Validation of a New HPLC Method for Determination of Midazolam and its Metabolites: Application to Determine its Pharmacokinetics in Human and Measure Hepatic CYP3A Activity in Rabbits

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Abstract
Midazolam (MDZ) is a commonly used Benzodiazepine in clinical practice. In addition, its metabolic oxidation is used as a surrogate marker for Cytochrome P450 (CYP) 3A enzyme activity as well. Thus, a new simpler method to measure MDZ and its metabolites is welcomed. Herein we report a new and simple HPLC method with ultraviolet detection for the simultaneous determination of midazolam and its hydroxyl metabolites using lorazepam as an internal standard. A liquid-liquid extraction was used to extract the compounds from rabbit hepatic microsomes and human plasma. The separation was performed on a Zorbax Eclipse XDB C18 column using a mobile phase composed of 0.05M Na2PO4 (pH 4.5) and acetonitrile mixture (67:33) pumped at 1.2 mL/min. The calibration curves showed good linearity with correlation coefficient higher than 0.999 for all analytes in the range 10-500 ng/mL. Accuracy in the measurement of quality control (QC) samples was in the range 95-106% of the nominal values. The intra-day and inter-day precision in the measurement of QC samples were less than 11% coefficient of variation. Although less sensitive than gas-chromatography-mass spectrometry (GC-MS), the proposed method was adequately sensitive to measure midazolam hydroxylase activity as a marker for CYP3A activity, and was applied to measure midazolam pharmacokinetics in human plasma.

Disciplines
Pharmacy and Pharmaceutical Sciences

Comments
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Valiation of a New HPLC Method for Determination of Midazolam and its Metabolites: Application to Determine its Pharmacokinetics in Human and Measure Hepatic CYP3A Activity in Rabbits

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ABSTRACT

Midazolam (MDZ) is a commonly used Benzodiazepine in clinical practice. In addition, its metabolic oxidation is used as a surrogate marker for Cytochrome P450 (CYP) 3A enzyme activity as well. Thus, a new simpler method to measure MDZ and its metabolites is warranted. Herein we report a new and simple HPLC method with ultraviolet detection for the simultaneous determination of midazolam and its hydroxy metabolites using lineazepam as an internal standard. A liquid-liquid extraction was used to extract the compounds from rabbit hepatic microsomes and human plasma. The separation was performed on a Zorbax Eclipse XDB-C18 column using a mobile phase composed of 0.05M Na2PO4 (pH4.5) and acetonitrile mixture (67:33) pumped at 1.2 mL/min. The calibration curves showed good linearity with correlation coefficient higher than 0.998 for all analytes in the range 10-500 ng/mL. Accuracy in the measurement of quality control (QC) samples was in the range 95-106% of the nominal values. The intra-day and inter-day precision in the measurement of QC samples was less than 11% coefficient of variation. Although less sensitive than gas-chromatography-mass spectrometry (GC-MS), the proposed method was adequately sensitive to measure midazolam hydroxylation activity as a marker for CYP3A, and was applied to measure midazolam pharmacokinetics in human plasma.

METHODS

HPLC

Institution

The HPLC system consisted of Waters model 2695 Alliance separation module, model 2565 photo-diode array detector and Empower data module (Waters Corporation, Milford, MA, USA). Chromatographic separation was carried out on Zorbax Eclipse XDB C18 column (150 x 4.6 mm I.D., 5 µm particle size). The column was kept at 25°C.

CHROMATOGRAPHIC CONDITIONS

The isocratic mobile phase consisted of 0.05M Na2PO4 (pH 4.5) adjusted with phosphoric acid and acetonitrile mixture (67:33) was run at a flow rate of 1.2 mL/min. Absorbance was monitored at 227 nm. This wavelength was found adequate to monitor MDZ, 1-OH MDZ, 4-OH MDZ, and LOR as indicated by using the PDA detector.

RESULT

Extraction recovery of MDZ, its metabolites and the internal standard (LOR) from spiked rabbit hepatic microsomes (n= 5), and extraction recovery of MDZ and LOR in spiked human plasma samples (n = 5).

Table 1. Extraction recovery of MDZ, its metabolites and the internal standard (LOR) from spiked rabbit hepatic microsomes (n = 5), and extraction recovery of MDZ and LOR in spiked human plasma samples (n = 5).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Nominal Conc. (ng/mL)</th>
<th>Average (n=5)</th>
<th>SD</th>
<th>% Accuracy</th>
<th>CV%</th>
<th>Average (n=10)</th>
<th>SD</th>
<th>% Accuracy</th>
<th>CV%</th>
</tr>
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<tbody>
<tr>
<td>1-OH MDZ</td>
<td>20</td>
<td>19.03</td>
<td>1.36</td>
<td>95.15</td>
<td>7.15</td>
<td>19.5</td>
<td>1.96</td>
<td>97.5</td>
<td>5.4</td>
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<tr>
<td>4-OH MDZ</td>
<td>100</td>
<td>105.3</td>
<td>6.3</td>
<td>105.3</td>
<td>6.3</td>
<td>106.2</td>
<td>5.7</td>
<td>106.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Human Plasma</td>
<td>20</td>
<td>20.8</td>
<td>2.1</td>
<td>104</td>
<td>10.1</td>
<td>20.0</td>
<td>1.7</td>
<td>100.5</td>
<td>6.2</td>
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<tr>
<td>LOR</td>
<td>20000</td>
<td>94.2</td>
<td>12.2</td>
<td>94.2</td>
<td>12.2</td>
<td>94.2</td>
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<td>±3</td>
<td>±6</td>
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<td>±6</td>
<td>±3</td>
<td>±6</td>
<td>±3</td>
<td>±6</td>
</tr>
</tbody>
</table>

MEASUREMENT OF HEPATIC CYP3A ACTIVITY IN RABBITS

MDZ hydroxylation activity was determined by quantification of 1-OH MDZ and 4-OH MDZ formation rates in rabbit hepatic microsomes. Preliminary experiments were conducted to determine linear metabolism formation kinetics with respect to MDZ-concentration, incubation time and microsomal protein concentration.

PHARMACOKINETIC ANALYSIS

The maximum plasma concentration (Cmax) and time to reach Cmax (Tmax) following MDZ administration were obtained directly from the individual plasma-concentration time data for MDZ. The area under concentration-time curve from time zero to infinity (AUC(∞)) was measured using linear trapezoidal summation with extrapolation. The terminal elimination rate constant (β) was estimated by linear least square regression analysis of the terminal log-linear portion of plasma-concentration time curve. The terminal elimination half-life (t1/2) was determined as ln(2)/β.

CONCT INFORMATION

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