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Development and Validation of A HPLC Analytical Assay Method for Amlodipine Besylate Tablet

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ABSTRACT

The objective of this work was to develop and validate analytical method for qualitative determination of amlodipine besylate in a tablet formulation. This method was performed with a cosmosil C18 column (250 mm X 4.6 mm, 5µ) at a flow rate of 1 mL/min and the run time is 10 min. The sample was analysed using acetonitrile: potassium dihydrogen orthophosphate buffer: methanol in the ratio of 44:46:10 and pH was adjusted to 3 with orthophosphoric acid and eluents were scanned using PDA detector at 239 nm. The retention time for Amlodipine was found to be 2.39 min. The method can be used for estimation of combination of these drugs in combined dosage form.

1.0 INTRODUCTION:

Amlodipine besylate belongs to the third generation antagonists which retain tissue selectivity and show favourable pharmacokinetic data [1]. The drug does not appear to cause postural hypotension, reflex tachycardia or cardiac conduction disturbances. Thus, amlodipine besylate provides an attractive therapeutic option for the treatment of hypertension, and offers potential for patients with angina pectoris [2].

Amlodipine is a member of 1, 4-dihydropyridine class of calcium antagonist approved for the treatment of heart diseases like hypertension and angina pectoris. It is a long acting calcium channel blocker that inhibits the influx of calcium ions into the vascular smooth muscle and cardiac muscle [3, 4]. Chemically amlodipine is 3-ethyl-5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydropyridine-6-methyl-3,5-dicarboxylate [5].

Literature survey reveals the availability of several methods for estimation of Amlodipine besylate [6-11] includes UV, HPLC as alone or in combination with other drugs. Furthermore these methods are not impressionable to achieve the high throughput study which can be possible by optimizing the method in such a way which includes shortest runtime with maximum selectivity. Hence, it can be maximum utilize for the analysis of formulation development and stability testing as well as at quality control laboratory for routine use.

2.0 MATERIALS AND METHODS:

A High Performance Liquid Chromatograph system, with LC solutions data handling system (WATERS ALLIANCE 2695 Separation module) with an auto sampler was used for the analysis. The data was recorded using WATERS EMPOWER software. A pure sample of Amlodipine was obtained from Wockhard Ltd. Aurangabad. HPLC grade Orthrophosphric acid, Acetonitrile and Methanol were procured from Qualigens fine chemicals. High pure water prepared by using Millipore Milli Q plus purification system.

2.1 Preparation of mobile phase

Several mobile phases at different ratios were used but no favorable results were obtained except for acetonitrile: potassium dihydrogen orthophosphate buffer: methanol (44:46:10), which gave an acceptable peak. 70 mM was selected as ionic strength for buffer solution at pH 3.0 to get a sharp symmetrical peak with good resolution.

2.2 Chromatographic conditions

WATERS C_{18} column 250 mm × 4.6 mm (5µm) with mobile phase as acetonitrile: 70mM potassium dihydrogen orthophosphate buffer: methanol (44:46:10) adjusted to pH 3 was used. Mobile phase flow rate was maintained at 1.0ml/min and detected at 239 nm. The retention time was 2.39 minutes.

2.3 Analysis of formulation

Preparation of buffer

3.40 gm of potassium dihydrogen phosphate was taken in1000 ml HPLC grade water mixed well and pH 3.0 adjusted with orthophosphoric acid. Buffer was filtered through 0.45 µ paper filter and degassed by sonication before use.

Preparation of Standard stock Solution:

100.0 mg of AMB working standard was weighed accurately and transferred into 100 ml volumetric flask that gives 1000 mcg solution. Different dilutions prepared according to requirement from the Standard solution.

Preparation of sample solution:

Weigh 20 tablets and crushed to fine powder. Tablet sample equivalent to 100mg was accurately weighed and transfer to 100 ml of volumetric flask volume adjusted with diluents i.e. 1000 µg/ml.

System Suitability Test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from six replicate injection of standard drug solution.

3.0 RESULTS:

3.1 Validation of the Developed Method

After chromatographic method development and optimization it was validated. The validation of an analytical method verifies that characteristics of the method satisfy the requirements of application domain. Proposed method was validated according to ICH guidelines for linearity, precision, accuracy sensitivity, and recovery (ICH Q2A, 2005).

1. Calibration curve (linearity)

Preparation of linearity solution:

A series of standard preparations of Amlodipine besylate was prepared over a range of 50% to 250% of the sample concentrations. For Amlodipine besylate, the range would be 50, 100, 150, 200 and 250 µg/ml. The result of linearity has shown in table 1 and Figure 1.

Table No. 1: Results of Linearity

S. No.	Linearity Level (%)	Vol. of stock Taken	Diluted	Concentration (ppm)	Avg. Area
1	50	5	100	50	1524976
2	100	10	100	100	2555554
3	150	15	100	150	3646350
4	200	20	100	200	4600680
5	250	25	100	250	5599702
		Correlation Coefficient			0.999
		Slope (m)			20389

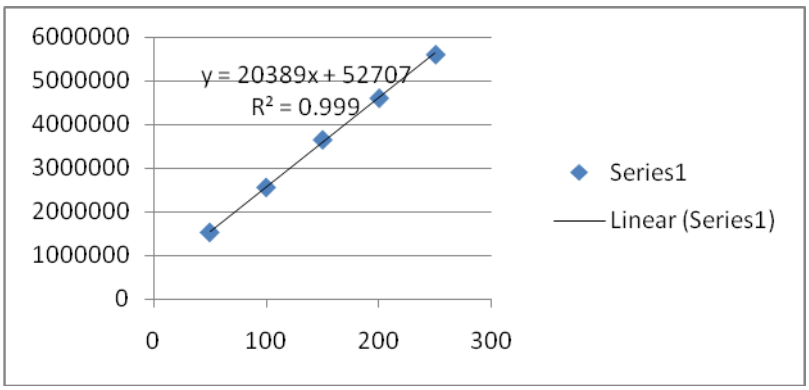

Fig. 1: Calibration curve of amlodipine besylate

2. Accuracy (Recovery): The result of accuracy has shown in table 2.

Table No. 2: Results of Accuracy

Concentration in %	Amt. of Drug added	Actual amount added	Area	Amt recovered	% recovery
50	24.90	17.83	2300582	17.90	100.4
50	25.20	18.04	2350725	18.29	101.4
50	25.30	18.12	2300895	17.90	98.8
100	49.99	35.80	4579056	35.64	99.5
100	50.10	35.88	4600832	35.81	99.8
100	50.20	35.95	4682983	36.45	101.4
150	75.00	53.71	6900582	53.71	100
150	75.00	53.71	6899382	53.70	100
150	75.00	53.71	6988456	54.39	101.3
				Average	100.2
				Std.Dev.	0.92
				%RSD	0.91

3. Precision

Repeatability precision:

In method precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results for a single aliqute. The result shown in table 3.

Table No.3: Results of Precision

Injection No.	Retention Time	Area
1	2.51	5503753
2	2.53	5504889
3	2.52	5569502
4	2.39	5470638
5	2.46	5545520
6	2.49	5530832
Mean		5518860
S.D.		38807.66
%RSD		0.706

4. Robustness

Standard preparation will be prepared as described under methodology and this solution will be injected as per methodology and under different chromatographic condition (variable/rugged parameters) as shown below.

4.1. High pH: The result shown in table 4.1.

Table No.4.1: Results of Robustness high pH

S. No.	Concentration	Area
1	250	5503723
2	250	5505623
3	250	5526723
4	250	5543212
5	250	5533254
6	250	5500023
Avg.		5518759.667
Std. Dev.		16437.52321
%RSD		0.297848143

4.2. Low pH: The result shown in table 4.2.

Table No..4.2 Results of Robustness low pH

S. No.	Concentration	Area
1	250	5543212
2	250	5511243
3	250	5534982
4	250	5553542
5	250	5512345
6	250	5600032
Avg.		5542559.333
Std.Dev.		32787.76298
%RSD		0.59156359

5. Limit of Detection

The lowest conc. of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental conditions simply indicates that the sample is below or above certain level.

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

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

$$LOD = 3.3*38807.66/20389$$

$$= 6.28\ \mu g/ml$$

6. Limit of Quantitation:

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is expressed as the concentration of analyte in the sample.

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

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

$$LOQ= 10*38807.66/20389$$

$$= 19.033\ \mu g/ml$$

4.0 SUMMARY AND CONCLUSION

In RP-HPLC method, the analyte were resolved by using isocratic programme and mobile phase Acetonitrile : Phosphate Buffer: Methanol (44:46:10),at a flow rate of 1ml/min, on HPLC auto-sampler system containing UV- visible detector with workstation Software and Cromosil C18 column (4.6 x 250 mm). The detection of Amlodipine Besylate was carried out at 239 nm. The method gave the good resolution and suitable retention time.

The results of analysis in all the method were validated in terms of accuracy, precision, linearity and range.

Validation:

Validation of these methods was performed as per the ICH guidelines for these following parameters.

1) **Accuracy -** Accuracy of the proposed method was ascertained from the recovery studies by standard addition method.

2) **Precision -** Replicate estimation by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed % R.S.D. <2.

3) **Robustness -** Studies were carried out for the different parameters like Change in pH. Results of estimation by proposed method are very much similar under variety of conditions. This study signifies the Robustness of the method under varying condition of its performance.

4) **Linearity and Range**- Amlodipine Besylate was found to be linear in the range of 50% to 150 % of test concentration with $R^2 \approx 1$ at selected wavelength.

RP-HPLC method for the estimation of Amlodipine besylate in tablet dosage form was found to be within the limits. Validation parameters like accuracy, precision and linearity showed low %RSD values which indicates that the method is precise and sensitive. Hence this method can be employed for laboratory works.

5.0 REFERENCES

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