# Supplementary Materials: High-Performance Liquid Chromatography–Fluorescence Detection Method for Ochratoxin A Quantification in Small Mice Sample Volumes: Versatile Application across Diverse Matrices Relevant for Neurodegeneration Research

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**Table S1**. Raw data of the linearity study of column A in the following ranges: 2.35 – 22.83 ng/mL and 22.83 – 228.33 ng/mL.

| | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| **Range 2.35 – 22.83 ng/mL** | | | |
| Curve equation[a] | y = 9.488x − 0.826 | y = 9.256x − 0.995 | y = 8.853x + 4.989 |
| $r^2$ | 0.995 | 0.994 | 0.993 |
| Slope limits (p=95%) | 8.652; 10.32 | 8.131; 10.382 | 7.944; 9.762 |
| Intercept limits (p=95%) | -10.63;8.976 | -9.77; 7.78 | -5.66; 15.65 |
| CV[c] of response factors (%) | 4.88 | 3.24 | 4.55 |
| Back-calculated RE[b] (%) | < 7.5 | < 4.2 | < 7.4 |
| **Range 22.83 – 228.33 ng/mL** | | | |
| Curve equation[a] | y = 8.639 x + 1.714 | y = 8.585x − 5.356 | y = 8.988x + 5.589 |
| $r^2$ | 0.999 | 0.998 | 0.998 |
| Slope limits (p=95%) | 8.264; 9.015 | 7.976; 9.195 | 8.482; 9.493 |
| Intercept limits (p=95%) | -41.84; 45.27 | -82.59; 71.87 | -53.10; 64.28 |
| CV[c] of response factors (%) | 4.03 | 4.14 | 4.39 |
| Back-calculated RE[b] (%) | < 7.3 | < 7.4 | <7.2 |

[a] y: peak area, x: concentration of OTA (ng/mL for plasma or ng/g for tissues).

[b] Relative error.

[c] Coefficient of variation.

**Table S2.** Raw data from the precision and accuracy study. The precision within-day was studied by analyzing some calibrators (2.35, 22.83, 228.33 ng/mL) in triplicate each day. The precision between-day was assured analyzing calibrators of these levels in three different days.

| $C_{nominal}$ (ng/mL) | Day 1 $C_{measured}$ (ng/mL) | CV[a] | A[b] (%) | Day 2 $C_{measured}$ (ng/mL) | CV[a] | A[b] (%) | Day 3 $C_{measured}$ (ng/mL) | CV[a] | A[b] (%) |
|---|---|---|---|---|---|---|---|---|---|
| **Range 2.35 – 22.83 ng/mL [c,d]** | | | | | | | | | |
| 2.35 | 2.18 | 4.91 | 7.4 | 2.28 | 3.48 | 2.8 | 2.21 | | 5.8 |
| | 2.40 | | 2.1 | 2.35 | | 0.1 | 2.29 | 4.08 | 2.7 |
| | 2.30 | | 2.0 | 2.19 | | 6.6 | 2.40 | | 2.2 |
| 22.83 | 23.15 | 2.17 | 1.4 | 20.62 | 10.7 | 9.7 | 22.00 | | 3.6 |
| | 22.83 | | 0.0 | 20.44 | | 10.5 | 22.06 | 2.09 | 3.4 |
| | 22.19 | | 2.8 | 20.13 | | 11.8 | 21.25 | | 6.9 |
| **Range 22.83 – 228.33 ng/mL [e,f]** | | | | | | | | | |
| 22.83 | 23.88 | 2.22 | 4.6 | 21.22 | 1.25 | 7.1 | 22.67 | 2.13 | 0.7 |
| | 23.54 | | 3.1 | 21.03 | | 7.9 | 22.73 | | 0.4 |
| | 22.86 | | 0.1 | 20.70 | | 9.3 | 21.87 | | 4.2 |
| 228.33 | 211.50 | 1.83 | 7.4 | 224.42 | 2.40 | 1.7 | 235.50 | 0.49 | 3.1 |
| | 208.68 | | 8.6 | 233.23 | | 2.1 | 234.84 | | 2.9 |
| | 216.34 | | 5.3 | 234.65 | | 2.8 | 237.11 | | 3.8 |

[a] Coefficient of variation (%).

[b] Accuracy (RE%).

[c] Equivalent range in plasma: 2.35 – 22.83 ng/mL (no dilution factor)

[d] Equivalent range in kidney, liver, brain and intestine tissue: 9.4 – 91.32 ng/g (dilution factor: 4).

[e] Equivalent range in plasma: 342.45 – 3424.95 ng/mL (dilution factor: 15)

[f] Equivalent range in kidney, liver, brain and intestine tissue: 91.32 – 913.32 ng/g (dilution factor: 4).

**Table S3.** Raw data from the recovery study. The repeatability of the process was studied by carrying out the complete recovery experiment for each matrix on 1 day (within-day) and on 3 different days (between-day).

| $C_{nominal}$ (ng/mL) | | 2.35 | | | 22.83 | | | 228.33 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 |
| Brain | Measure 1 | 68.05 | 65.98 | 57.79 | 73.60 | 90.35 | 75.40 | 87.50 | 94.40 | 81.92 |
| | Measure 2 | 68.01 | 70.26 | 55.81 | 83.41 | 85.04 | 87.00 | 79.47 | 100.82 | 86.83 |
| | Measure 3 | 61.98 | 71.60 | 69.16 | 81.68 | 93.72 | 77.34 | 88.85 | 107.49 | 88.55 |
| | **Within-day recovery (n=3, first three dataset)** | | | | | | | | | |
| | Mean | 66.01 | | | 79.56 | | | 85.27 | | |
| | CV[a] (%) | 5.3 | | | 6.6 | | | 5.9 | | |
| | **Between-day recovery (n=9)** | | | | | | | | | |
| | Mean | 65.40 | | | 83.06 | | | 90.65 | | |
| | CV[a] (%) | 8.6 | | | 8.2 | | | 9.8 | | |
| Liver | Measure 1 | 77.76 | 79.65 | 61.10 | 83.68 | 89.95 | 71.04 | 74.31 | 71.82 | 71.04 |
| | Measure 2 | 71.96 | 63.27 | 72.33 | 84.00 | 83.56 | 70.83 | 81.14 | 75.87 | 81.53 |
| | Measure 3 | 73.76 | 74.91 | 68.33 | 81.97 | 83.14 | 74.19 | 81.06 | 80.75 | 74.96 |
| | **Within-day recovery (n=3, first three dataset)** | | | | | | | | | |
| | Mean | 74.49 | | | 83.22 | | | 78.84 | | |
| | CV[a] (%) | 4.0 | | | 1.3 | | | 5.0 | | |
| | **Between-day recovery (n=9)** | | | | | | | | | |
| | Mean | 71.45 | | | 80.26 | | | 76.94 | | |
| | CV[a] (%) | 8.7 | | | 8.3 | | | 5.5 | | |
| Kidney | Measure 1 | 91.47 | 87.65 | 85.63 | 88.82 | 94.58 | 97.04 | 77.20 | 82.05 | 81.99 |
| | Measure 2 | 86.37 | 91.66 | 69.85 | 92.41 | 93.46 | 88.66 | 90.21 | 87.67 | 84.31 |
| | Measure 3 | 91.15 | 76.93 | 91.24 | 91.39 | 86.99 | 95.88 | 90.21 | 87.27 | 82.42 |
| | **Within-day recovery (n=3, first three dataset)** | | | | | | | | | |
| | Mean | 89.66 | | | 90.87 | | | 85.87 | | |
| | CV[a] (%) | 3.2 | | | 2.0 | | | 8.7 | | |
| | **Between-day recovery (n=9)** | | | | | | | | | |
| | Mean | 85.77 | | | 92.14 | | | 84.81 | | |
| | CV[a] (%) | 8.9 | | | 3.8 | | | 5.1 | | |
| Intestine | Measure 1 | 75.05 | 61.84 | 73.80 | 81.33 | 77.07 | 71.09 | 93.43 | 85.87 | 97.99 |
| | Measure 2 | 71.00 | 70.90 | 73.76 | 84.28 | 85.63 | 77.61 | 78.90 | 95.31 | 84.51 |
| | Measure 3 | 65.89 | 77.76 | 68.65 | 77.01 | 86.54 | 78.77 | 96.60 | 77.82 | 96.47 |
| | **Within-day recovery (n=3, first three dataset)** | | | | | | | | | |
| | Mean | 70.64 | | | 80.87 | | | 89.64 | | |
| | CV[a] (%) | 6.50 | | | 4.52 | | | 10.53 | | |
| | **Between-day recovery (n=9)** | | | | | | | | | |
| | Mean | 70.96 | | | 79.93 | | | 89.66 | | |
| | CV[a] (%) | 6.9 | | | 6.2 | | | 8.9 | | |
| Plasma | Measure 1 | 72.42 | 58.34 | 74.40 | 79.22 | 78.80 | 80.80 | 74.38 | 75.46 | 74.16 |
| | Measure 2 | 73.02 | 64.88 | 72.98 | 80.41 | 80.39 | 79.76 | 72.38 | 74.56 | 74.78 |
| | Measure 3 | 67.91 | 68.28 | 72.98 | 83.22 | 79.95 | 83.14 | 73.82 | 75.49 | 74.27 |
| | **Within-day recovery (n=3, first three dataset)** | | | | | | | | | |
| | Mean | 71.12 | | | 80.95 | | | 73.53 | | |
| | CV[a] (%) | 3.9 | | | 2.5 | | | 1.4 | | |
| | **Between-day recovery (n=9)** | | | | | | | | | |
| | Mean | 69.46 | | | 80.63 | | | 74.37 | | |
| | CV[a] (%) | 7.5 | | | 1.9 | | | 1.3 | | |

[a] Coefficient of variation.

**Table S4.** Results of the robustness study. Raw data of the linearity study of column B in the following ranges: 2.35 – 22.83 ng/mL and 22.83 – 228.33 ng/mL.

| | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| **Range 2.35 – 22.83 ng/mL** | | | |
| Curve equation[a] | y = 9.652x – 0.379 | y = 9.348x + 0.434 | y = 9.674x + 1.567 |
| $r^2$ | 0.991 | 0.993 | 0.997 |
| Slope limits (p=95%) | 8.490; 10.815 | 8.365; 10.330 | 8.963; 10.386 |
| Intercept limits (p=95%) | -14.01; 13.25 | -11.09; 11.95 | -6.78; 9.91 |
| CV[c] of response factors (%) | 6.13 | 6.55 | 4.20 |
| Back-calculated RE[b] (%) | < 10.7 | < 11.9 | < 6.5 |
| **Range 22.83 – 228.33 ng/mL** | | | |
| Curve equation[a] | y = 9.103 x + 1.949 | y = 8.979x – 17.909 | y = 9.240x – 7.715 |
| $r^2$ | 0.999 | 0.998 | 0.998 |
| Slope limits (p=95%) | 8.264; 9.015 | 8.438; 9.520 | 8.776; 9.705 |
| Intercept limits (p=95%) | -29.58; 33.47 | -80.68; 44.87 | -61.59; 46.16 |
| CV[c] of response factors (%) | 2.65 | 4.73 | 3.12 |
| Back-calculated RE[b] (%) | < 3.5 | < 11.3 | <8.1 |

[a] y: peak area, x: concentration of OTA (ng/mL for plasma or ng/g for tissues).

[b] Relative error.

[c] Coefficient of variation.

**Table S5.** Global calibration curves of column B obtained as a result of the linearity study in the following ranges: 2.35 – 22.83 ng/mL and 22.83 – 228.33 ng/mL. Eighteen data points were used for each calibration range.

| | Range 2.35 – 22.83 ng/mL | Range 22.83 – 228.33 ng/mL |
|---|---|---|
| Curve equation[a] | y = 9.54 x + 0.417 | y = 9.08x – 2.33 |
| $r^2$ | 0.995 | 0.999 |
| Slope limits (p=95%) | 8.72; 10.37 | 8.72; 9.44 |
| Intercept limits (p=95%) | -9.22; 10.05 | -44.25; 39.59 |
| CV[b] of response factors (%) | 4.59 | 4.82 |
| Back-calculated ER (%) | < 7.55 | < 5.0 |

[a] y: peak area, x: concentration of OTA (ng/mL for plasma or ng/g for tissues).

[b] Coefficient of variation.