CHAPTER III

Sensitive spectrophotometric methods for the assay of an antidepressant in bulk powder and dosage forms

Abstract

In this chapter, three simple, rapid and sensitive spectrophotometric methods have been proposed for the assay of trazodone hydrochloride (TRZH) in bulk powder and dosage forms. The methods are based on the oxidative coupling of TRZH with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in presence of iron (III) chloride as an oxidant [Method I] and formation of the ion-association complexes of TRZH with bromocresol green (BCG) in KCl-HCl buffer of pH 1.5 [Method II] and with bromothymol blue (BTB) in the same buffer of pH 2 [Method III]. The colored species exhibited absorption maxima at 598, 415 and 423 nm for methods I, II, and III, respectively. The systems obeyed Beer’s law in the ranges of 0.1-3.2, 0.9-17 and 0.2-14.5 µg/ml with molar absorptivity values of 0.92 x 10⁴, 1.66 x 10⁴ and 2.11 x 10⁴ l/mol/cm for MBTH, BCG and BTB, respectively. Common excipients did not interfere in the assay of TRZH in pharmaceutical formulations. The applicability of the methods was checked by analyzing various dosage forms. The results of analysis were subjected to t-test and F-test.

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GENERAL DRUG PROFILE

*Trazodone hydrochloride (TRZH)*

**Chemical name**

\[ \text{2-\{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl\} - 1,2,4 - triazolo [4,3 - a] pyridin - 3 -(2H)-one monohydrochloride} \]

**Molecular formula**

\[ \text{C}_{19}\text{H}_{23}\text{Cl}_{2}\text{N}_{5}\text{O} \]

**Molecular weight**

408.324

**Melting point**

86 - 87 °C

**Description**

White crystalline solid

**Solubility**

Readily soluble in water

**Category**

Antidepressant

INTRODUCTION

TRZH is an anti-depressant. It is generally used in the treatment of depressive disorders associated with insomnia and anxiety. The drug does not aggravate psychotic symptoms in patients with schizophrenia or schizoaffective disorders.

The official methods for the determination of TRZH are (i) potentiometric non-aqueous titration with perchloric acid\(^1\) and (ii) HPLC method using octadecyl silane column and methanol-0.01 M ammonium
phosphate buffer of pH 6 (60:40) as mobile phase. Several analytical methods have been reported for the assay of TRZH in pharmaceutical formulations. These include UV absorption measurement at 246 nm, ion-selective electrode, voltammetry, HPLC, capillary gas chromatography, gas chromatography mass spectrometry and thin layer chromatography. For single component preparations, the simplest assay method involves the direct measurement of absorbance at maximum wavelength (in UV region). TRZH is relatively weak UV absorbing compound and therefore the direct UV absorbance measurements at low concentration will be unreliable. Spectrophotometric, spectrofluorimetric and LC determination of TRZH has been reported. The reported spectrophotometric method does not discuss about the sensitivity, detection limits and stability of the method. Recently, Mohamed et al have reported spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochloride based on ion-pair formation with molybdenum and thiocyanate in pure and dosage forms. This method obeyed Beer's law in the concentration range of 2-28 μg/ml for TRZH. The effects of common excipients have not been investigated in both methods. This prompted us to develop simple, sensitive and accurate spectrophotometric methods for the determination of TRZH in pure and pharmaceutical formulations. These methods are based on the oxidative coupling of the TRZH with MBTH in presence of iron (III) as an oxidant [Method I] and formation of chloroform soluble ion-association complexes of TRZH with BCG [Method II] and with BTB [Method III].
EXPERIMENTAL

Preparation of reagents

Freshly prepared aqueous solutions of 0.4 % MBTH, 1 % iron(III) chloride, 0.1% BCG and 0.05% BTB were used in the study.

Standard drug solution

A stock solution of TRZH containing 250 µg/ml was prepared in distilled water. The solution was stable at room temperature.

Buffers

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

RECOMMENDED PROCEDURES

The following procedures were adopted for the assay of TRZH after thorough investigations of the various parameters involved in the formation of colored products.

Assay procedure for pure drug

Method I

MBTH (2 ml) was transferred into a series of 10 ml volumetric flasks. An aliquot of TRZH standard solution was added so that the final concentration of the drug was in the range of 0.1-3.2 µg/ml. Then 2 ml of ferric chloride solution was added and allowed to stand for 20 min. The volume was adjusted
to the mark with distilled water, mixed well and the absorbance was measured against a reagent blank at 598 nm. Calibration graph was constructed by plotting the values of absorbance versus concentration.

**Methods II and III**

An aliquot of the solution containing 9-170 μg for Method II or 2-145 μg for Method III of TRZH was transferred into a series of 125 ml separating funnels. A volume of 3 ml of KCl-HCl buffer of pH 1.5 for Method II or 3 ml of KCl-HCl buffer of pH 2 for Method III and 3 ml of BCG or 5 ml of BTB were added. Chloroform (10 ml) was added to each of the separating funnels, the contents were 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. The absorbances of yellow colored complexes were measured at 415 and 423 nm for BCG and BTB, respectively, against the corresponding reagent blank. The calibration graphs were constructed.

**Assay procedure for tablets**

Ten tablets were weighed and finely powdered. An amount of the powder equivalent to 100 mg of TRZH was transferred into a 100 ml beaker containing about 75 ml of distilled water. Using a mechanical stirrer, the powder was completely disintegrated in distilled water, diluted to the mark and filtered through Whatman filter paper No. 40. Further, 25 ml of the filtrate was diluted to 100 ml and a suitable amount of aliquot was taken and analyzed using the procedures given above.
RESULTS AND DISCUSSION

MBTH was introduced as a reagent for colorimetric determination of pharmaceutical compounds in 1961. When treated with an oxidizing agent, it undergoes oxidation with the loss of two electrons and one proton forming the electrophilic intermediate, which is believed to be the active coupling species.\textsuperscript{16,17} Further, the intermediate readily reacts with amine or phenol by electrophilic attack on the most nucleophilic site on the aromatic ring of the amine or phenol moiety and the resulting intermediate is spontaneously oxidized with an oxidant to form the colored species. The extent of oxidation of MBTH and the formation of oxidative coupling product depends upon the experimental conditions \textit{viz.}, pH, nature and concentration of oxidant, order of addition, temperature and time maintained during each addition. MBTH was employed as an analytical reagent in the assay of various class of compounds.\textsuperscript{18-26}

In the present investigation, MBTH and Fe (III) were used as analytical reagents for the determination of TRZH and the possible reaction mechanism is shown in Scheme 1.

In acidic buffer, the positively charged TRZH reacted with BCG and with BTB and formed respective ion-pair complexes. The probable reaction mechanisms are given in Scheme 2.

\textit{Spectral characteristics}

Method I is based on oxidative coupling of MBTH in presence of Fe (III) with TRZH. The colored product exhibited absorption maximum at
598 nm (Fig. 1.). In methods II and III, TRZH reacted with BCG and with BTB in acidic buffer and yielded chloroform soluble ion-pair complexes having absorption maxima at 415 and 423 nm, respectively (Fig. 1.).

**Standardization of experimental parameters**

In order to establish optimum reaction conditions necessary for the formation of colored species of maximum stability and sensitivity, the investigator has measured the absorbances of a series of solutions at \( \lambda_{\text{max}} \) value by varying one at a time and fixing the other. These optimum conditions are shown in the procedures.

**For Method I**

**Effect of MBTH concentration**

The effect of the concentration of MBTH was studied by measuring the absorbances at the specified wavelength as mentioned in the standard procedure for solutions containing a fixed concentration of TRZH and varying amounts of MBTH. A volume of 2 ml of 1% MBTH in a total volume of 10 ml was found to be sufficient to yield maximum color intensity. Low absorbance values were observed at higher concentrations of the reagent (Fig. 2).

**Effect of Fe\(^{3+}\) concentration**

The optimum volume of iron (III) chloride that yielded colored species of maximum intensity and stability was observed to be 2 ml of 1% (w/v) solution in a total volume of 10 ml of the reaction mixture (Fig. 3). However, at higher concentrations of oxidant, the colored species showed unstable and
low absorbance values. Further, iron (III) chloride solution made in 1 M HCl gave satisfactory results.

**Effect of reaction time**

Though the colored product was formed soon after the addition of reagents, it required 20 min to attain maximum intensity. The colored product was noticed to be stable for more than 10 h.

**For Methods II and III**

Optimum reaction conditions for quantitative formation of ion-pair complexes were established by performing a number of trials.

**Optimization of buffer**

Formation and stability of the ion-association complex depended on the type of buffer and its pH. This was investigated by using various buffers viz., 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). The colored species of maximum color intensity and stability were noticed in KCl-HCl (Clark & Lubs) buffer of pH 1.5 for BCG and in the same buffer of pH 2.0 for BTB (Fig. 4). Further, 3 ml of the buffer of respective pH produced the optimum colored species.

**Effect of concentration of reagent**

The effect of the reagent was studied by measuring the absorbances of solutions containing a fixed concentration of TRZH and varied amounts of the reagent separately. Maximum color intensity of the complex was achieved
with 3 ml of 0.1 % BCG or with 5 ml of 0.05 % BTB (Fig. 5). The absorbances decreased with increase in concentration of the respective reagent.

**Selection of organic solvent**

Several organic solvents were tried for effective extraction of the colored species from aqueous phase. Chloroform was found to be the most suitable extractant as it was noticed that only one extraction was adequate to achieve a quantitative recovery of the complex. Further, shaking times of 1 to 1.5 min produced constant absorbances.

**Order of addition of reagents**

It was observed that, there was no appreciable change in the absorbance or color of the product or stability of the complex even if the order of addition of the reactants was varied.

**Evaluation of accuracy and precision**

The accuracy of the proposed methods was assessed by analyzing six replicates of fixed amount of pure drug and the precision was established by determining the RSD for six replicate determinations on the same solution. The low % errors and the RSD values indicated the high accuracy and precision of the methods (Table 1).

**Optical characteristics of ion pair complexes**

To find whether the colored products formed in the proposed methods obeyed Beer’s law or not, the absorbances of a series of solutions containing varying amounts of TRZH were recorded. Beer’s law limits (Fig. 6), molar
absorptivity and Sandell's sensitivity values were evaluated and are shown in Table 1. Least-square regression analysis was also carried out for calculating the slope, intercept and correlation coefficient. The results are summarized in Table 1.

**Effect of temperature on the colored complexes**

It was found that the colored complexes were stable up to 34 °C. However, at higher temperatures, the absorbances increased due to evaporation of chloroform. Further, the complexes remained stable for 8.5 h and 6 h, respectively, at room temperature.

**Detection and quantification limits**

The detection limit (LOD) and Quantification limits (LOQ) for the proposed method were calculated as per the recommendation of Analytical Methods Committee. The LOD values were calculated to be 0.03, 0.27 and 0.06 μg/ml while LOQ values were observed to be 0.11, 0.92 and 0.21 μg/ml for methods I, II and III respectively.

**Interference studies**

The effects of common excipients and additives were tested for their possible interferences in the assay of TRZH. It was observed that the talc, glucose, starch, lactose, dextrose, gum acacia and magnesium stearate did not interfere in the determination of TRZH at the levels normally found in dosage forms (Table 2).
Recovery studies

The reliability and accuracy of the methods were further confirmed by recovery studies by standard addition method. For this, to a amount of the drug in the formulation (pre-analyzed), pure drug was added and the total amount of the drug was determined by the proposed methods. The percent recoveries were found to be quantitative (99.12-100.94 %).

Ruggedness

In order to check ruggedness of the proposed methods, six replicate determinations at different concentration levels of the drugs were carried out. The within-day and between-day RSD values were observed to be less than 0.9 % (Table 3). The high degree of reproducibility of results on different days indicated that proposed methods are fairly rugged.

Stoichiometry of the complexes

The stoichiometric ratio of drug to dye was determined by Job’s method of continuous variation using 1.0 x 10^{-4} M solutions of drug and respective reagent. The results indicated that the ratio of drug to reagent to be 1:1 (Fig. 7). It is also evident from reaction Scheme 2.

Analysis of pharmaceutical formulations, and statistical comparison of the results with official method

The proposed methods were successfully applied to the analysis of TRZH in commercial tablets. The performance of the proposed methods was assessed by calculation of t- and F-values. At 95% confidence level, the calculated t-
and F-values did not exceed the respective theoretical values thereby indicating that there was no significant difference in accuracy and precision between the proposed and official methods. The corresponding results are shown in Table 3.

CONCLUSIONS

The proposed methods are simple, accurate and economical with reasonable precision and accuracy. The method III is more sensitive compared to methods I and II. The proposed methods are found to be free from interference by common additives and excipients. The validity of the proposed methods is well demonstrated by analyzing the tablets containing TRZH. Hence, these methods could be readily adopted for routine quality control by pharmaceutical industries.
REFERENCES


23. A.V.S.S. Prasada, C.S.R. Lakshmib, C.S.P. Sastry, V.P. Uppuletia, 

   2001, 907.


Table 1. Optical characteristics, precision and accuracy data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values of</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MBTH</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>598</td>
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<tr>
<td>Beer's law limits (( \mu g/ml ))</td>
<td>0.1-3.2</td>
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<tr>
<td>Molar absorptivity (l/mol/cm)</td>
<td>( 0.92 \times 10^4 )</td>
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<tr>
<td>Sandell's sensitivity (ng/cm\textsuperscript{2})</td>
<td>44.5</td>
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<tr>
<td>Stability (h)</td>
<td>10.5</td>
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<tr>
<td>Correlation coefficient (R)</td>
<td>0.9936</td>
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<tr>
<td>Regression equation ((Y)) \textsuperscript{a}</td>
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<tr>
<td>Slope, b</td>
<td>0.215</td>
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<tr>
<td>Intercept, c</td>
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<tr>
<td>Relative standard deviation %) \textsuperscript{d}</td>
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<tr>
<td>% Error \textsuperscript{d}</td>
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<tr>
<td>Limit of detection (( \mu g/ml ))</td>
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<tr>
<td>Limit of quantification (( \mu g/ml ))</td>
<td>0.11</td>
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\textsuperscript{a} \( Y = bX + c \), where \( X \) is the concentration of drug in \( \mu g/ml \)

\textsuperscript{d} Average of six determinations
Table 2. Determination of TRZH\(^a\) in the presence of excipients and additives

<table>
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<tr>
<th>Material</th>
<th>Amount (mg)</th>
<th>% Recovery of TRZH ± RSD(^b)</th>
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<tr>
<td></td>
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<td>Method I</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>30</td>
<td>99.6 ± 0.82</td>
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<tr>
<td>Glucose</td>
<td>40</td>
<td>98.4 ± 0.94</td>
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<tr>
<td>Lactose</td>
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<td>98.3 ± 1.04</td>
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<td>Dextrose</td>
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<td>99.1 ± 0.89</td>
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<td>Starch</td>
<td>40</td>
<td>98.4 ± 1.10</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>40</td>
<td>99.6 ± 0.96</td>
</tr>
<tr>
<td>Talc</td>
<td>25</td>
<td>97.8 ± 0.93</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>25</td>
<td>98.6 ± 0.69</td>
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</table>

\(^a\) 2.5 µg/ml of TRZH taken

\(^b\) Average of five determinations
Table 3. Analysis of tablet, recovery and ruggedness of assay of TRZH by the proposed methods and their comparison with the Official method²

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drug present (mg)</th>
<th>Found* ± SD, % and their comparison with official method</th>
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<tr>
<td></td>
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<td>Official method</td>
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<tr>
<td>Commercial tablet</td>
<td>100</td>
<td>100.4 ± 0.78</td>
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<tr>
<td>Recovery</td>
<td>50</td>
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<tr>
<td>Between-day analysis</td>
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<td>Within-day analysis</td>
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<td>-</td>
</tr>
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</table>

* Average of six determinations
Scheme 1. Oxidative coupling reaction of TRZH with MBTH
Scheme 2. Probable reaction mechanism for formation of ion-association complexes of TRZH with BCG and BTB
Fig. 1. Absorption spectra of (a) TRZH (2.8 μg/ml)-MBTH (b) TRZH (10 μg/ml) - BCG complex and (c) TRZH (15 μg/ml) - BTB complex
Fig. 2. Effect of MBTH on the formation of oxidative coupling product

Fig. 3. Effect of FeCl₃ on absorbances of the oxidative coupling product
Fig. 4. Effect of pH of the buffer on absorbances of ion-association complexes of TRZH (10 ppm) with (a) BCG and (b) BTB

Fig. 5. Effect of reagent on absorbances of the ion-association complexes of TRZH (10 ppm) with (a) BCG and (b) BTB
Fig. 6. Beer’s law plots of TRZH for (a) MBTH (b) BCG and (c) BTB

Fig. 7. Composition (a)TRZH-BCP and (b) TRZH-BTB complex by Job’s method using 1x 10^{-4} M each of drug and reagent