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Original Research Article

Development and Validation of Reversed Phase High Performance Liquid Chromatographic Method for Determination of Amlodipine

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Abstract

Purpose: *To develop and validate a new sensitive and low-cost method for the analysis of amlodipine in tablet dosage form using reversed phase high performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection.*
Methods: *Standards and samples were prepared by dissolving amlodipine besylate standard or amlodipine tablets in mobile phase and sonicated for 5 min. The samples were analysed by RP-HPLC equipped with quaternary pump and auto-injector. Separation was achieved using C18 column, and the mobile phase consisted of ammonium acetate buffer containing 0.02 % triethylamine TEA (pH = 4, adjusted using glacial acetic acid) and acetonitrile in the ratio 60:40 v/v. The flow rate was 1 ml/min and a UV detector was used for the detection of amlodipine at a wavelength of 248 nm. The method was validated according to International Conference of Harmonization (ICH) guidelines.*
Results: *The retention time for amlodipine peak was 3.44 ± 0.41 min with a total run time of 6 min. The method was linear over the range of 0.5 - 40 µg/ml with coefficient of determination (R^2) of 0.999. Recovery was 98.09 - 100.19 %, and the method showed high precision and repeatability. All validated parameters were within the range of ICH requirements.*
Conclusion: *A new rapid sensitive and low-cost method has been developed and validated for the analysis of amlodipine in tablet dosage form.*

Keywords: *Amlodipine, Recovery, Repeatability, Precision, Reversed phase high performance liquid chromatography, Validation*

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INTRODUCTION

Amlodipine, 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester, a third generation dihydropyridine calcium antagonist. It is used in the treatment of hypertension and coronary artery disease (CAD) such as chronic stable angina, vasospastic angina (Prinzmetal's or Variant Angina) and angiographically-documented CAD [1]. It was firstly formulated by Pfizer under the brand name of Norvasc, but several generic versions are now available. Amlodipine was combined with several drugs in order to enhance their activities or to combine more activities.

Literature review shows different analytical methods for the analysis of amlodipine in pharmaceutical preparations or biological fluids either as a single drug or in combination with other drugs. Those analytical methods include: spectrophotometric methods with or without derivatization [2-7]. The derivative spectroscopy has also been applied for the determination of amlodipine and its photodegradant compound [8]. Chromatographic methods have been used widely for the analysis of amlodipine, including thin layer chromatography (TLC) methods [9-12] and HPLC methods [13,14]. Capillary electrophoresis methods were also used for the analysis of amlodipine in its formulations and biological fluids [15,16]. Amlodipine was also determined using liquid chromatography mass spectrometry (LC-MS) [17,18]. .

One of the most important requirements to prepare any new formula for existed drugs is to develop and validate an analytical method for the analysis of the drug in bulk and finished product. Some existed methods were tested for the analysis of new amlodipine tablet produced but they were found not to fulfil the requirements. Some of the low-cost methods reported before have low sensitivity, whereas the highly-sensitive methods are costly. The aim of this study was to develop and validate a new, low-cost, highly-sensitive and specific HPLC method for the analysis of amlodipine in tablets dosage form.

EXPERIMENTAL

All the chemicals were of analytical grade and the solvents were HPLC grade. Amlodipine besylate USP standard with potency of 99.8 % was purchased from Sigma. Acetonitrile, methanol, phosphoric acid, and triethylamine were obtained from Fisher Scientific. Ammonium acetate, glacial acetic acid, sodium hydroxide, hydrochloric acid, hydrogen peroxide, were all purchased from Merck. All solvent were filtered using vacuum filtration unit Sartoriuse (Sartorius Goettingen, Germany) with Nilon filters (0.45 µm). Mettler Toledo (USA) analytical balance was used. The pH of the buffers was monitored using Mettler Toledo pH meter.

Chromatographic conditions

HPLC (Agilent Technologies USA) equipped with Agilent 1200 series quaternary pump and vacuum degasser, an Agilent 1200 series auto-injector with 0.1 – 100 uL variable volume injector and an Agilent 1200 series variable wavelength detector (VWD). Data processing was performed using HP Chem-Station software. The samples were injected to the C18 Hypersil (3.5 µm, 2.1 × 100 mm) column and the mobile phase consisted of ACN: Ammonium acetate buffer 0.05 M containing 0.02 % TEA (40:60 v/v) and the pH of the buffer was adjusted to pH = 4 using glacial acetic acid. The flow rate was 1 ml/min and the detection of amlodipine besylate was monitored using UV detector at 248 nm wavelength.

Method development

Standard preparation

A stock solution of amlodipine with the concentration of 1 mg/ml was prepared by transferring 13.87 mg of amlodipine besylate (equivalent to 10 mg of amlodipine) into 10 ml volumetric flask and 2 ml of mobile phase was added and sonicated for 5 min and then cooled to the room temperature. The solution was diluted to the mark using mobile phase. Further dilutions with mobile phase were prepared to obtain suitable calibration curve standard in the linear range of 0.5-40 µg/ml.

Sample preparation

To prepare the tablet samples, 10 tablets were weight and grounded and then the equivalence weight of one tablet was transferred into 10 ml volumetric flask and mixed with 5 ml of mobile phase. The mixture was sonicated for 20 min, cooled to the room temperature and then the volume was brought to 10 ml using mobile phase. The mixture was mixed thoroughly and then filtered using Whatman filter paper no. 1. Further dilution was carried out using mobile phase before injection.

All stock solutions were kept in a refrigerator under 4 ^{o}C and they were stable for three months under such conditions.

Chromatographic analysis

Samples were analysed using Agilent 1200 Series RP-HPLC equipped with quaternary pump and auto injector. UV detector was used for the detection, and the wavelength was 248 nm.

Different types of column C8 and C18 were tested to choose the best for the analysis. All chromatographic parameters were evaluated for better separation, including mobile phase

composition, pH, flow rate and column temperature.

Method validation

The developed method was validated according to ICH guidelines [19] to ensure specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness of the method

Forced degradation studies

Forced degradation studies of the drug substance can help identify the likely degradation products, the stability and specificity of the analytical procedure.

Acid degradation studies

A volume of 9 ml of 0.1 M hydrochloric acid solution was added to 1 ml of amlodipine standard stock solution or to 1 ml of tablet stock solution. These solutions were divided into three parts and maintained under 4, 50, and 90 ^{o}C, the portions were allowed to stand for 6 days and analysed at intervals over 120 h after dilution to 10 µg/ml and neutralization using NaOH.

Alkali degradation studies

Sodium hydroxide 0.1 M was used for alkali degradation study. A volume of 9 ml of sodium hydroxide was added to 1 ml of amlodipine standard stock solution or to 1 ml of tablet stock solution. These solutions were divided to three parts and maintained at 4, 50, and 90 ^{o}C; the portions were allowed to stand for 6 days and analysed at intervals over 120 h after dilution to 10 µg/ml using mobile phase and neutralization using HCl.

Oxidization degradation studies

A volume of 9 ml of 3 % hydrogen peroxide was added to 1 ml of amlodipine standard stock solution or tablet stock solution. These solutions were divided to three parts and maintained at 4, 50, and 90 ^{o}C, the portions were allowed to stand for 6 days and analysed at intervals over 120 h after dilution to 10 µg/ml.

Temperature stress studies

A 1 ml of amlodipine standard stock solution or Amlodipine besylate tablet with concentration of 1 mg/ml of amlodipine besylate were maintained at 4, 50, and 90 ^{o}C for 6 days and analysed at intervals over 120 h.

Statistical analysis

All data were derived from at least three independent experiments. Statistical analysis of the data was carried out using Microsoft Excel 2010.

RESULTS

Chromatographic conditions

The optimized chromatographic conditions were as follow: the column was C18 Hypersil (3.5 µm, 2.1 × 100 mm) as it gave the best peak shape and the highest reproducibility and repeatability. Mobile phase was ACN: Ammonium acetate buffer 0.05 M (40:60 v/v) with pH = 4 adjusted using glacial acetic acid. The detector used in this study was UV/Vis, the wavelength was set at λ = 248 nm to reduce the noise which is generated by using lower wavelengths. The elution was performed at 30 ^{o}C with flow rate of 1 ml /min. The retention time (RT) of amlodipine under these conditions was 3.4 min and the full run time was optimized to 6 min; Figure 1.

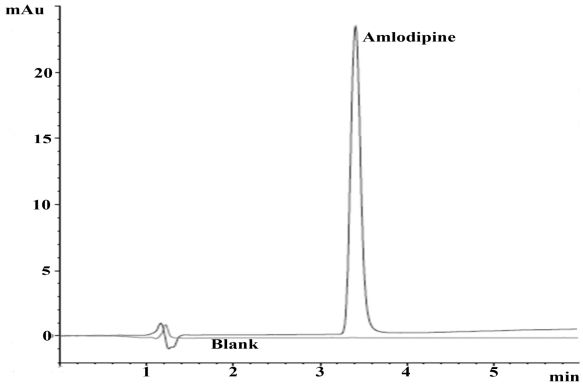

Figure 1: Chromatograms obtained from amlodipine besylate and placebo (blank)

Validation results

Specificity

The specificity of the method was determined by injecting the sample solution containing the placebo which was prepared by mixing the excipients without the amlodipine using same amount as the tablet. The method was specific for amlodipine upon running the placebo and degradation study. The results showed no interference from the excipients as shown in Figure 1. The results of forced degradation studies revealed no interference with the degradants peaks also.

Linearity

Linearity solutions were prepared at 7 concentration levels from 2.5 % to 200 % of analyte concentration. The method was linear over the range of 0.5 - 40 µg/ml with linear regression equation of y = 9.6362x - 1.7689 and a coefficient of determination R^2 of 0.999.

Accuracy

The accuracy of the method was carried out by spiking known amount of amlodipine besylate at seven concentration levels ranging from 50 % and 150 % of the label claim along with the excipients in triplicate. Samples were prepared following the sample preparation method described above. The recovered amounts were in the range of 98.1 - 100.19 % which is within the acceptable range according to ICH guidelines. The accuracy and recovery results are presented in Table 1.

Precision

Repeatability, intra-day and inter-day precision studies were carried out to ensure the precision of the method. Precision of the method was checked by carrying out six independent assays of amlodipine besylate test samples against the standard. For repeatability and intraday precisions, six replicate of standard solutions were injected within same day, while for inter-day precision, the samples were injected in different days. Two parameters were evaluated for precision studies which are area and retention time of the peak. The results are shown in Table 2.

Table 2: Precision (repeatability, inter-day and intraday) results

Parameter	Concentration	Retention time (min)
Repeatability	1.028	0.186
Intra-day RSD	1.187	0.416
Inter-day RSD	1.408	0.318

Limit of detection (LOD) and of quantification (LOQ)

LOD and LOQ were calculated using the slope and standard deviation of standard curve for low concentrations of amlodipine. LOD was 17.2 ng/ml while LOQ was 52.3 ng/ml.

System suitability

System suitability parameters were evaluated according to ICH guidelines such as USP tailing factor, plate number and capacity factor. The RSD for repeated sample run was evaluated also according to USP method for amlodipine. Table 3 shows the results for system suitability parameters and they are all within the acceptable range for ICH and USP guidelines.

Forced degradation studies

The results showed that amlodipine standard and amlodipine tablet were stable under room temperature, acid hydrolysis, base hydrolysis and solvent effect conditions as shown in Figure 3a, 3b, and 3c. The results also revealed that amlodipine standard and tablet are stable under 50 ^{o}C using base hydrolysis as it appears in Figure 3b. It is indicated in the results that both standard and tablets followed the same path of degradation up to 48 h indicating that the

Table 1: Accuracy and recovery of amlodipine in spiked samples

Drug	Concentration						
	50%	70%	80%	100%	120%	130%	150%
Amount added (mg)	5	7	8	10	12	13	15
Amount recovered (mg)	4.928	7.0135	8.011	9.815	11.808	12.752	14.715
Actual content (%)	98.551	100.193	100.140	98.150	98.405	98.096	98.106
RSD	1.884	1.787	0.998	0.786	0.924	0.257	0.480

Table 3: System suitability parameters results of amlodipine standard

System suitability parameter	USP tailing factor	Plate count number	Capacity factor K`	RSD for peak area for repeated run
Accepted range	1.022 < 2	2791 > 2000	5.880 > 2	1.028 < 2

excipients have no effect on the degradation of amlodipine in tablet form. After 48 h and the excipients showed some effect in the degradation process of amlodipine in tablet form using acid and solvent effect degradation and this effect appeared significantly after 120 h as shown in Figure 3a and 3c. For oxidization, about 50 % of amlodipine was degraded after 30 h under 4 and 50 ^{o}C (Figure 3d). Under 90 ^{o}C, full degradation was achieved using oxidization after 2 h (Figure 3d), while it needed 120 h to degrade the whole amlodipine under acid, base and solvent degradation as it is shown in Figure 3a, 3b and 3c.

Application of proposed method to commercial products

The method was applied for three commercial products which are: amlodine (S.Y.P. Malaysia), Vamlo (Ranbaxy) and iAmlo (formulated in-house at IIUM Pilot Plant). The samples were prepared according to sample preparation method described in experimental section and analyzed using the developed method. The results were shown in Table 4 and they were comparable with the reference method [1].

DISCUSSION

Among others, amlodipine is one of the products which has been studied for the quality control issues. This study was designed to develop and validate suitable in house analytical method for the analysis of amlodipine in the new formulation. From the results it can be claimed that the developed method was specific, accurate and reproducible. The detector used in this study was UV/Vis detector which

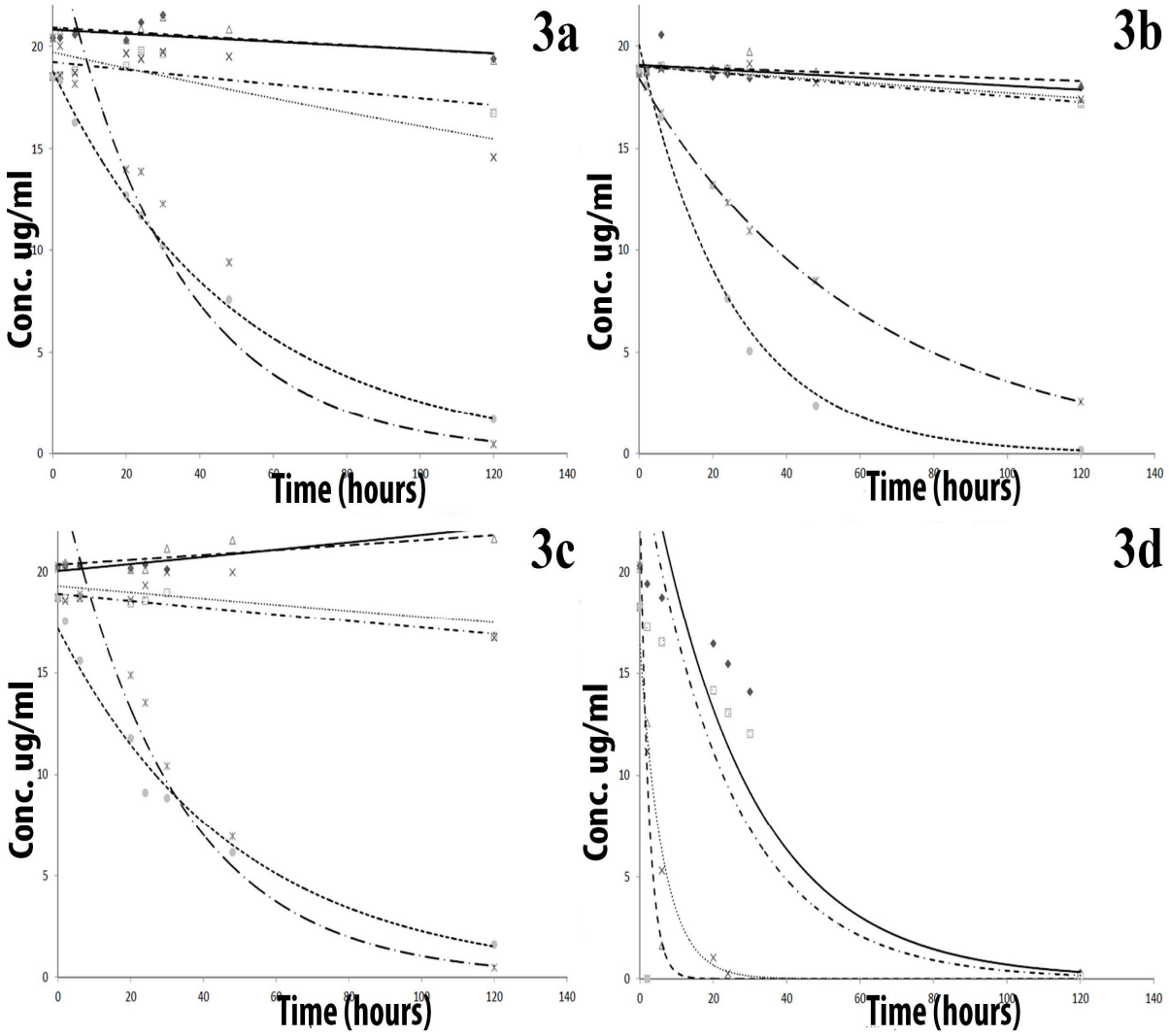

Figure 3: Forced degradation profiles for amlodipine standard and tablet under varying conditions: ♦, △ and * standard at 4, 50 and 90 ^{o}C; □, X, ○ tablets at 4, 50 and 90 ^{o}C

Table 4: Assay results for the determination of amlodipine in three commercial formulations

Dosage form		Labeled amount (mg)	Proposed method		Reference method
			Amount found (mg)	Amount found (%)	Amount found (%)
Anlodine	Sample 1	10	9.947	99.470	
	Sample 1	10	9.884	98.840	
	Sample 3	10	9.763	97.630	
	Mean(SD)		9.865(0.076)	98.650	
Vamlo	Sample 1	5	5.027	100.540	
	Sample 1	5	4.930	98.610	
	Sample 3	5	4.925	98.500	
	Mean(SD)		4.961(0.047)	99.220	
lAmlo	Sample 1	10	9.774	97.740	97.020
	Sample 1	10	10.315	103.150	99.360
	Sample 3	10	10.022	100.220	98.920
	Mean(SD)		10.036(0.220)	100.360	98.430(1.020)

ensures that the method is low-cost, and also that high reproducibility achieved. LOD and LOQ results from this study were comparable with those achieved using sophisticated technology. The method was linear over the range of 0.5 - 40 µg/ml with R^2 of 0.999 and the linearity was evaluated for lower concentrations to find LOD in the range of 0.1 - 5 µg/ml with R^2 = 0.999.

The method was evaluated for precision and reproducibility and the results were in the accepted range of ICH guidelines. The method was robust upon using different instruments or columns. The forced degradation studies showed that amlodipine is stable against acid and base hydrolysis in room temperature and 50 ^oC, while it is totally unstable due to oxidization by H_2O_2. Comparison of the results obtained in this study with results from reference methods indicate that this method is highly accurate and robust.

CONCLUSION

A robust, accurate, economic and precise method has been developed and validated according to ICH guidelines and is suitable for routine analysis of amlodipine in solid dosage forms.

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