Analyst® 1.6.1 Software

Scheduled MRM™ Algorithm Tutorial

AB SCIEX

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Scheduled MRM™ Algorithm Tutorial

Topics in this section:

Objectives

You 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.

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 you provide when creating the acquisition method. The *Scheduled* MRM algorithm functionality eliminates the requirement for multi-period experiments. You can also use it as a survey scan in an IDA experiment.

The algorithm provides enough points across the chromatographic peak to give you better peak detection and improved reproducibility. With this feature, you can also view data files with many MRM transitions by displaying the compound ID, analyte Integration Quality™ index, and IS (internal standard) Integration Quality index columns in the Results Table. For more information on the maximum number of transitions you can acquire with your instrument, see the release notes for the Analyst® software.

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

Figure 1-1 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.

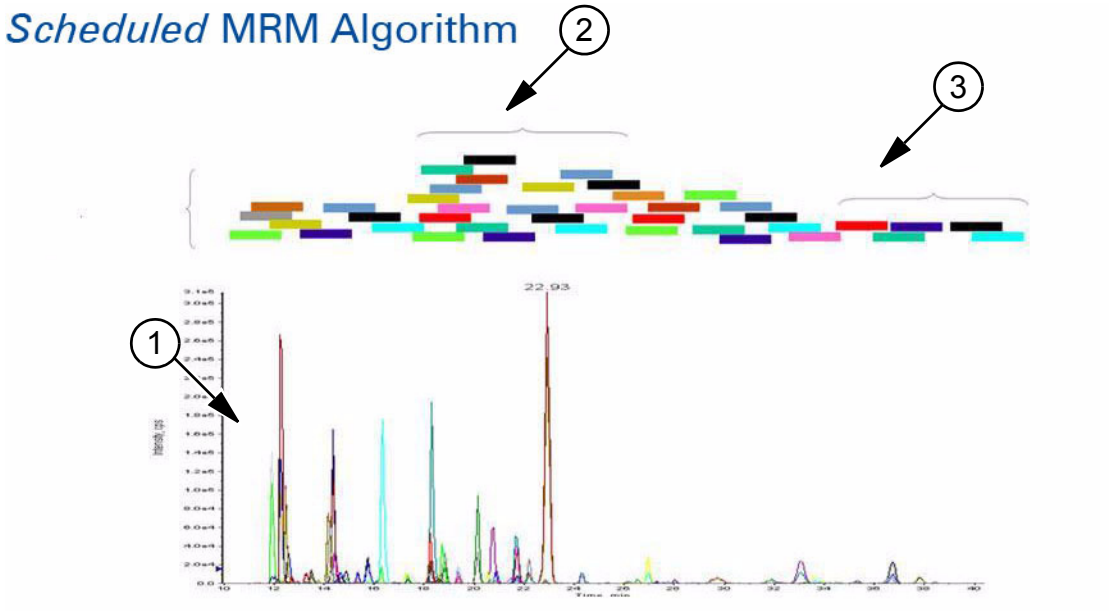


Figure 1-1 Typical example of a *Scheduled* MRM algorithm LC run

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

Related Documentation

- Instrument operator's guide for the instrument
- *Getting Started Guide* for the Analyst® software
- *Advanced User Guide* for the Analyst® software
- *Analyst Software 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 on creating methods using IDA)

If you are interested in proteomics and process large numbers of *Scheduled* MRM algorithm transitions, you can use the MultiQuant™ software for data processing. For more information, contact your AB SCIEX sales representative.

Technical Support

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

Prerequisites

You should know how 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

Creating a .csv or .txt File

You will need a file containing information about the MRM transitions to import into the mass ranges table of an acquisition method. Use the following criteria:

- The file must not contain any header, column, or row titles.
- The order and number of columns in the file must match the order and number of the columns in the mass ranges table.
- There must be no empty cells.

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

Creating a *Scheduled* MRM Algorithm Acquisition Method

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 (MRM)** and then select the **Scheduled MRM** check box.

   The following features are used to create the method:

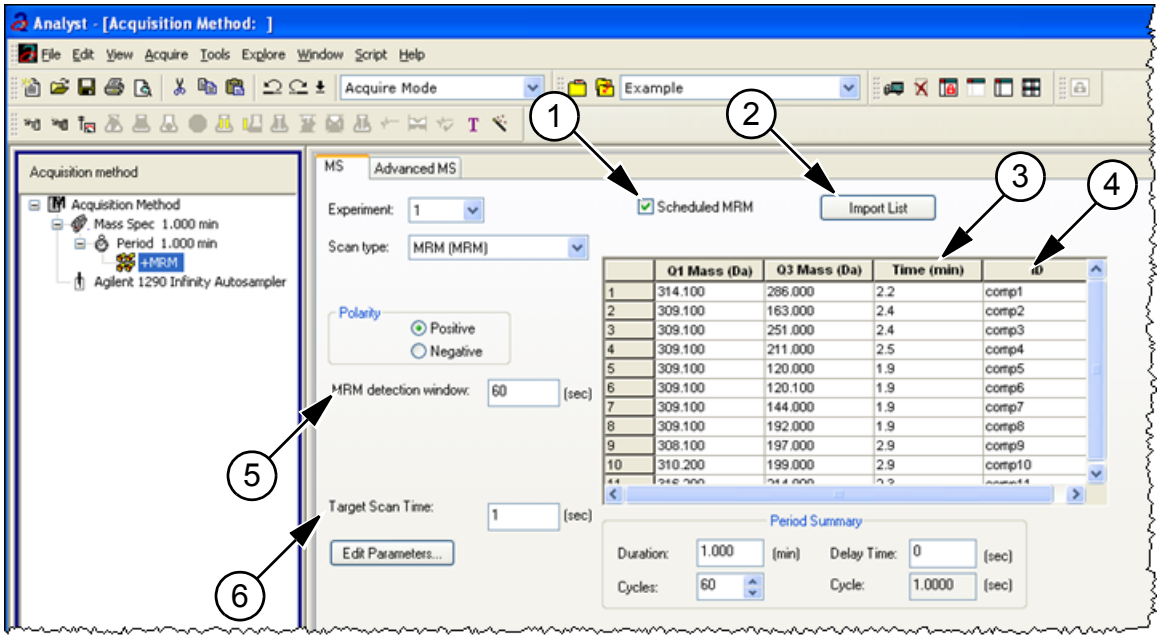


Figure 1-2 *Scheduled* MRM™ algorithm software features

Item	Description
1	**Scheduled MRM** check box: Select to use the *Scheduled* MRM algorithm feature.
2	**Import List** button: Click to import MRM transitions, time, ID, and compound-dependent parameters from a .txt or .csv file.
3	**Time (min)** column: Retention time; type the expected retention time in minutes for the corresponding MRM transition. This column displays the dwell time in msec for MRM methods.
4	**ID** column: (Optional) Type a compound ID for the transition of interest.
5	**MRM detection window (sec)** field. Type the amount of time for detection that surrounds the retention time for each transition.
6	**Target Scan Time (sec)** field. The total scan time is adjustable so that a specific number of points across the LC peaks can be targeted.

3. 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. 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** window that appears, select either a .csv (comma-separated values) or .txt (tab-separated values) file that contains the MRM transition information. After selecting the file, click **Open**. The file contents appear in the mass ranges table. For more information on creating files, see Creating a .csv or .txt File on page 5.

- 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 new column to the mass ranges table, right-click in the mass ranges table and then select a compound-dependent parameter.

4. 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 your LC chromatography as a guide to selecting the best *Scheduled* MRM algorithm parameters. Determine the width of a typical peak at base and then see Table 1-1 for the recommended settings. It is also important to consider the stability of your retention time in defining the MRM detection window.

   Table 1-1 *Scheduled* MRM Algorithm Parameters

Peak Width At Base	MRM Detection Window	Target Scan 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 unit time monitored is adjusted based on their retention time window. To maximize the dwell time used for each analyte and its signal-to-noise ratio, AB SCIEX recommends that you use a smaller, but reasonable, retention time window that allows your peak of interest to be captured. A value of 60 seconds is a good starting point. This 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 your expected retention time is 4.5 minutes, typing 60 seconds sets a detection window from 4 minutes to 5 minutes.

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

5. In the **Target Scan Time (sec)** field, type the target length of time for each cycle during the experiment (see Table 1-1.) This parameter helps to define the number of points across the chromatographic peak.

   You can use the width of your 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 15

data points over a 15-second peak, which will give you excellent accuracy and reproducibility.

Note: If the actual cycle time exceeds the user-specified Target Scan Time because of the number of transitions in the cycle, the time will increase for that specific cycle and then return to the original value.

6. Select the required values in the remaining fields of the acquisition method.
7. 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.

Creating an Acquisition Method using two *Scheduled* MRM Algorithm Scan Types

Use this procedure if you want to create an experiment that allows for switching.

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 (MRM)** and then select the ***Scheduled* MRM** check box.

The following features are used to create the method:

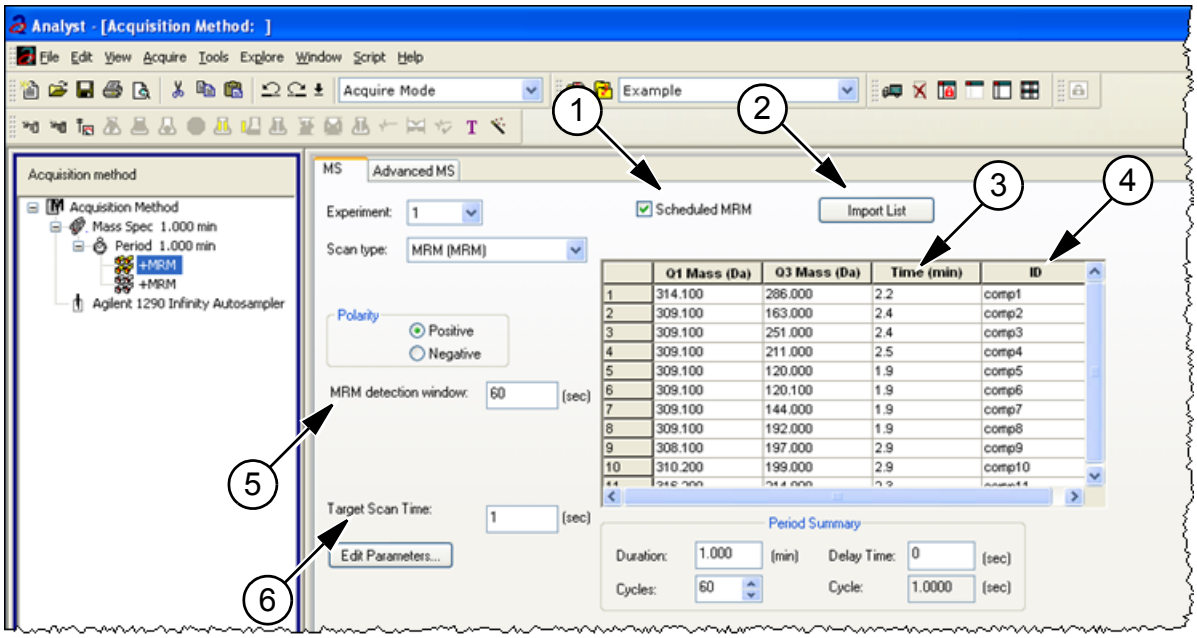


Figure 1-3 *Scheduled* MRM™ Algorithm Software Features

Item	Description
1	**Scheduled MRM** check box: Select to use the *Scheduled* MRM algorithm feature.
2	**Import List** button: Click to import MRM transitions, time, ID, and compound-dependent parameters from a .txt or .csv file.
3	**Time (min)** column: Retention time; type the expected retention time in minutes for the corresponding MRM transition. This column displays the dwell time in msec for MRM methods.
4	**ID** column: (Optional) Type a compound ID for the transition of interest.
5	**MRM detection window (sec)** field. Type the amount of time for detection that surrounds the retention time for each transition.
6	**Target Scan Time (sec)** field. The total scan time is adjustable so that a specific number of points across the LC peaks can be targeted.

3. 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. 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** window that appears, select either a .csv (comma-separated values) or .txt (tab-separated values) file that contains the MRM transition information. After selecting the file, click **Open**. The file contents appear in the mass ranges table. For more information on creating files, see Creating a .csv or .txt File on page 5.

- 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 new column to the mass ranges table, right-click in the mass ranges table and then select a compound-dependent parameter.

4. 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 your LC chromatography as a guide to selecting the best *Scheduled* MRM algorithm parameters. Determine the width of a typical peak at base and then see Table 1-2 for the recommended settings. It is also important to consider the stability of your retention time in defining the MRM detection window.

   Table 1-2 *Scheduled* MRM Algorithm Parameters

Peak Width At Base	MRM Detection Window	Target Scan 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 unit time monitored is adjusted based on their retention time window. To maximize the dwell time used for each analyte and its signal-to-noise ratio, AB SCIEX recommends that you use a smaller, but reasonable, retention time window that allows your peak of interest to be captured. A value of 60 seconds is a good starting point. This 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 your expected retention time is 4.5 minutes, typing 60 seconds sets a detection window from 4 minutes to 5 minutes.

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

5. In the **Target Scan Time (sec)** field, type the target length of time for each cycle during the experiment (see Table 1-1.) This parameter helps to define the number of points across the chromatographic peak.

   You can use the width of your 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 15

data points over a 15-second peak, which will give you excellent accuracy and reproducibility.

Note: If the actual cycle time exceeds the user-specified Target Scan Time because of the number of transitions in the cycle, the time will increase for that specific cycle and then return to the original value.

6. Select the required values in the remaining fields of the acquisition method.
7. In the **Acquisition method** pane, right-click **Period** and then click **Add experiment**.

   A second MRM scan type is created.
8. On the **MS** tab, select the **Scheduled MRM** check box.
9. Complete the acquisition method as described in steps 3 to 6.
10. 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.

Generating an Extracted Ion Chromatogram

If you open an MRM or *Scheduled* MRM™ algorithm .wiff file that has more than 2500 transitions, you will see the TIC instead of the 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 appear as an overlaid XIC display.
2. Click **Explore** > **Extract Ions** > **Use Dialog**

   The Extract Ions dialog appears showing each MRM transition in the selected data file, along with the corresponding expected retention time and compound ID, if entered.

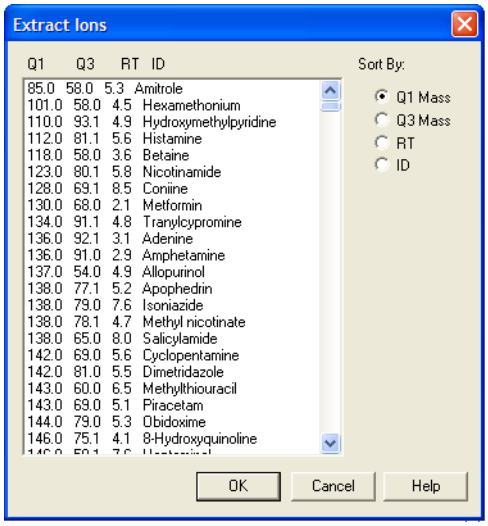


Figure 1-4 Extract Ions dialog

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

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

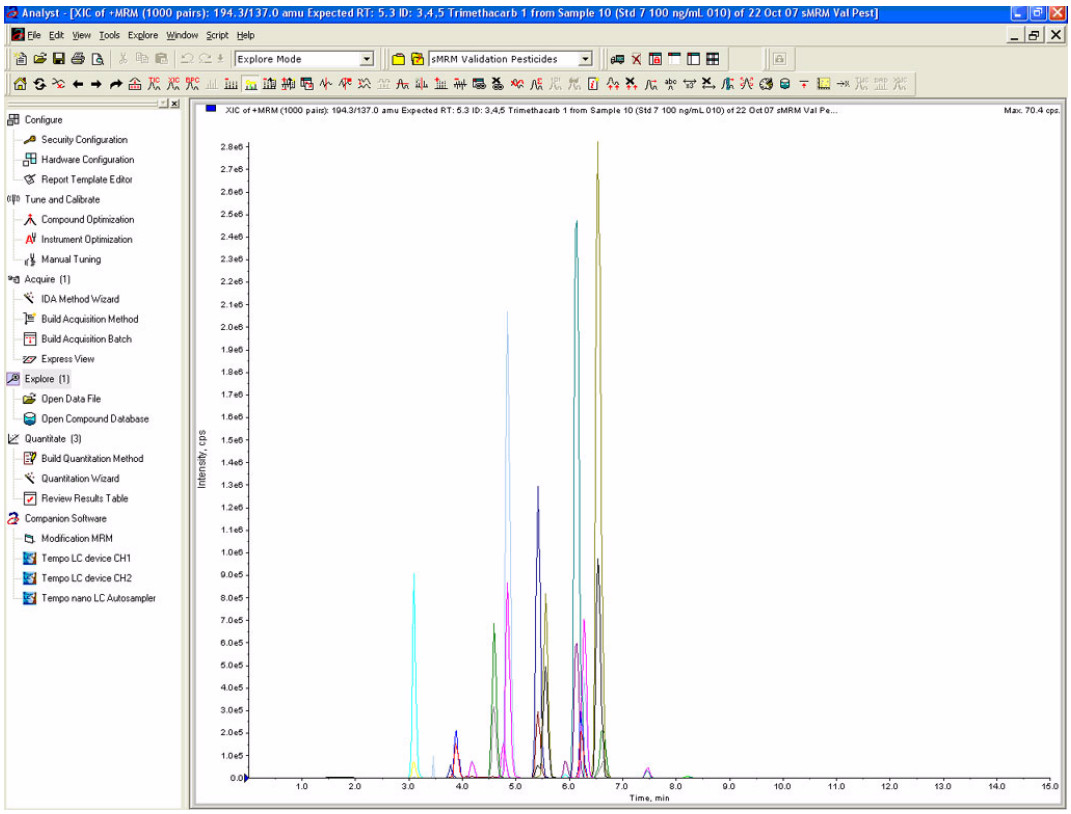


Figure 1-5 Example of an overlaid XIC that appears when multiple ions are extracted

Displaying MRM Transitions

1. Generate an XIC.
2. Right-click the XIC title to display the MRM transitions that are active in the displayed region (Figure 1-6.) Select the MRM transition of interest to display a label of the retention time in the chromatogram.

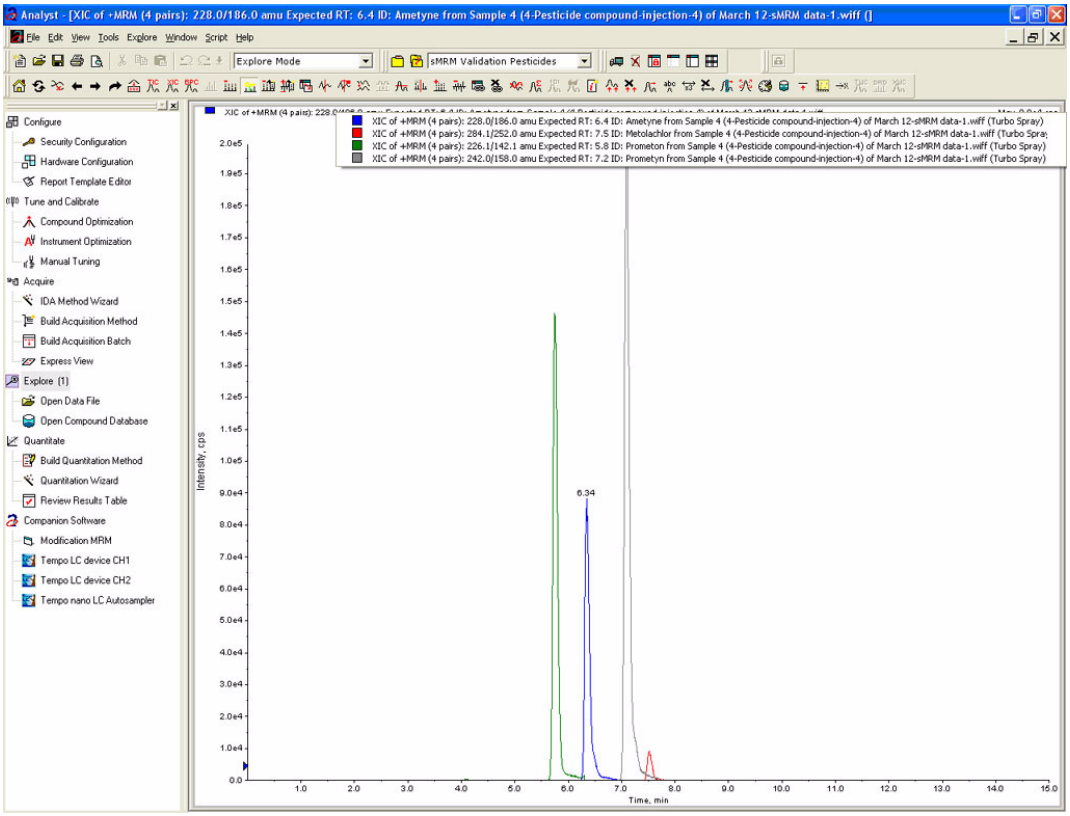


Figure 1-6 Active MRM transitions

3. Drag the cursor along the x-axis to zoom in on a specific time region.

   The XIC now rescales to the highest peak in the displayed data.
4. You can right-click the XIC title again to display the MRM transitions that are active in the specific time region.

   All transitions above the threshold and inside the zoomed in region are displayed. The title will be reduced to the number of transitions in the zoomed section.

Creating Quantitation Methods for more than 94 Transitions

Before you begin, make sure that you are using the recommended IntelliQuan MQ III algorithm. For more information on selecting algorithms, see the Help.

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

Note: Use the Quantitation Wizard to create a quantitation method when you are analyzing data that contains less than 94 transitions.

2. Select the data file and sample you just acquired and then click **OK**.

   A Name column appears in the Analytes table. Because the transitions were selected during the acquisition, this column is populated with the compound ID from the acquisition method.

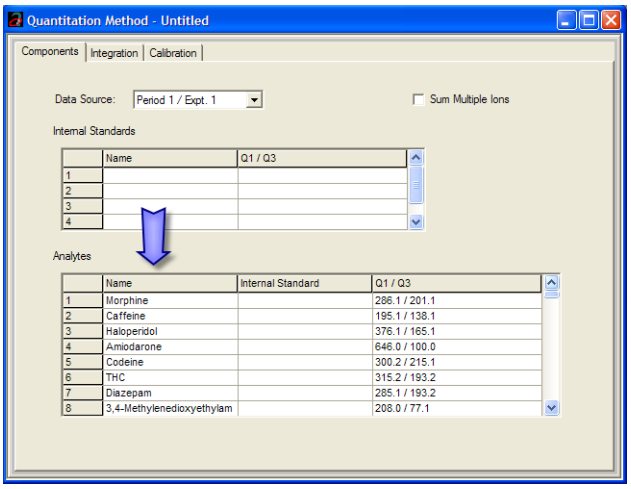


Figure 1-7 Quantitation Method: Components tab

Note: If you have multiple experiments, you must review each experiment by selecting it from the Data Source list in order for the analytes in the experiment to be used in the quantitation method. If there are many analytes, it may 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 quantitation set. Make sure that you select the quantitation method you just created.

Tip! If you have a large number of MRM transitions, you can 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.

Reviewing the Results Table

1. To see 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.

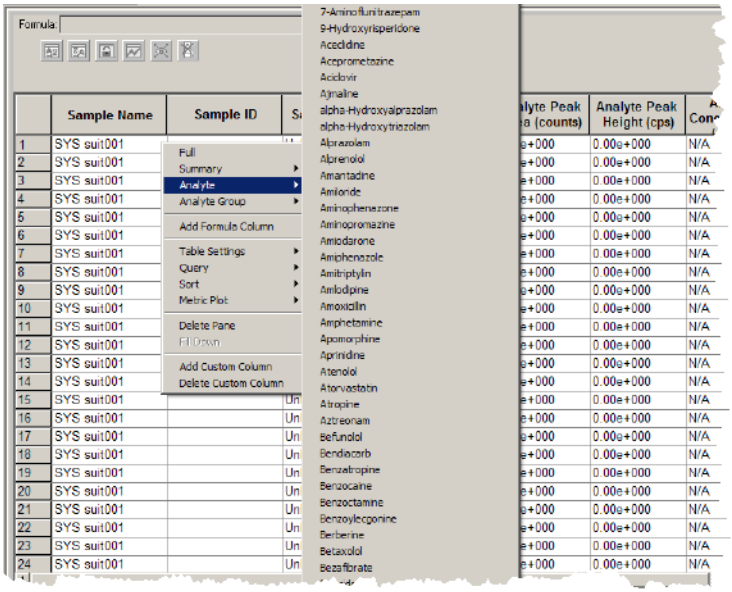


Figure 1-8 Results Table: analyte selection

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, in the drop-down list, select **Analyte**.
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 appears in that column.

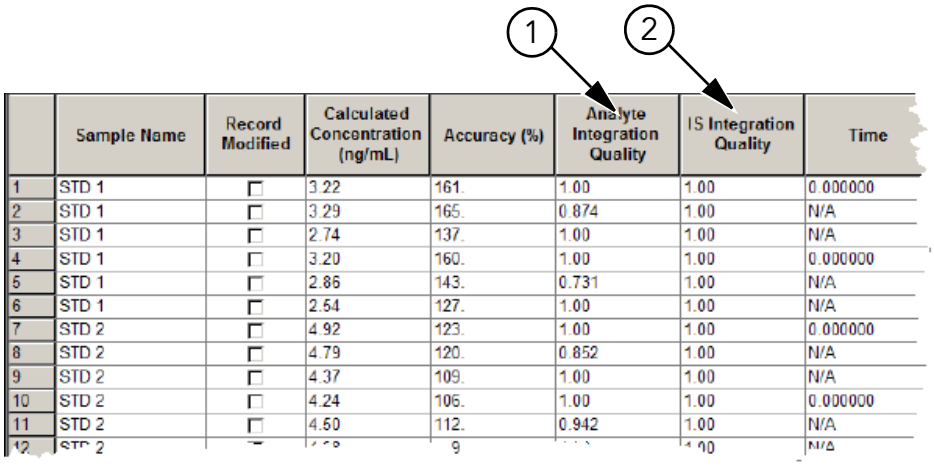
	Sample Name	Sample Type	File Name	Analyte Peak Name
1	SYS suit001	Unknown	Triple Quad\23 Aug	3,4-Methylenedioxyamphetamine
2	SYS suit001	Unknown	Triple Quad\23 Aug	3,4-Methylenedioxyethylamphetamine
3	SYS suit001	Unknown	Triple Quad\23 Aug	3,4-Methylenedioxymethamphetamine
4	SYS suit001	Unknown	Triple 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	Acepromet azine
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

Figure 1-9 Results Table: Analyte Peak Name column

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

    The two new 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 as you can easily see the peaks with low analyte Integration Quality™ index values for manual review. In addition, you can query the data for the analyte Integration Quality index values that are less than a value you consider acceptable in order to display and manually review a subset of the data.



	Sample Name	Record Modified	Calculated Concentration (ng/mL)	Accuracy (%)	Analyte Integration Quality	IS Integration Quality	Time
1	STD 1	☐	3.22	161.	1.00	1.00	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

Figure 1-10 Results Table Columns

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