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Improved Sequence Coverage by High Resolution Peptide Mapping

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Overview

Peptide mapping MS and MS/MS data analysis are crucial processes in biotherapeutic characterization. This process is usually time consuming and labor intensive. In proteomics, the peptide sequence is identified from a large database using data dependent acquisition and analysis. However, in the analysis of a well characterized therapeutic, there is a known sequence and a well defined list of modifications. This presents a different set of issues than those in proteomics. By applying mass calibration on the fly, we are able to obtain peptide mapping LC/MS/MS spectra with high mass accuracy (< 10 ppm). This not only increases the confidence of sequence determination, but also enables unified parameter settings for the MS/MS data processing software. In this method, we consolidate mass correction, charge recognition, spectrum deconvolution, and database searching into one automatic process. Results from this process can be filtered by user customization and the sequence coverage and PTMs will be revealed in the final report. Software automation shows great potential in biotherapeutic characterization by increasing both efficiency and accuracy in data analysis.

System Components

Waters® BioSuite™ Peptide Mapping MS/MS System

Waters® 2796 Bioseparations Module
Waters® 2487 Dual Wavelength Absorbance Detector
Waters® Micromass® Q-Tof micro™ Mass Spectrometer

Experimental

MS Conditions:

-Source = ESI(+)
-Capillary (kV) = 3.3
-Cone (V) = 35
-Temperature (°C)
  -Source = 150
  -Desolvation = 400
-Gas Flow (L/Hr)
  -Cone = 50
  -Desolvation = 500
-Scan Mode
  -MS Mode
-Collision Energy (V) = 6
-Dual Source
  -Lock Spray
-Lock Spray Reference
  -Leucine Enkephalin

MS/MS Conditions:

-Scan Mode
  -Survey Scan Mode
-Number of Components = 3
-Collision Energy
  -Use Charge State Recognition
-MS/MS Scan Duration
  -Scan Time (sec) = 1.9
  -Inter Scan Time (sec) = 0.1

HPLC Conditions:

Waters® BioSuite™ PA-A C_{18} 2.1 X 250 mm, 3 µm column
Mobile Phase A: 0.2% formic acid in water
Mobile Phase B: 0.2% formic acid in acetonitrile
Gradient: 0-40% Mobile Phase B in 300 mins

Results and Discussion

Tryptic digested human serum albumin (HSA) was used for this study. Peptide mapping LC/MS data of HSA is shown in Figure 1.

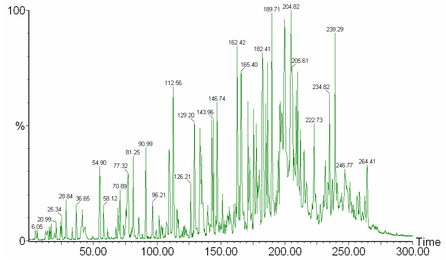


Figure 1: LC/MS Spectrum of Human Serum Albumin

Lock spray, which infuses reference standard via second electrospray probe was installed on a Q-Tof micro™ instrument to get accurate mass for LC/MS and LC/MS/MS experiments. The instrument is setup to take data at survey scan mode. Besides MS channel, four more channels including lock mass and three MS/MS channels are opened for data acquisition (Figure 2).

In survey scan MS mode, co-eluted peptide peaks were selected separately according to their intensities before being fragmented in collision cell. Collision energies used for MS/MS experiment is decided by the mass and the charge state of selected peptide ions. Parent ions with higher mass and/or higher charge state are subjected to higher collision energies.

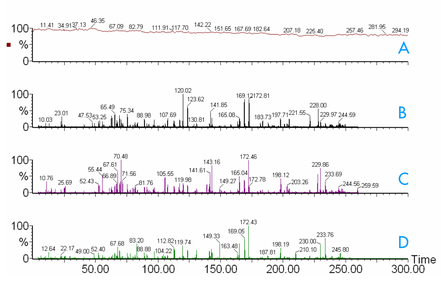


Figure 2: Different Channels of Q-Tof micro Survey Scan Mode
A: Lock Mass Channel; B, C, and D: MS/MS Channels

Analysis on peptide mapping LC/MS/MS data can be tedious when manually examining each peptide fragmentation. Figure 3 shows peptide sequencing by PepSeq™ program from BioLynx™ software. Before being manually transferred to PepSeq™, MS/MS spectra need to be retrieved from chromatograph and deconvoluted by MaxEnt 3 program. In Figure 3, all the matching b type ions are labeled with blue color and all the matching y type ions are labeled with red color.

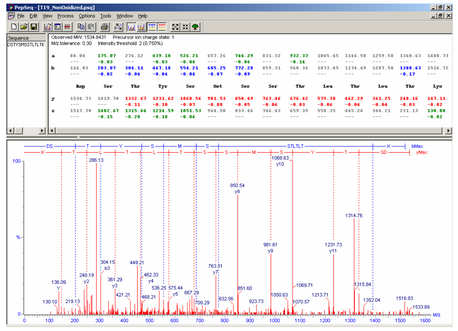


Figure 3: Peptide Sequencing by BioLynx™ PepSeq Program

For long gradient LC/MS/MS runs, mass errors at different elution time can be different due to variations of environmental conditions. This is not a problem for manual peptide sequencing because the parameters for each MS/MS data process can be setup individually. But it will cause problems on batch processing of peptide mapping MS/MS spectra.

By using lock spray in a peptide mapping MS/MS system, we were able to correct peptide fragment masses according to lock reference. This enables unified parameter settings for deconvolution of MS/MS spectra because all the peptides and their fragment ions at different elution time will have same mass accuracy after lock mass correction.

Waters ProteinLynx™ 2.05 software is capable of doing lock mass correction and deconvolution on peptide mapping LC/MS/MS data. As shown in Figure 4, raw survey scan data can be attached to a sample before being processed with optimized parameter settings. The processed data file will be saved in XML format for De Novo Sequencing or AutoMod Analysis.

Figure 4: LC/MS/MS Data Processing by ProteinLynx™

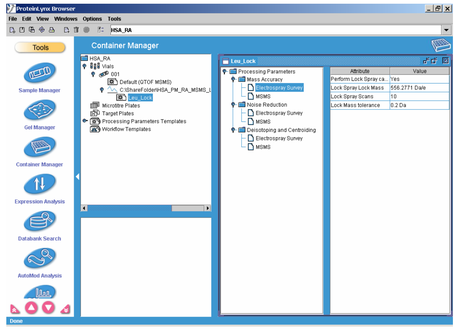
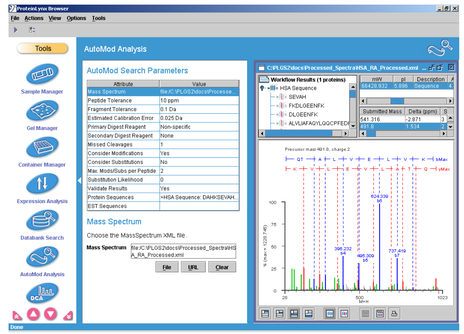


Figure 5: AutoMod Analysis on Processed LC/MS/MS Data



In biotherapeutic analysis, the sequences of analytes are known. AutoMod analysis is an ideal tool for matching peptide mapping data with known protein sequence and identifying possible post translational modifications (Figure5). After matching user input sequence with pre-processed LC/MS/MS data, AutoMod will provide a sequence coverage chart as shown in Figure 6 and a report with all modification products identified.

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1    DAHKSEVAHR  FKDLGEENFK  ALVLIAFAQY  LQQCPFEDHV  KLVNEVTEFA
51   KTCVADESAE  NCDKSLHTLF  GDKLCTVATL  RETYGEMADC  CAKQEPERNE
101  CFLQHKDDNP  NLPRLVRPEV  DVMCTAFHDN  EETFLKKYLY  EIARRHPYFY
151  APELLFFAKR  YKAAFTECCQ  AADKAACLLP  KLDELRDEGK  ASSAKQRLKC
201  ASLQKFGERA  FKAWAVARLS  QRFPKAEFAE  VSKLVTDLTK  VHTECCHGDL
251  LECADDRADL  AKYICENQDS  ISSKLKECCE  KPLLEKSHCI  AEVENDEMPA
301  DLPSLAADFV  ESKDVCKNYA  EAKDVFLGMF  LYEYARRHPD  YSVVLLLRLA
351  KTYETTLEKC  CAAADPHECY  AKVFDEFKPL  VEEPQNLIKQ  NCELFEQLGE
401  YKFQNALLVR  YTKKVPQVST  PTLVEVSRNL  GKVGSKCCKH  PEAKRMPCAE
451  DYLSVVLNQL  CVLHEKTPVS  DRVTKCCTES  LVNRRPCFSA  LEVDETYVPK
501  EFNAETFTFH  ADICTLSEKE  RQIKKQTALV  ELVKHKPKAT  KEQLKAVMDD
551  FAAFVEKCCK  ADDKETCFAE  EGKKLVAASQ  AALGL
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Figure 6: Sequence Coverage of HSA by AutoMod Analysis

Our AutoMod Analysis MS data for human serum albumin peptide mapping study shows about 97% sequence coverage without validation. On the other hand, AutoMod Analysis MS/MS data with validation (three consecutive amino acid sequence matching) shows about 50% sequence coverage.

Conclusions

- Using lock spray in peptide mapping LC/MS/MS experiments can dramatically increase mass accuracy to about 10 ppm
- High mass accuracy increases the confidence of sequencing and peptide variants identification
- ProteinLynx™ 2.05 is a powerful tool for lock mass correction and mass spectra deconvolution
- AutoMod Analysis can be used for peptide mapping LC/MS/MS data analysis, post translational modification identification, and sequence coverage analysis
- Combining lock mass reference with software automation increases both efficiency and accuracy on biotherapeutic characterization