Progesterone (P4 \[\text{pregn-4-ene-3,20-dione}\]) in mammals is a derivative of cholesterol and a precursor of aldosterone, cortisol and many other steroid hormones. Progesterone levels in adult humans are highest in pregnant women and nursing mothers. The most common uses of therapeutic progesterone are for reproductive system related disorders or dysfunctions. However, it is also postulated to be effective in treatment of multiple sclerosis due to its protective effects on brain myelin, and in treatment of skin conditions.

A progesterone test is done to:

- Help find the cause of infertility
- Monitor the success of medicines for infertility or the effect of treatment with progesterone. Help determine whether ovulation is occurring.
- Assess the risk of miscarriage.
- Monitor the function of the ovaries and placenta during pregnancy.
- Help diagnose problems with the adrenal gland and some types of cancer.

A sensitive LC-MS/MS method was developed for the determination of progesterone in human K2-EDTA plasma. The plasma samples were prepared through simple protein precipitation with 10 ng/mL d8-progesterone as the internal standard. The final supernatant was collected analyzed on the API 4000 LC-MS/MS system by electrospray ionization (ESI) mass spectrometry with multiple reaction monitoring (MRM) of positive ions. The MRM used precursor → product ions of \(m/z\) 315.3 → 109.1, and 323.4 → 113.1 to monitor progesterone and d8-progesterone, respectively. The ratio of progesterone product ion peak area to that of the internal standard were the responses used for quantitation.

The validation showed that the method was linear \((r^2 = 0.9984-0.9998)\) over the concentration range of 500 pg/mL to 1000 ng/mL for progesterone. No significant interference was observed in blank plasma.

The intra-day and inter-day accuracy for the determination of progesterone in plasma samples did not exceed 10.59% at the LLOQ and was within 6.89% at all other levels. The intra-day and inter-day precision for the determination of progesterone in plasma samples did not exceed 15.24% CV for low QC and 5.38% for other QCs. Progesterone extracted from plasma was stable at 4 °C (the temperature of the cooled auto-sampler) for at least 24 hours. Progesterone was stable in plasma after 3 freeze-thaw cycles. The selectivity, sensitivity, linearity, accuracy, precision and robustness of the method are sufficient for analysis of progesterone in human plasma samples, and demonstrate the performance of the LC-MS/MS system to be qualified.

Typical Calibration curve