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Using 9-Chloroacridine as A Chromogenic Reagent for The Determination of Tetracyclin

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Abstract

An accurate, cost-effective and simple method has been described for the quantitative determination of Tetracycline hydrochloride (TCs) in bulk and pharmaceutical samples using an aqueous medium. The proposed method is based on the nucleophilic substitution reaction of 9-chloroacridine (9-CA) as a chromogenic reagent with TCs in an aqueous solution forming a colored complex. The spectrum of the product shows maximum absorption at 385 nm against the blank. The linearity range of the calibration graph was (1-28) µg.mL^{-1} with a limited detection value (LOD) 0.138 µg.mL^{-1}. The results show the absence of interferences from the excipients compounds on the determination of the drug. The suggested method exhibited high reproducibility (RSD) 2.3 % with mean percentage recovery (R%) 99.95. Furthermore, the stability constant has been calculated to be 9.843×10^5 L.mol^{-1} and the reaction mechanism is proposed. A ratio of 1: 1 was found between the above drug and the reagent using Job and molar ratio methods. The accuracy and validity of the proposed method were further determined by performing a recovery experiment by the use of the standard addition method. It is found that the method does not require extraction process and it agree well standard addition method. Application of the suggested method to commercial pharmaceutical preparations (capsule and ointment) is presented.

Keyword: Nucleophilic substitution; Tetracycline hydrochloride; UV-VIS determination; 9-Chloroacridine.

1. Introduction

Antibiotics are one of the main common pharmaceutical products, used for the treatment of parasitic, fungal, and bacterial infections. Among antibiotics, tetracyclines are widely used in both human and veterinary therapy. Tetracyclines are broad-spectrum antibiotics that are effective against nearly all gram-positive and gram-negative organisms [1]. Simultaneously, because of its harmful effects on public health, concerns are raised regarding the residues of antibiotics in environmental and biological samples. Tetracycline could be used as an alternative medication for allergic people to a penicillin [2]. Although the tetracycline group is at the current of secondary position to the penicillins and cephalosporins, it is still an essential group of drugs because it is imperative to have at hand orally active, broad-spectrum antibiotics that are not structurally linked to the broad-spectrum penicillins [3, 4]. Antibiotic and non-antibiotic characteristics are both reduced by synthetic alteration of the lower periphery area. Biological targets, on the other hand, can be enriched by altering the upper peripheral zone, mainly locations C7 through C9 of the D Ring **Figure 1**. This has been changed with tetracycline semisynthetic compounds such as doxycycline and minocycline [3]. Many approaches have been described for the detection of TCs, in bulk material and pharmaceutical formulation including spectrophotometry[5-9], voltammetry[10-13], HPLC methods[14, 15], LC-MS methods[16-18], chemiluminescence[19, 20] and flow injection analysis methods[21-24]. Conversely; most of these approaches are either inadequately sensitive, time-consuming, tedious, suffered from interference compounds, used an organic solvent and required an extraction step[25] or limit linear range[26].

Spectrophotometry is a quantitative measurement of the reflection or transmission properties of a material as function of wavelength. The main benefit of these methods is labor consumption as a result of reducing the analysis time and reagent amount compared with classical manual procedures. Besides that, the spectrophotometric method

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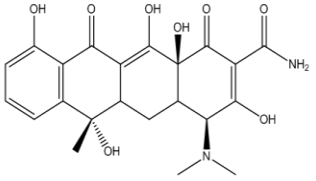
has an excellent precision. The interaction of TCs with 9-CA to give a highly colored substituted 9-chloroacridine has been noticed [27]. The unstable 9-chloro derivative was modified to stable 9-phenoxy derivative by reacting with an excess phenol. 9-chloroacridines could react with molten phenol (MPh) to produce stable 9-phenoxy intermediates[28]. The current work describes an easy and accurate spectrophotometric technique for estimation of TCs. The method is relying on the formation of a colored product by the reaction of TCs with 9-CA as chromogenic reagent without any derivatisation, extraction or catalysis. This resulted in an improvement of a colorimetric method that can be used to determine TCs in pharmaceutical dosage forms. Recently, one of the most serious environmental and human health problems is the production of antibiotics without control assay. Therefore, there is a need to develop a cost-effective method for the detection of antibiotics in their pharmaceutical preparations. The United States Pharmacopoeia describes an approach for the determination of tetracycline (TCs) and it is based on high-performance liquid chromatography (HPLC) technique[29], but this technique is highly-cost and requires an expert researcher. Therefore, the method adopted in the British pharmacopoeia requires a long time to perform the analysis process, as well the equipment in somewhat complicated and expensive[30]. This work design to be simple, fast, sensitive and cost-effective to be useful for the determination of Tetracycline hydrochloride in pharmaceutical formulation. The suggested procedure based on the spectrophotometric determination of the colored formed by the interaction between TCs and 9-chloroacridine in an aqueous medium.

2. Experimental

2.1 ApparatusSpectrophotometric and absorbance measurements were made in Shimadzu UV-Vis 1800 (Double-beam spectrophotometry) with 1 cm quartz cells. The pH of the solution was recorded using inolab pH 7110. Solutions were heated up by Elektro.mag and Mettler H 54AR was used for weighing.

2.2 Materials

All solvents (methanol, acetone and water) were high purity which is supplied by Fluka and BDH while 9-chloroacridine was provided by Eastman Chemical Co. Absolute ethanol was purchased from ROTH Co. The 9-chloroacridine reagent (2×10^{-3} M) prepared by dissolving 0.042g in 100 mL distilled water (DW). TCs 100 µg/mL as stock standard solutions were prepared by precisely weighed 0.01 g of TCs and dissolved in water into a volumetric flask (100 mL) and completed up to mark with DW. NaOH and HCl (1M) were prepared and diluted when needed it. All solvents and chemicals were used without extra purification.



4S,6S,12aS-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide

Figure 1. Structure of Tetracycline

2.3 Method Procedure

In an appropriate volumetric flask (25 mL), a quantity of an aqueous solution containing TCs was placed. The 9-CA solution containing 1.0 mL of ($2\ x10^{-3}$M) was added to volumetric flask. This solution was basified with 1 mL of ($2\ x10^{-5}$ M) of NaOH and water was added then shaken at a room temperature (30 Cº) for 10 min. The measurements were corrected for reagent blank in the method. One milliters of the solution was transferred into a quartz cell to check the absorbance at 385 nm against its blank. The 9-CA as a reagent was reacted quantitatively with TCs in basic medium to produce colored solution directly. It was clear to note that the color intensity increased when the reaction mixtures were left for 10 min in 30 Cº compare to the blank and show a highest absorption at 385 nm, which was applied in subsequent experiments.

2.4 Pharmaceutical Preparations

Capsules samples: The content of ten capsule (each capsule containing 250 mg TCs) were weighed accurately, crushed to powder and mixed well. A portion of the homogenized fine powder equivalent to one capsule was weighed and dissolved in few drops of $1\ x10^{-1}$ M HCl. After that, the solution transferred to 100 mL volumetric

flask and completed up to mark with DW to get solution of 2500 µg/mL and the procedure as described was followed.

Ointment samples: Homogenized mixture of five ointments tubes was prepared. After that, precisely weighed of ointment equivalent 25 mg of TCs was dissolved in 20 mL of diethyl ether (DEE) and extracted with 60 mL of 1 $x10^{-1}$ M HCl. The solution was filtered and transferred into a 100 mL volumetric flask, the additives compounds washed with 1 $x10^{-1}$ M HCl and diluted to get 250 µg/mL of TCs.

3. Results and Discussion

3.1 Studying the optimum reaction conditions

To get a high absorption value and the best condition for the product (Drug- reagent), numerous parameters such as concentration of reagent, time, temperature, type of base, volume of NaOH, order of addition of components on color intensity and type of surfactant were examined and optimized. All experiments were done using 4 µg/mL of TCs in the final volume (25 mL).

3.2 Reagent concentration

The 25 mL solution prepared by dissolving 0.0106 g of 9- CA then completed up to mark with ethanol absolute. As recommended in previous research, fresh reagent solution (9- CA) was prepared and used immediately[29]. It was found that 1.0 mL of 2×10^{-3} M solution of reagent with 4 µg/mL of the drug in alkaline medium showed highest absorbance value at 385 nm after 10 min at ambient temperature
Table 1.

3.3 Volume of Reagent

In order to set the volume of reagent, different samples that contains a variety of reagent volume has been prepared. It has been noticed that the 1 mL of reagent gives the highest absorbance

Table 2.

3.4 Type of Base

The reaction pH was wide-ranging between pH 2.5 and 11.5 by adding 0.01 M of HCl or NaOH. It was indicated that the sensitivity of complex was reduced when adding acid. Nevertheless, the pH of final solutions was checked as well in the absence of NaOH and HCl. To achieve high sensitivity value for (TCs-9-CA) complexes, different bases such as sodium hydroxide, ammonium hydroxide, sodium carbonate, and potassium hydroxide with a fixed volume (5×10-2 M) were tested by adding it to a fixed concentration of TCs. It was indicated that NaOH gave maximum colour intensity in 385 nm Figure 2. The results were compatible with the previous research which indicate that the reaction can be done in a basic medium[30, 31].

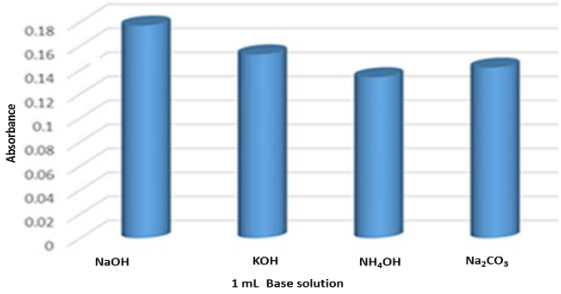


Figure 2. Type of Base

3.5 The volume of NaOH and pH value

The next step is to study the effect of basic volume on the absorbance different volumes of NaOH as shown in

Table 3. It was indicated that 1.5 mL of sodium hydroxide could give maximum color intensity and absorption value so it can be used in the subsequent experiments.

3.6 Effect of Buffer solution

As shown in **Error! Reference source not found.** different buffer solutions which have pH 11.2 (Carbonate-bicarbonate, borate-sodium hydroxide, and bicarbonate-sodium hydroxide) were also examined. However, the buffer has a negative effect on the absorbance intensity of the product.

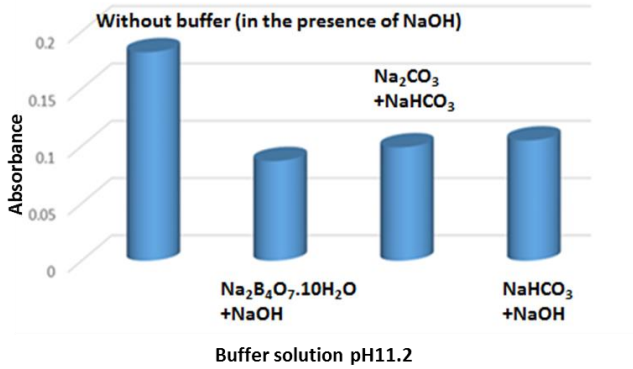


Figure 3. Effect of Buffer solution

3.7 Order *of addition Effect*

In order to obtain extraordinary absorbance intensity, the sequence addition of reagent (R), base (B), and the drug (S) must follow the instructions in the general procedure; however, the loss of color intensity is noted **Table 4**.

3.8 Surfactant addition effect

Effect of different surfactant agents such as cetylperydinum chloride (CPC), sodium dodecyl sulphate (SDS), Tween-80, cetyltrimethylammonium bromide (CTAB), and Triton x were verified. It was noticed that the addition of surfactant has a negative impact on the absorbance value and color intensity.

3.9 Stability time and the effect of temperature

The temperature effect on the absorbance intensity of the reaction products for TCs was studied at different temperatures at room temperature 30, 40, 50, and 60 ºC at the above stated optimal conditions. It was found that the higher absorbance was obtained at ambient temperature (30 °C) after 10 min (**Figure 4**). The experimental result shows that the absorbance intensity value decrease with increasing temperature and that 30 °C is the optimum temperature for the TCs-9-CA complex. However, a decreased in absorbance was noticed indicating dissociation of the product. The absorbance remained stable for 60 min **Figure 4**.

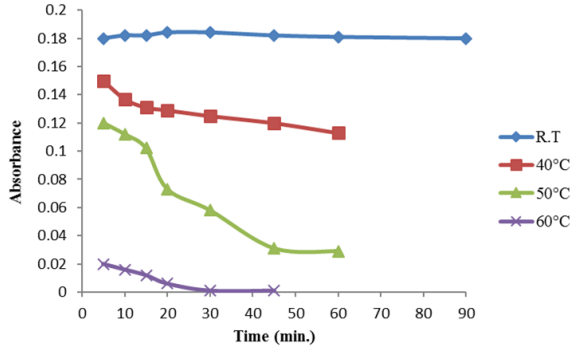


Figure 4. Effect of Tempreture and time

3.10 Final Spectrum

Under the optimum condition, the reagent was reacted directly with TCs in water solution and introduce light yellow colored solutions immediately. When the reaction mixture is kept at room temperature for 10 minutes, the intensity of the color increases. The complex shows highest absorption value at 385 nm, which is used in next experiments. However, blank solution shows no absorption at same wavelength **Figure 5**.

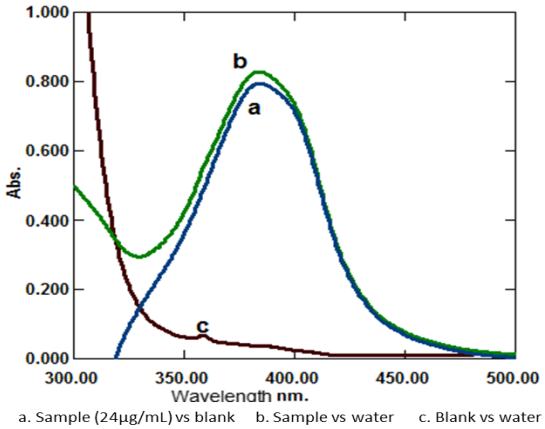


Figure 5. Final Spectrum

4. The calibration data

Under the optimized experimental reaction conditions, the calibration curve of products for TCs - 9-CA was created by plotting the relationship between absorbance and concentration, as shown in **Figure 6**. The linear regression coefficient (R^2) of this graph is 0.999. As shown in
Table 5, the developed method indicates high sensitivity.

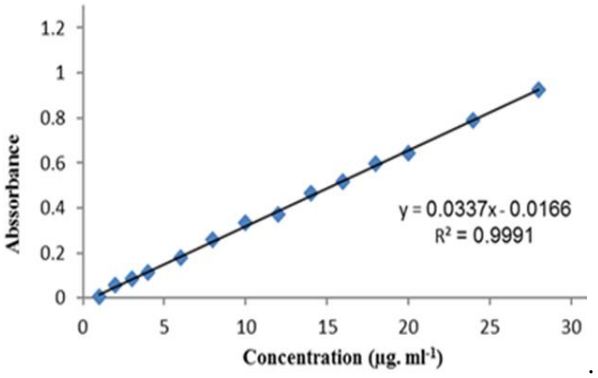


Figure 6. Calibration Curve

5. Precision and accuracy

The average recovery percentage (% recovery) and the relative standard deviation (RSD) are calculated for three different concentrations, with an average of five determinations. The results indicating that the proposed technique was precise and accurate as shown in

Table 6**Error! Reference source not found.**.

6. Nature of the colored product and stability constant

As described previously[32], the reaction stoichiometry for (Tetracycline - 9-CA) was calculated by Job and molar ratio methods using $1x10^{-4}$ M for (TCs - 9-CA). The results showed that the product was formed in the ratio of 1:1 (TCs -9-CA). As clearly presented in **Figure 7**, the results confirmed that 1:1 (TCs - 9-CA) was formed using both methods. It can be concluded that the phenolic hydroxyl group in the drug is responsible for the interaction and formation of the product.

Stability constant has been determined by comparing the absorbance of the solution containing same concentration

of the TCs and 9-CA (As) to one having an excessive amount of 9-CA (Am). According to the 1:1 ratio, the average of stability constant of the product was calculated via the following equations:

$$Kst = [1 - \alpha / \propto^2]/C$$

$$\alpha = Am - As/Am$$

Kst = the stability constant (mole/L), , C= the concentration of the product which is similar to the concentrations of TCs α= the dissociation degree. Nevertheless; the stability constant for three different concentrations was found 9.843×10^5 mol/L for Tetracycline-9-CA product indicating high stability **Table 7**.

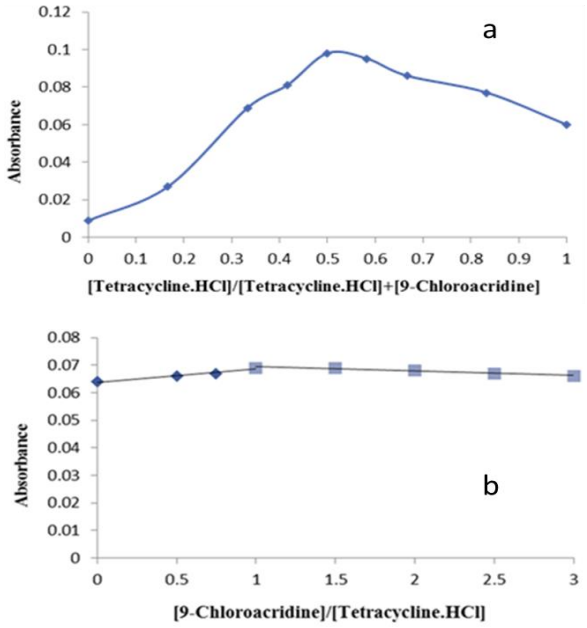


Figure 7. Job (a) and mole ratio (b) plots for the Tetracycline 9-CA

Effect of Interferences

Addition of some additives which frequently added by pharmaceutical companies were calculated by evaluating the absorbance of solutions containing a precise amount of drug (10 μg mL^{-1}) and various amounts of varied species in a final volume of (5 mL). It was confirmed that the studied excipients did not affect or interfere even in the presence of a 1000-fold excess

Table 8. However; an error of less than 5.0 % in the absorbance intensity was considered tolerable. Furthermore, the result points out that the additives material and common excipients do not inhibit with the determination of TCs concentration.

8. Proposed Mechanism

As can be seen in **Figure 8**, the nucleophilic attack occurs on the carbon atom number 9 in the reagent (9-chloroacridin) by the hydroxyl group of the drug (TCs) to form an etheric bond as shown in the diagram below[33, 34]. It has been recommended that the reaction produces more rapidly with the free phenolic group. It would be possible, consequently, to apply this analysis method for any hydroxyl-containing compound. Nucleophilic substitution can be applied to determine compound when the 9-position of the acridine ring bears a facile leaving group and substituted by hydroxyl group to form acridinium derivatives. Conversely; reaction mechanism was suggested as presented in **Figure 8**.

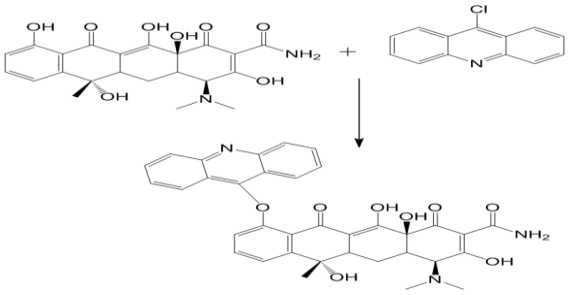


Figure 8. Mechanism of the proposed chemical reaction

9. Analytical application

The suggested method was applied successfully to estimate TCs in pharmaceutical preparations (capsule and ointment) using three different concentrations for each pharmaceutical. The average recovery % was in the range 98.98- 99.03 % indicating that the proposed method is accurate **Table 9**.

10. Recovery Study

The proposed method was validated and confirmed through a recovery study. As shown in **Figure 9**, standard addition method was used to evaluate recovery by adding two different concentration levels of pure TCs to the commercial form of the drug (Samacycline capsules (SDI)). The TCs concentration was estimated by the suggested method using 9-CA as an acceptor. The recovery percentage value shows that the proposed method affords good accuracy and the recovery value range from 99.98 to 99.03. Furthermore, the result reveals that the co-formulated materials and common additives do not interfere the determination process.

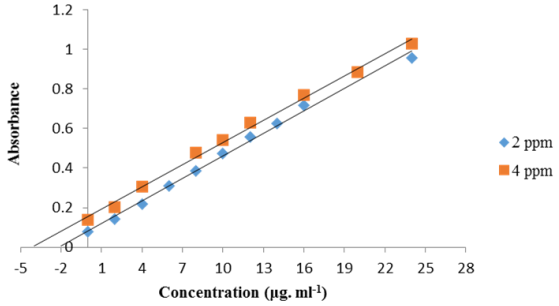


Figure 9. Standard addition curve to determine TCs capsule

Comparing the obtained resulted with published work[5, 30, 31, 35]. As can be seen in **Table** 10, the proposed method has higher sensitive than the cited works. In addition, there is no interferences have been noticed and it can be carried on an aqueous medium.

Table 1. The effect of 9-CA concentration on the absorption of 4µg /mL TCs

Conc.of 9-CA (M)	5×10^{-2}	1×10^{-2}	5×10^{-3}	2×10^{-3}	1×10^{-3}
Absorbance	Turbid	0.155	0.167	0.174	0.162
λ_{max}	--	388	386	385	382

Table 2. The effect of 9-CA Volume on the absorption value of 4µg /mL TCs

mL of 9-CA (2×10^{-3} M)	0.25	0.5	1.0	1.5	2.0	2.5

Absorbance	0.165	0.169	0.176	0.170	0.167	0.156

Table 3. Effect of NaOH volume

Volume of NaOH 5×10^{-2} M (mL)	Without	0.5	1.0	1.5	2.0	2.5
Absorbance	0.092	0.169	0.177	0.182	0.163	0.141
Final pH	6.4	10.5	10.9	11.2	11.5	11.8

Table 4. Order of Addition

Command Number	Reaction component	Absorbance
1	S+R+B	0.182
2	S+B+R	0.090
3	R+B+S	0.101

Table 5. Statistic data for the proposed method

Compound	Linearity range ($\mu g.mL^{-1}$)	Molar extinction coefficient ($L.mol^{-1}.cm^{-1}$)	LOD*	LOQ*	Slope	Intercept	Correlation coefficient (R^2)
Tetracycline hydrochloride	1.0-28	16206.33	0.138	0.462	0.033	0.0166	0.9991

Table 6. Precision and accuracy

Compound	Amount added ($\mu g.\ mL^{-1}$)	Recovery percentage (%)	Average recovery (%)	RSD* (%)
Tetracycline hydrochloride	2	100.00	99.95	2.5
	4	102.10		1.9
	6	97.77		2.4

Table 7. Stability constant for (Tetracycline-9CA) product

Compound	Conc. ($mol.L^{-1}$)	Absorbance As	Absorbance Am	α	Average Kst ($L.mol^{-1}$)
Tetracycline Hydrochloride	$2x10^{-6}$	0.021	0.050	0.580	$9.843x10^5$
	$4x10^{-6}$	0.045	0.077	0.415	
	$6x10^{-6}$	0.083	0.116	0.284	

Table 8. Effect of additives compounds on the absorbance intensity of 4 µg/mL of Tetracycline

Additives	Recovery percentage of 4 $\mu g.\ mL^{-1}$ of TCs. HCl per $\mu g.\ mL^{-1}$ of foreign added			
	100	500	750	1000
Glucose	100.65	104.26	104.57	103.94

Fructose	103.28	104.67	104.79	104.57
Sucrose	104.26	96.05	97.36	98.15
Starch	102.63	101.97	103.28	104.26
Talc	98.50	100.20	102.08	103.90
Mg-stearate	99.13	101.97	103.28	104.60

Table 9. Assay results of TCs pharmaceutical preparations using the proposed method

Pharmaceutical preparation	Certified value	Amount present ($\mu g.mL^{-1}$)	Drug content found* (mg)	Recovery* (%)	Average recovery (%)
Samacycline	250 mg	2	249.42	99.76	
capsule (SDI)		4	246.45	98.58	98.90
/Iraq		6	254.02	101.60	
Tetracycline	10 mg	2	10.12	101.20	
hydrochloride		4	9.91	99.10	100.05
ointment (India)		6	9.68	96.80	

***Average of five determinations

Table 10. Comparison of the suggested method with published methods

Parameters	9- Chloroacridin (Present method)	Cerium (VI) (Al-Sowdani,2006)	Sulphanilic acid (Ali et al.,2018)	2,4–dinitrophenyl hydrazine (Khaleel et al., 2020)
λmax (nm)	385	430	403	360
Temp.(°C)	RT	RT	RT	----
Development time	10	-------	10	5
Linearity(µg.mL)	1-28	5-35	9-80	0.1-9
Stability (min)	60	50	75	10
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	16206	10000	6536	12620
RSD≤	2.5	0.03	4.07	0.467
Solvent	Water	Methanol	Methanol	water
Application	Capsule and ointment	capsule	capsule	Capsule
Disadvantage	Limit linear range	Using an organic solvent and low sensitivity	Using an organic solvent and low sensitivity	Unknown temperature and Limit linear range

11.

Conclusion

The experimental results show that the suggested method can be applied successfully for the estimation of tetracycline as an antibiotics drug, with high sensitivity and reproducibility. The excellent R^2 and intercept value confirm the good linearity of the calibration curve. A comparative study of this procedure was made with the published spectrophotometric method. It can be concluding that the suggested method has high accuracy and

repeatability. Additionally, this method is simple, rapid, and does not require critical reactions, any pre-treatment and extraction steps which are attractive features for analytical pharmaceutical studies. The proposed method was applied successfully for the assay of the pharmaceutical preparation as capsule and ointment of tetracycline.

12. Acknowledgment

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13. Conflict of interest

The authors state that there is no conflict of interest.

14. References

1. C.K. Okamoto, C.W. Van den Berg, M. Masashi, R.M. Gonçalves-de-Andrade, D.V. Tambourgi, Tetracycline reduces kidney damage induced by loxosceles spider venom, Toxins, **9**, 90 (2017).
2. A.A. Lodhi, M.A. Murtaza, N.A.G. Haider, S.A. Junejo, M. Kamal, Role of tetracycline in corneal neovascularization, Pakistan Journal of Ophthalmology, **31**, 150-153 (2015).
3. H.K. Hami, R.F. Abbas, A. Jasim, D.A. Abdul Abass, M.A. Abed, A.A. Maryoosh, Kinetics study of Removal Doxycycline drug from aqueous solution using Aluminum Oxide surface, Egyptian Journal of Chemistry, **62**, 91-101 (2019).
4. J. Brown, D. Ireland, Structural requirements for tetracycline activity, Advances in Pharmacology, **15**, 161-202 (1978).
5. R.J. Ali, H.J.S. Hawezy, M.S. Abdullah, Spectrophotometric Determination of Tetracycline Hydrochloride Through Coupling with Sulphanilic Acid, Diyala Journal of Medicine, **15**, 15-22 (2018).
6. M.M. Abd, S.B. Dikran, A.K. Mahmood, 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 Science, **30**, 400-412 (2017).
7. L.F.M.M.H. Bakir, M.S.A. Aziz, Spectrophotometric Determination of Tetracycline, in pharmaceutical formulations by oxidative coupling, kirkuk university journal for scientific studies, **11**, 159-183 (2016).
8. R.S. Al-Kalissy, A.K. Mohammed, Spectrophotometric Method for the Determination of Tetracycline and Doxycycline by Oxidizing Coupling Reaction with 4-aminoantipyrine, Ibn AL-Haitham Journal For Pure and Applied Science, **28**, 154-168 (2017).
9. N.R. Ahmed, S.B. Edress, H.W. Yaseen, Assay of tetracycline in pharmaceutical preparations, spiked industrial wastewater and chicken meat samples using visible spectrophotometer technique, Basra J. Vet. Res., **17**, 252-264 (2018).
10. G. Guo, F. Zhao, F. Xiao, B. Zeng, Voltammetric determination of tetracycline by using multi-wall carbon nanotube-ionic liquid film coated glassy carbon electrode, Int. J. Electrochem. Sci, **4**, 1365-1372 (2009).
11. Y.A. Akeneev, G.B. Slepchenko, V.I. Deryabina, T.M. Gindullina, I. Maximchuk, T.I. Shchukina, Determination of tetracycline in honey by voltammetry, Procedia Chemistry, **15**, 355-359 (2015).
12. A.S. Lorenzetti, T. Sierra, C.E. Domini, A.G. Lista, A.G. Crevillen, A. Escarpa, Electrochemically Reduced Graphene Oxide-Based Screen-Printed Electrodes for Total Tetracycline Determination by Adsorptive Transfer Stripping Differential Pulse Voltammetry, Sensors, **20**, 76 (2020).
13. L. Devkota, L.T. Nguyen, T.T. Vu, B. Piro, Electrochemical determination of tetracycline using AuNP-coated molecularly imprinted overoxidized polypyrrole sensing interface, Electrochimica Acta, **270**, 535-542 (2018).
14. H. Filik, A. A Avan, S. Aydar, D. Ozyurt, B. Demirata, Determination of tetracycline on the surface of a high-performance graphene modified screen-printed carbon electrode in milk and honey samples, Current Nanoscience, **12**, 527-533 (2016).
15. B. Arabsorkhi, H. Sereshti, Determination of tetracycline and cefotaxime residues in honey by micro-solid phase extraction based on electrospun nanofibers coupled with HPLC, Microchemical Journal, **140**, 241-247 (2018).
16. B.S. Reddy, Y. Sudhakar, Y.S. Rao, P. Reddyprasad, N. Sreedhar, LC-MS/MS method development and validation to determine three tetracyclines and their epimers in shrimp samples, Orient. J. Chem., **33**, 2459-2469 (2017).
17. G. Alfredsson, C. Branzell, K. Granelli, Å. Lundström, Simple and rapid screening and confirmation of tetracyclines in honey and egg by a dipstick test and LC–MS/MS, Analytica Chimica Acta, **529**, 47-51 (2005).
18. U. Koesukwiwat, S. Jayanta, N. Leepipatpiboon, Validation of a liquid chromatography–mass spectrometry multi-residue method for the simultaneous determination of sulfonamides, tetracyclines, and pyrimethamine in milk, Journal of chromatography A, **1140**, 147-156 (2007).

19. G. Wang, H.C. Zhang, J. Liu, J.P. Wang, A receptor-based chemiluminescence enzyme linked immunosorbent assay for determination of tetracyclines in milk, Analytical biochemistry, **564**, 40-46 (2019).
20. C. Lau, J. Lu, M. Kai, Chemiluminescence determination of tetracycline based on radical production in a basic acetonitrile–hydrogen peroxide reaction, Analytica chimica acta, **503**, 235-239 (2004).
21. M.Q. Al-Abachi, H. Hadi, Flow injection spectrophotometric determination of Tetracycline hydrochloride in pharmaceutical samples, Irq. Nat. J. Chem., **55**, 243-251 (2014).
22. S. Palaharn, T. Charoenraks, N. Wangfuengkanagul, K. Grudpan, O. Chailapakul, Flow injection analysis of tetracycline in pharmaceutical formulation with pulsed amperometric detection, Analytica chimica acta, **499**, 191-197 (2003).
23. M.P. Rodríguez, H.R. Pezza, L. Pezza, Simple and clean determination of tetracyclines by flow injection analysis, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, **153** 386-392 (2016).
24. S. Liawruangrath, B. Liawruangrath, S. Watanesk, W. Ruengsitagoon, Flow injection spectrophotometric determination of tetracycline in a pharmaceutical preparation by complexation with aluminium (III), Analytical sciences, **22**, 15-19 (2006).
25. M.Y. Dhamra, N. Theia'a, T.S. Al-Ghabsha, Spectrophotometric determination of Terbutaline Sulphate and tetracycline hydrochloride via ion pair complex formation using Eosin Y, Pakistan Journal of Analytical & Environmental Chemistry, **15**, 9 (2014).
26. Y. Ni, N. Deng, S. Kokot, A simple kinetic spectrophotometric method for simultaneous determination of tetracyclines by use of chemometrics, Analytical Methods, **2**, 1302-1309 (2010).
27. J.T. Stewart, D.A. Settle, Colorimetric determination of isoniazid with 9-chloroacridine, Journal of pharmaceutical sciences, **64**, 1403-1405 (1975).
28. A.R. Benoit, Synthesis and evaluation of acridine and acridone based compound as anti-Cancer and anti-bacterial agents, (2013).
29. Y. Vander Heyden, K. Luypaert, C. Hartmann, D. Massart, J. Hoogmartens, J. De Beer, Ruggedness tests on the high-performance liquid chromatography assay of the United States Pharmacopeia XXII for tetracycline hydrochloride. A comparison of experimental designs and statistical interpretations, Analytica chimica acta, **312**, 245-262 (1995).
30. K. Al-Sowdani, Spectrophotometric determination of tetracycline in some pharmaceutical preparations Journal of Basrah Researches (Sciences), **32**, 43-48 (2006).
31. H. Hadi, G. Fadhil, Sensitive Spectrophotometric Determination of Tetracycline Hydrochloride Indosage forms using Sodium Nitroprusside and Hydroxylamine Hydrochloride, Al-Nahrain Journal of Science, **17**, 53-58 (2014).
32. F.H. Awad, Colorimetric Determination of Paracetamol Using 9-Chloroacridine Reagent: Application to Pharmaceutical Formulations, Pakistan Journal of Scientific & Industrial Research Series A: Physical Sciences, **63**, 71-78 (2020).
33. H. Barber, J. Wilkinson, V. Edwards, The reaction of 5-alkoxyacridines with bases and their salts, Journal of the Society of Chemical Industry, **66** 411-415 (1947).
34. I.A. Shehab, T. Al-Sabha, Spectrophotometric determination of Aspirin and Pyridoxine Hydrochloride using 9-Chloroacridin reagent in aqueous solution, EDUSJ, **29**, 60-74 (2020).
35. R.M. Khaleel, D.H. Mohammed, Spectrophotometric Determination of tetracycline hydrochloride Using 2, 4–dinitrophenyl hydrazine as Coupling Reagent, J. Phys. Conf. Ser., 012084 (2020).