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Research Article

A Stability Indicating RP-HPLC Method Development and Validation for Estimation of Atenolol Amlodipine Besylate Hydrochlorothiazide in their Combined Dosage Form

B. Naresh Kumar Reddy*[1], A. Dinakar Reddy[2]

[1]*PG Research Scholar, Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore, A.P.*
[2]*Professor, Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Nellore, A.P.*

Abstract

HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. Atenolol and Amlodipine besylate was freely soluble in water and alcohol. Hydrochlorothiazide was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100µg/ml. The % recovery of Atenolol, Amlodipine besylate and Hydrochlorothiazide were found to be in the range of 99.22%-100.11 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Good agreement was seen in the assay results of Pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Atenolol, Amlodipine besylate and Hydrochlorothiazide in Bulk drug and Pharmaceutical formulation.

Keywords: Atenolol, Amlodipine besylate and Hydrochlorothiazide, HPLC

Article Info

Corresponding Author
B. Naresh Kumar Reddy
Department of Pharmaceutical Analysis
Sun Institute of Pharmaceutical Education and Research, Kakupalli, Nellore, Andhra Pradesh, India.
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Contents

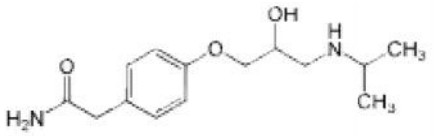
1. Introduction

Atenolol

IUPAC Name : 2-(4-{2-hydroxy-3-[(propan-2-yl) amino] propoxy} phenyl)acetamide

Chemical formula : $C_{14}H_{22}N_2O_3$

Structure :



Molecular weight : 266.3361

Cas No : 29122-68-7

Description: A cardio selective beta-adrenergic blocker possessing properties and potency similar to propranolol, but without a negative inotropic effect

Solubility : soluble in water

Melting Point : 158-160

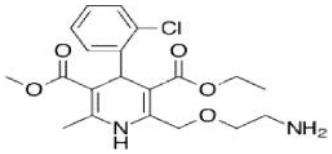
Pka : 9.6

Category: Antihypertensive Agents, Adrenergic beta-1 Receptor antagonists, sympatholytics, Anti-Arrhythmia Agents.

Mechanism of action: 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.

Brand name : Normiten, Myocord, Tenormin.

Amlodipine



Chemical name: 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

Molecular formula: $C_{20}H_{25}ClN_2O_5$

Molecular weight: 408.876 g/mol

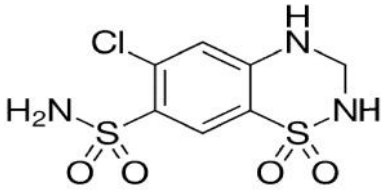
- **Categories:**
  ACE Inhibitors and Calcium Channel Blockers
- Agents Acting on the Renin-Angiotensin System
- Antianginal Agents

Mechanism of action:

Naltrexone is a pure opiate antagonist and has little or no agonist activity. The mechanism of action of naltrexone in alcoholism is not understood; however, involvement of the endogenous opioid system is suggested by preclinical data. Naltrexone is thought to act as a competitive antagonist at mc, κ, and δ receptors in the CNS, with the highest affintiy for the μ receptor. Naltrexone competitively binds to such receptors and may block the effects of endogenous opioids. This leads to the antagonization of most of the subjective and objective effects of opiates, including respiratory depression, miosis, euphoria, and drug craving. The major metabolite of naltrexone, 6-β-naltrexol, is also an opiate antagonist and may contribute to the antagonistic activity of the drug.

Branmd name: Amlobenz

Hydrochlorothiazide



Chemical Data

IUPAC Name: 6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ⁶,2,4-benzothiadiazine-7-sulfonamide

Chemical formula : $C_7H_8ClN_3O_4S_2$

Molecular weight : 297.739

CAS No : 58-93-5

Physical Data

Description: A thiazide diuretic often considered the prototypical member of this class. 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.

Brand Name: Hydrodiuril, Microzide, Esidrix, Oretic

2. Methodology

Table 1: Chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	0.45μ filter paper	Millipore	HPLC grade

Table2. List of chemicals use

S.no.	Name
1.	Atenolol working standard
2.	Amlodipine besylate working standard
3.	Hydrochlorothiazide working standard

Table 3. List of Instruments used

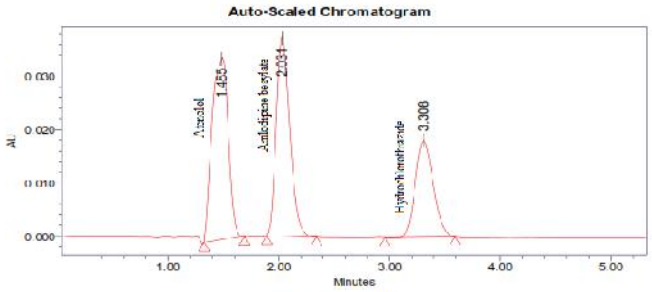
S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC-auto sampler –UV detector	Separation module2695, UV.detector2487	Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India

Method optimization trials:

Initial chromatographic conditions: There are several trails in selection of column and mobile phase solvents for the development of reliable method for simultaneous estimation of Atenolol, Amlodipine besylate and Hydrochlorothiazide. They are listed below.

Trial 1:

Mobile phase : Methanol: phosphate buffer P^H 3 (50:50)
Flow rate : 1 ml per min
Column : ThermoHypersil BDS, C18,(150 x 4..6 mm,5µm)
Detector wavelength : 260 nm
Detector : PDA
Column oven : Ambient
Injection volume : 10µl
Runtime : 8min
Rt : 1.485, 2.031 & 3.308



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Atenol	1.485	346212	34321	2.23	0.93	519	1
2	Amlodi	2.031	315344	37511	2.25	1.27	1295	1
3	Hydro	3.308	203947	18167	4.80	1.15	1952	1

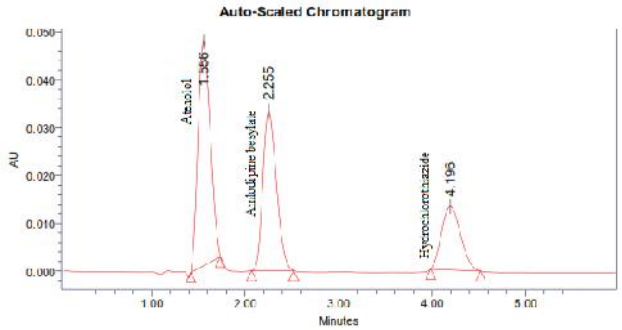
Fig.No.1 Chromatogram showing trail-1injection

Observation: The separation of analytical peaks is obtained but less resolution and plate count.

Trail 2:

Chromatographic conditions:

Mobile phase : Methanol: phosphate buffer P^H 3 (60:40)
Flow rate : 1 ml per min
Column : ThermoHypersil BDS, C18,(150 x 4..6 mm,5µm)
Detector wavelength : 260 nm
Detector : PDA
Column oven : Ambient
Injection volume : 10µl
Runtime : 8min
Rt : 1.556, 2.255&4.196



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Ateno	1.556	434937	47093	2.23	1.15	606	2
2	Amlodi	2.255	335440	33500	2.64	1.24	1125	2
3	Hydro	4.196	187031	13273	5.09	1.10	1913	2

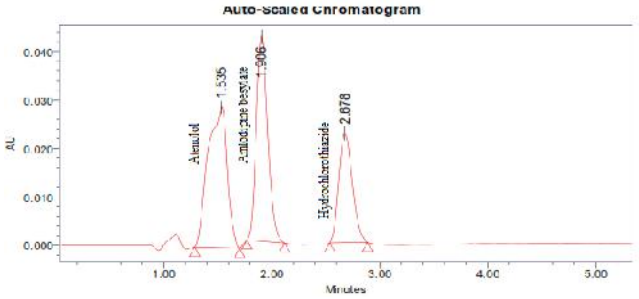
Fig.No.2 Chromatogram showing trail-2 injection

Observation: Peaks are separated but tailing has been observed.

Trial-3

Chromatographic conditions:

Mobile phase : Methanol: phosphate buffer P^H (65:35)
Flow rate : 1 ml per min
Column: ThermoHypersil BDS, C18,(150 x 4..6 mm,5µm)
Detector wavelength : 260 nm
Detector : PDA
Column oven : Ambient
Injection volume : 10ml
Run time : 8 min
Rt : 1.535, 1.9067&2.678



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Atenol	1.535	360947	29039	2.23	0.80	352	1
2	Amlodi	1.906	310417	42368	1.44	1.21	1520	1
3	Hydro	2.676	197065	22786	3.54	1.19	2087	1

Fig.No.3 Chromatogram showing trail-2 injection

Observation: Peaks were eluted but with less resolution peaks were seen. The chromatogram for trial 3.

Trial-4

Chromatographic conditions (Optimized Method)

Mobile phase : Methanol: phosphate buffer P^H (70:30)
Flow rate : 1 ml per min
Column: ThermoHypersil BDS, C18,(150 x 4..6 mm,5µm)
Detector wavelength : 260 nm
Column oven : Ambient
Injection volume: 10ml
Run time: 10 min
Rt: 1.763, 2.245& 3.171.

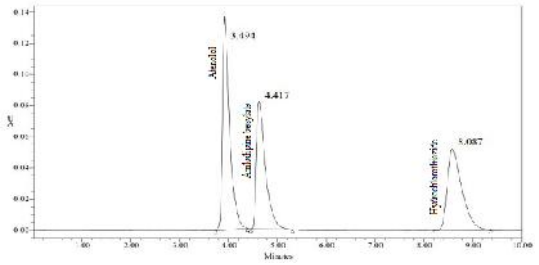


Fig.No.4. Chromatogram for Optimized Method.

Observation: Thus a method has been optimized where Atenolol, Amlodipine besylate and Hydrochlorothiazide. Both the components were eluted with good retention times & peak shapes.

Optimized method for atenolol, amlodipine besylate & hydrochlorothiazide.

Optimized equipment conditions:

Analytical conditions:

Mobile phase: Methanol: phosphate buffer P^H (70:30)
Flow rate : 1 ml per min
Column : ThermoHypersil BDS, C18,(150 x 4..6 mm,5µm)
Detector wavelength : 260 nm
Detector : PDA
Column oven : Ambient
Injection volume : 10ml
Run time : 10 min

Preparation of Standard Stock Solution

Weigh accurately about 10 mg of Atenolol, 10mg of Amlodipine besylate and 20mg of Hydrochlorothiazide were transferred into 100 ml volumetric flask and dissolve in 20ml of methanol and dilute with diluents to volume and mix.

Preparation of Working Standard Solution

Dilute 5ml of the above solution to 50ml with the diluents to obtain the concentration of 10µg/ml of Atenolol, 10µg/ml of Amlodipine besylate and 20µg/ml of Hydrochlorothiazide.

Sample preparation

Weigh 20 tablets and grind to fine powder in a dry mortar. Transfer 185 mg of the powder into a 100 ml volumetric flask. Add 20 ml of methanol and dissolve and add 25 ml of diluents and sonicate for 30 minutes and shake for 30 minutes. Dilute to volume with diluent and mix. Filter through 0.45µmembrane filter by discarding the first 5 ml. Dilute 5 ml of the above solution to 50 ml with the diluents.

Procedure:

The solutions of 100% level (i.e., solutions containing 10µg/ml of Atenolol, 10µg/ml Amlodipine besylate and 20µg/ml of Hydrochlorothiazide) which were previously prepared in duplicate were injected at the optimized method conditions and the chromatograms were recorded and the percentage drug content was calculated.

3. Results and Discussion

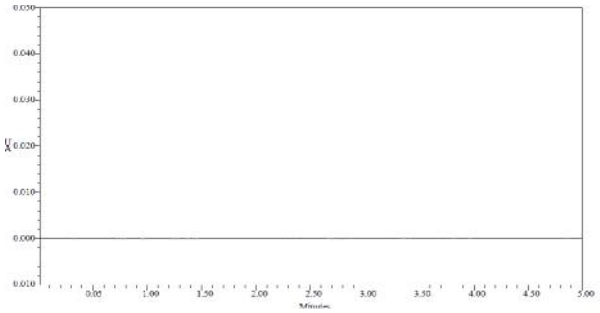


Fig.No.5. Chromatogram for blank

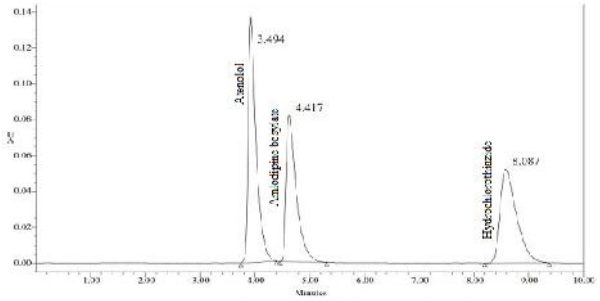


Fig.No.6. Chromatograph for System Suitability-1

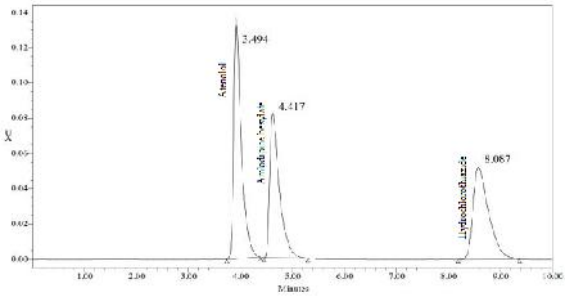


Fig.No.7. Chromatograph for System Suitability-2

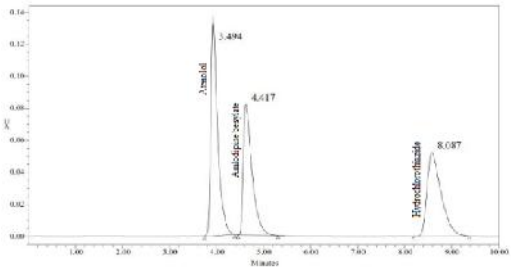


Fig.No.8. Chromatograph for System Suitability-3

Table: 4. Chromatogram values for System suitability of **Atenolol**

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	3.494	1250763	2487	1.62
2	3.494	1247867	2489	1.58
3	3.494	1255849	2496	1.64
Mean		1251360		
SD		3850.679		
% RSD		0.30722		

Acceptance Criteria:

1). Tailing factor Obtained from the standard injection is 1.7

2). Theoretical Plates Obtained from the standard injection is 2496

Table no: 5. Chromatogram values for System suitability of **Amlodipine besylate**

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.245	940627	2281	1.51	3.04
2	2.245	931161	2244	1.47	3.09
3	2.245	940306	2261	1.47	3.05
Mean		937364.7			
SD		5374.93			
% RSD		0.573409			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.51

2) Theoretical Plates Obtained from the standard injection is 2281

Table no-6. Chromatogram values for System suitability of Hydrochlorothiazide

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	8.087	932578	2508	1.25	12.95
2	8.087	934464	2594	1.25	13.23
3	8.087	920416	2567	1.23	13.16
Mean		929167.7			
SD		7598.933			
% RSD		0.817822			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.25

2) Theoretical Plates Obtained from the standard injection is 2594

Table: 7 Linearity results for Atenolol

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	839286
2	II	20 ppm	1067774
3	III	30 ppm	1246474
4	IV	40 ppm	1439994

5	V	50 ppm	1639065
Correlation Coefficient			0.999

Table: 8 Linearity results for Amlodipine besylate

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	626221
2	II	20 ppm	778750
3	III	30 ppm	931447
4	IV	40 ppm	1070162
5	V	50 ppm	1196060
Correlation Coefficient			0.999

Table: 9 Linearity results for Hydrochlorothiazide

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	631737
2	II	40 ppm	753615
3	III	60 ppm	899796
4	IV	80 ppm	1035191
5	V	100 ppm	1194356
Correlation Coefficient			0.999

Table 10: Chromatogram Values for Accuracy of Atenolol.

Sample No.	Accuracy	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 11: Chromatogram Values For Accuracy of Amlodipine besylate

Sample No.	Spike Level	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	10	9.8	98%	100%
		10	10.2	102%	
		10	10	100%	
2	100 %	20	19.8	99%	100%
		20	20.2	101%	
		20	20	100%	
3	150 %	30	29.6	98.66%	99.33%
		30	30	100%	
		30	29.8	99.33%	

Table 12: Robustness results for Amlodipine besylate

S.No	Drug	Mobile phase		
		More organic R_t	Organic R_t	Less organic R_t
1	**Hydrochlorothiazide Robustness Results**	4.705	4.754	4.802
USP Plate count		2482	2 2556	2030
USP Tailing		1.20	1.24	1.31

4. Conclusion

Atenolol and Amlodipine besylate was freely soluble in water and alcohol. Hydrochlorothiazide was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100 µg / ml. The % recovery of Atenolol, Amlodipine besylate and Hydrochlorothiazide were found to be in the range of 99.22 % - 100.11 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts.

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