International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Validation methods of amlodipine drug with comparative study of standard and sample by using UV-spectroscopy

Kollaboina Sekhar, Abbas S. Md, Chandra Mohan G, Eranna P, Hamana Naik D, Kalyan P, Mahesh T, Rajesh J, Varun B.*

Department of Pharmaceutical Analysis, Sri Lakshmi Venkateshwara Institute of Pharmaceutical Sciences, Kothapeta, Proddatur, Kadapa (dist.), Andhra Pradesh, India

Abstract

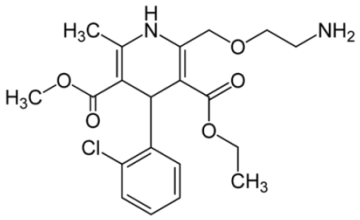
The present study describes validation method for amlodipine using UV-spectroscopy. During the validation process, the drug absorbance was measured from 336 – 338 nm. The method was validated in terms of Accuracy, Precision, Linearity, Ruggedness, Robustness, LOD, LOQ and Assay. The results of the analysis were validated statistically and by recovery studies. No interferences of impurities or excipients were detected during the validation procedure of drug in used formulation. Furthermore, the used method did not utilised any additional chemical reagents for color development with amlodipine. In conclusion, the validated method was very accurate, simple and less time consuming.

Keywords: Amlodipine; Validation; UV-spectroscopy; Comparative studies; Tablet; Analysis

Full length article **Corresponding Author*, e-mail: thummalakuntamahesh@gmail.com

1. Introduction

Validation is a process of checking or proving the validity or accuracy of the product. A wide variety of procedures, processes and activities need to be validated. The process validation is the collection and evaluation of data from the process design stage throughout production, which establish scientific evidence that a process can produce quality products. Anti-hypertensive is the class of drugs that is used to treat hypertension. Anti-hypertensive drugs are used to reduce the blood pressure such as stroke and myocardial infraction. There are many classes of anti-hypertensives, which lower blood pressure by different means. Among the most important and most widely used drugs are ACE inhibitors, calcium channel blockers, and thiazide diuretics etc. Calcium channel blockers are a group of medications that disrupt the movement of calcium through calcium channels. These drugs are used to lower blood pressure by lowering the movement of calcium into the cells of the blood vessels walls. It makes easier to pump the blood through the blood walls. As a result, the heart doesn't have to work as hard and blood pressure lowers. Calcium channel blockers include Amlodipine, Diltiazem, Verapamil, Nisoldipine, Isoptin, Felodipine, and Nicardipine etc. In the present study amlodipine is used.



IUPAC name

(RS)-3-ethyl 5-methyl 2[(2-amino ethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4dihydropyridine-3,5-dicarboxylate

Amlodipine, initially approved by the FDA in 1987, and is a popular anti-hypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers. Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has anti-oxidant properties and an ability to enhance the production of nitric oxide (NO). It is an important vasodilator that decreases the blood pressure. Amlodipine sold under the brand name Norvasc.

2. Materials and methods

The instrument used for the present study was genesis 10s UV-Visible spectrophotometry with quartz cell size- length 10 mm, diameter 45×12.5×12.5.

2.1. Method validation

2.1.1. Solubility test

Solubility test of the drug amlodipine was performed by using various solvents. The solvents include water, methanol, chloroform and acetone. But it was found that amlodipine soluble in methanol and water in the ratio of methanol: water (60:40).

2.1.2. Determination of λ_{max}

Preparation of stock solution

Accurately weighed 10 mg of amlodipine and transferred into 10 ml capacity volumetric flask; then added 5 ml of diluent (methanol) and sonicated the mixture for 20 min. Flasks were made up with diluent and labelled as standard stock solution. Aliquots of standard stock solution were pipetted out and suitably diluted with diluent to get the final concentration of 2-14 µg/ml. The solutions were scanned in spectrum mode from 200-400 nm wavelength range.

Preparation of sample stock solution

Twenty tablets were weighed and powdered. About 10 mg of amlodipine weighed and transferred into 10 ml of volumetric flask, then added 5 ml of diluents and sonicated mixture for 20 min, Final volume make up was using methanol and solution was filtered by HPLC filters before use.

Validation methods

In general, validation is defined as the process used for the confirmation of method validity. It is employed to check the suitability of the analytical procedure employed for a specific test. Validation is also considered as an integral part of any good analytical practice.

Accuracy

The nearness of the test result which is obtained by the true value or reference result is expressed by the accurate analytical procedure. The experiment was performed in triplicates at levels 80, 100 & 120 % of test concentration using amlodipine working standard as per the test method and measured absorbance of each solution thrice. The recovery results showed that the proposed method as an acceptable level of accuracy for SFS which was from 80-120 %.

Preparation of standard stock solution

Twenty tablets were weighed and powdered. About 10 mg of amlodipine was transferred into 10 ml capacity volumetric flask, and then added 5 ml of diluent (methanol) and sonicated mixture for 20 min. Final volume make up was using methanol and solution was filtered by HPLC filters before use.

Preparation of 80 % spiked solution

Powdered tablet equivalent to 8 mg of amlodipine weighed and transferred into a 10 ml capacity volumetric flask, and then added 5 ml of diluent (methanol) and sonicated mixture for 20 min, further the volume was made up of with diluent and filtered by HPLC filters. From this solution, 0.1 ml was taken into a 10 ml capacity volumetric flask and volume was made up to the mark with diluent.

Preparation of 100 % spiked solution

Powdered tablet equivalent to 10 mg of amlodipine weighed and transferred into a 10 ml capacity volumetric flask, then added 5 ml of diluent (methanol) and sonicated mixture for 20 min, further volume was made up with diluent and filtered by HPLC filters. From this solution, 0.1 ml was taken into a 10 ml volumetric flask and made up volume to the mark with diluent.

Preparation of 120 % spiked solution

Powdered tablet equivalent to 12 mg of amlodipine was weighed and transferred into a 10 ml volumetric flask, then added 5 ml of diluent (methanol) and sonicated mixture for 20 min. Further volume made up was with diluent and filtered by HPLC filters. From this solution, measured 0.1 ml into 10 ml capacity volumetric flask and made up volume to the mark with diluent.

Acceptance criteria

The percentage (%) recovery for each level should be between 98 to 102.

Precision

Precision of an analytical method is a measurement of its nearness of agreement between a series of measurement by carrying out the analysis from multiple sampling of the same identical sample under the certain situation. The Intra and Inter day precision of the marketed formulation was analysed on the same day at different time intervals on different days, respectively. Precision of the method was estimated by carrying out six independent assays of test samples of Amlodipine. The intermediate precision of the method also determined at different days with two different analysis in the laboratory.

Preparation of sample stock solution

Twenty tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of Amlodipine weighed and transferred into a 10 ml volumetric flask, then 5 ml of diluent was added and sonicated for 25 min. Final volume make up was using methanol and solution was filtered by HPLC filters before use.

Intra-day precision

Six working samples solution of 10 ppm were injected and percentage amount found was calculated. The percentage relative standard deviation (% RSD) was found to be 0.56 %. As the limit of precision was less than "2" the system precision was acceptable for this method.

Inter-day precision

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentration were prepared, and absorbance was measured (Table). Average area percentage relative standard deviation (% RSD) was 0.26 % for the drug under study. As the limit of precision was less than "2" the system precision was passed for this method.

Specificity

Analysis of marketed formulations was done to estimate the presence of impurities, degradants, and excipients in the sample and was compared with standard drug. For this λ_{max} and absorbance of reference standard solution and sample solution were measured.

Limit of detection [LOD]

Sample preparation

0.25 ml of standard stock solution was pipetted out and transferred to 10 ml capacity volumetric flask. The volume make up was with diluent. From the above solution 0.1 ml of amlodipine was transferred to 10 ml volumetric flask and volume make up was using same diluent. Detection limit of amlodipine in this method was found to be 0.27 µg/ml.

Limit of quantification

Sample preparation

0.25 ml of standard stock solution was pipetted out and transferred into 10 ml capacity volumetric flask and made volume up to the mark using diluent. From the above mixture, 0.3 ml of amlodipine, were transferred into 10 ml capacity volumetric flask and make up volume up to the mark with diluent. Quantification limit of the amlodipine in this method was found to be 0.082 µg/ml.

Robustness

Robustness of the method was determined by carrying out the analysis under different wavelengths (334–336 nm) using 10 ppm solution. Percentage relative standard deviation (% RSD) was calculated.

Ruggedness

Six working sample solutions of 10 ppm were injected and percentage relative standard deviation (% RSD) was found to be 0.34 %. As the limit of precision was less than "2" the system precision was passed for this method.

Assay

Twenty tablets were accurately weighed and a quantity of tablet powder equivalent to 5 mg of amlodipine besylate was weighed and then dissolved in 100 ml of methanol. The solution was further diluted to obtained final concentration of 25 µg/ml. The sample solution was analysed and % of drug content was determined from the absorbance using the regression equation obtained after calibration.

3. Results and discussions

The method followed for validation of amlodipine was found to be precise as the percentage standard deviation (% RSD) values for intra-day and inter-day were found to be 0.56 % and 0.26 %, respectively. First order kinetics values obtained at each added concentration indicated that the method was accurate. The validation method percentage recovery was ranging from 99.5-101.6 %. The LOD and LOQ were found to be within the limits indicating sensitivity of the method. The method was found to be of good robustness (0.3 %) and ruggedness (0.16 %) as the percentage recovery was less than 2%. Assay results indicated that the amount of drug was in good agreement with the label claim of the respective formulation. The results are given in the following tables.

Accuracy

Level of recovery (%)	Label claimed amount (mg)	Amount recovered (µg/ml)	% Recovery	Mean % recovery
80%	5	78.62	99.47	99.44
100%	5	99.56	99.65	99.44
120%	5	118.49	99.21	99.44

Precision

Intra-day

Sr. No	Absorbance
1	0.620
2	0.627
3	0.630
4	0.625
5	0.627
6	0.629
Mean	0.626
Standard Deviation	0.004
% RSD	0.568

Inter-day

Sr. No	Absorbance
1	0.622
2	0.618
3	0.621
4	0.622
5	0.620
6	0.618
Mean	0.620
Standard Deviation	0.003
% RSD	0.264

Limit of detection

Sr. No	Wavelength (nm)	Absorbance
1	396	0.022
2	368	0.021
3	363	0.020
4	361	0.016
5	356	0.015
6	353	0.014
7	242	0.011
8	338	0.010
9	320	0.010
10	315	0.009
11	312	0.006
12	309	0.005
13	307	0.003
14	305	0.001

Limit of quantification

Sr. No	Wavelength (nm)	Absorbance
1	397	0.012
2	371	0.005
3	370	0.014
4	360	0.010
5	358	0.006
6	355	0.004
7	348	0.007
8	347	0.004
9	347	0.008
10	342	0.085
11	337	0.098
12	336	0.075
13	335	0.078
14	330	0.081

Linearity

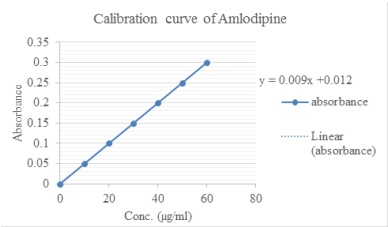
Sr. No	Concentration	Wavelength	Absorbance	% Linearity
1	10	0	0	10
2	20	320	0.062	20
3	30	322	0.102	30
4	40	326	0.167	40
5	50	328	0.198	50
6	60	330	0.247	60
7	70	332	0.256	70
8	80	341	0.267	80

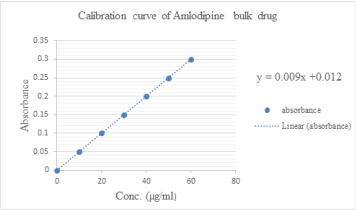
Ruggedness

Sr. No	Absorbance
1	0.623
2	0.624
3	0.619
4	0.625
5	0.621
6	0.622
Mean	0.622
Standard Deviation	0.002
% RSD	0.347

Robustness

Sr. No	Concentration	Absorbance
1	10	0.462
2	10	0.461
3	10	0.462
4	10	0.461
5	10	0.460
Mean	-	0.4612
SD	-	0.000748
% RSD	-	0.16218





4. Conclusions

The bulk and dosage forms were validated in terms of Accuracy, Linearity, Specificity, Precision, LOD, LOQ, Robustness, Ruggedness and Assay. The results of the study were validated statistically and by recovery studies. The validation results indicated that the amount of drug was in agreement with label claim of the formulation. It was observed that there was no interference of impurities or excipients during the validation of drug formulation. This study, thus explored the possibilities for determining pharmacokinetic profile of Amlodipine. The proposed spectroscopic methods were found to be simple, precise, highly accurate and less time consuming.

References

1. [2007] The Indian Pharmacopoeia Commission, Indian Pharmacopoeia, Ghaziabad, India, pp.714
2. Mishra P, Guptha Alka, Shah K [2007]. Simultaneous estimation of atorvastatin calcium and amlodipine besylate from tablets. IPCS 69:831-833.
3. Thomas Asha B [2004] simultaneous spectrophotometric estimation of amlodipine besylate and telmisartan in tablet dosage form. Der pharma chemical 4(2):725-730
4. ICH [2005] validation of analytical procedure: text and methodology. ICH Guideline Q2[R1], Switzerland.
5. Jain Nilesh [2010] spectrophotometric method and validation for Quantitative Estimation of Amlodipine besylate in bulk drug and their dosage form by using Hydrotropic Agent. Eurasian Anal Chem 5[3]:212-217.
6. Bernard Shyny [2011] spectrophotometric method of estimation of Amlodipine besylate using Hydrotropic solubilization. Journal of applied pharmaceutical sciences 1[9]:177-180.
7. ICH guidelines, for stability testing of new drug substances and products Q1A (R2) 2004.