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Research Article

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Development and validation of UV spectroscopy method for the estimation of prednisolone in bulk and dosage form

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ABSTRACT

Four simple, sensitive, rapid and accurate UV spectroscopic method has been developed for the estimation of Prednisolone in bulk and pharmaceutical dosage forms. A simple UV spectroscopic method was developed and validated for the estimation of Prednisolone using the solvent acetonitrile: methanol in ratio of 30:70. The linearity range for Prednisolone was obtained as 4-14 µg/ml and its wavelength of detection was 246 nm. The method was validated based on ICH guidelines. It is simple, sensitive, and reliable and results are reproducible. Hence useful for the routine analysis of Prednisolone.

Key words: Prednisolone, acetonitrile, methanol, UV spectrophotometric, linearity, validation parameter.

INTRODUCTION

Prednisolone,(11ß)-11, 17, 21-trihydroxypregna-1, 4diene-3, 20-dione [fig.1] is a steroidal drug with predominant glucocorticoid and low mineral corticoid activity.

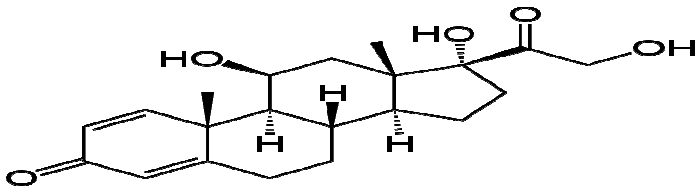

Figure 1 :Prednisolone (11ß)-11, 17, 21-trihydroxypregna-1, 4diene-3, 20-dione

It is mainly used for the treatment of a wide range of inflammatory and auto-immune diseases [1-2] suchas asthma, multiple sclerosis, rheumatoid arthritis, autoimmune hepatitis[3] etc. Prednisolone a very slightly water soluble glucocorticoid. So the potential of liquisolid systems to improve the dissolution properties of water-insoluble agents was investigated using prednisolone as the model drug in some study.[4] Prednisolone is also known as 'disease modifying anti-arthritic drugs' because of its anti-inflammatory [5] action by inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthetase [6]The aimof the present study is to develop two new simple, rapid, reliableand precise UV Spectrophotometric methods for analysis of Prednisolone. First method is based onmeasurement of UV absorbance of Prednisolone at 246 nm inmethanol: acetonitrile in ratio 70:30. The

main objective of the study is to develop and validate UV method so as to obtain an accurate, sensitive and precise for quantitative determination of prednisolone.

Validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical result. It is an integral part of any good analytical practice.[7]Prednisolonesoluble 1 in 30 parts of ethanol.Oral liquids of prednisolone, prednisolone sodium phosphate and prednisone are commercially available in many countries. Prednisone suspensions usually have a pH of 3 - 4.5 and contain 2 - 5% ethanol; solutions have a pH 2.6 - 3.6 and contain 4-6% ethanol. [8]There are many method such as HPLC, colorimetry, UV method were developed for the determination of Prednisolone in dosage form. [9-11]

The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy**,** Precision**,** Specificity**,** Limit of detection**,** Limit of quantitation, Linearity and range, Ruggedness**,** Robustness[12-15]As quality control process is not static some form of validation/verification should continue till the validated procedure is in use. It should not be a concept that once the method is initially developed and validated it is forgotten.

EXPERIMENTAL SECTION

Materials and Methods:
A UV method was developed for Predenisolone in bulk and pharmaceutical formulation in acetonitrile and methanol.

Reagents and chemicals:
Prednisolone: Pure drug.
Acetonitrile and Methanol as diluents in ratio of 30:70.

Stock solutions:
Preparation of standard stock solution of Prednisolone: (1000 µg /ml).
Stock solution of Predenisolone was prepared by weighing accurately 100mg of pure drug in to a 100ml volumetric flask and dissolved with acetonitrile: methanol in ratio of 30:70 to give a conc. of 1mg/ml.

Preparation of working standard solution of Prednisolone (100µg/ml).
The working solution of Prednisolone was prepared by further diluting the stock solution suitably with methanol to get a concentration of 100µg / ml.

Instrument:
All the experiments were carried out on SOMADZU UV-VIS1700 spectrophotometer using 1cm matched quartz cuvettes.

Parameter fixation:
Determination of λmax.
An absorption maximum or λmax is the wavelength at which maximum absorption takes place.

- **λmax of Predenisolone with diluents:**

The solution of Predenisolone were suitably diluted with diluent and subjected for determination ofλmax.Given in figure 2.

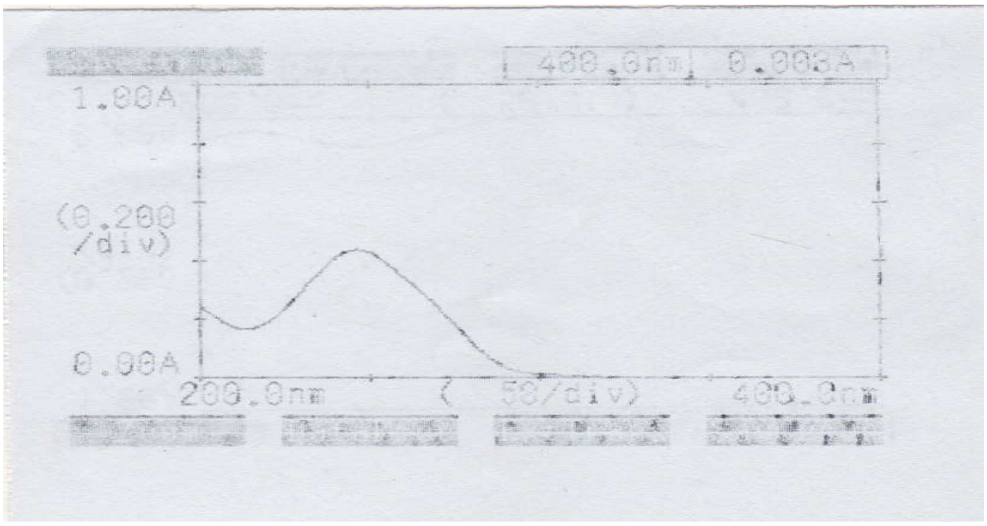

Figure 2: Predenisolone with diluents

Stability of sample:

The sample of 4µg/ml drug solution was prepared by suitable dilution with diluents and absorbance were taken at 246 nm against the blank. The stability of sample was found to be more than 10 hrs. As shown following Table no.1 and figure 3.

Table 1: stability of sample

Sr. No.	Concentration of drug solution (µg/ml)	Time (min)	Absorbance at 278 nm
1	4	0	0.172
2	4	30	0.172
3	4	60	0.171
4	4	90	0.170
5	4	120	0.170
6	4	240	0.169
7	4	360	0.167
8	4	480	0.166
9	4	600	0.165

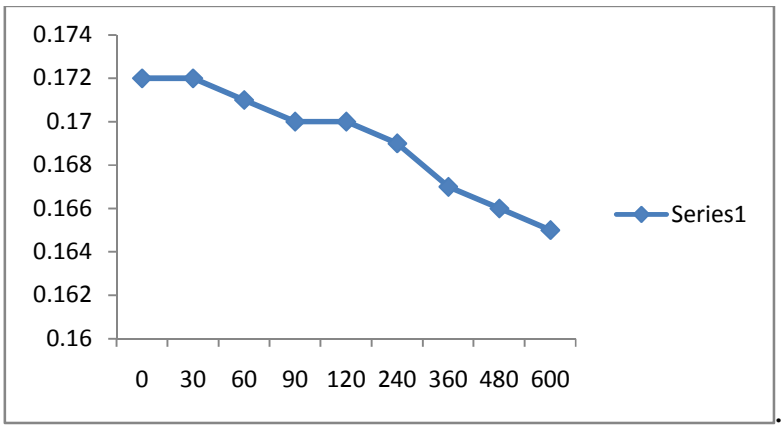

Figure 3: stability of sample

Linearity of samples:
From the working standard 0.4, 0.6, 0.8, 1, 1.2 and 1.4 ml of drug solution were placed in 6different 10ml volumetric flasks and volume was made up to the mark with acetonitrile: methanol mixture in ratio of 30:70 and their absorbance was measured against corresponding reagent blank at 246nm and result are recorded in table no.2 and figure no. 4. Linear response of drug over a range of 4-14µg/ml of the conc.

Table 2: Absorbance of different conc. of Predenisolone obeying beer's law

Sr. No	Concentration of drug taken (100 µg/ml)	Concentration Range (µg/ml) in 10ml	Absorbance At 246nm
1.	0.4 ml	4 µg	0.172
2.	0.6 ml	6 µg	0.27
3.	0.8 ml	8 µg	0.342
4.	1 ml	10 µg	0.439
5.	1.2 ml	12 µg	0.536
6.	1.4 ml	14 µg	0.638

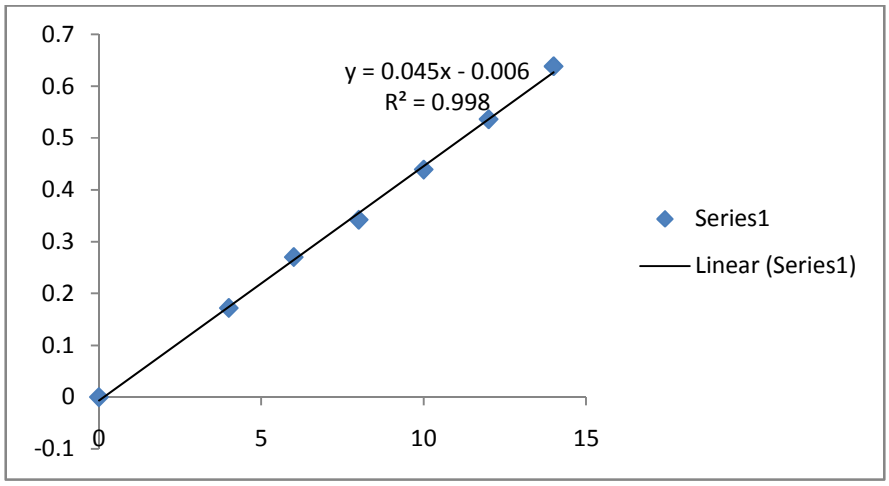

Figure 3: linearity of sample

Analysis of formulation:
Prednisolone was procured from the local market as tablets of strength 5 mg and marketed with brand name of WYSOLONE 5 and it was manufactured in India by Wyeth limited,Verna,Goa.. .

Preparation of solution:
20 tablets were weighed and crushed properly using a mortar and pestle. Then powder weight equivalent to 100mg was weighed and transferred to 100ml of volumetric flask and dissolved in acetonitrile: methanol mixture ratio in 30:70 and filter through whatmann filter paper in to another 100ml volumetric flask and make up to mark with same diluents which gives the solution of 1mg/ml conc., further dilution were performed to get a concentration of 100µg/ml. %Prednisolone is recorded in table 3.

Table 3: Assay Results of Marketed Formulation

Formulation	Actual concentration of Prednisolone (µg/ml)	Amount obtained of Prednisolone (µg/ml)	% Prednisolone
Tablet	5	4.9	98 %

Validation parameter:[7]
1. Linearity
A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance (by dilution of astandard stock solution) and using the proposed procedure.This method obeys the beer- lambert's law in the concentration range of 4-12 µg/ml. As given in table no.2.

2. Accuracy

Accuracy was established across the specified range of the analytical procedure.

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples. The results of determination of accuracy are given in table 4.

Table 4: Determination of Accuracy

Amt of sample Prednisolone µg/ml	Amt. of drug added Prednisolone µg/ml	Amt. of drug recovered Prednisolone µg/ml	% Recovery Prednisolone %
10	0	9.91	-
10	4	3.99	99.8
10	6	5.93	99.3
10	8	7.87	99.13

3. Repeatability :

Standard solutions of prednisolone (4, 6, 8, 10, 12 and 14 µg/ml) were prepared and a spectrum was recorded. Absorbance was measured at 246 nm taking the mixture of ammonium molybdate and water as the blank. The absorbance of the same concentration solution was measured six times and RSD was calculated. Repeatability data for prednisolone and sample of Prednisolone are recorded in table 5 and table 6.

Table 5: Repeatability data for Prednisolone at 246 nm

Concentration	4µg/ml	6µg/ml	8µg/ml	10µg/ml	12µg/ml	14 µg/ml
Absorption	0.172	0.27	0.342	0.439	0.536	0.638
	0.17	0.27	0.34	0.438	0.538	0.637
	0.17	0.273	0.342	0.435	0.544	0.638
	0.172	0.273	0.344	0.436	0.543	0.639
	0.173	0.269	0.345	0.435	0.534	0.635
	0.169	0.274	0.336	0.439	0.537	0.639
Mean.	0.171	0.271	0.341	0.437	0.538	0.637
Std. Dev.	0.001549	0.002074	0.003209	0.001897	0.003983	0.001506
Coefficient variation	0.0090	0.0076	0.0094	0.0043	0.0074	0.0023
% RSD	0.90	0.76	0.94	0.43	0.74	0.23

n = 6 determination

Table 6: Repeatability of sample application data for Prednisolone

Concentration	Prednisolone 5 µg/ml
Absorption	0.224
	0.221
	0.220
	0.221
	0.222
	0.224
Mean.	0.222
Std. Dev.	0.001673
Coefficient variation	0.0075
% RSD	0.75

n = 6 determinations

4. Limit of detection (LOD) & limit of quantitation (LOQ):

The limit of detection and quantification of the drugs were calculated with the standard deviation and slop. Its value described in table 7.

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

σ= standard deviation
s= slope of the calibration curve

Table 7: LOD AND LOQ

LOD	LOQ
0.16	0.53

5. Specificity and selectivity :
Prednisolone is specific and selective as given in table 8.

Table 8: Specificity and Selectivity study

Study	PREDNISOLONE
Specificity	Specific
Selectivity	Selective

6. Reproducibility :
Reproducibility is assessed by means of an inter-laboratory trial. The absorbance readings were measured at 246nm at different laboratory using anotherspectrophotometer and the values obtained were evaluated using t- test to verify their reproducibility.Reproducibility data for prednisolone at 246 nm is recorded in table 9.

Table 9: Reproducibility data for Prednisolone at 246 nm

Instrument 1 SIMADZU	Instrument 2 JASCO	Result of t test*	Inference
0.170 ± 0.001550	0.171 ± 0.001648	0.99	Not significant difference

** At 95% confidence interval*

7. Intra and inter day precision :
Variation of results within the day (intraday), variation of results between days (inter day) were analyzed. Intraday precision was determined by analyzing prednisolone for three times in the same day at 246 nm.

Inter day precision was determined by analyzing the drug different day for three days at 246 nm.Precision data for prednisolone at 246 nm is given in table 10.

Table 10: Precision data for Prednisolone at 246 nm

Conc. µg/ml	Intraday (n=3)	CV	% RSD
10	0.170 ± 0.001550	0.0091	0.91
20	0.271 ± 0.002074	0.0076	0.76
30	0.341 ± 0.003209	0.0094	0.94

Conc. µg/ml	Inter day (n=3)	CV	% RSD
10	0.169± 0.001520	0.0089	0.89
20	0.269 ± 0.001870	0.0069	0.69
30	0.340 ± 0.003100	0.0091	0.91

RESULTS AND DISCUSSION

In the methodprednisolone was estimated by using ultraviolet spectroscopic method. The method obeys beer-lamberts law in the concentration range of 4-14 µg/ml and its wavelength of detection was 246nm. Finally recovery studies were undertaken. The quantitative parameters for determination of prednisolone in pharmaceutical dosage form are listed in table 11.

Table 11: The quantitative parameters for determination of Prednisolone

Parameter	Result
λmax (nm)	246.0
Beer's law limits (µg/ml)	4-14
Molar absorptivity	4.366×10^{4}
Regression equation (y=bc+a) Slope (b) Intercept (a)	Y= 0.044+-0.0065 0.044 -0.0065
Correlation coefficient (r)	0.998
Relative standard deviation (%)	0.66
n	6

CONCLUSION

The proposed method is simple, selective and sensitive. The obtained and statistical parameters for determination of prednisolone that the proposed UV spectrophotometry method by is simple, accurate, fast and precise. The method showed acceptable linearity and accuracy. The proposed method is highly sensitive; therefore it could be used easily for the routine analysis of pure drugs and their vial formulations.

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