Development of a high-throughput Ultra Performance Liquid Chromatographic-Tandem Mass Spectrometric (UPLC-MS/MS) method for medroxyprogesterone acetate (MP 17 Acetate) quantification in human plasma

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Background: Medroxyprogesterone acetate (MPA) is a synthetic analog of the steroidal hormone progesterone. As an agonist of the progesterone receptor, MPA has primarily been used as a contraceptive agent, and is administered orally or as a long-acting subcutaneous or intramuscular injectable; the latter formulations are referred to as depot MPA (DMPA). Previous studies have assessed the pharmacokinetics of MPA and DMPA, and peak concentrations of long-acting MPA vary between 0.95 ng/mL to 2.5 ng/mL [1-3]. Current methods for MPA quantification require large sample volumes (>1.0 mL) and laborious sample preparation with extensive analytical run times. Therefore, a high-throughput liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method has been developed to circumvent these issues.

Methods: Human male plasma K_2EDTA was obtained from Bioreclamation (Bioreclamation IVT, Baltimore, MD) and Biological Specialty Corporation (Biological Specialty Corporation, Colmar, PA). MPA ($C_{24}H_{34}O_4$) and its isotopically labeled internal standard, Medroxyprogesterone d6 17 acetate, MPd6-17 acetate ($C_{24}H_{28}D_6O_4$), were obtained in powder form from Toronto Research Chemicals, Inc. (TRC, Toronto, ON) to generate calibration standards and quality control (QC) solutions. For extraction of MPA from plasma, 600 µL of sample was combined with 650 µL of water containing 1% acetic acid and 50 µL of MPd6-17 acetate in methanol and centrifuged for 5 min at 4˚C. Subsequently, 1300 µL of supernatant was added to a methanol pre-conditioned Oasis HLB 3cc Vac (60 mg) SPE cartridge (Waters Corporation, Milford, MA) attached to a vacuum manifold (Waters Corporation) Post-washing, cartridges were dried under vacuum pressure and eluted with mL of methanol containing 1% acetic acid. Eluents were transferred to 1 mL glass inserts in a 96-well plate, evaporated to dryness under a dry nitrogen stream, and reconstituted in 1:1 water:methanol and subjected to UPLC-MS/MS analysis using an Agilent Zorbax Eclipse Plus C18 5.0 µm, 2.1 x 50 mm

(Agilent, Wilmington, DE) column on an API 5500 mass analyzer (AB SCIEX, Foster City, CA) operated in selective reaction monitoring mode. The analytical run time is 4.0 min. The assay was validated in accordance with the recommendations endorsed by FDA Guidance for Industry, Bioanalytical Method Validation document.

Results: The analytical measuring range for MPA is 0.2 pg/mL to 10,000 pg/mL. Analytical metrics assessed included both intra- and inter-assay precision and accuracy, dilutional analysis studies, stability challenges in response to freeze-thaw cycles, post-extraction and in sample matrix, as well as matrix effects analysis and selectivity studies. Quality control (QC) samples prepared at the lower limit of quantitation, as well as low, mid and high QC levels yielded intra- and inter-assay coefficients of variation (%CVs) ranging from 1.0 to 10.5% and 4.2 to 16.3%, respectively; Intra and inter-assay accuracies ranged from -13.5 to 16.1% and -10.1 to 11.6%, respectively. Dilutional analysis studies demonstrate specimens can be diluted and quantified, and observed concentrations are within 10.7% of theoretical concentrations. Further, MPA is stable in sample matrix for 24 hours and following three freeze-thaw cycles when compared to QC samples immediately prepared and analyzed. Finally to assess the potential influence of endogenous compounds on analyte suppression or enhancement, matrix effects studies were performed following the guidelines of Matuszewski and colleagues [4]. While significant ion suppression was observed for MPA, the isotopically labeled internal standard also showed comparable suppression, and relative matrix effects were <5% at any tested concentration.

Conclusions: The described work highlights the development and validation of a rugged assay for MPA quantification in plasma. Compared to other methods, the method requires low sample volumes, avoids liquid-liquid extraction and has an analytical run time less than 5 minutes.

References:

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