3293

$n = 3$. Column: RP Select B, 125 × 4.0 mm I.D. (5 μm). Fluorimetric detection, $\lambda_{ex} = 230$ nm, $\lambda_{em} = 280$ nm.

Table 2
Recovery of **2** after extraction from serum and urine

	Concentration (ng/ml)	Average recovery (%)	S.E.M.
Serum:	17.8	81.5	0.993
	35.6	83.7	5.964
	71.2	84.5	2.991
	142.4	76.6	5.483
	284.8	74.9	5.621
Urine:	71.2	72.2	6.886
	142.4	89.0	6.084
	284.8	70.8	6.660
	569.7	76.0	5.531

77.0 ± 10.7%. During 24 h intervals about 4.5–8.5% of the applicated dose were eliminated as the carboxylic metabolite MDL 16.455. Also in urine unmetabolized terfenadine was not found during this study. Typical chromatograms of (a) blank urine and (b) a urine sample interval 2–4 h after oral administration of 120 mg racemic terfenadine are presented in Fig. 4.

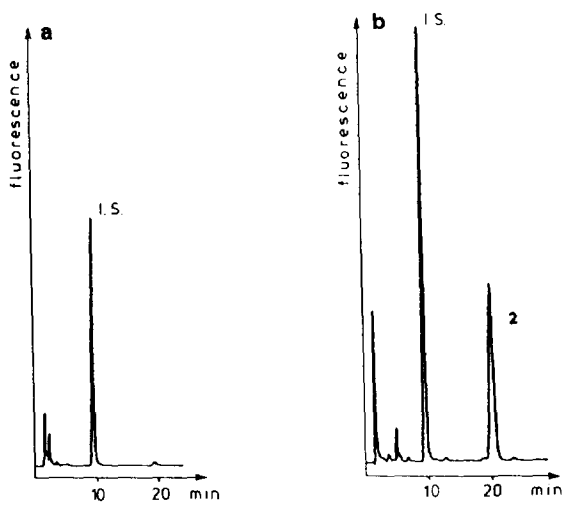

Fig. 3. Chromatograms of a blank serum spiked with the internal standard (I.S.) diphenylpyralin (a) and a serum sample 2.5 h after oral administration of 120 mg racemic terfenadine (b). Conditions: RP Select B column; 0.01 *M* phosphate buffer pH 2.5–methanol–acetonitrile (52:48:1.5); flow-rate, 1.0 ml/min; fluorimetric detection.

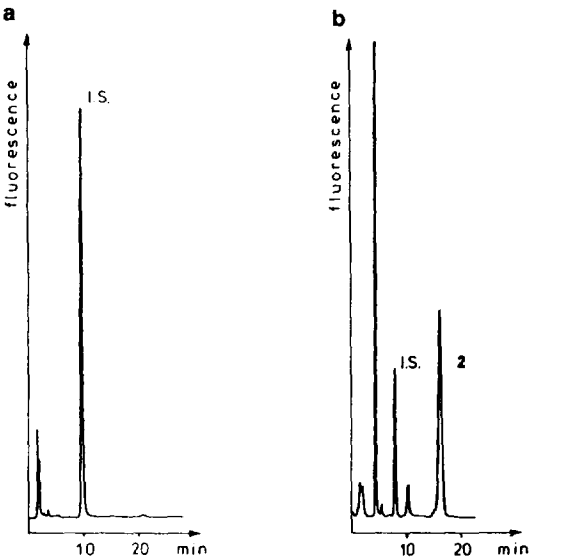

Fig. 4. Typical chromatograms of (a) blank urine containing the I.S. and (b) a urine sample interval 2–4 h after oral administration of 120 mg racemic terfenadine using an achiral HPLC system with a RP Select B column.

3.3. Determination of the enantiomeric ratio of MDL 16.455 (2) in serum and urine (chiral assay)

To exclude a discrimination one of the enantiomers during the extraction procedure the percentage of (*R*)-**2** was determined after extraction of racemic terfenadine from spiked serum and urine samples. Using a HPLC system with an Ultron ES-OVM column as a CSP the composition of the enantiomers after extraction was found to be 49.6–50.1% in both cases. Therefore, a correction of the determined enantiomeric ratios of **2** was not necessary.

In urine a non-stereoselective excretion of **2** was found (Table 3). Representative chromatograms of serum samples are given in Fig. 5. The elution order of the enantiomers of **2** was determined by incubation of terfenadine enantiomers with rat liver microsomes and analysing the extracts by HPLC on the Ultron ES-OVM col-

Table 3
Cumulative excretion (ng) and enantiomeric ration (*R*/*S*) of MDL 16.455 (**2**) in urine of three volunteers after oral application of 120 mg of terfenadine

Collection interval (h)	Volunteer					
	1		2		3	
	mg	*R*/*S*	mg	*R*/*S*	mg	*R*/*S*
0–2	0.88	1.11	0.74	1.35	2.60	1.27
2–4	2.36	1.02	1.79	1.11	3.87	1.00
4–6	3.80	1.03	3.05	1.11	4.96	1.11
6–8	4.72	1.03	4.04	1.09	7.45	1.07
8–10	5.03	0.90	4.74	1.00	8.15	0.96
10–12	5.40	0.79	5.06	1.09	8.46	0.86
12–24	n.d.	n.d.	6.66	0.85	10.2	0.78

n.d. = Not determined.

umn. The percentage of (*R*)-MDL 16.455 in serum was found to be significantly higher in the samples of all six volunteers over 24 h (Table 4).

4. Conclusion

The HPLC assays described are suitable methods for the quantification of MDL 16.455 in serum and urine as well as for the determination of the enantiomeric composition of the investigated samples. In serum samples the metabolite (*R*)-**2** predominates. However, the excretion of **2** in urine was found to be non-stereoselective.

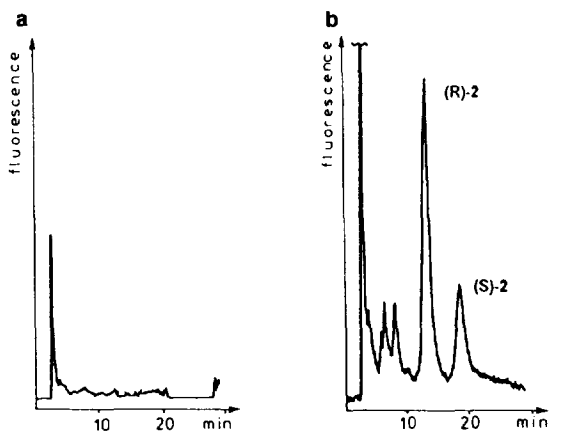

Fig. 5. Determination of the enantiomeric composition in serum using an Ultron ES-OVM column. Typical chromatograms of (a) blank serum and (b) a serum sample 0.5 h after oral administration of 120 mg racemic terfenadine.

Table 4
Averages of serum concentrations (ng/ml) and enantiomeric ratios (*R*/*S*) of MDL 16.455 (**2**) after oral application of 120 mg of terfenadine to six human volunteers

Time after application (h)	ng/ml	S.E.M.	*R*/*S*	S.E.M.
0.5	69	19	2.59	0.50
1.5	253	53	2.44	0.16
2.5	203	55	2.31	0.19
5.0	104	16	2.20	0.21
8.0	82	11	2.36	0.40
12.0	38	9.3	2.14	0.35
24.0	20	5.2	2.40	0.68

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