بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Validation of a Spectrophotometric Method for Amlodipine Besylate in a Tablet Form

التحقق من إستخدام طريقة المضوائية الطيفية في تقدير الأملودبين في هيئة أقراص دوائية

A Thesis submitted in partial fulfillment for the requirements Master degree in chemistry

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الاستفتاح

بسم الله الرحمن الرحيم

" الله نور السموات و الارض مثل نوره كمشكاة فيها مصباح المصباح في زجاجة الزجاجة كأنها كوكب دري يوقد من شجرة مباركة زيتونة لا شرقية و لا غربية يكاد زيتها يضئ و لو لم تمسسه نار نور على نور يهدي الله لنوره من يشاء و يضرب الله الامثال للناس و الله بكل شئ عليم"

(سورة النور الاية 35)

Dedication

To,.....

My Family,

My Uncle Muhammad,

Gamar aldin Muhammad,

And Friends.

Acknowledgment

Primarily I would like to thank God for enabling me to complete this research . Thank to my supervisors **Professor Dr Elmugdad Ahmed Ali.** Lots of thanks go to **Professor Dr Salah Sultan**, SAMF Corporation whose valuable guidance has helped me to accomplish this research at his laboratories. Their suggestion and instructions have contributed towards the completion of the project. Thank also **Rayan Abd Almoniem** for her continuous help.

ABSTRACT

The main objective of this work was to develop a new method for quantitative determination of **Amlodipine** in tablet pharmaceutical dosage Form by UV Spectrometry. The method was based on measuring the absorbance value of **Amlodipine** besylate at 237nm wavelength, using a mixture of methanol, acetonitrile, buffer solution (35:15:50) as a solvent. A sample of drug was dissolved in the mixture. The method for the quantitative determination of **Amlodipine** in tablet form conformed with the requirements of the ICH guidelines for the main validation parameters: selectivity, accuracy, linearity, Sensitivity, Precision and robustness. The results obtained in study clearly indicated that the developed UV Spectrometry method was fast, economical, simple, accurate and suitable for determination of **Amlodipine** in tablet form.

المستخلص

الهدف الرئيسي من هذه الدراسة التحقق من طريقة مضوائية طيفية لتعين الاملوديبين على هيئة اقراص ، تعتمد الطريقة على تقدير قيمة الامتصاص عند طول موجي 237 نانومتر، يسخدم خليط من الميثانول، الاسيتونيتريل و المحلول المنظم بنسبة (50:15:35) كمذيب، طريقة التعين الكلي للأمليوديبين على هيئة اقراص تثبت متطلبات نظام المؤتمر العالمي للتجانس وهي:الانتقائية والمصداقية و الخطية و الحساسية و الدقة و القوة. تشير النتائج التي تم الحصول عليها من الدراسة الي ان طريقة المطيافية للاشعة فوق البنفسجية سريعة و اقتصادية و دقيقة ومناسبة لتعين اللأمليوديبين على هيئة اقراص.

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List of Abbreviations

BP	British Pharmacopeia
HPLC	High Performance Liquid Chromatography
FDA	Food and Drug Administration
ICH	International conference on Harmonisation
IEC	International Electrotechnical Commission
ISO	International Organization for standardization
LOD	Limit of detection
LOQ	Limit of quantification
QC	Quality control
STDV	Standard deviation
RP-HPLC	Reverse phase high performance liquid chromatography
RM	Reference material
RSD	Relative standard deviation
UV/VIS	Ultraviolet / visible
U	Uncertainty
FT-IR	Fourier Transform Infrared Spectroscopy
USP	United States Pharmacopeia

Chapter One

Introduction &Literature review

Chapter one

1. Introduction & Literature review

1.1. Analytical chemistry

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and compounds are presenting the object in question. During this period significant contributions to analytical chemistry include the development of systematic element analysis by *Justus Von Liebig* systematized organic analysis based on the specific reaction of functional groups. The first instrumental analysis was flame emission spectrometry developed by *Robert Bunsen* and *Gustav Kirchhoff* who discovered rubidium (Rb) and caesium (Cs) in 1860 (Arikawa 2001). Most of the major developments in analytical chemistry take place after 1900. During this period instrumental analysis becomes progressively dominant in the field. In particular many of basic spectroscopic and spectrometric techniques were discovered in the in the early 20th century and refined in the late 20th century (Miller 2000). The separation sciences follow a similar time line of development and also become increasingly transformed into high performance instruments (Bartle 2002). In the 1970s many of these techniques began to be used together as hybrid techniques to achieve a complete characterization of samples. Starting in approximately the 1970s into the present day analytical chemistry has progressively become more inclusive of biological questions (bioanalytical chemistry), whereas it had previously been largely focused on inorganic or small organic molecules. Lasers have been increasingly used in chemistry as probes and even to initiate and influence a wide variety of reactions. The late 20th century also saw an expansion of the application of analytical from somewhat academic chemical questions to forensic, environmental,

industrial and medical questions, such as in histology (Laitinen 1989). Modern analytical chemistry is dominated by instrumental analysis. Many analytical chemistry focus on a single type of instrument. Academics tend to either focus on new applications and discoveries or on new methods of analysis.

1.1.1.Classical methods

Although modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are from traditional techniques many of which are still used today. These techniques also tend to form the backbone of most undergraduate analytical chemistry educational labs.

1.1.1.1.Qualitative analysis

A qualitative analysis determines the presence or absence of a particular compound, but not the mass or concentration. By definition, qualitative analysis do not measure quantity.

a-Chemical tests

b-Flame test

1.1.1.2.Quantitative analysis

-Gravimetric analysis

Gravimetric analysis involves determining the amount of material present by we-ighing the sample before and /or after some transformation (Peter & Skoog 2005).

-Volumetric analysis

Titration involves the addition of a reactant to a solution being analyzed until equivalence point is reached. Often the amount of material in the solution being analyzed may be determined. The most familiar to those who have taken chemistry during secondary education, is the acid-base titration involving a color chang-ing indicator (Peter & Skoog 2005).

1.1.2.Instrumental methods

1.1.2.1.Spectrscopy

Spectroscopy measures the interaction of the molecules with electromagnetic radiation. Spectroscopy consists of many different applications such as molecular absorption spectrometry, molecular fluorescence spectroscopy, atomic absorption spectroscopy, atomic emission, ultraviolet-visible spectroscopy, x-ray fluorescence spectroscopy, infrared spectroscopy, mass spectrometry, and so on (peter & sock 2005).

1.1.2.2.Electrochemical analysis

Electrochemical method measure the potential(volts) and/or current in electrochemical cell containing the analyte (Bard et al 2000)(Skoog et al 1988).

1.1.2.3.Chromatography

Chromatography is based on phase-equilibrium phenomena. The components of the analyte sample are caused to equilibrate between two phases, a mobile phase and a stationary phase. Because the mobile phase percolates through the stationary phase, rapid mass transfer takes place, and the mobile phase carries the components through the column to a detector. The velocity of this transfer is related to the equilibrium constant . Hence, only compatible combinations of mobile and stationary phase (Helmut & Alex 2001) .

Types of Chromatography:

- Column chromatography
- Ion exchange chromatography
- Gel permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography

- Thin layer chromatography
- Gas chromatography
- High pressure liquid chromatography

-High-pressure liquid chromatography HPLC:

Using these chromatography technique it is possible to perform structural, functional analysis, and purifictional of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino aci- ds, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, in HPLC, mobile phase passes through columns under 10-400 atmospheric presses, and with high (0.1-5cm/sec) flow rate. In this technique, u-se of small particles, and application of high pressure on the rate of solvent flow increase separation power, of HPLC and the analysis is completed within a short time (Helmut & Alex 2001).

1.2.Method validation

Method validation is an important requirement in the practice of chemical analysis. Most analytical chemists are aware of its importance, but why it should be done and is not always clear to them. Some analysts used to see method validation as something that can only be done in collaboration with other laboratories and therefore refrained from it. Requirement in standards such as ISO/IEC 17025 (ISO/IEC 2005), ISO 15189 (ISO 2012) and ISO 15195 (ISO 2003) have helped in clarifying this**. Method validation** is basically the process of defining an analytical requirement, and consideration of capabilities consistent with what the application requires. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose (ICH 2005).

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:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance. A brief description of the types of tests are considered as follows:

-Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard;

- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test;
- Assay procedures are intended to measure the analyte present in a given sample. The assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution) (ICH 2005).The objective of the

analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated.

Characteristics which should be considered are:

accuracy, precision, repeatability, intermediate precision, detection limit, specificity, quantitation, linearity, range.

1.3.Amlodipine Besylate Tablet

Hypertension is the preceding cause of premature deaths worldwide and is associated with an economic burden of billion dollars per year. Blood pressure (BP) is under control only in half of patients having this critical health problem, which involves 1 of every 3 people(Triggle 2007),(Taylor 1994). Uncontrolled hypertension leads to significant complications such as heart diseases, stroke, renal failure, and death. It has been reported that hypertension accounts for at least 45% of the deaths from heart diseases and 51% of the deaths from stroke (Abernthy 1992).Amlodipine besylate is an antihypertensive drugs. It is used as an anti-hypertensive and in the treatment of angina. It lowers the blood pressure, relaxes heart muscles and dilates the heart blood vessels to prevent spasm (Tripathi 2003). Amlodipine besylate, is a long-acting dihydropyridine class of calcium channel blocker, approved for treating hypertension (Blank 2005). Amlodipine is commonly used in the treatment of heart diseases like angina and hypertension (British National Formulary 2011).

Methods available for the determination of Amlodipine Besylate include [HPLC] (Zarghi 2005) (Bahrami2004) , HPLC (IIango 1997) simultaneous spectrophotometric determination (Sahu 2007) (Khan

2006) (Singhvi 1998), spectrofluorometric (Abdel-Wadood 2007), [LCMS] and stability indicating assay method (Kamat 2005).

The chemical name for Amlodipine Besylate is 3-ethyl -5-methyl -4 RSa-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6methyl-1,4-dicarboxylate benzene sulphonate (British pharmacopoeia 2008) in **Figer1-1**.

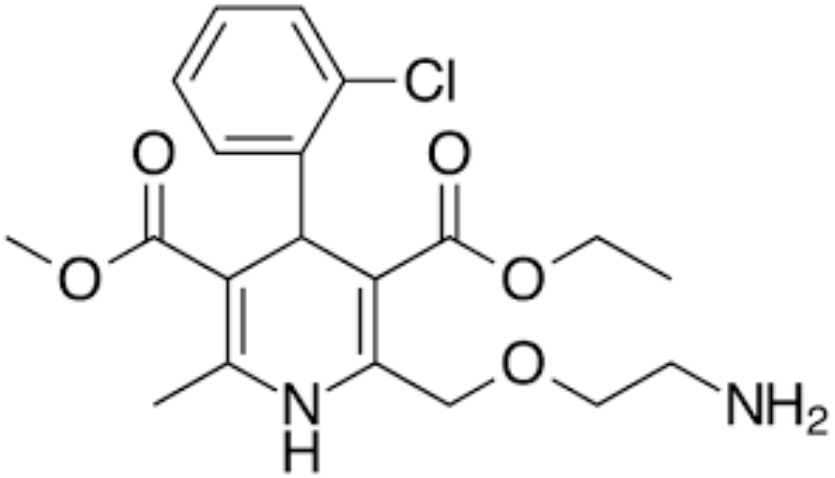


Figure 1-1 Structure of Amlodipine

1.4.UV Spectroscopy

Spectroscopy measures the interaction of molecules with electromagnetic radiat-ion. spectroscopy consists of many different application such as ultraviolet-visible and so on. A spectrometer is spectrophotometers are spectrometers that allow measurement of the ratio

of the radiant powers of two beams, a requirement to measure absorbance that A = logP0/P (Log P solvent/ P solution). Photometers use a filter for wavelength selection in conjunction with a suitable radiation transducer. Spectrophotometers offer the considerable advantage that the wavelength used can be varied continuously, making possible to record absorption spectra. Photometers have the advantages of simplicity, ruggedness, and low cost. Several dozen models of spectrophotometers are available commercially. Most spectrophotometers cover the UV/visible and occasionally the near-infrared region, while photometers are most often used for the visible region. Photometers find considerable use as detectors for chromatography, electrophoresis, immunoassays, or continuous flow analysis (Peter & sock 2005).

Ultraviolet and visible spectroscopy deals with the recording of the absorption of radiation in the ultraviolet and visible regions of the electromagnetic spectrum. The ultraviolet region extends from 10 to 400 nm. The absorption of electromagnetic radiations in the UV and visible regions induces the excitation of an electron from a lower to higher molecular orbital (electronic energy level). Since UV and visible spectroscopy involves electronic transition, it is often called *electronic spectroscopy*(Helmut & Alex 2001).

1.5.Previous Studies

Evaluation the pharmaceutical properties of three brands 0f Amlodipine (5mg) for local and international pharmaceutical companies was studied Rawnag, & Hussein (2015). A HPLC device was used in the assay test to determine the percentage of the labeled amount of amlodipine (active ingredient) in the portion of tablets or capsules. Results of assay test showed that values for Norvasc,

Mydipine, and Nordip were 97.83%, 95.31%, and 93.64%, respectively, which are within the acceptable range of 90-110% of the labeled amount of Amlodipine. UV-Visible Spectrometer was used in dissolution test to measure the percentage of dissolved amlodipine. Results of dissolution test showed that values for Norvasc, Myodipine, and Nordip were 99.795%, 99.415%, and 96.61, respectively, which are al within the acceptable range of not less than 75% of the labeled amount of dissolved amlodipine. All brands of Amlodipine Besylate showed satisfactory results for the chemical and physical tests.

Validation of methods of quantitative determination of amlodipine in tablets by liquid chromatography. Methods: was carried out the chromatographic analysis was performed on amlodipine liquid chromatography Agilent 1290 Infinity II LC System. Validation of methods of quantitative determination of amlodipine by HPLC tablets has been performed. It was established that the method proves the requirements of the state pharmacopoeia of Ukraine for the main validation parameters: specificity, accuracy, linearity, robasnist, conclusion; Dmytro & Liliya (2016) developed fast, economical, simple, accurate HPLC method for determination of amlodipine in medications.

Noshin (2015) formulated a combined oral dosage form of rosuvastatin calcium and amlodipine besylate and validate an analytical method to be adopted for both routine quality control assay and in vitro dissolution studies of the formulation. The proposed combination formulation has shown compatibility with the chosen excipients, verified through FT-IR study. A novel gradient RP-HPLC method was developed and validated according to the ICH guideline which was found to be suitable for the simultaneous estimation of

rosuvastatin calcium and amlodipine besylate from the formulation. The retention time of 2.7 and 6.08 min allows the analysis of large amount of samples with less mobile phase which makes the method encouraging which makes the combination formulation of rosuvastatin calcium and amlodipine besylate superior and effective in achieving patient compliance.

Chanchal (2017) has developed an inexpensive, easy, particular, sensible and reproducible spectrophotometric method has been developed and validated for the estimation of amlodipine in pure drug make Tablet formulation. Analysis was carried out at 338nm for pure drug amlodipine and 355nm for amlodipine make tablet formulation. The main purpose of the investigation was to measure that how much percentage of drug present in marketed tablet formulation for the estimation of amlodipine besylate tablet and amlodipine pure drug using method was assessed by dues studies and was found to be in range of 99.80% of amlodipine pure compound and 99.20% of amlodipine marketed tablet formulation. The LOD were found 0.132 and 0.141 µg/ml of amlodipine pure drug and LOQ were 0.416 and 0.427µg/ml of marketed tablet respectively. The result were validated found to be fair and met the admissible criteria.

Amlodipine besylate is a potent long-acting calcium channel blocking agent. Safila, Hina, Wardha, & Urooj (2014) has developed an efficient least time consuming and simple spectrophotometric method for the assay of Amlodipine has been used. The assay is based on the ultraviolet UV absorbance maxima at about 238nm wavelength of amlodipine using water as solvent. A sample of drug was dissolved in water to produce a solution containing Amlodipine. Similarly, various dilution were made. The absorbance of sample

prepared was measured at 238nm against the solvent blank and the assay was determined. In study a simple and quick assay method using U.V spectrophotometer has been used. The assay is based on measuring the absorbance of formulation of amlodipine dilution at the wavelength of 238nm. Four different dilution of 50ppm, 25ppm, 12.5ppm, 6.25ppm are prepared and their percent assay is calculated.

A novel stability indicating HPLC has been developed for estimation of amlodipine in tablet formulation. Koustubhmani& Chaturvedi (2005) the method was validated using specificity, stability in analytical solution, precision, accuracy and system suitability as parameters. The mobile phase consists of 0.05M ortho-phosphoric acid buffer, methanol and acetonitrile in the ratio of 50:35:15 and the results show that the method is reproducible and accurate. Degradation of amlodipine was performed in various condition and the resulting solution was analyzed on HPLC using column (150×4.6mm) with a detection maxima of 361nm. The method gave a good separation between drug and degradation peaks. Recovery studies gave results between 99.7 to 100.7% for 5mg tablets .

Amlodipine besylate is a potent calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina. Amlodipine besylate avoids the adverse effect of amlodipine in racemic mixtures. Richa &Saahil (2012) has developed a highly precise and cost effective RP-HPLC method with retention time of 2.60 minutes was developed for the estimation of amlodipine besylate in tablet dosage form, by fixing the parameters as WATERS C18 column 250mm×4.6mm(5um), with mobile phase as acetonitrile: 70mM potassium dihydrogen orthophosphate buffer: methanol

(15:30:55) and pH adjusted to 3.00 using OPA . Mobil phase flow rate was maintained at 1.0ml/min and detected at 240nm.

The chromatographic analysis was performed on Athena C18 column (250×4.6mm, 5µ particle size) with mobile phase consisting of methanol and phosphate buffer (pH 4) in the ratio of 70:30v/v, at a flow rate of 1ml/min and eluents monitored at 240nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per international conference on Harmonization (ICH) guidelines was carried out Kranthi &Srinivas (2014) . The retention times of amlodipine besylate and telmisartan were 2.3 and 3.4 min, respectively. The curves of peak area versus concentration, which was linear from 2.5µg/ml for amlodipine besylate and 20-120µg/ml for telmisartan, had regression coefficient (r2) greater than 0.998. The method had the requisite accuracy, precision, and robustness for simultaneous determination of amlodipine besylate and telmisartan in tablets. And force degradation also performed. The proposed method is simple, economical, accurate and precise.

A reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of amlodipine and metoprolol in marketed formulation was developed Sohan, Mohammed & Dinesh (2008). The determination was carried out on a kromasil C18 (250×4.6mm, 5µm) column using mobile phase of o.o2 M phosphate buffer solution: acetonitrile (70:30v/v, pH 3.0). The flow rate was 1.oml/min with detection at 221nm. The retention time for amlodipine was 2.57 min and for metoprolol 4.49 min. Amlodipine and metoprolol showed a linear response in the concentration range of 10-110 µg/ml. The correlation co-efficient (r

value) for amlodipine and metoprolol was 0.9992, respectively. The results of analysis have been validated statistically and by recovery studies. The percentage recoveries obtained for amlodipine and metoprolol ranges from 100.04 to 100.57 %.

Amlodipine besylate is a calcium channel blocker which is used in treatment of hypertension alone or in combination with other antihypertensive drugs like angiotensin-ll-receptor antagonists (ARA ll) group (Losartan potassium and Valsartan) or in combination with anti hyperlipidemic agent like Atorvastatin calcium. RP-HPLC method was developed for assay of these drugs. The method was performed by reversed phase high performance liquid chromatography using a mobile phase 0.01 M ammonium acetate buffer (pH 5.5): acetonitrile with detection at 240nm on a spherical monomeric C18column (250mm ×4.6mm, 5µm) at flow rate of 1.5ml/min. The proposed method was validation in terms of linearity ranged between {(2-12, 10-60, 16-96, 4-24µg/ml) corresponding levels of 20-120% w/w of the nominal analytical concentration } with linear regression equation were {(y =64.627 × -3.6383 (r = 0.9998), y = 75.385 × -8.3856 (r = 0.9997), y = 64.492 × -25.981 (r = 0.9998), y = 70.964 × -28.505 (r = 0.9998)}, accuracy {100.18 ±1.38, 100.79 ± 0.59, 100.45 ± 0.58 and 100.8± 1.69 %}, precision {99.29, 99.33, 99.30 and 99.30}, limits of detection {0.03, 0.18, 0.15, 0.007 µg/ml} and limits of quantitation {0.1, 0.54, 0.45, 0.024 µg/ml} for Amlodipine besylate, Losartan potassium, Valsartan and Atorvastatin calcium respectively. Method validation was developed following the recommendations for analytical method validation of ICH and FDA organization Hafez (2014).

Two rapid assay procedures based on visible spectrophotometry and high performance liquid chromatography (HPLC), Kanakapura (2005) have been developed for the determination of amlodipine besylate (ADB) in pharmaceutical formulations. Spectrophotometric method was based on the bromination of ADB with a known excess of bromated-bromide mixture in acid medium followed by the determination of surplus bromine by reacting with Metanil Yellow and measuring the absorbance at 530nm. The HPLC determination was carried out on reversed phase C18 column using 0.1% orthophosphoric acid (pH 3): acetonitrile (20:80) at a flow rate of 1.0 ml min^{-1} with UV- detection at 238nm. In the spectrophotometric method, the absorbance is found to increase linearly with increasing concentration of ADB, which is corroborated by the calculated correlation coefficient of 0.9975. The system obeys Beer's law for 1.25-7.50 µg ml^{-1} ADB with a molar absorptivity of 2.51×10^4 I $mol^{-1}cm^{-1}$ and a sandell sensitivity of 16.37ng cm^{-2}. The limits of detection and quantification are calculated to be 0.17 and 0.56 µg ml^{-1}, respectively. In the HPLC method, a rectilinear relationship was observed between 7.55- 241.6µg ml^{-1} ADB with a detection limits of 1.51 µg ml^{-1} and a quantification limit of 3.02 µg ml^{-1}. The statistical evaluation of the method was examined by determining intra-day and inter-day precision. The methods, when applied to the determination of ADB in tablets, gave satisfactory results. Accuracy and reliability of proposed methods were further ascertained by parallel determination by the reference method and by recovery studies.

Mir (2007) has developed a simple, rapid and precise method for the quantitative simultaneous determination of atenolol and

amlodipine in a combined pharmaceutical-dosage form. The method was based on High Performance Liquid Chromatography (HPLC) on a reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm×25cm & 0.5µm, using a mobile phase of ammonium acetate buffer (the pH was adjusted to 4.5 ± 0.05 with glacial acetic acid), acetonitrile and methanol (35:30:35 v/v). The buffer used in the mobile phase contains ammonium acetate in double-distilled water. The chromatographic conditions are flow rate of 1.5ml/min, column temperature at 40°C and detector wavelength of 237nm. Both the drugs were well resolved on the stationary phase and the retention times were around 1.5 minute for atenolol and 3.4 minute for amlodipine. The method was validated and shown to be linear for atenolol and amlodipine. The relative standard deviations for six replicate measurements in two 0.999963 and 0.999979, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets is always less than 2% and mean% error of active recovery not more than ± 1.5% . The method was validated for precision and accuracy. The proposed method was successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.

Nashwah (2011) has developed a spectrophotometric method for simultaneous determination of amlodipine (Aml) and Valsartan (Val) without previous separation. In this method amlodipine in methanolic solution was determined using zero order UV spectrophotometry by measuring its absorbency at 360.5 nm without any interference from Valsartan. Valsartan spectrum in zero order is totally overlapped with that of amlodipine. First, second and third

derivative could not resolve the overlapped peaks. The first derivative of the ratio spectra technique was applied for the measurement of Valsartan. The ration spectrum was obtained by dividing the absorption spectrum of the mixture by that of amlodipine, so that concentration of Valsartan could be determined from the first derivative of the ratio spectrum at 290 nm. Quantitative limits of amlodipine and Valsartan were 10-80 µg/ml and 20-180µg/ml respectively. The method was successfully applied for the quantitative determination of both drugs in bulk powder and pharmaceutical formulation.

Our study **Amlodipine** besylate is an operant long-acting calcium channel blocking agent.. An efficient least time consuming and simple spectrophotometric method for the assay of **Amlodipine** has been used. The assay is based on the ultraviolet UV absorbance maxima at about 237nm wavelength of **Amlodipine** using mixture from Methanol, Acetonitrile, Buffer (35:15:50) as solvent. A sample of drug was dissolved in mixture. The absorbance of sample preparation was measured at 237nm against the solvent blank and assay was determined. The results obtained proved to validate the method for quantitative determination of **Amlodipine** in tablet formulations utilizing the UV spectrometry. It was established that the method proves the requirements of the ICH guidelines for the main validation parameters: selectivity, accuracy, linearity, Sensitivity, Precision and robustness. As a conclusion: The results obtained in study clearly indicate that the developed UV Spectrometry method is fast, economical, simple, accurate and suitable for determination of **Amlodipine** in tablet formulations.

Objective

The main objective of this work was to develop a new method for quantitative determination of **Amlodipine** in tablet pharmaceutical dosage Form by UV Spectrometry.

Chapter Two

Materials & Methods

Chapter two

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Reference material for amlodipine Besylate (98.4%) from prudence pharma chem (India) ., Quality control sample., Purified water., Buffer solution., Methanol HPLC Filtered through 0.2 micron membrane (CH_3OH 99.8%)., In active material { Lactose , starch, PVP , Magnesium stearate , Talc}., Ortho phosphoric Acid (H_3PO_4 88.05%), triethylamine AR($C_{15}H_3N$ 99.50%), Acetonitrile for Preparative HPLC Filtered through 0.2 micron membrane (CH_3CN 99.8%)**.**

2.1.2. Instruments:

The technical data of the weight balance, UV spectrophotometer and pH meter are shown in table 2-1, 2-2, 2-3, respectively.

Table 2-1: Technical data of the AG balances

Readability	0.1mg
Maximum capacity	210g
Taring range	0…210g
Repeatability	0.1mg
Linearity	+0.2mg
Calibration weight, internal	200g
Calibration weight, external	50/100/200g

Table2-2: Technical data of the Zsimc UV

Model	UV-T500
Wavelength Range	200-1000nm
Wavelength Accuracy	-+nm
Wavelength Repeatability	0.5nm
Stability	0.002A/h @ 500nm
Data Output part	USB

Table 2-3: Technical data of the Fisher pH meter

Ranges Normal Mode: Expand Mode:	 0 to 14 pH or 0 to +- 1400 mV Any 1.4 PH or +- 140 mV span
Resolution Normal Mode: Expand Mode:	 0.01 pH or 10 mV 0.1 pH or 1 mV
Relative Accuracy Normal Mode: Expand Mode:	 +- 0.08 pH1 or +- 8 mV +-0.008 pH2 or 0.3 mV
Precision Normal Mode: Expand Mode:	 +-0.03 pH or +-3mV +-0.003 pH or +- 0.3 mV
Stability	+- 0.003 pH drift in 24 hr.

2.2. Methods

2.2.1.Buffer solution:

7 cm^3 from triethylamine was taken and added to 900 cm^3 purified water and the pH was adjusted to 3 by Ortho phosphoric acid, then completed to 1 liter by water.

2.2.2.Solvent mixture:

Methanol, Acetonitrile, Buffer (35:15:50) were taken.

2.2.3. Sampling

According to USP monograph , the average of 5 tablets was weighed and dissolved by 35 cm^3 of solvent mixture; methanol, acetonitrile, buffer(35:15:50), then it was put for 30 min in the shaker , then completed to 100 ml in volumetric flask (100 cm^3) , then transferred to centrifuge for 10 min , and the solution was filtered by using 0.45 Mm filter paper, and 10 ml from the filtrate were taken and completed by the solvent to 25 cm^3 volumetric flask for preparing 0.02mg/cm^3 Amlodipine besylate. The absorbance was read at wavelength 237 in the UV spectrum .This validation exercise was repeated to determine (selectivity , linearity , precision LOD , LOQ , Accuracy , and robustness. From 15thFebruaryto 7 th March 2020 (USP 2016).

2.2.4.Standard preparation

Standard solution was prepared by weighing of 0.2g of RM Amlodipine besylate and dissolved by solvent mixture and completed to the mark, it 1.4 cm^3 was taken to 100 cm^3 volumetric flask was completed to the mark, that prepare standard 0.02mg/cm^3 (USP 2016).

2.2.5.Placebo preparation

Placebo was prepared by weighing all inactive material that enter in manufacturing of the Amlodipine besylate 5mg and was dissolved by 35 cm^3 of solvent mixture; methanol, acetonitrile, buffer(35:15:50). it was put for 30 min in the shaker , completed in volumetric flask (100 cm^3) , transferred to centrifuge for 10 min , the solution was filtered by using 0.45 mm filter paper, and 10 cm^3 from the filtrated was taken in volumetric flask 25 cm^3, and completed by the solvent.

2.2.6. Method Validation:

2.2.6.1.Selectivity

0.02mg/cm^3 of the sample solution and standard 0.02mg/cm^3 and placebo were prepared, and the sample, placebo and standard were scanned.

2.2.6.2.Linearity

5 different concentration from sample were prepared, sequential 1.4 cm^3, 2.8, 4.2 cm^3, 5.5 cm^3,and 6.9 cm^3 was taken. As per the result table 2 ,and standard 0.02mg/cm^3 was prepared.

2.2.6.3.Precession

6 replicate of qc samples were prepared and the Abs of each one was read.

2.2.6.4.Accuracy

Accuracy is performed on 5 mg Amlodipine Besylate by preparing 9 samples by weighing and spiking placebo as described in the sample, preparation (the placebo remains 100% of method concentration in all samples). the placebo samples were spiked by active material at each of the three levels as per the method . three samples at 50 % were prepared also anther three samples at 100% were Prepared and in the some way

three samples at 150% were prepared. each sample was read three times and analyzed according to the analytical method. The samples were read from the lowest concentration to the highest concentration. The RSD % was Calculated for each individual weight at each level. The recovery of each individual sample weight was Calculated .

2.2.6.5.Robustness:

0.02mg/ml of sample and standard 0.02mg/ml were prepared. The Absorbance of each one at 237±2 nm.

Calculation of Assay of Amlodipine Besylate :

Actual concentration of sample was calculated by Calibration curve which obtained by linearity in **Figer {3-1}** . Then the Assay of Amlodipine Besylate calculated by sample and standard comparison by the following equation:

Assay of Amlodipine Besylate =
(Actual Con of sample / Actual Con of standard)*100

Chapter three

Results, Discussion& Conclusion

Chapter three

3. Result, Discussion & Conclusion

3.1. Results & Discussion

3.1.1. Selectivity

According to the recommended wavelength in USP pharmacopeia 237, Identity confirmation was carried out by preparing quality control sample, Standard solution of Amlodipine and placebo and was read at the specific wavelength in UV spectrometry and the results in **Table 3-1** show that the method was selective to Amlodipine.

Assay of Amlodipine Besylate =

(Abs of sample / Abs of standard)*100

Table 3-1 : selectivity calculation

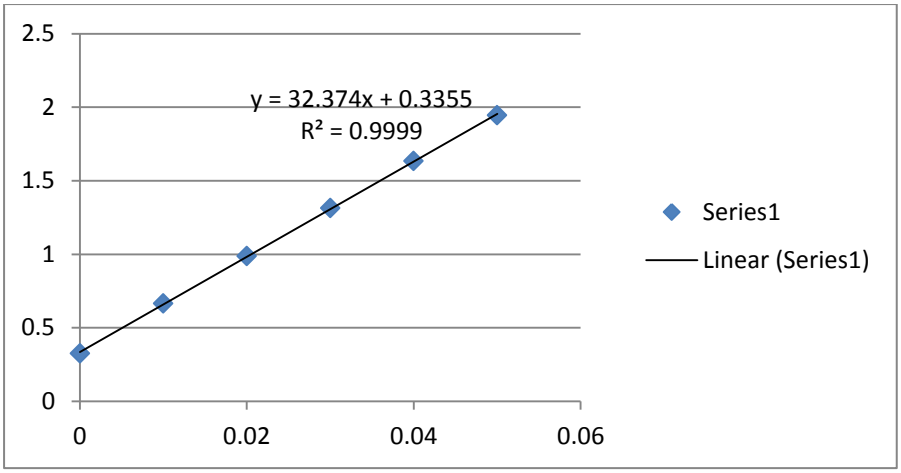
	Abs of sample	**Abs of standard**	**Abs of placebo**
	0.975	0.963	0.0012
Assay	101.25%	-	0.125%

According to the assay% of placebo and sample, it seemed that method was selective to the Amlodipine besylate because of small absorbance of placebo in the specific wavelength of Amlodipine besylate.

3.1.2.Linearity

The linearity was examined using a calibration curve obtained by using UV spectrometry with (5) different concentrations .**Figs 3-1** shows the calibration curve for Amlodipine with (5) calibration levels . **Table 3-2** show the concentrations of calibration standards for Amlodipine. With calibration coefficient better than 0.999.

The calibration curve is : y = ax+b : a is slope , and b is intercept .



Figs 3-1 : Calibration curve

Table 3-2: linearity

Concentration	Absorbance
0.00	0.326
0.01	0.664
0.02	0.987
0.03	1.313
0.04	1.634
0.05	1.945

From the calibration carve, the regression was 0.999, and it confirm the linear relation between the conc and the absorbance.

3.1.3.Precision

3.1.3.1 Repeatability

Through QC samples and one analyst used to test repeatability of the method . **Table 3-3** list the results of 6 QC , STDV and RSD .

Table 3-3: repeatability results

S.No.	Abs of samples
1	0.0175
2	0.0176
3	0.0178
4	0.0174
5	0.0175
6	0.0173
mean	0.0175
STDV	0.000169
RSD	0.9637%

-Acceptance criteria:

Calculated by excel the STDV &RSD , the value should be < 2 % .

RSD = (standard deviation / mean) * 100

3.1.3.2 Intermediate precision

Through QC samples and different day and different analyst used to test intermediate precision of the method . **Table 3-4**list the results of 6 QC samples, STDV and RSD.

Table 3-4: intermediate precision results

S.NO	Abs
1	0.0174
2	0.0175
3	0.0176
4	0.0177
5	0.0179
6	0.0173
7	0.0175
8	0.0176
9	0.0178
10	0.0174
11	0.0173
12	0.0175
Mean	0.0175
STDV	0.000192
RSD	1.0945%

-Acceptance criteria:

Calculate by excel the STDV &RSD , the value should be < 2 % .

After calculating the RSD of repeatability and intermediate precision, the RSD for the 12 sample was 1.0945%, and it is an acceptable results according to ICH guideline, and it insured that the method was precise.

3.1.4.Accuracy

dry spiking of Amlodipine RM to placebo used was to prepare 3 concentration level 0.01, 0.02 and 0.03 (50,100,150) then the Absorbance of each level by UV spectrometry was determined and the assay percentage was calculated and average Assay percentage were calculated which show that in **Table 3-5** the method was Accurate.

Assay = {(Average abs of sample / Average abs of STD) * (Actual Conc of STD / Actual Conc sample)} * 100

Table 3-5: Accuracy results

Theoretical CON	Actual CON of STD	Weight of STD	Mean Abs of STD	Actual CON of samples	Weight of samples	Mean Abs of samples	Assay %	Average	Criteria
Level 50 %	0.027793	200mg	0.939333	0.013897	3.5	0.470	100.071	**99.03415**	100±2
Level 100%	0.027793	200 mg	0.939333	0.02752	6.93	0.9181	98.731		100±2
Level 150%	0.027793	200 mg	0.939333	0.0413	10.4	1.371857	98.30008		100±2

Acceptance criteria:

- Each individual sample recovery should lie within the range of 98% to 102% .
- The average range of assay should be from 97 to 103 %

The assay percentage calculated by using reference value and the value that found by the practical and study the matrix effect for amlodipine besylate it shows that method give accurate results.

3.1.5.LOD&LOQ

Limit of detection(**LOD**) and limit of quantitation(**LOQ**)were calculated by the follow equation according to ICH guideline.

LOD = 3.3*Standard error of residual / slope

LOQ= 10* Standard error of residual / slope

The results obtained are shown in table 3-6.

Table 3-6 : LOD &LOQ results

Standard Error	Slope	LOD	LOQ
0.0198	31.97577	0.002044	0.006195

3.1.6.Robustness

Robustness of the method was determined by preparing standard, QC sample and the Abs at ± 2 of the specific wavelength was read, and the results in **Table (3-7)** show that the robustness of method.

Table 3-7 : robustness results

Abs of Sample	Abs of STD	Wavelength	Assay %
0.916	0.963	235	95.12
0.952		236	98.86
0.975		237	101.15
0.960		238	99.69
0.990		239	102.80

The assay of Amlodipine besylate was calculated at wavelength 237 ± 2 and the result was in the range which is recommended in the pharmacopeia USP 90-110, that insure the robustness of method

3.1.7.Estimation of expanded uncertainty

-Acceptance criteria:

Uncertainty is an interval associated with the measurement results which express the range of values that can be reasonably attributed to the quantity being measured. Measurement uncertainty is not a performance characteristic of a particular measurement procedure . but a property of the results obtained using that measurement uncertainty.

Type A :

- Calibration curve :

So $U_{(b)}$ = 0.0000577 Because of calibration curve co efficiency is omitted.

-Precision uncertainty :

Precision uncertainty = 0.000361

SO Type A Uncertainty =<u>0.000361</u>

Type B :

-Dilution

-use pipette 10 ml

(0.0577/10)×100

$U_{(P)}$ =0.5777

-use volumetric flask 100 ml = (0.0577/100)×100

$U_{(f)}$ = 0.0577

-use volumetric flask 25 ml = (0.0346/25)×100

$U_{(f)}$ =0.0346

-use volumetric flask 1000 ml

(0.2309/1000)×100

$U_{(f)}$ =0.02309

-Weight sample

Use balance to weigh the samples and standard to 0.0001 mg

So the **combined uncertainty**

$=\sqrt{Type\ A + Type\ B}$ = 0.2474

And the **Expanded uncertainty = combined uncertainty* coverage factor**

0.2474 * 1.99987 = **±0.49477**

3.2. Conclusion

A validation of methods of quantitative determination of amlodipine in.; accordance with USP pharmacopeia. It was established that the method meets the requirements for ICH guideline: Selectivity, linearity, LOD, LOQ, Accuracy, Precision and Robustness in the range 90-110%. The results obtained clearly indicated that the developed UV Spectrometric method is simple, fast, economical and suitable for determination of amlodipine in medicines.

Recommendation

- Adults: initial dose 5 mg once daily; maximum dose of 10 mg per day
- Geriatric and Debilitated patients: reduce initial dose to 2.5 mg once daily; maximum dose of 10 mg per day
- Adolescents and Children 6 years of age or older: 2.5 to 5 mg once daily; maximum dose of 5 mg per day
- Children 6 years of age or younger: 0.05 to 0.2 mg/kg per day; maximum dose 0.3 to 0.6 mg/kg per day

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