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Review Article

Estimation of Progesterone in Oil based Injection by UV- Visible Spectroscopy.

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ABSTRACT
Progesterone is steroidal hormone belongs to class of Progestogens. Progesterone is widely used for hormone replacement therapy. Literature reveals various chromatographic methods for the detection of progesterone in combination with various other steroidal drugs. Therefore it is necessary to develop method for rapid, simple and accurate method for estimation of progesterone as single component from oil based injection was elaborated. In proposed method calibration curve was obtained with correlation coefficient, 0.996. 2- Propanol was found to be suitable solvent for extraction of progesterone from oil based injection. UV detection was carried out at 244nm. Developed method was validated for precision, accuracy and linearity.

KEYWORDS
Progesterone, Analysis in injection, UV spectrophotometric analysis.

1. INTRODUCTION

Progesterone, pregn-4-ene-3, 20-dione is a C-21 steroid hormone belongs to class Progestogens and involved in the female menstrual cycle. The therapeutic dose is 100-200 mg of progesterone. Progesterone is widely used in hormone therapy in injection form and therefore it is necessary to establish a simple and accurate method for its identification and quantitative determination in pharmaceuticals. The literature reveals that gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS)[1-5], and high performance liquid chromatography (HPLC)[6-8] have been used for the simultaneous determination of progesterone, 17-hydroxyprogesterone and other 3-keto steroids.

In addition, detection of progesterone from capsule and injection dosage form by chromatographic technique have been reported.[9,10] However, no single method has been developed for estimation of progesterone as single component in injection dosage form by spectroscopic technique. In the present study, new, simple and selective UV spectrophotometric method was elaborated for the determination of progesterone in commercial oil based injection.

2. MATERIALS AND METHODS

2.1. Reagents

Micronized progesterone was obtained as gift sample from Puremed Biotech (Gujarat, India). Susten 100 mg was used as commercial formulation for the analysis. 2- Propanol was obtained from Molychem. All the other reagents were of analytical grade.

2.2. Spectrophotometry

The absorbtivity of Progesterone in 2- propanol was examined in the range of 200-400 nm and wavelength of maximum absorption was recorded. Progesterone exhibits λ_{max} at 244 nm.

2.3. Preparation of calibration curve in 2- propanol

Stock solution was prepared by dissolving 10 mg of Micronized progesterone in 100 ml of 2-propanol. From stock solution 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8 ,2 and 2.2 ml was transferred to volumetric flask and diluted to 10 ml using 2- propanol, to get final concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 µg/ mL. Each of the prepared solution was examined three times and calibration curve was obtained by plotting absorbance against concentration of each dilution. Regression equation was obtained using calibration curve.

2.4. Assay in dosage form

Accurately weighed amount of Progesterone oily injection equivalent to 100 mg of progesterone was transferred to test tube containing 10 ml of 2- propanol, and, after agitation for 10 minutes in an ultrasonic bath, stored at 4^0 C. The resultant was successively centrifuged for 15 min at 3000rpm. Finally, 10 µg/mL solutions were prepared after passing the supernatant through a cellulose acetate syringe filter (0.20 µm) & absorbance is recorded at λmax 244 nm.[11]

2.5. Precision and accuracy

Measurements for lower, middle and higher concentrations were repeated three times within the day to determine repeatability (intra-day precision), results for the study are summarized in Table 2. The percent relative standard deviation (% RDS) values were calculated. Recovery was calculated to investigate accuracy of the proposed method. The results for accuracy are summarized in Table 3.

3. RESULTS AND DISCUSSION

The proposed UV-spectroscopic method was found to be suitable for analysis of Progesterone in Injection form. 2- propanol was found to be appropriate solvent for extraction and analysis of drug in the formulation. For the quantitative analysis, linear calibration curve was obtained over the concentration range of 10-22 µg/ml. The parameters of calibration curve are, y = 0.046x+0.012, correlation coefficient r, 0.996. The results indicate good linear relationship between detector response and progesterone concentration. Excipients showed no interference with the results. The precision of the describe method is given in Table 2. For verification of Accuracy of the method, Recovery studies were carried out by analyzing mixtures of progesterone. The accuracy of the method is given in the Table 3.

The described spectroscopic method for analysis of progesterone in injection is precise, accurate and sensitive. The advantages of proposed method are it requires short time for sample preparation and thus for analysis.

4. CONCLUSION

The simple, rapid and reliable proposed methods were employed for the determination of progesterone in injection. The satisfying recoveries and low coefficient of variation suggests the suitability of proposed method for routine analysis of progesterone injection.

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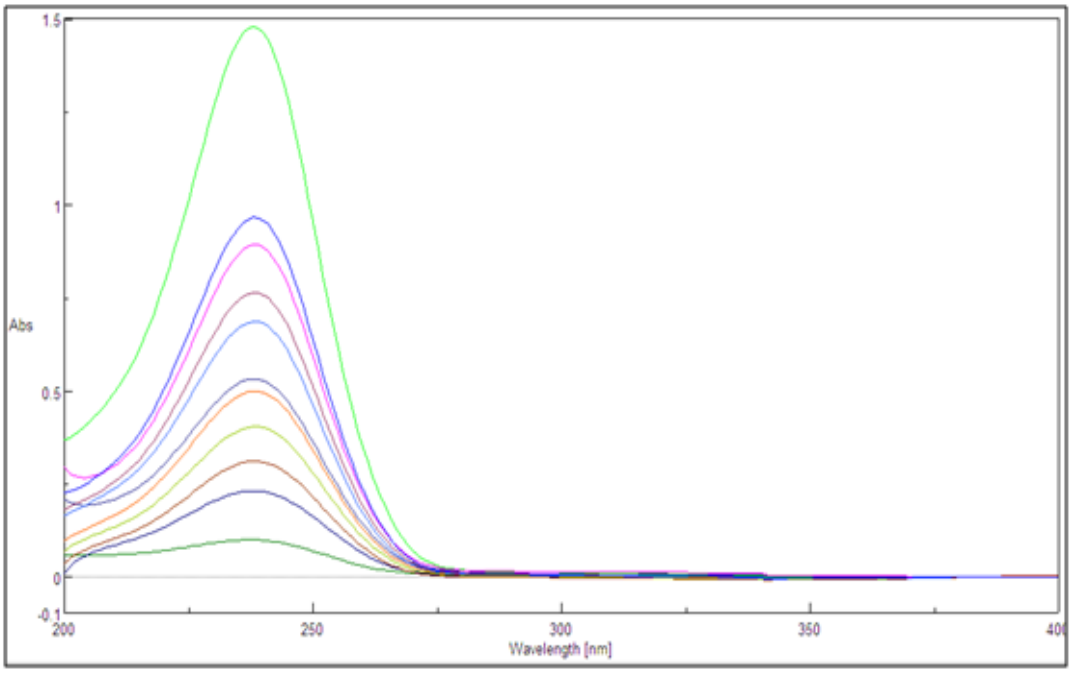

Fig.1- UV- Visible spectrum of Progesterone at different concentrations.

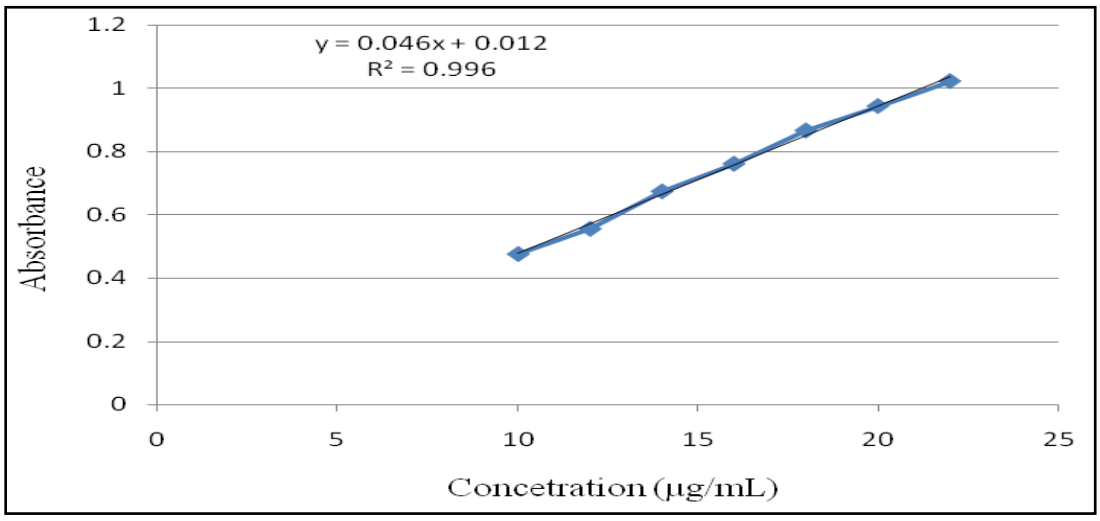

Fig. 2- Calibration curve of Progesterone in 2- Propanol

Table 1: Assay of Progesterone in pharmaceutical formulation (Injection)

Preparation	Label Claim (mg)	Percent Formula
Susten	100	99.60±0.5

Table 2: Intra-day precision and Intra-day error.

Interval	Amount Taken (µg/ml)	Amount Found (µg/ml)	e_r (%)	RSD (%)
Morning	10	9.99	0.1	1.2
	16	15.98	0.1	0.1
	22	21.97	0.1	0.1
Afternoon	10	9.97	0.3	1.3
	16	15.93	0.4	0.7
	22	21.93	0.3	0.1
Evening	10	9.9	1	0.2
	16	15.87	0.8	0.0
	22	21.90	0.4	0.3

e_r - Relative error.

Table 3: Recovery by standard addition method.

Amount of drug in formulation (mg)	Amount of drug added (%)	Recovery (%)[a]
100	**80**	**79.7±0.2**
	100	**99.7±0.2**
	120	**119.7±0.3**