

CONFERENCE

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TITLE

Determination of Morphine and Its Metabolites Morphine 3-Glucuronide and Morphine 6-Glucuronide in Human Plasma by LC-MS/MS

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ABSTRACT

Novel Aspect: We developed a rapid and simultaneous analysis method for Morphine and its metabolites Morphine 3-Glucuronide and Morphine 6-Glucuronide using LC-MS/MS.

Introduction

Morphine; and its metabolites Morphine 3-Glucuronide and Morphine 6-Glucuronide belong to a group of drugs known as opiates which possess high, potent analgesic actions. Morphine is one of the most effective drugs known for pain relief and is mostly used to treat moderate to severe pain. Morphine can also be used to ease pain before, during and after operations especially in cancer patients and other ailments. In our current study, we report the method for the determination of Morphine and its metabolites, Morphine 3-Glucuronide and Morphine 6-Glucuronide in human plasma employing solid phase extraction and LC-tandem MS. Our data indicates that the method reported here is rugged, sensitive, selective, accurate, and reproducible.

Methods

Calibration standards and quality controls for Morphine, Morphine 3-Glucuronide and Morphine 6 Glucuronide were prepared in pooled blank plasma extracted by solid-phase extraction using OasisTM 3cc (60 mg) cartridges with 200 μ L sample volume. Eluent collected in a clean collection plate, dried at 40°C and reconstituted with water. Morphine, its metabolites and their deuterated IS were separated on AQUASIL C18, 2x100 mm, 5 μ m, 100Å, Phenomenex using gradient conditions. Mobile phases water: Acetic Acid, at 100:0.01 (v: v) and Methanol: Acetic Acid, at 100:0.01 (v: v) at flow-rate of 0.250 mL/min are used to separate Morphine and its metabolites in ~ 8 minutes. The analytes were detected and quantified by MRM on Quattro Ultima, Micro Mass, Inc.

Preliminary results

A new assay based on reverse phase LC and tandem MS was developed and validated for the identification and quantification of Morphine, Morphine 3-Glucuronide and Morphine 6-Glucuronide in human plasma. The concentration range of 0.5-50 ng/mL for Morphine; 10-1000 ng/mL for Morphine 3-Glucuronide; and 1-100 ng/mL for Morphine 6-Glucuronide was validated. The intra and inter-day coefficients of variation (%CV) for Morphine were 1.9-9.9 and 3.8-10.4, respectively; for Morphine 3-Glucuronide were 1.5-3.6 and 2.1-5.3, respectively; and for Morphine 6-Glucuronide were 4.2-6.1 and 2.2-5.5, respectively. The stability of Morphine; Morphine 3-Glucuronide and Morphine 6-Glucuronide in human plasma was also assessed and found to be stable for 15 hours at room temperature, after 3 freeze/thaw cycles at -20°C and after 309 days of storage at -20°C. Morphine, Morphine 3-Glucuronide and Morphine 6-Glucuronide are stable during storage, processing, and analysis in human plasma samples. This method provides a useful tool in quantification of Morphine, Morphine 3-Glucuronide and Morphine 6-Glucuronide in pharmacokinetic and toxicological studies.