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RP-LC method for the determination of cetirizine in serum

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Abstract

The development and evaluation of HPLC method for quantifying cetirizine in human serum is described. The method involves liquid phase extraction of cetirizine in methylene chloride, adding diazepam as an internal standard, followed by separation on a reversed phase C_{18} Novapak column (150 × 3.9 nm; 4 μm), and employing a UV-detection set at 230 nm at ambient temperature. The mobile phase consists of a 13 mM phosphoric acid solution and acetonitrile (61:39 v/v) adjusted to pH 2.8 with 5 M NaOH. The assay is linear from 10 to 500 ng ml^{-1} with a detection limit of 5 ng ml^{-1} and a mean recovery of 96.5%. The applicability of this method in pharmacokinetic studies is evaluated.

Keywords: Cetirizine; Pharmacokinetics; Human serum; RP-HPLC

1. Introduction

Cetirizine, Zitek, or Zyrtec [1], Fig. 1A, are the approved name for a synthetic piperazine known as [2-[4[(*p*-chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy acetic acid dihydrochloride, with the formula of $C_{21}H_{25}ClN_2O_3.2HCl$ [1–3].

Cetirizine is a potent and well tolerated nonsedating antihistamine drug for the treatment of seasonal and prennial allergic rhinites and chronic urticaria [1–5].

Pharmacokinetic studies require a reliable chromatographic method for quantitation of cetirizine in serum. This requires a low detection limit (less than 10 ng ml^{-1}) to enable accurate and precise measurements of cetirizine in serum that were collected at the very early and late stages of the study. It should also be rapid (less than 4 min) so that the great number of serum samples can be examined in reasonable time. Moreover, it would have simple extraction procedure of cetirizine in serum, be performed at room temperature and based on readily available chemicals and conventional instruments. In this context, several methods of cetirizine analysis are reported in the literature. Including GC-NPD method with a detection limit of 20 ng ml^{-1} and a retention time of 8.5 min [1]. The time consuming derivetization and incubation in the sample preparation step and

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the longer retention time would make it less attractive for pharmacokinetic studies. A liquid chromatographic method with a detection limit of 50 ng ml^{-1} and a retention time of 6.4 min was also developed [3]. The method was based on precipitation of plasma with 25% prechloric acid solution, centrifuging for 3 min, injecting the clear supernatant into the chromatograph, and keeping the column temperature at 35°C. Despite the fact that the method has the advantage of simple extraction procedure. However, it lacked the sensitivity required for pharmaco-kinetic studies. Moreover, it did not employ an internal standard Adjust to pH 12 by addition of 1 M NaOH and was not performed at room temperature. Furthermore, it was recommended for samples containing more than 100 ng ml^{-1}. Other HPLC methods for analysis of cetirizine in bulk samples, pharmaceutical dosage forms and urine have also been reported [6,8]. TLC and radiolabeled methods have been reported as well [5,7,9], but they are not suitable for routine clinical tests.

In the present paper, we describe a reliable method for quantifying nanograms of cetirizine in serum. The method is based on liquid extraction of cetirizine in methylene chloride. The chromatographic conditions were optimized and the results of the validation in terms of linearity, accuracy, precision, recovery, detection, quantitation limits, and specificity are provided. The applicability of this method in pharmacokinetic studies is evaluated.

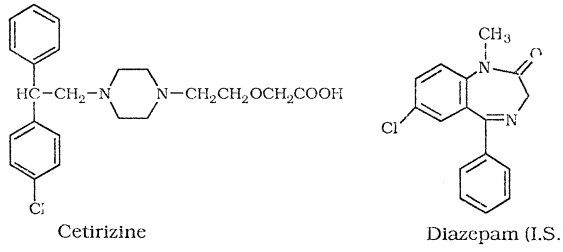

Fig. 1. Structural formula of cetirizine and diazepam.

2. Experimental

2.1. Materials

Cetirizine dihydrochloride and diazepam, internal standard and drugs used for specificity studies were obtained from Arab Pharmaceutical Manufacturing (APM, Jordan). All solvents and other reagents were purchased from Merck (Darmstadt, Germany) and water was double distilled. All solvents were HPLC grade, other chemicals and reagents were of analytical grade and used without further purification. Serum used for preparation of calibration standards was obtained by drawing blood from fasting healthy volunteers. The blood was allowed to clot at room temperature for 20 min before centrifugation. Serum was isolated and stored in clean-labeled vials at − 20°C.

2.2. Instrumentation

A VISTA 5500 HPLC system (Varian, CA, USA) was employed, it is consisted of a single piston, reciprocating pump driven by a stepper motor, a Valco injector equipped with a 100-μl loop and a UV-200 variable detector set at 230 nm connected to a 4290 Varian integrator. Separations were performed on a C_{18} analytical column (150 × 3.9 mm), 4 μm stationary phase (Novapak, Waters, USA). Analytical runs were processed by the Autolab Software Winner 386 system (Spectra Physics).

2.3. Dosing and sample collection

A single oral dose of a drug containing 10 mg cetirizine was administered to four healthy volunteers after fasting. Blood samples were drawn at 0.0 (pre dose), and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10, 12, 16, 20 h (post dose). Blood (10 ml) was collected by a syringe into a 15-ml centrifugating tube and allowed to clot at room temperature for 20 min before centrifugation. Serum from each sample was removed to clean-labeled vial and stored at − 20°C until assayed.

2.4. Preparation of standards

Stock solution of cetirizine or the internal standard was prepared by accurately weighing 10 mg of the compound into 5 ml distilled water and diluting to volume in a 10 ml volumetric flask to give a concentration of 1000 μg ml^{-1} cetirizine or internal standard. The cetirizine standard was sequentially diluted with distilled water to yield 0.2, 0.6, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 10 μg ml^{-1} working standard solutions for preparation of calibration curves. The internal standard stock solution was diluted with water to yield 0.6 μg ml^{-1} internal standard working solution. The stock solutions were stored at −20°C and the working standards were prepared freshly daily and stored at 4°C.

Serum standards were prepared by aliquoting 50 μl of each cetirizine working standard solution and 25 μl of internal standard working solution to 1.0 ml blank serum (drug free) in 10 ml glass tubes. The final standard concentrations for the calibration curve were 10, 30, 50, 75, 100, 150, 200, 300 and 500 ng ml^{-1} and the concentration of internal standard was 150 ng ml^{-1}. Assay validation samples were prepared by aliquoting cetirizine standards to produce concentration pools of 50, 300 and 500 ng ml^{-1}. These samples were stored at −20°C. Pharmacokinetic or assay validation samples were prepared by adding 1-ml of serum sample, 50 μl of water and 25 μl of internal standard working solution into glass tubes.

2.5. Sample preparation

The serum standards, assay validation samples or pharmacokinetic study samples were vortexed for 20 s followed by addition of 6.0 ml methylene chloride. The contents were vortexed again for one min, and the phases were separated by centrifugation for 5.0 min at 3200 rpm. The aqueous layer in each tube was discarded and the organic portion was transferred to a 10 ml tube and evaporated almost to dryness under nitrogen at 50°C. The residue in each tube was reconstituted in a 100 μl of the mobile phase and vortexed for 30 s before being transferred to a disposable polyethylene microcentrifuge tube (1.5 ml, Eppendrof), the latter was centrifuged for 30 s at 11 500 rpm. An appropriate fixed amount (50 μl) was finally injected directly into the HPLC system.

2.6. Chromatographic conditions

The separation was performed using an acetonitrile–water (39:61 v/v) mobile phase containing a 13 mM phosphate buffer. The pH was adjusted to pH 2.8 with 5 M NaOH. This phase was filtered through a 0.45-μm membrane and degassed by bubbling helium through. All analysis were performed at room temperature.

2.7. Assay validation

Linearity of the assay was demonstrated by running serum standards in triplicate at nine concentrations over the range 10–500 ng ml^{-1} for five consecutive days. Peak height ratios (cetirizine/internal standard) were plotted against cetirizine concentrations and analyzed using least square linear regression. Precision and accuracy were assessed in conjunction with the linearity studies using three spiked serum samples at each of three concentrations (nominally, 50, 300, 500 ng ml^{-1}). Measured concentrations were determined by application of the appropriate standard curve obtained on each occasion. Precision was assessed in terms of coefficient of variation.

Accuracy was determined from the bias calculations. Recovery of cetirizine from serum was assessed using spiked drug-free serum at three different concentrations, and was estimated by comparing the mean peak ratios of the extracted spiked serum to the mean peak height ratios of equivalent aqueous standard solutions. The internal standard was not extracted.

Specificity in relation to endogenous components was demonstrated by analysis of a series of randomly selected drug-free serum samples. In addition, commonly used drugs such as amoxycilline, caffiene and effidrene were also investigated for possible interference due to co-elution. The stability of cetirizine under conditions of storage and handling relevant to conduct of pharmacokinetic studies (8 h at room temperature and 21 days at −20°C) was also investigated.

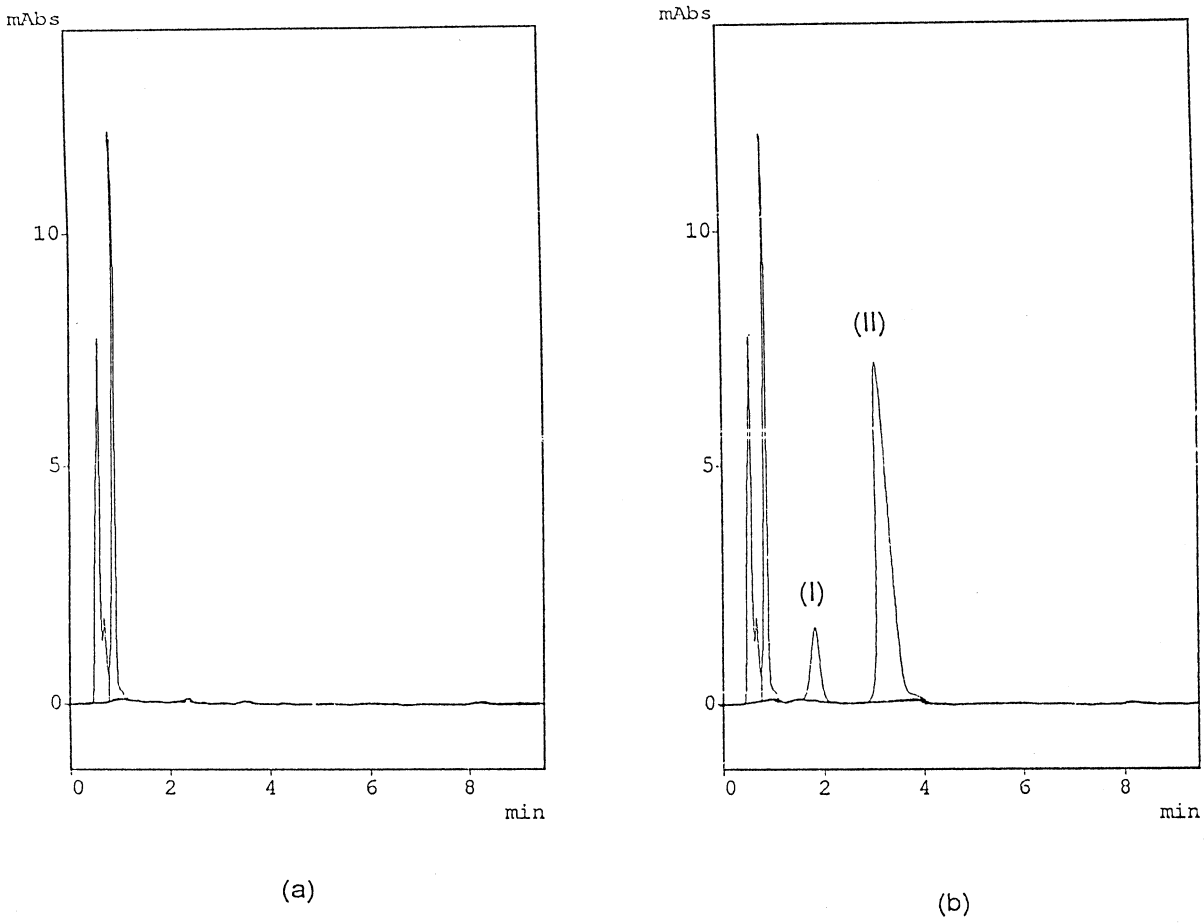

Fig. 2. Liquid chromatograms of drug-free serum and serum sample spiked with: (I) cetirizine (75 ng ml^{-1}), (II) diazepam (150 ng ml^{-1}).

3. Results and discussion

Results show that increasing the acetonitrile fraction in the mobile phase increases the relative peak height of cetirizine and decreases the retention times of cetirizine and the internal standard. But if this fraction exceeds 40%, some of the endogenous serum compounds starts precipitating inside the column, leading to higher column pressures and lower sensitivities. Lowering the pH gives shorter retention times for cetirizine and internal standard, but this lowering becomes insignificant for pH values lower than 2.8. Based on these results, the previously stated composition of mobile phase was selected.

A typical chromatogram for cetirizine is illustrated in Fig. 2. The retention times of cetirizine and internal standards are 1.69 and 2.9 min, respectively. The absence of interfering peaks and base line resolution between serum, cetirizine and internal standard peaks is obvious, (Fig. 2).

The assay was linear between 10 and 500 ng ml^{-1} with a typical calibration curve of $Y = (0.0027 \pm 4.8 \times 10^{-5})X + (0.0054 \pm 0.0012)$; the standard error of estimate, $S_{yx} = 0.0064$; and the correlation coefficient, $r = 0.998$. Symbols y and x stand for peak height ratio and concentration of the analyte (ng ml^{-1}), respectively. The coefficient of variation of the slope is less than 2% and the intercept includes zero. Intra and inter-batch pre-

Table 1
Intra-batch precision and accuracy for assay of cetirizine

Nominal cetirizine (ng ml^{-1})	Measured cetirizine (ng ml^{-1})	Precision (%)[a]	Accuracy (%)[b]
50	51.9	4.8	3.7
300	287.0	3.6	4.3
500	490.0	3.8	2.1

[a] Assessed in terms of the RSD of the measured concentrations in a replicate set ($n = 6$).
[b] Determined from bias, the mean relative error in a replicate set ($n = 6$).

Table 2
Inter-batch precision and accuracy for assay of cetirizine

Nominal cetirizine (ng ml^{-1})	Measured cetirizine (ng ml^{-1})	Precision (%)[a]	Accuracy (%)[b]
50	50.4	6.7	0.7
300	288.0	5.8	4.3
500	490.0	2.9	3.6

[a] Assessed in terms of the RSD of the measured concentrations in a replicate set ($n = 30$).
[b] Determined from bias, the mean relative error in a replicate set ($n = 30$).

cision and accuracy were assessed from six replicates at three nominal concentrations, (Tables 1 and 2). In both cases, the intra-batch and inter-batch precision and accuracy are less than 6%.

The extraction efficiency (recovery) of cetirizine in serum ranged from 93.8 to 97.3%, Table 3. The detection limit based on signal to noise ratio 3/1 was 5 ng ml^{-1}, and the limit of quantitation was 15 ng ml^{-1}. The data obtained for triplicate 15 ng ml^{-1} serum standards gave precision of 8.6% and accuracy of 7.5%. Stability results show no significant difference between measured and prepared cetirizine of spiked samples kept for 8 h at room temperature and 21 days at -21°C. A linear least squares fit of cetirizine measured vs. spiked concentrations gave a slope of 0.98 ± 0.03 and an intercept of 1.5 ± 3.5 ng. The standard error of estimates was 7.5 ng and the correlation coefficient 0.998.

Results of pooled drug-free serum showed no interfering endogenous peaks from serum Fig. 2. Commonly used drugs, as amoxycilline, ampicilin, caffiene and effidrene did not show co-elution interferences with either cetirizine or the internal standard, since their retention times were lower than one minute.

A typical cetirizine human serum concentration-time curve after administration of a drug containing 10 mg cetirizine is presented in Fig. 3. The concentrations ranged between 286 and 32 ng ml^{-1}, which are within the linear range of the method and the lowest concentration is twice the limit of quantitation. Analysis of serum samples following a 10 mg oral dose administrating to each of four subjects provided the following pharmacokinetic data: C_{max} 286 ± 32 ng ml^{-1}; $AUC_{0-\infty}$ 3382 ± 420 ng h ml^{-1}; $t_{1/2}$ 7.95 ± 0.45 h; and k_e 0.0872 ± 0.015 h^{-1}. These results compare favorably with previously published values of these parameters [10].

Table 3
Absolute recovery of cetirizine from human serum

Concentration (ng ml^{-1})	Mean peak height ratio		Recovery[a] (%)
	Aqueous	Serum	
50	0.144	0.135	93.8
300	0.832	0.785	97.3
500	1.410	1.330	95.8

[a] Six replicate analysis for each concentration.

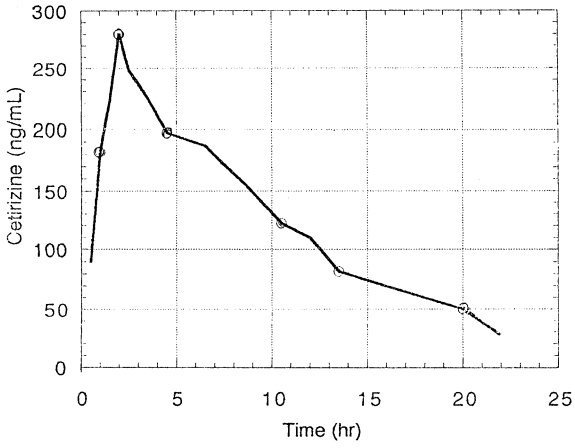

Fig. 3. Typical cetirizine human serum concentration–time profile after oral administration of 10 mg cetirizine.

4. Conclusion

We have described a sensitive, simple, rapid, selective and reliable chromatographic method for the analysis of nanograms of cetirizine in human serum. The described method is useful for the quantifying of cetirizine in real human serum samples. The extraction procedure is simple and the chromatographic separation of cetirizine and internal standard from serum matrix is achieved in less than 4 min. This method presents some advantages over other reported methods viz. shorter retention time, better limit of detection in spiked serum samples, good accuracy, precision and enough sensitivity for the quantitation of cetirizine in serum at all intervals after the dosing of the drug in pharmacokinetic studies.

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References

[1] E. Baltes, R. Coupez, L. Brouwers, J. Gobert, J. Chromatogr. 340 (1988) 149.
[2] Merk Index 11 (1989) 310.
[3] J. Moncrieff, J. Chromatogr. Biomed. Appl. 121 (1992) 128.
[4] C. Devos, M. Mallenx, E. Baltes, J. Delannoy, Ann. Allergy 55 (1985) 392.
[5] S.H. Snyder, A.M. Snowman, Ann. Allergy 59 (1987) 4.
[6] M.T. Rossel, R.A. Lefebvre, J. Chromatogr. Biomed. Appl. 565 (1991) 504.
[7] G.R. Maicke, J. Yeh, W.A. Awin, M. Clung, Ann. Allergy 59 (1987) 25.
[8] M.V. Suryanarayana, B.P. Reddy, G.L. Krupadonam, Indian Drugs 20 (1992) 605.
[9] S.G. Wood, B.A. John, Ann. Allergy 59 (1987) 31.
[10] R.A. Quercia, L. Broisman, Hospital-Formulary 29 (1994) 560.