Autotune Function and Gain Calibration in the Agilent 1100 Series LC/MSD

Technical Note

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Introduction

Tuning is the process of adjusting an instrument to ensure optimum performance. The Agilent 1100 Series LC/MSD quadrupole mass spectrometer features an automatic tuning program (Autotune) that performs this function without operator interaction. One of the essential functions within Autotune is gain checking and gain calibration. This note provides an overview of LC/MSD tuning and the Autotune function, and a more detailed look at gain calibration and adjustment within Autotune.

What is Tuning?

When the Agilent 1100 Series LC/MSD quadrupole mass spectrometer is used as a detector for a liquid chromatograph (LC), a mass spectrum is associated with each data point in the LC chromatogram. To obtain high quality, accurate mass spectra, the LC/MSD must be optimized to:

- Maximize sensitivity
- Maintain acceptable resolution
- Ensure accurate mass assignments

Tuning is the process of adjusting LC/MSD parameters to achieve these goals. The software provides two ways to tune the LC/MSD:

- **Autotune** is an automated tuning program that adjusts the LC/MSD parameters for optimal performance over the entire mass range
- **Manual Tune** allows the user to tune the LC/MSD by adjusting one parameter at a time until the desired performance is obtained. Manual tune is most often used when maximum sensitivity is required, when a restricted mass range is to be targeted, or when a tuning compound other than the standard calibrants is needed.

The product of tuning is a tune file (actually a directory) containing two collections of parameter settings: one for positive ionization and one for negative ionization. Whenever a tune file is used, the software automatically loads the settings appropriate for the ion polarity specified by the method. Autotune also generates a report.

During data acquisition, the parameters associated with ion formation are controlled by the data acquisition method. The parameters associated with ion transmission are controlled by the tune file assigned to the data acquisition method.

Parameters Adjusted During Tuning

The LC/MSD has two sets of parameters that can be adjusted. One set of parameters is associated with the formation of ions. These parameters control the spray chamber (electrospray, atmospheric pressure chemical ionization, or atmospheric pressure photoionization) and fragmentor. The other set of parameters is associated with the transmission, filtering, and detection of ions. These parameters control skimmer 1 or skimmer 2 (present in earlier versions of the instrument), the octopole, lenses, quadrupole mass filter, the iris for the high energy dynode (HED), and the electron multiplier (detector).

It is important to note that the two sets of parameters are not tied to each other. The spray chamber settings (which include the fragmentor) are, as the name implies, specific to the spray chamber and vary depending upon the sample. The optics parameters, on the other hand, are inside the mass spectrometer itself and are independent of the choice of spray chamber.

Tuning is primarily concerned with finding the correct settings for the parameters that control the transmission, filtering, and detection of ions. It is accomplished by introducing a calibrant into the LC/MSD and generating ions. Using these ions, the tune parameters are then adjusted to achieve sensitivity, resolution, and mass assignment goals. With a few exceptions, the parameters that control ion formation are not adjusted. They are set to fixed values known to be good for generating ions from the calibrant solution.

Autotune

Autotune is a program that optimizes the transmission, filtering, and detection of ions without user intervention.

Autotune:

- Calibrates the mass axis
- Adjusts the (mass) peak widths/resolution
- Adjusts the ion optics for optimum ion transmission
- Checks the abundance and, if necessary, performs gain calibration (adjusts detector)

Autotune can be performed for both ion polarities or for a single polarity (positive or negative). If dual polarity is chosen, autotune is initially performed for positive ion mode. The result is saved as part of the tune file atunes.tun. Next, autotune starts over and performs the same steps for negative ion mode. These results are also saved in atunes.tun. The tune file contains two sets of parameters—one set for positive ion mode and one set for negative ion mode.

If one of the single-polarity autotunes is chosen, the steps are the same except that only parameters for the specified polarity are adjusted.

Frequent tuning, automated or manual, is not required. Once tuned, the LC/MSD is very stable. Tuning is generally not needed more often than monthly. If problems related to tuning are suspected, the Check Tune program can be used to confirm that the LC/MSD is out of adjustment before retuning.

Ion transmission

Figure 1 shows a software-generated plot of ion abundance versus fragmentor voltage for three tuning ions from the calibrant solution supplied with the LC/MSD. As the name implies, the fragmentor controls the fragmentation of ions. Its voltage is compound-specific because of stability differences among various molecules. Note that the calibrant compound whose mass-to-charge-ratio (*m/z*) is 118 optimizes with a much lower fragmentor setting than the other compounds in the solution. This is why the fragmentor is always dynamically ramped during automatic tuning (autotune), meaning that the voltage applied to the fragmentor varies across the mass range. For data acquisition, the proper setting of the fragmentor depends on the sample to be analyzed.

Figure 2 shows a similar plot for lens 1, one of the optics parameters. Notice how the ramps co-optimize across the mass range. The optics parameters do not vary with the type of compound, LC flow rate, or any of the spray chamber parameter settings. They deal strictly with the focusing and detection of the ions themselves. The optics are designed for optimal transmission of all masses. Autotune sets the parameters to maximize the transmission of all masses.

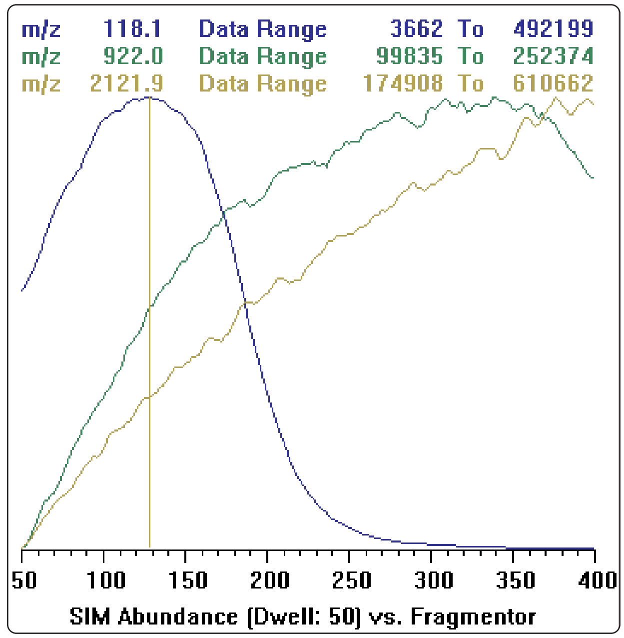

Figure 1. Plot of ion abundance versus fragmentor voltage

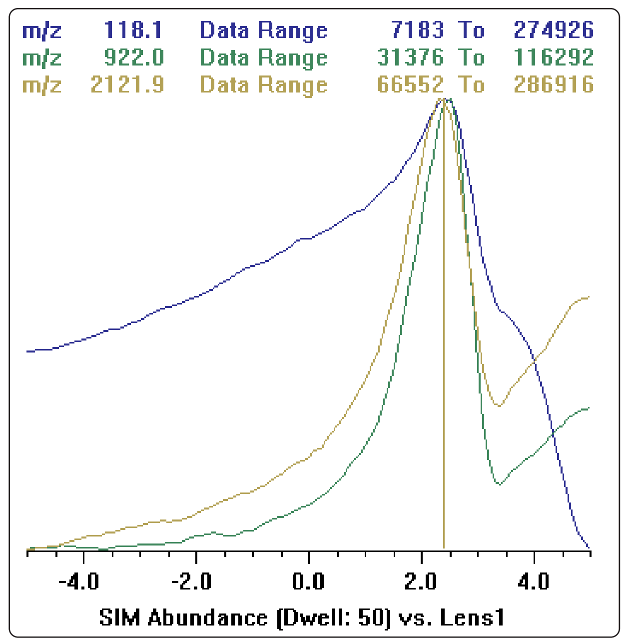

Figure 2. Plot of ion abundance versus lens 1 voltage

Gain calibration and adjustment

What is gain?

The HED electron multiplier receives an input current generated by the ions striking it, amplifies that current, and generates a proportional output current.

$$\text{Gain} = \frac{\text{Output current}}{\text{Input current}}$$

Gain is controlled by the electron multiplier voltage (EMV). The higher the EMV, the higher the gain. The relationship between EMV and gain is log linear. This linear relationship is common for all multipliers. Since the slope of this line is constant, the only adjustment that needs to be made is to the intercept. The gain calibration routine simply adjusts the value of the intercept for the specific instrument. The instrument-specific gain curve coefficients are then stored in the LC/MSD.

The use of gain enables the distribution of methods from instrument to instrument. In principle, using the same gain on two different instruments should result in the same signal response, making it easier to transfer methods from one instrument to another. Using gain also makes it easier to develop methods. Because the relationship between EMV and gain is log linear, a gain of 2.0 gives approximately twice the abundance of a gain of 1.0.

In general, the detector should be run at the lowest gain that still produces adequate abundance. High gains increase noise as well as signal and often result in poorer signal-to-noise ratios. Increasing the gain increases the EMV which shortens the life span of the electron multiplier.

As an electron multiplier ages, it slowly becomes less efficient. For a given ion current input, it generates a smaller and smaller output current (abundance). Low abundance caused by an aging electron multiplier is not easily distinguished from low abundance caused by poor ion generation or transmission (low ion current input). It may be tempting to compensate for poor ion generation or transmission by increasing the gain (thereby increasing the EMV) even though the electron multiplier is actually performing correctly at its previous gain. The increased gain will improve abundance, but may decrease the signal-to-noise ratio and will shorten the life span of the electron multiplier.

Gain calibration

A gain calibration checks the abundance of mass 622 for a gain setting of 1.0. If it is not within the target abundance range, the curve is adjusted. This means that the instrument consistently produces the same abundance for a gain setting of 1.0. The curve is linear, so that increasing the gain setting to 2.0 effectively doubles the resulting abundance.

Gain calibration during autotune

The autotune program checks ion abundances. If abundances are too low or too high, it automatically performs a gain calibration curve adjustment. The calibration curve is adjusted such that a gain setting of 1.0 produces the target signal abundance for mass 622.

Gain calibration shifts the gain curve so that a gain of 1, which is always used for positive ion autotune, occurs at an electron multiplier voltage that puts ion abundances within the autotune limits. If the multiplier setting exceeds 2600 volts, indicating decreasing electron multiplier performance, the user is warned that system maintenance may be required.

Gain calibration checking occurs only during the positive-ion portion of autotune. If ion abundances are too low or too high during the negative-ion portion of autotune, the gain is increased or decreased. Thus, the result of autotune is always a gain of 1 in positive mode but may be different (for example, 1.2) in negative mode. The reason for this is that there is only one gain curve for the instrument and it is constructed using the more commonly used positive ion mode. It does not make sense to perform gain compensation based on measurements in positive ion mode and then recompensate based on measurements in negative ion mode.

Conclusions

Tuning the LC/MSD is the process of finding the correct settings for parameters that control the transmission, filtering, and detection of ions. The LC/MSD has a sophisticated program for automatic tuning, as well as tools for manual tuning. Gain calibration, used with either tuning method, sets the electron multiplier voltage in a manner which simplifies method development and portability.

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Printed in the U.S.A. January 25, 2002
5988-5460EN