Rafidain Journal of Science

https://rsci.mosuljournals.com

Vol. 33, No. 1, pp. 110-120، 2024

Development of a Spectrophotometric Method to Assay Tetracycline Hydrochloride by Coupling with Diazotized p-Aminobenzoic Acid: Application to Pharmaceutical Forms

Rania I. Sheet **Salim A. Mohammed**
Department of Chemistry/ College of Science / University of Mosul

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Article information

Received: 1/ 1/ 2023
Revised: 20/ 3/ 2023
Accepted: 27/ 3/ 2023

DOI: 10.33899/rjs.2024.182835

corresponding author:
Rania I. Sheet
rania.ibr92@gmail.com

Salim A. Mohammed
salimsalih813@yahoo.com

ABSTRACT

This work comprises developing a sensitive and precise spectrophotometric procedure to analyze tetracycline hydrochloride in the bulk and in the drug forms. In this method, p-aminobenzoic acid reagent was diazotized with an equimolar $NaNO_2$ (sodium nitrite) solution in the existence of HCl (hydrochloric acid) to produce corresponding diazonium salt subsequently coupling with tetracycline hydrochloride in a basic solution of NH_4OH to produce a yellow azo dye which was water soluble and has a maximum absorption at 422 nm versus a reagent blank. Under optimal conditions, the linearity of Beer's law was estimated to cover the concentration range of 0.25-17.5 µg/ml with an exceptional determination coefficient (R^2= 0.9993) and molar absorptivity of 1.66×10^4 l/mol.cm. The calculated values of detection limit (LOD) and quantitation limit (LOQ) were equal to 0.012 and 0.040 µg/ml, respectively. The values of error percentage and precision (RSD%) ranges were calculated and found in the range of -0.08% to 3.3% and 0.16% to 1.59%, respectively. The stoichiometry of the product was set up to be 1:1 tetracycline hydrochloride: diazotized p-aminobenzoic acid. The mentioned procedure has been utilized successfully to the evaluation of tetracycline hydrochloride in capsules and ointment.

Keywords: Tetracycline, HCl, Diazotization, p-aminobenzoic acid, Spectrophotometric Method.

INTRODUCTION

Among broad-spectrum antibiotics, the tetracycline-series antibiotics occupy a leading place. They suppress Rickettsia, acid-resistant bacilli, the reproduction of gram-positive and gram-negative microorganisms and many infections (Mamani *et al.*, 2006). Tetracycline hydrochloride (TCH), is a yellow powder and chemically acknowledged as [(4S, 4aS, 5aS, 6S, 12aS)-4-dimethylamino-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1, 11- dioxo-1, 4-, 4a-, 5, 5a, 6, 11, 12a-octahydro-tetracene-2-carboxamide monohydrochloride] (The Japanese Pharmacopoeia, 2016). Fig. (1).

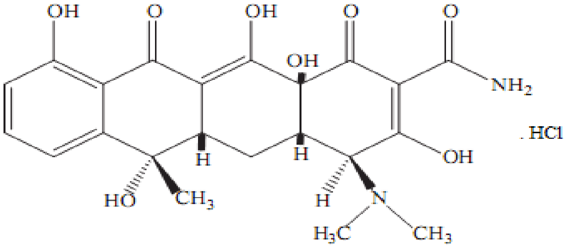


Fig. 1: The chemical structure of tetracycline hydrochloride

$C_{22}H_{24}N_2O_8.HCl$

M.Wt. = 480.9 g/mol

TCH is a synthetic agent used in human and veterinary therapy for hundreds of years. The study of the literature found that several different methods have been described for the direct and indirect valuation of the TCH, they included: high performance liquid chromatography (HPLC)-diode array detection (Vuran *et al.*, 2021), sulfur quantum dot probes (Lu *et al.*, 2021), HPLC–MS/MS (Pang *et al.*,2021), cyclic voltammetry using the reduced grapheme oxide (rGO) modified electrode associated with the flow-injection analysis (Faria *et al.*, 2019), fluorometric and electrochemical dual-mode nanoprobe for tetracycline (Hu *et al.*, 2020), a potentiometric sensor coupled to HPLC (Gil *et al.*,2022) and kinetic spectrophotometry (Alassaf *et al.*, 2019). However, most of these procedures need a skilled operation and expensive equipment.

For the analytical measurement of TCH in pharmaceutical formulations, UV-Visible spectrophotometry is still regarded as a cost-effective and feasible approach. TCH has also been measured using a variety of spectrophotometric techniques, both in bulk and in pharmacological preparations. Some of these methods included diazotization and coupling reaction which is based on the coupling of TCH with diazotized of p-nitroaniline (Al-Abbasi, 2009), 4-aminoantipyrine in a presence of cetylpyridinium chloride (Othman and Al-Ashow, 2012), anthranilic acid (Abd *et al.*,2017), sulphanilic acid (Ali *et al.*,2018). Others based on the oxidative coupling reactions of TCH with p-N, N-dimethylphenylenediamine and sodium meta periodate (Tella *et al.*, 2011), 2,4-dinitrophenylhydrazine (2,4-DNPH) with potassium periodate in a basic medium (Hameedi, 2021) and N, N-diethyl-p-phenylenediamine reagent in presence of oxidizing agent of N-bromosuccinimide in the alkaline medium (Mari *et al.*, 2016). Other spectrophotometric methods have also been developed for estimating TCH based on the complex formation with cerium (IV) (Al-Sowdani *et al.*, 2006), zirconium (IV) (Saenjum *et al.*, 2022) and yttrium (III) in the presence of cationic surfactant (Thanasarakhana *et al.*, 2011). As well as, charge transfer complex formation with chloranilic acid (Fahelelbom, 2008) and oxidation reaction with sodium hypochlorite (Ahmed *et al.*, 2018) have been used to determine TCH by spectrophotometric method. However, many of these methods suffer from various limitations, for instance, low sensitivity, low stability of the resulting product and time consuming.

In the recent investigation, a p-aminobenzoic acid (p-ABA) reagent was used to produce an easy, rapid, and inexpensive visible spectrophotometric method such as assaying TCH in both its pure and pharmacological forms. The approach was based on the formation of a yellow dye in a basic solution by the reaction of TCH with diazotized p-ABA reagent, which has been used to assay TCH in capsules and ointment.

EXPERIMENTAL

Apparatus

All measurements of absorbance and spectra of absorption were performed using a double-beam UV-visible spectrophotometer (JASCOV-630) with 1.0-cm glass cells. While a professional Benchtop pH meter (BP3001) was used to record the pH values.

Chemical materials and reagents

All chemicals used are of the highest purity available. TCH was provided by the state company for drug industries and medical appliances (SDI), Sammara, Iraq.

TCH working solution, 100 µg/ml. A 0.0100 g quantity of TCH was dissolved in 100 ml DW (distilled water) using a standard flask. This solution was stored in a brown container.

p-ABA solution, (7.29×10^{-4} M). A 0.0100 g p-ABA (Fluka) was weighed and dissolved in a tiny amount of DW in a 100 ml standard flask and with the same solvent the volume was made to the mark. The prepared solution was transferred to a dark container for preservation.

Sodium nitrite ($NaNO_2$) stock solution (7.29×10^{-3}M). A 0.05 g of $NaNO_2$ was dissolved in 100 ml of DW.

Sodium nitrite working solution (7.29×10^{-4} M). A 10.0 ml of the (stock $NaNO_2$) solution was diluted with DW to make the final volume 100 ml.

Hydrochloride acid solution (1M). This solution was made by taking 8.6 ml of concentrated hydrochloride acid (11.6 M) and the volume was diluted to 100 ml in a standard flask.

Ammonium hydroxide solution (1M). A 7.5 ml of the concentrated ammonium hydroxide solution (13.4M) was diluted with 100 ml of DW. The solution was then stored in a plastic container.

Essential method and standard curve

Into a sequence of 20 ml standard flasks, 1ml of 7.29×10^{-4}M p-ABA, an equimolar of $NaNO_2$ and 1.25 ml of 1M hydrochloric acid solutions were added to each flask. The solutions were mixed well and set aside at RT (laboratory temperature) for 20 min. An aliquot 0.05-3.5 ml of TCH working solution (100 µg/ml) was then added and followed by adding 3 ml of 1M ammonium hydroxide solutions. The final solutions were shake well and made to the mark with DW. At 422 nm, the absorbance of the produced azo dye was measured against a blank solution prepared by using the same way but without TCH. A standard calibration curve gives a straight line in the concentration range of 0.25-17.5 µg/ml TCH with R^2= 0.9993 as shown in Fig. (2). The molar absorptivity and Sandell's sensitivity were calculated and found to be 1.66×10^4 l/mol.cm and 0.0289 µg/cm^2, respectively. LOD and LOQ have been also calculated and found to 0.0120 and 0.040 µg/ml respectively.

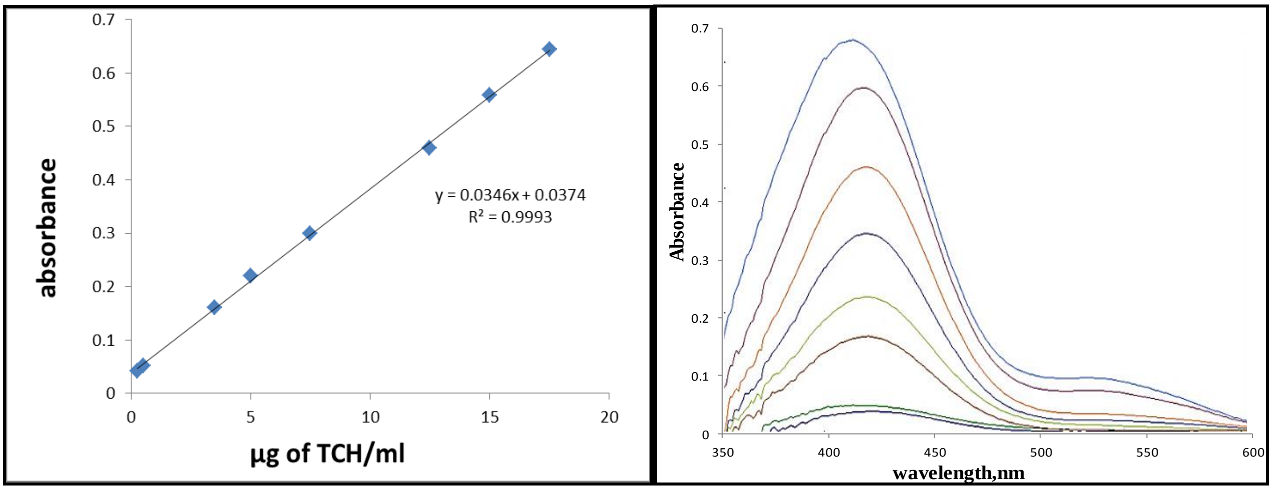


Fig. 2: The calibration curve and the absorption spectra for estimating TCH according to the suggested procedure

Procedure for assaying TCH in its pharmaceutical formulations

For TCH capsule

The contents of five capsules (each capsule contains 250 mg TCH) were weighed and mixed well. An accurate quantity of powder equivalent to 0.0100g TCH was taken and dissolved in 100 ml DW using a standard flask to obtain a solution of TCH its concentration of 100 µg/ml and an aliquot of this drug solution was analyzed by following the suggested method.

For TCH ointment

The content of three containers of TCH ointment is mixed very well and an accurate quantity equivalent to 0.0100g TCH was weighed and dissolved in a mixture of (3 ml ethanol and 50 ml DW). The mixture was warmed and filtered into 100 ml standard flask, the volume diluted up to the mark with DW (Othman and Al-Ashow, 2012), each ml of the diluted solution contains 100 µg of TCH and a portion of it was analyzed using the described method.

RESULTS AND DISCUSSION

Preliminary conditions

In this investigation, TCH was coupled with diazotized p-ABA in an alkaline solution to produce a yellow azo dye which shows highest absorption at 422 nm vs. blank solution. The resulting colour intensity was found to be proportional to the amount of TCH that originally exist in the solution.

Optimal reaction conditions

Influence of the type and amount of acid

The equation below describes how the diazotization process happens:

$$ArNH_2 + NaNO_2 + 2\ HX \longrightarrow ArN_2X + NaX + 2\ H_2O$$

An acid solution is necessary for the diazotization process. Therefore, the effect of several acid solutions in varying volumes (1M), including HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH, on the dye's absorbance has been studied. The volume of 1.25 ml of 1M HCl is the best, according to the experimental results in Fig. (3), the volume 1.25 ml of HCl was selected for the next experiments.

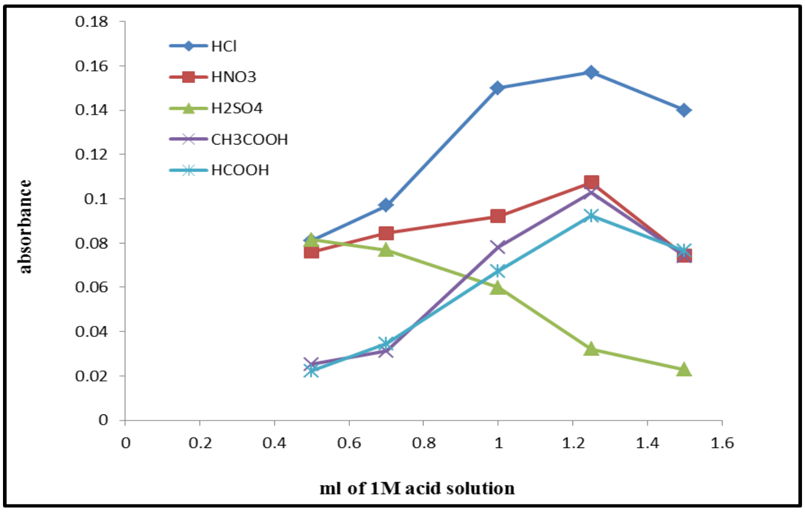


Fig. 3: Influence of various acids and their quantities on absorbance

Influence of p-ABA reagent amount

The influence of varying amounts (0.5 to 1.5ml) of p-ABA reagent (0.01%) on the absorption of the resulting azo dye has been tested. According to (Table 1), the result of 1.0 ml of p-ABA (0.01%) was found to be the optimum and provide highest absorbance with coefficient value (R^2=0.9995).

Table 1: Effect of reagent amount on the absorbance

ml of (p-ABA) solution (0.01%)	Absorbance / µg of TCH present in 20 ml							R^2
	20	**50**	**100**	**120**	**150**	**200**	**300**	
0.5	0.0433	0.0742	0.1241	0.1447	0.1816	0.2415	0.3367	0.9984
0.7	0.0512	0.0789	0.1387	0.1552	0.1943	0.2411	0.3314	0.9966
1.0	0.0766	0.1089	0.1619	0.1802	0.2057	0.2571	0.3563	**0.9995**
1.2	0.0637	0.0913	0.1521	0.1605	0.1917	0.2435	0.3367	0.9980
1.5	0.0441	0.0718	0.1192	0.1438	0.1743	0.2379	0.3398	0.9981

Influence of diazotization time

The influence of diazotized reaction time on the azo dye's absorption intensity was carried out by measuring the absorption at different periods of time at laboratory temperature (25±2C^0) and the results are illustrated in Fig. (4).

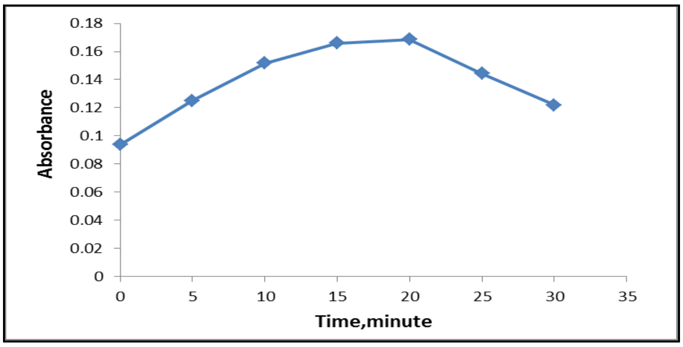


Fig. 4: Time effect of the diazotization reaction on absorbance

The results in Fig. (4) confirmed that the diazotization process needs 20 minutes to be completed and to give the highest value of absorbance.

Influence of base type and its amount

The influence of varying quantities (0.5- 4.0 ml) of various base solutions (sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate and ammonium hydroxide) on the intensity of the yellow dye have been studied. The data are listed in (Table 2) and reveal that the volume of 3 ml of ammonium hydroxide solution (1M) is the best because it provides the highest sensitivity.

Table 2: Influence of various bases and their quantities on absorbance

Type of base solution (1M)	Absorbance / ml of base added						pH
	0.5	**1.0**	**1.5**	**2.0**	**3.0**	**4.0**	
NaOH	0.0381	0.0630	0.0931	0.0760	0.0489	0.0355	12.9
KOH	0.0342	0.0404	0.0538	0.0330	0.0233	0.0214	12.9
Na_2CO_3	0.1105	0.1680	0.1335	0.1236	0.0943	0.0781	9.44
$NaHCO_3$	0.0245	0.0340	0.0861	0.1472	0.1489	0.1453	8.20
NH_4OH	0.0324	0.0783	0.1160	0.1479	**0.2242**	0.2008	9.90

Time effect on the colour development

Two different amounts 100 and 200 µg of TCH were used to investigate the effect of time on the azo dye's color stability at 422 nm. The absorbance was determined at varying times up to 60 minutes. The data in Fig. (5) show that the yellow azo dye that resulted from adding the alkaline solution of ammonium hydroxide appears immediately and is stable for at least 60 minutes at laboratory temperature (RT).

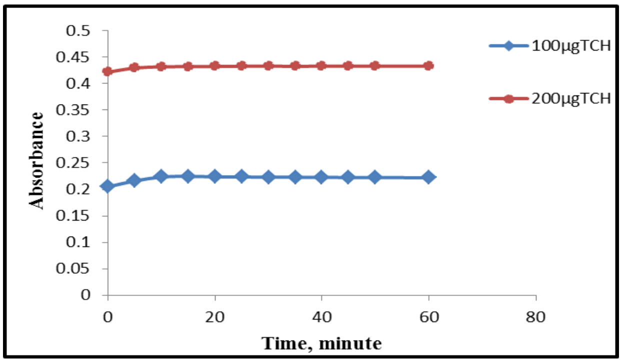


Fig. 5: Influence of colour development time on absorbance.

Absorption spectrum

By applying the ideal reaction conditions, 5µg/ml of TCH was reacted with the diazotized p-ABA reagent solution in an alkaline solution of NH_4OH to produce a yellow water-soluble azo dye which exhibits a high peak intensity with an absorption maximum at 422 nm versus the reagent blank solution Fig. (6).

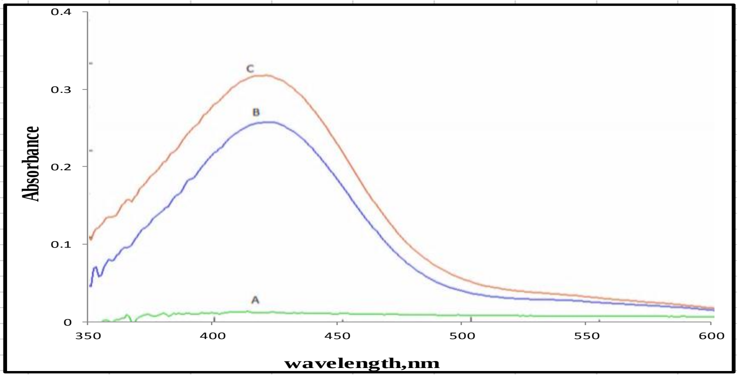


Fig. 6: Absorption spectra of 5µg/ml TCH measured against blank solution (A), DW (B) and blank solution against DW(C)

Nature of the resulting azo dye

Under the optimized conditions of the essential procedure, continuous variation and mole ratio methods (Delevic, 1997) were used to clarify the correlation ratio of TCH with the diazotized p-ABA. Both methods were carried out using the same concentrations of TCH and p-ABA solutions (2.079×10^{-4}M). The results of both methods are explained in Fig. (7) revealing that the dye was created by the reaction of TCH and diazotized p-ABA compound in a ratio of 1:1.

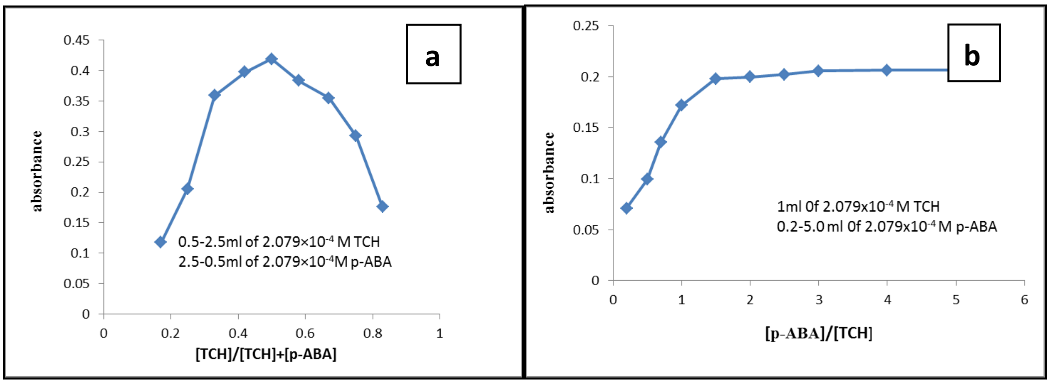
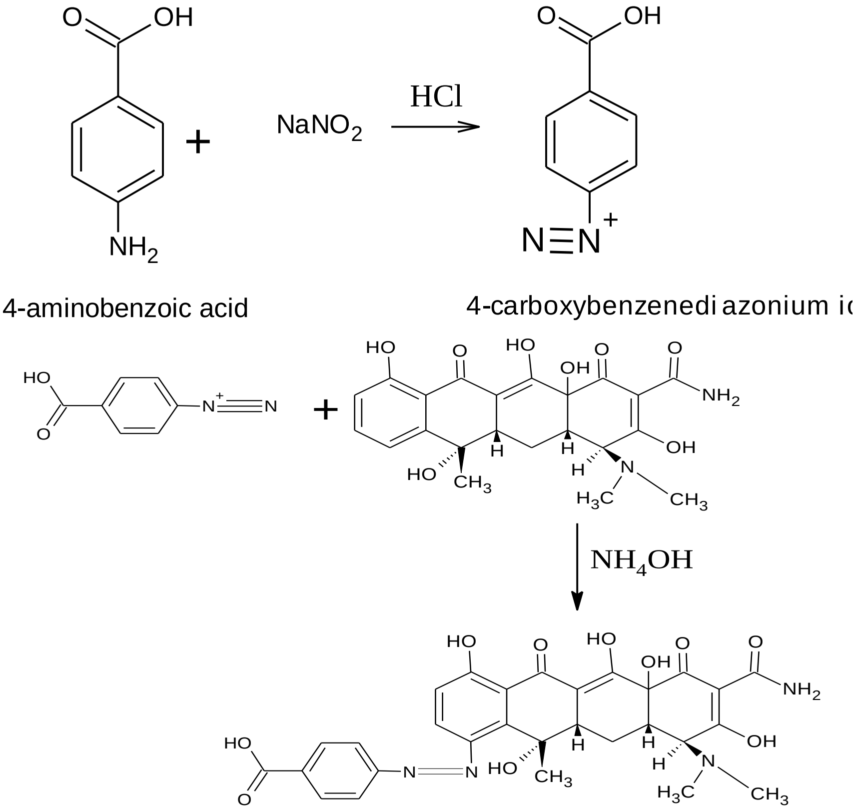


Fig. 7: Plots of (a) the continuous variation and (b) mole-ratio methods for TCH-diazotized p-ABA azo dye.

Mechanism of the reaction

A mechanism of interaction has been suggested which includes that p-ABA react with ($NaNO_2$) in presence of the strong acid like (HCl) to produce diazonium salt coupled with TCH in presence of NH_4OH solution and production of a yellow azo dye. The reaction's mechanism is illustrated in (Scheme 1).



Scheme 1: The reaction mechanism

Quantification

The concentration range of Beer's law, molar absorptivity (ε), recovery percent, RSD, LOD, and LOQ were determined and the data were listed in (Table 3). The Linearity of the method was also described by the regression equation and the corresponding determination coefficient (R^2) for TCH was estimated by using the suggested method and referred to a good linearity.

Table 3: Summary of optical characteristics and statistical data for the proposed method

Parameter	Information
Range of Beer 's law, µg/ml	0.25-17.5
λ max, nm	422
Molar absorptivity, l/mol.cm	1.66×10^{4}
Recovery range, %	99.96% to 101.47 %
Error percent range, %	-0.08% to 3.3 %
Precision (RSD), %	≤ 1.59
Determination coefficient (R^2)	0.9993
LOD, µg/ml	0.0120
LOQ, µg/ml	0.040
Slope (a)[#]	0.0346
Intercept (b)[#]	0.0374

[#] Regression equation: ($Y = a X + b$), X =TCH concentration in µg per ml.

Application

The suggested method has been applied for the analysis of TCH in its pharmaceutical forms (capsules and skin ointment) from different origins and at three different quantities (50, 100, 150 µg). The results listed in (Table 4) indicated that the developed method is a suitable for assaying TCH with an acceptable result.

To evaluate the results of the developed method a t-test has been investigated and the results in (Table 4) reveal that the values of the t-experiment are less than the t-tabulated value at the (95%) confidence level (Christian, 2004). The experimental results indicated that the difference is statistically not significant, which confirms the success of applying the proposed method to estimate TCH in its pharmaceutical forms.

Table 4: Determination of TCH in capsules and skin ointment

Pharmaceutical Form	Certified value	µg TCH Found*	RE. (%)*	Mean Reco. (%)	RSD (%)*	Measured value	Experim. t-value[a]
Apcycline (India)	250 mg/ Capsule	49.96	-0.08	99.98	0.16	249.95 mg	1.91
		100.035	0.035		0.41		
		149.97	-0.02		0.24		
Tetracycline (Iran)		49.96	-0.08	99.96	0.80	249.90 mg	1.48
		99.98	-0.02		0.48		
		149.96	-0.03		0.55		
Samacycline (SDI-Iraq)		50.02	0.04	100.014	0.66	250.035 mg	1.05
		99.99	-0.01		0.43		
		149.98	0.013		0.27		
Samacycline (SDI-Iraq)	3% skin ointment	50.04	0.08	101.47	1.19	3.044 %	2.19
		101.04	1.04		1.59		
		154.95	3.30		1.45		

*Average of five estimations, [a]t-value = $(\bar{x} - \mu)\frac{\sqrt{N}}{s}$, [a]Tabulated "t"value at 95% confidence level is equal to 2.776, degree of freedom (N= 4)

Evaluation of the results of the proposed procedure using the standard addition method

To evaluate the selectivity of the suggested procedure and its success in estimating TCH in its pharmaceutical forms, the standard addition method was applied for this purpose. The results exemplified in Fig. (8) and listed in Table 5 indicating that the standard additions method agrees well with the results of the proposed method without interferences and within a satisfactory range of error.

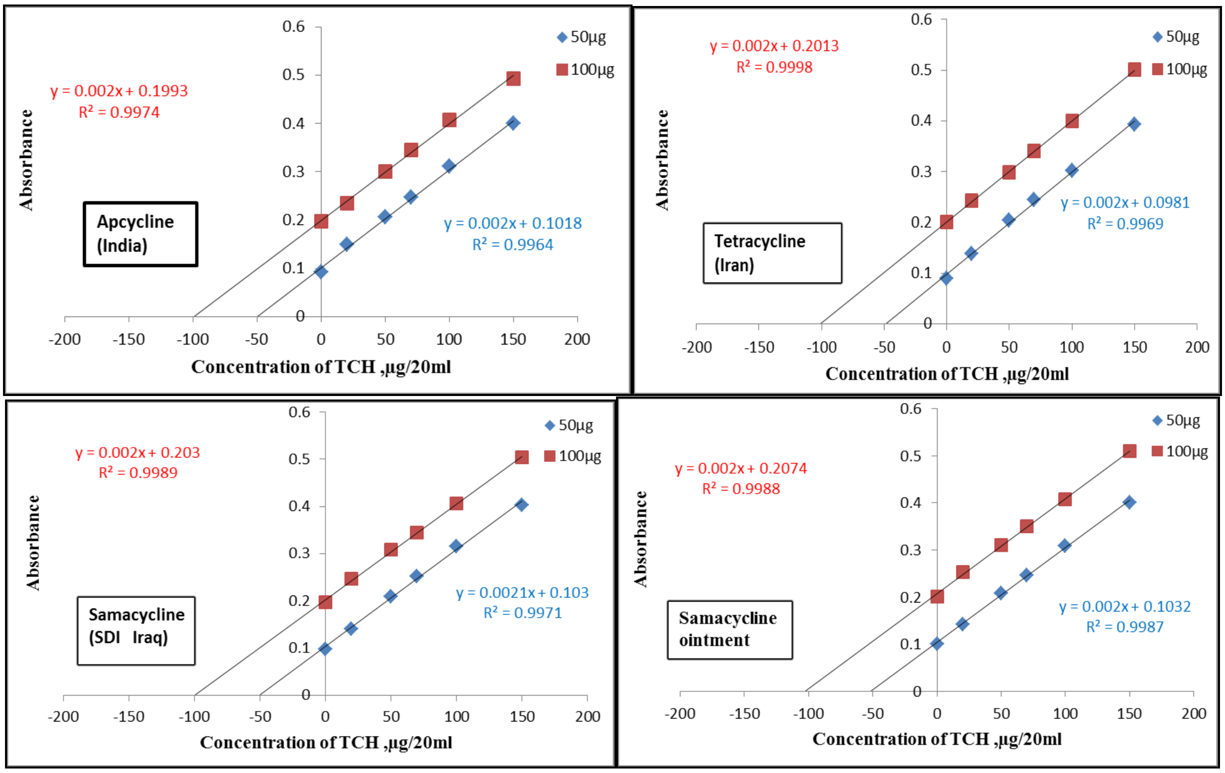


Fig. 8: Standard graphs of standard addition method for the analysis of TCH in pharmaceutical forms (capsules and skin ointment)

Table 5: Estimation of TCH in its pharmaceutical preparations using the standard addition method

Pharmaceutical preparation	Certified value	µg of TCH Present	µg of TCH Found	Recovery (%)*	Measured value
Apcycline (India)	250 mg / capsule	50	50.90	101.8	254.5 mg
		100	99.65	99.65	249.12 mg
Tetracycline (Iran)		50	49.05	98.10	245.25 mg
		100	100.65	100.65	251.62 mg
Samacycline (SDI-Iraq)		50	49.04	98.08	245.20 mg
		100	101.5	101.5	253.75 mg
Samacycline (SDI-Iraq)	3% Skin ointment	50	51.60	103.2	3.096 %
		100	103.7	103.7	3.11 %

CONCLUSION

In this investigation, TCH was coupled with diazotized p-aminobenzoic acid (p-ABA) in an alkaline solution to produce a yellow azo dye. This dye was stable and soluble in water and showed maximum absorption peak at 422 nm vs. blank solution. Beer's law was linear in the concentration range of 0.25-17.5 µg/ml with molar absorptivity of 1.66×10^4 l/mol.cm. The values of error percentage and precision (RSD%) were calculated and found in the range of -0.08% to 3.3% and 0.16% to 1.59%, correspondingly. The suggested approach for estimating TCH in pharmaceutical samples has the advantages of being sensitive, low-cost, and straightforward because it does not require a solvent extraction step or temperature control. The technique is also accurate and precise enough to replace the current spectrophotometric method and evaluation of TCH in ointment and capsules.

REFERENCES

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 J. for Pure Appl. Sci.*, **30**(1), 400-412. https://doi.org/10.30526/30.1.1088

Ahmed, N.R.; Edress, S.B.; Yaseen, H.W. (2018). Assay of tetracycline in pharmaceutical preparations, spiked industrial wastewater and chicken meat samples using visible spectrophotometric technique. *Bas. J. Vet. Res.*, **17**(2), 252- 264.

Al-Abbasi, K.M.H. (2009). Spectrophotometric determination of tetracycline by azo dye formation with diazotised p-nitroaniline-application to pharmaceutical preparations and biological fluids. *Raf. J. Sci.*, **20**(1), 61-74.

Alassaf, N.A.; Zankanah, F.H.; Hamody, A.S.; Dikran, S.B. (2019). Kinetic-spectrophotometric estimation of tetracycline in bulk and pharmaceutical forms. *IJDDT*, **9**(02),197-201.

Ali, R.J.; Hawezy, H.J.S.; Abdullah, M.S. (2018). Spectrophotometric determination of tetracycline hydrochloride through coupling with sulphanilic acid. *Diyala J. of Medicine*, **15**(2),15-22. https://doi.org/10.26505/DJM

AL-Sowdani, K.H.; AL-Abdullah, Z.T.; AL-Abdalaziz, B.A. (2006). Spectrophotometric determination of tetracycline in some pharmaceutical preparations. *J. Basrah Researches (Sciences)*, **32**(1), 43-48.

Christian, G.D. (2004). "Analytical Chemistry". John Wiley and Sons, 6th ed., Philadelphia, pp. 90-97.

De levic, R. (1997). “Principles of Quantitative Chemical Analysis”. Mc, Graw-Hill, International ed., Singapore, pp. 495-502.

Fahelelbom, K.M.S. (2008). Analysis of certain tetracyclines and oxytetracyclines through charge transfer complexation. *Am. J. Pharm. Toxicol.*, **3**(3), 212-218.

Faria, L.V.; Lima, A.P.; Araújo, F.M.; Lisboa, T.P.; Matos, M.A.C.; Munoz, R.A.A.; Matos, R.C. (2019). High-throughput amperometric determination of tetracycline residues in milk and quality control of pharmaceutical formulations: flow-injection versus batch-injection analysis. *Anal. Methods*, **11**(41), 5328-5336. https://doi.org/10.1039/C9AY01759C

Gil, R.L.; Amorim, C.M.P.G.; Montenegro, M.D.C.B.; Araújo, A.N. (2022). Cucurbit [8] uril-based potentiometric sensor coupled to HPLC for determination of tetracycline residues in milk samples. *Chemosens.*, **10**(98), 1-16. https://doi.org/10.3390/chemosensors10030098

Hameedi, I.T. (2021). Determination of tetracycline hydrochloride in pure and pharmaceutical samples via oxidative coupling reaction. *Mater. Today: Proceed.*,**42**(5), 2953-2958. https://doi.org/10.1016/j.matpr.2020.12.802

Hu, X.; Xu, Y.; Cui, X.; Li, W.; Huang, X.; Li, Z.; Shi, J. (2020). Fluorometric and electrochemical dual-mode nanoprobe for tetracycline by using a nanocomposite prepared from carbon nitride quantum dots and silver nanoparticles. *Microchimic. Acta*, **187**(1), 1-10. https://doi.org/10.1007/s00604-019-3828-4

Lu, H.; Zhang, H.; Li, Y.; Gan, F. (2021). Sensitive and selective determination of tetracycline in milk based on sulfur quantum dot probes. *RSC Advances*, **11**(37), 22960-22968. Doi: 10.1039/d1ra03745e

Mamani, M.C.V.; Farfán, J.A.; Reyes, F.G.R.; Rath, S. (2006). Simultaneous determination of tetracyclines in pharmaceuticals by CZE using experimental design. *Talanta.*, **70**(2), 236-243. https://doi.org/10.1016/j.talanta.2006.02.048

Mari, L.F; Bakir, M.H.; Aziz, M.S.A. (2016). Spectrophotometric determination of tetracycline, in pharmaceutical formulations by oxidative coupling. *KUJSS*, **11**(4), 159-183.

Othman, N.S.; Al-Ashow, J.R. (2012). Spectrophotometric determination of tetracycline by coupling with diazotised 4-aminoantipyrine in presence of cetylpyridinium chloride. *Raf. J. Sci.*, **23**(3), 72-84.

Pang, Y.H.; Lv, Z.Y.; Sun, J.C.; Yang, C.; Shen, X.F. (2021). Collaborative compounding of metal–organic frameworks for dispersive solid-phase extraction HPLC–MS/MS determination of tetracyclines in honey. *Food Chemistry*, **355,** 129411. https://doi.org/10.1016/j.foodchem.2021.129411

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. *J. Food Composit. Analys.*, **105**, 104215. https://doi.org/10.1016/j.jfca.2021.104215

Tella, E.D.; Taherunnisa, M.; Deepthi, G.K.; Choragudi, B.M.; Choragudi, B.R. (2011). Spectrophotometric determination of tetracyclines using p-N, N-dimethylphenylenediamine and sodium metaperiodate. *Rasayan J. Chem.*, **4**(3), 539-543.

Thanasarakhan, W.; Kruanetr, S.; Deming, R.L.; Liawruangrath, B.; Wangkam, S.; Liawruangrath, S. (2011). Sequential injection spectrophotometric determination of tetracycline antibiotics in pharmaceutical preparations and their residues in honey and milk samples using yttrium (III) and cationic surfactant. *Talanta,* **84(5)**, 1401– 1409. https://doi.org/10.1016/j.talanta.2011.03.087

The Japanese Pharmacopoeia (2016). “The Ministry of Health, Labor and Welfare”. 17th ed., English Version, 1670 p.

Vuran, B.; Ulusoy, H. I.; Sarp, G.; Yilmaz, E.; Morgül, U.; Kabir, A.; Soylak, M. (2021). Determination of chloramphenicol and tetracycline residues in milk samples by means of nanofiber coated magnetic particles prior to high-performance liquid chromatography-diode array detection. *Talanta,* **230**, 122307. https://doi.org/10.1016/j.talanta.2021.122307

تطوير طريقة طيفية لتقدير التتراسايكلين هيدروكلوريد بالاقتران مع بارا – أمينو حامض البنزويك المؤزوت – تطبيق في المستحضرات الصيدلانية

رانيه إبراهيم شيت **سالم علي محمد**

قسم الكيمياء/ كلية العلوم/ جامعة الموصل

الملخص

يتضمن هذا البحث تطوير طريقة طيفية حساسة ودقيقة لتقدير التتراسايكلين هيدروكلوريد في الوسط المائي وفي المستحضرات الصيدلانية. تعتمد هذه الطريقة على ازوتة الكاشف بارا- أمينو حامض البنزويك بتفاعله مع كمية مماثلة من نتريت الصوديوم بوجود محلول حامض الهيدروكلوريك لتكوين مركب الدايازونيوم الذي يقترن مباشرة مع التتراسايكلين هيدروكلوريد في وسط قاعدي من محلول هيدروكسيد الامونيوم لتكوين صبغة ازوية ذات لون اصفر ذائبة ومستقرة في الماء تعطي أعلى شدة امتصاص عند الطول الموجي (422) نانوميتر مقابل المحلول الصوري. و كانت الطريقة خطية تتبع حدود قانون بير في مدى التركيز (17.5−0.25) مايكروغرام/مللتر بمعامل تقدير (R^2= 0.9993) و امتصاصية مولارية مساوية الى(R^2= 0.9993) و (1.66×10^4) لتر/ مول. سم على التوالي. و كانت حساب قيمة حد الكشف (LOD) و قيمة حد التقدير الكمي (LOQ) مساوية الى (0.012) و (0.040) مايكروغرام/ مللتر على التوالي. بينما كان معدل قيم الخطأ النسبي وقيم التوافقية (RSD%) يتراوح بين (-0.08%) الى (3.3%) و(0.16%) الى (1.59%) على التوالي. وتم حساب النسبة المولية بين التتراسايكلين هيدروكلوريد والكاشف بارا- أمينو حامض البنزويك المؤزوت وكانت مساوية الى1:1. طبقت الطريقة المقترحة بنجاح في تقدير التتراسايكلين هيدروكلوريد في الكبسولات والمرهم.

الكلمات الدالة: تتراسايكلين هيدروكلوريد، الاقتران الازوي، بارا أمينو حامض البنزويك، الضوئية.