UPLC™ chromatograph coupled with a Q-Tof Premier™ mass spectrometer is utilized in this search algorithm (Fig. 10).

Alternate scanning LC-MS (LC-MS E)1,2 data from a nanoACQUITY UPLC™ is a hierarchical, protein-centric search algorithm containing three increasingly stringent modules (Fig. 15).

1. Raw Tryptic Peptide/Protein Identification: Time-resolved accurate mass, LC-MS data is matched directly to a protein database to produce multiple, tentative peptide identifications.

2. Stringent Tryptic Peptide/Protein Identification: Stringent search criteria mass and intensity-based phosphorylation attributes are used to score the peptide/protein identification, above a user-specified identification threshold.

3. Expanded Tryptic Peptide/Protein Identification: A subset database search of the identified proteins is performed to increase peptide sequence coverage of the identified proteins. The search criteria is also expanded to include corresponding in-source fragments, neutral losses (ROH and ROH+H) and post-translational modifications (acetylation, phosphorylation, deamination, ...).

CONCLUSIONS

A novel "Ion Accounting" algorithm for Protein Database Searches

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REFERENCES