SCIEX
The Power of Precision

Analyst MD Software

Scheduled MRM Algorithm Tutorial

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EC REP

Leica Microsystems CMS
GmbH Ernst-Leitz-Strasse
17-37 35578 Wetzlar
Germany

AB Sciex Pte. Ltd.
Blk33, #04-06 Marsiling Industrial Estate Road 3
Woodlands Central Industrial Estate, Singapore 739256

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Scheduled MRM Algorithm Tutorial

Objectives

Users will learn how to:

- Create a method to monitor *Scheduled* MRM algorithm transitions.
- View *Scheduled* MRM algorithm transitions in an extracted ion chromatogram.
- Analyze the quantitative data by creating the quantitation method and reviewing the Results Table.
- Create a *Scheduled* MRM Pro Algorithm Acquisition Method.
- Create an IDA *Scheduled* MRM Pro Algorithm Acquisition Method.

About the *Scheduled* MRM Algorithm

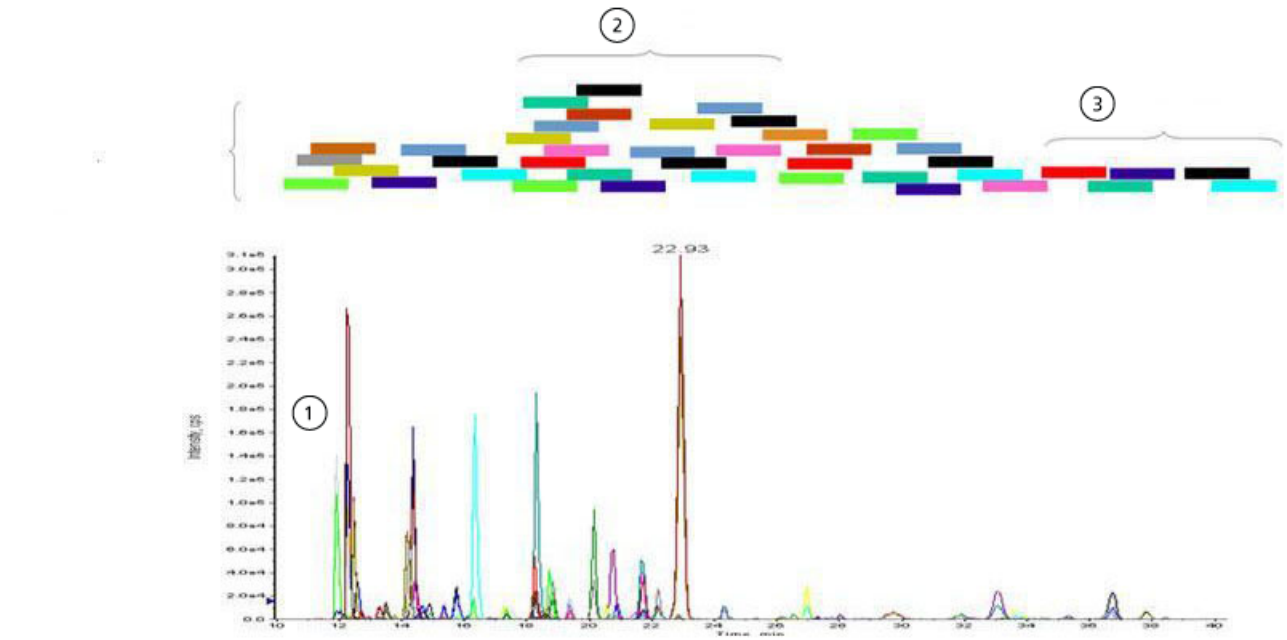
The *Scheduled* MRM algorithm aids in the acquisition of hundreds of compounds based on a list of multiple reaction monitoring (MRM) transitions, retention times, and compound IDs that are provided when the acquisition method is created. The *Scheduled* MRM algorithm functionality reduces the requirement for multi-period experiments. It can also be used as a survey scan in an IDA (Information Dependent Acquisition) method.

The algorithm maximizes points across the chromatographic peak to give better peak detection and improved reproducibility. With this feature, the user can also view data files with many MRM transitions by showing the compound ID, analyte Integration Quality index, and IS (internal standard) Integration Quality index columns in the Results Table. For SCIEX 3200MD systems, a maximum of 1000 transitions are supported by the *Scheduled* MRM algorithm. For SCIEX 4500MD and Citrine systems, a maximum of 4000 transitions are supported by the *Scheduled* MRM algorithm.

Note: The **Analyte Integration Quality Index** column and the **IS Integration Quality Index** column are also available for MRM data in the Results Table.

The following figure shows an example of a *Scheduled* MRM algorithm LC run. The number of MRM transitions monitored simultaneously varies during the LC analysis, but remains constant between injections.

Figure 1 Typical Example of a *Scheduled* MRM Algorithm LC Run



Item	Description
1	Monitored MRM transitions.
2	High number of MRM transitions monitored.
3	Low number of MRM transitions monitored.

To process a large number of transitions with the *Scheduled* MRM algorithm, use the MultiQuant MD software for data processing. For more information, contact a SCIEX sales representative.

Related Documentation

- *System User Guide* for the mass spectrometer
- *Advanced User Guide*
- *Scripts User Guide* (for more information about the Create Quan Methods From Text Files script and the Create Text File from Quan Method script)
- *Information Dependent Acquisition Tutorial* (for more information about creating methods using IDA)

- *Analyst Help*

Prerequisites

Prerequisites
Users should be able to: • Create an acquisition method • Submit a batch • Create a quantitation method, and create and review a Results Table. The following peripheral devices must be included in the hardware profile: • LC pump • Autosampler

Create a csv or txt File

Optionally, transition information for a *Scheduled* MRM method can be created and stored in a csv or txt file and can be imported into the mass ranges table of a *Scheduled* MRM Algorithm method. Use the following criteria for creating the csv or txt file:

- The file must not contain any header, column title, or row title.
- The order and number of columns in the file must match the order and number of the columns in the mass ranges table.
- For MRM and *Scheduled* MRM algorithm methods, there must be no empty cells. For *Scheduled* MRM Pro Algorithm methods, the **Window** and **Threshold** columns can be left blank to allow for use of default values. The **Group** column can also be left blank.

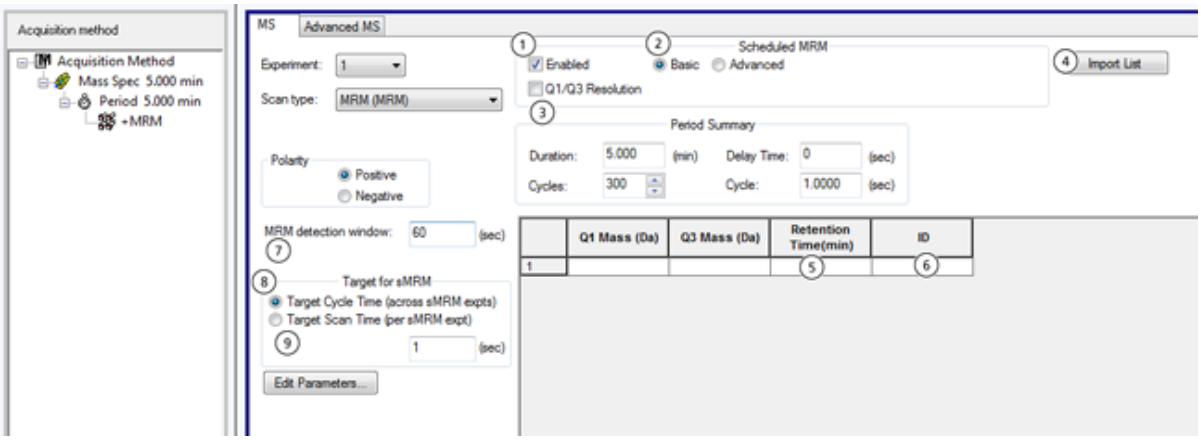
Make sure that the file is saved with either a csv or txt extension.

Create a *Scheduled* MRM Algorithm Acquisition Method

Note: For SCIEX 4500MD and Citrine systems, a maximum of 1,250 unscheduled MRM transitions and 4,000 MRM transitions with the *Scheduled* MRM algorithm can be acquired. For the SCIEX 3200MD systems, a maximum of 300 unscheduled MRM transitions and 1,000 MRM transitions with the *Scheduled* MRM algorithm can be acquired.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method** and then, in the Acquisition Method pane, select the **Mass Spec** icon.
2. Make sure that the selected **Scan Type** is **MRM** and then select the **Enabled** check box in the **Scheduled MRM** group. To create a method for the SCIEX 4500MD and Citrine systems, refer to the features in the figure: Figure 2. To create a method for the SCIEX 3200MD systems, refer to the features in the figure: Figure 3.

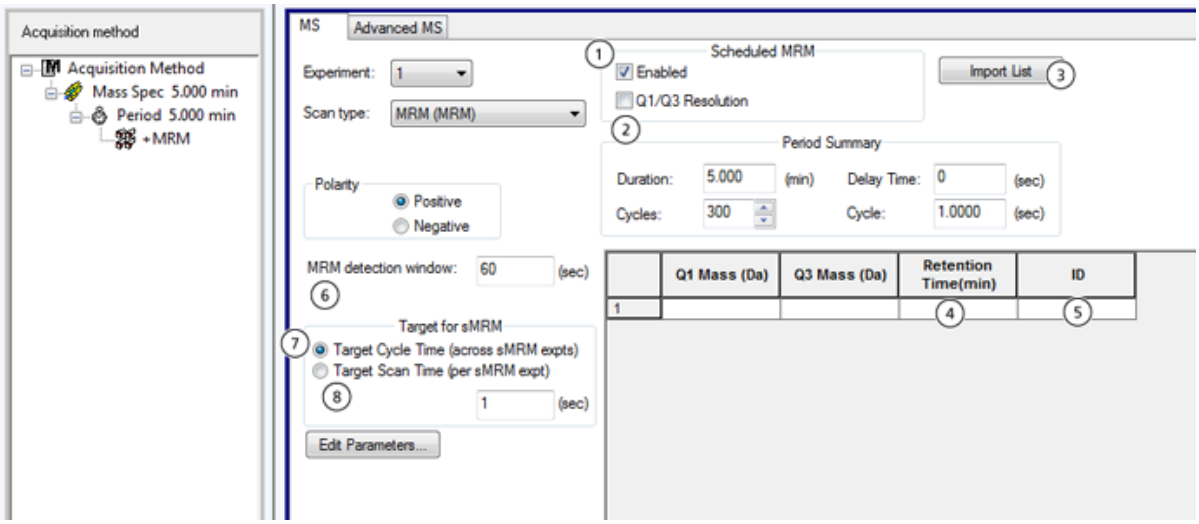
Figure 2 *Scheduled* MRM Algorithm Software Features for the SCIEX 4500MD and Citrine Systems



Item	Description
1	**Enabled** check box in the **Scheduled MRM** group: Select to enable the basic and advanced *Scheduled* MRM algorithm features.
2	**Basic** option: Select to enable the *Scheduled* MRM algorithm feature. Basic is the preset option.

Item	Description
3	**Q1/Q3 Resolution** check box: Select to apply different Q1 and Q3 resolution settings to each transition. When this option is selected, the **Q1 Resolution** column and the **Q3 Resolution** column are added to the mass ranges table.
4	**Import List** button: Click to import MRM transitions, time, ID, and compound-dependent parameters from a txt or csv file.
5	**Retention Time (min)** column: Type the expected retention time, in minutes, for the corresponding MRM transition. This column shows the dwell time in msec for MRM methods.
6	**ID** column: (Optional) Type a compound ID for the transition of interest.
7	**MRM detection window (sec)** field: Type the amount of time for detection that surrounds the retention time for each transition.
8	**Target Cycle Time (across sMRM experiments)** option: Select to specify and use the target cycle time for all of the *Scheduled* MRM algorithm experiments in the entire period or method. Selecting or clearing the option in one *Scheduled* MRM algorithm experiment automatically applies the same setting to the other *Scheduled* MRM algorithm experiment if there are two *Scheduled* MRM algorithm experiments in the method. A maximum of two *Scheduled* MRM algorithm experiments are allowed in one period or method. The target cycle time is adjustable so that a specific cycle time can be targeted throughout all cycles to get more evenly distributed data points across a peak regardless of a polarity switch between the concurrent transitions within a cycle. **Target Cycle Time** is the preset setting.
9	**Target Scan Time (per sMRM experiment) (sec)** field: Select to specify the target amount of time to use for the experiment in each cycle. The software will keep the total scan time for this experiment close to the target time in each cycle unless the minimum dwell time or the maximum dwell time is applied to some of the concurrent transitions. The target scan time is adjustable so that a specific number of points across the LC peaks can be targeted.

Figure 3 *Scheduled* MRM Algorithm Software Features for the SCIEX 3200MD Systems



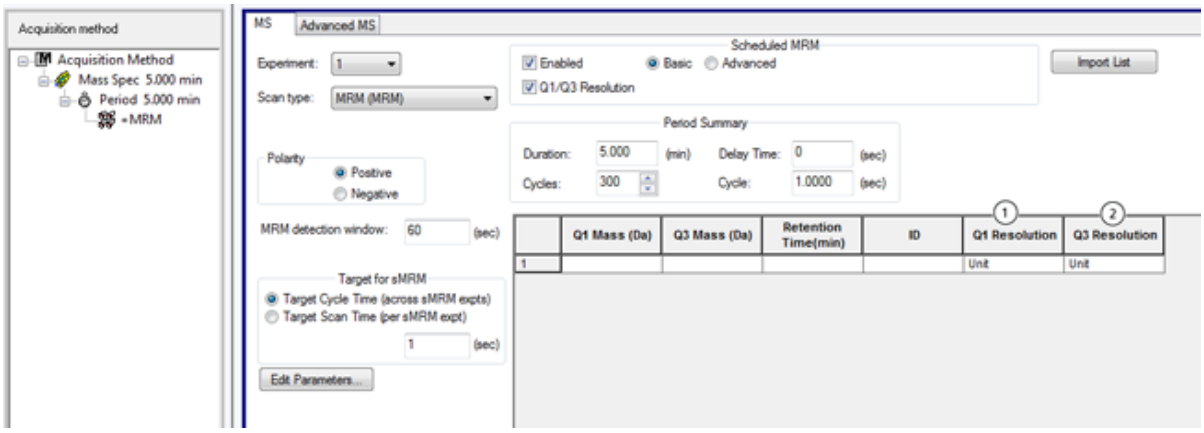
Item	Description
1	**Enabled** check box in the **Scheduled MRM** group: Select to enable the *Scheduled* MRM algorithm feature.
2	**Q1/Q3 Resolution** check box: Select to apply different Q1 and Q3 resolution settings to each transition. When this option is selected, the **Q1 Resolution** column and the **Q3 Resolution** column are added to the mass ranges table.
3	**Import List** button: Click to import MRM transitions, time, ID, and compound-dependent parameters from a txt or csv file.
4	**Retention Time (min)** column: Type the expected retention time, in minutes, for the corresponding MRM transition. This column shows the dwell time in msec for MRM methods.
5	**ID** column: (Optional) Type a compound ID for the transition of interest.
6	**MRM detection window (sec)** field: Type the amount of time for detection that surrounds the retention time for each transition.

Item	Description
7	**Target Cycle Time (across sMRM experiments)** option: Select to specify and use the target cycle time for all of the *Scheduled* MRM algorithm experiments in the entire period or method. Selecting or clearing the option in one *Scheduled* MRM algorithm experiment automatically applies the same setting to the other *Scheduled* MRM algorithm experiment if there are two *Scheduled* MRM algorithm experiments in the method. A maximum of two *Scheduled* MRM algorithm experiments are allowed in one period or method. The target cycle time is adjustable so that a specific cycle time can be targeted throughout all cycles to get more evenly distributed data points across a peak regardless of a polarity switch between the concurrent transitions within a cycle. **Target Cycle Time** is the preset setting.
8	**Target Scan Time (per sMRM experiment) (sec)** field: Select to specify the target amount of time to use for the experiment in each cycle. The software will keep the total scan time for this experiment close to the target time in each cycle unless the minimum dwell time or the maximum dwell time is applied to some of the concurrent transitions. The target scan time is adjustable so that a specific number of points across the LC peaks can be targeted.

3. To use different Q1 and Q3 Resolution settings for each transition, click **Q1/Q3 Resolution** in the Scheduled MRM group.

   Two columns are added to the mass ranges table: Q1 Resolution and Q3 Resolution.

Figure 4 Q1/Q3 Resolution Option Selected for the SCIEX 4500MD and Citrine Systems



Item	Description
1	**Q1 Resolution** column: The mode in which the Q1 scan separates closely spaced components. This is the resolving ability of the first quadrupole. Options include High, Unit, Low, or Open.
2	**Q3 Resolution** column: The mode in which the Q3 scan separates closely spaced components. This is the resolving ability of the third quadrupole. Options include are High, Unit, Low, or Open.

The **Resolution Q1** option and the **Resolution Q3** option on the Advanced MS tab are not available for use.

If the **Q1/Q3 Resolution** check box is selected in the Tune Method Editor, then the **Q1 Resolution** and the **Q3 Resolution** options on the Resolution tab are not available for use.

4. Complete the mass ranges table for each MRM transition of interest, using one of the following methods:

   - Type MRM transitions manually: Type the Q1 mass, Q3 mass, retention time, and compound ID for each transition of interest. For each transition, select values in the **Q1 Resolution** and **Q3 Resolution** columns. Right-click to add compound-dependent parameters as required. A maximum of four compound-dependent parameters can be added to the mass ranges table.
   - Import MRM transitions: Click **Import List** and, in the Open dialog, select either a comma-separated values (csv) or tab-separated values (txt) file that contains the MRM transition information. After selecting the file, click **Open**. The file contents are shown in the mass ranges table. For more information on creating files, refer to the section: Create a csv or txt File.
   - Copy and paste MRM transitions: Select the cells containing the required information from a csv or txt file and then press **Ctrl+C**. When pasting lines of information, select the first **Q1 Mass (Da)** cell in the mass ranges table and then press **Ctrl+V**.

   Note: Before importing or copying and pasting, make sure that the columns of data in the csv or txt file match those in the mass ranges table in the software. The number of columns and column order in the source file and destination table must be the same. Add, remove, or reorder the columns in the source file as required. To add a column to the mass ranges table, right-click in the mass ranges table and then select a compound-dependent parameter. For compound dependent parameters, the values must be in the allowable ranges for the selected polarity.

5. In the **MRM detection window (sec)** field, type the amount of time for detection that surrounds the retention time for each transition. This window should reflect the expected width of the chromatographic peak and the variability in the chromatographic retention time of the analyte such that the entire MRM peak is always in the window.

Use the LC chromatography as a guide to select the best *Scheduled* MRM algorithm parameters. Determine the width of a typical peak at base and then refer to the following table for the recommended settings. Make sure to consider the stability of the retention time in defining the MRM detection window.

Table 1 Recommended Settings for *Scheduled* MRM Algorithm Parameters

Peak Width At Base	MRM Detection Window	Target Scan Time or Target Cycle Time
30 seconds	90 seconds	2 seconds
15 seconds	60 seconds	1 second
10 seconds	30 seconds	0.5 seconds

For the *Scheduled* MRM algorithm, the number of analytes per cycle monitored is adjusted based on the retention time window of the analytes. To maximize the dwell time used for each analyte and its signal-to-noise ratio, we recommend using a smaller, but reasonable, retention time window that allows the peak of interest to be captured. A value of 60 seconds is a good starting point. This value is sufficient for chromatography that yields a peak width of 15 seconds and a potential retention time shift of 20 seconds both to the left and to the right of the peak.

For example, if the expected retention time is 4.5 minutes, then typing 60 seconds sets a detection window from 4 minutes to 5 minutes.

Note: If the **Retention Time** is set to 0, the software will monitor that transition for the full run time.

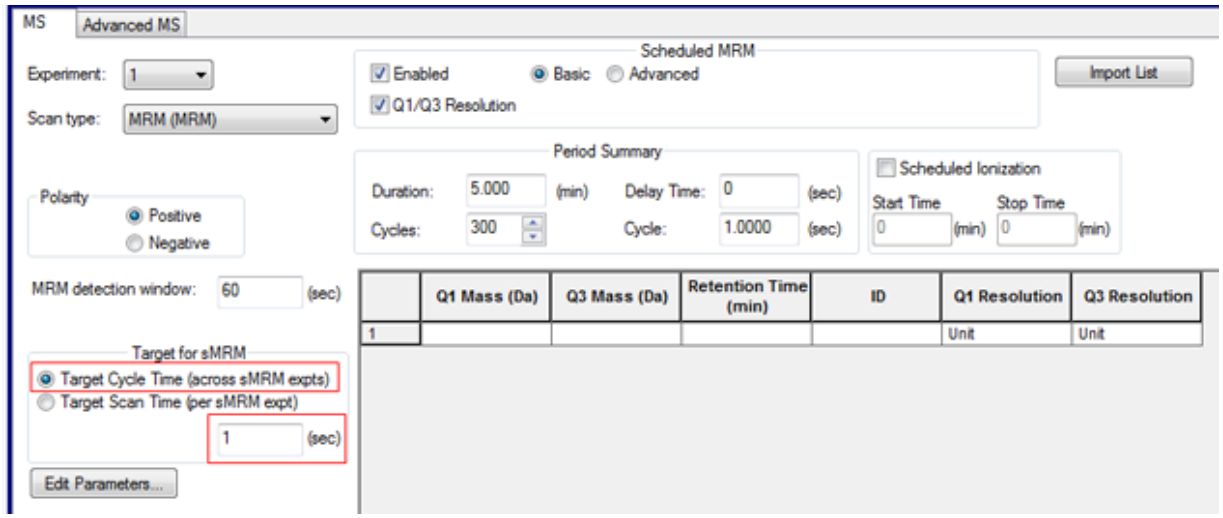
6. Do one of the following as required:

   - If required, leave the **Target Cycle Time (across sMRM expts)** option selected and then type a target cycle time, in seconds, as shown in the following figure. If the **Target Cycle Time** option is selected, then the software tries to apply the same cycle time to every cycle. However, the actual cycle time used for each cycle varies primarily depending on the sum of the dwell times of all of the transitions in the cycle.

     The software uses the target cycle time, polarity switch times, if there are any, and all of the pause times to calculate the dwell time for each transition within a cycle. The dwell time allotted for a transition depends on the calculated dwell time for each cycle in the detection window, whether the calculated dwell time is less than the minimum dwell time or greater than the maximum dwell time for some cycles, and how much it overlaps with each of the other transitions on the detection window. If the calculated dwell time is less than the minimum dwell time because of high concurrency or greater than the maximum dwell time because of low concurrency, then the minimum dwell time or the maximum dwell time will be used for that transition in that cycle for the next step of the calculation.

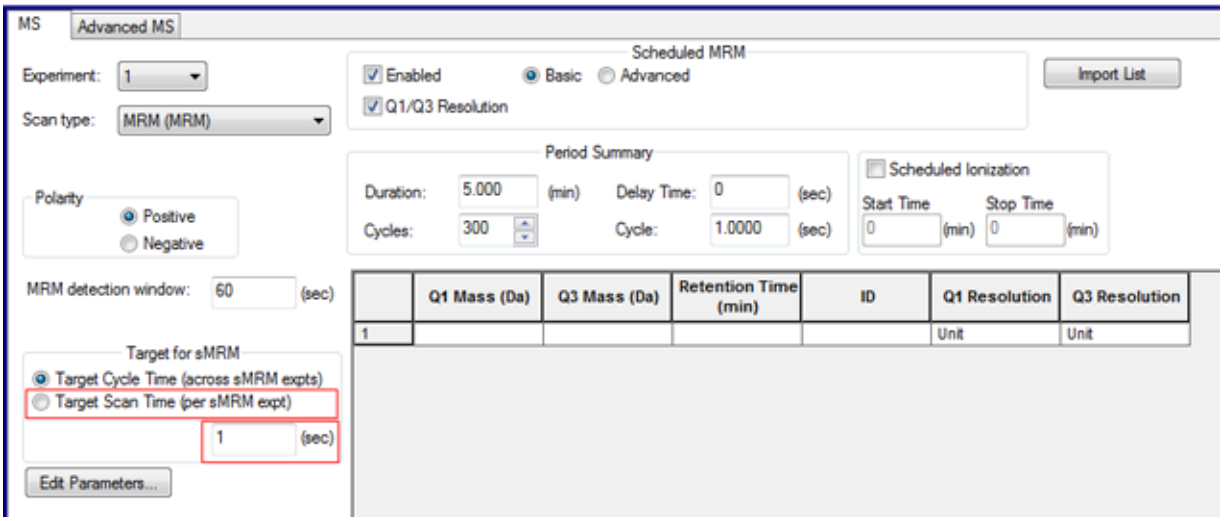
The average of the dwell times of all of the cycles for this transition is the final dwell time allotted for this transition.

Figure 5 Target Cycle Time (across sMRM expts)



- If required, click **Target Scan Time (per sMRM expt)**, and then type, in seconds, the target length of time for the experiment for each cycle as shown in the following figure. This parameter helps to define the number of points across the chromatographic peak.

Figure 6 Target Scan Time (per sMRM expt)



Use the width of the chromatographic peaks as a guide to help set this value. A value of 1 second is a good starting point for chromatography that yields a peak width of 15 seconds. In this case, a 1-second target scan time will generate approximately 15

data points over a 15-second peak when no minimum dwell time or maximum dwell time is being applied to any of the concurrent transitions within this detection window.

7. Provide the required values in the remaining fields of the acquisition method.
8. Save the acquisition method in the project from which the acquisition will run.

Note: The *Scheduled* MRM algorithm fields are also available in the Tune Method Editor.

Create an Acquisition Method using Two *Scheduled* MRM Algorithm Experiments

Use this procedure to create a method that supports polarity switching.

1. Create a *Scheduled* MRM Algorithm acquisition method using the steps in the section: Create a Scheduled MRM Algorithm Acquisition Method.
2. In the Acquisition method pane, right-click **Period** and then click **Add experiment**. A second MRM scan experiment is created.
3. On the MS tab, select one of the following options based on the mass spectrometer being used:
   - For the SCIEX 4500MD and Citrine systems, select the **Enabled** check box in the **Scheduled MRM** group. To create a regular *Scheduled* MRM Algorithm acquisition method, make sure that the **Basic** option is selected in the **Scheduled MRM** group. To create the method, refer to the features in the figure: Figure 2. To create a *Scheduled* MRM Pro Algorithm method, make sure the **Advanced** option is selected. To create this method, refer to the section: Create a Scheduled MRM Pro Algorithm Acquisition Method.
   - For the SCIEX 3200MD systems, select the **Enabled** check box in the **Scheduled MRM** group. To create the method, refer to the features in the figure: Figure 3.
4. Complete the acquisition method as described in the section: Create a Scheduled MRM Algorithm Acquisition Method.
5. Save the acquisition method in the project from which the acquisition will run.

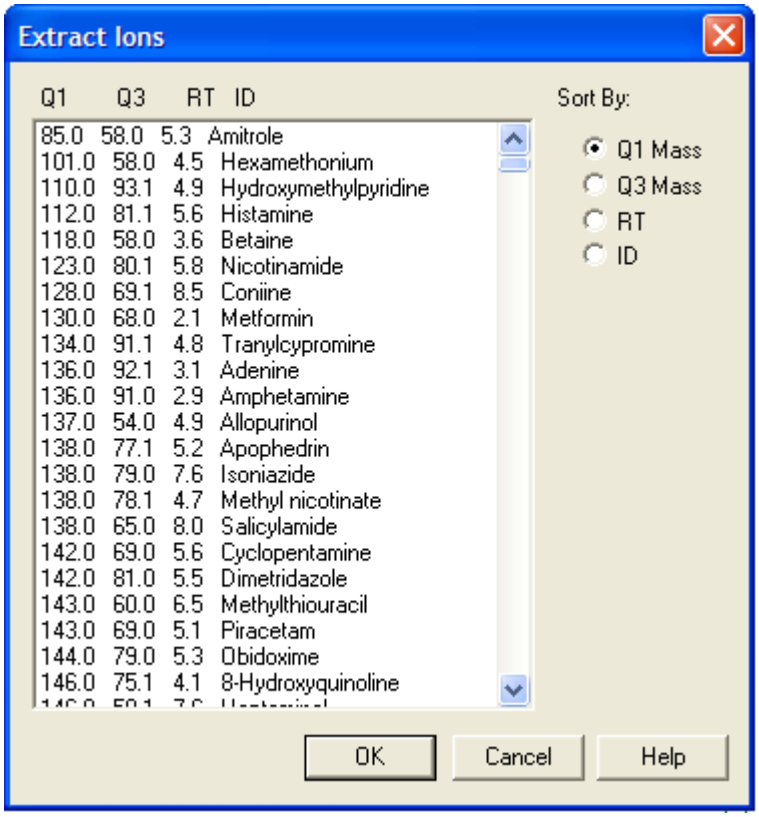
Note: For the SCIEX 3200MD and SCIEX 4500MD systems, the default settling time will be applied when there is a polarity switch in that cycle. For Citrine series of instruments, user configured settling time will be applied when there is a polarity switch in that cycle.

Generate an Extracted Ion Chromatogram

Note: If a *Scheduled* MRM algorithm wiff file containing more than 2500 transitions is opened, a TIC is shown instead of an XIC.

1. After generating *Scheduled* MRM algorithm data using the acquisition method created in the previous procedure, on the Navigation bar, under **Explore**, double-click **Open Data File** and then select the data file and sample.
   MRM and *Scheduled* MRM algorithm data are preset to be shown as an overlaid XIC.
2. Click **Explore** > **Extract Ions** > **Use Dialog**.
   The Extract Ions dialog opens, showing each MRM transition in the selected data file, along with the corresponding expected retention time and compound ID, if entered.

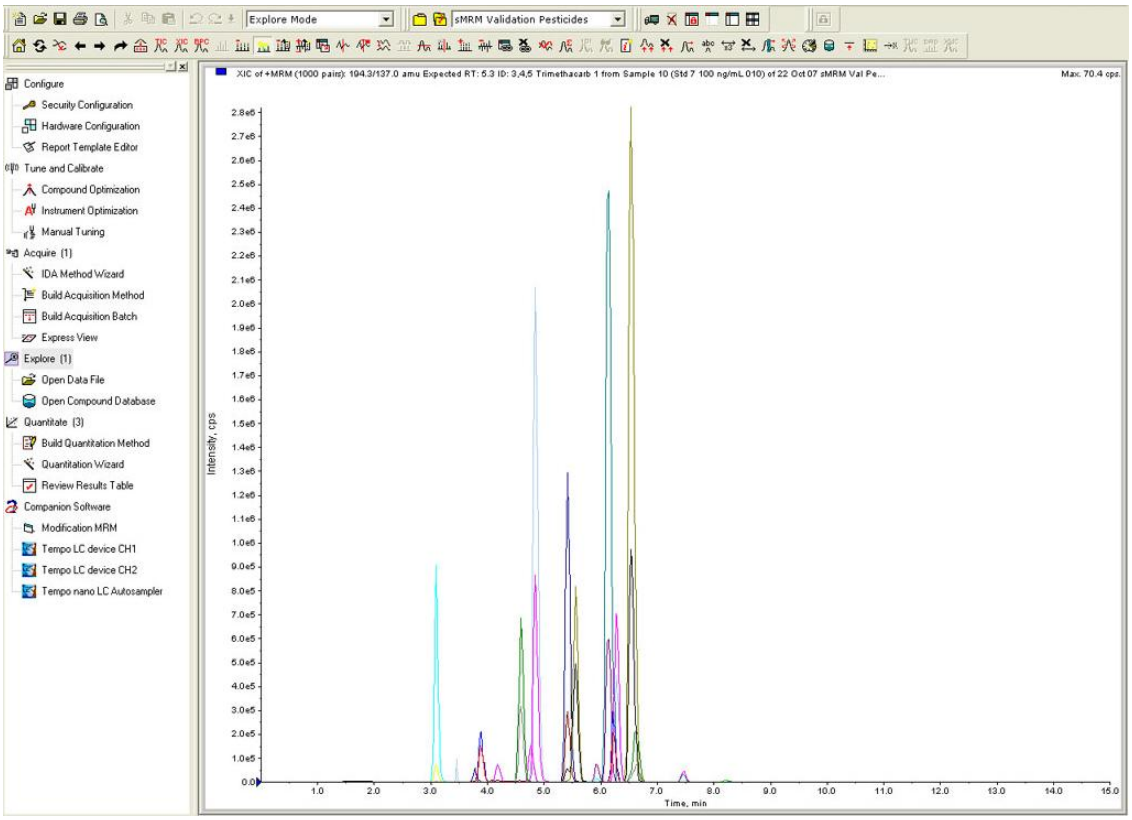
Figure 7 Extract Ions Dialog



3. Select to sort the list by Q1 Mass, Q3 Mass, RT (retention time), or compound ID.
4. Select one or more transitions.
5. Click **OK**.

The XIC is shown below the chromatogram and the Compound ID of the first selected transition is shown in the title.

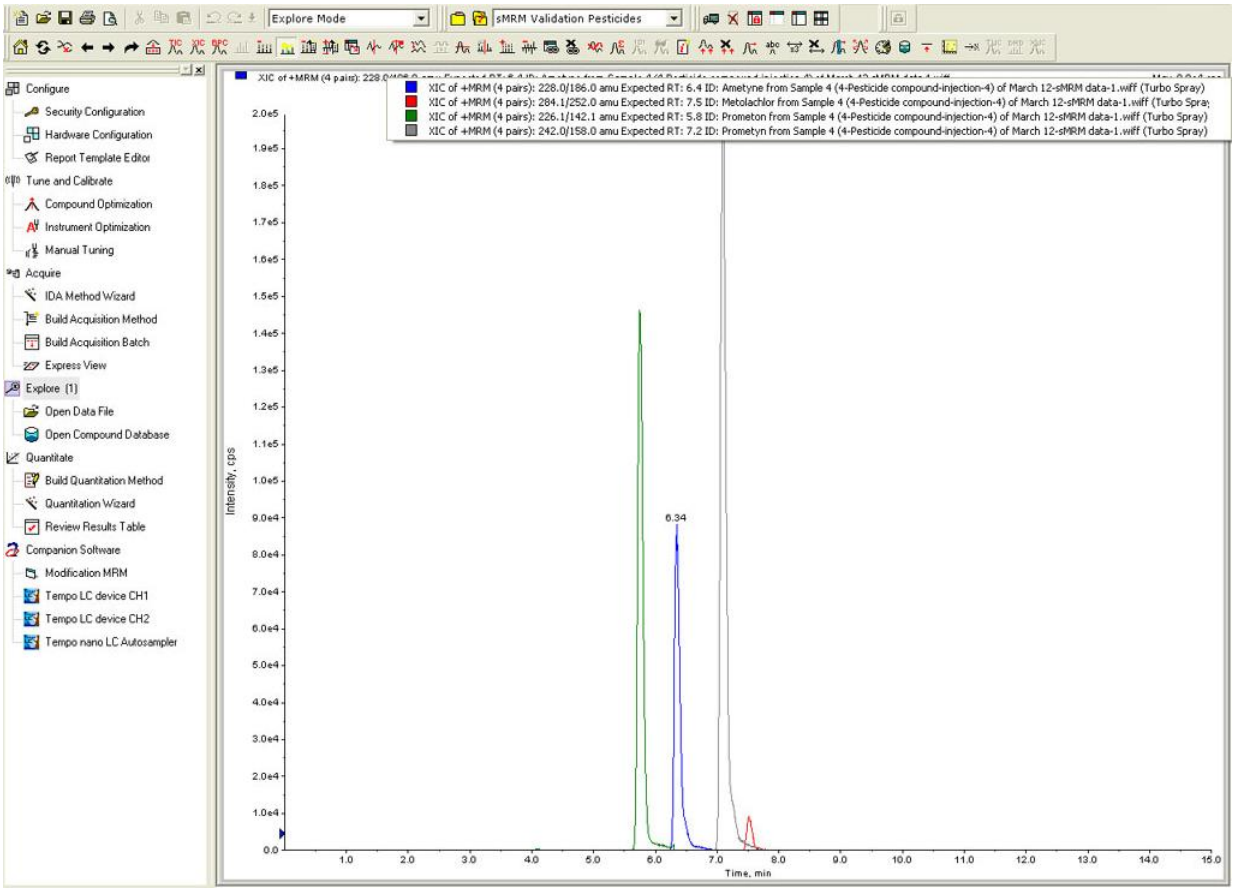
Figure 8 Example of an Overlaid XIC that Opens When Multiple Ions are Extracted



View MRM Transitions

1. Generate an XIC.
2. Right-click the XIC title to show the MRM transitions that are active in the region. Select the MRM transition of interest to show a label of the retention time in the chromatogram.

Figure 9 Active MRM Transitions



3. Drag the cursor along the X-axis to zoom in on a specific time region.
   The XIC rescales to the highest peak in the shown data.
4. Right-click the XIC title again to show the MRM transitions that are active in the specific time region.
   All transitions above the threshold and inside the zoomed in region are shown. The title will be reduced to the number of transitions in the zoomed section.

Create Quantitation Methods

Note: In the Analyst MD software, the Build Quantitation Method must be used to create a quantitation method for a data file that contains more than 94 transitions. The Quantitation Wizard can only create quantitation methods for data files with 94 transitions or less.

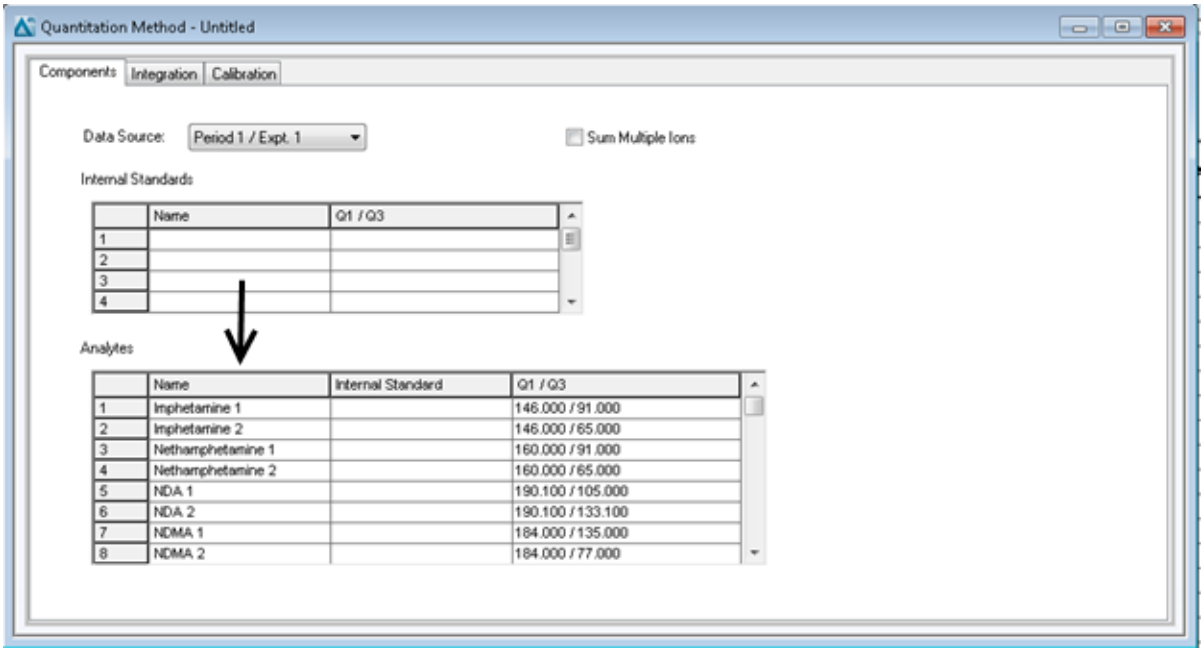
Before beginning, make sure that the recommended IntelliQuan-MQ III algorithm is being used. For more information on selecting algorithms, refer to the document: *Help*.

1. On the **Navigation** bar, under **Quantitate**, double-click **Build Quantitation Method**.

Note: The Quantitation Wizard can be used to create a quantitation method to analyze data that contains less than 94 transitions.

2. Select the data file and sample that was just acquired and then click **OK**.
A **Name** column is shown in the Analytes table. Because the transitions were selected during the acquisition, this column is populated with the compound ID from the acquisition method.

Figure 10 Quantitation Method: Components Tab



Note: If multiple experiments are included in the method, then review each experiment by selecting it from the **Data Source** list to allow the analytes in the experiment to be used in the quantitation method. If there are many analytes, it might take some time for the **Data Source** list to be populated.

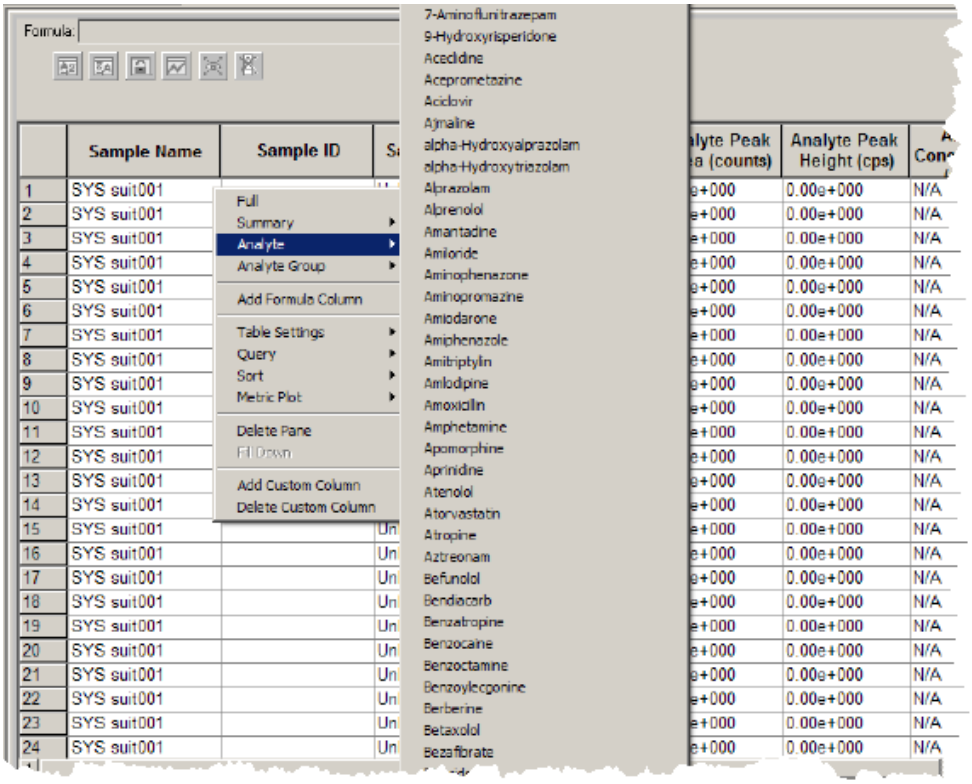
3. Set the required values in the remaining fields of the quantitation method and then save the quantitation method.
4. Use the Quantitation Wizard to create the Results Table. Make sure to select the quantitation method that was just created.

Tip! If the data file contains a large number of MRM transitions, then use the Create Quan Methods From Text Files script and the Create Text File from Quan Method script to create or modify a quantitation method. For more information, refer to the document: *Scripts User Guide*.

Review the Results Table

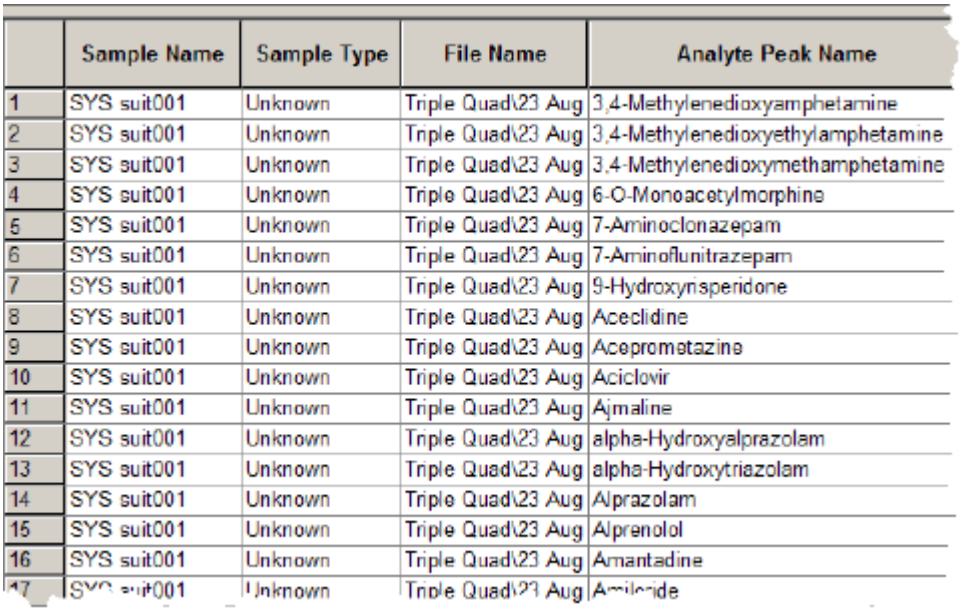
1. To view the results for a specific transition, right-click in the Results Table, select **Analyte** and then select the transition from the list of compound IDs.

Figure 11 Results Table: Analyte Selection



2. Right-click in the Results Table and then click **Table Settings** > **Edit** to open the Table Settings dialog.
3. Double-click **Columns** and then select **Analyte** from the list.
4. Beside **Analyte Peak Name**, select the **Shown** check box.
5. Click **OK** and then click **Done**.
   The **Analyte Peak Name** column is added to the Results Table and the compound ID of each transition is shown in that column.

Figure 12 Results Table: Analyte Peak Name Column



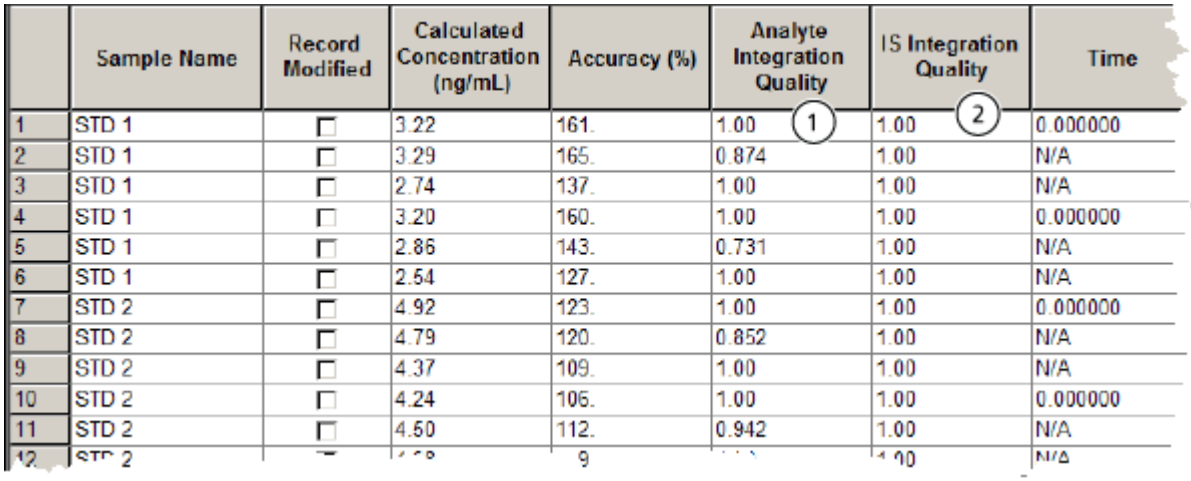
	Sample Name	Sample Type	File Name	Analyte Peak Name
1	SYS suit001	Unknown	Triple Quad\23 Aug	3,4-Methylenedioxyamphetamine
2	SYS suit001	Unknown	Triple Quad\23 Aug	3,4-Methylenedioxyethylamphetamine
3	SYS suit001	Unknown	Triple Quad\23 Aug	3,4-Methylenedioxymethamphetamine
4	SYS suit001	Unknown	Triple Quad\23 Aug	6-O-Monoacetylmorphine
5	SYS suit001	Unknown	Triple Quad\23 Aug	7-Aminoclonazepam
6	SYS suit001	Unknown	Triple Quad\23 Aug	7-Aminoflunitrazepam
7	SYS suit001	Unknown	Triple Quad\23 Aug	9-Hydroxyrisperidone
8	SYS suit001	Unknown	Triple Quad\23 Aug	Aceclidine
9	SYS suit001	Unknown	Triple Quad\23 Aug	Acepromatazine
10	SYS suit001	Unknown	Triple Quad\23 Aug	Aciclovir
11	SYS suit001	Unknown	Triple Quad\23 Aug	Ajmaline
12	SYS suit001	Unknown	Triple Quad\23 Aug	alpha-Hydroxyalprazolam
13	SYS suit001	Unknown	Triple Quad\23 Aug	alpha-Hydroxytriazolam
14	SYS suit001	Unknown	Triple Quad\23 Aug	Alprazolam
15	SYS suit001	Unknown	Triple Quad\23 Aug	Alprenolol
16	SYS suit001	Unknown	Triple Quad\23 Aug	Amantadine
17	SYS suit001	Unknown	Triple Quad\23 Aug	Amiloride

6. Right-click in the Results Table and then click **Table Settings** > **Edit** to show the **Analyte Integration Quality** and **IS Integration Quality** columns in the table.
7. Double-click **Columns** and then select **Analyte** from the list.
8. Beside **Analyte Integration Quality**, select the **Shown** check box, and then click **OK**.
9. Select **Internal Standard** from the list.
10. Beside **IS Integration Quality**, select the **Shown** check box.
11. Click **OK** and then click **Done**.

    The two columns are added to the Results Table. Integration quality indicates how well the peak is integrated. Values closer to 1 indicate well-integrated peaks. Smaller values can indicate that the peak is not well-integrated, that there is a large background, or that there might be another peak in the region.

    These columns facilitate peak review because the user can easily see the peaks with low analyte **Integration Quality** index values for manual review. In addition, the user can query the data for the analyte **Integration Quality** index values that are less than a value considered acceptable to show and manually review a subset of the data.

Figure 13 Results Table Columns



	Sample Name	Record Modified	Calculated Concentration (ng/mL)	Accuracy (%)	Analyte Integration Quality	IS Integration Quality	Time
1	STD 1	☐	3.22	161.	1.00 (1)	1.00 (2)	0.000000
2	STD 1	☐	3.29	165.	0.874	1.00	N/A
3	STD 1	☐	2.74	137.	1.00	1.00	N/A
4	STD 1	☐	3.20	160.	1.00	1.00	0.000000
5	STD 1	☐	2.86	143.	0.731	1.00	N/A
6	STD 1	☐	2.54	127.	1.00	1.00	N/A
7	STD 2	☐	4.92	123.	1.00	1.00	0.000000
8	STD 2	☐	4.79	120.	0.852	1.00	N/A
9	STD 2	☐	4.37	109.	1.00	1.00	N/A
10	STD 2	☐	4.24	106.	1.00	1.00	0.000000
11	STD 2	☐	4.50	112.	0.942	1.00	N/A
12	STD 2	☐		9		1.00	N/A

Item	Description
1	Analyte Integration Quality Index column
2	IS Integration Quality Index column

About the *Scheduled* MRM Pro Algorithm

The *Scheduled* MRM Pro algorithm feature is supported in the SCIEX 4500MD and Citrine systems.

The *Scheduled* MRM Pro algorithm adds advanced functionality to the *Scheduled* MRM algorithm. It improves the retention time robustness of experiments by allowing acquisition windows to be set for each transition in the acquisition method. Users can adjust individual windows for compounds that have broad LC peaks or large variation in their retention times.

In addition, the *Scheduled* MRM Pro agorithm includes the following functionality:

- The *Scheduled* MRM Pro algorithm also supports automatic window extension during acquisition. Users have the option of turning Dynamic Window Extension (DWE) on or off. Users can also set a different trigger threshold and DWE threshold for each individual transition. For example, a low trigger threshold and a high DWE threshold are more likely to trigger a secondary transition but avoid unnecessary dynamic window extension. When DWE is enabled, if a compound has shifted to a later retention time and the intensity has not dropped below its extension threshold after its retention time has passed, then the *Scheduled* MRM Pro algorithm automatically extends the detection window until its intensity drops below the threshold. The transition is monitored for another half of the method-specified detection window. The detection window after the extension is up to double the duration of the specified acquisition window. This feature allows smaller windows to be used,

but at the same time makes sure that every peak is captured in its entirety. It also increases method robustness to most shifts in retention time.

- The user can label multiple transitions for an analyte as either primary or secondary. Primary transitions are monitored throughout the acquisition window, while secondary transitions are only monitored after the associated primary transitions reach their trigger thresholds. This minimizes the cycle time by reducing the number of MRM transitions monitored. The acquisition time is focused on collecting data for the analytes that are present in a sample and not for analytes that are not present in the sample.
- The algorithm also supports the use of dwell weight. Dwell weight allows required dwell time to be expressed relatively. Highly abundant compounds can be assigned a low dwell weight, while less abundant compounds can be assigned a high dwell weight. During the run, the available dwell time is assigned based on this weight.
- The algorithm supports Dynamic Background Subtraction (DBS) for triggering secondary transitions. The DBS option is only available to non-IDA methods and experiments that use the *Scheduled* MRM algorithm. For these methods or experiments, when DBS is enabled, it is applied to the primary transitions to trigger secondary transitions in that experiment.

  For IDA methods that use the *Scheduled* MRM algorithm for survey scans, if DBS is enabled in the IDA Criteria, then it is applied to the primary transitions to trigger both secondary transitions and dependent scans.

  When both DWE and DBS are enabled for the *Scheduled* MRM algorithm experiment, DWE triggering depends on the non-processed data without DBS of the primary transitions.

  When DBS is enabled for an experiment that uses the *Scheduled* MRM algorithm, the secondary transitions, once triggered, will continue to acquire until their primary transitions stop acquiring.

Create a *Scheduled* MRM Pro Algorithm Acquisition Method

The *Scheduled* MRM Pro algorithm feature is only supported in the SCIEX 4500MD and Citrine systems.

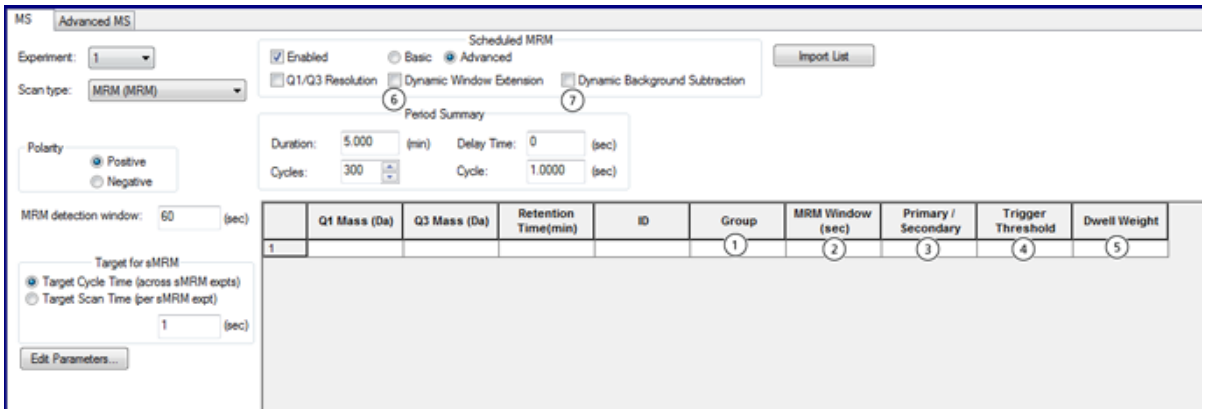
Use this procedure to create a *Scheduled* MRM Pro algorithm method in the Method Editor window. This type of method can also be created in the Manual Tune window.

1. Activate a hardware profile for the supported mass spectrometer. For Citrine systems, if required, select a different mass mode in the hardware profile and then activate the hardware profile.
2. Open an existing *Scheduled* MRM algorithm acquisition method or create one using the procedure in the section: Create a Scheduled MRM Algorithm Acquisition Method.

3. Select the **Advanced** option in the **Scheduled MRM** section on the MS tab in the Acquisition Method Editor. This enables the *Scheduled* MRM Pro algorithm feature.

   Five new columns are added to the mass ranges table, and two check boxes, **Dynamic Window Extension** and **Dynamic Background Subtraction** are shown in the **Scheduled MRM** section.

Figure 14 *Scheduled* MRM Pro Algorithm Parameters

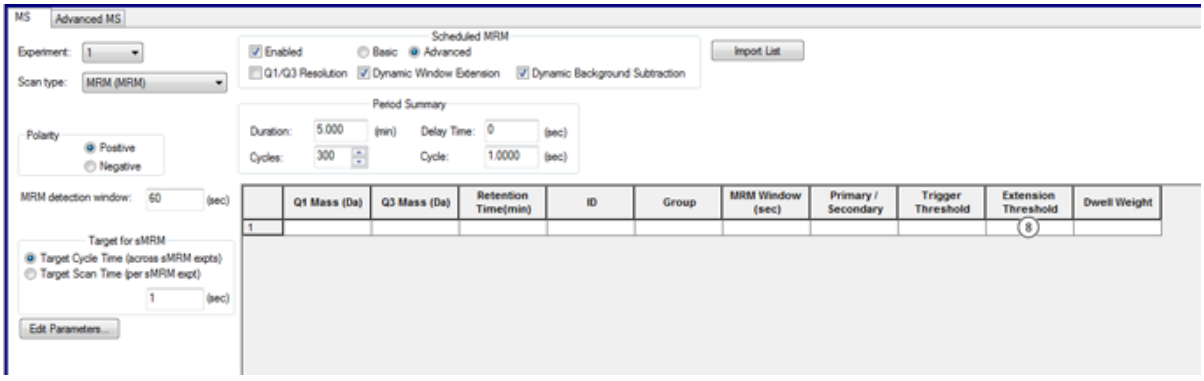


Item	Description
1	**Group column**: Use this column to group the transitions of a compound together. Assign the same name to all the transitions that are part of one group.
2	**MRM Window (sec)** field: Specify the length of time, centered around the retention time, that the transition will be monitored. Values for transitions in this field supersede the value in the method default window in the **MRM detection window** field. The **MRM Window** column is similar to the MRM detection window field but the value entered only applies to that specific transition. If left blank, the value is taken from the **MRM detection window** field.
3	**Primary/Secondary**: Use this column to specify whether a transition is primary or secondary. Primary transitions are monitored for the entire acquisition window. Secondary transitions are monitored within the acquisition window as long as all the primary transitions are above their individual thresholds. Assign the number `1` to the primary transitions and `2` to the secondary transitions in a group. There can be multiple primary and secondary transitions in a group.

Item	Description
4	**Trigger Threshold**: Specify the intensity that triggers secondary transitions. In IDA methods, **Trigger Threshold** is also used to trigger dependent scans. This value can be 0 or higher.
5	**Dwell Weight**: Use this column to specify whether a specific transition should have a longer scan time (dwell time) than other transitions. The default value is 1. Dwell weight allows the required dwell time to be expressed relatively. Highly abundant compounds can be assigned a low dwell weight (<1), while less abundant compounds can be assigned a high dwell weight (>1). The range for dwell weight is 0.1 to 10. During the run, the available dwell time is assigned based on this weight. **Note:** The total dwell weight equals the sum of dwell weights of all of the primary transitions plus half of the sum of the dwell weights of all of the secondary transitions in that cycle. However, the dwell time allocation still uses each individual dwell weight divided by the total dwell weight. For example, the dwell weight is 1 for both primary and secondary transitions for a method containing only two transitions with the same retention time. The total dwell weight is 1 + 0.5 = 1.5. The dwell time for both transitions will be the total dwell time that can be assigned to all of the concurrent transitions multiplied by 1/1.5. The total dwell time that can be assigned is calculated after deducting the pause times and the polarity switch times, if applicable, and then corrected by a factor of 1.2. All blank entries for dwell weight use the default 1. **Note:** When the calculated dwell time is not the same for all of the cycles for a transition throughout its retention time window, then the average dwell time of all cycles will be used for acquisition.

Item	Description
6	**Dynamic Window Extension**: Select to enable dynamic window extension in the *Scheduled* MRM Pro acquisition method. If dynamic window extension (DWE) is not used, then clear the **Dynamic Window Extension** check box. When DWE is selected, a new **Extension Threshold** column is added to the mass ranges table. If DWE is selected and if the peak intensity of a transition is still above the extension threshold after its retention time has passed, then the *Scheduled* MRM Pro algorithm automatically extends the detection window until the intensity drops below the extension threshold and the transition will be monitored for another half of the method-specified detection window. The acquisition window after the extension can be up to double the duration of the method-specified detection window.
7	**Dynamic Background Subtraction**: Select to use dynamic background subtraction in the *Scheduled* MRM Pro acquisition method. In an IDA method triggered by the Scheduled MRM Pro algorithm, the **Dynamic Background Subtraction** check box is not available in the *Scheduled* MRM Pro algorithm experiment if it is enabled in the IDA criteria. The **Dynamic Background Subtraction** check box in the IDA criteria, if selected, is used to trigger both secondary transitions and dependent scans.
8	**Extension Threshold**: Specify the threshold value for the dynamic window extension in the *Scheduled* MRM Pro acquisition method. This value can be zero or higher. Refer to the figure: Figure 15.

Figure 15 Dynamic Window Extension Selected - Extension Threshold Column



4. Complete the mass ranges table for each MRM transition by providing appropriate values in the five *Scheduled* MRM Pro algorithm columns. Use the following rules and the information in the figure: Figure 14.

- All of the transitions in the same group must be listed consecutively in the mass ranges table.
- All of the transitions in the same group must have the same retention time in the **Retention Time (min)** column.
- All of the transitions in the same group must have the same time in the **MRM Window (sec)** column.
- All of the primary transitions in the same group must be entered before the secondary transitions in a group. If a group has only one transition, then that transition must be the primary transition.

5. Save the acquisition method.
   The *Scheduled* MRM Pro algorithm acquisition method can only contain one period and up to two *Scheduled* MRM algorithm experiments.

Impact of *Scheduled* MRM Pro Algorithm in IDA

If an Information Dependent Acquisition (IDA) survey scan is performed using the *Scheduled* MRM Pro algorithm, then a dependent scan in the IDA method is only triggered when the intensities of all of the MRM transitions in a group are above their trigger thresholds. This improves the cycle time by eliminating false triggers of dependent scans.

Create an IDA *Scheduled* MRM Pro Algorithm Acquisition Method

1. Create a *Scheduled* MRM Pro algorithm using the procedure in the section: Create a Scheduled MRM Pro Algorithm Acquisition Method.
2. If required, to add an experiment of ER scan type, add it before adding the IDA Criteria.
3. Right-click the **Period** icon and then click **Add IDA Criteria Level**.
4. Specify the IDA Criteria parameters. Refer to the document: *Information Dependent Acquisition Tutorial*.
5. Right-click the **Period** icon and then click **Add experiment**.
6. On the MS tab, in the **Scan type** list, select a dependent scan type. For this example, select **Product Ion (MS2)** or **Enhanced Product Ion (EPI)**.

   Note: For all dependent scan types, the **Product Of** must be 30 Da.

7. If required, copy or add more dependent experiments.
   It depends on the IDA criteria, from X to Y most intense ions.

8. Specify the experiment parameters.
9. Save the acquisition method in the project from where the acquisition will run.

Note: During data acquisition using an IDA *Scheduled* MRM Pro algorithm method, the trigger threshold for each MRM transition in the method is used to trigger dependent scans instead of the IDA threshold.

Note: To optimize a *Scheduled* MRM algorithm method for the dwell time allocation, concurrency, and projected cycle time, install and use the sMRM Calculator script. For more information, refer to the document: *Scripts User Guide*.

View the *Scheduled* MRM Pro Algorithm Parameters in the File Info

After the acquisition of a sample is complete, the file information for the data file acquired with the *Scheduled* MRM Pro algorithm method shows all of the user-defined parameters for each transition. These parameters include the following:

- **MRM** (being scheduled or not)
- **Q1**
- **Q3**
- **Retention Time**
- **ID**
- **Group**
- **MRM Window (sec)**
- **Primary/Secondary**
- **Trigger Threshold**
- **Extension Threshold** (if the **Dynamic Window Extension** check box was selected
- **Dwell Weight**
- **Q1 Resolution**
- **Q3 Resolution** (if the **Q1/Q3 Resolution** check box was selected)

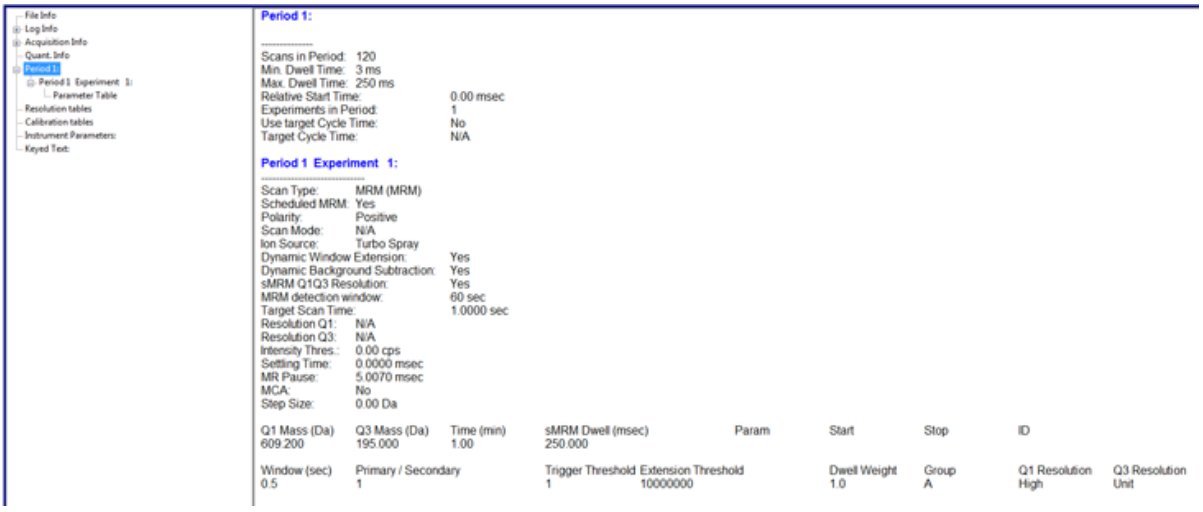
The **Dynamic Background Subtraction**, **Dynamic Window Extension**, **Dwell Time**, **Target Scan Time** or **Target Cycle Time** parameters are also shown.

1. On the **Navigation** bar, under **Explore**, double-click **Open Data File**.

The Select Sample dialog opens.

2. In the Data Files pane, select the wiff file.
3. In the Samples pane, select the sample.
4. Click **OK**.
   The data acquired from the sample opens.
5. To view the file information, click **Show File Info** on the toolbar.
   The File Information pane opens below the graph.
6. Expand the required period in the left pane of the File Information pane.
7. Click the required period experiment link.
   The values for the *Scheduled* MRM Pro algorithm parameters for each transition are recorded under the selected Period Experiment section in the right pane.

Figure 16 *Scheduled* MRM Pro Algorithm Parameters in File Info



Create a *Scheduled* MRM Basic or Pro Algorithm Acquisition Method with Scheduled Ionization

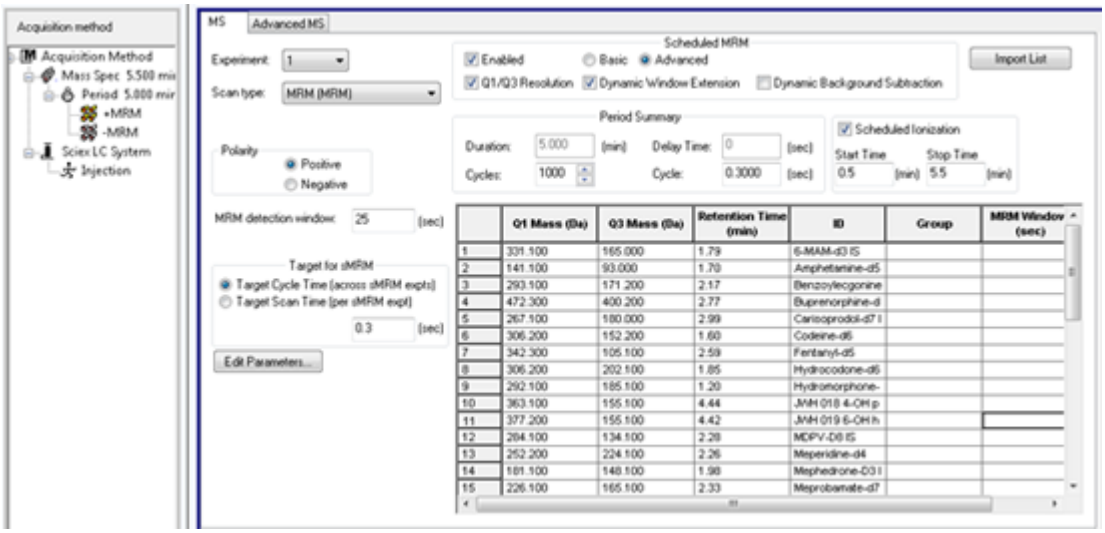
1. Create a *Scheduled* MRM Basic or Pro algorithm acquisition method.
2. Select any of the experiments in the method, and then select the **Scheduled Ionization** check box.
3. In the **Scheduled Ionization** group, type appropriate **Start Time** and **Stop Time**. Make sure that the peaks of interest will elute between the **Start Time** and **Stop Time**. If **Dynamic Window Extension** is also selected, then make sure that the **Stop Time** is after the latest

retention time in the mass table plus one and half of the detection window for this transition. Also make sure the acquisition method **Synchronization Mode** and the LC method are set the same as when **Scheduled Ionization** is not used.

Note: Scheduled Ionization is only available in single-period acquisition methods.

The following figure shows that the **LC Synchronization Mode** is used and the LC method is 7 minutes long. All of the peaks of interest elute after 0.5 minutes and before 5.0 minutes. Because the last eluted peak acquisition window might be extended by a full detection window, 5.5 minutes is used for the **Stop Time**. When **Scheduled Ionization** is used, an **IonSpray** voltage of 0 is applied before the **Start Time** and after the **Stop Time**. The **IonSpray** voltage set in the MS method is only applied between the **Start Time** and **Stop Time**. The **Scheduled Ionization** feature can reduce the risk of instrument contamination and thus decrease the mass spectrometer down time. For more information about **Scheduled Ionization**, refer to the document: *Advanced User Guide*.

Figure 17 *Scheduled* MRM Pro Algorithm With Scheduled Ionization



4. Save the acquisition method.

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