Simultaneous Measurement of Dehydroepiandrosterone (DHEA) and 17-Hydroxyprogesterone (17-OHP) as Biomarkers in Human Plasma Using UPLC-MS/MS

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**Purpose**
Dehydroepiandrosterone (DHEA) is a hormone produced by the body's adrenal glands. The body uses DHEA to make androgens and estrogens, the male and female sex hormones. 17-Hydroxyprogesterone (17-OHP) is an endogenous progestogen in the biosynthesis of other steroid hormones, including corticosteroids, androgens, and estrogens. In recent years, LC-MS/MS methods have been developed for determination of steroid hormones in human plasma. Many of them suffered bioanalytical issues such as peak tailing and limited sensitivity. In this study, we have developed a rugged method for simultaneous determination of DHEA and 17-OHP in human plasma.

**Methods**
Multiple lots of human plasma were screened and those with lower levels of DHEA and 17-OHP were pooled for the preparation of QC samples for the validation. The method utilized a liquid-liquid extraction and then derivatization procedure prior to LC-MS/MS analysis. Briefly, DHEA, 17-OHP and the added internal standards were extracted from 50 μL of human plasma using MTBE (methylterbutyl ether). The organic layer was transferred and dried under N2. The residue was reconstituted and derivatized with hydroxylamine. The resultant products were submitted for LC-MS/MS analysis.

The LC-MS/MS analysis was carried out on a Sciex Triple Quad 5500 mass spectrometer coupled with a Shimadzu UPLC system. The mass spectrometer was operated in positive ESI mode. The multiple reaction monitoring (MRM) transition was m/z 304.1→253.1 for DHEA, m/z 309.1→258.1 for DHEA-d₅, m/z 361.1→112.1 for 17-OHP and m/z 369.1→112.1 for 17-OHP-d₈, respectively.

**Results**
Due to presence of endogenous DHEA and 17-OHP in human plasma, surrogate matrix of BSA in PBS solution was used for the preparation of calibration standards. QC samples (LLOQ, LQC, MQC and HQC) were prepared in pooled authentic human plasma. Excellent linearity was obtained with a correlation coefficient ≥ 0.9958 for DHEA and ≥ 0.9981 for 17-OHP. The calibration curve range was from 0.020 ng/mL to 10 ng/mL (0.1 pg to 50 pg on-column). CVs and biases at LLOQ level were 7.1% and -4.8% for DHEA, and 6.7% and -7.5% for 17-OHP, respectively. CVs at all other QC levels were from 5.6 to 8.3% for DHEA and from 2.0 to 10.0% for 17-OHP. The biases at all other levels were from -1.6 to 1.5% for DHEA and from -4.0 to 5.8% for 17-OHP. Endogenous levels of screened multiple lots of human plasma are from 0.04 to 5.18 ng/mL for DHEA, and from 0.110 to 2.08 ng/mL for 17-OHP.

**Conclusion**
A sensitive and reproducible UPLC-MS/MS method has been validated for the simultaneous quantitation of steroid hormone DHEA and 17-OHP in human plasma. The validated method has been successfully used for the analysis of clinical study samples.