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# Short Communication

# Determination of cetirizine in serum using reversed-phase high-performance liquid chromatography with ultraviolet spectrophotometric detection

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ABSTRACT

A method using reversed-phase high-performance liquid chromatography with ultraviolet detection for the determination of cetirizine in serum is described. The method is sensitive down to 50 ng/ml (250-$\mu$l loop). Sample preparation involves only serum deproteination with perchloric acid and injection of the centrifuged supernatant. Elution is at pH 2.5 with acetonitrile–methanol–0.05 *M* phosphate buffer (33:9:58, v/v) on a 25 cm × 4.6 mm I.D. Spherisorb S5 ODS2 column. Detection is at 211 nm, its $\lambda_{max}$. For levels above 300 ng/ml the serum sample size is 100 $\mu$l and a 200-$\mu$l sample is necessary for concentrations less than 300 ng/ml. At the 2 $\mu$g/ml concentration the intra-assay relative standard deviation is better than 2.2%, whilst the inter-assay deviation is 2.6% over eight samples. At 200 ng/ml the intra-assay relative standard deviation is 6% over seven samples. Detector response is linear from 100 ng/ml to 10 $\mu$g/ml (100-$\mu$l loop).

## INTRODUCTION

Ceterizine (CTZ), [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid (Fig. 1), is a piperizine derivative and carboxylated metabolite of hydroxyzine. It is a potent antihistaminic drug with marked affinity for peripheral $H_1$-receptors [1]. Results from controlled clinical trials indicate that CTZ is an effective and well tolerated drug for seasonal and perennial allergic rhinitis and chronic idiopathic urticaria [2]. The standard dose of 10 mg daily appears to be as effective as the larger doses necessary with chlor-

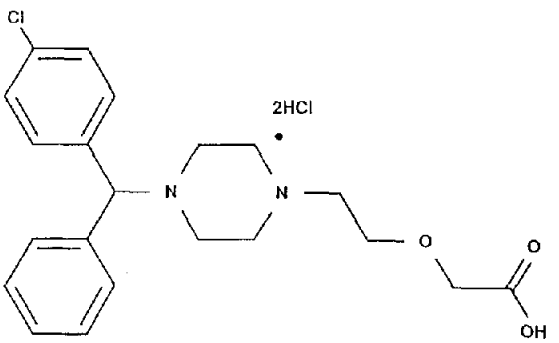


Fig. 1. Structure of cetirizine.

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pheniramine, terfenadine and hydroxyzine treatment and is less sedative in action.

To determine the pharmacokinetic parameters of CTZ in individuals, a rapid, reliable and readily available technique for its determination in body fluids is necessary. A gas chromatographic method, with a detection limit of 20 ng/ml, has been used to measure CTZ in plasma [3]. Whilst high-performance liquid chromatography (HPLC) is reported as the method used for the determination of [$^{14}$C]CTZ down to 10 ng/ml in plasma and 100 ng/ml in urine [4], and of non-radiolabelled CTZ at the same sensitivity [5], neither the chromatographic conditions nor the conditions of detection are reported in the former [4], whilst the latter [5] records only that a Brownlee $C_8$ column and UV–VIS detection at 230 nm were used. No further details on the methods used by these authors could be found in the literature.

Rosseel and Lefebvre [6] have recently reported the use of reversed-phase HPLC with UV detection at 230 nm for the determination of CTZ in the residue from 500 μl of urine after acidification and extraction into chloroform. The limit of detection was 20 ng/ml. Details of a thin-layer chromatographic method for determining the purity of aqueous [$^{14}$C]CTZ solutions and percentage metabolized drug in the urine have also been reported [5].

It was thus decided that an HPLC method which required less sample and a more rapid sample clean-up should be developed for the determination of CTZ in serum. Because of the moderate polarity of CTZ, an ODS column and UV detection were used for the investigation which is reported herein.

## EXPERIMENTAL

### *Reagents*

All solvents used were spectroscopic grade from Burdick and Jackson (Muskegon, MI, USA) and all water was purified by a Milli-Q system (Millipore, Milford, MA, USA). The CTZ standard was donated by U.C.B. (Basel, Switzerland). All other reagents were analytical-reagent grade. The elution buffer was 0.05 *M* ammonium phosphate, pH 2.5 containing 500 μl/l triethylamine and was prepared using 0.05 *M* ammonium dihydrogenphosphate, adding the triethylamine and adjusting to pH 2.5 with 85% (m/v) orthophosphoric acid.

### *Sample collection*

Human blood samples were collected in plain Vacu test tubes from the antecubital vein of medical student volunteers who were dosed orally with 20 mg of CTZ daily for seven days for steady-state levels and to ensure optimal receptor blockade prior to antigen challenge. After 15 min when clotting was complete, each sample was centrifuged and the serum transferred to a clean glass vial. All samples were stored at −18°C until assayed, within two weeks after collection.

### *Sample preparation*

A 100-μl serum volume was added to 50 μl of elution buffer in a borosilicate centrifuge tube, and the protein was precipitated with 50 μl of 25% perchloric acid, vortex-mixed for 10 s and centrifuged at 2000 *g* for 3 min. The clear supernatant was injected into the chromatograph through a 100-μl loop injector valve. For low-level samples a 250-μl loop was used and the sample size was increased to 200 μl of serum proportionately.

### *Standards preparation*

Standard solutions in elution buffer were prepared in the range 200 ng/ml to 10 μg/ml to give final serum concentrations of 100 ng/ml to 5 μg/ml. A 100-μl drug-free serum sample was added to 50 μl of standard solution in a centrifuge tube and gently shaken. Then 50 μl of 25% perchloric acid were added, the contents vortex-mixed for 10 s then centrifuged at 2000 *g* for 3 min and the supernatant was injected. A separate calibration curve from 50 ng/ml to 400 ng/ml was constructed for levels lower than 300 ng/ml, using 200 μl of serum instead of 100 μl. Standard solutions were stored at −18°C for up to five days.

*Chromatography*

HPLC was performed on a Spectra Physics 8100 liquid chromatograph with a Valco autoinjector valve fitted with a 100-$\mu$l loop. Separation was achieved on a 250 mm × 4.6 mm I.D. Spherisorb S5 ODS2 reversed-phase column preceded by a 10 mm × 4.6 mm I.D. laboratory-made guard column packed with Shandon Hyperspheres 5-$\mu$m ODS. Isocratic elution with acetonitrile–methanol–0.05 *M* ammonium phosphate elution buffer pH 2.5 (33:9:58, v/v) at 2 ml/min was performed at a column temperature of 35°C. The retention time for CTZ was 6.4 min. The dealkylated metabolite of CTZ eluted at 9.0 min.

*Detection*

A Spectra Physics SP8400 UV–VIS detector was used to detect CTZ at 211 nm, its $\lambda_{max}$ in mobile phase. For method development purposes the output was recorded simultaneously on a Perkin-Elmer 56 strip-chart recorder and a Spectra Physics SP4200 integrator. The concentrations of CTZ in serum were estimated on the basis of peak height from the calibration curve for the same detector.

## RESULTS AND DISCUSSION

Using the above method, good separation and detectability of CTZ in serum was obtained with minimal interference from serum components and metabolites (Fig. 2). The UV–VIS absorption spectrum for CTZ in mobile phase showed an absorption maximum at 211 nm and so this was selected as the detection wavelength. Detection was linear from 100 ng/ml to 10 $\mu$g/ml on the basis of peak height. The intra-assay relative standard deviation was 2.2% at 2 $\mu$g/ml and the inter-assay relative standard deviation was 2.5% at the same level using a 100-$\mu$l loop. Using a 250-$\mu$l loop the intra- and inter-assay relative standard deviations at 200 ng/ml were 6 and 9.5%, respectively. The detection limit was 20 ng/ml and the accuracy was better than 90%.

Whilst the sensitivity is less than that achieved using gas chromatography or using much larger samples and extensive extraction, the method is nonetheless sufficiently sensitive to follow the blood levels (100 ng/ml to 20 $\mu$g/ml) found for several hours after therapeutic oral and parenteral dosing and so provides a more rapid determination, requiring less sample than presently reported methods.

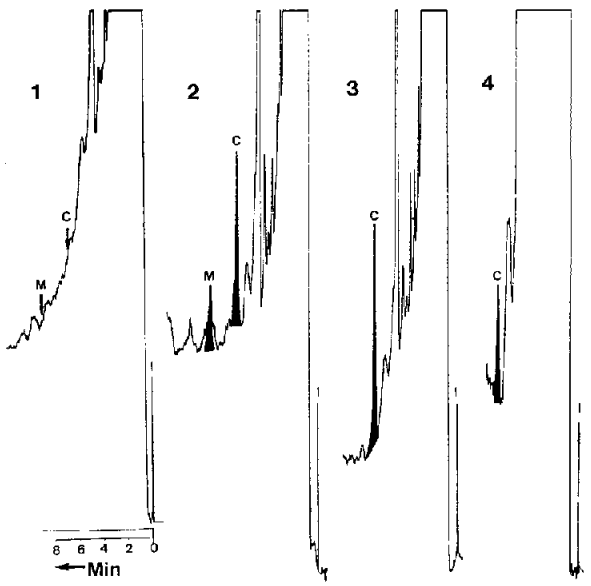


Fig. 2. Chromatograms of serum and standard samples. (1) Blank serum (100-$\mu$l loop, 0.01 a.u.f.s.); (2) 790 ng/ml steady-state serum after continuous dosing with 20 mg of cetirizine daily (100-$\mu$l loop, 0.01 a.u.f.s.); (3) blank serum spiked with 2 $\mu$g/ml cetirizine (100-$\mu$l loop, 0.02 a.u.f.s.); (4) blank serum spiked with 250 ng/ml cetirizine (250-$\mu$l loop, 0.01 a.u.f.s.). Peaks: C = cetirizine; I = inject; M = cetirizine metabolite.

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