UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PROGESTERONE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT:

Objective: The objective of the present work is to develop a new, simple, economical, precise, sensitive, linear, accurate, rapid UV Spectrophotometric method has been developed for the estimation of Progesterone in bulk and pharmaceutical formulation as per ICH guidelines.

Method: Spiked Progesterone arrangement was checked over UV-visible extends for its wavelength of greatest absorbance.

Results: The wavelength of most extreme absorbance for Progesterone was found to be 241.6 nm. The relationship coefficient over the concentration extend of 3-15µg/ml was found to be 0.9982. The LOD and LOQ of Progesterone were found to be 30.8722 and 93.5521 respectively. The 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 of Progesterone in Tablet formulation.

KEYWORDS: Progesterone, Methanol, UV-Visible Spectrophotometric method, Development, Validation.

INTRODUCTION:

Progesterone is a C-21 steroid hormone which one comes under important functions to reproduction. Progesterone chemically Known as P4(prgen-4-ene-3,20-dione) Drugs with Progesterone are used in humans for endometrial protection, dysfunctional exploiting, treatments pre- or- post menopause, pregnancy maintenance is helped for a reproduction treatment and prevention of premature birth .in veterinary drug, exogenous progesterone this is used special for a cattle in fixed- time artificial insemination protocol, aimed at the synchronization of estrus in women and improvement the Fertilization rates. The usage of estrus control the methods, besides facilitating the management of livestock, allows expanding usage of artificial insemination , accelerating genetic enhancement and bringing to the production of a meat and milk[1] . Progesterone is described chemically as its molecular formula $C_{21}H_{30}O_2$ its molecular weight 314.469g/mol. Respectively it is insoluble in water but soluble in Ethanol, Methanol, ethyl ether ,ethyl acetate .

The Aim of this study is to give a new sample, sensitive, precise and reproducible UV spectroscopic Method was developed for progesterone in tablet .progesterone is a yellowish substance.[2] The Structural formula is shown fig. 1.[3]
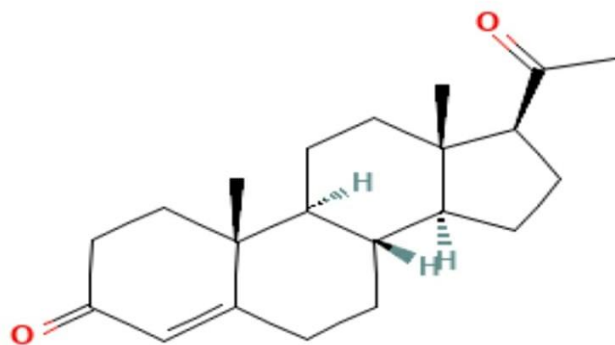
Fig.1: Chemical structure of Progesterone

Progesterone is a hormone used for a variety of functions including in that contraception , control of abnormal uterine pain, bleeding ,its maintain the pregnancy ,and prevention of endometrial hyperplasia.[4] A hormone that occurs natural in females ,and is essential for endometrial receptivity, for an embryo implantation , and its successful establishment of pregnancy. A low concentration of progesterone it's an insufficient response to the progesterone can cause infertility and Pregnancy loss is occur. [5] it is used in a various contraceptive preparations to prevent ovulation and then fertilization is occur.[6]in other formulations to promote and support pregnancy, see the Medroxyprogesterone acetate, Megesterol Acetate, Dydrogesterone and Hydroxyprogesterone

entries for information on various other forms of a progesterone. Is that made for a Pharmaceutical plant source like a starting material. It is chemically identified to progesterone of a human ovarian origin.

Is that available various a dosage form gelatinized capsules, vaginal gel, tablet form, vaginal insert form, and injectable form also available. All are used for a various purposes.[7,8,9,10.]

From literature review it's found that one method was reported on derivative spectrophotometry for simultaneous estimation of progesterone with degrading product on UV for impurity in Progesterone[11] Lot of work was done on UV method development for Progesterone in combination with other drugs[12,13]. There is also method reported HPLC, RP-HPLC,TLC of Progesterone with other drug on HPLC [14,15]. There is also method reported Bioanalytical LCMS method of Progesterone on human plasma [16]. But very few methods were reported on estimation of Progesterone in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Progesterone in tablet dosage forms. The aim of the study was to develop a simple, precise, linear, economic and accurate UV method for determination of Progesterone in tablet dosage forms.

Several methods have been reported in literature for the determination of Progesterone in the presence of other drugs which includes UV[17,18], HPLC-UV for stability indicating assay, impurities, in human plasma [19,20], HPLC- Fluorimetric detection[21,22], HPTLC[23], LC-MS[24,25]

The aim of present work was to develop simple, sensitive, specific spectrophotometric method for detection of Progesterone in bulk as well as pharmaceutical formulation.

MATERIALS AND METHOD:

Materials:

Progesterone was obtained as a gift sample from Sun pharma Industry, PVT,LTD.Panoli,Gujrat India. Tablets of Susten SR200 were purchased from local market; each tablet was labeled to contain 200 mg. All chemicals and regents were of analytical.

Instruments:

A Double beam UV Visible Spectrophotometer (Systronic-2201 Spectrophotometer) was used for the detection of absorbance, Sonicator (Microclean-1103), and Weighing Balance (SHIMADZU AY220) used for experimental purpose.

Chemical and Reagent:

Methanol, distilled water, Whatman filter paper were used.

Experimental Work:

METHOD DEVELOPMENT:

Selection of solvent:

Solubility of Progesterone was performed in solvent methanol and UV spectra of drug in this solution were recorded. The absorbance value of drug was higher at λ_{max} with methanol as a solvent. Hence, methanol was selected as a solvent for further investigation as it is more economical.

Determination of Wavelength:

An UV Spectrophotometric scanning (200-400) was carried out to select the wavelength (λ_{max}) for detection of Methanol, because of every drug should have adequate absorbance in the same solvent for the simultaneous determination.

Preparation of Standard stock solution:

Accurately weighed 10mg of Progesterone was transferred in to a 10 ml volumetric flask; dissolved in methanol and volume was made up to the mark with methanol.(Concentration 1000µg/ml). Further pipette out 1ml of Progesterone stock solution into a 10ml of volumetric flask and dilute up to the mark with diluents (conc.100µg/ml)

Preparation of Sample Stock Solution:

20 Tablet content were weighted and mix them in mortar pestle. Powder equivalent to 10 mg Progesterone weighted and transferred into the 10 ml volumetric flask and 5 ml of methanol sonicate for 10 minutes and make the volume to 10ml with methanol. Further pipette out 1 ml of Progesterone stock solution into a 10 ml of volumetric flask and diluted up to the mark with diluents. (Conc.100µg/ml).

Preparation of Calibration curve:

For calibration curve is the range of Concentration3-15 µg/ml.

Method Validation:

The developed method was validated as per ICH guidelines. The parameter ICH assessed were specificity, linearity, range, accuracy, precision (repeatability), LOD and LOQ.

1. **Linearity:**

    Five different concentration of Progesterone solutions were prepared and analyzed at wavelength 241nm. The regression coefficient was found to be 0.9982. The absorbance was found in limit,   in the limit 0-1 i.e.3-15µg/ml

2. **Range:**

The range of analytical method was decided for Progesterone (3-15µg/ml)

3. **Accuracy :**

The accuracy was determined by calculating % recovery of progesterone. It was carried out by adding known amounts of analyte corresponding to the concentration levels 80, 100, and 120% and results were expressed as % recovery.

4. **Precision:**

The precision of analytical method was studied by performing repeatability studies were carried out by estimating responses of working standard solution (Conc. Of Progesterone: 12µg/ml) for 6 times. The results were reported in terms of percentage relative standard deviation (%RSD)

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

LOD is the lowest amount of analyte in sample that can be easily but not necessarily quantified. LOD was calculated by following formula.

$LOD = 3.3 \times S_O / b$

Where S_o and b are the standard deviation of the response and the slope of the calibration curve.

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

LOQ is the lowest amount of analyte in sample that can be easily detected and quantified with suitable precision and accuracy. LOD was calculated by following formula.

$LOQ = 10 \times S_O / b$

**Assay:** Assay of tablets (Sample solution) was also calculated.

RESULT AND DISSCUSSION:

Selection of solvent

Solubility of Progesterone was performed in solvent methanol and UV spectra of drug in this solution were recorded. The absorbance value of drug was higher at λ_{max+} with methanol as a solvent. Hence, methanol was selected as a solvent for further investigation as it is more economical

Determination of Wavelength:

An UV Spectrophotometric scanning (200-400) was carried out to select the wavelength (λ_{max}) for detection of Progesterone, because of every drug should have adequate absorbance in the same solvent for the simultaneous determination. The wavelength of most extreme absorbance for Progesterone was discovered to be 241.6 nm.

DOUBLE BEAM SPECTROPHOTOMETER: 2201
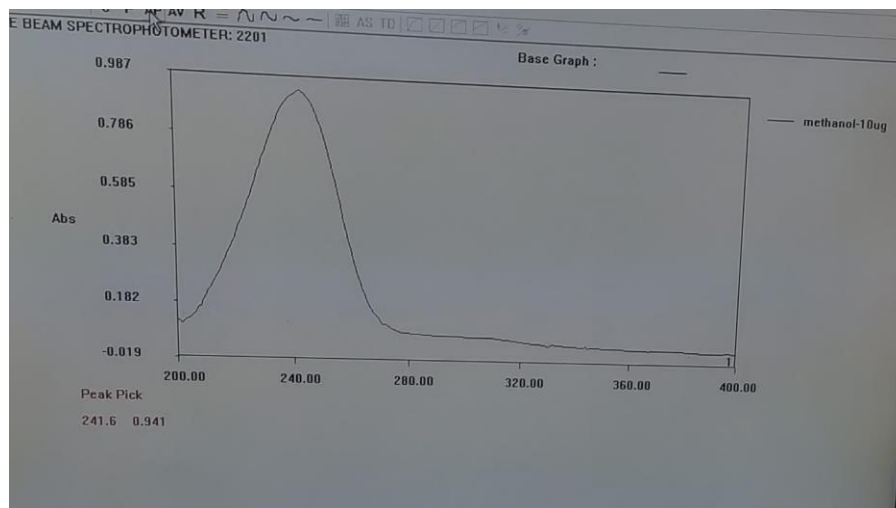
Fig: 2 UV –visible spectra of Progesterone.

Validation of method as per ICH guidelines:

1. Linearity:

Six different concentration of Progesterone were prepared and analyzed at wavelength 241.6 nm. The regression coefficient was found to be 0.9982nm. The absorbance was found in limit i.e. 0-1. (Table no 01).

Sr.No.	Concentration	Absorbance
1.	3	0.203
2.	6	0.418
3.	9	0.596
4.	12	0.780
5.	15	0.949
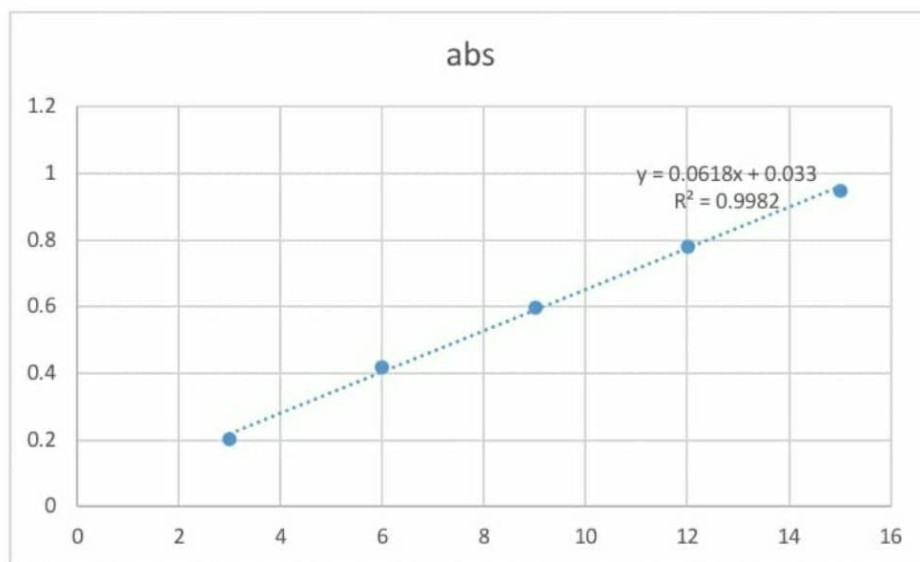
Fig 2. Calibration curve for Progesterone

Table: 2 Optimization parameter of Progesterone

Parameter	Method values
Wavelength detection	241.6nm
Beers law	3-15
Correlation Coefficient	0.9982
Regression coefficient	Y=0.0618x+0.033
Slope	0.0618
Intercept	0.033

2. Accuracy:

The concentration 10µg/ml was taken as 100 % recovery was found to be range 98%-101%. Hence forward the parameter was found to be validated.

Table 3:Result of accuracy

Name of Drug	Recovery Level in %	Concentration	Amount Recovered	% recovery with SD
Progesterone	80			**98.8**
	100%	10µg/ml	9.97	**99.08**
	120%			**100.8**

3. Range:

Range is an interval between maximum and lowest concentration limit of the analyte3-15µg/ml.

4. Precision:

In precision intra-day precision were performed at concentration (12µg/ml). The obtained results were found within limit i.e. less than 2% RSD.

Table 4: Result of intra-day precision

Sr. no.	Concentration	Absorbance
1		0.784
2		0.782
3		0.784
4	(12 µg /ml)	0.785
5		0.784
6		0.786
	SD	**0.001329**
	%RSD	**0.001695**

Table 5: Result of Inter- day precision

Sr. no	Concentration	Absorbance (Day 1)	Absorbance (Day 2)
1		0.882	0.976
2		0.884	0.979
3	(12 µg/ml)	0.884	0.979
4		0.882	0.981
5		0.881	0.978
6		0.880	0.980
SD		**0.001602**	**0.001722**
%RSD		**0.001816**	**0.00176**

5. Limit of Detection (LOD):

The limit of detection was found to be 30.8722 µg/ml.

3. Limit of Quantification:

The limit of quantification was found to be 93.5521µg/ml.

7. Assay:

Sample solution of concentration 10 µg/ml was analysed at wavelength 241nm and the % purity was calculated.

Table 7: Result of Assay

Formulation	Concentration(µg/ml)	Amount obtained(µg/ml)	% Purity
Susten SR 200mg Tablets	10	9.8	98

Conclusion:

An analyte UV spectrophotometric method was developed and validated thoroughly for quantitative determination Progesterone in Tablet formulation. The presented method simple, accurate, precise, easy, economic, reproducible gives an acceptable recovery of the analyte.

Acknowledgements:

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