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ORIGINAL ARTICLE

Spectrophotometric Determination of Tetracycline via Coupling with Diazotized m-Nitroaniline

Nabeel S. Othman[1] , Aws M. Nejres [2]* , Rasha J. Al-Ashow [3]

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1. **Department of Chemistry, College of Science, University of Mosul. Mosul, Iraq, e-mail: nsn20002004@uomosul.edu.iq .**
2. **(Corresponding Author) Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq, e-mail: aws.m.nejres@uomosul.edu.iq .**
3. **Ministry of Agriculture, Office of Agriculture Research,Mosul,Iraq, e-mail: Alashorasha15@gmail.com**
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***Corresponding Author Email:**
aws.m.nejres@uomosul.edu.iq

ABSTRACT:

Background: Tetracycline hydrochloride (TC-HCl) microgram quantities can be determined using a spectrophotometric technique that has been proposed.

Methods: The process involves reacting diazotized m-nitroaniline with Tetracycline hydrochloride (TC-HCl) in an alkaline media.

Results: At λ_{max} of 382.5 nm, the produced dye's obeyed Beer's law within the range of (1.0 – 30 µg.mL^{-1}). The sensitivity represented by molar absorptivity reached 1.66× 10^{4} l.mol^{-1}. cm^{-1} and Sandell sensitivity index of 2.8 × 10^{-2} µg.cm^{-2} . Depending on the concentration level, the color reaction had a relative standard deviation of 0.357 to 1.26% and a relative accuracy of -2.8 to 0.45%. It was also quite steady. The technique has been effective in of tetracycline-HCl in pharmaceutical.

Conclusions: Thus, this technique has been successfully proven to be effective in determining the concentration of Tetracycline hydrochloride (TC-HCl) compound within its pharmaceutical formulations.

KEYWORDS: TC-HCl, m-nitroaniline, azo dye, coupling reaction, spectrophotometric method

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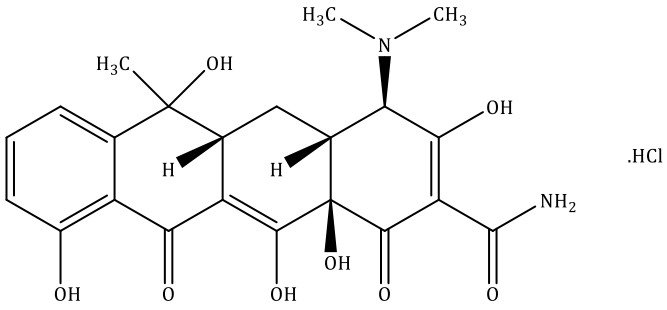
INTRODUCTION:

In recent years, many chemists have tried to find various methods that have been used to determine Chemicals in pharmaceutical preparations (Hussein & Othman, 2023; Faeji, Fasoro, & Oni, 2024).Tetracycline (TC-HCl) is one of the primary antibiotic groups used for veterinary purposes, human treatment, and agriculture, is the most often used throughout the world (Daghrir and Drogui 2013; López-Peñalver et al. 2010)· , discovered in the 1940s. Its first chemical structure was published in 1954 titled "The Structure of Aureomycin (Nelson and Stuart 2011). The chemical scaffold of (TC-HCl) is a versatile and adjustable structure that can interact with plentiful cellular targets (Zakeri and Gerard 2008). Consequently, it is used as an active ingredient in antibacterial products against a wide range of gram-positive/-negative bacteria with numerous therapeutic potentials (Tauch et al. 2000; Bahrami et al. 2012) and is used in the therapy of dysentery, pneumonia, mycoplasmas, gonorrhea, rickettsiae and other infectious diseases (Eliopoulo et al. 2003; Ian et al. 2001). It is available in the form of tablets, capsules, and suspension solutions, in addition to the rare form of injections. The ointment form covers the treatment of eye diseases and burns, and some of it is used in compound pharmaceutical formulations to expand its therapeutic effect (Bezruk et al. 2017). (TC-HCl) is a yellow hygroscopic powder, odorless, crystalline, with a bitter taste. It has the ability to dissolve in water (Egbuna 2019)· TC-HCl compound that has the ability to form complex compounds by reacting with metal ions. It has the chemical formula (4S,4aS,5aS,6S,12aS)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo1,4,4a,5,5a,6,11, 12a-octahydro-tetracene-2-car boxamide hydrochloride according to (IUPAC) (The Stationery Office 2009). Figure (1).



Chemical Formula: $C_{22}H_{25}ClN_2O_8$
Molecular Weight: 480.90

***Figure 1**. Structure of tetracycline. HCl*

The chemical structure of tetracycline includes aromatic rings, as well as, amide/methylamine and hydroxyl groups. Hydroxyl groups can be oxidized, while the other groups enter the electrophilic substitution reactions. In addition, in aqueous media, methylamine and hydroxyl groups present acid-

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base equilibria which could strongly influence the reactions of tetracycline (Benavides et al. 2017). Therefore, various methods have been reported in the literature for the determination of tetracycline in pure and its pharmaceutical formulations, including flow injection (Rufino et al. 2009; Rodríguez, Pezza et al. 2016; Townshend et al. 2005; Oteef and Idris 2023; Wangfuengkanagul, Siangproh, and Chailapakul 2004) , high performance liquid chromatography (HPLC) (Kargin et al. 2016; Peres et al. 2010), square wave voltammetry adsorptive stripping (Turbale et al. 2020) , and electrochemical techniques (Liu et al. 2018; Wang et al. 2011; Gan et al. 2014). But a lot of these call very pricey gear and expert operation. The most used spectrophotometric methods for determining tetracycline are: complexation with zirconium (Saenjum et al. 2022) , diazo-coupling with sulphanilic acid (Ali, R. J., et al. 2018) , charge transfer complex (Fahelelbom 2008) , and oxidative coupling (Hameedi 2021) estimation of area under the peak of acidic tetracycline (Alhfidh and Othman 2021) .

Several of these methods have one or more drawbacks, like poor selectivity, sensitivity, or extraction techniques. Therefore, a straightforward and precise approach for measuring tetracycline in various drugs is required for routine analysis. The present suggested procedures aim to determine TC-HCl in its formulation via safety rate is a high and inexpensive, easy, accurate and routine, method. The azo-dye and coupling reactions are used as a basis for work and are considered important reactions in the field of chemistry because of their highly stable results. Diazonium salts work as intermediates for the preparation of other compounds such as phenols, aryl halides (Hari and König 2013). Some azo compounds in biological research as tools for identifying proteins or nucleic acids(Mix, Aronoff, et al. 2016). These reactions were selected for the purpose of quantitative determination of (TC-HCl) in pharmaceutical preparations in this article.

MATERIALS and METHODS

Apparatus

For recording CECIL CE7200 UV-Visible spectrophotometer instrument with 1.0 cm matched quartz cells was used for all absorption measurements.

Reagent and Solution

Every chemical utilized is of the caliber of an analytical reagent. Pure TC-HCl (SDI Company, Sammara, Iraq), m-nitro aniline (Fluka, Germany) was used.

100 ml of distilled water was used to dissolve 0.0100 g of TC-HCl to prepare a solution with a

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concentration of 100 µg ml^{-1}. The appropriate amounts of sodium hydroxide at concentrations of (1M), and prepared varied interference solutions (Arabic Gum, Sucrose, Lactose, Starch, Cellulose) (1000 µg.ml^{-1}) by using distilled water as a dissolving and diluting solution.

To prepare the corresponding diazonium salt from m-nitroaniline: dissolving 0.0691 g of m-nitroaniline in 60 ml of distilled water and adding 3 ml of strong hydrochloric acid (11.8 M), the diazotized m-nitroaniline (5 mM) solution was ready. In the end step, the liquid was poured into a 100 ml volumetric flask placed in an ice bath for cool at 0–5 ^{0}C, after adding 0.0345 g of sodium nitrite, the mixture was forcefully agitated. Fill it to the mark with cold distilled water after five minutes. It remains stable until five days.

General procedure and calibration graph

A series of volumetric flasks, to which (0.1–3.0 ml) of TC-HCl (100 µg ml^{-1}) was added. Then, 1 ml of diazotized m-nitroaniline solution (5 mM) and 2 ml of sodium hydroxide solution (1 M) were added to each flask. The volume was then filled to mark with distilled water. At 382.5 nm, the absorbance of the dye produced by the reaction was measured against a reagent blank that contained the reaction mixture but without TC-HCl. According to Figure 2. The calibration graph was linear between 10 and 300 µg of TC-HCl/10 ml ≃ 1 and 30 µg.ml^{-1}. It has been determined that the apparent molar absorptivity was 1.66×10^4 l.mol^{-1}.cm^{-1}, and the Sandell sensitivity is 0.029 µg.cm^{-2}.

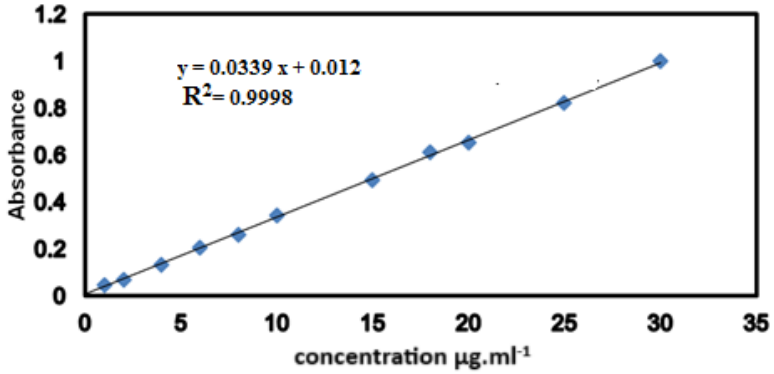

Figure 2. Calibration curve for absorbance TC-HCl using the proposed approach

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Determination of TC-HCl in pharmaceutical formulations

1. *A capsule* (250 mg); took five capsules, removed the cap, weighed them andmix the contents. Equivalent to 0.0100 g TC-HCl accurately weighed an amount of powder, then dissolved and transferred it to a 100 ml volumetric flask. by procedures used in the proposed approach for analyzing the pharmaceutical formulation, the samples were prepared for measurement.
2. *Ointment* (3%); four containers were mixed well, from which the equivalent of 0.0100 g of TC-HCl was accurately weighed, the weighed portion was completely dissolved in 3 ml of ethanol and then 50 ml of distilled water was added, followed by heating for 10-15 minutes and the mixture was filtered and transfer to a volumetric flask capacity 100 ml, and with distilled water the volume was fill to the mark, using the recommended estimation procedures for the pharmaceutical compound, their applicability to the pharmaceutical formulation was verified.

Results and Discussion

The effect of different parameters on the color development resulting from the diazotization of m-nitroaniline and then its coupling with tetracycline was investigated in order to select the optimum conditions to achieve the highest accuracy in the results.

The principle of method

The aromatic primary amine reacts with nitrous acid to form diazonium salt, a reaction called diazotization (Betelu et al. 2017). It is an exothermic reaction, so care must be taken during its preparation by controlling the reaction temperature (Schotten et al. 2020; Mahouche-Chergui et al. 2011). The nitrous acid used in the reaction is prepared immediately from the reaction of sodium nitrite in an aqueous solution with a strong acid such as hydrochloric acid or sulfuric acid, as it is unstable at normal temperatures, so it is prepared at temperatures below 5^{o}C (Sheng, Frurip, and Gorman 2015; Maseer and Najem 2023). The reaction suggested approach included the following steps (Figure 3,4):

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- Preparation of diazonium salt of m-nitroaniline:

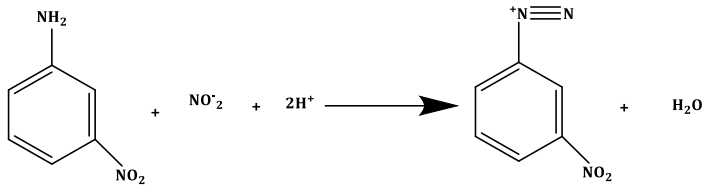
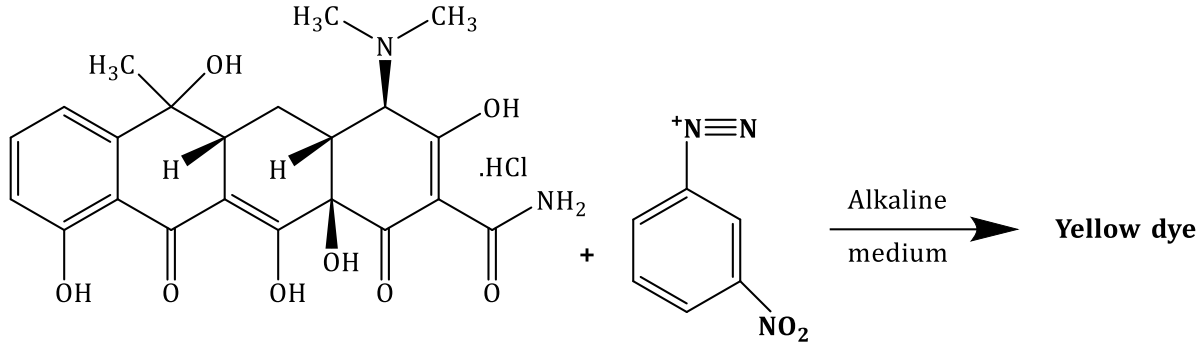


Figure 3. Preparation of diazonium salt

- Coupling of tetracycline with diazotized m-nitroaniline



tetracycline (TC-HCl)

diazotized m-nitroaniline

Figure 4. Coupling reaction

Selected of diazotized agents

Two diazotized agents were tested to select the appropriate one in an azo-dye reaction by neutralizing the wavelength and absorbance intensity under certain conditions. The results are shown in Table 1. The m-nitroaniline provides the highest absorbance intensity, the widest colour difference, and the optimal sensitivity. The result of this was select in subsequent experiments.

Table 1. selected of reagent (diazotized agents)

5mM Reagents	Absorbance	λ_{max}(nm)	$\Delta\lambda_{max}$(nm)	ε (l.mol^{-1}.cm^{-1})
m-Nitroaniline	0.345	382.5	122.5	1.86×10^{4}
p-Phenyldiamine	0.280	390	38	1.35×10^{4}

$\Delta\lambda_{max}$ = widest colour = λ_{max}Z- λ_{max}B where (Z: The dye, B: Blank)

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Effect of diazotized m-nitroaniline amount

To study the effect of 5 mM diazonium salt (diazotized m-nitroaniline) on the absorbance of the resulting dye colour, a series of various volumes (0.1-1.0 ml) of it were taken. Each volume separately added to a series of volumetric flasks containing various concentrations of tetracycline (10-30 $\mu g.ml^{-1}$). The results showed that the absorbance gave its highest intensity when utilizing a volume of 0.5 ml of diazonium salt.

Effect of basic solution

Through the initial experiments conducted between TC-HCl and the diazonium salt (m-nitroaniline), the dye appears only when the reaction medium is basic. As a result, different bases (weak/strong) were studied, as shown in Table 2. It is noted that 1M sodium hydroxide gives the highest intensity of the colored dye at 2 ml, the result was confirmed for subsequent use.

Table 2. Effect of various basic solutions on dye absorbance intensity

Amount of basic (ml)	Absorbance of basics concentrations (1M)			
	Sodium hydroxide	Potassium hydroxide	Sodium carbonate	Sodium bicarbonate
0.5	0.335	0.277	0.125	0.031
1.0	0.350	0.345	0.132	0.063
2.0	0.352	0.348	0.104	0.054
3.0	0.349	0.340	0.091	0.050
pH	(12.6- 12.9)	(12.3-12.80)	(10.78-10.90)	(8.97-9.22)

Stability of azo dye

To complete the limitation of the optimal conditions for the reaction, the effect of the time of development of the dye color resulting from the diazotized reaction and the stability of that color was studied by studying the absorbance measured at subsequent periods shown in Table 3. The results show that the formed color is stable for up to 60 minutes.

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Table 3. Stability of dye with subsequent periods

Time/min.	Absorbance/TC-HCl (µg.ml^{-1})		
	10	20	30
Immediately	0.340	1.000	0.648
5	0.341	0.997	0.649
10	0.342	0.998	0.647
20	0.341	0.995	0.645
30	0.342	0.996	0.644
40	0. 341	0.994	0.643
50	0. 341	0.995	0.640
60	0. 340	0.993	0.643

Final absorbance spectrum

An absorbance spectrum of the developed colored dye by coupling of diazotized m-nitroaniline with TC-HCl in 1M sodium hydroxide solution as a basic medium, under recommended conditions, against its blank show maximum absorbance at 382.5 nm in comparison to the reagent blank against distilled water which shows maximum absorbance at 255 nm with out-of-range absorbance value, as shown in figure 5. conducted under recommended conditions.

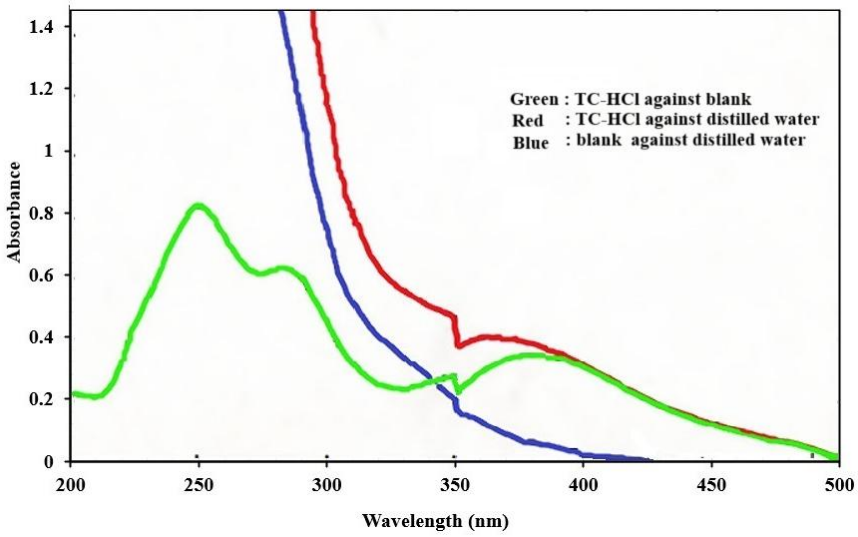

Figure 5. Absorbance spectrum of (5.0 µg.ml^{-1}) TC-HCl (as azo-dye)reaction

Reliability and Compatibility

To investigate the reliability (accuracy) and compatibility (precision) of the proposed approach for determined TC-HCl, two concentrations were selected within the calibration curve range, and mathematical calculations were performed. The results indicated that the proposed gave satisfactory

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results, as shown in Table 4.

Table 4. reliability (accuracy) and compatibility (precision)

TC-HCl (µg.ml^{-1})	Accuracy		Precision
	Recovery, %	Relative error, %	*RSD, %
5.0	100.51	0.51	1.290
10	101.75	1.75	0.707

Average of five determinations, *RSD; Relative standard deviation

Complexity type of azo-dye formed

Using the Joule and mole-ratio methods (Hargis 1988), it was possible to determine the type of binding between TC-HCl and the reagent, which was 1:1, confirming what was stated in previous studies (J Al-Ashow and S Othman 2012; Khaleel and Mohammed 2020; Khalafa and Othmanb 2023) . Therefore, the coupling of diazotized at the para position of TC-HCl, and the suggested structure of formed azo dye was as shown in figure 6.

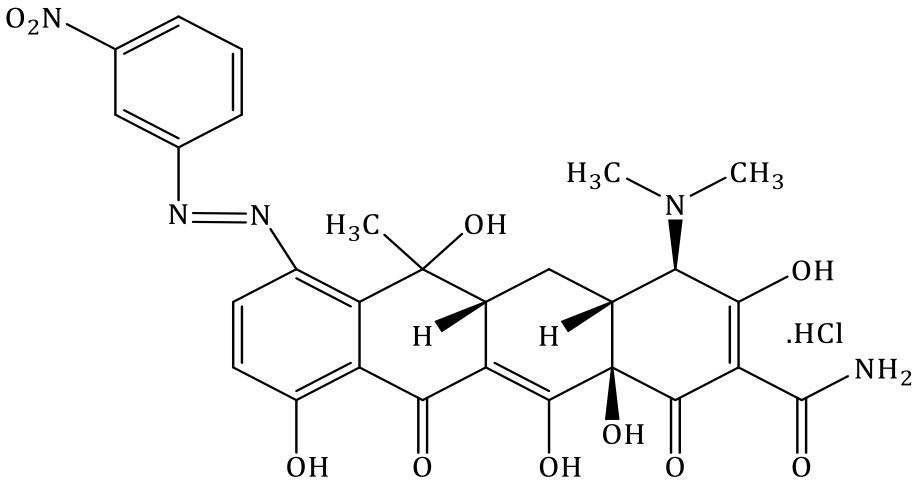

Figure 6. Yellow azo-dye

Effect of interferences

To invagination the effectiveness and compatibility of the suggested approach, used the standard addition method (Nejres and Najem 2022) , it was carried out by taking increasing concentrations of pure TC-HCl (0, 2.0, 4.0, 6.0, 8.0 and 10) and two constant concentrations of its pharmaceutical formulations (5.0 and 10 µg.ml^{-1}) each concentration individually The approach successfully determined the property without interferences effect, can be shown that in Figure 7.

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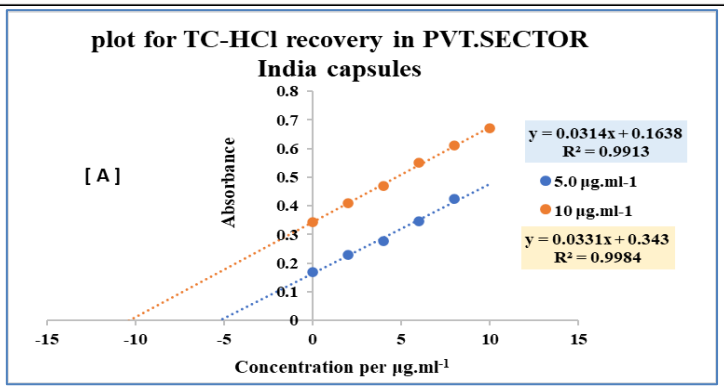

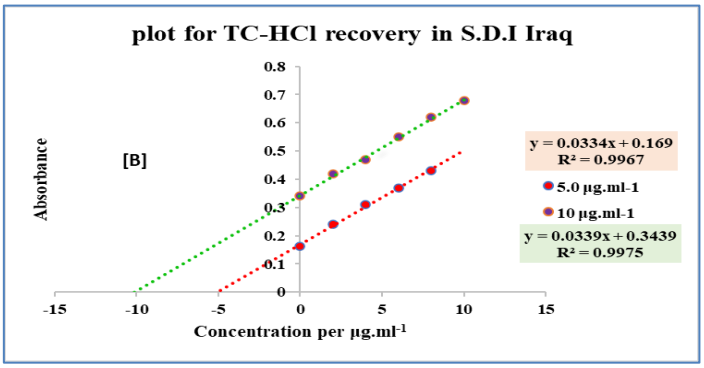

Figure 7. Standard addition method plot of TC-HCl capsules; a) PVT. SECTOR/Indian b) SDI /Iraq

The results shown in Table 5, extracted from Figure 7, indicated no additive effect in determining TC-HCl in their formulation and recoveries with the accepted analytical error.

Table 5. The measured of standard addition method in figure 7.

Pharmaceutical formulations	Concentration per µg.ml^{-1}		Recovery, %
	Amount taken	Amount measured	
Apecycline 250 mg Capsules (PVT.SECTOR India)	5.0	5.21	104.33
	10	10.36	103.62
Samacycline 250 mg Capsules (S.D.I/Iraq)	5.0	5.05	101.19
	10	10.14	101.44

Application the approach on pharmaceutical formulations

To verify the success of the proposed approach, it was applied to TC-HCl pharmaceutical formulations by taking concentrations within the range of the calibration curve, calculating the percentage recovery, and comparing the taken and measured amounts. The results in Table 6. showed that the proposed approach gave good results that can be relied upon in determining the pharmaceutical formulations, capsule, and skin ointment. Table 6. displays the findings. show that a successful recovery was

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achieved.

Table 6. Analytic applications of the suggested approach

Pharmaceutical formations	Concentration per μg.ml^{-1}		Recovery, %
	Amount taken	Amount measured	
Apecycline 250 mg Capsules (PVT.SECTOR India)	5.0	4.979	99.58
	10	9.967	99.67
Samacycline 250 mg Capsules (S.D.I/Iraq)	5.0	5.003	100.06
	10	10.054	100.54
Samacycline ointment 3% (S.D.I/Iraq)	5.0	5.008	100.16
	10	10.011	100.11

Average of five determinations

Validity of proposed approach

To verify the validity of the proposed approach,The results of the tabular t-test calculated by comparing the reading rate of the proposed method with the estimation method in the British Pharmacopoeia protocol(Prichard 2009), at 95% Confidence Interval of the Difference degrees of freedom; t-test 4.303 (Christian, G. D., Dasgupta, P. K., and Schug 2013) , showed no significant difference between the statistical values of the proposed method and the standard method Table 7.

Table 7. T-test value of pharmaceutical preparation TC-HCl of the proposed approach

Pharmaceutical formations	Recovery %		t-test*
	Amount measured	The-British Pharmacopoeia	
TC-HCl	100.60	99.55	0.81
	101.70		3.02

* $t = (x - \mu)\frac{\sqrt{N}}{s}$

Comparison of the proposed approach

The proposed approach was compared to previous studies of TC-HCl, as shown in Table 8, in which the most important parameters were reviewed, which shows that the method has a wide range of obey Beer's law, in addition to the accuracy, precision and sensitivity of the method.

Table 8. Comparison of the proposed approach

methods	λ_{max}(nm)	Range (μg.ml^{-1})	Recovery %	RSD %	Molar absorptivity (l.mol^{-1}.cm^{-1})×10^4	Sandal's sensitivity	Ref.

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						$\mu g.cm^{-2}$	
oxidation of chromium (VI)	543	1.2-30	NA	NA	1.03	0.0431	(Saleem, et al. 2020)
	610	2.0-60	NA	NA	0.63	0.0705	
TC-HCL- Ce(IV) complex	430	50-350	101	0.030	NA	NA	(AL-Sowdani, et al. 2006)
Sulphonation	435	2-40	96.6	0.142	0.831	0.0294	(Alhfidh and Othman 2021)
azo dye, coupling	419	0.5-50	99.58	0.295	1.461	NA	(Abd, Dikran, and Mahmood 2017)
chelate complex	430	5.0-160	100.56	0.917	0.289	NA	(Ali and Kamoon 2016)
		2.0-50	101.05	0.897		NA	
azo dye, coupling	382.5	1.0-30	101.13	0.80	1.66	0.0290	This work

CONCLUSION

TC-HCl could be determined in its pure and pharmaceutical form, using the proposed method, which was characterized as a simple and sensitive method with few steps that reduced the relative error, giving it good accuracy and precision (molar absorptivity reached 1.66×10^{4} $l.mol^{-1}.cm^{-1}$and sandell sensitivity index of 2.9×10^{-2} $\mu g\ cm^{-2}$. Depending on the wide concentration level, the color reaction had a relative standard deviation of 0.357 to 1.26%). This is confirmed by comparing it with spectrophotometric methods, where the results came out good with values close at times and superior at other times to those previous studies, in addition to the extent of obey with Beer's law and stability that enabled the researcher to use the method comfortably. Therefore, the method succeeded in all its aspects.

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CONFLICTS OF INTEREST STATEMENT

No conflicts of interest

References

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1. Abd, M. M., Dikran, S. B., & Mahmood, A. K. (2017). Spectrophotometric determination of tetracycline hydrochloride in pure form and pharmaceutical preparation by coupling with diazotized anthranilic acid. *Ibn AL-Haitham Journal for Pure and Applied Sciences, 30*(1), 400–412. https://doi.org/10.30526/30.1.1088.
2. AL-Sowdani, K. H., AL-Abdullah, Z. T., & AL-Abdalaziz, B. A. (2006). Spectrophotometric determination of tetracycline in some pharmaceutical preparations. *Journal of Basrah Researches (Sciences), 32*(1), 43–48. https://www.iasj.net/iasj/download/6acb684ad7295a89
3. Alhfidh, H. A. S., & Othman, N. S. (2021). Development of two spectrophotometric methods in determination of tetracycline. *Samarra Journal of Pure and Applied Science, 3*(4), 66–78. https://doi.org/10.54153/sjpas.2021.v3i4.307
4. Ali, R. J., Hawezy, H. J. S., & Abdullah, M. S. (2018). Spectrophotometric determination of tetracycline hydrochloride through coupling with sulphanilic acid. *Diyala Journal of Medicine, 15*(2), 15–22. https://doi.org/10.52865/AVWR7365
5. Ali, F., & Kamoon, R. A. (2016). Spectrophotometric determination of tetracycline hydrochloride in pharmaceutical preparations using rhodium (II) as a mediator metal. *International Journal of Research Pharmacy and Chemistry, 6*(2), 249–261. https://www.ijrpc.com/files/11-04-16/07-644.pdf
6. Bahrami, F., Morris, D. L., & Pourgholami, M. H. (2012). Tetracyclines: Drugs with huge therapeutic potential. *Mini-Reviews in Medicinal Chemistry, 12*(1), 44–52. https://doi.org/10.2174/138955712798868977
7. Benavides, J., Barrias, P., Piro, N., Arenas, A., Orrego, A., Pino, E., Villegas, L., Dorta, E., Aspée, A., & López-Alarcón, C. (2017). Reaction of tetracycline with biologically relevant chloramines. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 178*, 171–180. https://doi.org/10.1016/j.saa.2017.02.001
8. Betelu, S., Tijunelyte, I., Lecaque, L. B., Ignatiadis, I., Schnepf, A. C., Guenin, E., Bouchemal, N., Felidj, N., Rinnert, E., & Lamy De La Chapelle, M. (2017). Raman characterization of phenyl-derivatives: From primary amine to diazonium salts. *Journal of Organic & Inorganic Chemistry, 3*(1), 1–10. https://doi.org/10.1039/c0cs00179a
9. Bezruk, I., Vrakin, V., Savchenko, L., Materiienko, A., & Georgiyants, V. (2017). Development and validation of tetracycline hydrochloride assay procedure by spectrophotometry in compounded ointment. *Scripta Scientifica Pharmaceutica, 4*(1), 33–38. https://doi.org/10.14748/ssp.v4i1.2117
10. Christian, G. D., Dasgupta, P. K., & Schug, K. A. (2013). *Analytical chemistry* (7th ed.). John Wiley & Sons.
11. Daghrir, R., & Drogui, P. (2013). Tetracycline antibiotics in the environment: A review. *Environmental Chemistry Letters, 11*(3), 209–227. https://doi.org/10.1007/s10311-013-0404-8.
12. Egbuna, C. (2019). An unusual color change in tetracycline HCl powder – from drug to poison. *Medical Journal of Dr. D.Y. Patil University, 12*(2), 134–135. https://doi.org/10.4103/mjdrdypu.mjdrdypu_215_18
13. Eliopoulos, G. M., & Roberts, M. C. (2003). Tetracycline therapy: Update. *Clinical Infectious Diseases, 36*(4), 462–467. https://doi.org/10.1086/367622

14. Fahelelbom, K. M. S. (2008). Analysis of certain tetracyclines and oxytetracyclines through charge transfer complexation. *American Journal of Pharmacology and Toxicology, 3*(3), 212–218. https://doi.org/10.3844/ajptsp.2008.212.218
15. Faeji, C., Fasoro, A., & Oni, O. (2024). Bioactive compounds in *Phyllanthus amarus* leaf extract and its toxicity in the treatment of Newcastle disease. *Israa University Journal of Applied Sciences, 7*(2). https://doi.org/10.52865/JBVF9778
16. Gan, T., Shi, Z., Sun, J., & Liu, Y. (2014). Simple and novel electrochemical sensor for the determination of tetracycline based on iron/zinc cations–exchanged montmorillonite catalyst. *Talanta, 121*, 187–193. https://doi.org/10.1016/j.talanta.2014.01.002
17. Hameedi, I. T. (2021). Determination of tetracycline hydrochloride in pure and pharmaceutical samples via oxidative coupling reaction. *Materials Today: Proceedings, 42*, 2953–2958. https://doi.org/10.1016/j.matpr.2020.12.802
18. Hargis, L. G. (1988). *Analytical chemistry: Principles and techniques*. Prentice Hall.
19. Hari, D. P., & König, B. (2013). The photocatalyzed Meerwein arylation: Classic reaction of aryl diazonium salts in a new light. *Angewandte Chemie International Edition, 52*(18), 4734–4743. https://doi.org/10.1002/anie.201210276
20. Hussein, M. A., & Othman, S. N. (2023). Development of a new analytical method for estimation of trifluoperazine-HCl in pharmaceutical formulation. *Israa University Journal of Applied Science, 6*(2), 56–70. https://doi.org/10.52865/rwic5362
21. Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*(2), 232–260. https://doi.org/10.1128/mmbr.65.2.232-260.2001
22. Al-Ashow, J. R., & Othman, N. S. (2012). Spectrophotometric determination of tetracycline by coupling with diazotised 4-aminoantipyrine in presence of cetylpyridinium chloride. *Rafidain Journal of Science, 23*(3), 72–84. https://www.iasj.net/iasj/article/44397
23. Kargin, I. D., Sokolova, L. S., Pirogov, A. V., & Shpigun, O. A. (2016). HPLC determination of tetracycline antibiotics in milk with post-column derivatization and fluorescence detection. *Inorganic Materials, 52*(14), 1365–1369. https://doi.org/10.1134/S0020168516140065
24. Khalaf, N. M., & Othman, N. S. (2023). Diazo-coupling reaction in spectrophotometric determination of doxycycline in pure and its dosage forms. *Egyptian Journal of Chemistry, 66*(6), 171–177. https://doi.org/10.21608/ejchem.2022.152883.6619
25. Khaleel, R. M., & Mohammed, D. H. (2020). Spectrophotometric determination of tetracycline hydrochloride using 2,4–dinitrophenyl hydrazine as coupling reagent. *Journal of Physics: Conference Series, 1664*(1), 012084. https://doi.org/10.1088/1742-6596/1664/1/012084
26. Liu, X., Huang, D., Lai, C., Zeng, G., Qin, L., Zhang, C., Yi, H., et al. (2018). Recent advances in sensors for tetracycline antibiotics and their applications. *TrAC Trends in Analytical Chemistry, 109*, 260–274. https://doi.org/10.1016/j.trac.2018.10.011.
27. López-Peñalver, J. J., Sánchez-Polo, M., Gómez-Pacheco, C. V., & Rivera-Utrilla, J. (2010). Photodegradation of tetracyclines in aqueous solution by using UV and UV/H2O2 oxidation processes. *Journal of Chemical Technology & Biotechnology, 85*(10), 1325–1333. https://doi.org/10.1002/jctb.2435

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ISRAA UNIVERSITY JOURNAL OF APPLIED SCINCE (IUJAS)

28. Mahouche-Chergui, S., Gam-Derouich, S., Mangeney, C., & Chehimi, M. M. (2011). Aryl diazonium salts: A new class of coupling agents for bonding polymers, biomacromolecules and nanoparticles to surfaces. *Chemical Society Reviews, 40*(7), 4143–4166. https://doi.org/10.1039/C0CS00179A
29. Maseer, A., & Najem, M. (2023). Spectrophotometric determination of mesalazine in pure form and pharmaceutical formulations by diazotization and coupling with 2,7-dihydroxynaphthalene as a new coupling agent. *Hacettepe University Journal of the Faculty of Pharmacy, 43*(3), 195–203. https://doi.org/10.52794/hujpharm.1129852
30. Mix, K. A., Aronoff, M. R., & Raines, R. T. (2016). Diazo compounds: Versatile tools for chemical biology. *ACS Chemical Biology, 11*(12), 3233–3244. https://doi.org/10.1021/acschembio.6b00810
31. Nejres, A. M., & Najem, M. A. (2022). Use of mesalazine for the determination of dopamine and its pharmaceutical preparations by spectrophotometric method. *Israa University Journal of Applied Science, 6*(1), 228–245. https://doi.org/10.52865/AVWR7365
32. Nelson, M. L., & Levy, S. B. (2011). The history of the tetracyclines. *Annals of the New York Academy of Sciences, 1241*(1), 17–32. https://doi.org/10.1111/j.1749-6632.2011.06354.x
33. Oteef, M. D. Y., & Idris, A. M. (2023). Flow injection techniques for tetracycline quantification: A review. *Critical Reviews in Analytical Chemistry, 53*(2), 396–414. https://doi.org/10.1080/10408347.2021.1964343
34. Peres, G. T., Rath, S., & Reyes, F. G. R. (2010). A HPLC with fluorescence detection method for the determination of tetracyclines residues and evaluation of their stability in honey. *Food Control, 21*(5), 620–625. https://doi.org/10.1016/j.foodcont.2009.09.006
35. Prichard, J. E. (2009). *The British Pharmacopoeia*. Stationery Office Books.
36. Rodríguez, M. P., Pezza, H. R., & Pezza, L. (2016). Simple and clean determination of tetracyclines by flow injection analysis. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 153*, 386–392. https://doi.org/10.1016/j.saa.2015.08.048
37. Rufino, J. L., Weinert, P. L., Pezza, H. R., & Pezza, L. (2009). Flow-injection spectrophotometric determination of tetracycline and doxycycline in pharmaceutical formulations using chloramine-T as oxidizing agent. *Química Nova, 32*(7), 1764–1769. https://doi.org/10.1590/S0100-40422009000700016
38. Saenjum, C., Pattapong, N., Aunsakol, T., Pattananandecha, T., Apichai, S., Murakami, H., Grudpan, K., & Teshima, N. (2022). High sensitivity spectrophotometric determination of tetracycline with zirconium chelation by employing simultaneous injection effective mixing analysis (SIEMA): Tetracycline residue in honey. *Journal of Food Composition and Analysis, 105*, 104215. https://doi.org/10.1016/j.jfca.2021.104215.
39. Saleem, B. A. A., Al-Jarah, F. K., & Shekho, N. H. (2020). Spectrophotometric assay of tetracycline in pharmaceutical preparation. *Biochemical & Cellular Archives, 20*(2), 6743. https://linksshortcut.com/qFbmR.
40. Schotten, C., Leprevost, S. K., Yong, L. M., Hughes, C. E., Harris, K. D. M., & Browne, D. L. (2020). Comparison of the thermal stabilities of diazonium salts and their corresponding triazenes. *Organic Process Research & Development, 24*(10), 2336–2341. https://doi.org/10.1021/acs.oprd.0c00162

ISSN:2523 - 0522
Volume 8, Issue 1, April 2025
ISRAA UNIVERSITY JOURNAL OF APPLIED SCINCE (IUJAS)

41. Shalaby, A. R., Salama, N. A., Abou-Raya, S. H., Emam, W. H., & Mehaya, F. M. (2011). Validation of HPLC method for determination of tetracycline residues in chicken meat and liver. *Food Chemistry, 124*(4), 1660–1666. https://doi.org/10.1016/j.foodchem.2010.07.048
42. Sheng, M., Frurip, D., & Gorman, D. (2015). Reactive chemical hazards of diazonium salts. *Journal of Loss Prevention in the Process Industries, 38*, 114–118. https://doi.org/10.1016/j.jlp.2015.09.004
43. Tauch, A., Pühler, A., Kalinowski, J., & Thierbach, G. (2000). TetZ, a new tetracycline resistance determinant discovered in Gram-positive bacteria, shows high homology to Gram-negative regulated efflux systems. *Plasmid, 44*(3), 285–291. https://doi.org/10.1006/plas.2000.1489
44. British Pharmacopoeia Commission. (2009). *British Pharmacopoeia* (6th ed.). The Stationery Office.
45. Townshend, A., Ruengsitagoon, W., Thongpoon, C., & Liawruangrath, S. (2005). Flow injection chemiluminescence determination of tetracycline. *Analytica Chimica Acta, 541*(1), 103–109. https://doi.org/10.1016/j.aca.2004.11.013
46. Turbale, M., Moges, A., Dawit, M., & Amare, M. (2020). Adsorptive stripping voltammetric determination of tetracycline in pharmaceutical capsule formulation using poly(malachite green) modified glassy carbon electrode. *Heliyon, 6*(12), e05782. https://doi.org/10.1016/j.heliyon.2020.e05782
47. Wang, H., Zhao, H., Quan, X., & Chen, S. (2011). Electrochemical determination of tetracycline using molecularly imprinted polymer modified carbon nanotube-gold nanoparticles electrode. *Electroanalysis, 23*(8), 1863–1869. https://doi.org/10.1002/elan.201100049
48. Wangfuengkanagul, N., Siangproh, W., & Chailapakul, O. (2004). A flow injection method for the analysis of tetracycline antibiotics in pharmaceutical formulations using electrochemical detection at anodized boron-doped diamond thin film electrode. *Talanta, 64*(5), 1183–1188. https://doi.org/10.1016/j.talanta.2004.04.032
49. Zakeri, B., & Wright, G. D. (2008). Chemical biology of tetracycline antibiotics. *Biochemistry and Cell Biology, 86*(2), 124–136. https://doi.org/10.1139/O08-002.