Introduction to DMS Technology

This section describes Differential Mobility Spectrometry (DMS) technology and its benefits, and the role of modifiers. The DMS Off operation mode is also discussed here.

About DMS Technology

DMS is a method of separating ions based on the difference between ion mobility in high and low electric fields in gases at or near atmospheric pressure. DMS is a variant of IMS (ion mobility spectrometry).

In DMS technology, Separation Voltages (SV) are applied across the ion transport channel, perpendicular to the direction of the transport gas flow, as shown in Figure 1-1 on page 5. Due to the difference between high- and low-electric-field ion mobility coefficients, ions migrate toward the walls and leave the flight path. Their trajectory is corrected by a counterbalancing DC voltage called Compensation Voltage (COV).

Figure 1-1  DMS Technology

Instead of recording the flight time of an ion through the ion transport channel, the COV required to correct the trajectory of a particular ion is recorded for a range of SV amplitudes. The COV can be scanned to serially pass ions according to their differential mobility, or set to a fixed value to pass only the ion species with a particular differential mobility.

Certain combinations of SV and COV fields allow the target ion to pass through the SelexION™ device analytical region without colliding with the electrodes. Therefore, by scanning or fixing SV and COV, the SelexION device can operate in the following modes:

- A particular SV and COV combination can be selected, resulting in continuous filtration of particular ion species.
- When SV is fixed and COV scanned, a linear DMS spectra can be recorded.
Benefits of DMS Technology

- It enhances the quality of mass analysis and quantitative accuracy by reducing chemical noise, and by pre-separating ions of similar mass.
- It provides fast ion transit times and offers a transparent mode of operation (similar to when the SelexION™ device is not installed on the system) that allows all ions to be transmitted without discrimination when SV and COV are set to zero.
- It supports simultaneous transmission of ions of both polarities and subjects each to separation based on their differential mobility constants.

Role of Modifiers in DMS Technology

Modifiers can be added to the gas transporting the ions through the SelexION™ device to increase the peak capacity and separation power of this device. The presence of these modifiers, which are typically common chromatography solvents (for example, 2-propanol, acetonitrile, or methanol), affects the mobilities of ions in the device. Ions entering the mobility cell form clusters with the modifier molecules which alters their mobility characteristics. The clusters can form under low electric field conditions but fall apart again under high electric fields, which is referred to as the dynamic cluster-decluster model. The net effect of the cluster formation is that the differences between high- and low-field mobilities are amplified, yielding better separation power and increased peak capacity. Moreover, since cluster formation itself depends on the specific interaction between an analyte ion and a user-selected modifier, the use of modifiers offers an additional separation dimension to traditional chromatographic and mass spectrometric approaches. Further information on the effects of modifiers can be found in the following journal article: B.B. Schneider, T.R. Covey, S.L. Coy, E.V. Krylov, and E.G. Nazarov, *Anal. Chem.* 2010, 82, 1867-1880.

Because modifier use involves gas-phase ion chemistry, certain general behaviors can be expected. In positive ion mode, if the modifier has a greater proton affinity than the analyte ion, the charge may be stripped from the analyte resulting in lower signal but not necessarily lower signal-to-noise. Alternatively, if the modifier has greater gas-phase acidity than the analyte, the negative ion current will be reduced. Knowing the characteristics of the analyte and modifier can inform the appropriate choice of modifier. Using 2-propanol as a modifier is a good starting point for investigating the effect of modifiers on a given DMS separation because it has been observed to improve the separation power for a wide range of compounds.
Instrument Tuning

Before installing the SelexION™ device on the mass spectrometer, make sure that it is tuned and calibrated.

DMS Off Operation Mode

When the SelexION™ device is installed on the mass spectrometer, in some situations, users might need to acquire spectral data without using the SelexION device. Instead of removing the SelexION device from the system, users can use the DMS Off operation mode to acquire data. The Analyst® software provides a DMS Off operation mode, which allows the user to work in an environment similar to when the SelexION device is not installed on the mass spectrometer. However, in the DMS Off mode, signal intensities will be significantly decreased as compared to the signal intensities of data acquired with the SelexION device physically removed from the mass spectrometer.

To work in the DMS Off operation mode, select the DMS Off check box available on the MS tab in the Acquisition method window or on the MS tab in the Tune Method Editor window.

Figure 1-2  DMS Off Check Box in the Tune Method Editor

In the DMS Off operation mode, the following changes occur:

- Most of the DMS parameters are no longer available. Refer to Table A-2 on page 58. Only the DMS Temperature (DT) parameter is visible in the DMS Off mode, and it can be optimized in this mode to obtain the best sensitivity.
- The DMS parameters (SV, COV, and DMO) are removed from the parameter list in the Ramp Parameter Settings dialog.
- The default pause time between mass ranges becomes 5 ms.
Using the SelexION™ Device

A 5500 or 6500 series instrument with SelexION™ technology includes a Field Service Employee (FSE) installed controller module and associated electronics, along with a customer installed interface device.

Figure 2-1  6500 System with SelexION Technology

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controller module with side tray</td>
<td>FSE upgrade</td>
</tr>
<tr>
<td>2</td>
<td>IonDrive™ Turbo V ion source</td>
<td>Attached to the SelexION device</td>
</tr>
</tbody>
</table>

Note: The NanoSpray® ion source can be installed on a 5500 or 6500 series instrument equipped with SelexION technology. However, you will not be able to use modifiers. You can create acquisition methods that include modifier information and flows, but when executed the modifier pump will be disabled.
The SelexION device includes the following customer replaceable components:

- Ion mobility cell
- Adapter ring
- SelexION curtain plate

**Figure 2-2  SelexION Technology**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vacuum interface housing</td>
<td>FSE upgrade</td>
</tr>
<tr>
<td>2</td>
<td>Dual drain assembly</td>
<td>FSE upgrade</td>
</tr>
<tr>
<td>3</td>
<td>Ceramic orifice plate</td>
<td>FSE upgrade</td>
</tr>
<tr>
<td>4</td>
<td>Ion mobility cell</td>
<td>Customer installable</td>
</tr>
<tr>
<td>5</td>
<td>Adapter ring</td>
<td>Customer installable</td>
</tr>
<tr>
<td>6</td>
<td>SelexION curtain plate</td>
<td>Customer installable</td>
</tr>
</tbody>
</table>
Install the SelexION Device

Required Materials

• Powder-free gloves

**WARNING!** Hot Surface Hazard: Some surfaces of the vacuum interface and the ion source become hot during operation and should cool for at least 30 minutes prior to starting any maintenance procedures. With the IonDrive™ Turbo V ion source, you must let the ion source cool for at least 90 minutes before starting any maintenance procedures.

**Caution:** Potential System Damage: Make sure that the vacuum interface components are kept clean and free of chemical or dust contamination. To prevent contamination, always wear powder-free gloves when handling the vacuum interface components.

1. Complete any ongoing scans or click **Acquire > Abort Sample**.
2. Shut off the sample flow to the mass spectrometer.
3. In the Analyst® software, deactivate the active hardware profile.

**WARNING!** Hot Surface Hazard: With the IonDrive Turbo V ion source, only touch the blue plastic covering. Do not touch the tower or drain on the bottom of the source.

4. Rotate the two ion source latches upwards to the 12 o’clock open position, and then gently pull the ion source off the SelexION adapter ring.
5. Remove the curtain plate by pulling it straight off the vacuum interface. It is held in place by three retaining ball catches mounted on the orifice plate.

**Caution:** Potential System Damage: Handle vacuum interface components with care. They are very fragile and expensive.

6. Position the SelexION ion mobility cell on the SelexION orifice plate, making sure that the connections on the ion mobility cell are aligned with the orifice plate, then tighten the thumbscrews.
7. Position the SelexION adapter ring on the vacuum interface housing. Make sure that the two thumbscrews on the adapter ring fit into the receptacles in the vacuum interface housing, then tighten the thumbscrews.

**Note:** The adapter ring equipped with a locator pin on the bottom presses down onto a spring-loaded plate in the dual drain housing. You have to position the locator pin on the dual drain housing, tilt the adapter ring towards you, then tilt the top of adapter ring towards the instrument. If you are installing the adapter ring equipped with a latch (item 8), then proceed directly to step 8.
8. Align the pins of the SelexION curtain plate with the holes in the ion mobility cell, and then press the SelexION curtain plate onto the ion mobility cell.

9. Make sure the two source latches on the ion source are pointing upwards in the 12 o’clock open position.

10. Position the ion source on the SelexION adapter ring. Make sure the guide pins on the ion source fit into the receptacles in the adapter ring, then rotate the source.
latches downward to the 6:30 locked position to lock the ion source in place. Refer to Figure 2-4.

11. Turn on the power to the SelexION technology.

The power switch is located on the back of the SelexION controller module.

**Note:** Failure to deactivate the active hardware profile before turning the SelexION controller module on or off (to temporarily add or remove the SelexION technology to or from the system), may cause the mass spectrometer to enter an unstable state resulting in loss of control from within the Analyst software. If the hardware profile is not deactivated and the mass spectrometer enters this unstable state, control may be recovered by removing and re-seating the ion source, or by power cycling both the mass spectrometer and controller module and reactivating the hardware profile.

12. Purge the SelexION modifier pump before running any SelexION experiments using modifiers. Refer to **Purge the Modifier on page 27**.

**WARNING!** Toxic Chemical Hazard: Take caution when filling or re-filling modifier bottles with caustic hazardous or toxic chemicals.

**Caution:** Do not refill the modifier bottle while it is in the side tray. Disconnect the fluid line from the bottle, refill the bottle in a safe location, then reinstall the bottle and fluid line in the side tray.

### Remove the SelexION Device

Remove the SelexION device and install the standard curtain plate to restore the 5500 or 6500 series instrument to standard performance.

**Required Materials**

- Powder-free gloves

**WARNING!** Hot Surface Hazard: Some surfaces of the vacuum interface and the ion source become hot during operation and should cool for at least 30 minutes prior to starting any maintenance procedures. With the IonDrive™ Turbo V ion source, you must let the ion source cool for at least 90 minutes before starting any maintenance procedures.

**Caution:** Potential System Damage: Make sure that the vacuum interface components are kept clean and free of chemical or dust contamination. To prevent contamination, always wear powder-free gloves when handling the vacuum interface components.

1. Complete any ongoing scans or click **Acquire > Abort Sample**.
2. Shut off the sample flow to the mass spectrometer.
3. In the Analyst software, deactivate the active hardware profile.
4. Turn off the power to the SelexION technology.
   The power switch is located on the back of the SelexION controller module.

   **Note:** Failure to deactivate the active hardware profile before turning the SelexION controller module on or off (to temporarily add or remove the SelexION technology to or from the system), may cause the mass spectrometer to enter an unstable state resulting in loss of control from within the Analyst software. If the hardware profile is not deactivated and the mass spectrometer enters this unstable state, control may be recovered by removing and re-seating the ion source, or by power cycling both the mass spectrometer and controller module and reactivating the hardware profile.

5. Remove the adapter ring by loosening the thumbscrews, and then pull the adapter ring off the vacuum interface.

   **Note:** The adapter ring has a locator pin on the bottom that presses down onto a spring-loaded plate in the dual drain housing. You may have to tilt the top of adapter ring towards you when removing it from the vacuum interface. Refer to Figure 2-4.

6. Remove the SelexION curtain plate by pulling it straight off the ion mobility cell.

   **Caution:** Potential System Damage: Vacuum interface components are very fragile and expensive. Handle them with care.

7. Remove the SelexION ion mobility cell by loosening the thumbscrews, and pulling the ion mobility cell off the orifice plate. Refer to Figure 2-3.

8. Install the standard curtain plate.

   **Note:** You do not need to remove the SelexION orifice plate or the dual drain assembly. The IonDrive Turbo V ion source functions with the SelexION orifice plate, and uses the drain hole of the dual drain assembly closest to the interface housing.

9. Make sure the two source latches on the ion source housing are pointing upwards in the 12 o’clock open position.

10. Position the ion source on the vacuum interface. Make sure the guide pins on the ion source fit into the receptacles in the vacuum interface, and then rotate the source latches downward to the 6:30 locked position to lock the ion source in place.
Clean and Align the Ion Mobility Cell Electrodes

Clean and align the ion mobility cell electrodes when contamination is observed on the surfaces, or when repeated high-voltage discharge errors occur.

**Required Materials**

- Powder-free glove
- Small slotted screwdriver
- #600 sandpaper
- 50:50 methanol:water solution
- Spacer tool

**WARNING! Hot Surface Hazard:** Some surfaces of the vacuum interface and the ion source become hot during operation and should cool for at least 30 minutes prior to starting any maintenance procedures. With the IonDrive™ Turbo V ion source, you must let the ion source cool for at least 90 minutes before starting any maintenance procedures.

**Caution:** Potential System Damage: Make sure that the vacuum interface components are kept clean and free of chemical or dust contamination. To prevent contamination, always wear powder-free gloves when handling the vacuum interface components.

1. Remove the ion mobility cell. Perform steps 1 to 8 in **Remove the SelexION Device on page 14**.
2. Using the small slotted screwdriver, loosen the adjusting screws until the electrodes move freely in the ion mobility cell, and then remove the electrodes from the ion mobility cell.

**Figure 2-5 SelexION Ion Mobility Cell**
3. Using the #600 sandpaper, lightly scrub the flat surface of each electrode, then wash the electrodes with a 50:50 methanol:water solution.

4. Allow the electrodes to dry, then insert them into the ion mobility cell.

5. Slide the spacer tool through the gap in the front of the electrodes, then using the small slotted screwdriver, align the electrodes in the ion mobility cell, so that the spacer tool is centered in the ion mobility cell.

   Make sure that the spacer tools fits between the electrodes, then adjust the screws on each side of the ion mobility cell to center the electrodes.

6. Position the SelexION ion mobility cell on the SelexION orifice plate, and then tighten the thumbscrews. Refer to Install the SelexION Device on page 11.

### Clean the SelexION Technology Surfaces

Clean the external surfaces of the SelexION technology after a fluid spill, or when they become dirty.

- Using warm, soapy water and a soft cloth, wipe the external surfaces.
Optimize DMS Parameters

The DMS parameters must be optimized to obtain the best signal and separation of compounds of interest. For more information about DMS parameters, refer to DMS Parameters Descriptions on page 63.

The DMS parameters can be optimized in two ways in the Tune and Calibrate mode:

- You can use, as a starting point, an existing acquisition method that was created for the compound to be analyzed, and contains optimized compound parameters, source parameters, and LC flow rate and then optimize the DMS parameters.

  Or

- You can create a new acquisition method, optimize the compound parameters, source parameters, and the LC flow rate first, and then optimize the DMS parameters.

This section describes the second method of optimizing the DMS parameters. The first method is a subset of the second method described in this section.

Objectives

- Optimize the DMS parameters with and without a modifier in the Manual Tuning mode.
- Optimize the DMS parameters using compound optimization.

Optimize DMS Parameters in Manual Tuning

Section Objectives

- Create an acquisition method in the Manual Tuning mode and optimize DMS parameters without a modifier.
- Purge the modifier.
- Create an acquisition method and optimize DMS parameters in the Manual Tuning mode, with a modifier selected.

Note: To acquire data using a modifier, create the acquisition method and optimize the DMS parameters with the modifier selected in the method. The DMS parameters Separation Voltage (SV), Compensation Voltage (COV), and the DMS Offset (DMO) are affected by the choice of a modifier. Therefore, adding or changing the modifier or the modifier concentration after optimizing SV, COV, and DMO parameters will require a re-optimization of these parameters.

Prerequisites

- Make sure that the SelexION™ technology is installed on the mass spectrometer and the controller module is switched on.
Create an Acquisition Method and Optimize DMS Parameters without a Modifier

You can optimize the DMS parameters for your compound in the Manual Tuning mode and save them in a new acquisition method.

In this procedure you will create a new acquisition method and optimize the DMS parameters. A modifier is not being used in this scenario.


2. T-infuse (or split-infuse) the sample into the LC stream. Refer to T-Infuse Sample into LC Stream on page 66.


4. Select a scan type, type mass ranges, transitions, scan speed, and other required information. Optimize the compound and ion source parameters. Then, optimize the LC flow rate according to your chromatographic method setup. Refer to the Manual Optimization Tutorial.

5. Click the DMS tab.

Figure 3-1 Default DMS Parameter Settings in a 6500 Series Instrument

<table>
<thead>
<tr>
<th>Source/Gas</th>
<th>Compound</th>
<th>DMS</th>
<th>Resolution</th>
<th>Detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS Temperature Reached</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS Temperature (DT)</td>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modifier (MD)</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separation Voltage (SV)</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensation Voltage (COV)</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS Offset (DMO)</td>
<td>-3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS Resolution Enhancement (DR)</td>
<td>Open</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Before turning the SelexION controller module on or off (to temporarily add or remove the SelexION technology to or from the system), first deactivate the active hardware profile within the Analyst software. Failure to do so may cause the mass spectrometer to enter an unstable state resulting in loss of control from within the Analyst software. If the hardware profile is not deactivated and the mass spectrometer enters this unstable state, control may be recovered by removing and re-seating the Ion Source, or by power cycling both the mass spectrometer and controller module and reactivating the hardware profile.
All the DMS parameters have default values. The Separation Voltage (SV) and the Compensation Voltage (COV) are zero.

Optimize the DMS parameters to get the best separation and sensitivity. The following parameters need to be optimized in the specified order:

a. **DMS Temperature (DT)**: Click **Start** to test this parameter with the default value **Low**. For the test, use the default values for other DMS parameters (SV = 0; COV = 0; Modifier = None) or switch to the DMS Off mode by selecting the **DMS Off** check box on the MS tab.

![Figure 3-2 Tune Method Editor with Default DMS Parameter Settings in a 6500 Series Instrument](image)

Data is shown in the panes below the Tune Method Editor. If you are not satisfied with the signal obtained for the peak of interest, change the value of DT either to Medium or High and evaluate the signal again until you obtain the best signal.

**Note:** For each DT value change, allow the system to equilibrate for at least 10 to 15 minutes before optimizing DMS parameters.

b. **Modifier (MD)**: Make sure the value of this parameter is **None**.

c. **Separation Voltage (SV) and Compensation Voltage (COV)**: The SV and COV are tested together iteratively to find the SV and COV combination that gives the best signal and separation. Step SV through 0 to the maximum allowed SV range while ramping COV through the full COV range.

**Note:** The full COV range is –100 to 100 volts. However, most compounds optimize between –20 to 30 volts range in the absence of a modifier. Some compounds may optimize even higher than 30 volts. In the presence of a modifier, we recommend using at least –60 to 20 volts COV range because the negative COV shifts can be very large for some compounds. The maximum SV value is linked to DT and TEM parameters.

- Start with an **SV** value of **0**.
- Click **Edit Ramp**.
  The Ramp Parameter Settings dialog opens.
Optimize DMS Parameters

- Select Compensation Voltage in the Parameter field.
- Type –20 in the Start field.
- Type 30 in the Stop field.
- Use the default value in the Step field or type a required step size and then click OK.
  The Ramp Parameter check box is selected.
- Click Start.
  Data is shown in the panes below the Tune Method Editor. Check the maximum signal intensity and make a note of it. The COV value at which this maximum sensitivity is achieved should be zero.
- Change the SV value to a number between 0 and the maximum allowed SV range, for example 2000. Use SV in increments of 500 or more because the acceptable SV range is large.
- Use the same COV range for ramping and then click Start to evaluate the signal again.
  Data is shown in the panes below the Tune Method Editor. Check the maximum signal intensity and COV value at which the maximum signal was achieved, and make a note of it.
- Repeat the process of stepping SV and ramping COV, and make a note of the SV value that gives the best signal intensity. After the signal intensity starts to decrease, stop the process. Further fine tune the SV value by repeating the above process with smaller SV steps in the 100 to 200 volts range as used above, and ramping COV.
  After the optimal SV and COV pair is determined, set the SV and COV parameters to these values in your method.

d. **DMS Offset (DMO):** Ramp DMO for the optimized SV and COV combination. For example:
- Click Edit Ramp.
  The Ramp Parameter Settings dialog opens.
- Select DMS Offset in the Parameter field.
- Type –30 in the Start field.
- Type 30 in the Stop field.
- Use the default value in the Step field or type a number and click OK.
- Click Start.
  Data is shown in the panes below the Tune Method Editor. The allowed range for DMO is –100 to 100 volts.

e. **DMS Resolution Enhancement (DR):** (In 6500 series of instruments) For a single compound, the Open (no throttle gas is being added) DR value should be used. Other DR values Off, Low, Medium, or High are used when the separation of compounds needs to be improved. For example, when two compounds have close COV and need to be baseline separated, you can test the Off, Low, Medium, or High DR values to get the best separation. Select the
required value (Off, Low, Medium, or High) for the DR parameter and then click **Start** to ramp COV at a particular SV value to evaluate the signal and separation of the compounds. Test the different DR values until you are satisfied with the separation of the compounds.

**(In 5500 series of instruments)** For a single compound, the Off (no throttle gas is being added) DR value should be used. Other DR values Low, Medium, or High are used when the separation of compounds needs to be improved.

**Note:** For 6500 series of instruments, when the value of DR is set to either Off, Low, Medium, or High, gas flow is enabled between the SelexION device exit and the Orifice inlet into the mass spectrometer, which improves the resolution of the SelexION device. For 5500 series of instruments, DR value of Low, Medium, or High, enables the gas flow between the SelexION device exit and the Orifice inlet into the mass spectrometer.

Use of the DR parameter increases the residence time of ions in the SelexION™ ion mobility cell, leading to reduced sensitivity due to larger diffusional signal loss. When the DR value is changed, the pause time between mass ranges and the total scan time will be updated to account for ion transport through the SelexION ion mobility cell. When performing RF/DC scans (quadrupole scans), the pause time between mass ranges and the total scan time will be updated.

**Table 3-1** shows the different DR values and the corresponding Pause between mass ranges times.

### Table 3-1
**DR Values and the Corresponding Pause Between Mass Ranges Time**

<table>
<thead>
<tr>
<th>DR Values</th>
<th>6500 Series of Instruments</th>
<th>5500 Series of Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open (default) (Only available for 6500 series of instruments)</td>
<td>20 ms (default)</td>
<td>—</td>
</tr>
<tr>
<td>Off (default for 5500 series of instruments)</td>
<td>20 ms</td>
<td>20 ms</td>
</tr>
<tr>
<td>Low</td>
<td>30 ms</td>
<td>30 ms</td>
</tr>
<tr>
<td>Medium</td>
<td>40 ms</td>
<td>40 ms</td>
</tr>
<tr>
<td>High</td>
<td>50 ms</td>
<td>50 ms</td>
</tr>
</tbody>
</table>

**Tip!** The DR readbacks show the actual pressure in the Mass Spec Detailed Status dialog. Double-click the Mass Spec icon on the status bar to open this dialog.

6. After all the DMS parameters have been successfully optimized, save the acquisition method.
Create an Acquisition Method with a Modifier and Optimize DMS Parameters

Note: When using the NanoSpray® ion source, users will not be able to use modifiers. Users will be able to create acquisition methods that include modifier information and flows, but when executed the modifier pump will be disabled.

If the compounds of interest did not get adequately separated in the DMS mode, a modifier can be introduced to help separate these compounds. A modifier is a chemical that is added into the Curtain Gas™ flow to help improve the separation of compounds.

You can select a modifier and optimize the DMS parameters in the Manual Tuning mode and create an acquisition method to save the optimized parameters’ settings.

Before selecting a modifier for the first time or when changing the modifier, purge the modifier line. Refer to Purge the Modifier on page 27.

Prerequisite

- Make sure that there is sufficient modifier in the modifier bottle for your acquisition needs. For more information about calculating modifier consumption, refer to Calculate the Appropriate Modifier Consumption for a Batch on page 49.

To refill the modifier bottle, refer to Refill the Modifier Bottle on page 67.

Create an Acquisition Method with a Modifier Selected and Optimize DMS Parameters

1. T-infuse the sample into the LC stream. Refer to T-Infuse Sample into LC Stream on page 66.
3. Select a scan type, type mass ranges, transitions, scan speed, and other required information. Optimize the compound and source parameters. Then, optimize the LC flow rate according to your chromatographic method setup. Refer to the Manual Optimization Tutorial.

Note: Instead of creating an acquisition method, you can use, as a starting point, an existing acquisition method that was created for the compound to be analyzed in the absence of modifiers or in the presence of a different modifier than the one currently used. This acquisition method must contain optimized compound, source, and DMS parameters, and LC flow rate. However, you must re-optimize the DMS parameters for the method using a modifier.

4. Click the DMS tab.
5. Optimize the DT parameter as described in Create an Acquisition Method and Optimize DMS Parameters without a Modifier on page 20.
6. In the **Modifier (MD)** parameter, select a modifier from the list. Select one of the pre-defined modifiers, but if you want to use a modifier other than the pre-defined ones, select **Custom**. For information about the Custom modifier value, refer to step 8 on page 26.

**Figure 3-3  DMS Parameter Settings in a 6500 Series Instrument**

![Figure 3-3 DMS Parameter Settings in a 6500 Series Instrument](image)

The Modifier Composition (MDC) parameter is shown with its default value, which is set to Low.

**Figure 3-4  DMS Parameter Settings in a 6500 Series Instrument**

![Figure 3-4 DMS Parameter Settings in a 6500 Series Instrument](image)
7. In the **MDC** parameter, if required, select **High** to test the modifier concentration that would provide the optimal separation. Low is 1.5% or higher, and High is 3.0% concentration of modifier.

The Analyst® software uses the Curtain Gas flow value and the selected modifier’s density and molecular weight to calculate the modifier’s flow rate and apply that flow rate automatically.

8. If you selected **Custom** in the **Modifier (MD)** parameter in step 6 on page 25, then you need to provide the custom modifier’s density and molecular weight as shown in the following figure so that the Analyst software can calculate the flow rate for the modifier for the specified setting (Low or High) and apply it.

**Figure 3-5  DMS Parameter Setting in a 6500 Series Instrument**

- **Modifier Density (g/mL) (MDD)**: Specify the density of the custom modifier. The Analyst software uses this density along with the Modifier molecular weight and the Curtain Gas flow value to determine the custom modifier’s flow rate.

- **Modifier MW (MDW)**: Specify the molecular weight of the custom modifier in g/mol. The Analyst software uses the value of this parameter along with the Modifier density and the Curtain Gas flow value to determine the custom modifier’s flow rate.

**Tip!** You can view the modifier pump flow rate in the Mass Spec Detailed Status dialog. Double-click the Mass Spec icon on the status bar to view this dialog.
9. For the selected modifier and the MDC value, tune the SV, COV, and DMO parameters as described in Create an Acquisition Method and Optimize DMS Parameters without a Modifier on page 20, until you are satisfied with the signal and separation.

If you want to test a different modifier concentration (High or Low) of the same modifier (selected in step 6 on page 25), select a different MDC value (Low or High) and re-optimize the SV, COV, and DMO parameters as described in Create an Acquisition Method and Optimize DMS Parameters without a Modifier on page 20.

If you want to use a different modifier, first purge the modifier line, select a different modifier in the acquisition method, specify the MDC value for the new modifier, and then re-optimize the SV, COV, and DMO parameters as described in Create an Acquisition Method and Optimize DMS Parameters without a Modifier on page 20.

10. If needed, optimize the DR parameter as described in Create an Acquisition Method and Optimize DMS Parameters without a Modifier on page 20.

11. Save the acquisition method.

Real-Time Modifier Parameters Control

If you start acquiring data from Manual Tuning using the acquisition method created in the previous topic and then change the MDC value from Low to High or High to Low during acquisition, the Analyst® software immediately calculates the required modifier pump flow rate and then adjusts the modifier pump accordingly.

Similarly, if during acquisition you change the molecular weight or molecular density of the custom modifier specified in the acquisition method, the Analyst software immediately calculates the required modifier pump flow rate and then adjusts the modifier pump accordingly.

Purge the Modifier

Before using a new modifier, purge the modifier currently being used from the modifier pump. Purging the modifier will take around four minutes during which the mass spectrometer will be unavailable for use.

A modifier can be purged or cleared from the modifier pump from the Manual Tuning or Acquire mode when the SelexION™ device is switched on.

Purge the Modifier in the Manual Tune or Acquire mode when the SelexION Device is On

1. Make sure the SelexION controller module is switched on and activate a hardware profile. Refer to Creating Hardware Profiles and Projects in the Getting Started Guide.

2. Connect the bottle containing the other modifier to the modifier pump.

3. If you are already in the Manual Tuning or Acquire mode, go to step 4. If you are in the Acquire mode with samples in the acquisition queue, refer to Purge Modifiers from Acquire Mode with Samples in the Acquisition Queue on page 30. If you are not in either the Manual Tuning or Acquire mode, then on the Navigation bar, click Acquire or Tune and Calibrate.
4. Click the **Purge Modifier** icon on the toolbar.

**Figure 3-6  Purge Modifier Icon**

![Purge Modifier Icon](image)

5. Click the **Purge** button.

**Figure 3-7  Purge Modifier Dialog**

![Purge Modifier Dialog](image)

The Purge Modifier dialog opens. The Status shows as Ready, which indicates the system is ready to start the Purge process.

5. Click the **Purge** button.

The purge process starts. If the modifier purge was initiated from the Manual Tuning mode, the system is automatically entered into a non-Tuning mode, and the Reserve Instrument for Tuning icon becomes deselected on the toolbar.
The purge process enters the Initializing/Equilibrating mode. During the Initializing mode, an acquisition method and a batch file are created in the background. During the Equilibrating mode, the system is equilibrating.

The status in the Purge Modifier dialog then changes from Initializing/Equilibrating to Purging, which indicates that the modifier is being cleared from the modifier pump. During the modifier purge, the instrument scans and data is collected in the API Instrument>Data folder. After the purge is complete, the acquisition method, the batch file and the data are automatically removed.

During Purging, the Purge button changes to Abort. The expected purge time (four minutes) and the time elapsed in the Purge cycle are displayed. The Analyst® software automatically sets the DMS and source parameters to new values for four minutes (expected purge time). To view these values, refer to Values of DMS and Source Parameters Used During Modifier Purge on page 65.

**Tip!** You can view the status of the modifier purge process in the Mass Spec Detailed Status dialog. Double-click the Mass Spec icon on the status bar to view this dialog.
Purging the modifier from the modifier pump takes around four minutes. After the Purge process is complete, the status in the Purge Modifier dialog changes to Complete. The Abort button changes back to Purge. The system automatically re-enters the Manual Tuning mode if the modifier purge was initiated from the Manual Tuning mode. The values of some DMS and source parameters are reset. To view the reset values, refer to Values of DMS and Source Parameters After Modifier Purge is Completed or Aborted on page 66.

6. Click X to close the Purge Modifier dialog.

After modifier purge completion, allow the system to equilibrate for approximately 10 minutes with the required source conditions and the new modifier running before acquiring new samples.

Abort the Purge Process

1. In the Purge Modifier dialog, click Abort.

The purge cycle is terminated, and the values of some DMS and source parameters are reset. To view the reset values, refer to Values of DMS and Source Parameters After Modifier Purge is Completed or Aborted on page 66.

The Status changes to Aborted, and the Abort button changes to Purge.

If the modifier purge was initiated from the Manual Tuning mode, after abort, the system is set back into the Manual Tuning mode.

After the modifier purge is aborted, allow the system to equilibrate for approximately 10 minutes with the required source conditions and the new modifier running before acquiring new samples.

Purge Modifiers from Acquire Mode with Samples in the Acquisition Queue

If you are in the Acquire mode with samples in the acquisition queue, and select the Purge Modifier icon, a Queue Busy message is shown in the Purge Modifier dialog.
If you click Purge in the dialog, a message is shown indicating that the acquisition is currently in progress. For the purge to continue, complete or cancel the acquisition process and then make sure that no samples are waiting in the queue.

**Modifier Purge Status**

<table>
<thead>
<tr>
<th>Purge Status</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready</td>
<td>The system is ready to start the Purge process.</td>
</tr>
<tr>
<td>Initializing/Equilibrating</td>
<td>Creating method and batch files/System is equilibrating.</td>
</tr>
<tr>
<td>Purging</td>
<td>Modifier purge is in progress.</td>
</tr>
<tr>
<td>Aborted</td>
<td>The purge cycle is terminated.</td>
</tr>
<tr>
<td>Queue Busy</td>
<td>Acquisition in progress; Purge is not possible.</td>
</tr>
<tr>
<td>Complete</td>
<td>The purge process is complete.</td>
</tr>
</tbody>
</table>

**Optimize DMS Parameters using Compound Optimization**

The T-infusion optimization type in the Compound Optimization mode allows you to automatically optimize the following:

- DMS Parameters (SV, COV, and DMO)
- Compound and DMS Parameters (SV, COV, and DMO)

The FIA optimization type allows you to optimize only the COV parameter for a specified SV for the SelexION™ technology. It can also be used to optimize the source- and compound-dependent parameters.
Section Objectives

In this section, you will do the following:

- Optimize DMS parameters with T-Infusion.
- Optimize the compound and DMS parameters with T-Infusion.
- Optimize COV with FIA.

Prerequisite

- Before starting T-Infusion optimization, create an acquisition method that will be used as the starter acquisition method for T-Infusion optimization. This method should have the following:
  - An isocratic LC method (optional if an external isocratic LC pump is being used).
  - A syringe pump method (optional if an external syringe pump is being used).
  - An MRM or Q1 MI scan type.
  - Optimized source parameters.
  - Optimized DMS temperature parameter with default SV, COV, and DMO values.
  - Modifier type and flow may or may not be specified.
  - If an AAO driver or an integrated Shimadzu driver is used as LC pump, set the LC duration long enough for acquisition. The Analyst® software cannot modify the LC time.
  - Maximum number of compounds is 20.

Optimize DMS Parameters Only with T-Infusion Optimization Type

To automatically optimize the SV, COV, and DMO DMS parameters in T-Infusion, use the following procedure:

1. Make sure a hardware profile is active. If the starter method contains a syringe pump method or an LC pump method, the hardware profile must also contain a syringe pump or an LC pump, respectively. Refer to Creating Hardware Profiles and Projects in the Getting Started Guide.

2. T-infuse the sample into the LC stream. Refer to T-Infuse Sample into LC Stream on page 66.
3. On the Navigation bar, under **Tune and Calibrate**, double-click **Compound Optimization**.

*Figure 3-12 Instrument Settings Dialog in a 6500 Series Instrument*
4. On the **Instruments Settings** page, in the **Inlet** group, click **T-Infusion**.

**Figure 3-13 Instrument Settings Dialog in a 6500 Series Instrument**

![Instrument Settings Dialog]

5. In the **Default Acq. Method** field, select an acquisition method, which will be used as the starter method, from the list.

6. Make sure **DMS Only** is selected.

The Mass Spectrometer option (MS Analysis or MS/MS Analysis) is set based on the scan type in starter acquisition method.
7. Click **Next**.

**Figure 3-14 DMS Options Dialog**

![DMS Options Dialog Image]

The DMS Options dialog opens. The default Start, Stop, and Step values for the SV, COV, and DMO parameters are shown in the dialog.

8. In the **DMS Options** dialog, specify the values to use to optimize the SV, COV and DMO DMS parameters. Use the default values or a smaller range which can reduce the time required for the optimization process. The default step size is used unless a different step size is specified for SV, COV, and DMO. The allowed ranges for SV, COV, and DMO are listed in **Table 3-3 on page 35**.

**Table 3-3 Allowed Ranges for SV, COV, and DMO**

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Start</th>
<th>Stop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation Voltage (SV)</td>
<td>0</td>
<td>The maximum allowed SV based on the DT and TEM values specified in the starter acquisition method will be displayed in this field.</td>
</tr>
<tr>
<td>Compensation Voltage (COV)</td>
<td>–100</td>
<td>100</td>
</tr>
<tr>
<td>DMS Offset (DMO)</td>
<td>–100</td>
<td>100</td>
</tr>
</tbody>
</table>

COV is ramped at each level of SV. After COV ramping, DMO is ramped.
9. Click **Finish** to begin the optimization process.

The optimization process starts. The screen actively displays two windows, a text file window and an acquisition window. You may need to minimize one of them to see the other. The x-axis shows the parameter that is being optimized, for example COV, in volts. The y-axis shows the intensity in counts per second (cps). The text file window is updated as results are generated.

At the end of the optimization process, the combination of parameters that provides the highest signal intensity is saved. If the intensity is below the minimum value (100 cps), an error message will be displayed in the text file window.

After the optimization process is successfully completed, the optimized parameters are added into a copy of the starter method, and the new method is saved with the name: "[startmethodname]_DMS.dam", in the C:\Analyst Data\Projects\[Your_Project folder]\Acquisition Methods folder. The text file is saved in C:\Analyst Data\Projects\[Your_Project folder]\Log folder.

All the .wiff files generated during the optimization process are saved in the C:\Analyst Data\Projects\[Your_Project folder]\Data folder.

### Optimize Compound and DMS Parameters with T-Infusion Optimization Type

The Analyst® software provides an option to optimize both compound and DMS parameters together in the Compound Optimization mode. When this option is used, first the compound parameters are optimized in T-infusion using a similar workflow as the infusion optimization, and then the DMS parameters are optimized at the end similar to the DMS Only option.

1. On the Navigation bar, under **Tune and Calibrate**, double-click **Compound Optimization**.
2. On the **Instruments Settings** page, in the **Inlet** section, click **T-Infusion**.
3. In the **Default Acq. Method** field, select an acquisition method, which will be used as the starter method, from the list.
4. Click **Compound and DMS**.
   The Mass Spectrometer options are enabled.
5. Click **MS Analysis** or **MS/MS Analysis** based on the scan type selected in your default acquisition method.
6. Click **Next**.
   The Ions to use in MS/MS Analysis dialog opens.

**Note:** Most compounds optimize between –20 to 30 volts range in the absence of a modifier. Some compounds may optimize even higher than 30 volts. In the presence of a modifier, we recommend using at least –60 to 20 volts COV range because the negative COV shifts can be very large for some compounds.
7. On the **Ions to use in MS/MS Analysis** dialog, type the values for all the fields. Refer to the *Automatic Optimization Tutorial*.

8. Click **Criteria** next to the Auto Select option.

9. In the **Product Ion Auto Selection Criteria** dialog, type the values for all the fields. Refer to the *Automatic Optimization Tutorial*.

10. Click **OK** to save the changes to the selection criteria.

11. Click **Next**.

12. In the **Target Components** dialog, type the names of the compounds and their Q1 and Q3 masses. Refer to the *Automatic Optimization Tutorial*.

13. Click **Next**.

**Figure 3-15 DMS Options Dialog**

The DMS Options dialog opens. The default Start, Stop, and Step values for the SV, COV, and DMO parameters are shown in the dialog.

14. In the **DMS Options** dialog, specify the values to use to optimize the SV, COV, and DMO parameters. Use the default values or a smaller range that can reduce the time required for the optimization process. The default step size is used unless a different step size is specified for SV, COV, and DMO. The allowed ranges for SV, COV, and DMO are listed in Table 3-4.
COV is ramped at each level of SV. After COV ramping, DMO is ramped.

**Note:** Most compounds optimize between –20 to 30 volts range in the absence of a modifier. Some compounds may optimize even higher than 30 volts. In the presence of a modifier, we recommend using at least –60 to 20 volts COV range because the negative COV shifts can be very large for some compounds.

15. Click **Finish** to begin the optimization process.

The optimization process starts. The screen actively displays two windows, a text file window and an acquisition window. You may need to minimize one of them to see the other window. The x-axis shows the parameter that is being optimized, for example COV, in volts. The y-axis shows the intensity in counts per second (cps). The text file window is updated as results are generated.

At the end of the optimization process, the combination of parameters that provides the highest signal intensity is saved. If the intensity is below the minimum value (100 cps), an error message will be displayed in the text file window.

After the optimization process is successfully completed, the optimized parameters are added into a copy of the starter method, and the new method is saved with the name: "[startmethodname]_DMS.dam", in the C:\Analyst Data\Projects\[Your_Project folder]\Acquisition Methods folder. The text file is saved in C:\Analyst Data\Projects\[Your_Project folder]\Log folder.

All the .wiff files generated during the optimization process are saved in the C:\Analyst Data\Projects\[Your_Project folder]\Data folder.

**Optimize COV Through Flow Injection (FIA)**

COV is the only DMS parameter that can be optimized using the FIA method. The FIA method is also used to fine-tune the source- and compound-dependent parameters.

Before starting FIA optimization, you should already have created an acquisition method with optimized source, compound, and DMS parameters using the T-infusion method described in Optimize Compound and DMS Parameters with T-Infusion Optimization Type on page 36. Make sure that an autosampler is included in methods used for FIA optimization. This method will be used as the starter method for FIA.

**Optimize the COV Parameter Through FIA**

1. On the Navigation bar, under **Tune and Calibrate**, double-click **Compound Optimization**.

---

### Table 3-4 Allowed Ranges for SV, COV, and DMO

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Start</th>
<th>Stop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation Voltage (SV)</td>
<td>0</td>
<td>The maximum allowed SV based on the DT and TEM values specified in the starter acquisition method will be displayed in this field.</td>
</tr>
<tr>
<td>Compensation Voltage (COV)</td>
<td>–100</td>
<td>100</td>
</tr>
<tr>
<td>DMS Offset (DMO)</td>
<td>–100</td>
<td>100</td>
</tr>
</tbody>
</table>
2. On the **Instrument Settings** page, depending on the stack you are using, type the following:

<table>
<thead>
<tr>
<th>Field</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>FIA</td>
</tr>
<tr>
<td>Default Acq. Method</td>
<td>Starter acquisition method</td>
</tr>
<tr>
<td>Rack Code</td>
<td>Autosampler specific</td>
</tr>
<tr>
<td>Rack Position</td>
<td>Autosampler specific</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>Amount of sample to be injected in µL</td>
</tr>
<tr>
<td>Mass Spectrometer</td>
<td>MS/MS Analysis</td>
</tr>
</tbody>
</table>

3. Click **Next**.

4. On the **FIA Target Compounds** page, do not select the **Int. Std.** check box because any transition that is marked as an Internal Standard will not be optimized.

5. In the **Resolution** section, select **Unit** in both the **Q1 Resolution** and **Q3 Resolution** fields.

6. Click **Next**.

7. On the **FIA Source Parameters** page, if needed, for each parameter you want optimized, type at least two values to optimize and then select the check box in the **Optimize** column. Refer to the **Automatic Optimization Tutorial**.

8. Click **Next**.

9. On the **FIA Compound Parameters** page, if needed, for each compound, type the values to use to optimize for Declustering Potential, Collision Energy, and Collision Cell Exit Potential. Refer to the **Automatic Optimization Tutorial**.

10. On the same page, for each compound, type the values to optimize for the COV parameter in the **Values for Optimization** column separated by **semicolons (;)**. For example, 2.1;2.2;2.3;.

    **Note:** Most compounds optimize between −20 to 30 volts range in the absence of a modifier. Some compounds may optimize even higher than 30 volts. In the presence of a modifier, we recommend using at least −60 to 20 volts COV range because the negative COV shifts can be very large for some compounds.

11. Select the check box in the **Optimize** column for COV.

    The Total # of Injections and Total Sample Volume fields update automatically. The Mass Spec. Duration field shows the duration from the starter method selected in step 2.

12. Click **Finish** to begin the optimization process.

    The Analyst® software runs the specified values of source- and compound-dependent parameters including COV and selects the value with the highest response, to get the best signal for the compounds of interest. As the software proceeds through the optimization, it creates a FIA optimization report. The FIA
optimization report will report the optimal COV for the SV specified in the starter method for each compound.

13. The software generates a final optimized FIA acquisition method called "*_DMS.dam". You can open this method and save it using a simpler name.

To optimize COV at a different SV value, modify the starter method and then run FIA optimization again.

Instrument Optimization

Instrument optimization with the SelexION™ device installed should only be used for troubleshooting or correcting minor resolution or calibration issues. For full instrument optimization, we recommend that the SelexION ion mobility cell be removed from the ion source before performing instrument optimization.
Objectives

In this chapter, you will do the following:

- Create an acquisition method with fixed DMS parameters in the Acquire mode.
- Learn about the capability of the SelexION™ technology to separate isobaric compounds.
- Differentiate isobaric compounds in MRM and Scheduled MRM™ algorithm acquisition methods.
- Create a Scheduled MRM algorithm acquisition method with DMS parameters.
- Create an acquisition method to ramp COV in the batch acquisition mode.
- Learn about modifier consumption calculations.

Prerequisites

- Make sure that the SelexION™ technology is installed on the instrument and the controller module is switched on.

Create an Acquisition Method with Fixed DMS Parameters in the Acquire Mode

You can use this procedure if you already have the optimized values for all the DMS parameters and need to create a new acquisition method using these values.

3. Click Mass Spec in the Acquisition method pane.
4. Notice that the Ramp COV check box on the MS tab page is deselected. This is done to allow the use of fixed COV value and not ramp the COV parameter.
5. Select a scan type and then type, as applicable, the mass range, MRM transitions, dwell time, scan speed, and other needed information. Refer to the Manual Optimization Tutorial.
6. Right-click in the mass ranges table and then select Separation Voltage SV. A new column called SV (volts) is added to the mass ranges table.
7. Type the optimized SV value in the first row of the SV (volts) column.
8. Right-click in the mass ranges table and then select Compensation Voltage COV. A new column called COV (volts) is added to the mass ranges table.
9. Type the optimized COV value in the first row of the COV (volts) column.

10. Right-click in the mass ranges table and then select DMS Offset DMO.
    A new column called DMO (volts) is added to the mass ranges table.

11. Type the optimized DMO value in the first row of the DMO (volts) column.

12. Type the masses and other information including SV, COV, and DMO values for all the other compounds in the mass ranges table.

13. Click the Edit Parameters button.
    The Period 1 Experiment 1 parameter Table dialog opens.

14. Type the optimized values of the compound parameters on the Compound tab.
    Type the optimized values of the ion source and gas parameters on the Source/Gas tab. Make sure that the source temperature specified here is same as the one used for optimizing the DMS parameters. Refer to the Manual Optimization Tutorial.

15. Select the DMS tab and then select the required values for the DMS Temperature (DT), Modifier (MD), and DMS Resolution Enhancement (DR) parameters. Make sure that the DMS temperature specified here is same as the one used for optimizing the DMS parameters.

16. Click OK.

17. If required, provide values for the parameters of the peripheral devices included in the active hardware profile.

18. Save the acquisition method.
The pause time between mass ranges is tied to the DMS Resolution Enhancement (DR) value, but users can use their preferred value. However, the use of pause time between mass ranges lower than the default value (20 ms) set by the Analyst® software may result in signal loss.

**Note:** If you want to optimize different values for DMS parameters, you can do so by opening the acquisition method in the Manual Tuning mode and then optimizing the parameters again with different values.

### Separate Isobaric Compounds using the SelexION™ Technology

Isobaric compounds have the same nominal mass and, thus, cannot be distinguished by the mass spectrometer. The SelexION™ technology automatically eliminates crosstalk and separates isobaric compounds.

When creating an MRM acquisition method for multiple isobaric compounds, we recommend alternating isobaric masses with mass ranges of other compounds if possible, and avoid having isobaric masses in consecutive MRM transitions. For example if you have two isobaric compounds and two non-isobaric compounds in an MRM method, then first type the mass ranges of one isobaric compound, next the mass ranges of one non-isobaric compound, then the second isobaric compound, and finally the second non-isobaric compound. This non-consecutive arrangement of isobaric compounds removes any potential crosstalk between the isobars.

If consecutive isobars are unavoidable in a given method, the software will take precautions to avoid crosstalk at the expense of a slightly longer cycle time.

### Differentiate Isobaric Compounds in MRM and Scheduled MRM™ Algorithm Acquisition Methods

When saving an MRM, Q1 MI, Q3 MI acquisition method or a Scheduled MRM™ algorithm acquisition method containing two or more consecutive isobaric compounds with the same Q1 and Q3 masses, the Analyst® software will display a warning. The warning indicates that if you use this method for quantitation, then the chromatograms in the Results Table for the isobaric compounds will be identical and will correspond to only one of the isobaric compounds.

To resolve this conflict, in the acquisition method, differentiate the isobaric compounds by adding at least 0.001 Da to either the Q1 or Q3 mass of one (or more) of the isobaric compounds. For example, the Q1 masses of some consecutive isobaric compounds could be changed in the following manner: 700.000, 700.001, 700.002, and so on.

Another method to differentiate the isobaric compounds is to type the name of the isobaric compounds in the Compound ID field in the acquisition method. This will help you easily identify the isobaric compounds correctly while you are creating the quantitation method for these compounds.
If data analyses and quantitation will be done using the MultiQuant™ software, identical masses will be separately quantified based on their Compound ID.

**Use DMS Parameters in a Scheduled MRM™ Algorithm Acquisition Method**

The Analyst® software supports the use of DMS parameters with the Scheduled MRM™ algorithm to obtain the best signal and separation of the compounds of interest.

You can use this procedure when you already have the optimized values for all the DMS parameters and you need to create a new Scheduled MRM algorithm acquisition method.

**Note:** Instead of creating a new Scheduled MRM algorithm acquisition method, you can use, as a starting point, an existing acquisition method that contains optimized compound, source, and DMS parameters, and LC flow rate. To this acquisition method, just add the required information about MRM scheduling. Refer to Scheduled MRM Algorithm Tutorial.

**Use DMS Parameters in a Scheduled MRM Algorithm Acquisition Method**

3. Click Mass Spec in the Acquisition method pane.
4. Create a Scheduled MRM algorithm acquisition method. Refer to Scheduled MRM Algorithm Tutorial.
5. Add the optimized SV, COV, and DMO values to the mass ranges table for the MRM transition using the following steps:
   a. Right-click in the mass ranges table and select Separation Voltage SV from the right-click menu.
      A new column called SV (volts) is added to the mass ranges table. The optimized SV value is also added.
   b. Right-click in the mass ranges table and select Compensation Voltage COV from the right-click menu.
      A new column called COV (volts) is added to the mass ranges table. The optimized COV value is also added.
   c. Right-click in the mass ranges table and select DMS Offset DMO from the right-click menu.
      A new column called DMO (volts) is added to the mass ranges table. The optimized DMO value is also added.
6. Repeat step 5 for all the MRM transitions in the acquisition method.
7. Click Edit Parameters. The Period 1 Experiment 1 parameter Table dialog opens.
8. Type the optimized values of the compound parameters on the **Compound** tab. Type the optimized values of the ion source and gas parameters on the **Source/Gas** tab. Make sure that the source temperature specified here is the same value as the one used for optimizing the DMS parameters. Refer to the *Manual Optimization Tutorial*.

9. Select the **DMS** tab and select the required values for the DMS Temperature (DT), Modifier (MD), and DMS Resolution Enhancement (DR) parameters. Make sure that the DMS temperature specified here is the same value as the one used for optimizing the DMS parameters.

10. Click **OK**.

11. If required, provide values for the parameters of the peripheral devices included in the active hardware profile, and then save the acquisition method.

In a *Scheduled* MRM algorithm acquisition method, the pause time between mass ranges is tied to the DMS Resolution Enhancement (DR) value, but users can use their preferred value. However, the use of pause time between mass ranges lower than the default value (20 ms) set by the Analyst software results in signal loss.

### Create an Acquisition Method to Ramp COV in the Batch Acquisition Mode

For analysis of samples by infusion, for example metabolism using surface sampling techniques, the SelexION™ device can be used for separation of compounds instead of liquid chromatography (LC), and the ramping Compensation Voltage (COV) feature can be used to mimic the LC gradient.

In addition to the Manual Tuning mode, the COV parameter can also be ramped during batch acquisition by selecting the Ramp COV check box in the acquisition method in the Acquisition Method editor. The COV parameter is treated as a cycle-dependant parameter. The ramping COV functionality works for a single period DMS acquisition method.

Use this procedure when you already have the optimized values or acceptable default values for all the DMS parameters except COV.

### Create an Acquisition Method to Ramp COV during the Batch Acquisition Mode


3. Click **Mass Spec** in the Acquisition method pane.

4. Select a scan type and then, as applicable, type the mass range, MRM transitions, dwell time, scan speed, and other needed information for all the compounds. Refer to the *Manual Optimization Tutorial*.

5. Select the **Ramp COV** check box on the MS tab page and then provide the following COV values in the allowed COV range of –100 to 100 volts:
   - **Start**: Type the voltage at which the ramping starts.
• **Stop**: Type the voltage at which the ramping stops.

• **Step**: Set the size of the steps on each cycle.

**Note**: Most compounds optimize between –20 to 30 volts range in the absence of a modifier. Some compounds may optimize even higher than 30 volts. In the presence of a modifier, we recommend using at least –60 to 20 volts COV range because the negative COV shifts can be very large for some compounds.

**Figure 4-1  MS Tab**

6. Click the **Edit Parameters** button. The Period 1 Experiment 1 parameter Table dialog opens.

7. Type the optimized values of the compound parameters on the **Compound** tab. Refer to the **Manual Optimization Tutorial**.

8. Type the optimized values of the ion source and gas parameters on the **Source/Gas** tab. Make sure that the source temperature (TEM) specified here is the same value as the one used for optimizing the DMS parameters. Refer to the **Manual Optimization Tutorial**.

9. Select the **DMS** tab and do the following:

   • Select the required values for the **DMS Temperature (DT)**, **Modifier (MD)**, and **DMS Resolution Enhancement (DR)** parameters. Make sure that the DMS temperature specified here is the same as the one used for optimizing the DMS parameters.

   • Type the optimized SV value for all the compounds in the **Separation Voltage (SV)** field.

   • Type the optimized DMO value for all the compounds in the **DMS Offset (DMO)** field. Click **OK**.
10. If required, provide values for the parameters of the peripheral devices included in the active hardware profile.

11. Save the acquisition method. It can be used for ramping COV during batch acquisition of samples and acquiring data.

Create and Submit Batches for Data Acquisition


Modifier Consumption Calculation

The modifier pump flow rate for the pre-defined modifiers in the Analyst® software is calculated by the software using the modifier density and the modifier molecular weight. The density and molecular weight of the pre-defined modifiers in the Analyst software are shown in Table 4-1.
The Analyst software uses the following formula to calculate the modifier pump flow rate in µL/min:

**Figure 4-2  Formula to Calculate the Modifier Pump Flow Rate**

\[
\text{Modifier Pump Flow Rate} = \frac{(0.1596 \times \text{CUR} + 2.0543) \times \text{MDC} \times 0.04089 \times \text{MDW} \times 1000}{[(100 - \text{MDC}) \times \text{MDD} ]}
\]

Where:

- **MDW**: Modifier Weight (g/mol)
- **MDC**: Modifier Composition
- **MDD**: Modifier Density (g/ml)
- **CUR**: Curtain Gas™ flow value

**Example Modifier Consumption Calculation**

As an example, approximate modifier consumption calculations for all the pre-defined modifiers in the Analyst software at a Curtain Gas flow of 20 psi and at different modifier concentrations for a 24 hour batch is displayed in Table 4-2.

**Table 4-2 Approximate Modifier Consumption (in mL) at a Curtain Gas Flow of 20 psi for a 24 Hour Batch**

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Concentration</th>
<th>Low (1.5%) (mL/24hrs)</th>
<th>High (3.0%) (mL/24hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Propanol</td>
<td></td>
<td>360</td>
<td>730</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td></td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td>190</td>
<td>390</td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
<td>340</td>
<td>700</td>
</tr>
</tbody>
</table>
Calculate the Appropriate Modifier Consumption for a Batch

1. Determine the duration of the batch.
2. Determine the modifier pump flow rate from the Mass Spec Detailed Status dialog by clicking on the Mass Spec icon on the Status bar in the bottom right corner of the Analyst software window, while running the method from the Analyst software.
3. Multiply the modifier pump flow rate (µL/min) with the batch duration (minutes) to obtain the approximate volume of modifier required for a batch.

**Note:** We recommend using a 2 L bottle for the modifier when running long batches at high CUR value and high modifier concentration.
Objectives

- View DMS parameters in File Information while viewing data files.
- Create a quantitation method and generate Results Table.
- Quantify isobaric compounds.
- Report data.
- Review a data file acquired with the ramped COV parameter.
- Display a contour plot for data acquired with the ramped COV parameter.
- View ramped DMS parameters in file information while viewing data files.

Analyze Quantitative Data

View DMS Parameters Used for Acquisition in File Information While Viewing Data Files

The experimental conditions used to collect data are stored in the data file along with the results. You can see the DMS parameters that were used to acquire samples in the File Information of the data file.

   The Select Sample dialog opens.
2. In the Data Files pane, select the .wiff file you want to view.
3. In the Samples pane, select the sample you want to view and then click OK.
   The data acquired from your sample opens.
4. To view the file information, click the Show File Info icon.
   The File information pane opens below the graph.
5. Expand the required period in the left pane of the File Information pane and then click the required period experiment link.
   The start, stop, and step values of the COV parameter used during sample acquisition are recorded under the period section. The COV start and stop values for each compound are recorded under the period experiment section. All the DMS parameters (except COV) and modifier parameters used during the sample acquisition are recorded in the Parameter table section for the selected period and experiment.

Create Quantitation Methods and Generate Results Tables

You can create a quantitation method and generate a Results Table using the information available in the Quantitative Analysis module in the Getting Started Guide. You can also refer to the 5500 Series of Instruments System User Guide, the 6500 Series of Instruments System User Guide, and the Advanced User Guide.

Quantify Isobaric Compounds

While creating a quantitation method for isobaric compounds, it is much easier to identify them if their compound name was entered in the Compound ID field in the acquisition method. This is because the name of each isobaric compound (or any compound present in the acquisition method) is automatically entered next to its Q1/Q3 mass in the Analyte table when building a quantitation method.

If the compound name was not provided in the acquisition method for the isobaric compounds, then while creating the quantitation method, you will need to identify the isobaric compounds correctly and manually type their names next to their Q1/Q3 masses. For related information, refer to Differentiate Isobaric Compounds in MRM and Scheduled MRM™ Algorithm Acquisition Methods on page 43.

For quantifying isobaric compounds and generating Results Table for them, use the information available in the Quantitative Analysis module in the Getting Started Guide.

Report Data

You can create reports from a Results Table using the Reporter Software. Refer to the Reporter User Guide available at Start > All Programs > AB SCIEX > Reporter > Reporter Help.

If the MultiQuant™ software is used to quantitate data, the Reporter Software can be used to create reports.

Analyze Qualitative Data

Review a Data File Acquired with the Ramped COV Parameter

To review a data file acquired with the ramped COV parameter and an MRM scan type, use the following procedure:

1. Make sure that you are in the project folder where you saved the acquired data.
3. From the Data Files list, select the .wiff file containing the acquired data.
4. If required, in the Samples list, click a sample and then click OK.
The XIC for the selected data file opens. The x-axis displays the COV in volts and the y-axis displays the intensity in cps. The XIC for each compound is displayed in a different color.

5. Select a COV range in the XIC, right-click and select **Show Spectrum**.

The spectrum, with intensity on y-axis and Q1/Q3 masses in Da on x-axis, for the selected COV range is shown under the XIC. It shows the intensity of all the compounds that appear in the SelexION™ ion mobility cell in the selected COV range. The selected COV range is shown in the title of the open spectrum.
Display a Contour Plot for Data Acquired with the Ramped COV Parameter

The Analyst® software can display a contour plot for the data that is acquired using an acquisition method with ramped COV parameter and a full scan type. Contour plots provide visualization of the separation of the compounds. For more information about contour plots, refer to Contour Plots in Analyze and Process Data chapter in the Getting Started Guide, and Qualitative Data Analysis in Advanced User Guide.

2. In the Select Sample dialog, in the Data Files box, select a data file (.wiff file).
3. In the Samples pane, select a sample.
4. Click OK.

The TIC for the selected sample opens. The x-axis shows the COV in volts and the y-axis shows the intensity in cps.

5. Highlight the range you want to view in the contour plot. If you do not make a selection, you will view the entire range.
6. Right-click in the TIC and then select Show Contour Plot.
The contour plot for the selected sample is shown under the TIC window. The x-axis shows the COV in volts and the y-axis shows the m/z in Da.

7. To view the spectrum, select a region in the contour plot, right-click and then click **Show Spectrum**.

The spectrum for the selected COV range is shown below the contour plot window. It shows the intensity (y-axis) of all m/z values (x-axis) in the selected COV range. The selected COV range is shown in the title of the open spectrum.
View Ramped DMS Parameters in File Information While Viewing Data Files

The experimental conditions used to collect data are stored in the data file along with the results. You can see the ramped DMS parameters that were used to acquire samples in File Information of the data files.

   The Select Sample dialog opens.
2. In the Data Files pane, select the .wiff file you want to view.
3. In the Samples pane, select the sample you want to view and then click OK.
   The XIC for the selected sample opens.
4. To view the file information, click Show File Info icon on the toolbar.
   The File information pane opens below the TIC.
5. Click Period 1 in the left pane of the File Information pane.
   The start, stop, and step values used for the ramped COV parameter are displayed along with other information in the right pane of the File Information pane.
   Select the required period and experiment in the left pane to see the various experimental settings used in the acquisition method that were used to collect the data. All the DMS parameters used during the sample acquisition are recorded there too.
Objectives

• Create an Information Dependent Acquisition (IDA) method to ramp Compensation Voltage (COV) during batch acquisition mode.
• View the IDA data acquired with ramped COV in the IDA explorer.

Prerequisites

• Make sure that the SelexION™ technology is installed on the instrument and the controller module is switched on.

Create an IDA Acquisition Method to Ramp COV During Batch Acquisition

Instead of using liquid chromatography (LC) for analysis of samples, the SelexION™ device can be used with infusion to separate the compounds of interest and filter out the chemical noise or interference. With the SelexION device, the ramping COV feature of the Analyst® software can be used to mimic the LC gradient.

In addition to the Manual Tuning mode, the COV parameter can also be ramped during batch acquisition by selecting the Ramp COV check box in an acquisition method in the Acquisition Method editor. The COV parameter is treated as a cycle-dependant parameter. The ramping COV functionality works for a single period DMS acquisition method.

The IDA, Dynamic Fill Time (DFT), and Scheduled MRM™ algorithm methods without ramped parameter will run for the required method duration, but the IDA and DFT methods with ramped COV will run for the required number of cycles based on the ramping COV start, stop, and step values. The method run will terminate when the last step of COV is acquired, and not when the method duration is reached. The COV value is dynamically calculated and set for each cycle.

The COV, SV, DT, and DMO values of the MRM transitions in the survey scan (or any other supported IDA survey scans) are automatically propagated to the dependant scans in an IDA method. The same applies to a Scheduled MRM algorithm IDA method and to the DFT pre-scan for an MRM-triggered IDA method with DFT scans.

The modifier used in the survey scan of an MRM IDA method also gets automatically propagated to the dependant scans.

Use this procedure when you already have the optimized values for all the DMS parameters except COV.

Create an IDA Acquisition Method to Ramp COV During the Batch Acquisition Mode


2. Create an IDA method with an MRM scan as survey scan. Refer to the IDA Tutorial.
3. In the Acquisition method editor, in the open IDA method you created in the previous step, click **MRM** in the Acquisition method pane.

4. Select the **Ramp COV** check box on the MS tab page and then provide the following COV values in the allowed COV range of –100 to 100 volts:
   - **Start**: Type the voltage at which the ramping starts.
   - **Stop**: Type the voltage at which the ramping stops.
   - **Step**: Set the size of the steps on each cycle.

**Note**: Most compounds optimize between –20 to 30 volts range in the absence of a modifier. Some compounds may optimize even higher than 30 volts. In the presence of a modifier, we recommend using at least –60 to 20 volts COV range because the negative COV shifts can be very large for some compounds.

5. Click the **Edit Parameters** button.
The Period 1 Experiment 1 parameter Table dialog opens.

6. Type the optimized values of the compound parameters on the **Compound** tab. Refer to the *Manual Optimization Tutorial*.

7. Type the optimized values of the ion source and gas parameters on the **Source/Gas** tab. Make sure that the source temperature (TEM) specified here is the same as the one used for optimizing the DMS parameters. Refer to the *Manual Optimization Tutorial*.

8. Select the **DMS** tab and do the following:
   - Select the required values for the **DMS Temperature (DT)**, **Modifier (MD)**, and **DMS Resolution Enhancement (DR)** parameters. Make sure that the DMS temperature specified here is the same as the one used for optimizing the DMS parameters.
   - Type the optimized SV value for all the compounds in the **Separation Voltage (SV)** field.
   - Type the optimized DMO value for all the compounds in the **DMS Offset (DMO)** field.

9. Click **OK**.

10. If required, provide values for the parameters of the peripheral devices included in the active hardware profile.

11. Save the acquisition method. It can be used to ramp COV during batch acquisition and acquire data.
Create and Submit Batches for Data Acquisition


View IDA Data

If you are acquiring data using an IDA method, the data will not open in the IDA viewer until the acquisition has finished, but it will show in the Explore window during acquisition.

To make it easier to view IDA data, you can set the IDA Explorer as your preset viewer. You can use the IDA Explorer tab in the Appearance Options dialog to select the IDA Explorer that will be used when showing IDA data. You can also select the columns for the Mass-List List view pane. The IDA Explorer is preset to show IDA samples.

For more information, refer to View IDA data in the IDA Tutorial.

View the IDA Data Acquired with Ramped COV Parameter in the IDA Explorer

1. Make sure that you are in the project folder where you saved the acquired IDA data.
   The Select Sample dialog opens.
3. From the Data Files list, select the .wiff file containing the acquired IDA data.
4. In the Samples list, click the required sample.
5. Click OK.
The IDA Explorer opens. The left portion of the pane shows a list of masses sent to the dependent scan as a tree view, or as a list view. You can switch between views by selecting Tree View or List View at the bottom of the Mass List pane.

The right portion of the pane shows the acquired IDA data graphically. Notice that instead of time, the ramped COV range is shown on the x-axis in the TIC and XIC graphs.

6. Use the two buttons above the graph to switch between single graph and multiple graph displays. To view the active graph only, click **Show only active graph**. To return to multi-graph view, click **Show all the graphs**. To view all the graphs in Explorer, click **Export all the graphs to Explorer**.
Figure 6-1 Buttons in IDA Explorer

<table>
<thead>
<tr>
<th>Item</th>
<th>Button name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Show all the graphs</td>
</tr>
<tr>
<td>2</td>
<td>Show only active graph</td>
</tr>
<tr>
<td>3</td>
<td>Export all the graphs to Explorer</td>
</tr>
</tbody>
</table>
### DMS Parameters Descriptions

**Table A-1  DMS Parameters, Descriptions, and Possible Settings**

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Description</th>
<th>Possible Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS Temperature (DT)</td>
<td>The temperature applied on the ceramic heater that is mounted in the back of the curtain plate heat exchanger. For each DT value change, allow the system to equilibrate for at least 10 to 15 minutes before optimizing any DMS parameters. When the new temperature is reached, “DMS Temperature reached” is displayed above the DT field.</td>
<td>• Low (150 °C; Default)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Medium (225 °C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High (300 °C)</td>
</tr>
<tr>
<td>Modifier (MD)</td>
<td>A chemical that is added into the Curtain Gas™ flow to help improve the separation of ions. If the MD value is set to None, the MDC, MDD, and MDW parameters are not available. If the MD value is set to a value other than None, the MDC parameter becomes available. If MD is assigned the Custom value, the MDC, MDD, and MDW parameters become available.</td>
<td>• None (Default)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 2-Propanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Acetonitrile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Methanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Acetone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Custom</td>
</tr>
<tr>
<td>Modifier Composition (MDC)</td>
<td>The parameter that controls the modifier concentration. The Analyst® software will use the density and molecular weight of the selected modifier and the Curtain Gas flow value to calculate the modifier pump flow rate and apply that flow rate automatically.</td>
<td>• Off (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Low (default; 1.5% or higher)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High (3.0)</td>
</tr>
<tr>
<td>Modifier Density (g/mL) (MDD)</td>
<td>The density of a custom modifier. The Analyst software uses this density along with the Modifier molecular weight and the Curtain Gas flow value to determine the modifier pump flow rate.</td>
<td>Specify the density in g/mL for the modifier.</td>
</tr>
<tr>
<td>Modifier MW (MDW)</td>
<td>The molecular weight in g/mol of a custom modifier. The Analyst software uses the value of this parameter along with the Modifier density and the Curtain Gas flow value to determine the modifier pump flow rate.</td>
<td>Specify the molecular weight (g/mol) of the modifier.</td>
</tr>
</tbody>
</table>
### Table A-1  DMS Parameters, Descriptions, and Possible Settings (Continued)

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Description</th>
<th>Possible Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation Voltage (SV)</td>
<td>The peak to peak amplitude of the separation voltage waveform.</td>
<td>• 0.0 (Default)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Allowed range: 0 to the maximum SV value based on the DT and TEM parameters in each method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Specify the Separation Voltage.</td>
</tr>
<tr>
<td>Compensation Voltage (COV)</td>
<td>A DC offset potential between the two electrodes of the mobility device. For example, if COV is 5 volts, then one of the two electrodes has 5 volts higher potential applied than the other one.</td>
<td>• 0.0 (Default)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Allowed range: –100 to 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Specify the Compensation Voltage to be applied.</td>
</tr>
<tr>
<td>DMS Offset (DMO)</td>
<td>The voltage applied to the two electrodes of the SelexION™ ion mobility cell, relative to the orifice potential. For example, if the DMS offset potential is 5 volts and the orifice potential is at 100 volts, then the two electrodes in front of the orifice inlet have 105 volts potential. The DMS offset is a way to tune the transmission out of the SelexION ion mobility cell into the mass spectrometer.</td>
<td>• –3.0 volts in positive polarity; 3.0 volts in negative polarity (Default)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Access range: –100 to 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Specify the DMO value.</td>
</tr>
<tr>
<td>DMS Resolution Enhancement (DR)</td>
<td>The parameter that controls the flow of the nitrogen gas that can be enabled between the SelexION device exit and the orifice inlet into the mass spectrometer. In the 6500 series of instruments, when the value of DR is set to either Off, Low, Medium, or High, gas flow is enabled, which improves the resolution of the SelexION device. For the 5500 series of instruments, DR value of Low, Medium, or High, enables the gas flow that improves the resolution of the SelexION device. DR is also referred to as throttle gas.</td>
<td>For 6500 series of instruments: • Open (0; default) • Off (10) • Low (22) • Medium (34) • High (43) For 5500 series of instruments: • Off (0; default) • Low (10) • Medium (25) • High (40) DR can be set to the Operator mode in the Parameter Settings Editor, and the allowed range is 0 to 100.</td>
</tr>
</tbody>
</table>
State of DMS Parameters in the DMS Off Mode

Table A-2  State of DMS Parameters in the DMS Off Mode

<table>
<thead>
<tr>
<th>DMS Parameters</th>
<th>State in DMS Off Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS Temperature (DT)</td>
<td>Visible on UI (user interface); value = Low (default)</td>
</tr>
<tr>
<td>Modifier (MD)</td>
<td>Hidden; value = none</td>
</tr>
<tr>
<td>Separation Voltage (SV)</td>
<td>Hidden; value = 0</td>
</tr>
<tr>
<td>Compensation Voltage (COV)</td>
<td>Hidden; value = 0</td>
</tr>
<tr>
<td>DMS Offset (DMO)</td>
<td>Hidden; value = –3.0 volts in positive polarity; 3.0 volts in negative polarity</td>
</tr>
<tr>
<td>DMS Resolution Enhancement (DR)</td>
<td>Hidden; value = Open (for 6500 series of instruments); value = Off (for 5500 series of instruments)</td>
</tr>
</tbody>
</table>

Values of DMS and Source Parameters Used During Modifier Purge

Table A-3  DMS and Source Parameters Used During Modifier Purge

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Value Set During Modifier Purge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modifier Pump Flow Rate</td>
<td>1000 (µL/min)</td>
</tr>
<tr>
<td>Temperature (TEM)</td>
<td>300</td>
</tr>
<tr>
<td>DMS Temperature (DT)</td>
<td>225</td>
</tr>
<tr>
<td>DMS Resolution Enhancement (DR)</td>
<td>0</td>
</tr>
<tr>
<td>Separation Voltage (SV)</td>
<td>0</td>
</tr>
<tr>
<td>Compensation Voltage (COV)</td>
<td>0</td>
</tr>
<tr>
<td>DMS Offset (DMO)</td>
<td>3</td>
</tr>
<tr>
<td>Ion Source Gas 1 (GS1)</td>
<td>50</td>
</tr>
<tr>
<td>Ion Source Gas 1 (GS2)</td>
<td>50</td>
</tr>
<tr>
<td>Curtain Gas (CUR)</td>
<td>20</td>
</tr>
<tr>
<td>IonSpray Voltage (IS)</td>
<td>0</td>
</tr>
<tr>
<td>Collision Gas (CAD)</td>
<td>System Default</td>
</tr>
</tbody>
</table>
Values of DMS and Source Parameters After Modifier Purge is Completed or Aborted

Table A-4  DMS and Source Parameters Reset After Modifier Purge is Completed or Aborted

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Value After Modifier Purge is Completed or Aborted</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS Temperature (DT)</td>
<td>150</td>
</tr>
<tr>
<td>Temperature (TEM)</td>
<td>0</td>
</tr>
<tr>
<td>Modifier Pump Flow Rate</td>
<td>0</td>
</tr>
<tr>
<td>GS1</td>
<td>20</td>
</tr>
<tr>
<td>GS2</td>
<td>0</td>
</tr>
<tr>
<td>CUR</td>
<td>30</td>
</tr>
<tr>
<td>CAD</td>
<td>6 (for AB SCIEX Triple Quad™ 5500 and AB SCIEX Triple Quad 6500 systems); 9 (medium) for QTRAP® 5500 and QTRAP 6500 systems</td>
</tr>
</tbody>
</table>

WARNING! Toxic Chemical Hazard: Make sure that the sample tubing nut is tightened properly before operating this equipment. If the sample tubing nut is not tight, the sample may leak, and you may be exposed to dangerous chemicals.

1. Replace the two-way grounding union with a three-way grounding union (PN 018786: Fitting union 0.15 mm Bore; PN 018787: Fitting Tee Ring) on the ion source.
2. Connect the red PEEK tubing from the syringe pump to the grounding union on the ion source.
3. Connect the grounding union on the ion source to an LC pump.
4. Connect the sample tubing nut to the grounding union through a 30 cm piece of red PEEK tubing. Refer to Connect the Sample Tubing topic in the Turbo V™ Ion Source Operator Guide.

Tip! T-infusion (split-infusion) can also be performed using an external tee to connect the three lines. The LC and syringe streams connect to the tee, which in turn is connected to the two-way grounding union.

WARNING! Electrical Shock Hazard: Do not bypass the grounding union connection. The grounding union provides safety grounding between the mass spectrometer and the sample introduction device.
Refill the Modifier Bottle

Caution: Do not refill the modifier bottle while it is in the side tray.

1. Disconnect the fluid line from the modifier bottle in the side tray.
2. Refill the modifier bottle in a safe location taking the appropriate safety precautions if needed.
   
   ! WARNING! Toxic Chemical Hazard: Take caution when filling or re-filling modifier bottles with caustic hazardous or toxic chemicals.
   
3. Reinstall the modifier bottle in the side tray and reconnect the fluid line.
Troubleshooting

Issue #1

An error message is displayed about detection of discharge in the SelexION™ ion mobility cell during sample acquisition or equilibration.

Solution

Lower the SV by 100 volts and re-optimize the DMS Parameters.

When a discharge is detected in the SelexION ion mobility cell during sample acquisition, the Analyst® software aborts the sample acquisition and logs the error in the event log. If you double-click the aborted sample in the queue, an error message is displayed.

If a discharge is detected in the SelexION ion mobility cell, then to avoid repeating the discharge when using the same method, use lower Separation Voltage (SV) or DMS Temperature (DT) value in the acquisition method. The DMS parameters will need to be re-optimized for the new SV or DT value.

If you notice discharges with SV and DT combinations that have worked in the past, then clean and align the SelexION ion mobility cell electrodes. Refer to Clean and Align the Ion Mobility Cell Electrodes on page 16.

When the fault is cleared the instrument goes into the Standby mode.

Issue #2

When running FIA Compound Optimization using the Shimadzu LC System, the optimization process appears to have stalled.

Solution

The default duration for Shimadzu methods is 90 minutes. When tuning with a mass spectrometer scan duration shorter than the Shimadzu time program duration (as in the default tune method), you will not be able to stop the tune run using the Analyst® software after the mass spectrometer has finished scanning. Press Run on the controller to stop the run or change the default Shimadzu run duration to match the MS duration in the Shimadzu method editor.

Issue #3

When the SelexION controller module is turned on or off, the Mass Spectrometer icon in the Analyst software turns and remains red.

Solution

Before turning the SelexION controller module on or off (to temporarily add or remove the SelexION technology to or from the system), first disable the active hardware profile within the Analyst software. Failure to do so may cause the mass spectrometer to enter an unstable state resulting in loss of control from within the Analyst software. If the hardware profile is not
deactivated and the mass spectrometer enters this unstable state, control may be recovered by removing and re-seating the ion source, or by power cycling both the mass spectrometer and controller module and reactivating the hardware profile.
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