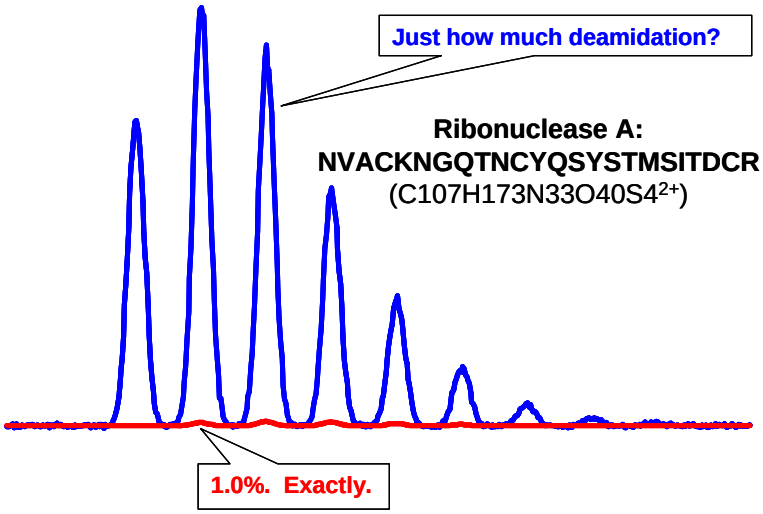
Cerno Application Note

Extending the Limits of Mass Spectrometry

Accurate Quantitation of Low Level Degradation Impurity for Biologics

In the development and production of large molecule drugs including oligos, peptides, and proteins, sensitive and accurate quantitation of deamidation/deamination impurity is of high importance during both the R&D phase and the manufacturing and distribution process. Since the deamidation/deamination product is formed by the addition of -OH group and the simultaneous loss of $-NH_2$ group [1-2], the native and the degradation impurity have very similar and mutually overlapping mass spectral responses. Adding to the complexity are the typical multiple charges from these larger molecules associated with ESI LC/MS and the need for impurity quantitation at a low concentration level of 1% or less.

While it may be possible to individually quantify these components through elaborate chromatographic separations such as ion chromatography [2], it is more desirable to measure and quantify this important degradation impurity under either direct infusion or faster LC separation where these components are either not separated at all or not completely separated, resulting in mutually overlapping mass spectral peaks. By using raw profile mode MS data and the unique Cerno MassWorks™ calibration that corrects not only the m/z but more importantly the MS peak shape [3], a mathematically exact solution could be found which would quantify the relative amounts of both the native and the degradation impurity. With the mixture search capability recently updated in Version 4.0 of MassWorks software (either CLIPS™ or sCLIPS™ [4]), accurate and sensitive quantitation of deamidation or deamination impurity can be achieved at concentration levels as low as 1%.



References

[1] Donato, A. D. et al, *J. Bio. Chem.* **1993**, 268, 4745-4751.
[2] Dionex Corp., Application Note 125, "Monitoring Protein Deamidation by Cation-Exchange Chromatography."
[3] Wang, Y.; Gu, M. *Anal. Chem.* **2010**, *82*, 7055-7062.
[4] Erve, J. C. L. et al, *J. Am. Soc. Mass Spectrom.* **2009**, 20, 2318-2333.

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