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Studies of the metabolism of the antihistaminic drug dimethindene by high-performance liquid chromatography and capillary electrophoresis including enantioselective aspects[1]

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Abstract

A reversed-phase high-performance liquid chromatography method for the determination of dimethindene and its main metabolites N-demethyldimethindene, 6-hydroxydimethindene and 6-hydroxy-N-demethyldimethindene in human urine was developed. The assay was also applied to the quantification of dimethindene-N-oxide in rat urine. Conjugates of the hydroxylated metabolites were determined after enzymatic deconjugation. Moreover the direct determination of dimethindene and its metabolites without prior extraction from urine was performed by capillary electrophoresis. The direct simultaneous determination of the enantiomers of dimethindene and N-demethyldimethindene was achieved on a Chiralcel OD column. Urinary data after oral administration of dimethindene are presented. The assays were used to study dimethindene and it metabolites in urine upon oral administration of the drug to rats and human volunteers.

Keywords: Dimethindene; N-Demethyldimethindene; 6-Hydroxydimethindene; 6-Hydroxy-N-demethyldimethindene; Dimethindene-N-oxide

1. Introduction

Dimethindene maleate, N,N-dimethyl-3-[1-(2-pyridinyl)-ethyl)-1H-indene-2-ethanamine maleate, an alkylamine derivative, is a potent H_1-receptor antagonist. As daily therapeutic doses for adults, only 3–6 mg are required. In vitro and in vivo animal studies demonstrated a higher affinity to the histamine receptor and a higher pharmacological activity for the *R*-(−)-enantiomer [1–3]. A stereoselectivity in the excretion of the enantiomers of dimethindene after oral administration of racemic dimethindene was found [4].

Dimethindene (Fig. 1, **1**) is almost completely metabolized. In addition with N-demethyldimethindene only 1 to 6% of the administered dose are found in urine, depending on the pH of the urine [4]. A further step of the metabolic pathway of dimethindene is the hydroxylation to 6-hydroxydimethindene found in vitro and in humans, and to 6-hydroxy-N-demethyldimethindene, found in vitro by Radler [6,7]. The presence of conjugates of hydroxylated metabolites has been reported [5,7]. The main route of excretion is the urine [5]. To date, there are no data available about the elimination rate of the hydroxylated metabolites and their conjugates

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[1] Dedicated to Professor Dr. R. Neidlein on the occasion of his 65th birthday.

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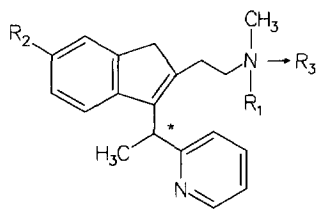

		R_1	R_2	R_3
dimethindene	1	CH_3	H	-
N-demethyldimethindene	2	H	H	-
6-hydroxydimethindene	3	CH_3	OH	-
6-hydroxy-N-demethyldimethindene	4	H	OH	-
dimethindene-N-oxide	5	CH_3	H	O
6-methoxy-N-demethyldimethindene	6	H	OCH_3	-

Fig. 1. Structures of dimethindene, N-demethyldimethindene, 6-hydroxydimethindene, 6-hydroxy-N-demethyldimethindene, dimethindene-N-oxide and 6-methoxy-N-demethyldimethindene.

in humans or animals and only a few data about the stereoselectivity of the phase I and II metabolism.

Thus, the present study was conducted to determine the metabolites of dimethindene in rat and human urine after oral administration of the drug. Moreover, the stereoselectivity of the excretion of dimethindene and its nonphenolic metabolite N-demethyldimethindene was determined.

2. Experimental

2.1. Chemicals

Dimethindene maleate was a gift from Zyma (Nyon, Switzerland). N-Demethyldimethindene, 6-hydroxydimethindene, 6-methoxy-N-demethyldimethindene and the *R*-(−)- and *S*-(+)-enantiomers of dimethindene were prepared as described by Radler [4,6]. The internal standard (I.S.) codeine was obtained from Merck (Darmstadt, Germany). Cyclohexane, ethanol, ethyl acetate, methanol, *n*-hexane and tetrahydrofuran were LiChrosolv reagents from Merck. Tetrahydrofuran was freshly distilled. The other chemicals were of analytical grade. Buffer solutions were prepared in double-distilled, deionized water and filtered (0.22 μm). β-Glucuronidase (purified, type H-1 from *Helix pomatia*, E.C. 3.2.1.31, 338 000 I.U./g) with arylsulfatase (E.C. 3.1.6.1, 16 600 I.U./g) was obtained from Sigma (Deisenhofen, Germany).

2.2. Synthesis of dimethindene-N-oxide

A solution of dimethindene base (4.82 mmol) in ethanol (70 ml) was treated with hydrogen peroxide (30%, v/v, 20 ml). After stirring for 24 h at room temperature the excess hydrogen peroxide was destroyed by adding manganese dioxide (15 g) while cooling to 5°C. After filtration the filtrate was adjusted to pH 8 after diluting with water (200 ml), extracted with diethyl ether and the water phase was evaporated to dryness. The residue, dissolved in ethanol and purified by flash chromatography over an RP 2 column with ethanol as eluent, was evaporated and the oily residue was dissolved in ethanol abs. (10 ml) and 0.6 g of maleic acid was added. The solution was cooled to 5°C and allowed to stand overnight. The resulting precipitate was filtered off and dried under reduced pressure. The yield was 47.8% (2.05 mmol) and the colourless needles had a m.p. of 137°C. The dimethindene-N-oxide maleate was characterized by ^1H and ^{13}C NMR, recorded on a Varian Gemini 200 (Bremen, Germany) and elemental analysis. The liquid chromatography–mass spectrometry (LC–MS) data were *m/z* (%)=309 (100) $[M^+ + H]$, 248 (6) $[M^+ - C_2H_6NO - H]$.

2.3. Synthesis of 6-hydroxy-N-demethyldimethindene

6-Hydroxy-N-demethyldimethindene was prepared by dissolving 6-methoxy-N-demethyldimethindene oxalate (0.38 mmol) in hydrobromic acid (47% v/v, 30 ml) and boiling under reflux conditions for three hours according to the procedure of Radler [6]. After diluting with water, the base was extracted with methylene chloride at pH 9 and the methylene chloride layer was evaporated. The oily residue was dissolved in ethanol and purified by flash chromatography over an RP 2 column with ethanol as eluent. The yield was 65.2% (0.25 mmol). 6-Hydroxy-N-

demethyldimethindene was characterized by LC–MS with the following data: m/z (%)=295 (100) [M^++H], 188 (8) [$M^+-C_7H_8N$], 160 (16) [$C_{11}H_{10}O^+$+2H].

2.4. Apparatus for high-performance liquid chromatography (HPLC)

The chromatographic system consisted of a Merck-Hitachi L 6200 liquid chromatograph (Darmstadt, Germany) for the gradient method and a Knauer HPLC pump 64 (Bad Homburg, Germany) for the chiral method, a Rheodyne sample injector Model 7125 (Latek, Eppelheim, Germany) equipped with a 20-μl loop, a Merck-Hitachi 655 A variable-wavelength detector and a Merck-Hitachi D 2000 Chromato-Integrator. The UV detector was set at 259 nm. The polarimetric determinations were performed on a Perkin-Elmer Type 241 polarimeter (Überlingen, Germany) with a flow-through cell (80 μl).

2.5. Achiral chromatography

The separation of dimethindene and its metabolites was performed on an RP Select B RP-8 stationary phase (5 μm particle size, 250×4 mm I.D.), with a 30×4 mm I.D. RP Select B RP-8 guard column from Merck. A combined linear and step elution gradient programme was used from the time of injection as follows:

Phosphate buffer solution (0.05 mol/l, pH 4.5)–methanol–tetrahydrofuran from 70:27:3 (v/v) to 70:27:3 in 18.9 min at a flow-rate of 0.5 ml/min, from 19 to 38 min 48:48:4 at a flow-rate of 0.45 ml/min.

2.6. Chiral chromatography

The simultaneous enantioseparation of dimethindene and N-demethyldimethindene was achieved on a Chiralcel OD column (10 μm particle size, 250×4 I.D.) with a 50×4.6 mm I.D. Chiralcel OD guard column from Baker (Gross-Gerau, Germany). The mobile phase was *n*-hexane–ethanol (94:6, v/v) containing 0.1% (v/v) diethylamine, and the flow-rate 0.6 ml/min. A LiChrosorb Diol guard column (7 μm particle size, 30×4 mm I.D., Merck) was additionally used.

2.7. Apparatus for capillary electrophoresis (CE)

A P/ACE 2100 CE system from Beckman Instruments (Munich, Germany) was used with an untreated fused-silica capillary (Grom, Herrenberg, Germany) of 40 cm effective length and 50 μm I.D.

Standard operating conditions: effective electric field, 400 V/cm; temperature, 20°C; sample introduction, low pressure for 1 s (ca. 1 nl sample volume); detection UV at 200 nm; anode, cathode and run buffers, 0.1 mol/l phosphate buffer solution (pH 3.2).

2.8. Apparatus for LC–MS

LC–MS was recorded on a Waters 600 HPLC multisolvent delivery system with a Waters 600-E-MS System Controller (Millipore, Bedford, MA, USA) and a mass spectrometer Finnigan MAT TSQ 7000 (Bremen, Germany) with atmospheric pressure chemical ionisation. Vaporizer, 90°C; source, 210°C. The chromatographic separation was achieved on an RP Select B RP-8 stationary phase (5 μm particle size, 250×4 mm I.D.), with a 125×4 mm I.D. RP Select B RP-8 guard column from Merck. The mobile phase consisted of 0.05 mol/l ammonia acetate buffer (pH 4.5)–acetonitrile (55:45, v/v) operated at a flow-rate of 0.6 ml/min.

2.9. Drug administration and sample collection

A 4-mg weight of dimethindene maleate was administered to one healthy male volunteer (27 years of age, 70 kg), as an aqueous solution (4 ml Fenistil Tropfen). Urine was collected before and every hour up to 6 h and then at 10, 15, 24 and 30 h after administration.

Three male RAI rats were treated with 200 mg/kg of racemic dimethindene maleate (rat weight: 247 g), *R*-(−)-dimethindene (rat weight: 243 g) or *S*-(+)-dimethindene (rat weight: 232 g) as aqueous solution given orally and the urine was collected before and at 3, 6 and 12 h after administration.

The samples were stored at −20°C until analysis.

2.10. Extraction procedure and determination of the conjugates in urine

For the quantitative determination of dimethindene and its metabolites 2, 3 and 4 on the RP Select B column, 50 μl of the solution of the I.S. codeine (80.0 μg/ml in 0.05 mol/l phosphate buffer pH 4.5–methanol, 1:1, v/v) and 0.5 ml of aqueous ammonia (25%) were added to a 2.0-ml volume of each human urine sample. Extraction was performed twice with 3 ml of cyclohexane–ethyl acetate (1:1, v/v) for 10 min and the tubes were centrifuged for 20 min. The organic layer was removed, two to four of these extracts of the urine samples taken 1, 24 and 30 h after administration were combined, and then evaporated under a stream of nitrogen. The residue was dissolved in 0.05 mol/l phosphate buffer (pH 4.5)–methanol (1:1, v/v) and 20 μl were analysed by HPLC.

For the determination of dimethindene and the metabolites 2, 3 and 4 in rat urine 200 μl of each sample were diluted with double-distilled water to 2 ml and extracted as described above. The determination of dimethindene-N-oxide was performed corresponding to this procedure by using ethyl acetate for extraction.

The hydrolysis of the conjugates was performed by diluting 1 ml of human and 50–200 μl rat urine samples respectively with 0.02 mol/l sodium acetate buffer (pH 4.6) to 2 ml and incubating with 1000 I.U. of β-glucuronidase with activity of arylsulfatase in 500 μl sodium chloride solution (0.2%, m/m) at 37°C for 24 h under stirring. The extraction was performed as described above.

For the determination of the enantiomeric ratio of dimethindene and N-demethyldimethindene, 2 ml of each urine sample were extracted with *n*-hexane as described above without addition of the I.S.. The residue was dissolved in 50 μl of the mobile phase and 20 μl were analysed by HPLC on the Chiralcel OD column.

2.11. Isolation of 6-hydroxy-N-demethyldimethindene and dimethindene-N-oxide

The isolation of 6-hydroxy-N-demethyldimethindene and dimethindene-N-oxide was performed by extraction with cyclohexane–ethyl acetate (1:1, v/v) or ethyl acetate from human and rat urine samples as described above. The residue was dissolved in 50 μl of the mobile phase used for LC–MS and 20 μl were analysed. The conditions used are given under Section 2.8. The molecular ion peak of 6-hydroxy-N-demethyldimethindene at *m/z* 295 $[M^+ + H]$ with the corresponding fragment ions with similar intensities were detected in human and rat urine samples. The molecular ion peak of dimethindene-N-oxide at *m/z* 309 $[M^+ + H]$ with the corresponding fragment ion of *m/z* 248 were detected with similar intensities in rat urine samples.

2.12. Enantiomeric elution order

The elution order of dimethindene enantiomers on the Chiralcel OD column was determined by chromatography of the pure enantiomers. Because the pure enantiomers of N-demethyldimethindene were not available, polarimetric determinations after chiral separation and rat urine samples after oral administration of 200 mg/kg *R*-(−)- or *S*-(+)-dimethindene were used to determine the elution order of the enantiomers.

3. Results and discussion

3.1. Quantitative determination by HPLC on RP Select B RP 8

Dimethindene and metabolites were quantified in spiked urine. Representative chromatograms of a human blank urine (a), a human urine sample (b), a human urine sample after enzymatic deconjugation (c) and a rat urine sample extracted with ethyl acetate (d) are shown in Fig. 2. No interferences occurred with the peaks of dimethindene, the metabolites and the I.S. codeine after extraction of urine samples with cyclohexane–ethyl acetate (1:1) or with ethyl acetate. Assay precision, reproducibility and calibration curves were determined in blank urine samples spiked with known amounts of the compounds. The concentrations for the calibration curves for dimethindene and the metabolites ranged from 0.5 to 390 μg/ml (Table 1). The equations for the standard curves were $y=0.873x-0.003$ for dimethindene, $y=1.011x-0.090$ for N-demethyldimethindene, $y=$

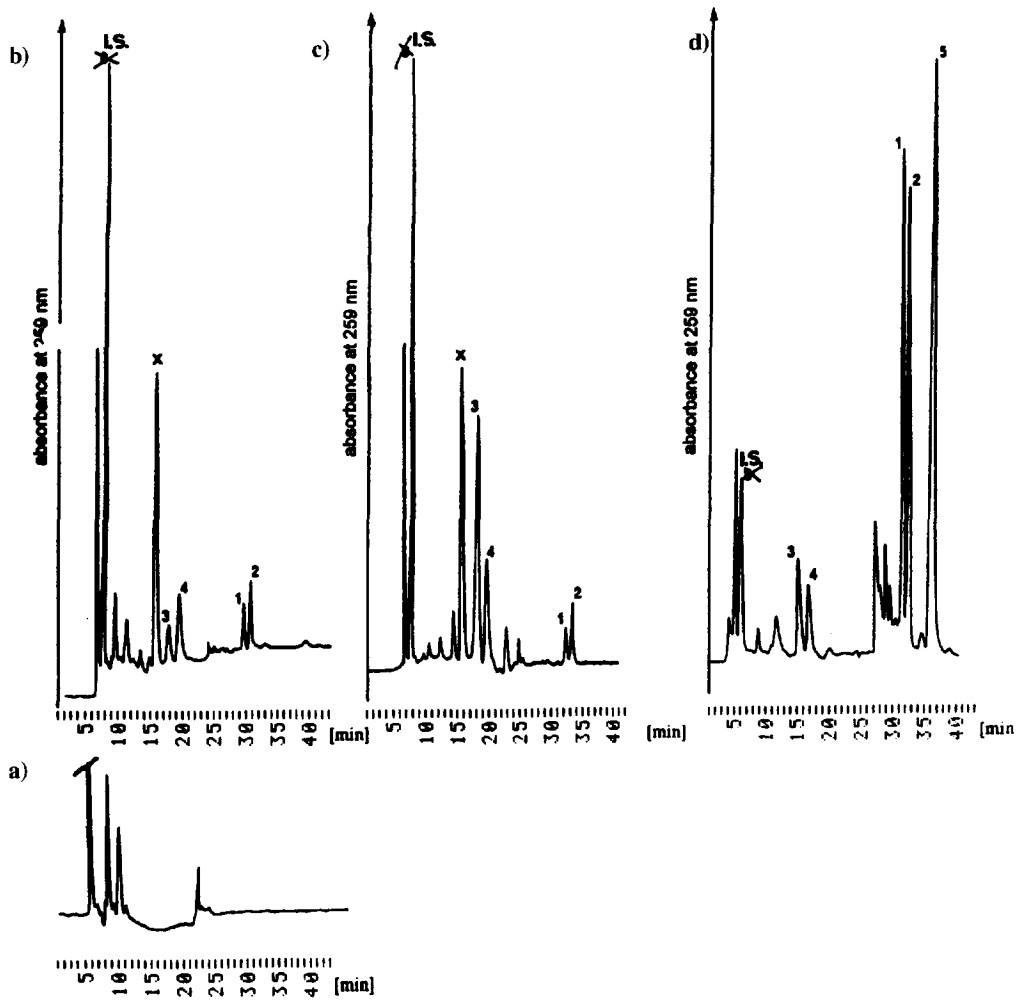

Fig. 2. Chromatograms of (a) a human blank urine, (b) a human urine sample, (c) a human urine sample enzymatically deconjugated (interval 10–15 h) and (d) a rat urine sample extracted with ethyl acetate (interval 3–6 h) after oral administration of dimethindene. Peaks: 1=dimethindene; 2=N-demethyldimethindene; 3=6-hydroxydimethindene; 4=6-hydroxy-N-demethyldimethindene; 5=dimethindene-N-oxide; I.S.=codeine; x=compound excreted with urine, not a metabolite of dimethindene. For chromatographic conditions see Section 2.

$0.736x-0.050$ for 6-hydroxydimethindene, $y=0.692x+0.038$ for 6-hydroxy-N-demethyldimethindene and $y=0.199x+0.002$ for dimethindene-N-oxide. For dimethindene and its metabolites the correlation coefficient exceeded 0.9997 except for 6-hydroxy-N-demethyldimethindene with a coefficient of 0.9976. The mean recovery(±S.D.) was 96.5±1.5% for dimethindene, 82.7±6.2% for N-demethyldimethindene, 96.0±4.6% for 6-hydroxydimethindene, 87.9±5.5% for 6-hydroxy-N-demethyldimethindene, extracted with cyclohexane–ethyl acetate (1:1, v/v) and 23.9±3.4% for dimethindene-N-oxide, extracted with ethyl acetate. The detection limit of the method was approximately 1 ng/ml urine for dimethindene and N-demethyldimethindene and 2 ng/ml urine for the other metabolites with a signal-to-noise ratio of 3. Due to impurities in 6-hydroxy-N-demethyldimethindene the calculation for this metabolite was performed with the equation of 6-hydroxydimethindene.

Table 1
Reproducibility of the HPLC assay

Amount spiked (μg)	Peak-area ratio, compound/codeine	R.S.D. (%)
Dimethindene (**1**)		
0.032	0.029	6.90
0.387	0.342	3.80
0.838	0.747	1.21
1.933	1.702	1.17
3.222	2.750	3.60
16.11	14.06	4.67
N-Demethyldimethindene (**2**)		
0.028	0.045	8.89
0.339	0.359	5.01
0.734	0.606	4.62
1.694	1.505	2.33
2.824	2.690	0.37
14.12	14.22	4.63
6-Hydroxydimethindene (**3**)		
0.039	0.027	7.41
0.464	0.340	3.24
1.006	0.712	3.79
2.321	1.505	2.33
3.868	2.822	1.38
19.33	14.19	4.97
6-Hydroxy-N-demethyldimethindene (**4**)		
0.039	0.022	4.55
3.137	1.991	5.32
5.930	4.325	1.90
9.311	6.795	1.41
13.259	9.028	2.97
16.40	11.33	3.84
Dimethindene-N-oxide (**5**)		
0.028	0.011	9.10
0.333	0.065	3.08
0.722	0.164	7.32
1.666	0.310	6.45
2.777	0.565	3.89
13.88	2.776	3.93

Values are mean±S.D., $n=4$. For structures of the compounds see Fig. 1.

3.2. Quantitative determination by CE

For the direct determination of urine samples without prior extraction rat urine samples were analysed by capillary electrophoresis [8,9]. Fig. 3 shows representative electropherograms of a blank urine spiked with dimethindene and the metabolites **2–5** (a), a blank urine (b) and a rat urine after oral administration of dimethindene maleate (c). No interferences were observed. The relationship between the concentration and the detector response was determined with concentrations from 15–2100 μg/ml in 0.005 mol/l phosphate buffer (pH 3.2) for dimethindene and the metabolites **2**, **3** and **5** (Table 2). A linear correlation was found for all compounds with equations of the calibration curve of $y=0.004x+0.006$ for dimethindene, $y=0.005x+0.017$ for N-demethyldimethindene, $y=0.004x+0.045$ for 6-hydroxydimethindene and $y=0.004x+0.036$ for dimethindene-N-oxide. For all compounds the correlation coefficient exceeded 0.9999. In human urine samples after oral application of 4 mg dimethindene, the concentrations of drug and metabolites were too low to be detected.

3.3. Urinary excretion in humans

The excretion of the drug and of its metabolites was analyzed in urine. The cumulative excretion of dimethindene and the metabolites **2**, **3** and **4** is shown in Fig. 4 for one volunteer after oral administration of 4 mg dimethindene maleate. The results are summarized in Table 3. After 30 h, only 3.6% of the administered dose were excreted as dimethindene and N-demethyldimethindene. The cumulative excretion of 6-hydroxydimethindene, 6-hydroxy-N-demethyldimethindene and their glucuronic and sulfate conjugates on the other hand was about 23% of the administered dose. The results indicate that conjugated 6-hydroxydimethindene is a main metabolite of dimethindene in humans.

3.4. Urinary excretion in rats

The cumulative excretion of dimethindene and its metabolites **2–5** in rat urine was about 9% and 11% respectively, with enzymatic deconjugation of the administered dose 12 h after oral application of 200 mg/kg dimethindene maleate. Dimethindene-N-oxide which is not present in human urine was detected as a main metabolite in the metabolism of dimethindene in rats. The results without enzymatic deconjugation were confirmed by direct determination without an extraction procedure by CE (Table 4).

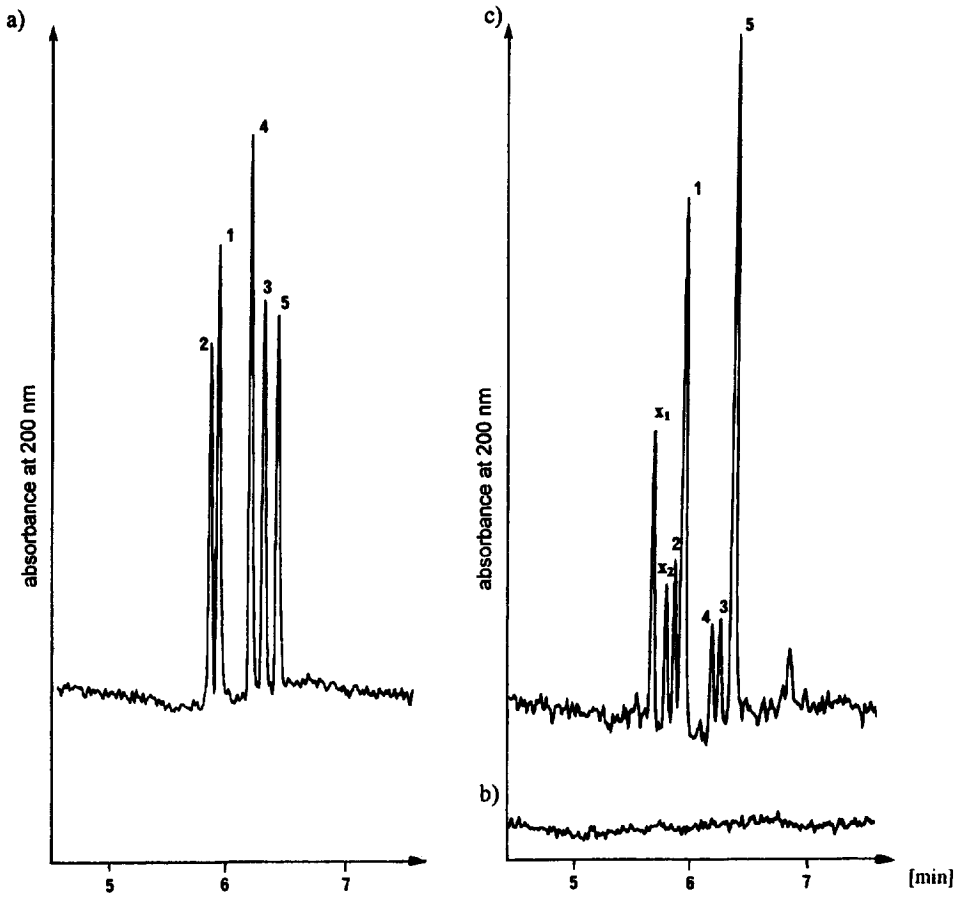

Fig. 3. Electropherograms of (a) a human blank urine spiked with dimethindene and metabolites, (b) a human blank urine and (c) a rat urine after oral administration of dimethindene (interval 0–3 h). Peaks: 1=dimethindene; 2=N-demethyldimethindene; 3=6-hydroxy-dimethindene; 4=6-hydroxy-N-demethyldimethindene; 5=dimethindene-N-oxide; x_1 and x_2=unknown metabolites. For conditions see Section 2.

*3.5. Determination of enantiomeric ratios of **1** and **2** in humans by HPLC*

The enantiomeric ratio of dimethindene and N-demethyldimethindene was simultaneously determined using a Chiralcel OD column. Thus, prior achiral separation on a cyano column which was necessary using an α_1-acid glycoprotein column was avoided [4]. Fig. 5 shows typical chromatograms of a blank urine (a), and a human urine sample after oral administration of 4 mg racemic dimethindene maleate (b). No interferences were observed with the peaks of the dimethindene and N-demethyldimethindene enantiomers. In order to identify the configuration of N-demethyldimethindene *R*-(−)-dimethindene with an ee (enantiomeric excess) of 91.2 was orally administered to rats. The second N-demethyldimethindene peak, which was polarimetrically determined to be levorotatory, was observed in the chromatogram (Fig. 5c). Because inversion of enantiomers is unlikely to occur during the N-demethylation it is concluded that (−)-N-demethyldimethindene is also *R*-configurated.

For calibration the enantiomers of dimethindene were combined to obtain mixtures of known enantiomeric ratios. Additionally, blank urine was spiked

Table 2
Reproducibility of the assay by capillary electrophoresis

Concentration (μg/ml)	Detector response	R.S.D. (%)
Dimethindene (**1**)		
15.2	0.061	7.63
152.1	0.602	5.87
1521	5.924	3.27
N-Demethyldimethindene (**2**)		
17.7	0.075	9.27
176.5	0.791	8.69
1765	8.180	4.86
6-Hydroxydimethindene (**3**)		
20.9	0.087	11.38
209.0	0.837	3.49
2090	7.591	7.32
Dimethindene-N-oxide (**5**)		
20.9	0.085	5.27
209.1	0.886	7.34
2091	8.176	2.17

Values are mean±S.D., $n=5$. For structures of the compounds see Fig. 1.

with different concentrations of racemic N-demethyldimethindene and analysed on different days for enantiomeric ratios. The mean determined enantiomeric ratio was 50.4% to 49.6% (*S/R*, S.D.= 1.26). The detection limits were less than 1 ng/ml urine for each enantiomer determined by extraction of human blank urine. The total amounts of dimethindene and N-demethyldimethindene were determined by achiral HPLC.

In agreement with Radler [4], in humans a stereoselectivity in the excretion of the enantiomers of dimethindene with a first preponderant excretion of *S*-(+)-dimethindene and a later reversion of this stereoselectivity was found (Fig. 6). The excretion of N-demethyldimethindene was highly stereoselective with a predominant elimination of the *R*-(−)-enantiomer. From 2 until 30 h after administration, the ratio of the *S*-(+)- to the *R*-(−)-enantiomer remained about 1:4.

Table 3
Excretion of dimethindene and its metabolites after oral administration of 4 mg dimethindene maleate to a male volunteer

	Free	After deconjugation
Dimethindene	1.59	Unchanged
N-Demethyldimethindene	1.96	Unchanged
6-Hydroxydimethindene	3.48	14.45
6-Hydroxy-N-demethyldimethindene	5.68	8.69
Total amount	12.71	26.69

Values are given as % by weight of the administered dose.

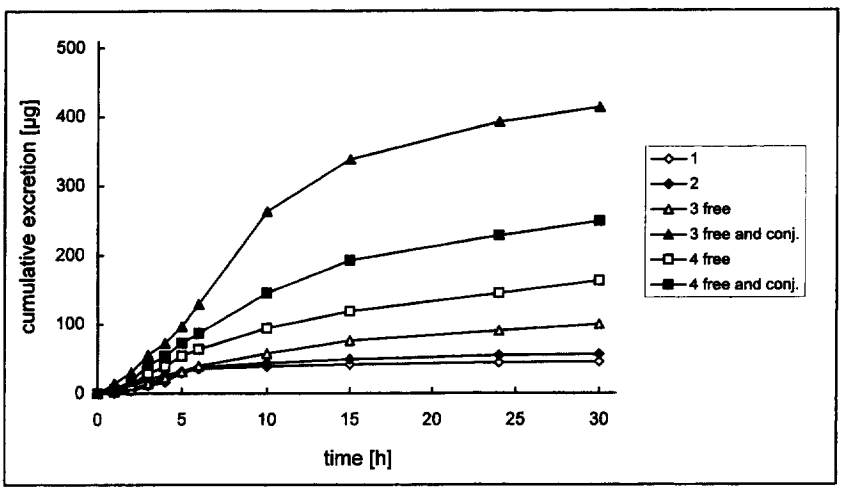

Fig. 4. Cumulative excretion curves of dimethindene and its metabolites after oral administration of dimethindene to one volunteer (conj.=conjugated). Compound numbering as in Fig. 1.

Table 4
Excretion of dimethindene and its metabolites after oral administration of 200 mg/kg dimethindene maleate to a male rat

	HPLC assay		CE assay
	Free	After deconjugation	Free
Dimethindene	1.26	Unchanged	1.44
N-Demethyldimethindene	0.69	Unchanged	0.90
6-Hydroxydimethindene	0.34	0.95	0.49
6-Hydroxy-N-demethyldimethindene	0.42	1.45	0.51
Dimethindene-N-Oxide	6.38	Unchanged	6.73
Total amount	9.09	10.73	10.07

Values are given as % by weight of the adminstered dose.

4. Conclusions

The HPLC assays developed in this study are selective, convenient and suitable for the quantification of dimethindene and its metabolites, including their conjugates, as well as for the analysis of the enantiomeric ratios of dimethindene and N-demethyldimethindene in urine samples. The CE meth-

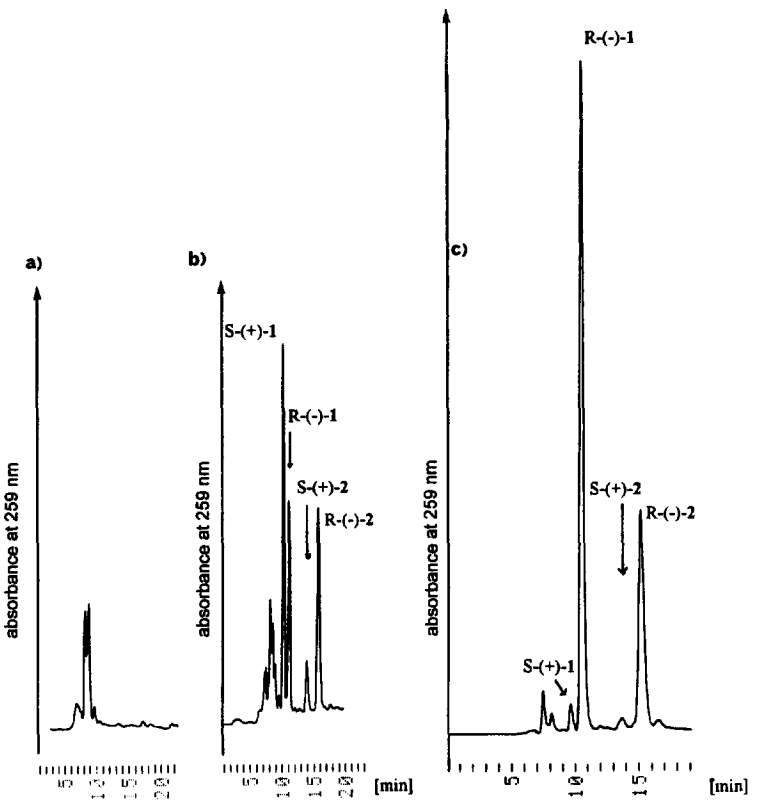

Fig. 5. Chromatograms of (a) a human blank urine and (b) a human urine sample after oral administration of 4 mg dimethindene maleate (interval 2–3 h) and (c) a rat urine sample after oral administration of 200 mg/kg *R*-(−)-dimethindene (ee=91.2, interval 3–6 h) on a Chiralcel OD column. Peaks: enantiomers of 1=dimethindene, 2=N-demethyldimethindene. For chromatographic conditions see Section 2.

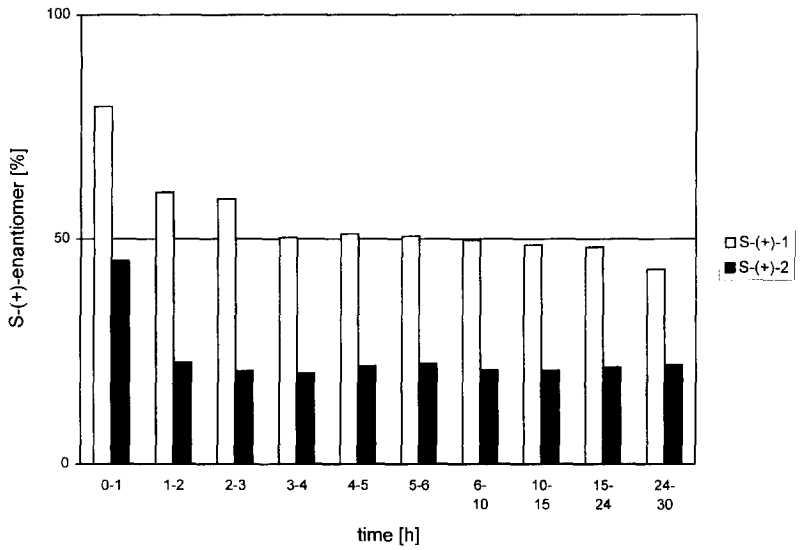

Fig. 6. Percentage of the *S*-(+)-enantiomers of dimethindene (**1**) and N-demethyldimethindene (**2**) in urine after oral administration of 4 mg dimethindene maleate to one volunteer.

od described is a simple method for the direct rapid determination of dimethindene and its metabolites from urine avoiding prior extraction steps. A high stereoselectivity of the excretion of demethyldimethindene was observed.

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