Sensitive Extractive Spectrophotometric Methods for the Determination of Trazodone Hydrochloride in Pharmaceutical Formulations

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Two simple, rapid and sensitive extractive spectrophotometric methods have been developed for the assay of trazodone hydrochloride (TRH) in pure and pharmaceutical formulations. These methods are based on the formation of chloroform soluble ion-association complexes of TRH with bromothymol blue (BTB) and with bromocresol purple (BCP) in KCl–HCl buffer of pH 2.0 (for BTB) and in NaOAc–AcOH buffer of pH of 3.6 (for BCP) with absorption maximum at 423 nm and at 408 nm for BTB and BCP, respectively. Reaction conditions were optimized to obtain the maximum color intensity. The absorbance was found to increase linearly with increase in concentration of TRH, which was corroborated by the calculated correlation coefficient values (0.9996, 0.9945). The systems obeyed Beer's law in the range of 0.2—14.5 and 0.2—14.1 μg/ml for BTB and BCP, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. No interference was observed from common excipients present in pharmaceutical formulations. The proposed methods are simple, accurate and suitable for quality control applications.

Key words spectrophotometric determination; trazodone hydrochloride

Trazodone hydrochloride, 2-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-1,2,4-triazolo[4,3-*a*]pyridin-3-(2*H*)-one monohydrochloride, is a anti-depressant (Fig. 1). It has been shown to be effective in patients with major depressive disorders and other subsets of depressive disorders. It is generally more useful in depressive disorders associated with insomnia and anxiety. This drug does not aggravate psychotic symptoms in patients with schizophrenia or schizoaffective disorders.

The official method for the determination of trazodone hydrochloride (TRH) is potentiometric non-aqueous titration with perchloric acid[1] and HPLC using octadecyl silane column and methanol–0.01 M ammonium phosphate buffer pH 6.0 (60 : 40) as mobile phase.[2] Analytical methods that are reported for the determination of TRH in pharmaceutical formulations include UV absorption measurement at 246 nm,[3] ion-selective electrode,[4,5] voltammetry[6,7] and HPLC.[2,8] Various chromatographic methods have been reported for the determination of TRH in biological fluids including HPLC,[9,10] capillary gas chromatography,[11] gas chromatography mass spectrometry[12] and instrumental thin layer chromatography.[13] Though modern methods of analysis (HPLC, GLC, NMR and Mass) for purity assay of any drug afford simplicity, speed, good specificity and excellent precision and accuracy, they involve sophisticated equipments, which are not in the reach of most laboratories and small-scale industries. Moreover, they pose problems of maintenance.

TRH is relatively weak UV absorbing compound and hence the direct UV absorbance measurements at low concentration will be unreliable. Recently, spectrophotometric, spectrofluorimetric and LC determination of TRH has been reported.[14] The reported spectrophotometric method does not discuss about the sensitivity, detection limits and stability of the method. More over, the effect of common excipients has not been investigated by spectrophotometric method. This prompted us to develop simple, sensitive and accurate spectrophotometric methods for the determination of TRH in pure and pharmaceutical formulations. These methods are sensitive and based on the formation of chloroform soluble ion-association complexes of TRH with BTB and with BCP in KCl–HCl buffer of pH 2.0 for BTB and in NaOAc–HCl buffer of pH 3.6 for BCP.

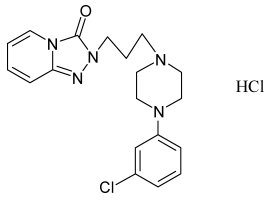

Fig. 1. Structure of Trazodone Hydrochloride

Experimental

Apparatus The absorption spectra were recorded on a double beam CARY 50-BIO UV–Visible spectrophotometer (Varian, Australia) with 1cm matched quartz cells. The pH measurements were made on a Schott Gerate pH meter CG 804.

Reagents and Chemicals All chemicals used were of analytical or pharmaceutical grade and quartz processed high-purity water was used throughout. Trazodone hydrochloride was obtained as a gift sample from Protec, Mumbai, India. Aqueous solutions of BTB (0.05%) and BCP (0.1%) were prepared separately in high purity water. Series of buffer solutions of KCl–HCl (pH=1.0—2.2), NaOAc–HCl (pH=1.99—4.92), NaOAc–AcOH (pH=3.6—5.6) and potassium hydrogen phthalate–HCl (pH=2.2—3.6) were prepared by following the standard methods.

A stock solution of TRH containing 250 μg/ml was prepared in distilled water. The solution is stable at room temperature. Commercial tablets of TRH were obtained from different firms.

Assay Procedure for Pure Drug An aliquot of the solution containing 2—145 μg (for BTB) or 2—141 μg (for BCP) of TRH were transferred into a series of 125 ml separating funnels. A volume of 3 ml of KCl–HCl buffer of pH 2.0 (for BTB) or 4 ml of NaOAc–AcOH buffer of pH 3.6 (for BCP), and 5 ml of BTB or 3 ml BCP 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 the yellow colored complexes were measured at 423 and 408 nm for BTB and BCP, respectively, against corresponding reagent blank. A calibration graph was plotted.

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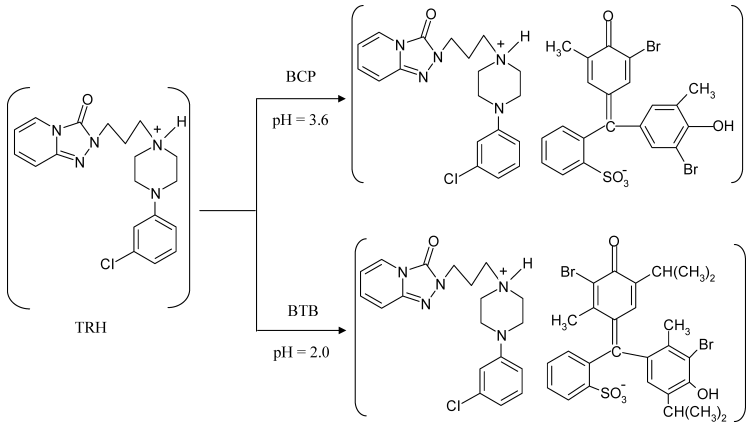

Fig. 2. Probable Reaction Mechanism for the Formation of Ion-Association Complexes of TRH with BCP and BTB

Assay Procedure for Tablets Six tablets were weighed and powdered. An amount of the powder equivalent to 100 mg of TRH was weighed into a 100 ml volumetric flask containing about 75 ml of distilled water. It was shaken thoroughly for about 15—20 min, filtered through a Whatman filter paper No. 40 to remove the insoluble matter and diluted to the mark with distilled water. A volume of 25 ml of the filtrate was diluted to 100 ml and a suitable aliquot was analyzed using the procedure given above.

Result and Discussion

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and hence, ion-pair extractive spectrophotometry has received a considerable attention for the quantitative determination of many pharmaceutical compounds.[15—18] TRH reacts with BTB and with BCP in acidic buffer to give chloroform soluble ion-association complexes (Fig. 2), which exhibit absorption maxima at 423 and at 408 nm for BTB and BCP, respectively (Figs. 3A, B). Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of TRH. The drug to dye stoichiometric ratio was determined by Job's method of continuous variation[19] and was found to be 1 : 1 with BTB as well as with BCP.

Optimization of Reaction Conditions Optimum reaction conditions for quantitative determination of ion-pair complexes were established *via* a number of preliminary experiments. It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl–HCl (pH=1.0—2.2), NaOAc–HCl (pH=1.99—4.92), NaOAc–AcOH (pH=3.6—5.6) and potassium hydrogen phthalate–HCl (pH=2.2—3.6). It was noticed that the maximum color intensity and constant absorbances were observed in KCl–HCl buffer (Clark and Lubs) of pH in the range of 1.9—2.1 (for BTB) and in NaOAc–AcOH buffer of pH in the range of 3.6—3.75 for BCP. Low absorbance values were observed for the buffers having more than or less than the above pH ranges with the respective reagent (BTB or BCP) since the ion-association complexes were not formed quantitatively. Moreover, these complexes were found to be less stable in the pH ranges other than mentioned above. Hence, Clark and Lubs buffer of pH 2.0 for BTB and NaOAc–AcOH buffer of pH 3.6 for BCP were selected for all subsequent measurements. Further, 3 ml of KCl–HCl buffer for BTB and

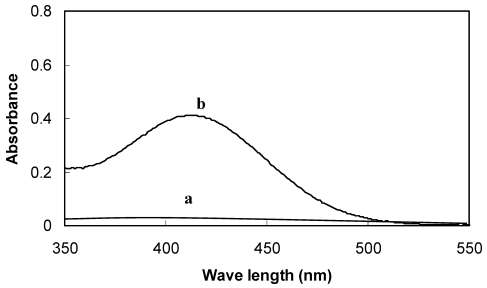

Fig. 3A. Absorption Spectrum of Reagent Blank (a) and Ion-Association Complex of TRH (8 μg/ml) with BTB (b)

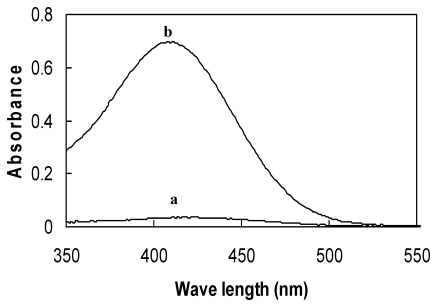

Fig. 3B. Absorption Spectrum of Reagent Blank (a) and Ion-Association Complex of TRH (13 μg/ml) with BCP (b)

4 ml of NaOAc–AcOH buffer for BCP gave maximum absorbances and reproducible results. The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of TRH and varied amounts of the respective reagent. Maximum color intensity of the complex was achieved with 5 ml of 0.05% BTB or with 3 ml of 0.1% BCP. Although a larger volume of the reagent had no pronounced effect on the complex formation, the absorbances increased slightly due to background of the colored reagent. Several organic solvents *viz*., chloroform, carbon tetrachloride, ethyl acetate, xylene, diethylether, butyl acetate, toluene, dichloromethane and chlorobenzene were tried for effective extraction of the colored species from aqueous phase. Only partial extraction of the complex was achieved with solvents other than chloroform. It was observed that only one extraction was adequate to achieve a quantitative recovery of the complex with chloroform. Shaking times of 0.5 to 2 min produced constant absorbances and hence a shaking time of 1 min was maintained throughout.

There was no appreciable change in the absorbance or color of the product even if the order of addition of the reactants was varied.

Effect of Temperature on the Colored Complexes The effect of temperature on colored complexes was investigated by measuring the absorbance values at different temperatures. It was found that the colored complexes were stable up to 35 °C. At higher temperatures, the drug concentration was found to increase due to volatile nature of the chloroform. As a result, the absorbances of the colored complexes increased. However, the complexes were stable for up to 6 h for BTB and for up to 6.5 h for BCP at room temperature.

Detection and Quantification Limits According to the Analytical Methods Committee,[20] the detection limit (LOD) is the concentration of TRH corresponding to a signal equal to the blank mean (YB) plus three times the standard deviation of the blank (SB). Quantification limits (LOQ) is the concentration of TRH corresponding to the blank mean plus ten times the standard deviation of the blank.

The LOD values were found to be 0.066 and 0.071 μg/ml for TRH with BTB and with BCP, respectively. The LOQ values were observed to be 0.219 and 0.236 μg/ml for TRH with BTB and with BCP, respectively. These values indicate that the BTB method is more sensitive compared to BCP method.

Quantification The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are given in Table 1. Regression analyses of Beer's law plots at their respective max values revealed a good correlation. Graphs of absorbances *versus* concentration showed zero intercept, and are 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 results are summarized in Table 1.

Recovery Studies Recovery studies were carried out by standard addition method. For this, known quantities of pure TRH 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 obtained were quantitative (99.63—99.89%), indicating good accuracy of the methods.

Interference Studies The effects of common excipients and additives were tested for their possible interferences in the assay of TRH. 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.

Ruggedness To ascertain the ruggedness of the methods, six replicate determinations at different concentration levels of the drugs were carried out. The within-day RSD values were found to be less than 1%. The values of between-day RSD for different concentrations of drugs obtained from determinations are given in Table 2, and indicate that the proposed methods have reasonable ruggedness.

Analysis of Pharmaceutical Formulations, and Statistical Comparison of the Results with Official Method[2] The proposed methods were successfully applied to the analysis of TRH in commercial tablet. The results of analysis of pharmaceutical formulations (Table 2) were compared statistically by Student t-test and by the variance ratio F-test with those obtained by reported method. The Student t-values at 95% confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and reported methods. It was also observed that the variance ratio F-values calculated for $p=0.05$ did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and Official methods.

Conclusions

Unlike the gas chromatographic and HPLC procedures, the spectrophotometer is simple and is not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers dis-

Table 1. Optical Characteristics, Precision and Accuracy Data

Parameter	BTB	BCP
λ_{max} (nm)	423	408
Beer's law limits (μg/ml)	0.2—14.5	0.2—14.1
Molar absorptivity (l mol^{-1} cm^{-1})	2.11×10^4	2.92×10^4
Sandell's sensitivity (ng cm^{-2})	19.315	13.982
Stability (h)	6	6.5
Correlation coefficient (R)	0.9996	0.9989
Regression equation (Y)[a]		
Slope, b	0.0434	0.0459
Intercept, c	0.0641	0.0679
Relative standard deviation (%)[b]	1.0526	0.8267
% Range of error [b] (95% confidence limit)	0.87	0.98
Limit of detection (μg ml^{-1})	0.066	0.071
Limit of quantification (μg ml^{-1})	0.219	0.236

a) $Y=bX+c$, where X is the concentration of drug in μg/ml. *b*) Average of six determinations.

Table 2. Analysis of Tablet, Recovery and Ruggedness of Assay of TRH by the Proposed Methods and Their Comparison with the Official Method[2]

Sample	Drug present (mg)	Found[a] ± S.D., % and their comparison with Official method		
		Official method	BTB method	BCP method
Commercial tablet	100	100.4±0.78	99.56±0.69 F=1.27 t=1.49	99.24±0.84 F=1.16 t=1.97
Recovery	100	—	99.38±0.46	99.23±0.55
Between-day analysis	100	—	99.12±0.87	100.11±0.59
Within-day analysis	100	—	99.66±0.67	99.84±0.98

a) Average of six determinations.

tinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of TRH in pure form and in pharmaceutical preparations.

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