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Research Article

Determination of cetirizine in human plasma and validation method using HPLC technique

Siti Hazar Binti Zuhri ^a, Abul Kalam Azad^b, Abdur Rashid Mia^b, ABM Helal Uddin ^{a*}, Mohamed Sufian Bin Mohd. Nawi ^a

^a Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia (IIUM), 25200 Kuantan, Pahang, Malaysia.

^b Advanced Drug Delivery Laboratory, Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia (IIUM), 25200 Kuantan, Pahang, Malaysia.

ABSTRACT

This research project is dedicated to analyze cetirizine in human plasma by using HPLC qualitatively and quantitatively, to make a reliable bioanalysis of this drug in human plasma with a goal to utilize it in pharmacokinetics studies. In this research project, cetirizine was extracted from plasma using liquid-liquid extraction by dichloromethane and ethyl acetate. The results in terms of accuracy and precision are compared. For both of the methods, amlodipine was used as the internal standard. Separation was carried out by Phenomenex C18 chromatography column. The mobile phase used here is 35:65, v/v of Acetonitrile to 0.3% triethylamine (TEA) buffer fixed at pH 3 by phosphoric acid, with flow rate of 1 mL/min. The column temperature was set at 30 °C. Variable wavelength detector (VWD) was used, and detection was set at 237 nm. The analysis was linear from 30 ng/mL to 500 ng/mL. Overall the recovery is more than 90% when using dichloromethane and more than 65% when using ethyl acetate.

Keywords: Bioanalysis, Method validation, Cetirizine, Human plasma, HPLC

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*Address for Correspondence:

Dr. ABM Helal Uddin, Associate Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia.

1. INTRODUCTION

Cetirizine is the most common used medicine among the people globally. However, to determine of Cetirizine in human plasma level is more complicated technique ¹. Therefore, the current approach was employed to develop and validate the suitable technique to determine its plasma level concentration. The present protocol was found the novel technique using HPLC.

Bioequivalence study requires bioanalytical method that is validated and reproducible. There are different bioanalytical methods available and one of them is using HPLC. Bioanalysis is used to describe quantitatively the substances like drugs or metabolite in biological matrices like urine or plasma. It consists of two main parts: sample pretreatment and detection of the compound ².

Cetirizine has hydrogen binding capacity and brain to blood transfer (efflux) are high causing lesser brain uptake. It has highly bounding capacity with plasma protein, mainly plasma albumin (mean=93%) and it has low apparent volume distribution, Vd contributes to low affinity for lean

tissues such as myocardium thus less tendency to cause cardiotoxicity. The average peak plasma concentration (C_{max}) is of 319 ng/mL for a single 10 mg dose ³.

The volume of distribution of cetirizine is low, meaning that its tissue distribution is quite restricted. Sample from biological matrices will give interferences to the response from analyte. These substances will precipitate in the interparticulate voids of stationary phase causing degradation and clogging. Sample pretreatment must be applied such as protein precipitation or extraction type ⁴.

The main objective of this study was to develop and validate the method to determine the Cetirizine in the human plasma using high performance liquid chromatography (HPLC). The method was successfully developed and validated. Cetirizine level in blank human plasma was determine using this newly developed method very accuracy and precisely.

2. MATERIALS AND METHODS

2.1 Instrumentation

An Agilent 1100 HPLC system was used, with an autosampler. Detection was done using VWD (variable wavelength detector) at 237 nm. Separation was done by Phenomenex C18 column.

2.2 Chromatographic condition

This chromatography system uses reversed phase system with C18 as stationary phase and 35:65, v/v of Acetonitrile to 0.3% triethylamine (TEA) buffer at pH 3 as the mobile phase ⁵. The column temperature was set at 30 °C. It is an isocratic separation with an analysis time of 10 minutes. Internal standard used is amlodipine.

2.3 Stock solution

Cetirizine reference standard (Sigma) of 10 mg dissolved in methanol. Then mobile phase was added to top up till 10 mL in volumetric flask. The stock solution concentration was 1 mg/mL or 1000 µg/mL. The same preparation steps were done to amlodipine (internal standard, IS) ⁶. IS stock solution being also 1000 µg/mL. Stock solutions were kept in fridge at 4 °C. Mobile phase was used to dilute the stock solution to make working standards.

2.4 Mobile phase

Mobile phase used for this method was 35:65, v/v of Acetonitrile to 0.3% triethylamine (TEA) buffer at pH 3. To prepare buffer, 1.5 mL of HPLC grade TEA diluted using Ultra-Pure (UP) water to make 500 mL solution ^{1,3}. Then, pH is fixed using phosphoric acid to reach pH 3.00. Mobile phase was filtered and degassed before being used for analysis. Fresh mobile phase was prepared daily.

2.5 Working standards

Stock solution diluted to become 100 µg/mL. Working standards constructed using 100µg/mL diluted stock to make: 0.6, 1.0, 2.0, 3.0, 4.0, 6.0 and 10.0 µg/mL of cetirizine working standards ⁸. Quality control standards consisted of 0.25, 1.5 and 2.5 µg/mL were prepared too. For IS, working standard of 6 µg/mL and QC standard of 0.75 µg/mL were prepared ⁹.

2.6 Sample preparation

1mL plasma with 50 µL working standard and 25 µL IS working standard was vortexed for 1 min. Then 6mL of dichloromethane (DCM) was added. This mixture was vortexed again for 2 minutes. Then, centrifuged at 4000 rpm for 10 minutes. The below layer was transferred to 10 mL glass tube. The collected fluid then evaporated under Nitrogen gas at 40 °C until dry. The residue was then reconstituted with 200 µL mobile phase. It was then vortexed for 30 seconds. Then, transferred to glass insert. After that, it was centrifuged again at 10000 rpm for 10 minutes. The supernatant was injected for analysis ⁶⁻⁸.

2.7 Calibration standard

Calibration standard was made by spiking 1mL plasma with 50 µL working standard and 25 µL IS working standard. In each of 1 mL of the blank plasma, 50 µL of 0.6, 1, 2, 3, 4, 6 and 10 µg/mL working standards are spiked into it ¹⁰.

Table 1: the concentration of working standards to be spiked into blank human plasma

Working standard concentration, µg/mL	Internal standard concentration, µg/mL
0.6	6
1	6
2	6
3	6
4	6
6	6
10	6

2.8 Quality control sample

For quality control sample, 1, 6 and 10 µg/mL working standard was spiked into 1mL of plasma, which after final reconstitution the concentration would be 0.25, 1.5 and 2.5 µg/mL. Same concentrations of standard were made to match the concentration of the QC samples. IS QC standard was prepared too.

Table 2: the QC samples were showing the spiked plasma concentration and final concentration with low, medium and high.

Working standard (Cetirizine µg/mL)	Spiked plasma concentration (1000 µL), ng/mL	Final concentration (reconstituted with 200 µL m.p.), µg/mL
1	50	0.25
6	300	1.5
10	500	2.5
Working standard (IS), µg/mL	Spiked plasma concentration (1000 µL), ng/mL	Final concentration (reconstituted with 200 µL m.p.), µg/mL
6	150	0.75

3. RESULTS AND DISCUSSION

3.1 Calibration curve

A calibration curve of plasma was prepared using the method stated above. Seven different concentrations of working standards was spiked into plasma, extracted using dichloromethane, dried and reconstituted again.

Table 3: the working standards used to spike into blank plasma to make a calibration standard.

Working standard µg/mL	(Cetirizine), ng/mL	Plasma concentration, ng/mL	
		Cetirizine	IS (Amlodipine)
0.6	30	150	
1.0	50	150	
2.0	100	150	
3.0	150	150	
4.0	200	150	
6.0	300	150	
10.0	500	150	

IS peak came out earlier around 5.4 minutes while cetirizine peak appeared around retention time of 7.4 minutes. The calibration curve was constructed by peak area ratio against concentration.

Table 4: showing the nominal plasma concentration against peak area ratio.

Plasma concentration, ng/mL	Peak area ratio (area of cetirizine/area of IS)
30	0.425
50	0.487
100	0.732
150	0.797
200	0.87
300	1.23
500	1.774

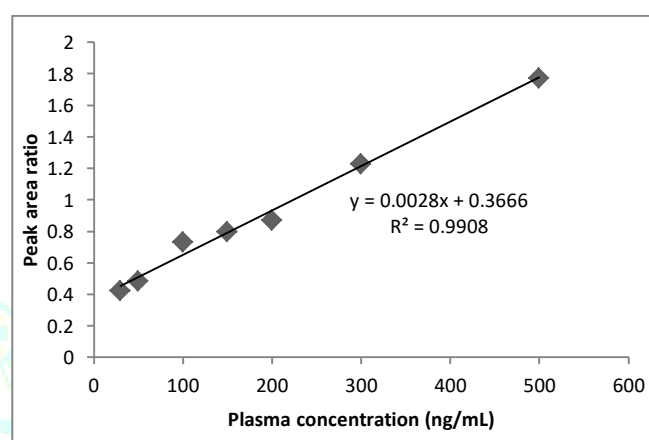


Figure 1: showing the calibration curve (peak area ratio vs. nominal concentration).

3.2 Linearity

The calibration curve was constructed with y axis represents the peak area ratio between cetirizine and IS, and x axis as the concentration. The curve equation is $y = 0.0028x + 0.3666$ with coefficient of determination, $R^2 = 0.9908$.

3.3 Selectivity

The selectivity of this analysis is represented by analysing blank plasma and compare it with plasma spiked with drug. The chromatogram of the blank plasma was free from any interference peaks at the cetirizine and IS retention times.

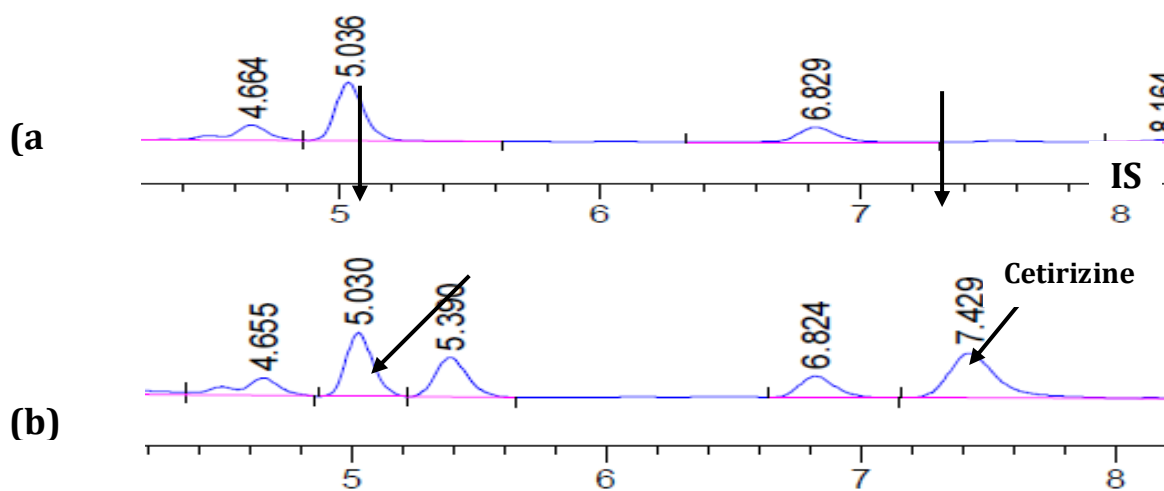


Figure 3: showing the chromatogram (a) blank plasma and (b) plasma spiked with Cetirizine and IS

3.3 Recovery

Recovery is calculated using this formula:

$$\text{Recovery}(\%) = \frac{\text{Extracted(plasma)}}{\text{Non-extracted (aqueous)}} \times 100\%$$

Mostly, the recovery of cetirizine in the plasma is more than 90% as stated below:

Table 5: showing the day recovery of analytes (cetirizine and amlodipine)

		QCL	QCM	QCH	QC(IS)
		250 ng/mL	1500 ng/mL	2500 ng/mL	750 ng/mL
Peak area	Plasma	4.08	33.8	37.6	25.27
	Aqueous	3.74	27.33	39.95	27.84
Recovery (%)		109.1	124	94	91

3.4 Precision

Precision was calculated using the formula:

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{N}}{N - 1}}$$

s = standard deviation

N = number of sample

x = measured concentration

Precision in percentage is a coefficient variation (CV) obtained from standard deviation and mean, which is calculated using the formula:

$$CV = \frac{s}{\text{mean}} \times 100\%$$

For intra-day precision, 3 sets of QC samples (low, medium and high) were analysed results shown in table below.

Table 6: showing the intra-day precision and accuracy of the analytes.

Samples number of	Concentration, ng/mL		Precision (%) CV (n=3)
	Nominal	Measured	
2	50	32.46	19
3	300	332.17	11.3
3	500	396.45	12.4

For inter-day precision, 3 sets of QC samples (low, medium and high) were analysed results shown in table below.

Table 7: showing the inter day precision of the analytes.

Samples number	Concentration, ng/mL		Precision (%)
	Nominal	Measured	
5	50	69.07	18.3
4	300	382.73	10.5
4	500	423	11.6

According to Bioanalytical Method validation guideline by the Centre for Drug Evaluation and Research (CDER), the accuracy and CV for QC samples should be within 15%. In our study it was observed QC medium and high concentrations were within the limit, but the low concentration was above 15%. So according to the guideline, the low concentration doesn't comply with the requirement.

4. CONCLUSION

The reported method has excellent recovery, selectivity and linearity (30-500 ng/ml) of the analyte from human plasma using dichloromethane as a solvent. It is also showed

satisfactory precession for medium and high concentration. The low concentration was the higher than the permissible limit by CDER.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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