Simultaneous Analysis of Tacrolimus, Sirolimus, Everolimus, and Cyclosporin A in Whole Blood Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Clinical Research

Authors

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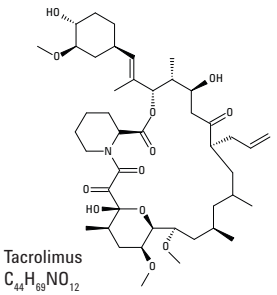
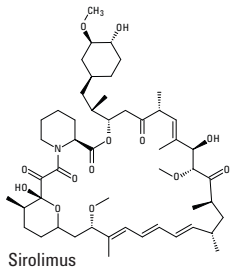
Introduction

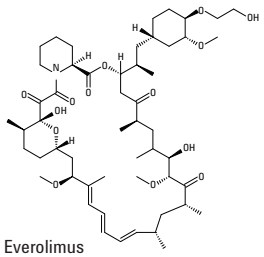
In many clinical research laboratories, liquid chromatography-mass spectrometry (LC/MS) methods for the analysis of Tacrolimus, Sirolimus, Everolimus, and Cyclosporin A have proven superior because of their increased sensitivity and selectivity. We have evaluated the ability of the Agilent RapidFire High-throughput Mass Spectrometry System, an ultrafast SPE/MS/MS system, to analyze a panel of these four drugs in whole blood. This system is capable of analysis times less than 13 seconds per sample. The results of this study demonstrate that the speed of the Agilent RapidFire/MS system complements the sensitivity and selectivity of mass spectrometry (MS), by producing significantly faster sample cycle times than LC/MS while yielding similar analytical results.



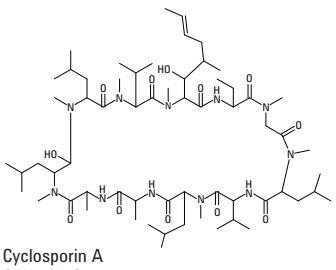
Tacrolimus
$C_{44}H_{68}NO_{12}$



Sirolimus
$C_{51}H_{79}NO_{13}$



Everolimus
$C_{53}H_{83}NO_{14}$



Cyclosporin A
$C_{62}H_{111}N_{11}O_{12}$

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Experimental

The RapidFire/MS/MS system consists of the following modules: an Agilent RapidFire 360, an Agilent 6460 Triple Quadrupole Mass Spectrometer, and MassHunter Qualitative Analysis B.04.00 and RapidFire Integrator Software.

RapidFire triple quadrupole conditions

Samples were analyzed at a rate of < 13 seconds per sample. Quantitative, qualitative, and internal standard ions were monitored simultaneously, in all experiments, for all four immunosuppressant drugs.

Chemicals and reagents

Whole blood was purchased from Pel-Freez Biologicals Rogers, AR. Quality control samples were purchased from UTAK Laboratories, Inc. Valencia, CA. Cyclosporin A-d4, everolimus-d4, and rapamycin-d3 (sirolimus-d3) were purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA. All other solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Sample preparation

Calibration standards for tacrolimus, sirolimus, everolimus (0.8–50 ng/mL), and cyclosporin A (15.6–1000 ng/mL) were prepared by spiking all four drugs into bovine whole blood. Commercially available quality control standards made in whole blood were also prepared according to the manufacturer's instructions. The standards and quality control samples were precipitated using a zinc sulfate and methanol solution containing the internal standards. Precipitated samples were gently mixed, centrifuged, and transferred to a 96-well plate for analysis.

Table 1. RapidFire/MS/MS conditions.

RapidFire conditions						
Buffer A	10 mM ammonium acetate with 0.09 % formic acid, 0.01 % trifluoroacetic acid; 1.5 mL/min flow rate					
Buffer B	50 % methanol; 1.5 mL/min flow rate					
Buffer C	10 mM ammonium acetate in methanol with 0.09 % formic acid, 0.01 % trifluoroacetic acid; 1.25 mL/min flow rate					
Injection volume	10 µL					
SPE Cartridge	Agilent RapidFire cartridge C (reversed-phase C18 chemistry, p/n: G9205A)					
RF State 1	sip sensor					
RF State 2	3000 ms					
RF State 3	2000 ms					
RF State 4	3500 ms					
RF State 5	500 ms					
Triple quadrupole conditions						
Gas temperature	225 °C					
Gas flow	9 L/min					
Nebulizer	40 psi					
Sheath gas temperature	325 °C					
Sheath gas flow	12 L/min					
Nozzle voltage	300 V					
Capillary voltage	4000 V					
Analyte	**Q1**	**Q3**	**Dwell**	**Fragmentor**	**CE**	**CAV**
Tacrolimus quantifier	821.9	768.5	10	145	17	6
Tacrolimus qualifier	821.9	786.5	10	145	13	6
Ascomycin	809.6	756.5	10	125	17	6
Everolimus quantifier	975.6	908.6	10	170	15	5
Everolimus qualifier	975.6	926.8	10	170	10	5
Everolimus-d4	979.6	912.7	10	180	10	5
Sirolimus quantifier	931.6	864.6	10	175	12	5
Sirolimus qualifier	931.6	882.2	10	175	8	5
Sirolimus-d3	934.6	864.5	10	180	18	5
Cyclosporin A quantifier	1219.8	1202.8	10	170	12	3
Cyclosporin A qualifier	1202.9	1184.8	10	200	30	3
Cyclosporin A-d4	1223.9	1206.9	10	170	12	3

Data analysis

RapidFire Integrator software was used for peak integration. The quantifier ion peak area count (AUC) of each analyte was normalized by the AUC of their respective internal standards. The data was subjected to linear regression with 1/x weighting.

Results and Discussion

Prepared calibration standards and commercially available quality controls were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Cyclosporin A had intra- and interday accuracies within 15 % and coefficient of variation values less than 6 % for all concentrations within the linear range (Table 2). This method had excellent linearity within the measured range of 7.8–1000 ng/mL with an R^2 value greater than 0.999 (Figure 1). The limit of quantification (LOQ) defined as the AUC reproducibility for three injections having a coefficient of variation of 20 % or less was determined to be 7.8 ng/mL. Signal-to-noise ratios were calculated by looking at peak to peak height and found to be greater than 100:1 at the LOQ concentration.

Similar results were observed for tacrolimus, everolimus, and sirolimus. All three analytes had intra- and interday accuracies within 15 % and coefficient of variation values less than 10 % for all concentrations within the measured range of 0.8–50 ng/mL (Tables 3–5). This method had excellent linearity for all three analytes with R^2 values greater than 0.995 (Figures 2–4). The LOQ for tacrolimus, everolimus, and sirolimus was 0.78 ng/mL. Signal-to-noise ratios were determined to be greater than 40:1 at the LOQ concentration for all three analytes. No significant carryover was measured for any of the drug analytes.

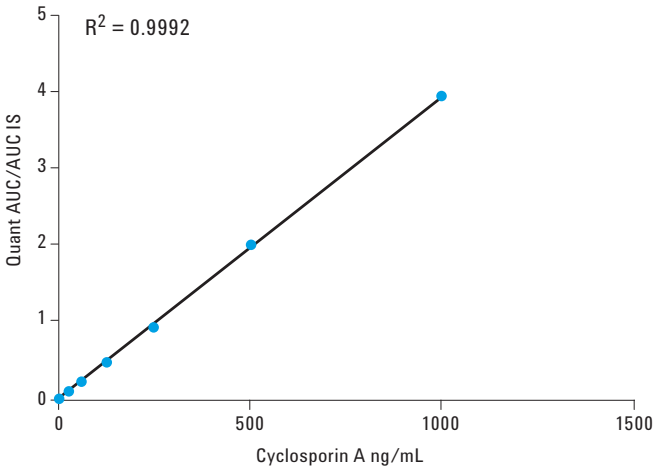

Figure 1. Representative standard curve for cyclosporin A.

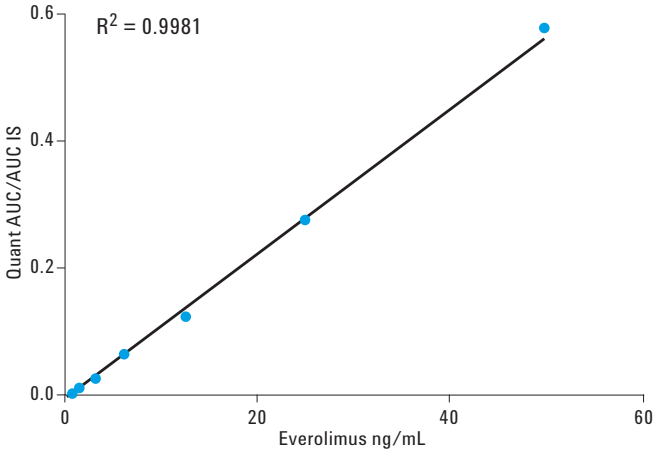

Figure 2. Representative standard curve for everolimus.

Table 2. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of cyclosporin A in whole blood.

Cyclosporin A	Intraday (n=3)		Interday (n=4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
7.8	5.4	113.1	3.8	111.6
15.6	3.6	95.9	2.4	97.5
31.3	0.4	94.2	0.7	93.4
62.5	2.2	95.3	1.2	95.7
125	0.5	98.9	4.5	101.2
250	4.9	99.8	1.3	98.9
500	4.4	104.3	1.7	102.3
1000	2.2	98.5	1.5	99.4
Low QC	1.0	100.7	3.8	98.0
Mid QC	5.0	93.9	3.4	96.2
High QC	2.3	97.5	3.7	98.0

Table 3. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of everolimus in whole blood.

Everolimus	Intraday (n=3)		Interday (n=4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
0.8	6.5	101.4	3.0	104.4
1.6	9.6	97.2	4.9	94.1
3.1	3.7	90.3	1.2	89.6
6.3	6.9	93.0	1.6	92.1
12.5	6.2	87.9	3.0	91.6
25	0.7	99.6	0.9	99.1
50	1.5	104.6	0.5	104.1
Low QC	8.1	103.2	4.2	98.4
Mid QC	4.3	95.7	2.8	93.5
High QC	9.7	94.3	4.5	95.7

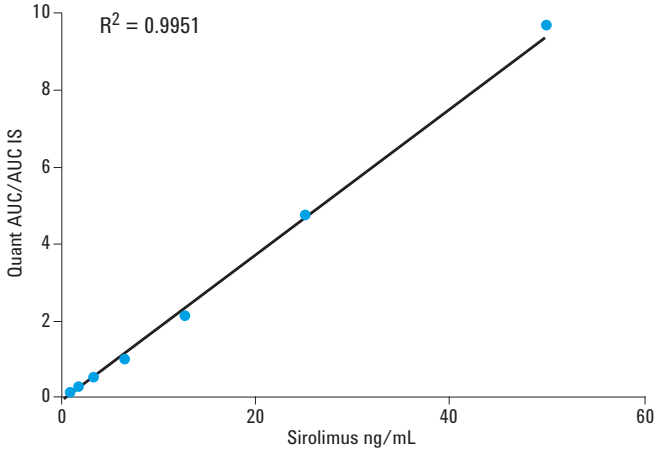

Figure 3. Representative standard curve for sirolimus.

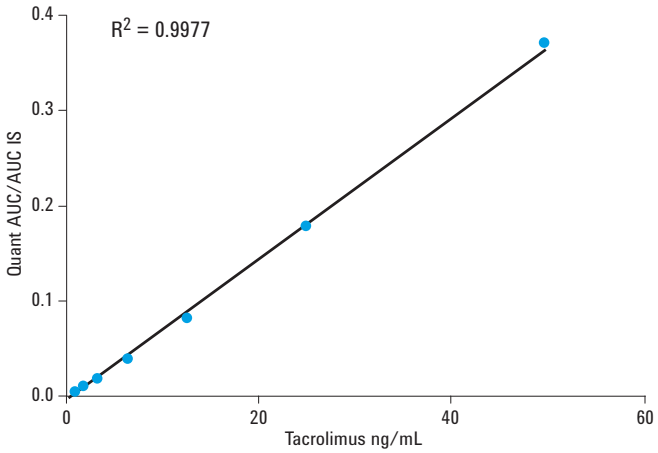

Figure 4. Representative standard curve for tacrolimus.

Table 4. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of sirolimus in whole blood.

Sirolimus	Intraday (n=3)		Interday (n=4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
0.8	2.8	108.9	2.6	106.2
1.6	4.4	94.0	4.9	96.1
3.1	8.3	92.2	6.4	85.3
6.3	6.2	86.9	6.0	91.9
12.5	9.6	90.6	3.3	91.6
25	5.6	96.2	2.2	95.4
50	1.9	106.2	0.6	106.1
Low QC	7.0	91.3	3.4	95.9
Mid QC	7.6	101.9	1.9	101.7
High QC	7.3	100.1	1.8	98.6

Table 5. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of tacrolimus in whole blood.

Tacrolimus	Intraday (n=3)		Interday (n=4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
0.8	7.0	97.4	8.1	106.7
1.6	5.2	97.3	4.6	91.5
3.1	1.9	90.1	3.4	88.9
6.3	1.1	91.9	3.0	88.8
12.5	1.3	91.8	2.6	92.2
25	1.4	98.3	1.9	98.0
50	0.9	104.4	1.0	105.0
Low QC	0.7	99.1	1.4	99.4
Mid QC	4.3	97.2	1.8	96.7
High QC	3.1	97.6	1.9	96.3

Conclusions

Cyclosporin A, everolimus, sirolimus, and tacrolimus were accurately and precisely measured in whole blood using an Agilent RapidFire/MS/MS System. All four drugs were simultaneously analyzed in a 12 MRM panel in less than 13 seconds per sample, providing a high-throughput method for measuring these analytes. This methodology is capable of throughputs greater than 270 samples per hour. Therefore, RapidFire/MS may be useful for the fast and efficient analysis of similar small molecule analytes in whole blood.

Acknowledgements

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Reference

1. Schlicht, K.E., Korman E.W., Miller V.P., Snozek C.L., Crow F. W., Langman L.J. and W A. LaMarr; High-Throughput Analysis of Tacrolimis in Whole Blood Using Ultrafast SPE/MS/MS. Poster #160 presented at the 59th ASMS Conference on Mass Spectrometry and Allied Topics, June 7th, **2011**, Denver, CO.

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