Determination of simvastatin in human plasma using ultra-performance liquid chromatography-tandem mass spectrometry

[1] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract A rapid and specific method has been developed for the determination of simvastatin in human plasma with ultra-performance liquid chromatography-tandem mass spectrometry. Simvastatin and the internal standard lovastatin were extracted from human plasma with diethyl ether-n-hexane-isopropanol (80:20:3) then separated on a Waters ACQUITY UPLC™ BEH C18 column (50 mm × 2.1 mm, 1.7 μm) with isocratic elution at a flow rate of 0.25 mL/min. The mobile phase was composed of 85% acetonitrile and 15% water containing 10 mmol/L ammonium acetate. Electrospray ionization ESI source was applied and operated in positive ion mode. Multiple reaction monitoring MRM mode with the transitions of m/z 419.2 → m/z 199.0 and m/z 405.0 → m/z 199.0 was used to quantify simvastatin and the internal standard respectively. The linear calibration curve was obtained in the concentration range of 0.051 – 20.4 ng/mL. The lower limit of quantification was 0.051 ng/mL. The inter-day and intra-day precision relative standard deviation were less than 10% and the accuracy relative error was within 2.7% – 0% calculated from quality control samples. The mean extraction recovery of simvastatin was 91.6%. The method was proved to be selective rapid and suitable for the pharmacokinetic study of simvastatin.

Key words ultra-performance liquid chromatography-tandem mass spectrometry, UPLC-MS/MS, simvastatin, plasma concentration.
1

1.1 Waters ACQUITY Ultra Performance LC™ Waters Quattro micro API ESI Masslynx 4.1

1.2 ACQUITY UPLC™ BEH C18 50 mm × 2.1 mm 1.7 μm Waters 10 mmol/L 0.25 mL/min 40 °C 20 μL

1.3 ESI[M⁺] 195.0 419.2 199.0 405.0 m/z 10 s 3.0 kV 20 V 500 L/h 15 eV

1.4 QC 100 μL 10 mL 0.051 0.102 0.204 0.510 0.045 10 20 4 ng/mL

1.5 3 0.153 0.08 16.3 ng/mL

Fig. 1 Product ion spectra of M + H⁺ of
a simvastatin and b lovastatin
2.2

将溶液加入空白血浆中，依同法操作，得色谱图。内标外，均按"标准曲线\$1.4’’操作，考察方法的基质效应，结果表明本方法无基质效应。

2.3

分别取人空白血浆$\times10^3$、$\times10^2$和$\times10^1$倍的辛伐他汀溶液，涡流混合，于$37^\circ\text{C}$下用氮气吹干，残渣用$0.1\%$甲醇流动相溶解，取$20\mu\text{L}$上样，进样分析，获得相应的峰面积，以每一浓度两种峰面积的比值计算提取回收率。其结果表明，血浆样品的内源性物质不干扰辛伐他汀的测定。

2.4

分别置于肝凝素处理的离心试管中，离心$10\text{min}$，去上清液，流速为$100 \mu\text{L}$/min，40℃、20μL溶液，40℃下用氮气吹干，残渣用$0.1\%$甲醇流动相溶解，取$20\mu\text{L}$上样，进样分析，获得相应的峰面积，以当日的标准曲线计算各浓度的质量控制样品，每一浓度进行$3$次冻融循环及长期放置的稳定性。结果表明，血浆样品的浓度，将$100\mu\text{L}$溶液，$1.4’’$项下操作，考察血浆样品浓度，将$100\mu\text{L}$溶液，$1.4’’$项下操作，保持稳定；在$3$天$4^\circ\text{C}$下，于$12$小时后$2\text{h}$，将$100\mu\text{L}$溶液，$1.5$项下操作，得色谱图。内标外，均按"标准曲线\$1.5’’操作，平均为$89.9\%$，$91.6\%$，$95.4\%$。

2.5

Table 1 Precision and accuracy of the method

<table>
<thead>
<tr>
<th></th>
<th>Simvastatin/ ng/mL</th>
<th>intra-day RSD/%</th>
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<th>Relative error/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>added</td>
<td>found</td>
<td>$n = 6$</td>
<td>$n = 3$</td>
<td></td>
</tr>
<tr>
<td>0.153</td>
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<td>7.0</td>
<td>7.2</td>
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<td>4.08</td>
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<td>16.24</td>
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2.6

分别取人空白血浆$500\mu\text{L}$，$1.4’’$项下操作，取$20\mu\text{L}$溶液，$1.4’’$项下操作，3天$4^\circ\text{C}$下，分别于$2\times4^\circ\text{C}$和$2\times4^\circ\text{C}$下，$12$小时后$2\text{h}$，将$100\mu\text{L}$溶液，$1.4’’$项下操作，得色谱图。内标外，均按"标准曲线\$1.4’’操作，平均为$89.9\%$，$91.6\%$，$95.4\%$。

2.7

Fig. 2 Chromatograms of simvastatin and lovastatin

- a. blank plasma
- b. blank plasma spiked with $4.08\text{ ng/mL}$ simvastatin and internal standard
- c. the plasma at $1\text{ h}$ after an oral dose of $40\text{ mg}$ simvastatin capsule.

1. lovastatin internal standard $t_R = 1.15\text{ min}$
2. simvastatin $t_R = 1.37\text{ min}$

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- a. Blank plasma
- b. Blank plasma spiked with 4.08 ng/mL simvastatin and internal standard
- c. Plasma at 1 h after oral dose of 40 mg simvastatin capsule.

1. Lovastatin internal standard $t_R = 1.15\text{ min}$
2. Simvastatin $t_R = 1.37\text{ min}$
30 min[8] | 800 0.1 mol/L | 2 | pH 4.5 | UPLC-MS/MS

2.7

Fig. 3 Plasma concentration-time of simvastatin in a subject after single oral dose of 40 mg simvastatin $\bar{X} = s/n = 20$