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Determination of the enantiomers of chlorpheniramine and its main monodesmethyl metabolite in urine using achiral–chiral liquid chromatography

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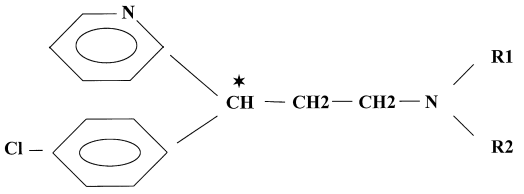
Abstract

The enantiomers of chlorpheniramine and its monodesmethyl metabolite were determined separately in urine by using a coupled achiral–chiral chromatographic system. The two enantiomers of the studied compound and the internal standard were separated from the biological matrix on a cyanopropyl column and reinjected into a chiral amylose AD column where the two enantiomers were separated and quantified by UV detection. The method was validated for chlorpheniramine and for the metabolite within the range 0–1000 ng/ml. It was also applied in a pilot pharmacokinetic study to samples from a volunteer given 8 mg of racemic chlorpheniramine by mouth. © 1998 Elsevier Science B.V.

Keywords: Enantionmer separation; Chlorpheniramine; Monodesmethylchlorpheniramine

1. Introduction

Chlorpheniramine (CPAM), also known as chlorphenamine (Fig. 1) is an antiallergic drug with H_1 antihistamine properties. It is considered in the World Health Organization standards as an essential drug due to its effectiveness and low cost. Therefore, it is distributed worldwide as a generic compound. CPAM is strongly metabolised in the liver by desmethylation. Two metabolites have been identified: monodesmethylchlorpheniramine (DCPM) and didesmethylchlorpheniramine (DDCPM). After a single oral therapeutic dose, DCPM and DDCPM



	R1	R2
CPAM	CH3	CH3
DCPM	CH3	H
DDCPM	H	H

Fig. 1. Chemical structures of chlorpheniramine (CPAM), the monodesmethyl metabolite (DCPM) and the didesmethyl metabolite (DDCPM). The asterisk represents the asymmetric carbon.

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cannot be detected in blood [1]. In urine, unchanged CPAM and DCPM each account for about 20% of the dose while DDCPM accounts for less than 3% [2,3].

CPAM, DCPM and DDCPM bear an asymmetric carbon on the propane chain. In a large number of commercial drug preparations, CPAM is used as the racemic mixture of the two enantiomers. Pharmacokinetic studies of CPAM enantiomers, performed on rats after bolus intravenous injection of the racemic mixture, showed that (−)-CPAM plasma concentrations are higher than those of the antipode [4], and that plasma protein binding [4] and metabolism [5] may be involved in this stereoselectivity. Nonstereoselective liquid chromatographic methods for the determination of plasma and urine concentrations have been described [2,3,6–8]. Chiral separation of CPAM enantiomers by high-performance liquid chromatography (HPLC) has also been reported. Partial resolution was obtained for CPAM and metabolite enantiomers in solution on an α1-acid glycoprotein column by HPLC [9] and with β-cyclodextrin (as a chiral additive) by capillary electrophoresis [10]. Complete resolution was obtained in solution, using carboxymethyl-β-cyclodextrin as the chiral additive, by means of capillary electrophoresis [11] and on an ovomucoid chiral column by HPLC [12].

We describe a coupled achiral–chiral HPLC method to separate CPAM and DCPM from a biological matrix on a cyanopropyl achiral column, followed by chiral resolution of the corresponding enantiomers on a Chiralpak AD® column.

2. Experimental

2.1. Chemicals and solvents

Racemic CPAM and (+)-CPAM, and the internal standards (+)-brompheniramine [(+)-BPAM] and amodiaquine [AMQ], were from Sigma Aldrich (Saint Quentin Fallavier, France). The monodesmethyl metabolite was kindly supplied by Schering Plough (Levallois-Perret, France). UV grade hexane and ethanol were from Carlo Erba (Paris la Défense, France). Diethylamine was from Merck (Strasbourg, France). Ethyl ether was from Prolabo (Paris, France). All other solvents and reagents were of analytical grade.

2.2. Stock solutions

Standard solutions of CPAM, DCPM and (+)-BPAM were prepared in ethanol at a concentration of 1 mg/ml. They were stable for at least 6 months at 4°C. Working solutions were prepared by dilution in ethanol to the required concentrations.

2.3. Achiral–chiral chromatographic system

The coupled method involved an achiral column connected to a chiral column via an EPS 130 HP2P automatic switching valve (Informatiques et Technologies, Le Blanc Mesnil, France). The achiral chromatographic system was based on a Waters WISP 717 Plus autosampler and a Thermo Separation Spectraseries P100 pump with a Nucleosil 5 μm cyanopropyl column (250×4.6 mm I.D.) (Life Sciences, Eragny, France).

The chiral chromatography system consisted of a Beckman A114 pump and an amylose Chiralpak AD® column (Mallinckrodt Baker, Noisy-le-Sec, France).

Two different mobile phases were used for the determination of CPAM and DCPM enantiomers using identical columns. Each mobile phase consisted of a mixture of hexane–ethanol–diethylamine [(96:4:0.05, v/v/v) for CPAM and (85:15:0.1, v/v/v) for DCPM] running at 0.5 ml/min and room temperature.

UV detection was performed after the chiral column step and used a Shimadzu SPD6A detector (set at 262 nm) connected to a Shimadzu CR-5A integrator.

The extractum was injected on the cyanopropyl column. The interferences were first discarded to waste. Just before coelution on the cyanopropyl column of the enantiomeric mixture of the compound of interest and the internal standard, the system was automatically switched in order to connect both achiral and chiral columns. The enantiomeric mixture and the internal standard were then driven from the cyanopropyl column to the Chiralpak AD® column, where the two enantiomers of the compound

of interest and the internal standard were separated and detected.

2.4. Extraction procedure

Extraction procedures were similar for CPAM and DCPM and were performed according to Athanikar et al. [8] with the following modifications: to 1 ml of urine we added the internal standard [200 μl of a solution of (+)-BPAM at 1 μg/ml for CPAM and 50 μl of a solution of AMQ at 1 μg/ml for DCPM], 250 μl of 5% potassium hydroxide and 5 ml of ethyl ether. The mixture was vortexed for 30 s and centrifuged for 10 min. The aqueous phase was frozen using dry ice in acetone. The organic phase was separated and 500 μl of 0.5% phosphoric acid was added. The mixture was vortexed for 30 s and centrifuged for 10 min. To the aqueous phase we added 250 μl of 5% potassium hydroxide and 5 ml of ethyl ether. The aqueous phase was frozen with dry ice in acetone and the organic phase was decanted and evaporated at 37°C under a stream of nitrogen. The residue was then reconstituted with 70 μl of mobile phase and 50 μl was injected into the chromatographic system.

2.5. Method validation

The method was separately validated for CPAM and the metabolite DCPM in urine.

Urine standards were prepared by spiking normal human drug-free urine with solutions of racemic CPAM and racemic DCPM to concentrations of 0, 50, 100, 250, 500, 750 and 1000 ng/ml. Linearity was determined on three calibration curves for the two enantiomers of CPAM and for the two enantiomers of DCPM. Calibration curves and analysis of linearity were performed for each enantiomer by using linear least-squares regression analysis by plotting ratios of the peak area of the separated enantiomer and the internal standard against the concentration of the enantiomer.

Reproducibility was studied by between-day and within-day validation. Five samples with a low (100 ng/ml of racemic CPAM and DCPM) and a high (750 ng/ml of racemic CPAM and DCPM) concentration were analyzed to calculate the within-day coefficients of variation. Day-to-day reproducibility was studied on three different days.

Samples containing theoretical amounts of racemic CPAM and its racemic metabolite DCPM at the same concentrations as those used for the calibration curve but spiked with other stock solutions were analyzed to determine accuracy.

The detection limit was evaluated with urine spiked with decreasing concentrations of CPAM and DCPM and was defined as the lowest concentration which could be determined with a precision better than 20%.

Recovery was determined by comparing the peak areas of the enantiomers of CPAM and DCPM in five spiked urine samples at low (50 ng/ml) and high (375 ng/ml) concentrations and the peak areas of the corresponding compounds after direct injection of ethanol solutions.

2.6. In vivo pilot pharmacokinetic study

A healthy subject received 8 mg of racemic chlorpheniramine maleate (1 tablet of CONTAC®, Smithkline Beecham, Pittsburgh, PA, USA). Urine samples were collected from 0 to 144 h after administration. Concentrations of CPAM and DCPM enantiomers were determined as described above.

3. Results and discussion

Direct injection of the extractum was tested on the amylose AD column after extraction. Due to interference by biological matrix components, the retention times of the enantiomers normally have to be increased, drastically diminishing the sensitivity of the method and the working life of the column. For this reason, we preferred a coupled achiral–chiral system. The backflush method, used to trap the racemic mixture in a guard column and to reinject it into the chiral column, did not improve resolution.

The mobile phase for the coupled system was limited to solvents compatible with the chiral Chiralpak AD® column, i.e., hexane, ethanol and 2-propanol at all possible proportions, and diethylamine up to 1%. Whatever the proportions of these solvents, it was not possible to elute both CPAM and

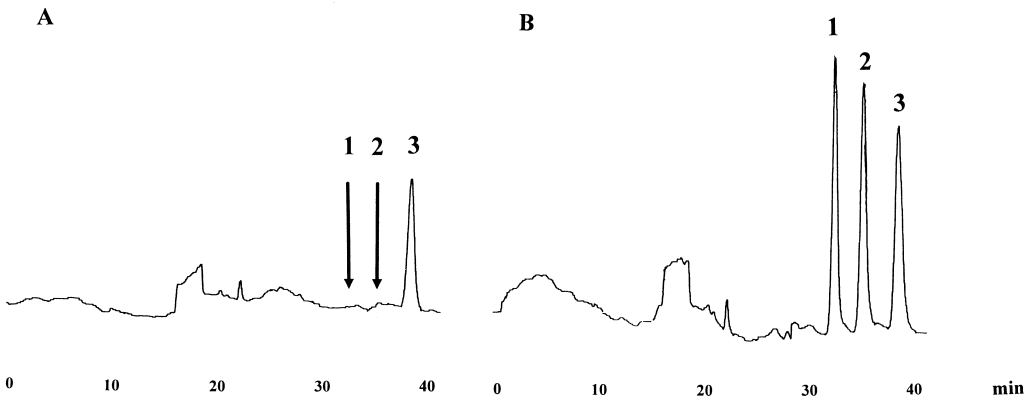

Fig. 2. Chromatograms obtained with a blank urine (A) and a urine sample spiked with 750 ng/ml of chlorpheniramine (CPAM) (B), where: 1, (−)-CPAM; 2, (+)-CPAM; 3, (+)-brompheniramine as internal standard.

DCPM from the achiral column with a similar mobile phase and with reasonable retention times. This is why we used different mobile phases for the analysis of CPAM enantiomers and DCPM enantiomers.

3.1. Chromatograms

The chromatograms obtained after extraction of a blank urine and a urine sample spiked with CPAM and DCPM are presented in Fig. 2 for CPAM and in Fig. 3 for DCPM.

Optical rotation of CPAM was identified by injection of its isolated (+)-enantiomer. Optical rotation of the enantiomers of DCPM was not known, and the two enantiomers were identified according to the order of elution on the amylose AD column (DCPM1 for the first eluted enantiomer and DCPM2 for the second).

3.2. Validation

Standard curves for CPAM and DCPM enantiomers were linear over the range investigated. The

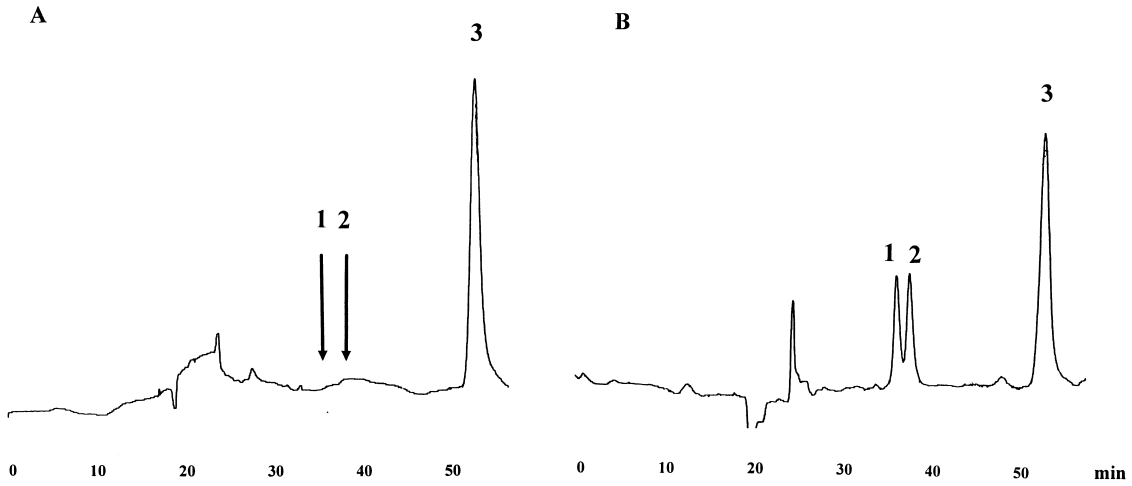

Fig. 3. Chromatograms obtained with a blank urine (A), a urine sample spiked with 250 ng/ml of desmethylchlorpheniramine (DCPM) (B), where: 1, DCPM1; 2, DCPM2; 3, amodiaquine as internal standard.

Table 1
Analytical parameters determined by linear least-squares regression in urine samples spiked with racemic CPAM and DCPM in the range of 0 to 1000 ng/ml

	Correlation coefficients	Regression coefficient	Intercept
(+)-CPAM	0.997	252.82	9.69
(−)-CPAM	0.998	248.43	0.45
DCPM1	0.994	500.73	0.22
DCPM2	0.995	446.99	3.90

correlation coefficients (r), regression coefficients and intercepts for the four compounds are presented in Table 1.

Limit of quantitation, within-day and between-day reproducibility and precision are given in Table 2. Accuracy values were 6.7, 7.9, 14.8, and 19.8% for (+)-CPAM, (−)-CPAM, DCPM1 and DCPM2, respectively. Recovery values (±C.V.) were 51.7 (±15.2)%, 53.2 (±18.1)%, 62.7 (±20.3)%, 65.2 (±22.7)% at the concentration 50 ng/ml and 66.7 (±16.3)%, 67.6 (±12.9)%, 81.1 (±15.2)%, 82.2 (±17.7)% at the concentration 375 ng/ml for (+)-CPAM, (−)-CPAM, DCPM1 and DCPM2, respectively.

3.3. Column performance

A single amylose AD column was used to validate the method and to analyse a large number of urine samples. The use of a coupled achiral–chiral system avoided direct injections of interfering compounds from the biological matrix and preserved the chiral stationary phase from rapid degradation.

After more than 1000 injections of extracted urine, the achiral cyanopropyl stationary phase showed deterioration that modified the retention times of the analyte and internal standard. After validation and injection of several urine samples, the achiral column had to be changed and the stability of the retention times and the switching system was totally restored.

3.4. In vivo application

After administration of 8 mg of racemic CPAM, concentrations of CPAM and DCPM enantiomers were determined in urine by using the present validated method. Concentrations of CPAM enantiomers and DCPM enantiomers are presented in Fig. 4. Slight differences were observed for DCPM enantiomers but not for CPAM enantiomers.

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Table 2
Limit of quantitation, within-assay precision and between-assay precision for CPAM enantiomers and DCPM enantiomers

Compound	Limit of quantitation (C.V.%)	Concentration (ng/ml)	Within-assay precision (%) (n=5)	Between-assay precision (%) (3 days)
(−)-CPAM	35	100	8.7	16.0
	(17.6)	750	12.2	15.9
(+)-CPAM	35	100	6.7	17.6
	(16.4)	750	8.5	15.8
DCPM1	35	100	9.9	12.9
	(12.9)	750	2.8	9.2
DCPM2	35	100	6.5	11.9
	(11.9)	750	3.2	9.9

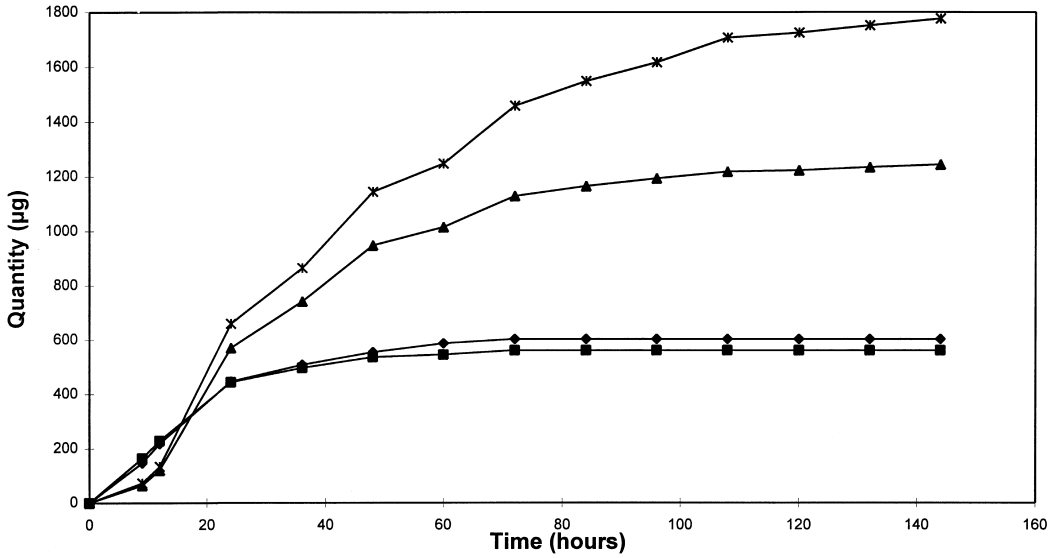

Fig. 4. Cumulative excreted amounts of chlorpheniramine enantiomers [(+)-CPAM and (−)-CPAM] and of desmethyl chlorpheniramine enantiomers [DCPM1 and DCPM2] after oral administration of 8 mg of racemic chlorpheniramine to a healthy volunteer, where: ♦, (+)-CPAM; ■, (−)-CPAM; ▲, DCPM1; and *, DCPM2

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