Cerno Application Note

Extending the Limits of Mass Spectrometry

Accurate Mass Compound Identification with Single Quadrupole GC/MS

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Single Quad GC/MS instruments are the workhorses of the environmental lab. They normally fall short for applications that require high mass accuracy. It is shown here that with proper calibration techniques, these instruments can indeed readily obtain high mass accuracies to within a few mDa and become powerful tools for unknown compound identification.

Introduction

Single quadrupole GC/MS have become workhorse instruments for environmental applications due to there reliability, cost advantage, ease-of-use, versatility in terms of the types of compounds, high sensitivity, and even portability or at least transportability. While sufficient for routine applications where a compound is known to belong to a given library (such as the 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[1,2], even at unit mass resolution, a high degree of mass accuracy can be achieved, making it possible for elemental composition search. 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 and with a larger instrument footprint. In order to achieve the necessary high mass accuracy, however, a more elaborate mass spectral calibration has to be performed outside of commercially available instrument systems at the present. Fortunately for GC/MS applications, such a calibration is greatly facilitated by the readily available on-board calibration standard, perfluorotributylamine (PFTBA), which can be software-controlled through a valve. Furthermore, with Electron Impact (EI) ionization typically 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 compound identification.

This application note will demonstrate the high mass accuracy measurement using the Agilent 5973N-*inert* MSD for the identification of pesticides through both their molecular ions, when available, and their fragment ions.

Experimental

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

MS conditions: the PFTBA and standard were acquired 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*.

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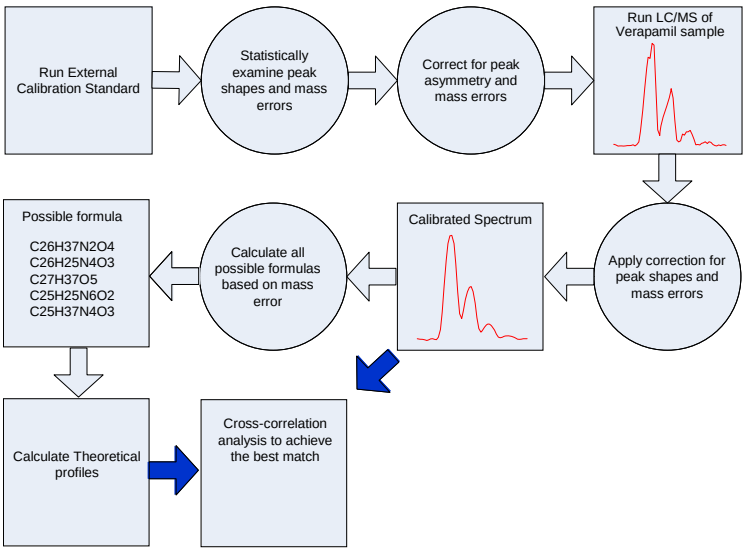

Figure 1. Data processing work flow for profile searching

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 the ON position. 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[3]. 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 mass values. 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[4], through the proposed elemental compositions.

Results

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 calibration 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 0.5mDa whereas the test mass errors are all within 2.4mDa.

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

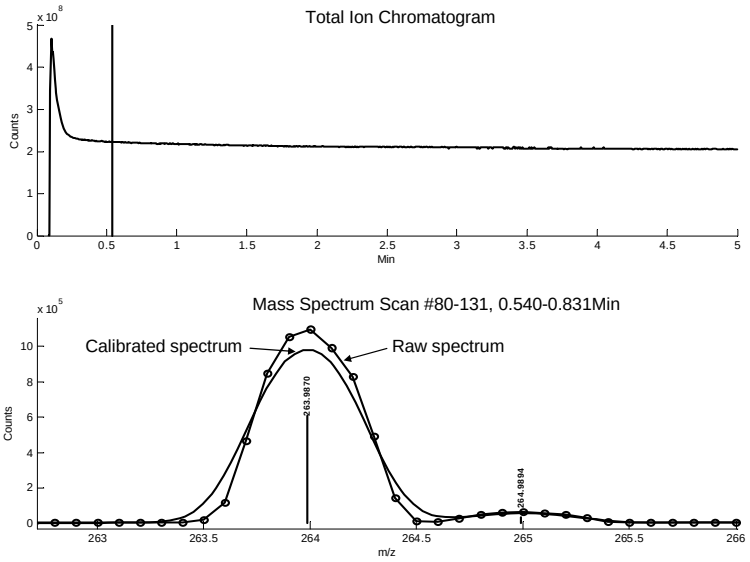

Figure 2. The raw and the calibrated mass spectrum for one of the calibration ions

Ions	Ion Formula	Exact Monoisotopic Mass (Da)	Calibration Scans		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	$C_9F_{20}N^+$	501.9711	501.9713	0.0002	501.9699	-0.0012

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

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 4mDa of the theoretical masses calculated from its elemental composition.

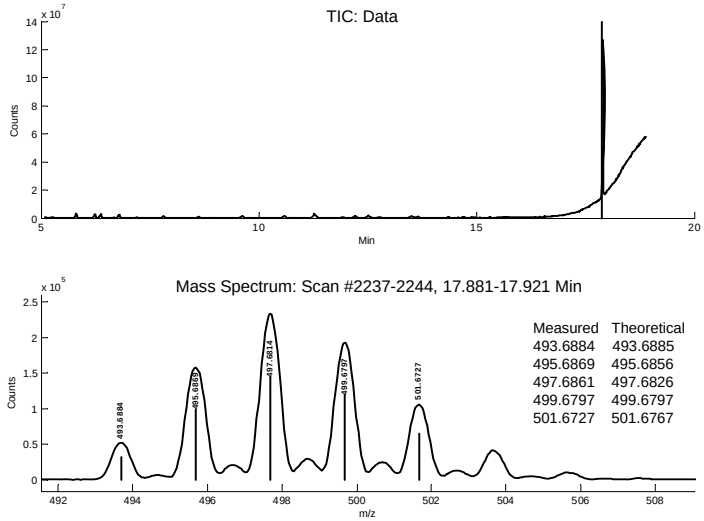

Figure 3. The calibrated mass spectrum for PCB 209 and the accurate masses reported for its five most intense isotopes.

While this molecular ion is known and can be easily verified with certainty, the identification of some of its EI fragments is more 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 $C_{12}C_8^+$ (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 $C_{12}C_8^+$ is the only correct ion formula for this fragment (Figure 4), in spite of its somewhat larger mass measurement error.

A small chromatographic peak at 12.52 min in Figure 5 is associated with a strong 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 $C_{13}H_9Cl_2^+$ (exact mass at 235.0081Da, or -2.3mDa

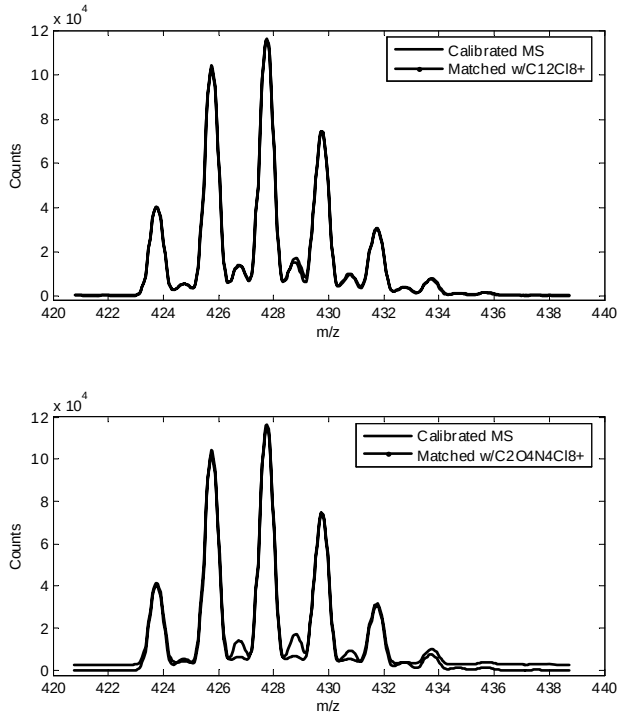

Figure 4. Isotope matching for the 1st (top) and 17th (bottom) hit from the elemental composition search on monoisotope masses.

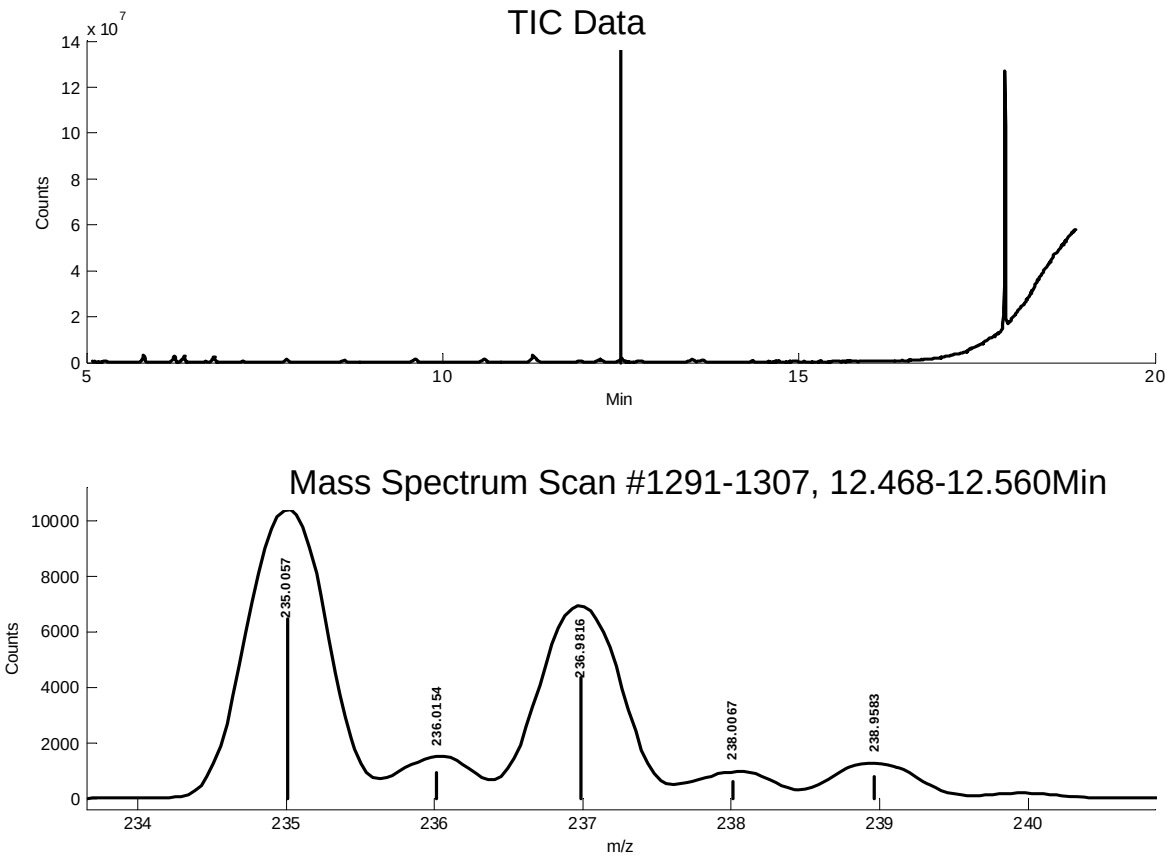

Figure 5. The accurate mass measurement for an EI fragment of pesticide p,p'-DDD

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 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 total ion signal. 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 Figure 6 (bottom). This list of possible candidates cn be further refined based on the knowledge of column chemistry to further the understanding of column bleeding.

Conclusion

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 using the external calibration

is accurate within 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.

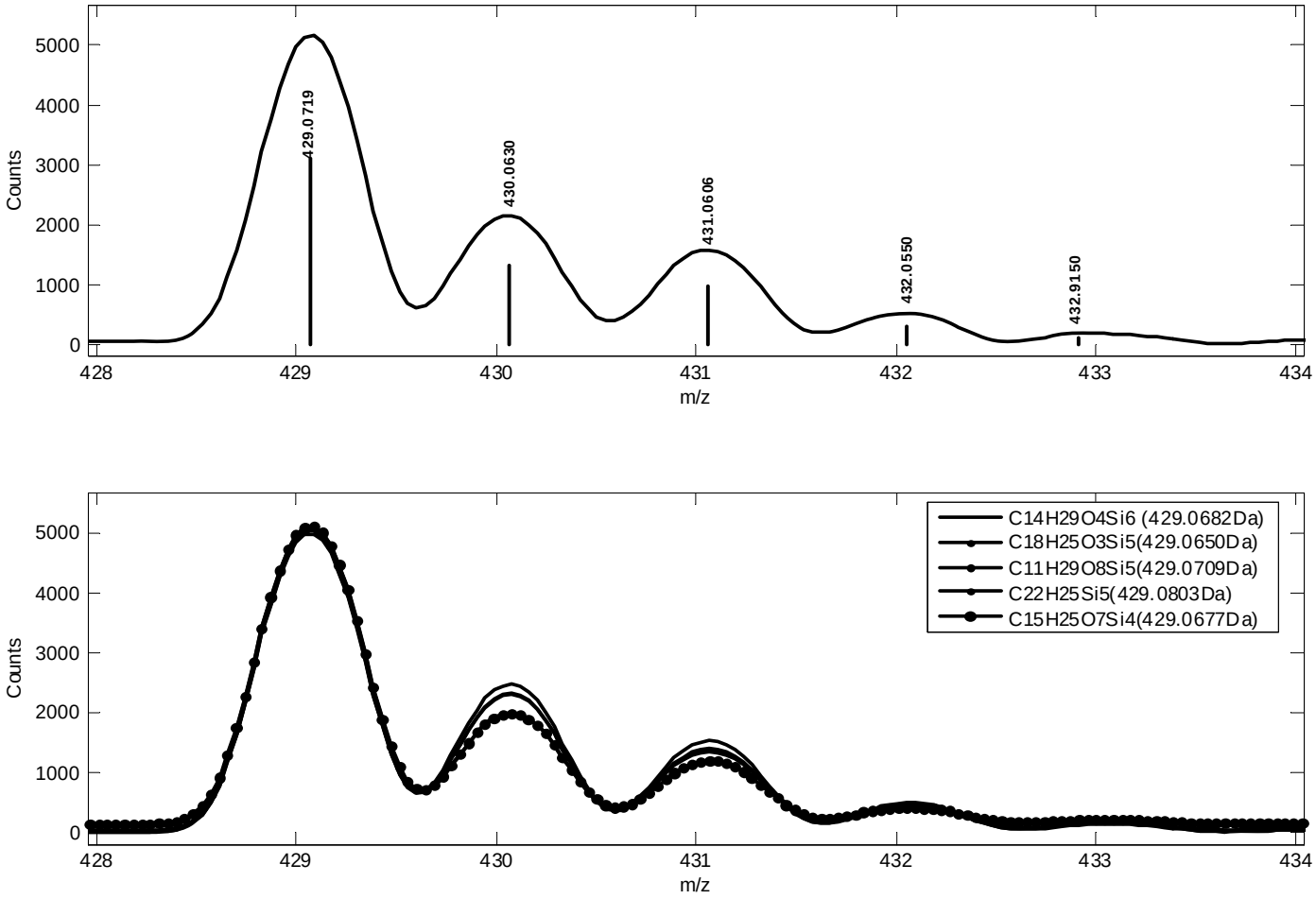

Figure 6. The ion isotope pattern related to the rise in total ion signal towards the end of a GC/MS run (top) and the theoretically calculated isotope patterns of five possible candidate ions (bottom)

[1]Gu M, Wang Y, Kuehl D. *Spectroscopy*, May, 2005.

[2]Gu M, Wang Y, Zhao X, Gu Z, Rapid Commun. Mass Spectrom. 2006; 20: 764–770.

[3]Cerno Bioscience, Danbury, CT 06810, USA

[4]Cerno Bioscience Application Note #101, May, 2006