Utilization of Spectrophotometric Technique for the Quantitative Evaluation of Doxycycline in its Pure form and its Therapeutic Doses

Basim Faisal Aldhalemy[1], Mohauman Mohammed AL-Rufaie[2]
[1], [2]Faculty of Science, Kufa University, Kufa Street 1, Najaf, Iraq

Summary
A rapid and easy spectrophotometric system has been proposed to determine Doxycycline Hyclate (DOX) in pharmaceutical formulations. The oxidation strategy consists of putting Doxycycline with an excess quantity of N-Bromo succinimide (NBS). That happens in an acidic medium and then estimates the amount of unreacted NBS by methylene blue at λ580 nm. The quantity of NBS interacting with the colourant is equivalent to the amount of the drug with a better molar absorption of 2.44×10^3 L. mol^{-1} cm^{-1}. The bear law scale was 5-25 $\mu g.ml^{-1}$, and Sandell's sensitivity index of 0.210 $g.cm^{-2}$. And also, the average relative error of the concentration is -0.503 %, and the relative standard deviation is 0.337%. The method was successfully applied in its quantifying to the determination of drug in pharmaceutical formulations.
Keywords: Therapeutic Doses; Doxycycline; Spectrophotometric Technique

1. Introduction

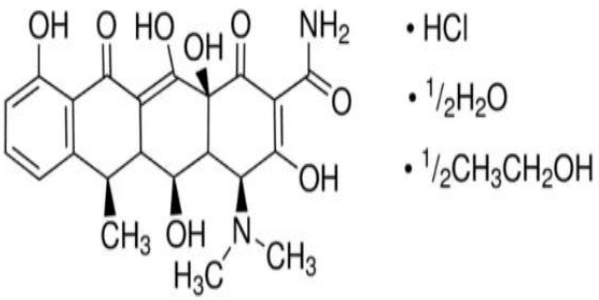

Figure1 : Chemical structure of Doxycycline Hyclate

Doxycycline Hyclate (DOX)] (C22H24N2O8. HCl. ½C2H5OH. ½H2O) Fig.1 a yellow, good water-soluble crystalline powder [1][2]. Its chemical composition is as follows: it belongs to the tetracycline group of drugs [3]. It inhibits matrix metalloproteinases (MMPs) [4], Which are found in various parasitic [5], viral,[6] and bacterial pathogens. DOX is used to treat many diseases, such as periodontitis, gum disease, degenerative rheumatic illnesses, degenerative vascular issues, diabetes mellitus, respiratory issues, diabetes mellitus, cardiovascular problems, fetopathy changes in the incubation period, alveolar bone misfortune and so on.[7] It treats diseases of animals such as poultry[8][9], birds [5], bees [10] and fish.[11]
For the determination of DOX, many Alternative techniques have been published in the literature. These alternative techniques include spectrophotometry [10]–[13], chromatography such as High-performance liquid chromatography[14]–[16] and liquid chromatography[17], Voltammetry[18], and Electrochemical Aptasensor.[19]
Under pharmaceutical applications, the technique of choice provides the benefits of simple and low-cost instruments available in all laboratories. The current research described a straightforward and sensitive spectrophotometric method for determining one phenolic drug. To determine DOX, DOX reacts with N-Bromo succinimide in an acidic medium. Then the amount of unreacted NBS is estimated by methylene blue to form a blue-coloured substance at λ 580 nm. The quantity of NBS interacting with the colourant is equivalent to the amount of the drug. An easy and quick analytical method is recommended for the simultaneous estimation of Doxycycline. This research aimed to create quality, reliable, sensitive and validated economic analytical methods for assessing Doxycycline in pure form and pharmaceutical formulations.
KEYWORDS: Doxycycline Hyclate, pharmaceutical formulations, Indirect Oxidation, N-Bromo succinimide, Methylene blue, spectrophotometric.
* E-mail: basimf.ali@uokufa.edu.iq ** Author to whom correspondence should be addressed. E-mail: muhaimin.alrufaie@uokufa.edu.iq

2. Material and Methods

Experiments Apparatus

The certain measurements were carried out using the 303 PD UV-Visible Spectrophotometer, Apel, Japan (Single beam) with 1cm quartz cells[1].

Reagents and solutions

Any chemical used has an analytical ranking. The State Company for Pharmaceutical Industries and Medical Equipment has acquired Doxycycline Hyclate (DOX).
Doxycycline solution, 250 µg mL^{-1} was prepared by dissolving 0.025 g of Doxycycline in a volumetric flask of 100 mL of deionized water.
Methylene blue dye solution, 1.6 x10-3 M was prepared by dissolving 0.051 g in a volumetric flask with 100 mL of deionized water.
N-Bromo succinimide solution, 1 x10-3 M was prepared by dissolving 0.0178 g of N-Bromo

doi.org/10.31838/hiv23.02.189 Received: 20.10.22, Revised: 29.11.22, Accepted: 13.12.22.

succinimide in a volumetric flask of 100 mL of deionized water.

Hydrochloric acid, Nitric acid, Sulfuric acid and Acetic acid solutions, 2M were prepared by diluting 16.66 mL, 17.89 mL, 10.88 mL and 11.49 mL of concentrated hydrochloric acid 30 %, Nitric acid 70%, Sulfuric acid 98% and glacial Acetic acid 99.6% in volumetric flasks into 100 mL of deionized water. A variety of formulations were studied, including metoclopramide as an effective ingredient. In Table 1, these are summarized[2].

Table 1: Studied pharmaceutical formulations for DOX.		
Pharmaceutical formulations	Declared composition	Company
DuraDox Tablets	Per 1 tablet 100 mg DOX	Julphar Co.
Doxydar Tablets	Per 1 tablet 100 mg DOX	Dar Aldawa Co.
Monodoks Tablets	Per 1 tablet 100 mg DOX	Deva Co.

Preparation solutions for tablets formulations

Furthermore, 0.025 g of the medication and diluted it with deionized water to 100 mL. A normal absolutely solutions were obtained by convenient dilution from studied solution as well as an advised procedure was exercised of the appreciation of DOC medicines.[20]

3. Results and Discussion

Analytical procedure

An increasing volume of Doxycycline solution (250 µg. mL^{-1}) was transmitted into a series of volumetric flasks (10 mL) to cover the range of the calibration graph (5 -25 µg. mL^{-1}). Then 0.5 mL of HNO_3 (2 M) and 0.5 mL of N-Bromo succinimide (0.001 M) were added and the solution was removed at temperature (10 °C) for 20 min. Eventually, after 10 min, 0.25 mL of methylene blue was applied. Then the flasks were diluted by deionized water to the level[3].

The absorbance was calculated at 580 nm compared to the blank reagent, prepared in the same way but including no medication. Doxycycline oxidation was used in the process by adding an excess of N-Bromo succinimide, then the unreacted N-Bromo succinimide was used to bleach the methylene blue colour. Then the absorption is estimated at 580 nm, which is linearly increased with increasing Doxycycline concentration.[21]

The factors affecting absorbance: To determine the optimal requirements for identifying Doxycycline in pharmaceutical preparations, the effect of different parameters on the production of colour has been tested. Effect of the volume of dye: in order to optimize the effective and optimal dye concentration (methylene blue) that can be calculated spectrophotometrically. The concentration of dye in the experimental tests is in Table 2.

Table 2: Dye volume influence on the product absorbance.		
No	Volume	Absorbance
1	0.1	0.287
2	0.2	0.412
3	0.3	0.604
4	0.4	0.722
5	0.5	0.890
6	0.6	0.932
7	0.7	0.931

The Effect of the volume of oxidant

The impact of various volumes from N-Bromo succinimide (0.1-0.7) mL of 0.001 M on the colour of Methylene blue dye was studied. It was researched without Doxycycline, table 3. It shows that 0.5 mL of N-Bromo succinimide solution was adequate to achieve optimum bleaching of the methylene blue dye pigment, so in subsequent experiments, it was suggested.

Table 3: Benefit of oxidant volume on absorbance of the substance.		
No	Volume	Absorbance
1	0.1	0.912
2	0.2	0.917
3	0.3	0.000
4	0.4	0.809
5	0.5	0.929
6	0.6	0.914
7	0.7	0.913

The acid Influence.

The influence of the acid on the oxidation of Doxycycline was tested. The study revealed that acidity approximately increases the absorption of the product. Also, acids such as HCl, CH3COOH, H2SO4 and HNO3 can improve the absorption of the colour produced by each discovered acid. HNO3 provided better absorption with the highest colour stability, so it was prescribed in the subsequent experiments with 0.5 mL (Table 4).

Table 4: Benefit of acid volume on absorbance of the product.		
No	Volume	Absorbance
1	0.1	0.639
2	0.2	0.645
3	0.3	0.775
4	0.4	0.806
5	0.5	0.940
6	0.6	0.938
7	0.7	0.935

Temperature effect

The influence of temperature on methylene blue's colour intensity has been recorded. When the formed colour was at 10 °C, a maximum absorbance was obtained, and the observed colour was less at lower or higher temperatures. Thus, for subsequent experiments, a temperature of 10 °C is recommended.

Sequence of addition

The sequence of addition of oxidant reagent (NBS) should be implemented as specified under the analytical procedure to achieve the best outcomes. Otherwise, colour intensity loss, as well as less stability, were observed (Table 5).

Table 5: Sequence of addition effect.		
No	Order of addition	Absorbance
1	Drug+Acid+NBS+Dye	0.893
2	Drug+NBS+Acid+Dye	0.907
3	NBS+Drug+Acid+Dye	0.901
4	Acid+NBS+Drug+Dye	0.922
5	Acid+Drug+NBS+Dye	0.933

The influence of time on the dye's oxidation and bleaching

The impact of time on Doxycycline oxidation and the time necessary for maximal methylene blue bleaching colour were investigated. Outcomes in **Table 6**. A sitting time of 10 min was required for the complete oxidation of Doxycycline, and 20 min for the bleaching of methylene blue dyes was necessary.

Table 6: The influence of time on the dye's oxidation and bleaching			
oxidation Time (min)	Absorbance	Dye bleaching Time(min)	Absorbance
0	0.860	0	0.953
5	0.880	5	0.948
10	0.954	10	0.944
15	0.945	15	0.941
20	0.915	20	0.934
25	0.915	25	0.934

Absorption spectra

When a DOX 0.5 mL diluted aqueous solution was mixed with 0.5 mL HNO_3 and 0.5 mL of N-Bromo succinimide, as well as 10 min after the continuation of the methyl blue with a volume of 0.6 mL was implemented and remained for 20 min, and severe blue is spontaneously generated. At 580 nm, the maximum absorption was seen in **Fig. 2**[22].

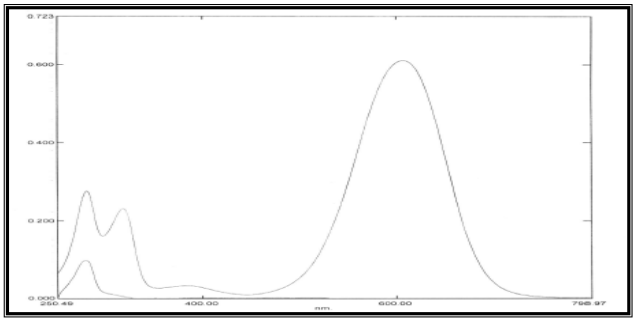

Figure2 : A) the maximum absorption for dye (methyl blue). B) the maximum absorption for drug.

Calibration graph

The linear relationship between absorption and Doxycycline concentration within the range 5-25 µg. mL^{-1}, with a solid determination coefficient value (R^2 = 0.9995), was obtained after the general procedure (Fig. 3).

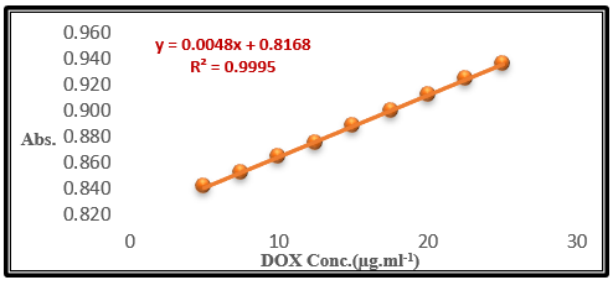

Figure 3:Calibration graph for the color product.

It tested the difference in the concentration effect on the absorption of the colour product. The sensitivity of the Sandell was observed as (0.212 g.cm 2). The molar absorptive was found to be 2.44 x 103 L. mol-1.cm-1 for the colour product. The equation LOD = 3 s/S and the equation LOQ = 10 s/S were verified by the limit of detection (LOD) and limit of quantitation (LOQ), where s is the standard deviation for the intercept; the slope of the intensity line was S (Table 7) [23].

Table 7: Analytical optical characteristics as reliability for the procedure tested.	
characteristics Values	characteristics Values
Molar absorptive (L. $mol^{-1}.cm^{-1}$)	2.44 $X10^3$
Regression equation (y = bx + a)	y = 0.0048x + 0.8168
Beer's Law limits (µg/mL) (x)	5-25
Sandell's sensitivity µg/cm^{-2}	0.214 g.cm^{-2}
Slop (b)	0.0048
Intercept (a)	0.8168
Correlation coefficients (R^2)	0.9995
(LOQ) quantification Limit (µg. mL^{-1})	1.892
(LOD) detection Limit (µg. mL^{-1})	6.305
Confidence limits with 0.01 level	±0.822
Confidence limits with 0.05 level	±0.628

Precision and accuracy

The precision as well as accuracy of the methodology was evaluated by five replicated tests of the pure drug solution at three separate Doxycycline concentrations, Outcomes in Table 8, to achieve professional precision and accuracy.[24]

Table 8: Precision and accuracy of the methodology evaluated.				
DOX conc. Present (µg. mL-1)	DOX conc. Found (µg. mL-1)	Erel%	Re%	R.S.D%
5	4.992	0.164	99.836	0.229
12.5	12.614	-0.910	100.910	0.361
25	25.191	-0.765	100.765	0.420
Mean		-0.506	100.506	0.337

Solvent Influence

Both polar and non-polar solvents were used in **Table 9** to select elegant solvents for Drug identification. Water was found to be a suitable solvent for DOX, providing optimal absorption with a defined drug concentration, whereas other solvents provided decreased absorption due to improper complex dissociation[23].

Table 9: Selected solvent influence on the absorbance.		
Selected Solvent	Absorbance	ε, $L.mol^{-1}.cm^{-1}$
Water	0.942	2.44 X103
Ethanol	1.068	0.604 X103
Methanol	1.224	1.559 X 103
DMSO	0.836	1.395 X 103
DMF	Two layers	
Chloroform	Two layers	
Toluene	Two layers	
DSM	Two layers	
Ethyl acetate	Two layers	
Butyl alcohol	Drip	

Interference influence

The influence of certain excipients explicitly labeled in pharmaceutical preparations was evaluated by Doxycycline determination in the presence of various excipients (Benzoic acid, Sucrose, Sodium disulfate, Magnesium stearate, Starch, Lactose, Calcium chloride, Fructose). Investigational findings revealed that there was no interference with the experimental technique by excipients, (Table 10).[25]

Table 10: Investigation of 25 $µg.mL^{-1}$ DOX in the presence of excipients.		
Interference	$E_{rel\%}$	Recovery%
Benzoic acid	0.924	99.076
Calcium chloride	1.458	98.542
Fructose	-1.214	101.214
Lactose	1.565	98.435
Magnesium stearate	0.496	99.504
Sodium bisulfate	0.283	99.717
Sodium sulfate	-0.893	100.893
Starch	0.283	99.717
Sucrose	-1.534	101.534

Doxycycline determination in medication formulations

In the evaluation of Doxycycline in numerous pharmaceutical formulations, the validation of the proposed methodology for quantitative detection of Doxycycline has been checked, in the **Table 11**, the obtained results were presented. F-test as well as student t-test population variance with those attained by British pharmacopeia at a confidence scale of 95 percent with three degrees of freedom, as seen in **Table 11**. The outcome suggested that the F-test as well as t-test were lower than the unrealistic account (F = 9.28, t = 2.45). The values (F = 7.927, t = 0.048) for the current technique. The estimates for the current technique (F = 7.927, t = 0.048) and also the values for the Typical Methodology showed that there was no substantial difference between the system developed as well as the typical methodology.[26][27]

Table 11: Evaluation of (DOX) in prescription medicines has been done in compliance with the official framework. Average of 3 determinations.					
Medicinal Products of (DOX)	Modified methodology		Typical methodology		Standard Quantities (t), (F)
	Recovery %	RSD %	Recovery %	RSD %	
Pure DOX	100.50	0.337	104.00	10.539	0.048
Dura Dox Tablets	100.67	1.009	105.88	9.428	(t)Value=2.45
Doxydar Tablets	99.35	0.852	104.70	15.385	7.927
Monodoks Tablets	101.56	1.555	101.41	6.804	(F)Value = 9.28

4. Conclusion

The proposed methodology identified the effective implementation of an easy, resilient as well as precise spectrophotometric method to determine Doxycycline employing N-Bromo succinimide as Doxycycline oxidant factor and also the unreacted N-Bromo succinimide bleached the methylene blue dye. In different pharmaceutical formulations, the process has been successfully extended to the evaluation of Doxycycline drug.

5. Acknowledgements

The author gratefully acknowledges the financial assistance of Kufa University's Supplementary Educational Fund, Faculty of Science, Department of Chemistry, Iraq.

References

JaKa MM, Dinh JM, McCann P, Katz AS, Kottke TE, Ziegenfuss JY. Promoting Fish Consumption Messages: Perspectives of Hmong Women of Childbearing Age. American Journal of Health Behavior. 2021;45(5):867-78. https://doi.org/10.5993/AJHB.45.5.7

Chen S. Effects of Coaches' Personality, Behavior, Approach and Role on Players Performance with the Mediating Role of Coaches' Training Method. Revista de Psicología del Deporte (Journal of Sport Psychology). 2021;30(1):285-95. Available from: https://www.rpd-online.com/index.php/rpd/article/view/409/274

Abeche CV, Khafif TC, Belizario GO, Silva TFd, Harkaly G, Gomide PIC, Serafim AdP. Personality traits and parenting styles in boys victims of sexual abuse: a pilot study. Archives of Clinical Psychiatry (São Paulo). 2021;48:105-10. https://doi.org/10.15761/0101-60830000000288

R. Alshaman et al., "Formulation and Characterization of Doxycycline-Loaded Polymeric Nanoparticles for Testing Antitumor/Antiangiogenic Action in Experimental Colon Cancer in Mice," Nanomaterials, vol. 12, no. 5, p. 857, Mar. 2022, doi: 10.3390/nano12050857.

K. C. Nicolaou and S. Rigol, "A brief history of antibiotics and select advances in their synthesis," J. Antibiot. (Tokyo)., vol. 71, no. 2, pp. 153–184, Feb. 2018, doi: 10.1038/ja.2017.62.

A. Dzimitrowicz et al., "Application of pulse-modulated radio-frequency atmospheric pressure glow discharge for degradation of doxycycline from a flowing liquid solution," Sci. Rep., vol. 12, no. 1, p. 7354, May 2022, doi: 10.1038/s41598-022-11088-w.

Y. N. Gholse, D. R. Chaple, and R. H. Kasliwal, "Development and Validation of Novel Analytical Simultaneous Estimation Based UV Spectrophotometric Method for Doxycycline and Levofloxacin Determination," Biointerface Res. Appl. Chem., vol. 12, no. 4, pp. 5458–5478, Oct. 2021, doi: 10.33263/BRIAC124.54585478.

T. Petkova, A. Yordanova, and A. Milanova, "Population Pharmacokinetics of Doxycycline, Administered Alone or with N-Acetylcysteine, in Chickens with Experimental Mycoplasma gallisepticum Infection," Pharmaceutics, vol. 14, no. 11, p. 2440, Nov. 2022, doi: 10.3390/pharmaceutics14112440.

R. Alexpandi et al., "Repurposing of Doxycycline to Hinder the Viral Replication of SARS-CoV-2: From in silico to in vitro Validation," Front. Microbiol., vol. 13, no. May, pp. 1–11, 2022, doi: 10.3389/fmicb.2022.757418.

S. Ghareghani et al., "Development and validation of a multi-residue method for determination of antimicrobial sulfonamides, macrolides and quinolones in Iranian feta cheese by SPE clean-up and LC-MS/MS," Int. Dairy J., vol. 137, p. 105506, Feb. 2023, doi: 10.1016/j.idairyj.2022.105506.

N. Mohammed, D. Adare Mengistu, A. Abdurehman, D. Belina, and S. Mengistu, "Determination of Tetracycline Residues in Kidney and Muscle of Beef Cattle Slaughtered in Dire Dawa and Harar Municipal Abattoirs, Eastern Ethiopia," Environ. Health Insights, vol. 16, 2022, doi: 10.1177/11786302221109720.

M. Zhao et al., "Simultaneous Determination of Four Tetracycline Antibiotics Residues in Chicken by Solid Phase Extraction Coupled with High Performance Liquid Chromatography," J. Food Res., vol. 11, no. 4, pp. 1–23, 2022, [Online]. Available: https://ideas.repec.org/a/ibn/jfrjnl/v11y2022i4p23.html

M. F. Lanjwani, N. Altunay, and M. Tuzen, "Preparation of fatty acid-based ternary deep eutectic solvents: Application for determination of tetracycline residue in water, honey and milk samples by using vortex-assisted microextraction," Food Chem., vol. 400, p. 134085, Jan. 2023, doi: 10.1016/j.foodchem.2022.134085.

[11] N. Xu et al., "The Pharmacokinetics of Doxycycline in Channel Catfish (Ictalurus punctatus) Following Intravenous and Oral Administrations," Front. Vet. Sci., vol. 7, Nov. 2020, doi: 10.3389/fvets.2020.577234.

[12] R. T. Kshirsagar, A. M. Tamboli, H. I. Shaikhmulani, H. T. Suryawanshi, and O. V. Khandare, "Simultaneous equation method for the estimation of ambroxol hydrochloride and doxycycline hydrochloride by UV spectrophotometry," Int. J. Mod. Pharm. Res., vol. 6, no. 7, pp. 48–53, 2022.

[13] C. Wang, Y. Huang, R. Zhou, L. Xie, and Y. Ying, "Rapid analysis of a doxycycline hydrochloride solution by metallic mesh device-based reflection terahertz spectroscopy," Opt. Express, vol. 28, no. 8, p. 12001, 2020, doi: 10.1364/oe.389517.

[14] R. Mashru and K. Nirupa, "Development and validation of UV-Spectrophotometric and RP-HPLC method for simultaneous estimation of Metformin and Doxycycline in bulk and synthetic mixture," J. Drug Deliv. Ther., vol. 11, no. 4, pp. 26–35, 2021, doi: 10.22270/jddt.v11i4-S.4964.

[15] A. Saghafi, R. Sanavi Khoshnood, D. Sanavi

Khoshnoud, and Z. Eshaghi, "Preconcentration and determination of four antibiotics in biological samples using nanofluid-assisted magnetic dispersive micro-solid-phase extraction coupled with high-performance liquid chromatography," Chem. Pap., vol. 76, no. 2, pp. 901–911, Feb. 2022, doi: 10.1007/s11696-021-01903-5.

[16] R. L. Gil, C. M. P. G. Amorim, M. da C. B. S. M. Montenegro, and A. N. Araújo, "Cucurbit[8]uril-Based Potentiometric Sensor Coupled to HPLC for Determination of Tetracycline Residues in Milk Samples," Chemosensors, vol. 10, no. 3, p. 98, Mar. 2022, doi: 10.3390/chemosensors10030098.

[17] S. Eugenia and G.-C. Fabio, "Determination of streptomycin and doxycycline using LC/MS towards an effective treatment against an experimental Brucella abortus infection in mice," J. Microbiol. Methods, vol. 194, 2022, doi: 10.1016/j.mimet.2022.106436.

[18] H. Sun, J. Wei, H. Wang, S. Wang, J. Jin, and Z. Zhang, "Electrochemical Behavior and Voltammetric Determination of Chloramphenicol and Doxycycline Using a Glassy Carbon Electrode Modified with Single-walled Carbon Nanohorns," Electroanal. Based Carbon Nanomater., vol. 34, no. 4, pp. 735–742, 2022, doi: 10.1002/elan.202100354.

[19] F. Guan et al., "Novel Electrochemical Aptasensor Based on Floral Zinc Oxide/Reduced Graphene Oxide/Colloidal Gold Composite for the Detection of Tetracycline, Doxycycline and Oxytetracycline in Milk," J. Electrochem. Soc., Oct. 2022, doi: 10.1149/1945-7111/ac9d6d.

[20] The British Pharmacopoeia. London: Her Majesty's Stationary office, 2009. pp.2134-2138,8722.

[21] US Pharmacopoeia. uS Pharmacopoeial Convention, 2007.pp.3244-3246.

[22] M. M. Al-Rufaie, A. A. Jawad, and H. M. Sadiq, "Colorimetric estimation for salbutamol sulphate in pure form and in different types of pharmaceutical," Int. J. Chem. Tech. Res., vol. 9, no. 11, pp. 432–442, 2016.

[23] M. M. Al-Rufaie, "Modern kinetic spectrophotometric procedure for estimation of furosemide drug as bulk form and in pharmaceuticals preparations," Curr. Issues Pharm. Med. Sci., vol. 29, no. 4, p. 184, 2016.

[24] M. M. Al-Rufaie, A. N. Al-Sharefy, and K. H. Kathem, "New spectrophotometric method for the determination chlorpromazine hydrochloride in pharmaceutical preparations by using oxidative coupling reaction," Int. J. Univ. Pharm. Biosci., vol. 2, no. 4, p. 19, 2013.

[25] K. A. T. Hanaa, M. M. Al-Rufaie, and Y. M. Zahraa, "Spectrophotometric determination of metoclopramide medicine in bulk form and in pharmaceuticals using orcinol as reagent.," Ovidius Univ. Ann. Chem., vol. 29, no. 2, pp. 85–89, 2018.

[26] K. Unal, I. Palabiyik, E. Karakan, and F. Onur, "Spectrophotometric determination of amoxicillin in pharmaceutical formulation.," Turk. J. Pharm. Sci., vol. 5, no. 1, pp. 1–6, 2008.

[27] M. M. Al-Rufaie, "Evolution and effectiveness of colorimetric approach for investigation of ceftriaxone medicine as a pure form and in formulation vials," J. Islam. Pharm, vol. 3, no. 2, pp. 16–24, 2018.