Cerno Bioscience MassWorks: Acquiring Calibration Data on Agilent GC/MSDs

Application Note

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Introduction

Cerno Bioscience MassWorks is a post-acquisition software solution for extending mass accuracy beyond the conventional limits and adding spectral accuracy to qualitative MS analysis. This innovative calibration algorithm [1] can extend mass assignment confidence and suggest probable empirical molecular formulae for either molecular or fragment ions to aid in compound identification [1,2]. To apply this approach, the data must be acquired in a suitable format and with associated calibration. This document presents a summary of how to collect the necessary "internal" and "external" calibration data in electron impact ionization (EI) mode on an Agilent GC/MSD using the MSD Productivity ChemStation software (ChemStation). Extension to the calibration in chemical ionization (CI) mode should be straightforward.

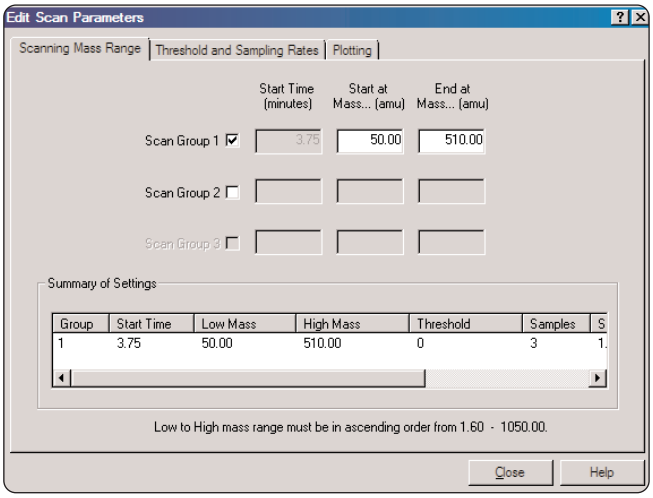
Agilent Technologies

Instrumental setup

For mass spectrometer detector (MSD) data to be suitable for the MassWorks software, it must be collected in raw scan mode. This feature is available on ChemStation G1701DA software versions D.02.00 and higher. To initiate raw scan mode, access the **MS SIM/Scan Parameters** screen (Figure 1). Set the **Acq Mode** to **Raw Scan**. If this item is not one of the options, check your version of the software. The other important parameters that are available in this menu are the **Solvent Delay** (which is the delay time in minutes before the acquisition begins) and the **EMV Mode**. It is highly recommended that **Gain Factor** be selected and used during acquisition. By using **Gain Factor**, the user can scale the signal directly which is very convenient for keeping signal ion currents within limits [3].

The usual parameters under **Edit Scan Parameters** must also be optimized. These are the amu scan range (**Start at Mass** and **End at Mass**), the threshold (which should be set to zero), and the number of samples (**Sampling Rate**)(Figure 2).

The usual rules apply. Set the mass range as short as possible to cover the compound spectra of interest and set the speed as low as possible (or the **Sampling Rate** as high as possible) to acquire data with the best statistics. MassWorks prefers between 7 and 25 scans over the chromatographic peak. Note also that if only the higher mass fragments are of interest, then the scan range can be narrowed and a higher starting mass (**Start at Mass**) can be used. To achieve 98% or better in Spectral Accuracy [4], a signal-to-noise ratio of at least 50:1 is needed when measured off the averaged mass spectrum across a chromatographic peak. This corresponds to about 17 times above the detection limit for the ion of interest in a given mode of acquisition, such as scan range, or scan speed. This must be explored in the context of the chromatography but on standard columns (0.25 mm i.d. and 30 m length), the sampling rate n is usually set to 2 or 3. Again, a threshold of zero is best for MassWorks.



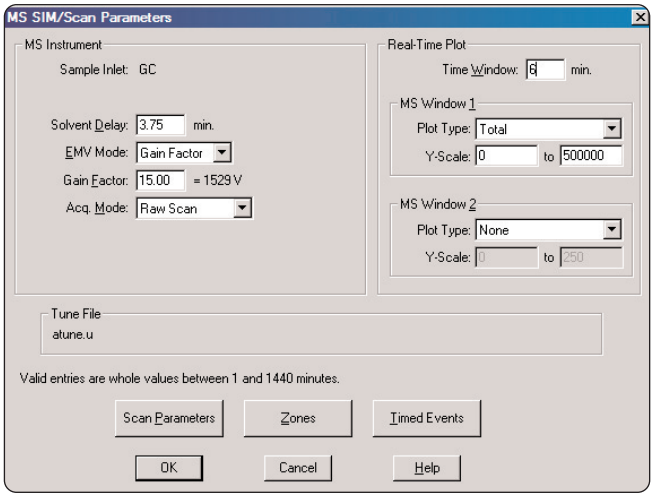


Figure 1. MS Parameters screen

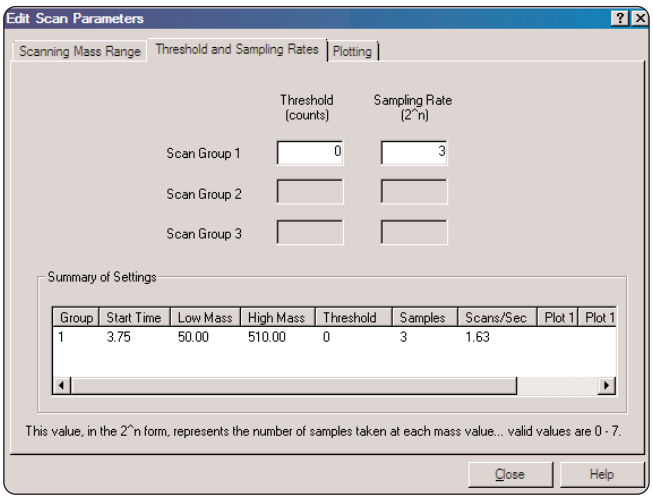


Figure 2. Scan parameters (upper) and threshold and sampling rate (lower) screens

Creating MassWorks calibration methods

The MSD EI calibration gas, PFTBA, is used to tune the MSD instrument (calibrate the ion *m/z* assignments, and set the peak widths) and is also used by MassWorks to generate the calibration data. Another agent (PFDTD) is used as a chemical ionization calibration gas. An external calibration is defined as a calibration gas acquisition alone and acquired as a separate run (method) before or after a set of samples is acquired for analysis by MassWorks. An internal calibration is defined as a calibration gas and sample acquisition within the same run and so is performed with each sample data acquisition. The heated, hyperbolic quadrupole of the Agilent MSD gives very stable mass assignments and an external calibration will serve for most applications [5-6]. For more automated and less frequent sample analysis, a method containing an internal calibration may be a better approach [2]. Directions and suggestions for both these approaches are detailed below.

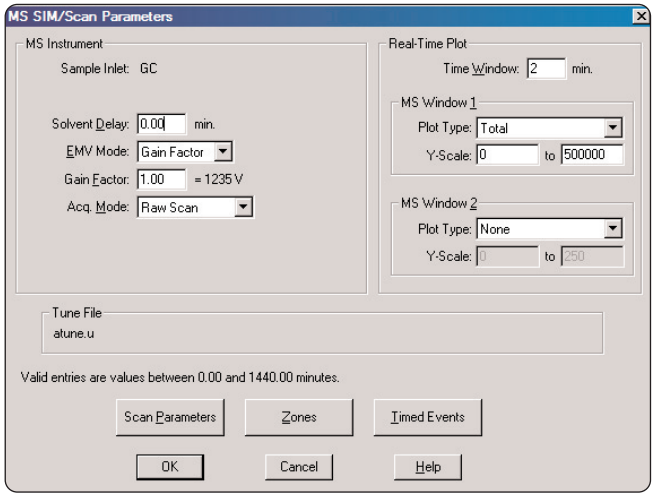


Figure 3 Example MS Parameters screen for external calibration method.

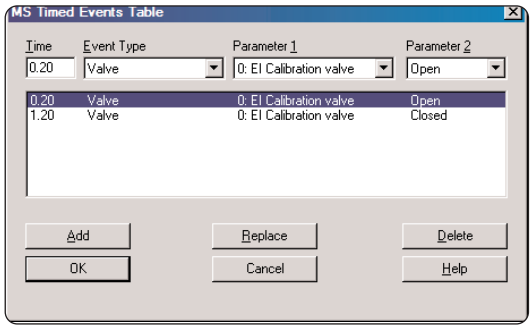


Figure 4. MS Timed Events Table which controls the EI & CI calibration gas.

Setting up an external EI calibration method

In this approach, a sample is not injected but an acquisition of the PFTBA calibration gas is made alone. Oven temperature is less important, and may be the starting temperature of the oven program, but the column flow must be the same as that during a usual method. In addition, all other MS parameters must be the same as those used in acquiring sample data, such as scan range, sampling rate, and threshold. The only exceptions are the **Solvent Delay** and the **Gain Factor**. Since nothing is injected the Solvent Delay can be 0 minutes and the Gain factor should be 1 (Figure 3).

Controlling PFTBA calibration gas:

1) In the **MS Parameters** window (Figure 1) click the **Timed Events** button to bring up the **MS Timed Events Table** (Figure 4).
2) Under **Time**, enter the number of minutes after the run has started that the PFTBA waits before turning on.
3) Select **Valve** under **Event Type**.
4) Select **Calibration Valve** under **Parameter 1**.
5) Select the **Open** state under **Parameter 2**.
6) Select the **Add** button to add that line to the **Timed Events Table**.

Repeat this procedure with the addition of a half-minute or longer before setting the state to **Closed**. The example shown in Figure 4 opens the valve at 0.20 min and closes the valve at 1.2 min.

In the **Instrument View**, under **Instrument**, select the top item of the menu, **Inlet/Injection Types** to get the **Inlet and Injection Parameters** screen (Figure 5). Set the **Injection Source** to **Manual**. Enter the **GC Edit Parameters** screen and set up an isothermal run, with column flow as in your usual method and a runtime of 1.5 minutes as in Figure 6. Be sure that the method has no pressure pulse or other change in column flow rate. The inlet screen should indicate either split or splitless with no pulse. Save the method.

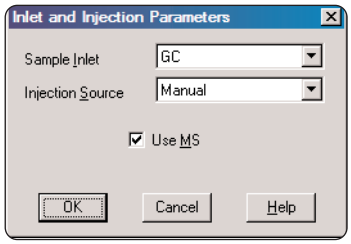


Figure 5. Inlet and Injection parameters, injection source set to manual.

Selecting **Run Method** will show the **Acquisition-Prepare to Inject** screen (Figure 7) and require that you press first the **Prep Run** and then the **Start** buttons on the GC.

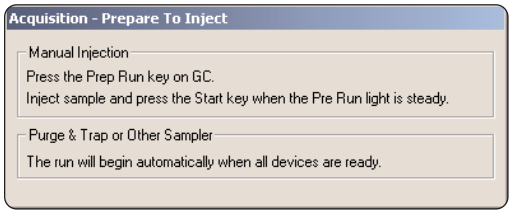


Figure 7. Run method, Acquisition - prepare to inject screen, manual injection information.

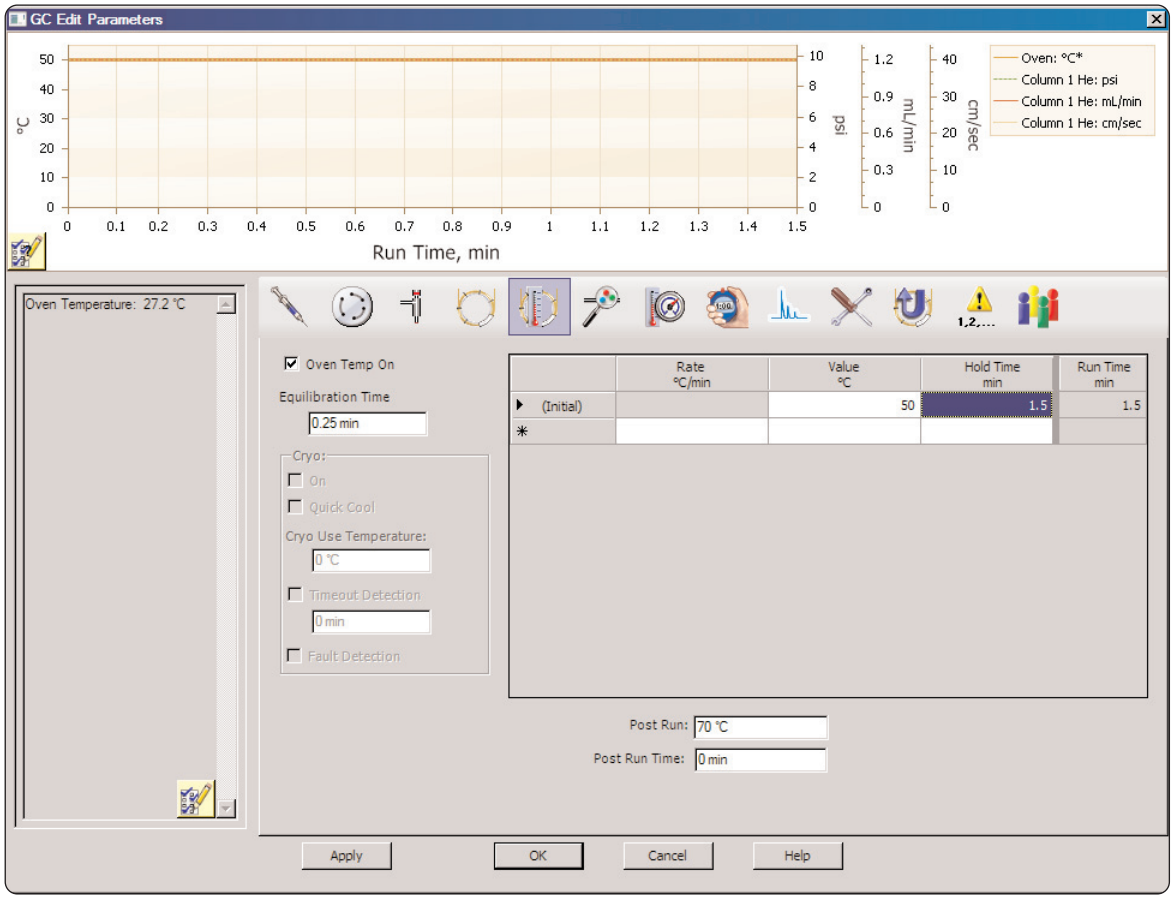


Figure 6. GC Edit Parameters screen: GC oven program.

When the data file is loaded in data analysis, this acquisition will exhibit a reconstructed total ion chromatogram (RTIC) and spectra similar to Figure 8. Notice there is an initial rise as the valve opens followed by a fall in signal to a plateau. It is important that the gain setting is not so high that the individual ion intensities seen in the spectrum (lower plot of Figure 8) reach >3 × 10^6 counts. Near saturation for the PFTBA or for the samples you acquire will lead to poor Spectral Accuracy producing poor results. Therefore in this method, the gain factor should be set to 1 or 2 and the calibration checked to insure that a sufficient signal is obtained at the tuning masses especially those of the isotopes. The gain factor can be raised to improve the signal up to the aforementioned limit.

Setting up an internal calibration method

In this approach, every acquired data file contains an acquisition of the PFTBA calibrant. This has an advantage in that each data file is always connected to a calibration or potential calibration. The disadvantage is that this occurs during the acquisition of a sample. Therefore, it must be assured that the PFTBA spectrum is not contaminated by an early-eluting solvent, high background (column bleed), or late-eluting analytes during the run. The best way to insure this is to perform the calibration at the end of the run after the oven has been cooled.

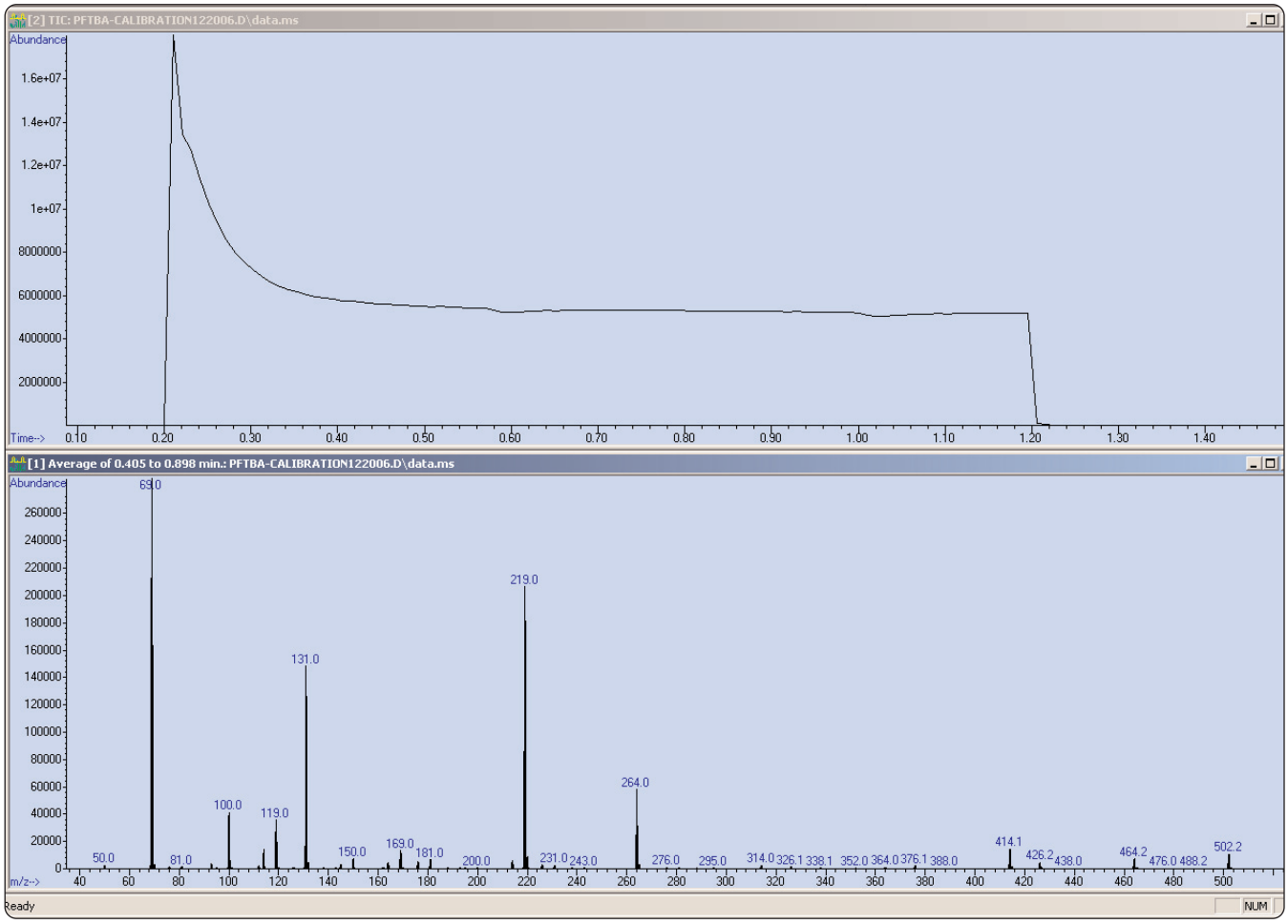


Figure 8. External PFTBA calibration data.

To do this, the user simply extends the GC oven and adds a ramp-down in temperature as shown in the example provided by Figure 9. This case is exaggerated, therefore, it is unnecessary to add as much time to the run due to improved cooling of the Agilent 7890A GC. The user then edits the MS parameters accordingly. Since acquisitions are usually at a high EMV, or gain factor setting, an extra line is added to lower the EMV setting during the PFTBA acquisition. This is shown in Figure 11 and will prevent excessive ion counts, which saturate the detector and distort the calibration. Note that the lowering of the EMV by about 300 or 200 V is to avoid saturation. Higher voltages may be necessary depending on the calibration ion signal. The best EM voltage value to target is that given on the instrument ATUNE report.

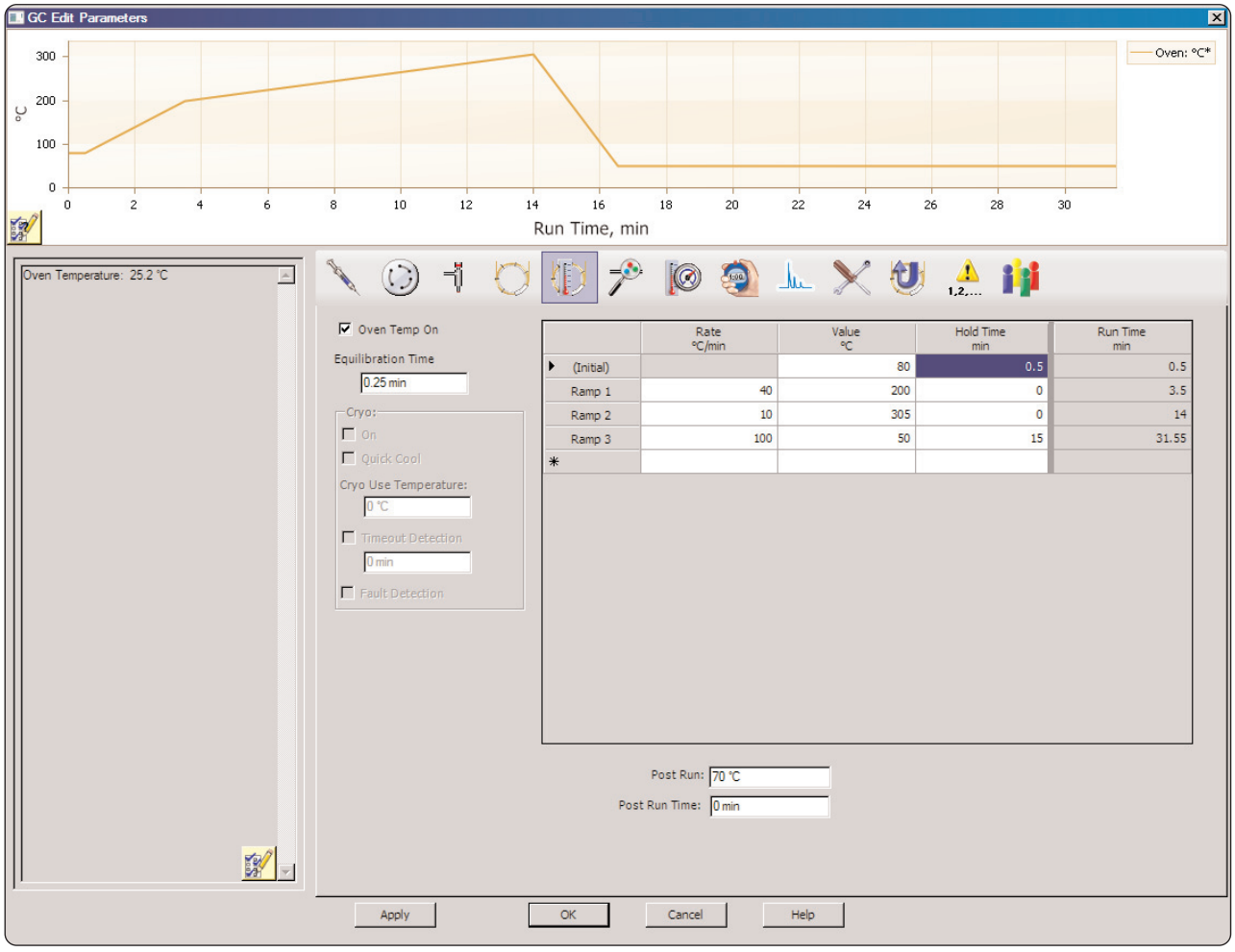


Figure 9. Extending the GC oven run for internal calibrations.

To obtain this voltage, look up the voltage provided in the ATUNE report, or the voltage set when the gain factor is equal to 1 in the **MS SIM/Scan Parameters** screen (Figure 10, lower). Subtract out the voltage for the given gain factor used in acquiring the analytes (Figure 10, upper). The difference is the value that should be entered into the **Parameter 1** field in Figure 11 and it is likely a negative number (-294V). This is a good starting point, and may be refined.

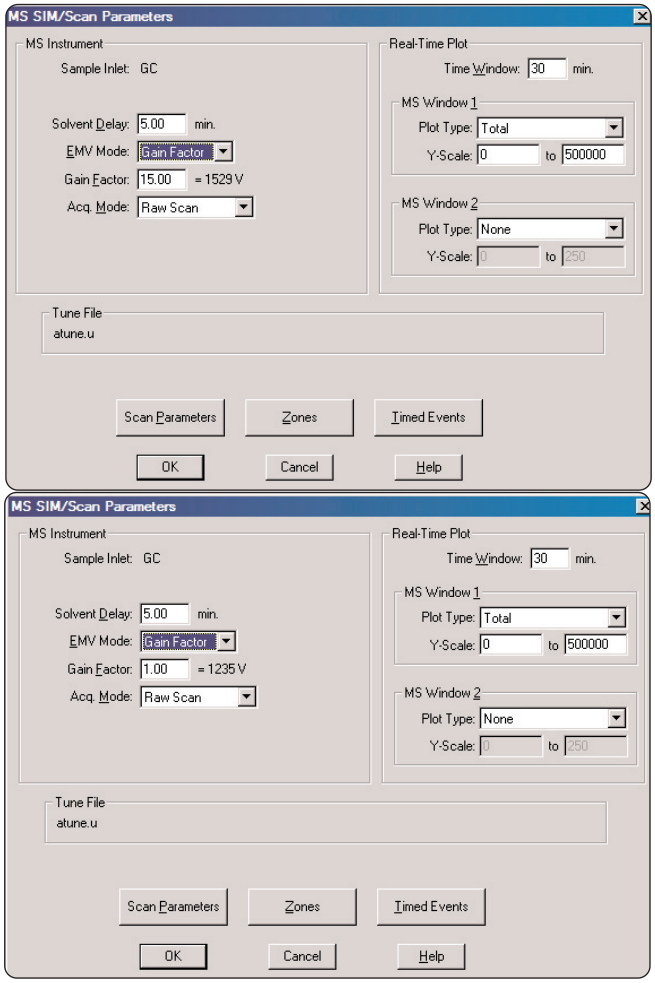


Figure 10. MS Parameters Screen for a sample acquisition – typical EMV setting and solvent delay (upper screen) and ascertaining the target voltage for the calibration gas section of the acquisition (lower screen).

An example of a sample acquisition with internal PFTBA calibration is shown in Figure 12. Note that the delay needs not be so long between the sample and the PFTBA calibration gas.

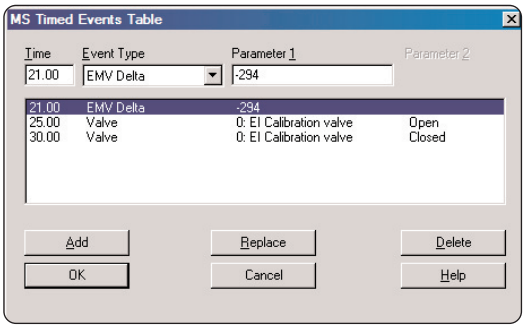


Figure 11. MS Timed Events screen - example for internal calibration.

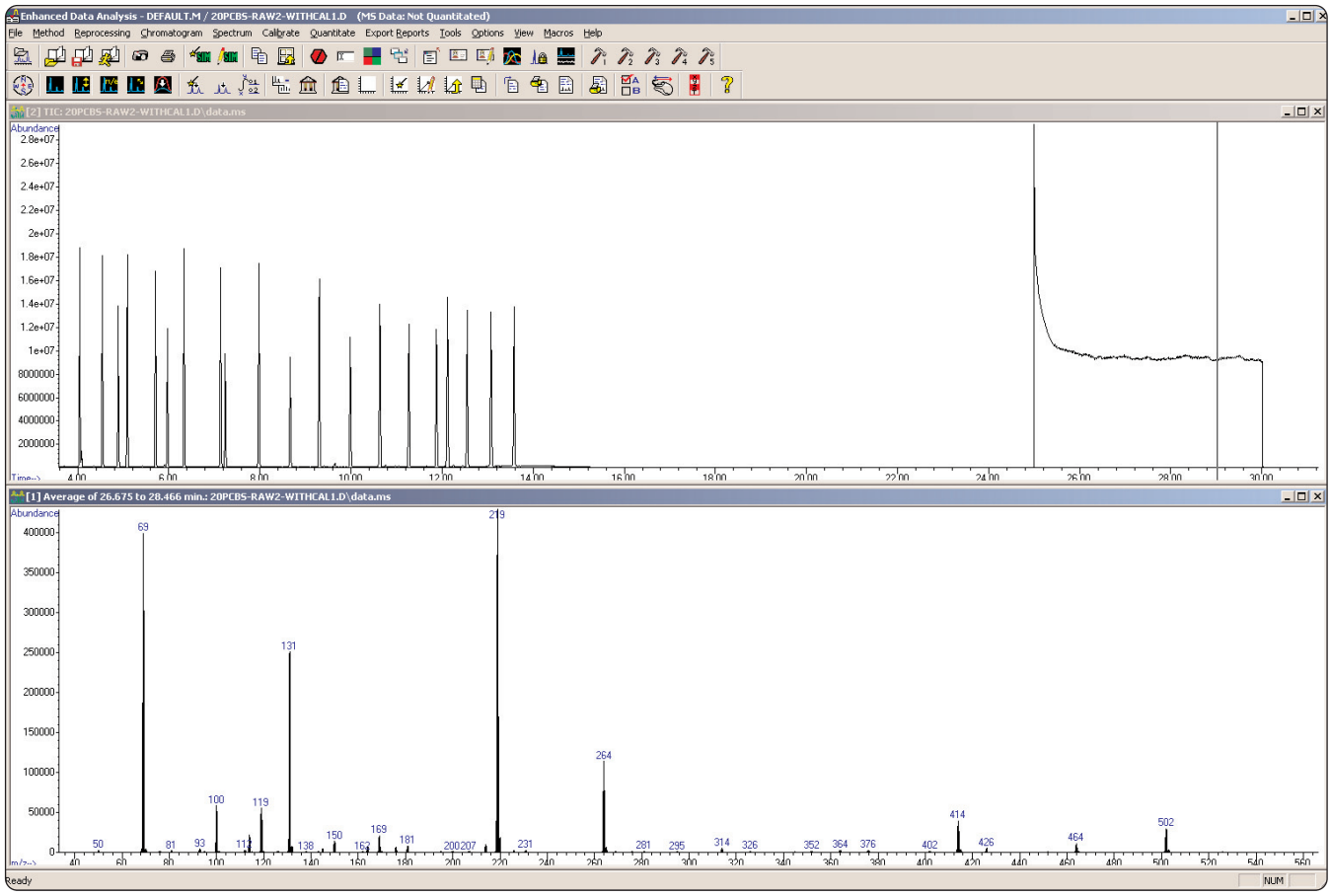


Figure 12. Internal Calibration at the end of a run.

Conclusion

Through the information provided in this note, the user can acquire datafiles containing necessary information for the MassWorks software. Using the calibration data and raw scan acquisition of samples, MassWorks will provide a high degree of mass accuracy in ion assignments allowing determination of the molecular empirical formula. The references provided cite some examples of the utility of this approach and the power of MassWorks in assisting compound identification. Since MassWorks handles chemical ionization (CI) data as well as that acquired in electron impact (EI) ionization mode, the combined approaches of EI and CI should supply insight into unknown compound molecular masses and, through analysis of fragments, helpful structural information.

References

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4. Erve J. C. L.; Gu, M.; Wang, Y.; DeMiao, W.; Talaat, R. E., JASMS, 2009, 20(11), 2058.
5. Wang Y, Prest H. Chromatography, 2006, 27(3), 135.
6. Wang Y, Gu M. Spectroscopy (MS Supplement), May, 2008.

Appendix

Useful tuning ions appearing in PFTBA: molecular formula of important EI fragments

Formula	M^+ Exact Mass[1]	$[M+1]^+$ Exact Mass	(relative abundance)
$C_{12}F_{24}N$	613.9648	614.9681	(13.7%)
$C_9F_{20}N$	501.9711	502.9745	(10.37%)
$C_8F_{16}N$	413.9775	414.9809	(9.26%)
$C_5F_{10}N$	263.9871	264.9905	(5.93%)
C_4F_9	218.9856	219.9890	(4.45%)
C_3F_7	168.9888	169.9922	(3.34%)
C_3F_5	130.9920	131.9954	(3.34%)
C_2F_5	118.9920	119.9954	(2.22%)
CF_3	68.9952	69.9986	(1.11%)
CF_2	49.9968	51.0002	(1.11%)

1. Note all extract masses in this Appendix are calculated without counting the gain or loss of an electron from the charge.

Useful tuning ions appearing in PFDTD: molecular formula of important PCI tuning fragments

Formula	M^+ Exact Mass
C_3F_7	168.9888
$C_5F_{10}O_1H$	266.9868
$C_{11}F_{22}O_3H$	598.9574

Useful tuning ions appearing in PFDTD: molecular formula of important NCI tuning fragments

Formula	M^- Exact Mass
C_3F_7O	184.9837
$C_6F_{13}O_2$	350.9691
$C_8F_{16}O_3H$	448.9670

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