Automatic and Seamless Calibration of a Quadrupole GC/MS for High Mass Accuracy and High Spectral Accuracy

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Overview

➤A software approach to automatic and seamless calibration for an Agilent single quadrupole GC/MS system for high mass accuracy and high spectral accuracy is described.

➤Formula determination by MassWorks can be carried out through ChemStation and has much simplified workflow by AutoCal.

➤The automatic calibration and formula ID is demonstrated by a blind test example.
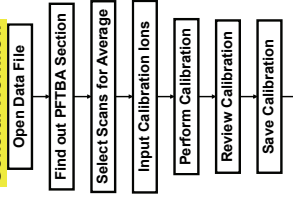
Introduction

During a comprehensive MS calibration, not only the m/z but also the MS peak shape is calibrated, resulting in a mathematically defined and symmetrical MS peak shape (1). Though such calibration has been shown to provide high enough mass accuracy and spectral accuracy on a quadrupole GC/MS system to enable elemental composition determination, it does involve an elaborate calibration procedure, requiring the introduction and measurement of calibration standards with known elemental compositions followed by the introduction and measurement of unknown samples. This paper describes a fully automated calibration scheme and the associated software to perform a seamless calibration behind the scene so that high mass accuracy and high spectral accuracy can be expected for all unknown samples at all times.

Methods

➤**Sample information**: Calibration standard PFTBA available as tune compound from the MSD instrument and a prepared sample containing an unknown peak at RT=8.7min.

➤**MS conditions**: the PFTBA and standard were acquired in "raw scan" mode (non-peak detected) at a scan speed 2^2 (A/D samples = 4) over a mass range of 40-550 m/z with ion threshold set to zero so as to acquire full continuum raw mass spectral data. As is typical for MSD, the MS source is heated to 230°C and the quadrupole is heated to 150°C, conditions shown to provide high calibration stability lasting as long as a week without requiring recalibration, based on a previous study on the system (2).

➤**Unknown GC Separation and Internal PFTBA Calibration**: After the 12min GC run, during which the unknown compound elutes at RT=8.7min, the EI Calibration Valve was programmed to be On at RT=12min and Off at RT=13.5min while the EMV Delta was set to minus 400V for the PFTBA data acquisition so as to avoid calibration ion signal saturation.

➤**Data acquisition**: The profile mode mass spectral scans were repeatedly collected for a total GC runtime of 12min. Similarly, the profile mode mass spectra of the PFTBA calibration standard were acquired immediately after the 12min GC runtime while the control valve was set at ON position and GC oven was programmed to Hold. Figure 3 shows both the TIC (top) and the averaged PFTBA mass spectrum (bottom) for the given Rt window marked up in the TIC.

➤**Data Analysis**: A comprehensive mass spectral calibration was conducted behind the scene automatically. It was created from the average of the PFTBA mass spectral scans within this time window using the MassWorks™ software from Cerno Bioscience and all available PFTBA (fragment) ions of sufficient abundances to cover the mass range of interest (40-550Da).

Results and Discussion

General Workflow

- Open Data File
- Find out PFTBA Section
- Select Scans for Average
- Input Calibration Ions
- Perform Calibration
- Review Calibration
- Save Calibration

Many Manual Operations

- Apply Calibration to a Data File
- Obtain Accurate Mass
- Perform CLIPS Search

Workflow with AutoCal

- Open Data File

AutoCal

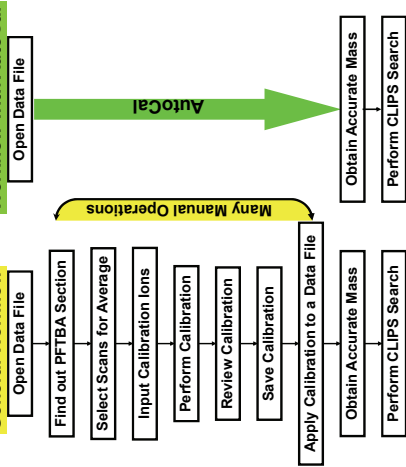- Obtain Accurate Mass
- Perform CLIPS Search

Figure 1. Simplified Workflow by AutoCal for Automatic Identification of Unknowns with a Single Quadrupole MS

The implementation of current MassWorks requires quite a few manual operations in the creation of the comprehensive MS calibration as described in Figure 1. It is desirable to automate the calibration procedures such that the high mass accuracy and high spectral accuracy can be obtained for formula determination as soon as the data files are opened. Through AutoCal, the underline technology to carry out the automation, the comprehensive calibration can be generated effortlessly as if it was performed at a firmware level. More importantly, the effective communication between MassWorks and ChemStation allows end users to launch AutoCal from ChemStation through its menu as shown in Figure 2. This is demonstrated through an analysis of a forensic sample where the elemental composition of an unknown was determined by both high mass accuracy and high spectral accuracy.

AutoCal first performed extracted ion chromatograms (XIC) for m/z 69 and 219, two fragments from the calibration compound of PFTBA, as shown in an insert of Figure 3. From the XIC, the scan numbers of the PFTBA spectra can be readily found and allow AutoCal to automatically take averaged spectra as highlighted in the insert for the calibration.

The comprehensive MassWorks calibration employed ten ions including the molecular ion of PFTBA and its fragments. Their elemental compositions and theoretically calculated exact masses are listed in Figure 4 as part of the MassWorks calibration report. The average of scans 1377-1593 (RT=12.11-13.37min) is used to build the calibration, which transforms a raw mass spectrum into a fully calibration mass spectrum, both of which are shown in Figure 4 as overlays for one of the calibration ions, C5F10N (m/z 263.9871). Once a mass spectral scan has been fully calibrated to have a known peak shape, the mass spectral peaks can be accurately determined even at unit mass resolution.
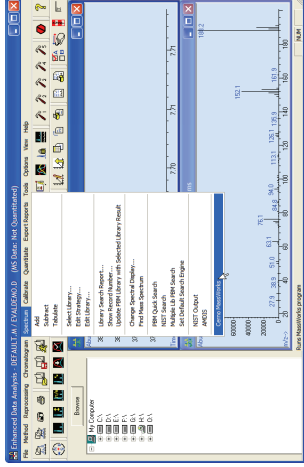

Figure 2. Access to MassWorks from ChemStation Menu

The unknown peak for the blind test eluted at RT = 8.7min and its averaged spectrum after applying the calibration is shown in Figure 5 for the largest ions at m/z 240. Since the ions are not known whether they are molecular ions or fragments, a universal CLIPS formula ID parameters with both even and odd electrons are used (Figure 6). A CLIPS formula determination on the 240Da ions resulted in 155 possible formulas, out of which C4H8N4O4S2+ is the top candidate with 98.8% Spectral Accuracy. If higher than 99% Spectral Accuracy is desired in order to better differentiate the correct formula candidate from the others on the list, a shorter mass spectral scan range could be used to increase the available signal to noise and therefore the Spectral Accuracy on this ion. This ion formula provides a nearly perfect match between the calibrated and the theoretical mass spectrum (Figure 5). At the conclusion of this blind test, it was confirmed that C4H8N4O4S2 is indeed the correct molecular formula of a highly toxic rodenticide, tetramethylenedisulfotetramine (TETS, DSTA, also called tetramine).
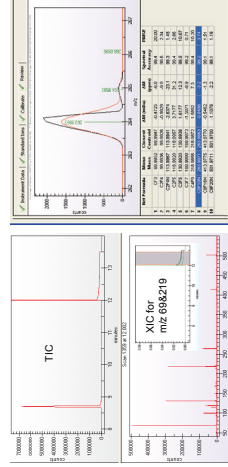

Figure 3. TIC (top) and the average PFTBA calibration spectrum (bottom) obtained from XIC for m/z 698219 (insert) from a single GC/MS acquisition.
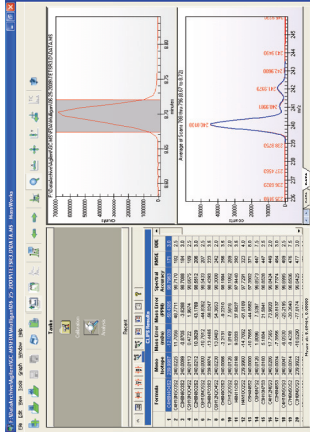

Figure 4. The raw (black) and the calibrated (red) mass spectra for the PFTBA calibration fragment ion C5F10N (exact mass 263.9871Da)

Figure 5 (above). Averaged MS (Red) for the largest ions from the unknown at RT=8.7min and the theoretical MS for C4H8N4O4S2 (Blue).

Figure 6 (left). MassWorks CLIPS formula ID parameters.

Conclusions

➤Automatic MS calibration for an Agilent single quadrupole GC/MS without user intervention to achieve high mass accuracy and high spectral accuracy for formula determination is demonstrated through a behind test. Both automatic calibration and the calibration by hand accomplished the same results (3).

➤By eliminating all manual operations for the calibration, AutoCal working behind the scene provides a calibration virtually equivalent to the calibration achieved at a firmware level. The end users now can have high mass accuracy and high spectral accuracy once the data files are opened.

➤Future work will include NIST library search utilizing high mass accuracy achieved by the quadrupole GC/MS for unknown identification.

References

(1) Y. Wang, M. Gu, The Concept of Spectral Accuracy for MS. Anal. Chem. 2010, 82, 7055.
(2) Y. Wang, M. Gu. Spectroscopy (MS Supplement), May, 2008.
(3) Application Notes 107, Cerno Bioscience