Agilent MassHunter Drug Analysis Mode Using MSD ChemStation Data Analysis

Workflow Guide

Review the Intelligent Sequencing Feature → Create the Quantitation Database → Update the Quant 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 MSD ChemStation Data Analysis, using the Drug Analysis Workflow mode in each program.

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 MSD ChemStation Data Analysis.

More common operations, not directly associated with Drug Analysis Workflow mode using 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 MSD ChemStation 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, reinject a specimen including reducing 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 Quantitation Database

Chapter 3 describes how to create a Quantitation Database for drug analysis from a example method included with the MSD ChemStation Data Analysis program. Using the sequence table to process the 4 calibration samples required for the calibration is covered. Procedures for updating compound parameters for an existing concentration level and for adding new concentration level is explained. Specialized integration parameters included with the example methods are also reviewed.

4 Update the Quantitative Analysis Method

Chapter 4 describes editing an entire method including the data acquisition and data analysis parameters. The parameters specific to drug mode are covered in detail.

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

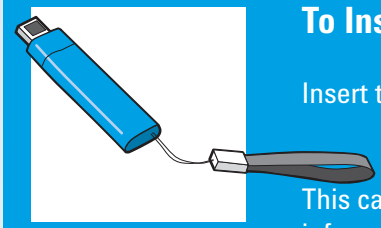
Accompanying your hardware and software is a comprehensive collection of manuals, videos, user applications, and method development tools. These are located on the:

- 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.

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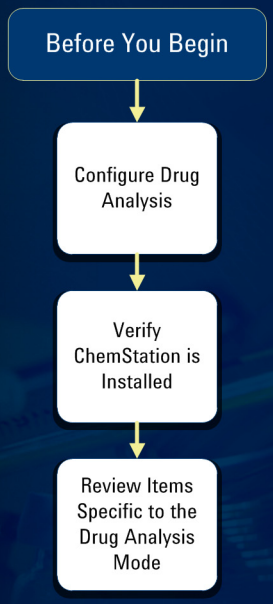
Before You Begin



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Configure the Programs

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

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

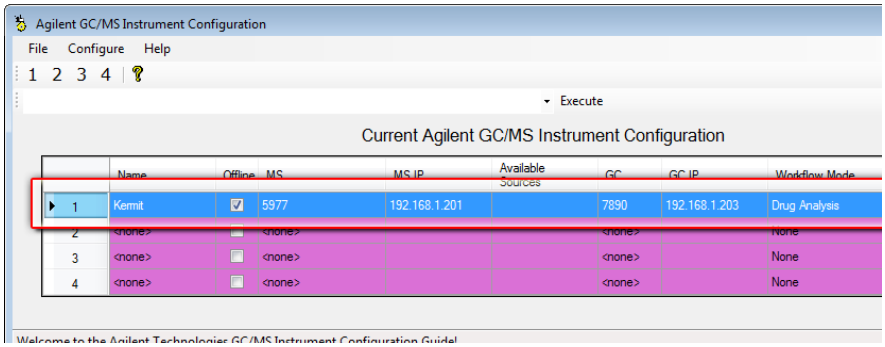
Here we are going to be using the **Drug Analysis Workflow Mode**. So, before doing anything else, you must set up the MassHunter GCMS Acquisition program and the MSD ChemStation Data Analysis program to run in the Drug Analysis Workflow Mode. Once this is done, when you open these programs, you will see elements that are unique to Drug Analysis, such as Intelligent Sequence parameters. In MSD ChemStation Data Analysis, you will also see elements that are unique to Drug Analysis. These include Drug Reports, DQ Edit, Drug ID, and others.

Select the Drug Analysis Workflow Mode

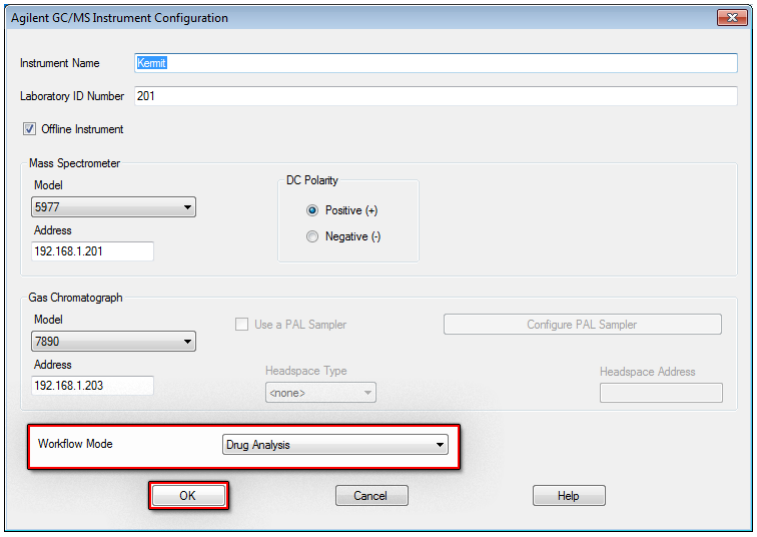
To configure an existing instrument to work in the Drug Analysis Mode:

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

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



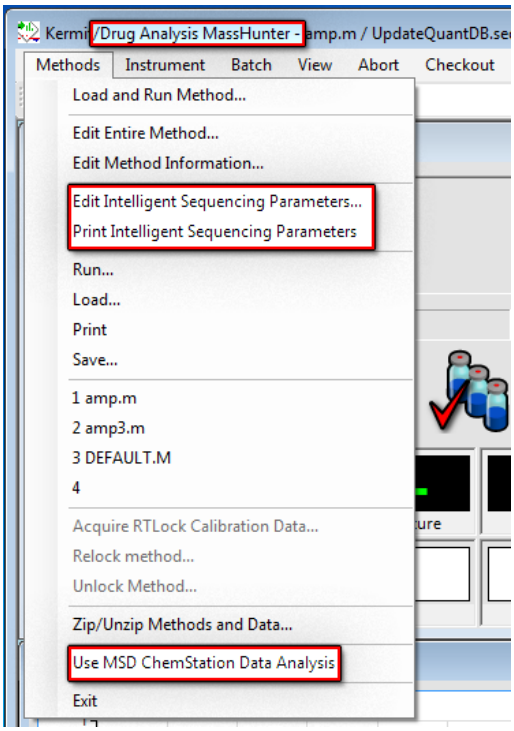
3. Select the **Drug Analysis Workflow Mode** and click **OK** to close the dialog.



4. Click **Yes** to confirm the configuration and exit the Agilent GCMS Configuration program.
5. Notice the items that are unique to the Drug Analysis Workflow mode.

The next time you start MassHunter GCMS Acquisition or MSD ChemStation Data Analysis, they will display the unique user interface elements that are added for 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 MSD ChemStation Data Analysis

Because this workflow uses MSD ChemStation Data Analysis, you must verify that MSD ChemStation Data 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 MSD ChemStation Data Analysis program, we want to use MSD ChemStation Data Analysis. Therefore, in MassHunter GCMS Acquisition, select: **Methods/Use MSD ChemStation Data Analysis**.

This will cause data analysis to be automatically done in MSD ChemStation Data Analysis rather than in MassHunter Quant. Remember:

- If this menu item is not selected, the system will use MassHunter Quant for automatic data analysis during a run.
- If this menu item is selected, the system will use MSD ChemStation Data Analysis.

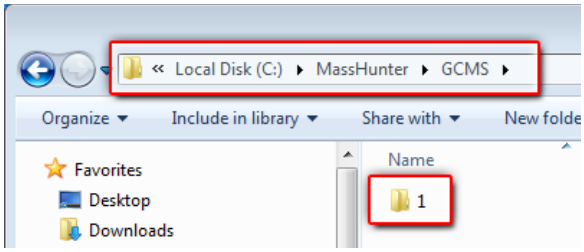
This menu item only exists when both MassHunter Quant and MSD ChemStation Data Analysis are installed on the same computer.

Understand the Directory Structure

1. Locate the instrument directories.

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

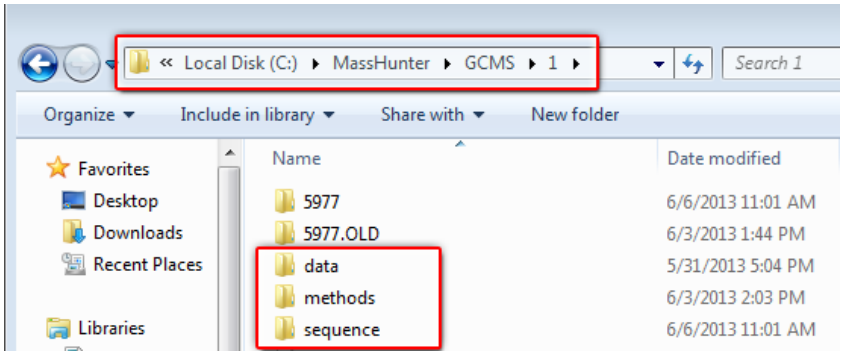
For each instrument you configure, MassHunter GCMS Acquisition will create a numbered directory corresponding to the instrument number. (***drive*:\MassHunter\GCMS\1** for example.)



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.



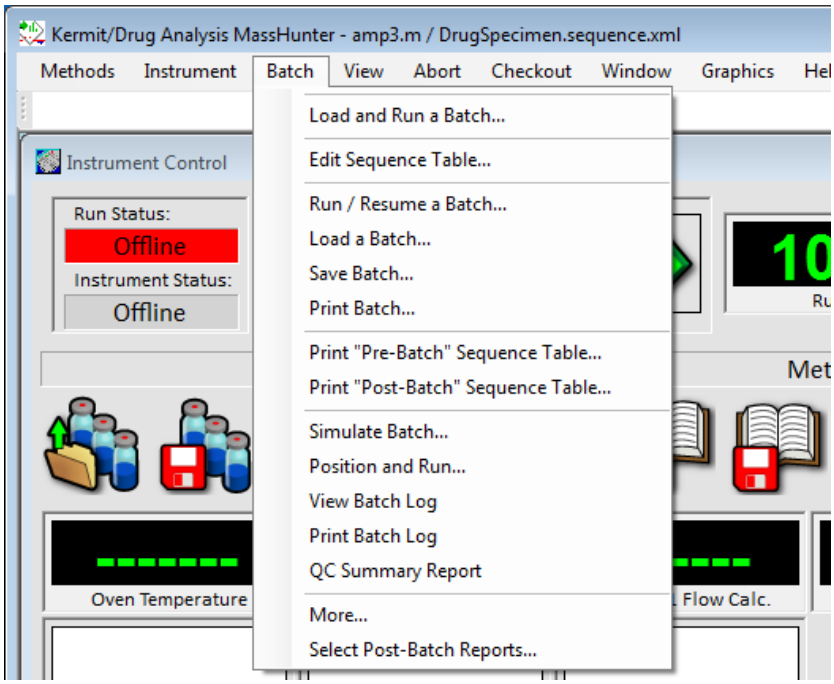
- **The data directory** contains the data from each batch run, stored in an individual directory.
- **The methods directory** contains all of your methods. Each method is stored in its own (.m) subdirectory, which contains all of the files for the method, such as compound lists, calibration tables, 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.

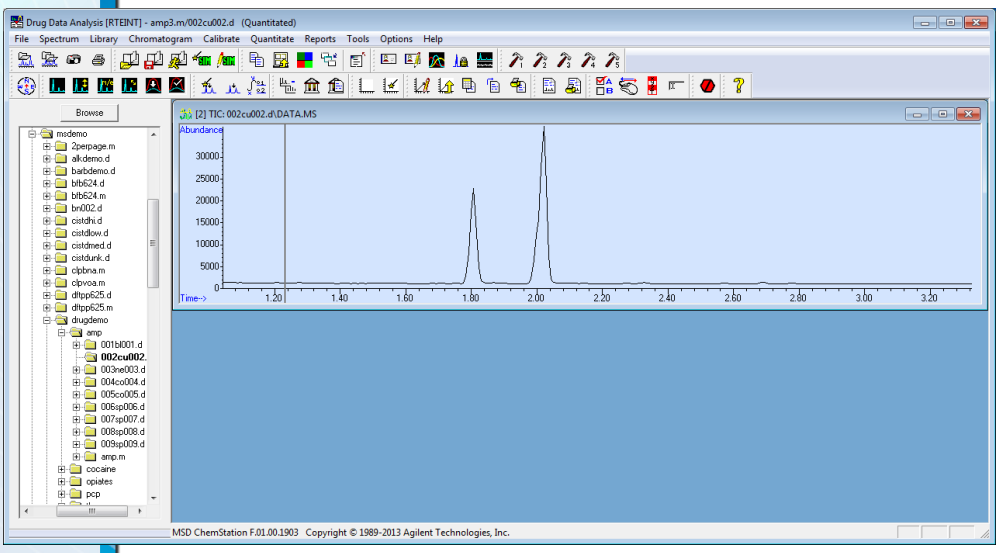
When MassHunter GCMS Acquisition is configured in the Enhanced Workflow mode, this menu is labeled **Sequence**, but contains similar options.



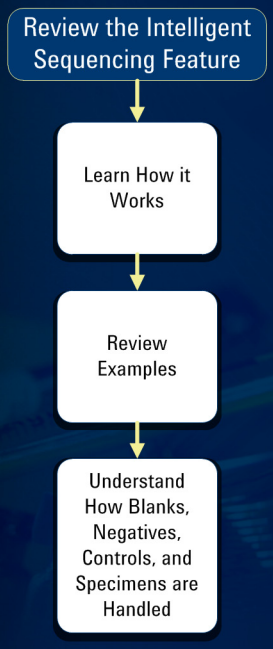
View Menu

If you have previously used MSD ChemStation Data Analysis in Drug Analysis Workflow mode you should already be familiar with the specific Drug Analysis Workflow elements in this UI.

To Access the MSD ChemStation Data Analysis, from the MassHunter Drug Analysis **View** menu, select **View\MSD ChemStation Data Analysis**. This will display the familiar MSD ChemStation Data Analysis program in Drug Workflow mode.



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.

- The acceptance criteria limits for Internal Standards, Controls, Negatives, and Blanks are set in the Data Analysis portion of the Method. See "Step 7: Enter your acceptable limits" on page 58.
- Intelligent Sequence Parameters are set in the Data Acquisition portion of the Method.

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.*

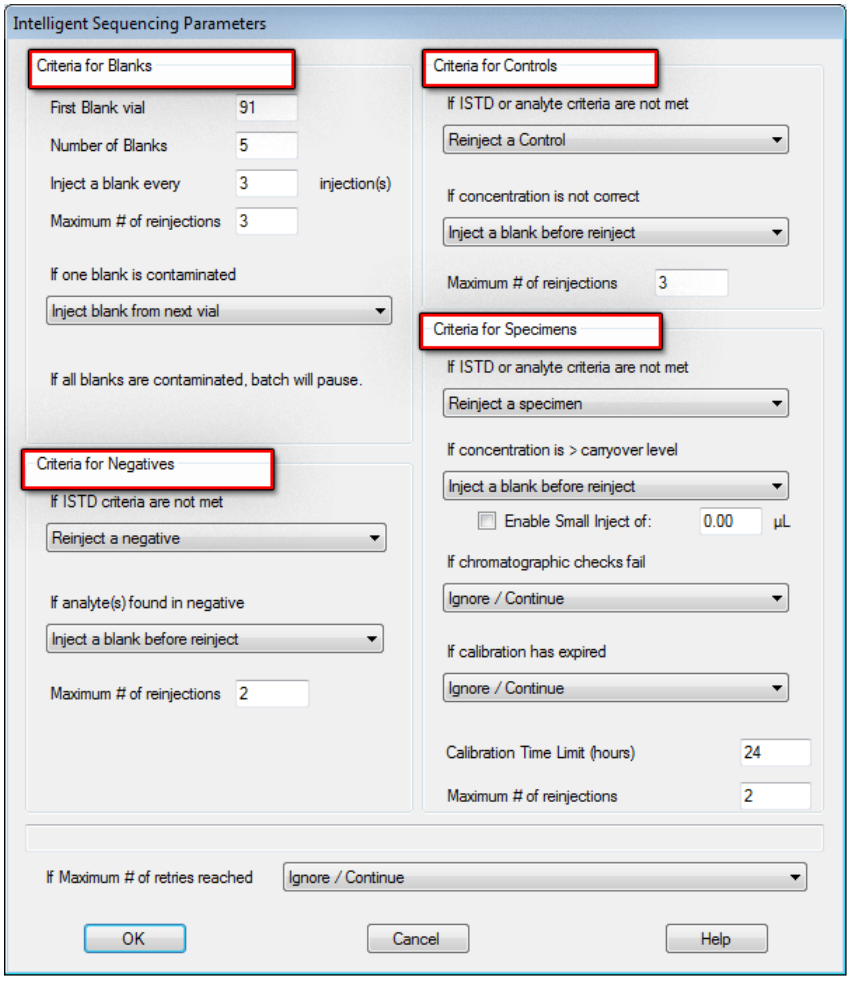
Details for how to complete the entries for each of the 4 sample types, highlighted in this example, are provided on the following pages:

- Blanks (See page 18)
- Negatives (See page 22)
- Controls (See page 22)
- Specimens (See page 28)

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

*See "Step 7: Enter your acceptable limits" on page 58 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 dialogs presented in the Data Analysis portion of your method. See "Step 7: Enter your acceptable limits" on page 58.

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 18)
- Negatives (See page 22)
- Controls (See page 22)
- Specimens (See page 28)

	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

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."*

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.

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.

MSD ChemStation Data 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 Maximum area/height count for blanks

  or...

- Finding a specified ISTD in a concentration greater than what is specified in the Minimum area/height count for ISTD

See "Step 7: Enter your acceptable limits" on page 58.

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 21 shows a flow chart of this process.

Intelligent Sequencing Parameters for Blanks

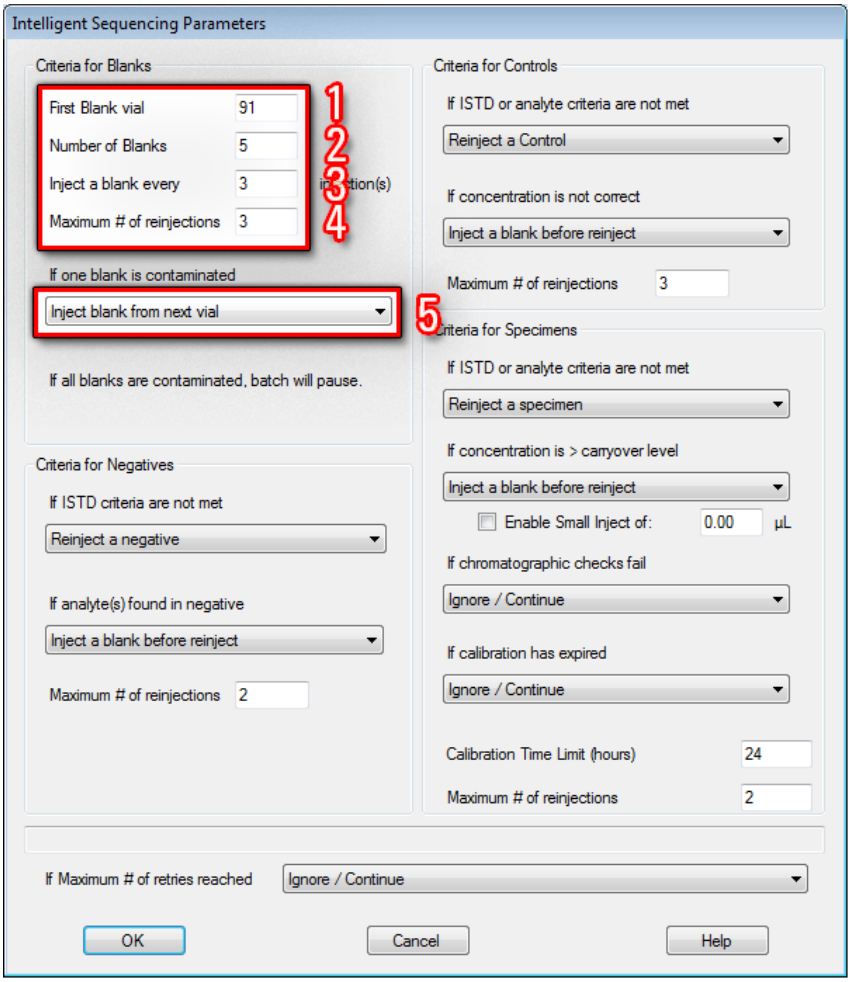
Highlighted fields are described below.

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 20 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 54 for details.

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

Automatic reset of the Blank cycle counter

Be aware:

1. 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 place blanks in any location in the sequence table. 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 22, and "How Intelligent Sequencing Processes Specimens" on page 28 for more details.
2. 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.

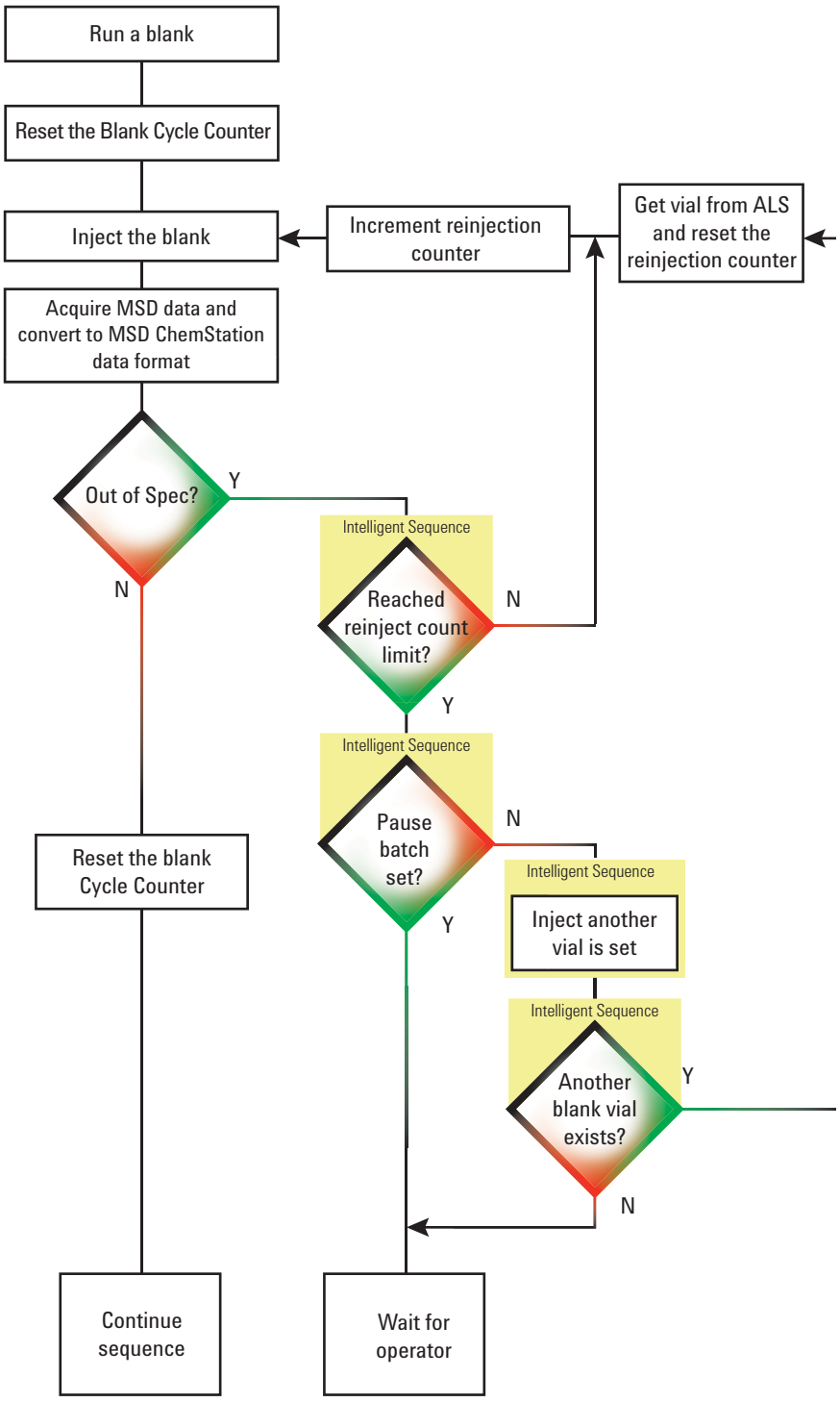


Figure 1 Blank Sample Processing Using Intelligent Sequencing

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 the MSD ChemStation Data 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: Enter your acceptable limits" on page 58.

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).

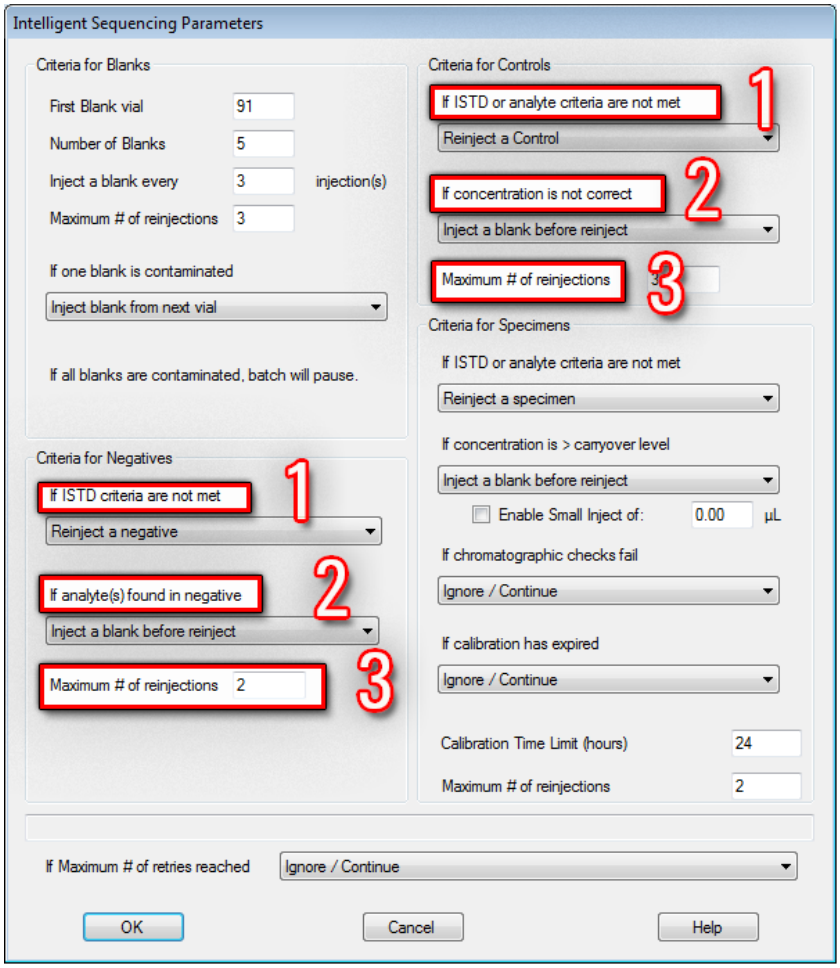
Figure 2 on page 24 shows a flow chart of this processing.

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 drop-down 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: Enter your acceptable limits" on page 58.



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.

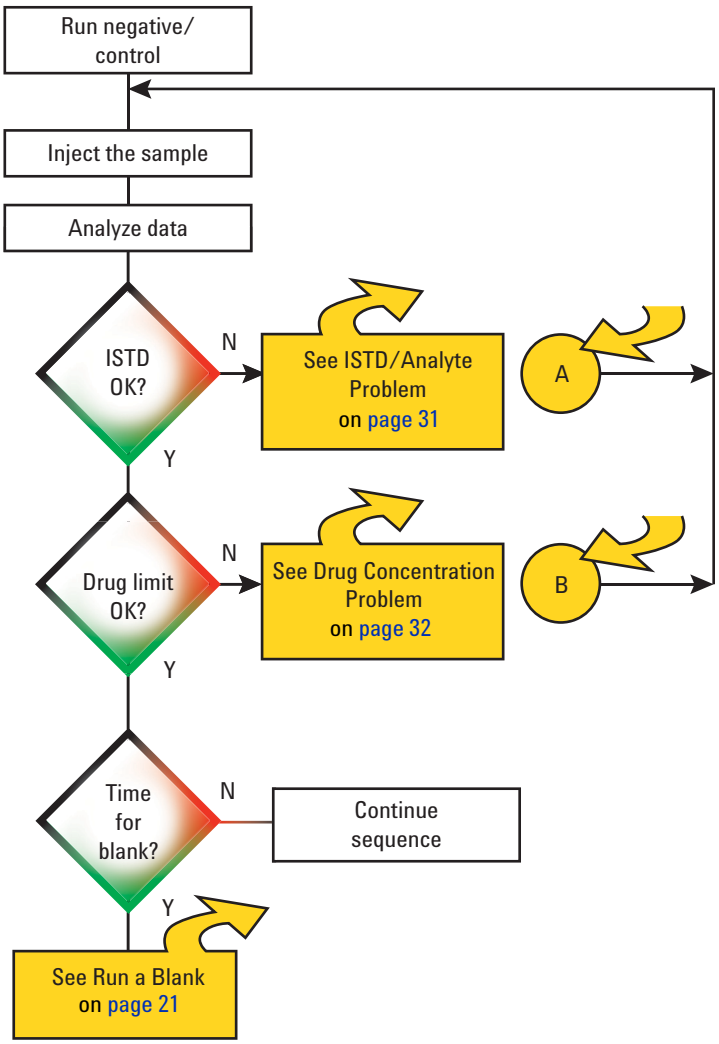


Figure 2 Negative and Control Sample Processing Using Intelligent Sequencing

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 24.

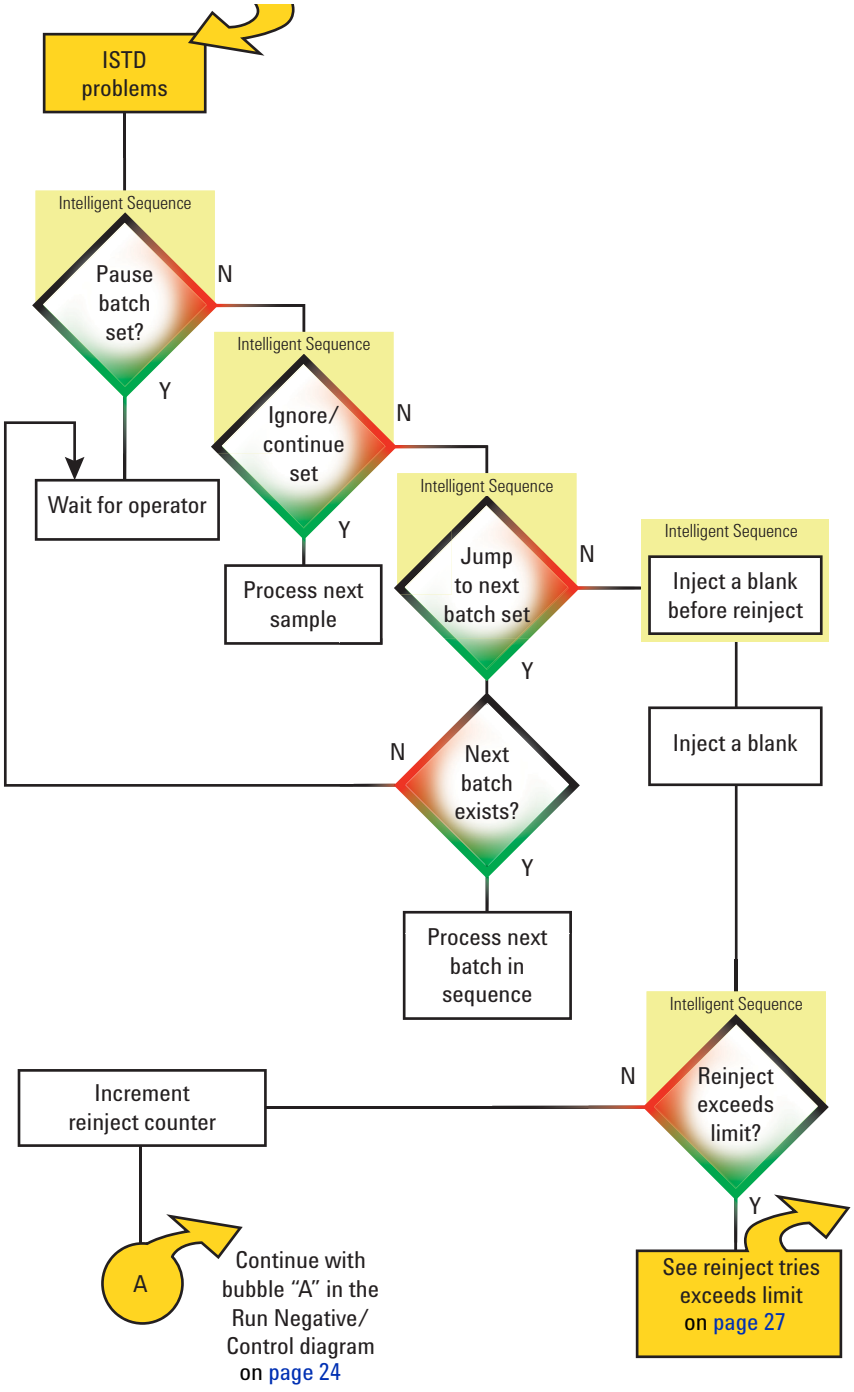


Figure 3 ISTD/analyte processing using Intelligent Sequencing

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 24.

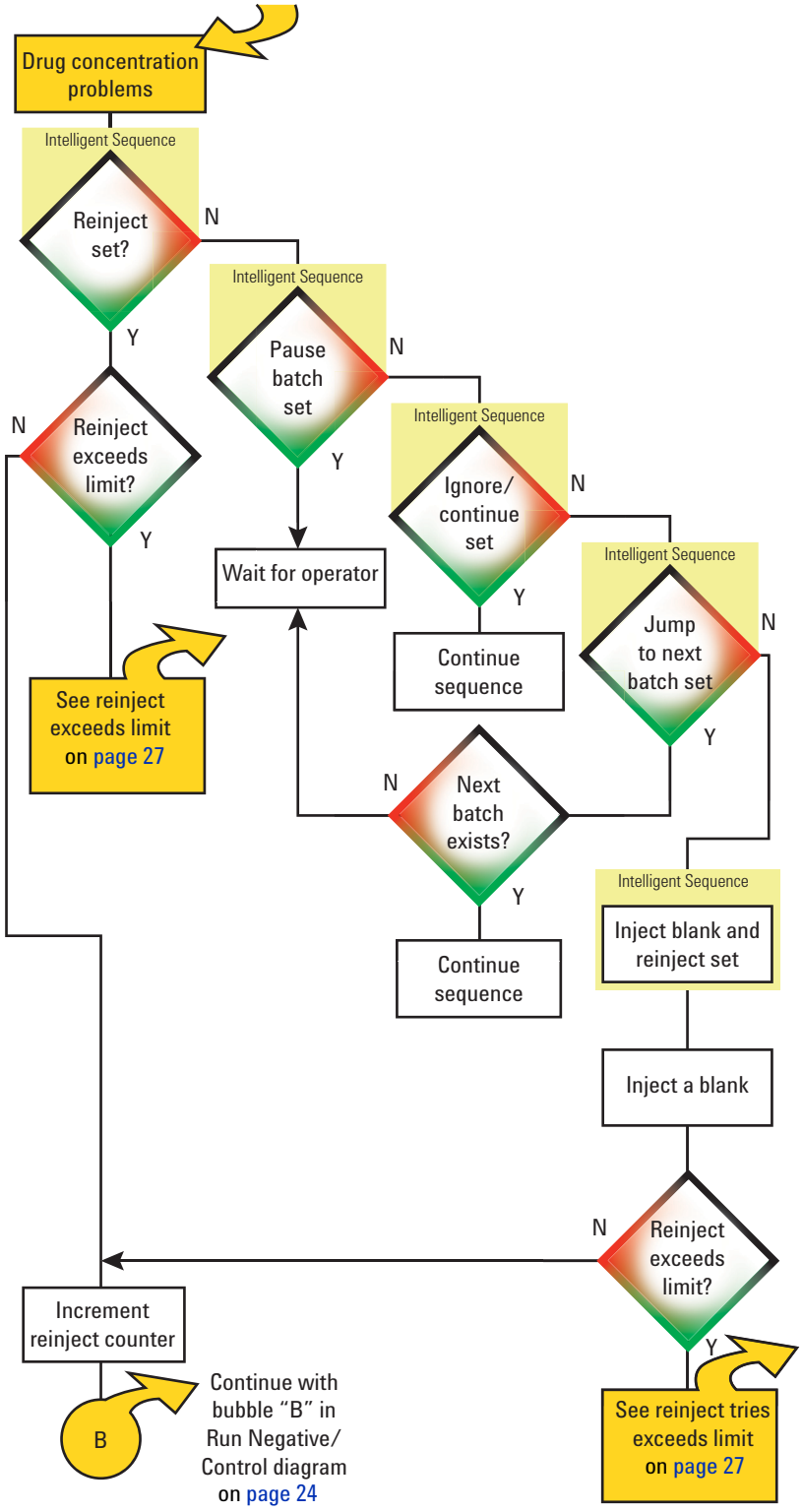


Figure 4 Drug concentration problem processing using Intelligent Sequencing

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.

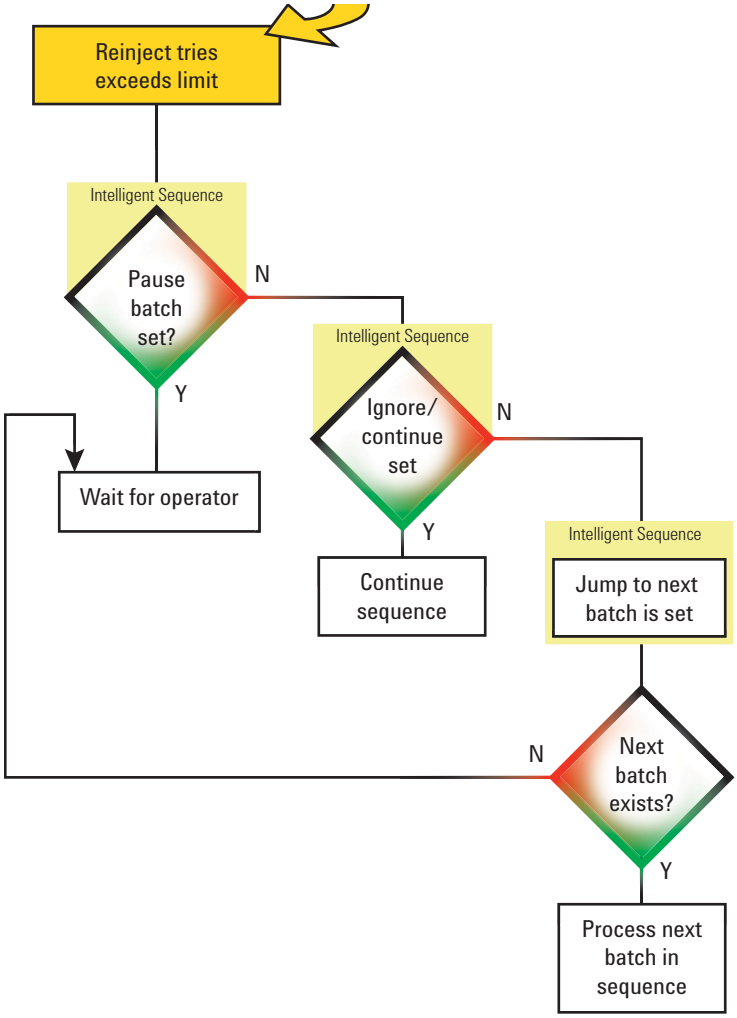


Figure 5 Reinject problem processing using Intelligent Sequencing

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, the data file is converted to MSD ChemStation Data Analysis data file format and sent to the MSD ChemStation Data Analysis for analysis. For a Specimen sample the MSD ChemStation Data Analysis 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 31 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 32 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 33 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 34 shows a flow chart of this process.

Finally it checks the **Maximum # of reinjections** 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 27.

Intelligent Sequencing Parameters for Specimens

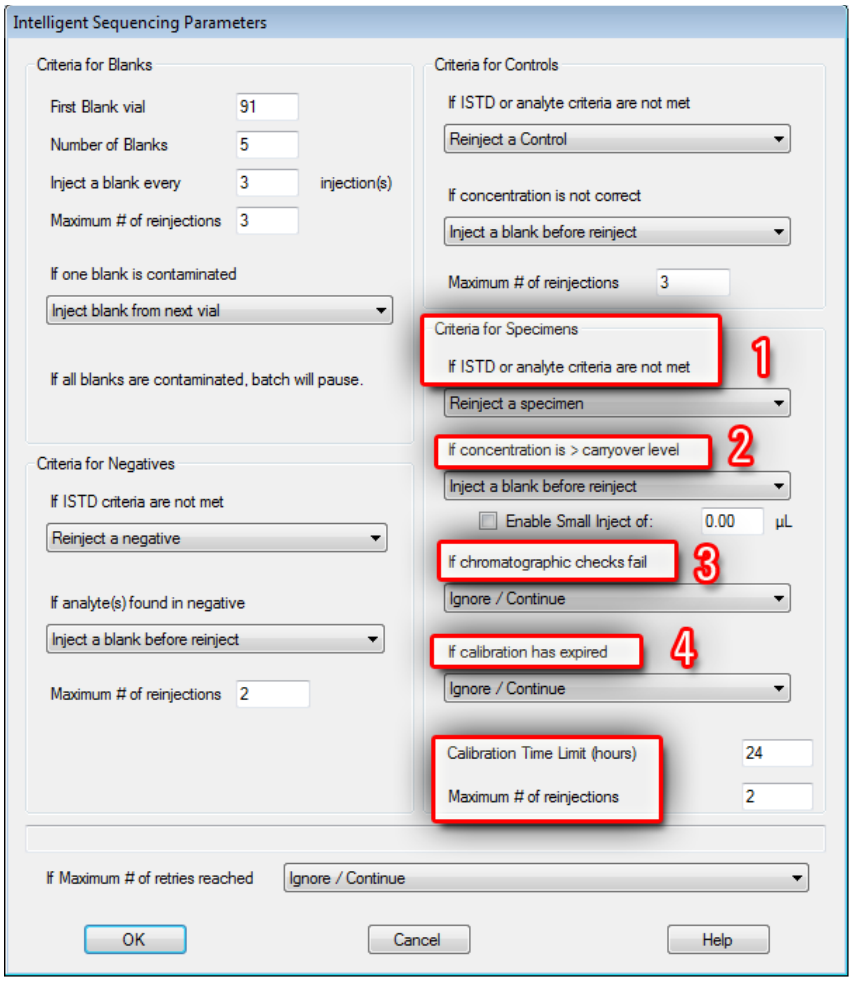
There are 6 areas to complete here. The options for sections 1, 2, 3, and 4 are shown in the table below.

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. The range is -1 to 9999. (-1 disables this parameter.)

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.

*Acceptable and unacceptable limits for internal standards and analytes are set in the Data Analysis portion of the method. See "Step 7: Enter your acceptable limits" on page 58.

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.

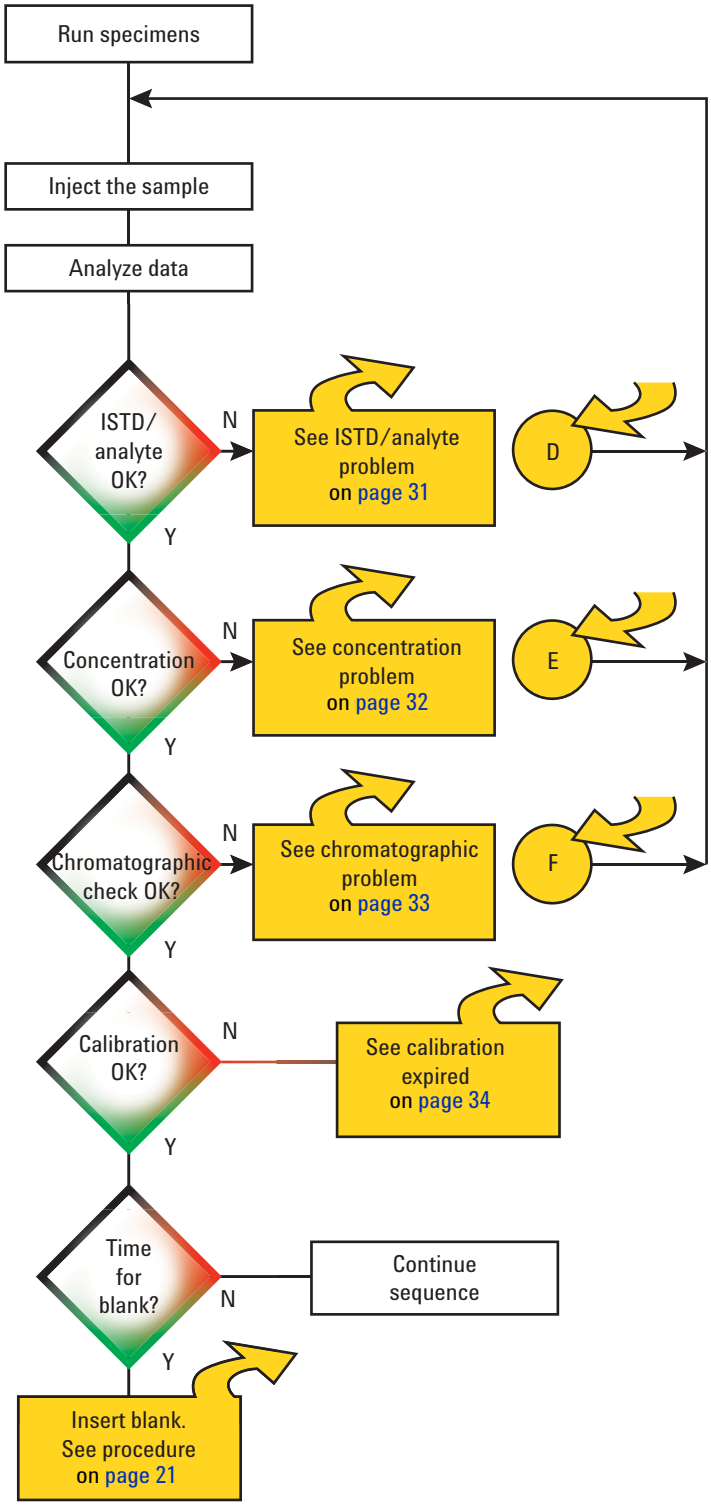


Figure 6 Specimen Sample Processing Using Intelligent Sequencing

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

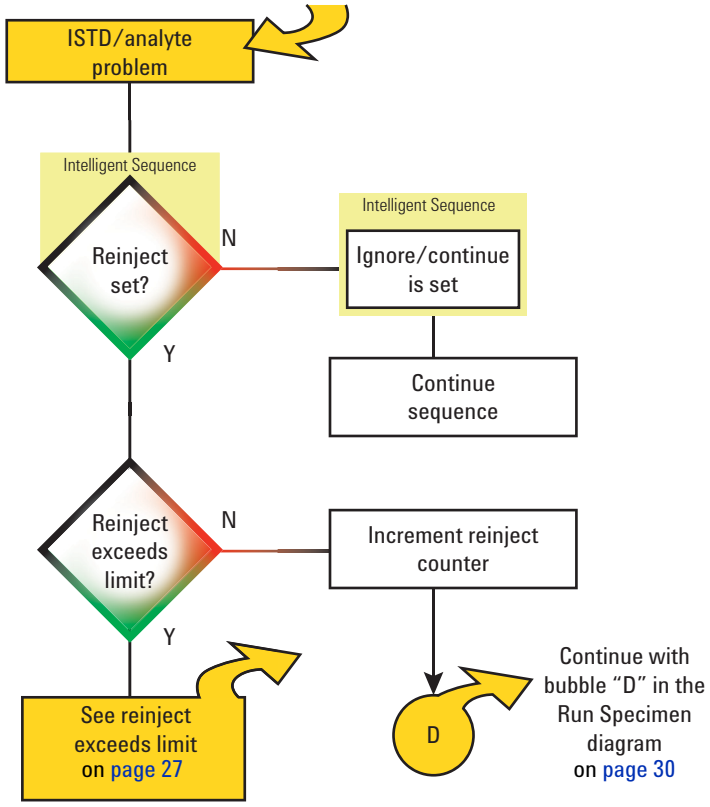


Figure 7 ISTD/analyte processing using Intelligent Sequencing

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

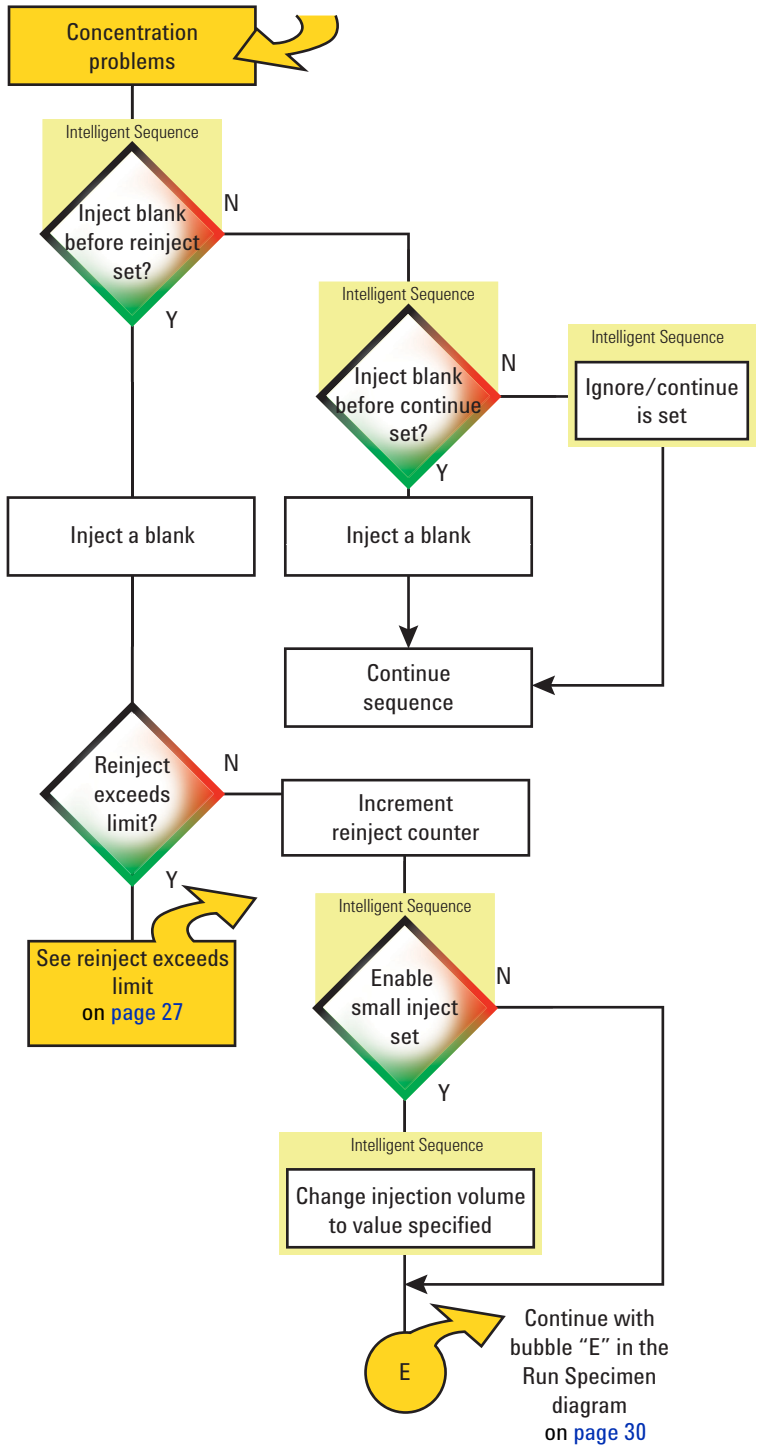


Figure 8 Drug concentration problem processing using Intelligent Sequencing

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.

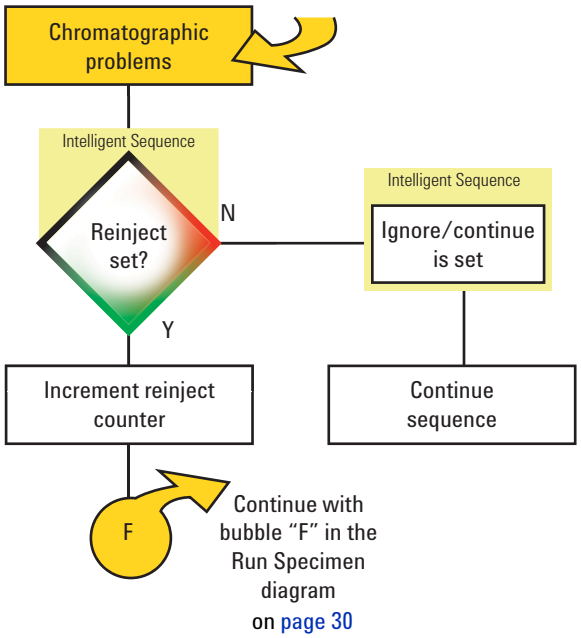


Figure 9 Chromatographic problem processing using Intelligent Sequencing

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

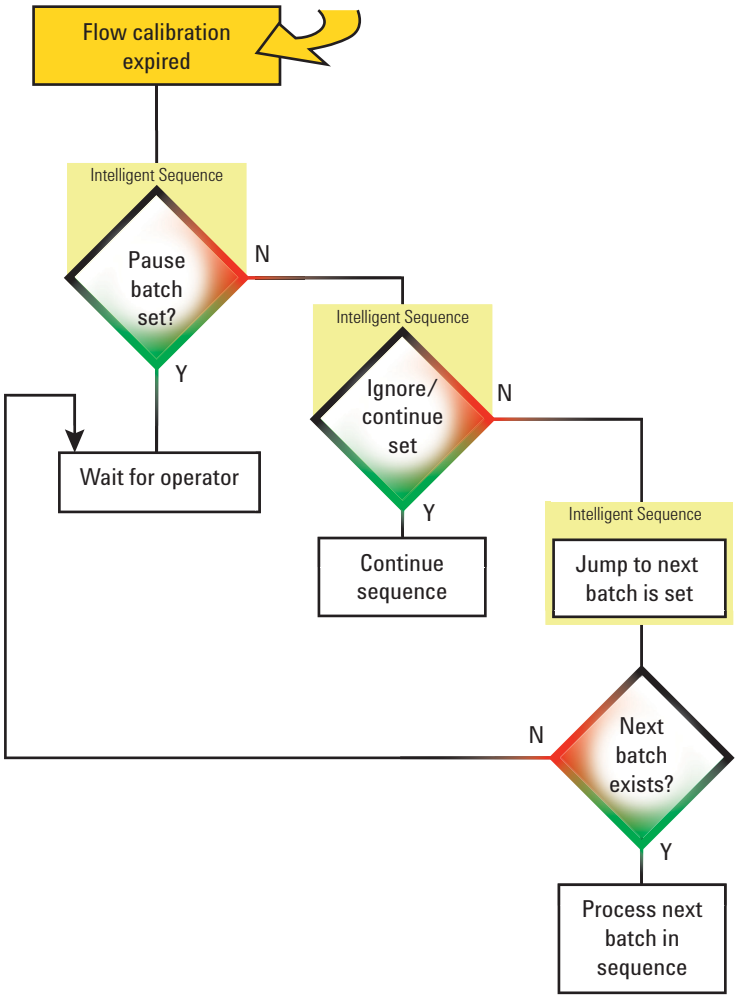


Figure 10 Calibration expired problem processing using Intelligent Sequencing

Maximum # of Retries

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

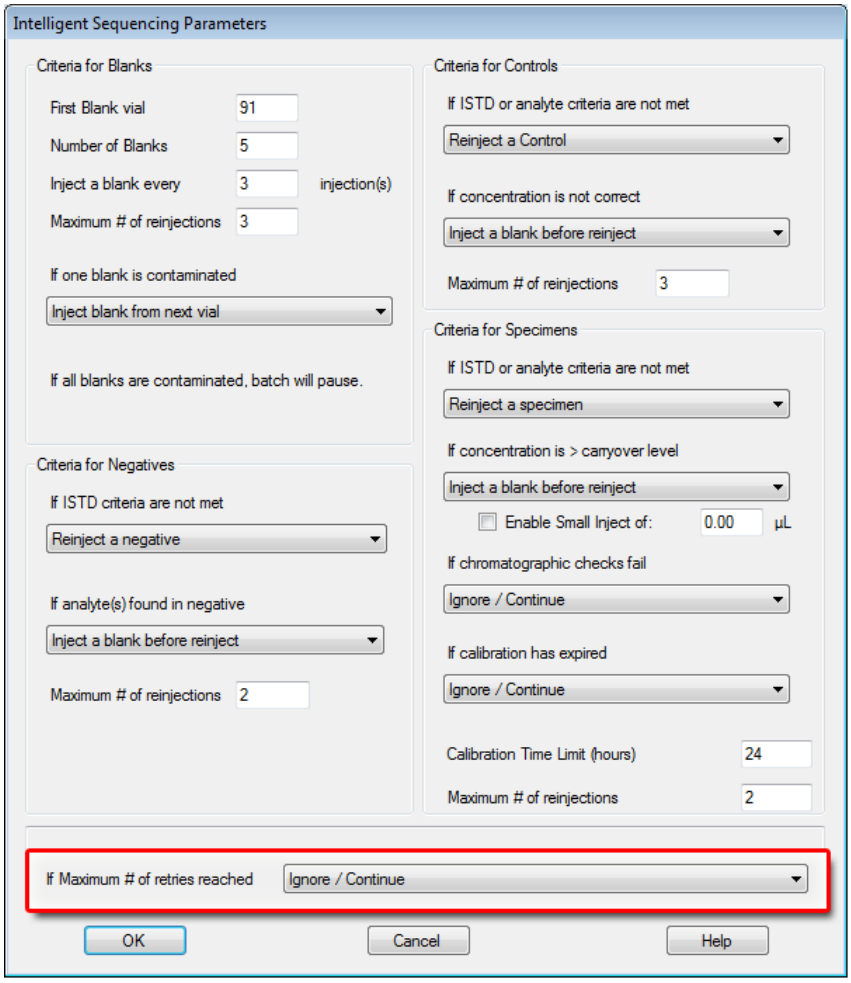
Here you will define what to do when any of the **"Maximum # of reinjections"** options is reached for:

- Blanks
- Negatives
- Controls
- Specimens

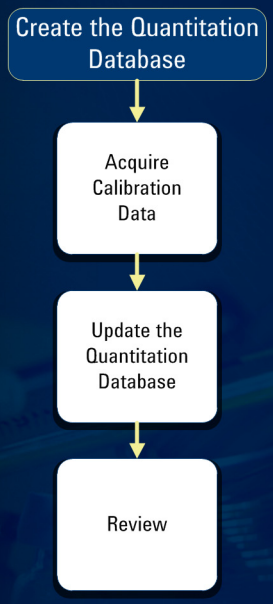
From the drop-down list box, select:

- **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 27 for a flow diagram of this process.



3
Create the Quantitation Database



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Overview

Before you create a Data Analysis method for use by Intelligent Sequencing, you must have a Data Acquisition method, optimized for your instrument, which will be used to acquire data for the drugs of interest.

Before being used to acquire data from specimens, this method will first be used to process calibration samples. These results are then added to the methods quantitation database to update the target and qualifier ions which are used to identify the drugs of interest, and the calibration curve, which is then used to quantitate the drugs analyzed.

The MSD ChemStation Data Analysis includes a set of quantitative analysis sample SIM methods for drugs of abuse. This is a good starting point for creating a quantative method for your analysis. These methods are located in the **MSDchem\msdemo\drugdemo** directory. Included are methods for:

- Amphetamines
- Cocaine
- Opiates
- PCP
- THC

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

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 positive in a specimen sample.

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

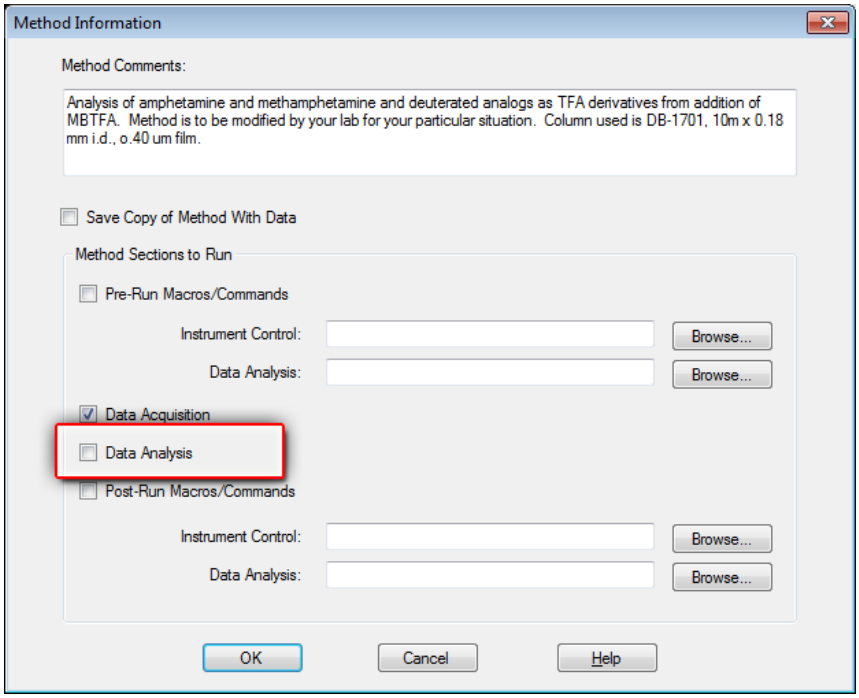
The following procedure describes how to edit one of the example methods for data acquisition of the calibration samples.

Step 1: Select a starting method and specify *Data Acquisition* only

1. Load one of the example methods in the **MSDchem\msdemo\drugdemo** directory (the one for Amphetamines (AMP.M) for example.

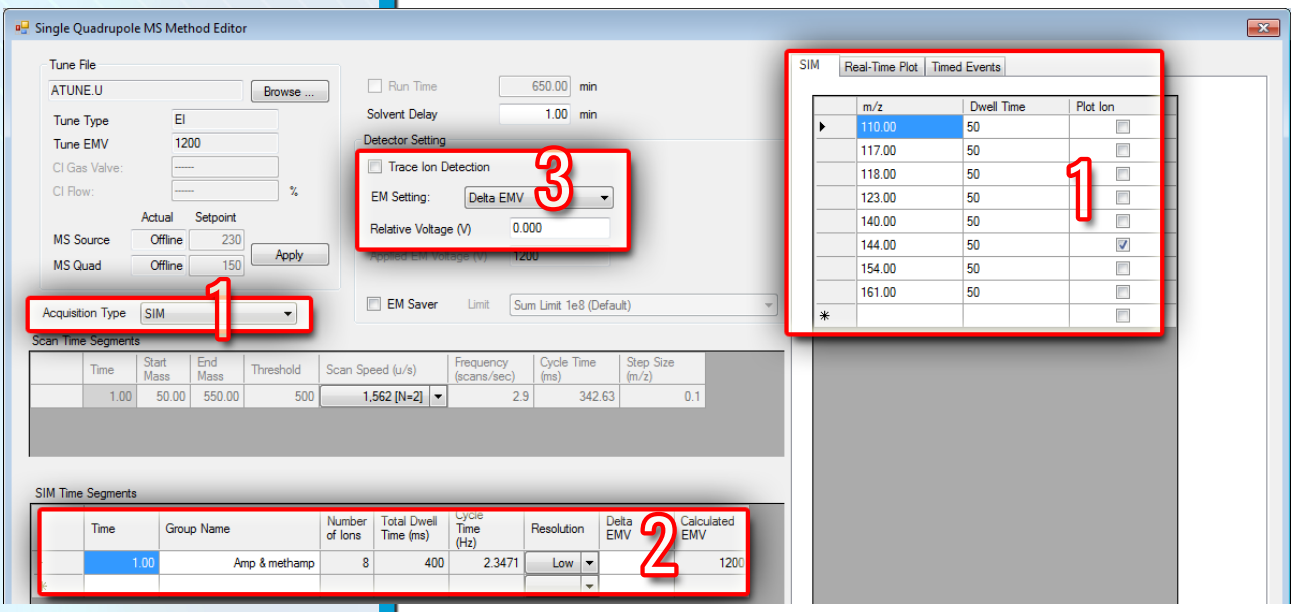
2. Disable Data Analysis for the run. Since the quantitation database does not yet specify the calibration levels needed for the drugs of abuse for which you are screening, you do not yet want to run the data analysis portion of the method.

   To disable data analysis for the run, in MassHunter GCMS Acquisition, select **Method/ Edit Method Information**, then clear the **Data Analysis** selection, and **Save** the method.



Step 2: Review the data acquisition parameters

1. Click the MS Parameters icon to access the Single Quadrupole MS Method Editor (MSME). Here you will see the MS acquisition parameters for the method you loaded. (The AMP.M method in this case.)
2. Update these settings, as well as the setting for the GC method parameters (See "Step 5: Complete the standard Instrument Acquisition dialogs" on page 55.) as required for your specific instrument and method, then save your method.



In this example SIM method, the ions specified are:

- 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.

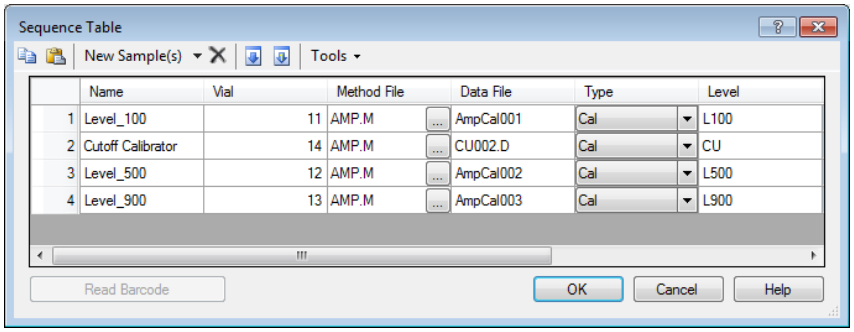
Note that, by default, the supplied method specifies a detector EM Setting in Delta EMV. However, today the preferred EM settings are expressed as a Gain Factor. To change this setting, click the drop-down listbox and select Gain Factor.

Step 3: Create the calibration run Sequence Table

1. Click the Edit Sequence icon in the Instrument Control window to open the STE.
2. Modify this table as appropriate to define your calibration samples.
3. Click **OK** to continue.

After optimizing the Data Acquisition parameters for your method, set up the calibration run.

The Sequence Table Editor (STE) is used to specify the processing of samples in a Batch.



The example Sequence Table shown here, is specifying:

Name - The name of each of the 4 samples in this Run

- 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.

Vial - The location of each of these vials in the autosampler tray (locations 11 through 14)

Method File - The method to use for each sample. In this case, the instrument method parameters are stored in the AMP.M method, so that is the method listed to be used for each sample.

Data File - Where the acquired data from the acquisition run is to be stored. In this case it will go into data files named AmpCal001, CU002, AmpCal002, and AmpCal003.

Type - The **Type** of each sample. **CAL** for each of these samples.

Level - The Calibration Level ID in the MSD ChemStation Quantitation method for this calibration sample.

Step 4: Prepare the calibration samples

Prepare calibration samples for creating a calibration curve of each drug of abuse compound in the Quantitation database.

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 calibration 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. Here those concentrations are 100, 250 (Cutoff calibrator), 500, and 900 ng/mL for both Amphetamine and MethAmphetamine. The cutoff calibration sample is also one of these points on the calibration curve.

Step 5: Prepare the cutoff 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.

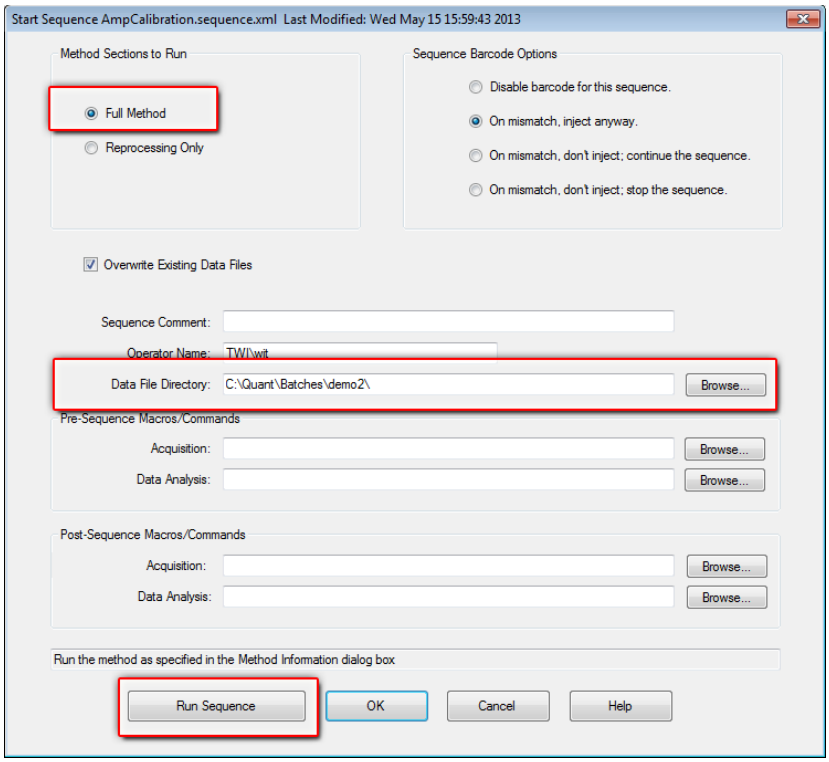
Spike the calibration sample with those ISTD compounds as done with the other calibration samples.

Step 6: Run the calibration Sequence Table

1. Click the Run Sequence icon to being this run.
2. On the **Start Sequence dialog** select **Full Method** and enter a batch subdirectory for your acquired data files.
3. Click **Run Sequence** to begin the automated acquisition.

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

This example assumes use of an automatic liquid sampler (ALS) which would require the 4 sample vials be placed in the sampler tray vial locations specified in the STE (The STE is illustrated in "Step 3: Create the calibration run Sequence Table" on page 41).



Step 7: Update the CU Level in the Quantitation Database

The quant database contained in the Amp.M method used in this example is modified by adding a calibration curve and also by updating the CU level parameters required for storing the cutoff sample data acquired for your instrument in your method. A review of the MSD ChemStation's Quant Database is covered later.

The cutoff level for a drug of abuse is the concentration below which the presence of a drug in a specimen is considered negative.

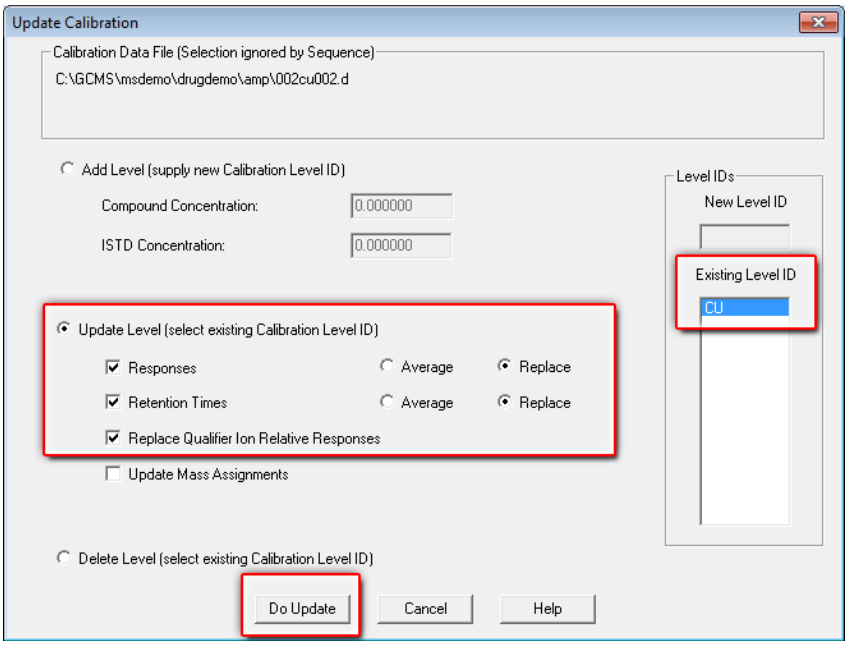
For example, using a validated GC/MS analysis, the HHS confirmatory test cutoff concentration for Amphetamine and Methamphetamine are both 250 ng/mL. If the concentration for these amphetamines in the specimen is below this value, the drug test is negative for amphetamines.

The acquired abundance and ion ratio identity for the compounds in this cutoff concentration must be present in the quantitation database to use during sample analysis.

The CU level is already set up for when you use the Amp.M method file provided with the MSD ChemStation Data Analysis. However, you need to update the parameters for this CAL level in Amp.M for your GC/MSD system. To do so:

1. Load the acquired data file for the cutoff sample into MSD ChemStation Data Analysis.
2. From the MSD ChemStation Data Analysis program click the Update Calibration dialog icon.

3. Select **Update Level**, and set the parameters as shown in this example.
4. Click **Do Update** to add this calibration data to the Quant database.



Step 8: Add CAL Levels to the Quantitation Database

1. Click the Update Calibration icon. The loaded file is shown as the Calibration Data File name displayed at the top of the dialog.

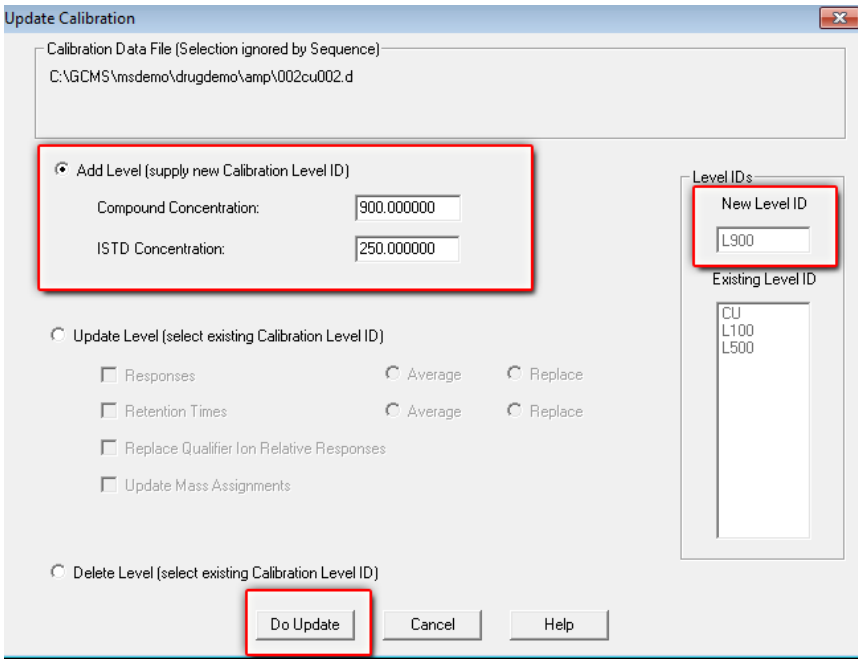
2. Select **Add Level (supply new Calibration Level ID)** and enter the known concentration in ng/mL for the drug compounds and ISTDs in this sample.

3. Enter the name for this first level (maximum of 4 characters) into the **New Level ID** parameter (e.g., L900). This exact name must be entered in the MassHunter STE Level column whenever you update these calibrations in the future.

4. Click **Do Update** to add this calibration data to the quantitation database and display the Compound Edit dialog.

5. Later we will look at the Compound Edit dialog but for now, click **OK** and save this new level entry, and exit the quantitation database Compound Edit dialog.

Load the first acquired CAL sample into MSD ChemStation Data Analysis. The calibration information in this data file will be added to the quantitation database using the Update Calibration dialog.



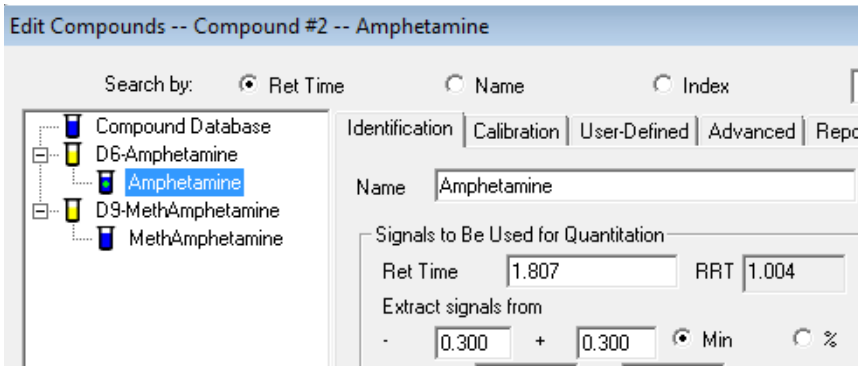
In this example we are adding a 4-point calibration curve to the method. Add the calibration data for the other two calibration points to the Quant Database as explained above.

Step 9: Review entries in the Quant Database UI

1. From the MSD ChemStation Data Analysis program, click the Quantitation database icon.

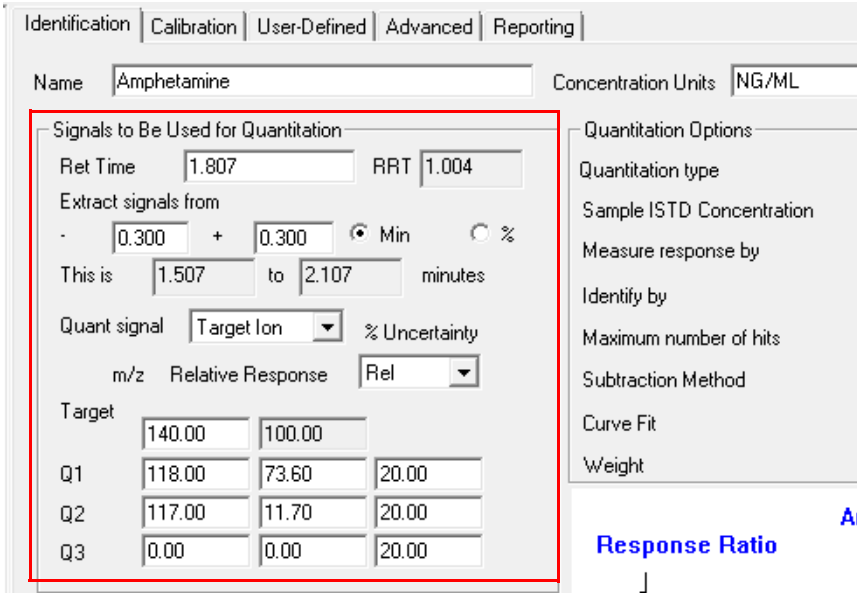
2. Select an ISTD or drug compound in this directory and its quantitation parameters are displayed.

Notice the compound directory tree on the left is structured by ISTD with drug compounds referencing this ISTD indented below each.



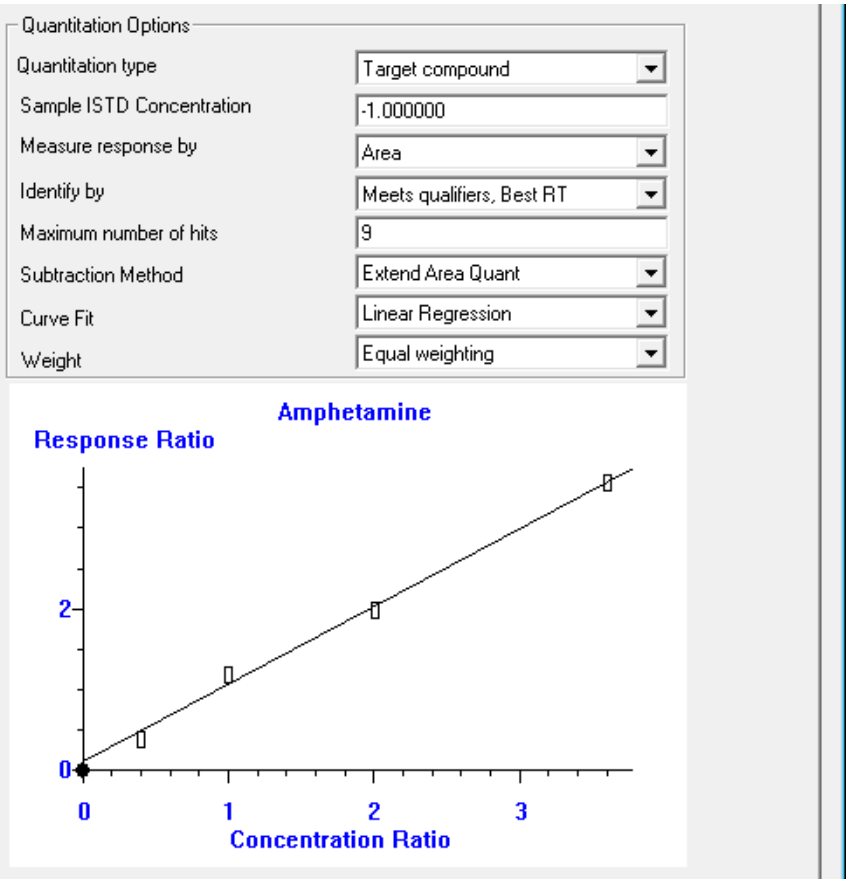
The **Signals to Be Used for Quantitation** shows the retention time (RT) and its window. This RT value and the ion ratios were replaced when you updated the CU level in the previous section. In the original Amp.M method, the target and qualifier ion values were specified, the Quant signal was set as target ion only, and the % uncertainty settings were defined.

3. Modify the **Signals to Be Used for Quantitation** entries, if required by your optimized acquisition method.



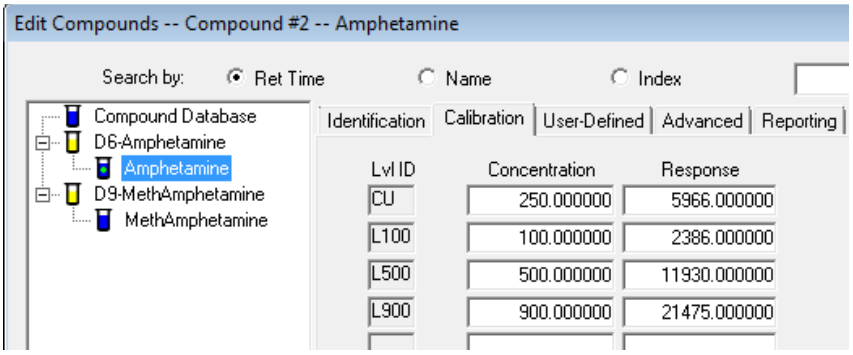
4. Review the Quantitation options.

The parameter settings for the **Quantitation Options** are all from the original MSD ChemStation's Data Analysis Amp.M example method. The Calibration curve shown is a plot of the ratios of concentration versus detector response of the 4-point calibration curve shown on the Calibration tab. Here our example specifies using Area for **Measure response by** for Amphetamine.

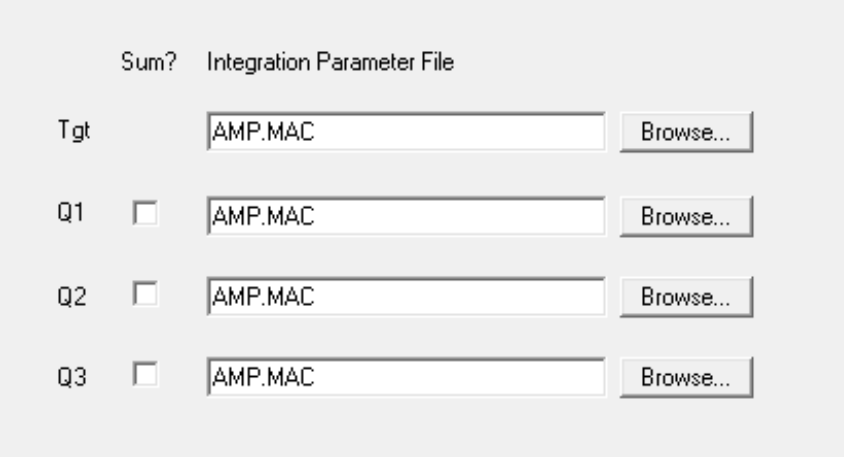


5. Select the **Calibration** tab to view the data points on the calibration curve.

The concentration and response for the CU level ID were updated for your acquired cutoff sample. The other levels were added to hold the data for the remaining entries in the 4-point calibration.



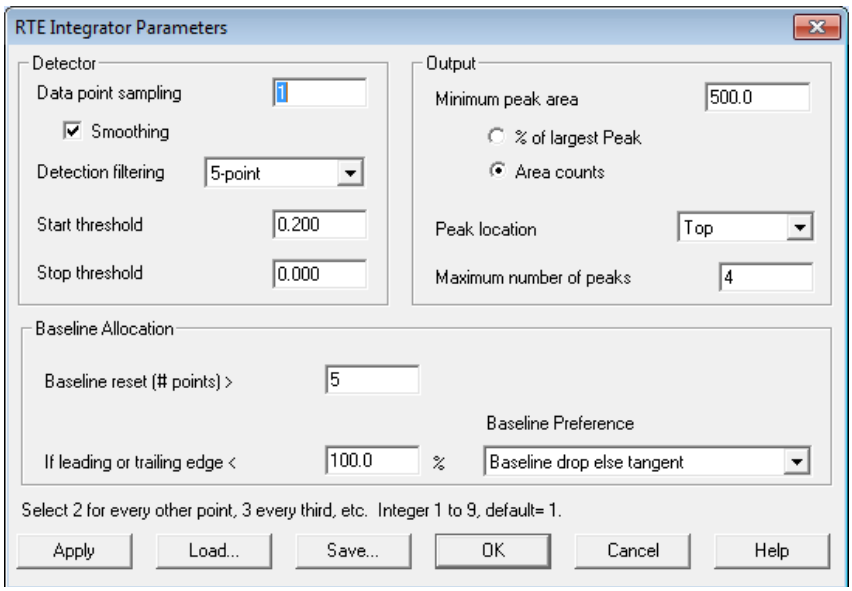
6. Select the **Advanced** tab to view the custom integration parameter files included with the Amp.M example method for integration of the target and its qualifier ions.



7. Exit the editor and examine the parameter settings contained in the AMP.MAC file.

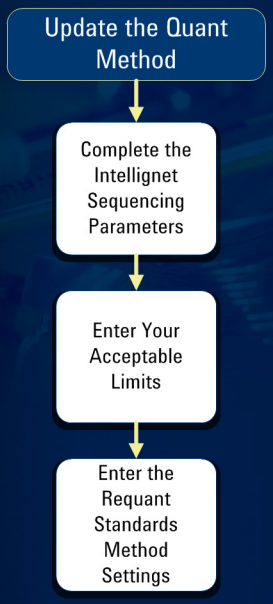
   Click the RTE Integration Parameters icon, then click **Load...** to load the AMP.MAC file and display its parameters in the dialog.

8. If required by your optimized method, edit these parameters and save this file with the method.



You now have a data acquisition and quantitative analysis method suitable for identification and quantitation for these drugs.

4
Update the Quantitative Analysis Method



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Step 1: Load a method with a valid Quant database

1. In MassHunter GCMS Acquisition, select **Methods/Load and Run Method...** and navigate to and select the method you wish to use.
2. Click **OK** to close the dialog.

Step 2: Select the parts of the method to edit

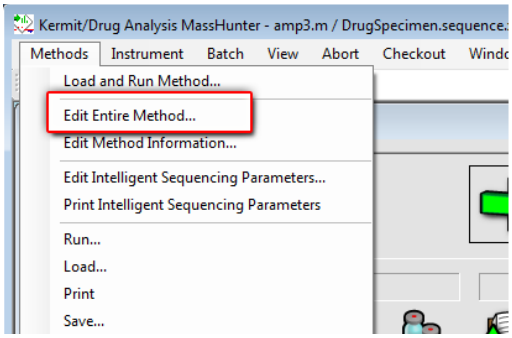
1. Select **Methods/Edit Entire Method**.
2. Check each item listed.

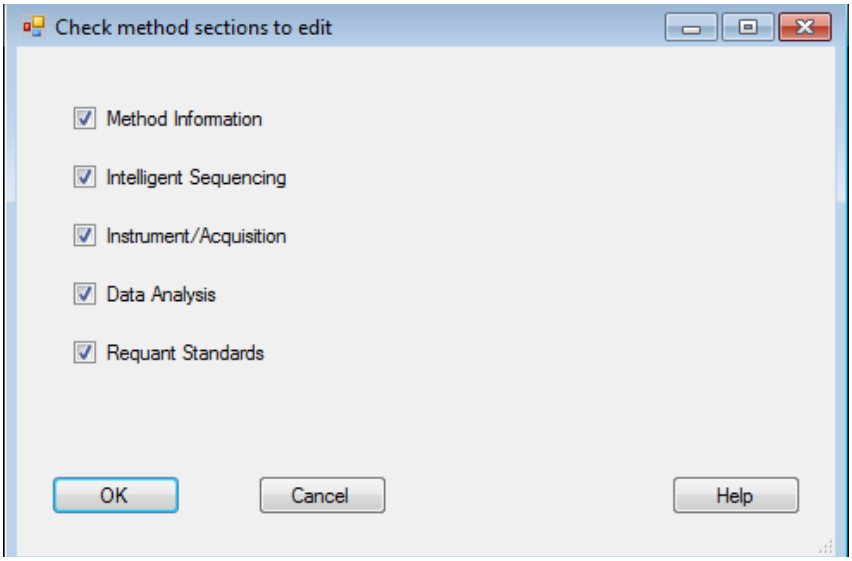
After you add the calibration curve to the quantitation database, you can update your method and add the intelligent sequencing parameters, as described here.

In our example, we will load the method containing the quant database we created in the last chapter.

Be sure to load a Quantative Analysis Method with a valid quant database. If you do not, when you reach the Data Analysis portion of the method, you will receive an error message.

During this process we will cover every aspect of Data Analysis methods.



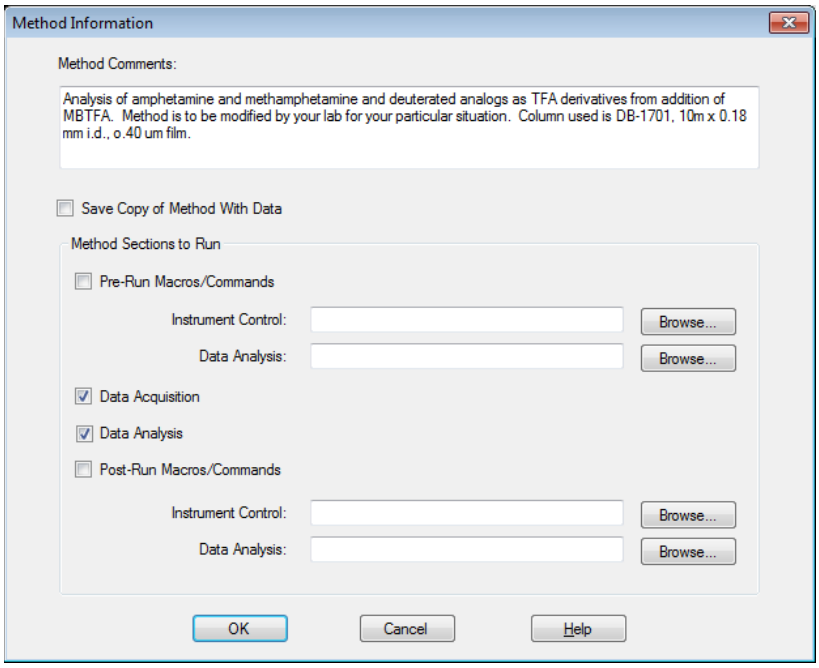


NOTE Intelligent Sequencing and Requant Standards are only available when running MassHunter GCMS Acquisition in the Drug Analysis Workflow mode.

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. Under **Method Sections to Run** select **Data Acquisition** and **Data Analysis**.
4. Click **OK** to display the Intelligent Sequencing Parameters dialog.

This dialog is completed in the same way for all MassHunter Modes, and is described in the MassHunter Familiarization guide found in online Help. Please refer to it 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

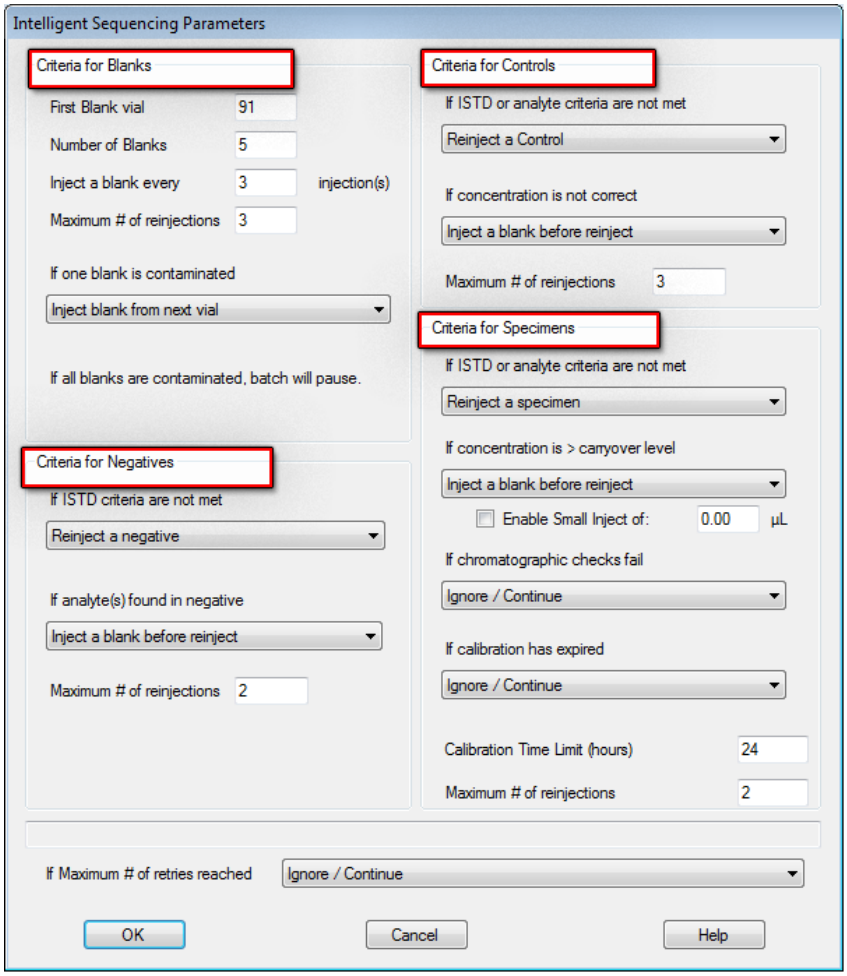
1. Complete the entries for each of the 4 sample types. See the following pages for more details on how to complete these entries:
   - Blanks (See page 18)
   - Negatives (See page 22)
   - Controls (See page 22)
   - Specimens (See page 28)
2. Click **OK** to display the Inlet and Injection Parameters dialog.

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

Included are criteria for 4 sample **Types**:

- Blanks
- Negatives
- Controls
- Specimens

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



Step 5: Complete the standard Instrument Acquisition dialogs

The next 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., Ehanced, 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.

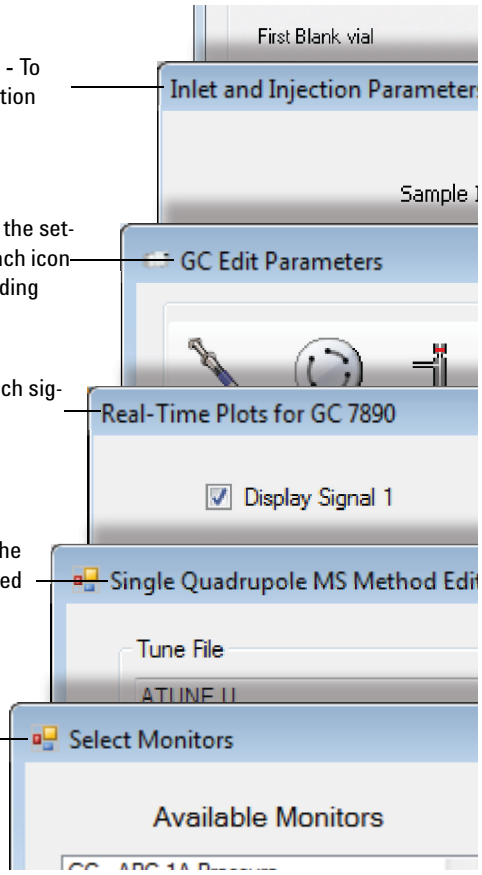
Inlet and Injection Parameters dialog - To select the sample type, inlet, and injection source.

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.

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

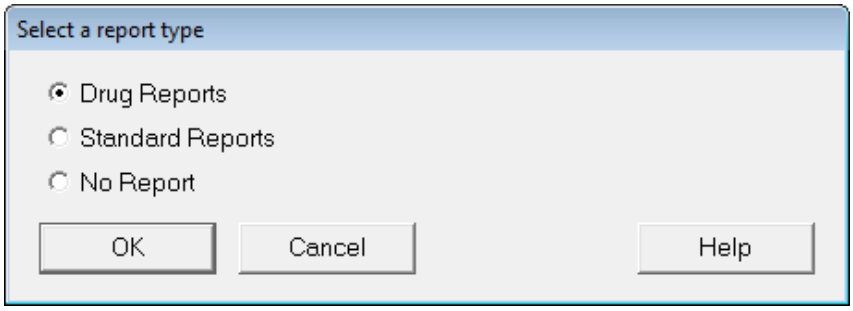


Step 6: Select your report type and format

1. Select a report type and click **OK** to continue.

   In this example we selected **Drug Reports**.

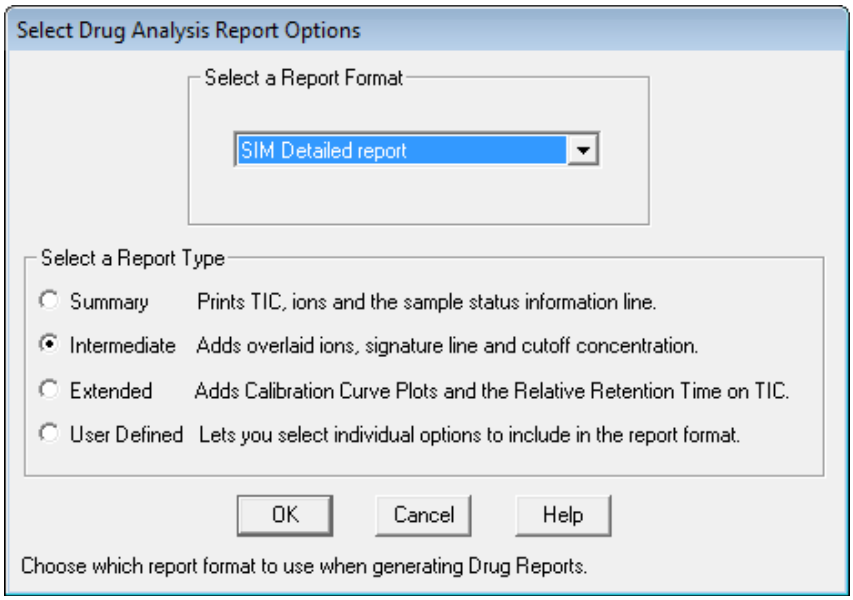
This is the first screen for the Data Analysis part of the method.



Since our example is using drug reports we are presented with the **Select Drug Analysis Report Options** dialog.

2. From the **Report Format** drop-down list, select the report format you would like.

   - If your method specifies SIM mode, only the SIM formats are listed.
   - In Scan mode, both SIM and Scan formats are given in the drop-down list.



NOTE This dialog is also accessed when you select **Reports / Set Report Options** in MSD ChemStation Data Analysis.

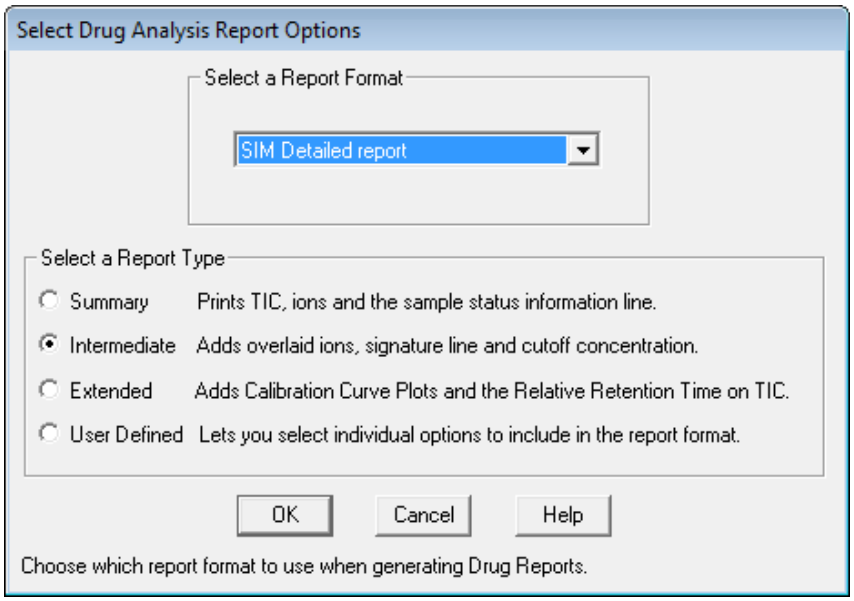
Report formats available in the **Select a Report** drop-down include:

- **SIM single drug / vertical ions**
  Reports the TIC and the selected ion chromatograms for the target compound and the ISTD. The selected ions are arranged vertically.
- **SIM single drug / horizontal ions**
  Reports the TIC and the selected ion chromatograms for the target compound and the ISTD. The selected ions are arranged horizontally.
- **SIM detailed report**
  Includes a full report with calibration information.

3. Select the level of detail you would like from the **Report Type** area. (Intermediate is selected in this example.)

4. Click **OK** to display the next data analysis section dialog.

- **SIM multi-compound report**
  Used when there is more than one compound and one ISTD. Report shows the TIC and the selected ion chromatograms for each compound. This format is a thumbnail format and is not the only report you can have if you have multiple compounds. It is just a more condensed version of the report, designed to maximize output and save paper.
- **Unknown Scan / Search / ISTD / Ions**
  In Scan mode, reports the TIC along with spectra of the unknown, top library hit, and ISTD; extracted ion chromatograms are displayed vertically
- **Unknown Scan / Search / Results / Ions**
  In Scan mode, reports the TIC along with spectra of the unknown and top library hit, tabulated library search results; extracted ion chromatograms are displayed vertically.
- **Scan Multi-Compound Report**
  In Scan mode, reports the top 3 hits from the library search for all integrated peaks in the TIC.
- **Blank Sample Report**
  Used in interactive mode only to produce a special report for blank sample types. Do not use this format when setting up the method. The blank report format is used automatically for blank samples during a run.



Step 7: Enter your acceptable limits

In the following series of dialogs, you will enter your acceptable limits for:

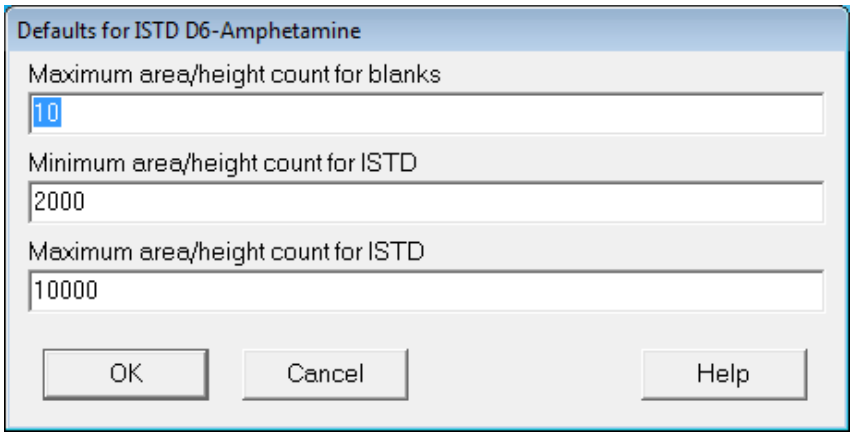
- Internal Standards
- Compounds
- Controls

Limits for Internal Standards

The first dialog that displays is for the first Internal Standard in your quant database.

This dialog is followed by dialogs where you will enter the Default for each drug Compound that references this ISTD.

If other ISTDs and referenced drug compounds are in the database this process is repeated until finished.



1. Set the **Maximum area/height count for blanks**. This is the maximum you will allow of this internal standard in blanks (which should contain no internal standard). (**10** in this example.)

2. Set the **Minimum area/height count for ISTD** (**2000** in this example).

3. Set the **Maximum area/height count for ISTD** (**10000** in this example).

When analyzing a Blank, the **Maximum area/height count for blanks** is the maximum acceptable count of this Internal Standard that may be detected before the blank is considered contaminated. If contaminated, the blank must be processed according to the settings in Intelligent Sequencing.

When analyzing any sample that contains this Internal Standard, the **Minimum area/height count for ISTD** is the minimum acceptable count for the presence of this ISTD. If the count is below this amount, the sample is outside the limits you set, and must be processed according to the settings in Intelligent Sequencing.

When analyzing any sample that contains this Internal Standard, the **Maximum area/height count for ISTD** is the maximum acceptable count for the presence of this ISTD. If the count is above this amount, the sample is outside the limits you set, and must be processed according to the settings in Intelligent Sequencing.

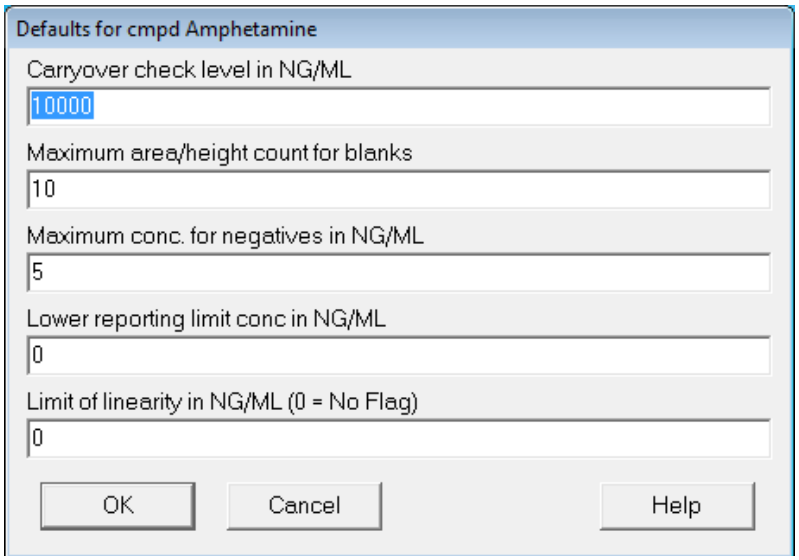
Limits for Compounds

1. Set the **Carryover check level in NG/ML**. (**10000** in this example.)
2. Set the **Maximum area/ height count for blanks**. (**10** in this example.)
3. Set the **Maximum conc. for negatives in NG/ML**. (**5** in this example.)
4. Set the **Lower reporting limit conc in NG/ML**. (**0** in this example.)
5. Set the **Limit of linearity in NG/ML (0 = No Flag)**. (**0** in this example.)

In this first dialog, enter the acceptable limits for the first compound you are screening for.

This is for the first compound associated with the Internal Standard described in the previous dialog (D6-Amphetamine).

A similar dialog will come up for each compound you have associated with that internal standard.



Carryover check level in NG/ML triggers the system to look for Excessive amount of compound. When analyzing any sample, If the calculated concentration of the drug of interest (Amphetamine in this case) is above this check level, the sample is considered overloaded and will be processed according to the criteria you set for Specimens in the Intelligent Sequencing Parameters dialog.

Maximum area/ height count for blanks sets the maximum acceptable count of the drug of interest in a blank (Amphetamine in this case). If the count of the drug of interest in a blank injection is above this limit, the blank is considered contaminated, and will be processed according to the settings in Intelligent Sequencing.

Maximum conc. for negatives in NG/ML sets the maximum concentration level of the drug of interest in a Negative (Amphetamine in this case). If the concentration exceeds this level, the sample will be processed according to the settings in Intelligent Sequencing.

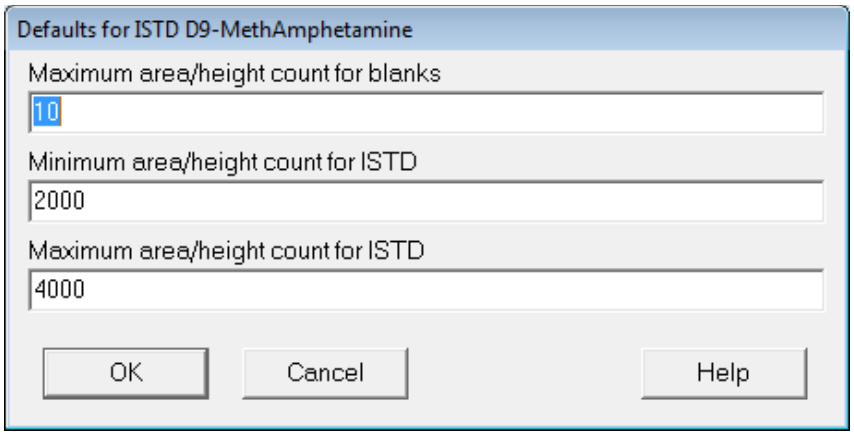
Lower reporting limit conc in NG/ML sets the lowest concentration level that will be reported for QC Summary Reports only. If the concentration level is below this value, it will not be reported.

Limit of linearity in NG/ML (0 = No Flag) sets the limit of linearity (LOL) that will be reported.

The next 2 dialogs are similar to the previous 2 but this time they are for methamphetamines.

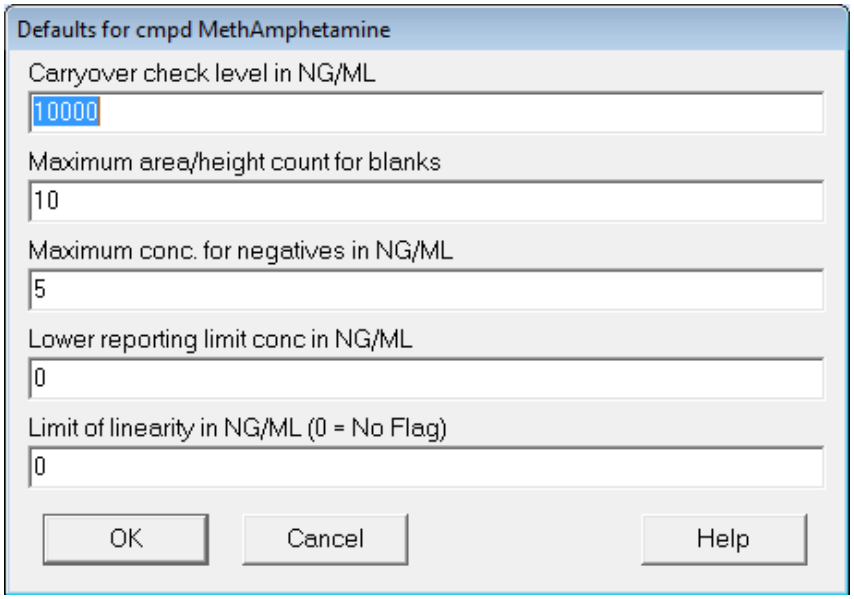
Defaults of the Internal Standards for the second compound

Complete this dialog in the same way as you did the previous compound.



Defaults for the second compound

Complete this dialog in the same way as you did for the first drug compound.



If other compounds reference the second ISTD, their default dialogs are now displayed.

This completes the dialog entries for ISTDs and drug compounds in our example.

Step 8: Enter the Control values for the first drug

1. Enter acceptable Low, Medium, and High Control values for the drug of interest.

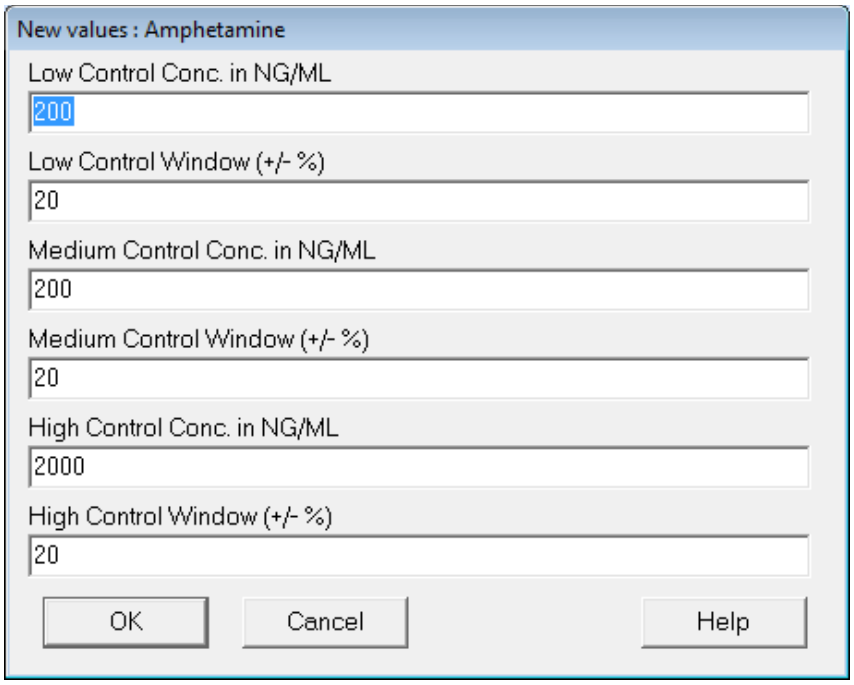
2. Click **OK** to continue.

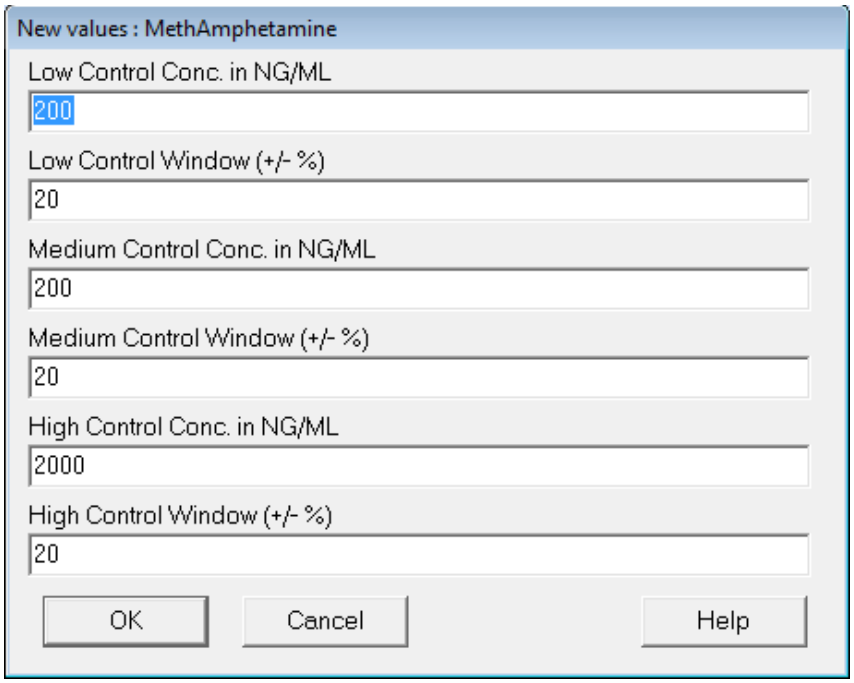
Step 9: Enter the Control values for the second drug

1. Enter acceptable limits for the second drug.

2. Click **OK** to continue.

In our example, if the Low Control is found to be between 160 and 240 NG/ML it is considered acceptable.

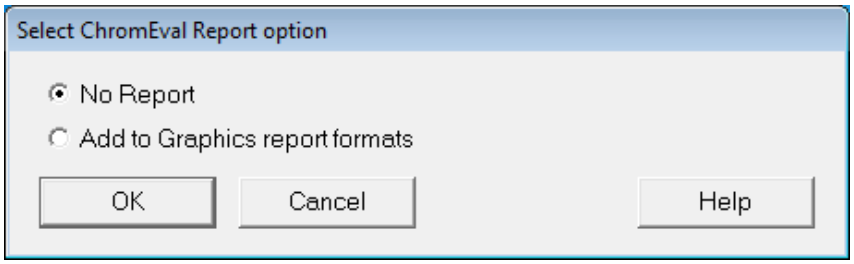




Step 10: Select the ChromEval Report option

1. Select **Add to Graphics Report formats** if you would like the Chromatographic evaluation results included.
2. Click **OK** to display the next dialog.

After entering your Control values, the **Select ChromEval Report Option** displays.

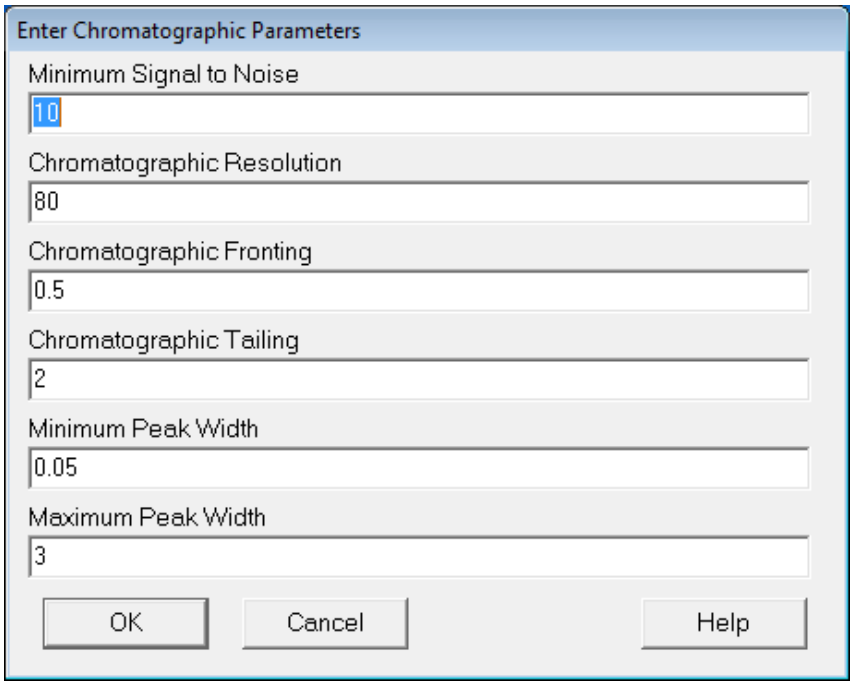


Step 11: Enter the Chromatographic Parameters

1. Set the acceptable chromatographic parameters.
2. Click **OK** to display the next dialog.

Here you will set the acceptable chromatographic parameters for any specimen.

When analyzing any specimen, if the chromatographic parameters are outside the limits you set here, the sample is considered unacceptable, and it will be processed according to the criteria you set in the Intelligent Processing parameters. See "Intelligent Sequencing Parameters for Specimens" on page 29.

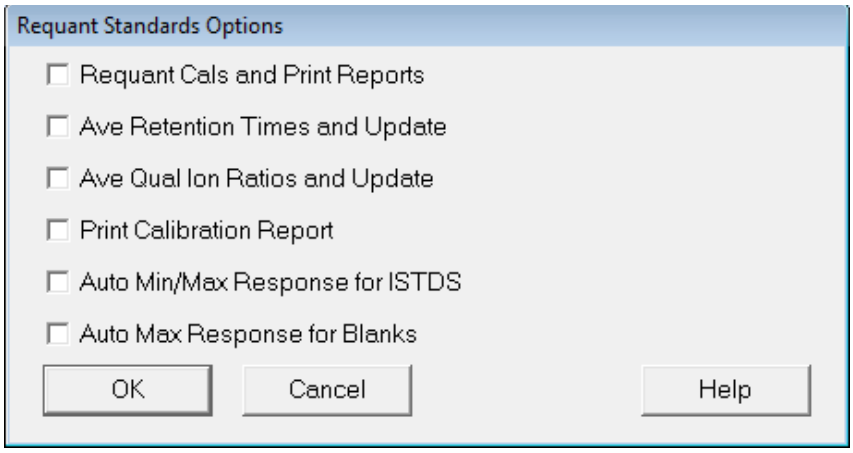


Step 12: Enter the Requant Standards method settings

1. Select one or more options from this list to perform when running a calibration standard.
2. Click **OK** to continue.

Finally, the Requant Standards dialog is displayed.

The Requant Standards process can requantitate your calibration standards and complete several other tasks that can be done on existing data, as described in this section.



Requant Cals and Print Reports

If this item is selected, the previously collected calibrators are requantitated using the **complete** calibration table and a report will be generated for each calibration run. The report style previously selected for the method will be used.

Ave Retention Times and Update

If this item is selected, the compound retention times in calibrators, as determined by the quantitation process, are averaged, and the expected retention time value of the Edit Compounds panel is updated. When this option is used, any retention time updates performed during the normal batch sequence process will be overwritten. Values used are rounded to two digits to the right of the decimal point before and after rounding is performed.

Ave Qual Ion Ratios and Update

If this item is selected, the qualifier ion ratios in calibrators, as determined by the quantitation process are averaged, and the values of the Edit Compounds panel are updated. The qualifier ratios are part of the Intelligent Sequencing decision process.

Print Calibration Report

An on/off selection. If this item is selected, the Calibration Summary Report will be printed.

AutoMin/Max Response for ISTDs

A dialog box is displayed when you select **Auto Min/Max Response for ISTDs** on the Requant Standards Options dialog box. This dialog lets you use response values of **all** internal standard compounds in **all** calibrators as determined by the quantitation process. These values will be used in the QC summary report for flagging samples that fall outside the limits.

(-) Percent of Ave Calibrators ISTDs for Min Allows you to specify the minimum percentage values to use for **all** ISTD compounds. This process will always expect the number entered is a percent value (for example, 50 = 50%).

(+) Percent of Ave Calibrators ISTDs for Max Allows you to specify the maximum percentage values to use for **all** ISTD compounds. This process will always expect the number entered is a percent value (for example, 50 = 50%).

The values you specify are used to update the minimum and maximum fields in the Defaults for ISTD dialog box seen in Data Analysis when you edit the Data Analysis portion of the method.

Auto Max Response for Blanks

A dialog box is displayed when you select **Auto Max Response for Blanks** on the Requant Standards Options dialog box. The values you specify are used in the QC summary report to flag blank sample types that lie above the specified limit, and for Intelligent Sequencing decisions for blank sample types.

Percent of Lowest Calibrator for Targets Lets you specify a percentage of the response values of target compounds in the lowest calibrator (as determined by the quantitation process) to update the maximum blank value on the User Defined tab of the Edit Compounds panel. This process will always expect the number entered is a percent value (for example, 50 = 50%).

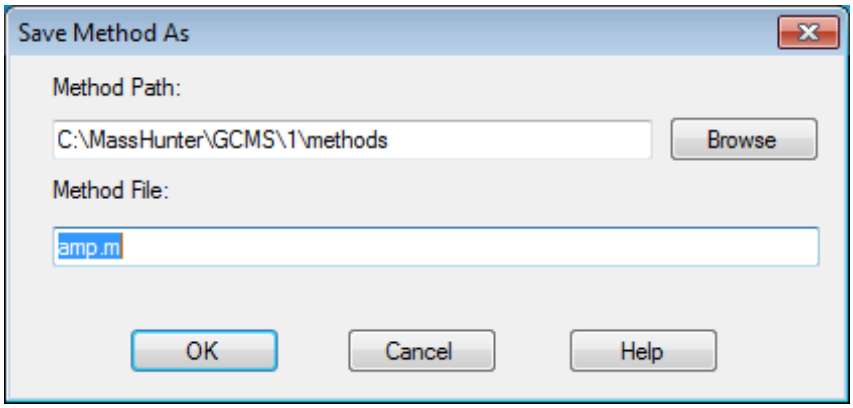
Percent of Average Calibrators for ISTDs Lets you specify a percentage of the response values for **all** calibrators (as determined by the quantitation process) to update the maximum blank value on the User Defined tab of the Edit Compounds panel. This process will always expect the number entered is a percent value (for example, 50 = 50%).

As calibrators are run as part of a batch (sequence), the actual method calibration is not complete until all calibrators have run. Some customers require that the individual calibration runs be reprocessed against the complete calibration table.

Calibrators are requantitated against the complete calibration curve based on a REQTSTDS keyword in the sequence table. This keyword **must** be entered after identified calibrators for correct operation. When this keyword is encountered, the sequencing code will execute the requant standards process based on the options selected.

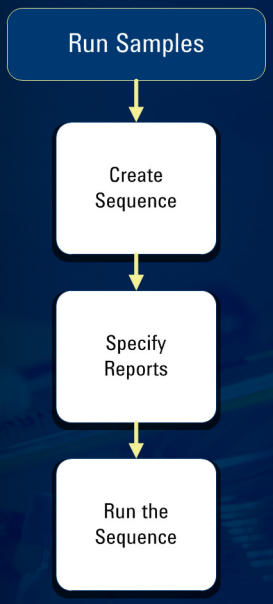
Step 13: Save the method

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



You are now ready to continue by creating a sequence table and running your samples using this method.

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

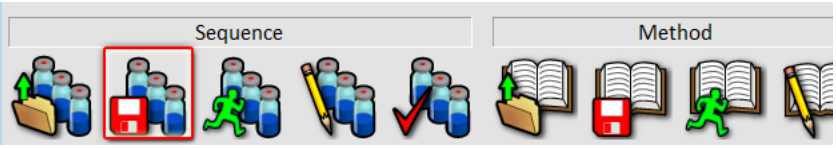
In this chapter we explain how to set up a Sequence Table with multiple batches of samples using the keyword **NewBatch**. This keyword indicates where the MassHunter should continue processing when a *Skip to next batch* command is generated by Intelligent Sequencing.

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.

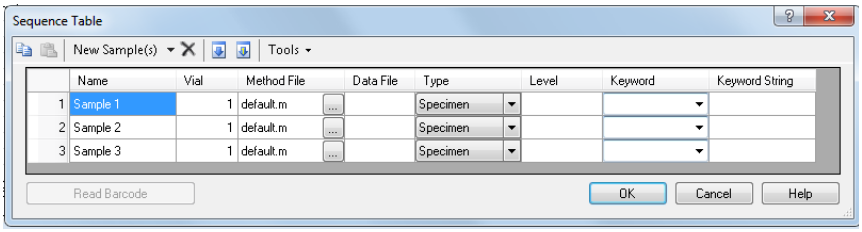


If you select a different directory for storing this sequence, that location will become the default storage directory location the next time you load or save a sequence.

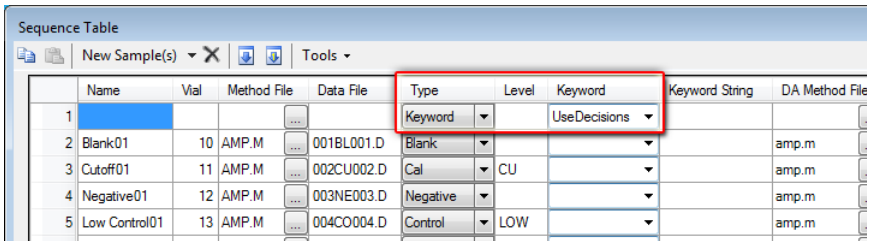
Step 2: Edit the Sequence Table

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**.

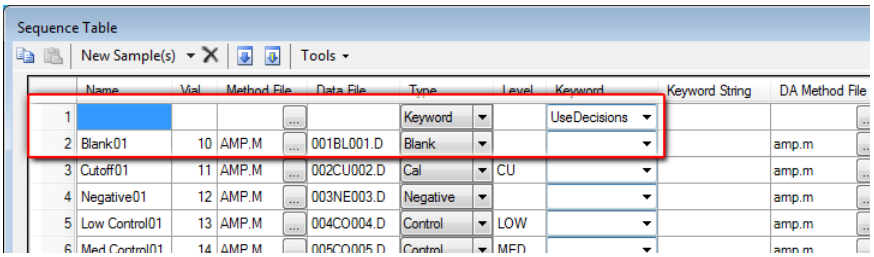


This command tells MassHunter GCMS Acquisition to send each sample's data file to MassHunter Quantitative Data Analysis for processing and wait for those results before running the next sample.

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.



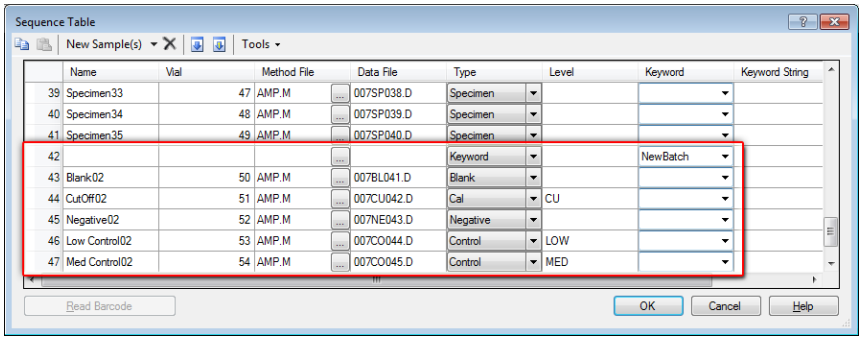
A typical sequence that includes multiple batches of specimens, starts out with a Blank sample that allows you to monitor for carryover contamination before you start to run specimens. This blank resets the Intelligent Sequencing cycle counter to zero.

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.

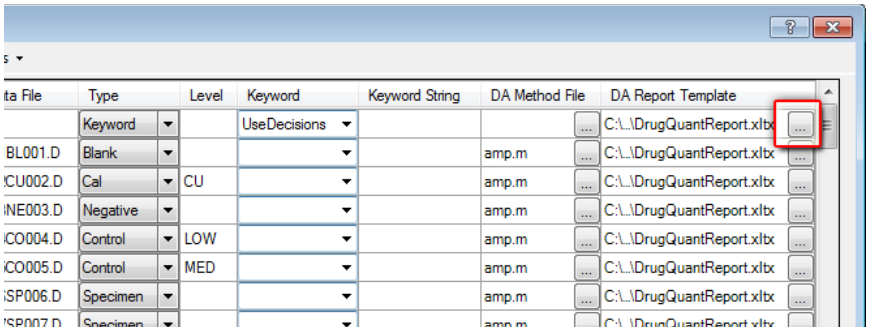
5. **To begin another batch in the same sequence table**, set the sample type to **Keyword** and select **NewBatch** from the **Keyword** dropdown. This batch, like the first, will use the MSD ChemStation auto naming batch feature.



Step 3: Specify reports

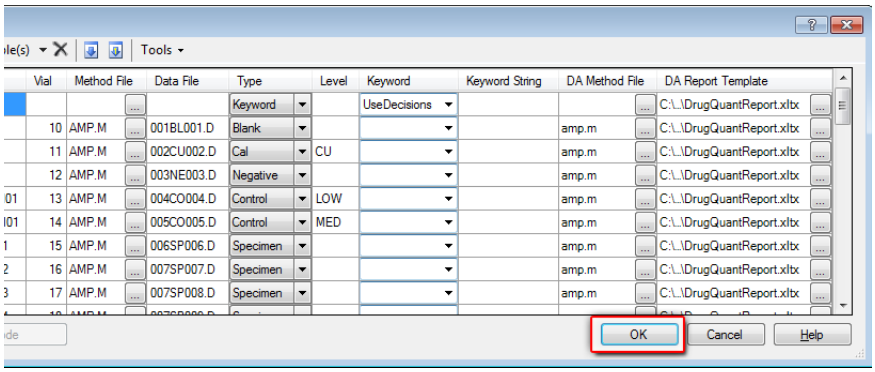
1. In the **DA Report Template** column, click the browse button and select a report template.

   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.

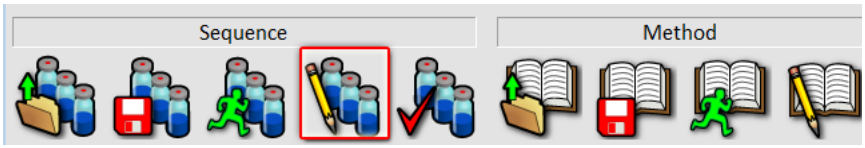
4. Notice that the name of the current sequence is displayed in the title bar.

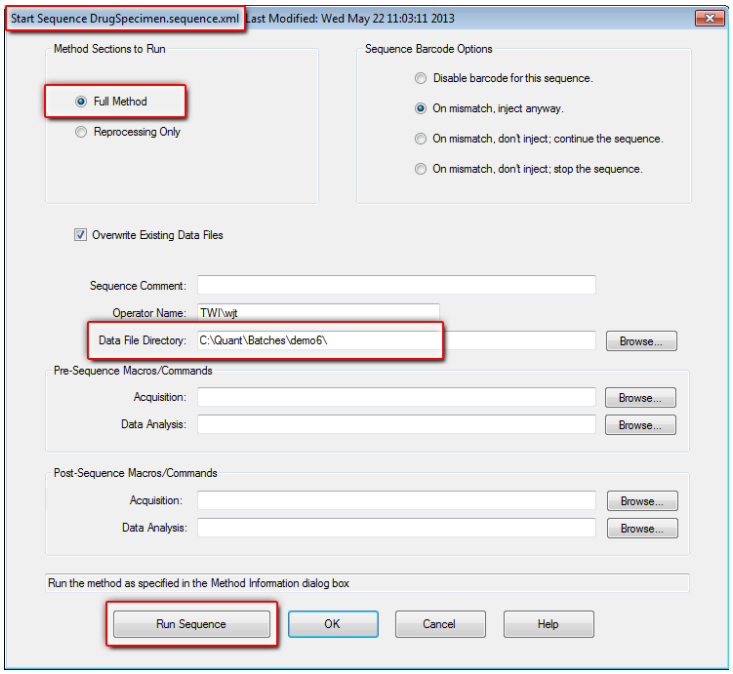
   For this Workflow, we are acquiring data, so we selected the **Full Method** option in the **Method Sections to Run** group box.

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.

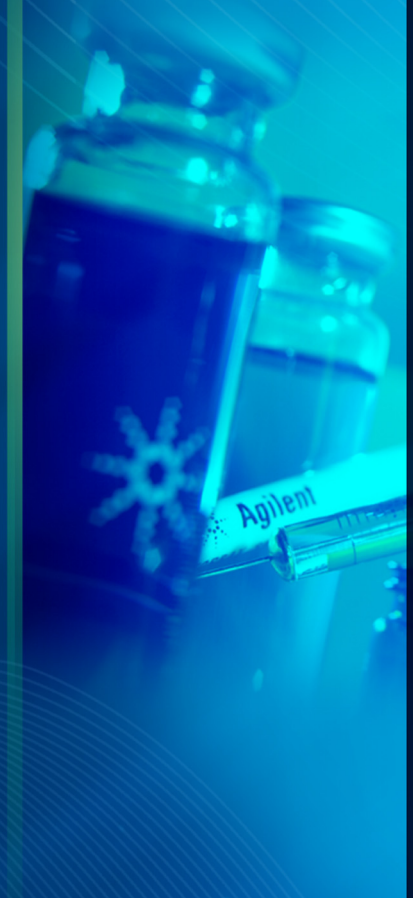
In the previous sections you learned how to create a sequence table containing multiple batches of specimens. Once your Sequence Table has been set up, and your vials are loaded into the sample tray, you may begin to process your samples as described here.

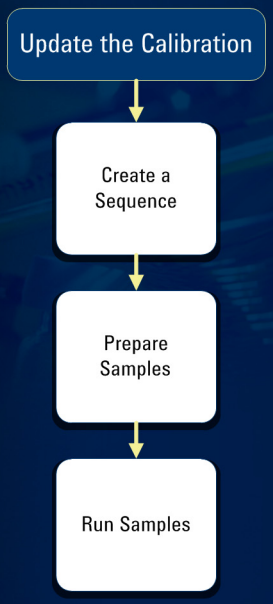




As each sample is analyzed, the results are compared with the parameters set in the method. If the results are outside the specified criteria, MassHunter GCMS Acquisition will continue the process based on the parameters specified in the Intelligent Sequencing portion of your method.

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.

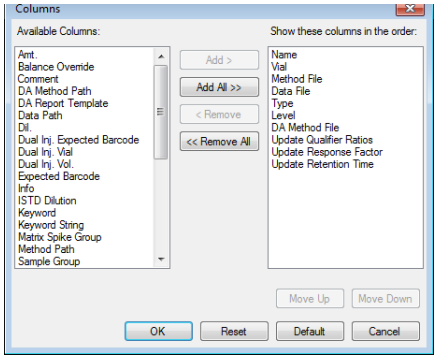
This chapter describes how the Sequence Table Editor (STE) in MassHunter GCMS Acquisition can be used to automate this process.

Step 1: Create the Sequence Table

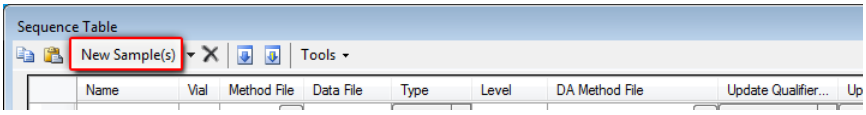
In the following steps we will describe how you can modify this table to define your calibration samples.

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.**

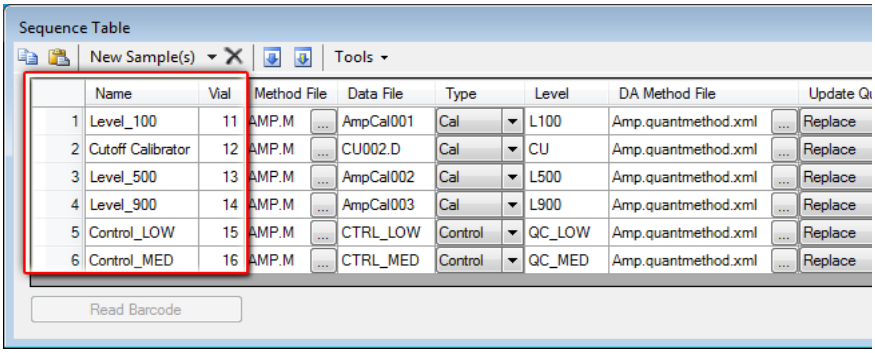


3. Click the **New sample(s)** menu three times to add three blank lines to the sequence table.



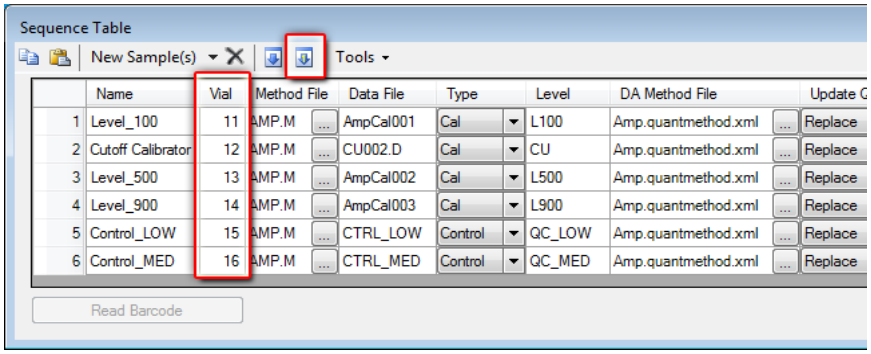
4. The example shown here illustrates a completed table. We will describe how it was created in the following steps.

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



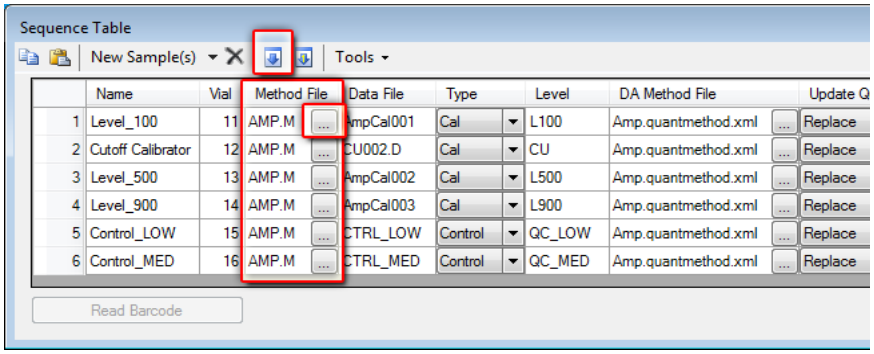
- Lines 1 through 4 are the 4 concentrations (**Levels**) required for a linear 4 point calibration curve for both Amphetamine and Methamphetamine. Line 2 is also a cutoff calibrator for these two drugs of abuse.
- Lines 5 and 6 are the control samples in our example.

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).



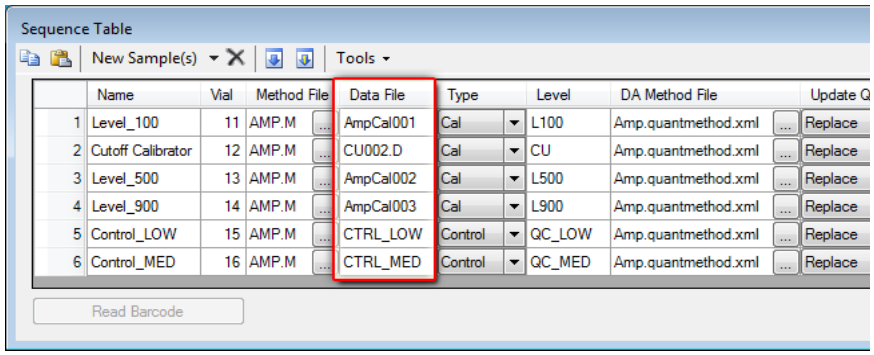
6. Click the browse icon next to the **Method File** name and select the method to use for each sample.

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



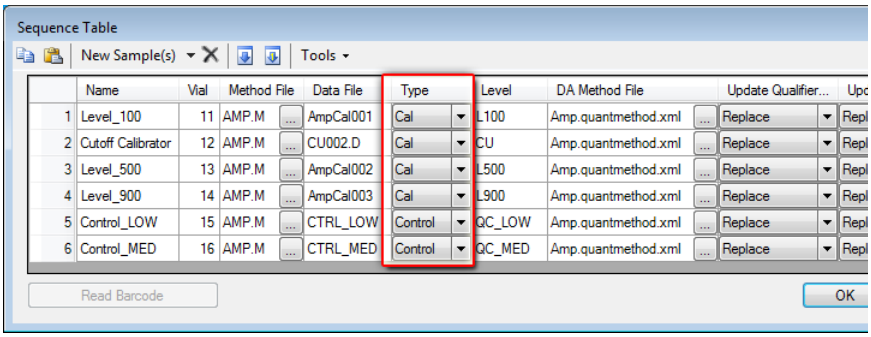
In this case, the instrument method parameters are stored in the AMP.M method, so that is the method to be used for each sample.

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



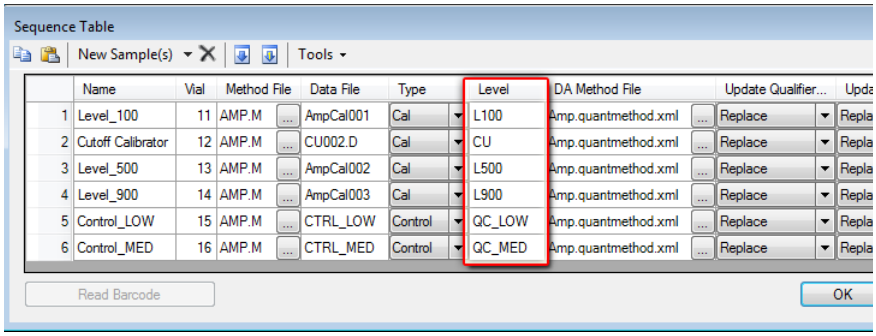
In this case the data will go into data files named AmpCal001, CU002, AmpCal002, AmpCal003, CTRL_LOW, and CTRL_MED.

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

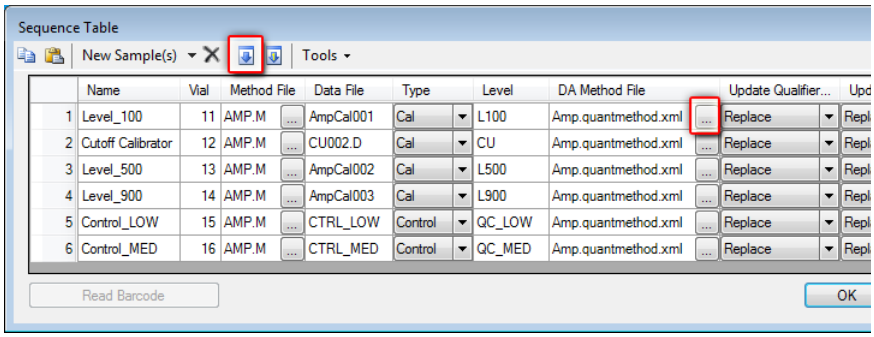


9. In the **Level** column, enter a level ID for each **CAL** and **Control** type sample.

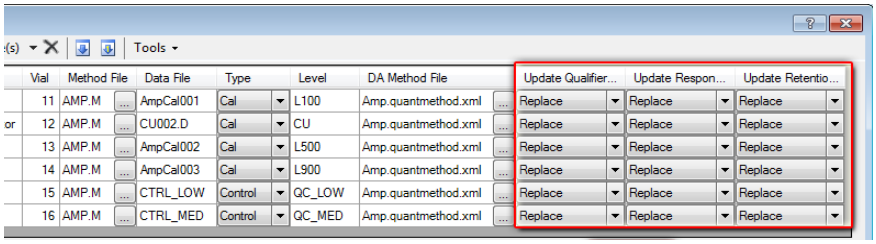
   This label must use *exactly the same name* as the corresponding concentration level ID in MassHunter Quantitative Analysis method. Capitalization is (is not) important, i.e. CU is not Cu.



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.



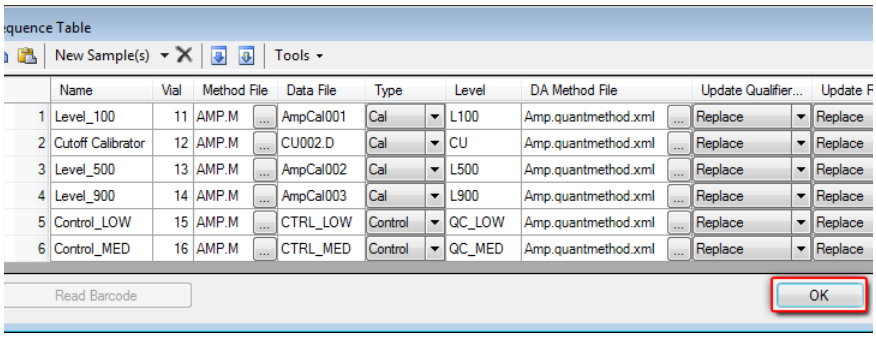
11. In the **Update Qualifier**, **Update Response**, and **Update Retention** columns, on line 1 select **Replace** from each dropdown. Copy this parameter to lines 2 through 6 for these three columns.



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.

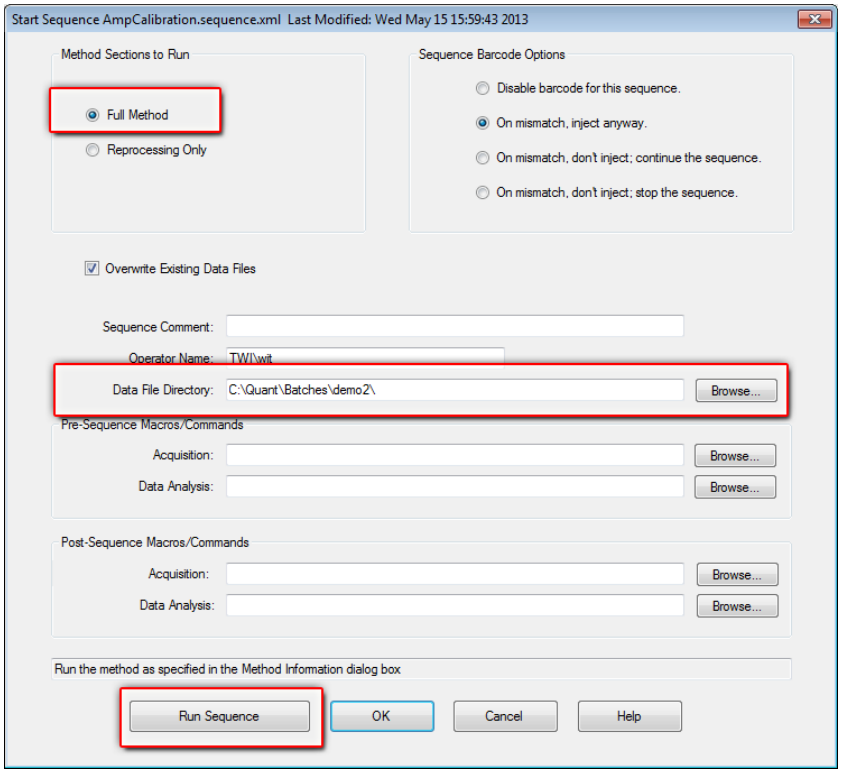
This example assumes use of an auto liquid sampler (ALS) which would require the 4 sample vials be placed in the sampler tray vial locations specified in the STE.

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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