UV-Visible Spectrophotometric Determination of Triflupromazine Hydrochloride using Cerium(IV) Ammonium Nitrate in Pure and Pharmaceutical Forms

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This study presents a novel, rapid, and cost-effective UV-visible spectrophotometric method for the quantitative determination of triflupromazine hydrochloride (TFM·HCl) in both pure and pharmaceutical forms. The method is based on the oxidative coupling reaction between TFM·HCl and diammonium cerium(IV) nitrate (CAN), resulting in the formation of a stable, colored complex. The experimental parameters influencing the reaction, such as pH, ion concentration, volume, reaction time, temperature, and order of addition, were optimized. Under the optimal conditions, the reaction product exhibited a maximum absorbance at 498.6 nm, with excellent adherence to the Beer–Lambert law over the concentration range of 2–70 μg mL^{-1}. The stoichiometric ratio between TFM·HCl and CAN was determined to be 1:1. The developed method demonstrated good sensitivity, with a calculated limit of detection (LOD) of 0.244 μg·mL^{-1} and a limit of quantification (LOQ) of 0.813 μg·mL^{-1}. Sandell's sensitivity was found to be 0.1075 μg·cm^{-2}. Moreover, a redshift of 220.6 nm was observed in the absorption spectrum of the product compared to the parent compound, confirming the formation of a new chromophore. These findings highlight the method's potential applicability in routine pharmaceutical analysis.

Keywords: spectrophotometric determination, triflupromazine hydrochloride, diammonium cerium (IV) nitrate, pharmaceutical analysis

Introduction

Phenothiazines represent a class of heterocyclic compounds that have been widely employed as antipsychotic agents since the mid-20th century. The introduction of chlorpromazine marked a pivotal advancement in psychiatric treatment, establishing phenothiazines as the primary therapeutic option for psychotic disorders between the 1950s and 1990s. Despite their early clinical success, these first-generation antipsychotics have gradually been supplanted by second-generation agents that exhibit improved safety profiles and reduced extrapyramidal side effects [1].

The therapeutic efficacy of phenothiazines, including triflupromazine hydrochloride (TFM·HCl), is attributed primarily to their antagonistic activity at dopamine D2 receptors. Additionally, they exhibit moderate affinity for other neurotransmitter receptors such as α1-adrenergic, M1-muscarinic, 5-HT2A-serotonergic, and H1-histaminergic systems [1,2]. Triflupromazine hydrochloride ($C_{18}H_{20}ClF_3N_2S$), a trifluoromethyl-substituted phenothiazine derivative, functions as both a centrally acting sedative and antiemetic. Its chemical structure is shown in Figure S1 [4]. It is commonly available as a white to pale crystalline powder with a slight odor, and its pharmacological activity is linked to its ability to inhibit central dopaminergic neurotransmission [3–5].

Despite its continued relevance in clinical practice, the reliable determination of TFM·HCl in pharmaceutical formulations remains analytically important, particularly for quality control and pharmacokinetic studies. Spectrophotometry offers a practical and sensitive approach for such analyses due to its operational simplicity, low cost, and availability in most analytical laboratories [6].

To date, several spectrophotometric methods have been reported for TFM·HCl determination involves oxidizing reagents that yield intensely colored products. For example, iron(III) has been used to produce a chromogenic reaction with an absorption maximum at 700 nm and a molar absorptivity of $2.72 \cdot 10^4$ L·mol^{-1}·cm^{-1} [7]. A method based on the chloramine-T–iodine–starch system has demonstrated improved sensitivity, with λ_{max} at 590 nm and $\varepsilon = 4.07 \cdot 10^4$ L·mol^{-1}·cm^{-1} [8]. Notably, potassium iodate has offered the highest molar absorptivity ($6.4 \cdot 10^5$ L·mol^{-1}·cm^{-1}), with detection possible at as low as 0.05 μg·mL^{-1} and a λ_{max} of 598 nm [9].

Although these approaches are effective, none have investigated the use of diammonium cerium(IV) nitrate (CAN) as an oxidizing agent for TFM·HCl analysis. CAN ($(NH_4)_2Ce(NO_3)_6$) is a widely used

oxidant in organic and coordination chemistry due to its high redox potential, commercial availability, and thermal stability [10]. Its molecular structure is illustrated in Figure S2 [13]. Moreover, it is relatively non-toxic, cost-effective, water-soluble, and easy to handle, making it particularly attractive for analytical applications [11]. Beyond organic synthesis, CAN has also found utility in the development of cerium-based photocatalysts and other advanced materials [12].

Given these advantages, the present study explores, for the first time, the reaction between TFM·HCl and CAN under controlled acidic conditions, aiming to develop a selective and robust UV-visible spectrophotometric method. The study further investigates the spectral properties of the resulting colored product and systematically evaluates the parameters influencing complex formation, including pH, temperature, reaction time, and reagent concentrations.

Experimental part

Chemicals and Reagents

Triflupromazine hydrochloride (TFM·HCl, purity 99%) was obtained from SDI and used as the reference compound. Diammonium cerium(IV) nitrate (CAN, 99%) was purchased from BDH and served as the oxidizing agent. Additional analytical-grade reagents acquired from BDH included acetone, potassium chloride, hydrochloric acid (37%), potassium hydrogen phthalate, dipotassium hydrogen phosphate, and sodium hydroxide. To assess method selectivity and potential matrix interference, several commonly used excipients were also tested, including glucose, sucrose, lactose, and starch, all with reported purity of 99%.

Siquil injectable solution (Cadia Healthcare Ltd., India), containing 5 mg·mL^{-1} of triflupromazine hydrochloride in aqueous solution, was used as a representative pharmaceutical sample for quantitative determination.

Instrumentation

Absorbance measurements were conducted using a Shimadzu UV-1650 PC (double-beam) and UV-2900 PC (single-beam) UV-Vis spectrophotometers. FT-IR spectra were recorded using a Shimadzu 8400S. pH values were measured with an Infitek pH meter (China). An analytical balance (Sartorius BL 210S) and water bath (GFL 1083, Germany) were used for sample handling and temperature control.

Preparation of solutions

Stock solutions of triflupromazine hydrochloride (TFM·HCl) and diammonium cerium(IV) nitrate (CAN) were freshly prepared in deionized water. A 250 μg·mL^{-1} TFM·HCl solution was obtained by dissolving 25 mg of the pure drug in a 100 mL volumetric flask and diluting to the mark. For the oxidizing reagent, a $1.0 \cdot 10^{-2}$ M CAN solution was prepared by dissolving 546 mg of the compound in 100 mL of deionized water. Working solutions of both reagents were prepared by serial dilution as required for spectrophotometric experiments.

To prepare the pharmaceutical formulation solution, 0.5 mL of *Siquil* injectable solution (containing 5 mg·mL^{-1} of TFM·HCl) was transferred into a 100 mL volumetric flask and diluted with deionized water to yield a final concentration of 250 μg·mL^{-1}. All solutions were protected from light and used within the same day to ensure stability.

Optimization of reaction conditions

A series of experiments was conducted to optimize the conditions for the formation of the colored product resulting from the reaction between TFM·HCl and CAN. Each parameter was varied independently while maintaining all other variables constant.

The effect of pH was investigated over the range of 1 to 8 using appropriate buffer solutions. Reaction mixtures were prepared by combining TFM·HCl (50 μg·mL^{-1}), CAN ($1.0 \cdot 10^{-3}$ M), and buffer solution in 10 mL volumetric flasks, followed by dilution to volume with deionized water. Absorbance was measured at 498.6 nm to determine the pH value that yielded the highest color intensity.

To determine the optimal CAN concentration, mixtures containing TFM·HCl (50 μg·mL^{-1}), pH 3 buffer, and CAN at concentrations ranging from $1.0 \cdot 10^{-5}$ to 2.5×10^{-4} M were prepared and analyzed under the same conditions.

The influence of reagent volume was assessed by varying the volume of CAN solution ($12.5 \cdot 10^{-5}$ M) from 0.5 to 4.0 mL, while keeping the volumes of TFM·HCl and buffer constant. The total volume was adjusted to 10 mL in all cases.

The reaction time was optimized by monitoring the absorbance at 498.6 nm over a 60-minute period, with measurements taken at 5-minute intervals. The point at which the absorbance plateaued was considered the optimal time for analysis.

The effect of temperature was studied by incubating the reaction mixture at different temperatures (15–50 °C) using a thermostatically controlled water bath. Absorbance was measured after 30 minutes of incubation to determine the temperature that produced the most stable and intense color.

Finally, the order of reagent addition was evaluated by preparing the mixtures with different sequences of component addition. The sequence that resulted in the highest absorbance at 498.6 nm under optimal pH, time, and temperature conditions was selected for subsequent procedures.

Calibration curve

A series of solutions with TFM·HCl concentrations from 2 to 70 μg·mL^{-1} was prepared using 2.5 mL of $12.5 \cdot 10^{-5}$ M CAN and 2 mL of pH 3 buffer in 10 mL volumetric flasks. Absorbance was measured at 498.6 nm under optimal time and temperature. A calibration curve was plotted and evaluated for linearity and conformity with Beer–Lambert's law.

Stoichiometry of the colored product

Job's method of continuous variation was used

to determine the stoichiometry. Equimolar solutions of TFM·HCl and CAN ($12.5 \cdot 10^{-5}$ M) were mixed in different volume ratios (1:9 to 9:1), with 2 mL of pH 3 buffer added and the total volume adjusted to 10 mL. Absorbance at 498.6 nm was measured, and the mole ratio was determined from the absorbance–composition plotd conformity with Beer–Lambert's law.

Results and discussions

UV-visible spectroscopy

The UV-Visible absorption spectra of the colorless drug TFM·HCl, the oxidant CAN, and the resulting colored product were recorded to evaluate their electronic transitions. As shown in Figure S3, TFM·HCl exhibited a maximum absorbance (λ_{max}) at 278 nm, while CAN showed λ_{max} at 287 nm, both measured against a water blank. The colored reaction product, formed by the interaction of TFM·HCl with CAN, displayed a prominent absorption band at 498.6 nm (orange solution), with a blank consisting of 2 mL of CAN and 8 mL of water. A significant bathochromic shift (redshift) of 220.6 nm and 211.6 nm was observed relative to TFM·HCl and CAN, respectively, confirming the formation of a new chromophoric species.

Optimization of experimental parameters

Effect of pH

The absorbance of the colored product was studied over a range of pH values (Figure 1a). Maximum intensity was observed at pH 3, beyond which a marked decrease in absorbance was noted. Therefore, pH 3 was selected as optimal for complex formation.

Effect of cerium(IV) ion concentration

To determine the optimal oxidant concentration, the cerium(IV) ion concentration varied from 1 to $25 \cdot 10^{-5}$ M, while keeping the TFM·HCl concentration constant at 50 μg·mL^{-1}. The highest absorbance was achieved at a concentration of $12.5 \cdot 10^{-5}$ M Ce(IV) (Figure 1b).

Effect of reaction time

The influence of reaction time on product stability was evaluated from 1 to 60 minutes (Figure 1d). Absorbance reached a maximum at 30 minutes and remained stable thereafter, indicating that adequate complex formation and stability had occurred.

Effect of temperature

The effect of temperature on the colored product was investigated over the range of 15 to 50 °C (Figure 1e). The highest absorbance intensity was observed at 20 °C, after which a decline in absorbance occurred. This reduction may be attributed to partial decomposition or increased solubility of the colored complex, which could potentially alter its spectral properties.

Effect of reagent addition sequence

To optimize the absorbance intensity, the effect of reagent addition sequence on the formation of the

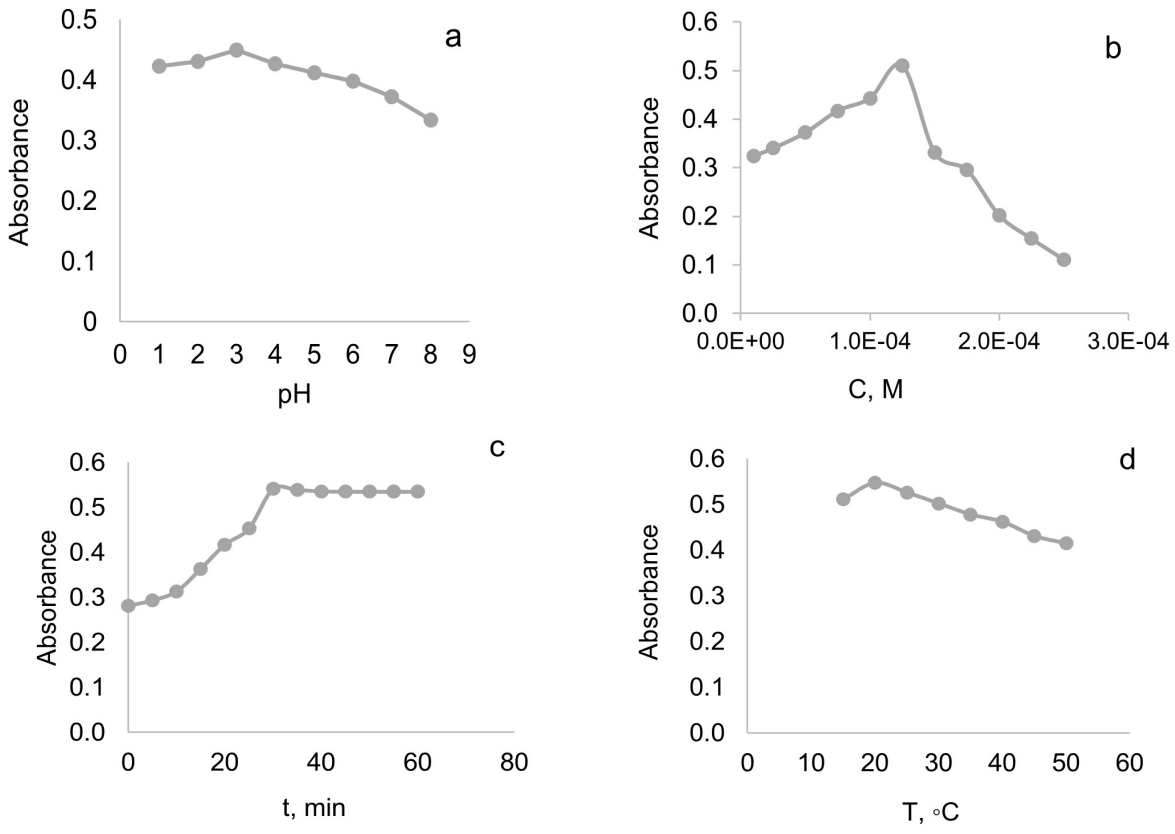

Figure 1. Effect of experimental parameters on the absorbance of the colored product: (a) pH; (b) concentration of cerium(IV) ions; (c) reaction time; (d) temperature.

colored product was investigated. Various sequences were tested, and the results revealed that the highest absorbance (0.537) was obtained when the reagents were added in the following order: CAN → TFM·HCl → acid (pH adjustment).

Calibration and analytical parameters

A calibration curve for the colored complex formed by the reaction of TFM·HCl with CAN was constructed under optimized experimental conditions, with absorbance measurements recorded at the λ_{max} of 498.6 nm (Figure S4). The method exhibited excellent linearity over the concentration range of 2–70 μg·mL^{-1}, with a correlation coefficient (R^2) of 0.9935, demonstrating compliance with Beer–Lambert's law within this range. The molar absorptivity (ε) was determined to be 3616.584 L·mol^{-1}·cm^{-1}, indicating moderate sensitivity of the chromophore. The Sandell sensitivity, calculated as 0.1075 μg·cm^{-2}, further supports the method's applicability for low-concentration detection. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.244 and 0.813 μg·mL^{-1}, respectively, confirming the method's high sensitivity and suitability for trace-level determination of TFM·HCl. The analytical parameters derived from the calibration curve are summarized in Table 1.

Table 1. Analytical parameters of the proposed spectrophotometric method.

Parameter	Value
λ_{max} (nm)	498.6
Regression equation	y = 0.0093x + 0.0853
Linear range (μg·mL^{-1})	2-70
Molar absorptivity (L·mol^{-1}·cm^{-1})	3616.584
Slope	0.0093
Correlation coefficient (R^2)	0.9935
Sandell's sensitivity (μg·cm^{-2})	0.1075
LOQ (μg·mL^{-1})	0.813
LOD (μg·mL^{-1})	0.244
Stoichiometric ratio	1:1

Accuracy and precision

The accuracy and precision of the proposed spectrophotometric method were assessed using TFM·HCl-spiked samples at three concentration levels: 20, 40, and 60 μg·mL^{-1}. As summarized in Table 2, the relative error (E%) did not exceed 2%, and the recovery percentages (R%) were within the acceptable range of 99.8–102.0%, indicating excellent analytical accuracy. All RSD values were below 1%, confirming excellent method precision. These results validate the reliability of the method for routine quantitative analysis of TFM·HCl in pharmaceutical formulations.

Interference study

The selectivity of the developed spectrophotometric method was assessed by evaluating the effect of potential interfering substances commonly present in pharmaceutical formulations. These included excipients, diluents, and flavoring agents, which were added at concentrations tenfold higher than that of the target analyte (TFM·HCl). The aim was to verify the method's robustness and its suitability for routine application in complex sample matrices. The absorbance values and corresponding recovery percentages are presented in Table 3. The data show that the presence of these substances had negligible impact on the absorbance of the colored product formed from the reaction of TFM·HCl with CAN. All recovery deviations were within ±1%, confirming that the method exhibits excellent selectivity and is unaffected by common formulation additives

Table 3. Effect of interfering substances on the absorbance and recovery of the TFM·HCl–CAN colored product.

Interfering substance	E%	R %
Benzyl alcohol	-0.73	100.8
Sodium chloride	+0.95	99.1

Stoichiometry of the complex

The stoichiometry of the complex formed between TFM·HCl and cerium(IV) was determined using the method of continuous variation (Job's method) under acidic conditions. The absorbance of a series of solutions containing varying molar ratios of drug and reagent was measured at the maximum wavelength of the colored product. As illustrated in Figure S5, the maximum absorbance was observed at a mole fraction of 0.5, indicating a 1:1 stoichiometric ratio between TFM·HCl and Ce(IV) ions in the complex.

Table 2. Precision and accuracy of the proposed spectrophotometric method for the determination of TFM·HCl.

Concentration (μg·mL^{-1})		E%	R%	RSD%
Nominal	Found			
20	20.40	2.00	102.0	0.84
40	40.31	0.78	100.8	0.63
60	60.28	0.47	100.5	0.75

Stability constant (β) determination

The stability constants (β) of the colored complex formed between TFM·HCl and Ce(IV) were determined using the molar ratio method, based on spectrophotometric data. Measurements were conducted at temperatures ranging from 15 to 50°C to evaluate the influence of thermal conditions on the stability of the complex.

The calculation of β was based on the absorbance values corresponding to the stoichiometric point and those obtained under excess reagent conditions. These values were used to estimate the degree of dissociation of the complex (α), which reflects the fraction of uncomplexed species in solutions. The stability constant β was then derived accordingly for each temperature point.

As presented in Table S1, the β values increased with temperature, reaching a maximum at 40 °C ($\beta = 3.86 \cdot 10^6$), indicating the highest stability of the complex at this condition. Slight decreases in stability were observed at higher and lower temperatures, suggesting that 40 °C represents the most favorable condition for complex formation. These results confirm that the TFM·HCl–CAN complex is thermodynamically stable and suitable for reliable analytical application.

Application

The method was successfully applied to commercial pharmaceutical formulation — *Siquil* injection containing 5 mg of TFM·HCl. The analysis was performed at three different concentration levels (10, 30, and 50 μg·mL^{-1}), and the results are summarized in Table 4. The method demonstrated excellent accuracy, with relative error (E%) values below 2%, and recovery percentages ranging from 99.8% to 101.2%. Precision was also satisfactory, with relative standard deviation (RSD%) values below 1% for all tested concentrations. These findings confirm the method's reliability and suitability for routine quality control of TFM·HCl in pharmaceutical formulations.

Table 4. Determination of TFM·HCl in a pharmaceutical dosage form by the proposed method.

Concentration (μg·mL^{-1})		R%	RSD%
Nominal	Found		
10	10.12	101.2	0.48
30	29.94	99.8	0.79
50	50.17	100.3	0.35

Proposed reaction mechanism

The spectrophotometric method developed in this study is based on a redox reaction between trifluoperazine dihydrochloride (TFM·HCl), a phenothiazine derivative, and cerium(IV) ammonium nitrate (CAN) under acidic conditions.

In acidic medium, the phenothiazine ring system of TFM·HCl becomes protonated at the nitrogen atom in the central thiazine ring. This protonation facilitates electron donation from the electron-rich aromatic system to the strong oxidizing agent, Ce(IV). As a result, an electron transfer occurs from TFM·HCl to Ce(IV), reducing cerium from the +4 to the +3 oxidation state (Ce^{3+}). Simultaneously, the phenothiazine molecule undergoes oxidation at the central ring, forming a highly conjugated cationic radical species. This oxidized form of TFM·HCl is responsible for the development of an intense orange color, with an absorption maximum at 498.6 nm, as observed in the UV-Vis spectrum. The 1:1 molar ratio between TFM·HCl and Ce(IV), determined by Job's method, supports the formation of a single-electron transfer complex. The resulting chromophore is stable under the optimized conditions and serves as the analytical basis for the quantification of TFM·HCl via spectrophotometry.

Conclusions

The present study demonstrated the successful application of UV-visible spectrophotometry for the quantitative determination of triflupromazine hydrochloride (TFM·HCl) via the formation of a colored complex with diammonium cerium(IV) nitrate (CAN). The reaction proceeded efficiently in an acidic medium, with a pH of 3 being found to be optimal for color development. The resulting chromophore exhibited a maximum absorbance (λ_{max}) at 498.6 nm, indicating a significant red shift of 220.6 nm compared to the native drug spectrum.

Stoichiometric analysis using the method of continuous variation confirmed a 1:1 molar ratio between TFM·HCl and CAN in the colored complex. The absorbance-concentration relationship followed Beer–Lambert's law over the range of 2–70 μg·mL^{-1}, affirming the linearity of the method. The complex exhibited high stability, with a maximum absorbance achieved after 30 minutes of reaction time.

Analytical performance parameters revealed high method sensitivity, with a calculated limit of detection (LOD) of 0.244 μg·mL^{-1} and limit of quantitation (LOQ) of 0.813 μg·mL^{-1}. The Sandell's sensitivity was found to be 0.1075 μg·cm^{-2}. These findings confirm that the developed method is simple, rapid, and reliable for the spectrophotometric determination of TFM·HCl in pharmaceutical formulations.

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