

## Overview

A simple method to determine the molecular weight of a biopolymer by calculating the ratio of mass-to-charge ( $m/z$ ) values of multiple-charged ions from a high resolution mass spectrometer is described.

A protein, Neupogen (Amgen) along with a generic version, was used as a model compound. The electrospray mass spectra were recorded on both an AB SCIEX API-4000 tandem mass spectrometer and a Thermo Electron LTQ Orbitrap XL mass spectrometer operated in the positive ion mode.

The molecular weights of Neupogen and a generic form were calculated and compared from data obtained from the two mass spectrometers. The data from the LTQ Orbitrap was much more accurate than that from the API-4000 system.

## Introduction

Electrospray ionization mass spectrometry (ESI-MS) with a unique feature of the ionization process has revolutionized the analysis of biopolymers in solution. The distribution of multiple-charged molecules produced by ESI-MS can be used to calculate the charge states, and subsequently, the molecular weight of a biopolymer compound. The spectrum acquired with a general triple quadrupole mass spectrometer shows low to moderate mass resolution, resulting in a relatively large error

in determination of the molecular weight through the deconvolution approach. In the quality control of protein production and evaluation of the bio-similarity of a protein, the molecular weight is a key parameter. We report herein a quick and simple method using an LTQ Orbitrap mass spectrometer to accurately determine the molecular weight of biopolymers.

## Results and Discussion

Based on the theory of Simin, etc.<sup>1</sup>, the low resolution mass spectrum of a protein can be used to determine the charge number  $z$  of each mass peak, and the molecular weight can then be calculated.

The following equation is used to calculate the molecular weight ( $M$ ) of a compound from the mass-to-charge ratio ( $R_z$ ) of multiple-charged ions that originate from the addition of charge-carrying species:

$$\text{Eq. 1. } R_z = (M + zm)/z$$

$$M = R_z \cdot z - zm$$

Here  $z$  is the number of charges and  $m$  is charge-carrying species (1 Da for  $H^+$ , 17 Da for  $NH_4^+$ , and 23 Da for  $Na^+$ )

## LC-MS/MS Conditions

### Mass Spectrometry 1

MS System: AB Sciex API-4000  
 Ionization Mode: Turbo Ion Spray® in Positive Mode  
 Ion Spray Voltage (IS): 4500 V  
 Temperature (TEM): 550 °C  
 Curtain Gas ( $N_2$ ) (CUR): 15  
 Collision Gas (CAD): 6  
 Turbo Gas: 35  
 Nebulas Gas: 35  
 Declustering Potential (DP): 70  
 Scan Range: 800 — 2000 Da.  
 Scan Time: 4 Sec.

### Mass Spectrometry 2

MS System: LTQ Orbitrap XL  
 Ionization Mode: ElectroSpray in Positive Ion Mode  
 Ion Spray Voltage (IS): 4500 V  
 Capillary Temperature: 300 °C  
 Aux Gas Flow Rate: 30  
 Sheath Gas Flow Rate: 60  
 Capillary Voltage: 9 V  
 Tube Lens: 100 V  
 Resolution: 60,000  
 Maximum Injection Time: 2 Sec.  
 Scan Range: 1000 — 2000 Da.

## Results and Discussion

An equation is derived by dividing the mass to charge ratios ( $R_z$ ) of two multiply charged ions ( $a$  and  $b$ ):

$$\text{Eq. 2. } [(R_z)_a z_a - (R_z)_b z_b] = m (z_a - z_b)$$

$$m = [(R_z)_a z_a - (R_z)_b z_b] / (z_a - z_b)$$

In this study, the  $m$  value is equal to or less than 2 (as shown in high resolution parts of Tables I and II). It indicates that the charge-carrying species is proton, and  $m$  should be 1. The equation 2 is then simplified to equation 3:

$$\text{Eq. 3. } M = R_z \cdot z - z$$

## Results and Discussion

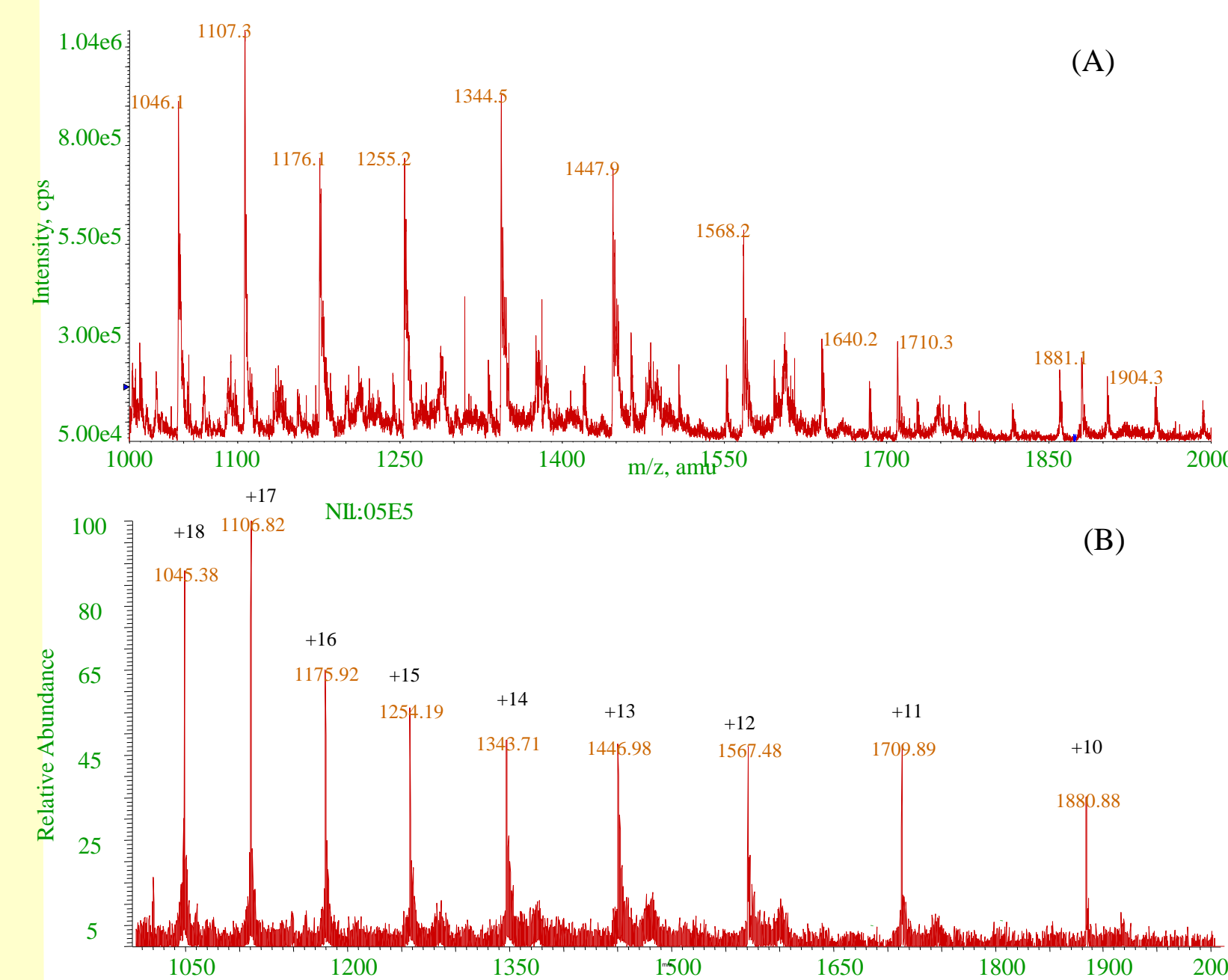


Figure 1. Mass spectra of Neupogen measure by AB Sciex API-4000 (A), and by Thermo Electron LTQ Orbitrap XL (B)

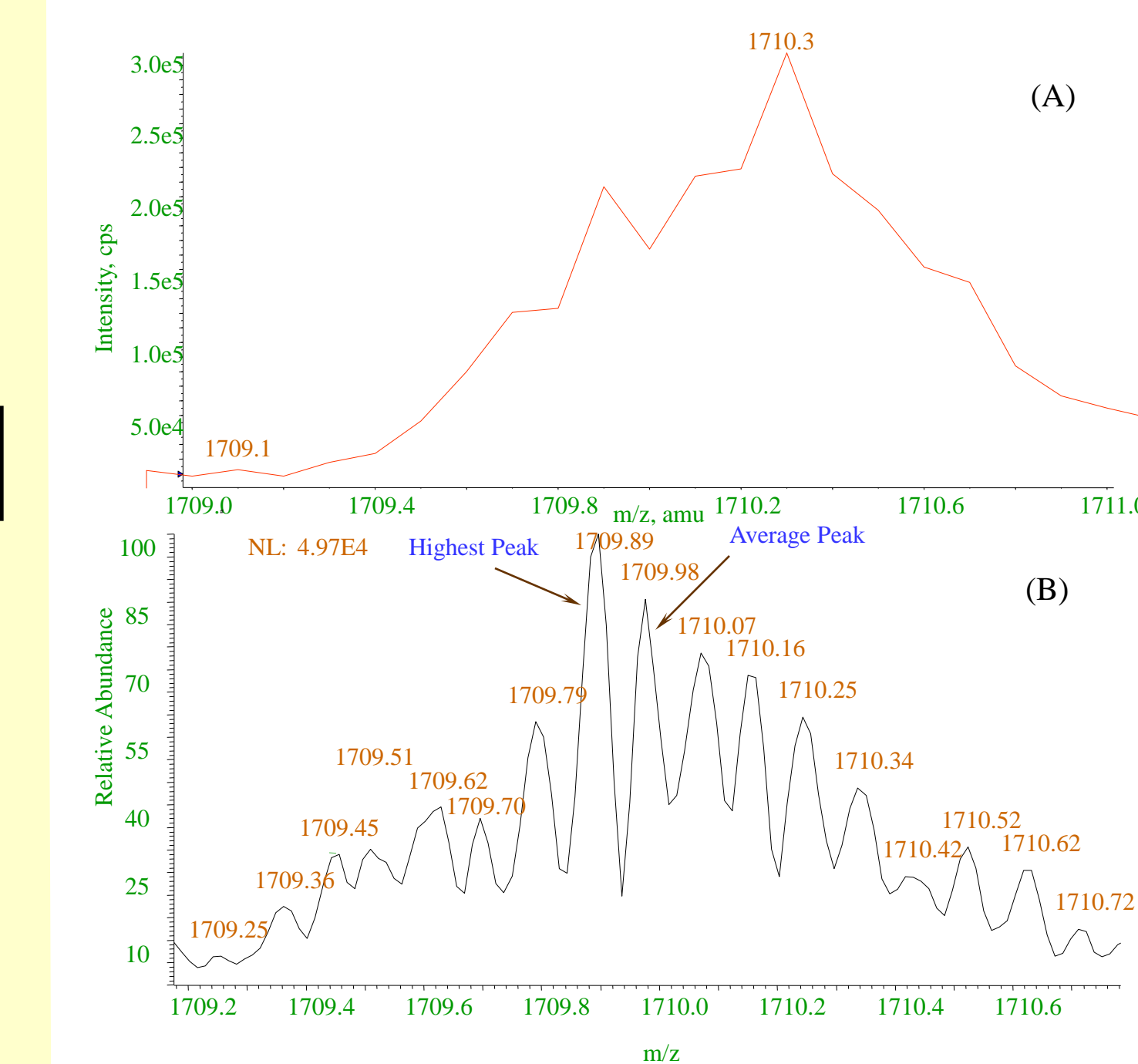


Figure 2. Expanded mass spectra (Carrying 11  $H^+$ ) of Neupogen measured by AB Sciex API-4000 (A), and Thermo Electron LTQ Orbitrap XL (B).

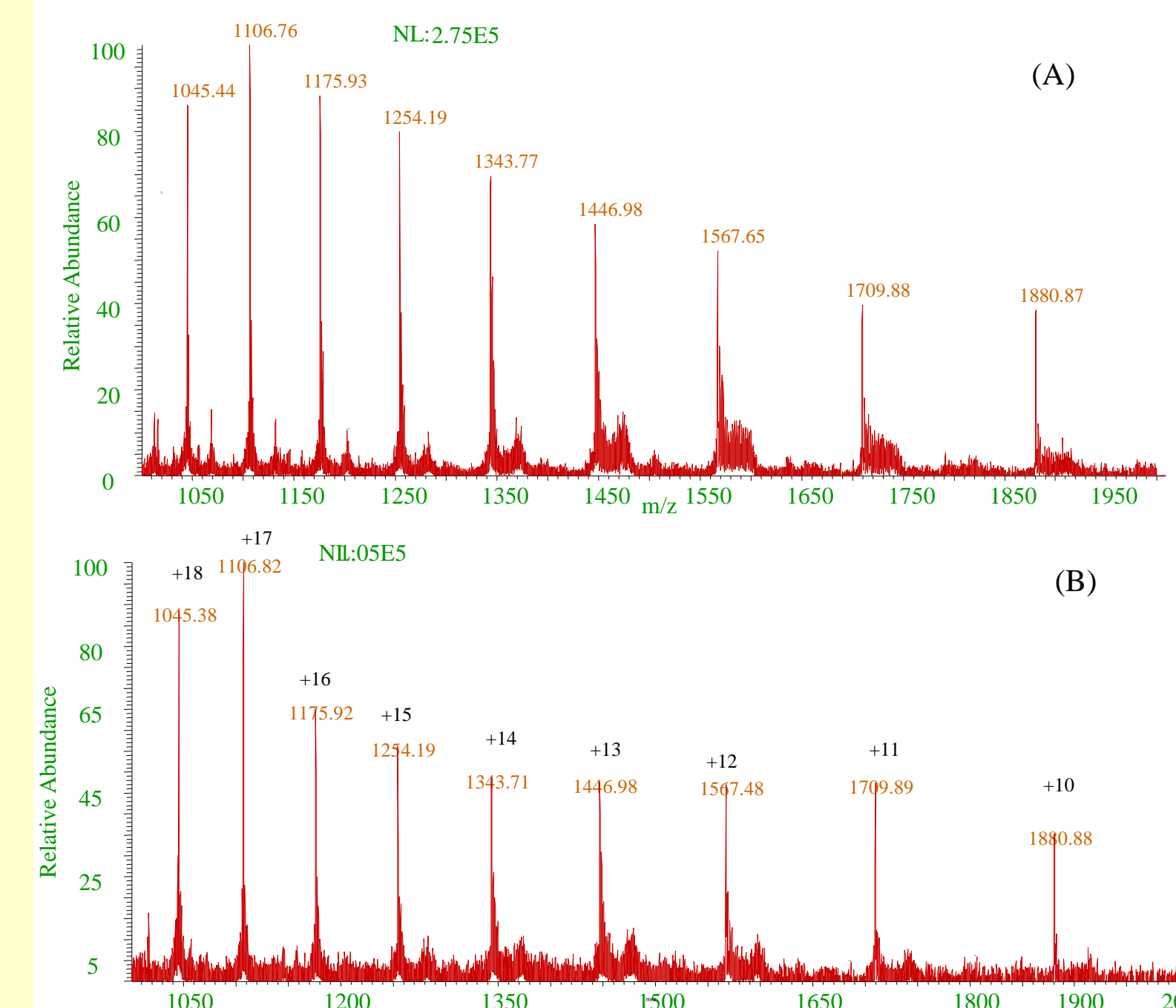


Figure 3. Mass spectra of generic Neupogen protein (A) and brand Neupogen (B) measure by Thermo Electron LTQ Orbitrap XL.

Table I. Data Summary of Neupogen Molecular Weight Measurement with API-4000 and LTQ Orbitrap Mass Spectrometers

With High Resolution MS				With Low Resolution MS			
$R_z$ (middle of Profile)	$z$	$m$	MW	$R_z$ (middle of Profile)	$z$	$m$	MW
1880.78	10	2	18797.8	1881.4	10	-1	18804.0
1709.98	11	1	18798.8	1710.3	11	5	18802.3
1567.56	12	0	18798.7	1568.2	12	4	18806.4
1446.98	13	1	18797.7	1447.9	13	0	18809.7
1343.71	14	2	18797.9	1344.5	14	5	18809.0
1254.26	15	1	18798.9	1255.2	15	1	18813.0
1175.92	16	1	18798.7	1176.8	16	-5	18812.8
1106.82	17	1	18798.9	1107.3	17	6	18810.7
1045.38	18	1	18798.8	1046.1	18	1	18811.8
Average:			18798.5	Average:			18808.5
SD (n-1)			0.50	SD (n-1)			3.81
%CV			0.0027	%CV			0.0203

Table II. Data Summary of Neupogen Generic Protein Molecular Weight Measurement with LTQ Orbitrap Mass Spectrometer

With High Resolution MS			
$R_z$ (middle of Profile)	$z$	$m$	MW
1880.77	10	2	18797.7
1709.97	11	1	18798.7
1567.57	12	0	18798.8
1446.98	13	1	18797.7
1343.70	14	2	18797.8
1254.26	15	1	18798.9
1175.93	16	0	18798.9
1106.76	17	2	18797.9
1045.44	18	1	18799.9
Average:			18798.5
SD (n-1)			0.75
%CV			0.0040

For a low resolution mass spectrum [Figure 1(A)] with non-resolved isotopic peaks [Figure 2(A)], the mass of the highest intensity peak position is assumed to be the theoretical average (i.e. weighted for all isotopes), and used to calculate the molecular weight. However, this may not be true for all measurements, because the experimental spectrum is normally not a perfect Gaussian distribution as predicted by the theory. On the other hand, with the help of the LTQ Orbitrap's high resolution power, all isotopic peaks for each multiple-charged state are resolved [Figure 2(B)]. A difference between the highest intensity mass and the average mass (approximate at the middle of the profile) is commonly observed. The observation that the highest-intensity peak that is assigned as the mass peak in the low resolution mass spectrum is not always in the middle of the profile is shown in Figure 2. This is possibly the main source of error introduced in molecular weight determination by a low resolution mass spectrometer.

The molecular weight and other parameter calculations are summarized in Table I. The  $m$  value from low resolution measurement is  $-5$ — $6$ , a larger variation compared with  $0$ — $2$  from the high resolution measurement. The charge-carrying species as proton could then be confirmed with  $m$  of  $0$ — $2$ .

High resolution mass data also show higher precision in molecular weight measurement with a %CV value of 0.0027% (Table I), which is approximately ten times lower than that measured with a low resolution MS (0.0203%).

The measured molecular weight of Neupogen is 18798.5 Da from high resolution MS (18799 nominal value), much more accurate compared with 18808.5 Da obtained from low resolution MS measurement.

The molecular weight of the generic protein of Neupogen was determined to be the same as that of Neupogen with the LTQ Orbitrap (Table II).

This data demonstrate that the LTQ Orbitrap is an excellent mass spectrometer for the determination of molecular weight of biopolymers.

## Reference

- Simin D. Maleknia, Kevin M. Downard, Charge Ratio Analysis Method: Approach for the Deconvolution of Electrospray Mass Spectra. *Anal. Chem.* 2005, 77, 111-119