Agilent Drug Discovery Screening

Workflow Overview
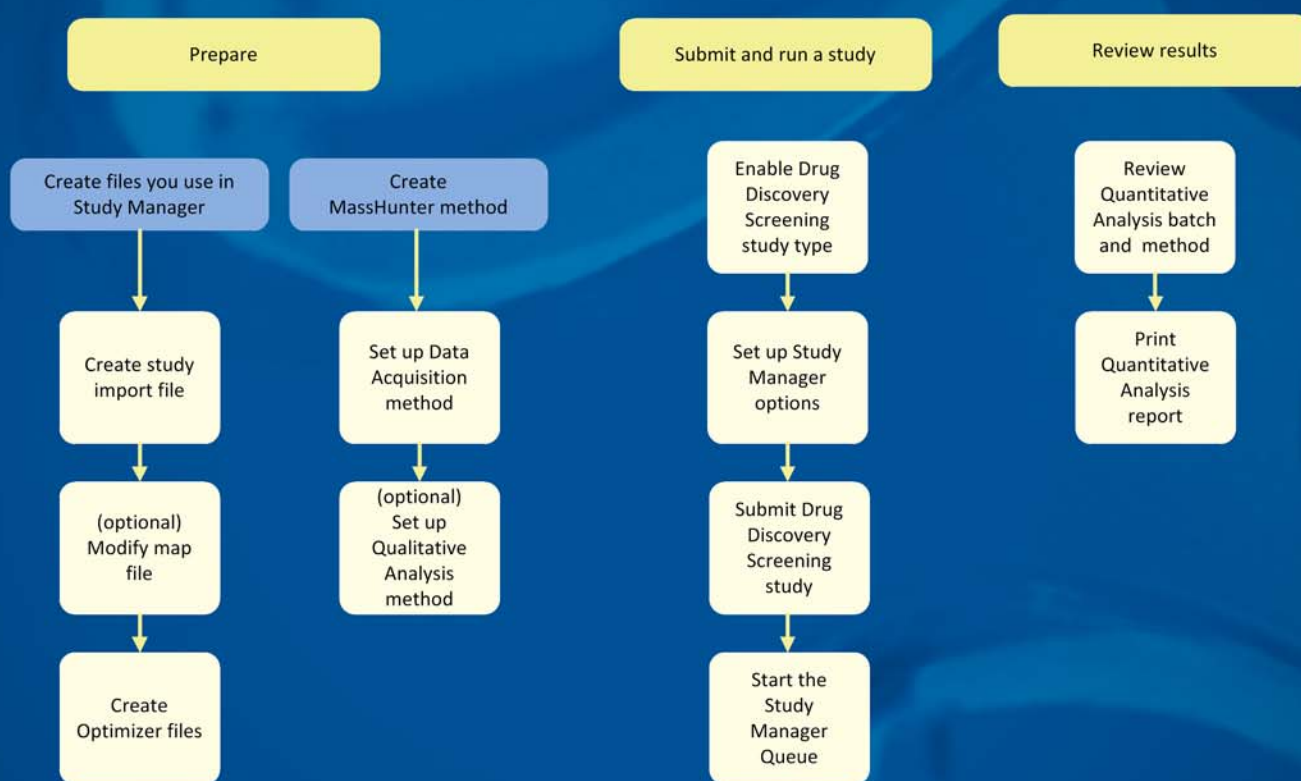
Prepare

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Agilent Technologies

Introduction

Many laboratories are working on discovering new drugs. One necessary step in this process is Drug Discovery Screening. Drug Discovery Screening encompasses all workflows involving measuring the properties of new chemical entities (NCEs) such as metabolic stability using liver microsomes. When you are doing drug discovery screening, you need to make sure to optimize the method for the compounds that you are screening.

The Agilent MassHunter Study Manager program has a specific study type that helps you with the process. You create an Excel spreadsheet that contains the information about your samples. Information from the Agilent MassHunter Optimizer program is used automatically to optimize method development. The worklist is created automatically from your Import File and from the Optimizer Database and Project that you select.

The MassHunter Study Manager program works together with the Agilent MassHunter Workstation Software - Data Acquisition program and the Agilent MassHunter Optimizer program to analyze the data from your Microsoft Excel import file. After you acquire the data, you run the Agilent MassHunter Quantitative Analysis program. When a Drug Discovery Screening study is run, a Quantitative Analysis method and batch are created automatically in the MassHunter Quantitative Analysis program. The information for the Quantitative Analysis method and batch is provided in the Import File in the Compound List worksheet.

Required Hardware and Software

Figure 1   *The workflow requires an Agilent LC and a Triple Quadrupole*

You use the following Agilent products to run the workflow described in this guide:
- Agilent LC System
  - Agilent 1200 Series LC system
  - Agilent 1200 Series Rapid Resolution LC system
  - Agilent 1220 Infinity Series LC system
  - Agilent 1260 Infinity Series LC system
  - Agilent 1290 Infinity Series LC system
- Agilent Mass Spectrometer
  - 6400 Series Triple Quadrupole LC/MS System
- Agilent software to control the instrument and analyze the data:
  - Agilent MassHunter Data Acquisition for QQQ version B.04.00 or higher
  - Agilent MassHunter Quantitative Analysis software version B.04.00 Service Pack 2 Pharma or higher

Preparing the Study Manager Files, the Optimizer Files and the LC/MS Method

The workflow includes creating Study Manager files and method development. See the *Agilent Drug Discovery Screening Workflow Guide* for details about the steps in this *Workflow Overview*.

You can instead use the Optimizer Automation study type if you want to optimize the compounds first and then run a Drug Discovery Screening study.

Create Study Manager files

You can submit a study by creating an input file in the Excel program. This section describes the format of this input file. You can modify the MAP file which is used to map the columns in your input file to the columns in the worklist. You use the MAP File Generator program to modify the MAP file. You can also optimize several of the method parameters using the Optimizer program.

Create the study import file

Sample List worksheet. The format of the Sample List worksheet in the Import File is the following:
*   The first line in the input file is usually the column headers. If not, then you set the **First lines as comments** box in the Settings tab of the Map File Generator program. The **First lines as comments** box is set to the number of lines to ignore at the beginning of the input file.
*   Each remaining line describes each sample in your study. The following columns are mandatory: **Sample Position**, **Sample Type**, **Data File Name**, **Sample Group**, **Quant Reference Flag**. These columns have a green background in the example below. You do not need to color code the import file. The color was only added to help identify the necessary columns.
*   The concentration column is used for the calibration samples.
*   The set of Sample Groups in Sample List must match the set of Compound Groups in Compound List.
*   One and only one Quant Reference Flag marked for each Sample Group
*   Calibration levels are created by scanning concentration levels. Calibration samples are optional.
*   The level name for each sample is set to a level corresponding to the concentration. It is possible that a batch does not contain any calibration samples even if an internal standard is present.

Figure 2   *The Sample List worksheet in the import file in Microsoft Excel*

Compound List worksheet. The format of the Compound List worksheet in the Import File is the following:

• The first line in the input file is the column headers. If not, then you set the **First lines as comments** box in the Settings tab of Map File Generator. The **First lines as comments** box is set to the number of lines to ignore at the beginning of the input file.

   • Each remaining line describes each compound in your study. The following columns are mandatory: **Compound Name**, **Sample Position** and **Compound Group**. These columns have a green background in Figure 3. Either **Compound Formula** or **Compound Mass** also has to be specified. These columns have a yellow background in Figure 3. You do not need to color code the import file. The color was only added to help identify the necessary columns.

• Very often a reference compound is added to every sample; therefore, this compound belongs in every compound group. To do this, you concatenate all the compound groups that it belongs to. For example, if the Compound Group is "A,B,C" and the compound is "reserpine", then "reserpine - A "is added to Compound Group A, and "reserpine - B will be added to Compound Group B and so on. Compound Group names which only contain a letter or a number work well when you need to concatenate Compound Group names.

• The ISTD Flag is optional if there is a reference compound in the Compound Group. The calibration samples in the corresponding sample group will be used to create the calibration curve. A single point calibration curve will be created if there are no calibration samples.

Figure 3   *The Compound List worksheet in the import file in Microsoft Excel*

(optional) Modify the map file

The Map File has multiple uses. The first use is to link the column headers in the Sample List sheet in your import file to the column names that are used in the Worklist within the Study Manager program and Acquisition program. If you want to customize the names of the columns in your Import file, you need to update the Map File.

The second use is to set up the columns in the Compound List. The compounds in the Compound List are used to generate the Quantitative Analysis batch file and method. The CompoundList sheet in the Map File links the column headers in the Compound List sheet to the columns used in the Quantitative Analysis program.

You usually do not need to modify the Map File. If you do want to modify the Map File, you use the Map File Generator program. See the *Agilent Drug Discovery Screening Workflow Guide* for details about modifying the map file.

Create Optimizer files

The Optimizer Software helps you optimize acquisition parameters. Specifically, it automates the selection of the best precursor ions, the optimization of the fragmentor voltage for each precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify.

To do this task, you first need to create the method to acquire the compounds. For more information, see "Set up the LC/MS Data Acquisition method" on page 5. You do not need to acquire the data file.

The fragmentor voltage for the 6490 is set automatically during Autotune. The fragmentor voltage for a 6490 is not optimized. The fragmentor parameters and results will not be displayed for a 6490 instrument.

a Start the MassHunter Optimizer software.

b Set the optimization parameters in the Optimization in the Optimizer Setup tab.

c Select the precursor ions in the **Precursor Ion Selection** tab.

d Select the product ions in the **Product Ion Selection** tab.

e Set up a compound list in the **Compound Setup** tab.

f Start the optimization process.

g Review the results.

Create the MassHunter method

You use the LC/MS Data Acquisition software to set the LC and Triple Quad parameters that you want to use for your analysis.

You probably do not need to use the MassHunter Qualitative Analysis program for this workflow. You can combine the Data Acquisition parameters and the Qualitative Analysis parameters in the same method.

Prepare the LC/MS system

a If you have not already done so, condition the analytical column that you will use for molecular weight confirmation.

b Tune the instrument. See the *Agilent MassHunter Data Acquisition Quick Start Guide* for more information.

c Purge the pump.

Set up the LC/MS Data Acquisition method

a Open the Data Acquisition program to access the pane for editing methods.

b Enter LC parameters appropriate for the analysis.

c   Enter MS parameters appropriate for the analysis.

d   Save the method.

(optional) Set up the Qualitative Analysis method

In a Drug Discovery Screening study, you probably do not need to set up a Qualitative Analysis method. If you do, please refer to the Agilent Qualitative Analysis Familiarization Guide (Agilent publication G3336-90007, Revision B, February 2011) for more information. If you do add a Qualitative Analysis method to the Data Acquisition method, then you need to select **Both Acquisition and DA** for the **Part of Method to Run** in the Worklist Run Parameters dialog box.

Submitting and Running a Study

1.   Enable the Drug Discovery Screening study type

a   Start the **Study Creator Enabler** program.

b   Enable the Drug Discovery Screening study.

c   (optional) Enable the the **Optimizer Automation**

2.   Set up Study Manager Options

a   Start the Study Manager program.

b   Set the Study Queue settings on the Settings tab. You can set the following options:
   •   Whether to pause or continue when an error occurs during a study.
   •   Whether to lock Data Acquisition when the Study Queue is running.
   •   Whether or not to acquire the data files in "locked mode".
   •   Whether or not to execute a standby script when the study is idle.

3.   Submit the Drug Discovery Screening study and start the Study Manager Queue

a   If necessary, start the Study Manager program.

b   Submit a Drug Discovery Screening study using an input file from Excel. You select the Drug Discovery screening study type in the Select Study Creator dialog box.

In this example, Custom was selected as the second Study Name option. Then, "DDS1" was typed in as the custom name.
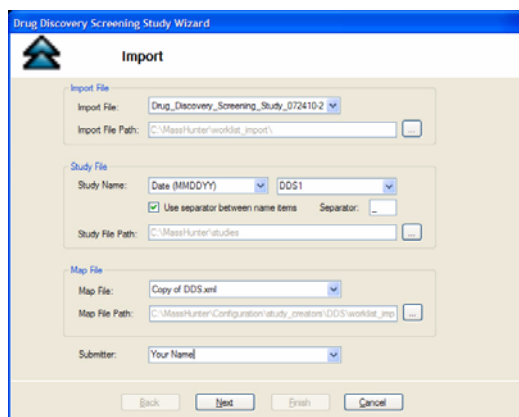
Figure 4   *Drug Discovery Screening Study Wizard - Import page*

c   Click the **Next** button. The Acquisition page is displayed. Select the **Method** and **Path**. You can also specify **Acquisition Method Parameters**.

d   Click **Next**. The Optimize page is displayed. Select the **Optimizer Database**, the **Optimizer Project** and either the **Positive Method** or the **Negative Method**.

e   Click **Next**. If the **Optimizer Project** or **Optimizer Database** has any errors, the errors are displayed in the Validation errors section. You need to fix any errors.

f   If no errors were found in the Optimize page, then the Worklist page is displayed. Review the worklist and make any necessary changes. Click the **Finish** button.

g   Click the **Start** button in the Home tab in the Ribbon.

For details about these procedures, see the *Agilent Drug Discovery Screening Workflow Guide*.

Reviewing Results in the Quantitative Analysis Program

You use the Quantitative Analysis program to review the Quantitative Analysis Batch and method which were created as part of the study. The Quantitative Analysis method is created with the information from the Compound List worksheet in the Import File (see "Create the study import file" on page 3).

Review the batch and method

a Open the batch file that is in the study folder.

b Click **View > Toolbars > Group Navigation** to add a toolbar to help you navigate through your results.

c Select the **Sample/Compound Group** in the Group Navigation toolbar. Then, only the compounds in a compound group that match the sample group are shown.

The Quantitative Analysis program tries to apply all compounds in the study to every sample. The filters allow you to only show the compounds in a compound group that match the sample group.

Unless you filter the results, you see the red exclamation mark as some compounds will not be found if they don't match the sample group. The red exclamation marks in Figure 5 can also mean that one (or more) of the values in that row of the batch table is not within the proper range. If you hover the mouse over the red exclamation mark, the error message is shown automatically.
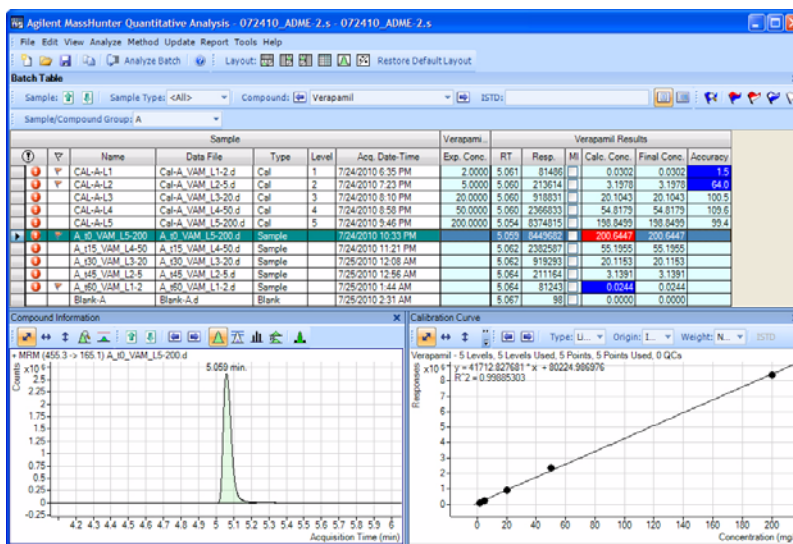

Figure 5 *MassHunter Quantitative Analysis program*

You can also use the Batch at a Glance program to review the Quantitative Analysis batch results. The Batch at a Glance program allows you to see the graphical results easily.
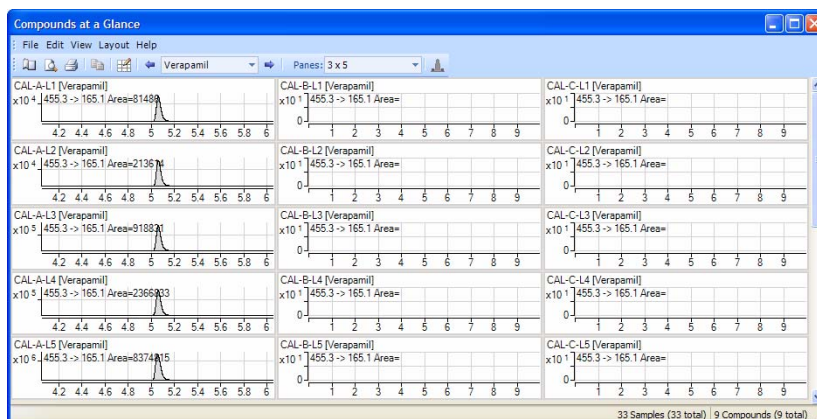
Figure 6   *MassHunter Compounds at a Glance program*

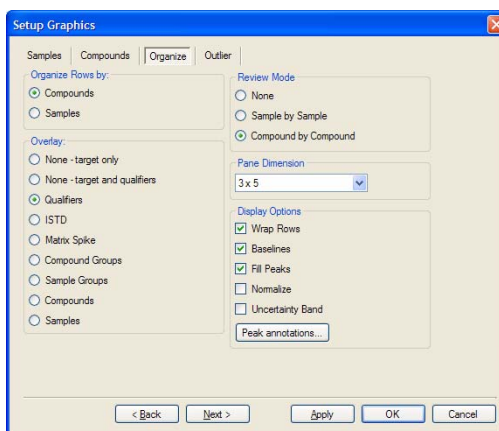You can change how the batch results are organized in the Setup Graphics dialog box.

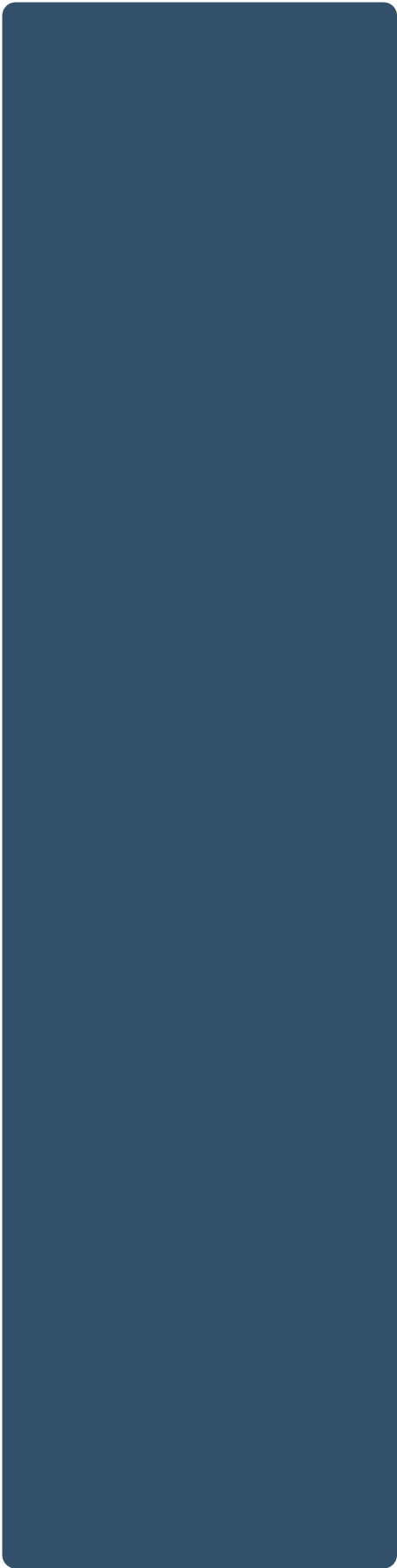**Figure 7**   *Organize tab in the Setup Graphics dialog box*

Print the Quantitative Analysis report

The Drug Discovery Screening template (DDS.xltx) is installed in the *Report Templates/Quant* folder. This report is designed to print out compounds that match the sample group. If you use a different template, the report will include many blank pages as the report prints out compounds that do not match the sample group.

You can modify a template to create a custom report. See the *Agilent MassHunter Workstation Software Reporting Familiarization Guide* or the *Agilent MassHunter Reporting Training DVD* for help on customizing a template.

For More Information

For details about these procedures, see the *Agilent Drug Discovery Screening Workflow Guide*. Visit our Web site at www.agilent.com/chem.

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