Quantitative Analysis of Post Translation Modification of Protein Therapeutics at a Subunit Level by Spectral Deconvolution

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Overview

- LC-MS analyses were performed on a IdeS digest of a NIST monoclonal antibody (mAb) reference materials.
- Spectral accuracy was evaluated under different data acquisition conditions to ensure high spectral accuracy can be achieved for collecting digested NIST mAb.
- Through peak shape calibration and spectral accuracy calculation, relative quantitation of computer simulated deamidated light chain subunits of mAb was performed.

Introduction

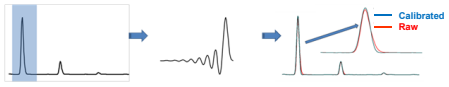
LC/MS/MS peptide mapping has been the method of choice to quantify post translation modification (PTM) of protein therapeutics. However, sample preparation and data processing often is timing consuming. More importantly, the most frequently used trypsin digestion process can introduce artificial deamidation, a false positive that could cause unnecessary concerns on the stability of the biologic medication under development. Alternatively, protein therapeutics can be digested by special enzyme Ides to generate mAb subunits very quickly and specifically without the possible deamidation artifacts. Based on well-established spectral accuracy approach to quantitation of deamidated peptides, we will explore feasibility to quantify important PTMs of protein therapeutics including deamidation at a subunits level.

Methods

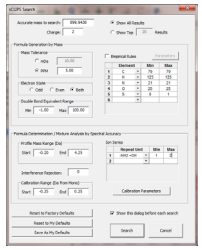
- **Samples:** NIST mAb reference materials were digested with Ides according manufacture protocol.
- **MS Data Acquisition and simulation:** LC-MS data were acquired in a profile mode with a mass range from *m/z* 200 to 2000 and resolving power of 70,000 and 140000 (FWHM) on Thermo Q-Exactive and Fusion Orbitrap mass spectrometers. To test our approach to quantitation of deamidated subunits of mAb, computer simulated light chain deamidated (1% to 10%) subunits based on commercially available mAb from Waters were prepared with MATLAB.
- **MassWorks data processing:** High resolution Orbitrap data files were opened directly by MassWorks to perform comprehensive calibration through CLIPS (Calibrated Lineshape Isotope Profile Search) based on the monoisotope peak. Through the calibration, spectral accuracy can be calculated and utilized to perform exact mixture analysis between calibrated spectra and theoretically calculated spectra for the purpose of quantitation of deamidated subunits mAb.

Results and Discussion

I. Illustration of the Approach with Deamidated Peptides

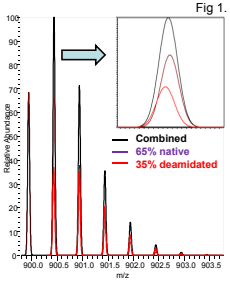


- A monoisotopic peak of the Ultramark is used as the calibration standard for both mass and peak shape calibration.
- The calibration standard should be as close in *m/z* and time of measurement to the unknown as possible to achieve the best spectral accuracy for quantitation of deamidated peptides or subunitsof mAb.



- **Calibrating for high spectral accuracy**
- **Exact mixture deconvolution**
- **Quantitative analysis**

Fig 1.



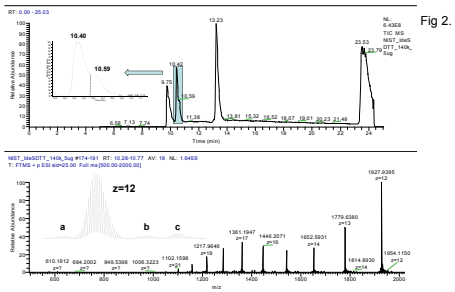
II. Evaluation of Spectral Accuracy with Various Conditions

Resolving Power	Micro scans	Scan rate (amu/s)	Spectral accuracy (%)		
			m/z 1022	m/z 1322	m/z 1722
70 K	3	2312.5	99.08	99.21	98.03
70 K	3	2371.8	99.37	98.82	97.56
70 K	10	709.2	99.07	99.15	97.89
70 K	10	711.5	98.94	99.30	97.94
70 K	10	685.2	99.20	99.08	97.79
140 K	3	1202.5	98.20	98.67	98.69
140 K	3	1185.9	98.65	98.65	98.26
140 K	10	370.0	98.59	98.58	98.60
140 K	10	355.8	98.79	98.46	98.25
140 K	10	354.4	98.45	98.42	98.35

In order to obtain accurate quantitation of deamidated subunits of mAb, it is essential to achieve high spectral accuracy on measured spectra. We performed a brief investigation on spectral accuracy with Ultramark under different levels of resolving power, scan rate, number of microscans, and different m/z values. As summarized in above table, High spectral accuracy of 98% or better were achieved for most of measurements.

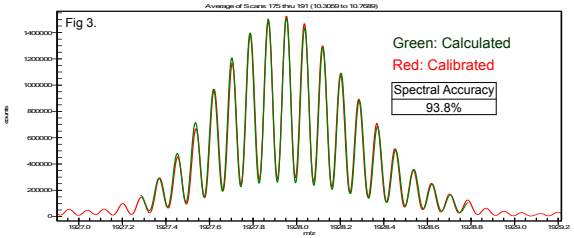
III. LC/MS of subunits of NIST mAb Reference Materials

Fig 2.



IV Spectral Accuracy of Light Chain Subunits of NIST mAb

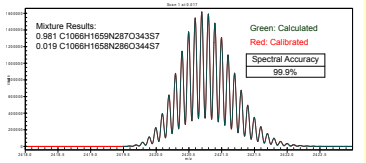
Fig 3.



The light chain subunits of NIST mAb reference materials were eluted at Rt about 10.4 min and observed with multiple charge states. As the most abundant ions carrying 12 charges, the multiple charged ion with m/z value of 1927.9 were calibrated by Ultramark ions at m/z 1922. Since the calibration ion at m/z 1922 achieved high spectral accuracy better than 98% and are very close to the subunits in m/z value, we expected high spectral accuracy for the spectra of the light chain subunits. However, only 93.8% spectral accuracy was found for the light chain subunits, which is far away from commonly accepted benchmark of 98% required for accurate quantitation of deamidated peptides or subunits of mAb. We have carefully investigated both elution profile and mass spectra of the subunits to try to understand what causes the poor spectral accuracy. Although the LC peak (the insert in Fig 2.) of the subunits does appear to have a shoulder indicating two possible components, elemental composition determination on either the left of the peak (Rt = 10.40) or the right of the peak (10.59) resulted in no difference from each other (data not shown). This suggests there might be two different isoforms of light chain subunits. In addition, a close look at averaged spectra of the light chain subunits shows some minor components appear at the m/z values close the major ions at m/z 1927.9, denoted as **a**, **b**, and **c**. Therefore, there would be a possibility that some minor unknown components co-eluted with the subunits to cause the poor spectral accuracy.

V. Quantitation of Simulated Deamidated Subunits of mAb

Although we successfully demonstrated spectral accuracy can be used to quantify deamidated peptides previously, deamidation quantitation at subunits level is very challenging as the relative spectral changes due to this modification become extremely small. We have generated computer simulated light chain subunit spectra with 2% deamidation. Calibrated by a peptide at m/z 2464, the computer simulated mixture spectra were deconvoluted to achieve spectral accuracy of 99.9% and to be accurately determined to have 1.9% deamidated subunits as compared with 2.0% prepared by computer simulation. While the results of this simulated data are encouraging, real tests will be to quantify deamidated subunits from biologics manufacture processes.



Conclusions

- High spectral accuracy has been found at high resolving power of 140K and under many other different data acquisition condition on a Fusion Orbitrap mass spectrometer.
- Even though high spectral accuracy has been achieved with Ultramark, data analysis on IdeS digested light chain subunits of NIST mAb reference materials with similar m/z values to the Ultramark calibration ions did not result in high spectral accuracy as expected. Further work needs to be done to investigate the cause to the poor spectral accuracy.
- Accurate quantitation of computer simulated deamidated subunits of mAb was obtained through spectral accuracy calculation.

References

1. Yongdong Wang and Ming Gu, The Concept of Spectral Accuracy for MS. Anal. Chem. 2010, 82, 7055–7062
2. Ming Gu, Accurate Quantitation of Deamidated Peptides by Mass Spectral Accuracy. Proceedings of the 61st ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN, 2013.
3. Michael Peddicord, Difei Qiu, Ming Gu, and Yongdong Wang. Accurate Quantitation of Deamidated Peptides to Accelerate Formulation Process Development in Therapeutic Proteins. Proceedings of 62nd Annual ASMS Conference. June 2014, Baltimore, MD