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A validated reversed phase HPLC assay for the determination of cetirizine in human plasma

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ABSTRACT

A simple and precise reversed-phase high performance liquid chromatography (HPLC) method for the determination of cetirizine in human plasma was developed and validated. Using omeprazole as an internal standard (IS), separation was achieved on Symmetry C18 column. The mobile phase, 10 mM monobasic potassium phosphate and acetonitrile (70:30, v:v) was delivered at a flow rate of 1.2 ml/min. The eluent was monitored spectrophotometricly at 230 nm. Plasma samples were deproteinized using a mixture of dichloromethane and hexane (75:25, v:v) and extracts were evaporated and reconstituted in mobile phase. No interference in blank plasma or of commonly used drugs was observed. The relationship between the concentration of cetirizine in plasma and peak area ratio of cetirizine to the IS was linear over the range of 0.04-2.0 μg/ml. The intra-day and inter-day coefficients of variation and bias were ≤ 8.0 % and ≤ 8.6 %, and ≤4.5% and ≤13.5%, respectively. The extraction recovery of cetirizine and the IS from plasma samples was ≥□91%. The method was applied to assess the stability of cetirizine under various conditions generally encountered in the clinical laboratory. Cetirizine in plasma was stable for at least 24 hr at RT, 7 weeks at -20 ºC; and after three freeze-thaw cycles. Cetirizine in processed samples was stable for at least 24 hr at RT or 48 hr at -20 ºC. Stock solution of cetirizine (0.1 μg/ml) in methanol was stable for 24 hr at RT or 7 weeks at -20 ºC.

KEYWORDS

Cetirizine;
Omeprazole;
Human plasma;
HPLC.

INTRODUCTION

Cetirizine, (±) - [2- [4- [(4-chlorophenyl) phenylmethyl] -1- piperazinyl] ethoxy] acetic acid, is a piperazine-derivative antihistamine used as a symptomatic treatment seasonal rhinitis[1,2]. The oral bioavailability of cetirizine is estimated to be greater than 70 %, with a mean peak plasma concentration of around 300 ± 20 ng/ml at about 1.1 hours after the ingestion of one 10 mg therapeutic dosage[3,4].

Several analytical methods have been reported for the determination of cetirizine hydrochloride in pharmaceutical formulations, individually or in combination with others agents such as pseudoephedrine and phenylpropanolamine[5-11]. Cetirizine hydrochloride levels in human plasma or serum have been mainly determined by HPLC-UV or LC-MS/MS[12-15]. Recently, a hyphenated technique based on a combination of solid

phase extraction and LC-MS/MS was developed for the quantative analysis of fexofenadine using cetirizine hydrochloride as an internal standard[16]. This method provided good results; however, the instrumentation involved is expensive and may not be readily available.

We report the development and validation of a simple and reliable HPLC method for the quantitative determination of cetirizine hydrochloride levels in the therapeutic range, using 500 µl of human plasma. The method was applied to determine the stability of cetirizine under various conditions encountered in the clinical laboratory.

EXPERIMENTAL

Apparatus

Chromatography was performed on HPLC (Waters Alliance 2487 Separation Module) consisting of quaternary pump, autosampler, column thermostat, and 2487 UV dual λ absorbance detector. A reversed-phase Symmetry C18 (4.6 x 150 mm, 5-µm) column in conjunction with a Guard Pak pre-column module with Symmetry C18, 5-µm insert (Waters Associates Inc, Milford, MA, USA) were used for separation. The data were collected with a Pentium IV computer using Millennium Chromatography Manager Software[17].

Chemicals and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Cetirizine hydrochloride (USP) was purchased from USP, Rockville, MD, USA, and omeprazole was obtained from Globalpharma, Dubai, UAE. Acetonitrile, methanol (HPLC grade) and potassium phosphate (monobasic) were purchased from Fisher Scientific, Fairlawn, NJ, USA. Water for HPLC analysis was generated by "reverse-osmosis" and further purified by passing through a Synergy Purification System (Millipore Co., Bedford, MA, USA). Drug-free human plasma was obtained from the blood bank of King Faisal Specialist Hospital & Research Centre (KFSHRC) Riyadh, Saudi Arabia.

Chromatographic conditions

The mobile phase composed of 10 mM potassium phosphate (monobasic), and acetonitrile (70:30, v:v). Before delivering into the system, the mobile phase was filtered through 0.45 µm polyetersulfone membrane and sonicated under vacuum for 5 minutes. The analysis was carried out under isocratic conditions using a flow rate of 1.2 ml/min at 23°C and a run time of 15 minutes. Chromatograms were recorded at 230 nm using a UV detector.

Preparation of standard and quality control samples

Stock solution of cetirizine hydrochloride (0.1mg) in water and omeprazole (0.1mg) in methanol were prepared, and diluted with blank human plasma or mobile phase to produce working solutions of 4.0 µg/ml and 5.0 µg/ml, respectively. Nine calibration standards in the range of 0.04-2.0 µg/ml were prepared in human plasma. Four quality control (QC) samples were prepared as follows: Lower Limit Quantification (LLQ): 0.04 µg/ml, low (LLQ X 3): 0.12 µg/ml, middle = Upper Limit Quantification (ULQ X 0.5): 1.0 µg/ml, and high (ULQ X 0.9):1.8 µg/ml, were prepared in human plasma. The solutions were vortexed for one minute, then 0.5 ml aliquots were transferred into teflon-lined, screw capped, borosilicate, 13 X100 mm glass culture tubes, and stored at -20 ºC until used.

Sample preparation

Aliquots of 0.5 ml of calibration curve samples or QC samples were allowed to equilibrate to room temperature. To each tube, 40 µl of the IS working solution (5 µg/ml in mobile phase) were added and the mixture was vortexed for 10 seconds. After the addition of 4 ml of a mixture of dichloromethane and hexanes (75:25, v:v), the sample was vortexed again for 1 min and then centrifuged for 20 min at 4200 rpm at room temperature. The organic layer was carefully collected into a clean tube and evaporated to dryness under a gentle steam of nitrogen on multiplace heating block at temperature not exceeding 40 ºC, and the residue was reconstituted in 250 µl mobile phase then centrifuged at 16000 rpm for 3 min at room temperature. The supernatant was transferred into an auto-sampler vial and 100 µl were injected into the HPLC system. The run time was 15 min.

Stability studies

A total of 40 aliquots of the following QC samples were used for stability studies: cetrizine 0.04, 0.12, and

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1.8 µg/ml. Five aliquots of each QC sample were extracted and immediately analyzed (baseline), five aliquots were allowed to stand on the bench-top for 24 hours at room temperature before being processed and analyzed (counter stability, 24 hours at room temperature), five aliquots were stored at –20 °C for seven weeks before being processed and analyzed (long term freezer storage stability), and five aliquots were processed, reconstituted, and stored at room temperature for 24 hours or 48 hours at –20 °C before analysis (autosampler stability). Finally, fifteen aliquots of each QC sample were stored at –20 C for 24 hours. They were then left to completely thaw unassisted at room temperature. Five aliquots of each sample were extracted and analyzed and the rest returned to –20 °C for another 24 hours. The cycle was repeated three times (freeze-thaw stability).

Method validation

The method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bioanalytical method validation guidance[18].The validation parameter included: specificity, linearity, accuracy, precision, recovery and stability.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Under the optimal experimental conditions, a mobile phase of 10 mM potassium phosphate (monobasic) and acetonitrile (70:30, v:v) and a flow rate of 1.2 ml/min, cetirizine, omeprazole and plasma components exhibited a well defined chromatographic separation within 15 minutes run. The retention times of cetirizine and omeprazole were around 5.8 and 11.8 respectively.

Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Potential interfering substances in plasma samples include endogenous components, metabolites, and decomposition products. We screened six batches of blank plasma and eight frequently used medications (namely: aspirin, acetaminophen, ranitidine, nicotinic acid, ascorbic acid, caffeine, ibuprofen, and diclofenac) for potential interference. No interference was found in plasma and none of the drugs co-eluted with cetirizine or the IS. Figure 1 depicts a representative chromatogram of a drug free human plasma used in preparation of standard and quality control samples.

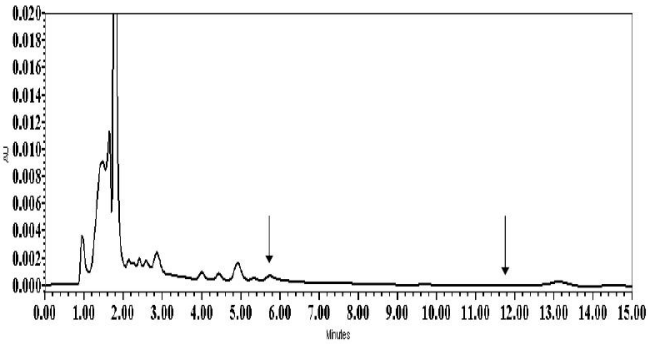

Figure 1 : Representative chromatogram of drug-free human plasma. The arrows indicate the retention times of cetirizine (5.8 min.) and omeprazole (11.8 min.)

Limit of quantification (LOQ) and linearity

The LOQ was defined as the lowest concentration on the calibration curve that can be determined with acceptable precision and accuracy (i.e., coefficient of

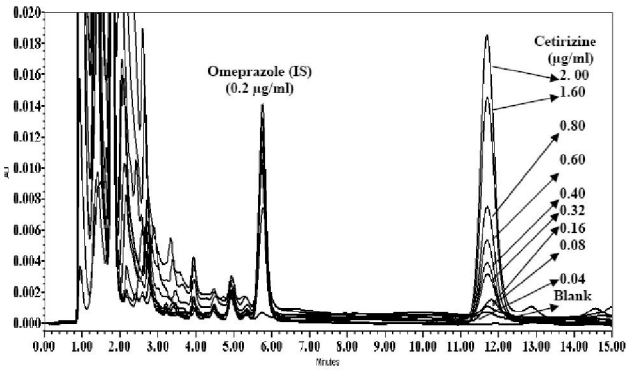

Figure 2 : Overlay of chromatograms of extracts of 0.5 ml human plasma spiked with the internal standard (IS) and one of ten concentrations of cetirizine, 0.00, 0.04, 0.08, 0.16, 0.32, 0.40, 0.60, 0.80, 1.60, and 2.00 µg/ml

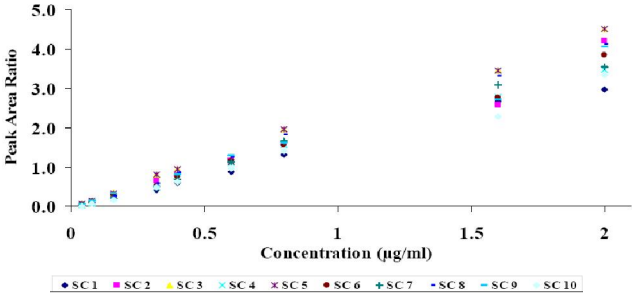

Figure 3 : Representative calibration curves used during the period of method validation. SC 1-10 standard curves 1 to10

TABLE 1 : Back-calculated cetirizine concentrations from ten calibration curves

Nominal Level (µg/ml)	Calculated Level (µg/ml)		CV (%)	Bias (%)
	Mean	SD		
0.04	0.0436	0.0055	12.6	+ 9.0
0.08	0.0800	0.0068	8.5	0
0.16	0.1587	0.0163	10.3	-0.8
0.32	0.3118	0.0340	10.9	-2.6
0.40	0.4277	0.0173	4.0	+7.0
0.60	0.6290	0.0490	7.8	+4.8
0.80	0.8849	0.0306	3.5	+10.6
1.60	1.5326	0.1227	8.0	-4.2
2.00	2.0450	0.0913	4.5	+2.3

SD, Standard deviation; CV, Standard deviation divided by mean measured concentration x100
Bias, measured level - nominal level divided by nominal level x 100

variation and relative error d" 20%). The LOQ of cetirizine in human plasma (response of analyte to blank > 5) was 0.04 µg/ml, whereas the lowest detection limit (LOD) was 0.01 µg/ml. Linearity of cetirizine was evaluated by analyzing ten curves of nine standard concentrations over the range (0.04-0.20 µg/ml) prepared in human plasma. Figure 2 represents an overlay of chromatograms of extracts of 0.5 ml human plasma spiked with the internal standard and one of nine concentrations of cetirizine. The peak area ratios were subjected to regression analysis. Figure 3 depicts ten calibration curves used over the period of method validation. The mean regression equation was $y = 1.9174\,x - 0.0200$. The suitability of the calibration curves was confirmed by back-calculating the concentration of cetirizine in human plasma from the calibration curves (TABLE 1). All calculated concentrations were well within the acceptable limits.

Accuracy and precision

According to predetermined criteria[18], accuracy and precision were determined for four QC concentrations in the expected range (0.04, 0.12, 1.0, and 1.8 µg/ml). The inter-day precision and accuracy of the assay were determined over three different days. The intra-day (n=10) and inter-day (n=20) precision was ≤ 8.0 % and ≤ 8.6 %, respectively. The intra-day and inter-day bias was in the range of -1.1 to 4.5 % and 0.7 to 13.5 %, respectively. The results are summarized in TABLE 2. The results indicate that the method was reliable within the studied concentration range.

TABLE 2 : Intra - and inter-day bias and precision of cetirizine assay

Nominal Level (µg/ml)	Measured Level (µg/ml)		CV (%)	Bias (%)
	Mean	SD		
Intra-day (n=10)				
0.04	0.0418	0.0033	8.0	+4.5
0.12	0.1188	0.0077	6.5	-1.0
1.0	0.9891	0.0436	4.4	-1.1
1.8	1.8036	0.1450	5.8	+0.2
Inter-day (n=20)				
0.04	0.0433	0.0037	8.5	+8.2
0.12	0.1362	0.0112	8.2	+13.5
1.0	1.0395	0.0706	6.8	+3.9
1.8	1.8128	0.1568	8.6	+0.7

SD, Standard deviation; CV, Standard deviation divided by mean measured concentration x100
Bias, measured level - nominal level divided by nominal level x 100

Recovery

The absolute recovery of cetirizine was assessed by direct comparison of absolute peak areas from plasma vs. mobile phase samples, using five replicates for each of the four QC concentrations (0.04, 0.12, 1.0 and 1.8 µg/ml). Similarly, the recovery of the IS was determined by comparing the peak areas of the IS in 5 aliquots of human plasma spiked with 0.2 µg/ml IS with the peak areas of equivalent samples prepared in mobile phase. The results are presented in TABLE 3. Mean recovery for cetirizine was 94%. The recovery

TABLE 3 : Recovery of cetirizine and the internal standard from 0.5 ml of human plasma

Concentration (µg/ml)	Human Plasma*	Mobile Phase*	Recovery (%)
Cetirizine 0.04	3667 (289)	4050 (495)	91
0.12	18984 (1495)	19880 (1190)	95
1.0	250849 (12810)	258382 (2579)	97
1.8	426879 (35104)	453131 (2999)	94
Internal Standard 0.2	86991(5406)	82029 (459)	106

*** Data are mean peak area (SD), n = 5**

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TABLE 4 : Stability of cetirizine under various clinical laboratory conditions

Stability (%)									
	*Plasma samples							**Stock Solution	
Nominal	Unprocessed		Processed		Freeze -Thaw			24 hrs RT	7 wks -20 °C
Level (µg/ml)	24 hrs RT	7 wks -20 °C	24 hrs RT	48 hrs -20 °C	Cycle 1	Cycle 2	Cycle 3		
0.12	96	91	96	96	113	92	100		
100								98	93
1.8	100	99	101	109	110	110	109		

Stability (%) = mean measured concentration (n=5) at the indicated time divided by mean measured concentration (n=5) at baseline x 100. *Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), after freezing at –20 °C for 7 weeks (7 wks –20 °C), or after 1 to 3 cycles of freezing at –20 °C and thawing at room temperature (freeze-thaw), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs RT) or 48 hours at –20 °C (48 hrs -20 °C).

**** Cetirizine stock solution, 0.1 mg/ml in water.**

of the IS was complete.

Stability

The stability of cetirizine in processed and unprocessed plasma samples under laboratory usual storage conditions was investigated. Stability data are summarized in TABLE 4. Stability of cetirizine was determined over three freeze and thaw cycles. Fifteen aliquots of each of two QC samples (0.12 and 1.8 µg/ml) were stored at –20 °C. After 24 hours, all aliquots were left to thaw unassisted at room temperature. When completely thawed, 5 aliquots of each QC sample were analyzed. The other aliquots were returned to –20 °C and kept for 24 hours. The same procedure was repeated three times. The concentrations in freeze-thaw samples were compared with the concentration of freshly prepared and analyzed samples. The result showed that cetirizine in plasma is resistant to at least three cycles of freeze and thaw (stability 100% to 109%). Fifteen aliquots of each of two QC samples (0.12 and 1.8 µg/ml) were processed. Five aliquots of each QC sample were analyzed immediately. The other aliquots were analyzed after being stored at room temperature for 24 hours or at –20 °C for 48 hours. The results showed that cetirizine is stable in processed samples for at least 24 hours at room temperature (96% to 101%) and for at least 48 hours at –20 °C (96% to 109%). Thirty aliquots of each of two QC samples (0.12 and 1.8 µg/ml) were prepared. Five aliquots of each QC sample were analyzed immediately. Five aliquots of each QC sample were allowed to stand on the bench-top for 24 hours at room temperature before extraction. The data indicate that cetirizine is stable in plasma for at least 24 hours at room temperature (96% to 100%). Five aliquots of each QC sample were stored at –20 °C for 1, 2, 5, or 7 weeks before analysis. The result showed that cetirizine in plasma is stable for at least 7 weeks at –20 °C (91% to 99%). Cetirizine in stock solution (0.1 mg/ml in water) was also stable at least for 24 hours at room temperature (98%) and at least for 7 weeks at -20 °C (93%).

CONCLUSION

In summary, the described HPLC method for cetirizine determination in human plasma is simple, precise, and accurate. It has been applied for studying cetirizine stability under various clinical laboratory conditions. It could be useful for clinical and biomedical investigations.

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