Accurate Mass Confirmation/Identification for Organic Synthesis with Single Quadrupole Mass Spectrometer

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Introduction

Quadrupole Mass Spectrometry (MS) coupled with GC, LC, or one of the many direct sampling techniques (e.g. DART, DESI, Nano Spray) is a common confirmation/identification tool for synthetic organic chemists. Unfortunately, typical quadrupole MS data only provide nominal mass values for a limited level of confirmation of the target compound being synthesized. It does not provide accurate enough information necessary for peer-reviewed publications, as it does not reasonably eliminate the possibility of mis-identification of the target compound, degradants, impurities, or side reaction products. Publication quality confirmation typically requires measuring the compound on a high resolution accurate mass instrument, which is not always convenient or available to all researchers.

Here we present an economical alternative, now accepted by ACS journals for organic synthesis confirmation [1-3], through a novel accurate mass calibration process [4] with single quadrupole GC/MS or LC/MS.

Methods

Utilizing a novel MS calibration process called TrueCal on unit mass resolution MS systems, up to 100X improvement in mass accuracy is obtained, from the typical 0.x to 0.00x Da, while achieving high spectral accuracy, a companion concept published in a front cover feature article in Analytical Chemistry, an ACS journal [4]. TrueCal works by acquiring a known set of standard ions in raw profile mode, such as those from the tune solution on Agilent LC/MSD or PFTBA tune gas on GC/MSD, and building a full calibration involving not only m/z but, more importantly, MS peak shape. The calibration thus obtained is then applied to a test or unknown sample for either unknown formula ID or known formula confirmation, as is the case in organic synthesis. The calibrated MS data have both accurate mass and high spectral accuracy, which quantitatively and accurately measures the spectral similarity between the calibrated and the true calculated MS including all isotopes, in a process called CLIPS (Calibrated Lineshape Isotope Profile Search). As a result, the accepted publications have reported both mass error and spectral accuracy (e.g., [2]).

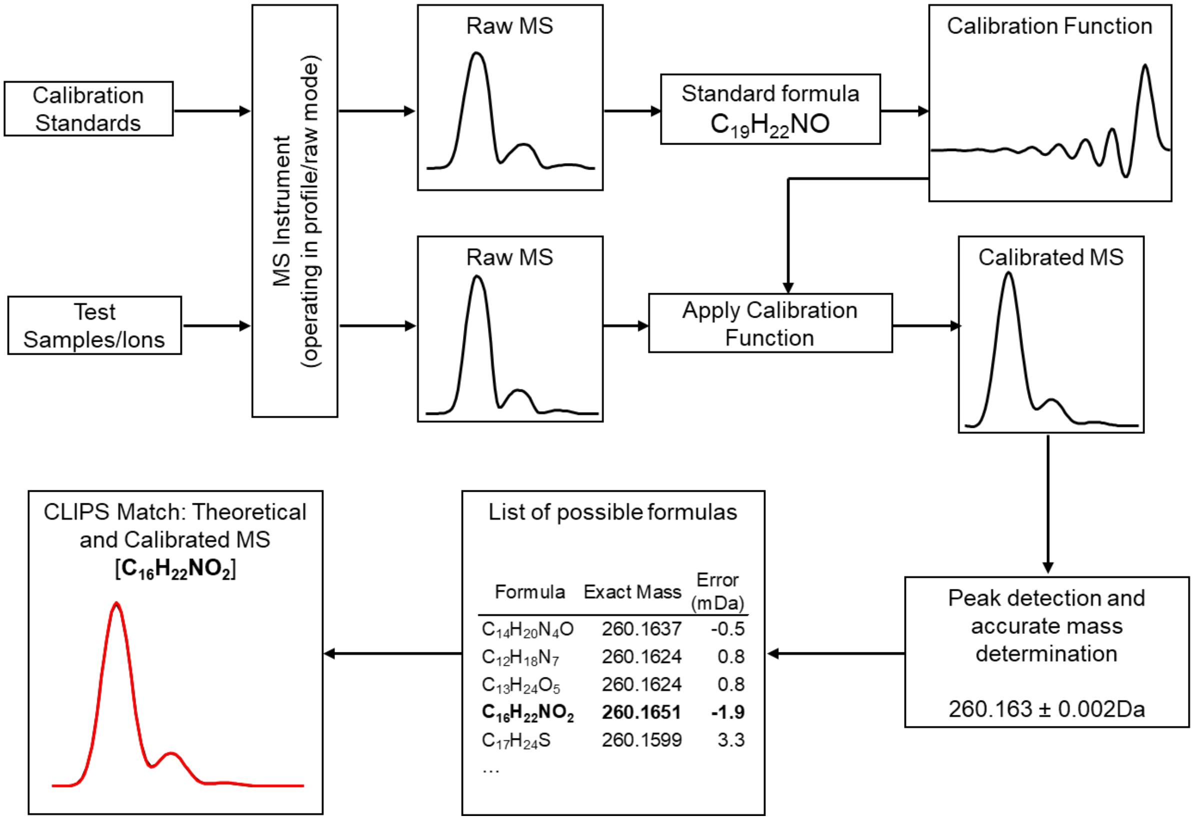
Experiments and Data Analysis

- ➢ Agilent GC/MSD: The readily available PFTBA calibration gas is turned on towards the end of a GC separation to acquire MS calibration data for accurate mass and spectral accuracy calibration, all internal to the same GC/MS run as the sample, without requiring any additional experiment or injection. To minimize spectral interferences from possible column bleeding and detector saturation, the GC oven is first cooled to the starting temperature and the detector voltage reduced by about 100V before the PFTBA valve is turned on. Raw scan or profile mode data without any ion threshold are acquired under the same MS scan conditions for both the standards and the sample.

- Agilent LC/MSD: There are multiple ways to acquire the required standard run data – acquire MS run data off the tune solution standard during manual tune, make a separate loop injection, or make a direct infusion of the tune solution. Again, (raw) scan or profile mode data without any ion threshold are acquired with full data storage under the same MS scan conditions for both the standards and the sample (reserpine for this experiment).

- Data Analysis: TrueCal MS calibration and data analysis are performed with MassWorks software commercially available from Cerno Bioscience, Norwalk, CT. With PFTBA turned on inside a GC/MSD run, a feature called AutoCal will kick off automatically during the data file opening process to perform the required MS calibration so that accurate mass and spectral accuracy become available once the data file is opened without any user intervention. In EI GC/MS, the accurate mass and spectral accuracy analysis is performed on the molecular ion and/or key fragments.

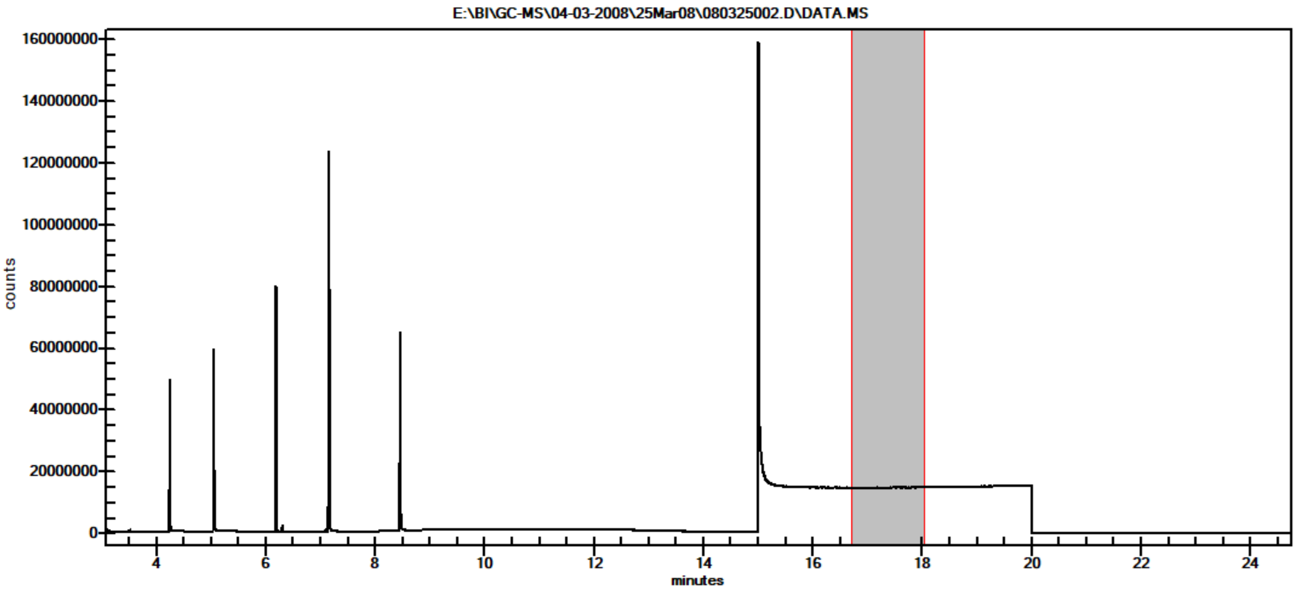
MassWorks Calibration + CLIPS Formula ID

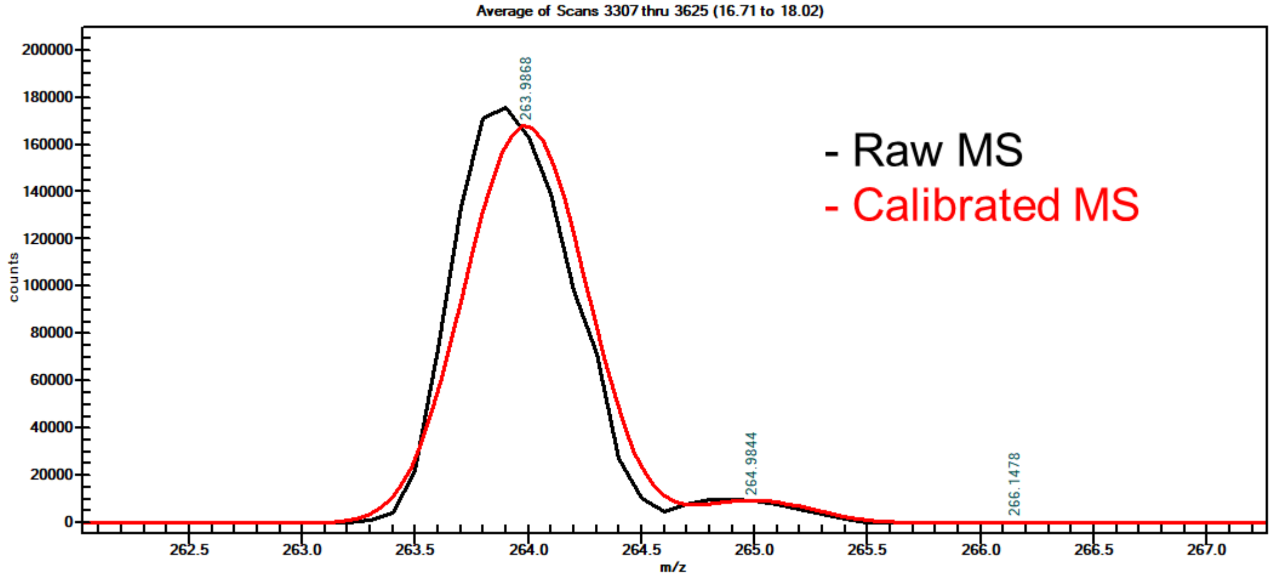


Formula	Exact Mass	Error (mDa)
$C_{14}H_{20}N_4O$	260.1637	-0.5
$C_{12}H_{18}N_7$	260.1624	0.8
$C_{13}H_{24}O_5$	260.1624	0.8
$C_{16}H_{22}NO_2$	**260.1651**	**-1.9**
$C_{17}H_{24}S$	260.1599	3.3
…		

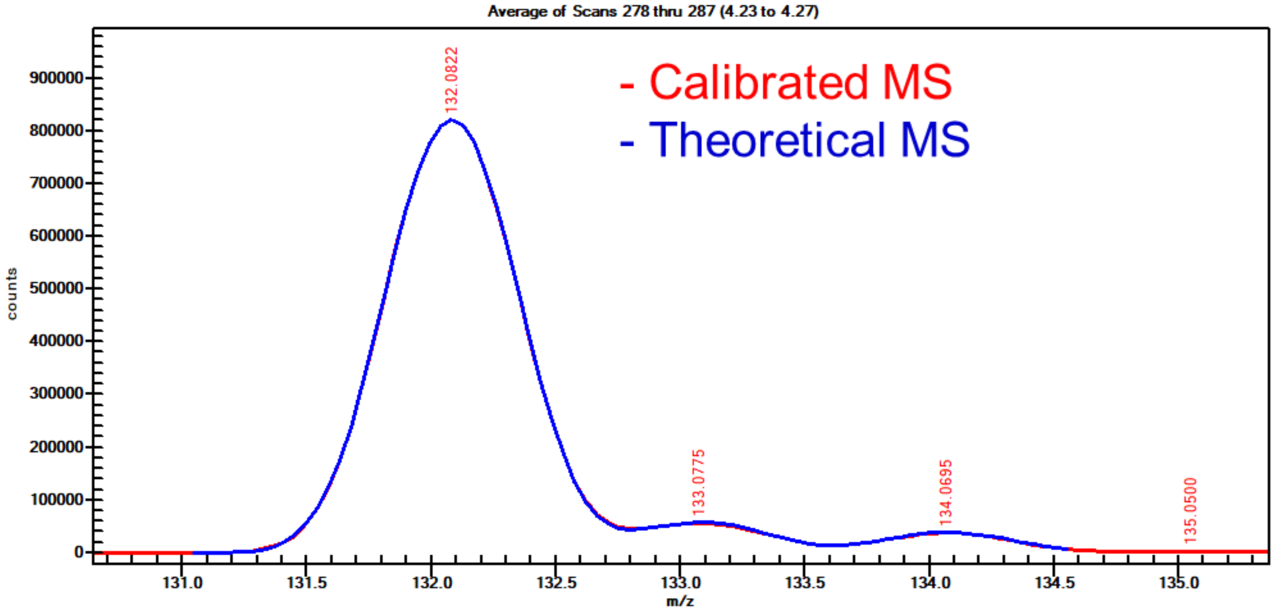
Results and Discussion

GC/MS Single Quad Application



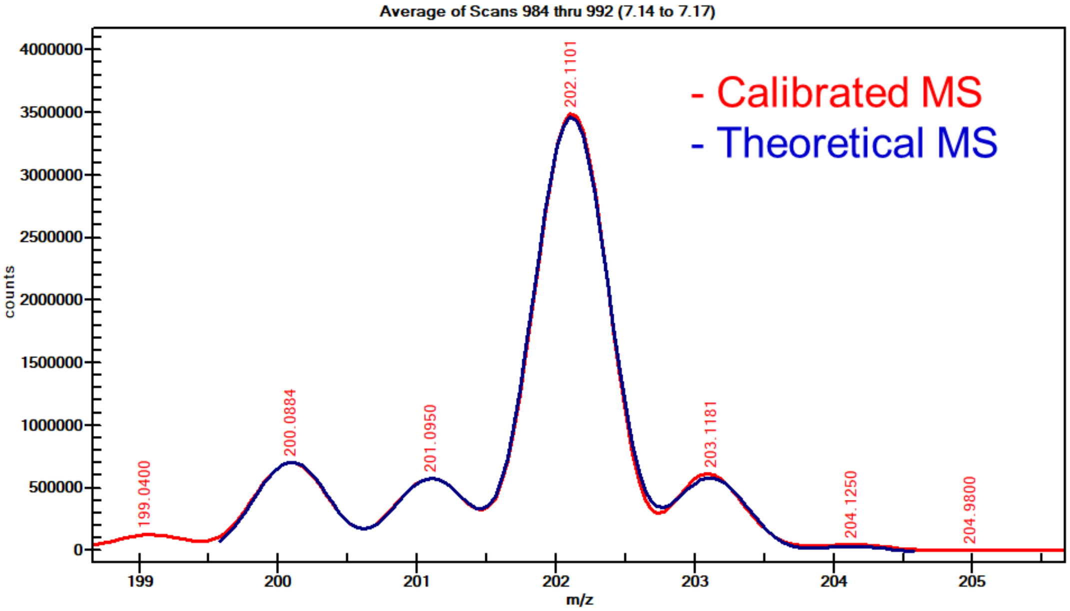


For each known ion fragments from the PFTBA standard, both mass and peak shape are adjusted as part of the calibration to enable highly accurate mass determination at unit mass resolution in the presence of isotope interferences, as shown for the C5F10N+ fragment ion (accurate mass 263.9868 vs exact mass 263.9871Da). This calibration is then applied to the unknown compounds #1-5 to test the CLIPS formula determination. When applied to the compound #1, the correct formula is correctly identified as the 1st hit with the highest spectral accuracy of 99.69%, even though the mass accuracy is only at 10mDa or 76ppm, demonstrating the superiority of spectral accuracy over mass accuracy. The high spectral accuracy reflects the near perfect match between the calibrated and theoretical MS and the difference between the two can be entirely accounted for by the fundamental ion counting noise.

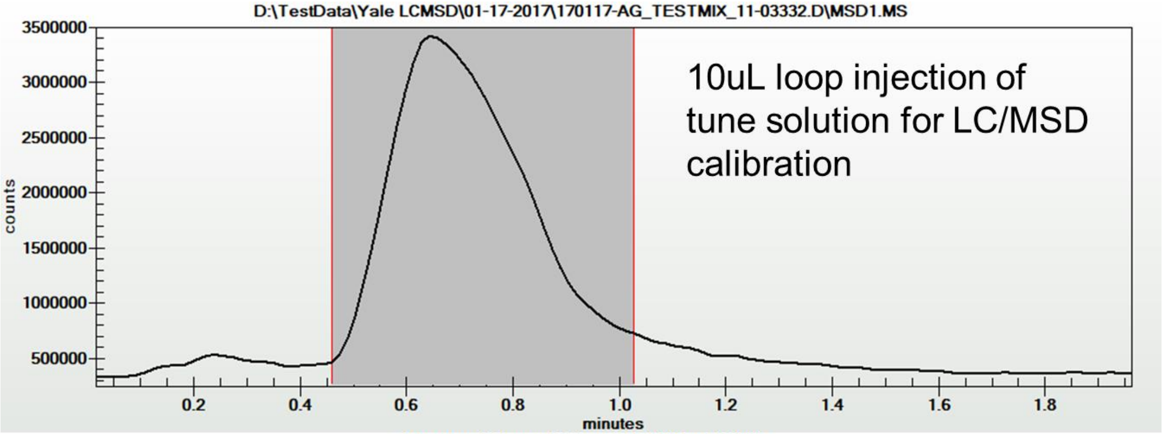


Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy (%)	RMSE	DBE
1	C5H12N2S	132.0721	10.1	76	99.69	913	1
2	C3H16O3S	132.0820	0.2	1	97.50	7,465	-4
3	C2H16N2O2S	132.0932	-11.0	-84	97.30	8,084	-4
4	C6H12O3	132.0786	3.6	27	96.95	9,118	1
5	C5H12N2O2	132.0899	-7.7	-58	96.88	9,340	1
6	C2H8N6O	132.0760	6.2	47	96.77	9,661	2
7	CH8N8	132.0872	-5.0	-38	96.57	10,250	2
8	CH12N2O5	132.0746	7.6	57	96.22	11,318	-3
9	H12N4O2S	132.0681	14.1	107	96.11	11,626	-3
10	H12N4O4	132.0859	-3.7	-28	95.95	12,129	-3
11	C8H8N2	132.0687	13.5	102	95.24	14,255	6
12	C2H16N2S2	132.0755	6.7	51	95.01	14,930	-4
13	C10H12	132.0939	-11.7	-89	94.33	16,958	5
14	H20O3S2	132.0854	-3.2	-24	92.82	21,476	-9
15	H20OS3	132.0676	14.6	110	90.06	29,734	-9

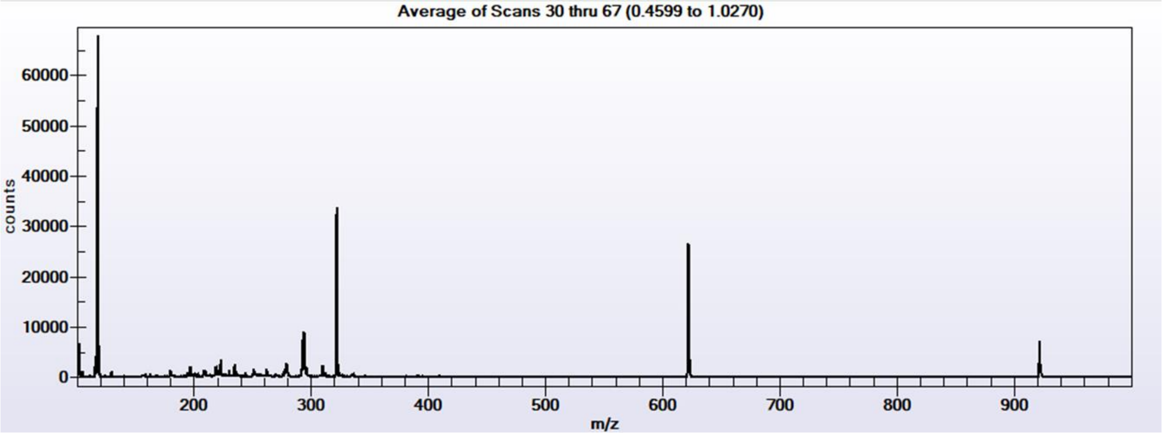
The type of interference exhibited by C16H10 (202Da) occurs frequently in EI mass spectrum. While the mass measurement will be biased due to the lack of resolving power, the Spectral Accuracy concept can be extended to account for mass spectral mixtures by including the interference ions so as to achieve similarly reliable formula determination with high spectral accuracy, as shown in the below graph.



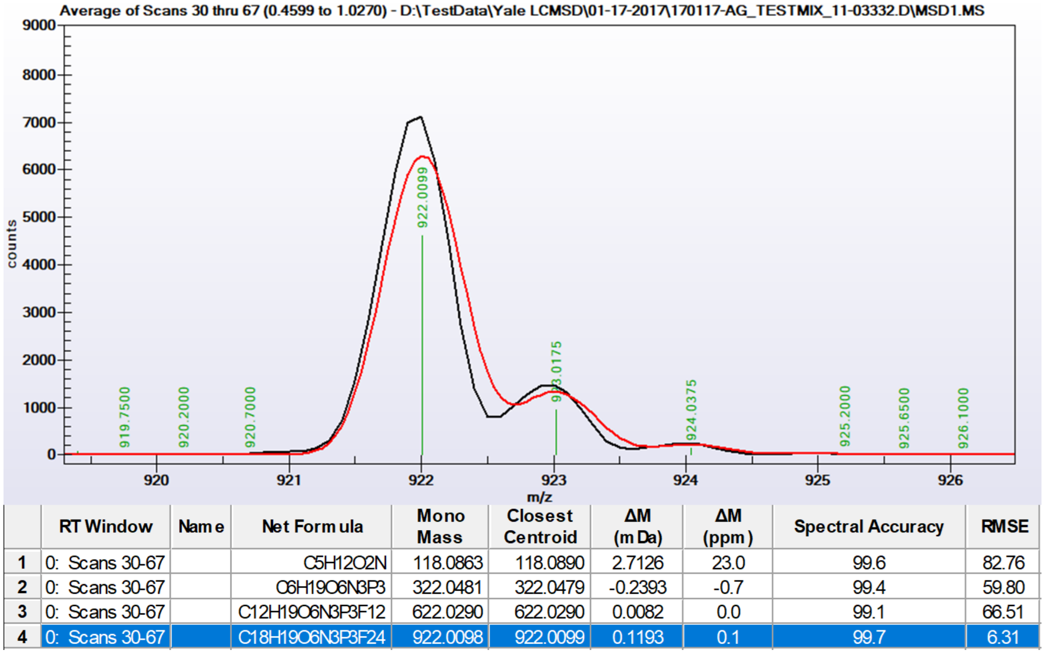
LC/MS Single Quad Application



10uL loop injection of tune solution for LC/MSD calibration



The calibration review page below shows the mass recovery for all standard ions within 5 mDa with the spectral accuracy all above 99.0%, demonstrating good hardware and calibration performance. This external calibration only needs to be performed per day under the same MS conditions and applied to all samples acquired during the same day.

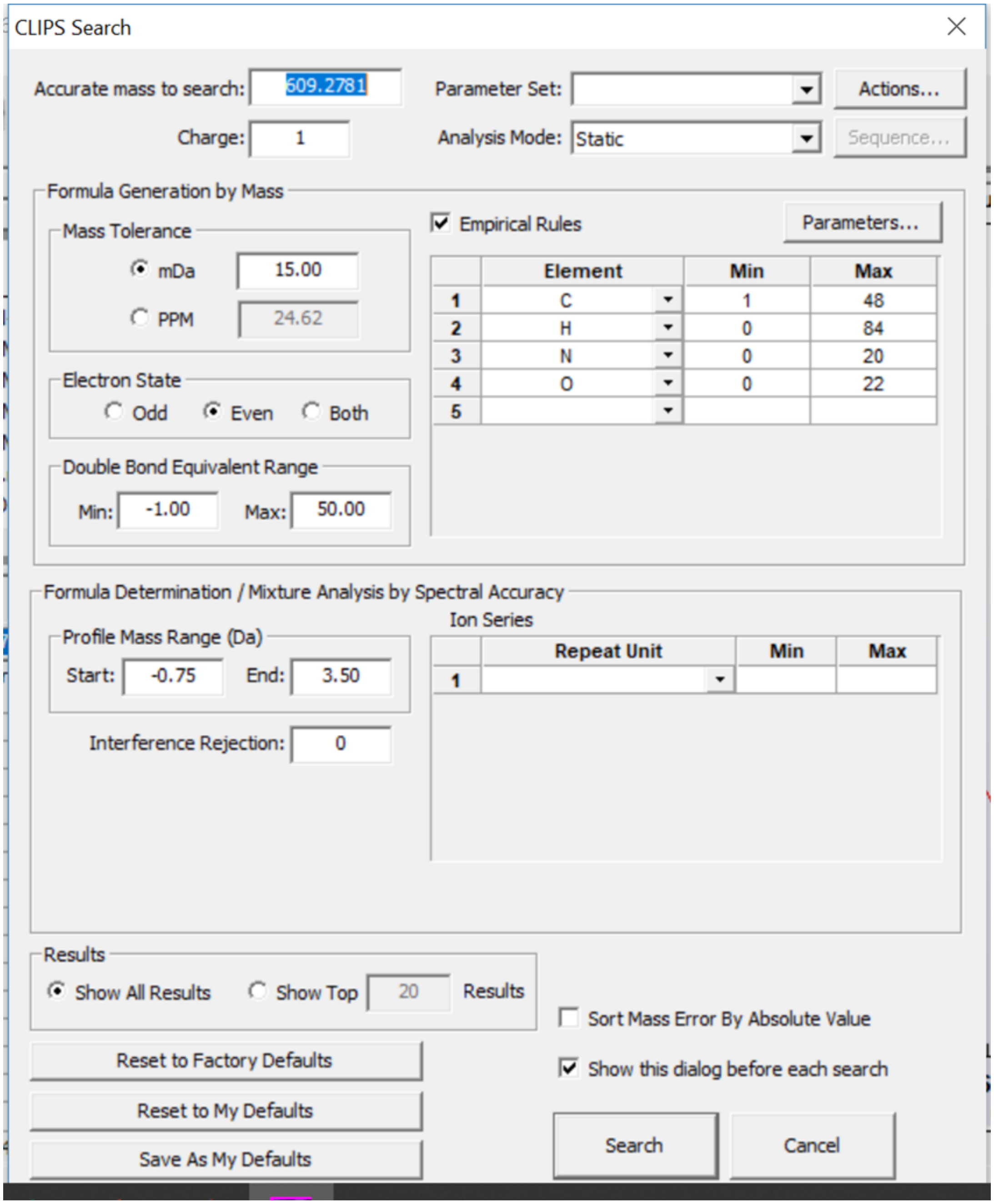


	RT Window	Name	Net Formula	Mono Mass	Closest Centroid	ΔM (mDa)	ΔM (ppm)	Spectral Accuracy	RMSE
1	0: Scans 30-67		C5H12O2N	118.0863	118.0890	2.7126	23.0	99.6	82.76
2	0: Scans 30-67		C6H19O6N3P3	322.0481	322.0479	-0.2393	-0.7	99.4	59.80
3	0: Scans 30-67		C12H19O6N3P3F12	622.0290	622.0290	0.0082	0.0	99.1	66.51
4	0: Scans 30-67		C18H19O6N3P3F24	922.0098	922.0099	0.1193	0.1	99.7	6.31

10 Repeated Injections of Reserpine Sample

Injection	Accurate Mass (Da)	Mass Error (mDa)	Mass Error (ppm)	Spectral Accuracy (%)
1	609.2781	-2.6	-4.2	99.06
2	609.2822	1.5	2.5	99.05
3	609.2824	1.7	2.9	99.01
4	609.2840	3.3	5.5	98.71
5	609.2781	0.4	0.7	98.98
6	609.2814	0.7	1.2	99.17
7	609.2802	-0.5	-0.8	98.82
8	609.2800	-0.7	-1.1	99.11
9	609.2781	-1.5	-2.4	98.82
10	609.2822	-0.2	-0.3	99.00
Average	609.2807	0.3	0.4	98.97
Std Dev	0.0021	1.7	2.8	0.15

Mass Accuracy + Spectral Accuracy on Quad
vs
Accurate Mass Only from HiRes MS



CLIPS Search

Accurate mass to search: 609.2781

Charge: 1

Parameter Set:

Analysis Mode: Static

Formula Generation by Mass

Mass Tolerance
● mDa 15.00
○ PPM 24.62

Electron State
○ Odd ● Even ○ Both

Double Bond Equivalent Range
Min: -1.00 Max: 50.00

☑ Empirical Rules

	Element	Min	Max
1	C	1	48
2	H	0	84
3	N	0	20
4	O	0	22
5			

Formula Determination / Mixture Analysis by Spectral Accuracy

Profile Mass Range (Da)
Start: -0.75 End: 3.50

Interference Rejection: 0

Ion Series

	Repeat Unit	Min	Max
1			

Results
● Show All Results ○ Show Top 20 Results

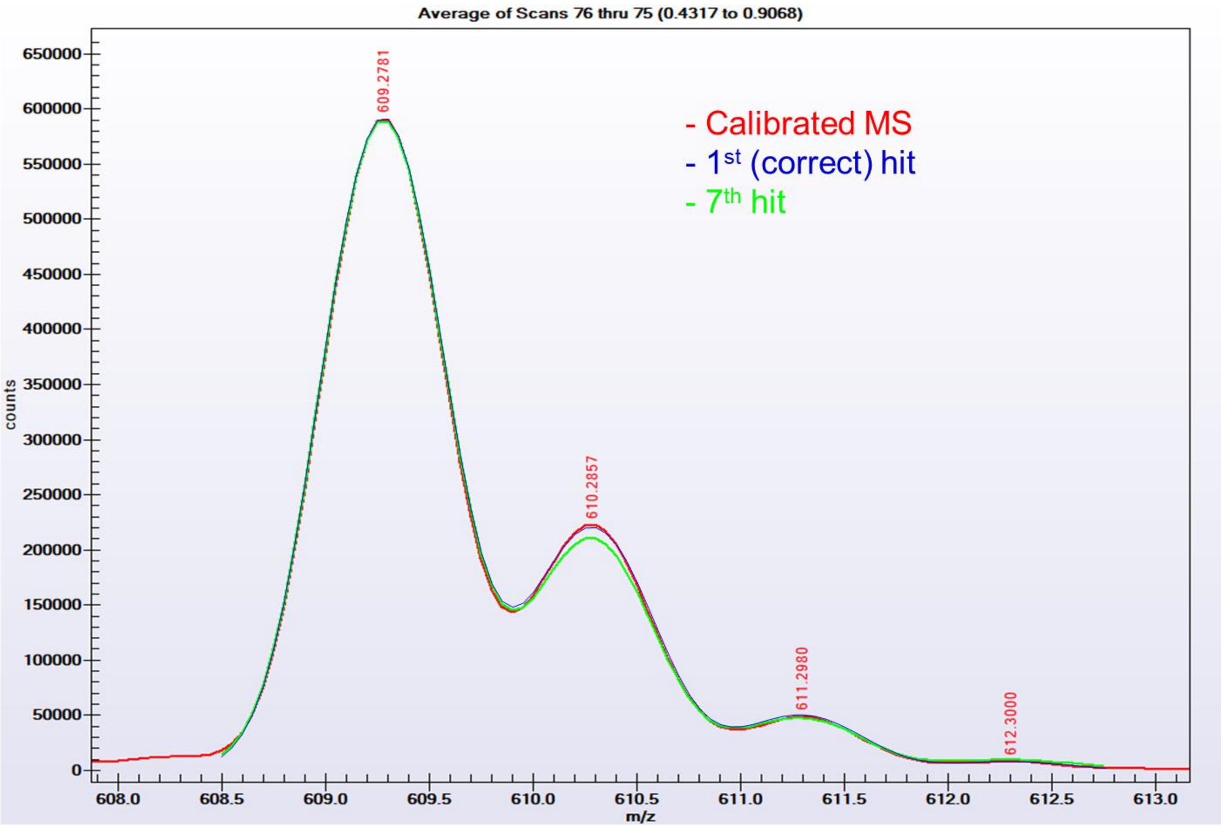
☐ Sort Mass Error By Absolute Value

☑ Show this dialog before each search

57 candidates within 15 mDa mass error window with the correct one on top of spectral accuracy list:

Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy (%)	RMSE	DBE
1	C33H41N2O9	609.2807	-2.6	-4.2	99.06	2,090	14.5
2	C34H41O10	609.2694	8.7	14.2	99.03	2,165	14.5
3	C32H41N4O8	609.2919	-13.8	-22.6	98.99	2,242	14.5
4	C31H33N10O4	609.2681	10.0	16.5	98.91	2,435	20.5
5	C30H33N12O3	609.2793	-1.2	-2.0	98.87	2,514	20.5
6	C29H33N14O2	609.2905	-12.4	-20.4	98.76	2,770	20.5
7	C30H37N6O8	609.2667	11.4	18.6	98.20	4,010	15.5
8	C27H29N16O2	609.2654	12.7	20.9	98.16	4,096	21.5
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
57	C7H37N20O13	609.2843	-6.2	-10.3	83.63	36,479	-0.5

While all top 6 hits may have similarly high spectral accuracy, the 7th and beyond are spectrally and statistically different:



HiRes MS with 5ppm mass error would have resulted 12 possible candidates, compared to 6 candidates within 15 mDa (25 ppm) combined with 98.5% spectral accuracy:

Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy (%)	RMSE	DBE
1	C29H37N8O7	609.2780	0.1	0.2	97.95	4,560	15.5
2	C14H33N20O8	609.2785	-0.4	-0.6	89.19	24,084	8.5
3	C45H37O2	609.2788	-0.7	-1.2	90.92	20,226	27.5
4	C13H37N16O12	609.2771	1.0	1.6	87.33	28,231	3.5
5	C30H33N12O3	609.2793	-1.2	-2.0	98.87	2,514	20.5
6	C28H41N4O11	609.2766	1.5	2.4	96.18	8,522	10.5
7	C26H29N18O	609.2766	1.5	2.4	97.89	4,705	21.5
8	C17H41N10O14	609.2798	-1.7	-2.8	88.93	24,665	2.5
9	C41H33N6	609.2761	2.0	3.2	92.56	16,576	28.5
10	C33H41N2O9	609.2807	-2.6	-4.2	99.06	2,090	14.5
11	C27H45O15	609.2753	2.8	4.6	94.28	12,746	5.5
12	C25H33N14O5	609.2753	2.8	4.6	96.36	8,104	16.5
13	C18H37N14O10	609.2812	-3.1	-5.0	90.81	20,470	7.5
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
57	C10H33N20O11	609.2632	14.9	24.4	85.99	31,223	4.5

Conclusions

- Quadrupole MS (either GC/MS or LC/MS) calibrated with MassWorks is capable of unknown elemental composition determination.
- For organic synthesis confirmation, when the sample is not terribly complex and sensitivity is rarely an issue, quadrupole MS with 15 mDa mass accuracy combined with 98.5% spectral accuracy seems to provide higher confidence confirmation than using the accurate mass alone available from higher resolution systems such as TOF or Orbitrap.
- Due to the relaxed requirement on mass accuracy, the calibration sample only needs to be acquired once every working day and externally to the test samples, which could even be scheduled for automatic injection early in the morning, e.g., at 5:00 am each day, suitable to the working schedule of an organic chemistry lab.
- The readily available and economical single quad MS makes it possible to have publishable elemental composition confirmation done on site in the organic chemistry lab.

Acknowledgement

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References

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