Analyst® 1.6 Software

Manual Optimization Tutorial

AB SCIEX

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Topics in this section:

Objectives

- Confirm the presence of compounds of interest.
- Determine the fragments for optimization.
- Optimize compound-dependent parameters.

About Manual Optimization

Manual optimization is used to optimize compound- and ion source-dependent parameters for the analyte. When you manually optimize for an analyte, you create an MS acquisition method in Tune and Calibrate mode. Based on the method of sample introduction you choose, you can add an LC method to the acquisition method so that infusion or LC can be used.

In general, when you optimize any parameter you want to obtain the lowest noise and the best signal. When adjusting the ion source parameters, an increase in the signal can result in an increase in the noise. If this occurs, readjust the parameters to lower the noise without sacrificing the signal.

Note: You cannot get a signal-to-noise calculation using infusion.

When optimizing ion source-dependent parameters, you must introduce the sample at flow rates that will be used during sample analysis, which means that you should use either FIA (flow injection analysis) or split infusion as the method of sample introduction. The only exception to this rule is the CAD gas, which is not an ion source parameter but is grouped with them. Because the CAD gas is used in the ion path for focusing and cooling ions, as well as collisionally activated dissociation, you can use infusion to introduce samples when you optimize CAD gas.

Before you optimize the ion source-dependent parameters, you should optimize the position of the ion source. For more information, see the appropriate ion source operator's guide.

About Scan Types

For this example, use the Q1 MS, Q1 MI, Product Ion, and MRM scan types. The Q1 MS scan type is used to confirm the presence of compounds of interest to optimize MS or pre-collision cell voltages. The Product Ion scan is used to determine the product ions of each compound. The MRM scan type is used to optimize the CE (collision energy) and CXP (collision cell exit potential) for each product ion or fragment. You can use the methods that you create in this tutorial for quantitative or qualitative analysis.

Related Documentation

- *Getting Started Guide* for the Analyst® software
- *Software Reference Guide* for the Analyst software
- Analyst software Help system
- The appropriate ion source guide

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

- The instrument is tuned and calibrated.
- There is an acquisition method.
- There is a hardware profile containing a syringe pump.

You must control the autosampler and injection valve manually, as these devices cannot be controlled through the system while it is in Tune and Calibrate mode.

The following solutions are suggested. You can use other solutions depending on your system, peripheral devices, and experiment.

- Mobile phase 1:1 acetonitrile:water + 2 mM ammonium acetate + 0.1% formic acid.
- 4-compound mix, consisting of reserpine, minoxidil, tolbutamide, and rescinnamine. Use a solution that is 49.9% acetonitrile, 50% deionized water, 0.1% formic acid as diluent.

Table 1-1 Compounds and Molecular Weights

Compound	m/z
Minoxidil	210.2

Table 1-1 Compounds and Molecular Weights (Continued)

Compound	m/z
Tolbutamide	271.3
Reserpine	609.4
Rescinnamine	635.4

Table 1-2 Estimates For Starting Concentrations

System	Concentration
API 2000™ system and QTRAP® system	500 ng/mL
API 3200™ system and 3200 QTRAP system	50 ng/mL
API 4000™ system and 4000 QTRAP system	10 ng/mL
API 5000™ system	10 ng/mL
AB SCIEX QTRAP 5500 system	10 ng/mL
AB SCIEX Triple Quad™ 5500 system	10 ng/mL

Manually Optimizing for an Analyte

After the acquisition method is created, you can optimize compound-dependent parameters using the Edit Ramp function or by manually editing the parameters in the Tune Method Editor. Ion source-dependent parameters can be optimized only by manually adjusting the parameters in the Tune Method Editor. Depending on the scan type you are using, different parameters are available to optimize.

Follow the procedures in the order given:

1. Confirming the Presence of Compounds on page 5
2. Optimizing Pre-collision Cell Voltages on page 7
3. Determining the Product Ions for Optimization on page 9
4. Optimizing Collision Energy for each Product Ion on page 10
5. Optimizing Collision Cell Exit Potential for each Product Ion on page 11

Confirming the Presence of Compounds

You should confirm the presence of compounds of interest before continuing with manual optimization.

1. Activate the hardware profile.
2. Infuse the compound in solution at a rate of 5 to 10 µL/min.
3. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning**.
4. From the method list, select **Syringe Pump Method**.
5. On the **Syringe Pump Method Properties** tab, type the parameters from the following table.

Table 1-3 Syringe Pump Method Properties Tab Parameters

Field	Value
Syringe Diameter	4.61 if a 1.0 mL syringe is used
Flow Rate	10
Unit	µL/min

6. Click **Start Syringe Pump**, if applicable.

7. From the method list, select **MS Method**.

8. On the **MS** tab, type the parameters from the following table.

Table 1-4 MS Tab Parameters

Field	Value
Scan type	Q1 MS (Q1)
Start (Da)	200
Stop (Da)	700
*Time (sec) (if available)	1
*Scan rate (Da/s) (if available)	1000
Duration (min)	3
* These fields are instrument-dependent.	

9. Click **Start**.

10. Wait for approximately one minute and then click **Stop** to make sure the flow is stable and you see ions.

11. Select the **MCA** check box and then, in the **Cycles** field, type **10**.

12. Click **Start**.

13. When the ten scans are complete, you should see the masses of the four compounds as ions.

> **Note:** The intensities of the compounds should be much higher than the smallest noise peaks, but not so high that you cannot see any noise peaks. In the first case, you may not be looking at a real compound. In the second case, you may have too high a concentration for the software to optimize properly.
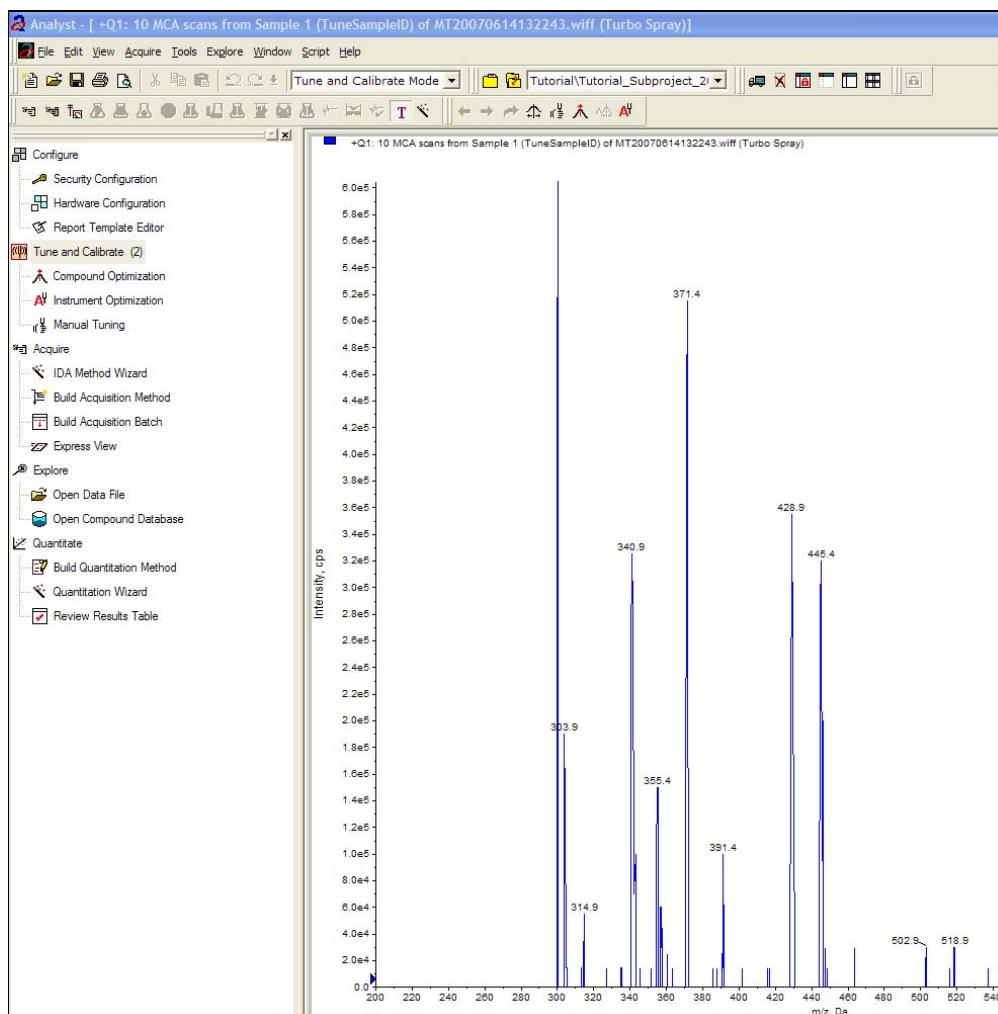
Figure 1-1   Intensities of the four compounds

14. Right-click the bottom-right spectral pane and then click **Open File**.

15. Find the compounds of interest and then write down the voltage values for the highest peaks. These values should be within 0.1 to 0.2 Da of the expected m/z. Use the voltage values in the next procedure.

Optimizing Pre-collision Cell Voltages

Declustering potential (DP), focusing potential (FP), entrance potential (EP), and collision cell entrance potential are pre-collision cell voltages. Optimization of these values involves gradually changing the voltage range while monitoring the signal intensity of the compound.

The potential applied to the orifice plate (OR) has the greatest effect on the amount of declustering in the orifice region of the interface. The DP is the difference between the orifice and the ground. The higher the potential difference, the greater the amount of declustering.

The working range of DP is typically 0 to 100 V, although it may be set higher (the maximum voltage depends on the instrument type and is controlled in the software).

The EP parameter controls the entrance potential, which guides and focuses the ions through the high-pressure Q0 region. It is typically set at 10 V for positive ions, or –10 V for negative ions, and affects the value of all the other instrument voltages. EP has a minor effect on compound optimization.

The CE parameter controls the potential difference between Q0 and Q2 (collision cell). It is used only in MS/MS-type scans. The CE is the amount of energy that the precursor ions receive as they are accelerated into the collision cell, where they collide with gas molecules and fragment. In Q1 and Q3 scans, the voltage applied to the collision cell is RO2 (collision cell rod offset).

1. Return to the **Tune Method Editor** and change the method to the Q1 Multiple Ions (Q1 MI) scan type.

2. In the mass table, type the parameters from the following table.

Table 1-5 Mass Table Parameters—Q1 Multiple Ions (Q1 MI)

Compound	Q1 Mass	Time
Reserpine	609.4	1
Minoxidil	210.2	1
Tolbutamide	271.3	1

Rescinnamine is not optimized because it is the internal standard. Start with reserpine for a simple case. You can repeat the manual optimization process for minoxidil.

3. Click **Edit Ramp**.

4. In the **Ramp Parameter Settings** dialog, select **Declustering Potential (DP)**.

It is important to go in order starting with DP and then continuing through the list. If you go out of order, you might not optimize all the parameters.

5. Type the required **Start**, **Stop**, and **Step** values. If you are unsure, then use the existing values. After you gain some experience with the Edit Ramp function, you might change these values to be more efficient.

6. Click **OK**.

7. Click **Start**.

8. Right-click the bottom-right XIC pane and then click **Open File**.

9. Monitor the TIC and mass spectrum. The value that gives the best signal per second for the ions of interest without distorting the peak shape is the optimal value.

10. Note the optimal value for the ion of interest.

11. Move the cursor to the mass table, right-click and then add the parameter just optimized. This adds a column to the table.

12. Add the optimized values to the appropriate row.

13. Repeat these steps for each mass in the acquisition method until you have a list of the optimal values for all the masses.

14. Repeat these steps to optimize for the other MS-specific parameters.

Table 1-6 MS Specific Parameters

Parameter	Comment
FP	Only used for the API 2000™ and API 3000™ systems. DP and FP should be done twice for best results.
EP	You rarely might optimize this parameter because it has a smaller effect.
CXP	Only used for the API 2000, QTRAP®, and API 3200™ systems.

Determining the Product Ions for Optimization

Perform this procedure one compound at a time using the optimized values that you obtained previously. The product ions are used to generate MRM transitions.

1. In the Tune Method Editor, close the XIC panes.

2. In the **Scan type** field, click **Product Ion (MS2)**.

3. Select the **Compound** tab and type the optimal value you noted previously.

4. On the **MS** tab, in the **Product Of** field, type **609.4**. This value is the mass assignment for reserpine that you noted previously.

5. Make sure the **Center / Width** check box is not selected.

6. In the mass table, type the following:

Table 1-7 Mass Table Parameters (Product Ion Scan)

Field	Value
Start (Da)	100
Stop (Da)	650
Time (sec)	2

7. Click **Edit Ramp**.

8. In the **Ramp Parameter Settings** dialog box, select **Collision Energy** and then type the required **Start**, **Stop**, and **Step** values. If you are unsure, then use the existing values. After you gain some experience with the Edit Ramp function, you might change these values to be more efficient.

9. Click **OK**.

10. Select the **MCA** check box.

11. Click **Start**.

12. Right-click the bottom-right XIC pane and then click **Open File**.

13. Pick the product ions with the greatest intensity. Note the product ion m/z to the first decimal point, such as 195.1. In the laboratory, you would select the top two or three product ions. Try this if you want a more realistic scenario.

    In the laboratory, it is typical to select two to three product ions for optimization. One is for quantitation, one is for confirmation, and the third product ion is used in case one of the other ions suffers from issues such as ion suppression in the matrix.

> **Note:** Make sure the highest peaks that you have chosen for optimization do not represent a common loss from the precursor ion, such as water. Also make sure the product ion is not too low in mass or else you may get interferences for that ion pair in the real samples or in the mobile phase when analyzing on column.

14. For more practice, repeat for minoxidil and tolbutamide.

Optimizing Collision Energy for each Product Ion

1. In the Tune Method Editor, close the XIC panes.

2. In the **Scan type** field, click **MRM**.

3. In the mass table, for each product ion, type the following:

   - In the **Q1 Mass** field, type the compound mass you noted previously, starting with reserpine.

   - In the **Q3 Mass** field, type the product ion or fragment mass assignment you noted previously. If optimizing more than one product ion continue entering other ion pairs in mass table.

   - In the **Time (ms)** field, type **100**.

4. In the mass table, if the compound parameters are not visible, right-click and then select the appropriate MS compound parameters you have optimized and then type the correct values.

5. Click **Edit Ramp**.

6. In the **Ramp Parameter Settings** dialog box, select **Collision Energy** and then type the required **Start**, **Stop**, and **Step** values. If you are unsure, then use the existing values. After you gain some experience with the Edit Ramp function, you might change these values to be more efficient.

7. Click **OK**.

8. Click **Start**.

9. Right-click the bottom-right XIC pane and then click **Open File**.

10. The value that gives the best signal for each ion pair of interest is the optimal value. To move between different XICs, right-click on the colored box on the upper left portion of the window and select the desired XIC.

11. Note the optimal value for the ion pair of interest.

12. Repeat if other product ions are monitored.

13. Return to the mass table, right-click and then select the parameter just optimized. This adds a column to the table.

14. Add the optimized values to the appropriate row.

15. Clear the **Ramp Parameter** check box and then save the method.

16. For more practice, repeat these steps for the remaining compounds.

> **Note:** If you save the method after adding new information, then you will not have to retype all the numbers again. To delete a line in the mass table, click the row number and then press Delete.

Optimizing Collision Cell Exit Potential for each Product Ion

Only used for the API 2000, QTRAP, and API 3200 systems.

1. In the Tune Method Editor, close the XIC panes.

2. Open the previously saved method. In the mass table, check the Q1 and Q3 values for the compound for which you would like to optimize CXP.

3. Select the **Compound** tab and then type the optimal DP and CE values you noted previously.

4. Click **Edit Ramp**.

5. In the **Ramp Parameter Settings** dialog box, select **Collision Cell Exit Potential (CXP)** and then type the required **Start**, **Stop**, and **Step** values. If you are unsure, use the existing values. After you gain some experience with the Edit Ramp function you might change these values to be more efficient.

6. Click **OK** and then click **Start**.

7. Right-click the bottom-right XIC pane and then click **Open File**.

8. The value that gives the best signal is the optimal value. Note the optimal value for the ion of interest.

9. In the mass table, right-click and then select the parameter just optimized. This adds a column to the table.

10. Repeat if other product ions were monitored.

11. Add the optimized values to the appropriate row.

12. Save the method.

13. Repeat for the other compounds if previously optimized.

Manually Optimizing the Ion Source and Gas Parameters

The ion source and gas settings must be set properly to make sure that the instrument stays clean and that the compounds of interest have been optimally put into the gas phase as ions.

The ion source and gas settings must be adjusted when the LC conditions change significantly.

To optimize the ion source and gas parameters, you typically set up a syringe pump with your compounds of interest and connect the line with a tee to the LC device. Controlling the pump can be done manually or through the Analyst® software.

Another way to manually optimize ion source and gas settings is to have the autosampler manually inject the compound of interest every 30 to 60 seconds while you manually vary the parameters in manual tuning to find the optimal settings.

Preparing the Ion Source

1. Set the horizontal micrometer to 5 mm.

2. Set the vertical micrometer on the ion source for your flow rate.

   For the Turbo V™ ion source, use the following parameters as a starting point. For more information, see the *Turbo V™ Ion Source Operator's Guide.*

   Table 1-8 Turbo V Ion Source Vertical Parameters

Flow rate	Initial vertical parameters
1 to 20 µL/min	10 mm
20 to 250 µL/min	5 mm
250 to 500 µL/min	2 mm
500 + µL/min	0 mm

   Depending on how you are running this optimization you may need to have a hardware profile with the LC pumps included.

Optimizing the Ion Source Parameters

In general, ion source parameters are optimized for best signal-to-noise for the compound of interest. The Curtain Gas™ interface supply is optimized at the highest setting without losing sensitivity. For more information about optimizing ion source parameters, see the appropriate ion source operator's guide.

Use the following procedure to optimize the Curtain Gas interface parameter. The main function of the Curtain Gas interface parameter is to prevent the contamination of the ion optics. The supply pressure should be 55 psi. The Curtain Gas interface parameter should always be maintained as high as possible without losing sensitivity. The value depends on the type of instrument and ion source.

Do not set the parameter below the starting value.

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

   The Tune Method Editor appears.

2. Click **File > Open**.

   The Open dialog appears.

3. In the **Files** list, click the acquisition method used to optimize the compound parameter and then click **OK**.

   The method appears in the Tune Method Editor.

4. Click the **Source/Gas** tab.

5. Using the ion source and gas flow guide, set all the ion source and gas parameters so that they are appropriate for the flow rate.

   > **Note:** These values are a starting point and should be optimized for best results. The values simply limit the amount of contamination into the mass spectrometer

6. Set the run time long enough so that many parameters can be adjusted; a good starting time is 15 minutes. You can change this time.

7. Click **Start**.

    Data appear in panes below the Tune Method Editor.

8. Note the signal of the peak of interest.

9. In the **Curtain Gas (CUR)** field, increase the value by five.

10. Continue increasing the Curtain Gas interface value until you find the highest value without losing sensitivity. As with most Source/Gas settings, if two values give the same result, then use the higher value.

11. Repeat these steps with the other Source/Gas parameters. When optimizing for these parameters, look for the setting that gives the highest signal-to-noise value. Depending on the instrument, you may not have all the parameters available.

Advanced Parameters

The following parameters should only be optimized by an experienced operator.

Optimizing AF2

The AF2 parameter controls the peak-to-peak voltage used to fragment the second precursor ion in an MS3 scan. The amount of excitation energy used depends on the compound and the desired amount of fragmentation.

- For both positive and negative ion modes, ramp AF2 from 0 to 200 mV, with a step value of 5 mV. Select the AF2 value that gives the best amount of fragmentation.

About Collision Energy Spread (CES)

For linear ion trap (LIT) instruments, the CES parameter controls the spread of collision energies used when filling the LIT and applies when you are using the AutoFrag feature. It is used with the Collision Energy (CE) parameter. The advantage of using a collision energy spread is that you do not have to optimize the collision energy.

By selecting the CE and CES parameters, low, medium, and high collision energies are used in a single scan to provide maximum information in the product ion spectra (low and high mass fragments). For example, if you use a CE value of 30 and a CES value of 15, then collision energies of 15, 30, and 45 will be used.

Table 1-9 Icons in Tune and Calibrate Mode

Icon	Name	Function
	Calibrate from spectrum	Click to open the Mass Calibration Option dialog box and use the active spectrum to calibrate the instrument.
	Manual Tune	Click to open the Manual Tune Editor.

Table 1-9 Icons in Tune and Calibrate Mode (Continued)

Icon	Name	Function
	Compound Optimization	Click to optimize for a compound using infusion by 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.