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# Determination of Progesterone in Pharmaceutical Preparations by UV, First-order Derivative Spectrophotometry and Gas Chromatography

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**ABSTRACT**

The determination of progesterone in pure and capsule form was accomplished in this work using new, simple and quick procedures by UV spectrophotometry, first-order derivative spectrophotometry and gas chromatography. To increase the sensitivity of the suggested methods, it was necessary to optimize the solvent system, the detection wavelength, and the chromatographic conditions. The linear regression equations for the UV spectrophotometry, first-order derivative spectrophotometry and gas chromatography were y=0.0536x+0.0002, y=0.1362x+0.0014, and y=1.8217x-1.239, respectively, as determined by the least square regression approach. Under the chosen experimental conditions, no interference was found. The suggested methods are extremely accurate and precise. When the suggested methods' findings were compared to those of two published reference methods, there was statistically no discernible difference. Therefore, the methods are applicable to the measurement of progesterone in pharmaceutical formulations.

***Keywords:*** Progesterone, Spectrophotometry, Gas chromatography, Validation.

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## Introduction

Steroid hormones, which are produced naturally in the gonads, placenta, and adrenal glands from cholesterol, are crucial for the regulation of many bodily processes. Low levels of them are found in the body, and alterations in them have been linked to conditions like diabetes, cancer, and even diabetes [1]. According to World Anti-Doping Agency standards [2-4], steroids are a crucial component of an athlete's biological passport. Therefore, it is thought that determining steroids accurately is a crucial task in order to avoid inaccurate results and investigate the mechanisms of diseases linked to steroid hormones.

Progesterone (Fig. 1) is a steroid hormone that is produced from cholesterol [5]. Progestagens or gestagens are the collective names for progesterone and other progestational substances. Progestagens are medically prescribed for use as male and female contraceptives, indicating a risk to fertility when exposure is unintended or used for other purposes. Progesterone is produced during the synthesis of steroid hormones in both males and females, as well as serving as an early intermediate. Exogenous progesterone exposure results from medicinal usage (contraception, prevention of premature labor, menstrual problems), use of cosmetics and supplements, and exposure through environmental media. Progesterone is most frequently used therapeutically for dysfunctional uterine bleeding or amenorrhea, for contraception (either alone or in combination with other hormones, such as estradiol or mestranol in oral contraceptives), and for hormone replacement therapy in post-menopausal women [6,7].

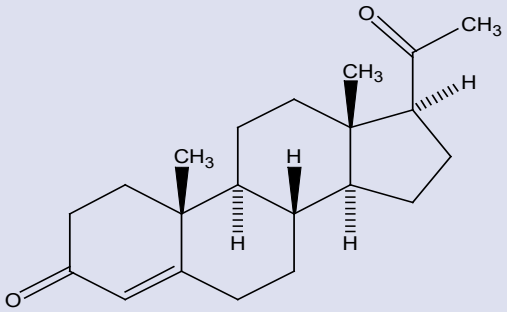


Figure 1. Structure of progesterone

Progesterone is widely used in hormone therapy, hence a quick and accurate way to identify and measure it in pharmaceutical formulations is needed. GC-MS [8-12] and HPLC [13-17] methods have all been described for progesterone in the literature. The analysis of progesterone in pharmaceutical formulations using UV spectrophotometry [18, 19] and derivative spectrophotometry [20] methods has been covered in a number of studies in the literature.

The first-order derivative spectrophotometry ($^1$D) method has not yet been covered in any articles in the literature. Spectrophotometric techniques are known for being quick, easy to use, and accessible, making them excellent for use in regular preparation analysis.

The use of flame ionization detection (FID) for progesterone determination together with simple, affordable, and reasonable analysis time were attempted to be demonstrated using the GC method.

Therefore, the goal of this study was to ascertain whether sample pre-treatment was required for the UV, 1D spectrophotometry and GC-FID methods to detect progesterone in pharmaceutical preparations. According to the International Conference on Harmonization's criteria, the developed methods were validated [21]. Statistics were used to compare the outcomes of developed and validated methods with two reference methods [18,19].

## Materials and Methods

### Chemicals

A progesterone reference standard with a purity of 99.9% was generously given by Riedel De Haen. Methanol of the analytical grade was bought from Merck (Germany). Local Turkish suppliers provided the Progestan® soft capsule (Kocak Pharm. Ind., Turkey). It was said that each Progestan® soft capsule contained 100 mg of progesterone and titanium dioxide as an excipient.

### Equipments

It was carried out using a Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ). Standard and sample solutions' UV and $^1$D spectra (N=6. Δλ=4.0 nm) between wavelength ranges of 200-300 nm.

An Agilent 6890 N Network GC with a flame ionization detector, an Agilent 7683 series autosampler, an Agilent chemstation, and an HP-5 column with a 0.25 µm film thickness (30 m x 0.320 mm I.D.) was used to execute the GC-FID system. Temperatures for the injection and detector are 250 and 300 °C, respectively. A split injection of 2 µL was performed. Throughout the experiment, the flow rate of the carrier gas ($N_2$) was held constant at 2 mL/min.

### Preparation of Standard Solutions

For UV and $^1$D methods, a stock solution (100 µg/mL) of progesterone was made by dissolving 10 mg of it in 100 mL of methanol. Daily working solutions (WS) containing 2, 4, 6, 8, 10 and 12 µg/mL of progesterone were prepared by methanol dilution of the stock solution. The WS was prepared each day through analysis. To conduct the study, solutions were moved to quartz cells. Before use, the stock solution was warmed to room temperature from its -20 °C storage location in a glass flask. Aliquots of progesterone standard solution were added to QC samples to develope final concentrations of 3, 7 and 11 µg/mL.

For GC-FID method, progesterone stock standard solution was made in methanol at a concentration of 100 µg/mL and kept at -20 °C for storage. Working standard solutions were developed from the stock solutions. For progesterone (n=6), a calibration graph was drawn in the range of 0.25, 0.5, 0.75, 1.25, 2.5, 5, 7.5, 10, 15, 20 and 30 µg/mL. The stock solution was diluted with methanol for QC samples containing progesterone at concentrations of 1, 12.5 and 25 µg/mL.

### Data Analysis

The Statistical Product and Service Solutions (SPSS) for Windows, version 15.0, was used for all statistical computations. Calculated P values of 0.05 or less were used to determine whether a correlation was statistically significant.

## Results and Discussion

### Development and Optimization of the Method

#### *Optimization of spectrophotometric conditions*

The experimental parameters, such as the solvent, the level of derivation, the wavelength range, and smoothing, were improved in order to construct sensitive UV and $^1$D spectrophotometric methods. UV and $^1$D spectrophotometry were used to evaluate the 200-300 nm wavelength region, and this produced the best findings. Different solvent systems, including methanol, ethanol, water and acetonitrile were tested in this assay either singly or in varied ratios.

The final choice was made because of methanol's sensitivity, interference, simplicity of fabrication, suitability for pharmaceuticals, content estimate, and cost, in that order. Due of its lack of toxicity, methanol was used in this study. In methanol at a concentration of 2-12 µg/mL, progesterone's UV and $^1$D spectra are shown in Figs. 2 and 3, respectively. Any spectrum may be used to identify this substance. The greatest peak of progesterone's UV spectra was found to be at 240 nm. The $^1$D spectra of progesterone showed a high peak at 231 nm and a low peak at 252 nm (Fig. 3).

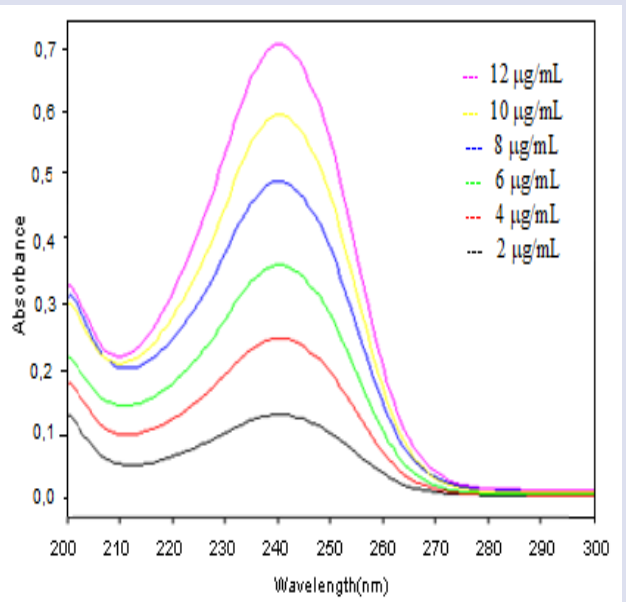


Figure 2. Spectra obtained from UV method

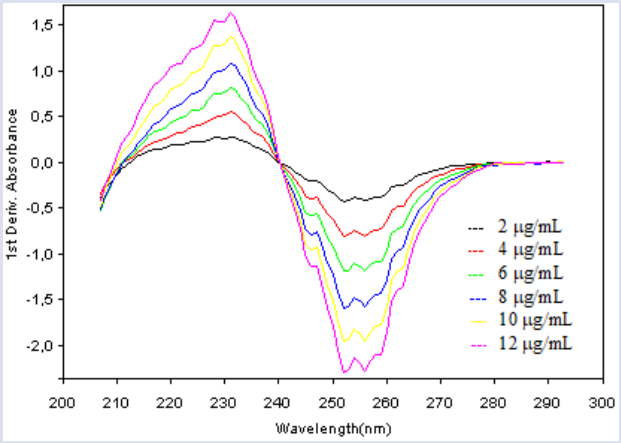


Figure 3. Spectra obtained from $^{1}$D method

### *Optimization of chromatographic conditions*

Based on the chemical characteristics of progesterone, a method for measuring it was developed. Methanol was utilized as the diluent since progesterone is a polar molecule and methanol is a polar solvent. For the separation of this analyte, HP-5 capillary column used in the study.

The boiling point served as the foundation for the GC-FID parameters employed in the method development. The temperature of the detector and injection port were set at 250 and 300 °C, respectively. For the GC oven, various temperature regimens were examined. The optimal temperature program was chosen for a decent resolution at the conclusion of this experiment. The GC oven's temperature programs, which had a 12-minute run length, were as follows: beginning temperature 150 °C, hold for 1.5 min; medium temperature 260 °C, hold for 5 min; ramp rate 50 °C/min; final temperature 270 °C, hold for 3.3 min; ramp rate 10 °C/min.

A 44 mL per minute hydrogen flow was ensured by setting the head pressure. Split mode (20:1) was selected. Progesterone had a retention duration of almost 9.7 min and a well-defined peak. No additional method optimization was necessary. Fig. 4 displays a typical chromatogram produced using standard progesterone.

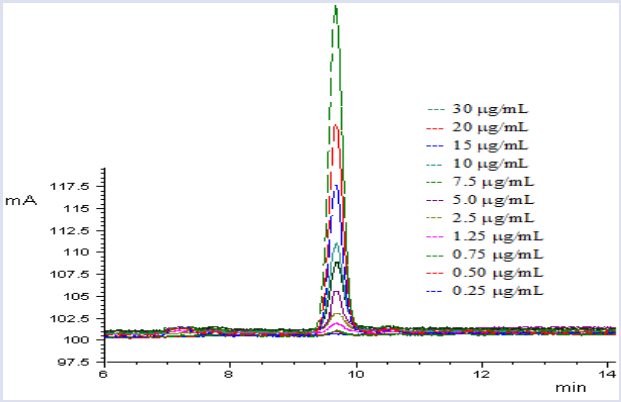


Figure 4. GC-FID chromatograms of progesterone

### *Validation of the Method*

ICH Q2B guidelines were followed while determining the validation parameters [21]. These criteria include specificity, linearity, precision, accuracy, recovery, limit of detection (LOD), limit of quantification (LOQ), ruggedness and stability.

### *Specificity*

In this study, it was investigated the potential interferences of common excipients and additives. The control samples were prepared and examined. At the concentrations present in dosage forms, there is no evidence of any interference from these chemicals. The excipient employed in this formulation was one that the pharmaceutical industry employs most frequently. Arachite oil, lecithin, gelatin, glycerin, and titanium oxide are common tablet additives that may interfere with the method's specificity. These exceptions had no negative effects on the suggested methods. The procedure might be specific in accordance with the findings of the analysis.

### *Linearity*

The working solutions were scanned at 200-300 nm against a similarly prepared blank in the UV and $^{1}$D spectrophotometry methods. Calibration curves were drawn using the 240 nm wavelength for UV and the 231 and 252 nm wavelengths for the $^{1}$D method. Six analyses were performed at each concentration level in a six-level calibration series. The absorbance values were plotted vs progesterone concentrations for UV and $^{1}$D spectrophotometry method.

For all calibration curves, UV and $^{1}$D methods demonstrated satisfactory linearity in the concentration range of 2-12 µg/mL. The least-square regression technique was used to obtain the regression equations (Table 1).

Progesterone concentrations between 0.25 and 30 µg/mL were tested for the GC-FID method to determine the linearity of peak area response vs concentration. The calibration curve's accuracy was evaluated using the correlation coefficient. The method's linearity was shown by the calibration equation which was derived from six repeat trials. All of the calibration curves' correlation coefficients were consistently higher than 0.99.

Table 1. Results of regression analysis of proposed methods

| Methods | Range (µg/mL ) | λ (nm) | LR[a] | R | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|---|---|
| UV | 2-12 | 240 | y=0.0536x+0.0002 | 0.9998 | 0.15 | 0.50 |
| $^1$D | 2-12 | 231 | y=0.1362x+0.0014 | 0.9998 | 0.15 | 0.50 |
| | 2-12 | 252 | y=0.1880x+0.0597 | 0.9996 | 0.15 | 0.50 |
| GC-FID | 0.25-30 | - | y=1.8217x -1.239 | 0.9997 | 0.08 | 0.25 |

λ: Wavelength, [a] Based on six calibration curves LR: Linear regressionequation, R: Coefficient of correlation

### *Precision and accuracy*

Precision and accuracy both within and between days are used to describe repeatability. It was assessed using an examination of three different progesterone concentrations on three separate days. To assess the accuracy of these methods, six replicate determinations at three distinct concentrations (3, 7 and 11 µg/mL) were made using the UV method at 240 nm and the $^1$D spectrophotometry method at 231 and 252 nm. All samples were made from scratch. The acceptable RSD% values for the UV and $^1$D spectrophotometry methods were 2.98% and 3.76% (n=6), respectively, for the within-day precision, and 3.13% and 4.27% (n=6), respectively, for the between-day precision (intermediate precision) (Table 2). According to Table 2, the accuracy of UV and $^1$D spectrophotometry procedures yielded acceptable relative error (RE%) levels of 2.71 and 2.67 (n=6), respectively.

The repeatability (within-day) and intermediate precision (between-day) of the GC-FID method were used to assess its level of precision. Three separate concentrations which served as QC samples were examined six times. The concentrations were 1.0, 12.5 and 25.0 µg/mL. Within-day precision's RSD value was 1.56%, and between-day precision's value was 1.81%. Within-day and between-day accuracy biases (relative error-RE%) were 2.00% and 3.00%, respectively. Table 2 provides a summary of these figures. The data gathered using the suggested methods showed that each method devised had a reasonable level of precision and accuracy.

Table 2. Precision and accuracy of proposed methods (n=6)

| Method | λ (nm) | Added (µg/mL) | Within-day | | | Between-day | | |
|---|---|---|---|---|---|---|---|---|
| | | | Found±SD (µg/mL) | Accuracy RE% | Precision RSD% | Found±SD (µg/mL) | Accuracy RE% | Precision RSD% |
| UV Method | $A_{240\ nm}$ | 3.0 | 2.95±0.06 | -1.67 | 1.93 | 3.04±0.07 | 1.33 | 2.14 |
| | | 7.0 | 7.14±0.21 | 2.00 | 2.98 | 7.19±0.23 | 2.71 | 3.13 |
| | | 11.0 | 10.88±0.17 | -1.09 | 1.57 | 10.84±0.23 | -1.45 | 2.10 |
| $^1$D Method | $^1D_{231\ nm}$ | 3.0 | 3.06±0.01 | 2.00 | 0.23 | 3.08±0.09 | 2.67 | 3.18 |
| | | 7.0 | 7.10±0.14 | 1.43 | 1.93 | 7.13±0.21 | 1.86 | 2.93 |
| | | 11.0 | 11.24±0.42 | 2.18 | 3.76 | 11.28±0.48 | 2.55 | 4.27 |
| | $^1D_{252\ nm}$ | 3.0 | 3.06±0.05 | 2.00 | 1.57 | 3.07±0.09 | 2.33 | 2.77 |
| | | 7.0 | 7.17±0.16 | 2.43 | 2.27 | 7.18±0.25 | 2.57 | 3.45 |
| | | 11.0 | 11.20±0.22 | 1.82 | 1.97 | 11.23±0.34 | 2.09 | 3.04 |
| GC-FID | | 1.0 | 1.02±0.01 | 2.00 | 1.28 | 1.03±0.02 | 3.00 | 1.65 |
| | | 12.5 | 12.40±0.19 | -0.80 | 1.56 | 12.30±0.22 | -1.60 | 1.81 |
| | | 25.0 | 24.90±0.22 | -0.40 | 0.87 | 25.10±0.29 | 0.40 | 1.14 |

### *Recovery*

The common addition methodology was used to verify the proposed spectrophotometry methods' accuracy. The 5.0 µg/mL concentration of capsule solution was mixed with the three different concentrations of pure sample solution (2.5, 7.5 and 12.5 µg/mL) before being analyzed. The following equation was used to compute the analytical recovery of the assay samples with the added standard.

Recovery % = $[(C_t-C_u) / C_a] \times 100$

Where $C_t$ is the total amount of the analyte that was measured, $C_u$ is the amount of the analyte in the formulation, and $C_a$ is the amount of the pure analyte that was added to the formulation. Table 3 contains the findings of the commercial capsule analysis and the recovery investigation. The average percent recoveries were quantified as 100.3% for the UV method and 99.8% for the $^1$D method, confirming the methodologies' high degree of accuracy. There was no evidence of any interference from common excipients.

Analytical recovery investigations were carried out by adding pre-analyzed samples of commercial dosage form to predetermined concentrations of pure medicines. The recovery was studied at three different concentration levels (4.0, 9.0 and 20.0 µg/mL). The accuracy of the GC-FID method was evaluated, and the interference of formulation additives was investigated. By comparing the concentrations obtained from the spiked samples with the actual added concentrations, the percent analytical recovery values were computed. The results are listed in Table 3.

**Table 3.** Results of recovery studies by standard addition method (n=6)

| Method | Amount taken (µg/mL) | Amount added (µg/mL) | Total amount found (µg/mL) (mean ±SD) | Recovery±RSD (%) |
|---|---|---|---|---|
| UV | 5.0 | 2.5 | 7.55±0.10 | 100.60±1.33 |
| | | 7.5 | 12.49±0.15 | 99.90±1.22 |
| | | 12.5 | 17.25±0.28 | 98.60±1.64 |
| $^1D_{231\ nm}$ | 5.0 | 2.5 | 7.49±0.11 | 99.90±1.51 |
| | | 7.5 | 12.51±0.35 | 100.10±2.81 |
| | | 12.5 | 17.48±0.13 | 99.90±0.75 |
| GC-FID | 1.0 | 4.0 | 5.08±0.12 | 101.60±2.38 |
| | | 9.0 | 9.92±0.19 | 99.20±1.94 |
| | | 20.0 | 20.90±0.41 | 99.50±1.97 |

### *LOD and LOQ*

The calibration standards were used to get the LOD and LOQ values for the indicated technique [22]. Table 1 provides a summary of the findings. For both techniques of preparing standard progesterone solutions, the LOQ and LOD values were discovered to be 0.50 µg/mL and 0.15 µg/mL, respectively. All RSD values were discovered to be less than 5%.

Serial progesterone stock solution dilutions were used to analyze the LOD and LOQ values in the GC-FID method in order to achieve signal to noise ratios of 3:1 for LOD and 10:1 for LOQ. The LOD and LOQ values for the analyte were determined to be, respectively, 0.075 and 0.25 µg/mL (Table 1).

### *Ruggedness*

The same instruments and standard standard solutions were used in this study by a separate analyst to assess the concentration of progesterone (Table 4).

Table 4. The results of analyses of progesterone by a different analyst (n=3)

| Method | Added (µg/mL) | Found (µg/mL) (Mean±SD) | % Recovery | % RSD |
|---|---|---|---|---|
| UV | 4.0 | 4.10±0.09 | 102.50 | 2.21 |
| | 8.0 | 7.92±0.17 | 99.20 | 2.17 |
| | 12 | 11.93±0.13 | 99.40 | 1.09 |
| $^1D_{231\ nm}$ | 4.0 | 4.09±0.09 | 102.00 | 2.15 |
| | 8.0 | 8.01±0.12 | 100.10 | 1.53 |
| | 12.0 | 12.19±0.17 | 101.60 | 1.37 |
| GC-FID | 1.25 | 1.26±0.02 | 100.80 | 1.83 |
| | 5.0 | 4.97±0.14 | 99.40 | 2.86 |
| | 15.0 | 14.93±0.13 | 99.50 | 0.89 |

No statistically significant discrepancies between the operators were found in the results, indicating the ruggedness of the developed methods.

### *Stability*

Progesterone solutions of 2.0, 4.0 and 10.0 µg/mL concentrations as well as stock solution were maintained in the refrigerator and at ambient temperature for four days to test the stability of progesterone standard solutions spectrophotometrically. The stability measurements were then performed. By comparing these measures to those of standards and expressing the results as a percentage deviation, the results were assessed. Progesterone solutions were stored for three days in the refrigerator and two days at room temperature to test their stability. Under either circumstance, a significant change in concentration was not discovered. Additionally, it was discovered that the stock solution remained stable for a week in the refrigerator.

Stability experiments using the GC-FID method showed that the samples were stable at room temperature, 4 and -20 °C for 24 hours (short-term refrigeration) and for 72 hours (long-term refrigeration). These stability studies' findings fall within the 90-110% acceptable range (Table 5).

Table 5. Stability of progesterone in solution (n=3)

| Stability (%) | | Room temperature stability (Recovery %±RSD) | | Refrigeratory stability +4 °C (Recovery %±RSD) | | Frozen stability - 20 °C (Recovery %±RSD) | |
|---|---|---|---|---|---|---|---|
| Method | Added (µg/mL) | 24 h | 72 h | 24 h | 72 h | 24 h | 72 h |
| UV | 2.0 | 99.5±3.1 | 101.3±4.0 | 99.4±3.1 | 101.2±4.0 | 97.4±4.2 | 101.2±3.9 |
| | 4.0 | 101.4±3.0 | 99.2±3.5 | 98.2±4.2 | 98.2±3.2 | 101.2±2.9 | 98.6±4.1 |
| | 10.0 | 98.4±3.0 | 98.6±2.1 | 101.3±2.6 | 99.6±3.8 | 102.3±1.7 | 101.4±2.1 |
| $^1D_{231\ nm}$ | 2.0 | 99.8±2.9 | 101.2±3.0 | 99.6±3.4 | 100.4±3.0 | 98.2±3.5 | 100.3±3.3 |
| | 4.0 | 100.1±2.8 | 99.6±3.1 | 99.2±3.9 | 98.9±2.3 | 99.4±3.4 | 99.4±1.6 |
| | 10.0 | 101.3±1.8 | 102.3±2.1 | 101.4±2.7 | 99.8±2.6 | 99.5±2.4 | 99.6±2.4 |
| GC-FID | 5.0 | 99.9±1.4 | 101.3±1.2 | 99.8±2.2 | 100.3±2.1 | 99.4±2.2 | 99.3±2.2 |
| | 10.0 | 99.1±1.4 | 98.9±2.6 | 99.4±2.4 | 99.7±1.2 | 99.7±2.4 | 99.6±1.1 |
| | 20.0 | 99.8±2.0 | 99.6±2.4 | 101.3±2.8 | 101.2±1.9 | 101.2±3.1 | 99.7±2.2 |

### Procedure for Pharmaceutical Preparations

Ten progesterone-containing capsules from Progestan were accurately weighed and finely ground. The precise weight of a powdered tablet containing 100 mg of progesterone was combined with methanol, and the mixture was ultrasonically dissolved for 15 minutes at room temperature. The resulting solution was then diluted with methanol to attain the proper concentration before being filtered through a 0.45 µm nylon 25 mm filter (Figs. 5-7).

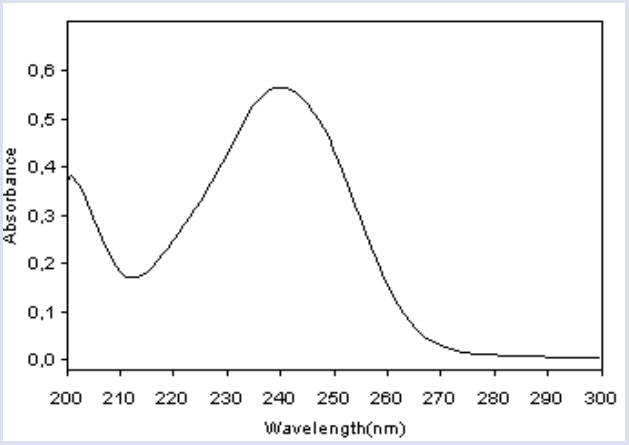


Figure 5. UV spectrum of Progestan® soft capsule solution (10 µg/mL)

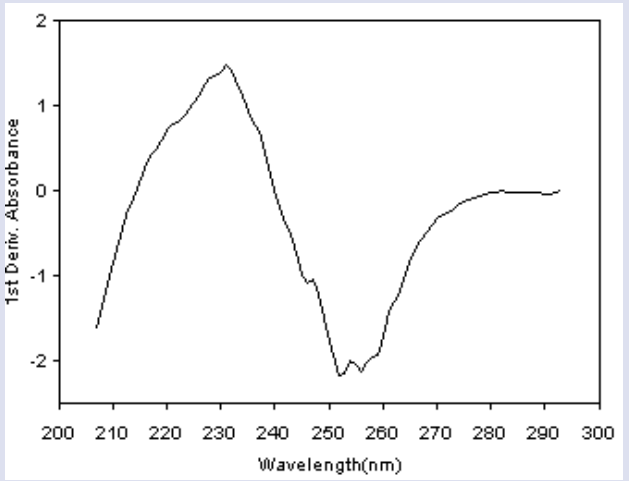


Figure 6. $^{1}$D spectrum of Progestan® soft capsule solution (10 µg/mL).

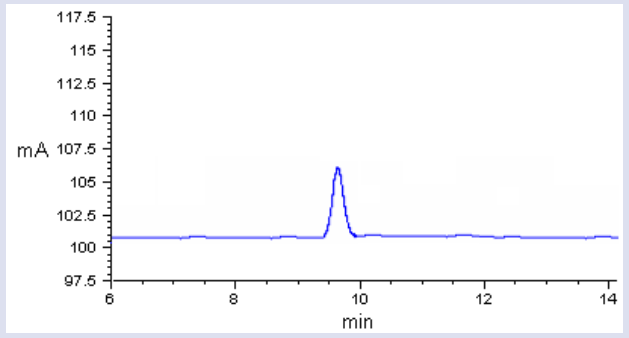


Figure 7. GC-FID chromatogram of progestan capsule solution (10 µg/mL).

### Comparison of the Methods

Due to their speed and simplicity, UV and $^{1}$D spectrophotometry methods have been widely used in the QC analyses of pharmaceutical preparations. These methods don't call for the pricey equipment needed for chromatographic methods or the time-consuming sample preparation steps like filtration and degassing required for HPLC operations. Derivative spectrophotometry methods can also be used to analyze a wide range of completed goods, with the benefit of resolving complex issues thanks to precise slope differentiation and producing a clean spectrum.

The spectrophotometry method in the visible range used in pharmacopoeias for the quantitative determination of norgestrel and norethisterone tablets [22] and hydroxyprogesterone in injection solutions [13,22] is based on the creation of derivatives, typically through a reaction with isonicotinic acid hydrazide. As a result, the quantitative measurement of progestogen hormones in substances and pharmaceutical preparations is frequently carried out using spectrophotometric methods. One major flaw in this method is that it can only be used to establish the general composition of the main component and a few admixtures. With visible-range spectrophotometry, progesterone and hydroxyprogesterone capronate in oil solutions are especially simple to assess for progestogen hormones in various pharmaceutical preparations.

This study uses UV and $^{1}$D spectrophotometry to quantify progesterone in capsules and in pure forms with minimal sample preparation. In this study, UV and $^{1}$D spectrophotometry methods were used to quickly, accurately and simply assess progesterone in pharmaceutical preparations. Progesterone's absorptivity in methanol was tested between 200 and 300 nm, and the UV method's "max value" (location of peak's maximum absorbance) was noted at 240 nm. A maximum peak and a low peak for the $^{1}$D technique were measured at 231 and 252 nm, respectively. Progesterone in capsules was determined using the developed spectrophotometric methods (Table 6).

Table 6. Determination of progesterone in capsule (100 mg progesterone/capsule)

| Commercial Preparations | Method | λ (nm) | Found±SD (mg) | Recovery (%) | RSD (%) | Confidence Interval |
|---|---|---|---|---|---|---|
| Progestan® soft capsule | UV | 240 | 101.1±0.1 | 101.1 | 0.09 | 101.0-101.2 |
| | $^{1}$D | 231 | 99.7±0.5 | 99.7 | 0.53 | 99.6-100.1 |
| | GC-FID | - | 99.3±0.2 | 99.3 | 0.24 | 98.2-102.1 |

In Figs. 5 and 6, respectively, the UV and $^{1}$D spectra obtained from Progestan® soft capsules are displayed. It was clear that the drug solution's maximum and

minimum wavelengths were identical to those of conventional solutions. Additionally, two reference methods from the literature [18,19] were statistically compared with the suggested UV and $^1$D spectrophotometry methods.

Progesterone in capsules is analyzed using UV spectrophotometry and HPLC methods as one of the reference methods, and progesterone in commercial formulations is determined using HPLC and derivative spectrophotometry methods as the second reference method.

Progesterone's absorptivity in methanol: water (80:20) was tested in the first reference method [18], and the max value (position of maximum absorbance of a peak) was noted at 254 nm. These groups also operated in the HPLC system $C_{18}$ (250x 4.0 mm, I.D. 5 µm) column with methanol as the mobile phase.

Progesterone had been measured using liquid chromatography and derivative spectrophotometry methods in both experimental micellar systems and commercial pharmaceutical formulations [19]. The spectrophotometric experiments had been carried out at 240 nm employing direct UV spectra.

The derivative values that were employed for quantitative calculations were determined as the difference between the heights of the first derivative spectra's maximum at 253.6 nm and minimum at 227.2 nm. In addition, Pucci et al. [19] conducted liquid chromatography using the $C_8$ (Res Elut, 150x/4.6 mm, I.D. 5 µm) column, a mobile phase made up of 2-propanol and a pH 2.5, 30 mM phosphate buffer, flow rate of 1.5 mL/min, and UV detection carried out at 254 nm.

The proposed UV and $^1$D spectrophotometry methods were linear over the concentration range of 2-12 µg/mL. The LOD and LOQ for both methods were found to be 0.15 and 0.50 µg/mL, respectively. For both of the suggested procedures, the RSD% values obtained from within-day and between-day testing of quality control samples ranged from 0.25% to 3.50%, suggesting high accuracy and precision. The average recovery value for progesterone in 100 mg capsule composites ranged from 91.7 to 101.1%.

The new UV and $^1$D spectrophotometry methods were used to analyze a pharmaceutical formulation that didn't need its samples to be separated or treated beforehand. Over the concentration ranges of 5-30 µg/mL and 2-50 µg/mL, respectively, the first and second reference techniques had been confirmed to be linear. The average recovery values in the first and second reference methods were 98.6-100.8% (for capsules) and 98.4-101.7% (for oily injection, capsules, and alcoholic intramuscular solution), respectively. The RSD% values ranged from 0.1-0.4% and from 0.3-1.1%, respectively. The second reference method's LOQ and LOD values were discovered to be 2.0 µg/mL and 1.5 µg/mL, respectively.

With a 95% confidence level and five degrees of freedom, the findings were statistically compared using the Student's t-test (for accuracy) and variance F-test (for precision) with reference methodology [18,19]. The outcomes demonstrated that there was no appreciable difference between the suggested and reference methods for P-values greater than P=0.05 (Table 7).

Table 7. Statistical comparison (Student *t*-test and *F*-test) of two reference methods with proposed methods

| | Method | n | X | RSD (%) | Std. Error Mean | *t*-values | P-Values |
|---|---|---|---|---|---|---|---|
| First comparison | Proposed UV method | 10 | 101.1 | 0.09 | 1.29 | 2.15 | 0.098 |
| | First reference method | 10 | 97.9 | 1.66 | 0.96 | | |
| Second comparison | Proposed UV method | 10 | 101.1 | 0.09 | 1.29 | 0.02 | 0.988 |
| | Second reference method | 10 | 101.3 | 1.71 | 0.99 | | |
| First comparison | Proposed $^1$D method | 10 | 99.7 | 0.53 | 1.39 | 2.17 | 0.098 |
| | First reference method | 10 | 101.9 | 1.67 | 0.99 | | |
| Second comparison | Proposed $^1$D method | 10 | 99.7 | 0.53 | 1.39 | 0.02 | 0.998 |
| | Second reference method | 10 | 101.7 | 1.81 | 0.99 | | |
| Third comparison | Proposed UV method | 10 | 101.1 | 0.09 | 1.29 | $F_c$=1.43 | |
| | Proposed $^1$D method | 10 | 99.7 | 0.53 | 1.39 | | |
| | Proposed GC-FID method | 10 | 99.3 | 0.24 | 0.08 | $F_t$=3.29 | |

n:Number of determination, X: Mean, RSD: Relative standard deviation, $t_c$: Calculated t values, $H_o$: Hypothesis: No statically significant difference exists between two methods $t_t > t_c$; $H_o$ hypothesis in accepted ($\alpha$=0.05), $F_c$: Calculated F values, $F_t$: Tabulated F values, $H_o$: Hypothesis: no statitically significant difference exists between methods $F_t > F_c$; $H_o$ hypothesis in accepted ($\alpha$=0.05)

Additionally, the recently proposed methods for measuring progesterone in capsules were contrasted with existing spectrophotometric methods. Methods were discovered to have benefits like great performance (RSD <2% for pure samples and dose form) and quick

reaction. As a result, it can be used in place of the current procedures.

Additionally, to our knowledge, there isn't a published GC-FID method for determining progesterone levels in pharmaceutical preparations. In order to determine progesterone in pharmaceutical preparations without the need for sample pre-treatment, we needed to design a new GC-FID method. The suggested techniques are also extremely efficient for measuring progesterone in pharmaceutical preparations. Recovery studies utilizing the conventional addition method were presented to demonstrate the viability of the suggested procedures. To do this, designed tablets were spiked with a known quantity of a reference medication, and the nominal value of the drug was calculated using the suggested approach.

## Conclusions

Using newly developed and validated UV, $^{1}$D spectrophotometry and GC-FID methods that were simple, rapid, dependable, specific, accurate, and precise, progesterone in pharmaceutical formulations was found in the current investigation. Progesterone in its pure form and in its formulation can be routinely analysed using the suggested procedures without separation or interference. They can be used for dissolution or related research as well. The use of UV and $^{1}$D spectrophotometry techniques, on the other hand, can be advantageous for routine examination of formulations and raw materials as well as sample analysis during accelerated stability tests.

## Conflicts of interest

The author states that did not has conflict of interests

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