Sensitive Spectrophotometric Determination of Trifluoperazine Hydrochloride via Ion-Association with Aniline Blue in Acidic Medium

Mirela Tong*. and Joan Febles
Sultan Qaboos University, Oman
*Corresponding author: Mirela2017@gmail.com

Received: 10 Sep 2023 | Accepted: 25 Nov 2023 | Published: 1 Jan 2024

ABSTRACT

A simple, sensitive, and cost-effective spectrophotometric method was developed for the determination of Trifluoperazine hydrochloride (TFPH) in an acidic medium using aniline blue as a chromogenic reagent. The method is based on the formation of an ion-association complex resulting from electrostatic interactions between the protonated drug and the anionic dye, leading to a measurable decrease in dye absorbance. The discoloration process was monitored at a maximum wavelength (λ_max) of 592 nm.

Key experimental parameters, including pH, reagent concentration, reaction time, and temperature, were systematically optimized to achieve maximum sensitivity and stability. Under the optimized conditions, a linear relationship was established between the decrease in absorbance (ΔA) and TFPH concentration over the range of 0.5–7.0 μg mL^{-1}, with a correlation coefficient (R^2) of 0.9962.

The method demonstrated satisfactory analytical performance, with an average recovery of 97.80%, a limit of detection (LOD) of 0.3319 μg mL^{-1}, and a limit of quantification (LOQ) of 1.1065 μg mL^{-1}. Precision and accuracy were confirmed by relative standard deviation (RSD%) values ranging from 0.35 to 2.79 and relative error (RE%) between −1.2 and −3.2.

The proposed method was successfully applied for the quantitative determination of TFPH in pharmaceutical tablet formulations, showing high accuracy, precision, and suitability for routine quality control analysis without the need for complex instrumentation or extraction steps.

Keywords : *Aniline blue, Ion association complex , Spectrophotometric method, Trifluoperazine Hydrochloride .*

Introduction:

Trifluoperazine hydrochloride (TFPH) is a well-established antipsychotic drug belonging to the phenothiazine class, commonly prescribed for the management of schizophrenia and other psychotic disorders. It exerts its pharmacological action by modulating neurotransmitter balance in the central nervous system, thereby improving cognitive clarity, reducing anxiety, and alleviating hallucinations. Although it may provide short-term relief of anxiety symptoms, it is not considered a first-line treatment for anxiety disorders [1]. Structurally, TFPH contains a piperazine side chain and is classified as a group III phenothiazine derivative, exhibiting physicochemical and therapeutic properties comparable to chlorpromazine [2]. Chemically, it is designated as 10-(3-(4-methyl-1-piperazinyl)propyl)-2-trifluoromethylphenothiazine dihydrochloride. The drug appears as a white to pale yellow crystalline powder, hygroscopic in nature, freely soluble in water and moderately soluble in alcohol [3].

Despite its therapeutic efficacy, TFPH may induce several adverse effects, including gastrointestinal discomfort, dermatological reactions, lethargy, nausea, jaundice, excessive perspiration, tachycardia, and urinary disturbances [4]. Consequently, the development of reliable, sensitive, and accessible analytical methods for its determination in pharmaceutical formulations remains of significant importance.

Various analytical techniques have been reported for the determination of TFPH, including spectrophotometric methods based on oxidative coupling reactions [5–10], peak area measurements [11], and ultraviolet spectrophotometry [12]. In addition, more advanced techniques such as indirect spectrofluorometry [13], indirect atomic absorption spectrometry [14], flow injection analysis [15], high-performance liquid chromatography (HPLC) [16–17], and electrochemical methods [18–20] have also been employed. While these methods offer satisfactory sensitivity and selectivity, some of them require sophisticated instrumentation, complex procedures, or high operational costs.

Ion-association complex formation represents an important approach in analytical chemistry for the determination of pharmaceutical and organic compounds. These complexes are formed through electrostatic interactions between oppositely charged ions, without the formation of covalent bonds. Organic dyes are frequently utilized as ion-pairing reagents due to their ability to form stable and measurable complexes with drug molecules [21]. Typically, such reactions result in the formation of a new species with absorbance directly proportional to analyte concentration at a characteristic wavelength [22]. However, a less common analytical behavior involves a decrease in absorbance intensity of the dye upon complex formation as the analyte concentration increases [23–24].

In this context, the present study introduces a spectrophotometric method based on the latter mechanism, involving the interaction of TFPH with aniline blue dye in an acidic medium. The ion-association complex is primarily formed through electrostatic attraction between the protonated tertiary amine groups of TFPH and the anionic functional groups of aniline blue. This interaction leads to a measurable decrease in the absorbance of the dye at 592 nm, which is exploited for the quantitative determination of the drug.

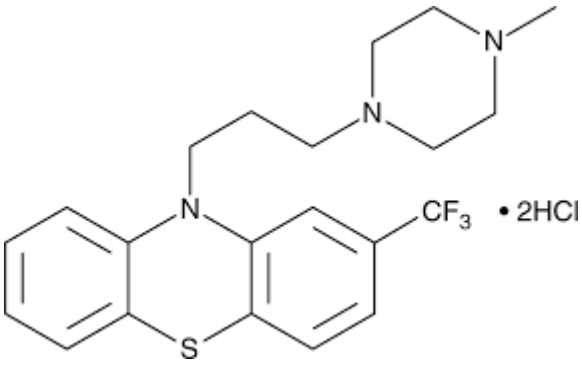


Figure1: Chemical structure of Trifluoperazine Hydrochloride.

Materials and Methods

All chemicals and reagents used in this study were of high analytical purity and utilized without further purification. Aniline blue dye (Fluka), Trifluoperazine Hydrochloride (SDI), and hydrochloric acid (Scharlau) were employed throughout the experiments.

A stock solution of Trifluoperazine Hydrochloride (1000 µg/mL) was prepared by accurately dissolving 0.1000 g of the drug in 100 mL of distilled water using a volumetric flask. The solution was stored in an opaque vial to prevent light degradation. A working solution (50 µg/mL) was subsequently prepared by transferring 5 mL of the stock solution into a 100 mL volumetric flask and diluting to the mark with distilled water.

Aniline blue dye solution (100 µg/mL) was prepared by dissolving 0.0100 g of the dye in 100 mL of distilled water. Hydrochloric acid solution (0.01 M) was prepared by first diluting concentrated HCl (12.1 M), where 8.26 mL was added to a small volume of distilled water and diluted to 100 mL to obtain a 1 M solution. Then, 1 mL of this solution was further diluted to 100 mL with distilled water to yield the desired 0.01 M concentration.

Tablet samples of Trifluoperazine Hydrochloride were prepared from two commercial formulations: STELLASIL (5 mg/tablet) and IRALZIN (5.9 mg/tablet). Ten tablets from each formulation were accurately weighed, finely powdered, and homogenized. The powdered samples were dissolved in distilled water, transferred to 50 mL volumetric flasks, and diluted to volume. The solutions were sonicated for 30 minutes to ensure complete dissolution and then filtered. Appropriate aliquots (5 mL for STELLASIL and 4.2 mL for IRALZIN) were further diluted to 100 mL with distilled water to obtain solutions containing 50 µg/mL of Trifluoperazine Hydrochloride.

Buffer solutions were prepared as follows: phthalate buffer was obtained by mixing 50 mL of potassium hydrogen phthalate (0.1 M) with 38.8 mL of HCl (0.1 M), followed by dilution to 100 mL and adjustment of pH to 2.5. Glycine-HCl buffer was prepared by mixing 50.0 mL of glycine (0.1 M) with 16.2 mL of HCl (0.2 M) and adjusting the pH to 2.5. Citric acid–NaOH buffer solution was prepared by mixing 10 mL of citric acid (2 M) with 3.0 mL of NaOH (2 M), diluting to 100 mL, and adjusting the pH to 2.5 [25].

Absorbance measurements and spectral analysis were carried out using a double-beam SHIMADZU UV–Vis spectrophotometer (UV-1900i) equipped with 1 cm path length glass cells. The pH of all solutions was measured using a BP3001 pH meter, while a BEL analytical balance was used for precise weighing.

Results and Discussion

The effect of different types of acids on ΔA (the difference in absorbance between the dye alone and the dye in presence Trifluoperazine Hydrochloride) were studied and noted that HCl gave the best results as shown in Table **1**.

Table1.Effect of various types of acids on ΔA

Acid used1ml ,0.01M	HCl	H_2SO_4	H_3PO_4	CH_3CO_2H
ΔA	0.316	0.314	0.297	0.276

without acid ΔA = 0.067

Then different volumes of HCl (0.01M) were added to 50 µg/mL of Trifluoperazine Hydrochloride followed by adding 2.5 mL of dye and standing for 5 minute before diluting with distilled water to the mark, the results are illustrated in Figure **2**.

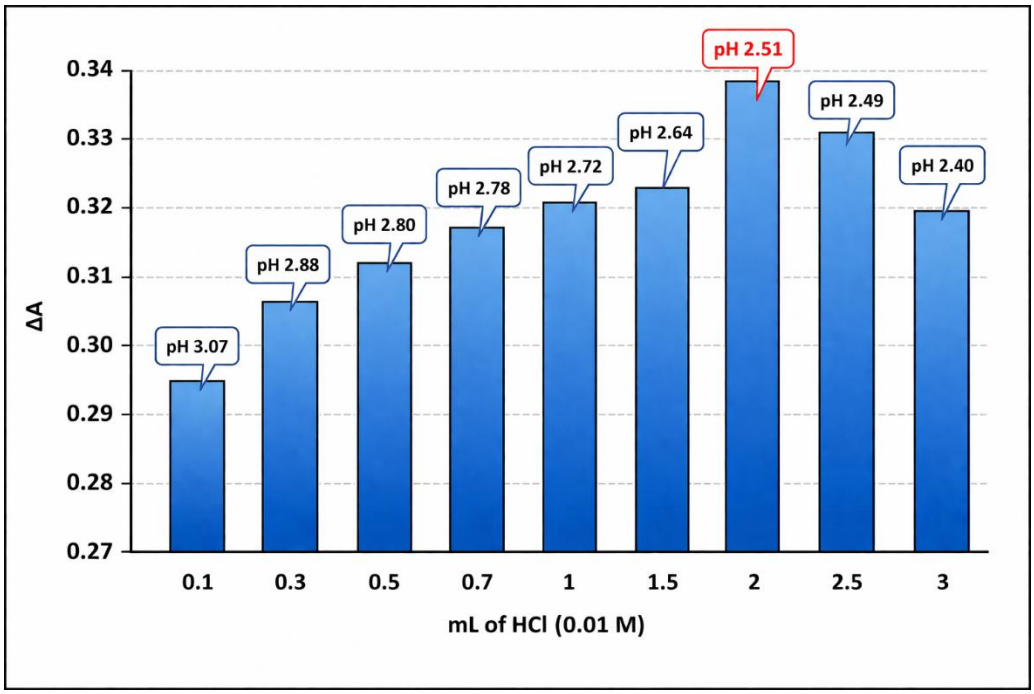


Figure 2.Effect of the amount of HCl on absorbance of formed ion association complex

Figure **2** indicates that the volume of 2 ml of HCl (0.01 M) and pH 2.5 gave the highest different in absorbance intensity of ion association formed, and accordingly, the effect of the medium pH was studied by adding different volumes from various buffer solution of pH 2.5 as shown in Table **2**.

Table 2. Effect of Medium Type and Buffer Volume on Final pH and Absorbance (ΔA)

Type of Medium	Buffer Volume (mL)	Final pH	ΔA
HCl (0.01 M)	2	2.51	0.340
Citric acid–NaOH buffer	1	2.71	0.334
Citric acid–NaOH buffer	2	2.68	0.300
KH–Phthalate buffer	1	2.83	0.328
KH–Phthalate buffer	2	2.74	0.334
Glycine–HCl buffer	1	2.72	0.329
Glycine–HCl buffer	2	2.68	0.304

The results showed that the use of buffer solutions did not improve ΔA, so 2 mL of HCl (0.01M)was adopted in subsequent experiments.The effect of aniline blue quantity (1-3mL) with different concentrations of Trifluoperazine Hydrochloride (1.5-6 µg/mL) in presence of 2 mL of HCl (0.01M) was studied . The highest ΔA and correlation coefficient were obtained when 2.5 mL of aniline blue was used.

The results were showed in Figure **3**

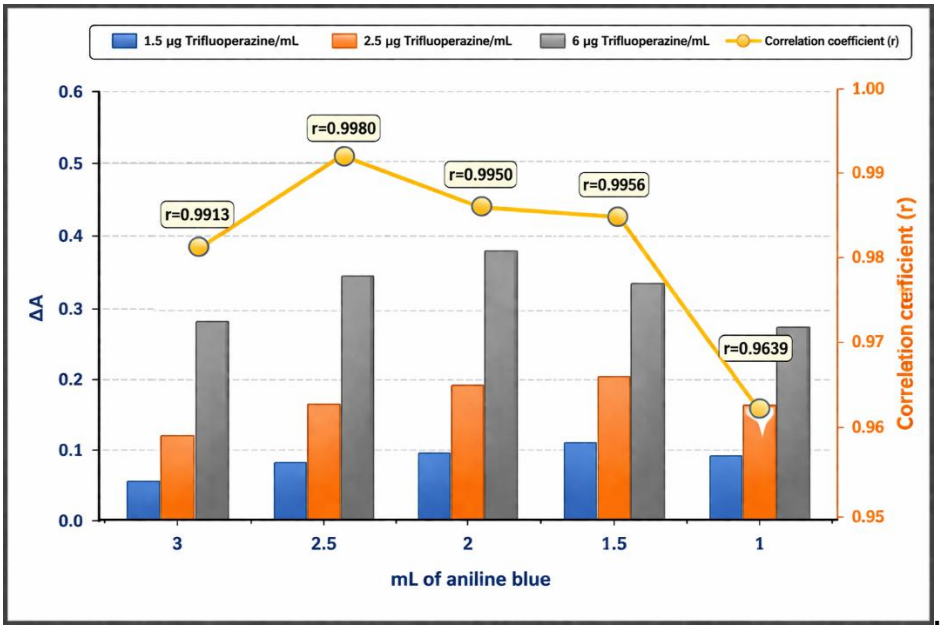


Figure 3.Effect quantity of aniline blue on ΔA

The effect of time on ΔA before diluting was studied, as shown in Figure **4.**

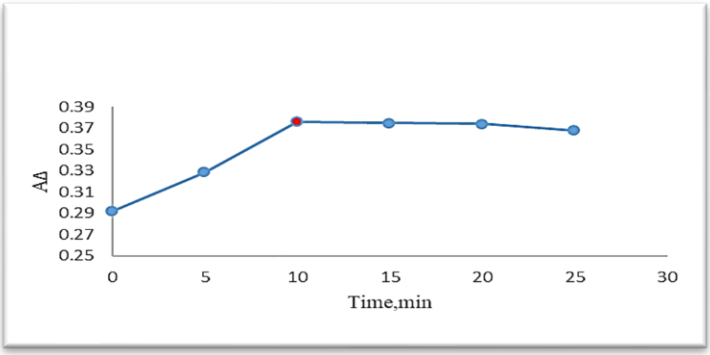


Figure 4. Effect of time before diluting to the mark

The results showed that the highest ΔA was obtained after 10 minutes of adding the dye to trifluoperazine hydrochloride in acidic medium, so this time was recommended before dilution with distilled water to the mark.

The effect of temperature on ion association complex formation and stability has been studied over a period of 60 minutes at three temperatures: 0 °C, RT(23±1°C) and 40 °C. The most stable results were obtained at room temperature for at least one hour. Figure**5**.

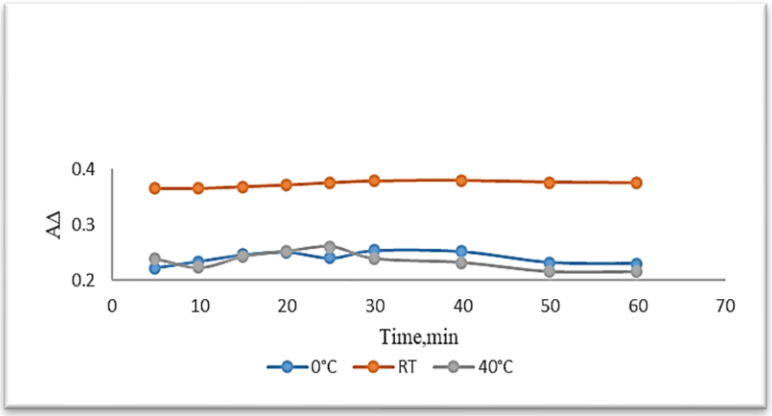


Figure5.The effect of temperature with standing time on ΔA

Calibration graph:

According to optimal conditions that have been obtained above, the calibration graph was prepared by adding 2.5 mL of 100 µg/mL aniline blue to different concentrations of Trifluoperazine Hydrochloride which contain 2 mL of (0.01M) HCl then standing 10 minutes at room temperature and completed the volume to the mark with distilled water then the absorption spectrum of each solution was done and the maximum absorption at 592 nm was fixed in the constructed calibration graph (Figure**6**).

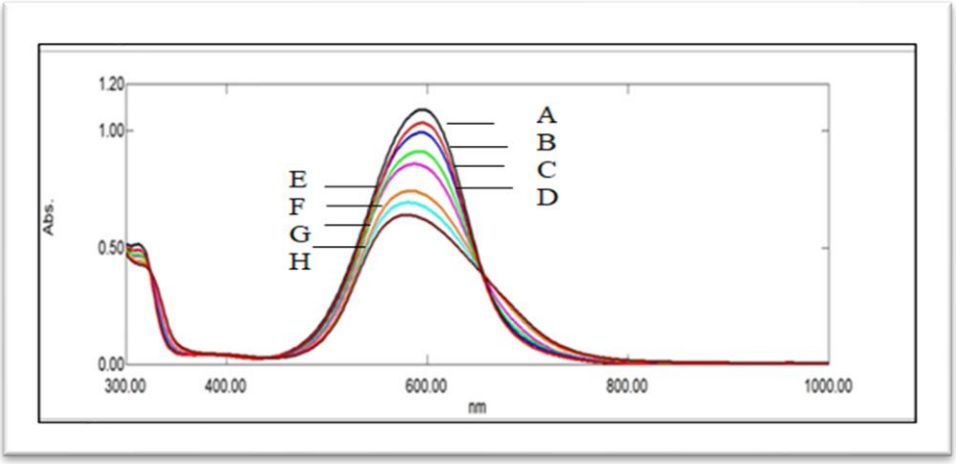


Figure6.Absorption spectra for (A) dye, (B)inpresence of 0.5,(C)1.5,(D)2.5, (E)3.5,(F)5.0,(G)6.0,and(H)7.0 µg/mL of Trifluoperazine Hydrochloride

A linear relationship was obtained over a concentration of 0.5-7.0 µg Trifluoperazine Hydrochloride /ml), as shown in Figure **7** with molar absorptivity 3.4 x 104 $L.mol^{-1}.cm^{-1}$.

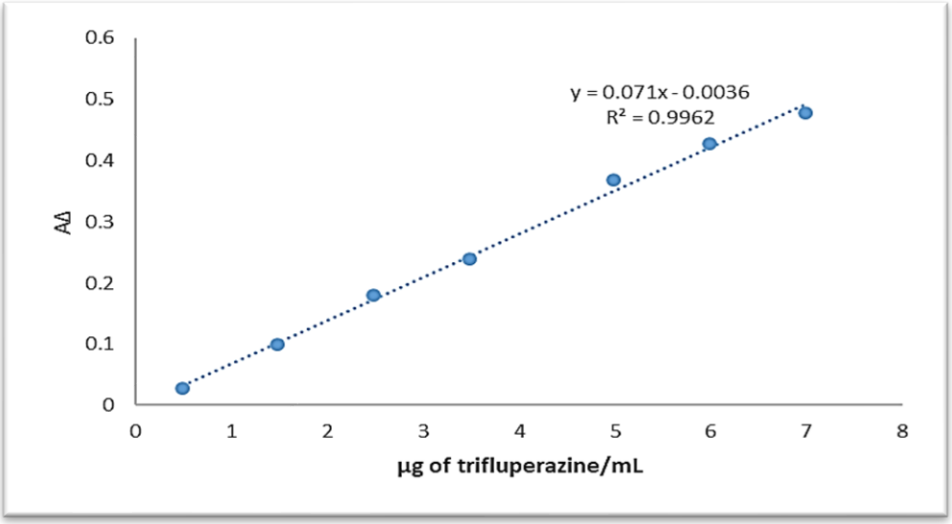


Figure 7.Calibration graph for Trifluoperazine Hydrochloride determination according to the proposed method.

The proposed method yielded favourable outcomes in terms of recoveries, accuracy (expressed as Er%), and precision (expressed as Relative Standard Deviation (RSD%), as illustrated in Table **3**.

Table 3. Accuracy and precision of the method

Amount taken (µg/mL)	Amount found (µg/mL)	Recovery, %	Relative error,%	Relative standard deviation,%
2.5	**2.42**	**96.80**	**-3.20**	**2.79**
5.0	**4.94**	**98.80**	**-1.20**	**0.35**

The nature of ion association complex:

The slope ratio method [26] was followed in order to find the molar compositional ratio of the ionic association complex which formed between the drug under investigation and aniline blue dye in acidic medium via using equimolar solutions ($2.08x10^{-4}$ M).

Two standard curves were drawn for Trifluoperazine Hydrochloride and aniline blue, the first (A)was done by fixing the volume of aniline blue dye, and taking gradient volumes of the drug, the second (B) involved fixing the volume of the drug and taking gradient volumes of the dye to obtain two linear graphs, as shown in Figure **8**:

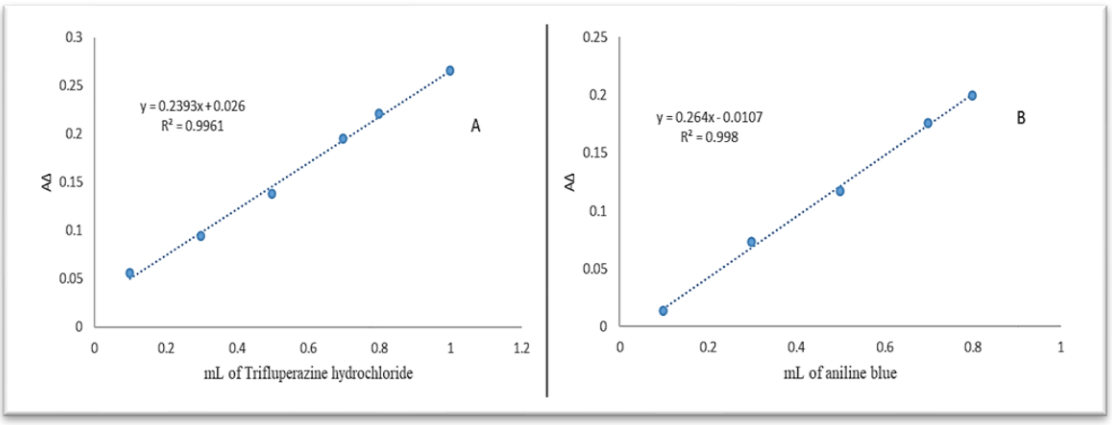
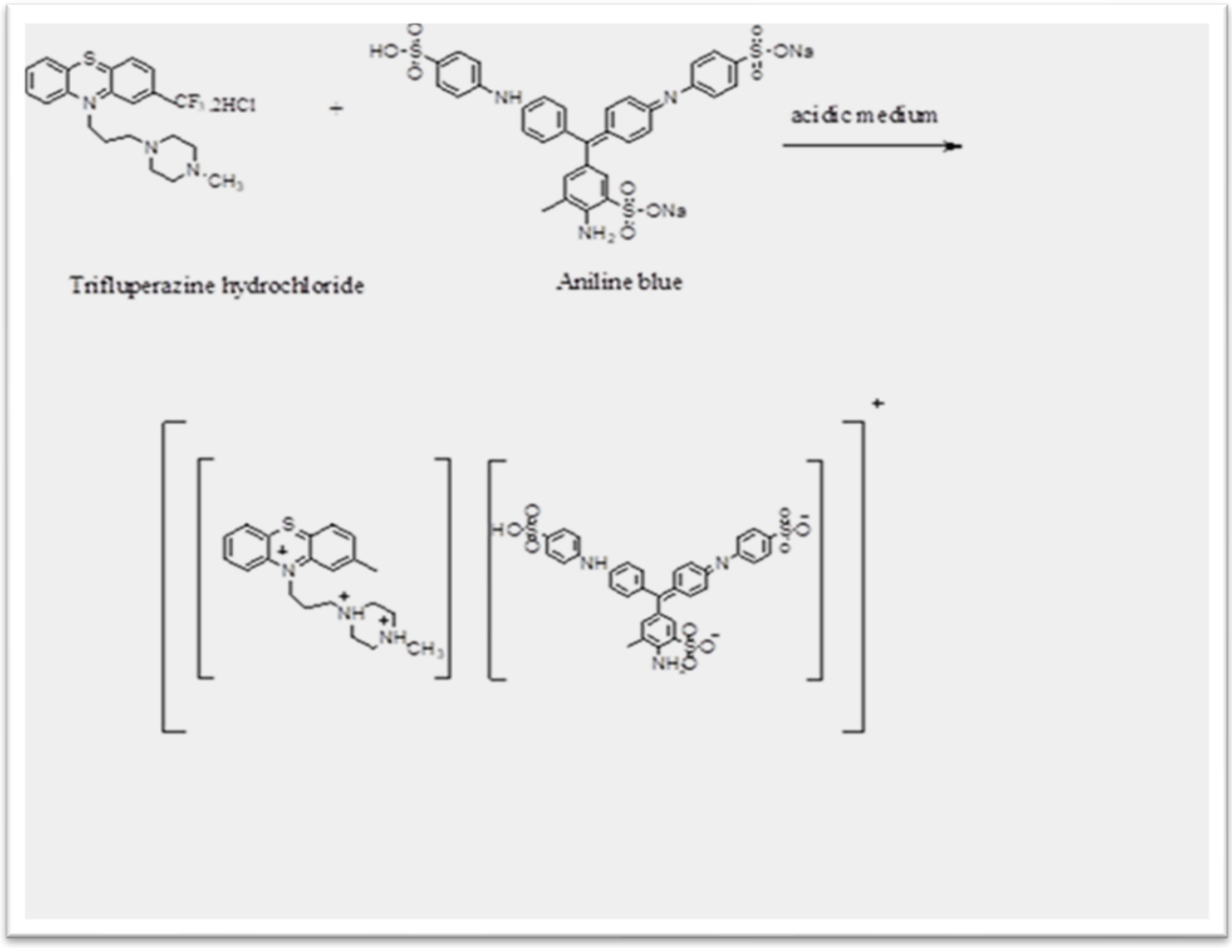


Figure 8.Calibration graph for Slope ratio method

According to the results which obtain from Figure 8, It was found that the ratio of the complex formed was 1:1(drug: dye), therefore, the proposed chemical reaction for the ionic association complex as in scheme **1**:



Scheme1. Chemical reaction for ion association complex

Method application:

The present method was applied to determine Trifluperazine Hydrochloride in tablet form under selected optimum conditions which was replicated four times and yielded favorable outcomes as in Table **4**:

Table 4.Application of the method

	Trifluoperazine hydrochloride (µg/mL)		Recovery,%	RSD,%
	Taken	Found		
Iralzin tablets (5mg-SDI)	2.5	2.50	100.00	3.81
	5.0	4.98	99.60	0.85
Stellasil tablets 5.9mg- kahira pharmaceuticals and chemical industries company	2.5	2.59	103.60	2.14
	5.0	4.95	99.00	0.65

Standard addition method:

The standard addition method was used to estimate the drug content in tablets. Two concentrations of 1.5and 2.5 µg/ml were taken from the solutions of the pharmaceutical preparations, followed by adding different concentrations of Trifluperazine Hydrochloride solution under study in its pure form, provided that it does not exceed the maximum estimate range in the calibration curve, noting that one of the volumetric flasks should contain only a known concentration of the pharmaceutical solution without the pure compound. These additions were made to two series of 10 ml volumetric flask. The additions were made according to the optimal conditions, and the absorbance of the solutions was read at the wavelength of 592 nm, Figure **9** and Table **5** show the results were obtained:

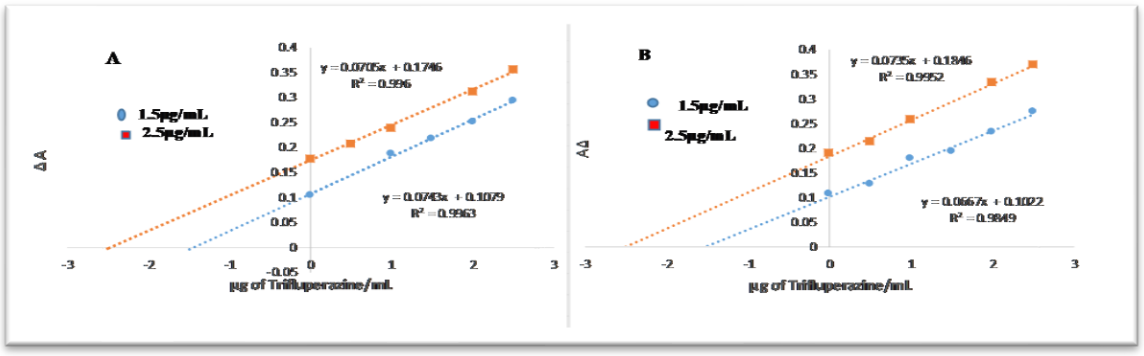


Figure9. Stander addition method curve for determining Trifluoperazine in tablet.(A)Iraqi company, (B)Egyptian company

Table 5. Stander addition method forr determining Trifluoperazine Hydrochloride

	Trifluoperazine Hydrochloride (µg/mL) Taken	Found	Recovery, %	Drug content (mg)
Iralzin tablets (5mg-SDI)	1.5	1.45	96.66	4.83
	2.5	2.47	98.80	4.94
Stellasil Tablets (5.9mg-Egypt)	1.5	1.53	102.00	6.01
	2.5	2.51	100.40	5.92

Conclusion:

The suggested method is simple, specific and, it does not require an extraction or any expensive solvents or temperature control. This method applied successfully to the analysis of Trifluoperazine Hydrochloride in tablet form with good accuracy and precision.

References:

[1] Muhammad, Sh. N., Muhammad, N.R.,Salman, B. I. H., Hisham, N. A.,Aziz, U., Tanzeela ,F., "How Synthetic Drugs Work" , (2023),215-253.

[2] Claire, D.,"xPharm: The Comprehensive Pharmacology Reference", (2007),1-6.

[3] British Pharmacopoeia, Pharma. Press London,(2022),*2*, 1227.

[4] Shelagh, M., Deborah, M., The Complete Pill Guide, Barnes and Noble books ,New York,(2003),1053.

[5] Hamzah, M. J., Taqi, R. M., Hasan, M. M., & Al-Timimi, R. J. (2017). Spectrophotometeric Determination of Trifluoperazine HCL in Pure Forms and Pharmaceutical Preperations. IJPCR, 9(5), 337-342. doi: 10.25258/ijpcr.v9i5.8593

[6] Al-Rufaie, M.M., Kathem, K.H.(2014).New spectrophotometric method for determination trifluoperazine hydrochloride in pharmaceutical preparations by using oxidative coupling reaction. World *J. Pharm. Res.*,*3*,1202-1214.

[7] Jalal, M. T. (2020). Spectrophotometric determination of trifluoperazine hydrochloride in pharmaceutical preparations by oxidative coupling reaction. *Syst. Rev. Pharm*., 11(6), 58-68. doi:10.31838/srp.2020.6.12

[8] Maadh, T., Kamal, M. M. (2016).Spectrophotomertic determination of triflouperazine hydrochloride using oxidative coupling reaction.*Inter. J. Innovative Res. in Tech*.: 23-27.

[9] Al_Taiee, A. K., Al_Doury, M. F. S., & Al_Rashidy, A. A. (2018). Spectrophotometric determination of Trifluoperazine Hydrochloride by oxidative coupling reaction with 4-amino benzoic acid using potassium iodate. Tikrit Journal of Pharmaceutical Sciences, 13(1), 1-10.

[10] Jalal, M.T., Al_Taiee, A. K., Al_Doury, M. F. S., & Al_Rashidy, A. A. (2018). Spectrophotometric determination of Trifluoperazine Hydrochloride by oxidative coupling reaction with 4-amino benzoic acid using potassium iodate. *Tikrit Journal of Pharmaceutical Sciences*, 13(1), 1-10.

[11] Hussein, A. M., & Othman, N. S. (2022). Spectrophotometric Determination of Trifluoperazine Hydrochloride Using Peak Area. African Journal of Advanced Pure and Applied Sciences (AJAPAS), 21-27.

[12] Nief ,R. A. (2014).Ultraviolet spectrophotometric determination of trifluoperazine. HCl in pharmaceutical preparations and environmental wastewater samples: Application to content uniformity testing, *Res.& Rev. J. Pharma. Analysis.* 2347-2340.

[13] Rawaa, A. Z. ;Elham, S. S.(2024).Indirect Spectrofluorometric Determination of Trifluperazine hydrochloride, Promethazine hydrochloride, Dimetindene Maleate in Pharmaceuticals by Ion Association Complex Formation with Phloxine B Dye , *College of Basic Education Research Journal*, ,*20*,819-843.

[14] Ameen, W. Q.;Zuhair ,A. K.; Ashraf S.R. (2011). An Indirect Atomic Absorption Spectrophotometric Determination of Trifluoperazine Hydrochloride in Pharmaceuticals ,*Arabian J. Sci. and Eng.*,*36*,553–563.

[15] Abdurhman, M. T. , Mahmoud, K. M. (2017). Flow injection spectrophotometric determination of trifluoperazine hydrochloride using oxidative coupling reaction, *International Journal of Current Research*,Vol. 9(4), 49218-49222.

[16] Shetti ,P. , Venkatachalan, A. (2011).Determination of Trihexaphenydyl HCl , Trifluoperazine HCl and Chloropromazine HCl from Blood Plasma, *J Pharm Biomed Sci* ,2230 – 7885.

[17] Patel, S. K., Patel, N. J. (2009) .Simultaneous RP-HPLC estimation of trifluoperazine hydrochloride and chlordiazepoxide in tablet dosage forms, *Indian J. of Pharm. Sci*., vol. 71, no. 5, 545-547.

[18]Stankovi´c ,D.,Dimitrijevi´c ,T.,Kuzmanovi´c ,D.,Krsti´c ,M.P.,Petkovic,B.B. (2015). Voltammetric determination of an antipsychotic agent trifluoperazine at a boron-doped diamond electrode in human urine, *RSC Adv.*, ,*5*,107058-107063.

[19] Ahmed, K. H., Suhaam, T. A.,Bahruddin ,S.,Suad, M .A.(2009). Potentiometric sensors for the determination of trifluoperazine hydrochloride in pharmaceutical preparations, *Anal. Sci*.,*25*,1295-9. doi: 10.2116/analsci.25.1295.

[20] Mohammed , S., Al-Jawadi, E., A.,M. (2022). Electrochemical Sensing for Trifluoperazine Determination at a PTH/MWCNTs Film-Modified Graphite Electrode ,*Egyptian Journal of Chemistry* ,*65*,13-17.

[21]Florea, M.,Ilie, M. (2017). Ion-pair spectrophotometry in pharmaceutical and biomedical analysis: Challenges and perspectives. Spectroscopic Analyses–Developments and Applications. *InTech*, Croatia,173-192.

[22] Zeena, Z. A. ; Maysam , H. A. (2021).Spectrophotometric and Spectrofluorimetric Determination of Terazosin in Tablets by Eosin Y ,*Baghdad Science Journal*,18,775-783.

[23]Rawaa,A.,Z.,Development of spectrophotometric and fluorimetric methods with the greenness evaluation for determination of some drug compounds using some basic and acidic *dyes,*Ph.D.Thesis,University of Mosul,2023.

[25]Perrine, D. D.; Dempsey, B., Buffer for pH and Metal ion control, John Wiley & Sons Inc., USA, (1974),134 148.

[26] Ali ,K. E. A., Preparation of phenacylchloride, morpholinophenacyl and N-Piperidinophenacyl oximes and study of their complexation with Copper (II) and Cobalt (II) ions, M. Sc. Thesis, University of Khartoum,1999.

[24]Mohammed,T.,A., (2019). Spectrophotometric and fluroimetric determination of some Antibiotics and enoxaparin in pharmaceutical preparations using Evans blue and acriflavine dyes, M.Sc. Thesis, University of Mosul,2019.