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HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND ATENOLOL AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A Simple, specific and sensitive an isocratic Estimation by RP-HPLC analytical Method were developed and validated for the quantification Amlodipine. And Atenolol Quantification was achieved by using the mobile phase (55 volumes of mixed phosphate buffers ph-4.0: 45 volumes of acetonitrate sonicate for 10 mins for removing gases). Inertsil BDS C18 250×4.6mm ID, 5μm Particle size was used as stationary phase. The flow rate was 1.0 ml/min. Measurements were made at a isobestic wavelength of 220nm. The average retention times for Atenolol and Amlodipine was found to be 6.060 & 2.593 min. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear Atenolol and Amlodipine from 60-140μg/ml & 6-14 μg/ml for respectively. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Atenolol and Amlodipine.

KEYWORDS: ATENOLOL, AMLODIPINE, HPLC, UV, CAN.

DRUG PROFILE

Amlodipine

Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina.

Mechanism of Action

Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. Signal amplification is achieved by calcium-induced calcium release from the sarcoplasmic reticulum through ryanodine receptors. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation. The vasodilatory effects of amlodipine result in an overall decrease in blood pressure. Amlodipine is a long-acting CCB that may be used to treat mild to moderate essential hypertension and exertion-related angina (chronic stable angina). Another possible mechanism is that amlodipine inhibits vascular smooth muscle carbonic anhydrase I activity causing cellular pH increases which may be involved in regulating intracelluar calcium influx through calcium channels.

ATENOLOL

Mechanism of Action

Like metoprolol, 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

Need For the Study
Analytical Method Development for Pharmaceutical Formulations
Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .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. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Analytical method development provides the support to track the quality of the product from batch to batch.

Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase.

Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task.

METHOD DEVELOPMENT OF ATENOLOL AND AMLODIPINE

Trial - 1
Chromatographic conditions
Mobile phase: Potassium phosphate buffer: Methanol
Ratio: 55:45
Column: Zodiac C18 (250×4.6× 5μ)
wavelength: 220 nm
Flow rate: 1.0ml/min
pH: 5.8

Preparation of standard stock solution of AMLODIPINE
50mg of AMLODIPINE was weighed in to 500ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 μg/ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard stock solution
Weigh accurately 5 mg of ATENOLOL and 50 mg of AMLODIPINE in 50 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From the above stock solution pipette out 1ml of the solution and transfer to 10ml volumetric flask and make up the volume with mobile phase. This solution is used for recording chromatogram.

Result for ATENOLOL and AMLODIPINE by using mobile phase.

S.No.	Name	Rt (min)	Peak Area	Asymmetry	Efficiency	Resolution
1	AMLODIPINE	2.537	334.491	1.538	3133	-
2	ATENOLOL	2.997	3287.075	1.969	2313	2.137

Observation
- The Efficiency was satisfactory for both ATENOLOL and AMLODIPINE.
- But, Resolution was very low for ATENOLOL and AMLODIPINE. The details are given in table 8.3.1 and figure 8.3.1, Hence it was not taken for optimization.

Trial- 2
Chromatographic conditions
Mobile phase: Triethylamine in water: ACN
Ratio: 62:38
Column: ODS Borosil (250×4.6 ×5μ)
Wavelength: 220nm
Flow rate: 1.0ml/min
pH: 4.0

S.No.	Name	Rt (min)	Peak Area	Asymmetry	Efficiency	Resolution
1	AMLODIPINE	3.677	378.493	1.310	955	-
2	ATENOLOL	4.100	24.210	3.048	4533	1.177

Observation

- The resolution for ATENOLOL and AMLODIPINE were very low and USP plate count was very low for Atenolol.
- The details are given in the table 8.3.2 and figure 8.3.2, Hence it was not taken for optimization.

Trial-5: (Optimized)
Chromatographic conditions
Mobile phase: Mixed Phosphate Buffer: ACN
Ratio: 55:45
Column: Inertsil BDS C18 column (250×4.6mm× 5μ)
Wavelength: 220 nm
Flow rate: 1.0ml/min
pH: 5.0

S.No.	Name	Rt (min)	Peak Area	Asymmetry	Efficiency	Resolution
1	AMLODIPINE	2.593	323.207	2.176	5365	-
2	ATENOLOL	6.060	2225.385	1.345	4203	13.450

Observation: The efficiency was good for both ATENOLOL and AMLODIPINE, and the retention time and resolution was also satisfactory.

Table No.7.9.9.2: Assay Results.

	AMLODIPINE (5mg)		ATENOLOL (50mg)	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	312.900	317.362	2148.568	2153.195
Injection-2	320.146	315.955	2146.138	2166.818
Injection-3	315.731	312.02	2150.744	2151.623
Injection-4	316.723	318.27	2160.914	2159.717
Injection-5	316.877	312.645	2181.788	2141.013
Average Area	316.475	315.250	2157.63	2154.473
Tablet average weight	300.2		300.2	
Standard weight	5		50	
Sample weight	301.2		301.2	
Label amount	5		50	
std. purity	99.8		99.7	
Amount found in mg	4.95		49.61	
Assay(%purity)	99.08		99.22	

Observation

The amount of AMLODIPINE and ATENOLOL present in the taken dosage form was found to be 99.08% and 99.22% respectively.

METHOD VALIDATION

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Results for system suitability of AMLODIPINE(5mg)
Table 8.1.1: Injection.

	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.593	318.648	5365	1.818
2	2.573	319.324	4884	1.835
3	2.563	319.881	5242	1.718
4	2.533	318.495	4671	1.846
5	2.543	319.486	4771	1.756
6	2.567	319.750	5255	1.706
Mean	2.5620	319.264	-	-
SD	0.0215	0.573	-	-
%RSD	0.84	0.18	-	-

Results for system suitability of ATENOLOL(50mg)
Table 8.1.2: Injection.

	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	6.060	2216.293	4203	1.345	13.450
2	6.023	2204.889	4153	1.370	13.240
3	5.980	2199.833	4093	1.352	13.256
4	6.010	2199.586	4021	1.337	13.205
5	6.000	2198.585	4121	1.327	13.265
6	6.017	2204.017	4144	1.345	13.385
Mean	6.015	2203.867	-	-	-
SD	0.027	6.603	-	-	-
%RSD	**0.44**	**0.30**	-	-	-

Acceptance criteria
1. The % RSD for the retention times of AMLODIPINE (5mg) and ATENOLOL (50mg) Peaks from 6 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of AMLODIPINE (5mg) and ATENOLOL (50mg) peaks from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the AMLODIPINE (5mg) and ATENOLOL (50mg) peaks is not less than 2000.
4. The Tailing factor (T) for the AMLODIPINE (5mg) and ATENOLOL(50mg) peak is not more than 2.0.

Observation
The % RSD for the retention times and peak area of AMLODIPINE (50mg) and ATENOLOL (5mg) were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

DISCUSSION

A simple and selective LC method is described for the determination of AMLODIPINE (5mg) and ATENOLOL (50mg) tablet dosage forms. Chromatographic separation was achieved on a Inertsil BDS C18 column using mobile phase consisting of a mixture of Mixed Phosphate buffer(KH_2PO_4+K_2HPO_4) pH:4.0: Acetonitrile (55:45v/v),with detection of 220 nm. Linearity was observed in the range 6-14 µg /ml for AMLODIPINE (r_2 =0.9983) and 60-140µg /ml for ATENOLOL(r_2 =0.9983) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

SUMMARY AND CONCLUSION

S No	Parameters	Amlodipine(5mg)	Atenolol(50mg)
1	Calibration range mcg/ml	5mg	50mg
2	Optimized wavelength	220nm	220nm
3	Mobile phase	KH2PO:ACN55:45	KH2PO:ACN55:45
4	Column	Inerstsil BDS C18 (250mm 4.6mm 5µ)	Inerstsil BDS C18 (250mm 4.6mm 5µ)
5	Retention time	2.593	6.067
6	Regression equvation y*	y=25.825X+44.389	y=19.843X+137.21
7	Correlation coefficient (r2)	0.9983	0.9983
8	% Recovery	99.97%	99.18%
9	% RSD	0.84%	0.44%
10	Lod (mcg /ml)	0.4	5.26
11	Loq (mcg/ml)	1.22	15.94

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of AMLODIPINE(5mg) and ATENOLOL(50mg) was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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