Case Study III: Peptide Structural Characterization

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Human Insulin UPLC-MS Method Development and Structural Characterization of Normal and Modified Forms

CHALLENGE: Human Insulin Analysis and Structural Characterization
SOLUTION: UPLC – UV – High Resolution Accurate Mass MS Analysis and MSMS Structural Characterization Workflow

Mass Spec Lab Capabilities in Peptide and Protein Analysis

UPLC – high resolution MS enabled detection of insulin to part per billion (nM) concentration level (Slide 6)

High mass accuracy and mass resolution allow deconvolution of insulin charge states and determination of ion elemental compositions (Slides 7 and Slide 8)

Xevo G2S Quadrupole-Time of Flight (TOF) high resolution MS allows collision induced dissociation of selected signals (MS/MS) to yield interpretable highly accurate fragment masses (Slide 9+)

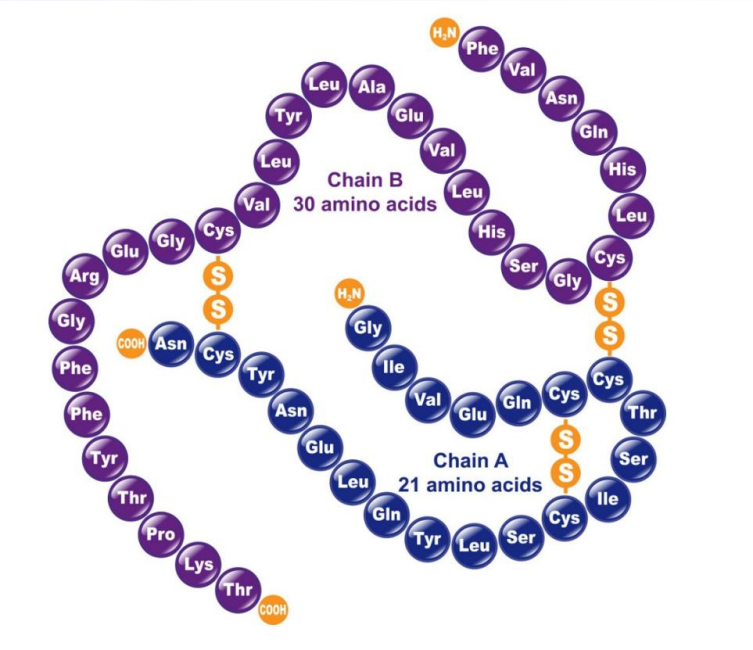
Mass Spec Lab instrument, software capabilities and in-house expertise offer thorough characterization of peptide and protein containing samples

Insulin Project Summary

- UPLC-MS method development and accurate intact mass analysis of native and modified insulin
- Charge state characterization and isotopic envelope based calculation of average delta mass of the modification
- Reduction and alkylation (R&A) of native and modified insulin (unknown)
- Comparison of UPLC-MS traces of R&A native and modified insulin
- Demonstration of MSMS capability for structural elucidation of native and modified insulin typically achieved by software processing, shown here in raw data form
- Identification of the modified amino acid residue section of the modified insulin

Mass Spec Lab instrument, software capabilities and in-house expertise offer thorough characterization of peptide and protein containing samples

Human Insulin Sequence

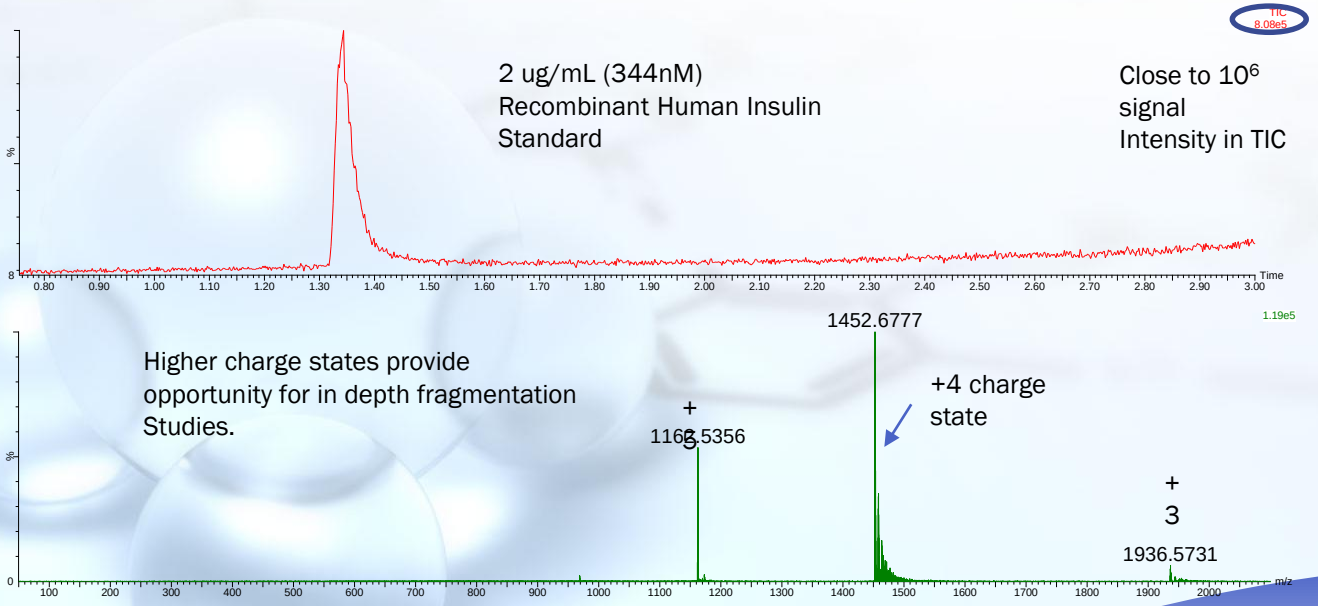


http://www.interactive-biology.com/wp-content/uploads/2012/05/Human-Insulin-Protein-Structure-917x1024.jpg

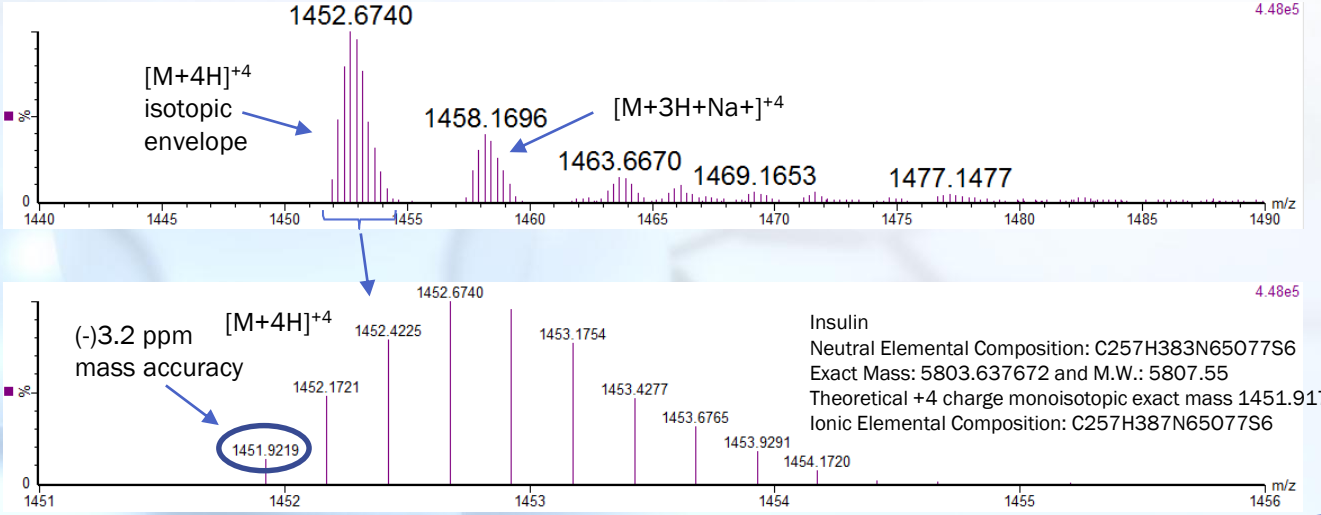
A chain GIVEQCCTSICSLYQLENYCN -> elemental composition $C_{99}H_{155}N_{25}O_{35}S_4$ monoisotopic mass 2382.0000 amu

B chain FVNQHLCGSHLVEALYLVCGERGFFYTPKT -> elemental composition $C_{158}H_{234}N_{40}O_{42}S_2$ monoisotopic mass 3427.6846 amu

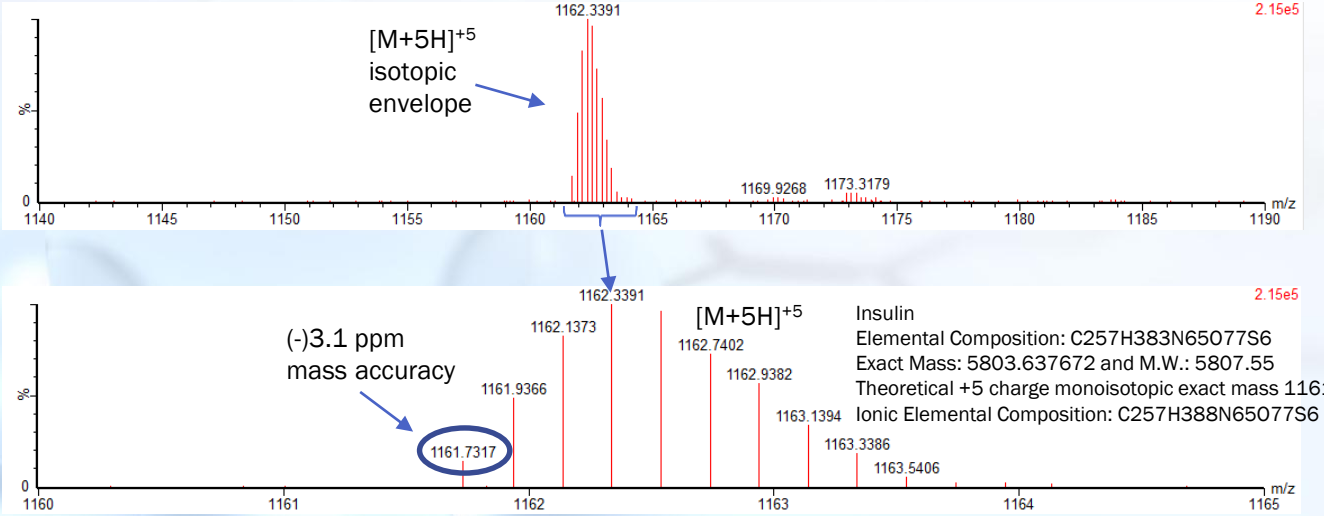
MSL Detection Sensitivity to PPB Level



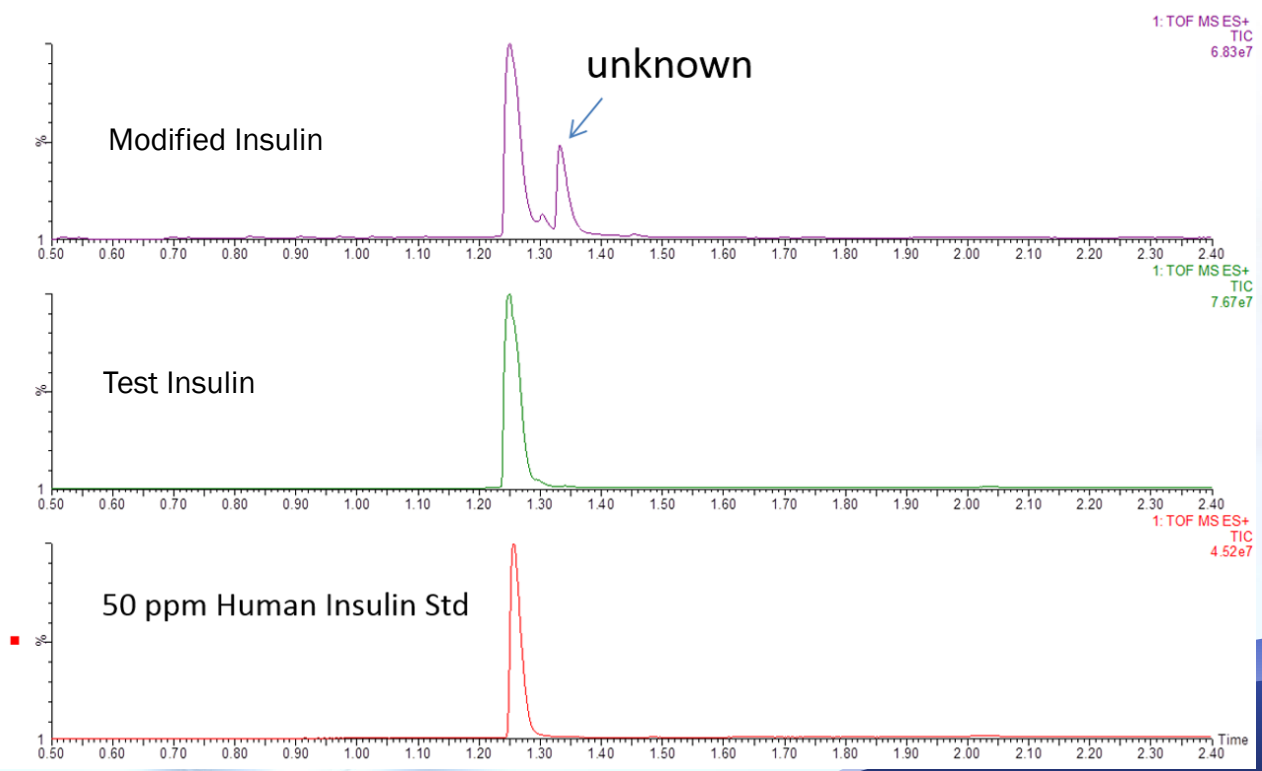
High Mass Accuracy and Resolution for Deconvolution of Insulin +4 Charge State



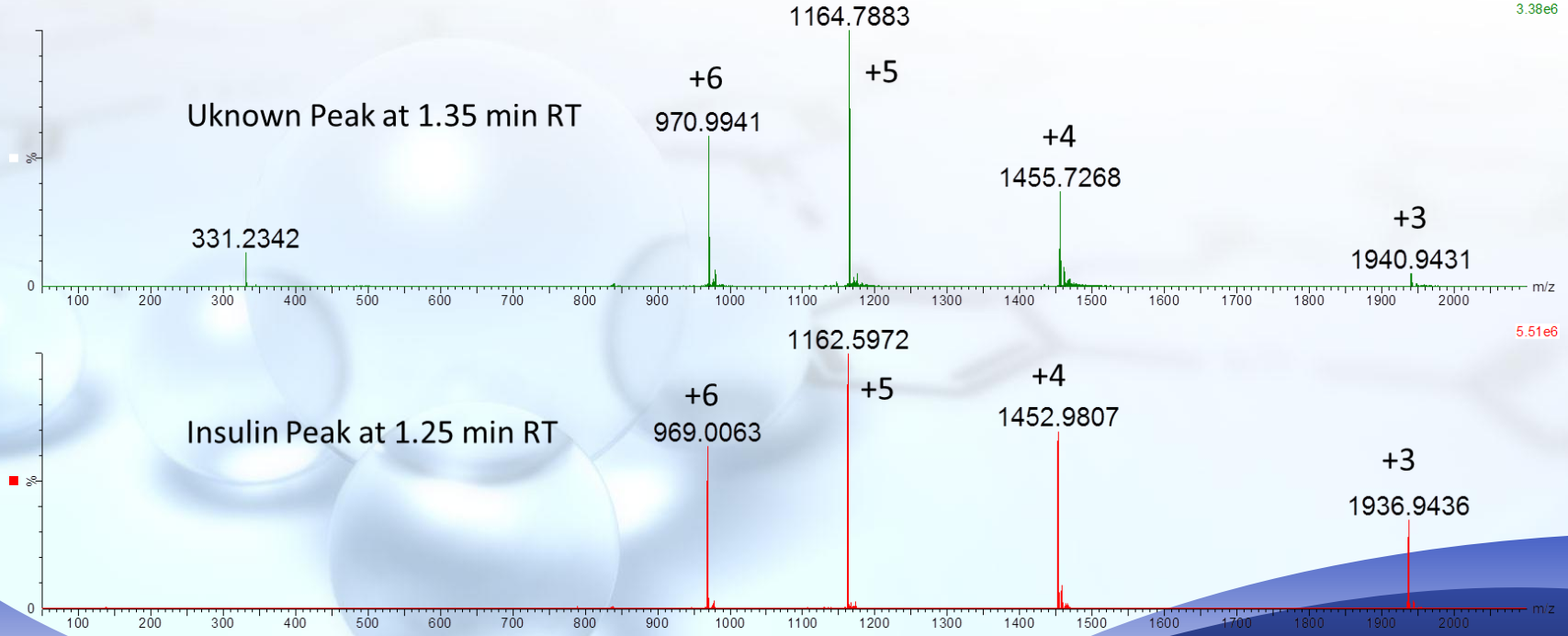
High Mass Accuracy and Resolution for Deconvolution of Insulin +5 Charge State



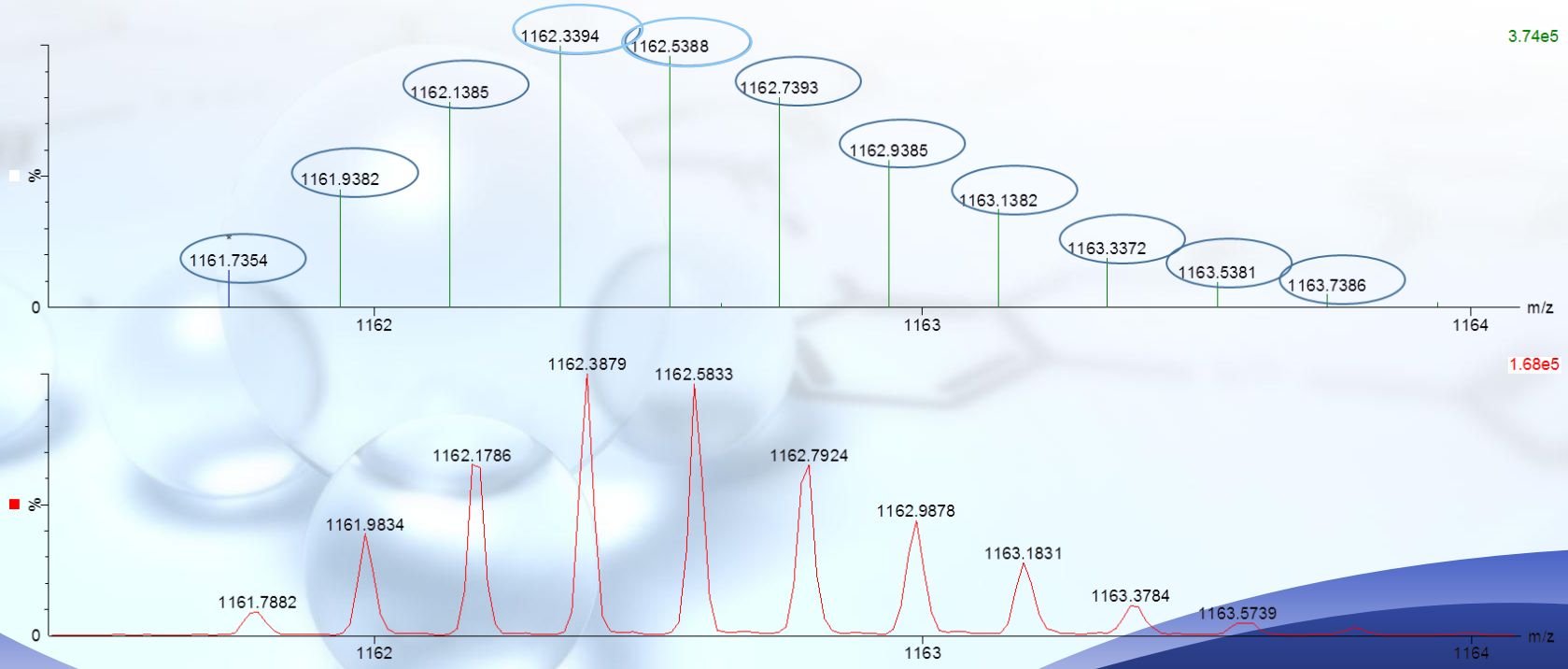
TIC Chromatograms of 50 ppm Human Insulin Standard, Insulin, and Modified Insulin Samples



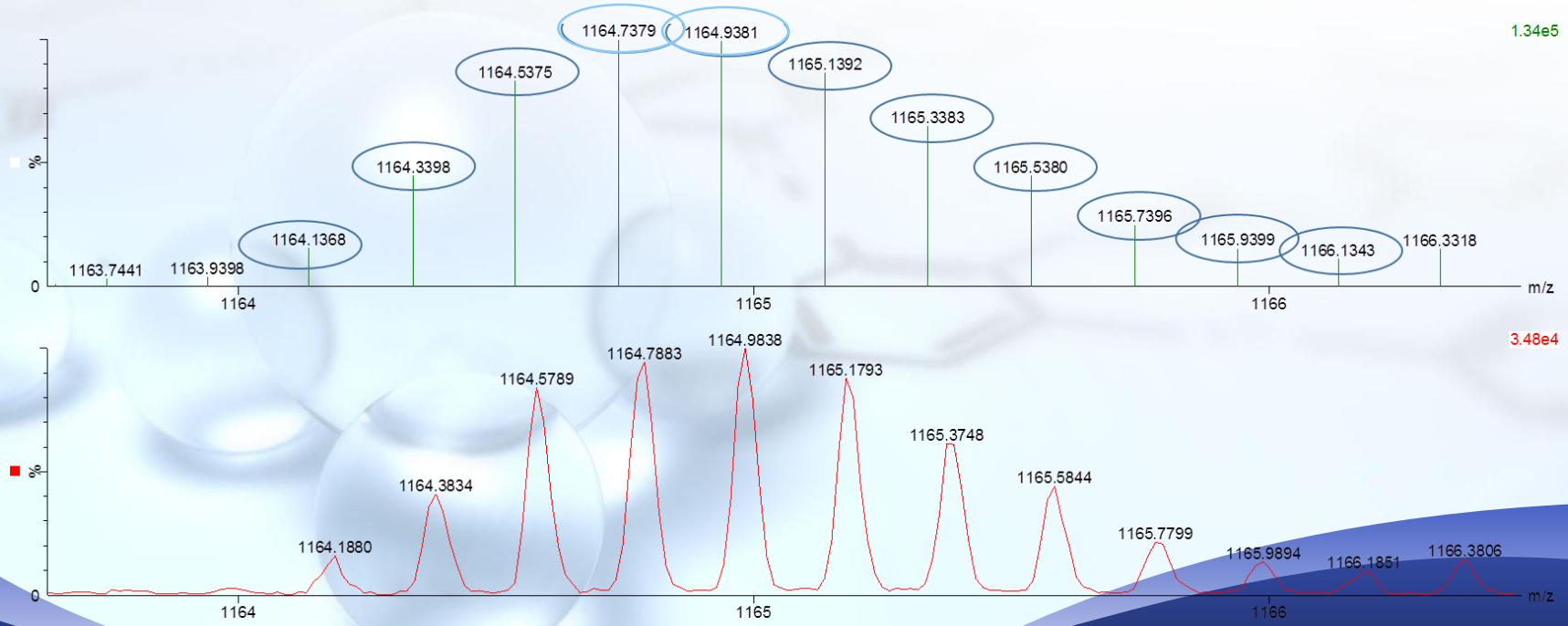
Combined MS Spectra of Insulin Peak at 1.25 min and Unknown Peak at 1.35 min



Combined Continuum and Centroided MS Spectra of +5 Charge Insulin Signal at 1.25 min Retention Time



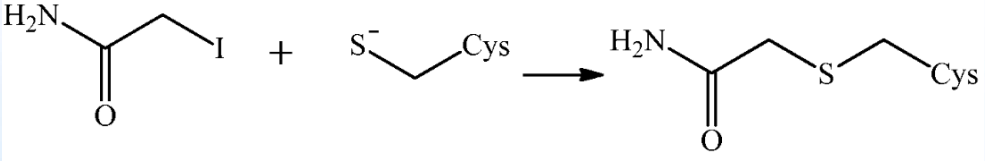
Combined Continuum and Centroided MS Spectra of +5 Charge Unknown Signal at 1.35 min Retention Time



Comparison Table of Recalculated Centroided Masses of the +5 Isotopic Envelope of Insulin and Unknown Peaks

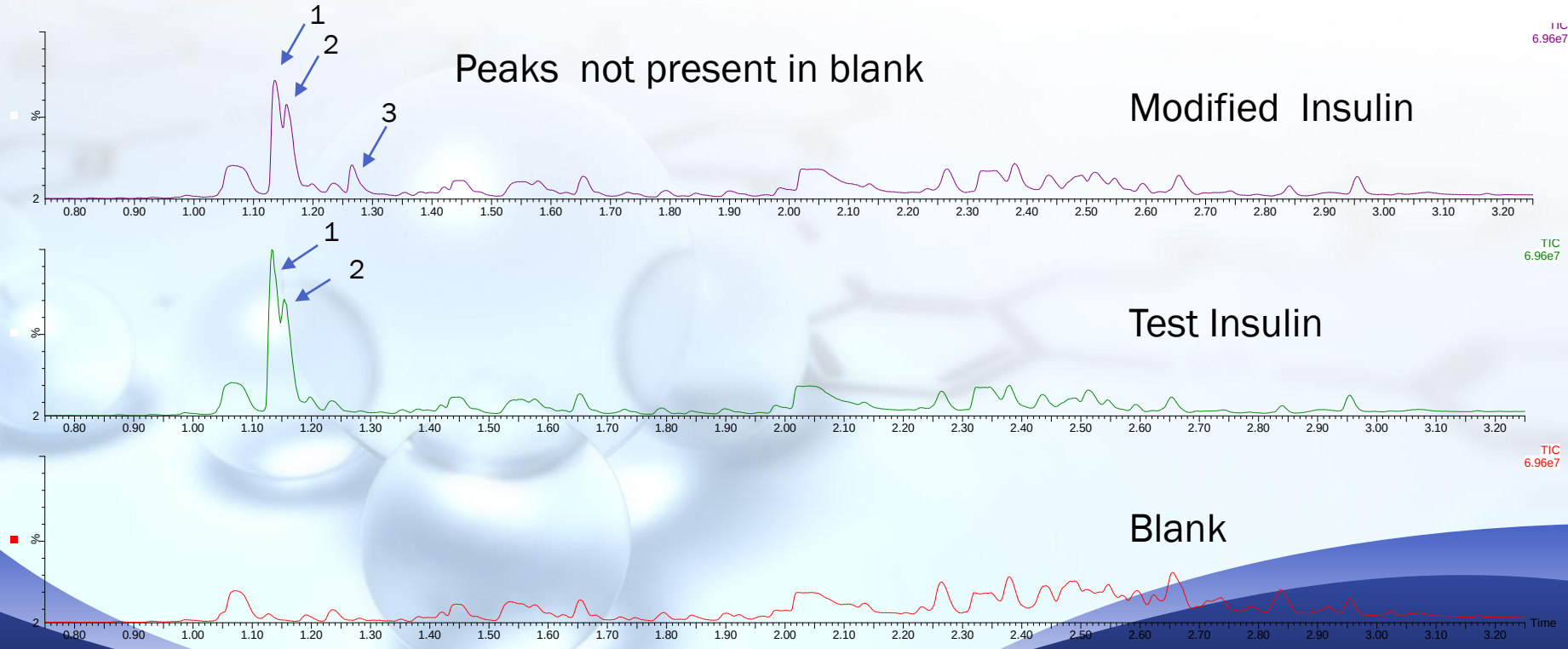
Centroided Data						Mass
Insulin			Unknown			Difference
m/z	m/z x 5		m/z	m/z x 5		
1161.7354	5808.6770		1164.1368	5820.6840		12.0070
1161.9382	5809.6910		1164.3398	5821.6990		12.0080
1162.1385	5810.6925		1164.5375	5822.6875		11.9950
1162.3394	5811.6970		1164.7379	5823.6895		11.9925
1162.5388	5812.6940		1164.9381	5824.6905		11.9965
1162.7393	5813.6965		1165.1392	5825.6960		11.9995
1162.9385	5814.6925		1165.3383	5826.6915		11.9990
1163.1382	5815.6910		1165.5380	5827.6900		11.9990
1163.3372	5816.6860		1165.7396	5828.6980		12.0120
1163.5381	5817.6905		1165.9399	5829.6995		12.0090
1163.7386	5818.6930		1166.1343	5830.6715		11.9785
					Average	11.9996

Reduction Alkylation of Insulin and Unknown

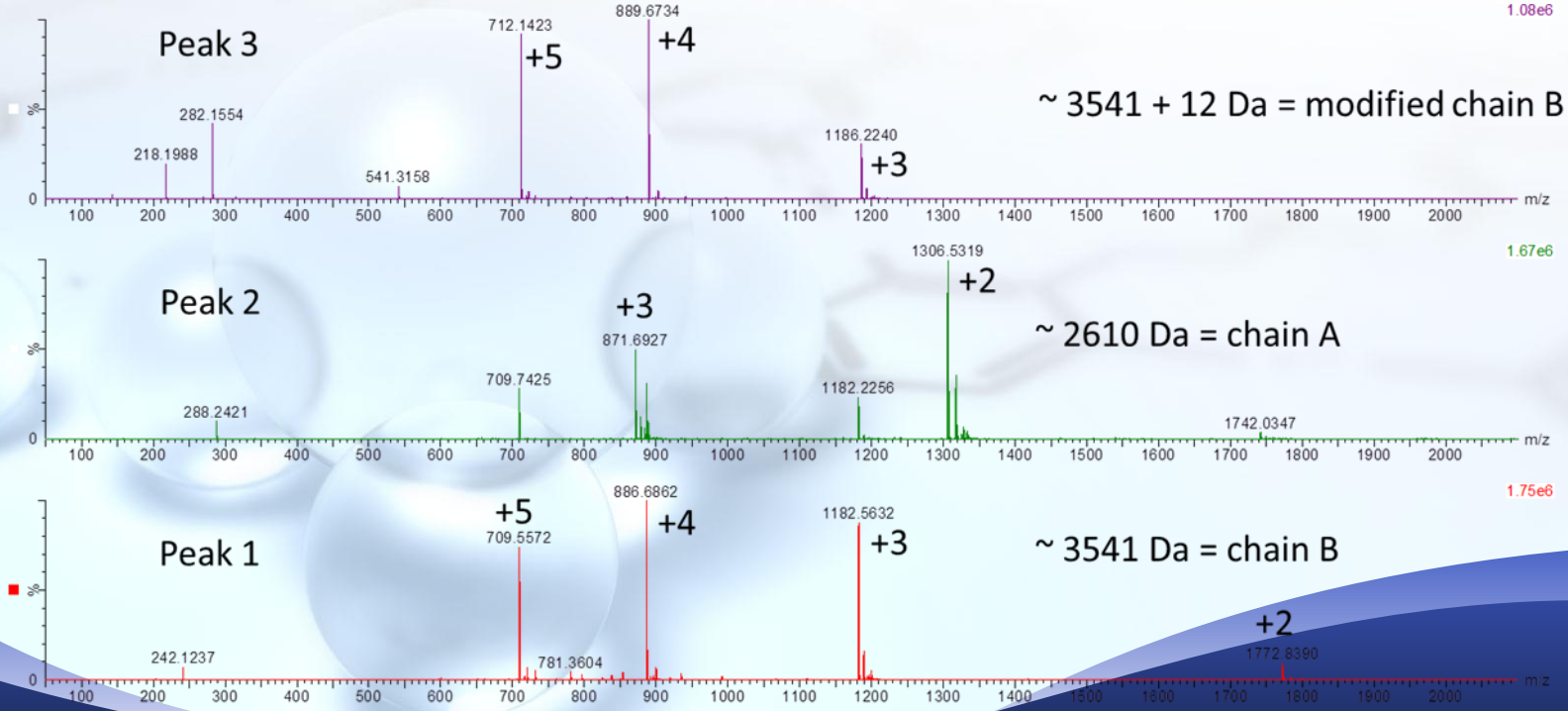


To find the site of modification, the insulin and modified insulin samples were **reduced and alkylated** as shown in reaction scheme above. Iodoacetamide alkylates the reduced disulfide bond by an addition of acetamide with elemental composition C2H4NO with monoisotopic exact mass of 58.0293. The difference between protonated sulfide (-SH) and acetamide alkylated sulfide (-SC2H4NO) corresponds to **57.0215 exact monoisotopic mass**. The knowledge of the exact mass of the alkylating group aids in the prediction of accurate mass of intact insulin and modified insulin chain molecular ions and their fragments.

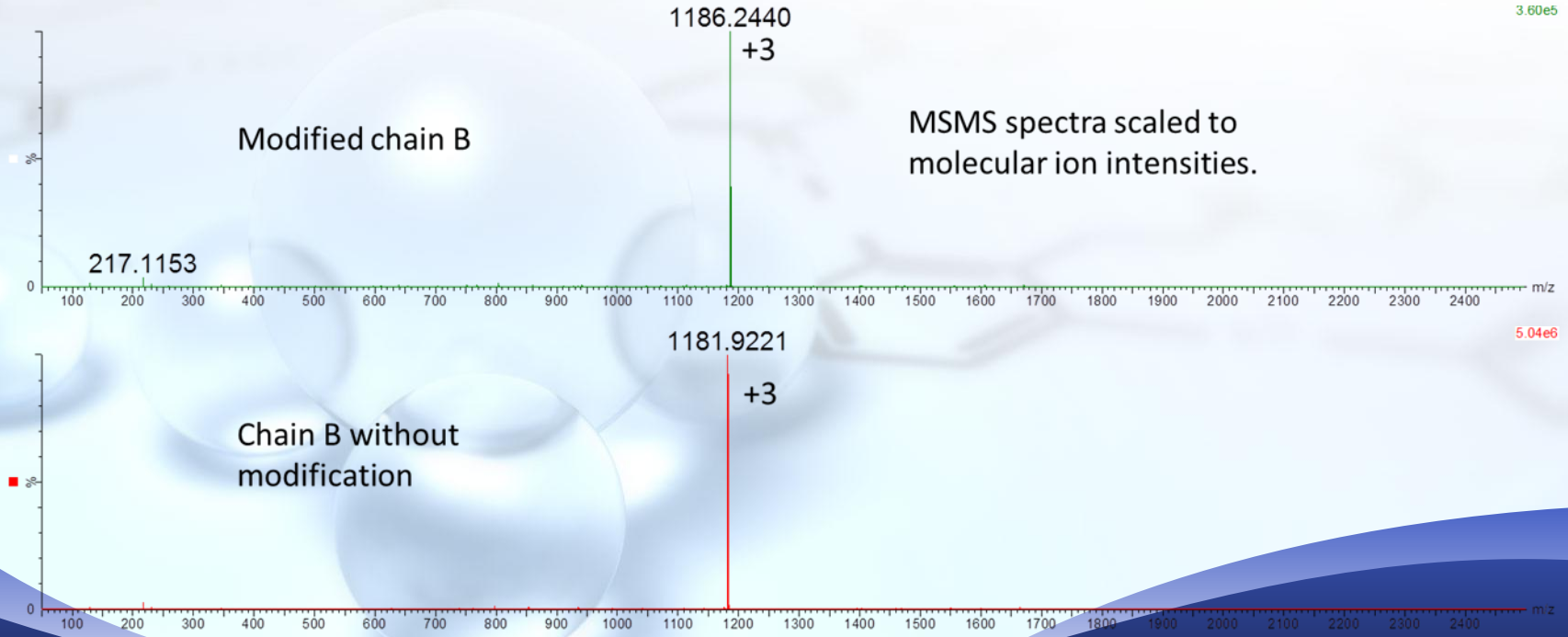
TICs of Reduced and Alkylated Blank, Insulin and Modified Insulin Samples



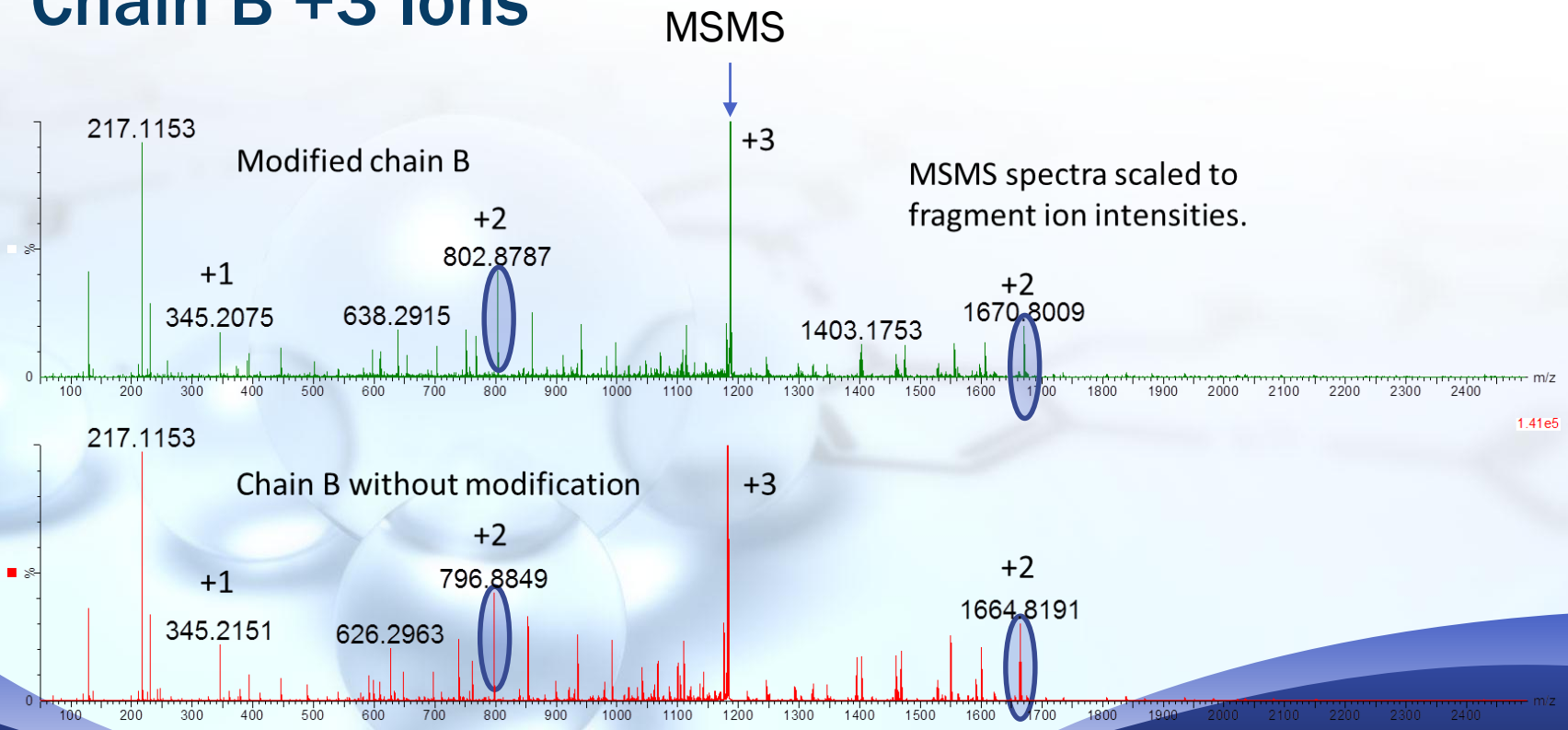
Combined MS Spectra of Peaks 1,2, and 3 of Reduced and Alkylated Modified Insulin Sample



CID MSMS Spectra Scaled to Intensity of Triply Charged Molecular Ions Selected for MSMS



MSMS Spectra of Insulin and Modified Insulin Chain B +3 Ions



Predicted Monoisotopic Accurate Masses of Singly Charged b and y Fragment Ions of Insulin Chain B

Sequence: **FVNQHLCGSHLVEALYLVCGERGFFYTPKT**, pI: **6.90375**

Fragment Ion Table, monoisotopic masses

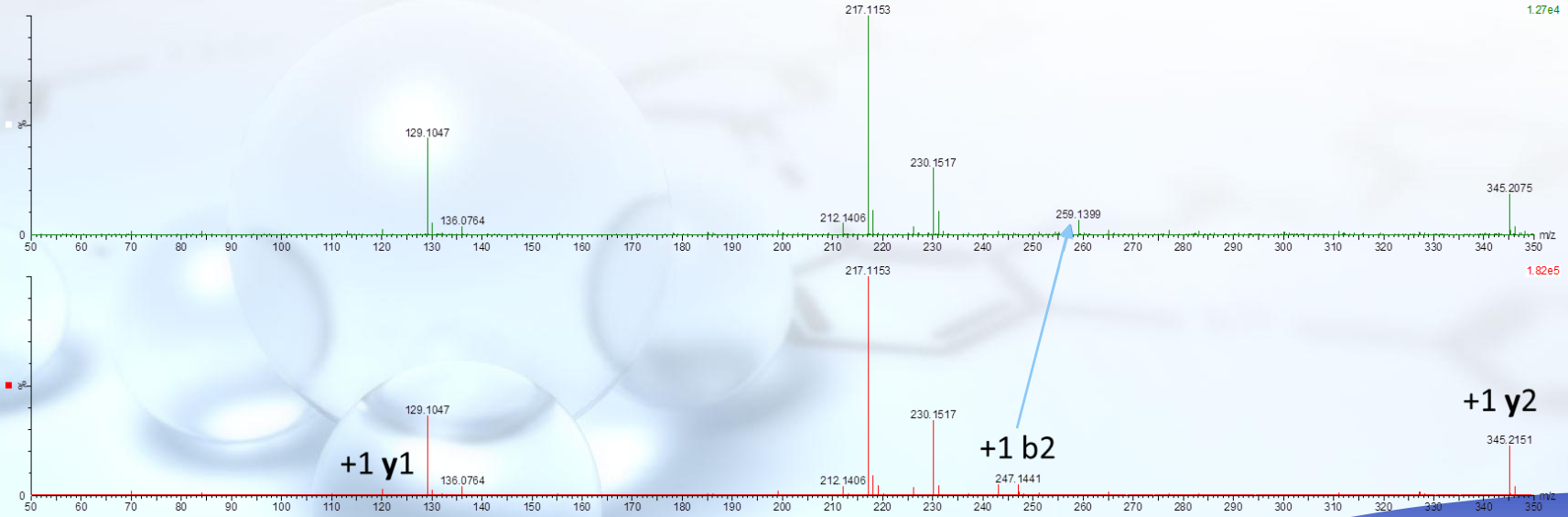
Seq	#	B	Y	# (+1)
F	1	148.07628	3542.73536	30
V	2	247.14470	3395.66695	29
N	3	361.18762	3296.59854	28
Q	4	489.24620	3182.55561	27
H	5	626.30511	3054.49703	26
L	6	739.38918	2917.43812	25
C	7	899.41983	2804.35406	24
G	8	956.44129	2644.32341	23
S	9	1043.47332	2587.30195	22
H	10	1180.53223	2500.26992	21
L	11	1293.61629	2363.21101	20
V	12	1392.68471	2250.12694	19
E	13	1521.72730	2151.05853	18
A	14	1592.76441	2022.01594	17
L	15	1705.84848	1950.97882	16
Y	16	1868.91181	1837.89476	15
L	17	1981.99587	1674.83143	14
V	18	2081.06428	1561.74737	13
C	19	2241.09493	1462.67895	12
G	20	2298.11640	1302.64830	11
E	21	2427.15899	1245.62684	10
R	22	2583.26010	1116.58425	9
G	23	2640.28156	960.48314	8
F	24	2787.34998	903.46167	7
F	25	2934.41839	756.39326	6
Y	26	3097.48172	609.32485	5
T	27	3198.52940	446.26152	4
P	28	3295.58216	345.21384	3
K	29	3423.67712	248.16108	2
T	30	3524.72480	120.06611	1

Mass/Charge Table

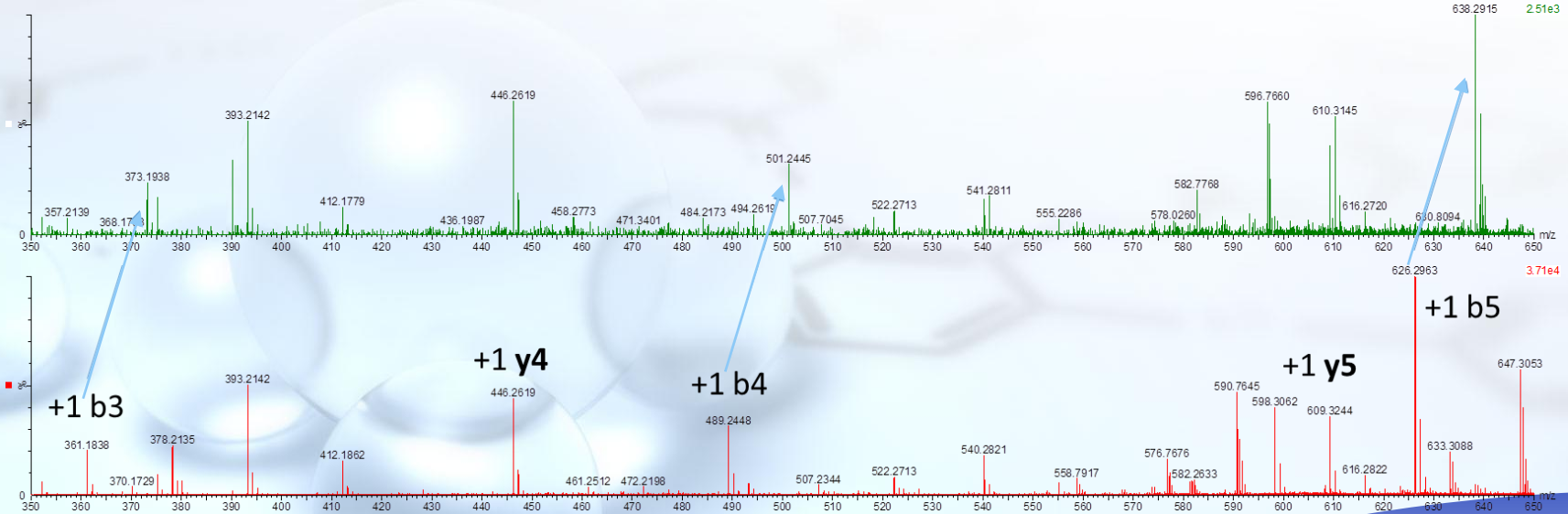
	Mass	
	Mono	Avg
(M)	3541.72754	3544.00173
$(M+H)^{+}$	3542.73536	3545.00967
$(M+2H)^{2+}$	1771.87162	1773.00883
$(M+3H)^{3+}$	1181.58370	1182.34188
$(M+4H)^{4+}$	886.43974	887.00841

Modifications:
* To residue C added the value 57.021464

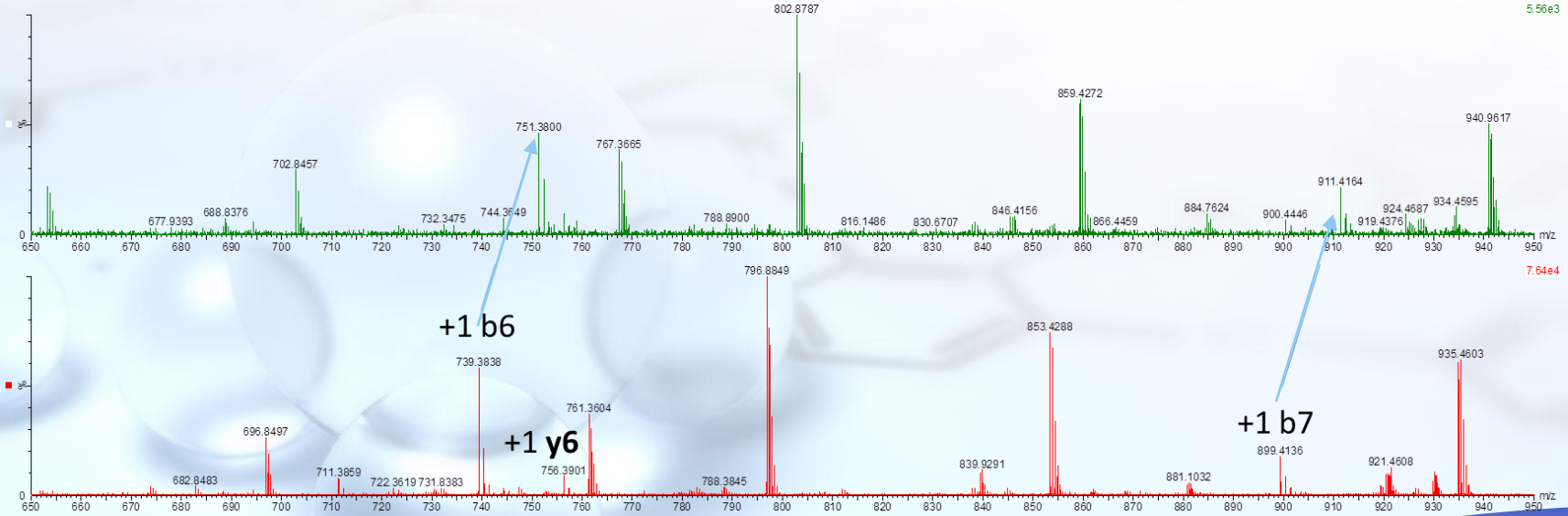
MSMS Spectra of Native and Modified Chain B (50 – 350 m/z Mass Range)



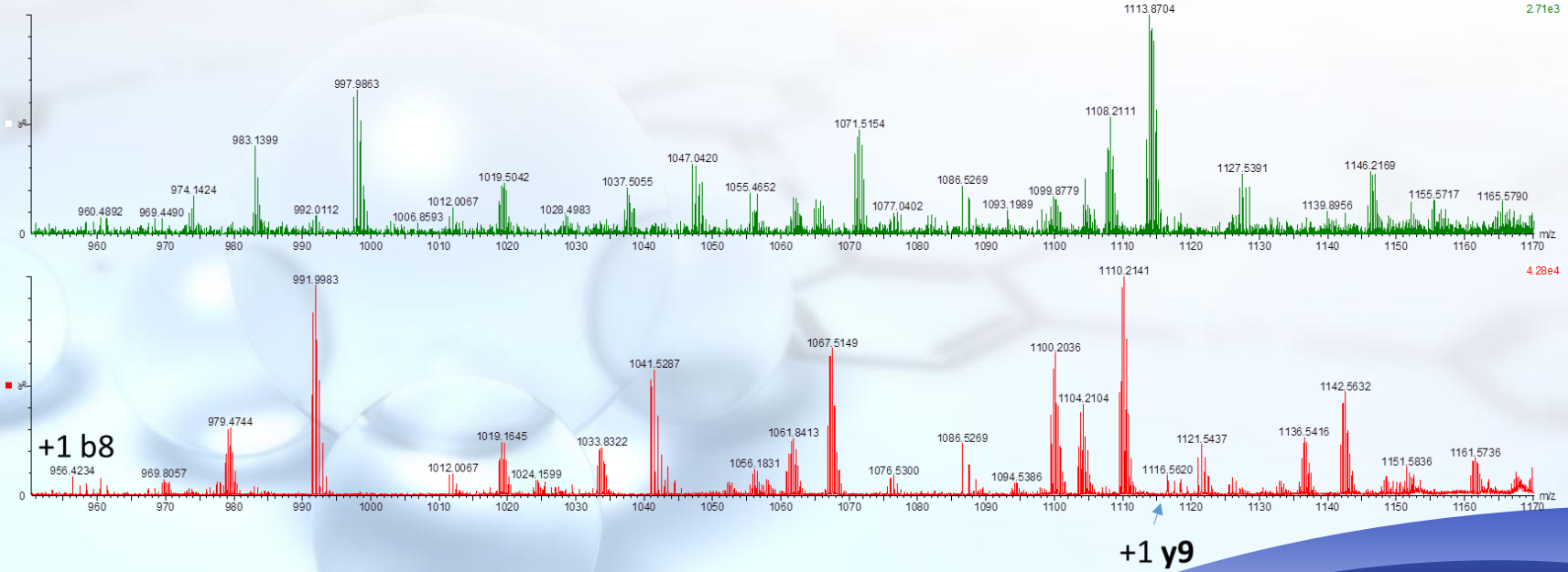
MSMS Spectra of Native and Modified Chain B (350 – 650 m/z Mass Range)



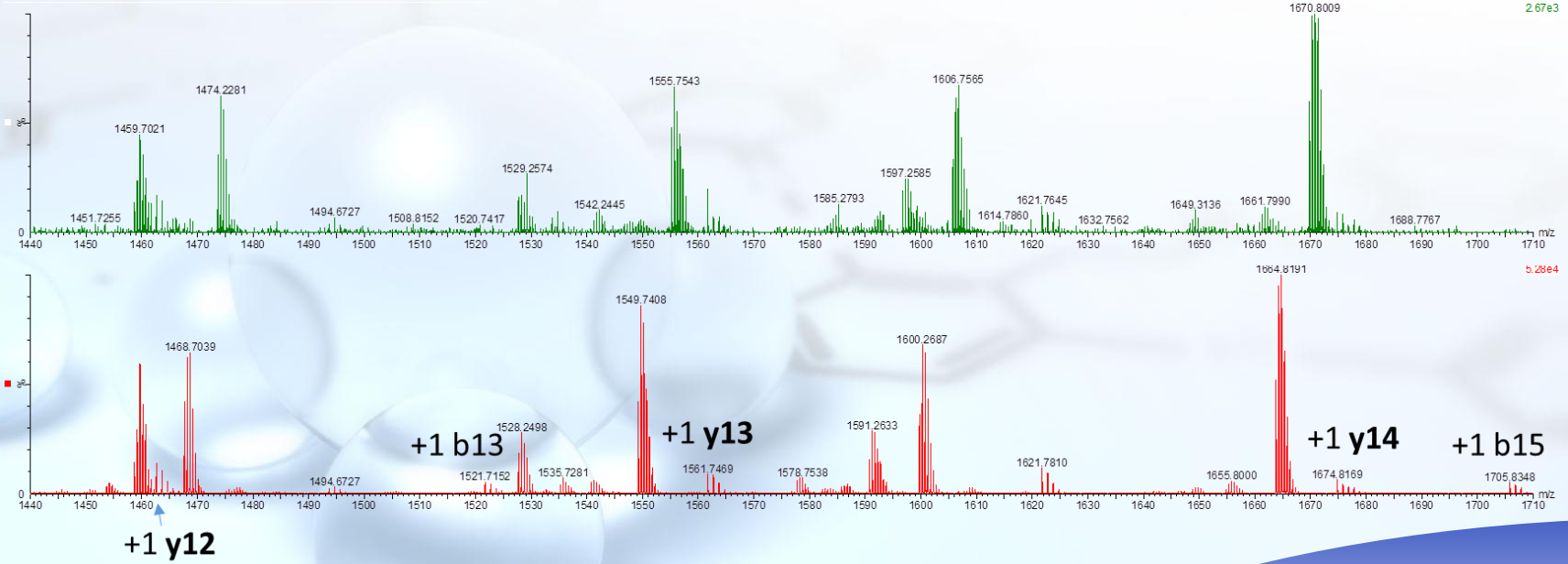
MSMS Spectra of Native and Modified Chain B (650 – 950 m/z Mass Range)



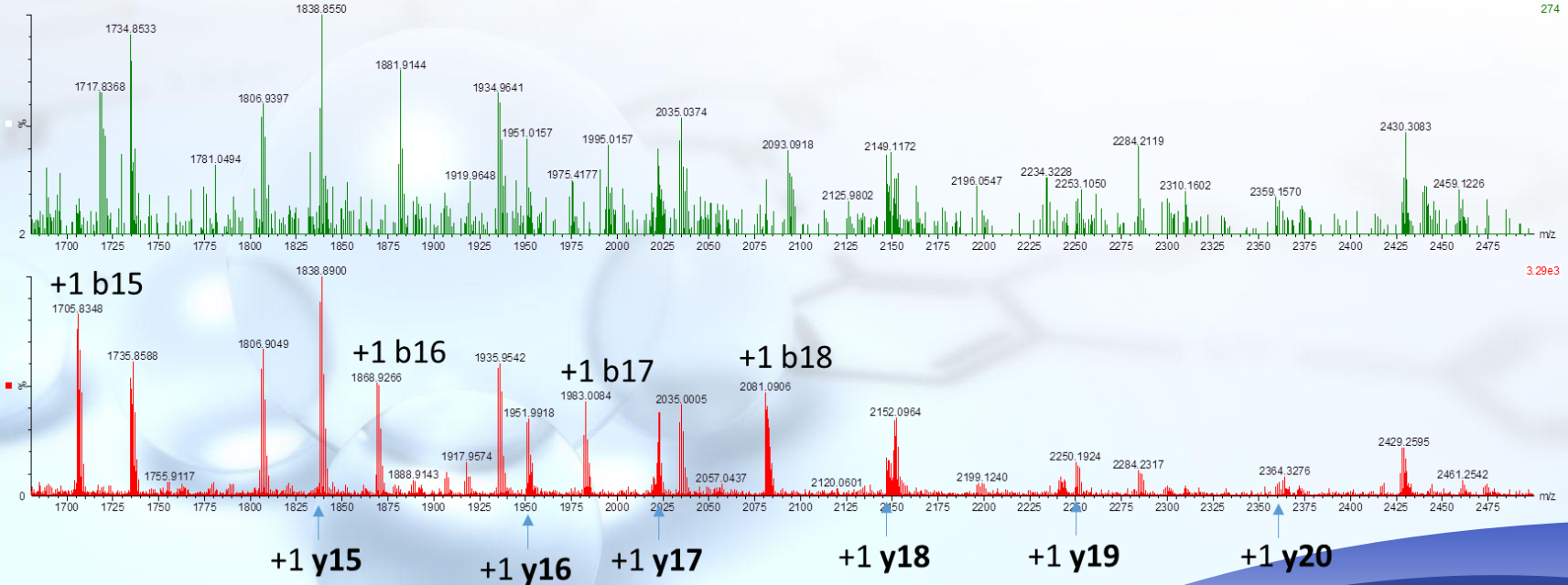
MSMS Spectra of Native and Modified Chain B (950 – 1170 m/z Mass Range)



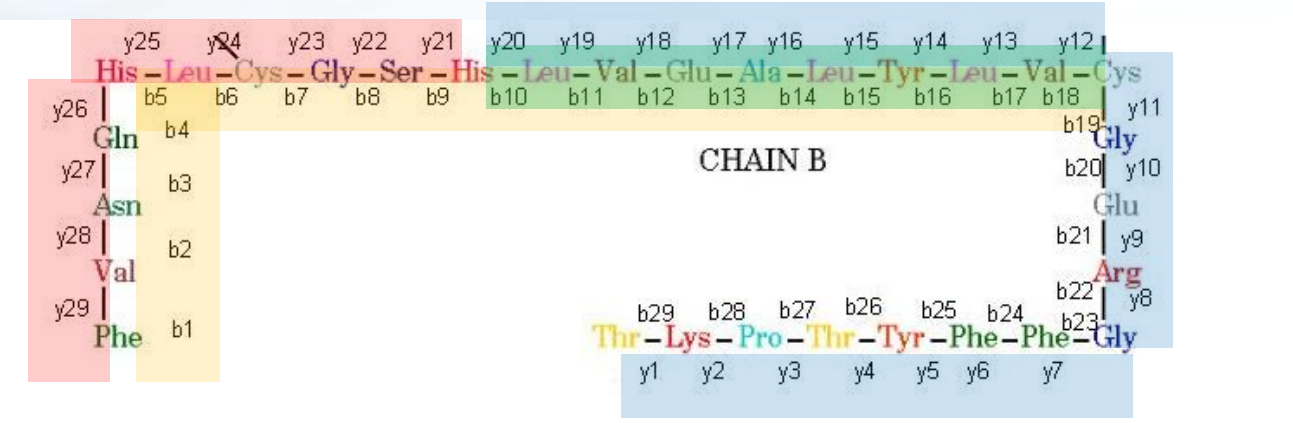
MSMS Spectra of Native and Modified Chain B (1440 – 1710 m/z Mass Range)



MSMS Spectra of Native and Modified Chain B (1700 – 2500 m/z Mass Range)



MSMS Fragment Analysis Localizing Insulin Modification Site to b2 Fragment at Amino Terminus (Val-Phe)



Indicates b fragments where unmodified mass (UM) and UM+12 amu modified masses detected in insulin and sponge treated insulin MSMS spectra, respectively.

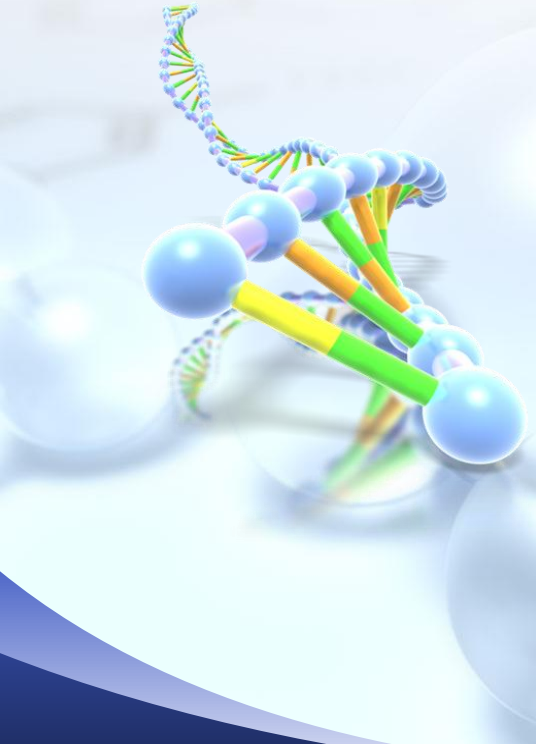
Indicates y fragments which where detected with unmodified mass (UM) in both insulin and sponge treated insulin. **Modification not indicated in this region.**

Overlap region of green and yellow. **Modification not present** in green overlap region since blue y fragments rule out modification.

Modification located at shortest fragment detected in red region. b2 was shortest fragment detected thus modification **detected in NH_2-Phe-Val fragment.**

Questions?

More Information?



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