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Identification of Therapeutic Peptide and its Impurities

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Introduction

Therapeutic peptides gained a lot of interest and attention due to various advantages over protein drug therapies due their small size (< 40-50 amino acids), ease of synthesis and its biological and chemical diverse activity. There are over 60 peptide drugs that have been approved in the USA, Europe, and Japan; over 150 are in active clinical development.

Peptides tend to have additional process impurities e.g. amino acid deletion or insertion, and degradation pathways e.g., oxidation and deamidation. These modifications are critical for product quality and need to be monitored.

In this study different therapeutic peptides were used viz. Liraglutide, Teriparatide, Abaloparatide and Exenatide for identification of peptide API's based on sequence confirmation using Accurate Mass, high resolution for Intact and MSMS Fragments.

Figure 1. Agilent 6545XT AdvanceBio LC/Q-TOF

The Agilent 6545XT AdvanceBio LC/Q-TOF has several features to enhance the sensitivity and dynamic range of the analysis. Agilent AJS Source improves analytical sensitivity and SWARM tuning help to improve resolution and applications based sensitivity.

Experimental

All the peptide samples were provided by MSN Laboratories Pvt Ltd and was analyzed on Agilent 6545XT AdvanceBio LC/Q-TOF. The LC separation method was developed using the AdvanceBio Peptide Mapping column to separate the impurities. Identification and confirmation of the peptide and impurities based on sequence matching algorithms of MassHunter BioConfirm B.07 Software using its Synthetic peptide workflow. This workflow has all the features to detect the intact mass and related missing/additional amino acids, oxidation and deamidation.

Workflow

1. Acquisition of sample by MS Only and AutoMSMS in ESI Positive mode
2. Extract the list of compounds using MFE and compile the list
3. Create the sequence of the peptide with modifications in BioConfirm
4. Match the theoretical sequences with the extracted list of experimental compounds
5. Confirm the peptide intact mass and impurities by Accurate Mass and MSMS data and annotate the identification

Figure 2. Agilent BioConfirm Synthetic Peptide Workflow

The peptide drugs are unique with certain chemical modification to the amino acids e.g. Lysine of Liraglutide is modified with Palmotyl-Glut, presence of an unnatural amino acid Aib at position 29 of Abaloparatide. These modifications are defined in the sequence manager of the BioConfirm B.07 software tool.

Results and Discussion

The therapeutic peptides were identified by accurate mass and sequence matched with less than 5 ppm error and the related impurities were identified based on the corresponding mass differences, which are due to either missing or additional amino acids, or oxidation and deamidation of specific amino acids

The high resolution and mass accuracy provides very good isotopic fidelity to confirm the peptide and its impurities at intact levels with multiple charge states. The data was deconvoluted and matched with theoretical sequence input in the BioConfirm B.07 Software to get the identification and confirmation.

Various impurities were identified in all the samples including the oxidation and deamidation of amino acids which might lead to typical product or process related aspects.

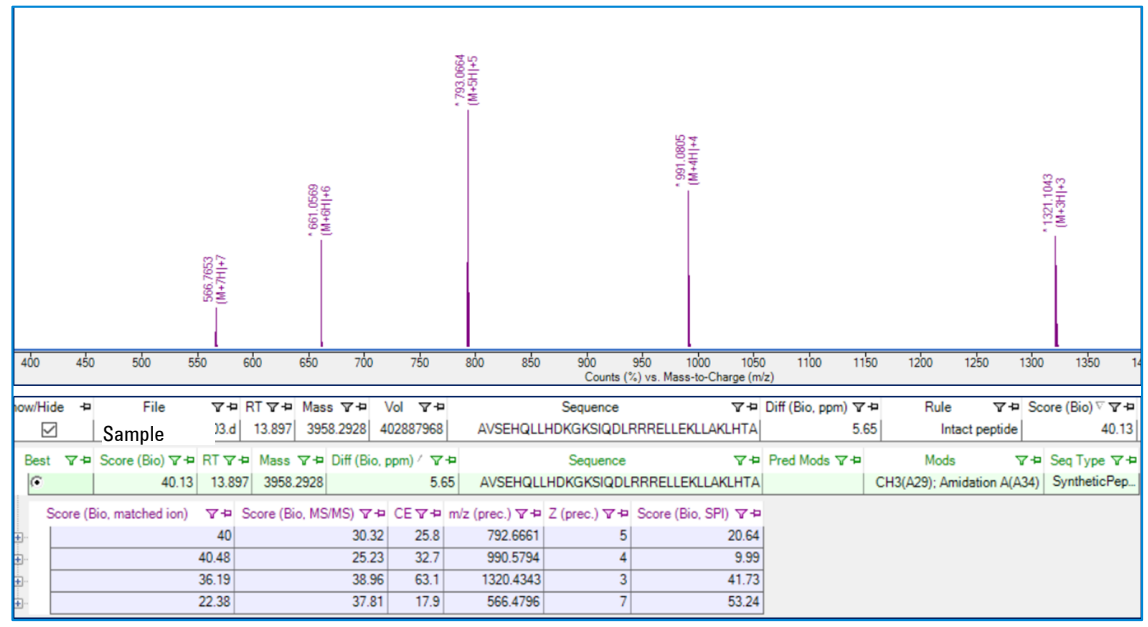

Figure 5. The intact mass and different charge states identified by BioConfirm for Abaloparatide

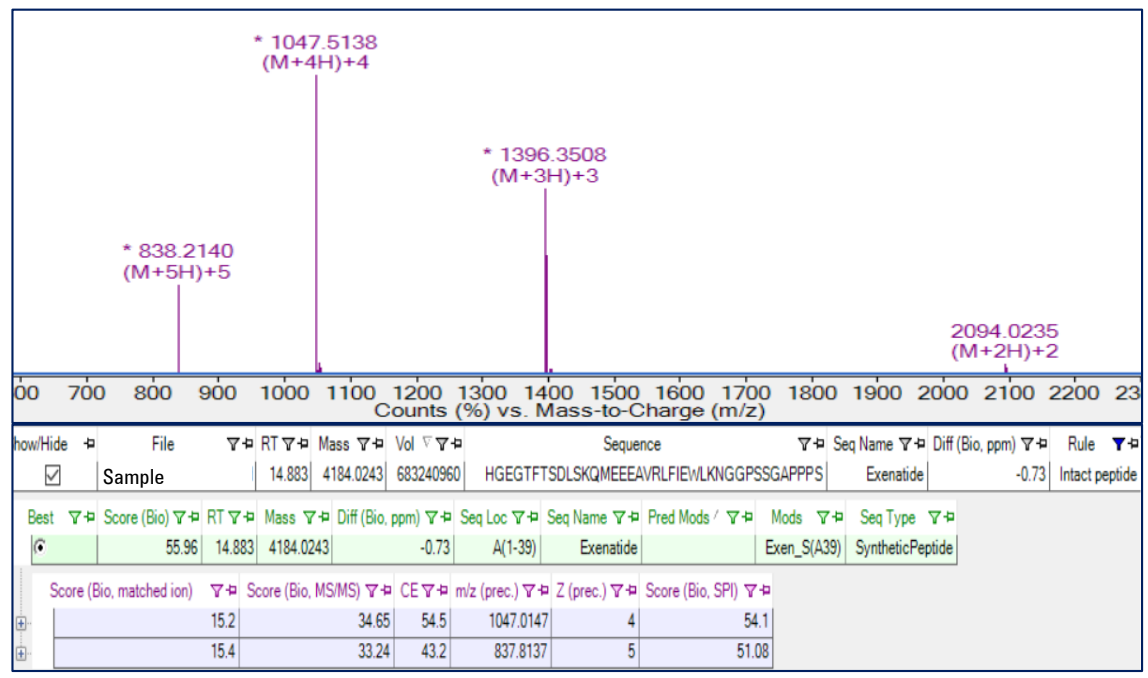

Figure 6. The intact mass and different charge states identified by BioConfirm for Exenatide

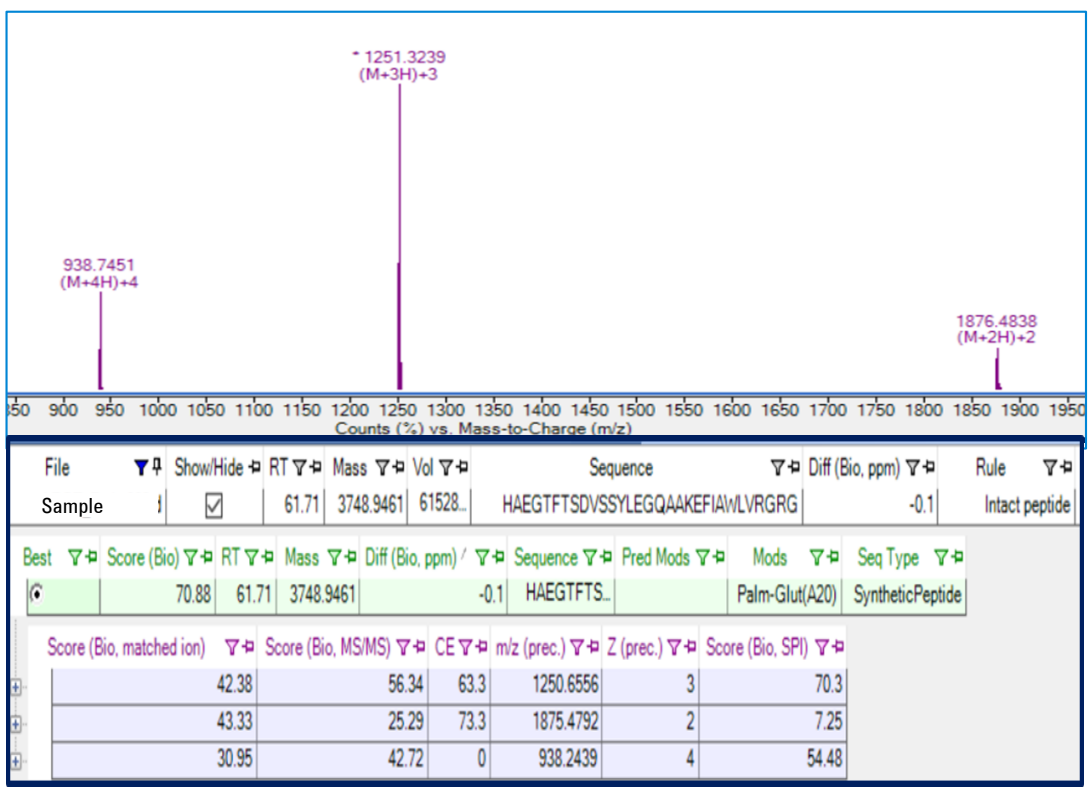

Figure 3. The intact mass and different charge states identified by BioConfirm for Liraglutide

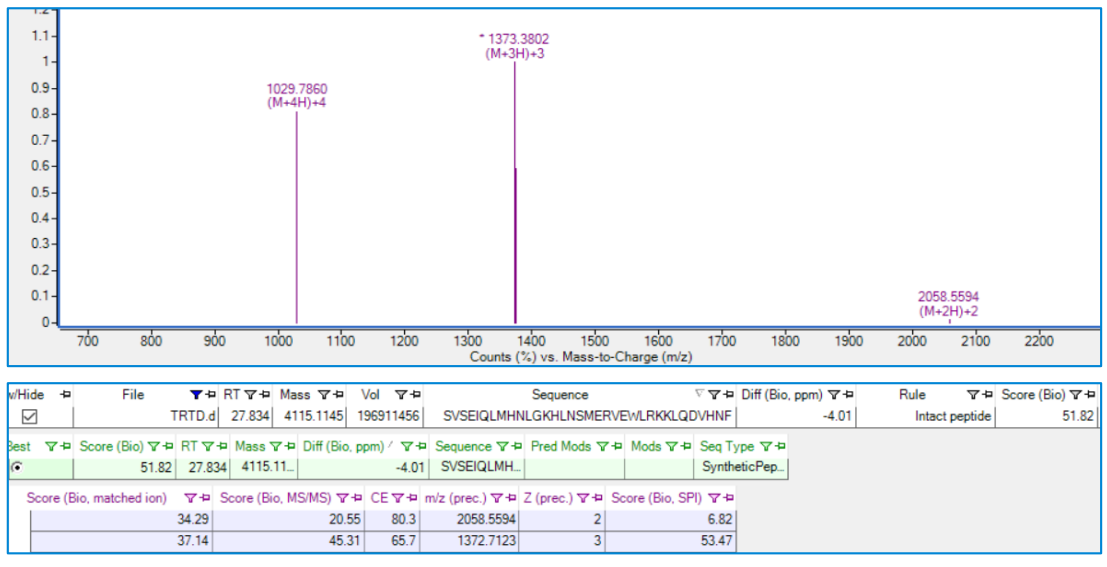

Figure 4. The intact mass and different charge states identified by BioConfirm for Teriparatide

Liraglutide			
Impurity	**Predicted Modification**	**Mass**	**ppm**
Missing Amino Acid	T5	3647.89	0.27
Missing Amino Acid	G31	3691.91	3.26
Missing Amino Acid	E21	3619.89	2.28
Teriparatide			
Impurity	**Predicted Modification**	**Mass**	**ppm**
Missing Amino Acid	R20	3959.01	4.84
Missing Amino Acid	H9	3978.45	4.12
Oxidation	M8	4131.106	4.63
Deamidation	N10	4116.1	3.97
Abaloparatide			
Impurity	**Predicted Modification**	**Mass**	**ppm**
Extra Amino Acid	K27	4086.36	1.17
Missing Amino Acid	A1	3887.23	0.03
Exenatide			
Impurity	**Predicted Modification**	**Mass**	**ppm**
Extra Amino Acid	M15	4315.06	0.08
Missing Amino Acid	Q13	4055.973	1.28
Oxidation	M14	4200.028	1.39

Figure 7. Summary of few impurities identified in different samples

Results and Discussion

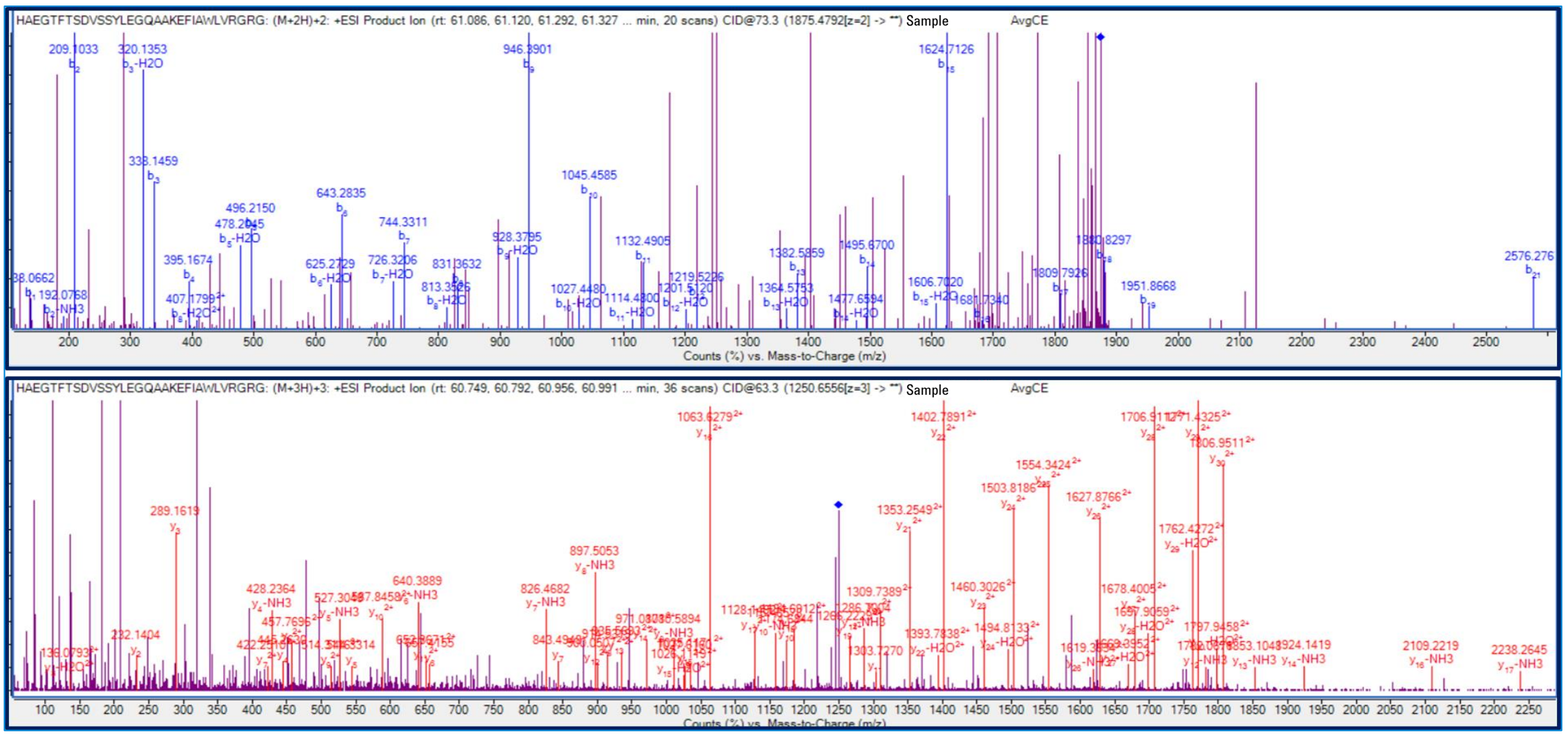

Figure 8 . The MSMS Fragmentation pattern for Liraglutide peptide with coverage of both b and y ion series

	INTACT					Missing T5			Missing T(5)	
RT	Mass	Diff (ppm)	Pred Mods	Mods	RT	Mass	Diff (ppm)	Pred Mods	Mods
61.71	3748.9461	-0.1		P-Glut(A20)	61.029	3647.8978	-0.27	T	P-Glut(A20)
m/z (prec.)	Z (prec.)	File			m/z (prec.)	Z (prec.)	File		
1250.6556	3	Sample			1216.9724	Sequence	Sample		
Ion	Sequence	Diff (ppm)	m/z (prod.)	Z (prod.)	Ion	Sequence	Diff (ppm)	m/z (prod.)	Z (prod.)
b3	HAE	-4.4	338.1459	1	b3	HAE	-3.7	338.1459	1
b4	HAEG	-4.2	395.1674	1	b4	HAEG	1.2	395.1674	1
b5	HAEGT	-3.4	496.215	1	b5	HAEGF	-2.1	542.2358	1
b6	HAEGTF	-3.8	643.2835	1	b6	HAEGFT	-6.2	643.2835	1
b8	HAEGTFTS	-9.7	831.3632	1	b8	HAEGFTSD	-7.4	845.3424	1
y25	TSDVSSYLEGQAAKEFIAWLVRGRG	-3.9	1554.3424	2	y25	TSDVSSYLEGQAAKEFIAWLVRGRG	-3.4	1554.3424	2
y27	TFTSDVSSYLEGQAAKEFIAWLVRGRG	-4.7	1678.4005	2	y28	EGFTSDVSSYLEGQAAKEFIAWLVRGRG	-7.1	1720.9087	2
y28	GTFTSDVSSYLEGQAAKEFIAWLVRGRG	-3.3	1706.9112	2	y29	AEGFTSDVSSYLEGQAAKEFIAWLVRGRG	4.2	1756.4272	2

Figure 9. Missing T(5) impurity in Liraglutide : Comparison of the b/y ions from the MS/MS spectrum for modified b5 and y27 ions shows an effective change over those ions in unmodified peptide, suggesting that Threonine, T is missing at position 5.

The Accurate mass MSMS data provides the ion series and the effective difference of mass due to shifts/modifications of amino acids, as annotated by the BioConfirm Software.

Conclusions

Using Accurate Mass MS, Therapeutic peptides and impurities can be detected and confirmed

The MSMS data of the peptides confirms the site of modifications

Excellent resolution and isotopic fidelity provides accurate data for confirmation and sequence matching

Several peptide drugs were identified and impurities characterized using this workflow

References

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