ELSEVIER

Journal of Chromatography B, 736 (1999) 231–235

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

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

J. Macek*, P. Ptáček, J. Klíma

Pharmakl s.r.o., U Vojenské Nemocnice 1200, CZ-16200 Prague 6, Czech Republic

Received 1 July 1999; received in revised form 7 October 1999; accepted 7 October 1999

Abstract

A high-performance liquid chromatographic method for the quantitation of cetirizine in human plasma is presented. The method is based on liquid–liquid extraction with dichloromethane and reversed-phase chromatography with spectrophotometric detection at 232 nm. Gradient elution was used to remove late eluting peaks. Diazepam was used as the internal standard. The limit of quantitation was 10 ng/ml using 0.5 ml of plasma. Within-day and between-day precision expressed by relative standard deviation was less than 10% and inaccuracy did not exceed 8%. The assay was applied to the analysis of samples from a pharmacokinetic study.

Keywords: Cetirizine

1. Introduction

Cetirizine belongs to the piperazine class of antihistamines. Due to its structure cetirizine negligibly penetrates central nervous system and has less anticholinergic side effects compared with other antihistamines [1].

Despite its wide use, only a few methods for the determination of cetirizine in body fluids can be found in the literature. High-performance liquid chromatographic determination in urine with a limit of quantification 100 ng/ml was described [2]. A gas chromatographic method for the determination of cetirizine in plasma required large volumes of organic solvents for extraction and the subsequent derivatization step, the limit of quantification being 20 ng/ml [3]. Cetirizine determination in serum using high-performance liquid chromatography (HPLC) [4] involved only deproteination of the sample, but the limit of quantification (50 ng/ml) makes the method inapplicable for pharmacokinetic studies at therapeutic doses. The high-performance thin-layer chromatographic assay for measurement of cetirizine in human plasma [5] suffers from the same limitation.

The aim of this study was to develop an HPLC method for cetirizine determination in plasma sensitive enough for pharmacokinetic studies.

2. Experimental

2.1. Chemicals

Methanol (for chromatography) and dichloromethane (analytical-reagent grade) was manufactured by Merck (Darmstadt, Germany). Diazepam (internal

*Corresponding author. Fax: +420-2-3115-933.
E-mail address: pharmakl@mbox.vol.cz (J. Macek)

0378-4347/99/$ – see front matter
PII: S0378-4347(99)00464-8

standard) was obtained in a local pharmacy in the form of tablets (2 mg tablets, Diazepam 2 Slovakofarma, Slovak Republic).

2.2. Apparatus

All HPLC instruments were obtained from Thermo Separation Products (Riviera Beach, FL, USA). The system consisted of a membrane degasser, pump ConstaMetric 4100, automatic sample injector AS 3000, spectrophotometric detector UV2000 and data station with PC1000 software, version 2.5. The separation was performed on a 150×3.2 mm I.D. column filled with Nucleosil 100-3 C_{18}, particle size 3 μm (Watrex, Prague, Czech Republic). A pre-column of 10×4 mm I.D. packed with Nucleosil 120-5 C_{18}, particle size 5 μm, was used.

Gradient elution was employed according to the values in Table 1.

The mobile phase buffer was 30 m*M* potassium dihydrogenphosphate, pH of the buffer was adjusted to 6.8 with potassium hydroxide. The column temperature was 40°C. The ultraviolet detector was operated at 232 nm and the time constant was set to 2 s.

2.3. Standards

Stock solutions of cetirizine were made by dissolving approximately 15 mg in 10 ml of water. Separate solutions were prepared for calibration curve and quality control samples. Further solutions were obtained by serial dilutions of stock solutions with water.

Table 1
Gradient elution program

Time (min)	Methanol (%)	Buffer (%)	Flow rate (ml/min)
0.0	62.0	38.0	0.5
7.5	62.0	38.0	0.5
7.6	87.5	12.5	0.5
11.0	87.5	12.5	0.5
11.1	62.0	38.0	0.5
13.0	62.0	38.0	0.7
18.0	62.0	38.0	0.5
21.0	62.0	38.0	0.5

One Diazepam 2 mg tablet was suspended in 10 ml of methanol in the ultrasonic bath. The mixture was centrifuged at 2600 *g* for 10 min, the supernatant was diluted 1:9 with methanol and used as the internal standard. The solution was stable for one month, all samples and standards were analysed with the same solution of internal standard.

All solutions were stored at +4°C and protected from light.

2.4. Preparation of the sample

The samples were stored in the freezer at −18°C and allowed to thaw at room temperature before processing. A 10-μl volume of internal standard solution was added to 0.5 ml of plasma (400 ng of diazepam per 1 ml of plasma) and the tube was briefly shaken. Then the mixture was vortex mixed with 4 ml of dichloromethane for 2 min at 1500 rpm. The tube was centrifuged for 5 min at 2600 *g*, the lower organic phase was transferred to another tube and evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in 100 μl of methanol–phosphate buffer (58:42, v/v). The sample was transferred to the polypropylene autosampler vial and 20 μl was injected into the chromatographic system.

2.5. Calibration curves

The calibration curve was constructed in the range 10–1100 ng/ml to encompass the expected concentrations in measured samples. The calibration curves were obtained by weighted linear regression (weighing factor $1/y^2$): the ratio of cetirizine peak height to diazepam peak height was plotted vs. ratio of cetirizine concentration to that of internal standard in ng/ml. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

2.6. Limit of quantitation

Limit of quantitation (LOQ) was defined as the lowest concentration at which the precision expressed by relative standard deviation (RSD) is better than 20% and accuracy expressed by relative difference of the measured and true value is also

lower than 20%. Six identical samples were analysed for the determination of the LOQ.

3. Results and discussion

3.1. Chromatography

Cetirizine produces a sharp and symmetric peak when chromatographed on reversed-phase using methanol or acetonitrile as organic modifier and phosphate buffer in the pH range 2.5–7.0. The mobile phase composition was optimised to separate cetirizine from the endogenous interferences. As late eluting peaks prolonged the run time to an unacceptable period, gradient elution was established. It should be noted, however, that although gradient elution starts at 7.5 min, cetirizine and internal standard are chromatographed under isocratic conditions, because the gradient delay due to volume of the mixer is ca. 6 min at a flow-rate of 0.5 ml/min.

The method selectivity was demonstrated on six blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks. A typical chromatogram of blank plasma is shown in Fig. 1 and the chromatogram of a plasma sample from a volunteer 4 h after administration of 10 mg of cetirizine is shown in Fig. 2. The concentration of cetirizine was 131.1 ng/ml.

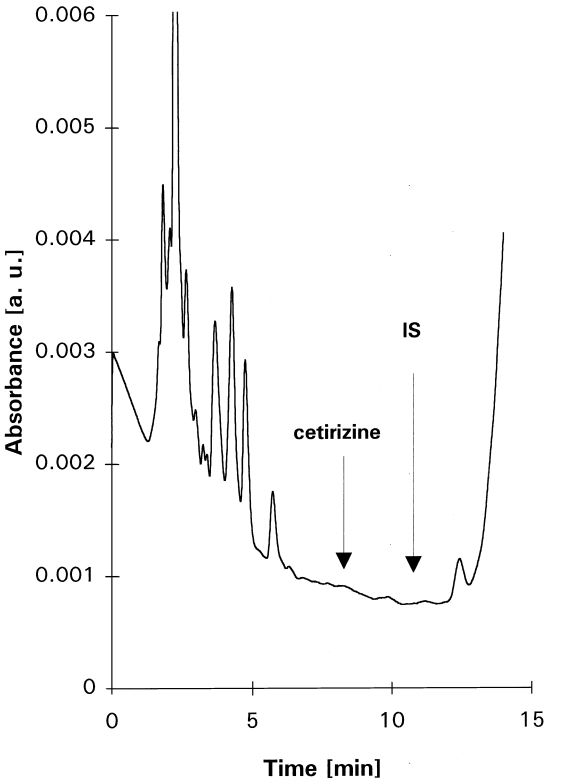

Fig. 1. Typical chromatogram of drug-free human plasma. The arrows indicate the retention time of cetirizine and diazepam (I.S.).

3.2. Linearity and limit of quantitation

The calibration curves were linear in the studied range. The calibration curve equation is $y=bx+c$, where y represents the cetirizine peak height to diazepam peak height ratio and x represents the ratio of cetirizine concentration to that of internal standard. The mean equation (curve coefficients±standard deviation) of the calibration curve ($n=10$) obtained from six points was $y=0.380$ (± 0.018)$x+0.0013$ (± 0.0019) (correlation coefficient $r=0.9978$).

The LOQ was 10.09 ng/ml. The precision, characterised by the RSD, was 6.2% and accuracy, defined as the deviation between the true and the measured value expressed in percents, was −1.5% at this concentration ($n=6$).

3.2.1. Intra-day precision

The intra-day precision of the method is illustrated in Table 2. Six sets of quality control samples (low, medium and high concentration) were analysed with calibration samples in a single run. The precision was better than 10% and the bias did not exceed 4% at all levels.

3.2.2. Inter-day precision and accuracy

The inter-day precision and accuracy was evaluated by processing a set of calibration and quality control samples (three levels analysed twice, results averaged for statistical evaluation) on six separate days. The samples were prepared in advance and stored at −18°C. The respective data are given in Table 3. The precision was better than 6% and the bias did not exceed 4% at all levels.

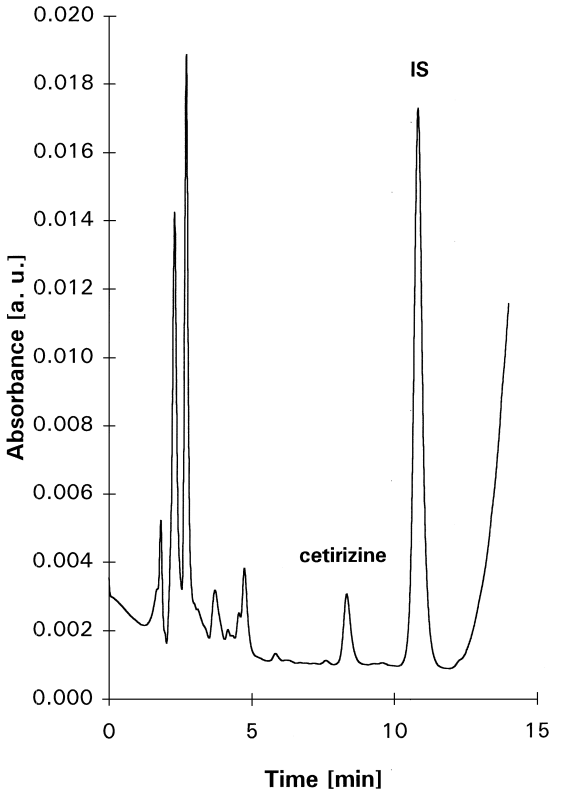

Fig. 2. Chromatogram of a plasma sample from a volunteer 4 h after administration of 10 mg of cetirizine. The respective concentration was 131.1 ng/ml.

Table 2
Intra-day precision and accuracy

n[a]	Concentration (ng/ml)		Bias (%)	RSD (%)
	Added	Measured		
6	20.17	19.42	−3.7	9.5
6	131.1	128.3	−2.1	2.8
6	912.9	927.1	1.6	2.7

[a] n=Number of samples.

Table 3
Inter-day precision and accuracy

n[a]	Concentration (ng/ml)		Bias (%)	RSD (%)
	Added	Measured		
5	20.17	19.39	−3.9	5.3
6	131.1	129.1	−1.5	5.0
6	912.9	909.7	−0.4	5.9

[a] n=Number of days.

3.2.3. Stability study

3.2.3.1. Freeze and thaw stability. Stock solutions of a low and high concentration samples were prepared. The solutions were stored at −18°C and subjected to three thaw and freeze cycles. During each cycle triplicate 0.5-ml aliquots were processed, analysed and the results averaged. The results are shown in Table 4. The concentrations found are well within the allowed limit ±15% of nominal concentration, indicating no significant substance loss during repeated thawing and freezing.

3.2.3.2. Processed sample stability. Two sets of samples with a low and a high concentration of cetirizine were analysed and left in the autosampler at ambient temperature. The samples were analysed using a freshly prepared calibration samples four days later. The results are presented in Table 4. The processed samples are stable at room temperature for four days.

3.2.3.3. Long term stability. Two sets of samples (low and high concentration of cetirizine) were stored in the freezer at −18°C for 25 days. The samples were then analysed using freshly prepared calibration samples. The results are within the acceptable ±15% limit of the nominal concentration (see Table 4). The samples are stable at −18°C for the studied period.

3.3. Application to biological samples

The proposed method was applied to the determination of cetirizine in plasma samples for the purpose of the bioequivalence study. Plasma samples were periodically collected up to 38 h after oral administration of a 10 mg single dose to 26 healthy male volunteers. Fig. 3 shows the mean plasma concentration of cetirizine. The plasma level of cetirizine reached its maximum 1 h after administration and thereafter the plasma level declined with an elimination half-time of ca. 7 h. These values agree with previously published reports [1]. The extrapolated fraction of the area under the curve (AUC) from 0 to infinity accounted only for 8% which indicates a suitability of the analytical method for pharmacokinetic studies.

Table 4
Stability of the samples

Sample C (ng/ml)	n[a]	Freeze and thaw stability					
		Cycle 1		Cycle 2		Cycle 3	
		Measured	Bias (%)	Measured	Bias (%)	Measured	Bias (%)
49.49	3	45.92	−7.2	46.45	−6.1	54.04	9.2
912.9	3	831.3	−8.9	861.3	−5.7	924.2	1.2
Sample		Processed sample stability					
		Concentration found (ng/ml)	RSD (%)	Bias (%)			
New	6	28.48	4.8	−7.7			
4 days old	6	29.53	5.2	−4.2			
New	6	927.1	2.7	1.6			
4 days old	6	958.1	4.0	4.9			
Sample C (ng/ml)		Long-term stability					
		Concentration found (ng/ml)	RSD (%)	Bias (%)			
50.10	6	56.45	7.0	12.7			
966.1	6	1104	7.3	14.2			

[a] n=Number of samples.

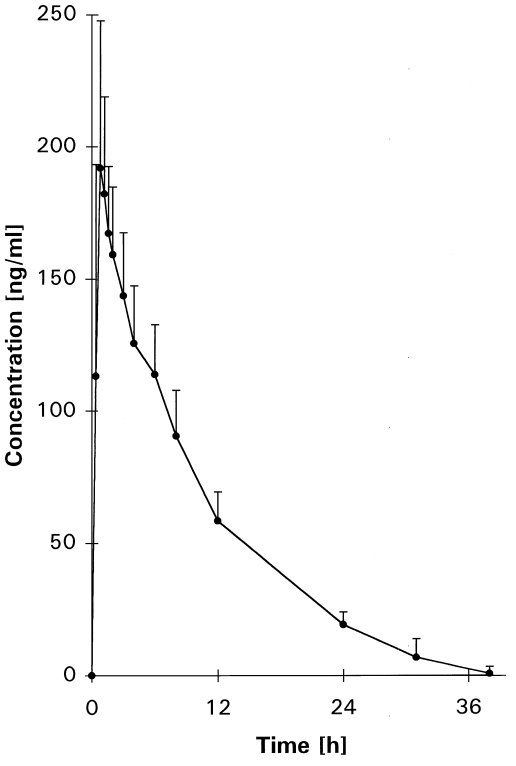

Fig. 3. Mean plasma concentrations of cetirizine after 10 mg single oral dose (26 healthy volunteers).

4. Conclusions

The validated method allows determination of cetirizine in the 10–1100 ng/ml range. The sample preparation is simple and rapid and requires only 0.5 ml of plasma. The precision and accuracy of the method are well within limits required for bioequivalence study methods. The limit of quantification 10 ng/ml permits the use of the method for pharmacokinetic studies.

References

[1] D.M. Campoli-Richards, M.M.T. Buckley, A. Fitton, Drugs 40 (1990) 762.
[2] M.T. Rosseel, R.A. Lefebvre, J. Chromatogr. 565 (1991) 504.
[3] E. Baltes, R. Coupez, L. Brouwers, J. Gobert, J. Chromatogr. 430 (1988) 149.
[4] J. Moncrief, J. Chromatogr. 583 (1992) 128.
[5] K.K. Pandya, R.A. Bangaru, T.P. Ghandi, I.A. Modi, B.K. Chakravarthy, J. Pharm. Pharmacol. 48 (1996) 510.