International Journal of Web of Multidisciplinary Studies
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
website: https://ijwos.com OPEN ACCESS E-ISSN : 3049-2424
Volume 3 Issue 7 , July 2026 DOI: https://doi.org/10.71366/ijwos03072665696

METHOD DEVELOPMENT AND VALIDATION OF AMLODIPINE BESYLATE BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD

K. SUJATHA[1], K.SAHANI[2], K.ANJALI[3], K.SRIMAHALAXMI[4], K.RAVINDER[5], SK.YASMIN[6]
[1] *Asst. Professor, Dept of Pharmaceutical Analysis, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana,508376.*
[2,3,4,5,6] *B Pharm IV Year, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana,508376.*

Article Info

Article History:

Published: 28 July 2026

Publication Issue:
Volume 3, Issue 7
July-2026

Page Number:
584-603

Corresponding Author:
K. SUJATHA

Abstract:

Methods were reported for the estimation of n in amlodipine besylate dosage forms. UV spectroscopic study was reported for amlodipine besylate in a single dosage form. So the present study was aimed at the development of a simple, accurate, rapid, and economical UV spectrophotometric assay method for the determination of amlodipine besylate in bulk and tablet formulation. The solvent selected for stock solution preparation was water, and the required concentrations were prepared using the same solvent. The λmax for detection of amlodipine besylate was found to be 360nm. In this method, amlodipine besylate followed linearity in the concentration range of 1, 2, 3, 4, 5, 6, 7, 8, and 9 µg/mL with a regression coefficient (r^2) of 0.999. The percent drug content was found to be 101.60057 ± 2.0815391 for amlodipine besylate API formulation. The method was found to be accurate and precise, as indicated by recovery studies, with recoveries close to 100% and RSD less than 2.
Inter-day precision: 0.1368
Intraday precision: 1.135
LOD: 0.387269 µg/mL LOQ:1.1735424µg/mL.

Keywords: Amlodipine Besylate, API formulation, Quantitative and Qualitative Analysis

1. INTRODUCTION

Analytical chemistry: Analytical chemistry is concerned with the science of chemical identification and determination of the composition of pharmaceutical substances, materials, and their chemical structures. The main aim of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Thus, analytical chemistry provides the methods and tools required for insight into our material world.

Analytical chemistry is a sub-discipline of chemistry that has a broad mission of understanding the chemical composition of all matter and developing the tools and experiments to make either qualitative or quantitative measurements. Analytical chemists work to improve the reliability of existing techniques to meet the demands for better chemical measurements that arise constantly in our society. They adapt proven methodologies to new kinds of materials or to answer new questions about their composition. They carry out research to discover completely new principles of measurement and are at the forefront of the utilization of major discoveries such as lasers and microchip devices for practical purposes. They make important contributions to many other fields as diverse as forensic chemistry, archaeology and space science.

Classification of analytical technique

According to the means by which an analytical aim can be achieved, the analytical methods can be divided into the following types.

Qualitative analysis

When a completely unknown sample is presented to an analyst, the first requirement is usually to ascertain what substances are present in it, or if there is any impurity present in the sample, then it should be detected by modern analytical techniques. eg.

Spectrophotometric analysis, X-ray diffraction, X-ray adsorption, Thermal analysis, electron microscopy, etc.

Quantitative analysis

Quantitative analysis is needed to quantify the amount of substance present in a sample. The main techniques employed in quantitative analysis are based on:

The quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction or ascertaining the amount of reaction product obtained.

- Appropriate electrical measurements.
- The measurements of certain spectroscopic properties.
- The characteristic movement of a substance through a defined medium under controlled conditions.

Validation: The validation or verification of a method follows a standardized set of experimental tests which produce data related to validation parameters. Validation is an act of proving that any procedure, process, equipment, material, activity, or system performs as expected under a given set of conditions and also gives the required accuracy, precision, sensitivity, and ruggedness. The process by which this is done should be written down as a standard operating procedure (SOP). Once methods have been validated or verified, they should be formally authorized for routine use in the laboratory by the responsible person.

When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly when carried out by the same or different persons, in the same or different laboratories, using different reagents and different equipment.

Major parameters of the analytical method validation

It is important for one to understand the parameters or characteristics involved in the validation process. Various analytical methods are used for the examination of pharmaceutical materials. Not all the characteristics referred to above will need to be considered in all cases. In view of assay validation, analytical methods may be broadly classified as follows:

Quantitative methods for drug testing require the following set of validation parameters to be determined:

1. Specificity/selectivity.
2. Limit of detection(LOD).
3. Precision (under within-laboratory repeatability and/or within-laboratory reproducibility conditions).
4. Linearity and working range.

5. Accuracy (bias) (under within-laboratory repeatability and within-laboratory reproducibility conditions).
6. Recovery.
7. Uncertainty of measurement.
8. Stability

Ultraviolet-Visible spectroscopy: UV-VIS spectroscopy plays an important role in analytical chemistry and has widespread application in chemistry, physics, and life sciences. The UV-Vis method is very useful in quality control of pharmaceutical products due to the potential of the great majority of drugs to absorb energy in these wavelengths. The absorption of UV-visible radiation occurs through the excitation of electrons within the molecular structure to a higher energy state. Although the selectivity depends on the chromophore of the drug, the method presents a series of applications: quantification of drugs in formulations where there is no interference from excipients, pKa determination, release of drugs from formulations with time in dissolution testing, monitoring of the reaction kinetics of drug degradation, and identification of drugs starting from the UV Spectrum.

Absorbance and concentration: Beer's law: When monochromatic electromagnetic radiation passes through a thin layer of sample, of thickness *dx,* it experiences a decrease in power of *dP*. The fractional decrease in power is proportional to the sample's thickness and the analyte's concentration, *C;* thus.

$-dP/p=\alpha Cx$ **(a)**

Where *P* is the power incident on the thin layer of sample, and α is a proportionality constant. Integrating the left side of equation (a) from $P = P_0$ to $P = P_T$, and the right sidefrom$x$=0to $x=b$, where *b* is the sample ' several thickness gives:

Converting from ln to log, and

$$-\int_{P=P_0}^{P=P_T}\frac{dP}{P}=\alpha C\int_{x=0}^{x=b}dx$$

$$\ln\left(\frac{P_0}{P_T}\right)=\alpha bC$$

$$A=abC \qquad \textbf{(b)}$$

Where *a* is the analyte's absorptivity with units of cm^{-1} $conc^{-1}$. When concentration is expressed using molarity, the absorptivity is replaced by the molar absorptivity, **e** (with units of cm–1 M–1)

$$A=\varepsilon bC \qquad \textbf{(c)}$$

The absorptivity and molar absorptivity give, in effect, the probability that the analyte will absorb a photon of given energy. As a result, values for both *a* and **ε depend** on the wavelength of electromagnetic radiation.

Equations b and c establish a linear relationship between absorbance and concentration, known as the Beer–Lambert law, or more commonly, as **Beer's law.** Calibration curves based on Beer's law are used routinely in quantitative analysis**. (*Harvey*, 2005)**

(a) Limitations of Beer's Law

According to Beer's law, a calibration curve of absorbance versus the concentration of analyte in a series of standard solutions should be a straight line with an intercept of 0 and a slope of *ab* or e*b.* In many cases, however, calibration curves are found to be nonlinear. Deviations from linearity are divided into three categories: fundamental, chemical, and instrumental**.** Beer's law is a limiting law that is valid only for low concentrations of analyte. There are two contributions to this fundamental limitation to Beer's law. At higher concentrations, the individual particles of analyte no longer behave independently of one another. The resulting interaction between particles of analyte may change the value of**.**

Another contribution is that the absorptivity, *a,* and molar absorptivity, **e**, depend on the sample's refractive index. Since the refractive index varies with the analyte's concentration, the values of ***a*** and **e will** change. For sufficiently low concentrations of analyte, the refractive index remains essentially constant, and the calibration curve is linear.

Chemical Limitations to Beer's Law

Chemical deviations from Beer's law can occur when the absorbing species is involved in an equilibrium reaction.

Instrumental Limitations to Beer's Law

There are two principal instrumental limitations to Beer's law.

The first limitation is that Beer's law is strictly valid for purely monochromatic radiation, i.e., for radiation consisting of only one wavelength; even the best wavelength selector passes radiation with a small, but finite effective bandwidth. Hence, polychromatic radiation always gives a negative deviation from Beer's law.

Stray radiation is the second contribution to instrumental deviations from Beer's law; For small concentrations of analyte, *Pstray is* significantly smaller than *P*0 and *P*T, and the absorbance is unaffected by the stray radiation. At higher concentrations of analyte, however, *Pstray is* no longer significantly smaller than PT, and the absorbance is smaller than expected. The result is a negative deviation from Beer's law**. (*Sharma,* 1999; *Christian*,2008).**

The determination of an analyte's concentration based on its absorption of ultraviolet or visible radiation is one of the most frequently encountered quantitative analytical methods. One reason for its popularity is that many organic and inorganic compounds have strong absorption bands in the UV/Vis region of the electromagnetic spectrum. In addition, analytes that do not absorb UV/Vis radiation, or that absorb such radiation only weakly, frequently can be chemically coupled to a species that does. e.g, nonadsorbing solutions of Pb^{2+} can be reacted with dithizone to form the red Pb–dithizonate complex.

An additional advantage to UV-Vis absorption is that in most cases it is relatively easy to adjust experimental and instrumental conditions so that Beer's law is obeyed.

Developing a quantitative method

In developing a quantitative analytical procedure, the conditions under which Beer's law is obeyed must be established. Method development involves selecting the wavelength or wavelengths that yield the best results for a particular analysis on a particular instrument. Here, best is defined by such parameters as accuracy, precision, sensitivity,

linearity, range, selectivity, and ruggedness. Method development for single-component, multiple-component, and mixture analysis is given below. (a)Single component. First, the most appropriate wavelength for the analysis is determined from an absorption spectrum. In most cases, the best wavelength corresponds to an absorption maximum because it provides greater sensitivity and is less susceptible to

Instrumental limitations to Beer's law due to the lack of monochromatic radiation. Second, if an instrument with adjustable slits is being used, then an appropriate slit width needs to be chosen. The absorption spectrum also aids in selecting a slit width. Finally, a calibration curve is constructed to determine the range of concentrations for which Beer's law is valid. Additional considerations that are important in any quantitative method are the effect of potential interference and establishing an appropriate blank. The concentration of a single analyte is determined by measuring the absorbance of the sample and applying Beer's law using any of the standardization methods. The most common methods are the normal calibration curve and the method of standard additions. Single-point standardizations also can be used, provided that the validity of Beer's law has been demonstrated.

Validation: Analytical method validation is now required by regulatory authorities for marketing authorizations, and guidelines have been published. It is important to isolate analytical method validation from the selection and development of the method. The most widely applied validation characteristics are accuracy, precision (repeatability and intermediate precision), specificity, detection limit, quantitation limit, linearity, range, robustness and stability of analytical solutions.

(a) *Linearity: Linearity is the ability of the method to produce test results that are proportional, either directly or by a well-defined mathematical transformation, to the concentration of analyte in samples within a given range. For UV-visible measurements, the usual linear relationship is Beer's law. Some instrumental and sample parameters can cause deviations from Beer's law, and significant quantitative errors can result if the calibration curve is not accurately characterized. However, because these deviations are wavelength dependent, selection of the appropriate wavelength can minimize their influence on results.*

A statistical method must be applied to find the best fit of the calibration curve to the data and, in a second step, to determine which type of calibration curve gives the best fit. The statistical method most often used is linear regression, which is also known as the least squares method. To compare two calibration curves, a measure of the goodness of the fit of the standards to the line is required. Several statistical values, including the correlation coefficient, standard error of regression, and uncertainty, can be used to obtain this measurement.

(b) *Accuracy: Accuracy of a method is the degree of agreement between an individual test result generated by the method and the true value. A graphical plot of the quantitative results versus wavelength enables quick evaluation; the best range must be determined through trial-and-error variation of the wavelength range and through comparison of the calculated results with actual values.*

Under normal conditions, relative errors of 1–5% are easily obtained with UV-VIS absorption. Accuracy is usually limited by the quality of the blank.

In the latter case, the interferant may react to form an absorbing species, giving rise to a positive determinate error. Interferents also may prevent the analyte from reacting, leading to a negative determinate error. With care, it may be possible to improve the accuracy of an analysis by as much as an order of magnitude.

(c) *Precision:* Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. A statistical value is required to determine precision. Standard deviation, percent relative standard deviation (obtained by dividing the standard deviation by the average value and multiplying by 100), and confidence interval are the most popular tools for assessing the precision or repeatability of a set of values. To determine the precision of a method, a set of typically 10–20 samples with the same concentration is prepared. These samples are then measured, and the amount of analyte is calculated. The

standard deviation of the results is a measure of the precision. In absorption spectroscopy, precision is limited by indeterminate errors, or instrumental "noise," introduced when measuring absorbance. Precision is generally worse with very low absorbance due to the uncertainty of distinguishing a small difference between P0 and PT, and for very high absorbance when PT approaches 0. We might expect, therefore, that precision will vary with transmittance.

When the absorbance is too high, precision can be improved by resetting 100% *T* using a standard solution of analyte whose concentration is less than that of the sample **(*Willard et al*.,1986).**

(d) *Selectivity:* Selectivity is rarely a problem in molecular absorption spectrophotometry. In many cases it is possible to find a wavelength at which only the analyte absorbs or to use chemical reactions in a manner such that the analyte is the only species that absorbs at the chosen wavelength. When two or more species contribute to the measured absorbance, a multicomponent analysis is still possible.

(e) *Sensitivity:* The sensitivity of a molecular absorption analysis is equivalent to the slope of a Beer's-law calibration curve and is determined by the product of the analyte's absorptivity and the path length of the sample cell. Sensitivity is improved by selecting a wavelength when absorbance is at a maximum or by increasing the path length. Sensitivity refers to the response obtained for a given amount of analyte and is often denoted by two analytical factors: the limit of detection (LOD) and the limit of quantification (LOQ).

The LOD is the lowest concentration of analyte that can be detected but not necessarily quantified in sample matrices. In general, the LOD isthe pointat which the signal from the analyte value(mg/L) is equal to the noise. Measurement number is equal to three times the noise in the measurement. Measurement results from some spectrophotometers list standard deviations based on the noise in the measurement. The LOD is approximately three times the standard deviation. The LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy in sample matrices. To calculate the LOQ, the acceptable limits of precision and accuracy (which depend on the objectives for the analysis) must be defined. The tools described above can then be used to determine the acceptable limits. A better way to determine the wavelength or wavelengths of optimum sensitivity is to measure the spectrum of a sample of low concentration several times. The average and percent relative standard deviation of the measured values at each wavelength are then calculated. The wavelength with the lowest percent relative standard deviation likely will yield the best sensitivity.

(f) *Ruggedness:* Ruggedness is the degree of reproducibility of test results obtained by analyzing the same samples under a variety of normal test conditions. The method should not be affected by changes in time or place. The reproducibility of the method should be established under various conditions. The ruggedness of an analytical method is determined by analyzing subsamples of a homogeneous sample in different laboratories and on different instruments. These tests should be performed by different analysts under operational and environmental conditions that may vary but that fall within the specified parameters for the method. In single-component quantification, the selection of wavelengths is more difficult with derivative spectra than with absorbance spectra since both positive and negative peaks are present. The even-order derivatives have a peak maximum or minimum at the same wavelength as the absorbance spectrum, but for the odd-order derivatives, this wavelength is a zero- crossing point. For accurate results, the sample to be analyzed must contain only the absorbing component for which the calibration has been performed. If the sample is a solution, a pure sample of the solvent should be used as a blank.

Requirements

(a) Sample handling: Molecular UV/Vis absorption is routinely used for the analysis of trace analytes in macro and meso samples. Major and minor analytes can be determined by diluting samples before analysis, and concentrating a sample may allow for the analysis of ultratrace analytes.

For accurate results, the sample to be analyzed must contain only the absorbing component for which the calibration has been performed. If the sample is a solution, a pure sample of the solvent should be used as a blank. It may be possible to correct for an interfering component with a second wavelength. The simple simultaneous equations and

least squares methods yield accurate results only if calibration is performed using pure standards or mixtures of standards for each component in the sample that contributes to the UV spectrum. The unknown sample must not have any additional absorbing capacity**(*Willard et al*., 1986).**

(b) *Instrument:* Instruments using monochromators for wavelength selection are called spectrometers. In absorbance spectroscopy, where the transmittance is a ratio of two radiant powers, the instrument is called a spectrophotometer. The simplest spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator. Currently, research-grade UV-VIS absorption instruments come in two configurations.

The first is called a scanning spectrophotometer because it measures the intensity of transmitted light of a narrow bandpass, and scans the wavelength in time in order to collect a spectrum. Because absorption is a ratiometric measurement, these instruments generally require the user to measure two spectra, one sample and one blank. The blank should be identical to the sample in every way except that the absorbing species of interest is not present. This can be done either consecutively with a single-beam instrument followed by the ratio calculation, or simultaneously with a dual-beam instrument. The simplest

Spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator. The dual-beam method is faster and has the added advantage that lamp drift and other slow intensity fluctuations are properly accounted for in the ratio calculation. Collecting spectra with scanning spectrophotometers is slow, but the instruments often have very high resolving power owing to the use of photomultiplier tube detectors, which can be used with very narrow slit widths.

The maximum resolution of the instrument is 1 nm. The real advantage of the instrument is its speed. A single spectrum can be taken in about 0.1 seconds. Double- beam instruments are more versatile than single-beam instruments, being useful for both quantitative and qualitative analyses **(*Christian*,2008).**

Calibration of a UV-Visible Spectrometer

Calibration is the most important step in analysis. Good precision and accuracy can only be obtained when a good calibration procedure is adopted. In spectrophotometric methods, the concentration of a sample cannot be measured directly, but is determined using a physical measuring quantity 'y' (absorbance of a solution).

An unambiguous empirical or theoretical relationship can be shown between this quantity and the concentration of an analyte. The calibration between y = g(x), the calibration function, can be obtained by fitting an adequate mathematical model through the experimental data. The most convenient calibration function is linear, goes through the origin, and is applicable over a wide dynamic range. In practice, however, many deviations from the ideal calibration line may occur.

Wavelength Calibration

Measure the spectrum of the solution using the UV/Vis Photodiode Array Spectrometer and check the apparent wavelength of the absorption peaks, which should be at 287.0, 361.1, 450.8, 537.0, and 640.4 nm. Use the Peak/Spectrum function to annotate each peak, λ_{max}spectrum, and the wavelength report to determine the exact wavelength maxima.

Photometric Calibration

Basic potassium chromate is recommended as a photometric standard by the National Bureau of Standards. Solutions containing 0.04, 0.03, 0.02, and 0.01 g/l K_2CrO_4 in 0.05 N KOH are used for calibration. Measure the spectra of these solutions on the spectroscope and read the absorbance at 370nm.

The absorbance of the most concentrated solution should be 0.9914. Any relative deviation of over 1% from linearity or from the proper slope is cause for concern.

Instrument self-test

Full performance verification of a spectrophotometer is time- consuming and thus normally done only at periodic intervals. These intervals are determined according to the stability of the instrument. To ensure that any deviations from performance occurring between verifications are detected in a timely fashion, the instrument should be equipped with self-test routines that can be run daily. These routines should include a check

of the electronic and optical operation of the spectrophotometer as well as wavelength accuracy checks with one or both lines from the deuterium lamp.

Single-component quantification is normally performed by measuring with the same instrument a standard or series of standards followed by an unknown. This calibration process should eliminate instrumental bias, making absolute wavelength accuracy and absolute photometric accuracy relatively unimportant. On the other hand, photometric reproducibility is essential for precise results. If measurements are performed only at the absorbance maximum, wavelength reproducibility is also of little importance because the rate of change of absorbance with wavelength is low. However, if a wavelength on the side of the band is used, wavelength reproducibility becomes very important. Finally, the instrumental linear range is critical, as the calibration process relies on a linear relationship. Accurate multicomponent analyses require excellent S/N, especially if the simple simultaneous equations method is used.

In the least squares method, data from the sides of absorbance bands are incorporated into the calculation, making excellent wavelength reproducibility essential as well.

(c) *Liquid samples Cells: UV-visible spectroscopy is used primarily to measure liquids or solutions. This mode is simpler and allows more accurate quantitative analysis than do reflectance measurements on solids. With this technique, a cell must contain the liquid or solution in the spectrophotometer sample area*

(d) *Material: Ideally, cells would be completely transparent at all wavelengths since any absorbance from the cell itself reduces the effective linear dynamic range for the sample.*

The cells lowest in cost are made of plastic. Glass cells are slightly more expensive than plastic cells but are more durable and, with proper care, can provide years of use.

(e) *Solvent: The ideal solvent for the preparation of sample solutions would dissolve all types of compounds, would be nonflammable and nontoxic, and would be completely transparent at all wavelengths. Distilled water approaches the ideal but is not suitable for many nonpolar organic compounds. Table 1 lists some of the most commonly used solvents. Except for water, these solvents all exhibit a cut-off wavelength in the UV range below which they absorb too strongly for sample measurements to be performed (Willard et al.,1986).*

Table No.1.3: Properties of some common solvents

Solvent	**Polarity**	**Cut-off wavelength**
Distilled water	78.5	<195
Hexane	1.9	199

Ethanol (absolute)	24.3	207
Methanol	32.6	210
Cyclohexane	2.0	211
Chloroform	4.8	246
Dimethylsulfoxide	None	270
Acetone	20.7	331

Factors influencing absorbance: Several factors, including the solvent used as well as the concentration, pH, and temperature of the sample, can affect the position and intensity of absorption bands of molecules. These parameters should be controlled to ensure maximum precision when comparing spectra measured under different conditions.

Effect of solvent: The polarity of a solvent can modify the electronic environment of the absorbing chromophore; the maximum (max) for polar compounds is usually shifted with the change in the polarity of the solvent. In general, the magnitude of the shift can be correlated with solvent polarity. For comparative analysis, a single solvent should be used for all measurements. (Sharma,1999)

Effect of concentration: Concentration normally affects only the intensity of bands. At higher concentrations, however, molecular interactions (for example, dimerization) may cause changes in the shape and position of the absorbance band. These changes in turn affect the linearity of the concentration versus absorbance relationship and may lead to inaccurate quantitative results.

Effect of pH: The effects of pH on absorbance spectra can be very large and result primarily from the shifting of equilibrium between two different forms. For example, pH indicators visually change color at different pH values. If the spectrum of the sample under study is found to be affected by pH, a buffer should be used to control this parameter. Note, however, that most buffers themselves exhibit significant absorbance, which may affect the wavelength range over which measurements can be performed.

Effect of temperature: Temperature also can affect UV-visible measurements. Simple expansion of the solvent, especially for some organic solvents, may be sufficient to change the apparent absorbance and thereby the accuracy of quantitative results. In addition, temperature may affect equilibria, which can be either chemical or physical (Sharma, 1999).

Weak absorbance: A recurring problem in analytical chemistry is low sensitivity, which may be due to low concentrations of the sample or to very weak absorptivity of the analyte. At low absorbances, noise in the measurement results in a loss of precision such that any single measurement may be inaccurate. Reducing the noise level directly improves the precision of results. Noise specification should be a key parameter in selecting a spectrophotometer, but note that varying the measurement parameters will reduce the noise level.

Strong absorbance: When samples absorb too strongly, the linear dynamic range of the instrument is exceeded, and the relationship between absorbance and concentration becomes nonlinear. The easiest solution to this problem is to dilute the sample to an absorbance level within the linear dynamic range. With solid samples, however, this is not possible. Moreover, any sample handling step, even dilution, introduces error and thus may be better avoided. An alternative is to select one or more wavelengths on the side of the absorbance band, where absorptivity is lower. When the wavelength of the absorbance maximum (241 nm) is used for calibration, a maximum concentration of approximately 0.26 mg/mL can be measured with reasonable accuracy. However, switching to the absorbance on the side of the band (266 nm) enables concentrations of up to 5 mg/mL to be measured with equal accuracy. A prerequisite for use of this technique is excellent wavelength reproducibility.

3. Applications

Other indirect quantification

(a) **Chemical derivatization:** Because many compounds exhibit either very weak or no absorbance in the UV or visible regions, several methods using chemical derivatization have been developed. Such methods usually involve adding an organic reagent, which forms a complex with strong absorptivity. The final stage of measurement closely resembles that of the direct methods. With this technique, the choice of an appropriate reagent can significantly enhance both sensitivity and selectivity **(*Sharma,*1999).**

(b) *Spectrophotometric titrations: In volumetric analyses, the color changes that signify the endpoint of a titration are most often detected through visual inspection. This process is inherently subjective and can be a source of error. The use of a spectrophotometer for endpoint detection introduces objectivity into the analysis and lends itself to automation(Willard et al.,1986).*

(c) *Enzyme kinetic assays: Direct UV-visible analysis of one component in biological matrices is difficult. Interference from other components often makes direct measurement of a specific property, such as absorbance. Enzyme assays can be used in the indirect analysis of one compound or a group of compounds in a complex matrix. If the enzyme is carefully selected, any change in the sample following addition of the enzyme will result only from the reaction of the specific compound or compounds.*

(d) *Environmental Applications: Methods for the analysis of waters and wastewaters relying on the absorption of UV-VIS radiation are among some of the most frequently employed analytical methods.*

(e) *Clinical Applications: UV-VIS molecular absorption is one of the most commonly employed techniques for the analysis of clinical samples. The barbiturates are extracted from a sample of serum with CHCl3, and extracted from the CHCl3 into 0.45 M NaOH (pH » 13). The absorbance of the aqueous extract is measured at 260 nm. (Harvey, 2000)*

(f) *Industrial Analysis: UV-VIS molecular absorption is used for the analysis of a diverse array of industrial samples, including pharmaceuticals, food, paint, glass, and metals. Many pharmaceutical compounds contain chromophores that make them suitable for analysis by UV/Vis absorption.*

Qualitative analysis: UV-visible spectroscopy can be used to determine many physicochemical characteristics of compounds and thus can provide information as to the identity of a particular compound.

(a) *Identification-spectra and structure: UV-visible spectra generally show only a few broad absorbance bands. Compared with techniques such as infrared spectroscopy, which produces many narrow bands, UV-visible spectroscopy provides a limited amount of qualitative information. Most absorption by organic compounds results from the presence of p (that is, unsaturated) bonds. A chromophore is a molecular group usually containing a p bond. When inserted into a saturated hydrocarbon (which exhibits no UV-visible absorbance spectrum), it produces a compound with absorption between 185 and 1000 nm. The presence of an absorbance band at a particular wavelength often is a good indicator of the presence of a chromophore.*

(b) *Confirmation of identity: Although UV-VIS spectra do not enable absolute identification of an unknown, they frequently are used to confirm the identity of a substance through comparison of the measured spectrum with a reference spectrum.*

(c) *Protein and nucleic acid melting temperature.: The absorbance spectra of proteins result largely from the presence of the aromatic amino acids tryptophan, tyrosine, and phenylalanine. A protein at room temperature has a specific tertiary structure or conformation that in turn creates a specific electronic environment for the aromatic amino acids.*

(d) Enzyme activity: The activity of an enzyme is a measure of its effectiveness as a catalyst. The concentration of enzyme in an impure preparation can be expressed in terms of units per milliliter, and the specific activity of the preparation can be expressed as units per milligram of protein. As an enzyme is purified, the specific activity increases to a limit (that of the pure enzyme. UV/Vis molecular absorption is routinely used in the analysis of narcotics and for drug testing. One interesting forensic application is the determination of blood alcohol using the Breathalyzer test

4. DRUG PROFILE:

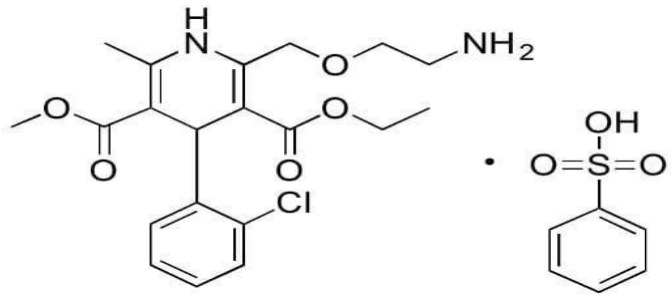
Name: Amlodipine besylate

Structure:

Molecular Weight:567.05 g/mol

Chemical Formula: C26H31ClN2O8S

IUPAC NAME: benzenesulfonic acid;3-O-ethyl 5-O-methyl 2-(2- aminoethoxymethyl) 4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate



Solubility:

- Highly soluble in acidic and physiological aqueous media.
- Slightly soluble in pure water.

Mechanism of Action: Amlodipine is a dihydropyridine calcium channel blocker. It inhibits the influx of calcium ions through L-type calcium channels in vascular smooth muscle and cardiac muscle.

- Causes relaxation of arterial smooth muscle.
- Produces peripheral vasodilation.
- Reduces peripheral vascular resistance (afterload).
- Lowers blood pressure.
- Increases oxygen supply to the heart and is useful in angina pectoris.

Distribution:

- Well absorbed after oral administration.
- Bioavailability: 64–90%.
- Extensively distributed throughout the body.
- Volume of distribution (Vd): approximately 21 L/kg.
- About 93–98% bound to plasma proteins.
- Crosses into tissues gradually, contributing to its long duration of action.

Elimination:

- Extensively metabolized in the liver to inactive metabolites.
- Mainly metabolized by CYP3A4 enzyme.
- Elimination half-life: 30–50 hours.
- About 60% is excreted in urine (mostly as metabolites).
- Around 20–25% excreted in feces.
- Only about 10% is excreted unchanged in urine

5. LITERATURE REVIEW :

- Patel R. et al., 2021. Developed and validated a simple UV-Visible spectrophotometric method for estimation of Amlodipine Besylate in bulk and tablet dosage forms. The drug was dissolved in methanol and scanned in the UV region. Amlodipine Besylate showed maximum absorbance (λmax) at 238 nm. The method obeyed Beer-Lambert's law in the concentration range of 5–30 µg/ml and showed good accuracy, precision, and reproducibility. International Journal of Pharmaceutical Sciences and Research, Vol. 12, Issue 4, pp. 2100–2105.

- Sharma P. et al., 2019. Proposed a UV spectrophotometric method for quantitative estimation of Amlodipine Besylate. A standard stock solution was prepared by dissolving 10 mg of the drug in 100 mL methanol to obtain a 100 µg/ml concentration. Further dilutions were made, and absorbance was measured at 238 nm. The method was validated according to ICH guidelines and found to be linear, accurate, and precise. Asian Journal of Pharmaceutical Analysis, Vol. 9, Issue 2, pp. 95–100.

- Kumar S. et al., 2017. Developed a validated UV-Visible spectroscopic method for determination of Amlodipine Besylate in pharmaceutical dosage forms. The standard solution was prepared in methanol and analyzed at λmax

238 nm. Linearity was observed in the range of 2–20 µg/ml with satisfactory recovery studies (98–102%). The method was simple, economical, and suitable for routine quality control analysis. World Journal of Pharmacy and Pharmaceutical Sciences, Vol. 6, Issue 8, pp. 1205–1212.

METHODOLOGY:

Method validation:

Validation of an analytical method is the process by which it is established by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Method validation is performed to ensure that an analytical methodology is accurate, specific, reproducible, and nigged over the specific range that an analyte will be analysed.

Preparation of Standard Stock Solution: Accurately weigh 10 mg of pure Amlodipine Besylate reference standard. Transfer it into a 10 mL volumetric flask. Add about 5 mL of the solvent and sonicate or shake to dissolve the drug completely. Make up the volume to the 10 mL mark with the solvent. Standard Working Solution (10 µg/mL): Pipette 1 mL of the stock solution into a 10 mL volumetric flask. Dilute to the mark with the solvent to get a concentration of 100 µg/mL. From this intermediate solution, pipette 1 mL into a 10 mL volumetric flask and dilute to volume.This results in a final working standard solution of 10 µg/mL.

Preparation of sample solution: Sample (Tablet) Preparation. Weigh 20 tablets and calculate their average weight. Crush them into a fine, uniform powder using a mortar and pestle. Weigh an amount of powder equivalent to 10 mg of Amlodipine Besylate. Transfer into a 10 mL volumetric flask, add 5 mL of solvent, and sonicate for 10–15 minutes to extract the drug completely. Filter the solution using Whatman filter paper. Pipette 1 mL of the filtrate into a 10 mL volumetric flask and dilute to the mark with solvent. Take further dilutions as necessary to bring the final sample concentration into the Beer's Law range (10 µg/mL).

Linearity: The linearity study of Amlodipine besylate was performed by preparing standard solutions of 1,2,3,4,5,6,7,8,9,µg/ml. The calibration graph of concentration versus absorbance is plotted for Amlodipine besylate.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from the multiple sampling of the same homogeneous sample under the prescribed condition. The precision of the method was evaluated by intraday and interday variation studies. In intraday studies, working solutions of standard and sample were analysed thrice in a day, and the percentage relative standard deviation (RSD) was calculated. In case of inter-day variation studies, the working solution of standard and sample were analysed on three consecutive days, and the percentage relative standard deviation (% RSD) was calculated. To check the degree of repeatability of the methods, suitable statistical conditions evaluation was carried out. Six samples of the tablet formulation were analysed for the repeatability study.

Accuracy: Recovery studies were carried out at three different levels (80%, 100% and 120%) by standard addition as per ICH guidelines to ascertain the accuracy of the proposed method. As per the label claim, the tablet contains 5mg of Amlodipine besylate. To perform a recovery study at 80%, tablet powder is weighed to about 56 mg, containing equivalent to 50mg of Amlodipine besylate, and to this is added standard 80mg of Amlodipine besylate. The mixture is triturated well, and from this powder, a portion is taken and added to a 50ml volumetric flask; then the solvent is added to prepare a solution of amlodipine besylate. To perform a recovery study at 100%, tablet powder is weighed to about 106 mg, containing an equivalent to 100mg of Amlodipine besylate, and to this is added standard 100mg of amlodipine besylate. The mixture is triturated well, and from this powder is taken and added into 100ml of volumetric flask, and make 8ml concentration solution of Amlodipine besylate. To perform a recovery study, 120% tablet powder weighing about 106 mg containing equivalent to 100mg of Amlodipine besylate is taken, and to this standard 100mg of Amlodipine besylate is added.

The mixture is triturated well, and from this powder is taken and added into 100ml of volumetric flask, and by serial dilution technique, the required concentration solution is prepared containing 8µg/ml.

Limit of Detection: 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. The limit of detection is calculated by using the formula DL-3.3a/S where, e is the SD of the response, and S is the slope of the calibration curve.

Limit of Quantitation: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit may be expressed as QL- 10 a/S, where a is the SD of the response, and S is the slope of the calibration curve.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate changes in method parameters, an indication of its reliability during normal usage. It is the ability to provide accurate and precise results under a variety of conditions.

2. RESULT AND DISCUSSION

Selection of wavelength: Scan the standard solution in a UV spectrophotometer between 200nm to 400nm on spectrum mode, using diluents as a blank. Amlodipinebesylateλmax at360 nm. The proposed analytical method is simple, accurate, and reproducible.

Method validation:

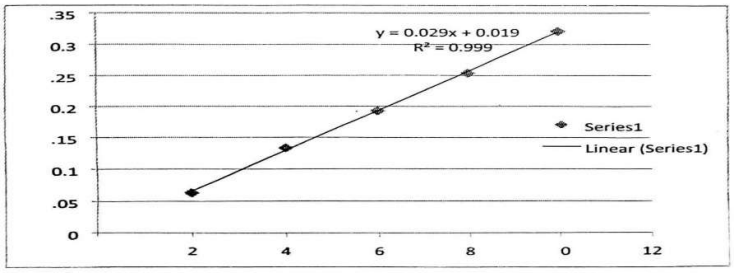
Linearity: Seven-point calibration curves were obtained in a concentration range from 1-9 Amlodipine besylate ppm for l. The response of the drug was found to be linear in the investigated concentration range, and the linear regression equation was Y=0.029x+0.019 with a correlation coefficient of 0.999.

LINEARITY, LOD & LOQ OF Amlodipine besylate:

Concentration	ABS	FOUND CONC(µg/ml)	%RECOVERY
2	0.0763	1.9758621	98.793103
4	0.1373	4.0793103	101.98276
6	0.201	6.2758621	104.5977
8	0.2547	8.1275862	101.59483
10	0.312	10.103448	101.03448
MEAN			**101.60057**
SD			**2.0815391**
SE OF INTERCEPT			**0.0034033**
SD OF INTERCEPT	**SD OF INTERCEPT*n**		**0.9309209**
LOD	**3*3(SD OF INTERCEPT/SLOPE)**		**0.387269**
LOQ	**10*(SD OF INTERCEPT/SLOPE)**		**1.1735424**

STANDARD GRAPH OF AMLODIPINE BESYLATE:

Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. LOD can be defined as the smallest level of analyte that gives a measurable response. The detection limit is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. 3 ways to usually determine it.



1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope. The limit of detection may be expressed as

LOD = 3.3 *(Standard error of the intercept/ Slope)

Limit of Quantification: The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. It is usually determined in 3 ways.

1. Based on Visual Evaluation

2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope. The limit of Quantification be expressed as;

LOQ = 10*(Standard error of the intercept/ Slope) LOD and LOQ were calculated according to the formula: LOD=0.387269µg/ml

LOQ=1.1735424µg/ml

Precision: Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per the normal weight taken for analysis. Repeat the analysis x times.

Calculate the % assay, mean assay, % deviation, % relative standard deviation, and % RSD. The developed method was found to be precise as the % RSD values for the repeatability and intermediate precision studies were 0.307% and 0.591%, respectively. **PRECISION:**

CONCENTRATIONS	**Absorbance**	**%Assay**
1	0.4951	101
2	0.4955	100.0
3	0.4975	100.6
4	0.4979	100.3
5	0.4992	100.22
6	0.4965	100.20
Average area	0.4965	100.33
Standard deviation	0.0015488	0.337
%RSD	0.31	0.34

PRECISION OF AMLODIPINE BESYLATE :

INTRADAY :

Conc.	**Absorbance**			**Mean**	**SD**	**% RSD**	**Mean % RSD**
	0 hr	**3 hrs**	**6 hrs**				
6ml	0.19656	0.19343	0.20026	0.19675	0.0005948	0.3028	0.2035
6ml	0.2553	0.2668	0.2655	0.2704	0.0004545	0.1729	
6ml	0.3610	0.3569	0.3549	0.3576	0.0004848	0.135	

INTERDAY

Conc.	Absorbance			Mean	SD	% RSD	Mean % RSD
	0 hr	3 hrs	6 hrs				
6ml	0. 1957	0.2019	0.1976	0.1984	0.0003525	0.1639	0.3682
6ml	0.267	0.2713	0.2735	0.2706	0.0004295	0.1587	
6ml	0.36536	0.3670	0.3602	0.3641	0.0004984	0.1368	

ACCURACY: Accuracy of the method is ascertained by the standard addition method at 3 levels. Standard quantities equivalent to 80%, 100%, and 120% are to be added to the sample. The results showed that the best recoveries (99.92-100.94%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

CONC	LEVEL %	ABS	MEAN	SD	%RSD
10 µg/ml	80%	0.1016	0.1013	0.00361	0.3563
		0.1014			
		0.1009			
10 µg/ml	100%	0.1728	0.1736	0.00985	0.56733
		0.1747			
		0.1733			
10 µg/ml	120%	0.2056	0.2047	0.00794	0.38775
		0.2041			
		0.2044			

LOD AND LOQ:

parameter	Concentration in ppm
LOD	0.38729ppm
LOQ	1.173542ppm

LOD AND LOQ: The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions, the analytical conditions should be suitably controlled, or a precautionary statement should be included in the procedure. The result of the robustness study of the developed assay method was established. The results showed that during all variance conditions, the assay value of the test preparation solution was not affected, and it was in accordance with that of the actual. System suitability parameters were also found satisfactory; hence, the analytical method was concluded as robust.

ROBUSTNESS OF AMLODIPINE BESYLATE :

S.NO	**CONC (µg/ml)**	**355nm**	**360nm**	**365nm**
1	8 µg/ml	0.2552	0.2779	0.2737
2		0.2555	0.2764	0.2731
3		0.2553	0.2763	0.2719
MEAN		0.2553	0.2768	0.2729
SD		0.000153	0.000896	0.000017
%RSD		0.0599	0.323	4.336

Ruggedness:

ANALYST-1			**ANALYST-2**	
SNO	**ABSORBANCE**	**%ASSAY**	**ABSORBANCE**	**%ASSAY**
1	0.3516	100.4	0.3501	100.2
2	0.3579	100.1	0.3573	100
3	0.3538	100.1	0.3531	99.9
4	0.3520	99.9	0.3522	99.9
5	0.3510	99.8	0.3514	99.9
6	0.3550	100.1	0.3546	100
Average Area	0.3535	100.2	0.3531	100.2
Standard Deviation	0.00259		0.00255	

	0.74%		0.72%	

4. CONCLUSION

- Amlodipine besylate tablet formulation validated according to ICH guidelines performed linearity, range, LOD, LOQ, Accuracy, precision (Intraday and Interday), and Robustness. Linearity was found in the range of 1-9 µg/ml which obeys Beer-Lambert's law, and the regression found that R^2= 0.999.
- Amlodipine besylate showed intraday precision with %RSD less than 2% (0.2035).
- Interday precision: Amlodipine besylate showed %RSD <2 (0.3682).
- In accuracy data, the formulation showed <2% RSD, which was acceptable. In robustness, all the formulations showed %RSD within the limit. From the above data, we can conclude that the formulations obey all parameters.
- The validation study shows that the developed UV method is linear, accurate, rapid, precise, robust, and inexpensive with an acceptable correlation coefficient, % RSD, and standard deviations, which make it versatile and valuable for the determination of amlodipine besylate in bulk or pharmaceutical dosage forms.

References

1. Ewing G. W., Instrumental Methods of Chemical Analysis, McGraw-Hill Publishing Company Inc., 2nd Ed., 1960: 3.
2. Lurie, S. Ira and Wittwer, D. John Jr., HPLC in Forensic Chemistry, vii.
3. Skoog D. A., West D. M. and Holler F. J., Fundamentals of Analytical Chemistry, Saunders College Publishing, New York, 6th Ed., 713.
4. Jeffery G. H., Bassett J., Medham J. and Denney R. C., Vogel's Textbook of Quantitative Chemical Analysis, English Language Book Society/Longman, 5th Ed., 1989: 668.
5. International Conference on Harmonization, Draft Guideline on Validation of Analytical Procedures, Definitions and Terminology, Federal Register (26), 1995: 11260.
6. United States Pharmacopoeia, Vol. I & II, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 2000: 2149.
7. United States Pharmacopoeia, Vol. I & II, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 2000: 1923.
8. Pitea M., Ghiran Doina, Mureșan Ana, Medicamente antiinflamatoare nesteroidiene, Editura Dacia, Cluj-Napoca. 1997, 231–237.
9. Goodman & Gillman's, The Pharmacological Basis of Therapeutics, Tenth Edition, New York, 2001, 1567–1573.
10. W.H. Zhu, G. High, Electrophoresis, 22 (2001), 1394–1398.
11. J.P. Wolbach, D.K. Lloyd, I.W. Wainer, J. Chromatogram. A, 914 (2001), 299–314.
12. Therapeutic Drugs, Volume 2, C. Dollery (ed.), Churchill Livingstone, London, UK, 1991, pp. 25–27.
13. Understanding NSAIDs: From Aspirin to COX-2, G.A. Green, M.D., Clinical Cornerstone Sports Medicine, 3. (2001) 50–59
14. USP XXIII (1995): The United States Pharmacopoeia. Shabir GA. J Pharm Biomed Anal. 2004; 34:207–13. [PubMed]
15. Denyer SP, Baird RM. Preservatives: Registrations and regulatory affairs—Guide to Microbiological Control in Pharmaceuticals. West Sussex: Ellis Horwood; 1990. pp. 314–40.

16. Pharmaceuticals. West Sussex: Ellis Horwood; 1990. pp. 314–40.
17. Pérez-Lozano P, García-Montoya E, Oriols A, Minarro M, Tico JR, Sune-Negre JM. J Pharm Biomed Anal. 2005; 39:920–7.
18. S'Sottofatori E, Anzaldi M, Balbi A, Tonello G. J Pharm Biomed Anal. 1998; 18:213–7.
19. Dvorak J, Hajkova R, Matysova L, Novakova L, Koupparis MA, Solich P. J Pharm Biomed Anal. 2004; 36:625–9.
20. Popovic G, Cakar M, Agbaba D. J Pharm Biomed Anal. 2003; 33:131–6.
21. Hajkova R, Slouch P, Dvorak J. J Pharm Biomed Anal. 2003; 32:921–7.
22. Koundourellis JE, Malliou ET, Broussali TA. J Pharm Biomed Anal. 2000; 23:469–75.
23. Shabir GA, Lough WJ, Shafique AA, Shar GQ. J Liq Chromatogr Relat Technol. 2006; 29:1223–33.
24. Dalibor S, Jitka HR, Ferreira LC, Maria C, Montenegro BS. J Pharm Biomed Anal. 2006; 40:287–93.
25. Kokoletsi MX, Kafkala S, Tsiaganis M. J Pharm Biomed Anal. 2005; 38:763–7.
26. Belgaied JE, Trabelsi H. J Pharm Biomed Anal. 2003; 33:991–8.
27. Mahuzier PE, Altria KD, Clark BJ. J Chromatogr A. 2001; 924:465–70.
28. Lee MR, Lin CY, Li ZG, Tsai TF. J Chromatogr A. 2006; 1120:244–51. Ross JR. Pharm. Sci. 63:594–9 (1974).
29. El-Sadek M., El-Adl S., Abou-Kull M., Sakr S.M.: Talanta 40, 585 (1993).
30. Eman K.M., Ali A.M.A., Maali N.A.E. Talanta 41, 639 (1994).
31. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. 14th ed. Merck & Co. Research Laboratories, Division of Merck and Co. Inc., Whitehouse Station, NJ, 2006, 1603.
32. Published by Merck Research Laboratories, Division of Merck and Co. Inc., Whitehouse Station, NJ, 2006, 1603.
33. Indian Pharmacopoeia 1996, Volume 1. Published by the Controller of Publications, Delhi, 424.
34. Qin Lua, Christine L.C., Greg E.C. Analytical Chimica Acta 572 (2006), 205–211.
35. Rosso A., Zuccaro S. Journal of Chromatography A 825, 1998, 96–101.
36. Sutherland F.C.W., Smits M.J., Herbst L., Hundt H.K.L., Swart K.J., Hundt A.F. Journal of Chromatography A,
37. 949, 2002, 71–77.
38. Blano M., Coello J., Iturriaga H., Maspoch S., Alaoui-Ismaili S., Fresenius ' Journal of Analytical Chemistry. Volume 357, Number 7, March 1997, 967–972.
39. Hassan Y., Aboul E., Dal A.G., Tuncel M. Il Farmaco, Volume 58, Issue 6, June 2003, 419–422.
40. Nader R., Lafi M., Sakamoto M., Masuyuki. Therapeutic Drug Monitoring, Volume 19, Issue 2, April 1997, 175–178.
41. Jalón E.G., Josa M., Campanero M.A., Sanoyo S., Ygartua P. Journal of Chromatography A, Volume 870.
42. Journal of Chromatography A, Volume 870, Issues 1–2, 18 February 2000, 143–149.

Issues 1–2, 18 February 2000, 143–149.

43. Bempong D.K., Bhattacharyya L. Journal of Chromatography A, Volume 1075, Issues

1–2, 6 May 2005, 341.

44. A. Chandrarey and R. Sharma. Indian Journal of Chemical Technology, Vol. 17, May 2010, 229–232.