Determination of sitagliptin phosphate in rat plasma by ultra high performance liquid chromatography-tandem mass spectrometry

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Abstract An ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the determination of sitagliptin phosphate in rat plasma was established. The blank rat plasma sample added with sitagliptin phosphate and the internal standard (fluoxetine) solution were prepared. Methanol was added in the sample for the deproteinization. Then the sample was vortex-mixed and centrifuged. The clear supernatant was used for the analysis of UPLC-MS/MS. A Thermo Hypersil Gold C18 column (50 mm × 2.1 mm, 1.9 μm) was employed with a guard column of Phenomenex Security Guard C18 column (4 mm × 3.0 mm) and the column temperature was set at 35 °C. The gradient elution of acetonitrile and water containing 0.05% v/v formic acid as mobile phases was performed at a flow rate of 200 μL/min and a rapid separation was completed in 5 min. The electrospray was operated in the positive ionization mode and the sitagliptin phosphate and fluoxetine were identified by selected reaction monitoring (SRM) mode and the monitoring ions of them were m/z 408. 0→235. 0 and m/z 310. 0→148. 0 respectively which were used to qualify and quantify the targets.
by the method of matrix-matched standard solution. The calibration curve showed good linearity within the concentrations of 1 to 1000 μg/L, \( r = 0.999 \) for the limit of detection was 0.2 μg/L. The mean recoveries were from 85% to 115% at the spiked levels of 50, 500 μg/L, the relative standard deviations (RSDs) of intra- and inter-day of variation were both less than 15%, which can meet the determination requirements of biological samples. Then the method was initially used for the determination of sitagliptin phosphate in SD rat plasma after the administration of a single intravenous injection dose of sitagliptin phosphate. The method is rapid, sensitive, convenient and reproducible in the determination of sitagliptin phosphate and can be used for the pharmacokinetics research of sitagliptin phosphate in plasma.

**Key words** ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), electrospray ionization (ESI), sitagliptin phosphate, rat plasma

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**1.2 仪器**

- TSQ Quantum Access LQ (Thermo Fisher)
- Mettler-Toledo AX205
- Mettler-Toledo FP-114F Advantec PWU-400
- Eppendorf minispin
- Eppendorf
- Kubota 3740

**1.3 药品与试剂**

- Sitagliptin phosphate, DPP-4 inhibitor, HbA1c, FPG, HbA1c, 43-70, Xcalibur 2.0.7
- Xcalibur 2.0.7
- Thermo Fisher Scientific
- Advantec PWU-400
- Eppendorf
- Kubota 3740

**1.4 实验部分**

1.4.1 仪器

- HPLC-MS/MS
- UPLC-MS/MS

**1.5 样品前处理**

- HPLC-MS/MS
- UPLC-MS/MS
- 200 μL
- 1.5 mL
- 10 μL
- 30 s
- 400 μL
- 30 s
- 1.0 mg/L
- 1.0 g/L
- 4 °C
- 14000 r/min
- 5 min
- 0.2 μm
样品均采用上述样本处理方法。取等量的空白血浆，按上述方法配制含磷酸西他列汀的标准曲线样品。取等量的空白血浆，按上述方法配制含磷酸西他列汀和氟西汀各自稳定的准分子离子峰，然后对它们分别量取的磷酸西他列汀和氟西汀(内标液的空白血浆样品)和待测血浆样品(添加内标液的空白血浆样品)、单空白样品(不加内标液的空白血浆样品)、双空白样品(前加内标液)的标准储备液，配制成质量浓度为0.079 μg/L的血浆基质的标准样品各一个浓度的血浆基质的低、中、高浓度的标准工作液，通过注射泵以3 L/min的流速将二者分开进入预柱，预柱的柱温为30 °C。流动相：甲醇-水(甲酸)相为乙腈，流速为0.2 mL/min，扫描宽度为0.05% v/v的喷雾电压为29.5 V，离子传输管温度为35 °C。进样量为10 μL。

1.5 质谱条件的优化

Thermo Hypersil Gold C_{18} 50 mm × 2.1 mm，1.9 μm Phenomenex Security Guard C_{18} 4 mm × 3.0 mm超高效液相色谱条件

流动相A:甲醇；流动相B:0.05% v/v甲酸水溶液，流速为1.5 mL/min，柱温为30 °C。(0 ~ 1.5 min [30% A]，1.5 ~ 2 min [30% A]，85% A) 1.5 min，3.5 ~ 5 min [30% A]，5 min 35 °C。进样量为10 μL。

1.6 移液

采用电喷雾离子源(AEI5B)、电喷雾离子源(AEI5B)、电喷雾离子源(AEI5B)模式下进行一级质谱分析(子离子扫描，得到磷酸西他列汀和内标物的部分质谱检测参数。其他质谱条件的优化，确定了最佳的质谱检测条件。在此基础上，将杆质谱仪连接，选择各自的监测离子对(准分子离子和特征碎片离子)，对离子传输管温度、透镜电压、鞘气压、辅助气压等条件进行优化，以获得最佳的进样条件。优化后的参数条件见表1。

<table>
<thead>
<tr>
<th>Drug</th>
<th>Precursor ion</th>
<th>Product ion</th>
<th>Collision energy/ eV</th>
<th>Tube lens offset/ V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaglipin</td>
<td>408.0</td>
<td>234.9</td>
<td>18</td>
<td>120</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>310.0</td>
<td>148.1</td>
<td>7</td>
<td>90</td>
</tr>
</tbody>
</table>

1.7 QC

为了获得较好的分离度和峰形，本文分别对流动相添加成分、洗脱条件和流速进行了优化。发现等度洗脱与梯度洗脱方式对色谱峰形的影响。等度洗脱时，目标物峰与内标峰均拖尾，峰形较宽且分辨率低。

2.1 UPLC

1.0 g/L的磷酸西他列汀和氟西汀(内标液的空白血浆样品)和待测血浆样品(添加内标液的空白血浆样品)、单空白样品(不加内标液的空白血浆样品)、双空白样品(前加内标液)的标准储备液，配制成质量浓度为0.079 μg/L的血浆基质的标准样品各一个浓度的血浆基质的低、中、高浓度的标准工作液，通过注射泵以3 L/min的流速将二者分开进入预柱，预柱的柱温为30 °C。流动相：甲醇-水(甲酸)相为乙腈，流速为0.2 mL/min，扫描宽度为0.05% v/v的喷雾电压为29.5 V，离子传输管温度为35 °C。进样量为10 μL。

2.2 UPLC

2.3 UPLC
2.4 UPLC

2.5 准确度、精密度与回收率

2.6 检出限
磷酸西他列汀在大鼠血浆中保持稳定。条件(以加标回收率计)以及日内及日间精密度(以日间准确度分别为5.6%3.4% 5.7%/% 28%. 85% ~ 115% ± 15% ± 15% ± 28%. 长期冻存下保存的稳定性及冻融稳定性方法进行样品前处理、测定。每个添加水平重复操作,计算方法的准确度和精密度结果见表(见表).

### Table 2 Preparations\(\) accuracies and average recoveries for the determination of sitagliptin phosphate in rat plasma matrix

<table>
<thead>
<tr>
<th>Spiked/ µg/L</th>
<th>Intra-day ( n = 6 )</th>
<th>Inter-day ( n = 5 )</th>
<th>Recovery ( n = 6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found mean ± SD/ µg/L</td>
<td>Precision RSD/ %</td>
<td>Accuracy/ %</td>
</tr>
<tr>
<td>1</td>
<td>0.92 ± 0.049</td>
<td>5.3</td>
<td>92.64</td>
</tr>
<tr>
<td>5</td>
<td>5.17 ± 0.22</td>
<td>4.2</td>
<td>103.4</td>
</tr>
<tr>
<td>50</td>
<td>50.36 ± 1.91</td>
<td>3.8</td>
<td>100.7</td>
</tr>
<tr>
<td>500</td>
<td>503.93 ± 24.58</td>
<td>4.9</td>
<td>100.8</td>
</tr>
</tbody>
</table>

### Table 3 Effects of temperature and stocking conditions on the stability of sitagliptin phosphate in rat plasma

<table>
<thead>
<tr>
<th>Spiked/ µg/L</th>
<th>Short-term stock</th>
<th>Room temperature</th>
<th>Long-term stock ( -70 \degree C )</th>
<th>3 Cycles of freeze and thaw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found mean ± SD/ µg/L</td>
<td>Precision RSD/ %</td>
<td>Accuracy/ %</td>
<td>Found mean ± SD/ µg/L</td>
</tr>
<tr>
<td>5</td>
<td>5.06 ± 0.14</td>
<td>2.8</td>
<td>101.1</td>
<td>5.24 ± 0.23</td>
</tr>
<tr>
<td>500</td>
<td>501.42 ± 7.1</td>
<td>1.4</td>
<td>100.3</td>
<td>496.73 ± 22.17</td>
</tr>
</tbody>
</table>

#### 2.7.1

<table>
<thead>
<tr>
<th>25 \degree C</th>
<th>0 h</th>
<th>4 h</th>
</tr>
</thead>
</table>

#### 2.7.2

<table>
<thead>
<tr>
<th>14 \degree C</th>
<th>2000 µg/L</th>
<th>10 000 µg/L</th>
</tr>
</thead>
</table>

#### 2.7.3

<table>
<thead>
<tr>
<th>70 \degree C</th>
<th>3 h</th>
<th>21 h</th>
</tr>
</thead>
</table>

#### 2.8

<table>
<thead>
<tr>
<th>6 h</th>
<th>4 h</th>
<th>10 min</th>
</tr>
</thead>
</table>

#### 2.9

<table>
<thead>
<tr>
<th>9 mg/kg</th>
<th>6 h</th>
<th>4 h</th>
</tr>
</thead>
</table>
Determining the concentration of sitagliptin phosphate in plasma. The study optimized pretreatment methods, chromatographic conditions, and mass spectrometric conditions. The samples were pretreated using methanol to precipitate proteins, and this method was simple and had a high recovery rate. The addition of formic acid to the mobile phase improved ionization efficiency, improved peak shape, and increased sensitivity. The use of positive ionization mode for mass spectrometry detection demonstrated high selectivity and was suitable for batch analysis of clinical samples. This method can effectively eliminate endogenous substance matrix effects. The method has high sensitivity, is operationally simple, fast, and accurate, and can be used for preclinical pharmacokinetics and bioequivalence studies. Moreover, it can provide reference for large-scale clinical sample research.

<table>
<thead>
<tr>
<th>Spiked/μg/L</th>
<th>Intra-day (n = 6)</th>
<th>Inter-day (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found ± SD/μg/L</td>
<td>Precision RSD/%</td>
</tr>
<tr>
<td>2000</td>
<td>2019.26 ± 40.70</td>
<td>2.0</td>
</tr>
<tr>
<td>10000</td>
<td>10149.01 ± 259.04</td>
<td>2.6</td>
</tr>
</tbody>
</table>

a: The sample was processed with 4-fold dilution. b: The sample was processed with 20-fold dilution.

**Fig. 2** Chromatograms of a plasma sample of SD rat after the administration of a 9 mg/kg single intravenous injection dose of sitagliptin phosphate in SRM mode