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Stability Indicating Assay Method Development and Validation of Amlodipine Besylate in Bulk and Tablet Dosage form by UV Spectroscopy and RP-HPLC

Rubeen Dadakalandar Nadaf[1], Sudhahar Dharmalingam[2], Shweta Harshal Zalte[3], Shyma Sundar Sahu[4], Swarnima Pandey[5], Deepak Sharma[6], Shanmugam Vippamakula[7], Awinish Kumar Shrivastaw[8], Raveesha Peeriga[9], Ramanlal N. Kachave[10*]

[1]KAHER's Dr. Prabhakar Kore Basic Science Research Center, Belagavi, Karnataka, India

[2]Department of Pharmaceutical Chemistry and Analysis, Nehru College of Pharmacy, Thiruvilwamala, Thrissur, Kerala, India

[3]Assistant Professor, Matoshri College of Pharmacy, Eklahare, Nashik, 422003, Maharashtra, India

[4]School of Pharmacy, Centurion University of Technology and Management, Odisha, India

[5]Principal, Apex College of Pharmacy, NH 87, Village Kaushaganj, Tahsil Bilaspur, Rampur, 244923, Uttar Pradesh, India

[6]Professor, Department of Pharmaceutical Analysis, Institute of Pharmacy, Assam Don Bosco University, Tapesi Assam, 782402, India

[7]Department of Pharmaceutics, MB School of Pharmaceutical Sciences (Erstwhile Sree Vidyaniketan College of Pharmacy), Mohan Babu University, Tirupati, Andhra Pradesh, India

[8]Assistant Professor, Maharana Pratap College of Pharmacy, Mandhana, Kanpur, 209217, Uttar Pradesh, India

[9]V. V. Institute of Pharmaceutical Sciences, Seshadri Rao knowledge Village, Gudlavalleru -521356, Krishna District, Andhra Pradesh.

[10]GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research Nashik, Affiliated Savitribai Phule Pune University, Pune, Nashik, Maharashtra, 422005, India

***Corresponding author: Ramanlal N. Kachave,** GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research Nashik, Affiliated Savitribai Phule Pune University, Pune, Nashik, Maharashtra, 422005, India

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KEYWORDS
Amlodipine Besylate,
UV Spectroscopy,
RP-HPLC,
Stability indicating assay method

ABSTRACT:

A simple, cost effective, highly precise and robust stability indicating UV Spectroscopy and RP-HPLC method was developed for the estimation of Amlodipine in bulk and tablet dosage form. The chromatographic separation was achieved on Agilent C18 (250mm×4.6mm, 5µm) with mobile phase methanol: water in the ratio of 80:20 v/v with flow rate of 1ml/min at detection wavelength 238nm using Photodiode array detector. The method was validated for linearity, precision, accuracy, LOD, LOQ and robustness. The linearity range by UV Spectroscopy was found to be 10-30µg/ml and by RP-HPLC method was found to be 10-50µg/ml with correlation coefficient of 0.999 for both the methods. The relative standard deviation values for precision, accuracy and robustness were less than 2%. The LOD was found to be 1.84µg/ml and 1.56µg/ml for UV Spectroscopy and RP-HPLC method respectively. The LOQ was found to be 5.59µg/ml and 4.81µg/ml for UV Spectroscopy and RP-HPLC method respectively. Amlodipine was subjected to stress conditions of degradation including acid degradation, alkaline degradation, Oxidation, photolysis and thermal degradation. The method is simple, reliable, sensitive and precise which could separate the drug and their degraded product under various stress conditions,

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thus it can be employed as stability indicating method for the determination of Amlodipine in bulk and tablet dosage form.

Introduction

Amlodipine Besylate (AB) is chemically 3-ethyl 5-methyl (4RS) 2-[(2 aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulfonate (fig.1) It is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, angina pectoris and prinzmetal variant angina. Amlodipine is an intrinsically long acting, vasoselective calcium channel antagonist that inhibits calcium ion influx across the cell membranes selectively, with a great effect on vascular smooth muscle cells than on cardiac muscle cells. It is well absorbed after oral administration (1). Oral administration of 10 mg dose resulted into high absolute bioavailability of 64%. It has large volume of distribution (2). Amlodipine is 97% bound to plasma proteins. Metabolism of amlodipine is extensive but relatively slow. Grapefruit juice can alter the oral pharmacokinetics due to irreversible inactivation of intestinal CYP3A4.Around 62% of amlodipine is recovered from urine and the remainder in the faces after I.V administration(3,4). The literature survey revealed that Amlodipine besylate was analyzed in combination with other drug (5-9). Besides only one analytical technique was preferred for analysis. In the present research work amlodipine besylate is being analyzed individually and by UV Spectroscopy as well as RP-HPLC method and the method is developed and validated as per ICH guidelines (10-15). Further forced degradation study was also carried out for the same(16-19).

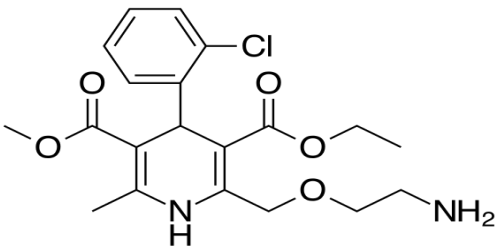

Figure 1. Amlodipine Besylate

Experimental

Chemicals

Amlodipine Besylate (API) was kindly provided by Amrutvahini College of pharmacy. Amlip-5 (5 mg AB) tablet was purchased from local pharmacy. Methanol, Hydrochloric acid, Sodium hydroxide, Hydrogen Peroxide was obtained from Merck. All reagents used, were of analytical grade except methanol which was HPLC grade. HPLC grade water was obtained through a Milli-Q system (Merck Millipore).

HPLC instrumentation

The chromatographic system used to perform development and validation of this method consisted of an Agilent technology 1260 Infinity II RP-HPLC instrument equipped with quaternary pump, sample injector with a 20µL loop, Agilent C_{18} column (250mm i.d ×4.6 mm, 5µm particle size) and PDA detector was used. The mobile phase consisted of methanol: water in the ratio of 80:20 v/v at a flow rate of 1ml/min. The detection was performed at wavelength 238 nm.The column was maintained at ambient temperature (25°C) and an injection volume of 20µl was used. The mobile phase was filtered through 0.45µm nylon membrane filter paper prior to use. The HPLC system equipped with open lab software for data acquisition and quantification of peaks.

Preparation of stock and standard solutions

Stock solution of Amlodipine Besylate was prepared by transferring accurately weighed 10 mg of Amlodipine Besylate into a 100 ml volumetric flask and small amount of mobile phase was added. The mixture was sonicated for 5 min and solution was diluted with distilled water for UV Spectroscopy method and with the mobile phase for RP-HPLC method to prepare 100 µg/ml of standard stock solution. Aliquots of the standard stock solutions were transferred using bulb pipettes into 10ml volumetric flasks and the solutions were made up to volume with mobile phase to yield.

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Preparation of marketed stock solution

Ten tablets were weighed and powdered. Weighed powder equivalent to 10 mg of drug was transferred into 100 ml volumetric flask and small quantity of mobile phase was added. The solution was sonicated for 15 min and then filtered through nylon membrane filter paper (0.45µ) and the final volume was made with mobile phase. Then further concentrations of dilutions were prepared in triplicate.

Development of UV Spectroscopy method

The solvent system for method development was selected depending upon the solubility of drug in different solvents. According to the solubility and economical point of view initially methanol was used to dissolve the drug and final volume was made up with distilled water for method development. The standard stock solution and further dilutions were prepared and scanned over the range of 200-400 nm. The spectrum was recorded and λ max was observed for Amlodipine Besylate. It was found to be 238 nm.

Development of RP-HPLC method

Agilent C18 (250mm×4.6mm, 5µm) column maintained at ambient temperature (25°C) was used for the method development and validation of Amlodipine Besylate. The composition and flow rate of mobile phase were changed to optimize the separation conditions. A mobile phase consisting of methanol: water in the ratio of 80:20 v/v at a flow rate of 1ml/min was selected for further studies after several trials and chromatographic runs (20-22). Under the described chromatographic conditions the peak was well defined, has good shape with theoretical plates as per the acceptance criteria (23). The chromatogram for Amlodipine Besylate is shown in Figure 2.

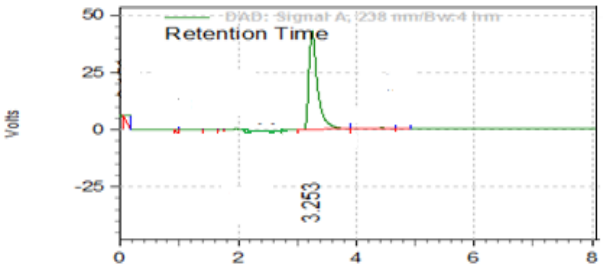

Figure 2. Chromatogram for Amlodipine Besylate

Forced degradation studies

The forced degradation studies were conducted on API by acid and alkaline degradation, oxidative, photolytic and thermal degradation.

Acid degradation

Accurately 10 mg of Amlodipine Besylate drug was weighed and transferred into 100 ml volumetric flask and volume was made up to mark with mobile phase (100 µg/ml). From the above solution, 2 ml was pipette out into 10 ml volumetric flask and volume was made with 0.1N HCL (20µg/ml).The flask was kept at room temperature for 3 hrs. The solution was filtered through 0.45µ syringe filter and injected in HPLC and peak area was measured. Shown in Figure 3.

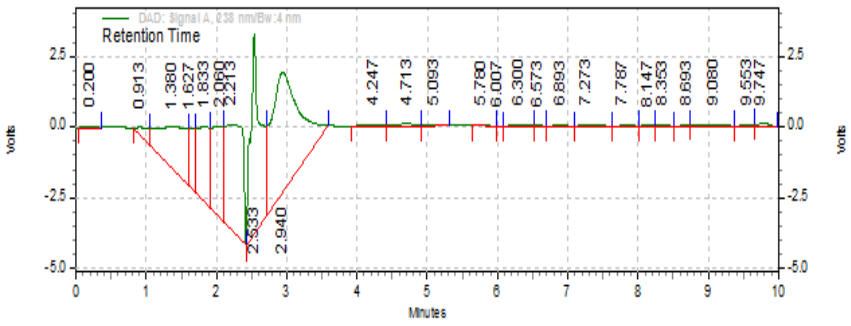

Figure 3. Chromatogram for acid degradation

Alkaline degradation

Accurately 10 mg of Amlodipine Besylate drug was weighed and transferred into 100 ml volumetric flask and volume was made up to mark with mobile phase (100 µg/ml). From the above solution, 2 ml was pipette out into 10 ml volumetric flask and volume was made with

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0.1N NaOH (20µg/ml).The flask was kept at room temperature for 3 hrs. The solution was filtered through 0.45µ syringe filter and injected in HPLC and peak area was measured. Shown in Figure 4

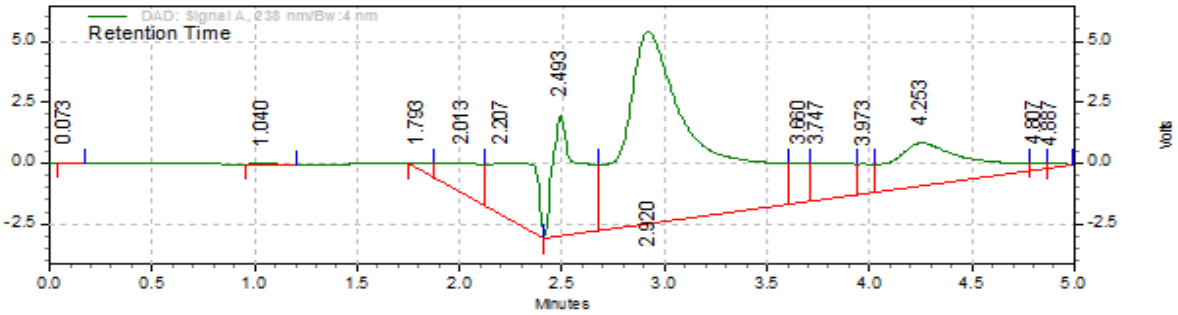

Figure 4. Chromatogram for alkaline degradation

Oxidative degradation

Accurately 10 mg of Amlodipine Besylate drug was weighed and transferred into 100 ml volumetric flask and volume was made up to mark with mobile phase (100 µg/ml).From the above solution, 2 ml was pipette out into 10 ml volumetric flask and volume was made with 3% hydrogen peroxide (20µg/ml).The flask was kept at room temperature for 3 hrs. The solution was filtered through 0.45µ syringe filter and injected in HPLC and peak area was measured. Shown in Figure 5

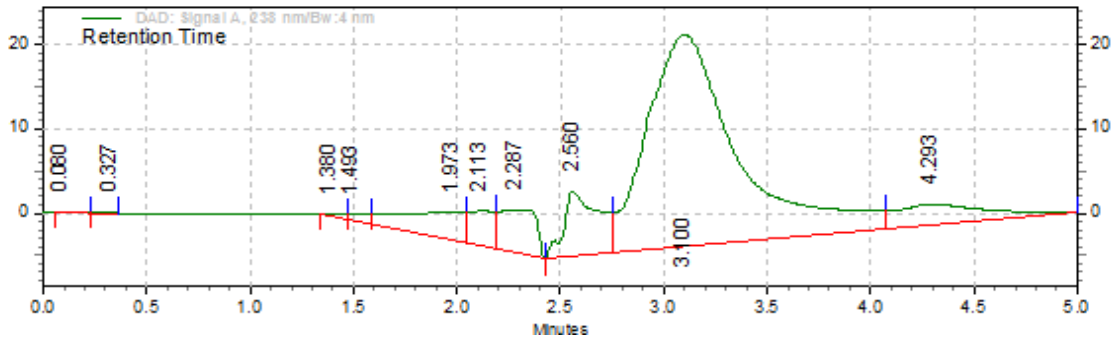

Figure 5. Chromatogram for oxidative degradation

Photolytic degradation

Accurately 10 mg of Amlodipine Besylate drug was weighed and transferred into 100 ml volumetric flask and volume was made up to mark with mobile phase (100 µg/ml).From the above solution, 2 ml was pipette out into 10 ml volumetric flask and volume was made with mobile phase (20µg/ml).The flask was kept in UV chamber for 14 hrs. The solution was filtered through 0.45µ syringe filter and injected in HPLC and peak area was measured. Shown in Figure 6

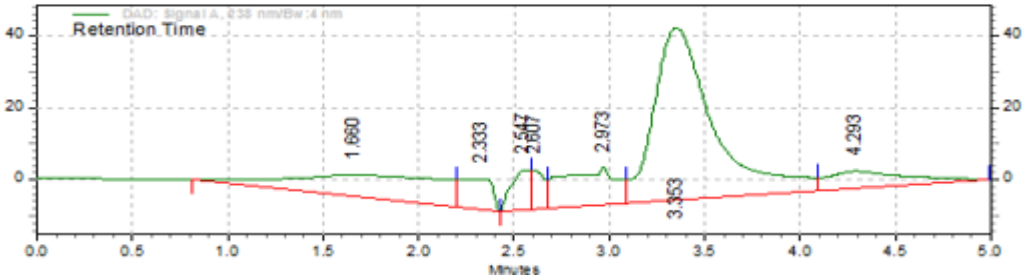

Figure 6. Chromatogram for photolytic degradation

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Thermal degradation

Accurately 10 mg of Amlodipine Besylate drug was weighed and transferred into 100 ml volumetric flask and volume was made up to mark with mobile phase (100 µg/ml). From the above solution, 2 ml was pipette out into 10 ml volumetric flask and volume was made with mobile phase (20µg/ml).The flask was kept in oven at 80ºC for 7 hrs. The solution was filtered through 0.45µ syringe filter and injected in HPLC and peak area was measured. Shown in Figure 7.

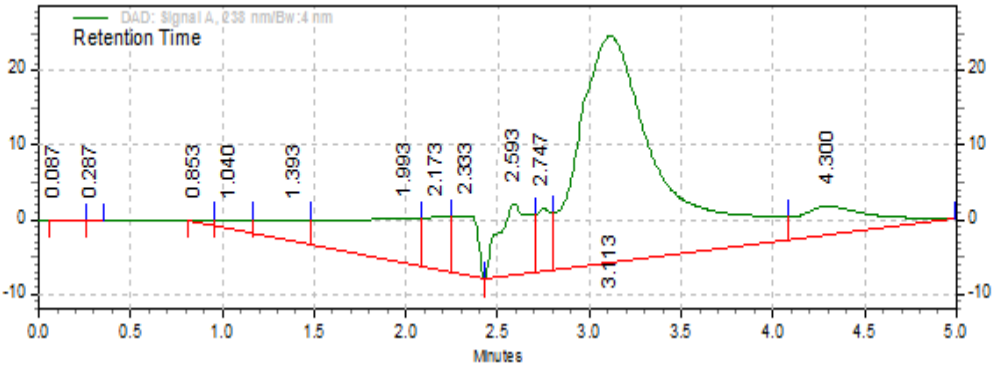

Figure 7. Chromatogram for thermal degradation

Result and discussion

Method validation (20-21)

The method was validated with respect to parameters such as linearity, precision, accuracy, robustness, limit of quantitation (LOQ) and limit of detection (LOD).

System suitability parameters

System suitability parameters were assessed for theoretical plates, asymmetry and retention time. The data is shown in Table 1 for the same.

Table No. 1 System suitability parameters

Sr. No.	Parameters	Amlodipine Besylate
1.	Peak Area	907845
2.	Theoretical plate	2850
3.	Retention time	3.253 min
4.	Asymmetry	1.3

Linearity

Linearity was established by using standard solutions prepared to produce solutions of five different concentrations with five replicates and linear regression analysis of calibration curve. The constructed calibration curves were linear over the concentration range of 10-30µg/ml by UV Spectroscopy and 10-50 µg/ml by RP-HPLC method. Correlation coefficient was found to be 0.999 in both the methods. The linearity data is shown in Table 2.

Table No. 2 Table for linearity Amlodipine Besylate

Parameters	UV Visible Spectroscopy Method	HPLC Method
λ_{max} wavelength (nm)	238	238
Linearity range (µg/ml)	Oct-30	Oct-50
Slope (m)	0.034	22837
Intercept (C)	0.006	27403
Regression coefficient r^2	0.999	0.999

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Precision

The precision of the method was evaluated by performing repeatability. For that, three concentrations 10, 15, 20 µg/ml with three replicates of each were analysed for UV Spectroscopy and three concentrations 10, 20, 30µg/ml with three replicates of each for RP-HPLC method. Intermediate precision was performed by evaluating inter-day variation and analyst to analyst variation in case of UV method and inter-day variation in case of RP-HPLC method. The precision data is shown in Table 3.

Table No. 3 A Table for precision data

Parameters	UV Visible Spectroscopy		HPLC	
	S.D	%RSD	S.D	%RSD
Repeatability	0.712	0.719	0.912	0.926
Inter-day precision	0.861	0.884	1.36	1.40
Analyst to analyst variation	1.21	1.23	-	-

LOD and LOQ

The LOD and LOQ were for both the methods were carried out by evaluating the standard deviation of the response and the slope of the calibration curve. The LOD was found to be 1.84µg/ml and 1.56µg/ml for UV Spectroscopy and RP-HPLC method respectively. The LOQ was found to be 5.59µg/ml and 4.81µg/ml for UV Spectroscopy and RP-HPLC method respectively.

Robustness

The robustness of the method was evaluated by making small deliberate changes in the solvent concentration in case of UV method and by changing flow rate to ±1 ml/min in RP-HPLC method. Results are shown in Table 4.

Table 4 Table for robustness

Parameters	S.D	%RSD
Change in solvent ratio	1.12	1.15
Change in flow rate		
0.9 ml/min	0.38	1.43
1 ml/min	1.65	1.68
1.1 ml/min	1.72	1.72

Accuracy

The accuracy of the method was determined by performing tablet pre analysis and then by preparing solutions of different concentrations that is 80%, 100% and 120% for both the analytical methods. Results are shown in Table 5.

Table 5 Table for accuracy Amlodipine Besylate

Parameters	UV Visible Spectroscopy		HPLC	
	S.D	%RSD	S.D	%RSD
Tablet pre analysis	0.208	0.205	0.51	0.60
80%	0.85	0.99	1.39	1.43
100%	1.24	1.40	1.34	1.44
120%	1.54	1.56	1.27	1.32

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Forced degradation

The forced degradation studies were carried out on API. The API was found to be more sensitive to acid degradation than alkaline degradation. The % degradation in acid was found to be 47.01% and 43.79% in case of alkaline degradation. In oxidative degradation the degradation was found to be 44.35%, in photolysis it was found to be 50.66% and in case of thermal degradation was found to be 54.75%.The forced degradation data is shown in Table 6

Table No.6 Data for forced degradation

Stress conditions	%Degradation
Acid degradation	47.01
Alkaline degradation	43.79
Oxidative degradation	44.35
Photolytic degradation	50.66
Thermal degradation	54.75

Conclusion

In this study, the developed stability indicating chromatographic assay method was found to be suitable for determination of Amlodipine Besylate in the presence of degradation product and in pharmaceutical tablet dosage form. It was found to be simple, rapid, economical and precise which could separate the drug and their degraded product formed under various stress conditions. The method developed was evaluated for validation parameters and the results were within the acceptance criteria as per ICH guidelines. Thus, the method can be used for routine analysis of Amlodipine Besylate in bulk and tablet dosage form.

Funding

Nil

Conflict of Interest

None

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