A Sensitive Method for Quantification of Pemetrexed in Human Plasma Using High-Performance Liquid Chromatography Coupled with Mass Spectrometry

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Introduction

Pemetrexed, a novel anticancer agent, is approved for treatment of pleural mesothelioma and non-small cell lung cancer. It has also demonstrated agent activity against a variety of solid tumors including breast cancer. The primary mechanism of action is inhibition of three enzymes involved in purine and pyrimidine synthesis—thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycaminide ribonucleotide formyltransferase (GARFT)—thus preventing DNA and RNA synthesis. To support clinical studies, a highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of Pemetrexed in human plasma.

Methods

**Extraction**
Solid phase extraction (Oasis HLB 96-well plate, Waters)

**Chromatographic Conditions**
- Column: Gemini C18, 50 x 2 mm, 5-µm particle size, Phenomenex
- Mobile Phase A [MA]: 10 mM Ammonium bicarbonate in water, pH 8
- Mobile Phase B [MB]: Methanol:Water (80:20, v:v)
- Mobile Phase C [MC]: Acetonitrile:Water (90:10, v:v)
- Flow Rate: 0.6 mL/min for mobile [MA] and [MB]; 1 mL/min for [MC]

**Mass Spectrometer Parameters**
- Mass Spectrometer: Sciex API 5000
- Ionization: Positive Ion Electrospray (ESI+)
- Mode: MRM
- Pemetrexed: 428.4 → 281.1
- Pemetrexed-d5 (IS): 433.5 → 281.1

Results

**Figure 2.** Structure of Pemetrexed and its internal standard.

**Figure 3.** LLOQ chromatograms of Pemetrexed and its internal standard in human plasma.

**Figure 4.** ULOQ chromatograms of Pemetrexed and its internal standard in human plasma.

**Figure 5.** Linearity of the curve from 0.100 ng/mL to 100 ng/mL in human plasma.

**Table 1.** Precision and Accuracy of Pemetrexed in Human Plasma Calibration Standard (3 P&A runs, n=6)

<table>
<thead>
<tr>
<th>ng/mL</th>
<th>Std1</th>
<th>Std2</th>
<th>Std3</th>
<th>Std4</th>
<th>Std5</th>
<th>Std6</th>
<th>Std7</th>
<th>Std8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.2</td>
<td>1</td>
<td>5</td>
<td>25</td>
<td>50</td>
<td>85</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>RSD (%)</td>
<td>4.2</td>
<td>3.4</td>
<td>3.0</td>
<td>2.9</td>
<td>1.8</td>
<td>1.4</td>
<td>2.1</td>
<td>7.3</td>
</tr>
<tr>
<td>% Bias</td>
<td>-1.8</td>
<td>3.5</td>
<td>-1.1</td>
<td>0.8</td>
<td>9.0</td>
<td>-24.5</td>
<td>1.1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

**Table 2.** Precision and Accuracy of Pemetrexed in Human Plasma Quality Control Samples (3 P&A runs, n=18)

<table>
<thead>
<tr>
<th>ng/mL</th>
<th>QC</th>
<th>LQC</th>
<th>MQC</th>
<th>HMQC</th>
<th>HQC</th>
<th>Dil QC (20-400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>5</td>
<td>25</td>
<td>80</td>
<td>1000</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>4.7</td>
<td>3.7</td>
<td>2.7</td>
<td>2.8</td>
<td>2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>% Bias</td>
<td>7.0</td>
<td>3.6</td>
<td>-0.6</td>
<td>-0.8</td>
<td>-1.4</td>
<td>-2.3</td>
</tr>
</tbody>
</table>

**Table 3.** Recovery and Matrix Effect in Human Plasma

<table>
<thead>
<tr>
<th>ng/mL</th>
<th>Pemetrexed</th>
<th>Pemetrexed-d5</th>
<th>Pemetrexed</th>
<th>Pemetrexed-d5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.300</td>
<td>71.6</td>
<td>68.8</td>
<td>0.936</td>
<td>NA</td>
</tr>
<tr>
<td>80.0</td>
<td>69.5</td>
<td>70.4</td>
<td>1.02</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Conclusions**

- A sensitive method was developed and validated following the regulated bioanalysis guidelines.
- The LLOQ of 0.1 ng/mL was achieved, which is about 25 to 100-fold lower than the currently available methods.
- The sample volume was reduced to 150 µL, and the extraction time was reduced to approximately 3 hours and the run time per sample was reduced to 5.5 minutes, which are significant improvements comparing to the currently available methods.
- The method showed good precision, accuracy and selectivity.
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