EVALUATING MULTIPLEX FRAGMENTATION AND ION MOBILITY SEPARATIONS TO IMPROVE THE QUALITY OF RAPID LC/MS PEPTIDE MAPS FOR BIO THERAPEUTICS PROTEINS.

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OVERVIEW

- LC/MS peptide mapping remains the fundamental technique for defining primary structure for biotherapeutic proteins.
- Improvements in separations, mass detection, and informatics have reduced typical map acquisition times to below 90 min., and further reduced data processing from days to hours.
- While traditional maps are invaluable for biotherapeutic characterization, they lack the throughput required of effective screening tools for early (clone screening, QbD) and late (Formulations, Stability) development activities.
- Modern TOF analyzers have enabled the rapid collection of accurate mass peptide mapping data, but accurate mass only peptide identifications can be ambiguous, and constitute insufficient evidence for confident verification of new variants.
- Here, we investigate how MS3 multiplexed fragmentation and ion mobility LC/MS could provide more confident fast peptide map assignments.

METHODS

- Analytical-scale UPLC-QTof MS methods were developed for rapid (5-10 min gradient), and typical (90 min) peptide maps of a biotherapeutic IgG1 monoclonal antibody.
- Mass precursor and multiplexed fragmentation data were acquired using LC/MSQ acquisition methodology.
- For conventional 90 min acquisitions, the precursor fragmentation (MS/MS) duty cycle was 1 sec, equally divided, but reduced to 0.5 sec cycle time during fast map runs to facilitate precursor identification, quantification, and chromatographic interchange of precursors to their fragments.
- In selected experiments, SYNAPT ion mobility functionality was enabled (HDMS), permitting additional ion separation prior to TOF MS fragmentation, other parameters unchanged.
- Automated data processing was accomplished using the Biopharmalyx 1.3.2 software package. Coverage maps shown include peptides with ≥2 confirmatory b/y fragments (>10 ppm error precursor and fragments, 1 missed cleavage).

UNDERSTANDING LC/HDMS ANALYSIS

- LC/MS data acquisition: The Triwave device acts as a simple collision cell and alternates between low (MS) and elevated (MS3) energies to collect precursors and multiplexed fragmentation ion data in a single experiment.
- LC/HDMS data acquisition: The central Ion Mobility region is activated to enable gas phase peptide precursor ion separations (based on mass, charge, shape of peptides). Ion mobility drift resolved peptides are fragmented in the Transfer region prior to TOF MS analysis.

CONCLUSIONS

- Sub-10 min LC/MS5 mAb peptide maps can yield high sequence coverage peptide maps, containing accurate mass fragmentation data sufficient to validate accurate mass peptide assignments.
- Higher spectral background and greater incidence of chimeric fragmentation are seen as map times compress.
- This chirmeray does not preclude automated data analysis using physical quality metrics (e.g. min number or % of b/y ion intensity) to confirm peptide assignment.
- Ion Mobility capabilities of the SYNAPT HDMS-QToF (LC/HDMS) reduced or eliminated fragment ion chimerism and improved visual data clarity, but were not essential for MS5 fragment ion confirmation in these studies.

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