

# The Robustness of Formula Determination on a Single Quadrupole GC/MS

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## Introduction

The quadrupole GC/MS is a widely available workhorse instrument in the modern analytical laboratory. It would be highly desirable to have accurate mass capability on these otherwise conventional single quad GC/MS systems. Different from other earlier work on quadrupole accurate mass measurement, which requires either massive sampling (1) or tuning the system to higher than unit mass resolution (2), we had demonstrated that an elaborate calibration involving both m/z and peak shape enables accurate mass measurement on a quadrupole MS under routine GC and MS conditions. This allows for elemental composition determination for either molecular ions and/or its fragments ion to facilitate the identification and structural elucidation of unknown compounds (3-4). Since this calibration scheme involves both mass accuracy and spectral accuracy, a question typically arises concerning the stability of such an extensive calibration and its implications for practical applications. This paper will study the robustness of such an approach through the observation of long term stability in reported accurate masses and peak shapes, with and without re-tuning the MS instruments. In addition, limit of identification (the lowest amount of a compound necessary to elucidate its structure) is also evaluated via mass spectral library search, mass accuracy and spectral accuracy.

## Methods

Five commercially available organic compounds are selected from various compound classes, including aromatics/hydrocarbons/pesticide as unknowns to be tested. An Agilent 5973N GC/MS system is programmed to perform a 12min GC/MS acquisition followed by oven cooling and PFTBA valve opening timed-event for the acquisition of PFTBA standard spectrum, all within the same GC/MS experiment. The analysis of this same mixture will be repeated in 4 different modes: (1) within the same day; (2) within two days; (3) within 10 days; (4) within 29 days. The PFTBA data acquired within each run can be applied to the GC/MS scans within the same run for internal calibration or to those scans from another run for external calibration to evaluate the calibration stability. During these experiments, the mass spectrometer was tuned once to allow for the study of MS tuning on mass accuracy and spectral accuracy. For limit of identification a series of dilutions was made from approx. 12 ng down to 0.08 ng on column. The solutions were tested using the above "raw" acquisition mode for mass and spectral accuracy evaluation and scan acquisition mode for Nist98 MS Library search.

## Experiments and Data Analysis

GC conditions: Cool on-column injection with oven track on, 1 µL/inj, 50°C hold for 1min, ramp 40°C/min to 300°C, hold for 5min, -100°C/min to 50°C for 10min, 1.2 mL/min flow rate, Phenomenex ZB-1MS 30m x 250µm column with 0.25µm film thickness.

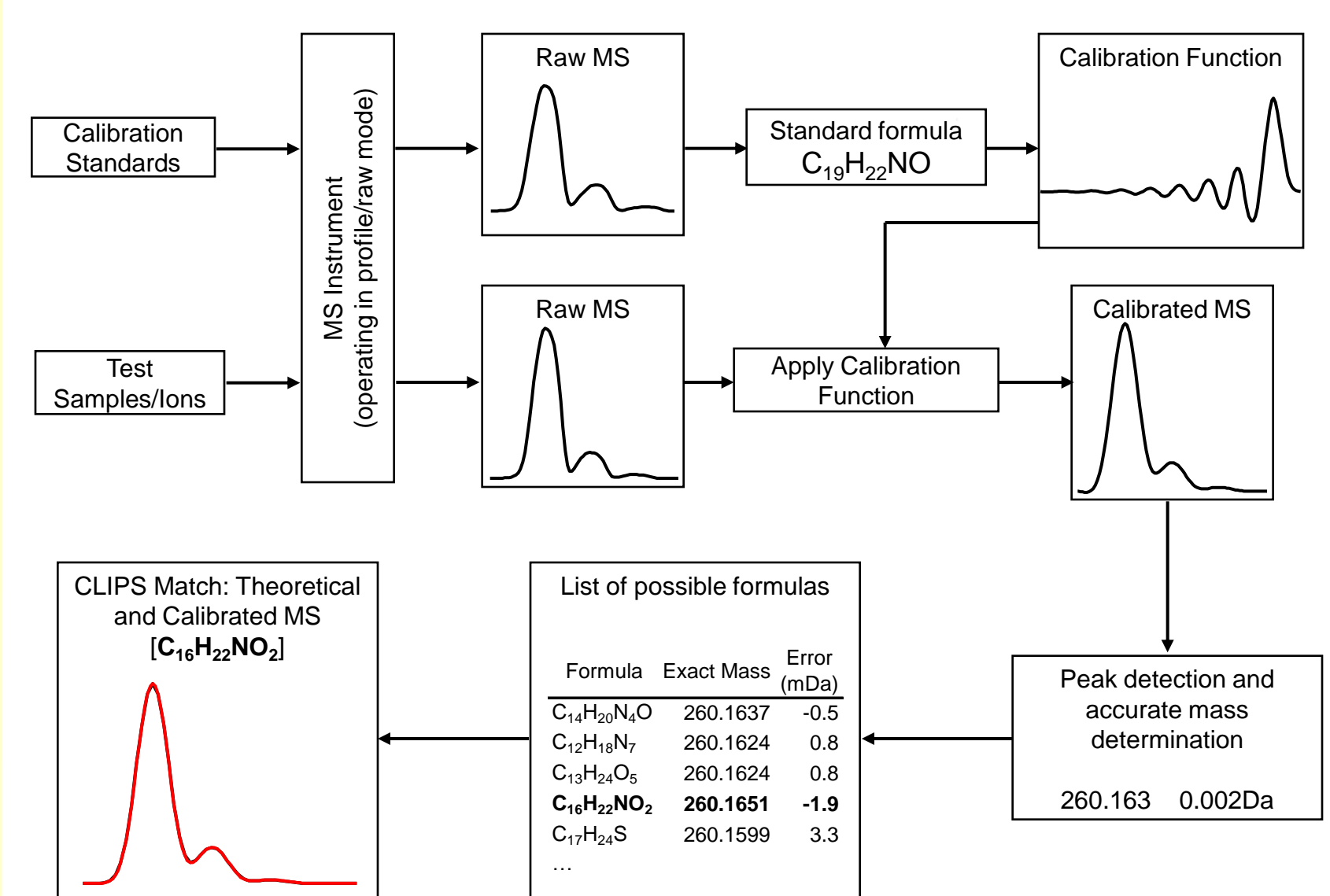
MS conditions: MS data were acquired on an Agilent 5973 MSD in "raw" mode (non-peak detected) at a scan speed 2<sup>2</sup> (A/D samples = 4) over a mass range of 50-400 m/z. Calibration valve was turned on @15min and off @20 min.

Sample composition: (1) 3,3-Tetramethyl-2-thiourea, C5H12N2S, 98% Pure, Aldrich, 12.92/1.29ng; (2) 1,3-Diacetyl Benzene, C10H10O2, 99% Pure, Aldrich, 12.66/1.266ng; (3) 2,4-Diphenyl-4-methyl-1-pentene, C18H20, 97% Pure, Aldrich, 9.80/0.98ng; (4) Pyrene, C16H10, 98% Pure, Lancaster, 11.99/1.20ng; (5) Hexacosane, C26H54, 99% Pure, Aldrich, 9.66/0.97ng.

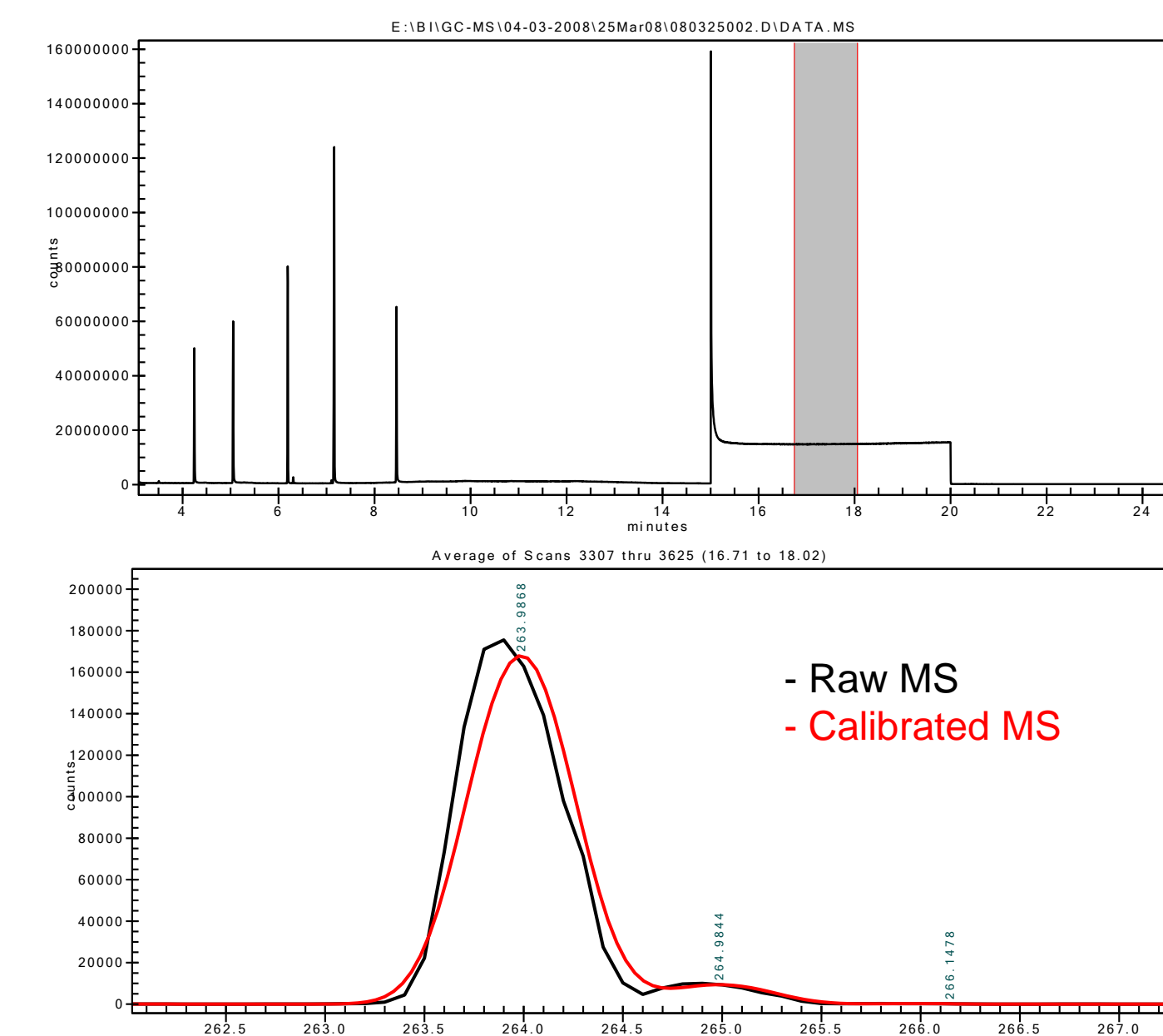
Limit of Identification: The above experiments were repeated at 6 different concentrations (12ng down to 0.08ng) for both "raw" and "scan" mode data acquisition to allow for comparison to Nist98 MS Library Search.

The Premise for MassWorks processing: MassWorks calibrates both the mass position and the mass spectral peak shape function, a key for achieving high mass accuracy. When applied to raw mass spectral data, the raw mass spectrum can be transformed into its calibrated version with mass spectral peaks located at accurate mass positions. Furthermore, the mass spectral peak shape would also be transformed in the same process to a mathematically definable function, a key for achieving high spectral accuracy and CLIPS formula ID.

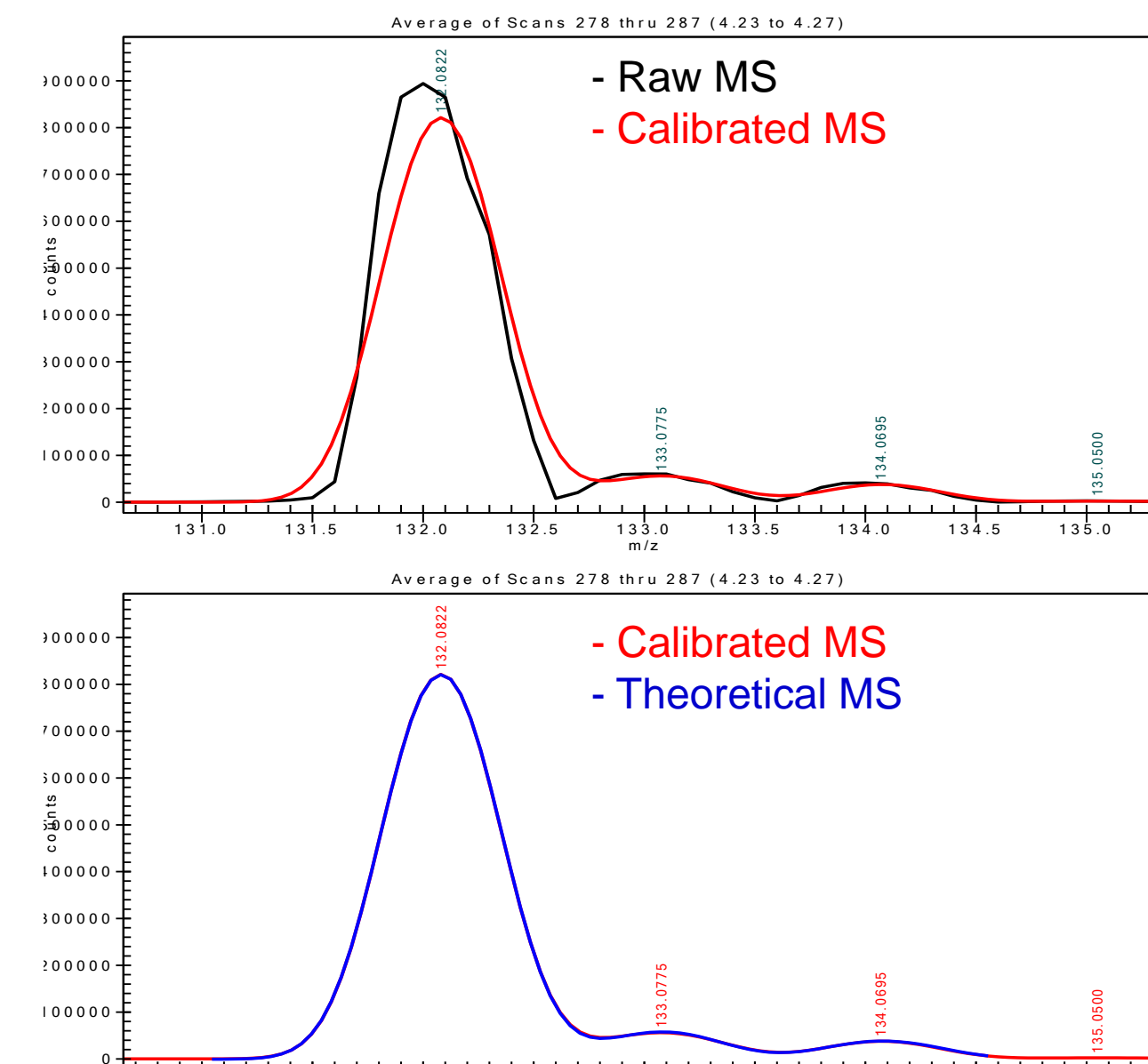
## MassWorks Calibration+CLIPS Formula ID



## Results and Discussion



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 (@12.92ng), the correct formula is correctly identified as the 1<sup>st</sup> 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.



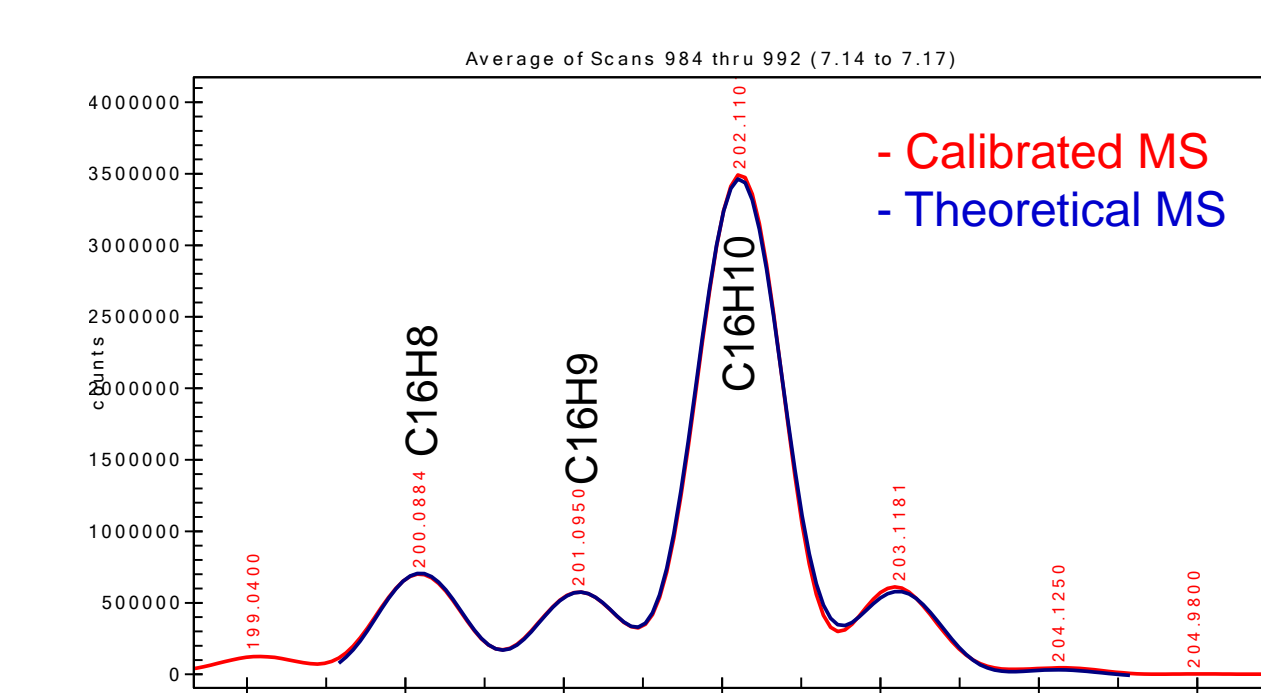
Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy (%)	RMSE	DBE
1	C5H12N2S	132.0721	10.1	76	99.69	913	1
2	C3H16O3S	132.0820	0.2	1	97.50	7,465	-4
3	C2H16N2O2S	132.0932	-11.0	-84	97.30	8,084	-4
4	C8H12O3	132.0786	3.6	27	96.95	9,118	1
5	C5H12N2O2	132.0899	-7.7	-58	96.88	9,340	1
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	132.0677	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	H2OOS2	132.0854	-3.2	-24	92.82	21,476	-9
15	H2OOS3	132.0676	14.6	110	90.06	29,734	-9

To test out the calibration stability over time and over different mass spectrometer tunes, the above calibration was applied as both internal standards and external standards to the 5 compounds within the same run and from other runs on different days where a new MS tune is performed for each. The CLIPS formula determination results listed in the following table shows that excellent Spectral Accuracy can be attained with internal calibration which leads to unique formula ID for all five compounds, even for C16H10 and C26H54 each with its own complications. As soon as a new MS tune is performed, however, both mass accuracy and Spectral Accuracy would be compromised with inferior formula determination results.

Formula	Nominal Mass (Da)	Conc (ng)	Acquisition Date	Tune Date	Calibration Date	Calibration Type	Mass Error (mDa)	Spectral Accuracy (%)	Rank (Total)
C5H12N2S	132	12.92	Day 1	Day -3	Day 1	Internal	10	99.70	1 (50)
C10H10O2	162	12.66	Day 1	Day -3	Day 1	Internal	10	99.28	1 (84)
C18H20	236	9.80	Day 1	Day -3	Day 1	Internal	8	99.35	1 (194)
C16H10	202	11.98	Day 1	Day -3	Day 1	Internal	32*	97.87	1 (137)
C26H54	366	9.66	Day 1	Day -3	Day 1	Internal	34*	96.17	1 (387)
C5H12N2S	132	12.92	Day 2	Day 2	Day 1	External	-34	95.36	2 (55)
C10H10O2	162	12.66	Day 2	Day 2	Day 1	External	-33	94.98	2 (83)
C18H20	236	9.80	Day 2	Day 2	Day 1	External	-27	93.33	2 (202)
C16H10	202	11.98	Day 2	Day 2	Day 1	External	-6	94.65	1 (148)
C26H54	366	9.66	Day 2	Day 2	Day 1	External	3	88.31	4 (421)
C5H12N2S	132	1.29	Day 9	Day 8	Day 1	External	49	95.31	2 (47)
C10H10O2	162	1.27	Day 9	Day 8	Day 1	External	47	93.52	4 (78)
C18H20	236	0.98	Day 9	Day 8	Day 1	External	46	91.42	1 (183)
C16H10	202	1.20	Day 9	Day 8	Day 1	External	50	93.34	1 (135)
C26H54	366	0.97	Day 9	Day 8	Day 1	External	86	84.10	51 (625)

\*Has significant interference from (M+H)+ and (M+H)2+  
\*Very weak molecular ion observed

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 required (20K or better), the Spectral Accuracy concept can be extended to include the interference ions so as to achieve similarly reliable formula determination, as shown in the next graph.



Although the MS tune has a profound impact on both the mass and spectral accuracy, the same excellent formula ID results as these from Day 1 can be obtained, after a new PFTBA calibration on Day 18 (see table below). This new calibration would last for more than 11 days into Day 29 when no degradation of mass accuracy or spectral accuracy was observed. After 19 days (Day 37), however, a systematic mass shift of about -20mDa became evident. Remarkably, the Spectral Accuracy hardly changed, leading to similarly excellent formula determination results in the presence of this systematic mass shift.

Formula	Nominal Mass (Da)	Conc (ng)	Acquisition Date	Tune Date	Calibration Date	Calibration Type	Mass Error (mDa)	Spectral Accuracy (%)	Rank (Total)
C5H12N2S	132	1.29	Day 18	Day 10	Day 18	Internal	-2	99.53	1 (52)
C10H10O2	162	1.27	Day 18	Day 10	Day 18	Internal	-4	98.99	1 (85)
C18H20	236	0.98	Day 18	Day 10	Day 18	Internal	-5	97.83	1 (195)
C16H10	202	1.20	Day 18	Day 10	Day 18	Internal	0	98.89	1 (147)
C26H54	366	0.97	Day 18	Day 10	Day 18	Internal	35	85.74	1 (387)
C5H12N2S	132	1.29	Day 29	Day 10	Day 18	External	-2	99.47	2 (52)
C10H10O2	162	1.27	Day 29	Day 10	Day 18	External	-7	97.41	1 (196)
C18H20	236	0.98	Day 29	Day 10	Day 18	External	-2	97.41	1 (196)
C16H10	202	1.20	Day 29	Day 10	Day 18	External	-3	99.28	1 (149)
C26H54	366	0.97	Day 29	Day 10	Day 18	External	6	82.16	1 (421)
C5H12N2S	132	1.29	Day 37	Day 10	Day 18	External	-22	98.94	1 (93)
C10H10O2	162	1.27	Day 37	Day 10	Day 18	External	-18	98.35	1 (85)
C18H20	236	0.98	Day 37	Day 10	Day 18	External	-25	97.06	1 (202)
C16H10	202	1.20	Day 37	Day 10	Day 18	External	-24	98.20	1 (150)
C26H54	366	0.97	Day 37	Day 10	Day 18	External	-14	84.07	1 (439)

To study the limit of identification, the stock solution (~ 10 ng/µL) is diluted 5 times in a series and injected into the GC/MS system for both profile mode data acquisition for MassWorks CLIPS) and scan mode data acquisition for Nist98 mass spectral library search. The following TIC plots show two such chromatograms.

## Conclusions

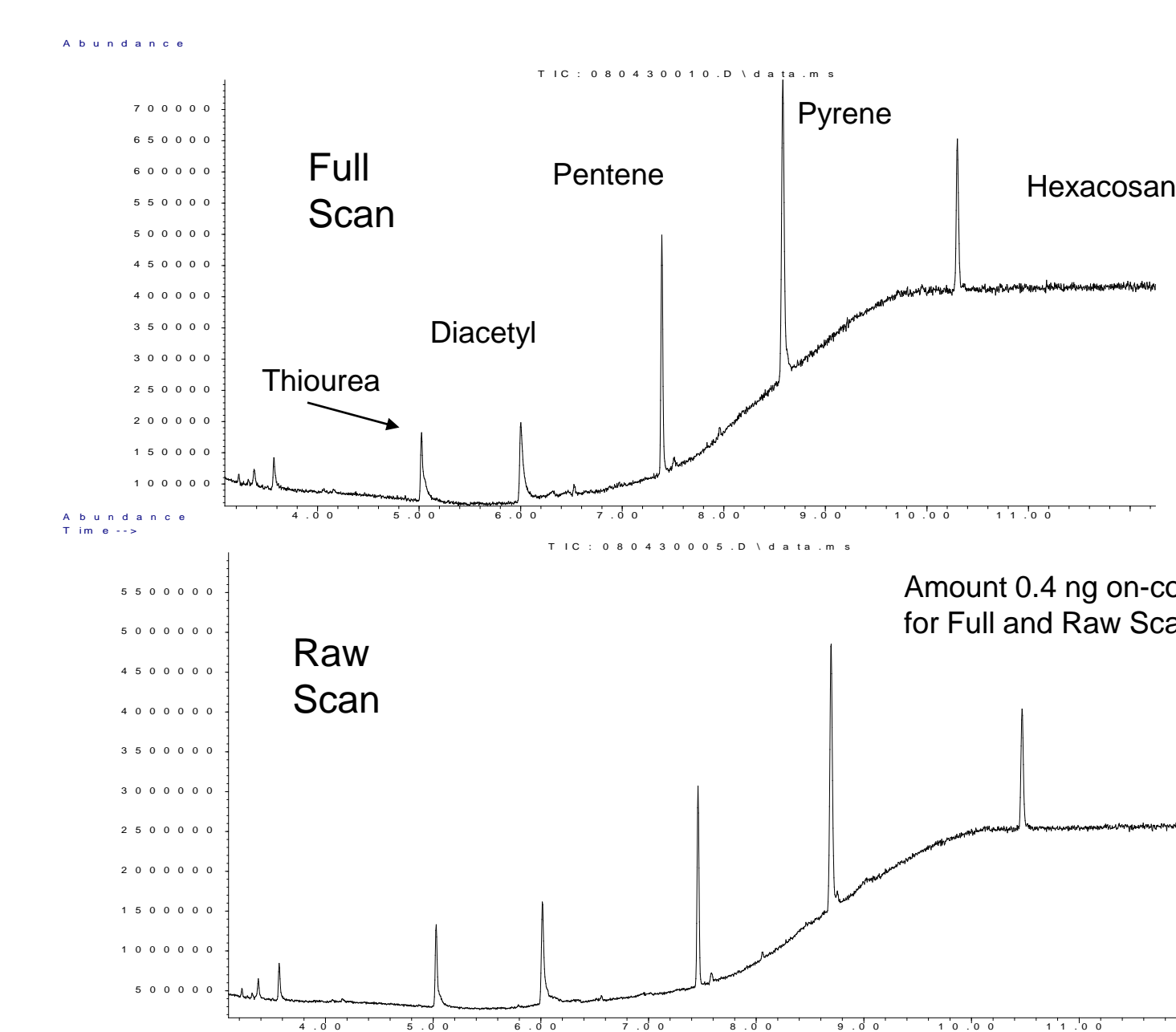
With PFTBA calibration performed to each GC/MS run, mass accuracy better than 10mDa can be achieved and Spectral Accuracy of better than 99% can be achieved to help attain reliable formula determination.

The mass calibration is stable after 11 days without significantly compromising formula determination.

While mass calibration is more susceptible to time related variations, the Spectral Accuracy has been shown to be robust enough for formula determination after 19 days.

MS tune changes both mass calibration and Spectral Accuracy (MS peak shape) and recalibration is therefore required after the tune in order to maintain the same formula determination capability.

The limit of identification is at about 0.4 ng on column for all five compounds tested, comparable to NIST MS library search. The spectral accuracy needs to be above 98.0% to have correct formula as the top two hits.



The table below compares the library match quality index with the Spectral Accuracy for all 5 compounds at the 6 different concentration levels. The results are very similar between the two with both performing well down to Level 2 (0.38 ng) and having difficulty for Hexacosane starting at Level 5 (2.4 ng). It should be pointed out that the formula determination is based on a single molecular ion without the use of any mass spectral library and is therefore amenable for the formula ID of unknown compounds not even included in the library.

Dilution (Approx. Conc. ng on column)	Tetramethyl Thiourea		1,4-Diacetyl Benzene		2,4-Diphenyl-4-methyl-1-pentene		Pyrene		Hexacosane	
	Library Match Quality	Spectral Accuracy % (Rank)	Library Match Quality	Spectral Accuracy % (Rank)	Library Match Quality	Spectral Accuracy % (Rank)	Library Match Quality	Spectral Accuracy % (Rank)	Library Match Quality	Spectral Accuracy % (Rank)
Stock 10ng	90	99.12 (2)	94	98.55 (1)	96	98.24 (1)	96	97.46 (1)	96	97.07 (1)
Level 5 6ng	91	98.71 (2)	94	97.05 (1)	96	95.54 (1)	96	98.06 (1)	94	81.62 (66)
Level 4 2.4ng	90	98.94 (2)	97	98.32 (1)	96	95.94 (1)	96	98.33 (1)	97	82.24 (36)
Level 3 0.96ng	90	99.10 (2)	94	98.21 (1)	96	97.81 (1)	96	98.39 (1)	93	93.31 (7)
Level 2 0.38ng	64	99.06 (2)	94	98.32 (1)	95	97.54 (1)	95	97.70 (1)	76	93.61 (4)
Level 1 0.08ng	50	96.79 (20)	70	95.21 (6)	90	83.40 (2)	89	97.59 (1)	Not Found	Not Found

## Acknowledgement

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