**Supporting Information**

**Rapid determination of immunosuppressive drug concentrations in whole blood by Coated Blade Spray-Tandem Mass Spectrometry (CBS-MS/MS)**

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| --- | --- | --- | --- | --- | --- |
| **Compound** | **Polarity** | **Precursor (m/z)** | **Product (m/z)** | **Collision Energy (V)** | **RF Lens (V)** |
| Tacrolimus | Positive | 821.488 | 768.35 | 19.86 | 85 |
| Tacrolimus-d2C1 | Positive | 824.522 | 771.481 | 20.416 | 85 |
| Sirolimus | Positive | 931.54 | 864.425 | 16.522 | 85 |
| Sirolimus-d3 | Positive | 934.606 | 864.497 | 16.067 | 88 |
| Everolimus | Positive | 975.578 | 908.454 | 16.421 | 87 |
| Everolimus-d4 | Positive | 979.609 | 912.528 | 15.511 | 88 |
| Cyclosporine A | Positive | 1219.8 | 1202.729 | 16.32 | 96 |
| Cyclosporine A-d4 | Positive | 1223.87 | 1206.854 | 11.4 | 97 |

**Table S1** Mass spectrometry parameters used to monitor each ISD.

**Table S2** Calibration points for levels of tacrolimus, everolimus, and sirolimus.

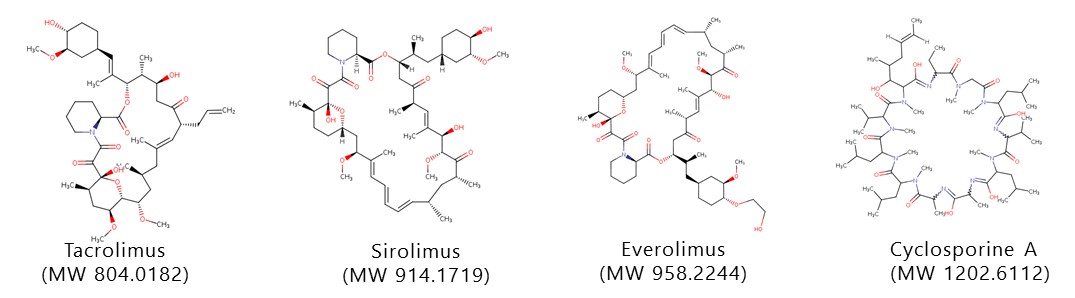
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Calibration points** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| ng/mL | 1 | 2.5 | 5 | 10 | 15 | 25 | 50 |

**Table S3** Calibration points for levels of Cyclosporine A.

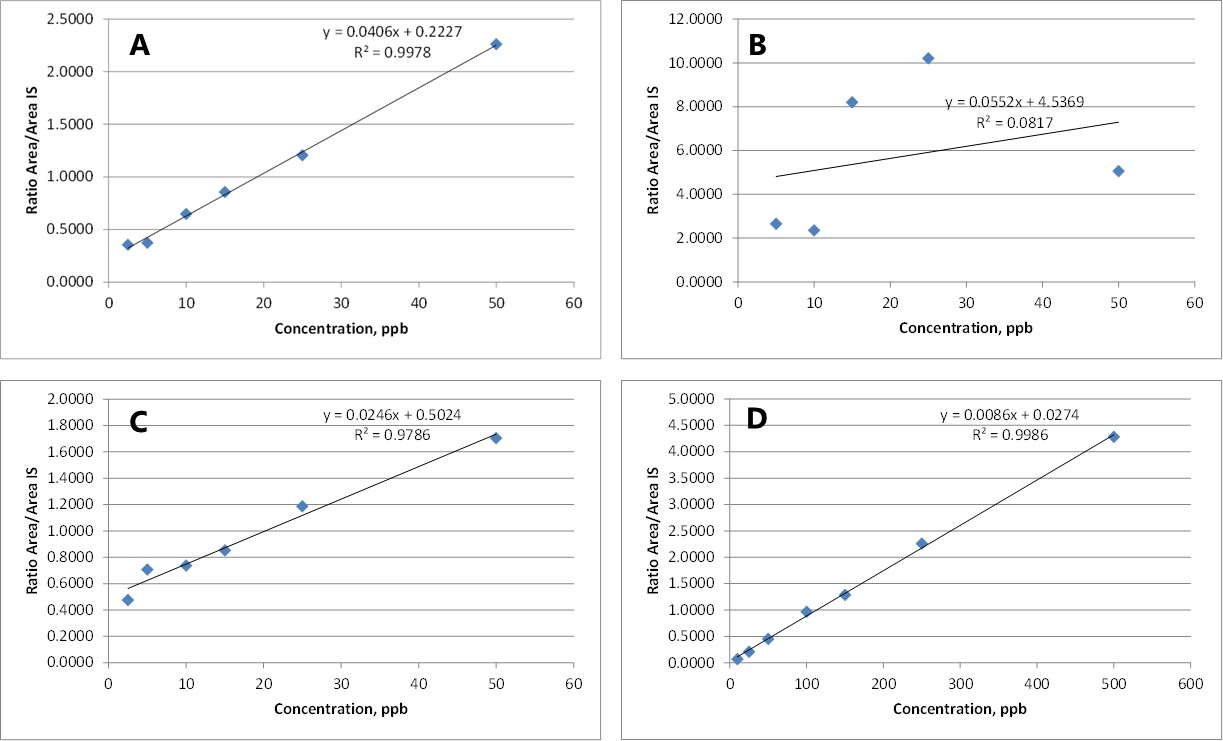
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Calibration points** | **1** | **2** | **3** | **4** | **5** | **6** | **7\*** |
| ng/mL | 10 | 25 | 50 | 100 | 150 | 250 | 500 |

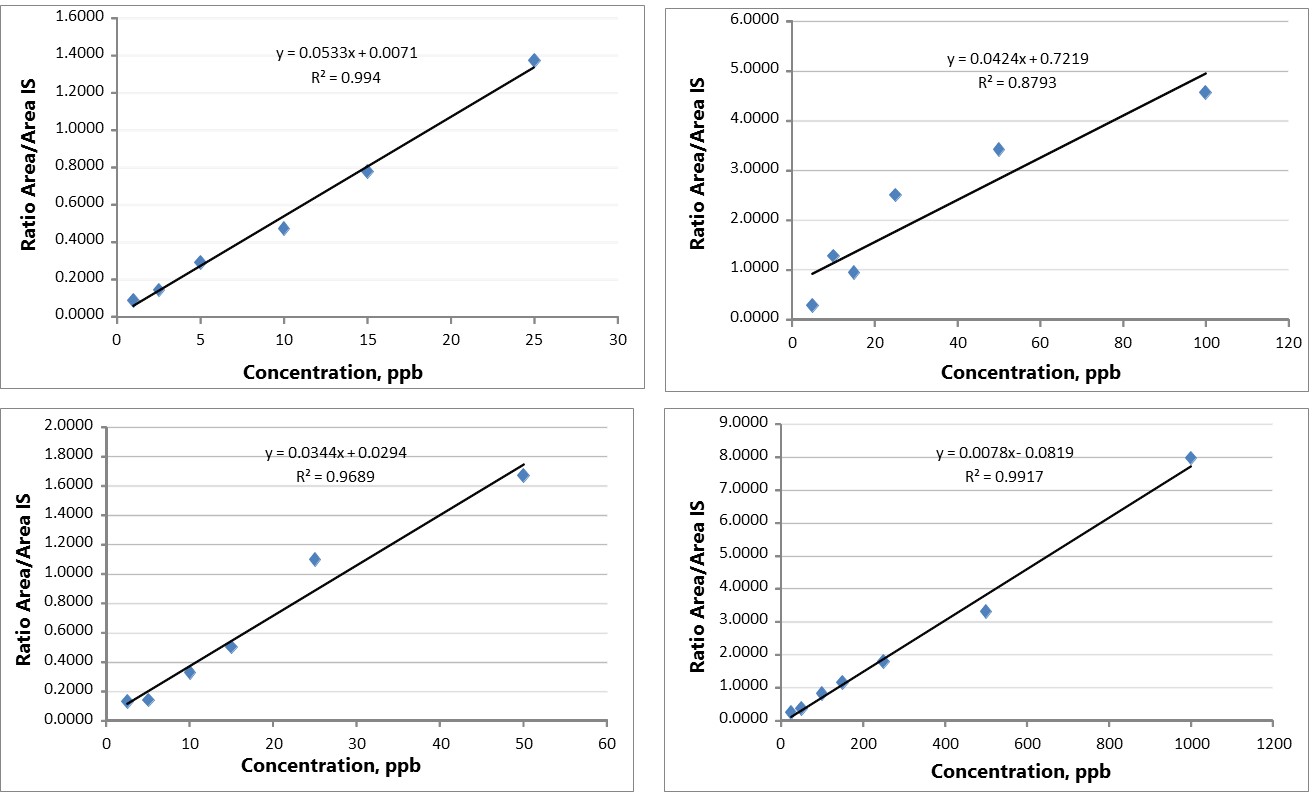
**Table S4** Liquid check quality control (QC) standards acquired from Bio-Rad.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Calibration points [ng·mL-1]** | **Level 1** | **Level 2** | **Level 3** | **Level 4** |
| Cyclosporine | 55.6 | 179 | 324 | 699 |
| Everolimus | N/A | 3.14 | 6.78 | 17.6 |
| Sirolimus | 3.62 | 7.74 | 13.1 | N/A |
| Tacrolimus | 3.94 | 9.05 | 17.0 | 26.7 |

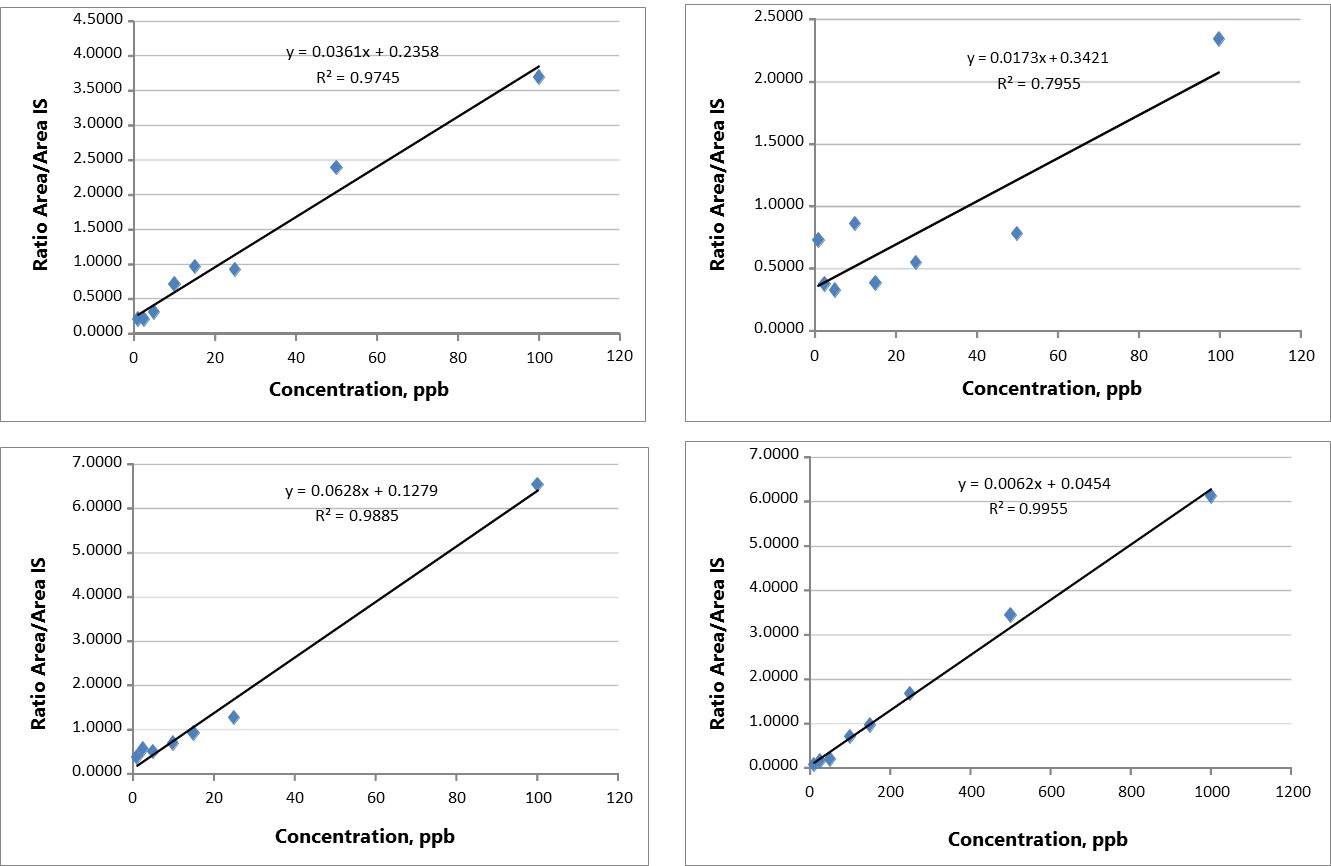


**Figure S1** Chemical structure of the target analytes.

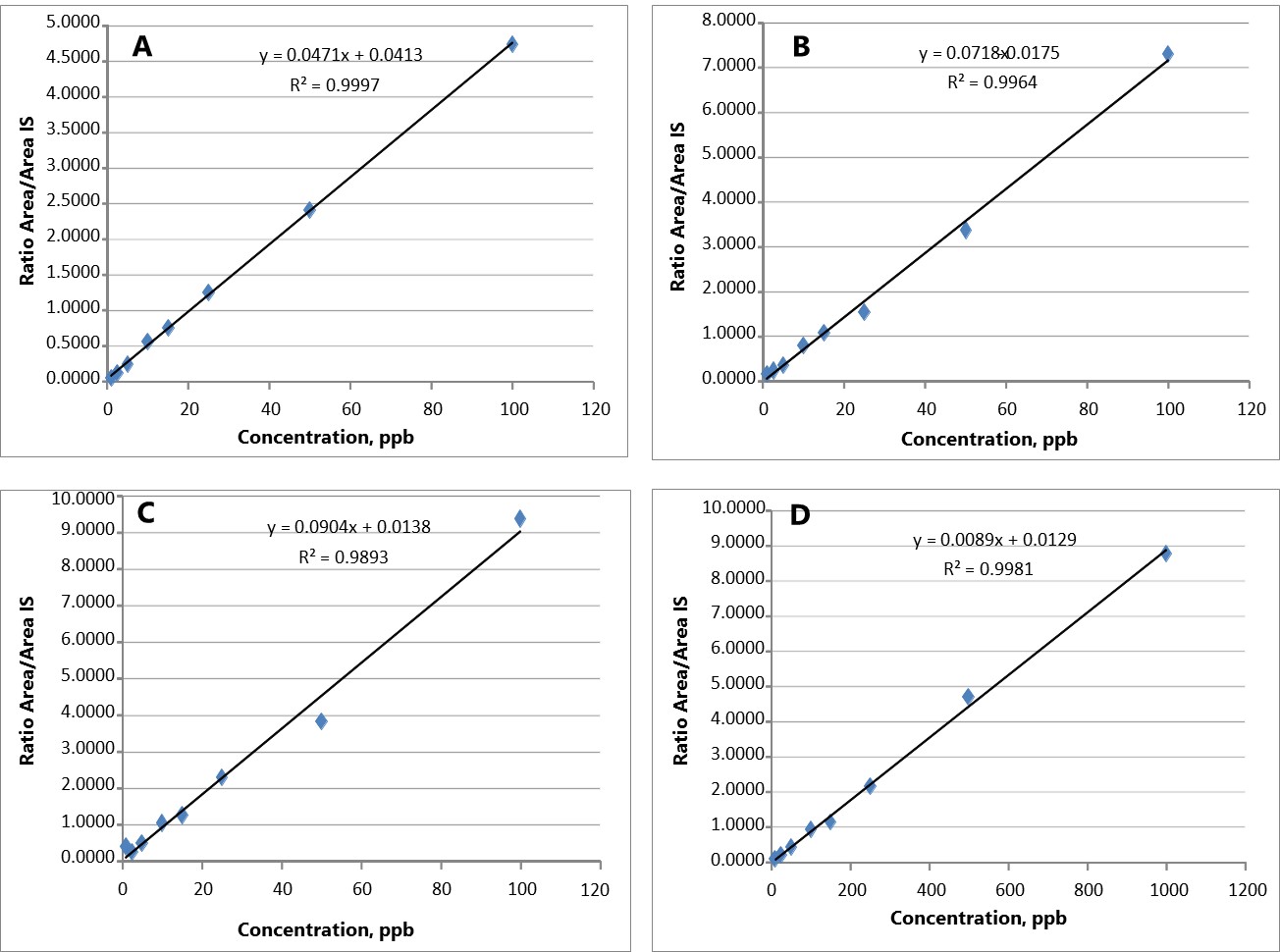
**Figure S2** Quantitative determination of (A) TAC, (B) SIR, (C) EVR, and (D) CycA. Extractions were performed from 100 µL of whole human blood pre-mixed with 100 µL of a 0.1M ZnO4-solution. 20 min of extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each. 



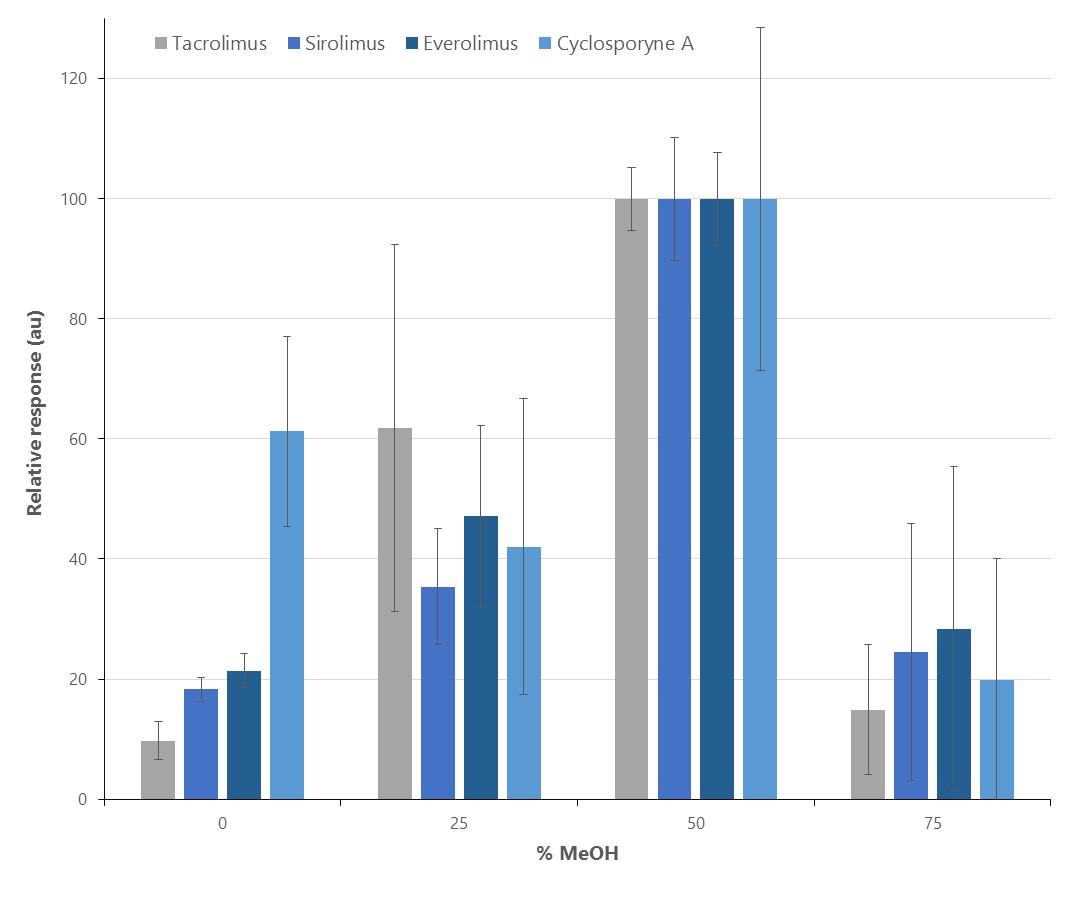
**Figure S3** Quantitative determination of (A) TAC, (B) SIR, (C) EVR, and (D) CycA. Extractions were performed from 200 µL of whole human blood pre-mixed with 500 µL of a 0.1M ZnO4-solution and 500 µL of LC-MS water. 30 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.



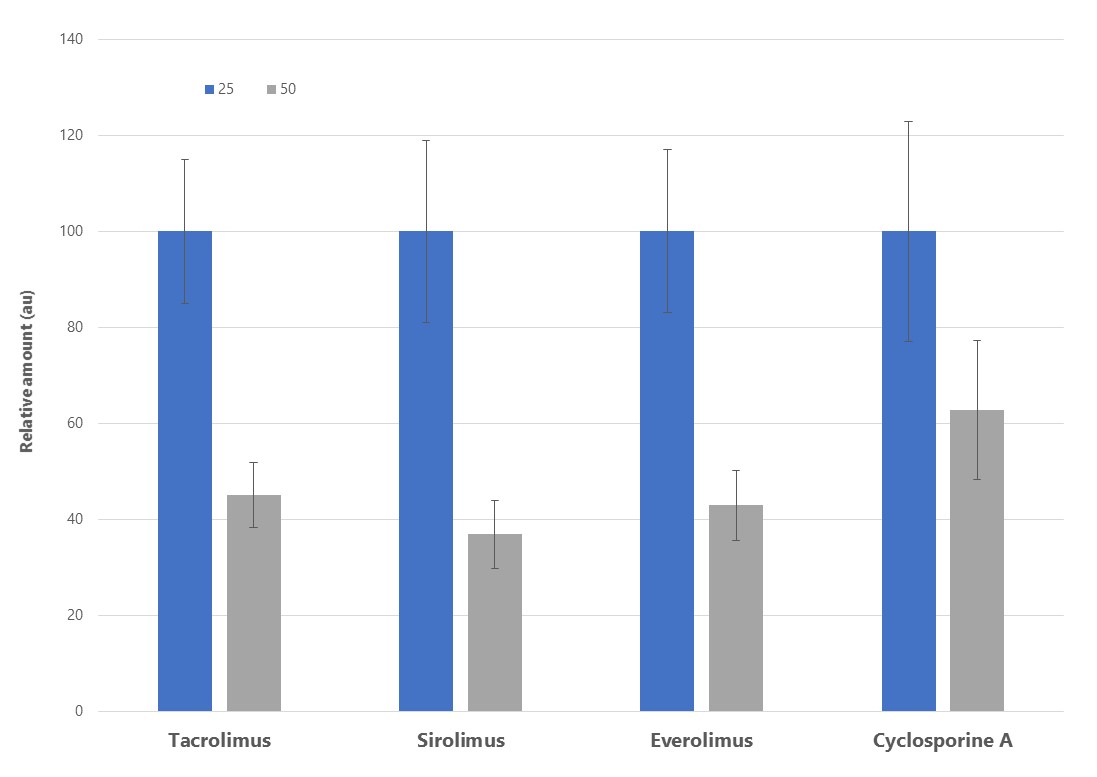
**Figure S4** Quantitative determination of (**A**) TAC, (**B**) SIR, (**C**) EVR, and (**D**) CycA. Extractions were performed from 200 µL of whole human blood frozen (-80 °C, 1h) and thawed. 30 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.

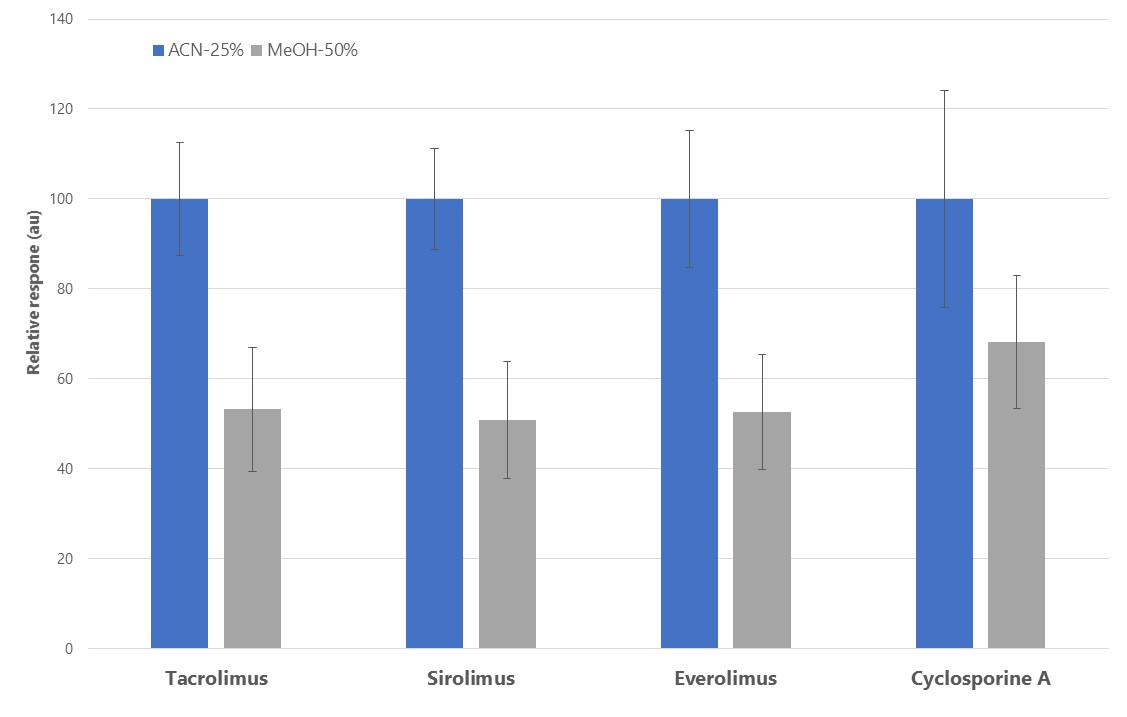


**Figure S5** Quantitative determination of (**A**) TAC, (**B**) SIR, (**C**) EVR, and (**D**) CycA. Extractions were performed from 300 µL of human plasma. 30 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.

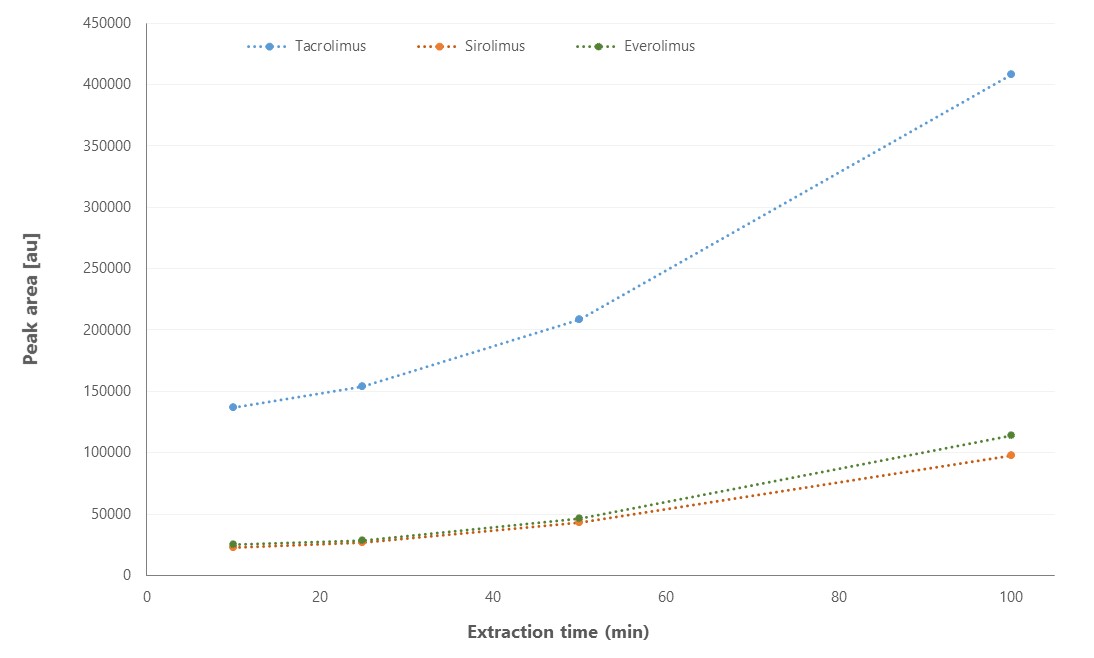


**Figure S6** Optimization of organic content (MeOH, %) required to achieve the highest instrumental response. Extractions were performed from 100 µL of whole human blood spiked at 50 ng mL-1 with TAC/SIR/EVR and 500 ng mL-1 for CycA, and pre-mixed with a 0.1M ZnO4-solution and MeOH according to their respective ratios. 30 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.

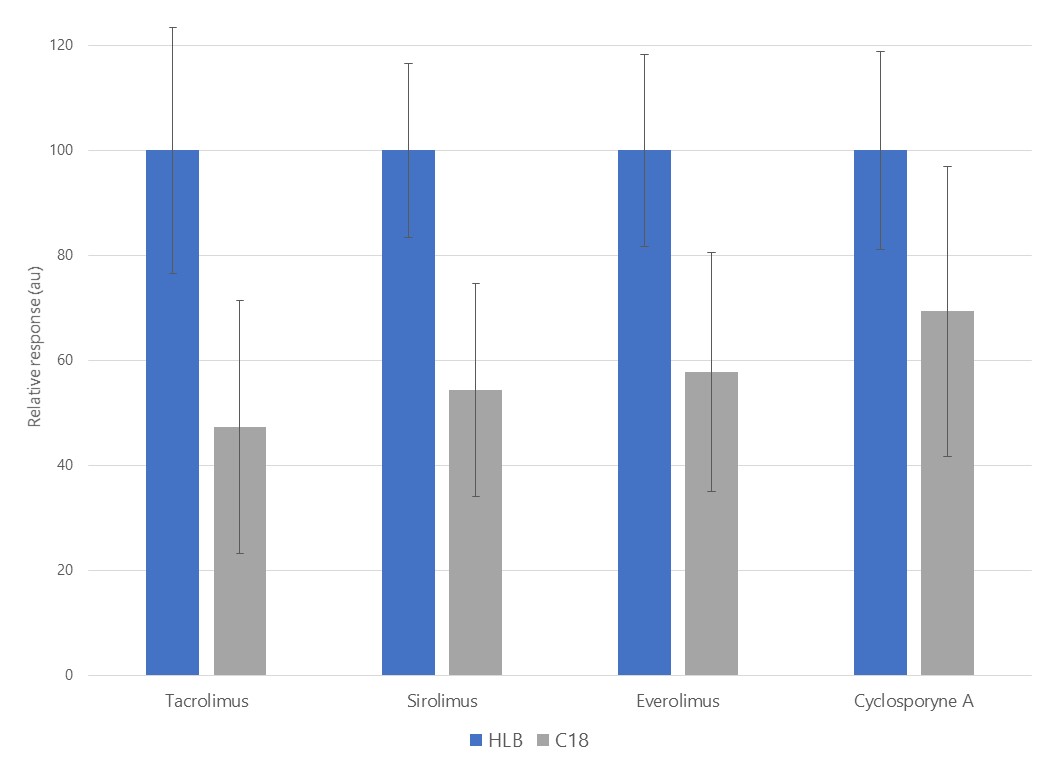
**Figure S7** Optimization of organic content (ACN, %) required to achieve the highest instrumental response. Extractions were performed from 100 µL of whole human blood spiked at 50 ng mL-1 with TAC/SIR/EVR and 500 ng mL-1 for CycA, and pre-mixed with a 0.1M ZnO4-solution and CAN according to their respective ratios. 30 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.



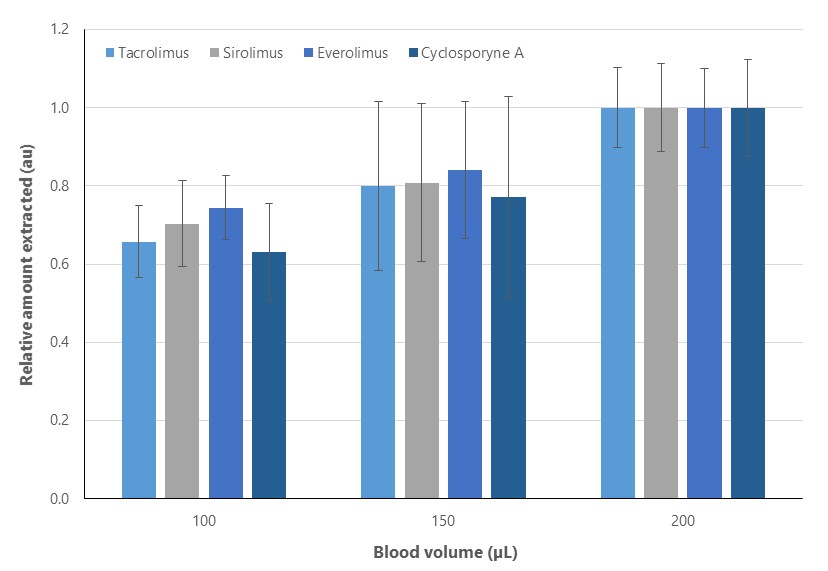
**Figure S8** Comparison between two different organic solvent denaturing mixtures: 25% ACN versus 50% MeOH.



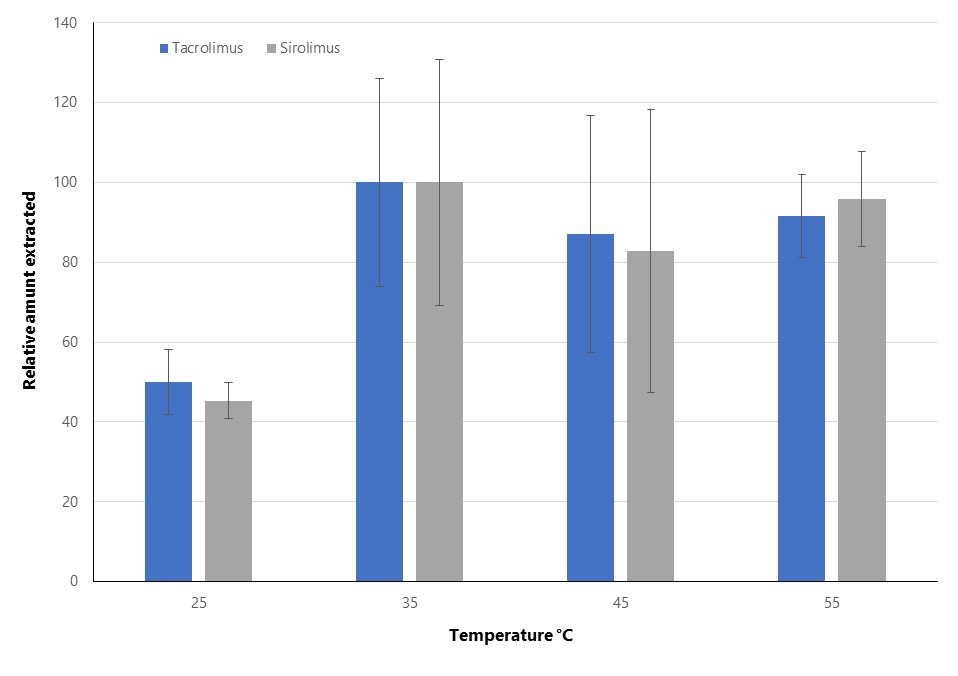
**Figure S9** Extraction time profile for three of the ISDs (10, 25, 50, 100 min). Extractions were performed from whole human blood spiked at 50 ng mL-1 with TAC/SIR/EVR, and pre-mixed with a 0.1M ZnO4-solution and ACN (25%) according to their respective ratios.



**Figure S10** Comparison of two different coating chemistries for the extraction of ISDs from a modified blood-matrix. Extractions were performed from whole human blood spiked at 50 ng mL-1 with TAC/SIR/EVR and 500 ng mL-1 for CycA, and pre-mixed with a 0.1M ZnSO4-solution and ACN (25%) according to their respective ratios. 90 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.



**Figure S11** Comparison of three different blood sample volumes for the determination of ISDs from a modified blood-matrix. Extractions were performed from whole human blood spiked at 50 ng mL-1 with TAC/SIR/EVR and 500 ng mL-1 for CycA, and pre-mixed with a 0.1M ZnSO4-solution and ACN (25%) according to their respective ratios. 90 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.

**Figure S12** Comparison of four different extraction temperatures for the determination of ISDs from a modified blood-matrix. Extractions were performed from whole human blood spiked at 50 ng mL-1 with TAC/SIR/EVR and 500 ng mL-1 for CycA, and pre-mixed with a 0.1M ZnSO4-solution and ACN (25%) according to their respective ratios. 90 min extraction at 2000 rpm was followed by three rinsing steps in fresh water of 5s each.