SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR AMLODIPINE BESYLATE AND NEVIRAPINE IN BULK AND TABLET DOSAGE FORM USING ABSORPTION RATIO METHOD

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ABSTRACT
A simple, economic, and accurate absorption ratio method was developed for the simultaneous estimation of Amlodipine besylate (AML) and Nevirapine (NEV) in bulk and tablet dosage form. 0.1M HCl was used as a diluent to dissolve AML and NEV. The absorptions were observed at 252 nm (isobestic point) and 295 nm (λmax of NEV), which were selected based on overlapping spectra of AML and NEV. The linearity range was found to be 3-7 µg/ml at 252 nm for AML ($r^2=0.9995\pm0.0005$) and 295 nm for NEV ($r^2=0.9985\pm0.0012$). The method was found to be simple, precise, accurate and rapid for the simultaneous determination of AML and NEV in bulk and tablet dosage form using absorption ratio method. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

Keywords: Amlodipine besylate, Nevirapine, Spectrophotometric analysis.

INTRODUCTION
Amlodipine besylate (AML) is chemically 2 - [(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid, 3-ethyl, 5-methyl ester besylate mono benzene sulphonate.$^{[1]}$ Amlodipine belongs to a class of cardiovascular drugs, which is used to treat hypertension and chronic stable angina. It is a long-acting dihydropyridine, L-type, calcium channel protein inhibitor and blocker. The mechanism of action of amlodipine is that
amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction by inhibiting the influx of calcium in smooth muscle cells.[2] Its molecular formula and molecular weight are C\textsubscript{26}H\textsubscript{31}ClN\textsubscript{2}O\textsubscript{8}S and 567.1 g/mol, respectively.[3] The structural formula of AML is in Figure 1. Literature survey reveals few analytical methods for the determination of Amlodipine alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. Spectrophotometry\textsuperscript{[4-10]}, HPLC\textsuperscript{[11-16]} and HPTLC\textsuperscript{[15-18]}.

Nevirapine (NEV) is chemically 11-cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido[3, 2-b: 2′, 3′-e] [1, 4] diazepin-6-one. Nevirapine is a white to off-white crystalline powder with the molecular weight of 266.30 g/mol and the molecular formula C\textsubscript{15}H\textsubscript{14}N\textsubscript{4}O\textsuperscript{[3,19]}.

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes. A survey of literature revealed that simultaneous analytical methods are not available for the drug combination AML and NEV. Hence it is proposed to develop new methods for the assay of AML and NEV in pharmaceutical dosage forms adapting UV visible spectrophotometry. The objective of the study was to develop a simple and accurate method for the determination of AML and NEV simultaneously using absorption ratio method by UV-spectrophotometry in pharmaceutical dosage form.

MATERIALS AND METHODS

Materials
AML and NEV obtained from pharmaceutical market were of analytical grade. A commercial sample AML and NEV tablets were procured from local market and used within their shelf-life period. Hydrochloric acid (S. D. Fine Chemical Limited, Mumbai) was of pharmaceutical or analytical grades.
Instrumentation
Quantitative estimation was performed on Labindia UV 3000+ double beam UV visible spectrophotometers (Maharashtra, India) with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UV-Win (Maharashtra, India) software was used along with quartz cuvette for the λmax and absorption prediction.

Trial and error method
To develop a suitable and robust absorption ratio method for the determination of AML and NEV, different diluents like methanol, 0.1M NaOH, etc., were tried based on the solubility and functional group present in the compound. Finally 0.1M HCl was selected as a diluent due to its reproducible results. Absorbance was measured at selected λmax (252 nm and 295 nm) based on the overlap spectrum of both drugs. The data were collected and analysed with software (Labindia UV-Win, Maharashtra, India) in a computer system.

Preparation of Standard Stock Solutions of AML and NEV
Stock solution of AML(1000 µg/mL) was prepared by dissolving 100 mg of drug in 100 ml of volumetric flask containing 50 mL of 0.1M HCl. The solution was sonicated for about 15 minutes and then made up to 100ml with 0.1M HCl. From the stock solution, 1mL was pipette out and transferred into the 10mL volumetric flask to get 100 µg/mL concentrations. From the second dilution, 0.5mL was pipette out and transferred into the 10mL volumetric flask to get 5 µg/mL concentrations. The same procedure was followed for NEV standard. The final solutions of both standard drugs solutions were scanned individually and spectra obtained were overlapped. From the overlap spectrum, two wavelengths were selected. Among the two, 295 nm is a λmax of NEV and 252 nm is an isobestic point (The wavelength at which both the drugs show same absorbance). Then the absorbance was measured at 252 nm and 295 nm and calculated the absorptivity from the formula \( \varepsilon = \frac{A}{cl} \) where A is absorbance; c is concentration; l is path length.

Preparation of standard mixture
From 100 µg/mL of AML and NEV standard second dilution, 0.5ml was pipette out individually and mixed in 10 ml volumetric flask then it was made up to the mark with 0.1M HCl. Absorbance was measured at selected λmax (252 nm and 295 nm).
Preparation of tablet Stock Solutions of AML and NEV

20 AML tablets were weighed and powdered. The amount of powder equivalent to 100 mg of AML was weighed and transferred into the 100 ml of volumetric flask containing 50 ml of 0.1M HCl. The solution was sonicated for about 20 minutes and then made up to volume with 0.1M HCl. The solution was filtered using 0.25 µ filter paper and vacuum-associated filtration unit. From the filtrate, 1ml was pipette out and transferred into the 10 ml volumetric flask then made up to the mark with 0.1M HCl to get 100 µg/ml concentration. 20 NEV tablets were weighed and powdered. The amount of powder equivalent to 100 mg of NEV was weighed. Then same procedures were followed to get the 100 µg/ml concentration.

Preparation of tablet mixture

From 100 µg/ml concentration of tablet second dilutions, 0.5ml was pipette out individually and transferred combine in to a 10 ml volumetric flask then it was made up to the mark with 0.1M HCl. The amount of drug present in pharmaceutical formulation was calculated using the following formula

\[ Cy = \frac{A_1}{ax_1} - C_x \]

\[ C_x = \frac{(Q_m - Q_y)}{(Q_x - Q_y)} \times \frac{A_1}{ax_1} \]

where, \( Cy \) is a concentration of NEV in mixture; \( C_x \) is a concentration of drug AML in mixture; \( Q_x \) (absorption ratio of AML) = \( ax_2/ax_1 \); \( Q_y \) (absorption ratio of NEV) = \( ay_2/ay_1 \); \( Q_m \) (absorption ratio of mixture) = \( A_2/A_1 \); \( A_1 \) is absorption at 252 nm in mixture; \( A_2 \) is absorption at 295 nm in mixture; \( ax_1 \) and \( ax_2 \) are absorptivities of AML at 252 nm and 295 nm respectively. \( ay_1 \) and \( ay_2 \) are absorptivities of NEV at 252 nm and 295 nm, respectively.

Validation

The described method has been validated for the assay of AML and NEV using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with absorbance. Six different concentrations of AML and NEV drug mixtures (3 to 7 µg/ml of each drug in the mixture) were employed i.e., 3, 4, 5, 6, 7 µg/ml. All solutions were scanned and absorbance measured at 252 nm and 295 nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of AML and NEV (5 µg/ml) on the same day. Ruggedness is determined by the same solutions were scanned using different Instruments (Labindia UV 3000+, Elico SL 210). The precision of each
method was ascertained separately from the absorbance obtained by actual determination of
five replicates of a fixed amount of drug (5µg/mL). The %RSD (percentage relative standard
deviation) was calculated for precision and ruggedness. The accuracy of the method was
shown by analyzing the model mixtures containing 80,100 and 120% of both AML and NEV
along with 5 µg/mL of placebo. After the measurement, the Amount found and individual
recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ)
were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation
/slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the
same method with different normality of HCl.

RESULTS

Table 1: Data for Precision and ruggedness of AML and NEV.

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<th>Precision</th>
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<tr>
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<td>AML</td>
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NEV – Nevirapine; AML – Amlodipine; SD- Standard deviation; %RSD- percentage relative standard
deviation

Table 2: Data for accuracy of AML and NEV

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% content
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SD- Standard deviation; %RSD- percentage relative standard deviation

**Fig. 1:** Structures of drugs (a) AML and (b) NEV

**Fig. 2:** Overlap spectrogram of standard drugs AML, NEV and their mixture.
An absorption ratio method procedure was proposed as a suitable method for the analysis of drugs AML and NEV in dosage forms. A typical overlap spectrogram of standard AML and NEV and their mixture is shown in Figure 2. The $\lambda_{\text{max}}$ was found to be 252 nm and 295 nm. The regression equation for the method at 252 nm for AML was found to be $y = 0.6897x - 0.00346$ (slope, intercept and correlation coefficient were found to be $0.6897 \pm 0.5565$, $-0.00346 \pm 0.0016$ and $0.9995 \pm 0.0005$) and linear over Beer’s range 3-7 µg/ml. The regression equation for the method at 295 nm for NEV was found to be $y = 0.01545x + 0.0045$ (slope, intercept and correlation coefficient were found to be $0.0154 \pm 0.0004$, $0.0045 \pm 0.0023$ and $0.9985 \pm 0.0012$) and linear over Beer’s range 3-7 µg/ml. The linearity graph of AML and
NEV mixtures is shown in Figure 3. A typical overlap spectrogram of different concentration of mixture of AML and NEV is shown in Figure 4.

The percentage of content of NEV and AML in tablet dosage form was 104.72±0.8972% and 95.27±1.6916%, respectively. The precision and ruggedness were determined using the % RSD of the absorbance for five replicate preparations of the drug. The %RSD of precision and ruggedness of NEV were found to be 1.18 and 1.67 respectively; for AML were 0.88 and 1.48 respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 1. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 80%, 100% and 120% of standard solution of drug AML and drug NEV and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 102.05±1.39, 96.86±1.14 and 92.13±0.21%w/w for 80%, 100% and 120% respectively for AML. The mean percentage recoveries were found to be 101.64±0.809, 103.81±0.94 and 91.56±1.074%w/w for 80%, 100% and 120% respectively for NEV. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. Accuracy data are presented in Table 2. LOD for NEV and AML was found to be 0.505 µg and 0.00796µg, respectively. LOQ for NEV and AML was found to be 1.530µg and 0.0242 µg, respectively. Robustness was performed by following the same method with 0.05M, 0.1M and 0.15M HCl. Percentage content of NEV and AML in 0.05M HCl diluents were 83.093±1.277 and 83.57±0.647 respectively; for 0.15M HCl were 90.14±0.504 and 90.69±1.086 respectively. The results of robustness shown that in 0.05M and 0.15M HCl, percentage content was reduced between 83 to 91%.

**DISCUSSION**

The developed method can be used for routine analysis because the linearity found in NEV and AML is nearby to 1 that is 0.998 and 0.999 which shows the good regression for linearity. Maximum recovery is obtained by this developed method and the mean percentage recoveries for each component are nearby 100%. So, method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. The spectrophotometric assay methods employed in our study indicated less interference from excipients used in formulation by the percent recoveries values. Most of the existing methods\[4-6, 20-22\] consumed expensive reagents for individual drug analysis. But the method we developed involves chemicals like Hydrochloric acid, and
distilled water, which are very simple, economical and also easily available. And also our proposed method requires less time for the determination of AML and NEV simultaneously compared to other methods and even these other methods required reagents which is costly and time taking for the reaction.

CONCLUSION
The presented method was found to be precise, sensitive and accurate. This method has simple sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of AML and NEV in pharmaceuticals.

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REFERENCES


