BioGecko Vol 12 Issue 03 2023

ISSN NO: 2230-5807

Method Development and Validation of Prednisolone acetate in Simulated Tear Fluid and methanol by UV Spectrophotometric

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Abstract:
Prednisolone acetate is a form of prednisolone that has been ester-bonded to an acetate functional group. (1) Background: The purpose of this work was to create and validate a UV/Visible spectrophotometric method for determining prednisolone acetate in its pure form that was simple, sensitive, precise, and accurate. (2) Method: Prednisolone acetate was spectrophotometrically determined using a UV Spectrophotometer with a couple off of quartz cells and a 10 mm path length. (3) Results: Methanol (y = 0.077x + 0.01 and R2 = 0.999) and STF (Y=.024x+.033) were discovered to have linear regression equations. The re-analyzed prednisolone acetate solutions produced in methanol and STF had an accuracy of 98.28-103.5 percent and 100.41-106.39 percent, respectively. After six determinations, the relative standard deviation (percent RSD) was 2.61 percent at 2g/ml for methanol and 2.20 percent at 5g/ml for STF. (4) Conclusion: This spectrophotometrical method is reproducible, extremely basic, accurate, and sensitive.
Keywords: UV/Visible spectrophotometric, precise, accurate, methanol, Prednisolone acetate

Introduction:

Prednisolone acetate is a glucocorticoid that is used to treat a wide range of endocrine, inflammatory, and immunological problems, as well as to treat neoplastic illnesses. Prednisolone acetate is a prednisolone molecule that is ester-bonded to an acetate functional group. In 1955, the FDA approved prednisolone acetate. Prednisolone acetate is prescribed to treat allergy and problems of the neurological system, kidney, lungs, haematology, gastrointestinal tract, rheumatology, or infectious diseases. Prednisolone acetate is also prescribed for organ transplant patients, as well as those suffering from endocrine or neoplastic problems.

The glucocorticoid receptor is occupied by corticosteroids, which reduce pro-inflammatory impulses while enhancing anti-inflammatory signals. A relatively brief half-life of 2-3 hours is possessed by prednisolone acetate. Because individuals may need doses that are higher than what the body naturally generates, corticosteroids have a wide therapeutic windowCorticosteroid users should be aware of the possibility of hypothalamic-pituitary-adrenal axis suppression and increased susceptibility to infections. Corticosteroids diminish vasodilation, capillary permeability, and leukocyte migration to inflammatory areas in the short term. The glucocorticoid receptor is bound by corticosteroids, which affects gene expression in a number of ways over the course of hours to days.

Glucocorticoids boost anti-inflammatory genes like interleukin-10 and by inhibiting NF-Kappa B and other inflammatory transcription factors, arachidonic acid derivatives are prevented from being formed. Low dosages of corticosteroids are anti-inflammatory, but excessive doses weaken the immune system. Long-term high glucocorticoid doses bind to the mineralocorticoids.

UV spectrophotometry is a preferred approach for quantitative drug testing because it is quick, accurate, and repeatable. The goal of the study was to develop an intraocular delivery system for prednisolone acetate to the anterior and posterior segments of the eye, and the literature evaluation revealed that a straightforward approach for UV measurement of prednisolone acetate in simulated tear fluid was lacking. As a result, so, the current goal for the conclusion is to come up with and test a way to measure the amount of prednisolone acetate in STF and methanol using a UV-visible spectrophotometer.

As a result, the existing conclusion goal is to design and evaluate a technique for estimating prednisolone acetate in STF and methanol using a UV-visible spectrophotometer.

Chemical and Raw Materials

Chemicals

Prednisolone acetate was offered as a free sample by Sun Pharmaceutical Industries Ltd. (Ponta Sahib, Himachal Pradesh). We bought, sodium bicarbonate, potassium phosphate, potassium chloride, potassium phosphate and calcium chloride from Rankem, Gurgram, India. Additional compounds were used, and they were of an analytical quality. The solvent utilized was methanol, and it was of pure analytical grade.

Solvent Selection

Methanol and STF are employed as solvents because medicines are soluble in both.

Technique and Procedures

Instrumentation used for Method development

Prednisolone acetate's spectrophotometric analysis was carried out utilising a UV- Visible Spectrophotometer (Shimadzu, Kyoto, Japan) equipped with two quartz cells and a 10 mm path length.

Determination of lambda max (ʎmax)

A 10µg/ml prednisolone acetate test solution was produced in methanol and then scanned against a blank at a ʎ max of 200 to 400nm The produced solution's highest absorbance level was recorded, and this value was treated as absorption maxima that would be utilized to build the standard curve.

Preparation of calibration curve of prednisolone acetate in simulated tear fluid

Simulated tear fluid consist of 6.78 gm of NaCl, 1.38 gm of KCl, 2.18 gm of $NaHCO_3$ and 0.0843gm of $CaCl_2$ dissolved in deionized water, volume made up to 1L and adjusted the pH to 7.4 with the same.

Stock solution as standard

Stock solution for STF

Stock solution I for STF

Prednisolone acetate 25 milligram in the flask of 25 milliliter with STF was dissolved to create a primary standard stock solution of 1 mg/ml (1000µg/ml), and the resulting solution had a concentration of 1000µg/ml. The principal stock solution was kept chilled.

Stock solution II for STF

A 100µg/ml secondary stock solution was created by transferring a 10 ml aliquot of the prepared primary standard stock solution into a 100 ml volumetric flask. A 10ml volumetric flask was then filled with STF to make up the capacity after aliquots of the various test solutions (2–10) µg/ml were transferred inside.

Stock solution for Methanol

Stock solution I for Methanol

Prednisolone acetate 25 milligram in the flask of 25 milliliter with Methanol was dissolved to create a primary standard stock solution of 1 mg/ml (1000µg/ml), and the resulting solution had a concentration of 1000µg/ml. The principal stock solution was kept chilled.

Stock solution II for Methanol

A 100µg/ml secondary stock solution was created by transferring a 10 ml aliquot of the prepared primary standard stock solution into a 100 ml volumetric flask. A 10ml volumetric flask was then filled with methanol to make up the capacity after aliquots of the various test solutions (5–30) µg/ml were transferred inside.

Calibration Curve of prednisolone acetate in methanol and STF

Prednisolone acetate calibration curve was prepared in methanol and STF. Aliquots of 0.2,0.4,0.6, 0.8, 1.0 ml of solution withdrawn from stock solution using calibrated pipette and volume adjusted to 10 ml with methanol to obtain aliquots of 2,4,6,8,10µg/ml of both methanol. Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml of solution withdrawn from stock solution using calibrated pipette and volume adjusted to 10 ml with simulated tear fluid obtain aliquots of 5,10,15,20,25,30µg/ml of STF(Fig:1). Calibration curve was prepared by measuring absorbance of these solutions against blank under UV spectrophotometer at 243nmThe process was carried out three times, It was noted the mean absorption.

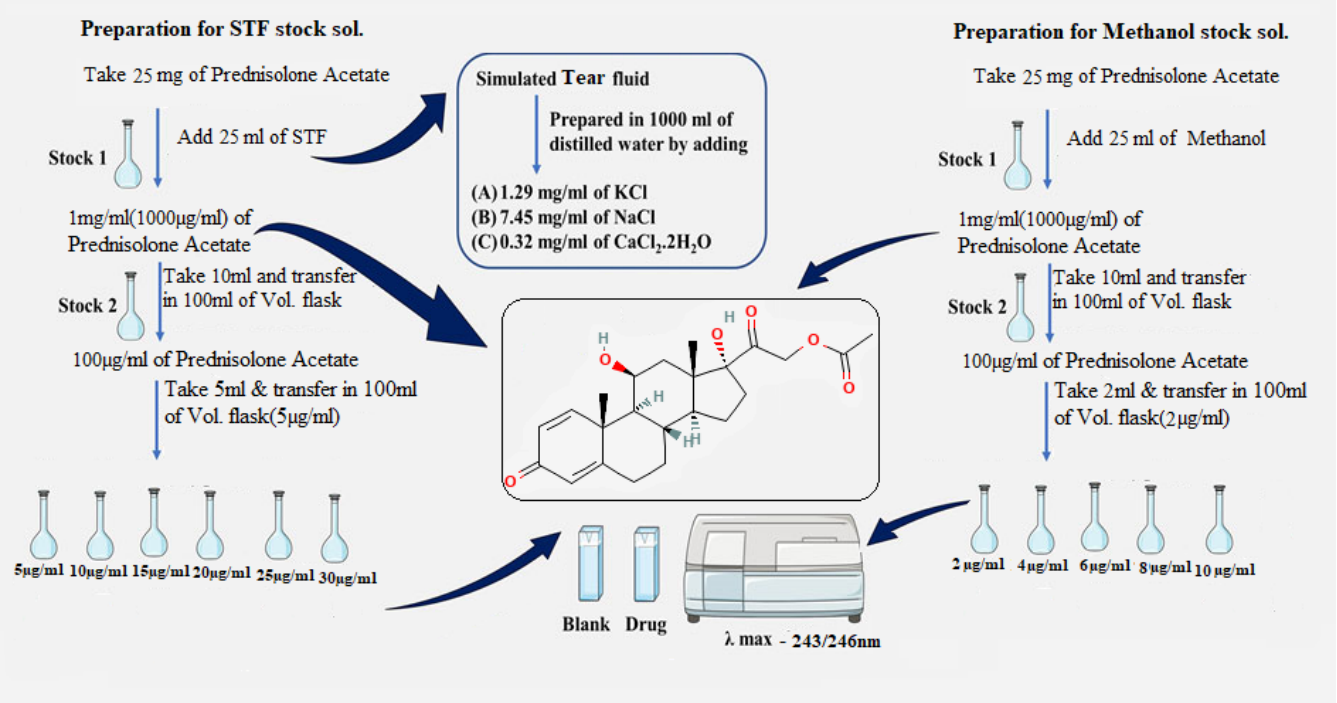

Figure 1: Preparation of Prednisolone Acetate solution of Methanol and STF

Parameters for Analytical Validation

Linearity

The capacity of a validation technique to directly or indirectly, through an appropriate mathematical transformation, produce observed concentration values that are directly proportional to the theoretical concentration of analyzed in the measured samples is known as linearity. The dependent variable (absorbance Y) and the independent variable (concentration X) were used to create the calibration curve absorbance vs. concentration

Accuracy

An analytical process is said to be accurate if the test results are reasonably near to the theoretical value. The test solutions are analyzed using the suggested techniques, and the calculated observed concentrations are compared to the theoretical concentrations by the regression line equation derived as of generated calibration standard curve. There are three levels of percentage analyzed recovery studies: 50, 100, and 150 percent.

Precision

The instrument's accuracy was evaluated using repeated scans and measurements of the absorbance of solutions (n=6) containing prednisolone acetate (2g/ml and 5g/ml) in methanol and STF without modifying any of the parameters of the proposed spectrophotometry method.

Intra-day & Inter-day Precision Percent RSD intra-day and inter-day was used to gauge how precise the method was. For intraday precision, prednisolone acetate (2g/ml and 5g/ml) in methanol and STF were used three times a day; for interday precision, they were utilised three times on three different days.

Limit of Quantification & Limit of Detection

LOD is defined as the lowest concentration of analyte that can be accurately and precisely detected in a sample under specific experimental conditions. Under particular circumstances and for a particular set of experimental parameters, LOD values for a given sample are particular. It's a quantitative technique whose outcomes depend on the technique, the tool, and additional factors. The LOQ is almost ten times more than the blank and is defined as quantifiable minimum concentration of an assayed sample within a group of samples.

RESULTS & DISCUSSION

Determination of UV absorbance maxima

Spectrophotometric studies were carried out in order to determine the λmax of Prednisolone acetate at different physiological pH i.e. simulated tear fluid (pH 7.4), 0.00001% (w/v) solution in methanol scanned in the range of 260nm and 360nm (Table 6), it shows experimental absorption maxima at 243nm which complies with the literature value of 243 nm (Clark's analysis Vol. II).

S No.	Media	Literature(λmax)	Experimental(λmax)
1	0.00001% (w/v) solution of Prednisolone acetate in methanol	243nm	243 nm
2	Simulated tear fluid (pH 7.4)	--------	246 nm

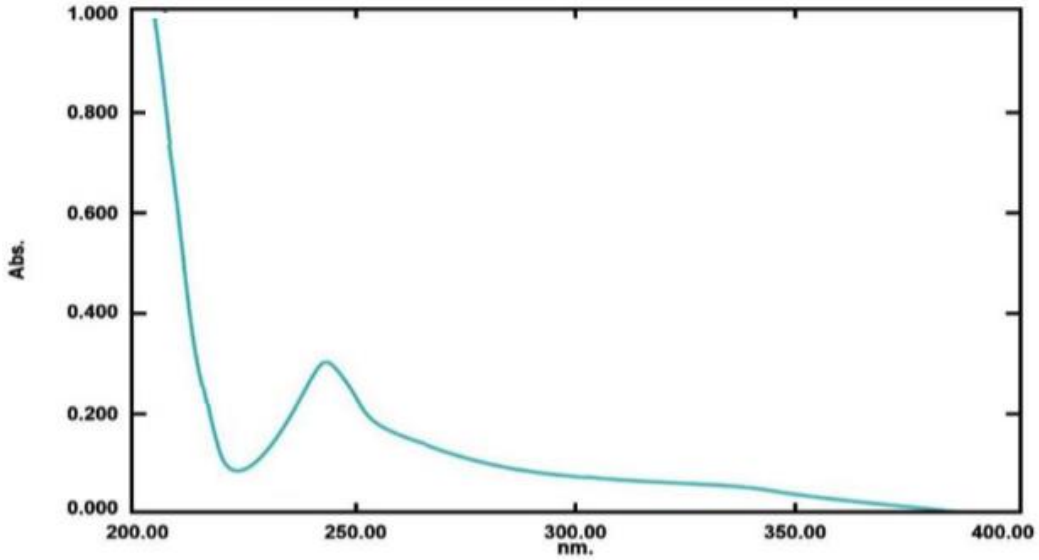

Figure 2: Scanning of Prednisolone acetate in methanol at Wavelength 243

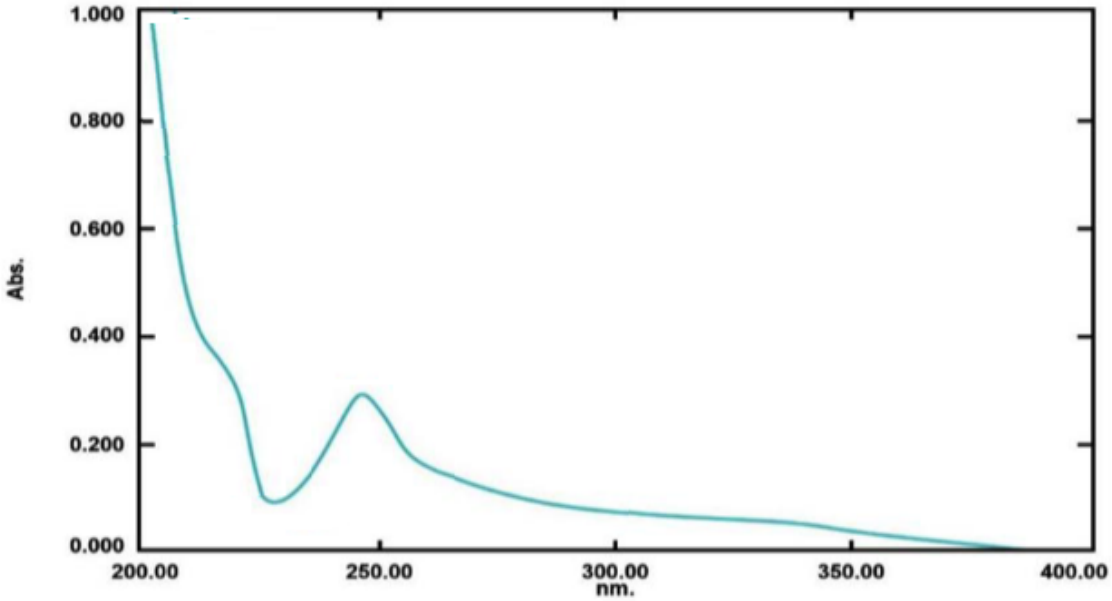

Figure 3: Scanning of Prednisolone acetate in STF at Wavelength 246

Calibration Curve of prednisolone acetate in methanol and STF

standard calibration curves the mean absorbance values (n=5) of different concentrations in range of 5-30 µg/milliliter as well as 2-10 µg/ milliliter of stock solution in STF and methanol was prepared and the absorbance were plotted against concentration.

Table:1 Observation of prednisolone acetate in methanol

S.No	Concentration(µg/ml)	Absorbance
1	0	0

2	2	0.173
3	4	0.328
4	6	0.465
5	8	0.616
6	10	0.778

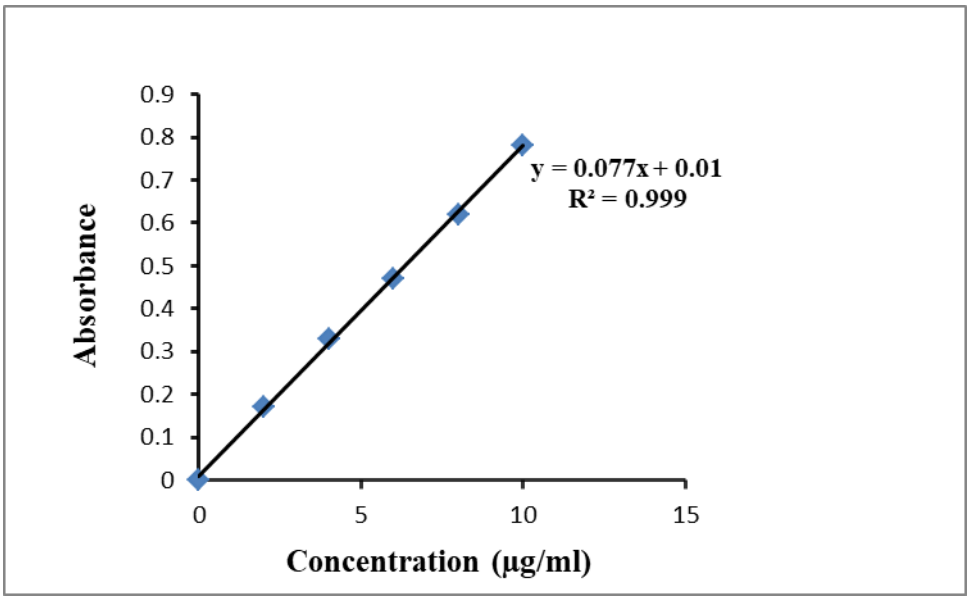

Fig 3 calibration curve of prednisolone acetate in methanol

Table:2 Observation of prednisolone acetate in STF

S. No	Concentration (µg/ml)	Absorbance
1	**0**	**0**
2	**5**	**0.164**
3	**10**	**0.283**
4	**15**	**0.394**
5	**20**	**0.521**
6	**25**	**0.642**
7	**30**	**0.778**

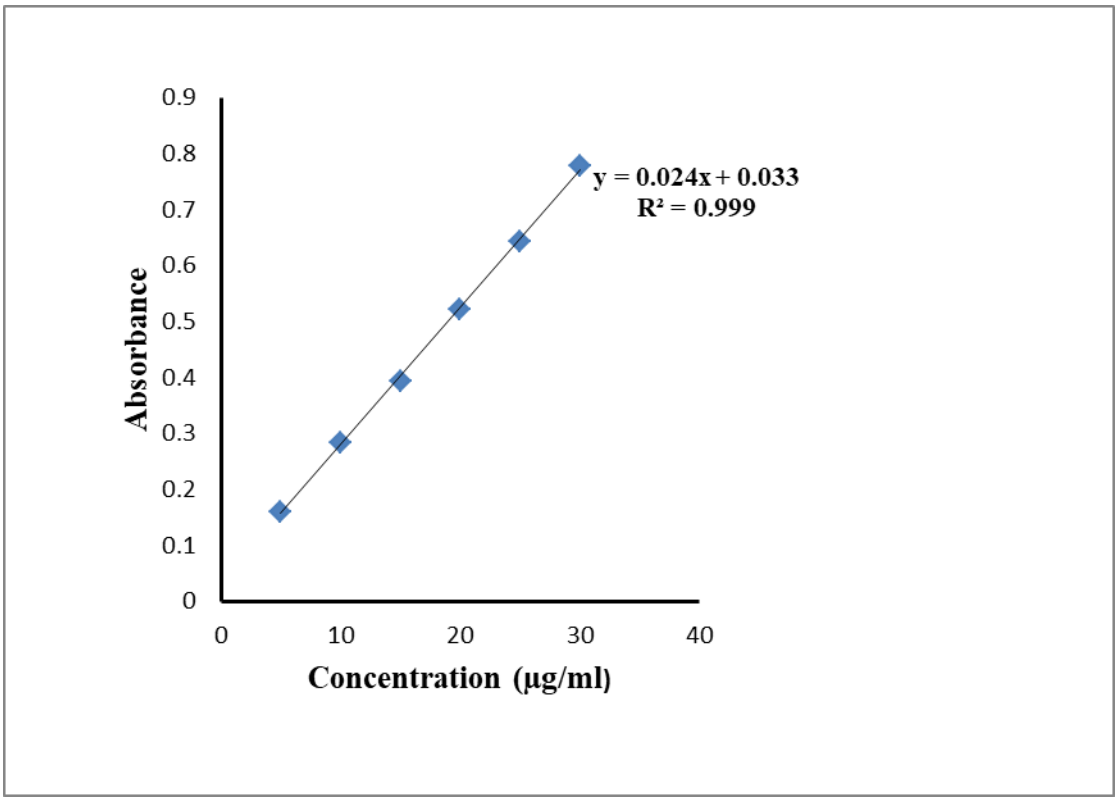

Fig 4 calibration curve of prednisolone acetate in STF

Linearity

The results of linear regression of prednisolone acetate solutions in methanol and STF over the range of (2-10) g/ml demonstrate high linearity and low standard deviation limits, indicating that the solutions are consistent. It was discovered that methanol was the linear regression equation. (y = 0.077x + 0.01 and R^2 = 0.999) and STF (Y=.024x+.033) and R^2 = 0.999 as shown in Table 3.

Table 3: Statistical Parameters for methanol and STF

Statistical Parameters	STF	Methanol
Average Abs*	0.164	0
	0.283	0.173
	0.394	0.33
	0.522	0.47
	0.643	0.62
	0.778	0.780
Concentration Range (µg/ml)	5-30	2-10
Straight Line Equation	y = 0.024x + 0.033	y = 0.077x + 0.01
Correlation Coefficient (R2)	0.999	0.999
LOD	1.733	0.304
LOQ	5.253	0.922

Accuracy

As shown in the table, the accuracy of re-analyzed prednisolone acetate solutions produced in methanol and STF was 98.28-103.5 percent and 100.41-106.39 percent, respectively. The findings of recovery trials indicate the method's efficacy shown in Table 4

Precision

Following six determinations, the Relative Standard Deviation (%RSD) was 2.61% at 2µg/milliliter for methanol and 2.20% at 5µg/milliliter for STF (see Table 4).

Table 4: Precision Data for methanol and STF

Precision	**Concentration((µg/ml))**		**RSD%**	
	STF	**Methanol**	**STF**	**Methanol**
Intraday	5	2	2.20	2.61
Intraday	5	2	0.922	1.72
Repeatability	5	2	2.20	2.61
Recovery				
Methanol	**Conc. Taken (µg/ml)**	**found conc.**	**RSD%**	**Recovery%**
	2	2.113	2.612	105.63
	4	4.134	1.373	103.35
	6	5.913	0.869	98.56
	8	7.870	0.744	98.38
	10	9.983	1.107	99.83
STF	5	5.39	2.49	107.8
	10	10.4	2.04	104.3
	15	15.1	0.94	100.4
	20	20.3	0.44	101.7
	25	24.7	2.48	98.7
	30	31.1	0.20	103.5

LOD and LOQ

LOD and LOQ were used to determine the sensitivity of the proposed approach, which were determined to be 1.733 and 5.253 in methanol and 0.304 and 0.922 in STF, respectively, as shown in Table 3.

Conclusion

This UV-spectrophotometric method is extremely easy, precise, reproducible, and sensitive. To test prednisolone acetate, the UV approach was devised. According to the validation technique, this is an appropriate method for quantifying them in the formulation. It is also used in the routine quality control of formulations that contain this entire component.

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