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Ultra–violet spectrophotometric method for estimation and validation of amlodipine in bulk and tablet formulation

Abstract

In the present work an inexpensive, easy, mercurial, particular, sensible and reproducible and spectrophotometric method has been developed and validated for the estimation of amlodipine in pure drug and Marketed Tablet Formulation. Analysis was carried out at 338nm for pure drug amlodipine and 355nm for amlodipine marketed tablet formulation. The main purpose of the investigation was to measure that how much percentage of drug present in marketed tablet formulation for the estimation of amlodipine besylate tablet and amlodipine pure drug using methanol as a solvent, a simple method has been developed. The accuracy of the method was assessed by dues studies and was found to be in spectrum of 99.80% of amlodipine pure compound and 99.20% of amlodipine marketed tablet formulation. The LOD were found 0.132 and 0.141µg/ml of amlodipine pure drug and LOQ were 0.416 and 0.427µg/ml of marketed tablet respectively. The result were validated found to be fair and met the admissible criteria.

Keywords: amlodipine, validation, spectroscopy, tablet

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Bina Gidwani, Laxmikant Patel, Anshita Gupta, Chanchal Deep Kaur
Shri Rawatpura Sarkar Institute of Pharmacy, India

Correspondence: Chanchal Deep Kaur, Associate Professor and Vice–Principal of Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg (C.G.), India, Email: dr.chanchaldeep@gmail.com beenagidwani@gmail.com

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Introduction

Validation is a process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in production or testing maintains the desired level of compliance at all stages.[1] A wide variety of procedures, processes, and activities need to be validated, the field of validation is divided into a number of subsections including the following: (Smith Alice E. et al 1996).[47]

Process validation is defined as the collection and evaluation of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products.[3] Anti–hypertensives are a class of drugs that are used to treat hypertension (high blood pressure). Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as stroke and myocardial infarction. Evidence suggests that reduction of the blood pressure by 5mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease.[4] There are many classes of anti–hypertensives, which lower blood pressure by different means. Among the most important and most widely used drugs are thiazide diuretics calcium channel blockers, ACE inhibitors,angiotensin II receptor antagonists (ARBs), and beta blockers.[5]

Calcium channel blockers are drugs used to lower blood pressure. They work by slowing the movement of calcium into the cells of the heart and blood vessel walls, which makes it easier for the heart to pump and widens blood vessels. As a result, the heart doesn't have to work as hard, and blood pressure lowers.Calcium channel blockers are prescription medications that relax blood vessels and increase the supply of bloodand oxygen to the heart while also reducing the heart's workload. Examples of calcium channel blockers include Amlodipine (Norvasc), Diltiazem (Cardizem, Tiazac), Felodipine, Isradipine, Nicardipine (Cardene SR), Nifedipine (Procardia), Nisoldipine (Sular), Verapamil (Calan, Verelan, Covera–HS) etc. In the present study amlodipine and its besylate salt is used. Structures are shown in Figure 1 & 2.

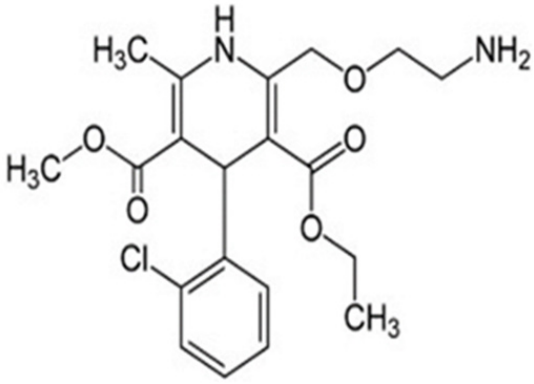

Figure 1 Chemical structure of Amlodipine.

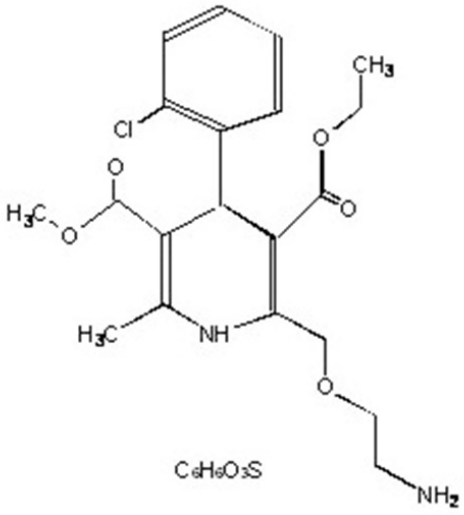

Figure 2 Chemical structure of Amlodipine Besylate.

Materials

Chemicals and solvent

Amlodipine was supplied as a gift sample by Vigor Pharmaceuticals Pvt. Ltd., Mumbai, Maharashtra (India). The marketed formulation tablets of amlodipine besylate 5mg Amlip Cipla Company were purchase from local market. Extra pure methanol was purchased from E. Merck Ltd, Mumbai, India. All other chemicals and solvents used for the study were of analytical grade.

Apparatus

Digital balance, FTIR instrument, double beam UV Visible spectrophotometer (1800) with resolution of 1nm & 0.5mm slit width and a pair of 1cm matched quartz cells was used to measure absorbance of the resulting solutions.[6]

Experimental work

Solubility studies: In order to find an ideal solvent in which the drug amlodipine was completely soluble, solubility studies were carried out. Various solvents like distilled water, methanol, ethyl alcohol, 0.1M HCL, Chloroform, Acetic acid, Acetone etc. Firstly, for the measurement of solubility firstly 5 mg of pure drug taken in a measuring cylinder and 1ml of distilled water is added, then add 2, 3, 5, 10, 30, 100….and this procedure flowing to all organic solvents.[7]

Preparation of standard stock solution

By dissolving 10mg amlodipine in 10ml methanol, standard solution of amlodipine was prepared. Later it was transferred into a 100ml volumetric flask and volume was made up to mark with methanol to make the solution of 100µg/ml concentration.[8,9]

Table 1 The solubility criteria as per BP

Very soluble	Less than 1 Part
Freely soluble	From 1 to 10 parts
Soluble	From 10 to 30 parts
Sparingly soluble	From 30 to 100 parts
Slightly soluble	From 100 to 1000 parts
Very slightly soluble	From 1000 to 10000 parts
Practically soluble	More than 10000 parts

Preparation of working standard solution

In order to obtain working standard solution of amlodipine, prepared stock solution were further diluted with methanol. The absorbance of each solution was then measured at 338nm with methanol as blank. Accurately weight 10mg of pure drug taken in clean dry 100ml of volumetric flask and dissolved in small volume of 10ml methanol in 100ml volumetric flask and volume make up into the 100ml up to the mark. The concentration range 10, 20, 30, 40, 50mcg/ml were prepared from stock solution of pure drug than calibration curve was plotted and the correlation coefficient was calculated. Same procedure flowed for the tablet formulation. Graph was plotted by taking concentration of drug on X–axis and absorbance on Y–axis.[10]

Determination of maximum wavelength (λmax)

By scanning within range of 200–400nm of a particular concentration of amlodipine solution is against a corresponding reagent blank, maximum wavelength is determined.[9–11]

Analysis of marketed tablet and pure drug of amlodipine

Twenty commercial tablets were emptied and powdered. Firstly 10mg amlodipine powder was accurately weighed and then transferred to 10mL volumetric flask and sonicated to dissolve the drug completely. Then it was filtered through 0.45µ filter and the volume is made up to 10mL with methanol to get a concentration of 1mg/mL stock solution. Furthermore, 1.0mL of the above stock solution was pipetted into a 10mL volumetric flask and diluted up to the mark to obtain required concentrations of amlodipine. The absorbance of the solution was measured at 338nm bulk compound and marketed tablet 355nm and amount of drug recovered was determined.[9]

Method validation

As per the International Conference on Harmonization (ICH) guidelines Validation of an analytical method is done Q2 (R1) (ICH, 2005).[12] In general, validation is defined as the process used for the confirmation of its validity i.e. the analytical procedure employed for a specific test is suitable for its intended use. Also, validation is considered as an integral part of any good analytical practice.[13] The results of method validation are used to judge the quality, trustworthy and regularity of analytical result. The USP has published specific guidelines for method validation for compound evaluation. According to USP, there are eight important parameters for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity and range, Ruggedness, Robustness.[14]

Accuracy

The nearness of the test result which is obtained by the true value or reference result are expressed by the accurate analytical procedure. The recovery test or experiments for accuracy were performed by taking known amount of the drug (amlodipine) in the place. According to ICH guidelines dues studies were carried out by applying the standard addition method.[15] The experiment was performed at three different levels i.e. 80%, 100% and 120% of standard concentration of amlodipine. The solutions were prepared according to the above mentioned procedure and then analyzed. The percentage of recovery were calculated from the calibration curve. All the experiments were performed in triplicate.[9]

Precision

Precision (repeatability) of an analytical method is a measurement of its nearness of agreement (degree of scatter) 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 analyzed on the same day at different time intervals and on different days respectively. Precision of the method (intra–day precision) was estimated by carrying out six independent assays of test samples of amlodipine. The intermediate precision (inter–day precision) of the method was also determined at different days with two different analysis in the laboratory.

Linearity

Test result are obtained by analytical method in which linearity is its ability, which are directly proportional to the concentration of analyte in the sample within a given range. From this standard stock solution various concentration of the solution were prepared and the absorbance was measured. Regression equation and correlation coefficient were determined by plotting the graph between absorbance and corresponding concentration.[16,17]

Citation: Bina G, Laxmikant P, Anshita G. Ultra–violet spectrophotometric method for estimation and validation of amlodipine in bulk and tablet formulation. *J Anal Pharm Res.* 2017;4(6):187–195. DOI: 10.15406/japlr.2017.04.00125

Specificity

Analysis of Marketed formulations (tablet of amlodipine) was done to estimate the presence of impurities, degradants and excipients in the sample and was compared with standard drug. For this the λmax and absorbance of reference standard solution and sample solution was measured.[18]

Robustness and ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[12] Robustness is evaluated during the development phase and is depended on the type of procedure used in the study. Moreover, robustness of sample shows the reliability of an analysis with respect to the variations in parameters of the method used in study. To determine the ruggedness the same procedure was carried by another analyst and the results was compared with the same previous procedure. In the present work, Robustness of the proposed method was determined by changing the λmax of the analysis by ±1.0nm. % Mean recovery (± % confidence interval) as well as % relative error was reported.[19,20]

Sensitivity and limit of detection (LOD) and limit of quantitation (LOQ)

Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantitation. According to ICH guidelines, the limit of detection is the lowest amount of analyte in a sample that can be detected and the limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.[9,16] We have considered standard deviation of the responses and the slope for calculation of LOD and LOQ. They are expressed as under:

$$LOD = \frac{3.3\sigma}{S} \quad LOQ = \frac{10\sigma}{S}$$

Where σ =the standard deviation of y–intercepts of regression line.

S = the slope of the calibration curve.

The LOD and LOQ were determined separately through calibration curve. The residual standard deviation of a regression line or the standard deviation of y–intercepts of regression lines were used to calculate the LOD and LOQ.[9]

Assay

Twenty tablets were accurately weighed and a quantity of tablet powder equivalent to 5mg of amlodipine besylate was weighed and then dissolved in 100ml methanol. The solution was then filled and further diluted to obtained final concentration of 25mcg/ml. The sample solution was analyzed and the % of drug content was determined from the absorbance using the regression equation obtained in the calibration.[21]

Results and Discussion

Solubility studies

Determination of the solubility studies of marketed tablet formulation and vent was added & later on in the same way solvent is increased like 2, 3, 4, 5, 10, 30….& the results were determined as given in Table 2.

Table 2 Solubility study of pure amlodipine and marketed tablet

S.no.	Solvent	Pure Drug Amlodipine	Marketed Tablet Formulation
1	Distilled Water	Poor soluble	Poor soluble
2	Methanol	Freely Soluble	Freely Soluble
3	Ethyl alcohol	Soluble	Soluble
4	0.1M HCL	Freely Soluble	Freely Soluble
5	Chloroform	Slightly soluble	Slightly soluble
6	DSMO	Freely soluble	Freely soluble
7	Benzene	Poor soluble	Poor soluble
8	Acetic acid	Soluble	Soluble

Analysis of absorbance maxima pure drug amlodipine

The absorption curve showed characteristic absorption maxima at 238nm for amlodipine (pure drug). The resulting spectrum (graph between absorbance and wavelength) is shown in Figure 5.2.1.

Analysis of absorbance maxima marketed tablet formulation

The absorption curve showed characteristic absorption maxima at 355nm for amlodipine (marketed tablet formulation). The resulting spectrum (graph between absorbance & wavelength) is shown in Figure 4.[22–28]

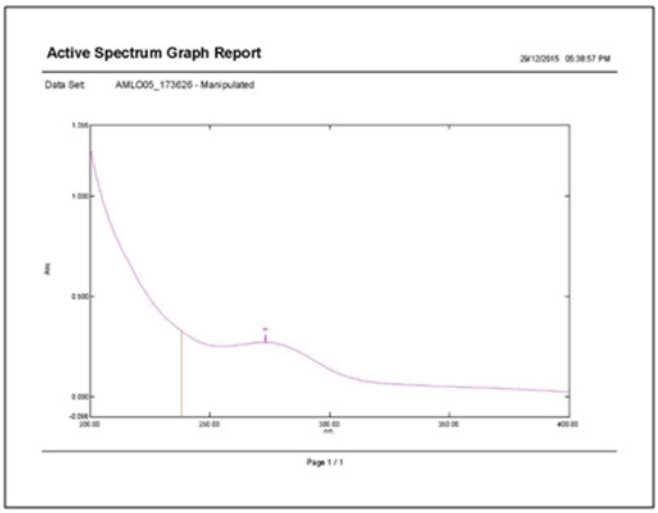

Figure 3 λ max of amlodipine (Pure drug) by UV-spectrophotometer (Shimadzu 1800).

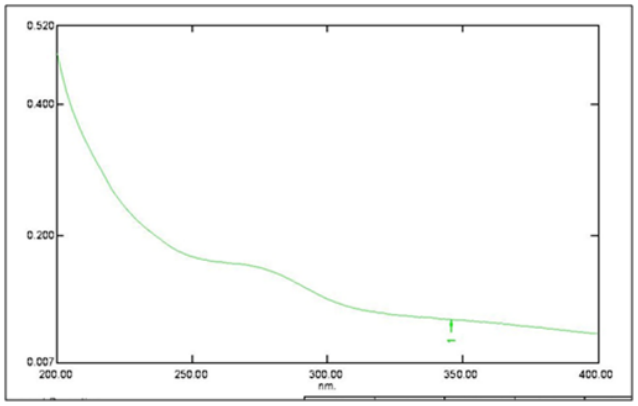

Figure 4 λ max of amlodipine (tablet formulation) by UV-spectrophotometer (Shimadzu 1800).

Preparation of calibration curve of pure drug

The calibration curve was plotted by taking different concentration of drug on x–axis and absorbance on y–axis and is shown in Figure 5 of pure drug amlodipine. The drug (amlodipine) obeys Beer's law in the concentration range of 10–50µg/ml with coefficient of correlation (R^2) = 0.997.[29–32]

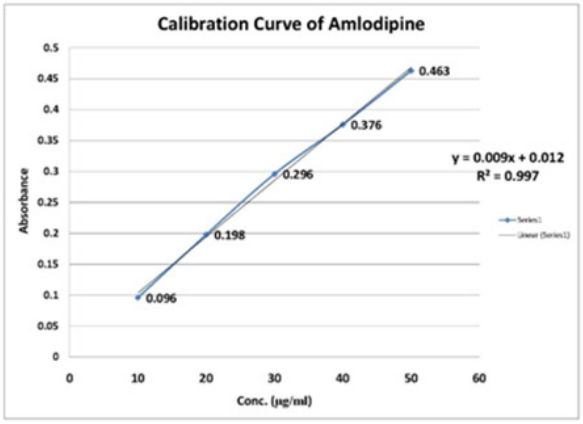

Figure 5 Calibration curve of amlodipine bulk compound.

Preparation of calibration curve of marketed tablet formulation

The calibration curve was plotted by taking different concentration of drug on x–axis and absorbance on y–axis and is shown in Figure 6 marketed tablet formulation. The drug (amlodipine) obeys Beer's law in the concentration range of 10–50µg/ml with coefficient of correlation (R^2) = 0.990. And wavelength is 355nm.[33,34]

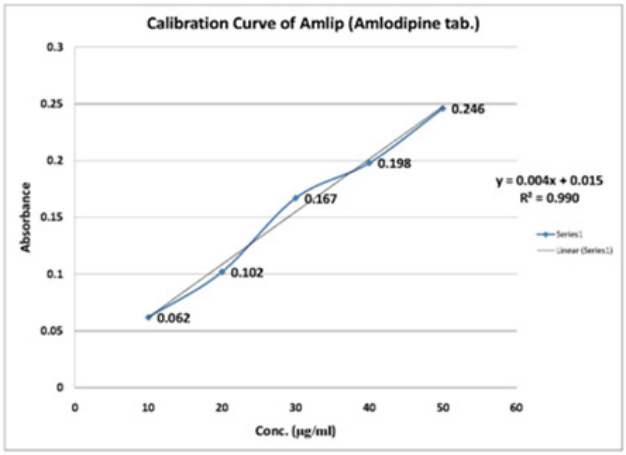

Figure 6 Calibration curve of amlodipine marketed tablet formulation.

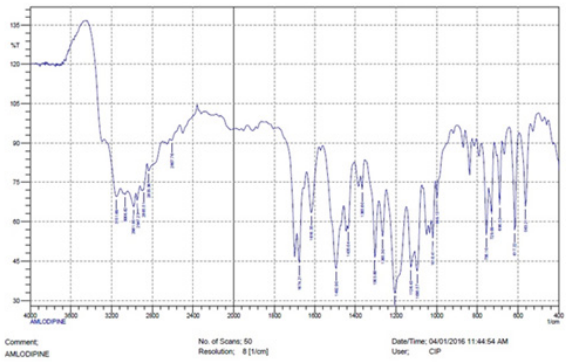

Figure 7 IR spectra of pure amlodipine compound.

FTIR study of pure drug

FTIR studies of pure amlodipine drug by using of FTIR instrument. The same amount of drug are used for measurement and KBr used as a alkali halide mixture compound. The range fixed in 4000–200nm. And there functional group and wavelength is determine. Results of functional groups are shown in Table 3.[35–37]

Table 3 FTIR of pure amlodipine drug

S. No.	Functional group	Wavelength (nm)
1	Alkanes. a) C – H stretching Methyl group (-CH2) b) C – C stretching c) C – H bending	3000-2840 1200-1800 1385-1380
2	Alkenes. a) C=C unconjugated b) C=C conjugated	1667-1640 1650-1600
3	Alkynes. a) C – H	700-610
4	Mononuclear aromatic hydrocarbons a) C – H bending b) C – H stretching	1300- 1000 3100-3000
5	Aldehydes. a) C=O stretching b) C – H stretching	1740- 1720 2830- 2695
6	Amides. a) N – H bending	1655- 1620
7	Amines. a) N – H stretching b) C – N stretching	1650- 1580 1250-1020
8	Organic halides. a) C – Br	690-515

Analysis of pure drug amlodipine and tablet formulation

The commercially available bulk compound and marketed tablet of amlodipine were analyzed and the % recovery of bulk drug in formulation ranged from 99.6–99.8% and formulated tablet range obtained from 98.60–98.80. The results are shown in Table 4 and 4.1. From the results it can be concluded that the % drug content in marketed formulations is almost similar to pure drug.

Table 4 Analysis of the tablet formulation

Formulation	Label Claimed Amount (mg)	Amount Recovered			% drug Recovered	Mean	Standard Deviation	% RSD
Amlip	5 mg	4.93	4.95	4.94	98.6			
Amlip	5 mg	4.94	4.94	4.93	98.8	4.92	0.0216	0.43906
Amlip	5 mg	4.89	4.91	4.92	97.8			

Table 4.1 Analysis of the pure drug

Formulation	Label Claimed Amount (mg)	Amount Recovered			% drug Recovered	Mean	Standard Deviation	% RSD
Amlodipine	5 mg	4.98	4.97	4.99	99.6			
Amlodipine	5 mg	4.99	4.98	4.99	99.8	4.98	0.00817	0.00164
Amlodipine	5 mg	4.97	4.97	4.98	99.4			

Citation: Bina G, Laxmikant P, Anshita G. Ultra–violet spectrophotometric method for estimation and validation of amlodipine in bulk and tablet formulation. *J Anal Pharm Res.* 2017;4(6):187–195. DOI: 10.15406/japlr.2017.04.00125

Accuracy of pure drug amlodipine

The accuracy of the proposed method was estimated through recovery studies at three levels i.e. 80, 100 and 120%. The results of the recovery studies and its statistical evaluation are summarized in Table 5. The recovery values for amlodipine pure drug ranged from 99.12 to 99.8%.[38–41]

Table 5 Evaluation of accuracy study of pure drug amlodipine

Level of Recovery (%)	Label Claimed Amount (mg)	Amount of Pure Drug (mg)	% Recovery	Statistical Analysis		
				Mean (%)	SD	% RSD
80	5	4.1	102.5			
80	5	3.97	99.25	100.1	0.0665	0.0664
80	5	3.95	98.75			
100	5	4.96	99.02			
100	5	4.97	99.4	99.07	0.01247	0.0125
100	5	4.94	98.8			
120	5	5.97	99.5			
120	5	5.96	99.33	99.33	0.4714	0.00474
120	5	5.96	99.33			

Accuracy of pure amlodipine & marketed tablet formulation

To determine the accuracy of the proposed method, recovery study is carried out by adding different amount 80%, 100%, 120% of tablet formulation sample of amlodipine besylate within the linearity range and results obtained are shown in Table 5.1. The recovery values of amlodipine tablet formulation range is all most similar to the pure drug.[42–45]

Table 5.1 Evaluation of accuracy study of tablet formulation

Level of Recovery (%)	Label Claimed Amount (mg)	Amount of Pure Drug (mg)	% Recovery	Statistical Analysis		
				Mean (%)	SD	% RSD
80	5	3.96	99			
80	5	3.97	99.25	99.08	0.007417	0.004760
80	5	3.96	99.0			
100	5	4.99	99.80			
100	5	4.97	99.40	99.53	0.009428	0.009418
100	5	4.97	99.40			
120	5	5.98	99.60			
120	5	5.96	99.30	99.43	0.008165	0.008211
120	5	5.97	99.40			

Precision of pure drug amlodipine and tablet formulation

The developed UV–spectroscopic method found to be precise values is the %RSD of (pure drug) values of the repeatability and intermediate precision studies were <0.0083% and <0.0972%, respectively and tablet formulation values of the repeatability and intermediate precision studies were <0.0291% and <0.00479%, respectively. The results of intermediate inter–day summarized in Table 6 & 6.1 and intra–day precision study are summarized in Table 7 & 7.1.[46–50]

Table 6 Evaluation of intraday precision study (pure drug)

Time	Amount of Drug (mg) Claimed	Amount of Drug Recovered	Statistical Analysis		
			Mean (%)	SD	% RSD
		4.94			
Morning	5 mg	4.93	98.6	±0.004714	0.00478
		4.93			
		4.91			
Afternoon	5 mg	4.89	98	±0.008165	0.00832
		4.9			
		4.89			
Evening	5 mg	4.88	97.6	±0.004714	0.00482
		4.88			

Table 6.1 Evaluation of intraday precision study (tablet formulation)

Time	Amount of Drug (mg) Claimed	Amount of Drug Recovered	Statistical Analysis		
			Mean (%)	SD	% RSD
		4.79			
Morning	5 mg	4.62	93.2	±0.094163	0.10103
		4.57			
		4.73			
Afternoon	5 mg	4.51	92.466	±0.0899	0.09722
		4.63			
		4.59			
Evening	5 mg	4.25	88.333	±0.13888	0.15722
		4.41			

Table 7 Evaluation of inter-day precision study of pure drug

Day	Amount of Drug (mg) Claimed	Mean Amount of Drug Recovered	Statistical Analysis*		
			Mean (%)	SD	% RSD
Day 1	5 mg	4.69	93.8	±0.0301	0.032
Day 2	5 mg	4.56	91.2	±0.0296	0.0291
Day 3	5 mg	4.44	88.8	±0.281	0.0301

*n=3 (average of 3 determinations).

Table 7.1 Evaluation of inter-day precision study of tablet formulation

Day	Amount of Drug (mg) Claimed	Mean Amount of Drug Recovered	Statistical Analysis*		
			Mean (%)	SD	% RSD
Day 1	5 mg	4.94	99	±0.008165	0.00824
Day 2	5 mg	4.92	98.4	±0.004714	0.00479
Day 3	5 mg	4.79	95.8	±0.008165	0.00852

Linearity of pure drug amlodipine and tablet formulation

The linearity of the method was demonstrated over the concentration range of 10–50mcg/ml of concentration. Accurately weight 10mg of pure drug taken in clean dry 100ml of volumetric flask and dissolved in small volume of 10ml methanol in 100ml volumetric flask and volume make up into the 100ml upto the mark. The concentration range 10, 20, 30, 40, 50mcg/ml were prepared from stock solution of pure drug than calibration curve was plotted and the correlation coefficient was calculated. Same procedure flowed for the tablet formulation drug. The linearity of the response of the drug (amlodipine) was observed in concentration range from 10 –50µg/ml and it obeyed Beers law (Table 8).[51,52]

Table 8 Linearity data pure drug amlodipine

Concentration (µg/ml)	Mean Absorbance (±SD)
0	0
10	0.096
20	0.198
30	0.296
40	0.376
50	0.463

Table 8.1 Linearity data tablet formulation

Concentration (µg/ml)	Mean Absorbance (±SD)
0	0
10	0.062
20	0.102
30	0.167
40	0.198
50	0.246

Linearity data tablet formulation

The linearity of the method was demonstrated over the concentration range of 10–50mcg/ml of concentration. Accurately weight 10mg of tablet formulation drug taken in clean dry 100ml of volumetric flask and dissolved in small volume of 10ml methanol in 100ml volumetric flask and volume make up into the 100ml upto the mark. The concentration range 10, 20, 30, 40, 50mcg/ml were prepared from stock solution of pure drug than calibration curve was plotted and the correlation coefficient was calculated. And calibration curve for amlodipine tablet $Y = 0.004x + 0.015$. The calibration curve was found to be linear with the correlation coefficient (R^2) = 0.990.

Specificity of pure drug amlodipine and tablet formulation

The presence of excipients in the formulation does not interfere with the analysis of drug. Thus, the proposed method was found specific and selective for the drug. The results are shown in Table 9 of pure drug and marketed tablet formulation Table 9.1.

Citation: Bina G, Laxmikant P, Anshita G. Ultra–violet spectrophotometric method for estimation and validation of amlodipine in bulk and tablet formulation. *J Anal Pharm Res.* 2017;4(6):187–195. DOI: 10.15406/japlr.2017.04.00125

Table 9 Specificity study of pure drug

Sr. No	Drug Amount Claimed (mg)	Amount of Drug Recovered	% Drug Recovered	Mean	SD	%RSD
1	5	4.98	99.6			
2	5	4.99	99.8	99.73	±0.004714	0.004726
3	5	4.99	99.8			

Table 9.1 Specificity study of marketed tablet formulation

Sr. No	% Excipient	Drug Amount Claimed (mg)	Amount of Drug Recovered	% Drug Recovered	Mean	SD	%RSD
1	5	5	4.89	97.8			
2	10	5	4.75	95.01	95.27	±0.09843	0.10331
3	15	5	4.65	93			

Robustness of pure amlodipine and tablet formulation

The evaluation data for robustness study at 10µg/ml concentration is summarized in Table 10 for pure drug & 10.1 for marketed tablet formulation.

Table 10 Evaluation data of Robustness study pure drug

Sr. No	Concentration			
	Absorbance at 337nm	Absorbance at 338nm	Absorbance at 336nm	Absorbance at 339nm
1	0.462	0.462	0.461	0.462
2	0.461	0.462	0.461	0.462
3	0.462	0.462	0.460	0.461
4	0.460	0.461	0.459	0.459
5	0.461	0.459	0.459	0.460
Mean	0.4612	0.4611	0.460	0.4608
SD	0.000748	0.001166	0.000894	0.001160
% RSD	0.16218	0.25287	0.19434	0.25173

Table 10.1 Evaluation data of Robustness study tablet formulation

Sr. No	Concentration			
	Absorbance at 355nm	Absorbance at 356nm	Absorbance at 354nm	Absorbance at 357nm
1	0.371	0.371	0.370	0.371
2	0.370	0.369	0.369	0.371
3	0.371	0.369	0.368	0.370
4	0.369	0.368	0.368	0.369
5	0.369	0.368	0.367	0.370
Mean	0.370	0.3693	0.3684	0.3701
SD	0.000894	0.001095	0.00102	0.000894
% RSD	0.24162	0.2964	0.27687	0.24162

Ruggedness of pure drug amlodipine and tablet formulation

The evaluation data for ruggedness study at 10µg/ml concentration is summarized in Table 11 for pure compound & Table 11.1 for tablet formulation.

Table 11 Ruggedness study of pure drug

Concentration	Absorbance at 338nm
	Analyst 1
10µg/ml	0.458
10µg/ml	0.458
10µg/ml	0.457
10µg/ml	0.456
10µg/ml	0.456
Mean	0.4570
SD	0.000849
%RSD	018577

Table 11.1 Ruggedness study of marketed tablet formulation

Concentration	Absorbance at 355nm
	Analyst 1
10µg/ml	0.365
10µg/ml	0.365
10µg/ml	0.363
10µg/ml	0.363
10µg/ml	0.362
Mean	0.3636
SD	0.0012
%RSD	0.33003

LOD and LOQ of pure drug amlodipine and tablet formulation

The detection limits and quantitation limits of amlodipine (Pure) were found to be 0.132 and 0.416µg/ml respectively And The detection limits and quantitation limits of amlodipine (bulk) were found to be 0.141 and 0.437µg/ml respectively These results prove that microgram quantity of the drug sample can also be determined accurately and precisely.

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Assay

Twenty tablet were accurately weighed and a quantity of tablet powder equivalent to 5mg of amlodipine besylate was weighed and dissolved in 100ml methanol. The solution was then filled and diluted further to obtained final concentration of 25mcg/ml. The sample solution was analyzed and the % drug content was determined from the absorbance using the regression equation obtained in the calibration. So the analyzed the marketed tablet and pure drug and resulting that the % purity of pure and tablet formulation is similar. Results are shown in the Table 12.

Table 12 Assay of marketed tablet formulation

Drug	Label claim (mg/tab)	Amount estimated (mg/tab)	% purity
Amlodipine pure drug	5 mg	4.99 4.98 4.99	99.8
Tablet formulation	5 mg	4.96 4.98 4.97	99.2

The equation of the calibration curve for amlodipine pure compound was Y = 0.009x + 0.012, the calibration curve was found to be linear with the correlation coefficient (R2) = 0.997.

Formula used for calculation

$$\%\,Relative\ standard\ deviation = \frac{Standard\ deviation\ of\ measurements}{mean\ of\ measurements} X100$$

$$\%\,Recovery = \frac{Amount\ found}{Amount\ added} X100$$

$$Amount\ found = \frac{Mean\ test\ absorbance}{Mean\ standard\ absorbance} X\ standard\ concentration$$

$$Amount\ added = weight\ /\ volume$$

Calculation of limit for stability study:

$$Limit = \frac{Absorbance\ of\ standard\ initial - absorbance\ of\ standard\ at\ time\ interval}{absorbance\ of\ standard\ initial} X100$$

Summary & conclusion

The quantitative estimation & validation parameters of the pure drug and marketed tablet were compared. The quantitative estimation of pure drug and marketed tablet formulation was compared with the absorption maxima of the pure drug and marketed tablet formulation. Then calibration curve is plotted pure drug and marketed tablet formulation separately. Then for the assessment of various functional groups present in FTIR studies were performed. Various functional groups and peak were analyzed and compared with the standard which proved that the drug was Amlodipine. Also, the assay of the marketed tablet formulation was determined in which 99.2% of the drug was obtained and pure drug amlodipine 99.8%. Then validation parameter was also determined according to USP 8 parameters like Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity, Ruggedness, and Robustness. These all parameters were studied between the pure drug and marketed tablet formulation. And resulting were the all validation parameter values similar to the marketed tablet formulation.

On the basis of the results obtained, it can be concluded that Quantitative method for estimation of drug in pure form and in commercially available marketed formulations is simple, accurate, precise, specific, selective, cost–effective, convenient, reproducible and reliable which meets the required acceptance criteria. This analytical method can be used for its intended purpose. For all the validation parameters, the values of standard deviation and %relative standard deviation were low which indicate higher degree of precision. This method can be successfully employed in future for routine estimation, quantification and quality control of pharmaceutical dosage forms.

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