Application Note

PROTEIN ANALYSIS

Via Manual Injection using a Compact Mass Spectrometer with extended mass range (CMS-L)

Advion

INTRODUCTION

The CMS-L is a high performance, easy to use, single quadrupole mass spectrometer with an extended mass range analysis capability to *m/z* 2000 – ideal for large molecule applications such as the analysis of proteins. Its small foot print makes it the instrument of choice for space restricted laboratories and is more affordable than comparable MS systems. The CMS-L brings the analytical benefits of mass spectrometry to more scientists than ever before.

A MS detector measures the mass to charge ratio (*m/z*) of an analyte ion, with electrospray ionization resulting in singly (or multiply) charged ions such as $(M+H)^+$, called the molecular ion. Larger molecules such as peptides and proteins, can carry more than one charge, often carrying charges of 10-50 resulting in several ions in multiple charge states such as $(M+19H)^{19+}$, $(M+20H)^{20+}$, $(M+21H)^{21+}$, etc. The resulting mass spectral pattern is commonly referred to as a 'charge envelope' with signals showing a characteristic spacing and intensity distribution. This MS data can be used to calculate the average mass of the analyte by using the approach shown below:

Using neighboring ion signals of the same analyte but sequential charge state, one can calculate the average mass of the analyte in question using the following two equations:

1. $Q_1=(x_2-1)/(x_1-x_2)$ and 2. $M_{ave}=Q_1*(m_1-1)$

Where: $x_1=m_1/z_1$ and $x_2=m_2/z_2$. Note $z_2=z_1+1$; hence being the lower observed *m/z* value of the pair because of the higher charge

RESULTS

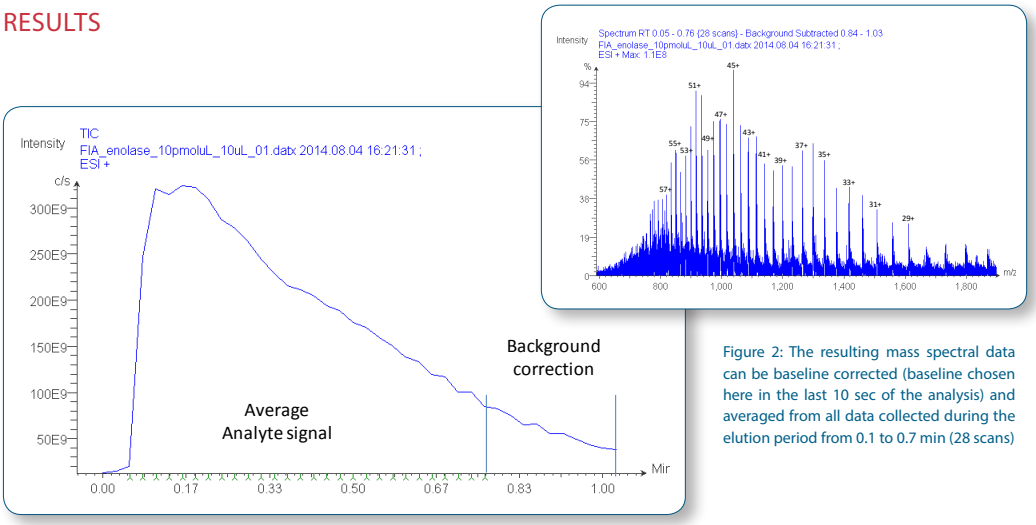

Figure 1 shows a simple protein analysis example using Manual Injection Analysis (MIA) with the CMS-L for the analysis of Enolase (Sigma Aldrich E6126; Enolase from bakers yeast, 10 µL injection of a 10 pmol/µL solution). The analyte is transported to the ESI sprayer via a 100 µL/min solvent flow of 30/15/55 iso-propanol, acetonitrile and water containing 0.1 vol% formic acid. The TIC increases significantly a few seconds after injection and declines within the 1 min analysis window as the protein elutes from the system.

Figure 2: The resulting mass spectral data can be baseline corrected (baseline chosen here in the last 10 sec of the analysis) and averaged from all data collected during the elution period from 0.1 to 0.7 min (28 scans)

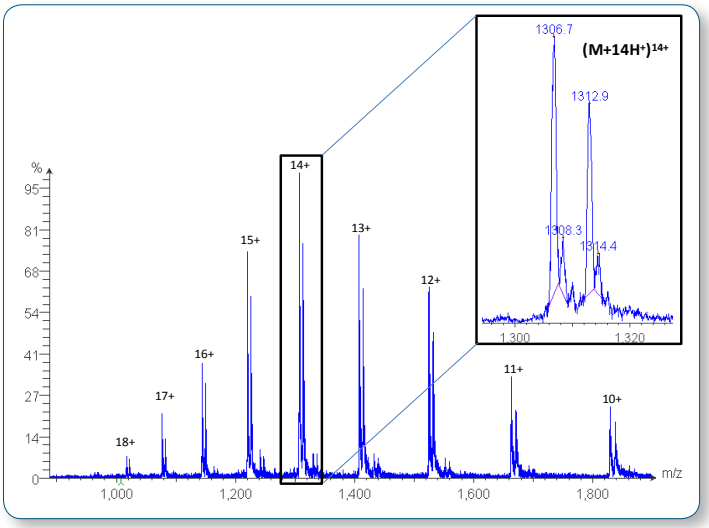

Figure 3 shows a second example of an MIA/CMS-L analysis of beta-lactoglobulin (Sigma Aldrich L3908; 10 µL of a 55 pmol/µL solution injected). This protein is a mixture of the A and B variant of the LACB_bovine protein and shows a distinct charge envelope in the *m/z* range from 1000 to 2000 with charges from 18^+ to 10^+. Highlighting one of the charge states clearly shows two major ion species and additional minor protein forms. Calculation of the average mass of the two major ion envelopes (Table 2) results in an average mass for LACB_bovine B variant of 18,280±1 and 18,366±1 for the A variant. These results are in good agreement with theoretical values for the LACB_bovine AA 17-178 chain of 18,281 and 18,367 respectively [2, 3].

m/z	Calculated Q	Calculated M_{ave}
1016.47		
1076.35	17	18,281
1143.52	16	18,280
1219.70	15	18,281
1306.74	14	18,280
1407.17	13	18,280
1524.35	12	18,280
1662.79	11	18,280
1828.81	10	18,278
	Average	**18,280**
	SD	**1**

m/z	Calculated Q	Calculated M_{ave}
1021.28		
1081.43	17	18,367
1148.82	16	18,365
1225.45	15	18,367
1312.92	14	18,367
1413.82	13	18,367
1531.46	12	18,366
1670.64	11	18,366
1837.52	10	18,365
	Average	**18,366**
	SD	**1**

Table 2

Table 1: Enolase forms an extensive charge envelope in the *m/z* range from 800 to 2000. Applying the aforementioned equations for analysis of the charge states and molecular mass (Table 1) the average protein mass can be determined to be 46,683±5. This represents a good experimental fit to the theoretical average mass of 46,685 of the 2-437 amino acid chain of ENO1_yeast[1, 2].

m/z	Calculated Q	Calculated M_{ave}
805.88		
819.91	57	46,678
834.53	56	46,678
849.65	55	46,676
865.65	54	46,691
881.93	53	46,689
898.73	52	46,682
916.41	51	46,686
934.75	50	46,688
953.74	49	46,684
973.59	48	46,684
994.18	47	46,679
1015.88	46	46,684
1038.35	45	46,681
1062.07	44	46,687
1086.70	43	46,685
1112.59	42	46,687
1139.50	41	46,679
1168.11	40	46,684
1197.98	39	46,682
1229.60	38	46,687
1262.71	37	46,683
1297.75	36	46,683
1334.92	35	46,687
1373.81	34	46,676
1415.68	33	46,684
1459.76	32	46,680
1506.92	31	46,684
1556.90	30	46,677
1610.39	29	46,672
	Average	**46,683**
	SD	**5**

SUMMARY

Manual Injection Analysis using the expression CMS-L is a fast, powerful, yet simple tool to determine, confirm or characterize the mass of a relatively pure protein analyte.

LITERATURE

[1] http://www.uniprot.org search for P00924 (ENO1_YEAST)

[2] http://web.expasy.org/peptide_mass/ for mass calculation from protein amino acid sequence

[3] http://www.uniprot.org search for P02754 (LACB_BOVIN)