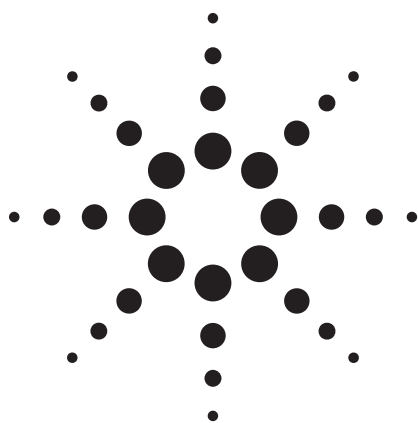


Enhancements to Gain Normalized Instrument Tuning: Understanding the Benefits and Features



Technical Overview

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Introduction

The automated optimization of ion detection in a mass spectrometer (MS), commonly referred to as “autotuning,” has been a tremendous simplification in MS operation. This critical operation has become so routine that it is taken for granted. Convenient as this may be, the approach to setting the voltage of the electron multiplier in the detector was usually left to operator experience. The ChemStation G1701EA (E.00.xx) software introduced the capacity to produce Gain Normalized tunes at Gains of 10^5 , 10^6 , or 1.5×10^6 [1]. The new ChemStation G1701EA (E.02.xx) software has enhanced this approach by adding more choices to the possible detector Gain selections and expanded Gain Normalization to include chemical ionization modes. While the E.00 revision allowed tunefiles to be Gain Normalized, E.02 allows the user to create Gain Normalized methods. This technical overview describes the advantages of Gain Normalization in detail, how it has been implemented in the ChemStation software, and how customers can specify Gain settings in their methods.

Mass Spectrometer Tuning and Detector Gain

Figure 1 shows a schematic diagram of a quadrupole MS with emphasis on the ion detection system (detector), which is composed of the high-energy dynode and electron multiplier. In performing a tune or autotune, MS systems introduce a volatile calibrant and adjust the MS parameters to meet some criteria such as the abundances of certain calibrant ions. Historically that is how the 597X

series of instruments have tuned. (Software allows the user to change or adjust these tuning targets through the Tune Wizard.) After the ion optics are optimized and calibrant ion peak widths are adjusted, the target calibrant fragment ion abundances are achieved by adjusting the voltage applied to the electron multiplier (EM). The voltage applied across the EM amplifies the very small ion current emerging from the mass filter [i] to produce a much larger electron current [I] to meet the target signal abundances. A measure of the degree of amplification of this very small current to the higher, more easily quantified current is called the multiplier “Gain” (that is, $\text{Gain} \equiv I/i$). This process produces an electron multiplier voltage (EMV) based on the target calibrant ion abundancies in the source which is derived from the relatively high pressure of the calibrant gas (for example, PFTBA). In general, analysts adjust this voltage to meet the analytical requirements such as linear range, detection limits, etc., in their method parameters. For analysts who are usually interested in determining compounds at very low levels, the habit is to add an additional incremental voltage beyond that obtained in autotuning on the calibrant; for example, ATUNE.U+400V. This produces a Gain more appropriate to trace analysis and is reflected in higher signals than the case where the ATUNE.U voltage is used alone. So indirectly the Gain is being adjusted to meet the requirements. However, problems in this approach arise in several forms, such as differences between instruments or loss of signal as EMs “age.”



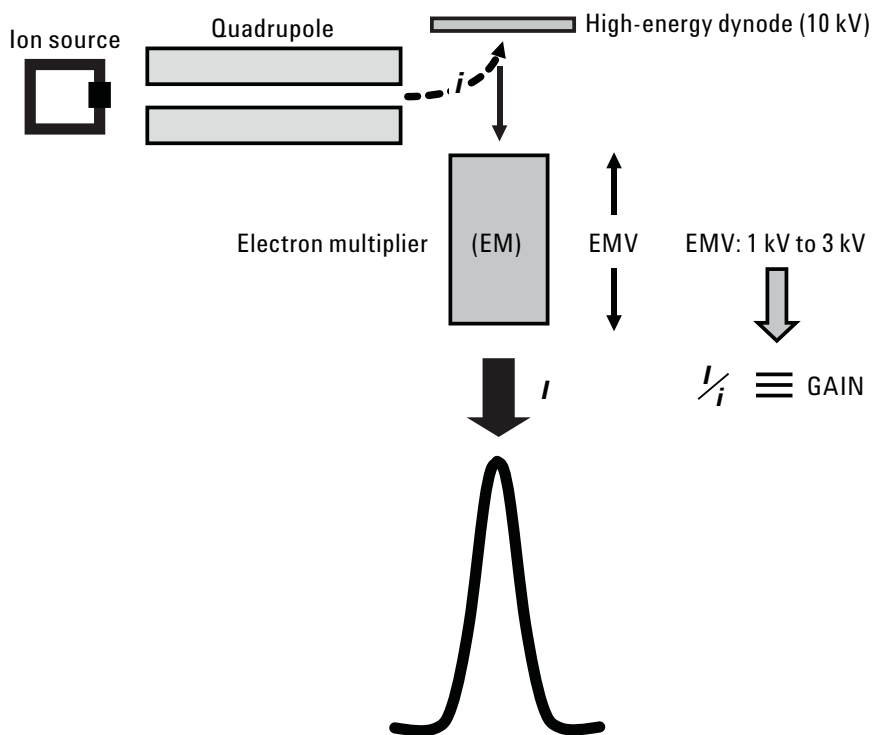


Figure 1. MS schematic and detector Gain.

Consider Figure 2, which illustrates the relationship of detector signal versus the voltage applied for a “new” EM and an older, “aged” EM. The left curve shows the response of a typical new EM. At 1,000 V, the EM voltage meets the target abundances set in the autotune (ATUNE). To meet the analytical requirements of trace concentration detection, 400 V are added to the tune voltage to produce a high signal (S_{new}). However, after some period of use or unintentional abuse, the detector character changes and the signal versus EMV curve shifts. The tuning process targets the same abundances but requires a higher voltage of 1,700 V to meet them. The method still calls for the addition of 400 V but as can be seen in the graph, the signal produced is lower than before. This produces problems. This falloff in response can initiate a lot of troubleshooting activities, such as source cleaning, column maintenance, etc., when in fact the aging of the detector is responsible.

Another way of looking at this situation is to consider the new and aged EMs as belonging to two different GC-MS systems. One system may be wrongly considered a higher sensitivity system than the other. The message is simply, “Because detectors do differ and ‘age’ over time, the same voltage setting will *not* always produce the same signal.”

Alternatively, from the point of view of Gain, signal at a particular Gain setting does not depend on the detector age. Figure 3 shows a Gain versus Signal curve for both a new and an aged detector. For both the new and aged detector, the voltage is adjusted to *produce* a fixed Gain, here selected as 10×10^5 , which in turn produces the same signal ($S_{\text{aged}} \cong S_{\text{new}}$). Again, the message is simply, “Even if detectors differ and ‘age’ over time, the same detector Gain will produce the same signal.” Gain Normalized methods maintain response over time.

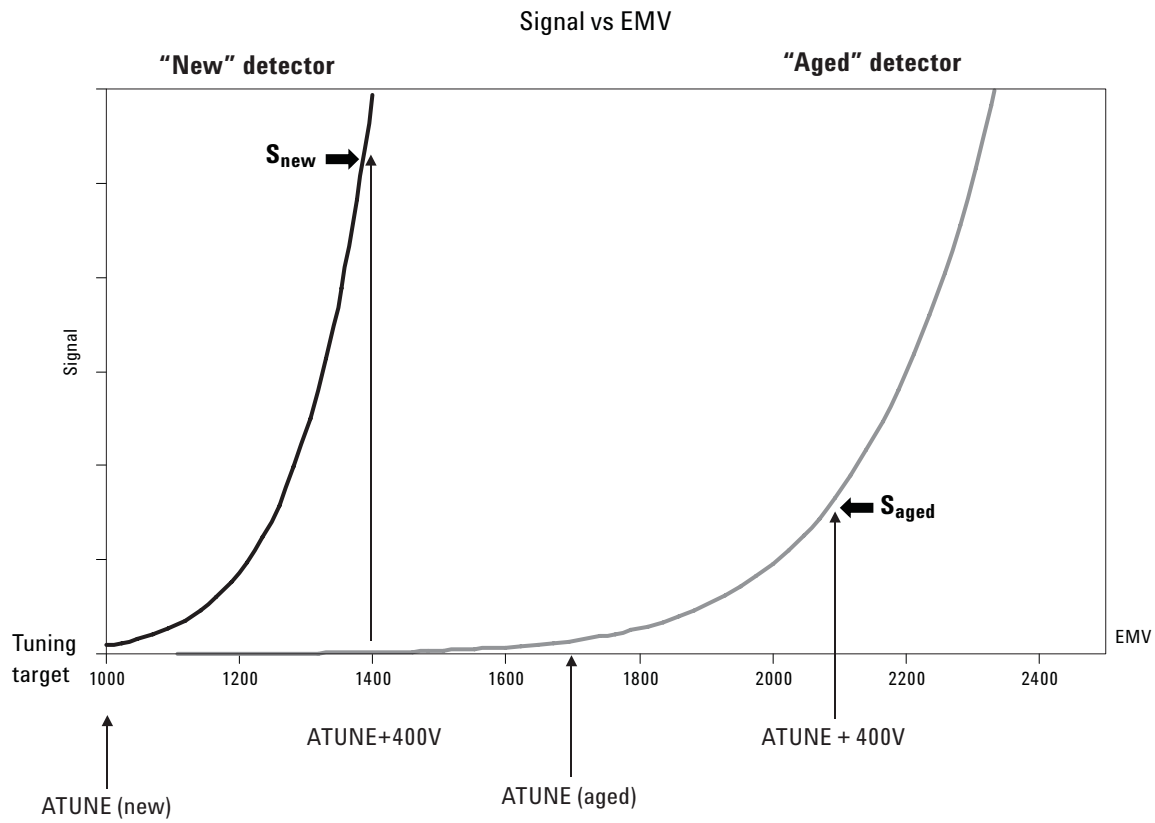


Figure 2. Weakness in using EM voltages: signal versus applied detector voltage.

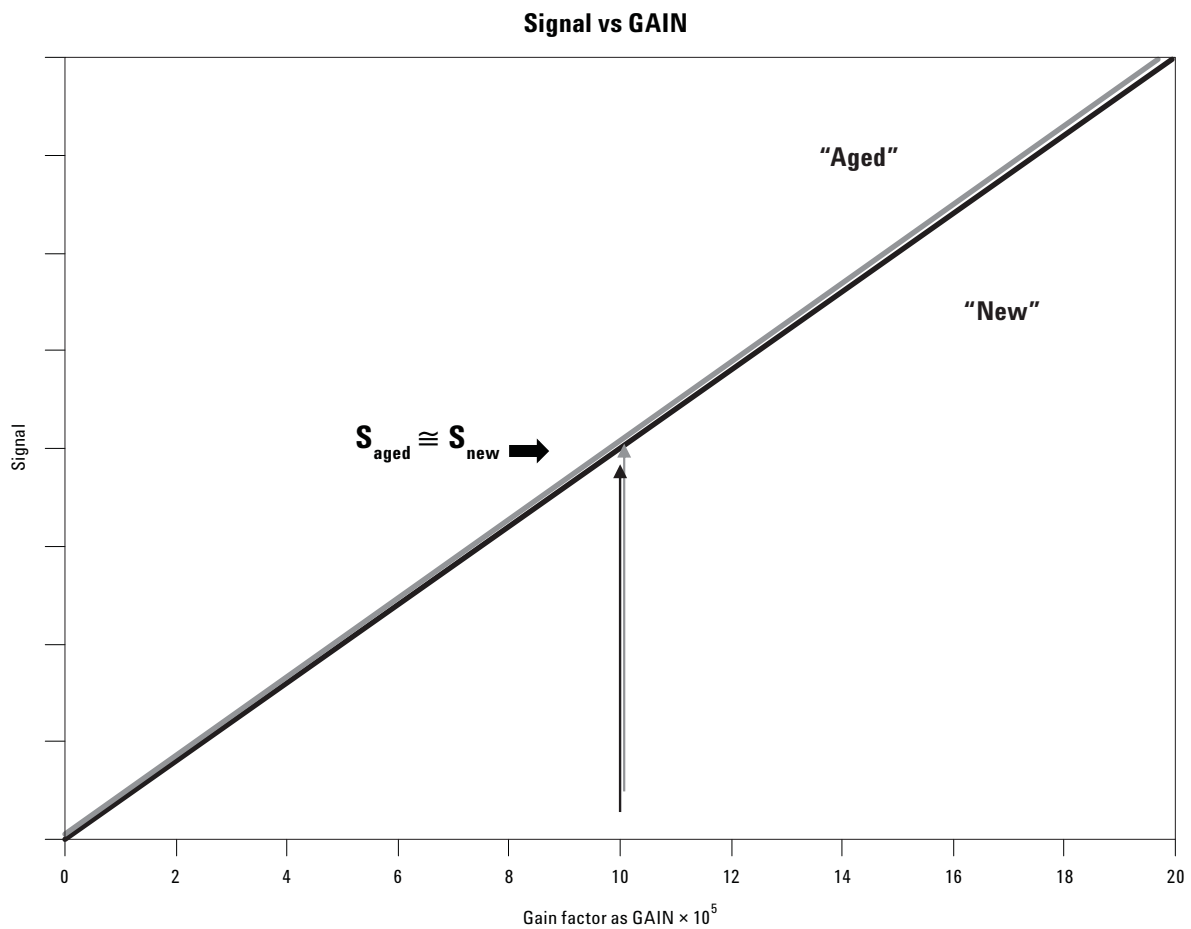


Figure 3. The advantage of Gain Normalization: signal versus Gain factor.

The situation experienced by analysts can be seen in Figure 4. The left panel shows an overlay using data from analysis of PCBs via SIM acquired with a new detector in a method using a fixed Gain (1.5×10^6) and another method using a voltage added to the tuning voltage (ATUNE + n V). When the detector is new the PCB responses match (actually because the Gain factors match). However, the right-hand panel shows the results of applying exactly the same methods with a detector that has aged. Now the method using ATUNE + n V does not provide the same response, but the Gain Normalized method does produce the same result as before. Again, the argument extends to a comparison of more than one instrument and explains why nominally equivalent instruments can produce very different results.

Everything can be summarized in one idea, “Gain Normalized methods provide more consistent response over time and between instruments.”

To summarize some advantages of this approach:

- **Better consistency for compound responses.** After a source cleaning, column servicing, or EM replacement, an instrument retuned to the same Gain as previously used will show compound responses more similar to and more consistent with those obtained prior to maintenance.
- **Better agreement between different instruments for the same compounds.** If instruments are tuned with the same criteria and to the

same Gain, they will show better agreement in compound responses among the instruments.

- **Better diagnostics in tuning and troubleshooting.** For example, more exacting water and air criteria can be developed by tuning to a particular Gain and then examining the air and water background. When inspecting a chromatogram acquired under a fixed Gain it becomes easier to decide whether column bleed profiles have increased or decreased over time. Further, if during tuning the voltage applied becomes excessive or is not sufficient to reach a fixed Gain, it is clear that this multiplier has reached the end of its life. And most importantly, if compound responses, before and after servicing or even just between tunes, are not similar, this indicates that there is a problem likely in the GC portion of the GC-MSD that needs attention. This is invaluable for establishing operation criteria.
- Most significantly, **users have more direct control over their methods’ performance** because signal is directly proportional to Gain. The approaches to creating Gain Factor Normalized methods described below will illustrate this further.

Creating Gain Factor Normalized Methods

The new ChemStation G1701EA (E.02.xx) software enables Gain Normalized methods. These methods use Gain factors instead of the usual voltage

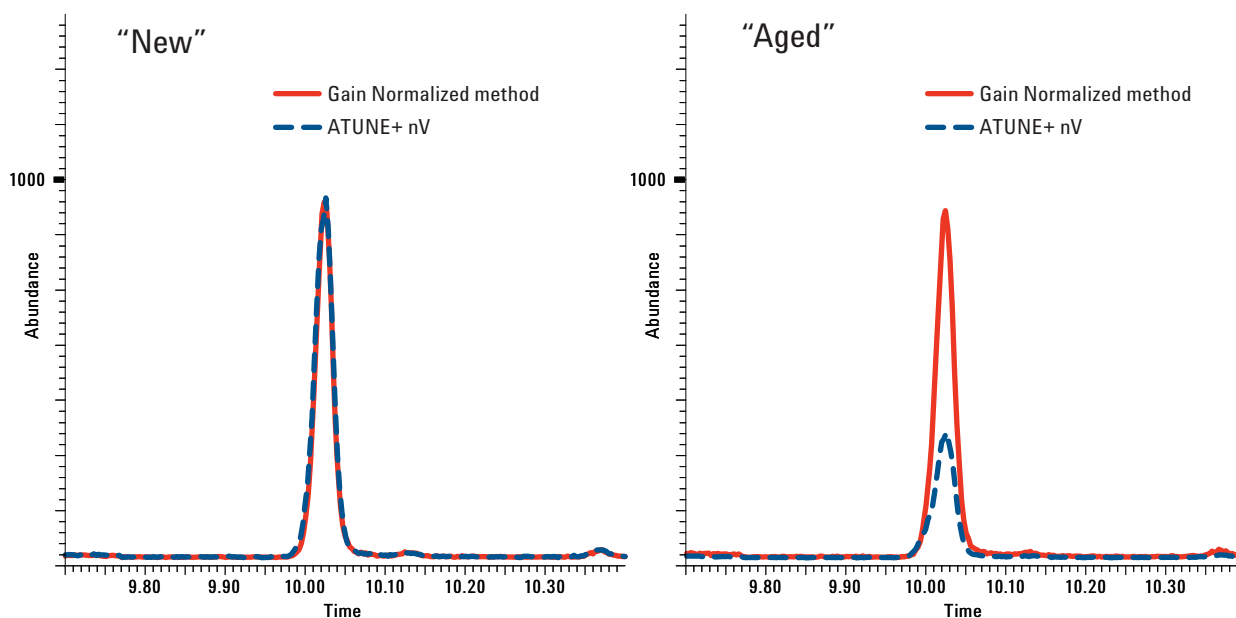


Figure 4. Gain Normalized methods versus EM voltage adjusted methods.

adjustments to tune files. Figure 5 shows a section of the new **MS parameters** panel. The rearranged panel allows the EM setting to be in the relative (**REL**) or absolute (**ABS**) modes of the past or the **EM Mode** can be selected to use **Gain Factor** mode. In Gain Factor mode, the user can select any Gain factor between 0.25 and 25 in increments of 0.01 and enter this value in the box next to **Gain Factor**. The Gain factors are in multiples of 10^5 ; that is, a Gain factor of 15 means the detector will be set to produce a Gain of 15×10^5 or 1.5 million. Based on the last calibration of the EM voltage versus Gain curve, the EM voltage setting that achieves the selected Gain factor is displayed. The user can note this voltage to keep track of the EM life. When the method is saved, the Gain factor is saved and the method becomes a Gain Factor Normalized method.

How Gain Factors Are Determined by the Software

Every time the ChemStation G1701EA (E.02.xx) software generates an Autotune, it also establishes a relationship between the EM Gain and the EM voltage. Alternatively, if a manual tune is made, the user can update the calibration between EM Gain and EM voltage by executing **Update EMV Gain Coefficients** under **Parameters** in the **TUNE** view. Because these coefficients are updated with every autotune, using Gain Normalized methods is as simple as setting the Gain factor in the **MS Parameters** and making the tune.

What Gain Factor Should Be Used?

Table 1 gives some recommended Gain factor ranges and a value for trace analytical applications.

Ionization mode	Recommended Gain factor ranges	Settings for trace analysis
Electron impact (EI) ionization	0.5 to 15	15
Positive chemical ionization (PCI)	0.5 to 2	2
Negative chemical ionization (NCI)	1 to 14	14

For example, for the EI checkout method run on 1 picogram of octafluoronaphthalene at installation of the GC-MSD, the Gain factor is set to 15 since low concentrations are being measured and high sensitivity is required.

Determining a Proper Gain Factor

For existing methods

If users have an existing method that works well for their analysis, they can find the Gain factor they are presently using by following these steps:

1. Examine the method and determine what voltage offset is applied relative to the method's Tune file value. For example, say it is Atune.U and the EM voltage is relative and equal to -200 V.

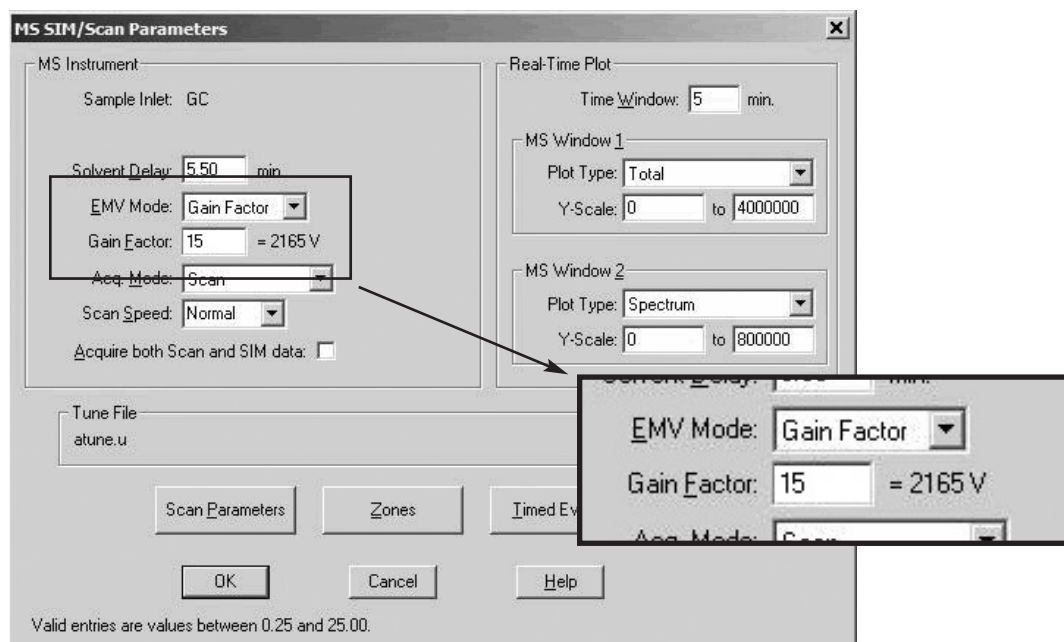


Figure 5. New MS parameters panel.

2. Enter the **TUNE** view, and under **Parameters** select **Manual Tune**, then change the EMV setting to the resulting EM voltage calculated in the method by the offset. For example, if ATUNE.U has a final tune voltage of 1,200 V, then the method would be executed under:

$$\text{Atune.U} - 200 \text{ V} = 1,200 \text{ V} - 200 \text{ V} = 1,000 \text{ V.}$$

So the tune value EM setting is set to 1,000 V. Hit OK and leave the Tune panel.

3. While still in the **Tune View**, under **File** select **Generate Report**. This will execute the tune values and create a printed tune report. In the lower right corner of the tune report the EM Gain will be cited, and below it will be the Gain factor.

For method development

If users are interested in finding the optimum Gain factor for their method, there are several approaches depending on the goals of the analysis. If the method involves measuring very low concentrations, the recommended Gain factors given in Table 1 will be a good guideline. In cases where trace analysis is not the goal and higher concentrations are involved, the following approach will help.

- 1) Tune the instrument. In the GC-MS method, set the Gain factor to 1.0 and acquire the highest concentration standard.
- 2) Examine the data file and check the reconstructed total ion current (RTIC) chromatogram for the peaks of greatest height. Two cases emerge:
 - a) Analyte peak heights are low, $\ll 10^6$ counts, and more signal is needed. Determine the peak height of the highest peak in the RTIC chromatogram. Target a new RTIC height abundance of 2×10^6 and calculate the needed Gain factor via (equation 1):

$$\frac{(\text{Present Peak Height})/(\text{Present Gain Factor})}{= (\text{New Peak Height}/\text{New Gain Factor})}$$

$$\Rightarrow (\text{New Gain Factor}) = (2 \times 10^6)/[(\text{Present Peak Height})/(1.0)]$$

Go into the method (MS Parameters) and change to the new Gain factor and reacquire the high concentration standard. The new data should present analyte peaks with heights close to 2 million counts.

To confirm that this method is suitable, the ability to detect the analytes in the lowest concentration standard should be examined and the Gain factor adjusted accordingly.

The user will find that for concentrations less than 10 ng, a Gain factor near the trace analysis suggestion of Table 1 will be suitable in most cases.

As an illustration of this calculation, consider Figure 6. When acquired with a Gain factor of 1.0, the RTIC chromatogram of the high-concentration standard produces a highest peak for an analyte of 180k counts. To get about 2 million counts for the analyte, rearrange and use equation 1 above:

$$[1.0] \times (2 \times 10^6 \text{ counts}) / (180 \times 10^3 \text{ counts}) \cong 11 \text{ (the new Gain factor)}$$

Changing the Gain factor from 1.0 to 11 in the method and reacquiring the standard results in a new peak height of 2.04 million counts.

It is important to note that the Gain factor should actually be optimized using the extracted ion current for the most abundant ion signal. Also, 2 million counts may be considered as an approximation actually more applicable and pertinent to the reconstructed extracted ion current (REIC) chromatogram than the RTIC chromatogram as many compounds show a great deal of fragmentation.

- b) There is excessive signal and chromatographic peaks or REIC chromatograms are flat-topped (“clipped”).

Reduce the Gain factor to 0.25 and reinject the high concentration standard and examine the analyte REIC chromatograms. The REIC for the ions should be < 4 or 5 million counts. This situation is shown in Figure 7. If the REIC is too much lower than about 4 million, raise the Gain factor proportionally. If it is still very high (> 6 million counts), consider other method changes, such as a higher split in injection, etc., or sample preparation changes like dilution.

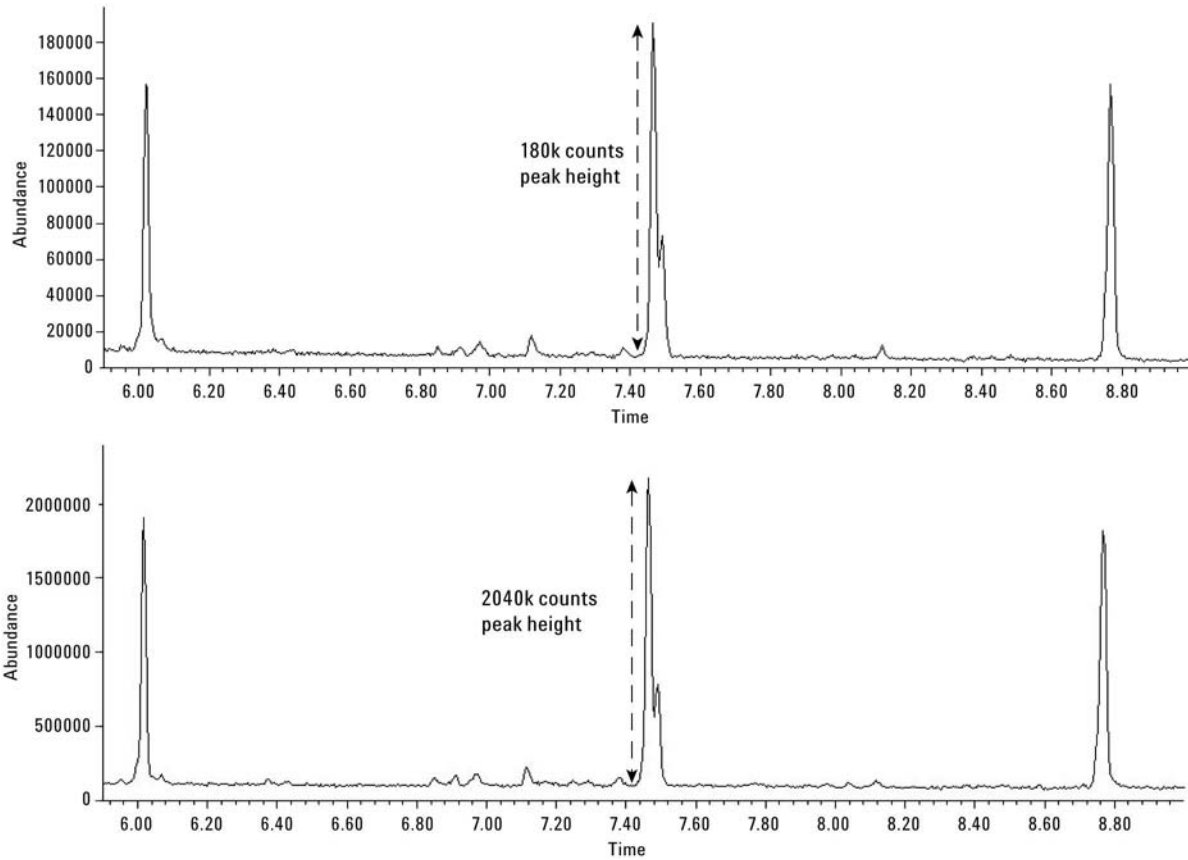


Figure 6. Using Gain factors for method optimization. Upper panel: RTIC of standard acquired at Gain factor 1.0 has a highest peak of 180k counts. Lower panel: Same standard reacquired with calculated Gain factor of 11 to produce target of 2×10^6 counts.

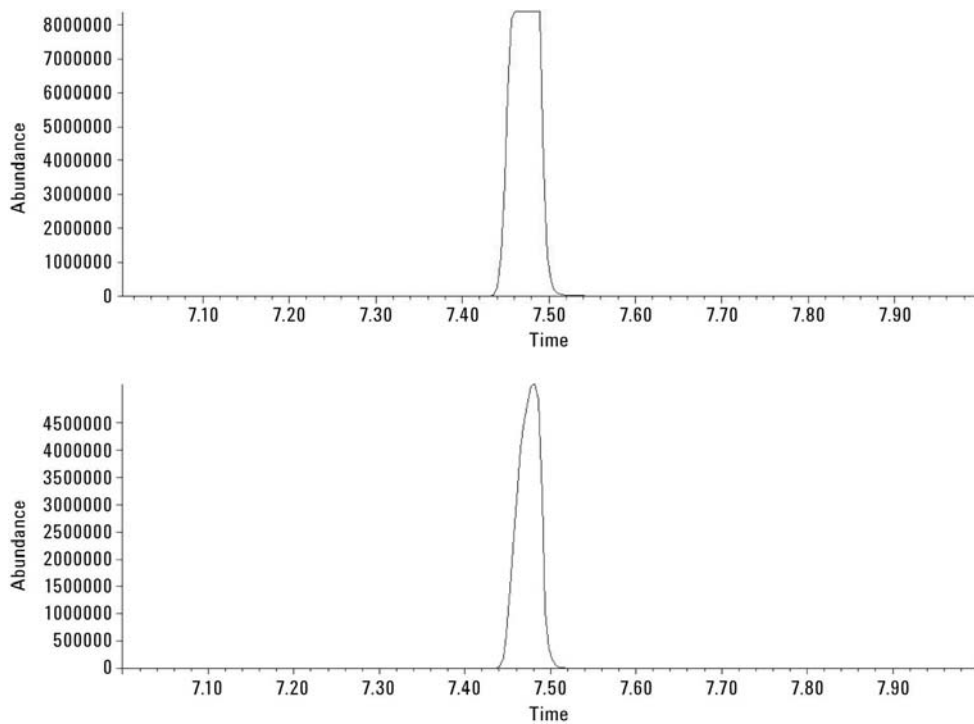


Figure 7. Using Gain factors for method optimization: handling excessive signal. Upper panel, reconstructed extracted ion chromatogram (REIC) of most abundant component showing "clipped" peak acquired at Gain factor 1. Lower panel, reacquired at Gain factor 0.25.

Summary

Gain Normalized methods have many advantages but the most important can be summarized by stating that signal is directly proportional to Gain setting (for example, doubling the Gain factor will double the signal). Users are accustomed to changing electron multiplier voltage settings; however, unlike Gain, signal is not directly proportional to the EM voltage. Because of this proportionality and the fact that despite detector aging, Gain can be consistently maintained up until the demise of the EM itself, Gain Normalized methods provide the user with:

- Better consistency in compound responses over time
- Better correspondence between instruments
- More diagnostic insights
- Better optimization of methods
- Simple “Tune and Use” methods

More advanced usage, such as in calibration curves based on Gain factors and other compound response-related issues, is also possible.

References

1. J. Kernan, H. Prest, “The 5975C Series MSDs: Normalized Instrument Tuning,” Agilent Publication 5989-6050EN

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