

Thermo Scientific GC-MS Data Acquisition Instructions for Cerno Bioscience MassWorks™ Software

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- Accurate Mass
- Elemental Composition Determination
- Unknown Identification
- Mass Spectral Calibration
- Single Quadrupole GC-MS
- Triple Quadrupole GC-MS
- Spectral Accuracy

Introduction

As a post-acquisition data processing tool, Cerno Bioscience MassWorks provides unique capability to achieve high mass accuracy and spectral accuracy on a unit mass resolution mass spectrometer. This innovative technology [1-3] performs calibration on not only m/z values but also mass spectral peak shape to enable a quadrupole instrument such as ISQ Series GC-MS to determine elemental composition of either molecular ions or fragments to facilitate compound identification [4, 5] or structural elucidation of pharmaceutical impurities. To apply this approach, the data must be acquired in a suitable format and with either internal or external calibration. This article describes step-by-step procedures on how to acquire calibration standard data in electron impact (EI) ionization mode on Thermo Scientific GC-MS systems using the Chromeleon 7.2, TraceFinder or Xcalibur data acquisition platforms. Nearly identical procedures can be applied to data collection for the calibration standard in chemical ionization (CI) mode by selecting the CI reagent gas in the mass spectrometer's method option.

General Requirements

For accurate mass measurements through MassWorks software, data acquisition on Thermo Scientific quadrupole GC/MS systems must be performed in full scan and **Profile** mode with **Threshold** set to zero. To achieve the best mass accuracy and spectral accuracy, additional MS scanning parameters should be optimized to maximize signal to noise ratio. The mass range should be set as narrow as possible to cover only the ions of interest, while **Dwell Time** should be long enough to allow at least 5-10 MS scans for a given GC peak. To achieve 98% or better in Spectral Accuracy [2, 3], a signal to-noise ratio of at least 50:1 is needed when measured off the averaged mass spectrum across a GC peak. This corresponds to about 17 times above the detection limit for the ion of interest.

Setup for an internal calibration method

An internal calibration in this context is defined as a calibration gas, Perfluorotributylamine (PFTBA), and sample acquisition within the same data file. The data collection on PFTBA is typically obtained after GC separation of unknown components from the sample. Thermo Scientific's software

allows the user to do this incredibly easily by simply setting an On and Off time in the MS method very similar to how you would modify the CI gas or flow for chemical ionization.

Step 1 - The GC Ramp: The first part of acquiring PFTBA standard spectrum is to make sure the GC goes back to its initial starting temperature, or something similar to it. Failure to go back down could lead to increased column bleed and higher background levels thus contaminating the standard PFTBA spectrum.

Program your oven ramp as you would normally. In this example (Figure 1), the oven ramp is from 40 to 325 °C, but at the end of the ramp we set another temperature of 50 °C so it ramps back down. Be careful not to ramp down too quickly or else the GC cannot physically cool off in time, we suggest a starting rate of approximately 20 °C/min. After the GC reaches the end temperature, hold it there for about 5 minutes so that the mass spec's calibration gas can be turned on. Typical analyses only require about 1min total for the calibration gas to be turned on.

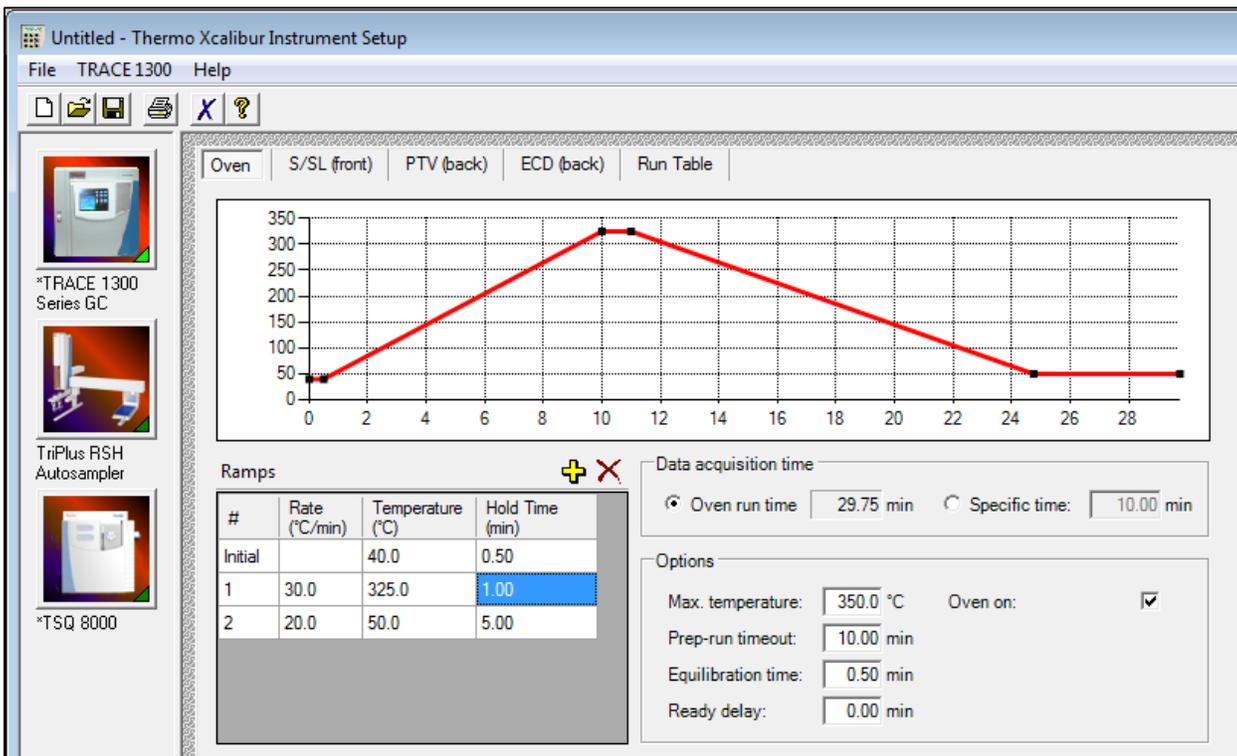


Figure 1.A GC method for acquiring an internal calibration standard

Step 2 - The MS Method: To configure the mass spectrometer properly you need to set a few variables including threshold, scanning mode, dwell time, and timed events as illustrated through following screenshots. Please note that these screenshots show a TSQ 8000 triple-quad method, the same values and features can be found on the ISQ Series single quad. The detail procedures are as follows.

Right-click on the **Scans** header (over Tune file name, SRM, SIM, and Scan mases, etc.) to show the additional fields available, then select "Data Type: Centroid", this will allow choosing **Profile**, Centroid or Nominal data (Figure 2). Select "**Profile**" for all rows so that the raw spectra are stored.

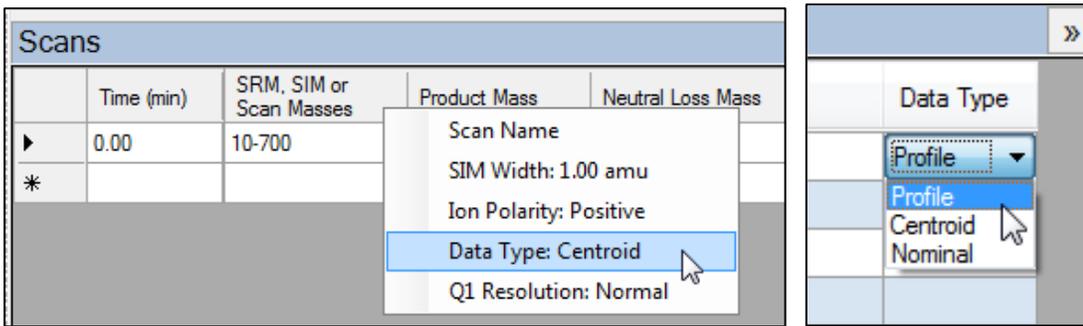


Figure 2. Setting up profile mode (Right-click header menu for Data type and left-click to select profile) Perform the same right-click over the **Groups** header to select “Calibraton Gas:Off” (Figure 3). Now go back to the oven ramp and figure out when the GC reaches the final temperature, in this example it is at approximately 25 min, so we will use 26 min as our starting point to give the column a bit of equilibration time at the new temperature. In the first line of the **Scans** table, enter 0.00 min as the starting time, 10-700 as the mass range, 0.096 seconds as the **Dwell time**, and make sure **Profile** is selected as the data type. The **Dwell time** of 0.096 s is used here because with the electronic stabilization it comes out to almost 0.1 s, although slower dwell times (0.15 s, 0.2 s etc.) could potentially yield better mass spectral data. On the right side under the Groups tab, make sure the Calibration Gas is set to Off.

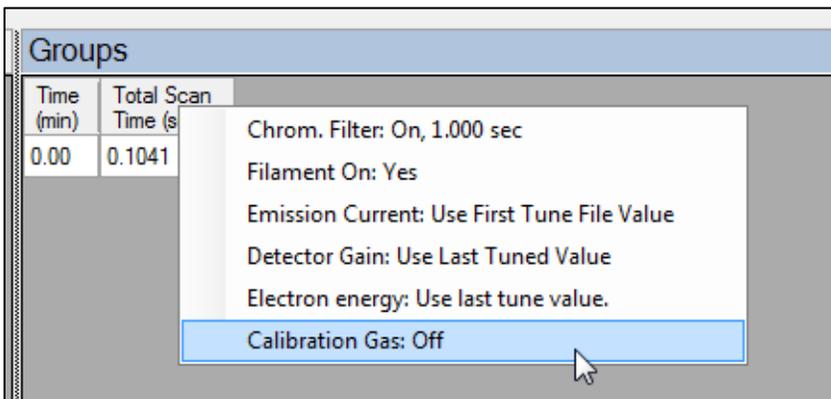


Figure 3. Setting up time events for acquiring calibration gas (Right-click header menu for Groups and left-click to select calibration gas On or Off)

Repeat this procedure to add a second line exactly the same as the first except for the time (26 min) and the Calibration gas set to EI. Repeat this for a third line with the time set to 27 min and the calibration gas set back to Off. Note that **Threshold** check box (Figure 4) should be either unchecked or checked with value set to zero.

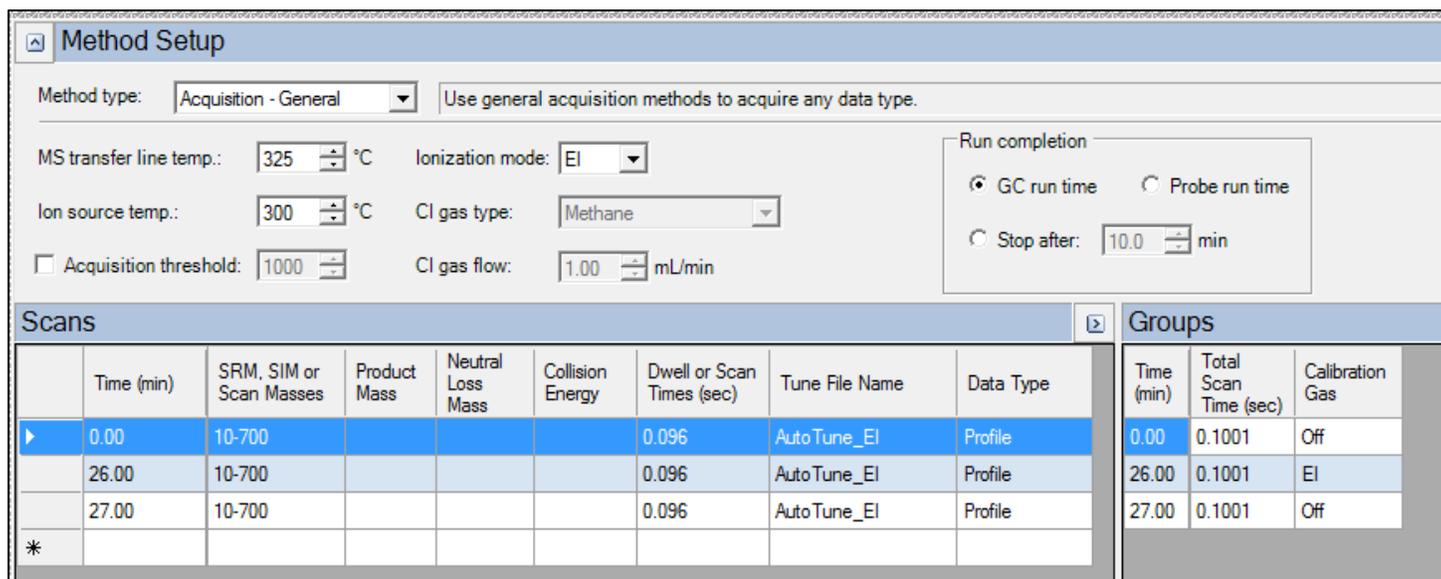


Figure 4. Finished MS method for internal calibration

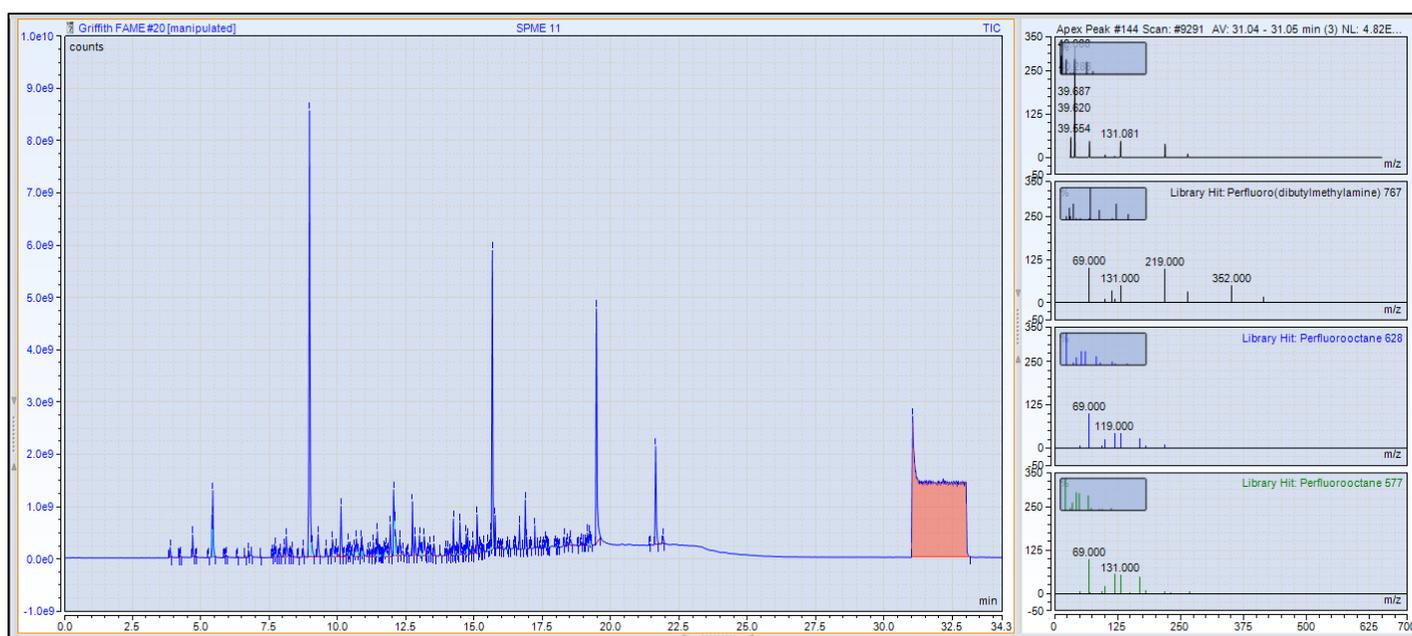


Figure 5. TIC of GC-MS separation including both unknown components and internal calibration standard PFTBA

Setup for an external calibration method

An external calibration is defined as a calibration gas (PFTBA) and sample acquired separately in different data files. The data collection on PFTBA employs the same MS scanning parameters as those used for internal calibration and has similar setup for GC program and time events to control PFTBA as shown in below screenshots.

Step 1 - The GC Ramp: This is actually very easy. Set a basic ramp and hold it at a low isothermal temperature for a few minutes. In this example we're going to hold the oven at 50 °C for 4 min total (Figure 6).

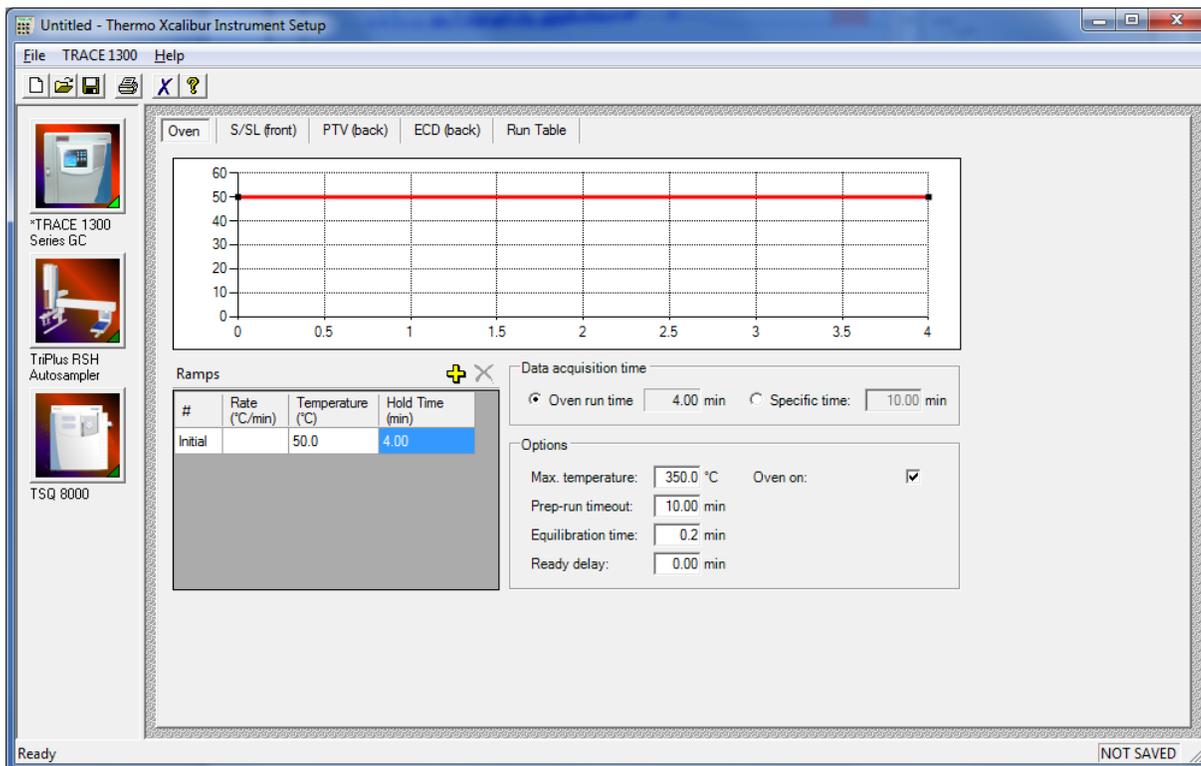


Figure 6. A GC method for acquiring an external calibration standard

Step 2 - The MS Method: This is almost identical to the previous (internal) calibration style, but simpler. Instead of trying to coordinate the calibration gas On/Off time with the oven cool down cycle, we just wait for a short period of time (e.g., 1 min in Figure 7) to let everything settle and then turn on the calibration gas for 1 min before turning it off.

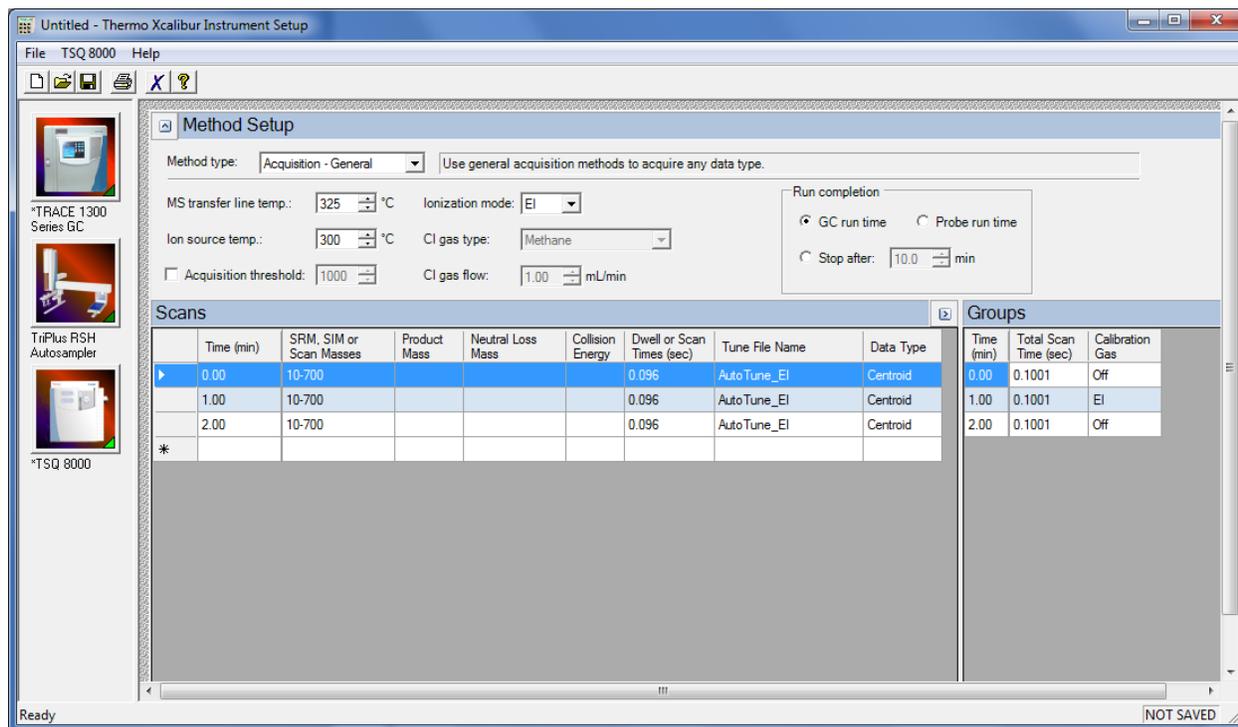


Figure 7. Finished MS method for external calibration

Chromeleon 7.2:

Step 1 - The GC Ramp: The above steps will work to set up a Chromeleon method as well. Figure 8 shows what a Chromeleon GC method for internal calibration would look like.

The screenshot displays the Chromeleon Chromatography Studio interface for a Whisky Analysis (Sequence) method. The main window shows the 'Trace_1310 Oven Settings' configuration. The temperature profile is graphed as follows:

Time [min]	Temperature [°C]	Rate [°C/min]	Target value [°C]	Hold time [min]
0.000	40.0	Run		
0.500	40.0	0.00	40.0	0.50
11.000	325.0	30.00	325.0	1.00
29.750	50.0	20.00	50.0	5.00
29.750	50.0	New Row		
29.750	50.0	StopRun		

The interface also includes a table of method parameters and various toolbars:

Parameter	Value	Range
Prep Run Timeout	30.00	[0.00...999.99 min]
Oven equilibration time	0.20	[0.00...999.99 min]
Ready delay	0.00	[0.00...99.99 min]
Cryogenics enabled	<input type="checkbox"/>	
Cryogenics threshold		[40...200 °C]

Figure 8. A GC method for acquiring an internal calibration standard with Chromeleon

Figure 9 shows what a Chromeleon GC method for an external calibration looks like.

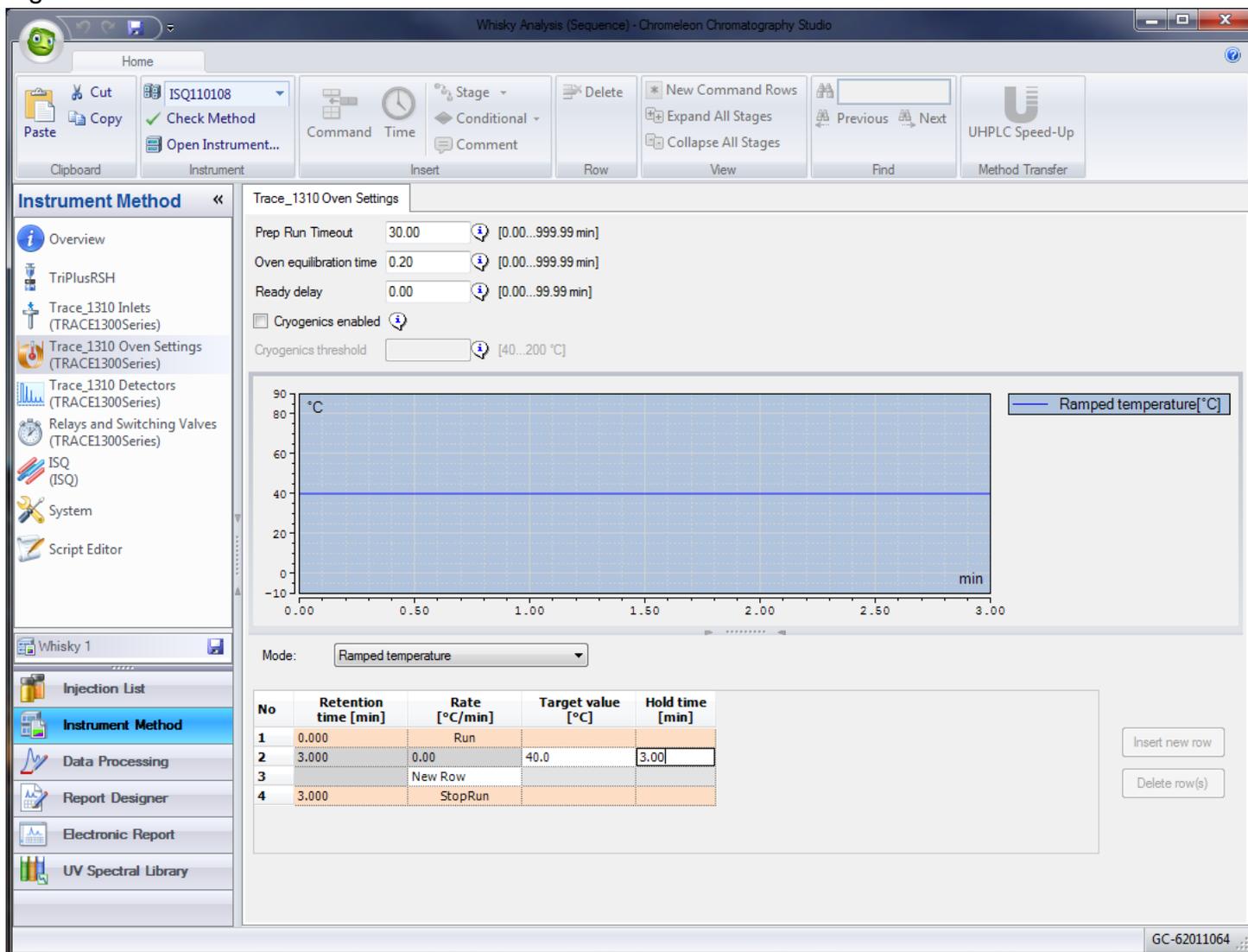


Figure 9. A GC method for acquiring an external calibration standard with Chromeleon

Step 2 - The MS Method: The dialog boxes for Chromeleon 7.2 are very similar to those for the Xcalibur or TraceFinder, so users who are familiar with those software platforms will feel right at home. By clicking on the ISQ (or TSQ) on the left-hand side of the Instrument Method Navigation page you can open the mass spec's method parameters. In this example, we will show how to acquire an external calibration. Set the first time to 0 min to let the mass spec acquire some background data before turning on the calibration gas. Pick a mass range wide enough to cover the ions of interest in PFTBA spectrum, and then select a **Dwell Time** (0.096 s dwell or 0.1 s total scan times are recommended), then pick your tune file name and lastly set the Data Type to **Profile**. Over on the right side's Groups box, make sure the calibration gas is set to Off. Repeat the first line two more times, change the time to 1.00 min and the calibration gas to EI for the second line, and the time to 2.00 min and calibration gas to Off for the third line. Figure 10 shows what a Chromeleon MS

method for an internal calibration looks like.

The screenshot displays the 'Whisky Analysis (Sequence) - Chromeleon Chromatography Studio' window. The 'Instrument Method' tab is active, showing the 'Method Setup' section. The 'Method type' is set to 'Acquisition - General'. The 'MS transfer line temp.' is 325 °C, 'Ionization mode' is EI, 'Ion source temp.' is 300 °C, 'CI gas type' is Methane, and 'CI gas flow' is 1.00 mL/min. The 'Acquisition threshold' is 1000. The 'Run completion' options are 'GC run time' (selected) and 'Probe run time'. The 'Stop after' is set to 10.0 min. The 'Scans' table shows four scans at 0.00, 1.00, 2.00, and 2.00 min, all with a mass list of 10-700, dwell of 0.096 sec, and tune file 'AutoTune_EI'. The 'Groups' table shows three groups at 0.00, 1.00, and 2.00 min, all with a total scan time of 0.1 sec and calibration gas 'Off'. The 'Calibration Gas' column is highlighted in blue for the 2.00 min group.

Time (min)	Mass List or Range (amu)	Dwell or Scan Times (sec)	Tune File Name	Data Type
0.00	10-700	0.096	AutoTune_EI	Profile
1.00	10-700	0.096	AutoTune_EI	Profile
2.00	10-700	0.096	AutoTune_EI	Profile
2.00			AutoTune_EI	Profile

Time (min)	Total Scan Time (sec)	Calibration Gas
0.00	0.1	Off
1.00	0.1	EI
2.00	0.1	Off

Figure 10. Finished MS method for internal calibration with Chromeleon

References

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