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

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Abstract

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 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.

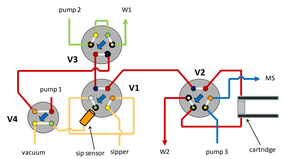
Methods: MS methods for tacrolimus and its internal standard ascomycin were optimized for analysis by QQQ MS. Calibration standards for tacrolimus (2-50 ng/ml) were prepared in bovine whole blood. The whole blood samples were mixed with water and precipitated using a zinc sulfate and methanol solution 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.

Results: 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 windows which were found to be greater than 30 to 1. These analytical results are comparable to those using LC-MS/MS, however the analysis time for SPE/MS/MS was approximately 10 times faster. Blinded human samples will be evaluated to further investigate this method.

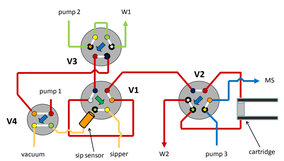
Conclusions: Based on these results, Tacrolimus can be accurately and precisely measured in whole blood using ultra-fast SPE/MS/MS at rates of 9.5 seconds per sample. While the analytical results were comparable to LC-MS/MS, the analysis time was approximately 10 times faster. This novel methodology is capable of throughputs >370 samples per hour.

Introduction

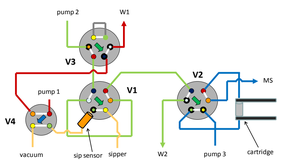
States 1&4: Aspirate & Re-equilibrate



State 2: Load/Wash



State 3: Elute



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

Agilent 6460 Settings

Source Parameters

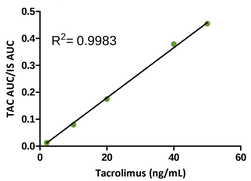
Ionization mode	ESI + Agilent Jet Stream
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	11 L/min
Nebulizer pressure	45 psi
Nozzle voltage	500 V
Capillary voltage	3500 V

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

Representative Standard Curve



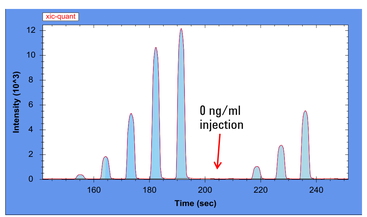
Tacrolimus Conc (ng/mL)	Accuracy* (%) Intraday (n=3)	Precision* (%) Intraday (n=3)	Accuracy* (%) Interday (n=4)	Precision* (%) Interday (n=4)	Quant/Qual AUC
2	106.3	3.5	105.2	2.6	3.4
10	94.6	1.2	96.0	3.8	3.1
20	96.6	7.4	97.0	4.0	3.4
40	101.1	3.3	100.3	4.5	3.5
50	101.3	1.9	101.6	2.8	3.4
UTAK 1 (4.9)	99.1	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

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. Both quantifier and qualifier ion values had excellent linearity within the measured range of 2-50 ng/ml with an R^2 value greater than 0.99. The signal window was found to be greater than 30 to 1. Carryover was evaluated by injecting a 0 ng/ml sample containing internal standard after the highest standard concentration. Carryover was determined to be 0%.

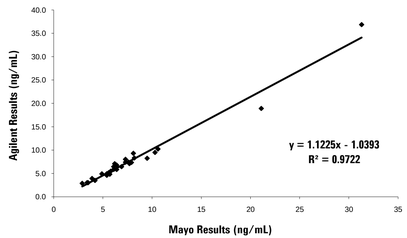
Note: The method described in this presentation is for research purposes only and not approved for diagnostic use

Representative Standard Curve MS trace



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.
- 0% carryover was determined after the highest standard curve concentration
- 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 10 times faster. This novel methodology is capable of throughputs >370 samples per hour.