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Development and Validation of the UV-Spectrophotometric Method for Determination of Progesterone in Bulk and in Formulation

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ABSTRACT

Objective: The goal of the current work is to provide a brand-new, affordable, exact, sensitive, linear, accurate, and quick UV Spectrophotometric method for the determination of progesterone in bulk and pharmaceutical formulation in accordance with ICH requirements. **Method:** Ethanol is used as a solvent and the absorbance of the drug was measured at $\lambda_{max.}$ **Results**: The maximum absorbance of progesterone obtained at wavelength of 240 nm. Calibration curve plotted in range of 2-10 µg/ml show the linearity with the regression coefficient of 0.998 and the equation y = 0.0393x + 0.0819. The accuracy was found to be in the range of 97.9-98.9%, the intra-day and inter-day precision %RSD value was 0.27 & 0.57 respectively and the LOD and LOQ were 0.0185 µg/ml& 0.61 µg/ml respectively. **Conclusion:** The method was found to comply all the validation parameters as per ICH guidelines.

INTRODUCTION:

A steroid hormone, progesterone plays crucial roles in reproduction. Humans utilize progesterone-containing medications for endometrial protection, dysfunctional exploitation, pre- or postmenopausal therapies, pregnancy maintenance in assisted reproduction therapy, and prevention of preterm delivery. Exogenous progesterone is utilized specifically for cattle in fixed-time artificial insemination protocols in veterinary medicine with the goal of synchronizing female estrus and enhancing fertilization rates. In addition to making livestock management easier, the use of estrus cycle control methods speeds up genetic modification, increases meat and milk production, and expands the use of artificial insemination, speeds up genetic improvement, and increases the production of meat and milk. Chemically, progesterone is characterized by its molecular weight and molecular formula, $C_{21}H_{30}O_2$.

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.

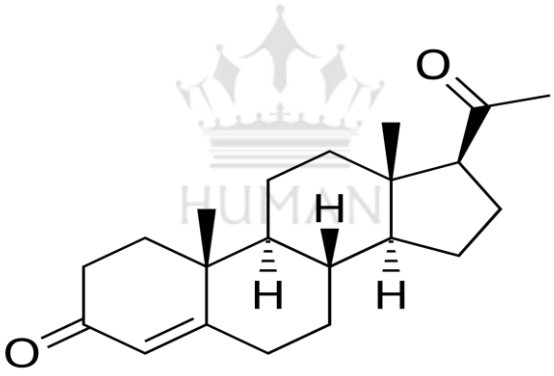

Fig. No. 1: Chemical Structure of Progesterone

It is available in a variety of dose forms, including gelatinized capsules, vaginal gel, tablets, vaginal inserts, and injectable forms. Each is employed for a different purpose. One approach was described on derivative spectrophotometry for simultaneous measurement of progesterone with degradation product on UV for impurity in progesterone, according to a study of the literature. Lots of study has been put into developing UV methods for progesterone when combined with other medications. Progesterone with other drugs has been documented using HPLC, RP-HPLC, and TLC on HPLC. Additionally, a bioanalytical LCMS method of progesterone analysis on human plasma has been published. However, relatively few techniques for estimating progesterone in tablet dose form for UV spectroscopic approach were published.

MATERIALS AND METHOD:

Materials and Reagents

Progesterone was received from Sun pharma Industry, PVT, LTD. Panoli, Gujarat, India as a gift sample. Whatman filter paper, distilled water and ethanol are used.

Instruments:

The instruments used are Weighing Balance (SHIMADZU AY220), Sonicator (Microclean-1103), and Double Beam UV Visible Spectrophotometer (Systronic-2201 Spectrophotometer) were used for the method development.

METHOD DEVELOPMENT:

Selection of solvent:

Solubility of progesterone was checked in different solvent like water, ethanol, methanol, DMF and progesterone was soluble in ethanol.

Preparation of Standard stock solution:

Progesterone 10 mg was added into a 10 ml volumetric flask and mixed with enough ethanol to dissolve it and dilute up to the mark. (1000 µg/ml).1ml of the stock solution was diluted up to 10mlwith ethanol to get a concentration of 100 µg/ml.

Preparation of Sample Stock Solution:

The contents of 20 tablets were weighed and combined in a mortar and pestle. 10 mg of weighed progesterone powder was transferred to 10 ml volumetric flask with 5 ml of ethanol, which was then sonicated for 10 minutes and dilute up to mark. Pipette 1ml of the stock solution was diluted up to 10ml with ethanol to get a concentration of 100 µg/ml.

Determination of absorption maxima (λmax)

Scan the 100µg/ml concentration of standard solution over the range of 200-400 nm. Measure the wavelength at which progesterone shows maximum absorption (λmax). Hence the maximum absorption was found at 240 nm.

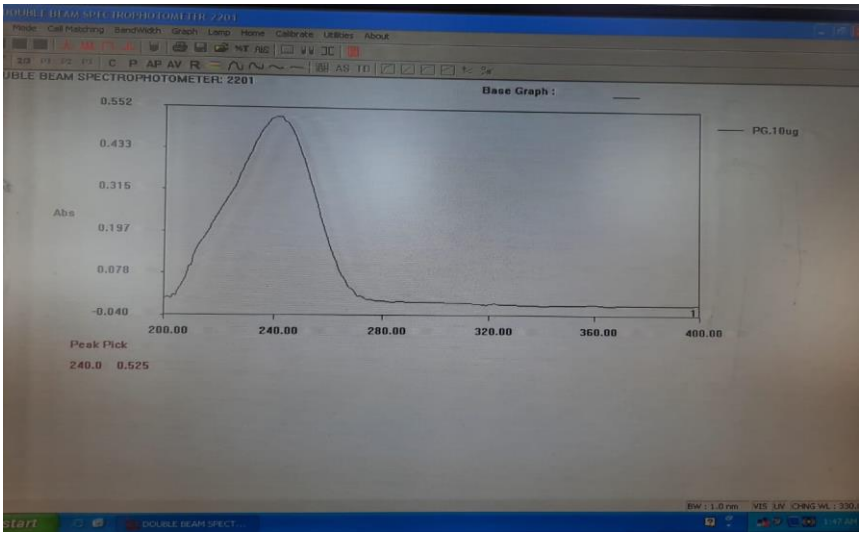

Fig. No. 2: Scan analysis of Progesterone

Method Validation:

The developed method is validated according to ICH guidelines(Q2R1). The parameters assessed were LOD, LOQ, accuracy, precision (repeatability), range, linearity, and specificity.

1. Linearity and Range

At a wavelength of 240 nm, five different concentrations of progesterone solutions were made and examined. The regression coefficient was found to be 0.9988 within the range of 2-10 µg/ml. Pipette out 0.2, 0.4, 0.6, 0.8, 1 ml from the standard solution of 100 µg/ml and transfer to 10 ml volumetric flask to get the concentration of 2,4, 6, 8, 10 µg/ml respectively.

2. Accuracy:

By calculating the progesterone recovery percentage, the accuracy was ascertained. Results were expressed as a percentage recovery after adding known amounts of analyte to concentration levels of 80, 100, and 120%.

3. Precision:

The reproducibility of the analytical method was investigated by estimating the responses of the working standard solution (Conc. of Progesterone: 10 µg/ml) six times. The findings were presented as percentages of relative standard deviation (%RSD).

4. Limit of Detector (LOD):

LOD is the smallest amount of analyte in a sample that is readily quantifiable but not always. LOD was computed using the formula below.

$$LOD = 3.3SO/b$$

Where so and b are the response's standard deviation and calibration curve's slope, respectively.

5. Limit of Quantification (LOQ):

The lowest amount of analyte in a sample that can be quickly and accurately identified and quantified is known as the limit of quantification, or LOQ. LOD was computed using the formula below.

$$LOQ=10\ SO/b$$

RESULTS AND DISSCUSSION

Validation of method as per ICH guidelines:

1. Linearity:

Table No. 1: Result of linearity

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.158
2.	4	0.245
3.	6	0.312
4.	8	0.398
5.	10	0.474

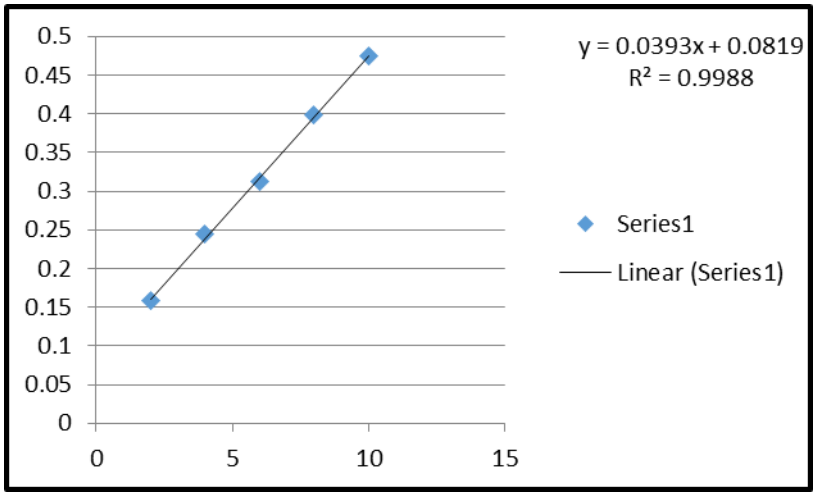

Fig. No. 3: Calibration curve for Progesterone

Table No. 2: Optimization Parameter of Progesterone

Parameter	**Method values**
Wavelength detection	240nm
Beers range	2-10
Correlation Coefficient	0.9988
Regression coefficient	Y=0.0393x+0.0819
Slope	0.0393
Intercept	0.0819

2. Accuracy:

Accuracy was calculated at three levels these are 80%, 100%, 120% by standard addition method. Accuracy was found to be 97.9% - 98.9%.

Table No. 3: Result of Accuracy

Sr. No.	% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	80	8	7.85	98.1
2	100	10	9.89	98.9
3	120	12	11.75	97.9

3. Range:

Progesterone shows linearity in the range of (2–10 µg/ml).

4. Precision:

Precision is defined as an analytical procedure is to define the closeness of agreement between a sample of measurements obtained from multiple sampling of the same homogenous sampling in specific conditions. Precision was determined by taking six readings of 10µg/ml concentration intra-day and inter-day. The relative standard deviation (% RSD) was calculated.

Table No. 4: Result of intra-day precision

Sr. No.	**Concentration**	**Absorbance**
1	(10 µg /ml)	0.889
2		0.882
3		0.887
4		0.885
5		0.884
6		0.886
Mean		**0.8855**
SD		**0.002429**
% RSD		**0.274307**

Table No. 5: Result of Inter-day precision

Sr. no	**Concentration**	**Absorbance (Day 1)**	**Absorbance (Day 2)**
1	(10 µg/ml)	0.889	0.918
2		0.882	0.916
3		0.887	0.921
4		0.885	0.914
5		0.884	0.929
6		0.886	0.917
Mean		**0.8855**	**0.919167**
SD		**0.002429**	**0.005345**
% RSD		**0.274307**	**0.579066**

The % RSD value for intra-day and inter-day precision is < 2% (0.274 & 0.579 respectively) which is in the limit hence the precision parameter is validated.

5. Limit of Detection (LOD):

The minimum amount of progesterone to detect was found to be 0.0185µg/ml.

6. Limit of Quantification:

The minimum amount of progesterone to quantify was found to be 0.61µg/ml.

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.

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