

Characterisation of protein glycosylation by ESI LC-MS/MS using a precursor ion discovery method on a Q-ToF mass spectrometer.

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Asparagine (N-) linked glycosylation is one of the most common, complex, and highly variable post-translational modification. As glycoforms are the product of a series of biochemical modifications, perturbations within a cell can have profound effects on their structure. As glycosylation also plays an important role in cell signaling and recognition it's detection and characterisation are of great importance.

Here we present a method for the detection and partial characterisation of glycopeptides. A proteolytic digest is analysed by reverse phase HPLC-ESI using a Q-ToF mass spectrometer. The instrument is switched at one-second intervals between low and high collision energy on the collision cell. The quadrupole operates in the non-selective rf only mode. The first data set at low energy (7eV) shows only the normal pseudo molecular ions. The second at higher energy shows their fragments. Wherever a specified product occurs in the high-energy data all its possible precursors are revealed by the corresponding 7eV data. Upon detection of the carbohydrate oxonium ions at m/z 204 (HexNAc), 366 (HexHexNAc) and 274/292 (NeuAc) the instrument is switched into MS/MS mode and ions from the low energy spectra are selected by the quadrupole for fragmentation. Ions failing to produce the carbohydrate product ions are passed over, whilst precursor ions showing the glycopeptide related fragment ion(s) are fragmented for a user specified time. Exact mass values for glycopeptide ions can be used to determine compositions, using MS/MS data for confirmation.

This method is suitable for N- and O-linked glycosylation, providing peptide specific glycoform information.
