

Characterization of Antibody Drug with Ultra High Performance Mass Spectrometer

–Identification of complete amino acid sequence and modification analysis are available ! –

Toray Research Center has introduced an ultra high performance mass spectrometer for the first time as a CRO in Japan, and realized complete amino acid sequence and post-translational modification analysis of antibody drugs. It enabled us to conduct efficient deamidation analysis.

1. Pretreatment

Reductive alkylation of Cys
↓
Buffer exchange
↓
Protein digestion

We perform optimal fragmentation of proteins using both enzymatic digestion and chemical cleavage.

2. LC/MS/MS measurement of digested samples

Ultra-high performance liquid chromatography mass spectrometer was introduced for the first time as a Japanese CRO.

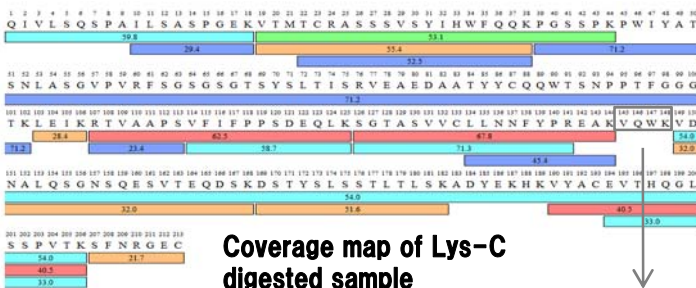


Measurement error for peptide (< 2000 Da) is lower than 1 ppm.

MSⁿ analysis is also available.

Orbitrap Fusion Lumos
©Thermo Fisher Scientific

3. Identification of complete amino acid sequence



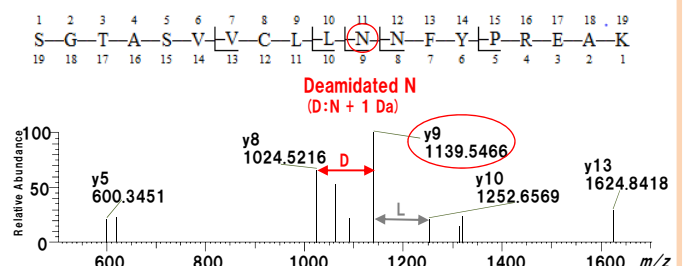
Coverage map of Lys-C digested sample

We use multiple digestion conditions for peptidization to get 100% amino acid sequence coverage in order to meet requests from customers.

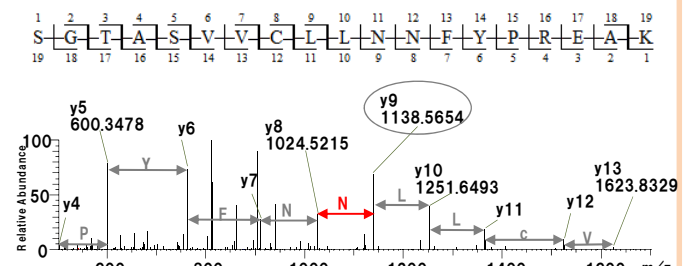
4. Deamidation analysis

(MS/MS of 126–144 peptide)

Accurate identification of deamidated sites (Asn to Asp) in two adjacent N (Asn) has been realized.



Product ion spectrum and assignment result of 126–144 peptide including a deamidated site



Product ion spectrum and assignment result of 126–144 peptide

5. Calculation of deamidation ratio

In the antibody drug, 7 out of 16 Asn sites were found to be deamidated, where deamidation ratios were 1 ~ 11% (calculated from peak intensity).

We can evaluate deamination, which affects the activity and stability of proteins.

We have been challenging to realize difficult characterization of biopharmaceuticals using Ultra High Performance Mass Spectrometer and professional pretreating techniques based on our abundant experiences. We can also work on various tests under “Standards of Reliability of Application Data”.