Improving Intact Mass Analysis of Large Molecules with Single Quadrupole MS through Spectrally Accurate Charge Deconvolution

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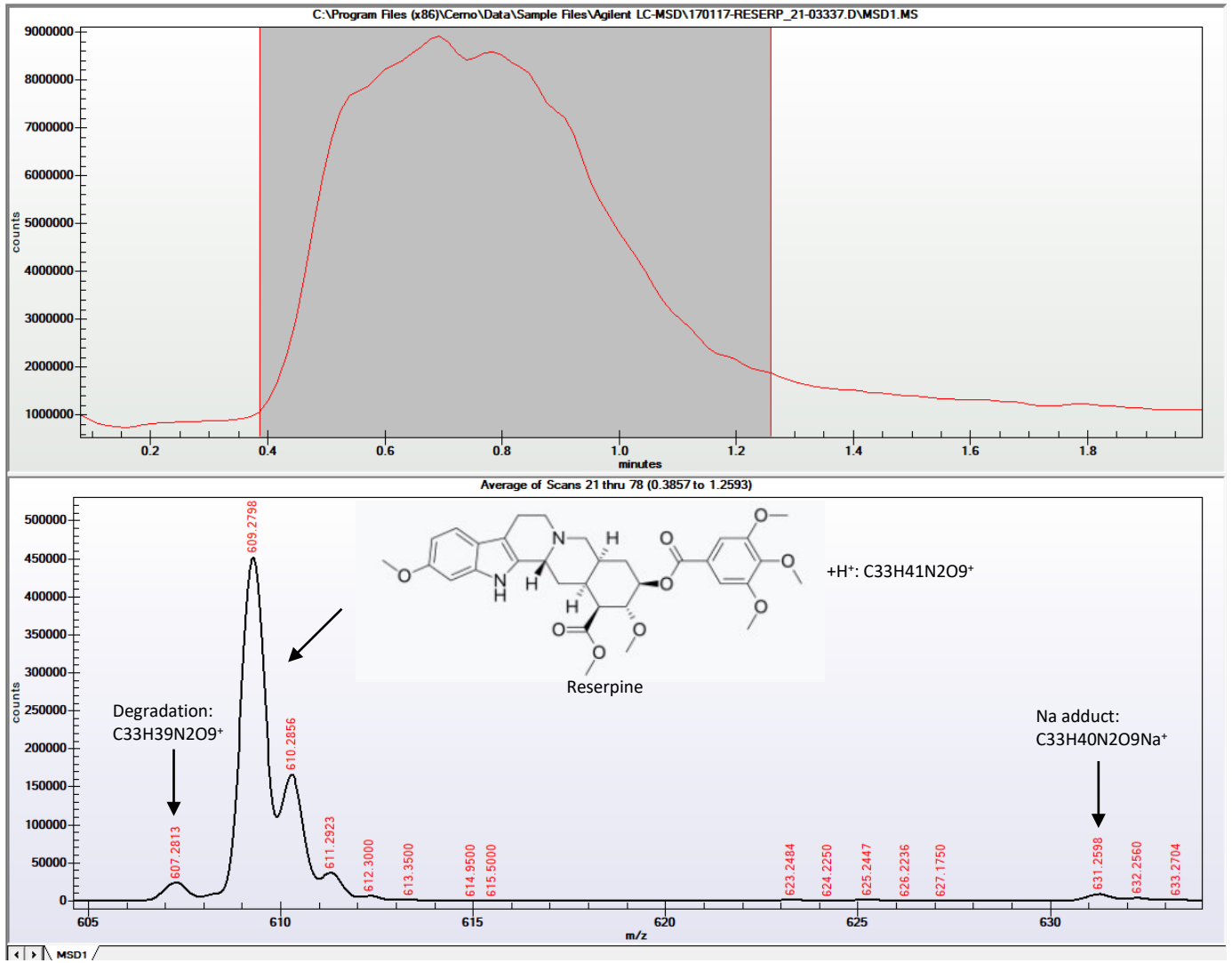
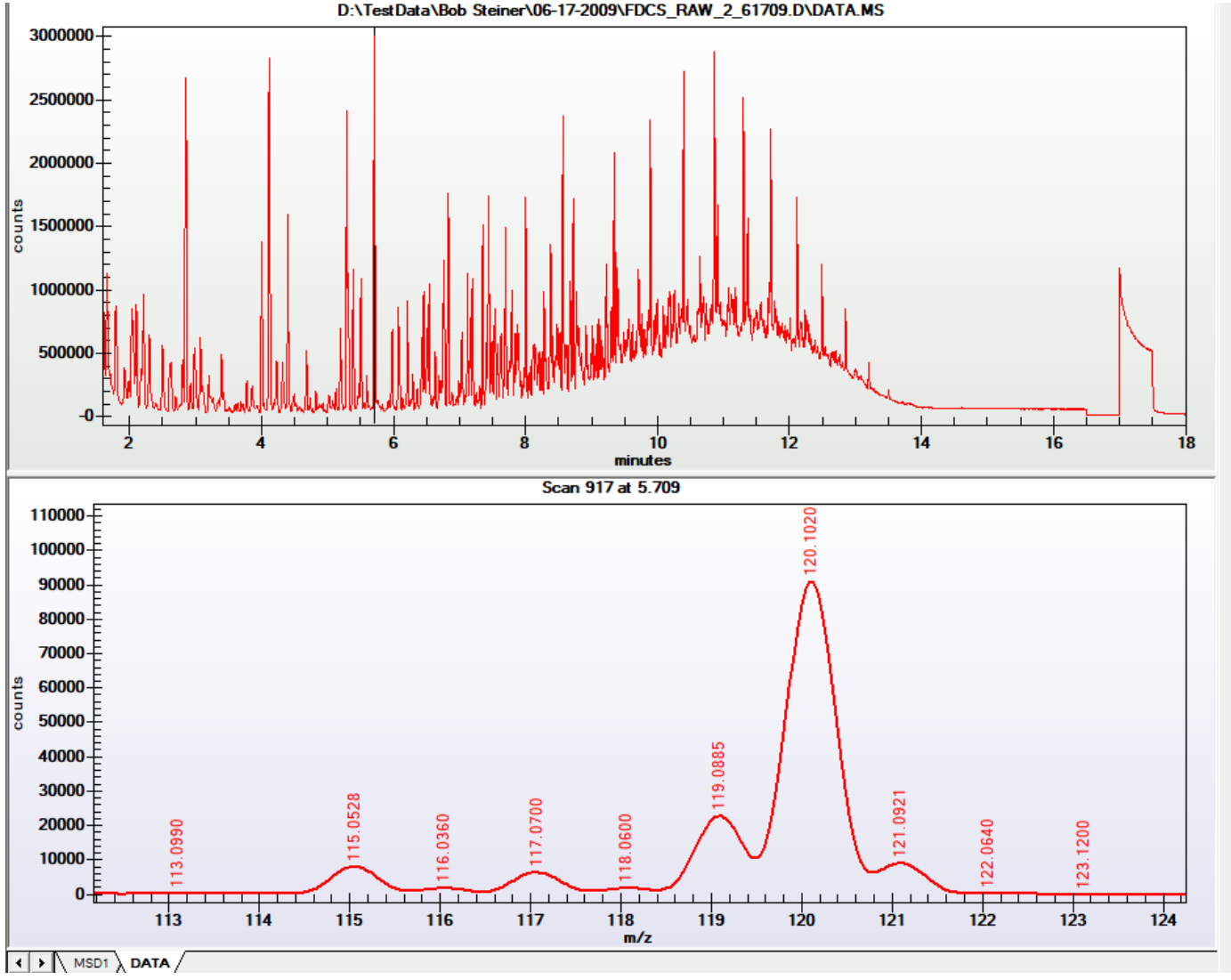
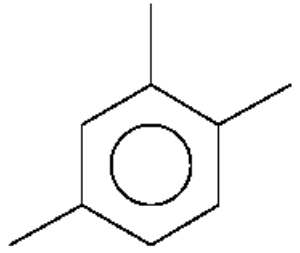
MS Detected Ion Species Not Separated by LC



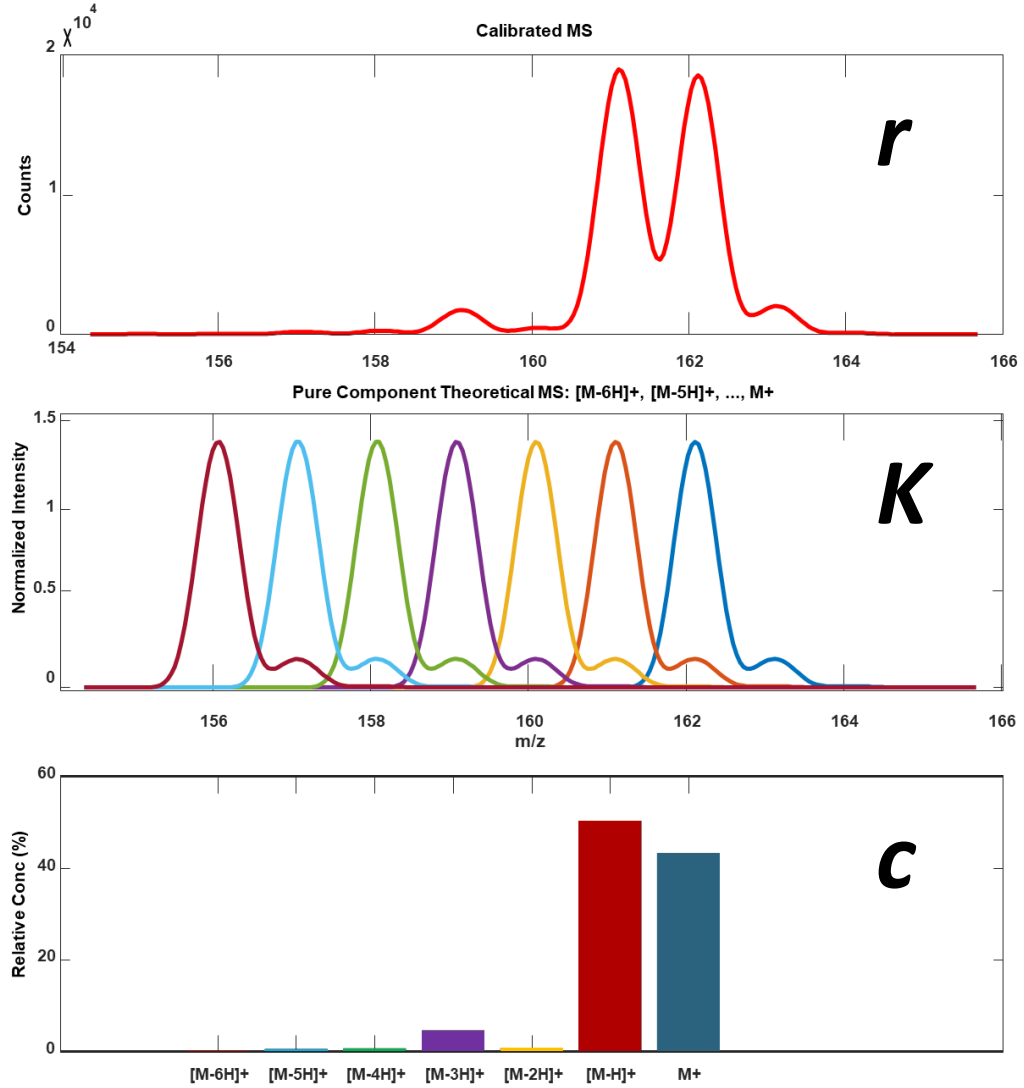
EI MS Detected Ion Species Not Separated by GC





Loss of up to 5 H's under 70eV

Potential of Multiple Linear Regression (MLR) for Ion Mixtures Deconvolution



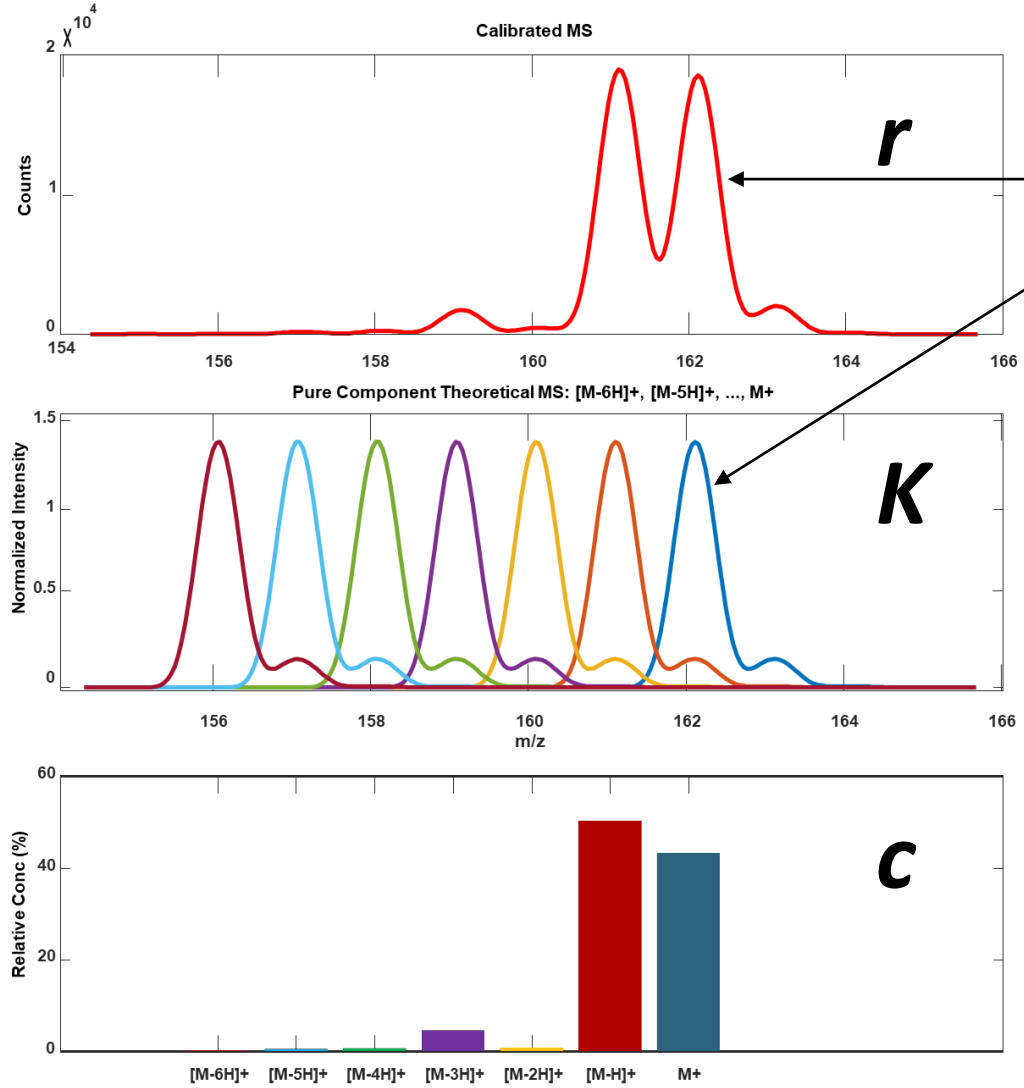
$$\boldsymbol{r = Kc + e}$$

$$\boldsymbol{\hat{r} = Kc}$$

Where $\boldsymbol{e}$ is fitting error

$\boldsymbol{\hat{r}}$ is fitted mass spec

A Fundamental Requirement: Consistent MS Spectral Peak Shape between r and K



MS Spectral Peak Shape

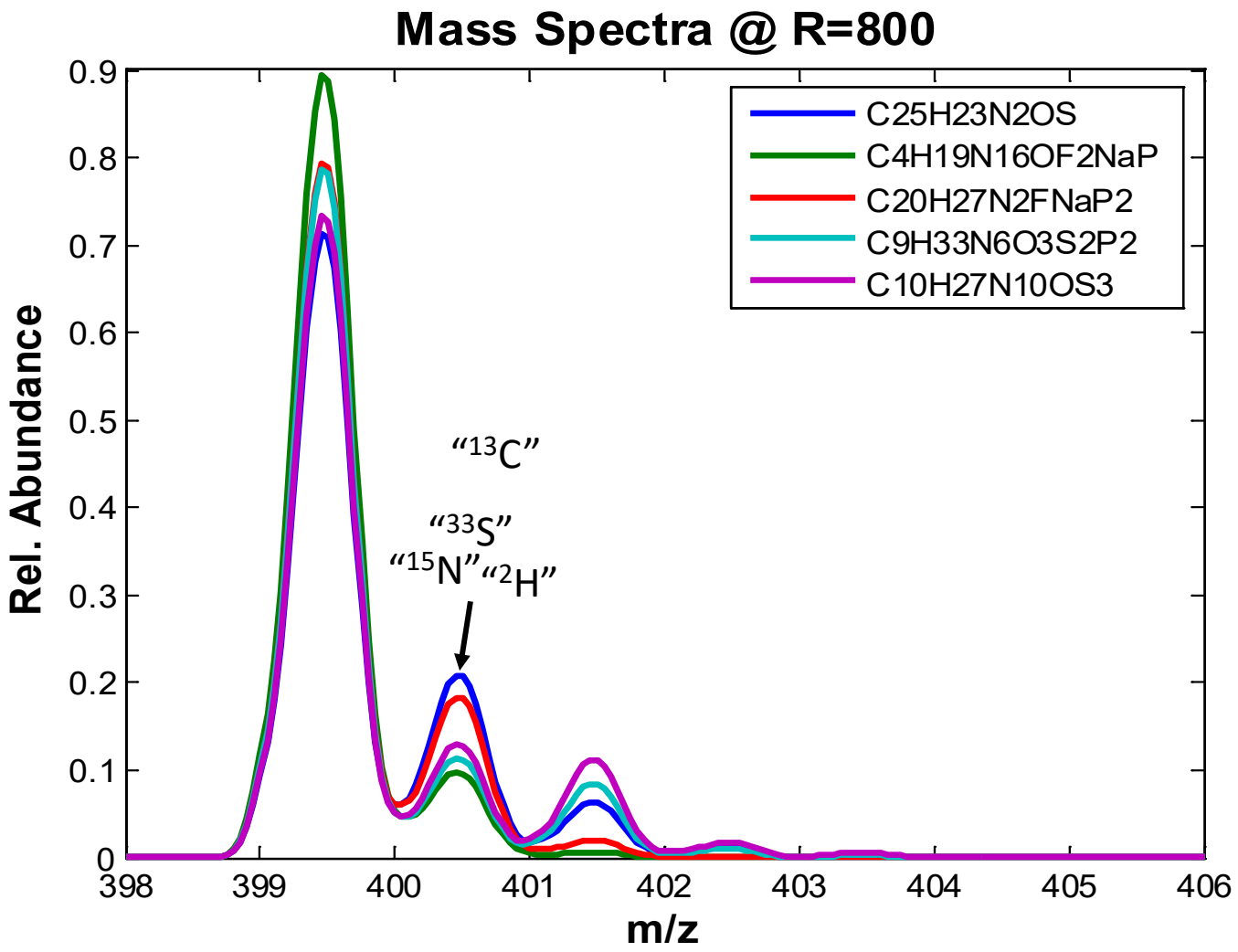
$$r = Kc + e$$

$$\hat{r} = Kc$$

Where e is fitting error

$\hat{r}$ is fitted mass spec

The Concept of Spectral Accuracy for Mass Spectrometry



- Much more and complete information in raw profile mode
- Possibility of formula ID on a single quad

Wang, Y. et al *Anal. Chem.* **2010**, *82*, 7055-7062 (17)

A Very Different Type of Calibration for Mass Spectrometry

TrueCal: True & Fully Calibrated MS

Raw Profile MS and True MS

m/z-Only Calibration
(conventional calibration)

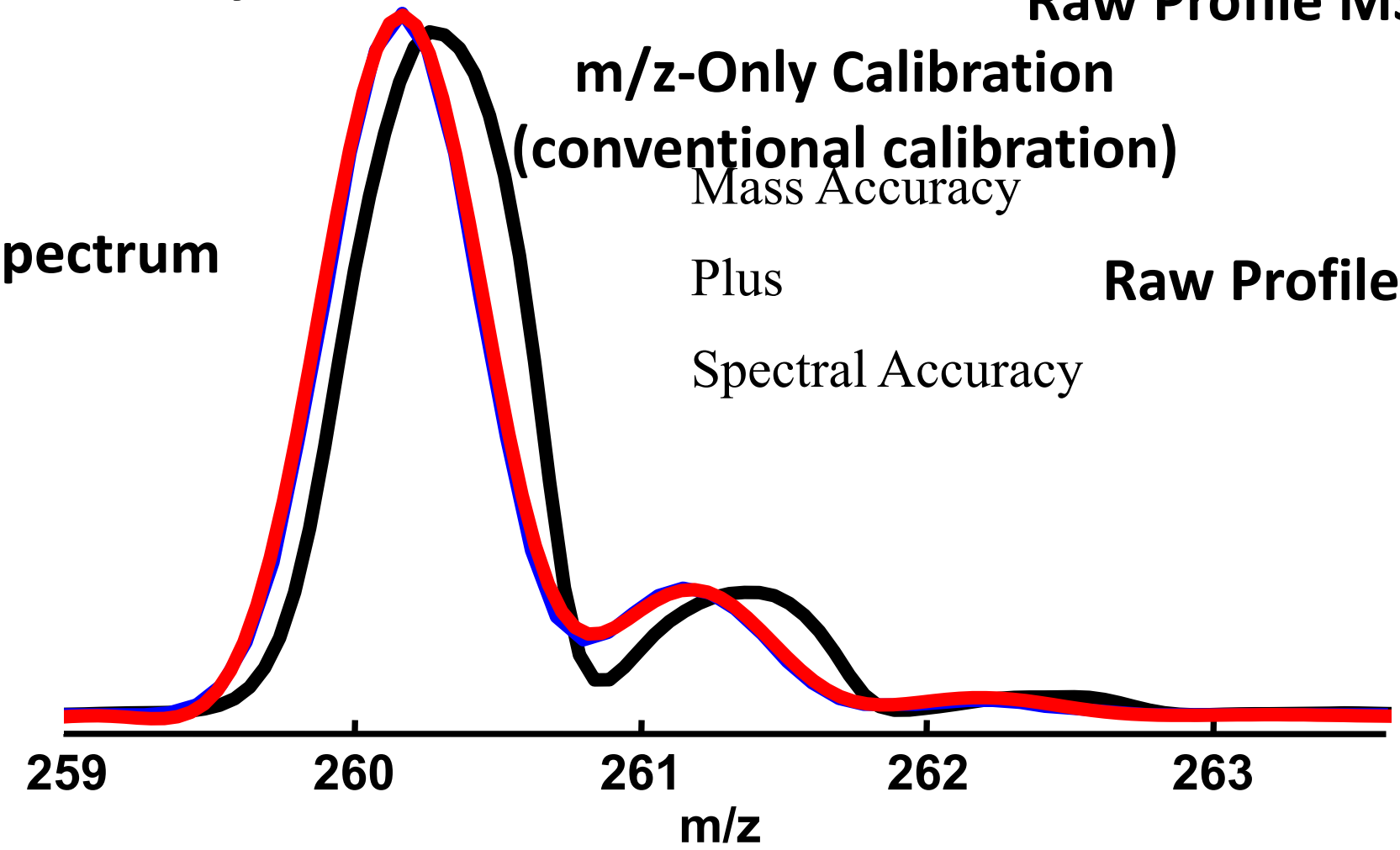
Mass Accuracy

Plus

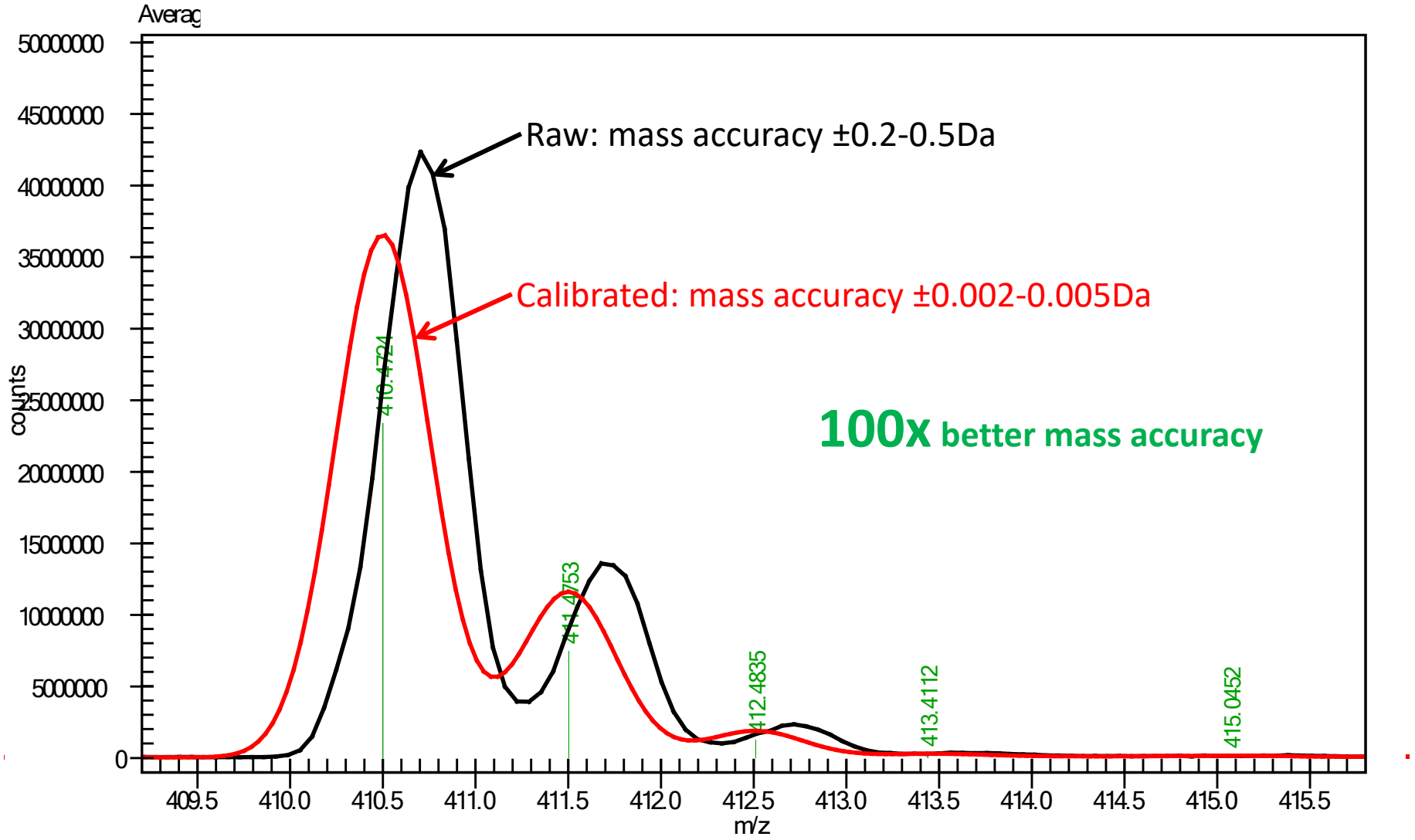
Spectral Accuracy

True Mass Spectrum

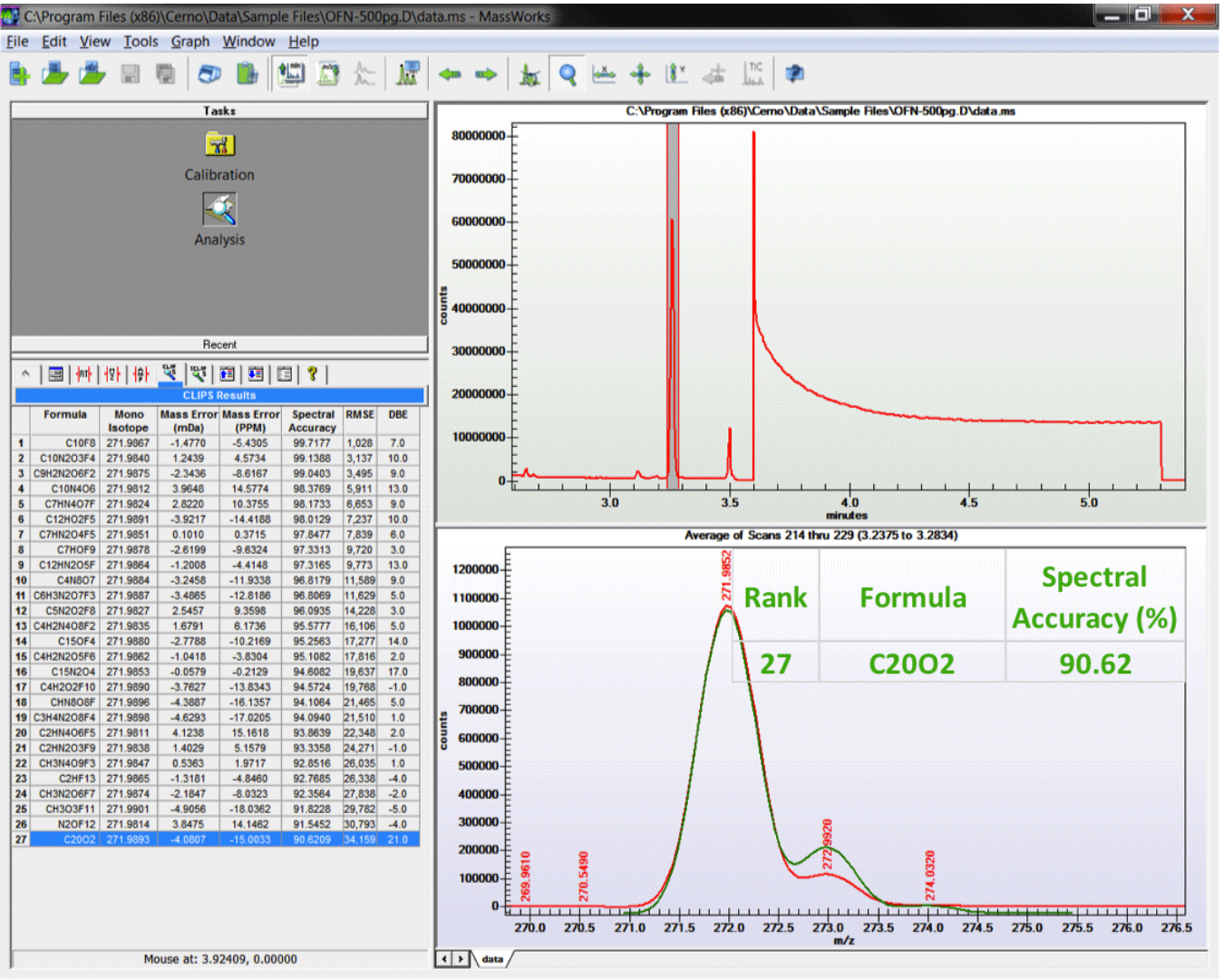
Raw Profile Mass Spectrum



Dramatic Gain in Mass Accuracy



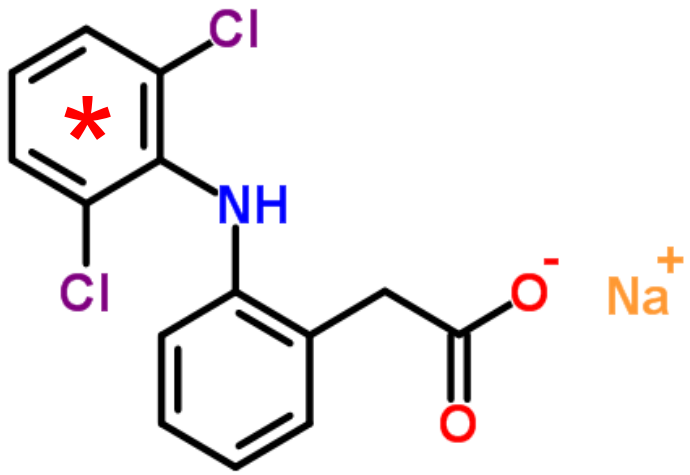
Spectral Accuracy to Complete the Analysis



	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C10F8	271.9867	-1.4770	-5.4305	99.7177	1,028	7.0
2	C10N2O3F4	271.9840	1.2439	4.5734	99.1388	3,137	10.0
3	C9H2N2O6F2	271.9875	-2.3436	-8.6167	99.0403	3,495	9.0
4	C10N4O6	271.9812	3.9648	14.5774	98.3769	5,911	13.0
5	C7HN4O7F	271.9824	2.8220	10.3755	98.1733	6,653	9.0
6	C12HO2F5	271.9891	-3.9217	-14.4188	98.0129	7,237	10.0
7	C7HN2O4F5	271.9851	0.1010	0.3715	97.8477	7,839	6.0
8	C7HOF9	271.9878	-2.6199	-9.6324	97.3313	9,720	3.0
9	C12HN2O5F	271.9864	-1.2008	-4.4148	97.3165	9,773	13.0
10	C4N8O7	271.9884	-3.2458	-11.9338	96.8179	11,589	9.0
11	C6H3N2O7F3	271.9887	-3.4865	-12.8186	96.8069	11,629	5.0
12	C5N2O2F8	271.9827	2.5457	9.3598	96.0935	14,228	3.0
13	C4H2N4O8F2	271.9835	1.6791	6.1736	95.5777	16,106	5.0
14	C15OF4	271.9880	-2.7768	-10.2169	95.2563	17,277	14.0
15	C4H2N2O5F6	271.9862	-1.0418	-3.8304	95.1082	17,816	2.0
16	C15N2O4	271.9853	-0.0579	-0.2129	94.6082	19,637	17.0
17	C4H2O2F10	271.9890	-3.7627	-13.8343	94.5724	19,768	-1.0
18	CHN8O8F	271.9896	-4.3887	-16.1357	94.1064	21,465	5.0
19	C3H4N2O8F4	271.9898	-4.6293	-17.0205	94.0940	21,510	1.0
20	C2HN4O6F5	271.9811	4.1238	15.1618	93.8639	22,348	2.0
21	C2HN2O3F9	271.9838	1.4029	5.1579	93.3358	24,271	-1.0
22	CH3N4O9F3	271.9847	0.5363	1.9717	92.8516	26,035	1.0
23	C2HF13	271.9865	-1.3181	-4.8460	92.7685	26,338	-4.0
24	CH3N2O6F7	271.9874	-2.1847	-8.0323	92.3564	27,838	-2.0
25	CH3O3F11	271.9901	-4.9056	-18.0362	91.8228	29,782	-5.0
26	N2OF12	271.9814	3.8475	14.1462	91.5452	30,793	-4.0
27	C20O2	271.9893	-4.0807	-15.0033	90.6209	34,159	21.0

- For the 1st time, measured MS directly compared to theoretical MS
- Thus born the brand-new concept of Spectral Accuracy
- Applicable to both Quad and/or TOF

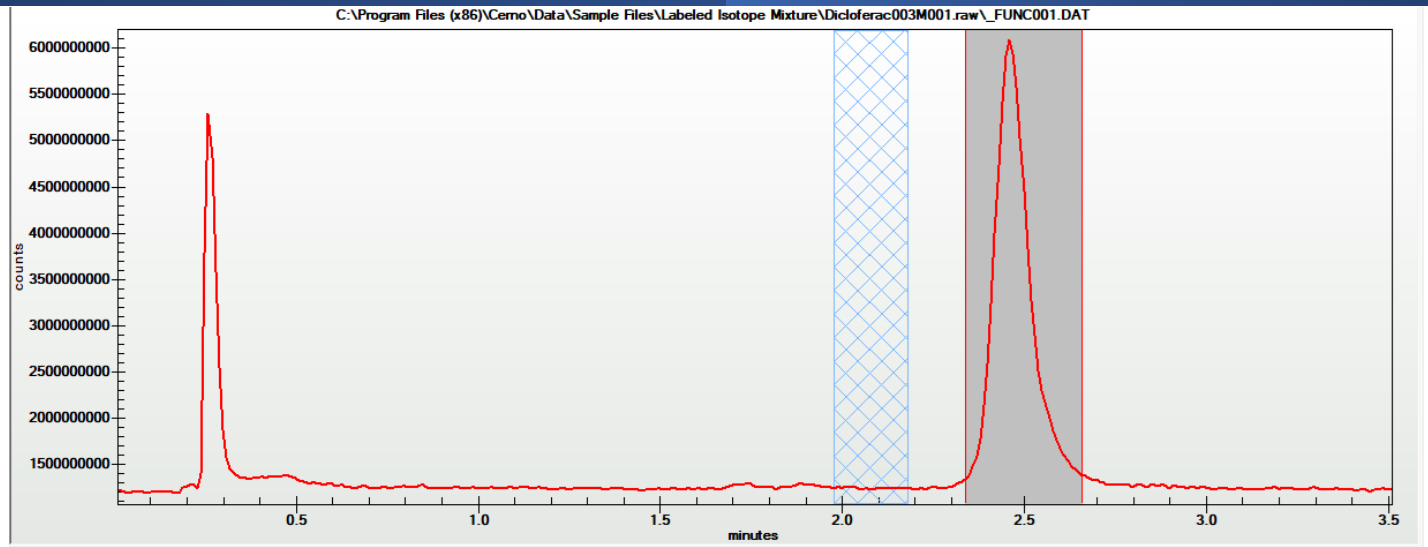
Labelled Compounds Not Separated by LC

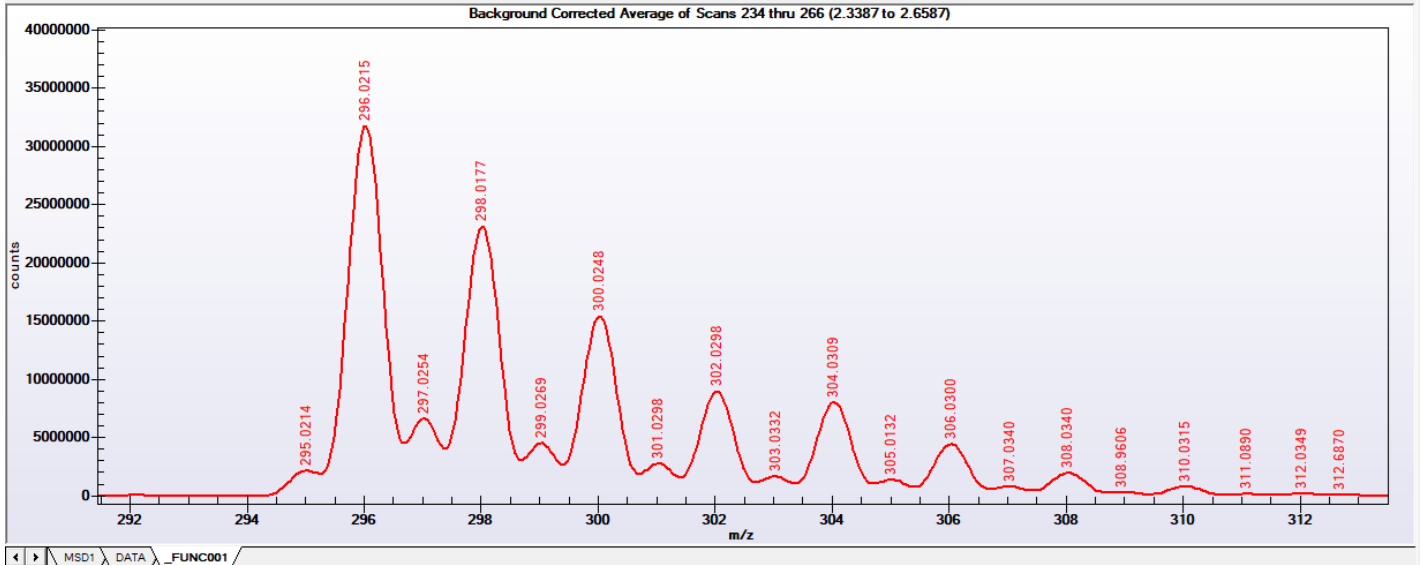


Diclofenac Sodium
C14H10Cl2NNaO2

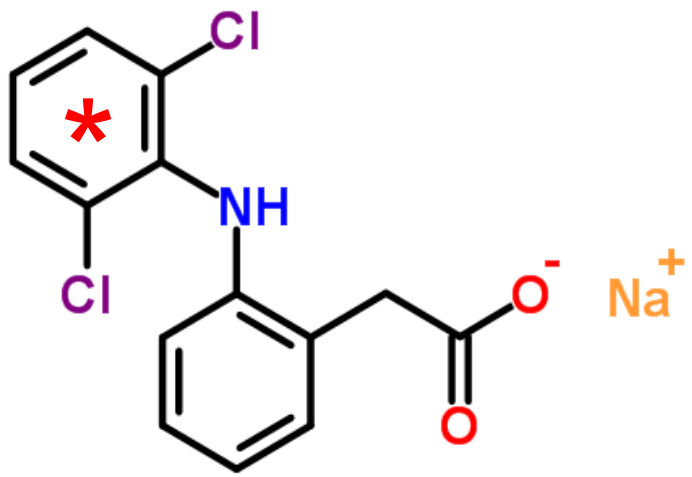
[14C] labeled
SA=62.7 mCi/mmol

7 possible labelled compounds:
Native, 1[14C] - 6[14C]





Implementation in MassWorks Softawre



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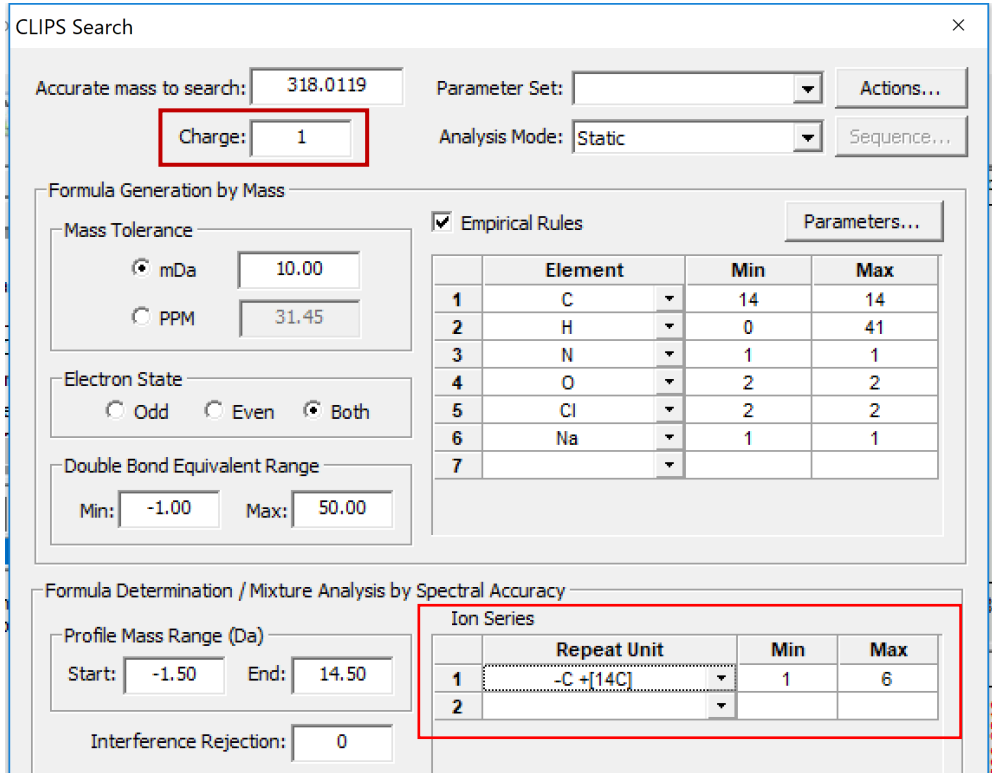
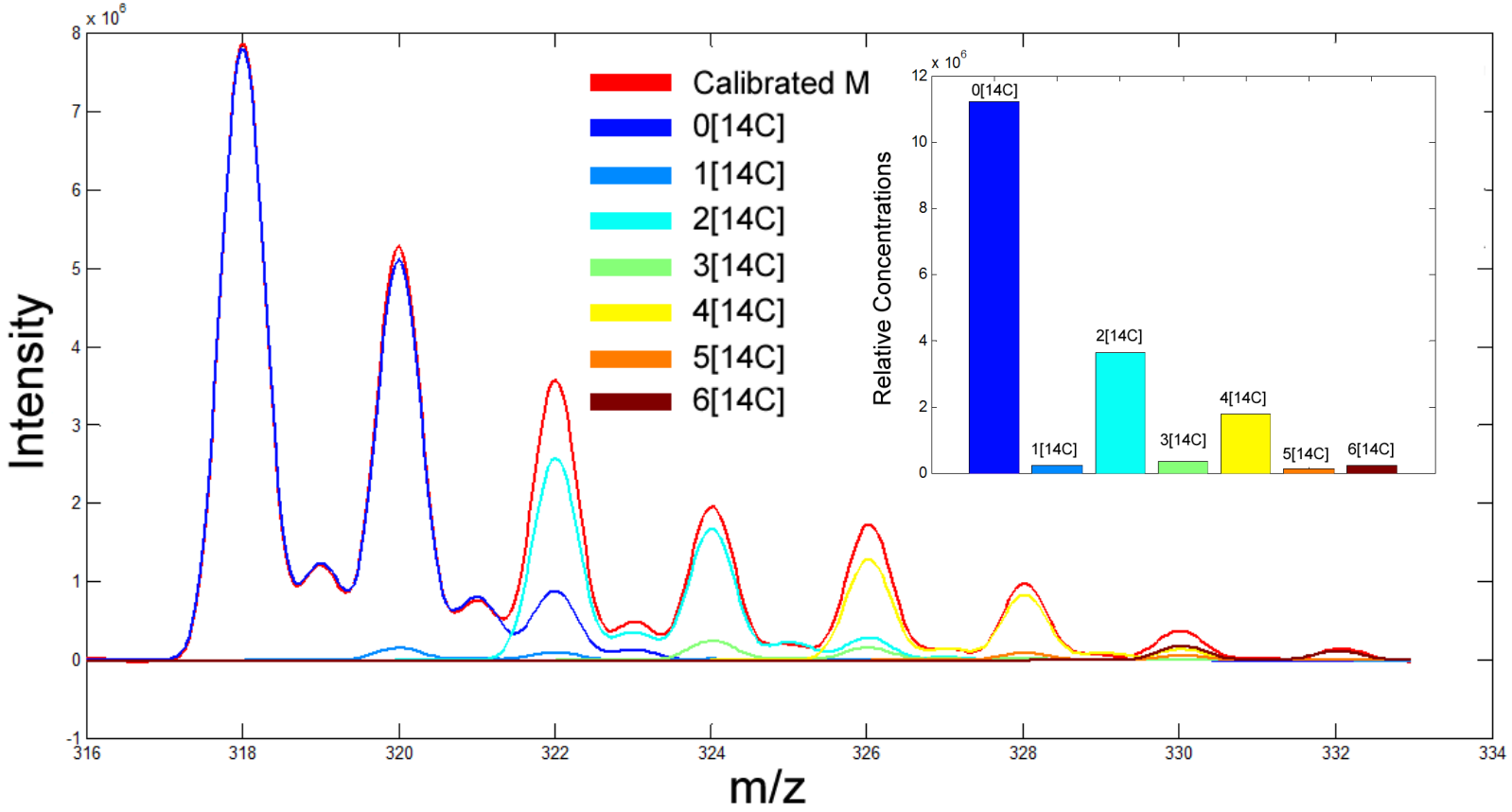


Rapid Commun Mass Spectrom. 2021;e9103.
https://doi.org/10.1002/rcm.9103

Accurate Relative Quantitation Achieved



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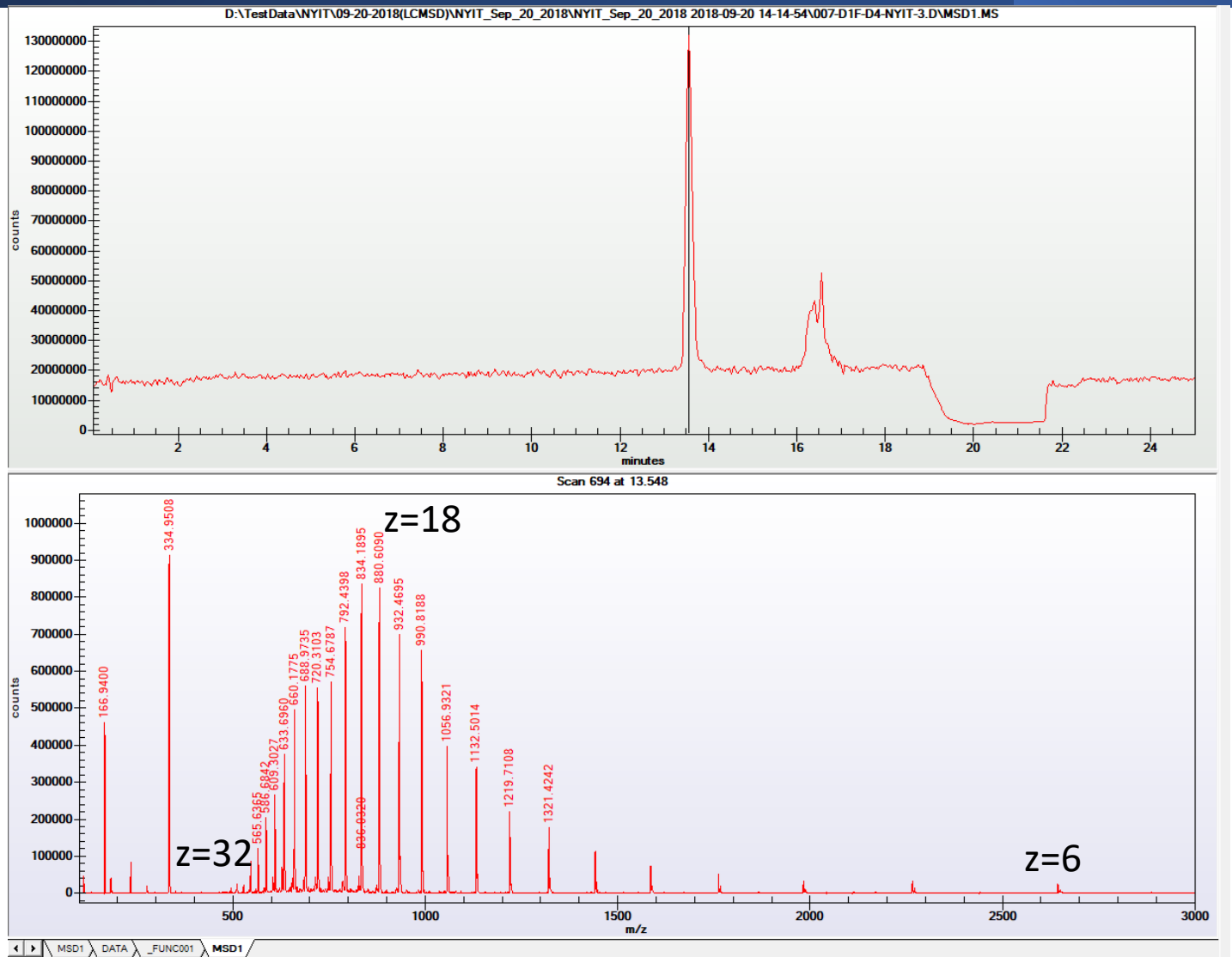
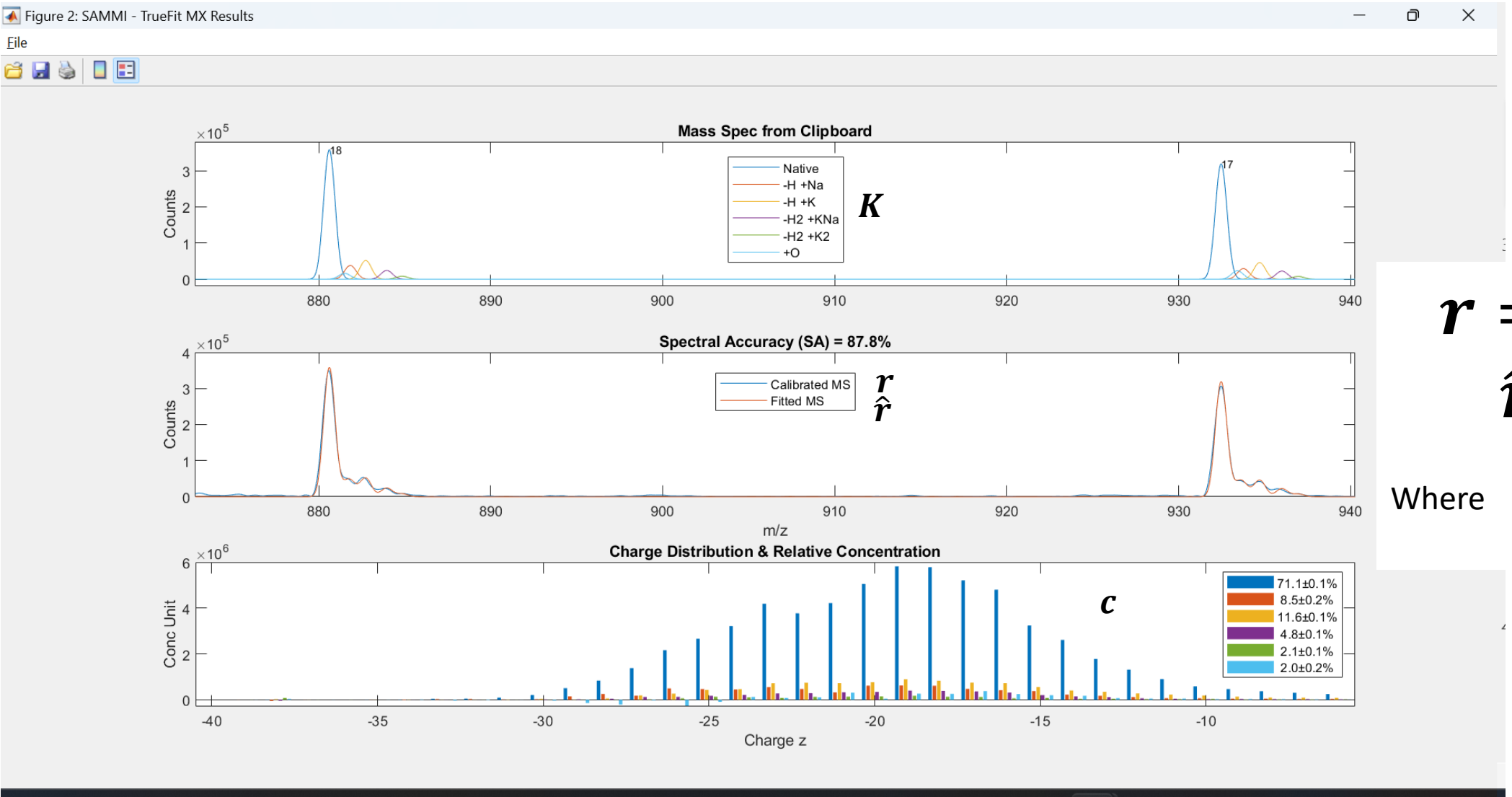
Multiply-charged ESI- Ions of 50-nt RNA Measured across Wide m/z Range



SAMMI: Spectrally Accurate Modeling of Multiply-charged Ions



$$\boldsymbol{r = Kc + e}$$

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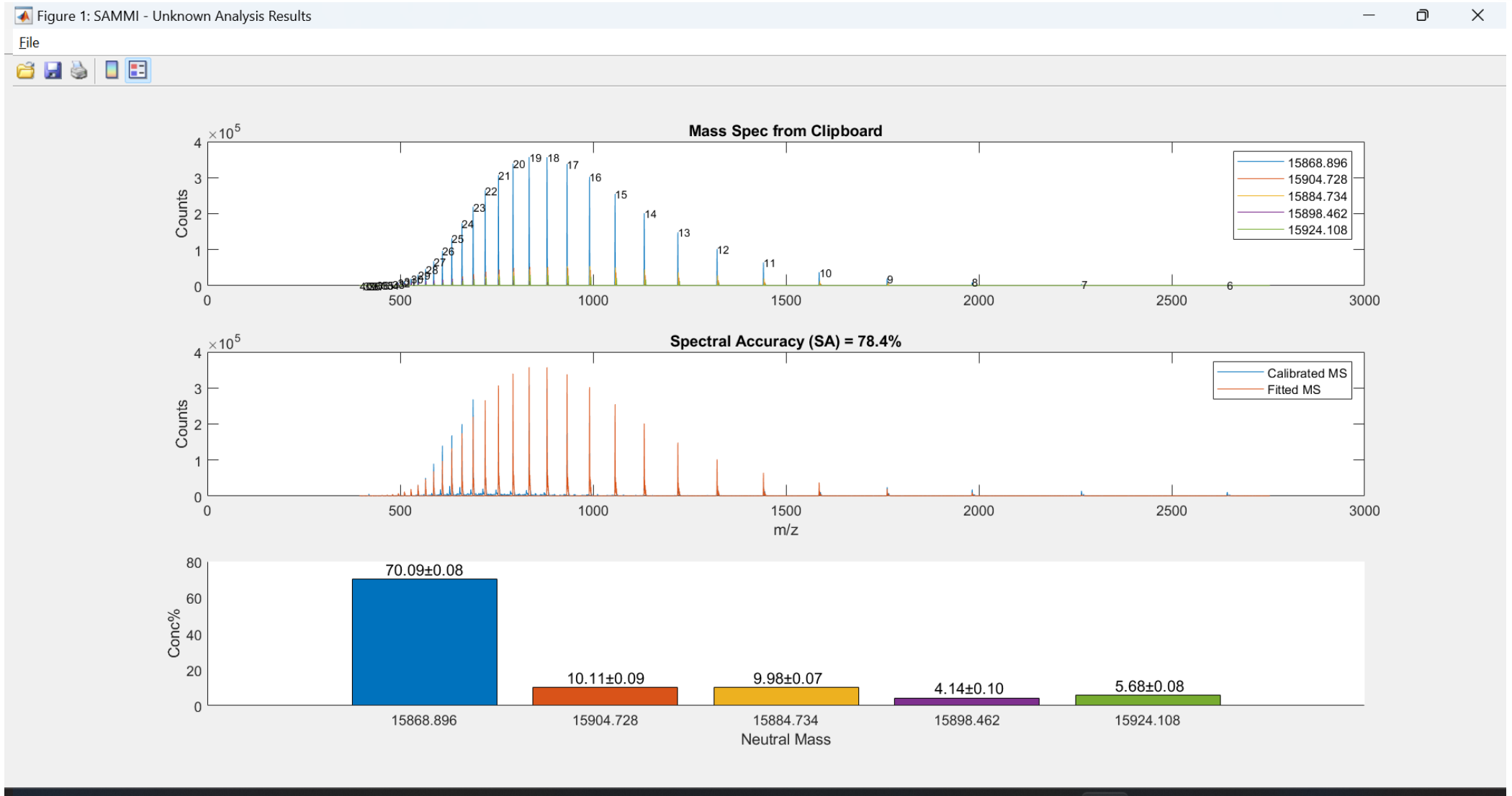
Expanding from Confirmation of Known into Intact Mass Determination of Unknown RNA Molecule



Conclusion

- Spectral Accuracy is critical for accurate ion mixture deconvolution: GC, LC, small or large molecules
- TrueCal is one reliable way to achieve both accurate mass and spectral calibration
- Large molecule confirmation is a direct extension of spectrally accurate ion deconvolution to cover multiple different charges in wide m/z ranges
- Intact mass determination of large unknown molecules feasible within 1Da accuracy on quadrupole MS, approaching HiRes MS performance
- More info: Pittcon Booth #2922 demo, info@cernobioscience.com