SIMULTANEOUS DETERMINATION OF Amiloride Hydrochloride AND Torsemide IN COMBINED PHARMACEUTICAL DOSAGE FORM USING SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Two simple, rapid and precise UV spectroscopic methods namely Simultaneous equation (method 1), Q-absorbance ratio (method 2) and have been developed for the simultaneous determination of Amiloride HCl (AML) and Torsemide (TOR). In method 1, both the drugs exhibit good linearity over the concentration range of 2-14 μg/ml and 4-28 μg/ml of AML and TOR at 361 (r²=0.9996 and 0.9993 respectively) and 285 (r²=0.998 and 0.9993 respectively) wavelengths respectively. Method 2 involves the formation of Q-absorbance equation using the absorptivity values at 330.0 nm (isoabsorptive point) (r²=0.9993 and 0.999 respectively) and 285 nm (λmax of TOR) (r²=0.9992 and 0.9991 respectively) and Beer’s Lambert’s law was obeyed over the concentration range of 2-14 μg/ml and 4-28 μg/ml respectively. The proposed methods were validated according to ICH guidelines for evaluation of accuracy, precision, sensitivity etc. In conclusion, the proposed methods are novel, simple, accurate, precise, sensitive, rapid and economically viable methods that do not require any prior separation procedure. The proposed two methods hold potential for simultaneous determination of AML and TOR in tablet dosage form.

Keywords: Amiloride HCl (AML), Torsemide (TOR), Spectrophotometric method, simultaneous Equation method, Absorption ratio method, validation.

INTRODUCTION

Hypertension is defined conventionally as blood pressure >140/90 mmHg. Sustained
hypertension damages heart, kidney, blood vessels & brain which leads to ischemic heart disease, congestive heart failure, renal failure & stroke. Congestive heart failure occurs when cardiac output is insufficient to meet the demands of tissue perfusion.

Amiloride HCl is chemically N-Amidino-3, 5-diamino-6-chloropyrazine carboxamide (figure I(a)). Amiloride HCl is a potassium sparing diuretic & antihypertensive agent. It reduces the amount of water and sodium retained by the body. Amiloride HCl is used to restore normal serum potassium levels in patients who develop hypokalemia. Amiloride HCl is mostly used in conjunction with other drugs to lower blood pressure. Amiloride HCl is official in Indian Pharmacopoeia, British Pharmacopoeia and United State Pharmacopoeia. Potentiometric titration method is official method for the estimation. Many methods like UV-Visible spectroscopy, Derivative Spectroscopy and RP- HPLC methods were reported for estimation of Amiloride HCl.

Torsemide is chemically 1-[4-(3-methylanilino) pyridin-3-yl]sulfonyl-3-propan-2-yl urea (figure I(b)). Torsemide is a pyridine-sulfonylurea type loop diuretic mainly used in the management of edema associated with congestive heart failure. It is also used at low doses for the management of hypertension. Torsemide is official drug in United state Pharmacopoeia. HPLC method is official method for the estimation. Many methods like UV spectroscopy, RP-HPLC and RP- UPLC methods were reported for estimation of Torsemide.

![Figure I: Chemical structures of (a) Amiloride HCl (b) Torsemide](image)

The combination of Amiloride HCl and Torsemide is available in tablet dosage form. Amiloride HCl & Torsemide combination is used in treatment for edema associated with congestive heart failure & hypertension. HPLC method was reported for estimation of combination of Amiloride HCl Torsemide in pharmaceutical dosage form.
MATERIALS AND METHODS

Instrument
A Shimadzu model UV-1800 double beam UV-visible Spectrophotometer, attached to a computer software UV probe 2.34, with a spectral width of 1 nm and pair of 1 cm matched quartz cells was used. Shimadzu analytical balance (Sartorius, Gottingen, Germany), and Ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used throughout the practical. Class ‘A’ volumetric glassware were used.

Reagents and Materials
Methanol (AR grade) was used as solvent. Amiloride HCl bulk powder was kindly gifted by West Coast Pharma Pvt. Ltd. Torsemide was gifted by Intas pharmaceuticals Pvt. Ltd.

Preparation of Standard stock solution
Accurately weighed standard drug of AML(10mg) and TOR (10mg) were transferred to a separate 100ml volumetric flask, dissolved in methanol. The Volume was adjusted with the same up to mark to give final concentrations of AML(100µg/ml) and TOR(100µg/ml).

Preparation of Calibration curve (method 1)
From the Standard solutions of AML (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml) and standard solutions of TOR (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 ml) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol and mixed.

The absorbances of the solutions were measured at 361.0 nm and 285.0 nm against taking methanol as a blank. The absorptivity coefficients of each drug at both wavelengths were determined and substituted in their equation to obtain concentration of both drugs. The concentration of each compound in the mixture was calculated from the following simultaneous equations (18).

\[ C_{AML} = A_2ay_1 - A_1ay2 / ax_2ay_1 - ax_1ay2 \] .......... 1.

\[ C_{TOR} = A_1ax_2 - A_2ax2 / ax_2ay_1 - ax_1ay2 \] .......... 2.

Where, \( C_{AML} \) and \( C_{TOR} \) are concentration of AML and TOR respectively; \( A_1 \) and \( A_2 \) are absorbance of mixture at 361 nm and 285 nm respectively; \( ax_1 \) and \( ax_2 \) are absorptivity coefficient of AML at 361 nm and 285 nm respectively; \( ay_1 \) and \( ay_2 \) are absorptivity coefficient of TOR at 361 nm and 285 nm respectively.
Preparation of Calibration curve (method 2)
From the Standard solutions of AML (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4ml) and standard solutions of TOR (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8ml) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with Methanol and mixed.

The over line spectrum of AML and TOR, one wavelength was selected for the estimation of both drugs, which is known as iso-absorptive point (at 330.0 nm) and one was $\lambda_{\text{max}}$ of one drug. The dilutions of standard and sample solutions were prepared. The Absorptivity values were determined at 330.0 nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using following formula\(^{(18)}\),

$$
C_X = \frac{(Q_M - Q_Y) \times A_1}{(Q_X - Q_Y) \times aX_1} \quad \text{AND} \quad C_Y = \frac{A_1}{aX_1 - C_X}
$$

Where, $A_1$ & $A_2$ are the absorbance of the mixture at 330.0 nm & 285.0 nm respectively; $aX_1$ and $aY_1$ are absorptivities of AML and TOR respectively at 330.0 nm; $aX_2$ and $aY_2$ are absorptivities of AML and TOR respectively at 285 nm; $Q_M=A_2/A_1$, $Q_X= aX_2/ aX_1$ and $Q_Y= aY_2/ aY_1$.

VALIDATION OF METHODS\(^{(19)}\)
Proposed methods were validated in accordance with ICH guidelines Q2 (R1) for evaluation of various parameters; linearity, limit of detection, limit of quantification, precision and accuracy.

Linearity
Calibration curves were plotted over a concentration range of 2-14 µg/ml and 4-28 µg/ml for AML and TOR respectively. The calibration curves were constructed by plotting absorbances Vs concentrations.

2.2 Method precision (repeatability)
The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions ($n=6$) of AML and TOR (6 µg/ml and 12 µg/ml respectively) without changing the parameters for the simultaneous equation method.
2.3 Intermediate precision (reproducibility)
The intraday and interday precisions of the proposed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days, different concentrations of standard solutions of AML (2, 6 & 12 µg/ml) and TOR (4, 12 and 24 µg/ml). Results were reported in terms of RSD.

2.4 LOD and LOQ
The limit of detection (LOD) and limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline: The LOQ may be expressed as

\[
\text{LOQ} = \frac{10 \sigma}{S}
\]

Where, \( \sigma \) = the standard deviation of the response
\( S \) = the slope of the calibration curve

The LOD may be expressed as:

\[
\text{LOD} = \frac{3.3 \sigma}{S}
\]

Where, \( \sigma \) = the standard deviation of the response
\( S \) = the slope of the calibration curve

2.5 Accuracy (Recovery study)
The accuracy of the methods was determined by calculating recoveries of AML and TOR by the standard addition method. Known amounts of standard solutions of AML and TOR were added at 80, 100 and 120 % levels to prequantified sample solutions of AML and TOR (6 and 12 µg/ml respectively). The amounts of AML and TOR were estimated by applying the obtained values to the simultaneous equation method.

2.6 Analysis of AML and TOR in Pharmaceutical dosage form
In pharmaceutical dosage form, both drugs AML and TOR in ratio of 1:2. The absorbance was measured at 361.0 and 285.0 nm (Simultaneous equation) and 330.0 and 285 nm (Q-ratio) for quantification of AML and TOR, respectively. The amounts of AML and TOR
present in sample solutions were determined by fitting the response into the simultaneous equation and Q-ratio method for AML and TOR.

RESULTS AND DISCUSSION
Simultaneous equation method
The standard solutions of AML and TOR were prepared separately in Methanol. They were scanned in the wavelength range of 200-400nm. Data were recorded at an interval of 1 nm. Maximum absorbance was obtained at 361.0 nm and 285.0 nm for AML and TOR, respectively. Select these two analytical wavelengths for determination of AML and TOR, respectively shown in figure II. These two wavelengths can be employed for the determination of AML and TOR without any interference from their combined pharmaceutical dosage form.

Q-ratio method
The standard solutions of AML and TOR were prepared separately in methanol. They were scanned in the wavelength range of 200-400nm. Data were recorded at an interval of 1 nm. The over line spectrum of AML and TOR, one wavelength was selected for the estimation of both drugs, which is known as iso absorptive point (at 330.0 nm) shown in figure II and one was λmax of one drug. The dilutions of standard and sample solutions were prepared. The Absorptivity values were determined at 330.0 nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using the formula.

![Figure II: Overlaid spectra of AML and TOR](image-url)
Validation of the proposed method

The proposed methods have been validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The calibration curves were constructed for the proposed methods according to their respective concentration ranges and were found to be linear over the concentration range for AML and TOR with acceptable regression coefficient as shown in Table I for three proposed methods.

Linearity

Linear correlation was obtained between absorbance versus concentrations of AML and TOR in the ranges of 2-14 µg/ml and 4-28 µg/ml, respectively for both the methods. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression.

Table I: Regression analysis data for the proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
<td>TOR</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>361</td>
<td>285</td>
</tr>
<tr>
<td>Linear correlation (µg/ml)</td>
<td>2-14</td>
<td>2-14</td>
</tr>
<tr>
<td>Molar absorptivity (1 mole⁻¹ cm⁻¹)</td>
<td>645.7</td>
<td>627.068</td>
</tr>
<tr>
<td>Reg. equation (Y= mx + c)</td>
<td>0.0621x+0.0109</td>
<td>0.0566x+0.0264</td>
</tr>
<tr>
<td>Slope=m</td>
<td>0.0621</td>
<td>0.05</td>
</tr>
<tr>
<td>Intercept=c</td>
<td>0.0019</td>
<td>0.0264</td>
</tr>
<tr>
<td>Correlation Coefficient (r²)</td>
<td>0.9996</td>
<td>0.9992</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.345</td>
<td>0.281</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.985</td>
<td>0.852</td>
</tr>
</tbody>
</table>

* Average of five determination, LOD=Limit of detection, LOQ=Limit of quantification

Table I give LOD and LOQ value of AML and TOR. The precision data for the both methods is given in Table II. Recovery study performed by spiking the standard solution at 80,100 and 120%, less than 2 % RSD indicate the recovery study was acceptable (Table-III).
Table II: Precision data for AML and TOR by proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
<td>TOR</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>361</td>
<td>285</td>
</tr>
<tr>
<td>Repeatability (%RSD, n=6)</td>
<td>0.195</td>
<td>0.180</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>0.497-1.742%</td>
<td>0.214-1.056%</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>0.278-1.171%</td>
<td>0.186-0.801%</td>
</tr>
</tbody>
</table>

*n= Number of determination

Table III: Recovery data for AML and TOR by Proposed methods

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount present in mixture (µg/ml)</th>
<th>Amount Added (%)</th>
<th>% Recovery ± %RSD</th>
<th>Amount present in mixture (µg/ml)</th>
<th>Amount Added (%)</th>
<th>% Recovery ± %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method I</td>
<td>Method II</td>
<td></td>
<td>Method I</td>
<td>Method II</td>
</tr>
<tr>
<td>AML</td>
<td>6</td>
<td>80%</td>
<td>99.86±1.17</td>
<td>6</td>
<td>80%</td>
<td>98.80±0.64</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>100%</td>
<td>98.58±0.86</td>
<td>6</td>
<td>100%</td>
<td>101.1±1.25</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>120%</td>
<td>98.02±0.68</td>
<td>6</td>
<td>120%</td>
<td>99.07±1.2</td>
</tr>
<tr>
<td>TOR</td>
<td>12</td>
<td>80%</td>
<td>98.27±0.66</td>
<td>12</td>
<td>80%</td>
<td>98.59±0.80</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100%</td>
<td>98.29±0.48</td>
<td>12</td>
<td>100%</td>
<td>100.93±1.13</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>120%</td>
<td>98.76±0.45</td>
<td>12</td>
<td>120%</td>
<td>98.78±1.04</td>
</tr>
</tbody>
</table>

* Average of three determination

Assay of pharmaceutical dosage form (Tablet) by proposed methods

The proposed validated methods were successfully applied for the determination of AML and TOR in their combined dosage forms. Results are given in table IV. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine analysis of Amiloride HCl and Torsemide in pharmaceutical dosage form.

Table IV: Assay for the Pharmaceutical dosage (Tablet) form for proposed methods

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Amount taken</th>
<th>Amount found</th>
<th>% label claim</th>
<th>Amount found</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
<td>TOR</td>
<td>AML</td>
<td>TOR</td>
<td>AML</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>10</td>
<td>5.07</td>
<td>10.17</td>
<td>101.4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>10</td>
<td>5.02</td>
<td>10.19</td>
<td>100.4</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>10</td>
<td>5.1</td>
<td>10.03</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>10</td>
<td>5.04</td>
<td>10.07</td>
<td>109.9</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>5.02</td>
<td>9.94</td>
<td>100.4</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>10</td>
<td>5.006</td>
<td>10.025</td>
<td>100.1</td>
</tr>
<tr>
<td>Mean</td>
<td>5.042</td>
<td>10.07</td>
<td>100.8</td>
<td>100.7</td>
<td>5.02</td>
</tr>
<tr>
<td>SD</td>
<td>0.0757</td>
<td>0.9415</td>
<td>0.725</td>
<td>1.481</td>
<td>0.045</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.501</td>
<td>0.9415</td>
<td>0.718</td>
<td>1.471</td>
<td>0.915</td>
</tr>
</tbody>
</table>
CONCLUSION
Based on the results, it can be concluded that the method has linear response in the range of 2– 14 and 4-28 µg/ml for Amiloride HCl and Torsemide. Less than 2 %RSD indicate that UV-spectroscopic methods are accurate and precise.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with prepared ratio of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Amiloride HCl and Torsemide.

The method can be used for the routine analysis of for Amiloride HCl and Torsemide in combined pharmaceutical dosage form.

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We are thankful to West Coast Pharma Pvt. Ltd, Ahmedabad & Intas Pharmaceutical Pvt. Ltd for providing Amiloride HCl and Torsemide respectively for research. The authors are highly thankful to Babaria Institute of Pharmacy, Vadodara, Gujarat, India for providing all the facilities to carry out the research work.

REFERENCE


