M1025

A SENSITIVE METHOD FOR THE DETERMINATION OF PROGESTERONE IN HUMAN PLASMA BY LC-MS-MS

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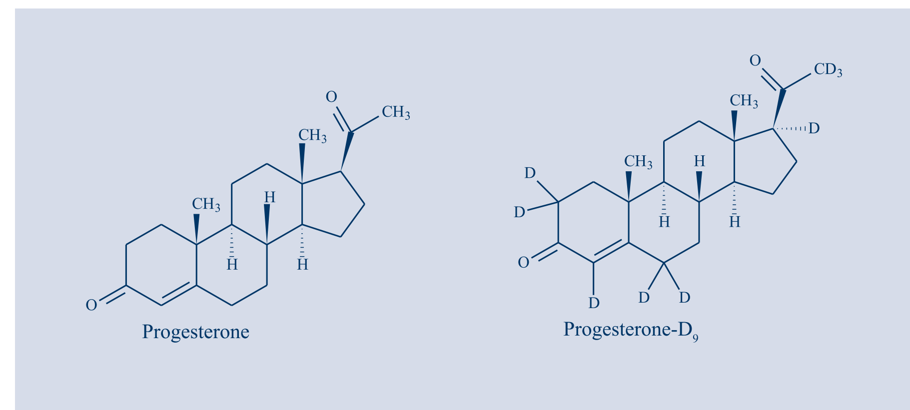


Abstract:
Progesterone is an active principle of the corpus luteum, secreted during the latter half of the menstrual cycle. The synthetic progestin, progesterone, is indicated in the treatment of secondary amenorrhea due to hormonal imbalance in the absence of organic pathology, such as fibroids or uterine cancer. A method for the analysis of progesterone suitable for pharmacokinetic investigations of a 200 mg capsule was developed and validated. The method was validated with a quantitative range of 0.100 to 50.0 ng/mL from 0.500 mL of human plasma. The plasma sample was fortified with the internal standard, progesterone-D_9, prior to extraction. Buffered human plasma (pH=7) was extracted with an ethyl acetate/hexane (1:1) mixture. The extract was injected onto a Monochrom Silica 4.6 x 100 mm HPLC column with a mobile phase of ethyl acetate/hexane/isopropanol (100:200:10.0) at 1.4 ml/min. The eluant was analyzed by a Sciex API 4000 LC-MS-MS in heated nebulizer mode with a run time of 2.0 minutes. Mass spectra were acquired in positive ion mode with multiple reaction monitoring using the mass transition of m/z 315→97. Three validation runs were performed each on separate days. Precision (CV) and accuracy (%bias) across all levels of the calibration range, including the LLOQ, were within ±12.8%. Although measurable endogenous progesterone levels were noted in some blank plasma lots, the matrix effect on analytical recovery was unaffected. Stability of progesterone in plasma was established for 24 hours at room temperature, 5 cycles of freezing and thawing, and 22 hours in the final extract at room temperature. This accurate method requires few extraction steps leading to high throughput and good precision. With one LC-MS-MS instrument, an analyst can routinely analyze 500 samples per day.

Objective:
Develop and validate an efficient and rugged method for the analysis of study samples from progesterone clinical trials utilizing progesterone.

Introduction:
A rugged and sensitive method for the analysis of progesterone for pharmacokinetic investigation was developed and validated. The quantitative range is 0.100 to 50.0 ng/mL of progesterone in human plasma. This presentation provides details of the method and validation.

Methodology:

Chemicals
Progesterone and the internal standard, progesterone-D_9, were purchased with a reported chemical purity of greater than 99%. All other chemicals were AR grade. Solvents used were HPLC grade.

Solutions
Stock solutions of progesterone and its stable labeled internal standard were prepared in acetonitrile/water (1:1) and stored at approximately 4ºC. Intermediate solutions were prepared from these stock solutions and a series of spiking standard solutions were made to cover the method quantitation range. Quality control (QC) samples in EDTA human plasma were prepared at three different concentrations (40.0, 10.0 and 0.300 ng/mL progesterone). The QC samples were aliquotted at 0.500 mL and stored at -20ºC.

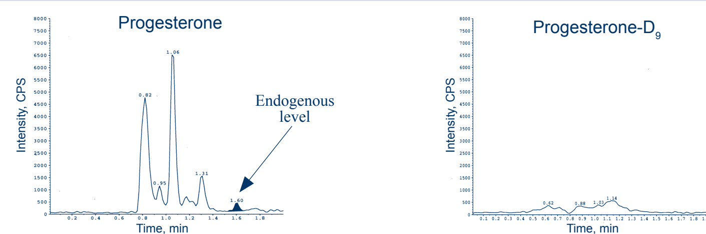
Extraction
- 0.500 mL of EDTA human plasma
- 20.0 µL of working internal standard solution
- 0.500 mL of 1.0 M sodium acetate solution
- Extract with 3.00 mL of ethyl acetate/hexane (1:1)
- Centrifuge and transfer clear upper organic layer to clean test tube
- Evaporate to dryness
- Reconstitute in 100 µL of mobile phase
- Analyze by LC-MS-MS

Chromatographic System
The LC-MS-MS system consisted of a Shimadzu LC pump, Perkin-Elmer autosampler and SCIEX API 4000 mass spectrometer with a heated nebulizer interface. The mass spectrometer was optimized for the intensity of a progesterone signal in positive ion mode. The ion transitions monitored were as follows: progesterone (m/z 315→97) and progesterone-D_9 (m/z 324→100).

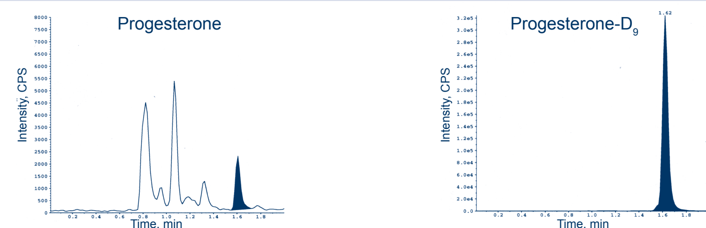
Extracts were injected on the HPLC system with a column flow rate of 1.40 mL/min. The chromatographic separation was achieved by a 3 micron, 4.6 x 100 mm Monochrom Silica column with a mobile phase consisting of ethyl acetate/hexane/isopropanol (100:200:10.0).

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy
Mean of Three Validation Days

Amount Added, ng/mL	0.100	0.200	1.00	2.00	5.00	25.0	45.0	50.0
Mean Found, ng/mL	0.0991	0.204	0.977	1.98	5.13	25.3	44.5	49.6
CV (%)	0.8	1.9	2.7	2.9	1.7	4.2	2.2	0.50
% Bias	-0.9	2.0	-2.3	-1.0	2.6	1.2	-1.1	-0.8

LLOQ and QC Precision and Accuracy
Mean of Three Validation Days (n=18)

Amount Added, ng/mL	0.100	0.300	10.0	40.0
Mean Found, ng/mL	0.105	0.311	10.0	38.4
CV (%)	11.7	6.1	4.5	4.1
% Bias	5.0	3.7	0.0	-4.0

Stability

Concentration, ng/mL	0.300	40.0
	Mean % Change	
BTS, 24 hrs	5.3	-2.5
FTS, 5 cycles	3.7	-4.5
XTS, 22 hrs	1.7	-0.5
LTS, 111 days @ -20°C	12.3	-1.0

The linear quantitation range was established from 0.100 to 50.0 ng/mL using freshly fortified calibration samples. Precision and accuracy of the method was determined to be acceptable after evaluating LLOQ samples and QC samples over three days of validation. Inter-day precision (%CV) at the LLOQ was 11.7%. Accuracy for the LLOQ was 5.0%. Precision of QC samples ranged from 4.1 to 6.1%, with accuracy ranging from –4.0 to 3.7%. Stability of QC samples in plasma during normal handling was acceptable after stressing with multiple freeze-thaw cycles, room temperature exposure, and an extended term in -20ºC frozen storage. Extraction recovery was 85.5%. No significant chromatographic interferences of 18 common OTC drugs were detected at the retention times of progesterone or the internal standard. There was measurable endogenous progesterone peaks in some blank plasma lots, however, the matrix effect on analytical recovery was unaffected. Progesterone is endogenous to human plasma; therefore, pre-dose samples may have measurable levels.

Conclusion:
This method demonstrates good ruggedness over the stated quantitation range coupled with excellent sensitivity.