Separation of glycopeptides and their glycoforms using HILIC columns and UPLC/MS² system

A failure to characterize the glycosylation of a therapeutic protein means that changes in their efficacy may be poorly understood. Comparison of HILIC and RP-LC results is shown indicating where RP methods alone would not be sufficient.

A novel Ultra Performance Liquid Chromatography (UPLC) method with complete separation of glycopeptides from non-glycosylated tryptic peptides as well as UV quantification of glycan microheterogeneity.

Mass spectrometry detection (UPLC/MS²) provides simultaneous qualitative and quantitative information (peptide mapping and glycosylation analysis).

RESULTS

1. Figure 1 illustrates that RP-UPLC is capable of partially resolving glycosylated peptides.

2. Figure 2 shows HILIC separation of an IgG peptide map. Note that glycosylated peptides are better retained than non-glycosylated ones.

3. Figure 3A shows that glycosylated peptides are well resolved in HILIC mode. Relative quantification is possible at 280 nm using peptide UV absorbance. In cases of incomplete resolution, the MS signal can be used for relative quantification (Figures 3B and 3C).

4. In Figures 3B and 3C the extracted ion chromatograms (XIC) confirm the presence of a GIb isomer of the peptide and is therefore invisible in the UV and TIC traces.

5. Quantitation by MS XIC or UV is comparable (see Table in Figure 1A and 1B). The relative ratio of glycosylation peaks is also in good agreement with LC-fluorescent analysis of 2AB labeled released glycans (data not shown).

6. The elevated energy MS trapezoidal MS/E analysis can tentatively assign glycosylated peptides via their retention time and MS ion pattern (fragments with m/z 204.10 and 366.15 Da).

CONCLUSIONS

1. Novel Glycan HILIC columns packed with sub 2 µm amide sorbent developed for separation of 2-AB labeled glycans are also useful for analysis of peptide maps and glycosylated peptides in particular.

2. UPLC/MS² analysis can tentatively assign glycosylated peptides via their retention and MS ion pattern (fragments with m/z 204.10 and 366.15 Da).

3. Quantitative analysis of glycosylation can be performed by UV (detecting peptide absorbance).

4. Incompletely resolved glycoforms can be quantified using MS signal.