Available online on 21.11.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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A Novel Derivatization Ultraviolet Spectrophotometric Method for the Determination of Amlodipine Besylate Using Benzoyl Chloride

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ABSTRACT

The present research work aims to develop a novel ultraviolet UV spectrophotometric method for the determination of Amlodipine Besylate using Benzoyl Chloride as a derivatizing agent, which is simple, rapid, sensitive, selective, and accurate method for the spectrophotometric determination of Amlodipine Besylate in powder form. Synthesis is based upon the Schotten Baumann Reaction. In this method, derivatization of aliphatic amine group of Amlodipine Besylate carried out with benzoyl chloride and aqueous sodium hydroxide (NaOH).The λmax was found to be 237 and 226nm for assay of Amlodipine Besylate and synthesised product respectively. The linearity was found in concentration range of 1-10 µg/ml. The correlation coefficient (r^2)was found 0.9985. The regression equation, intercept (a) and slope (b) was found as Y=0.0762x - 0.0077, 0.0077 and 0.0762 respectively. Method was developed and validated as per ICH guidelines for linearity, accuracy, precision, LOD, LOQ, interday and intraday. The LOD and LOQ for estimation of Amlodipine besylate were found as 0.2367, 0.7178 respectively. Recovery of Amlodipine besylate was found to be 93.30%.The proposed method is found to be simple, rapid, selective and highly sensitive than most of the Spectrophotometric methods available in literature.

Keywords: Derivatization, Ultraviolet spectrophotometry, Amlodipine besylate, Validation, Synthesis.

Article Info: Received 19 Sep 2019; Review Completed 23 Oct 2019; Accepted 28 Oct 2019; Available online 21 Nov 2019

Cite this article as:

Patel MSN, Shaikh SN, Khan MJA, Deshmukh NI, Khan MDSA, Kazi UZ, Khan SJ, A Novel Derivatization Ultraviolet Spectrophotometric Method for the Determination of Amlodipine Besylate Using Benzoyl Chloride, Journal of Drug Delivery and Therapeutics. 2019; 9(6):173-178 http://dx.doi.org/10.22270/jddt.v9i6.3632

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INTRODUCTION

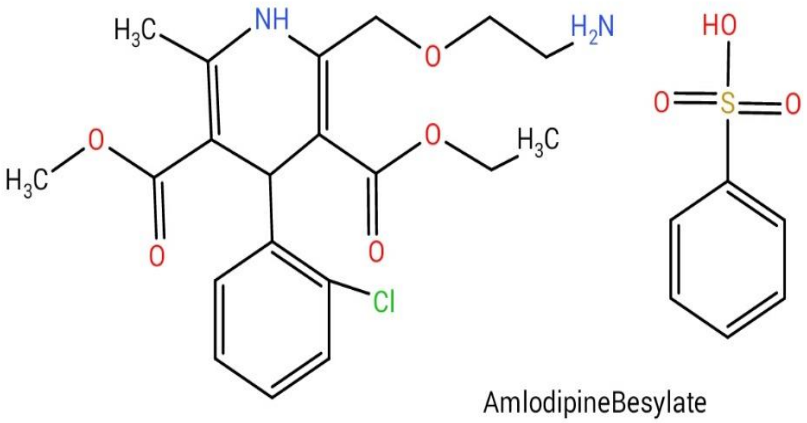
Amlodipine Besylate is 3-ethyl 5 methyl(4RS)-2-[(2aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate.[1]

Amlodipine Besylateis a dihydropyridine calcium-channel blocker that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and angina.[2]

However, latest findings have revealed that its efficacy is not only limited to the treatment of cardiovascular diseases as it has shown to possess antioxidant activity and plays an important role in apoptosis. Therefore, it is also employed in the treatment of cerebrovascular stroke, neurodegenerative diseases, leukemia, breast cancer, and so forth either alone or in combination with other drugs.[3]Amlodipine is used in the management of hypertension1 and coronary artery disease. Itis available in several officials in Pharmacopoeia. Literature survey reveals that, Spectrophotometric methods, HPLC, HPTLC, UPLC.[4]Amlodipine Besylate is freely soluble in methanol, slightly soluble in 2-propanol and water and sparingly soluble in ethanol 95**%.**[5] Amlodipine Besylate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{26}H_{31}ClN_2O_8S$, calculated on the anhydrous basis. Category. Antihypertensive; antianginal.[6]

It is marketed as the benzene sulfonic acid salt (besylate). Amlodipine was approved in September 2007 as a combination product with olmesartan (Azor), an angiotensin II receptor antagonist, for the treatment of hypertension. Amlodipine is also marketed as a combination therapy with atorvastatin under the tradename Norvasc for the management of high cholesterol and high blood pressure.[7]

Molecular structure:



AmlodipineBesylate

Molecular weight: 567.1 gm/mol

Molecular formula: $C_{26}H_{31}ClN_2O_8S$

Melting point: 199-201 ^0C

MATERIALS AND METHODS

Instruments

A double beam Shimadzu UV-1800 series spectrophotometer was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell at fast scanned speed and fixed slit width of 1.0 nm. All weighing of ingredients were done on ohaus digital weighing balance.[8]

Reagents and standards

All the ingredients are taken from Ali – Allana College of Pharmacy Akkalkuwa (Pharmaceutical Chemistry laboratory) like Amlodipine Besylate, Methanol, NaOH, etc.

Preparation of Standard Stock Solution

About 100 mg of Amlodipine Besylate and Synthesised Product was weighed accurately and transferred in to 100 ml of volumetric flask respectively. The volume was made up to 100 ml using distilled water to obtain a solution that has a concentration 1000 µg/ml. 1 ml of above solution was taken and diluted up to 10 ml using distilled water to obtain a solution that has a concentration 100 µg/ml. The above 10 ml solution was taken and diluted up to 100 ml using distilled water to obtain a solution that has a concentration 10 µg/ml. From above stock solution 1ml, 2 ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml solution were taken in separate volumetric flask and diluted using distilled water up to 10 ml to each to obtain solution that has concentration 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml and 10µg/ml respectively.

Calibration Curve

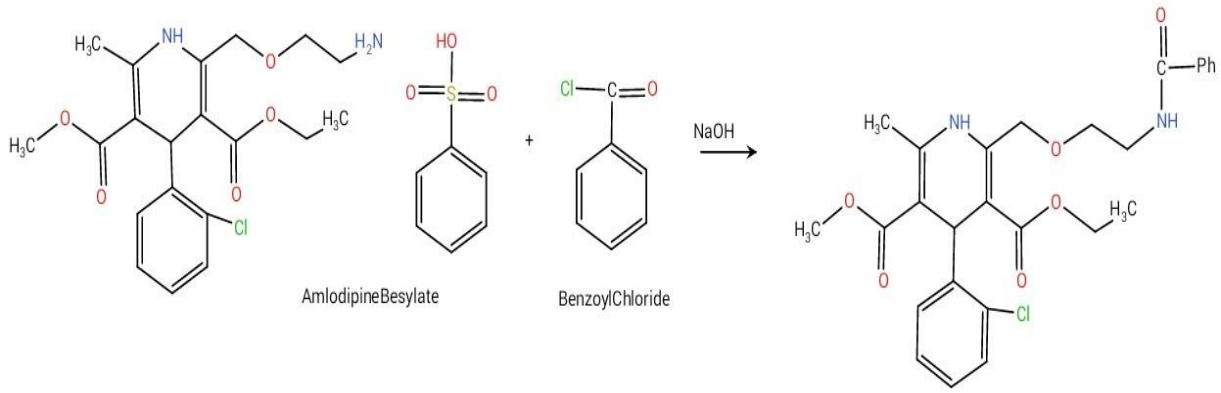
The standard solutions of Amlodipine Besylate and Synthesised Product were diluted with methanol individually to get the concentration of 10µg/ml and 10µg/ml respectively and were scanned in the UV range 400-200nm. The λmax of both the drugs were found to be 237nm and 226 nm respectively. The spectral data was processed to obtain first order derivative spectrum at wavelength interval of 2 nm and scaling factor 10 for the range of 400-200nm with scanning speed of 400nm/min. It was observed that Amlodipine Besylate shows zero crossing at 237nm while Synthesised Product shows zero crossing at 226nm. At zero crossing point of Amlodipine Besylate (237nm), Synthesised Product showed a measurable dA/dλ whereas at zero crossing point of Synthesised Product(226nm), Amlodipine Besylate showed a measurable dA/dλ. Hence the wavelengths 237nm and 226nm were selected as analytical wavelengths for determination of Amlodipine Besylate and Synthesised Product first order derivative method respectively.[9]

Preparation of Sample Solution

About 50 mg of Amlodipine Besylate and Synthesised Product pure drug was weighed accurately and transferred in to 50 ml of volumetric flask respectively. The volume was made up to 50 ml using distilled water to obtain a solution that has a concentration 1000 µg/ml. 1 ml of above solution was taken and diluted up to 10 ml using distilled water to obtain a solution that has a concentration 100 µg/ml. The above 10 ml solution was taken and diluted up to 100 ml using distilled water to obtain a solution that has a concentration 10 µg/ml. From above stock solution 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml of Amlodipine Besylate and Synthesised Product were taken and diluted up to 10ml to obtain 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml and 10µg/ml respectively.

Chemical Derivetization

Reaction

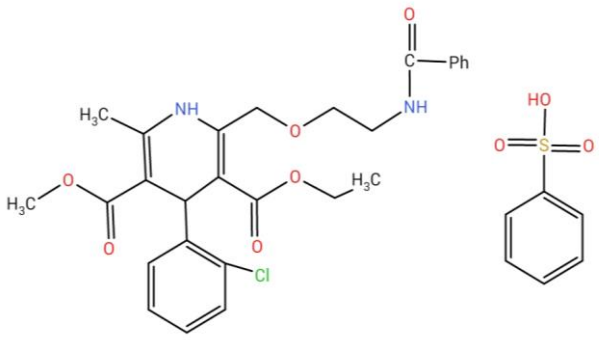


Procedure

1. Place 1.0gm (0.99 ml) of amlodipine besylate and 9.61 ml of 10% aqueous sodium hydroxide solution in an iodine flask and then add 1.35 ml (1.65) of benzoyl chloride, stopper the flask and shake vigorously for 10 min. heat is evolved in the reaction. The crude benzoyl derivative separates as white powder.
2. When the reaction is complete i.e. when the odour of benzoyl chloride can no longer be detected (smell cautiously) make sure that reaction mixture is alkaline and dilute it with 4.42 ml of water.
3. Filter off the product with suction on a small Buchner fullel, break up the mass (if necessary) wash well with water, and drain
4. Recrystallise from hot alcohol (methylated spirit) filter the hot solution through a hot water funnel or through a warm Buchner funnel. Collect the crystals which are separated and dry in the air or in the steam oven.
5. Report the yield and melting point
6. The yield of benzanilide is 0.59 gm.
7. The % practical yield is 70%.[10]

Synthesized Product

Molecular structure:



Molecular formula: $C_{33}H_{35}ClN_2O_9S$

Molecular weight: 671.1

Solubility: Methanol

Melting point: 108-110 ^{0}C

Amides

In recent year with the introduction of new peptides coupling reagent in organic synthesis, the method of amide synthesis have been significantly advanced. But the two step acylation, activation of carboxylic acid a reaction with amines have been often used in nonpeptide chemistry. Acid chloride are generally recognised as key intermediate for acylation, for conversion into many other function groups along with amides such as anhydride, ester and ketones. An acylation with technique of protecting group in multistep synthetic processes, important synthetic routes for many bio active compounds such as vitamins, agrochemicals, xanthine's and in combinational peptides synthesis.[11]

Validation

The UV Spectrophotometric methods were validated as per ICH Q2A guidelines. Parameters like Sensitivity, Linearity, Range, Accuracy, Precision, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness were evaluated.[12]

Linearity:

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range was found to be 1-10 µg/ml for Paracetamol and 1-10 µg/ml for Phenylephrine HCl. The linearity data for method is presented in Table1.[13]

Accuracy:

To check the accuracy of the proposed method recovery studies were carried out at 80, 100, 120 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are reported in Table1.[14]

Precision:

Precision was determined by studying the repeatability and intermediate precision.

Interday and Intraday precision:

The interday and intraday precision was determined by assay of the sample solution on the interday precision was determined by assay of the sample solution on the same day and on different days at different time interval respectively.[15]

Limit of detection (LOD) and Limit of Quantification (LOQ):

The Limit of Detection (LOD) is the smallest concentration of analyte that give the measurable response. LOD was calculated using the following formula and shown in Table no. 1.

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

Where,

S = Slope of calibration curve

SD = standard deviation of the response.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table no….

LOQ = 10 (SD/ **S)**

Where,

S = slope of calibration curve

SD = standard deviation of the response.[16]

RESULT AND DISCUSSION

The absorption spectrum of Product was measured in the range 200–400 nm against the blank solution Methanol similarly prepared. The standard solution show maximum absorbance at λ max for each three systems as recorded in Table 1. And the method was validated by studying the following parameters. The optimum conditions for UV spectroscopy method has been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of products on the absorbance of the sample and colour species. Beer's law limits, molar absorptivity and % relative standard deviation are summarized in Table 1. The regression analysis using the method of least squares was made for the slope (b), intercept(a) and correlation coefficient (r^2)obtained from different concentrations are given in Table 1. The results showed that the method have reasonable precision. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical powder forms analyzed by the proposed methods. The percentage recoveries are given in Table 1.

Table No. 1: Optimum conditions, Optical characteristics and Statistical data of the regression equation in UV method with product

Sr. No.	Parameter	Proposed method
1.	Regression equation	Y=0.0762x – 0.0077
2.	Slope	0.0762
3.	Correlation coefficietion	0.9985
4.	Linear range(ppm)	1-10µg/ml
5.	Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.765
6.	Limit of detection (LOD)	0.2367
7.	Limit of quantification (LOQ)	0.7178
8.	Recovery	93.3%
9.	Lambda max (nm)	226nm
10.	Intercept (a)	0.0077

Compare peaks of Amlodipine Besylate with synthesised product

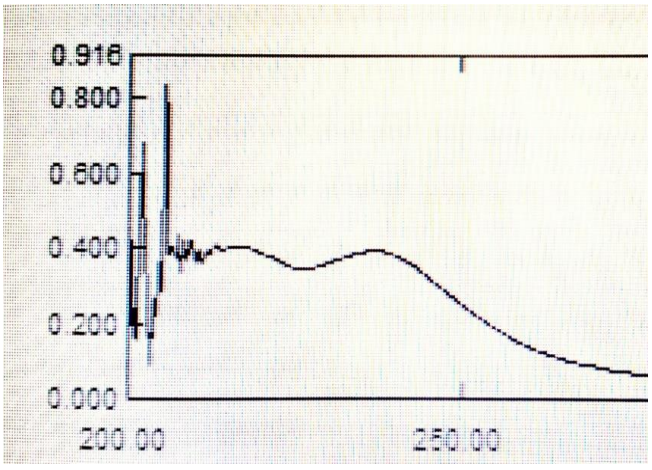


Figure no. 1: Peak of Amlodipine Besylate

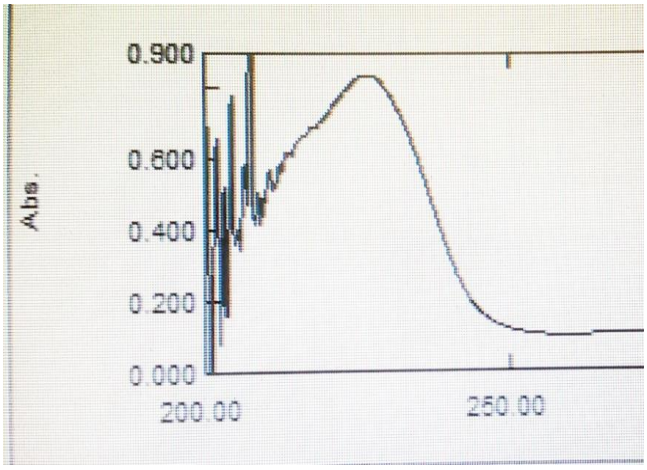


Figure no. 2: Peak of synthesised product

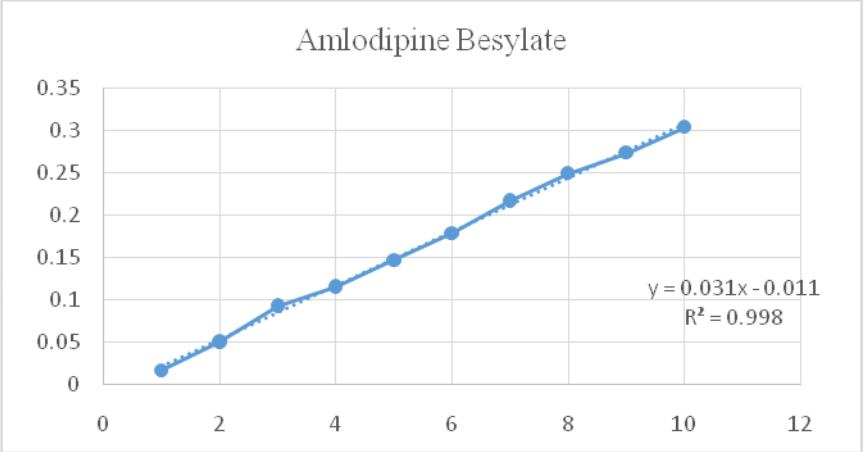
The above peaks of Amlodipine Besylate and synthesised product denoted that is the synthesised product peak is higher than the Amlodipine Besylate peak.

Calibration curve of Amlodipine Besylate and Product

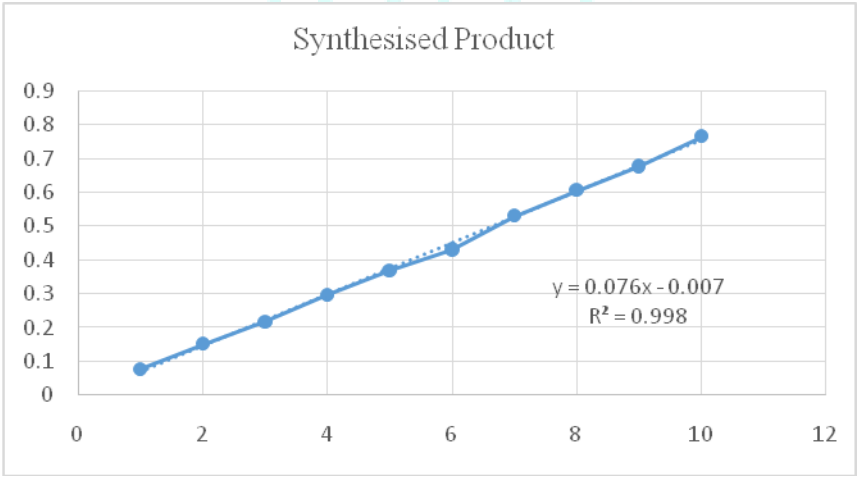
A calibration curve constructed with our method at the absorbance at 237nm, showed linearity in the range 1 - 10 µg/mL for Amlodipine Besylate and at 226nm 1 – 10 µg/mL for Product, with r2 of 0.998 and 0.9985 respectively. The wavelength of 237 nm was selected for Amlodipine Besylate because the two absorption maxima in the UV region (359.6 nm and 285 nm) were not suitable. The wavelength of 226nm was selected for product because the two absorption maxima in the UV region (271.4 nm and 260.8 nm) were not suitable.

Table No. 3: Calibration curve of Amlodipine Besylate and Product

Sr. No.	Concentration	Amlodipine Besylate	Product
1.	1 µg/ml	0.016	0.077
2.	2 µg/ml	0.050	0.151
3.	3 µg/ml	0.092	0.216
4.	4 µg/ml	0.115	0.297
5.	5 µg/ml	0.146	0.368
6.	6 µg/ml	0.178	0.450
7.	7 µg/ml	0.216	0.530
8.	8 µg/ml	0.249	0.606
9.	9 µg/ml	0.273	0.676
10.	10 µg/ml	0.303	0.765



Graph no. 1: Calibration curve of Amlodipine Besylate



Graph no. 2: Calibration curve of Product

CONCLUSION

The proposed method is found to be simple, rapid, selective and highly sensitive than most of the spectrophotometric methods available in literature. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus the method can be adopted as an excellent spectrophotometric method. It is therefore recommended as an alternative method for routine quality control of Amlodipine Besylate.

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