Direct and Accurate Quantitation of Labeled and Un-Labeled Ions with LC/MS

Jianwei Zhao, Peter L. Wang, Dawei Zhou & Xinping Fang

XenoBiotic Laboratories Inc.
Plainsboro, NJ, USA

药明康德 WuXi AppTec | XenoBiotic Laboratories, Inc. A subsidiary of Lab Testing Division, WuXi AppTec Inc.

Ming Gu & Yongdong Wang
Cerno Bioscience
Norwalk, CT, USA

cerno BIOSCIENCE

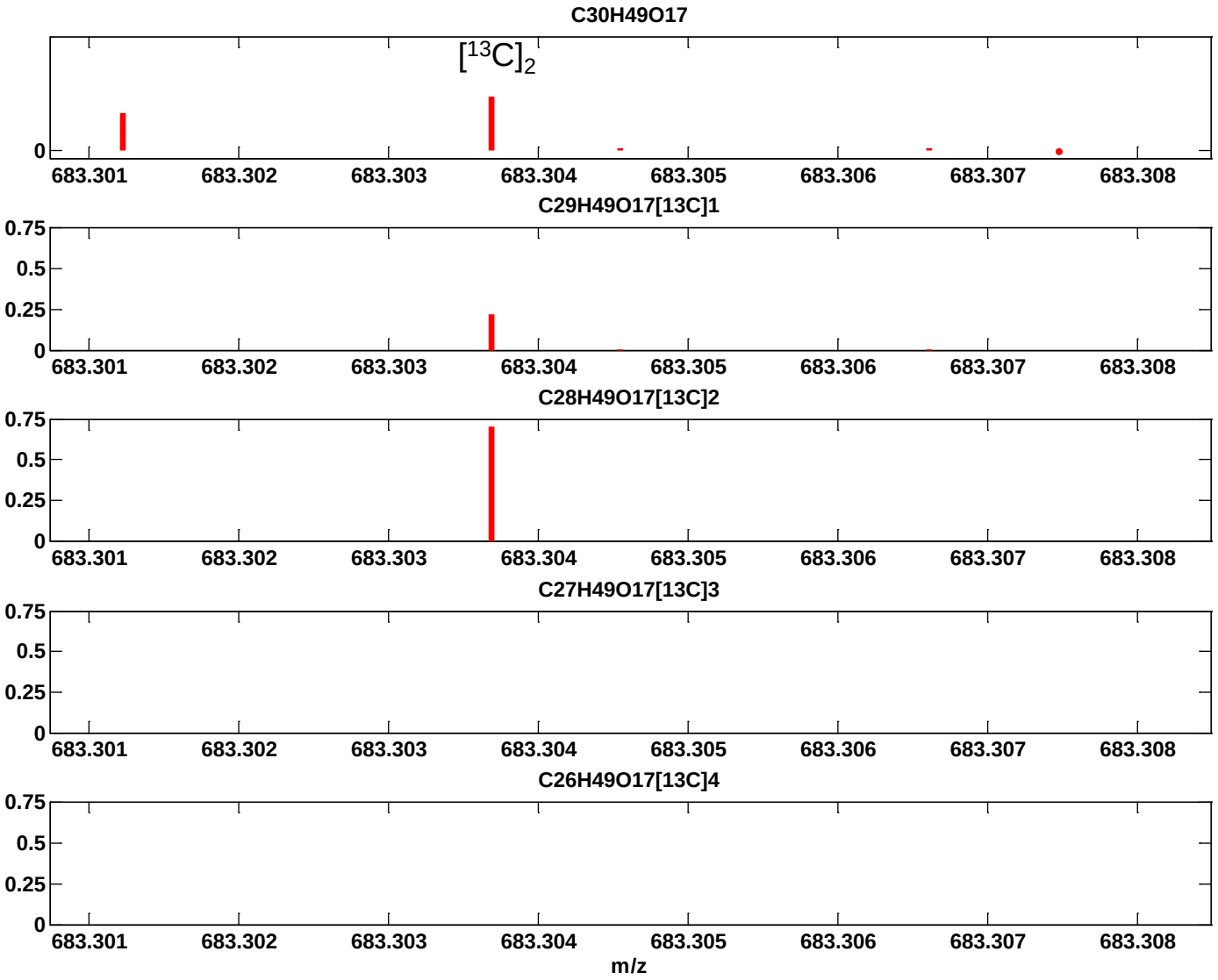
Isotope Labeling for Metabolism Studies

- ^{2}H replacement: internal standard for LC/MS/MS quant
- ^{13}C labeling: internal standard/metabolic flux analysis
- ^{3}H and ^{14}C labeling: radioactive tracer
  - Application in drug ADME studies
  - Golden standard in determining mass balance
  - Specific activity needs to be accurately determined for quantitation
- A common challenge: chromatographic co-eluting

Labeled & Un-Labeled Quant

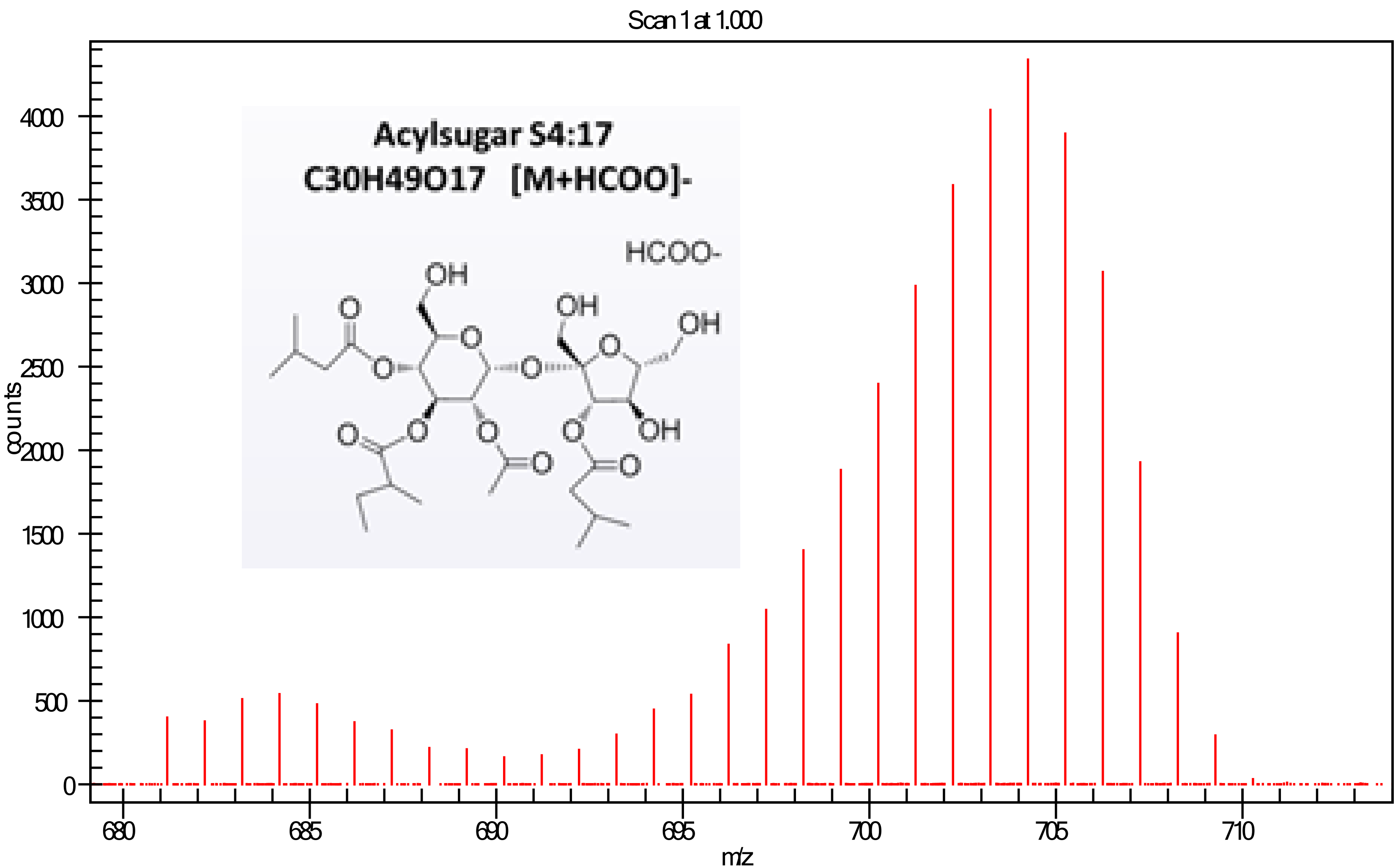
- Radiolabeling: radioactivity detector
  - Sensitive and highly selective
  - Special handling / lab certification
  - Special/separate radio-detector
  - LC/UV/Fraction collection: tedious / time-consuming
- NMR
  - Sensitivity issue
  - Multiple replacements and resulting mixtures
- MS detection
  - Universal: incl. 14C
  - Signal overlapping: multiple labels for m/z separation
  - QA/QC: the number or mixture of labels?

^{13}C Labeling: Spectral Overlapping

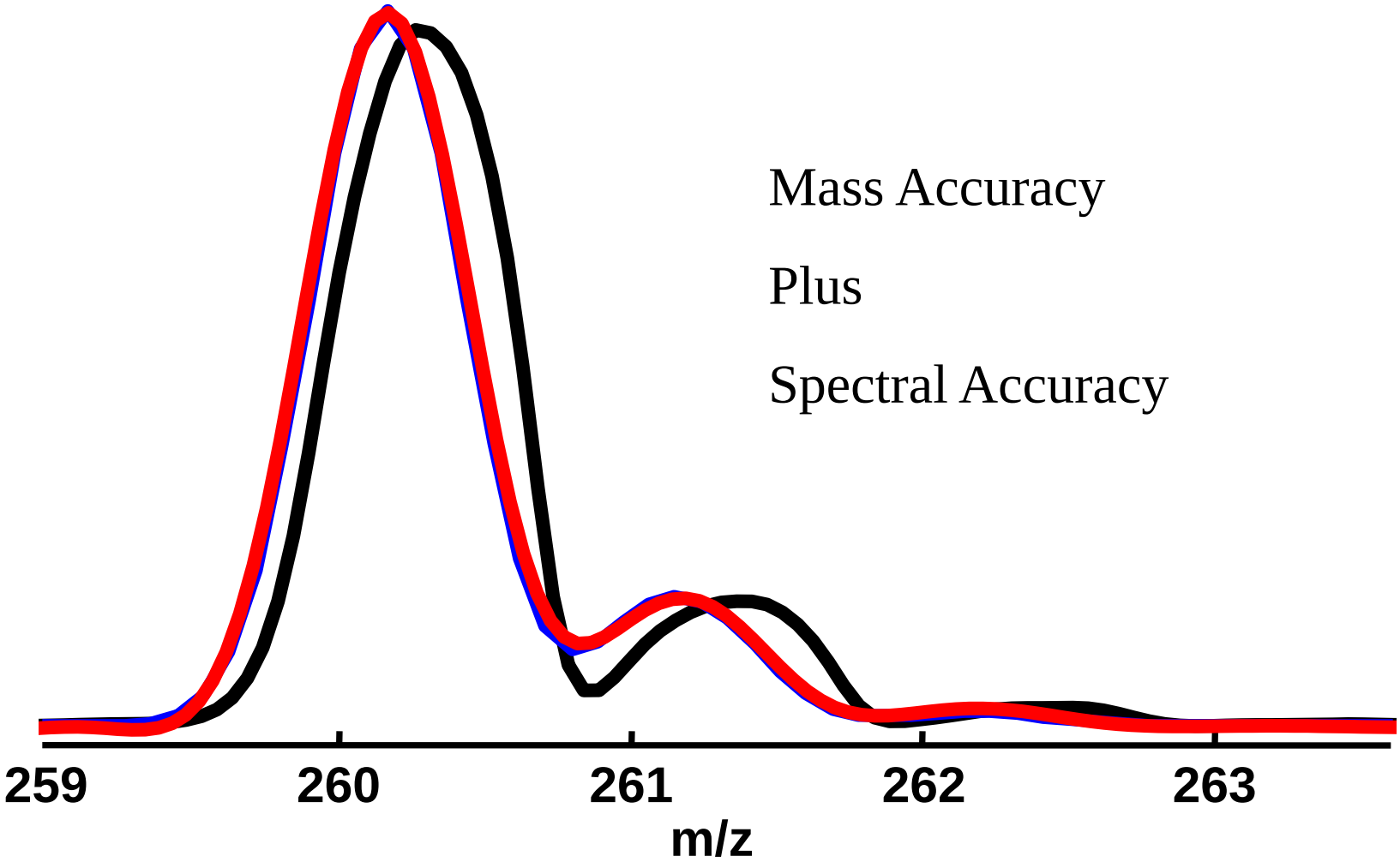


Wang, Z. & Jones A. D. et al, 63rd ASMS Poster, MP#410

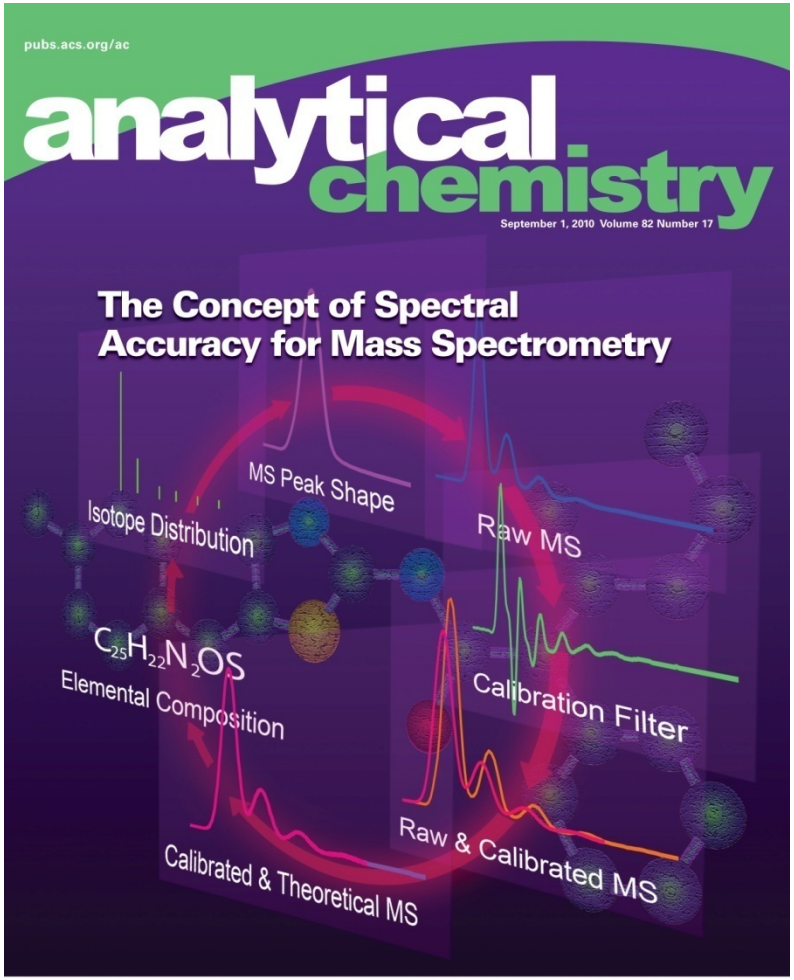
Actual LC TOF Data: Profile vs Centroid



Wang, Z. & Jones A. D. et al, 63rd ASMS Poster, MP#410


Mass Accuracy
Plus
Spectral Accuracy
259
260
261
262
263
m/z

Further Technical Details



Sept 1, 2010, Vol. 82, No. 17

Analyzing Mutually Overlapping Ion Signals

r – Calibrated MS sampled at *n* m/z points (*n x 1*)
to a defined MS peak shape

K – Theoretical MS for all *p* ions included (*n x p*)
with the same defined MS peak shape

c – Concentrations for all *p* ions included (*p x 1*)

e – Least squares fitting residual/error (*n x 1*)

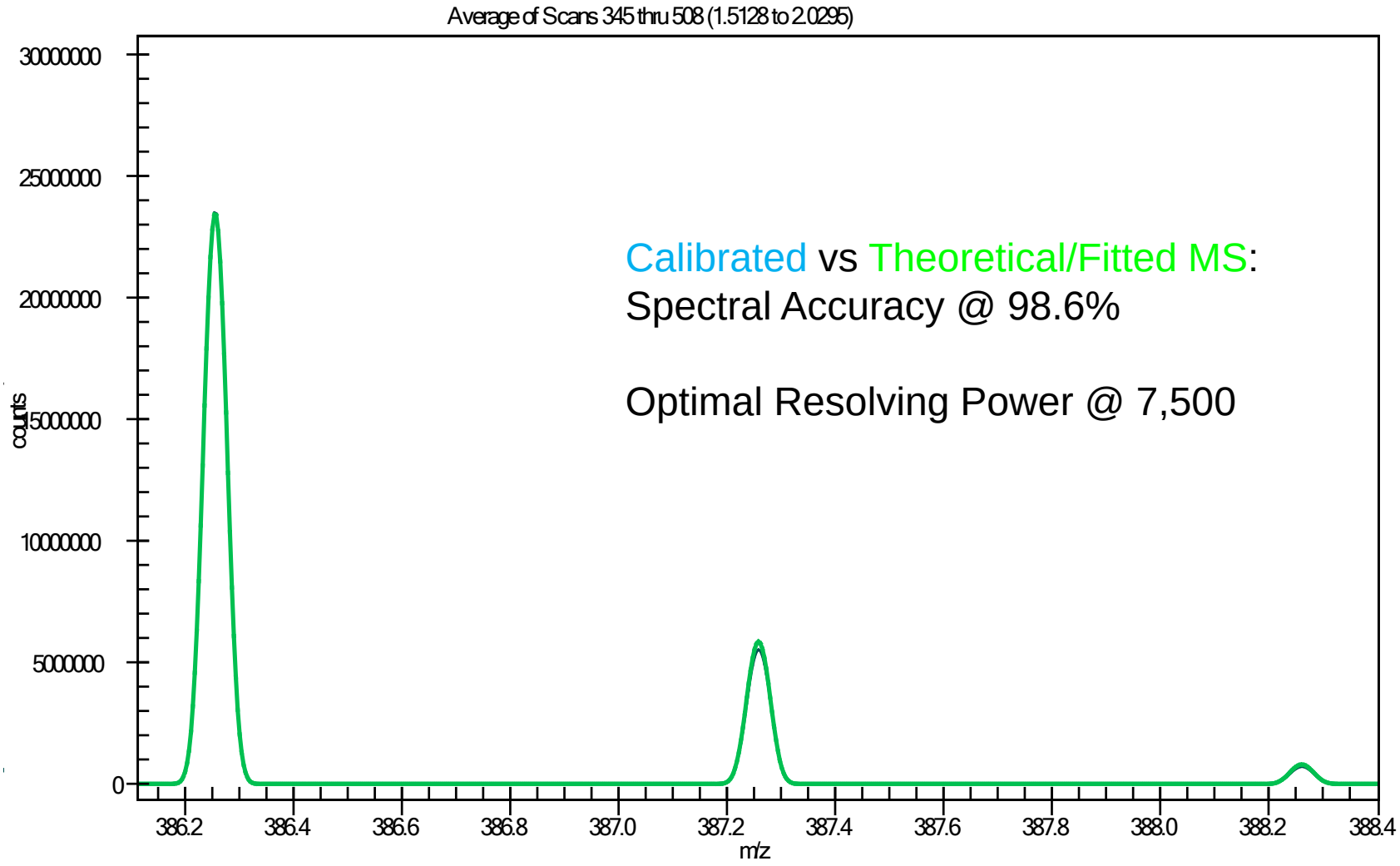
Seeking **c** via matrix inversion:

$$\mathbf{r} = \mathbf{K}\mathbf{c} + \mathbf{e}$$

$$Spectral \quad Error\,(\%) = \frac{\|\mathbf{e}\|_2}{\|\mathbf{r}\|_2} \times 100$$

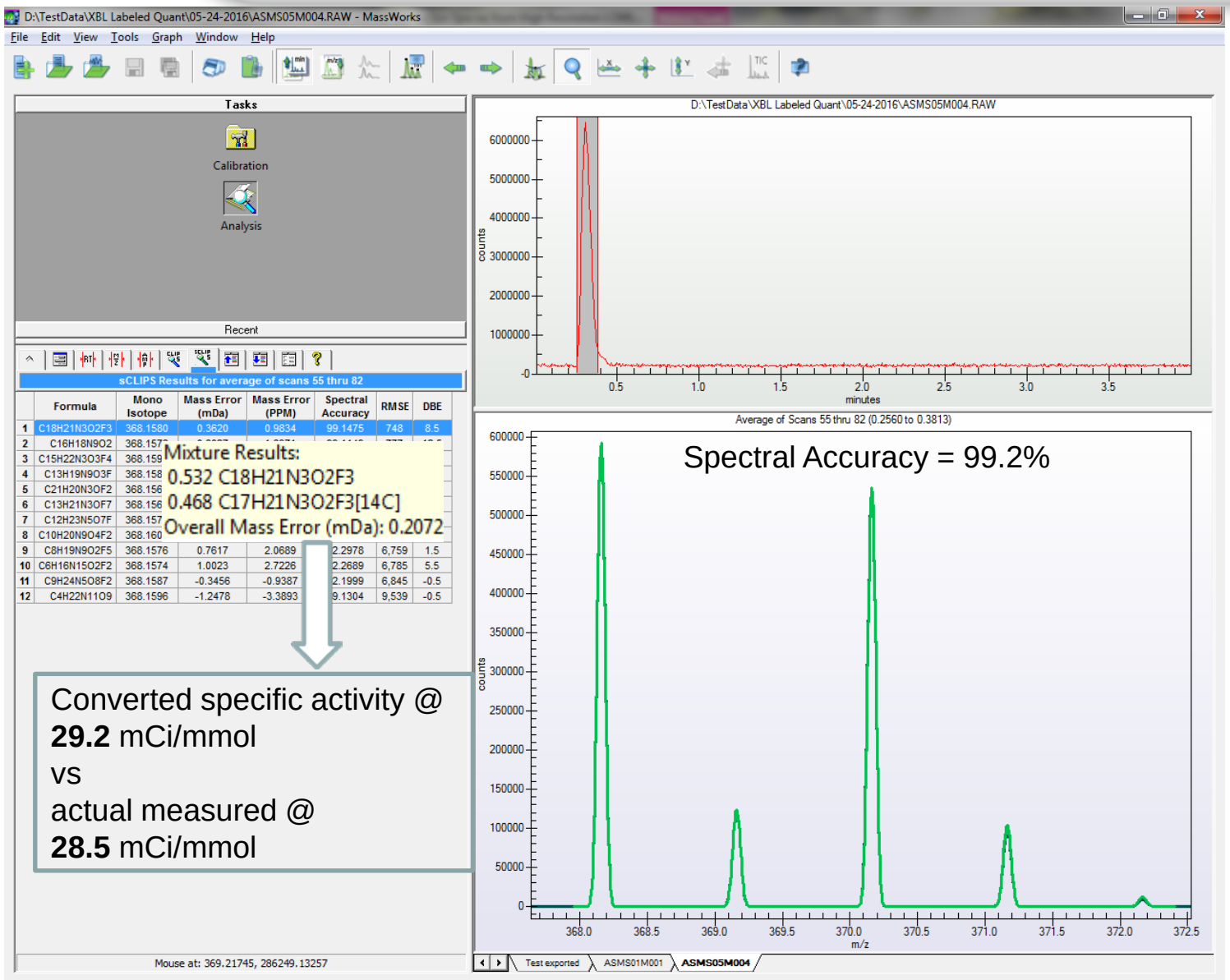
$$Spectral \quad Accuracy(\%) = \left(1 - \frac{\|\mathbf{e}\|_2}{\|\mathbf{r}\|_2}\right) \times 100$$

Optimal Resolving Power for Spectral Accuracy: LTQ Orbitrap

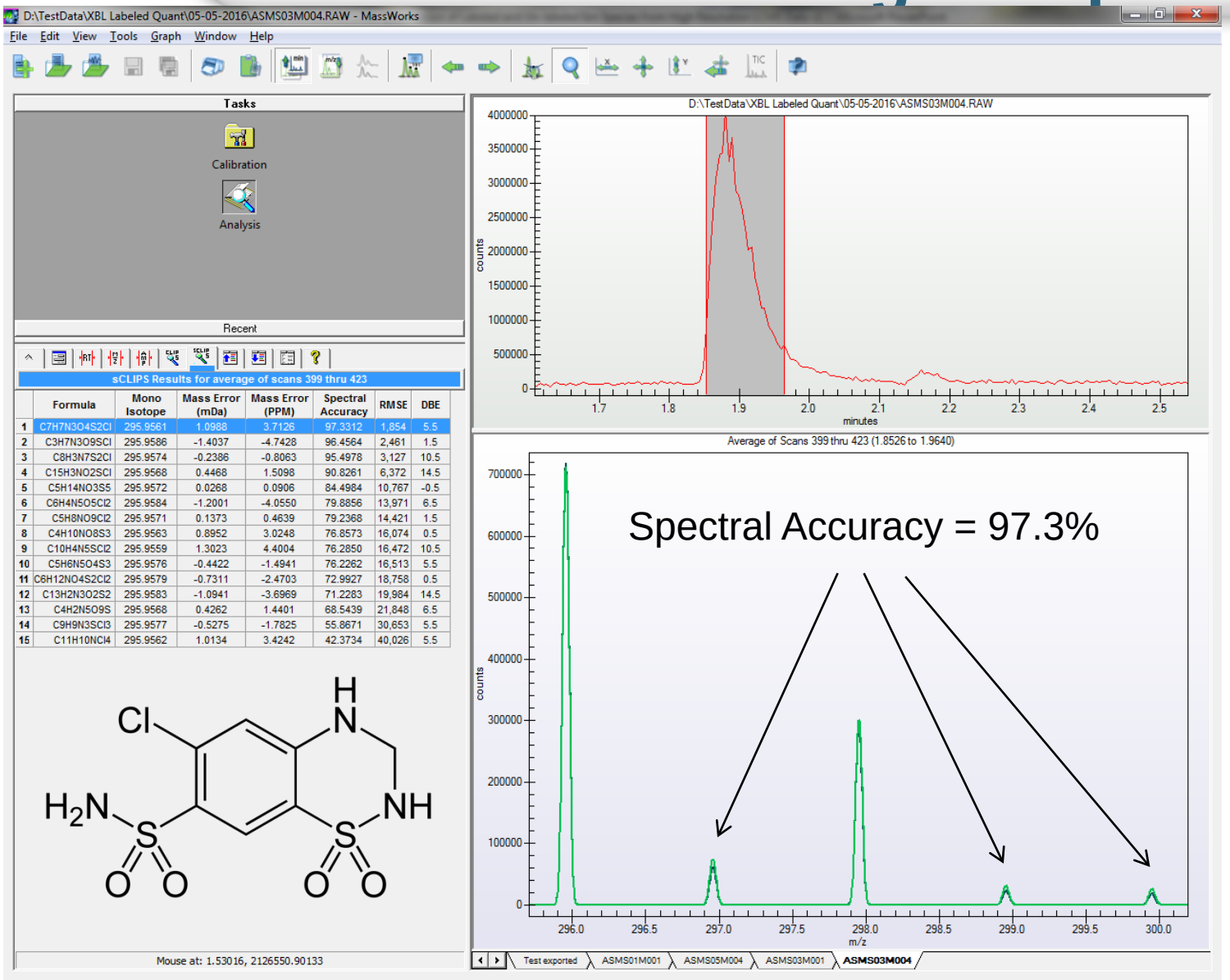


Resolving power reference: Erve, J. C. L. et al, JASMS, 2009, 20, 2058

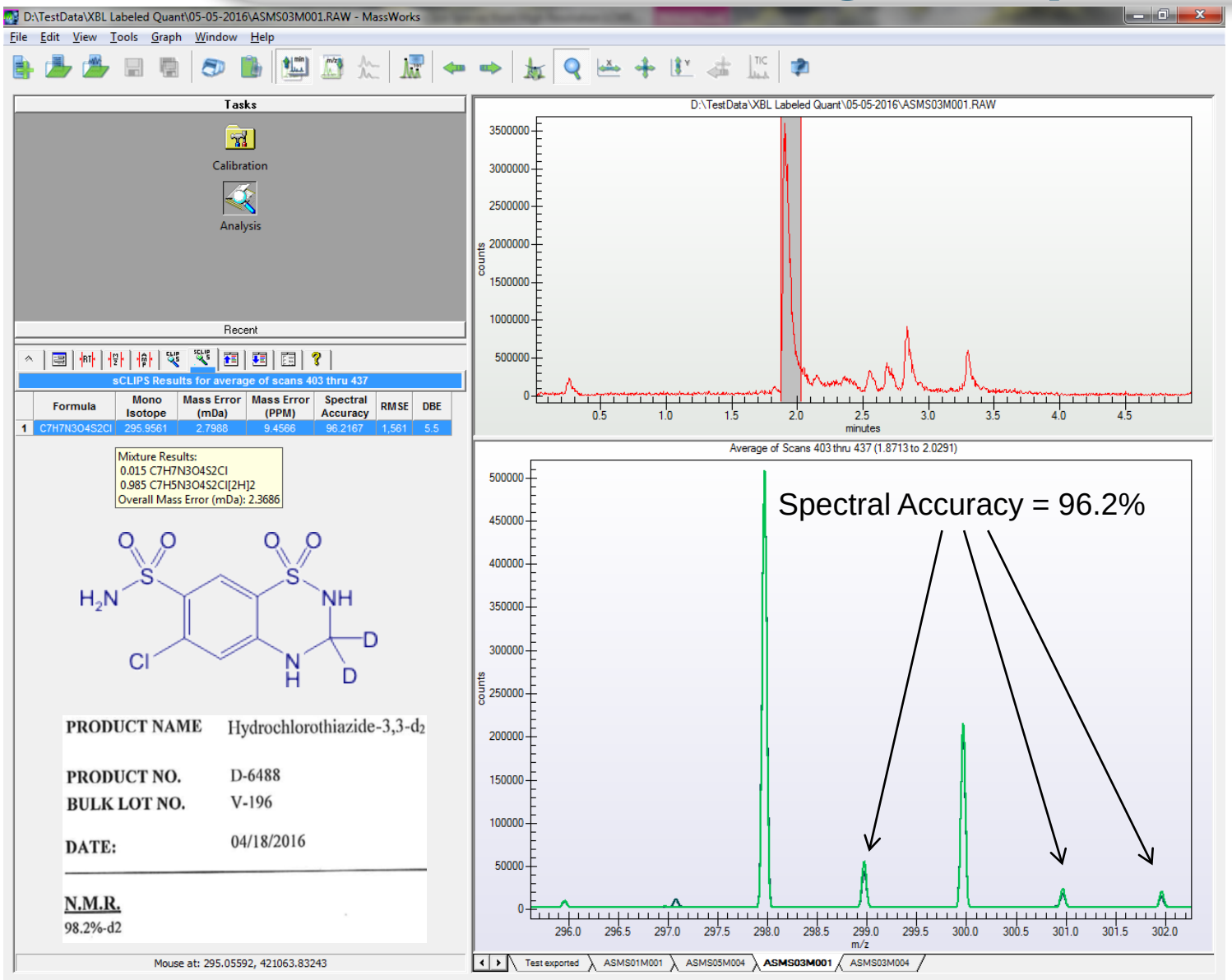
Single 14C Labeling: Compound with C/H/N/O/F



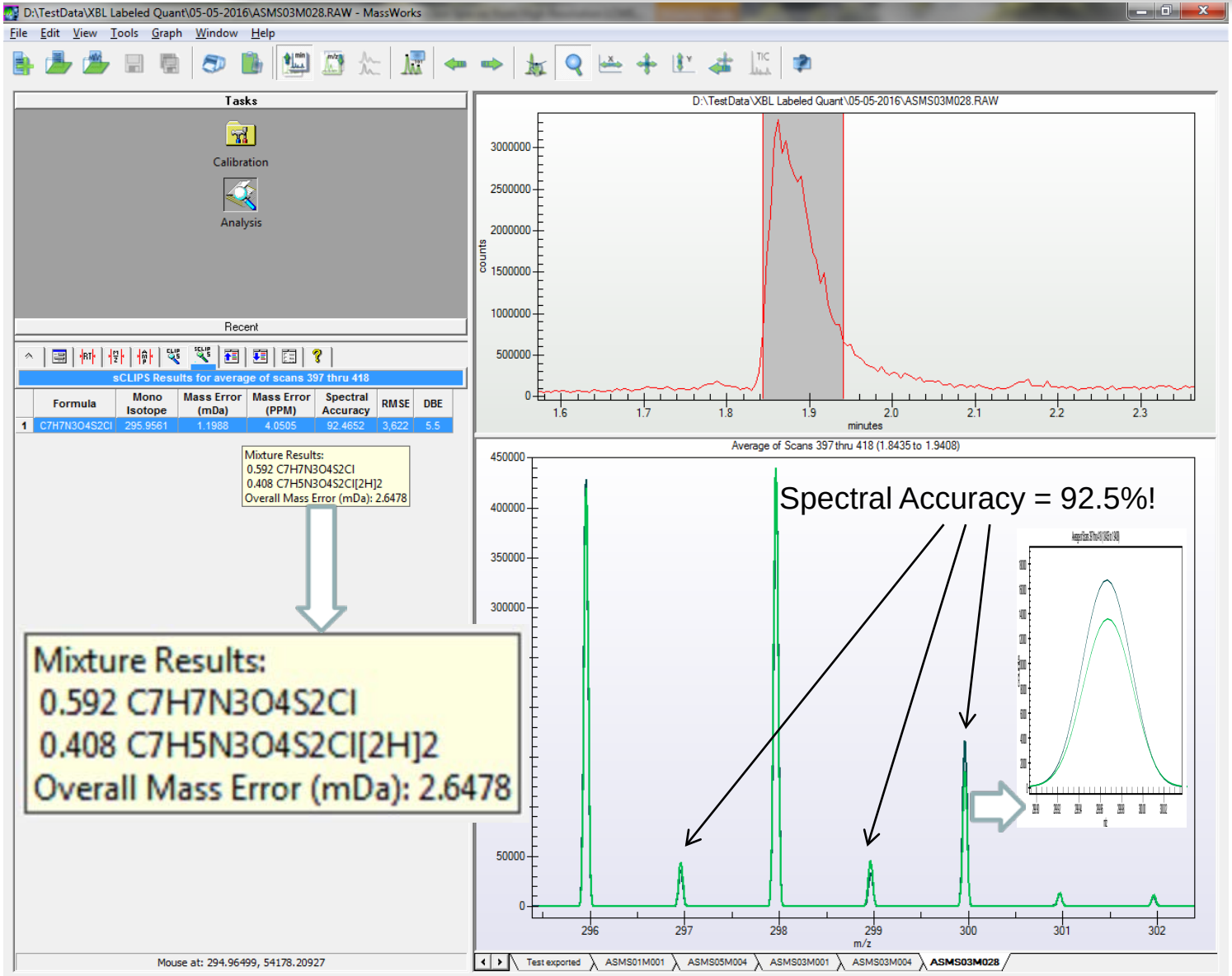
Un-labeled Cl-Containing Compound



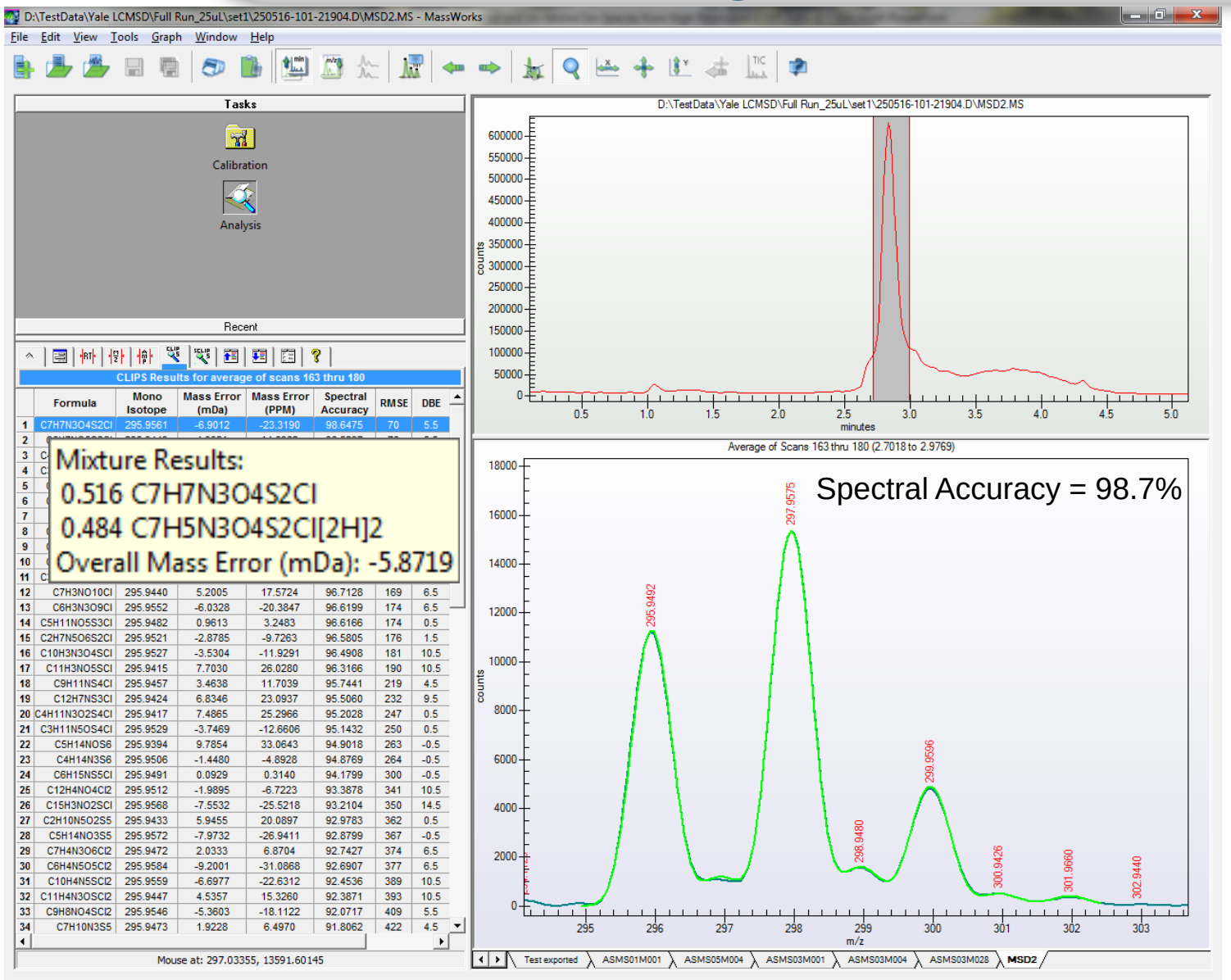
D2 Labeled Cl-Containing Compound?



50:50 Mix: Native & D2 Labeled Cl-Containing Compound?

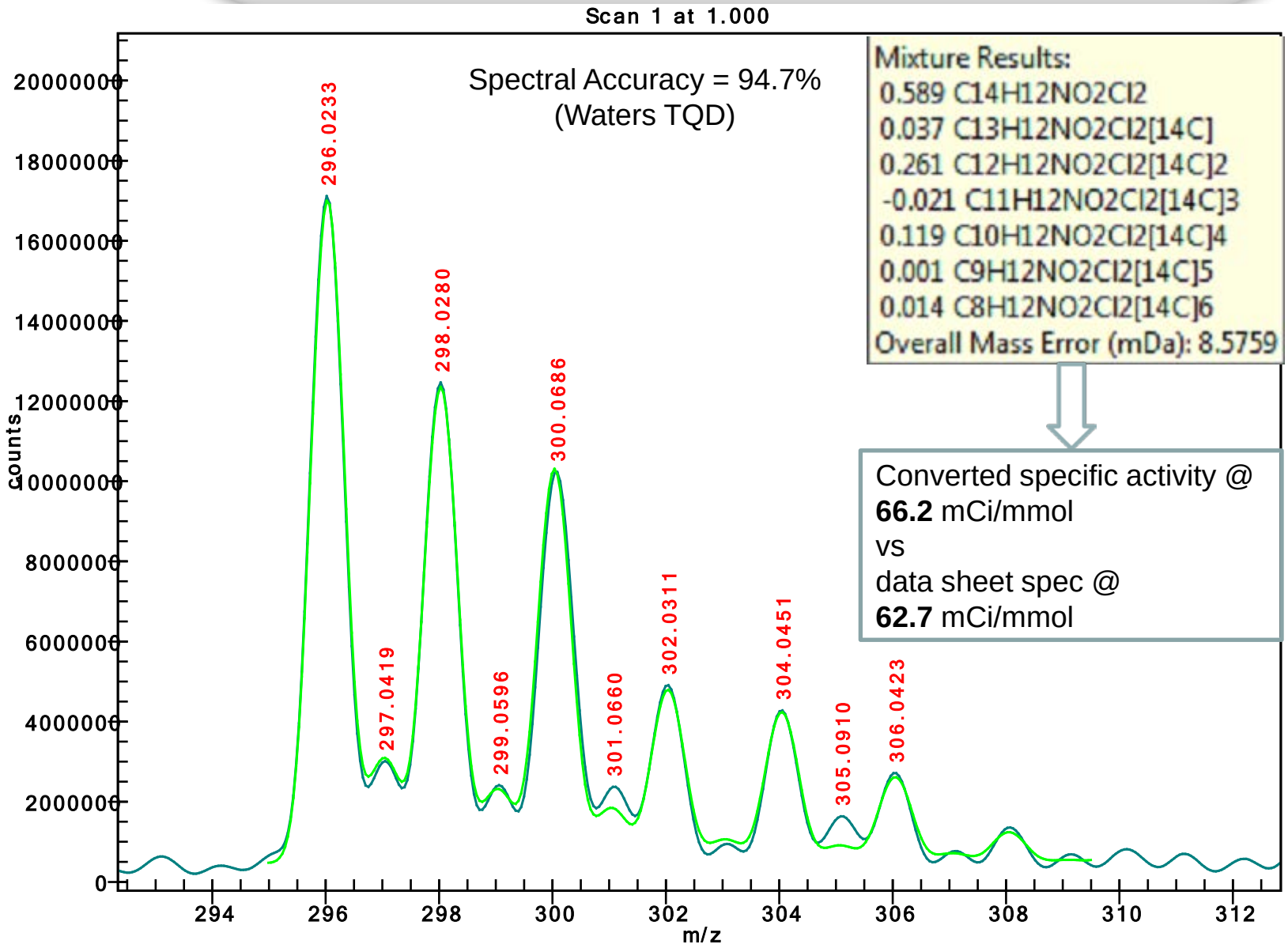


50:50 Mix: Quick Check with Single Quad LC/MSD



	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C7H7N3O4S2Cl	295.9561	-6.9012	-23.3190	98.6475	70	5.5
12	C7H3NO10Cl	295.9440	5.2005	17.5724	96.7128	169	6.5
13	C6H3N3O9Cl	295.9552	-6.0328	-20.3847	96.6199	174	6.5
14	C5H11NO5S3Cl	295.9482	0.9613	3.2483	96.6166	174	0.5
15	C2H7N5O6S2Cl	295.9521	-2.8785	-9.7263	96.5805	176	1.5
16	C10H3N3O4SCl	295.9527	-3.5304	-11.9291	96.4908	181	10.5
17	C11H3NO5SCl	295.9415	7.7030	26.0280	96.3166	190	10.5
18	C9H11NS4Cl	295.9457	3.4638	11.7039	95.7441	219	4.5
19	C12H7NS3Cl	295.9424	6.8346	23.0937	95.5060	232	9.5
20	C4H11N3O2S4Cl	295.9417	7.4865	25.2966	95.2028	247	0.5
21	C3H11N5OS4Cl	295.9529	-3.7469	-12.6606	95.1432	250	0.5
22	C5H14NOS6	295.9394	9.7854	33.0643	94.9018	263	-0.5
23	C4H14N3S6	295.9506	-1.4480	-4.8928	94.8769	264	-0.5
24	C6H15NS5Cl	295.9491	0.0929	0.3140	94.1799	300	-0.5
25	C12H4NO4Cl2	295.9512	-1.9895	-6.7223	93.3878	341	10.5
26	C15H3NO2SCl	295.9568	-7.5532	-25.5218	93.2104	350	14.5
27	C2H10N5O2S5	295.9433	5.9455	20.0897	92.9783	362	0.5
28	C5H14NO3S5	295.9572	-7.9732	-26.9411	92.8799	367	-0.5
29	C7H4N3O6Cl2	295.9472	2.0333	6.8704	92.7427	374	6.5
30	C6H4N5O5Cl2	295.9584	-9.2001	-31.0868	92.6907	377	6.5
31	C10H4N5SCl2	295.9559	-6.6977	-22.6312	92.4536	389	10.5
32	C11H4N3OSCl2	295.9447	4.5357	15.3260	92.3871	393	10.5
33	C9H8NO4SCl2	295.9546	-5.3603	-18.1122	92.0717	409	5.5
34	C7H10N3S5	295.9473	1.9228	6.4970	91.8062	422	4.5

Ring 14C Labeled Compounds



Conclusions

- MS quant of overlapping multiple labeled species: feasible, accurate, universal, sensitive & convenient
- Spectral accuracy calibration is key
  - To ensure good results
  - To diagnose problems with questionable results
- Quadrupole MS systems with properly calibration: dependable and robust (even walk-up systems)
- HiRes MS requires special attention to spectral accuracy
- Other mixture analysis:
  - Mixture identification/quantitation: CO/N_2, deamidation/deamination, HDX MS, metal-organics etc
  - M+ and (M-H)+ in EI mode

Further Info & Acknowledgement

- www.cernobioscience.www (Anal. Chem. front cover)
- www.cernobioscience.com/resources.html (apps)
- www.cernobioscience.com/technology.html (tech demo)
- info@cernobioscience.com (contact)
- Booth # 129

Acknowledgement

- Many early adopters
- Many “out-of-box” scientists/researchers
- Yale West Campus Analytical Core Facility: Terence Wu