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Quantitative Analysis of Amlodipine Tablets in the Libyan Market Using Ultraviolet Spectrophotometry

Mohammad S Faisal[1*], Mustafa S Sawan[2], Zrien Naz[1], Aya M Haweel[1], Nada A Bin Khalil[1]
1. College of Pharmacy, Alasmarya University, Zliten, Libya;
2. College of Pharmacy, Elmergib University, Al-Khums, Libya

ABSTRACT

A simple, precise, and accurate UV-spectrophotometric method was developed for the quantitative analysis of amlodipine (AMD) in various tablet brands available in Libya. This method was used to determine AMD at its maximum absorbance (λmax) of 238 nm in methanol. The method demonstrated linearity in the concentration range of 5-30 µg/mL for AMD drugs, with a notably high correlation coefficient (r2 = 0.9992). The detection limit and quantification limit were determined to be 1.62 and 4.88 µg/mL, respectively. The suitability of the developed method for quantitative determination of drug was proved by ICH validation. The method was effectively employed to analyze amlodipine tablets available in the Libyan market.

KEYWORDS: *UV spectrophotometer, Amlodipine, Validation, ICH*

** Corresponding author:* faisalhamdard07@gmail.com

INTRODUCTION:

Amlodipine besylate is a commonly used drug for treating hypertension and angina (1,2). Chemically, it is a 2-[(2-aminoethoxy) methyl] -4-(2-chlorophenyl)-3ethoxy carbonyl-5- methoxycarbonyl-6-methyl- 1,4-dihydropyridine benzene sulfonate (Figure.1), it acts by inhibiting calcium channels, which prevents the influx of calcium ions into vascular smooth muscles and cardiac muscles. As a result, it affects their contractile process and leads to reduced blood pressure. It is more effective than β-blockers in treating variant angina because it prevents and reverses coronary spasms, resulting in increased blood flow and improved myocardial oxygen supply (3,4). Additionally, amlodipine selectively inhibits arterial vascular smooth muscle cell proliferation, preventing progressive narrowing of the arteries (5,6). Amlodipine besylate is officially recognized in the British Pharmacopoeia (7). It is a chiral calcium antagonist currently used as a racemate (a 1:1 mixture of R-(+) - and (S)-(–)-amlodipine) (8).

The literature survey reveals that spectrophotometric methods (9-21), HPLC (22,23), HPTLC (24,25), and LC-MS/MS methods (26-28) have been used. In the present study, an attempt has been made to develop a UV Spectrophotometric method for the determination of Amlodipine besylate in bulk and tablets available in the Libyan market using methanol as a solvent. The advantage of the UV method over other sophisticated methods is that it does not require the extraction procedure for sample preparation, there is no need to add an internal standard, and it avoids the procedures usually associated with chromatographic methods (10). It is less time-consuming and economical. The spectroscopy method requires only a wavelength scan (11), so it can be utilized for frequent analysis of Amlodipine in pharmaceutical dosage forms more than the other sophisticated methods.

MATERIAL AND METHODS:

MATERIALS:

Pure amlodipine was procured from Nawah scientific Centre, Egypt. Double beam UV spectrophotometer (SPECORD 200 PLUS, Analytik Jena GmbH, Germany), was used for all absorbance measurements, Analytical electronic balance, two different brand of Amlodipine Besylate 5mg tablet was (Bristol laboratories Ltd UK) and was purchased from local Libyan market. Analytical grade methanol.

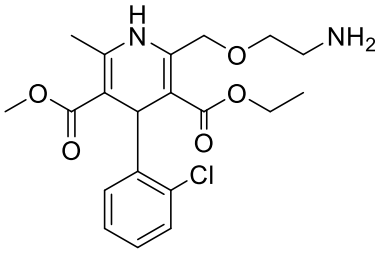

Figure 1: Chemical structure of amlodipine

METHOD VALIDATION:

The method was validated in accordance with the ICH guidelines for the validation of analytical procedures, with the aim of determining the linearity, sensitivity, precision, robustness, and accuracy of the analyte (29). To assess the method's validity and reproducibility, a known quantity of the pure drug was added to the analysed tablet powder sample, and the mixture was analysed for drug content using the proposed method. The recovery was assessed by using standard addition method. The prenasalized samples of amlodipine was spiked with the extra 0, 80, 100 and 120 % of the standard amlodipine and the mixtures were analysed by the proposed method. The experiment was performed in triplicate. The % recovery of samples, % RSD was calculated at each concentration level as shown in Table 1.

Table 1. Recovery by accuracy

S. No	Level of % recovery	Amount of Drug (µg/mL)	Amount of standard added (µg/mL)	Total amount	Amount found	% Recovery
1	80	10	8	18	17.6	97.78
2	100	10	10	20	20	100
3	120	10	12	22	22.3	101.36

PRECISION:

As per the ICH Q2 (R2) guidelines, precision was evaluated at two levels: repeatability and intermediate precision. Repeatability was determined as intraday variation of sample application, while intermediate precision was evaluated through inter-day variation for the determination of amlodipine at three different concentration levels of 8, 10, and 12 µg/mL, each performed in triplicate (Table 2). The method's reproducibility was examined by measuring six equal concentrations of amlodipine (10 µg/mL), prepared from a standard stock solution as shown in Table 3.

Table 2: Intermediate Precision of amlodipine

Precision	Concentration (µg/mL)	Absorbance ± SD	%RSD
Intraday	8	0.3167 ± 0.0031	0.95
	10	0.3711 ± 0.0020	0.49
	12	0.4994 ± 0.00194	0.38
Intraday	8	0.3267 ± 0.0030	0.92
	10	0.3744 ± 0.0035	0.95
	12	0.4920 ± 0.0064	1.31

Table 3: Repeatability Amlodipine

Amlodipine	Absorbance	Average Absorbance ±SD	%RSD
10 µg/ mL	0.3665	0.3664 ± 0.0033	0.92%
	0.3681		
	0.3625		
	0.3691		
	0.3621		
	0.3701		

SENSITIVITY AND LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):

The sensitivity of the method was evaluated based on the limit of detection (LOD) and the limit of quantitation. As per the ICH guidelines, the LOD is defined as the smallest quantity of the analyte in a sample that can be detected, while the limit of quantitation is the smallest quantity of the analyte in a sample that can be quantitatively determined with appropriate precision and accuracy [9,16]. The determination of LOD and LOQ was done using the slope of the calibration curve and σy/x of the blank sample by the following formulae:

$$LOD=(3.3\sigma(y/x))/S$$

$$LOD=(10\sigma(y/x))/S$$

where σ(y/x) is the standard deviation of the blank response, and S is the slope of the calibration curve.

The LOD and LOQ took into account the standard deviation of the responses and the slope.

The detection limit of a specific analytical procedure refers to the smallest quantity of the analyte that can be detected within a sample, although it may not be quantified as an exact value. The LOD of AMD by the proposed method was found to be 1.627 µg/mL.

QUANTITATION LIMIT (LOQ):

The quantitation limit of a specific analytical procedure is the minimum concentration of an analyte in a sample that can be quantified with acceptable accuracy and precision. The LOQ of AMD by the proposed method was found to be 4.883 µg/mL 4.883 µg/mL.

LINEARITY:

The linearity of an analytical method refers to its ability to obtain test results that are directly proportional to the concentration of the analyte in the sample. The calibration curve of amlodipine was linear over the range of 5 – 30 µg/mL (Table 4 & Figure 2)

Table 4: Linearity data for Amlodipine

S. No	Drug Concentration µg/ mL	Mean Absorbance
1	5	0.2009 ± 0.010
2	10	0.3664 ± 0.018
3	15	0.5274 ± 0.027
4	20	0.722 ± 0.036
5	30	1.0447 ± 0.053

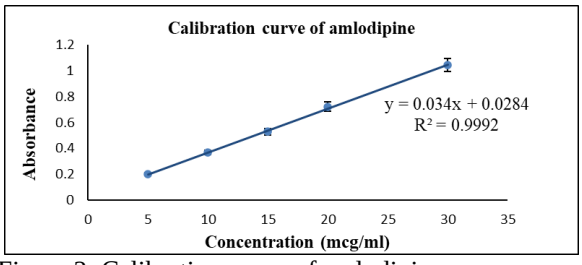

Figure 2: Calibration curve of amlodipine

Table 5: Spectrophotometric Analysis Parameters for Amlodipine

Parameter	λ_{max} 238 nm
Beer's Law	5-30 µg/mL
Regression equation	Y =0.034x + 0.0284
Slope	0.034
Intercept	0.0284
Correlation coefficient r^2	0.9992
Molar absorptivity	0.149×10^5
LOD	1.627 µg/mL
LOQ	4.883 µg/mL

SPECIFICITY:

The specificity of the method was established by measuring the interference, if any, observed due to the methanol solvent and excipients at the wavelength maxima of amlodipine. No significant absorbance due to methanol was observed at 238 nm. The method is specific for the intended analysis

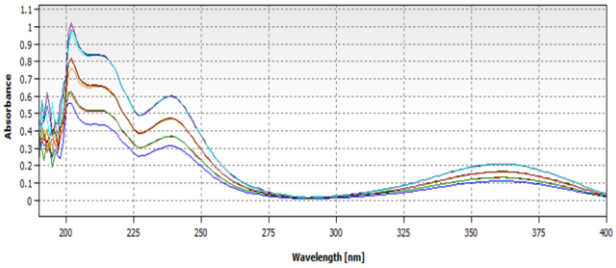

Figure 3: Overlay spectrum of amlodipine besylate

ASSAY METHOD FOR AMLODIPINE TABLETS:

Two commercial amlodipine products, Amlor (Viatris, Spain) and Amlodipine (Bristol laboratories, United Kingdom), were acquired from a local market. The mean weight of each tablet was determined by weighing 20 tablets and then grinding them into a fine powder. The powder, equivalent to 100 mg of Amlodipine, was weighed and transferred to a 100 ml volumetric flask. Then, 70 ml of methanol was added to the flask and the mixture was sonicated for 10 minutes to dissolve the drug. The volume was then adjusted to 100 ml with distilled water. The solutions were filtered through Whatmann filter paper No. 41, with the initial few milliliters being discarded. The filtrates were diluted with distilled water to achieve a concentration of 10 µg/mL of amlodipine besylate. The absorbance at 238 nm was measured, and the drug quantity in the sample solutions was calculated using the slope and intercept values from the calibration curve (Table 5). This experiment was conducted three times to verify its reproducibility. The results of the tablet formulation analysis are documented in Table 6.

Table 6: Analysis data for tablet formulation

Sample	Batch number	Label claim	Amount obtained	Percentage	Relative standard deviation
Amlodipine	A622004B	5 mg	4.95	99%	0.52%
Amlor	B739002F	5 mg	5.1	102%	0.38%

RESULTS:

Amlodipine, which dissolves more readily in methanol than in water (European Pharmacopoeia), was quantified using methanol as the solvent. This solvent was used to extract and dissolve amlodipine besylate from its powdered tablet form. The results demonstrated a strong correlation between the estimated quantities and the amounts stated by the manufacturers. The average percentage of label claims for Amlodipine and Amlor were 99% and 102% respectively (Table 6), closely approximating 100 with low standard deviation values, which validates the accuracy of the proposed method. The results also showed a low percentage of RSD for precision and reproducibility, indicating that the proposed method is both precise and reproducible. The suggested method exhibits specificity, as evidenced by the lack of methanol's interference with the drug. The equation Y=0.034x+0.0284, derived from the linear regression of absorbance on concentration, and a correlation coefficient of 0.9992 (Table 5), further validate the method's reliability. Bernard et al., 2011 and Naveed et al., 2014 has also reported the similar result with high accuracy and precision, with a relative standard deviation of less than 2% (12 & 4). Given these attributes, the method is suitable for routine analysis.

CONCLUSION:

The method employed in this research for quantifying Amlodipine besylate from tablet formulations is simple, precise, accurate, sensitive, and reproducible. This proposed method could be employed for regular analysis in quality control laboratories.

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