DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ATENOLOL AND AMLODIPINE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT
Amlodipine and Atenolol drugs are one of the most commonly used hypertensive drugs in India. The history we observed in a 30 years old woman patients used amount of amlodipine 5mg and atenolol 50mg combination of a 100 multiple doses. The test pulse rate was 58 / min and blood pressure 76/40 mmHg. Advance serum creatine at 1.2 mg / dl during the 5 days increased to 4.8 mg / dl. The patient initiated the immunological perspective on the improper. As the patient uses the beta blocker and the calcium channel blocker, plasmapheresis, calcium gluconate infusion, glucagon, intravenous lipid emulsion, high dosage of insulin dextrose infusion and continuous low-efficiency dialysis have been used. Continue serum creatine levels, hypertension and urinary medicine were supervised, which was improved in the next 1 week and the patient was discharged. Hence we report this interesting content over the combination of Amlodipine and Atenolol drug.

INTRODUCTION
The pharmaceutical industry as a vital segment of the health care system conduct research, manufacture and market pharmaceutical and biological product for the treatment and diagnosis of disease.

Even increasing pathways of research in pharmaceutical industries has resulted in the emergence of novel and competent formulations in the market. Some of these dosage forms are highly potent. Whereas, some contain impurities. Such developments naturally require
precise, easy and sensitive methods of chemical analysis, as quality is very important for a pharmaceutical product. Since it involves life, unlike other consumer goods there is no second quality in medicines. Quality can only be achieved through the quality assurance department.

**QUALITY ASSURANCE**

Quality assurance is defined as the activity of providing to all concerned, the evidence needs to establish confidence that the quality function is being performed adequately. It covers all matters, which individually or collectively influence the quality of a product. It ensures that quality is built in to the product, beginning with research and development through production and testing of the final product.

**QUALITY CONTROL**

Quality control department is responsible for the day-to-day control of quality within a company. The department does the analytical testing of the incoming raw materials, intermediate and finished product as well as the inspection of packaging components.

In analytical chemistry it is of prime important to gain information about the qualitative and quantitative composition of substances and chemical species that is to find out what a substance is composed of and exactly how much.

In qualitative analysis, information regarding the presence or absence one or more component of the sample is taken in to account, where as in quantitative analysis, amount of the sample present is considered.

The quantitative execution of chemical reaction is the basis of the traditional or classical methods of chemical analysis. Gravimetric analysis, titrimetric and volumetric methods have come to be known as classical or chemical method of analysis.

Instrumental or physiochemical methods of chemical analysis have now become the backbone of the experimental chemistry. These methods may be used by the analytical chemist to save time and to avoid chemicals separation or to obtain increased accuracy.

Instrumental methods are generally classified into two broad two groups

- Electrochemical
- Spectral (optical) methods.
Electrochemical methods of analysis involve the measurement of current, voltage or resistance in relation to the concentration of a certain species in solution.

Techniques which can be employed under this heading are-

- Voltametry
- Coulometry
- Potentiometry
- Conductometry

**Optical methods are two types**

- Absorption methods
- Emission methods

Absorption Spectrophotometry is the measurement of the absorption of electromagnetic radiation of definite and narrow wavelength range by molecules and atoms of a chemical substance.

Absorption methods are classified according to the wavelength as

- Visible Spectrophotometry
- Ultraviolet Spectrophotometry
- Infrared Spectrophotometry
- Absorption Spectrophotometry
- Nephelometry and turbidimetry methods

Emission methods involve in subjecting the sample to heat or electrical treatment. So that atoms are raised to excited status causing them to emit energy and the intensity of this emitted energy is measured. The common excitation is

- Emission spectroscopy
- Flame photometry
- Fluorimetry

**SPECTROPHOTOMETRY**

Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter resulting in the absorption or emission of energy in discrete amounts called quanta. It is one of the most powerful tools available for the study of atomic and molecular structure and is used in the analysis of a wide range of samples.
Absorption Spectrophotometry is the measurement of the absorption of electromagnetic radiation of definite and narrow wavelength range by molecules and atoms of a chemical substance. Techniques frequently employed in pharmaceutical analysis include Atomic Absorption, Fluorescent, Infrared, Ultraviolet and visible Spectrophotometry.

Atomic Spectrophotometry is the measurement of the intensity of emission of absorption of light at particular wavelength by the atomic vapors of certain metals, generated by introducing into flame solutions containing ions of such metals.

Fluorescence Spectrophotometry is the measurement of the intensity of emission of fluorescent light emitted by a chemical substance while it is being exposed to Ultraviolet, Visible, or other electromagnetic radiation.

The wavelength range available for these measurements extends from the wavelength of the Ultra violet through the Infra red. For convenience of reference, this special range is roughly divided into the Ultra violet (185nm to 380nm), the visible (380nm to 780nm), The near infra red 780nm to 3000nm, and the far Infra Red 3000nm to 4000nm.

The Infra Red absorption spectrum is unique for any given chemical compound with the exception of optical isomers, which have identical spectra. However, Polymorphism may sometimes show differences in the Infra Red spectrum of a given compound in the solid state.

Because of the large numbers of maxima in an Infra Red absorption spectrum, possible to measured qualitative composition without prior separation.

**QUANTITATIVE ANALYSIS IN SPECTROSCOPY**

The spectroscopy assay of drugs rarely involves the measurement of absorbance of sample containing only one absorbing component.

Quantitative analysis of drugs in UV spectroscopy is undertaken for both single component and multi component formulations.

Single component analysis may be done by the use of Standard absorptive value
- Calibration graph
- Single or double point of standardization methods
In standard absorptive value method the use of standard A (1%, 1cm) or E values are used in order to determine its absorptivity values. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. A. In calibration graph method the absorbance of a number of standard solutions of the reference substance at concentrations encompassing the sample concentration are measured and a calibration graph is constructed. The concentration of analyte in the sample solution is read from the graph as the concentration corresponding to the absorption of the solutions.

The single point procedure involves the measurement of the absorbance of a sample solution and standard solution of the reference substances. The concentration of the substance in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

\[
C_{\text{test}} = \frac{A_{\text{test}} \times C_{\text{std}}}{A_{\text{std}}}
\]

Where \(C_{\text{test}}\) and \(C_{\text{std}}\) are the concentrations in the sample and standard solutions respectively and \(A_{\text{test}}\) and \(A_{\text{std}}\) are the absorbance’s of the sample and standard solutions respectively.

This method is preferred when assaying substances that obey Beer’s law and for which a reference standard of adequate purity is available.

**SIMULTANEOUS ANALYSIS OF DRUGS**

The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the recipe sample formulation is available to the analyst.

The identity and concentration of the interferents are known and the extent of interference in the assay may be determined. Alternatively, interference, which is difficult to quantify, may be arise in the analysis of formulation excipients. Unwanted absorption from these sources is termed irrelevant absorption and if not removed, imparts a systemic error to the assay of drugs in the sample.

Irrelevant absorption may be due to impurities, formulation additives, degradation products, etc. These types of interference are normally negligible, but in certain cases it may be
significant, particularly if the amount of the drug present is very less compared to formulation additives and such interference by formulation additives cannot be overlooked.

This type interference can be eliminated by
- Geometrical – correction method
- Orthogonal polynomial function method
- Differential spectroscopic method

The second type of interference is due to the presence of two or more drugs in a formulation. In such multi component formulation, the components mutually interfere with other in their estimation. For the simultaneous estimation of drugs in such formulation, many methods have been suggested.

They are
- Simultaneous equation method
- Absorbance method
- Derivative spectroscopy method
- Chemical derivation method
- Multi component mode of analysis

**SIMUATIONEOUS EQUATION METHOD**
If a sample contains two absorbing species each of which absorbs at the \( \lambda \) max of the other, it may be possible to determine both the drugs by the technique of simultaneous equation or vierodt’s method.

Criteria for obtaining maximum precision, based upon absorbance ratios have been suggested that place limits on the relative concentration of the components of the mixture. The ratio should lie outside the range of 0.1 – 0.2 for the precise determination of the two drugs respectively. These criteria are satisfied only when the \( \lambda \) max of the two components are reasonably dissimilar. An addition criterion is that the two components do not interact chemically, there by negating the initial assumption that the total absorbances should always be found in the development of a new application of this technique.

**ABSORABANCE RATIO METHOD**
This method depends on the property that for a substance which obeys Bear’s law at all
wavelength, the ratio of absorbance’s at any two wavelengths is a constant value independent of concentration and path length. The two different dilutions of the sample give the same absorbance ratio this ratio is referred as Q value.

CHEMICAL DERIVATISATION METHOD

The indirect Spectrophotometric assays are based on the conversion of the analyte by a chemical reagent to a derivative that has different spectral properties. When an excess of reagent is used, to ensure complete conversion, the absorbance of the derivative is usually, but not always proportional to the concentration of the analyte. The majority of indirect spectrophotometric procedures involve the conversion of the analyte to a derivative that has a longer λ max and a higher absorptivity.

This method is employed for of the following reasons.

- If the analyte absorbs weakly in the UV region, a more sensitive method of assay is obtained by converting the substance to a derivative with a more intensely absorbing chromophores.
- The interference from irrelevant, absorption may be avoided by converting the analyze to a derivative, which absorbs in the visible region, where irrelevant absorption is negligible.
- Indirect Spectrophotometric procedure are also used to improve the selectivity of the assay of an ultraviolet absorbing substance in a sample that contains other UV – absorbing components.

MULTICOMPONENT MODE OF ANALYSIS

Two or more substances can be analyzed by this method, if the absorption spectra of the two components are different that two wavelength can be found at which each substances absorbs light without interferences from the other, the problem reduced in multi component analysis, since neither component interferes with the analysis of the other. In general both the substances will absorb light at the same wavelengths, but if their absorb light at the same wavelength, but if their absorption spectra are markedly different, the mixture can still be analyzed.

First determine the absorption spectra of two pure compounds. These spectra are compare, absorbance values to a common basis as molar absorptivities, and superimposing the spectra. two analytical wavelength are selected such that the difference in absorption by the compound is maximum at these wavelengths, with the absorptivity for the compound A being
greater than that of B t one wavelength and less at the each other.

The next step in the analysis is to make Beer’s law plots, using solutions of the pure substance, for each compound at each wavelength. All quantities being and evaluated at the same wavelength should be line. From the slope of intercept of the line, the concentration of the two compounds may be determined

CONCLUSION
Hence spectroscopic method is the most accurate method for determining the concentration of substance in solutions. It may be regarded as a refined filter photoelectric photometer, which permits the use of continuously variable and more monochromatic bands of light.

ANAYTICAL METHOD DEVELOPMENT
Developing a method involves the study of following.

INVESTIGATION OF THE CHEMICAL STRUCTURE
Chemical structure with reference to the reactive functional group of the selective drugs was studied in detail. This is essential for selection of an analytical reagent is based on its reactivity with the appropriate functional survey.

LITERATURE SURVEY
An extinctive survey of available literature was undertaken in order to enumerate reported methods for analysis of experimental drug.

STANDARDIZATION
Carefully planned study of the variable was conducted to determine the optical condition of the reaction.

ANALYSIS OF THE COMMERCAIAL DOSAGE FORMS
The proposed method in applied to the analysis of various marketed formulations.

STATISTICAL ANALYSIS AND VALIDATION
Statistical analysis and validation were performed to justify the suitability of the methods and statistical analysis to justify reliability and reproducibility of the proposed methods.

ANALYTICAL METHOD VALIDATION
Jay Breaux, Kelvin Jones and P.L. Erre, Boular analytical methods development and
validation play an important role in discovery, development and manufacture of pharmaceutical, Quality control laboratories are used to ensure the identity, purity, potency and performance of drug products.

Validation is defined as establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification quality characteristics.

- It is an integral part of the quality control system.
- Current good manufacturing practice requires assay validation.

PARAMETERS USED FOR ASSAY VALIDATION

The validation of the assay procedure was carried out using the following parameters.

SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix, etc., (component) which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure.

ACCURACY

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

PRECISION

The precision of an analytical procedure express the closeness of agreement between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed condition. The precision of an analytical procedure is usually expressed as the variance, standard deviation or variation of a series of measurements.

REPEATABILITY

Repeatability expresses the precision under the same operating condition over a short interval of time Repeatability is also termed as intra assay precision.

LIMIT OF DETECTION (LOD)

LOD is the lowest concentration of the substance can detect but not necessarily indicates that
the quality. LOD simply sample is below or above a certain level.

**LIMIT OF QUANTITATIVE (LOQ)**

LOQ is the lowest concentration of the substance that can be estimated quantitatively with acceptable, accuracy and reliability by the proposed method. LOQ is determined by analysis of sample containing decreasing quantity of the substance and determining the lowest level at which acceptable level of accuracy and precision is attained.

**LINEARITY**

The linearity of an analytical procedure is its ability (with in a given range) to obtain test results, which are directly proportional to the concentration (amount) of analytical in the sample.

**RANGE**

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

**STATISTICAL PARAMETERS USED FOR ANALYSIS**

The quantitative results were subjected to the following statistical analysis;

- Sample mean (S.M)
- Standard deviation (S.D)
- Relative standard deviation (R.S.D)
- Standard error of mean (S.E)

The formulas for the statistical parameters are

$$S. M = \frac{X_1 + X_2 + X_3 ... ... + X_n}{n}$$

$$S. D = \sqrt{\frac{\sum(X_i - X)^2}{n - 1}}$$

$$R. S. D = \frac{S. D}{mean} \times 100$$

$$S. E = \frac{S. D}{\sqrt{n}}$$
AIM AND OBJECTIVE OF THE WORK
The present work is aimed to
1. Develop a UV Spectrophotometric method for the simultaneous estimation of Atenolol and Amlodipine.
2. To standardize the develop method.
3. Analyze the marketed formulation for the reliability and accuracy.
4. Perform the recovery studies for the developed UV-spectroscopic.
5. Validate the developed method for their accuracy, precision and reproducibility as per ICH guidelines.

STMULTAEOUS ESTIMATION OF ATENOLOL AND AMLODIPINE

EXPERIMENTAL WORK
In this study, a combination of tablet containing Atenolol and Amlodipine were subjected to the following method of analysis for quantitative estimation using reference standard and Amlodipine.

METHODS SPECTROPHOTOMETRY
The Spectrophotometric analysis was carried out by the following method.

SIMULTANEOUS EQUATION METHOD
➢ Procedure
➢ Results and discussion

DRUG PROFILE INTRODUCTION
The combination of Atenolol and Amlodipine is affixed dose combination has been introduced in India, having therapeutic profile composition.
Each film coated tablet contains
Atenolol: 50mg
Amlodipine: 5mg

ATENOLOL CHEMICAL STRUCTURE

![Figure 1: Structure of Atenolol.](image)
Molecular Formula: C_{14}H_{22}N_{2}O_{3}
Molecular weight: 266.366 g/ml.
Appearance: white crystalline powder
Solubility: soluble in dehydrated alcohol, poorly soluble in alkaline solution, soluble in water: 26.7 mg per ml
Pka values: 9.6 Melting point: 160°C

**MECHANISM OF ACTION**
Atenolol competes with sympathomimetic neurotransmitters such as catecholamine for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension. Higher doses of atenolol also competitively block beta (2) - adrenergic responses in the bronchial and vascular smooth muscles.

Therapeutic category:
- Anti hypertensive agent

Therapeutic indications:
It is indicated for the treatment of
- Hypertension
- Angina
- Long QT syndrome
- Acute myocardial infraction
- Supra ventricular tachycardia
- Ventricular tachycardia
- Migraine.

Dosage and administration: 50mg oral-once a day.
AMLODIPINE CHEMICAL STRUCTURE

Molecular formula: C20H25ClN2O5 Molecular weight: 408.879g/mol
Chemical name: (RS)-3-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl -1,4-dihydropyridine -3,5-dicarboxylate.
Appearance: white crystalline powder
Solubility: slightly soluble in water and sparingly soluble in ethanol Pka values: 8.58
Melting point: 239°

MECHANISM OF ACTION
Amlodipine inhibits the movement of calcium ions into vascular smooth muscle cell and cardiac muscle cells. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells. Negative inotropic effects can be detected in vitro, but such effects have not been seen in intact animals at therapeutic doses. Serum calcium concentration is not affected by Amlodipine. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. As a calcium channel blocker, Amlodipine is expected to inhibit the currents of L-type cav1.3channels in the zonaglomerulosa.

The mechanisms by which Amlodipine relieves angina include:
- Stable angina.
- Prinzmetal’s angina
- Amlodipine has additionally been found to act as an antagonist of the mineralocorticoid
receptor, or as an antimineralocorticoid.

Therapeutic category: along acting calcium channel blocker.

Usual adult dose 2.5,5 and 10mg of Amlodipine for oral administration.

ATENOLOL AND AMLODIPINE LITERATURE SURVEY

1. Manzoorahmed et al., Developed a method fosimultaneous estimation of atenolol and hydrochlorothiazide in combined dosage form by UV spectrophotometric methods.
2. N. Fernandes et al., dual wavelength and simultaneous equation spectrophotometric methods for estimation of atenolol and indapamide in their combined dosage form.
3. K.V Lalitha Devi et al., UV spectrophotometric method development and validation for the determination of atenolol and losartan potassium by quality analysis.
4. A. Lakshmi Devi et al., performed a new RP-HPLC method development and validation for the dissolution studies of atenolol and chlorthalidone in immediate release tablet dosage forms.
5. Manishamasih et al., Spectrophotometric simultaneous estimation of amlodipine besylate and losartan potassium in tablet dosage forms.
6. S. Dey et al., spectrophotometric method for simultaneous determination of atenolol and atorvastatin in tablet dosage forms.
7. Bilalyilmaz et al., Determination atenolol in pharmaceutical preparation by zero, first, second, and third – order derivative spectrophotometric methods.
9. Afaf Abou – elkhei et al., performed derivative ratio, isobestic point, factorized absorbptivity and bivariate spectrophotometric determination of atenolol and chlorthalidone.
10. Lalitha g et al, development a method and performed and its validation for the analysis of atenolol in tablet dosage form by UV spectroscopy.
11. Gurram Bhagath Kumar Goud et al., performed simultaneous estimation of atenolol and chlorthalidone in bulk and combined pharmaceutical dosage form by UV spectroscopy.
12. Tengli AR et al., developed a method and performed validation of tablet dosage form containing losartan, atenolol and hydrochlorothiazide using internal standard by RP –
HPLC.


**SIMULTANEOUS EQUATION METHODS OF ANALYSIS**

**INTRODUCTION**

When a sample contains two adsorbing drugs X and Y, each of which absorbing at the wavelength of the other, it is a possible to determine both the drugs at the same time without separating them from each other by simultaneous equation method.

This method employs the formation and the solving of simultaneous equation of the two drugs in combined dosage form once the equation parameters are set out, then it is just required to measure the absorbance of the sample solution at wavelength of two drugs. Then the measured absorbance of the sample solution are substituted in the equation and solved for the determination of to drugs.

**DATA REQUIRED FOR THE CONSTRUCTION OF SIMULTANEOUS EQUATION**

The following steps should be followed

1. The wavelength of two drugs should be found out using the reference standards of the two drugs.
2. The calibration curve should be plotted for the each drug and the linearity range should be found out.
3. The absorbance values of each reference drugs at the two wavelengths of the two drugs should be measured and their absorptivity values should be calculated.
4. The absorbance values of the tablet formulation at the two wavelength should be measured and recorded.

A set of two simultaneous equation were framed using the absorptivity as give below

\[
A_1 = ax_1C_x + ay_1C_y \\
A_2 = ax_2C_x + ay_2C_y
\]
Where

\[ ax_1 \text{ and } ax_2 = \text{Absorbance values of compound } x \text{ at } \lambda_1 \text{ and } \lambda_2 \text{ respectively.} \]

\[ ay_1 \text{ and } ay_2 = \text{Absorbance values of compound } y \text{ at } \lambda_1 \text{ and } \lambda_2 \text{ respectively.} \]

\[ C_x \text{ and } C_y = \text{concentration of components } X \text{ and } Y \text{ in diluted sample.} \]

\[ A_1 \text{ and } A_2 = \text{absorbance values of diluted sample at } \lambda_1 \text{ and } \lambda_2 \text{ respectively} \]

By applying the crammer’s rule and matrices to the above two equation, concentration \( C_x \) and \( C_y \) can be obtained as

\[
\text{Concentration of } X(c) = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1y_2} \\
\text{Concentration of } Y(c) = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1y_2}
\]

**Criteria for obtaining maximum precision**

1. The ratio \( A_2/A_1 \text{ and } ay_2ay_1 \) should lie outside the range 0.1-2.0 for the precise determination of two drugs \( X \text{ and } Y \)

2. The \( \lambda \text{ max} \text{ of } x \text{ and } y \) should be dissimilar.

3. The two components should not react chemically.

4. The additively of the individual absorbance of the two components should be confirmed to the total absorbance of the sample i.e.,

Total absorbance of sample = absorbance of \( X \) + absorbance of \( Y \)

**MATERIALS AND METHODS**

**INSTRUMENTATION**


2. UV-single beam spectrophotometer- Genesys 10S Thermo scientific.

**Reagents**

Methanol

6.8 pH phosphate buffer

Reference standards.

**Atenolol and Amlodipine supplier**

Received s gift samples from Ranbaxy laboratories, gurgaum and the authenticity and purity of the samples were certified by the same.
Table 1: Marketed formulation.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>BRAND NAME</th>
<th>COMPANY NAME</th>
<th>MFG. DATE</th>
<th>EXPIRY DATE</th>
<th>BATCH NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Angicem Beta</td>
<td>Blue cross</td>
<td>01/2019</td>
<td>03/2019</td>
<td>AGB 1519</td>
</tr>
</tbody>
</table>

STANDARDISATION OF THE METHOD

In developing a quantitative method for determining an unknown concentration of analyte by absorption spectrophotometry. Certain parameters have to be established. The important are

- Absorption maximum (λ max).
- Beer’s law concentration range.
- Stability of absorbance.

Preparation of standard stock solution

The standard stock solution of both and atenolol were prepared separately by dissolving 100 mg of amlodipine and 1000 mg of atenolol in 100 mL standard flask separately using 6:4 methanol: 6.8 pH phosphate buffer as a solvent to give a concentration of 1000 mg/ml respectively. These were then used for establishing the following parameters.

Absorption maximum

The stock solution were suitably diluted with 6:4 methanol : 6.8 pH phosphate buffer as to contain 25 micrograms /ml solution these solution were scanned in the UV region between 400-200 nm and found that amlodipine exhibited wavelength maxima at 238 nm and atenolol exhibited wavelength at 273 nm.

Beer’s law concentration range

The stock solutions were suitably diluted with 6:4 pH phosphate buffer get concentration range from 1-1000 mcg/ml for amlodipine and 1-1000 mg/ml for atenolol respectively. The solution were scanned in the UV region between 400-200 nm and their absorbance were measured at respective λ max points.

Using the absorbance values against concentration the calibration curve was plotted. From the graph it was found that amlodipine obeys beers law between 10-50 mcg /ml. The regression analysis was carried out for the regression line which estimates the degree of linearity.

Stability of absorbance

The stability of the solution was checked by measuring the absorbance at regular intervals of
time. It was observed that the absorbance remained stable for a period of more than 120 min.

which is sufficient to carry the project.

ANALYSIS OF FORMULATION
PROCEDURE
Preparation of the standard solution
The stock solution were suitably diluted with 6:4 methanol; 6.8 pH phosphate buffer acid so as to contain 225 mcg /ml of amlodipine and 25 mcg /ml of atenolol the solution were scanned in the UV region between 400-200 nm and found that amlodipine has exhibited wavelength maxima at 238 nm and atenolol at 273 nm respectively.

Absorptivity values of both of the drugs were calculated from the mean of five independent determinations by the following formula.
Absorptivity (a) = A/ BC

Where
A= absorbance B= path length
C = concentration in g/100 ml

Assay of tablet formulation
20 tablets were finely powdered and accurately weighed quantity of powder equivalent to about 100 mg tablet powder to a 100 ml standard flask. the content of the flask was mixed with 6:4 methanol: 6.8 pH phosphate buffer and shaken to dissolve the active ingredients and then made up to the volume with the same solvent.

The solution was the filtrate was further diluted with 6:4 methanol: 6.8 phosphate buffer to give a final drug concentration of 25 mcg/ml. absorbance values of sample solution was recorded at 238 nm and 273 nm as A1 and A2 respectively.

Concentration of the two drug samples were determine by using the following equation.

\[
\text{Concentration of } X \ (C_x) = \frac{A_{2y1A1y2}}{ax2 \ ay2 - ax1 \ ay2} \\
\text{Concentration of } Y \ (C_y) = \frac{A_{2ax1A1y2}}{ax2 \ ay1 - ax1 \ ay2}
\]
The analysis was carried out on five replicates for tablet formulation. The amount of drug present in the tablet formulation was calculated as follows.

\[
\text{Amount of drug present} = \frac{\text{concentration \times \text{dilution factor \times \text{average weight}}}}{\text{weight taken}}
\]

VALIDATION OF THE METHOD

The proposed method is validated for the following parameters.

Assay

The absorbance and absorptivity values of drugs at wavelength 210nm and 225nm were found from the following table 2.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Name of the drug</th>
<th>λ1 273nm</th>
<th>λ2 2238 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atenolol</td>
<td>13.78</td>
<td>14.573</td>
</tr>
<tr>
<td>2</td>
<td>Amlodipine</td>
<td>15.083</td>
<td>15.296</td>
</tr>
<tr>
<td>3</td>
<td>Tablet</td>
<td>2.288</td>
<td>0.793</td>
</tr>
</tbody>
</table>

Concentration of Atenolol (x) = 28.3mcg/ml
Concentration of Amlodipine(y) = 30.3mcg/ml.

Table 3: Percentage purity values of Atenolol and Amlodipine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount found</th>
<th>Label claim</th>
<th>Percentage purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>28.3</td>
<td>28</td>
<td>101.06</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>30.3</td>
<td>30</td>
<td>99.178</td>
</tr>
</tbody>
</table>

Spectrum: atenolol.dsp
Description: spectrum
Operator: SLVPS-PC\slvps
Created: 3/14/2019 3:26:46 PM.

Spectrophotometer: GENESIS 10S UV-VIS
Serial number: 2L9P082004 Firmware: 4.002
Baseline: 3/14/2019 3:26:46 PM.
Figure: Spectrum of Atenolol.

Figure: Spectrum of Amlodipine.

Figure: Spectrum of combined dosage form.
Linearity

The linearity of a method is ability to obtain test that are directly proportional to the sample concentration over a given range. The absorbance values against concentration were plotted to get a standard calibration curve. The linearity of calibration curve (absorbance V/s concentration) in pure drug solution was checked over the concentration ranges of 10-50 µg/ml and 10-50µg/ml for Atenolol and Amlodipine respectively. the regression line relating standard concentration of drugs using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained.

\[ y = 0.068x + 0.0302, \]

\[ R^2 = 0.9996 \] for Atenolol and \[ y = 0.0149x - 0.0033, \]

\[ R^2 = 0.9994 \] for Amlodipine. The linearity plots were shown in table No. 4

Table 4: Linearity plots of Atenolol and Amlodipine.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (mcg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Atenolol</td>
</tr>
<tr>
<td>01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>02</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>03</td>
<td>20</td>
<td>0.7</td>
</tr>
<tr>
<td>04</td>
<td>30</td>
<td>0.4</td>
</tr>
<tr>
<td>05</td>
<td>40</td>
<td>0.8</td>
</tr>
<tr>
<td>06</td>
<td>50</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Acuracy

The accuracy of the method was determined by recovery experiments. The accuracy studies were carried out at three concentration i.e., 50%, 100%, 150%. The three concentration of solution were prepared and absorbance are recorded for three times. The percentage recovery was calculated from the data obtained and the results were tabulated in table 5.

Table 5: Accuracy values of Atenolol and Amlodipine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>QC conc. (µg/ml)</th>
<th>Amount drug added</th>
<th>Amount of drug found (µg/ml) mean±S.D</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>10</td>
<td>50%</td>
<td>0.572±0.08</td>
<td>0.652</td>
<td>99.85%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>0.607 ±0.1</td>
<td>0.507</td>
<td>99.996%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150%</td>
<td>17.3±0.05</td>
<td>0.35</td>
<td>100.153%</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>10</td>
<td>50%</td>
<td>0.002±0.08</td>
<td>0.082</td>
<td>100.041%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>0.048±0.05</td>
<td>0.098</td>
<td>100.025%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150%</td>
<td>15.24±0.05</td>
<td>0.19</td>
<td>99.933%</td>
</tr>
</tbody>
</table>

Repeatability testing

The linearity precision studies were studied by six replicate measurements of 25µg/ml Atenolol and 25µg/ml for Amlodipine at two wavelength maxima. Statistical evaluation revealed that relative standard deviation of drugs was less than 2.0. The results were mentioned in table 6.

Table 6: Repeatability testing values if Atenolol and Amlodipine.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Atenolol</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance at 273nm</td>
<td>Absorbance at 238nm</td>
</tr>
<tr>
<td>Mean</td>
<td>RSD</td>
<td>Mean</td>
</tr>
<tr>
<td>01</td>
<td>2.264</td>
<td>0.2</td>
</tr>
<tr>
<td>02</td>
<td>2.251</td>
<td>0.2</td>
</tr>
</tbody>
</table>
**Range**
Beer’s law limit for Atenolol from linearity data = 2.307 Beer’s law limit for Amlodipine from linearity data = 2.09.

**RESULTS AND DISCUSSION**
The UV spectra of Atenolol and Amlodipine are presented in figure 3 and 4 respectively. The absorption maxima (wavelength) were observed at 273nm and 238nm for Atenolol and Amlodipine respectively.

Obey née to Beer’s law was confirmed by the linearity of the calibration curve of Atenolol and Amlodipine. Which are represented in figure 6 and 7 respectively.

Atenolol shows the linearity in the concentration ranges of 10 to 50mcg/ml. The data recording the calibration curves are given in a table absorptivity values for Atenolol and Amlodipine were calculated and are presented in table 2.

The quantitative estimation was carried out on tablet formulation by taking a concentration of 25mcg/ml. The data regarding the quantitative estimation in table 3. The tablet formulation shows the percentage purity of 102.8% w/w for Atenolol and 101.4% for Amlodipine.

The validation of the proposed simultaneous equation method was further confirmed by linearity, precision and recovery studies. $R^2$ value for linearity plot of Atenolol was 0.9996. $R^2$ values for linearity plot of Amlodipine was 0.9994. The range for Atenolol and Amlodipine was 10-50mcg/ml and 10-50mcg/ml. Hence the method developed was linear for specified range.

The recovery data is given in the table 5. The percentage recovery values vary from 99.5% to 101.7% for Atenolol and 100.2% to 101.5% amlodipine. This serves as a good index of accuracy.

**CONCLUSION**
The quantitative results obtained were subjected to statistical analysis to find out standard deviation and standard error values. The relative standard deviation values are below 2%, indicating the precision of the method. The validation of the proposed simultaneous equation method was confirmed by recovery studies.
From the results of the proposed method it is evident that the method which is developed is simple, specific and precise and can be employed in the routine analytical work.

REFERENCES