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Application Data Sheet

No.41

LC-MS
Liquid Chromatograph Mass Spectromrter

Rapid Analysis of Immuno-suppressants Using Triple Quadrupole LC-MS/MS

This report exemplifies an ultra high speed analysis of 4 immuno-supressants (Tacrolimus, Everolimus, Rapamycin, Cyclosporin A) in less than 120 seconds by Shimadzu UFMS Triple Quadrupole Mass Spectrometer LCMS-8050 with negative ionization mode. Combination of Nexera X2 and LCMS-8050 provides you much faster run time than ever you experienced without sacrificing the quality of results.

Including two internal standrads such as Ascomycin and Cyclosporin D, total 6 compounds were measured in 1.8 minutes. The structure of each compounds is illustrated below. In spite of low injection volume (1.5 uL) was utilized, this ultra high speed method achieves 1 ng / mL as the limit of quantitation for all compounds.

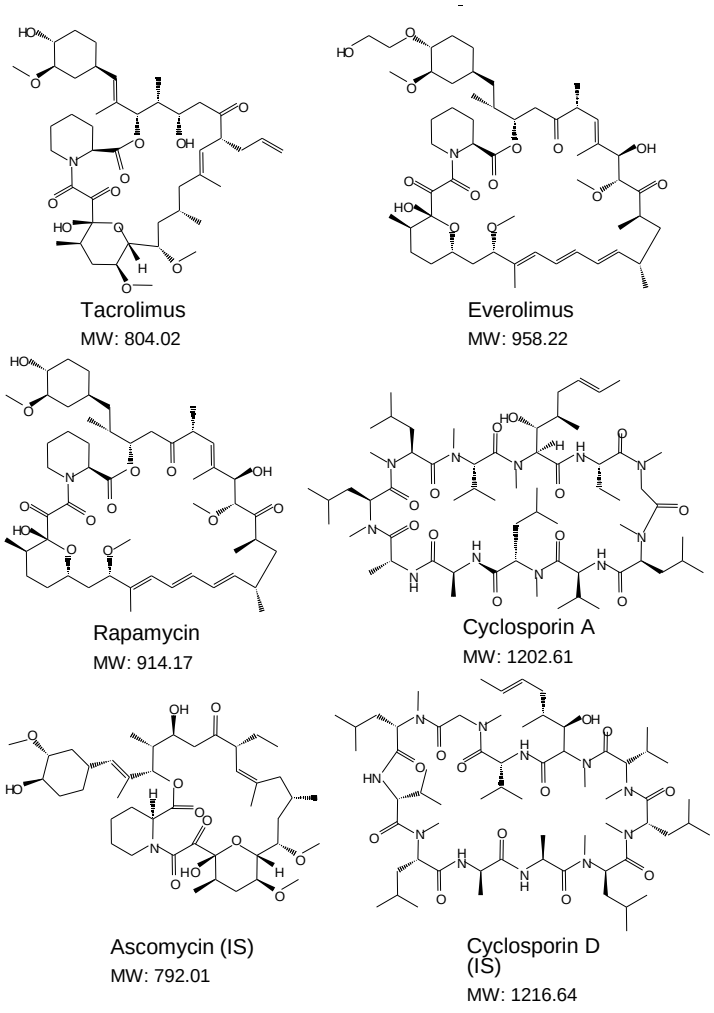


Figure 1. Structure of immuno-supressants and internal standards

Table 1. Experimental Conditions

HPLC conditions (Nexera X2)	
Analytical Columun	: YMC-Triart C18 (30 mmL. X 2 mmI.D. , 1.9 µm)
Mobile phase A	: 1 mmol/L Ammonium acetate aqueous solution
Mobile phase B	: 1 mmol/L Ammonium acetate methanol solution
Time program	: 60 % B. (0 min) → 75 % B. (0.10 min) → 95 % B. (0.70 – 0.90 min) → 60 % B. (0.91 – 1.80 min)
Flow rate	: 0.45mL / min.
Injection volume	: 1.5 µL
Column oven temp.	: 65 ℃
MS conditions (LCMS-8050)	
Ionization mode	: ESI（Negative)
Applied voltage	: -4.5 ~ -3 kV
Nebulizer gas	: 3.0L / min.
Drying gas	: 5.0 L / min.
Heating gas	: 15.0 L / min.
Interface temp.	: 400℃
DL temp.	: 150℃
Heat block temp.	: 390℃

Table 2. MRM Transitions

Peak No.	Compounds	Polarity	Precursor (*m/z*)	Product (*m/z*)
1	Tacrolimus	-	802.70	560.50
2	Everolimus	-	956.80	365.35
3	Rapamycin	-	912.70	321.20
4	Ascomysin (IS)	-	790.40	548.20
5	Cyclosporin A	-	1200.90	1088.70
6	Cyclosporin D (IS)	-	1215.10	1102.60

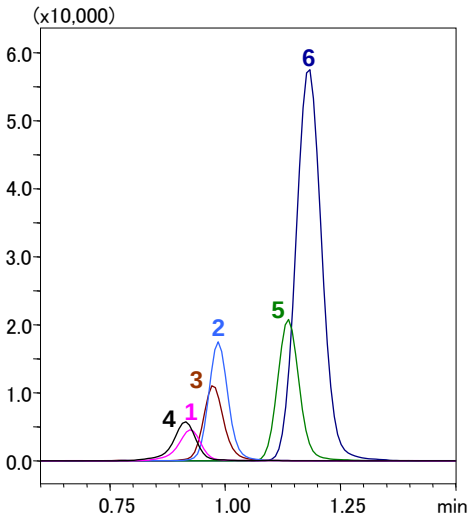
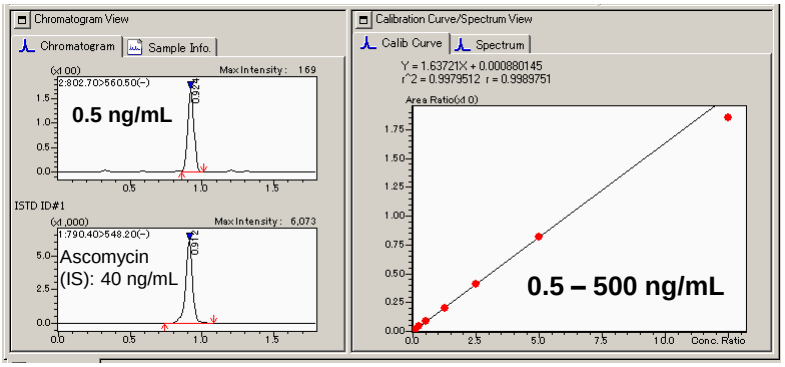
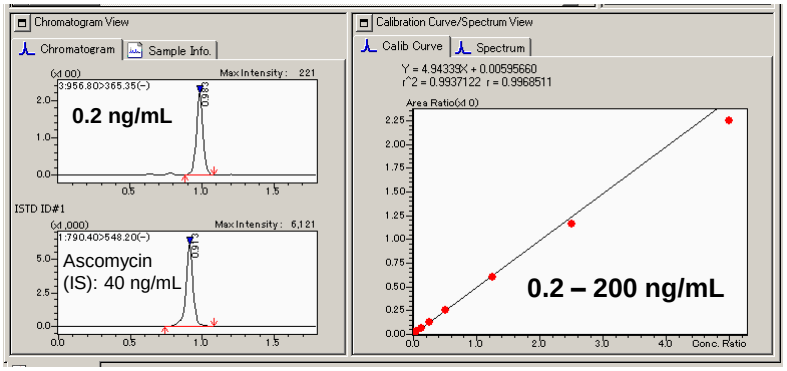


Figure 2. Representative MRM chromatograms at 20 ng/mL

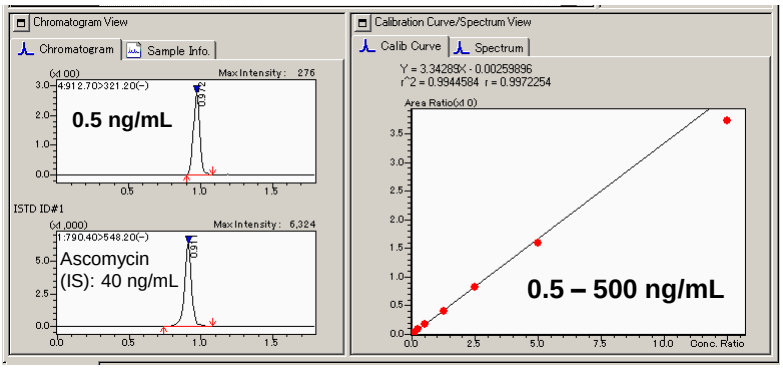
Tacrolimus



Everolimus



Rapamycin



Cyclosporin A

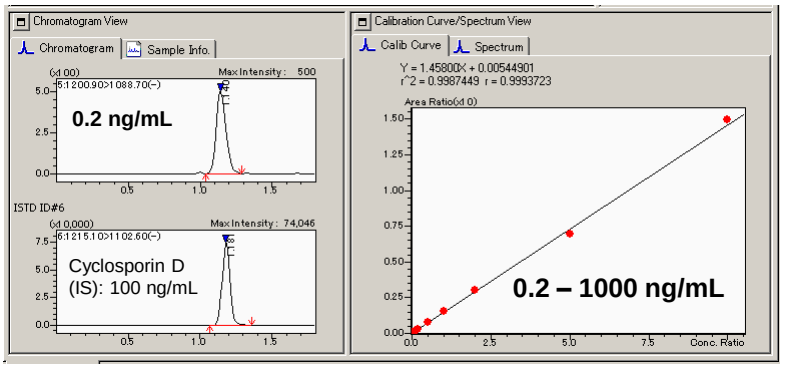


Figure 3. Results of 4 immuno-suppressants: Calibration curve and MRM chromatogram at lowest calibration points and internal standard (bottom)

Figure 3. has shown a calibration curve and chromatogram at lowest calibration level for each 4 immuno-suppressants. Table 3 illustrated a reproducibility and accuracy across actual therapeutic range of each immuno-suppresants we have simultaneously measured in 1.8 minutes.

In high speed measurement condition, we have achieved a required sensitivity and enough dynamic range for all analytes which fulfilled with the purpose of this kind of assay. Additionally, accuracy of each analytes was ranging from 85 to 115% and area reproducibility at the lowest calibration level of each analytes was less than 20%.

Table 3. Reproducibility and Accuracy

Compounds	Sample Conc. (ng/mL)	%RSD (n = 6)	Accuracy (%)
Tacrolimus	0.5	19.2	98.1
	2	11.7	103.8
	500	4.94	90.5
Everolimus	0.2	14.1	100.5
	5	3.73	102.7
	200	2.79	90.9
Rapamycin	0.5	7.55	96.2
	5	7.38	100.4
	500	3.93	89.1
Cyclosporin A	0.2	13.5	102.5
	10	2.78	99.6
	1000	2.20	102.4

Acknowledgement

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