Application Note 00623

Deconvolution of Multiply Charged Ions on Varian's 500-MS LC Ion Trap Mass Spectrometer

Mahalakshmi Rudrabhatla, Ph.D., Varian, Inc.

Introduction

Recent advances in genomic and proteomic discovery have increased the need for analytical tools to characterize biomolecules. Liquid chromatography with electro spray ionization and ion trap mass spectrometry is well established for the analysis of biomolecules. The analysis of proteins, peptides and oligonucleotides result in multiply-charged mass peaks that need to be deconvoluted to produce an average molecular weight of the species. An accurate deconvolution algorithm is required because any mass measurement errors for the multiply-charged species are multiplied by the mass peak charges.

In this work, the use of Varian's Mass ID (multiply-charged ion deconvolution) software for the deconvolution of biomolecules was evaluated. This software features a novel deconvolution algorithm for determining the molecular weights of biomolecules with high mass accuracy.

Instrumentation

Varian 500-MS ion trap mass spectrometer equipped with an ESI source

Varian Prostar™ 210 solvent delivery system (2)

Varian Prostar 430 autosampler

Materials and Reagents

All the protein standards and HPLC reagents were purchased from Sigma –Aldrich Co, St. Louis, MO. Oligonucleotides were purchased from Integrated DNA Technologies Coralville, IA 52241.

Sample Preparation

The stock solutions of proteins were prepared in 0.1% TFA in water. Oligonucleotide stocks were prepared by dissolving them in 0.1 mM TEAA, pH 7.0. All the stocks were stored at 4 °C until further use.

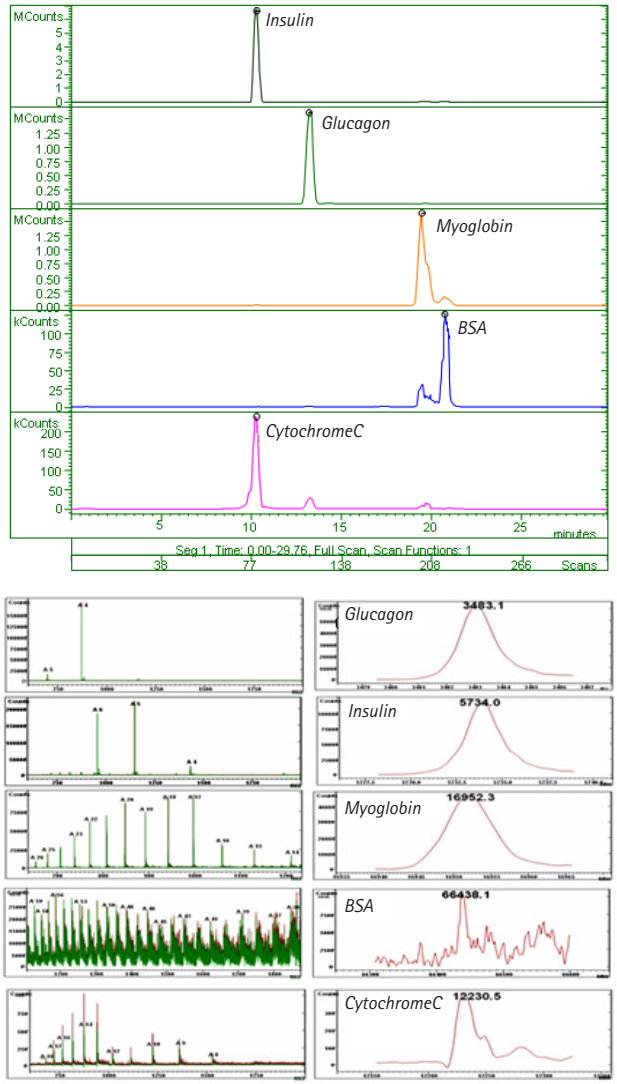

Figure 1 Extracted LC/MS chromatograms (top), Original and deconvoluted spectra (bottom) of Glucagon, Insulin, Myoglobin, BSA, and CytochromeC. Injection volume 10 ul on 500 MS LC ion trap mass spectrometer.

LC Conditions

Column	Pursuit C18 column, 5 µm, 150 mm x 2.1 mm ID (Varian part No A2000150x030)
Buffer A	0.1% HCOOH in water
Buffer B	0.1% HCOOH in 100% methanol
Injection volume	10-100 µl

A gradient of 10-70% buffer B was used with a flow rate of 0.2 ml/min depending upon the protein used.

API Conditions

The API conditions varied depending upon the molecular weight and charge state of the biomolecules analyzed on the mass spectrometer. They are given below:

Ionization Mode	ESI (positive and negative)
API Drying Gas	15-30 psi at 150-350 °C
API Nebuilizing Gas	50 psi
Needle	± 3500-4500 V
Capillary	60-300 V
Shield	± 600 V
RF Loading	80-100%

Discussion

The accurate molecular weights of all the proteins used for this study were obtained from Swiss Prot (protein knowledge base hosted by SID Switzerland) by entering the accession numbers corresponding to the respective proteins.

Name	Expected mass	Deconvoluted mass	Mass error (%)
Glucagon	3482.75	3483.1	0.010
Insulin	5733.4	5734.0	0.010
Cytochrome C	12230	12230.5	0.004
Myoglobin	16950	16952.3	0.013
BSA	66430	66438.1	0.012

Table 1 Mass error (%) of Insulin, Glucagon, Cytochrome C, Myoglobin and BSA after deconvolution

The suitability of Mass ID software was tested for the deconvolution of ESI MS spectra of proteins ranging from low to high molecular weights. A protein mix consisting of insulin (34.8 fmol/µl), glucagon (57.2 fmol/µl), cytochrome C (817.6 fmol/µl), myoglobin (11.79 fmol/µl) and BSA (15.06 fmol/µl) was separated by LC/MS on 500 MS LC ion trap mass spectrometer. The molecular weights of proteins in the mix ranged from ~3400 to ~66000 Daltons. The ESI MS spectra obtained by averaging the extracted ion chromatograms of individual proteins were deconvoluted by Mass ID software. The extracted LC/MS chromatograms, original and deconvoluted spectra of the above proteins are given in Figure 1. The mass errors (difference between the expected and the deconvoluted mass) for all these proteins were calculated and are given in Table 1.

As seen from the Table 1, the mass error for all these five proteins (~3400 to 66,000 Daltons) was found to be less than 0.014% (0.004%-0.013%) indicating that Mass ID software is a powerful tool for protein accurate molecular weight confirmation. Proteins ranging from extremely low to high molecular weight were easily deconvoluted to their accurate main mass with this software. Unlike other deconvolution software, high mass accuracies were obtained for very low molecular weight proteins (Glucagon-3482.75) by Mass ID software.

The efficiency of Mass ID software in deconvoluting mixtures of proteins was tested. A mixture of Rnase A, Carbonic anhydrase and Lysozyme was separated on 500-MS LC ion trap mass spectrometer. The LC/MS TIC chromatogram obtained from the separation of these three proteins on the 500-MS was processed as a single spectrum. The complex spectral mixture obtained by averaging the TIC chromatogram was deconvoluted using the Mass ID software to obtain the molecular weights of all the three proteins. (Figure 2). The mass error (%) for all the three proteins was calculated and is given in table 2. The mass error (%) was found to be very low (0.006%-0.033%) and is comparable with the deconvolution of individual proteins. This indicates the suitability of Mass ID software for the deconvolution of complex mixtures of proteins.

Name	Expected mass	Deconvoluted mass	Mass error (%)
Rnase A	13682.2	13680.0	0.016
Carbonic anhydrase	29021.6	29019.6	0.006
Lysozyme	14694	14698.9	0.033

Table 2 Mass error (%) of Rnase A, Carbonic Anhydrase and Lysozyme

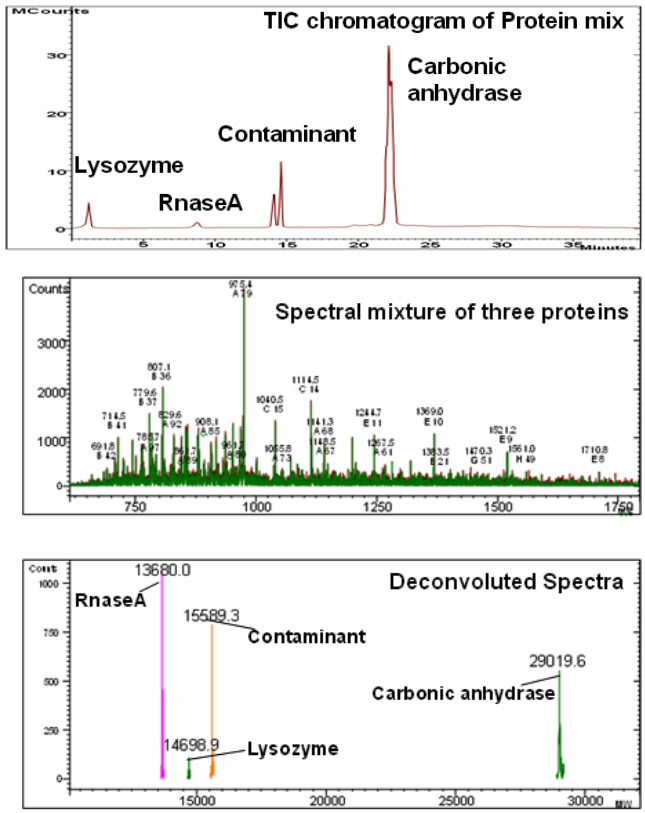

Fig 2 LC/MS TIC chromatogram of a mixture of Rnase A, Carbonic Anhydrase and Lysozyme. The original and deconvoluted spectra are given below.

In the next step, Mass ID software was evaluated for the processing of low signal to noise spectra of large heterogeneous proteins. Transferrin, a large heterogeneous protein was infused at a concentration of 100 fmol/µl. The original spectrum had a heavy baseline noise (Figure 3). A background correction of 5 Da was applied to remove the background noise completely before the actual deconvolution. The background corrected spectrum was then deconvoluted to its main mass. The resultant mass error was very low (0.018%) showing the importance of back ground correction prior to deconvolution. If not effectively removed, baseline noise can cause mis-assignment of charge states, particularly on low S/N spectra.

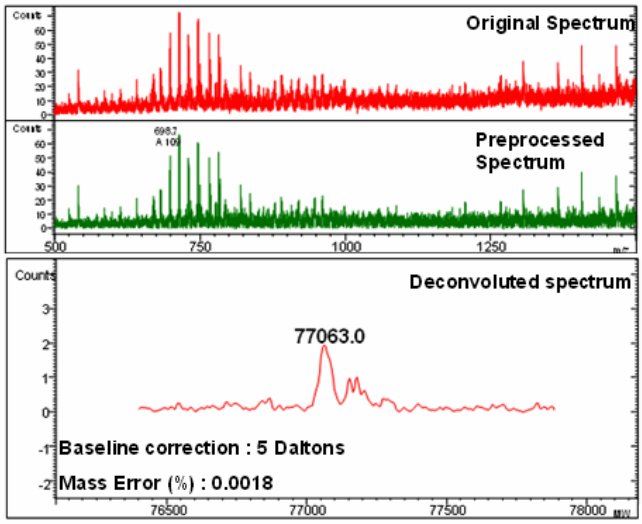

Fig 3 The effect of baseline correction on the deconvolution of low signal-to-noise spectra of a heterogeneous protein Transferrin.

The baseline correction algorithm of Mass ID software is very effective in removing the baseline noise hump that is generally observed in the ESI MS spectra of large proteins (Figure 4). The baseline hump of the BSA ESI MS spectrum was flattened by applying the baseline correction algorithm. This resulted in proper assignment of charges thus leading to the deconvolution of BSA with very high mass accuracy.

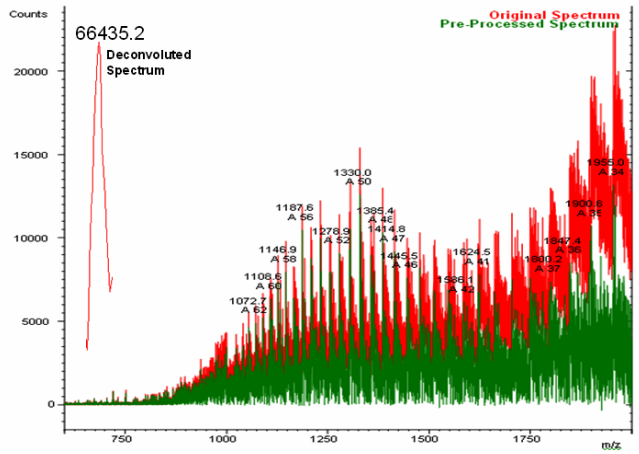

Fig 4 The effectiveness of background correction in removing the back ground hump for BSA is depicted in this figure

The suitability of Mass ID software for oligonucleotide quality control on a 500-MS was evaluated. A 31-mer oligonucleotide (MW: 9555.1) in both the desalted and un-desalted forms at 25 fmol/µl was infused on a 500-MS LC ion trap mass spectrometer. The spectra obtained were deconvoluted. Figure 5 gives the original and deconvoluted spectra of 31-mer desalted (1) and un-desalted (2).

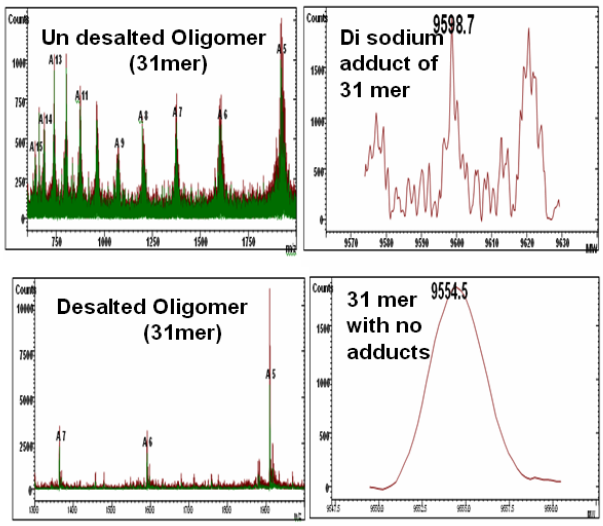

Fig 5 Deconvolution of desalted and un-desalted oligomer (25 fmoles/µl)

As seen from the figure, the desalted oligonucleotide deconvoluted to its main mass and the un-desalted oligonucleotide was deconvoluted to a mass equivalent to its disodium adduct of its main mass.

The deconvolution of proteins with adducts was also tested. Insulin (174 fmol/µl) was infused on a 500-MS LC ion trap mass spectrometer. The ESI MS spectrum of insulin displayed sodium adducts. It was deconvoluted to its original mass and sodium adducts of its original mass. This indicates that Mass ID software can deconvolute species with adducts to its adducted accurate main mass (Figure 6).

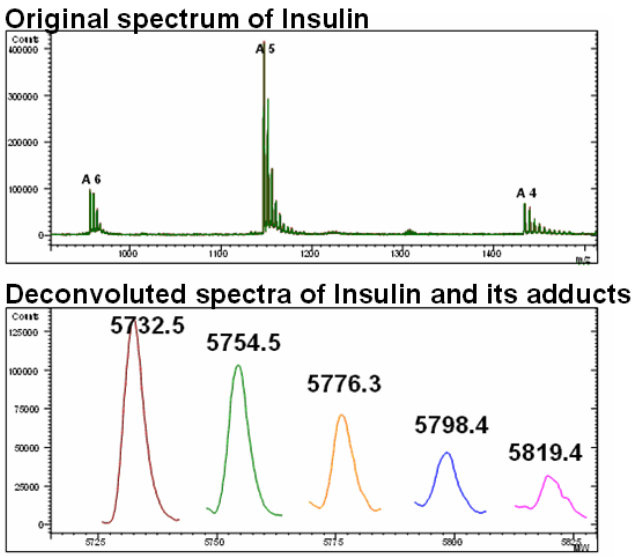

Fig 6 Deconvolution of Insulin adducted to sodium (174 fmoles/µl)

The suitability of Mass ID software for the deconvolution of centroid and profile spectra was evaluated. Figure 7 gives the deconvolution of Cytochrome C in both the profile and centroid modes. From the figure, it can be seen that Mass ID software was very effective in deconvoluting both the profile and centroid ESI MS spectra.

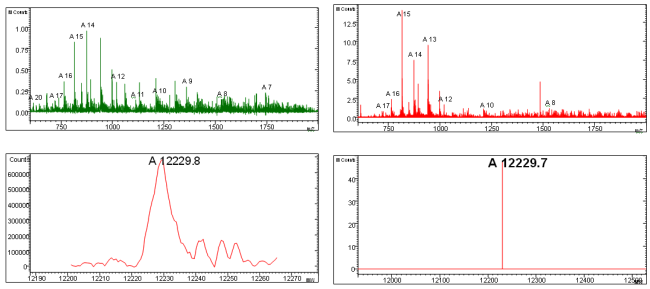

Figure 7 Centroid and Profile deconvolution of Cytochrome C.

Conclusion

Mass ID software is very effective in deconvoluting multiply-charged spectra of proteins, peptides and oligonucleotides that require high mass accuracy. Further, the 500-MS LC ion trap mass spectrometer coupled with Mass ID software is an effective tool for the low ppm analysis of peptides, proteins and oligonucleotides that require high resolution.