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CONFERENCE

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TITLE

Determination of Low Concentration of Oxymorphone and 6β-hydroxyoxymorphone in Human Plasma by LC-MS/MS

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ABSTRACT

Novel Aspect: An assay required high sensitivity and chromatographically baseline separation of polar analytes using high pH stable column Gemini-NX.

Introduction

Oxymorphone is a semi-synthetic opioid agonist and a Schedule II drug. 6β-hydroxyoxymorphone is a major metabolite of oxymorphone. Due to assay requirements to support a bioequivalence study, a highly sensitive assay at the lowest limit of quantitation (10 pg/mL) is required for both analytes. In addition, it is necessary to chromatographically baseline separation of these two analytes due to potential “cross-talk” resulting from isotopic distribution overlap. We report here a development and validation of an LC/MS/MS assay for oxymorphone and 6β-hydroxyoxymorphone in human plasma.

Methods

Human plasma samples (400 µL) were spiked with internal standard and immediately processed by solid-supported liquid-liquid extraction cartridge on Tomtec and extracted with 1400 µL of ethyl acetate. The supernatant was transferred and evaporated to dryness under nitrogen. The extracts were then reconstituted with 200 µL reconstitution solvent (Type I water) for LC/MS/MS analysis. Chromatographic separation was achieved with gradient elution on a Gemini-NX column (50x2.0 mm, 5 µm) under high pH mobile phase condition with a total run time of 8 minutes. Detection was by positive turboionspray tandem mass spectrometry on Sciex API4000.

Preliminary results

The LC/MS/MS assay was validated over a range of 10 to 3000 pg/mL for oxymorphone and 6β-hydroxyoxymorphone in human plasma by using 400 µL of the human plasma. The precursor > product ion transitions under multiple reaction monitoring (MRM) were: m/z 302.2 $(M+H)^+ \rightarrow$ 227.20 (for oxymorphone), and m/z 304.30 $(M+H)^+ \rightarrow$ 268.2 (for 6β-hydroxyoxymorphone). Quality control samples at 4 concentrations were used to determine precision and accuracy. Intra-assay precision ranged from 1.4 to 10.9% for oxymorphone and 2.2 to 10.1% for 6β-hydroxyoxymorphone, while Inter-assay precision ranged from 2.2 to 8.1% for oxymorphone and 3.6 to 8.4% for 6β-hydroxyoxymorphone. The Intra-assay accuracy (defined as % error from nominal) ranged from -9.2 to 0.7% for oxymorphone and -6.9 to 1.2% for 6β-hydroxyoxymorphone and the Inter-assay accuracy ranged from -6.7 to -1.7% for oxymorphone and -4.1 to 0.2% for 6β-hydroxyoxymorphone. The analytes have been shown to be stable in plasma for 19 hours at room temperature and after 6 freeze/thaw cycles. The analytes are also stable in processed sample for 140 hours at 4°C. In addition, the selectivity, dilution reproducibility, matrix effect and recovery were also demonstrated. The assay was shown to be sensitive, selective, accurate, reproducible and reliable. The method is currently being used to support a bioequivalence study. The incurred sample reproducibility assessment showed that 100% and 98.7% of the samples evaluated met the pre-specified acceptance criteria for oxymorphone and 6β-hydroxyoxymorphone, respectively.