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CONFERENCE

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TITLE

Quantitative Analysis of 17-Desacetyl Norgestimate (Norelgestromin) in Human Plasma by HPLC-MS/MS

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ABSTRACT

Novel Aspect: Low sensitivity, and interference issues, were overcome by optimizing gradient, extraction method and column selection, while maintaining short run times.

Introduction

17-Desacetyl Norgestimate is a metabolite of Norgestimate. Norgestimate is a molecule used in hormonal contraceptives and is a form of progesterone, which is a female hormone important for the regulation of ovulation and menstruation. 17-Desacetyl Norgestimate is pharmacologically active with a similar profile to Norgestimate. Norgestimate is used with estradiol (a female sex hormone that is involved in the development and maintenance of the female reproductive system), to treat the symptoms of menopause such as hot flashes, and vaginal dryness, burning, and irritation. It is also used to prevent osteoporosis.

Methods

An LC-MS/MS assay for the determination of 17-Desacetyl Norgestimate in K_2EDTA human plasma was validated. Human plasma samples (500 µL) were spiked with internal standard Norelgestromin-d_6, (6 ng/mL) processed by liquid-liquid extraction and analyzed using reversed phase HPLC with Turbo Ion Spray MS/MS detection. Positive ions for 17-Desacetyl Norgestimate were monitored in MRM mode. 17-Desacetyl Norgestimate was resolved on a Luna Phenyl Hexyl 50 x 2.1 mm 5µ column. Total run time was 5 minutes with a retention time of 1.9 min. The stability of 17-Desacetyl Norgestimate in K2EDTA human plasma under various storage conditions using stability samples (n = 5) prepared at low and high concentrations of 60 and 8000 pg/mL was evaluated.

Preliminary results

The LC-MS/MS assay was validated over the range of 20 to 10000 pg/mL. Analyte-to-IS peak area ratios for the standards were used to create a linear calibration curve using $1/x^2$ weighted least-squares regression analysis. Quality control samples (n = 6) at 4 concentrations were used in one Intraday and two Inter-day runs to determine precision and accuracy. Intra day and Inter day precision were 1.5 to 10.3 %CV and 2.3 to 8.0 % CV, respectively. Accuracy for Intra day and Inter day ranged from -6.3 to 3.3 % and -5.2 to 3.1 % difference from nominal concentration, respectively. The extraction recovery at low, middle and high concentrations using five replicates was 71.2 – 83.9%. Matrix effect was evaluated at low, middle and high concentrations using five replicates and ranged from 102.6 to 109.6. Selectivity of the method was proven by extracting and analyzing six individual lots of blank Human plasma with and without spiking 17-Desacetyl Norgestimate at the LLOQ level. Dilution test was performed to ensure that human plasma samples could be diluted without affecting final concentration. Long term solution stability (LTSS) in plasma at both -20°C and -70°C was established for 154 days and stock solution stability in methanol was established for 285 days at -20°C.