

Using Single Quadrupole GC/MS to Identify Compounds with Accurate Mass in Environmental Laboratories

ENVIRONMENTAL
ANALYSIS

By Yongdong Wang, Cerno Bioscience, Danbury, CT 06810, USA and Harry Prest, Agilent Technologies, Santa Clara, CA 95051, USA

With proper calibration techniques, Single Quad GC/MS instruments can become powerful tools for unknown compound identification in environmental labs. As shown here, these instruments which would normally fall short for applications requiring high mass accuracy, can readily obtain high mass accuracies to within a few mDa.

Introduction

Though sufficient for routine applications where a compound is known to belong to a given library (such as the NIST MS library), single quadrupole GC/MS are typically not used for unknown or new compound identification, due to their nominal unit mass resolution and lack of tandem MS capabilities. As this experiment will show, by combining novel calibration techniques with sound mathematical principles, significant improvements can be made to MS data. With proper calibration techniques, these workhorse instruments which are readily used for environmental applications, due to their reliability, cost advantage, ease-of-use, versatility for use with various types of compounds, high sensitivity, and even portability or at least transportability, are expanded, enhanced, and greatly extended.

For the purpose of compound identification, elemental composition determination is a capability typically reserved for higher resolution systems such as qTOF or FTMS with a larger instrument footprint and at a much higher cost. At present, in order to achieve the necessary high mass accuracy, a more elaborate mass spectral calibration has to be performed outside of commercially available instrument systems. Fortunately for GC/MS applications, through a software-controlled valve, such a calibration is greatly facilitated by the readily available on-board calibration standard, perfluorotributylamine (PFTBA). Furthermore, additional information for the compound identification 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. As previously demonstrated[1,2,] even at unit mass resolution, a high degree of mass accuracy can be achieved, making it possible for elemental composition determination.

Using the Agilent 5973N-inert MSD for the identification of pesticides through both their molecular ions, when available, and their fragment ions, this application note will demonstrate the high mass accuracy measurement achievable with this otherwise routine instrumentation.

Experimental

Sample information: Calibration standard PFTBA and a 17-compound organochlorine pesticide standard (1ng/ul) also containing approximately 50 ng/ul PCB 209 (decachlorobiphenyl, C12Cl10).

MS conditions: the PFTBA and standard were acquired in "raw" mode (non-peak detected) at a scan speed of 2² (A/D samples = 4) over a mass range of 50-550 m/z.

Data acquisition and analysis: The general flow of the data processing is shown in Figure 1. The mass spectra of the PFTBA calibration standard were acquired in profile mode continuously for five minutes during the infusion process while the control valve was at the ON position. The profile mode mass spectral scans were repeatedly collected during the GC separation process similarly, during the GC/MS sample analysis, for a total runtime of 19 minutes. 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]. Key for achieving high mass accuracy, this unique calibration process calibrates both the mass position and the mass spectral peak shape function. This calibration was then applied to each scan 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 highly 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 determined elemental compositions.

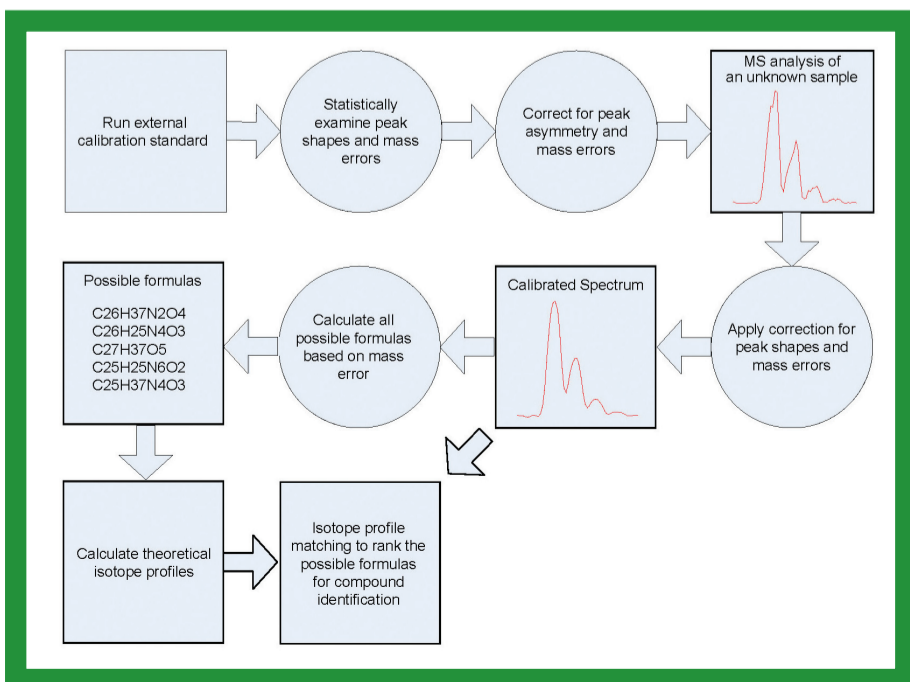


Figure 1. The general flow of MassWorks calibration and its MS analysis process

Results

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 ₂ F ₄ ⁺	99.9936	99.9931	-0.0005	99.9922	-0.0014
Frag #3	C ₂ F ₄ N ⁺	113.9967	113.9965	-0.0002	113.9943	-0.0024
Frag #4	C ₂ F ₅ ⁺	118.9920	118.9919	-0.0001	118.9910	-0.0010
Frag #5	C ₃ F ₅ ⁺	130.9920	130.9915	-0.0005	130.9901	-0.0019
Frag #6	C ₃ F ₇ ⁺	168.9888	168.9887	-0.0001	168.9869	-0.0019
Frag #7	C ₄ F ₉ ⁺	218.9856	218.9858	0.0002	218.9847	-0.0009
Frag #8	C ₅ F ₁₀ N ⁺	263.9871	263.9870	-0.0001	263.9852	-0.0019
Frag #9	C ₇ F ₁₄ N ⁺	363.9807	363.9811	0.0004	363.9819	0.0012
Frag #10	C ₈ F ₁₈ N ⁺	413.9775	413.9778	0.0003	413.9761	-0.0014
Frag #11	C ₉ F ₁₈ N ⁺	463.9743	463.9746	0.0003	463.9732	-0.0011
Molecular Ion	C ₉ F ₂₀ N ⁺	501.9711	501.9713	0.0002	501.9699	-0.0012

Including the molecular ion of PFTBA, twelve ions are selected for the comprehensive MassWorks calibration. Their theoretically calculated exact masses and elemental compositions 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 test mass errors are all within 2.4mDa whereas calibration mass errors are all within 0.5mDa.

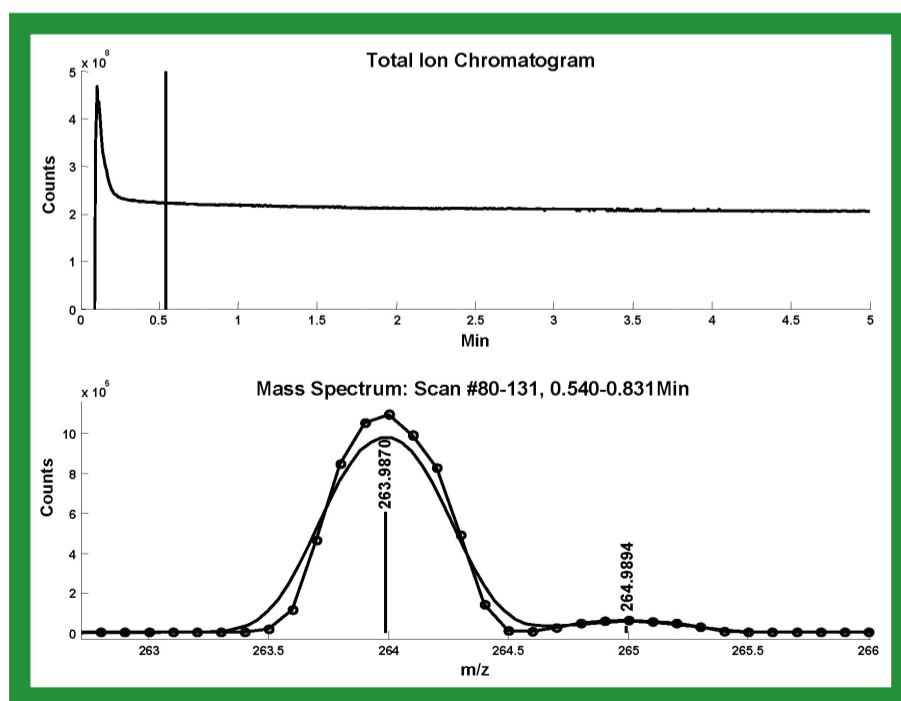


Figure 2. The raw and the calibrated mass spectrum for one of the calibration ions

The results in Table 1 show good mass accuracy on the calibration ions themselves over a five minute time period, although 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. As a true test of mass spectral calibration, the GC/MS analysis of the pesticide mixture will present 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 eight 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 elemental compositions.