UV SPECTROPHOTOMETERIC METHOD DEVELOPMENT AND VALIDATIONFOR ESTIMATION OF PROGESTERONE IN BULK AND TABLET DOSAGE FORM

[1]Prof. Deepak Bhosale, [1]Lagamanna Koli*.

[1] Student, Department of Pharmaceutics,

Dakshin Solapur Taluka Shikshan Mandal's College of Pharmacy, Solapur-413004, Maharashtra, India.

[2] Professor, Department of Pharmaceutics,

Dakshin Solapur Taluka Shikshan Mandal's College of Pharmacy, Solapur -413004, Maharashtra, India.

ABSTRACT:

Objective: The objective of the present work is to develop a new, simple, sensitive and precise UV spectrophotometric method for Progesterone in bulk and pharmaceutical formulation as per ICH guidelines.

Method: The UV spectrophotometric method has been developed using by Dimethylformamide : Methanol as solvent to determine the Progesterone in bulk and pharmaceutical formulation. The λ_{max} of Progesterone in Dimethylformamide: Methanol was found to be 322.4 nm.

Results: The drug was proved linear in the concentration range of 2-10 µg/ml and regression coefficient was found to be 0.9994. The LOD and LOQ of Progesterone was found to be 0. 776063 and 2.3571 respectively. This method was successfully applied to Progesterone in marketed formulation and results were in good agreement with label claims.

Conclusion: Depending on the results, the given method can be successfully applied for assay of Progesterone in Tablet formulation.

KEYWORDS:

Progesterone, Validation, Specificity, LOD, LOQ

Introduction

Progesterone is a steroid hormone with important functions to reproduction. Drugs with progestogens are used in humans for endometrial protection, dysfunctional exploiting, treatments in pre- or postmenopause, pregnancy maintenance in helped reproduction treatment, and prevention of premature birth. In veterinary drug, exogenous progesterone is used specially for cattle in fixed-time artificial insemination protocol, aimed at the synchronization of estrus in women and improvements in fertilization rates. The usage of estrus cycle control methods, besides facilitating the management of livestock, allows expanding the usage of artificial insemination, accelerating genetic enhancement and bringing improvements to the production of meat and milk.[1-4]
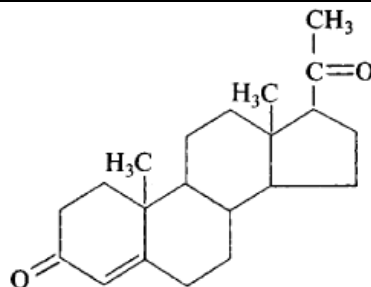
Figure 1 Chemical structure of Progesterone

Molecular formula and weight of Progesterone is $C_{21}H_{30}O_2$ and 314.469g/mol respectively. Chemical name of Progesterone. Progestogen is insoluble in water but soluble in DMF, chloroform, ethyl ether, ethanol, ethyl acetate. The aim of this study is to give a new, simple, sensitive, precise and reproducible UV spectroscopic method was developed for Progesteron in Tablet formulation.

MATERIALS AND METHODS:

Materials:

Progesterone was obtained as gift sample, Dimethylformamide and Methanol used in validation were of laboratory grade.

Instruments**:**

Sonicator (Microclean-1103), UV-Visible spectrophotometer (Systronic 2201), Analytical balance (Shimadzu AY220).

Experimental:

Preparation of standard stock solution:

Accurately weighed 10 mg of Progesterone was transferred to a 10 ml volumetric flask; dissolved in Dimethylformamide: Methanol (70:30) and volume was made upto the mark with Dimethylformamide : Methanol (70:30). (Conc: 1000μg/ml)

Working Standard:

Add 0.1 ml of standard stock solution in 10 ml volumetric flask and add 5 ml of Dimethylformamide : Methanol (60:40), mix for 2 min and make up the volume upto 10 ml with Dimethylformamide : Methanol. (Conc: 10 μg/ml) Selection of analytical wavelength was done by scanning above solution in the range 200-800 nm

Procedure for plotting calibration curve:

For calibration curve, in a series of 10 ml volumetric flasks, 0.2, 0.4, 0.6, 0.8 and 1ml of standard stock solution of 100μg/ml was pipetted out separately and the volume was made upto the mark using Dimethylformamide : Methanol (60:40). The absorbance was measured at wavelength 322.4 nm against the blank solution.

A.   Sample stock solution:
20 Tablet content were weighed and mix them in mortar and pestle. Powder weight equivalent to 10 mg Progesterone was weighed and transferred into the 10 ml volumetric flask and add 5 ml of Dimethylformamide : Methanol (70:30), sonicate for 10 minutes and make the volume to 10 ml with Dimethylformamide : Methanol. (Conc: 1000μg/ml)

B.   Sample solution:
0.5 ml of above solution was then transferred into a 10 ml volumetric flask and 5 ml of Dimethylformamide : Methanol was added, sonicate for 10 minutes and make the volume upto 10 ml with the Dimethylformamide : Methanol and analysed at 322.4 nm. Then % purity of Progesterone was calculated. (Conc: 50 μg/ml)

RESULTS AND DISCUSSION:

The absorption spectrum shows λ_{max} of Progesterone at 322.4 nm. The proposed method was validated according to ICH Q2 R1 guidelines for validation of analytical procedure.
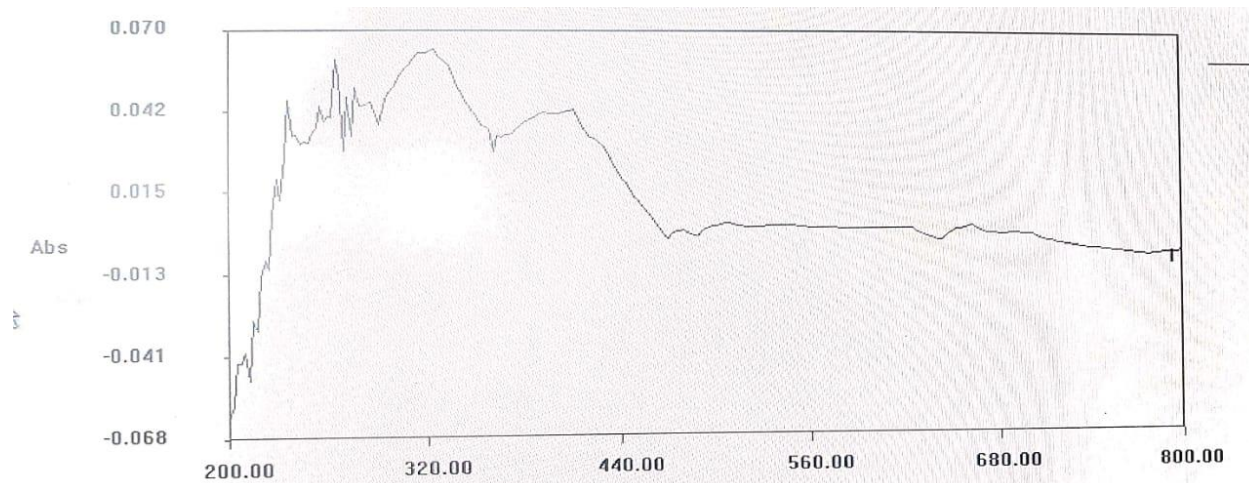

Figure 2. UV scan of Progesterone

1. **Linearity:[5]**

Five different concentrations of Progesterone were prepared and analysed at wavelength 322.4 nm. The regression coefficient was found to be 0.9994. The absorbance was found in limit i.e. 0-1. (table no 1)

Table 1

Sr.No	Conc (µg/ml)	Abs
1	2	0.113
2	4	0.231
3	6	0.354
4	8	0.462
5	10	0.591

ABS   y = 0.0593x - 0.0058
        R² = 0.9994
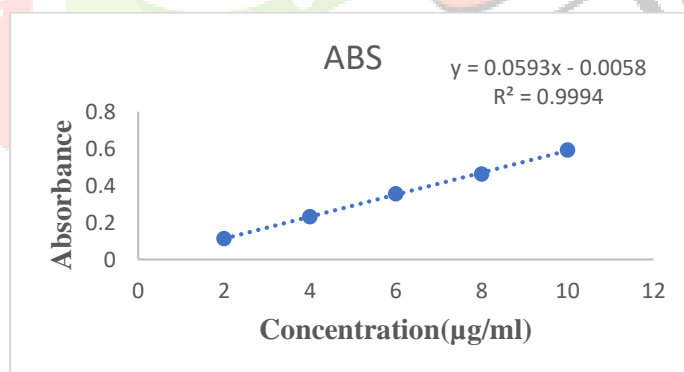
Fig 3. Linearity

Table no 2: Optimization parameters of Progesterone

Parameters	Method values
Maximum Wavelength	322.4
Beers law	2-10µg/ml
Correlation Coefficient	0.9994
Regression Equation	Y=0.0594x + 0.0059
Slope	0.0594
Intercept	0.0059

2.   **Accuracy:**

The concentration 2,4,6µg/ml was taken as 50,100,150% and % recovery was found to be in range 99%-101%. Henceforward the parameter was found to be validated.

Table 3: Results of Accuracy

Name of Drug	Recovery Level in %	Concentration	Amount Recovered	% recovery with SD
Progesterone	50	2µg/ml	2.01	100.8±0.577
	100	4µg/ml	4.03	100.75±0.25
	150	6µg/ml	5.97	99.6±0.096

3.   **Range:**

Range is an interval between maximum and lowest concentration limit of the analyte i.e. 2-10 µg/ml.

4.   **Precision:**

In precision intra-day and inter-day precision were performed at concentration (6µg/ml). The obtained results were found within limit i.e., less than 2%RSD.

Table 4: Results of Intra-day Precision

Sr. no.	Concentration	Absorbance
1		0.354
2		0.356
3	(6µg/ml)	0.358
4		0.353
5		0.351
6		0.355
SD		**0.00243**
%RSD		**0.68519**

Table 5: Results of Inter-day precision

Sr.no.	Concentration	Absorbance (Day1)	Absorbance (Day2)
1		0.354	0.355
2		0.356	0.355
3	(6µg/ml)	0.358	0.355
4		0.353	0.354
5		0.351	0.354
6		0.355	0.351
SD		**0.00243**	0.00154
%RSD		**0.68519**	0.4376

5.   **Limit of Detection (LOD):**

It was calculated by ANOVA technique. The limit of detection was found to be 0.776063 µg/ml.

6.   **Limit of Quantification (LOQ):**

The limit of quantification was found to be 2.3517µg/ml.

7.   **Ruggedness:**

The change in analyst with same environmental and concentration condition didn't affect the results.

Table 6: Results of Ruggedness

Concentration	Absorbance (Analyst1)	Absorbance (Analyst2)
10μg/ml	0.591	0.592
	0.589	0.593
	0.592	0.589
	0.591	0.59
	0.59	0.591
	0.591	0.592
Average	**0.590667**	0.591166667
SD	**0.001033**	**0.00147196**

Assay:

Sample solution of concentration 50 μg/ml was analysed at wavelength 322.4 nm and the % purity was calculated.

Table 7: Result of Assay

Formulation	Labeled Amount	Amount obtained	% purity
Prominal 100mg Tablets	1 mg	99.48	99.48%

CONCLUSION:

An analytical UV spectrophotometric method was developed & validated thoroughly for quantitative determination of Progesterone in Tablet Formulation. The presented method was found to be simple, precise, accurate, and reproducible gives an acceptable recovery of the analyte.

ACKNOWLEDGEMENT:.

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