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Intact Mass Analysis using LC/MS

Introduction

Intact mass analysis is the assessment of a protein's total molecular weight by mass spectrometry (MS) without prior digestion or fragmentation of the protein. High resolution MS allows the average molecular weight of large proteins, e.g. monoclonal antibodies (mAbs), to be measured with accuracy better than ± 0.005%. The observed mass can then be compared to the expected mass for a given amino acid sequence. Differences between the expected mass and observed mass may indicate any of these possible biological conditions:

a) the expected protein contains unexpected amino acids in the expressed sequence

b) N or C term truncations

c) post translational modifications

d) internal cleavage events

e) poorly folded proteins (SEC)

If the observed deconvoluted molecular weight can be accounted for, then intact mass analysis can serve as assurance of protein identity.

Why use Intact Mass Analysis?

Intact mass analysis is a powerful tool for protein characterization of biotherapeutics during the development phase of pharmaceutical research. Using intact mass analysis, Spectrus can assess homogeneity, subunit diversity, chain-paring fidelity, and purity and stability of your biotherapeutic [1]. Additionally, Spectrus techniques also survey for post-translational modifications such as N-terminal pyroglutamic acid formation, C-terminal lysine clipping, and N-glycosylation in your sample.

Methods in Brief

Depending on the research requirement, protein samples can be analyzed in native, reduced and/or deglycosylated conditions.

- **Native analysis** is performed in the conditions you provide. Native analysis reveals the total molecular weight of your protein, as well as any degradation or contaminant proteins that may be present.
- **Reduced analysis** can be performed by treating samples with a reducing agent, such as dithiothreitol (DTT). DTT breaks disulfide bonds within a protein, enabling characterization of individual subunits and allowing for a more specific structural analysis.
- **Deglycosylated analysis** reduces heterogeneity of samples associated with glycosylation by treating samples with a glycosidase such as PNGase F. This allows for higher resolution analysis of the protein or subunits.

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Proof of Concept

To demonstrate our ability to characterize intact protein, Spectrus characterized each of the proteins present in the Thermo Scientific™ Pierce™ Intact Protein Standard Mix, a commercially available protein standard. Spectrus deconvoluted each protein in this protein mix with a mass accuracy of ± 1Da.

The Thermo Scientific™ Pierce™ Intact Protein Standard mix was reconstituted in a solution of 10% Acetonitrile/90% Water, 0.1% Formic Acid (v/v/v) before being analyzed with our in-house developed method.

Table 1. Thermo Scientific™ Pierce™ Intact Protein Standard Mix

Protein Name	**UniProt Protein Accession**	**Theoretical Average Mass (Da)**	**Theoretical Monoisotopic Mass (Da)**
Human IGF-I LR3	P05019 (40-118)	9,111.47	9,105.35
Human Thioredoxin	Q99757(60-166)	11,865.52	11,858.04
Streptococcus dysgalactiae Protein G	P06654(223-413)	21,442.61	21,429.76
Bovine Carbonic Anhydrase II	P00921	28,981.29	28,963.69
Streptococcus Protein AG *(chimeric)*	P02976, P19909	50,459.74	50,429.85
Escherichia coli Exo Klenow	P00582(324-928)	68,001.15	67,959.43

Chromatography was preformed using a Dionex™ UltiMate™ 3000 HPLC in tandem with a Thermo Q Exactive™ Hybrid Quadrupole-Orbitrap™

- Stationary Phase:
  Agilent™ PLRP-S 1000Å 5µm, 2.1x50mm
- Mobile Phase A:
  Water, 0.1% Formic Acid
- Mobile Phase B:
  Acetonitrile, 0.1% Formic Acid

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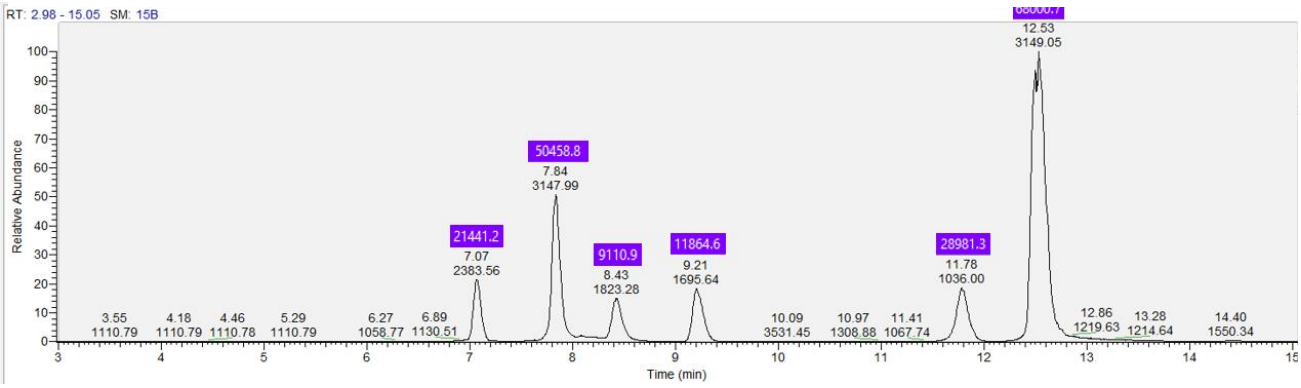
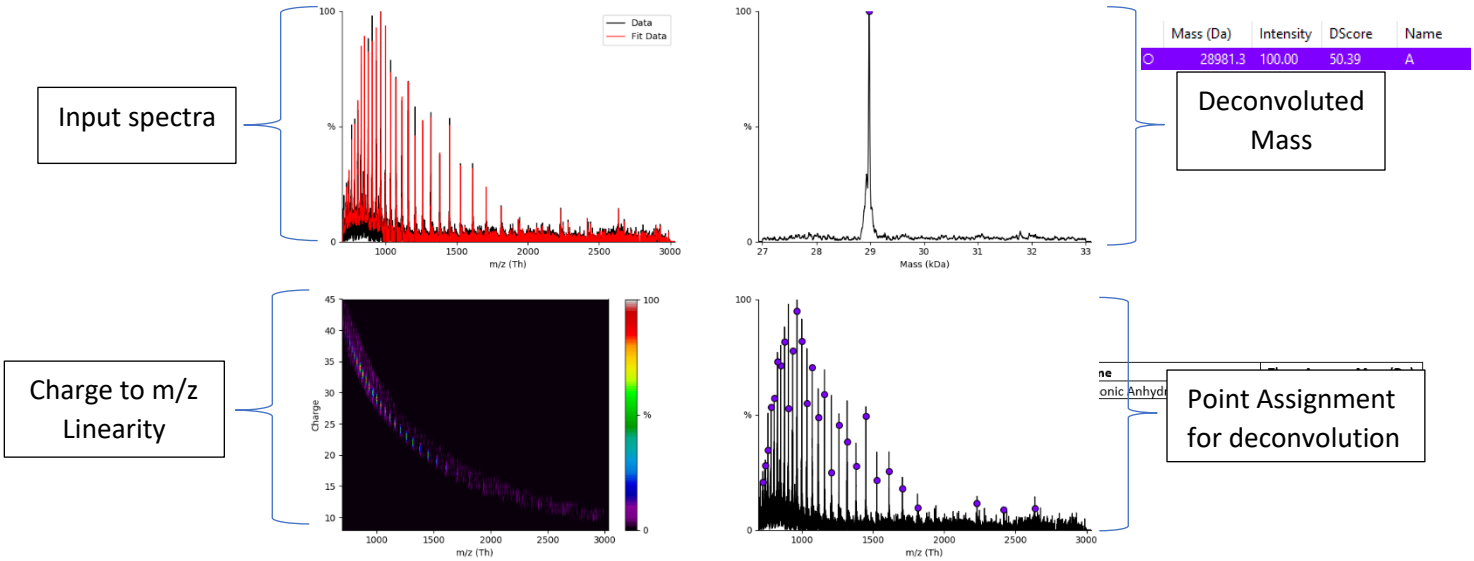
Summary of Findings

Figure 1. Chromatographic Profile of Pierce™ Intact Protein Standard Mix



Using Spectrus developed chromatographic method, scientist achieved baseline separation of each of the 6 proteins present in the Thermo Scientific™ Pierce™ Intact Protein Standard Mix.



Using UniDec: Universal Deconvulation of Mass Spectra, an open-source Bayesian deconvolution software [2], Spectrus is able to deconvolute the spectra observed during chromatography into an average neutral mass with a degree of accuracy of ±2 Daltons.

Why Spectrus is the right is choice for Intact Mass Analysis

Spectrus' combination of experience in sample preparation together with state of the art mass spectrometry instrumentation allows Spectrus to provide the highest degree of confidence in molecular weight determination of your biomolecules. Spectrus' is an expert in the deconvolution of complex overlapping spectra due to post translational modifications and has the experience to deconvolute your most challenging spectra

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Literature

[1] S. Vimer, G. Ben-Nissan and M. Sharon, "Mass Spectrometry Analysis of Intact Proteins from Crude Samples," *Analytical Chemistry,* vol. 92, no. 19, pp. 12741-12749, 6 10 2020.

[2] M. T. Marty, A. J. Baldwin, E. G. Marklund, G. K. Hochberg, J. L. Benesch and C. V. Robinson, "Bayesian Deconvolution of Mass and Ion Mobility Spectra: From Binary Interactions to Polydisperse Ensembles," *Analytical Chemistry,* vol. 87, no. 8, p. 4370–4376, 23 03 2015.