

**Table S1**

Response models and statistical parameters obtained from ANOVA for CCD.

| Response                 | Regression model                                                        | Model p-value | %C.V. | Adequate precision |
|--------------------------|-------------------------------------------------------------------------|---------------|-------|--------------------|
| R <sub>AX</sub>          | 1.14-0.067A-0.22B+0.048AB-0.016 A <sup>2</sup><br>+0.054B <sup>2</sup>  | < 0.0001      | 2.43  | 33.13              |
| R <sub>CX</sub>          | 1.69-0.086A-0.3B+ 0.1AB+ 0.00075A <sup>2</sup><br>+ 0.066B <sup>2</sup> | < 0.0001      | 1.38  | 52.99              |
| T <sub>FX</sub><br>(min) | 17.10-0.83A-3.09B+0.65AB+0.062 A <sup>2</sup><br>+0.51 B <sup>2</sup>   | < 0.0001      | 1.77  | 41.60              |

**Table S2**

The system suitability test results of the developed method for determination of CL,AX,SB,TZ,AP,CX and FX .

| Compound | Retention time (min) <sup>a</sup> | Capacity factor (K') | Selectivity ( $\alpha$ ) <sup>b</sup> | Resolution ( $R_s$ ) <sup>b</sup> | Tailing factor | %RSD <sup>c</sup> of retention time | Plate Count |
|----------|-----------------------------------|----------------------|---------------------------------------|-----------------------------------|----------------|-------------------------------------|-------------|
| CL       | 3.91                              | 2.25                 | 1.33 ( $a_1$ )                        | 2.10( $b_1$ )                     | 1.10           | 1.53                                | 2532        |
| AX       | 4.80                              | 3.00                 | 1.33 ( $a_2$ )                        | 2.40( $b_2$ )                     | 0.98           | 1.66                                | 3459        |
| SB       | 6.00                              | 4.00                 | 1.43( $a_3$ )                         | 6.56( $b_3$ )                     | 1.01           | 1.50                                | 5190        |
| TZ       | 8.12                              | 5.75                 | 1.17( $a_4$ )                         | 5.67( $b_4$ )                     | 1.10           | 0.62                                | 6870        |
| AP       | 9.30                              | 6.75                 | 2.20( $a_5$ )                         | 21.77( $b_5$ )                    | 0.99           | 0.75                                | 8900        |
| CX       | 19.10                             | 14.90                | 1.04( $a_6$ )                         | 1.51( $b_6$ )                     | 0.98           | 0.52                                | 10234       |
| FX       | 19.82                             | 15.50                |                                       |                                   | 1.01           | 0.40                                | 11526       |

a :The retention time of unretained peak is 1.2 min.

b:  $a_1$ ,  $b_1$ : are  $\alpha$  and  $R_s$  calculated for AX-CL.

$a_2$ ,  $b_2$ : are  $\alpha$  and  $R_s$  calculated for SB-AX.

$a_3$ ,  $b_3$ : are  $\alpha$  and  $R_s$  calculated for TZ-SB.

$a_4$ ,  $b_4$ : are  $\alpha$  and  $R_s$  calculated for AP-TZ.

$a_5$ ,  $b_5$ : are  $\alpha$  and  $R_s$  calculated for CX-AP.

$a_6$ ,  $b_6$ : are  $\alpha$  and  $R_s$  calculated for FX-CX.

c: Relative standard deviation.

**Table S3**

Characteristic parameters for the regression equations of the proposed HPLC method for determination of CL,AX,SB,AP,CX and FX in plasma and urine.

| Parameters                                         | CL                        |                          | AX                        |                          | SB                     |                        | AP                     |                        | CX                     |                        | FX                     |                        |
|----------------------------------------------------|---------------------------|--------------------------|---------------------------|--------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|                                                    | Plasma                    | Urine                    | Plasma                    | Urine                    | Plasma                 | Urine                  | Plasma                 | Urine                  | Plasma                 | Urine                  | Plasma                 | Urine                  |
| Calibration range ( $\mu\text{g mL}^{-1}$ )        | 0.3-20                    | 0.3-20                   | 0.6-15                    | 0.6-20                   | 0.7-15                 | 0.7-15                 | 0.6-15                 | 0.6-20                 | 0.1-15                 | 0.1-15                 | 0.1-15                 | 0.1-15                 |
| Regression equation(Y) <sup>a</sup> :<br>Slope (b) | 0.0848                    | 0.0978                   | 0.0520                    | 0.0580                   | 0.0132                 | 0.0108                 | 0.033                  | 0.043                  | 0.096                  | 0.122                  | 0.105                  | 0.134                  |
| Standard deviation of the slope ( $S_b$ )          | 0.0013                    | 0.0008                   | 0.0011                    | 0.0006                   | 0.0002                 | 0.0003                 | 0.0005                 | 0.0007                 | 0.0008                 | 0.0012                 | 0.0011                 | 0.0019                 |
| Relative standard deviation of the slope (%)       | 1.58                      | 0.87                     | 2.08                      | 1.07                     | 2.03                   | 2.49                   | 1.78                   | 1.77                   | 0.84                   | 0.96                   | 1.00                   | 1.45                   |
| Confidence limit of the slope <sup>b</sup>         | 0.0835-<br>0.0861         | 0.0969-<br>0.0986        | 0.051-<br>0.053           | 0.057-<br>0.058          | 0.0128-<br>0.0134      | 0.0106-<br>0.0111      | 0.0323-<br>0.0334      | 0.042-<br>0.044        | 0.095-<br>0.097        | 0.121-<br>0.123        | 0.103-<br>0.106        | 0.132-<br>0.135        |
| Intercept (a)                                      | -0.0041                   | -0.0037                  | -0.0024                   | -0.0025                  | 0.0010                 | 0.0011                 | -0.0013                | 0.0032                 | 0.0022                 | 0.0028                 | 0.0036                 | 0.0016                 |
| Standard deviation of the intercept ( $S_a$ )      | -0.0016                   | -0.00144                 | -0.00093                  | -0.00097                 | 0.000389               | 0.000428               | -0.00051               | 0.001245               | 0.000856               | 0.001089               | 0.001401               | 0.000623               |
| Confidence limit of the intercept <sup>b</sup>     | -0.00565<br>-<br>-0.00255 | -0.0051-<br>-<br>-0.0023 | -0.00331<br>-<br>-0.00149 | -0.0034<br>-<br>-0.00156 | 0.001378 -<br>0.000622 | 0.001516 -<br>0.000684 | -0.00179 -<br>-0.00081 | 0.004409 -<br>0.001991 | 0.003032 -<br>0.001368 | 0.003858 -<br>0.001742 | 0.004961 -<br>0.002239 | 0.002205 -<br>0.000995 |
| Correlation coefficient (r)                        | 0.9999                    | 0.9997                   | 0.9996                    | 0.9999                   | 0.9996                 | 0.9995                 | 0.9997                 | 0.9997                 | 0.9999                 | 0.9999                 | 0.9999                 | 0.9999                 |

<sup>a</sup>Y = a + bC, where Y is the response (peak area ratio), a is the intercept, b is the slope, and C is the concentration of compounds ( $\mu\text{g mL}^{-1}$ ) in human urine and plasma.

<sup>b</sup> 95% confidence limit.

**Table S4**

ANOVA (showing lack of fit calculation) for CL,AX,SB,AP,CX and FX in plasma and urine.

| Comp. | Matrix | Source of variation | Sum of squares         | degree of freedom | Mean sum of squares     | F-Ratio |
|-------|--------|---------------------|------------------------|-------------------|-------------------------|---------|
| CL    | Plasma | Total               | 5.51                   | 21                | 0.26                    | 1.75    |
|       |        | Regression          | 5.43                   | 2                 | 2.72                    |         |
|       |        | Residual            | 4.05x 10 <sup>-4</sup> | 19                | 2.13x 10 <sup>-5</sup>  |         |
|       |        | Replicate           | 1.01x 10 <sup>-4</sup> | 7                 | 1.44 x 10 <sup>-5</sup> |         |
|       |        | Lack of fit         | 3.04x 10 <sup>-4</sup> | 12                | 2.53x 10 <sup>-5</sup>  |         |
|       | Urine  | Total               | 4.25                   | 21                | 0.20                    | 1.84    |
|       |        | Regression          | 4.16                   | 2                 | 2.08                    |         |
|       |        | Residual            | 8.33x 10 <sup>-4</sup> | 19                | 4.38 x 10 <sup>-5</sup> |         |
|       |        | Replicate           | 2.00x 10 <sup>-4</sup> | 7                 | 2.86 x 10 <sup>-5</sup> |         |
|       |        | Lack of fit         | 6.33x 10 <sup>-4</sup> | 12                | 5.27x 10 <sup>-5</sup>  |         |
| AX    | Plasma | Total               | 1.10                   | 21                | 0.05                    | 1.21    |
|       |        | Regression          | 1.08                   | 2                 | 0.54                    |         |
|       |        | Residual            | 3.08x 10 <sup>-4</sup> | 19                | 1.62 x 10 <sup>-5</sup> |         |
|       |        | Replicate           | 1.00x 10 <sup>-4</sup> | 7                 | 1.43x 10 <sup>-5</sup>  |         |
|       |        | Lack of fit         | 2.08x 10 <sup>-4</sup> | 12                | 1.73x 10 <sup>-5</sup>  |         |
|       | Urine  | Total               | 1.92                   | 21                | 0.09                    | 0.04    |
|       |        | Regression          | 1.93                   | 2                 | 0.96                    |         |
|       |        | Residual            | 1.06x 10 <sup>-4</sup> | 19                | 1.91 x 10 <sup>-5</sup> |         |
|       |        | Replicate           | 1.00x 10 <sup>-4</sup> | 7                 | 1.43x 10 <sup>-5</sup>  |         |
|       |        | Lack of fit         | 5.80x 10 <sup>-6</sup> | 12                | 4.83x 10 <sup>-7</sup>  |         |
| SB    | Plasma | Total               | 0.073                  | 21                | 3.51 x 10 <sup>-3</sup> | 3.38    |
|       |        | Regression          | 0.071                  | 2                 | 3.59 x 10 <sup>-2</sup> |         |
|       |        | Residual            | 2.05x 10 <sup>-5</sup> | 19                | 1.08 x 10 <sup>-6</sup> |         |
|       |        | Replicate           | 3.01x 10 <sup>-6</sup> | 7                 | 4.30 x 10 <sup>-7</sup> |         |
|       |        | Lack of fit         | 1.75x 10 <sup>-5</sup> | 12                | 1.45x 10 <sup>-6</sup>  |         |
|       | Urine  | Total               | 0.048                  | 21                | 2.32 x 10 <sup>-3</sup> | 1.31    |
|       |        | Regression          | 0.041                  | 2                 | 2.06 x 10 <sup>-2</sup> |         |
|       |        | Residual            | 3.25x 10 <sup>-4</sup> | 19                | 1.71 x 10 <sup>-5</sup> |         |
|       |        | Replicate           | 1.00x 10 <sup>-4</sup> | 7                 | 1.43 x 10 <sup>-5</sup> |         |
|       |        | Lack of fit         | 2.25x 10 <sup>-4</sup> | 12                | 1.87x 10 <sup>-5</sup>  |         |

**Table S4: continued**

| Comp. | Matrix | Source of variation | Sum of squares        | degree of freedom | Mean sum of squares   | F-Ratio |
|-------|--------|---------------------|-----------------------|-------------------|-----------------------|---------|
| AP    | Plasma | Total               | 0.43                  | 21                | $2.06 \times 10^{-2}$ | 1.48    |
|       |        | Regression          | 0.41                  | 2                 | $2.05 \times 10^{-2}$ |         |
|       |        | Residual            | $3.55 \times 10^{-4}$ | 19                | $1.87 \times 10^{-5}$ |         |
|       |        | Replicate           | $1.00 \times 10^{-4}$ | 7                 | $1.43 \times 10^{-5}$ |         |
|       |        | Lack of fit         | $2.55 \times 10^{-4}$ | 12                | $2.12 \times 10^{-5}$ |         |
|       | Urine  | Total               | 0.78                  | 21                | $3.73 \times 10^{-2}$ | 2.04    |
|       |        | Regression          | 0.75                  | 2                 | $3.75 \times 10^{-1}$ |         |
|       |        | Residual            | $4.51 \times 10^{-4}$ | 19                | $2.30 \times 10^{-5}$ |         |
|       |        | Replicate           | $1.00 \times 10^{-4}$ | 7                 | $1.43 \times 10^{-5}$ |         |
|       |        | Lack of fit         | $3.51 \times 10^{-4}$ | 12                | $2.92 \times 10^{-5}$ |         |
| CX    | Plasma | Total               | 3.76                  | 21                | 0.18                  | 3.03    |
|       |        | Regression          | 3.73                  | 2                 | 1.86                  |         |
|       |        | Residual            | $1.24 \times 10^{-4}$ | 19                | $6.52 \times 10^{-6}$ |         |
|       |        | Replicate           | $2.00 \times 10^{-5}$ | 7                 | $2.86 \times 10^{-6}$ |         |
|       |        | Lack of fit         | $1.04 \times 10^{-4}$ | 12                | $8.66 \times 10^{-6}$ |         |
|       | Urine  | Total               | 6.21                  | 21                | 0.29                  | 1.87    |
|       |        | Regression          | 6.22                  | 2                 | 3.11                  |         |
|       |        | Residual            | $1.77 \times 10^{-4}$ | 19                | $9.31 \times 10^{-6}$ |         |
|       |        | Replicate           | $4.20 \times 10^{-4}$ | 7                 | $6.00 \times 10^{-6}$ |         |
|       |        | Lack of fit         | $1.35 \times 10^{-4}$ | 12                | $1.12 \times 10^{-5}$ |         |
| FX    | Plasma | Total               | 4.48                  | 21                | 0.21                  | 3.42    |
|       |        | Regression          | 4.47                  | 2                 | 2.23                  |         |
|       |        | Residual            | $1.44 \times 10^{-4}$ | 19                | $7.59 \times 10^{-6}$ |         |
|       |        | Replicate           | $2.10 \times 10^{-5}$ | 7                 | $3.00 \times 10^{-6}$ |         |
|       |        | Lack of fit         | $1.23 \times 10^{-4}$ | 12                | $1.03 \times 10^{-5}$ |         |
|       | Urine  | Total               | 7.53                  | 21                | 0.36                  | 1.96    |
|       |        | Regression          | 7.42                  | 2                 | 3.71                  |         |
|       |        | Residual            | $8.72 \times 10^{-4}$ | 19                | $4.59 \times 10^{-5}$ |         |
|       |        | Replicate           | $2.00 \times 10^{-4}$ | 7                 | $2.86 \times 10^{-5}$ |         |
|       |        | Lack of fit         | $6.72 \times 10^{-4}$ | 12                | $5.60 \times 10^{-5}$ |         |

The critical value of F-Ratio is 3.57 at  $\alpha = 0.05$

**Table S5**

Accuracy and precision of the proposed method in plasma and urine.

| Cpd | Matrix | conc<br>( $\mu\text{g mL}^{-1}$ ) | Intra-day                  |      |       | Inter-day        |      |       |
|-----|--------|-----------------------------------|----------------------------|------|-------|------------------|------|-------|
|     |        |                                   | Mean $\pm$ SD <sup>a</sup> | RSD% | RE%   | Mean $\pm$ SD    | RSD% | RE%   |
| CL  | Plasma | 0.3                               | 0.29 $\pm$ 0.01            | 3.44 | -3.33 | 0.31 $\pm$ 0.01  | 3.22 | 3.33  |
|     |        | 5                                 | 5.18 $\pm$ 0.18            | 3.45 | 3.60  | 5.32 $\pm$ 0.29  | 5.38 | 6.40  |
|     |        | 20                                | 20.26 $\pm$ 0.36           | 1.77 | 1.33  | 20.50 $\pm$ 0.87 | 4.22 | 2.50  |
|     | Urine  | 0.3                               | 0.32 $\pm$ 0.02            | 5.75 | 6.67  | 0.33 $\pm$ 0.03  | 8.57 | 10.00 |
|     |        | 5                                 | 5.14 $\pm$ 0.31            | 6.09 | 2.80  | 5.26 $\pm$ 0.36  | 6.80 | 5.20  |
|     |        | 20                                | 20.20 $\pm$ 0.29           | 1.44 | 1.00  | 20.72 $\pm$ 0.82 | 3.97 | 3.60  |
| AX  | Plasma | 0.6                               | 0.61 $\pm$ 0.02            | 2.92 | 1.67  | 0.63 $\pm$ 0.03  | 5.50 | 5.00  |
|     |        | 5                                 | 5.12 $\pm$ 0.27            | 5.24 | 2.40  | 5.24 $\pm$ 0.29  | 5.50 | 4.80  |
|     |        | 15                                | 15.32 $\pm$ 0.37           | 2.42 | 2.13  | 15.84 $\pm$ 0.74 | 4.65 | 5.60  |
|     | Urine  | 0.6                               | 0.61 $\pm$ 0.01            | 2.32 | 1.67  | 0.63 $\pm$ 0.04  | 6.75 | 5.00  |
|     |        | 5                                 | 5.12 $\pm$ 0.27            | 5.24 | 2.40  | 5.38 $\pm$ 0.52  | 9.69 | 7.60  |
|     |        | 20                                | 20.36 $\pm$ 0.50           | 2.45 | 1.80  | 20.90 $\pm$ 0.91 | 4.36 | 4.50  |
| SB  | Plasma | 0.7                               | 0.71 $\pm$ 0.02            | 3.07 | 1.43  | 0.75 $\pm$ 0.04  | 4.95 | 7.14  |
|     |        | 5                                 | 5.06 $\pm$ 0.13            | 2.65 | 1.20  | 5.34 $\pm$ 0.48  | 8.94 | 6.80  |
|     |        | 15                                | 15.38 $\pm$ 0.52           | 3.39 | 2.53  | 15.78 $\pm$ 1.30 | 8.24 | 5.20  |
|     | Urine  | 0.7                               | 0.71 $\pm$ 0.01            | 1.83 | 1.43  | 0.73 $\pm$ 0.04  | 4.84 | 4.29  |
|     |        | 5                                 | 5.10 $\pm$ 0.22            | 4.38 | 2.00  | 5.26 $\pm$ 0.37  | 7.06 | 5.20  |
|     |        | 15                                | 15.22 $\pm$ 0.30           | 1.99 | 1.47  | 15.74 $\pm$ 1.29 | 8.25 | 4.93  |
| AP  | Plasma | 0.6                               | 0.61 $\pm$ 0.01            | 2.21 | 1.67  | 0.63 $\pm$ 0.03  | 5.42 | 5.00  |
|     |        | 5                                 | 5.10 $\pm$ 0.22            | 4.38 | 2.00  | 5.40 $\pm$ 0.38  | 7.05 | 8.00  |
|     |        | 15                                | 15.30 $\pm$ 0.42           | 2.77 | 2.00  | 15.90 $\pm$ 1.20 | 7.56 | 6.00  |
|     | Urine  | 0.6                               | 0.61 $\pm$ 0.01            | 1.80 | 1.67  | 0.63 $\pm$ 0.04  | 5.96 | 5.00  |
|     |        | 5                                 | 5.12 $\pm$ 0.18            | 3.49 | 2.40  | 5.34 $\pm$ 0.33  | 6.15 | 6.80  |
|     |        | 15                                | 15.28 $\pm$ 0.39           | 2.55 | 1.87  | 15.74 $\pm$ 0.82 | 5.23 | 4.93  |
| CX  | Plasma | 0.1                               | 0.11 $\pm$ 0.01            | 8.60 | 5.00  | 0.10 $\pm$ 0.01  | 8.60 | 4.00  |
|     |        | 5                                 | 5.06 $\pm$ 0.13            | 2.65 | 1.20  | 5.38 $\pm$ 0.52  | 9.69 | 7.60  |
|     |        | 15                                | 15.34 $\pm$ 0.47           | 3.04 | 2.27  | 15.78 $\pm$ 1.30 | 8.24 | 5.20  |
|     | Urine  | 0.1                               | 0.10 $\pm$ 0.01            | 8.60 | 3.00  | 0.11 $\pm$ 0.01  | 7.75 | 10.00 |
|     |        | 5                                 | 5.10 $\pm$ 0.22            | 4.38 | 2.00  | 5.28 $\pm$ 0.39  | 7.38 | 5.60  |
|     |        | 15                                | 15.22 $\pm$ 0.32           | 2.10 | 1.47  | 15.68 $\pm$ 1.31 | 8.34 | 4.53  |
| FX  | Plasma | 0.1                               | 0.10 $\pm$ 0.01            | 5.27 | 3.00  | 0.11 $\pm$ 0.01  | 8.44 | 10.00 |
|     |        | 5                                 | 5.08 $\pm$ 0.18            | 3.52 | 1.60  | 5.36 $\pm$ 0.50  | 9.29 | 7.20  |
|     |        | 15                                | 15.38 $\pm$ 0.38           | 2.45 | 2.53  | 15.94 $\pm$ 1.23 | 7.70 | 6.29  |
|     | Urine  | 0.1                               | 0.10 $\pm$ 0.01            | 5.27 | 0.00  | 0.11 $\pm$ 0.01  | 8.44 | 10.00 |
|     |        | 5                                 | 5.08 $\pm$ 0.18            | 3.52 | 1.60  | 5.34 $\pm$ 0.47  | 8.74 | 6.80  |
|     |        | 15                                | 15.28 $\pm$ 0.27           | 1.76 | 1.87  | 15.70 $\pm$ 1.29 | 8.23 | 4.73  |

<sup>a</sup> Mean and S.D. for five determinations.

**Table S6**

a) Factors for robustness study and b) responses measured.

a)

| Independent factors                  | Levels    |          |           |
|--------------------------------------|-----------|----------|-----------|
|                                      | Level(-1) | Level(0) | Level(+1) |
| A: Percentage of acetonitrile (ACN%) | 12.5      | 14       | 15.5      |
| B: pH                                | 3.2       | 3.4      | 3.6       |
| C:Flow rate(mL/min)                  | 0.8       | 1.0      | 1.2       |
| D:Wavelength of detection(nm)        | 223       | 225      | 227       |

b)

| Std | Run | A    | B   | C   | D   | Peak area $\pm$ SD |                 |                 |                   |                  |                   |                  |
|-----|-----|------|-----|-----|-----|--------------------|-----------------|-----------------|-------------------|------------------|-------------------|------------------|
|     |     |      |     |     |     | CL                 | AX              | SB              | TZ                | AP               | CX                | FX               |
| 5   | 1   | 15.5 | 3.2 | 1.2 | 223 | 1124997 $\pm$ 155  | 442728 $\pm$ 77 | 179999 $\pm$ 33 | 2849507 $\pm$ 27  | 1145633 $\pm$ 45 | 1602171 $\pm$ 103 | 2219288 $\pm$ 97 |
| 1   | 2   | 15.5 | 3.2 | 0.8 | 227 | 1041043 $\pm$ 167  | 604000 $\pm$ 86 | 242181 $\pm$ 71 | 4078643 $\pm$ 49  | 1646138 $\pm$ 34 | 2268083 $\pm$ 67  | 3170040 $\pm$ 89 |
| 7   | 3   | 12.5 | 3.6 | 0.8 | 227 | 1216924 $\pm$ 45   | 437435 $\pm$ 39 | 332930 $\pm$ 66 | 3718039 $\pm$ 57  | 1250233 $\pm$ 98 | 1960766 $\pm$ 45  | 2647352 $\pm$ 74 |
| 2   | 4   | 12.5 | 3.2 | 0.8 | 223 | 1400120 $\pm$ 56   | 696108 $\pm$ 64 | 237832 $\pm$ 53 | 4087964 $\pm$ 87  | 1564232 $\pm$ 54 | 2260818 $\pm$ 98  | 3129712 $\pm$ 89 |
| 4   | 5   | 12.5 | 3.6 | 1.2 | 223 | 1117057 $\pm$ 44   | 566450 $\pm$ 81 | 335166 $\pm$ 90 | 2920781 $\pm$ 93  | 1177674 $\pm$ 75 | 1670888 $\pm$ 99  | 2338590 $\pm$ 89 |
| 8   | 6   | 12.5 | 3.2 | 1.2 | 227 | 713288 $\pm$ 43    | 525508 $\pm$ 74 | 138965 $\pm$ 65 | 2750135 $\pm$ 122 | 1843903 $\pm$ 86 | 1428869 $\pm$ 121 | 1920785 $\pm$ 96 |
| 6   | 7   | 15.5 | 3.6 | 1.2 | 227 | 1107349 $\pm$ 98   | 464559 $\pm$ 88 | 206161 $\pm$ 96 | 2999913 $\pm$ 99  | 1480172 $\pm$ 80 | 1635662 $\pm$ 65  | 2283656 $\pm$ 84 |
| 3   | 8   | 15.5 | 3.6 | 0.8 | 223 | 1657374 $\pm$ 89   | 651389 $\pm$ 63 | 255213 $\pm$ 74 | 3563632 $\pm$ 36  | 1498645 $\pm$ 98 | 2105079 $\pm$ 47  | 2943144 $\pm$ 98 |
| 9   | 9   | 14.0 | 3.4 | 1   | 225 | 971749 $\pm$ 96    | 735675 $\pm$ 56 | 172859 $\pm$ 56 | 3111107 $\pm$ 102 | 1036796 $\pm$ 67 | 1603811 $\pm$ 88  | 2303097 $\pm$ 94 |

**Table S7**

ANOVA results for robustness test. A 5% level of significance was desired.

| Factors | Response (Peak area) |        |      |        |      |        |                      |        |      |        |       |        |       |        |
|---------|----------------------|--------|------|--------|------|--------|----------------------|--------|------|--------|-------|--------|-------|--------|
|         | CL                   |        | AX   |        | SB   |        | TZ                   |        | AP   |        | CX    |        | FX    |        |
|         | F                    | p*     | F    | p*     | F    | p*     | F                    | p*     | F    | p*     | F     | p*     | F     | p*     |
| A       | 1.75                 | 0.2564 | 0.57 | 0.7022 | 0.95 | 0.3855 | 1.58                 | 0.3760 | 0.01 | 0.9445 | 0.32  | 0.6006 | 0.68  | 0.4572 |
| B       | 5.03                 | 0.0884 | 0.03 | 0.8677 | 3.98 | 0.1169 | 4.2×10 <sup>-4</sup> | 0.9846 | 0.81 | 0.4203 | 0.14  | 0.7319 | 0.10  | 0.7636 |
| C       | 6.76                 | 0.0966 | 0.18 | 0.6962 | 1.57 | 0.2781 | 0.61                 | 0.4779 | 0.12 | 0.7420 | 19.56 | 0.0115 | 19.67 | 0.0114 |
| D       | 11.17                | 0.0588 | 1.21 | 0.3324 | 0.28 | 0.6237 | 2.96                 | 0.055  | 0.89 | 0.3987 | 0.46  | 0.5354 | 0.75  | 0.4366 |

\*p-value should be less than 0.05 to be statistically significant.



**Table S8**

Stability analysis of CL,AX,SB,AP,CX, FX and TZ (I.S).

| Conc.<br>(µg/mL) |    | Stock soln. at |       | Stock soln. at |      | Short- term |      | Long- term |       |        |       | Freeze- thaw cycles |       |        |       | Post-preparative sample |       |        |       |       |       |
|------------------|----|----------------|-------|----------------|------|-------------|------|------------|-------|--------|-------|---------------------|-------|--------|-------|-------------------------|-------|--------|-------|-------|-------|
|                  |    | 25°C           |       | 4°C            |      | Plasma      |      | Urine      |       | Plasma |       | Urine               |       | Plasma |       | Urine                   |       | Plasma |       | Urine |       |
|                  |    | RE%            | RSD%  | RE%            | RSD% |             |      |            |       |        |       |                     |       |        |       |                         |       |        |       |       |       |
| TZ               | 1  | 4.67           | 4.81  | 5.00           | 4.76 | RE%         | RSD% | RE%        | RSD%  | RE%    | RSD%  | RE%                 | RSD%  | RE%    | RSD%  | RE%                     | RSD%  | RE%    | RSD%  | RE%   | RSD%  |
|                  | 15 | 3.56           | 1.62  | 2.22           | 3.71 |             |      |            |       |        |       |                     |       |        |       |                         |       |        |       |       |       |
| CL               | 1  | 7.67           | 10.02 | 9.33           | 9.21 | 6.67        | 2.86 | 5.00       | 5.30  | 4.00   | 6.93  | 12.00               | 6.44  | 8.67   | 2.13  | 6.00                    | 6.54  | 5.00   | 5.95  | 4.67  | 5.84  |
|                  | 15 | 4.00           | 1.28  | 2.67           | 2.60 | 2.44        | 3.34 | -2.00      | 1.18  | -1.11  | 0.39  | -2.22               | 1.57  | -2.44  | 1.97  | -2.67                   | 1.81  | -2.89  | 2.21  | -3.11 | 2.10  |
| AX               | 1  | 8.33           | 2.66  | 13.00          | 5.38 | 8.33        | 9.61 | 6.00       | 11.48 | 5.67   | 11.83 | 6.00                | 11.48 | 6.33   | 11.14 | 7.67                    | 10.19 | 5.67   | 12.85 | 5.33  | 12.96 |
|                  | 15 | 2.89           | 1.63  | 3.78           | 2.07 | 2.44        | 2.63 | -2.00      | 2.45  | -1.33  | 3.38  | -1.56               | 3.73  | -1.78  | 3.42  | -2.00                   | 3.12  | -2.22  | 3.50  | -2.00 | 3.79  |
| SB               | 1  | 4.00           | 5.09  | 6.00           | 4.99 | 12.67       | 5.71 | 12.33      | 6.06  | 11.67  | 6.84  | 11.33               | 7.26  | 8.00   | 3.21  | 8.67                    | 3.83  | 10.33  | 1.38  | 10.67 | 1.88  |
|                  | 15 | 3.33           | 1.71  | 2.67           | 2.60 | 2.89        | 1.63 | 2.67       | 1.95  | 2.89   | 1.63  | 2.67                | 1.95  | 2.22   | 2.10  | 2.00                    | 2.26  | 2.22   | 2.10  | 3.33  | 1.71  |
| AP               | 1  | 4.67           | 4.81  | 4.00           | 5.09 | 8.33        | 1.41 | 8.67       | 1.41  | 9.33   | 0.53  | 8.67                | 1.41  | 8.00   | 1.60  | 7.67                    | 1.93  | 8.00   | 2.45  | 8.67  | 1.41  |
|                  | 15 | 3.11           | 1.98  | 2.89           | 2.28 | 2.44        | 2.63 | 3.11       | 2.69  | 3.78   | 1.62  | 3.56                | 1.97  | 4.00   | 2.22  | 3.78                    | 2.07  | 4.00   | 2.22  | 4.22  | 2.42  |
| CX               | 1  | 6.33           | 4.44  | 7.00           | 3.37 | 5.00        | 4.36 | 5.67       | 4.27  | 6.33   | 3.30  | 4.00                | 5.35  | 3.67   | 5.49  | 3.33                    | 5.67  | 4.00   | 5.35  | 6.00  | 5.74  |
|                  | 15 | 4.00           | 1.70  | 1.78           | 5.49 | 3.33        | 1.71 | 3.11       | 1.98  | 4.00   | 1.28  | 1.78                | 4.00  | 1.56   | 3.96  | 1.56                    | 3.62  | 2.89   | 4.12  | 3.11  | 4.30  |
| FX               | 1  | 6.33           | 3.80  | 7.33           | 2.34 | 8.00        | 1.85 | 5.00       | 6.67  | 4.33   | 7.81  | 14.33               | 8.13  | 13.67  | 8.81  | 13.33                   | 9.18  | 14.33  | 8.13  | 13.00 | 9.57  |
|                  | 15 | 3.11           | 1.35  | 3.56           | 2.07 | 2.00        | 4.58 | 1.33       | 4.31  | -1.11  | 5.73  | -1.33               | 5.89  | -1.56  | 6.14  | -1.11                   | 5.65  | -1.33  | 5.89  | 2.44  | 5.46  |

**Table S9**

a) Factors examined in the screening phase (fractional factorial design), b) Matrix of the design.

a)

| Independent factors                 | Levels    |          |           |
|-------------------------------------|-----------|----------|-----------|
|                                     | Level(-1) | Level(0) | Level(+1) |
| A-Percentage of acetonitrile (ACN%) | 10        | 15       | 20        |
| B-pH                                | 3         | 5        | 7         |
| C-Column temperature (°C)           | 25        | 30       | 35        |
| D-Percentage of TFA (TFA %)         | 0.05      | 0.10     | 0.15      |

b)

| Std order | Run order | Experimental design |        |        |        | Experimental set-up |    |          |       |
|-----------|-----------|---------------------|--------|--------|--------|---------------------|----|----------|-------|
|           |           | Factor              | Factor | Factor | Factor | ACN%                | pH | Temp. °C | TFA % |
|           |           | 1                   | 2      | 3      | 4      |                     |    |          |       |
| 1         | 1         | -1                  | -1     | -1     | -1     | 10                  | 3  | 25       | 0.05  |
| 7         | 2         | -1                  | 1      | 1      | -1     | 10                  | 7  | 35       | 0.05  |
| 5         | 3         | -1                  | -1     | 1      | 1      | 10                  | 3  | 35       | 0.15  |
| 3         | 4         | -1                  | 1      | -1     | 1      | 10                  | 7  | 25       | 0.15  |
| 8         | 5         | 1                   | 1      | 1      | 1      | 20                  | 7  | 35       | 0.15  |
| 6         | 6         | 1                   | -1     | 1      | -1     | 20                  | 3  | 35       | 0.05  |
| 2         | 7         | 1                   | -1     | -1     | 1      | 20                  | 3  | 25       | 0.15  |
| 4         | 8         | 1                   | 1      | -1     | -1     | 20                  | 7  | 25       | 0.05  |
| 9         | 9         | 0                   | 0      | 0      | 0      | 15                  | 5  | 30       | 0.10  |

**Table S10**

Central composite design for the HPLC response optimization.

| Standard<br>order | Run<br>order | Factors |     | Responses       |                 |                 |
|-------------------|--------------|---------|-----|-----------------|-----------------|-----------------|
|                   |              | ACN%    | pH  | R <sub>AX</sub> | R <sub>CX</sub> | T <sub>FX</sub> |
| 9                 | 1            | 7.9     | 5.0 | 1.8             | 1.2             | 18.0            |
| 3                 | 2            | 10.0    | 7.0 | 1.4             | 1.0             | 15.2            |
| 6                 | 3            | 10.0    | 3.0 | 2.3             | 1.5             | 22.5            |
| 2                 | 4            | 15.0    | 5.0 | 1.7             | 1.2             | 17.2            |
| 4                 | 5            | 15.0    | 5.0 | 1.6             | 1.2             | 17.3            |
| 10                | 6            | 15.0    | 7.8 | 1.4             | 0.9             | 13.4            |
| 8                 | 7            | 15.0    | 2.2 | 2.2             | 1.6             | 20.0            |
| 7                 | 8            | 20.0    | 3.0 | 1.8             | 1.3             | 19.3            |
| 5                 | 9            | 20.0    | 7.0 | 1.5             | 1.0             | 14.6            |
| 1                 | 10           | 22.1    | 5.0 | 1.7             | 1.0             | 16.0            |

