AN AUTOMATED LC/MS DATA ANALYSIS USING BIOPHARMALYNX ; A CASE STUDY FOR CHARACTERIZATION OF THERAPEUTIC INTERFERON PROTEIN

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Joomi Ahn, Ying Qing Yu, Martin Gilar, and John Gebler
Waters Corporation; Milford, MA

OVERVIEW

- Therapeutic Interferon proteins were analyzed to demonstrate the utility of BiopharmaLynx™ software.
- Fast data process, accurate protein MW determination and peptide mapping to confirm protein sequence were demonstrated.
- Efficient data interpretation was achieved using automated features for peptide peak annotation and data comparison.

INTRODUCTION

Interferon(IFN) α proteins are a group of genetically related cytokins with a wide spectrum of biological activities including antiviral, antiproliferative, and immunomodulatory properties (1-2). The goal of the study is to chracterize and compare the recombinant Interferon α-2b proteins harvested from different cell growth conditions.

Intact Interferon protein and the enzymatically digested protein were analyzed using Liquid Chromatography-Time of Flight Mass Spectrometry (LC/TOF-MS). LC/TOF-MS has been extensively used for biotherapeutic drug development. Conventionally, LC/TOF-MS data generated for these studies is interpreted manually or being processed with a limited automation to confirm protein masses and peptide maps. They are often time-consuming processes and offer limited information due to the high complexity nature of the data.

BiopharmaLynx™, a new application manager, significantly reduces data analysis time for LC/TOF-MS peptide mapping and intact protein analyses. It automatically annotates the peaks from LC/TOF-MS data. This software also automates batch data processing of multiple LC/MS experiments. The display tools for data interpretation in user friendly interface are some of the features designed to reduce the burden of data analysis.

Waters BiopharmaLynx™, ACQUITY UPLC®, and SYNAPT™ Mass Spectrometry System.

METHODS

LC condition

- Intact Protein LC condition

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
Flow : 200 µL/min.
Gradient : 35 –40 %B over 15 min.
Column Temperature : 40 °C

- Peptide Map LC Condition

Waters ACQUITY UPLC System
Waters ACQUITY UPLC BEH 300 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
Flow : 200 µL/min.
Gradient : 0-50 %B over 120min.
Column Temperature : 60 °C
Weak wash : 95 % buffer A / 5 % buffer B
Strong wash : 20 % buffer A / 80 % buffer B

MS condition

Waters SYNAPT™ Mass Spectrometry System
Ionization Mode : ESI Positive
Capillary Voltage : 3.0 kV
Cone Voltage : 35 V
Desolvation Temperature : 250 °C
Desolvation Gas : 350 L/hr
Source Temperature : 120 °C
Acquisition : 50-1700 m/z for peptide,
50-4000 m/z for intact protein
Lockmass : 100 fmol/µL Glu-Fibrinopeptide B directly infused

Sample preparation

Production batches of recombinant human interferon α-2b protein were used. *A control IFN was expressed in cell condition X and an analyte was expressed in cell condition Y.*
For peptide mapping, a control and one of analytes were dissolved in 50 mM ammonium bicarbonate and denatured with 0.025 % RapiGest™ SF (Waters), then reduced and alkylated with DTT and iodoacetamide (Sigma) respectively. Finally the protein was digested with trypsin (Promega).
For intact protein analysis, the samples were dissolved in 50 mM ammonium bicarbonate and directly injected into a column.

BiopharmaLynx™ 1.1 Application Manager

LC/MS data were processed in BiopharmaLynx 1.1. The screen captures from BiopharmaLynx browser were shown in the results section.

RESULTS

INTACT PROTEIN ANALYSIS

Figure 1. **Raw spectra** of intact IFN control and analyte

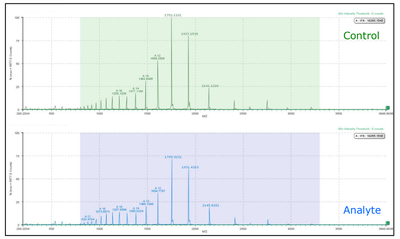
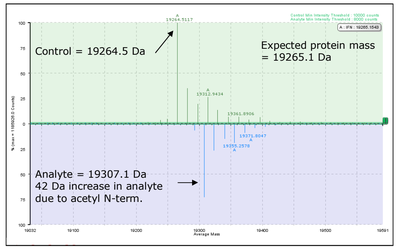


Figure 2. **Processed spectra** in mirror view



The processed spectrum is a centroid MaxEnt1 spectrum.

Figure 3. **Peak match data table**

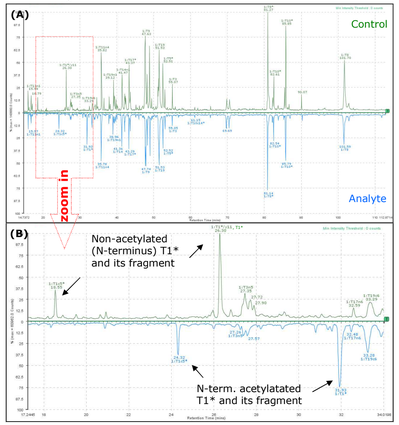
Chromatogram | Spectrum | Coverage Map | Protein Digests | Peak Match Data

▲¹	...	Modifiers	Calculated Protein Mass (Da)	Control Intensity ...	Analyte Intensity ...
IFN			19265.2	1185925.0	43870.5
IFN	Oxidation M(1)	Control	19281.1	417330.0	
IFN	Oxidation M(2)		19297.1	232133.7	
IFN	Oxidation M(3)		19313.1	309943.9	
IFN	Oxidation M(4)		19329.1	161971.1	
IFN	Oxidation M(5)		19345.1	78938.3	
IFN	Acetyl N-TERM(1)		19307.2		1369475.6
IFN	Acetyl N-TERM(1),Oxidation M(1)	Analye	19323.2		499505.6
IFN	Acetyl N-TERM(1),Oxidation M(2)		19339.2		247443.1
IFN	Acetyl N-TERM(1),Oxidation M(3)		19355.2		303450.3
IFN	Acetyl N-TERM(1),Oxidation M(4)		19371.1		138679.7
IFN	Acetyl N-TERM(1),Oxidation M(5)		19387.1		67256.1

The intact protein analysis revealed that the control is the expected IFN and its oxidized forms. The analyte was confirmed as acetylated IFN and oxidized form of acetylated IFN.

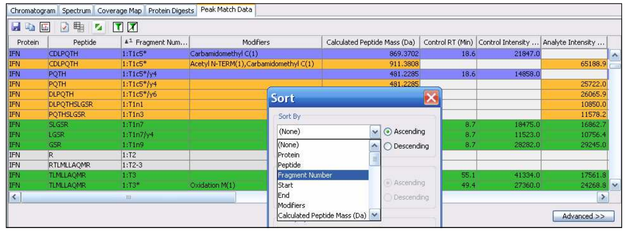
PEPTIDE MAPPING ANALYSIS

Figure 4. **Automated peak annotation** in raw TIC chromatograms in Mirror view



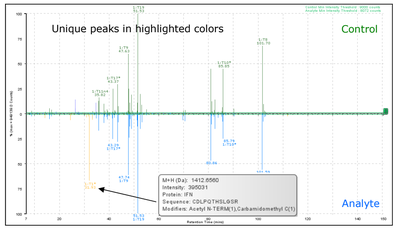
(A) Peptide maps of control and analyte in chromatogram comparison view. The tryptic peptides were annotated with protein digest fragment numbers (T_{no}), e.g., T1 is the first tryptic peptide from N-terminus. (B) Enlarge the area to view the differences in the control and analyte chromatograms. T1 indicated the modified form of T1. The control T1 was carbamidomethylated on N-terminus cysteine and analyte T1 was carbamidomethylated and acetylated on the same cysteine (see Figure 6 for the Interferon amino acid sequence).*

Figure 5. **Peak match data table**



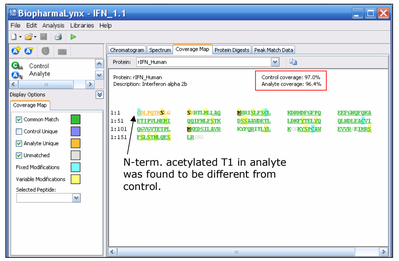
The peak match data table display contains processed results for all detected LC/MS components, including protein and peptide annotations produced by the targeted protein search results. This tabular display of peptides can be customized, sorted by any category, and have intensity thresholds applied to eliminate false identifications. In addition the table can be exported to excel spreadsheet.

Figure 6. **Processed chromatogram** in highlighted view



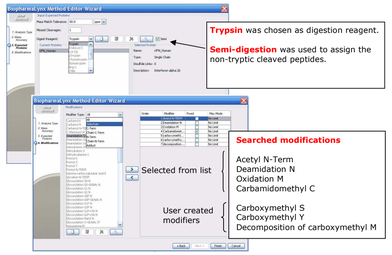
The processed chromatogram provides simpler view. Each "stick" represents the summed intensity (ion counts) of all isotopic peaks for all detected charge states of that peptide over the full chromatographic elution profile.

Figure 7. **Coverage Map**



96.4 % and 97.0 % coverage were achieved in control and analyte respectively. The color coded coverage view provides additional information, such as peptides that have been matched, modified and unmatched

Figure 8. Proteolytic enzyme selection and searched modifications in Method Editor



Method editor can be used to batch process LC/TOF-MS peptide map data. It includes the ability to select the proteolytic enzyme and search the modifications.

CONCLUSION

- Intact protein study revealed that the analyte was acetylated IFN and its oxidized forms.
- Peptide mapping analysis reconfirmed the acetylation in the analyte sample and identified the modification site to be the N-terminus cysteine.
- The primary sequence of recombinant IFN was confirmed up to 97 % of peptide coverage. Various protein post translation modifications were identified in both control and analyte.
- BiopharmaLynx proved to be fast and efficient for automated data analysis for therapeutic protein characterization.

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)

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS