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Optimization and Validation of Spectrophotometric Methods for the Determination of Prednisolone in Chitosan Nanoparticles

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Abstract: This study validated a UV spectrophotometric method for quantifying prednisolone in pharmaceutical formulations. The method was selective, linear, accurate, precise, and compatible with filtration. The determined linearity range 0.6-25 μg/mL was based on a correlation value of 0.9996. Accuracy was determined through recovery trials, confirming recovery within 98.5%–101.5% of the initial value, with %RSD of less than 2.0 for all levels. Precision was meticulously evaluated through intra-day and inter-day trials, revealing a relative standard deviation (RSD) of less than 2%, attesting to the method's remarkable accuracy. In addition, the study also evaluated the wavelength of maximum absorption for prednisolone (246 nm) and the method's selectivity against commonly used excipients in pharmaceutical formulations. The method was also stable under different conditions, including temperature, pH, and light. This study demonstrated that the UV spectrophotometric method is reliable and robust for quantifying prednisolone in pharmaceutical formulations. It is simple, rapid, and cost-effective, making it a valuable tool for quality control and research. This study successfully achieved its stated goal of developing a simple, rapid, and cost-effective UV spectrophotometric method for quantifying prednisolone in pharmaceutical formulations with good selectivity, linearity, accuracy, and precision. This offers advantages over the standard pharmacopeial high-performance liquid chromatography (HPLC) method in being a simpler, faster, and more cost-effective analytical method while retaining satisfactory accuracy and precision.
Keywords: ultraviolet spectrophotometry, prednisolone quantification, drug, method validation.

壳聚糖纳米粒子中泼尼松龙测定的分光光度方法优化及验证

摘要:
本研究验证了一种紫外分光光度法，用于定量药物制剂中的泼尼松龙。该方法具有选择性、线性、准确度、精密度，并且与过滤兼容。确定的线性范围0.6-25微克/毫升基于0.9996的相关值。通过回收试验确定准确度，确认回收率在初始值的98.5%–101.5%范围内，所有水平的相对标准偏差%均小于2.0。通过日内和日间试验对精密度进行了细致评估，结果显示相对标准偏差(相对标准偏差)小于2%，证明了该方法的卓越准确性。

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此外，该研究还评估了泼尼松龙的最大吸收波长(246纳米)以及该方法对药物制剂中常用赋形剂的选择性。该方法在不同条件下也很稳定，包括温度、pH值和光照。本研究表明，紫外分光光度法可靠且耐用，可用于定量药物制剂中的泼尼松龙。它简单、快速、经济高效，是质量控制和研究的宝贵工具。本研究成功实现了既定目标，即开发一种简单、快速、经济高效的紫外分光光度法，用于定量药物制剂中的泼尼松龙，具有良好的选择性、线性、准确度和精密度。与标准药典高效液相色谱(高效液相色谱)方法相比，该方法具有优势，是一种更简单、更快速、更经济高效的分析方法，同时保持了令人满意的准确度和精密度。

关键词：紫外分光光度法，泼尼松龙定量，药物，方法验证。

1. Introduction

Synthetic methylprednisolone, or prednisolone, is a corticosteroid with potential applications in the treatment of inflammation, autoimmune disorders, and cancer. This versatile compound is available in various pharmaceutical formats such as pills, injections, lotions, and ointments [1, 2]. Ensuring accurate dosing for patients requires a dependable method to detect the quantity of prednisolone in these formulations [3–5].

Among the range of analytical techniques available for prednisolone determination, high-performance liquid chromatography (HPLC), gas chromatography (GC), and ultraviolet (UV) spectrophotometry (SPR) are noteworthy [6–9]. Among these, UV spectrophotometry is a rapid and cost-effective means of quantifying prednisolone concentrations. This approach leverages the compound's capacity to absorb ultraviolet light, enabling straightforward and swift measurements. Because the absorbance of prednisolone correlates with its concentration [10], it facilitates accurate quantitative analysis.

HPLC and UV spectrophotometric methods have been developed to estimate salbutamol and prednisolone simultaneously, demonstrating linearity in concentration ranges and validating results through static recovery studies [3]. In another study, HPLC and UV spectroscopic methods were developed and validated for the determination of prednisolone sodium phosphate. These methods were established using the analytical quality by design (AQbD) approach in accordance with the ICH Q8 guidelines. The validation of these methods showcases their reliability and accuracy in pharmaceutical analysis [11]. UV spectroscopic routine analysis uses a solvent with a 246-nm detection wavelength for precise and sensitive estimation of prednisolone in bulk and pharmaceutical dosage forms [12]. Two updated chemometric models, PCA and PLS, were developed for determining prednisolone and esomeprazole levels in spiked human plasma using UV spectrophotometric data [13].

This study employed a validated UV spectrophotometric method to determine prednisolone concentrations in pharmaceutical preparations, i.e., chitosan nanoparticles (data are under preparation for publication). The investigation demonstrated the method's effectiveness in terms of linearity, accuracy, precision, and selectivity. The method's reliability, reproducibility, and capability to discern prednisolone in pharmaceutical formulations were established through validation studies. The proposed novel UV spectrophotometric approach further enhances the ability to measure prednisolone concentrations in drug formulations. This method offers greater precision, accuracy, and selectivity than previous techniques [10, 12], making it a valuable tool for evaluating prednisolone concentrations in diverse pharmaceutical formulations. The method presented in this study is an optimized UV spectrophotometric technique that provides a simpler, quicker, and more cost-effective alternative to the standard HPLC method. Using the inherent UV absorbance properties of prednisolone, the proposed method achieves high accuracy and precision, as evidenced by the recovery rates and relative standard deviations (RSDs) within acceptable ranges. This advancement in the analytical approach not only streamlines the process of prednisolone measurement in pharmaceutical formulations but also ensures reliable quality control, thereby supporting the efficacy and safety of corticosteroid-based therapies. The method's robustness against various conditions, including temperature, pH, and light, further underscores its potential as a valuable tool for routine pharmaceutical analysis.

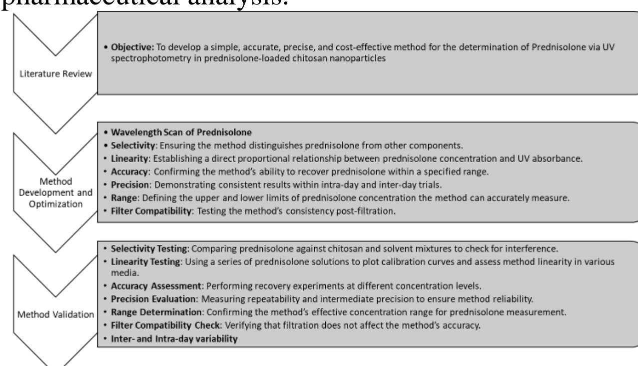

Fig. 1 Flowchart of the research methodology (The authors)

2. Methods

2.1. Optimization and Validation of the UV Method for Prednisolone

The UV analytical method was validated to establish its appropriateness for quantifying prednisolone within the specified formulation. The validation encompassed selectivity, linearity, accuracy, precision, range, and compatibility with filtration, adhering to the criteria set forth by the International Conference on Harmonization (ICH) guidelines Q2(R1) 2005 [14].

2.2. Wavelength Scanning of Prednisolone

Approximately 10.0 mg of prednisolone was transferred into a 10.0 mL volumetric flask. From this, an appropriate 0.5-mL portion was taken and transferred to another 10.0-mL volumetric flask, then further diluted to the mark using a mixture of ethanol and distilled water. Subsequently, 0.5 mL of this solution was transferred to a 5.0-mL volumetric flask and diluted to the mark with ethanol-distilled water. This process yielded prednisolone concentration of 5 µg/mL. The resulting solution was then placed into a 1-cm quartz cell and scanned across the UV-Vis range (200-700 nm) to determine the wavelength of maximum absorption for the UV detectors.

2.3. Stock and Standard Solution Preparation

Stock solutions were created by dissolving 50.0-mg prednisolone in a 25.0-mL volumetric flask. A standard solution was then prepared by diluting this solution with 100.0 mL of a mixture of ethanol and distilled water in a 1:1 ratio, resulting in a concentration of 0.06 mg/mL. Subsequently, various dilutions were derived from the standard solution to achieve the desired concentration range from 0.6 to 25 µg/mL.

2.4. UV Method Validation

2.4.1. Selectivity

Selectivity assesses the analyte in the presence of anticipated accurate components, which is a key focus [15, 16]. Three distinct samples were analyzed to establish selectivity: a prednisolone standard, a 1:1 mixture of ethanol and distilled water, and an excipient “chitosan” without the active ingredient. Each sample underwent UV scanning, and the resulting spectra were compared to identify and evaluate potential interferences.

2.4.2. Linearity in the Ethanol-Distilled Water Mixture

Linearity signifies the analytical method’s capability to generate outcomes directly proportional to the concentration (amount) of the analyte in the sample, falling within a specific range [17]. A series of nine prednisolone solutions with varying concentrations was prepared to examine the linearity of the reactions. Each solution, containing an accurately measured 30.0 mg of prednisolone within a 25.0-mL volumetric flask, was subsequently dissolved and diluted using a 1:1 water-ethanol mixture. Furthermore, 5.0 mL of this solution was diluted with a 100.0-mL mixture to achieve a 0.06-mg/mL concentration. The next step involved preparing a sequence of dilutions ranging from 0.1 to 4.16 mL, each of which was mixed to 10 mL. The concentrations were 0.6, 3, 6, 6.6, 7, 7.2, 7.8, 10, and 25 µg/mL. They were subjected to triplicate analyses. Calibration curves were generated by plotting the absorbance (Abs) against the prednisolone concentration, and linear regression equations were derived using the least squares method.

$$A = \varepsilon b C \quad (1)$$

where A is the absorbance, ε is the absorptivity, b is the length of the light path, and C is the concentration.

2.4.3. Linearity in Diluted Acetic Acid

To assess the linearity of prednisolone in diluted acetic acid (AA), we followed the identical procedure outlined in Section 2.4.2 to create the standard solutions. However, we dissolved prednisolone in the ethanol-distilled water mixture instead of dissolving it in the diluted AA solution.

2.4.4. Accuracy

The accuracy of the analytical technique was evaluated on the basis of the extent of agreement between the measured value and a recognized conventional or reference value [18]. A recovery experiment was conducted to determine the method's accuracy. This involved investigating recovery at three levels: 10%, 100%, and 120% of the standard solutions. Three replicates were prepared for each level using the excipient chitosan, resulting in nine determination solutions. These solutions were then subjected to measurement, and the percentage recovery was calculated using Equation (2).

$$\%\text{Recovery} = \frac{\text{Abs(R)}}{\text{Abs (S)}} \times \frac{\text{C(S)}}{\text{C(R)}} \times 100\% \quad (2)$$

where Abs(R) is the absorbance of the recovery sample solution, Abs (S) is the absorbance of a standard solution, C(R) is the concentration of the recovery sample, and C(S) is the concentration of the standard solution.

2.4.5. Precision

Repeatability exemplifies accuracy within a short timeframe while maintaining uniform operational circumstances. This concept, known as intra-assay precision, conveys the same notion [19]. Six samples with identical composition (6 g/mL) were subjected to absorbance measurements to establish procedure reproducibility. Subsequently, the mean, standard deviation, and RSD were calculated. An acceptable level of precision is indicated by a RSD not exceeding 2%.

2.4.6. Intermediate Precision

Intermediate precision expresses within-laboratory variations: different days, different analysts, and different equipment [19]. The intermediate precision was obtained by measuring the absorbance of six samples used in the reparability test, containing 6 µg/mL, and then, the average, standard deviation, and RSD were calculated. This procedure was repeated on different days (inter-day) and by different persons, including the partner (inter-person). Finally, we compared these data with the data obtained from the repeatability test.

2.4.7. Range

The range of an analytical technique is the period between the higher and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which the analytical procedure has been proven to have a satisfactory degree of precision, accuracy, and linearity [12].

2.4.8. Filter Compatibility

Filtration compatibility was examined using a 6-µg/mL standard prednisolone solution. This solution was analyzed using UV spectroscopy without filtration; then, the same solution was filtered using a 0.45-µm PTFE syringe filter and analyzed using UV spectroscopy. The Abs of the filtered solution was compared to that of the non-filtered solution according to Equation (3):

(3)

3. Results and Discussion

3.1. Wavelength Scanning of Prednisolone

The wavelength of the prednisolone solution was determined to be 246 nm, as shown in Fig. 2. This value matches the results found in the literature [12].

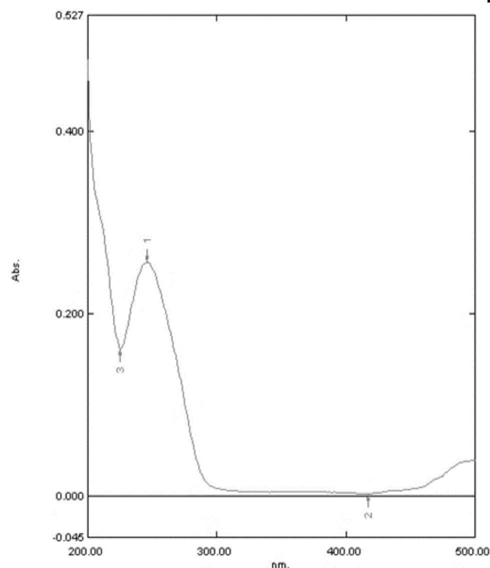

Fig. 2 UV-Vis scan of prednisolone in ethanol and water (1:1) at the sample concentration of 5 µg/mL (The authors)

3.2. Validation of the UV Method

The spectrophotometric method was validated using a UV instrument (Shimadzu®). The UV method was validated according to the ICH guidelines Q2(R1) [20] regarding selectivity, linearity, precision, intermediate precision, recovery, and range.

3.2.1. Selectivity

The selectivity results were determined by scanning the prednisolone solution, ethanol and water (1:1), polymer chitosan, and the spectra shown in Fig. 3.

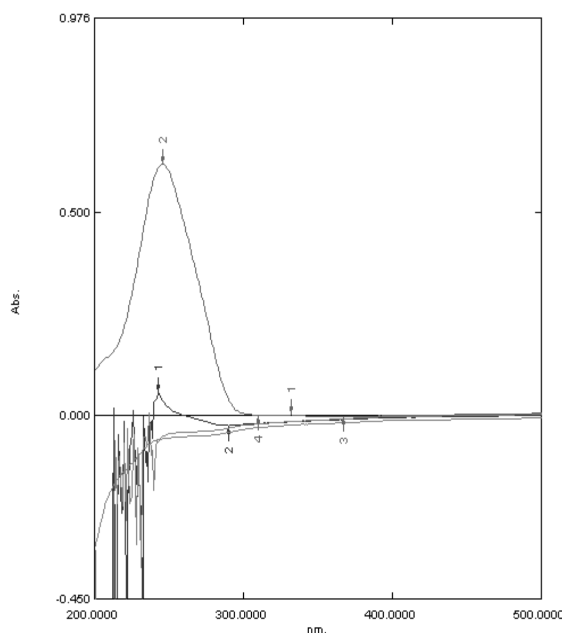

Fig. 3 Selectivity spectra of samples: prednisolone, chitosan, diluted AA, and ethanol-distilled water (The authors)

The selectivity results demonstrate the ability to assess the analyte in the presence of excipients. According to ICH guidelines Q2(R1) [20], Fig. 3 shows no interfering peaks between prednisolone, chitosan, and ethanol-distilled water, indicating that the method is selective and, as a result, the analytical method can be selective toward the analyte.

3.2.2. Linearity in the Ethanol-Distilled Water Mixture

Linearity is the ability of the system to obtain test results that are directly proportional to the concentration of analytes in the sample within a given range [20]. At nine different concentrations, validated parameters were assessed for PRED, as shown in Table 1 and Fig. 4, showing the calibration curve obtained by plotting the absorbance against the concentration.

Table 1 Absorbance of different concentrations of prednisolone in ethanol-distilled water (The authors)

Sr. No.	Volume of working standard taken (ml)	Concentration of prednisolone in µg/mL	Absorbance at 246 nm (± standard deviation)
1	0.1	0.6	0.036 ± 0.0006

Continuation of Table 1			
2	0. 5	3	0.15 ± 0.0025
3	1	6	0.274 ± 0.0025
4	1.1	6.6	0.314 ± 0.0035
5	1.16	7	0.339 ± 0.0021
6	1.2	7.2	0.35 ± 0.004
7	1.3	7.8	0.375 ± 0.0031
8	1.66	10	0.481 ± 0.004
9	4.16	25	1.195 ± 0.006

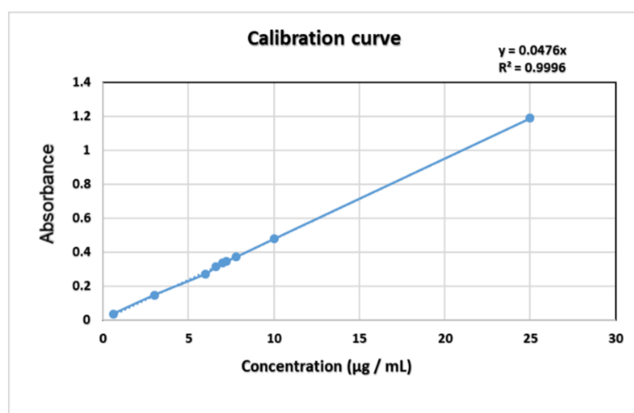

Fig. 4 Calibration curve for prednisolone in ethanol-distilled water: concentration against Abs (The authors)

The concentration of PRED dissolved in ethanol-distilled water ranged from 0.0006 to 0.025 mg/mL. A calibration curve was then generated, as depicted in Fig. 4. Based on the evidence presented, it can be confidently stated that the calibration curve exhibits linearity and a proportional relationship between the analyte concentration and Abs values. This conclusion is supported by the linear calibration curve of PRED within the specified range, which demonstrated a strong correlation coefficient $R^2 = 0.9996$, approaching unity ($R^2 = 1$).

3.2.3. Linearity in Diluted Acetic Acid

At different concentrations, validated parameters were assessed for prednisolone, as shown in Table 2 and Fig. 5 showing the calibration curve obtained by plotting the Abs against the concentration.

Table 2 Absorbance of different concentrations of prednisolone in diluted AA (The authors)

Sr. No	Volume of working standard taken (ml)	Concentration of prednisolone in µg/mL	Absorbance at 246 nm (± standard deviation)
1	0. 5	3.0	0.135 ± 0.0021
2	0.66	4.0	0.182 ± 0.0025
3	0.75	4.5	0.2 ± 0.0025
4	0.83	5.0	0.235 ± 0.0025
5	0.91	5.5	0.251 ± 0.0025
6	1.0	6.0	0.273 ± 0.0032
7	1.1	6.6	0.302 ± 0.0046
8	1.3	7.8	0.364 ± 0.0031
9	1.41	8.5	0.394 ± 0.0025
10	1.66	10.0	0.458 ± 0.0025
11	4.16	25.0	1.161 ± 0.0085

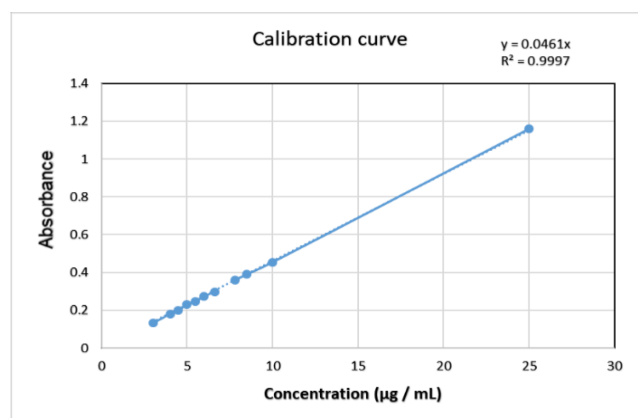

Fig. 5 Calibration curve for prednisolone in diluted AA: concentration against Abs (The authors)

The concentration of prednisolone dissolved in diluted AA ranged from 3 to 25 µg/ml; the calibration curve obtained is shown in Fig. 5. There is sufficient evidence to conclude that the calibration curve is linear and that there is a proportional relationship between analyte concentration and Abs values because the calibration curve of prednisolone was linear in the specified range and had a good correlation coefficient $R^2 = 0.9997$, which was close to unity ($R^2 = 1$).

3.2.4. Linearity in pH 1.2 Buffer Solutions

At six different concentrations, validated parameters were assessed for PRED, as shown in Table 3 and Fig. 6 showing the calibration curve obtained by plotting the Abs against the concentration.

Table 3 Absorbance of different concentrations of PRED at pH 1.2 (The authors)

Sr. No	Volume of working standard taken (ml)	Concentration of prednisolone in µg/mL	Absorbance at 246 nm (± standard deviation)
1	0. 24	0.003	0.13 ± 0.0025
2	0.4	0.005	0.217 ± 0.004
3	0.44	0.0055	0.237 ± 0.0025
4	0.48	0.006	0.255 ± 0.0026
5	0.56	0.007	0.304 ± 0.004
6	0.624	0.0078	0.335 ± 0.0051

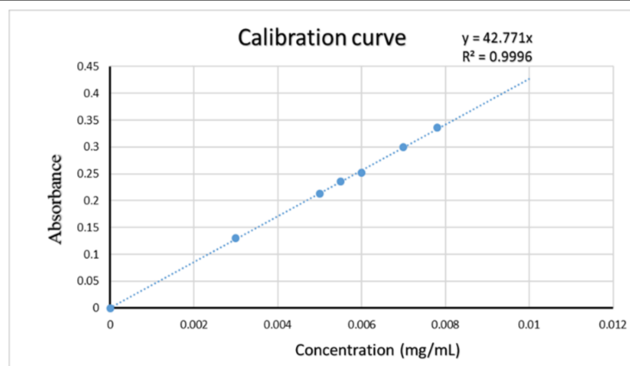

Fig. 6 Calibration curve for PRED at pH 1.2: concentration against Abs (The authors)

3.2.5. Accuracy

Three concentration levels (10%, 100%, and 120%) were analyzed in accordance with the ICH guidelines Q2(R1) 2005. Each concentration level was measured in triplicate, and the average, SD, and RSD were calculated for each level. The results are shown in Table 4.

Table 4 Recovery results of three levels of prednisolone, n = 3 (The authors)

Level of recovery	Preparation No.	% Recovered	Average percentage recovered	%RSD
10%	1	99.98	99.15	1.46
	2	97.48		
	3	99.98		
100%	1	100.04	100.76	0.76
	2	101.57		
	3	100.67		
120%	1	98.96	98.46	0.51
	2	98.46		
	3	97.96		

The average recovery should be in the range of 97%−103% to indicate a good recovery. The drug concentrations in the sample and standard were calculated, and the percentage recovery was calculated using Equation 2. The percentage recovery was found to be between 98.46% and 100.76%, and the %RSD for each level was NMT 2, which is in the acceptable range.

3.2.6. Precision

Precision was determined as repeatability and intermediate precision (the inter-day and inter-person precision).

3.2.7. Repeatability

The repeatability of a standard sample with a concentration of 6 μg/mL was tested using six replicates; the results are shown in Table 5.

Table 5 Intra-day repeatability results for six samples of prednisolone in the same formula (The authors)

Concentration (μg/ml)	Absorbance	Mean	SD	%RSD
6.0	0.271	0.266833	0.003656	0.266833
	0.265			
	0.269			
	0.262			
	0.264			
	0.27			

Repeatability results showed that the %RSD for the six samples was 1.37%. The %RSD value obtained was NMT 2, indicating that the method was precise because the results were within the acceptance criteria.

3.2.8. Intermediate Precision

The standard sample was analyzed in six replicates over the course of two days to assess analyst-to-analyst variation; the results are shown in Table 6.

Table 6 Results of intermediate precision for six samples of prednisolone in the same formula (The authors)

Inter-day	Inter-day	Inter-person
Concentration (μg/ml)	6.0	6.0
Absorbance	0.263	0.265
	0.269	0.269
	0.262	0.266
	0.267	0.271
	0.259	0.267
	0.267	0.272
Mean	0.2645	0.268333
SD	0.003782	0.002805
%RSD	1.429692	1.045251

Intermediate precision (inter-day and inter-person)

results are shown in Table 6, where the %RSD was 1.43% and 1.05%, respectively. The %RSD values obtained were NMT 2, indicating that the method was precise because the results were within the acceptance criteria.

3.2.9. Range

Different plots were prepared in triplicate using concentrations ranging from 0.6 to 25 μg/mL. The procedure adheres to the Beer-Lambert law within a concentration range of 0.6–25 μg/mL.

3.2.10. Filter Compatibility

The compatibility of filtration with the standard prednisolone solution was assessed, yielding results of 102.7% ± 1.9 (mean ± SD, n = 3). After the analysis, it was found that PTFE filters were suitable for filtration (Fig. 7).

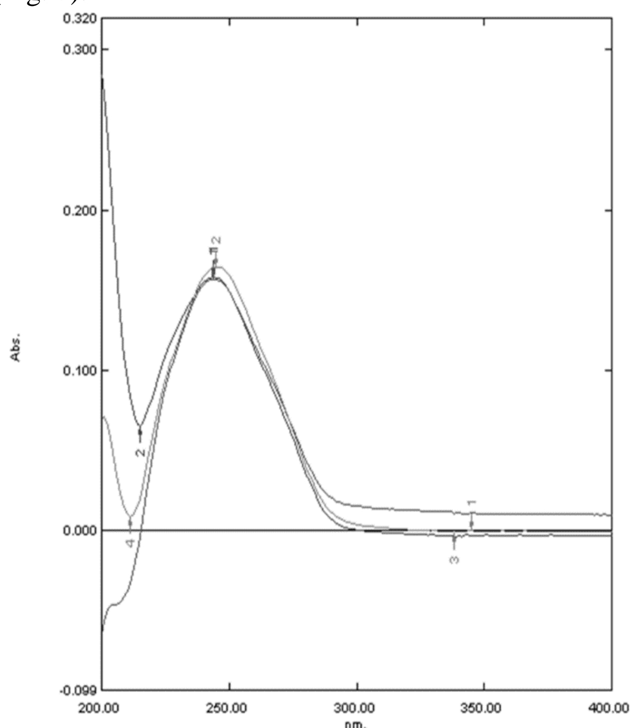

Fig. 7 Filter compatibility (The authors)

4. Conclusion

This study successfully developed and validated a UV spectrophotometric method for the quantification of prednisolone in chitosan nanoparticles. The method demonstrated high selectivity, linearity, accuracy, and precision, with a linearity range from 0.6 to 25 μg/mL and a correlation value of 0.99962. Recovery trials confirmed accuracy within 98.5%−101.5% of the initial value, and precision was evidenced by a %RSD of less than 2.0 for all levels.

Compared with other analytical techniques such as HPLC and GC, the UV spectrophotometric method offers a simpler, faster, and more cost-effective alternative for prednisolone quantification in pharmaceutical formulations. The method's robustness and reliability are comparable to the standard pharmacopeial HPLC method, yet it requires less operational complexity and resources.

The validated UV spectrophotometric method provides a practical and efficient tool for routine quality control of prednisolone in various pharmaceutical forms. Its simplicity and cost-effectiveness make it accessible to laboratories with limited resources, potentially improving quality assurance processes in the pharmaceutical industry.

It is recommended that the newly developed UV spectrophotometric method be considered as an alternative to more complex and resource-intensive methods for prednisolone analysis. Future research should focus on applying this method to a broader range of pharmaceutical compounds and exploring its potential for in-line quality control during the manufacturing process. In addition, further studies could investigate the method's applicability in different environmental conditions and its integration into automated systems for pharmaceutical analysis.

References

[1] ZIHLIF M., ABUSARA O. H., AL-QEREM W., AL-IBADAH M., MAHAFZA T. M., AL-AKHRAS F. M., and MAHAFZA N. T. CRHR1 polymorphism at rs242941, rs242940, and rs72834580: association of symptoms improvement with intranasal corticosteroids in allergic rhinitis Jordanian patients. *Drug Metabolism and Personalized Therapy,* 2024, 38(4): 331-338. https://doi.org/10.1515/dmpt-2023-0014

[2] HAMED R., ABU KWIAK A. D., AL-ADHAMI Y., HAMMAD A. M., OBAIDAT R., ABUSARA O. H., and HUWAIJ R. A. Microemulsions as lipid nanosystems loaded into thermoresponsive in situ microgels for local ocular delivery of prednisolone. *Pharmaceutics,* 2022, 14(9): 1975. https://doi.org/10.3390/pharmaceutics14091975

[3] CHITLANGE S. S., CHATURVEDI K. K., and WANKHEDE S. B. Development and validation of spectrophotometric and HPLC method for the simultaneous estimation of salbutamol sulphate and prednisolone in tablet dosage form. *Analytical & Bioanalytical Techniques,* 2011, 2(1): 117. https://doi.org/10.4172/2155-9872.1000117

[4] DAYYIH W. A., HAILAT M., AL HUJRAN T. A., MAGHARBEH M., ZAKARAYA Z., AL TAMIMI L., ABURUMMAN A. M., ABUMANSOUR H., and AWAD R. Spectrophotometric analysis of empagliflozin tablets as SGLT2 inhibitors in pharmaceutical samples. *Journal of Applied Pharmaceutical Science,* 2022, 12(10): 140-146. https://doi.org/10.7324/JAPS.2022.121015

[5] JARRAR Y., MOSLEH R., HAWASH M., and JARRAR Q. Knowledge and Attitudes of Pharmacy Students towards Pharmacogenomics among Universities in Jordan and West Bank of Palestine. *Pharmacogenomics and Personalized Medicine,* 2019, 12: 247-255. https://doi.org/10.2147/PGPM.S222705

[6] LOO J. K., BUTTERFIELD A. G., MOFFATT J., and JORDAN N. Analysis of prednisolone in plasma by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications,* 1977, 143(3): 275-280. https://doi.org/10.1016/S0378-4347(00)81807-1

[7] GUPTA V. D. High-pressure liquid chromatographic evaluation of aqueous vehicles for preparation of

prednisolone and prednisone liquid dosage forms. *Journal of Pharmaceutical Sciences,* 1979, 68(7): 908-910. https://doi.org/10.1002/jps.2600680734
[8] SHIBASAKI H., NAKAYAMA H., FURUTA T., KASUYA Y., TSUCHIYA M., SOEJIMA A., YAMADA A., and NAGASAWA T. Simultaneous determination of prednisolone, prednisone, cortisol, and cortisone in plasma by GC–MS: estimating unbound prednisolone concentration in patients with nephrotic syndrome during oral prednisolone therapy. *Journal of Chromatography B,* 2008, 870(2): 164-169. https://doi.org/10.1016/j.jchromb.2008.03.003
[9] SINGH G., KUMAR D., SHARMA D., SINGH M., and KAUR S. Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Prednisolone and 5-Amino Salicylic Acid in Tablet Dosage form. *Journal of Applied Pharmaceutical Science,* 2012, 2(7): 222-226. https://doi.org/10.7324/JAPS.2012.2736
[10] SHAREEF N. B. Direct determination of prednisolone by derivative UV spectroscopy. *Rafidain Journal of Science,* 2008, 19(5): 38-46. https://doi.org/10.33899/rjs.2008.41452
[11] BHUSNURE O. G., GHOLVE S., MANOJ B., TODKAR V., and GIRAM P. S. Analytical Method Development and Validation of Prednisolone Sodium Phosphate by QbD Approach. *IOSR Journal of Pharmacy and Biological Sciences,* 2015, 10(6), Ver. III: 64-75. https://doi.org/10.9790/3008-10636475
[12] KASHYAP R., SUBRAHMANYAM E. V. S., and SHARBARAYA A. R. Development and validation of UV spectroscopy method for the estimation of prednisolone in bulk and dosage form. *Journal of Chemical and Pharmaceutical Research,* 2012, 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
[13] FARID N. F., MAGDY M. A., ANWAR B. H., and ABDELHAMID N. S. Multivariate model update chemometric methods for determination of prednisolone and esomeprazole in spiked human plasma: A comparative study. *Journal of AOAC International,* 2022, 105(1): 317-322. https://doi.org/10.1093/jaoacint/qsab114
[14] BABER N. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). *British Journal of Clinical Pharmacology,* 1994, 37(5): 401–404. https://doi.org/10.1111/j.1365-2125.1994.tb05705.x
[15] HABASH I. W., AL-SHDEFAT R. I., HAILAT M. M., DAYYIH W. A., and ABU DAYYIH W. A stability indicating RP-HPLC method development for simultaneous estimation of alogliptin, pioglitazone, and metformin in pharmaceutical formulations. *Acta Poloniae Pharmaceutica-Drug Research,* 2020, 77(4): 549-562. https://doi.org/10.32383/appdr/125774
[16] ALHUSBAN A. A., HAMMAD A. M., ALZAGHARI L. F., SHALLAN A. I., and SHNEWER K. Rapid and sensitive HPLC–MS/MS method for the quantification of dopamine, GABA, serotonin, glutamine and glutamate in rat brain regions after exposure to tobacco cigarettes. *Biomedical Chromatography,* 2023, 37(1): e5513. https://doi.org/10.1002/BMC.5513
[17] AHMAD R., HAILAT M., ZAKARAYA Z., AL MEANAZEL O., and ABU DAYYIH W. Development and Validation of an HPLC Method for the Determination of Meloxicam and Pantoprazole in a Combined Formulation. *Analytica,* 2022, 3(2): 161-177. https://doi.org/10.3390/analytica3020012
[18] AHMAD R., HAILAT M., JABER M., ALKHAWAJA B., RASRAS A., AL-SHDEFAT R., MALLAH, E., and ABU DAYYIH W. RP-HPLC method development for simultaneous estimation of empagliflozin, pioglitazone, and metformin in bulk and tablet dosage forms. *Acta Poloniae Pharmaceutica – Drug Research,* 2021, 78: 305-315. https://doi.org/10.32383/appdr/139635
[19] ALKATHER Z., HAILAT M., AL-SHDEFAT R., and ABU DAYYIH W. Development and validation of HPLC method for five gliptins in pharmaceutical dosage forms in finished marketed products. *Current Pharmaceutical Analysis,* 2021, 17(10): 1263-1271. https://doi.org/10.2174/1573412917999201102212635
[20] SOMATEK. *Validation of Analytical Procedures: Text and Methodology.* 2014. http://somatek.com/wp-content/uploads/2014/06/sk140605h.pdf

参考文:

[1] ZIHLIF M.、ABUSARA O. H.、AL-QEREM W.、AL-IBADAH M.、MAHAFZA T. M.、AL-AKHRAS F. M. 和 MAHAFZA N. T. 促红细胞生成素受体1多态性在章斯242941、章斯242940和章斯72834580：约旦过敏性鼻炎患者症状改善与鼻内皮质类固醇的关系。药物代谢与个性化治疗，2024，38(4): 331-338。https://doi.org/10.1515/dmpt-2023-0014
[2] HAMED R.、ABU KWIAK A. D.、AL-ADHAMI Y.、HAMMAD A. M.、OBAIDAT R.、ABUSARA O. H. 和 HUWAIJ R. A. 微乳剂作为脂质纳米系统装入热响应原位微凝胶中，用于局部眼部递送泼尼松龙。药剂学，2022，14(9)：1975。https://doi.org/10.3390/pharmaceutics14091975
[3] CHITLANGE S. S.、CHATURVEDI K. K. 和 WANKHEDE S. B. 开发和验证分光光度法和高效液相色谱法，用于同时估算片剂剂型中的硫酸沙丁胺醇和泼尼松龙。分析与生物分析技术，2011，2(1): 117。https://doi.org/10.4172/2155-9872.1000117
[4] DAYYIH W. A.、HAILAT M.、AL HUJRAN T. A.、MAGHARBEH M.、ZAKARAYA Z.、AL TAMIMI L.、ABURUMMAN A. M.、ABUMANSOUR H. 和 AWAD R. 采用分光光度法分析药物样品中作为新加坡运输部2抑制剂的恩格列净片。应用药学杂志，2022， 12(10): 140-146。https://doi.org/10.7324/JAPS.2022.121015
[5] JARRAR Y.、MOSLEH R.、HAWASH M. 和 JARRAR Q. 约旦和巴勒斯坦西岸大学药学专业学生对药物基因组学的知识和态度。药物基因组学和个性化医疗，2019年，1

2：247-255。https://doi.org/10.2147/PGPM.S222705

[6] LOO J. K.、BUTTERFIELD A. G.、MOFFATT J. 和 JORDAN N. 高效液相色谱法分析血浆中的泼尼松龙。色谱杂志乙：生物医学科学与应用，1977年，143(3)：275-280。https://doi.org/10.1016/S0378-4347(00)81807-1

[7] GUPTA V. D. 高压液相色谱评价用于制备泼尼松龙和泼尼松液体剂型的水性载体。《药学杂志》，1979年，68(7)：908-910。https://doi.org/10.1002/jps.2600680734

[8] SHIBASAKI H.、NAKAYAMA H.、FURUTA T.、KASUYA Y.、TSUCHIYA M.、SOEJIMA A.、YAMADA A. 和 NAGASAWA T. 通过气相色谱-质谱法同时测定血浆中的泼尼松龙、泼尼松、皮质醇和可的松：估算口服泼尼松龙治疗期间肾病综合征患者体内的游离泼尼松龙浓度。色谱杂志乙，2008，870(2)：164-169。https://doi.org/10.1016/j.jchromb.2008.03.003

[9] SINGH G.、KUMAR D.、SHARMA D.、SINGH M. 和 KAUR S. 问-吸光度比分光光度法同时测定片剂中的泼尼松龙和5-氨基水杨酸。《应用药学杂志》，2012年，2(7)：222-226。https://doi.org/10.7324/JAPS.2012.2736

[10] SHAREEF N. B. 通过导数紫外光谱法直接测定泼尼松龙。《拉菲丹科学杂志》，2008年，19(5)：38-46。https://doi.org/10.33899/rjs.2008.41452

[11] BHUSNURE O. G.、GHOLVE S.、MANOJ B.、TODKAR V. 和 GIRAM P. S. 通过质量保证方法开发和验证泼尼松龙磷酸钠的分析方法。IOSR药学和生物科学杂志，2015年，10(6)，第III版：64-75。https://doi.org/10.9790/3008-10636475

[12] KASHYAP R.、SUBRAHMANYAM E. V. S. 和 SHARBARAYA A. R. 开发和验证用于估算散装和剂型泼尼松龙的紫外光谱法。《化学与制药研究杂志》，2012年，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

[13] FARID N. F.、MAGDY M. A.、ANWAR B. H. 和 ABDELHAMID N. S. 多变量模型更新化学计量法测定人血浆中的泼尼松龙和埃索美拉唑：一项比较研究。美国化学学会国际杂志，2022年，105(1)：317-322。https://doi.org/10.1093/jaoacint/qsab114

[14] BABER N. 人用药品注册技术要求协调国际会议(脑出血)。英国临床药理学杂志，1994， 37(5): 401–404。https://doi.org/10.1111/j.1365-2125.1994.tb05705.x

[15] HABASH I. W.、AL-SHDEFAT R. I.、HAILAT M. M.、DAYYIH W. A. 和 ABU DAYYIH W. 一种稳定性指示反相高效液相色谱方法开发，用于同时估计药物制剂中的阿格列汀、吡格列酮和二甲双胍。波兰药学学报-药物研究， 2020， 77(4): 549-562。https://doi.org/10.32383/appdr/125774

[16] ALHUSBAN A. A.、HAMMAD A. M.、ALZAGHARI L. F.、SHALLAN A. I. 和 SHNEWER K. 快速灵敏的液相色谱串联质谱法方法用于定量大鼠大脑区域接触烟草香烟后多巴胺、氨基丁酸、血清素、谷氨酰胺和谷氨酸。生物医学色谱，2023年，37(1): e5513。https://doi.org/10.1002/BMC.5513

[17] AHMAD R.、HAILAT M.、ZAKARAYA Z.、AL MANAZEL O. 和 ABU DAYYIH W. 开发和验证用于测定复方制剂中美洛昔康和泮托拉唑的高效液相色谱方法。分析，2022，3(2)：161-177。https://doi.org/10.3390/analytica3020012

[18] AHMAD R.、HAILAT M.、JABER M.、ALKHAWAJA B.、RASRAS A.、AL-SHDEFAT R.、MALLAH、E. 和 ABU DAYYIH W. 开发用于同时估算散装和片剂剂型中的恩格列净、吡格列酮和二甲双胍的反相高效液相色谱方法。波兰药学学报–药物研究，2021，78：305-315。https://doi.org/10.32383/appdr/139635

[19] ALKATHER Z.、HAILAT M.、AL-SHDEFAT R. 和 ABU DAYYIH W. 开发和验证成品中药物剂型中五种格列汀类药物的高效液相色谱方法。当前药物分析，2021，17(10)：1263-1271。https://doi.org/10.2174/1573412917999201102212635

[20]索玛泰克。分析程序的验证：文本和方法。2014年。http://somatek.com/wp-content/uploads/2014/06/sk140605h.pdf