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Determination of progesterone in commercial formulations and in non conventional micellar systems

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Abstract

Progesterone was determined in commercial pharmaceutical formulations and experimental micellar systems by means of two analytical methods based on liquid chromatography and derivative spectrophotometry. The chromatographic analysis, with ultraviolet detection at 245 nm, was carried out on a C8 column using a mobile phase composed of 2-propanol and a pH 2.5, 30 mM phosphate buffer. Derivative spectrophotometry (DS) used the difference between the values of the first derivative at 227.2 and 253.6 nm. Both methods require only a simple extraction procedure of progesterone from the formulations before analysis. The high-performance liquid chromatography (HPLC) procedure allows for the quantitative determination of progesterone in all pharmaceutical formulations tested (oily and alcoholic injectable solutions, gel preparations and soft capsules) and also of the newly-developed polymeric micellar system. On the contrary, the derivative spectrophotometric method is not suitable for the pharmaceutical formulation containing estradiol and for the new micellar systems. The results obtained with the two methods are in good agreement and always satisfactory in terms of precision and accuracy.

Keywords: Progesterone; Liquid chromatography; Derivative spectrophotometry; Pharmaceutical dosage forms; Micellar systems

1. Introduction

Progesterone, pregn-4-ene-3,20-dione (Fig. 1), is a luteo-hormone, and a precursor of many steroidal hormones including gluco-corticoids, mineral corticoids, androgens and estrogens [1].

Progesterone is widely used in therapy. The most frequent therapeutic uses of progesterone are for dysfunctional uterine bleeding or amenorrhoea [2,3], for contraception (either alone or with e.g. estradiol or mestranol in oral contraceptives) and, in combination with estrogens for hormone replacement therapy of post-menopausal women [4–6].

Moreover, many papers report the antitumoral efficacy of progesterone in the treatment of breast, endometrial and prostate cancers [7–10].

Progesterone does not have appreciable activity when administered by the oral route; in fact, when

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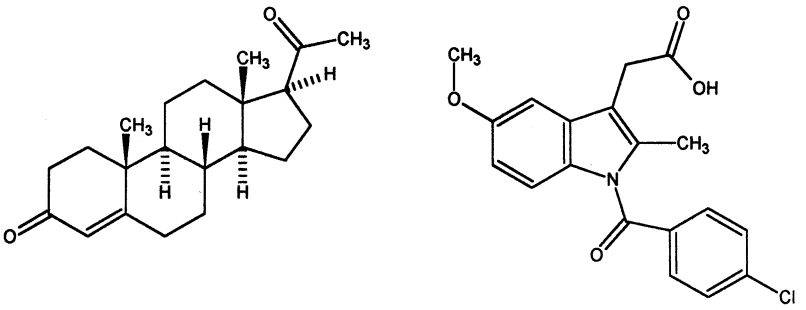

PROGESTERONE (PRG) **INDOMETHACIN (I.S.)**

Fig. 1. Chemical structure of progesterone and indomethacin (I.S.).

ingested, it is readily inactivated by the liver undergoing an extensive first-pass hepatic metabolism [11]. For this reason, progesterone is usually administered as an oily intramuscular injection, or as vaginal gel, pessaries and suppositories. However, an oral micronized preparation of progesterone is also available [12], which seems to be very effective for the treatment of premenstrual syndrome [13]. Moreover, new non conventional microparticulate systems (spheres and capsules) or micellar systems (amphiphilic polymers supporting the drug self-assembling as a function of environmental medium) are also proposed with the aim of increasing the drug availability [14].

Several papers are available in the literature on the analysis of progesterone in pharmaceutical formulations. Most of them have only investigated the quantitative determination of progesterone in oily injectable solutions by means of derivative spectrophotometry [15], circular dichroism spectroscopy [16] and linear sweep polarography [17]. A direct spectrophometric method was used for the determination of progesterone in aqueous and oily injectable solutions after derivatization with isoniazide [18]. Some of these methods require laborious extraction steps and are time consuming. Progesterone in oily injectable solutions was also determined by means of high-performance liquid chromatography (HPLC) systems using water-methanol or alcoholic mixtures [19–21] as mobile phases. The USP method [21], however, has some disadvantages because the column has to be thermostatted at 40 °C, and high percentages of various organic solvents are necessary.

In the present paper, a selective and sensitive HPLC method was developed and compared with a feasible spectrophotometric method for the quantitative determination of progesterone in different pharmaceutical formulations containing progesterone. In particular, four commercial preparations (oily and alcoholic injectable solutions, gel preparations, soft capsules) and one experimental micellar preparation were tested.

The chromatographic method gave satisfactory results in terms of precision and accuracy, which were in good agreement with those obtained by the derivative spectrophotometric procedure.

2. Experimental

2.1. Chemicals

Progesterone reference standard and indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid, Fig. 1), used as the internal standard (I.S.), were purchased from Sigma Chemicals (St. Louis, MO, USA). The pharmaceutical formulations containing progesterone, namely: Prometrium® (Rottafarm S.r.l., Monza, MI, Italy), Prontogest® (A.M.S.A. S.r.l., Rome, Italy), Progestogel® (Lusofarmaco S.p.A., Milan, Italy) and Menovis® (Teofarma S.r.l., Pavia, Italy) were purchased from a pharmacy. Acetonitrile, dichlor-

omethane, chloroform, 1-propanol, 2-propanol and methanol, HPLC grade, were from Carlo Erba (Milan, Italy). Sodium hydroxide and 85% (w/w) *ortho*-phosphoric acid were from E. Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore (Milford, MA, USA) MilliQ apparatus.

For the preparation of the micelles the following materials were used: polyvinyl alcohol (PVA, MW = 10 000 and 15 000 D, 80% hydrolyzed) from Sigma Chemicals; ethanol, oleoyl chloride and *N*-methylpyrrolidone from Fluka (Basel, Switzerland).

2.2. Polymeric micelles preparation

Substituted polyvinyl alcohol was prepared by dissolving the polymer in *N*-methylpyrrolidone at 50 °C and, subsequently, adding the substituent, oleoyl chloride. Stirring was carried out at room temperature for 24 h. The solution obtained was supplemented with water to induce precipitation of the substituted polymer. The solid obtained was washed with water and dried under vacuum to constant weight. The degree of substitution of the polymer was determined by elemental analyses performed using H^1-NMR.

The polymer and progesterone in a 5:2 (w/w) ratio were dissolved in ethanol and rapidly injected in an aqueous phase during ultrasonication. The resulting micelle suspension was then diluted with a 1.7% aqueous solution of $PVA_{15\,000}$ and nebulised and dried by means of spray-drying (110 °C inlet temperature, 60 °C outlet temperature).

2.3. Standard solutions

The stock solutions (1 mg ml^{-1}) of progesterone and I.S. were prepared by dissolving 20 mg of compound in 20 ml of methanol. Progesterone standard solutions at different concentrations were prepared by diluting suitable volumes of the stock solution with 2-propanol for the spectrophotometric method and with the mobile phase for the HPLC method. The stock solutions of the analyte in methanol were stable in freezer (−20 °C) for at least 1 month (spectrophotometric evaluation), while the standard solutions were prepared daily from the stock solutions, in order to avoid any alteration of the drug.

The I.S. at the concentration of 150 ng ml^{-1} was added to the standard solutions which were analyzed by means of the HPLC method.

2.4. Apparatus and experimental conditions

2.4.1. Derivative spectrophotometry (DS)

For the spectrophotometric assays of Prometrium®, Prontogest® and Progestogel® a JASCO (Tokyo, Japan) Uvidec-610 double-beam spectrophotometer and quartz cuvettes (1 cm optical path) were used.

The spectrophotometric assays, using the direct UV spectra, were performed at the wavelength of 240 nm.

The values of derivative used for quantitative determinations were calculated as the difference between the height of the maximum at $\lambda = 253.6$ nm and that of the minimum at $\lambda = 227.2$ nm of the first derivative spectra. 2-propanol was used as the blank solution for all measurements.

2.4.2. High-performance liquid chromatography (HPLC)

The chromatographic apparatus consisted of a Beckman (Palo Alto, CA, USA) Programmable Solvent Module 126 chromatographic pump and a Beckman Programmable Detector 166 spectrophotometric detector set at 245 nm. During the analysis of Menovis® the detector was set at 245 nm from 0 to 6 min and at 230 nm from 6 min to the end of the chromatographic run since this pharmaceutical formulation contains also estradiol benzoate as a second active principle.

Separation was obtained on a Varian (Harbor City, CA, USA) reversed phase column (Res Elut, C8 150 × 4.6 mm, I.D. 5 μm,) kept at room temperature and connected with a precolumn (Res Elut, C8 30 × 4.6 mm I.D., 5 μm, Varian). The injection was carried out through a 20 μl loop. The mobile phase was composed of a mixture of 2-propanol-phosphate buffer (pH 2.5; 30 mM) with an apparent pH* of 3.0 (1:1, v/v). The mobile phase was filtered through a Millipore membrane filter (nylon, 47 mm diameter, 0.2 μm pore size) and degassed by an ultrasonic apparatus (Trans-

sonic T-310 apparatus from Elma GmbH, Singen, Germany) before use. The flow rate was kept at 1 ml min^{-1}. Data were handled by means of a BECKMAN GOLD 7.11 software.

2.5. Extraction procedure

The four commercialized pharmaceutical formulations analyzed were:

Prontogest®: 1 ml oily injection solution containing 100 mg of progesterone and benzyl alcohol and ethyl oleate as excipients.
Prometrium® soft capsules for oral and vaginal route containing 100 mg of micronized progesterone in an emulsion composed by peanut oil, soybean lecithin, gelatine, glycerol and titanium oxide.
Progestogel®: gel for vaginal route containing 1 g of progesterone in 100 g of carboxypolymethylen, triethanolamine, ethanol and pure water.
Menovis®: alcoholic intramuscular solution containing 50 mg of progesterone and 5 mg of estradiol benzoate in 0.8 ml of benzyl alcohol and ethanol.

The solutions (1 mg ml^{-1}) of Prontogest®, Progestogel® and Menovis® were prepared treating a suitable amount of the pharmaceutical formulation, corresponding to 20 mg of progesterone, with 20 ml of methanol. The methanolic mixtures after vortexing for 5 min were then centrifuged for 15 min at 3000 rpm. Finally, the supernatants were filtered through a cellulose acetate syringe filter (0.20 μm, Albet-Jacs). These solutions had a progesterone nominal concentration of 1000 μg ml^{-1}.

The Prometrium® stock solution (1 mg ml^{-1}) was prepared by removing, as completely as possible, the contents of 20 capsules and mixing. An accurately weighed portion of the emulsion, equivalent to 20 mg of progesterone, was transferred into a test tube with 20 ml of 2-propanol, and, after agitation for 10 min in an ultrasonic bath, it was stored for 5 min at 4 °C. It was successively centrifuged for 15 min at 3000 rpm. Finally, the supernatant was filtered through a cellulose acetate syringe filter (0.20 μm, Albet-Jacs).

The experimental pharmaceutical formulation is a polymeric micellar system containing progesterone (3.5%) in a micellar core of oleoyl chloride substituted $PVA_{10\,000}$ (polyvinyl alcohol) and coated with $PVA_{15\,000}$. The stock solution of experimental formulation (1 mg ml^{-1}) was prepared by dissolving a suitable amount of polymeric micelles in a water:2-propanol (50:50, v/v) mixture, then filtering through a cellulose acetate syringe filter (0.20 μm, Albet-Jacs).

Working solutions at different concentrations were prepared from these stock solutions, by diluting with 2-propanol for the spectrophotometric method and diluting with mobile phase for the HPLC method.

A suitable amount of I.S. was added to each formulation sample before extraction, in order to obtain a final I.S. concentration after the extraction procedure of 150 ng ml^{-1}.

2.6. Method validation

2.6.1. Linearity

For the DS method, a ten-point calibration curve was set up on standard solutions in the 2–50 μg ml^{-1} concentration range by plotting derivative absorbance values against the respective progesterone concentrations (expressed as μg ml^{-1}).

For the HPLC method, a ten-point calibration curve was set up on standard solutions in the 25–500 ng ml^{-1} concentration range plotting the progesterone/I.S. peak area ratios (a dimensionless number) against the corresponding progesterone concentrations (expressed as ng ml^{-1}).

According to USP guidelines [22], the LOD and LOQ values were obtained analyzing standard solutions at several concentrations and determining the lowest concentrations that can be reliably detected and quantitated (Coefficient of variation, $CV < 20\%$), respectively. These values were then validated injecting samples at known concentrations.

The amount found of declared was calculated analyzing sample solutions from pharmaceutical formulations having a nominal progesterone con-

centration of 5, 10 and 15 μg ml^{-1} (DS method) or 50, 250 and 500 ng ml^{-1} (HPLC method) and interpolating the measurements on the respective calibration curves. The progesterone concentrations thus found were then compared with the nominal concentrations.

2.6.2. Precision

Progesterone solutions extracted from the four pharmaceutical formulations were prepared and analyzed six times within the same day to obtain the repeatability, and six times over different days to obtain the intermediate precision, according to USP 24 requirements [22].

Each assay was carried out at three different nominal concentrations of progesterone, namely: 2.5, 10.0 and 20.0 μg ml^{-1} for the DS method and 50, 250 and 500 ng ml^{-1} for the HPLC method. The relative standard deviation percentages (R.S.D.%) of the data obtained were calculated.

2.6.3. Accuracy

The accuracy of the methods was evaluated by means of recovery assays, adding known amounts of the reference compound powder to a known amount of each pharmaceutical formulation, in order to obtain three different levels of addition. For the DS method, amounts of 5.0, 7.5 and 10.0 μg ml^{-1} of progesterone were added to the pharmaceutical formulations, obtaining final concentrations of 10.0, 12.5 and 15.0 μg ml^{-1}. For the HPLC method, amounts of 50, 100 and 250 ng ml^{-1} of progesterone were added to the pharmaceutical preparations, obtaining final concentrations of 300, 350 and 500 ng ml^{-1}. The samples were analyzed and the mean recovery, as well as the repeatability, was calculated on six assays for each concentration added.

3. Results and discussion

3.1. Extraction procedure optimization

Different assays were carried out in order to find a common procedure to extract progesterone from the four different pharmaceutical formulations. Different solvents were tested, namely: methanol, acetonitrile, dichloromethane, chloroform, 1-propanol or 2-propanol, and the resulting solutions were analyzed by spectrophotometry. However, not all of these assays gave satisfactory results for all the four formulations. For this reason methanol was used for the extraction of progesterone from Prontogest®, Progestogel® and Menovis®, and 2-propanol for the pretreatment of Prometrium®. These feasible extraction procedures of progesterone from the four pharmaceutical formulations did not lead to any interference for the HPLC method, and to only a small interference for the spectrophotometric method which was eliminated using the first derivative spectra.

These very simple and fast extraction procedures of progesterone from commercial formulations were used for both analytical methods.

3.2. DS method

The spectrum of a 10 μg ml^{-1} progesterone standard solution in 2-propanol is shown in Fig. 2a. An intense absorbance band in the UV region, with maximum at 240 nm, is apparent. Preliminary assays demonstrated that the direct spectrophoto-

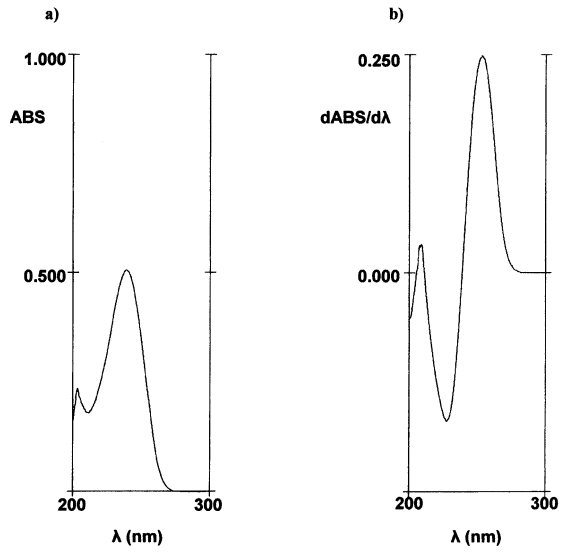

Fig. 2. Ultraviolet spectra of a 10 μg ml^{-1} progesterone standard solution in 2-propanol against a blank of 2-propanol (a) and first derivative spectrum of the same solution (b).

metric analysis of the drug in the dosage forms was complicated by background interference from the formulation matrix. This spectral interference lead to an over-estimation of the concentration of PRG: the amounts found of declared, in fact, were always higher than 120%. For this reason, several assays were carried out using the first and second derivative spectra; best results were obtained when first derivative of the absorbance was used. Fig. 2b shows the first derivative spectrum of a progesterone standard solution.

Good linearity was obtained on standard solutions in the 2–50 μg ml^{-1} progesterone concentration range. The conditions used here are very different from those reported in another paper [15] dealing with the analysis of several hormones, which gives only a few data for method validation. The linearity equation was $y = -0.0069 + 0.0095\,x$ ($r_c = 0.9994$), where x is the progesterone concentration (expressed as μg ml^{-1}) and y is the difference between the values of the first derivative at 227.2 and 253.6 nm. The LOQ was 2 μg ml^{-1} and the LOD 1.5 μg ml^{-1}.

Precision assessed on standard solutions was satisfactory: R.S.D.% mean values of 1.5% (repeatability) and 1.7% (intermediate precision) were found for six replicates at concentrations of 2.5, 10 and 20 μg ml^{-1}.

Menovis® and the experimental micellar formulation could be analyzed by HPLC only, since the former contains progesterone in combination with estradiol benzoate (which has similar absorbance spectrum) and the latter shows strong interference due to the formulation matrix.

The first derivative spectra of the formulation sample solutions are morphologically identical to those of standard solutions having the same concentration. Fig. 3 shows (a) the ultraviolet direct spectrum of a Prometrium® sample solution (nominal concentration of 10 μg ml^{-1}) and (b) the first derivative spectrum of the same solution.

The data obtained are summarized in Table 1. As one can see all assays gave satisfactory results: the mean amount found of declared was always between 98.4 and 100.3% for the three formulations analyzed by means of DS method.

Precision assays were carried out analyzing the extracts of the three pharmaceutical formulations

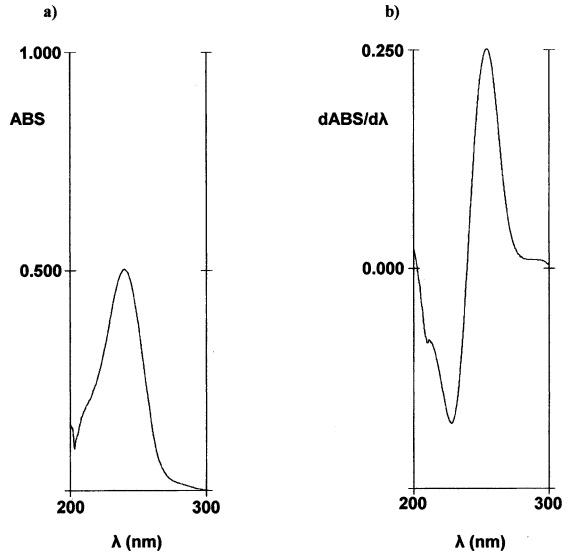

Fig. 3. Ultraviolet spectra of a Prometrium® sample solution (nominal concentration: 10 μg ml^{-1}) (a) and first derivative spectrum of the same solution (b).

in order to evaluate the R.S.D.% data of intraday and interday assays. Table 1 reports the precision data: the R.S.D. values were between 0.3 and 1.1% for the repeatability while the intermediate precision was always better than 1.2%, respectively.

The accuracy of the method was calculated by means of recovery studies. The results are reported in Table 2. The high recovery values (the mean recovery was 99.5%) indicate the fine accuracy of the proposed DS method.

The precision of the recovery assays (repeating the procedure six times) was also satisfactory; in fact, the values of R.S.D.% intraday, calculated on six trials, varied between 0.3 and 0.6.

3.3. HPLC method

Preliminary chromatographic experiments for the determination of progesterone were carried out starting from chromatographic conditions already reported in the literature [24] and precisely a C18 column (300 × 4 mm, I.D. 5 μm) as the stationary phase and as the mobile phase a mixture of H_2O-2-propanol (72:28, v/v) (flow rate of 1.5 ml min^{-1}, detection at 254 nm).

Table 1
Quantitative determination of progesterone in pharmaceutical formulations by means of DS method

	Formulation and concentration analyzed (μg ml^{-1})	% Found of declared (intraday)[a]	Repeatability R.S.D.%	% Found of declared (interday)[a]	Intermediate precision R.S.D.%
Prometrium®	5	99.2	0.9	99.6	1.0
	10	99.8	1.1	99.9	1.2
	15	99.0	0.6	98.8	0.5
Prontogest®	5	100.2	0.6	100.2	0.7
	10	100.2	0.2	99.9	0.7
	15	100.0	0.2	100.3	0.8
Progestogel®	5	98.4	0.8	99.6	0.4
	10	100.0	0.7	99.8	0.3
	15	100.0	0.3	99.8	0.3

[a] $n = 6$.

In order to reduce the analysis time (which was about 21 min using the method reported in [24]), a shorter and less hydrophobic column (Res Elut, C8 150 × 4.6 mm I.D., 5 μm) was chosen as the stationary phase, and the optimal composition of the mobile phase was investigated. The 2-propanol amount was varied from 10 to 70% (in 10% steps). Best results were obtained using a 50% value of 2-propanol. Using as the mobile phase a H_2O-2-propanol (50:50, v/v) mixture with a flow rate of 1 ml min^{-1}, the peak of progesterone is detected in less than 6.0 min. To better separate the peak of the compound chosen as the I.S. (i.e. indomethacin) from that of progesterone, a pH 2.5, 30 mM phosphate buffer was used instead of water. This also lead to a more symmetric peak shape of progesterone. The UV detector was set at 245 nm, because progesterone Vis–UV spectra, in the phosphate buffer–2-propanol medium exhibit a maximum of absorbance at 245 nm.

Linearity was observed between 25 and 500 ng ml^{-1} of progesterone. The equation of the calibration line, obtained by the least-square regression was: $y = 0.0242 + 0.0083\ x$, where x is the progesterone concentration, expressed as ng ml^{-1}, and y is the ratio between the area of progesterone and that of the I.S.. The linear correlation coefficient, r, was 0.9995. The LOQ (quantitation limit) value was 20 ng ml^{-1} and the LOD (detection limit) value was 10 ng ml^{-1}. The precision values were very satisfactory and the assays carried out on three different progesterone concentrations gave R.S.D.% values lower than 1.5 for repeatability and lower than 1.6 for the intermediate precision ($n = 6$).

The chromatogram of a Prometrium® extract having a nominal concentration of 250 ng ml^{-1} of

Table 2
Accuracy of the DS method

	Formulation and concentration added (μg ml^{-1})	Recovery%[a]	Repeatability R.S.D.%[a]
Prometrium®	5	101.7	0.6
	7.5	98.0	0.5
	10	99.2	0.3
Prontogest®	5	101.7	0.6
	7.5	98.8	0.5
	10	99.2	0.3
Progestogel®	5	98.8	0.5
	7.5	98.8	0.4
	10	99.2	0.3

[a] $n = 6$.

progesterone is shown in Fig. 4. It is apparent that the peak of progesterone is very neat, with a retention time of 4.5 min and well separated from that of indomethacin (150 ng ml^{-1}), used as the I.S., which has a retention time of 3.3 min. The overall morphology of the chromatogram is nearly identical to that of a standard solution at the same concentration.

The chromatogram of an extract from Menovis® having a nominal concentration of 250 ng ml^{-1} of progesterone is shown in Fig. 5. The presence of a third peak, due to estradiol benzoate (25 ng ml^{-1}) which is the second active principle of the formulation, is detected at 230 nm. Thus, this method seems to be promising for the simultaneous determination of progesterone and estradiol, but this is not the topic of this paper.

The extraction procedure of the drug from the pharmaceutical formulations is very simple and feasible; it is based only on the one step treatment of the formulation with methanol or 2-propanol as

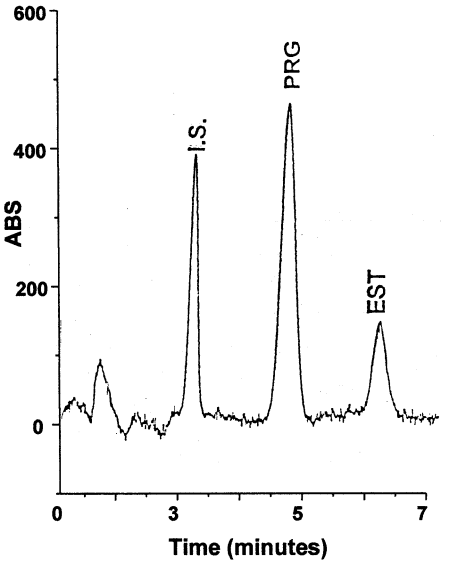

Fig. 5. Chromatogram of a Menovis® sample solution (progesterone nominal concentration of 250 ng ml^{-1} and estradiol nominal concentration of 25 ng ml^{-1}) containing 150 ng ml^{-1} of I.S. (indomethacin). Detection at 245 and 230 nm. Other conditions as in Fig. 4.

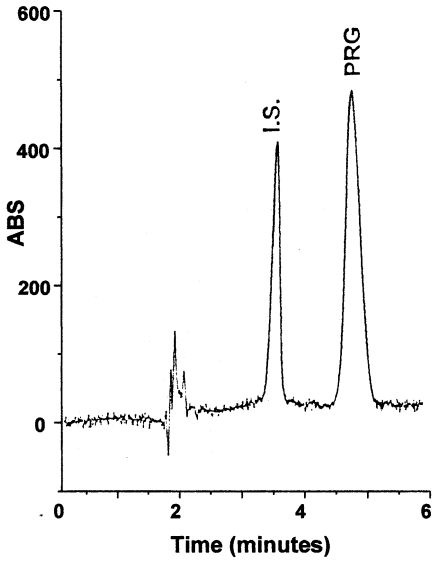

Fig. 4. Chromatogram of a Prometrium® sample solution (progesterone nominal concentration of 250 ng ml^{-1}) containing 150 ng ml^{-1} of I.S. (indomethacin). Conditions: RP C8 column, 150 mm length, 4.6 mm I.D., 5 μm particle size. Mobile phase: 2-propanol–phosphate buffer (30 mM; pH 2.5) (50:50, v/v, pH* 3.0). Flow rate: 1.0 ml min^{-1}. Detection at 245 nm.

reported in the Section 2. In fact, no interference from excipients was revealed in the formulations analyzed.

The percentage found of label claim, reported in Table 1, is always better than 98.6%, this indicates that the pharmaceutical formulations were accurately prepared by manufacturers in accordance with the claimed value, and within the limit prescribed by U.S.P. XXIV [23].

Precision assays were carried out analyzing extracts of the four pharmaceutical formulations in order to evaluate the R.S.D.% data of intraday and interday assays. For this purpose several extracts of each formulation at different concentrations were analyzed. The obtained data were very satisfactory as reported in Table 3. In fact, the R.S.D. values were equal or less than 1.7% for the repeatability, better than 1.9% for the intermediate precision.

The accuracy of the method was evaluated by means of recovery studies, and the results of these assays are reported in Table 4. The high recovery

Table 3
Quantitative determination of progesterone in pharmaceutical formulations by means of HPLC method

	Formulation and concentration analyzed (ng ml^{-1})	% Found of declared (intraday)[a]	Repeatability R.S.D.%	% Found of declared (interday)[a]	Intermediate precision R.S.D.%
Prometrium®	50	98.6	0.9	99.5	1.2
	250	99.1	0.5	99.4	0.5
	500	99.5	1.1	99.5	0.1
Prontogest®	50	98.7	1.7	100.0	1.0
	250	100.9	0.7	99.3	1.7
	500	99.5	0.5	99.7	0.9
Progestogel®	50	99.8	0.7	99.7	0.6
	250	100.1	0.7	100.2	0.7
	500	100.2	0.2	100.1	0.4
Menovis®	50	99.1	1.6	98.7	1.7
	250	99.3	1.1	99.2	1.0
	500	98.6	1.2	99.1	1.8
Polymeric micelles	50	103.9	1.7	102.8	1.9
	250	102.0	1.5	102.1	1.8
	500	102.8	1.2	103.0	1.5

[a] $n = 6$.

values (the mean recovery is 100.2%) demonstrate a quantitative recovery of the analyte indicating the fine accuracy of the proposed HPLC method.

The precision of the recovery assays was also satisfactory; in fact, the values of R.S.D.% intraday, calculated on six trials for each pharmaceutical formulation, varied between 0.2 and 1.7.

3.4. Comparison of the analytical methods

Comparison of the performance of the two methods implemented shows that, as expected, the HPLC method is much more sensitive than the DS method (linearity was 2–50 μg ml^{-1} for the latter and 25–500 ng ml^{-1} for the former; LOQ

Table 4
Accuracy of the HPLC method

	Formulation and concentration added (ng ml^{-1})	Recovery%[a]	Repeatability R.S.D.%[a]
Prometrium®	50	100.5	0.3
	100	100.1	0.8
	250	99.9	0.2
Prontogest®	50	99.6	0.7
	100	101.0	0.6
	250	98.8	0.7
Progestogel®	50	100.3	0.3
	100	99.4	0.3
	250	100.2	0.3
Menovis®	50	99.1	0.4
	100	99.6	0.3
	250	100.1	0.4
Polymeric micelles	50	101.1	1.7
	100	104.8	1.5
	250	99.3	1.2

[a] $n = 6$.

values were 2 μg ml^{-1} and 20 ng ml^{-1}, respectively), of about two orders of magnitude.

However, within their respective linearity range and for the formulations analyzed by the two methods, the values of drug found of declared are in very good agreement (the highest difference between the two methods is 1.5%), with R.S.D.% values for precision which are similar and very low (0.2–1.2% for the DS method, 0.2–1.7% for the HPLC method).

The accuracy of the DS method tends to be lower than that of the HPLC method, but always within 2.5% of the latter.

Thus, the results obtained with the two methods developed seem to be largely equivalent.

3.5. Determination of drug content in polymeric micelles

The use of 2-propanol to extract the active principle from the experimental preparations gave poor recovery results. For this reason, water was added to 2-propanol in order to solubilise the polymeric matrix and the coating. Several water:2-propanol ratios were tried, from 0 to 70% of water; best results were obtained using a 50:50 (v/v) mixture, which was then used for all subsequent assays.

Fig. 6 shows the chromatogram of an extract obtained from the polymeric micellar system, having a nominal concentration of 250 ng ml^{-1} of progesterone. As one can see, the peaks of progesterone and I.S. are clearly detected and no interference due to the formulation matrix is present.

The percentage of drug found of declared (Table 3), is always better than 104%, indicating a good preparation of the polymeric micelles.

Precision assays were carried out analyzing extracts of the experimental formulations. For this purpose several extracts at different concentrations (50, 250 and 500 ng ml^{-1}) were analyzed. The R.S.D. values were better than 1.7% for repeatability, better than 1.9% for intermediate precision, as reported in Table 3.

The accuracy of the method was evaluated by means of recovery studies carried out in the same way as described for the commercial formulations, above. The high recovery values (mean recovery 99.3–104.8%) indicate a quantitative recovery of the analyte; the results are reported in detail in Table 4.

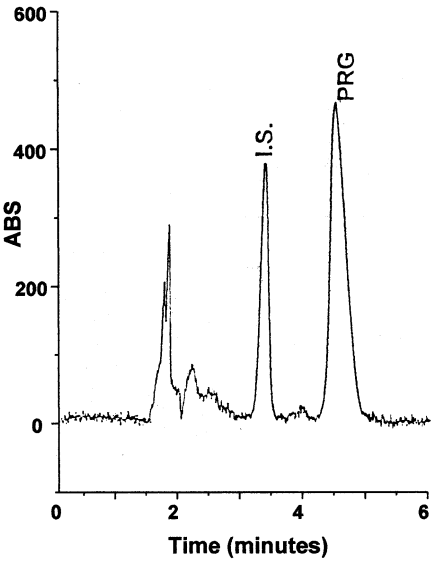

Fig. 6. Chromatogram of an extract obtained from the polymeric micellar system (progesterone nominal concentration of 250 ng ml^{-1}) containing 150 ng ml^{-1} of I.S. (indomethacin). Other conditions as in Fig. 4.

The precision of the recovery assays was also satisfactory; in fact, the values of R.S.D.% for repeatability, calculated on six trials, varied between 1.2 and 1.7%.

The results reported show clearly that the active principle can be accurately dispersed in the new micellar system. Assays are in progress, in order to apply this HPLC method to the evaluation of progesterone release from the micellar system at different pH values.

4. Conclusion

The developed HPLC method allowed the analysis of progesterone in different kinds of pharmaceutical preparations, both commercial and non-conventional micellar systems, giving very satisfactory results in terms of repeatability, intermediate precision and accuracy. Compared with the HPLC methods reported in literature, the

proposed method allows analysis of progesterone in pharmaceutical preparations in a shorter time period [19] (within 5 min), with better precision and higher sensitivity [20], and moreover it needs minor amounts of organic solvents [21].

Hence, the HPLC method seems to be suitable for the quantitative determination of progesterone in pharmaceutical formulations, including an injectable preparation containing progesterone in association with estradiol benzoate. The DS method shows the same good precision and accuracy but does not have suitable selectivity for the quantitative determination of progesterone in the presence of estradiol benzoate and in the polymeric micellar systems.

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