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Determination of Progesterone in Capsules by High-Performance Liquid Chromatography and UV- Spectrophotometry

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ABSTRACT

The rapid, simple, and accurate chromatographic (high-performance liquid chromatography) and spectrophotometric method for the determination of progesterone in capsule was elaborated. Methanol was found to be a suitable extraction solvent. The samples were chromatographed on Linchrocart C18 column and UV detection at 254 nm. The elution was achieved isocratically with a mobile phase of methanol - water (80:20, v/v). The method was validated for precision, linearity, accuracy, and limit of detection.

Key words: Analysis in capsules, progesterone, reversed-phase chromatography, UV-spectrophotometry

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INTRODUCTION

Progesterone, pregn-4-ene-3, 20-dione [Figure 1], is a C-21 steroid hormone involved in the female menstrual cycle. The therapeutic dose is 100-200 mg of progesterone. Progesterone is widely used in hormone-therapy and therefore it is necessary to establish a simple and accurate method for its identification and quantitative determination in pharmaceuticals. The literature shows that gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS),[1-5] and high performance liquid chromatography (HPLC)[6-8] have been reported for the simultaneous determination of progesterone, 17-hydroxyprogesterone and other 3-keto steroids. However, no single method has been developed for progesterone in single component form. In the present study, new, simple and selective HPLC and UV spectrophotometric methods were elaborated for the determination of progesterone in commercial dosage forms.

Experimental details

Reagents

progesterone was purchased from Tanya Biotech (Punjab, India). The marketed formulation used was Prolin 100

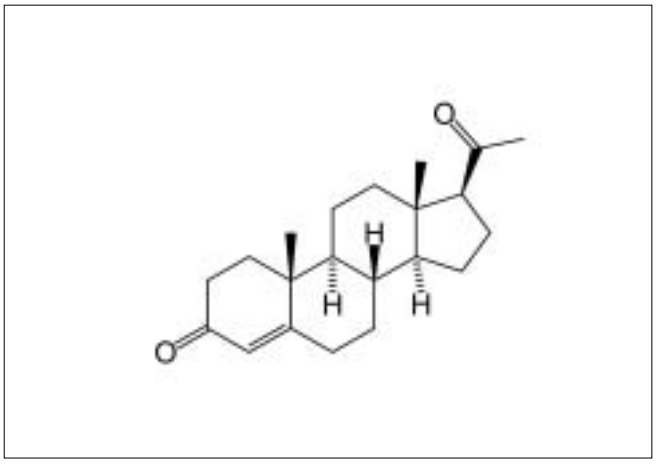

Figure 1: Structure of progesterone

Table 1: Assay of drug in pharmaceutical formulations (Capsules)

Preparation	Label claim	Percent founda		Student t-valueb		F-valuec	
	(mg)	HPLC	UV	HPLC	UV	HPLC	UV
1	100	99.9±0.4	99.8±0.5	0.15	0.03	2.74	1.87
2	150	99.9±0.3	99.8±0.3	0.24	1.70	1.98	1.41

aMean ± SD, n = 8, bTabulated value at 95% confidence level is 2.365, cTabulated value at 95% confidence level is 3.79

capsule, which contain 100 mg of Progesterone. Methanol for chromatography obtained from Merck Ltd, New Delhi, India, was used. Water used was of HPLC grade water from Merck Ltd, New Delhi, India. All the other reagents were of analytical grade.

Instrumentation

Liquid chromatography: A Merck Hitachi HPLC system consisting of a L-7110 pump, and a L-7400 variable wavelength detector (UVVIS) was used. Manual injections were made using a Rheodyne injectable valve (20 µl loop). The detector wavelength was set at 254 nm. The chromatographic separations were performed at ambient temperature on a Linchrocart C18 ((250 × 4.0 mm), 5µm). The mobile phase was a mixture of methanol and water (80:20, v/v), filtered, and flowing at the rate of 1 ml/min. The data were collected and analyzed with Winchrom software.

Spectrophotometry: A thermospectronic model of Elico India SL-159 UV/VIS spectrophotometer with 1 cm matched quartz cells was used for the spectrophotometric method.

Solutions: Stock solutions (1.0 mg/ml) of progesterone were prepared by dissolving appropriate amount in methanol: water. These solutions were stable for 48 h at room temperature. The working solutions of 0.01 mg/ml for progesterone were prepared by diluting the stock solutions with the methanol: water.

Method 2 Chromatographic Method

Preparation of the calibration curve of the drug

From stock solutions of progesterone 1 ml was taken and diluted up to 10 ml. From this solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml volume was transferred to 10 ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives a standard drug solution of 5, 10, 20, 25, 30 µg/ml concentration. Each of the standard drug solutions was injected three times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Assay in dosage form

Twenty capsules were accurately weighed, and the average capsule mass was calculated. To a fine powder the amount equivalent to 100 mg (after a declaration) of progesterone was diluted with mobile phase in 100 ml volumetric flasks. Further 0.1 ml of this solution was taken and diluted up to 10 ml to obtain a final concentration of 10 µg/ml of progesterone The sample was injected using a 20 µl fixed loop into the column. All measurements were repeated five times for each concentration. The results are shown in Table 1.

Method 2 Spectrophotometry

The absorptivity of progesterone in methanol: water was examined in the range 200–400 nm and the λmax value (position of maximum absorbance of a peak) was recorded. The spectrum exhibits a maximum at 254 nm.

Preparation of the calibration curve of the drug

From stock solutions of progesterone, dilutions were made to give a standard drug solution of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 g/ml concentration. Each of the standard drug solutions was injected three times and the mean absorbance of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve and the calibration curve was obtained.

Assay in dosage form

Twenty capsules were accurately weighed and the average capsule mass was calculated. To a fine powder and amount equivalent to 100 mg (after a declaration) of progesterone was diluted with mobile phase in 100 ml volumetric flasks. Finally, the diluted sample of 10 µg/ml was taken and absorbance was measured by using the spectrophotometer

Table 2: Intra-day precision and intra-day error of the methods

Method	Amount taken (µg/ml)	Amount found (µg/ml)	e_r (%)	RSD (%)
HPLC	0.5	0.49	0.8	0.4
	1.50	1.49	0.13	0.2
	2.50	2.49	0.1	0.2
UV	3.0	2.99	0.15	0.1
	3.50	3.49	0.11	0.10
	4.00	3.99	0.25	0.1

e_r – relative error. CL – confidence limits at 95% confidence level for seven degrees of freedom.

Table 3: Recovery by the standard addition method

Preparation	HPLC			UV		
	Amount of drug in formulation (mg)	Amount of drug added (mg)	Recovery (%)[a]	Amount of drug in formulation (mg)	Amount of drug added (mg)	Recovery (%)[a]
1	100	8	102.3 ± 0.3	100	8	99.6 ± 0.4
	100	10	100.0 ± 0.4	100	10	100.3± 0.6
	100	12	100.0 ± 0.8	100	12	100.0± 0.3
2	150	12	99.3 ± 0.6	150	12	98.6 ± 0.9
	150	15	99.6 ± 0.3	150	15	99.7 ± 0.6
	150	18	100.2 ± 0.8	150	18	100.8± 0.7

[a]Mean ± SD, n=3

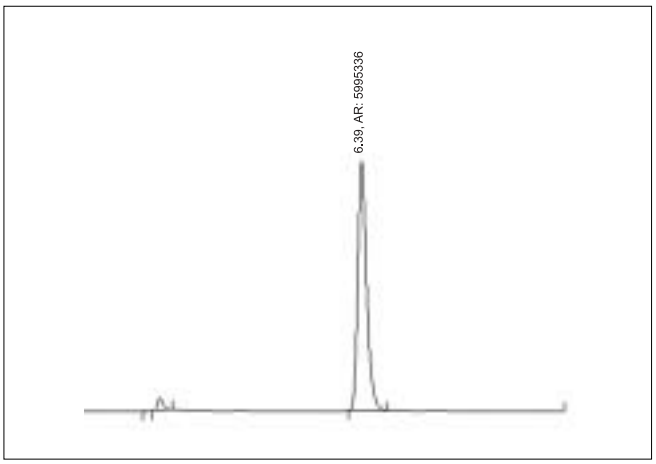

Figure 2: Typical chromatogram of Progesterone under described HPLC conditions

at 254 nm. The concentration of progesterone was found out by using regression equation.

RESULTS AND DISCUSSION

A reversed-phase isocratic procedure was proposed as a suitable method for the analysis of Progesterone in capsules. A mixture of methanol: water (80:20, v: v) at a flow rate of 1 ml/min was found to be an appropriate mobile phase allowing adequate and rapid separation of analyte (retention time 6.39 min). As shown in Figure 2, the substances were eluted forming well-shaped, symmetrical single peak, well separated from the solvent front. For quantitative applications, a linear calibration curve was obtained over the working concentration range of 5–30 μg/ml. The parameters of the calibration graph were *y* =0.3992*x* 2 0.0914, correlation coefficient *r*, 0.9997. The results indicate a good linear proportionality between the detector response and the concentration of progesterone. Methanol was chosen for the extraction from capsule because it is an excellent solvent for the analyte and is suitable for the reversed phase made of chromatography. The selectivity of the chosen chromatographic system was also ascertained. Excipients showed no interferences with the determination of progesterone and the internal standard. The precision of the elaborated methods is given in Table 2. In order to verify the accuracy of the described methods, recovery studies were carried out by analyzing model mixtures of progesterone. The recovery of progesterone was evaluated from 50 to 150% of the labeled tablet amount. The accuracy of the methods is given in Table 3. The described HPLC method of determination of progesterone in tablets is precise, sensitive, and accurate. The advantages of the proposed method are its short analysis time and a simple procedure for sample preparation. The results of the determinations of progesterone showed good precision and accuracy of the spectrophotometric method. When Student's *t*-test at the 95% confidence level was applied to compare the results obtained by the HPLC and the spectrophotometric method, the calculated *t* values did not exceed the tabulated one.

CONCLUSION

The rapid, simple, and fairly reliable proposed methods were employed for the determination of progesterone in tablets. The satisfying recoveries and low coefficients of variation confirm the suitability of both proposed methods for the routine analysis of progesterone in tablets.

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