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Analytical method development and validation for the estimation of chlorthalidone and atenolol by RP-HPLC

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ABSTRACT

Separation of Atenolol and Chlorthalidone was successfully achieved thermo, C_{18}, 250X4.6mm, 5µm, or equivalent in an isocratic mode utilizing 0.1M KH_2PO_4: Methanol (65:35) at a flow rate of 1.0ml/min and elute was monitored at 256nm, with a retention time of 3.346 and 3.931 minutes for Atenolol and Chlorthalidone respectively. The method was validated and their response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Atenolol and 50µg/ml to150 µg/ml for Chlorthalidone. The values of the correlation coefficient were found to 0.999 for Atenolol and 0.999 for Chlorthalidone respectively. The LOD and LOQ for Atenolol were found to be 0.110 and 0.366 respectively. The LOD and LOQ for Chlorthalidone were found to be 0.0818 and 0.2728 respectively. This method was found to be a good percentage recovery for were found to be 100 and 100 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precision, Specificity, and Robustness.

Keywords— *Chlorthalidone, Atenolol, High-performance liquid chromatography*

1. INTRODUCTION

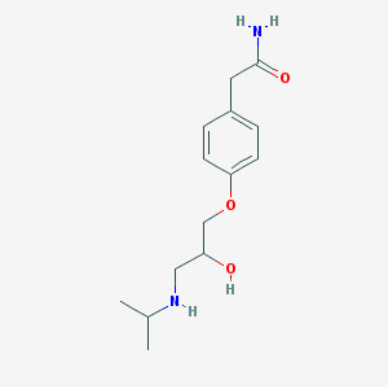

Fig. 1: Structure of Atenolol

Atenolol: The management of hypertension and long-term management of patients with angina pectoris.

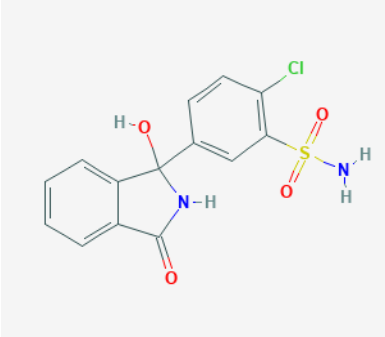

Fig. 2: Structure of Chlorthalidone

2. EXPERIMENTAL PROCEDURE

2.1 Instruments

WATERS HPLC, Model: Waters 2695, Photo diode array detector (PDA), with an automated sample injector, Electronic balance, Ultra-sonicator, heating mantle, pH meter**.**

2.2 Reagents

Potassium dihydrogen phosphate (KH_2PO_4), Water, Methanol, Orthophosphoric acid (OPA), Atenolol, Chlorthalidone.

3. PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

3.1 Standard solution

Accurately weigh and transfer 50.00 of Atenolol and 12.5 Chlorthalidone into 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with Methanol.

Transfer the above solution 1ml into 10ml volumetric flask dilute to volume with Methanol.

3.2 Sample solution

Commercially available 20 tablets were weighed and powdered the powdered equivalent to the 460.00mg of Atenolol and Chlorthalidone of active ingredients were transferred into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with Methanol.

Transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through a 0.45µm filter before injecting into the HPLC system.

4. PREPA RATION OF MOBILE PHASE

Weigh accurately 13.609g KH_2PO_4 in 1000 ml beaker add 500 ml HPLC grade Water and finally makeup with 1000ml using HPLC grade water.

Transfer the above prepared KH_2PO_4 buffer and Methanol is mixed in the proportion of (65:35). They are mixed and sonicated for 20min.

5. RESULTS AND DISCUSSION

Table 1: Parametres and the optimized methods

Parameters	Optimized method
Mobile Phase	K_2HPO_4: Methanol (65:35)
Column	THERMO, C_{18}, 250X 4.6mm, 5µm
Flow Rate	1.0ml/Min
Temperature	25˚C
Wavelength	266nm
Injection Volume	10µl
Retention Time	Atenolol:3.816, Chlorthalidone:3.931

5.1 Validation parameters

System suitability, Accuracy, Linearity, Precision, LOD, LOQ, Robustness, Specificity

5.2 System suitability

Tailing factor for the peaks due to Atenolol and Chlorthalidone standard solution should not be more than 2.0. Theoretical plates for the Atenolol and Chlorthalidone peaks in standard solution should not be less than 2000.

5.3 Precision

% RSD of peak areas was calculated for a various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that the method is precise**.**

5.4 Accuracy

The measured value was obtained by the recovery test. Spiked amount of both the drug was compared against the recovery amount % Recovery was 100.1% for Atenolol and 99.71% for Chlorthalidone. All the results indicate that the method is highly accurate**.**

5.5 Linearity

The linearity of the method was determined at five concentration levels from 50-150(µg/ml). The calibration curve was constructed by plotting peak area versus concentration the slope and intercept values of Atenolol Y=2682x & R^2=0.780 and Chlorthalidone Y=9847x & R^2=0.780.

5.6 Robustness

The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce significant changes in analytical results.

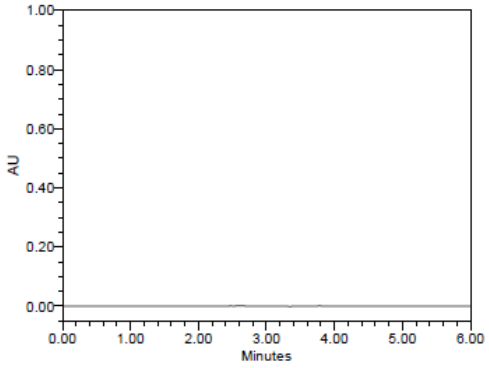

Fig. 3: Chromatogram for blank

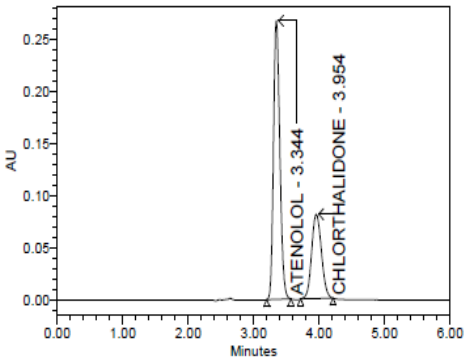

Fig. 4: Chromatogram for sample

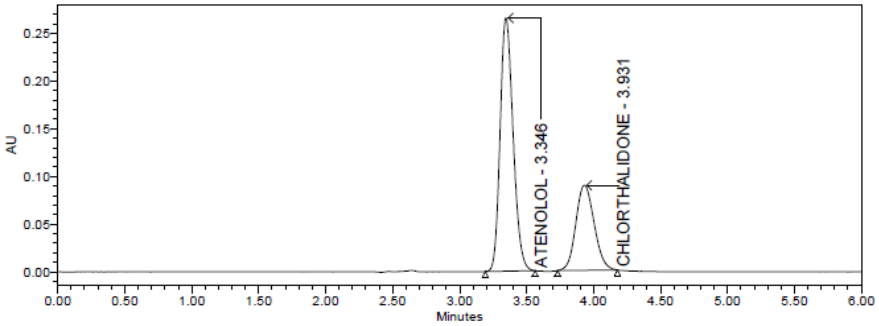

Fig. 5: Chromatogram for standard

Table 2: System suitability data for atenolol and Chlorthalidone

Parameter	Atenolol	Chlorthalidone	Acceptance criteria
Retention time	3.816	3.931	+-10
Theoretical plates	5683	3979	>2500
Tailing factor	1.21	1.12	<2.00
% RSD	0.5	0.4	<2.00

5.7 Specificity

Table 3: Specificity Data for Atenolol and Chlorthalidone

S. no	Sample name	Chlorthalidone area	Rt	Atenolol Area	Rt
1	Standard	847546	3.931	1788532	3.346
2	Sample	863604	3.954	1809142	3.344
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

5.8 Linearity

Table 4: Linearity data for Atenolol

S. no	Conc.(µg/ml)	RT	Area
1.	50	3.504	900767
2.	75	3.349	1351747
3.	100	3.341	1800158
4.	125	3.333	2252538
5.	150	3.327	2703980
The correlation coefficient (r^2)			0.780
Intercept			2682

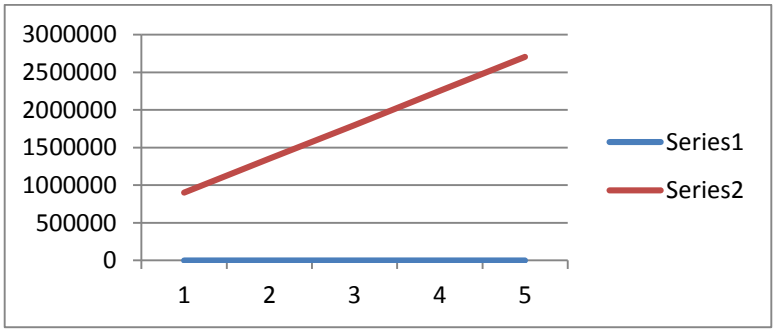

Fig. 6: Linearity plot for Atenolol

Table 5: Linearity Data for Chlorthalidone

S. no	Conc. (µg/ml)	RT	Area
1.	50	4.177	431991
2.	75	3.937	647468
3.	100	3.921	863529
4.	125	3.910	1070305
5.	150	3.903	1290452
The correlation coefficient (r^2)			0.78
Intercept			9847

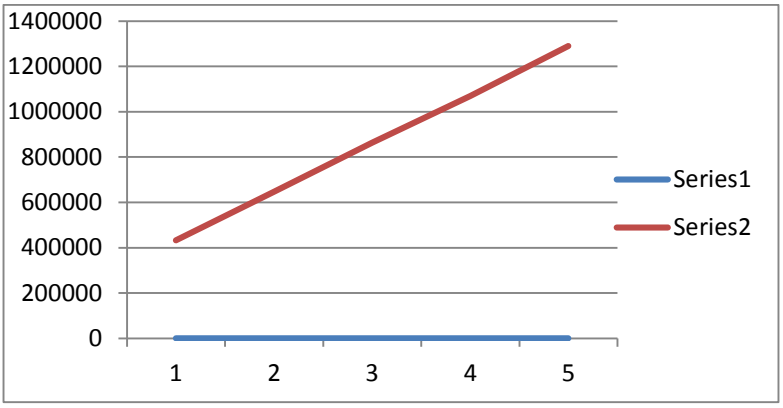

Fig. 7: Linearity plot for Chlorthalidone

5.9 PRECISION

Table 6: Precision Data for Atenolol

S. no	RT	Area	% Assay
Injection1	3.344	1809142	101.12
Injection 2	3.363	1806458	100.97
Injection 3	3.343	1802017	100.72
Injection 4	3.339	1809370	101.13
Injection 5	3.337	1807260	101.01
Injection 6	3.338	1807632	101.03
Mean			100.96
Std. Dev.			0.30
% RSD			0.30

Table 7: Precision Data for Chlorthalidone

S. no	RT	Area	% Assay
injection1	3.954	863604	99.47
injection2	3.957	863618	99.47
injection3	3.923	863331	99.43
injection4	3.917	863715	99.48
injection5	3.914	863867	99.50
injection6	3.914	863956	99.51
Mean			99.47
Std. Dev.			0.06
% RSD			0.06

5.10 Accuracy

Table 8: Accuracy data for Chlorthalidone

S. no	Accuracy level	Injection	Sample area	RT
1	50%	1	431223	3.929
		2	431008	3.927
		3	431494	3.927
2	100%	1	863253	3.910
		2	863253	3.909
		3	863066	3.914
3	150%	1	1294261	3.907
		2	1295052	3.906
		3	1292290	3.906

Table 9: Accuracy Data for Atenolol

S. no	Accuracy level	Injection	Sample area	RT
1	50%	1	900185	3.345
		2	900254	3.344
		3	900263	3.345
2	100%	1	1808029	3.336
		2	1804315	3.335
		3	1804752	3.337
3	150%	1	2707167	3.329
		2	2704334	3.331
		3	27012296	3.329

5.11 LOD

Table 10: LOD Data for Atenolol and Chlorthalidone

S. no	Sample name	RT	Area
1	Atenolol	3.347	101791
2	Chlorthalidone	3.931	88201

5.12 LOQ

Table 11: LOQ Data for Atenolol and Chlorthalidone

S. no	Sample name	R_t	Area
1	Atenolol	3.342	499427
2	Chlorthalidone	3.926	261551

5.13 Robustness

Table 12: Robustness Data for Chlorthalidone

Parameter	RT	Theoretical plates	Tailing Factor
Decreased flow rate (0.8ml/min)	4.805	3896	1.00
Increased flow rate (1.2ml/min)	3.268	3808	0.96
Decreased temperature (20^0c)	4.800	3866	1.00
Increased temperature (30^0c)	3.276	3867	0.98

Table 13: Robustness data for Atenolol

Parameter	RT	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	4.134	6034	1.23
Increased flow rate (1.2ml/min)	2.781	5352	1.22
Decreased temperature (20^0c)	4.131	6049	1.22
Increased temperature (30^0c)	2.784	5348	1.22

6. SUMMARY

Table 14: Summary Report for Atenolol

S. no	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time % RSD	 5683 1.21 3.364 0.5	 Not less than 2500 Not more than2 Not more than 2%
2	Specificity a) Blank interference b) Placebo interference	 Specific	 Specific
3	Method precision (% RSD)	0.15	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient (r^2)	50-150 mcg/ml 0.780	 Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	 100.32% 100.05% 100.04%	 97 - 103%
6	Robustness a) Flow rate variation b) Temperature variation	All the system suitability parameters are within the limits.	

Table 15: Summary report for Chlorthalidone

S. no	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 3979 1.12 3.931 0.4	 Not less than 2500 Not more than2 Not more than 2%
2	Specificity c) Blank interference d) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.3	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	50-150 mcg/ml 0.780	 Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	 99.74% 99.65% 99.74%	 97.00 – 103.00%
6	Robustness c) Flow rate variation d) Temperature variation	All the system suitability parameters are within the limits.	

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