Immunosuppressants in human blood

Illustration of analytical performance for immunosuppressants in human blood.

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze immunosuppressants in human blood matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and methods

The Citrine MS/MS system was controlled, and data processed using Analyst MD software, version 1.6.3. Blood calibrators, controls and samples were processed using the following conditions:

Sample preparation: Sample preparation was performed using Diagnotix's immunosuppressants' reagent set [https://www.diagnotix.com/en/products/immunosuppressiva] according to the manufacturer's specifications. A 50 µL blood sample spiked using the set of calibrators was used for the procedure.

Liquid chromatography conditions: Chromatographic separation was achieved using a Phenomenex Kinetex C18 column. Mobile phases A and B from the reagent set were used. The total run time was 3 minutes at a flow rate of 500 µL/min. The injection volume was 25 µL.

Mass spectrometry conditions: Mass spectrometry analysis was performed using the Citrine Triple Quad MS/MS system, operating in positive electrospray mode. Compound-dependent parameters were optimized by infusion.

Results

Analytical performance statistics including the concentration range evaluated, accuracy and precision (n=4 replicates), as well as signal-to-noise ratio (S/N) and linearity (r^2) are shown in Table 1. Chromatograms of the compounds evaluated utilizing the described method are shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Table 1. Performance statistics for the analysis of immunosuppressants in human blood. Measured range (ng/mL), % accuracy, %CV, S/N ratio and linearity for the immunosuppressants. Values for the lowest calibrator and over the measured ranges were used, as appropriate.

Compound	Range (pg/mL)	% Accuracy	%CV	S/N*	Linearity (r^2)
Sirolimus	1.60-52.7	107.33	2.1	76.9:1*	0.99881
Cyclosporin A	20-1297	96.95	10.2	70.6:1*	0.99797
Tacrolimus	1.43-49.5	95.37	5.8	96.8:1*	0.99595
Everolimus	1.40-46.9	102.50	5.7	205.8:1*	0.99834

*S/N ratio calculated using a peak-to-peak algorithm for lowest matrix calibrator measured.

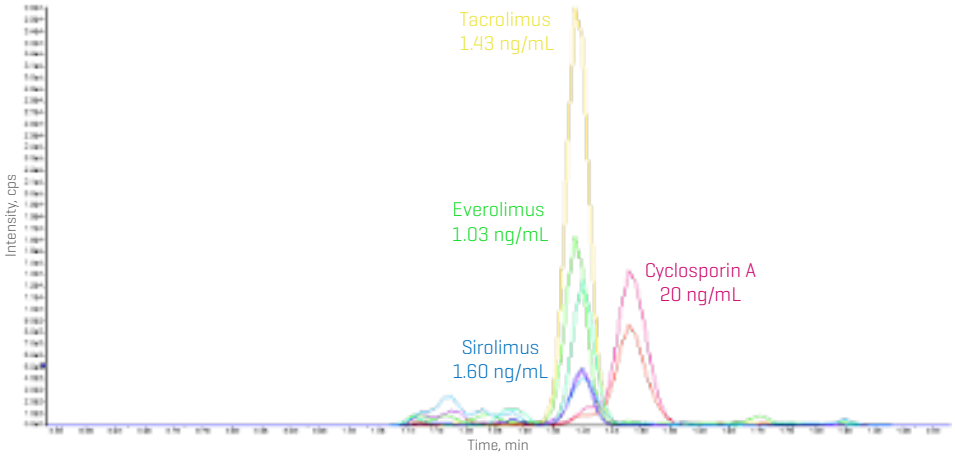

Figure 1. Chromatogram of sirolimus (blue), cyclosporin A (purple), tacrolimus (yellow) and everolimus (green) extracted from blood matrix. Chromatogram of calibration standards in matrix for sirolimus at 1.60 ng/mL, cyclosporin A at 20 ng/mL, tacrolimus at 1.43 ng/mL and everolimus at 1.40 ng/mL shows a S/N of 76.9:1 for sirolimus, 70.6:1 for cyclosporin A, 96.8:1 for tacrolimus and 205.8:1 for everolimus based on a peak-to-peak algorithm.

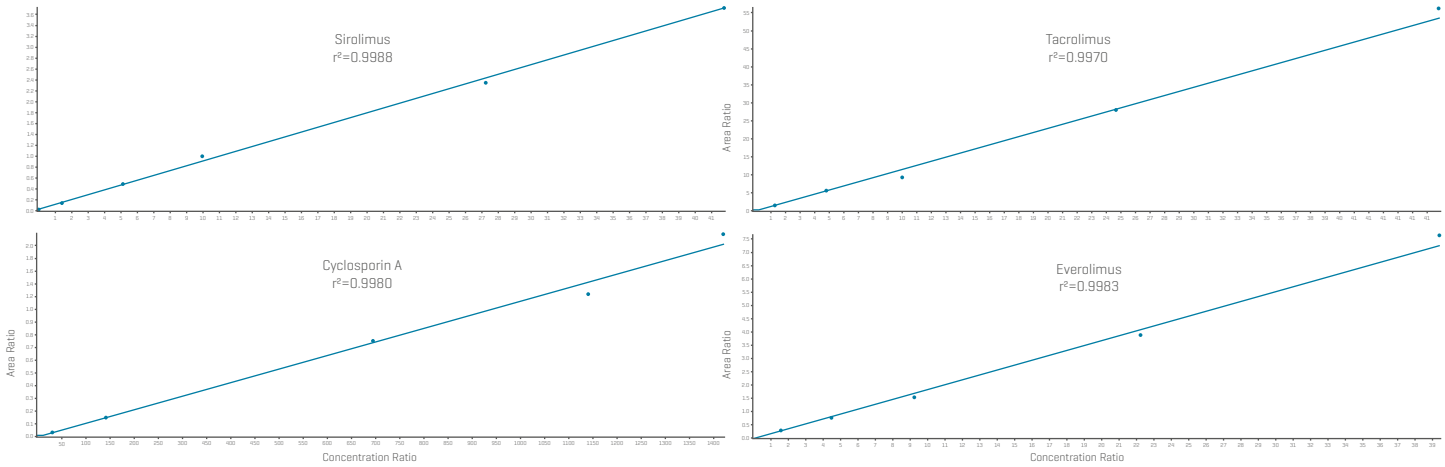

Figure 2. Linear calibration curves for sirolimus (top left), cyclosporin A (bottom left), tacrolimus (top right) and everolimus (bottom right) extracted from blood matrix. The calibration curves were run across the following concentration ranges (1.60-52.7 ng/mL for sirolimus, 20-1297 ng/mL for cyclosporin A, 1.43-49.5 ng/mL for tacrolimus and 1.40-46.9 ng/mL for everolimus). The curves were generated using linear regression and 1/x weighting for all four compounds, resulting in r^2 values of 0.9988 for sirolimus, 0.9980 for cyclosporin A, 0.9970 for tacrolimus and 0.9983 for everolimus, respectively.

Conclusions

Based on the above performance testing, the following results were obtained:

Sensitivity: Analytical sensitivity was investigated with a series of calibration standards prepared as described and showed a S/N of 76.9:1 for sirolimus, 70.6:1 for cyclosporin A, 96.8:1 for tacrolimus and 205.8:1 for everolimus, at the lowest matrix calibrator measured (1.60 ng/mL for sirolimus, 20 ng/mL for cyclosporin A, 1.43 ng/mL for tacrolimus and 1.40 ng/mL for everolimus), calculated using a peak-to-peak algorithm.

Assay linearity: Linearity was assessed in matrix over the following concentration ranges: 1.60-52.7 ng/mL for sirolimus, 20-1297 ng/mL for cyclosporin A, 1.43-49.5 ng/mL for tacrolimus and 1.40-46.9 ng/mL for everolimus. The r^2 values were 0.9988, 0.9980, 0.9970 and 0.9983, respectively.

Accuracy: At the lowest matrix calibrators measured (1.60 ng/mL for sirolimus, 20 ng/mL for cyclosporin A, 1.43 ng/mL for tacrolimus and 1.40 ng/mL for everolimus), the % accuracy was 107.33% for sirolimus, 96.95% for cyclosporin A, 95.37% for tacrolimus and 102.50% for everolimus, determined by 4 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

Reproducibility: At the lowest matrix calibrators measured (1.60 ng/mL for sirolimus, 20 ng/mL for cyclosporin A, 1.43 ng/mL for tacrolimus and 1.40 ng/mL for everolimus), the precision (%CV) was 2.1% for sirolimus, 10.2% for cyclosporin A, 5.8% for tacrolimus and 5.7% for everolimus, determined by 4 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

In these experiments, the Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for the quantitation of immunosuppressants in blood matrix.