Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMITRIPTYLINE HCL AND CHLORDIAZEPOXIDE IN BULK DRUG AND COMBINED PHARMACEUTICAL DOSAGE FORM.

MONA KARIA*1, BHARGAV GOHEL2

ABSTRACT

A simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method has been developed for the simultaneous determination of Amitryptiline HCl and Chlordizepoxide in combined dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Amitryptiline HCl and Chlordizepoxide show an isoabsorptive point at 220 nm in 0.1 N HCl. The second wavelength used is 246 nm, which is the λ-max of Chlordizepoxide in 0.1 N HCl. The linearity was obtained in the concentration range of 5-10 μg/ml for Amitryptiline HCl and 5-10 μg/ml for Chlordizepoxide. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of Chlordizepoxide. The method was successfully applied to pharmaceutical dosage form because of no interference. The results of analysis have been validated by recovery studies.

KEY WORDS

Amitriptyline HCl (AMI), Chlordiazepoxide (CHLOR), 0.1 N HCl, Absorbace ratio method, Isoabsorptive point.

AFFILIATION

1. *Ms.Mona P. Karia, B.Pharm, Atmiya Institute of Pharmacy, ‘Meet Heigths’, Flat No-401, Panchnath Plot-17, Near Limbda chowk, Rajkot, Gujarat, India


Address for Correspondence:

Email: mona.karia@yahoo.in, Mobile No: 9426788066,
INTRODUCTION

Amitriptyline hydrochloride (Figure 1-A) 10, 11-dihydro-N, N-dimethyl-5H-dibenzo[a, d]cycloheptane-Δ5γ-propylamine hydrochloride is used commonly in combination with chlordiazepoxide (Figure 1-B) 7-chloro-2-(methyl-amino)-5-phenyl-3H-1, 4-benzodiazepine-4-oxide [1-3]. The mixture of these two drugs is used for the treatment of patients with moderate to severe depression associated with moderate to severe anxiety.

Fig. 1: Chemical structure (A) Amitriptyline HCl (B) Chlordiazepoxide.

Amitryptiline HCl is official in IP, BP and USP. In IP’07 and USP’07 describe potentiometry method for its estimation. [3, 5] Chlordiazepoxide official in IP’07 describes potentiometry method for its estimation. [3] So many methods like UV, HPLC, and RP-HPLC are available for estimation of AMI and CHLOR individually and in combination with other drugs. [6-10]

According to detailed survey of analytical literature not even a single analytical procedure describes a simple and satisfactory UV spectrophotometric method for simultaneous determination of AMI and CHLOR in their combined dosage forms by using 0.1 N HCl as a solvent. So the objective of this work was to develop simple, precise and rapid spectrophotometric methods for combination drug products containing AMI and CHLOR.

The validation procedures followed the International Conference on Harmonization (ICH) guidelines evaluating the parameters like linearity, precision, accuracy; detection limit and quantitation limit [11].

MATERIALS AND METHODS

An UV-Visible double beam spectrophotometer (héλios Alpha, Model - V 7.09) having two matched quartz cells with 10 mm light path. All weighing were done on electronic balance (Contech, Model-CA34). AR grade HCl was purchased from Chemdyes Corporation, Ahmedabad. Amitriptyline HCl and Chlordiazepoxide reference standard was provided as gift sample by Reliance Formulation Pvt. Ltd., Ahmedabad, Gujarat. The commercial fixed dose combination product (RELIDEP PLUS-H) was procured from the local market.

Preparation of Standard Stock Solution of AMI and CHLOR

Accurately weighed quantity 100 mg of AMI and CHLOR were transferred inti separate 100 ml volumetric flask, dissolved and diluted up to mark with 0.1 N HCl (100 ml). This will give a stock solution having strength of 1000 µg/ml of each.
Preparation of Working Standard Solution of AMI and CHLOR

100 µg/ml of CHLOR and AMI solution were prepared by diluting 10 ml of stock solution to 100 ml with 0.1 N HCl in separate 100 ml volumetric flask.

Suitable aliquots of this solution were diluted up to the mark with 0.1 N HCl to get the concentration range of 5, 6, 7, 8, 9 and 10 µg/ml for CHLOR and 5, 6, 7, 8, 9 and 10 µg/ml for AMI.

Selection of Analytical Wavelength

From working standard solution of AMI (100 µg/ml) and CHLOR (100 µg/ml) prepare 10 µg/ml for AMI and CHLOR both. The scanning for solution of AMI and CHLOR were carried out in the range of 200-400 nm against using 0.1 N HCl as a blank. The maximum absorption (λmax) of CHLOR was found at 246 nm and iso-absorptive point at 220 nm. Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths.

Preparation of calibration curve

Standard solutions of AMI in the concentration range of 5 to 10 µg/ml obtained by transferring (0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml) of AMI working standard solution (100 µg/ml) to the series of 10 ml volumetric flasks and standard solutions of CHLOR in the concentration range of 3 to 10 µg/ml were obtained by transferring (0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml) of CHLOR working standard solution (100 µg/ml) to the series of 10 ml volumetric flasks. Then volume was adjusted up-to mark with 0.1 N HCl. All dilutions were scanned in wavelength range of 200 nm to 400 nm. The absorbances were plotted against the respective concentrations to obtain the calibration curves.

Quantification

Construct the chromatogram of the analytes, calculate the absorbance and plot calibration curve absorbance against concentration in µg/ml.

Concentration of analyte is calculated using linear equation,

\[ y = mx + c \]

Where,

- \( y \) = absorbance of the analyte
- \( m \) = slope of the calibration curve
- \( x \) = concentration of the analyte in sample in µg/ml
- \( c \) = intercept of the calibration curve

**METHODODOLOGY**

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the λmax of one of the two components.
Fig. 2: Overlain Spectrum of CHLOR and AMI showing iso-absorptive point in 0.1 N HCl

From the overlain spectra of two drugs (as shown in figure 2), it shows that AMI and CHLOR having iso-absorptive point at 220 nm. The second wavelength used is 246 nm, which is the λmax of CHLOR. Working standard solutions having concentration 5, 6, 7, 8, 9 and 10 µg/ml for AMI and 5, 6, 7, 8, 9 and 10 µg/ml for CHLOR were prepared and the absorbance at 220 nm (iso-absorptive point) and 246 nm (λmax of CHLOR) were measured and absorptivity coefficient were calculated using calibrations curve.

RESULT AND DISCUSSION

Validation parameters

Validation of developed method was carried outs as per ICH guideline. Parameters such as Linearity and range, Accuracy, Precision, LOD and LOQ were taken up as tests for analytical method validation.

Linearity and Range

Appropriate volume of aliquot from CHLOR and AMI working standard solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with 0.1 N HCl to give a solutions containing 5-10 µg/ml CHLOR and 5-10 µg/ml AMI. The absorbances of solution were then measured at 220 nm and 246 nm. The calibration curves were constructed by plotting average absorbances versus concentration and the regression equations were calculated (n=5).
Straight line equations were obtained from these calibration curves.

**Table No. 1: Regression Characteristics of Chlordiazepoxide and Amitryptaline:**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AMI</th>
<th>CHLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>220</td>
<td>246</td>
</tr>
<tr>
<td>Linearity (µg/ml)</td>
<td>5-10</td>
<td>5-10</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>[ y = 0.0846x + 0.1417 ]</td>
<td>[ y = 0.0367x + 0.2032 ]</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0846</td>
<td>0.0367</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.9918</td>
<td>0.9925</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.1417</td>
<td>0.2032</td>
</tr>
<tr>
<td>S.D. of Intercept</td>
<td>0.0113</td>
<td>0.0155</td>
</tr>
</tbody>
</table>

**Precision**

Precision of the method was determined in the terms of Repeatability, Intraday and Interday precision. Repeatability (% RSD) was assessed by analyzing est drug solution within the calibration range, six times on the same day. Intraday variation (% RSD) was determined by analysis of this solution three times on the same day. Interday variation (% RSD) was determined by analysis of this solution on three different days.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

They were calculated as 3.3 \( \sigma/S \) and 10 \( \sigma/S \) respectively. Where \( \sigma \) is the standard deviation of the response (y-intercept) and S, is the mean of the slope of calibration plot.

**Table No. 2: Validation results for Chlordiazepoxide and Amitryptaline:**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AMI</th>
<th>CHLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>246</td>
<td>220</td>
</tr>
<tr>
<td>Repeatability (%RSD) (n=6)</td>
<td>0.1372</td>
<td>0.1309</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>0.8540-1.3036</td>
<td>0.6328-0.7648</td>
</tr>
</tbody>
</table>
Recovery Studies

Recovery studies were done so as to check the accuracy of the method. Known amounts of standard solutions of CHLOR and AMI were added to pre-quantified saplsolutions of CHLOR and AMI and absorbance were determined at 220 nm and 246 nm. Concentration of the drug in the mixture was calculated using the equations. The analysis was done in a set of 3 replicates.

Table No. 3: Result of Recovery Studies for CHLOR in pharmaceutical dosage form:

<table>
<thead>
<tr>
<th>Amount of CHLOR in mixture (µg/ml)</th>
<th>Amount of Std CHLOR added (µg/ml)</th>
<th>Total amount of CHLOR (µg/ml)</th>
<th>Total amount of CHLOR found (µg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
<td>7.2</td>
<td>7.22±0.0784</td>
<td>100.27</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8</td>
<td>7.89±0.0410</td>
<td>98.63</td>
</tr>
<tr>
<td>4</td>
<td>4.8</td>
<td>8.8</td>
<td>8.79±0.1593</td>
<td>99.85</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]
Table No. 4: Result of Recovery Studies for AMI in pharmaceutical dosage form:

<table>
<thead>
<tr>
<th>Amount of AMI in mixture (μg/ml)</th>
<th>Amount of Std AMI added (μg/ml)</th>
<th>Total amount of AMI (μg/ml)</th>
<th>Total amount of AMI found (μg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
<td>7.2</td>
<td>7.16±0.2817</td>
<td>99.44</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8</td>
<td>7.90±1.1013</td>
<td>98.74</td>
</tr>
<tr>
<td>4</td>
<td>4.8</td>
<td>8.8</td>
<td>8.69±0.7247</td>
<td>98.77</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]

Application of Proposed Method to Pharmaceutical dosage form

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 25 mg of Chlordiazepoxide and 10 mg of Amitryptiline HCl into a 100 mL volumetric flask, added 50 ml of 0.1 N HCl and mechanically stirred for 20 minutes. This solution was filtered through the Whatmann filter paper No. 41 and residues were washed with 0.1 N HCl. The filtrate and washings were combined and volume was made-up to 100 ml with 0.1 N HCl. 0.3 ml from above stock solution is transferred to 100 ml volumetric flask and dilute to 100 ml with 0.1 N HCl to get final concentration as 7.5 ppm of Chlordizepoxide and 3 ppm of Amitryptiline HCl.

Table No. 5: Analysis of CHLOR and AMI in pharmaceutical dosage form:

<table>
<thead>
<tr>
<th>Pharmaceutical dosage form</th>
<th>CHLOR</th>
<th>AMI</th>
<th>CHLOR</th>
<th>AMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>10</td>
<td>101.12±1.0963</td>
<td>99.44±1.6311</td>
</tr>
</tbody>
</table>

[*=mean value of 5 determination]

CONCLUSION

The results obtained by applying the suggested procedures, it is proved that the proposed method is accurate, precise, simple, sensitive, selective and rapid and can be applied successfully in routine analysis for the estimation of AMI and CHLOR in bulk and pharmaceutical dosage form. The developed method was validated as per ICH guidelines.
ACKNOWLEDGEMENT

The authors are thankful to Smt. R. D. Gardi B. Pharmacy College, Rajkot, Gujarat, India for providing necessary facilities to carry out this work.

REFERENCES


