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Note

Gas chromatographic method for the determination of cetirizine in plasma

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Cetirizine (CTZ), [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]-ethoxy]acetic acid, is a new antihistaminic drug [1–3], currently marketed under the trade name Zyrtec®. It is indicated for the treatment of perennial and seasonal allergic rhinitis as well as chronic urticaria. Standard therapy is 10 mg once a day. In order to perform pharmacokinetic studies in humans and to monitor plasma levels during clinical trials, the feasibility of an assay designed to determine CTZ in plasma following therapeutic doses was examined. Since the molecular structure includes a carboxylic group, as shown in Fig. 1 (structure 1), and because of the molecular nitrogen content, CTZ was analysed by a gas chromatography (GC) method involving esterification and nitrogen–phosphorus flame-ionization detection. This paper describes the development and the validation of this method. The assay was used to measure plasma levels in healthy volunteers who received single daily doses of 10 mg of CTZ dihydrochloride.

EXPERIMENTAL

Reagents, solvents and materials

CTZ dihydrochloride and ucb 26294, the internal standard (I.S.) (Fig. 1, structure 2), were from UCB (Brussels, Belgium). Chloroform (analytical reagent, AR) and *n*-propanol (AR) were purchased from Merck (Darmstadt, F.R.G.); toluene (spectrophotometric grade) was from Janssen Chimica (Beerse, Belgium); sulphuric acid (AR) was from UCB.

Glass extraction tubes (20 ml) and Reacti-vials (5 ml) were obtained from Oedenkoven (Brussels, Belgium). The column for chromatography was an Ul-

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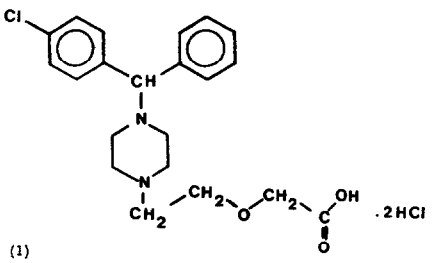

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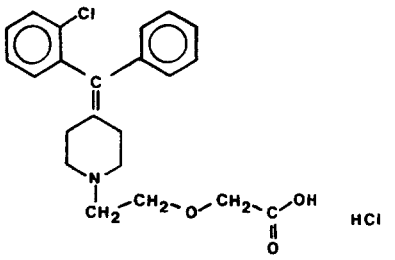

(2)

Fig. 1. Structures of cetirizine (1) and I.S. (2).

traperformance cross-linked methylsilicone (0.17 μm film) fused-silica column, purchased from Hewlett-Packard (Brussels, Belgium).

Aqueous solutions were prepared in reagent-grade water obtained from a Milli-Q^{TM} system (Millipore, Brussels, Belgium).

Apparatus

The apparatus used to measure CTZ concentrations was a Hewlett-Packard 5880A gas chromatograph fitted with a nitrogen–phosphorus flame-ionization detector and with a split–splitless injector operating in the split mode (split ratio 10:1). The detector signal was recorded and integrated on a level 4 integrator, part of the 5880A Hewlett-Packard gas chromatograph. The concentrations of CTZ in test samples were calculated using the peak-height ratio technique, and determined relative to a calibration curve.

Chromatographic conditions

The 25 m $\times$ 0.31 mm I.D. fused-silica column was coated with a 0.17-μm film of methylsilicone stationary phase. The detector temperature was set at 300 °C, the injector at 285 °C and the oven at 260 °C. The carrier gas used was oxygen-free helium at a velocity of 35 cm/s, and the detector make-up helium flow-rate was 20 ml/min. Peak heights were recorded on a 5880 Hewlett-Packard integrator.

Standard solutions

A stock solution of I.S. (10 mg) was prepared in water (10 ml), and the internal working standard was prepared in water at a concentration of 10 μg/ml (this working standard provided a concentration of 500 ng/ml of plasma). A stock

solution of CTZ dihydrochloride (10 mg) was prepared in water (10 ml), and the working standards were prepared in water at concentrations of 0.1, 0.5, 1, 2, 4, 6 and 10 μg/ml (these working standards were used to provide concentrations of 10 to 1000 ng/ml of plasma).

Extraction procedure

To the plasma (1 ml) were added the internal working standard (50 μl), citrate buffer (1 *M*, pH 5, 1 ml) and chloroform (10 ml). The solution was shaken for 10 min on a mechanical shaker. The phases were separated by centrifugation, and the organic phase was transferred to a screw-capped Reacti-vial (5 ml) and evaporated at 40°C to dryness under a gentle stream of oxygen-free nitrogen.

Derivatization procedure

To the dry residue from the chloroform extract, toluene (900 μl), concentrated sulphuric acid (2 μl) and *n*-propanol (100 μl) were added. The vials were sealed and incubated in an oven for 60 min at 100°C. After cooling, glycine–sodium chloride–sodium hydroxide buffer (0.3 *M*, pH 10, 1.5 ml) was added, and the vial contents were mixed on a Vortex mixer and centrifuged to separate the phases. Portions of the upper layer (3 μl) were injected into the gas chromatograph.

Calibration curves

Standard calibration curves for the measurement of CTZ were constructed by the analysis of extracted and derivatized samples of blank plasma to which the working standards of CTZ (100 μl) and the internal working standard (50 μl) had been added. Calibration standards (at least six samples covering the concentration range) were prepared with each batch of test samples and were used to construct daily calibration lines using least-squares regression analysis of peak-height ratio against concentrations of CTZ dihydrochloride.

The validity of the calibration line was checked daily by the analysis of quality control samples, spiked with known concentrations of CTZ dihydrochloride (A = 40 ng/ml, B = 100 ng/ml and C = 400 ng/ml). At least two of these samples were included with each batch of test samples and relative standard deviations were calculated.

Extraction recoveries

The extraction recoveries of the I.S. were determined from plasma by adding the I.S. (100 μl) to samples of blank plasma, which were then extracted. CTZ was added to the extracts, which were then derivatized and injected into the gas chromatograph. The peak-height ratios were compared with those obtained from derivatized non-extracted standard solutions at the same concentration.

The extraction recoveries of CTZ were determined from plasma by adding CTZ working standard (1 and 10 μg/ml, 100 μl) to samples of blank plasma, which were then extracted. I.S. was added to the extracts, which were then derivatized and injected into the gas chromatograph. The peak-height ratios were compared with those obtained from derivatized non-extracted standard solutions at the same concentration.

$$\text{Recovery} = \frac{\text{peak-height ratio in extract}}{\text{mean peak-height ratio of solution}} \times 100\%.$$

Reproducibility

The intra-day reproducibility of the assay was assessed from the coefficients of variation (C.V.) of the means of daily recoveries obtained from the quality control samples. The inter-day reproducibility of the assay was assessed from the C.V. of the means of six replicate analyses of the same three quality control samples determined within six working days.

Test samples

Plasma samples were collected during a pharmacokinetic study in sixteen healthy volunteers after a single 10-mg oral dose of CTZ dihydrochloride and after repeated oral administration for ten days at 10 mg daily. Blood samples were collected before dosing and again 10, 20, 40, 60 and 90 min and 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h after dosing. Plasma samples were stored at −20°C until taken for analysis.

RESULTS AND DISCUSSION

The chromatogram resulting from the injection of a derivatized extract from a blank plasma sample obtained from a volunteer not exposed to CTZ is shown in Fig. 2A. The retention times of CTZ and I.S. were 8.50 and 7.80 min, respectively. Fig. 2B shows a chromatogram resulting from the injection of a derivatized extract obtained from a plasma sample collected 2 h after dosing from a volunteer given a single 10-mg oral dose of CTZ dihydrochloride. Comparison of Fig. 2A and 2B illustrates the absence of interference from endogenous compounds in plasma for the CTZ peak. Moreover, there was no interference at the retention time of the I.S. The least-squares regression equations of calibration curves, as well the coefficients of correlation (r) obtained on nineteen consecutive working days, together with results from quality control samples, are presented in Table I. Coefficients of correlation were always higher than 0.992, indicating good linearity over the range of concentrations examined. Mean daily relative deviations for quality control samples were less than ±4%. Aliquot fractions of the three quality control samples were stored in the same conditions as test samples (deep-freezer, −20°C) over a period of nineteen working days. Freshly prepared calibration curves were used on each working day. Similar results were obtained for the quality control samples over the whole period of analysis, indicating that CTZ in these conditions was stable, at least over a period of one calendar month corresponding to nineteen working days.

The limit of detection was 20 ng/ml; this was sufficient to measure drug levels following therapeutic doses of CTZ.

The extraction recovery of CTZ determined from plasma was 89% for the two tested concentrations. Statistical analysis by one-way analysis of variance demonstrated that there was no significant difference ($P > 0.05$) among the mean

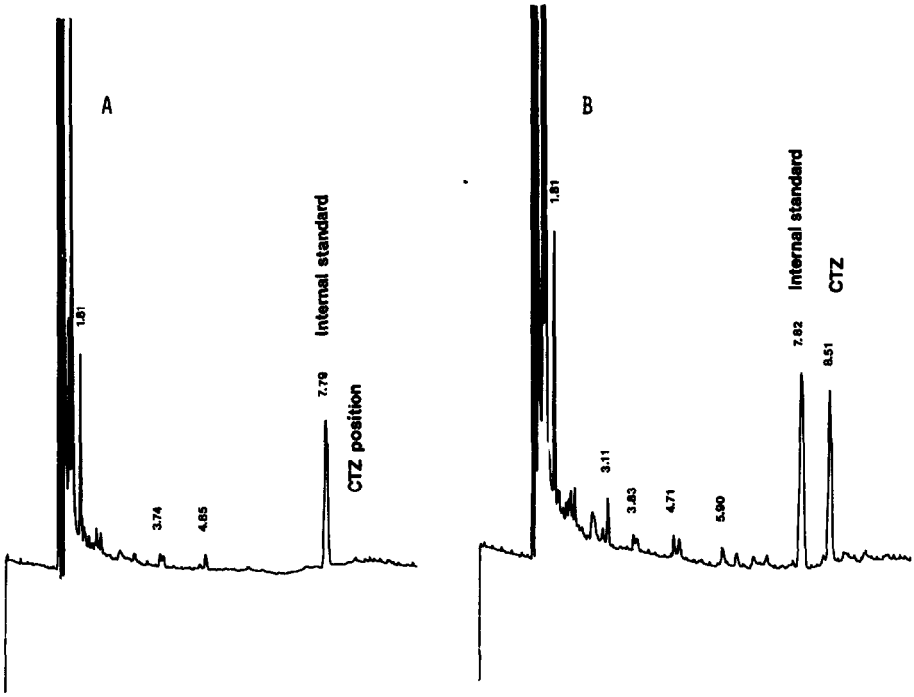

Fig. 2. Chromatograms of (A) plasma extract from a volunteer not dosed with CTZ, spiked with 500 ng/ml I.S.; (B) plasma extract from a volunteer given a single 10-mg oral dose of CTZ, spiked with 500 ng/ml I.S., collected 2 h after dosing.

recoveries of CTZ. Therefore, these data suggest that extraction efficiencies were concentration-independent over the range of drug concentrations (100–1000 ng/ml) investigated. Extraction recovery of I.S. was calculated to be 74%. Since concentrations of CTZ were calculated from calibration lines of extracted CTZ, the results were automatically corrected for systematic losses during analysis.

The intra-day variability of the assay for each of six consecutive days, as well as the inter-day variability over the same six days, are presented for quality control samples A, B and C in Table II. The C.V. values for both inter- and intra-day variability were all less than 3.1%, except the one measured on day 5 which was 6.1%.

The mean plasma levels in sixteen volunteers after a single dose and on the tenth day of a chronic daily treatment of CTZ dihydrochoride are shown in Fig. 3. After the single dose, the plasma concentrations were above the limit of detection in twelve out of sixteen volunteers after 10 min, and in all the volunteers between 20 min and 24 h. The plasma concentrations were in all cases above the limit of detection from the first sampling time up to 24 h after medication on the tenth day of treatment. At 36 h after medication, concentrations over 20 ng/ml could only be measured in three and ten volunteers after the single dose and after a ten-day treatment, respectively. These data indicate that the assay is suitable for measuring concentrations after therapeutic doses of the drug.

Means ($\pm$S.D.) of peak concentrations of 318 ± 44 and 362 ± 41 ng/ml oc-

TABLE I

REGRESSION EQUATIONS AND r VALUES FOR CALIBRATION CURVES AND DAILY QUALITY CONTROL DATA

Working day	Regression equation	r	Mean daily quality control data (% deviation)
1	$0.00342x+0.03734$	0.9987	−3.9
2	$0.00331x+0.02078$	0.9990	+1.3
3	$0.00324x+0.04638$	0.9970	−0.1
4	$0.00353x+0.03910$	0.9967	−2.9
5	$0.00311x+0.07396$	0.9968	+0.4
6	$0.00355x+0.06288$	0.9964	−0.4
7	$0.00329x+0.06676$	0.9969	+0.3
8	$0.00254x-0.01351$	0.9993	+0.5
9	$0.00340x-0.01633$	0.9987	+3.8
10	$0.00308x-0.01637$	0.9975	+2.8
11	$0.00285x+0.02780$	0.9923	+1.9
12	$0.00244x-0.00738$	0.9969	−0.5
13	$0.00271x+0.00097$	0.9936	+1.5
14	$0.00226x+0.03439$	0.9984	−0.5
15	$0.00302x+0.02976$	0.9980	+0.1
16	$0.00281x+0.00332$	0.9925	−1.3
17	$0.00299x+0.04243$	0.9963	+1.1
18	$0.00303x-0.00003$	0.9995	+0.1
19	$0.00304x+0.00079$	0.9998	−1.2
Mean	$0.00303x+0.02279$		
S.D.	$0.00036x$ 0.02881		

TABLE II

REPRODUCIBILITY DATA OF CETIRIZINE ASSAYS MEASURED IN PLASMA

Day/concentration	Recovery*; (mean ± S.D.) (%)	Deviation (mean ± S.D.) (%)	C.V. (%)
Intra-assay variation (n=3)			
Day 1	99.5 ± 2.3	−0.5 ± 2.3	2.3
Day 2	100.1 ± 1.4	0.1 ± 1.4	1.4
Day 3	98.7 ± 1.5	−1.3 ± 1.5	1.5
Day 4	98.9 ± 1.7	−1.1 ± 1.7	1.7
Day 5	100.1 ± 6.1	0.1 ± 6.1	6.1
Day 6	98.8 ± 2.5	−1.2 ± 2.5	2.5
Inter-assay variation (n=6)			
40 ng/ml	100.2 ± 2.2	0.2 ± 2.2	2.2
100 ng/ml	99.5 ± 2.6	−0.5 ± 2.6	2.6
400 ng/ml	98.3 ± 3.1	−1.7 ± 3.1	3.1

* Adjusted recovery (concentrations were calculated from calibration lines of extracted cetirizine and were corrected for systematic losses of both cetirizine and internal standard during analysis).

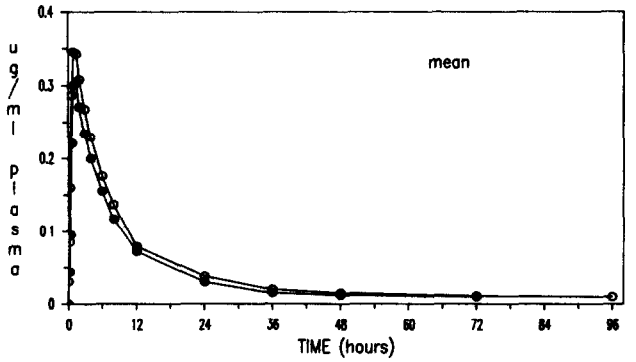

Fig. 3. Plasma kinetics of CTZ at day 1 (●) and day 13 (○), after a single oral daily dose of 10 mg of CTZ dihydrochloride.

curred, respectively, at mean (±S.D.) times of 1.19±0.25 and 1.14±0.27 h after a single dose and after ten days chronic administration. The mean (±S.D.) residence times of CTZ were 10.9±2.2 and 11.2±1.8 h, respectively, after a single dose and after chronic treatment. The apparent body clearance, assuming complete absorption, was a mean (±S.D.) of 0.980±0.151 ml/min/kg.

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