The National Ribat University

Faculty of Graduate Studies & Scientific Research

Development and validation of UV Spectrophotometery method for estimation of amlodipine in capsules and tablets form

A thesis submitted in partial fulfillment of the requirements for master Degree in Drug Quality Control

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الآية

بسم الله الرحمن الرحيم

قال تعالي: " يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات والله بما تعملون خبير"

سورة المجادلة الآية 11

Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those who were close to my heart.

My humble effort dedicates to my sweet and loving.

Mother, father, sister and husband also my little son

Whose affection, love, encouragement and prays of day and night make me able to get such success and honor.

I also dedicate this thesis with special thanks to my sincerely friends, who have always encouraged me and believed in me.

I finally this thesis is dedicated to every teacher, doctor and professor who has taught me letter in my life, without them I could not have reached this level.

Acknowledgment

This research project would not have been done without ALLAH and the after support of many people.

I would like to express my deepest gratitude to my supervisor **prof. Elrasheed Ahmed Gadkariem**for his unwavering support and collegiality throughout this project.

Special thanks to **Dr. Imad Osman Abu Reid** for his valuable advice and support.

I wish to thank staff in the quality control department at national Ribat University

Abstract

Background:

Amlodipine is one of the most used antihypertensive drugs.

The quality control of such drugs (safety and efficacy) is considered of high important to obtain high quality drug.

Development of a simple easily conducted method using UV-spectrophotometry is consider of great value.

Objective of this work to develop a simple UV-spectrophotometry, validated method for analysis of amlodipine in its dosage forms marketed in Sudan.

Methodology:

Weight equivalent to 70mg of amlodipine was accurate weighted, transferred to 100ml volumetric flask, sonicated for 15 minutes using mixture of DMSO:Water in ratio 50:50% v/v, allowed to cool filtered and finally the volume was completed to 100ml.then 1ml from above solution was transferred to 10ml volumetric flask and the volume completed to the mark using same solvent.

From this solution 2ml was transferred into 10 ml volumetric flask and volume completed to the mark with same solvent (14µg/ml). The absorbance was measured at 365nm and the content % of was calculated. Using beer-lambert law.

Method validation parameters was conducting according to ICH guidelines (linearity, precision, accuracy, LOD, LOQ).

Standard addition method was carried to evaluate % recovery of the sample and of the added standard.

Results:

Regression analysis of Beer's law plot of the results obtained for analysis of the drugs showed correlation (r=0.9993) in a concentration linearity range 7-35µg/ml; at wavelength 365nm.

The developed method proved to be precise by the results (RSD% is less than 2%)

The LOD and LOQ values were found 1.54µg/ml and 4.69µg/ml respectively.

The assay results of the three brands of amlodipine besylate was found to 99.50±0.77 (n=3), 101.00±0.68 (n=3),101.00±0.93 (n=3).

Conclusion:

The developed Uv-spectrophotometery method was proved to be simple, sensitive, accurate and precise for determination of the selected drugs.

Recommendation:

This method been very simple and accurate, inexpensive cost, recommended to be used to in state of expensive and time consuming chromatographic and also for colorimetric method which need specific reagents.

المستخلص

ملخص

خلفية:

أملوديبين هو واحد من خافضات الضغط الأكثر استخداما.

وتعتبر مراقبة جودة هذه الأدوية (السلامة والفعالية) مهمة جدا للحصول على دواء عالي الجودة.

ويعتبر تطوير طريقة بسيطة وسهلة الاستخدام باستخدام طيف الأشعة فوق البنفسجية ذات قيمة كبيرة.

الهدف من هذا العمل لتطوير طيف الأشعة فوق البنفسجية بسيطة، طريقة التحقق من صحة لتحليل أملوديبين في أشكالها غالينيك تسويقها في السودان.

المنهجية:

كان وزنه يعادل 70 ملغ من أملوديبين وزنها بدقة، نقل إلى 100 مل قارورة الحجمي، سونيكاتد لمدة 15 دقيقة باستخدام 50: المياه المبردة دمسو: خليط المياه. وأخيرا تم الانتهاء من حجم إلى 100 مل. تم نقل 1 مل من الحل أعلاه إلى V / V 50٪ قارورة حجمية 10 مل وتم ملء حجم للعلامة باستخدام نفس المذيبات.

من هذا الحل، تم نقل 2 مل إلى قارورة حجمية 10 مل واستكمل حجم مع نفس المذيبات (14 ميكروغرام / مل). تم قياس الامتصاصية في 365 نانومتر وتم حساب المحتوى٪. استخدام قانون البيرة لامبرت.

وكانت معلمات التحقق من الطريقة وفقا للمبادئ التوجيهية إش (الخطي والدقة والدقة، لود، لوق).

تم إجراء طريقة الإضافة القياسية لتقييم نسبة الاسترداد للعينة والمعيار المضاف.

النتائج:

في نطاق خطي **(**r = 0.9993أ ظهر تحليل الانحدار مرة قانون بير للنتائج التي تم الحصول عليها لتحليل الأدوية الارتباط (.nmمل؛ في الطول الموجي 365 / µgتركيز 7-35

أثبتت الطريقة المتقدمة أن تكون دقيقة بالنتائج (رسد٪ أقل من 2٪)

مل على التوالي. / µgمل و 4.69 / µgتم العثور على قيم لود و لوق 1.54

تم العثور على نتائج مقايسة العلامات التجارية الثلاث من بيسيلات الأملوديبين إلى 99.50 ± 0.77 (ن = 3)، 101.00 ± 0.68 (ن = 3)، 101.00 ± 0.93 (ن = 3).

استنتاج:

وقد ثبت أن طريقة طيف الأشعة فوق البنفسجية المتقدمة لتكون بسيطة وحساسة ودقيقة لتحديد الأدوية المختارة.

تم التحقق من صحة هذه الطريقة على أساس المبادئ التوجيهية ، انها بسيطة وموثوق بها .وبالتالي مفيدة للتحليل الروتيني لأملوديبين.

توصية:

وكانت هذه الطريقة بسيطة جدا ودقيقة، و غير مكلفة، وأوصت لاستخدامها كبديل من الكروماتوغرافي لانها مكلفة وتستغرق وقتا طويلا وأيضا بديل لطريقة اللونية التي تحتاج الكواشف محددة.

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Chapter One

Introduction & Literature Review

Chapter Two

Materials and Methods

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Discussion Conclusion & Recommendation

1. Introduction& Literature Review

1.1 Analytical spectroscopy

Analytical spectroscopy is the science of determining how much of a substance is present in a sample by accurately measuring how much light is absorbed or emitted by atoms or molecules within it. Different types of spectroscopy are available, depending on the type or wavelength of electromagnetic radiation absorbed or emitted by the atom or molecule. [1]

1.1.1. Methods of Analysis

Before the beginning of the twentieth century most quantitative chemical analyses used gravimetric or titrimetric as the analytical method.

With these methods, analysts achieved highly accurate results, but were usually limited to the analysis of major and minor analytes. Other methods developed during this period extended quantitative analysis to include trace level analytes. [2]

1.1.2. Applications of UV spectrophotometry in pharmaceutical analysis

Qualitative analysis through spectrophotometric methods achieves fast and accurate results using only small sample quantities. This fast and effect instrumentation has become an essential tool in the pharmaceutical industry due to its adaptability and economic value. Qualitative analysis has proven highly useful in many major forms of organic compounds and helps to ensure patient health and safety.

The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law. [3]

1.1.2.2. Beer-Lambert law

The measurment of light absorption by a solution of molecules is governed by the Beer-Lambert Law , which is written as follows:

$$A = \varepsilon\, b\, c$$

Where, A is known as the absorbance and is measure of the amount of light absorbed by sample.

ε is constant known as molar extinction or extinction coefficient and is the absorbance of a 1M solution of the analyte.

b=path length of the cell (in cm) usually 1cm.

c=concentration of the analyte in moles/liter. [4]

In pharmaceutical products, concentration and amounts are usually expressed in grams or milligrams rather than in moles and thus for the purposes of the analysis of these Products, the Beer-Lambert equation is written in the following form:

When c is in gm. /100 ml, then the constant is called A (1%, 1 cm)

$$A=A1\%1cmbc.$$

A is the measured absorbance; A (1%, 1 cm) is the absorbance of a 1% w/v(1g/100ml) solution in a 1cm cell; b is the path length in cm; and c is the concentration of the sample in g/100ml. [4]

1.1.3. Methods of drug assay

There are two methods of using spectroscopic measurements in drug analysis, the absolute and the comparative methods of assay.

In the UK and Europe, the Beer–Lambert equation tends to be used in what is called the absolute method of assay. In this procedure the absorbance is measured experimentally and the Beer–

Lambert equation is solved for *c*, the drug concentration. For this reason, the British Pharmacopoeia and European Pharmacopoeia quote A1% values in drug monographs.

In the US Pharmacopoeia, the comparative method of assay is preferred. In this type of assay, a standard solution of the drug to be analyzed is prepared, the absorbance of the sample and the standard are measured under identical conditions, and the concentration of the sample

is calculated from the relationship $\frac{A\,[test]}{A[STD]} = \frac{[Test]}{[STD]}$

where [test] is the concentration of the sample and [STD] is the concentration of the prepared standard. The comparative method of assay has the advantage that it can be used even if the drug undergoes a chemical reaction during the assay (e.g. formation of a colored derivative to allow measurement in the visible region of the spectrum), but suffers from the disadvantage

that an authentic sample of the drug in question must be available for comparison. [2]

1.1.4. Method validation

Validation is concerned with assuring that a measurement process produces valid measurements; Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice.

A measurement process producing valid measurements for an intended application is fit for purpose. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. [5]

Analytical methods need to be validated or revalidated:

_ before their introduction into routine use;

_ whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and

_ whenever the method is changed and the change is outside the original scope of the method. [5]

Types of Analytical Procedures to be validated:

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

1- Identification tests;

2- Quantitative tests for impurities' content;

3- Limit tests for the control of impurities;

4- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product [5].

1.1.5. Analytical characteristics used in method validation

1.1.5.1. Accuracy

"The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found". [6]

1.1.5.2. **Specificity**

"Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Lack of specificity of an individual procedure may be compensated by other supporting analytical procedure(s)" [6].

1.1.5.3. Precision

"The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample[7].

1.1.5.4. Repeatability

"Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment [6].

1.1.5.5. Intermediate Precision

"Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory [6].

1.1.5.6. Linearity

"The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample". [6]

1.1.5.7. Range

"The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity." [6]

1.1.5.8. Detection limit

"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. [6]

1.1.5.9. Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy."[6]

1.1.5.10. Standard Addition

If no adequate placebo can be prepared, a known amount of drug substance can also be added to an authentic batch of drug product (standard addition). Of course, in this case, only the range above the nominal content is accessible. In order to provide practically relevant information, the upper limit of the investigated spiking range should not greater than 150 %. Because the precision is concentration dependent, the percentage recovery calculation should be based on the overall amount of active present in the batch, and the spiked amount. [6]

Selected drug:

1.1.6 Amlodipine besylate:(ADB)

Is besylate salt of amlodipine, synthetic dihydropyridine with antihypertensive and antianginal effect.

1.1.6.1. Chemical properties

IUPAC name: benzene sulfonic acid; it's 3-o-ethyl 5-o-methyl 2-(2-amino ethoxy methyl) -4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.[7]

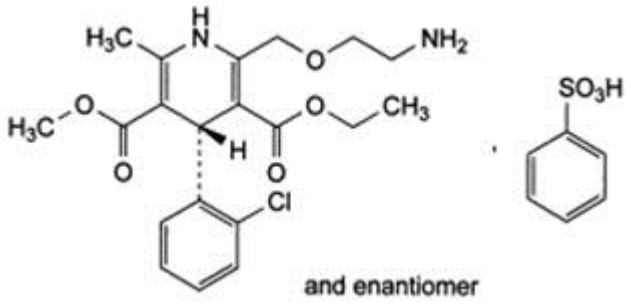


Figure (1) chemical structure of amlodipine besylate

Molecular formula: C26H31ClN2O8S

Molecular weight: 569.05

1.1.6.2. physical properties

White or almost white powder, slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol, slightly soluble in 2-propanol. [8]

1.2. Literature Review

Over the last decades, an increasing number of generic drug formulations have been released on pharmaceutical market. These products are generally analyzed with compendia methods.

Most methods developed for ADB in pharmaceutical form are have been applied for its determination in combined dosage forms and a limited number of them have been reported for the quantification of drug in single dosage forms.

Uv-Visible spectrophotometry is widely used for the assay of ADB in dosage form.

The assay procedure listed in European pharmacopeia describes a reversed phase high performance liquid chromatography method (HPLC.) for the determination of drug in bulk and pharmaceutical formulation. [9]

The official USP for the active pharmaceutical amlodipine assay in amlodipine besylate tablets is based on isocratic reversed phase LC analysis with uv absorption at wavelength about 237 nm.[10]

Another method based on several chromatographic techniques like liquid chromatography tandem-mass spectrometry [11], high performance thin layer chromatography [12] , unicellar electro kinetic chromatography [13] , packed column supercritical fluid chromatography [14] , and HPLC with photometric [15] and electrochemical detection [16] system have been used for the quantitative determination of amlodipine in pharmaceuticals . However, majority of HPLC procedures are applicable to combined dosage forms, and even those procedures used for assay in single dosage forms have either narrow range of applicability (0.5-16µg/ml) or poor sensitivity (2-10 mg/ml).

Other methods reported for the determination of ADB in formulations include UV-spectrophotometry [17], difference uv -spectrophotometry [18], fluorimetry [19] , and anodic stripping voltammetry [20].

-Other procedures based on ion-pair complex followed by extraction [21], derivatization [22], redox [23], oxidative coupling [21] and complex formation reactions [23] have been proposed by several researches.

Table 1: colorimetric methods reported for the assay of amlodipine:

Serial no.	Reagent/s	λ max. (nm)	Linear range (µg/ml)	remarks	Reference.
1	Bromothymol blue	405	40-45	Involved extraction	21
2	Bromocresol green	410	0-80	Involved extraction	24
3	Chloranil	362	1-70	Requires rigid PH control	25
4	Ascorbic acid	530	1-140	Requires boiling at 100±1 c for 25 min .	26
5	NaOH	456	20-100	Requires non-aq. Medium	27
6	2,3-dichloro-5,6 dicyano-1, 4-benzoquinone.	580	1-125	Uses non-aq.medium .	26
7	1,2-naphthaquinone-4 sulfonic acid.	477	1-80	Required strict PH control	25
8	1,2-naphthaquinone-4 sulfonic acid.	462	10-80	Involved incubation at 50®C for 20 min, requires strict PH control.	23
9	Ninhydrin	595	10-60	Uses non-aq.medium	28

another spectrophotometric methods involved treating a fixed amount of bromate–bromide solution in acid medium with ADB solution with determination of the unreacted bromine by treating affixed amount of metanil yellow dye solution and measuring the absorbance at 530nm. [29]

Moreover, ADB in pharmaceutical preparation was determined using charge transfer phenomena via complexation with tetracyanoethylene (TCNE) AND 7,7,8.8-tetra-cyanoquinodimethane (TCNQ) which are known to yield charge transfer complex with a variety of electron donors and to use these reagents for the quantitive determination of the drug in its dosage forms. [30]

1.3. Main objectives & Scope of the work:

1.3.1. Rational

Amlodipine besylate is most commonly used in Sudan for treatment of cardio-vascular disease. Most methods of analysis of this drug are either expensive, time consuming or required number of reagents.

In developing countries, development of simple, cost effective methods of analysis using the most available Uv-spectrophotometer is highly preferred

1.3.2 Objective

- Development of simple and reliable Uv-spectroscopic method for estimation of amlodipine in formulations.
- Validation of developed method according to the ICH guidelines.
- Application of the validated developed for assay of different brands of amlodipine marketed In Sudan.

2. Materials and Methods

2.1. Materials

Table 2: Materials:

NO	Chemical	Company	Origin
1	Dimethyl sulphoxide, extra pure USP, assay (99.5%). Batch no: GB003367 M.F.G:6/2016 E.X.P:6/2020	Scharalu chemie	European union
2	Amlodipine besylate working standard. Batch no: AB15423 M.F.G:10/2015 E.X.P:10/2017	Amipharma-laboratories	Sudan
3	Brand (A) capsule, equivalent to amlodipine10mg. Was purchased from local pharmacy. Batch no: D944, M.F.G:10/16 ,E.X.P:10/18	Tabuk-pharmaceutical	Sudan
4	Brand (B), equivalent to amlodipine 10mg Batch no: G605014, M.F.G: 10/16, E.X.P:09/18	Zydus	India
5	Brand (C), equivalent to amlodipine 10mg. Batch no: AMNH0179 M.F.G: 04/17 E.X.P:09/19	Micro labs	India
6	Laboratory Distilled water	Alribat university-faculty of pharmacy	Sudan

2.2 Instruments and Equipments

Table 3. Instruments and equipments:

NO	Instrument	Company	Origin
1	UV-min-1240 spectrophotometer	Shimazdu	Japan
2	Electronic balance	Shimazdu	Japan
3	Conical flask, measuring cylinder , volumetric flask, micro syringe	ISO lab	Germany
4	Filter paper	Bright	china
5	Bandelin ultrasonic RK100	Berlin	Germany

2.3. Methodology

2.3.1. Selection of suitable solvent

69.44mg of amlodipine besylate working standard (equivalent to 50mg amlodipine) was accurately weighed and transferred to 100ml volumetric flask and dissolved in either of the following solvents (dimethyl sulphoxide {DMSO}, methanol, mixture of DMSO:Water in ratio 50:50% v/v, and mixture of Methanol:Water in ratio 50:50%v/v); then were sonicated for 15 minutes, allowed to cool and finally completed to the mark with same solvent. 5ml from these solutions were transferred to 100ml volumetric flask and completed to the mark with the same solvent to obtain concentration (25µg/ml) of amlodipine; then the prepared amlodipine solution was scanned using uv-visible spectrophotometer between range 200-600 nm to select wavelength of maximum absorbance.

2.3.2. Selected Solvent preparation

250 ml DMSO and 250 ml distilled water were accurately measured, mixed to obtain mixture solution DMSO: Water in ratio 50:50%v/v.

2.3.3. Preparation of standard solution

- 97.22 mg of amlodipine besylate working standard was accurately weighed and transferred to 100ml volumetric flask and dissolved in solvent mixture (DMSO: WATER) in ratio 50:50%v/v. then were sonicated for 15 minutes, allowed to cool and finally completed to the mark with same solvent to obtain solution (**A)** (700µg/ml) of amlodipine. Then 1ml from solution (A) were transferred to 10 ml volumetric flask and completed to the mark with the same solvent (solution **B** 70 µg/ml)

2.3.4 Construction of calibration curve

five serial dilution were prepared by take 1, 2, 3,4, and 5ml from solution (B) were transferred to 10ml volumetric flask the volume was completed to the mark with the same solvent to obtain solutions of a concentration range (7,14,21,28 and35µg/ml) respectively. The absorbance of each solution was measured at λ max 365nm. Calibration curve was constructed by plotting the concentration against the absorbance.

2.3.5. LOD and LOQ

LOD and LOQ were calculated from linear regression equation using the following formula

LOD= 3.3sd/slope ,

where sd: is standard deviation of the absorbance (Y intercept) ,slope : of linear equation

LOQ= 10sd/slope

2.4. Validation

2.4.1. Determination of precision

2.4.1.1. Repeatability

Three replicates of Amlodipine standard solutions each (14,21,28µg/ml) were prepared, the absorbance of the prepared solutions was measured at λ max 365nm and RSD% were calculated.

2.4.1.2. Intraday precision

Three replicates of Amlodipine standard solutions each (14,21,28µg/ml) were prepared, the absorbance of each was measured 2 times in the same day. The RSD% was calculated.

2.4.1.3. Inter day precision

Three replicates of Amlodipine standard solutions each (14,21,28µg/ml) were prepared, the absorbance of each was measured on different days. The RSD% was calculated.

2.4.2. Accuracy

Determined by using standard addition method at three levels of standard of quantity equivalent to 50%,100%, and 150% added to pre-analyzed amlodipine sample. Then the absorbance of each solution was measure , after that recovery and percentage of recovery of the sample and added standard were calculated.

2.3.8. Assay of amlodipine brands

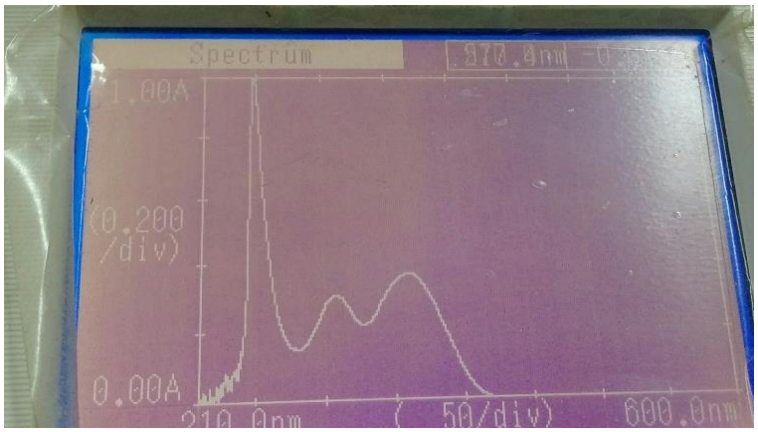
10 capsules from Brand (A) , 10 tablets from each of Brand (B) and Brand (C) (containing 10mg of amlodipine) were weighed and the average weight of each was determined .From powder of capsules and for tables ,a weight equivalent to 70mg of amlodipine was transferred to

100ml volumetric flask, sonicated for 15 minutes using mixture of DMSO:Water in ratio 50:50and allowed to cool , filtered and finally the volume was completed to 100ml.then 1ml from above solutions was transferred to 10ml volumetric flask ,completed to the mark with the same solvent.

From this solutions 2ml was transferred into 10 ml volumetric flask and volume completed to the mark. The absorbance was measured and the content % of each brand was calculated.

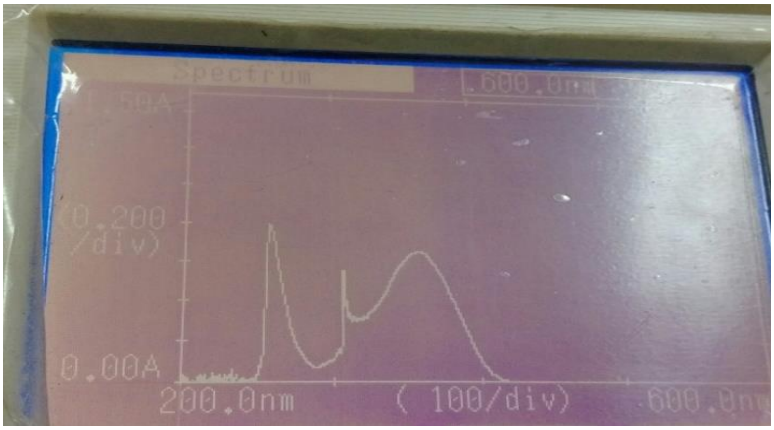
3. Results

3.1. Selection of the solvent, that shows better amlodipine wavelength.



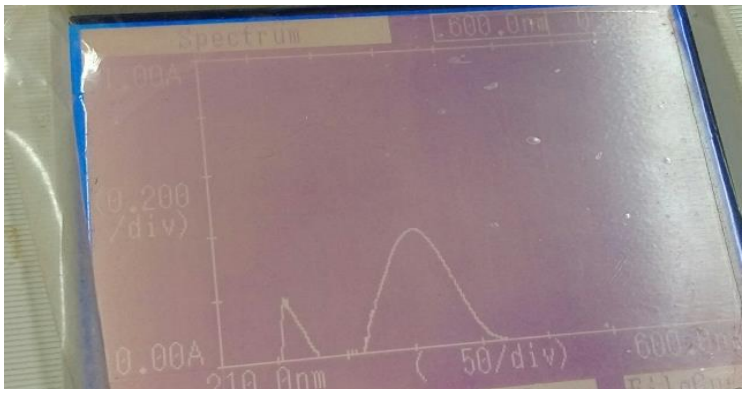
wavelenght	Absorbance
365nm	0.418

Figure 2: Spectrum for amlodipine 25µg/ml in mixture DMSO:water in ratio 50:50%v/v.



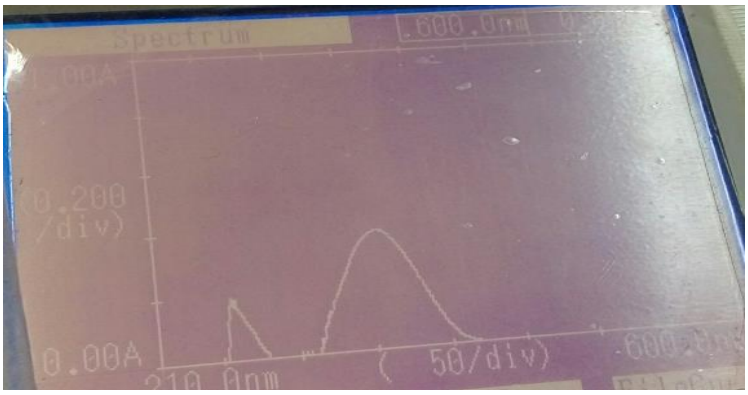
wavelenght	Absorbance
360nm	0.410

Figure 3: Spectrum for amlodipine 25µg/ml in mixture methanol: water in ratio 50:50%v/v.



wavelenght	Absorbance
360nm	0.430

Figure 4: Spectrum for amlodipine 25µg/ml in DMSO.



wavelenght	Absorbance
360nm	0.430

Figure 5: Spectrum for amlodipine 25µg/ml in Methanol.

3.2. Construction of Calibration Curve

The following tables and figures represent the linearity data within the range 7-35 µg/ml

Table (4): Concentration vs. Absorbance for linearity study using DMSO-Water:

Sr. no.	Volumes take from stock solution (7mg/ml)	Conc. Range (µg/ml)	Absorbance at 365nm
1	10 µl	7	0.120
2	20 µl	14	0.218
3	30 µl	21	0.330
4	40 µl	28	0.445
5	50 µl	35	0.565

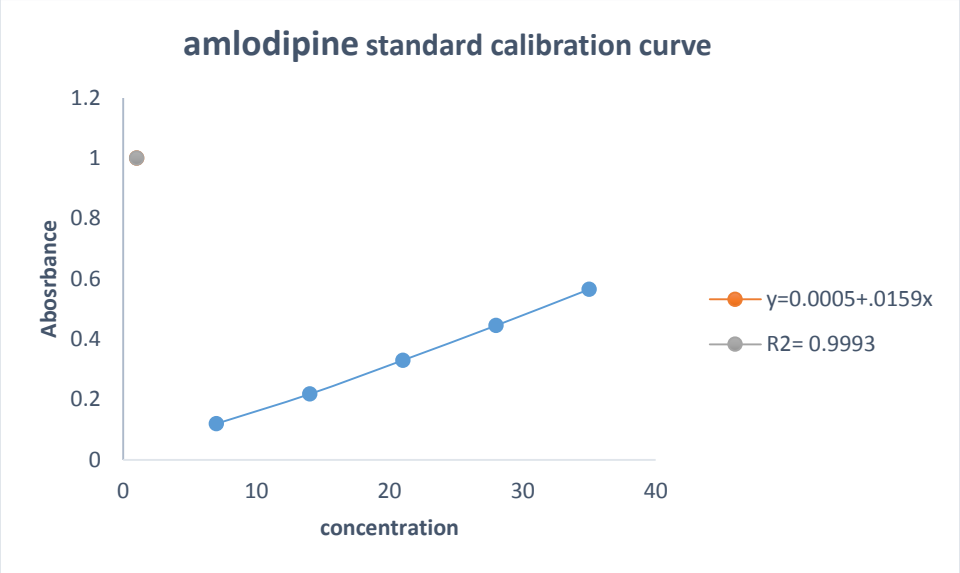


Figure 6: Calibration curve of Amlodipine standard solutions against Absorbance

3.3 Precision

Precision was performed in terms of repeatability, intraday & interday measurements.

3.3.1. Repeatability

Table 5: Repeatability study

Sample conc.(µg/ml)	14		21		28	
	Abs.	Assay	Abs.	Assay	Abs.	Assay1

	0.218	13.68	0.328	20.59	0.436	27.39
	0.215	14.12	0.328	20.59	0.440	27.64
	0.225	13.93	0.329	20.66	0.437	27.45
average	0.222	13.91	0.329	20.62	0.438	27.49
Recovered conc. %	99.36		98.18		98.19	
SD	0.0158		0.036		0.131	
RSD%	1.59		0.176		0.476	

3.3.2. Intraday precision

Table 6: Intraday precision

Sample conc.(µg/ml)	**14**		**21**		**28**	
	Abs.	**Assay**	**Abs.**	**Assay**	**Abs.**	**Assay1**
	0.215	13.49	0.330	20.72	0.441	27.70
	0.218	13.68	0.328	20.59	0.438	27.52
	0.223	13.99	0.335	21.04	0.440	27.64
average	0.219	13.72	0.331	20.78	0.440	27.62
Recovered conc. %	98.01		98.98		98.64	
SD	0.254		0.227		0.096	
RSD%	1.85		1.09		0.35	

Table 7: Intraday precision

Sample conc.(µg/ml)	**14**		**21**		**28**	
	Abs.	**Assay**	**Abs.**	**Assay**	**Abs.**	**Assay1**
	0.220	13.81	0.330	20.72	0.438	27.52
	0.226	14.18	0.333	20.91	0.438	27.52
	0.224	14.04	0.335	21.04	0.440	27.64
average	0.223	14.04	0.333	20.89	0.440	27.57
Recovered conc. %	100.10		99.48		98.42	
SD	0.192		0.158		0.073	
RSD%	1.37		0.758		0.264	

3.3.3. Interday precision

Table 8: Interday precision at day 2

Sample conc.(µg/ml)	**14**		**21**		**28**	
	Abs.	**Assay**	**Abs.**	**Assay**	**Abs.**	**Assay1**

	0.216	13.55	0.347	21.79	0.446	28.02
	0.223	13.99	0.348	21.85	0.442	27.77
	0.223	13.99	0.338	21.23	0.438	27.52
average	0.221	13.85	0.342	21.62	0.442	27.77
Recovered conc. %	98.91		103.77		99.17	
SD	0.254		0.346		0.252	
RSD%	1.84		1.60		0.906	

3.4. Accuracy:

The following table and figure represent the accuracy data:

Table 9: Accuracy study by standard addition method

samples (14µg/m)	**50% (7µg/ml)**	**100% (14µg/ml)**	**150% (21µg/ml)**
0.220	0.337	0.446	0.550
0.222	0.333	0.440	0.551
0.222	0.330	0.439	0.561
0.221	**0.333**	**0.442**	**0.554**
found conc.	7.09	13.99	21.08
Recovery %	101.29	99.90	100.36

Calculation of the % recovery for the sample:

$$Y = 0.0158X + 0.221$$

Considering y = 0

˜ X = 0.221/0.158= 13.99µg/ml

˜ % = 13.99/14*100= 99.93%

Or The Calculation can be done considering Y values for the % recovery for the standard addition Y values as 0.333 (7µg/ml) or 0.442 (14µg/ml)

Or 0.554 (21µg/ml). table (9**)**

Y (0.333) =0.0158X+0.221

X= 0.333-0.221/0.0158=7.09 µg/ml

% recovery**=**7.09/7*100= 101.29%

Y (0.442); X= 13.99, % recovery = 99.90%

Y (0.554); X= 21.08, % recovery = 100.36

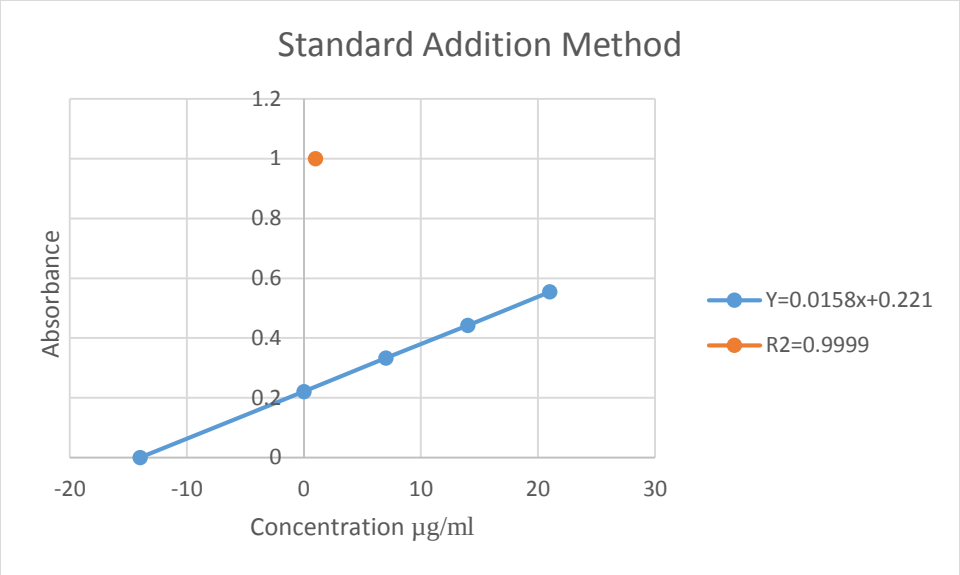


Figure 7: Plot Diagram of Standard Addition Method

3.5. LOD & LOQ:

LOD & LOQ were calculated from the linear regression equation suing the following formulas.

LOD $= \frac{3.3 \times SD}{Slope}$ **limit of detection =3.3×.**$\frac{0.00749}{.0159}$**= 1.54µg/ml**

LOQ was calculated from the linear regression equation suing the following formulas

$$\mathbf{LOQ} = \frac{\mathbf{10 \times SD}}{\mathbf{Slope}}$$

So limit of quantification =**10×.**$\frac{0.00749}{.0159}$ = **4.69µg/ml**

3.6. Assay results of Amlodipine besylate of marketed formulations

The following table represent results assay of amlodipine from different formulation brands

Table 10: Comparative studies between different brand of amlodipine 10 mg assay results:

Standard 14µg/ml	Brand (A) capsules 14µg/ml		Brand (B) tabs 14µg/ml		Brand (C) tabs 14µg/ml	
	Abs.	Assay	Abs.	Assay	Abs.	Assay
0.218	0.220	98.61	0.225	100.85	0.226	101.30
0.218	0.223	99.95	0.227	101.75	0.227	101.75
0.217	0.223	99.95	0.224	100.40	0.223	99.95
SD	0.77		0.69		0.94	
Assay	99.50±0.77		101.00±0.69		101.00±0.94	

Table 11: Analysis of variance (ANOVA) for comparison between three brands of amlodipine

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
LOTENSE	3	0.666	0.222	3E-06
amlong	3	0.676	0.225333	2.33E-06
amlodac	3	0.676	0.225333	4.33E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.22E-05	2	1.11E-05	3.448276	0.100701	5.143253
Within Groups	1.93E-05	6	3.22E-06			
Total	4.16E-05	8				

4.1. Discussion

Development of analytical methods is crucial to counteract for limitation of official methods in term of cost, sensitivity, selectivity and accuracy to assure for delivery of drugs with adequate quality and safety to the patients.

Amlodipine is one of the most frequently prescribed antihypertensive drugs to manage various cardiovascular disorders in Sudan due to efficacy and safety. It is formulated in tablets, capsules dosage forms ranging from 5 and 10 mg of amlodipine base.

Previous methods reported for assay of amlodipine included chromatographic and colorimetric methods. These methods are considered expensive, time consuming, and required number of solvents and reagents. All these methods also required long procedure to optimization the conditions. Table (1)

Development and validation of simple, sensitive, feasible, reliable and reproducible spectrophotometric method is preferred because of the availability of spectrophotometers specially in developing countries.

Different solvents were tried for dissolving amlodipine including :(DMSO:Water mixture in ratio of 50:50% v/v, DMSO, Methanol, Methanol:Water mixture in ratio of 50:50% v/v).

Scanning of amlodipine solutions in these different solvents revealed λ maximum of amlodipine at 365nm for the DMSO:Water mixture in ratio 50:50% v/v (figure2), at 360nm for the Methanol:Water mixture in ratio of 50:50% v/v (figure 3), at 360nm for DMSO (figure 4),and at 360nm for Methanol (figure 5).

During the selection of suitable solvent for dissolving amlodipine the DMSO:Water mixture in ratio of 50:50%v/v was found the best ; because showed highest wavelength (365nm) to ensure freedom from any interference or excipients.

In study carried by J. M. G. Cowie AND P. M. Toporowski (1961) they proved that system DMSO-water belongs to the class of solutions possessing numerous solute water bonds which are stronger than the hydrogen bonds between water molecules. This suggest higher solubility properties for this mixture. Many binary liquid systems exhibit non-linear viscosity isotherms

which pass through a minimum. However, a smaller group possess a maximum, indicating some form of liquid-liquid interaction, and the DMSO-Water system was found to belong to this latter class. [31]

Also this can suggest that mixture can be considered as hydrotropic solvent (The Hydro tropes are a class of chemical compounds that cause a several fold increase in the solubility for sparingly soluble solute under normal conditions). [32]

The method was validated as per ICH Q2B (R1) guidelines. The validation parameter included: in table (5) and figure (6) reflect the results of linearity; tables (6-9) reflect the results of precision, table (10) and figure (7) reflect the results of accuracy, section 3.5 reflect the result of LOD and LOQ.

It is evident from results of validation study that, the method was accurate, sensitive, selective, and precise for spectroscopic estimation of Amlodipine as RSD was less than 2%.

Also the precision of this methods was evaluated statistically regarding standard deviation of the three brands studied above all values obtained by this method lie between the accepted SD values for assay (less than 2%), and the assay results were found as follows:

- Brand A 99.50±0.77 (n=3).
- Brand B 101.00±0.69 (n=3).
- Brand C 101.00±0.94 (n=3).

The results of the % recovery of the sample and the added standard deduced from data of table (9) indicate absence of interference in the assay of amlodipine. as all results of RSD% less than 1%.

The Null hypothesis theory test i.e: ANOVA of the three brands of amlodipine showed no significant difference between them. (the calculated f-value 3.49 is less than f-value 5.14 and

calculated p-value 0.10 is greater than p-value 0.05) this indicate the Null hypothesis theory is valid.

These above results also prove that developed method can have considered a quick and inexpensive method to replace chromatography for the analysis of amlodipine dosage formulations at very low cost, a characteristic required in many countries where expensive equipments are not afforded.

4.2. Conclusion

The method was found to be sensitive, simple, precise and accurate. Hence it can reasonably be concluded that the developed spectroscopic method is accurate and reproducible for the analysis of Amlodipine dosage forms.

4.3. Recommendation

1- Application of this method for analysis of amlodipine in Quality Control laboratories.

2- the accuracy and simplicity of the method suggest it's suitability in cases quick results are demanded e.g. as in-process analysis procedures in industrial steps, or as routine method of analysis for amlodipine.

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