MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 43 (3): 30-36 (September 2024) https://mjs.um.edu.my

DEVELOPMENT OF A SPECTROPHOTOMETRIC DETERMINATION OF PREDNISOLONE IN DIFFERENT DOSAGE FORMS

Hasan M. Luaibi[1a], Khalid Waleed S. Al-Janabi[2b], Ali Khalil Mahmood[3b], Takleef Dheyab Sallal[4c], and Tayser Sumer Gaaz[5d,e]*

Abstract: A sensitive, accurate, and affordable colorimetric method was developed for assaying prednisolone (PRZ) in various medicinal forms. The procedure involves the oxidation of PRZ by ferric ions, followed by complexation of the resulting ferrous ions with ferricyanide to produce a greenish-blue product. Common complexation conditions were thoroughly investigated. The mole ratio of $FeCl_3 \cdot 6H_2O$ to $K_3Fe(CN)_6$ was 8:1. The proposed mechanism of complexation was suggested and considered. Various parameters were optimized, including the reduction of the colorimetric reaction temperature to 50°C and the duration of heating and analysis to 20-30 minutes. The calibration curve was linear over the range of 1-60 µg/mL. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.5 µg/mL and 1 µg/mL, respectively. Spiking actual samples with standard PRZ showed recoveries within the 97.3-100.1% range. The method exhibited high precision, with an RSD% of less than 1.5%. Additionally, the study confirmed that common pharmaceutical excipients did not interfere. Real medicinal samples, including tablets, syrup, eye drops, and creams, were successfully examined for direct analysis of PRZ using the developed methodology, demonstrating its suitability for routine analysis of various PRZ-containing drug formulations.

Keywords: *colorimetric analysis, UV-Vis, derivatization, pharmaceuticals, prednisolone, steroid determination.*

1. Introduction

Prednisolone Prednisolone (PRZ), a synthetic steroid, inhibits the release of inflammatory chemicals in the body. It is five times more potent than cortisone acetate, with less sodium and fluid retention, which mitigates stomach issues. PRZ and prednisone are corticosteroids frequently used to treat inflammatory and immunological diseases. PRZ is humans' active form of prednisone (Xu et al., 2007). It is commonly used to treat inflammation, ulcerative colitis, gland disorders (endocrine), lupus, allergic disorders, psoriasis, and other conditions affecting the eyes, skin, nervous system, lungs, blood cells, or stomach (Albayati & Jassam, 2019). However, it should be avoided in patients with fungal infections and in those taking certain drugs, including vitamins and herbal products. Additionally, PRZ reduces the immune system's response to various syndromes, such as swelling, discomfort, and allergy symptoms (Schuster et al., 2005).

Synthetic corticosteroids are incorporated in many pharmaceutical preparations, leading to numerous studies on their assay within different pharmaceutical mixtures. Balaji et al. (2008) developed a voltammetric approach using a β-cyclodextrin-modified carbon paste electrode to test PRZ along with other corticosteroids (Balaji et al., 2008). Colorimetric absorption spectroscopy is generally used for regular analytical quantifications, provided the active material's allowable percentage in the remedy is not less than ±10% (Evtifeeva et al., 2016).

Traditional chromatographic methods (Raut et al., 2014; Razzaq et al., 2017), HILIC (Hydrophilic Interaction Liquid Chromatography) (Ali & Rasheed, 2020), and spectrophotometric methods (Kashyap et al., 2012) have been employed for PRZ determination. El Gammal et al. evaluated PRZ in various pharmaceutical mixtures using micellar liquid chromatography (El Gammal et al., 2018), while Lemus Gallego et al. used micellar electrokinetic chromatography (Lemus Gallego & Pérez Arroyo, 2003). Gas chromatography and a mass spectrometer have also been used to quantify PRZ (Iannella et al., 2019). Primpray et al. separated and quantified steroids, including PRZ, in adulterated herbal medications using electrochemical analysis (Primpray et al., 2019).

Another study employed the quality-by-design tactic (QbD) to develop a spectroscopic technique for PRZ assay by varying seventeen parameters (Bhusnure et al., 2015).

The spectrophotometric quantification method for PRZ was validated using identical model blends analyzed by one analyst on various spectrometers in two different laboratories. Evtifeeva et al. quantified PRZ spectroscopically directly at 243.5 nm without derivatization (Evtifeeva et al., 2016). Additionally, PRZ was

Authors information:
[a]Department of Renewable Energy, College of Energy and Environmental Sciences, Al-Karkh University of Science, Baghdad, IRAQ. E-mail: hasan.luaibi@gmail.com[1]
[b]Department of Chemistry, College of Education for Pure Sciences/ Ibn Al-Haitham, University of Baghdad, Baghdad, IRAQ. E-mail: Khalid.janabi@gmail.com[2]; ali.khalil.mahmood@gmail.com[3]
[c]Ministry of Education, Baghdad, IRAQ. E-mail: sallaltakleef535@gmail.com[4]
[d]Technical College Al-Musaib, Al-Furat Al-Awsat Technical University, Babil, IRAQ. E-mail: taysersumer@atu.edu.iq[5]
[e]Prosthetics and Orthotics Engineering Department, College of Engineering and Technologies, Al-Mustaqbal University, Babylon, IRAQ. E-mail: tayser.sumer.gaaz@uomus.edu.iq[5]
*Corresponding Author: tayser.sumer.gaaz@uomus.edu.iq

Received: June 12, 2023
Accepted: October 26, 2023
Published: September 30, 2024

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)

determined by dropping the mercury electrode (DME) on the differential pulse voltammeter (Smajdor et al., 2016).

Derivative spectrometry is a significant analytical practice that avoids overlapping spectra in chemical mixtures (Abdul Kareem & Al-Janabi, 2018; Al-Janabi, 2013; Al-Janabi et al., 2020c, 2020a, 2020b; Hammod, Al-Janabi, et al., 2020; Hannoun & Al-Janabi, 2020; Radhi & Al-Janabi, 2020). A study reported a colorimetric procedure for assaying dexamethasone after fluoranil derivatization (Al-Janabi et al., 2020b).

The current work aimed to demonstrate a simple, sensitive, and economically viable colorimetric method for quantifying PRZ within pharmaceutical formulations without preliminary separation steps. The method is based on the oxidation of PRZ in an acidic medium with ferric ions, followed by the complexation of ferrous ions with ferricyanide. This approach offers stability and high sensitivity, and common excipients present in pharmaceutical preparations did not interfere with the PRZ assay.

2. Materials and Method

Apparatus

Spectrophotometric analyses were performed using a double beam UV-Visible Spectrometer (T80+ PG Instruments Ltd., UK), with a path length of 1 cm, and quartz cuvettes.

Material

All reagents and materials were of analytical grade. Prednisolone powder, with a purity of 99.8%, was obtained from the State Company for Drug Industry and Medical Appliances (SDI), Samarra, Iraq.

Preparing the Stock and Working Solutions:

Standard working solutions of PRZ were prepared in methanol at concentrations of 1, 2, 5, 10, 20, 30, 40, 50, and 60 µg/mL. Standard solutions of potassium hexacyanoferrate (III), $K_3Fe(CN)_6$, referred to hereinafter as potassium ferricyanide, and ferric chloride $FeCl_3{\cdot}6H_2O$, were prepared in distilled water at a concentration of 0.5% (w/v) individually.

An aliquot of 10 mL of each PRZ standard was mixed with 2 mL of 4N sulfuric acid, followed by 2 mL of ferric chloride (0.5% w/v), and 0.5 mL of ferrate solution (0.5% w/v). The mixture was warmed to 50°C in a water bath for 25±5 minutes, with intermittent shaking, before being diluted with distilled water. The resulting colored products were stable for over an hour and examined at 778 nm.

Preparation of Sample Solutions from Pharmaceutical Dosage Forms

Pharmaceutical samples were prepared for colorimetric PRZ quantification, comparing the medicine's designated dosage to the analyzed results. Sample preparation varied based on the physical state of the medicine, such as tablets, creams, or drops, and was then subjected to colorimetric analysis as outlined in the suggested procedure.

An exact amount of powdered tablets, equivalent to the pure form of PRZ, was dissolved in 10 mL of methanol (Dikran et al., 2017). The mixture was filtered and rinsed with 10 mL of alcohol. The filtrate and washing were diluted with distilled water in a 100 mL volumetric flask to the required concentration. For creams, 5 g of cream was weighed and gently heated, swirling in 50 mL of methanol. Eye drops were diluted directly with methanol. The original concentration of PRZ in the eye drops was simulated by diluting various aliquots of pre-prepared solutions with methanol (Mahmood, 2017). Recovery was intensively studied by spiking the drug (tablet and syrup) with different amounts of standard PRZ solutions over the range of 1-20 µg/mL.

Optimum settings were obtained by adjusting one factor at a time while keeping the others unchanged. The influence of ferric chloride and ferricyanide concentrations was studied as a function of absorbance, maintaining the PRZ amount constant. Maximum absorbance was achieved with a concentration of 4.0×10^{-2} M for $FeCl_3{\cdot}6H_2O$ and 5.0×10^{-3} M for $K_3Fe(CN)_6$, which were therefore adopted as optimal.

3. Results and Discussion

PRZ was directly assayed in the UV region of the spectrum at a peak maximum of 236.4 nm (Figure 1). However, this absorbance presents a significant drawback, as it interferes with many other active medicinal compounds that absorb in the same spectral range. To mitigate this issue, PRZ is derivatized to produce a colored product that absorbs in the visible region, a technique known as a bathochromic shift, or redshift (Skoog et al., 2017). This approach overcomes interference from other compounds. The common reversible redox system of ferricyanide/ferrocyanide (Mortimer, 2017) was utilized as the basis for detecting PRZ spectrophotometrically, demonstrating no interference with common excipients.

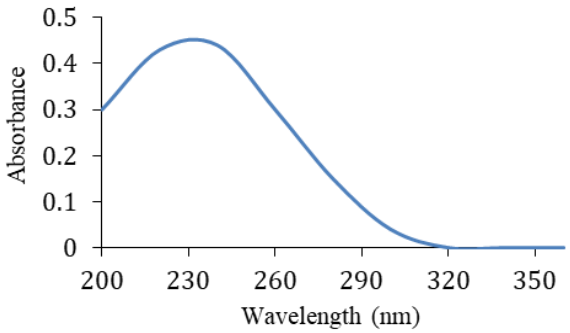

Figure 1. The UV absorption spectrum of the PRZ solution with a maximum peak of 236.4 nm.

The proposed method demonstrates no interaction between the active compound of PRZ and common excipients or other additives. The method's efficacy for quantifying pharmacological formulations was evaluated, with assay outcomes reflecting accuracy and precision. The investigated PRZ is oxidized by ferric chloride ($FeCl_3{\cdot}6H_2O$) in an acidic medium, producing ferrous (II) ions (Singh & Verma, 2008) (Figure 2).

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)

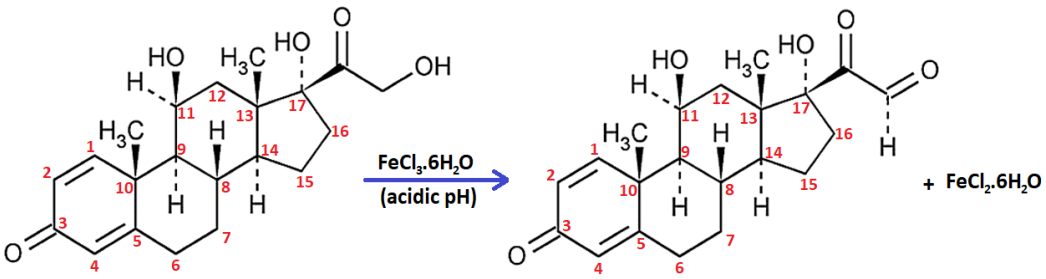

Figure 2. The oxidation of PRZ by ferric chloride to produce the PRZ dione derivative and ferrous chloride in an acidic medium

The complexation conditions for the proposed procedure of Singh & Verma (2008) were thoroughly studied and optimized. The mole ratio of $FeCl_3 \cdot 6H_2O$ to $K_3Fe(CN)_6$ was determined to be 8:1. The mechanism of the complexation reaction was considered and suggested. Improvements were observed in various parameters, such as lowering the colorimetric reaction temperature to 50 °C and reducing the time between heating and analysis to 20-30 minutes. A linear calibration curve was constructed over the 1–60 µg/mL range.

The LOD and LOQ were determined to be 0.5 µg/mL and 1 µg/mL, respectively. Real medicinal samples of tablets, syrup, eye drops, and creams containing PRZ were spiked and analyzed with standard PRZ, yielding recoveries between 97.3% and 100.1%.

In the acidic medium, ferric ion acts as an oxidizing agent for PRZ. The reduction of ferric ion to ferrous ion by PRZ involves the oxidation of the 2-hydroxyacetyl side chain on C17 of PRZ into two adjacent carbonyl groups, forming a dione. FTIR analysis confirms the formation of the oxidized PRZ dione derivative through the appearance of a sharp C-H stretching peak at 2725 cm^{-1} and a C=O stretching peak at 1711 cm^{-1}, as shown in Figure 3.

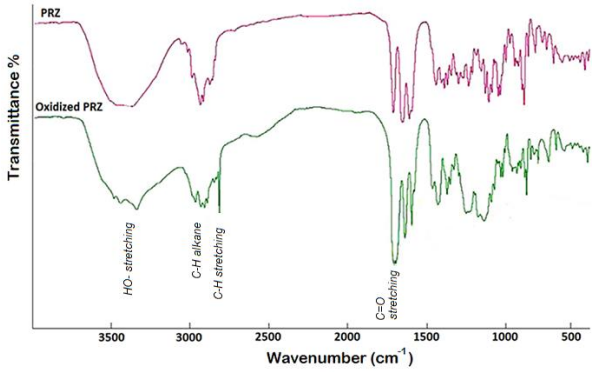

Figure 3. An overlaid FTIR spectra of PRZ and PRZ oxidized dione derivative confirming the formation of PRZ dione derivative by the appearance of a sharp peak of C-H stretching at 2725 cm^{-1} and C=O stretching peak at 1711 cm^{-1}

The ferrous (II) ion resulting from the PRZ oxidation reacts with potassium ferricyanide to produce a bluish-green complex of potassium ferrocyanide (Lillie & Donaldson, 1974) (Figure 4), which has an absorbance maximum at 778 nm, as shown in Figure 5.

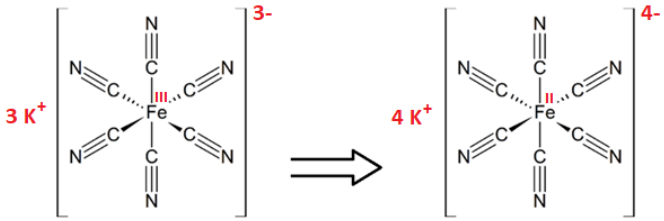

Figure 4. Reduction of Ferricyanide to Ferrocyanide (Mortimer, 2017).

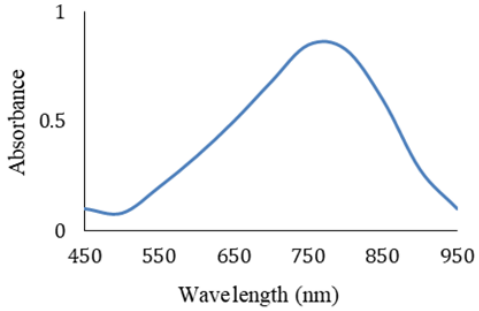

Figure 5. UV/Vis spectrum for the resulting colored complex with maximum absorbance at 778 nm.

The optimal amount of $FeCl_3 \cdot 6H_2O$ relative to $K_3Fe(CN)_6$ was determined by adding different concentrations of $FeCl_3 \cdot 6H_2O$ to a fixed amount (5.0×10^{-3} M) of $K_3Fe(CN)_6$. Spectrophotometric analyses were performed against a blank containing the same ratio of reagents, with the same amount of $FeCl_3 \cdot 6H_2O$ added to both the sample and the blank each time. The optimal ratio was found to be 8:1 (Figure 6).

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)

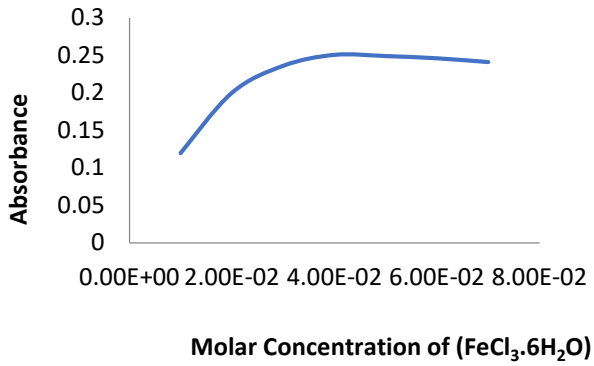

Figure 6. Effect of adding different concentrations of $FeCl_3.6H_2O$ to 5.0×10^{-3} M of $K_3Fe(CN)_6$

The color reaction between 4.0×10^{-2} M $FeCl_3 \cdot 6H_2O$ and 5.0×10^{-3} M $K_3Fe(CN)_6$ occurred at room temperature; however, color development was faster at elevated temperatures. The absorbance maximum was detected within 20 to 30 minutes of heating at 50 °C, after which the color began to gradually fade, as shown in Figure 7. The anticipated cause of the color fading is the thermal decomposition of the colored complex.

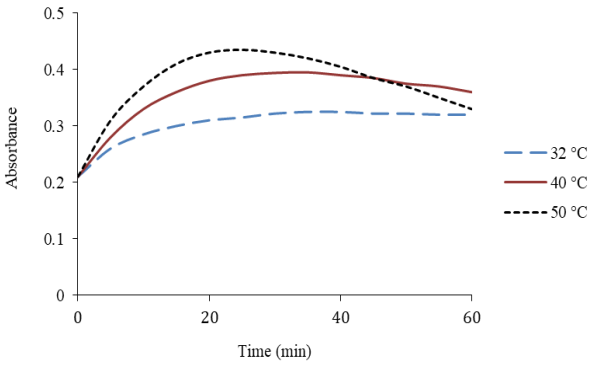

Figure 7. The influence of reaction temperature on absorbance as a function for time and color development.

PRZ aliquots (20 µg/mL) were analyzed using the proposed method with ten replicates. The mean absorbance value was 0.2506 AU, with a relative standard deviation (RSD%) of 0.75%. After optimizing the spectrophotometric analysis conditions, a calibration curve was established, demonstrating linearity in accordance with Beer's law over the range of 1 to 60 µg/mL. The optimal temperature for the colorimetric reaction was found to be 50 °C, with the measurement optimal within 20-30 minutes after the onset of the colorimetric reaction at λ_max of 778 nm. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.5 and 1 µg/mL, respectively, with a correlation coefficient of 0.9989 (Figure 8). Additionally, statistical data were calculated for three PRZ concentrations (5, 10, 40 µg/mL) with five replicates each. The RSD was less than 1.5%, and the relative errors ranged from -0.520% to +1.002% (Table 1).

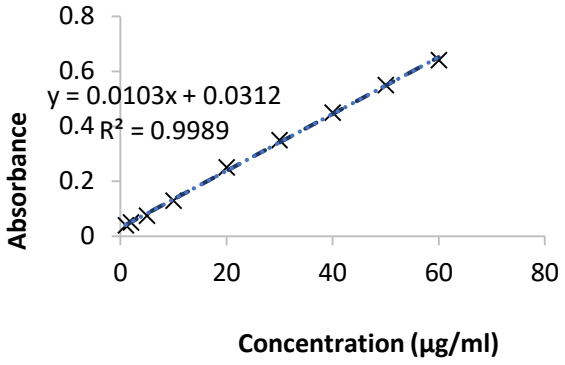

Figure 8. PRZ calibration curve under optimal conditions, linearity obtained for the range (1 to 60 µg/mL).

Table 1. Some statistical parameters for the proposed method

Concentration (µg/mL)		Rel. Error %	RSD%
Taken	Found*		
5	5.0501	+1.002	1.4022
10	9.9480	-0.520	0.9601
40	39.778	+0.555	0.8125

* Average of five measurements.

Assaying Different Forms of PRZ Pharmaceuticals:

The stability of the colored product was found to be over 1 hour when left at room temperature. The method was applied to quantify PRZ in tablets, including its pure form and in proprietary drugs from various pharmaceutical manufacturers. The readings, taken with n=5 replicates, were reproducible. The analysis results, as shown in Table 2, indicate no significant differences compared to the dosages prescribed by the manufacturers.

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)

Table 2. Quantification of PRZ tablets from various manufacturers using the proposed approach.

Brand name	Generic name	Dosage (mg)	Found* (mg)	Error (mg)	RSD%
SDI, Iraq	Prisolone 5	5	4.87	-0.13	1.023
Accord, UK	Prednisolone 5	5	5.03	+0.03	0.902
Wockhardt, UK	Prednisolone 5 mg	5	4.98	-0.02	0.888
Care Formulation Lab, India	PREDTORE (eye drop) 1%	5	4.89	-0.11	1.303
GPO, Thailand	Prednisolone (Cream) 0.5%	10	9.84	-0.16	1.390

* Mean value of n = 5.

Using widely available chemicals, such as ferric chloride as the color agent and sulfuric acid as the acidity modifier, renders this method highly cost-effective. The overall duration of the proposed procedure is less than one hour, while still maintaining high sensitivity and accuracy.

The method was validated for precision, linearity, recovery, and accuracy. By analyzing a series of standard PRZ solutions within the specified range, the linearity of the proposed method for assaying PRZ was evaluated. Quantification was performed according to the experimentally validated parameters.

Separate analyses at various concentrations of PRZ were conducted to assess the repeatability of the readings and the precision of the method. The resulting readings exhibited a relative standard deviation (RSD%) of less than 1.5%, indicating excellent precision.

The method's selectivity, including the matrix effect, was also investigated. Solutions of PRZ in two different pharmaceutical matrices (tablets and syrup) were spiked with varying amounts of pure PRZ. The resulting measurements demonstrated the method's high selectivity, as there was no interference from excipients in the assay of PRZ using the recommended procedure. The recoveries obtained (n=3) ranged from 97.3% to 100.1%, reflecting satisfactory precision of the method, as shown in Table 3.

Table 3. Various amounts of pure PRZ were spiked into real pharmaceutical samples of PRZ (n = 3).

PRZ dosage forms	Initial concentration (µg/mL)	After Spiking (µg/mL)	Found (µg/mL)	Relative error (%)	Recovery (%)
Tablet	10	11	10.96	0.36	99.6%
Tablet	10	13	12.98	0.15	99.8%
Tablet	10	15	14.82	1.20	98.8%
Tablet	10	20	19.95	0.25	99.8%
Tablet	10	30	30.03	0.10	100.1%
Syrup	10	11	10.70	2.73	97.3%
Syrup	10	13	12.80	1.54	98.5%
Syrup	10	15	14.73	1.80	98.2%
Syrup	10	20	19.70	1.50	98.5%
Syrup	10	30	29.95	0.17	99.8%

4. Conclusion

The proposed colorimetric method has been validated for the assay of PRZ in both its pure form and commercial pharmaceutical preparations. The method demonstrated accuracy, reproducibility, and cost-effectiveness for PRZ quantification. The results obtained were satisfactory. Additionally, the method does not require a separation step, as there is no interference from common excipients. Therefore, the developed technique is suitable for routine analysis of prednisolone.

5. References

Abdul Kareem, R. Q., & Al-Janabi, K. W. S. (2018). Determination of the Degree of Consumption (DoC) of Lube Engine Oils Using Fluorescence Spectroscopy. *Ibn AL- Haitham Journal For Pure and Applied Science*, *31*(1), 124. https://doi.org/10.30526/31.1.1860.

Albayati, T. M., & Jassam, A. A. A. (2019). Synthesis and characterization of mesoporous materials as a carrier and release of prednisolone in drug delivery system. *Journal of Drug Delivery*

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)

Science and Technology, *53*, 101176. https://doi.org/10.1016/j.jddst.2019.101176.

Ali, A. S., & Rasheed, A. S. (2020). Application of hydrophilic interaction chromatography methods for prednisolone acetate determination in their pure and tablet, syrup dosage forms. *Plant Archives*, *20*(1), 2807–2812. http://plantarchives.org/20-1/2807-2812%20(6042).pdf.

Al-Janabi, K. W. S. (2013). Quantification of Acrylamide content in Potato Chips and Iraqi "Harissa." *Ibn Al-Haitham Journal for Pure and Applied Sciences*, *26*(3), 236–244.

Al-Janabi, K. W. S., Mahmood, A. K., & Luaibi, H. M. (2020a). Determination of the Dissociation Constants of Metformin from a Second Derivative UV Spectrum. *International Journal of Research in Pharmaceutical Sciences*, *11*(1), 790–796. https://pharmascope.org/index.php/ijrps/article/view/1896.

Al-Janabi, K. W. S., Mahmood, A. K., & Luaibi, H. M. (2020b). Development of a simple colorimetric determination of dexamethasone. *International Journal of Drug Delivery Technology*, *10*(2), 255–258. https://doi.org/10.25258/ijddt.10.2.12.

Al-Janabi, K. W. S., Mahmood, A. K., & Luaibi, H. M. (2020c). Quantitative Analysis of Some Aromatic Amino Acids By Spectrophotometric Mathematical Derivatization. *Biochemical and Cellular Archives*, *20*(2), 6435–6439.

Balaji, K., Reddy, G. V. R., Reddy, T. M., & Reddy, S. J. (2008). Determination of prednisolone, dexamethasone and hydrocortisone in pharmaceutical formulations and biological fluid samples by voltammetric techniques using b-cyclodextrin modified carbon paste electrode. *African Journal of Pharmacy and Pharmacology*, *2*(8), 157–166. https://doi.org/https://doi.org/10.5897/AJPP.9000096.

Bhusnure, O. G., Manoj, B., Todkar, V., & Giram, P. S. (2015). Analytical Method Development and Validation of Prednisolone Sodium Phosphate by QbD Approach. *IOSR Journal of Pharmacy and Biological Sciences Ver. III*, *10*(6), 2319–7676. https://doi.org/10.9790/3008-10636475.

Dikran, S. B., Mohammed, A. K., & Mahmood, A. K. (2017). Uni and Multivariate Optimization for the Spectrophotometric Determination of Cimetidine Drug via Charge-Transfer Complex Formation. *Ibn AL-Haitham Journal For Pure and Applied Science*, *28*(3), 113–128.

El Gammal, R. N., Hammouda, M. E. A., El-Wasseef, D. R., & El-Ashry, S. M. (2018). Simultaneous determination of gatifloxacin and prednisolone in their bulk powder, synthetic mixture and their combined ophthalmic preparation using micellar liquid chromatography. *Journal of Chromatographic Science*, *56*(4), 367–374. https://doi.org/10.1093/chromsci/bmy011..

Evtifeeva, O. A., Proskurina, K. I., Ganeva, E. V., & Zhukova, T. V. (2016). Evaluation of Metrological Characteristics for Quantitative Spectrophotometric Determination of Prednisolone by an Optical Absorbance Method. *Pharmaceutical Chemistry Journal*, *49*(12), 847–853. https://doi.org/10.1007/s11094-016-1385-2.

Hammod, N. M., Al-Janabi, K. W. S., & Hasan, S. A. (2020). Determination of some volatile organic compounds in the water produced at al-ahdab oilfield in the governorate of Wasit, Iraq using headspace SPE-GC-FID. *Indian Journal of Forensic Medicine and Toxicology*, *14*(1), 994–999. https://doi.org/10.37506/v14/i1/2020/ijfmt/193034.

Hammod, N. M., Al-janabi, K. W. S., & Hasan, S. A. (2020). TRACKING OF THE EXISTENCE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN WATER RESOURCES AROUND AND AWAY FROM AL-AHDAB OIL FIELD IN WASIT GOVERNORATE OF IRAQ. *Plant Archives*, *20*(2), 5047–5052.

Hannoun, W. A., & Al-Janabi, K. W. S. (2020). Spectrophotometric determination of isopropamide iodide based on ion-pair complex formation with thymol blue. *Indian Journal of Forensic Medicine and Toxicology*, *14*(2), 1043–1049. http://medicopublication.com/index.php/ijfmt/article/view/3045.

Iannella, L., Botrè, F., Colamonici, C., Curcio, D., & de la Torre, X. (2019). Development and validation of a method to confirm the exogenous origin of prednisone and prednisolone by GC-C-IRMS. *Drug Testing and Analysis*, *11*(11–12), 1615–1628. https://doi.org/10.1002/dta.2715.

Kashyap, R., Subrahmanyam, E. V. S., & Sharbaraya, A. R. (2012). Development evelopment and validation of UV spectroscopy method for the estimation of prednisolone in bulk and dosage form. *Journal of Chemical and Pharmaceutical Research*, *4*(2), 1090–1096. https://www.jocpr.com/articles/development-and-validation-of-uv-spectroscopy-method-for-the-estimation-of-prednisolone-in-bulk-and-dosage-form.pdf

Lemus Gallego, J. M., & Pérez Arroyo, J. (2003). Simultaneous Determination of Hydrocortisone and Zn-Bacitracin by Spectrophotometric Derivative and Multivariate Methods. *Microchimica Acta*, *141*(3–4), 133–141. https://doi.org/10.1007/s00604-002-0940-6

Mahmood, A. K. (2017). Development Of Two Different Spectrophotometric Methods For The Determination Of Atropine Drug In Pure Form And Pharmaceutical Preparations. *Ibn AL-Haitham Journal For Pure and Applied Science*, *25*(3), 226–241.

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)

Mortimer, R. J. (2017). Spectroelectrochemistry, Applications. In *Encyclopedia of Spectroscopy and Spectrometry* (pp. 160–171). Elsevier. https://doi.org/10.1016/B978-0-12-803224-4.00288-0

Primpray, V., Chailapakul, O., Tokeshi, M., Rojanarata, T., & Laiwattanapaisal, W. (2019). A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines. *Analytica Chimica Acta*, *1078*, 16–23. https://doi.org/10.1016/j.aca.2019.05.072.

Radhi, N. A. A., & Al-Janabi, K. W. S. (2020). Spectrophotometric determination of Di phenyl hydramine HCl in pure and pharmaceutical formulations using Thymol blue. *Annals of Tropical Medicine and Public Health*.

Raut, G. S., Shirkhedkar, A. A., Ugale, V. G., & Surana, S. J. (2014). Simultaneous determination of Prednisolone acetate and Moxifloxacin hydrochloride in bulk and in eye drop using RP-HPTLC. *Journal of Liquid Chromatography and Related Technologies*, *37*(4), 528–537. https://doi.org/10.1080/10826076.2012.749495.

Razzaq, S. N., Ashfaq, M., Khan, I. U., Mariam, I., Razzaq, S. S., & Azeem, W. (2017). Simultaneous determination of dexamethasone and moxifloxacin in pharmaceutical formulations using stability indicating HPLC method. *Arabian Journal of Chemistry*, *10*(3), 321–328. https://doi.org/10.1016/j.arabjc.2014.11.016.

Schuster, D., Laggner, C., & Langer, T. (2005). Why drugs fail-a study on side effects in new chemical entities. *Current Pharmaceutical Design*, *11*(27), 3545–3559.

Singh, D. K., & Verma, R. (2008). Spectrophotometric Determination of Corticosteroids and Its Application in Pharmaceutical Formulation. *Iranian Journal of Pharmacology & Therapeutics*, *7*(1), 61–65. http://ijpt.iums.ac.ir/article-1-155-en.html.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). *Principles of Instrumental Analysis*. Cengage Learning. https://books.google.iq/books?id=D13EDQAAQBAJ.

Smajdor, J., Piech, R., & Paczosa-bator, B. (2016). *A Novel Method of High Sensitive Determination of Prednisolone on Renewable Mercury Film Silver Based Electrode*. 394–400. https://doi.org/10.1002/elan.201500262.

Xu, J., Winkler, J., & Derendorf, H. (2007). A pharmacokinetic/pharmacodynamic approach to predict total prednisolone concentrations in human plasma. *Journal of Pharmacokinetics and Pharmacodynamics*, *34*(3), 355–372. https://doi.org/10.1007/s10928-007-9050-8.

DOI:https//doi.org/10.22452/mjs.vol43no3.4
Malaysian Journal of Science 43(3): 30-36 (September 2024)