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Molecular Weight Confirmation of a Peptide Using MS Spectral Deconvolution for OpenLab CDS and the Agilent InfinityLab LC/MSD XT System

Authors

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Abstract

Intact mass analysis of proteins and peptides by liquid chromatography/mass spectrometry (LC/MS) is a valuable tool in biopharmaceutical research. This application note shows the development of a molecular weight confirmation method for a peptide sample using the MS Spectral Deconvolution feature of Agilent OpenLab CDS version 2.8.

Data were acquired on an Agilent 1290 Infinity II LC coupled to the Agilent InfinityLab LC/MSD XT using the latest version of OpenLab CDS. A recombinant peptide sample was analyzed, and its intact molecular weight, as well as an oxidized species molecular weight, were confirmed by deconvolution of the mass spectra.

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Introduction

Intact mass by LC/MS is a common analytical workflow used to quickly confirm a biomolecule's molecular weight. Large biomolecules such as proteins, peptides, and oligonucleotides have multiple ionizable functional groups, and during electrospray ionization take on multiple charge states, leading to a complicated mass spectrum. This characteristic spectrum, often referred to as a charge envelope, can be used to back calculate the molecular mass of the originating species by a process called deconvolution.

Although it is common to use high-resolution accurate mass detection and spectral deconvolution for intact mass determination, unit mass instruments may also be used when mass accuracy is secondary to robustness. For molecular weight confirmation workflows, unit mass instruments are easier to operate and present a more cost-effective solution than high resolution instruments[1,2].

This application note shows how Agilent OpenLab CDS can be used with an Agilent InfinityLab LC/MSD XT system to automate the molecular weight confirmation of a recombinant 42 amino acid polypeptide, gastric inhibitory peptide-1 (GIP-1). Spectral deconvolution was used to quickly confirm the molecular weight of the peptide. Additionally, an oxidized species of GIP-1 was identified and confirmed using the deconvolution feature. This demonstrates the utility of the LC/MSD XT and the MS Spectral Deconvolution feature to increase confidence in assigning a specific modification to an event such as oxidation without the need for high resolution mass spectrometry.

Experimental

Instrumentation

An Agilent 1290 Infinity II and LC/MSD XT were used to analyze the peptide samples. The complete instrument setup consisted of the following modules:

- Agilent 1290 Infinity II flexible pump (G7104A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1290 Infinity II DAD (G7117B)
- Agilent InfinityLab LC/MSD XT (G6135C) with electrospray ionization (ESI) source (G1948B)

LC method parameters applied for the analysis are shown in Table 1, and MS parameters shown in Table 2.

Table 1. Agilent 1290 Infinity II LC method parameters.

Parameter	Value	
Column	ZORBAX RRHT StableBond 2.1 x 50 mm, 1.8 µm. 80Å, 80 °C	
Mobile Phase A	0.1 % formic acid in water	
Mobile Phase B	0.1 % formic acid in acetonitrile	
Gradient	Time (min)	%B
	0	5
	5	40
	5.5	5
Flow Rate	0.5 mL/min	
Injection Volume	5 µL, standard wash	
Detection UV	285 nm, 4 nm bandwidth, ref. 360 nm	
Acquisition Rate	2.5 Hz	

Table 2. Agilent InfinityLab LC/MSD XT parameters

Parameter	Value
Ion Source	ESI
Polarity	Positive
Drying Gas Temperature	350 °C
Gas Flow	10 L/min
Nebulizer Pressure	35 psi
Capillary Voltage Positive	4,000 V
Scan Range (m/z)	400-2,000
Scan Time (ms)	936
Fragmentor Voltage	200 V
Gain	3
Threshold	0
Storage Mode	Profile

Software

OpenLab CDS version 2.8 with MS Spectral Deconvolution capabilities was used to operate the instrument and perform the analysis. The algorithm used in OpenLab CDS for spectral deconvolution is optimized for simplifying spectra of multiply charged molecules obtained from unit mass instrumentation. The deconvolution parameters needed only slight adjustments for the target analyte, as it produced a good-quality spectrum with highly abundant peaks. Table 3 summarizes the basic settings chosen for the target analyte. A detailed overview over the capabilities of MS Spectral Deconvolution is available in the application note[3] describing the feature.

Table 3. MS Spectral Deconvolution settings

Automatic Deconvolution	
Run Automatic Deconvolution	Checked
All Peaks	Selected
TIC top (n) Peaks	3
Basic Settings	
Use m/z Range	Unselected
Low/High Molecular Weight	1000-8000
Maximum Charge	10
Minimum Peaks in Set	3
Advanced Settings	
MW Agreement (0.01%)	5 (0.05%)
Absolute Noise Threshold	1000
Relative Abundance Threshold (%)	10%
MW Algorithm	Curve Fit
MW Algorithm Threshold	40%
Envelope Threshold (%)	50%

Chemicals

Acetonitrile, formic acid, hydrogen peroxide, and LC/MS grade water were purchased from VWR (Bruchsal, Germany). Recombinant human GIP-1 was purchased from GenScript (Piscataway, NJ, USA). Unless stated otherwise, samples were prepared from lyophilized GIP-1 to a concentration of 1 mg/mL stock solution by diluting with de-ionized water, aliquoted to 50 µL in sample tubes, and kept frozen at -20 °C until use. To force oxidation of GIP-1, a 50 µL aliquot was diluted to 50 µg/mL using a 2% hydrogen peroxide solution and incubated for five minutes at room temperature.

Results and discussion

LC/MS analysis of a stored aliquot of GIP-1 showed two peaks in the total ion current (TIC) chromatogram (Figure 1a). The mass spectra of the peaks at 3.8 and 4.0 minutes displayed distinctive charge envelope patterns common to large molecules (Figure 1b). The deconvolution algorithm reported an apparent mass of 4998.7 Da and 4982.7 Da, respectively, for the main components under the two peaks (Figure 1c). The larger peak at 4.0 minutes was therefore identified as the unmodified peptide GIP-1 with a theoretical mass of 4983.6 Da. The delta mass of the main component in the earlier eluting peak amounts to 16 Da, which suggested an oxidized species of GIP-1.

To confirm the identity of the impurity peak as an oxidation product, a second aliquot was treated with 2% hydrogen peroxide to force oxidation. Additionally, a fresh standard was prepared from lyophilized GIP-1 and analyzed immediately without cold storage to rule out the storage as a source for the observed impurity. The peroxide-treated sample displayed a single peak at 3.8 minutes (Figure 2a) and a deconvoluted mass spectrum that matched the one for the suspected oxidized impurity shown in Figure 1b. The freshly prepared GIP-1 sample showed two peaks as before (Figure 2a), but with a different ratio of the peak areas. Consequently, it was concluded that the impurity observed in the GIP-1 samples was an oxidation product that was naturally present in the samples. Although a possible relationship to storage conditions could not be ruled out, it was not investigated further.

With the observed components identified, the method was then extended to include an automatic PDF report, as shown in Figure 3. The report template presents the deconvolution results per peak and summarizes calculated masses for components and their relative abundance in the spectrum, providing a clear mass readout per peak. This increases the confidence of correct identification of the species without relying on retention time alone, or requiring manual calculations based on known charge states.
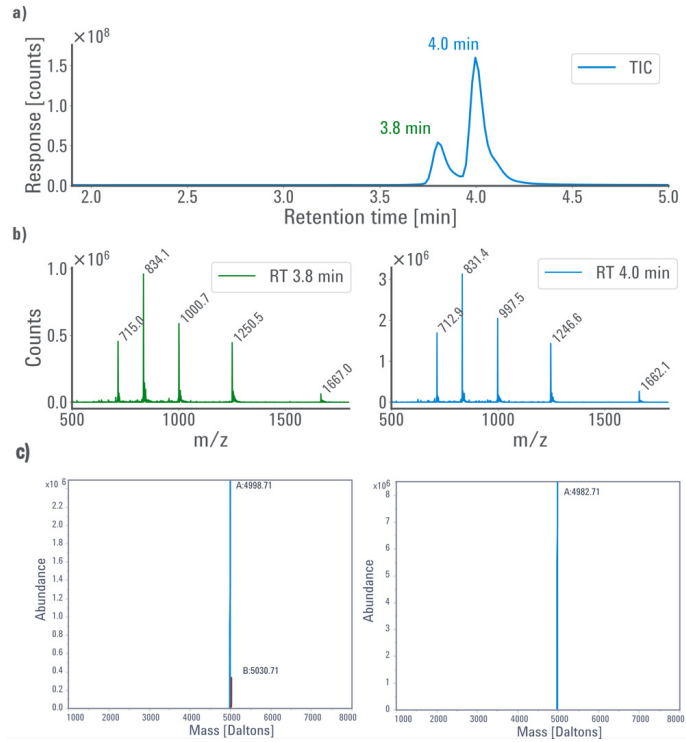
Figure 1. A) Total ion current chromatogram of GIP-1 frozen aliquot (100 µg/mL in water). B) Extracted mass spectra for peaks at 3.8 and 4.0 minutes, respectively. C) Deconvoluted mass spectra for the two identified peaks.
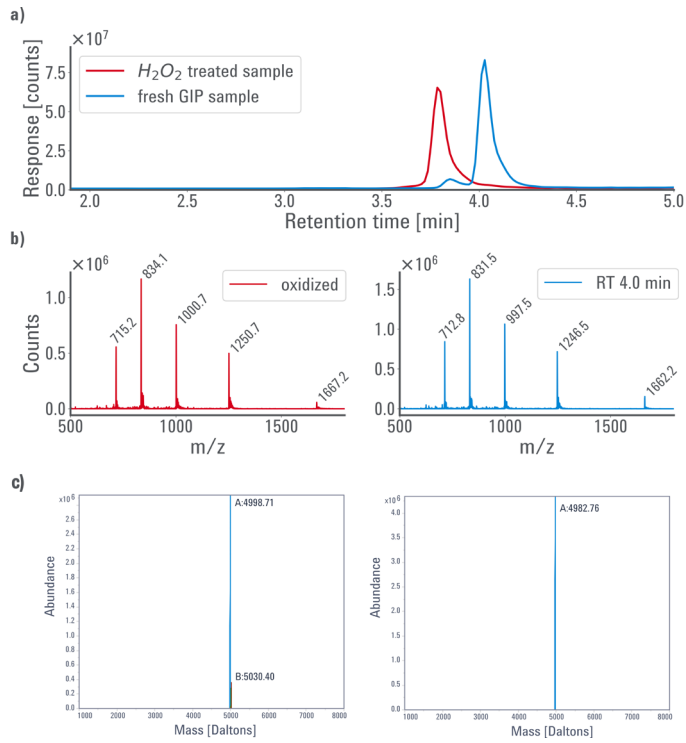

Figure 2. A) Overlaid TIC chromatograms of a GIP-1 sample prepared fresh from lyophilized standard and a hydrogen peroxide treated aliquot (50 µg/mL each). B) Extracted mass spectra for the respective peaks in A). C) Deconvoluted mass spectra for the peroxide-treated sample, and in comparison, the one for GIP-1 prepared fresh.

Deconvolution of peak at RT: 3.801

Signal: MS1 +TIC SCAN ESI Frag=200V Gain=3.0

Spectrum: MS1 +Scan ESI (rt: 3.801 min) Frag=200V Gain=3.0
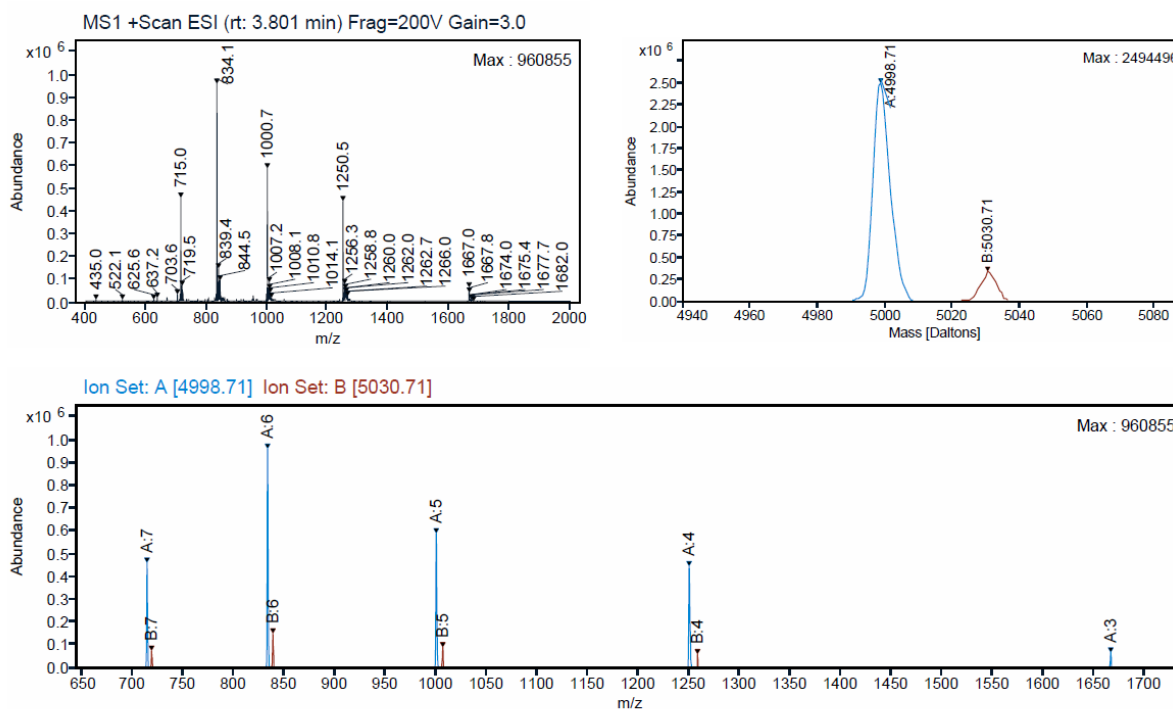
Component	Mass	Absolute Abundance	Relative Abundance (%)	Relative Quantitation (%)
A	4998.71	2494496	100.00	88.19
B	5030.71	333957	13.39	11.81

Figure 3. Excerpt from an automatic report showing the deconvolution results for the peak of the oxidized GIP-1 species. The report gives a detailed view of the mass spectrum, the deconvolution result, the ions considered in deconvolution, and a table with estimates of the relative abundance of the two components.

Conclusion

The combination of the Agilent 1290 Infinity II LC system with the Agilent LC/MSD XT and Agilent OpenLab CDS version 2.8 with MS Spectral Deconvolution is a powerful tool to perform peptide analysis on a rugged platform. The automatic deconvolution enables mass confirmation for a target peptide, as well as its impurities, improving confidence in assigning the correct species. The deconvolution process can be run interactively through the CDS interface during method development, with various options to control the output. The fully automated workflow, where a report can be generated without user intervention, makes it easy to transition to higher throughput analyses. This versatility makes OpenLab CDS an attractive option for both research and routine quality control laboratories where efficiency, accuracy, and time-to-result depends on the instrumentation and associated software.

References

1. Fast and Simple Protein Molecular Weight Confirmation Using the Agilent InfinityLab LC/MSD XT Mass Selective Detector. Agilent Technologies application note, publication number 5994-0188EN, **2019**.

2. Critical Quality Attribute Monitoring of mAbs at the Intact and Subunit Levels Using a Cost-Effective, Simple and Robust LC/MS Solution. Agilent Technologies application note, publication number 5994-1349EN, **2019**.

3. Unit Mass Spectral Deconvolution for Molecular Weight Confirmation of Large Molecules. Agilent Technologies technical note, publication number 5994-6928EN, **2023**.

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