CHAPTER VIII

Spectrophotometric assay of an anti-allergic agent in pure and pharmaceutical preparations using thymol blue

Abstract

A simple, rapid and sensitive extractive spectrophotometric method has been described for the assay of cinnarizine (CNR) in pure and pharmaceutical formulations in the present study. The method is based on the formation of ion-pair complex of CNR with thymol blue (TB) in NaOAc-AcOH buffer of pH 3.6. Reaction conditions were optimized to obtain the colored species of maximum intensity and stability. The system obeyed Beer's law in the range of 0.6-15.8 µg/ml. Various analytical parameters have been evaluated and the results have been supported by statistical data. The comparison of the results of analysis by the proposed method with those of official method revealed good agreement between two methods.

The results recorded in this chapter are published in Journal of Chinese Chemical Society, 54, 63-68, (2007).
GENERAL DRUG PROFILE

*Cinnarizine (CNR)*

**Chemical name**

(E)-(diphenylmethyl)-4-(3-phenylprop-2-enyl) piperizine

**Structure**

![Structure of Cinnarizine](image)

**Molecular formula**

C$_{26}$H$_{28}$N$_{2}$

**Molecular weight**

368.514

**Description**

White crystalline solid

**Solubility**

Easily soluble in water

**Category**

Anti-allergic, Calcium channel blocker, Histamine H$_{1}$ Antagonist

INTRODUCTION

CNR is known to improve the cerebral blood flow. It is widely used orally for the treatment of cerebral apoplexy, cerebral arteriosclerosis and post-traumatic cerebral symptoms. It is used for the control of nausea and vomiting$^{1}$. It is also known as an antihistamine and a calcium channel blocker.

The official method mentioned in European Pharmacopoeia$^{2}$ for the determination of CNR in bulk sample involves a non-aqueous titration method. Monitoring of CNR in biological fluids has been carried out mainly by HPLC with UV$^{3}$ or fluorescence detection$^{4}$. CNR in different pharmaceutical preparations was assayed by various analytical methods viz., GC$^{5}$, HPLC$^{6-8}$.
HPTLC\textsuperscript{9}, electrochemical\textsuperscript{10-12} and spectrophotometric methods\textsuperscript{13-24}. Some of these spectrophotometric methods are laborious\textsuperscript{13,14} or applicable to higher concentration of the drug\textsuperscript{15,20} or less sensitive.\textsuperscript{21-23} The spectrophotometric method\textsuperscript{24} described by Abdine et al., does not discuss about the stability, detection and quantification limits. In view of the above, the investigator has developed a novel spectrophotometric method for the assay of CNR.

**EXPERIMENTAL**

**Reagent**

Aqueous solution of TB (0.01 %) was employed in the study.

**Buffers**

Different buffer solutions were prepared by following the standard procedures as mentioned in Chapter II.

**Pure drug solution**

A stock solution of CNR containing 250 \( \mu \text{g/ml} \) was prepared by dissolving it in minimum amount of ethanol and then diluting with distilled water. The working solution was prepared by suitable dilution of the stock solution with water as and when required. The solution was noticed to be stable at room temperature.

**RECOMMENDED PROCEDURES**

Following procedure was recommended for the determination of CNR after carrying out the preliminary experiments.
**Assay procedure bulk sample**

An aliquot of the solution containing 6-158 μg of CNR was transferred into a series of 125 ml separating funnels. A volume of 3 ml of NaOAc-AcOH buffer of pH 3.6 followed by 3 ml of TB was added. Chloroform (10 ml) was added, shaken well and left at room temperature for a minute. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. Absorbances were recorded at 405 nm against the reagent blank and the calibration graph was constructed.

**Analysis of tablets**

Ten tablets were weighed and powdered. An amount of the powder equivalent to 100 mg of CNR was weighed into a 100 ml volumetric flask containing about 75 ml of distilled water. It was shaken thoroughly for about 15-20 min and diluted to the mark. It was filtered through a Whatman filter paper No. 40. A volume of 10 ml of the filtrate was taken and diluted to 50 ml. An appropriate amount of the aliquot was taken and analyzed using the procedure given above.

**RESULTS AND DISCUSSION**

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of several drugs. CNR reacted with TB in the acidic buffer and produced yellow colored ion-pair complex. The probable reaction mechanism for the formation of complex is given in the reaction Scheme 1.
Spectral characteristics of ion-association complex

The absorption spectrum of the complex was scanned on a spectrophotometer in the wavelength region of 300-600 nm against the reagent blank. The complex exhibited maximum absorption at 405 nm. Under the experimental conditions, the reagent blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of CNR (Fig. 1).

Standardization of reaction conditions

In order to establish the conditions necessary for the quantitative formation of ion-association complex with maximum sensitivity and stability, the investigator has performed several experiments. This was done by measuring the absorbances of a series of solutions at 405 nm by varying one and fixing the other parameters. These conditions were incorporated in the procedure.

Optimization of pH of buffer

The effect of pH was studied by extracting the colored complex in presence of various buffers such as KCl-HCl (pH=1.0-2.2), NaOAc-HCl (pH=0.65-5.2), NaOAc-AcOH (pH=3.6-5.6) and potassium hydrogen phthalate-HCl (pH=2.2-3.6). It was observed that the maximum color intensity and reproducible results were observed in NaOAc-AcOH buffer (Clark and Lubs) of pH 3.6. Low absorbance values were observed at higher pH values. Further, 3 ml of the Clark and Lubs buffer of pH 3.6 showed maximum absorbances.
**Effect of TB on formation of complex**

The maximum color intensity of the complex was achieved with 3 ml of 0.01 % TB (Fig. 2). Low absorbance readings were noticed with volume of the reagent for less than or more than 3 ml.

**Selection of extractant**

Among chloroform, carbon tetrachloride, ethyl acetate, xylene, diethyl ether, butyl acetate, toluene, dichloromethane and chlorobenzene tried for quantitative extraction of the colored species, chloroform was found to be the most suitable solvent. Further, it was noticed that only one extraction was adequate to achieve a quantitative recovery of the complex with chloroform with a shaking time of 0.5-1.0 min.

**Order of addition of reagents**

Experiments were carried out to examine the effect of order of addition of reagents on stability of ion-association complex. It was clear from the experiments that there was no appreciable change in sensitivity or stability of the colored complex even if the order of addition was altered.

**Precision and accuracy**

The precision of the method is ascertained from the absorbance values obtained by actual determination of five replicates of fixed amount of CNR. The percent relative standard deviation was calculated for the proposed method and is presented in Table 1.
To determine the accuracy of the proposed method, five replicates of known amount of the drug within the Beer's law limit were taken and analyzed by the proposed method. The percent error was calculated and the same is given in Table 1.

**Optical characteristics of ion pair complex**

Beer's law limit (Fig. 3), molar absorptivity and Sandell’s sensitivity values were calculated and are given in Table 1. Slope, intercept and the correlation coefficient values were also evaluated using the least squares regression analysis and the results are recorded in Table 1.

**Effect of temperature on colored complex**

The effect of temperature on colored complex was examined and it was found that the colored complex was stable upto 34 °C. At higher temperatures, the absorbances increased due to the evaporation of chloroform. However, the complex remained stable for more than 8 h at room temperature.

**LOD and LOQ values**

The limit of detection was found to be 0.18 µg/ml while the limit of quantification was noticed to be 0.61 µg/ml for the proposed method (Table 1).

**Interference Studies**

It was observed that the talc, glucose, starch, lactose, dextrose, gum acacia and magnesium stearate did not interfere in the determination at the levels normally found in dosage forms thereby revealing that the proposed method could be applied for the assay of CNR in presence of above excipients and additives. The results are summarized in Table 2.
Recovery studies

For recovery studies, known quantities of pure CNR were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The average percent recovery values were found to be quantitative (99.76 - 99.89 %) indicating good accuracy of the method.

Ruggedness

To ascertain the ruggedness of the method, five replicate determinations at different concentration levels of the drug were carried out. The within-day RSD values were found to be less than 1.0 %. Between day precision values recorded in Table 3 revealed that the proposed method has reasonable ruggedness.

Stoichiometry of the complex

The drug to dye stoichiometric ratio was determined by Job’s method of continuous variation using 1.0 x 10^-4 M solution of each of drug and TB. The results revealed that 1 mole of the drug combined with 1 mole of the dye to form an ion-pair complex (Fig. 4).

Analysis of pharmaceutical preparations and statistical comparison of the results with official method

The proposed method was successfully applied to the analysis of CNR in commercial tablet. The performance of the method was assessed by calculating
t- and F-values (Table 4). The calculated t-values at 95 % confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and official methods. Further, it was also observed that the variance ratio F-values calculated for p=0.05 did not exceed the theoretical value revealing thereby that there was no significant difference in precision between the proposed and official methods.

**CONCLUSIONS**

The reagent utilized in the proposed method is cheaper and readily available. The procedure does not involve any critical reaction conditions or tedious sample preparation. The results were reproducible and accurate. The method is found to be free from interference by common additives and excipients. The wide applicability of the new procedure for routine quality control is well established by the assay of CNR in pure form and in tablets. The t- and F-values revealed that there is no significant difference between the proposed and official methods. So, the proposed method could be employed as a better alternative to the existing methods.
REFERENCES


Table 1. Optical characteristics, precision and accuracy data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>405</td>
</tr>
<tr>
<td>Beer's law limits (( \mu g/ml ))</td>
<td>0.6-15.8</td>
</tr>
<tr>
<td>Molar absorptivity (l/mol/cm)</td>
<td>( 1.64 \times 10^4 )</td>
</tr>
<tr>
<td>Sandell's sensitivity (ng/cm(^4))</td>
<td>22.22</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>8.5</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.9996</td>
</tr>
<tr>
<td>Regression equation (( Y )) (^a)</td>
<td></td>
</tr>
<tr>
<td>Slope, ( b )</td>
<td>0.0429</td>
</tr>
<tr>
<td>Intercept, ( c )</td>
<td>0.0306</td>
</tr>
<tr>
<td>Relative standard deviation (%) (^d)</td>
<td>2.12</td>
</tr>
<tr>
<td>% Error (^d)</td>
<td>1.06</td>
</tr>
<tr>
<td>Limit of detection (( \mu g/ml ))</td>
<td>0.18</td>
</tr>
<tr>
<td>Limit of quantification (( \mu g/ml ))</td>
<td>0.61</td>
</tr>
</tbody>
</table>

\(^a\) \( Y = bX + c \), where \( X \) is the concentration of drug in \( \mu g/ml \)

\(^d\) Average of five determinations
**Table 2.** Determination of CNR<sup>a</sup> in presence of excipients and additives

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount (mg)</th>
<th>% Recovery of CNR ± RSD&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium stearate</td>
<td>30</td>
<td>98.8 ± 0.82</td>
</tr>
<tr>
<td>Glucose</td>
<td>40</td>
<td>99.6 ± 0.91</td>
</tr>
<tr>
<td>Lactose</td>
<td>40</td>
<td>98.7 ± 0.94</td>
</tr>
<tr>
<td>Dextrose</td>
<td>40</td>
<td>98.8 ± 1.04</td>
</tr>
<tr>
<td>Starch</td>
<td>30</td>
<td>100.2 ± 0.89</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>40</td>
<td>99.2 ± 1.10</td>
</tr>
<tr>
<td>Talc</td>
<td>40</td>
<td>99.5 ± 0.96</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>25</td>
<td>97.4 ± 0.93</td>
</tr>
</tbody>
</table>

<sup>a</sup> 5 µg/ml of CNR taken  
<sup>b</sup> Average of five determinations

**Table 3.** Between-day precision of the assay of CNR by the proposed method

<table>
<thead>
<tr>
<th>Formulation (Tablet)</th>
<th>Amount taken/µg</th>
<th>Amount found*/µg</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cintigo&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25</td>
<td>24.62</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>73.74</td>
<td>0.92</td>
</tr>
<tr>
<td>Cinzan&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25</td>
<td>24.90</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>74.07</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*Average of five determinations  
<br><sup>a</sup>Wallace Pharmaceuticals Ltd., India  
<br><sup>b</sup>FDC Limited, India
Table 4. Determination of CNR in pharmaceutical preparations by the proposed method and its comparison with official method $^2$

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug present (mg)</th>
<th>Found(a) ± RSD, % and its comparison with official method</th>
<th>Official method</th>
<th>TB method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial tablet</td>
<td>100</td>
<td>99.66 ± 0.73</td>
<td></td>
<td>99.66 ± 0.59</td>
</tr>
<tr>
<td>Recovery</td>
<td>100</td>
<td>-</td>
<td>F =1.53, t = 1.48</td>
<td>99.78 ± 0.86</td>
</tr>
<tr>
<td>Within-day analysis</td>
<td>100</td>
<td>-</td>
<td></td>
<td>99.88 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
<td></td>
<td>99.22 ± 0.96</td>
</tr>
</tbody>
</table>

$^a$ Average of five determinations
Fig. 1. Absorption spectra of (a) reagent blank (b) and ion-association complex of CNR (9 pg/ml) with TB.

Fig. 2. Effect of TB on the absorbances of ion-association complex of CNR (10 ppm)
Fig. 3. Beer's law plot of CNR

Fig. 4. Composition of CNR-TB complex by Job's method using $1 \times 10^{-4}$ M solution of each of drug and reagent

161
Scheme 1. Probable reaction mechanism for the formation of complex of CNR with TB