Agilent MassHunter Drug Analysis Mode Using Quantitative Analysis

Workflow Guide

Review the Intelligent Sequencing Feature → Create the Data Acquisition Method → Create the Quant Analysis Method → Run Samples

Agilent Technologies

Notices

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In This Guide...

This workflow guide describes how a method can be created to perform data acquisition in MassHunter GCMS Acquisition and data analysis in MassHunter Quantitative Analysis, using the Drug Analysis Workflow mode. Also, for those users interested in migrating from MSD ChemStation to MassHunter Quantitative Analysis, Chapter 4 includes a section on converting an existing MSD ChemStation Method to a MassHunter Quantitative Analysis method.

This document emphasizes the use and understanding of Intelligent Sequencing features in MassHunter Drug Workflow mode, which are not currently covered in other documents created for MassHunter GCMS Acquisition and Quantitative Analysis.

More common operations, not directly associated with Drug Analysis Workflow mode and Intelligent Sequencing, such as setting GC Parameters and real-time plot displays, are briefly discussed here, but are covered in more detail in both online Help and Familiarization Guides. Please refer to the online Help for more details on these topics and for a link to an unabridged version of the MassHunter GCMS Acquisition Software for 5975/5977 Series GC/MSD Familiarization Guide (G1701-90110).

A brief summary of chapter contents for this Workflow Guide follows.

1 Before You Begin

Chapter 1 describes how to set up your MassHunter GCMS Acquisition and MassHunter Quantitative Data Analysis programs for using the Drug Workflow mode user interface (UI). Items specific to this UI are then reviewed.

2 About Intelligent Sequencing

Chapter 2 describes Intelligent Sequencing and how it uses keywords to initiate actions based on results from Data Analysis. These actions include injecting blanks to reduce sample carryover, reinjecting a specimen including reducing the injection volume when its concentration is over a predefined limit, skipping to another batch, or just waiting for an operator to intervene. Because a number of actions may be employed within a given sequence, flow diagrams are included to aide in following the decision process.

3 Create the Data Acquisition Method

Chapter 3 describes how to set up a SIM method for data acquisition. A Data Acquisition method must exist prior to the creation of a Quantitative Data Analysis method that uses Intelligent Sequencing.

4 Create the Quantitative Analysis Method

Chapter 4 describes how to create a Drug Quant method, compatible with Intelligent Sequencing, by editing an existing method. It starts out by describing how you can translate an existing MSD ChemStation method into a MassHunter Quantitative Analysis method, if desired. It then proceeds with setting up calibration curves for

various drugs and setting parameters required by Intelligent Sequencing. This includes setting the allowable specifications used during analysis and the set up of control samples used for monitoring the calibration state.

5 Run Samples

Chapter 5 explains how to set up and run a Sequence Table with multiple batches of samples using the keyword **NewBatch**. This keyword indicates where MassHunter should continue processing when a *Skip to next batch* command is generated by Intelligent Sequencing.

6 Update the Calibration

Chapter 6 describes how the Sequence Table Editor (STE) in MassHunter GCMS Acquisition can be used to automate the process of updating the calibration table. As required by governing regulations, the calibration stored in a method must be updated when a specified time has elapsed or when a quality control sample indicates an unacceptable deviation from the stored calibration curve.

Where to Find More Information

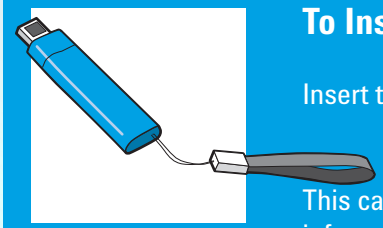
Accompanying your hardware and software is a comprehensive collection of manuals, videos, user applications, and method development tools. These are located on the:

- Agilent GC and GC/MS Manuals and Tools DVD set
- Agilent GC/MS Software Information and Manuals memory stick

To Install Your Hardware Library

Insert Disk 1 into your DVD drive and follow the prompts.

This can be installed by anyone who has authority to copy information onto the receiving computer.



To Install Your Software Library

Insert the memory stick into a USB port and follow the prompts.

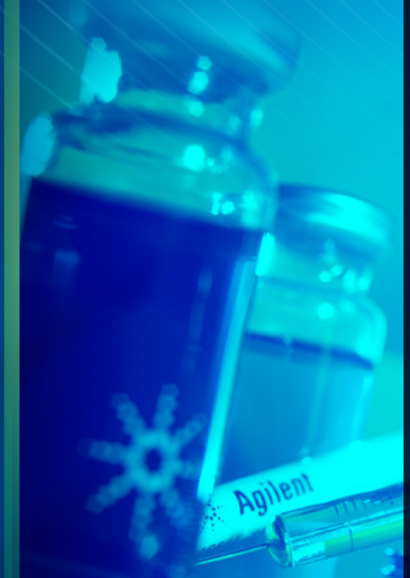
This can be installed by anyone who has authority to copy information onto the receiving computer.

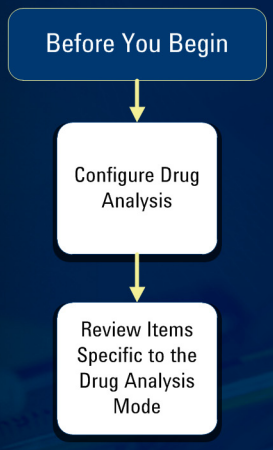
Contents

5 Run Samples

6 Update the Calibration

1
Before You Begin





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Configure MassHunter GCMS Acquisition for Drug Analysis

Depending on your instrument, MassHunter GCMS Acquisition and MassHunter Quantitative Analysis may be set up to run in several Workflow Modes, including:

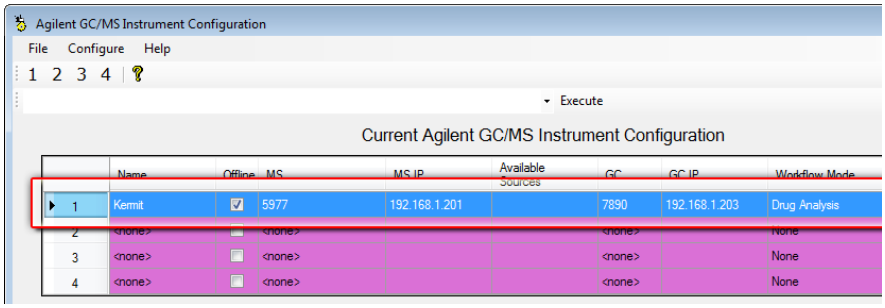
- Enhanced
- Drug Analysis
- EnviroQuant (EPA)
- Aromatics in Gasoline

Here we are going to be using the **Drug Analysis Workflow Mode**. So, before doing anything else, you must set up the MassHunter GCMS Acquisition program and the MassHunter Quantitative Analysis program to run in the Drug Analysis Workflow Mode.

To reconfigure an existing GC/MSD instrument to work in the Drug Analysis Mode:

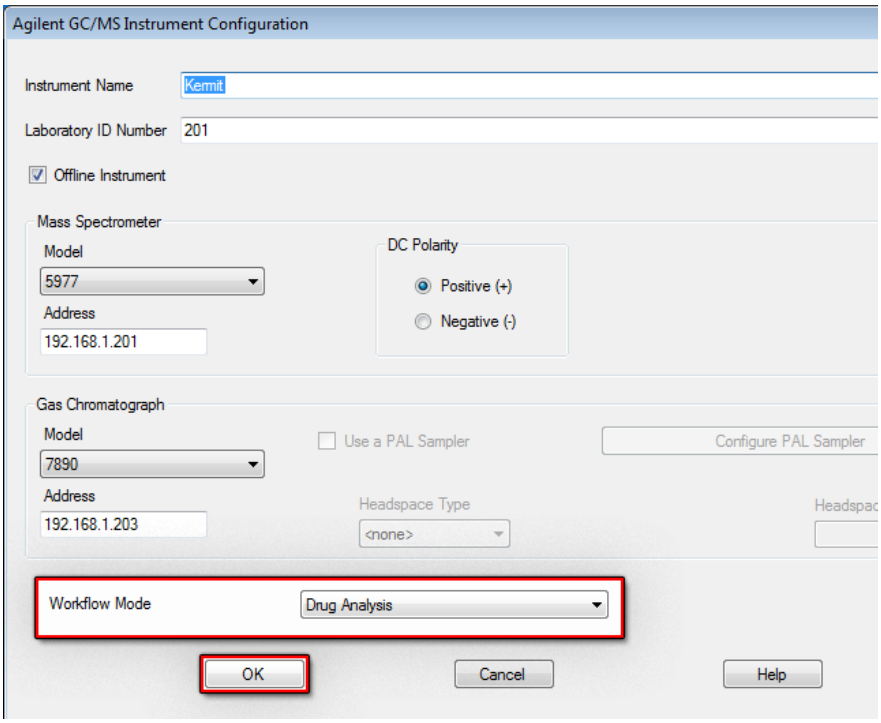
1. Double-click the GCMS Configuration desktop icon to launch the Agilent GC/MS Configuration program.

2. Select the instrument name that you will be running to acquire the data. Instrument **1** is selected in this example.

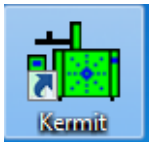


3. Select the **Drug Analysis Workflow Mode** and click **OK** to close the dialog.

   Click **Yes** to confirm the configuration and exit the Agilent GC/MS Configuration program.

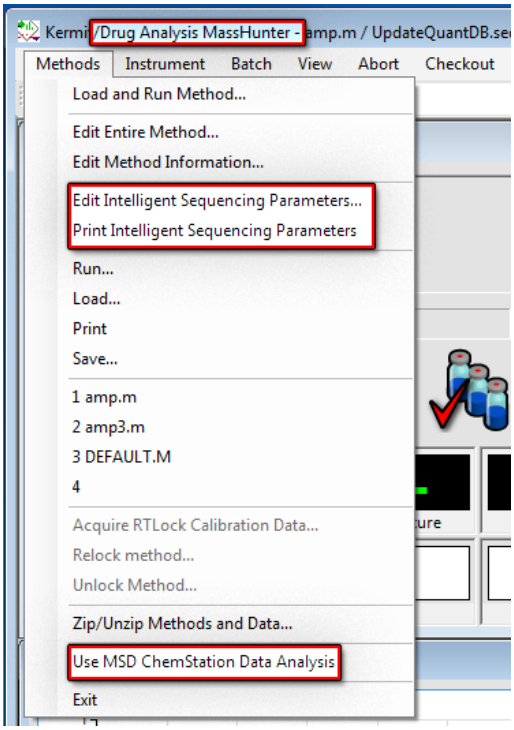


4. Double-click the Instrument icon to launch MassHunter GCMS Acquisition.



5. Notice the items that are unique to the Drug Analysis Workflow mode.

The next time you start MassHunter GCMS Acquisition you will see Drug Analysis in the Title Bar, and under the Methods menu, you will see options for **Intelligent Sequencing Parameters**, and possibly one for **Use MSD ChemStation Data Analysis**.

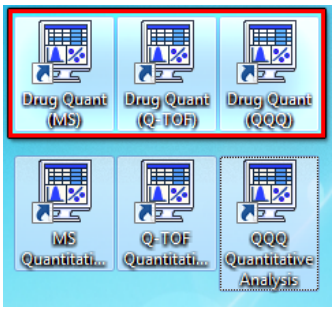


Configure MassHunter Quant for Drug Analysis

Check for the Startup icon

When MassHunter Quantitative Analysis is installed, a group of icons for starting Quantitative Analysis, similar to those shown here, is placed on the windows desktop.

To begin MassHunter Quantitative Analysis you simply double-click on the applicable icon.

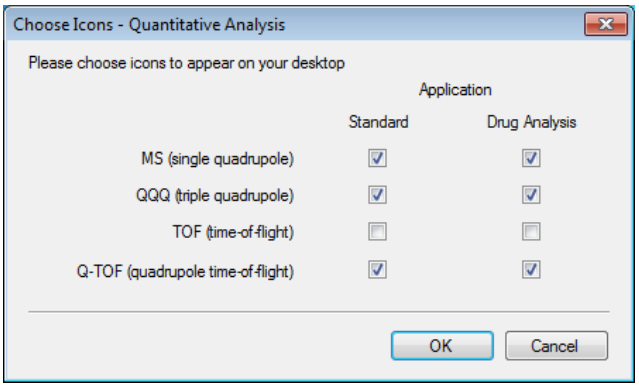


For example, to start a Quantitative Analysis session for Triple Quadrupole Data in the Drug Quant workflow mode you would click the desktop icon labeled **Drug Quant (QQQ)**. The Quant program is then optimized for Triple Quadrupole Data in the Drug Quant workflow mode.

Add a startup icon

1. From the windows Start menu select **Agilent\MassHunter Workstation\Quant Tools\Setup Desktop Icons.**
2. Check the **Drug Analysis** mode for your instrument(s).
3. Click **OK** to close the dialog and add your newly selected startup icon(s) to the windows desktop.

If you do not see a desktop icon labeled **Drug Quant** for your instrument, add it as follows.



In this example, both the Standard and Drug Analysis modes are selected for MS, Triple Quadrupole, and QTOF instruments.

Confirm the Data Analysis program can communicate with Data Acquisition program

This workflow uses:

- MassHunter GCMS Acquisition to acquire data
- MassHunter Quantitative Analysis (with Intelligent Sequencing) for Data Analysis

Intelligent sequencing uses automated processing and feedback between these two programs as it processes each sample. Therefore, be sure that:

MassHunter Quantitative Analysis is installed on the same PC as the MassHunter GCMS Acquisition program that will acquire the data.

If MassHunter Quant is installed on the same PC as the MassHunter GCMS Acquisition program, by default, it is generally configured to be used for data analysis of MassHunter GCMS Acquisition files. However, to be sure, in MassHunter GCMS Acquisition, select **Method** and, if the **Use MSD ChemStation Data Analysis** option is listed in the menu, be sure that it is **not** checked. (This menu item only exists when both MassHunter Quant and MSD ChemStation Data Analysis are installed on the same computer.)

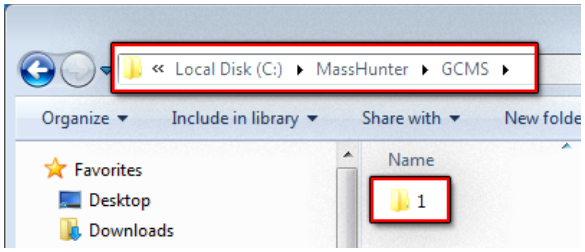
Remember, if this menu item is selected, the system will use MSD ChemStation for data analysis, and for this workflow that is not what you want. This workflow uses MassHunter Quantitative Analysis for Data Analysis.

Understand the Directory Structure

1. Locate the instrument directories.

You can configure and run up to four instruments with MassHunter GCMS Acquisition.

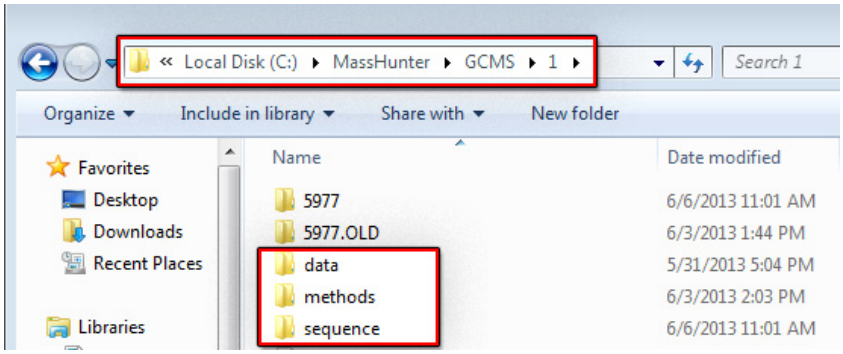
For each instrument you configure, MassHunter GCMS Acquisition will create a numbered directory corresponding to the instrument number (***drive***:**\MassHunter\GCMS\1** for example).



Under each instrument directory (**1** shown here), you will see a default data, methods, and sequence subdirectory, as shown in the next example.

2. Review the default data, methods, and sequence directories.

These are the recommended and default locations for your data, methods, and sequences. Your files can be located here or you can locate these files anywhere that is accessible to these programs.



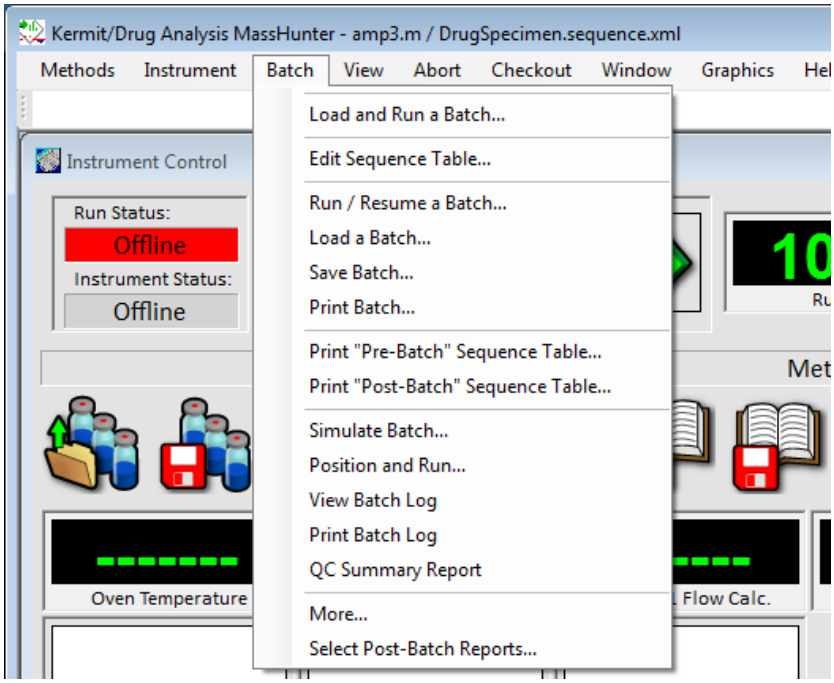
- **The data directory** contains the data from each batch run, stored in an individual directory.
- **The methods directory** contains all of your methods. Each method is stored in its own (.m) subdirectory, which contains all of the files for the method, such as compound lists, calibration tables, reports, etc.
- **The sequence directory** contains all of your sequence files. (The MassHunter GCMS Acquisition format sequence files use the (.sequence.xml) extension.)

Review the Batch Menu

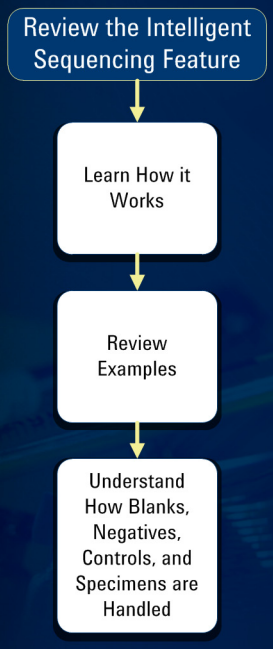
The few menu items that are unique to MassHunter GCMS Acquisition when it is configured in the Drug Analysis Workflow mode are described in this section.

When MassHunter GCMS Acquisition is configured in the Drug Analysis Workflow mode, the **Batch** menu contains the options associated with loading, editing, and running batches and sequences, plus access to Drug Analysis QC reports.

When MassHunter GCMS Acquisition is configured in the Enhanced Workflow mode, this menu is labeled **Sequence**, but contains similar options.



2
About Intelligent Sequencing



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What is Intelligent Sequencing?

Definition - Intelligent Sequencing capabilities provided in the Drug Analysis Workflow mode of MassHunter GCMS Acquisition, automatically adjust the sequence it is running based on the quantitative and qualitative results of each analyzed sample.

How does it work?

Intelligent Sequencing will analyze each sample as it is processed, then, if that analysis does not meet the criteria set for your analysis, the system will perform the corrective actions defined in Intelligent Sequencing. For example it could, reinject the sample, inject a blank, skip to the next batch, or pause the sequence.

Generally speaking, when processing a sequence using the Intelligent Sequence option the following steps occur.

1. The sequence table identifies the samples to be run, what *Type* of samples they are (i.e., Blank, Negative, Control, or Specimen), and what method to use to process each sample.
2. When the sequence begins, the data for the first sample is collected and analyzed.
3. The results of the analyzed sample are compared to the criteria limits you specify in your method.
4. **If the data are within your acceptable limits**, the next sample is processed.
5. **If the data are outside your acceptable limits,** the decisions specified in the Intelligent Sequencing portion of the method are used to continue or pause your batch. Using those instructions, the system may:
   - **a** Inject a blank before continuing with the next sample in the sequence
   - **b** Pause the sequence
   - **c** Re-inject the sample
   - **d** Skip to the next batch
   - **e** Etc...

The criteria you use, and therefore, the decisions Intelligent Sequencing makes, may be based on agency regulations, good laboratory practices, or simply the unique needs of your laboratory.

Where are limits and parameters defined?

Intelligent Sequencing analyzes each of the different sample types in a different way, based on the criteria you specify in the method. When you create your method, these criteria are entered in two places.

- In the Data Analysis portion of the Method you will set the acceptance criteria limits for Internal Standards, Controls, Negatives, and Blanks. See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61.
- In the Data Acquisition portion of the Method you will set the Intelligent Sequencing Parameters as described in the next section.

Intelligent Sequence Parameters

Here you will define how you want the sequence to proceed if an analyzed sample (Blank, Negative, Control, or Specimen) falls outside the acceptable limits you specified.*

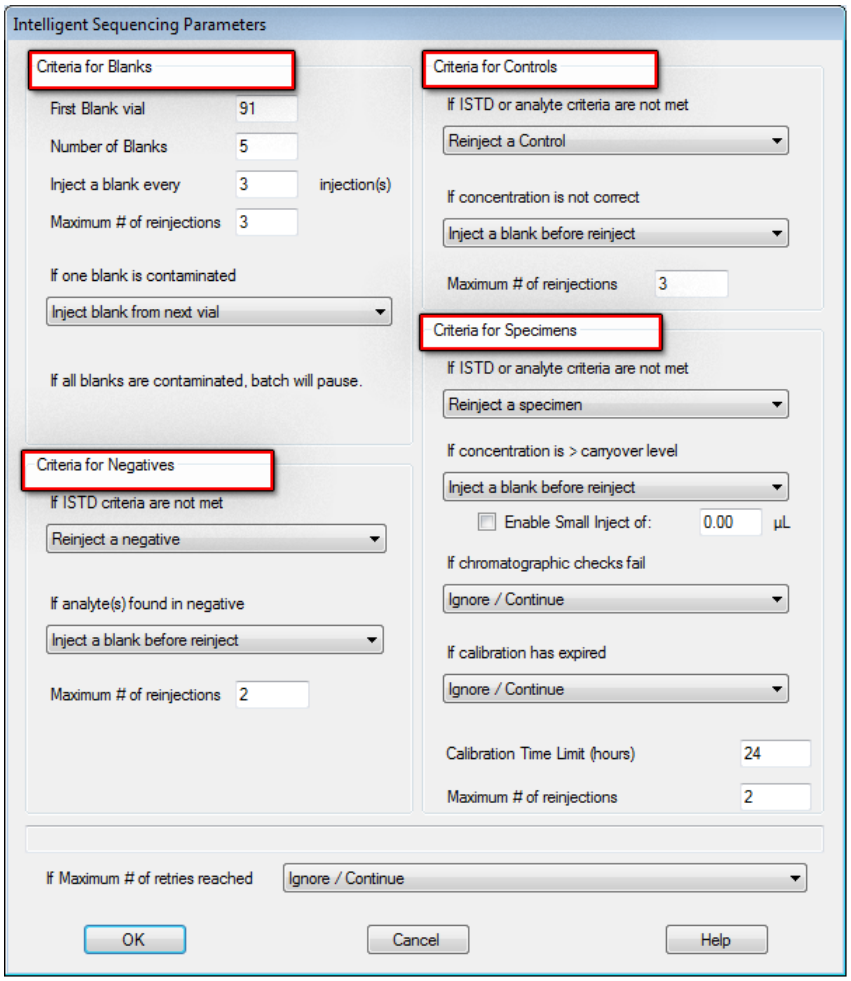
Details for how to complete the entries for each of the 4 sample types, highlighted in this example, are provided on the following pages:

- Blanks (See page 21)
- Negatives (See page 25)
- Controls (See page 25)
- Specimens (See page 31)

You may access this dialog box at any time by selecting **Methods/Intelligent Sequencing Parameters.**

*See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61 for details on how to set up your acceptable limits for these sample types.

This dialog is unique to MassHunter GCMS Acquisition configured for the Drug Analysis Workflow mode.



What actions can be taken by Intelligent Sequencing?

As each sample is analyzed with Intelligent Sequencing, the sample results will be used to modify the next sequence action if some, predefined, criteria are met. The Table below shows the possible sequence modifications that can occur with Intelligent Sequencing.

- If they are within the acceptable limits, the sequence continues.
- If they are outside the acceptable limits, Intelligent Sequencing can take one of the actions shown in the table below.

Acceptable Limits are defined in the Quantitative Data Analysis method. See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61 for details on setting acceptable limits.

Actions to take are defined in the Intelligent Sequencing dialog presented in the Intelligent Sequencing portion of your method. See the pages listed below for more details on completing the entries for each of these 4 sample types:

- Blanks (See page 20)
- Negatives (See page 24)
- Controls (See page 24)
- Specimens (See page 30)

	Sample type			
Actions available based on the sample type	**Negative**	**Control**	**Specimen**	**Blank**
Reinject ... Reinjects the Negative, Control, or Specimen and then tests those results before continuing.	✓	✓	✓	
Inject a blank before reinject Injects a blank before the system reinjects the specimen.	✓	✓	✓	
Ignore/Continue Allows the system to ignore the result and proceed with remainder of batch sequence.	✓	✓	✓	
Jump to Next Batch Jumps to the next line in the Sequence Table labeled with the keyword **NewBatch.** If there is none, the batch will pause.	✓	✓	✓	
Inject a blank before continuing Ensures that the GC/MS system is flushed with a blank before it injects the next vial.			✓	
Pause batch Pauses and waits for operator intervention either to end or continue the sequence. The injection counter for blanks is reset whenever a batch is paused.	✓	✓	✓ *	✓
Inject blank from next vial Injects a blank from the next blank vial position and continues with remainder of the sequence.				✓

* When calibration for a specimen has expired, the batch can be paused.

What are some examples of how Intelligent Sequencing can be used?

Intelligent Sequencing allows quality guidelines to be imposed without operator input. Here are a few examples.

Example 1: Carryover Limit Exceeded

Instead of having an operator examine every injection as it occurs and manually injecting a blank when needed, you may set the criteria which specifies:

> *If a specimen is found to contain a drug above the carryover limit, automatically inject a blank before continuing with the next sample in the Sequence Table.*

This can save you valuable laboratory time by:

- not having to re-extract specimens contaminated by carryover
- not having to rerun specimens contaminated by carryover
- allowing the instrument operator to perform other duties

Example 2: Drug Found in the Negative

With Intelligent Sequencing if a drug of interest is found in the negative sample, the batch will be stopped early and corrective action can be taken before the bad negative invades the entire batch.

Consider the case when a negative is found to contain the drug of interest above the maximum concentration allowed for negatives. If Intelligent Sequencing is not used in this situation (and the instrument operator is not present to make the appropriate decision), then the batch will continue to run. The bad negative will invalidate the entire batch. Thus, all samples in the batch sequence will have to be reextracted and rerun. In this case you may set Intelligent Sequencing criteria to:

> *"Pause if the drug of interest is found in the negative."*

Important Considerations

Intelligent Sequencing decisions need to be set with care. If, for example, a negative is found to contain the drug of interest, and the decision criteria are set to "inject a blank before reinjection" and the number of reinjections is set to 9, the run may be as follows:

- original injection of the negative,
- a blank injection (because drug was found in the negative),
- a reinjection of the negative (because you set Intelligent Sequencing to re-inject),
- followed by a blank injection (if the re-injected negative still contained drug)
- and a negative rejection (if each re-injected negative contained drug)

This run would be repeated 8 more times for a total of 19 injections, wasting valuable time. Thus, it is important to set the Intelligent Sequencing decisions very carefully.

How are Blanks handled?

Definition of a Blank Sample Type

For DrugQuant, a Blank is a sample that does not contain any analytes or ISTD. A Blank is generally made up of a solvent and does not normally undergo the same extraction and preparation procedures as the other sample types. This sample type can, for example, be used in a sequence between valid specimens to evaluate carry-over contamination from a previous sample, or to flush the system before injecting another sample.

You can physically place a blank sample in any tray of the autosampler, or you can use the Intelligent Sequencing feature which injects a blank at your predefined settings.

How Intelligent Sequencing Processes Blanks

Before processing any sample, the system first checks to see if it is time to inject a blank. If the injection count indicates it is time to inject a blank, the blank is injected and processed before this sample is processed. If the count is below the blank inject value the system injects the next sample and then increments the blank injection counter.

When a Blank is injected, data are acquired by the MSD and sent to MassHunter Quantitative Analysis for analysis.

MassHunter Quantitative Analysis checks the analysis to verify the results are within your Blank sample acceptance criteria. For a Blank, this includes:

- Finding a specified target compound in a concentration above what is specified in the Carryover Amount outlier

  Or

- Finding a specified ISTD in a concentration greater than what is specified in the Blank Response outlier

See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61.

If the analysis shows that the results are within your specified limits:

a The Blank cycle counter is initialized. This counter is used by Intelligent Sequencing to keep track of the number of non-blank injections allowed before automatically injecting a blank.

b The next sample in the batch is processed.

If the analysis indicates that the results are outside your specified limits, the system processes the sample based on the parameters you set in the Intelligent Sequencing parameters dialog (shown below).

Figure 1 on page 23 shows a decision flow chart for a Blank sample.

Intelligent Sequencing Parameters for Blanks

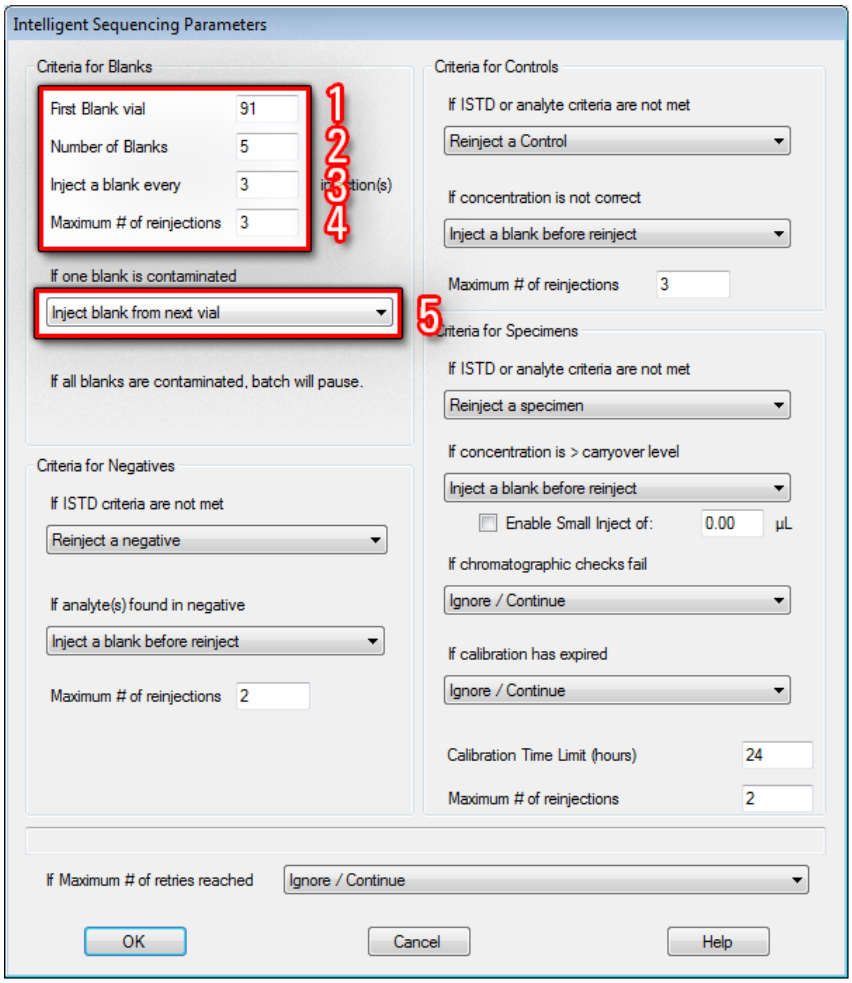
Highlighted fields are described below.

1. Where the first Blank vial is located in your autosampler tray. (Position **91** in this case.)
2. How many contiguous Blank vials you have on the tray (1-10). (This example is showing **5**. So locations 91 through 95 contain Blank vials.)
3. When you want a Blank injected. (***After every 3*** injections, in this example.) See "Example – Effects of resetting the Blank counter" on page 22 for more details.)
4. The number of times the system can consecutively reinject a Blank when the blank sample analysis is out of spec (0-9). (This example shows **3**.)
5. What to do if one blank is contaminated. Options include:

   Pause batch - Waits for operator intervention.*
   Inject blank from next vial - Gets the next Blank, makes he injection and does the analysis once again. If there are no more Blank vials, the batch is paused and the system waits for operator intervention.

The first group box in the Intelligent Sequencing dialog is dedicated to setting the Intelligent Sequencing parameters for Blanks. You may access this dialog in MassHunter GCMS Acquisition through the **Edit Entire Method** option, or by selecting **Methods/Edit Intelligent Sequencing Parameters.**

Here you will define the Blank vial locations and capacity, the default frequency of blank injection and the actions of the sequence if the blank is contaminated.



Criteria defining what constitutes a Contaminated Blank are set in the Data Analysis portion of the method. See "Step 4: Complete the Intelligent Sequencing parameters" on page 42 for details.

* The injection counter for Blanks is reset whenever a batch is paused.

Automatic reset of the Blank cycle counter

Be aware:

- The Blank cycle counter resets to zero after ANY Blank is run.
  Blanks may be injected at times other than those specified by the default frequency. For example, you may have Intelligent Sequencing inject a blank automatically when your criteria are not met for Negatives, Controls, and Specimens. Also, you may choose to physically place blanks in any location in the autosampler tray. When these blanks are processed, similar to when a blank is injected based on the default frequency setting, the blank cycle counter resets back to zero. See"How Intelligent Sequencing Processes Negative and Control Samples" on page 24, and "How Intelligent Sequencing Processes Specimens" on page 30 for more details.
- Paused batches ALWAYS reset the Blank counter to zero.

Example – Effects of resetting the Blank counter

If this field is set to 4, and there is a specimen with signs of **carryover in your batch**, the Blank counter resets to zero after every 4 injections, plus after the sample that shows carryover, and as shown below.

1 Sample (1)
2 Sample (2)
3 Sample (3)
4 Sample (4)
5 Blank (runs automatically after 4 injections)

Blank counter resets to zero

1 Sample (5)
2 Carryover Sample (6) (Causes a Blank to be run because of the criteria you set.)
3 Blank

Blank counter resets to zero

1 Sample (7)
2 Sample (8)
3 Sample (9)
4 Sample (10)
5 Blank (runs automatically after 4 injections)

This process occurs whenever a black is run. A Blank is run at two times:

- When Intelligent Sequencing says it is time to run a Blank
- When the Sequence Table identifies the sample being run as a Blank.

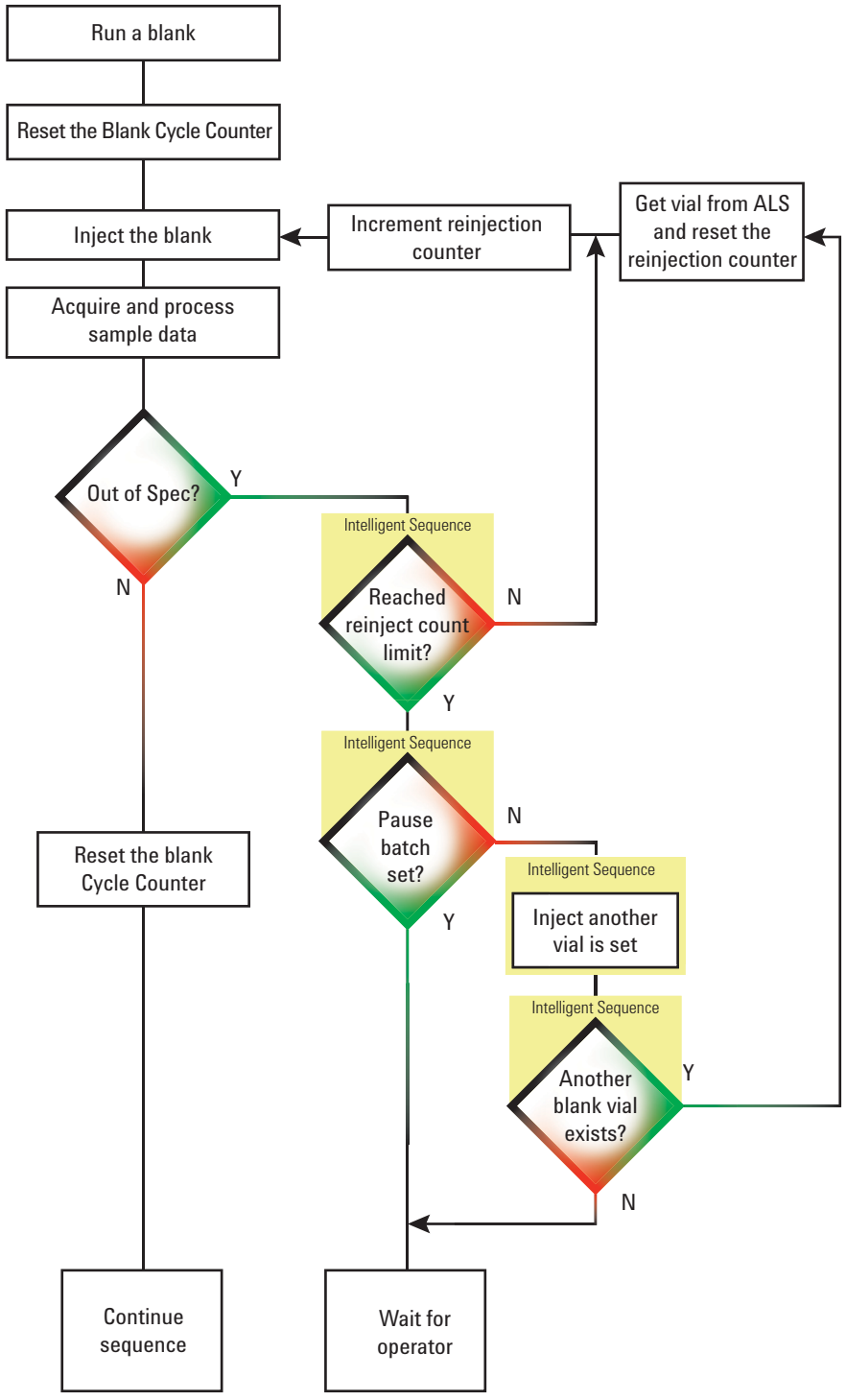


Figure 1 Blank Sample Processing Using Intelligent Sequencing

How are Negatives and Controls handled?

Definitions

A Negative Sample is a sample that contains an internal standard but no drug of interest (target compound). This sample type is generally prepared by the adding an internal standard to a clean sample matrix (urine, plasma). The negative sample goes through the same extraction and preparation procedures as all other sample types in the same batch sequence.

A Control Sample is a clean sample matrix (urine, plasma, etc.) that has been spiked with known amounts of the drug of interest and internal standard. A Control sample is a sample used in the batch to determine the integrity of the calibration and the performance of the instrument.

How Intelligent Sequencing Processes Negative and Control Samples

Before processing any sample, the system first checks to see if it is time to inject a blank. If the injection Blank cycle counter indicates it is time to inject a blank, the blank is injected and processed before this sample is processed. If the count is below the blank inject value the system injects the next sample and then increments the Blank cycle counter.

When the system processes a sample labeled as a **Negative** or a **Control**, data is acquired from the GC/MS Instrument and sent to MassHunter Quantitative Analysis for analysis. For Negative and Control samples, the processed results are checked for two things, the amount of:

- ISTD in the sample
- Drug compound ID (ion ratios) and amount in the sample

If the criteria are within your specified limits as set in your method, the system continues as usual with the next sample in the Sequence. See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61.

If the criteria are outside your specified limits, the system processes the sample based on the parameters you set in the Intelligent Sequencing Parameters dialog (shown below).

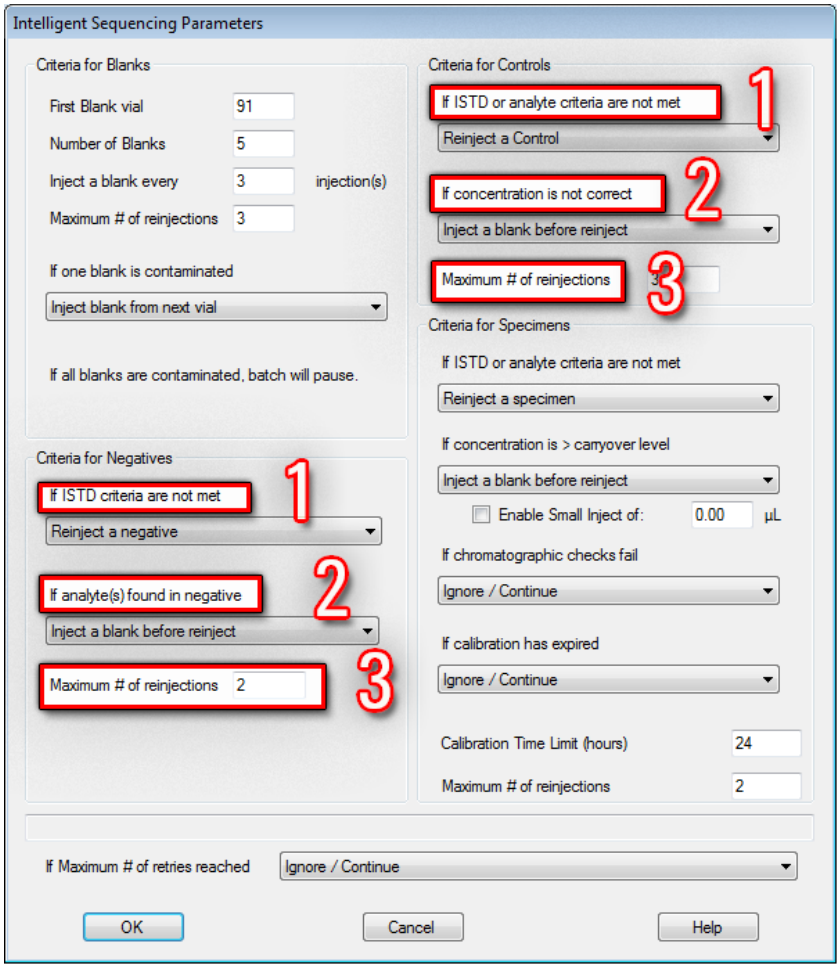
Figure 2 on page 26 shows a flow chart of this processing.

Intelligent Sequencing Parameters for Negatives and Controls

In the **Criteria for Negatives** group box and the **Criteria for Controls** group box, you will select from dropdown lists to define what actions should be taken if the sample was shown to contain Internal Standard or Analyte(s) outside the limits you specified in your method*.

There are three areas to complete in each group. The options listed for each are shown below.

*Acceptable and unacceptable limits for Internal Standards and Analytes are set in the Data Analysis portion of the method. See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61.



Criteria for Negatives	Criteria for Controls	Options include
1 If ISTD criteria are not met	1 If ISTD or analyte criteria are not met	**Reinject a negative (or control).** Reinjects the sample from the same vial. **Pause batch.** Waits for operator intervention to resolve the problem. **Ignore/Continue.** Ignores the result and proceeds with the remainder of the batch sequence. **Jump to Next Batch.** Jumps to the next line in the Sequence Table labeled with the keyword **NewBatch.** If there is none, the batch will pause.
2 If analytes are found in the Negative	2 If concentration is not correct	**The same** as those specified for the **ISTD**, *plus* **Inject a blank before reinject.** A blank is injected to remove contamination and then the sample is injected again and analyzed.
3 Maximum # of reinjections	3 Maximum # of reinjections	Here you will identify the maximum number of reinjections to make from this vial. That is, how many times you would like to re-inject and retest this sample. The range is 0 – 9. **If the maximum number is reached**, Intelligent Sequencing will continue based on the criteria you specify in the last field in this dialog box, labeled If **Maximum # of retries reached**.

This process occurs whenever a sample labeled as a negative or control is run.

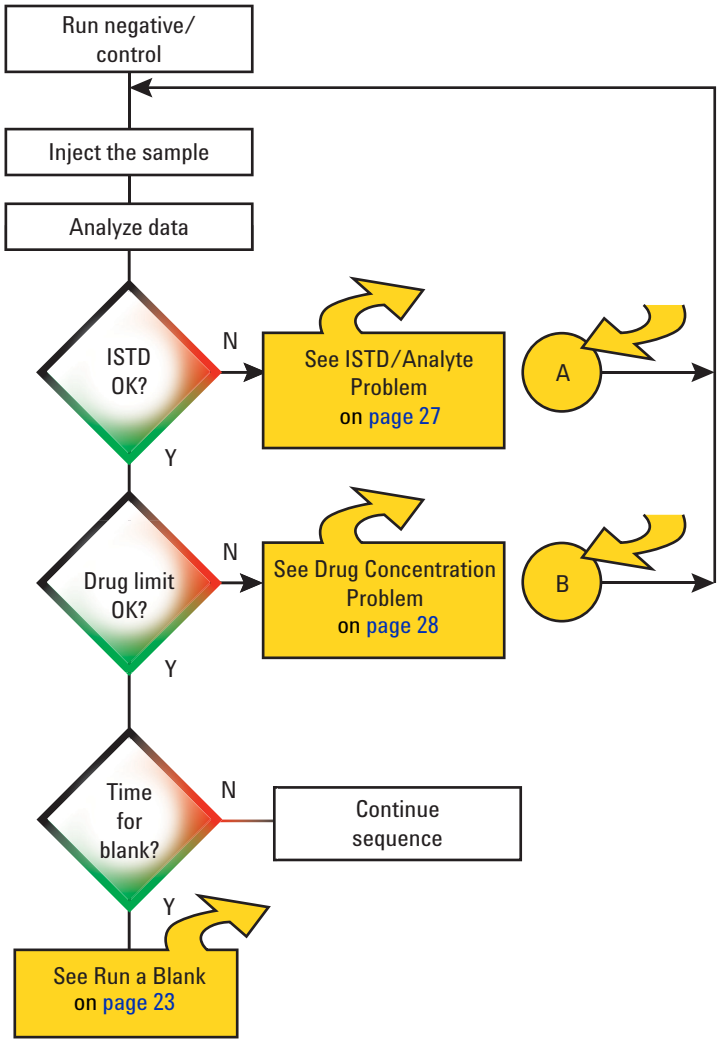


Figure 2 Negative and Control Sample Processing Using Intelligent Sequencing

This occurs whenever the internal standard is found to be outside your acceptable limits in a negative or control sample. The beginning of this process is shown in Figure 2 on page 26.

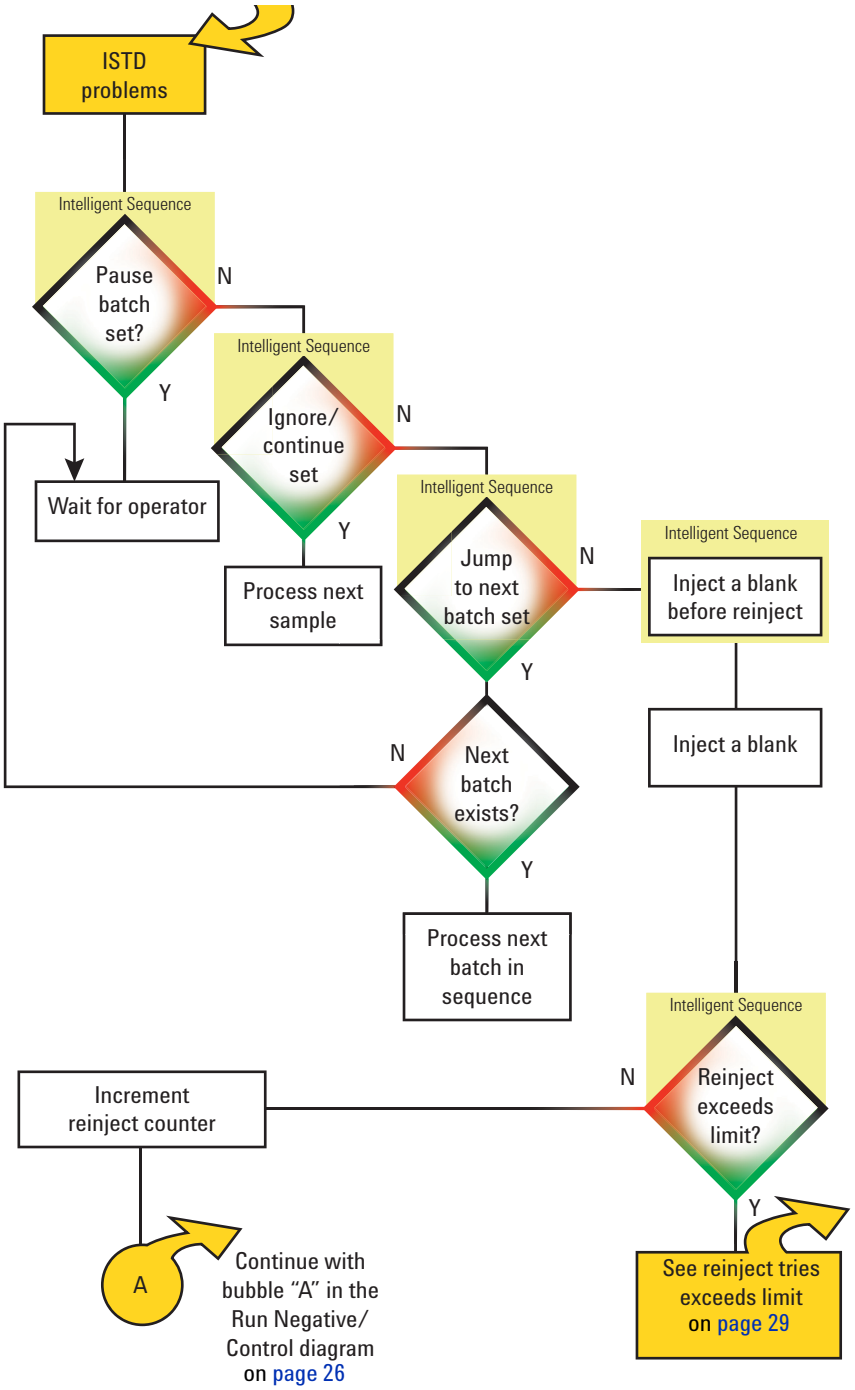


Figure 3 ISTD/analyte processing using Intelligent Sequencing

This occurs whenever the drug concentration is found to be outside your acceptable limits in a negative or control sample. The beginning of this process is shown in Figure 2 on page 26.

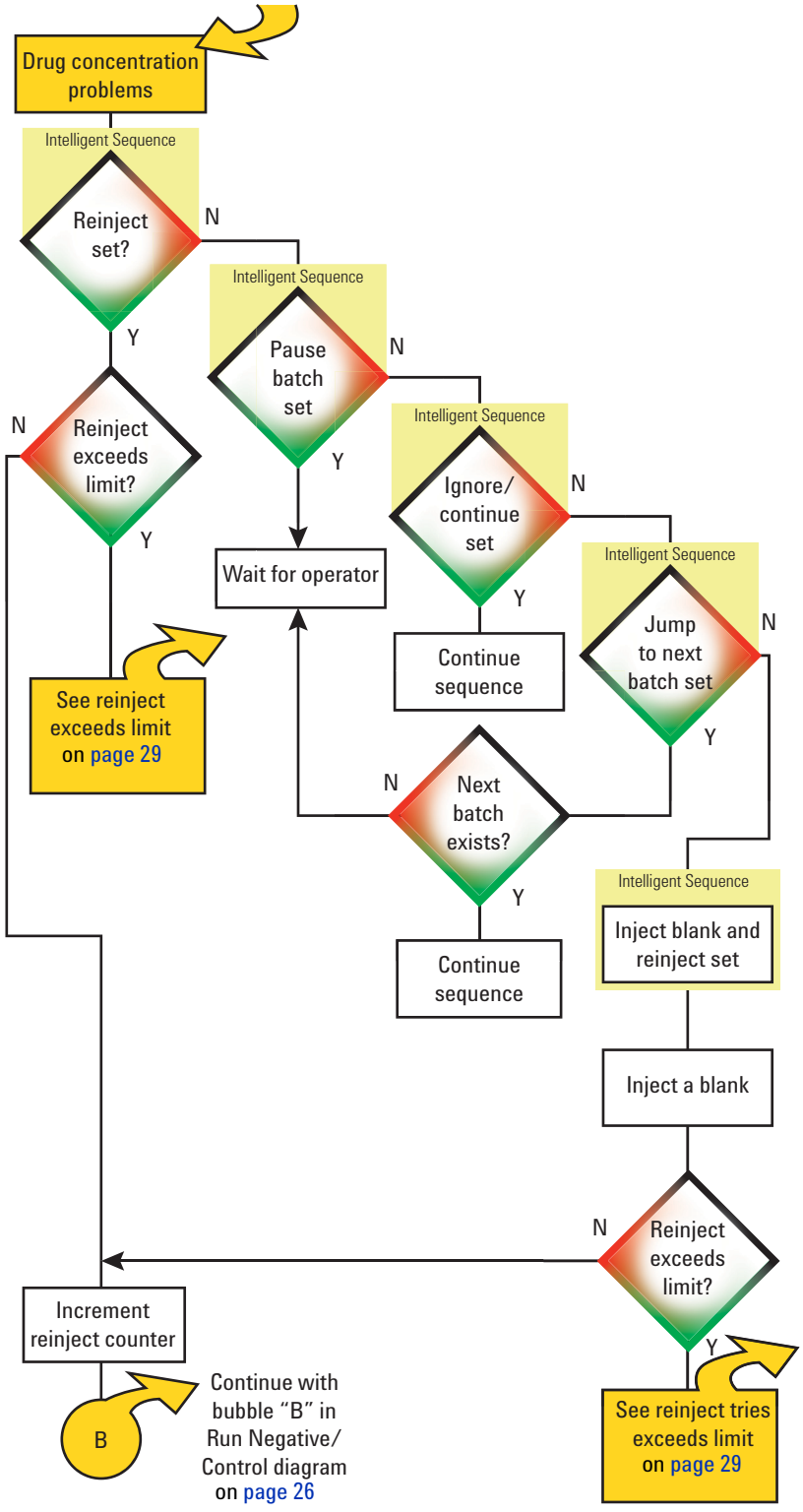


Figure 4 Drug concentration problem processing using Intelligent Sequencing

This process occurs whenever Intelligent Sequencing has specified to reinject a sample but there are no more reinjections available to process. This can happen when, for example, there are no more blank vials available for processing.

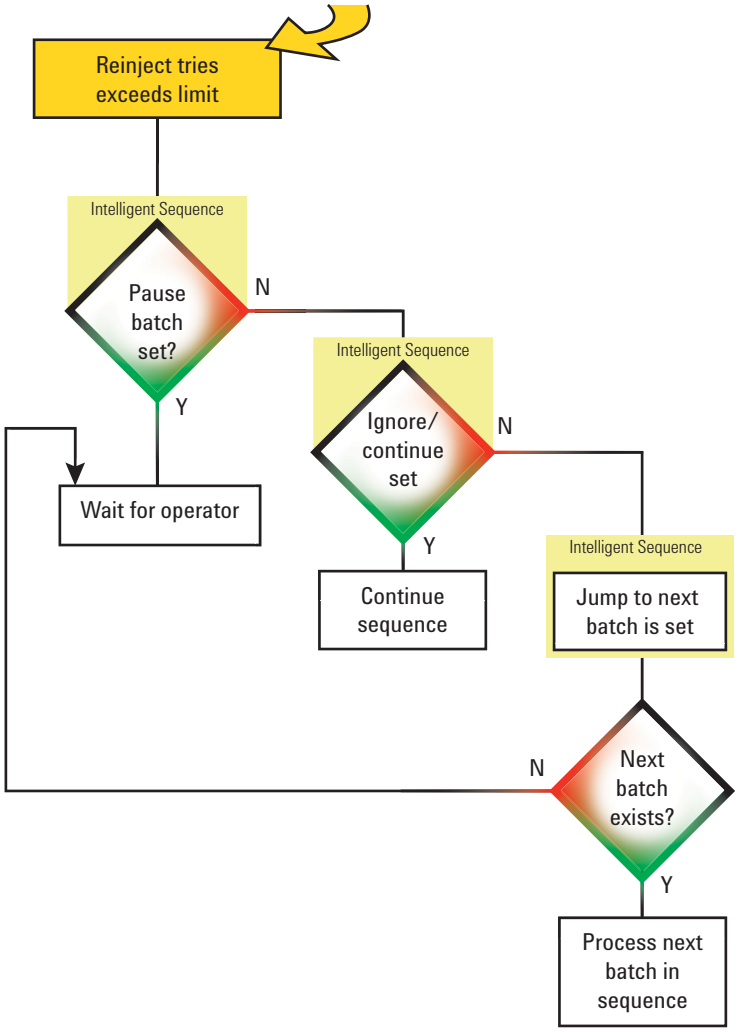


Figure 5 Reinject problem processing using Intelligent Sequencing

How are Specimens handled?

Definition of a Specimen Sample Type

A Specimen sample type is a sample to be analyzed for the drug of interest.

An Internal Standard is generally spiked into the specimen and then the specimen undergoes the same extraction and preparation procedures as the other samples in the batch.

Identification of the drug of interest and the internal standard is based upon ion ratios and retention times being within your specified limits. If identification criteria are met, then the concentration of the drug of interest is calculated based on the internal standard recovery.

How Intelligent Sequencing Processes Specimens

When the system processes a sample labeled as a Specimen, data is acquired from the GC/MS system and sent to MassHunter Quantitative Analysis for processing. For a Specimen sample MassHunter GCMS Acquisition checks the analysis for a number of items.

Checks the analysis to see if the ISTD is within the specified tolerance and the drug criteria is met. If the ISTD is outside the limits you specified in your method, the system processes the sample as described in the Intelligent Sequencing Parameters settings. Figure 7 on page 33 shows a flow chart of this process.

Checks to see if the concentration of drug is greater than the carryover level allowed. The concentration is compared to maximum allowable concentration entered for the method. If it is greater than this value, it proceeds as specified in the Intelligent Sequencing parameters. Figure 8 on page 34 shows a flow chart of this process.

After checking the concentration level, the system checks the **Chromatographic parameters** in the method for minimum signal to noise, resolution, fronting, tailing, and minimum/maximum peak width. If any of these parameters are out of your specified limits the system proceeds as specified in the Intelligent Sequencing parameters. Figure 9 on page 35 shows a flow chart of this process.

Then the system checks to see if **Calibration time** limit in hours has expired according to limit set in Intelligent Sequencing Criteria for Specimens. Figure 10 on page 36 shows a flow chart of this process.

Finally it checks the **Maximum # of reinjection**s permitted. The specimen sample can only be reinjected the specified number of times set in Intelligent Sequencing Criteria for Specimens. Once that number of reinjections is reached, the system will proceed as specified in Intelligent Sequencing parameters. Figure 5 on page 29.

Intelligent Sequencing Parameters for Specimens

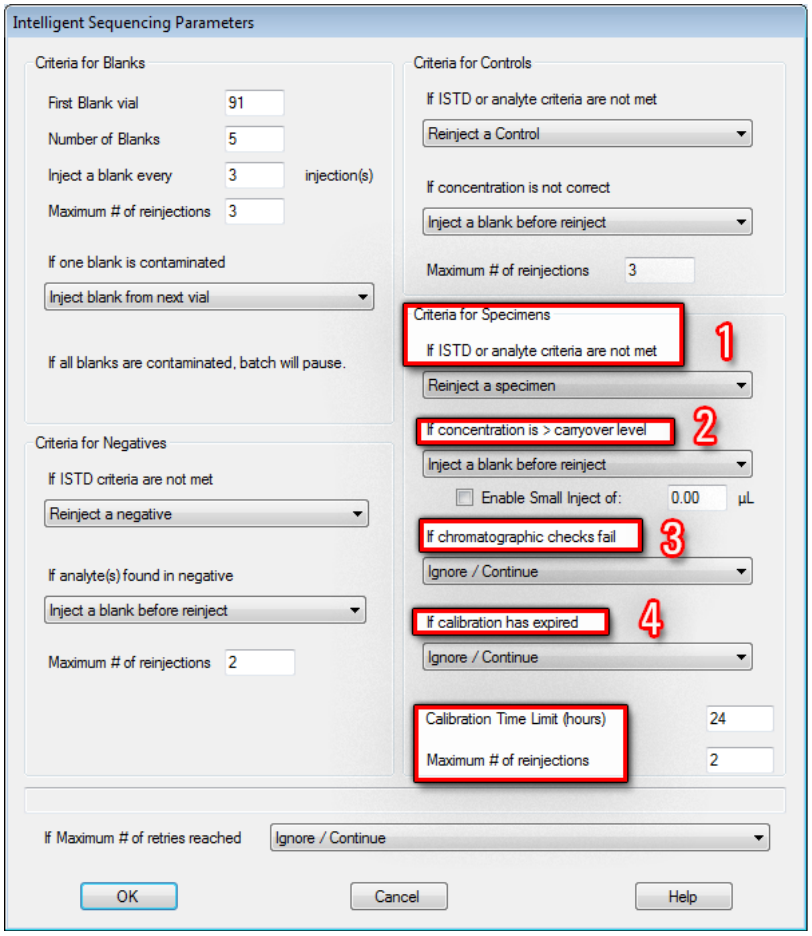
There are 6 areas to complete here. The options for sections 1, 2, 3, and 4 are shown in the table below.

In the **Calibration Time Limit (hours)** box, enter the maximum calibration time limit, in hours. Calibrations that exceed this number of hours are considered expired. (-1 disables this parameter.) This example shows **24**. Enter what is appropriate for your method.

In the **Maximum # of reinjections** box, enter the number of reinjections to allow from this vial. That is, how many times you would allow the system to re-inject and retest this sample. The range is 0 to 9. This example show **2**. Yours may be different, depending on your method.

*Acceptable and unacceptable limits for internal standards and analytes are set in the Data Analysis portion of the method. See "Step 7: Set up outliers for intelligent sequencing decisions" on page 61.

In the Criteria for Specimens group box you will select from dropdown lists to define what Intelligent Sequencing should do when the results of a Specimen, do not meet your criteria.*



Options include	1 ISTD or analyte criteria are not met	2 Concentration is > carryover level	3 Chromatographic checks fail	4 Calibration has expired
Ignore/Continue Ignores the result and proceeds with the remainder of the batch sequence.	✓	✓	✓	✓
Reinject the specimen Reinjects the s from the same vial.	✓		✓	
Pause batch Waits for operator intervention to resolve the problem.				✓
Jump to Next Batch Jumps to the next line in the Sequence Table labeled with the keyword **NewBatch.** If there is none, the batch will pause.				✓
Inject a blank before reinject A blank is injected to remove contamination and then the sample is injected again and analyzed.		✓		
Inject a blank before continuing A blank is injected to remove contamination and then the next sample in the batch is injected and analyzed.		✓		

This process occurs each time a specimen is processed.

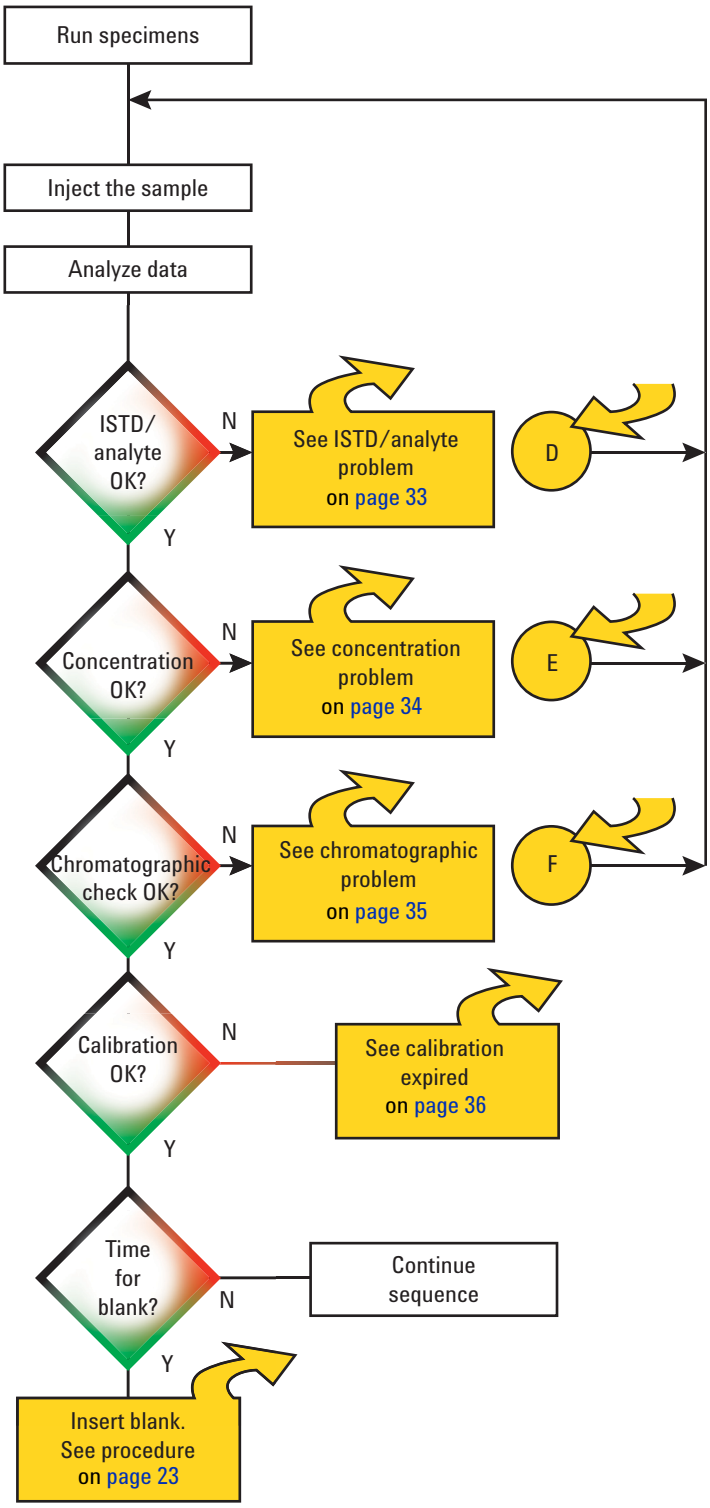


Figure 6 Specimen Sample Processing Using Intelligent Sequencing

This process occurs when an internal standard or analyte found in a specimen is outside your acceptable limits.

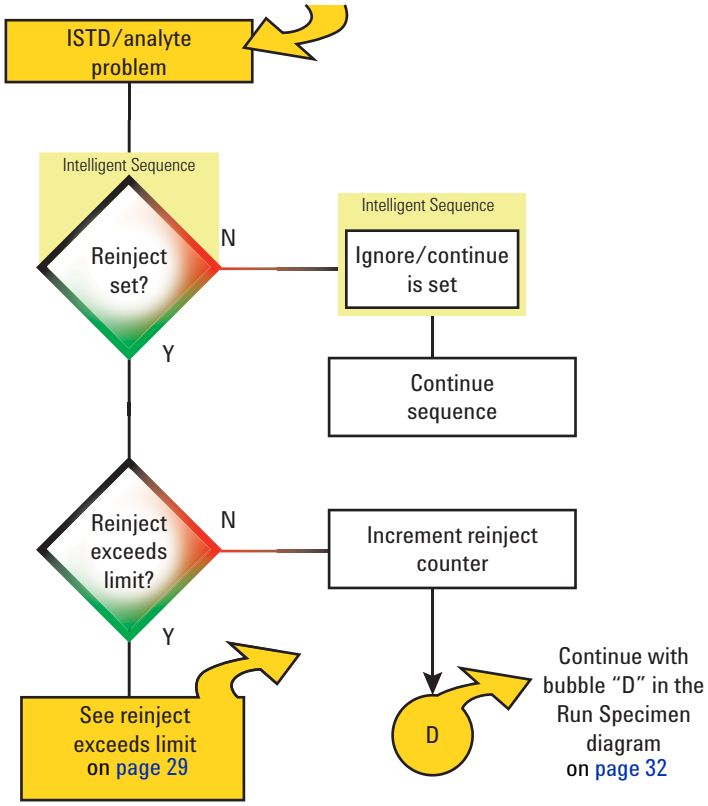


Figure 7 ISTD/analyte processing using Intelligent Sequencing

This process occurs when the concentration of analyte found in a specimen is outside the acceptable carryover concentration range.

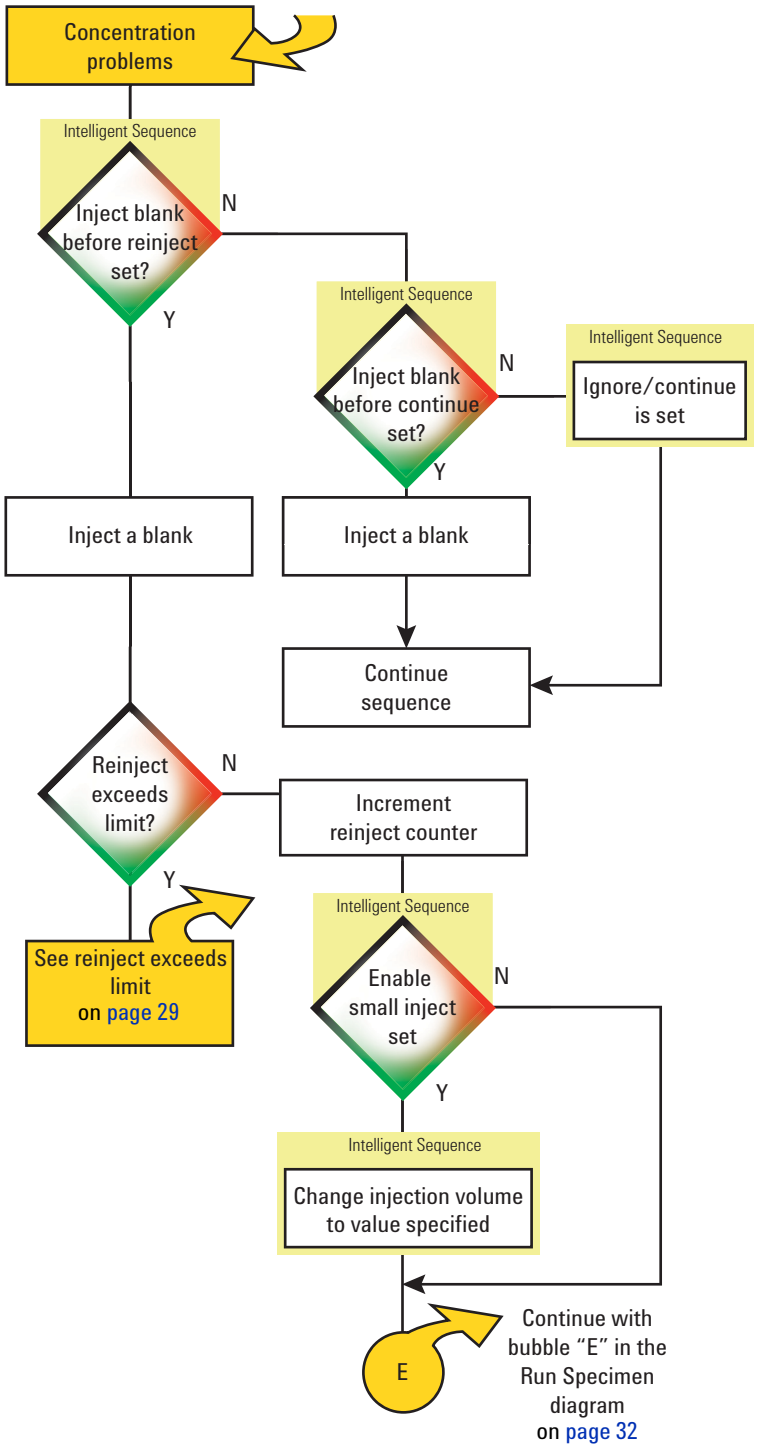


Figure 8 Drug concentration problem processing using Intelligent Sequencing

This process occurs when the signal to noise, peak resolution, peak symmetry, or peak width of a chromatogram for a specimen are outside your acceptable limits.

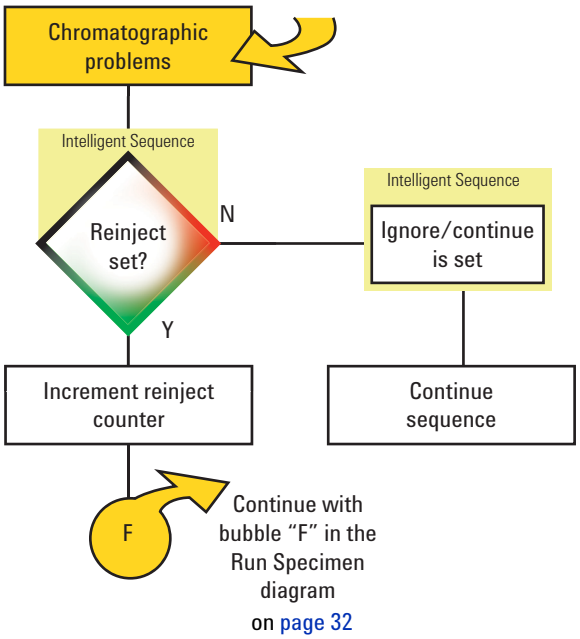


Figure 9 Chromatographic problem processing using Intelligent Sequencing

This process occurs if, when running a specimen, the system recognizes the calibration has expired.

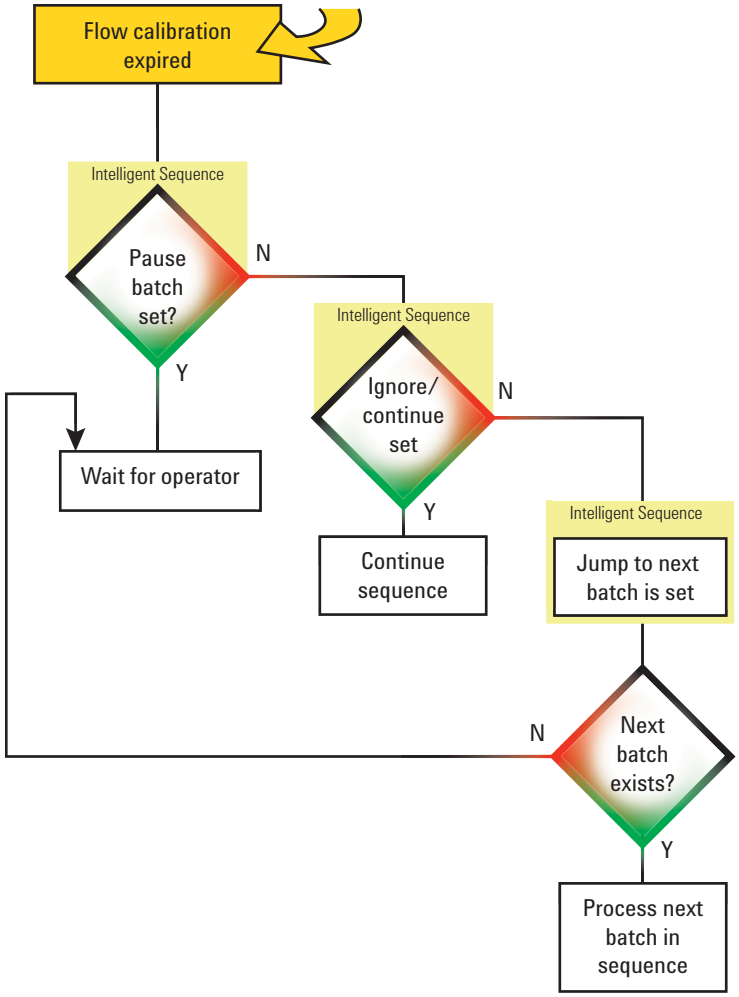


Figure 10 Calibration expired problem processing using Intelligent Sequencing

Maximum # of Retries

The last box on the Intelligent Sequencing Parameters dialog is the **Maximum # of retries reached** box.

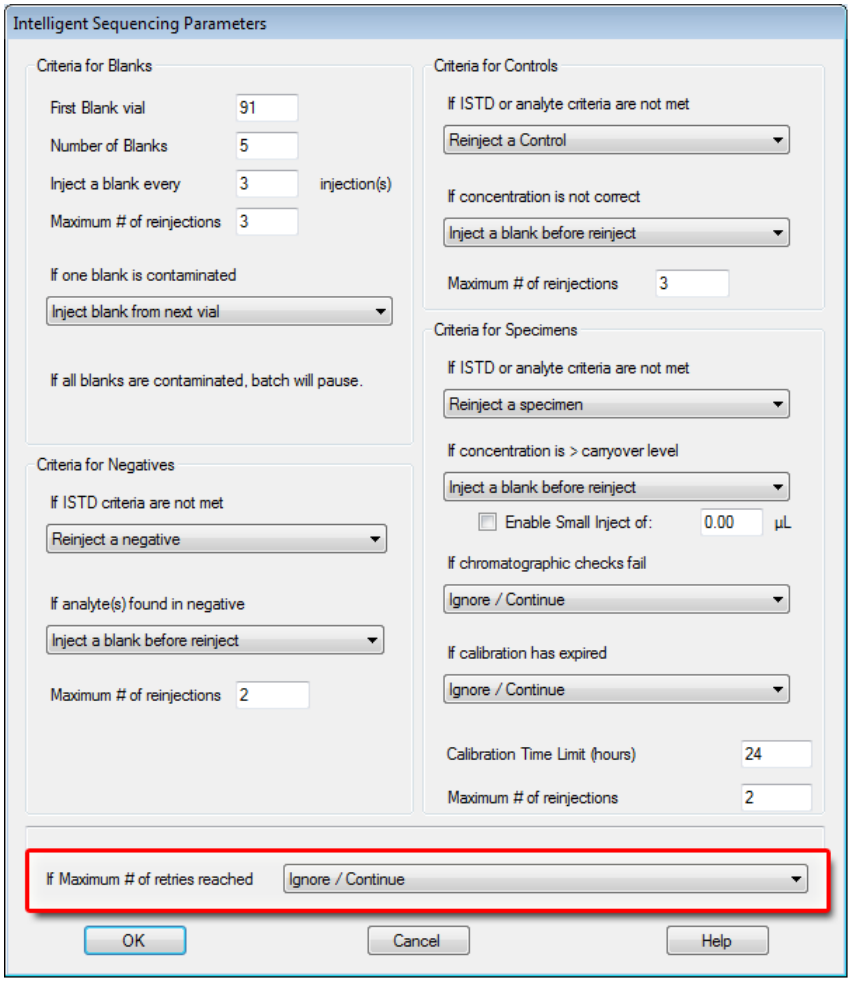
Here you will define what to do when any of the **"Maximum # of reinjections"** options is reached for:

- Blanks
- Negatives
- Controls
- Specimens

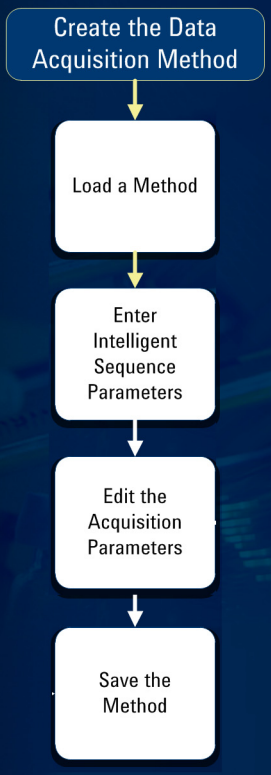
From the drop-down list box, select:

- **Ignore/continue** – which allows the system to ignore the result and proceed with the remainder of the batch sequence.
- **Pause Batch** – which pauses the batch and waits for manual intervention either to end or continue the sequence.
- **Jump to Next Batch** – Jumps to the next batch. The next batch MUST have been specified by the NewBatch keyword in the Sample Log Table, otherwise the batch will pause.

See Figure 5 on page 29 for a flow diagram of this process.



3
Create the Data Acquisition Method



Agilent Technologies

Step 1: Load a data acquisition method

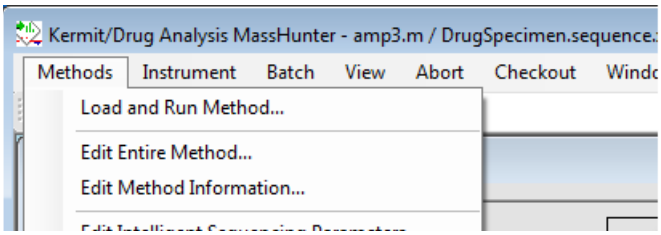
The following procedure describes how to create a SIM data acquisition method for acquiring calibration sample data. Here we are assuming that you have previously created and optimized a scan method with good chromatographic properties that identifies the drugs target and qualifier ions and their ratios. We will use this method as a starting point and convert it to a SIM method along the way.

1. From the Instrument Control view menu select **Methods/ Load and Run Method...** then navigate to the optimized scan method created for your instrument, and select it.
2. Click **OK.** to close the dialog.

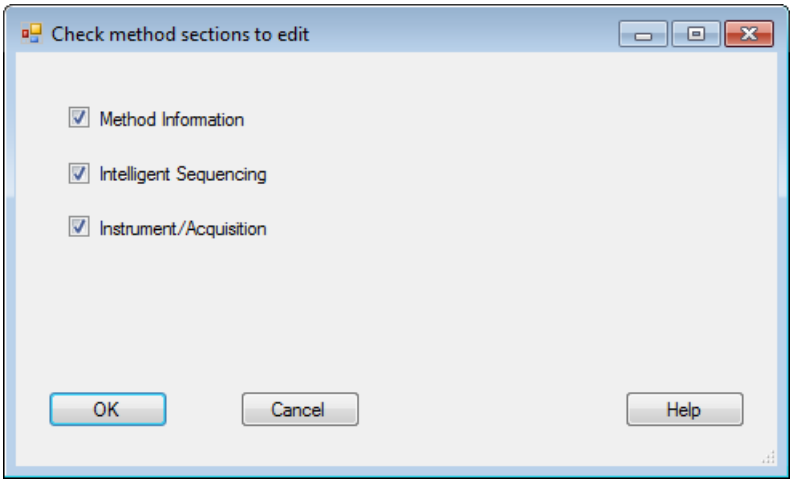
Step 2: Select the parts of the method to edit

During this process we will cover every part of a data acquisition method.

1. Select **Methods/Edit Entire Method**.



2. Check each item listed.

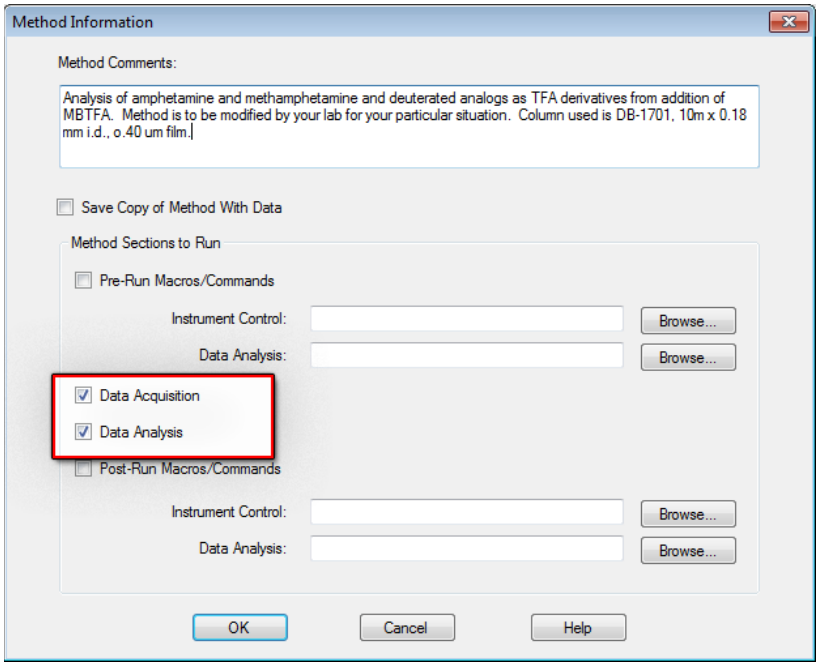


3. Click **OK** to display the Method Information dialog.

Step 3: Describe the method and where it is saved

1. Provide a description of the method in **Method Comments**.
2. Decide whether or not to save a copy of this method in the batch data folder.
3. Select **Data Acquisition** and **Data Analysis** for the run. Although the MassHunter Quantitative Analysis method does not yet exist, you will want to run the data analysis portion of the method when it is available.
4. Click **OK** to display the Intelligent Sequencing Parameters dialog.

This is the same for all MassHunter Modes, and is described in the MassHunter Familiarization guide found in online Help. Please refer to the Familiarization guide for more details.



Note – The Data Analysis method cannot be edited in the Data Acquisition program. The data analysis method can only be created or edited in the MassHunter Quantitative Analysis program.

Step 4: Complete the Intelligent Sequencing parameters

You may access this dialog box at any time by selecting **Methods/Edit Intelligent Sequencing Parameters**.

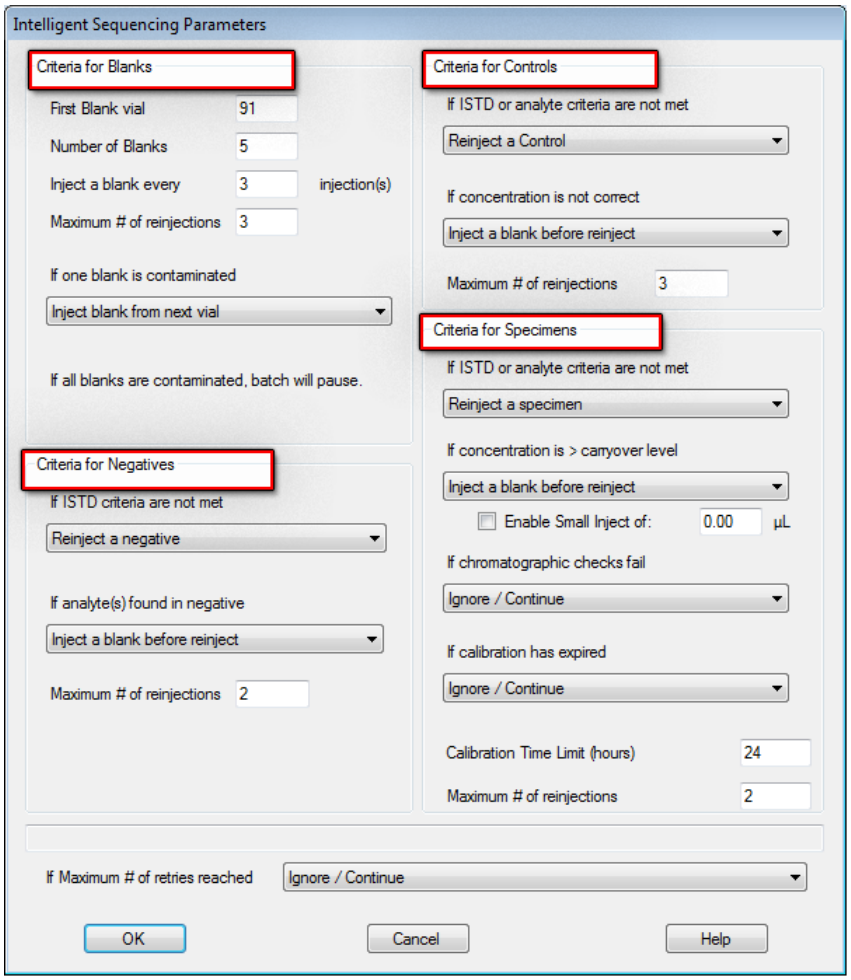
Intelligent Sequencing is unique to the MassHunter Drug Analysis Workflow mode. You will not see this dialog box unless you are running MassHunter GCMS Acquisition in the Drug Analysis Workflow mode.

Here you will define how you want the sequence to proceed if an analyzed sample (Blank, Negative, Control, or Specimen) falls outside the acceptable limits you specified.* Details for how to complete the entries for each of the 4 sample types are provided on the pages listed below:

- Blanks (See page 20)
- Negatives (See page 24)
- Controls (See page 24)
- Specimens (See page 30)

*For details on how to set up your acceptable limits for these sample types, see "Step 7: Set up outliers for intelligent sequencing decisions" on page 61.

When you have completed these parameters, click **OK** to display the Inlet and Injection Parameters dialog.



Step 5: Complete the Standard Instrument Acquisition dialogs

Edit the remaining Instrument Acquisition parameters. The remaining 5 dialogs in the Data Acquisition portion of the method are completed in the exactly the same way for all Work flow Modes (i.e., Enhanced, Drug Quant, Gasoline, etc.), and are described in detail the MassHunter Familiarization guide and in online Help. Please refer to that documentation for more details on those dialogs. Each time you complete a dialog's entries and click OK the next dialog is opened for edit.

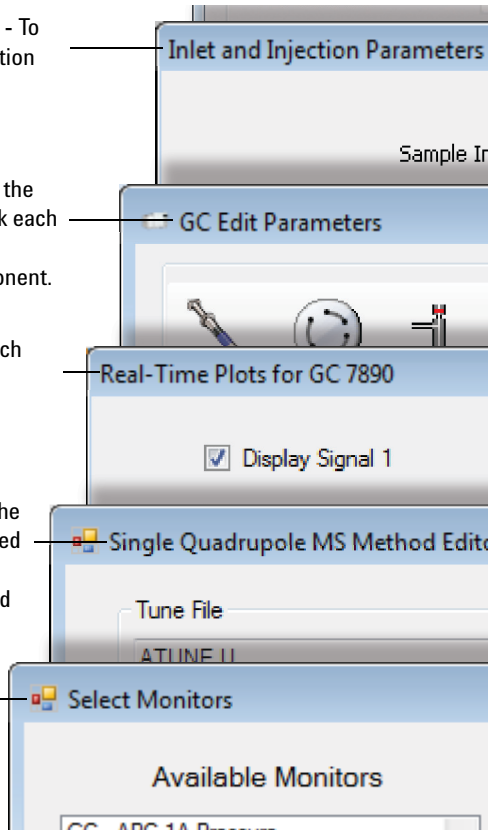
Inlet and Injection Parameters dialog - To select the sample type, inlet, and injection source.

GC Edit Parameters dialog - To define the settings for your GC. Here you will click each icon to display and complete the corresponding window for each component.

Real-Time Plots dialog - To select which signals you want displayed.

MS Method Editor dialog - To define the Tune File, SIM, Real-Time Plot, and Timed Events, settings, using the single quadrupole or triple quadrupole method editor.*

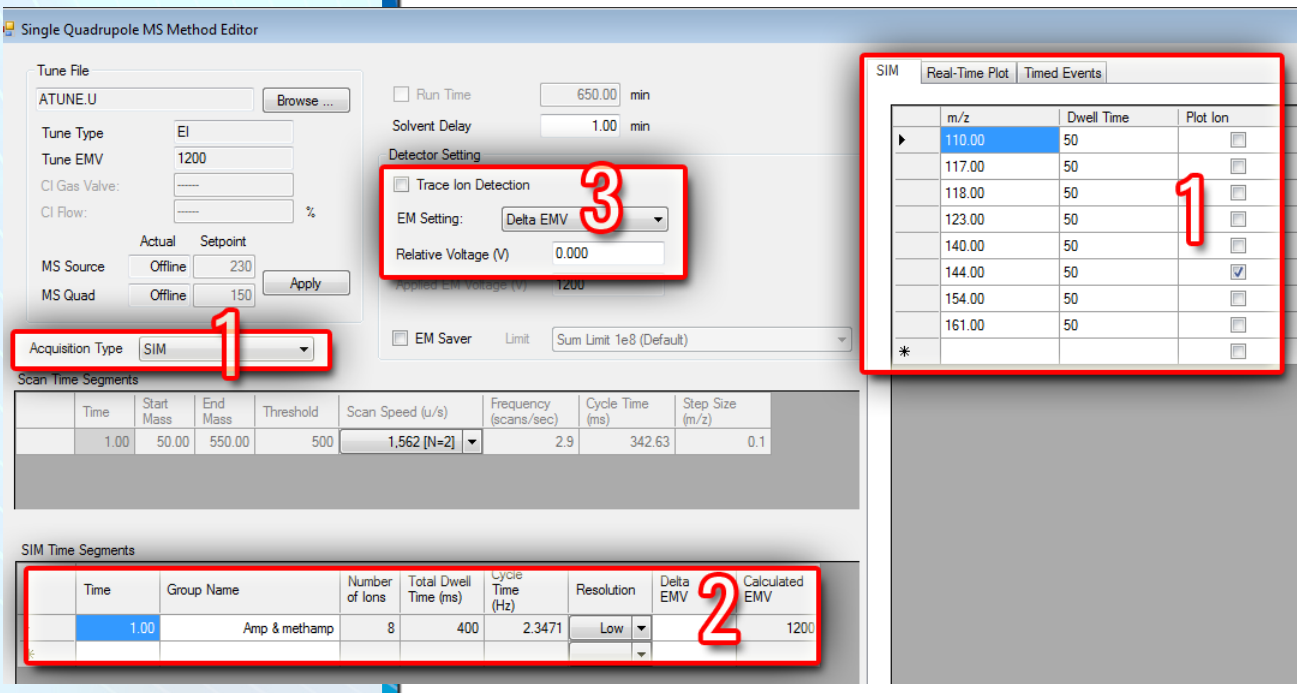
Monitors dialog - To define the MS monitors you wish to display.



*The MS Method Editor dialog is covered in more detail in "Step 6: Create a SIM method from a Scan method" on page 44.

Step 6: Create a SIM method from a Scan method

We will edit the MS Method Editor, as described here, to create an example SIM data acquisition method suitable for acquiring Amphetamine, MethAmphetamine, and their ISTDs.



Select **SIM** as the **Acquisition Type**. 1

In this example SIM method, enter the ions specified here in the SIM tab as shown in the dialog:

- D6-Amphetamine (144, 123)
- Amphetamine (140, 118, 117)
- D9-MethAmphetamine (161, 123)
- MethAmphetamine (154,118, 110)

The eight ions used in this method are specified in a single SIM time segment which has a starting time of 1 min. This means that the solvent has passed through the instrument at 1 minute. When optimizing these settings for your own method, adjust this value for the time that the solvent peak passes through your instrument. 2

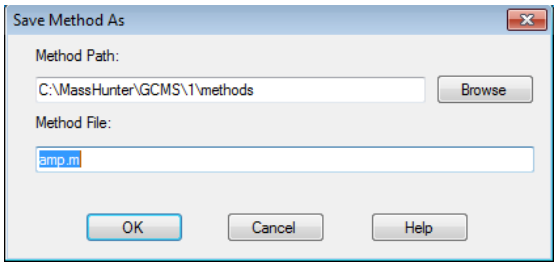
Set the Detector **EM Setting** to **Gain Factor** which is the Agilent preferred setting. The gain Factor is initially set to provide the same EMV as obtained for the most recent tune. 3

Step 7: Save the Method

1. Enter a new name for this method.

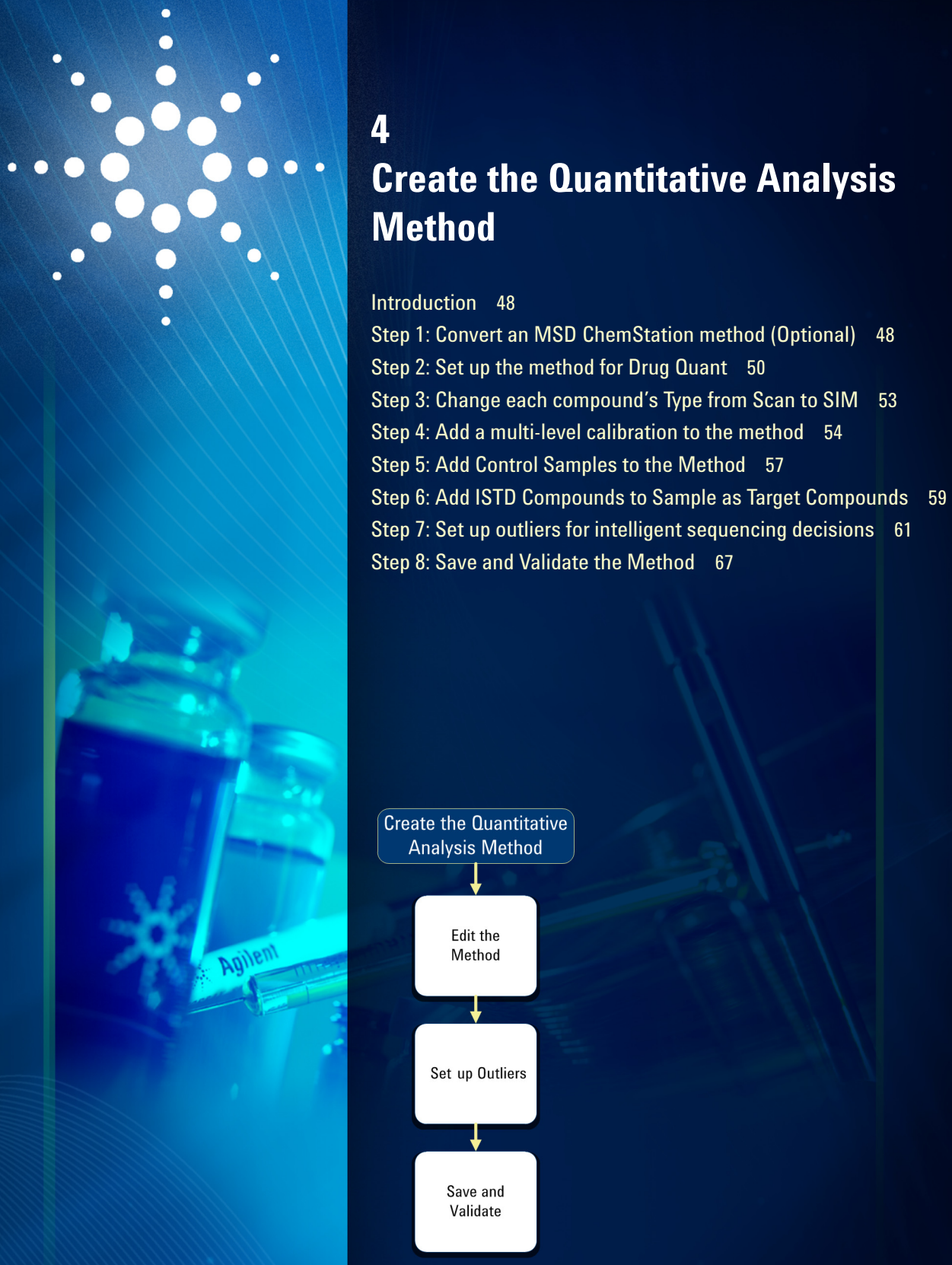
2. Click **OK** to save the method and close the dialog.

When you have completed each of the above dialogs, Save your method.



You are now ready to continue by creating a Drug Quant method.

4
Create the Quantitative Analysis Method



Agilent Technologies

Introduction

In this chapter you will learn how to create a Drug Quant method that is compatible with Intelligent Sequencing.

Step 1 is an optional step. It describes how to translate an existing MSD ChemStation method into a MassHunter Quantitative Analysis method, if desired. If you are not migrating an existing MSD ChemStation method into MassHunter Quantitative Analysis, you should skip this step and begin with Step 2.

Steps 2 through 8 describe how to set up a calibration and define your acceptable limits for Blanks, Negatives, and Controls found in samples. If a sample falls outside the limits specified here, MassHunter will use the settings defined in the Intelligent Sequencing dialog to complete the sequence, as described in Chapter 2.

Also included in this chapter are details for setting up control samples used for monitoring the calibration state.

Step 1: Convert an MSD ChemStation method (Optional)

For those users interested in migrating from MSD ChemStation to MassHunter Quantitative Analysis, this step describes how to convert an existing MSD ChemStation method to a MassHunter Quantitative Analysis method using the **GC MSD Translator** tool.

If you do not want to migrate an existing MSD ChemStation method into the MassHunter Quantitative Analysis program, skip this step and create the Quant method manually by closely following the examples shown here, starting with "Step 2: Set up the method for Drug Quant" on page 50.

1. Install the GC MSD Translator.

   If the **GC MSD Translator** tool is not installed, install it using the **setup.exe** program found in the **GCMS Translator** directory located on the **Agilent MassHunter Workstation GC/MS Supplemental Software** disk.

2. Select the starting SIM method.

   There are 5 SIM methods supplied with your MSD ChemStation. These methods, located in the **MSDchem\msdemo\drugdemo** directory, are for:
   - Amphetamines
   - Cocaine
   - Opiates
   - PCP
   - THC

   These methods use the MSD ChemStation RTE integrator in Data Analysis with customized integration parameters specific to the drugs addressed. The RTE integrator is recommended for use with MSD data when using the MSD ChemStation.

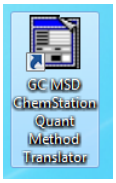
   The quantitation database in these demo drug methods consists of data from a single cutoff calibration sample. The cutoff calibration sample defines the minimum concentration considered to be a positive result in a specimen sample.

   The MSD ChemStation Quant Database we will use in our example is included in the **AMP.M** method, for drugs of abuse, This is a good starting point for creating a quantative method for your analysis.

   To use these methods after you convert them, you will need to edit the method parameters to suit your particular analysis requirements. You may also need to enter the calibration curve for each drug of abuse compound.

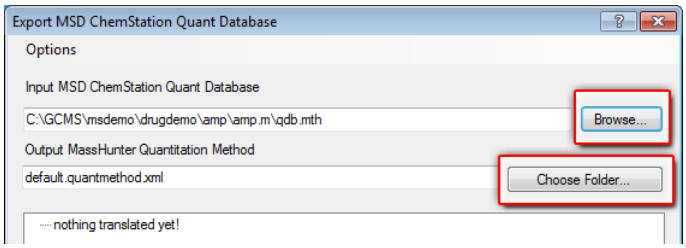
3. From the **GCMS\msdemo\drug-demo\amp** directory where your MSD ChemStation is installed, select the AMP.M method directory and copy it to a suitable location on the computer containing the **GC MSD Translator** tool.

4. Click the **GC MSD ChemStation Quant Method Translator** tool icon to start the program.

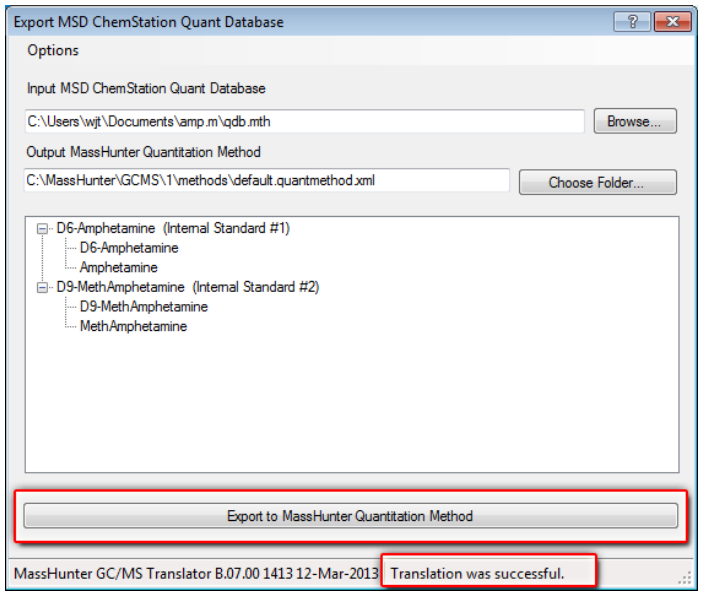


5. Click **Browse** and select the **qdb.mth** file from the MSD ChemStation method folder (i.e., AMP.M).

6. Click **Choose Folder...** and select a path for the translated file.



7. Click **Export to MassHunter Quantitation Method**.

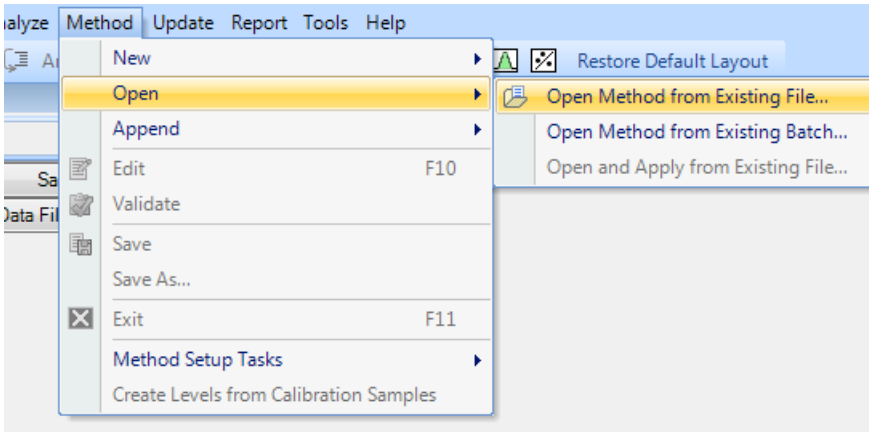


8. If necessary, copy this new method (**default.quant-method.xml**) to the computer where the MassHunter Quantitative Analysis program is located.

Notice that the bottom right corner of the dialog now indicates **Translation was successful**. The data structure now shows the Amphetamine drugs grouped under its referenced ISTD and MethAmphetamine grouped under its ISTD.

Step 2: Set up the method for Drug Quant

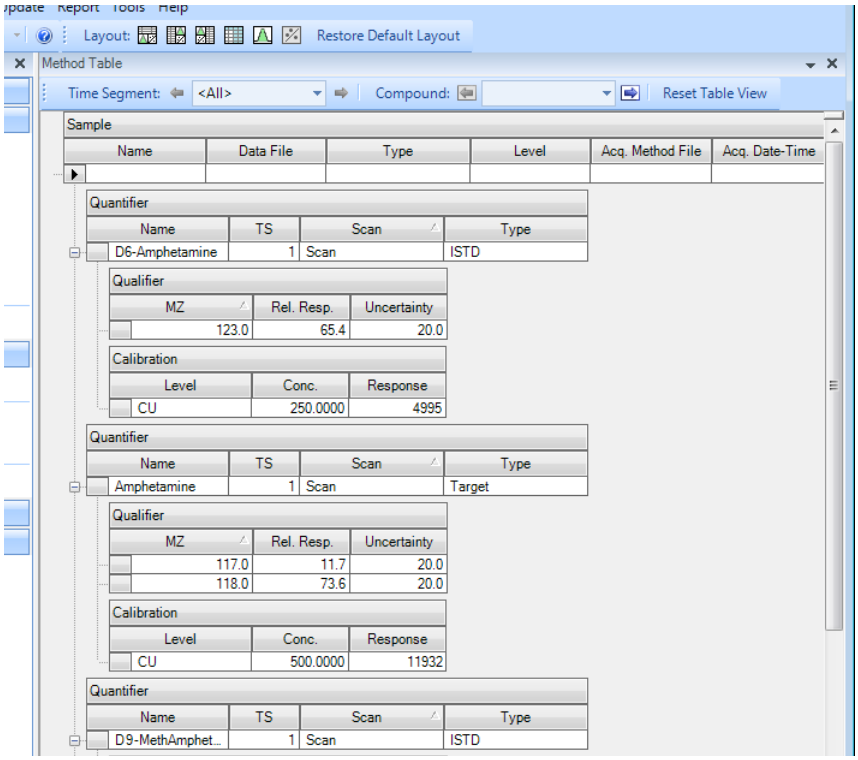
1. In MassHunter Quantitative Analysis, select **Method/ Open Method from Existing File**.



If you did not convert a method, as described in the previous section, see the online Help and the Familiarization manual (accessible from online Help) for details on creating a quant method manually, then closely follow the examples in this chapter to setup the method for Drug Quant using Intelligent Sequencing.

2. Select the **default.quant-method.xml** file that you converted in the last section, or the quant method you created manually, and click **OK** to close the dialog and enter the Quant Method Editor with this method loaded.

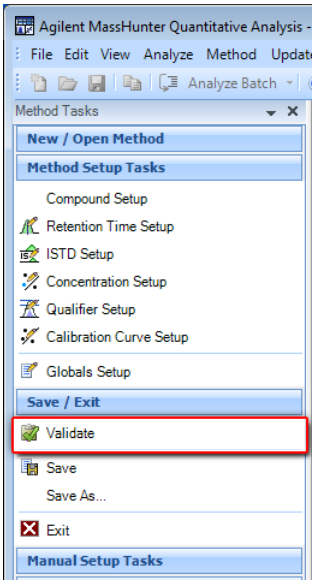
On the next few pages, we describe how to add several levels to these calibrations so that the Target drugs will have a 4 point linear calibration curve.

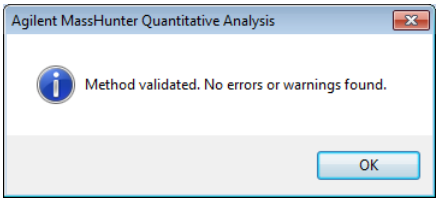


Here notice the sample structure.

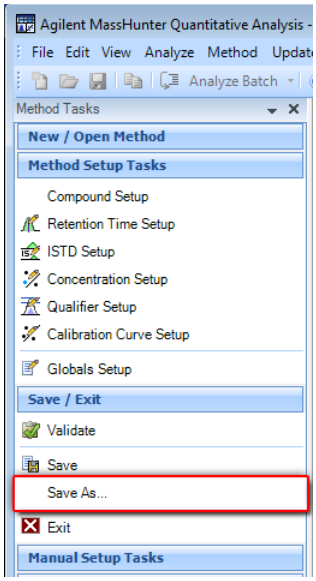
- The first element is the ISTD quantifier **D6-Amphetamine**, its qualifier, and a single CU level concentration.
- Next is the **Amphetamine** Target quantifier, its qualifiers, and a single CU level concentration.

3. In the **Save/Exit** section of Method Tasks area click **Validate**. A dialog displays indicating your method is valid for MassHunter GCMS Acquisition.





4. In the **Save/Exit** section, click **Save As** and save this method as **Amp.quantmethod.xml**.

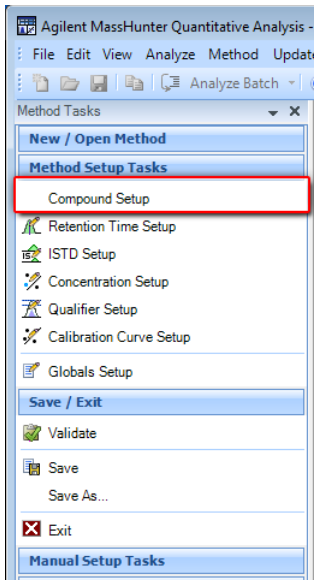


Step 3: Change each compound's Type from Scan to SIM

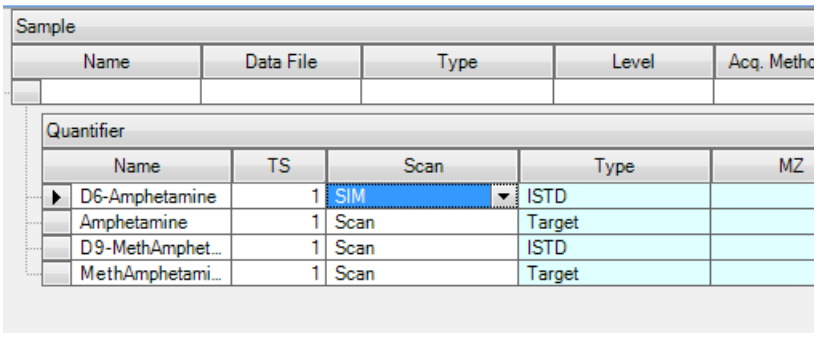
Skip this step if you did not convert an MSD ChemStation method.

The method translator program does not register the SIM data type automatically. To resolve this:

1. With your method loaded, in the **Method Setup Tasks** section, click **Compound Setup**.



2. In the first line of the **Scan** column select **SIM**.

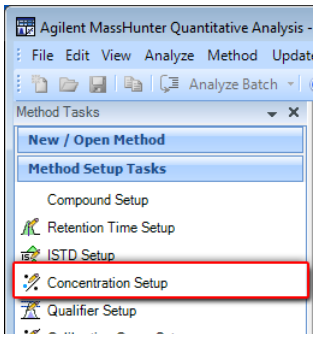


3. Right-click on this SIM entry and select **Fill Down** from the context menu. All scans types are now correctly identified as **SIM**.

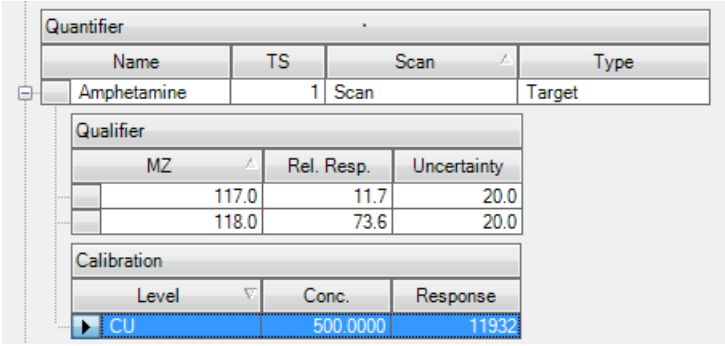
Step 4: Add a multi-level calibration to the method

Our translated method contains a single calibration level, the CU level. In this step **we are adding 3 more calibration points** to this single point calibration to the target compounds. Later we will add control samples to this method.

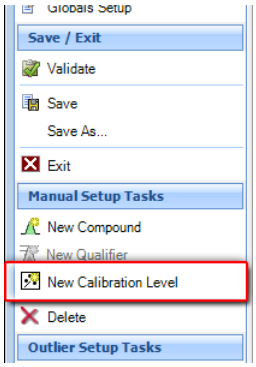
1. With your method loaded, in the Method Setup Tasks section, click **Concentration Setup**.



2. Select the **CU Level** line in the **Amphetamine Calibration** table.



3. In the Manual Setup Tasks section, click the **New Calibration Level** icon **3 times** to add three lines to the Calibration table.



4. Enter your calibration levels.

5. Enter the Response for these levels.

6. Use the above process to add these same levels to the Meth-Amphetamine Target quantifier (not shown).

7. Add these same levels to both ISTDs.

For our example, shown below, we entered the level labels and concentration amount in ng/mL in the **Level** and **Conc** columns. Note that we are also changing the concentration of the CU Level to 250 ng/mL.

Quantifier

Name	TS	Scan	Type	Units
Amphetamine	1	SIM	Target	NG/ML

Calibration

Level	Conc.	Response	Enable
L100	100.0000		☑
L400	400.0000		☑
L900	900.0000		☑
CU	250.0000	11932	☑

The **Response** column for all **Levels** will be updated later using calibration samples run from a sequence. For now we can use a linear response proportional to that of the **CU** level (11932/500 counts/conc).

Quantifier

Name	TS	Scan	Type	Units
Amphetamine	1	SIM	Target	NG/ML

Calibration

Level	Conc.	Response	Enable
L100	100.0000	2386	☑
L400	400.0000	9545	☑
L900	900.0000	21478	☑
CU	250.0000	5966	☑

For each Level use an ISTD concentration of 250 ng/mL.
(Only D6-Amphetamine ISTD is shown here.)

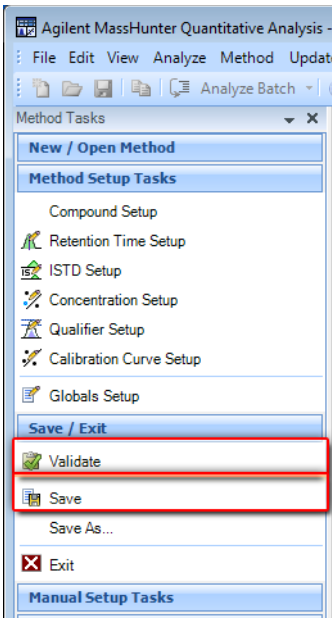
Quantifier

Name	TS	Scan	Type	Units
D6-Amphetamine	1	SIM	ISTD	NG/ML

Calibration

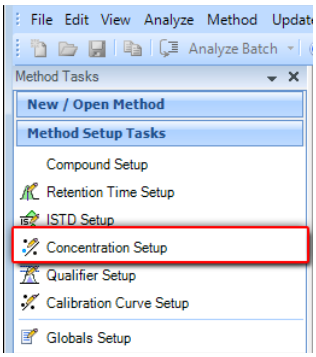
Level	Conc.	Response	Enable
L900	250.0000	4995	☑
L400	250.0000	4995	☑
L100	250.0000	4995	☑
CU	250.0000	4995	☑

8. Validate and save the method.



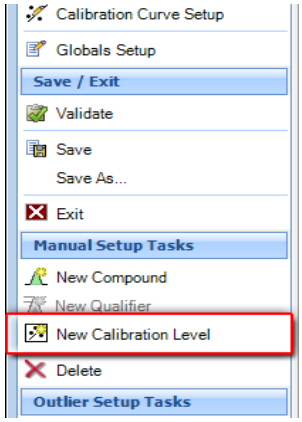
Step 5: Add Control Samples to the Method

1. With your method loaded, in the **Method Setup Tasks** section, click **Concentration Setup**.



2. Select the last line in the Calibration Table for D6-Amphetamine.

3. In the **Manual Setup Tasks** section, click **New Calibration Level** twice to add two data entry lines for new levels.



4. Fill in these levels as shown here with new controls labeled **QCLow** and **QCMed**. Since this is an ISTD the concentration will be 250 ng/mL for both.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	Units
D6-Amphetamine	1	SIM	ISTD	NG/ML

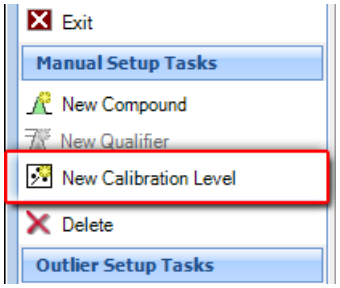
Calibration

Level	Conc.	Response	Enable
QCLow	250.0000	4995	☑
QCMed	250.0000	4995	☑
CU	250.0000	4995	☑
L100	250.0000	4995	☑
L400	250.0000	4995	☑
L900	250.0000	4995	☑

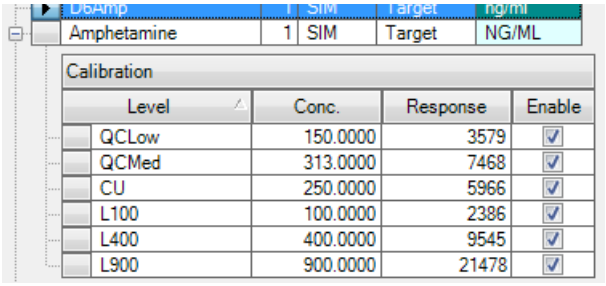
5. Use the above procedure to add these controls to the other ISTD.

6. Select the last line in the Calibration Table for Amphetamine.

7. In the **Manual Setup Tasks** section, click **New Calibration Level** twice to add two data entry lines for new levels.



8. Fill in these levels as shown here with new controls labeled **QCLow** and **QCMed**.



Amphetamine	1	SIM	Target	NG/ML

Calibration

Level	Conc.	Response	Enable
QCLow	150.0000	3579	☑
QCMed	313.0000	7468	☑
CU	250.0000	5966	☑
L100	100.0000	2386	☑
L400	400.0000	9545	☑
L900	900.0000	21478	☑

The concentrations here are set for a regulation requiring a control set at 40% below and 25% above the cutoff (**CU**) level.

9. Using a procedure similar to the preceding steps, add these two controls to the other target compounds.

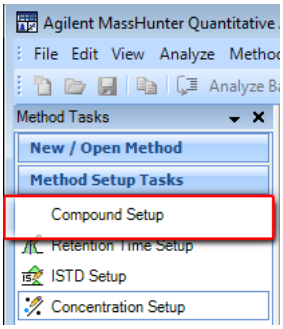
Step 6: Add ISTD Compounds to Sample as Target Compounds

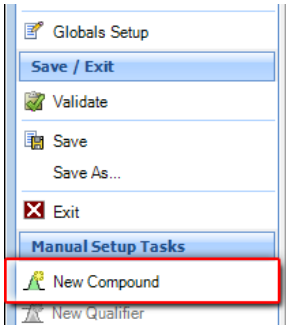
1. With your method loaded, in the **Method Setup Tasks** section, click **Compound Setup**.

2. In the **Manual Setup Tasks** section, click **New Compound** twice to add two lines to the Quantifier table.

3. Enter the compound Name, Scan, Type, MZ and RT, Criteria as shown here for D6Amp and D9MethAmp.

MassHunter GCMS Acquisition cannot process a carryover ISTD response in a Blank sample run as it does with a carryover Target compound response in a Blank. Therefore, it is necessary to set up additional compounds with the same ion identity as the ISTD we want to monitor for carryover when running a Blank sample but identify them as Target compounds.





For our example, we are adding D6-Amphetamine and D9-MethAmphetamine to our method as target compounds under a different compound name.

mple

Name	Data File	Type	Level	Acq. Method File	A

Quantifier

Name	TS	Scan	Type	MZ	RT	Ion Polarity	Criteria
D6-Amphetamine	1	SIM	ISTD	144.0	1.799	Positive	Close RT with Qu
Amphetamine	1	SIM	Target	140.0	1.807	Positive	Close RT with Qu
D9-MethAmphetamine	1	SIM	ISTD	161.0	1.998	Positive	Close RT with Qu
MethAmphetamine	1	SIM	Target	154.0	2.021	Positive	Close RT with Qu
D6Amp	1	SIM	Target	144.0	1.799	Positive	Close RT with Qu
D9MethAmp	1	SIM	Target	161.0	1.998	Positive	Close RT with Qu

Note that other than the name and Type, they are identical to the two ISTDs.

4. In the **Method Setup Tasks** section, click **Qualifier Setup**.

5. Select the new compound D6Amp in the Quantifier table.

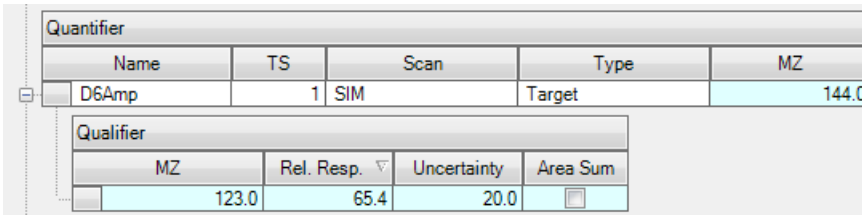
6. In the **Manual Setup Tasks** section, click **New Qualifier** to add a Qualifier Table under the D6Amp quantifier with one qualifier entry line.

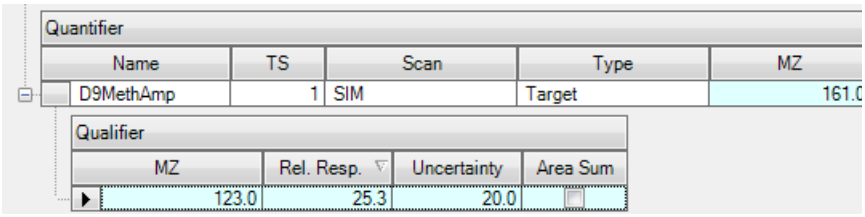
7. Enter the **MZ**, **Rel**. **Resp**., and **Uncertainty** for the **D6Amp** qualifier as shown here.

8. Repeat similar to the above steps to add the **D9MethAmp** qualifier as shown here.

9. Validate the method and save it.

For our example, we are copying the D6-Amphetamine qualifier entries here.





Step 7: Set up outliers for intelligent sequencing decisions

Here we set up the allowable specifications for our sample analysis. When a specification entered here is not met, a decision entered in Intelligent Sequencing is carried out by MassHunter GCMS Acquisition. Leave an entry blank if you do not want MassHunter GCMS Acquisition to report or act on that outlier.

Set Limits for ISTDs in Blank Samples

As previously discussed in "Step 6: Add ISTD Compounds to Sample as Target Compounds" on page 59, MassHunter GCMS Acquisition cannot process an ISTD response for a Blank sample type directly. MassHunter GCMS Acquisition can process a Target compound response for a Blank sample type so we set up the D6Amp and D9MethAmp target compounds in the previous step for that purpose.

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Blank Response** and enter the acceptable maximum response for each ISTD compound found in a Blank sample type. The example shows the entry of acceptable carryover responses for the ISTDs in a Blank sample.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	Max. Blank Resp.
D6-Amphetamine	1	SIM	ISTD	
Amphetamine	1	SIM	Target	
D9-MethAmphetamine	1	SIM	ISTD	
MethAmphetamine	1	SIM	Target	
D6Amp	1	SIM	Target	10
D9MethAmp	1	SIM	Target	10

Set the Acceptable Response Range for an ISTD in a Negative Sample

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **ISTD Response** and enter the High and Low response for each ISTD compound found in a Negative sample type.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	ISTD Resp. Limit Low	ISTD Resp. Limit High
MethAmphetamine	1	SIM	Target		
D9-MethAmphetamine	1	SIM	ISTD	2000	4000
D9MethAmp	1	SIM	Target		
D6-Amphetamine	1	SIM	ISTD	2000	10000
D6Amp	1	SIM	Target		
Amphetamine	1	SIM	Target		

Set an Acceptable Carryover Concentration Range for Target Compounds

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Carryover Amount** and enter the High and Low amount in ng/mL for each compound found in any sample type.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

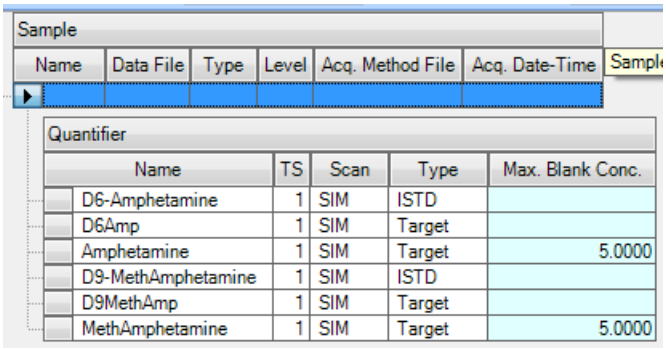
Name	TS	Scan	Type	Amt. Limit Low	Amt. Limit High
D6-Amphetamine	1	SIM	ISTD		
D6Amp	1	SIM	Target		
Amphetamine	1	SIM	Target	0	1E+04
D9-MethAmphetamine	1	SIM	ISTD		
D9MethAmp	1	SIM	Target		
MethAmphetamine	1	SIM	Target	0	1E+04

Set Concentration Limits for Target Compounds in Negative Samples

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Negative Concentration** and enter the acceptable response in ng/mL for each Target compound found in a Negative sample type.

This example shows the entry of acceptable carryover responses for the target compounds in a Negative sample.



Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	Sample

Quantifier

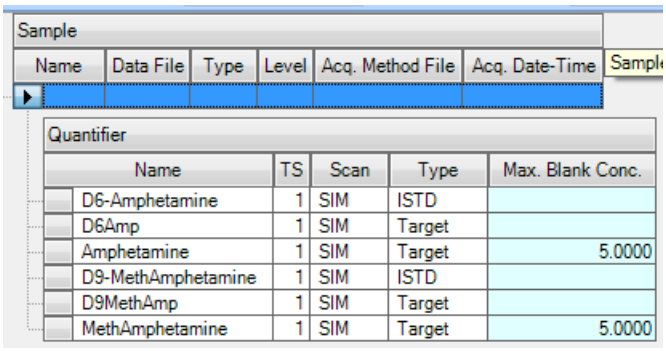
Name	TS	Scan	Type	Max. Blank Conc.
D6-Amphetamine	1	SIM	ISTD	
D6Amp	1	SIM	Target	
Amphetamine	1	SIM	Target	5.0000
D9-MethAmphetamine	1	SIM	ISTD	
D9MethAmp	1	SIM	Target	
MethAmphetamine	1	SIM	Target	5.0000

Set an Acceptable Concentration for Target Compounds in Negatives

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Negative Concentration** and enter the acceptable response in ng/mL for each Target compound found in a Negative sample type.

This example shows the entry of acceptable carryover concentrations in ng/mL for the target compounds in a Negative sample.



Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	Sample

Quantifier

Name	TS	Scan	Type	Max. Blank Conc.
D6-Amphetamine	1	SIM	ISTD	
D6Amp	1	SIM	Target	
Amphetamine	1	SIM	Target	5.0000
D9-MethAmphetamine	1	SIM	ISTD	
D9MethAmp	1	SIM	Target	
MethAmphetamine	1	SIM	Target	5.0000

Set the Concentration Linearity Limit

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Limit of Quantitation** and enter the lowest concentration in ng/mL for each Target compound found in a Negative sample type.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	LOQ
D6-Amphetamine	1	SIM	ISTD	
D6Amp	1	SIM	Target	
Amphetamine	1	SIM	Target	150
D9-MethAmphetamine	1	SIM	ISTD	
D9MethAmp	1	SIM	Target	
MethAmphetamine	1	SIM	Target	150

Setup Minimum Signal to Noise for Specimen Samples

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Signal-to-Noise Ratio** and enter acceptable values for the target compounds.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	Min. S/N
D6-Amphetamine	1	SIM	ISTD	
D6Amp	1	SIM	Target	
Amphetamine	1	SIM	Target	10.00
D9-MethAmphetamine	1	SIM	ISTD	
D9MethAmp	1	SIM	Target	
MethAmphetamine	1	SIM	Target	10.00

Setup Chromatographic Resolution for Specimen Samples

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Peak Resolution** and enter acceptable values for the target compounds.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	Resolution Limit
D6-Amphetamine	1	SIM	ISTD	
D6Amp	1	SIM	Target	
Amphetamine	1	SIM	Target	80.0
D9-MethAmphetamine	1	SIM	ISTD	
D9MethAmp	1	SIM	Target	
MethAmphetamine	1	SIM	Target	80.0

Setup Chromatographic Symmetry for Specimen Samples

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Peak Symmetry** and enter acceptable values for the target compounds. A value of 1.0 indicates perfect symmetry. A Limit Low less than 1 indicates allowable fronting and a Limit High greater than 1 indicates allowable tailing.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	Symmetry Limit Low	Symmetry Limit High
D6-Amphetamine	1	SIM	ISTD		
D6Amp	1	SIM	Target		
Amphetamine	1	SIM	Target	0.50	2.00
D9-MethAmphetamine	1	SIM	ISTD		
D9MethAmp	1	SIM	Target		
MethAmphetamine	1	SIM	Target	0.50	2.00

Set Allowable Peak Width for Specimen Samples

a With your method loaded, in the **Method Setup Tasks** section, click **Outlier Setup Tasks**.

b Click **Peak Full Width Half Maximum** and enter an acceptable peak width range in minutes for the target compounds.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time

Quantifier

Name	TS	Scan	Type	FWHM Limit Low	FWHM Limit High
D6-Amphetamine	1	SIM	ISTD		
D6Amp	1	SIM	Target		
Amphetamine	1	SIM	Target	0.050	3.000
D9-MethAmphetamine	1	SIM	ISTD		
D9MethAmp	1	SIM	Target		
MethAmphetamine	1	SIM	Target	0.050	3.000

Step 8: Save and Validate the Method

1. With your method loaded, in the **Method Setup Tasks** section click **Save / Exit**.

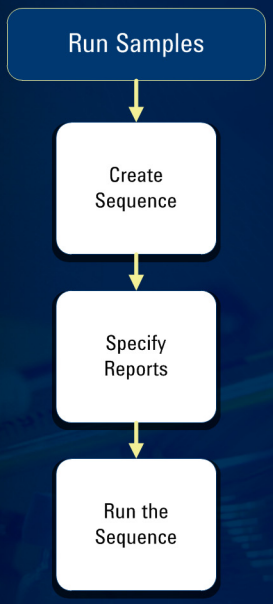
2. Click **Validate** to verify that you method is valid. If no problems exist click OK to acknowledge the verification message. If problems exist, a linked problem message displays in the **Method Error List** window located below the **Method Table**.

3. Click **Save As** and save this method in the directory you use for storing master methods used by automated sequences.

At this point our quantitation method contains all the settings required by Intelligent Sequencing when analyzing specimens for the presence of Amphetamines and MethAmphetamines. What remains is to replace the current compound responses that originated in our translated method with calibrated responses from the real analytical system.

See Chapter 6 for details on how to update the calibration.

5
Run Samples



Agilent Technologies

Introduction

In MassHunter GCMS Acquisition, data acquisition is automated using the sequence table.

The Sequence Table defines the:

- Name of the sample
- Method to be used for data acquisition and analysis
- Sample Type being analyzed
- Location in which to save your results
- Optional instructions for sample processing (Keyword/Keyword string)
- ALS vial location
- Batch samples and optional name
- Reports to be run

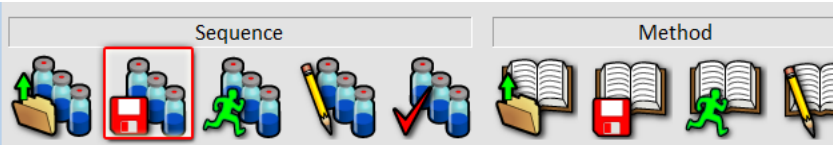
In this chapter we explain how to set up a Sequence Table with multiple batches of samples using the keyword **NewBatch**. This keyword indicates where the MassHunter should continue processing when a *Skip to next batch* command is generated by Intelligent Sequencing.

The following example consist of HHS required Quality Control samples followed by an HHS allowable number of specimens.

Step 1: Load the default Sequence

1. In the Data Acquisition Instrument Control view, click the Load Sequence icon then select the default.sequence.xml file from your instrument directory sequence folder.

2. Save this sequence as a new name in the sequence folder of the instrument directory.

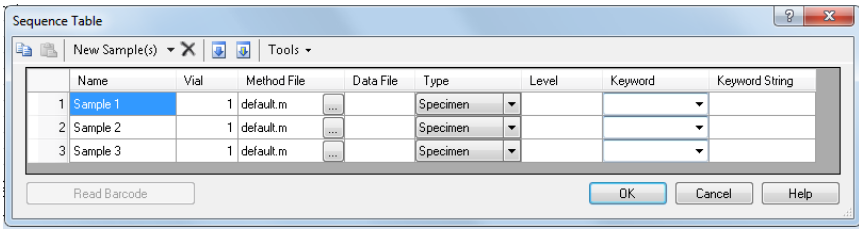


If you select a different directory for storing this sequence, that location will become the default storage directory location the next time you load or save a sequence.

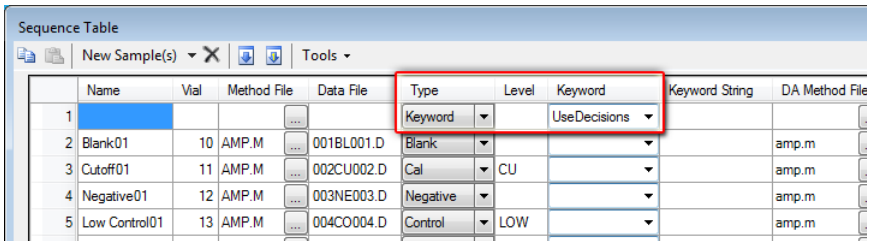
Step 2: Edit the Sequence Table

1. Click the Edit Sequence icon to open the Sequence Table for editing.

2. To begin, from the **Tools** menu, select **Add/Remove Columns** and add the DA Method File and DA Report Templates columns.



3. From the **Keyword** dropdown, select **Use Decisions**.

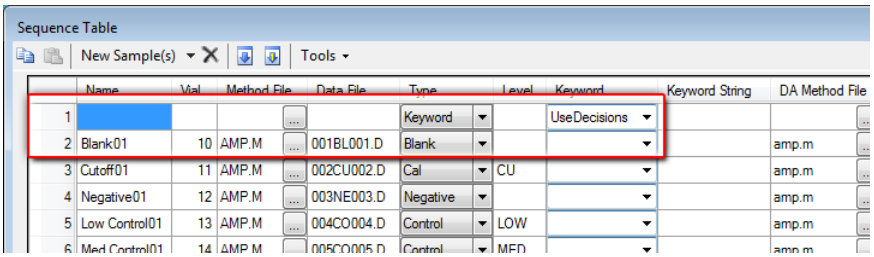


This command tells MassHunter GCMS Acquisition to send each sample's data file to MassHunter Quantitative Data Analysis for processing and wait for those results before running the next sample.

During processing, if MassHunter GCMS Acquisition finds the sample is out of spec it reviews the actions specified in **Intelligent Sequencing** and implements your previously defined decision.

In this example we are not defining the batch name (see BatchDir keyword in online help). The system names the batch directory using a unique default name consisting of date, and time, for example: 2013-06-11-1640.b. See the online help for more information.

4. Start the first batch on the first line after the Keyword you just entered.



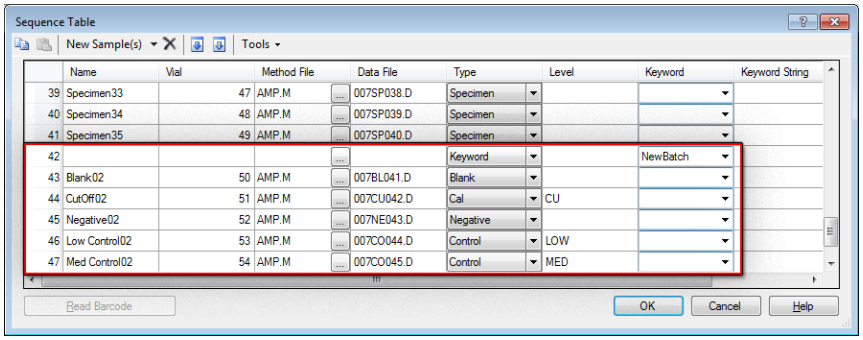
A typical sequence that includes multiple batches of specimens, starts out with a Blank sample that allows you to monitor for carryover contamination before you start to run specimens. This blank resets the Intelligent Sequencing cycle counter to zero.

For an HHS certified laboratory this is usually followed by:

- A cutoff calibration sample (**Type** = **CAL**, **Level** = **CU** to match the level label entered in the MassHunter Quantitative Analysis method)
- A negative sample (**Type**=**Negative**)
- A positive control set at 25% above the drug cutoff (**Type**=**Control**, **Level** = **MED**)
- A control set at or below 40% of the drug cutoff concentration (**Type**=**Control**, **Level** = **LOW**).

With a criteria that 10% of all samples in the batch must be quality control samples, these QC samples can be followed by up to 36 specimen samples.

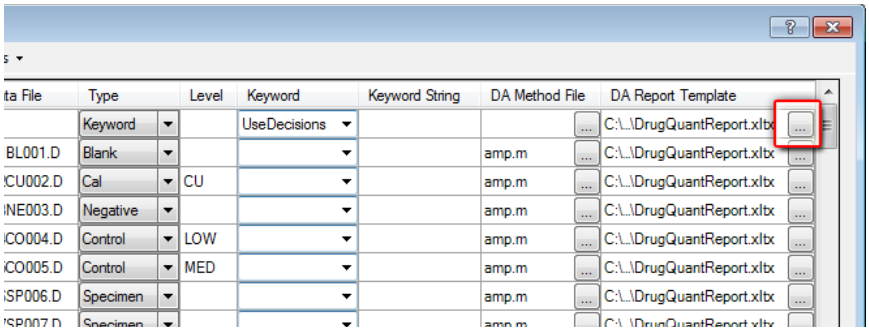
5. **To begin another batch in the same sequence table**, set the sample type to **Keyword** and select **NewBatch** from the **Keyword** dropdown. This batch, like the first, will use the MSD ChemStation auto naming batch feature.



Step 3: Specify reports

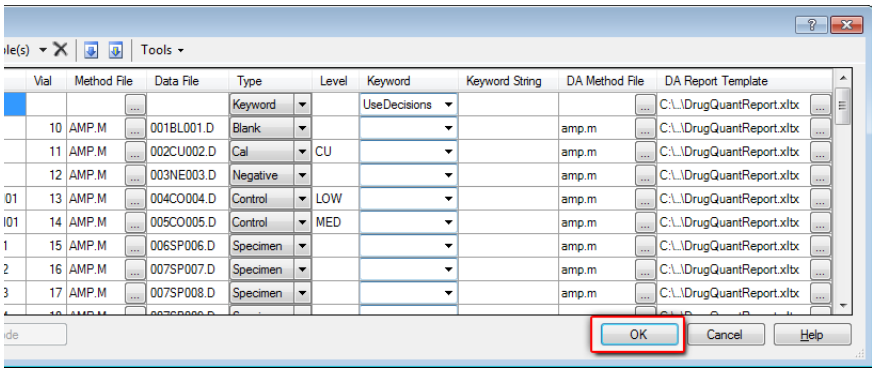
1. In the **DA Report Template** column, click the browse button and select a report template.

   This template is used to generate a report automatically with each sample.



Step 4: Save the Sequence Table

1. Once you have completed entering all your samples in the Sequence Table, click **OK** to close the dialog.



2. Save the completed Sequence Table.

Step 5: Run the Sequence

1. Load a previously created sequence table containing your batches of specimens.

2. Click the view icon to view the currently loaded sequence table and edit if needed.

3. With your sequence loaded, click the Start Sequence icon display the Start Sequence dialog.

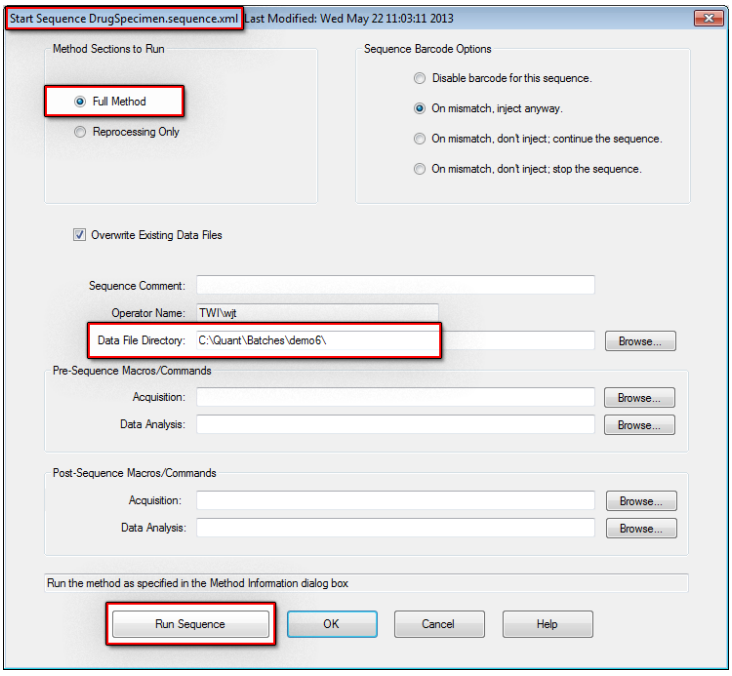
4. Notice that the name of the current sequence is displayed in the title bar.

   For this Workflow, we are acquiring data, so we selected the **Full Method** option in the **Method Sections to Run** group box.

5. For the **Data File Directory** enter the path where you would like the batch directories to be created. Each automatically created batch directory will contain the acquired data files and a copy of the method used to acquire the data.

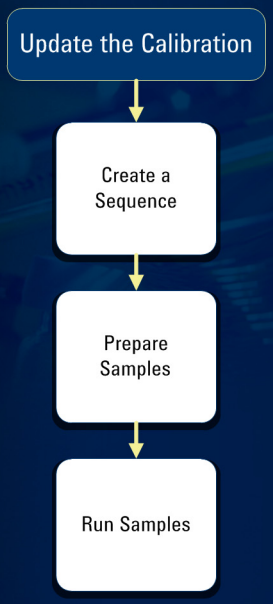
6. Click **Run Sequence** to begin processing all samples in this sequence.

In the previous sections you learned how to create a sequence table containing multiple batches of specimens. Once your sequence table has been set up, and your vials are loaded into the sample tray, you may begin to process your samples as described here.



As each sample is analyzed, the results are compared with the parameters set in the method. If the results are outside the specified criteria, MassHunter GCMS Acquisition will continue the process based on the parameters specified in the Intelligent Sequencing portion of your method.

6
Update the Calibration



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Introduction

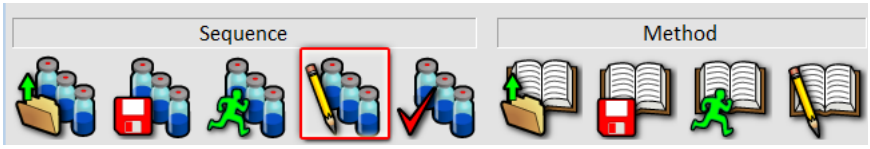
As required by governing regulations, the calibration curve stored in a method must be updated when a specified time has elapsed or when a quality control sample indicates an unacceptable deviation from the stored calibration curve.

This chapter describes how the Sequence Table Editor (STE) in MassHunter GCMS Acquisition can be used to automate this process.

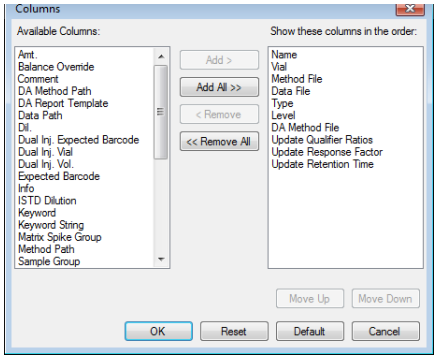
Step 1: Create the Sequence Table

In the following steps we will describe how you can modify this table to define your calibration samples.

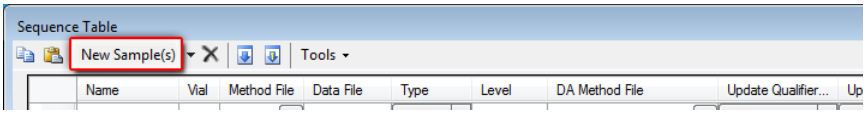
1. In the Instrument Control window, click the Edit Sequence icon to open the STE.



2. From the **Tools** menu select **Add/ Remove Columns** and edit your columns to be similar to those shown here, then click **OK.**

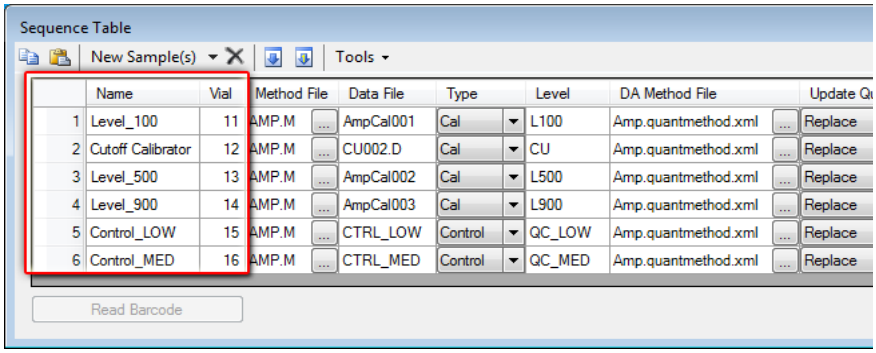


3. Click the **New sample(s)** menu three times to add three blank lines to the sequence table.



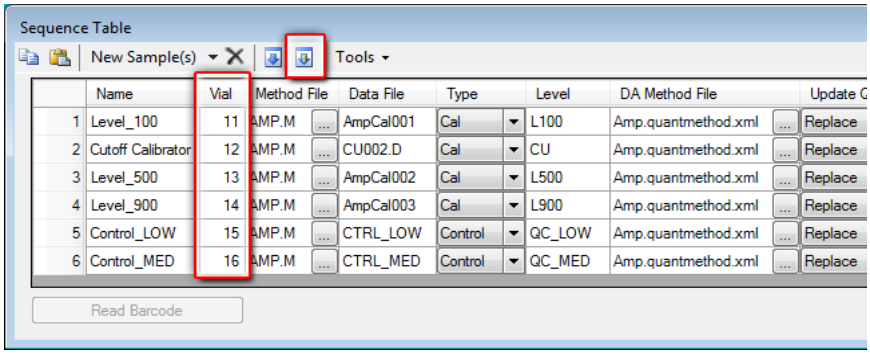
4. The example shown here illustrates a completed table. We will describe how it was created in the following steps.

   To begin, in the **Name** column, enter a sample name for each sample.



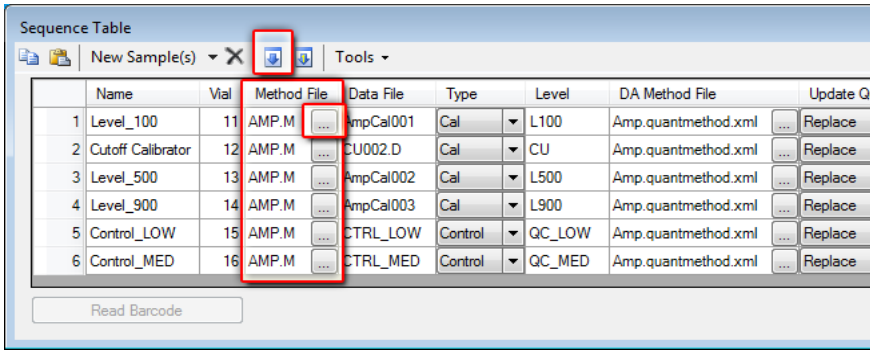
- Lines 1 through 4 are the 4 concentrations (**Levels**) required for a linear 4 point calibration curve for both Amphetamine and Methamphetamine. Line 2 is also a cutoff calibrator for these two drugs of abuse.
- Lines 5 and 6 are the control samples in our example.

5. In the **Vial** column, enter the location for the first sample (11 here), then click the increment icon (highlighted here), to automatically fill-down the vial numbers, (12 through 16 shown here).



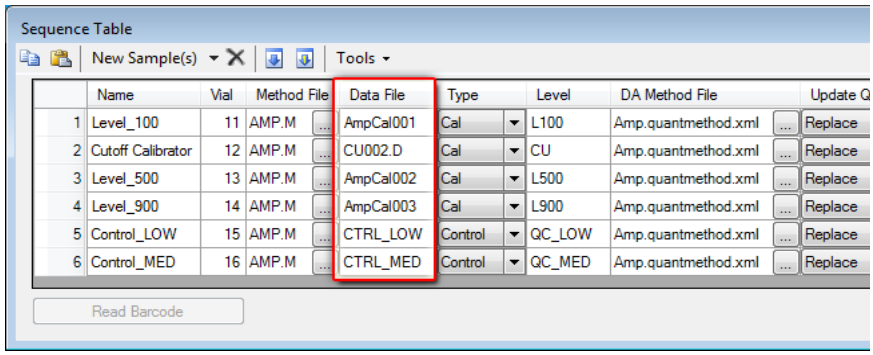
6. Click the browse icon next to the **Method File** name and select the method to use for each sample.

   With your cursor on the first line, click the copy icon to copy this method name to each line in the table.



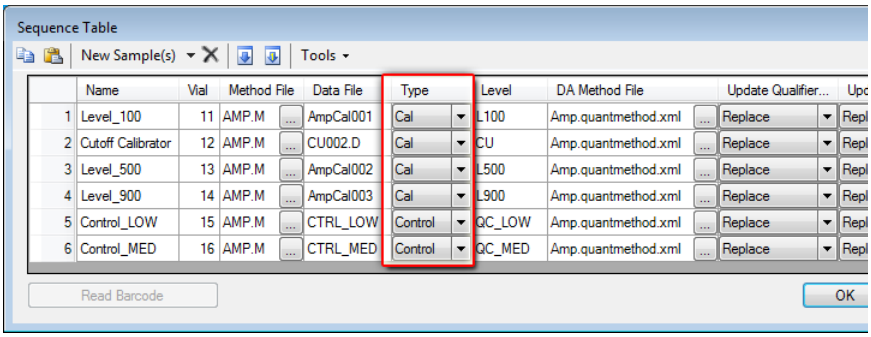
In this case, the instrument method parameters are stored in the AMP.M method, so that is the method to be used for each sample.

7. In the **Data File** column, enter a destination file name for the data acquired in the acquisition run.



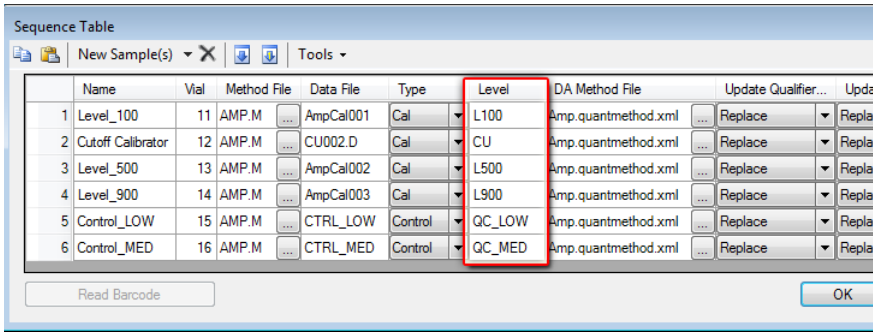
In this case the data will go into data files named AmpCal001, CU002, AmpCal002, AmpCal003, CTRL_LOW, and CTRL_MED.

8. In the **Type** column, select **CAL** from the dropdown list for lines 1 through 4 and select **Control** for lines 5 and 6.

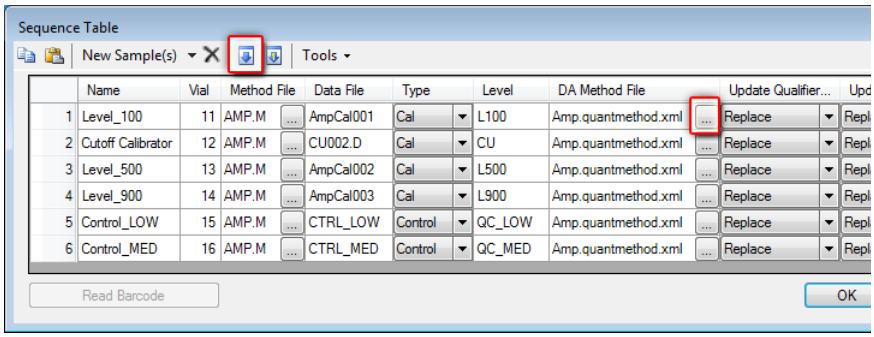


9. In the **Level** column, enter a level ID for each **CAL** and **Control** type sample.

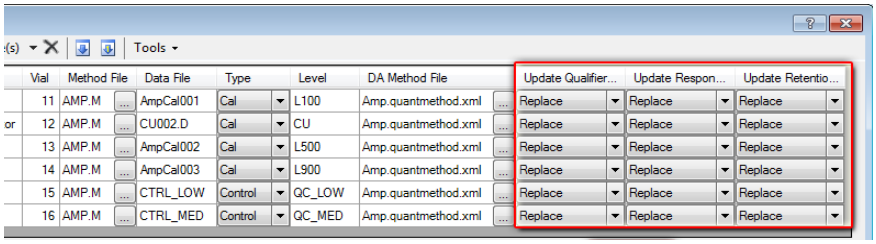
   This label must use *exactly the same name* as the corresponding concentration level ID in MassHunter Quantitative Analysis method. Capitalization is (is not) important, i.e. CU is not Cu.



10. In the **DA Method File** column, click the browse button on line 1, and select the MassHunter Quantitative Analysis method created in the last chapter.
    With the cursor still in this cell, click the copy icon to copy this method to all other samples in the table.



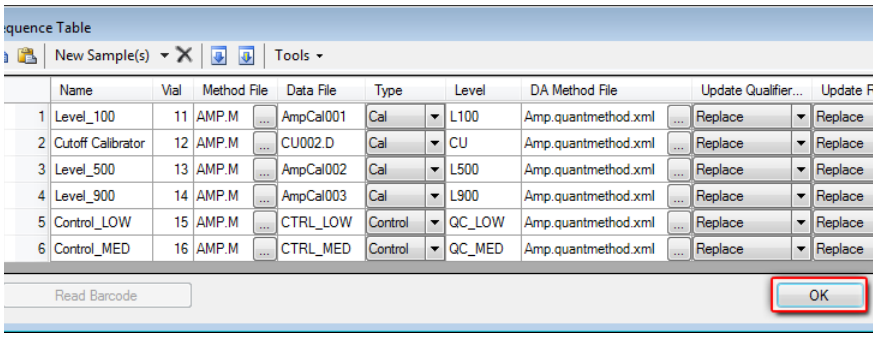
11. In the **Update Qualifier**, **Update Response**, and **Update Retention** columns, on line 1 select **Replace** from each dropdown. Copy this parameter to lines 2 through 6 for these three columns.



The drop-down selections in these columns allow you to:

- **Replace** the existing parameter in the Quantitative Analysis method with the results from that sample analysis
- **Average** the result with the parameter in the Quantitative Analysis method
- **No Update** to analyze the CAL sample without updating the parameters in the Quantitative Analysis method

12. Click **OK** to close the sequence table.



13. Save this sequence as a new name. Enter a new name for the sequence and save it to your instrument directory sequence folder.

Step 2: Prepare the calibration samples

Prepare calibration samples for creating a calibration curve of each drug of abuse compound in the MassHunter Quantitative Analysis method.

Each calibration sample should contain the drug of abuse compounds in concentrations that cover the expected linear range of each drug's calibration curve.

Each sample of a specified concentration should be spiked with those ISTD compounds referenced by each drug of abuse contained in the sample. In our example we are using 250 ng/mL of each ISTD in each of our CAL standards.

Our example uses a 4 point calibration requiring 4 concentrations of the compounds in individual vials. Here those concentrations are 100, 400, and 900 ng/mL for both Amphetamine and MethAmphetamine. The cutoff calibration sample contains the drugs and ISTD's for the 250 ng/mL level and is the second point on the calibration curve.

Step 3: Prepare the cutoff calibration sample

Prepare a cutoff calibration sample containing each drug of abuse in the concentration level required by the governing regulation. In our example the cutoff sample contains 250 ng/mL of both Amphetamine and Methamphetamine.

Spike the sample with those ISTD compounds as done with the other calibration samples.

Step 4: Load the calibration sample vials in the ALS

Once you have created your Sequence Table, place the vials into the sample tray and run the batch.

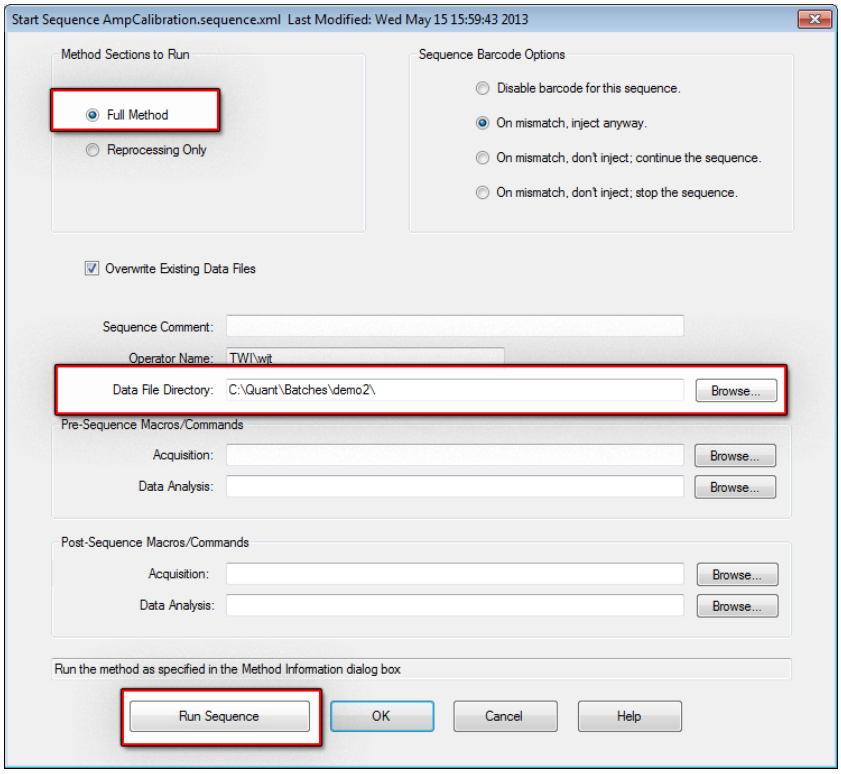
This example assumes use of an auto liquid sampler (ALS) which would require the 4 sample vials be placed in the sampler tray vial locations specified in the STE.

Step 5: Run the Calibration Sequence

1. With the newly created sequence loaded, click the **Run Sequence** icon.

2. On the Start Sequence dialog, select **Full Method** and enter a batch subdirectory for your acquired data files.

   When the run is completed, the data files for will be stored in the **Data File Directory**.



3. Click **Run Sequence** to begin the automated acquisition.

4. When the run is complete, in MassHunter Quantitative analysis **open this Batch** of 6 samples with the updated method and review the Calibration curve for linearity.

5. Enter the Quant Method Editor and examine these updated results in the method table.

6. Exit MassHunter Quantitative Analysis.

The data for a sample is acquired and analyzed before the next sample in the table is processed. The quantitative method is updated by replacing the qualifier ratios, detector response, and retention time of the quantitative ion with the actual results from the analysis of the sample. This is done for the level specified for this sample in the Sequence table.

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