**Intact plasma quantification of the large therapeutic lipopeptide bulevirtide**

Max Sauter[1,2], Antje Blank[1,2], Felicitas Stoll[1,2], Natalie Lutz[1,2], Walter E. Haefeli[1,2], Jürgen Burhenne[1,2,*]

[1] Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

[2] German Center for Infection Research (DZIF), Heidelberg Partner Site, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

## Supplementary Information

Table S1: Optimized MS/MS parameters for the detection of bulevirtide using heated ESI and SRM in the positive ion mode.

| **Parameter** | **High concentration assay** | **Low concentration assay** |
|---|---|---|
| **MS system** | **Xevo TQ-S** | **Xevo TQ-XS** |
| Capillary voltage [kV] | 0.8 | 1.8 |
| Cone voltage [V] | 12 | 20 |
| Source temperature [°C] | 150 | |
| Desolvation temperature [°C] | 600 | |
| Cone gas (N2) flow [L/h] | 150 | |
| Desolvation gas (N2) flow [L/h] | 1000 | |
| BLT mass transition SRM [m/z] | 1080.8 → 1155.2 | |
| IS mass transition SRM [m/z] | 1086.6 → 1162.4 | |
| Collision gas (Ar) flow [mL/min] | 0.15 | |
| Collision energy [V] | 17 | 22 |

ESI: Electrospray ionization; IS: internal standard; SRM: selected reaction monitoring.

Table S2: Recovery data of the plasma validation.

| QC level | Recovery [%] | | | SIL-IS normalized recovery [%] | | |
|---|---|---|---|---|---|---|
| | Plasma lot # | | | | | |
| | Pool | Hem | Lip | Pool | Hem | Lip |
| QC A | 51.3 | 24.3 | 57.8 | 102 | 126 | 101 |
| QC B | 53.0 | 13.4 | 14.8 | 107 | 98 | 119 |
| QC C | 51.5 | 19.3 | 53.2 | 95 | 95 | 95 |
| QC D | 53.8 | 25.8 | 58.1 | 95 | 100 | 98 |
| QC E | 52.2 | 12.5 | 15.8 | 111 | 135 | 108 |
| QC F | 52.6 | 12.9 | 14.1 | 115 | 168 | 108 |
| Low IS | 53.6 | | | 95 | 100 | 98 |
| High IS | 49.3 | | | | | |

Hem: hemolytic plasma; IS: internal standard; Lip: lipemic plasma; Pool: pooled plasma.

N = 3 replicates at each QC concentration.

Table S3: Matrix effect data.

| QC level | Matrix effect [%] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plasma lot # | | | | | | | | |
| | #12 | #13 | #14 | #16 | #17 | #18 | Pool | Hem | Lip |
| QC A | 44.1 | 54.2 | 49.5 | 44.0 | 55.4 | 57.1 | 55.4 | 56.5 | 18.6 |
| QC B | 75.6 | 77.0 | 72.8 | 72.3 | 80.3 | 79.9 | 82.3 | 80.3 | 50.5 |
| QC C | 36.7 | 45.0 | 38.8 | 43.2 | 48.9 | 49.6 | 44.2 | 48.4 | 17.1 |
| QC D | 36.3 | 43.1 | 37.4 | 41.6 | 46.2 | 45.6 | 43.6 | 41.6 | 15.9 |
| QC E | 96.3 | 102.2 | 97.2 | 92.0 | 98.2 | 96.0 | 91.3 | 96.4 | 56.3 |
| QC F | 90.0 | 94.5 | 93.5 | 88.2 | 94.0 | 90.1 | 86.8 | 90.9 | 55.8 |

Hem: hemolytic plasma; Lip: lipemic plasma; Pool: pooled plasma.

N = 3 replicates at each QC concentration.

Table S4: IS-normalized matrix effect data.

| QC level | IS normalized matrix effect [%] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plasma lot # | | | | | | | | |
| | #12 | #13 | #14 | #16 | #17 | #18 | Pool | Hem | Lip |
| QC A | 107.3 | 107.3 | 108.5 | 98.8 | 107.3 | 97.6 | 108.5 | 106.1 | 107.3 |
| QC B | 109.5 | 107.4 | 105.4 | 106.8 | 106.8 | 106.1 | 108.8 | 110.8 | 113.5 |
| QC C | 94.6 | 97.1 | 92.0 | 97.6 | 99.3 | 97.3 | 97.4 | 103.9 | 103.7 |
| QC D | 96.8 | 95.7 | 93.2 | 94.5 | 96.2 | 95.8 | 98.6 | 100.2 | 100.7 |
| QC E | 93.6 | 94.5 | 94.6 | 95.7 | 96.0 | 100.7 | 101.0 | 98.6 | 101.7 |
| QC F | 92.3 | 94.5 | 95.3 | 96.8 | 98.0 | 99.5 | 101.6 | 99.3 | 100.8 |

Hem: hemolytic plasma; IS: internal standard; Lip: lipemic plasma; Pool: pooled plasma.

N = 3 replicates at each QC concentration.

Table S5: Calibration curves of validation batches calculated with linear regression and $1/x^2$ weighting.

| Validation batch | $R^2$ | Equations |
|---|---|---|
| #1 high | 0.9985 | y = 0.01884 x + 0.00079 |
| #2 high | 0.9985 | y = 0.01945 x + 0.00094 |
| #3 high | 0.9944 | y = 0.01856 x + 0.00067 |
| #1 low | 0.9962 | y = 0.0915 x + 0.0012 |
| #2 low | 0.9946 | y = 0.1013 x + 0.0005 |
| #3 low | 0.9894 | y = 0.0961 x + 0.0018 |

Table S6: Accuracy and precision data for the determination of minimally diluted plasma QC with spiked calibration samples.

| QC level | Accuracy [%] | Precision [% CV] |
|---|---|---|
| QC B | 107.8 | 3.5 |
| QC E | 104.4 | 4.1 |
| QC F | 108.2 | 1.5 |

N = 3 replicates at each QC concentration.

Table S7: Cross-validation of low and high concentration assays.

| Volunteer # | Sampling time [h] | Original analysis [ng/mL] | Reanalysis [ng/mL] | Deviation from mean [%] |
|---|---|---|---|---|
| 1 | 0.25 | 6.30 | 7.58 | 18.5 |
| 1 | 0.5 | 9.12 | 11.02 | 18.9 |
| 1 | 1 | 14.7 | 16.89 | 13.8 |
| 1 | 2 | 18.9 | 19.87 | 5.0 |
| 1 | 4 | 11.4 | 13.63 | 17.8 |
| 1 | 6 | 8.20 | 10.0 | 20.1 |
| 1 | SS-0 | 46.2 | 52.5 | 12.8 |
| 2 | 0.25 | 11.0 | 13.2 | 17.9 |
| 2 | 0.5 | 13.8 | 16.6 | 18.2 |
| 2 | 1 | 15.1 | 15.8 | 4.3 |
| 2 | 2 | 17.4 | 19.7 | 12.2 |
| 2 | 4 | 13.6 | 13.0 | 4.8 |
| 2 | 6 | 9.54 | 9.9 | 4.0 |
| 2 | SS-0 | 1.56 | 1.73 | 10.1 |
| 3 | 0.25 | 20.7 | 18.9 | 9.0 |
| 3 | 0.5 | 25.7 | 27.8 | 7.9 |
| 3 | 1 | 24.4 | 23.1 | 5.3 |
| 3 | 2 | 20.0 | 30.6 | 42.0 |
| 3 | 4 | 19.1 | 18.7 | 2.1 |
| 3 | 6 | 17.4 | 17.1 | 1.7 |
| 3 | SS-0 | 0.856 | 0.722 | 17.0 |
| 4 | 0.25 | 11.0 | 14.4 | 26.5 |
| 4 | 0.5 | 14.0 | 17.7 | 23.6 |
| 4 | 1 | 12.9 | 16.8 | 26.4 |
| 4 | 2 | 9.24 | 10.3 | 11.3 |
| 4 | 4 | 5.06 | 5.94 | 16.1 |
| 4 | 6 | 5.53 | 5.88 | 6.2 |
| 4 | SS-0 | 6.92 | 5.71 | 19.2 |
| 5 | 0.25 | 20.6 | 22.7 | 9.7 |
| 5 | 0.5 | 23.4 | 28.6 | 20.1 |

| | | | | |
|---|---|---|---|---|
| 5 | 1 | 23.8 | 29.9 | 22.8 |
| 5 | 2 | 23.5 | 26.2 | 10.7 |
| 5 | 4 | 18.8 | 21.2 | 12.1 |
| 5 | 6 | 15.2 | 17.5 | 14.1 |
| 5 | SS-0 | 17.9 | 22.8 | 24.1 |
| 6 | 0.25 | 9.49 | 10.1 | 6.3 |
| 6 | 0.5 | 12.7 | 14.2 | 11.0 |
| 6 | 1 | 18.5 | 18.6 | 0.4 |
| 6 | 2 | 22.7 | 24.6 | 7.9 |
| 6 | 4 | 14.4 | 15.4 | 6.6 |
| 6 | 6 | 9.10 | 7.60 | 18.0 |
| 6 | SS-0 | 7.34 | 7.23 | 1.5 |

SS: single dose at steady-state.

Table S8: Incurred sample reanalysis of the high concentration assay.

| Volunteer # | Sampling time [h] | Original analysis [ng/mL] | Reanalysis [ng/mL] | Deviation from mean [%] |
|---|---|---|---|---|
| 1 | 0.25 | 6.30 | 7.39 | 15.9 |
| 1 | 0.5 | 9.12 | 9.68 | 6.0 |
| 1 | 1 | 14.7 | 16.1 | 8.9 |
| 1 | 2 | 18.9 | 20.5 | 8.2 |
| 1 | 4 | 11.4 | 12.9 | 12.7 |
| 1 | 6 | 8.20 | 9.37 | 13.3 |
| 1 | SS-0 | 46.2 | 49.9 | 7.8 |
| 1 | SS-0.25 | 55.4 | 60.4 | 8.7 |
| 1 | SS-0.5 | 82.4 | 87.0 | 5.4 |
| 1 | SS-1 | 150 | 148 | 1.1 |
| 1 | SS-2 | 217 | 242 | 10.8 |
| 1 | SS-4 | 316 | 329 | 4.0 |
| 1 | SS-6 | 294 | 312 | 5.9 |
| 2 | 0.25 | 11.0 | 11.6 | 4.6 |
| 2 | 0.5 | 13.8 | 14.8 | 7.2 |
| 2 | 1 | 15.1 | 13.9 | 8.3 |
| 2 | 2 | 17.4 | 18.1 | 3.9 |
| 2 | 4 | 13.6 | 13.2 | 3.2 |
| 2 | 6 | 9.54 | 10.1 | 5.5 |
| 2 | SS-0 | 1.56 | 1.82 | 15.5 |
| 2 | SS-0.25 | 12.4 | 12.6 | 1.8 |
| 2 | SS-0.5 | 25.9 | 26.9 | 3.7 |
| 2 | SS-1 | 27.3 | 29.6 | 8.2 |
| 2 | SS-2 | 55.0 | 56.5 | 2.6 |
| 2 | SS-4 | 66.1 | 71.5 | 7.9 |
| 2 | SS-6 | 43.6 | 43.4 | 0.3 |

SS: single dose at steady-state.

Table S9: Incurred sample reanalysis of the low concentration assay.

| Volunteer # | Sampling time [h] | Original analysis [ng/mL] | Reanalysis [ng/mL] | Deviation from mean [%] |
|---|---|---|---|---|
| 1 | 0.25 | 7.58 | 7.71 | 1.7 |
| 1 | 0.5 | 11.0 | 11.1 | 0.7 |
| 1 | 1 | 16.9 | 16.5 | 2.3 |
| 1 | 2 | 19.9 | 24.7 | 21.7 |
| 1 | 4 | 13.6 | 14.0 | 2.7 |
| 1 | 6 | 10.0 | 10.9 | 8.2 |
| 1 | SS-0 | 52.5 | 56.9 | 8.0 |
| 2 | 0.25 | 13.2 | 14.0 | 6.2 |
| 2 | 0.5 | 16.6 | 19.7 | 17.3 |
| 2 | 1 | 15.8 | 15.9 | 0.9 |
| 2 | 2 | 19.7 | 22.1 | 11.7 |
| 2 | 4 | 13.0 | 16.5 | 23.7 |
| 2 | 6 | 9.93 | 12.0 | 18.9 |
| 2 | SS-0 | 1.73 | 2.07 | 18.1 |
| 4 | 0.25 | 14.4 | 13.8 | 4.0 |
| 4 | 0.5 | 17.7 | 19.5 | 9.4 |
| 4 | 1 | 16.8 | 18.0 | 6.7 |
| 4 | 2 | 10.3 | 13.6 | 27.2 |
| 4 | 4 | 5.94 | 6.66 | 11.4 |
| 4 | 6 | 5.88 | 7.00 | 17.4 |
| 6 | 0.25 | 10.1 | 11.9 | 16.3 |
| 6 | 0.5 | 14.2 | 16.2 | 13.3 |
| 6 | 1 | 18.6 | 24.8 | 28.7 |
| 6 | 2 | 24.6 | 24.2 | 1.5 |
| 6 | 4 | 15.4 | 16.5 | 7.0 |
| 6 | 6 | 7.60 | 12.4 | 48.0 |
| 6 | SS-0 | 7.23 | 8.87 | 20.3 |

SS: single dose at steady-state.

Table S10: Accuracy and precision data for the freeze-and-thaw stability determination.

| QC level | Accuracy [%] | Precision [% CV] |
|---|---|---|
| QC B | 99.6 | 1.5 |
| QC E | 107.7 | 3.0 |
| QC F | 86.5 | 0.4 |

N = 3 replicates at each QC concentration.

Table S11: Accuracy and precision data for the 14 days (-20 °C) plasma stability determination.

| QC level | Accuracy [%] | Precision [% CV] |
|---|---|---|
| QC B | 108.7 | 0.6 |
| QC E | 107.2 | 0.8 |
| QC F | 108.8 | 1.0 |

N = 3 replicates at each QC concentration.

Table S12: Accuracy and precision data for the 24 h autosampler stability determination.

| QC level | Accuracy [%] | Precision [% CV] |
|---|---|---|
| QC A | 88.8 | 3.1 |
| QC B | 98.3 | 5.7 |
| QC C | 105.9 | 3.5 |
| QC D | 111.8 | 4.3 |
| QC E | 95.9 | 1.7 |
| QC F | 93.1 | 2.2 |

N = 3 replicates at each QC concentration.

Table S13: Accuracy and precision data for the 7-week stock solution stability determination.

| QC level | Accuracy [%] | Precision [% CV] |
|---|---|---|
| QC B | 101.6 | 5.8 |
| QC C | 107.5 | 1.5 |
| QC D | 110.2 | 1.7 |

N = 3 replicates at each QC concentration.