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SPECTROPHOTOMETRIC DETERMINATION OF TRIFLUOPERAZINE HYDROCHLORIDE USING DIAMMONIUM CERIUM NITRATE IN ITS PURE AND PHARMACEUTICAL FORM

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Abstract: Trifluoperazine hydrochloride (TFPH) is widely used in the treatment of some diseases such as hysteria, vomiting and schizophrenia. This study aims to develop a fast, easy, sensitive, accurate and economical spectrophotometric method for the determination of TFPH. This method included the reaction of TFPH with Diammonium Cerium Nitrate (CAN) in a medium with a pH value of 1 to form a complex. Parameters influencing the stability of the resulting complex (i.e. time; and temperature) were also investigated. The method depended on the UV spectrum at the wavelength of (499 nm) and the correlation coefficient of (R^2= 0.9826). The results showed that the method has a good accuracy as (Rec% = 98.925), detection limit (0.408) and quantitative limit (1.361). The linearity range was 2-80 µg/mL. Moreover, this method has been successfully applied for the determination of the drug in its pure form and in its pharmaceutical preparation.

Keywords: *Spectrophotometric Determination; Trifluoperazine Hydrochloride; Diammonium Cerium Nitrate*

Introduction

Trifluoperazine hydrochloride (TFPH) is a phenothiazine antipsychotic used as an antidepressant commonly prescribed to treat mental disorders and to relieve symptoms of schizophrenia.[1] The TFPH is

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10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-10H-phenothiazine dihydrochloride[2] with structure shown in Figure 1. TFPH is a white powder that is soluble in water at a temperature of 20 and has a melting point of 240 ºC.[3] TFPH is commonly utilized in the treatment of hysteria, depression, nausea, vomiting and schizophrenia.[4] The side effects of TFPH are mainly dose-dependent, and declining the total orally administered dose reduces the severity of the toxicity, so TFPH can be considered a good candidate for an infectious drug delivery system due to its low oral bioavailability.[5]

TFPH is a powerful chelator of many biologically substantial charged metal ions such as (V^{5+}, Pt^{4+} and Pd^{2+}).[6] This compound is characterized by its attractive features represented by the chemical structure of the phenothiazine derivatives to which TFPH belongs, which prompted analytical chemists to use it extensively both as a chemical reagent[7] and to develop simple and rapid procedures for its detection.[8]

The formal method for estimating TFPH is either using a crystal violet index or a non-aqueous titration with perchloric acid, determination of the end point voltage.[9] Several methods have been used in the literature in determining TFPH such as Spectrophotometric,[3,10,11] Spectrofluorometric,[12] HPLC,[13] RP-HPLC,[14] Electrochemistry,[15] Colorimetric,[16] Multi Walled Carbon Nanotubes[17] and flow injection analysis.[18]

On the other hand, the processes of analysis and determination of drugs and the use of drug synthesis techniques have helped remarkably in the treatment, diagnosis and even prevention of diseases in individuals.[19-21] Detection techniques should have a low detection limit because these techniques may focus on analyzing small quantities of biological samples such as blood, urine and serum.[19,22]

The ease of use of spectrophotometric methods and the availability of its devices have made it an important technique for wide-ranging applications in various fields.[23] UV-visible spectroscopic methods have several advantages,[24] including: their reliability and speed in determining the composition of diverse mixtures of drug complexes, their ability to solve overlapping spectra of complexes appropriately and quickly, low operating costs as they can be operated with less quantities of analytical solvents, in addition to the fact that they produce few wastes.

Thus, this study aims to determine TFPH spectrophotometrically in its pure and pharmaceutical form. The method involved the reaction of TFPH (in acidic medium) with Diammonium Cerium Nitrate (CAN) to form a complex. CAN is among the most important oxidants in organic synthesis due to its high stability and commercial availability.[25] It is also useful in entering and removing protective groups (by Lewis acid catalysis or single electron transfer) as well as acting as a proper reagent for the generation of radicals from acidic CH substrates.[26] Parameters affecting the stability of the resulting complex, as well as the biological activity of the drug and the complex against different types of bacteria were also studied. It is believed that this method is promising for its ease of application and reproducibility, as well as for its high accuracy.

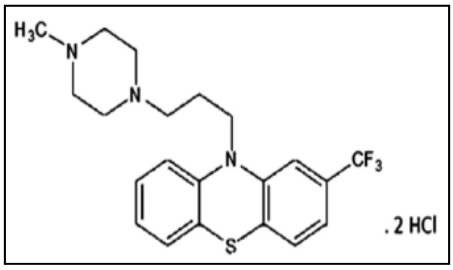

Figure 1. Trifluoperazine hydrochloride: molecular weight, 480.43 g/mole.[26]

Experimental Program

Apparatus and materials

Apparatus

1. Double beam UV-Visible: UV-Visible spectrophotometer Type UV-1650 PC from Shimadzu Company was used. The double beam UV-Vis instrument was used for quantitative analysis to measure the absorbance intensity of the complex under different conditions (to determine the optimal conditions for the complex under study).
2. Single beam UV-Visible: UV-Visible spectrophotometer Type UV-2900 PC from Shimadzu Company was utilized. The single beam UV-Vis instrument was used for qualitative analysis to determine the maximum wavelength absorbance of the resulting-colored solution.
3. pH meter.
4. Electronic balance.
5. Water bath with GFL 1083 was used.

Materials

1. TFPH drug ($C_{21}H_{26}C_{l2}F_3N_3S$) produced by BDH company with purity of 99% was used.
2. Diammonium Cerium (IV) Nitrate ($Ce(NH_4)_2(NO_3)_6$) purchased from BDH company with purity of 99% was utilized.
3. TFPH tablet (5 mg) purchased from SDI Samarra, Iraq was employed.
4. Distilled water.

Preparation of solutions

1. Pure TFPH drug (250 $\mu g \cdot mL^{-1}$). An amount (0.025 g) of TFPH was taken and dissolved in distilled water using a volumetric flask (100 mL).

2. Diammonium Cerium (IV) Nitrate (0.01 M). This solution was prepared by taking a weight of diammonium cerium nitrate (0.54626 g) and dissolving it in a volumetric flask of 100 mL of distilled water.
3. Aqueous solution of TFPH from pharmaceutical tablet (5 mg). It was prepared by taking a weight equivalent to a concentration (250 $\mu g \cdot mL^{-1}$), which is estimated at (1.1625 g) and dissolving it in a volumetric flask of 100 mL of distilled water.

Results And Discussion

The maximum wavelength of the complex produced by the reaction of diammonium cerium nitrate at a concentration of (2×10^{-3} M) with TFPH at a concentration of (50 $\mu g \cdot mL^{-1}$) was measured. The resulting color was orange and the maximum absorbance was recorded at a wavelength of (499 nm) because of increasing the electronic transitions (Hassouna et al. 2012). The UV-Vis spectrum of the complex resulted from the reaction of TFPH and CAN is presented in Figure 2.

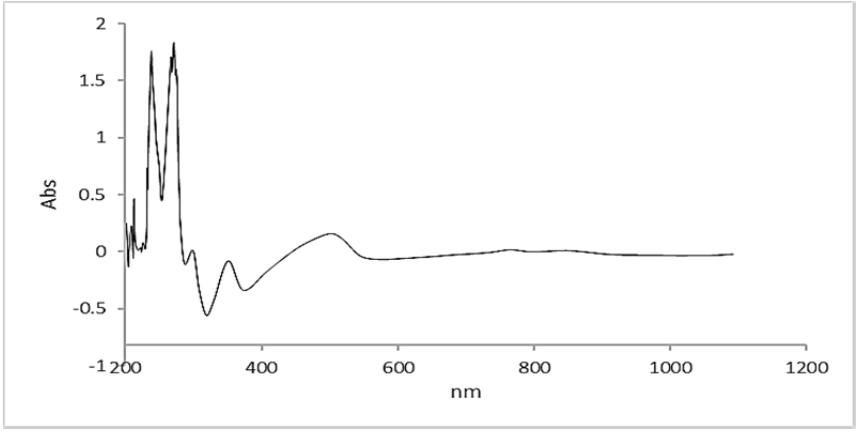

Figure 2. The UV-Vis spectrum of the complex produced from the reaction of TFPH and CAN.

The impact of different parameters on the absorption intensity of TFPH-CAN complex, as presented in the following sections, was studied and the optimal reaction conditions were chosen.

The best concentration of diammonium cerium nitrate

The effect of diammonium cerium nitrate concentration on the complex was studied with a range of concentrations ranging from (2 to 40 $\times 10^{-5}$ M). Results indicated that the best absorbance was recorded at the concentration of (10×10^{-5} M) as presented in Figure 3.

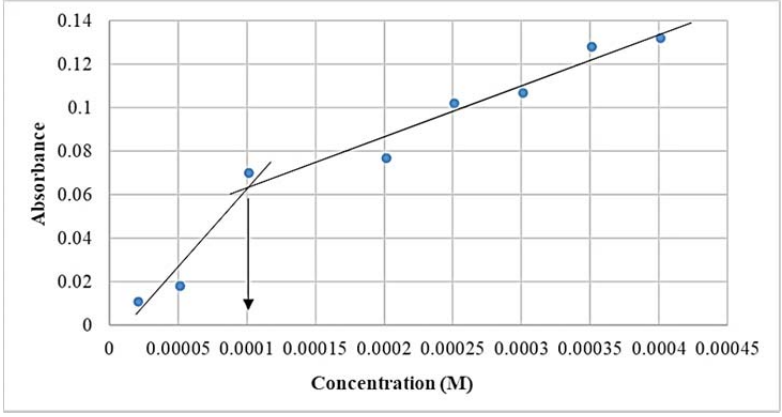

Figure 3. The absorbance curve of solutions of the product formed by the reaction of TFPH with CAN at different concentrations of CAN.

Effect of pH

The effect of pH values with a range between 1 - 9 on the resulting complex was studied, and the best absorbance was found at pH = 1 as shown in Figure 4. Then, as the pH value increased and the acidity decreased, the absorbance was decreased because of the salt formations between the acidic drug and the basic media [18]. As a result, a pH value of 1 was adopted in the later studies.

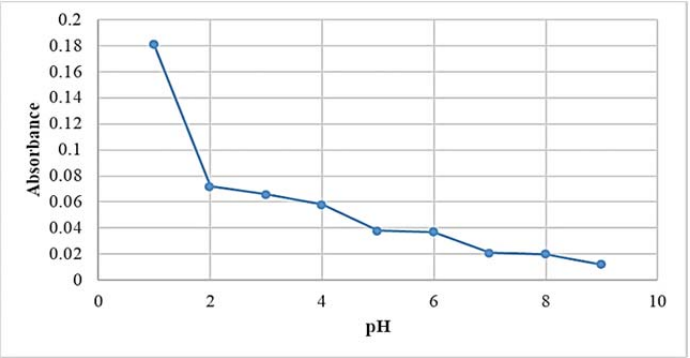

Figure 4. The effect of pH value on the absorbance of the TFPH-CAN product.

Effect of Time

The effect of time on the complex was measured for a time ranging from 5 to 60 minutes, and the absorbance corresponding to each time was measured, as shown in Figure 5. The results showed that the best absorbance was recorded after 20 minutes from the start of the reaction. After that, the absorbance was stable for a while and then, with time, the absorbance began to decrease and the stability of the complex was declined. Other meanings, the optimum time of reaction was 20 minutes. It can be seen from Figure 5 that the absorbance at times of 10 and 15 minutes is not much different from that at a time of 20 minutes. However, beyond the time of 20 minutes, the absorbance values tend to decline significantly, thus, it was chosen.

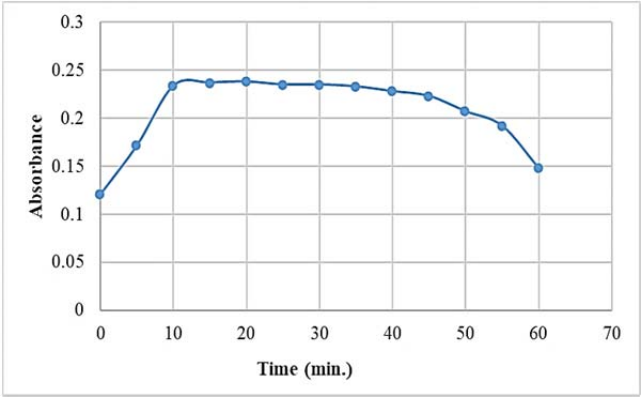

Figure 5. The effect of time in minutes on the absorbance of the TFPH-CAN product.

Effect of Temperature

The temperature of the complex was measured in a range of 15 to 50 °C, and the absorbance was measured at each temperature. The best absorbance was observed at 20 °C, as shown in Figure 6. Thereafter, the absorbance started to decrease with increasing temperature due to the dissociation of the resulting complex and the change in the value of λ max [13]. The stability time of the complex was more than 2 hours. Therefore, it is recommended to perform the reaction at a temperature of (20 °C).

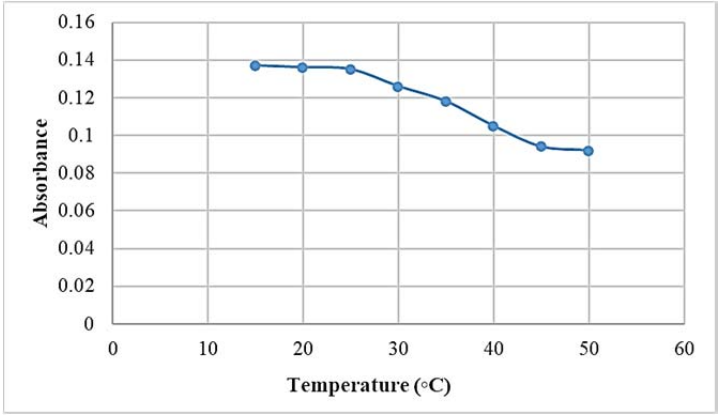

Figure 6. The effect of temperature on the absorbance of the TFPH-CAN product.

Effect of the best addition sequence

The effect of the complex addition sequence was studied, as the absorbance was measured at each different sequence. The sequence of addition is shown in Table 1. Results revealed that the highest absorbance value was recorded at the addition sequence (drug - distilled water - element - pH). Therefore, this sequence was adopted in the posterior measurements. which that produced from total oxidation for the drug and more stable for the color product.

Table 1. The sequence of addition of solutions and the corresponding absorbance.

No.	Addition sequence	Absorbance
1	Drug + Element + pH + Distilled Water	0.081
2	Drug + pH + Element + Distilled Water	0.077
3	Element + Drug + pH + Distilled Water	0.079
4	Element + pH + Drug + Distilled Water	0.078
5	Drug + Distilled Water + Element + pH	0.087
6	Element + Distilled Water + Drug + pH	0.082

Calibration curve

A calibration curve was constructed (see Figure 7) for the complex resulting from the reaction of TFPH drug with diammonium cerium nitrate by taking a range of different concentrations of the drug. It can be seen from Figure 7 that the concentrations that yielded to Beer-Lambert's law (linear calibration curve) were within the range (2-80 µg/mL) and the correlation coefficient (R^2) was 0.9826. This R^2 value, statistically, indicated that the constructed curve has high linear characteristics. Analytical values related to the calibration curve for drug determination can be seen in Table 2. It was found that the molar absorptivity was 1729.548 L/mole.cm while the calculated Sandell's sensitivity was 0.2778 µg/cm^2. Furthermore, the limit of detection (LOD) and limit of quantitation were 0.408 µg/mL (less than the lower value of linear range that obeyed to Beer-Lambert's law) and 1.361 µg/mL respectively.

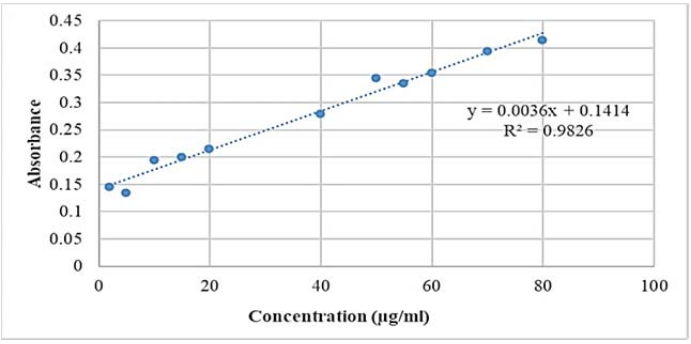

Figure 7. The calibration curve of the TFPH-CAN product.

Table 2. Analytical values related to the calibration curve for drug determination.

Parameter	Value
λ max (nm)	499
Slope	0.0036
Regression equation	y = 0.0036x + 0.1414
Molar absorptivity (L.mole-1.cm-1)	1729.548
Linear range (µg/mL)	2-80
Correlation coefficient (R^2)	0.9826
LOD	0.408
LOQ	1.361
Sandell's sensitivity (µg/cm^2)	0.2778
Stoichiometric ratio	1:1

Complex stoichiometry of TFPH and diammonium cerium (IV) nitrate in aqueous solution

In this study, the stoichiometric determination of the complex was determined by molar ratio and continuous variation methods as shown in the Figures 8 and 9 respectively. The results showed that the ratio between the drug and diammonium cerium nitrate was (1:1).

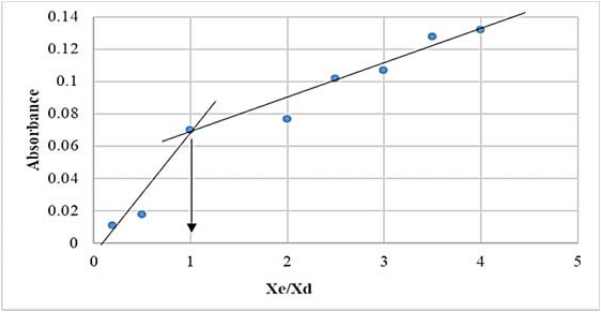

Figure 8. The molar ratio method of the TFPH-CAN product.

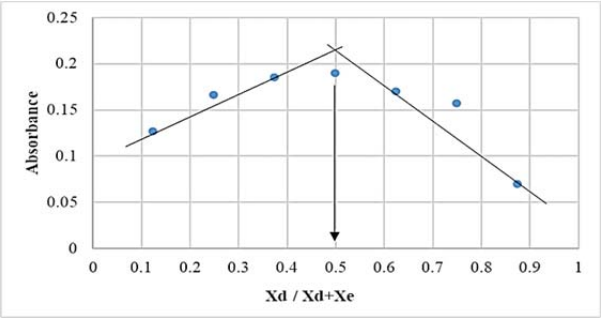

Figure 9. The continuous variation method of the TFPH-CAN product.

The mechanism of reaction can suggest between the drug under study and the used in its estimation, as well as the presence of the oxidizing factor and the acidic medium as shown in the linear equations, based on the result of mole ratio and which studied nature compound by reviewing the literature.[27,28]

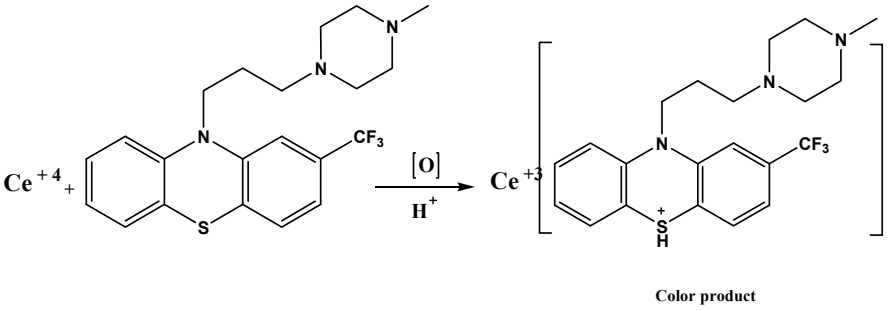

Figure 10. The suggested mechanism of reaction for the TFPH-CAN product.

Precision and accuracy

The precision and accuracy of this method have been studied. The precision represents the convergence between the analytical and real values and is expressed by the relative error percentage (Erel%) or percentage of recovery (Rec%) according to the following equations:

$$\text{Erel\%} = \frac{A-R}{R} \times 100 \quad (1)$$

$$\text{Rec\%} = 100 \pm \text{Erel\%} \quad (2)$$

where,

A: Analytical value;

B: Real value.

Accuracy represents the degree of convergence between the repeated readings of the same concentration and can be found by the relative standard deviation (RSD) according to the following equation:

$$\text{RSD} = \frac{\text{SD}}{\bar{x}} \times 100$$

where,

SD: Standard deviation;

$\bar{x}$: Arithmetic mean.

The results of precision and accuracy are shown in Table 3. Seven replicates have been depended in calculating RSD. It was found that minimum value of the recovery was 97.78 which corresponded to a relative error of 0.775%. This indicated that the adopted spectrophotometric method had a good accuracy and acceptable precision.[26,27]

Table 3. Precision and accuracy results.

Concentration (µg/mL)				
Present	**Found**	**RSD%**	**Rec%**	**E%**
20	20.44	2.22	97.78	0.775
60	59.33	-1.11	101.11	0.469
70	70.44	0.635	99.365	0.421

Interferences effect

Excipients, diluents and flavoring agents are among the many interrelated substances expected in pharmaceutical preparations.[29] These substances may have an effect on the absorption of the complex resulting from the interaction of the drug with the reagent or the element, so it is important to study their effect. Table 4 shows the absorbance of some of these interfering materials and the rate of recovery (%). From the values presented in the table above, it is clear that these materials have no effect on the absorbance of the complex, since the recovery change was less than ±5%.

Table 4. The effect of additions existence, at a concentration of 500 ppm, on the absorbance of TFPH-CAN complex.

Interference	**Error %**	**Recovery %**
Lactose	+1.235	98.765
Starch	-0.485	100.485
$Cacl_2$	+1.533	98.467
Sucrose	+1.630	98.370
Glucose	-1.154	101.154
Benzoic acid	-1.124	101.124
ascorbic acid	-1.273	101.273
Fructose	+1.435	98.565

The pharmaceutical application

The pharmaceutical application was carried out by using a homogeneously grinded pooled sample of ten tables (5 mg active substance each tablet) provided by SDI Company, Iraq. An amount of powder was taken equivalent to (250 $\mu g \cdot mL^{-1}$) and dissolved in distilled water, then filtered and placed in a volumetric bottle of 100 mL capacity, and the volume was completed to the mark. The rest of the concentrations were prepared from it according to the dilution law and a calibration curve was made and the accuracy and precision were measured as shown in the Table 5. Seven replicates were tested for each concentration level. The results showed that there is a good agreement between the suggested method and the results acquired for the pharmaceutical preparation, as the recovery percentage was not less than 98%.It was calculated By liner equation of the calibration carve.

Table 5. The accuracy and precision of the pharmaceutical application.

Concentration (µg/mL)				
Present	**Found**	**RSD%**	**Rec%**	**E%**
10	10.2	2	98	0.839
20	20.2	1	99	0.525
50	49.6	-0.8	100.8	0.448

Conclusions

This paper includes proposing a spectrophotometric (UV-Vis) method for the determination of TFPH in its pure and pharmaceutical form using diammonium cerium nitrate. Depending on what was obtained through the results, the proposed method can be applied effectively in both forms of the drug: pure and pharmaceutical forms. The potential application

of this method is limited to only determining the purity of selected TFPH active substance or to its' determination from pharmaceutical formulations. No external quality assurance or quality control tests were performed for the method performance parameters. Thus, this can be considered a potential limitation in the manuscript.

References

1. Saad, A. S.; Mohamed, M. A.; Koshek, S. H.; et al. Assessment of the Oxidative Degradation Pathway for Trifluoperazine Hydrochloride and Isopropamide Iodide through a Validated Spe-HPLC Methods and Application in Human Plasma. *Int. J. Appl. Pharm. Biol. Res.* **2017**, *2*, 10-19.

2. Prashanth, K. N.; Swamy, N.; Basavaiah, K. Extraction-Free Ion-Pair Methods for the Assay of Trifluoperazine Dihydrochloride in Bulk Drug, Tablets, and Spiked Human Urine Using Three Sulfonphthalein Dyes. *J. Appl. Spectrosc.* **2014**, *81*, 893-902.

3. Al_Rashidy, A. A. M.; Al_Doury, M. F. S.; Al_Taiee, A. K. A. Spectrophotometric Determination of Trifluoperazine Hydrochloride by Oxidative Coupling Reaction with 4-Amino Benzoic Acid Using Potassium Iodate. *Tikret J. Pharm. Sci.* **2018**, *13(1)*.

4. Jalal, M. T. Spectrophotometric Determination of Trifluoperazine Hydrochloride in Pharmaceutical Preparations by Oxidative Coupling Reaction. *Syst. Rev. Pharm.* **2020**, *11*, 58-68.

5. Gahiwade, H. P.; Patil, M. V; Tekade, B. W.; et al. Formulation and In-Vitro Evaluation of Trifluoperazine Hydrochloride Bi-Layer Floating Tablet. *Int. J. Pharm. Biol. Sci.* **2012**, *2*, 166-172.

6. Seubert, A.; Saad Rasheed, A. Separation of Metal-Trifluoperazine Hydrochloride Complexes Using Zwitterionic Ion Chromatography (ZIC) Coupled Online with ICP-AES. *Curr. Pharm. Anal.* **2017**, *13*, 328-333.

7. Qasim, A. W.; Khammas, Z. A. A. An Indirect Atomic Absorption

Spectrometric Determination of Trifluoperazine Hydrochloride in Pharmaceutical Formulations Based on Chelate Formation with Palladium (II). *E-Journal Chem.* **2010**, *7*, S433-S441.

8. Waleed, A. Q.; Khammas, Z. A. A.; Al-Ayash, A. S.; et al. An Indirect Atomic Absorption Spectrophotometric Determination of Trifluoperazine Hydrochloride in Pharmaceuticals. *Arab. J. Sci. Eng.* **2011**, *36*, 553-563.

9. Abbas, S. S.; Zaazaa, H. E.; Abdelkawy, M.; et al. Spectrophotometric Determination of Isopropamide Iodide and Trifluoperazine Hydrochloride in Presence of Trifluoperazine Oxidative Degradate. *Drug Test. Anal.* **2010**, *2*, 168-181.

10. Ahmed, N. R. Ultraviolet Spectrophotometric Determination of Trifluoperazine. HCl in Pharmaceutical Preparations and Environmental Wastewater Samples: Application to Content Uniformity Testing. *Res Rev J Pharm Anal* **2014**, *3*, 30-34.

11. Hassouna, M. E. M.; Adawi, A. M.; Ali, E. A. Extractive Spectrophotometric Determination of Chlorpromazine and Trifluoperazine Hydrochloride in Pharmaceutical Preparations. *Egypt. J. Forensic Sci.* **2012**, *2*, 62-68.

12. Omar, M. A.; Mohamed, A.; Nagy, D. M.; et al. Highly Sensitive Spectrofluorimetric Procedure for the Assay of Phenothiazine Derivatives in the Presence of Their Sulfoxide Oxidized Product. *Luminescence* **2020**, *35*, 1134-1141.

13. Dhabab, J. M.; Al-Ameri, S. A. H.; Taufeeq, A. H. Separation and Determination of Trifluoperazine and Prochlorperazine in Pharmaceutical Preparations by HPLC. *J. Assoc. Arab Univ. Basic Appl. Sci.* **2013**, *13*, 14-18.

14. Chaudhary, A. B.; Raval, R. J.; Vaghela, K.; et al. Development and Validation of Analytical Method for Simultaneous Estimation of Chlordiazepoxide, Trifluoperazine Hydrochloride and Trihexyphenidyl Hydrochloride in Tablet Dosage Form. *Int. Bull. Drug Res.* **2016**, *6*, 1-6.

15. Stanković, D.; Dimitrijević, T.; Kuzmanović, D.; et al. Voltammetric Determination of an Antipsychotic Agent Trifluoperazine at a Boron-Doped Diamond Electrode in Human Urine. *RSC Adv.* **2015**, *5*, 107058-107063.

16. Ahmed, N. R.; Hasoon, L. 'Colorimetric Trifluoperazine. HCL Assay in Tablets and Industrial Wastewater. *World J. Pharm. Pharm. Sci.* **2018**, *7*, 198-1205.

17. Konoz, E.; Sahebi, H.; Paridar, T. Perconcentration and Determination of Trifluoperazine Hydrochloride in Biological Samples by Using Multi Walled Carbon Nanotubes as Sorbent. *Int. J. Pharm. Technol.* **2016**, *8*, 16779-16789.

18. Mahmoud, K. M.; Abdurhman, M. T. Flow Injection Spectrophotometric Determination of Trifluoperazine Hydrochloride Using Oxidative Coupling Reaction. *Int. J. Curr. Res.* **2017**, *9*, 49218-49222.

19. Safaei, M.; Shishehbore, M. R. A Review on Analytical Methods with Special Reference to Electroanalytical Methods for the Determination of Some Anticancer Drugs in Pharmaceutical and Biological Samples. *Talanta* **2021**, *229*, 122247.

20. Mohamed, M. A.; Fayed, A. S.; Hegazy, M. A. et al. Fully Optimized New Sensitive Electrochemical Sensing Platform for the Selective Determination of Antiepileptic Drug Ezogabine. *Microchem. J.* **2019**, *144*, 130-138.

21. Zarezadeh, A.; Rajabi, H. R.; Sheydaei, O. et al. Application of a Nano-Structured Molecularly Imprinted Polymer as an Efficient Modifier for the Design of Captopril Drug Selective Sensor: Mechanism Study and Quantitative Determination. *Mater. Sci. Eng. C* **2019**, *94*, 879-885.

22. Khorshed, A. A.; Khairy, M.; Elsafty, S. A. et al. Disposable Screen-Printed Electrodes Modified with Uniform Iron Oxide Nanocubes for the Simple Electrochemical Determination of Meclizine, an Antihistamine Drug. *Anal. Methods* **2019**, *11*, 282-287.

23. Hamzah, M. J.; Taqi, R. M.; Hasan, M. M. et al. Spectrophotometeric Determination of Trifluoperazine HCL in Pure Forms and Pharmaceutical Preperations. *Int. J. Pharm. Clin. Res.* **2017**, *9*, 337-342.

24. Gupta, D.; Bhardwaj, S.; Sethi, S.; et al. Simultaneous Spectrophotometric Determination of Drug Components from Their Dosage Formulations. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2022**, 120819.

25. Dhakshinamoorthy, A. Cerium (IV) Ammonium Nitrate: A Versatile Oxidant in Synthetic Organic Chemistry. *Synlett* **2005**, *2005*, 3014-3015.

26. Abdelrahman, M. M.; Abdelaleem, E. A. Mean Centering of Ratio Spectra and Successive Derivative Ratio Spectrophotometric Methods for Determination of Isopropamide Iodide, Trifluoperazine Hydrochloride and Trifluoperazine Oxidative Degradate. *J. Saudi Chem. Soc.* **2016**, *20*, S153-S160.

27. Al-Ward, H. S. Spectrophotometric Micro Determination of Promethazine Hydrochloride in Pharmaceutical Preparations via Oxidative Coupling Reaction with Sulphanilamide and in the Presence of Ferric Chloride. *J. Um-Salama Sci.* **2005**, *2*, 110-119.

28. Alizadeh, N.; Rezakhani, Z. Extractive Spectrophotometric Determination of Ketoconazole, Clotrimazole and Fluconazole by Ion-Pair Complex Formation with Bromothymol Blue and Picric Acid. *J. Chil. Chem. Soc.* **2012**, *57*, 1104-1108.

29. AL-Da'amy, M. A. A.; AL-Moswi, R. F. A sensitive Colorimetric Method for the Determination of Methyldopa in Pharmaceutical Preparation via Oxidative Coupling Organic Reaction with Thiamine Hydrochloride in the Presence of Potassium Periodate. *Kerbala J. Pharm. Sci.* **2013**, *6*, 69-77.