AUTOMATED LC/MS DATA ANALYSIS FOR COMPARISON OF ALPHA-INTERFERON PRODUCED USING TWO RECOMBINANT EXPRESSION SYSTEMS

Joomi Ahn, Asish B. Chakraborty, Ying Qing Yu, Scott J. Berger
Biopharmaceutical Sciences, Waters Corporation; Milford, MA

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OVERVIEW

- Two samples of Interferon alpha-2b were prepared using distinct recombinant expression systems.
- The interferon produced by the two recombinant systems were compared using intact mass and LC/MS^E peptide map analysis.
- BiopharmaLynx™ 1.2 software was used to automate the possessing of the resulting data sets, and facilitate efficient comparative analysis for the two samples.
- The results of this indicate that the two expression systems produce distinct protein products, where N-terminal acetylation constitutes the primary site of structural differentiation

INTRODUCTION

Interferons (IFNs) are a cytokine family with a wide spectrum of biological activities including antiviral, antiproliferative, and immunomodulatory properties. (1-2). For this study, two preparations of interferon (alpha-2b class) were produced using different recombinant expression systems. The study of these two preparations was accomplished using LC/TofMS analysis of the intact proteins, and LC/MS^E analyses of the respective tryptic digests.

In the past, LC/MS data generated by such studies was processed using a time-consuming semi-manual approaches, where scientists undertook the primary activity of simplifying the raw data to a list of components and intensities, or for the informatic assignment of these components to a therapeutic protein. This poster will describe the use of BiopharmaLynx™ software workflows for automating both phases of data processing, and for facilitating comparative analysis of the two interferon preparations.

Waters ACQUITY UPLC® and SYNAPT™ Mass Spectrometry System. Data was acquired by MassLynx 4.1, and processed by the BiopharmaLynx 1.2 MassLynx Application Manager

INTACT PROTEIN ANALYSIS

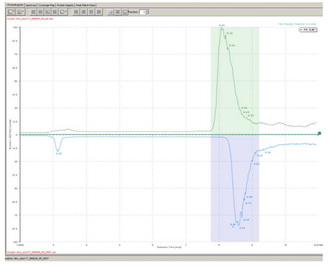


Figure 1. Mirror view of the Total Ion Chromatograms for IFN produced by Expression System 1 (Top) and Expression System 2 (Bottom) shows difference in protein retention.

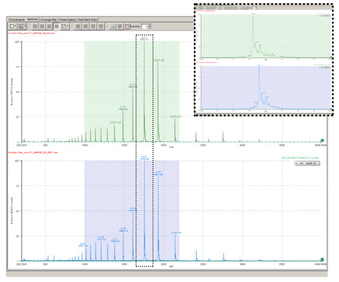


Figure 2. Similar charge envelopes are observed for IFN produced by Expression System 1 (Top) and Expression System 2 (Bottom). A closer look at the most intense charge state shows that the protein variants produced in System 2 are shifted higher in mass than those produced by System 1.

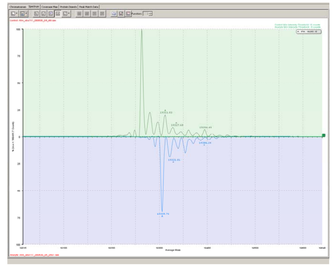


Figure 3. The mirror view of the MaxEnt1 deconvoluted spectrum for IFN produced by Expression System 1 (Top) and Expression System 2 (Bottom) shows a +42 Da mass difference for the most intense components. A series of +16 Da (Oxidation) peaks can be seen for both samples.

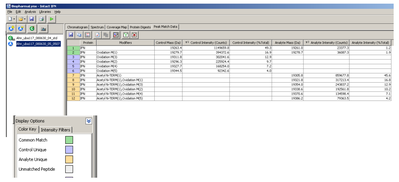


Figure 4. Results from the intact mass study indicate that IFN produced by Expression System 1 is consistent with the expected product, and that protein produced by Expression System 2 is consistent with modification by N-terminal acetylation. The presence of an N-terminal Cys and increase in retention are consistent with this potential assignment. This can be confirmed by peptide mapping. A series of oxidation products is also observed for each IFN preparation.

PEPTIDE MAPPING ANALYSIS

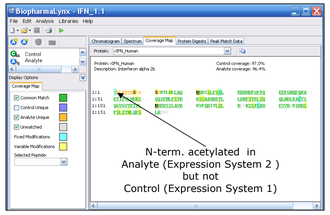
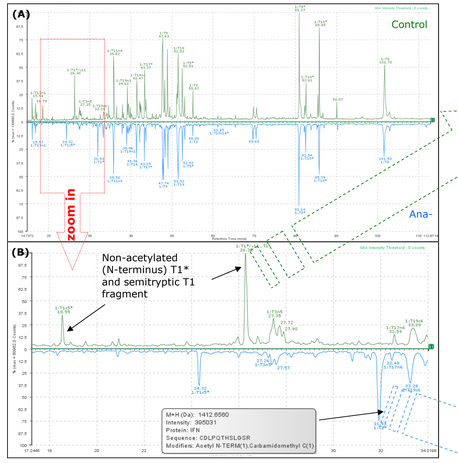
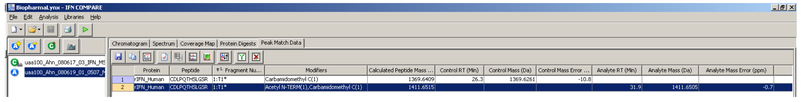


Figure 5. The LC/MS^E peptide map confirms that IFN preparations differ primarily by a +42 Da modification on the T1 (N-terminal) peptide, consistent with results from intact mass analysis.

- ~97% protein coverage obtained on both maps
- Primary differences mapped to two early eluting peaks (see red box on right).
- These peaks corresponded to the T1 (N-terminal) peptide and a semi-tryptic T1c5 fragment (with loss of c-terminal 5 AA) of the T1 peptide.





VALIDATING N-ACETYLATION USING MS^E FRAGMENTATION DATA

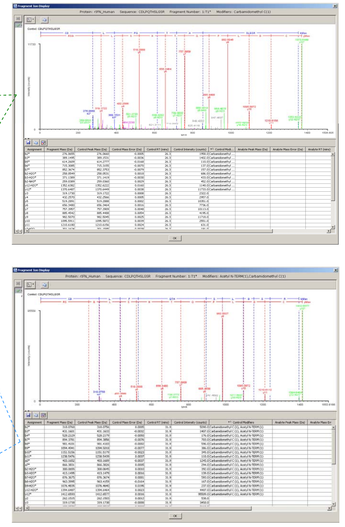


Figure 6. MS^E fragmentation data confirms the unmodified T1 peptide from Expression System 1 IFN (TOP) and the N-acetylated T1 IFN peptide from Expression System 2.

CONCLUSIONS

- BiopharmaLynx 1.2 was used to automatically process intact mass and LC/MS^E peptide mapping data sets for two preparations of Interferon alpha 2b produced by distinct expression systems.
- The intact mass analysis indicated a +42 Da mass difference and increased protein retention by RP chromatography.
- The most likely interpretation of the intact mass data (N-acetylation was confirmed using accurate mass tryptic peptide maps for the two IFN preparations.
- MS^E fragmentation data was used to further validate the presence of the modification, and it's localization to the protein N-terminus.
- Significant improvements in laboratory productivity can be achieved using BiopharmaLynx to automate processing of LC/MS data during biotherapeutic comparability studies.

References

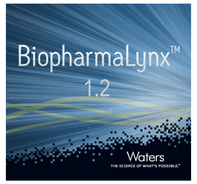
1. S. Nagata, N. Mantei, and C. Weissmann. The structure of one of the eight or more distinct chromosomal genes for human interferon-α, Nature 287:401-408 (1980).
2. D. V. Goeddel, D. W. Leung, T. J. Dull, M. Gross, R. M. Lawn, R. McCandliss, P. H. Seeburg, A. Ullrich, E. Yelverton, and P. W. Gray. The structure of eight distinct human leukocyte interferon cDNAs. Nature 290:20-26 (1981)

Sample Preparation
Samples were dissolved in 50 mM ammonium bicarbonate and directly analyzed.

Chromatography
Waters ACQUITY UPLC® System
Waters ACQUITY UPLC™ BEH 300 C4, 1.7 µm 300 Å, 2.1 x 50 mm column
Mobile phase A : 0.1% Formic acid in water
Mobile phase B : 0.1% Formic acid in acetonitrile
Gradient : 35 –40 %B (15 min @ 200 µL/min)
Column Temperature : 40 °C

Mass Spectrometry
Waters SYNAPT™ Mass Spectrometry System
Ionization Mode : ESI Positive
Capillary Voltage : 3.0 kV, Cone Voltage : 35 V, Source Temperature : 120 °C
Desolvation Temperature : 250 °C, Desolvation Gas : 350 L/hr
Acquisition Range: 50-4000 m/z
Lockmass : 100 fmol/µL Glu-Fibrinopeptide B

Informatics



Sample Preparation
Samples were denatured in (0.025% RapiGest™ SF 50 mM ammonium bicarbonate), then reduced (DTT) and alkylated (IAA) prior to overnight tryptic digestion.

Chromatography
Waters ACQUITY UPLC® System
Waters ACQUITY UPLC™ BEH 300Å C18, 1.7 µm 300 Å, 2.1 x 150 mm column
Mobile phase A : 0.1% Formic acid in water
Mobile phase B : 0.1% Formic acid in acetonitrile
Gradient : 0 –50 %B (120 min @ 200 µL/min)
Column Temperature : 60 °C

Mass Spectrometry
Waters SYNAPT™ Mass Spectrometry System
Ionization Mode : ESI Positive
Capillary Voltage : 3.0 kV, Cone Voltage : 35 V, Source Temperature : 120 °C
Desolvation Temperature : 250 °C, Desolvation Gas : 350 L/hr
Acquisition Range: 50-1700 m/z
Lockmass : 100 fmol/µL Glu-Fibrinopeptide B

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