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Research Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC FOR THE SIMULTANEOUS ESTIMATION OF ATENOLOL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The reverse phase high performance liquid chromatography (RP-HPLC) method of Atenolol and Hydrochlorothiazide is individually available in United State of Pharmacopoeia-27 (USP-27) but no reference is available for combined estimation of Atenolol and Hydrochlorothiazide in tablets formulation. The aim of our present work was to develop a precise and validated RP-HPLC method for the simultaneous determination of Atenolol and Hydrochlorothiazide in tablets formulation. The quantification was carried out by using Zorbax SB-CN (250 x 4.6 mm), 5µm column in isocratic mode with mobile phase, Water: Buffer: Methanol (50:35:15). The flow rate was 1.2 ml/min. The peak purity of Atenolol and Hydrochlorothiazide were 0.999 and 1.000 respectively. Ruggedness and robustness of method were performed and the percentage relative standard deviation (RSD) was found below 2.0%. The percentage recovery was found in the range of 98% to 102% at three different levels. Calibration curves were linear over studies ranges with correlation co-efficient found between the range of 0.99 to 1.00. Sample and standard solution stability study was performed over 21 h at room temperature and found stable. The percentage deviation was below 2.0%.

KEY WORDS: Atenolol; Hydrochlorothiazide; RP-HPLC method; Combination Tablets.

INTRODUCTION

Monotherapy with various antihypertensive agents is not always sufficient to control the bloodpressure, and concomitant use of two or more drugs is necessary in 50% of the hypertensive patient[1-4.] The primary goal of any antihypertensive therapy is therefore achievement of normotension, without the addition of intolerable side effects, which can be accomplished by combining drug with different mechanism of action. A combination of hydrochlorothiazide and atenolol in the form of tablet or capsule is widely used for moderate to severe hypertension not controlled by a single antihypertensive agent and also in older patient who have low Renin levels[5-8]. In the stepped-care approach this combination is a first line antihypertensive drug[9]. Atenolol is a cardioselective beta-adrenoreceptor blocking agent without partial agonist or membrane stabilizing action[10]. Atenolol also decreased Renin release from the kidney. While Hydrochlorothiazide is a Thiazide diuretic, act as an antihypertensive drug by decreasing Nacl reabsorption from the luminal side of epithelial cells in the distal convulated tubule by blocking Na/Cl transporter[11]. It also eventually reduced blood volume, reduced venous pressure and reduced preload. In combination with Atenolol, Hydrochlorothiazide has additive effect like direct vasorelaxant effect on resistance vessel[12]. Plasma half-life of Atenolol is 6-7 hrs and it is incompletely absorbed from gastrointestinal tract. Hydrochlorothiazide has plasma half-life between 5 to 15 hrs and fairly rapidly absorbed from gastrointestinal tract, excreted unchanged by urine[13]. A co-administration of Atenolol with Hydrochlorothiazide produced its significant prolongation of half-life due to decrease in Hydrochlorothiazide elimination. The official monographs describe the procedure for individual assay of Hydrochlorothiazide, Atenolol as well as amiloride hydrochloride and Hydrochlorothiazide combination[14]. There are reports on the derivative spectrophotometric methods for the simultaneous determination of amiloride hydrochloride and hydrochlorothiazide[11]. However, no spectrophotometric method has been reported for the quantitative determination of Atenolol and Hydrochlorothiazide drugs from their combined formulations.

MATERIAL AND METHODS

Chemicals and Materials:

Atenolol was obtained from Suchem Laboratories India and Hydrochlorothiazide was obtained from Ipca Laboratories India. Methanol, Potassium dihydrogen ortho phosphate and Triethyl amine (HPLC grade) were purchased from Spectrochem and E-Merck Limited. In-house purified water (USPgrade) was used throughout the study.

Instrumentation:

Shimadzu 2010C integrated high performance liquid chromatographic system was used for this experiment. Shimadzu 2010C system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Zorbax SB-CN (250X4.6 mm), 5 µm was used as a stationary phase.

HPLC Condition:

Column	Zorbax SB-CN (250X4.6 mm), 5µm
Detector	286 nm

Injection volume	20 µl		
Flow rate		1.2 ml/min	
Temperature		30˚	
Run time	30 min		
Mobile phase (50:35:15)		Water: Buffer:	Methanol

Buffer preparation:
Weigh 3.4 g potassium dihydrogen ortho phosphate in to 1.0 l volumetric flask. Then add 2.5-ml triethylamine, shake well and make volume up to mark with HPLC grade water. Adjust pH 5.0 with dilute ortho phosphoric acid solution.

Diluent:
Use mobile phase as a diluent

Standard preparation:
Standard stock solutions were prepared in methanol and further for second dilution, dilute it with diluent to make final concentration Atenolol 100 µg and Hydrochlorothiazide 50 µg respectively.

Sample preparation:
Weigh accurately tablets powdered equivalent to about 125 mg Atenolol, 62.5 mg of Hydrochlorothiazide in to 250 ml volumetric flask. Add about 150 ml methanol and sonicate it for 25 minute to dissolve. Filtered it through 0.45 µ HVLP nylon filter and made further dilution 5.0 ml to 50.0 ml with mobile phase.

RESULTS

The detection wavelength was chosen at 286 nm for Atenolol and Hydrochlorothiazide in tablet dosage form has better absorption and sensitivity at this wavelength. However, to achieve the better separation of Atenolol and Hydrochlorothiazide in the present combination, the mobile phase chromatogram was shown in Fig. 1(a), (b) and (c), which illustrate the separation of both active ingredients in this system. The isocratic HPLC method was adopted to analyze both components in a single run.

System suitability and system precision:
System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in table 1.

Linearity and calibration curve:
The linearity of the calibration curve was determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in table 2.

Specificity:
There was no interference from sample placebo and peak purity of Atenolol and Hydrochlorothiazide were 0.999 and 1.000. It showed that developed analytical method was specific for the analysis of Atenolol and Hydrochlorothiazide in tablet dosage form.

Standard and sample solution stability:
Standard and sample solution stability was evaluated at room temperature for 22 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 22 h at room temperature.

Method precision:
The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in table 3.

Method accuracy:
To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in table 4.

Method robustness:
Robustness of the method was determined by small deliberate changes in pH, flow rate, Organic phase ratio of mobile phase and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 5.

Method Ruggedness:
Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in table 6 [1], [2] and [3].

Table 1.
System suitability and system precision

Compound	Retention time (Mean ± SEM)	n	k'		R	T	α	
Atenolol	5.02 ± 0.0224	5818	1.01		-	1.67	-	
HCTZ	7.67 ± 0.0016		9847	2.06		9.26	1.32	2.04

HCTZ= Hydrochlorothiazide, n= Theoritical plates, k'= Capacity Factor, R= Resolution, T= Asymetry α = Selectivity

Table 2.
Characteristics of the analytical method derived from the standard calibration curve

Compound	LOD µg/ml	LOQ µg/ml	Linearity range n=(6)	Correlation co-efficient µg/ml	Residual std. regression	Slope of regression σ	S

Atenolol	1.5	2.5	50 to 150	0.99999	0.43650	1.8149		
HCTZ		0.37	1.24	12.6 to 75.6		0.99999	2.20464	19.58537

HCTZ= Hydrochlorothiazide, OD= Limit of detection, LOQ= Limit of quantification

Table 3.
Method precision

Compound	Concentration µg/ml (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
Atenolol	100	5.02 ± 0.0224	97.4 ± 0.5984	1.5
HCTZ	50	7.67 ± 0.0016	98.4 ± 0.6850	1.7

HCTZ = Hydrochlorothiazide

Table 4.
Method accuracy

Level (mg)	Drug Added (n=3)	Drug recovered (Mean ± SEM) (n=3)	% Assay	% RSD of Assay (mg)
For Atenolol				
50%	125.01	124.21	99.4 ± 0.2449	0.3
100%	249.70	248.99	99.7 ± 0.3674	0.5
150%	374.51	374.92	100.1 ± 0.0408	0.1
For HCTZ				
50%	31.17	31.11	99.8 ± 0.0	0.1
100%	124.95	124.14	99.4 ± 0.1224	0.2
150%	187.68	186.08	99.1 ± 0.0	0.1

HCTZ = Hydrochlorothiazide

Table 5.
Method robustness

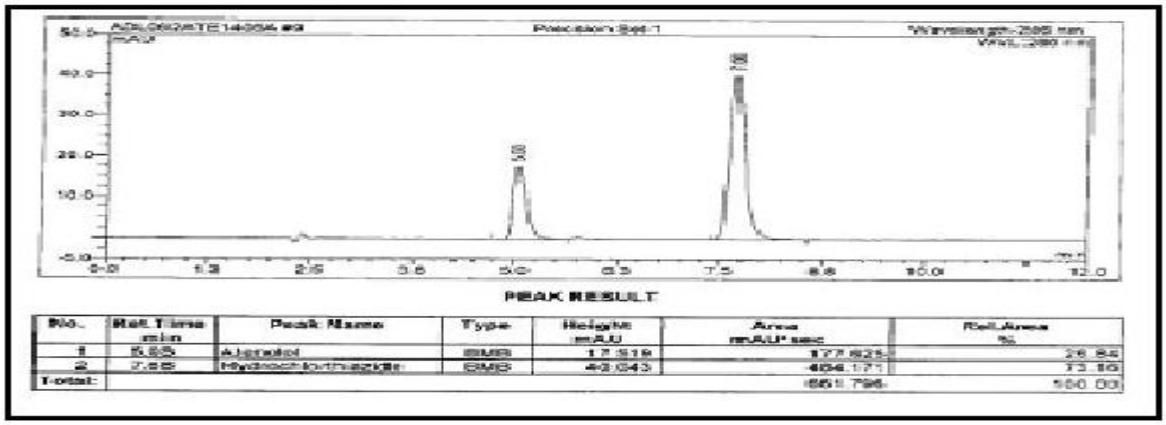
Compound	% RSD in Normal and Changed condition (n=5)			
For Temperature	**% RSD Normal**	**% RSD (-5°C)**	**% RSD (+5°C)**	
Atenolol	0.1	0.1	0.2	
HCTZ	0.1	0.02	0.1	
For pH	**% RSD Normal**	**% RSD (-0.2 unit)**	**% RSD (+0.2 unit)**	
Atenolol	0.1	0.1	0.7	
HCTZ	0.1	0.1	0.1	
Flow Rate	**% RSD Normal**	**% RSD (-10%)**	**% RSD (+10%)**	
Atenolol	0.1	0.1	0.3	
HCTZ	0.1	0.1	0.1	
Mobile phase ratio	**% RSD Normal**	**% RSD (-2%)**	**% RSD (+2%)**	
Atenolol	0.1	0.2	0.1	
HCTZ	0.1	0.2	0.1	

HCTZ = Hydrochlorothiazide

Table 6.
Method ruggedness

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1	**Analyst-1, Instrument-1 & Column-1**	
Atenolol	97.4 ± 0.5237	1.5
HCTZ	98.4 ± 0.5572	1.7
Day 2	**Analyst-2, Instrument-2 & Column-2**	
Atenolol	97.8 ± 0.3284	0.8
HCTZ	98.6 ± 0.1354	0.4

HCTZ = Hydrochlorothiazide



PEAK RESULT

No.	Ret.Time min	Peak Name	Type	Height mAU	Area mAU*sec	Rel.Area %
1	5.65	Atenolol	BMB	17.519*	177.625*	26.84
2	7.65	Hydrochlorothiazide	BMB	42.043	484.171	73.16
Total:					661.796	100.00

Fig: 1(a)

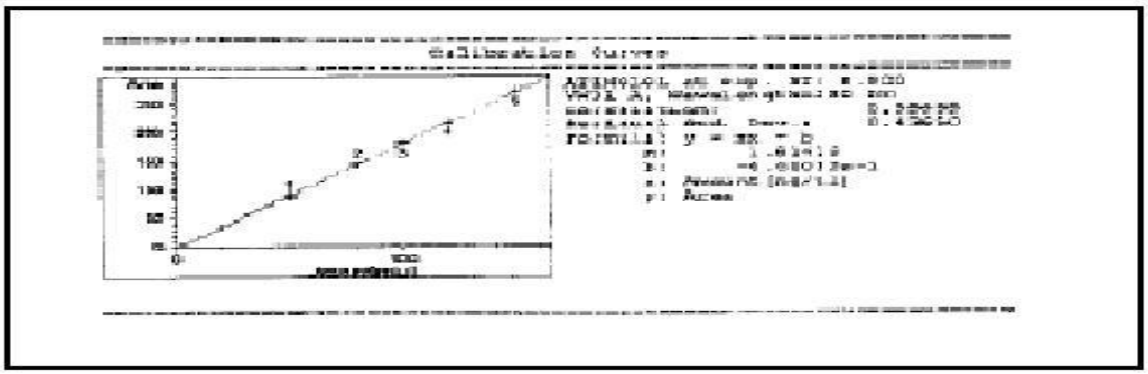


Fig 1(b)

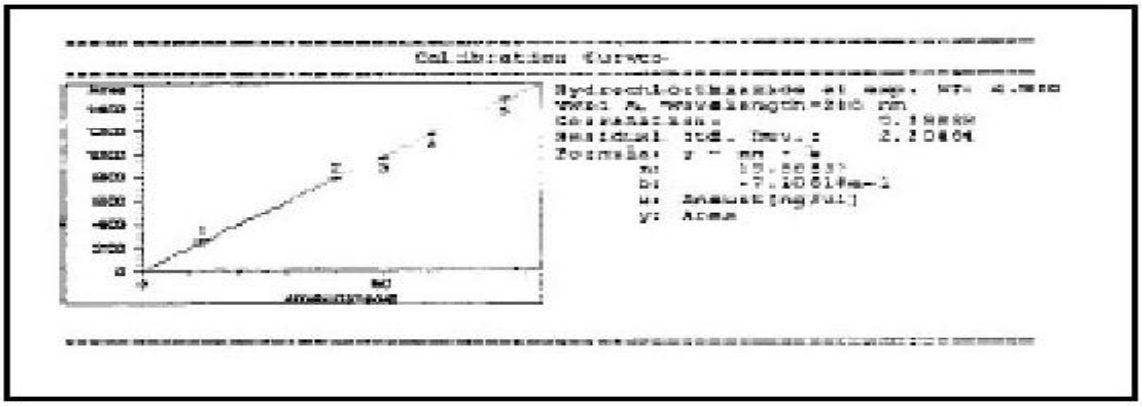


Fig 1 (c)

DISCUSSION

The method described enables to the quantification of Atenolol and Hydrochlorothiazide in film-coated tablets. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility.

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REFERENCES

1. Os I, Hotens T, Dollerup J, Mogensen CE. Comparison of the Combination of Enalapril and a very low dose of Hydrochlorothiazide with Atenolol in-patients with mild-to-moderate Hypertension. Amer J Hypertens 1991; 10:899-904.

2. Schmieder E. Telmisartan/hydrochlorothiazide combination therapy in the treatment of essential hypertension. Expert Opin Pharmacother 2004; 5:2303–10.

3. Collins R, Peto R, MacMohan S. Blood pressure, stroke and coronary hearth disease. Part 2, short-term reduction in blood pressure: overview of randomized drug trials in their epidemiological context. Lancet 1990; 335:827-38.

4. Suharmi S, Santoso B, Mulyono. Influence of atenolol on the pharma-cokinetics and diuretic effects of hydrochlorothiazide in healthy volunteers. Eur J Pharmacol 1990; 183:2375-76.

5. Vander Does R, Widmann L, Horrmann M, Machwirth M, Stienen U. Suppl. Efficacy and safety of carvedilol in comparison with atenolol in hypertensive patients pretreated with hydrochlorothiazide. Eur J Pharmacol 1990; 38:147-152.

6. CVN Prasad, Parihar C, Sunil K, Parimoo P. Simultaneous determination of Amiloride HCL, hydrochlorothiazide and atenolol in combined formulation by derivative Spectroscopy. J Pharm Bio Anal 2004; 5:2303-10.

7. MCF Ferraro, Castellano PM, kaufmana TS. Chemometric determination of amiloride hydrochloride, atenolol, hydrochlorothiazide and timolol maleate in synthetic mixtures and pharmaceutical formulation. J Pharm Bio Anal 2004; 34:305-14

8. Parrissi-poulou M, Reizopolou V, Kouppans M, Machers P. Second derivative UV Spectrophotometric determination of hydrochlorothiazide and amiloride - hydrochlorothiazide combination tablets. Int J Pharma 1989; 51:169-74

9. Annonymous, United States Pharmacopoeia (USP-27), Asian addition Published by the United States Pharmacopoeia Convention Inc, USA; 2004, p. 222-6.

10. Rapado-Martinez I, Garcia-Alvarez-Coque MC, Villanueva-Camanas RM. Performance of micellar mobile phases in reversed-phase chromatography for the analysis of pharmaceuticals containing beta-blockers and other antihypertensive drugs. Analyst 1996; 121:1677-82.

11. Reynolds JGF, Martindale. The Extra Pharmacopoeia, The Pharmaceutical Press, 29th ed, London, UK, 1989, pp. 783–785.

12. Annonymous, British Pharmacopoeia, Published by HMSO, London; 2008, p. 509-11.

13. Nevin Erk. Spectrophotometric analysis of Hydrochlorothiazide and Valsartan. Analytical Letters, 2002; 35: 283-302.

14. Bhushan R., Deepak Gupta, Shravan Kumar Singh. Liquid chromatographic separation and UV determination of certain antihypertensive agents. Biomed Chromato. 2005; 20:217-224.

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