A new method development and validation of dual wavelength UV Spectrophotometric method for Simultaneous estimation of Atenolol and Amlodipine besylate in combined dosage form

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INTRODUCTION

Atenolol (ATN) is chemically 4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl acetamide, is beta-blocker seem to be equally effective as an antihypertensive, anti-anginal and antiarrhythmic drug. It is widely used cardiovascular drug in combination with Amlodipine. Amlodipine Besylate (AMN) is chemically 3-ethyl-5-methyl-(4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylatebenzene sulphonate, is calcium channel blocker used as potent coronary and peripheral vasodilator and in bradycardia.

Literature survey reveals that various analytical methods have been reported for the assay of atenolol and amlodipine besylate in pure form and in pharmaceutical formulations. Non aqueous titration method is specified in Indian Pharmacopoeia for the assay of atenolol. While British Pharmacopoeia described liquid chromatography method for the assay of amlodipine besylate. Other methods such as derivative spectroscopy2,3, simultaneous spectroscopic estimation4,5,HPLC6-11,RP-HPLC12,13,Colourimetry, gas chromatography, difference spectroscopy14, HP-TLC15 were reported for the estimation of atenolol and amlodipine in individual formulations and...
combined dosage forms. An attempt was made to develop simple, accurate, precise, reproducible, economic and organic solvent free method for simultaneous estimation of both these drugs in combined dosage form.

Dual wavelength spectroscopy offers an efficient method for analyzing a component in presence of an interfering component. For elimination of interferences, dual analytical wavelengths were selected in a way to make the absorbance difference zero for one drug in order to analyze the other drug. The difference in absorbance at 230 nm (A1) and 242nm (A2) was zero for Amlodipine besylate, so A1 - A2 were used to analyze Atenolol whereas the difference in absorbance at 263 nm (A3) and 277 nm (A4) was zero for Atenolol and hence, A3 - A4 were used to analyze Amlodipine besylate [1, 2].

MATERIALS AND METHOD:

Apparatus

Instrument used was an UV-Visible double beam spectrophotometer, make: LABINDIA (model UVWIN-3000+) with a pair of 1 cm matched quartz cells. All weighing was done on Elico analytical balance (Model AU-220). Calibrated glassware’s were used throughout the work.

Reagents and chemicals

Pure drug samples of ATN and AMN were obtained as gift samples from Hetero Labs, Hyderabad. Methanol from Merk, water was used as solvent.

Marketed formulation

The marketed formulation studied was AMLOKIND tablet manufactured by Cipla pvt limited. Each tablet contains 50 mg Atenolol and 5 mg Amlodipine besylate.

Method development

UV spectrophotometric method for estimation Atenolol and Amlodipine besylate was carried by the dual wavelength spectroscopic method [3-5].

Determination of solubility

Atenolol and Amlodipine besylate solubility were tested in different organic and aqueous solvents. Both are soluble in methanol and sparingly soluble in water [6].

Standard solutions and calibration graphs for UV measurement

Standard stock solution of Atenolol and Amlodipine besylate were prepared in methanol(1000µg/ml) (1st stock). From this solution 1 ml was taken and diluted to 10 ml with water(100µg/ml)(2nd stock). Calibration standards at six levels were prepared by diluting 2nd stock standard solutions in the concentration range of 5-30 µg/ml and 1-6 µg/ml for Atenolol and Amlodipine besylate respectively. Samples in triplicates were made for each concentration. Calibration graph was plotted by taking concentration on x-axis and absorbance on y-axis [7, 8].

Dual wavelength method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration [9-12].

Study of overlain spectra and selection of wavelength

By appropriate dilutions from the working standard solutions of 100 µg/ml of ATN and 100 µg/ml of AMN, the solutions of ATN (5-30 µg/ml) and AMN (1-6 µg/ml) were prepared working standard solutions were scanned from 200 to 400 nm to select the wavelengths for estimation. The maximum absorbance at λmax for Atenolol and Amlodipine besylate was 273 nm and 243 nm respectively in methanol. From the overlain spectrum shown in figure the wavelengths selected for estimation of Atenolol were 230 nm and 242 nm, where Amlodipine besylate has no absorbance difference at that wavelengths and for Amlodipine besylate they were 263 nm and 277 nm, where Atenolol has no absorbance difference. Different concentrations of Atenolol and Amlodipine besylate were prepared, and then run in entire range from 200 to 400 nm. The drugs obey the Beer’s law in the concentration range of 1-60µg/ml, 1-60 µg/ml for Atenolol and Amlodipine besylate respectively [13, 14].

Assay of tablet formulation by dual wavelength Spectrophotometry

Twenty tablets of AMLOKIND were weighed and finely powdered and tablet powder equivalent to 10mg of both Atenolol and Amlodipine besylate is weighed and extracted with methanol in a 100 ml volumetric flask. The flask was sonicated for 15 min and volume was made up to the mark with methanol. 1ml was transferred into a 10ml volumetric flask and the volume was made up to the mark with water, and 1ml of above solution is added to 10ml volumetric flask and made up to the mark with water, finally the
solution is filtered by using syringe filter to obtain 10μg/ml of Atenolol and 1μg/ml of Amlodipine besylate. The absorbance of the solution was measured under UV spectrophotometer. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated [15, 16].

**Method validation**

Method validation was performed in terms of sensitivity, specificity, linearity, LOQ, LOD, precision, accuracy and robustness [17].

**Linearity**

The linearity of calibration curves in pure solution was checked over the concentration ranges of about range of 5-30 μg/ml and 1-6 μg/ml for Atenolol and Amlodipine besylate respectively. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained: Y=0.007x + 0.016; R²=0.998 for Atenolol at 273 nm and Y=0.048x - 0.012; R²=0.998 at 243 nm for Amlodipine besylate respectively. The mean and correlation coefficient of standard curves (N=3) were calculated.

**Precision**

The precision of the developed method was assessed in terms of repeatability and intermediate precision by analyzing replicate QC standard sample of 2μg/ml, 4μg/ml, 6μg/ml for Amlodipine besylate and 10μg/ml, 20μg/ml, 30μg/ml for Atenolol. The % R.S.D values of the results corresponding to the absorption values were expressed for intraday precision and on 3 days for inter-day precision. The intra and inter-day accuracy and precision were calculated and results were presented in the table. Precision of the analytical method was found to be reliable based on %R.S.D.

**Accuracy**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at spiking levels of 80%, 100% and 120%
of test concentration. Results of assay and recovery were presented in the table.

**Specificity**

For determining Specificity of the method, a tablet dosage form was analysed. These results demonstrate that there was no interference from other materials in the tablet formulation therefore, confirm the specificity of the method.

**Limit of detection (LOD), Limit of Quantification (LOQ)**

Limit of detection was found to be $0.237 \mu g/ml$ for Atenolol at 273 nm and $0.04 \mu g/ml$ for Amlodipine besylate at 243 nm respectively and Limit of Quantification was found to be $0.824 \mu g/ml$ for Atenolol at 273 nm and $0.1212 \mu g/ml$ for Amlodipine besylate at 243 nm respectively.

**RESULTS AND DISCUSSION**

The selected drugs Atenolol and Amlodipine besylate were estimated by using simultaneous estimation by dual wavelength spectroscopic method as per ICH guidelines. The method was validated for all validation parameters as per ICH guidelines. The linearity range for Atenolol and Amlodipine besylate was 5-30 $\mu g/ml$ and 1-6 $\mu g/ml$, with $R^2$ value of 0.9987 and 0.9983 respectively. The % RSD for intraday and interday precision and interday precision was <2%. The method has been validated in assay of tablet dosage forms. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits, with % recovery 98-102 (i.e., within acceptable range 98-102%). The assay results were found to be 99.13% and 98.01% (within acceptable limits).

**CONCLUSION**

An accurate and precise dual wavelength spectroscopic method has been developed and validated for the analysis of Atenolol and Amlodipine besylate in tablet dosage form. The percentage recovery and found concentration of active ingredient in pharmaceutical formulations showed that the amount of drug present is consistent with the label claim. Hence, this method is very useful simple and accurate for estimation of Atenolol and Amlodipine besylate in tablet dosage form.

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### Optimized method parameters for Dual Wavelength Spectrophotometry

**Method parameters Optimized parameters**

| Solvent       | Methanol          |
|---------------|-------------------|
| Scanning range| 200 nm to 450 nm  |
| Scan speed    | Medium            |
| Analytical wavelengths for the determination of ATN | 230nm and 242nm |
| Analytical wavelengths for the determination of AMN | 263nm and 277nm |

### Linearly of Atenolol and Amlodipine besylate by dual wavelength method

| S.No | Atenolol | Amlodipine besylate |
|------|----------|---------------------|
|      | Concentration | Absorbance at 230nm-242nm | Concentration | Absorbance at 263nm-277nm |
| 1    | 5         | 0.117               | 1             | 0.006               |
| 2    | 10        | 0.255               | 2             | 0.015               |
| 3    | 15        | 0.377               | 3             | 0.024               |
| 4    | 20        | 0.481               | 4             | 0.035               |
| 5    | 25        | 0.610               | 5             | 0.043               |
| 6    | 30        | 0.720               | 6             | 0.054               |

Regression Value $R^2$: 0.9987 0.9983
Regression equation: $Y = 0.023x + 0.008$ $Y = 0.009x - 0.004$

### Assay

| Formulation | Drug       | Label claim | Amount Found ± SD       | Assay   | % RSD  |
|-------------|------------|-------------|-------------------------|---------|--------|
| AMLOKIND    | Atenolol   | 50 mg       | 49.565±0.307591mg        | 99.13%  | 0.61   |
|             | Amlodipine besylate | 5mg | 4.855 ±0.10253mg         | 98.01%  | 2.08   |
Linearity curve of Atenolol:

\[ y = 0.023x + 0.008 \]
\[ R^2 = 0.9987 \]

Linearity curve of Amlodipine besylate:

\[ y = 0.009x - 0.004 \]
\[ R^2 = 0.9983 \]

Precision studies

| Sample | Intraday precision | Interday Precision |
|--------|--------------------|--------------------|
|        | Atenolol at 273 nm | Atenolol at 273 nm |
|        | Amlodipine besylate at 243 nm | Amlodipine besylate at 243 nm |
| Conc. (µg/ml) | %RSD | Conc. (µg/ml) | %RSD | Conc. (µg/ml) | %RSD | Conc. (µg/ml) | %RSD |
| 1 | 10 | 1.103 | 2 | 1.403 | 10 | 0.935 | 2 | 1.257 |
| 2 | 20 | 0.628 | 4 | 0.881 | 20 | 0.852 | 4 | 0.845 |
| 3 | 30 | 0.670 | 6 | 0.202 | 30 | 0.659 | 6 | 0.540 |
## Recovery studies

| S.No. | Name of the drug          | Amount of sample (µg/ml) | Recovery level | Amount of drug added (µg/ml) | Amount found (µg/ml) | % Recovery |
|-------|---------------------------|--------------------------|----------------|-----------------------------|---------------------|------------|
| 1     | Atenolol                  | 10                       | 80%            | 8                           | 17.913              | 99.05      |
|       |                            |                          | 100%           | 10                          | 20.152              | 101.07     |
|       |                            |                          | 120%           | 12                          | 22.240              | 101.09     |
| 2     | Amlodipine besylate       | 1                        | 80%            | 0.8                         | 1.821               | 101.16     |
|       |                            |                          | 100%           | 1.0                         | 1.984               | 99.02      |
|       |                            |                          | 120%           | 1.2                         | 2.156               | 98.01      |

## Summary of the present method

| S.No. | Parameter                        | Atenolol | Amlodipine besylate |
|-------|----------------------------------|----------|---------------------|
| 1     | \(\lambda_{\text{max}}\)       | 273 nm   | 243 nm              |
| 2     | Beer’s limit (µg/ml)             | 5-30     | 1-6                 |
| 3     | Regression equation at 230nm-242nm |          | at 263nm-277nm     |
|       | Slope                            | 0.023    | 0.009               |
|       | Intercept                        | 0.008    | 0.004               |
|       | Co-relation coefficient          | 0.9987   | 0.9983              |
| 4     | LOD (µg/ml)                      | 0.272    | 0.040               |
| 5     | LOQ (µg/ml)                      | 0.824    | 0.121               |
| 6     | Precision                        | <2%      | <2%                 |
| 7     | % Recovery                       | 99.05-101.09 | 98.01-101.16 |
| 8     | Wavelengths selection            | 263 nm & 277 nm | 230 nm & 242 nm |
| 9     | Sandal sensitivity               | -0.3043µg/ml | 0.5555µg/ml |

## LOD and LOQ

| S.NO | Parameter     | Atenolol | Amlodipine besylate |
|------|---------------|----------|---------------------|
| 1    | LOD (µg/ml)   | 0.272    | 0.040               |
| 2    | LOQ (µg/ml)   | 0.824    | 0.121               |
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