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ORIGINAL ARTICLE

Aniline Blue as a Chromogenic Reagent for Spectrophotometric Determination of Trifluoperazine Hydrochloride

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Competing Interests:
The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

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ABSTRACT:

Background: *Trifluoperazine hydrochloride is a group 3 phenothiazine antipsychotic used primarily to treat schizophrenia and psychotic disorders by rebalancing brain chemicals and reducing symptoms like anxiety and hallucinations .*

Methods: *A novel method for determining of trifluoperazine hydrochloride has been established in an acidic medium using aniline blue dye.*

Results: *A linear decrease in dye absorbance was observed with increasing drug concentration as a result of electrostatic attraction in ion association formation. The effects of various experimental parameters such as solution pH, time, temperature ,and dye concentration on decreasing the absorbance of the blue dye were investigated in detail at λ_{max} 592 nm, and a good linear relationship was obtained between ΔA and the concentration of trifluoperazine hydrochloride in the range of 0.5 -7.0 µg/mL with a determination coefficient of 0.9962, recovery average % of 97.80% ,Limit of detection(LOD) and limit of quantification(LOQ) values are 0.3319 µg.ml^{-1}and 1.1065 µg.ml^{-1} respectively. The relative Error RE% of -1.2 to -3.2% and a relative standard deviation RSD % of 0.35 to 2.79%.*

Conclusion:
This proposed method had been successfully applied to determine of trifluoperazine hydrochloride in tablet form accurately and precisely.

KEYWORDS: Aniline blue, Ion association complex, Spectrophotometric method, Trifluoperazine hydrochloride.

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INTRODUCTION:

Trifluoperazine hydrochloride is an antipsychotic medication. Psychotic disorders and schizophrenia are among the mental conditions for which this drug is administered. Trifluoperazine hydrochloride keeps people active and happy, reduces anxiety, and enhances their capacity for clear thought. Trifluoperazine hydrochloride is also used to treat hallucinations. It aids in the brain's rebalancing of different natural chemicals. Short-term anxiety relief has also been seen with this drug. However, it is not the initial course of treatment for anxiety. (Muhammad et al., 2023) It has a piperazine side chain and is categorized as a group 3 phenothiazine. Its general properties and therapeutic uses are similar to chlorpromazine (Claire, 2007). The scientific name of the drug is 10-(3-(4-Methyl-1-piperazinyl)propyl)-2-rifluoromethylphenorhiazine Di-hydrochloride (Figure 1). It is a white to pale yellow, crystalline powder, hygroscopic, freely soluble in water, soluble in alcohol. (British Pharmacopoeia, 2022).

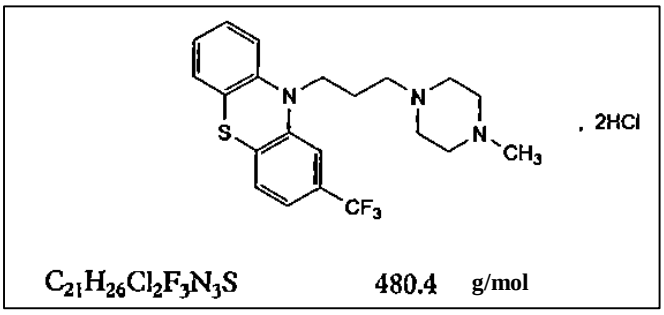

Figure1. Chemical structure of trifluoperazine hydrochloride

Trifluoperazine hydrochloride was determined with different analytical techniques, such as; spectrophotometric methods depending on oxidative coupling reaction (Al_Taiee et al., 2018; Hamzah et al., 2017 ;Jalal, 2020), area under the peak(Hussein and Othman, 2022), and oxidation-reduction reaction with 4,7diphenyl 1,10-phenanthroline(Hussein and Othman 2023). Also, other types of techniques have been used such as: derivative spectrophotometric, TLC densitometric and HPLC in presence of its hydrogen peroxide induced-degradation product(El-Gindy et al., 2002), indirect atomic absorption method (Ameen et al., 2011), Flow injection analysis (Abdurhman and Mahmoud, 2017), high performance liquid chromatography (Shetti and Venkatachalan, 2010; Patel and Patel, 2009), and Electrochemical methods (Stankovi´c et al., 2015; Ahmed et al., 2009; Mohammed and Al-Jawadi, 2022).

Ionic association complexes are widely used in analytical chemistry for the determination of many pharmaceutical and organic compounds which are formed when ion pairs of oppositely charged ions are held together by Coulomb attraction without the formation of a covalent bond. Organic dyes have

been used in the formation of ionic pair complexes to estimate pharmaceutical compounds (Florea and Ilie, 2017). Ion association complex reactions either formed at wavelength different about the wavelength of organic dye(reagent), and this type of reaction is the most common and the absorbance of the complex formed proportional directly with the concentration of the sample (Nejres & Najem, 2022; Al abdali and Al Fakri, 2021), and another type very rare based on decrease of the absorbance of ion association complex with increasing the concentration of sample (Sun et al. 2006a; Sun et al. 2006b; Sun et al. 2007).

The proposed method in this study is an example of the last type of reaction, which occurs due to the interaction of trifluoperazine hydrochloride and aniline blue in an acidic medium. The formed complex is mainly due to the electrostatic interaction between the tertiary amino groups in trifluoperazine hydrochloride and the anionic functional groups of aniline blue under acidic medium, causing a decrease in the absorbance of aniline blue at 592 nm.

EXPERIMENTAL

Instruments

A double beam SHIMADZU UV-VIS spectrophotometer (UV-1900i) with two glass cells which have a 1 cm light path were used to measure absorbance and spectrum. The pH of solutions under examination were measured using a BP3001 pH meter, and for exact, weight a BEL-Sensitive balance was used.

Chemical reagents:

Trifluoperazine hydrochloride solution (1000 µg/mL) was prepared by dissolving 0.1000 g (S.D.I-Iraq) in precisely 100 mL of distilled water in a volumetric flask and keep in an opaque vial then to prepare 50µg/mL from the above solution,5 mL was transferred and completed to the mark of 100 ml in a volumetric flask with the same solvent.

Aniline blue dye solution, (100 µg/mL) was prepared by dissolving 0.0100 g of pure reagent(Fluka) in precisely 100 mL of distilled water in a volumetric flask.

Hydrochloric acid solution, (0.01N) was prepared by adding 8.26 ml of concentrated acid (12.1 N Scharlau) to a small amount of distilled water in a volumetric flask of 100 ml and then the volume was made up to the mark with distilled water to prepare 1M of Hydrochloric acid solution then 1 mL from this solution was transferred to 100 mL volumetric flask and completed the volume to the mark with the same volume.

Trifluoperazine hydrochloride tablets,50µg/mL were prepared by taking ten tablets of each type ,STELLASIL 5 mg/tablet and Ten tablets of IRALZIN 5.9 mg/tablet), finely powdered and mixed

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homogeneously then dissolved with distilled water, and the volumes were completed to the mark in a 50 mL volumetric flasks and sonicated for 30 min for further dissolving then filtered, after that 5 mL from STELLASIL solution and 4.2 mL from IRALZIN solutions were diluted individually to 100 mL with the same solvent to prepare 50 µg/mL trifluoperazine hydrochloride of each solution.

RESULTS AND DISCUSSION

The effect of different types of acids on ΔA (△A=A-A°, A: absorbance of dye against distilled water, A°: absorbance of dye in presence trifluoperazine hydrochloride against distilled water) 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,0.01N	HCl	H_2SO_4	H_3PO_4	CH_3CO_2H	Without acid
A	1.100	1.109	1.110	1.073	0.901
A^0	0.784	0.795	0.813	0.797	0.824
ΔA	0.316	0.314	0.297	0.276	0.077

Then different volumes of HCl (0.01N) were added to 50 µg 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**.

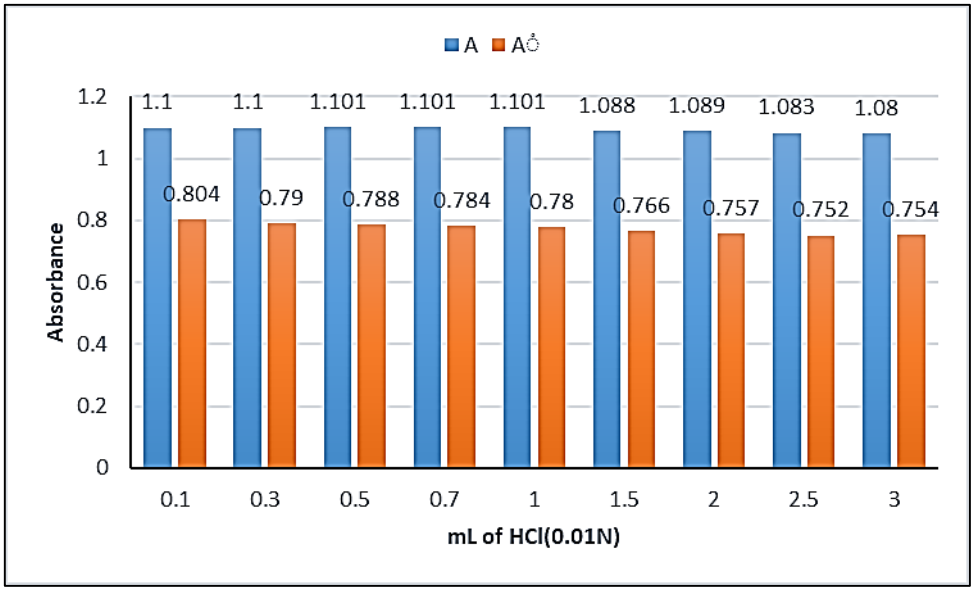

Figure 2. Effect of the amount of HCl on ion association formation

Figure 2 indicates that the volume of 2 ml of HCl (0.01 N) gave the highest value of ΔA (0.332) at pH

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2.5, and accordingly, the effect of various buffer solution at pH 2.5 was studied by adding different volumes of them, as shown in Table 2.

Table 2. Effect of buffer solutions

Type of medium	mL of buffer	A	A°	ΔA	Final pH
Citric acid-NaOH buffer	1	1.094	0.765	0.329	2.71
	2	1.088	0.788	0.300	2.68
KH-Phthalate buffer	1	1.045	0.717	0.328	2.83
	2	1.040	0.706	0.334	2.74
Glycine-HCl buffer	1	1.052	0.723	0.329	2.72
	2	1.007	0.703	0.304	2.68

The results showed that the use of buffer solutions did not improve ΔA, so 2 mL of HCl (0.01N) 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 (0.01N) was studied. The highest ΔA and determination coefficient (0.9960) were obtained when 2.5 mL of aniline blue was used. The results were showed in Figure 3

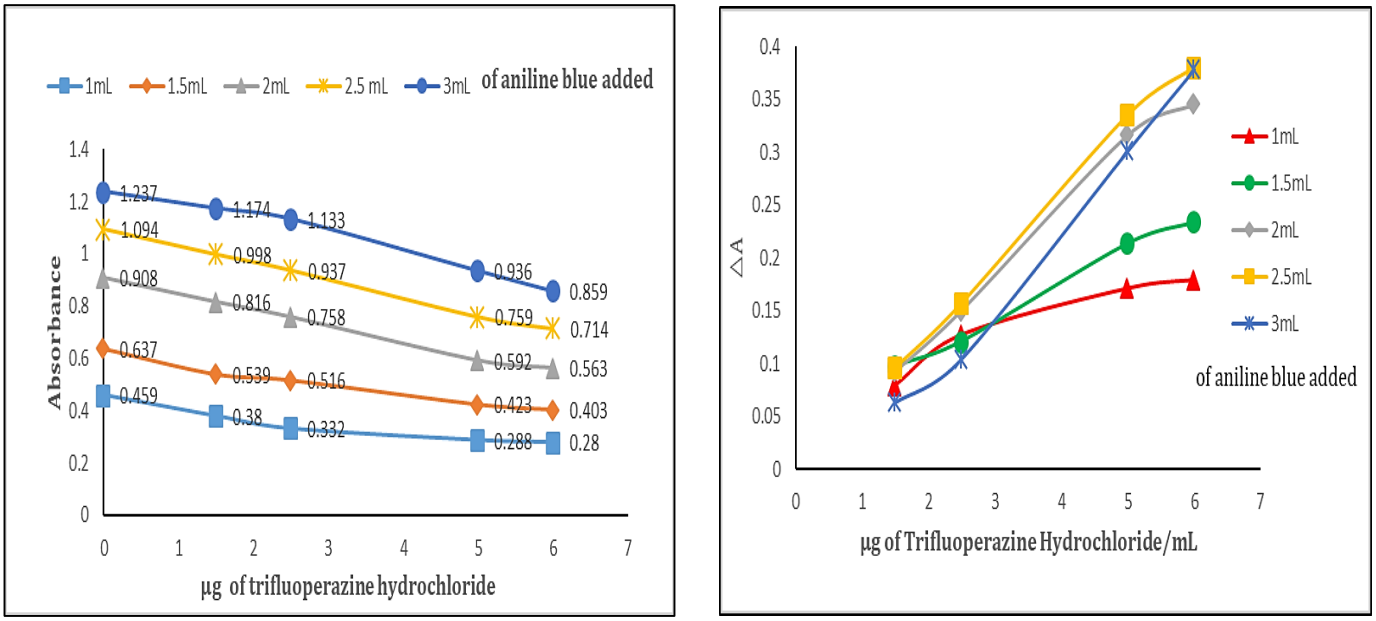

Figure 3. Effect quantity of aniline blue

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

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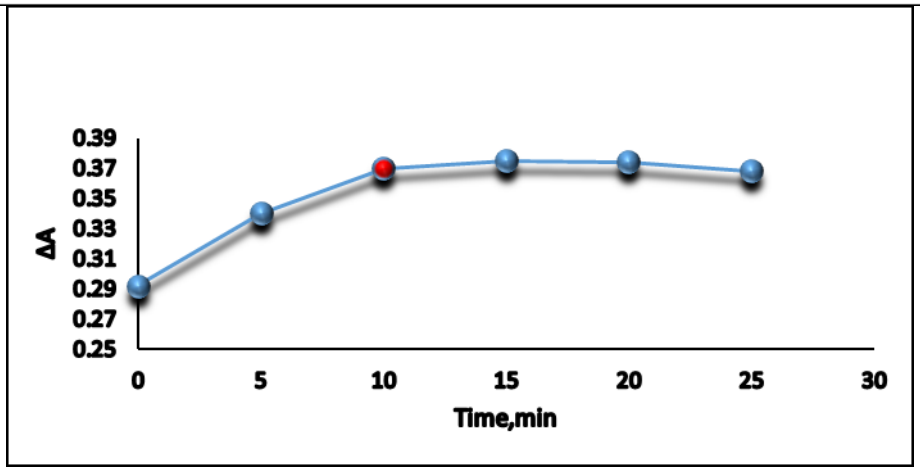

Figure 4. Effect of time before diluting the solution

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. In low temperature there is weak interaction and also in 40°C a decreasing in absorbance of aniline blue dye. The most stable results were obtained at room temperature for at least one hour (Table 3).

Table 3. The effect of temperature with standing time

Temperature, °C		Standing time, min								
		5	10	15	20	25	30	40	50	60
0	A	1.044	1.044	1.056	1.055	1.055	1.046	1.046	1.044	1.046
	A°	0.822	0.822	0.822	0.809	0.804	0.806	0.792	0.792	0.804
RT	A	1.104	1.104	1.104	1.104	1.100	1.104	1.104	1.104	1.104
	A°	0.739	0.739	0.739	0.736	0.729	0.729	0.726	0.725	0.728
40	A	1.082	1.082	1.063	1.060	1.051	1.046	1.024	1.013	0.996
	A°	0.844	0.844	0.840	0.817	0.799	0.786	0.785	0.781	0.780

Final absorbance spectrum

After optimizing all factors that affect the formation of ion association complex, the final spectra of aniline blue alone and in presence 5 µg/mL of trifluoperazine hydrochloride was obtained with acceptable absorbance at 592 nm, as shown in Figure 5.

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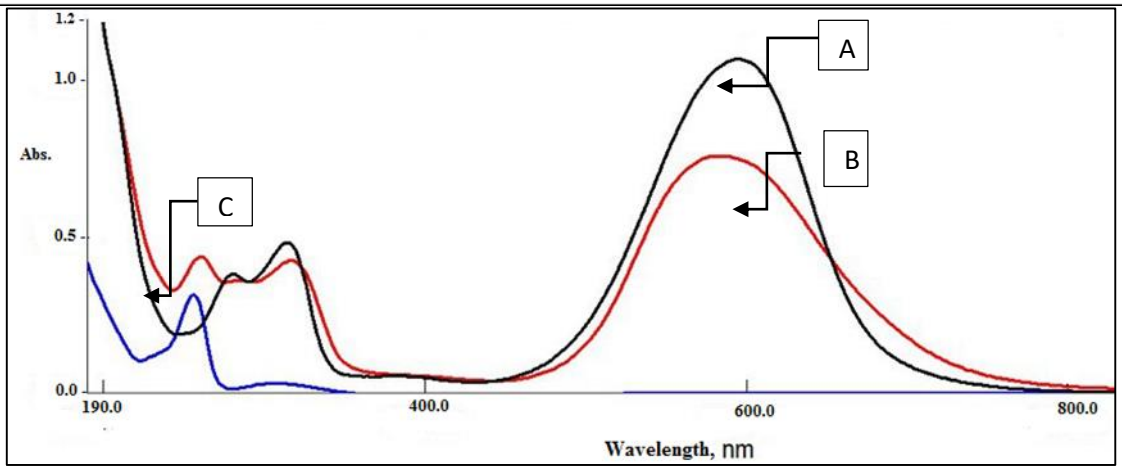

Figure5. The final absorption spectrum of 25 µg/ml of aniline blue against distilled water (A) in presence 5 µg/ml of trifluoperazine hydrochloride (B), 5 µg/ml of trifluoperazine hydrochloride against distilled water (C)

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 (0.01N) 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 in two methods (Figure**6**).

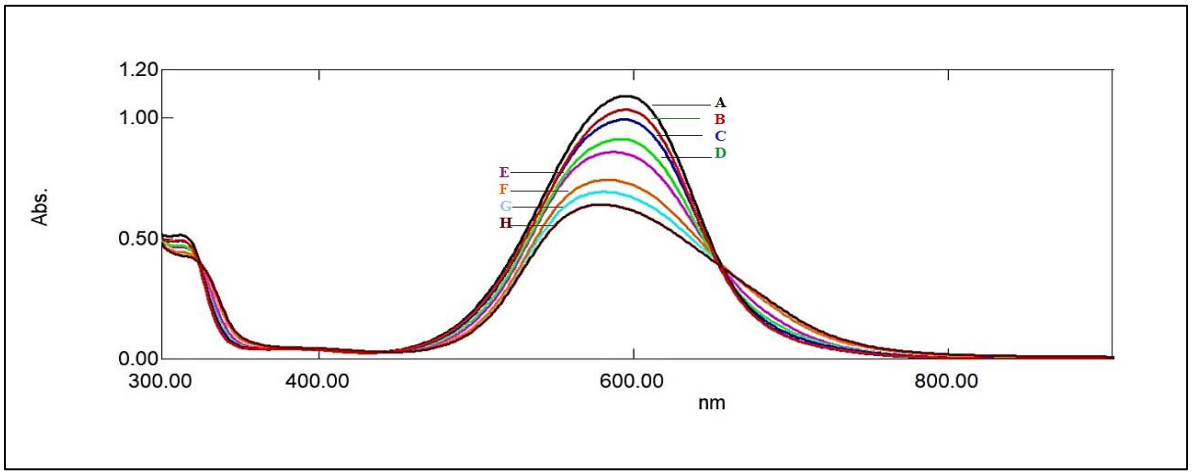

Figure6.Absorption spectra for (A) dye, (B)in presence of 0.5,(C)1.5,(D)2.5, (E)3.5, (F)5.0,(G)6.0 and(H)7.0 µg/mL of trifluoperazine Hydrochloride.

A linear relationship which was obtained over a concentration of 0.5-7.0 µg/ml of trifluoperazine hydrochloride either directly proportional with △A Figure7(A), or inversely proportional with absorbance (absorbance of dye in presence trifluoperazine hydrochloride) as shown in Figure7(B).

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The values of molar absorptivity and Sandell index were 3.4 x 10^4 L.mol^{-1}.cm^{-1} , 0. 0141μg.cm ^{2}, respectively.

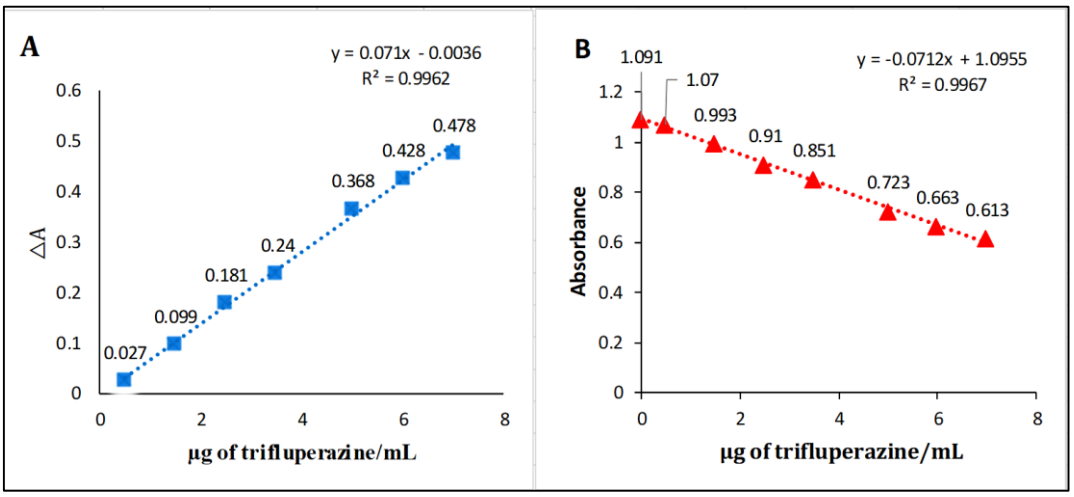

Figure 7.Calibration graph for trifluoperazine hydrochloride determination according to the proposed method

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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 4.

Table 4. 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

* Average of four determinations.

Statistical analysis

The nature of ion association complex:

The slope ratio method 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:

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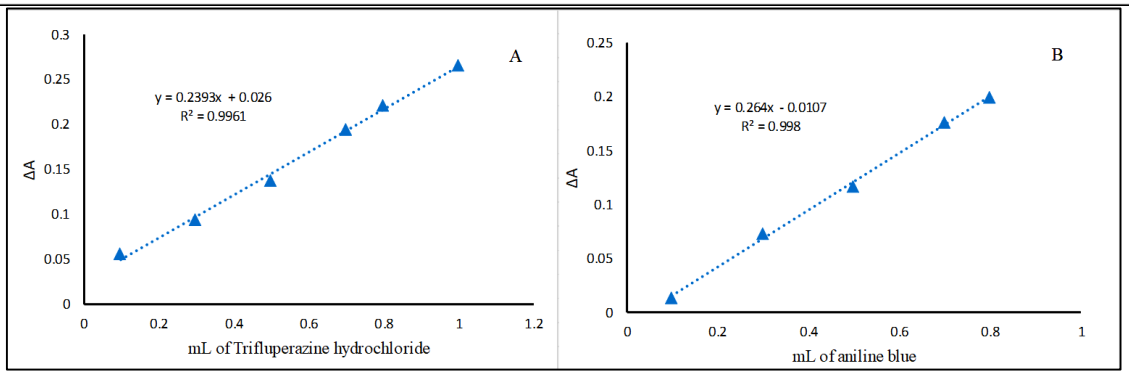
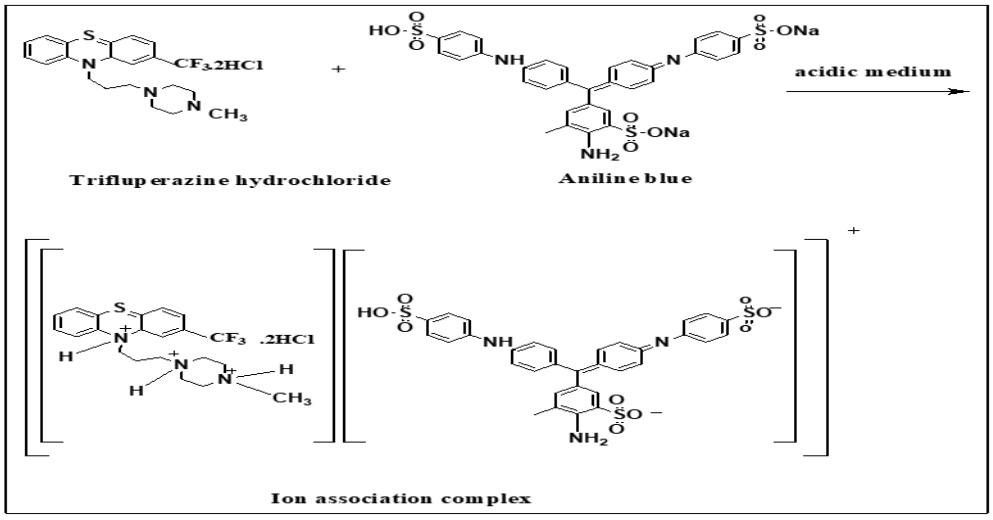


Figure 8. Calibration graph for Slope ratio method

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. Also, the results of the suggested method had been examined statistically using t-test (Skoog and wast 2013) and yielded favorable outcomes as in Table 5.

The results show that, at the 95% confidence level for four replicates, the experimental t-test is less than the theoretical value (t=3.18), showing that there is no discernible difference between the certified value and the determined amount.

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,

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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 6 show the results were obtained:

Table 5. Application of the method

	Trifluoperazine hydrochloride (µg/mL)		Recovery*,%	t-test
	Taken	Found		
Iralzin tablets (5mg-SDI)	2.5	2.48	99.20	2.44
	5.0	4.96	99.20	1.85
Stellasil tablets 5.9mg- kahira pharmaceuticals and chemical industries company	2.5	2.52	100.80	0.76
	5.0	4.95	99.00	2.96

*Average of four determinations.

Table 6. Stander addition method for determining trifluoperazine hydrochloride

	Trifluoperazine hydrochloride (µg/mL)		Recovery%	Drug content (mg)
	Taken	Found		
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

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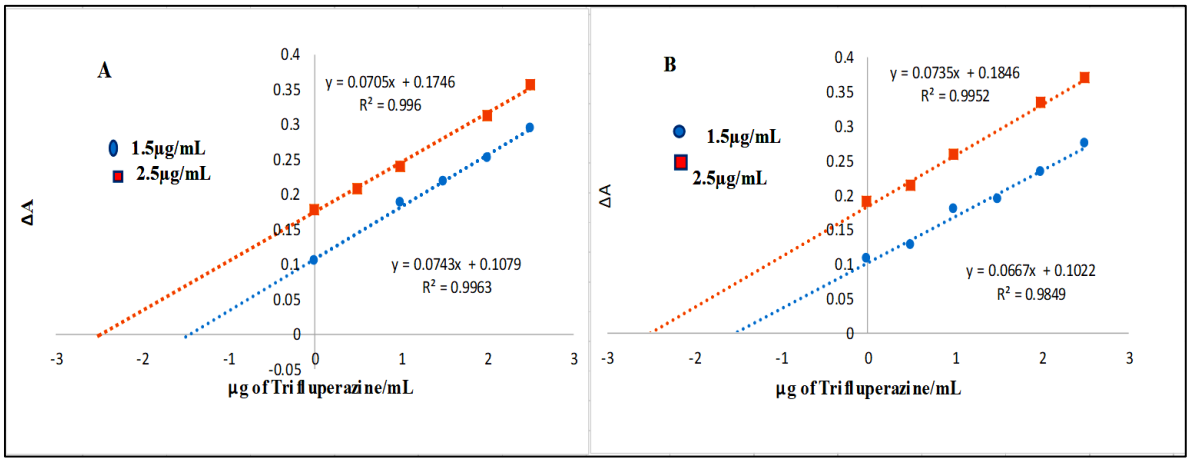

Figure9. Stander addition method curve for determining trifluoperazine in tablet. (A)Iraqi compai (B)Egyptian company

Comparison of the proposed approach

A comparison of the analytical variables for the proposed method which suggested for determination of trifluoperazine hydrochloride with other spectrophotometric methods used in the literature was made , as the proposed method is no less important, sensitive, and acceptable results compared to other methods(Table 7).

The present method is more sensitive than other methods in comparison according to the value of molar absorptivity and can be determined lower concentration of trifluoperazine hydrochloride.

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.

Table 7. Comparison with literatures

Analytical parameters	Present method	Literature method(Ahmed and Ahmed 2022)	Literature method(Nasr and Al-Rufaie 2022)	Literature Method (Hussein and Othman 2023)
Reagent	Aniline blue	Naphthalene 1,5-diamine	Diammonium Cerium Nitrate	4,7- Diphenyl 1,10-phenanthroline in the presence of Ferric[III]ion
Type of reaction	Ion association complex	Oxidative coupling	Complex formation	Oxidation-reduction
$\lambda_{max\ (nm)}$	592	535	499	533
Linearity, µg/mL	0.5-7.0	10-50	2-80	5-100
Temperature	At room temperature	At room temperature	At room temperature	50 °C
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	3.4×10^4	0.79×10^4	0.17×10^4	0.44×10^4
RE%	-1.2 to -3.2	-0.2 to -0.5	0.421 to 0.775	-0.96 to 0.98
RSD%	0.35 to 2.79	0.56 to 0.23	-1.11 to 2.22	0.31 to1.21
Dye's color	Blue	Violet	Orange	……
Sandell Index ($\mu g.cm^{-2}$)	0. 0141	0.0602	0.2778	……
Determination coefficient	0.9962	0.9992	0.9826	0.9993

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