CHAPTER II

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

Abstract:

Two simple, rapid and sensitive spectrophotometric methods have been developed for the determination of trazodone hydrochloride (TRZH) in bulk powder and pharmaceutical formulations. The methods are based on the formation of chloroform soluble ion-association complexes of TRZH with bromocresol purple (BCP) in NaOAc-AcOH buffer of pH 3.6 [Method I] and with methyl orange (MO) in NaOAc-HCl buffer of pH 3.29 [Method II]. The colored species were found to obey Beer’s law in the concentration ranges of 0.2-14.1 µg/ml and 1-20 µg/ml with molar absorptivity values of $2.92 \times 10^4$ and $1.37 \times 10^4$ l/mol/cm for BCP and MO at 408 and 422 nm, respectively. The effects of common excipients were investigated in the assay of TRZH in pharmaceutical formulations. The applicability of the methods was examined by analyzing various pharmaceutical preparations. The results have been subjected to t-test and F-test.

The results of this Chapter have been published in Chemical and Pharmaceutical Bulletin (Japan), 54 (7) 968-971, 2006 and in Journal of Serbian Chemical Society, 71 (7) 829-837, 2006.
GENERAL DRUG PROFILE

Trazodone hydrochloride (TRZH)

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

Structure:

Molecular formula: C_{19}H_{23}Cl_{2}N_{5}O

Molecular weight: 408.32

Melting point: 86 - 87 °C

Description: White odorless crystalline solid

Solubility: Readily soluble in water and organic solvents

Category: Antidepressant
INTRODUCTION

TRZH is a triazolopyridine derivative with antidepressant effect [1]. 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. In animals, TRZH selectively inhibits serotonin uptake by brain synaptosomes and potentiates the behavioral changes induced by the serotonin precursor, 5-hydroxytryptophan. 

*In vitro* studies in human liver microsomes show that trazodone is metabolized to an active metabolite, m-chlorophenylpiperazine (mCPP) by cytochrome P450 3A4 (CYP3A4). Other metabolic pathways that may be involved in metabolism of trazodone have not been well characterized.

The Official methods of assay of TRZH include potentiometric non-aqueous titration with perchloric acid [2] and HPLC method using octadecyl silane column and methanol-0.01 M ammonium phosphate buffer of pH 6 (60:40) as mobile phase [3]. In view of its biological importance, several analytical methods including UV absorption measurement at 246 nm [4], spectrofluorimetry [5], ion-selective electrode [6,7], voltammetry [8,9], HPLC [3,10-12], capillary gas chromatography [13], gas chromatography mass spectrometry [14], thin layer chromatography [15], AES and AAS [16] methods have been reported for the assay of TRZH in pharmaceutical formulations. For single component preparations, the simplest assay method involves the direct measurement of absorbance at maximum wavelength in UV region. Since, TRZH is relatively weak UV absorbing compound and its
determination in UV region at low concentrations may not be reliable. Gindy et al have reported spectrophotometric, spectrofluorimetric and LC determination of TRZH [17]. This spectrophotometric method does not discuss about the sensitivity, detection limits and stability. Recently, spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochloride based on ion-pair formation with molybdenum and thiocyanate in pure and dosage forms has been described by Mohamed et al [18]. The proposed methods have not investigated the effects of common excipients in the essay of TRZH. This prompted us to develop simple, sensitive and accurate spectrophotometric methods for the determination of TRZH in pure and pharmaceutical formulations. The proposed methods are based on the formation of chloroform soluble ion-association complexes of TRZH with BCP in NaOAc-AcOH buffer of pH 3.6 [Method I] and with MO in NaOAc-HCl buffer of pH 3.29 [Method II].

**EXPERIMENTAL**

*Preparation of reagents*

Freshly prepared aqueous solutions of each of 0.1% BCP and MO 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 observed to be stable at 28 °C.
Buffers

The following buffers were prepared using standard methods [19-21] :

1. NaOAc-HCl buffers of pH 0.65-5.2 using 1M each of NaOAc and HCl.

2. NaOAc-AcOH buffers of pH 3.6-5.6 (by mixing appropriate volumes of 
   0.2 M each of NaOAc and AcOH).

3. Potassium hydrogen phthalate-HCl buffers of pH 2.2-3.6 from 0.1M 
   each of potassium hydrogen phthalate and HCl.

4. KCl-HCl buffers of pH 1.0-2.2 (by mixing appropriate volumes of 
   0.2 M each of KCl and HCl).

RECOMMENDED PROCEDURES

After a detailed and systematic study of various parameters involved in 
the formation of ion-association complexes (as described under results and 
discussion), the following procedures were adopted for the assay of TRZH.

Assay procedure for pure drug sample

Methods I and II

Suitable amounts of aliquots of pure drug solution containing 2-141 µg 
for Method I or 10-200 µg for Method II were transferred into a series of 
125 ml separating funnels. A volume of 4 ml of NaOAc-AcOH buffer of 
pH 3.6 for Method I or 5 ml of NaOAc-HCl buffer of pH 3.29 for Method II 
and 3 ml of BCP or 2 ml of MO were added. It was diluted to 25 ml with 
distilled water. 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 recorded at 408 nm and at 422 nm for BCP and MO, respectively, against the corresponding reagent blank. The calibration graphs were constructed by plotting the values of absorbance versus concentration.

**Assay procedure for tablets**

Ten tablets were weighed and finely powdered. An amount of the powder equivalent to 50 mg of TRZH was transferred into a 250 ml beaker containing about 60 ml distilled water. Using a mechanical stirrer, the powder was completely disintegrated in distilled water, diluted to 100 ml and filtered through Whatman filter paper No. 40. Further, 10 ml of the filtrate was diluted to 50 ml and a suitable amount of aliquot was taken and analyzed following the procedures given above.

**RESULTS AND DISCUSSION**

Extractive spectrophotometric methods have been commonly employed in the assay of various class of drugs. These are popular for their sensitivity. Hence, ion-pair extractive spectrophotometry has received a considerable attention for quantitative determination of several pharmaceutical compounds [22-25].

The positively charged TRZH reacted with BCP and with MO and yielded respective ion-pair complexes in acidic buffer. These complexes were found to be quantitatively extractable into chloroform. The probable reaction mechanism showing the formation of ion-pair complexes is shown in Reaction Scheme 1.
**Spectral characteristics**

In order to determine the wavelength of maximum absorption ($\lambda_{\text{max}}$) of TRZH-dye complex formed in the proposed method, suitable amount (within Beer’s law limit) of the drug was taken and the reaction product was developed following the procedure. The absorption spectrum was scanned on a spectrophotometer in the wavelength region of 300-600 nm against the reagent blank. The complexes exhibited absorption maxim at 408 nm and 422 nm for BCP and MO, respectively. The corresponding reagent blank showed a negligible absorbance at respective $\lambda_{\text{max}}$ (Fig. 1) thereby permitting good analytical conditions for the assay of TRZH. Hence, all the subsequent measurements were carried out at the corresponding $\lambda_{\text{max}}$.

**Optimization of reaction conditions**

In order to establish optimum reaction conditions necessary for the formation of ion-pair complexes of maximum stability and intensity, the absorbances of a series of solutions at the respective $\lambda_{\text{max}}$ value were recorded by varying one at a time and fixing the rest. These optimum conditions have been incorporated in the procedures for quantitative determination.

**Selection of buffer**

It was noticed that the formation, intensity and stability of the ion-association complex depended on the type of buffer used and its pH. This was examined by employing various buffers *viz.*, KC1-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). Stable and intense colored complexes were formed in NaOAc-AcOH buffer of pH 3.6 for BCP and in NaOAc-HCl buffer of pH 3.29 for MO (Fig. 2). Further, the effect of volume of the buffer was investigated. It was noticed that 4 ml of NaOAc-AcOH buffer of pH 3.6 and 5 ml of NaOAc-HCl buffer of pH 3.29 yielded the optimum colored species (Fig. 3).

**Effect of concentration of reagent**

In order to examine the effect of the reagent, the absorbances of solutions containing a fixed concentration of TRZH and varied amounts of the respective reagent were measured, separately. It was observed that the maximum color intensity of the complex was obtained with 3 ml of 0.1% BCP or with 2 ml of 0.1% MO (Fig. 4). The absorbance values decreased with increase in concentration of the respective reagent.

**Selection of organic solvent**

Selection of a suitable organic solvent is an important step in extractive spectrophotometric determination of a drug. In the present study, several organic solvents viz., toluene, carbon tetra chloride, benzene, ether, chloroform and dichloromethane were employed for quantitative extraction of the colored complexes from aqueous phase. Among these solvents, chloroform was found to be the most suitable extractant. Further, it was noticed that only one extraction was sufficient to achieve a quantitative recovery of the complex. A shaking time of 1-1.5 min yielded constant absorbance throughout.

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**Order of addition of reagents**

In the present investigation, it was seen that there was no appreciable change in the absorbance or color of the species or stability of the complex even if the order of addition of the reactants was varied.

**Evaluation of accuracy and precision**

The precision of the proposed method was ascertained from absorbance values obtained by actual determination of six replicates of 10 µg/ml of drug for BCP and 12 µg/ml of drug for MO. The low values of percent relative standard deviation recorded in Table 1 indicated good precision of the proposed methods.

In order to determine the accuracy of the proposed method, six replicates of 10 µg/ml of drug for BCP and 12 µg/ml of drug for MO were taken and analyzed. The percentage errors were calculated and are given in Table 1. These values revealed that the proposed methods were reasonably accurate.

**Optical characteristics of ion pair complexes**

In order to examine the range in which the colored ion-association complexes obeyed Beer's law, the absorbances of a series of solutions containing increased amounts of drug were measured at corresponding $\lambda_{\text{max}}$ against the respective reagent blank. Beer’s law plots are shown in Fig. 5. Regression analysis of Beer’s law plot revealed a good correlation between absorbance and concentration of colored product in the concentration range.
given in Table 1. The graph of absorbances versus concentration showed almost zero intercept and is described by regression equation, $Y = bX + c$ (where $Y$ is the absorbance of a 1 cm layer, $b$ is the slope, $c$ is the intercept and $X$ is the concentration of the drug in µg/ml obtained by least-squares method.

The values of slope, intercept and correlation coefficient are shown in Table 1. Molar absorptivity and Sandell's sensitivity values were calculated from Beer's law data and the results are recorded in Table 1. Higher values of molar absorptivity indicated good sensitivity of the methods.

**Effect of temperature on the colored complexes**

The dependence of stability of ion-association complexes on temperature was investigated by recording the absorbance of the solution at different temperatures. It was noticed that the colored complexes were stable up to $34^\circ$C. However, at higher temperatures the absorbances increased due to evaporation of chloroform. Further, the complexes remained stable for 6.5 h and 8.25 h, for methods I and II, respectively, at $28^\circ$C.

**Detection and quantification limits**

According to the Analytical Methods Committee [26], the detection limit (LOD) is the concentration of drug corresponding to a signal equal to the blank mean ($Y_B$) plus three times the standard deviation of the blank ($S_B$). Quantification limits (LOQ) is the concentration of TRZH corresponding to the blank mean plus ten times the standard deviation of the blank. The LOD and LOQ values for the proposed method were calculated and are summarized in Table 1.
Interference studies

The selectivity and possible analytical applications of the proposed methods were investigated by studying the effects of excipients and other substances, which often accompany with TRZH in various pharmaceutical formulations. This was carried out by adding different amounts of foreign substances to known amount of the drug and color was developed following the procedure described earlier. Generally, a compound was considered to be interfered with the determination if the observed absorbance values differed by more than ±2% from that for the drug alone. In the present study, the effects of talc, glucose, starch, gum acacia, sodium alginate, lactose, dextrose and magnesium stearate in the assay of TRZH were examined. The corresponding results are shown in Table 2. It was evident that the excipients and other substances did not interfere in the assay of drug at the levels found in formulations as evident from high percent recovery values. Thus, the proposed methods were noticed to be free from interferences by various substances.

Recovery studies

The reliability and accuracy of the methods were further confirmed by recovery studies by standard addition method. For this, known quantities of pure TRZH 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 recoveries ranged from 98.92 to 101.86%.
**Ruggedness**

In order to examine ruggedness of the proposed methods, six replicate determinations at different concentration levels of the drug were carried out and RSD values for within-day and between-day results were calculated. These values were found to be less than 1.0% (Table 3). The low values of RSD revealed high degree of reproducibility of results. Hence, the proposed methods were considered to be fairly rugged.

**Stoichiometry of the complexes**

The stoichiometric ratio of drug to dye in the complex was determined by Job’s method of continuous variation [27] using $1.25 \times 10^{-4}$ M solutions of each of drug and dye. The results indicated that the ratio of drug to reagent to be 1:1. It is also evident from Reaction Scheme 1.

**Analysis of pharmaceutical formulations and statistical comparison of the results with official method [3]**

The utility of the proposed method for the assay of TRZH in tablet was examined. The results of assay of tablet containing TRZH are summarized in Table 3. Further, these results were compared statistically by Student t-test and by the variance ratio F-test with those of official method. It was observed that the Student t-values at 95% confidence level did not exceed the theoretical value thereby indicating that there was no significant difference between the accuracy of the proposed and official methods. Further, the variance ratio F-values calculated for $p=0.05$ did not exceed the theoretical value thereby
revealing that there was no significant difference between the precision of 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. Unlike the gas chromatographic and HPLC procedures, the spectrophotometric methods are simple and of not high cost. In spectrophotometric analysis, the importance lies in the chemical reaction upon which the procedure is based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of great interest in analytical pharmacy as it offers distinct possibility in the assay of a particular active component in complex dosage forms. The reagents employed in the present study are cheaper, readily available and the procedures do not involve tedious sample preparations. Further, the method is free from interference by common additives and excipients. The applicability of the new procedures for routine quality control is well demonstrated by the assay of TRZH in pure form and pharmaceutical preparations. Hence, the proposed methods could be employed for quality assurance.
REFERENCES


Table 1. Optical characteristics, precision and accuracy data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BCP</th>
<th>MO</th>
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<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>408</td>
<td>422</td>
</tr>
<tr>
<td>Beer's law limits (µg/ml)</td>
<td>0.2-14.1</td>
<td>1-20</td>
</tr>
<tr>
<td>Molar absorptivity (l/mol/cm)</td>
<td>(2.92 \times 10^4)</td>
<td>(1.37 \times 10^4)</td>
</tr>
<tr>
<td>Sandell's sensitivity (ng/cm(^2))</td>
<td>13.982</td>
<td>29.59</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>6.50</td>
<td>8.25</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.9989</td>
<td>0.9994</td>
</tr>
<tr>
<td>Regression equation (Y) (^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0459</td>
<td>0.0336</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0379</td>
<td>0.0032</td>
</tr>
<tr>
<td>Relative standard deviation (%) (^d)</td>
<td>0.83</td>
<td>0.89</td>
</tr>
<tr>
<td>% Error (^d)</td>
<td>0.98</td>
<td>1.09</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.071</td>
<td>0.31</td>
</tr>
<tr>
<td>Limit of quantification (µg/ml)</td>
<td>0.236</td>
<td>1.03</td>
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</table>

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

\(^d\) Average of six determinations.
Table 2. Determination of TRZH in presence of excipients and additives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (mg)</th>
<th>% Recovery of TRZH ± % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method I&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>40</td>
<td>99.39 ± 0.82</td>
</tr>
<tr>
<td>Glucose</td>
<td>40</td>
<td>99.41 ± 0.94</td>
</tr>
<tr>
<td>Lactose</td>
<td>30</td>
<td>99.82 ± 1.04</td>
</tr>
<tr>
<td>Dextrose</td>
<td>30</td>
<td>100.74 ± 0.89</td>
</tr>
<tr>
<td>Starch</td>
<td>30</td>
<td>100.24 ± 1.10</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>40</td>
<td>99.44 ± 0.96</td>
</tr>
<tr>
<td>Talc</td>
<td>30</td>
<td>98.96 ± 0.93</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>30</td>
<td>99.05 ± 0.69</td>
</tr>
</tbody>
</table>

<sup>a</sup> 10.0 μg/ml of TRZH was taken (n = 5).

<sup>b</sup> 12.0 μg/ml of TRZH was taken (n = 5).
Table 3. Analysis of tablet, recovery and ruggedness of assay of TRZH by the proposed methods and their comparison with the Official method [3].

<table>
<thead>
<tr>
<th>Drug (Tablet)</th>
<th>Label claim (mg per tablet)</th>
<th>%Recovery ± %RSD and their comparison with official method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Official method</td>
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<tr>
<td>Trazalon a</td>
<td>100</td>
<td>100.4 ± 0.78</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>%Recovery ± %RSD</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Between-day analysis</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Within-day analysis</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

* Average of six determinations.

a Marketed by Sun pharmaceuticals Ltd., India.

Tabulated t - value of 95% confidence limit level is 2.57 for n = 6.

Tabulated F- value of 95% confidence limit level is 5.05 for n = 6.
**Reaction Scheme 1.** Probable reaction mechanism for formation of ion-association complexes of TRZH with BCP and MO
Fig. 1. Absorption spectra of (a) TRZH (14 μg/ml)-BCP (b) corresponding reagent blank, (c) TRZH (14 μg/ml) - MO complex and (d) respective reagent blank.

Fig. 2. Effect of buffer pH on the absorbances of the colored complexes of (a) TRZH (10 μg/ml) – BCP and (b) TRZH (14 μg/ml) - MO.
Fig. 3. Effect of volume of buffer on the absorbances of colored complexes of (a) TRZH (10 µg/ml) - BCP and (b) TRZH (14 µg/ml) - MO.

Fig. 4. Effect of reagent on the absorbances of the colored complexes of (a) TRZH (10 µg/ml) - BCP and (b) TRZH (14 µg/ml) - MO.
Fig. 5. Beer's law plots of TRZH for (a) BCP and (b) MO.