SCIEX
The Power of Precision

Analyst MD Software

Manual Tuning Tutorial

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Manual Tuning Tutorial

Users will learn how to manually tune and calibrate a mass spectrometer in quadrupole mode and in linear ion trap (LIT) mode.

Prerequisites
Users should be able to: • Build an acquisition method. • Submit a batch. The following peripheral devices and equipment are suggested: • An active hardware profile containing the mass spectrometer and syringe pump. • PPG or appropriate tuning solutions.

About Tuning

Tuning maximizes the resolution and intensity performance of the mass spectrometer. Do the following when tuning a mass spectrometer:

- Adjust the resolution offsets values to adjust the intensity and resolution of calibrant masses (for quadrupole mode only).
- Select the masses to be calibrated. If needed, masses can be added and removed from the calibration list.
- Create one or more unique calibration standard sets. A calibration standard set should have at least two compounds for the low and high ends of the mass range of interest.

As the mass spectrometer is tuned and calibrated, changes to the configuration are saved in an `InstrumentData` file in the API Instrument folder. The preset parameters in the API Instrument method folder should be used because they were optimized by the Field Service Employee (FSE) during installation.

Table 1 Tuning Frequency

	Calibration		Resolution Optimization	
Scan Type	**Frequency**	**Manual/ Automated**	**Frequency**	**Manual/ Automated**
Q1 and Q3	3 to 6 months	Both	3 to 6 months	Both
LIT	Every two weeks, as required	Both	3 to 6 months	Automated only

Resolution and Sensitivity

The software uses pre-defined unit, high, low, and open resolution values for quadrupole mode. For quadrupole scans, there is a balance between resolution and sensitivity. The wider the peak, the more intense the peak will be. The opposite is true for narrower peaks. LIT (linear ion trap) resolution and sensitivity are not connected because of how the LIT functions.

The following offsets for low and open resolution are typical, but they can be changed according to standard operating procedures.

- Low resolution (voltage decrease from unit resolution): 0.03
- Open resolution (voltage decrease from unit resolution): 0.30

Tip! Resolution settings can be checked or edited on the Resolution tab in the Tuning Options dialog. To open the dialog, in **Tune and Calibrate** mode, click **Tools** > **Settings** > **Tuning Options**.

Manual Adjustment of the Resolution

The software has four resolution parameters for the quadrupoles: unit, high, low, and open. The peak widths are set at 0.7 (± 0.1 Da FWHH (Full Width at Half Height)) for unit and 0.5 (± 0.1) for high resolution. This is done by adjusting the resolution offsets. The software calculates the low and open resolution parameters from the unit resolution parameter. Adjustments to the resolution offsets are made from the Resolution tab in the Tune Method Editor.

The Q1 resolution is varied for LIT scans depending on the scan used. The Q1 resolution is fixed for ER and EMS scans. The Q1 resolution for ER scans is preset to open, which allows a reasonable width of masses into the LIT.

For EPI and MS3 scans, set Q1 resolution at any of the selected resolution parameters. In general, this is set to unit resolution, but it can be set at a lower resolution either to allow a larger

mass window into the collision cell and show more isotopes or to increase sensitivity in the same way as running an MRM scan at a low resolution.

For LIT scan modes, the resolution is affected by scan speed. In general, the slower the scan speed, the better the resolution.

Adjustment of the Resolution on LIT Instruments

The resolution of a peak is determined by the mass of that peak and its peak width. In LIT mode, the resolution depends on how fast the ions are being mass-selectively ejected from the LIT. To change the sensitivity and resolution of the LIT scan types, use the Instrument Optimization feature. Refer to the document: *System User Guide* or the *Analyst MD Software Help*.

Mass Calibration

Mass calibration is the process of assigning the correct mass-to-charge values to mass peaks. By performing a mass calibration using a calibration standard, such as polypropylene glycol (PPG), compare the results with a previous calibration to determine how close the mass-to-charge values for the observed peaks are to the theoretical values. Update the previous calibration or, more typically, replace it with the new calibration.

Select multiple masses when calibrating Q1, Q3, and all LIT scans for each polarity. The results are stored in a calibration table. When a mass calibration is performed, the calibration table is updated with new digital-to-analog converter (DAC) values from the new calibration. DAC values for masses already in the calibration table are updated. All data for masses not calibrated in the current calibration are retained but not used. If the mass calibration is replaced, then all of the previous calibration values for all of the masses selected for use are replaced.

Perform a mass calibration using a newly acquired spectrum, or use a spectrum from a stored data file.

While performing a mass calibration, the software:

1. Finds the largest peak in the search range for each selected mass.
2. Obtains the mass, intensity, and peak width values.
3. Compares the observed mass with the expected mass and calculates the shift, if any.
4. Compares the peak width with the target peak width.
5. Compares the intensity with the previous calibration.
6. Shows the results in a graph and text format.

7. Saves the calibration table in the instrument data file in the `<Drive>:\Analyst Data\Projects\API Instrument\Instrument Data` folder.

Manual Mass Calibration

After manually adjusting the Q1 and Q3 quadrupole resolutions, check the calibration. Changes to the parameters during a resolution optimization can affect the previous mass calibration.

The calibration report shows three graphs: the mass shift, the peak width, and the intensity difference.

- The mass shift graph shows the difference between the measured masses from the current calibration and the actual masses from the reference table.
- The peak width graph shows the peak width for each mass as compared to the target width selected in the acquisition method.
- The intensity difference graph shows the intensity difference between the previous calibration and the current calibration.

Tuning and calibrating should only be performed by experienced operators and tuning parameters should only be performed by FSEs.

Technical Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the website at sciex.com.

Manually Tune and Calibrate in Quadrupole Mode

To properly tune and calibrate the mass spectrometer, adjust the resolution and perform a mass calibration.

For each scan type using a particular calibration solution, a different acquisition method is required. Different calibration solutions are used for positive and negative mode calibration. If the analysis is a subset of both the scan type and the polarity, then the user can also tune just one quadrupole, one polarity, or one resolution type instead of performing the complete tuning and calibration process for all combinations of quadrupole, polarity, and resolution type.

Follow the procedures in the order given:

1. Select an Acquisition Method

2. Adjust the Resolution
3. Perform Mass Calibration in Quadrupole Mode

Select an Acquisition Method

1. Create a project to save the calibration methods and results.
   This can be specific for tuning or it can be part of a working project.
2. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning**.
3. Create an appropriate acquisition method in Manual Tune or go to the **API Instrument** folder and then open the acquisition method for calibration if a 3200MD series of instrument is used.

   Tip! (**Only applicable to 3200MD series of instruments**) The software is not installed with the default acquisition methods for optimizing at high resolution. The only difference, however, between these methods and the methods for unit resolution (Q1PosPPG.dam, Q1NegPPG.dam, Q3PosPPG.dam, and Q3NegPPG.dam) is the resolution type. The first time the mass spectrometer is tuned and the high resolution methods are required, open the unit resolution methods, change the resolution type to high, and then save them with a different name, such as Q1PosPPG_high.dam.
4. Infuse 5 µL/min to 10 µL/min of either the PPG solution for tuning in Q1 and Q3 positive mode, or the PPG 3000, the solution for tuning in Q1 and Q3 negative mode.

   Tip! Copy the method so that the original method can be kept for the FSE.
5. Select a new project from the project list and then save the method using the same name.

Adjust the Resolution

Make sure that the spray is stable.

1. In the Tune Method Editor, on the MS tab, make sure that the **MCA** check box is selected.
2. In the **Period Summary** section, in the **Cycles** field, type `10`.
3. Click **Start**.
   A mass spectrum pane is shown at the bottom of the Manual Tune window.
4. After the mass spectrometer is in Idle state, right-click in the mass spectrum pane, and then click **Open File**.
   In a new window, one mass spectrum data pane is shown for each mass in the method.
5. On one of the data panes, right-click and then click **List Data**.

A new pane opens listing the data for the spectrum. This pane contains the Data List, Calibration Peak List, and Peak List tabs.

6. Open the Calibration Peak List tab.

   Tip! This tab is shown only if the option to show it is selected. To show the Calibration Peak List tab, click **Tools** > **Settings** > **Appearance Options.** On the Miscellaneous tab, select the **Show Mass Calibration Peak List** check box and then click **OK**.

7. If the target masses in the Calibration Peak List do not match the masses shown, right-click the **Calibration Peak List**, click the appropriate **Reference** list, and then click **Use as Reference**.
8. Examine the data in the **Calibration Peak List**.

   Note: If the values in the **Width (Da)** column are all 0.7 ± 0.1 Da for unit resolution or 0.5 ± 0.1 Da for high resolution, then the resolution is acceptable.

   - If the resolution is acceptable, then continue with step 14.
   - If the values are not within the required tolerance, then continue with step 9.

9. Close the panes and then, in the Tune Method Editor, open the Resolution tab.
10. Click **Advanced**.

    The Resolution Table dialog opens. This dialog lists the calibration peak masses and their resolution offset values for that scan.

11. For each mass that does not fall within the peak width criteria of 0.7 ± 0.1 Da for unit resolution or 0.5 ± 0.1 Da for high resolution, adjust the offset as follows:

    - If the peak is too wide, then increase the offset by 0.05 or less.
    - If the peak is too narrow, then decrease the offset by 0.05 or less.

12. Click **Apply**.
    The changes are saved to the InstrumentData file.
13. Click **Close**.
14. Repeat steps 1 through 8 for every mass peak until all of the peaks in the Calibration Peak List meet the peak width criteria of 0.7 ± 0.1 Da for unit resolution or 0.5 ± 0.1 Da for high resolution.
15. Click **Close**.

Perform Mass Calibration in Quadrupole Mode

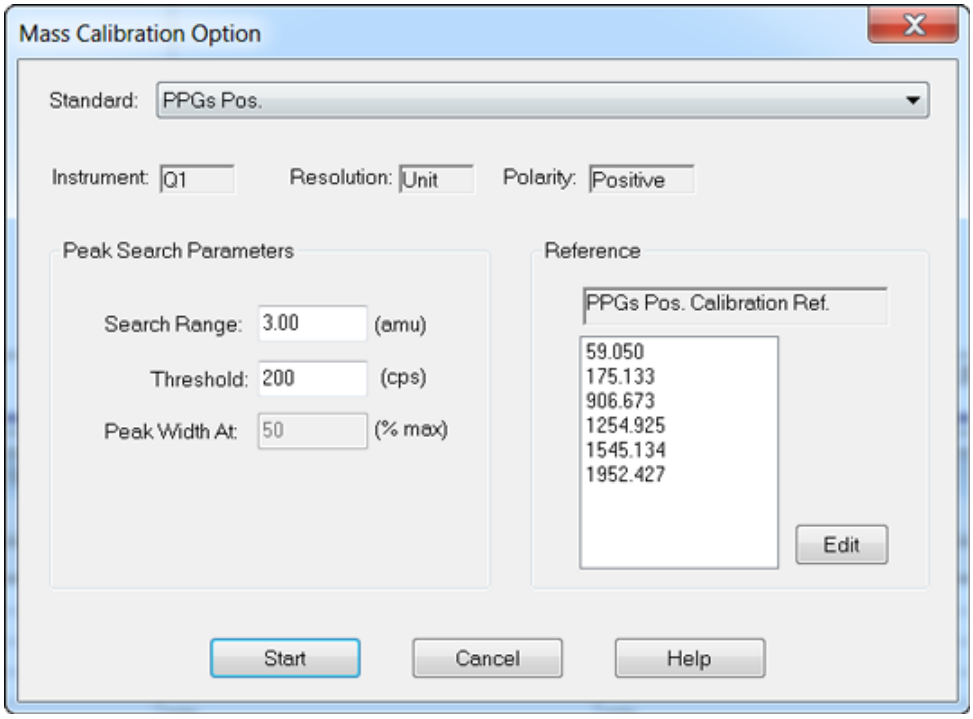
1. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning**.

2. In the Tune Method Editor, on the MS tab, make sure that the **MCA** check box is selected.
3. In the **Period Summary** section, in the **Cycles** field, type `10`.
4. Click **Start**.
   A mass spectrum pane is shown at the bottom of the Manual Tune window.
5. After the mass spectrometer is in Idle state, right-click in the mass spectrum pane, and then click **Open File**.
   In a new window, one mass spectrum data pane is shown for each mass in the method.
6. On one of the data panes, right-click and then click **List Data**.
   A new pane opens showing the data for the spectrum. This pane contains the Data List, Calibration Peak List, and Peak List tabs.
7. Open the Calibration Peak List tab.

   Tip! This tab is shown only if the option to show it is selected. To show the Calibration Peak List tab, click **Tools** > **Settings** > **Appearance Options.** On the Miscellaneous tab, select the **Show Mass Calibration Peak List** check box and then click **OK**.
8. Examine the data in the Calibration Peak List tab. If the value in the **Mass Shift (Da)** column is greater than 0.1 Da for any of the masses, then proceed to the next step. Otherwise, the mass calibration is complete.

   Note: Peak labels in the graph are the apex values but the peak values listed in the **Found At** column in the Calibration Peak List tab are centroid values. If a peak is not perfectly symmetrical, then the apex value and the centroid value for the same peak might be slightly different. The more accurate centroid values are used for calibration.
9. Click anywhere in one of the mass spectrum panes.
10. Click **Tools** > **Calibrate from Spectrum.**
    The Mass Calibration Option dialog opens.

Figure 1 Mass Calibration Option Dialog



11. In the **Standard** list, click **PPGs Pos**. or **PPGs Neg**. according to the polarity indicated by the acquisition method used.
12. The preset Peak Search Parameters are appropriate for most situations. If you want to change them, then click a field and then type the new values.

    Note: On mass spectrometers with a larger mass range, PPG peaks around 2000 can give peaks where the most intense isotope is not the first isotope, which can cause calibration issues. If this happens, then narrow the Search Range to 0.8.

13. Verify that the masses listed in the Reference list match those for which data is acquired. If the masses match, then proceed to the next step. If the masses do not match, then do the following:

    a. Click **Edit**.

       The Reference Table dialog opens.

    b. Match the masses in the **Reference** list with the masses for which data is acquired by selecting or clearing the check boxes in the **Use** column.

    c. Click **Update Ref.** to save the changes.

    d. Click **Close**.

14. Click **Start** to begin the mass calibration.

- The software finds the largest peak in the search range for each mass and determines the mass, intensity, and peak width values.
- The software compares the mass with the expected mass and determines the mass shift, if any, compares the peak width with the target peak width, and compares the intensity with the previous calibration.
- The software shows the mass calibration results graphically and in report form.

15. If the software has not selected the correct peak for the target mass, then exclude the point from the calibration.

Tip! On the point to be excluded, right-click and then click **Exclude**.

16. Click **Window** and then click the calibration results.
A text version of the Calibration report is shown.
17. In the Calibration report window, do one of the following:
    - To update values for the changed masses and to add the values from any new masses to the existing mass calibration, click **Update Mass Calibration**. Only the existing calibration values for masses that were calibrated on are overwritten.
    - To completely replace the existing masses and values with the new masses and values, click **Replace Mass Calibration**. All of the existing calibration values are overwritten and any mass not calibrated on is removed from the calibration table.
18. For the mass calibration changes to take affect, click **Save**.
19. Click **Close**.
20. Perform a 10 scan MCA to check the calibration.

Note: Repeat the calibration procedure if necessary.

Manually Calibrate the Mass Spectrometer in LIT Mode

To calibrate the mass spectrometer in LIT mode, perform a mass calibration for the scan speeds in both positive and negative mode.

Follow the procedures in the order given:

1. Select the Acquisition Method to Manually Calibrate in LIT Mode
2. Perform Mass Calibration in LIT Mode

Select the Acquisition Method to Manually Calibrate in LIT Mode

1. Infuse the Agilent Mix or PPG 3000 at 5 µL/min to 10 µL/min.
2. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning**.
3. (3200MD QTRAP systems). Create a proper Enhanced Resolution acquisition method at a selected scan rate or click **File** > **Open**.
4. In the **Files** list, select a method from the **API Instrument** > **Acquisition Methods** > **QTRAP3200**.
5. Click **OK**.
   The Tune Method Editor shows the details of the selected method.
6. Select a new project from the project list and then save the method data.

Perform Mass Calibration in LIT Mode

1. In the Tune Method Editor, on the MS tab, make sure that the **MCA** check box is selected.
2. On the MS tab, select the polarity and scan rate.
3. On the MS tab, in the **Period Summary** section, in the **Cycles** field, type `50`.
4. Click **Start**.
   A mass spectrum pane is shown at the bottom of the Manual Tune window.
5. After the MS icon shows Idle, in the mass spectrum pane, right-click and then click **Open File**.
   A new window opens with each ion of interest in a separate pane.
6. On one of the data panes, right-click and then click **List Data**.
   A new pane opens showing the data for the spectrum. This pane contains the Data List, Calibration Peak List, and Peak List tabs.
7. Open the Calibration Peak List tab.

   Tip! This tab is shown only if the option to show it is selected. To show the Calibration Peak List tab, click **Tools** > **Settings** > **Appearance Options.** On the Miscellaneous tab, select the **Show Mass Calibration Peak List** check box and then click **OK**.
8. Right-click in the Calibration Peak List table and then in the right-click menu, make sure the correct reference table is selected.

   If the correct reference table is not selected, then select the correct reference table and click **Use as Reference**.

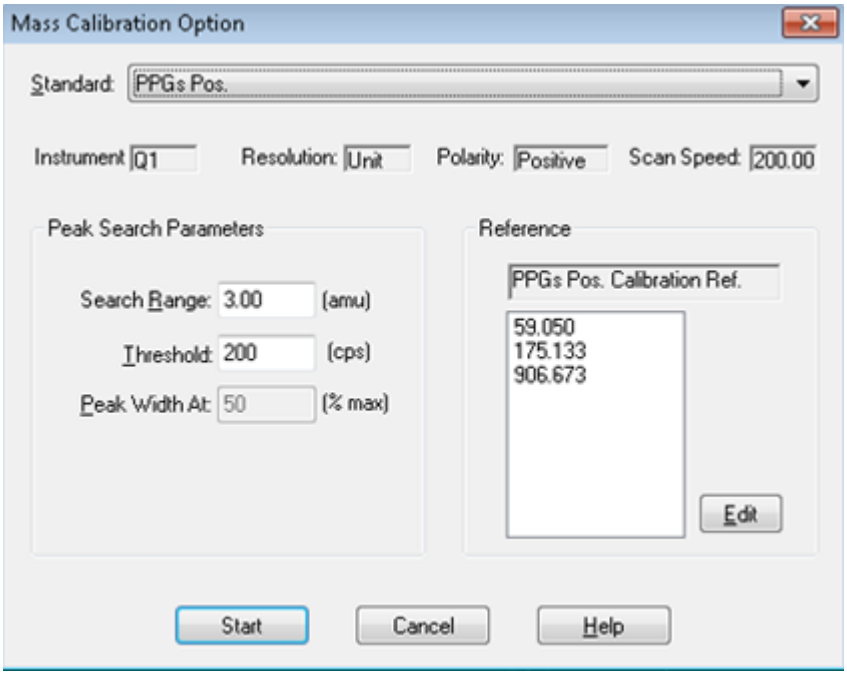
Note: If all of the masses are not shown in the **Calibration Peak List** tab, then right-click in the Calibration Peak List table. In the right-click menu, place the cursor on the reference table in use, and then in the submenu, click **Edit Reference Table**. In the Reference Table Edit dialog, check the **Use** box for the masses to be shown in the Calibration Peak List tab, and then click **Update Ref**.

9. Examine the data in the Calibration Peak List tab. If the value in the **Mass Shift (Da)** column is greater than 0.1 Da for any of the masses, then proceed to the next step. Otherwise, the mass calibration is complete.

Note: Peak labels in the graph are the apex values but the peak values listed in the **Found At** column in the Calibration Peak List tab are centroid values. If a peak is not perfectly symmetrical, then the apex value and the centroid value for the same peak might be slightly different. The more accurate centroid values are used for calibration.

10. Click anywhere in one of the mass spectrum panes.
11. Click **Tools** > **Calibrate from Spectrum.**
    The Mass Calibration Option dialog opens.

Figure 2 Mass Calibration Option Dialog



12. In the **Standard** list, if PPG was used as the standard solution, then click **PPGs Pos. LIT Ref.** or **PPGs Neg. LIT Ref.** according to the polarity indicated by the acquisition method used.
13. To change the Peak Search Parameters, click a field and then type the new values.

The preset Peak Search Parameters are appropriate for most situations.

14. Check that the masses listed in the **Reference** list match those for which data is acquired.

    If the masses match, proceed to the next step. If the masses do not match, complete the following steps:

    a. Click **Edit**.

       The Reference Table dialog opens.

    b. Match the masses in the **Reference** list with the masses for which data is acquired by selecting or clearing the check boxes in the **Use** column.

    c. Click **Update Ref.** to save the changes.

15. Press **Start** to begin the mass calibration.

    - The software finds the largest peak in the search range for each mass and determines the mass, intensity, and peak width values.
    - The software compares the mass with the expected mass and determines the mass shift, if any, compares the peak width with the target peak width, and compares the intensity with the previous calibration.
    - The software shows the mass calibration results graphically and in report form.

    Note: Do not use the middle peak width graph indicators, that is, the dotted lines. These were created for quadrupole scans and do not apply to LIT scans.

16. Click **Window** and then click the calibration results.
    A text version of the Calibration report is shown.

17. Examine the slope variation values. They must be 1.000 ± 0.002. There will be an **N/A** for the lowest data point because a point cannot have a slope.

18. If the difference is greater than 0.002, then do not calibrate the mass spectrometer. Contact SCIEX Support at sciex.com/request-support.

19. If the slope variation numbers are good, then go to the Calibration report window.

20. In the Calibration report window, do one of the following:

    - To update values for the changed masses and to add the values from any new masses to the existing mass calibration, click **Update Mass Calibration**. Only the existing calibration values for masses that were calibrated on are overwritten.
    - To completely replace the existing masses and values with the new masses and values, click **Replace Mass Calibration**. All of the existing calibration values are overwritten and any mass not calibrated on is removed from the calibration table.

21. For the mass calibration changes to take affect, click **Save**.

22. Click **Close**.
23. Perform a 10 scan MCA to check the calibration.

Note: Repeat the calibration procedure if necessary.

Tune and Calibrate Mode Icons

Icon	Name	Description
	**Calibrate from spectrum**	Opens the Mass Calibration Option dialog and uses the active spectrum to calibrate the mass spectrometer.
	**Manual Tune**	Opens the Manual Tune Editor.
	**Compound Optimization**	Optimizes for a compound using infusion by FIA.
	**Instrument Optimization**	Verifies the instrument performance, adjusts the mass calibration, or adjusts mass spectrometer settings.
	**View Queue**	Shows the sample queue.
	**Instrument Queue**	Shows a remote instrument.
	**Status for Remote Instrument**	Shows the status of a remote instrument.
	**Reserve Instrument for Tuning**	Reserves the instrument for tuning and calibrating.
	**IDA Method Wizard**	Starts the IDA Method Wizard.

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- SCIEX Now Learning Hub

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