



ThermoFisher
S C I E N T I F I C

Comprehensive Workflows for High Performance Quantitation Utilizing High Resolution Accurate Mass

Keeley Murphy,
Thermo Fisher Scientific

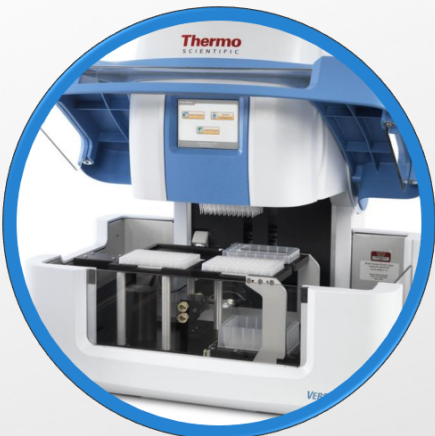
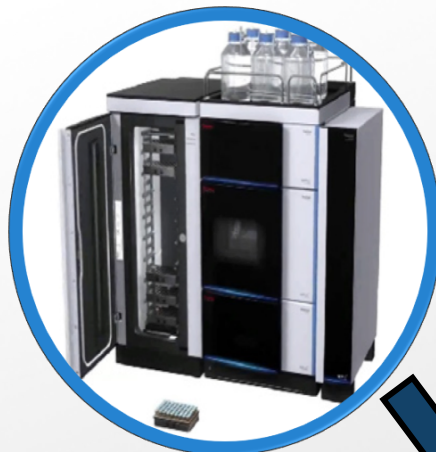
The world leader in serving science

Insulin Quantitation Workflow

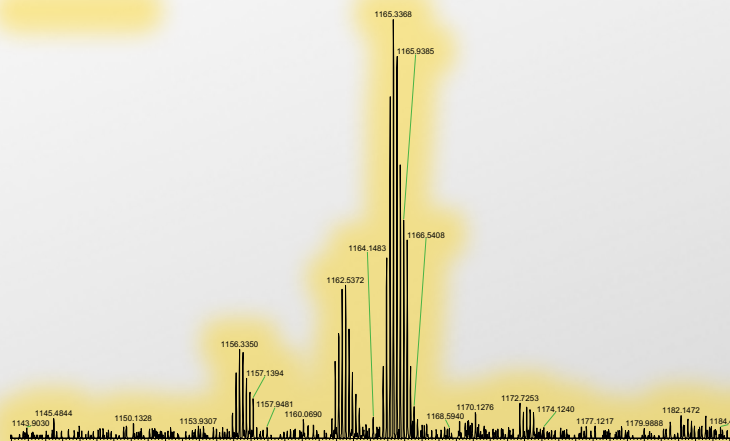
Software Solution



Chromatographic Separation



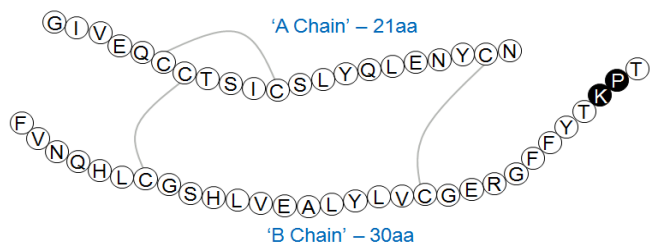
Sample Cleanup



High Resolution Accurate Mass

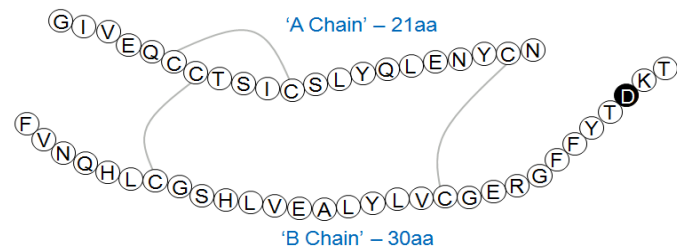
Insulin Overview

Humalog®



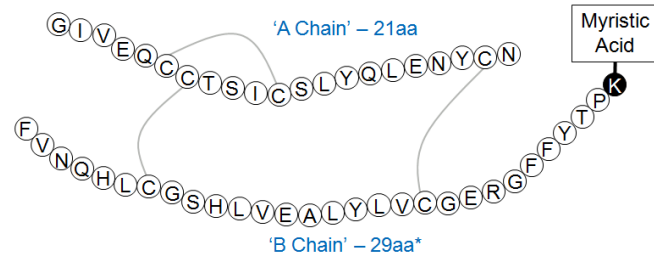
Insulin Lispro, Exact Mass – 5803.6371

NovoRapid®, NovoLog®



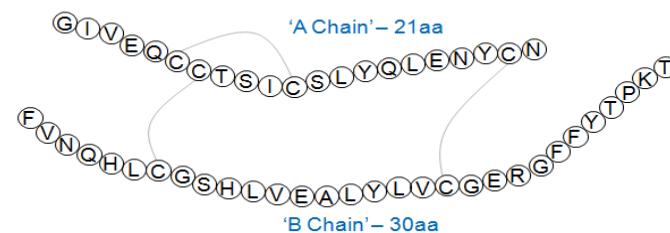
Insulin Aspart, Exact Mass – 5821.6113

Levemir®, Novo Nordisk



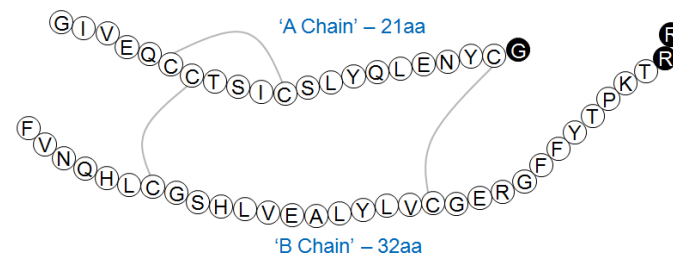
Insulin Detemir, Exact Mass – 5912.7878

Human Insulin



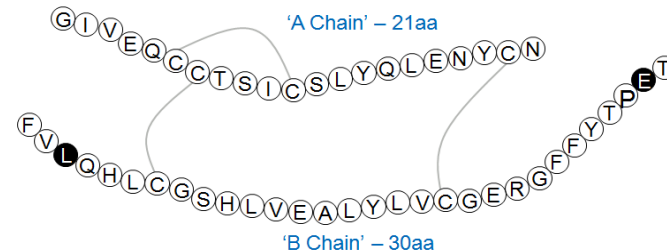
Insulin Human, Exact Mass – 5803.6371

Lantus®



Insulin Glargine, Exact Mass – 6058.8179

Apidra®

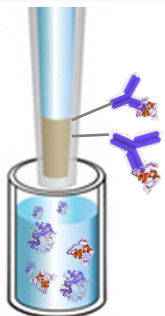


Insulin Glulisine, Exact Mass – 5818.6368

Sample Clean Up - MSIA

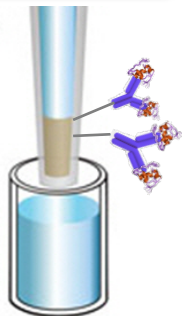
MSIA Workflow (biological matrix to data)

Capture



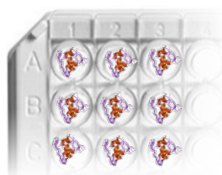
Analytical Sample

Wash



Wash Buffer

Elute



Eluted analyte

Analytical - Detection

Mass Spectrometry

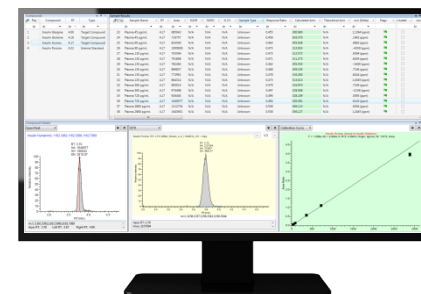


Pre-Analytical (fully-automated)



Acquire

Report



TraceFinder – Insulin Data

Sample Clean Up - MSIA

Three Step Clean Up

1) Sample Prep

-500uL Plasma

- Add IS to Plasma Sample

2) Analyte Capture and Clean Up

-10mM PBS

-Water

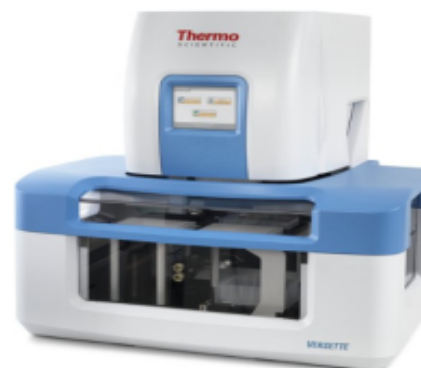
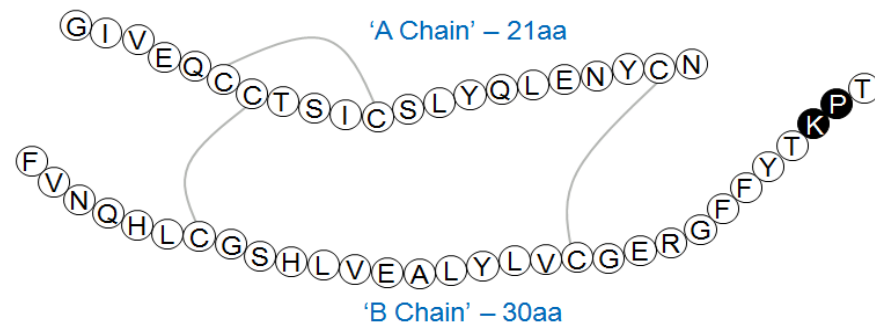
3) Analyte Elution

-63uL of Elution Solution

-21uL of water

Total processing time of ~ 90 minutes

Humalog®





Pinpoint 1.4

- Method Development
- Provides tools for evaluation preliminary data
- Refinement of of targeted quantitative methods.



TraceFinder 4.1

- Comprehensive and quantitative workflows
- Customized Data Visualization
- Flexible Report Generation

TraceFinder 4.1 – Routine Quantitation

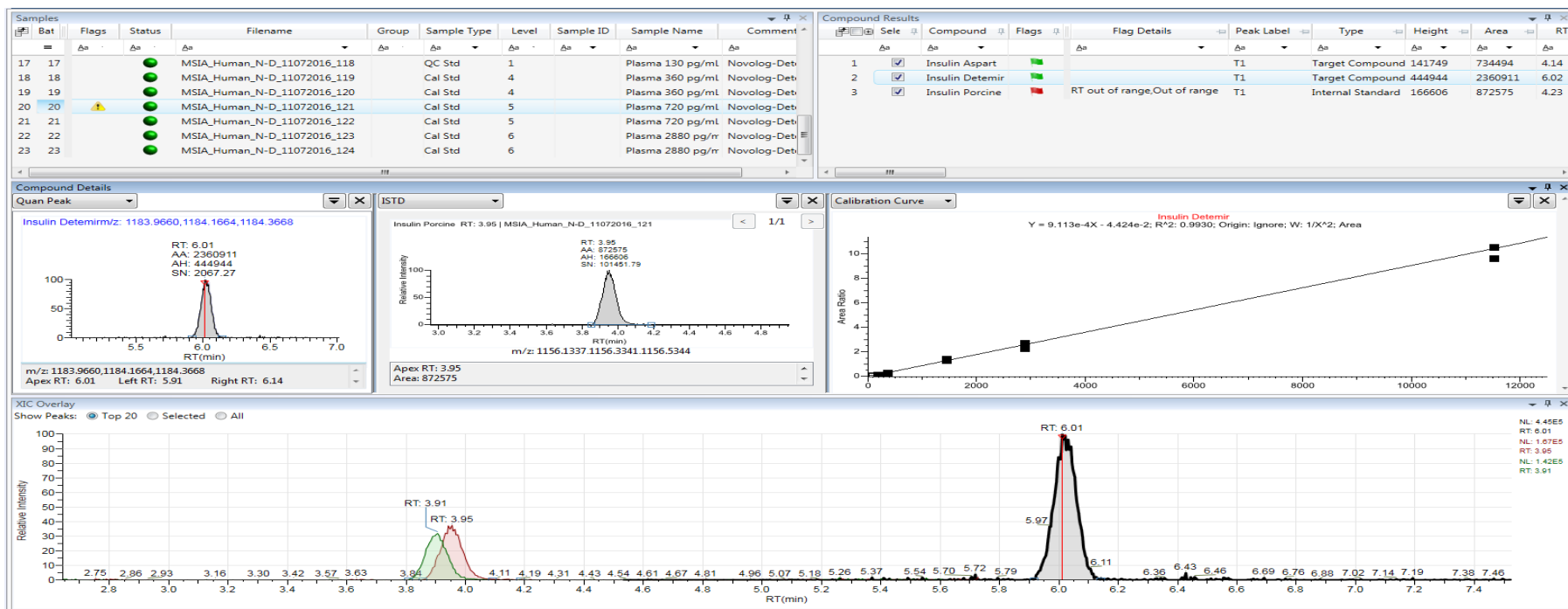
- Database Driven compound management
- Optimized for Streamlined Data Review and Reporting
- Customizable Views Maximum Efficiency



TraceFinder™ 4.1
Optimized for Quantitation

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Thermo
SCIENTIFIC



Chromatographic Separation – Vanquish UPHLC

- Fully Biocompatible System
- 1500 Bar Pump
- New sealing system for higher up time
- Charger Module for Increased Sample Capacity
- Fingertight check valves and Viper technology provide tool free maintenance



High Resolution Accurate Mass – Q Exactive

PERFORMANCE



Q Exactive Focus

- Orbitrap analyzer
- Mass Range m/z 50 - 3000
- Mass Accuracy $<1\text{ppm}$
- Max. Mass Resolution $>70,000$
- Scan speed up to 12Hz MS and 12 Hz MS/MS
- Polarity switching
- Top 3 ddMS2
- SIM Multiplexing
- Refined workflows that are optimal for routine laboratories

BioPharma Option



Q Exactive & Q Exactive Plus

- Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy $<1\text{ppm}$
- Max. Mass Resolution $>140,000$
- Scan speed up to 12Hz MS and MS/MS
- Spectral Multiplexing
- AQT & AABG (QE Plus only)
- Optional Intact Protein Mode and Enhanced Resolution (280k) for QE Plus only

Q Exactive HF

- Ultra High Field Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy $<1\text{ppm}$
- Max. Mass Resolution $>240,000$
- Scan speed up to 18Hz MS and MS/MS
- Spectral Multiplexing
- AQT & AABG
- Optional Intact Protein Mode

Added VALUE for Customer

Three Types of Quantitative Analyses

- **Full MS**

- Selectivity comes through resolution
- Parent mass sensitivity, but limited by background
- Minimal method development



- **SIM**

- Selectivity comes through resolution
- Ultimate sensitivity
 - Interference reduction through quad isolation
- Very little optimization needed



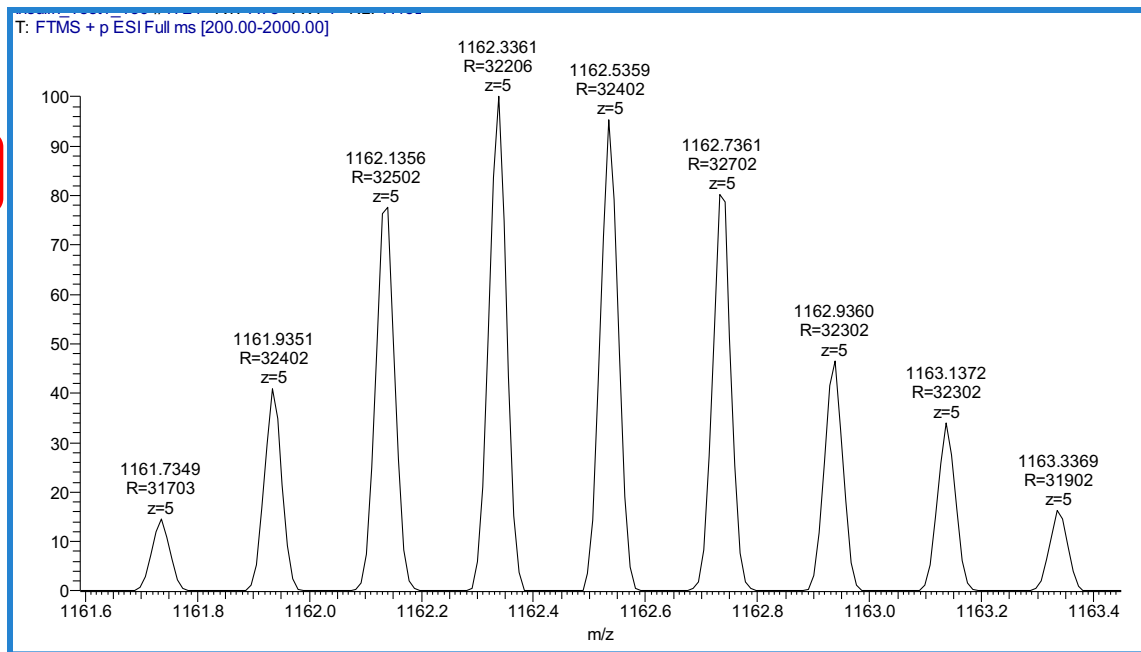
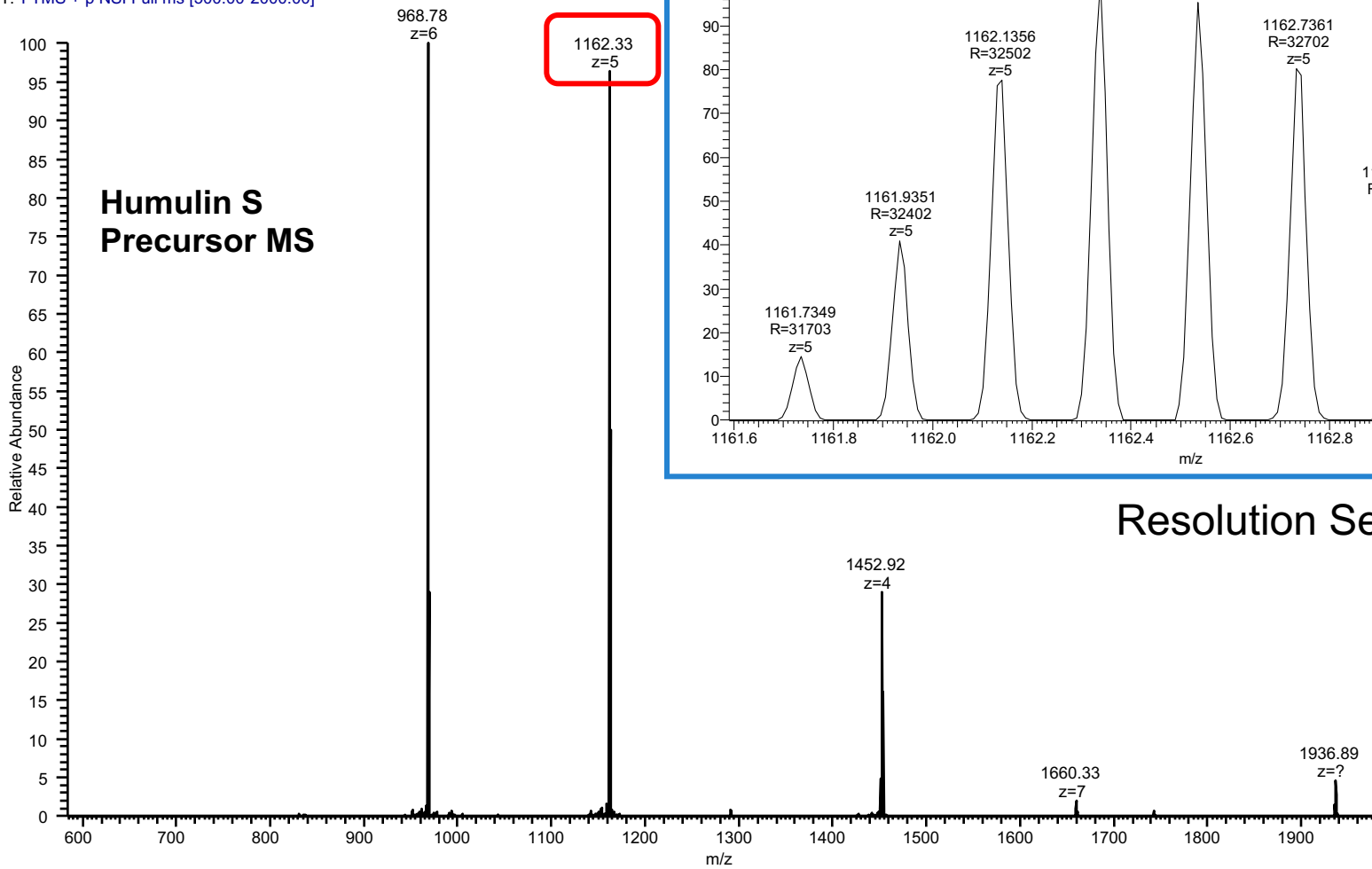
- **PRM**

- Highest level of selectivity (resolution & transition)
- Full MS/MS spectrum acquired in a single scan
- Choose transitions to quantitate on post-acquisition



Full Scan HRAM MS of Charge States and Isotopes

Insulin_Human_tMS2_1 #1428-1445 RT: 11.64-11.72 AV: 3 NL: 7.42E8
T: FTMS + p NSI Full ms [500.00-2000.00]



Resolution Setting = 70,000



Full Scan Analysis – Results

Full Scan Quantitation Results for Insulin Glargine and Insulin Glulisine

STD Conc (pM)	Mean (5-curves)	Stdev	%CV	Accuracy
0	7.42	1.02		
7.5	10.56	0.95	9.04%	40.80%
15	16378	1.42	8.46%	11.87%
30	28.96	1.12	3.85%	-3.46%
60	58.41	1.61	2.75%	-2.66%
120	115.93	1.96	1.69%	-3.39%
240	232.65	2.80	1.20%	-3.06%
480	473.25	14.41	3.04%	-1.41%
960	963.31	6.47	0.67%	0.34%

Calibration Curve Results

STD Conc (pM)	Mean (3 controls x 5-curves)	Stdevp	%CV	Accuracy
50.00	51.21	1.33	3%	2.43%

Intra-Day Reproducibility

STD Conc (pM)	Mean (3 controls x 5-curves)	Stdevp	%CV	Accuracy
50.00	51.07	0.81	2%	2.15%

Inter-Day Reproducibility

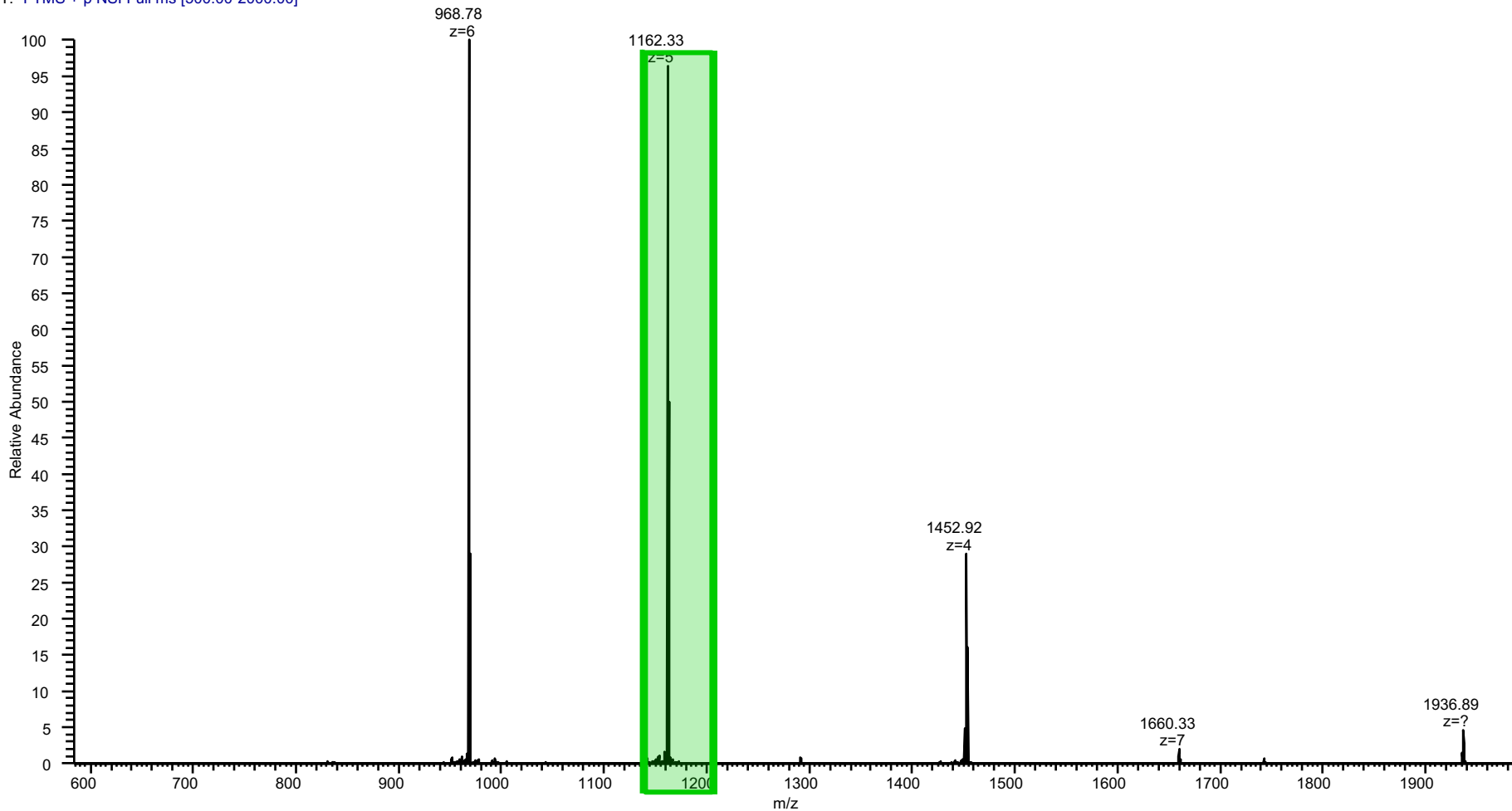
Sample	Spike Conc. (pM)	Exp. Conc. (pM)	Average (pM)	Exp Recovery Conc. (pM)	% Yield
Neat 1	0.00	43.79	44.59		
Neat 2		45.59			
Neat 3		44.38			
Low 1	19.50	65.08	64.11	19.52	100.12%
Low 2		63.65			
Low 3		63.61			
Medium 1	199.50	241.19	237.56	192.97	96.73%
Medium 2		239.80			
Medium 3		231.70			
High 1	919.50	960.91	928.63	884.05	96.14%
High 2		905.35			
High 3		919.64			

Spike and Recovery Study

- LOQ = 90pg/mL
- Simplified Data Acquisition
- Comprehensive Coverage
- Large Injection Volume

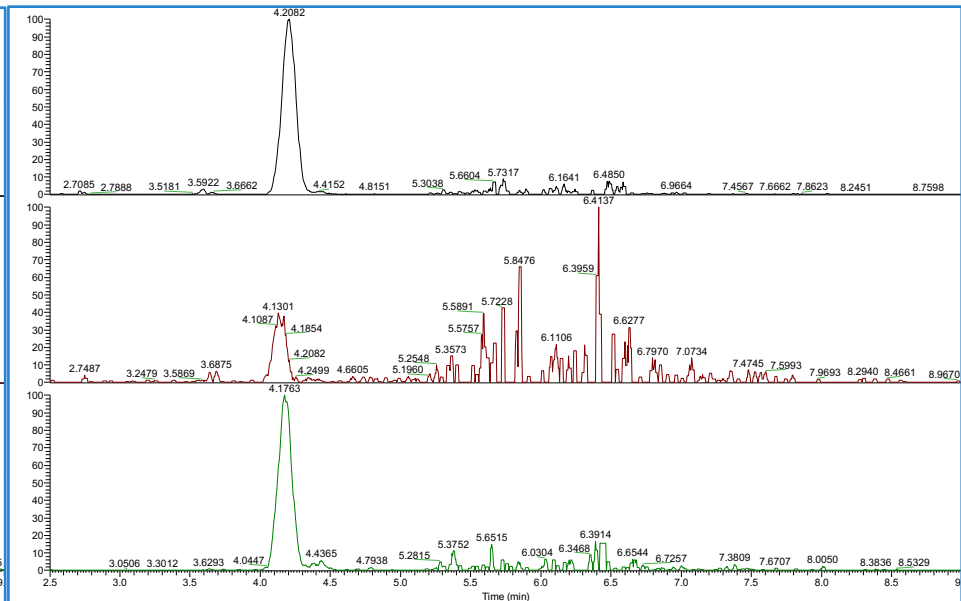
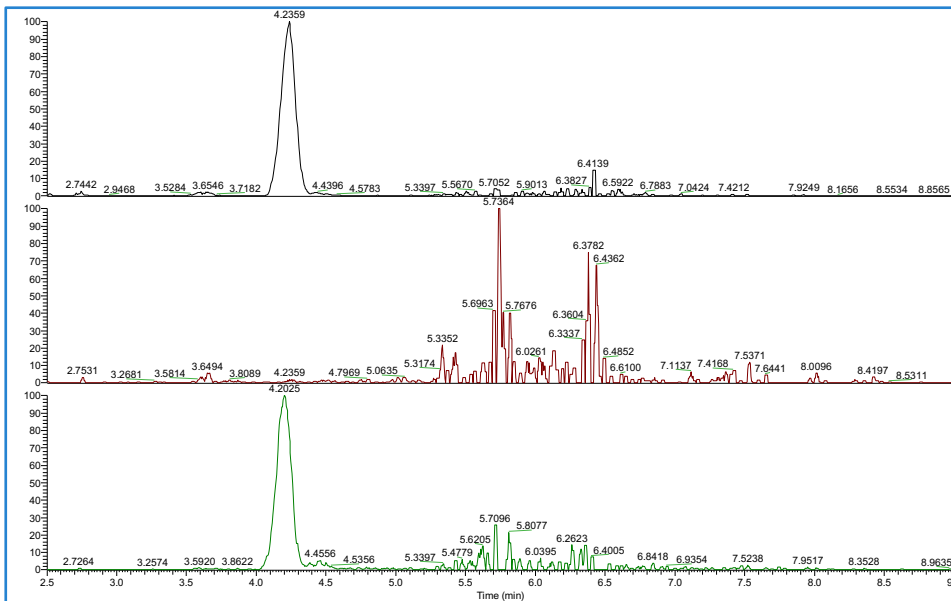
Sensitivity Improvement – SIM Analysis

Insulin_Human_tMS2_1 #1428-1445 RT: 11.64-11.72 AV: 3 NL: 7.42E8
T: FTMS + p NSI Full ms [500.00-2000.00]



Glulisine at 25 pg/mL (4.3 pM)

Glulisine 35uL Injection Volume

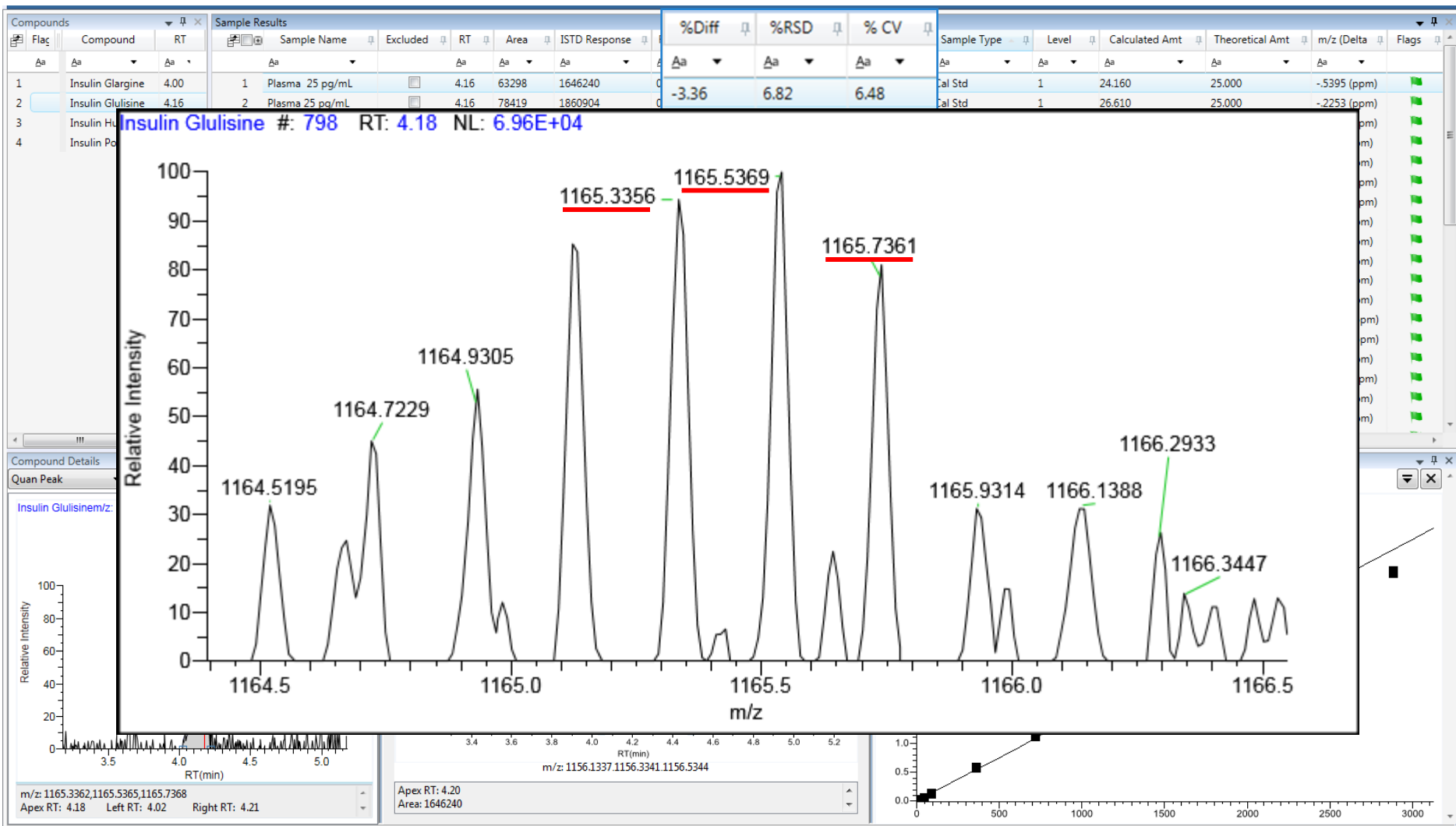


Glulisine Plasma Blank

Glulisine at 25 pg/mL



Insulin Glulisine at 25pg/mL

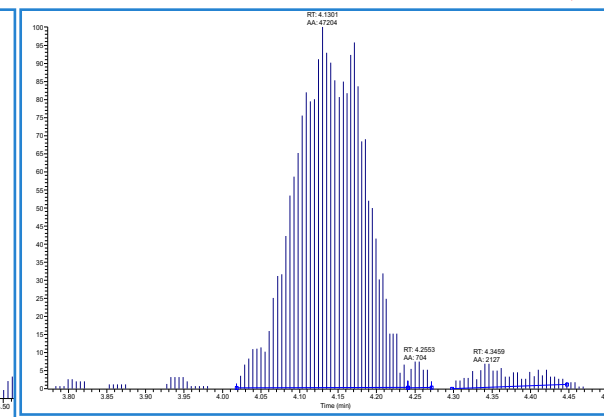
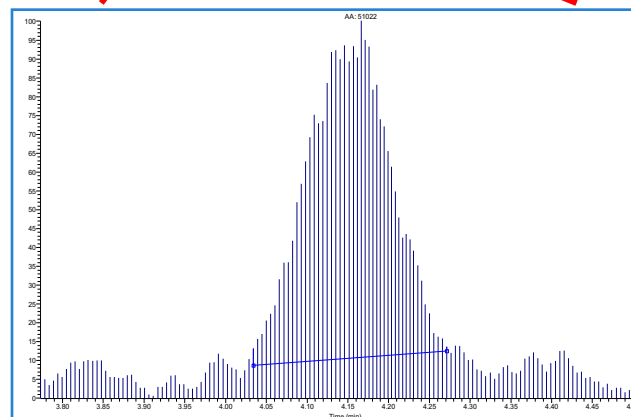
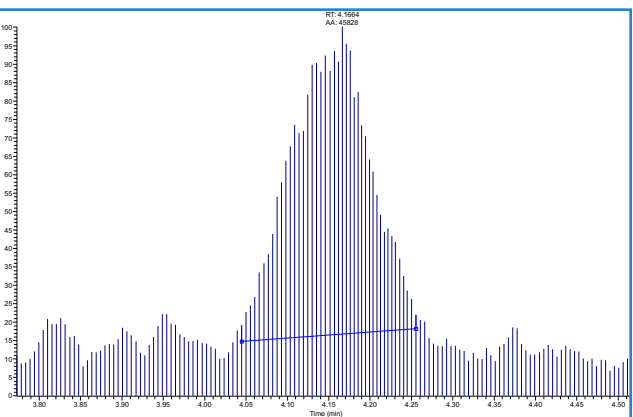
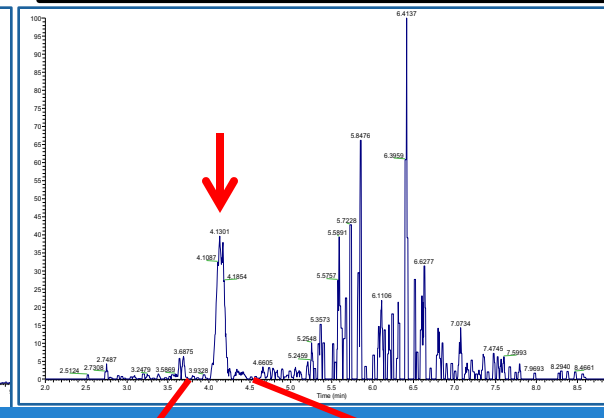
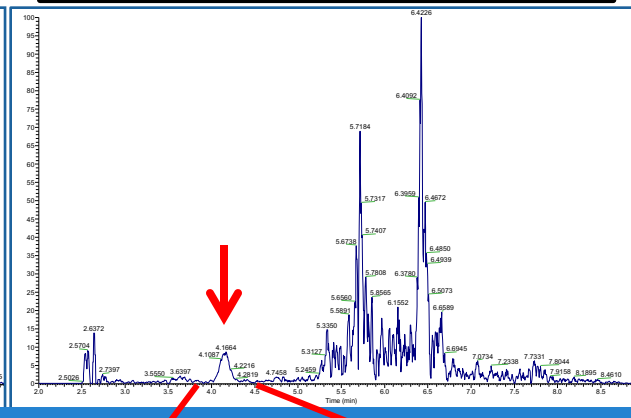
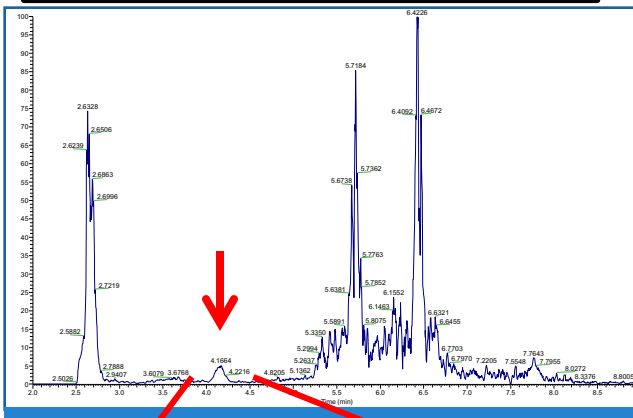


Mass Accuracy – Glulisine at 25 pg/mL

Mass Tolerance = 50 ppm

Mass Tolerance = 25 ppm

Mass Tolerance = 5 ppm

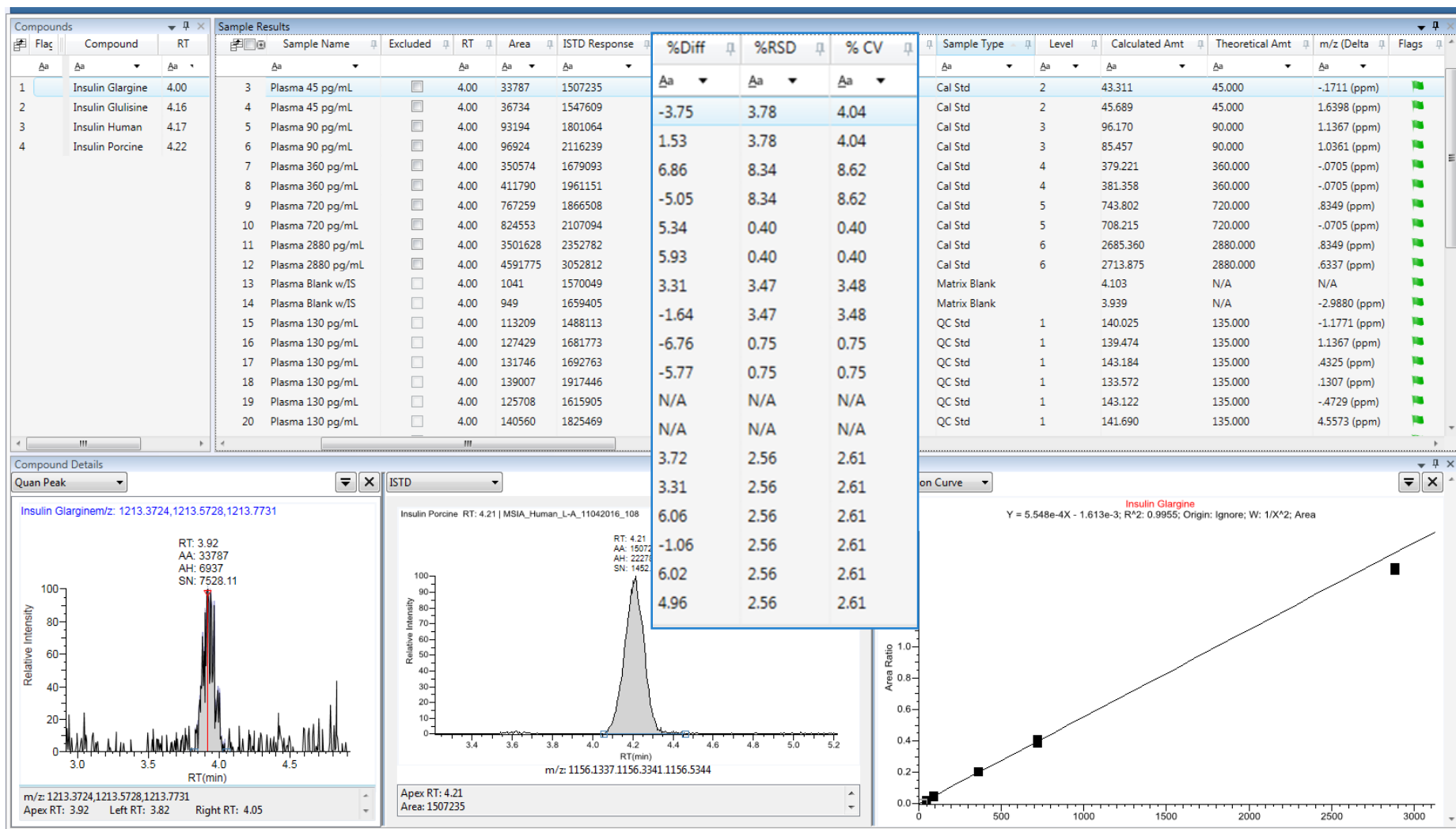


Mass Tolerance = 50 ppm

Mass Tolerance = 25 ppm

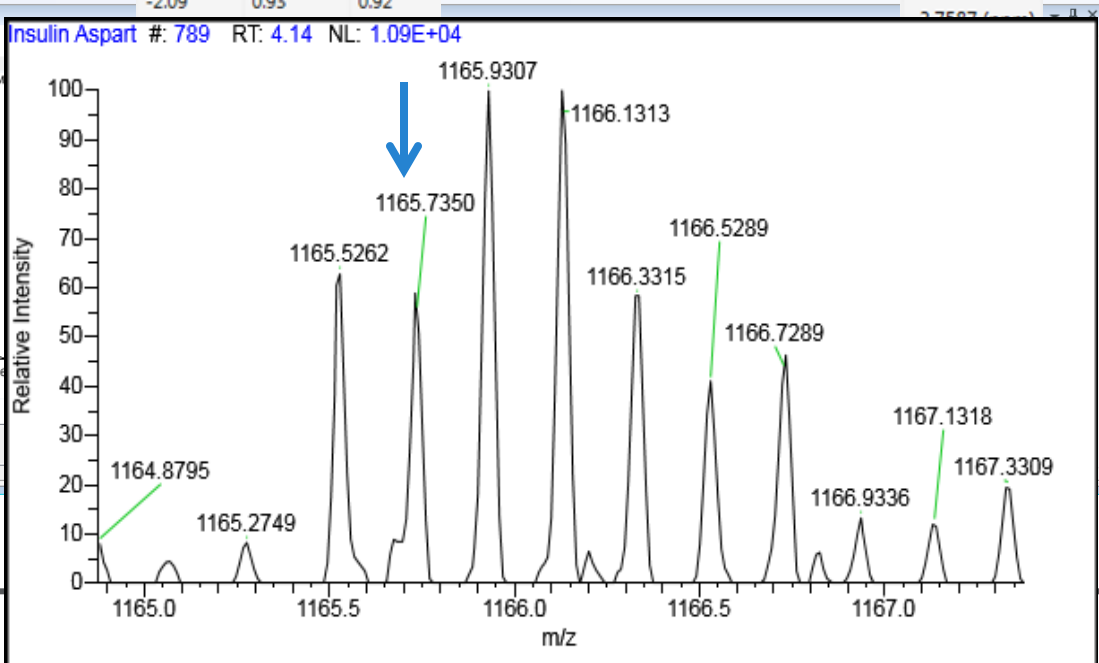
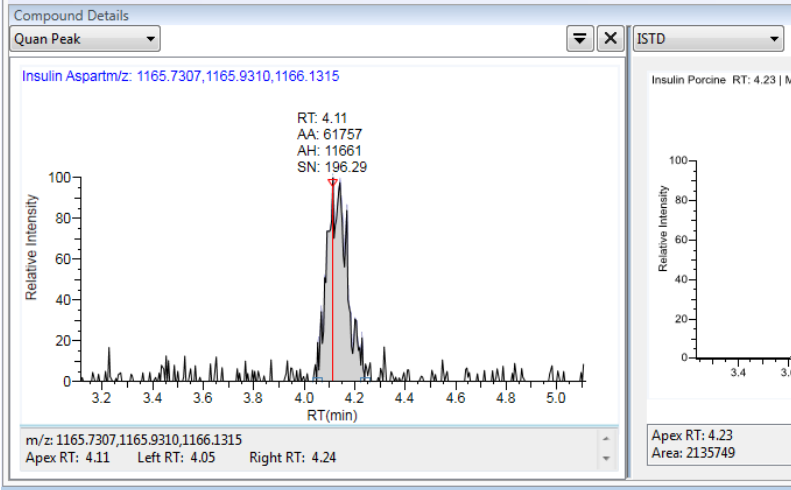
Mass Tolerance = 5 ppm

Insulin Glargine at 45pg/mL

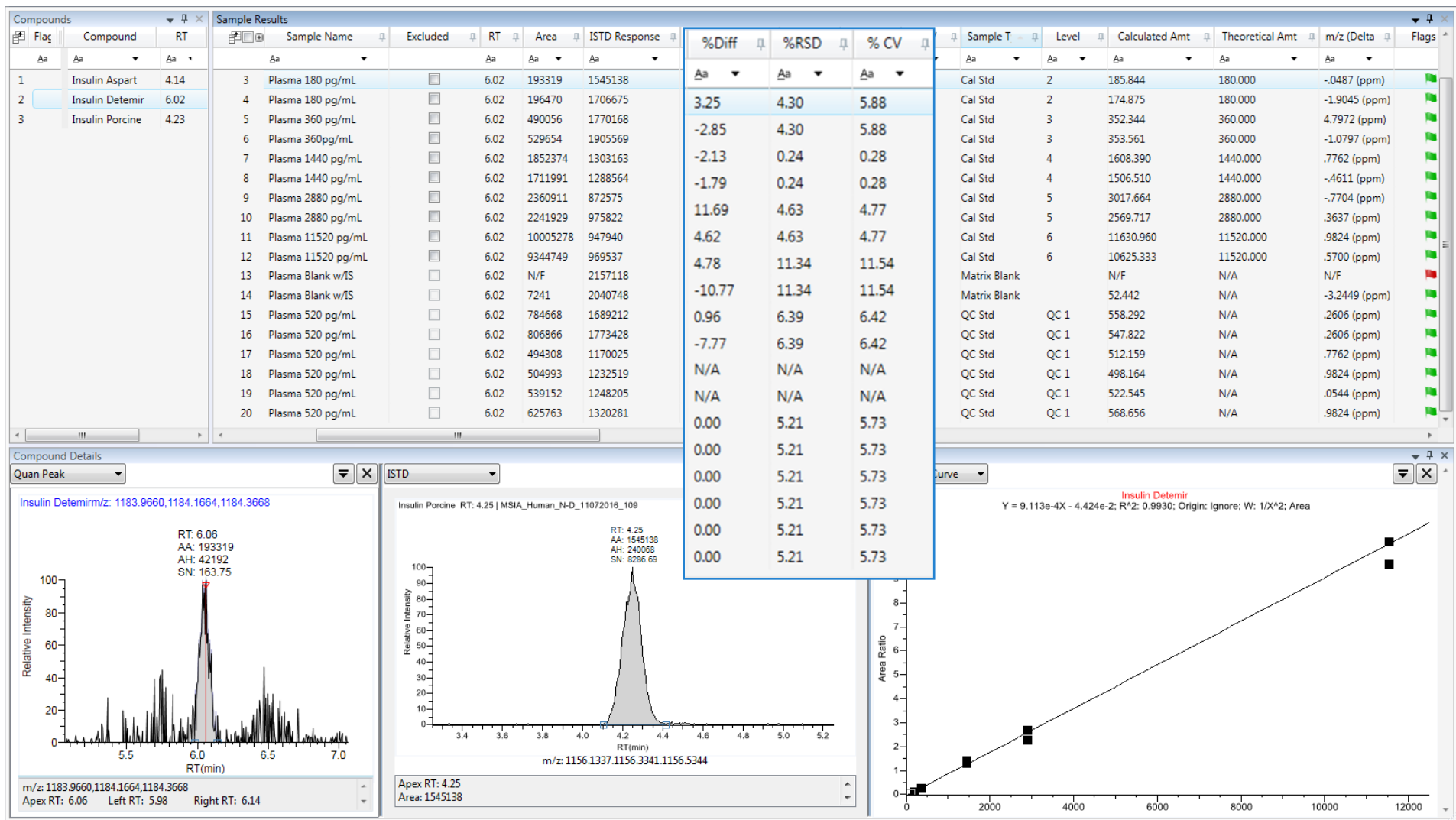


Insulin Aspart at 25pg/mL

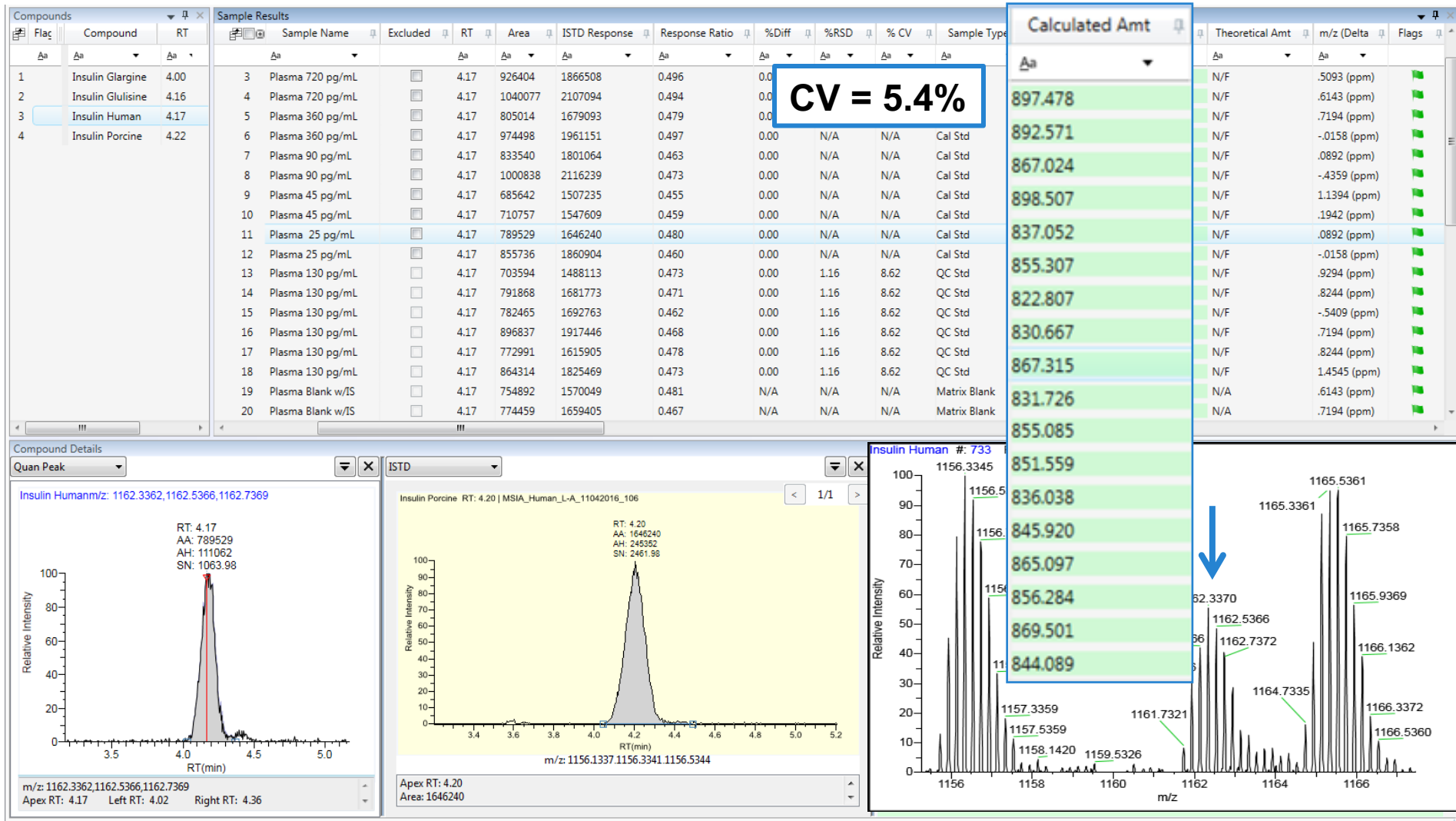
Compounds			Sample Results										Sample Type				Calculated Amt		Theoretical Amt	m/z (Delta)
Flg	Compound	RT	Sample Name	Excluded	RT	Area	ISTD Response	Response R	%Diff	%RSD	% CV	Sample Type	Level	Calculated Amt	Theoretical Amt					
1	Insulin Aspart	4.14	1 Plasma 25 pg/mL	<input type="checkbox"/>	4.14	61757	2135749	0.029	-7.37	5.90	5.62	Std	1	23.157	25.000					
2	Insulin Detemir	6.02	2 Plasma 25 pg/mL	<input type="checkbox"/>	4.14	75924	2425035	0.031	0.69	5.90	5.62	Std	1	25.172	25.000	.5346 (ppm)				
3	Insulin Porcine	4.23	3 Plasma 45 pg/mL	<input type="checkbox"/>	4.14	85967	1545138	0.056	1.47	2.63	2.57	Std	2	45.663	45.000	2.2101 (ppm)				
			4 Plasma 45 pg/mL	<input type="checkbox"/>	4.14	98464	1706675	0.058	1.47	2.63	2.57	Std	2	47.395	45.000	3.6761 (ppm)				
			5 Plasma 90 pg/mL	<input type="checkbox"/>	4.14	203126	1770168	0.115	5.32	2.63	2.57	Std	3	95.451	90.000	-2.6068 (ppm)				
			6 Plasma 90 pg/mL	<input type="checkbox"/>	4.14	211285	1905569	0.111	6.06	2.46	2.43	Std	3	92.190	90.000	-0.937 (ppm)				
			7 Plasma 360 pg/mL	<input type="checkbox"/>	4.14	601041	1303163	0.461	2.43	2.46	2.43	Std	4	387.266	360.000	-0.937 (ppm)				
			8 Plasma 360 pg/mL	<input type="checkbox"/>	4.14	576190	1288564	0.447	7.57	2.20	2.19	Std	4	375.424	360.000	-1.984 (ppm)				
			9 Plasma 720 pg/mL	<input type="checkbox"/>	4.14	734494	872575	0.842	4.28	2.20	2.19	Std	5	707.776	720.000	-1.984 (ppm)				
			10 Plasma 720 pg/mL	<input type="checkbox"/>	4.14	818133	975822	0.838	-1.70	0.28	0.28	Std	5	704.954	720.000	.6394 (ppm)				
			11 Plasma 2880 pg/mL	<input type="checkbox"/>	4.14	2988893	947940	3.153	-2.09	0.28	0.28	Std	6	2654.474	2880.000	-4.299 (ppm)				
			12 Plasma 2880 pg/mL	<input type="checkbox"/>	4.14	3023332	969537	3.118	-7.83	0.78	0.78	Std	6	2625.234	2880.000	.6394 (ppm)				
			13 Plasma Blank w/IS	<input type="checkbox"/>	4.14	605	2157118	0.000	-8.85	0.78	0.78	trix Blank		-0.961	N/A	-4.078 (ppm)				
			14 Plasma Blank w/IS	<input type="checkbox"/>	4.14	2097	2040748	0.001	N/A	N/A	N/A	trix Blank		-0.332	N/A	.6394 (ppm)				
			15 Plasma 130 pg/mL	<input type="checkbox"/>	4.14	267483	1689212	0.158	N/A	N/A	N/A	Std	1	132.172	135.000	.7441 (ppm)				
			16 Plasma 130 pg/mL	<input type="checkbox"/>	4.14	285523	1773428	0.161	N/A	N/A	N/A	Std	1	134.406	135.000	1.1629 (ppm)				
			17 Plasma 130 pg/mL	<input type="checkbox"/>	4.14	189724	1170025	0.162	-2.09	0.93	0.92	Std	1	135.378	135.000	N/A				
			18 Plasma 130 pg/mL	<input type="checkbox"/>	4.14	196175	1232519	0.159				Std	1	132.861	135.000					
			19 Plasma 130 pg/mL	<input type="checkbox"/>	4.14	109102	1249205	0.150				Std	1	132.477	135.000					



Insulin Detemir at 180pg/mL



Insulin Human Endogenous _Ext Curve (Glargine) TF



Summary Table

Full Scan (500-2000 m/z)

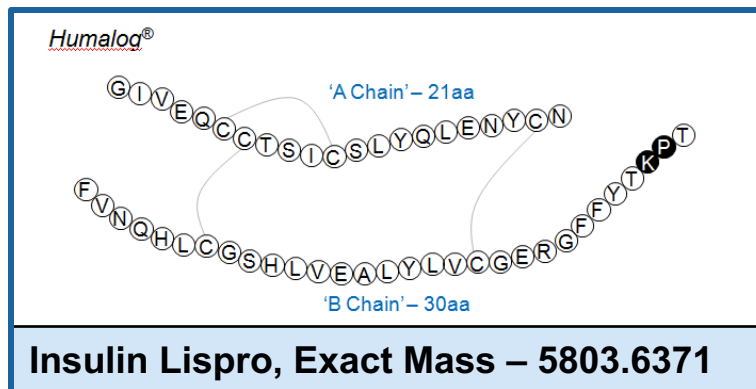
Insulin Analog	Conc. Range pg/mL	Conc. Range pM	Linear Fit (1/x ²)	Mean QC Accuracy (n=6)
Insulin Glulisine	90 – 5,720	15 - 960	0.9943	103.7
Insulin Glargine	90 – 5,720	15 - 960	0.9955	102.2

SIM

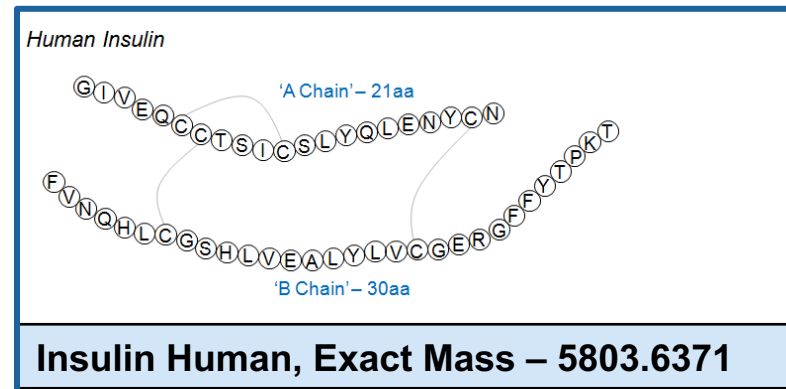
Insulin Analog	Conc. Range pg/mL	Conc. Range pM	Linear Fit (1/x ²)	Mean QC Accuracy (n=6)
Insulin Glulisine	25 - 2,880	4.3 - 496	0.9943	103.7
Insulin Glargine	25 - 2,880	4.3 - 496	0.9955	102.2
Insulin Aspart	45 - 2,88	7.7 - 496	0.9947	98.7
Insulin Detemir	180 - 11,520	30 - 1,986	0.9930	98.8



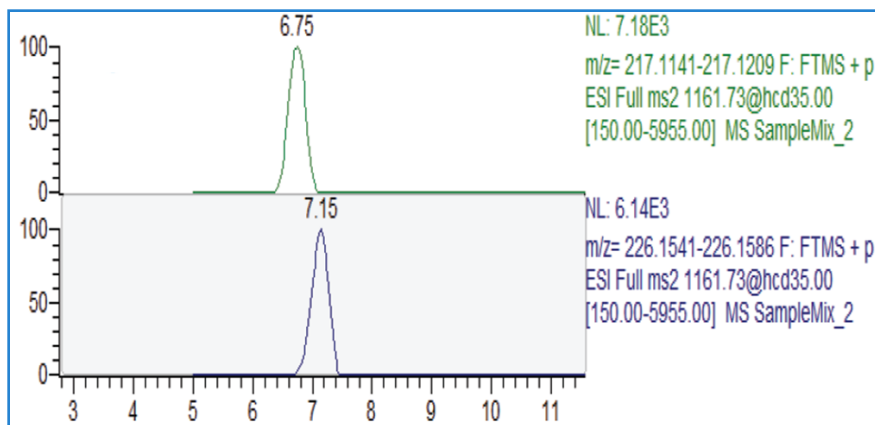
Insulin Lispro and Insulin Endogenous



1162.3362 → 217.1182

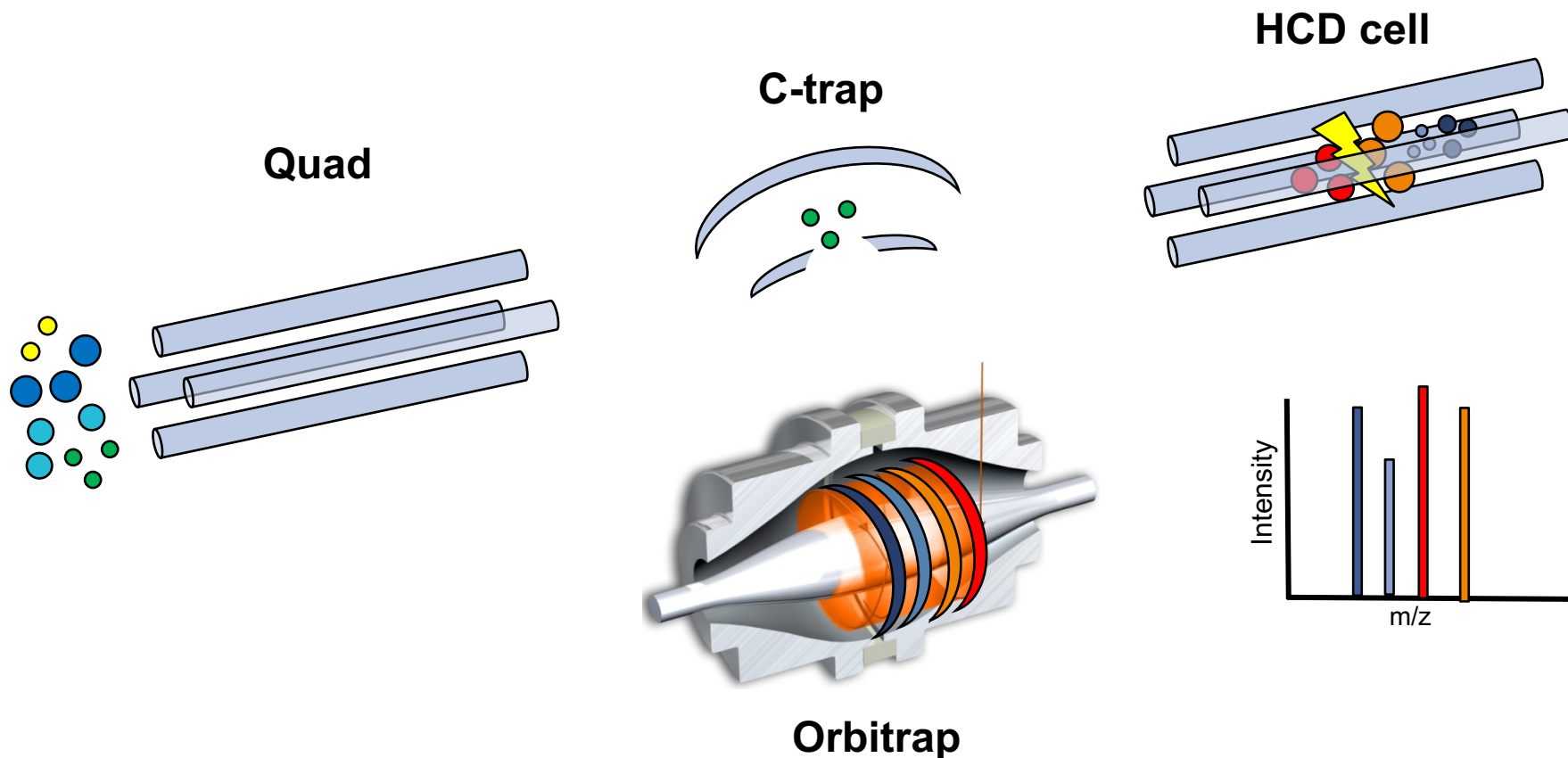


1162.3362 → 226.1548



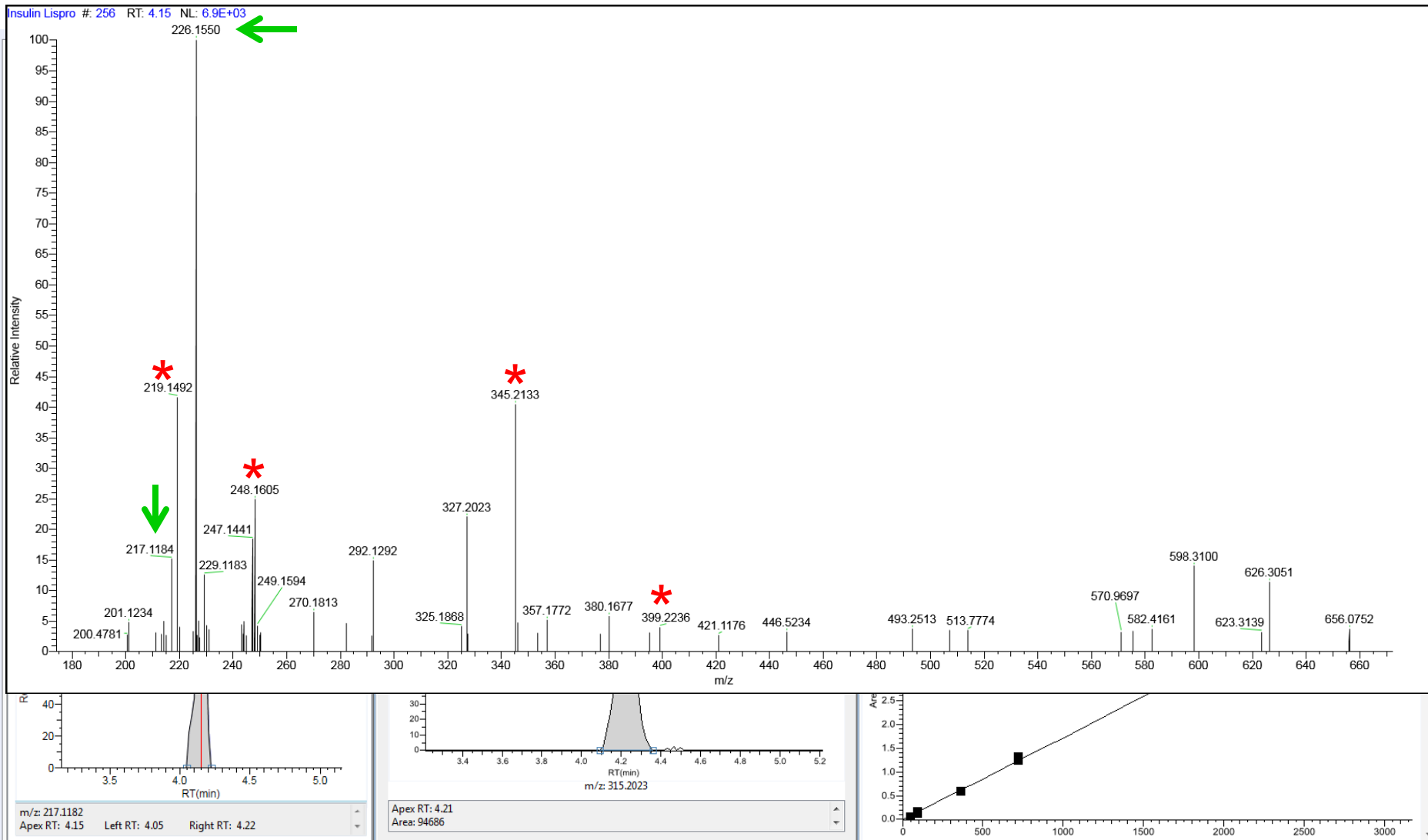
- PRM
 - Simultaneous Fragment Collection
 - Normalized Collision Energy
 - Additional Noise Reduction

PRM – SRM-like Quantitation



- Simultaneous detection of all fragment ions
- Specificity from extracting narrow mass tolerances around fragment ions
- Sensitivity from summing the areas of multiple transitions

Insulin Lispro at 45pg/mL – PRM



Summary Table

Insulin Analog	Conc. Range pg/mL	Conc. Range pM	Linear Fit ($1/x^2$)	Mean QC Accuracy (n=6)
Insulin Glulisine	25 - 2,880	4.3 - 496	0.9943	103.7
Insulin Glargine	25 - 2,880	4.3 - 496	0.9955	102.2
Insulin Aspart	45 - 2,880	7.7 - 496	0.9947	98.7
Insulin Detemir	180 - 11,520	30 - 1,986	0.9930	98.8
Insulin Lispro	45 - 2,880	7.7 - 496	0.9917	103.9



Summary

- Automated Sample Preparation
 - Optimized Protocol
 - Minimized Reagent Prep
- Robust Chromatographic Separation
 - Simple LC Operation
 - Robust Column Performance
- Flexible HRAM Operation
 - Full Scan, SIM, PRM
- High Sensitivity for Insulin Analogs
 - Low pg/mL LOQ



Acknowledgments

- David Sarracino
- Susan Abbatiello
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- Jonathan Josephs
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- Rainer Bauder
- Maciej Bromirski

