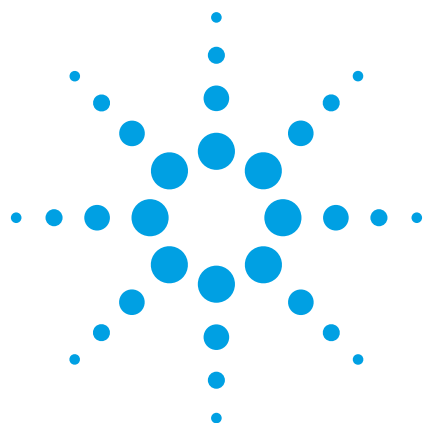




Agilent MassHunter BioConfirm B.02.00 Software

High-confidence intact protein and peptide characterization

Agilent MassHunter BioConfirm B.02.00 software utilizes enhanced deconvolution algorithms and a powerful new molecular feature extraction tool to facilitate efficient and accurate molecular weight determination of intact proteins and peptides in both simple and complex samples. Data matching to user-defined protein, protein digest and peptide sequences is supported by the platform. The MassHunter BioConfirm B.02.00 software package is designed to enhance MS characterization of recombinant expression products using Agilent Accurate-Mass TOF and Q-TOF LC/MS systems.

Fast, Accurate Confirmation of Intact Proteins and Peptides

Agilent MassHunter BioConfirm B.02.00 software couples powerful data processing algorithms with sophisticated sequence and database matching tools to enable fast, accurate identification of intact proteins, peptides, and their variants in simple and complex mixtures. Compatible with Agilent's line of sensitive, high-resolution Accurate-Mass TOF and Q-TOF instruments, MassHunter BioConfirm B.02.00 is tailored for recombinant protein and peptide analysis, and is especially relevant for the biopharmaceutical industry.

Enhanced Resolution and Mass Accuracy for Intact Protein Analysis

BioConfirm B.02.00 software employs unique and improved maximum entropy deconvolution algorithms to generate

protein molecular weight information from raw, multiply-charged input MS spectra. A new "singlet width" parameter enables users to input an estimate of the mass spectral peak width, which significantly improves the resolution and cleanliness of the deconvoluted spectrum (**Figure 1**). Further control over the completeness of the maximum entropy deconvolution (using "number of iterations") has been added to allow users to produce higher resolution output. Quality filters distinguish random noise peaks, which are typical with maximum entropy processing, from true compounds, thereby enabling deconvolution of more complex data. Compound-based navigation allows users to easily access and visualize sample-relevant raw data, deconvoluted spectra, and chromatograms.

Our measure is your success.



Large Molecular Feature Extraction for Complex Mixture Analysis

Maximum entropy processing of complex protein mixtures may be limited by the inability to identify an appropriate averaged raw spectrum, especially where there are many overlapping chromatographic peaks. MassHunter BioConfirm B.02.00 software provides the new Large Molecule Feature Extractor (LMFE) algorithm that is specifically designed for the analysis of complex mixtures of intact proteins or large oligonucleotides. Unlike maximum entropy deconvolution,

LMFE does not require input of a target mass range, nor does it require distinct chromatographic peaks from which a spectrum can be extracted. Data processing with the LMFE algorithm proceeds with exceptional speed and yields substantially more information for complex intact protein mixtures than maximum entropy deconvolution (**Figure 2**).

Protein Sequence Creation

The Sequence Editor tool, now integrated with MassHunter Qualitative Analysis software, allows users to

define protein sequences and site-specific modifications for data matching and characterization of target proteins, protein variants and post-translational modifications. The software also automatically assigns immunoglobulin glycoforms. Sequences from previous versions of BioConfirm software are compatible with the Sequence Editor feature in BioConfirm B.02.00.

Protein Digest Characterization

MassHunter BioConfirm B.02.00 supports the analysis of protein digests. The Sequence Editor feature facilitates

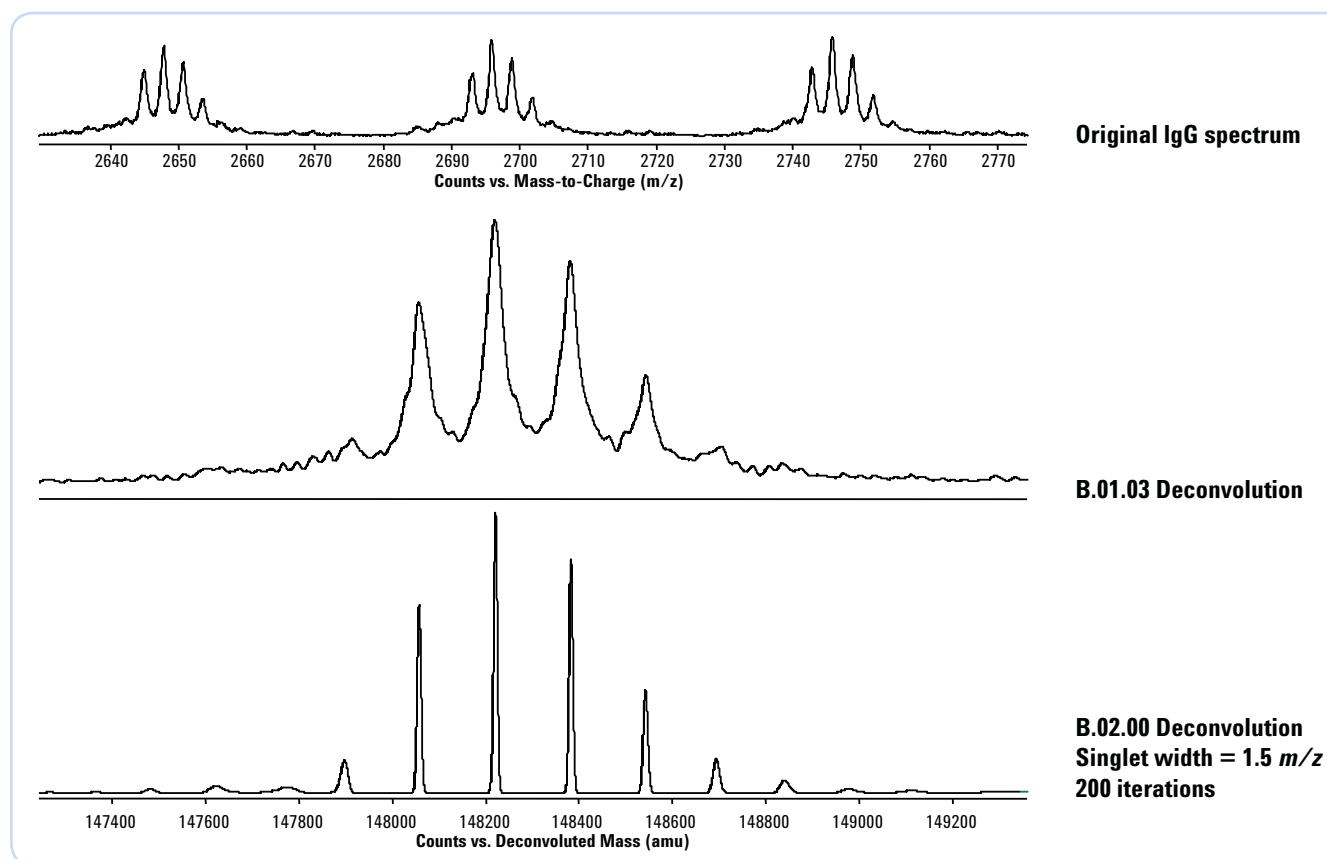


Figure 1. The optimized maximum entropy algorithms in MassHunter BioConfirm B.02.00 improve resolution and mass accuracy.

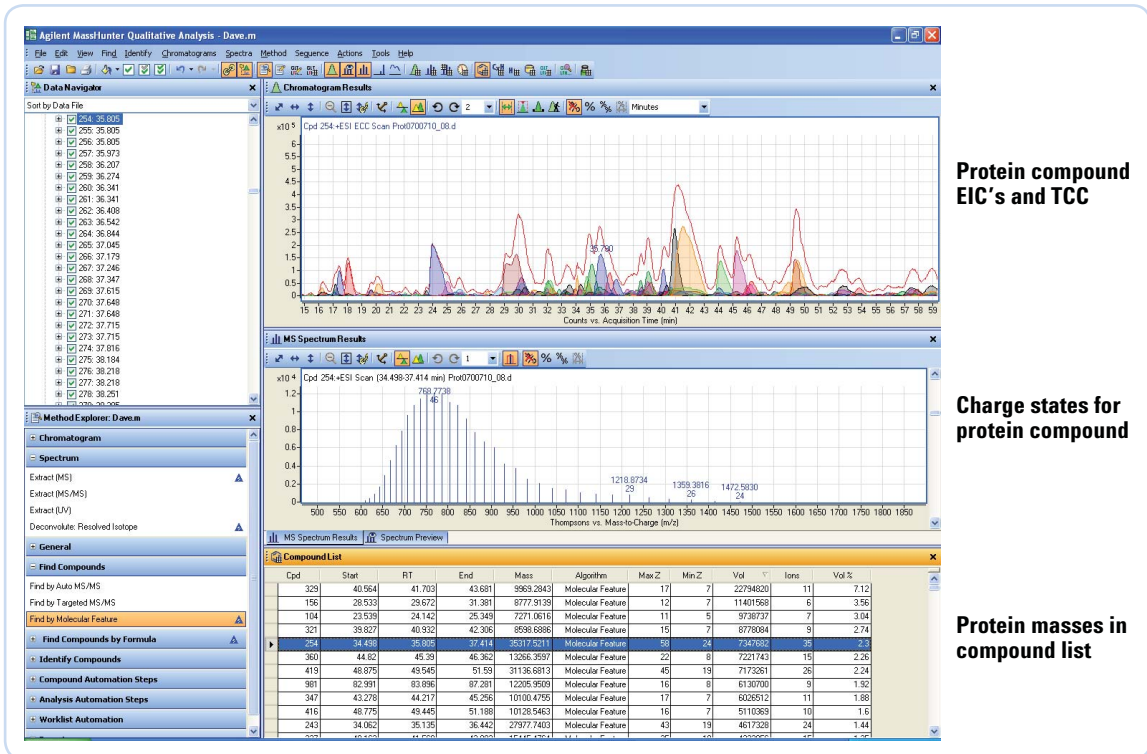


Figure 2. LMFE processing of a mixture of *E. coli* intact proteins generates a list of 682 identifiable compounds within 15 minutes. In comparison, maximum entropy deconvolution yields 140 compounds in 90 minutes.

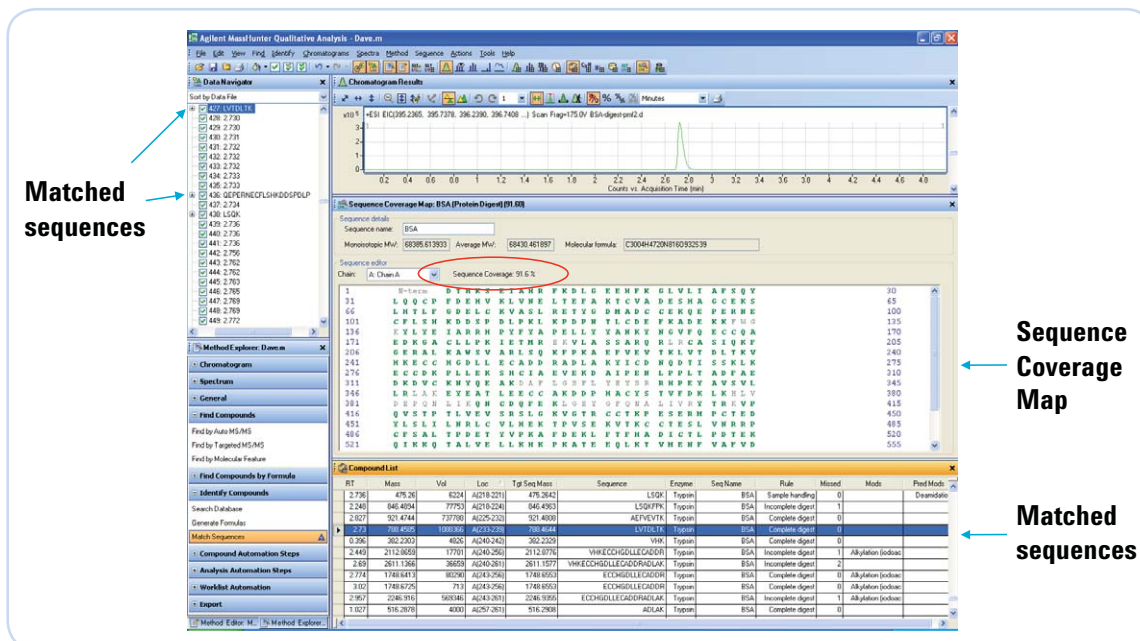


Figure 3. MassHunter BioConfirm B.02.00 software facilitates protein digest analysis and extensive sequence coverage.

the creation of protein digest sequences with site-specific modifications. Matching of protein digest data to defined sequences produces a sequence coverage map (**Figure 3**). Peptides with disulfide links can be matched to confirm the location of disulfide bridges within a protein.

Synthetic Peptide Confirmation

BioConfirm B.02.00 software provides a rapid and accurate means for verifying the integrity and purity of synthetic peptides. The nature of structural errors that may occur during synthesis, such as unremoved blocking groups, missing amino acids and extra amino acids, are accurately characterized.

Superior MS Platforms for Intact Proteins and Synthetic Peptides

Integration of MassHunter BioConfirm B.02.00 software with Accurate-Mass TOF and Q-TOF systems ensures optimal MS characterization of intact proteins and synthetic peptides. Agilent Accurate-Mass TOF and Q-TOF instruments deliver excellent sensitivity, mass accuracy, and mass resolution, as well as up to five orders of in-spectrum dynamic range. Incorporation of Agilent Jet Stream technology further enhances sensitivity at optimal LC flow rates, while the use of HPLC-Chip/MS ensures maximum sensitivity for nanoflow methods. The wide dynamic range and exceptional sensitivity of the Accurate-Mass TOF and Q-TOF MS platforms enable MassHunter BioConfirm B.02.00 software to identify as many trace-level targets and low-abundance impurities as possible in the presence of complex samples.

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