UV-Visible Spectrophotometric Estimation of Amlodipine in Pharmaceutical Dosage Form

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Abstract:
A simple spectrophotometric method based on single wavelength spectroscopy has been developed for the Amlodipine in different pharmaceutical dosage forms. The method is based on the simple solubility of Amlodipine in methanol. The absorbance maximum of Amlodipine was measured at wavelength 241nm and 529nm for the UV method and visible method. Both the methods obeyed Beer-Lambert’s law over the concentration range 2-10 µg/ml and 0.5-3 µg/ml. The proposed method was successfully applied to the determination of Amlodipine in pharmaceutical dosage forms and the results tallyed well with the label claim.

Keywords: Amlodipine, UV spectrophotometer, Alizarin Red-S method, Amlokind, Amlostat.

Introduction:
Amlodipine belongs to a class of drugs called calcium channel blockers which relaxes (widens) blood vessels and improves blood flow. It is used to treat high blood pressure (hypertension) or chest pain (angina) and other conditions caused by coronary artery disease. This medication is for use in adults and children above 6 years old. Its chemical name is 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-5-pyridine dicarboxylate monobenzene sulphonate and has an empirical formula of C_{20}H_{25}ClN_{2}O_{5}C_{6}H_{6}O_{3}S and a molecular weight of 408.879 g/mol. The structural formula is:

![Figure 1: Structure of Amlodipine](image)
2. Materials and Methods

2.1 Instrumentation
Techcomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Ultrasonicator (1.5L) was used to sonicate the standard and formulation. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

2.2 Reagents, Standard and samples:
Working standard sample Amlodipine was obtained from well reputed research laboratory, formulation sample was purchased from local pharmacy. Spectrophotometric coloring reagent i.e Alazirin Red S reagent (ARS reagent) was purchased from Merk chemicals pvt limited, Mumbai, India.

2.3 Preparation of standard stock solution:
Standard stock solution of Amlodipine pure drug was prepared by accurately weighing about 10mg of each drug in 10ml volumetric flask. The drugs were dissolved with 5ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent; results 1000µg/ml solution was obtained. From this 1ml was taken and diluted to 10ml to get a concentration of 100µg/ml. From 100µg/ml solution 2ml was taken and made up to 20ml to get a final working stock solution of 10µg/ml required concentrations or dilutions needed for UV and visible estimation was prepared from 10µg/ml solution.

2.4 Preparation of Formulation Sample:
10 tablets from each of the brand selected for assay estimation of Amlodipine was grinded till to get a fine powder and homogenously mixed using a mortar and pestle. From the powder, an amount of the powder equivalent to 10mg of Amlodipine was weighed and was dissolved in 10ml of Methanol. The solution was sonicated for 10min to complete extraction of drugs in Methanol. The solution was centrifuged at 4000 rpm for 10 min; the clear supernatant was collected and was filtered through whatmann filter paper. From this solution selected concentration was prepared by proper dilution. Similar procedure was followed for the preparation of remaining branded tablets separately. The prepared solutions were used for the assay of Amlodipine.

3. UV Spectrophotometric estimation:
3.1 Selection of solvent for solubility:
The drug Amlodipine was practically insoluble in Water. Hence water was not used for the preparation of drug solutions. We prepared different soluble solvents of Amlodipine like methanol, Acetonitrile etc in a fixed dilute solution and absorbance of solution was measured. Finally solvent Methanol and its dilutions with water was show improved absorbance compared to other solvents. Hence standard drug was soluble in methanol and necessary required dilutions were prepared with water as diluents for spectrophotometric estimation.

3.2 Selection of wavelength maxima:
Suitable maximum absorbance for the estimation of Amlodipine was identified by scanning the absorbance in spectrum mode within the wavelength region of 400-200nm in three different dilute solutions. In all the solutions the drug absorbed maximum wavelength at 241nm. Hence 241nm was found to be suitable wavelength for the estimation of Amlodipine.
3.3 Construction of calibration curve:
From the prepared standard stock solution, a series of calibration standards were prepared by selected dilutions. From the stock solution, 1µg/ml, 2, 4, 6, 8, 10µg/ml was prepared. The absorbance of the prepared solutions was measured at 241nm against a regent blank. At each concentration triplet readings were measured and mean value was used for the Construction of calibration curve. Calibration curve was constructed by taking concentration of the prepared solution on x-axis and corresponding absorbance on y-axis.

3.4 Formulation analysis:
The absorbance of the prepared formulation solution in all the brands was measured at 211nm in triplets separately. The average absorbance value was used for the formulation estimation of Amlodipine. The % assay estimated in the prepared sample solutions by substituting the absorbance values in the regression equation.

4.0 Visible Spectrophotometric estimation:

4.1 Preparation of Reagents:
ARS solution: weigh 200 mg of ARS and is dissolved in 100ml of distill water.
HCL Solution: dissolve 8.6 ml of concentrated hydrochloric acid in 1000ml of distill water.

4.2 Method procedure:
In a series of 125 ml separating funnels containing aliquots of standard drug solution was taken. To this 6ml of HCl solution and 2ml of ARS solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 529nm against a similar reagent blank. Organic layer attains orange red color while the blank is colorless.

4.3 Formulation Assay:
From the prepared 10µg/ml of the sample solution, 1ml was taken and the method procedure as describes above was applied. After the development of the color, the absorbance of the separated chloroform layer was measured at 529nm against a similar reagent blank. The resultant absorbance values were used for the estimation of Amlodipine in the formulation assay. The % assay estimated in the prepared sample solutions by substituting the absorbance values in the regression equation.

5.0 Results and Discussion:
Amlodipine showed maximum absorbance at 241 nm and 529nm for UV method and visible method. Based on the experimental data the standard calibration curve was plotted (Fig.5.2) The absorbance range was found to be 2-10 µg/ml for UV method and 0.5-3.0 µg/ml for the ARS method. As these solutions obeyed Beer-Lambert’s law and the content of drug was calculated from the equation y = 0.0285x - 0.003 with correlation coefficient of 0.9989 and y = 0.042x - 0.057 with correlation coefficient of 0.998. Wavelength scanning result was shown in figure 5.1.
Figure 5.1: Wavelength scanning spectrum of Amlodipine in UV region and ARS method.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>UV method</th>
<th>ARS method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration µg/ml</td>
<td>Average Absorbance</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.055</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.108</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.172</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.222</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.283</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Slope | 0.0285 | Slope | 0.0422 |
Intercept | 0.003 | Intercept | 0.0576 |
Correlation | 0.9989 | Correlation | 0.998 |

Table 5.1: Calibration Curve Results of Amlodipine in UV Method and ARS method
5.1 Formulation Assay:
The absorbance of the prepared formulation solutions was measured and from the resultant sample values % assay was calculated. In the entire brand under study, % assay was found to be more than 98%. High amount of drug was estimated in Amlokind brand whereas low amount of Amlodipine was estimated in Amlostat brand in both the methods. Hence the followed method was successfully applied for the estimation of Amlodipine. Results of the assay studies were shown in table 5.2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Brand</th>
<th>Dosage (mg)</th>
<th>Amount Prepared (µg/ml)</th>
<th>%Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV method</td>
<td>Amlokind</td>
<td>10</td>
<td>10</td>
<td>99.29</td>
</tr>
<tr>
<td></td>
<td>Amlostat</td>
<td>5</td>
<td>10</td>
<td>98.87</td>
</tr>
<tr>
<td>ARS method</td>
<td>Amlokind</td>
<td>10</td>
<td>10</td>
<td>99.73</td>
</tr>
<tr>
<td></td>
<td>Amlostat</td>
<td>5</td>
<td>10</td>
<td>98.91</td>
</tr>
</tbody>
</table>

Table 5.2: Formulation results of Amlodipine in UV Method and ARS method

6.0 CONCLUSION

One visible and one UV spectrophotometric method was followed for the estimation of Amlodipine in different marketed formulations. Both the methods have different calibration ranges. Both the methods can successfully estimate the amount of drug in formulations with high accuracy. Formulation excipients do not interfere in the estimation in both uv and visible region. Hence more than 98% assay was found in both the estimation methods.
7.0 REFERENCES