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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF ATENOLOL AND HYDROCHLORTHIAZIDE IN COMBINED SOLID DOSAGE FORM

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ABSTRACT

Objective: To develop a simple, selective and rapid stability indicating reversed phase high performance liquid chromatographic (HPLC) method for the analysis of Atenolol and hydrochlorothiazide in combined in combined pharmaceutical dosage form as per ICH guidelines.

Methods: The separation was achieved from C18 column at 25oc with a mobile phase consisting of methanol: phosphate buffer in the ratio of 70:30 v/v with a flow rate of 0.5 ml/min (UV detection at 226 nm) and the retention time was about 2.3 min for Atenolol and 2.9 for hydrochlorothiazide. This method was selective to Atenolol and hydrochlorothiazide and also describes the analysis of degraded products of Atenolol and hydrochlorothiazide.

Results: ATN and HCZ were found to be linear in the concentration range of 20 – 100 µg/ml and 10 – 50 µg/ml respectively. Amount of ATN and HCZ were found in the range from 99.14 – 101.56% and 95.44 – 99.80% respectively. The amounts of degradation products for ATN were found to be 9.45% (in acidic condition), 34.9% (in basic condition), 0.15% (in oxidized condition), 4.55% (in thermal condition), 2.65% (in UV degradation at 254 nm) and 2.4% (in UV degradation at 366 nm). The amounts of degradation products for HCTZ were found to be 0.5% (in acidic condition), 8.0% (in basic condition), 8.1% (in thermal condition), 5.5% (in UV degradation at 254 nm) and 4.0% (in UV degradation at 366 nm).

Conclusion: The developed stability indicating assay method was found to be simple, accurate, sensitive, précised, specific and rapid. This method can be applied for routine quantitative analysis of Atenolol and hydrochlorothiazide in bulk and pharmaceutical formulation like tablets. This method was also capable to separate the degradation product of both drugs. Hence, it can be used to check the quality of product after different storage conditions and also in stress degradation study.

Keywords: Atenolol, Hydrochlorothiazide, Reverse phase HPLC, Isocratic elution, Validation, Stability indicating.

INTRODUCTION

Atenolol is a selective β1 receptor antagonist, a drug belonging to the group of beta blockers and primarily used in cardiovascular diseases [1]. Atenolol is one of the most widely used β-blockers in the United Kingdom and was once the first-line treatment for hypertension [2-5]. Hydrochlorothiazide is a diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water [6]. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. Hydrochlorothiazide is a calcium-sparing diuretic, meaning it can help the body gets rid of excess water while still keeping calcium. The only HPLC method of the combination formulation of atenolol and hydrochlorothiazide in stability indicating method suffers the drawback of splitting of the peak and sometimes tailing and fronting effect occurred. Literature survey reveals that no RP-HPLC methods could be found for simultaneous estimation and stability study of Atenolol and hydrochlorothiazide in combined dosage forms by using methanol: buffer (phosphate buffer, pH4) in the ratio of 70:30v/v as solvent system[7-9]. developed stability indicating assay method was found to be simple, accurate, sensitive, précised, specific and rapid. This method can be applied for routine quantitative analysis of Atenolol and hydrochlorothiazide in bulk and pharmaceutical formulation like tablets. This method was also capable to separate the degradation product of both drugs. Hence, it can be used to check the quality of product after different storage conditions and also in stress degradation study.

Experimental

Chemicals and reagents

Atenolol (ATN) working standard has been supplied by Cadila Healthcare Pvt. Ltd. (Sikkim, India) and hydrochlorothiazide (HCZ) working standard has been supplied by Cadila Healthcare Pvt. Ltd. (Sikkim, India). Water (HPLC grade), methanol (HPLC grade), chloroform, di-sodium hydrogen phosphate, concentrated hydrochloric acid, sodium hydroxide, hydrogen peroxide, glacial acetic acid were purchased from Merck India. All chemicals were analytical grade and used as received. Combined formulation of Atenolol and hydrochlorothiazide is available as a tablet with brand name ATN-H with label claimed 50mg Atenolol and 25mg hydrochlorothiazide.

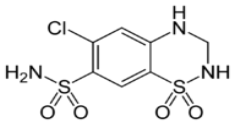

Fig. 1: Hydrochlorothiazide

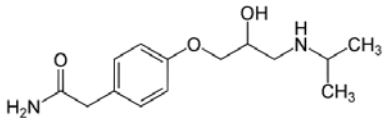

Fig. 2: Atenolol

Chromatographic condition

Chromatographic separation was achieved by using a Waters HPLC system manufactured by Waters Corporation, Milford, MA 01757 U. S. A with Waters 515 HPLC pump and waters 2489 UV/Visible detector and data processor 'Empower'. Isocratic elution was performed using methanol: phosphate buffer (70:30, v/v) with flow rate 0.5 mL/min. 20 µL of sample was injected into the HPLC system.

Preparation of calibration curve- As the selected formulation was in the ratio of 2:1 respectively for ATN and HCZ, so 2 ml of the solution from 200µg/ml of Atenolol and 1 ml of the solution from 100µg/ml of Hydrochlorothiazide were pipetted out and transferred to 10 ml volumetric flask and the volume was made up to the mark by HPLC grade methanol. Finally the concentration was 20:10µg/ml (v/v). By the similar way 40:20µg/ml (v/v), 60:30 µg/ml (v/v), 80:40 µg/ml (v/v) and 100:50 µg/ml (v/v) samples were prepared for calibration.

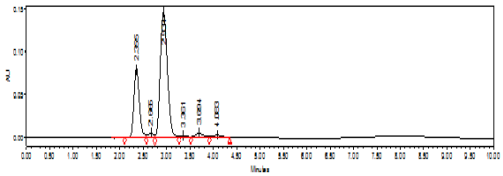

Fig. 3: Chromatogram of combined standard of concentration 20: 10 µg/ml (v/v)

Table 1: Calibration curve for ATN & HCZ

Conc (µg/ml)	Peak area							±SD	%RSD
	I	II	III	IV	V	VI	Mean		
20	960002	960000	960002	960001	960002	960000	960001.16	0.983	0.00010
40	1691608	1691607	1691607	1691609	1601608	1691607	1691607.66	0.816	0.000048
60	2605921	2605920	2605922	2605920	2605920	2605921	2605920.66	0.816	0.000031
80	3474563	3474560	3474561	3474560	3474562	3474561	3474561.16	1.169	0.000033
100	4343202	4343200	4343202	4343202	4343201	4343201	4343200.66	0.816	0.000019

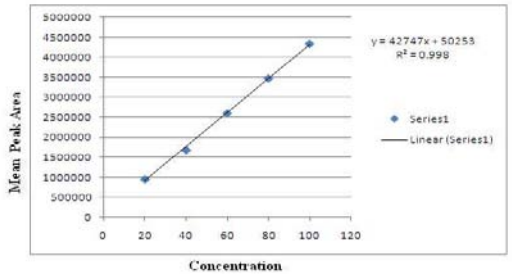

Fig. 4: Calibration of Atenolol

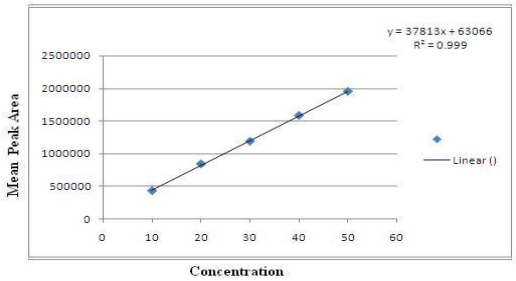

Fig. 5: Calibration of Hydrochlorothiazide

Estimation of ATN and HCZ in tablet formulation by RP-HPLC method

20 tablets were accurately weighed and crushed to fine powders. Accurately weighed quantity of 330.5mg powder containing 50mg ATN and 25mg HCZ transferred into a 100 ml volumetric flask and volume was made up to 100 ml with HPLC grade methanol. The solution was then filtered through whattman filter paper. Finally the concentration of the solution was 1000 µg/ml and 500 µg/ml respectively for ATN and HCZ. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of the respective drugs. The solution was filtered and the filtrate was diluted with mobile phase 20 µL of these solutions were injected into the system and the peak area was recorded from the respective chromatogram.

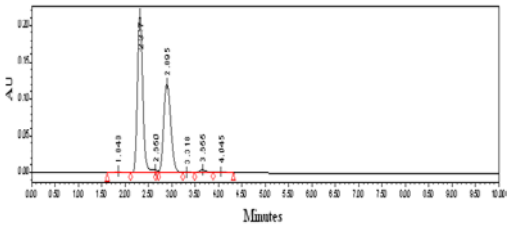

Fig. 6: Chromatogram of formulation of concentration 20:10 µg/ml (v/v)

Table 2: Assay results of tablet formulations by RP-HPLC method

S. No.	Amount present (mg/tablet)		Amount found (mg/tablet)		% Amount found	
	ATN	HCZ	ATN	HCZ	ATN	HCZ
1	50	25	50.21	24.46	100.29	97.76
2	50	25	50.32	24.47	100.69	97.95
3	50	25	50.13	23.85	100.15	95.41
4	50	25	49.53	23.93	99.17	95.75
5	50	25	50.75	24.37	101.54	97.38
6	50	25	50.74	24.93	101.52	99.79
Mean			50.28	24.34	100.56	97.34
± SD			0.461779	0.39753	0.923558	1.590119
% RSD			0.9184	1.633	0.9184	1.633

Validation of the proposed method

The method was validated for the following parameters linearity, precision, accuracy, selectivity, robustness, limit of quantization (LOQ), limit of detection (LOD) and system suitability. The concentration ranges; 10, 20, 30, 40, 50, 60 and 100 µg/ml for atenolol were selected as linearity range. Similarly, the concentration ranges; 5, 10, 15, 20, 25, 30 and 50 µg/ml for HCZ were selected as linearity range.

Recovery studies were carried out by standard addition method by adding known amount of ATN and HCZ (reference standard) separately to the reanalyzed sample at three different concentration levels i. e. 80%, 100%, and 120% of assay concentration and percentage recovery were calculated.

Intra-day precision was determined by analyzing the sample solution of formulation 20:10µg/ml in linearity range at three different time intervals on same day. Inter-day precision was

determined by analyzing the sample solution of formulation 20:10 μg/ml in linearity range on three consecutive days (n=3).

Robustness of the assay method was evaluated by observing the effect of change in the pH of mobile phase and flow rate on the retention time, tailing factor, theoretical plates and resolution were studied. The sample solutions of formulation were prepared and analyzed at different pH (3.9, 4.1) of the mobile phase at different flow rate (0.45, 0.55 ml/min). Detection limit and quantization limit were determined based on the standard deviation of y – intercepts of six calibration curves and average slope of six calibration curves.

Table3: Results of Intraday precision

Concentration of atenolol (µg/ml)	Mean Peak area at different time interval	% RSD	Concentration of Hydrochlorothiazide (µg/ml)	Mean Peak area at different time interval	% RSD
10	415090.66	0.000368	20	872988.66	0.000661
10	415087.66	0.000139	20	872988.33	0.000174
10	415090.33	0.000278	20	872988.33	0.000175

Table 4: Results of Interday precision

Concentration of atenolol (µg/ml)	Mean peak area of Atenolol on 3 different days	% RSD	Concentration of Hydrochlorothiazide (µg/ml)	Mean peak area of Atenolol on 3 different days	% RSD
20	872988.66	0.000661	10	415089.66	0.000368
20	872988.33	0.000174	10	415090.66	0.000368
20	872988.33	0.000175	10	415087.66	0.000139

Table 5: Results of Statistical Validation data for Accuracy

Level of % Recovery	Mean* (% recovery)		± SD		% RSD	
	ATN	HCZ	ATN	HCZ	ATN	HCZ
80%	100.39	104.86	0.277128	0.057735	0.276	0.055
100%	100.65	104.7	0.1	0.70946	0.099	0.6776
120%	100.63	105.26	0.104083	0.1	0.1034	0.095

Table 6: Result of Robustness for variation in pH

pH	Analyte	Retention time*(min)	Tailing factor(T)	Theoretical plate(N)	Resolution (R)
3.9	ATN	2.306	1.44	2665	3.85
	HCZ	2.880	1.22	2545	
4.1	ATN	2.313	1.48	2895	4.1
	HCZ	2.891	1.25	2623	
Required limit			T < 2	N > 2000	R > 2
Flow rate (ml/min)	Analyte	Retention time*(min)	Tailing factor(T)	Theoretical plate(N)	Resolution (R)
0.45	ATN	2.332	1.34	2701	3.9
	HCZ	2.915	1.28	2567	
0.55	ATN	2.303	1.4	2876	4.23
	HCZ	2.926	1.31	2640	
Required limit			T < 2	N > 2000	R > 2

Forced degradation study

In order to determine whether the analytical method for the assay was stability indicating, tablets, pure active pharmaceutical ingredient (API) of ATN and HCZ were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid/ base hydrolysis, oxidation, thermal degradation, UV degradation, as mentioned in ICH Q1A (R2).

Acid decomposition was carried out in 1M HCl and alkaline degradation was conducted using 0.1M NaOH and refluxed for 30 min at 80o c. After cooling the solutions were neutralized and diluted with mobile phase.

Solutions for oxidative stress studies were performed by using 3% H2O2 at a concentration at

80o C for 6 hr and diluted accordingly with the mobile phase.

For thermal degradation ten tablets were weighed, crushed and powder equivalent to 10 mg of ATN and 5 mg of HCZ, was taken in a Petri dish and was kept in a hot air oven for 6 hrs at 60 °C. For UV degradation at 366 nm ten tablets were weighed, crushed and powdered equivalent to 10 mg of ATN and 5 mg of HCZ, was taken in a petri dish and was kept in a UV chamber at 366 nm for 6 hrs at 60 °C.

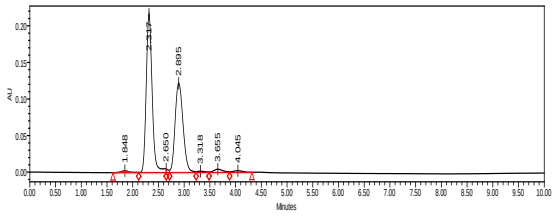

Fig. 7: Chromatogram of acid degradation of 20: 10 µg/ml of formulation

Table 7: Results of forced degradation study of tablet formulation by RP-HPLC method

	Drugs	% Assay of drugs after degradation	% Degradation
HCL/1.0 N/ 6 hrs	ATN	90.55	9.45
	HCZ	99.50	0.5
NaOH/5.0 N/ 6 hrs	ATN	65.10	34.9
	HCZ	92	8
H_2O_2/75%/ 6 hrs	ATN	99.85	0.15
Thermal/ 6 hrs	ATN	95.45	4.55
	HCZ	91.90	8.1
UV at 254 nm/ 6 hrs	ATN	97.35	2.65
	HCZ	94.50	5.5
UV at 366 nm/ 6 hrs	ATN	97.60	2.4
	HCZ	96.00	4

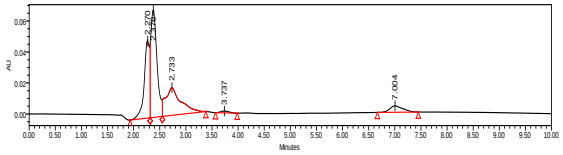

Fig. 8: Chromatogram of basic degradation of 20: 10 µg/ml of formulation

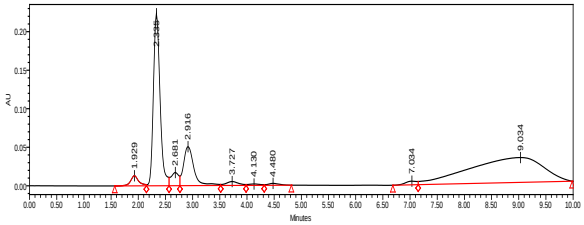

Fig. 9: Chromatogram of thermal degradation of 20: 10 µg/ml of formulation

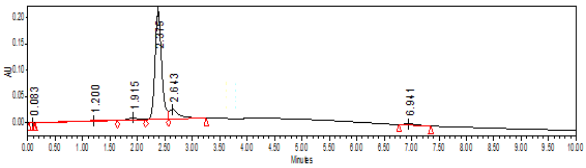

Fig. 10: Chromatogram of H_2O_2 degradation of 20: 10 µg/ml of formulation

CONCLUSION

The developed stability indicating assay method found to be simple, accurate, sensitive, précised, specific and rapid. This method can be applied for routine quantitative analysis of atenolol and hydrochlorothiazide in bulk and pharmaceutical formulation like tablets. This method was also capable to separate the degradation product of both drugs hence it can be used to check quality of product after different storage condition and in stress degradation study.

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CONFLICT OF INTERESTS

Declared None

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