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## Reverse Phase HPLC Method for the Simultaneous Determination of Cetirizine, Verapamil/Diltiazem and Amlodipine

Hina Shamshad<sup>a</sup>, Asia Naz<sup>b</sup> and Agha Zeeshan Mirza<sup>c,\*</sup>

<sup>a</sup>Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi, 75270, Pakistan

<sup>b</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi

<sup>c</sup>Chemistry Department, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, Saudi Arabia

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Determination of cetirizine, diltiazem, or verapamil and amlodipine in an active and in dosage formulations has been performed using a simple RP-HPLC method. Rosuvastatin is used in this novel RP-HPLC method as an internal standard to improve the selectivity of the method. At 230 nm, the separation was performed using a mobile phase consisting of methanol, acetonitrile, and water mixture in a ratio of 65:5:30, and pH at 2.8 allowed improved separation and faster times of analysis. ICH guidelines have pursued the validation of the method by assessing accuracy, precision (%RSD > 2), and linearity (>0.999). The retention times of diltiazem, verapamil, amlodipine, cetirizine, and rosuvastatin was found to be 2.5, 3.2, 4.1 and 6 minutes, respectively. The specificity of the proposed method was good as no interference of excipients of the tablets was observed in the analysis. The developed method could be used for routine quality control and in biological samples for the analysis of these drugs.

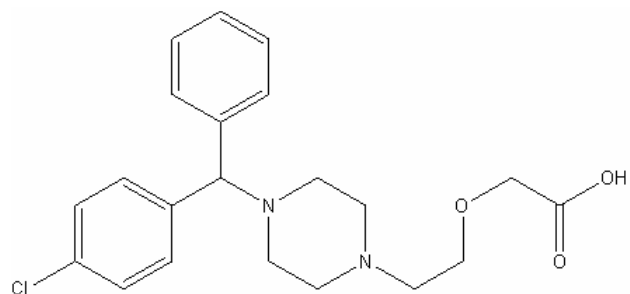
**Keywords:** Cetirizine, Verapamil, Amlodipine, Rosuvastatin, RP-HPLC

### INTRODUCTION

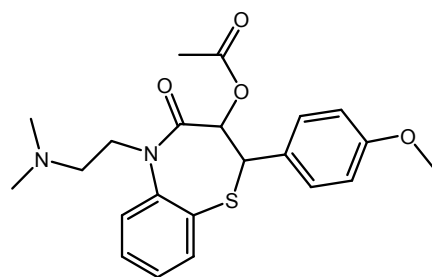
Cetirizine dihydrochloride (Fig. 1a) is an H<sub>1</sub> blocker used as an anti-allergic, diltiazem (benzothiazepines) (Fig. 1b), verapamil (phenylalkylamines) (Fig. 1c) and amlodipine (dihydropyridine) (Fig. 1d) are the drugs belonging to calcium channel blockers, prescribed mostly for hypertension. Calcium-channel blockers were found to have inhibitory effects on histamine secretions [1]. Moreover, rhinitis was also found to be strongly associated with systolic blood pressure and hypertension in men [2]. In another study, calcium blockers were found to be as much potent as the H<sub>1</sub> antagonist. It was suggested that certain slow calcium channel blockers may be of significance in the cure of asthma and different allergic disorders [3]. It was also reported that nasal catarrh is related to hypertension; the risk of hypertension linked with asthma and hay fever was particularly high in Negroes [4].

Numerous methods of HPLC have been reported for the determination of calcium blockers. García, *et al.* reported simultaneous analysis of diltiazem, verapamil, nifedipine, and nitrendipine with their metabolites through the HPLC method. The mobile phase used in this method consisted of methanol, ammonium acetate, acetonitrile, and triethylamine [5]. An HPLC method was reported for the analysis of amlodipine and valsartan simultaneously in samples for liver perfusion studies. The linearity of the method was in the range of 0.05–60 µg ml<sup>-1</sup> [6]. Vora and Kadav developed an HPLC method for bisoprolol fumarate and amlodipine besylate in formulation. Linearity was established in the range of 8–33 µg ml<sup>-1</sup> [7]. Chaudhari *et al.* described the RP-HPLC method for estimation of the stability in the formulation for atorvastatin and amlodipine [8]. A method has been reported for the simultaneous determination of amlodipine besylate with benazepril hydrochloride in a single formulation [9]. In another study, determination of amlodipine was performed in the presence of gliquidone and pioglitazone [10]. A

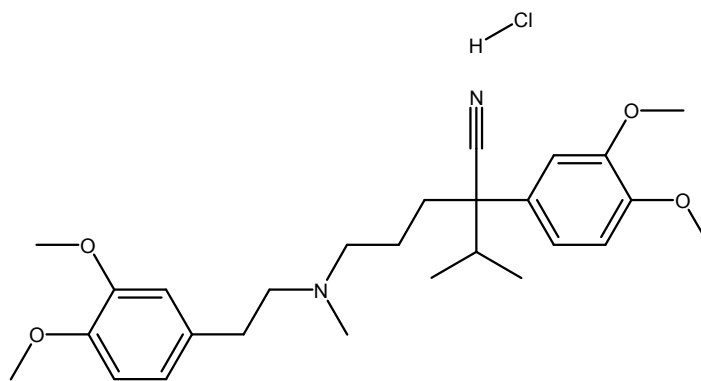
\*Corresponding author. E-mail: dr.zeeshan80@gmail.com



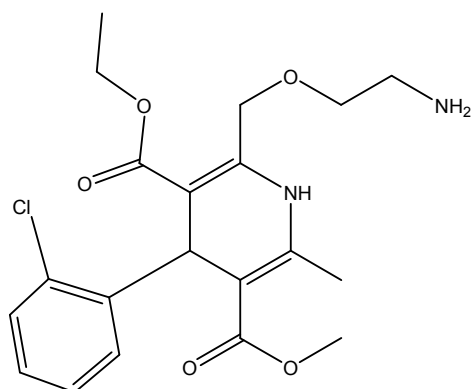
**Fig. 1a.** Cetirizine dihydrochloride.



**Fig. 1b.** Diltiazem.



**Fig. 1c.** Verapamil.



**Fig. 1d.** Amlodipine.

stability-indicating simultaneous method was proposed to determine amlodipine, irbesartan and atorvastatin [11] and also verapamil with gliquidone and pioglitazone [12]. Several HPLC methods were also reported for cetirizine alone and with other drugs [13-16] however, no method for simultaneous determination of cetirizine in the presence of calcium blockers has been reported. Accordingly, a need for a simultaneous method for the estimation of cetirizine in the presence of calcium blockers was felt. Hence, an estimation of cetirizine in presence of diltiazem, verapamil, and amlodipine using HPLC was developed and validated. Two different methods of verapamil and amlodipine with anti-diabetic drugs has been reported by our groups. For the optimization of the method, we have performed variation in experimental conditions. To get the improved peak and shorter run time, a different composition of mobile phases aqueous/organic ratio was examined. The choice of internal standard make easy the accuracy calculation.

## EXPERIMENTAL

### Materials and Instrumentation

The standards of cetirizine dihydrochloride, verapamil, and amlodipine were a kind gift of different pharmaceutical companies. The internal standard rosuvastatin was kindly gifted by PharmEvo (Private) Limited. Zyrtec<sup>®</sup> (10 mg), verapamil (Calan<sup>®</sup> 40 mg) from Wilson's Pharma (Pvt.) Ltd., Sofvasc<sup>®</sup> 5 mg (amlodipine besylate) from Wilson's Pharma (Pvt.) Ltd. tablets were purchased from a local pharmacy. HPLC grade methanol and orthophosphoric acid used were from Merck, Germany. De-ionized water was freshly prepared in the laboratory. Shimadzu HPLC system, Purospher<sup>®</sup> star, RP-18 column (5  $\mu$ m, 25  $\times$  0.46 cm), and UV-Vis detector were used.

### Methods

**Chromatographic conditions.** The chromatographic conditions were set using methanol:acetonitrile:water mixture in a ratio of 65:5:30. The pH of 2.8 was adjusted for the mobile phase using orthophosphoric acid and 230 nm was used to detect all drugs.

**Solution preparations.** 10 mg of cetirizine dihydrochloride, diltiazem/verapamil, amlodipine, and

rosuvastatin were accurately weighed and transferred to a 100 ml volumetric flask. Then, 30 ml of the diluent (methanol:acetonitrile:water 65:5:30) was added to dissolve the drugs, the solution was diluted up to the mark with the same solvent and was mixed. The final concentration was 100  $\mu$ g ml<sup>-1</sup> for all the drugs. Dilution from the standard solution was performed in the range of 2.5-50  $\mu$ g ml<sup>-1</sup> to obtain working solutions for all the drugs and then were added to each of these internal standards (5  $\mu$ g ml<sup>-1</sup>).

**Assay in formulations.** The 20 tablets of each of the medicine were powdered to check the content in the formulations, and powder equivalent to 10  $\mu$ g ml<sup>-1</sup> of each drug (cetirizine, diltiazem/verapamil, amlodipine and rosuvastatin) was separately prepared. The drugs after dissolving in 30 ml of the diluents were made up to the mark then sonicated and filtered through a filter paper (Whatman No.40). Aliquots of the solution were diluted accordingly to obtain solutions with final concentration ranging between 2.5-50  $\mu$ g ml<sup>-1</sup> for cetirizine, diltiazem/verapamil, amlodipine, and rosuvastatin. In each solution, internal standard (5  $\mu$ g ml<sup>-1</sup>) was also added.

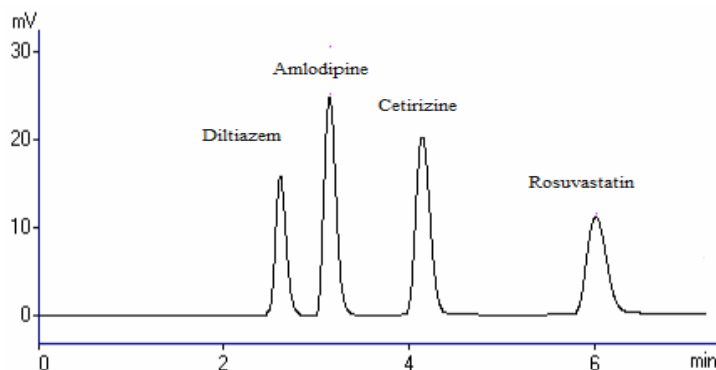
**Wavelength selection.** 230 nm was assessed as the isosbestic point for the detection of all drugs with an acceptable sensitivity.

**Method validation.** Method validation was performed according to ICH guidelines [17]. Ten replicates of a standard solution were injected and the peak area of each injection was evaluated. The chromatograms showed that the good specificity of the method as all components present in the sample matrix are well resolved. Precision was assessed by four representative samples on each of 2 days. The method accuracy for all the drugs was evaluated at three different concentrations, 8, 10, 12  $\mu$ g ml<sup>-1</sup> (n = 6). The stability of analyte in the solvent was determined by injecting the standard and sample solutions and detector response was noted as 'peak areas'. The solution was allowed to stand at ambient temperature for 24 h and then the solutions were analyzed against the freshly prepared standard.

## RESULTS AND DISCUSSION

### Optimization of Method

The literature survey revealed that no simultaneous



**Fig. 2.** Representative chromatogram of drugs in standard.

HPLC determination of cetirizine in the presence of calcium blockers is reported. So, a method was developed and validated to estimate cetirizine in the presence of diltiazem, verapamil, and amlodipine. It was observed that for verapamil the two mobile phase systems were resulted in poor resolution of the peak. Hence, three mobile phase systems were used. Less time and better separation were achieved by using methanol:acetonitrile: water in a ratio of 65:5:30 and pH adjusted to 2.8. However, the pH of the mobile phase was not decreased further than 2.8 to prevent column corrosion and poor separation of amlodipine and cetirizine. The rosuvastatin was chosen as an internal standard since it can eliminate the possible interferences due to the excipients of the dosage forms.

**Chromatographic conditions.** The mobile phase consisting of methanol acetonitrile and water mixture in a ratio of 65:5:30 and pH at 2.8 allowed improved separation and faster times of analysis. The analysis was conducted at 230 nm using a UV detector. The retention times of diltiazem, verapamil, amlodipine, cetirizine, and rosuvastatin were found to be 2.5, 3.2, 4.1 and 6 min, respectively. The retention time of diltiazem was found to be the same as that of verapamil (2.5 min) hence it was determined separately (Fig. 2).

### Range and Linearity

The six working solutions in the range of 2-50  $\mu\text{g ml}^{-1}$  for each analyte were prepared in which 5  $\mu\text{g ml}^{-1}$  of rosuvastatin (internal standard) was also added. Each solution was injected in triplicate and the calibration

curves were constructed. The correlation coefficients were calculated (Table 1) and linear regression equations are shown below,

$$\text{Verapamil} \quad y = 21054x + 30493$$

$$\text{Diltiazem} \quad y = 7938.8x + 41587$$

$$\text{Amlodipine} \quad y = 18130x + 27731$$

$$\text{Cetirizine} \quad y = 16145x + 58960$$

### Specificity

The specificity was performed by preparing the analytical spiked samples of each analyte. It was observed that the signal was represented only by the analytes and chromatograms showed very fine peaks of each analyte. There were no considerable changes in the area under curve or retention time evidently indicated the specificity of the proposed method.

### Accuracy and Precision

Accuracy (Table 2) study was performed by spiking 80, 100 and 120% of working standard solutions. The precision of the method was satisfactory as RSD% was not more than 2%. These results indicated that the individual recovery of all the drugs ranged between 98.72-101.52%. Precision (Tables 2 and 3) was measured in terms of repeatability of application and measurement. The RSD% of peak areas for all the drugs was found to be very low.

**Table 1.** Regression Statistics and Sensitivity of the Method

Drugs	R <sup>2</sup>	LOQ ( $\mu\text{g ml}^{-1}$ )	LOD ( $\mu\text{g ml}^{-1}$ )
Diltiazem	0.999	0.061	0.018
Verapamil	0.999	0.003	0.001
Amlodipine	0.995	0.081	0.011
Cetirizine	0.999	0.011	0.002

**Table 2.** Accuracy and Precision

Drugs	Conc. (%)	RSD (%)	Recovery (%)
Diltiazem	80	1.18	98.72
	100	0.94	101.53
	120	0.533	100.39
Verapamil	80	1.65	99.4
	100	0.23	100.06
	120	0.32	98.87
Amlodipine	80	1.03	98.82
	100	0.822	101
	120	0.71	100.39
Cetirizine	80	1.39	100.71
	100	0.8	98.73
	120	0.87	100.98

**Robustness, Ruggedness and %Recoveries**

The %deviation from mean assay value showed the robustness and ruggedness. No degradation of any analyte was observed in the formulations in the proposed method (Table 4) while the RSD% value indicated the suitability. The recoveries of drugs in the presence of serum for all the drugs were tested to check the presence of components that

could interfere with the drugs being analyzed (Table 5). The chromatograms of blank plasma and spiked plasma samples proved the satisfactory specificity and selectivity of the proposed procedure.

**CONCLUSIONS**

The proposed method enabled the determination

**Table 3.** Intermediate Precision of the Method

Conc. ( $\mu\text{g ml}^{-1}$ )	Diltiazem		Verapamil		Amlodipine		Cetirizine	
	RSD		RSD		RSD		RSD	
	(% )		(% )		(% )		(% )	
	Interday	Intraday	Interday	Intraday	Interday	Intraday	Interday	Intraday
2.5	0.23	0.26	0.74	0.98	0.99	0.3	0.09	0.55
5	0.56	0.59	0.56	0.45	0.32	0.1	0.94	0.87
15	0.12	0.59	0.83	1.35	1.59	0.16	1.31	1.35
20	0.39	0.69	1.31	1.54	1.87	0.23	0.96	0.92
25	0.55	0.87	0.55	0.88	0.89	0.39	0.48	0.98
50	0.2	0.85	0.12	0.97	1.03	0.17	0.6	1.56

**Table 4.** %Recoveries of Different Brands

Conc. ( $\mu\text{g ml}^{-1}$ )	Diltiazem		Verapamil		Amlodipine		Cetirizine	
	Recovered	Found	Recovered	Found	Recovered	Found	Recovered	Found
	(%)	( $\mu\text{g ml}^{-1}$ )	(%)	( $\mu\text{g ml}^{-1}$ )	(%)	( $\mu\text{g ml}^{-1}$ )	(%)	( $\mu\text{g ml}^{-1}$ )
2	98	1.96	99.95	1.95	102.11	2.00	99.52	1.99
5	98.97	4.94	105.21	4.94	100.43	5.02	94.24	4.71
15	98.59	14.78	104.11	14.78	101.20	15.18	98.14	14.72
20	99.85	19.97	102.73	19.97	99.34	19.86	97.49	19.49
25	100.28	25.07	101.76	25.07	101.17	25.29	98.54	24.63
50	101.21	50.60	100.25	50.60	96.58	48.29	104.39	52.19

of cetirizine, verapamil, and amlodipine in standard, in dosage formulations with good sensitivity and specificity. This validated method could have a wide application in the therapeutic field. It can be effectively and efficiently used in analytical and research laboratories to save the time and unnecessary use of chemicals as short run time and

preparation of a single sample for the determination of all other drugs.

#### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

**Table 5.** Analysis in Serum

Conc. ( $\mu\text{g mL}^{-1}$ )	Recovered (%)					
	2	5	15	20	25	50
Diltiazem	98.52	101.21	98.75	100.12	100.09	101.36
Verapamil	95.26	100.44	96.31	99.88	97.41	96.53
Amlodipine	98.65	100.74	100.09	102.99	108.54	104.75
Cetirizine	94.66	100.36	101.02	102.66	107.45	100.88

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