UV Spectrophotometric Determination of Hydrochlorothiazide and Olmesartan Medoxomil in Pharmaceutical Formulation

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Abstract: A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of hydrochlorothiazide and olmesartan medoxomil in combined dosage form by UV spectrophotometric method. UV spectrophotometric method includes simultaneous equation method (Method I) 271.5 nm and 257.0 nm \( \lambda_{\text{max}} \) of both the drugs were selected, absorbance Ratio method (Method II) 261.5 nm an isoabsorptive wavelength and 257.0 nm were selected for estimation of hydrochlorothiazide and olmesartan medoxomil respectively and Three wavelength method (Method III), two wavelengths were selected such that hydrochlorothiazide give same absorbances (263.8 and 278.4 nm) at two selected wavelength while third wavelength (316.5 nm) was such that olmesartan gives nearly zero absorbance. The two drugs follow Beer’s law over the concentration range of 5-25 \( \mu \)g/mL. The % recoveries of the both the drugs were found to be nearly 100 % representing the accuracy of the proposed methods. Validation of the proposed methods was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of hydrochlorothiazide and olmesartan medoxomil in combined dosage form.

Keywords: Hydrochlorothiazide, Olmesartan medoxomil, UV spectroscopy, Simultaneous Equation method, Absorbance Ratio method.

Introduction

Hydrochlorothiazide (HTZ) is a drug used in the treatment of hypertension. It blocks the reabsorption of \( \text{Na}^+ \) in the distal convoluted tubules by inhibiting the luminal membrane
bound Na⁺/Cl⁻ co-transport system. HTZ, chemically it is 6-chloro-3,4-dihydro-2H-1,2,4-
benzothiadiazine-7-sulphonamide1, 1 dioxide. Olmesartan medoxomil (OLM) is used in the
treatment of hypertension. It is angiotensin II receptor antagonists. OLM, chemically it is
2,3-dihydroxy- 2-butenyl -4- (1-hydroxy-1-methylethyl)- 2-propyl-1- [p-(o-1H-tetrazol-5-yl-
phenyl) benzyl] imidazole-5-carboxylate, cyclic 2,3-corbionate. Literature survey revealed
that the methods reported earlier were only for the analysis of single drug like UV-
spectrophotometry3 for HTZ and LC4, UV5 and Capillary electrophoresis6 for determination
of OLM. In combination methods are reported for HTZ with telmisartan7 irbesartan8,
valsartan9 and lisinopril10 while OLM with ramipril11 for their determinations in
pharmaceutical formulation. HPTLC12 method is reported for the HTZ with OLM
combination. The paper presents two simple, accurate, reproducible, rapid and economical
methods for simultaneous analysis of the two components in tablet formulation.

**Experimental**

SHIMADZU double beam UV visible spectrophotometer (model 1700) with 1 cm matched
quartz cuvettes were used for all absorbance measurements. Shimadzu AUX 220 balance
was used for weighing the samples. All the chemicals used were of AR grade. Double
distilled water and Whatman filter paper (no.41) were used throughout the experimental
work.

Multicomponent tablet olmetor-H (HTZ 12.5 mg and OLM 20.0 mg) manufactured by
Hetero drugs ltd, Dist- Baddi, Himachal Pradesh. All chemicals and reagents used were of
analytical grade.

**Standard stock solution**

Standard stock solutions of HTZ and OLM were prepared individually in methanol having
concentration of 100 µg/mL and 160 µg/mL respectively. Aliquot portions of the stock
solutions were diluted individually with distilled water to get final concentration of 10
µg/mL and 16 µg/mL for HTZ and OLM respectively. These working standard solutions
were scanned in the range of 400-200 nm in 1.0 cm cell against solvent blank.

**Determination of absorptivity value**

The solutions of each drug in triplicate were read against solvent blank at the selected
wavelengths and A(1% 1 cm) value were calculated using below formula:

\[
Absorptivity, A (1\% 1\ cm) = \frac{\text{Absorbance at selected wavelengths}}{\text{Concentration in g / 100 mL}}
\]

**Study of linearity**

Stock solutions each of HTZ and OLM having concentration of 100 µg/mL were prepared.
Aliquots of each solution were appropriately diluted and the final dilutions were read at the
selected wavelengths individually and in their combination. The correlation coefficient was
found to be less than 1. The method was first applied to standard laboratory mixture which
yielded encouraging results and the proposed methods were applied to marketed
formulation.

**Assay**

Twenty tablets were weighed and average weight was calculated. The tablets were triturated
thoroughly and mixed. Tablet powder equivalent to 12.5 mg of hydrochlorothiazide (~20.0
mg of olmesartan medoximil, on the basis of label claim) was transferred to 50.0 mL
volumetric flask, dissolved by sonication for 15 min. with sufficient quantity of methanol and volume was made up to mark with methanol. The content was filtered through Whatman filter paper (no.41). A 10.0 mL portion of the above filtrate was further diluted to 50.0 mL with distilled water. A 10.0 mL portion of this solution was further diluted to 50.0 mL with distilled water.

The final dilution of all sample solutions were read at the selected wavelengths using blank methanol with distilled water. The amount of each drug was estimated by proposed methods using the following formulae:

**Simultaneous equation method (Method I)**

\[
C_{HTZ} = A_2a_{y1} - A_1a_{y2} / a_{x2}a_{y1} - a_{x1}a_{y2}
\]

\[
C_{OLM} = A_1a_{x2} - A_2a_{x1} / a_{x2}a_{y1} - a_{x1}a_{y2}
\]

where:

- \(a_{x1}\) = The absorptivity of HTZ at 257.0 nm, \(a_{x2}\) = The absorptivity of HTZ at 271.5 nm
- \(a_{y1}\) = The absorptivity of OLM at 257.0 nm, \(a_{y2}\) = The absorptivity of OLM at 271.5 nm

**Absorbance ratio method (Method II)**

\[
C_{HTZ} = \frac{Q_m - Q_x}{Q_x - Q_y} \times \frac{A}{a_x} \quad C_{OLM} = \frac{Q_m - Q_y}{Q_y - Q_x} \times \frac{A}{a_y}
\]

Where:

- \(Q_m\) = Absorbance ratio of sample mixture at 257.0/261.5 nm
- \(Q_x\) = Ratio of absorptivity of OLM at 257.0/261.5 nm
- \(Q_y\) = Ratio of absorptivity of HTZ at 257.0/261.5 nm
- \(a_x\) = Absorptivity of HTZ at 257.0, \(a_y\) = Absorptivity of OLM at 261.5
- \(A\) = Absorbance of sample mixture at isobestic wavelength (261.5 nm)

**Three wavelength method (Method III)**

\[
(C_{OLME}) \times Cu = \frac{A_u}{A_s} \times Cs
\]

Cu = Concentration of unknown, Cs = Concentration of standard

As = \((A1 - A2)\), were \(A1\) = Absorbance of standard OLM at 263.8 nm
\(A2\) = Absorbance of standard OLM at 278.4 nm
Au = (A1-A2), were A1 = Absorbance of sample OLM at 263.8 nm
A2 = Absorbance of sample OLM at 278.4 nm

\( C_{HUZ} \), \( Cu = \frac{Au}{As} \times Cs \)

Cu = Concentration of unknown, Cs = Concentration of standard
As = Abs. Of standard at 316.5 nm, Au = Abs. of unknown at 316.5 nm

The results are shown in Table 1 for all proposed methods.

**Method validation**

Validation of the proposed methods was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines.

**Accuracy**

Recovery studies were carried out at four different levels by adding the pure drug to previously analysed tablet powder sample. From the amount of drug total drug found, percentage recovery was calculated by proposed three methods and results are shown in Table 2a and 2b.

<table>
<thead>
<tr>
<th>Amount of pure drug added, mg</th>
<th>Amount of pure drug recovered, mg</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTZ</td>
<td>OLM</td>
<td>HTZ</td>
</tr>
<tr>
<td>2.6</td>
<td>3.9</td>
<td>2.62</td>
</tr>
<tr>
<td>5.0</td>
<td>7.4</td>
<td>5.1</td>
</tr>
<tr>
<td>7.3</td>
<td>11.6</td>
<td>7.39</td>
</tr>
<tr>
<td>10.0</td>
<td>15.7</td>
<td>9.95</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± S.D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% R.S.D (CV)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2b. Results of recovery studies.**

<table>
<thead>
<tr>
<th>Amount of pure drug added, mg</th>
<th>Amount of pure drug recovered, mg</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTZ</td>
<td>OLM</td>
<td>HTZ</td>
</tr>
<tr>
<td>2.4</td>
<td>3.7</td>
<td>2.43</td>
</tr>
<tr>
<td>4.8</td>
<td>7.2</td>
<td>4.82</td>
</tr>
<tr>
<td>7.2</td>
<td>11.1</td>
<td>7.27</td>
</tr>
<tr>
<td>9.0</td>
<td>14.0</td>
<td>8.98</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± S.D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% R.S.D (CV)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Method I- Simultaneous equation method, Method-II- Absorbance ratio method, Method III- Three wavelength method.*

**Precision**

**Inter-day precision**

It was done by analysing the solutions by same analyst on alternate days till 5th day. The % RSD is shown in Table 3. Results indicate that the solution is stable up to 1 day, thereafter degradation may have taken place leading lower percent label claim.
**Intra day precision**

It was done by analysing the solutions by same analyst within a day. The % RSD is shown in Table 3. Results indicate that the solution is stable up to 4 h and thereafter, degradation may have taken place in the solution.

**Table 3. Results of ruggedness studies.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean percent of label claim ±%R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method I</td>
</tr>
<tr>
<td></td>
<td>HTZ</td>
</tr>
<tr>
<td>Precision</td>
<td>97.63±0.80</td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td></td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>87.66±10.41</td>
</tr>
<tr>
<td>Analyst- Analyst</td>
<td>101.40±0.12</td>
</tr>
</tbody>
</table>

*Method I- Simultaneous equation method, Method-II- Absorbance ratio method, Method III- Three wavelength method.*

**Linearity and range**

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim of HTZ were taken in a series of 50 mL volumetric flasks and dilutions were made as under sample solution. The graphs of % label claim vs. absorbance were plotted, were found to be linear.

**Ruggedness**

It was done by analysing the samples solutions by three different analysts. The % RSD by proposed methods is shown in Table 3 and was found to be within limits.

**Stability**

Sample solution: Accurately weighed quantities of tablet content equivalent to about 12.5 mg HCTZ, were transferred to 50.0 mL volumetric flask. These entire samples were stored for 24 h under following different conditions.

At 50 °C after addition of 1.0 mL of 1N HCl (Acid)
At 50 °C after addition of 1.0 mL of 1N NaOH (Alkali)
At 50 °C after addition of 1.0 mL of 6% H₂O₂ (Oxide)
At 60 °C (Heat)

After the specified period, the results were analysed by proposed methods and results are shown in Table 4.

**Table 4. Results of stability studies.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent of un-degraded drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method I</td>
</tr>
<tr>
<td></td>
<td>HTZ</td>
</tr>
<tr>
<td>1N HCl</td>
<td>75.5</td>
</tr>
<tr>
<td>1N NaOH</td>
<td>----</td>
</tr>
<tr>
<td>6% H₂O₂</td>
<td>69.7</td>
</tr>
<tr>
<td>60 °C</td>
<td>97.3</td>
</tr>
</tbody>
</table>

*Method I- Simultaneous equation method, Method-II- Absorbance ratio method, Method III- Three wavelength method.*
Results and Discussion

The study of spectrum recorded (Figure 1) showed that the HTZ exhibited two $\lambda_{\text{max}}$ at 271.6 and 316.5 nm while OLM showed at 257.0 nm. Simultaneous Equation was constructed using 271.5 nm and 257.0 nm $\lambda_{\text{max}}$ of both the drugs. Absorbance Ratio method utilises 261.5 nm an isoabsorptive wavelength and 257.0 nm for estimation of hydrochlorothiazide and Olmesartan medoxomil respectively and Three-wavelength method, two wavelengths were selected such that hydroclorthiazide give same absorbance (263.8 and 278.4 nm) at two selected wavelength while third wavelength (316.5 nm) was such that olmesartan gives nearly zero absorbance. The difference in absorbance of olmesartan at the former two selected wavelength was used for estimation of OLM while at latter wavelength HCTZ was estimated. The two drugs follow Beer’s law over the concentration range of 5-25 µg/mL. The values of relative standard deviation were found satisfactory and the recovery studies were close to 100%. The stability studies showed that the drugs are susceptible to stress condition that indicates, the drugs are not intrinsically stable towards such conditions.

![Over lain spectrum of hydrochlorothiazide and olmesartan medoximil.](image)

**Figure 1.** Overlain spectrum of hydrochlorothiazide and olmesartan medoxomil.

Conclusion

The proposed methods were found to be accurate, simple, rapid and reproducible. Thus all the methods can be applied in the routine analysis of the hydrochlorothiazide and Olmesartan medoxomil in formulations.

Acknowledgments

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References
