Ultrafast Analysis of Tacrolimus in Whole Blood Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

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Introduction

In many clinical research laboratories, liquid chromatography/mass spectrometry (LC/MS) methods of analysis of immunosuppressant drugs have proven superior because of their increased sensitivity and selectivity. We have evaluated the ability of an ultrafast SPE/MS/MS system, the Agilent RapidFire High-throughput Mass Spectrometry System, to analyze the immunosuppressant drug tacrolimus in whole blood. This system is capable of analysis times of <10 seconds per sample. This study demonstrates that the speed of the Agilent RapidFire/MS system can complement the sensitivity and selectivity of MS by producing significantly faster sample cycle times than LC/MS while yielding similar analytical results.

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Experimental

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

RapidFire triple quadrupole conditions

Samples were analyzed at a rate of <10 seconds per sample, using the conditions shown in Table 1. Quantitative and qualitative ions for tacrolimus and the internal standard (ascomycin) were monitored simultaneously in all experiments (Table 1).

Chemicals and reagents

Whole Blood was purchased from Lampire Biological Products, Ottsville, PA. Quality control samples were purchased from UTAK Laboratories, Inc. Valencia, CA.

Sample preparation

Calibration standards for tacrolimus (2–50 ng/mL) were prepared in bovine whole blood. Commercially available quality control standards made in whole blood were also prepared. The human samples were mixed with water and precipitated using a zinc sulfate and methanol solution containing the internal standard. Precipitated samples were gently mixed, centrifuged, and transferred to a 96-well plate for analysis.

Data analysis

RapidFire Integrator software was used for peak integration. The quantifier ion 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.

Table 1. RapidFire conditions.

RapidFire conditions						
Buffer A	Water with 0.09 % formic acid, 0.01 % trifluoroacetic acid; 1.5 mL/min flow rate					
Buffer B	Acetonitrile with 0.09 % formic acid, 0.01 % trifluoroacetic acid; 1.25 mL/min flow rate					
Injection volume	10 µL					
SPE cartridge	Agilent RapidFire cartridge F (reversed-phase phenyl chemistry, p/n: G9205A)					
RF state 1	sip sensor					
RF state 2	3,000 ms					
RF state 3	3,000 ms					
RF state 4	500 ms					
Triple quadrupole conditions						
Gas temperature	350 °C					
Gas flow	8 L/min					
Nebulizer	45 psi					
Sheath gas temperature	400 °C					
Sheath gas flow	11 L/min					
Nozzle voltage	500 V					
Capillary voltage	3,500 V					
	Q1	Q3	Dwell	Fragmentor	CE	CAV
IS	809.6	756.5	100	125	17	3
Quantifier	821.9	768.5	100	145	17	6
Qualifier	821.9	786.5	100	145	13	6

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. Tacrolimus (both the quantifier and qualifier ions) had intra- and inter-day accuracies within 15 % and coefficient of variation values less than 10 % for all concentrations within the linear range (Table 2). This method had excellent linearity within the measured range of 2–50 ng/mL with an R^2 value greater than 0.995 (Figure 1). Carryover was assessed by analyzing the AUC of a blank injection immediately following the highest standard curve concentration and calculated as a % of the mean peak area of the 2 ng/mL standard. No significant carryover (< 1 %) was seen using this method. Signal-to-noise ratios were calculated looking at peak-to-peak height and found to be greater than 30:1 at 2 ng/mL.

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

Tacrolimus (ng/mL)	Intraday % accuracy (n=3)	Intraday % precision (n=3)	Interday % accuracy (n=4)	Interday % precision (n=4)
2	106.3	3.5	105.2	2.6
10	94.6	1.2	96	3.8
20	96.6	7.4	97	4
40	101.1	3.3	100.3	4.5
50	101.3	1.3	101.6	2.8
UTAK1 (4.9)	99.9	5.3	95.9	4.6
UTAK2 (14.2)	90.1	2.6	93.8	6.1
UTAK3 (28)	96.5	0.5	96.6	5

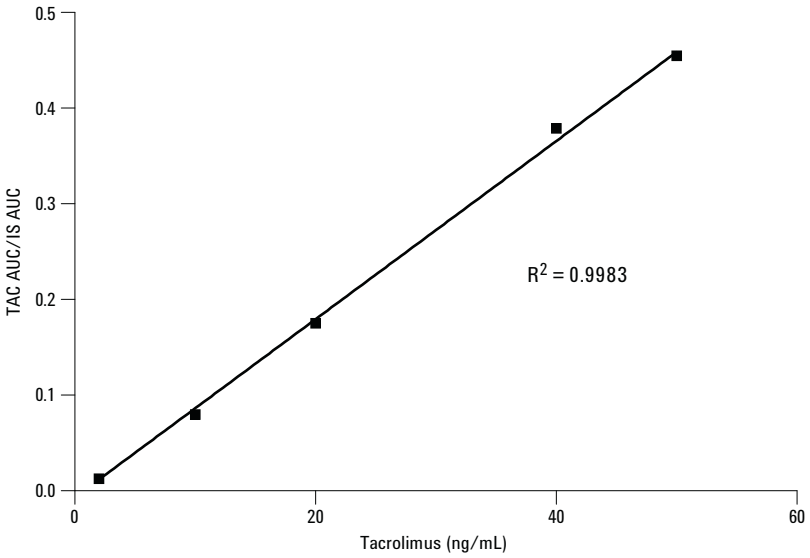

Figure 1. Representative standard curve for Tacrolimus.

To further evaluate this method, 30 blinded human samples were analyzed for tacrolimus. The human samples were determined to have tacrolimus values ranging from < 2 to 36.9 ng/mL. The results obtained using a RapidFire/MS/MS system were compared to the results determined independently on the same samples at the Mayo Clinic using a traditional LC/MS/MS method. A very good correlation between the two methods was found with an R^2 value greater than 0.97 (Figure 2).[1]

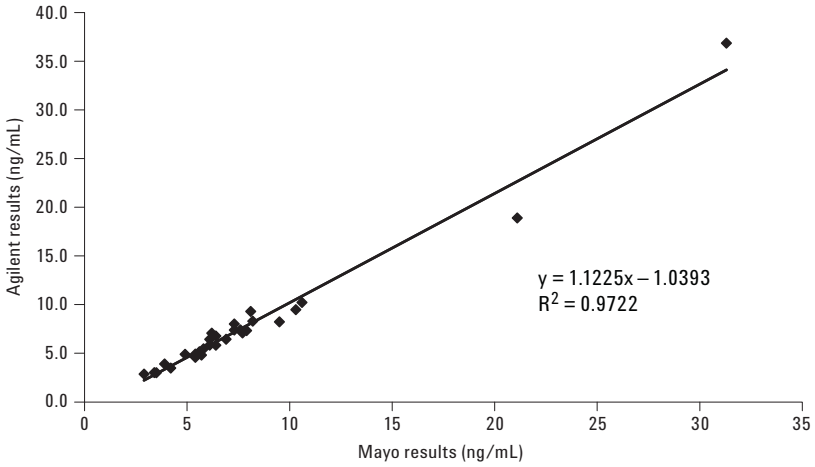

Figure 2. Correlation between RapidFire/MS/MS and LC/MS/MS for blinded human samples.

Conclusions

Tacrolimus was accurately and precisely measured in whole blood using an Agilent RapidFire/MS/MS System. Samples were analyzed at less than 10 seconds per sample, providing a high-throughput method of detection of this analyte. While the analytical results of blinded human samples were comparable to LC/MS/MS, the analysis time was approximately 20 times faster. This methodology is capable of throughputs > 370 samples per hour. RapidFire/MS/MS may be useful for the fast and efficient analysis of similar small molecule analytes in whole blood.

Acknowledgements

The authors gratefully acknowledge Frank Crow, Eric Korman, Christine Snozek, and Loralie Langman at the Mayo Clinic, Rochester, Minnesota for proving technical assistance and the blinded human samples used in this study.

Reference

1. Schlict, K.E. *et al.* High-Throughput Analysis of Tacrolimus 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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Published in the USA, March 23, 2012
5990-9994EN

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