

THE EVALUATION OF ABBREVIATED CALIBRATION CURVES FOR SIROLIMUS QUANTIFICATION BY HPLC-MASS SPECTROMETRY

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We have previously reported a quantitative method using HPLC-mass spectrometry for the immunosuppressant drug sirolimus to support Phase II and III clinical trials [1]. For regulatory purposes the method used an 8-point calibration curve (0.2 to 100 µg/L) with weighted $1/x^2$ regression for calculation of concentrations. In this study we investigated the analytical performance of single-point linear through zero calibration (10, 25 and 80 µg/L) and 2-point calibration (0.2 with 10, 25 and 80 µg/L) against the original 8-point calibration to determine sirolimus concentrations.

Nine batches of samples were selected at random from ongoing clinical studies. The performance of each calibration type was assessed using 3 quality controls (0.5, 20, 75 µg/L) analysed within each batch. The accuracy (%) and precision (%CV in brackets) of this investigation is shown below:

Quality Control	8-point	Single-point (linear through 0)				2-point ($1/x^2$)		
	($1/x^2$)	10	25	80	0.2, 10	0.2, 25	0.2, 80	
0.5	-4.8 (6.6)	+0.4 (5.5)	0.0 (8.2)	1.2 (5.2)	-4.6 (6.0)	-5.0 (7.6)	-4.2 (6.6)	
20	-2.0 (4.7)	-2.0 (5.3)	-2.4 (8.1)	-1.2 (4.3)	-1.8 (5.3)	-2.4 (8.1)	-1.3 (4.2)	
75	-6.3 (2.9)	-6.1 (3.8)	-6.7 (4.7)	-5.3 (3.7)	-5.9 (3.9)	-6.5 (4.7)	-5.3 (3.7)	

All calibration methods showed acceptable accuracy and precision when compared to results obtained from the 8-point calibration method. The 2-point standard curve approach did not provide improved accuracy over the single point calibration. As sirolimus is expected to have a therapeutic range of approximately 2 to 20 µg/L for trough blood samples, the single-point calibration using the 10 µg/L standard was evaluated to calculate patient samples analysed in the 9 batches. These values were compared with results obtained from the 8-point calibration ($y = 1.02x - 0.223$, $r^2 = 0.995$, $n = 147$). The mean bias between results was -0.15% (range: -8.3% to 7.7%).

The single-point calibration can be used in place of the 8-point calibration for the quantitation of sirolimus by HPLC-mass spectrometry. The use of a single-point calibration would decrease chromatographic run time by 70 min (cf 8-point), thus increasing sample throughput and facilitating stat analysis in the clinical setting.

The authors wish to acknowledge Wyeth Australia Pty Ltd for financial support of this study.