Analyst® 1.6.1 Software

Manual Tuning Tutorial

Topics in this tutorial:

Objectives

You will learn how to manually tune and calibrate an instrument in quadrupole mode and linear ion trap (LIT) mode.

About Tuning

Instrument tuning maximizes the resolution and intensity performance of the instrument. You can do the following when you tune an instrument:

- 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 you tune and calibrate the instrument, changes to the instrument configuration are saved in data files in the API Instrument folder. The preset parameters in the API Instrument method folder should be used because they were optimized by the FSE during installation.

Table 1-1 Tuning Frequency

Scan Type	Calibration		Resolution Optimization	
	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

About 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

The value of the voltage decrease from the *unit* resolution field may vary from instrument to instrument, but for small molecule applications this value should be set to 0.03 for *low* and 0.3 for *open*. For proteomics applications, the recommended setting is 0.08 to 0.15 for *low* and 0.3 for *open*. The voltage decrease for *low* resolution is greater for proteomics applications to allow for more isotopes from a multiply-charged profile to be displayed as compared to the setting for small molecule applications. The software uses this value to calculate the value of low or open resolution in a method.

> **Tip!** To check or edit the resolution settings, in **Tune and Calibrate** mode, click **Tools > Settings > Tuning Options**.

About 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) 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. You can adjust the resolution offsets 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, EMS, and EMC scans. The Q1 resolution for ER scans is preset to *open*, which allows a reasonable width of masses into the LIT.

For EPI, MS3, and TDF scans, you can set Q1 resolution at any of the selected resolution parameters. In general, this is set to *unit* resolution, but it can be set at lower resolution to either let a larger mass window into the collision cell and show more isotopes or to increase sensitivity the same way you would if you were running an MRM scan at a *low* resolution.

For LIT scan modes, the resolution is determined by scan speed. In general, the slower the scan speed is, the better the resolution will be.

About 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. For more information, see the *Getting Started Guide* or Help.

About 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 PPG, you can 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. You can then update the previous calibration or, more typically, replace it with the new calibration.

You can select multiple masses when calibrating Q1, Q3, and all LIT scans for each polarity. The results are stored in a calibration table. When you perform a mass calibration, you are updating the calibration table with new DAC (digital-to-analog converter) 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. If you choose to replace the mass calibration, all previous calibration values for all masses are replaced.

You can perform a mass calibration using a newly acquired spectrum, or you can 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 determines the shift, if any.

4. Compares the peak width with the target peak width.

5. Compares the intensity with the previous calibration.

6. Displays 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.

About Manual Mass Calibration

After you have manually adjusted the Q1 and Q3 quadrupole resolutions, you should check the calibration. Changes to the parameters during a resolution optimization can effect the previous mass calibration.

The calibration report displays 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.

In this tutorial, you will learn how to tune and calibrate an instrument. Tuning and calibrating should only be performed by experienced instrument operators and tuning instrument parameters should only be performed by FSEs.

Related Documentation

- *Getting Started Guide* for the Analyst® software (for tuning solutions and concentrations)
- *Advanced User Guide* for the Analyst software
- Analyst software Help system
- Operator's guide for the instrument

Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the Web site at www.absciex.com.

Prerequisites

You should be able to:

- Build an acquisition method
- Submit a batch

The following peripheral devices and equipment are suggested:

- A hardware profile containing the mass spectrometer and syringe is active
- PPG tuning solutions

Manually Tuning and Calibrating in Quadrupole Mode

To properly tune and calibrate the instrument, you must adjust the resolution and perform a mass calibration.

For each scan, a different acquisition method is required. Different calibration solutions are used for positive and negative mode calibration. If your analysis is a subset of both the scan type and the polarity, then you 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:

Selecting an Acquisition Method

Before you begin, make sure the active hardware profile contains the mass spectrometer and a syringe.

1. Create a project that you can keep calibration methods and results in. This can be specific for instrument tuning or it can be part of a working project.

2. On the Navigation bar, under **Tune**, double-click **Manual Tuning**.

3. Go to API Instrument folder and open the acquisition method for calibration.

> **Tip!** 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 you tune the instrument and require the high resolution methods, you can 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 to 10 µL/min of either PPG solution for tuning Q1 and Q3 in positive mode, or PPG 3000, solution for tuning in Q1 and Q3 negative mode.

> **Tip!** Copy the method so that you can keep the original method for the FSE.

5. Select a new project from the project list and then save the method using the same name.

Adjusting the Resolution

Before you begin this procedure, make sure that you have a stable spray.

1. In the **Tune Method Editor**, on the **MS** tab, make sure that the **MCA** check box is selected.

2. In the **Period** section, in the **Cycles** field, type **10**.

3. Click **Start**.

   A mass spectrum pane appears at the bottom of the Manual Tune window.

4. After the MS icon displays Idle, in the mass spectrum pane, right-click and then click **Open File**.

   A new window appears. In it, one mass spectrum data pane appears for each mass in the method.

5. On one of the data panes, right-click and then click **List Data**.

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

6. Click the **Calibration Peak List** tab.

   > **Tip!** This tab appears only if the option to display it is selected. To display 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 displayed, 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. 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 values are not within the required tolerance, then continue with the next step.

9. Close the panes and then, in the **Tune Method Editor**, click the **Resolution** tab.

10. On the **Resolution** tab, click **Advanced**.

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

11. For each mass that does not fall into 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** to save the changes to the instrument data file and then click **Close**.

13. Repeat the preceding steps for every mass peak until all peaks in the calibration list meet the peak width criteria of 0.7 ±0.1 Da for unit resolution or 0.5 ±0.1 Da for high resolution.

14. Click **Close**.

Performing 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** section, in the **Cycles** field, type **10**.

4. Click **Start**.

   A mass spectrum pane appears at the bottom of the Manual Tune window.

5. After the MS icon displays Idle, in the mass spectrum pane, right-click and then click **Open File**.

   In a new window, one mass spectrum pane appears for each mass in the method.

6. (Optional) You can create a table directly from a graphical pane. On one of the data panes, right-click and then click **List Data**.

   A new pane appears listing the data for the spectrum.This pane contains three tabs: **Data List**, **Calibration Peak List**, and **Peak List**.

   • Click the **Calibration Peak List** tab.

   > **Tip!** This tab appears only if the option to **display** it is selected. To display 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**.

   • Examine the data in the calibration peak list. 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.

7. Click anywhere in one of the mass spectrum panes.

8. Click **Tools > Calibrate from Spectrum**.

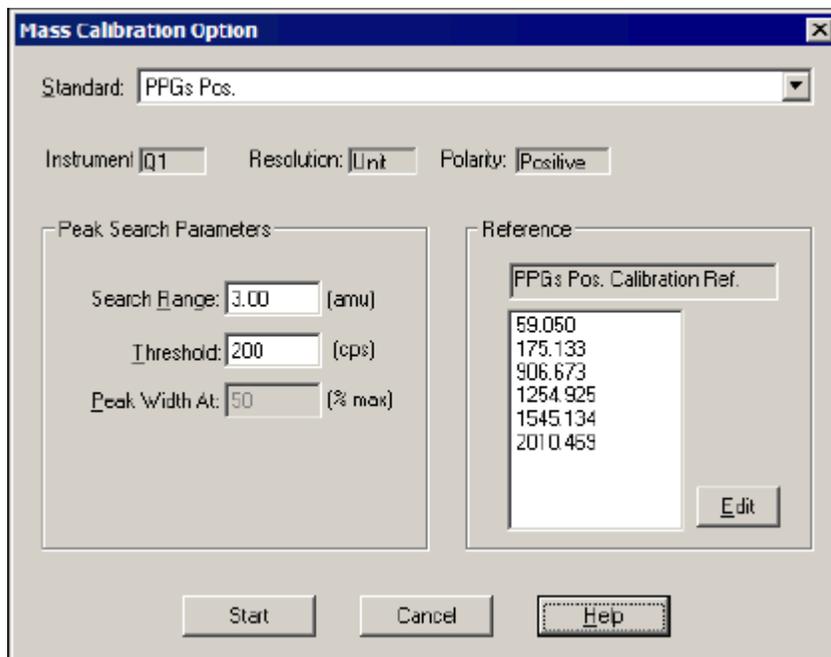   The Mass Calibration Option dialog appears.

Figure 1-1   Mass Calibration Option dialog

9. In the **Standard** list, click **PPGs Pos.** or **PPGs Neg.** according to the polarity indicated by the acquisition method used. The preset Peak Search Parameters are appropriate for most situations.

> **Note:** On instruments 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 you can narrow the Search Range to 0.8.

10. Check that the masses listed in the Reference list include the masses you want to use for calibration. If they match, proceed to the next step. If they do *not* match, then do the following:

    •   Click **Edit**.

    The Reference Table dialog appears.

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

    •   Click **Update Ref.** and then click **Close**.

11. 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 displays the mass calibration results graphically and in report form.

12. If the software has not selected the correct peak for the target mass, then you can exclude the point from the calibration. On the point to be excluded, right-click and then click **Exclude**.

13. Click **Window** and then click the calibration results. This is a text version of the Calibration report.

14. 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.

15. If the difference is greater than 0.002, then do not calibrate the instrument. Contact AB SCIEX support.

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

17. 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 table.

18. For the mass calibration changes to take effect, click **Save** and then click **Close**.

19. Perform a 10 scan MCA to check the calibration. Repeat the calibration procedure if necessary.

Manually Calibrating the Instrument in LIT Mode

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

Follow the procedures in the order given:

Selecting the Acquisition Method for Manually Calibrating in LIT Mode

Before you begin, make sure the active hardware profile contains the mass spectrometer and a syringe.

1. Infuse 5 to 10 µL/min using of Agilent Mix or PPG 3000.

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

3. Make sure that you are using the API Instrument project.

4. Click **File > Open**.

5. In the **Files** list, select a method.

6. Click **OK**.

   The Tune Method Editor displays the details of the selected method.

7. Select a new project from the project list and then save the method data.

Performing 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 appears at the bottom of the Manual Tune window.

5. After the MS icon displays Idle, in the mass spectrum pane, right-click and then click **Open File**.

   A new window appears 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 will appear listing the data for the spectrum. This pane contains three tabs: Data List, Calibration Peak List, and Peak List.

7. Click the **Calibration Peak List** tab.

   > **Tip!** This tab appears only if the option to display it is selected. To display 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. 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.

9. Click anywhere in one of the mass spectrum panes.

10. Click **Tools > Calibrate from Spectrum**.

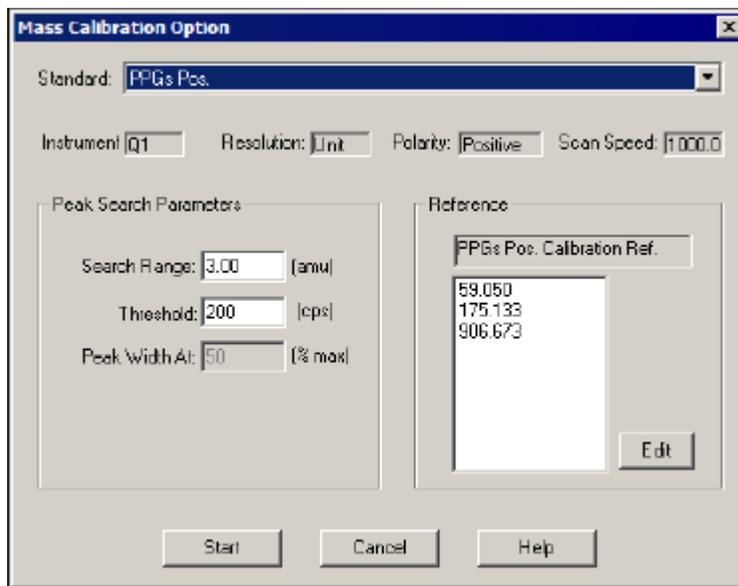   The Mass Calibration Option dialog appears.

Figure 1-2   Mass Calibration Option dialog

11. In the **Standard** list, click **PPGs Pos. LIT Ref.** or **PPGs Neg. LIT Ref.** according to the polarity indicated by the acquisition method you are using.

12. The preset Peak Search Parameters are appropriate for most situations. If you want to change them, click a field and then type the new values.

13. Check that the masses listed in the Reference list match those for which you acquired data. If they match, proceed to the next step. If they do not match, complete the following steps.

   • Click **Edit**.

     The Reference Table dialog appears.

   • Match the masses in the Reference list with the masses you acquired data for by selecting or deselecting the check boxes in the **Use** column.

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

14. 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 displays the mass calibration results graphically and in report form.

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

15. Go to the window menu and select the calibration results. This is a text version of the Calibration report.

16. Examine the slope variation values. they need to be 1.000 plus or minus 0.002. There will be an N/A for the lowest data point since a point cannot have a slope.

    If the difference is greater than 0.002 then there is either a problem with the instrument or the peak could be incorrectly labelled. Do not Calibrate the instrument if this situation occurs. contact an expert user or AB technical support.

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

18. 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 table.

19. For the mass calibration changes to take effect, click **Save** and then click **Close**.

20. Perform a 10 scan MCA to check the calibration. Repeat the calibration procedure if necessary.

Table 1-2 Icons in Tune and Calibrate Mode

Icon	Name	Function
	Calibrate from spectrum	Click to open the Mass Calibration Option dialog and use the active spectrum to calibrate the instrument.
	Manual Tune	Click to open the Manual Tune Editor.
	Compound Optimization	Click to optimize for a compound using infusion of FIA.
	View Queue	Click to view the sample queue.
	Instrument Queue	Click to view a remote instrument station.
	Status for Remote Instrument	Click to view the status of a remote instrument.
	Reserve Instrument for Tuning	Click to reserve the instrument for tuning and calibrating.
	IDA Method Wizard	Click to start the IDA Method wizard.