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HPLC Method validation for estimation of Atenolol and Indapamide in tablet dosage form

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Abstract

The present High Performance liquid chromatographic method is to determine Atenolol and Indapamide from its formulation. Various experiments were carried out to establish the method. The mobile phase was methanol : water (55:45) with 0.1% v/v of Ammonium Hydroxide is found to be ideal for the estimation of Atenolol and Indapamide. The elution order was as followed (Atenolol – 7.5min, Indapamide – 8.9 min). The values of linearity, Precision and standard deviation show that the proposed method was reproducible, accurate and precise.

Keywords: Method Validation, HPLC, Tablet dosage form

Introduction

Administration of two or more drugs at a time becomes imperative for several therapeutic reasons and there exist a number of drug combinations, which have proved to be effective due to combined mode of action in the body. The combined dosage forms are complex in nature during the process of estimation. It is important to confirm that one component does not interfere with the estimation of the other. [1]

Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at β(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 β(2)-adrenergic responses in the bronchial and vascular smooth muscles. [2-3]

Indapamide blocks the slow component of delayed rectifier potassium current (IKs) without altering the rapid component (IKr) or the inward rectifier current. Specifically it blocks or antagonizes the action the proteins KCNQ1 and KCNE1. Indapamide is also thought to stimulate the synthesis of the vasodilatory hypotensive prostaglandin PGE2.

In the current scenario, development of analytical method plays an important role. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and final products obtained meet the required specifications. [2-3]

The drugs and drug formulations introduced into the market may be either new entities or partial structural modifications of the existing ones or novel dosage forms or multicomponent dosage forms. Very often, there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias.

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The continuous and wider usage of these drugs, reports new toxicities, patient resistance under these conditions, standards and analytical procedures for these drugs may not be available in pharmacopoeias. Therefore it becomes necessary to develop newer analytical methods. [4-5] Considering all these views the drug diac and its formulations from different therapeutic segments that are presently being marketed were selected for the present study.

Material and Methods [6-8]

Working Standard: The drug **"Atenolol and Indapamide"** was procured from industry. **Drug Sample:** ATEN-D tablet was purchased from market Atenolol : 50 mg & Indapamide : 2.5 mg)**. Instruments Used:** Shimadzu LC 20AD HPLC System. **Chromatographic Condition:** Column : Phenomenex C_{18} Analytical Column (25×0.46 cm, i.d, 5 µm); Flow rate: 1.0ml/min**;** Injection Volume: 20µl**;** Wavelength used: 260nm**;** Mobile Phase: Methanol:Water (55:45) with 0.1%v/v Ammonium Hydroxide.

Method validation of Aten-D tablet by RP-HPLC

Validation of method is carried out by using following validation parameters.

- Selectivity & Specificity
- Linearit
- Precision
- Limit of detection
- Limit of Quantitation or Quantification
- Change in Analyst

Specificity and system suitability

Stationary phase **:** C_{18} 250 mm X 4.6 mm, 5µ, Inertsil ODS 3V.

Mobile phase **:** 825.0 ml of Methanol (HPLC grade), 675 ml of water (HPLC grade) was taken separately filtered through membrane nylon filters of size 4.5 µ, to the filtered solution 1.5 ml of Ammonium Hydroxide Solution was added and the mixed solution was sonicated for 15 minutes and filtered through membrane nylon filters of size 4.5 µ.

Detector parameter: UV at wavelength 260 nm.

Flow rate **:** 1 ml/min.

Injection volume **:** 20 µl

Column oven temperature : 25^0 C.

Mode **:** Isocratic

Elution order: Atenolol and Indapamide.

Retention time: Atenolol 7.5 min., Indapamide 8.99 min.

Blank **:** Methanol.

Run Time **:** 12 minutes.

Linearity

Preparation of Stock Solution A:

Weigh accurately 50mg of Atenolol and 2.5mg of Indapamide into 10 ml of volumetric flask. Add sufficient amount of methanol, sonicate, cool and dilute upto mark with methanol.

Stock Solution B:

Take 1ml of Stock Solution A in a 100ml volumetric flask and dilute it upto 100ml with methanol.

Sample 1:

Take 1ml of stock solution A in 10ml of volumetric flask with pipette and dilute it with methanol upto the mark.

Sample 2:

Take 1ml of Sample 1 solution in 10 ml of volumetric flask with pipette and dilute it with methanol upto the mark.

Sample 3:

Take 1ml of sample 2 solution in 10 ml of volumetric flask with pippete and dilute it upto mark with methanol.

Sample 4:

Take 9ml of stock solution B in 10ml of volumetric flask with pipette and dilute upto 10ml with methanol.

Precision

Repeatability of Injection:

Preparation of solution:

From the Stock Solution C 1ml was pippeted out in 10ml of volumetric flask and the volume was made upto 10 ml with methanol.

Limit of detection

The limit of detection (LOD) was calculated using following formulae:

$$LOD = 3.3(SD)/S$$

Limit of quantification

The Limit of Quantification was calculated using following formulae:

$$LOQ = 10\ (SD)/S,$$

Robustness (Change in Analyst):

Results and Discussion

The objective of present work was to validate method for estimation of Atenolol and Indapamide in tablet formulation. Validation of method is carried out by using validation

parameters viz., Selectivity & Specificity, Linearity, Precision, Limit of detection, Limit of Quantitation or Quantification and Change in Analyst

Table 1: Data for Specificity test of Atenolol

Sample Name	Area µAU*sec Atenolol	Retention Time Atenolol	Similarity factor for Atenolol
STD 1	1428838	7.580	
STD 2	1427258	7.572	
STD 3	1428844	7.580	1.00
%RSD	0.064	0.061	

Table 2: Data for Specificity test of Indapamide

Sample Name	Area µAU*sec Indapamide	Retention Time Indapamide	Similarity factor for Indapamide
STD 1	708991	8.986	
STD 2	707950	8.988	
STD 3	702786	9.004	0.996
%RSD	0.470	0.110	

Table 3: System Suitability

Parameter	**Acceptance Criteria**	**Atenolol**	**Indapamide**
Tailing Factor	**NMT 2**	1.253	1.002
Capacity Factor	**NLT 2**	2.03	2.52
Similarity Factor	**0.98 to 1.02**	1.0	0.996
%RSD of STD A for Area	**NMT 2**	0.064%	0.470%
%RSD of STD A for Retention time	**NMT 2**	0.061%	0.110%

Table 4: Linearity of Standards for Atenolol

Sample Name	Area µAU*min Atenolol	Concentration Atenolol
Sample 4	3699	4.5
Sample 3	24941	5
Sample 2	201532	50
Sample 1	1428838	500

Table 5: Linearity of Standards for Indapamide

Sample Name	Area µAU*min Indapamide	Concentration Indapamide
Sample 4	1934	0.225
Sample 3	14940	0.25
Sample 2	103114	2.5
Sample 1	708991	25

Table 6: Summary of Linearity

Name	Correlation Coefficient
Atenolol	0.99
Indapamide	0.99
Acceptance Criteria	**NLT 0.99**

Table 6: Repeatability of sample:

For Atenolol:

Concentration (in ppm)	Retention time	RSD in %	Area	RSD in %	Avg RSD in %
500	7.580 7.572 7.580	0.061	1428838 1427258 1428844	0.064	
50	7.612 7.609 7.608	0.027	202460 201577 203032	0.362	**0.4083**
5	7.608 7.596 7.600	0.080	24941 25043 25328	0.799	

For Indapamide:

Concentration (in ppm)	Retention time	RSD in %	Area	RSD in %	Avg RSD in %
25	8.986 8.988 9.004	0.110	708991 707950 702786	0.470	
2.5	8.989 9.001 8.989	0.077	103114 103405 103449	0.176	**0.5723**
0.25	9.000 8.997 9.001	0.051	14940 14815 14625	1.072	

Table 7: Data of Repeatability Test for Atenolol

Sample Name	Retention time	Area µAU*sec Atenolol	Area %
Sample 1	7.580	1428838	66.836
Sample 2	7.572	1427258	66.844
Sample 3	7.580	1428844	67.031
Mean	7.577	1428313	66.904
% RSD	0.061	0.064	0.165

Table 8: Data of Repeatability Test for Indapamide

Sample Name	Retention time	Area µAU*sec Indapamide	Area %
Sample 1	8.986	708991	33.164

Sample 2	8.988	707950	33.156
Sample 3	9.004	702786	32.969
Mean	8.993	706576	33.096
% RSD	0.110	0.470	0.333

Table 9: Summary of Repeatability

Parameter	**Acceptance Criteria**	Atenolol	Indapamide
%RSD of Area	**NMT 2**	0.064	0.47
Similarity Factor	**0.98 to 1.02**	0.98	1.00

Table 10: LOD for Atenolol and Indapamide

Sample Name	**LOD**
ATENOLOL	0.025 µg/ml
INDAPAMIDE	0.03 µg/ml

Table 11: LOQ for Atenolol and Indapamide

SAMPLE	**LOQ**
ATENOLOL	0.07 µg/ml
INDAPAMIDE	0.09 µg/ml

Table 12: Data for Change in Analyst:
Data for Atenolol

Sr. no	**Analyst**	**Sample**	**Amount of drug present(mg)**	**Amount of drug found(mg)**	**Area**	**% Label Claim**	**Tailing factor**
1	Analyst1	Sample1	50	48.55	4794325	97.1%	1.741
2	Analyst2	Sample2	50	49.50	4785357	99.0%	1.744
3	Analyst3	Sample3	50	49.40	4784689	98.8%	1.742
	%RSD				0.163		0.057

Data for Indapamide

Formulation Sample

Sr.no	**Analyst**	**Label Claim(mg)**	**Content of drug found (mg)**	**Sample**	**Area**	**% Label Claim**	**Tailing factor**
1	Analyst 1	2.5	2.42	Sample1	1975164	97.1%	0.675
2	Analyst2	2.5	2.57	Sample2	1997984	103.0%	0.683
3	Analyst3	2.5	2.52	Sample3	1991474	101.1%	0.673
	%RSD				0.163		0.616

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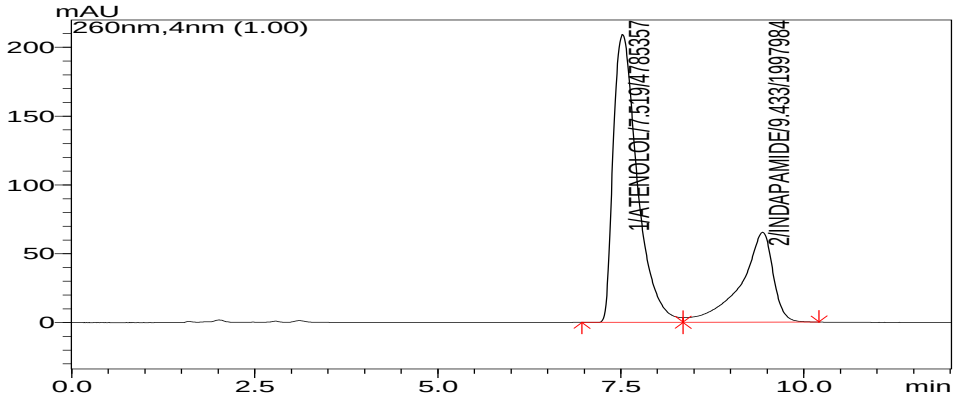

Table 13: Linearity for Atenolol in tablet

Sample Name	Concentration Atenolol	Area µAU*min Atenolol
Sample 1	5000	4794325
Sample 2	500	517772
Sample 3	50	106067
Sample 4	5	82465

Table 14: Linearity for Indapamide

Sample Name	Concentration Indapamide	Area µAU*min Indapamide
Sample 1	250	1995270
Sample 2	25	212740
Sample 3	2.5	41731
Sample 4	0.25	33185

Table 15: Summary of Linearity

Name	Correlation Coefficient
Atenolol	0.99
Indapamide	0.99
Acceptance Criteria	**NLT 0.99**

Conclusion

The present High Performance liquid chromatographic method is to determine Atenolol and Indapamide from its formulation. Various experiments were carried out to establish the method. The mobile phase was methanol : water (55:45) with 0.1% v/v of Ammonium Hydroxide is found to be ideal for the estimation of Atenolol and Indapamide. The elution order was as followed (Atenolol – 7.5min, Indapamide – 8.9 min). The values of linearity, Precision and standard deviation show that the proposed method was reproducible, accurate and precise.

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