Quantitation of Midazolam and 1’-Hydroxymidazolam in Human Plasma Using API-4000 LC-MS/MS Systems with Higher Specificity and Lower Background Noise

Guangchun Zhou, Nicole Roenker, and Yong-Xi Li
Medpace Bioanalytical Laboratories, 5365 Medpace Way, Cincinnati, OH 45227

Overview

Using liquid-liquid extraction procedure, a sensitive and specific liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying Midazolam and 1’-Hydroxymidazolam in human plasma is described. In this method, the drug was extracted from a 0.1 ml of human plasma using simple extraction method. Separation was performed on a reverse phase C8 column. Detection was achieved using a ABSCIEX API-4000 system in the positive ion mode along with multiple reaction monitoring (MRM).

This method has been successfully applied to clinical pharmacokinetic studies.

Introduction

Midazolam is a short-acting drug in the benzodiazepine class of medicines that slow down the nervous system. In recent years, some analytical methods have been developed for human plasma using simple extraction method. Separation of poor chromatograms. In this study, the positive ESI multiple ion monitoring (MRM) method has been successfully applied to clinical pharmacokinetic studies. MRM provides higher specificity and lower background noise.

Midazolam is a CNS depressant, which are medicines that slow down the nervous system. In recent years, some analytical methods have been developed for human plasma using simple extraction method. Separation of poor chromatograms. In this study, the positive ESI multiple ion monitoring (MRM) method has been successfully applied to clinical pharmacokinetic studies. MRM provides higher specificity and lower background noise. A rapid, simple and specific LC-MS/MS method has been developed and validated for quantifying Midazolam and 1’-Hydroxymidazolam in human plasma.

Methods

Sample Preparation:
Plasma samples were extracted by using the 108-µl aliquot of plasma. After extraction, the extracts must be centrifuged at 14,000 rpm for about at least 10 minutes. The extract was then transferred to LC vials for LC-MS/MS or stay in the 96-well plate for the analysis.

Liquid Chromatography:

Pump: Shimadzu UFLC LC-20A
Autosampler: Shimadzu UFLC SIL-20ACcs
System Controller: Shimadzu CBM-20A
Analytical Column: C8 column, 2.0 x 50 mm, 5 µm
Gradient: The analytes were eluted using a gradient of mobile phase A (0.1% formic acid) and mobile phase B (0.1% formic acid in methanol) from 25% to 95% mobile phase B in 3 minutes.
Injection Volume: 5 µL.

Mass Spectrometry:
MS System: AB/Sciex API-4000
Condition: LC/MS/MS, MRM transition: Midazolam: 291.2 to 119.2 (Lower) with Unit Resolution, mass for Midazolam-d4: 292.2 to 119.2 (Lower) with Unit Resolution, mass for 1’-Hydroxymidazolam: 242.2 to 110.1 (Lower) with Unit Resolution.

Conclusions

Excellent linearity was obtained with a correlation coefficient ≥ 0.9977 for Midazolam and ≥0.9987 for 1’-Hydroxymidazolam. The high dynamic calibration range was reached due to eliminated background noise. (Table I, Figures 1 to 3).

For Midazolam, including LLOQ, the inter-day CV ranged from 2.5% to 4.3% and the biases of the means ranged from -3.3% to 0.5%. For 1’-Hydroxymidazolam, including LLOQ, the inter-day CV ranged from 2.6% to 11.3% and the biases of the means ranged from -6.0% to 1.6%. These results also indicate that the liquid-liquid extraction method is more suitable than protein precipitation extraction method for Midazolam and 1’-Hydroxymidazolam analysis in human plasma.

Figure 1. Ion chromatograms of blank plasma (Upper) and 0.5 ng/mL Midazolam extracted from plasma (Lower) with Unit Resolution.

Figure 2. Ion chromatograms of blank plasma (Upper) and 0.5 ng/mL 1’-OH Midazolam extracted from plasma (Lower) with Unit Resolution.

Figure 3. Typical Calibration Curve of Testosterone (Left) and 1’-Hydroxymidazolam (Right) in Human Plasma.

Table I. Validation Data Summary for Midazolam

<table>
<thead>
<tr>
<th>Condition</th>
<th>Accuracy &amp; Precision</th>
<th>Precision (%)</th>
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</thead>
<tbody>
<tr>
<td>LLOQ</td>
<td>99.9 ± 1.6</td>
<td>1.2</td>
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<tr>
<td>Low</td>
<td>99.9 ± 1.5</td>
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<tr>
<td>Median</td>
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<tr>
<td>High</td>
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Table II. Validation Data Summary for 1’-OH Midazolam

<table>
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<th>Condition</th>
<th>Accuracy &amp; Precision</th>
<th>Precision (%)</th>
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<tbody>
<tr>
<td>LLOQ</td>
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<tr>
<td>Low</td>
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<tr>
<td>Median</td>
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<td>2.0</td>
</tr>
<tr>
<td>High</td>
<td>99.9 ± 1.0</td>
<td>2.0</td>
</tr>
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A rapid, simple and specific LC-MS/MS method has been developed and validated for quantifying Midazolam and 1’-OH Midazolam with a lower limit of quantitation of 0.5 ng/mL from a 0.1 ml plasma sample.