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High Performance Liquid Chromatographic Estimation of Atenolol, Hydrochlorothiazide and Amlodipine in their Combined Tablet Dosage form

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ABSTRACT

A *simple, new, precise and accurate high performance liquid chromatography method is developed and validated for the estimation of atenolol, hydrochlorothiazide, amlodipine as the bulk drug and in pharmaceutical dosage forms. Chromatographic separation of the drugs was performed on Phenomenex C8 (250 x 4.6 mm; 5 µm particle size) analytical column as the stationary phase. The solvent system consisted of 0.1M Ammonium acetate and Methanol in the ratio of 60:40 (v/v) as mobile phase. Evaluation of the separated drugs was performed using a PDA detector covering the range of 200-400 nm. All the three drugs were resolved with the retention time of 3.276 min, 6.045 min and 8.287 min for atenolol, hydrochlorothiazide, amlodipine, respectively. The method was validated with respect to linearity, sensitivity, precision, accuracy and robustness in accordance with ICH guidelines. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding good and reproducible result.*

Keywords: *Atenolol; Hydrochlorothiazide; Amlodipine; High-performance liquid chromatography; Simultaneous Determination.*

INTRODUCTION

Atenolol [1, 2] is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers, a class of drugs used primarily in cardiovascular diseases. Chemically, atenolol is described as 2-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy} phenyl)acetamide. Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension. Higher doses of atenolol also competitively block beta (2)-adrenergic responses in the bronchial and vascular smooth muscles.

Hydrochlorothiazide [3, 4] is a thiazide diuretic often considered the prototypical member of this class. Chemically, hydrochlorothiazide is described as 6-chloro-1,1-dioxo-3, 4-dihydro -2H-1λ^6,2,4-benzothiadiazine-7-sulfonamide. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium.It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. It inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption

Amlodipine [5, 6] is a long-acting 1,4-dihydropyridine calcium channel blocker. Amlodipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. Chemically, amlodipine is described as 3-ethyl 5-methyl2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4 -dihydropyridine-3,5-dicarboxylate. Amlodipine inhibits calcium ions into vascular smooth muscle cells and cardiac muscle cells. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells.

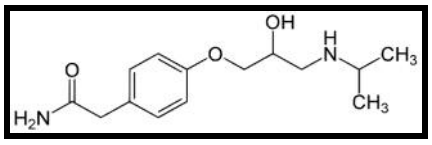

Fig. 1: Chemical structure of atenolol

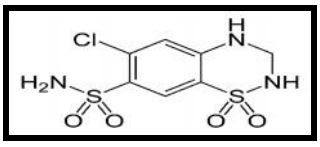

Fig. 2: Chemical structure of hydrochlorothiazide

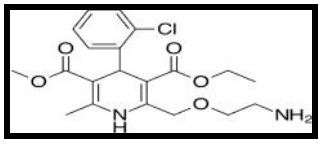

Fig. 3: Chemical structure of amlodipine

The literature reports, many methods for simultaneous quantitative determination of atenolol, hydrochlorothiazide and amlodipine in bulk, tablet dosage form, capsule dosage form and human plasma. These methods include simultaneous estimation of atenolol, hydrochlorothiazide and amlodipine by UV spectrophotometry [7-11], HPTLC [12-14], HPLC [15-21] and LC-ESI-MS/MS [22].

The aim of the present investigation is to develop and validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of atenolol, hydrochlorothiazide and amlodipine in bulk and in its combined pharmaceutical formulation.

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MATERIALS AND METHODS

Apparatus:

A Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used in the present investigation.

Mobile phase:

The solvents and chemicals used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used was 0.1M Ammonium Acetate and Methanol in the ratio of 60:40 *v/v*. Before use, the mobile phase was filtered through millipore membrane filter and degassed for 15 minutes by sonication.

Chromatographic conditions:

Phenomenex C8 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of atenolol, hydrochlorothiazide, and amlodipine. The column temperature was maintained at 30±1^{0}C. The separation was carried out under isocratic elution. The flow rate was maintained as 1.0 ml/min. The injection volume was 10 µl. The eluents were detected at 268nm.

Standard solutions:

The standard stock solution was prepared by dissolving 100mg atenolol, 50 mg of hydrochlorothiazide, and 10 mg of amlodipine in 100 ml mobile phase. Working standard solutions equivalent to 50-150 µg/ml atenolol, 25-75 µg/ml hydrochlorothiazide and 5-15µg/ml amlodipine was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

Sample Solution:

Ten tablets were weighed and crushed to a fine powder. The powder equivalent of 50mg atenolol, 25 mg of hydrochlorothiazide, and 5 mg of amlodipine was taken in a 100 ml volumetric flask containing 20 ml of mobile phase, sonicated for 20 minute and made up to mark with the same solvent. The resultant mixture was filtered through 0.45 µm filter paper. The filtrate was diluted appropriately with the mobile phase to get a final concentration of 100mg atenolol, 50 mg of hydrochlorothiazide, and 10 mg of amlodipine.

RESULTS AND DISCUSSION

HPLC parameters optimization:

So as to study the simultaneous elution of more than one drug under isocratic conditions, different chromatographic conditions (type of the column, mobile phase composition, flow rate and pH) have been investigated. The objective of the simultaneous HPLC method development was to achieve a peak tailing factor <2, USP plate count ≥ 2000, retention time in between 4,7 and 9 minutes, along with good resolution. This objective was obtained using mobile phase consisting of 0.1M Ammonium acetate – Methanol in the proportion of (60/40, *v/v*). The pH of the mobile phase was adjusted to 4.5with acetic acid. Under the above described conditions, the analyte peaks were well defined, resolved and free from tailing. The tailing factors were <2 for both the peaks. The elution orders were atenolol (retention time- 3.276 min), hydrochlorothiazide (retention time- 6.045 min) and amlodipine (retention time- 8.287 min) at a flow rate of 1.0 ml/min (Fig. 4). The optimum wavelength for detection was 268 nm at which much better detector responses for the selected drugs were obtained.

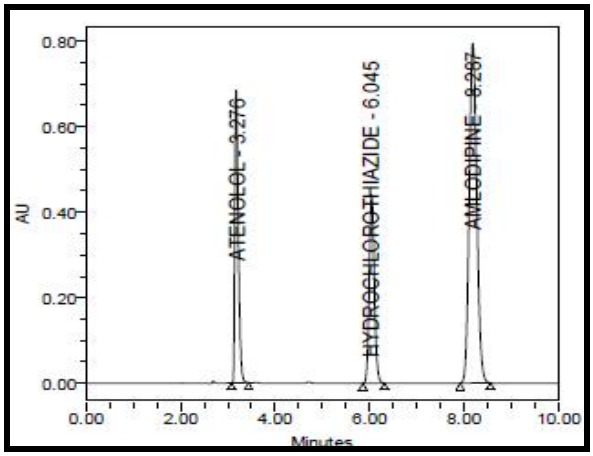

Fig. 4: Typical chromatogram of atenolol, hydrochlorothiazide and amlodipine

Method validation:

The optimized RP-HPLC method for simultaneous assay of atenolol, hydrochlorothiazide and amlodipine was validated according to ICH guidelines [24] with respect to system suitability, linearity, sensitivity, accuracy, precision and robustness.

System suitability:

Prior to analysis, the chromatographic system must satisfy system suitability test requirements. System suitability test was assessed from five replicate injections of the standard solution containing 100, 50 and 10 µg/mL atenolol, hydrochlorothiazide, and amlodipine, respectively. All the three peaks were well resolved and the precision of injections for all the peaks were acceptable. The percent relative standard deviation of the atenolol, hydrochlorothiazide, and amlodipine peaks area responses were determined to be less than 1. The USP tailing factor and USP plate count were also calculated. The results of system suitability in comparison with the required limits are shown in Table 1 and are found to be within the accepted limits.

Table No. 1: System suitability test of the HPLC method

Parameters	Results			Recommended limits
	Atenolol	Hydrochlorothiazide	Amlodipine	
Retention time	3.270	6.045	8.207	-
Peak area	2673844 (% RSD – 0.6)	2515289 (% RSD – 0.5)	6564436 (% RSD – 0.4)	RSD ≤1
USP resolution	-	16.96	8.60	> 1.5
USP plate count	8944	14896	13041	> 2000
USP tailing factor	**1.35**	**1.13**	**1.10**	**≤ 2**

Linearity and range:

The linearity of the method was determined by analyzing five different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 50-150 µg/ml atenolol, 25-75 µg/ml hydrochlorothiazide and 5-15µg/ml amlodipine. The parameters such as a regression equation and regression coefficient are given in Figures 4 and 5. The results show a good correlation between the peak areas of the drugs and their corresponding concentrations.

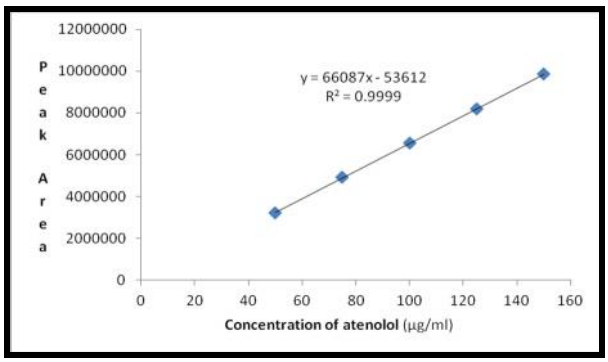

Fig. 5: Linearity curve of atenolol

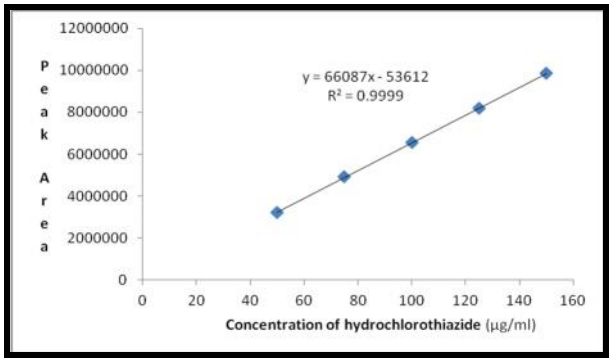

Fig. 6: Linearity curve of hydrochlorothiazide

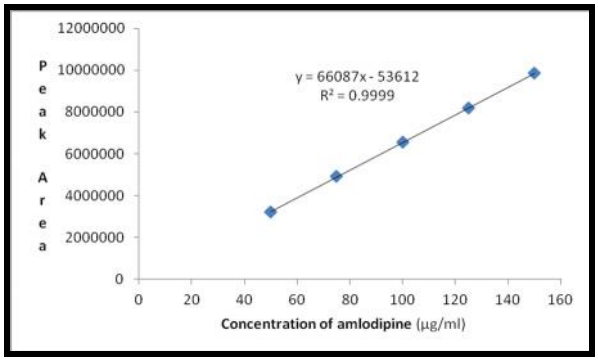

Fig. 7: Linearity curve of amlodipine

Sensitivity:

The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ) according to ICH guidelines. The results are summarized in Table 2. The low values of LOD and LOQ demonstrate the sufficient sensitivity of the method. The chromatograms of atenolol, hydrochlorothiazide, and amlodipine at LOD and LOQ levels are presented in Fig. 8 & 9.

Table No. 2: Sensitivity of the HPLC method

Parameters	Results		
	Atenolol	Hydrochlorothiazide	Amlodipine
LOD	3.224	5.947	7.957
LOQ	**3.209**	**5.943**	**7.950**

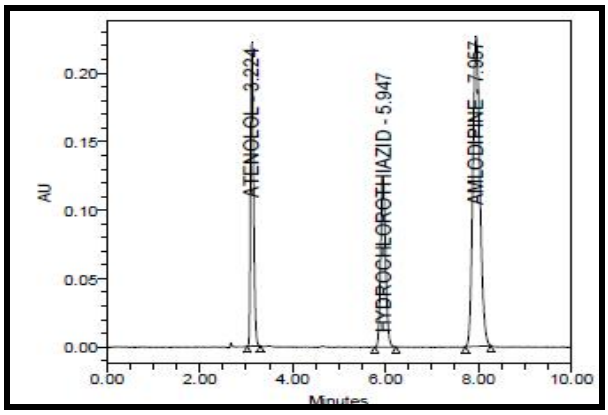

Fig. 8: Chromatogram of atenolol, hydrochlorothiazide and amlodipine at LOD level

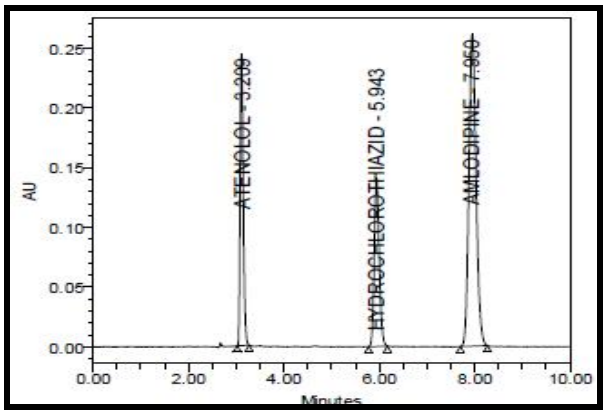

Fig. 9: Chromatogram of atenolol, hydrochlorothiazide and amlodipine at LOQ level

Precision:

Precision was determined by injecting six standard solutions of atenolol (100µg/ml), hydrochlorothiazide (50µg/ml), amlodipine(10µg/ml). The peak areas were determined. Relative standard deviationn of peak areas of the two drugs was then calculated to represent precision. The results are summarized in Table 3. The low % RSD values indicated that the method was precise.

Table No. 3: Precision of the HPLC method

Atenolol		Hydrochlorothiazide		Amlodipine	
Peak area	% RSD	Peak area	% RSD	Peak area	% RSD
2674023	0.07	2511366	0.13	6567053	0.03
2675620		2517819		6567542	
2674543		2518644		6563780	
2674859		2514431		6568987	
2675394		2519362		6568488	
2679589		2513718		6563779	

Accuracy:

Accuracy of the method was evaluated by recovery studies at three concentration (50%, 100%, and 150%) levels by standard addition method. The mean percentage recoveries obtained were shown in Table 5. The good % recovery values showed that the method was highly accurate.

Table No. 4: Accuracy of the HPLC method

Drug	Spiked Level	µg/ml added	µg/ml found	% Recovery	% Mean
	50%	49.500	49.42	100	100
	50%	49.500	49.65	100	
	50%	49.500	49.41	100	
	100%	99.000	99.12	100	

Atenolol	100%	99.000	99.20	100	100
	100%	99.000	99.30	100	
	150%	148.500	148.71	100	100
	150%	148.500	148.87	100	
	150%	148.500	148.92	100	
Hydrochlorothiazide	50%	25.000	24.96	100	100
	50%	25.000	24.91	100	
	50%	25.000	24.94	100	
	100%	50.000	49.93	100	100
	100%	50.000	49.83	100	
	100%	50.000	49.78	100	
	150%	75.000	74.64	100	100
	150%	75.000	74.63	100	
	150%	75.000	74.65	100	
Amlodipine	50%	4.950	4.97	100	100
	50%	4.950	4.98	101	
	50%	4.950	4.98	101	
	100%	9.900	9.94	100	100
	100%	9.900	9.94	100	
	100%	9.900	9.93	100	
	150%	14.850	14.90	100	100
	150%	14.850	14.90	100	
	150%	14.850	14.90	100	

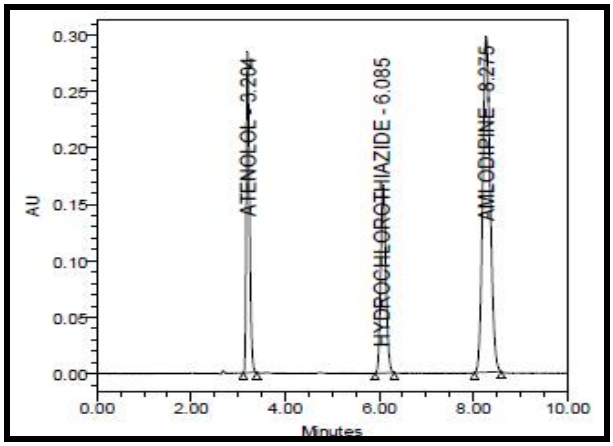

Fig. 10: Chromatogram of atenolol, hydrochlorothiazide and amlodipine at 50% level

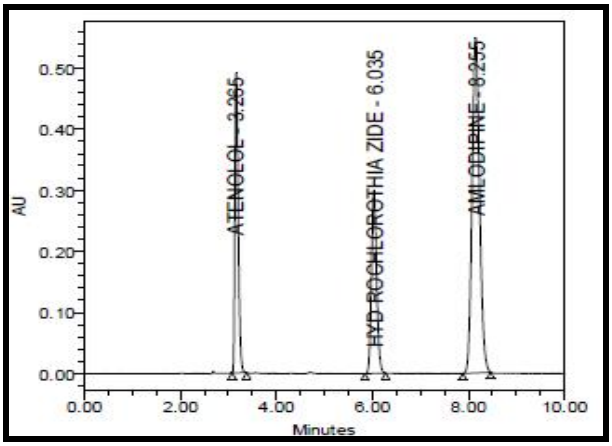

Fig. 11: Chromatogram of atenolol, hydrochlorothiazide and amlodipine at 100% level

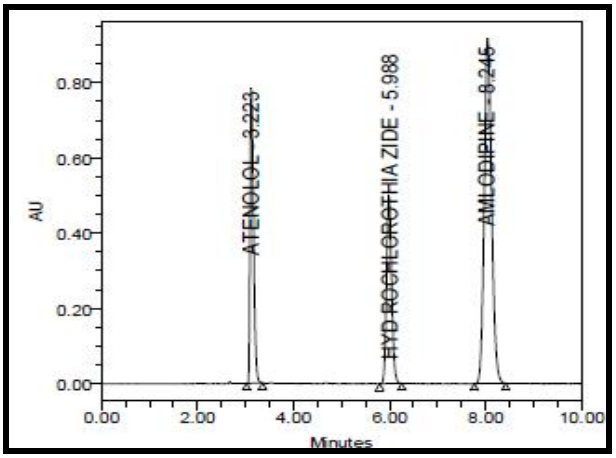

Fig. 12: Chromatogram of atenolol, hydrochlorothiazide and amlodipine at 150% level

Robustness:

In order to show the robustness of the method, system suitability parameters were evaluated at different flow rate and different column temperature. The parameters used to define robustness are retention time, USP tailing factor and USP plate count. The results showed (Table 5) that slight variations in method parameters had a negligible effect on the analysis.

Table 5: Robustness of the method

Drug	Parameter	Retention time	Peak area	USP Plate Count	USP Tailing
Atenolol	**Flow 1**	3.678	3336907	10475	1.56
	Flow 2	2.550	2287621	9054	1.48
	Temperature 1	3.097	2716365	9784	1.49
	Temperature 2	3.057	2723318	11273	1.50
Hydrochlorothiazide	**Flow 1**	7.182	3079758	16945	1.16
	Flow 2	5.080	2132119	14209	1.17
	Temperature 1	5.838	2527449	15907	1.15
	Temperature 2	5.726	2520887	16771	1.17
Amlodipine	**Flow 1**	9.634	8317788	14387	1.14
	Flow 2	6.762	5669515	12088	1.15
	Temperature 1	7.837	6795404	13475	1.14
	Temperature 2	**7.568**	**6827416**	**14555**	**1.16**

CONCLUSION

A RP-HPLC method has been reported for simultaneous estimation atenolol, hydrochlorothiazide, and amlodipine. The proposed method gives good resolution of the above said drugs. The validation of developed method was done as per ICH guidelines and proved that method to be simple, sensitive, precise, accurate and robust. The validated method was successfully applied to the determination of commercially available pharmaceutical dosage form. Hence, the method can be used for the routine quality control analysis of pharmaceutical dosage forms containing atenolol, hydrochlorothiazide, and amlodipine.

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