High-Throughput Analysis of Tacrolimus in Whole Blood Using Ultra-fast SPE-MS/MS

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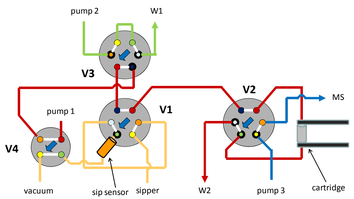
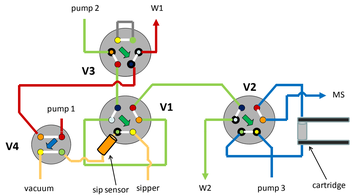
Introduction

In many clinical research laboratories, liquid chromatography-mass spectrometry (LC/MS) methods of analysis of immunosuppressant drugs have proven superior to current analytical measurement technologies because of their increased sensitivity and selectivity. We have evaluated the ability of an ultra-fast SPE/MS/MS system (Agilent RapidFire High-throughput Mass Spectrometry System) to analyze the immunosuppressant drug tacrolimus in whole blood. The results demonstrate the superior speed of SPE/MS/MS to complement the superior sensitivity and selectivity of MS with significantly faster sample cycle times than LC/MS while yielding similar analytical results.

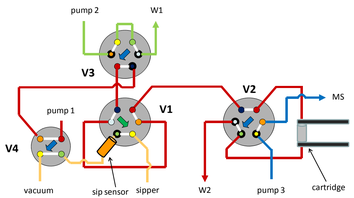
States 1&4: Aspirate & Re-equlibrate



State 3: Elute



State 2: Load/Wash



RapidFire Conditions		
Cycle durations (ms)	State #1 aspirate	600
	State #2 load/wash	3000
	State # 3 elute	3000
	State # 4 re-equilibrate	500
Solvents	Solvent A: water + 0.09% formic acid + 0.01% TFA	
	Solvenet B: acetonitrile + 0.09% formic acid + 0.01% TFA	
Column	Phenyl	

Experimental

MS methods for tacrolimus and its internal standard ascomycin were optimized for analysis by QQQ MS. Calibration standards for tacrolimus (2-50 ng/ml) were prepared in bovine whole blood. The whole blood 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. Samples were analyzed at a rate of 9.5 seconds per sample using an Agilent RapidFire high-throughput mass spectrometry system coupled to an Agilent 6460 mass spectrometer. The SPE method consisted of a Phenyl column and elution with 100% acetonitrile. Data analysis was performed using RapidFire Integrator software. This methodology is capable of throughputs >370 samples per hour.

Agilent 6460 Settings

Source Parameters

Ionization mode	ESI + Agilent Jet Stream
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	11 L/min
Nebulizer pressure	45 psi
Nozzle voltage	500 V
Capillary voltage	3500 V

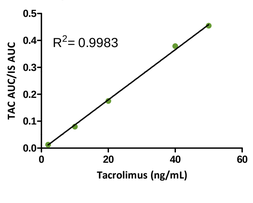
Acquisition Parameters (Positive Mode)

Transition	Precursor Ion	Product Ion	Dwell (ms)	Frag. (V)	CE (V)	CAV
IS	809.61	756.5	100	125	17	3
Quantifier	821.91	768.5	100	145	17	6
Qualifier	821.91	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 had both intra- and inter-day accuracies within 15% and coefficient of variation values less than 10% for all concentrations within the linear range. This method had excellent linearity within the measured range of 2-50 ng/ml with an R^2 value greater than 0.99. Blank whole blood was treated and analyzed in the absence of internal standard in the same manner as the other samples to establish signal-to-noise which was found to be greater than 30 to 1.

Representative Standard Curve

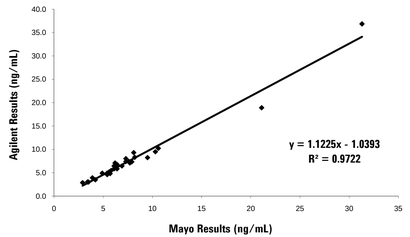


Tacrolimus Conc (ng/mL)	Accuracy* (%) Intraday (n=3)	Precision* (%) Intraday (n=3)	Accuracy* (%) Interday (n=4)	Precision* (%) Interday (n=4)	Quant/Qual AUC
2	106.3	3.5	105.2	2.6	3.4
10	94.6	1.2	96.0	3.8	3.1
20	96.6	7.4	97.0	4.0	3.4
40	101.1	3.3	100.3	4.5	3.5
50	101.3	1.9	101.6	2.8	3.4
UTAK 1 (4.9)	99.1	5.3	95.9	4.6	3.7
UTAK 2 (14.2)	90.1	2.6	93.8	6.1	3.6
UTAK 3 (28)	96.5	0.5	96.6	5.0	3.5

*1/x weighing factor

Results and Discussion

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



Tacrolimus	Mayo ng/mL	Agilent ng/mL	% Difference
P1	3.4	3.0	-11.1%
P2	4.9	4.9	0.1%
P3	6.4	6.8	6.0%
P4	6.2	7.1	14.1%
P5	5.8	5.5	-5.7%
P6	5.4	4.6	-15.0%
P7	9.5	8.2	-13.3%
P8	2.9	2.9	-0.9%
P9	6.1	5.8	-4.2%
P10	7.3	7.4	1.3%
P11	7.3	8.0	9.9%
P12	8.2	8.3	1.3%
P13	10.6	10.2	-3.5%
P14	6.9	6.4	-6.5%
P15	5.4	4.9	-8.8%
P16	< 2.0	< 2.0	0.0%
P17	6.4	5.8	-8.8%
P18	10.3	9.5	-7.9%
P19	8.1	9.3	14.9%
P20	5.7	4.8	-15.4%
P21	5.6	5.2	-7.7%
P22	6.1	6.4	5.6%
P23	4.2	3.5	-16.9%
P24	3.9	3.9	0.4%
P25	31.3	36.9	17.8%
P26	3.5	3.0	-13.9%
P27	7.9	7.3	-7.1%
P28	7.6	7.4	-2.5%
P29	7.7	7.1	-7.8%
P30	21.1	18.9	-10.4%

Conclusions

- Tacrolimus was accurately measured in whole blood using a RapidFire-MS system at a rate of 9.5 seconds per sample
- Intra- and inter-day accuracies were determined to be within 15% and precision values less than 10% for all concentrations within the linear range (2-50 ng/mL).
- Both quantifier and qualifier transitions for tacrolimus had excellent linearity with R^2 values >0.998.
- Blinded human samples analyzed using the RapidFire-MS system correlated well with identical samples ran independently using a traditional LC/MS/MS system
- Analytical results are comparable to LC/MS/MS with an analysis time that is approximately 20 times faster
- This novel methodology is capable of throughputs >370 samples per hour.