SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND NIACIN IN TABLET DOSAGE FORM

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ABSTRACT

Two UV spectrophotometric methods were developed and validated for quantitative determination of atorvastatin (ATR) and niacin (NIA) in tablet dosage form. Method I is based on the simultaneous equation, and method II is based on the absorbance ratio method. The absorption maxima were found to be at 246 nm and 262 nm in methanol for the atorvastatin and niacin respectively. Beer's law is obeyed in the concentration range of 5-25 μg/ml with correlation coefficient within range of 0.996 - 0.999 for both the drugs. The accuracy of the methods was assessed by recovery studies and found to be 99.77 ± 0.34 and 99.97 ± 0.17 by the simultaneous equation method, where as 99.78 ± 0.49 and 99.98 ± 0.39 by the absorbance ratio method for atorvastatin and niacin respectively.

KEYWORDS: Simultaneous equation method, absorption ratio method, atorvastatin, niacin

INTRODUCTION

Atorvastatin (ATR) is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent. Chemically ATR is \([R^*-(R^*, R^*]-2(4-fluorophenyl) -B,B dihydroxy -5-(1-methylethyl)-3-phenyl-4 -[(phenyl amino) carbonyl] -1H- pyrrole-1- heptanoic acid\) (Figure 1). ATR is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3- methyl gultaryl -coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. ATR is official in Indian Pharmacopoeia. Literature survey revealed several methods based on different techniques like spectrometric method, HPLC, HPTLC, LCMS/MS for its determination in pharmaceuticals and its metabolites in serum.

Niacin (NIA) chemically designated as pyridine 3 carboxylic acid (Figure 2) which reduce triglyceride levels, is also effective for increasing serum HDL levels. A number of analytical methods have been developed for its determination in pharmaceutical formulations or in biofluids either alone or in combination with other drugs. These include determination of niacin by HPLC, flow injection TLC, HPTLC, capillary electrophoretic and tandem mass spectrometry.

A combination of ATR and NIA is commercially available in tablet dosage form. Literature survey reveals that RP-HPLC method is available for the simultaneous determination of these drugs in combination which is not UV spectrophotometric method is available. So we communicate here rapid and cost effective quality control tool for their routine quantitative analysis in pure and combined dosage forms by UV-spectrophotometry.

MATERIAL AND METHODS

Instrumentation

A UV–Visible double beam spectrophotometer of Jasco Model: V-630, with a fixed bandwidth 2 nm and a pair of 1cm matched quartz cell were used for all spectrophotometric measurements.

Selection of common solvent

After assessing the solubility of both drugs in different solvents methanol was selected as a common solvent for developing spectral characteristics.

Preparation of standard solution

The standard stock solutions of ATR and NIA were prepared by dissolving 10 mg of each drug in 40 ml of methanol and final volume was adjusted with methanol to get a solution containing 100 μg/ml of each drug.

For the selection of analytical wavelength, standard solution of ATR (20 μg/ml) and NIA (20 μg/ml) were prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the entire UV range to determine λmax of both the drugs. The λmax of ATR and NIA were found to be 246 nm and 262 nm, respectively and 258 nm as λmax of common absorbance (isosbestic wavelength). A series of standard solutions were prepared having concentration in the range of 5-25μg/ml for both ATR and NIA. The absorbance of resulting solutions was measured at 246 nm, 258 and 262 nm, and calibration curves were plotted. Both the drugs obeyed linearity in the concentration range under study. The standard calibration curve of ATR and NIA are shown in Figure 3 and 4.

Method I: Simultaneous equation method

This method of analysis was based on the absorption of ATR and NIA at the wavelength maximum of each other. Two wavelengths selected for the development of simultaneous equations were 246 nm and 262 nm which were λmax of ATR and NIA respectively. The absorbances of ATR and NIA were measured at the selected wavelengths. The absorbitivity values E (1%, 1cm) were determined for both the drugs at the selected wavelengths. These values were mean of five estimations. Overlain spectra of ATR and NIA are shown in Figure 5 respectively. The concentration of both drugs in mixture can be calculated by using following equations-

\[ C_2 = \frac{A_2ax_2 - A_2ay_2}{ay_2 - ax_2} \quad \text{Eq (1)} \]

\[ C_3 = \frac{A_3ax_3 - A_3ay_3}{ay_3 - ax_3} \quad \text{Eq (2)} \]
Where, $A_1$ and $A_2$ are absorbances of mixture at 246 and 262 nm respectively. $\alpha_1$ and $\alpha_2$ are absorptivities of ATR at 246 and 262 nm respectively. $\alpha_1$ and $\alpha_2$ are the absorptivities of NIA at 246 and 262 nm respectively. Cx and Cy are the concentrations of ATR and NIA respectively.

Analysis of marketed formulation
Twenty tablets were accurately weighed; average weight was determined and finely powdered. An accurately weighed quantity of tablet powder equivalent to 20 mg of ATR was transferred to 100 ml volumetric flask and dissolved by sonication with sufficient quantity of methanol and volume was made to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. 1 ml portion of the filtrate was taken in 10 ml volumetric flask and final volume was adjusted with methanol. The above mixture was analyzed at 246, 258 and 262 nm wavelengths and values of the absorbance were substituted in respective equations (Eqn. 1 to 4) to obtain the content of ATR and NIA respectively. The result of analysis is mentioned in Table 1.

Method II: Absorption ratio method
In quantitative assay of two components by absorption ratio method (Q-analysis), absorbances were measured at the isobestic wavelength (258 nm) and maximum absorption of one of the two components. From overlay spectra of ATR and NIA shown in Figure 5, absorbances were measured at the selected wavelengths of 258 nm (isobestic wavelength) and 246 nm (wavelength of maximum absorption of ATR). From the following sets of equations, the concentration of each component in sample solution can be calculated. $C_x = (Q_1 - Q_2) \times A_1 / (Q_1 - Q_2) \times A_1$ ...

RESULTS AND DISCUSSION
The developed methods for simultaneous estimation of ATR and NIA were validated as per ICH guidelines.

Accuracy
To check the accuracy of the developed methods and to study the interference of formulation additives, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). The results of recovery studies expressed as percent recovery were satisfactory and are presented in Table 2.

Intermediate precision (inter-day and intra-day precision)
The reproducibility of the proposed methods was determined by analyzing tablets at different time intervals on same day (Intra-day assay precision) and on three different days (Inter-day assay precision). The results are presented in Table 3.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ were separately determined based on the standard deviation of y-intercept of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were determined by visual methods as suggested in ICH guidelines, which were found to be as per given in Table 3.

CONCLUSION
The proposed UV spectrophotometric methods were tested and validated for various parameters according to ICH guidelines and can be used for routine analysis of atorvastatin and niacin in pharmaceutical dosage forms as a quality control tool.

REFERENCES
1. Indian Pharmacopoeia, Published by the Government of India, Ministry of Health and Family Welfare. The Indian Pharmacopoeia Commission: New Delhi; 2010.
### Table 1: RESULT OF TABLET ANALYSIS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg/tablet)</th>
<th>Amount of drug estimated (mg/tablet)</th>
<th>% Label claim estimated ± S.D.*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method I</td>
<td>Method II</td>
</tr>
<tr>
<td>ATR</td>
<td>10</td>
<td>10.01</td>
<td>9.96</td>
</tr>
<tr>
<td>NIA</td>
<td>375</td>
<td>374.77</td>
<td>374.62</td>
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</tbody>
</table>

* Mean of six determinations

### Table 2: RESULT OF RECOVERY STUDIES

<table>
<thead>
<tr>
<th>Method</th>
<th>Level of recovery (%)</th>
<th>% Recovery ± S.D. #</th>
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<tbody>
<tr>
<td>I</td>
<td>80</td>
<td>99.75 ± 0.0456</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.01 ± 0.0345</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>99.89 ± 0.2321</td>
</tr>
<tr>
<td>II</td>
<td>80</td>
<td>99.55 ± 0.2341</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.99 ± 0.1679</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>100.01 ± 0.2451</td>
</tr>
</tbody>
</table>

# Mean of three determinations. SD: Standard deviation.

### Table 3: OPTICAL CHARACTERISTICS AND VALIDATION PARAMETERS

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>ATR</th>
<th>NIA</th>
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<tr>
<td>λ_max (nm)</td>
<td>246</td>
<td>258</td>
</tr>
<tr>
<td>Concentration range (µg/ml)</td>
<td>5-25</td>
<td>5-25</td>
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<tr>
<td>Regression equation</td>
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<tr>
<td>(y = mx + c)</td>
<td>0.0533</td>
<td>0.0445</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.042</td>
<td>0.023</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0475</td>
<td>0.0097</td>
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<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9964</td>
<td>0.9988</td>
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<tr>
<td>LOD (µg/ml)</td>
<td>0.2823</td>
<td>0.201</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.8554</td>
<td>0.609</td>
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<tr>
<td>Precision (COV*)</td>
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</tr>
<tr>
<td>Interday (n = 3)</td>
<td>0.1507</td>
<td>0.0572</td>
</tr>
<tr>
<td>Intraday (n = 3)</td>
<td>0.1705</td>
<td>0.5816</td>
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</table>

* COV: Coefficient of variance

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Fig 1. Chemical structure of ATR

Fig 2. Chemical structure of NIA
Fig. 3: Standard calibration curve of ATR

Fig. 4: Standard calibration curve of NIA

Fig. 5: Overlaid spectra of ATR and NIA

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