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Short Communication

Determination of cetirizine in human urine by high-performance liquid chromatography

M. T. ROSSEEL* and R. A. LEFEBVRE

Heymans Institute of Pharmacology, University of Ghent Medical School, De Pintelaan 185, B-9000 Ghent (Belgium)

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ABSTRACT

A high-performance liquid chromatographic method for the determination of the histamine H_1-receptor antagonist cetirizine in human urine was developed. Cetirizine and the internal standard are extracted from acidified (pH 5) urine (0.5 ml) into chloroform and the organic layer is evaporated to dryness. The residue is chromatographed on a Spherisorb 5ODS-2 column using Pic A (5 m*M* aqueous tetrabutylammonium phosphate)–methanol–tetrahydrofuran (33:65:2, v/v) as the mobile phase with ultraviolet detection (230 nm). The calibration graph is linear from 0.1 to 10 μg/ml and using 0.5 ml of urine the detection limit is 20 ng/ml. The within-run relative standard deviation is $<6\%$ and the accuracy is within 10% of the theoretical value at concentrations between 0.1 and 10 μg/ml in urine. There is a good correlation (r = 0.99606) with a previously described capillary gas chromatographic assay.

INTRODUCTION

Cetirizine is a non-sedating H_1-receptor antagonist [1] used in patients with urticaria and allergic rhinitis. After a single oral administration of 10 mg of [^{14}C]cetirizine dihydrochloride, 60% of the dose was recovered in the 24-h urine, mainly as the unchanged drug; one metabolite, formed by O-dealkylisation of the cetirizine side-chain, was detected in plasma and faeces but not in urine [2].

A capillary gas chromatographic (CGC) method for the determination of cetirizine in plasma has been published [3]; we have previously applied this method to determine the plasma and urinary concentrations of cetirizine [4]. We have now developed a rapid high-performance liquid chromatographic (HPLC) method with UV detection for this purpose. The reproducibility and accuracy of the HPLC method were compared with those of the CGC method on urine samples from healthy volunteers receiving 5 mg of cetirizine orally.

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EXPERIMENTAL

Materials

Cetirizine hydrochloride (UCB P071), the O-dealkylated cetirizine metabolite (UCB P026) and the internal standard (UCB J028) (Fig. 1) were kindly supplied by UCB (Brussels, Belgium). HPLC-grade methanol and tetrahydrofuran were from Carlo Erba (Milan, Italy).

Pic A (5 m*M* aqueous tetrabutylammonium phosphate) was obtained from Waters Assoc. (Milford, MA, U.S.A.). All other reagents were of analytical-reagent grade from Merck (Darmstadt, Germany). Water, doubly distilled in glass, was passed through a 0.45-μm filter (Type HA; Millipore, Bedford, MA, U.S.A.).

Stock solutions of cetirizine (5 mg/ml) and the internal standard (5 mg/ml) were prepared by dissolving accurately weighed amounts in 0.1 *M* hydrochloric acid. The solutions were stable for at least three months when stored at 4°C. Working standard solutions of cetirizine and a working solution of the internal standard were made by diluting the stock solutions with water.

Instrumentation and chromatography

The chromatographic system consisted of a Varian (Sunnyvale, CA, U.S.A.) Star 9010 solvent delivery system set at 1.0 ml/min. The mobile phase was Pic A–methanol–tetrahydrofuran (33:65:2, v/v). The analytical column was a Spherisorb 5ODS-2 (25 cm × 4.6 mm I.D.) from Chrompack (Antwerp, Belgium).

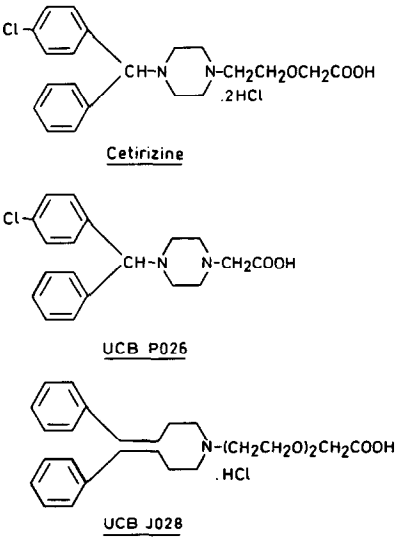

Fig. 1. Structures of cetirizine, the O-dealkylated cetirizine metabolite (UCB P026) and the internal standard (UCB J028).

Injections were made with a Model 7125 manual injector (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 10-μl loop. The eluent was monitored with a Perkin-Elmer (Norwalk, CT, U.S.A.) Model LC 235 diode-array detector set at 230 nm and connected to a Hewlett-Packard (Avondale, PA, U.S.A.) Model 3390 A integrator in the peak-height mode.

Extraction procedure

To a 10-ml glass-stoppered centrifuge tube containing 0.5 ml of urine, the internal standard (25 μl of 100 μg/ml standard solution) was added followed by 1 ml of citrate buffer (1 *M*, pH 5) and 6 ml of chloroform. After shaking horizontally for 10 min, the mixture was centrifuged at 3015 *g* for 10 min at 4°C. The organic layer was transferred into a 6-ml silanized conical glass tube and evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was dissolved in 100 μl of the mobile phase. An aliquot (10 μl) of this solution was injected into the column.

Comparison of the HPLC and CGC methods

Urine was collected from twelve healthy volunteers (six males, six females; age range 21–29 years) at 0–6, 6–12, 12–24 and 24–48 h after oral intake of 5 mg of cetirizine. A sample of 20 ml was stored at −20°C until analysis for the cetirizine concentration by the CGC method [3,4] and by the HPLC method.

Calibration graphs

Calibration standards were prepared by spiking drug-free urine with known amounts of cetirizine (0.1–10 μg/ml) and the internal standard. Calibration graphs were obtained by plotting the peak-height ratio (of cetirizine to the internal standard) (y) against the concentration (x) of cetirizine in urine and calculating the regression line by least-squares linear regression analysis. Unknown concentrations in urine samples were determined by comparison of peak-height ratios (of cetirizine to the internal standard) with those of the calibration standards. These standards were included with each batch of unknown samples.

RESULTS AND DISCUSSION

Chromatograms

Typical HPLC traces for 0.5 ml of pre-drug urine, 0.5 ml of pre-drug urine spiked with 0.1 μg/ml cetirizine and 0.5 ml of urine obtained from the same volunteer 12–24 h after administration of 5 mg of cetirizine are shown in Fig. 2.

The chromatographic conditions achieved full resolution over the whole concentration range of cetirizine (0.1–10 μg/ml). Cetirizine and the internal standard eluted at 6.17 and 5.53 min, respectively. Drug-free human urine showed no interference.

Injection of an aqueous sample of the O-dealkylated cetirizine metabolite

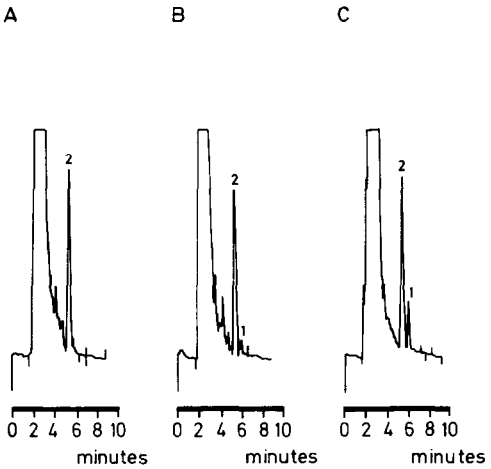

Fig. 2. HPLC of cetirizine in urine. (A) Pre-drug urine sample spiked with 5 μg/ml internal standard. (B) Pre-drug urine sample spiked with 0.1 μg/ml cetirizine and with 5 μg/ml internal standard. (C) Urine sample from the same subject collected over the 12–24 h period following oral administration of 5 mg of cetirizine and spiked with 5 μg/ml internal standard. The calculated cetirizine concentration was 1.15 μg/ml. Peaks: 1 = cetirizine; 2 = internal standard.

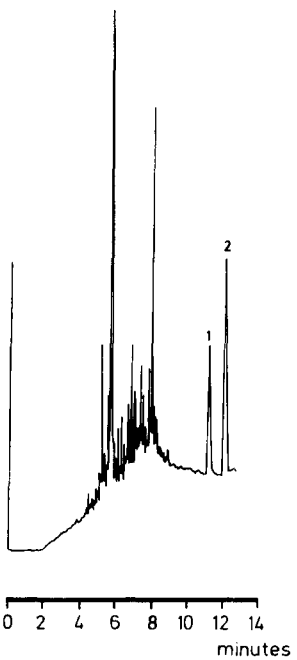

Fig. 3. Same sample as in Fig. 2C, but analysed by CGC and spiked with 5 μg/ml internal standard. The calculated cetirizine concentration was 1.20 μg/ml. Peaks: 1 = cetirizine; 2 = internal standard.

yielded a retention time of 5.75 min. The peak was not found in the urine samples.

A typical CGC trace for the same urine sample as in Fig. 2C is shown in Fig. 3. The gas chromatographic conditions were as described previously [4].

Extraction efficiency

By comparing the peak heights of cetirizine, the O-dealkylated citirizine metabolite and the internal standard in urine processed through the extraction procedure with those in aqueous solutions injected directly into the chromatographic system, it was ascertained that the mean ± S.D. (n = 6) absolute drug recovery from urine was 78.8 ± 2.9, 73.6 ± 3.4 and 94.5 ± 3.7%, respectively, for concentrations of 0.1–10 μg/ml.

Detections limit

At a signal-to-noise ratio of 3, the minimum detectable concentration of cetirizine in urine was 20 ng/ml under the conditions used.

Within-run and between-run precision and accuracy

The within-run precision and accuracy were evaluated from the analysis of five urine samples for each of five cetirizine concentrations (range 0.1–10 μg/ml). The within-run relative standard deviation (R.S.D.) was <6%. The within-run accuracy was within 10% of the theoretical value at each concentration (Table I). The between-run reproducibility and accuracy of the assay were demonstrated by an R.S.D. of 5.3% (n = 13) and a mean result of 1.89 μg/ml using pooled human urine spiked with cetirizine at 2 μg/ml and analysed over three weeks.

Calibration graphs

The calibration graphs for cetirizine showed a linear response over the range evaluated (0.1–10 μg/ml). Typical calibration graphs give a mean regression (n = 15) of $y = (0.01373 \pm 0.00638)x + (0.26514 \pm 0.00738)$, $r = 0.99927 \pm 0.00033$, where y = peak-height ratio, x = concentration of cetirizine and r = correlation coefficient.

TABLE I

WITHIN-RUN PRECISION AND ACCURACY FOR THE DETERMINATION OF CETIRIZINE IN URINE (n = 5)

Concentration added (μg/ml)	Precision (R.S.D., %)	Accuracy (%)
0.1	5.3	109.0
0.2	3.5	105.4
1.0	3.5	99.2
5.0	3.6	102.2
10.0	3.1	98.9

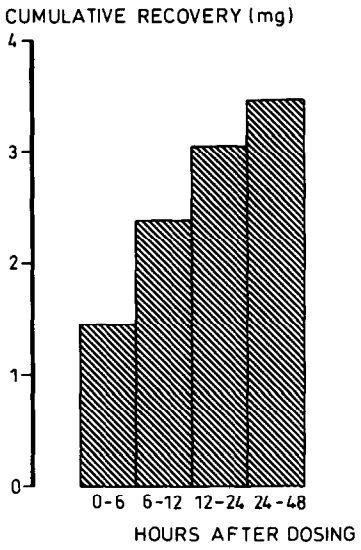

Fig. 4. Recovery of cetirizine from urine from a volunteer after oral intake of 5 mg of cetirizine.

Application

As an example, the cumulative urinary recovery observed in a healthy subject after oral administration of 5 mg of cetirizine is shown in Fig. 4.

Comparison of the HPLC and CGC methods

Urine samples (n = 48) obtained from twelve healthy subjects who participated in a pharmacokinetic study and received an oral dose of 5 mg cetirizine were split and determined by HPLC and CGC. There was a good correlation of the results from the two methods (r = 0.99606) over the concentration range obtained (0.12–7.10 μg/ml).

CONCLUSION

The proposed HPLC technique is reproducible and accurate. It requires less technical expertise and is less time-consuming than the CGC method, as in the latter method cetirizine and the internal standard have to be derivatized after extraction and the CGC analysis time is longer.

ACKNOWLEDGEMENT

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