Development of an LC-MS/MS Method for the Determination of Morphine, Oxycodone, and Hydrocodone in Human Plasma

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Introduction

Opioids such as morphine, oxycodone, and hydrocodone have been used for pain management therapy for many years. With opioid use, there is an increasing need to monitor and quantitate the concentration of opioids in patients to determine possible therapeutic ranges, drug interactions and dosing in special populations. Opioids have been monitored in multiple types of matrices including plasma, urine, and oral fluid. Many laboratories have monitored opioids and other drugs with different techniques, including LC-MS/MS; however, there is no established protocol of sample preparation or detection. Creating a simple and robust LC-MS/MS method will allow for a more unified detection protocol of opioids in human plasma.

Methods

Drug-free human K2 EDTA plasma samples were spiked with known concentrations of a mixture of the opioids in solution along with their stable labeled isotopic internal standards to perform protein precipitation . The samples were then vortexed, centrifuged, and dried down under nitrogen. Next, dried samples were reconstituted in 95:5:0.1 water: methanol: formic acid and vortexed and centrifuged again. Samples were injected (20 µL) onto a Vanquish UPLC with a Phenomenex Synergi™ Polar-RP (4 µm, 80Å, 2 mm x 50 mm) column coupled to a Thermo Fisher Fortis mass spectrometer. Samples were eluted using solvent A: 0.1% FA in water, and solvent B: 0.1% FA in methanol, with a flow rate of 0.4 mL/min using a gradient elution.

Preliminary Data

The target linear range for this method is 1.0 ng/ml to 500 ng/mL. Calibration curves were generated for all three opioids with R^2 values of 0.9953, 0.9934, and 0.9916 for morphine, oxycodone, and hydrocodone, respectively. Retention times of the analytes are 1.02 minutes for morphine, 1.90 minutes for oxycodone, and 1.97 minutes for hydrocodone. A full method validation, adopted from the US FDA Guidance for Industry- Bioanalytical Method Validation, is currently being performed, with all relevant data to be included. This data includes accuracy and precision, stability, selectivity, and matrix effect studies.

Novel Aspect

Establishing a robust, simple extraction and quantitative LC-MS/MS method for the determination of morphine, oxycodone, and hydrocodone in human plasma.

Conflict of Interest Disclosure

The authors declare no competing financial interest.