Technical Review

Accurate Mass Measurement on Real Chromatographic Time Scale with a Single Quadrupole Mass Spectrometer

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Abstract

On a unit mass resolution mass spectrometer such as a single quadrupole MS, it has been widely accepted that the mass (m/z) can only be measured to 0.1−0.5 Da accuracy, allowing for only rough mass confirmation for qualitative analysis. A novel mass spectral calibration technique, MSIntegrity™ implemented in MassWorks™, is introduced to comprehensively calibrate not only the mass axis but also the mass spectral peak shape function. With this comprehensive mass spectral calibration, a high level of mass accuracy on the level of 0.00x Da can be achieved even at unit mass resolution on a true chromatographic time scale, which will now enable the elemental composition determination of unknown ions or ion fragments using a conventional mass spectrometer hyphenated with chromatographic separation. This identification capability is further enhanced by a uniquely accurate approach for elemental composition determination through the use of isotope distribution information, CLIPS™.

Keywords: Accurate mass, mass spectrometry (MS), unit mass resolution, identification, elemental composition, isotope distribution, calibration

Introduction

Single quadrupole GC/MS has become a workhorse instrument in environmental and other applications due to its reliability, cost advantage, ease−of−use, versatility in terms of the types of compounds, high sensitivity, and even portability or at least transportability [1]. While sufficient for routine applications where a compound is known to belong to a given library, e.g., NIST MS library, these instruments are typically not used for unknown or new compound identification, due to their nominally unit mass resolution and lack of tandem MS capabilities.

As has been shown elsewhere [2−3], even at unit mass resolution, a high degree of mass accuracy can be achieved, making it possible to determine the elemental composition of unknown ions or fragments and greatly facilitate the identification of metabolites or other compounds. Elemental composition search for the purpose of compound identification is a capability typically reserved for higher resolution systems such as qTOF or FTMS at a much higher cost with a larger instrument footprint [4−6]. In order to achieve the necessary high mass accuracy at the conventional unit mass resolution, a more elaborate and comprehensive mass spectral calibration has to be performed outside the commercially available instrument systems at the present. Fortunately for GC/MS applications, such an elaborate calibration is quite feasible by the readily available on−board calibration standard, e.g., perfluorotributylamine (PFTBA), typically software−controllable through a valve. Furthermore, with Electron Impact (EI) ionization widely available on GC/MS systems, a molecular ion in many cases is fragmented into quite a few observable fragment ions which can also be measured with high mass accuracy, providing additional information for the accurate identification of the molecular ion and

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the elucidation of its possible structures.

This short paper will demonstrate such high mass accuracy measurement using Agilent 5973 N−*inert* MSD for the identification of pesticides through both their molecular ions when available and their fragment ions, to greatly enhance the capabilities of a conventional single quadrupole MS system, which lacks the tandem MS capability and the high resolving power otherwise required of highly confident identification work.

Experimental

Sample information: Calibration standard PFTBA and a 17−compound organochlorine pesticide standard (1 ng/ul) mixture also containing approximately 50 ng/ul PCB 209 (decachlorobiphenyl, $C_{12}Cl_{10}$).

MS conditions: the PFTBA and the mixture were acquired in "raw" mode (non−peak detected) at a scan speed 2ˆ2 (A/D samples = 4) over a mass range of 50−550 *m/z*.

Data acquisition and analysis: Figure 1 shows the general flow of the data processing. The profile mode mass spectra of the PFTBA calibration standard were acquired continuously for 5 min during the infusion process while the control valve was at ON position. Similarly, during the GC/MS analysis of the sample mixture, the profile mode mass spectral scans were repeatedly collected during the GC separation process for a total runtime of 19 min. An elaborate and comprehensive mass spectral calibration can be created from the average of the PFTBA mass spectral scans within a given time window using the MassWorks™ software from Cerno Bioscience. This unique calibration process calibrates both the mass and the mass spectral peak shape function, the key for achieving high mass accuracy. This calibration function 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 mass values. Peak detection can then be applied to reliably and accurately calculate the mass locations for molecular ions/their fragment ions for the purpose of compound identification by searching within a small mass tolerance window for a list of possible formulas. This list of possible formulas can be further refined and greatly shortened through the Calibrated Line−shape Isotope Profile Search or CLIPS™, also available in MassWorks, which utilizes whole isotope profiles to determine an elemental composition, a highly selective capability made uniquely possible by the comprehensive MS calibration performed [7].

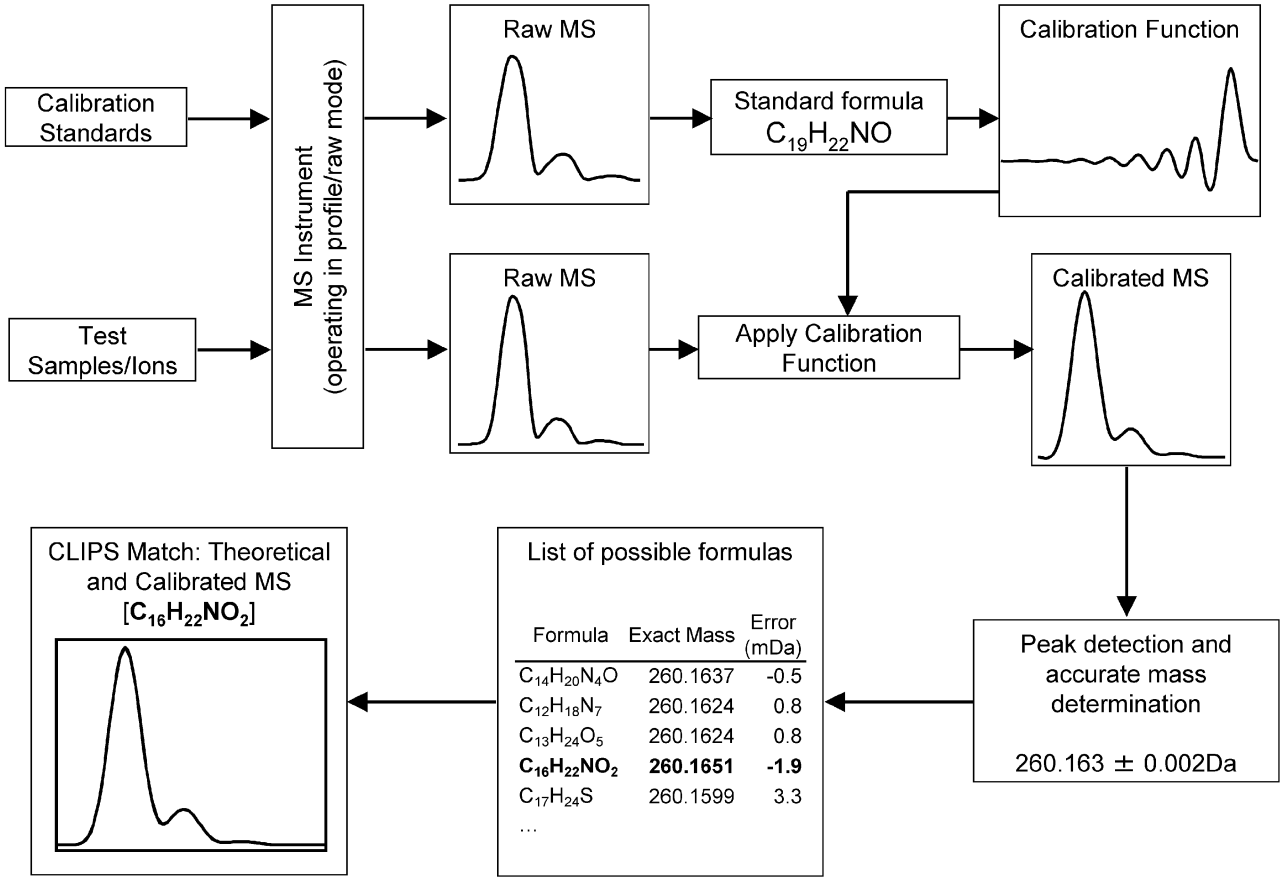

Figure 1. The general flow of MassWorks calibration and its elemental composition determination process

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

Ions	Ion Formula	Exact Monoisotopic Mass (Da)	Calibration Scans #80−131		Test Scans #684−764	
			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	$C_2F_4^+$	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	$C_2F_5^+$	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	$C_4F_9^+$	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	$C_7F_{14}N^+$	363.9807	363.9811	0.0004	363.9819	0.0012
Frag #10	$C_8F_{16}N^+$	413.9775	413.9778	0.0003	413.9761	−0.0014
Frag #11	$C_9F_{18}N^+$	463.9743	463.9746	0.0003	463.9732	−0.0011
Molecular Ion	$C_9F_{20}N^+$	501.9711	501.9713	0.0002	501.9699	−0.0012

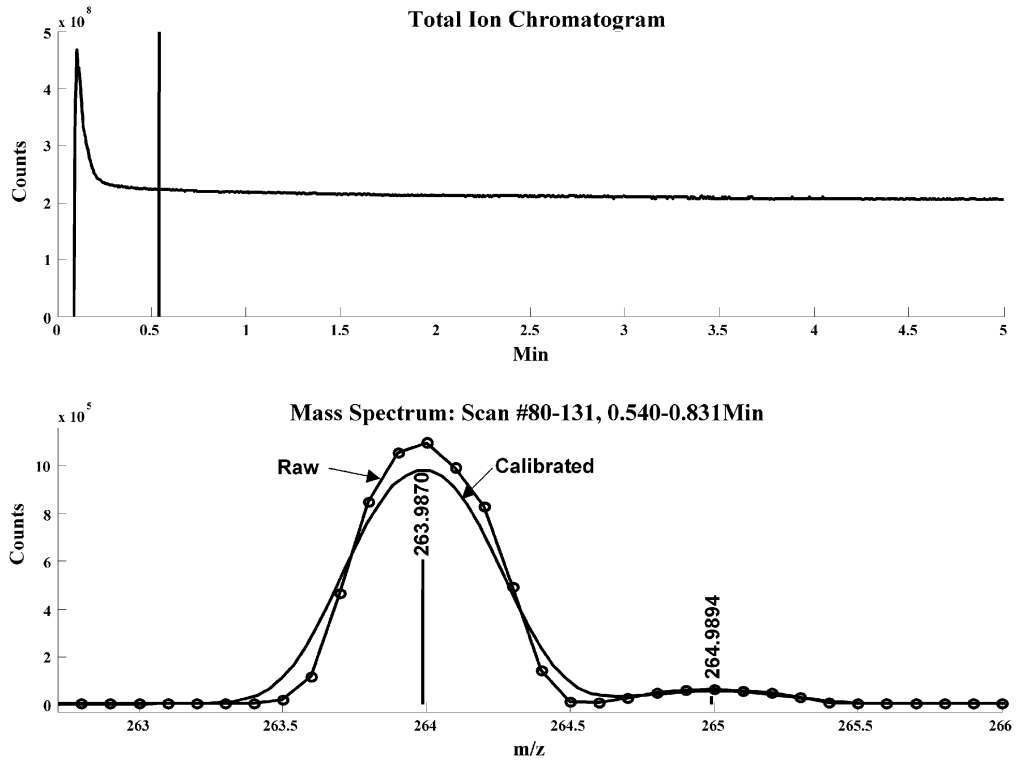

Figure 2. The raw and the calibrated mass spectrum for one of the calibration ions

Results and Discussion

Twelve ions including the molecular ion of PFTBA are selected for the comprehensive MassWorks calibration. Their elemental compositions and theoretically calculated exact masses are listed in Table 1. The average of scans 80−131 is used to build the calibration, which transforms the raw mass spectral scan into a fully calibrated mass spectral scan, both of which are shown in Figure 2 for one of the calibration ions. The calibration thus built can be applied to all the scans to check for the mass accuracy within this run itself. Once a mass spectral scan has been fully calibrated, mass spectral peaks can be accurately determined even for this unit mass resolution data. Table 1 lists the calculated masses and mass errors for all 12 calibration ions for the calibration scans (acquired early in the run) as well as for test scans (acquired later in the run). It can be seen that the calibration mass errors are all within 0.5 mDa whereas the test mass errors are all within 2.4 mDa.

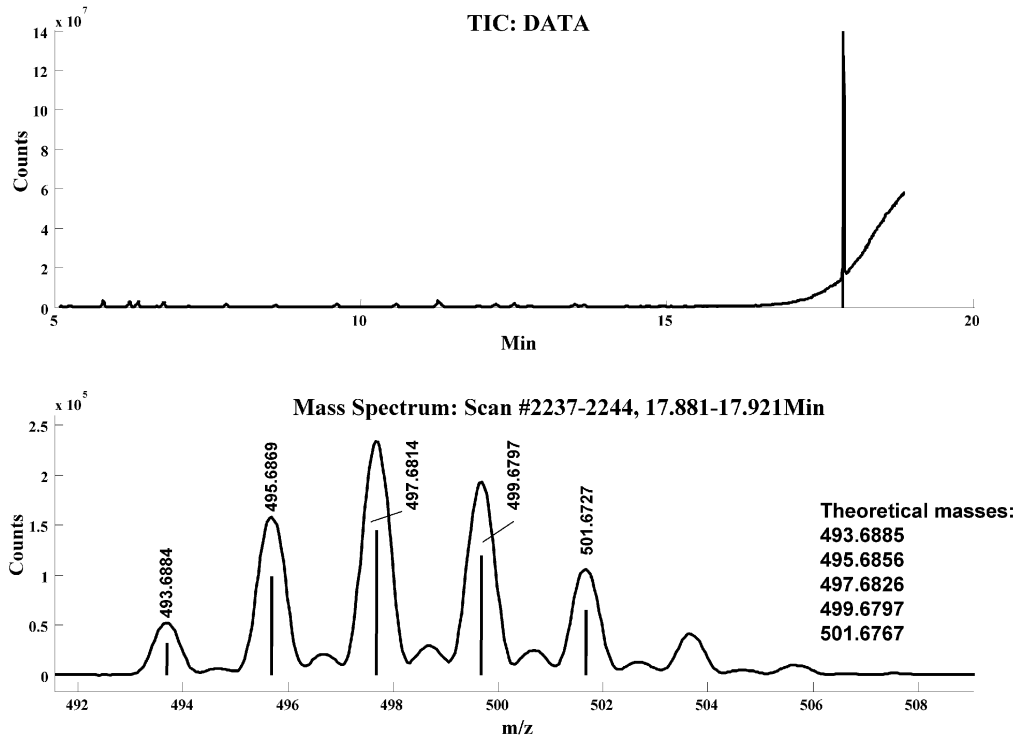

Figure 3. The calibrated mass spectrum for PCB 209 and the accurate masses reported for its five most intense isotopes

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 from a different 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. Figure 3 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 4 mDa 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 interesting. For the ion fragment around 424 Da, the accurate mass for the monoisotopic mass is reported as 423.7428 Da, an elemental composition search with C, H, N, O, and Cl as possible elements lists $C_{12}C_8^+$ (exact mass at 423.7503 Da) as the 17th candidate with −7.5 mDa mass error. When the whole isotope distribution is taken into consideration in CLIPS match, however, $C_{12}C_8^+$ becomes the top hit on the list and is indeed the only correct ion formula for this fragment (Figure 4), in spite of its somewhat larger mass measurement error.

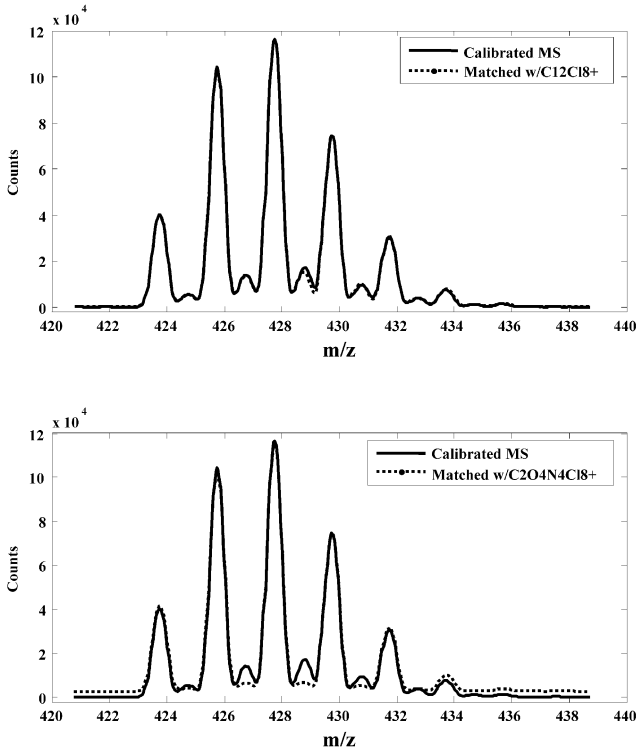

Figure 4. Isotope matching for the 17 th (top) and the 1 st (bottom) hit from the elemental composition search based only on monoisotope masses

A small chromatographic peak at 12.52 min in Figure 5 is associated with a strong ion signal around 235 Da, the accurate monoisotopic mass is reported as 235.0057 Da based on the average of 7 scans. An elemental composition search based on this re-

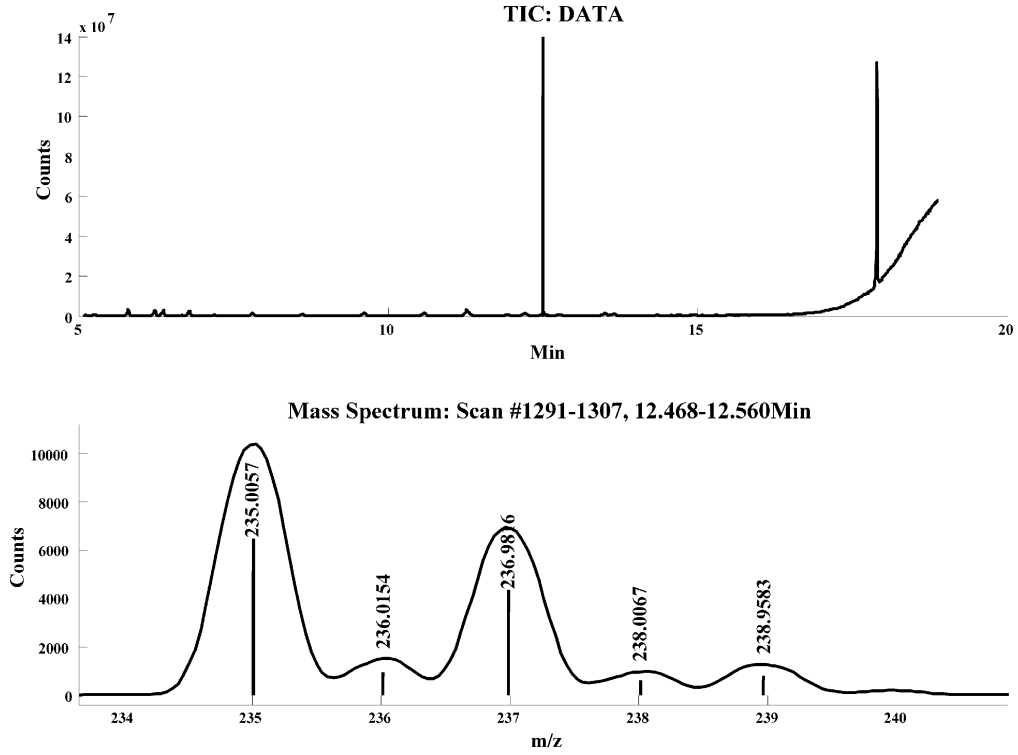

Figure 5. The accurate mass measurement for an EI fragment of pesticide p,p'−DDD

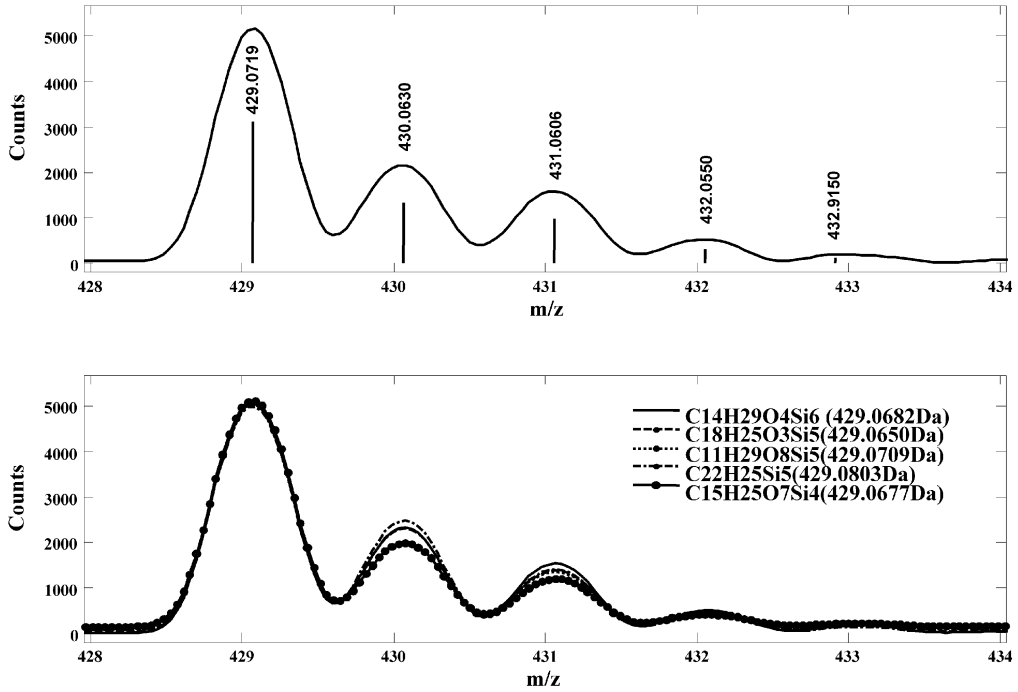

Figure 6. The ion isotope pattern related to the rise in total ion signal towards the end of a GC/MS run (top) and the theoretically calculated isotope patterns of five possible candidate ions (bottom)

ported monisotopic mass with C, H, N, O, and Cl as possible elements lists $C_{13}H_9Cl_2^+$ (exact mass at 235.0081 Da, or −2.4 mDa mass error) as the 21st hit. The subsequent refinement through CLIPS match moves it to the top of the list, revealing 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 unknown compounds, a section corre-

sponding to the rise in TIC towards the end of the GC/MS run shown in Figure 5 is averaged before accurate mass measurement to help identify possible GC column materials bleeding out of the system. Figure 6 (top) shows a section of the averaged mass spectrum that is correlated with the rise in TIC signal. With the accurate masses identified, an elemental composition search with possible elements C, H, N, O, and Si combined with CLIPS reveals a few possible candidates with their theoretical isotope patterns shown in Figure 6 (bottom). This list of possible candidates can be further refined based on the knowledge of column chemistry to improve the understanding of column bleeding.

Conclusions

This application example demonstrates that the comprehensive and elaborate 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 combined with CLIPS can greatly enhance the elemental composition determination for the purpose of truly unknown compound identification from GC/MS experiments.

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