Cerno Application Note

Extending the Limits of Mass Spectrometry

AutoCal™:
Fully Automated Calibration for Accurate Mass and High Spectral Accuracy

The unique comprehensive mass spectral calibration approach published in a front cover feature article in Analytical Chemistry [1] and implemented in the award winning MassWorks™ software provides up to 100 times improvement in mass measurement accuracy on a conventional quadrupole mass spectrometer system, e.g., Agilent GC/MSD. By combining the mass measurement accuracy of mDa mass error (standard deviation, at 50:1 signal to noise ratio) and the high spectral accuracy of more than 97.0%, elemental composition determination can be achieved on these mass spectrometer systems on real chromatographic time scale.

The elaborate mass spectral calibration process typically involves selecting appropriate calibration standards of sufficient ion abundance to cover the m/z range of interest, acquiring raw profile mode data with zero ion threshold from the calibration standards under the same mass spectral operating conditions (typically contained in the MS tune file such as starting mass, ending mass, scan rate etc.) as the unknown sample, manually performing the MassWorks calibration process to create a calibration file to be saved, and applying the calibration file thus created to the sample data for analysis. While this process is neither complicated nor difficult, it does require a multi-step manual process and some basic user training.

Fortunately for all commercial GC/MS instruments operating in EI+ mode, the selection and operation of a calibration standard have been elegantly addressed through the readily available perfluorotributylamine (PFTBA) calibration gas carried on board the instrument for MS tuning purpose. Furthermore, on some systems such as GC/MSD, the acquisition of PFTBA mass spectrum is fully programmable as part of the GC and MS method setup [2]. It is considered the most ideal to have the PFTBA acquired at the end of a GC run after the GC oven has been cooled down to the starting temperature when temperature programming is used for GC separation. This not only minimizes possible interferences from GC column bleeding but also ensures that the standard data and sample data be all contained within the same GC/MS run, greatly simplifying the data management and tracking. Even though some MS systems have been shown to be stable from one to three weeks at a time [3-4], without requiring PFTBA recalibration for each run, the acquisition of PFTBA inside a GC/MS run comes with very little cost of either time or effort.

In the soon-to-be-released version 4.0 of the MassWorks software, a brand new feature called AutoCal™ has been introduced to automatically search for the presence of PFTBA calibration data inside a GC/MS run acquired with MSD, average the relevant mass spectra for PFTBA, select the PFTBA standard ions within the acquired m/z range, perform the actual calibration, inspect the calibration report for possible calibration issues, save the calibration file successfully created, and finally applying the calibration file to the acquired GC/MS data so that accurate mass can be obtained seamlessly without any user intervention. This AutoCal process can be launched either through a MassWorks menu added to the ChemStation software (Figure 1) or within MassWorks upon opening a GC/MSD data file (Figure 2). This represents Cerno Bioscience's first effort at making MassWorks easy to use so that accurate mass and high spectral accuracy can be obtained fully automatically through a single push button operation.

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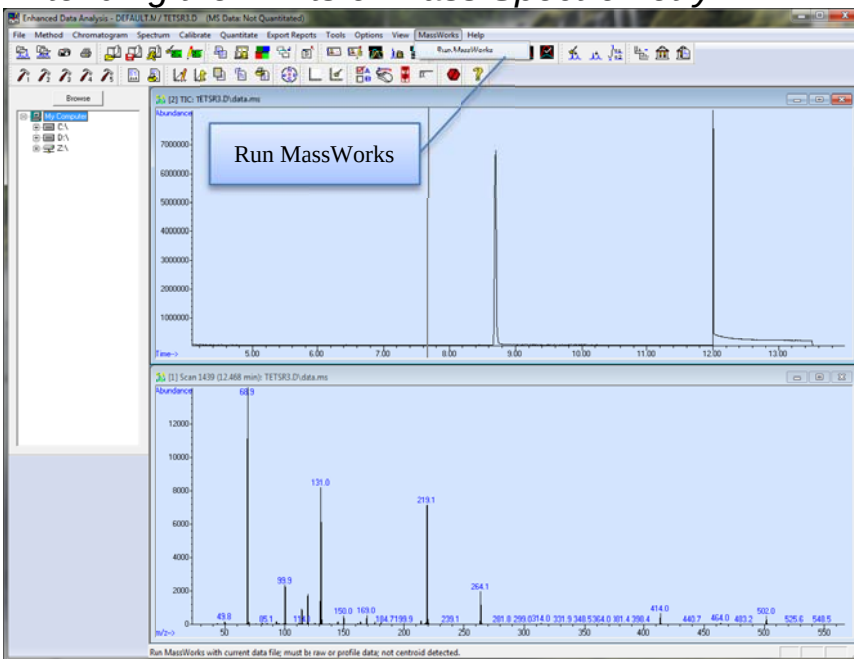

Figure 1. MassWorks menu button added to ChemStation to launch MassWorks from within ChemStation and perform AutoCal in a single operation

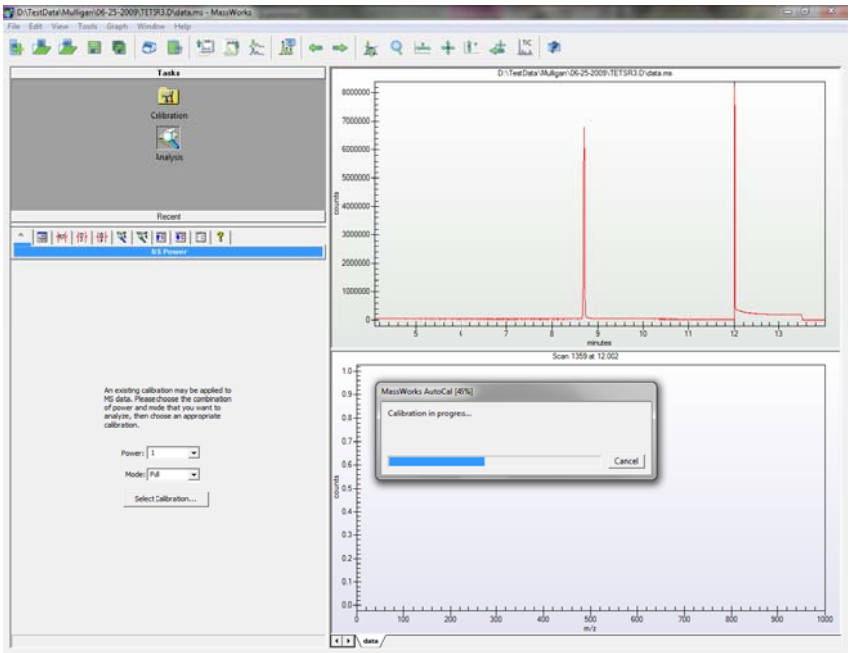

Figure 2. MassWorks detecting a GC/MSD data file containing PFTBA data and starting AutoCal process to perform all steps of calibration and provide accurate mass and high spectral accuracy results without any user intervention

References

[1] Wang, Y.; Gu, M. *Anal. Chem.* **2010**, *82*, 7055-7062.
[2] Prest, H.; Wang, Y. Agilent Application Note 5990-4966EN, *November*, 2009.
[3] Wang, Y.; Gu, M. *Spectroscopy (MS Supplement).* **2008**, *May*, 25-29.
[4] Mullis, J.; Qiu, F.; Wang, Y. The Robustness of Formula Determination on a Single Quadrupole GC/MS, *Proceedings of 55th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO, June 1-55*, **2008**.

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