## Original Research Article

# Spectrophotometric Determination of Trimipramine in Tablet Dosage Form via Charge Transfer Complex Formation

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## Abstract

**Purpose**: To develop and validate simple, rapid and sensitive spectrophotometric procedures for determination of trimipramine in tablet dosage form.
**Methods**: The methods were based on the interaction of trimipramine as n-electron donor with the $\sigma$-acceptor, iodine and various $\pi$-acceptors, namely: chloranil (CH), chloranilic acid (ChA), 2,3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ), and 7, 7,8, 8 tetracyanoquinodimethane (TCNQ), to form charge transfer complexes. The complexes obtained were measured spectrophotometrically at 292, 220, 520, 302, and 824 nm for $I_2$, CH, ChA, DDQ, and TCNQ, respectively. Different variables affecting the reaction were carefully studied and optimized.
 **Results:** Beer's law was obeyed over the concentration ranges 1 - 5, 5 - 50, 15 - 100, 5- 50, and 10 -75 ppm for $I_2$, CH, ChA, DDQ, and TCNQ respectively, with apparent molar absorptivities of $7.1 \times 10^4$, $0.3 \times 10^4$, $1.6 \times 10^4$, $0.26 \times 10^4$, and $0.1 \times 10^4$ $l\ mol^{-1}cm^{-1}$ respectively. The proposed methods were successfully applied to the determination of trimipramine with good accuracy and precision.
**Conclusion:** The results demonstrated that the developed methods are as accurate, precise and reproducible as the pharmacopeial method. The methods would be valuable for routine application in quality control.

*Keywords: Charge-transfer complex, Trimipramine, Spectrophotometry (T and S)*

## INTRODUCTION

Trimipramine maleate, 5-(3-dimethylamino-2-methylpropyl)-10, 11-dihdro-5H-dibenz [b, f] azepine acid maleate (Figure 1) [1], is a tri-cyclic antidepressant agent with an anxiety-reducing and sedative activity that substantiates its efficacy in the treatment of primary insomnia. Due to its pharmacological profile, trimipramine might be active as an antipsychotic. The pharmacological activity of trimipramine and its inherent toxicity has prompted the development of several methods for its determination.
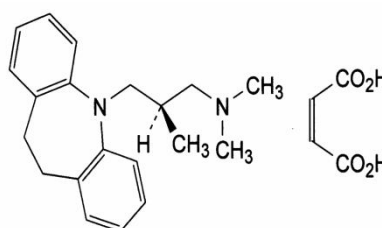


**Figure 1:** Chemical structure of trimipramine maleate

Various methods cited in literature for its determination in pharmaceutical formulations involve potentiometry [2], amperometry [2], voltammetry [3], high performance liquid chromatography (HPLC) using either RP-chromatography [4] or enantiomeric separation [5,6], HPLC-mass spectrometry [7], capillary electrophoresis (CE) [8], and spectrophotometry [9-12]. However, most of these methods involve time-consuming procedures, derivatization and/or sophisticated instruments that are not available in most quality control laboratories.

Spectrophotometry represents an attractive common technique adequate for solving many analytical problems, particularly when using the capabilities of modern instruments available nowadays. However, few spectrophotometric methods based on its oxidation have been reported for the analysis of trimipramine [9-12].

The molecular interaction between electron donors and acceptors are generally associated with formation of charge-transfer complexes [13,14], which absorb radiation in both ultra-violet and visible region. The rapid formation of the charge - transfer complexes by the electron donating compound with various acceptors lead to their utility in the development of simple and convenient spectrophotometric methods for these compounds. The aim of the present study was directed to investigate the charge transfer reaction with trimipramine which has not yet been reported. The application of $I_2$, CH, ChA, DDQ and TCNQ were selected due to their high electron affinities to achieve the spectrophotometric determination of trimipramine in its pure samples and in dosage form.

## EXPERIMENTAL

### Apparatus

A double beam ultraviolet-visible spectrophotometer (Thermo Scientific, England) with 1-cm quartz cells was used.

### Materials and reagents

Iodine (resublimed) was obtained from Riedel De-Haen AG. Germany; 0.5 % of its solution in chloroform was stable for at least 1 week at 4°C. Chloranil, ChA and DDQ were purchased from Merck, Schuchardt, Munich, Germany: 1 mg/ml solution of each of them was prepared in acetonitrile. The solution was prepared fresh daily. 7,7,8,8 tetracyanoquinodimethane was obtained from Aldrich Chemical Co., Milwaukee, USA, and its solution (2 mg/ml) was prepared in

acetonitrile and was stable for at least 1 week at 4 °C. Pure trimipramine maleate( $\geq$ 98%) was purchased from Sigma (St. Louis, Mo, USA, 1 mg/ml solution (in acetonitrile) of which was prepared freshly daily. Working solutions of trimipramine (100 and 50 µg/ml) were obtained by suitable dilutions of the stock solution with acetonitrile. All solvents and other chemicals used were of analytical reagent grade.

### Trimipramine-iodide change transfer complex method

Aliquots of the standard drug solutions covering the concentration ranges were transferred into a series of 50 ml measuring flask, followed by 5 ml of 0.5 % of iodine solution. The solutions were kept aside for 30 min, diluted to volume (50 ml) with chloroform and mixed well. The absorbance of the resulting solution was measured at 292 nm against a reagent blank prepared simultaneously.

### Preparation of test solution from tablets

Twenty tablets (Surmontil capsule) were weighed, powdered, mixed thoroughly, and a quantity of the powder equivalent to about 10 mg of trimipramine was dissolved in chloroform. The solution was filtered through Whatman filter paper (no. 41) into a 100 ml volumetric flask and diluted to the mark with chloroform. An appropriate aliquot was then taken in a 50 ml volumetric flask such that the final concentration was within the test range (1-5 µg/ml). The assay of trimipramine content was carried out as described in a previous section (trimipramine-iodide charge transfer complex).

### General procedure for π-acceptor

One milliliter of the standard or sample solution of trimipramine was transferred into a 10 ml calibrated flask. One milliliter (1.5 ml in the case of TCNQ) of the acceptor solution was added, the reaction was allowed to proceed at room temperature (25 ± 5 °C) for 30 min (in the case of CH) and heated at 70 °C for 20 min (in the case of TCNQ). The reaction, in the case of ChA and DDQ, was achieved instantaneously. The solutions were diluted to volume with acetonitrile. The absorbance of the resulting solutions were measured at the wavelengths of maximum absorption of 220, 520, 302 and 842 nm for CH, ChA, DDQ and TCNQ, respectively, against reagent blank treated similarly. The calibration graph was prepared by plotting absorbance *versus* concentration of trimipramine.

## Validation of the proposed method

Under the specified optimum reaction conditions, the calibration curve for trimipramine was constructed. Regression equation for the data was derived using least-squares method. Each concentration of standard solution was tested five times, and the mean absorbance obtained which was then plotted versus concentration.

### *Determination of accuracy and ruggedness of method*

The precision and accuracy of the method were investigated based on inter-day variation (repeatability) assessment by analyzing trimipramine using six replicates within the limit of quantification range. The precision and accuracy of the method were expressed as RSD and recovery of the measured concentration, respectively. Also reproducibility (within day or intraday variation) was investigated.

The ruggedness of the spectrophotometric method was evaluated by carrying out the analysis using different analysts (operator) and different instruments on different days.

## RESULTS

### Effect of reagent concentration

When various concentrations of acceptor were added to a fixed concentration of trimipramine it was found that 5 ml of 0.5 % w/v solution of iodine, 1.0 ml of 0.1% solution of either CH, ChA or DDQ, and 1.5 ml of 0.2 % w/v of TCNQ were sufficient for the production of maximum and reproducible absorbance intensity. Higher concentrations of the reagents did not affect the absorbance intensity. Figures 2-4 show the absorption curves of different charge transfer reagents.

### Effect of reaction time and temperature

The optimum reaction time was determined by monitoring the color developed at room temperature (25 ± 5 °C). Complete color development was attained instantaneously (with ChA and DDQ) and after 30 min for iodine and CH. The developed colors remained stable at room temperature for at least for 3 hr except for iodine (30 min) where the color decreased dramatically after 30 min resulting in higher imprecision of the reading.

TCNQ formed an intense chromogen with trimipramine when heated at 70 °C for 20 min. However at room temperature, only a pale color

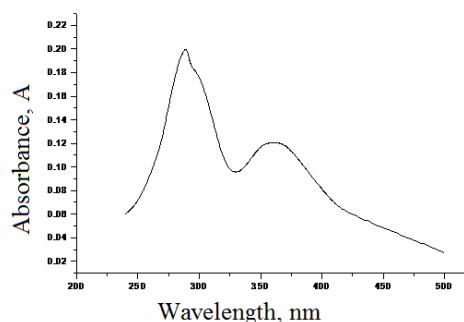appeared and full color development was achieved after 1 h.



**Figure 2:** Absorption spectrum of trimipramine solution (3 µg/ml) with iodine as charge transfer complex
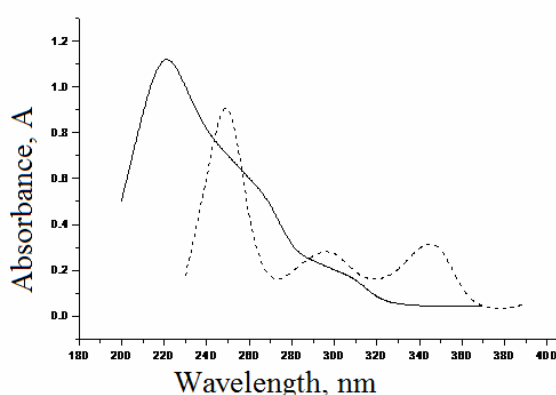


**Figure 3:** Absorption spectrum of a solution of trimipramine (20 and 25 µg/ml) in case of CH( — ), and DDQ(-------).
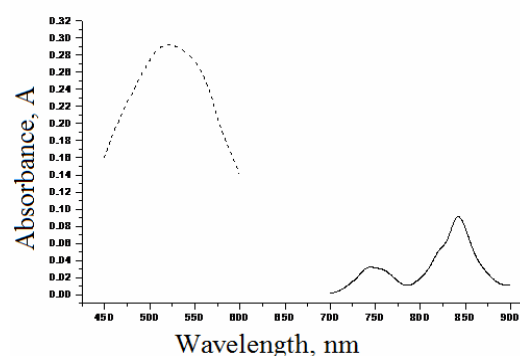


**Figure 4:** Absorption spectrum of a solution of trimipramine (25 µg/ml) using ChA (-----) and TCNQ (—) as charge transfer reagent.

### Stoichiometry of the reaction

Job's method of continuous variation [15] was used for determining the molar ratio of trimipramine to each of the analytical reagents employed in the charge-transfer reactions. These ratios were 1:1 in all cases. This indicates that only one site is possible for the formation of the complex.

## Effect of diluting solvent

In order to select the most appropriate solvent, the reaction was carried out in different solvents, acetonitrile, acetone, ethanol, water, DMF and DMSO. The results indicate that acetonitrile was the most suitable dilution solvent because it afforded excellent solvating power for CH, ChA, DDQ, and TCNQ reagents and gave high absorbance. Acetonitrile was considered as an ideal solvent for the other π–acceptors by offering maximum sensitivity, this was attributed to its high dielectric constant which promote maximum yield of radical anions and high solvation power for the acceptors [16].

## Limit of quantification (LOQ) and of detection (LOD)

The LOD and LOQ were determined [17] using the formula: LOD or LOQ = K.S.D. a/b where K= 3 for LOD and 10 for LOQ. S.D.a is the standard deviation of the intercept, and *b* is the slope. LOQ and LOD results are shown in Table 1. Beer's law displayed a linear response over the concentration range of 1-5, 3- 50, 15- 100, 5 - 50 and 10-75 ppm for $I_2$, CH, ChA and TCNQ, respectively. The limit of quantification was 1.0, 5, 15, 5 and 10 ppm for iodine, CH, ChA, DDQ and TCNQ, respectively, while the limit of detection was 0.35, 1.5, 5.0, 1.5 and 3.0 ppm with correlation coefficient was 0.9995, 0.9991, 0.997, and 0.988 for iodine, CH, ChA, DDQ and TCNQ, respectively.

## Precision and accuracy of the method

The RSD of less than 3.0 % were observed for repetitive measurements on three different daytime periods using two different instruments and two operators. The results obtained (Table 2) are within the acceptance range of < 3.0 % (precision) and 2.0 % (accuracy).

## *Interferences*

Interference studies were carried out in order to investigate the effect of commonly encountered compounds that are present in trimipramine dosage form. It was found that, the proposed methods could be applied to determine trimipramine in a dosage form. The good recovery data obtained in tablets analysis using the proposed and official method (Table 3) indicate that there was no interference from the excipients present in the tablet dosage form.

Thus, tablets excipients such as starch, lactose and glucose did not interfere with the developed method.

## *Ruggedness*

The results of the ruggedness indicated that the method is capable of producing results with high precision.

## *Robustness*

The robustness of the method was demonstrated by varying the experimental factors that can affect the potential response. The optimized parameters were interchanged within the range of 1 – 10 % of the optimum recommended conditions. Preliminary inspection of the results (RSD, 0.3 - 0.4 %) under various (reagent concentration, reaction time, and stability of the resulting charge complex) conditions suggests that the method was fairly robust at the optimum conditions.

**Table 1:** Quantification parameters for the formed CT-complexes

| Parameters | $I_2$ | CH | ChA | DDQ | TCNQ |
|---|---|---|---|---|---|
| Beer`s law limits, µg/ ml | 1-5 | 5 - 50 | 15-100 | 5-50 | 10-75 |
| Lower limit of quantification (LLQ)µg/ml) | 1.0 | 5.0 | 15.0 | 5.0 | 10 |
| Lower limit of detection (LLD), (µg/ml) | 0.33 | 1.66 | 5.0 | 1.660 | 3.3 |
| Intercept (*a*) | -0.0359 | 0.0449 | -0.0093 | 0.0674 | -0.005 |
| Slope (*b*) | 0.1193 | 0.0302 | 0.0029 | 0.0063 | 0.0023 |
| Correlation coefficient, ($r^2$) | 0.9995 | 0.9991 | 0.9970 | 0.997 | 0.998 |
| Molar absorptivity (l mol$^{-1}$ cm$^{-1}$) | $7.1\times10^4$ | $1.6\times10^4$ | $0.13\times10^4$ | $0.26\times10^4$ | $0.1\times10^4$ |
| Sandell`s sensitivity (µg cm$^{-2}$) | 0.0057 | 0.0256 | 0.315 | 0.157 | 0.4105 |

*\* A = b x ± a,   where a = intercept, b = slope, and x = concentration (µg/ml)*

**Table 2:** Accuracy and precision data for the proposed method

| CT Reagent | Drug taken (µg/ml) | Found* (µg/ml) | R (%) | RSD (%) | Error (%) |
|---|---|---|---|---|---|
| $I_2$ | 1.0 | 0.97 | 98.0 | 0.82 | 2.0 |
| | 3.0 | 2.98 | 98.0 | 0.81 | 2.0 |
| | 5.0 | 4.97 | 99.1 | 0.82 | 0.9 |
| CH | 5.0 | 4.98 | 99.6 | 2.5 | 0.4 |
| | 25.0 | 24.90 | 99.6 | 2.0 | 0.4 |
| | 50.0 | 49.90 | 99.8 | 2.0 | 0.2 |
| ChA | 10.0 | 9.95 | 99.5 | 2.8 | 0.5 |
| | 50.0 | 49.95 | 99.9 | 2.6 | 0.1 |
| | 100.0 | 99.85 | 99.8 | 2.5 | 0.2 |
| DDQ | 5.0 | 5.90 | 98.0 | 2.9 | 0.7 |
| | 30.0 | 29.94 | 99.8 | 2.6 | 0.2 |
| | 50.0 | 49.95 | 99.9 | 2.5 | 0.1 |
| TCNQ | 10.0 | 9.95 | 99.5 | 2.8 | 0.5 |
| | 50.0 | 49.98 | 99.9 | 2.6 | 0.1 |
| | 75.0 | 74.5 | 99.3 | 2.6 | 0.1 |

* n = 6

**Table 3:** Determination of trimperamine maleate in tablets using the official method and charge transfer complexes

| Parameter | Recovery ± RSD (%) | | | | | |
|---|---|---|---|---|---|---|
| | $I_2$ | CH | ChA | DDQ | DCNQ | Official method |
| Label claim ( mg/tablet) | 98.8 ± 0.81 | 98.5± 2.5 | 97.8± 2.6 | 98.0 ± 2.6 | 98.1 ± 2.8 | 97.85 ± 0.31 |

## Applications

The reliability of the proposed method for the determination of the drug in a dosage form was first assessed for the pure solution. Determination of trimipramine in the pure drug solution (n = 6) by spectrophotometric method gave average recovery values of 98.0 - 99.6% with relative standard deviation of 0.81 - 2.80 % (see Table 2). This indicates high accuracy of the proposed methods. The results obtained for the formulation by both the proposed spectro-photometric methods and the official titrimetric method [18] are given in Table 3. These data suggest that the proposed method can be carried out on actual products with the accuracy as the official titrimetric method.
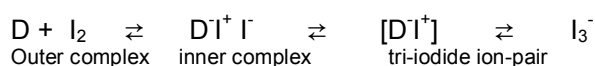
## Statistical data

Comparison of the experimental means for the two methods (developed and official methods) was carried out using the null hypothesis of $|t|_2$ for $p = 0.05$ (n = 5). It was found that $|t|_2 = 1.9$, 2.3, 2.0, 1.9 and 2.4 for each pair, respectively, which is less than the tabulated value ($|t|_2 = 3.36$) [19]. No significant difference was found between the two methods, which indicated that the proposed method is as accurate as the official method. Comparison of the precision of the proposed method with the official method to estimate the random errors of the two sets of data (Table 3) was also carried out using the

two-tailed F-test [19]. Based on this, it is clear that the experimental $F_{4,4}$ values are 3.7, 4.2 , 4.5 , 3.7 and 4.5, respectively. These values are obviously less than the tabulated value of $F_{4, 4}$ for $p = 0.05$ and n = 5 (6.38) [19]. This proves that the results obtained by the two methods are not subject to random errors.

## DISCUSSION

It is known that the color of iodine in chloroform is violet. This color was immediately changed to lemon yellow and the absorption spectrum of trimperamine-iodine reaction product showed absorption peaks at 292 and 365 nm (Fig 2) with the ionized structure, $DI^+ \ldots \ldots H_3^-$ [20].

This complex originates from the early intermediate outer complex, $D \ldots \ldots I_2$. The immediate change of the violet color of iodine in chloroform to lemon yellow upon reaction with trimperamine suggests charge transfere complex formation.

$$D + I_2 \rightleftarrows DI^+ I^- \rightleftarrows [DI^+] \rightleftarrows I_3^-$$
Outer complex   inner complex      tri-iodide ion-pair

The theoretical bases and application of charge transfer complex formation have been studied [20]. The ultraviolet region was scanned for the new band and the maximum absorption was found to occur at 292 nm. To make use of this complex formation for the determination of trimperamine, the concentration of iodine must

be suitable for the quantitative reaction, and should not be much higher than trimperamine concentration. To avoid the formation of intermolecular complexes with a consequent positive deviation from Beer's law, 5ml of 0.5 %w/v iodine solution was found to be adequate. The absorbance should be measured 30 min after the addition of the reactants to attain stable readings.

The interaction of trimipramine with CH, ChA, DDQ and TCNQ as $\pi$ acceptors in non-polar solvents such as dichloroethane produced colored charge-transfer complexes with low molar absorptivity values. In polar solvents such as acetonitrile, complete electron transfer from trimipramine (D), as an electron donor, to the acceptor moiety (A) took place with the formation of intensely colored radical ions with high molar absorptivity values,  according the following:

$$D + A \; \rightleftarrows \; (D\text{-}A) \; \rightleftarrows \; D^{\cdot\, +} + A^{\cdot\, -}$$
$$\text{complex} \qquad \text{redicals ions}$$
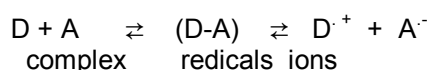
The dissociation of the donor-acceptor complex (D-A) was promoted by the high ionizing power of the polar solvent acetonitrile [20]. The predominant chromogen with chloranil in acetonitrile is a colorless radical anion exhibiting strong absorption maxima at 220 nm. This band is attributed to the formation of a radical anion, while the predominant chromogens showing different absorption maxima at 520, 302 and 842 nm for ChA,  DDQ, and TCNQ, respectively. The predominant chromogen with TCNQ in acetonitrile is the bluish-green colored radical anion exhibiting strong absorption maxima at 842, 825, and 742 nm. These bands are attributed to the formation of the radical anion, TCNQ $^{\cdot\, -}$, which was probably formed by the dissociation of an original donor-acceptor complex (D-A) with trimiparmaine.

## CONCLUSION

The suggested procedures, using $\sigma$ and $\pi$ acceptors, have been demonstrated to be suitable for the spectrophotometric analysis of trimipramine in formulated products. The methods have the advantage of being simple, accurate, sensitive and suitable for routine quality control of trimipramine in dosage form without any interference from excipients.

## ACKNOWLEDGEMENT

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