Simultaneous determination of exogenous phosphocreatine and its metabolite creatine in rabbit plasma using ion-pair reversed-phase high performance liquid chromatography

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Abstract: A method for simultaneous determination of exogenous phosphocreatine (PCr) and its metabolite creatine (Cr) in rabbit plasma was developed by using an ion-pair reversed-phase high performance liquid chromatography (IP-RP-HPLC). The pharmacokinetics (PK) of PCr was also investigated. In the IP-RP-HPLC method, a Kromasil C₁₈ column was used with methanol and phosphate buffer containing tetrabutylammonium hydrogen sulfate (TBA) ion-pair reagent as the mobile phases in a gradient elution mode while changing detection wavelength and flow rate. The internal standard method was used to quantify PCr and Cr and the baseline subtraction method was applied. The calibration curves showed good linearity ranged from 10 to 7500 mg/L for PCr and from 10 to 1500 mg/L for Cr and the correlation coefficients were greater than 0.999. The methodology validation showed high specificity precision and recovery with the intra-day and inter-day relative standard deviations of not more than 6.2% accuracies of 96.5% – 102.4% and extraction recoveries of more than 92%. After intravenous injection of PCr the concentration-time profile can be best described by two-compartment model with elimination half time of 20.4 ± 2.7 min apparent volume of distribution of 0.179 ± 0.037 L/kg and clearance rate of 0.019 ± 0.002 L/kg·min. The Cr appeared rapidly with time to maximal concentration of 30 min elimination half time of 43.7 ± 4.5 min.

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The results of practical application showed that this bio-analytical method can completely meet the requirements for PK study of PCr in rabbit plasma.

**Key words**: ion-pair reversed-phase high performance liquid chromatography [IP-RP-HPLC], pharmacokinetics, phosphocreatine, creatine, rabbit plasma.

Table 1 Composition of mobile phases, elution mode, flow rate and detection wavelength

<table>
<thead>
<tr>
<th>Time/min</th>
<th>Composition of mobile phase/%</th>
<th>Flow rate/mL/min</th>
<th>Detection wavelength/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A 100 0 0 1.0 210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>A 100 0 0 1.0 210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0 82 0 1.2 260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0 82 0 1.2 260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>100 0 0 1.0 210</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. 0.2% KH$_2$PO$_4$ + 0.08% tetrabutylammonium hydrogen sulphate pH 3.0; B. buffer A adjusted to pH 7.5 with 1 mol/L NaOH C. methanol.

1.3

1.3.1

Agilent 1100

PCr Cr

15 g/L Cr

100 200 500 1 000

5 000 25 000 5 000 mg/L Cr 1 000 200 500

1 000 3 000 7 500 15 000 mg/L Cr

-20 °C

1.3.2

IS

TMP

10 mL
10 μL[H 2 O 30 μL] 0.5 min[6%] 160 μL[4°C] 0.5 min[10 min] 4°C 2 min[150 μL] 2 mol/L[20 μL] pH[HPLC] 4°C 0.6 mL[H 2 O] 3 000 r/min[10 min] 200 μL[1.5] HPLC

2.1

PCR 500 mg/kg

2.2

PCR 20 min

2.3

PCr Cr IS

1.4

PCR Cr IS

3

Quality control QC

1.5

PCR 200 mg/L Cr 750 100 20 mg/L

3 QC

HPLC

PCR Cr IS

3

PCR Cr IS

RSD 96.5%~102.4%

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linear equation</th>
<th>r</th>
<th>Linear range/ LOQ/</th>
<th>LOQ/</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>( Y = 0.0092X + 0.0019 )</td>
<td>0.9999</td>
<td>10~7500</td>
<td>10</td>
</tr>
<tr>
<td>Cr</td>
<td>( Y = 0.0131X + 0.0193 )</td>
<td>0.9992</td>
<td>10~1500</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3  Intra-day and inter-day precisions RSDs for analysis of PCr and Cr in QC samples n = 5

<table>
<thead>
<tr>
<th>Component</th>
<th>Added/ Found/</th>
<th>RSD/</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>20</td>
<td>4.2</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.1</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2500</td>
<td>3.6</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>20</td>
<td>2.0</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.7</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>4.5</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 4  Extraction recoveries of PCr and Cr in QC samples and the method accuracy X ± SD n = 5

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Added/ Found/</th>
<th>Extraction recovery/</th>
<th>Accuracy/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/L</td>
<td>mg/L</td>
<td>%</td>
</tr>
<tr>
<td>PCr</td>
<td>20</td>
<td>19.7 ± 0.8</td>
<td>93.2 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.4 ± 5.1</td>
<td>93.1 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>2500</td>
<td>2555.9 ± 93</td>
<td>93.6 ± 2.7</td>
</tr>
<tr>
<td>Cr</td>
<td>20</td>
<td>19.80 ± 0.4</td>
<td>92.7 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>96.5 ± 5.6</td>
<td>93.5 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>768.4 ± 34.5</td>
<td>93.7 ± 3.3</td>
</tr>
</tbody>
</table>

2.4  Methodology

3  Sample preparation

3.1  QC samples

QC samples are prepared by spiking stock solutions of PCr and Cr into blank plasma to achieve concentrations at 0.25, 1.0, 5.0, and 20.0 μg/mL. Each concentration level is prepared in triplicate and analyzed in a single run.

3.2  Stability

QC samples are stored at -20°C and analyzed at 0, 1, 2, 4, and 24 hours after preparation. Stability is assessed by comparing the peak areas of the QC samples to the internal standard at each time point.

2.5  HPLC

HPLC separation is performed using a C18 column (5 μm, 150 x 4.6 mm). The mobile phase is a gradient elution of 60:40 (v/v) water:acetonitrile. The flow rate is set at 0.3 mL/min, and detection is performed at 254 nm.

2.6  Results and discussion

2.6.1  Pharmacokinetic analysis

The concentration-time profiles for PCr and Cr are fitted to a two-compartment open model using non-linear regression analysis. The parameters obtained are the apparent volume of distribution (Vd), clearance (CL), elimination half-life (t1/2), and apparent maximal concentration (Cmax).

- PCr: Vd = 0.179 ± 0.037 L/kg, CL = 0.019 ± 0.002 L/kg/min, t1/2 = 20.4 ± 2.7 min, Cmax = 573.80 mg/L
- Cr: Vd = 0.181 ± 0.032 L/kg, CL = 0.019 ± 0.002 L/kg/min, t1/2 = 21.0 ± 2.9 min, Cmax = 573.80 mg/L

3  Conclusion

The results indicate that the method is accurate, precise, and stable. The stability data show that QC samples are stable for at least 24 hours at -20°C. The pharmacokinetic parameters obtained suggest that PCr and Cr are rapidly eliminated from the plasma after intravenous injection.

Fig. 2 Concentration-time curves of exogenous PCr and its metabolite Cr in plasma of rabbits after intravenous injection of PCr 500 mg/kg
动相

合物及

尾的 现 象。经 过 筛 选,最 终 选 定

药代动力学的研究

间;高

性物质,而我们的目的是要定量测定血浆中的外源

得所建立的方法学的

分仍是一大难题。最好的方法是将外源性药物进行

用有机溶剂萃取富集,只能采用强酸沉淀蛋白的方

同位素标记,但易造成污染,且操作难度大,花费高,
尤其不适合人体药代动力学研究,故很少应用。本

文采用基线扣除法,即用给药后样品中测得的内源

性与外源性化合物总量减去给药前空白样品中测得

的内源性化合物的量,得到样品中外源性化合物的

定量方法的选择

两种化合物均为内源性生理活

动性与外源性化合物的出峰有利,但是将延长

在酸中稳定性差,室温下很快

...（分）进行

分解,故样品处理时需进行低温操作,包括所有的器

外源性

血浆样品制备条件的选择

及其代谢物

对它们的色谱行为亦有影响,低

%& $#

作为流动相,既可以满足两种目标化

中和至中性。本文所用的样品预处理方法

高至

的出峰,但会延长

在

级水平。由于

胡萝卜素、肌酸等

摩尔吸光系数低,使

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