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Indirect Spectrophotometric Method for the Quantitative Estimation of Tetracycline Hydrochloride in the Pharmaceutical Formulations

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Abstract

The objective of this work was to create an easy, fast, and accurate spectrophotometric approach for the assaying tetracycline hydrochloride in the bulk and in its pharmaceutical dosage forms. In this recommended approach tetracycline hydrochloride was oxidized with a known excess of an oxidant reagent N-bromosucinimide in an acidic medium and the excess of oxidant N-bromosucinimide was then reduced by indigo carmine dye and the absorbance of the residual indigo carmine dye was measured at wavelength of 610 nm. All the variables which affecting the conditions such as, influence of the amount of the oxidant, indigo carmine dye, acid concentration, oxidation time and the concentration limits of Beer′s law were adjusted and studied carefully. The optimal conditions showed the color of the product solution was stable for more than 30 minutes. The standard calibration curve was linear in the variety of concentrations from 0.2 to 4.5 µg/ml of tetracycline hydrochloride with a good determination coefficient (R^2) 0.9985 and molar absorptivity of 7.7190×10^4 l/mol.cm. The LOD and LOQ values were evaluated and instituted to be 0.0043 and 0.0142 µg/ml, respectively. The suggested approach was successfully used to assay tetracycline hydrochloride in the available dosage forms, such as capsules and skin ointment. The precision (RSD) was calculated and found to be better than 0.721 %, whereas the values of recovery percent and relative errors were in the range of 99.76% to 100.33% and -0.45% to 0.48% respectively, without interfering from any common excipients.

Keywords: Tetracycline hydrochloride; UV-Visible spectrophotometry; N-bromosuccinimide Indigo carmine dye

Introduction

Tetracycline hydrochloride (TCH), antibiotics are one of the largest groups of pharmaceutical substances used worldwide for treating diseases in humans as well as for preventing diseases and promoting growth in livestock (Jeong *et al.*, 2010). 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). TCH is a yellow powder and chemically acknowledged as [(4S, 4aS, 5aS, 6S, 12aS)-4-dimethylamino-3,6,10,12,12 apentahy-droxy - 6 - methyl- 1, 11- dioxo- 1, 4-, 4a-, 5, 5a, 6, 11, 12a - octahy- drotetracene-2-carboxamide monohydrochloride] (The Japanese Pharmacopoeia, 2016) (Fig.1).

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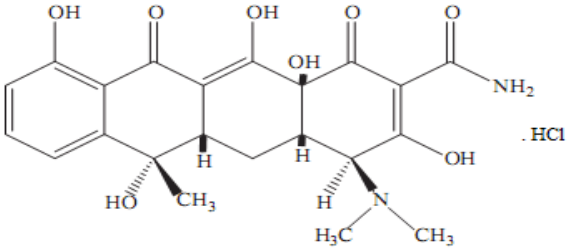

Fig.1 : The chemical structure TCH (M.Wt.= 480.935 g/mol)

Various analytical techniques for the estimation of TCH in the pharmaceutical formulations have been described in the literature, these included: HPLC-diode array detection (Vuran *et al.*, 2021), HPLC–MS/MS (Pang *et al.*, 2021), fluorescent probe based on sulfur quantum dots (SQDs) (Lu *et al*., 2021), cyclic voltammetry using the reduced graphene-oxide (rGO) modified electrode associated with flow-injection analysis (Faria *et al.*, 2019), fluorometric and electrochemical dual-mode method (Hu *et al.*, 2020), HPLC-ion-selective electrode (Gil *et al.*, 2022) and kinetic spectrophotometry (Alassaf *et al.*, 2019). However, most of these methodologies required post-column derivatization, skilled operation and an expensive equipments.

UV-Visible spectrophotometry was also employed to assay TCH due to its simplicity of instrumentation, cost-effectiveness, versatility of use and feasible approach. Variety of spectrophotometric methodologies have been reported to assay TCH in the bulk and in the pharmacological preparations. Most of these methods included diazo-coupling reaction which based on the coupling of TCH with diazotized of 4-aminoantipyrine in presence of cetylpyridinium chloride (Othman and Al-Ashow, 2012), sulphanilic acid (Ali *et al.*,2018), and anthranilic acid (Abd *et al.*, 2017). Others based on the oxidative coupling reactions using, N,N-diethyl-p-phenylenediamine reagent in presence of N-bromosuccinimide (NBS) as an oxidant (Mari *et al.*, 2016), p-N,N-dimethylphenylenediamine and sodium meta periodate (Tella *et al.*, 2011), 2,4-dinitrophenylhydrazine (2,4-DNPH) and potassium periodate in a basic medium (Hameedi, 2021) and p-aminoantipyrine in presence of NBS (Sheet and Mohammed, 2023). Other spectrophotometric approaches have been developed for assaying TCH in aqueous medium based on the formation of soluble complexes with cerium(IV) (Al-Sowdani et al, 2006), yttrium (III) in presence of cationic surfactant (Thanasarakhana *et al.*, 2011) and zirconium (IV) (Saenjum *et al.*, 2022). In addition, the charge transfer reaction of TCH with chloranilic acid (Fahelelbom, 2008) and redox reaction with sodium hypochlorite (Ahmed *et al*, 2018) for estimating TCH were also reported.

The goal of the recent investigation is to develop an indirect spectrophotometric approach for determining TCH in the bulk and in its pharmaceutical forms by adding an excess amount of NBS in order to oxidize TCH. The residual NBS amount was then used for bleaching indigo carmine dye and the absorbance of the blue final solution was measured at wavelength of 610 nm. which is directly proportional to the concentration of TCH.

Experimental

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Instrumentation

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

Chemical materials and standard solutions

The chemical materials and analytical reagents used were of a high degree of purity and the standard material of TCH powder was obtained from the state company for drug industries, Samara-Iraq (SDI).

Standard solution of TCH (500 µg/ml)

An accurately 0.0500 g of pure TCH was weighed and dissolved in about 10 ml distilled water (dw) and the volume was then completed with same solvent to 100 ml using a calibrated and saved in a dark bottle. Working solution in a concentration of 60 µg/ml was also prepared by diluting an appropriate volume of TCH standard solution with dw and kept it in a brown container.

NBS solution (2×10^{-3} M)

A 0.0356g of NBS (Fluka) was weighed and dissolved in a 100 ml dw using calibrated flask.

Indigo carmine dye solution (5×10^{-4} M)

It was prepared by dissolving 0.0233 g of indigo carmine dye with dw in a 100 ml calibrated flask.

Hydrochloric acid solution (1M)

This solution was made by diluting an appropriate volume of the concentrated hydrochloric acid (11.6 M) with dw.

Essential procedure

An increasing volumes of the working solution of TCH (3 µg/ml) were pipetted into a series of 20 ml calibrated flasks covering the concentration range 0.2-4.5 µg/ml. To each, 1ml of 1M hydrochloric acid and 1 ml of 2×10^{-3} M NBS oxidizing agent were added and mixed thoroughly. After waiting for 15 minutes in order to complete the oxidation of TCH, a 1.7 ml of 5×10^{-4} M indigo carmine dye solution was then added. The flasks were shaken and maintained at room temperature (20±2 Cº) for 5 minutes. Finally, the flasks were filled with dw to the mark, and mixed well. The absorbance of each flask was measured at wavelength of 610 nm against reagent blank solution.

Calibration curve

According to the optimal conditions, a linear calibration curves was obtained in the concentration range of 0.2-4.5 µg/ml TCH. A molar absorption coefficients and the Sandell's sensitivity index were estimated and found to be 7.7190×10^{4} l/mol.cm and 0.00623 µg/cm^2, correspondingly. Fig.(2) explains the calibration curve and absorption spectra of TCH measured by following the suggested method.

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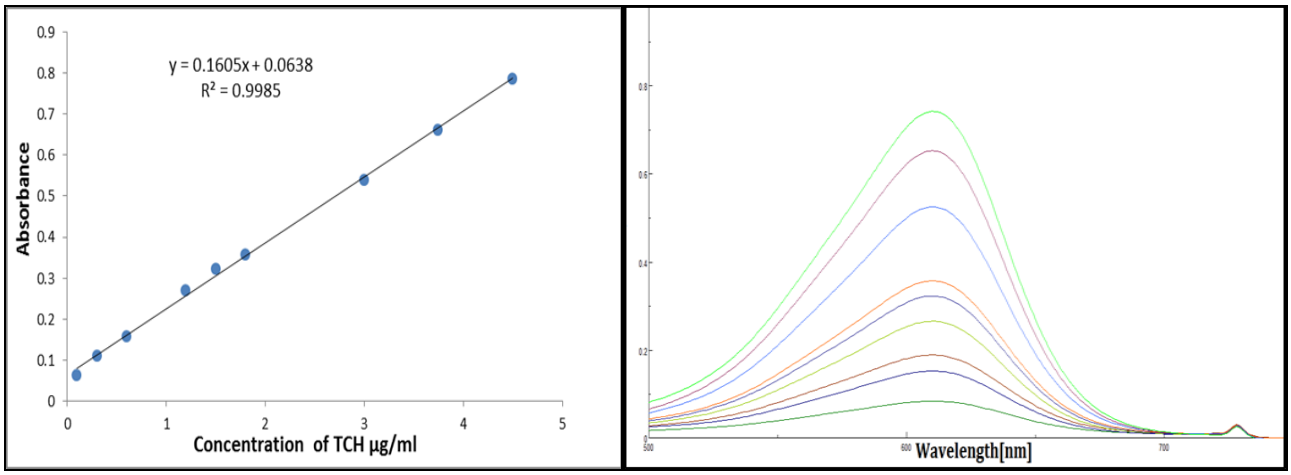

Fig. 2: The calibration curve and the absorption spectra for estimating TCH according to the suggested procedure

Analysis of TCH in the pharmaceutical forms

TCH capsule analysis

To prepare a solution of medicine in concentration of 100 µg/ml TCH, the contents of five capsules [each capsule contains 250 mg of TCH (except for tetrasiklin which contains 500 mg per capsule)] were mixed and homogenized well. An precise amount of medicine equivalent to 0.0100 g TCH was weighed and dissolved in a 100 ml dw using a calibrated flask. The estimation of TCH in the drug solution was worked by taking an aliquot of it and analysed by following the described procedure.

TCH ointment analysis

Three containers contenting TCH ointment were homogenized and mixed thoroughly. An exact quantity of the mixed ointment equivalent to 0.0100 g TCH was weighed and dissolved with mixture of 3 ml ethanol and 50 ml dw. The mixture solution was warmed , filtered into a 100 ml calibrated and filled to the mark with dw to obtain a solution of TCH in a concentration of 100 µg/ml (Othman and Al-Ashow, 2012), and an aliquot of the TCH ointment solution was taken and estimated according to the suggested method.

Results And Discussion

Chemistry

The determination of TCH was involved two steps:

In step (1) TCH was oxidized with an excess of NBS in acidic medium.

TCH + NBS → oxidized form of TCH + NBS
excess (colourless) residual (colourless)

In step (2): The indigo carmine dye was bleached by the residual amount of NBS and converted it to a colorless product at the same media.

NBS + indigo carmine → oxidized form of indigo carmine + indigo carmine (residual)
residual blue colourless product blue (λmax= 610 nm)

The solution of the residual indigo carmine dye shows maximum absorption at wavelength of 610 nm which proportional to the TCH concentration (Fig. 3).

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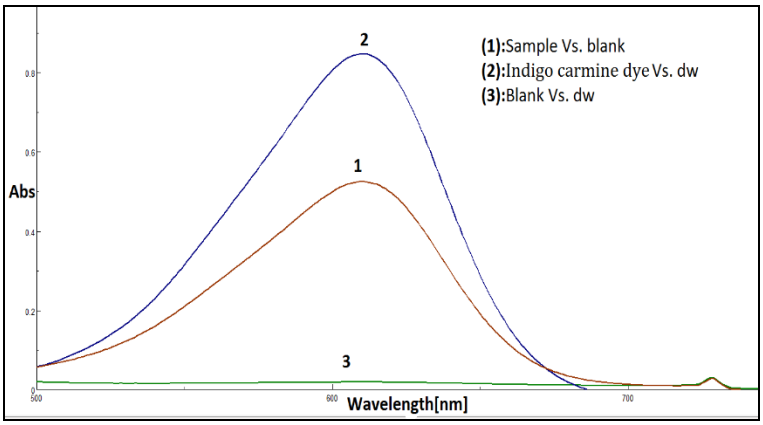

Fig. 3: Absorption spectra of 3 µg/ml TCH measured according to the recommended procedure

Optimization of the reaction conditions

All experimental variables affecting mainly on the sensitivity and stability of the blue colour of the residual indigo carmine dye were optimized. The subsequent experiments were performed using 60 µg of working TCH solution in a final volume of 20 ml calibrated flask and the absorbance of the blue colour was recorded at the wavelength 610 nm against its blank solution.

Influence of indigo carmine dye amount

In order to find the appropriate amount of the indigo carmine dye that can be used in the reaction, the influence of several quantities from 0.25 to 2.0 ml of indigo carmine dye solution on the absorbance was carried out. The absorbance was measured at the improved wavelength 610 nm against dw and the results are shown in Fig.4.

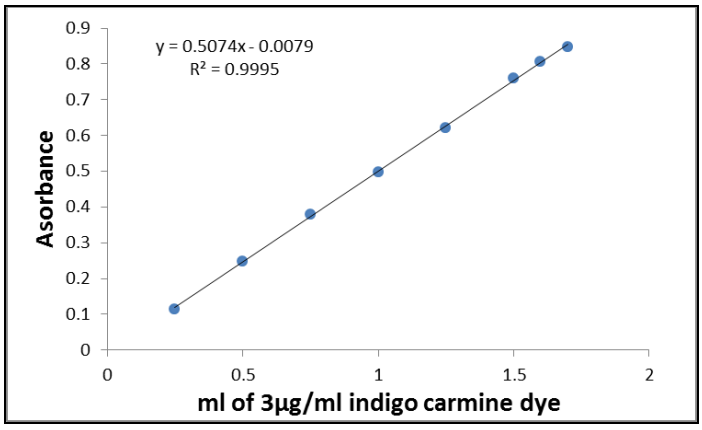

Fig. 4: Standard curve of indigo carmine dye

The results in Fig. 4 reveal that the linearity of the curve is continued to a volume of 1.7 ml of the indigo carmine dye, with determination coefficient (R^2) equal to 0.9995 and a greater amount of the dye cause a negative deviation from Beer's law. Therefore, a 1.7 ml of the dye solution is chosen as the optimum amount for the reaction in the subsequent experiments.

Selection of the proper oxidizing agent

The influence of 1ml of 2×10^{-3} M of various oxidizing agents such as, N-chlorosuccinimide (NCS), sodium periodate ($NaIO_4$), potassium periodate (KIO_4), potassium iodate (KIO_3) and

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NBS on the absorbance of 3 µg/ml TCH was investigated. The results are illustrated in Fig.5 indicate that the oxidizing reagent of NBS is suitable for oxidizing indigo carmine dye, thus it was chosen for the next investigations.

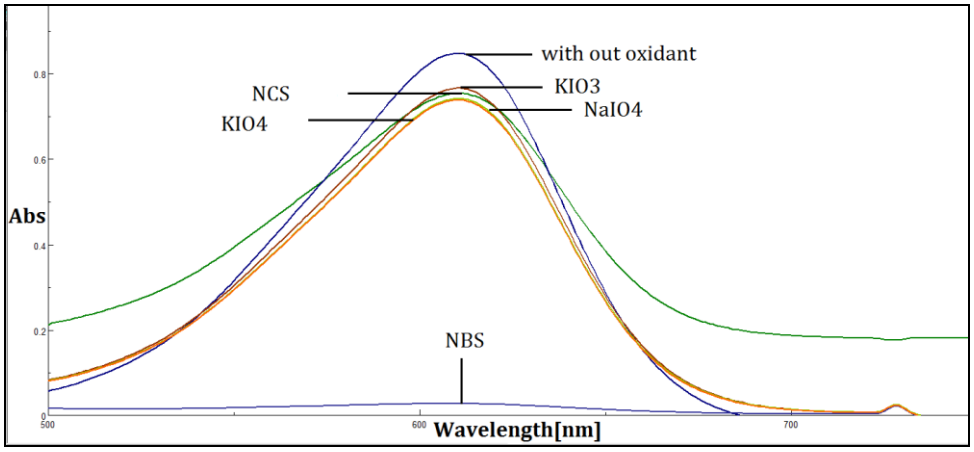

Fig. 5 : Effect of the oxidizing agents on absorbance

Effect of the amount of NBS

After selecting the ideal amount of the indigo carmine dye solution, The effect of diverse quantities 0.2 -1.5 ml of NBS (2×10^{-3}) M was studied in acid solution of 1M HCl. The results in Fig. 6 illustrate that a 1 ml of NBS is the optimal amount to reach almost complete dye color bleaching. Therefore, it has been proven and relied upon in subsequent experiments.

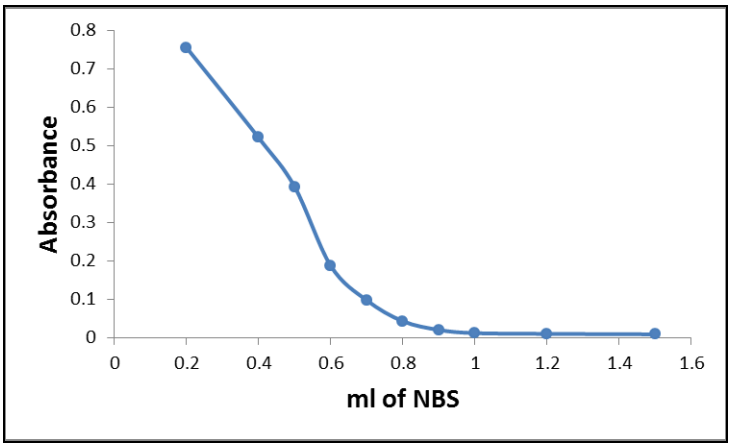

Fig. 6: Effect of NBS amount on bleaching the colour of the indigo carmine dye

Effect of acid type and its concentration

The effect of 1ml of various strong and weak acids (1M) on the absorbance of the residual indigo carmine dye was studied. The results are listed in Table 1.

Table 1: Effect of acid type on absorbance

Type of 1M acid	HCl	H_2SO_4	HNO_3	HCOOH	CH_3COOH
Absorbance	0.4133	0.2247	0.2042	0.0016	0.3572

The results in Table 1 show that the hydrochloric acid solution is still the best acid because, it gave the highest absorbance compared to the rest of other acid solutions. Accordingly, the effect of different concentrations of HCl solution on the absorbance of the dye was diagnosed (Table 2).

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Table 2: Effect of acid concentration on absorbance

Concentration of HCl (M)	0.5	1.75	1.0	1.25	1.50	2.0
Absorbance	0.2874	0.2722	0.4138	0.4113	0.4068	0.3981

The results summarized in Table 2 indicated that the concentration of 1M HCl is the optimal concentration, so it was maintained in the subsequent experiments.

Oxidation time effect

It is necessary to investigate the ideal times that are required for the oxidation of TCH and indigo carmine dye with NBS . The influence of different intervals of time 5-30 minutes on the absorbance of the oxidation of TCH and 1-20 minutes for the oxidation of indigo carmine dye with NBS was investigated. The results in Table 3 explained that the oxidation process of TCH with NBS requires at least 15 minutes to be completed , whereas, the oxidation of indigo carmine dye with the same oxidant needs 5 minutes..

Table 3: Effect of the oxidation reaction time

Time needed to oxidize indigo carmine dye (min.)	Absorbance / minute to oxidize TCH					
	1.0	5.0	10.0	15.0	20.0	30.0
Immediately	0.3211	0.3473	0.3507	0.3475	0.3264	0.3177
2.0	0.3249	0.3487	0.4228	0.423	0.4155	0.4451
5.0	0.3275	0.3522	0.5175	0.5206	0.5188	0.4861
10.0	0.4313	0.4396	0.4442	0.4258	0.4065	0.3660
15.0	0.4108	0.4136	0.4122	0.4041	0.3913	0.3656

Effect of temperature

The effect of different temperatures (5, room temp, 40 and 50 Cº) on the absorbance of the residual indigo carmine dye using a water bath with thermostatic control was performed. According to the results in Fig.7, the reaction is not temperature dependent because , the absorbance remains nearly constant for an 30 minutes at room temperature (20±2 °C)

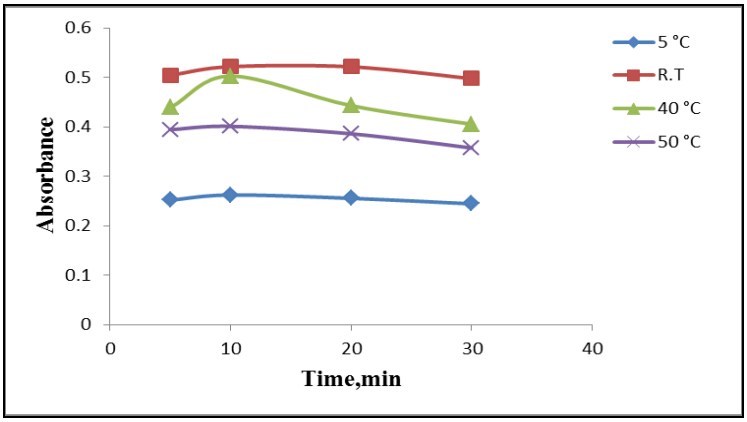

Fig. 7: Temperature effect on absorbance

Quantification

The limits of Beer's law, molar absorptivities, Sandell's sensitivities, accuracy (recovery %), and precision (RSD) of the proved methods was evaluated. The linearity of the suggested methods was also described by the equation of regression, as well as the corresponding

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determination coefficient (R^2) for TCH was calculated. The limits of detection (LOD) and quantitation (LOQ) are found according to the rules guidelines (Valcarcel Cases *et al*., 2018). All results are summarized in Table 4, which indicate that the proposed method are sensitive, precise and accurate.

Table 4: Summary of physical characteristics and arithmetical data for the suggested approach

Factors	Informations
Range of Beer 's law, µg/ml	0.2-4.5
λmax, nm	610
Molar absorptivity , l/mol.cm	7.7190×10^4
Recovery range , %	99.76% to 100.33%
Error percent range, %	-0.45% to 0.48%
Precision (RSD), %	≤ 0.721
Determination coefficient (R^2)	0.9985
LOD*, µg/ml	0.0043
LOQ*, µg/ml	0.0142
Slope (a)$^{\#}$	0.1605
Intercept (b)$^{\#}$	0.0638

Application

The suggested method was applied for the estimation of TCH in its pharmaceutical formulations (capsules and ointment) for four different concentrations 20,40 and 70 µg TCH/20 ml. The results are listed in Table 5 reveal that the proposed procedure has a good agreement and with the declared content.

To evaluate the results of the development method a t-test has been carried out. The results in Table 5 show that the values of t-exp. are less than the t-tabulated value at 95% confidence level and for four degrees of freedom (N = 4) (Christian *et al.*, 2014). This it means the difference is statistically not significant, which confirms the success of the proposed method to assay TCH in its drugs.

Table 5: Determination of TCH in capsules and skin ointment

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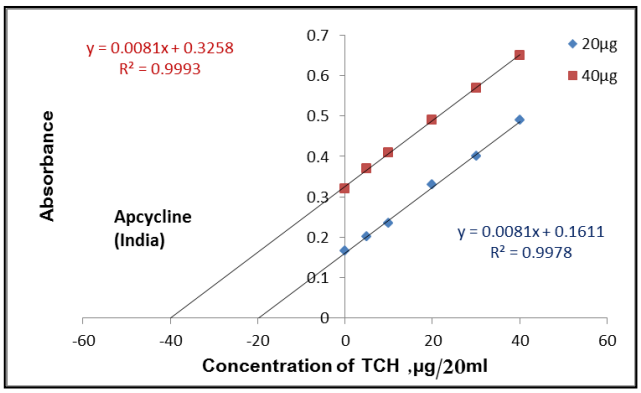
*Average of five estimations , [a]$t \pm = (\bar{x} - \mu)\frac{\sqrt{N}}{s}$, [a]Tabulated "t" value at 95% confidence level is equal to

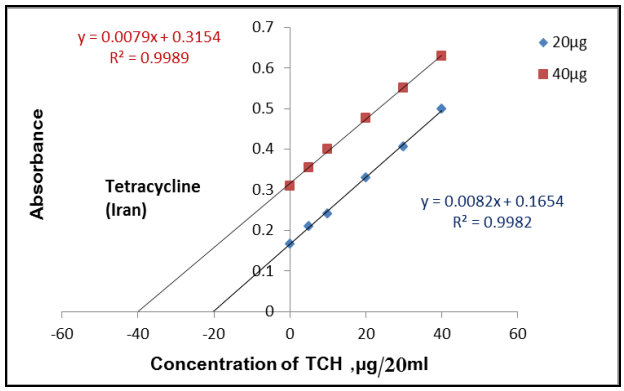
Pharmaceutical Form	Certified value	CH Found (µg)*	R.E. (%)*	Average of Rec. (%)	RSD (%) (N=5)	Measured value	t- exp[a]
Apcycline (India)	250 mg/capsule	19.91 39.96 69.19	-0.45 -0.10 -1.16	99.76	0.721 0.027 0.209	249.41	1.39
Tetracycline (Iran)		20.04 39.85 70.07	0.20 -0.37 -0.16	99.89	0.252 0.038 0.546	249.77	1.86
Samacycline (SDI-Iraq)		20.03 40.02 70.06	0.15 0.05 0.09	100.10	0.189 0.015 0.012	250.25	0.87
Tetrasiklin (Gensenta Turkey)	500 mg/capsule	20.04 39.94 70.18	0.20 - 0.15 0.26	100.11	0.284 0.025 0.019	500.53	1.66
Samacycline (SDI-Iraq)	3% skin ointment	20.06 40.19 70.14	0.30 0.48 0.20	100.33	0.197 0.074 0.029	3.010 %	2.39

2.776

Evolution of the suggested method

To prove the efficiency and credibility of the proposed method to estimate TCH and to ensure that it is free from the interference of additives, a standard additions method was performed. The results shown in Fig.8 and listed in Table 6 indicate that there is a high agreement between the standard addition method and the suggested approach for the analysis of TCH in the pharmaceutical forms.





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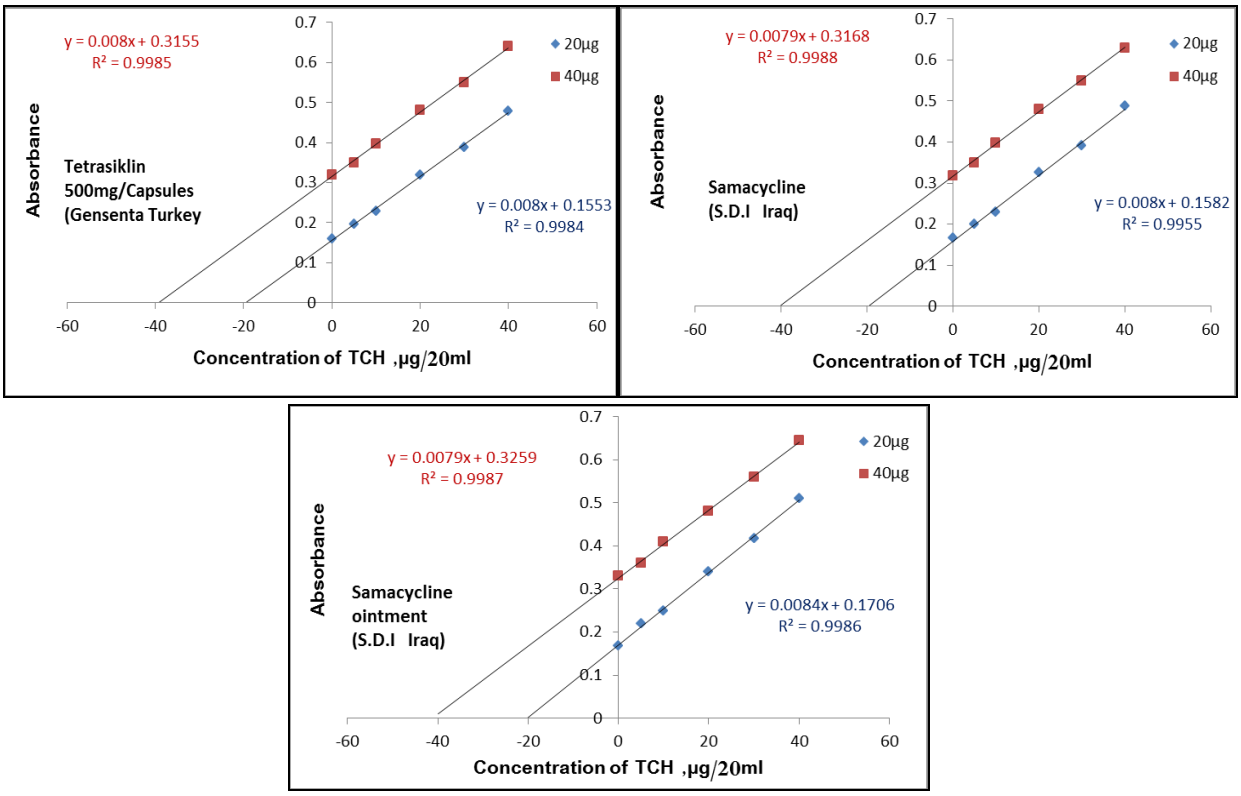

Fig. 8: Calibration graphs of standard addition method for analysis of TCH in pharmaceutical forms

Table 6: Estimation of TCH in the pharmaceutical forms using standard addition method

Pharmaceutical preparation	Certified Value	TCH Present (µg)	TCH Found (µg)	Recovery (%)	Measured value
Apcycline (India)	250 mg/capsule	20	19.89	99.45	248.63 mg
		40	40.22	100.55	251.38 mg
Tetracycline (Iran)		20	20.17	100.85	252.13 mg
		40	39.92	99.80	249.50 mg
Samacycline (S.D.I Iraq)		20	19.78	99.90	249.75 mg
		40	40.10	100.25	250.63 mg
Tetrasiklin (Gensenta Turkey)	500 mg/capsule	20	19.41	97.05	485.25 mg
		40	39.44	98.60	493.00 mg
Samacycline (S.D.I Iraq)	3% Skin Ointment	20	20.31	101.55	3.047 %
		40	41.25	103.13	3.094 %

Conclusion

This search describes a simple indirect UV-Visible spectrophotometric method. The suggested approach has the advantages of being sensitive, low-cost, accurate and precise enough to replace the current spectrophotometric method. It does not involve pre-extraction nor temperature control. The suggested approach is also selective and free from the common excipients ; hence it can be used routinely for the analysis of TCH in pharmaceutical forms.

References

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Received 25 January 2023; Accepted 05 February 2023

[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 Jour. for Pure & Appl. Sci.*, **30**(1), 400-412.

[2]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.

[3]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.

[4]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.

[5]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.

[6]Christian, G.D. ; Dasgupta ; P.K. ; Schug, K. (2014). Analytical chemistry, 7th Edn., Hoboken, NJ:Joh Wiley and Sons, Inc., New Yourk , p.84

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

[8]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.

[9]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. *Chemosensors*, **10**(98), 1-16.

[10] Hameedi, I.T. (2021). Determination of tetracycline hydrochloride in pure and pharmaceutical samples via oxidative coupling reaction. *Materials Today: Proceedings*, **42**(5), 2953-2958.

[11] 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. *Microchimica Acta*, **187**(1), 1-10.

[12] Jeong, J., Song, W., Cooper, W. J., Jung, J., & Greaves, J. (2010). Degradation of tetracycline antibiotics: mechanisms and kinetic studies for advanced oxidation/reduction processes. *Chemosphere*, ***78***(5), 533-540.

[13] 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.

[14] 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.

[15] 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.

[16] 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.

[17] 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.

[18] 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.

[19] Sheet, R.I. ; Mohammed, S.A. (2023). Improved a spectrophotometric estimation of tetracycline hydrochloride in pharmaceutical preparations through oxidative coupling reaction. *BioGecko*, **12**(2), 1223-1234.

[20] 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.

[21] 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.

[22] The Japanese Pharmacopoeia (2016). 17th Edn, English Version, The Ministry of Health, Labor and Welfare, p.1670.

[23] Valcarcel Cases, M. ; lopez-Lorente, A.I. ; Lopez-Jimenez, M. (2018). Principles of Analytical Chemistry. In foundations of Analytical Chemistry, Springer, Cham. pp.3-52.

[24] 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.