

## supplementary materials

### Comparison of LC–MS<sup>3</sup> and LC–MRM method for quantifying Voriconazole and its application in therapeutic drug monitoring of human plasma

#### 2.4 Method Validation

Specificity is evaluated by analyzing plasma samples from six healthy volunteers. Selectivity is also investigated by spiking blank sample with IS and blank sample with **LOQ** to check the signals in the channel of the **voriconazole**. The linearity is examined using calibration standards in plasma samples at concentration ranging from 0.25 to 20 µg/mL on three non-consecutive days. An acceptable determination coefficient ( $R^2=0.9996$ ) should be attained. The **LOQ** is defined as the lowest concentration of the calibration curves. The reproducibility at **LOQ** level is evaluated by consecutively injecting six processed samples at **LOQ** level and comparing its precision and accuracy against the established linearity. A maximum of  $\leq 20\%$  variation is accepted.

Extraction recovery and matrix effect of analyte and IS are determined by analyzing six samples at three different levels (0.5, 2.5 and 10.0 µg/mL). In this validation, three separate sets sample are prepared. Set A was made by spiking QC samples and IS in to blank plasma samples. Set B is prepared by adding **voriconazole** and IS at the same concentrations after the plasma extraction process (post-extraction addition). Set C is acquired by adding QC working solutions and IS into water. Recovery (%) =  $A/B * 100$ , and Matrix effect (%) =  $B/C * 100$ .

Precision (relative standard deviation, RSD) and accuracy (relative error, RE) are assayed by analyzing six sets of replicates of **LOQ** and QC samples. The intra-assay

precision and accuracy are evaluated by analyzing these samples on the same day, while inter-assay precision and accuracy are evaluated over three different days. The precision should not exceed 15% (20% for **LOQ**) of RSD and accuracy should be within 85–115% (80–120% for **LOQ**) of the nominal concentration.

The stability is evaluated for **voriconazole** in human plasma by analyzing QC samples with 0.5, 2.5 and 10 µg/mL at different storage conditions. Long term stability is evaluated for plasma samples after storage at –80 °C for 2 weeks. Short term stability was studied on analysis of QC samples after three freeze-thaw, and at room temperature (RT) for 8 h. Processed samples stored at 8 °C for 6 h are reanalyzed for auto sampler stability. Under all conditions, a maximum loss of 15% of **voriconazole** concentration was acceptable stability.

Table S1. Concentrations of **voriconazole** in 42 human plasma samples **are** analyzed by HPLC-MS<sup>2</sup> and HPLC-MS<sup>3</sup> methods

| Sample ID | HPLC-MS <sup>2</sup> (µg/mL) | HPLC-MS <sup>3</sup> (µg/mL) | (HPLC-MS <sup>2</sup> /HPLC-MS <sup>3</sup> )% |
|-----------|------------------------------|------------------------------|--|
| sample1   | 3.68                         | 3.41                         | 92.66  |
| sample2   | 1.05                         | 1.20                         | 114.29   |
| sample3   | 2.02                         | 2.08                         | 102.97   |
| sample4   | 0.72                         | 0.65                         | 90.72  |
| sample5   | 2.86                         | 2.37                         | 82.87  |
| sample6   | 1.97                         | 2.01                         | 102.03   |
| sample7   | 2.55                         | 2.16                         | 84.71  |
| sample8   | 3.58                         | 2.92                         | 81.56  |
| sample9   | 0.90                         | 0.89                         | 98.23  |
| sample10  | 1.51                         | 1.67                         | 110.60   |
| sample11  | 1.05                         | 0.91                         | 86.57  |
| sample12  | 3.13                         | 2.64                         | 84.35  |
| sample13  | 1.71                         | 1.76                         | 102.92   |
| sample14  | 2.32                         | 2.68                         | 115.52   |
| sample15  | 4.52                         | 3.75                         | 82.96  |
| sample16  | 1.1                          | 1.09                         | 99.09  |

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|----------|------|------|--------|
| sample17 | 1.77 | 1.74 | 98.31  |
| sample18 | 0.84 | 0.77 | 109.09 |
| sample19 | 2.82 | 3.16 | 112.06 |
| sample20 | 1.84 | 1.78 | 96.74  |
| sample21 | 2.32 | 2.39 | 103.02 |
| sample22 | 3.55 | 3.98 | 112.11 |
| sample23 | 1.03 | 1.05 | 101.94 |
| sample24 | 1.97 | 2.02 | 102.54 |
| sample25 | 1.16 | 0.92 | 83.64  |
| sample26 | 2.85 | 2.86 | 102.14 |
| sample27 | 2.09 | 1.97 | 94.26  |
| sample28 | 2.15 | 2.55 | 118.60 |
| sample29 | 3.32 | 3.58 | 107.83 |
| sample30 | 0.93 | 0.90 | 97.84  |
| sample31 | 2.25 | 2.51 | 111.56 |
| sample32 | 0.91 | 1.10 | 116.54 |
| Sample33 | 3.53 | 3.13 | 88.67  |
| Sample34 | 2.55 | 2.71 | 106.27 |
| Sample35 | 2.39 | 2.32 | 97.07  |
| sample36 | 5.63 | 5.52 | 98.05  |
| sample37 | 1.28 | 1.10 | 85.94  |
| sample38 | 2.40 | 2.77 | 115.42 |
| sample39 | 0.98 | 1.14 | 116.33 |
| Sample40 | 2.58 | 2.82 | 109.30 |
| Sample41 | 2.07 | 1.84 | 88.89  |
| Sample42 | 2.46 | 2.32 | 94.31  |

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