Evaluating Analytical Performance of Tacrolimus LC-MS/MS Assay Using Ascomycin Versus Tacrolimus-C13D2 Internal Standards

Kwaku Twum, Philip Bates, Nichole Korpi-Steiner.

Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Introduction & Background

- Therapeutic monitoring of whole blood tacrolimus concentration via liquid chromatography-tandem mass spectrometry (LC-MS/MS) remains essential to reduce the risk of rejection of a transplant organ.
- Conventional LC-MS/MS approaches for tacrolimus measurement have utilized the ascomycin, a structural analog, as internal standards. Recently, deuterated isotopically labeled tacrolimus internal standards have become increasingly commercially available, which may provide improved performance over ascomycin.
- This study evaluated the following method performance for measurement of tacrolimus in using ascomycin and deuterated tacrolimus (from Toronto Research Chemicals) as internal standards.
  - ❖ Precision
  - ❖ Accuracy
  - ❖ Linearity of calibrators and
  - ❖ Interference of matrix

Methods

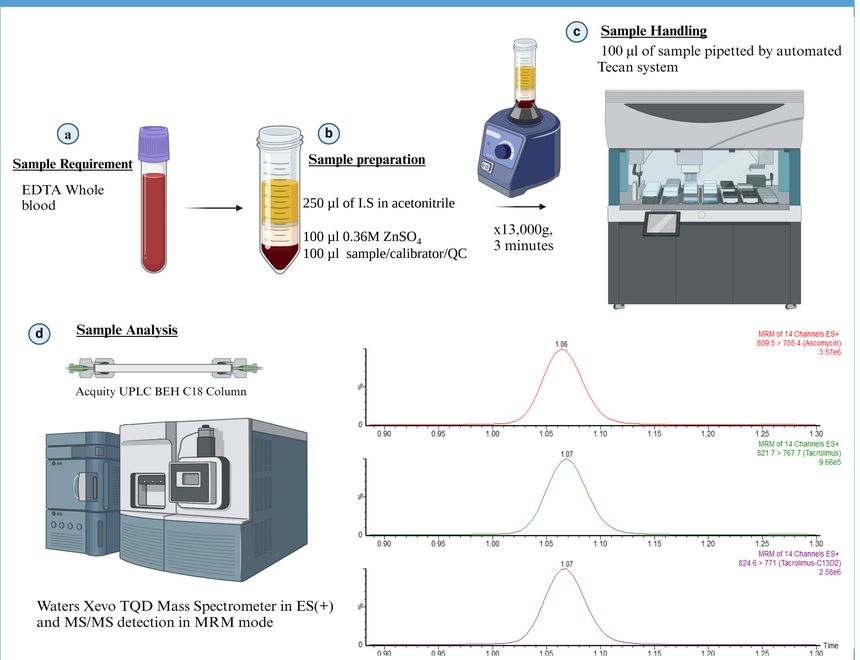

Figure 1: Pictural representation of sample processing and method for analyzing Tacrolimus from EDTA whole blood sample and a sample chromatogram

Table 1: Optimized primary and secondary mass transitions from Tacrolimus LC-MS/MS method

Compound	Retention Time (mins)	Quantitative Mass Transition	Qualitative Mass Transition
Tacrolimus	1.07	821.7 > 767.7	821.7 > 785.7
Ascomycin	1.06	809.5 > 755.4	-
Tacrolimus-C13D2	1.07	824.6 > 771.0	824.6 > 789.0

Results - Precision

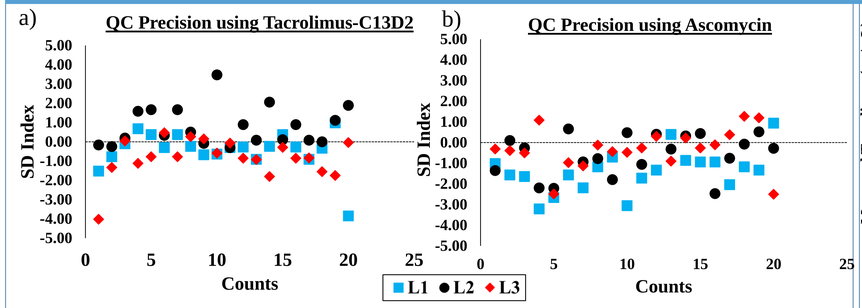

Figure 2: Levey-Jennings chart showing SDI of three levels of QC data obtained using a) Tacrolimus-C13D2 and b) Ascomycin as internal standards.

Table 2: QC statistics for 20 data points over 20 days

Compound	QC Levels	Mean (ng/mL)	Standard Deviation	Coefficient of Variation (%)
Tacrolimus-Tacro-C13D2 IS	1	3.52	0.30	8.40
	2	13.54	0.36	2.66
	3	20.41	0.75	3.67
Tacrolimus-Ascomycin IS	1	3.47	0.13	3.68
	2	12.97	0.50	3.87
	3	20.42	1.89	9.27

Results - Method Comparisons

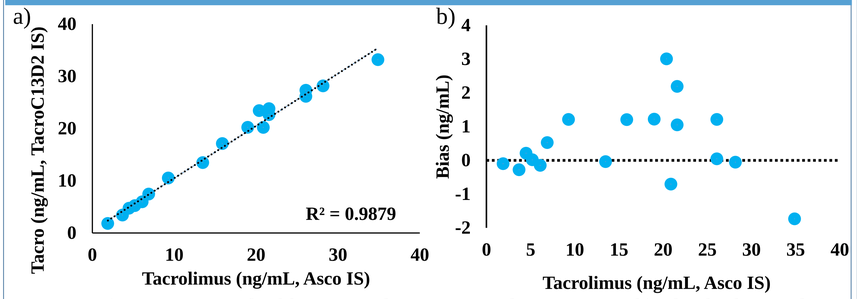

Figure 3: a) Comparison of calibrator results using tacrolimus assays, b) Bland-Altman plot comparing results between internal standards.

Table 3: Results of residual PT samples using Tacrolimus assays.

PT Samples	Group Mean	Group SD	Tacro (ng,mL – Asco IS)	Tacro (ng/ mL-TacroC13D2 IS)	Tacro (Asco IS) SDI	Tacro (TacroC13D2) SDI
1	4.43	0.49	4.02	4.66	-0.8	0.5
2	21.35	1.65	20.44	17.72	-0.6	-2.2
3	10.80	0.85	9.97	10.63	-1.0	-0.2
4	10.96	0.85	10.09	7.82	-1.0	-3.7
5	21.43	1.57	19.88	18.43	-1.0	-1.9
6	11.02	0.87	9.94	9.66	-1.2	-1.6
7	4.43	0.36	4.05	3.1	-1.1	-3.7
9	21.40	1.29	19.42	17.51	-1.5	-3.0

Linearity of Calibrators

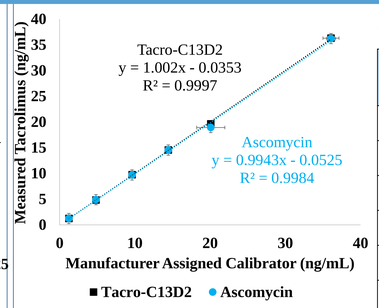

Table 4: Average calibrator results over 10 runs

Manufacturer Assigned Concentration	Tacro (ng/mL,TacroC 13D2 IS)	Tacro (ng/mL,Asco IS)	%Bias (Tacro-C13D2 IS)	%Bias (Asco IS)
1.23	1.23	1.23	-0.08	-0.24
4.84	4.83	4.89	-0.12	1.10
9.63	9.75	9.71	1.18	0.77
14.45	14.55	14.54	0.67	0.63
20.09	19.63	18.93	-2.34	-6.14
36.08	36.30	36.22	0.59	0.37

Figure 4 : Linearity of Calibrator response using Tacrolimus assays

Matrix effects

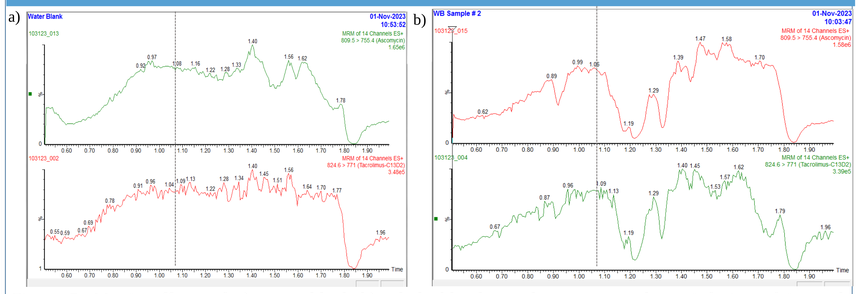

Figure 5: Matrix effects of a) water blank b) whole blood sample, on post column infusion of internal standards, dash lines = approximate internal standard retention time

Table 5: Matrix effects of water and whole blood matrices, on post extraction addition of Tacrolimus and internal standards.

	Tacrolimus			Tacrolimus-C13D2			Ascomycin		
	Peak Area	Conc. ng/mL	Matrix Effect (%)	Peak Area	Conc. ng/mL	Matrix Effect (%)	Peak Area	Conc. ng/mL	Matrix Effect (%)
Water 1	6230	18.0		2398	24.0		2468	25.2	
Matrix 1	8274	17.8	99%	3183	20.0	83%	3582	25.2	100%
Matrix 2	8846	18.0	100%	3711	23.9	100%	3989	27.5	109%
Matrix 3	8440	17.4	97%	3388	22.6	94%	3365	23.1	92%
Matrix 4	8200	17.3	96%	3275	21.4	89%	3405	22.7	90%
Matrix 5	7788	16.5	92%	3206	23.2	97%	3346	24.1	96%
Matrix 6	7727	16.6	92%	2980	20.3	85%	3183	22.4	89%
Matrix 7	6035	17.8	99%	2103	22.7	95%	2338	24.7	98%
Matrix 8	7870	17.8	99%	3118	23.6	98%	2900	22.9	91%
Matrix 9	7225	17.6	98%	2937	21.3	89%	3114	23.5	93%
Matrix 10	6827	18.4	102%	2306	21.0	88%	2703	26.5	105%

Conclusions

Whole blood tacrolimus measurement via LC-MS/MS demonstrated comparable analytical performance using tacrolimus-C13D2 or ascomycin as an internal standard. Linearity, imprecision, and accuracy in tacrolimus measurements showed acceptable performance for both internal standards, though a negative bias in measured tacrolimus compared with peers was observed. In evaluating the choice of internal standard, other factors such as cost and ease of obtaining pure isotopically labeled analog must be also considered.

References

1. Immunosuppressant Drug Monitoring User's Guide. Waters Corporation, Manual VI.I.
2. CLSI. Liquid Chromatrography-Mass Spectrometry Methods; Approve Guidelines. CLSI document C62-A. Wayne, PA: Clinical and Laboratory Standards Institute. 2014.
3. ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Immunosuppressive Agents, Waters Application Note, 2018.