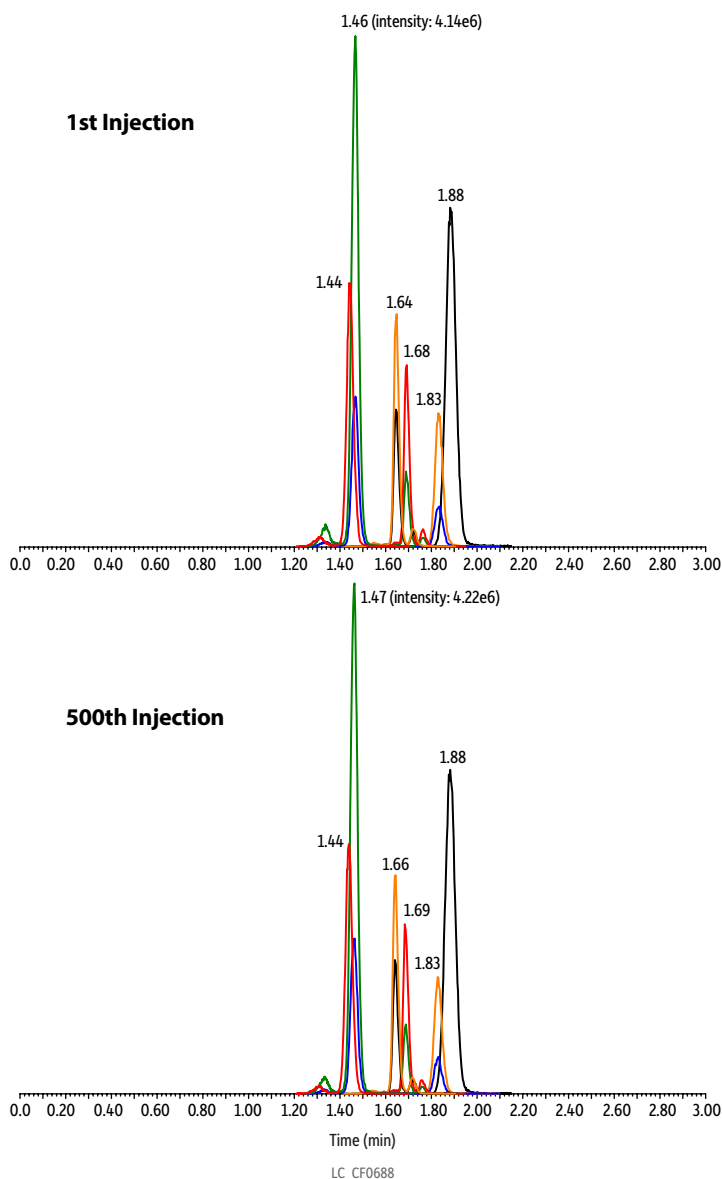


Column Lifetime Evaluation for Immunosuppressive Drug Analysis



Peaks	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Ascomycin	10	809.53	756.50
2. Tacrolimus	25	821.61	768.51
3. Sirolimus	25	931.63	864.57
4. Everolimus	25	975.68	908.62
5. Cyclosporin A	25	1219.83	1202.87
6. Cyclosporin D	100	1233.91	1216.88

See chromatograms for retention times.

Column Raptor Biphenyl (cat.# 9309A52)
 Dimensions: 50 mm x 2.1 mm ID
 Particle Size: 2.7 µm
 Pore Size: 90 Å
 Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
 Temp.: 70 °C
 Inj. Vol.: 5 µL
Mobile Phase
 A: 0.05% Formic acid, 5 mM ammonium formate in water
 B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	40	60
2.00	0.5	0	100
2.01	0.5	40	60
3.00	0.5	40	60

Max Pressure: 305 bar
Detector MS/MS
 Ion Mode: ESI+
 Mode: MRM
Instrument 571

Sample Preparation

Human whole blood was fortified with 4 analytes at 25 ng/mL. For quantitation, cyclosporin D was used as the internal standard for cyclosporin A and ascomycin was used as the internal standard for tacrolimus, sirolimus, and everolimus. The blood sample (2.5 mL) was mixed with 5 mL of precipitation solution (1:4 v/v 0.2 M ZnSO₄:methanol) containing 50 ng/mL of cyclosporin D and 5 ng/mL of ascomycin. The mixture was vortexed for 20 seconds at 3000 rpm and then centrifuged for 10 minutes at 4300 rpm. The supernatant was directly injected (5 µL) for analysis.