SCIEX
The Power of Precision

Analyst MD Software

Scripts User Guide

IVD-IDV-05-4988-C

January 2022

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

Contents

Foreword 1

Audience

This guide is intended for customers and Field Service Employees (FSEs).

Technical Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the website at sciex.com.

Scripts 2

This document explains how to install and use Analyst MD software scripts. It also provides an overview of the uses of each script and how to uninstall a script, if required.

Install or Uninstall Scripts

Some scripts are automatically installed when the Analyst MD software is installed.

The remaining scripts are available in the Scripts folder.

Scripts must be installed to use them. Refer to the section: Install a Script.

Install a Script

1. Do one of the following:
   - Browse to the `<Drive>:\Program Files\Analyst\Scripts` folder on the computer.
   - Browse to the `Extras\Scripts` folder on the software DVD, if available, or in the unzipped software web download package.
2. Open the Scripts folder.
3. Do one of the following:
   - For the sMRM Calculator script, double-click **sMRM Calculator Setup.exe**.
   - For all of the other scripts, double-click **ScriptRunner.exe**.
4. Follow the on-screen instructions to install the scripts.
   The installed scripts are available from the **Script** menu.

Uninstall a Script

Note: Do not uninstall the DFTTracker and MRM3 Optimization scripts. If these scripts are removed, then the Analyst MD software must be installed again to access these scripts.

To uninstall the script, do the following:

- For the Create Quan Methods From Text Files, Create Text File from Quant Method, and MSServiceLog scripts, browse to the `<drive>:\Analyst Data\Projects\API Instrument\Processing Scripts` folder and then delete the script dll manually.
- For the sMRM Calculator script, do the following:
  - On the Windows 7 operating system: Click **Start** > **All Programs** > **Control Panel** > **Programs and Features**.
  - On the Windows 10 operating system: Click **Start** > **Control Panel** > **Programs and Features**.
  - Right-click **sMRM Calculator** and then click **Uninstall**.
  - Follow the on-screen instructions.

Create Quantitation Methods and Text Files

The Create Text File From Quan Method script exports a quantitation method to a tab-delimited text file. The Create Quan Method From Text Files script imports the information contained in a tab-delimited text file to a Quantitation Method File (qmf). Currently, the Build Quantitation Method component in the Analyst MD software does not support this functionality.

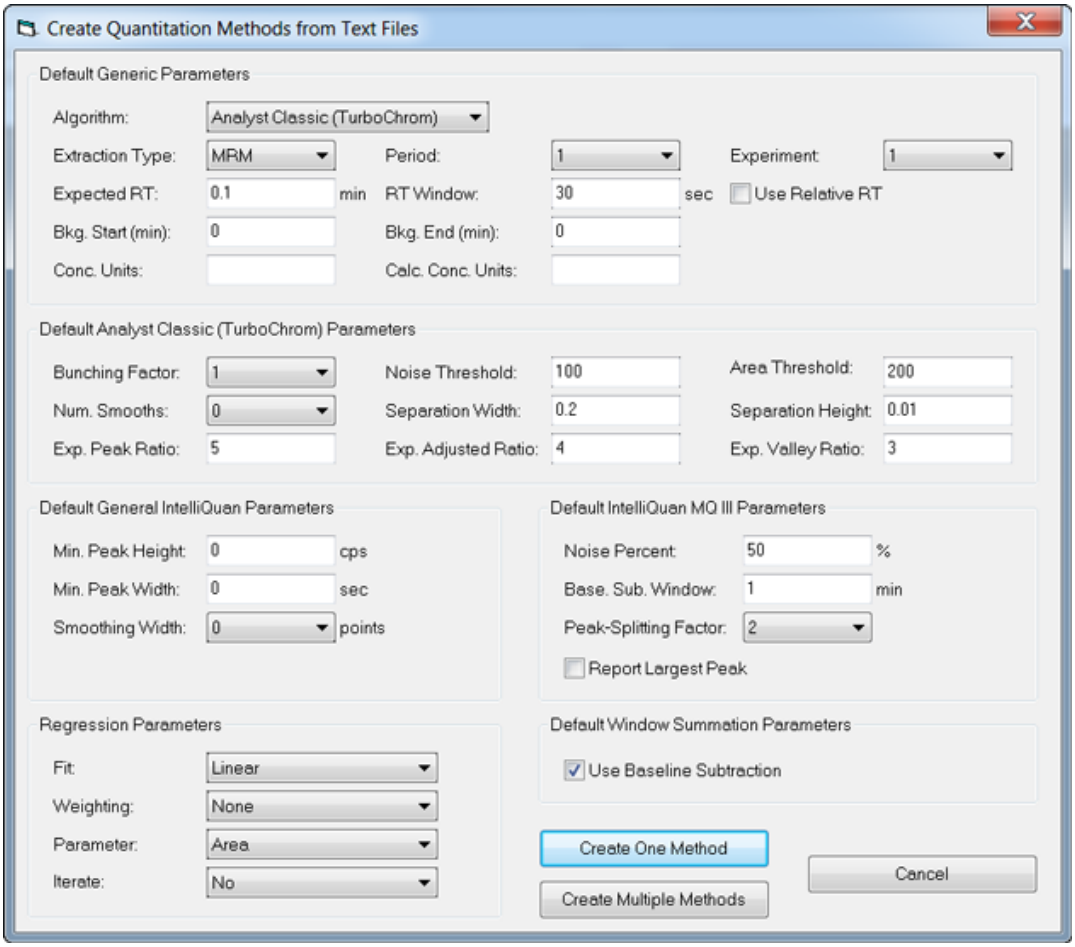
The Create Text File from Quan Method script creates a text file representation of a quantitation method file. A column for each required field is created in the text file if the **Export all columns** check box is selected. If the check box is not selected, then the script generates the text file with columns only for the fields where the field value is not the same for all peaks.

The Create Quan Method From Text Files script specifies default values for any of the non-required fields in the text file such as integration algorithm or regression parameters. For more information, refer to the section: Text File Format.

Use the Create Quan Methods From Text Files Script

1. Click **Script** > **Create Quan Methods From Text Files**.

Figure 2-1 Create Quantitation Methods from Text Files Dialog



2. Use the parameters in the Default Generic Parameters section to create a quantitation method. The **Algorithm**, **Extraction Type**, **Period**, and **Experiment** fields are not available in the Analyst MD software. Set the following parameters as required:

   - From the **Algorithm** list, select a peak-finding algorithm. The Window Summation algorithm sums all the intensities in the retention threshold and will not find any peaks.
   - From the **Extraction Type** list, select the type of data that will be integrated.
   - From the **Period** and **Experiment** lists, select the period number and experiment number.

   The Default Analyst Classic Parameters, Default General IntelliQuan Parameters, Default IntelliQuan MQ III Parameters, and the Default Window Summation Parameters groups contain the parameters that are used by the algorithm selected in the **Algorithm** field.

3. Select the **Use Baseline Subtraction** check box to have the Window Summation algorithm sum the intensities to the horizontal line at the minimum intensity of the data points within the summation window, as opposed to summing down to the intensity zero.
4. In the Regression Parameters section, select the regression information.

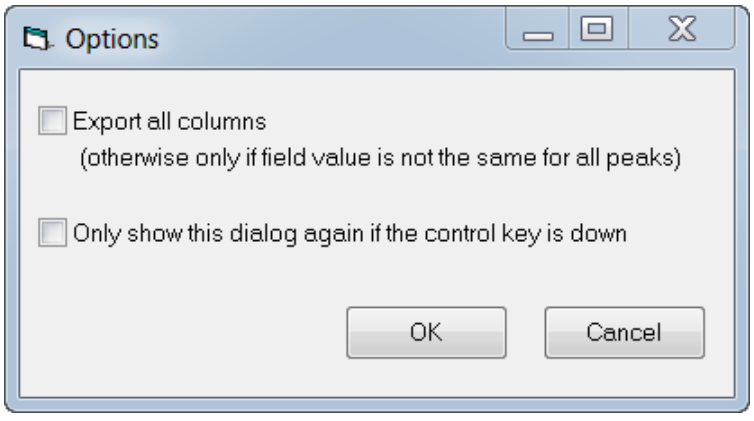
   The information specified here is applied to every analyte peak. Unlike the previous parameters, it is not possible to indicate this information in the text files. Therefore, the same regression parameters are applied to all analytes. For a full description of the parameters, refer to the document: *Help*.
5. To create one quantitation method, click **Create One Method**, browse to the text file that will be used to create the quantitation method, and then click **Open**.
   A quantitation method qmf file with the same file name as the txt file is created if the text file is in the correct format and contains the required columns. The created quantitation method is stored in the `Quantitation Methods` folder under the current working project in the Analyst MD software, regardless of the location of the text file.
6. To create multiple methods from multiple text files, click **Create Multiple Methods**, browse to the folder where the text files are located, and then click **OK**.

   A quantitation method qmf file with the same file name as the txt file is created for each individual text file in that folder if they are in the correct format and contain the required columns. The created quantitation methods are stored in the `Quantitation Methods` folder under the current working project in the Analyst MD software, regardless of the location of the text files.

Use the Create Text File from Quan Method Script

1. Create and save a quantitation method in the Analyst MD software.
2. Click **Script** > **Create Text File from Quan Method.**

Figure 2-2 Options Dialog



3. Select the **Export all columns** check box and then click **OK**.
4. Browse to and then select the quantitation method (qmf) file.
5. **Navigate** to and then select the location of the text file.
   The script generates the text file with all columns. If the **Export all columns** check box was not selected in step 3, then the script only generates the text file with columns for the fields where the field value is not the same for all peaks.

Text File Format

The text files used to create the quantitation methods (Create Quan Methods from Text Files) and generated from the methods (Create Text File from Quan Method) are in the following format:

- Separate the various fields with tab characters and end each line with a carriage return or line feed character.
- The first row of the file should contain column headings. All of the columns shown in the following table marked as Required must be present. The remaining columns are optional. The actual order of the columns is not important.
- Each subsequent line should contain the information as shown in the table for either one analyte or an internal standard peak.

Table 2-1 Text File Formats

Column Name	Required	Description
Peak Name	Yes	The name of the analyte or internal standard peak.
First Mass	Yes	For MRM data, the Q1 mass for the peak. For full-scan data, the starting mass for the XIC to integrate. For Q1 MI or Q3 MI data, the mass.
Second Mass	Maybe	This field is required when integrating full-scan or MRM data, but not for Q1 MI or Q3 MI data. For MRM data, this is the Q3 mass for the peak. For full-scan data, it is the ending mass for the XIC to integrate.
Extraction Type	No	The type of data to integrate. If present, this should be one of: 0 - MRM data 1 - Q1 MI or Q3 MI data 2 - full-scan data

Table 2-1 Text File Formats (continued)

Column Name	Required	Description
Is IS	No	Specifies whether the current peak is an internal standard or an analyte. TRUE if the peak is an internal standard. Otherwise, FALSE. If this column is not present, then all peaks defined are assumed to be analytes. **Note**: Internal standard peaks should be defined first in the text file before any analyte peaks that use that IS.
IS Name	No	For analyte peaks, specifies the name of the corresponding internal standard (if any). If a given analyte will not use an internal standard, then leave the contents of this field empty. For internal standard peaks themselves, the contents of this field are ignored.
Period	No	The period number for the peak (from 1 to the number of periods in the data).
Experiment	No	The experiment number for the peak (from 1 to the maximum number of experiments in the period).
Use Relative RT	No	For analyte peaks that are using an internal standard, specifies whether or not the expected retention time is relative to that of the IS. TRUE if so. Otherwise, FALSE. The contents of this field are ignored for other peaks, but must still contain either TRUE or FALSE.
Conc Units	No	The concentration units.
Calc Conc Units	No	The calculated concentration units.
Bkg Start	No	Start time, in minutes, for the peak background. This parameter does not affect the peak integration in any way, however, it does affect how the noise, and hence S/N, is calculated.
Bkg End	No	End time, in minutes, for the peak background.
Expected RT	No	The expected retention time, in minutes, from 0 to 1666.
RT Window	No	The retention time window, in seconds, from 1 to 1000.

Table 2-1 Text File Formats (continued)

Column Name	Required	Description
Algorithm	No	Specifies which peak-finding and integration algorithm should be used. If present, this should be one of: 0 - Analyst Classic (TurboChrom) 1 - IntelliQuan - IQA II (Automatic) 2 - IntelliQuan - MQ III 3 - Window Summation
Bunching Factor	No	(TurboChrom algorithm) The bunching factor for the peak, from 1 to 100.
Num Smooths	No	(TurboChrom algorithm) The number of smooths, from 0 to 10.
Noise Threshold	No	(TurboChrom algorithm) The noise threshold, from 1-6 to 19.
Area Threshold	No	(TurboChrom algorithm) The area threshold, from 1-6 to 112.
Separation Width	No	(TurboChrom algorithm) The separation width, from 0 to 5.
Separation Height	No	(TurboChrom algorithm) The separation height, from 0 to 1.
Exp Peak Ratio	No	(TurboChrom algorithm) The exponential peak ratio, from 1 to 16.
Exp Adjusted Ratio	No	(TurboChrom algorithm The exponential adjusted ratio, from 2 to 16.
Exp Valley Ratio	No	(TurboChrom algorithm) The exponential valley ratio, from 1 to 16.
Min Height	No	The minimum allowed peak height, from 0 to 116, when using the IntelliQuan algorithm.
Min Width	No	(IntelliQuan algorithm) The minimum allowed peak width, from 0 to 116, in seconds.
Smooth Width	No	(IntelliQuan algorithm) The half-width of the Savitzky-Golay smoothing filter, from 0 to 20.

Table 2-1 Text File Formats (continued)

Column Name	Required	Description
MQ III Noise Percent	No	(IntelliQuan algorithm) The noise percentage when the MQ III option is used. This should be an integer from, 0 to 100.
MQ III Baseline Sub Window	No	(IntelliQuan algorithm) The baseline subtraction window, from 0 to 10 minutes, when the MQ III option is used.
MQ III Peak Splitting Factor	No	(IntelliQuan algorithm) The peak-splitting factor, from 0 to 10, when the MQ III option is used.
MQ III Use Largest	No	(IntelliQuan algorithm) Specifies whether the largest peak when the MQ III option is used, within the retention time window, or the peak whose retention time is closest to that expected is reported. TRUE to use the largest peak and FALSE to use the closest.
Summation Baseline Sub	No	(Special window summation algorithm) Specifies whether the area should be integrated to the intensity=0 line or to the intensity value of the least intense data point within the window. TRUE if area should be integrated to the intensity value of the least intense data point, otherwise, FALSE if the area should be integrated to the intensity=0 line.

The following table shows an example text file for full-scan data. The text file contains tabs between the columns and a carriage return at the end of each line.

Table 2-2 Example Text File for Full-Scan Data

Peak Name	First Mass	Second Mass	Bunching Factor
Analyte Peak 1	500.1	500.7	1
Analyte Peak 2	812	813	2
Analyte Peak 3	400	401	3

The following table shows another example for MRM data. The Analyte Peak 1 is configured to use the specified internal standard and Analyte Peak 2 does not use an internal standard.

Table 2-3 Example Text File for MRM Data

Peak Name	Is IS	IS Name	First Mass	Second Mass
IS Peak 1	TRUE	—	500.1	413.2

Table 2-3 Example Text File for MRM Data (continued)

Peak Name	Is IS	IS Name	First Mass	Second Mass
Analyte Peak 1	FALSE	IS Peak 1	600.2	382.1
Analyte Peak 2	FALSE	IS Peak 1	400	312.1

The following table contains a mixture of full-scan and MRM data in different experiments:

Table 2-4 Example Text File for MRM Data

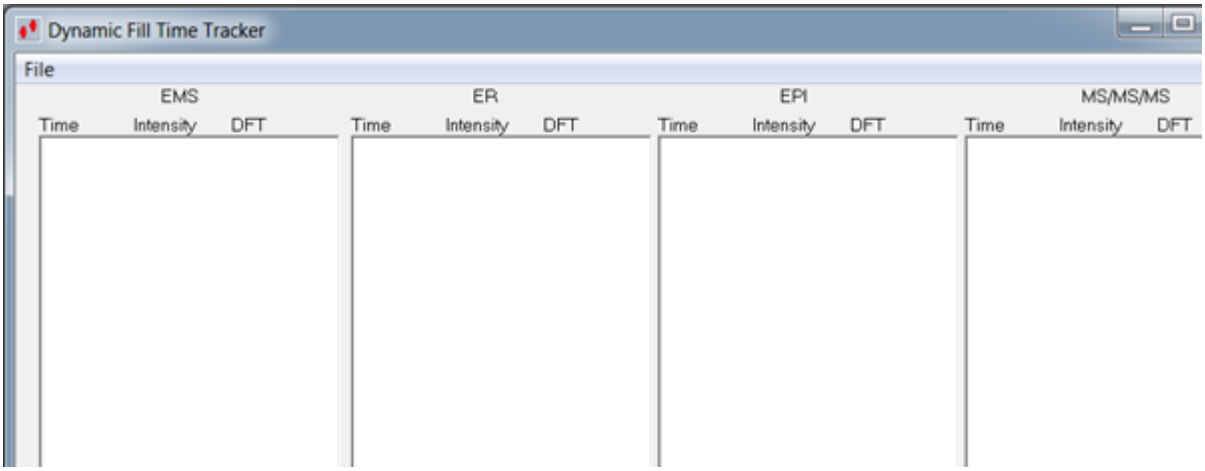
Peak Name	Extraction Type	Experiment	First Mass	Second Mass
Analyte Peak 1	0	1	500.1	413.2
Analyte Peak 2	0	1	600.2	382.1
Analyte Peak 3	2	2	812	813
Analyte Peak 4	2	2	400	401

DFT Tracker

The Dynamic Fill Time (DFT) Tracker script tracks the DFT settings used during the QTRAP system scans. Use the script to determine the optimal fill time for linear ion trap (LIT) mode to obtain high data quality over a wide dynamic range. The DFT Tracker monitors the following LIT scan types: Enhanced MS (EMS), Enhanced Resolution (ER), Enhanced Product Ion (EPI), and MS/MS/MS (MS3).

- Click **Script** > **DFTTracker**.

Figure 2-3 Dynamic Fill Time Tracker Dialog



DFT Tracker monitors the dynamic changes to the fill time occurring during a real-time run.

The system dynamically calculates the time required to fill the linear ion trap. For abundant compounds, a short fill time reduces the space charge effects by limiting the number of ions in the ion trap. A longer fill time increases weak signals by allowing the ions to accumulate.

- Click **File** > **Save** to save the tracked fill time.
- Click **File** > **Clear** to clear the tracked fill time.
- Click **File** > **Always On Top** to keep the Dynamic Fill Time Tracker window on top of all of the other open windows or applications.
- Click **File** > **Exit** to exit the DFT Tracker script.

MRM3 Optimization Script

Use this script for quantitation analysis on QTRAP systems to provide increased specificity and, therefore, improved detection when quantifying analytes in complex matrices. This script is designed to generate an optimal MS3 acquisition method by infusion. The script performs the following optimization steps:

- Confirm precursor mass
- Optimize transmission to collision cell
- Determine the major fragment ions
- Optimize the Collision Energy (CE) for each fragment ions
- Perform MS3 scans on each fragment ion
- Optimize Excitation Energy (AF2) for all MS3 scans
- Generate a report
- Save all data and acquisition methods

The script can also be used in qualitative applications to generate collections of MS/MS and MS3 spectra for compounds in a semi-automated way (that is, one compound at a time).

MRM3 Optimization Window Overview

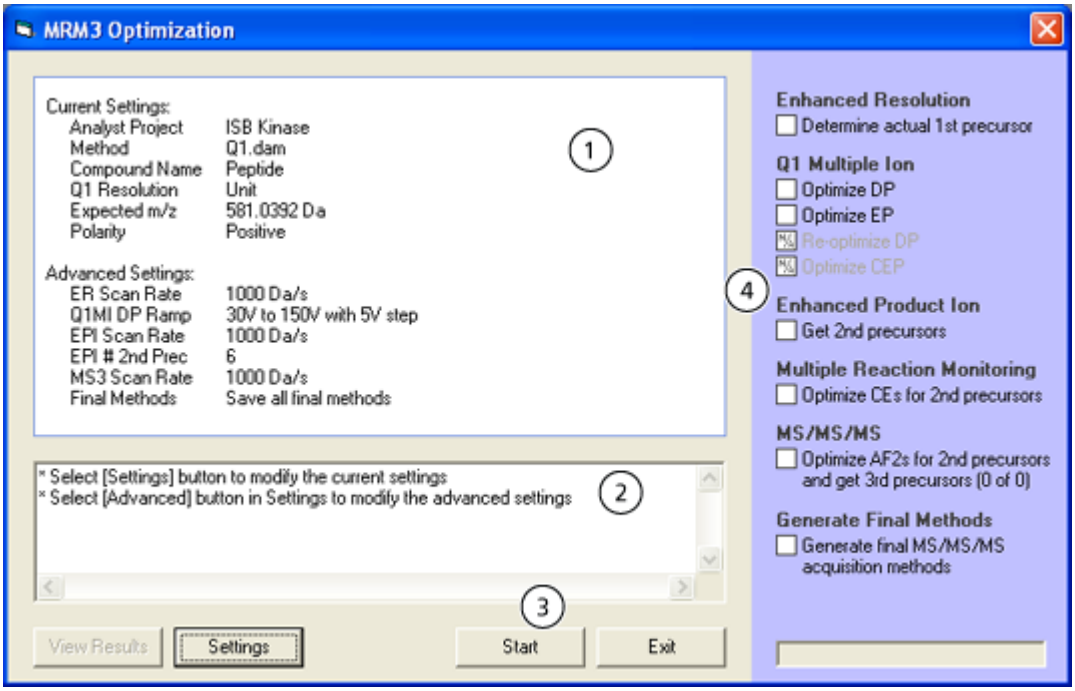
Use the controls in the MRM3 Optimization window to navigate. The window also shows the optimization results as they are generated. The following is an overview of the various sections in this window.

Table 2-5 MRM3 Optimization Window

Field	Description
Status Window	When the script is first started, this window shows the current optimization settings that will be used for optimization. When the optimization is started, spectral information is shown in this window.
Log File	Shows the results found during optimization in text format. Each entry found in this section is also added to the generated Log.txt file.
Overall Progress	Shows the overall optimization progress.
Main Controls	Contains all of the main functions associated with the setting and execution of the optimization process.

- Click **View Results**, to open and review the file using Microsoft Notepad. After the optimization is completed, a Results.txt file is automatically generated and saved.
- Click **Settings** to open a window to type compound information required for the optimization process.
- Click **Start** to initiate the optimization process. During optimization, this button is renamed to **Abort**, which can be clicked to stop the optimization process.

Figure 2-4 MRM3 Optimization Window



Item	Description
1	Status pane
2	Log file
3	Main controls
4	Overall progress

Set the Preferences

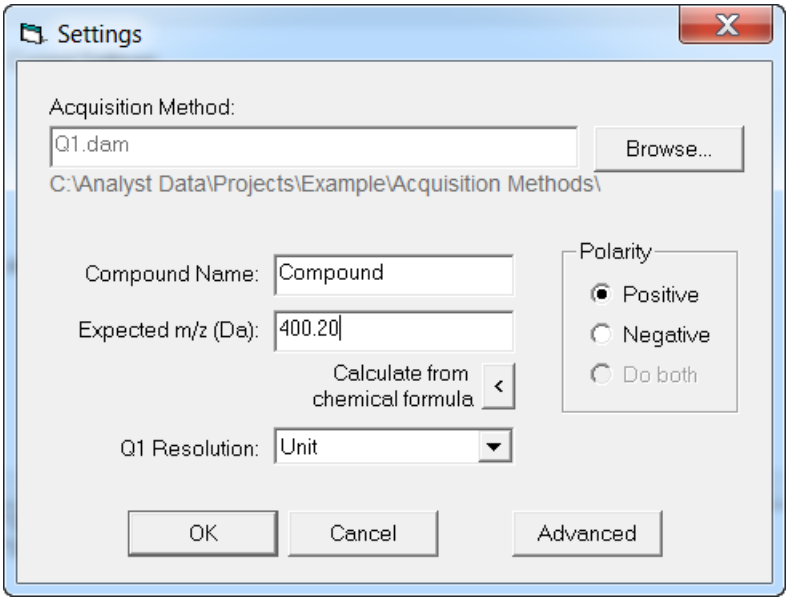
The Settings dialog opens automatically every time the script is launched.

1. Click **Browse** to browse to the starter acquisition method. This method contains the source conditions to be used for the optimization.
2. In the **Compound Name** field, type a descriptive compound name. This name is used as a prefix to all of the acquisition methods and data files generated.
3. In the **Expected m/z (amu)** field, type the expected mass-to-charge ratio (*m/z*) for the compound. If the *m/z*values of the compound is not known, then click **Calculate from chemical formula** to calculate it from the chemical formula of the compound. Refer to the section: Calculate m/z.
4. In the **Q1 Resolution** field, select a Q1 Resolution to be used for MS/MS and MS3.
5. In the **Polarity** group, click a polarity, which can differ from the starter method. The **Do both** option is currently not supported.
6. To modify some of the settings used by the optimization process, click **Advanced**. Refer to the section: Use the Advanced Settings Dialog.
7. To verify and use the updated settings, click **OK**.

Use the Script

1. Build a starter acquisition method if one does not already exist. The starter method should be a Q1 acquisition method created in Manual Tune and should contain the source conditions required for the tuning process because these are not optimized by the script.
2. Save the method in the `Acquisition Methods` folder of the required project where all generated files will be saved.
3. Click **Script** > **MRM3 Optimization**.

Figure 2-5 Settings Dialog



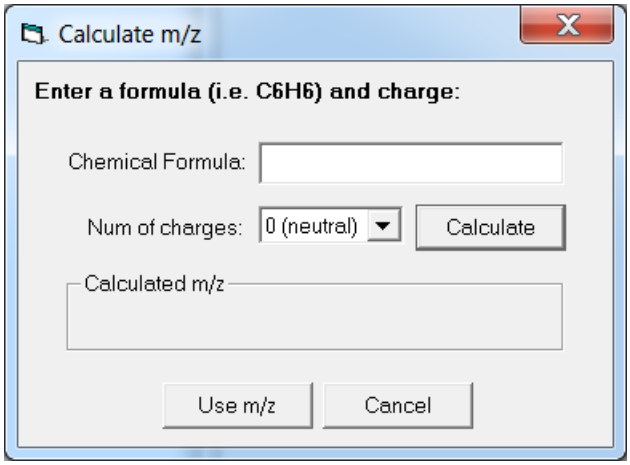
4. Enter the compound information required for the optimization process and then click **OK** on the Settings dialog.
5. To initiate the optimization process, click **Start** in the MRM3 Optimization window.

Calculate *m/z*

The *m/z* calculator is accessed through the Settings dialog.

1. In the MRM3 Optimization window, click **Settings**.
   The Settings dialog opens.
2. Click **Calculate from chemical formula**.

Figure 2-6 Calculate m/z Dialog



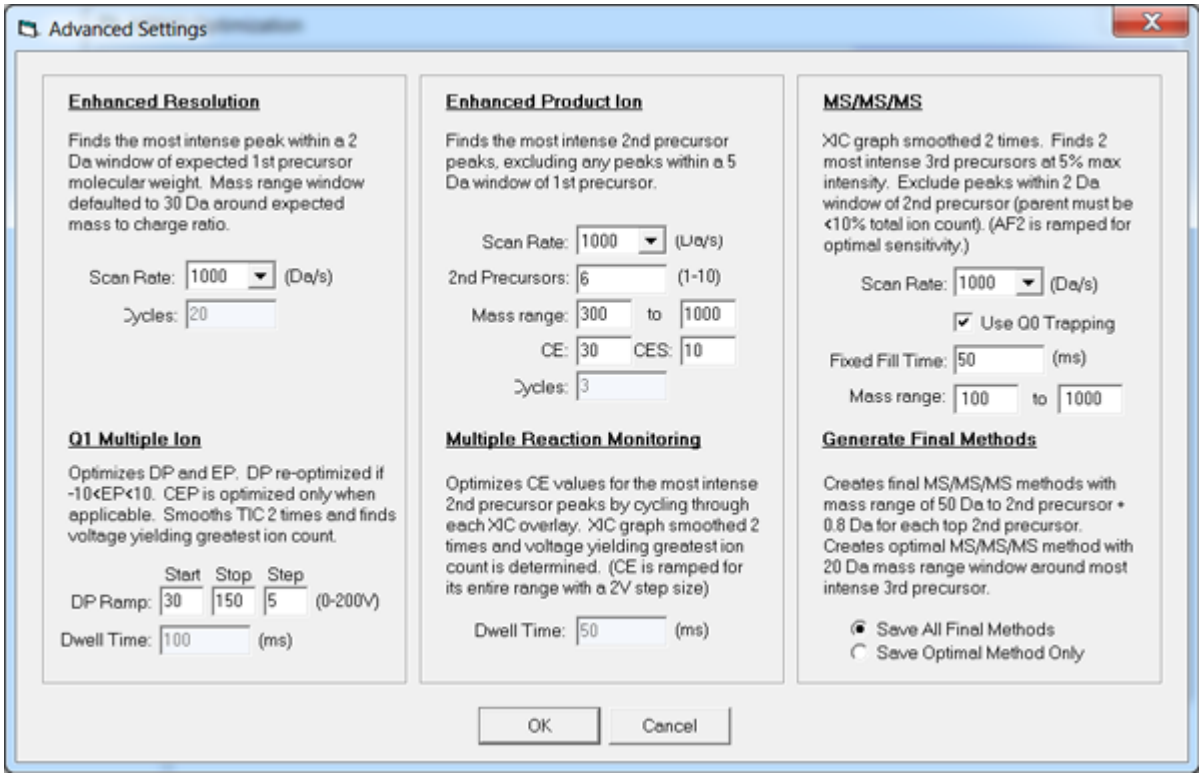
3. In the **Chemical Formula** field, type the chemical formula of the compound. Use capital letters for elements. The chemical formula for peptides is also entered in this dialog.
4. In the **Num of charges** field, click the number of charges.
5. To calculate the *m/z* for the entered chemical formula and charge, click **Calculate**.
6. To close the calculator and update the **Expected m/z (amu)** field in the Settings dialog with the calculated *m/z*, click **Use m/z**.

Use the Advanced Settings Dialog

In this dialog, a description for each of the optimization steps is provided. Some of the settings can be modified to customize the optimization.

1. In the MRM3 Optimization window, click **Settings**.
   The Settings dialog opens.
2. Click **Advanced**.

Figure 2-7 Advanced Settings Dialog



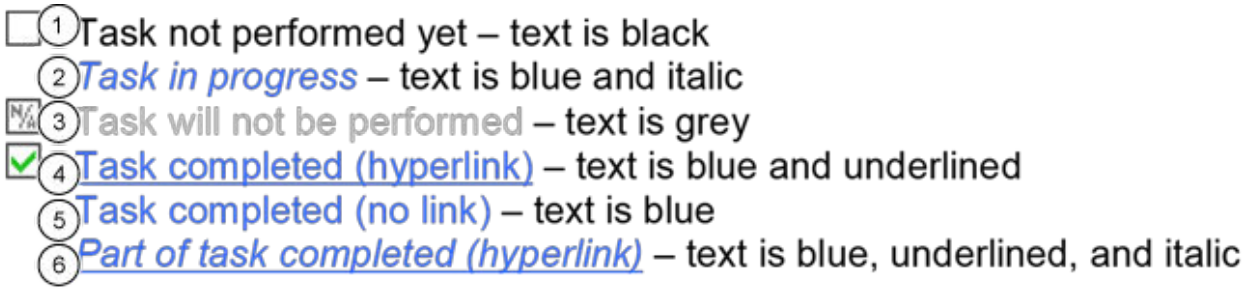
3. In the **Scan Rate** fields in the Enhanced Resolution, Enhanced Product Ion and MS/MS/MS groups, select a scan rate for **ER**, **EPI**, and **MS3**.
4. In the **Q1 Multiple Ion** group, in the **DP Ramp** fields, type the declustering potential (DP) range for optimization. The range is expressed in absolute values and the appropriate polarity is automatically applied based on the selection made in the Settings dialog.
5. In the **Enhanced Product Ion** group, do the following:
   - In the **2nd Precursors** field, type the maximum number of second precursors (fragment ions) used for MS3 optimization. Type a number between 1 and 10.
   - In the **Mass range** field, type a mass range for the second precursors that will be selected for MS3 optimization.
   - In the **CE** field, type a collision energy value and in the **CES** field, type a collision energy spread value that will provide a good MS/MS spectrum from which fragment ions can be selected.
6. To generate all of the final MS3 methods for each second precursor and the optimal MS3 method for quantitation analysis, in the **Generate Final Methods** group, click **Save All Final Methods**. Click **Save Optimal Method Only** to save only the optimal MS3 method (most sensitive for quantitation).

7. Click **OK** to accept the updated Advanced Settings.

Optimization in Progress

When the optimization is started, Manual Tune in the Analyst MD software is automatically stopped. While the script is running, all of the functions in the software can still be used. A Log.txt file is also updated as each part of the optimization procedure is completed. To stop the script at any time, click **Abort**. Refer to the following figures for examples of the script. In the Overall Progress section, the Checklist images and text fonts represent different statuses that are described in the following section.

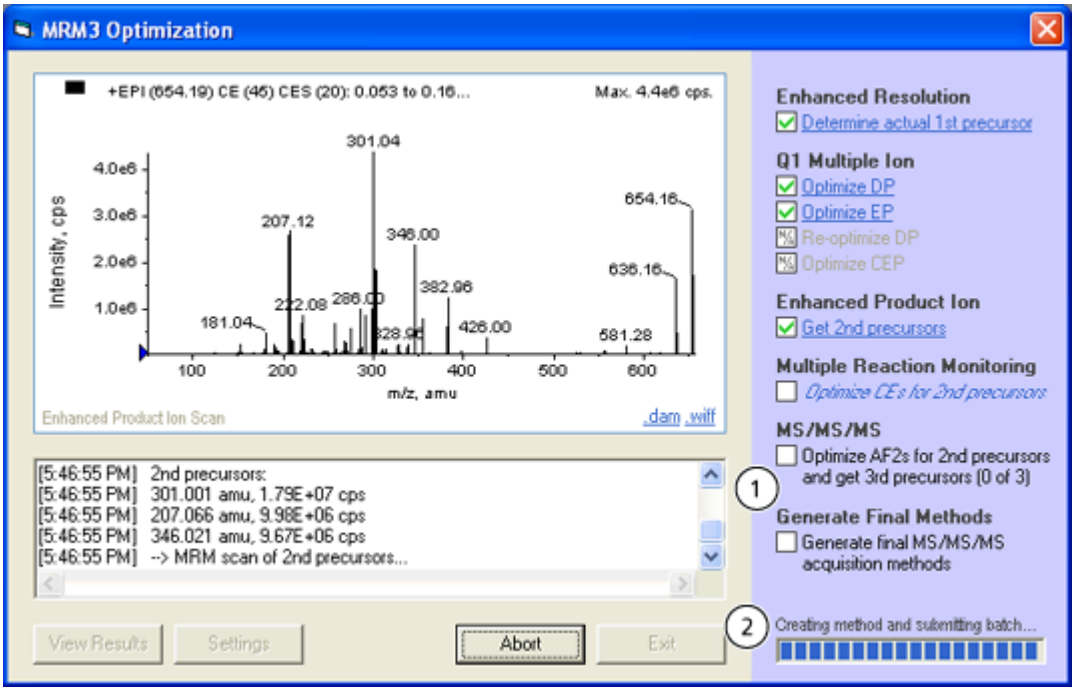
Figure 2-8 Status Examples



Item	Description
1	Tasks not performed yet - text is black
2	Task in progress - text is blue and italic
3	Task will not be performed - text is blue and underlined
4	Task completed (hyperlink) - text is blue and underlined
5	Task completed (no link) - text is blue
6	Part of task completed (hyperlink) - text is blue, underlined, and italic

When the text is underlined, click it like a web page hyperlink and the corresponding spectrum or chromatogram is shown. The text found under MS/MS/MS also shows the MS3 scan number that is being performed because it is possible to have between 1 and 10 scans. The Overall Progress section also includes a Message area. In this area, a progress bar shows the current step progress. Above the progress bar, various messages are shown such as the time and other statuses for the current optimization step.

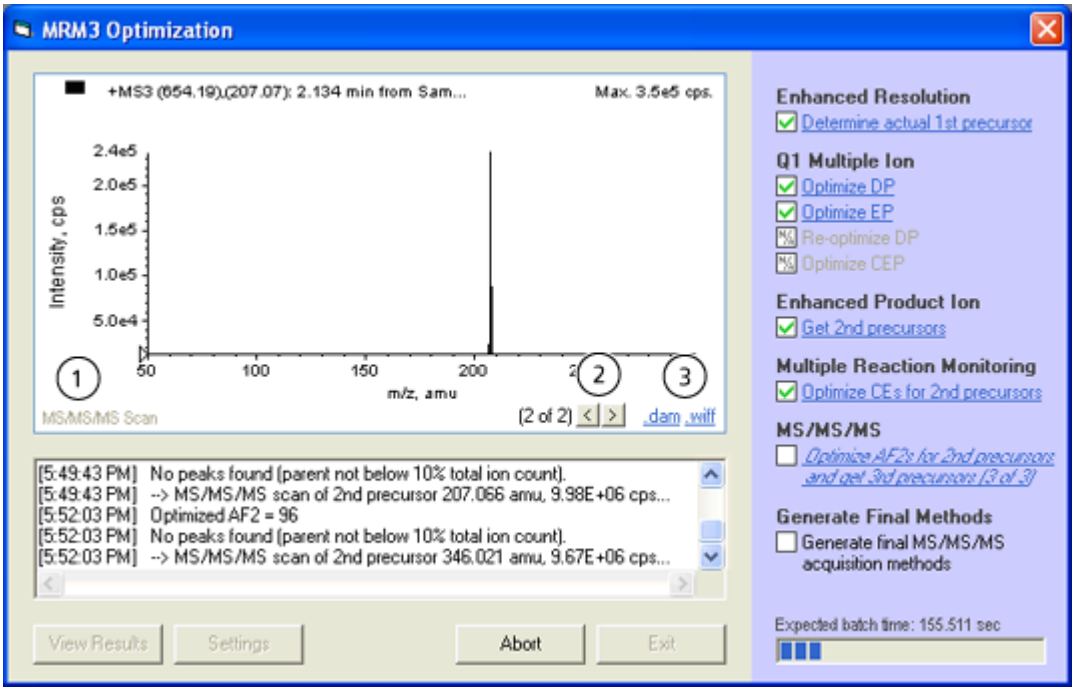
Figure 2-9 MRM3 Optimization Window After an EPI Scan



Item	Description
1	Checklist
2	Message

In the spectral status window, the previously generated spectrum or chromatogram is shown. When one of the checklist items is selected, the corresponding graph is shown. The scan type name indicates which scan is currently being shown. For each completed step, it is possible to open the acquisition method (dam) or data file (wiff) associated with the graph shown. If an MS/MS/MS scan is shown, then use the buttons to cycle through the different MS3 scans.

Figure 2-10 MRM3 Optimization Window During an MS3 Scan



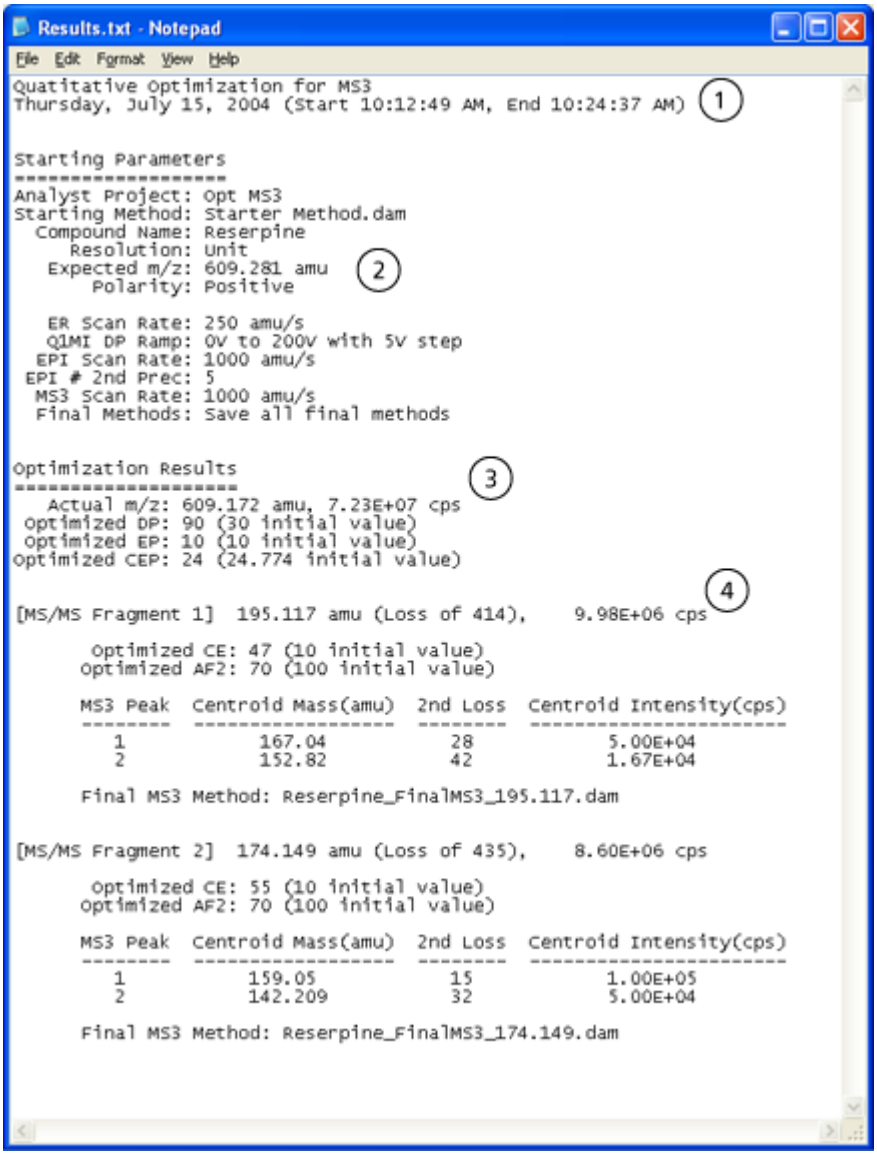
Item	Description
1	Scan type
2	Buttons to cycle through different MS3 scans
3	Links

Optimization Complete

When the quantitative optimization for MS3 is completed or stopped, a Results.txt file is generated. This file is automatically opened in Microsoft Notepad. Click **View Results** from the MRM3 Optimization window to view the file. The various parts of the Results.txt file are described below.

- **Time and Duration**: Shows the date and time duration of optimization.
- **User Starting Conditions**: Shows the settings and Advanced Settings in this section.
- **Optimization Conditions Found**: Shows the optimal conditions found during the ER and Q1MI scans.
- **MS3 Fragments Found and Associated Losses**: Shows the fragments and optimal conditions (collision energy and excitation energy) as well as associated losses found for the EPI scan and MS3.

Figure 2-11 Optimization Report



Item	Description
1	Time and duration
2	User starting conditions
3	Optimization conditions found
4	MS3 fragments found and associated losses

All of the generated acquisition methods have a descriptive file name in the format [supplied compound name] + [scan type] + [*m/z*] + dam. These methods are saved in the same folder as the starter acquisition method.

All of the data, Log.txt, and Results.txt files are saved in a Data subfolder that is created in the same project as the starter acquisition method. The subfolder has the format [supplied compound name] + OptMS3 + ([date], [time]). The data files have the format [supplied compound name] + [scan type] + [*m/z*] + wiff.

Detailed Description of Script Logic: Initialization

This section describes each phase of the optimization process. All scans are performed with the number of scans to sum set to 3.

Before performing any optimization scans, the MRM3 Optimization script performs the following initialization steps. If an error occurs during any of these steps, the script stops the optimization process.

1. Make sure that the Analyst MD software is running.
2. Load the starter acquisition method to determine if it is valid and to check the device type.
3. Create a new `Data` subfolder to store the wiff files.
4. Create the Log.txt file.

Enhanced Resolution Scan

This step confirms the mass of the ion used for optimization. The ER scan is performed for 20 cycles at the specified scan rate. The most intense peak within ± 1 amu of the expected first precursor *m/z* value is then selected. As in the Analyst MD software, this scan is performed with a 30 amu mass range around the specified *m/z* value. For multiply charged species, the C12 ion is determined in this step.

Q1 Multiple Ion Scan

This step optimizes transmission of the ion of interest up to the collision cell. This is performed using a Q1 MI scan. The script first optimizes the DP parameter by performing the scan at the specified DP ramp. Optimize the EP parameter by ramping it from 1 V to 12 V (–12 V to –1 V for negative mode), with 0.5 V step. If the optimal EP is less than 10 V (greater than –10 V for negative mode), then DP is re-optimized. The CEP parameter is also optimized by ramping from 0 V to 100 V (–100 V to 0 V for negative mode) with 2 V step. In determining the optimal voltage, graphs are smoothed two times and the voltage yielding the greatest ion count is used. Dwell Time for each scan is set to 100 ms.

Enhanced Product Ion Scan

This step selects the fragment ions that will be used for MS3 optimization. This is performed using an EPI scan for three cycles at the selected scan rate. Specify an optimal CE for the compound to be analyzed. If the optimal CE is unknown, then specify a CES value so that a range of CE settings are used. The most intense second precursor peaks are then found, excluding any peaks within ± 2.5 amu window of first precursor. The number of second precursors to use is selected in the Advanced Settings. The mass range from which the second precursors are selected is specified by the user.

Multiple Reaction Monitoring Scan

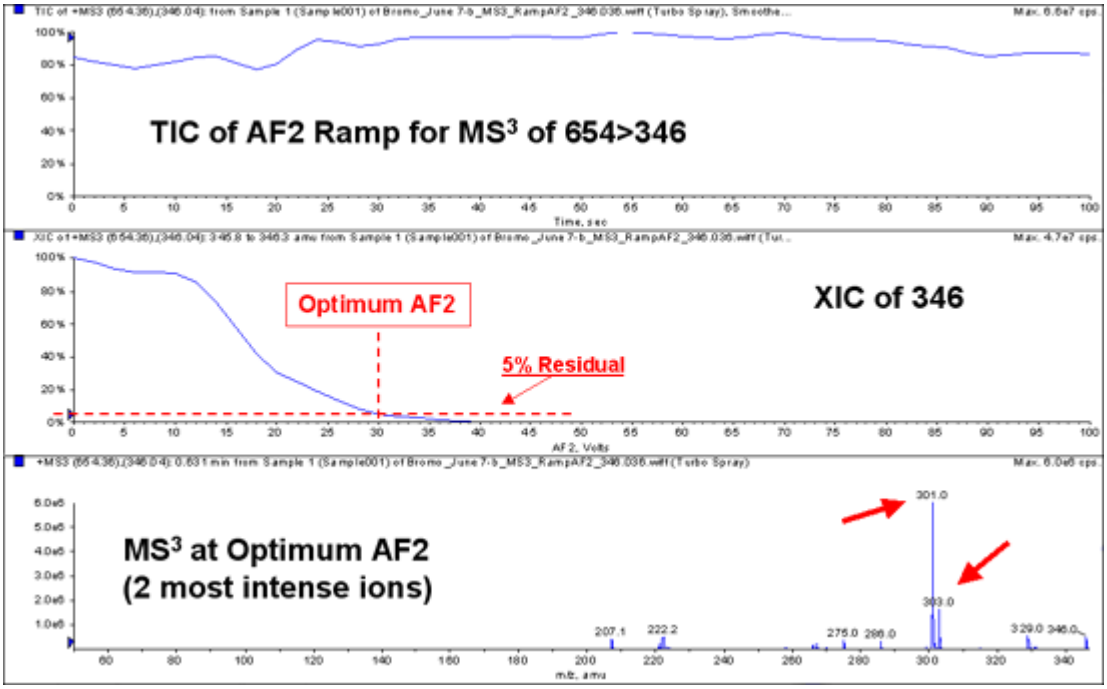
This step optimizes the collision energy for each of the fragment ions selected from the EPI scan. This is performed using MRM scans. Use CE ramps of 5 to 130 V (–130 to –5 V in negative mode) with 2 V step and Dwell Time of 50 ms. Each overlaid graph is then smoothed two times and the voltages yielding the greatest ion count are used as the optimal CE values.

MS/MS/MS (MS3) Scan

The script performs an MS/MS/MS (MS3) scan for each chosen second precursor at the specified scan rate and with an AF2 ramp of 0 to 100 V with 2 mV step for both polarities. The fill time of the scan is set, and Q0trapping can be turned on for maximum sensitivity if required. The lower limit of the mass range for the MS/MS/MS (MS3) scan can be specified, and the upper limit is second precursor + 5 amu.

The generated graphs are smoothed twice and the optimal AF2, as shown in the following figure, is obtained when the residual intensity of the second precursor (based on XIC) is at 5% of its maximum intensity. The spectrum at this AF2 value is then used to find the two most intense second-generation fragment ions, excluding peaks within ± 1 amu of the second precursor. If the second precursor *m/z* value is greater than 10% of the total ion count, then no fragments from that spectrum is used. This condition exists because if the second precursor *m/z* value is greater than 10%, then there is insufficient fragmentation.

Figure 2-12 How AF2 is Determined



Generate Final Methods

After the optimization scans are performed, the script generates the final MS/MS/MS methods. If the **Save Optimal Method Only** option is clicked in the Advanced Settings dialog, then only an optimal MS/MS/MS method with ± 10 amu around the most intense second generation fragment ion is created. If the **Save All Final Methods** option is clicked, then the optimal method as well as an MS/MS/MS method for each of the top second precursors are created using a mass range from the user-defined lower limit to an upper limit of (second precursor + 5) amu.

MSServiceLog Script

By default, readbacks from a mass spectrometer are recorded in the MS Service log file. Use the MSServiceLog script to turn off the recording of the readbacks or to start recording the readbacks from the instrument in the MS Service log file. The MSServiceLog script is only applicable to the 4500MD and Citrine systems systems.

The MSServiceLog script can be used without an active hardware profile but any changes made to the MS Service log settings take effect only after the hardware profile is reactivated.

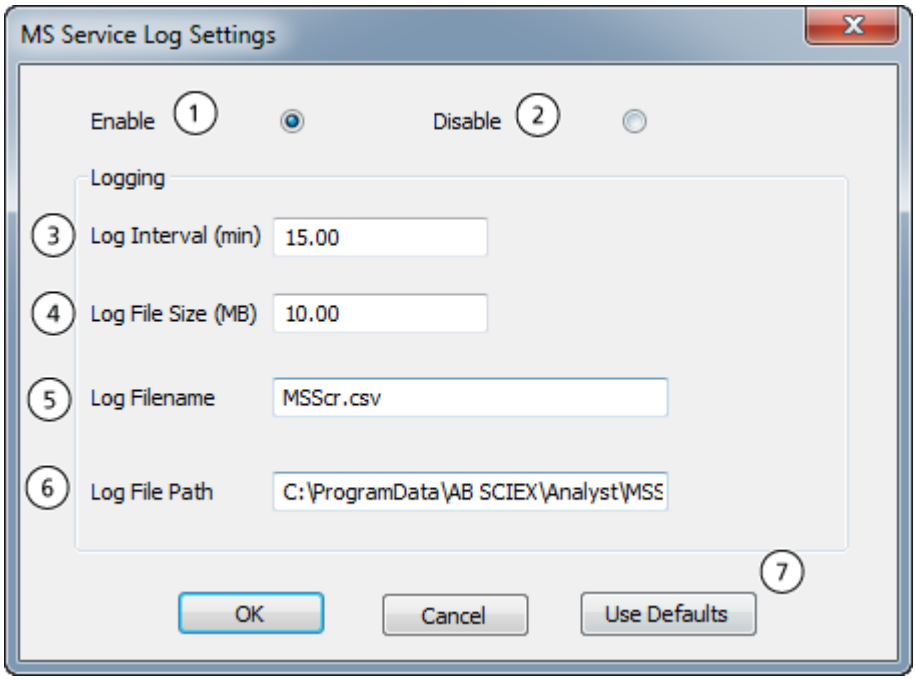
Install the Script

Refer to Install a Script.

Use the Script

1. Deactivate the hardware profile.
2. Click **Script** > **MSServiceLog**.

Figure 2-13 MS Service Log Settings Dialog



Item	Name	Description
1	**Enable**	Select to start recording the readbacks from the mass spectrometer to the MS Service log file using the MSServiceLog script.
2	**Disable**	Select to turn off the recording of the readbacks from the mass spectrometer to the MS Service log file using the MSServiceLog script.

Item	Name	Description
3	**Log Interval (min)**	Specify how frequently, in minutes, the readbacks from the mass spectrometer will be recorded to the MS Service log file. The default value is 15 minutes and the allowed range is from 1 minute to 1440 minutes.
4	**Log File Size (MB)**	Specify the size of the log file. The default size is 10 MB, and the allowed range is 1 MB to 1000 MB. There can be up to two log files: • The current log file, where the readbacks from the instrument are recorded. • The archived log file. When the current log file reaches the specified size, it is archived with an predefined archive filename, and a current log file is created to record the readbacks with the log file name specified in the MS Service Log Settings dialog.
5	**Log Filename**	Specify a name for the log file. The accepted file extensions are csv, txt, or log.
6	**Log File Path**	Specify the location where the log file is stored. Make sure that the new location is created inside the default location `C:\ProgramData\ABSCIEX\Analyst\MSServiceLog`.
7	**Use Defaults**	Click to revert to the preset values in all the fields in the dialog.

3. Click **Disable** to turn off the recording of the readbacks in the MS Service log file.
4. Click **Enable** to start recording the readbacks from the mass spectrometer to the MS Service log file.
5. To change the values in other fields in the MS Service Log Settings dialog, refer to the figure: Figure 2-13.
6. Click **OK** the apply the changes.

sMRM Calculator

Use the sMRM Calculator script for a visual representation of a *Scheduled* MRM algorithm acquisition method. The script uses four graphs to show an overview of the MRM transition, its

concurrency, its projected cycle time, and the dwell time to be applied to it. Refer to the figure: Figure 2-15. To achieve a suitable arrangement of the transitions over the run time, change the parameter values, such as **Maximum Dwell**, **Minimum Dwell**, **Target sMRM Cycle Time** or **Target sMRM Scan Time**, **Window Width**, **MRM Pause Time**, and **Settling Time**, in the script dialog. The four graphs are updated accordingly. Repeat this process until the required arrangement of the transitions is achieved.

Note: If **Target Cycle Time** is selected in the original method, then it cannot be changed to **Target Scan Time** in the script dialog. If **Target Scan Time** is selected in the original method, then it cannot be changed to **Target Cycle Time** in the script dialog.

Note: The **Settling time** option can only be modified for the Citrine systems in the sMRM Calculator script dialog.

Install the Script

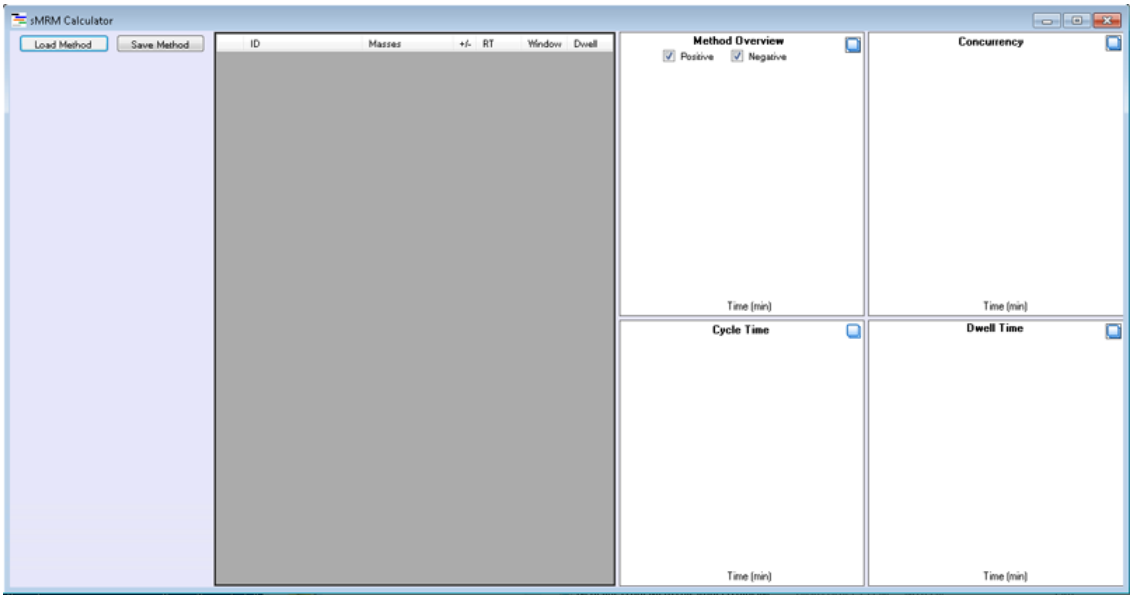
Refer to Install a Script.

Use the Script

Prerequisites
• Make sure that the Analyst MD software is open and a hardware profile is activated. • Make sure that a *Scheduled* MRM algorithm acquisition method is already created.

1. Click **Script** > **sMRM Calculator**.
   The **sMRM Calculator** dialog opens.

Figure 2-14 sMRM Calculator Dialog



2. Click **Load Method** to select an existing *Scheduled* MRM algorithm acquisition method. The **Open** dialog opens.

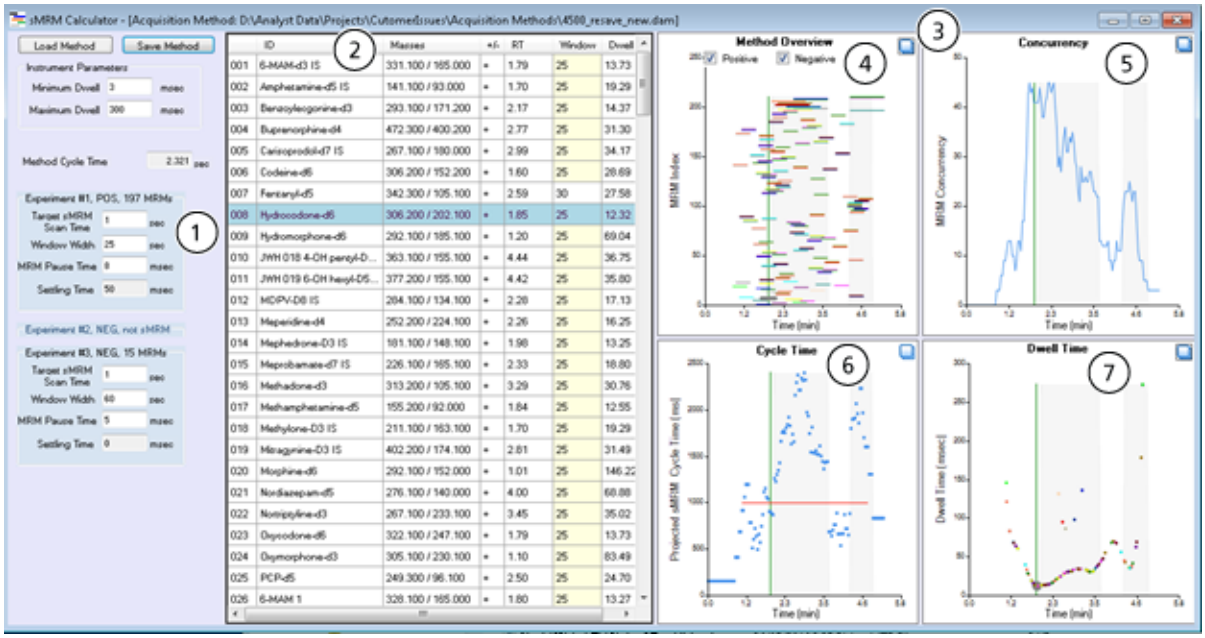
Note: Only an acquisition method that contains *Scheduled* MRM algorithm experiments and for the active mass spectrometer in the currently selected project can be opened in the sMRM Calculator script. Only the details for *Scheduled* MRM algorithm experiments are shown. The non-*Scheduled* MRM algorithm experiments are labeled as not *Scheduled* MRM in the script.

---

3. Select the *Scheduled* MRM algorithm acquisition method and then click **Open**.

   The selected acquisition method opens in the **sMRM Calculator** dialog. The file path of the open acquisition method file is shown in the title of the dialog.

Figure 2-15 Acquisition Method Opened in the sMRM Calculator Dialog



Item	Description
1	The left pane contains instrument and *Scheduled* MRM algorithm parameters. The parameters shown in this pane change depending on the acquisition method opened. If the arrangement of the transitions is not suitable in the four graphs in the right pane, then change the editable parameters and settings in the left pane. The affected columns in the table and the graphs are updated accordingly. The parameter values can be modified within the allowable range until a suitable arrangement of transitions is achieved. For example, if the value in the **Target sMRM Scan Time** field is changed, then the dwell time is recalculated and updated in the table, and the graphs are also updated accordingly. For example, if the value in the **Windows Width** field is changed, then this value is updated in the **Window** column for all of the transitions that use this global setting. The dwell time for all of the transitions is recalculated and updated in the table. The graphs in the right pane are also updated accordingly. For transitions with their own detection window settings in a *Scheduled* MRM Pro algorithm acquisition method, updating the global setting **Window Width** in the left pane does not update the values in the **Window** column for these transitions in the table. **Note:** The fields that show as grey in the left pane are not editable and the value cannot be changed.

Item	Description
2	The index, compound ID, Q1 and Q3 masses, polarity, window width, retention time, and dwell time are shown in the middle pane. The default view is in order by index number. To rearrange the view based on the information in the other columns, click the title of one of the seven columns: **index**, **ID**, **Masses**, **+/-**, **RT**, **Window**, and **Dwell**. The middle pane refreshes, showing the information sorted in the alphanumerical or numerical order of the selected column. For methods for SCIEX 4500MD and Citrine systems, the window width for all transitions in that *Scheduled* MRM algorithm experiment can also be edited in the table. The dwell time in the table and the graphs in the right pane are updated accordingly. Editing the window width in the table converts a *Scheduled* MRM algorithm acquisition method to a *Scheduled* MRM Pro algorithm acquisition method. **Note:** The window width that uses the global setting from the left pane has a yellow background. After the window width in the table is manually modified for an individual transition or if it already uses the advanced window width that is specific for its transition, then the background color for that cell changes to white.
3	The right pane shows all of the *Scheduled* MRM algorithm transitions contained in the loaded *Scheduled* MRM algorithm acquisition method graphically as four different types of graphs. • The selected MRM transition in the table is depicted by the green vertical line in the graphs. • The light grey areas in the graphs represent the retention time zones where there is polarity switch in each cycle. • Tool tips in each graph show X- and Y-values for the transition under the cursor. For the Method Overview and Dwell Time graphs, the compound ID is also shown in the tool tips. • Clicking an MRM transition in the Method Overview graph selects that transition in the other three graphs and the table.
4	The first graph, Method Overview, shows all of the transitions and the detection window of each transition. The X-axis shows the retention time. The Y-axis shows the MRM index number, which is the order in which the transitions were entered in the method.

Item	Description
5	The second graph, MRM Concurrency, shows the retention time on the X-axis and the MRM transition concurrency at each retention time on the Y-axis.
6	The third graph, Projected sMRM Cycle Time, plots the projected cycle time over retention time. The red line represents the **Target Cycle Time**, if it is used. If the **Target Scan Time** is used, then the value of the red line is the sum of the **Target sMRM Scan** time of all of the *Scheduled* MRM algorithm experiments in the method. **Note:** More data points are expected for transitions for which the **Projected sMRM Cycle Time** is much lower than the **Target Cycle Time** or the sum of the **Target Scan Time** (where the red bar is). Fewer data points are expected for transitions for which the **Projected sMRM Cycle Time** is much higher than the **Target Cycle Time** or the sum of the **Target Scan Time** (where the red bar is).
7	The fourth graph shows the dwell time for each transition at its retention time. The X-axis shows the retention time. The Y-axis shows the dwell time to be applied.

4. Change the parameter values as required to optimize the method to achieve a better distribution of the **Projected sMRM Cycle Time**.
5. Click **Save Method**.
   The **Save Method File** window opens.

   The changes to the method can be saved in the original acquisition method or can be saved as a new acquisition method. If the changes are saved to the original acquisition method, then the original parameter values are overwritten by the new values.
6. Type a new file name or select the original method and then click **Save**.
7. Open the saved acquisition method in the **Acquisition Method Editor** to view the new changes.

   If the original method was open in the **Acquisition Method Editor**, then the method must be closed and opened again.
8. Click the **X** in the upper-right corner of the **sMRM Calculator** dialog to close the dialog.

Contact Us

Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX Now Learning Hub

SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
- sciex.com/request-support

CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

This version of the document supercedes all previous versions of this document.

To view this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to https://get.adobe.com/reader.

Contact Us

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

To find hardware product documentation, refer to the *Customer Reference* DVD that comes with the system or component.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.