

## **Exactive Plus MS & Immunosuppressant Drugs**

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# Immunosuppressant Drugs

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- **Objective: Demonstrate the ability of the Thermo Scientific Exactive Plus high-performance bench-top Orbitrap mass spectrometer to analyze immunosuppressant drugs in whole blood for clinical research.**
- **Utilize the Thermo Scientific ClinSpec Immunosuppressant Test kit.**
- **Compare quantitative analysis results with SRM analysis obtained from a triple quadrupole mass spectrometer.**

# ClinSpec Immunosuppressant Test Kit

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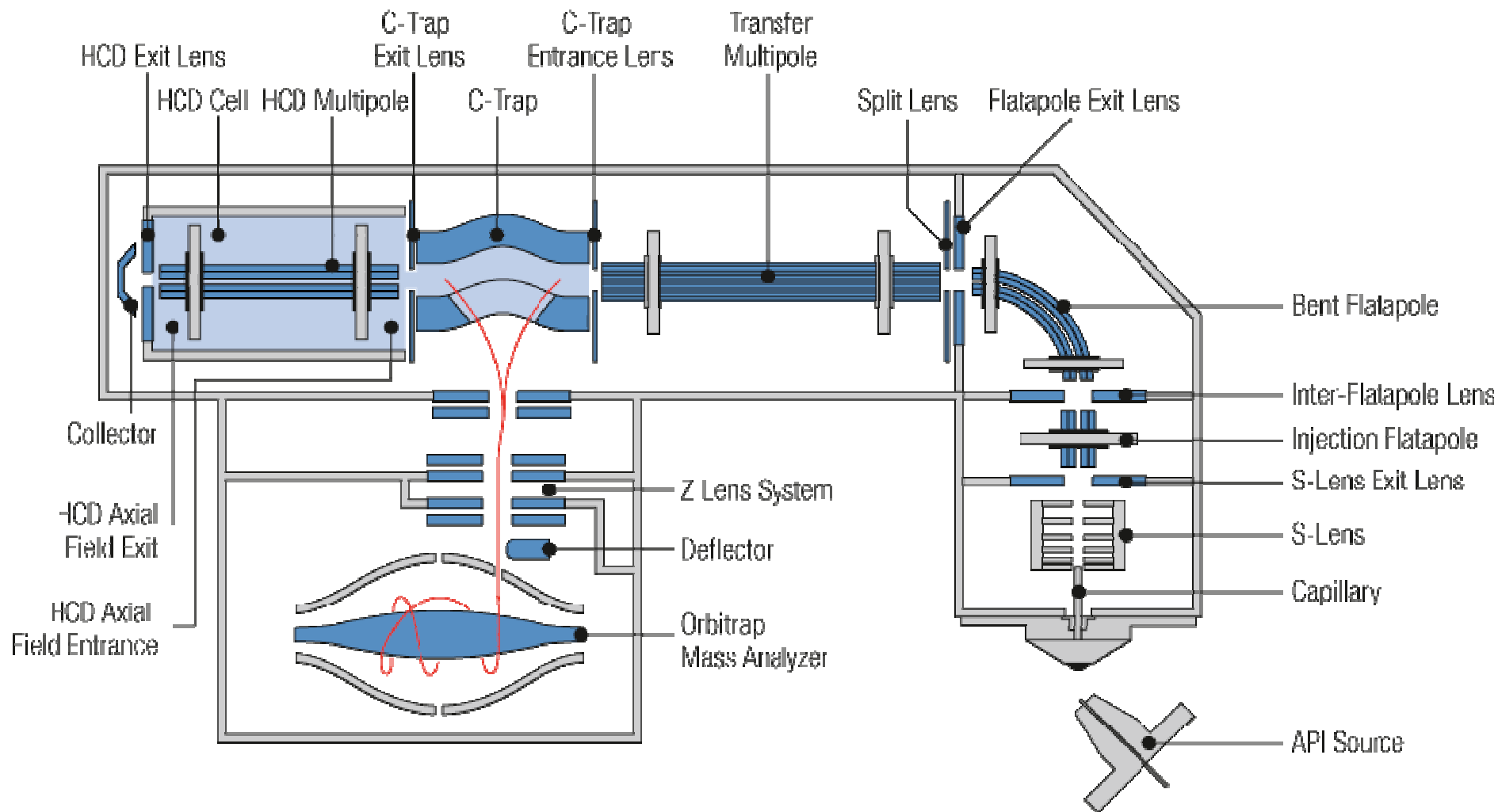
- **The ClinSpec™ Immunosuppressant Test kit was developed for use by research laboratories to analyze Tacrolimus, Sirolimus, Everolimus, and Cyclosporine A in whole blood specimens by LC-MS/MS.**
- **It consists of six different calibrator levels, and up to 5 quality control levels, internal standard and extraction reagent.**
- **Here we are using the same kit to analyze for these compounds using the Exactive Plus™ high-resolution, accurate-mass (HR/AM) mass spectrometer.**

# Exactive Plus MS: Exciting New Technology for Routine Markets

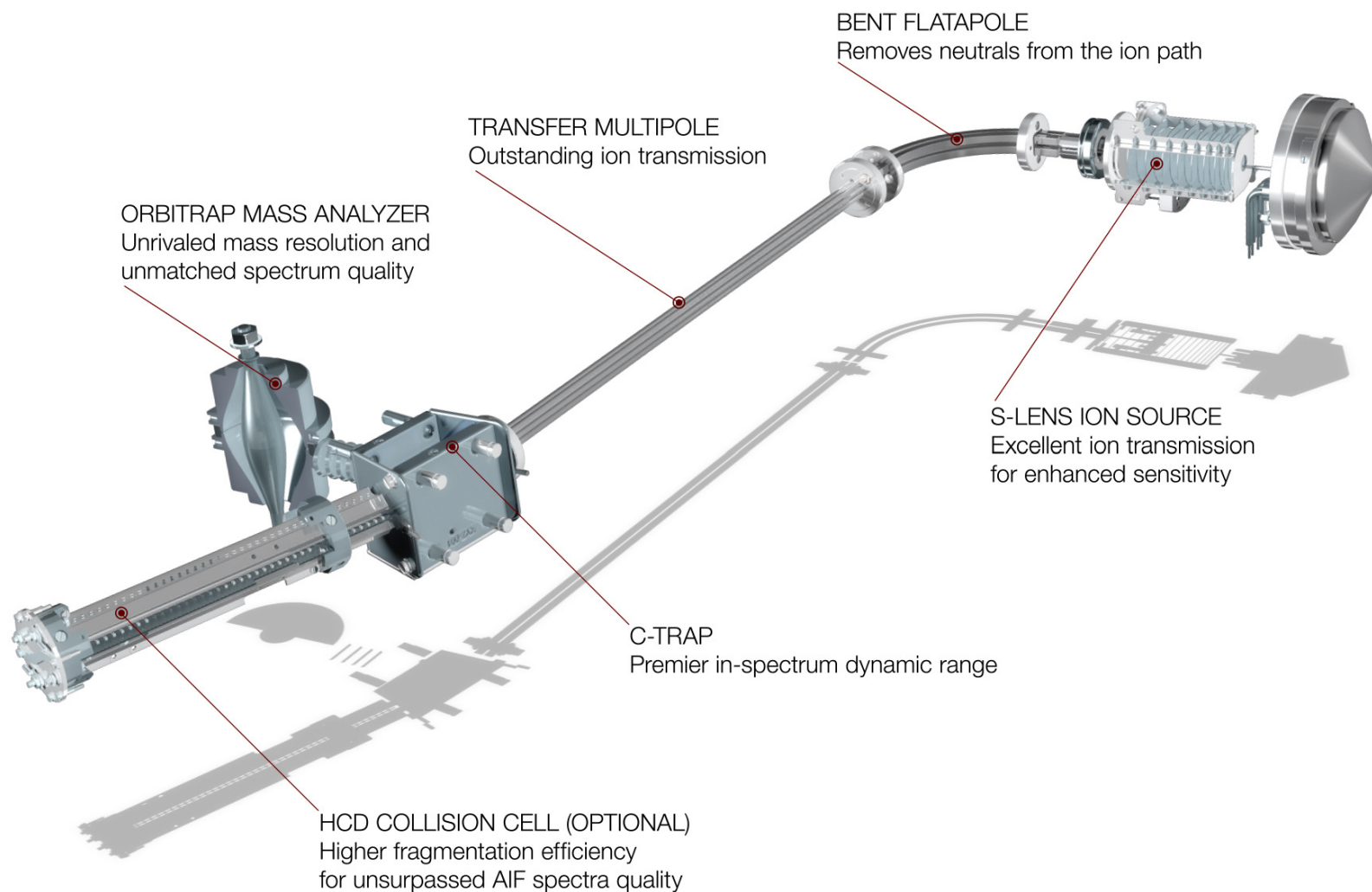
- **APCI source**
- **Mass range of  $m/z$  50 – 6000**
- **Maximum resolution of 140,000 (FWHM) at  $m/z$  200**
- **Mass accuracy < 1 ppm (internal)**
- **Polarity switching full cycle < 1 sec at 35,000 resolution**
- **Intrascan dynamic range > 5000:1**
- **HCD cell for All Ion Fragmentation (AIF)**
- **On-site upgradable to Q Exactive MS**



# Exactive Plus MS: Schematic Diagram



# Exactive Plus MS: Based on Q Exactive Platform



# Sample Preparation

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- **Samples are prepared per instructions in the ClinSpec kit.**
  - Mix 50  $\mu\text{L}$  of blood with 150  $\mu\text{L}$  of 40 mM  $\text{ZnSO}_4$  in 66% methanol containing internal standards
    - Ascomycin as internal standard for Tacrolimus, Sirolimus and Everolimus
    - Cyclosporine D as internal standard for Cyclosporine A
  - Shake for 30 minutes at room temperature
  - Centrifuge at 13,000 rpm for 10 minutes
  - Transfer supernatant to an autosampler vial, cap, and inject 50  $\mu\text{L}$  onto HPLC system

# ClinSpec Calibrators and Controls

	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6
Cyclosporine A	10	25	70	200	750	1500
Tacrolimus, Sirolimus, Everolimus	1	2	5	10	20	30

	Control 1	Control 2	Control 3	Control 4
Cyclosporine A	30	125	375	700
Tacrolimus, Sirolimus, Everolimus	3	12	25	NA



# Liquid Chromatography

- **Thermo Scientific Accela 600 pump**
- **Thermo Scientific Accela open autosampler**
- **Mobile phases:**
  - (A) 10 mM ammonium formate with 0.1% formic acid in water
  - (B) 10 mM ammonium formate with 0.1% formic acid in methanol
  - (C) acetonitrile:1-propanol:acetone (45:45:10)
- **Column: Javelin C18 guard column, 5  $\mu\text{m}$ , 10 x 2.1 mm**
- **Column temperature: 80° C**
- **Divert valve to MS 0.7-1.2 min**
- **Gradient (see table) →**

Start (min)	Sec	$\mu\text{L}/\text{min}$	%A	%B	%C
0.00	15	800	70	30	
0.25	15	800		100	
0.50	36	800		100	
1.10	24	1000			100
1.50	20	1000	70	30	
1.83	10	800	70	30	
2.00	1	800	70	30	

# Instrument Tune, Scan and Source Parameters

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## Scan Parameters

1. Mass range: 800 – 4000  $m/z$
2. Resolution: 70k
3. Polarity: Positive
4. Microscans: 1
5. Lock mass: Off
6. AGC target: Balanced ( $1e^6$ )
7. Max inject time: 200 ms

## Source Parameters

1. APCI source
2. Sheath gas flow rate: 15
3. Aux gas flow rate: 17
4. Sweep gas flow rate: 1
5. Discharge current: 4.6  $\mu A$
6. Capillary temp: 275° C
7. S-Lens RF level: 75 V
8. Vaporizer temp: 300° C

***No compound tuning required - just plug and play***

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# Analytical Performance Verification Plan

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- **Process and analyze a calibration curve and 3-6 replicates of each QC level.**
- **Repeat the above analysis on three days to obtain inter-analysis statistics.**
- **Compare statistics to those given in ClinSpec product insert.**
- **Analyze and compare results from samples previously analyzed utilizing a triple stage quadrupole instrument.**

# Data Analysis

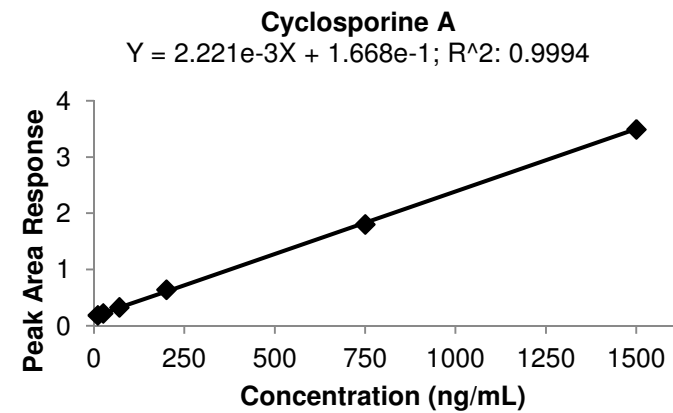
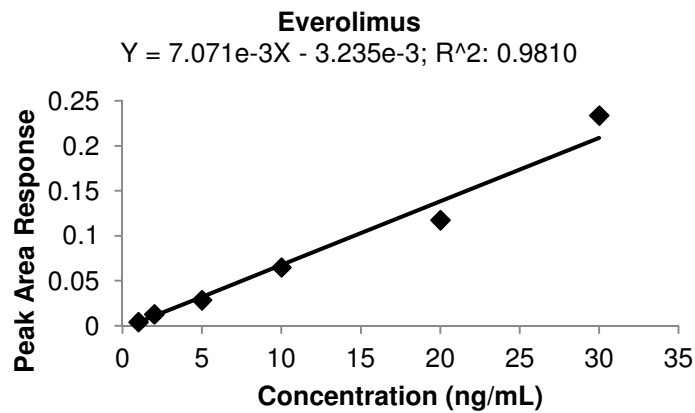
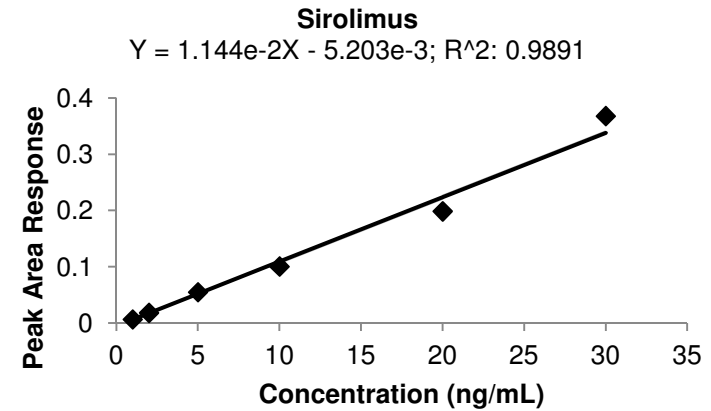
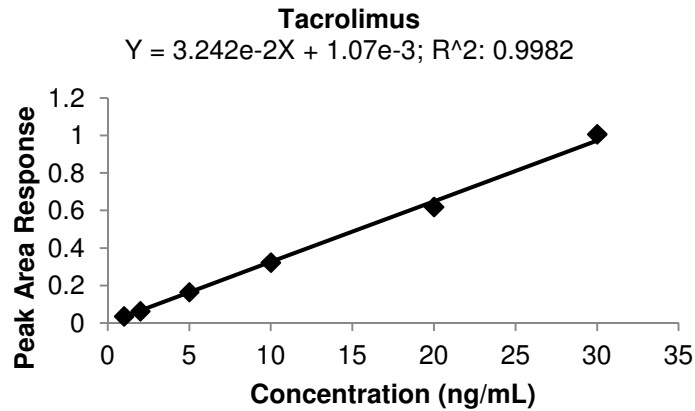
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- All data was acquired and processed using Thermo Scientific TraceFinder software.
- All of the compounds form ammoniated adducts with exact masses are listed below.

Compound	m/z	Compound	m/z
Ascomycin	809.5158	Everolimus	975.6152
Tacrolimus	821.5158	Cyclosporine A	1,219.8752
Sirolimus	931.5890	Cyclosporine D	1,233.8908

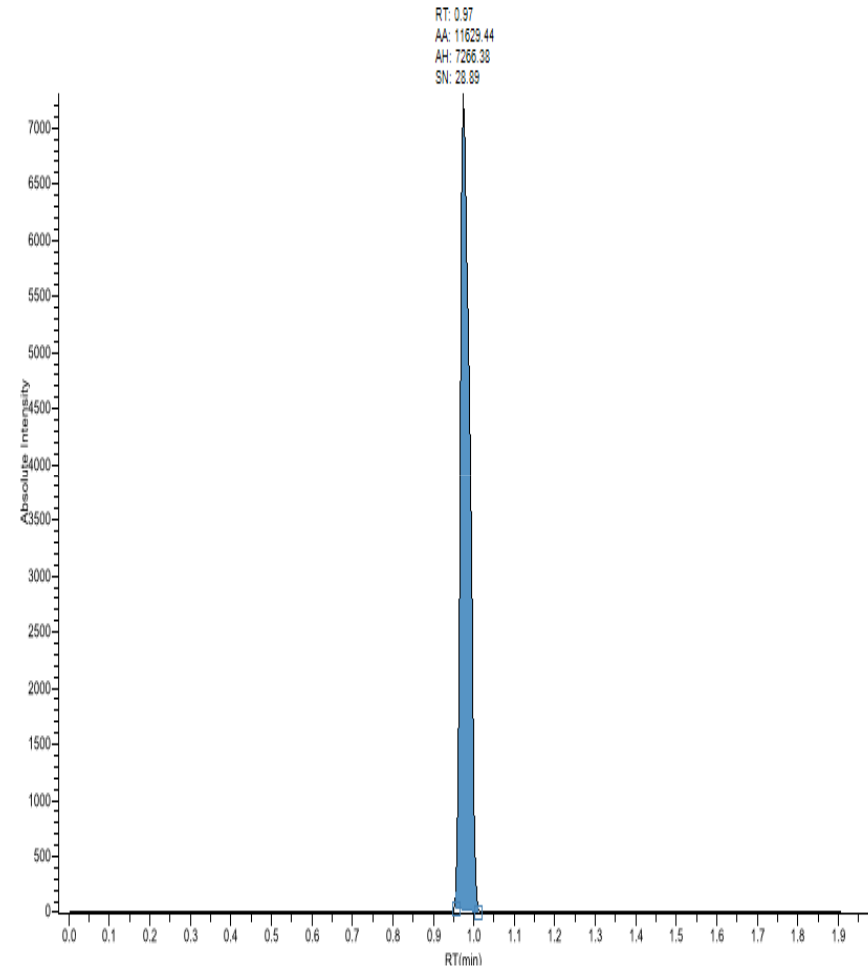
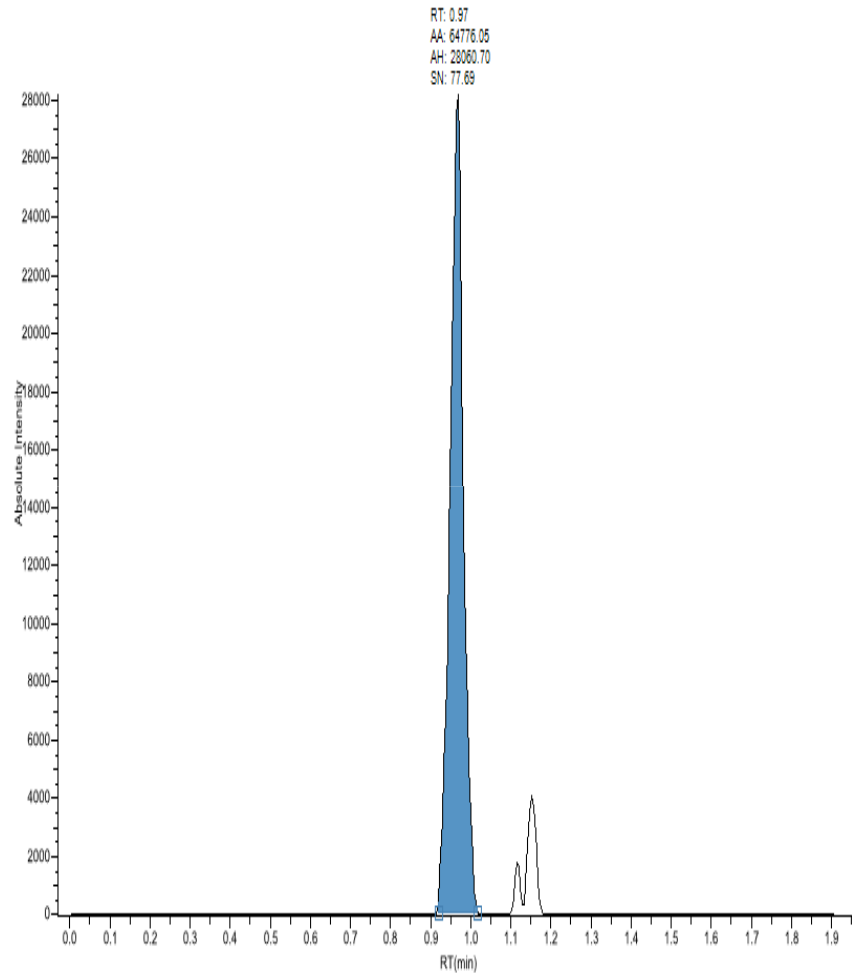
- Chromatograms for individual compounds were extracted from the full-scan data with a mass tolerance of 5 ppm.

# Linearity

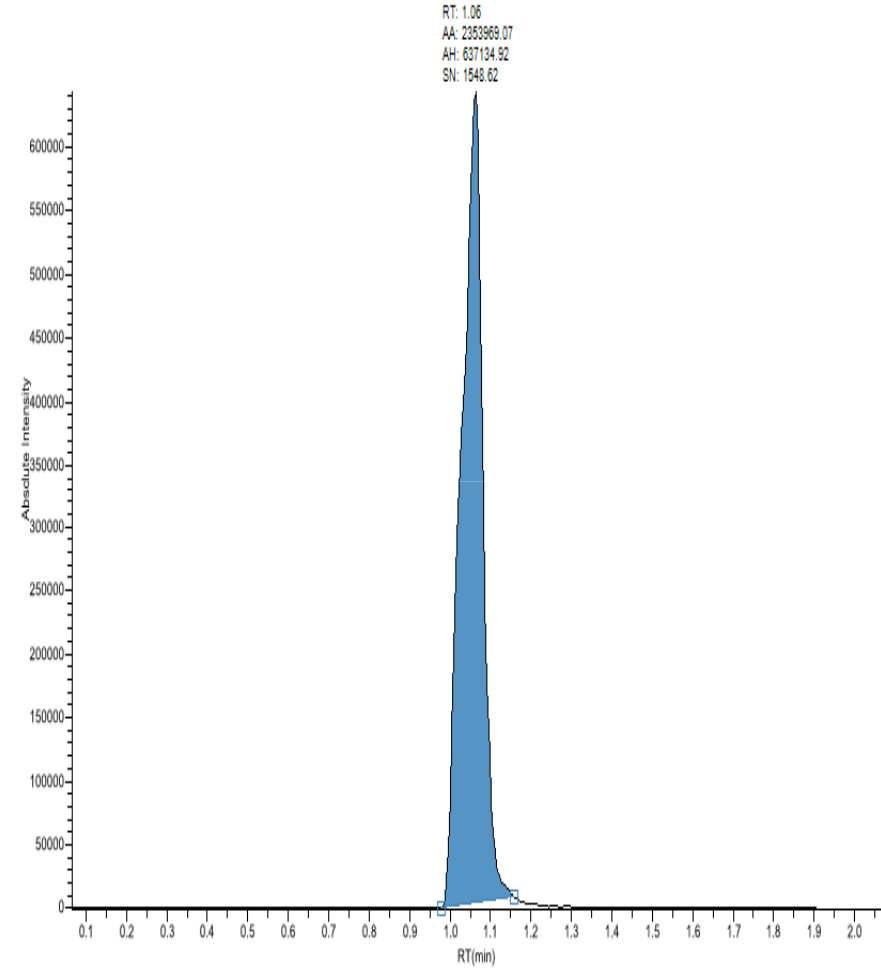
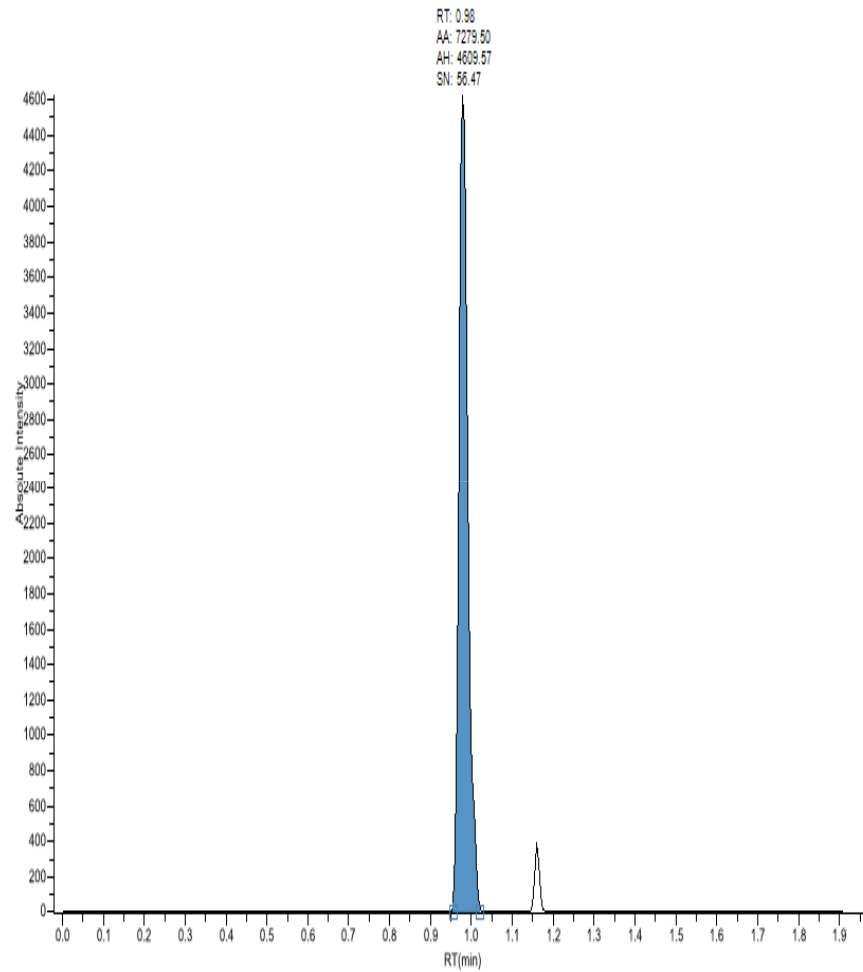


***All calibration standards back-calculate within  $\pm 15\%$ .***

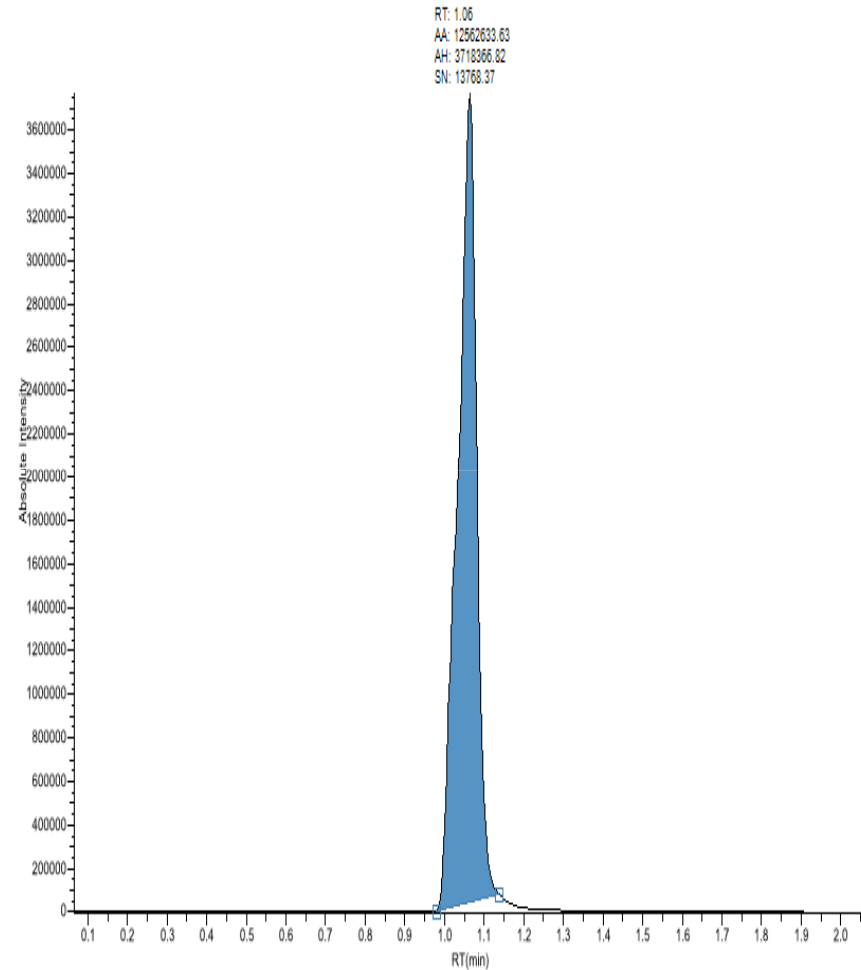
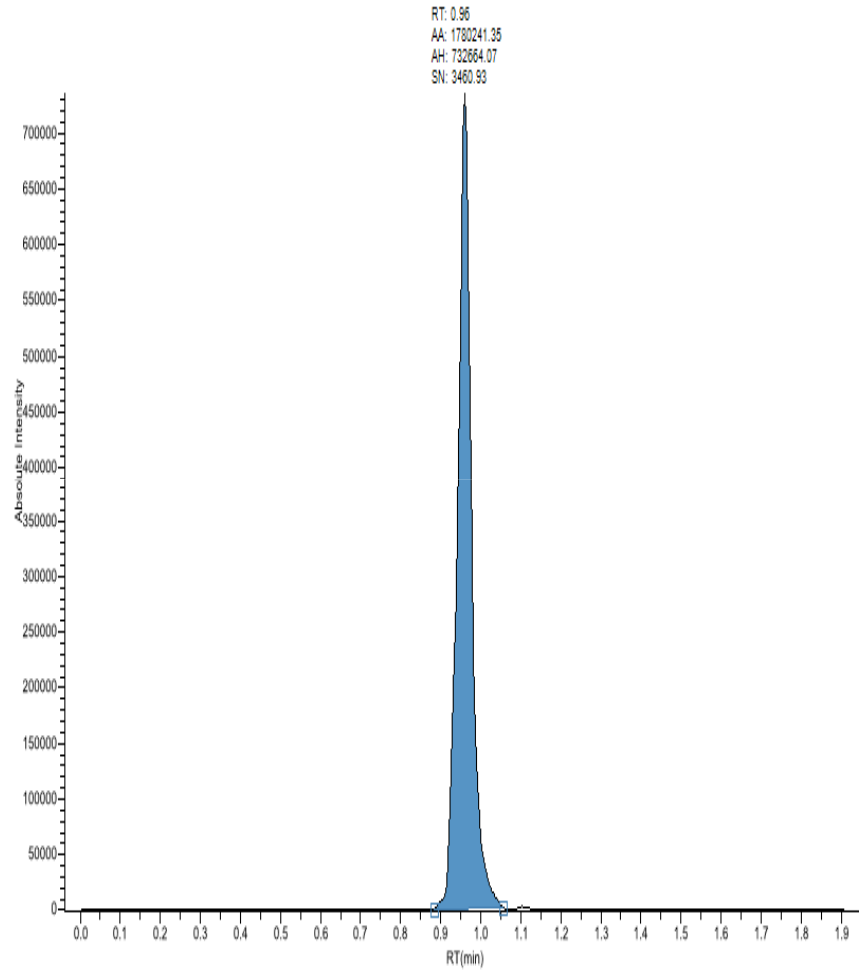
# Chromatography at LOQ (Tacrolimus and Sirolimus)



# Chromatography at LOQ (Everolimus and Cyclosporine A)



# Chromatography (Ascomycin and Cyclosporine D)





# Quality Controls

Tacrolimus			
	QC1	QC2	QC3
ng/mL	3	12	25
Mean	2.90	11.4	24.5
Std Dev	0.19	0.3	1.0
%Diff	-3.20	-4.60	-1.85
% CV*	6.6/10.3	2.2/6.1	4.2/5.9
n	13	13	11

Everolimus			
	QC1	QC2	QC3
ng/mL	3	12	25
Mean	3.28	10.7	23.7
Std Dev	0.61	1.0	1.9
%Diff	-9.17	-10.9	-5.18
% CV*	18.6/14.1	9.5/9.5	8.1/7.7
n	13	13	11

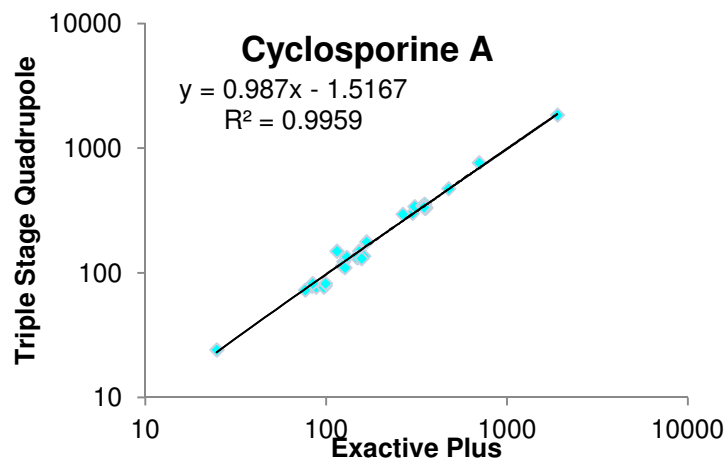
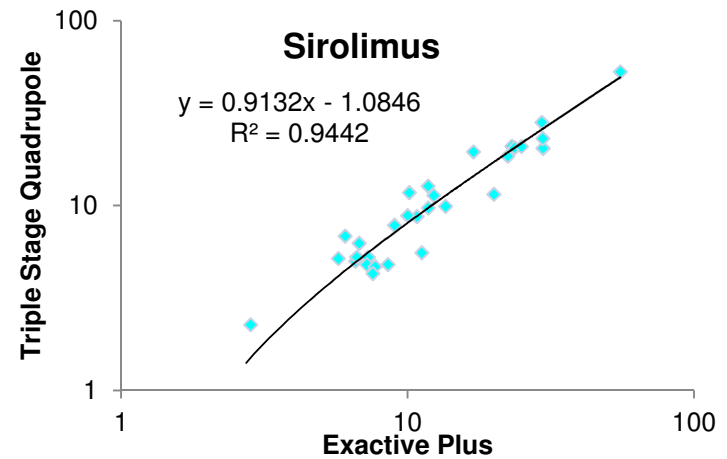
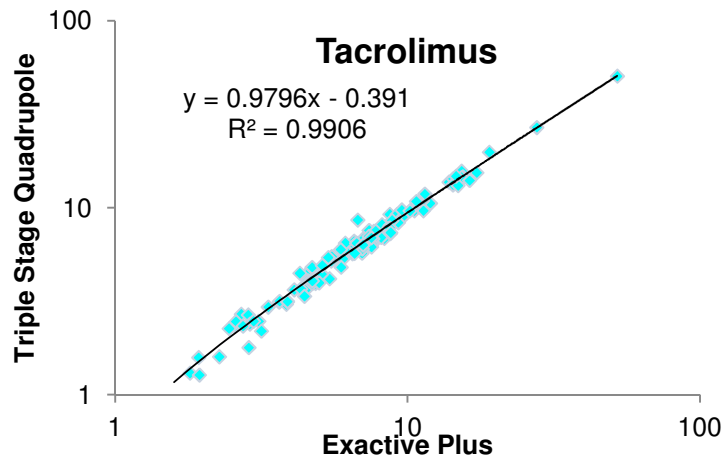
Sirolimus			
	QC1	QC2	QC3
ng/mL	3	12	25
Mean	3.02	10.8	21.3
Std Dev	0.39	0.8	1.0
%Diff	0.691	-10.1	-14.7
% CV*	12.9/13.7	7.6/9.0	4.7/8.3
n	13	13	11

Cyclosporine A				
	QC1	QC2	QC3	QC4
ng/mL	30	125	375	700
Mean	31.5	121	381	757
Std Dev	2.2	6	15	29
%Diff	5.12	-3.20	1.71	8.10
% CV*	7.1/7.7	4.9/5.9	4.0/6.6	3.8/6.2
n	13	13	11	13

***Exactive Plus does as good as or better than product insert.***

***\*CV from this study/%CV from product insert***

# Cross-Platform Comparison



- No donor values were available for Everolimus

***Exactive Plus shows excellent correlation with a 3Q instrument.***

# Summary

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- **Exactive Plus instrument gives results as good as a triple quadrupole instrument.**
- **Excellent choice for smaller clinical research labs that need a versatile instrument that can handle multiple analyses.**