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Determination of the stereoselective aspects in in-vitro and in-vivo metabolism of the analgesic meptazinol by high-performance liquid chromatography

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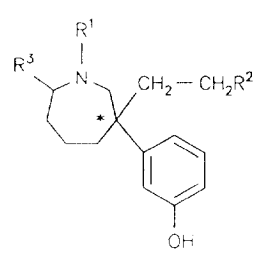
Abstract

A reversed-phase high-performance liquid chromatographic method to separate meptazinol and its phase I metabolites has been developed using a LiChrosper 100 CN column and a mobile phase of trimethylammoniumacetate buffer (pH 5.5)–acetonitrile–methanol. Quantification of meptazinol and N-desmethylmeptazinol in biological samples was achieved by extraction with organic solvents and chromatographic analysis (detection limit 0.4 and 0.25 μg/ml, respectively). Afterwards the enantiomeric ratio of the two compounds was determined on a Chiral AGP column with a mobile phase of phosphate buffer (pH 7.0)–acetonitrile ($\alpha = 1.29$ and 1.49, respectively). In-vitro metabolism data after incubation of the racemic compound and the enantiomers with liver supernatant and microsomes of different species are presented. Finally urinary data of two volunteers after oral application of the racemic drug were determined.

1. Introduction

Meptazinol, 3-(3-ethylhexahydro-1-methyl-1H-azepin-3-yl)phenol, is an opioid-type analgesic with mixed agonist–antagonist properties. It is used for the treatment of severe postoperative, obstetric or cancer pain [1]. For therapy only the racemate is available. The enantiomers of meptazinol have been prepared by crystallization with tartaric acid [2]. The (−)-enantiomer is a more potent antagonist than the (+)-enantiomer [3] and inhibits additionally acetylcholinesterase [4]. This cholinergic effect contributes to the analgesic activity. Both enantiomers have opioid-agonist properties.

In spite of the good enteral absorption, the oral bioavailibility is only ca. 9%, because the drug undergoes extensive first-pass metabolism. Meptazinol (1, Fig. 1) is rapidly conjugated with glucuronic acid or PAPS (3′-phosphoadenosine-5′-phosphosulfate). Besides, oxidative metabo-



	R^1	R^2	R^3
1	$-CH_3$	−H	−H
2	−H	−H	−H
3	$-CH_3$	−OH	−H
4	$-CH_3$	−H	=O
5	$-CH_2CH_3$	−H	−H

Fig. 1. Structures of meptazinol (1), N-desmethylmeptazinol (2), 3-hydroxyethylmeptazinol (3), 7-oxomeptazinol (4), and the internal standard (5).

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lism results in N-desmethylmeptazinol (2), 3-hydroxyethylmeptazinol (3) and 7-oxomeptazinol (4). Meptazinol is mostly excreted by the urine [5].

Although the pharmacological properties of the enantiomers of meptazinol have been examined, to date no stereoselective studies of phase I and phase II reactions have been presented. In the pharmacokinetics of meptazinol stereoselective aspects should be taken into account to evaluate the usefulness of administration of the single enantiomers.

Quantification of meptazinol in biological samples is described in several articles already published [6–9]. None of these methods, however, refers to a resolution of meptazinol and its phase I metabolites.

In this paper, we describe an improved approach for the preparative resolution of meptazinol. Additionally, an HPLC method to separate meptazinol and its phase I metabolites and quantitation of compounds 1 and 2 in biological samples is presented. The present method of achiral separation was developed based on the method of Frost [6]. Enantiomeric resolution of compounds 1 and 2 was achieved by chiral HPLC. These methods were applied to determine the enantiomeric ratio after incubation with liver preparations from rat and rabbit and in human urine.

2. Experimental

2.1. Chemicals

Meptazinol was a gift from Wyeth Pharma (Münster, Germany), N-desmethylmeptazinol (2), 3-hydroxyethylmeptazinol (3), 7-oxomeptazinol (4) and the internal standard N-desmethyl-N-ethylmeptazinol (5) were provided by Wyeth Research (Taplow, Maidenhead, UK). Acetonitrile, methanol, ethyl acetate and hexane were LiChrosolv reagents (Merck, Darmstadt, Germany). The other chemicals used in the experiments were of analytical grade. Buffer solutions were prepared in double distilled, deionized water and filtered through a 0.22-μm filter.

2.2. Apparatus

The chromatographic system consisted of a LC-6A liquid chromatograph (Shimadzu, Duisburg, Germany), a Rheodyne sample injector (Model 7125, Rheodyne) with a 50-μl loop, a SPD-6A variable-wavelength detector (Shimadzu), a SCL 6B system controller (Shimadzu) and a CR-6A chromato-integrator (Shimadzu). A wavelength of 220 nm was chosen for the analyses.

2.3. Achiral chromatography

For separation of meptazinol and its phase I metabolites, a LiChrospher 100 CN column (5 μm particle size, 250 × 4 mm I.D., Merck) with a LiChrospher 60 CN guard column (10 μm particle size, 30 × 4 mm I.D.) were used. The eluent was acetonitrile–methanol–1% triethylammonium–acetate buffer (pH 5.5) (15:5:80, v/v). The flow-rate was 0.5 ml/min. Fig. 2 shows a representative chromatogram.

2.4. Chiral chromatography

Resolution of racemic meptazinol and N-desmethylmeptazinol was achieved with a Chiral-AGP column (ChromTech, Norsborg, Sweden). This commercially available column (100 × 4 mm, I.D.) consists of human α_1-acid glycoprotein covalently immobilized on silica. Additionally a LiChrosorb NH_2 guard column (particle size 10 μm, 30 × 4 mm I.D.) was used. A mobile phase of 0.01 *M* phosphate buffer (pH 7.0)–acetonitrile (95:5, v/v) enabled the simultaneous enantioseparation of compounds 1 and 2.

2.5. Preparative separation of meptazinol enantiomers

Meptazinol was obtained from the aqueous solution of its hydrochloride as a white precipitate by alkalinization and 2.7 mmol of the racemic meptazinol base were dissolved in 3 ml

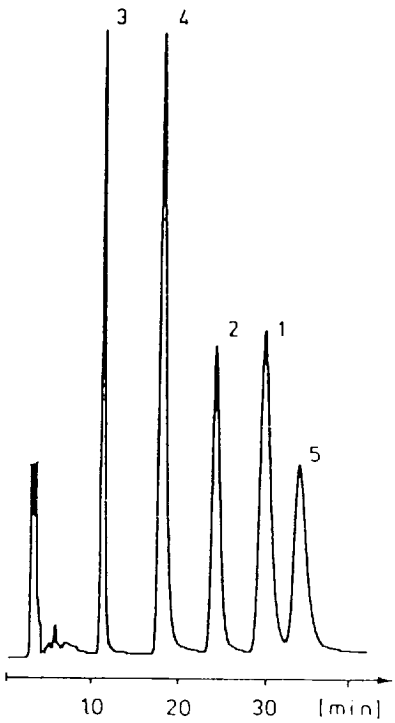

Fig. 2. Chromatogram of the achiral separation of meptazinol (1), N-desmethylmeptazinol (2), 3-hydroxyethylmeptazinol (3), 7-oxomeptazinol (4), and the internal standard ethylmeptazinol (each compound: 0.5 μg on column). Chromatographic conditions see Experimental.

of anhydrous ethanol. (−)-Di-O,O′-*p*-toluoyl-D-tartaric acid (1.5 mmol) was added and the mixture was allowed to crystallize for 24 h at 20°C. After 3 recrystallizations, the pure diastereomeric salt was collected, decomposed by alkalinization and extracted with diethyl ether. The ether solution of the enantiomeric base was concentrated by evaporation. With HCl gas, dried over sulphuric acid, the pure enantiomeric (−)-hydrochloride was obtained and identified by mass spectrometry, elemental analysis, NMR and melting point (215°C). The yield was 47.0%, the specific rotation of the (−)-hydrochloride was $[\alpha]_D = -9.00°$ ($c = 0.15$, H_2O, 20°C).

Accordingly, by crystallisation with (+)-di-O,O′-*p*-toluoyl-L-tartaric acid and further treatment as described above the (+)-meptazinol hydrochloride was obtained with a melting point of 210°C. The yield was 49.9%, the specific rotation of the (+)-hydrochloride was $[\alpha]_D = +9.98°$ ($c = 0.15$, H_2O, 20°C). The enantiomeric purity of both enantiomers was determined by HPLC analysis on the Chiral AGP column and was at least 98%.

This method proved to be superior to the method described in the literature [2], since it gave a better reproducible and higher yield.

2.6. In-vitro metabolism studies

Preparation of liver microsomes and supernatant and incubation assay

Livers from New Zealand rabbits and Sprague–Dawley rats were used. Some of the rats were treated with phenobarbitone (50 mg/kg for 6 days). The fresh livers were homogenized at 4°C and fractionated by a classical set of centrifugation steps according to the method of Dayer [10]. Liver preparations were stored at −80°C before use. Protein concentrations were determined according to Bradford [11] and cytochrome P 450 according to Omura and Sato [12].

The following incubation scheme turned out to be most suitable: To variable amounts of meptazinol-HCl, racemate and enantiomers respectively, dissolved in Tris buffer (pH 7.8 at 20°C), 0.006 μmol $MgCl_2$ and the liver preparation were added and the final volume adjusted to 2 ml with buffer. Incubation was started with addition of 0.98 μmol NADPH. Samples were incubated and stirred for 30 min at 37°C. The reaction was terminated by placing the vial in an ice bath and by adding the organic extraction solvent.

Determination of meptazinol and N-desmethylmeptazinol in liver microsome or supernatant preparations by achiral chromatography

After incubation, internal standard (5) was added and samples were buffered with 1 ml of NH_3/NH_4Cl buffer (1 *M*, pH 10.0). A 5-ml volume of a mixture of *n*-hexane–ethylacetate (50:50, v/v) was used for extraction. Four ml of the organic layer were removed and evaporated under a stream of nitrogen. The residue was dissolved in 50 μl of double distilled water and

20 μl were analysed by the achiral chromatographic system.

Determination of the enantiomeric ratio in in-vitro metabolism studies

For the determination of the enantiomeric ratio of unchanged meptazinol and N-desmethylmeptazinol the extraction was the same as for achiral analysis. A 20-μl aliquot was analysed by HPLC on the Chiral AGP column.

For calibration mixtures of pure enantiomers with a known enantiomeric ratio ranging from 90:10% to 10:90% (+)/(−) were prepared from solutions containing 123 μg/ml of one enantiomer. Aliquots of 20 μl were injected (i.e. 2.46 μg on column). Each mixture was analysed three times on the chiral HPLC column. A linear relationship was found and there were no differences between the enantiomers during extraction (Table 1). Concentrations beyond a 10:90 (−)/(+) ratio lead to difficulties in quantitation.

In-vivo metabolism study

Two healthy male volunteers received one Meptid tablet containing 231.24 mg meptazinol hydrochloride corresponding to 200 mg of meptazinol base. Urine was collected at 2-h intervals up to 12 h after administration and from 12 to 24 h. Urine volumes and pH were measured immediately, and the samples were stored at −20°C up to 5 weeks. No decomposition or change in the enantiomeric ratio was observed within this time period.

Table 1
Calibration of *S*/*R* ratios for meptazinol enantiomers

Theoretical (%)		Found (%)		R.S.D.(%)
(+)-1	(−)-1	(+)-1	(−)-1	
10.03	89.97	10.79	89.21	5.96
20.05	79.95	17.96	82.04	0.79
30.07	69.93	27.11	72.89	0.31
40.08	59.92	37.93	62.07	0.66
50.08	49.92	49.93	50.07	0.84
60.08	39.92	59.27	40.73	8.95
70.07	29.93	67.72	32.28	2.34
80.05	19.95	76.92	23.08	2.13
90.03	9.97	86.80	13.20	0.97

Values are given in %, $n = 3$; aliquots (20 μl) of a solution containing 123 μg/ml of the mixture of enantiomers were injected (= 2.46 μg on column).

2.7. Enzymatic deconjugation

Quantitation of conjugation products of meptazinol (glucuronides, sulphates) was performed indirectly by enzymatic deconjugation. To deconjugate phase II metabolites an assay according to Murray et al. [13] was modified by enhancing the quantity of enzymes.

Urine samples were incubated for 24 h with β-glucuronidase/sulphatase H_2 from *Helix pomatia* (Sigma, Deisenhofen, Germany). The enzyme concentration was adapted to the metabolite concentration (interval 0–4 h 2000 I.U./ml urine, 4–8 h 1000 I.U./ml, 8–24 h 500 I.U./ml).

Instead of undergoing enzyme incubation some samples were refluxed for 4 h after adding 20 ml of 1 *M* HCl. These drastic conditions lead to complete hydrolysis of the conjugates without changing meptazinol. The enzymatic assay and the assay with acid hydrolysis provided the same data [14]. Therefore enzymatic hydrolysis was proved to be complete.

Determination of meptazinol, N-desmethylmeptazinol and their conjugates by achiral chromatography

Aliquots of untreated urine samples and enzymatically deconjugated samples were buffered with 1 ml of NH_3/NH_4Cl buffer (1 *M*, pH 10.0) and extracted, after addition of internal standard, with 5 ml of *n*-hexane–ethylacetate (30:70, v/v). After vigorous stirring for 10 min, 4 ml of the organic layer were collected and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl of double distilled water and 20 μl were used for achiral HPLC. Total conjugation products of meptazinol and N-desmethylmeptazinol were calculated from these assays.

Determination of the enantiomeric ratios in untreated and deconjugated urine samples

Samples were prepared as for the achiral analysis except for the addition of the internal

standard. In order to avoid contamination of the protein column by impurities from the urine samples, compounds 1 and 2 were fractionated on the achiral column. These HPLC fractions were extracted with 2 × 5 ml of ethylacetate, the organic layer evaporated to dryness under a stream of nitrogen and the residue dissolved in 50 μl of double distilled water and analysed by chiral HPLC.

3. Results and discussion

3.1. Preparative separation

Both enantiomers of meptazinol were obtained by fractional crystallization of their diastereomeric salts with (+)- or (−)-di-O,O'-p-toluyl-tartaric acid.

3.2. In-vitro metabolism

Determination of meptazinol and N-desmethylmeptazinol in liver microsome or supernatant preparations by achiral chromatography

After incubation meptazinol was partially metabolised. In male Sprague–Dawley rat liver preparations, N-desmethylmeptazinol was the main metabolite, whereas 7-oxomeptazinol was not detected. N-desmethylmeptazinol is of special interest being the only metabolite with analgesic activity and toxic side effects [14]. Metabolism required the presence of NADPH and could be inhibited by addition of KCN or metyrapone. Therefore biotransformation is Cytochrome P 450-mediated. Fig. 3a shows the chromatogram of an incubation with microsomes of non-induced rats and a comparative sample without NADPH. Without NADPH there is no conversion of meptazinol (Fig. 3b).

Precision and accuracy of the assay were examined in blank liver preparations spiked with known amounts of meptazinol and N-desmethylmeptazinol, simulating concentrations from 0.313 to 5.015 μg/ml of compound 1 or 0.157 to 2.510 μg/ml of compound 2. Each concentration was analysed three times. Table 2 summarizes the data. For both compounds a linear correlation between detector signal and concentration was found. The following correlations were

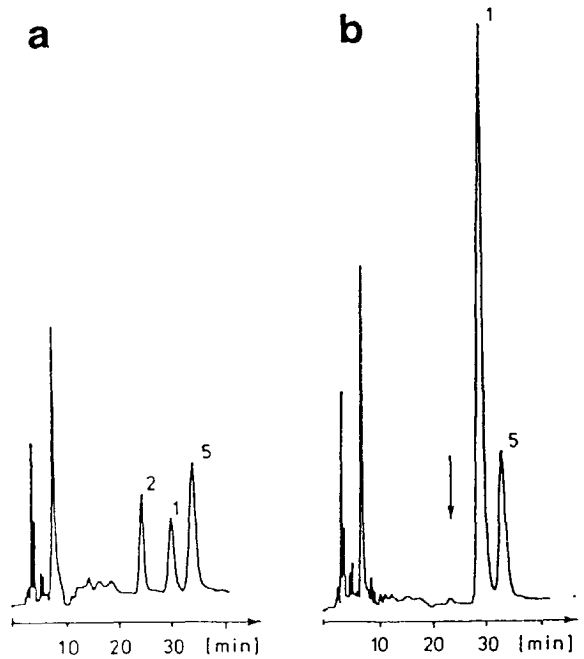

Fig. 3. Chromatograms of the achiral phase after incubation of 6.25 μg meptazinol-HCl with liver microsomes from non-induced rats (a) and without NADPH (b). Chromatographic conditions see Experimental. Peak: 2 = N-desmethylmeptazinol (2); 1 = meptazinol (1); 5 = internal standard (5).

Table 2
Reproducibility for meptazinol and N-desmethylmeptazinol in liver homogenates

Compound 1		Compound 2	
Spiked (μg/ml)	Found (mean ± S.D.) (μg/ml)	Spiked (μg/ml)	Found (means ± S.D.) (μg/ml)
0.313	0.290 ± 0.005	0.157	0.156 ± 0.08
0.627	0.662 ± 0.03	0.314	0.296 ± 0.02
1.254	1.194 ± 0.03	0.628	0.621 ± 0.01
2.508	2.580 ± 0.08	1.255	1.299 ± 0.05
5.015	4.991 ± 0.18	2.510	2.492 ± 0.03

$n = 3$.

calculated: $y = 0.759465x + 0.055454$, $r = 0.9992$ for compound 1; $y = 0.580036x - 0.00945$, $r = 0.9993$ for compound 2.

Values of recovery were obtained by comparison of peak areas in liver preparation samples, spiked with different amounts of 1 or 2 after extraction and standard solutions. The recovery (± S.D.) of meptazinol (mean value of $n = 15$, $c = 0.3$–5 μg/ml) was 76.4% (± 3.7%), and that for N-desmethylmeptazinol (mean value of $n = 15$, $c = 0.15$–2.5 μg/ml) was 58.9% (± 3.5%). The quantitation limit was 0.392 μg/ml for meptazinol and 0.256 μg/ml for N-desmethylmeptazinol.

After incubation with liver fractions of rabbits, there was also a substantial biotransformation of meptazinol.

Incubation of meptazinol enantiomers

Incubation of enantiomers gave different results depending on species, phenobarbitone induction and incubation conditions: With rabbit microsomes or supernatant more of the (−)-meptazinol was metabolized. Accordingly the enantiomer of 2 formed from (−)-meptazinol dominated after incubation with microsomes from rats. (+)-Meptazinol turnover was more intense. However, after incubation with supernatant (−)-meptazinol turnover was stronger (especially for phenobarbitone induced livers, the difference between the enantiomers after incubation with non-induced supernatant was only marginal). Table 3 presents some of the data.

Incubation of the racemate and determination of the enantiomeric ratio

After incubation of meptazinol racemate sample preparation was the same as for achiral chromatography (except the addition of the internal standard). Incubation with rabbit liver microsomes and supernatant resulted in a predominance of unchanged (+)-meptazinol rang-

Table 3
Incubation of meptazinol-HCl with different liver preparations

Liver preparation	Substrate	Unchanged 1 after incubation (μg)	S.D.	Compound 2 after incubation (μg)	S.D.
Non-induced rabbit microsomes	rac 1	1.295	0.097	–	–
	(+)-1	1.383	0.091	–	–
	(−)-1	1.202	0.103	–	–
Non-induced rabbit supernatant	rac 1	1.695	0.073	–	–
	(+)-1	2.041	0.087	–	–
	(−)-1	1.075	0.068	–	–
Non-induced rat microsomes	rac 1	1.567	0.095	1.301	0.071
	(+)-1	1.332	0.083	1.102	0.083
	(−)-1	1.789	0.165	1.459	0.060
Non-induced rat supernatant	rac 1	2.327	0.086	0.954	0.067
	(+)-1	2.347	0.194	0.758	0.057
	(−)-1	2.318	0.057	1.145	0.057
Phenobarbitone-induced rat microsomes	rac 1	0.049	0.002	0.932	0.054
	(+)-1	0.035	0.002	0.826	0.071
	(−)-1	0.057	0.004	0.991	0.063
Phenobarbitone-induced rat supernatant	rac 1	0.016	0.0015	0.343	0.028
	(+)-1	0.018	0.0017	0.239	0.021
	(−)-1	0.016	0.0017	0.431	0.034

Conditions: 30-min incubation of 6.25 μg meptazinol-HCl at pH 7.8.

ing from 56.31:43.69 to 62.28:37.72%. Incubation with liver supernatant resulted in a higher stereoselectivity.

Fig. 4 shows representative chromatograms of a blank microsome sample (a), after incubation of racemic meptazinol with non-induced rat microsomes (b), and with phenobarbitone induced rat microsomes (c). Again, results differed according to species, phenobarbitone induction and incubation parameters. After incubation with non-induced rat liver preparations meptazinol remained approximately racemic. Only at a high pH of 8.6 a significant predominance of (+)-meptazinol in microsome samples was observed. In contrast, incubation with liver fractions from phenobarbitone induced rats resulted in a predominance of (+)-meptazinol and the 2-enantiomer formed from (+)-meptazinol. Exemplary data are presented in Table 4 and demonstrated by Fig. 5.

Stereoselective aspects in in-vitro metabolism could be demonstrated [15]. N-Desmethylation of meptazinol is not the only stereoselective reaction.

3.3. Comparison of the incubation of enantiomers and the racemate

For rabbit liver preparations the incubation of enantiomers and the racemate lead to comparable results. The (−)-meptazinol turnover was always higher. Incubation with rat liver preparations gave varying results. After incubation of the enantiomers with microsomes (+)-meptazinol turnover was dominant, while with supernatant (−)-meptazinol turnover dominated. For all preparations more of the N-desmethylmeptazinol enantiomer formed from (−)-meptazinol was detectable. After incubation of the racemate more of the (−)-meptazinol was metabolized, differences being only evident after incubation with phenobarbitone-induced liver preparations. Here the N-desmethylmeptazinol enantiomer formed from (+)-meptazinol always dominated. This discrepancy between the enantiomers and the racemate may be explained by a mutual inhibition of the metabolism of enantiomers, if the racemate is incubated, a phenomenon already observed with other drugs.

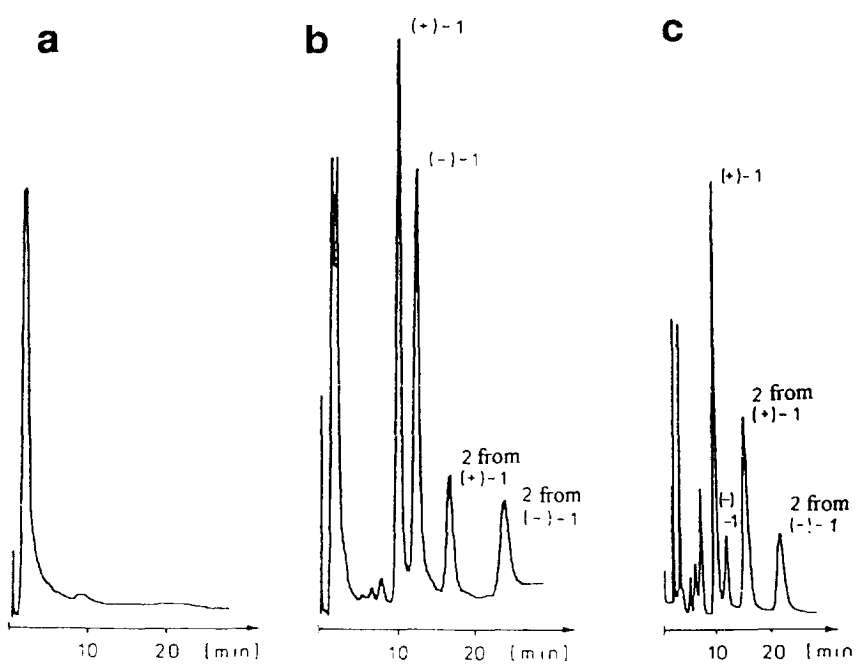

Fig. 4. Blank sample (a), determination of enantiomeric ratio after incubation of 6.25 μg meptazinol-HCl with liver microsomes of non-induced rats (b), and phenobarbitone induced rats (c). Chromatographic conditions see Experimental.

Table 4
Enantiomeric ratio after incubation of racemic meptazinol-HCl with different liver preparations

Liver preparation	Substrate (μg)	pH	(+)-1 (%)	(−)-1 (mean ± R.S.D.) (%)	2 from (+)-1 (%)	2 from (−)-1 (%)
Non-induced rabbit microsomes	12.5	7.8	56.71 ± 2.33	43.29 ± 3.06	–	–
	25.0	7.8	56.31 ± 0.99	43.69 ± 1.28	–	–
Non-induced rabbit supernatant	12.5	7.8	62.13 ± 1.06	37.87 ± 1.74	–	–
	25.0	7.8	62.28 ± 3.98	37.72 ± 6.75	–	–
Non-induced rat microsomes	12.5	7.8	49.58 ± 0.89	50.42 ± 0.88	50.20 ± 1.98	49.80 ± 2.00
	12.5	8.6	55.63 ± 1.43	44.37 ± 1.79	55.22 ± 2.60	44.37 ± 1.79
Non-induced rat supernatant	12.5	7.8	47.76 ± 1.66	52.24 ± 1.51	49.76 ± 4.14	50.24 ± 4.10
Phenobarbitone-induced rat microsomes	12.5	7.8	62.23 ± 2.37	37.77 ± 3.91	70.02 ± 0.90	29.98 ± 2.10
	25.0	7.8	79.84 ± 2.39	20.16 ± 9.45	66.46 ± 3.67	33.54 ± 7.27
Phenobarbitone-induced rat supernatant	25.0	7.8	78.01 ± 2.21	21.99 ± 7.84	68.50 ± 1.16	31.50 ± 2.51

Ratio of unchanged meptazinol (1) and of N-desmethylmeptazinol (2).

3.4. *In-vivo metabolism study*

Determination of urinary excretion

After administration of one Meptid tablet genuine samples and deconjugated samples were analysed on the LiChrospher 100 CN column. Fig. 6 shows chromatograms of blank urine (a) and a deconjugated sample collected 6 h after administration (b).

Calibration curves were obtained by spiking blank urine with known amounts of 1 and 2. Reproducibility and precision of the assay were determined (Table 5). The data were used to calculate urinary concentrations and cumulative

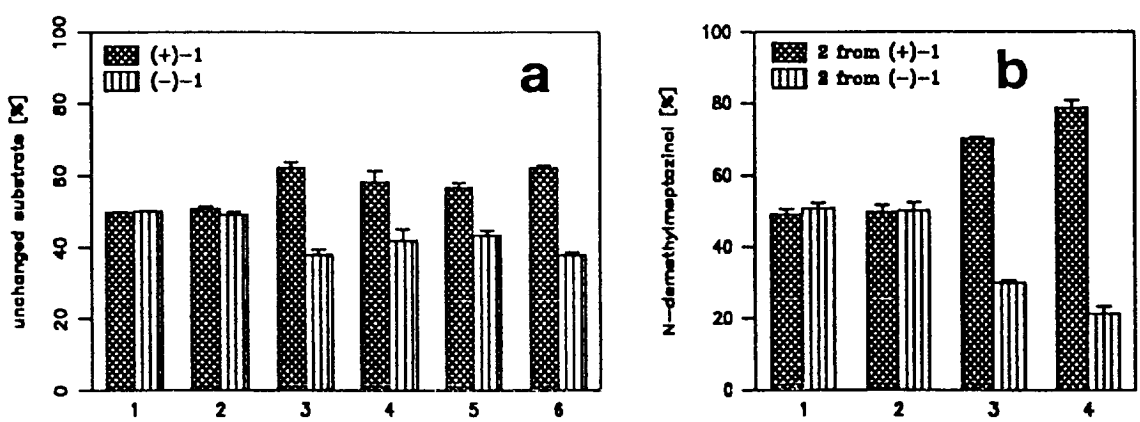

Fig. 5. (a) Enantiomeric ratio of unchanged meptazinol in different liver fractions; (b) enantiomeric ratio of N-demethylmeptazinol in different liver fractions. (1) Non-induced rat microsomes; (2) non-induced rat supernatant; (3) phenobarbitone-induced rat microsomes; (4) phenobarbitone-induced rat supernatant; (5) non-induced rabbit microsomes; (6) non-induced rabbit supernatant.

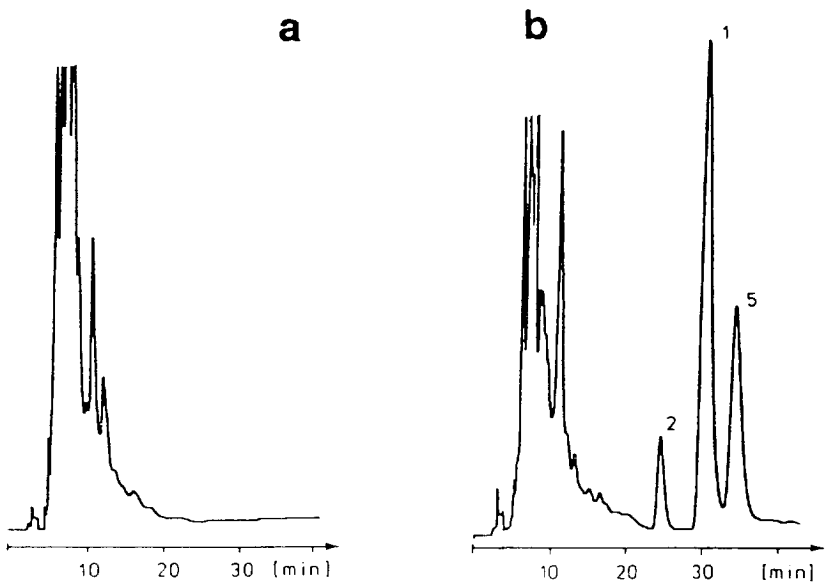

Fig. 6. Chromatogram of blank urine (a) and an urine sample after enzymatic deconjugation (b). Chromatographic conditions see Experimental. Retention times of 1,2 and I.S. were 30.92, 26.99 and 35.41 min, respectively.

excretion using the following correlations: $y = 0.201612x + 0.019321$, $r = 0.9996$ for 1; $y = 0.130665x - 0.03154$, $r = 0.9971$ for 2.

Cumulative excretion of unchanged metazinol, meptazinol conjugates and N-desmethylmeptazinol conjugates were 0.61, 36.41 and 7.40% of the applied dose for volunteer 1 and 0.66, 42.07 and 6.12% for volunteer 2. The amount of unconjugated N-desmethylmeptazinol was too low to quantify (<0.05%).

Table 5
Reproducibility for meptazinol and N-desmethylmeptazinol in human urine

Compound 1		Compound 2	
Spiked (μg/ml)	Found (mean ± S.D.) (μg/ml)	Spiked (μg/ml)	Found (mean ± S.D.) (μg/ml)
0.783	0.812 ± 0.02	0.298	0.276 ± 0.02
1.565	1.610 ± 0.03	0.595	0.560 ± 0.04
3.13	3.019 ± 0.14	1.191	1.106 ± 0.07
6.26	6.263 ± 0.34	2.381	2.093 ± 0.14
12.52	12.071 ± 0.39	4.763	4.650 ± 0.17
25.04	25.770 ± 1.63	9.525	9.647 ± 0.67
50.08	49.832 ± 1.60		

$n = 3$.

Determination of the enantiomeric ratios

The resolution of meptazinol and N-desmethylmeptazinol was achieved by chiral HPLC after fractionation of urine samples without addition of I.S. as shown before. Fig. 7 shows representative chromatograms of blank urine (a), meptazinol in an unchanged urine sample (b), and meptazinol in an urine sample after deconjugation (c). Both volunteers excreted more unchanged (−)-meptazinol with interindividual differences in the enantiomeric ratio. After enzymatic deconjugation the enantiomeric ratios of 1 and 2 were determined. For both volunteers (+)-meptazinol predominated especially in the first intervals. More of the N-desmethylmeptazinol enantiomer from (+)-meptazinol was formed, the stereoselectivity increased during the time after administration. Table 6 shows these data and Fig. 8 presents the cumulative excretion curves of the enantiomers calculated from the analysis data.

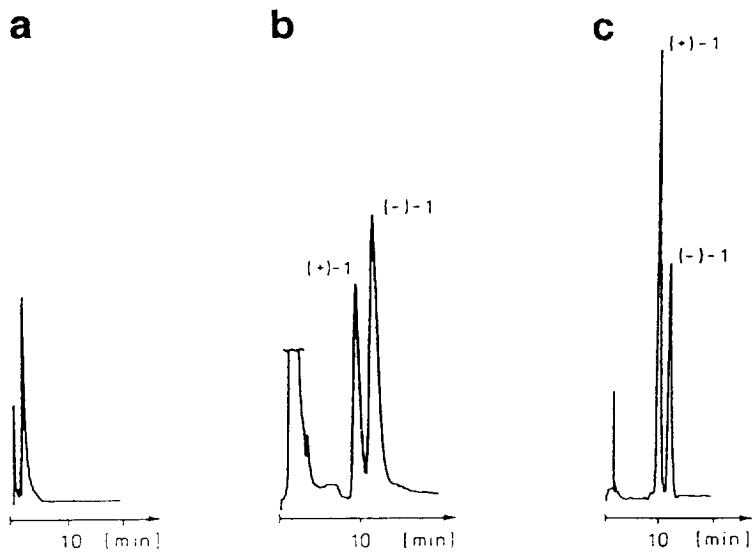

Fig. 7. Chromatogram of blank urine (a), the determination of enantiomeric ratio in human urine before (b), and after deconjugation (c) on Chiral AGP. Chromatographic conditions see Experimental.

4. Conclusions

An HPLC method to determine meptazinol, its active metabolite and indirectly their conjugation products including their enantiomeric ratios in biological samples was developed. Stereoselective aspects in in-vitro metabolism could be demonstrated, depending on the species, phenobarbitone induction and other experimental criteria. Additionally, this biotrans-

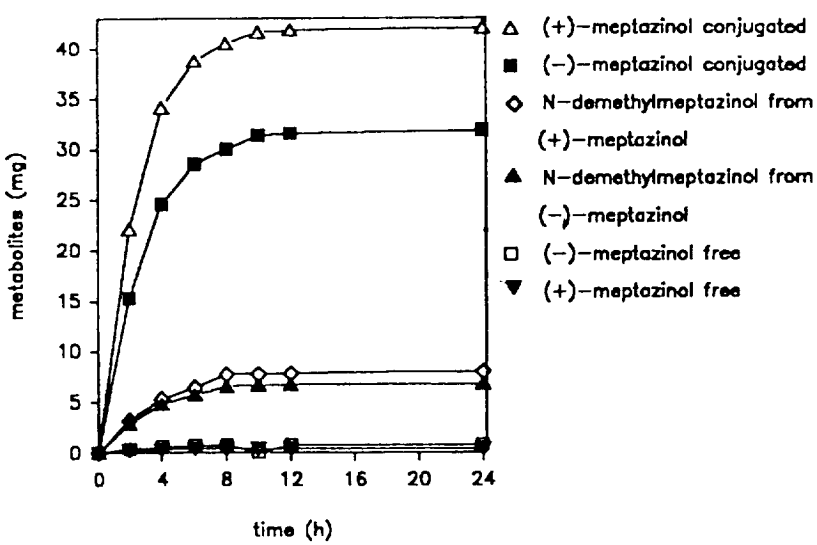

Fig. 8. Graphical representation of cumulative excretion of conjugated meptazinol (1) and conjugated N-desmethylmeptazinol (2) in human urine. Only very small amounts of nonconjugated meptazinol enantiomers were detected.

Table 6
Enantiomeric ratio after oral administration of racemic meptazinol-HCl in human urine

Patient	Interval (h)	Unconjugated meptazinol (mean ± R.S.D.) (%)		Meptazinol after deconjugation (mean ± R.S.D.) (%)	
		(+)-1	(−)-1	(+)-1	(−)-1
(a) Enantiomeric ratio of unconjugated meptazinol and meptazinol after deconjugation					
1	0–2	34.97 ± 4.45	65.03 ± 2.39	58.49 ± 1.02	41.51 ± 1.44
	2–4	36.86 ± 0.76	63.14 ± 0.45	55.03 ± 1.04	44.97 ± 1.27
	4–6	36.65 ± 2.06	63.35 ± 1.19	53.40 ± 3.56	46.60 ± 4.08
	6–8	34.28 ± 3.73	65.72 ± 1.95	52.76 ± 2.28	47.24 ± 2.54
	8–10	42.34 ± 4.00	57.66 ± 2.94	52.39 ± 1.19	47.61 ± 1.31
	10–12	n.d.	n.d.	49.87 ± 0.19	50.13 ± 0.19
	12–24	n.d.	n.d.	44.53 ± 2.02	55.47 ± 1.63
2	0–2	49.54 ± 1.55	50.46 ± 1.52	59.08 ± 2.79	40.92 ± 4.02
	2–4	47.87 ± 2.07	52.13 ± 1.90	53.30 ± 0.73	46.70 ± 0.83
	4–6	45.63 ± 0.65	54.37 ± 0.54	62.05 ± 1.89	37.95 ± 3.09
	6–8	46.57 ± 2.66	53.43 ± 2.32	60.69 ± 2.47	39.13 ± 3.82
	8–10	45.13 ± 1.16	54.87 ± 0.95	59.52 ± 1.06	40.48 ± 1.56
	10–12	n.d.	n.d.	51.79 ± 2.46	48.21 ± 1.27
	12–24	n.d.	n.d.	51.02 ± 1.30	48.98 ± 1.35

		N-Desmethylmeptazinol after deconjugation	
		2 from (+)-1 (mean ± R.S.D.) (%)	2 from (−)-1 (mean ± R.S.D.) (%)
(b) Enantiomeric ratio of N-desmethylmeptazinol (2)			
1	0–2	52.73 ± 0.33	47.27 ± 0.37
	2–4	52.19 ± 0.84	47.81 ± 0.92
	4–6	52.71 ± 1.25	44.29 ± 1.57
	6–8	56.23 ± 2.09	43.77 ± 2.68
	8–10	69.43 ± 2.98	30.57 ± 6.76
2	0–2	54.70 ± 0.88	45.230 ± 1.07
	2–4	59.36 ± 3.59	40.64 ± 5.25
	4–6	68.55 ± 2.97	31.45 ± 6.48
	6–8	85.01 ± 0.34	14.99 ± 1.93
	8–10	85.00 ± 0.66	15.00 ± 3.74

formation was investigated in urine samples of two human volunteers.

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