DEVELOPMENT AND VALIDATION OF A UV SPECTRPHOTOMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF NIFEDIPINE AND ATENOLOL IN COMBINED DOSAGE FORM

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INTRODUCTION

Nifedipine and Atenolol are available in tablet in combined dosage form in ratio 1: 5 ratio. Nifedipine is 1,4-dihydropyridine derivative used as Ca++ channel blocker, antianginal, and coronary vasodilator. Atenolol is β-adrenoceptor antagonist used as hypertensive. Both the drugs are acting on cardiovascular system. Both the drug simultaneously analysed by multicomponent analysis method using simultaneous equation method.

Both the drugs show the absorbance in UV region in the range 200-400 nm. Nifedipine and Atenolol have \( \lambda_{max} \) 341.2 nm and 273.8 nm respectively. Some of the paper shows that the compound was identified by taking the IR spectra. The method is applied for laboratory mixture and marketed tablet. The developed method was validated as per ICH guidelines using the parameter such as accuracy, linearity and range, ruggedness, limit of detection & quantification, robustness, and precisión. Precision was analysed by taking Reading interday and Intraday. Ruggedness was analysed by different analyst and robustness by changing the solvent composition.

KEY WORDS: Simultaneous Equation Method, Validation, Nifedipine, Atenolol.

MATERIALS & METHOD

Drugs
Nifedipine and Atenolol suplaid bey Cipla & Loopin Pharmaceuticals Pvt. Ltd. Mumbai.

Marketed preparation
The brand name of marketed preparation is Nilol, Presolar, Betatrop manufactured by Cipla Pharmaceuticals Pvt. Ltd. Baddi, & Sun pharma Pvt. Ltd containing Nifedipine 20mg and 50 mg Atenolol.

Reagents and chemicals
All reagents and chemicals purchased from Loba chemicals.

1. Methanol (AR grade)
2. Water (distilled)

Instruments
a) Spectrophotometer Double beam UV–visible spectrophotometer{(UV1800 PC (Japan)- Shimadzu)

b) Analytical balance Shimadzu aw 220

c) IR PerkinElmer Spectrum 65 FT-IR spectrometer

EXPERIMENTAL

Identification of drugs
Identificacin of Nifedipine and Atenolol is carried out by taking M.P. (NIF 172°C & ATN 147°C) and IR Spetra.

ESTIMATION OF NIFEDIPINE AND ATENOLOL BY UV-VISIBLE SPECTROSCOPY

Simultaneous equation method

Standard solutions: Nifedipine & Atenolol stock solution
An accurately weighed quantity of NIF & ATN equivalent to 100 mg was dissolved separately in 100 ml methanol in 100 ml volumetric flask and volume was made up to the mark. (1000 \( \mu \)g/ml).

Study of spectra and selection of wavelength
The solution of conc. 10 \( \mu \)g/mL of each drug was prepared and scanned in the range of 400-200 nm. The wavelengths selected for estimation of drugs were 341.2 nm as \( \lambda_{max} \) of Nifedipine and 273.8 nm as \( \lambda_{max} \) of Atenolol.

Study of Beer-Lamberts law
It was found that Std. laboratory mixture obeys Beer-Lamberts law in series of concentration between 2-10 \( \mu \)g/ml for NIF and 5-25 \( \mu \)g/ml For ATN.

Analysis of laboratory mixture by proposed method

Accurately weighed quantities of NIF 25 mg and ATN 25 mg were taken in 25 ml volumetric flask and dissolved in methanol by vigorous shaking. The volume was made up to the mark with water to get final concentration of about 20 \( \mu \)g/ml NIF and 50 \( \mu \)g/ml ATN. The absorbances of the resulting solutions were measured at 341.2 nm and 273.8 nm in 1 cm cell against blank. Amount of each drug was determined using simultaneous equation as followings

\[
C_X = \frac{A_x}{ax_1 - ax_2} = \frac{ay_1}{ay_2} --------- (1)
\]

\[
C_X = \frac{A_x}{Ax_1 - Ax_2} = \frac{Ay_1}{Ay_2} --------- (2)
\]

Where, \( C_X \) & \( C_Y \) = Concentration of ATN in \( \mu \)g/mL & NIF in. \( \mu \)g/mL respectively.

ax1 & ax2= Absorptivy of ATE at 273.8 nm & NIF at 341.2 nm respectively.

ay1 & ay2= Absorptivy value of ATN at 273.8 nm & NIF at 341.2 nm respectively.

A1 & A2= Absorbance of laboratory mixture at 273.8 nm & 341.2 nm.

\%

Estimation \[ = \frac{C_x \times 100}{W} \] ——— (3)

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ABSTRACT
Simultaneous estimation and validation of developed method of Nifedipine (NIF) and Atenolol (ATN) in combined dosage form as well as in laboratory mixture is studied under this paper. Nifedipine and Atenolol are used in combined dosage form for Cardiovascular System Diseases. The compound was identified by taking the IR spectra. The method is applied for laboratory mixture and marketed tablet. The developed method was validated as per ICH guidelines using the parameter such as accuracy, linearity and range, ruggedness, limit of detection & quantification, robustness, and precisión. Precision was analysed by taking Reading interday and Intraday. Ruggedness was analysed by different analyst and robustness by changing the solvent composition.

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Where,

\[ C = Cx \text{ or } Cy = \text{Conc. of ATE or NIF in. } \mu\text{g/mL}, \ D = \text{Dilution factor} \]

\[ W = \text{Weight of drug either NIF or ATN in laboratory mixture} \]

Results of estimation of drugs in laboratory mixture are shown in Table No. 1.

VALIDATION OF PROPOSED METHOD²-¹² 
Validation of analytical methods as per ICH and USP guidelines

The analytical methods are classified into-
- Identification tests.
- Quantitative tests for impurity content-Limit tests for the control of impurities.
- Quantitative tests of the active moiety in bulk drug substances or drug products other selected component(s) in the drug product

Important Terms and Properties to be considered for Measurements².¹³

RECOVERY STUDIES¹⁰

Recovery study was done by standard addition method.

Preparation of standard solution

An accurately weighed 50 mg of pure NIF and ATN were dissolved in 50 ml methanol separately and volume was made up to the mark to obtain concentration of 1 mg/mL.

Preparation of sample solution

An accurately weighed quantity of preanalysed laboratory mixture powder equivalent to 20 mg of NIF was taken in 100 ml volumetric flask; to it standard solutions of NIF and ATN were added in different proportions. Then volume was adjusted up to the mark with water. Solution was filtered through Whatman filter paper No. 42. The aliquot portions of the filtrate were further diluted to get final concentration. The absorbances of sam solutions were measured at 341.2 nm and 273.8 nm in 1 cm cell against blank. The content of drug was calculated using same formula as in marketed formulation. The % recovery was then calculated by using formula No.4

\[ \% \text{ Recovery} = \left[ \frac{A}{B + C} \right] \times 100 \]

Where,

\[ A = \text{Total amount of drug estimated}, \]
\[ B = \text{Amount of drug found on preanalysed basis}, \]
\[ C = \text{Amount of pure drug added} \]

The results of recovery study are shown in Table No.2

Validation paramenters

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by the method to the true value. It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded in Table No.2.

Precision

Precision of an analytical method is the degree of agreement among individual results when the method is applied repeatedly to multiple readings of a homogeneous sample. It is expressed as S.D. or R.S.D. of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

Intraday

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The % label claim was calculated using same formula as for marketed formulation analysis. Results and statistical data are shown in Table No. 3.

Interday

Same procedure was performed as under marketed formulation analysis and absorbance of same samples were recorded on different days. The % label claim was calculated using same formula as for marketed analysis. Results and statistical data are shown in Table No.4.

Ruggedness

The studies of ruggedness were carried out under following conditions

Different Analyst

The sample solutions were prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation. Results and statistical data are shown in Table No.5

Limit of Detection & Quantification

Limit of Quantification

It is the lowest concentration of analyte in the sample that can be detected but not necessarily quantified under the stated experimental conditions. It is expressed as signal to noise ratio of 2:1 or 3:1. Limit of detection can be calculated using following equation as per ICH guidelines.

\[ \text{LOD} = 3.3 \times \text{N/S} \]

Where, N is the standard deviation of the measurements of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy condition. It is expressed as signal to noise ratio of 10:1.

\[ \text{LOQ} = 10 \times \text{N/S} \]

Where, N is the standard deviation of the measurements of the drug and S is the slope.

Robustness

Robustness of the method was study by deliberated variation of the analytical parameter such as solvent composition i.e (75; 25) solvent composition of METHONOL: WATER.

Linearity and range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim were taken and dilutions were done appropriately to obtain a concentration in the range of 80-120% of the test concentration and absorbance were recorded at 341 nm and 273.8 nm. NIF and ATN were found to be linear in 80% - 120% of the test concentration. The plot of linearity and range is shown in Fig. no. 2.

RESULTS AND DISCUSSION

NIF & ATE has estimated at 341.2nm & 273.8nm in the solution of methanol. In this method drugs obey Beer’s law in the concentration range of 2-10 μg/ml for NIF & 5 -25 μg/μl. The method was validated as per the ICH and USP guidelines. The results of recovery study were found to be within the prescribed limit of 98 - 102 %, proving the accuracy and showing that the method is free from interference from excipients. The results are shown in Table No.2. For precision & ruggedness the proposed method was repeated under different conditions like different time, on different day and by different analyst. The values of SD or RSD are within the prescribed limit of 2 %, showing high precision of the method, as shown in Table No. 3&4. During the linearity study it was observed that absorbance values of NIF and ATE in the marketed
formulation were linear in the range of 80 % to 120 % of the test concentration with R2 close to one for this method of analysis. From the study of validation parameters namely accuracy, precision (SD and RSD), ruggedness (interday, intraday and different analyst), specificity, linearity and range, it was observed that the method is specific, accurate, precise, reproducible and rugged.

CONCLUSION

The proposed method is simple for simultaneous analysis of nifedipine and atenolol in combined formulation. Two sampling wavelength 341.2nm and 273.8nm were used for analysis of NIF and ATE. The proposed method of analysis was validated by analyzing the laboratory prepared samples. The results were satisfactory. The mean recovery was 99.5% for NIF and 101.66% for ATE respectively. The present method is quick and cost effective as compared to chromatographic techniques. Therefore, it can be concluded that the proposed method provides an alternative procedure for the quality control of NIF and ATE in pharmaceutical formulations.

REFERENCES


**TABLE NO.1: RESULT OF ESTIMATION OF NIF AND ATN IN LABORATORY MIXTURE**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Wt. of pure drug (g)/25ml</th>
<th>Absorbance at</th>
<th>% Estimation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIF</td>
<td>ATN</td>
<td>34.2 nm</td>
</tr>
<tr>
<td>1</td>
<td>0.0253</td>
<td>0.0260</td>
<td>0.152</td>
</tr>
<tr>
<td>2</td>
<td>0.0258</td>
<td>0.0255</td>
<td>0.158</td>
</tr>
<tr>
<td>3</td>
<td>0.0260</td>
<td>0.0253</td>
<td>0.155</td>
</tr>
<tr>
<td>Mean</td>
<td>0.0257</td>
<td>0.0257</td>
<td>0.155</td>
</tr>
<tr>
<td>± S.D.</td>
<td>0.00785</td>
<td>0.00759</td>
<td>0.0075</td>
</tr>
<tr>
<td>C. V.</td>
<td>0.9797</td>
<td>0.9989</td>
<td>0.9989</td>
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</table>

*n = 3

**TABLE NO.2: RESULTS OF RECOVERY STUDIES OF NIF AND ATN**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Amount present in formulation</th>
<th>Amount added µg/ml</th>
<th>Total drug µg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIF</td>
<td>ATN</td>
<td>NIF</td>
<td>ATN</td>
<td>341.2 nm</td>
</tr>
<tr>
<td>1</td>
<td>80%</td>
<td>20</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>100%</td>
<td>20</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>80%</td>
<td>20</td>
<td>50</td>
<td>24</td>
</tr>
</tbody>
</table>

Mean | 99.5 | 101.66 | ± S.D. | 1.285 | 0.984 |
| C. V. | 1.290 | 0.968 | *n = 3 |
### TABLE NO. 3: RESULT AND STATISTICAL DATA OF INTRADAY STUDY

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>CONC (µg/ml)</th>
<th>Absorbance at</th>
<th>% Label claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIF</td>
<td>ATE</td>
<td>NIF</td>
</tr>
<tr>
<td></td>
<td>341.2 nm</td>
<td>273.8 nm</td>
<td>NIF</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>50</td>
<td>0.321</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>50</td>
<td>0.318</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>50</td>
<td>0.325</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>0.5859</td>
</tr>
<tr>
<td>± S.D.</td>
<td></td>
<td></td>
<td>0.0293</td>
</tr>
<tr>
<td>C.V</td>
<td></td>
<td></td>
<td>0.0293</td>
</tr>
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</table>

n*=3

### TABLE NO. 4: RESULT AND STATISTICAL DATA OF INTERDAY STUDY

<table>
<thead>
<tr>
<th>Day</th>
<th>CONC (µg/ml)</th>
<th>Absorbance at</th>
<th>% Label claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIF</td>
<td>ATE</td>
<td>NIF</td>
</tr>
<tr>
<td></td>
<td>341.2 nm</td>
<td>273.8 nm</td>
<td>NIF</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>50</td>
<td>0.325</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>50</td>
<td>0.321</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>50</td>
<td>0.316</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>19.55</td>
</tr>
<tr>
<td>± S.D.</td>
<td></td>
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<tr>
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n*=3

### TABLE NO. 5: RESULT OF DIFFERENT ANALYST STUDY

<table>
<thead>
<tr>
<th>Analyst</th>
<th>CONC (µg/ml)</th>
<th>Absorbance at</th>
<th>% Label claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIF</td>
<td>ATE</td>
<td>NIF</td>
</tr>
<tr>
<td></td>
<td>341.2 nm</td>
<td>273.8 nm</td>
<td>NIF</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>50</td>
<td>0.302</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>50</td>
<td>0.298</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>50</td>
<td>0.305</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>20.34</td>
</tr>
<tr>
<td>± S.D.</td>
<td></td>
<td></td>
<td>0.7024</td>
</tr>
<tr>
<td>C.V</td>
<td></td>
<td></td>
<td>0.3632</td>
</tr>
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n*=3

### TABLE NO. 6: RESULT IF DETERMINATION OF LOD & LOQ

<table>
<thead>
<tr>
<th>DRUG NAME</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIFEDIPINE</td>
<td>0.273 µg/ml</td>
<td>0.824 µg/ml</td>
</tr>
<tr>
<td>ATENOLOL</td>
<td>0.159 µg/ml</td>
<td>0.483 µg/ml</td>
</tr>
</tbody>
</table>

### TABLE NO. 7: RESULT OF DIFFERENT SOLVENT RATIO STUDY

<table>
<thead>
<tr>
<th>RATIO</th>
<th>CONC (µg/ml)</th>
<th>Absorbance at</th>
<th>% Label claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIF</td>
<td>ATE</td>
<td>NIF</td>
</tr>
<tr>
<td></td>
<td>341.2 nm</td>
<td>273.8 nm</td>
<td>NIF</td>
</tr>
<tr>
<td>75:25</td>
<td>20</td>
<td>50</td>
<td>0.235</td>
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<tr>
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<td>20</td>
<td>50</td>
<td>0.240</td>
</tr>
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<td>90:10</td>
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<tr>
<td>C.V</td>
<td></td>
<td></td>
<td>0.0418</td>
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</table>

*n=3
FIGURE NO. 1: OVERLAIN SPECTRA OF NIFEDEPINE AND ATENOLOL

FIGURE NO. 2: THE PLOT OF LINEARITY AND RANGE FOR NIFEDIPIINE AND ATENOLOL

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