SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF ATENOLOL AND HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM BY SIMULTANEOUS EQUATION, ABSORPTION RATIO AND FIRST ORDER DERIVATIVE SPECTROSCOPY METHODS

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ABSTRACT
Three sensitive, precise, accurate and simple UV spectrophotometric methods have been developed for simultaneous estimation of Atenolol (ATN) and Hydrochlorothiazide (HCT) in tablet dosage forms. Method A involved simultaneous equation method. The two wavelengths 225 nm ($\lambda_{max}$ of ATN) and 271.5 nm ($\lambda_{max}$ of HCT) were selected for the formation of Simultaneous equations. Whereas method B involved formation of Q-absorbance equation at isobestic point (232nm). Method C is First order Derivative Spectroscopy method in which derivative amplitudes were measured at selected wavelengths (243 nm for ATN and 225 nm for HCT). Linearity was observed in the concentration range of 2.0-20, 2.0-32, 2.0-20 µg/ml for ATN and 0.5-5.0, 0.5-8.0, 0.5-5.0 µg/ml for HCT by method A, B and C respectively. The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

Keywords: Atenolol, Hydrochlorothiazide, simultaneous estimation, validation.

INTRODUCTION
Atenolol1 (Fig 1) is an antihypertensive, antianginal, and antiarrhythmic drug. Chemically, it is 4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl acetamide. The Indian Pharmacopoeia describes non-aqueous titration method for the assay of atenolol. UV Spectroscopy2-3, reversed phase HPLC4-6 and HPTLC7 are few of the methods reported in literature for the analysis of atenolol with other drugs.

Figure 1: Atenolol

Hydrochlorothiazide (Fig 2) 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine- 7-Sulphonamide 1, 1-dioxide, is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride and water.

UV Spectroscopy8-11, Ratio Spectra Derivative Spectrophotometry12, HPLC12-14 and HPTLC14 methods are reported for simultaneous estimation of Hydrochlorothiazide in combined dosage form.

No method has been reported for the estimation of Atenolol (ATN) and Hydrochlorothiazide (HCT) in combined dosage form. Present work emphasizes on the quantitative estimation of Atenolol and Hydrochlorothiazide in their combined dosage form by UV Spectroscopic methods.

MATERIALS AND METHODS
Instrumentation
A Double beam UV-Visible spectrophotometer (Jasco V 530) with 10 mm matched quartz cells was used. All weighing were done on single pan balance (Shimadzu).

Reagents and chemicals
ATN and HCT reference standards were kindly provided by Emcure Pharmaceuticals Pvt. Ltd, Pune. Analytical grade methanol was purchased from Merck Specialities Private Ltd., Mumbai. All the reagents were of analytical grade.

Tablets were purchased from local market each containing 50 mg of ATN and 12.5 mg of HCT. ATN and
HCT are available in the ratio of 4:1 respectively in the formulation and were used in same ratio for preparation of calibration curves.

**Determination of absorptivity values**

Standard stock solutions of ATN (100 µg/ml) and HCT (100 µg/ml) were prepared in methanol. For the selection of analytical wavelength solutions of ATN (10 µg/ml), HCT (10 µg/ml) were prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the spectrum mode from 200 to 400 nm. From the overlain spectra of these drugs [Figure 3], wavelengths 225 nm ($\lambda_{\text{max}}$ of ATN), 271.5 nm ($\lambda_{\text{max}}$ of HCT) and 232 nm (isosbestic point) were selected for analysis.

**Determination of Linearity**

Standard stock solutions of pure drugs containing 100 mg of ATN and 25 mg of HCT/100 ml were prepared in methanol. The working standard solutions were obtained by dilution of the stock solution in methanol. Series of solutions with conc. 2-32 µg mL$^{-1}$ and 0.5 – 8.0 µg mL$^{-1}$ of ATN and HCT respectively were used to determine linearity by three methods. Solutions were scanned and Beers Lamberts law limit was determined.

**Formulation analysis**

For estimating ATN and HCT in tablet formulation, twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 100 mg of ATN and 25 mg of HCT was transferred to 100 ml volumetric flask. 50 ml of methanol was added and sonicated for 15 min and volume was made up to the mark with methanol. The solution was then filtered through Whatmann filter paper No. 41. Appropriate aliquots were taken for further analysis.

**Method A: Simultaneous Equation Method**

Sample stock was appropriately diluted with methanol to obtain final concentration of 6 µg/ml for ATN and 1.5 µg/ml for HCT. Absorbance of diluted sample solution was measured at selected wavelengths. The concentration of drugs was determined by using the Equations 1 and 2.

Using absorptivity values following Eqns. were developed for determining concentration of ATN and HCT in tablet sample solution.

$A_1 = 61.07\ C_{\text{ATN}} + 117.2\ C_{\text{HCT}} (1)$

$A_2 = 8.249\ C_{\text{ATN}} + 66.50\ C_{\text{HCT}} (2)$

where $A_1, A_2$ are absorbances of the tablet sample solution at 225 and 271.5 nm, respectively.

$C_{\text{ATN}}$ is the concentration of ATN in gms/lit

$C_{\text{HCT}}$ is the concentration of the HCT gms/lit

**Method B: Absorption Ratio Method (Q Method)**

For Q method, 232 nm (isosbestic point) and 271.5 nm ($\lambda_{\text{max}}$ of HCT) were selected as wavelengths of...
measurements. Concentrations of ATN and HCT were determined using following equations.

\[ C_{ATN} = \frac{Qm - Q_{HCT}}{A_{1}} \div \frac{Q_{ATN} - Q_{HCT}}{a_{ATN}} \]

\[ C_{HCT} = \frac{Qm - Q_{ATN}}{A_{1}} \div \frac{Q_{HCT} - Q_{ATN}}{a_{HCT}} \]

Where

\[ Qm = \frac{A_{2}}{A_{1}} \]

\[ Q_{ATN} = \frac{a_{ATN2}}{a_{ATN1}} \]

\[ Q_{HCT} = \frac{a_{HCT2}}{a_{HCT1}} \]

A₂ = Absorbance of Mixture at 271.5nm

A₁ = Absorbance of Mixture at 232 nm

\[ a_{ATN1} = \text{absorptivity of ATN at 232 nm (40.21)} \]

\[ a_{HCT1} = \text{absorptivity of HCT at 232 nm (41.66)} \]

\[ a_{ATN2} = \text{absorptivity of ATN at 271.5 nm (8.249)} \]

\[ a_{HCT2} = \text{absorptivity of HCT at 271.5 nm (66.50)} \]

**Method C First Order Derivative Spectroscopy**

Standard solutions of both drugs (1-20 µg/ml) were scanned separately in the range of 200-400 nm. These spectrums were converted to first order derivative spectra (Figure 6) by using derivative mode with 21 data point. For this method, 225 nm and 243 nm were selected as wavelengths of measurements for HCT and ATN respectively. There was proportionate increase in amplitude at 225 and 243 nm for HCT and ATN respectively.

![Figure 6: It shows Overlay of Derivative Spectra of ATN and HCT](image)

**Table 1: It shows Linearity data for ATN and HCT for all three methods**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ATN</th>
<th>HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (µg mL⁻¹)</td>
<td>2.0-20</td>
<td>2.0-20</td>
</tr>
<tr>
<td>Correlation Coefficient (R²)</td>
<td>0.997</td>
<td>0.999</td>
</tr>
</tbody>
</table>

**Table 2: It shows Assay results for the determination of ATN and HCT in its tablets by the proposed methods**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (µg mL⁻¹)</th>
<th>Amount Found (µg mL⁻¹)</th>
<th>% label Claim</th>
<th>S. D. (±)</th>
<th>Amount Found (µg mL⁻¹)</th>
<th>% label Claim</th>
<th>S. D. (±)</th>
<th>Amount Found (µg mL⁻¹)</th>
<th>% label Claim</th>
<th>S. D. (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATN</td>
<td>50</td>
<td>49.35</td>
<td>98.7</td>
<td>0.39</td>
<td>48.92</td>
<td>97.84</td>
<td>0.72</td>
<td>49.86</td>
<td>99.72</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>12.25</td>
<td>98.00</td>
<td>0.52</td>
<td>12.3</td>
<td>98.4</td>
<td>0.59</td>
<td>12.41</td>
<td>99.28</td>
<td>0.43</td>
</tr>
<tr>
<td>HCT</td>
<td>50</td>
<td>49.35</td>
<td>98.7</td>
<td>0.39</td>
<td>48.92</td>
<td>97.84</td>
<td>0.72</td>
<td>49.86</td>
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<tr>
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<td>12.5</td>
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<td>98.00</td>
<td>0.52</td>
<td>12.3</td>
<td>98.4</td>
<td>0.59</td>
<td>12.41</td>
<td>99.28</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**Table 3: It shows Result of Recovery studies by the proposed methods**

<table>
<thead>
<tr>
<th>Amount added (µg mL⁻¹)</th>
<th>Amount recovered (Method A)</th>
<th>% Recovery (Method A) ± S.D.</th>
<th>Amount recovered (Method B)</th>
<th>% Recovery (Method B) ± S.D.</th>
<th>Amount recovered (Method C)</th>
<th>% Recovery (Method C) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATN</td>
<td>2.0</td>
<td>1.94</td>
<td>97.0 ± 0.54</td>
<td>1.95</td>
<td>97.5 ± 0.49</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>3.93</td>
<td>98.25 ± 0.72</td>
<td>3.92</td>
<td>98.0 ± 0.58</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>5.94</td>
<td>99.0 ± 0.47</td>
<td>5.88</td>
<td>98.0 ± 0.51</td>
<td>5.95</td>
</tr>
<tr>
<td>HCT</td>
<td>0.5</td>
<td>0.491</td>
<td>98.20 ± 0.51</td>
<td>0.492</td>
<td>98.4 ± 0.49</td>
<td>0.496</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.983</td>
<td>98.30 ± 0.45</td>
<td>0.986</td>
<td>98.6 ± 0.57</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.492</td>
<td>99.46 ± 0.39</td>
<td>1.486</td>
<td>99.06 ± 0.65</td>
<td>1.493</td>
</tr>
</tbody>
</table>

n=6
RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of ATN and HCT in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data as given in Table 3 and 4. The % R.S.D. as indicated in Table 4 was found to be less than 2, which indicates the validity of methods.

Linearity was observed by linear regression equation method for ATN and HCT in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The assay results obtained by proposed methods as shown in Table 2 are in fair agreement, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. It can be easily and conveniently adopted for routine quality control analysis. These methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

CONCLUSION

Simple UV spectrophotometric methods were developed for the simultaneous determination of Atenolol and Hydrochlorothiazide in bulk and tablet formulation without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The present methods succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in coformulated formulation.

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of ATN and HCT. It can therefore be concluded that use of these methods can save much time and money and they can be with accuracy.

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REFERENCES


Table 4: It shows Intra and Interday Precision

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Intraday Precision Method A</th>
<th>Intraday Precision Method B</th>
<th>Interday Precision Method C</th>
<th>Interday Precision Method A</th>
<th>Interday Precision Method B</th>
<th>Interday Precision Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATN</td>
<td>0.49 0.59</td>
<td>0.65 0.68</td>
<td>1.17 1.20</td>
<td>1.17 1.18</td>
<td>0.41 0.42</td>
<td>0.52 0.61</td>
</tr>
<tr>
<td>HCT</td>
<td>0.74 0.86</td>
<td>0.47 0.51</td>
<td>1.75 1.79</td>
<td>1.14 1.15</td>
<td>0.57 0.62</td>
<td>0.45 0.49</td>
</tr>
</tbody>
</table>

n=6

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