

**Table 1.** Standard calibration curves of cyclosporine A calculated by the UHPLC-MS/MS method.

| Range  | Slope (SD)     | Intercept (SD) | <i>r</i> |
|--|----------------|----------------|----------|
| Methanol<br>0.001-2.5 (µg/mL)                                    | 3.073 (0.127)  | 0.001 (0.003)  | 0.994    |
| Blood<br>0.001-2.5 (µg/mL)                                       | 8.149 (0.678)  | 0.030 (0.028)  | 0.996    |
| Liver <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0015-3.7 (µg/g)     | 14.097 (1.666) | 0.016 (0.006)  | 0.998    |
| Spleen <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0060-15.1 (µg/g)   | 7.471 (0.314)  | 0.014 (0.006)  | 0.998    |
| Kidney <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0045-11.2 (µg/g)   | 14.410 (0.613) | 0.012 (0.008)  | 0.998    |
| Lung <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0081-20.3 (µg/g)     | 8.005 (0.835)  | 0.009 (0.003)  | 0.996    |
| Intestine <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0022-5.5 (µg/g) | 7.836 (1.117)  | 0.008 (0.005)  | 0.998    |
| Heart <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0101-25.2 (µg/g)    | 6.450 (1.000)  | 0.011 (0.004)  | 0.994    |
| Brain <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0026-6.5 (µg/g)     | 10.720 (1.487) | 0.004 (0.004)  | 0.998    |
| Stomach <sup>a</sup><br>0.001-2.5 (µg/mL)<br>0.0066-16.4 (µg/g)  | 7.212 (1.536)  | 0.002 (0.001)  | 0.998    |

<sup>a</sup>Concentrations of tissues are expressed as µg/mL tissue homogenate. The equivalence in µg/g tissue is given in the second line.

**Table 2.** Accuracy, precision and between- and within- day measured concentrations for analysis of cyclosporine A quality controls by the UHPLC-MS/MS method.

| Matrix                 | Conc.<br>( $\mu\text{g/mL}$ ) | Measured concentration ( $\mu\text{g/mL}$ , mean $\pm$ S.D.) |         |                           |         | Accuracy<br>(bias %) | Precision (CV)                   |                              |
|------------------------|-------------------------------|--|---------|---------------------------|---------|----------------------|----------------------------------|------------------------------|
|                        |                               | Between-day (5 batches, n=21)                                |         | Within-day (1 batch, n=5) |         |                      | Between-day<br>(5 batches, n=21) | Within-day<br>(1 batch, n=5) |
|                        |                               | Mean   | SD      | Mean                      | SD      |                      |                                  |                              |
| Methanol               | 0.001                         | 0.00100  | 0.00005 | 0.00097                   | 0.00006 | -2.65                | 5.50                             | 5.77                         |
|                        | 0.003                         | 0.00306  | 0.00020 | 0.00295                   | 0.00015 | -1.80                | 6.39                             | 5.18                         |
|                        | 1.5                           | 1.46568  | 0.11242 | 1.35848                   | 0.05636 | -9.43                | 7.67                             | 4.15                         |
|                        | 2                             | 1.91466  | 0.15061 | 1.75294                   | 0.03146 | -12.35               | 7.87                             | 1.79                         |
| Blood                  | 0.001                         | 0.00099  | 0.00008 | 0.00100                   | 0.00008 | 0.50                 | 8.03                             | 8.02                         |
|                        | 0.003                         | 0.00280  | 0.00018 | 0.00270                   | 0.00013 | -10.01               | 6.33                             | 4.92                         |
|                        | 1.5                           | 1.42530  | 0.11066 | 1.40398                   | 0.11017 | -6.40                | 7.76                             | 7.85                         |
|                        | 2                             | 1.92910  | 0.15725 | 1.98038                   | 0.20177 | -0.98                | 8.15                             | 10.19                        |
| Liver <sup>a</sup>     | 0.001                         | 0.00097  | 0.00006 | 0.00099                   | 0.00006 | -0.60                | 6.23                             | 6.39                         |
|                        | 0.003                         | 0.00290  | 0.00019 | 0.00302                   | 0.00020 | 0.80                 | 6.47                             | 6.65                         |
|                        | 1.5                           | 1.43778  | 0.06443 | 1.41420                   | 0.05821 | -5.72                | 4.48                             | 4.12                         |
|                        | 2                             | 1.91775  | 0.10919 | 1.90058                   | 0.07760 | -4.97                | 5.69                             | 4.08                         |
| Spleen <sup>a</sup>    | 0.001                         | 0.00099  | 0.00006 | 0.00102                   | 0.00005 | 1.56                 | 5.89                             | 4.58                         |
|                        | 0.003                         | 0.00305  | 0.00019 | 0.00315                   | 0.00013 | 4.93                 | 6.24                             | 4.09                         |
|                        | 1.5                           | 1.54094  | 0.06850 | 1.55464                   | 0.07691 | 3.64                 | 4.45                             | 4.95                         |
|                        | 2                             | 2.05017  | 0.10382 | 2.09398                   | 0.05937 | 4.70                 | 5.06                             | 2.84                         |
| Kidney <sup>a</sup>    | 0.001                         | 0.00099  | 0.00006 | 0.00098                   | 0.00007 | -1.58                | 5.81                             | 6.77                         |
|                        | 0.003                         | 0.00298  | 0.00019 | 0.00289                   | 0.00018 | -3.64                | 6.28                             | 6.29                         |
|                        | 1.5                           | 1.48875  | 0.10638 | 1.40118                   | 0.08605 | -6.58                | 7.15                             | 6.14                         |
|                        | 2                             | 1.96596  | 0.13026 | 1.83016                   | 0.03118 | -8.50                | 6.63                             | 1.70                         |
| Lung <sup>a</sup>      | 0.001                         | 0.00104  | 0.00008 | 0.00108                   | 0.00006 | 7.99                 | 7.63                             | 5.17                         |
|                        | 0.003                         | 0.00279  | 0.00016 | 0.00266                   | 0.00004 | -11.32               | 5.84                             | 1.44                         |
|                        | 1.5                           | 1.45115  | 0.14113 | 1.37534                   | 0.08889 | -8.31                | 9.73                             | 6.46                         |
|                        | 2                             | 1.89224  | 0.21649 | 1.74432                   | 0.06739 | -12.78               | 11.44                            | 3.86                         |
| Intestine <sup>a</sup> | 0.001                         | 0.00099  | 0.00006 | 0.00101                   | 0.00004 | 0.87                 | 5.88                             | 3.69                         |
|                        | 0.003                         | 0.00300  | 0.00018 | 0.00318                   | 0.00005 | 5.99                 | 6.13                             | 1.49                         |
|                        | 1.5                           | 1.51636  | 0.08029 | 1.45500                   | 0.10010 | -3.00                | 5.29                             | 6.88                         |
|                        | 2                             | 1.96950  | 0.11312 | 1.97050                   | 0.10770 | -1.48                | 5.74                             | 5.47                         |
| Heart <sup>a</sup>     | 0.001                         | 0.00101  | 0.00006 | 0.00102                   | 0.00007 | 1.54                 | 5.55                             | 6.43                         |
|                        | 0.003                         | 0.00296  | 0.00017 | 0.00299                   | 0.00023 | -0.47                | 5.60                             | 7.85                         |
|                        | 1.5                           | 1.53956  | 0.08761 | 1.44026                   | 0.10444 | -3.98                | 5.69                             | 7.25                         |
|                        | 2                             | 2.06784  | 0.13069 | 2.10030                   | 0.15485 | 5.02                 | 6.32                             | 7.37                         |
| Brain <sup>a</sup>     | 0.001                         | 0.00100  | 0.00006 | 0.00097                   | 0.00004 | -2.52                | 5.53                             | 4.18                         |
|                        | 0.003                         | 0.00291  | 0.00013 | 0.00297                   | 0.00013 | -1.13                | 4.62                             | 4.33                         |
|                        | 1.5                           | 1.45232  | 0.08648 | 1.48638                   | 0.13211 | -0.91                | 5.95                             | 8.89                         |
|                        | 2                             | 1.99141  | 0.11251 | 2.11942                   | 0.06915 | 5.97                 | 5.65                             | 3.26                         |
| Stomach <sup>a</sup>   | 0.001                         | 0.00101  | 0.00006 | 0.00101                   | 0.00007 | 1.03                 | 6.17                             | 6.50                         |
|                        | 0.003                         | 0.00308  | 0.00017 | 0.00313                   | 0.00016 | 4.25                 | 5.41                             | 4.97                         |
|                        | 1.5                           | 1.48640  | 0.08477 | 1.48016                   | 0.05099 | -1.32                | 5.70                             | 3.45                         |
|                        | 2                             | 1.98460  | 0.14326 | 1.96186                   | 0.10753 | -1.91                | 7.22                             | 5.48                         |

<sup>a</sup>Concentrations of tissues expressed as  $\mu\text{g/mL}$  tissue homogenate

**Table 3.** Matrix effect and extraction recovery of cyclosporine A (CyA) in whole blood and tissues.

| Matrix    | Conc. ( $\mu\text{g/mL}$ ) | Matrix effect (%) | Extraction recovery of CyA (%) |
|-----------|----------------------------|-------------------|--------------------------------|
| Blood     | 0.003                      | 64.61             | 98.72                          |
|           | 1.5                        | 58.62             | 98.86                          |
|           | 2                          | 58.27             | 99.28                          |
| Liver     | 0.003                      | 8.43              | 89.78                          |
|           | 1.5                        | 7.25              | 86.08                          |
|           | 2                          | 11.94             | 92.23                          |
| Spleen    | 0.003                      | 21.80             | 99.90                          |
|           | 1.5                        | 25.60             | 89.22                          |
|           | 2                          | 19.04             | 70.54                          |
| Kidney    | 0.003                      | 0.02              | 73.30                          |
|           | 1.5                        | 9.67              | 83.14                          |
|           | 2                          | 4.47              | 82.64                          |
| Lung      | 0.003                      | 17.97             | 82.96                          |
|           | 1.5                        | 22.71             | 87.73                          |
|           | 2                          | 23.78             | 88.66                          |
| Intestine | 0.003                      | 15.44             | 89.48                          |
|           | 1.5                        | 14.67             | 96.75                          |
|           | 2                          | 14.00             | 99.99                          |
| Heart     | 0.003                      | 18.56             | 87.32                          |
|           | 1.5                        | 21.18             | 83.84                          |
|           | 2                          | 20.95             | 83.20                          |
| Brain     | 0.003                      | 17.15             | 77.19                          |
|           | 1.5                        | 16.06             | 76.48                          |
|           | 2                          | 12.10             | 73.71                          |
| Stomach   | 0.003                      | 5.47              | 84.43                          |
|           | 1.5                        | 11.66             | 82.35                          |
|           | 2                          | 4.60              | 81.96                          |

**Table 4. Post-extraction** stability of cyclosporine A in whole blood and liver under different conditions.

|   | QC2 (0.003 µg/mL)                                  |      |                           | QC4 (2 µg/mL)                                      |      |                           |
|---|--|------|---------------------------|--|------|---------------------------|
|   | Measured concentration<br>(µg/mL)<br>(mean ± S.D.) | CV   | Accuracy deviation<br>(%) | Measured concentration<br>(µg/mL)<br>(mean ± S.D.) | CV   | Accuracy deviation<br>(%) |
| <b>Stability in extracted blood sample</b>        |  |      |                           |  |      |                           |
| Autosampler Stability (72 h)                      | 0.0026±0.0001                                      | 2.28 | -12.23                    | 1.9003±0.0924                                      | 4.86 | -5.0                      |
| Short-term stability<br>(6 h at room temperature) | 0.0033±0.0001                                      | 3.16 | 10.36                     | 2.1735±0.1144                                      | 5.27 | 8.7                       |
| <b>Stability in extracted liver sample</b>        |  |      |                           |  |      |                           |
| Autosampler Stability (72 h)                      | 0.0030±0.0002                                      | 5.68 | -0.60                     | 1.7484±0.0493                                      | 2.82 | -12.5                     |
| Short-term stability<br>(6 h at room temperature) | 0.0026±0.0002                                      | 6.94 | -13.13                    | 1.9743±0.0313                                      | 1.58 | -1.3                      |

<sup>a</sup>Concentrations of liver expressed as µg/mL tissue homogenate