LC-MS/MS Identification of Impurities Present in Synthetic Peptide Drugs

Emily Lecky, Anna Meljon, Christopher Walker, Peter Smith, Alan Thompson, John Malone
Almac Sciences, 20 Seagoe Industrial Estate, Craigavon, Northern Ireland, BT63 5QD

Background

The presence of impurities can affect the safety and efficacy of a drug substance or API. For most peptide pharmaceuticals, the API can be clearly characterised, however the identification of peptide related impurities can be challenging due to their complexity. A correct identification of the impurities is critical for the development and optimisation of the API manufacturing process. Presented here is an example of a synthetic peptide related impurity characterisation performed at Almac. The peptide API and impurity sequences were elucidated using accurate mass measurements and MS/MS fragmentation. Using embedded algorithms in the MassHunter Software, the accurate mass measurements allowed for the determination of the molecular formula of impurities. MS/MS fragmentation profiling allowed for the determination of the peptide and impurity sequences, as well as the presence and the location of modifications.

Accurate Mass Determination by LC-ESI-MS

The analysis was performed using an Agilent 6530 Q-TOF tandem mass spectrometer coupled to an Agilent 1290 Infinity UHPLC system. The accurate mass was determined by MS analysis with an accuracy below 2 ppm. The peptide and impurities were observed as multiply charged ion species, which were recognised by the MassHunter software and deconvoluted to give the final parent masses (Figure 1). The software algorithm 'Extract by Molecular Feature' was used to suggest modifications to the API which matched the impurity masses and isotopic distributions.

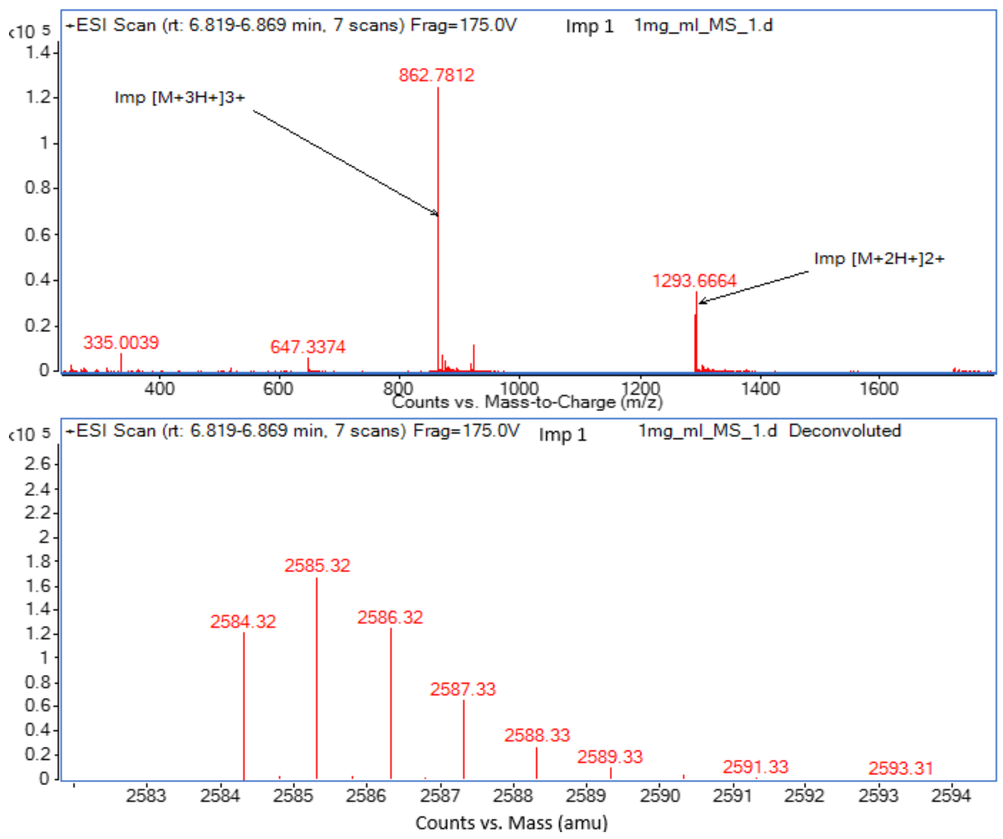

Figure 1: Mass Spectrum (top) and Deconvoluted Mass Spectrum (Bottom) of the Peptide Impurity.

Peptide Sequencing by LC-ESI-MS/MS

The peptide and impurity precursor ions identified during the accurate mass determination were fragmented in the tandem mass spectrometer and the product ions analysed. The collision energy was optimised to obtain the most informative MS/MS spectrum. The fragment ions were analysed by 'Find by Molecular Feature' and 'Define and Match Sequences' algorithms to match the empirical fragment ions with the theoretical fragments. The ions were detected within a 20 ppm accuracy window.

Based on the accurate mass and the fragmentation pattern of an impurity, the software can suggest modifications of the API consistent with the impurity features and assign probability scores for each modification. Several fragments consistent with the loss of proline were suggested by the software.

BioConfirm Fragment Analysis

The resulting list of MS/MS fragments were processed using the 'Extract by Molecular Feature' algorithm in MassHunter and then matched with the theoretical list of daughter ions for the API within single digit ppm mass difference (Table 1). The accurate mass measurement gives an indication of the type of modification whilst the MS/MS fragmentation data can be used to identify the location of the modification.

Table 1: Peptide Impurity Precursor Ion and Corresponding Product Ions

Precursor Ion				m/z	Z
$[M+H]^+$				862.45	2
Product Ion	**m/z**	**m/z (prod.)**	**Diff (Bio, ppm)**	**Sequence**	**Z (prod.)**
b_8	8X9.5113	8X9.5091	2.5	KX^1KVEX^2SP	1
b_{11}-NH_3	11X2.6522	11X2.6477	3.8	$KX^1KVEX^2SPX^3X^4S$	1
b_{18}-NH_3	6X8.3316	6X8.3338	-3.4	$KX^1KVEX^2SPX^3X^4SDX^5INX^6SP$	3
b_{20}	10X3.5912	10X3.5944	-2.9	$KX^1KVEX^2SPX^3X^4SDX^5INX^6SPX^7I$	2
b_{21}	11X8.1181	11X8.1157	2.1	$KX^1KVEX^2SPX^3X^4SDX^5INX^6SPX^7IE$	2
b_{22}	8X8.0948	8X8.0992	-5.3	$KX^1KVEX^2SPX^3X^4SDX^5INX^6SPX^7IEH$	3
y_{16}	9X7.4416	9X7.4394	2.4	$PX^3X^4SDX^5INX^6SPX^7IEHX^8$	2
y_{19}-H_2O	104X.9891	104X.9874	1.6	$EX^2SPX^3X^4SDX^5INX^6SPX^7IEHX^8$	2
y_{21}-H_2O	7X6.0456	7X6.0485	-3.8	$KVEX^2SPX^3X^4SDX^5INX^6SPX^7IEHX^8$	3
y_{22}	8X9.7471	8X9.7467	0.5	$X^1KVEX^2SPX^3X^4SDX^5INX^6SPX^7IEHX^8$	3

BioConfirm Peptide Sequencing

Peptide MS/MS daughter ions were compared with the list of theoretical product ions and the matched ions were annotated (Figure 2). The fragment ion abundance, the ion mass accuracy and the mass of an intact peptide are used to generate the hypothetical impurity sequence. The coverage of the peptide impurity sequence is displayed in Figure 3.

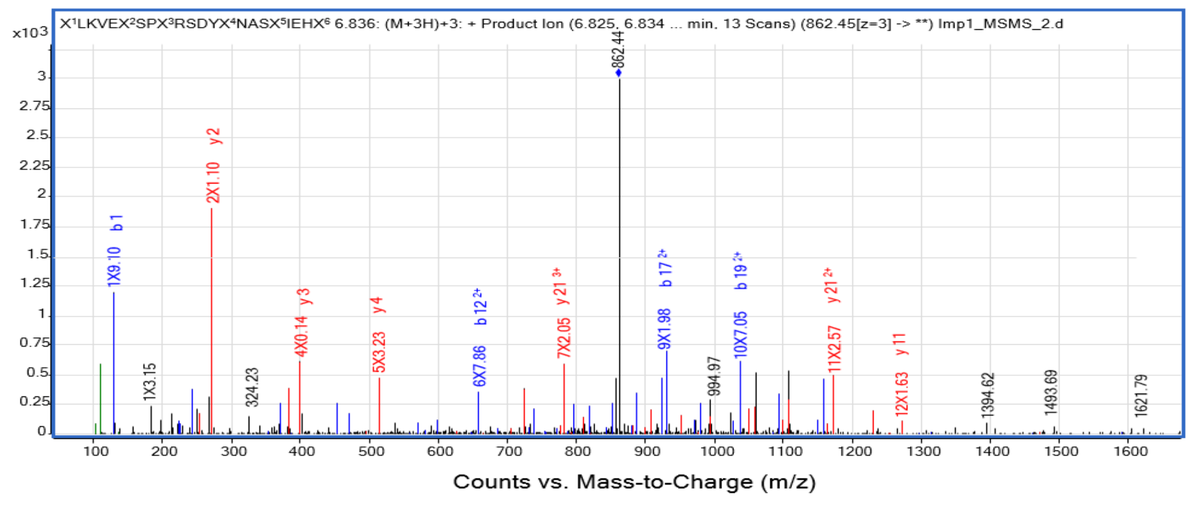

Figure 2: Example of Interpreted Product Ion Spectrum with b and y Ion Assignments.

X^1	K	V	E	X^2	S	P	X^3	X^4	S	D	X^5	I	N	X^6	S	P	X^7	I	E	H	X^8
b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	b_{10}	b_{11}	b_{12}	b_{13}	b_{14}	b_{15}	b_{16}	b_{17}	b_{18}	b_{19}	b_{20}	b_{21}	b_{22}
y_{22}	y_{21}	y_{20}	y_{19}	y_{18}	y_{17}	y_{16}	y_{15}	y_{14}	y_{13}	y_{12}	y_{11}	y_{10}	y_9	y_8	y_7	y_6	y_5	y_4	y_3	y_2	y_1
Sequence Confirmed by LC-MS/MS																					

Figure 3: Summary of Sequence Coverage.

Conclusion

The impurity of interest present in the peptide API was determined by LC-MS/MS to be a truncate, which had a terminal proline deletion. The methodology utilised both accurate mass measurements and the fragmentation profiles of the analytes to provide the exact peptide impurity structure.

Note: X^1-X^8 amino acids were masked for IP purposes.