INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS
VOL. 4 ISSUE 1, 2016; 1 –11; http://ijpda.com; ISSN: 2348-8948

RESEARCH ARTICLE

DESIGN, DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR AMLODIPINE-COPPER COMPLEX USING UV –VIS SPECTRO-PHOTOMETRY

PRASHANT L PINGALE, GARIMA KUMAR

Assistant Professor, Department of Pharmaceutics, GES's Sir Dr. M.S. Gosavi College of Pharmaceutical Education & Research, Nashik, Maharashtra, India 422005.

Date Received: 26th December 2015; Date Accepted: 4th January 2016 Date published: 15th January 2016

Email: prashant.pingale@gmail.com

Abstract: To develop spectrophotometric method for the determination of amlodipine besylate by forming complex with copper ion. Secondly, to develop a method that would be an easy, economical and accurate method as compared to other processes. The materials used were of laboratory grade and the method used was complexation with the potent API (Amlodipine besylate). The results showed that the complex was developed by using acetate buffer pH4.7 in a fixed ratio (1:1.25) with copper sulphate. Thus, it was concluded that the complex formation with copper reduced the photolytic degradation as observed in the bulk analysis of amlodipine by using UV Vis spectrophotometry. The developed method was found to be simple, accurate and economical for routine analysis.

Key words: Amlodipine, UV Vis- spectrophotometry, Photodegradation, Complexation

INTRODUCTION:

The objective was to develop a method that would reduce Photodegradation and thus, reduce the development of photo degraded products of amlodipine which do not have any pharmacological activity. Amlodipine besylate is a calcium channel blocker used for treatment of hypertension and angina pectoris; it is a very potent drug and is marketed as the benzene sulfonic acid salt commonly known as besylate. It is a 1, 4-Dihydropyridine calcium channel antagonist; thus it has a high tendency to get degraded when exposed to light. It gets degraded by oxidative aromatization of dihydropyridine fragment to the pyridine moiety. It is one of the main degradation pathways of molecules belonging to 1, 4- dihydropyridine family. They get degraded both in solution and in solid state. Drugs of this family are known to absorb intensively in the UV-A region and are also known to be photolabile.

As the API is photo-labile it becomes difficult to carry out routine analysis. It is also observed that the photo-degraded products of amlodipine do not have any pharmacological activity; thus it becomes necessary to reduce the photo-degradation of the API for pharmaceutical dosage form analysis. For this purpose the API needs to be protected by using protective agents. The complex formation is also a method which helps to reduce this photolabile property of the API.[8]

Literature survey showed that various attempts are made to form complexes with different methods and complexing agent which could reduce the photo-degradation of amlodipine. These could be explained as follows:

- Amlodipine inclusion complexes: The inclusion complexes were formed with β-cyclodextrin by using cyclic voltammetry and square wave voltammetry which studies the electrochemical activity of the complex formed. It was reported that photo-degradation was reduced by this method. This method is tedious, expensive and time consuming; thus could not be applied for routine analysis.
- Amlodipine charge transfer complex: The charge transfer complexes were formed by using p-chloranilic acid in 1, 4 dioxan dissolved in chloroform. It was not concluded that the method reduced photo-degradation. The method was applied to pharmaceutical dosage form and it was found that simple excipients did not interfered when analysed using Uv- spectrophotometry.
- Amlodipine complex with ninhydrin: Amlodipine complex was formed in this case with ninhydrin in N, N- Dimethyl formide (DMF) and was analysed spectrophotometrically.The method cannot distinguish the photo-degraded products as both the agent and products contain amino group; thus the determination is required to be done under conditions where light is completely avoided.

From these literature surveys, it was concluded that the photo-degradation of amlodipine can be avoided by complex formation but these methods cannot be employed for routine analysis as they require special equipment or chemicals or a special condition.In the present study, an attempt is made to overcome photolytic degradation of the API alongwith keeping the method simple, less time consuming and also economical. Amlodipine – copper complex is formed by using acetate buffer pH 4.7 instead of organic solvent such as methanol. [8,10,12]

MATERIALS AND METHOD:

Spectral runs were made carried on Perkin Elmer UV/Vis spectrophotometer Lambda 25 was employed. Glasswares used for each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid then were rinsed thoroughly with double distilled water. These were then dried in hot air oven at a suitable temperature. This was basically done to avoid any kind of impurities which could lead to problems while developing of the method. Amlodipine besylatereference standard for the bulk analysis were provided as a gift sample from industry. The complexing agent copper sulphate and sodium trihydrate acetate were of analytical grade and were procured from Ranekem. Other reagents like Methanol were of HPLC grade, Distilled water, 0.1N Hydrochloric acid from analytical reagents, 0.1N NaOH Ranekem, 3% H_2O_2 from analytical reagents were all of analytical grade. All the solutions prepared were protected from light by keeping them in dark and wereanalysed on the day of preparations.

EXPERIMENTAL WORK

Preliminary solubility studies: A small quantity of Amlodipine drug was dissolved in various solvents such as Methanol, Distilled water, various buffers, 0.1N HCl, 0.1N NaOH. By the solubility studies it was determined that amlodipine was freely soluble in Methanol, Slightly soluble in Distilled water and Acetate buffer pH 4.7 and also other solvents. [7]

Preparation of Standard stock solution: The stock solution was prepared by weighing 10 mg of Amlodipine; this formed a solution of 1000µg/ml. From the stock solution 1 ml was withdrawn in a 10 ml volumetric flask which was made up with the mixture reagent solution (Acetate buffer pH4.7 and copper sulphate) to obtain a concentration of 100µg/ml solution. Different aliquots of the solution was taken in the range 1-2ml in 10 ml volumetric flask and the volume was made up withthe mixture reagent solution (Acetate buffer pH4.7 and copper sulphate) to obtain the concentrations 10-20µg/ml; scanning ranges were finalized and it was scanned in the ranges 500-300 nm, to determine the complex formation of the API.

Determination of λmax: From the stock solution a working standard was prepared. The absorption spectrum for amlodipine-copper complex was recorded by using 10µg/ml and the maximum absorbance was found to be at 365 nm. The calibration curve for amlodipine–copper complex was prepared in concentration range of 10-20 µg/ml at the selected wavelength by diluting the stock solution of the API. The plots of beer's law were plotted in figure 1 and the absorbance values are given in table I

Table 1: Absorbance values of calibration curve

Concentration (µg/ml)	Absorbance (A)
0	0
10	0.159
12	0.177
14	0.202
16	0.226
18	0.256
20	0.272

Preparation of Amlodipine-Copper complex

For preparation of Amlodipine copper complex a study of suitable solvent in which the complex could be formed the solvents were selected on the basis of their solubility of the API and the complex was formed in liquid form and was determined by the chromic shifts observed in the UV Spectrum. [1]

Procedure For this purpose the complex formation was performed with methanol and distilled water first, no results were found the complex was not formed in these solvents. After this the third trial was performed using a mixture of the complexing agents (Copper sulphate and Acetate buffer pH 4.7) in a specific ratio of 1:1.25. For the purpose of the study the complex was formed in all the three solvents using following procedure: [5]

- ✓ 1 ml of stock solution was added to a 10 ml volumetric flask
- ✓ To this the complexing agents were added in the sequence copper sulphate (2 ml) and then acetate buffer pH 4.7 (2.5 ml)
- ✓ This was then made up to the required volume using the solvents methanol, distilled water and mixture for each trial respectively

Method Validation:

The method was validated with reference to linearity, range, accuracy and precision, and Robustness

Linearity

Linearity was performed by taking aliquots of 1, 1.2, 1.4, 1.6, 1.8,2 ml from a 100µg/ml stock solution and diluted upto the mark with the mixture of copper sulphate and acetate buffer in ratio 1:1.25.The final concentration of the

complex was found to be in the range of 10-20µg/ml. Calibration curve is as shown in figure 1.

Accuracy

The accuracy of the proposed method was tested by recovery studies at 80%, 100%, and 120%. This was performed by addition of a known amount of complex concentration of 10µg/ml. The recovery studies were performed to determine the accuracy of the proposed method. The results are shown in table 2.

Precision

The precision is measure of either the degree of reproducibility orrepeatability of analytical method which is expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements. It is used as an indication of random error in analytical method development. For this reason inter and intra-day precision is done using six replicates to check repeatability (precision) of the proposed method.

Robustness

The robustness of the method was carried out by changing the ratio of the solvent system of the proposed method of copper sulphate and acetate buffer pH4.7 as 1:1 and 1.25:1. [26]

SIAM (Stability indicating analytical method)

A stock solution containing 10 mg amlodipine in 10 ml of mixture of copper sulphate and acetate buffer pH 4.7 was prepared; which was diluted to prepare a stock of concentration 100µg/ml. The stock prepared was used for determination of forced degradation of complex under different conditions to provide an indication of the stability-indicating propertyand specificity of the proposed method. In all degradation studies, the absorbance of the complex was measured and the amount ofdegraded complex was calculated.[17] The procedures for various degradation studies are as follows:

Acidic degradation:

For this type of degradation studies 0.1 N HCl was used. 1 ml of the stock solution was taken in a 10 ml volumetric flask and was then made upto 10 ml with 0.1 N HCl and was kept for heating at 60°C for 4 hrs. The samples were withdrawn at 0,1,2,4 hrs and absorbance was determined to analyse the extent of degradation of the complex.

Alkali degradation:

For this type of degradation 0.1N NaOH was used.1 ml of stock solution was taken in a 10 ml volumetric flask and was then made upto 10 ml with 0.1 N NaOH and was kept for heating at 60°C for 4 hrs. The samples were withdrawn at 0,1,2,4 hrs and absorbance was determined to analyse the extent of degradation of the complex.

Oxidative Degradation:

For this type of degradation 3% H_2O_2 was used.1 ml of stock solution was taken in a 10 ml volumetric flask and was then made upto 10 ml with 3% H_2O_2 and was kept for heating at 60°C for 4 hrs. The samples were withdrawn at 0,1,2,4 hrs and absorbance was determined to analyse the extent of degradation of the complex.

Neutral degradation:

For this type of degradation methanol was used.1 ml of stock solution was taken in a 10 ml volumetric flask and was then made upto 10 ml with methanol and was kept for heating at 60°C for 4 hrs. The samples were withdrawn at 0,1,2,4 hrs and absorbance was determined to analyse the extent of degradation of the complex.

Photolytic degradation:

For photolytic degradation, the samples of complex were exposed to sunlight for 48 hrs. The samples were withdrawn at intervals 0, 12, 24, 36, 48.The blank was taken as the mixture of complexing agent in ratio 1:1.25.The samples were analysed using UV at the λmax. [4]

RESULTS

Selection of suitable solvent system and Method development: Selection of the best solvent system was based on the solubility of the complex and also absorbance which was determined by taking UV scan. The solvents which were choosen for study showed following results.

Methanol :

Methanol was used as the solvent for complex formation. For determination of the complex formation three types of UV scan were taken; First, drug in the reagent; Second, only the reagent; Third, the final scan of the complex which is formed in the reagent or the diluting solvent. The results of UV scan were found as follows:

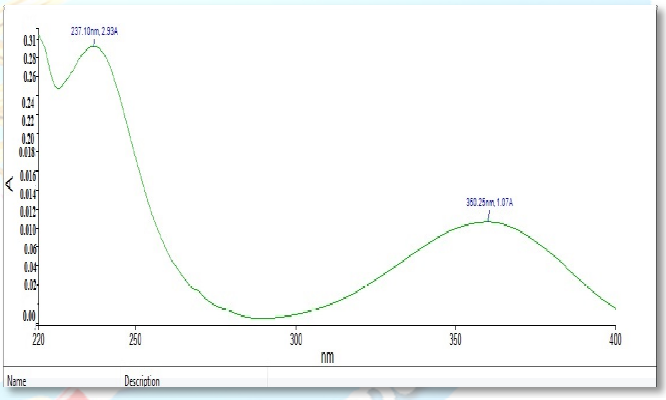


Fig 1: UV Scan of drug in methanol

From the Fig 1 it was observed that the drug (Amlodipine besylate) has two λ_{max} at 237.10nm and 360.25 nm. So, the further scans should differ from these peaks to confirm the formation of complex; as the complexing agent copper sulphate is a coloured substance a red or blue shift would be observed which would confirm the formation of complex.

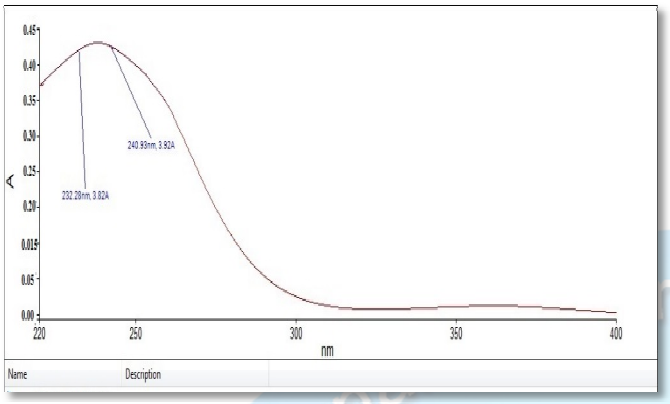


Fig 2: UV Scan of complex in methanol

From Fig 2 it was observed that the scan was wavy and also that it has two λ_{max} at 232.28 nm and 240.93 nm. The peaks observed were nearby that of drug alone and also the reagent. Thus, it was inferred that no complex was formed as there was no shift observed and the peaks were inferred to be of the drug and reagent alone. No change was observed which clearly determines that no complex is formed.

Distilled water:

In the case of distilled water again the UV scans were taken to determine whether the complex is formed in the distilled water; which could turn out to be economical. For this purpose again three type of scan were carried out; First, only drug in distilled water; Second, only reagent (Distilled water); Third, the complex formed in distilled water.

From Fig 3, it was observed that the complex formed showed a peak at 232.06 nm which was almost close to-that of the reagent alone. Thus, it could be concluded that no differentiated peak was observed from the reagent peak; the observed peak could not be of the complex formed. Hence no complex is formed using distilled water.

Mixture of copper sulphate and acetate buffer pH 4.7 (1:1.25)

In the case of mixture again the UV scans were taken to determine whether the complex is formed in the mixture; which could turn out to be economical. For this purpose again three type of scan were carried out; First, only drug in mixture; Second, only reagent (mixture of copper sulphate and acetate buffer pH 4.7 in ratio 1:1.25); Third, the complex formed in mixture.

From Fig 4, it was observed that the UV scan showed a shift to the right side. The drug in reagent showed a peak at 232.92 nm and the reagent alone showed at 231.06 nm. The UV scan showed a peak at 365 nm; this confirmed the shift towards right and thus, the formation of amlodipine-copper complex was confirmed in the mixture of copper sulphate and acetate buffer pH 4.7 (in a specific ratio 1:1.25).For further confirmation of whether the complex formed is stable the concentration of stock solution were varied from 10-20 µg/ml.

From Fig. 5, it can be inferred that the complex formed is stable as varying the concentration of stock solution does not shift the λ_{max} from 365 nm. Also the peaks observed at 365 nm were observed to be flat at all concentrations. Thus, it was confirmed that the complex is formed. Thus, the complex formation is confirmed and the method was further validated and also the stress degradation studies were done to infer the stability of the proposed method for routine analysis.

Validation of method:

The method was validated with reference to linearity, range, accuracy and precision and Robustness

Range: The range of the developed method was found to be 10 -20µg/ml as inferred from linearity graph.

Linearity: From the Fig 6, it can be concluded that the linearity was found to be $R^2 = 0.9922$; with the regression equation to be y=0.0137x+ 0.0088 in the concentration range 10 -20µg/ml.

Accuracy: The accuracy of proposed method was validated and the results were tabulated in table 2 and the figure 6 graphically represents the same.

From Table 2, it was observed that the proposed method was accurate and the % Recovery values were within the limits range which showed that the proposed method was accurate and the graphical representation in Fig 7 confirms the results obtained.

Precision: The precision was carried out as intra-day and inter-day precision. The results are tabulated in Table 3

From Table 3, it was can be concluded that all the % RSD values were less than 2 % which was found to be within limits and thus the proposed method was having good precision.

Robustness: The method was validated for robustness; to study whether a slight change in concentration of reagent affects the developed method or not. The concentration of the agent was changed for three ratio's 1:1.25, 1:1, 1.25:1 respectively for determining the robustness of method. The results are tabulated in Table 4.

From Table 4, it was observed that all the values of % RSD were within limits i.e. less than 2%; thus it can be concluded that the proposed method was robust. Thus, the results showed that the proposed method was well linear, accurate, and robust and had precision. The other concern was the stability of the formed complex for routine bulk analysis of amlodipine.

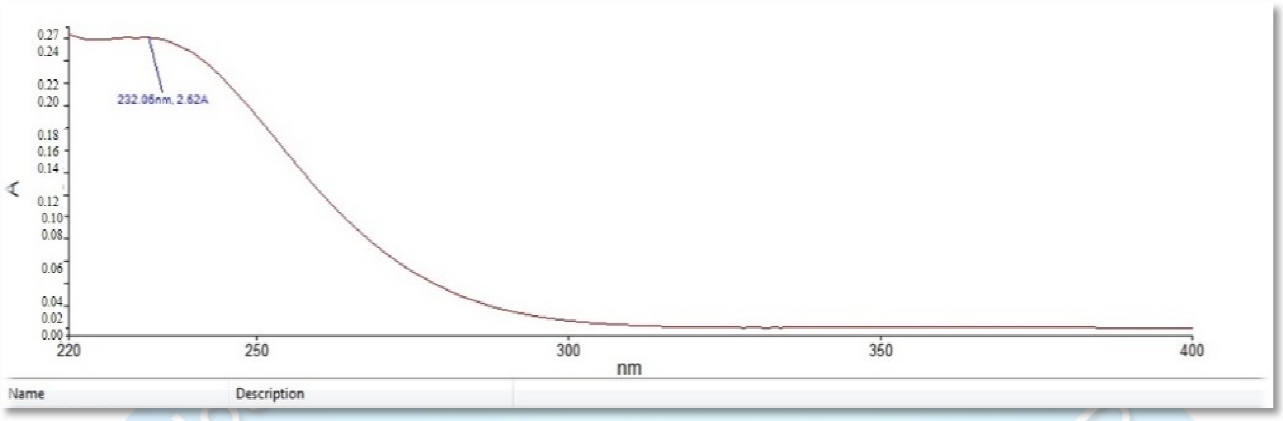


Fig 3: UV Scan of complex in distilled water

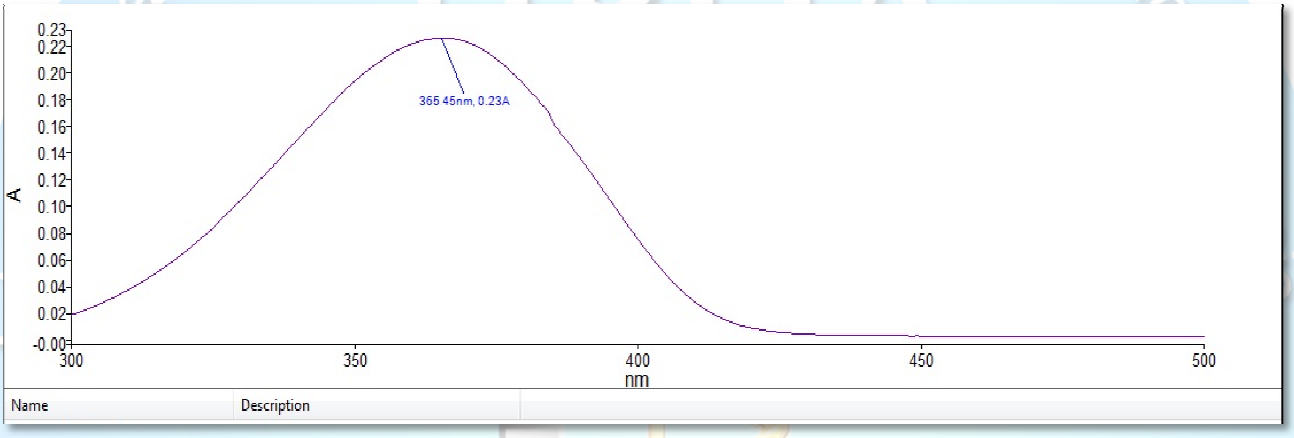


Fig 4: UV Scan of complex in the reagent mixture

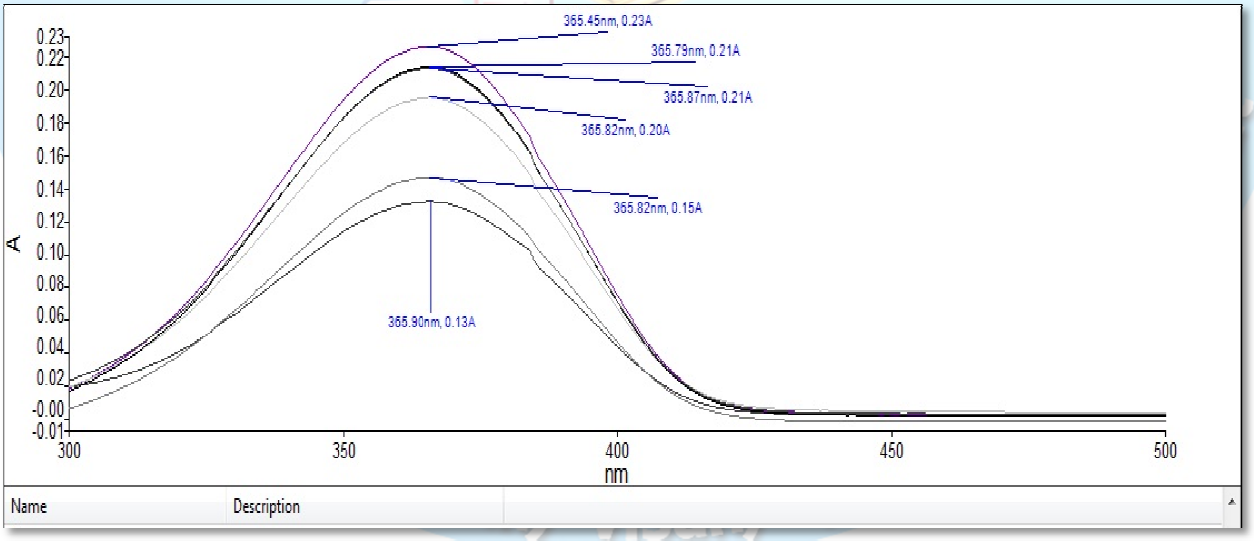


Fig 5: UV Scan at different concentration of stock solution of API

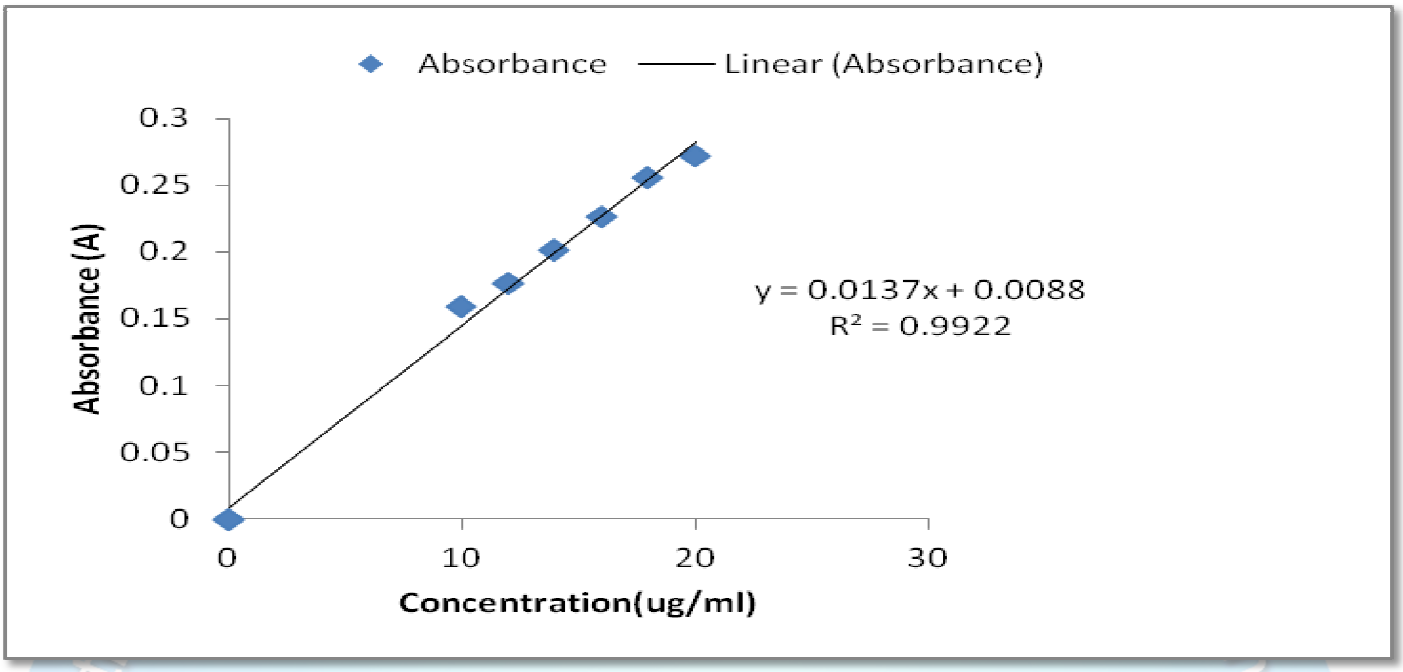


Fig 6: Linearity graph for proposed method

Table 2: Results of accuracy (n=3)

Level	Absorbance	Amount recovered (mg)	% Recovered	Mean	SD	%RSD
80%	0.222	15.56				
	0.225	15.78	101.56	0.22	0.003	0.015
	0.229	16.0				
100%	0.275	19.43				
	0.272	19.21	97.15	0.27	0.003	0.01
	0.278	19.64				
120%	0.333	23.66				
	0.329	23.37	97.99	0.33	0.002	0.006
	0.331	23.5				

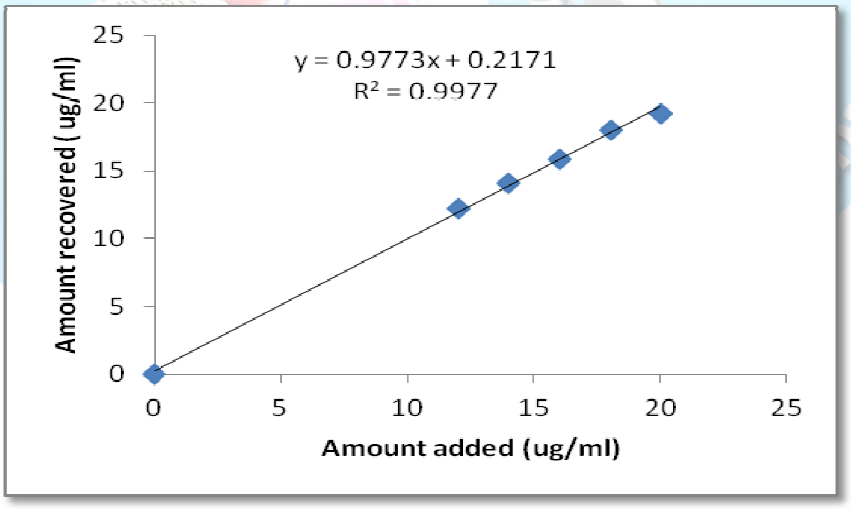


Fig 7: Accuracy recovery graph

Table 3: Results of Intra-day and Inter-day precision (n=3)

Concentration (ppm)	Intra-day precision			Inter-day precision		
	Mean	SD	%RSD	Mean	SD	%RSD
10	0.11	0.002	0.021	0.15	0.002	0.012
12	0.13	0.001	0.01	0.14	0.0025	0.017
14	0.14	0.003	0.02	0.19	0.002	0.013
16	0.18	0.0025	0.013	0.21	0.002	0.011
18	0.19	0.002	0.01	0.24	0.002	0.008
20	0.22	0.0015	0.006	0.26	0.003	0.011

Table 4: Results of Robustness (n=3)

Concentration (ppm)	Mean	SD	%RSD
10	0.08	0.002	0.028
12	0.10	0.0015	0.014
14	0.11	0.004	0.035
16	0.14	0.003	0.020
18	0.15	0.0035	0.023
20	0.17	0.0025	0.014

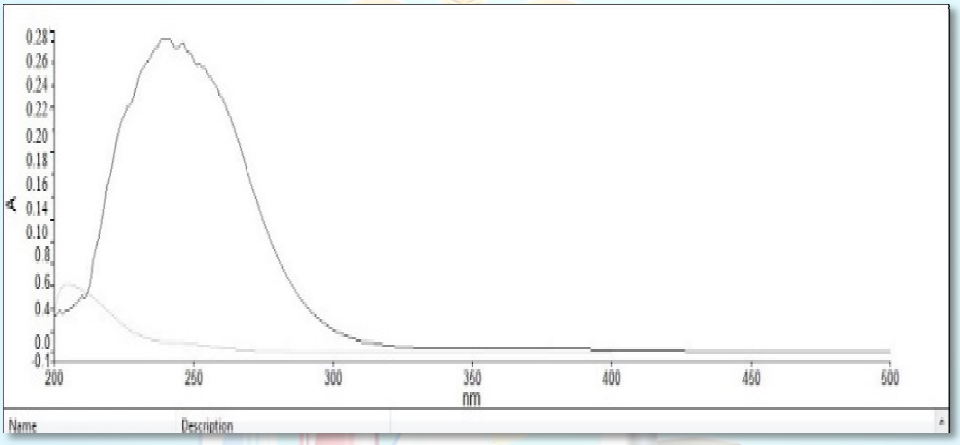


Fig 8: UV Scan of photolytic degradation of drug

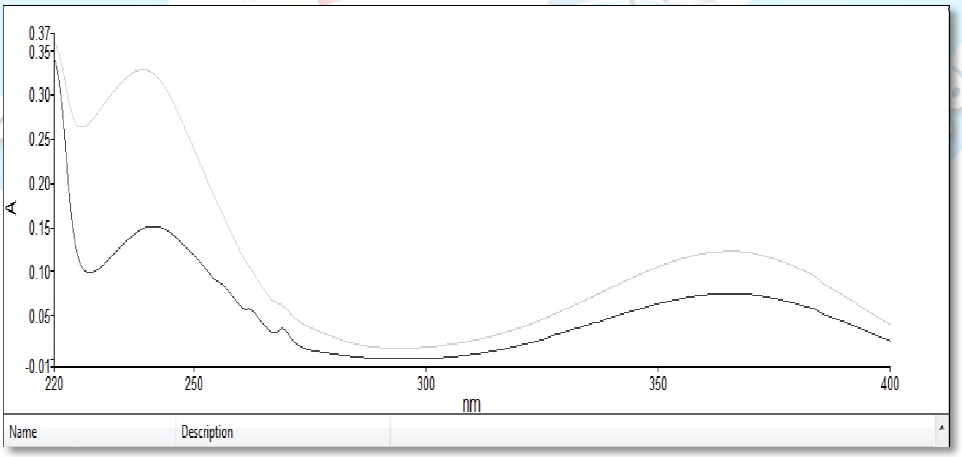


Fig 9: UV Scan of photolytic degradation of Complex

SIAM Results :(Forced degradation studies)

Photolytic degradation:

The UV Scan were taken at 0 hrs and then after 48 hrs and then were overlay in one graph and compared as the amount of drug degraded after completion of study.

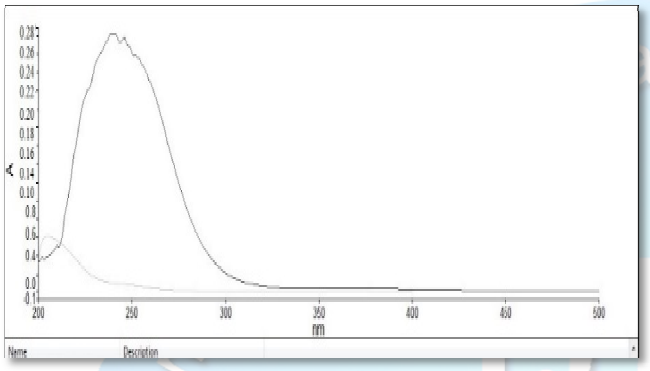


Fig 8: UV Scan of photolytic degradation of drug

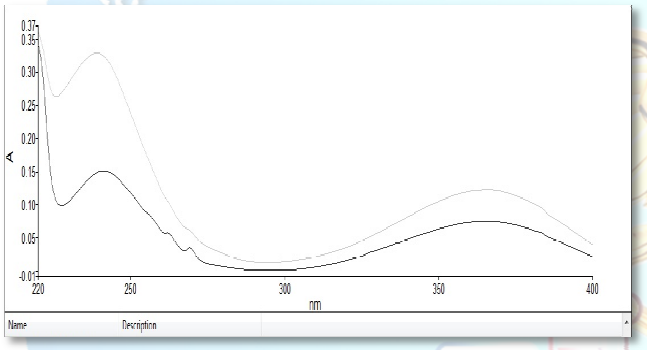


Fig 9: UV Scan of photolytic degradation of Complex

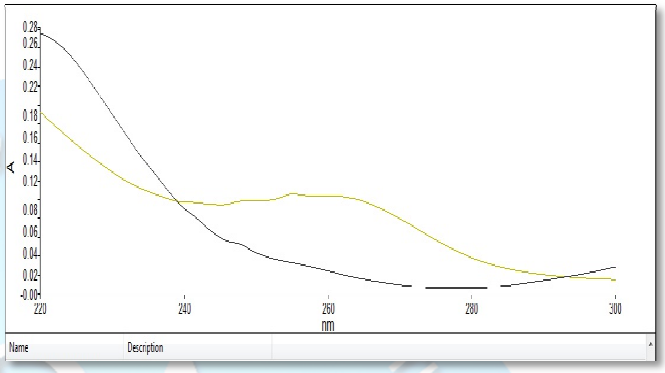
The observations of the degradation in Fig 8 and Fig 9 were tabulated at different time intervals in table 5. The % degraded at a particular time interval is shown in the tables. This gives an idea of the maximum amount of drug and also the complex get degraded.

Table 5: Observations of drug and complex degradation by photolysis

Time (hrs.)	%Degradation	
	Drug	Complex
0	0	0
12	22.32	15.18
24	30.73	21.02
36	39.90	27.59
48	46.78	34.16

The results observed in table 5 were compared graphically and from this it could be concluded that the drug degradation was found to be greater than the complex degradation.

Thus, from these observations it can be concluded that the photolytic degradation of the API was reduced by the formation of complex.



Acid Degradation:

Fig 10: UV Scan of drug degradation in 0.1N HCl

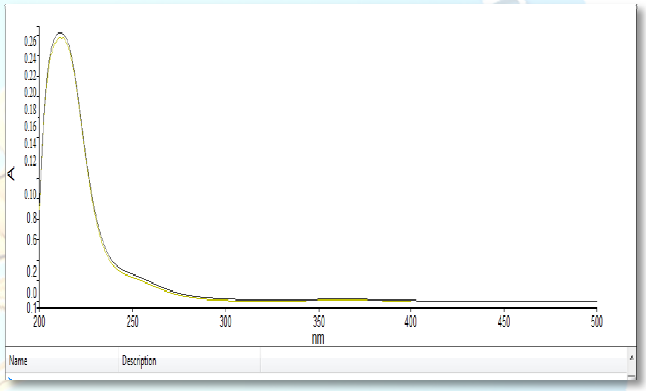


Fig 11: UV Scan of complex degradation in 0.1N HCl

The observations of the degradation in Fig 10 and Fig 11 were tabulated at different time intervals in table 5. The % degraded at a particular time interval is shown in the tables. This gives an idea of the maximum amount of drug and also the complex get degraded.

The results observed in table 6, were compared graphically and from this it could be concluded that the drug degradation was found to be greater than the complex degradation. Thus, from these observations it can be concluded that the acid degradation of the API was reduced by the formation of complex to some extent.

Alkali degradation:

The observations of the degradation in Fig 12 and Fig 13 were tabulated at different time intervals in table 6. The % degraded at a particular time interval is shown in the tables. This gives an idea of the maximum amount of drug and also the complex get degraded.

The results observed in table 6 were compared graphically and from this it could be concluded that the drug de-

gradation was found to be greater than the complex degradation. Out of the total time of degradation up till 2 hrs the % degradation of both drug and complex was almost same and after that the drug. Thus, from these observations it can be concluded that the alkali degradation of the API was reduced by the formation of complex to some extent.

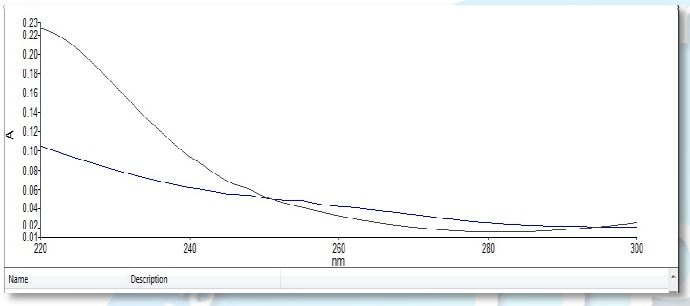


Fig 12: UV Scan of drug degradation in 0.1N NaOH

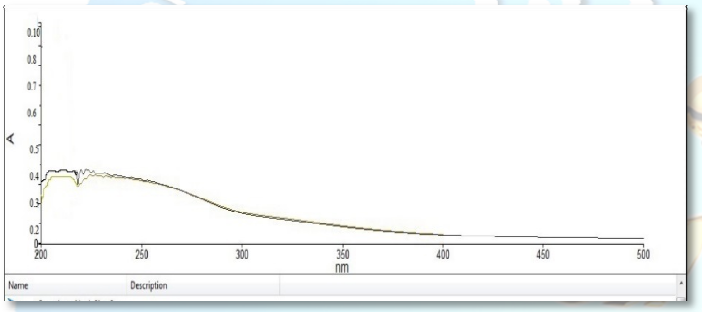


Fig 13: UV Scan of complex degradation in 0.1N NaOH

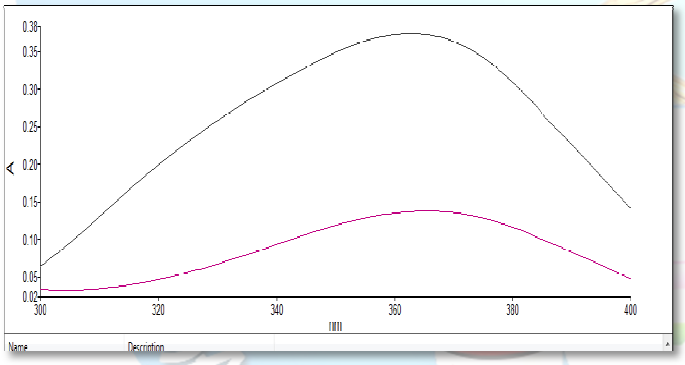


Fig 14: UV Scan of drug degradation in 3% H_2O

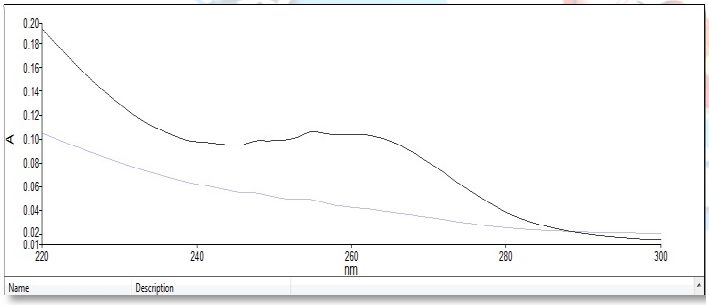


Fig 15: UV Scan of complex degradation in 3% H_2O_2

Oxidative Degradation:

The observations of the degradation in Fig 14 and Fig 15 were tabulated at different time intervals in table 6. The % degraded at a particular time interval is shown in the tables. This gives an idea of the maximum amount of drug and also the complex get degraded.

The results observed in table 6 were compared graphically and from this it could be concluded that the drug degradation was found to be greater than the complex degradation. Thus, from these observations it can be concluded that the oxidative degradation of the API was reduced by the formation of complex to some extent.

Neutral degradation:

The observations of the degradation were tabulated at different time intervals in table 6. The % degraded at a particular time interval is shown in the tables. This gives an idea of the maximum amount of drug and also the complex get degraded.

The results observed in table 6 were compared graphically and from this it could be concluded that the drug degradation was found to be greater than the complex degradation. It can be inferred that the drug degradation is very high compared to complex formed degradation. Thus, from these observations it can be concluded that the neutral degradation of the API was reduced by the formation of complex to some extent.

DISCUSSION:

The results above interpreted that there is a possibility of forming complex with copper-sulphate of amlodipine besylate. This method is easy and economical could be used to develop on a more accurate scale i.e. HPLC. The results obtained are discussed or interpreted as the neutral degradation of the complex was more than any other form of degradation and photolytic degradation was controlled to certain extent it needs to be further improved. The detail interpretation is done along the results revealed. The proposed method was for the development of an analytical method by complex formation with copper ion; the analysis of amlodipine was studied for bulk. From the results obtained of the bulk analysis of amlodipine by complex formation with copper ion, it was observed that the photolytic degradation reduced as compared to without complex formation. The validation results revealed that the proposed method is accurate and reproducible and also the proposed method was also observed not to be affected by slight variation in the proposed method; which means the method is robust. All the % RSD were found to be less than 2% which showed that the proposed method could be employed for routine analysis. The SIAM studies were performed to check the stability of the complex formed as it was observed in liquid state; this makes it extremely important to check for stability as the chances of getting degraded increases un-

der such circumstances The SIAM results that are the study indicating the stability of analytical method revealed that the formed complex of amlodipine with copper ion (C^{+2}) was stable and does not degrade to a great extent under various stress conditions.

Table 6: Observations of drug and complex degradation by Acid, Alkaline, Oxidation and Neutral photolysis

Time (hrs)	Degradation Mechanism							
	Acid		Alkaline		Oxidation		Neutral	
	Drug	Complex	Drug	Complex	Drug	Complex	Drug	Complex
0	0.91	0	0	0	0	0	92.69	0
1	17.99	4.19	2.99	4.40	24.15	4.52	95.18	8.16
2	24.82	6.46	28.47	8.10	68.30	31.89	135.08	9.50
4	36.21	11.76	47.57	29.65	69.76	40.84	140.07	13.50

Conclusion:

The proposed method was found to be simple, accurate, and precise. It is an economical method for routine bulk analysis of a very potent API (Amlodipine besylate); which is a photolabile drug and needs to be protected from photo degradation as the photo-degraded product of the API do not have any kind of pharmacological activity. The proposed method validation showed that all the results have % RSD less than 2% from which it can be concluded that the method is precise, robust and thus, can be applied for routine analysis. The SIAM studies showed that the complex so formed is stable under certain type of stress conditions. Mainly out of the stress conditions the photolytic degradation showed that by complex formation the degradation of amlodipine API could be reduced by forming complex with 0.2%w/w copper sulphate in acetate buffer pH 4.7.

References:

1. Hassan S.S., Zaidi F.A. Development and Validation of Analytical Method for Losartan-Copper Complex Using UV-Vis Spectrophotometry. Tropical Journal of Pharmaceutical Research. 2013; 12: 407-411.
2. Dash A.K., Mishra D.S. Method Development, Validation and Stability Study of Griseofulvin in Bulk and Pharmaceutical Dosage form by UV Spectrometric Method. Asian J. Pharm. Res. 2012; 2: 66-69.
3. Rani S.K., Swapna A, Padma A. A New Spectrophotometric Method for the Estimation of Amlodipine Besylate and its Stress Degradation Studies. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011; 2:470.
4. Xavier C.M., Basavaiah K.S. Development and Validation of Two Stability-Indicating Uv-Spectrophotometric Methods for the Determination of Repaglinide in Bulk and Dosage Forms. International Journal of ChemTech Research. 2013; 5:72-79.
5. Cesme M., Tarinc D., Golcu A. Spectrophotometric Determination of Metoprolol Tartrate in Pharmaceutical Dosage Forms on Complex Formation with Cu (II). Pharmaceuticals. 2011; 4:964-975.
6. Ramadan A.A., Dahhan H.M. Novel Formation Three Complexes of Cefixime-Copper using Acetate–Acetic acid Buffer and Determination of Cefixime in Pure and Pharmaceutical Dosage Forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5:469-477.
7. Pradhan K.K. Mishra U.S. Stress Degradation Studies on Valsartan and Development of a Validated Method by UV Spectrophotometric in Bulk and Pharmaceutical Dosage Form. Journal of pharmaceutical and biomedical sciences. 2011; 8:52-57.
8. Singh R., Rehman Z.U. Current Trends in Forced Degradation Study for Pharmaceutical Product Development. J Pharm Educ Res. 2012; 3:54-62.
9. Basavaiah K., Chandrasekhar U., Prameela H.C. Sensitive Spectrophotometric Determination of Amlodipine and Felodipine using Iron (III) and Ferricyanide. Farmaco.2003; 58:141- 148.
10. Rahman N., Singh M., Hoda M.N. Application of Oxidants to the Spectrophotometric Determination of Amlodipine Besylate in Pharmaceutical Formulations. IL Farmaco. 2004; 59: 913–919.
11. Abdellatef H.E., Magda M. A., Taha E.A. Spectrophotometric and Atomic Absorption Spectrometric Determination of Ramipril and Perindopril through Ternary Complex Formation with Eosin and Cu (II). Journal of Pharmaceutical and Biomedical Analysis.1999; 18:1021–1027.
12. Rahman N., Hoda M.N. Validated Spectrophotometric Methods for the Determination of Amlodipine Besylate in Drug Formulations using 2, 3-dichloro5,6-dicyano 1,4-benzoquinone and Ascorbic Acid. Journal of Pharmaceutical and Biomedical Analysis.2003; 31 :381-392.
13. Gavini R., Puranik S.B., Kumar G.V.S. Simultaneous Estimation of Amlodipine and Losartan by UV-method in Bulk Drug and Tablet Dosage Formula-

tion. Archives of Applied Science Research. 2012; 4:2206-2212.
14. Jadhav K.V., Dhamecha D.L. Stability Indicating Stress Degradation Studies of Lafutidine using UV Spectrophotometric Method. Pharmaceutical Methods. 2013; 4: 21-25.
15. Manthena V.S., Varma A.M., Garg S. Rapid and Selective UV Spectrophotometric and RP-HPLC Methods for Dissolution Studies of Oxybutynin Immediate Release and Controlled Release Formulations. Journal of Pharmaceutical and Biomedical Analysis.2004; 36:669–674.
16. Kalyanaramu B., Raghubabu K. Development of New Analytical Method for Determination of Raloxifene Hydrochloride in Formulations Based on Charge-Transfer Complex Formation. International Journal of Analytical and Bioanalytical Chemistry. 2011; 1:29-33.
17. Bakshi M., Singh S. Development of Validated Stability Indicating Assay Methods: Critical Review. Journal of Pharmaceutical and Biomedical Analysis. 2002; 28:1011-1040.
18. Walash M.I., Metwally M.E., Eid M., Shaheny R.N. Spectrophotometric Determination of Risedronate in Pharmaceutical Formulations via Complex Formation with Cu (II) Ions: Application to Content Uniformity Testing. International journal of Biomedical Science.2008; 4:303-308.
19. Kumar D. P., Samy K., Madhu Kumar C. Colorimetric Determination and Validation of Amlodipine Besylate in Pure and Tablet Dosage Form. International Journal of Research Pharmaceutical and Nano Sciences. 2013; 2:245-250.
20. Rahman N., Syed N. H. Spectrophotometric Method for the Determination of Amlodipine Besylate with Ninhydrin in Drug Formulations. IL Farmaco.2001; 56:731-735.
21. Stoiljkovic Z.Z., Jovanovic V.M. The Electrochemical Investigation of Inclusion Complexes of Nifedipine and Amlodipine with β-Cyclodextrin and (2-Hydroxypropyl)-β-Cyclodextrin. Int. J. Electrochem. Sci. 2001; 8:9543-9557.
22. Bhargavi P., Chandana B. Visible Spectrophotometric Method for the Estimation of Amlodipine Besylate in Tablet Dosage Forms. Journal of Pharmacy Research.2011; 4:4001-4002.
23. Derayea S.M., Spectrophotometric Determination of Amlodipine and Nicardipine in Pharmaceutical Formulations via Binary Complex Formation with Eosin Y. Journal of Applied Pharmaceutical Science. 2012; 2:84-89.
24. Patil V.P. New Eco-friendly Validated Spectrophotometric Method for the Estimation of Amlodipine Besylate in Bulk Drug Using Ninhydrin. Asian Journal of Biomedical and Pharmaceutical Sciences.2012; 3:14-19.
25. Jampana P.K. Visible Spectroscopic Method for Estimation of Amlodipine Besylate in Tablets. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2014; 4:173-177.
26. Jadhav S.B. Difference Spectroscopic Method for the Estimation of Amlodipine Besylate in Bulk and in Formulation. International Journal of Pharmaceutical and Chemical Sciences.2013; 2:1213-1217.
27. Kirtansinh G.N. Application of Analytical Techniques in Preformulation Study: A Review. International Journal of Pharmaceutical & Biological Archives. 2011; 2:1319-1326.
28. Validation of Analytical Procedure: Text and Methodology, ICH Harmonized Tripartite Guideline, Q2 (R1), 2005:1-3.
29. Joshi H.S. Development and Validation of Stability Indicating HPLC Assay Method for Simultaneous Determination of Amlodipine Besylate, Olmesartan Medoxomil and Hydrochlorothiazide in Tablet Formulation. Der Pharmacia Sinica. 2013; 4:145-152.
30. Singhvi I. Visible Spectrophotometric Methods for the Estimation of Amlodipine Besylate in Tablets. Indian Journal of Pharmaceutical Sciences. 1998; 2:309-310.