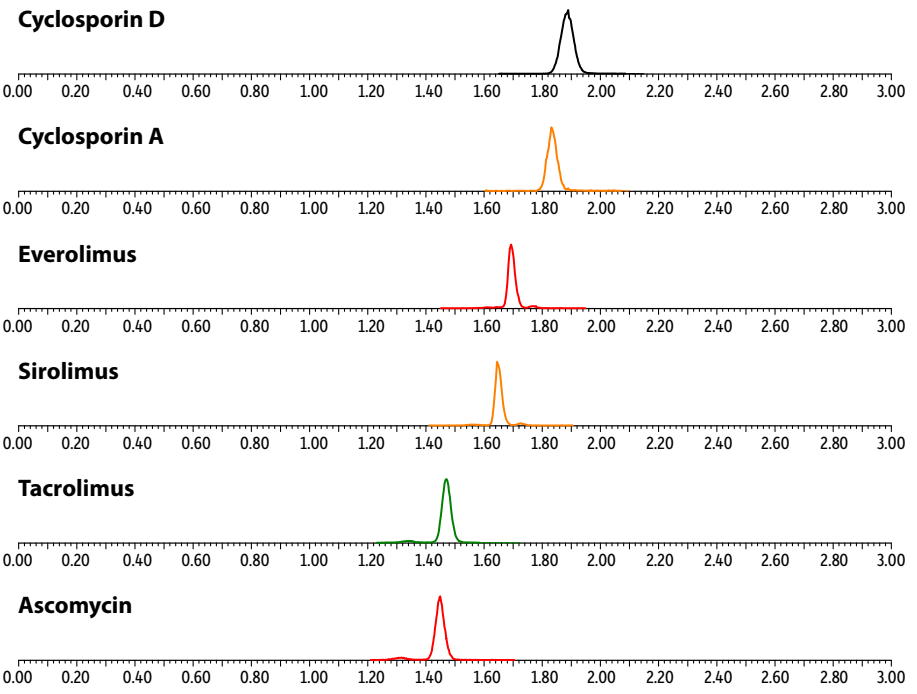
Immunosuppressive Drugs in Blood on Raptor Biphenyl by LC-MS/MS



Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Ascomycin	1.45	10	809.53	756.50
2. Tacrolimus	1.47	10	821.61	768.51
3. Sirolimus	1.64	10	931.63	864.57
4. Everolimus	1.69	10	975.68	908.62
5. Cyclosporin A	1.83	10	1219.83	1202.87
6. Cyclosporin D	1.89	100	1233.91	1216.88

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 UHPLC
Sample Preparation Human whole blood was fortified with 4 immunosuppressive drugs at 10 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 (100 µL) was mixed with 200 µL 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.