Processing Raw Mass Spectral Data for High Mass Accuracy

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Introduction

In qualitative MS analysis, there is a growing need to measure masses at higher and higher accuracy (5ppm or even 100ppb) to allow for unambiguous molecular identification in metabolism, biomarker discovery, and proteomics research. In quantitative MS analysis, constant efforts are being made to lower the MS detection limit and increase the quantitative accuracy. Careful examination of MS systems and the complete MS analysis process, however, reveals that the mass accuracy in a given MS system is largely limited by the mass calibration schemes currently available and the detection limit is most likely imposed by the heuristic peak detection methods used. This paper will introduce a systematic approach to achieve both comprehensive mass spectral calibration and robust peak detection.

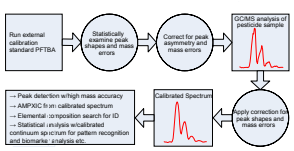
Methods

A comprehensive mass spectral calibration is performed through the use of an instrument calibration standard containing multiple known ions covering the mass spectral range of interest. Continuum mass spectral profile data can be acquired where the mass spectral response of each known ion encodes two pieces of critical calibration information, the mass shift and the corresponding peak shape deviation. A set of digital filters can then be numerically established to compensate for both the mass shift and the peak shape deviation and applied to successive MS scans to convert raw continuum data into their fully calibrated version. Much higher mass accuracy and significantly more reliable peak detection can be achieved on data thus calibrated, even on a unit mass resolution GC/MS system with single quadrupole.

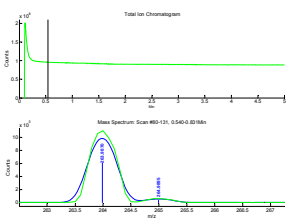
Experiments and Data Processing

- Sample information: Calibration standard PFTBA and 17 compound organochlorine pesticide sample (1ng/ul) also containing approximately 50 ng/ul PCB 209 (decachlorobiphenyl, $C_{12}Cl_{10}$).
- MS conditions: the PFTBA and standard were acquired on an Agilent 5973N-inert MSD in "raw" mode (non-peak detected) at a scan speed 2^2 (A/D samples = 4) over a mass range of 50-550 m/z.
- The profile mode mass spectra of the PFTBA calibration standard were acquired continuously for 5 min during its infusion. Similarly, during the GC/MS sample analysis, the profile mode mass spectral scans were repeatedly collected during the GC separation process for a total runtime of 19 min. A comprehensive mass spectral calibration can be created from the average of the PFTBA mass spectral scans within a given time window using MassWorks™ software.
- This unique calibration process calibrates both the mass position and the mass spectral peak shape function, a key for achieving high mass accuracy. This calibration was then applied to each scans in the GC/MS data file to transform each raw mass spectrum into its calibrated version with a mathematically defined symmetric peak shape located at accurate masses. Peak detection can then be applied to reliably and accurately calculate the mass locations for molecular ions and their fragment ions for the purpose of compound identification, with or without isotope profile matching (Refs 1 & 2). Other applications of the calibrated continuum MS spectrum includes elemental composition search, multivariate statistical analysis etc.

MassWorks Calibration & Data Processing



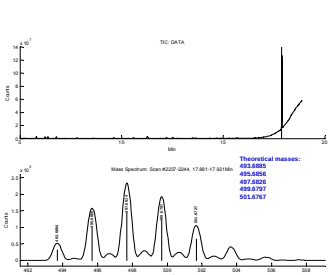
Results and Discussion



For each ion in the calibration sample, both mass and peak shape are adjusted as part of the calibration to achieve mass calibration, peak shape standardization, and noise filtering, all in one operation. The mass spectrum after such calibration can be readily peak-analyzed with high mass accuracy. Table I below shows the calculated mass positions for all the ions included in the calibration from the calibration segment itself as well as a test segment towards the end of the 5min infusion run shown in above graph.

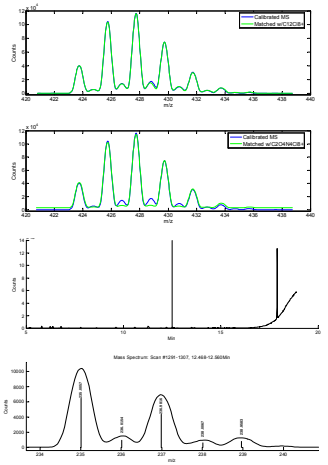
Table 1. Calibration Ions from PFTBA Standard and Calibration Mass Errors

Ions	Ion Formula	Exact Monoisotopic Mass (Da)	Calibration Scans #80-131		Test Scans #584-764	
			Calculated Mass (Da)	Mass Error (Da)	Calculated Mass (Da)	Mass Error (Da)
Frag #1	CF_3^+	68.9952	68.9952	0.0000	68.9943	-0.0009
Frag #2	$C_2F_3^+$	99.9936	99.9931	-0.0005	99.9922	-0.0014
Frag #3	$C_2F_4N^+$	113.9967	113.9965	-0.0002	113.9943	-0.0024
Frag #4	$C_3F_5^+$	118.9920	118.9919	-0.0001	118.9910	-0.0010
Frag #5	$C_3F_5^+$	130.9920	130.9915	-0.0005	130.9901	-0.0019
Frag #6	$C_3F_7^+$	168.9888	168.9887	-0.0001	168.9869	-0.0019
Frag #7	$C_4F_8^+$	218.9856	218.9858	0.0002	218.9847	-0.0009
Frag #8	$C_5F_{10}N^+$	263.9871	263.9870	-0.0001	263.9852	-0.0019
Frag #9	$C_7F_{10}N^+$	363.9807	363.9811	0.0004	363.9819	0.0012
Frag #10	$C_8F_{10}N^+$	413.9775	413.9778	0.0003	413.9761	-0.0014
Frag #11	$C_8F_{12}N^+$	463.9743	463.9746	0.0003	463.9732	-0.0011
Molecular Ion	$C_8F_{13}N^+$	501.9711	501.9713	0.0002	501.9699	-0.0012

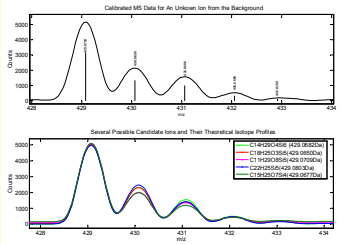


The calibration when applied to the calibration ions within the same standard run results in mass errors within 2.4mDa (Table 1), an unusually high mass accuracy for a single quadrupole mass spectrometer. Although the results in Table 1 show good mass accuracy on the calibration ions themselves over a 5 min time period, a more stringent test would be to apply this calibration to other MS scans containing different set of ions from another run, preferably on a true chromatographic time scale. The GC/MS analysis of the pesticide mixture will serve as a true test of mass spectral calibration, its applicability across different runs and on ions other than the calibration ions on a real chromatographic time scale. Above graph shows the accurate masses reported for the average of 8 mass spectral scans corresponding to the chromatographic elution profile of PCB 209. As can be seen, the reported accurate masses all come within 4mDa of the theoretical masses calculated from its elemental composition.

While this molecular ion is known and can be easily verified with certainty, the identification of some of its EI fragments will be more challenging and interesting. For the ion fragment around 424Da, the accurate mass for the monoisotopic mass is reported as 423.7428Da, an elemental composition search with C, H, N, O, and Cl as possible elements lists C12C8+ (exact mass at 423.7503Da) as the 17th candidate with -7.5mDa mass error. When the whole isotope cluster is taken into consideration, however, it becomes obvious that C12C8+ is the only correct ion formula for this fragment (shown in next graph), in spite of the somewhat larger measurement error on its monoisotopic mass.



A small chromatographic peak at 12.52 min in above graph is associated with an ion signal around 235Da, the accurate monoisotopic mass is reported as 235.0057Da based on the average of 7 scans. An elemental composition search based on this reported monisotopic mass with C, H, N, O, and Cl as possible elements lists C13H9Cl2+ (exact mass at 235.0081Da, or -2.3mDa mass error) as the 21st hit. The subsequent isotope profile matching reveals that this indeed is the only correct ion formula for a well known EI fragment of the pesticide p,p'-DDD.



To demonstrate the application of accurate mass measurement for the identification of compounds, a section towards the end of the GC/MS run shown in previous graph is averaged before accurate mass measurement to help identify possible GC column materials bleeding out of the system. Above graph (top) shows a section of the averaged mass spectrum that is correlated with the rise in TIC signal towards the end of the GC run. With the accurate masses identified, an elemental composition search with possible elements C, H, N, O, and Si combined with isotope profile matching reveals a few possible candidates with their theoretical isotope patterns shown in the above bottom graph. This list of possible candidates can be further refined based on the knowledge of column chemistry so that a good understanding of column bleeding may be gained, a subject of on-going research.

Conclusions

This application example demonstrates that the comprehensive mass spectral calibration involving both mass and peak shape is capable of achieving high mass accuracy on a single quadrupole GC/MS system at unit mass resolution. The calibration can be conveniently built with the on-board calibration standard through infusion measurement and is applicable to a real GC/MS run on a true chromatographic time scale. The mass shift due to such external calibration is within only a few mDa. The comprehensive calibration including the peak shape can greatly enhance the elemental composition search for the purpose of compound identification in GC/MS experiments.

Acknowledgement

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References

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