Extending the Concept of Spectral Accuracy to the Deconvolution of Multiply Charged Large Molecules

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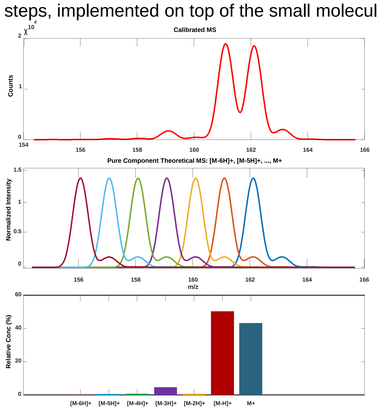
Introduction

Spectral accuracy measures how well an actual mass spectrum, typically acquired in full spectral profile mode including all significant isotopes after elaborate peak shape calibration, matches that theoretically calculated from a known or proposed elemental composition. It has proven to be quite useful for small molecule analysis, above and beyond mass accuracy alone[1-3]. For large molecules under ESI, a distribution of different charge states results in a series of related ions measured across a wide m/z range. These multiply charged ions typically require a multiple charge deconvolution step to back out the intact molecular mass for identification / relative quantitation.

A new approach, **S**pectrally **A**ccurate **M**odelling of **M**ultiply-charged **I**ons (**SAMMI**), is demonstrated here to extend the concept of spectral accuracy and apply it to the analysis of large molecules, as a spectrally interpretable alternative to the conventional multiple charge deconvolution, typically shrouded in some form of mystery and thus hard to understand, relate to, or diagnose.

Method

By delineating and mathematically modeling the physical, chemical, and instrumental factors, namely charge state, isotope distribution, MS instrument peak shape (beyond just the resolution), and spectral overlaps from adducts/modifications, SAMMI works in three discrete yet closely related steps, implemented on top of the small molecule mixture analysis in MassWorks CLIPS[4]:



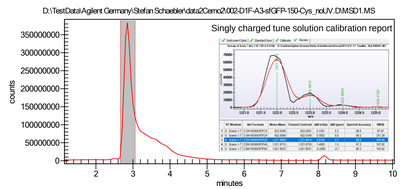
(1) Utilize a known and pure standard (either singly or multiply charged) to calibrate the profile mode MS data for both mass and spectral accuracy with the MassWorks software tool[4] to standardize/ remove instrument-specific factors from MS data;

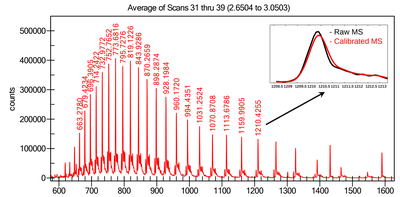
(2) Include all the charges, the native ions, possible modifications or labeled isotopes[5], and the corresponding isotope distributions, all under the same calibrated and known MS peak shape function and compute their theoretical pure component MS;

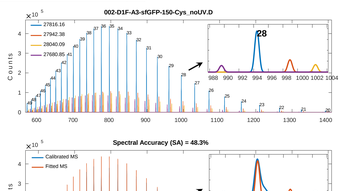
(3) Perform a Multiple Linear Regression (MLR) to fit all pure component MS in the least squares sense to the calibrated sample MS data for both identification and relative quantitation, directly in the actual measured MS (m/z) space without the need for any additional and oftentimes burdensome or even questionable multiple charge deconvolution.

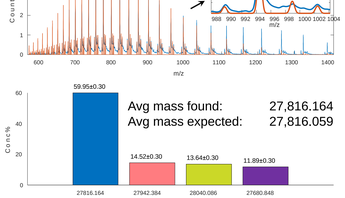
LowRes LC-MS: Unknown Intact Mass Determination

Agilent LC/MSD XT with Univ. Zurich Protein Sample





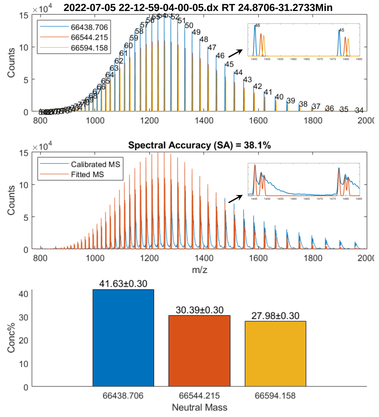




MassWorks mass & spectral calibration using the tune standard solution resulting in meaningful & significant adjustment to the profile mode MS data ready for SAMMI.

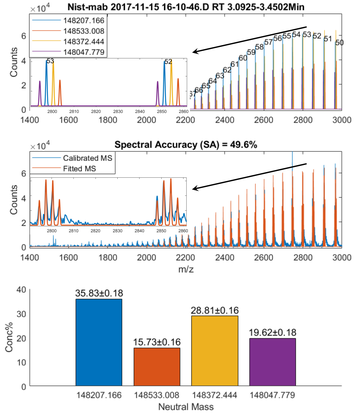
After identifying 4 components and their respective intact masses & charges, SAMMI performs MLR fit to the calibrated MS to obtain their relative concentrations.

Agilent Infinity LC/MSD with Ohio State BSA Sample



Biological samples such as BSA contain a continuous distribution of modified and related molecules as seen in the long tails for each charge. Nonetheless, SAMMI is able to determine the intact masses of the major species present.

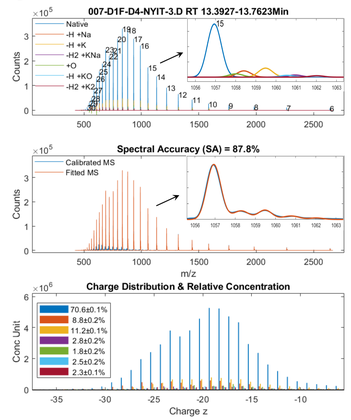
Agilent LC/MSD XT with NIST mAb Standard



Without a proper calibration standard available, we used the calibration from the Ohio State on the left instead. The intact mass assignment accuracy is therefore not expected to be high / optimal. This however does demonstrate the higher end of the mass range for SAMMI on a LowRes LC/MS system.

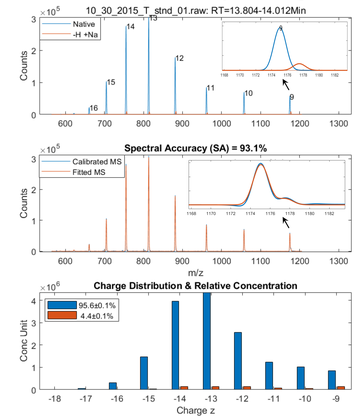
LowRes LC-MS: Confirmation, Modification, and Relative Quantitation

Agilent LC/MSD XT with NY Inst. Tech. 50nt RNA



- Avg mass found 15,868.893 vs 15,869.444 expected
- Nearly perfect spectral fitting achievable[6-7]
- High end single quad able to differentiate & quantify various adducts and oxidation
- Ability to detect and quantify deamination has been shown previously[8] but more systematic test/evaluation needed to establish the detectable % deamination and the achievable RSD%
- Consistent with previous observations[7], the adduct formation (especially K-adduct) is highly charge-dependent

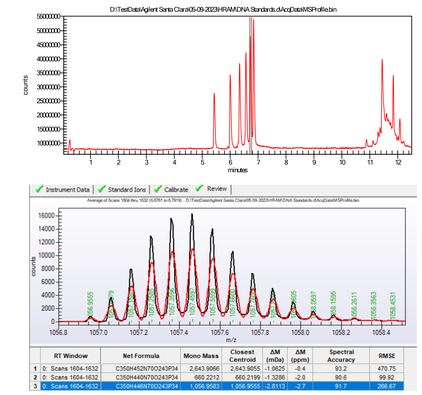
Waters Qda with PolyT 35nt Oligo

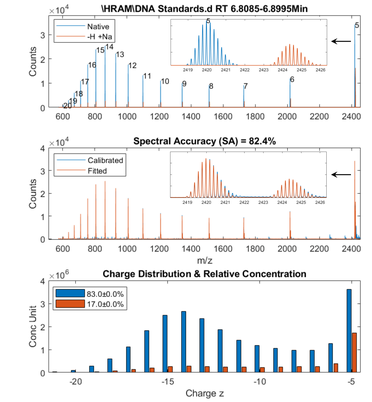


- Avg mass found 10,585.370 vs 15,584.794 expected
- A mass and spectral calibration using the conveniently available and multiply charged PolyT 15nt is feasible
- Reasonable spectral fitting achievable
- Low end single quad able to differentiate & quantify sodium adducts and likely other modifications of similar or larger mass difference
- Consistent with previous observations on higher end systems[7], the adduct formation seems to be charge-dependent

Application to HiRes LC-MS

Agilent LC/MS qTOF with PolyT Oligo Standard





- This PolyT oligo standard sample contains 15-, 20-, 25-, 30-, 35-, and 40-nt
- The 35-nt PolyT oligo with -4, -10, and -16 charges covers the m/z range of interest and was used as the MassWorks calibration standard, not to improve mass accuracy here for qTOF but to introduce Spectral Accuracy for accurate full spectral analysis
- The 40-nt PolyT was then used as a test molecule for SAMMI's confirmation analysis
- Good Spectral Accuracy was observed for each of the expected multiply charged ions including all significant isotopes
- The overall spectral error of 1-82.4%=17.6% comes mostly from the co-existing impurities from ~ m/z 2,300 and 900 which were not included in MLR model for this study
- Both the native and Na adduct can be accurately accounted for with their relative concentrations calculated for each and every charge state
- The formation of Na adduct is highly dependent on the charge state and any assumption otherwise would have resulted in large systematic errors

Conclusion

Accurate and scientifically transparent determination of multiply charged biomolecules is feasible, as long as the physical, chemical/biochemical, and instrumental factors that contribute to the mass spectral accuracy have been properly accounted for. Full spectral MS calibration involving mass spectral peak shape can significantly simplify the process while increasing the analysis accuracy, with either the low-end or high-end single quadrupole MS. The same also applies to HiRes LC/MS as shown by the qTOF data presented. From the various data sets and instrument systems tested, it is clear that each charge state has to be analyzed separately, in order to accommodate the varying amount of metal adducts and/or other modifications at different charges.

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