Gubbs Mass Spec Utilities / Quick Calc™ 7.x.x

GMSU/QC 6 and earlier

Supported Platforms

Thermo® XDKOEM 1.0.2.15
Thermo® XDKOEM 2.1.0.25
Sciex® Analyst™ 1.3.1 - 1.5

User Manual

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Version 13.1 (September 18, 2009)
C:\GubbsInc\WebSite\GubbsInc\GubbsInc03\files\GMSU_UserManual_14.doc

Table of Contents

Section **Page Number**

Table of Contents

Section **Page Number**

Table of Contents

Section **Page Number**

Table of Tables

Table **Page Number**

Table of Figures

Figure **Page Number**

Table of Figures

Figure **Page Number**

1 Introduction

Gubbs Mass Spec Utilities (GMSU/QC) is a suite of utilities that enhance the ability of scientists to perform post-acquisition processing of data acquired in a high throughput (HT) environment.

GMSU/QC uses either Microsoft® (MS) Access or MS SQL Server as a datastore. MS Access may be used to quickly setup a GMSU/QC instance for user testing or production use. MS SQL Server may be used if a more secure and possibly more efficient environment is desired. The MS Access database can be directly upgraded to SQL Server without loss of data. If users desire custom information reports, data stored in the database is easily accessible.

To perform chromatographic functions, GMSU/QC uses the software development kit components provided by Sciex and Thermo. Therefore, when users optimize chromatography, they can expect most of the features available in the native chromatography data system (CDS) will be available in GMSU/QC. In the same manner, it can be expected that the GMSU/QC chromatographic features will differ between Sciex data and Thermo data, though every effort has been made to ensure the differences are minimal.

GMSU/QC may be used in a multi-user environment. There are several configuration possibilities for multi-user environment which are described in more detail in the GMSU/QC Administration and Installation Manual. Briefly, the GMSU/QC database and acquired mass spectrometer raw data files may be stored in a common location accessible to others (either a shared file server directory or a shared local directory). In both instances, it is most optimal to place the GMSU/QC Access database on a filer server accessible to all GMSU/QC users.

For system requirements and installation procedures, please refer to the GMSU/QC Administration and Installation Manual.

The most current version of this manual may be found by browsing to www.gubbsinc.com and navigating to the GMSU/QC page.

2 Definitions

Term	Definition
AUC	Area Under the Curve (See Section 28)
CDS	Chromatography Data System
Cmax	Concentration Maximum (See Section 28)
GCV	Generic Chromatographic Viewer (See Section 27)
GMSU/QC	Gubbs Mass Spec Utilities
HPLC	High Performance Liquid Chromatograph
HT	High Throughput
LT	Low Throughput
MS	Microsoft®
QQ	Thermo QuickQuan™
RT	Retention Time

3 High Throughput (HT) vs. Low Throughput (LT) Acquisition

One of the salient features of GMSU/QC is that it can process HT acquired data as well as LT acquired data.

Typically, mass spectrometry data acquisition systems require that compound(s) of interest occur once in a chromatographic analysis. Therefore, in order to analyze a 12-point assay, scientists had to acquire twelve separate 'samples'. This is referred to as LT acquisition in the GMSU/QC system.

In HT acquisition, users open the chromatography data system (CDS) data acquisition window for as long as needed and inject and acquire all assay time points in a single chromatographic run. The benefits of HT acquisition [1 - 4] include:

- The ability to view all chromatographic peaks simultaneously to check for possible instrumental or sample handling problems and/or trends
- When combined with multiple-column switching techniques, increases throughput by decreasing the time between sample injections and minimizes communication problems that can occur between instrument and data acquisition system.

NOTE: Currently HT acquisition data processing is supported on the Sciex platform in the Hepatic Clearance and Permeability modules only.

It must be noted here that half of the benefit of HT acquisition is addressed by the 'Display HT Chromatography' feature of GMSU/QC (see Section 9.9). If normal LT acquisition is performed, users may click on the 'Display HT Chromatography' button to view all the injections (or samples) of an assay in a single chromatogram. GMSU/QC retrieves the XY data of each chromatographic injection/sample and concatenates the data end-to-end in a text file. Both ABI and Thermo chromatographic display components used by GMSU/QC allow text data to be loaded and viewed as a chromatogram. See the GMSU/QC Users Manual – Display HT Chromatography section for more information.

4 Data File Convention

Throughout this manual, the term "Data File" is used.

In the Sciex environment, the Data File is the .wiff file.

In the Thermo environment, the Data File is the .sld file. Even though the .sld file doesn't actually contain raw data (.raw files), the .sld contains the directory path reference to the .raw files. Since the .sld file "manages" the location of the .raw files, the .sld will be referred to as the Data File.

5 Data Acquisition Method Assumptions

GMSU/QC makes the following assumption concerning the configuration of Analyte and Internal Standard transition ions within the data acquisition method (applies to single-analyte assays):

The Analyte is listed first in the experiment transition ion section.
The Internal Standard is listed second in the experiment transition ion section.

If the opposite is true or the Chromatography Data System (CDS) lists the transition ions in order of compound molecular weight, then the user has the ability to assign the appropriate transition ions to Analyte and Internal Standard using the Menu item 'Assign Transition Ions' located in each module.

6 Multiple Analyte Experiments

The 'Assign Transition Ions' feature can also be used to prepare data in which data for multiple analytes have been acquired (e.g. in cassette dosing experiments or looped experiments).

Please note that this process is automated in the Thermo environment in which a valid processing method (.pmd) has been configured in the sequence file (.sld). The .pmd can be created manually using Thermo Xcalibur™, automatically using Thermo QuickQuan™, or exported with the .sld from Thermo LCQuan™. Please see the relevant help files inside any of these CDS's for further information.

7 Data Acquisition Data File Content Rules

Data file content must conform to certain rules in order to be successfully processed by GMSU/QC. The rules differ slightly depending on the data file.

7.1 Sciex

- Sciex data must be stored in a single .wiff file
- The .wiff file may contain data only from a single assay
- An 'assay' is defined as the injections/samples that make up the data set for that analysis (e.g. Hepatic Clearance).
- If the HPLC sequence contains a second assay, that assay must be stored in a different .wiff file

The following table is shown for a 5 injection/sample Hepatic Clearance sequence in which samples 1-5 are for Rat and 6-10 are for a second Rat experiment.

Sample Name	Data File
Sample001	Rat01
Sample002	Rat01
Sample003	Rat01
Sample004	Rat01
Sample005	Rat01
Sample006	Rat02
Sample007	Rat02
Sample008	Rat02
Sample009	Rat02
Sample010	Rat02

Notice that the data file names are different for the two assays.

Note that it is allowable to have several different analytes in each injection (e.g. cassette dosing, looped experiments).

7.2 Thermo

Similar to Sciex data, Thermo data (.raw files) must be grouped by assay. Thermo acquired data cannot be stored in a single data file; therefore, from the perspective of GMSU/QC, the Thermo sequence file (.sld) acts to group Thermo data by assay. The rules vary slightly depending on the CDS used to acquire the data

Note that Thermo applications generate several date-time stamped .sld's in the course of data acquisition and data processing. GMSU/QC ignores the date-time stamped .sld's.

- An 'assay' is defined as the injections/samples that make up the data set for that analysis (e.g. Hepatic Clearance).

7.2.1 Xcalibur™ data

- An .sld may contain only from a single assay
- If the HPLC sequence contains a second assay, that assay must be configured in a separate .sld and submitted to the sequence queue separately.

The following table is shown for a 5 injection/sample Hepatic Clearance sequence in which samples 1-5 are for Rat. The second table is samples 1-5 are for a second Rat experiment. The two .sld's would need to be submitted to the acquisition queue separately.

Sequence Name: Rat01	
Sample Name	**Path**
Sample001	C:\Xcalibur\Data\Rat01
Sample002	C:\Xcalibur\Data\Rat01
Sample003	C:\Xcalibur\Data\Rat01
Sample004	C:\Xcalibur\Data\Rat01
Sample005	C:\Xcalibur\Data\Rat01

Sequence Name: Rat02	
Sample Name	**Path**
Sample001	C:\Xcalibur\Data\Rat02
Sample002	C:\Xcalibur\Data\Rat02
Sample003	C:\Xcalibur\Data\Rat02
Sample004	C:\Xcalibur\Data\Rat02
Sample005	C:\Xcalibur\Data\Rat02

Please note the following:

- Even though it is permissible to save assay data to the same path, saving assay data to different paths will reduce the possibility of GMSU/QC errors in locating data.
- The two .sld's must have different names.
- It is allowable to have several different analytes in each injection (e.g. cassette dosing, looped experiments).

7.2.2 QuickQuan™ (QQ) data

QuickQuan is a highly specialized application allowing users to efficiently and quickly setup the instrument for high throughput data acquisition. QQ has an added benefit in that processing methods (.pmd's) are generated automatically and will be processed automatically by GMSU/QC. See Section 8 for a further discussion of processing methods.

The only limitation with QQ data is that the QQ sequence must be configured such that there is always a 1-to-1 relationship in generated .sld's-to-.pmd's (please see Section 7.2.4 – Rooney Method for an exception to this rule).

For example, the following QQ sequence will generate two data subdirectories, each containing one .sld and a corresponding .pmd

Normal Sequence

New Sequence	Drug Set	Sample Name
☒	QuickCalc	Sample01
☐	QuickCalc	Sample02
☐	QuickCalc	Sample03
☒	Labetalol	Sample01
☐	Labetalol	Sample02
☐	Labetalol	Sample03

The following example would not be allowed. The following QQ sequence will generate a single data subdirectory with two .sld's and two corresponding .pmd's. Users must open this data using the Rooney Method described in Section 7.2.4.

Rooney Method Sequence

New Sequence	Drug Set	Sample Name
☒	QuickCalc	Sample01
☐	QuickCalc	Sample02
☐	QuickCalc	Sample03
☐	Labetalol	Sample04
☐	Labetalol	Sample05
☐	Labetalol	Sample06

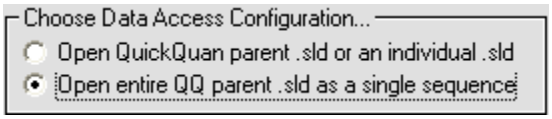
7.2.3 LCQuan™ data

GMSU/QC cannot process LCQuan data directly. Therefore, users must first export a desired .sld from within LCQuan. GMSU/QC then may open the exported .sld.

Users must ensure that, within LCQuan, the .pmd is also exported with the .sld.

7.2.4 Rooney Method data

Users may wish to generate large sequences containing several analytes, but to view the entire sequence as a single batch. Users have two options. We again will use the examples given in Section 7.2.2. In both options, the data should be opened using the Data Access Configuration option of 'Open entire QQ parent .sld as a single sequence', which will be referred to as Selection 2 in the following discussion.



The following QQ sequence will generate a single data subdirectory with two .sld's and two corresponding .pmd's.

Rooney Method Sequence

New Sequence	Drug Set	Sample Name
☒	QuickCalc	Sample01
☐	QuickCalc	Sample02
☐	QuickCalc	Sample03
☐	Labetalol	Sample04
☐	Labetalol	Sample05
☐	Labetalol	Sample06

If this data is opened using Selection 2, the entire sequence will be viewed as a single data file.

In another example, the following QQ sequence will generate two data subdirectories, each containing one .sld and a corresponding .pmd

Normal Sequence

New Sequence	Drug Set	Sample Name
☒	QuickCalc	Sample01
☐	QuickCalc	Sample02
☐	QuickCalc	Sample03
☒	Labetalol	Sample01
☐	Labetalol	Sample02
☐	Labetalol	Sample03

If this data is opened using Selection 2, the entire sequence will be viewed as a single data file, rather than being parsed into two data files.

A drawback to both of these options is that if the drug set listed in the processing method (.pmd) has more than one target, only the first target of each .pmd will be shown.

8 Thermo Processing Methods

There is an advantage to using Thermo data in that GMSU/QC can retrieve information from processing methods (.pmd's). GMSU/QC pulls as much information as it can from the files available to it from each CDS environment. Table 1 shows a comparison between Thermo and Sciex listing the information that GMSU/QC can retrieve automatically vs information that needs to be configured manually.

Table 1 Thermo/Sciex Automatic Information Retrieval Comparison

Information Component	Thermo [1]		Sciex	
	Automatic	Manual	Automatic	Manual
Analyte Name	X			X
Internal Standard Name	X			X
Analyte/IntStd Association	X			X
Compound association with transition ion set	X			X
Calibration/QC Level	X		X	
Calibration/QC Concentration	X			X

[1] With a correctly matching processing method

Though GMSU/QC contains the tools needed to configure manually all the Information Components listed, that action can become quite tedious, especially when throughput is ramped to >50 different analytes/species/assays per day.

If an incorrect (or no).pmd is configured in the Thermo .sld, then all the Information Components must be configured manually.

9 Conserved GMSU/QC Features

Each GMSU/QC module has several features that are identical (or nearly identical). These features are discussed here. See Figure 1 for a module that has examples of these features.

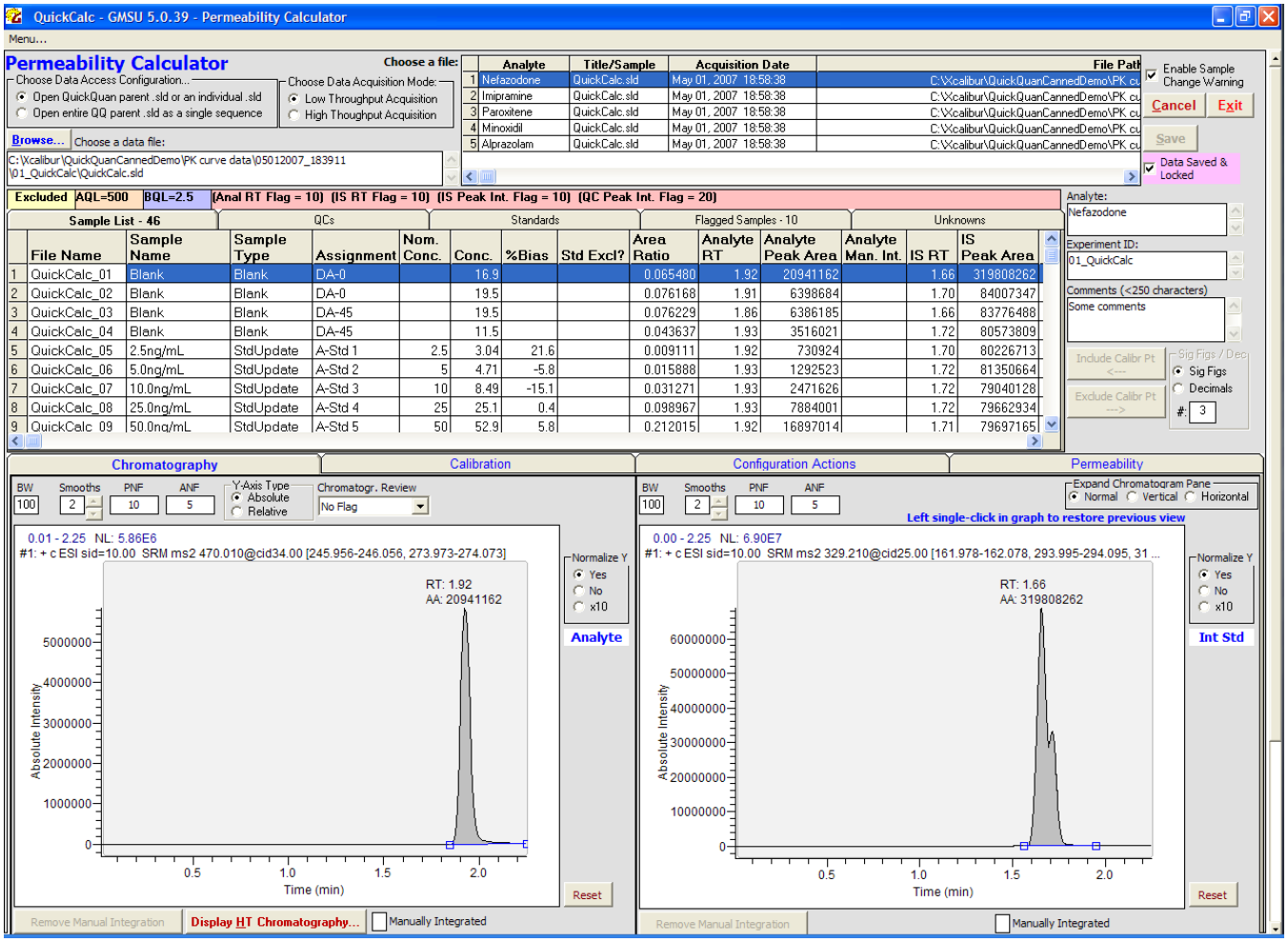


Figure 1 Permeability Calculator Example

9.1 Choose Data Access Configuration

These options govern how GMSU/QC will load data. The results of the options differ between Sciex data and Thermo data.

The default setting for this frame is configured in the Hepatic Clearance Calculator Configuration Settings in the Configuration Utility – Hepatic Clearance tab – Data File Access Mode item.

9.1.1 Sciex data

- Users may choose to load a single data file or all data files within a directory.
- If 'Open a single data file…' is chosen, then a single data (and any multiple analytes configured with that single data file) will be listed in the 'Choose a File' grid at the top of the window.
- If 'Open all data files within a directory…' is chosen, then all the data files in the same directory as the selected data file (and any multiple analytes configured with any of the data files) will be listed in the 'Choose a File' grid at the top of the window.

9.1.2 Thermo data

9.1.2.1 Open a data file or a QuickQuan parent .sld

A a single data file (and any multiple analytes configured with that data file) will be listed in the 'Choose a File' grid at the top of the window.

9.1.2.2 Open all data files within a directory

- If the data file is generated from XCalibur or LCQuan, then all the data files in the same directory as the selected data file (and any multiple analytes configured with any of the data files) will be listed in the 'Choose a File' grid at the top of the window.
- If the data file is generated from QQ, then a single data file will be listed in the 'Choose a File' grid at the top of the window. Any data related to multiple analytes configured with that data file will be shown in the Sample List (or chromatography list in the Hepatic Clearance module). This is advantageous in the Generic Chromatographic Viewer module because an entire sequence of data may be reviewed simultaneously. However, this is detrimental in any of the other modules since the data would not be able to be processed correctly.

9.2 Choose Data Acquisition Mode

HT Acquisition is supported with ABI data in both Hepatic Clearance and Permeability modules. HT Acquisition is supported currently with Thermo data in the Generic Chromatographic Viewer module only.

Users must select whether the data file being loaded is LT or HT. If the data file does not exist in the database, users will be prompted to assign the data as LT or HT (see Figure 2).

Figure 2 HT or LT acquisition type assignment

The default setting for this frame is configured in Configuration Utility – [module] tab – LT or HT Acquisition parameter. The installation default value is 'LT'.

If desired, an administrator can make this option frame invisible. The visibility of this frame is configured in Configuration Utility – [module] tab – Show Low Throughput/High Throughput Window parameter. The installation default value is 'No' (option frame not visible).

If the frame is INVISIBLE, new data will be processed automatically according to the Configuration Utility LT or HT setting and users will not be prompted to assign data.

9.3 Browse…

The default path for the Browse button is configured in the Configuration Utility – [module] tab – Default Data Directory field.

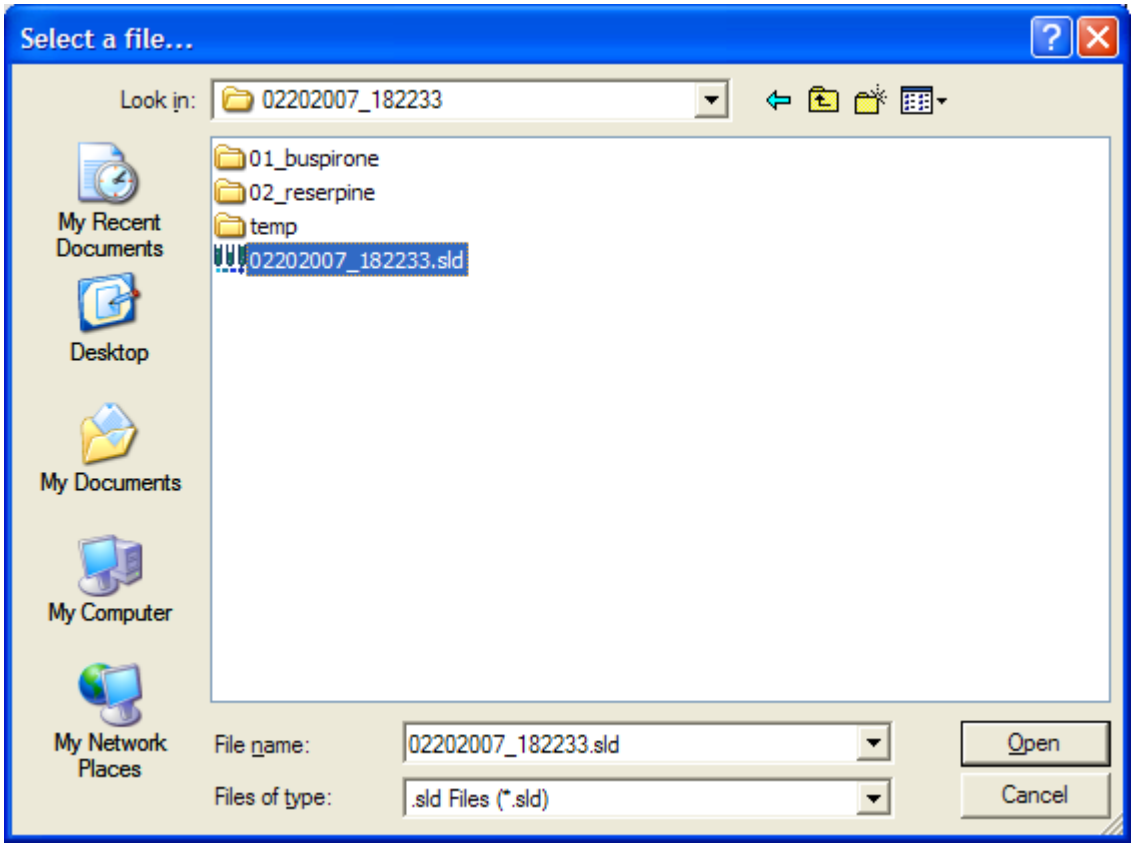


Figure 3 Browse… Window

- Choose the Browse… button to browse to a raw data file or raw data file directory.
- The 'Files of type' dropdown box content will include .sld, .wiff, or both (if both CDS environments are loaded on the workstation).
- If both CDS environments are loaded on the workstation, then the sequence order of the 'Files of type' dropdown box content is governed by Configuration Utility – [Module] – Select Default CDS Environment entry. If 'Thermo' is chosen, then '.sld' appears first in the 'Files of type' dropdown box.
- Data will be loaded into the 'Choose a File' grid (see Section 9.4, Figure 5) and the first file will be processed. The number of rows in the grid very depending on the selection of Choose Data Access Configuration (see Section 9.1). The column contents of the grid are explained in 9.4.

Once data has been loaded, GMSU/QC makes initial database table records and records five user identification records: Initial Date, Initial User ID, Initial User Name, Modification Date, Modification UserID, and Modification User Name. In this scenario, the Initial and Modification userid, username, and date information would be the same. The information may be viewed in the Choose a File grid.

	Initial Date	Initial UserID	Initial User Name	Modification Date	Modification UserID	Modification User Name
1	6/22/2007 1:48:06 PM	Gubbs	Larry Elvebak	6/22/2007 2:47:38 PM	Gubbs	Larry Elvebak
2	6/22/2007 1:48:06 PM	Gubbs	Larry Elvebak	6/22/2007 1:48:06 PM	Gubbs	Larry Elvebak
3	6/22/2007 1:48:07 PM	Gubbs	Larry Elvebak	6/22/2007 1:48:07 PM	Gubbs	Larry Elvebak
4	6/22/2007 1:48:07 PM	Gubbs	Larry Elvebak	6/22/2007 1:48:07 PM	Gubbs	Larry Elvebak
5	6/22/2007 1:48:07 PM	Gubbs	Larry Elvebak	6/22/2007 1:48:07 PM	Gubbs	Larry Elvebak

Figure 4 Choose a File Grid: User Identification

9.4 Choose a File Grid

	Analyte	Title/Sample	Acquisition Date	
1	Nefazodone	05012007_183911.sld	May 01, 2007 18:58:38	\\GUBBSLAP03\Xcalibur\QuickQuanCa
2	Imipramine	05012007_183911.sld	May 01, 2007 18:58:38	\\GUBBSLAP03\Xcalibur\QuickQuanCa
3	Paroxitene	05012007_183911.sld	May 01, 2007 18:58:38	\\GUBBSLAP03\Xcalibur\QuickQuanCa
4	Minoxidil	05012007_183911.sld	May 01, 2007 18:58:38	\\GUBBSLAP03\Xcalibur\QuickQuanCa
5	Alprazolam	05012007_183911.sld	May 01, 2007 18:58:38	\\GUBBSLAP03\Xcalibur\QuickQuanCa

Figure 5 Choose a File Grid

After the user loads data, the Choose a File grid is filled with data. This grid has several columns that contain useful information. The following describes the columns:

Column Heading	Description
Analyte	Analyte Name. • When data is initially loaded, GMSU/QC assigns Analyte Name as the data file name (minus the .wiff or .sld extension). If a .pmd file is present (for Thermo data), Analyte Name is retrieved from the .pmd file. • Once data has been loaded, users may modify the Analyte Name by modifying the contents of the window Analyte text box (see Section 9.12.1). When the Analyte text box is modified, the grid cell Choose a File grid cell updates automatically. • A third way to modify the Analyte Name is through the Assign Transition Ions feature (see Section 9.16).
Title/Sample	Sample Name or Sample Title. GMSU/QC assigns Sample Name/Title depending on environment and acquisition type. • For Thermo data, the Sample Name/Title is the .sld file name. • For Sciex data, LT Acquisition, the Sample Name/Title is the .wiff file name. • For Sciex data, HT Acquisition, the Sample Name/Title is the Sample value retrieved from the .wiff file.

Column Heading	Description
Acquisition Date	The acquisition date/time of the first sample in the data file
File Path	The directory path of the data file.
Sample #	For HT Acquisition. The sample number within the .wiff file.
HT/LT	Denotes acquisition type, either HT or LT Acquisition
A_PI	Analyte parent ion
A_DI	Analyte daughter ion
IS_PI	Internal Standard parent ion
IS_DI	Internal Standard daughter ion
TotTrans#	Total number of unique transitions listed in the data file
AnalTrans#	The transition ion item number (retrieved from the list of transition ions) of the Analyte
ISTrans#	The transition ion item number (retrieved from the list of transition ions) of the Internal Standard
Deactivated	Field not available at this time
TransSet#	Transition ion set number of the selected Analyte file
Analyte Filter	For Thermo data only. The filter string for Analyte
IS Filter	For Thermo data only. The filter string for Internal Standard
ID	The database record ID for the selected file
Initial Date	The date/time that the selected file was initially loaded in GMSU/QC
Initial UserID	The network User ID of the user who initially loaded the selected file
Initial User Name	The network User Name of the user who initially loaded the selected file
Modification Date	The date/time that the selected file was last modified in GMSU/QC
Modification UserID	The network User ID of the user who last modified the selected file
Modification User Name	The network User Name of the user who last modified the selected file

9.5 Sample Change Warning boxes

'Enable Sample Change Warning – Chromatogram' and 'Enable Sample Change Warning – Results' (for Hepatic Clearance module) warning boxes are checked by default. If the user attempts to select a new file in the 'Choose a File' grid, but hasn't saved the Chromatograms or Results (for Hepatic Clearance module), one or two warnings are displayed.

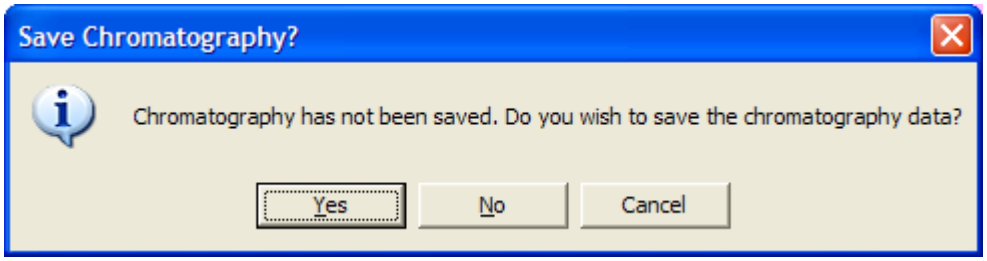


Figure 6 Example of a Sample Change Warning

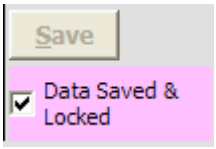
- If the user chooses 'Yes', the data is Saved and the next file is displayed.
- If the user chooses 'No', the data is Not Saved and the next file is displayed.
- If the user chooses 'Cancel', the action is canceled and the original data file remains displayed.

These warnings may become annoying if initial data review is all that is desired. By deselecting one or both of these warnings, the warning messages will be disabled.

9.6 Save Data

The Hepatic Clearance Calculator module and Permeability Calculator module have two save events: Save Chromatography and Save Results. All other modules have one save event: Save.

Data not saved/locked



Data saved/locked

When data is in a NOT Saved/Locked state, the Save button is enabled and the Data Saved&Locked checkbox is disabled. In this state, users are free to modify data.

When data is in a Saved/Locked state, the data is locked, cannot be modified, and can only be viewed. If the user wishes to modify saved data, then the user must first de-select the Data Saved&Locked checkbox.

When a save action occurs, the Modification user information for that data file (see Section 9.3, Figure 4) is updated.

9.7 Cancel

If a module has a Cancel button and that button is clicked, the underlying data for that record will be reset to its preceding Saved data.

9.8 Sample List

All modules except the Hepatic Clearance Calculator have a sample list. The sample list shows columns of sample-related data. The column headings of the sample list may be modified quickly and easily by clicking the Configuration Actions – Create/Edit Table Heading Set button. See Section 10 for more information.

Excluded | AQL=500 | BQL=2.5 | (Anal RT Flag = 10) (IS RT Flag = 10) (IS Peak Int. Flag = 20) (QC Peak Int. Flag = 20)

Sample List | QCs | Standards | Flagged Samples | Unknowns

	Sample Name	File Name	Sample Type	Nom. Conc.	Conc.	%Bias	Analyte RT	Analyte Peak Area	IS RT	IS Peak Area	Std Excl?	Area Ratio	Dose Form	DF Instance
1	Blank	QuickCalc_01	Blank		0.185		1.92	243663	1.66	315857929		0.00077143		
2	Blank	QuickCalc_02	Blank		0.207		1.91	70146	1.70	83395653		0.00084112		
3	Blank	QuickCalc_03	Blank		0.185		1.87	64033	1.66	82843803		0.00077294		
4	Blank	QuickCalc_04	Blank		0.107		1.95	42806	1.72	79972536		0.00053526		
5	2.5ng/mL	QuickCalc_05	StdUpdate	2.5	2.50	-0.1	1.92	620899	1.70	79393388		0.00782054		
6	5.0ng/mL	QuickCalc_06	StdUpdate	5	5.39	7.7	1.93	1335400	1.72	80533121		0.01658200		
7	10.0ng/mL	QuickCalc_07	StdUpdate	10	10.2	2.3	1.93	2443638	1.72	78339048		0.03119310		
8	25.0ng/mL	QuickCalc_08	StdUpdate	25	26.8	7.0	1.93	6326033	1.72	78917873		0.08015970		
9	50.0ng/mL	QuickCalc_09	StdUpdate	50	52.2	4.5	1.92	12092980	1.71	79029293		0.15301896		

Figure 7 Sample List

The sample list has several tabs that filter data to aid the user in viewing the data (see Figure 7).

In addition, the sample list has several flags that are color coded and blink if configured to do so:

9.8.1 Excluded (Yellow)

If the sample list contains calibration standards, the sample row will be yellow if the calibration standard has been excluded by the user (see Section 9.13.3).

9.8.2 AQL (Orange)

If the sample list contains calibration standards, the sample row will be orange if the calculated concentration is above the highest accepted calibration standard concentration.

9.8.3 BQL (Blue)

If the sample list contains calibration standards, the sample row will be blue if the calculated concentration is below the lowest accepted calibration standard concentration.

9.8.4 Miscellaneous Assay Flags (Red)

The Miscellaneous Flag displayed criteria settings are obtained from the Assay Flag Criteria settings (see Section 9.14.3). Row colors for these flags are shown only if the respective sample list Required Table Heading is shown:

Flag	Required Table Heading	Additional Table Headings	
Anal RT Flag	Analyte RT Flag	Analyte RT Ave	%Analyte RT Diff
IS RT Flag	IS RT Flag	IS RT Ave	%IS RT Diff
IS Peak Int. Flag	IS Int. Flag	IS Area Ave	%IS Area Diff
QC Peak Int. Flag	QC Int. Flag	QC Area Ave	%QC Area Diff

9.8.4.1 Anal RT Flag (Analyte Retention Time Flag)

- The Analyte RT Ave column contains the average Analyte retention time (RT) of all samples
- The %Analyte RT Diff column shows the % difference between the actual RT and the average RT
- If the % difference value is outside the displayed Flag percentage then the sample row will be pink.

9.8.4.2 IS RT Flag (Internal Standard Retention Time Flag)

- The IS RT Ave column contains the average IS retention time (RT) of all samples
- The % IS RT Diff column shows the % difference between the actual RT and the average RT
- If the % difference value is outside the displayed Flag percentage then the sample row will be pink.

9.8.4.3 IS Peak Int. Flag (Internal Standard Peak Integration Flag)

- The IS Area Ave column contains the average IS peak area of all samples
- The % IS Area Diff column shows the % difference between the actual peak area and the average peak area
- If the % difference value is outside the displayed Flag percentage then the sample row will be pink.

9.8.4.4 QC Peak Int. Flag (Quality Control Sample Peak Integration Flag)

- The QC Area Ave column contains the average QC peak area of all samples
- The % QC Area Diff column shows the % difference between the actual peak area and the average peak area
- If the % difference value is outside the displayed Flag percentage then the sample row will be pink.

9.9 Display HT Chromatography

(For LT acquisition data only)

Each GMSU/QC module contains a 'Display HT Chromatography' button. When the user clicks this button, GMSU/QC will look for an existing text file containing pseudo HT data. If the text file cannot be found, GMSU/QC will generate one (one each for Analyte and Internal Standard) by concatenating the XY data retrieved from each data set sample. The generated text file is displayed in the corresponding environment's (either Sciex or Thermo) graphing tool. The result is that the chromatographic peaks of the entire assay may be reviewed in a single chromatogram. For example, Figure 8 shows a 16-injection assay acquired in the normal LT manner, but displayed in a HT manner.

The advantage to this display is that users may review the entire assay at once. This type of view makes it obvious if there is a problem with a sample (e.g. very low signal for an Int Std or Analyte) or the assay (e.g. erratic signal for the Int Std), which may point to problems in sample preparation or problems in the HPLC instrument.

These types of observations can be easily missed in the traditional chromatographic review method in which individual chromatograms are reviewed (because each peak is typically normalized in the CDS window, hiding the fact that the peak signal may be attenuated) or peak area lists are reviewed (because it is tedious to generate the list and the review may miss small or erratic peak areas).

There is an additional advantage of the HT Display if Thermo data is being viewed. Integration parameters may be optimized at the HT level if Thermo data is viewed (see Figure 9). This is advantageous over the traditional manner of optimizing integration parameters because the user can see the effect of the optimization on all peaks immediately, rather than having to inspect each peak after an optimization event.

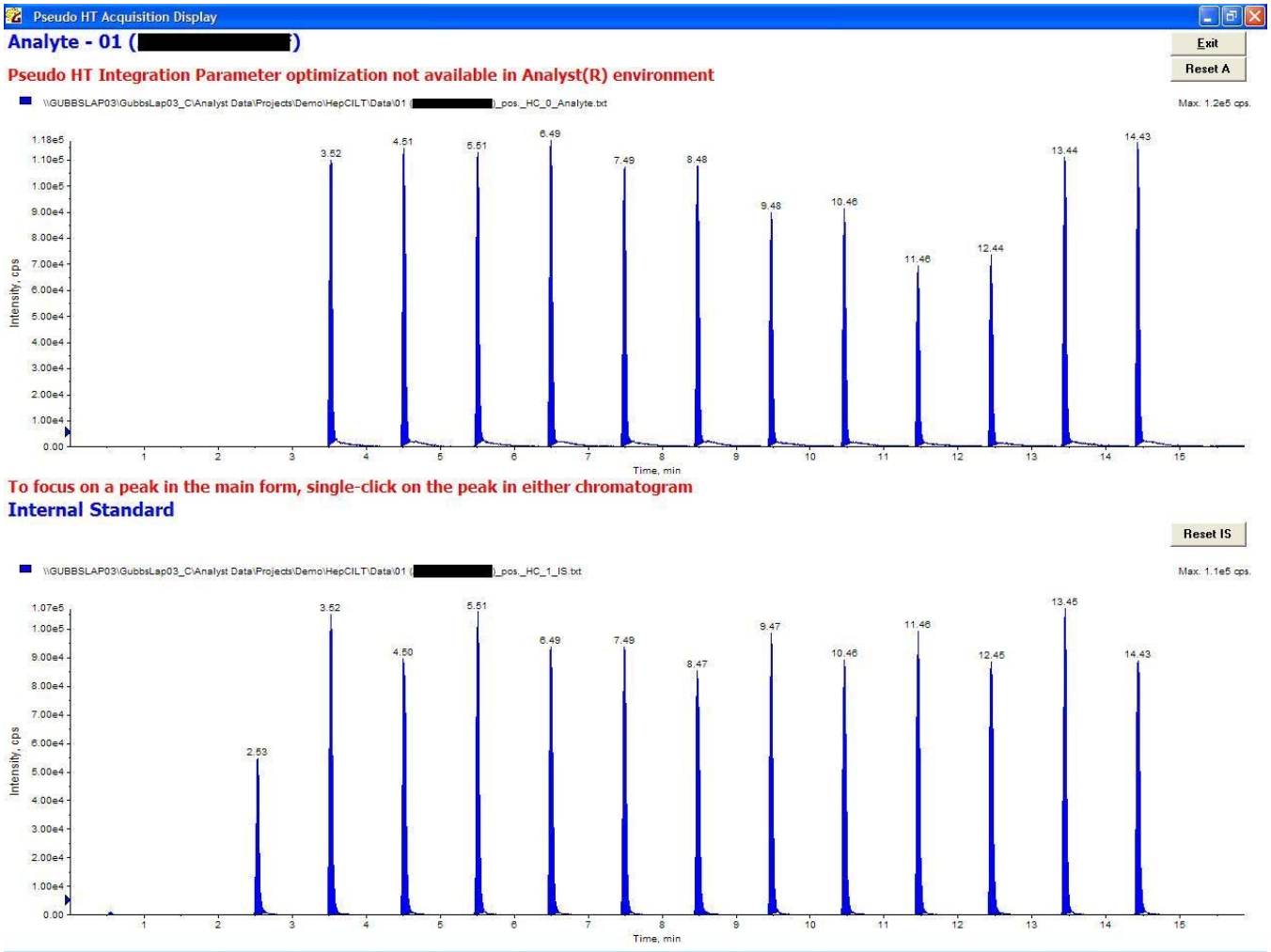


Figure 8 Display HT Chromatograph – Sciex Data

Figure 9 Display HT Chromatograph – Thermo Data

9.10 Chromatographic Display

All GMSU/QC modules display chromatography in essentially the same manner. One window (either top or left) shows the Analyte while the other (either bottom or right) shows the Internal Standard.

9.11 Chromatographic Processing Functions

To perform chromatographic functions, GMSU/QC uses the software development kit components provided by Sciex and Thermo, respectively. Therefore, when users optimize chromatography, they can expect the features available in the native chromatography data system (CDS) will be behave similarly in GMSU/QC. In the same manner, it can be expected that the GMSU/QC chromatographic features will differ between Sciex data and Thermo data, though every effort has been made to ensure the differences are minimal.

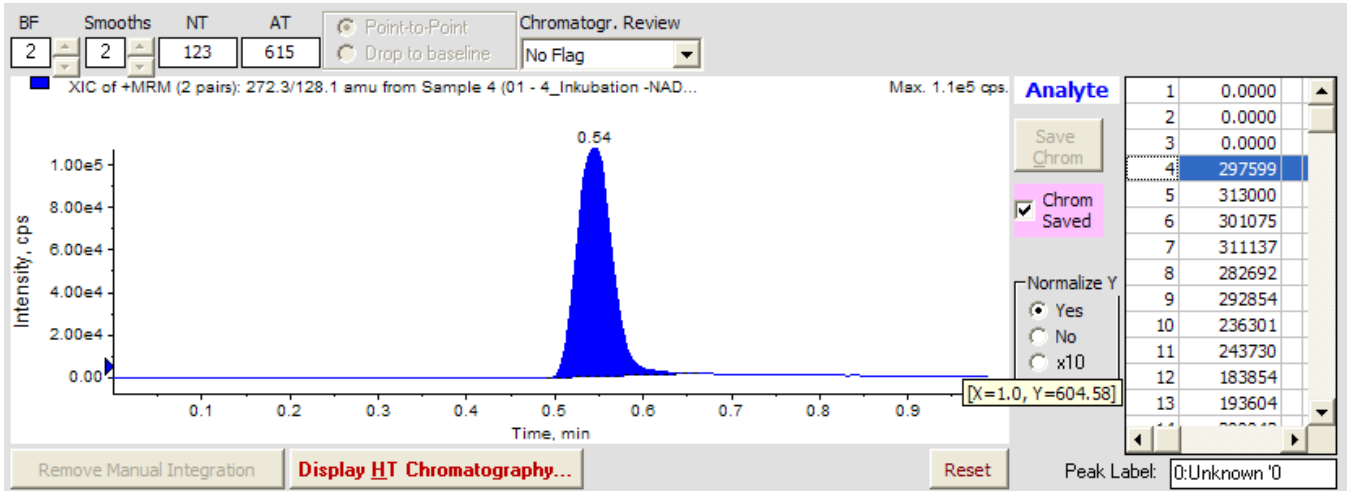


Figure 10 Example chromatogram window – Sciex

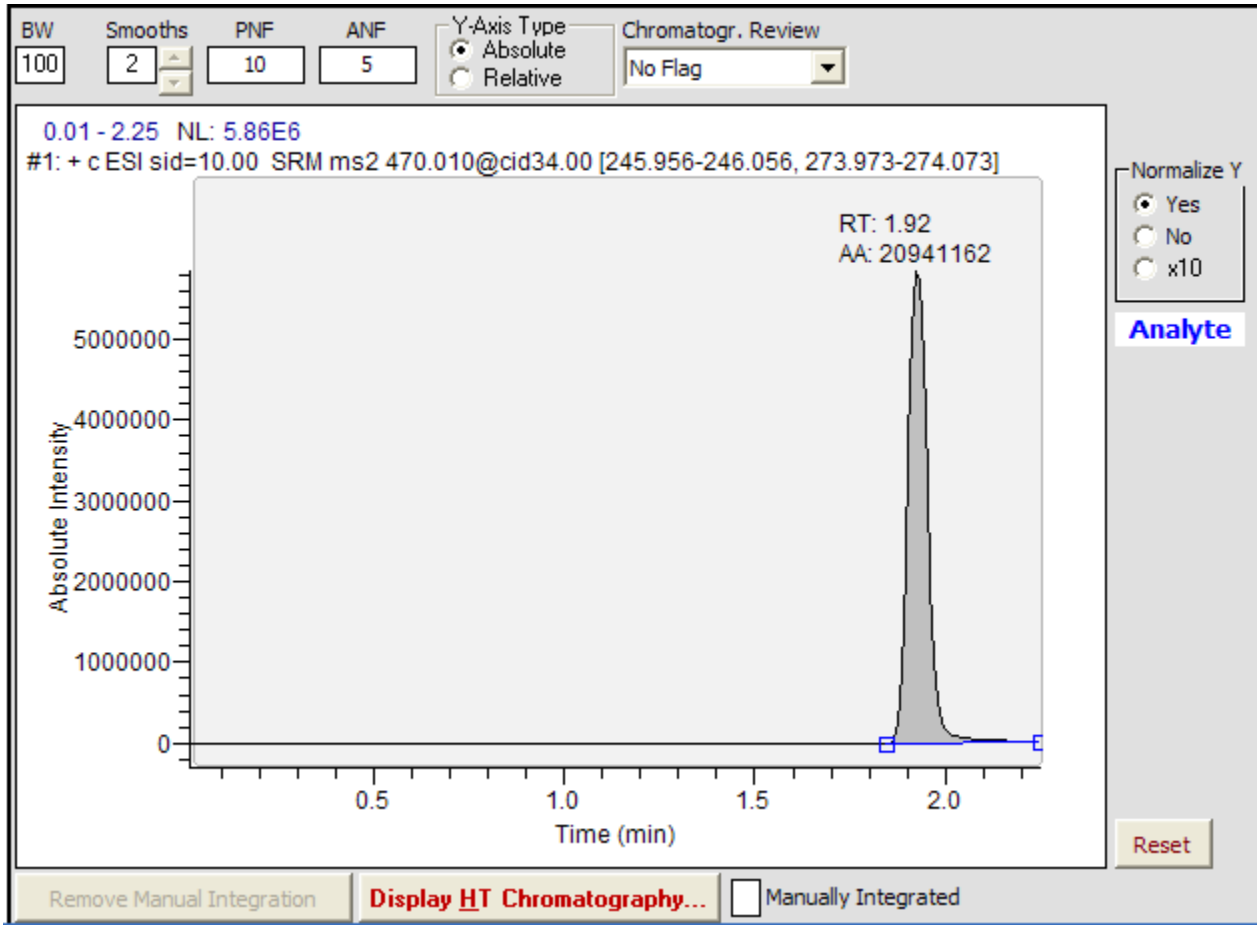
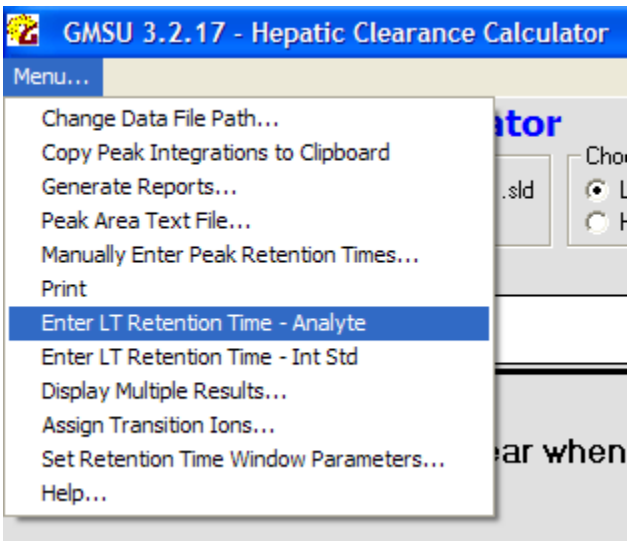


Figure 11 Example chromatogram window - Thermo

Users may perform the following events in a chromatogram:

Event	Description
BF (Bunching Factor)	Sciex only. Bunches points by the number displayed. Changes to BF are made to all samples.
BW (Baseline width)	Thermo only. Changes to BW are made to all samples.
Smooths	Smooths data by the factor displayed. Changes to Smooths are made to all samples.
NT/PNF	Noise Threshhold/Peak Noise Factor (Sciex/Thermo) Changes to NT or PNF are made to all samples.
AT/ANF	Area Threshhold/Area Noise Factor (Sciex/Thermo) Changes to AT or ANF are made to all samples.
Point-to-Point Drop to Baseline	Sciex only. Will apply the setting to a manual integration event.
Y-Axis Type	Thermo only. Will set the Y-Axis to Absolute or Relative
Chromatogr. Review	Choose different review states. The contents of this dropdown box are configured in Configuration Utility – Flags.
Peaks Button	Thermo only. Will display all peaks found in the chromatogram.
Save Chrom Button Chrom Saved Checkbox	Hepatic Clearance Calculator and Permeability Calculator modules only. Saves the chromatographic portion of the data. See Section 9.6.
Normalize Y	Will normalize the Y-Axis to the setting chosen.
Reset Button	Will reset the chromatographic window to full scale.
Display HT Chromatography Button	Self explanatory. See Section 9.9.
Remove Manual Integration Button	Will remove the manual integration settings of a peak (if manually integrated).

9.11.1 Assign expected peak retention time (Analyte or Internal Standard)



Choose Menu – Enter LT Retention Time – (Analyte/Int Std)

The user will be prompted to enter the expected retention time. The entered time will be applied to all samples.

The following features are slightly different between the Thermo and Sciex environments:

9.11.2 Sciex data

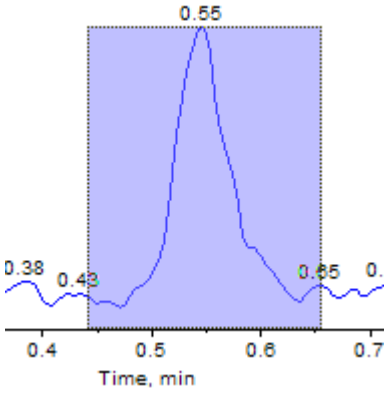
9.11.2.1 Zoom X-Axis

The x-axis may be zoomed by left-clicking and dragging in the X-AXIS TITLE AREA of the chromatogram.

9.11.2.2 Zoom Y-Axis

The y-axis may be zoomed by left-clicking and dragging in the Y-AXIS TITLE AREA of the chromatogram.

9.11.2.3 Manually integrate peak



The peak may be manually integrated by left-clicking and dragging from the desired start time to the desired end time.

In the Hepatic Clearance module and Permeability module, the peak that has been manually integrated is annotated with an "M" in the chromatography data table to the left of the chromatogram.

In all other modules, a manually integrated peak is denoted in two ways:

- The Sample List columns 'Analyte Man Int' or 'IS Man Int', if displayed, will contain an "M"
- The 'Manually Integrated' checkbox at the lower right of the chromatogram will be checked.

9.11.2.4 Re-identify peak (LT acquisition only)

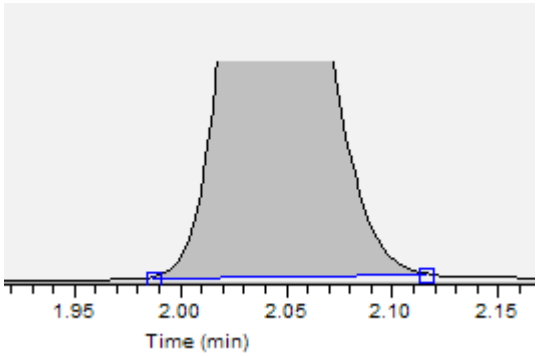
Right-mouse-click on the peak you wish to be identified as the peak

9.11.3 Thermo data

9.11.3.1 Zoom X- and Y-Axis

The x-axis and y-axis may be zoomed by left-clicking and dragging in the PLOT AREA of the chromatogram.

9.11.3.2 Manually integrate peak



The peak may be manually integrated by left-clicking and dragging either the blue start-peak box or the blue end-peak box to the desired start/end times.

In the Hepatic Clearance module and Permeability module, the peak that has been manually integrated is annotated with an "M" in the chromatography data table to the left of the chromatogram.

In all other modules, a manually integrated peak is denoted in two ways:

- The Sample List columns 'Analyte Man Int' or 'IS Man Int', if displayed, will contain an "M"
- The 'Manually Integrated' checkbox at the lower right of the chromatogram will be checked.

9.11.3.3 Add a peak

Right click on the chromatogram plot area and the cursor converts to a Thermo Add Peak cursor. Click and drag from left to right on the base line where the desired peak is located.

This action results in a single manually-integrated peak for the chromatogram.

9.12 Miscellaneous Features

All modules except the Hepatic Clearance Calculator and the Permeability Calculator have an area to the right of the Sample List that contains miscellaneous configuration features:

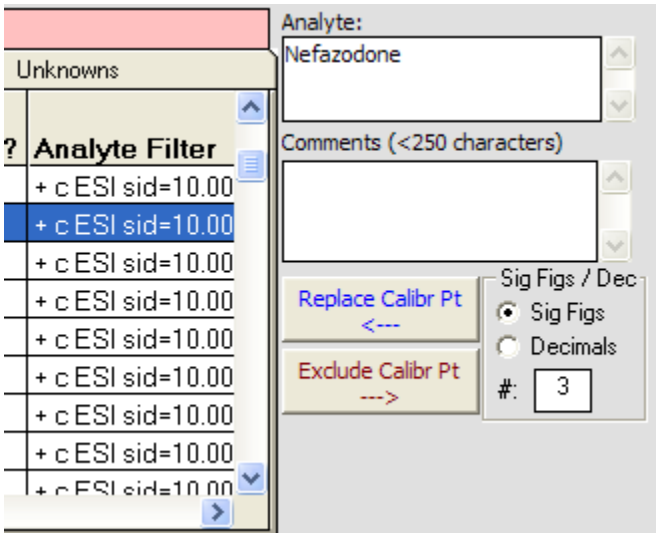


Figure 12 Miscellaneous Configuration Features

9.12.1 Analyte

Users may modify the Analyte Name in this text box. See Section 9.4 for additional details.

9.12.2 Comments

Users may enter comments in the Comments text box.

9.12.3 Replace Calibr Pt Button

To Replace excluded calibration points, users select one or more excluded samples in the sample list, then click the Replace Calibr. Pt. button.

9.12.4 Exclude Calibr Pt Button

To Exclude calibration points, users select one or more samples from the sample list to exclude, then click on the Exclude Calibr. Pt. button.

9.12.5 Sig Figs/Dec

Users may choose to display values based on significant figures or decimal places. Users enter the number of significant figures or decimal places in the text box. Data is updated immediately upon any change in this setting.

9.13 Calibration

All modules except the Hepatic Clearance Calculator and the Permeability Calculator have a Calibration window. The calibration window contains items to configure calibration parameters, such as regression type, regression weighting, Use IS, and concentration units. Note that, for Thermo data that contains a valid .pmd, concentration units are entered automatically.

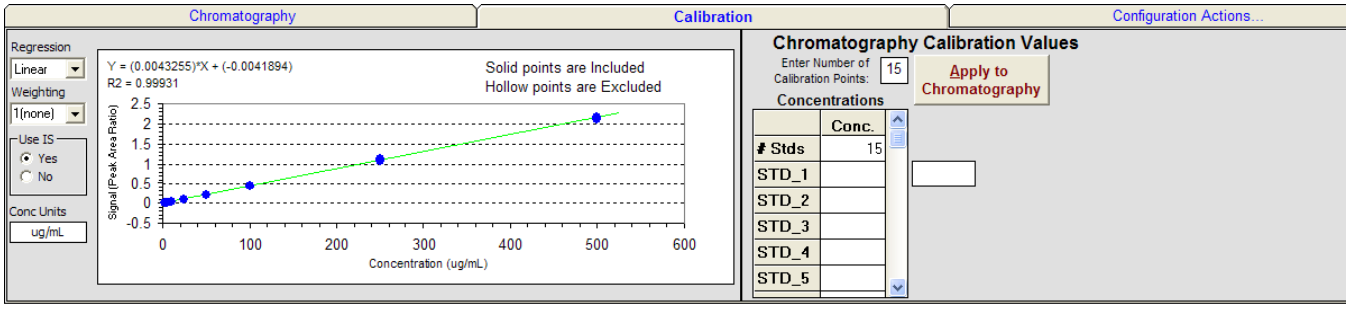


Figure 13 Calibration Tab

9.13.1 Calibration Discussion

GMSU/QC will retrieve calibration level information from the underlying data files (.wiff or .sld). The calibration level information is shown in Sample List Calibr Level table heading.

If the retrieved Sample Type shown in the Sample List is incorrect, users may modify this value in the Sample Type column. In addition, users may enter calibration (and QC) concentrations in the Nom Conc. column (nominal concentration).

Sample Type	Nom. Conc.
Blank	
StdUpdat	2.5
StdUpdate	5

Sample Type	Nom. Conc.
Blank	
StdUpdate	2.5
StdUpdate	5

Alternatively, users may use the Chromatography Calibration Values feature to enter calibration concentration values.

9.13.2 Chromatography Calibration Values

The Chromatography Calibration Values feature allows users to enter calibration values for calibration levels and/or sample types and Apply to Chromatography. The manner in which the concentration values are applied to the sample list depends on the presence of calibration levels.

If GMSU/QC was able to retrieve calibration levels from the underlying data, then STD_1, STD_2, etc. correspond to Level 1, Level 2, etc. For example, when the Apply to Chromatography button is clicked, then STD_1 concentrations are entered in any row with a Level 1 calibration level.

If GMSU/QC was unable to retrieve calibration levels from the underlying data, then STD_1, STD_2, etc. corresponds to the first Standard Sample Type, the second Standard Sample Type, etc. For example, if nine standard concentrations are configured in the Chromatography Calibration Values section, then these concentrations will be entered for the first nine Standard Sample Types. If, for example, there are eighteen Standard Sample Types, then the feature will start over: STD_1 = Standard Sample Type #10, STD_2 = Standard Sample Type #11, etc.

An advantage of using Thermo data is GMSU/QC can access Thermo processing methods (.pmd). If a correct .pmd is contained in the Thermo .sld, then GMSU/QC will retrieve the calibration level and calibration concentration (as well as QC concentrations) and enter the values automatically into the Sample List. This can be an enormous time saver if more than, say, ten assays are performed at once. See Section 8 for more discussion of Thermo .pmd's.

9.13.3 Excluding/Including calibration standards

All GMSU/QC modules that have calibration features contain buttons that allow users Exclude or Replace calibration points.

To Exclude calibration points, users select one or more samples in the sample list to exclude, then click on the Exclude Calibr. Pt. button.

To Replace excluded calibration points, users select one or more excluded samples in the sample list, then click the Replace Calibr. Pt. button.

In both actions, concentrations and parameters (e.g. clearance or AUC) are updated immediately.

9.14 Configuration Actions

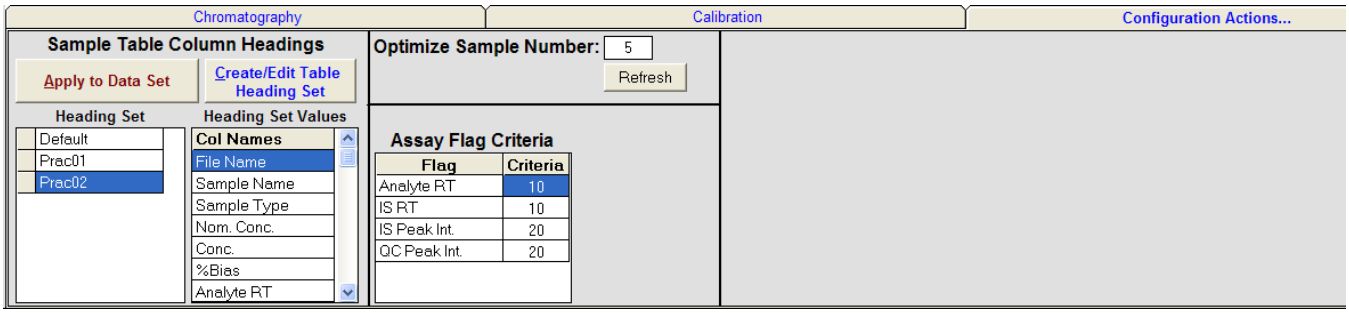


Figure 14 Configuration Actions

All modules except the Hepatic Clearance Calculator and the Permeability Calculator have a Configuration Actions window.

9.14.1 Sample Table Column Headings

9.14.1.1 Apply to Data Set Button

Users may quickly change the Sample List table headings by either double-clicking on a Heading Set entry or by choosing a Heading Set entry, then clicking the Apply to Data Set button.

9.14.1.2 Create/Edit Table Heading Set

Users may modify the Heading Set by clicking on the Create/Edit Table Heading set Button. The Create/Edit Table Heading Window is displayed. See Section 10 for further details.

9.14.2 Optimize Sample Number

When GMSU/QC initially processes loaded data, it must pick a sample in which to determine the expected compound retention time. The Optimize Sample Number box displays the sample number that gets chosen for processing.

Notes:

- The initial contents of this box is determined by the Configuration Utility – [Module] – Sample Number to Evaluate Chromatography field.
- The user may reprocess data by modifying the value in this box and clicking the Refresh button
- If the number in the box is greater than the number of samples in the data set, then the last sample in the data set is used to determine expected compound retention time.

9.14.3 Assay Flag Criteria

Assay Flag Criteria (see Section 9.8.4) specific to the chosen data file may be entered here. The default values for each flag for newly-loaded data are retrieved from the respective Configuration Utility – [Module] – Flag Criteria entries for that module

9.15 Generate Reports

There are two ways to generate reports in GMSU/QC. Figures of actual Generate Reports windows can be found within the individual modules User Manual sections.

9.15.1 Console – Generate Reports

Each Console module sub-level contains a button to go directly to the Generate Reports window for that module. For example, Figure 15 shows the Hepatic Clearance Calculator Reports button.

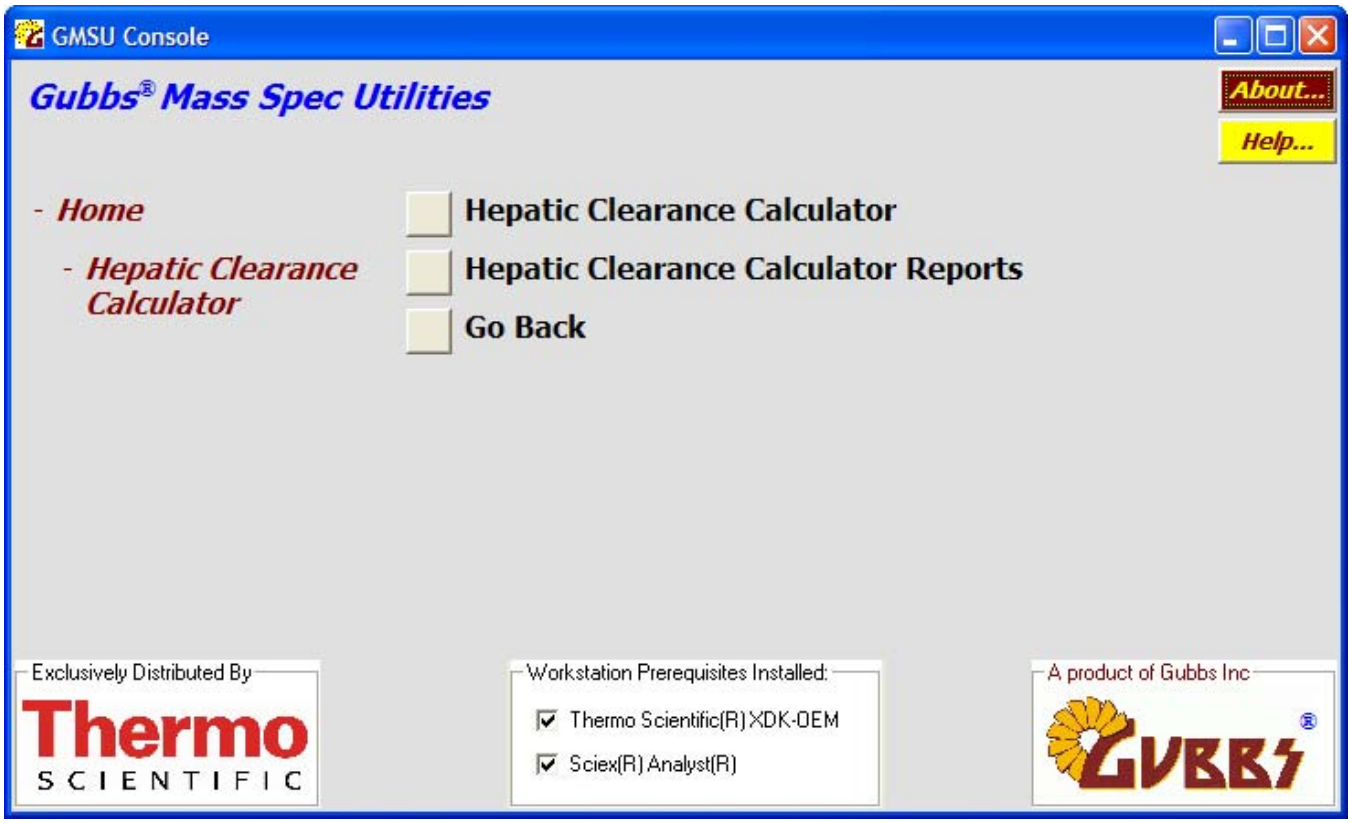


Figure 15 Example Console – Report Generation entry way

9.15.2 [Module] – Menu – Generate Reports…

Within each module, users may choose Menu – Generate Reports… to go to the Generate Reports window.

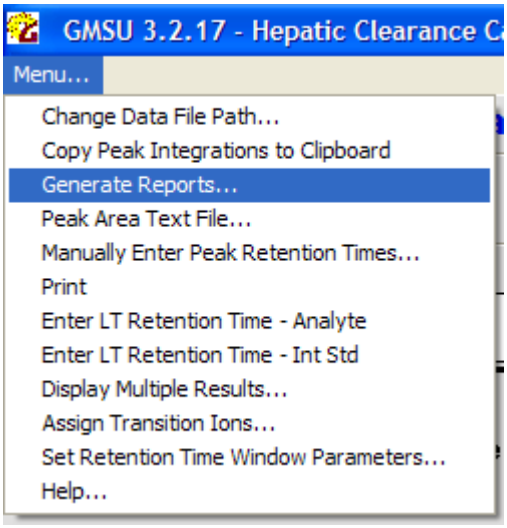


Figure 16 Menu – Generate Reports

9.16 Menu Items

Each module has a Menu from which additional functions can be performed. Some of these items are conserved in all modules and will be discussed here. Figure 17 shows the Generic Chromatographic Viewer Menu as an example.

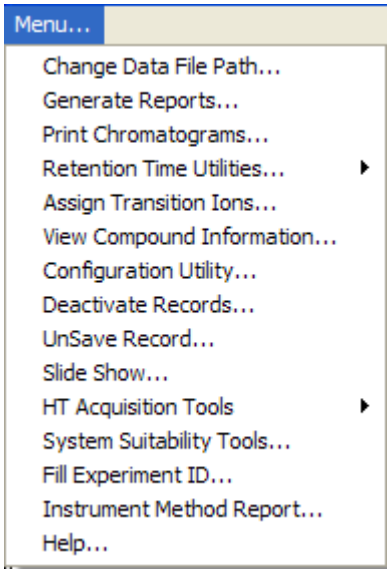


Figure 17 Generic Chromatographic Viewer Module Menu Items

9.16.1 Change Data File Path…

Under construction

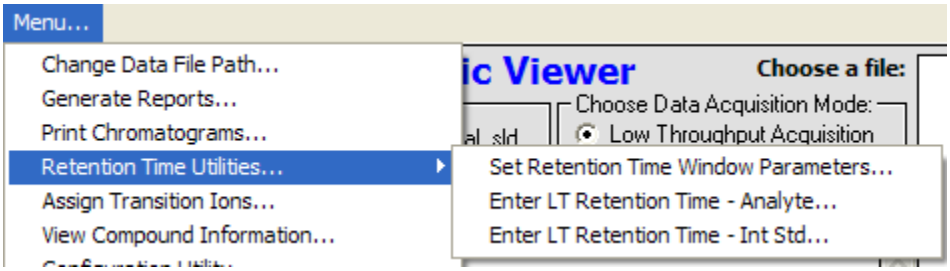
9.16.2 Generate Reports…

Opens the Generate Reports window for the module

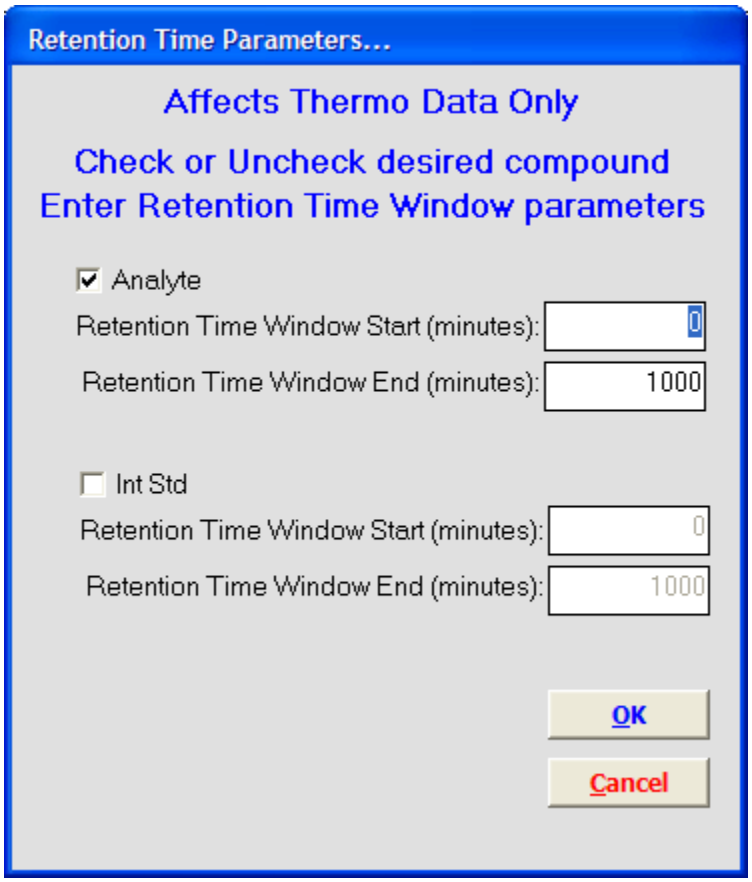
9.16.3 Print Chromatograms…

For Thermo only. Will generate a Word™ document containing figures of chromatograms.

9.16.4 Retention Time Utilities

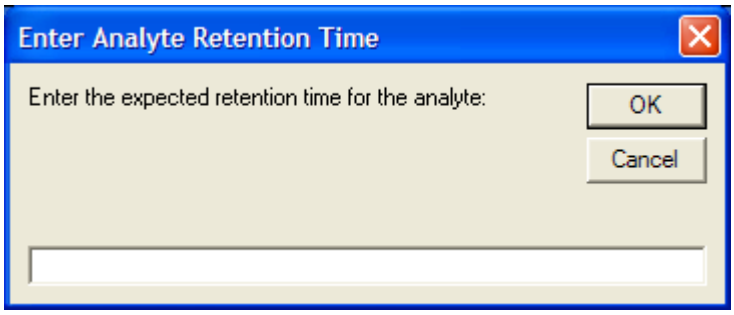


9.16.4.1 Set Retention Time Window Parameters…



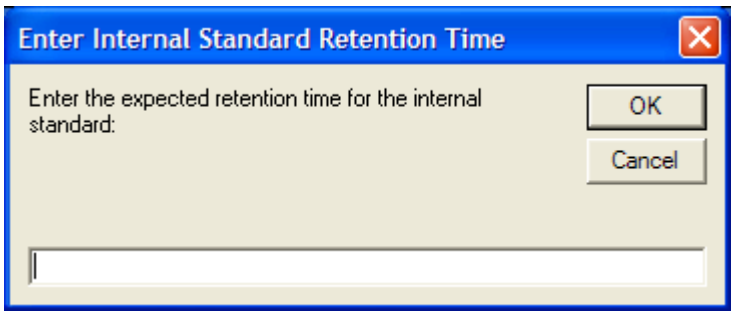
For Thermo data only. When the intensity of Analyte and/or Internal Standard peaks are low, then it is sometimes hard for GMSU/QC to distinguish expected (e.g. highest intensity) peaks from contaminant peaks. This function allows users to enter a retention time window to instruct GMSU/QC to look only within the retention window range to look for the most intense peak.

9.16.4.2 Enter LT Retention Time – Analyte…



For LT Acquisition only. This function is used to set the expected retention time of the analyte for LT data.

9.16.4.3 Enter LT Retention Time – Int Std…



For LT Acquisition only. This function is used to set the expected retention time of the internal standard for HT data.

9.16.5 Assign Transition Ions…

See Section 9.17 for details about assigning transition ions.

9.16.6 View Compound Information…

For Thermo only. Displays several tables of compound, sequence, and processing method information useful for debugging. Used specificially by GMSU/QC developers.

9.16.7 Configuration Utility…

Will open the Configuration Utility window and select the appropriate module tab.

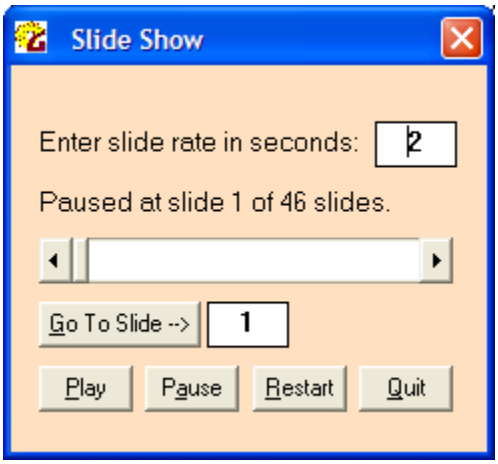
9.16.8 Deactivate Records…

Users may deactivate/activate records such that they do or do not appear in the Generate Reports window print queue. See Section 10.2 for further discussion.

9.16.9 UnSave Record…

This action will convert the Saved status of the record to False. The data is reprocessed as if it was a newly opened data file.

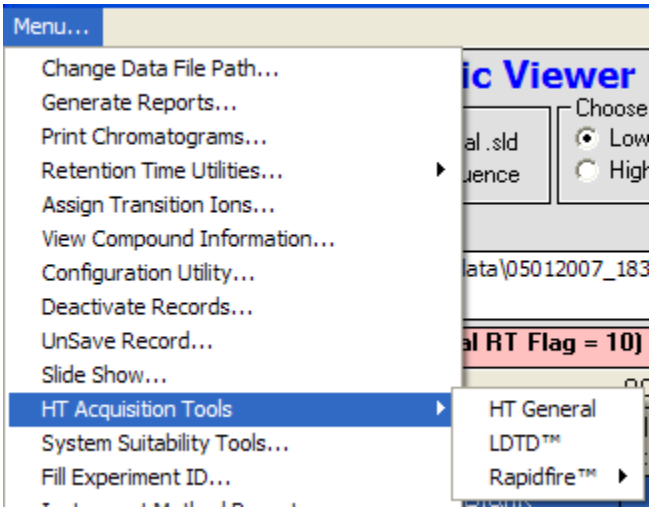
9.16.10 Slide Show…



Action will automatically scroll through the sample list chromatograms.

9.16.11 HT Acquisition Tools

For Thermo data only. Available only in the Generic Chromatographic Viewer. HT Acquisition is discussed in Section 3. GMSU/QC provides tools for the efficient processing of HT Acquisition data.

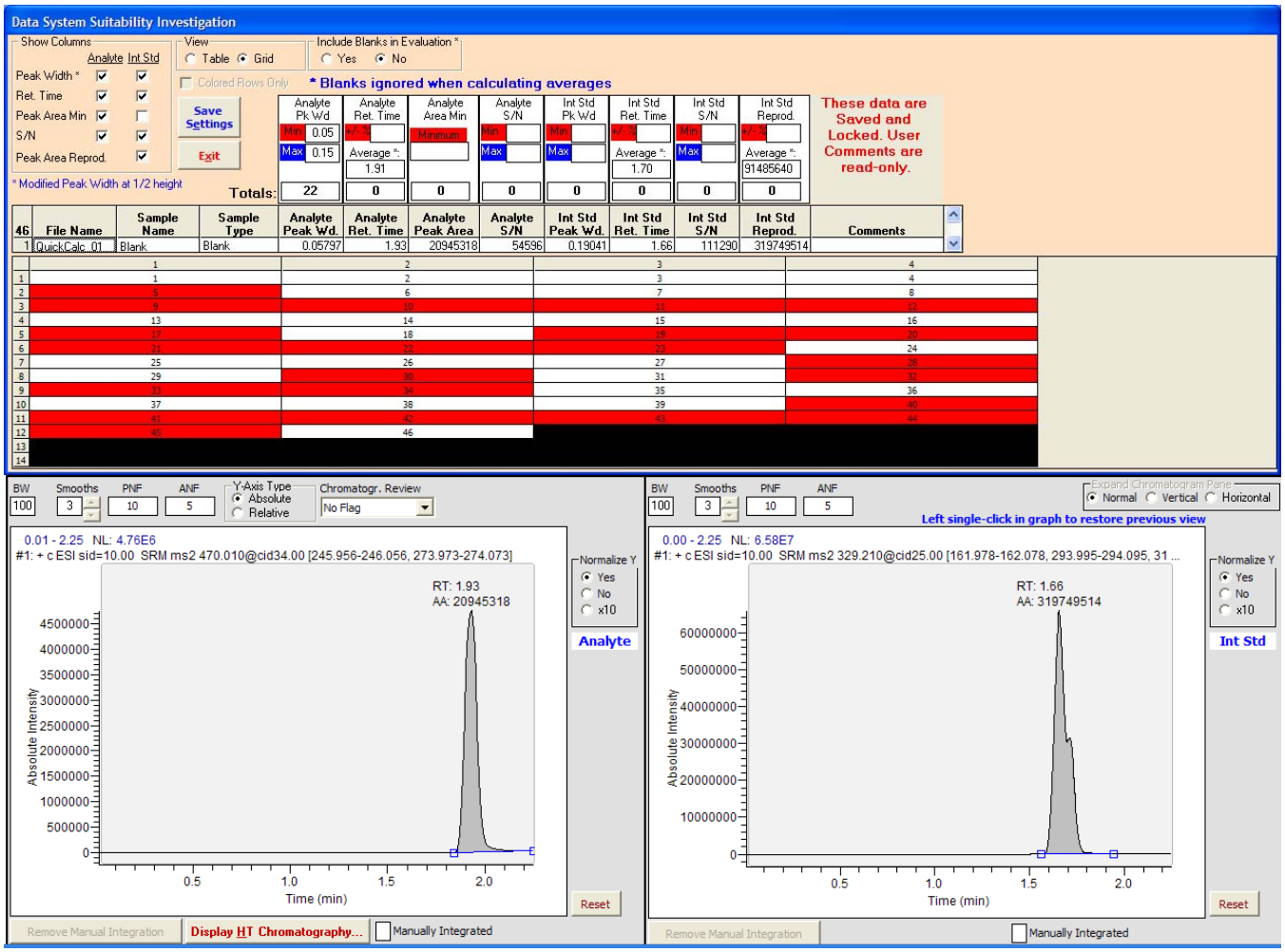


HT General refers to in-house processes for HT Acquisition.
LDTD™ refers to Phytronix LDTD HT Acquisition apparatus
RapidFire™ refers to Biotrove RapidFire HT Acquisition apparatus

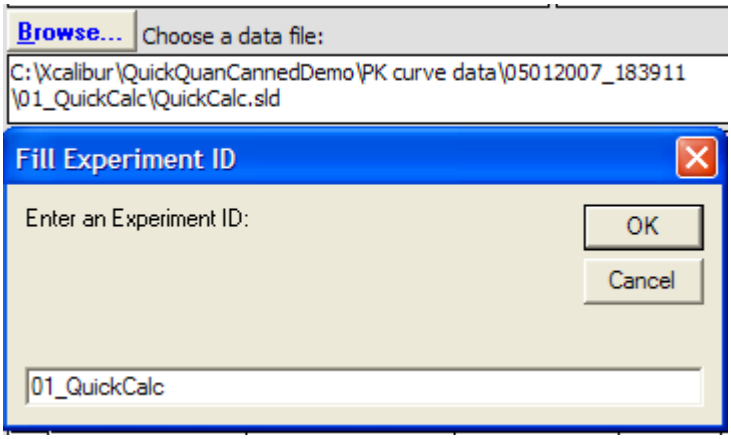
Chromatographic methods are created (if needed) to apply to HT Acquisition data. See Section 11 for discussion of the Chromatographic Method Designer.

9.16.12 System Suitability Tools…

The System Suitability Tool allows user to set several settings to determine if samples pass or fail criteria. This tool is useful for reviewing sequences containing a large number of samples (e.g. 384).



9.16.13 Fill Experiment ID…



Often times users need to tag several data files as part of group. This feature will automatically enter the user-entered Experiment ID into the Experiment ID field of every data file contained in the Choose a File table.

The default value for the Experiment ID is the subdirectory that contains the data file.

9.16.14 Instrument Method Report

Thermo data only. This feature will retrieve instrumental method from a .raw file in the sequence and display that information (along with some sequence information). The information may be exported to clipboard or Word™ document.

9.16.15 Help

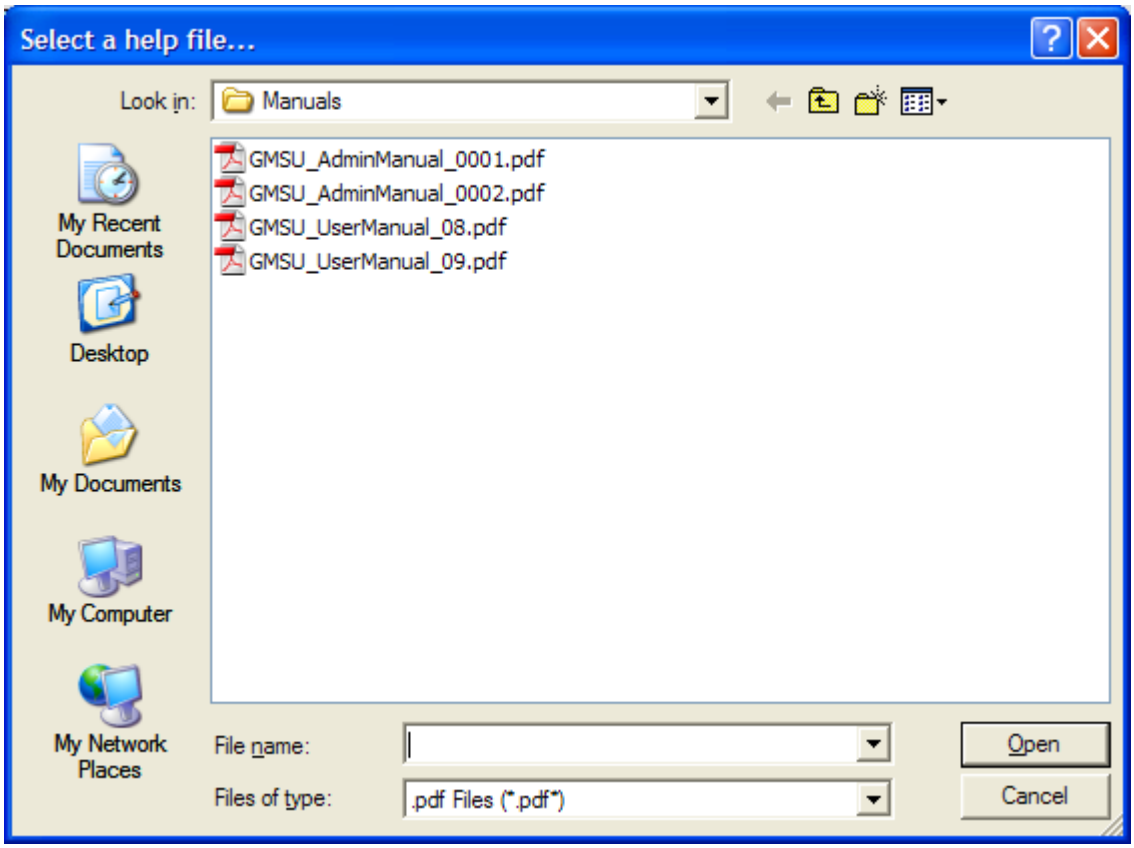


Figure 18 Help Window

By choosing Help, users will be prompted to open one of several Help files

9.17 Assign Transition Ions

9.17.1 Sciex data

When data is initially loaded, GMSU/QC looks in the instrument mass spec experiment and retrieves the first two transitions. GMSU/QC assigns the first transition as the Analyte and the second as the Internal Standard. If the acquired data is the opposite, then the user has the option to assign the appropriate transition to Analyte and Internal Standard. In addition, the user may create new Ion Sets if the data contain multiple analytes.

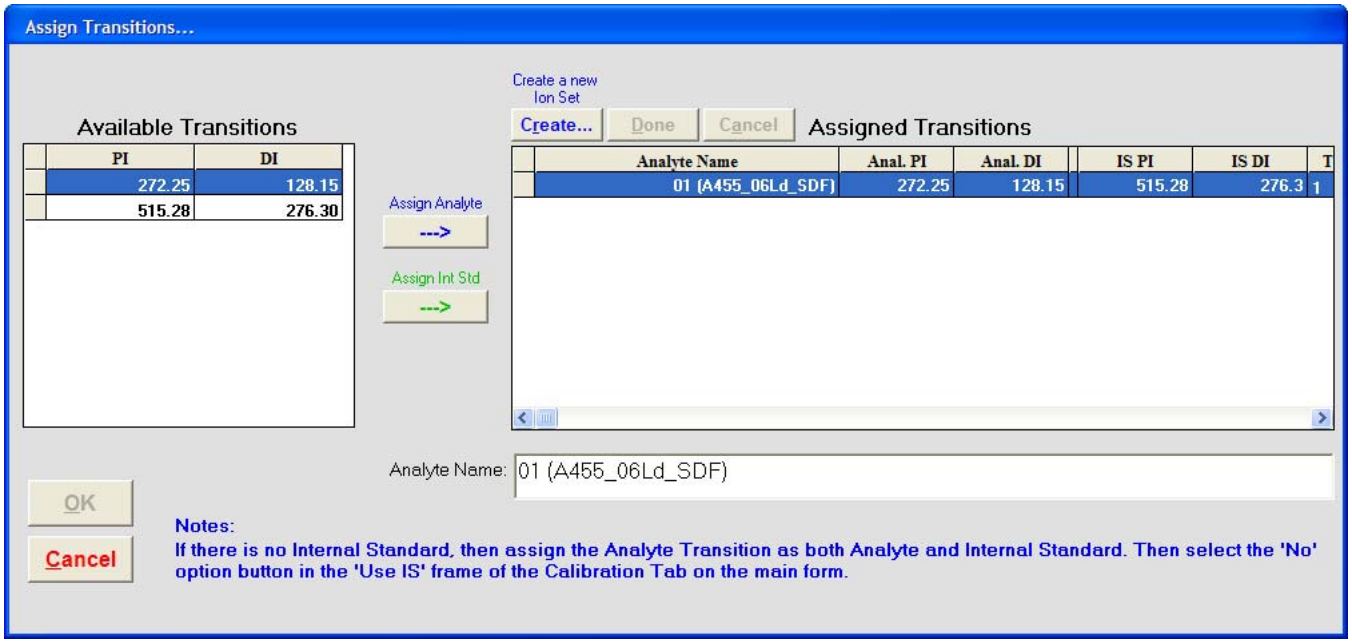
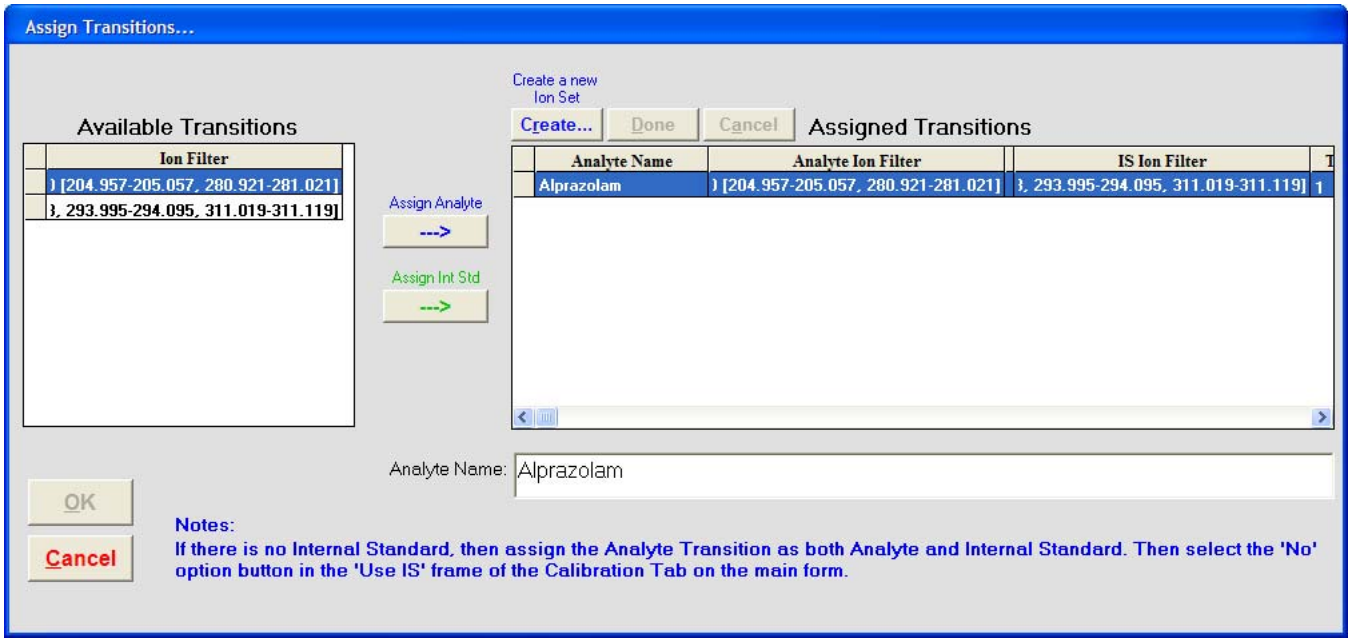


Figure 19 Assign Transition Ions Window – Sciex Data

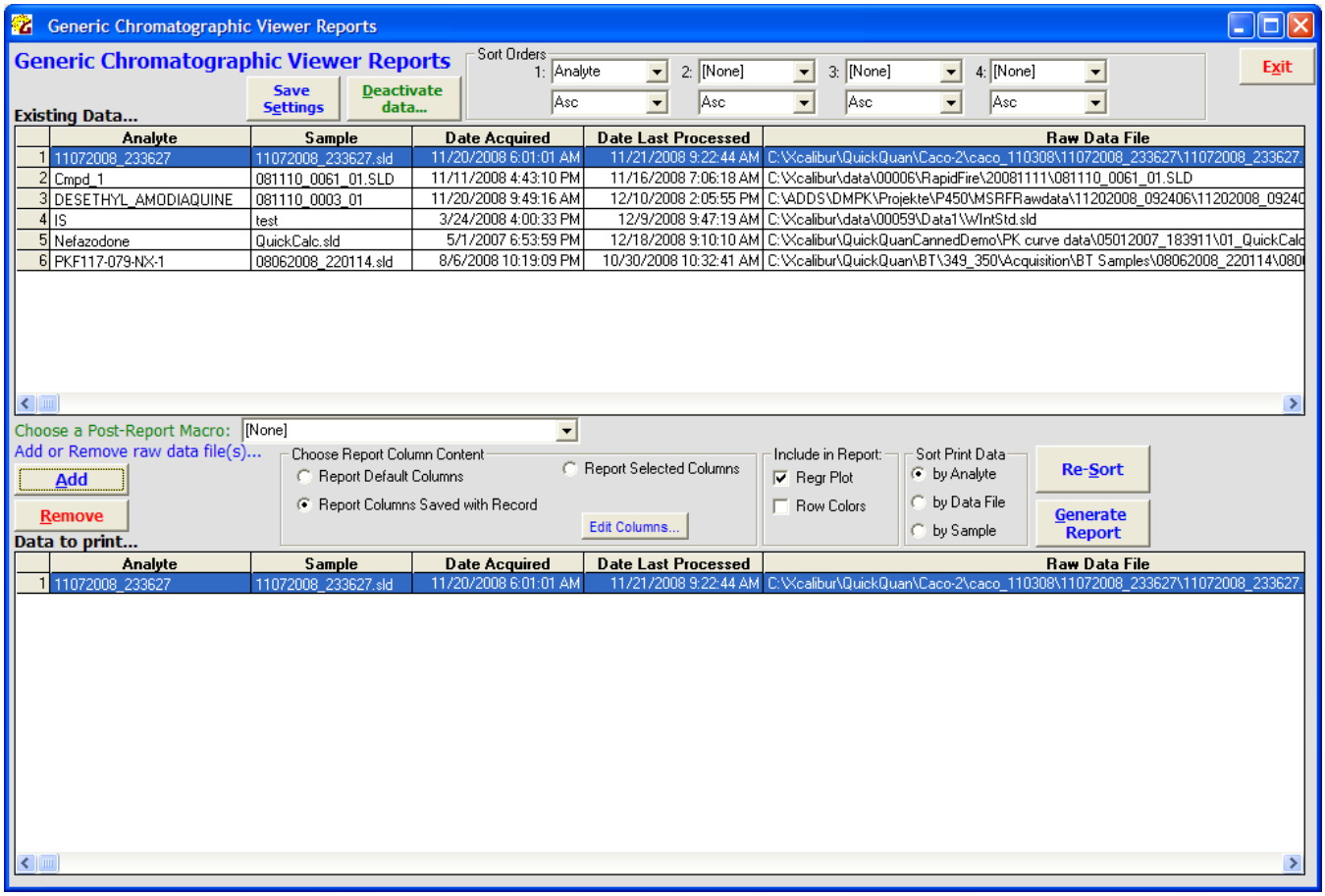
9.17.2 Thermo data

When data is initially loaded, GMSU/QC retrieves the first two transitions from the list of filter strings associated with each sample in the .sld. GMSU/QC assigns the first transition as the Analyte and the second as the Internal Standard. If the acquired data is the opposite, then the user has the option to assign the appropriate transition to Analyte and Internal Standard. In addition, the user may create new Ion Sets if the data contain multiple analytes.



10 Conserved GMSU/QC Reports Features

Each GMSU/QC module Reports window has several features that are identical (or nearly identical). These features are discussed here. The figure below shows the Generic Chromatographic Viewer module Reports window that has examples of these features.



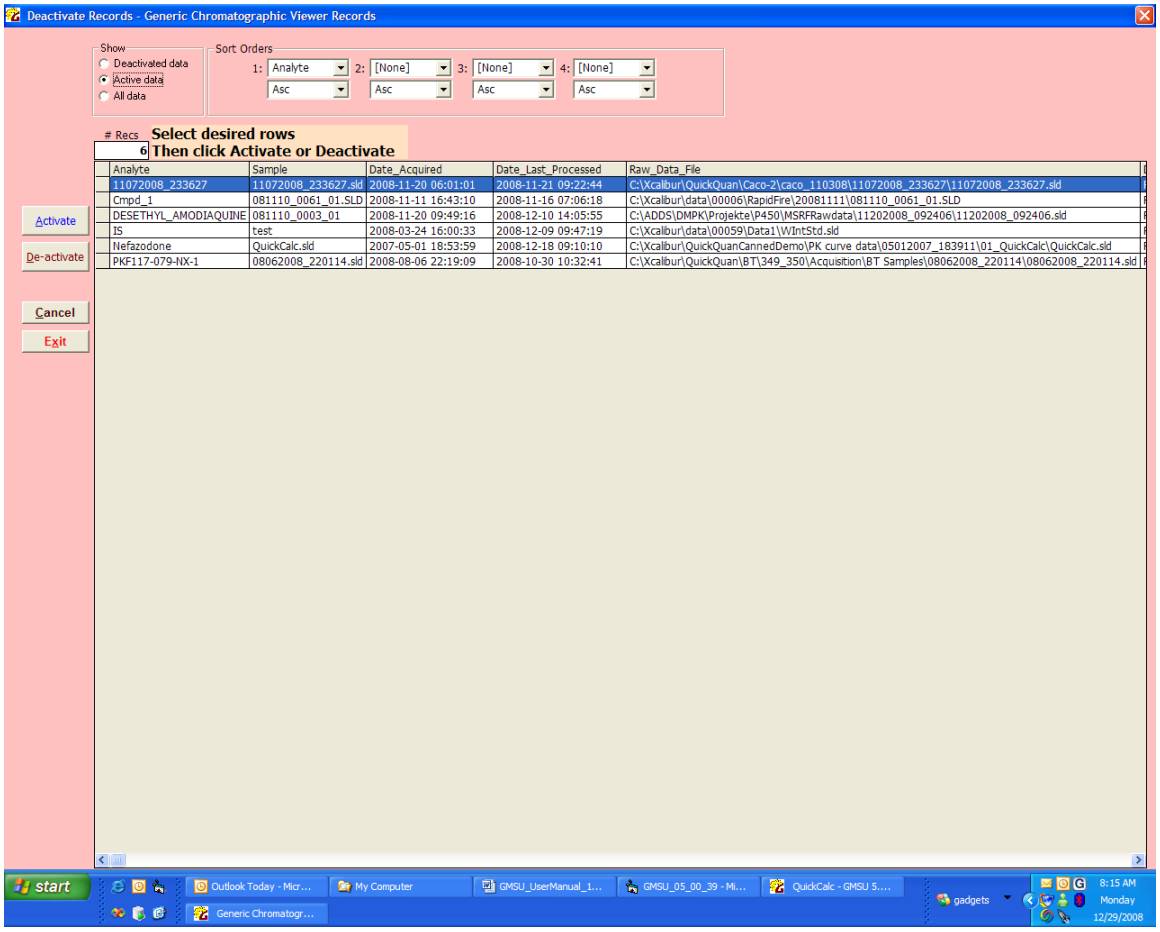
10.1 Save Settings

The Save Settings feature allows the user to save the settings (e.g. Sort Orders, Choose a Post-Report Macro, Include in Report, etc.) configured by the user. After executing Save Settings, those settings will be applied the next time the Reports window for that module is opened.

10.2 Deactivate Data

The Existing Data table is populated with data that has been Saved. Over time, this table can become quite large and filled with data that already has been reported. The Deactivate Data feature allows users to mark data as 'deactivated and not be listed in the Existing Data table.

Click the Deactivate Data button to show the Deactivate Records window:



To deactivate data:

- choose one or more rows
- click on the De-activate button.

To re-activate data

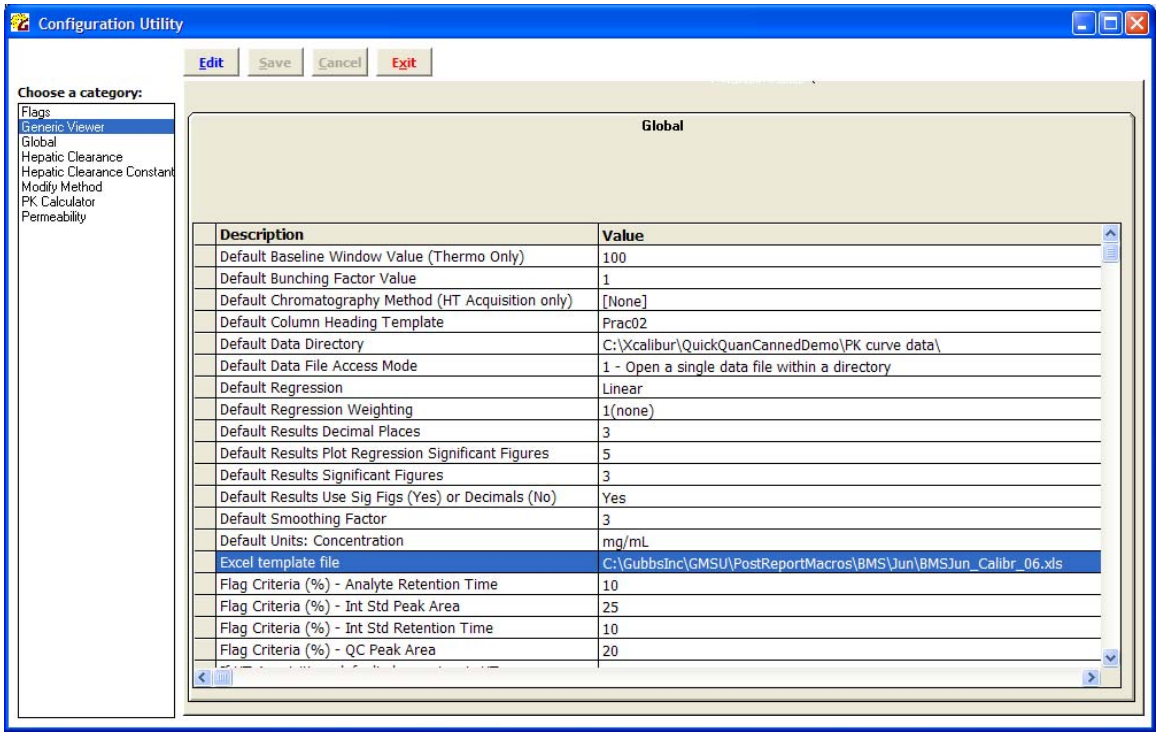
- choose the Deactivated data button in the Show option group
- choose one or more rows
- click on the Activate button

10.3 Sort Orders

Users may choose up to four sort orders to view data.

10.4 Choose a Post-Report Macro

Users may choose an appropriate Excel file template (or [None]) to use as a Post-Report macro. The dropdown box is populated with all Excel files that are contained in the Windows® directory of the 'Excel template file' entry of the module chosen in the Configuration Utility (see Section 14 for more information about the Configuration Utility).



11 Chromatographic Method Designer

Often times HT Acquisition data (see Section 3) may require a chromatographic method be developed in order to specify accurate peak retention times or to apply sample specific information to existing HT Acquisition data.

Depending on the acquisition source, there are two main deficiencies of HT Acquisition data:

A: Sample injection time and frequency information is may or may not be available

If sample injection time and frequency information is not known, then GMSU/QC cannot determine when or how many peaks should be contained in the HT chromatogram. GMSU/QC will simply report the number of peaks it can find using its normal integration algorithm – a number which is often correct if there are 25 or fewer peaks, but is most often incorrect if there are a large number (> 100) peaks.

B: Sample specific information may or may not be available

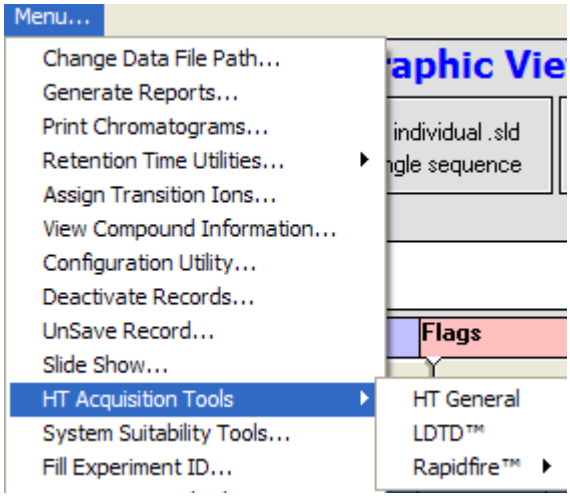
HT Acquisition data does not allow the data acquisition system to record sample specific information (e.g. sample name, sample type, sample ID, nominal concentration, etc.)

Fortunately, GMSU/QC has features (Chromatographic Method Designer) that assist the user in defining the number and expected times of peaks, as well as adding or importing sample specific information.

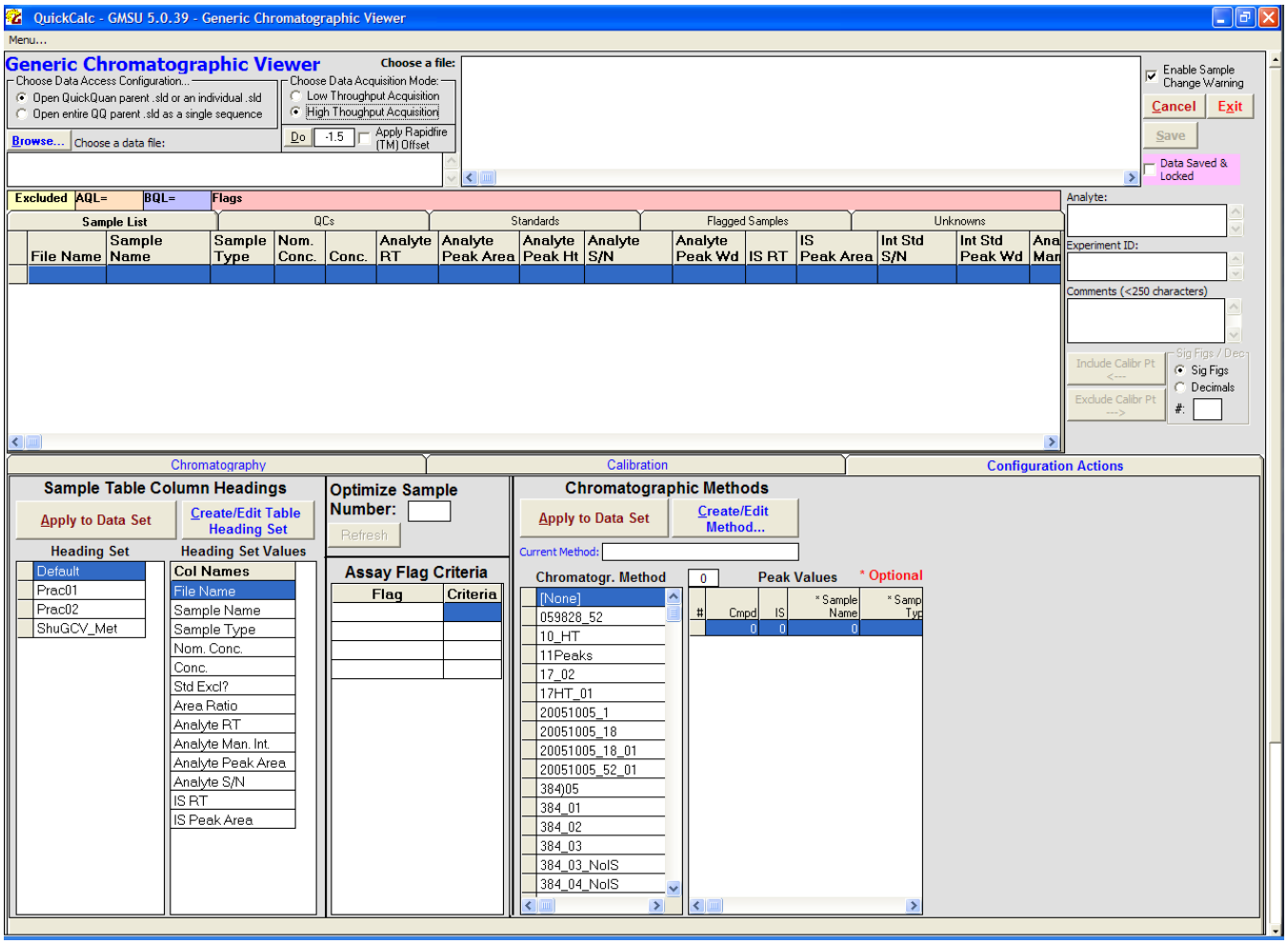
The following table contains comments about the deficiencies (A or B) in the HT Acquisition techniques

	HT General	LDTD	RapidFire
A	No means for obtaining injection times or number of expected peaks	No means for obtaining injection times or number of expected peaks	Generates a text file containing injection times and number of expected peaks
B	No means for obtaining sample specific information	No means for obtaining sample specific information	No means for obtaining sample specific information

The Chromatographic Method Designer can be accessed either through the Menu – HT Acquisition Tools – HT General (or LDTD) items.



Or the Designer may be obtained by clicking the Create/Edit Method button of the Configuration Actions tab (visible only if HT data is opened).



11.1 Chromatographic Method Designer window

The Chromatographic Method Designer is shown below.

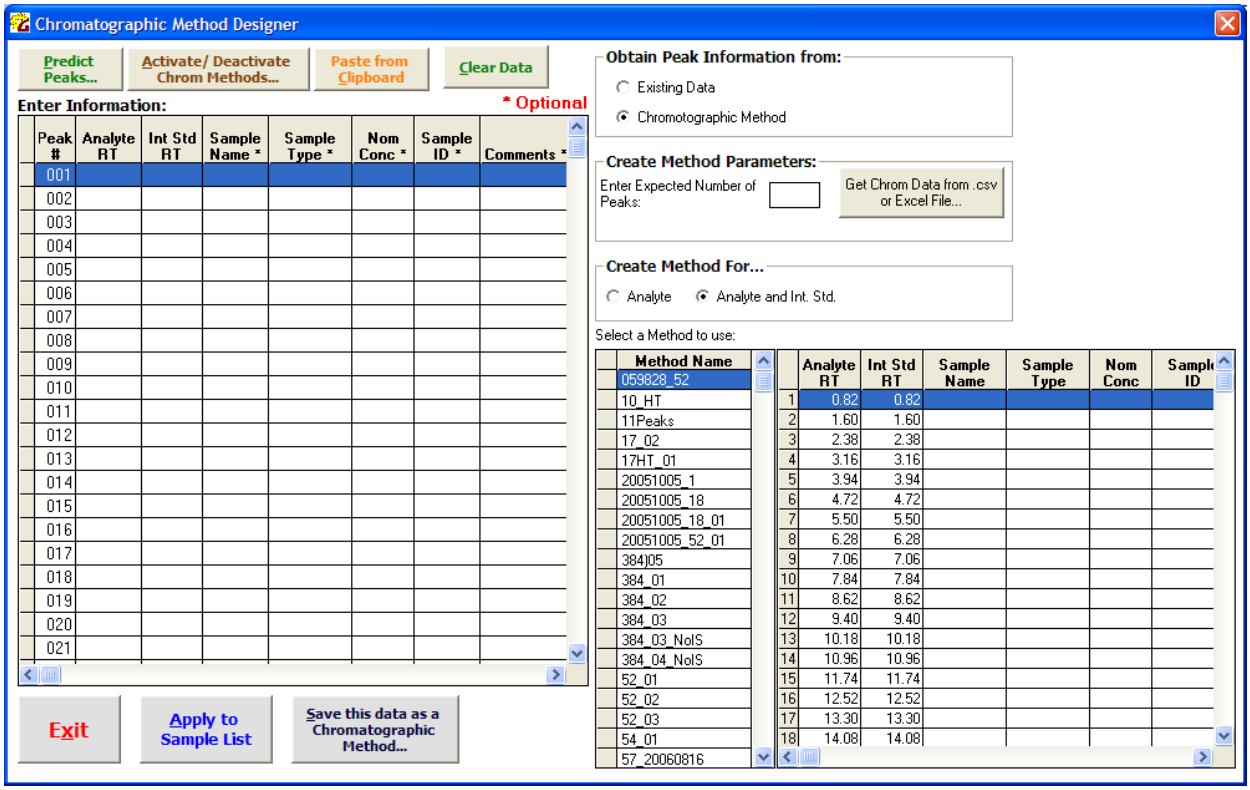


Figure 20 Chromatographic Method Designer Window

Users may choose to enter retention time for Analyte, Internal Standard, or both, and sample specific information, in the left portion of the window. If desired, the user may save this retention data as a new chromatographic method by clicking the 'Save this data…' button.

If desired, the user may use retention data from an existing saved sample by selecting the 'Existing Data' option button located in the 'Obtain Retention Data from:' option group. If the user chooses this option, then clicks on a sample in the 'Select a Sample to retrieve retention data' list, retention data from that sample will be automatically entered into the appropriate boxes (see Figure 21).

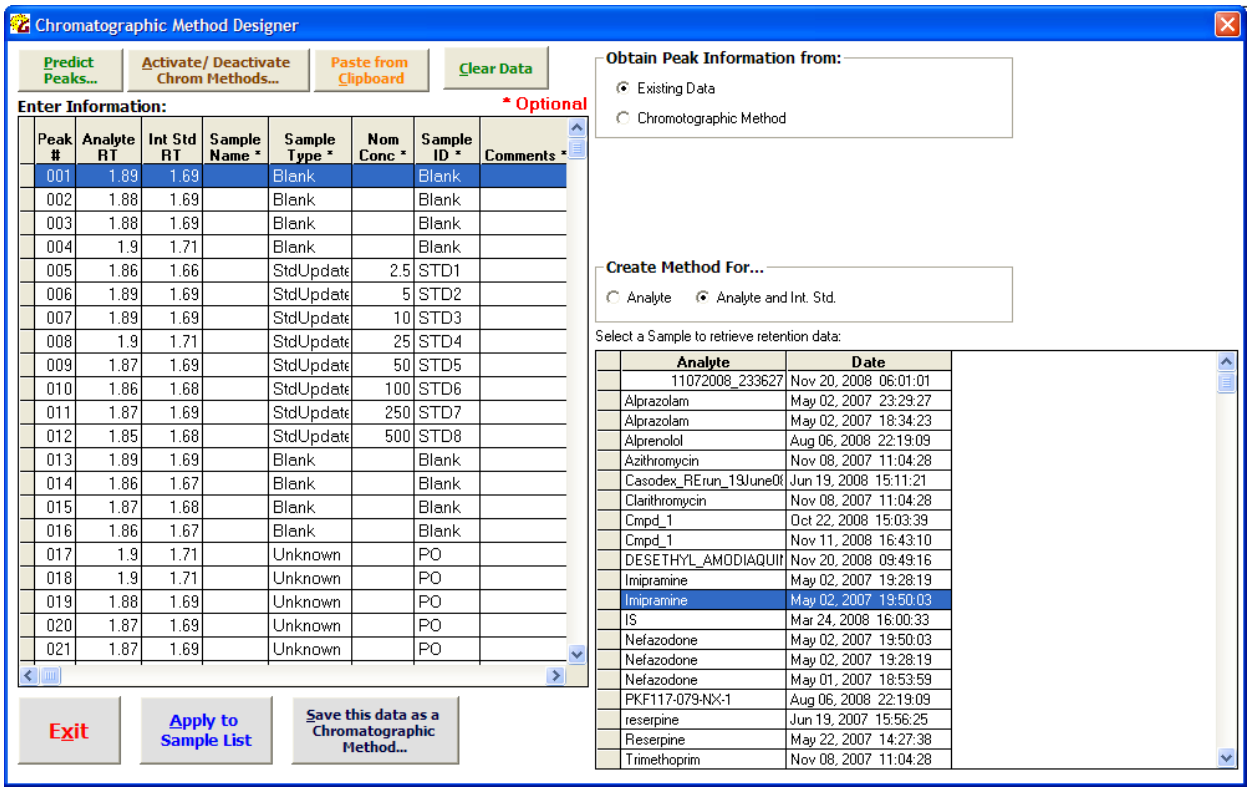


Figure 21 Use peaks from an existing sample

Alternatively, the user may wish to apply retention data from an existing chromatographic method by selecting the 'Chromatographic Method' option button located in the 'Obtain Retention Data from:' option group. If the user chooses this option, then clicks on a method in the 'Select a Method to use' list, retention data from that sample will be automatically entered into the appropriate boxes (see Figure 22).

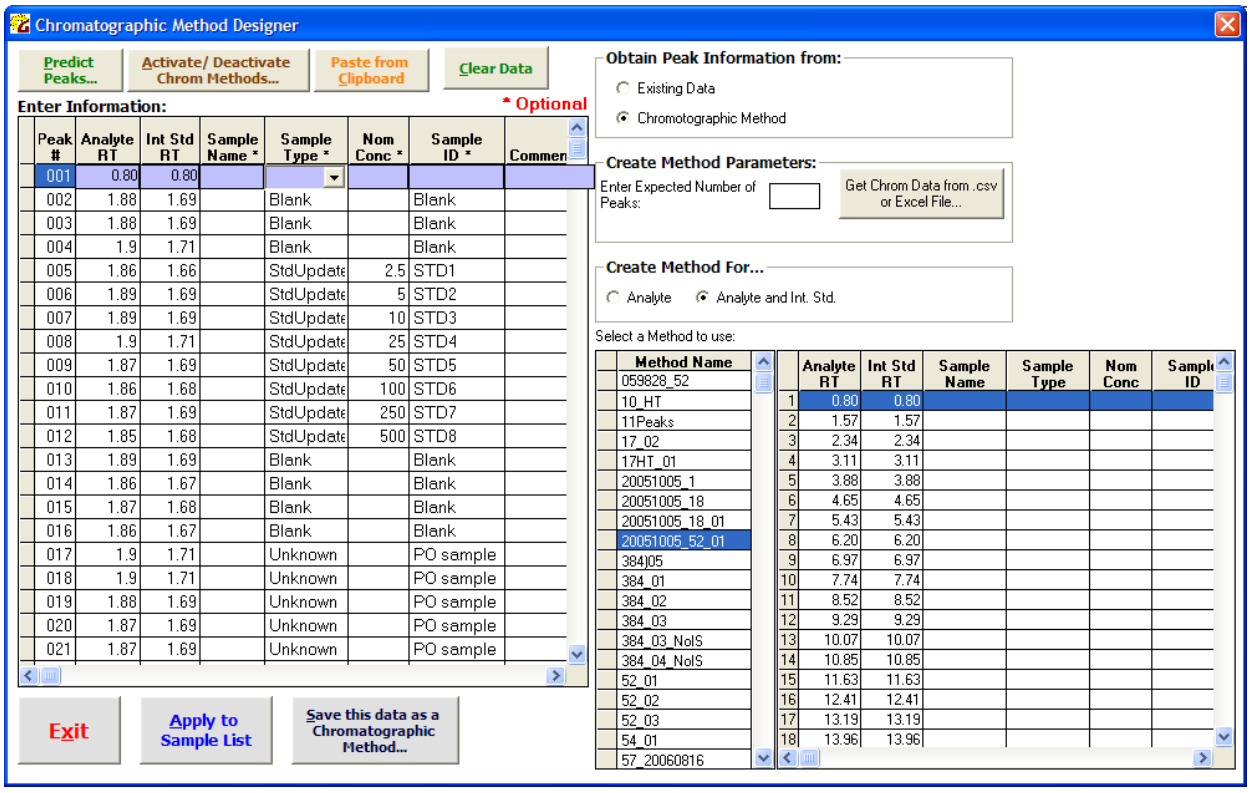


Figure 22 Use peaks from an existing chromatographic method

11.2 Paste from Clipboard… button

Alternatively, the user may copy/paste from an Excel file (Paste form Clipboard… button) the retention time and/or sample specific information. The Excel file must have 7 columns. In the example below, the first row contains the names of the column information. In this example, the user would copy rows 2 – 15, columns 1 – 7.

TargetRT	IntStdRT	SampleName	SampleType	NomConc	SampleID	Comments
0.12	0.12	Sample001	Blank		'0012	
0.36	0.36	Sample002	Blank		'0013	
0.6	0.6	Sample003	Standard	10	'0014	
0.84	0.84	Sample004	Standard	20	'0015	
1.09	1.09	Sample005	Standard	30	'0016	
1.32	1.32	Sample006	Unknown		'0017	
1.57	1.57	Sample007	Unknown		'0018	
1.81	1.81	Sample008	Unknown		'0019	
2.05	2.05	Sample009	Unknown		'0020	
2.33	2.33	Sample010	Unknown		'0021	
2.56	2.56	Sample011	Unknown		'0022	
2.8	2.8	Sample012	QC	20	'0023	
3.04	3.04	Sample013	QC	20	'0024	
3.29	3.29	Sample014	Blank		'0025	
3.52	3.52	Sample015	Blank		'0026	

11.3 Create Method Parameters

11.3.1 Enter Expected Number of Peaks

When the user enters a number here, the number of rows in the Enter Information table will be set to this number.

11.3.2 Get Chrom Data from .csv or Excel File… button

Users may import retention and/or sample specific data from an existing Excel or .csv file. The format of the file should conform to that shown in the table in Section 11.2.

11.4 Final action

After retention and/or sample specific data has been entered, the user may save the information (if desired) as a chromatographic method to be applied to future day.

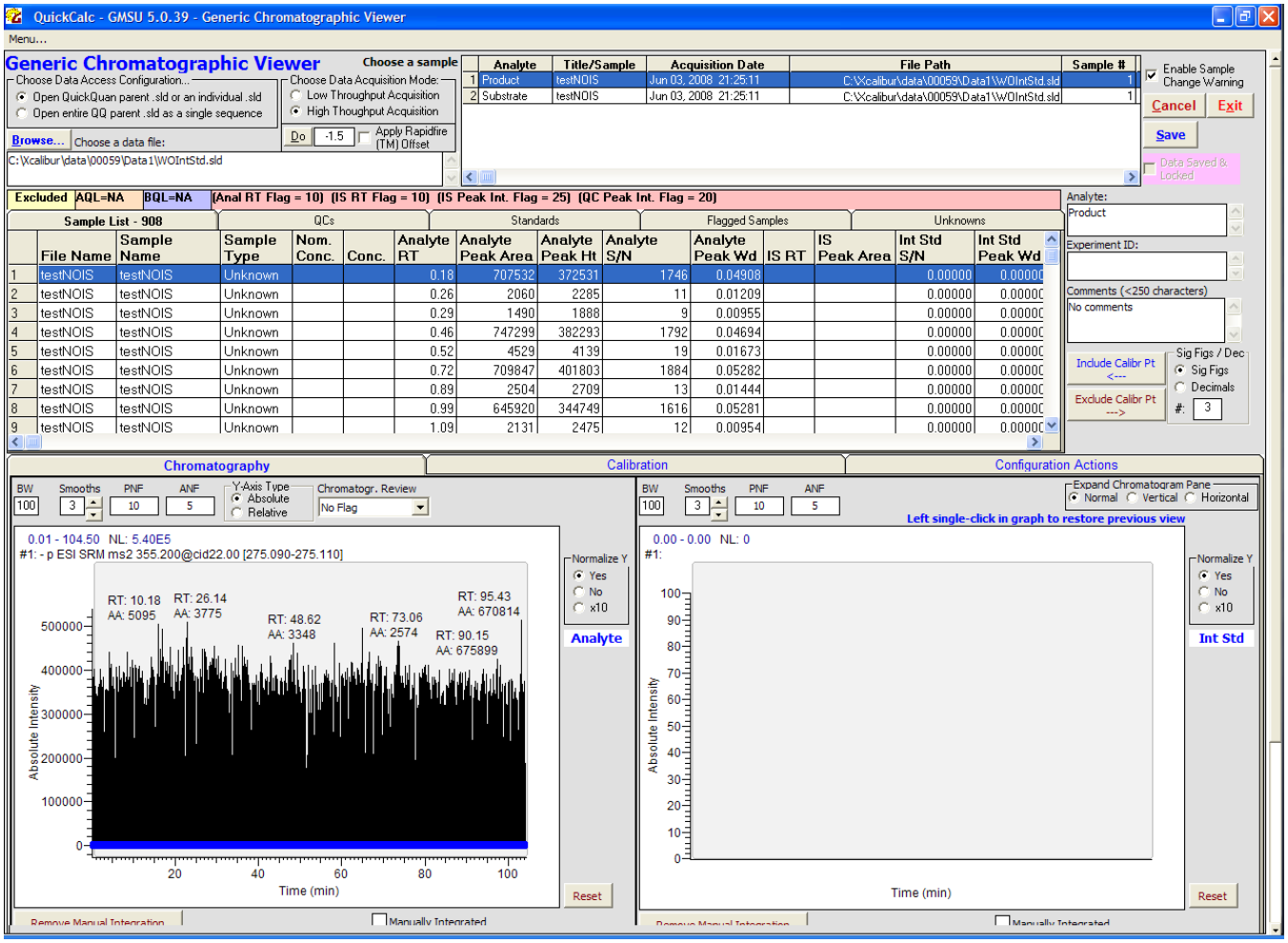
Finally, the user may apply this information to the underlying Sample List:

- the user clicks on the ‘Apply to Chromatogram’ button
- the retention and/or sample specific data are entered into the appropriate chromatographic data grids
- the GMSU/QC integration algorithm is called to calculate peak areas (if retention time has been applied).

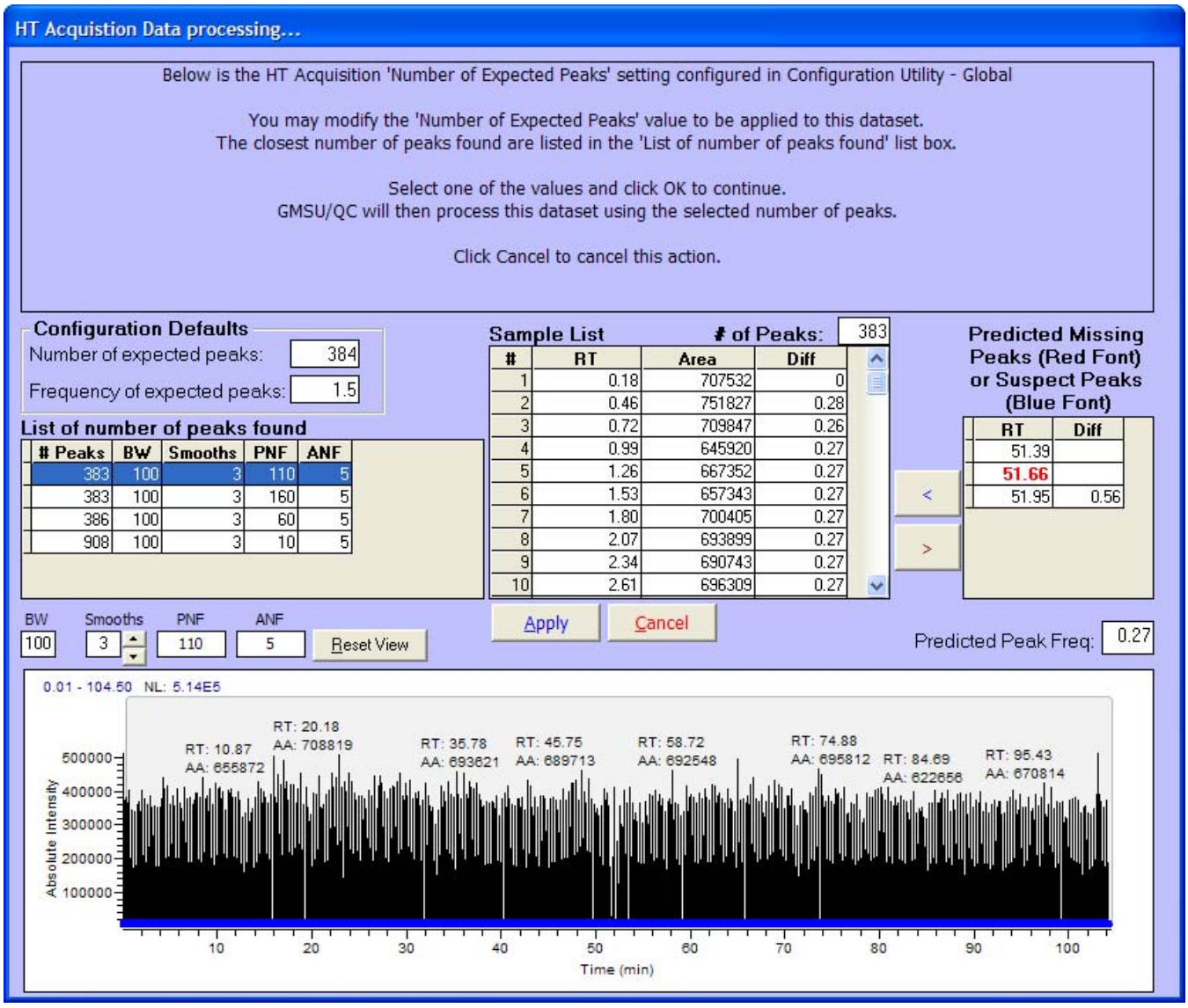
11.5 Predict Peaks…

When the HT Acquisition file contains a large number of peaks and no injection time information exists, the GMSU/QC peak identification algorithm becomes less reliable and may not predict the correct number of peaks.

In the example shown, the data is from 384 injections. Since no injection time information exists, GMSU/QC attempts to indentify the correct number of peaks. As is shown, the number of predicted peaks (908) is incorrect.

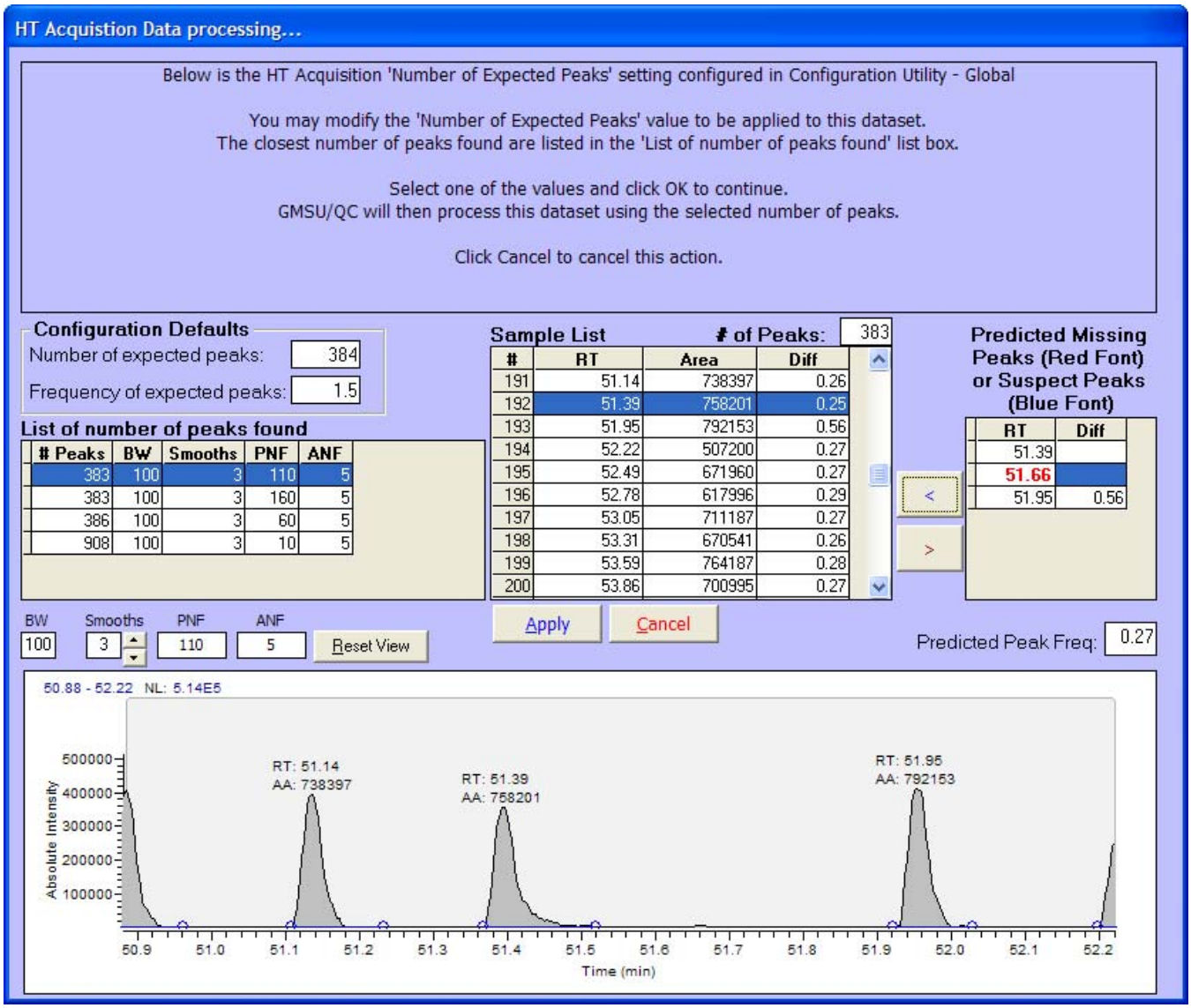


The Predict Peaks feature will predict the identity of peaks based on user-defined number of peaks entry, then optimizing the PNF variable to retrieve one or more sample lists with the number of peaks closest to the number expected. The choices of sample lists are listed in the List of Number of Peaks Found table. Users may select the different choices to investigate.

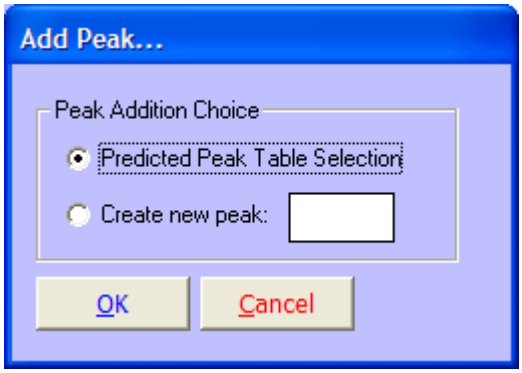


The Predict Missing Peaks table will give suggestions of missing peaks (in red font) or incorrect peaks (in blue font). In the example above, the closest integration variable choice contains 383 peaks. Predict Peaks suggests that peak RT 51.66 be added.

If the user clicks on the cell 51.39 or 51.95, the chromatogram will be expanded in this area to show indeed that a peak probably belongs at 51.66.

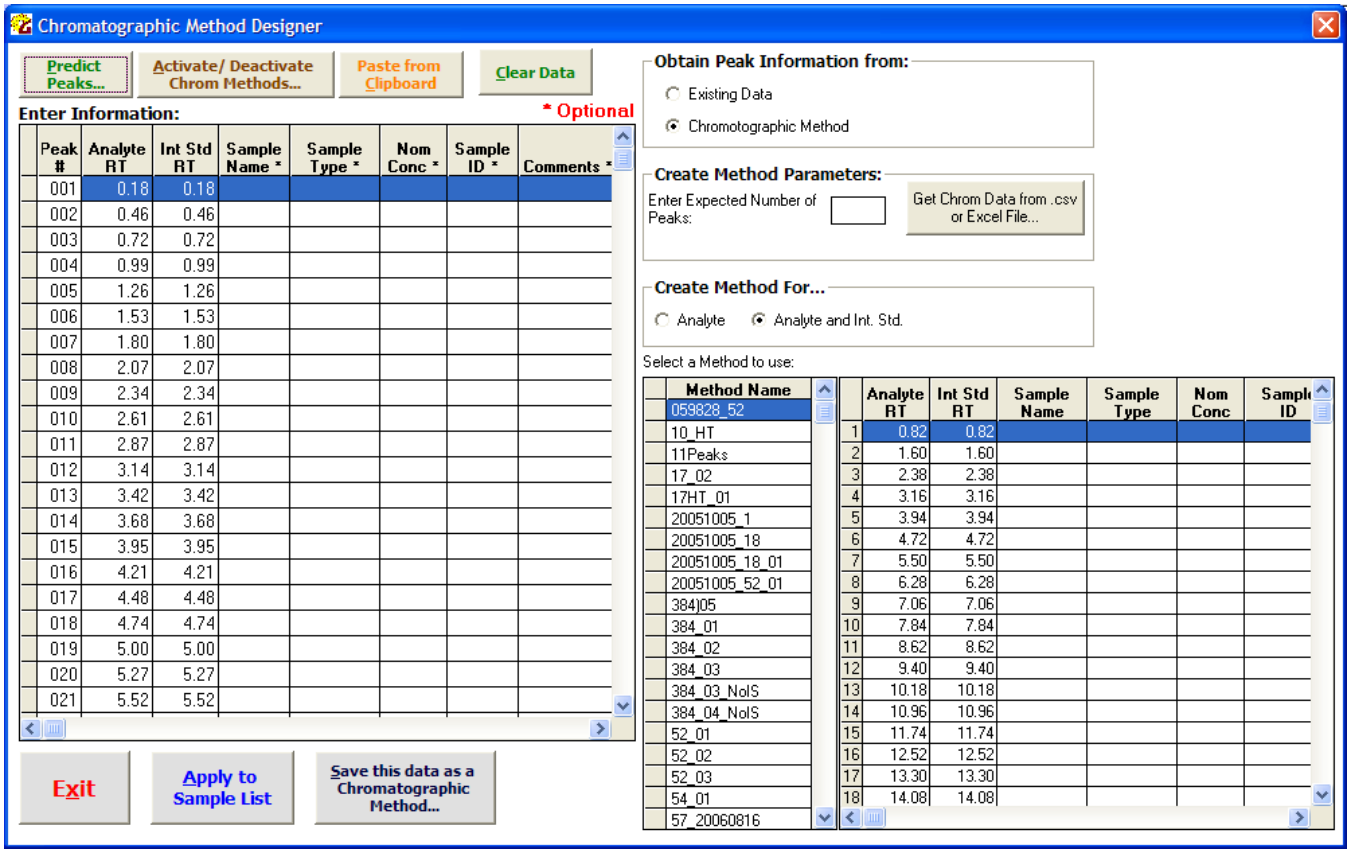


The user may add the peak at 51.66 by selecting 51.66 from the list, then clicking the blue lessthan arrow button. The user is given the choice to add the Predicted Peak to the sample list. Alternatively, the user may enter a user-defined peak by selecting the Create New Peak option and entering a new retention time.

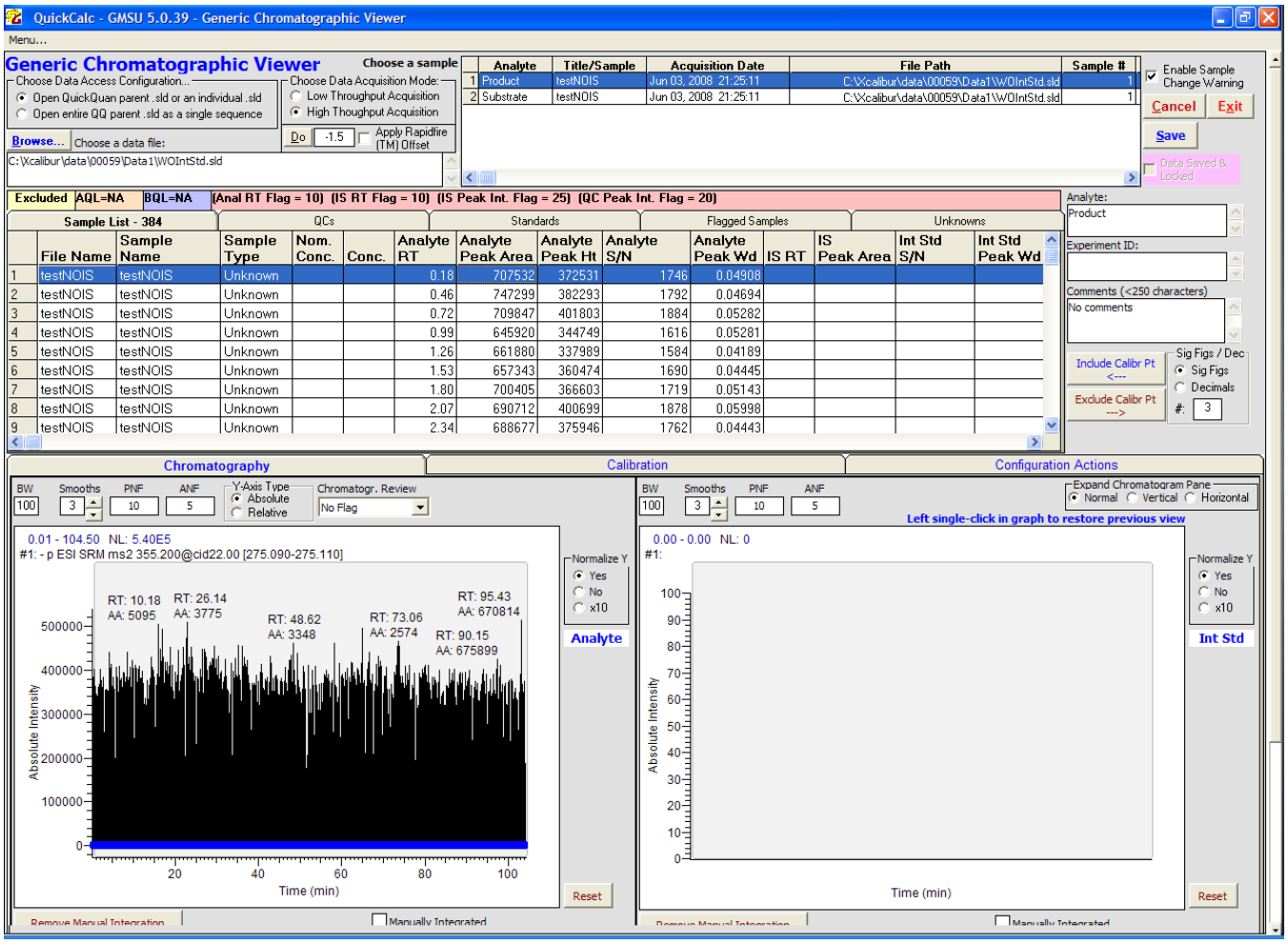


Users may also remove peaks from the Sample List by selecting an item from the Sample List and clicking the red greaterthan arrow button.

Once satisfied, the user clicks the Apply button, which will bring the user back the Chromatographic Method Designer window with the retention time information populated in the Enter Information table.



The user may then save the information as a chromatographic method, then apply the data to the underlying sample list. As shown below, the sample list now contains 384 samples.



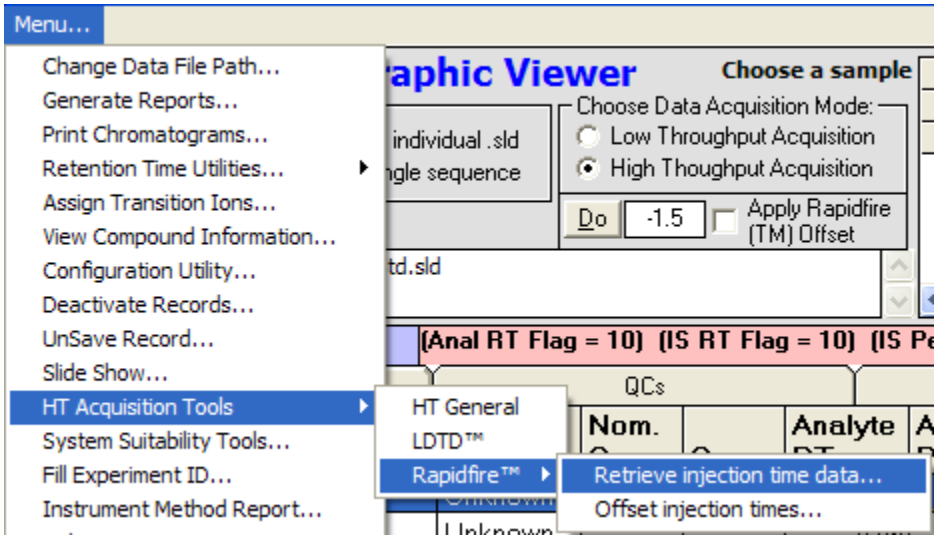
Users may then save that data as a new chromatographic method and/or apply the information to the underlying Sample List.

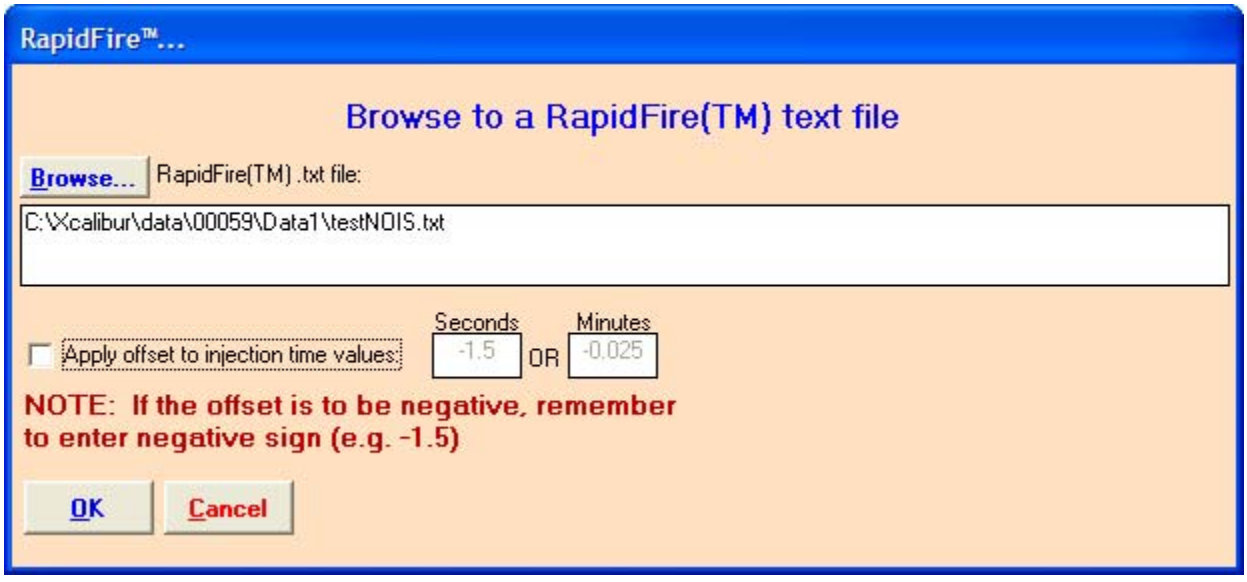
11.6 RapidFire™ tools

Thermo data only.

Biotrove RapidFire generates a text file containing injection times. If the text file is located in the same directory as the .raw file and has the same name as the .raw file, GMSU/QC will automatically open it, generate a chromatographic method, and apply the method to the underlying sample list.

If the text file is located elsewhere and/or does not have the same name as the .raw file, then the user may browse to and open the text file by executing Menu – HT Acquisition Tools – Rapidfire – Retrieve injection time data.





Note that sometimes the injection time information may be inaccurate by several seconds, which can possibly result in inaccurate predicted peaks. The user is offered the option to enter an offset (in seconds or minutes) to apply to the text file injection time information.

12 Design Sample Table Window

All modules except the Hepatic Clearance Calculator have a Design Sample Table window that may be accessed through the Configuration Actions tab – Create/Edit Table Heading Set button (see Section 9.14.1.2).

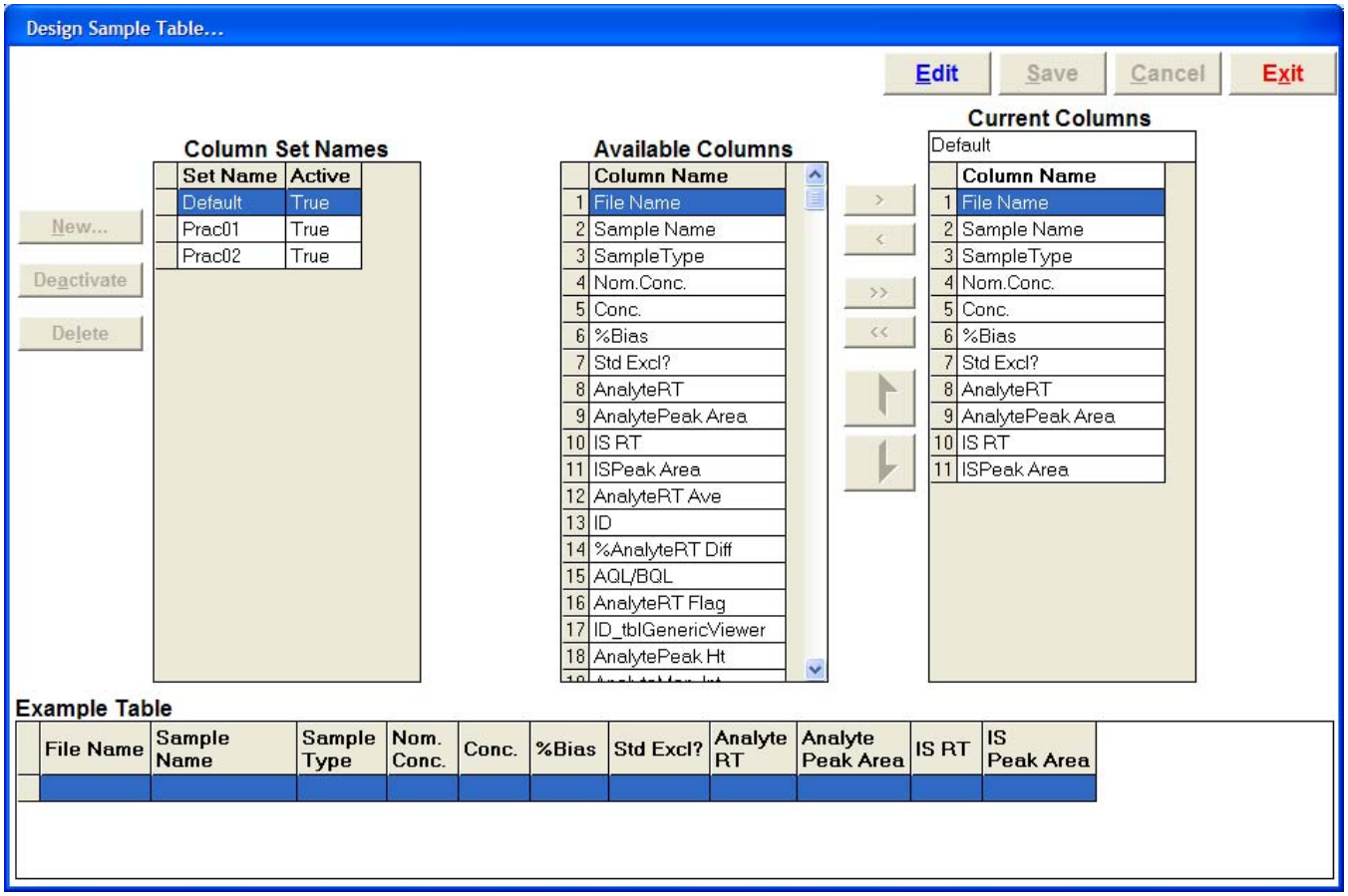


Figure 23 Design Sample Table

The window initially appears in a read-only mode. In order to perform an action (New, Deactivate, Delete), the user must:

- Select a Set Name in the Column Set Names table
- Click on Edit
- Perform Actions
- Click Cancel if the user wishes to discard actions OR
- Click Save if the user wishes to save actions

The window contains the following sections:

Section	Description
Column Set Names	A list of all Column Set Names. GMSU/QC comes preconfigured with Default
Available Columns	A list of all available columns for the Sample List for the module that calls the window
Current Columns	Shows the currently configured columns for the Column Set Name selected in the Column Set Names table
Example Table	Shows the layout of the Sample List table for the Column Set Name selected in the Column Set Names table

12.1 Procedure

12.1.1 Add New Column Set Name

- Click the Edit Button
- Click the New Button
- When prompted, enter the name of the new Set Name
  - The Current Columns table will be filled with Default values
- To add columns to the Current Columns table, select one or more rows in the Available Columns table and click the Add Row (>) Button
- To remove columns to the Current Columns table, select one or more rows in the Current Columns table and click the Remove Row (<) Button
- Order the columns in the desired order by selecting items and click the Up (↑) or Down(↓) Buttons

12.1.2 Edit a Column Set

- Choose a Set Name from the Column Set Name
- Click the Edit Button
- Modify the contents of Current Columns table as needed according to the procedures described in Section 12.1.1.

12.1.3 Delete a Column Set Name

- Choose a Set Name from the Column Set Name
- Click the Edit Button
- Click the Delete Button

The Column Set Name is now deleted. If any Saved data has been saved with this Column Set Name, the Set Name in the data set will revert to the Column Set Name 'Default' when data is loaded in a module.

12.1.4 Deactivate a Column Set Name

- Choose a Set Name from the Column Set Name
- Click the Edit Button
- Click the Deactivate Button

The Column Set Name is now deactivated. The Set Name will not be listed in the [Module] – Configuration Actions tab – Sample Table Column Headings section – Heading Set table. However, if any Saved data has been saved with this Column Set Name, the Set Name in the data set will remain unchanged when data is loaded in a module.

13 Console

The GMSU/QC Console is the module from which users navigate to other modules. The Console contents may differ slightly from what is shown.

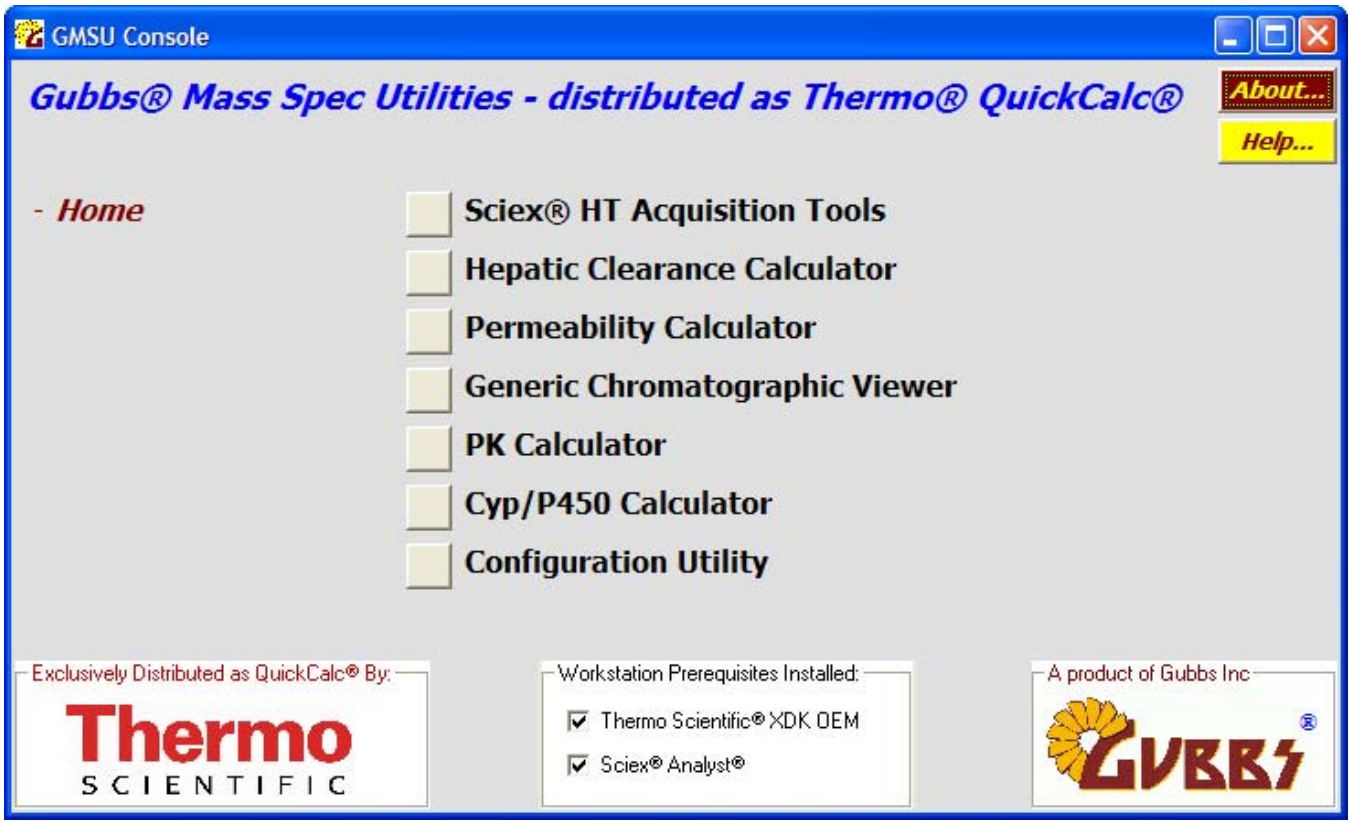


Figure 24 GMSU/QC Console

Users click on the appropriate buttons either to open a utility or to navigate to an additional hierarchical level. Please note that the Sciex HT Acquisition Tools apply are useable only by Millennium Pharmaceuticals in Cambridge, MA.

14 Configuration Utility

It is recommended that administrators and/or power users peruse all the options listed in the Configuration Utility. The utility sections contain many parameters that are configurable by the user.

Administrators use the Configuration Utility to configure such things as global variables, contents of module dropdown lists, and units (e.g. mg/mL) of different parameters. Please see the GMSU/QC Administration and Installation Manual for further details.

15 Sciex HT Acquisition Tools

This is a set of tools that are helpful to Sciex Analyst users when performing pre-acquisition actions. Please note that these tools support only the internal processes of Millennium Pharmaceuticals in Cambridge, MA.

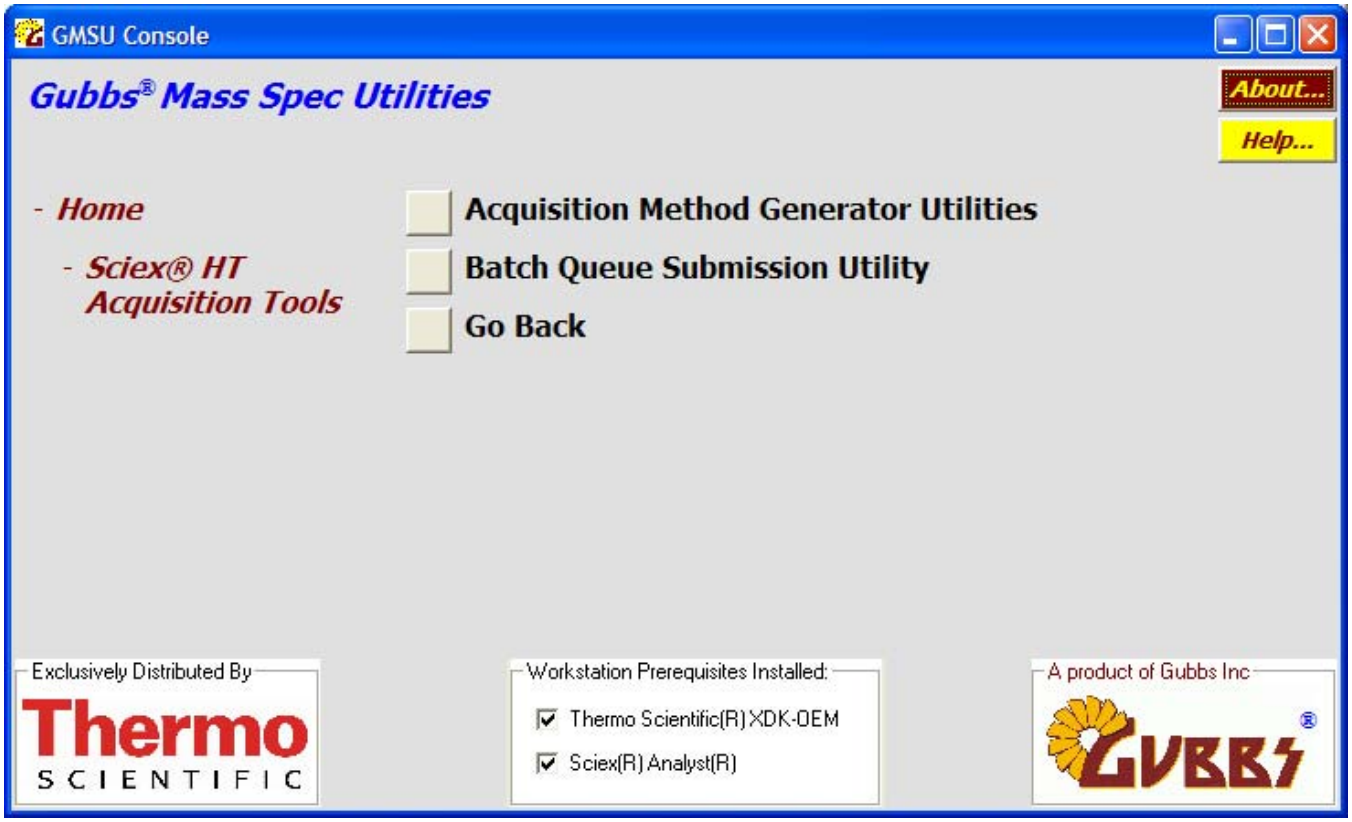


Figure 25 Sciex HT Acquisition Tools

16 Acquisition Method Generator Utilities

Please note that this tool supports only the internal processes of Millennium Pharmaceuticals in Cambridge, MA.

The Acquisition Method Generator Utilities are a suite of utilities that are focused on specific tasks involved in acquisition method generation. The available Method Generator Utilities are described as follows:

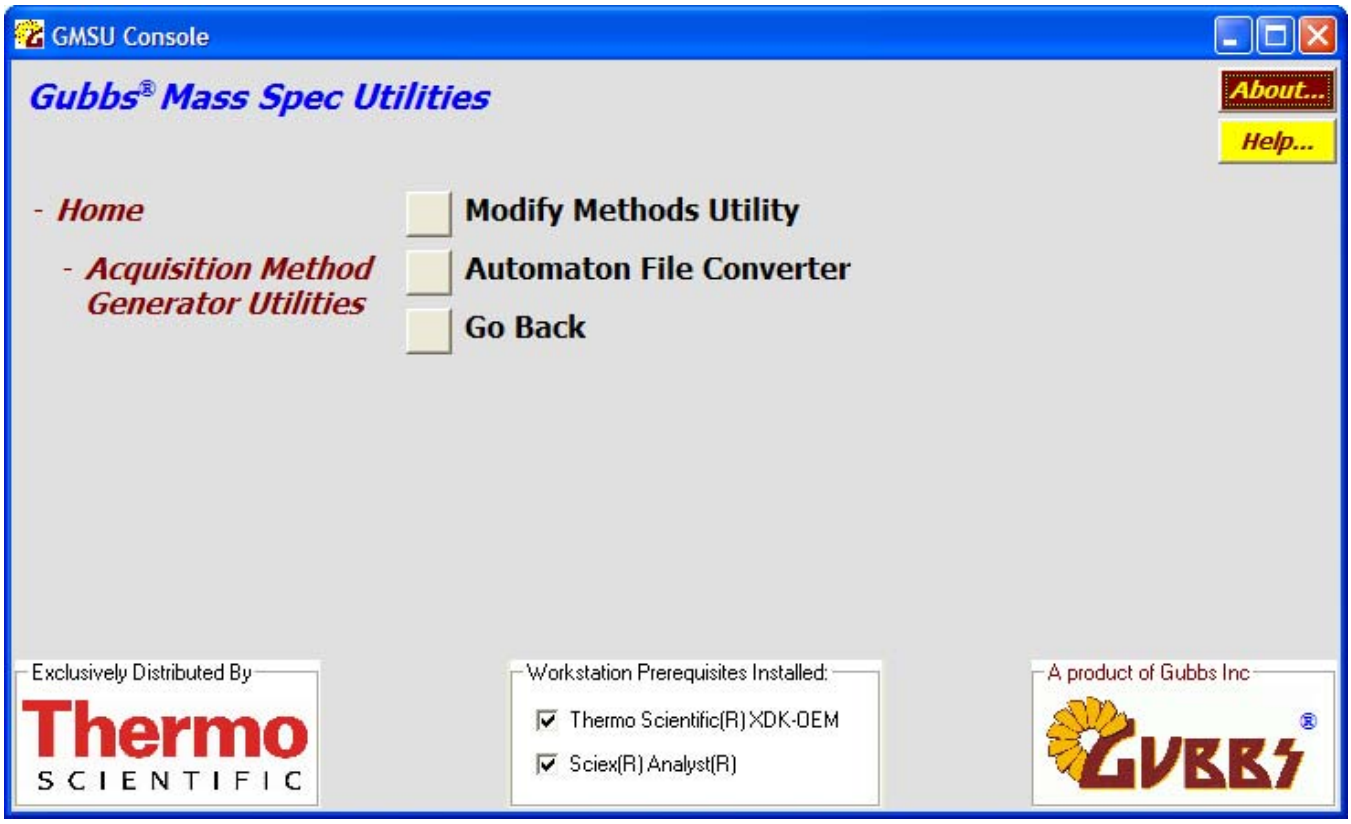


Figure 26 Acquisition Method Generator Utilities

17 Modify Methods Utility

Please note that this tool supports only the internal processes of Millennium Pharmaceuticals in Cambridge, MA.

The Modify Methods Utility performs several functions:

- Allows users to modify common method variables of existing methods in batch mode based on a user-specified text/Excel file or all methods within a directory.
- Allows users to add an Internal Standard (second transition in a period) to existing methods (form MRM methods only)

The following describes the procedure for using the MRM Method Generator utility.

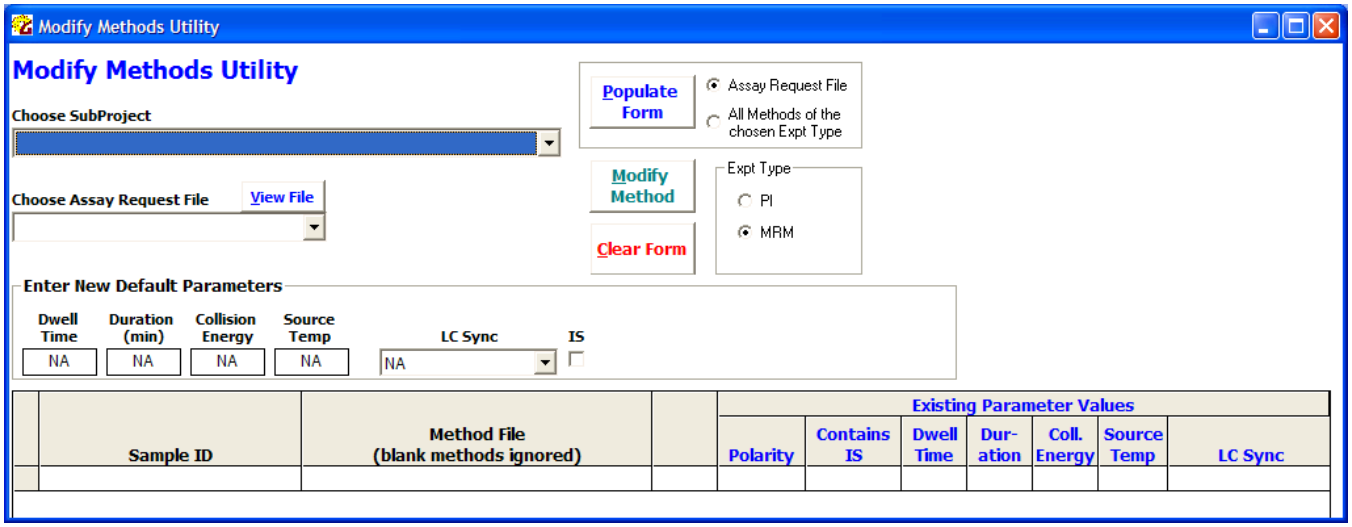


Figure 27 Modify Methods Utility

- Choose a 'Populate Form' option; either 'Assay Request File' or 'All Methods of the chosen Expt Type'.

  If 'All Methods of the chosen Expt Type' is chosen, then the 'Choose Assay Request File' dropbox becomes disabled and is unneeded.
- Choose an Experiment Type option from the 'Expt Type' frame.

  If Product Ion (PI) is chosen, then 'IS' checkbox is hidden and is not available.
- Choose subproject
- Choose Assay Request File from the dropbox
- Enter values for desired variables
- If an Internal Standard is to be added, select the IS checkbox.

  Two dropdown boxes will appear for Internal Standard method file source configuration.

  Both dropdowns must be completed (see Figure 28).

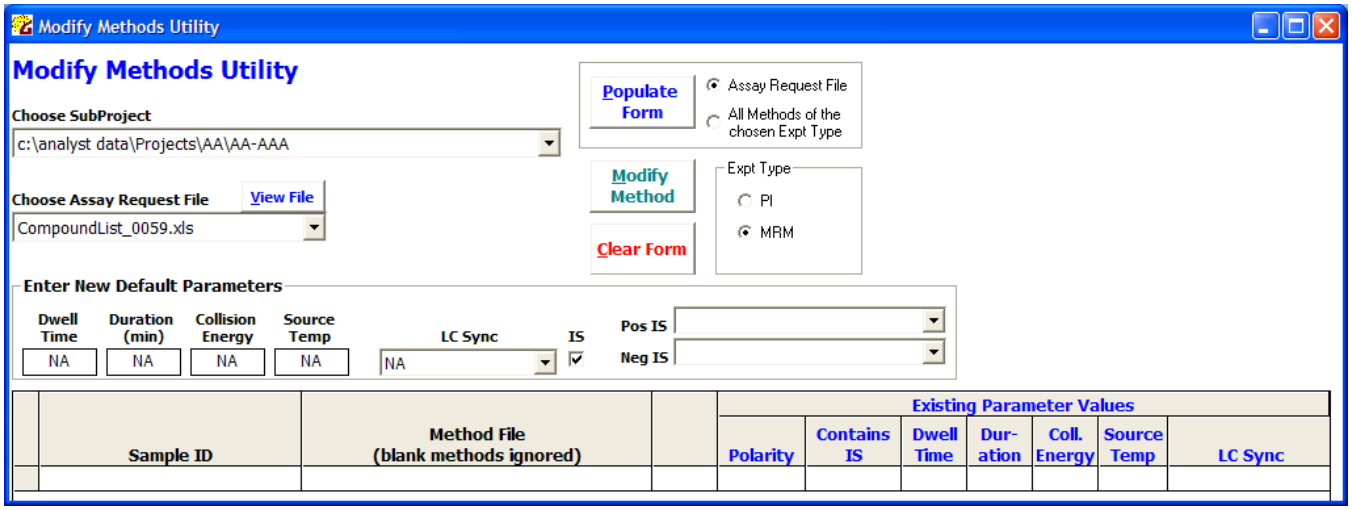


Figure 28 Choose Internal Standard Method File Source

17.1 Click on Populate Form to preview data

A populated window is displayed (see Figure 29).

If a method of the type chosen is not found, then the 'Method File' field for that analyte will be blank and ignored when the 'Modify Method' button is clicked.

The existing parameters of the existing methods are shown in 'Existing Parameter Values' columns.

If the method already contains an internal standard, it will be replaced with the Internal Standard information chosen when the 'Modify Method' button is clicked.

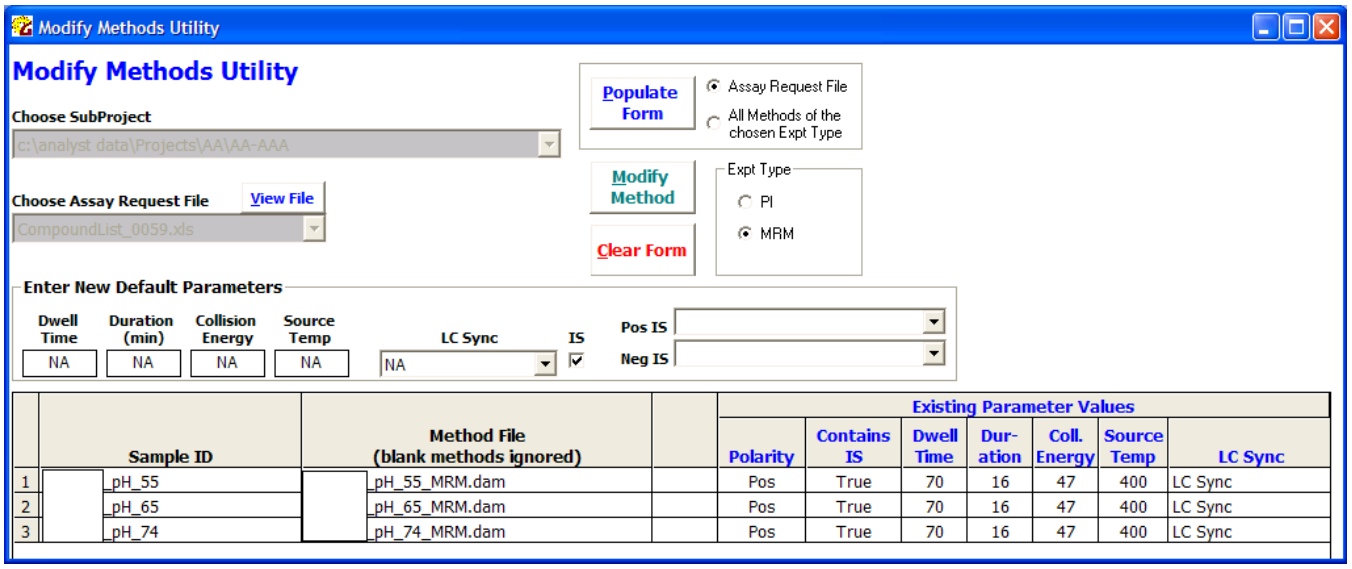


Figure 29 Populated Modify Methods Window

17.2 Click the 'Modify Method' button

The method parameters are updated accordingly and are reflected in the table.

Methods that are identified as Pos have positive Internal Standard method transitions added; while methods that are identified as Neg have negative Internal Standard method transitions added.

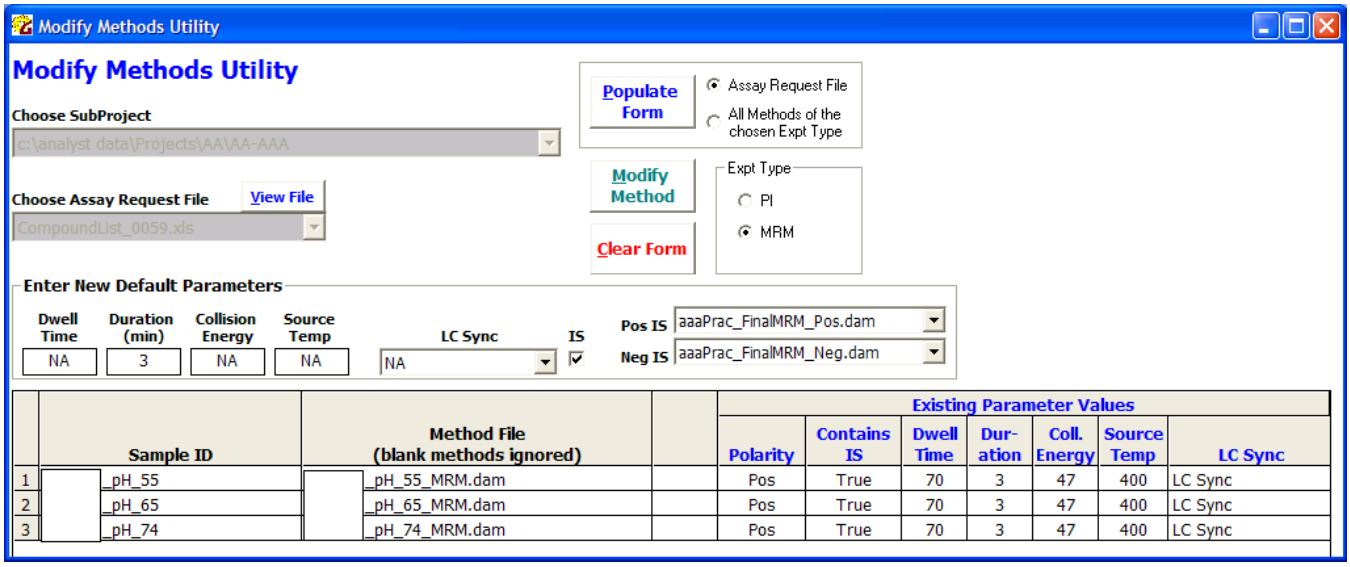


Figure 30 Results of 'Modify Method' button click

18 Automaton File Converter Utility

Please note that this tool supports only the internal processes of Millennium Pharmaceuticals in Cambridge, MA.

Utility for Sciex equipment only.

The Sciex Automaton application allows users to generate autotuned acquisition method files in batch mode based on a user inputted text file. Unfortunately, the Automaton utility generates files with filenames that are a combination of previously-mentioned text filename and sample order. Therefore, when a user is searching for method files to configure in an instrumental sequence, the user needs to interpret with a cross-reference table the name of the file to determine which method file is associated with a given analyte.

It would be beneficial if the method file names included the analyte name, thus eliminating any ambiguity involved in determining the identity of the method file.

The Automaton File Converter renames Automaton-generated files to a filename that includes the analyte name.

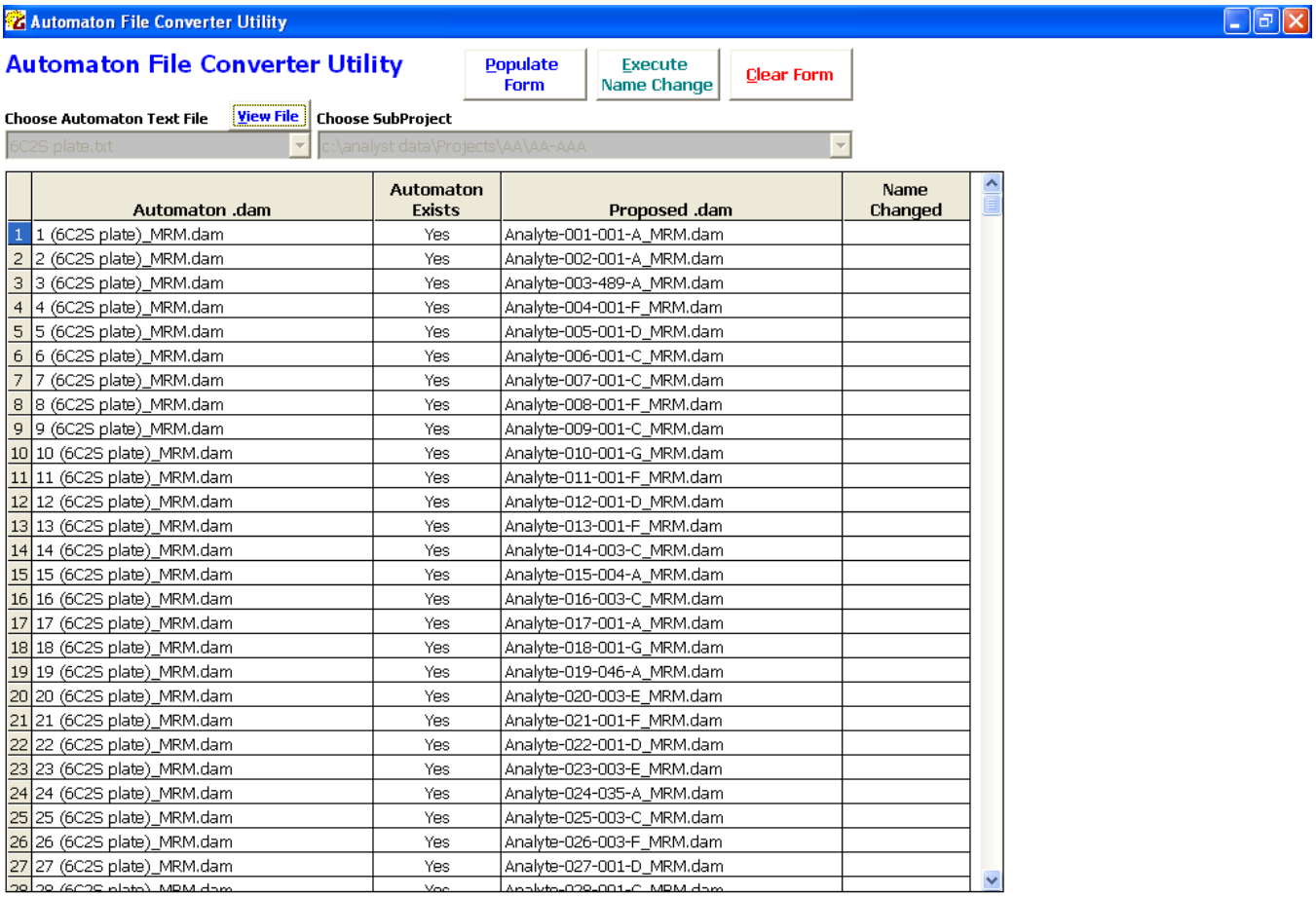
The following describes the procedure for using the Automaton File Converter Utility.

- Choose an Automaton Text File
- Choose a SubProject
- Click on 'Populate Form'

  The form is populated according to the following rules (see Figure 31).

  If an Automaton-generated method file that corresponds with the chosen Automaton text file **EXISTS**, then:
  - the 'Automaton.dam' column row is populated with the Automaton-generated method filename
  - the 'Automaton Exists' column row is populated with 'Yes'
  - the 'Proposed .dam' column row is populated with the new proposed filename

  If an Automaton-generated method file that corresponds with the chosen Automaton text file **DOES NOT EXIST**, then
  - the 'Automaton.dam' column row is left blank
  - the 'Automaton Exists' column row is populated with 'No'.
  - the 'Proposed .dam' column row is populated with the new proposed filename, though this will have no effect on the next actions.



	Automaton .dam	Automaton Exists	Proposed .dam	Name Changed
1	1 (6C2S plate)_MRM.dam	Yes	Analyte-001-001-A_MRM.dam	
2	2 (6C2S plate)_MRM.dam	Yes	Analyte-002-001-A_MRM.dam	
3	3 (6C2S plate)_MRM.dam	Yes	Analyte-003-489-A_MRM.dam	
4	4 (6C2S plate)_MRM.dam	Yes	Analyte-004-001-F_MRM.dam	
5	5 (6C2S plate)_MRM.dam	Yes	Analyte-005-001-D_MRM.dam	
6	6 (6C2S plate)_MRM.dam	Yes	Analyte-006-001-C_MRM.dam	
7	7 (6C2S plate)_MRM.dam	Yes	Analyte-007-001-C_MRM.dam	
8	8 (6C2S plate)_MRM.dam	Yes	Analyte-008-001-F_MRM.dam	
9	9 (6C2S plate)_MRM.dam	Yes	Analyte-009-001-C_MRM.dam	
10	10 (6C2S plate)_MRM.dam	Yes	Analyte-010-001-G_MRM.dam	
11	11 (6C2S plate)_MRM.dam	Yes	Analyte-011-001-F_MRM.dam	
12	12 (6C2S plate)_MRM.dam	Yes	Analyte-012-001-D_MRM.dam	
13	13 (6C2S plate)_MRM.dam	Yes	Analyte-013-001-F_MRM.dam	
14	14 (6C2S plate)_MRM.dam	Yes	Analyte-014-003-C_MRM.dam	
15	15 (6C2S plate)_MRM.dam	Yes	Analyte-015-004-A_MRM.dam	
16	16 (6C2S plate)_MRM.dam	Yes	Analyte-016-003-C_MRM.dam	
17	17 (6C2S plate)_MRM.dam	Yes	Analyte-017-001-A_MRM.dam	
18	18 (6C2S plate)_MRM.dam	Yes	Analyte-018-001-G_MRM.dam	
19	19 (6C2S plate)_MRM.dam	Yes	Analyte-019-046-A_MRM.dam	
20	20 (6C2S plate)_MRM.dam	Yes	Analyte-020-003-E_MRM.dam	
21	21 (6C2S plate)_MRM.dam	Yes	Analyte-021-001-F_MRM.dam	
22	22 (6C2S plate)_MRM.dam	Yes	Analyte-022-001-D_MRM.dam	
23	23 (6C2S plate)_MRM.dam	Yes	Analyte-023-003-E_MRM.dam	
24	24 (6C2S plate)_MRM.dam	Yes	Analyte-024-035-A_MRM.dam	
25	25 (6C2S plate)_MRM.dam	Yes	Analyte-025-003-C_MRM.dam	
26	26 (6C2S plate)_MRM.dam	Yes	Analyte-026-003-F_MRM.dam	
27	27 (6C2S plate)_MRM.dam	Yes	Analyte-027-001-D_MRM.dam	

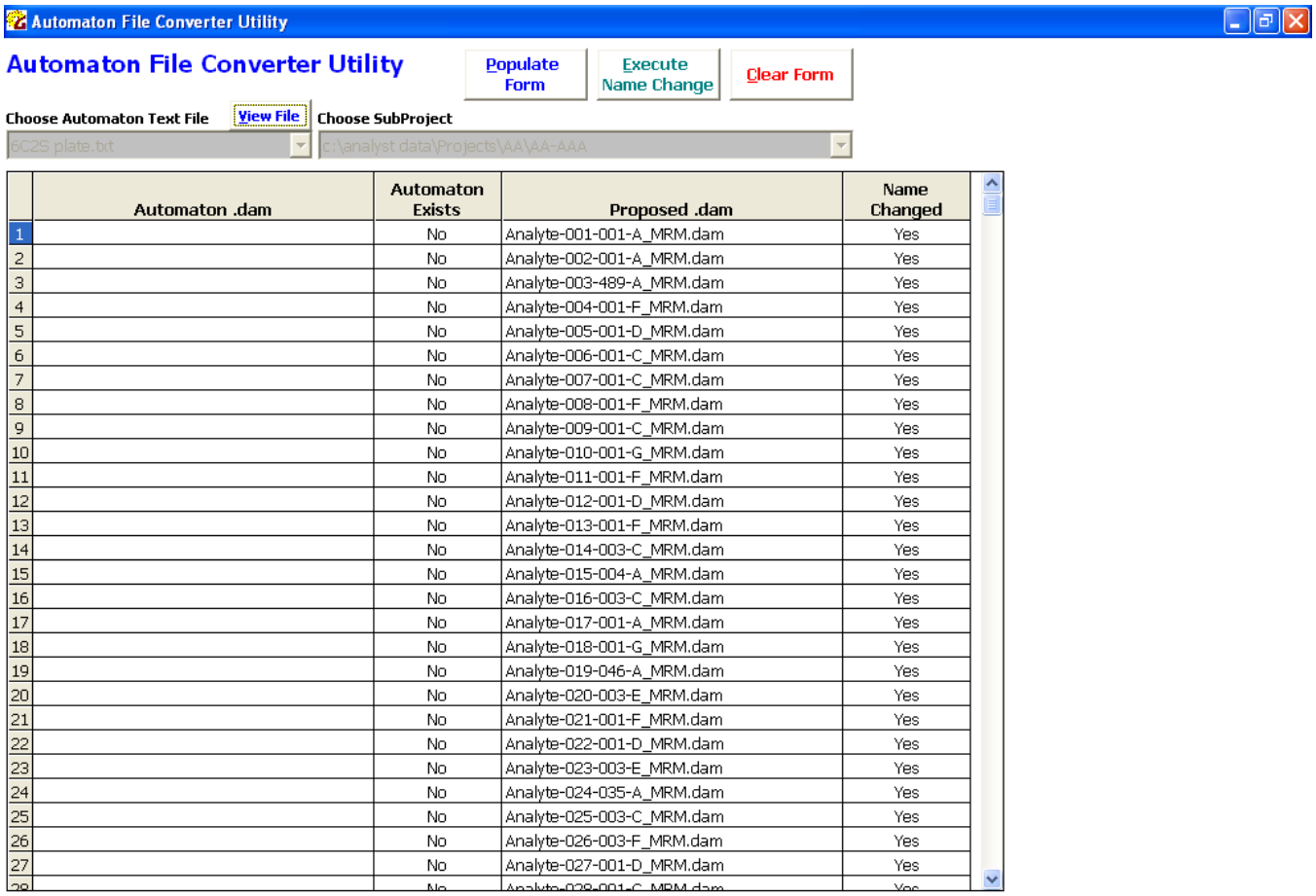
Figure 31 Automaton File Converter Utility

- Click on ‘Execute Name Change’ button

  When the ‘Execute Name Change’ button is clicked, only the rows that have an entry in the ‘Automaton .dam’ column are processed.

  The application changes the filename of the method file, then checks the directory to ensure that the filename has been changed.

  When the name change has been verified, ‘Yes’ will be entered in the appropriate row of the ‘Name Changed’ column and the ‘Automaton .dam’ column row value will be cleared (see Figure 32).



	Automaton .dam	Automaton Exists	Proposed .dam	Name Changed
1		No	Analyte-001-001-A_MRM.dam	Yes
2		No	Analyte-002-001-A_MRM.dam	Yes
3		No	Analyte-003-489-A_MRM.dam	Yes
4		No	Analyte-004-001-F_MRM.dam	Yes
5		No	Analyte-005-001-D_MRM.dam	Yes
6		No	Analyte-006-001-C_MRM.dam	Yes
7		No	Analyte-007-001-C_MRM.dam	Yes
8		No	Analyte-008-001-F_MRM.dam	Yes
9		No	Analyte-009-001-C_MRM.dam	Yes
10		No	Analyte-010-001-G_MRM.dam	Yes
11		No	Analyte-011-001-F_MRM.dam	Yes
12		No	Analyte-012-001-D_MRM.dam	Yes
13		No	Analyte-013-001-F_MRM.dam	Yes
14		No	Analyte-014-003-C_MRM.dam	Yes
15		No	Analyte-015-004-A_MRM.dam	Yes
16		No	Analyte-016-003-C_MRM.dam	Yes
17		No	Analyte-017-001-A_MRM.dam	Yes
18		No	Analyte-018-001-G_MRM.dam	Yes
19		No	Analyte-019-046-A_MRM.dam	Yes
20		No	Analyte-020-003-E_MRM.dam	Yes
21		No	Analyte-021-001-F_MRM.dam	Yes
22		No	Analyte-022-001-D_MRM.dam	Yes
23		No	Analyte-023-003-E_MRM.dam	Yes
24		No	Analyte-024-035-A_MRM.dam	Yes
25		No	Analyte-025-003-C_MRM.dam	Yes
26		No	Analyte-026-003-F_MRM.dam	Yes
27		No	Analyte-027-001-D_MRM.dam	Yes
28		No	Analyte-028-001-C_MRM.dam	Yes

Figure 32 Changed Automaton Filenames

19 Batch Queue Submission Utility

Please note that this tool supports only the internal processes of Millennium Pharmaceuticals in Cambridge, MA.

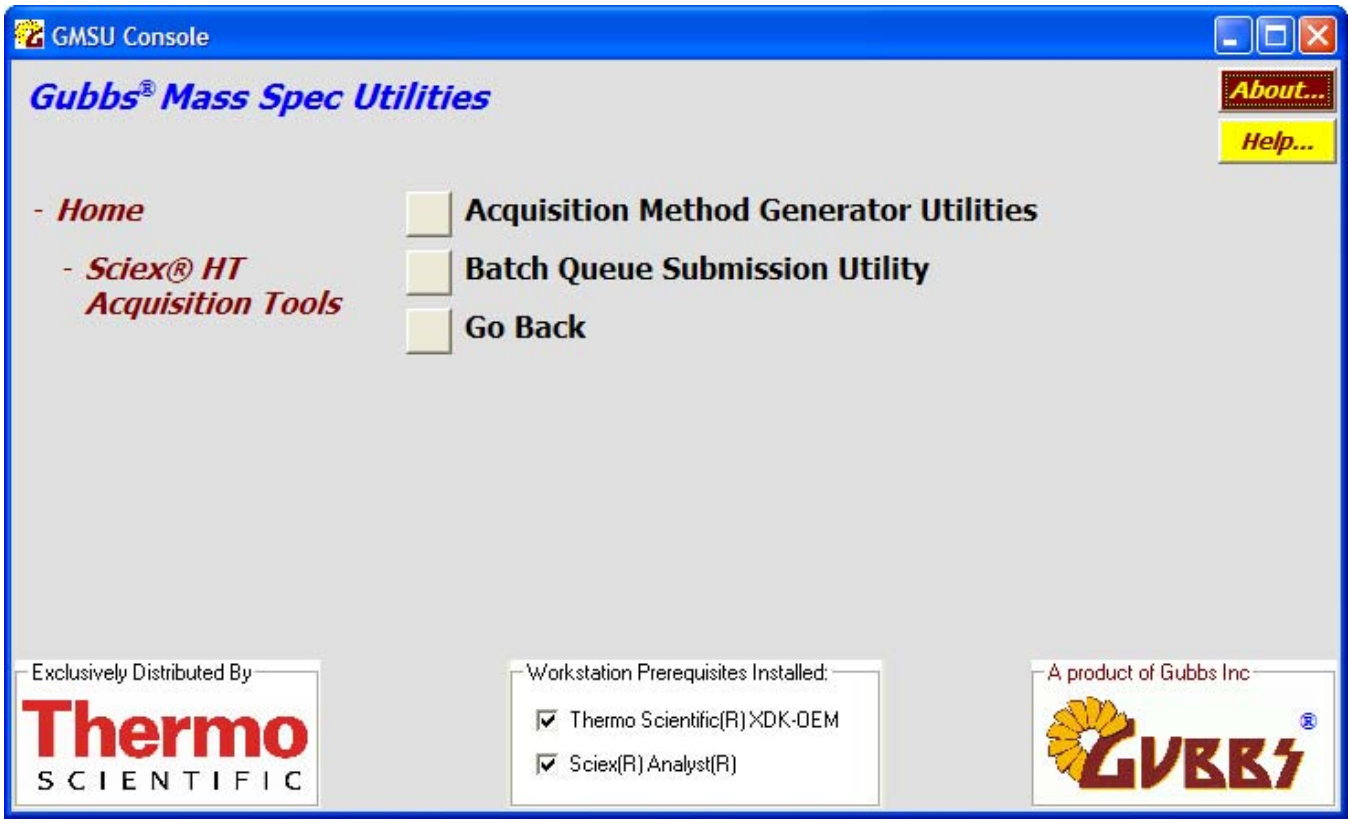


Figure 33 Batch Queue Submission Utility

The Batch Queue Submission Utility allows users to automatically create a batch sequence that whose samples contain existing unique data acquisition methods (.dam) and collect the data into a single raw data file (.wiff). This is useful in a high throughput environment in which tens to hundreds of samples are acquired in one analytical sequence. The Sciex alternative to this utility requires manually entering/choosing the correct method file for each sample.

After preparing a batch and before submitting, the user may save the batch by clicking the 'Save the Batch' button. As expected, the user may load this batch again at a later time.

The following describes the procedure for using the Batch Queue Submission Utility.

- Choose an Assay Request File
- Choose a SubProject
- Choose a Batch Type option from the Batch Type frame.

- Enter Set Name, Identifier #, Batch Owner, and raw data filename (if Batch Type is Final Expt)
- Click on the 'Populate Form' button

  The 'Method File' column will be filled with the appropriate existing method files based on the contents of the Assay Request File. If the expected method file does not exist, the 'Method File' column row entry will be blank.

  If the Batch Type was PI or MRM, then the '.wiff File Name' column row entry will be created corresponding to the sample name (see Figure 34). If the Batch Type was 'Final Expt', then the '.wiff File Name' column will be named according to the user entry described in Section 0 (see Figure 35).
- Click on Submit Batch

  When the user is ready to submit the batch to the queue, the 'Submit Batch' button is pushed.

  Note: Rows that have blank entries in the 'Method File' column will be ignored and not submitted.

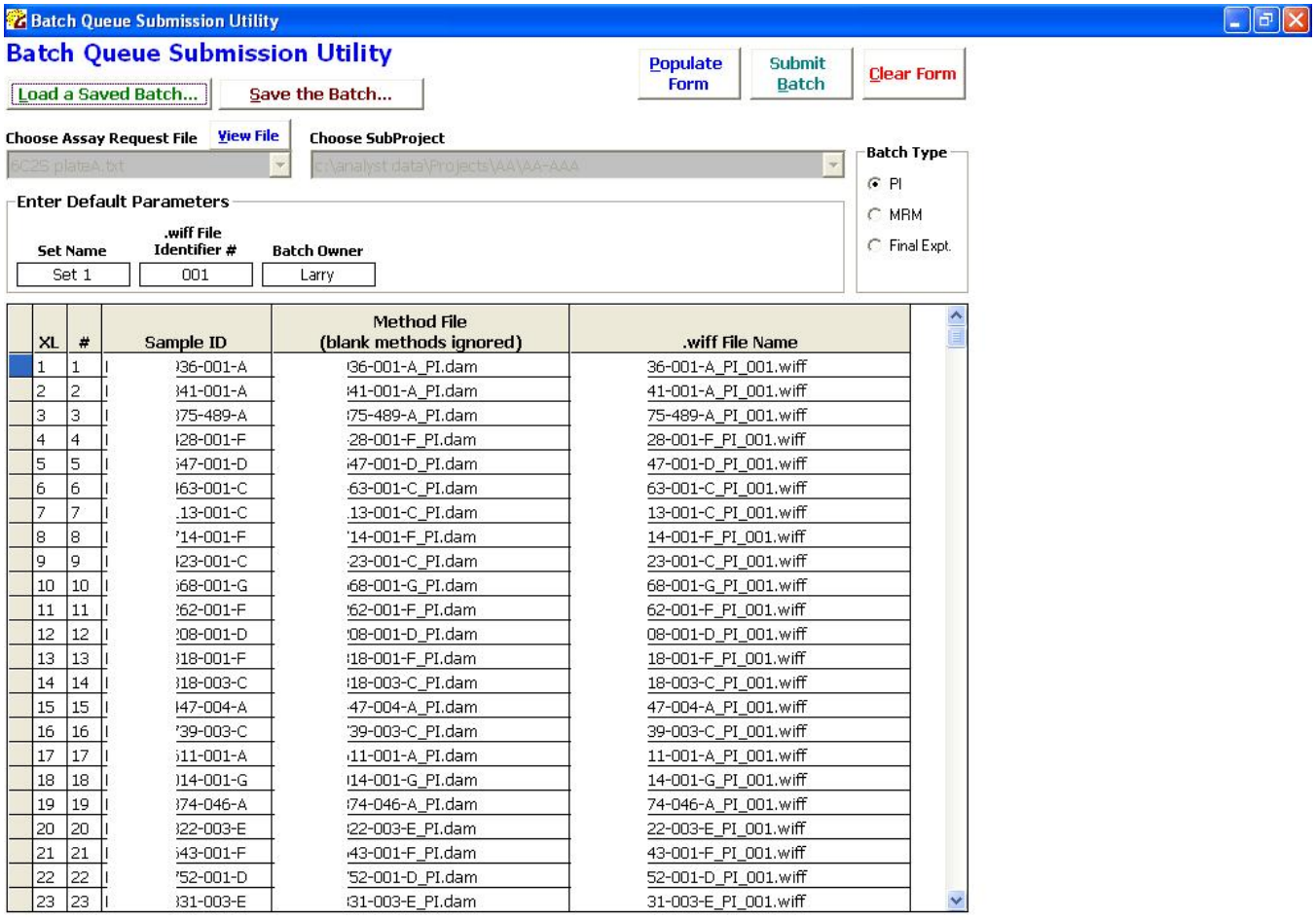


Figure 34 Populate Form – PI/MRM Experiment

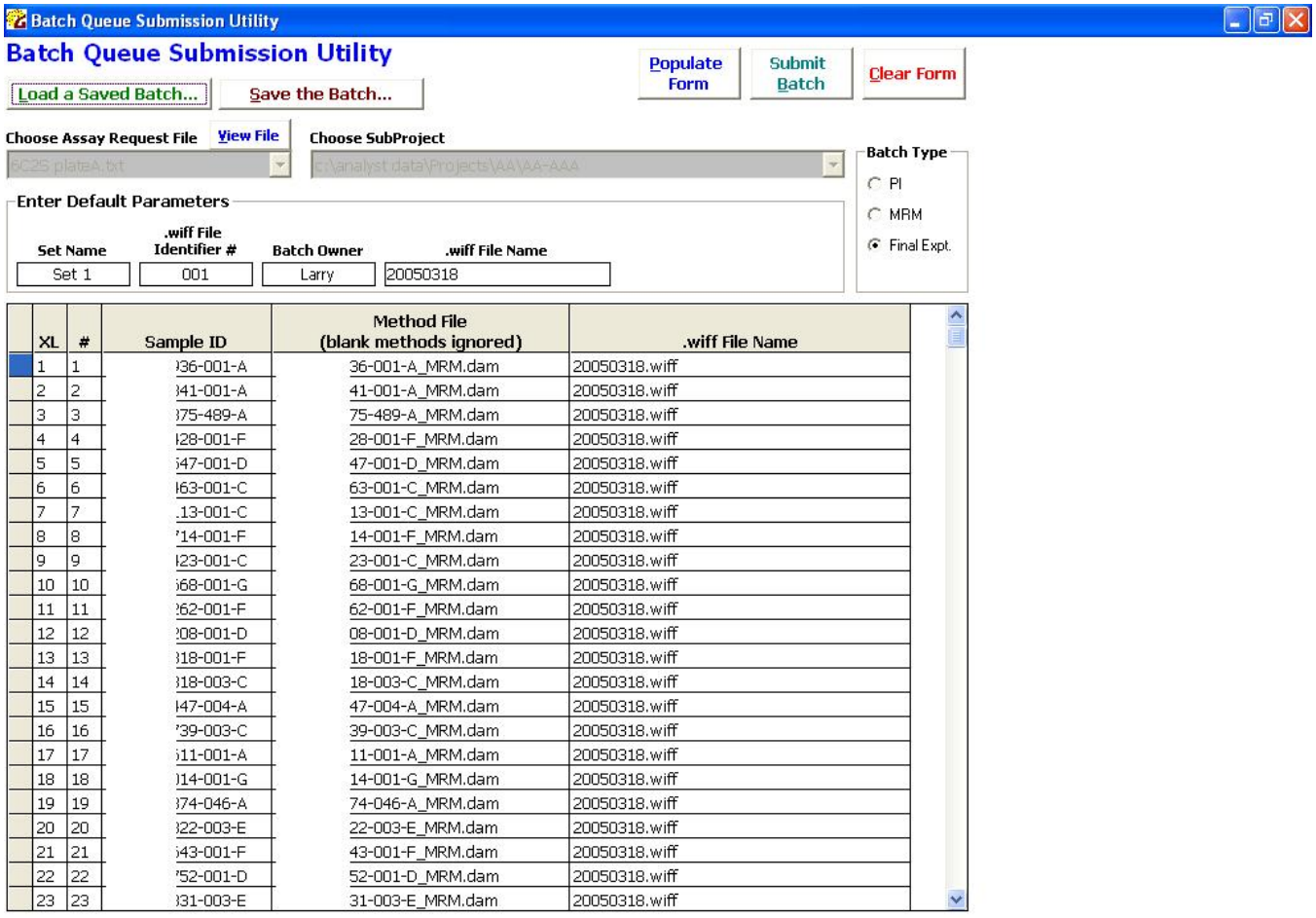


Figure 35 Populate Form – Final Expt Experiment

20 Hepatic Clearance Calculator - Overview

The Hepatic Clearance Calculator consists of three panes (see Figure 36). Users use the top pane (Section 21) to choose data files, configure default settings, choose samples, and choose Warning settings. Users use the bottom left pane to review and optimize chromatographic integration. Users use the bottom right pane (Section 22) to process and optimize Hepatic Clearance results.

In addition, Hepatic Clearance Calculator contains a Menu item that allows users to perform such actions as generate reports and copy peak integration data to the clipboard. See Section 23 for further discussion of the Menu item.

20.1 Hepatic Clearance Calculations

The Hepatic Clearance Calculator uses the following the calculations to generate clearance values.

20.1.1 Constants

In order to calculate clearance and associated values, species/experiment specific constants must be chosen (See the Administration and Installation Manual, Sections 26.9, 26.10, and 26.11 for instructions on configuring species specific constants).

For the purposes of this discussion, the following constants and units will be used:

Species:	Rat
Experiment:	Microsomes
A: Liver wt/Body wt (g/Kg):	45
B: Protein wt/Incubation vol (mg/mL):	0.5
C: Protein wt/Liver wt (mg/g):	45
D: Hepatic Blood Flow (L/hr/Kg):	3.3

20.1.2 Clearance Values

For the purposes of this discussion, the value units shown in the table below will be used. Please note that GMSU/QC will include unit conversion factors int IHC, PHC, and eH formulae if the used constants have different units than expected in the value (an example shown in the formula for IHC)/

Abbr.	Value	Formula
NA	Half Life (sec):	Half-life (t1/2) = Log10(2)/(-Slope)
IHC	Intrinsic Hepatic Clearance (L/hr/Kg)	IHC = (-2.303) * Slope * A * C / B * CF[1]
PHC	Predicted Hepatic Clearance (L/hr/Kg)	PHC = (IHC * D) / (IHC + D)
Eh	Eh:	Eh = PHC / D
LPR	Last Point Remaining %	LPR = (10 ^ (lp * Slope + Yint) / (10 ^ Yint)) * 100 where lp = last data point in assay

[1] CF = (60 min / 1 hr) * (1 L / 1000 mL)

20.1.3 Experimental Slope

Clearance Values are based on the slope generated from a plot of experimental values where the Y-axis = Log10(% Parent Remaining) and X-axis = time (minutes). The Y-axis is displayed as such in order that all displayed data is normalized. Please note that the slope generated from this plot is intrinsically equal to the slope generated if the Y-axis were equal to instrumental signal (e.g. peak area ratio).

20.1.4 Example data

Section 24.2 contains example data used to generate clearance values

The following sections describe the procedures for using the Hepatic Clearance Calculator.

21 Hepatic Clearance Calculator – Initial Settings

The initial state of the Hepatic Clearance Calculator is a screen lacking any data (Figure 36). Users first must set default constants, then choose a data file for processing. The following describes the steps for performing these actions:

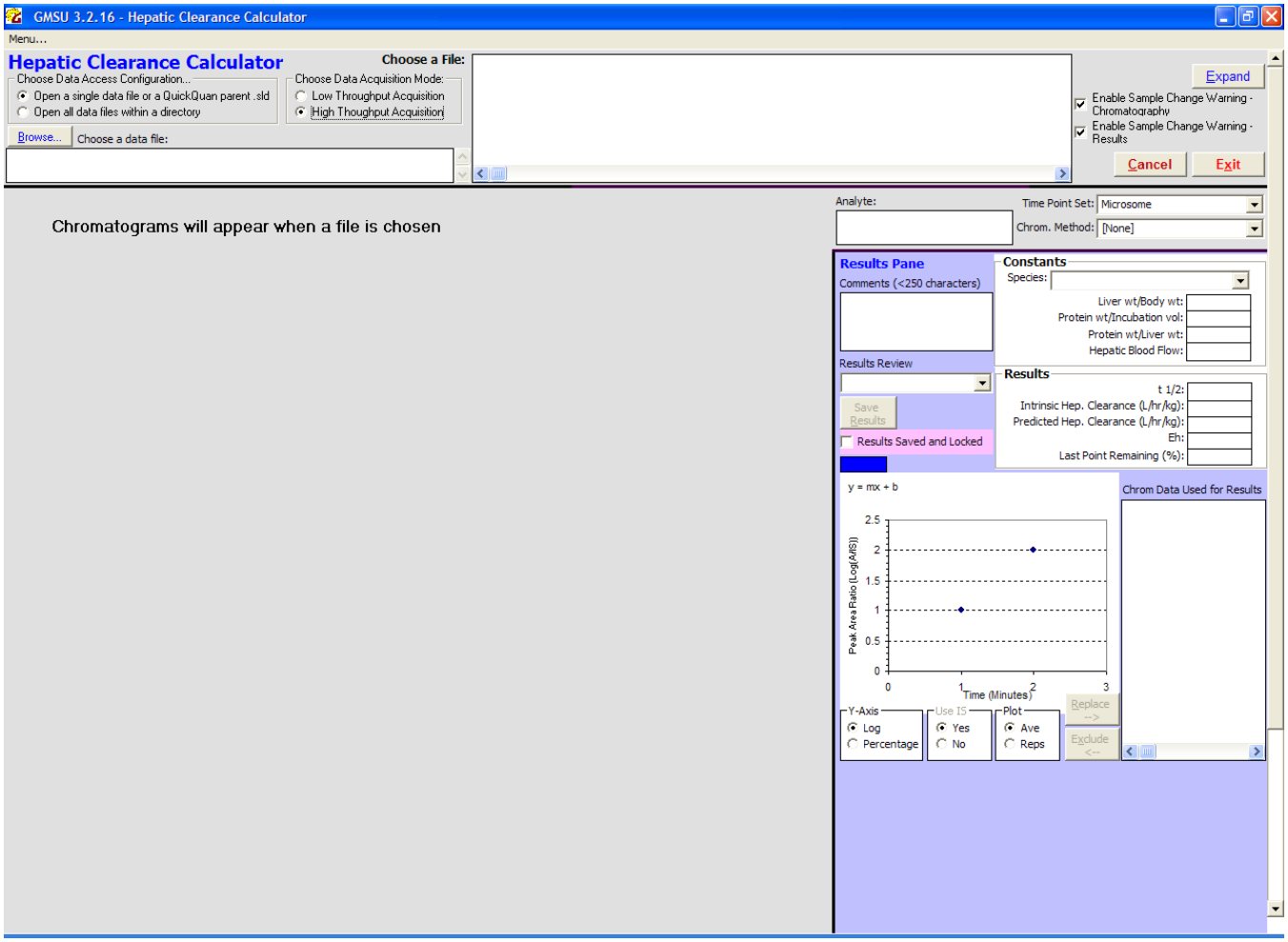


Figure 36 Hepatic Clearance Calculator – before opening data

21.1 Loading data and displaying and optimizing chromatography.

Please see Section 9 for a description of the procedures for loading data and displaying and optimizing chromatography.

21.2 Time Point Set

The Time Point Set contains assay time points to be applied to the chosen data file.

Ensure an appropriate Time Point Set is displayed here. Time Point Sets are created and configured in Configuration Utility – Hepatic Clearance Constants – Experiment Time Points tab. When a data file is opened, the default Time Point Set is retrieved from the setting found in Configuration Utility – Hepatic Clearance tab – Default Time Point Set item.

Please note that when the Hepatic Clearance Module is shown initially, the value displayed in the Time Point Set dropdown box is locked. In order to change it, the user must change the setting in Configuration Utility. Once data has been loaded, then the Time Point Set selection may be changed (if the data is not Locked and Saved).

21.3 Chrom. Method

Note: This field is shown only if the High Throughput Acquisition option is chosen in the Choose Data Acquisition Mode frame box. This applies only to HT acquisition data.

The Chrom. Method (chromatography method) is a chromatographic method containing expected chromatographic retention times to be applied to the chosen data file. This method may be applied to the data file if the GMSU/QC automatic peak identification algorithm is not successful (for, e.g., data files with low signal).

Ensure an appropriate Chrom. Method is displayed here ([None] if not used). The default Chrom. Method is retrieved from the Hepatic Clearance Calculator Configuration Settings in the Configuration Utility. Chrom. Methods are created and configured in the Hepatic Clearance Module via the Menu – Manually Enter Peak Retention Times… feature (see Section).

Please note that when the Hepatic Clearance Module is shown initially, the value displayed in the Chrom. Method dropdown box is locked. In order to change it, the user must change the setting in Configuration Utility. Once data has been loaded, then the Chrom. Method selection may be changed (if the data is not Locked and Saved).

21.4 Constants - Species

Ensure the appropriate species-specific experiment constant set is displayed here. Constants Sets are created and configured in Configuration Utility – Hepatic Clearance Constants – Existing Experiments tab. When a data file is opened, the default Constant Set is retrieved from the setting found in Configuration Utility – Hepatic Clearance tab – Default Initial Species item.

21.5 Choose a Sample

Users may choose a sample in the ‘Choose a File grid either by selecting with the mouse or using the arrow keys when the ‘Choose a File grid has focus.

If chromatograms and/or results have not been saved when the user scrolls through samples, the user will be prompted with warning messages to choose to save chromatograms or results. These warnings may be turned off be de-selecting the appropriate checkboxes located to the right of the ‘Choose a File’ grid (see Section 9.1).

If a sample is of incorrect format or if data file is of incorrect format, an error message will be displayed.

See Figure 37 for an example of loaded HT acquisition data and Figure 38 for an example of loaded LT acquisition data. Notice that, in HT data, the entire chromatogram is shown (if not, click on the Reset button to reset the chromatogram to full scale).

To inspect a single peak, the user may click on an entry in the chromatographic data table to the right of the chromatogram. When clicked, the chromatogram zooms on the chosen peak and looks much like Figure 38.

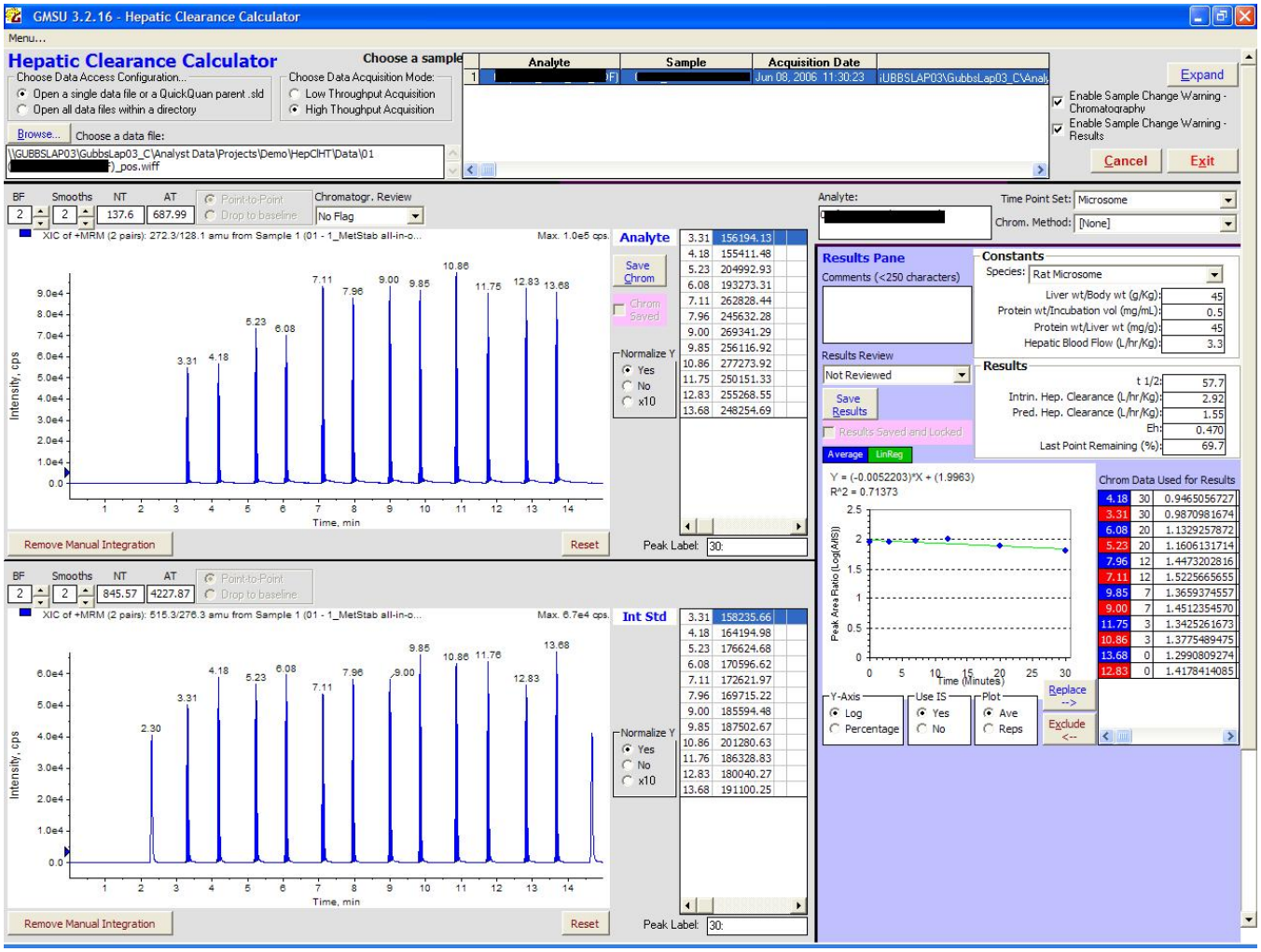


Figure 37 Loaded raw data – HT acquisition data

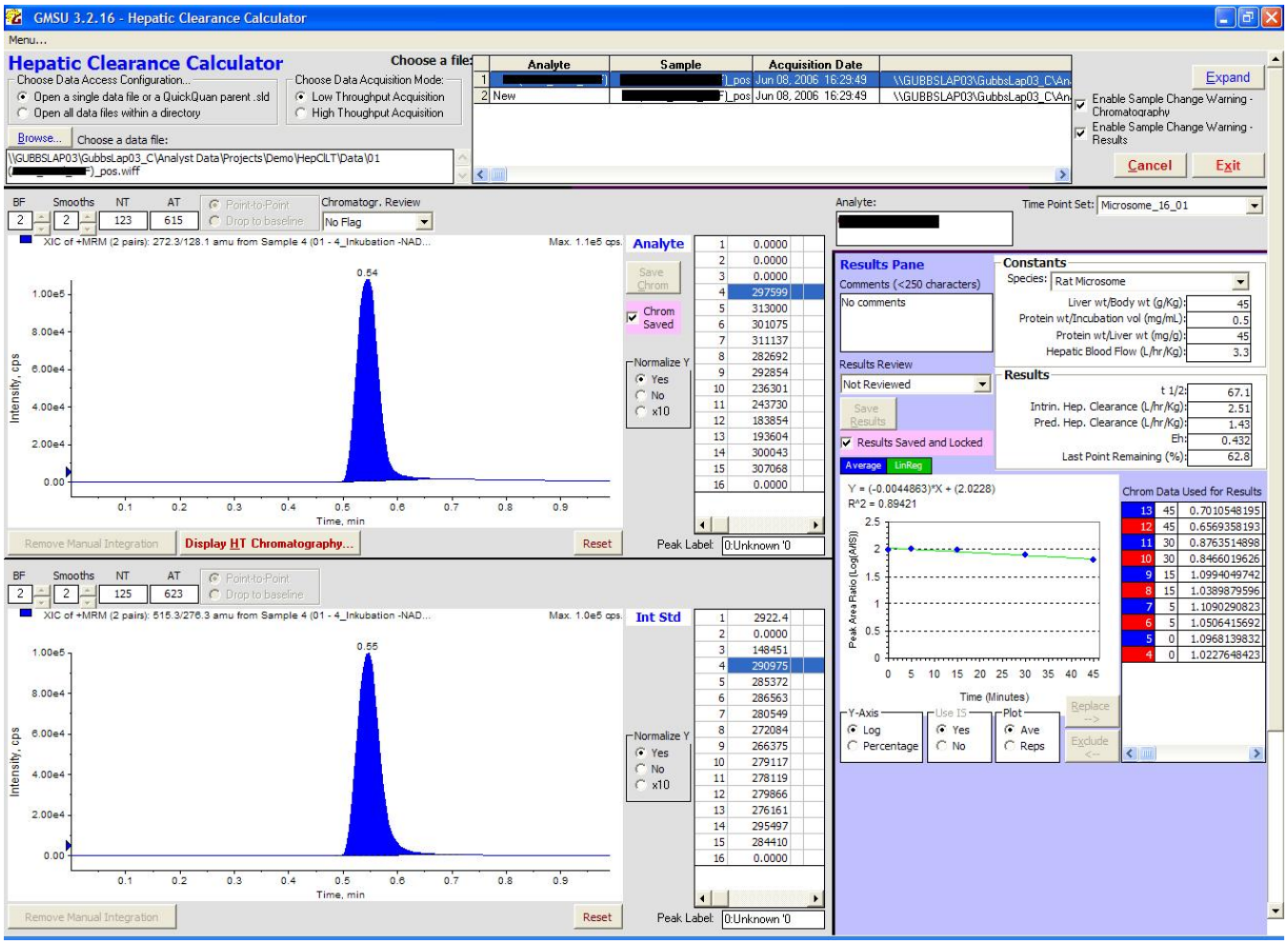


Figure 38 Loaded raw data – LT acquisition data

22 Hepatic Clearance Calculator – Results Pane

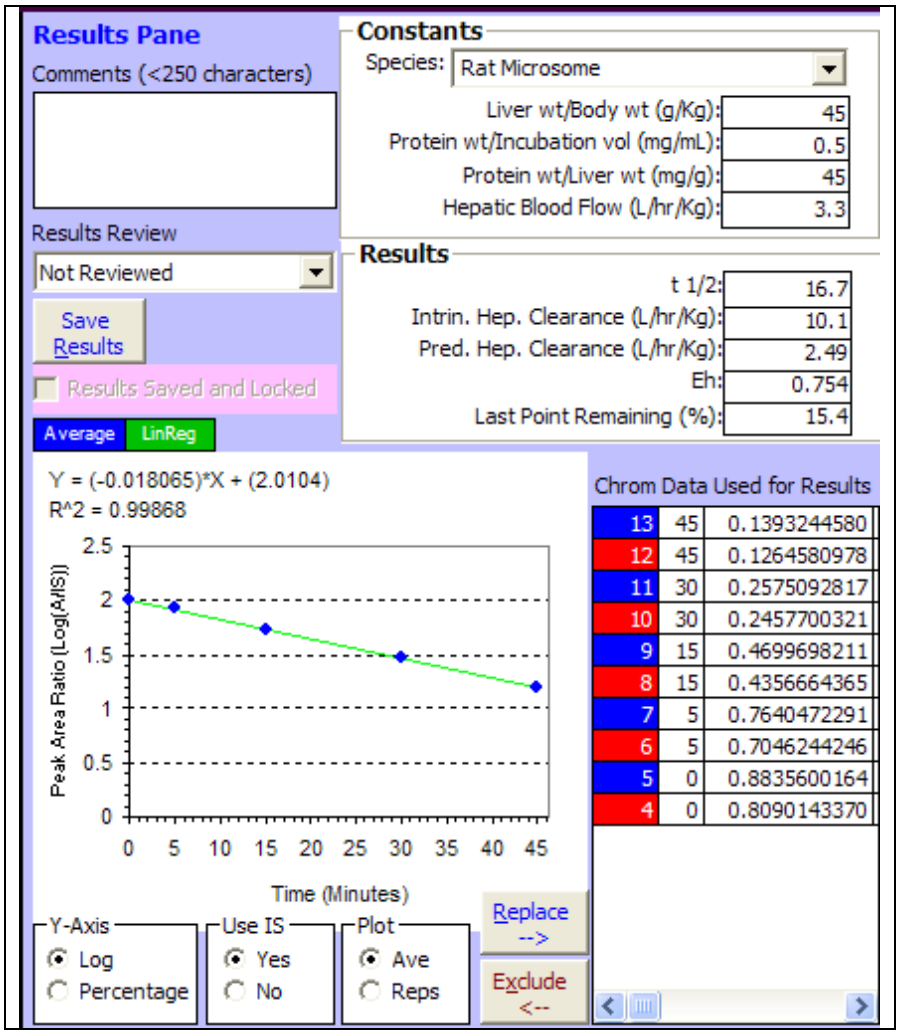
The Hepatic Clearance Calculator Results bottom right pane allows users to perform several functions:

- Automatically generates Hepatic Clearance Results based on selected species-specific Experiment Constants.
- Allows the user to optimize Hepatic Clearance Results by selecting the choice of using or not using internal standard peak area ratios.
- Allows the user to optimize Hepatic Clearance Results by excluding data points.
- Allows the user to select Results Review assignments and comments.

The following describes the functionality of the Hepatic Clearance Calculator – Results section.

22.1 Overview

The right Results Pane contains functionality for users to optimize and process data to obtain Hepatic Clearance Results.



- 'Comments' box allows users add comments about sample. Comments may be included in Reports.
- 'Results Review' box allows the user to choose a Review Status.
- 'Save Results' button allows users to save/lock the data such that it can't be changed unless manually unlocked.
- 'Results Saved and Locked' checkbox allows users to unlock data for further processing.
- 'Species' dropdown box allows users to associate samples with a specific species
- The 'Chrom. Data Used for Results' shows the retention data, time point, and peak area or ratio data for each data point.
- Y-Axis frame allows users to change the Y-Axis of the display.
- 'Use IS' frame allows users to choose to use Internal Standard peak area ratios or not.
- 'Plot' frame allows users to display average or individual replicates in the regression plot.
- 'Exclude' and 'Add' buttons allow users to exclude and restore data points.

Figure 39 Hepatic Clearance Results Pane Overview

Please note that Constants labels and the units for all Constants and Results values are configurable in the Configuration Utility – Hepatic Clearance Constants – Existing Experiments tab.

The following describes the functions available in the Hepatic Clearance Results pane. Please note that the Reset button shown in the following figures is no longer available.

22.2 Use IS Option Frame

Users may choose to use (Yes option) or not use (No option) internal standard area ratios by selecting the appropriate option button. The y-axis label will update accordingly.

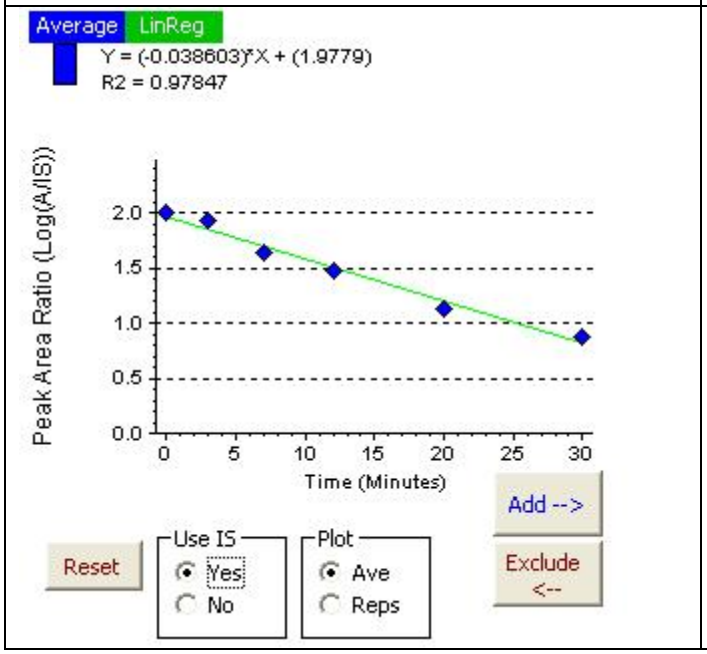
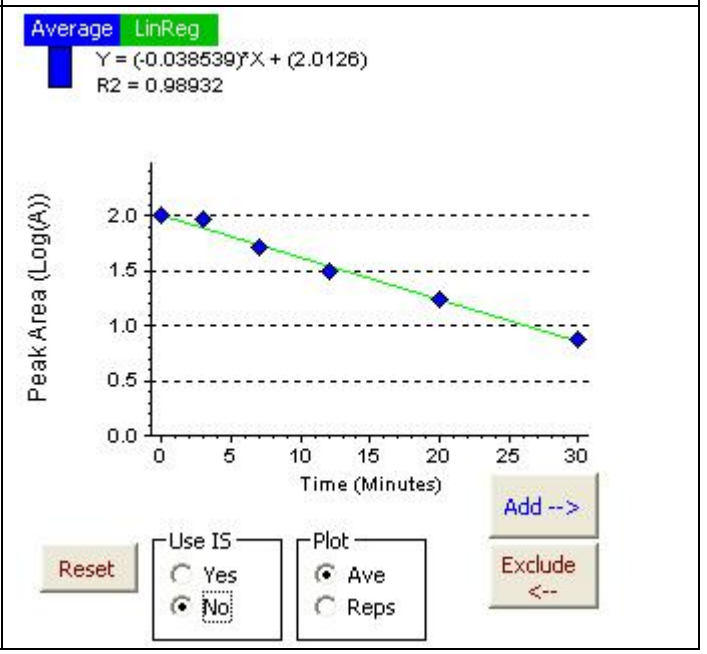
Use Internal Standard	Don't Use Internal Standard
	

Figure 40 Use IS Frame Options

22.3 Plot Option Frame

Users may choose to display average replicate points (Ave option) or individual replicates (Reps option). The individual replicates view is useful to identify individual outliers.

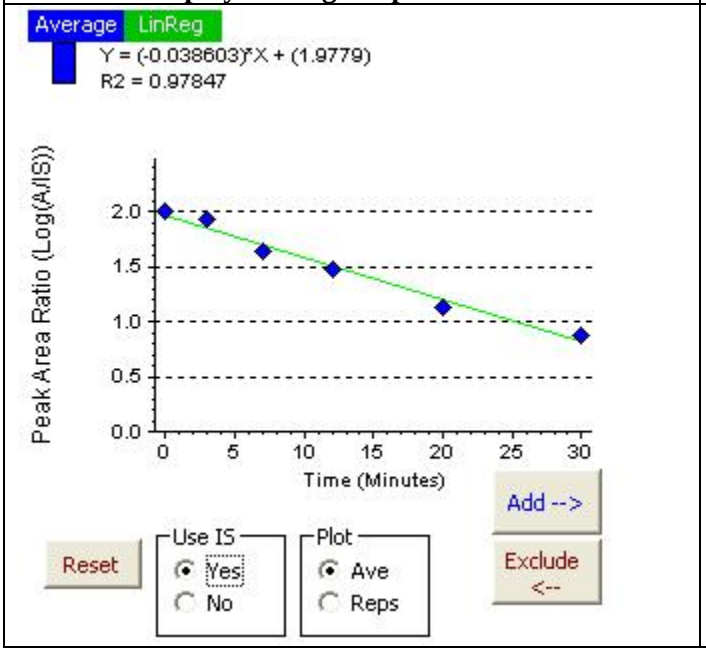
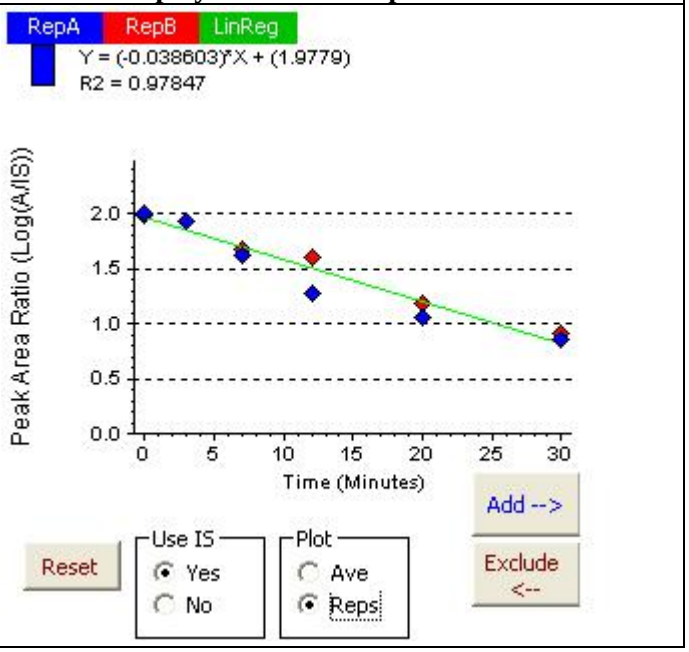
Display Average Replicate Points	Display Individual Replicate Points
	

Figure 41 Plot Frame Options

22.4 Constants Dropdown Box

Hepatic Clearance value calculations contain constants that are specific to species and assay. These constant values are configured by an administrator in Configuration Utility. The constants set for each sample is chosen by the user. If a user changes the species constant set, the Hepatic Clearance Values will update automatically.

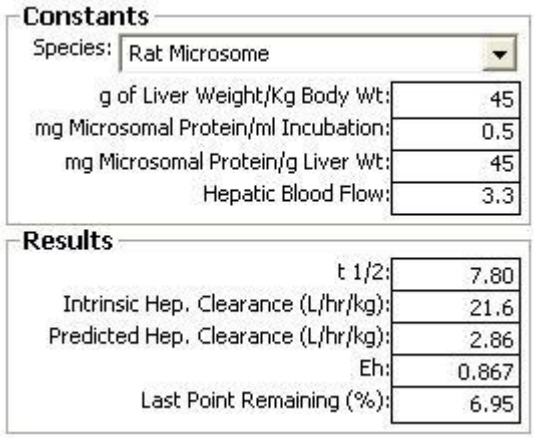
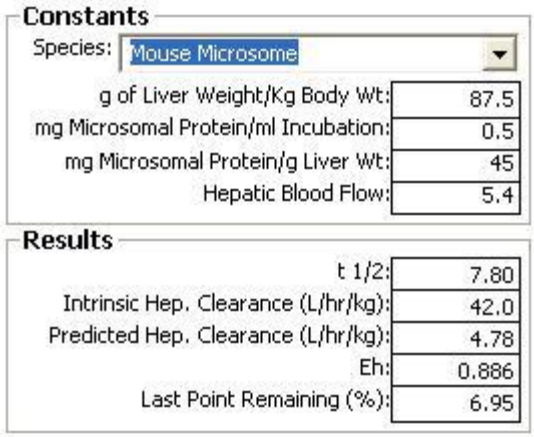
Results with Rat Microsome	Results with Mouse Microsome
	

Figure 42 Species Constants Settings

22.5 Exclude Data Points

Users may exclude data points if desired by selecting a data point to exclude, then clicking on the 'Exclude' button. Results will be updated automatically.

For example, if the red and blue 30 minute replicate in Figure 43 are to be excluded, the user would select the two 30 minute time points in the 'Chrom. Data Used for Results' grid and click the Exclude button.

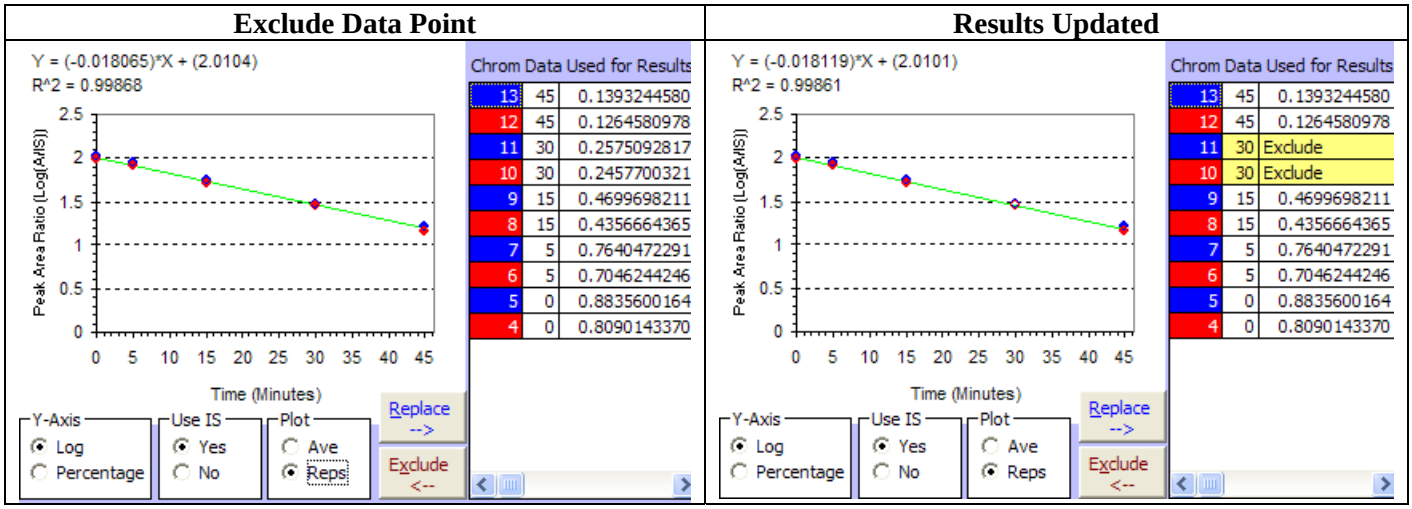


Figure 43 Excluding Points

Notice that the excluded time points on the graph convert from solid circles to hollow circles.

Note: Users may exclude as many data points as desired given that two points are remaining to generate a regression.

22.6 Restore Data Points

Similar to the Exclude procedure, users may restore excluded data points by selecting the data points to restore, then clicking on the 'Add' button. Results will be updated automatically.

22.7 Individual Time Point Comments

The user may record comments concerning individual time points by selecting one or more time points in the 'Chrom Data Used for Results' table and right-clicking. The user will be prompted for a dialog box to enter comments about the selected data points.

22.8 Comments

Users may enter comments about the data set.

22.9 Review

After optimization of Results data, users may classify the results by choosing a value for 'Results Review' dropdown box. The contents of the dropdown box include 'Not Reviewed', 'Acceptable', and 'Unacceptable'.

Selecting the proper review value is important with respect to Reports. Users are allowed to filter Reports based on the 'Review' field contents.

22.10 Save Results

When the user is satisfied that Results processing is complete, the user locks the Results by clicking on 'Save Results'. This disables all functions relating to chromatographic integration and results processing and the 'Results Saved' checkbox becomes selected. In addition, Results data are stored in the GMSU/QC data store.

If the user attempts to further process Saved Results, an error message will be displayed. In order to further process Saved Results, the user must deselect the 'Results Saved' checkbox.

23 Hepatic Clearance Calculator – Additional Functions

Additional Hepatic Clearance Calculator functions are found under the menu Menu. The following sections describe these functions. Functions that are conserved between modules and described in Section 9.16 will not be described here.

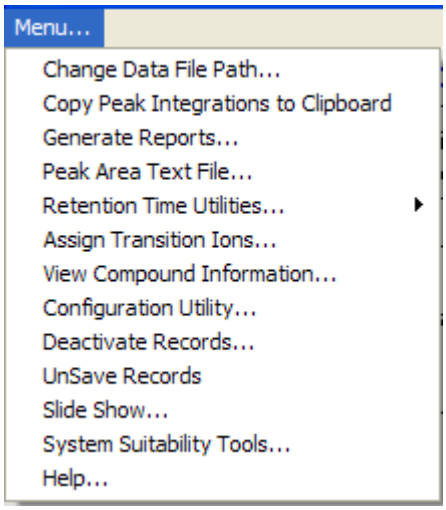


Figure 44 Hepatic Clearance Module Menu Items

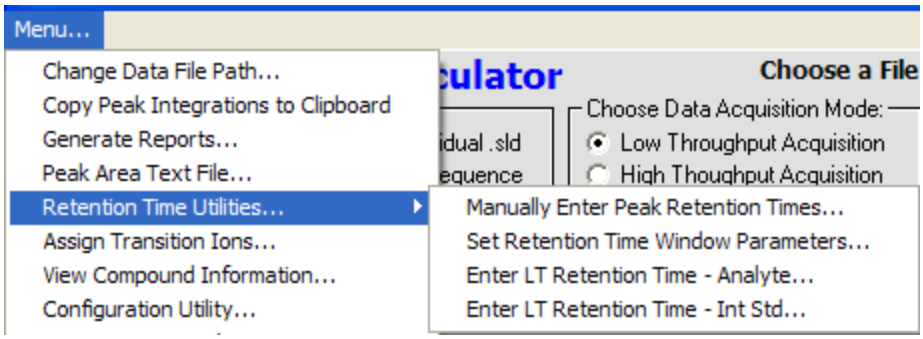
23.1 Copy Peak Integrations to Clipboard

Copies the Analyte and Internal Standard peak areas to the clipboard.

23.2 Peak Area Text File

The 'Peak Area Text File' function will export Analyte and Internal Standard time point and peak area data to a comma-delimited text file. The text file header includes 'Date of Report', 'Raw Data File Path', 'Analyte Name', and 'Acquisition Time'.

23.3 Manually Enter Peak Retention Times



See Section **Error! Reference source not found.** for further discussion of this feature.

23.4 Enter LT Retention Time – Analyte

See Section 9.16.1

23.5 Enter LT Retention Time – Int Std

9.16.4.3

23.6 Display Multiple Results

Under development.

23.7 Assign Transition Ions

See Section 9.16.5.

23.8 Set Retention Time Window Parameters

See Section 9.16.4.1

23.9 Help

See Section 9.16.12.

24 Hepatic Clearance Calculator – Reports

Users may generate Microsoft® Word or Excel reports based on user-defined choices. Most features of the Report window has been described in Section 10, while the other features are self-explanatory. The reports module may be activated from the GMSU/QC Console – Hepatic Clearance Calculator Reports or from the Hepatic Clearance Calculator Menu – Generate Reports (see Section 23.1). Figure 45 shows the Hepatic Clearance Calculator Reports window.

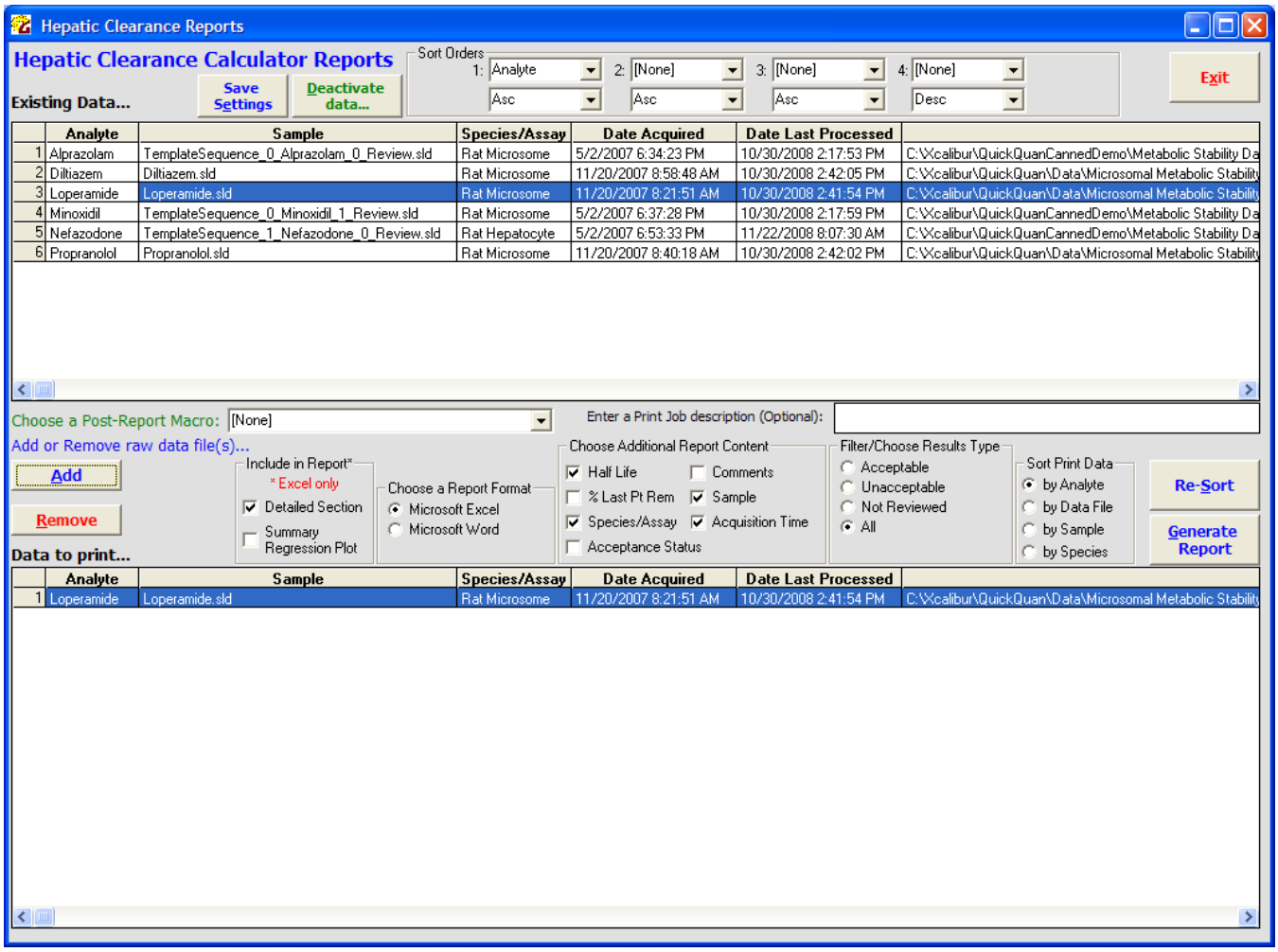


Figure 45 Hepatic Clearance Calculator Reports Module

Two styles of reports may be generated:

24.1 Normal Report

A Normal report contains a single page containing the default and chosen additional fields. The default fields displayed in a Report include:

- Analyte
- Intrinsic Hepatic Clearance
- Predicted Hepatic Clearance
- Eh

Additional fields may be added by selecting them from the Choose Additional Report Contents frame.

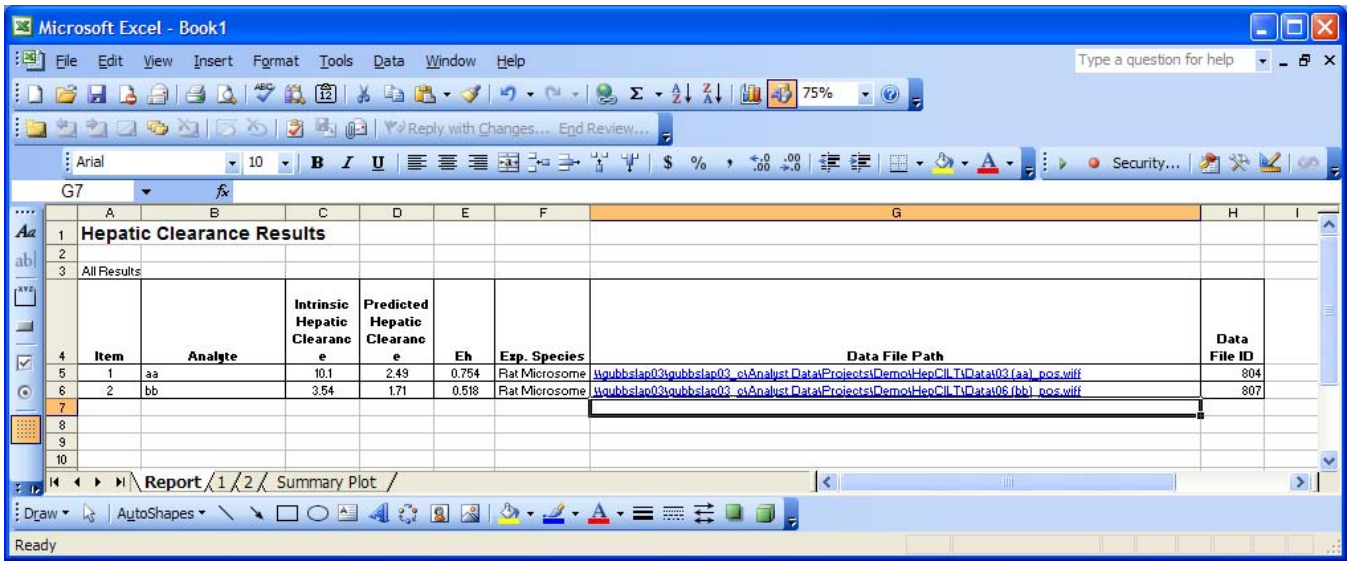


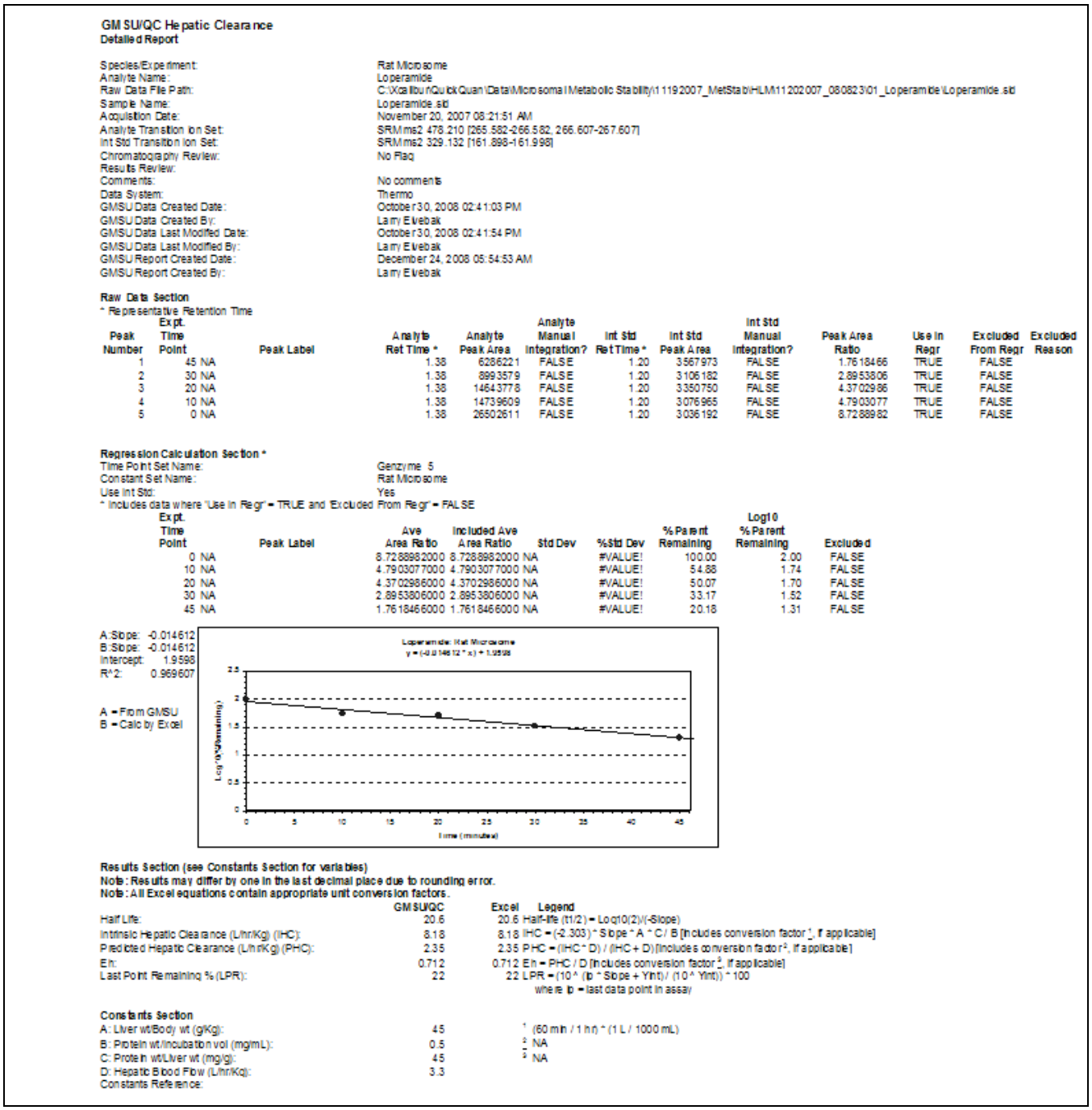
Figure 46 Hepatice Clearance – Normal Report

Note: Samples that have a 'Last Point Remaining %' value of greater then the CutOff value will display in the Report special text values specific to a species and experiment (all configured by an administrator in the Configuration Utility). For example, if a sample is has a 'Last Point Remaining %' of greater than 90% and the species experiment is Rat Microsome, the Intrinsic Hepatic Clearance, Predicted Hepatic Clearance and Eh values would be reported as '<0.85', '<0.68', and '<0.21' (or some other configured text values).

The CutOff value is configured as 'Last Point Remaining Value' in the Hepatic Clearance Calculator window of the Configuration Utility (see the Configuration Section of Installation and Administration Manual).

24.2 Detailed Report

A Detailed Section Report (Excel only, see Figure 47) will contain the normal report page, plus an extra worksheet for each item in the print queue. The detailed page contains all the information used to generate the clearance values.



GMSU/QC Hepatic Clearance
Detailed Report

Species/Experiment:	Rat Microsome
Analyte Name:	Loperamide
Raw Data File Path:	C:\Xcalibur\QuickQuan\Data\Microsomal Metabolic Stability\11192007_MetStab\HLM11202007_080823\01_Loperamide\Loperamide.sld
Sample Name:	Loperamide.sld
Acquisition Date:	November 20, 2007 08:21:51 AM
Analyte Transition Ion Set:	SRM ms2 478.210 [265.582-266.582, 266.607-267.607]
Int Std Transition Ion Set:	SRM ms2 329.132 [161.898-161.998]
Chromatography Review:	No Flag
Results Review:	
Comments:	No comments
Data System:	Thermo
GMSU Data Created Date:	October 30, 2008 02:41:03 PM
GMSU Data Created By:	Larry Elvebak
GMSU Data Last Modified Date:	October 30, 2008 02:41:54 PM
GMSU Data Last Modified By:	Larry Elvebak
GMSU Report Created Date:	December 24, 2008 05:54:53 AM
GMSU Report Created By:	Larry Elvebak

Raw Data Section
* Representative Retention Time

Peak Number	Expt. Time Point	Peak Label	Analyte Ret Time *	Analyte Peak Area	Analyte Manual Integration?	Int Std Ret Time *	Int Std Peak Area	Int Std Manual Integration?	Peak Area Ratio	Use In Regr	Excluded From Regr	Excluded Reason
1	45 NA		1.38	6286221	FALSE	1.20	3567973	FALSE	1.7618466	TRUE	FALSE	
2	30 NA		1.38	8993579	FALSE	1.20	3106182	FALSE	2.8953806	TRUE	FALSE	
3	20 NA		1.38	14643778	FALSE	1.20	3350750	FALSE	4.3702986	TRUE	FALSE	
4	10 NA		1.38	14739609	FALSE	1.20	3076965	FALSE	4.7903077	TRUE	FALSE	
5	0 NA		1.38	26502611	FALSE	1.20	3036192	FALSE	8.7288982	TRUE	FALSE	

Regression Calculation Section *

Time Point Set Name:	Genzyme 5
Constant Set Name:	Rat Microsome
Use Int Std:	Yes

* Includes data where 'Use In Regr' = TRUE and 'Excluded From Regr' = FALSE

Expt. Time Point	Peak Label	Ave Area Ratio	Included Ave Area Ratio	Std Dev	%Std Dev	% Parent Remaining	Log10 % Parent Remaining	Excluded
0 NA		8.7288982000	8.7288982000	NA	#VALUE!	100.00	2.00	FALSE
10 NA		4.7903077000	4.7903077000	NA	#VALUE!	54.88	1.74	FALSE
20 NA		4.3702986000	4.3702986000	NA	#VALUE!	50.07	1.70	FALSE
30 NA		2.8953806000	2.8953806000	NA	#VALUE!	33.17	1.52	FALSE
45 NA		1.7618466000	1.7618466000	NA	#VALUE!	20.18	1.31	FALSE

A:Slope: -0.014612
B:Slope: -0.014612
Intercept: 1.9598
R^2: 0.969607

A = From GMSU
B = Calc by Excel

Results Section (see Constants Section for variables)
Note: Results may differ by one in the last decimal place due to rounding error.
Note: All Excel equations contain appropriate unit conversion factors.

	GMSU/QC	Excel Legend
Half Life:	20.6	20.6 Half-life (t1/2) = Log10(2)/(-Slope)
Intrinsic Hepatic Clearance (L/hr/Kg) (IHC):	8.18	8.18 IHC = (-2.303) * Slope * A * C / B [Includes conversion factor [1], if applicable]
Predicted Hepatic Clearance (L/hr/Kg) (PHC):	2.35	2.35 PHC = (IHC * D) / (IHC + D) [Includes conversion factor [2], if applicable]
Eh:	0.712	0.712 Eh = PHC / D [Includes conversion factor [3], if applicable]
Last Point Remaining % (LPR):	22	22 LPR = (10 ^ (lp * Slope + Yint) / (10 ^ Yint)) * 100 where lp = last data point in assay

Constants Section

A: Liver wt/Body wt (g/Kg):	45	[1] (60 min / 1 hr) * (1 L / 1000 mL)
B: Protein wt/Incubation vol (mg/mL):	0.5	[2] NA
C: Protein wt/Liver wt (mg/g):	45	[3] NA
D: Hepatic Blood Flow (L/hr/Kg):	3.3	
Constants Reference:		

Figure 47 Hepatic Clearance – Detailed Report

24.3 Summary Regression Plot

If Detailed Section is chosen, the user has the additional choice of generating a Summary Regression Plot in which the regressions of all the items in the print queue are plotted on one plot.

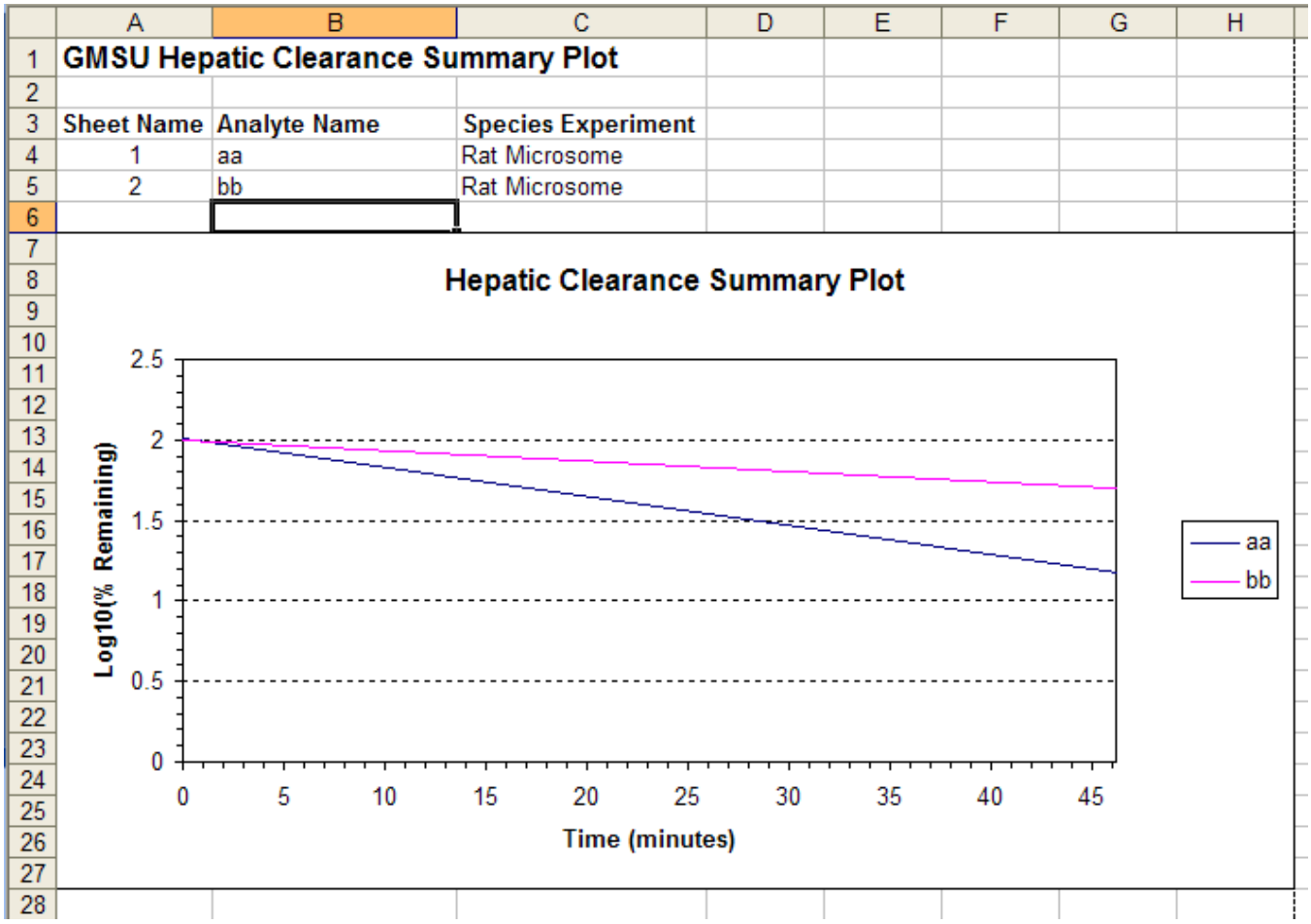
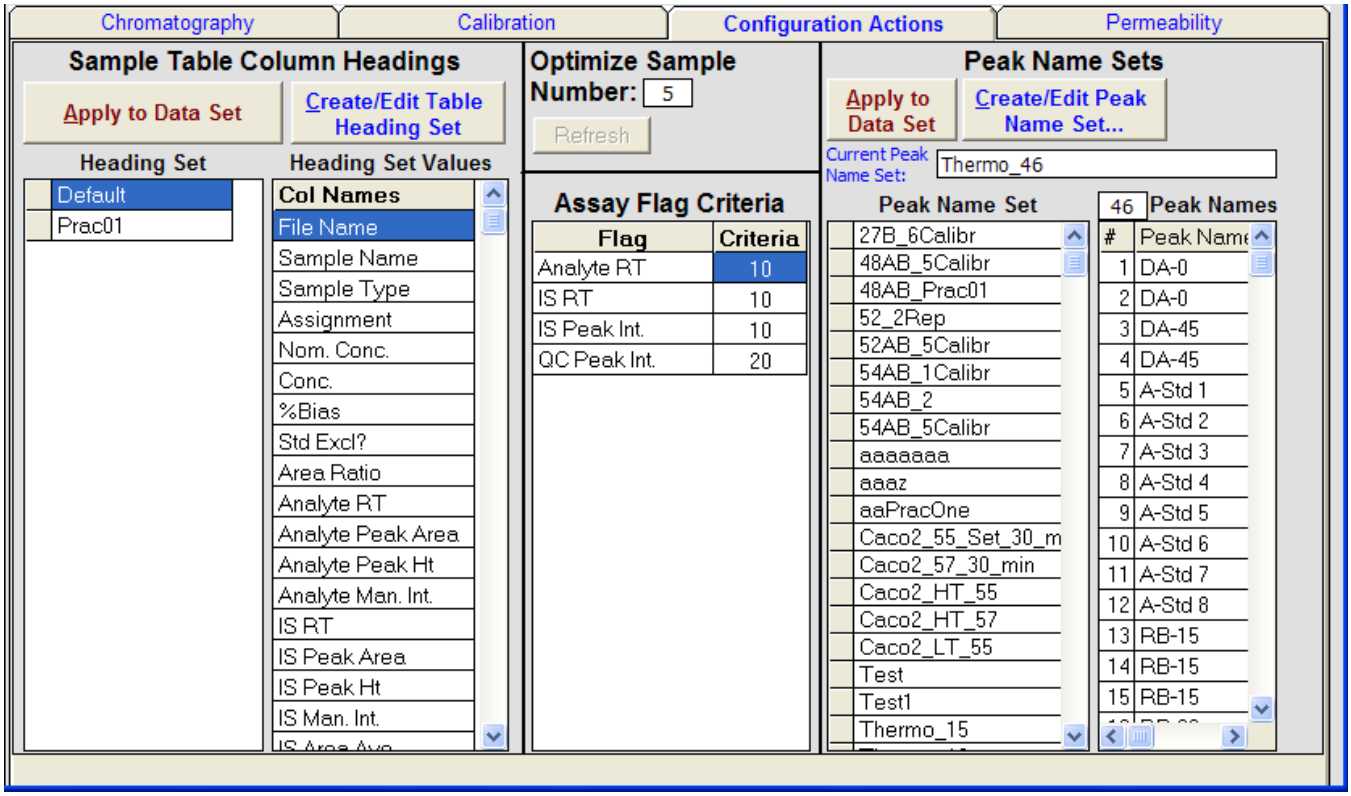


Figure 48 Hepatic Clearance – Summary Regression Plot

25 Permeability Calculator

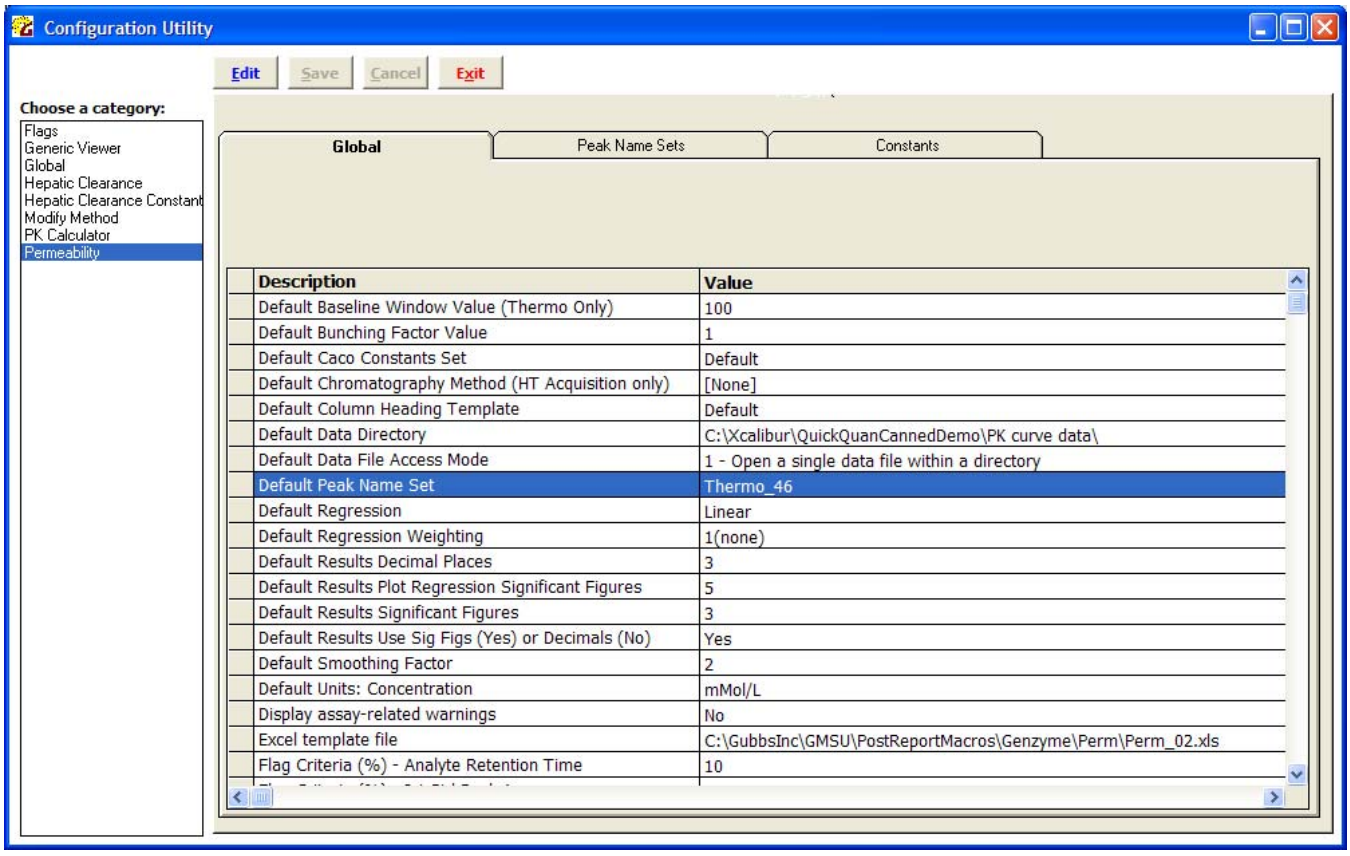
With the exception of a portion of the Configuration Actions tab, the Chromatography, Calibration, and Configuration Actions tabs are exactly the same as the Generic Chromatographic Viewer (GVC). The user is referred to the GVC section (Section 27) and the section on Conserved Features (Section 9) for a description on the use of these tabs and features.

25.1 Configuration Actions – Peak Name Sets



A permeability assay requires that samples be assigned appropriate sample types. Users must apply a Peak Name Set by selecting one and clicking the Apply To Data Set button.

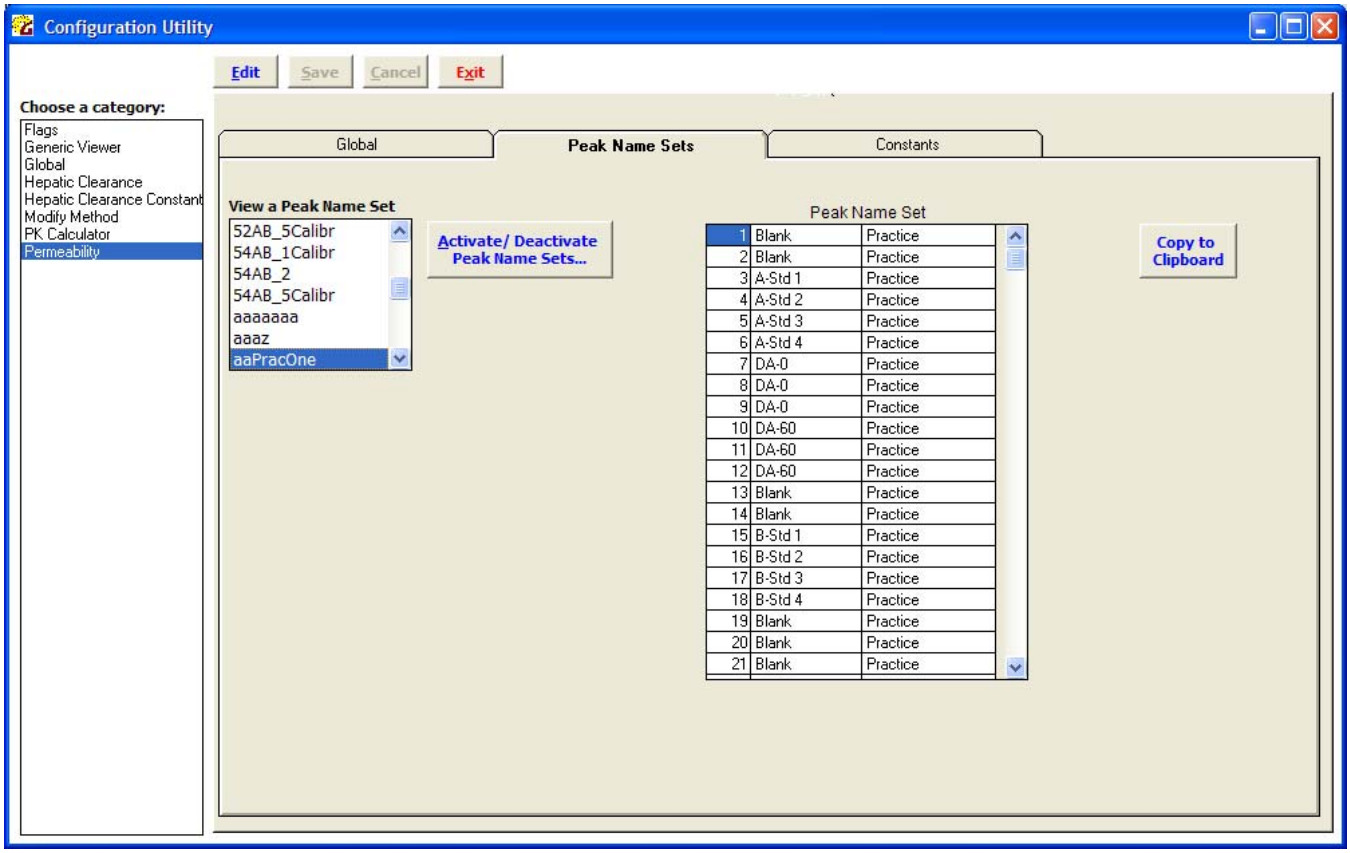
Alternatively, a default Peak Name Set may be configured in the Configuration Utility – Permeability – Global – Default Peak Name Set entry. This Peak Name Set will be applied to new or unsaved data when opened in the Permeability Calculator.



To create a new Peak Name set, click the Create/Edit Peak Name Set button (see section 25.1.1).

25.1.1 Create/Edit a Peak Name Set

When the Click/Edit Peak Name Set button is clicked, the Configuration Utility – Permeability – Peak Name Sets window is displayed.

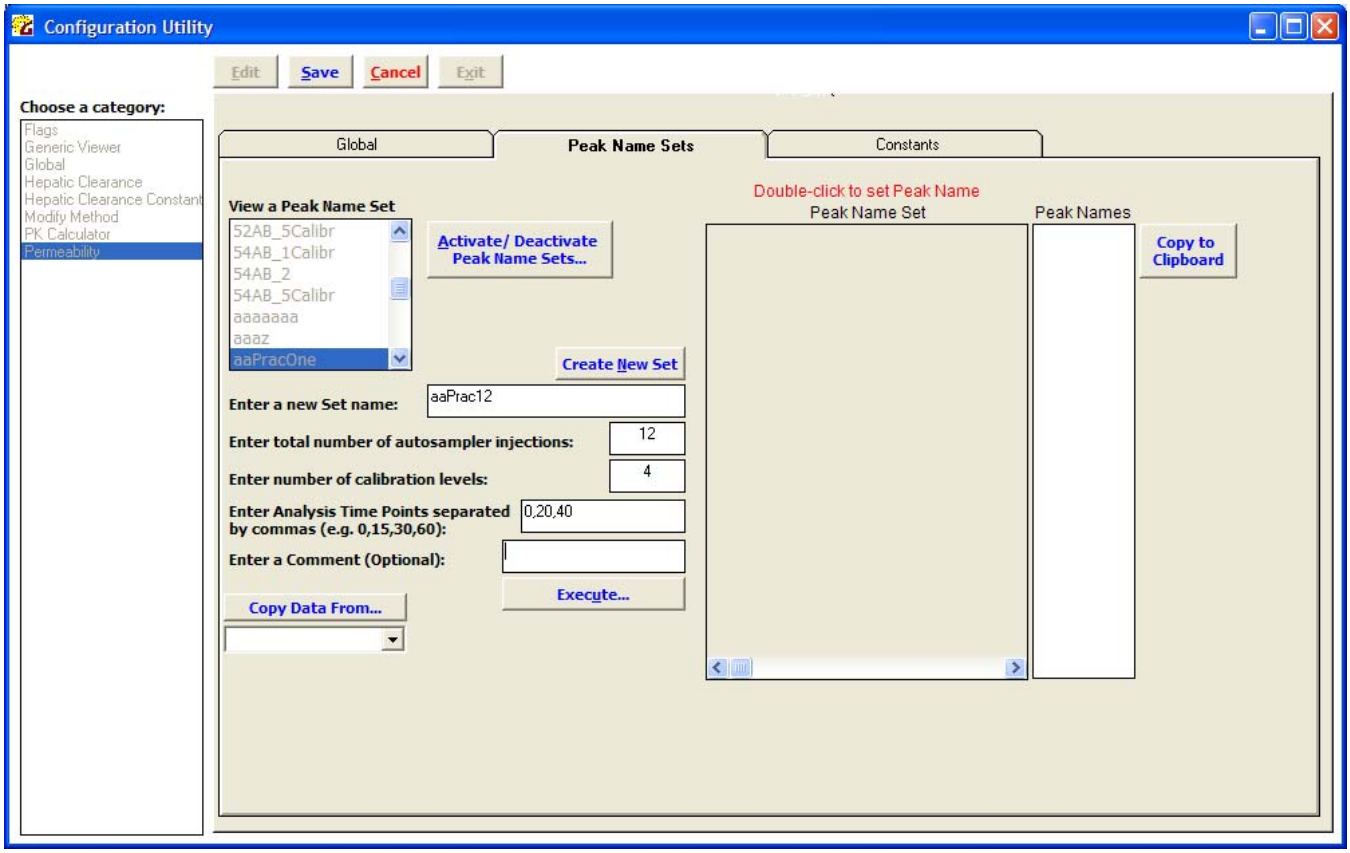


25.1.1.1 Create a new Peak Name Set

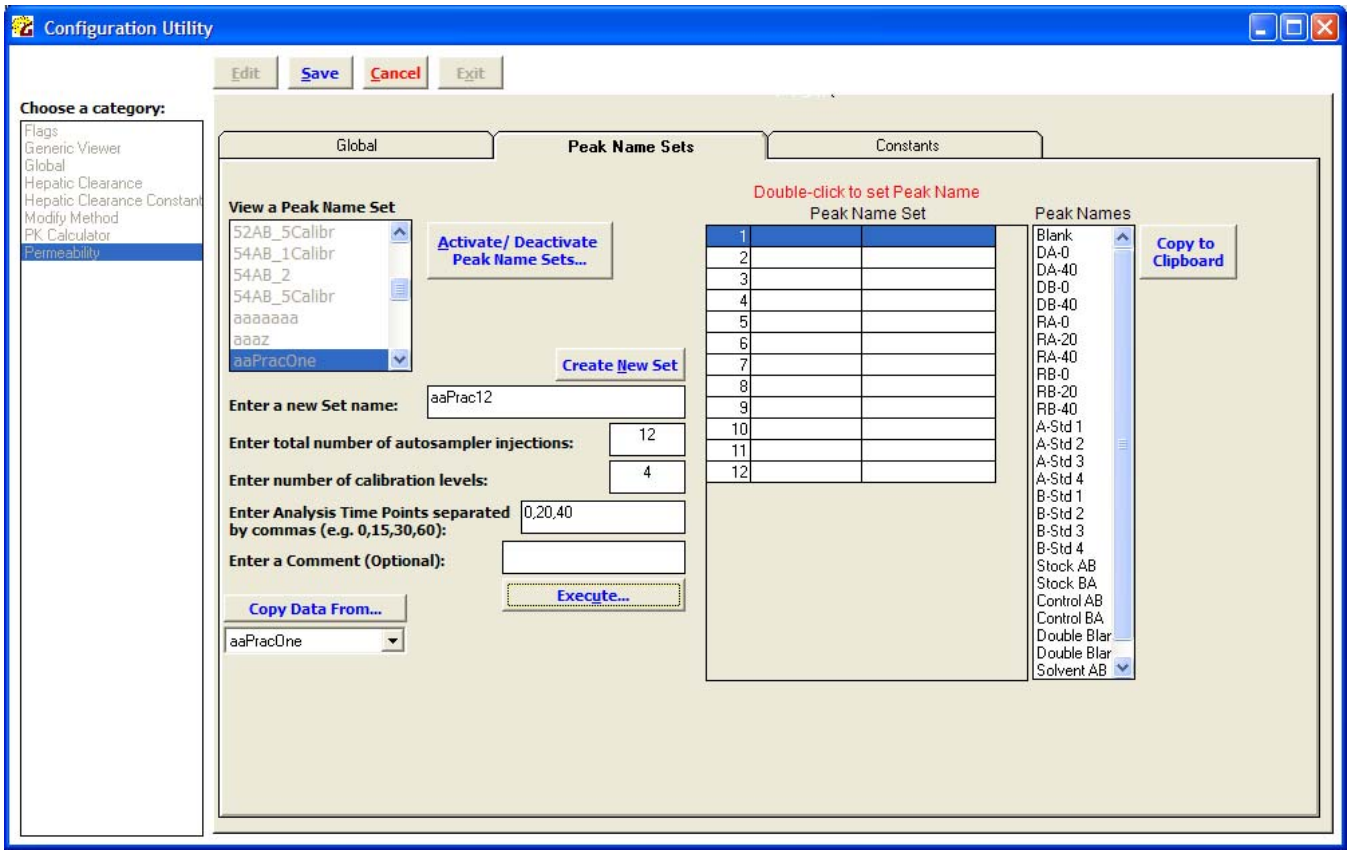
Note: See Section 25.1.1.2 for instructions on how to create a new Peak Name Set based information from an existing Peak Name Set.

To create a new Peak Name Set, perform the following actions:

- Click the Edit button to go into Edit mode
- Click the Create New Set button
- Complete the required entries:
  - Enter a new Set name
  - Enter total number of autosampler injections
  - Enter number of calibration levels
  - Enter Analysis Time Points…
  - Enter a Comment (optional)



- When complete, click the Execute button
  - The Peak Name Set table will be populated with the number of rows as defined in the 'Enter total number of autosampler injections' field
  - The Peak Names list will populate with sample identifiers that follow required GMSU/QC syntax rules



- Enter the peak name assignments (2nd column of the Peak Name Set table) by:
  - Select the Peak Name identifier in the Peak Names list
  - Select the row to be modified in the Peak Name Set table
  - Double-click the 2nd cell of the selected row – this action will synchronize the identifier in the Peak Name Set
  - Repeat until the entire table is filled.

- Save the Peak Name Set by clicking the Save button

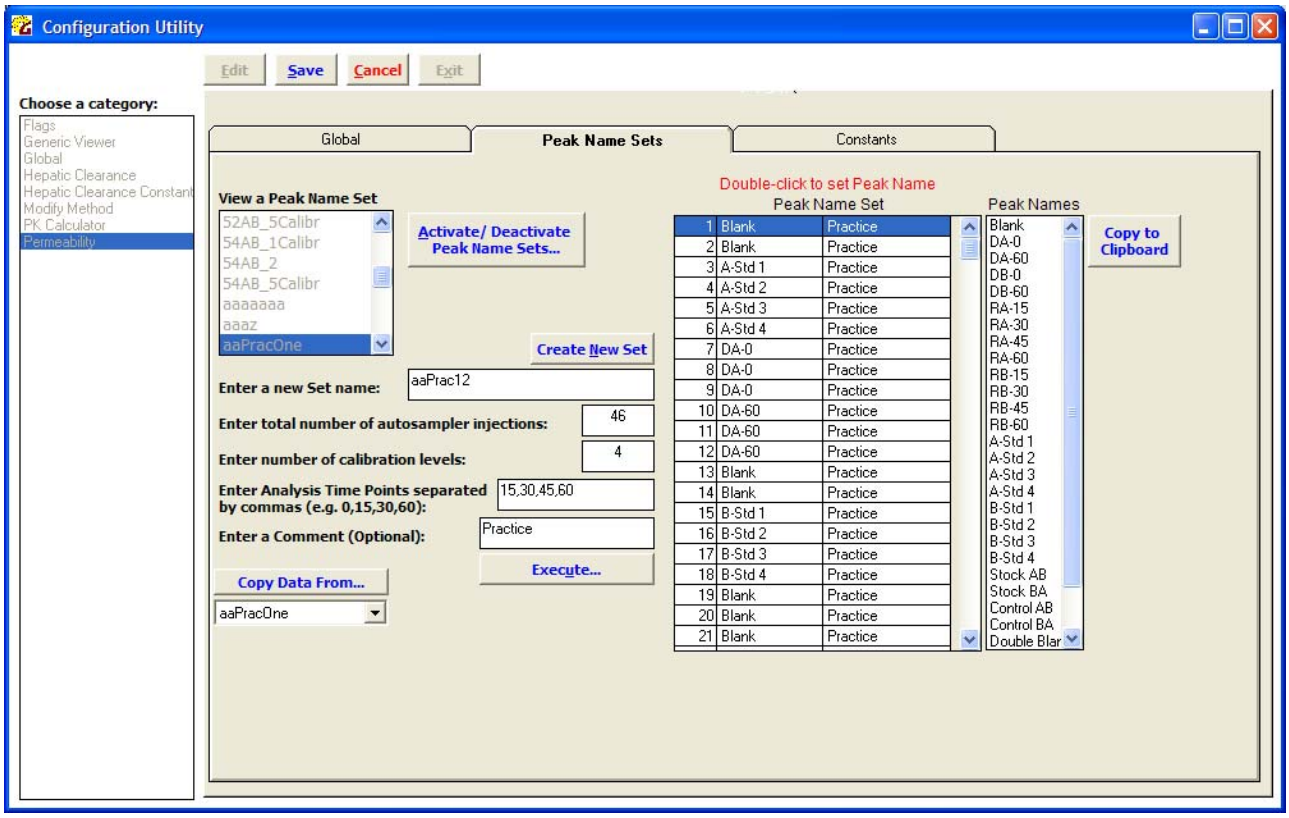
The different sample identifiers in a permeability assay include:

Sample Type	Description
Standard	A calibration curve standard. For example, 'A-Std 1' would mean the first set, or A-side, (A-Std 1) of a calibration standard set and the first calibration level (A-Std 1)
DA / DB	Donor side sample for a specific time point used in the calculation of Mass Balance and Sink Condition. E.g, DA-0 would be the Donor A-side 0 minute time point sample, while DB-60 would be the Donor B-side 60 minute time point sample.
RA / RB	Receiver side sample for a specific time point. E.g. RA-15 would be the Receiver side 15 minute time point sample, while RB-60 would be the Receiver side 60 minute sample.

25.1.1.2 Create a new Peak Name Set based on an existing Peak Name Set

To create a new Peak Name Set based on an existing Peak Name Set, perform the following actions:

- Click the Edit button to go into Edit mode
- Click the Create New Set button
- Enter a New Set Name in the appropriate field
- Choose an existing Peak Name Set from the 'Copy Data From' dropdown box
- Click the Copy Data From button
  - The fields and tables will be populated with the information from the chosen Peak Name Set
  - The user may now change parameters, such as increase/decrease the number of autosampler injections or change the Analysis Time Points
- Save the Peak Name Set by clicking the Save button

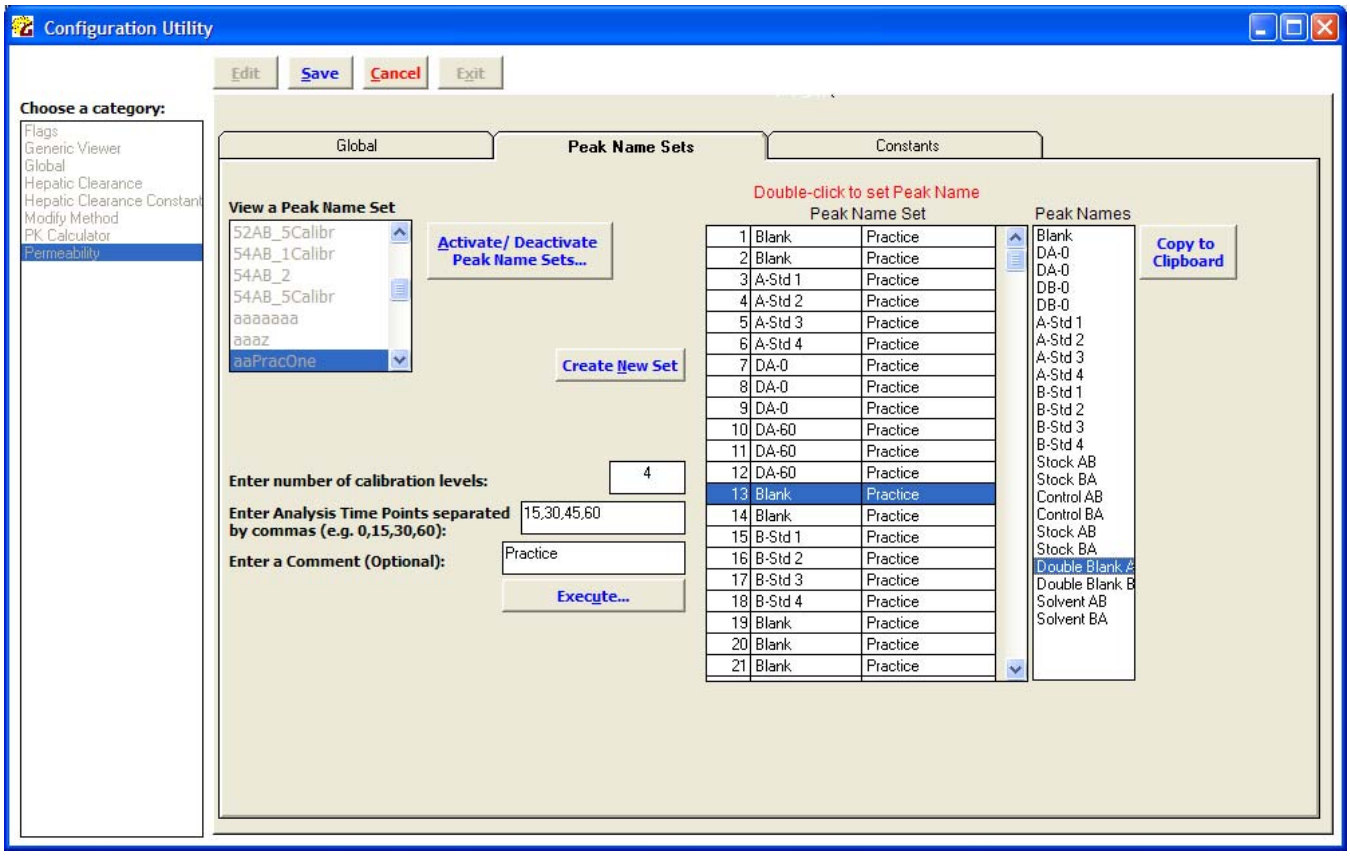


25.1.1.3 Edit an existing Peak Name Set

Note: The number of injections (rows in the Peak Name Set table) is not an editable field. In order to add injections to an existing Peak Name Set, the user must create a new Peak Name Set.

To edit an existing Peak Name set, perform the following actions:

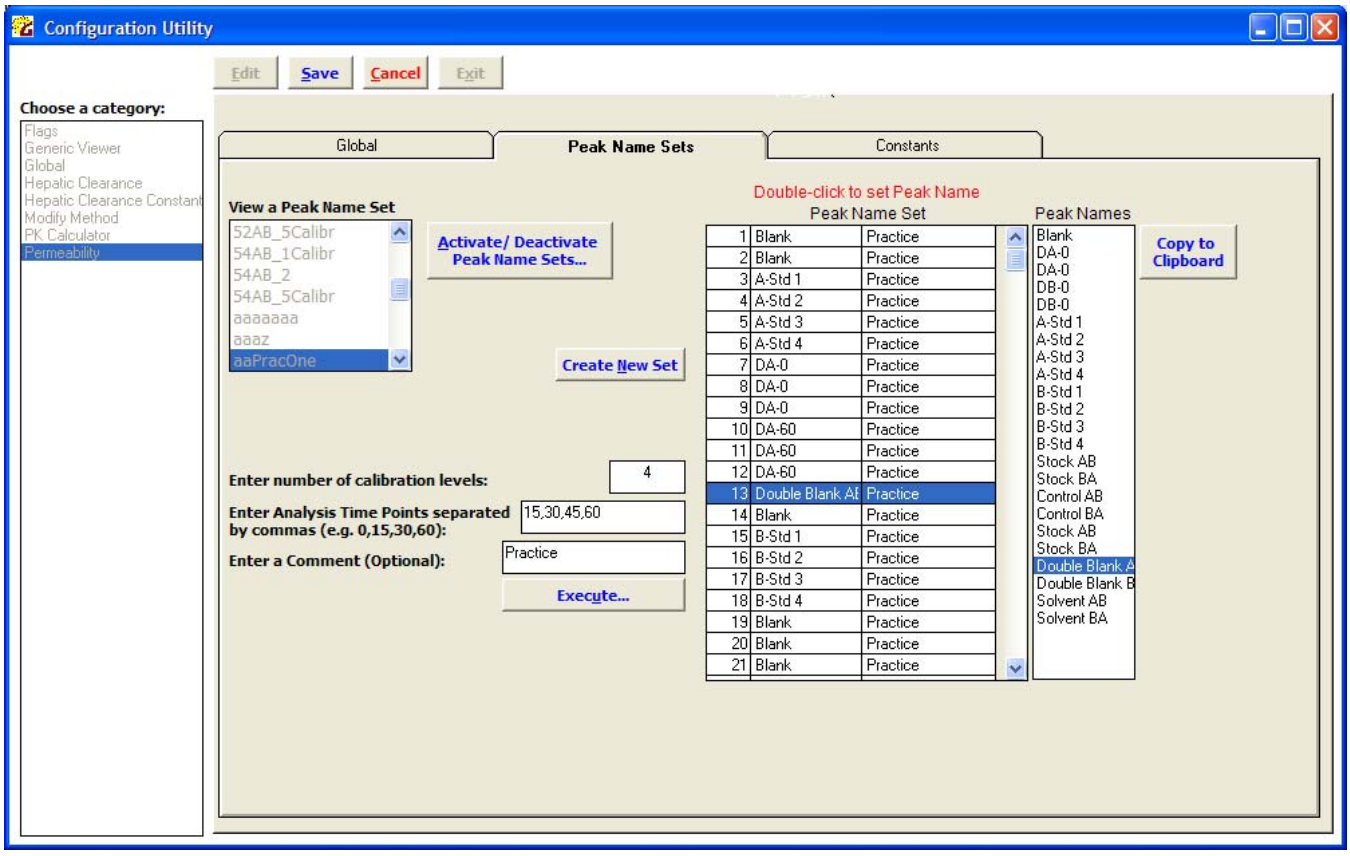
- In the 'View a Peak Name Set' table, choose a Peak Name Set to edit
- Click on the Edit button to go into Edit mode



- Modify the contents of the following fields:
  - Enter number of calibration levels
  - Enter Analysis Time Points….
  - Enter a comment (optional)

- Click the Execute button:
  - This action will update the contents of the Peak Names list

- Modify the peak name assignments (2nd column of the Peak Name Set table) by:
  - Select the Peak Name identifier in the Peak Names list
  - Select the row to be modified in the Peak Name Set table
  - Double-click the 2nd cell of the selected row – this action will synchronize the identifier in the Peak Name Set
- Save the Peak Name Set by clicking the Save button



25.2 Permeability Calculator Instructions

Instructions for the use of the Permeability Calculator are as follow:

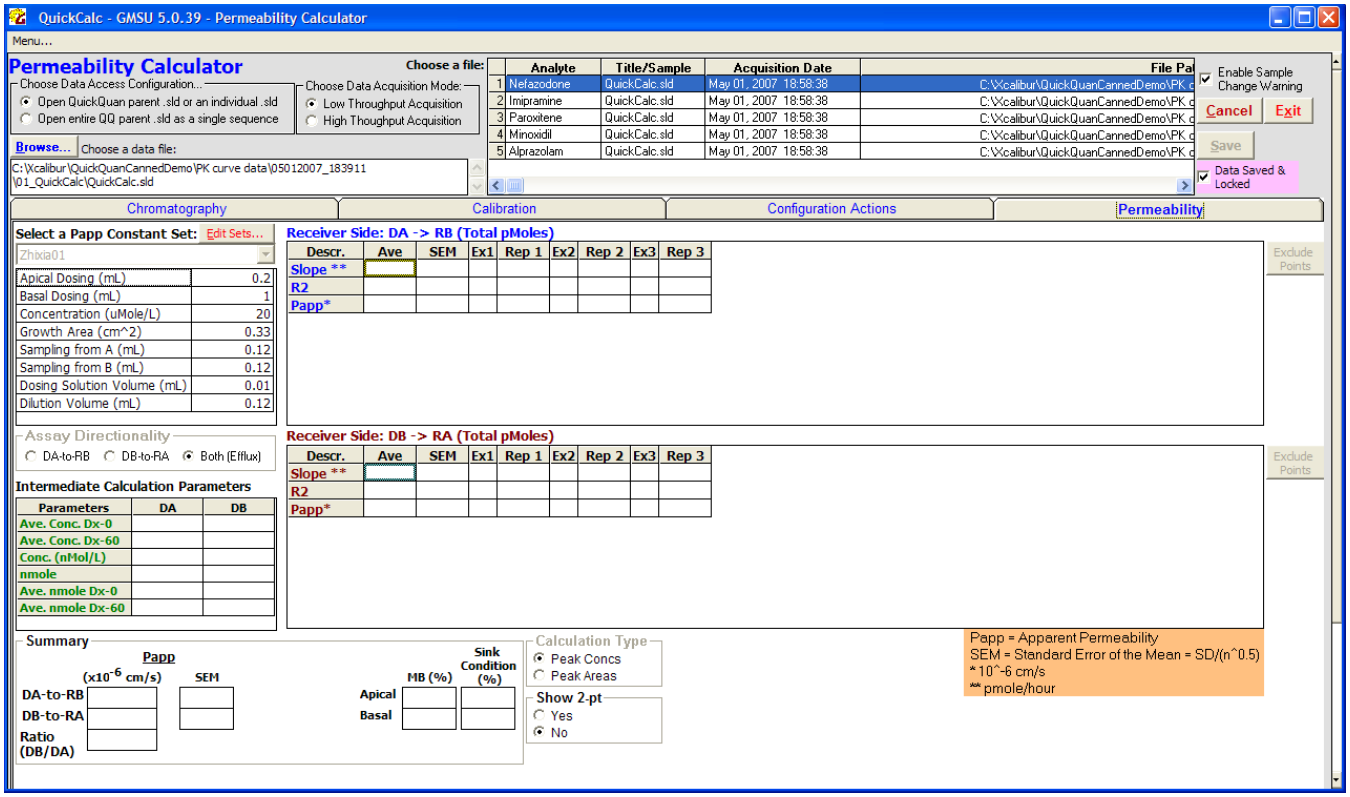


Figure 49 Permeability Calculator – Permeability Tab

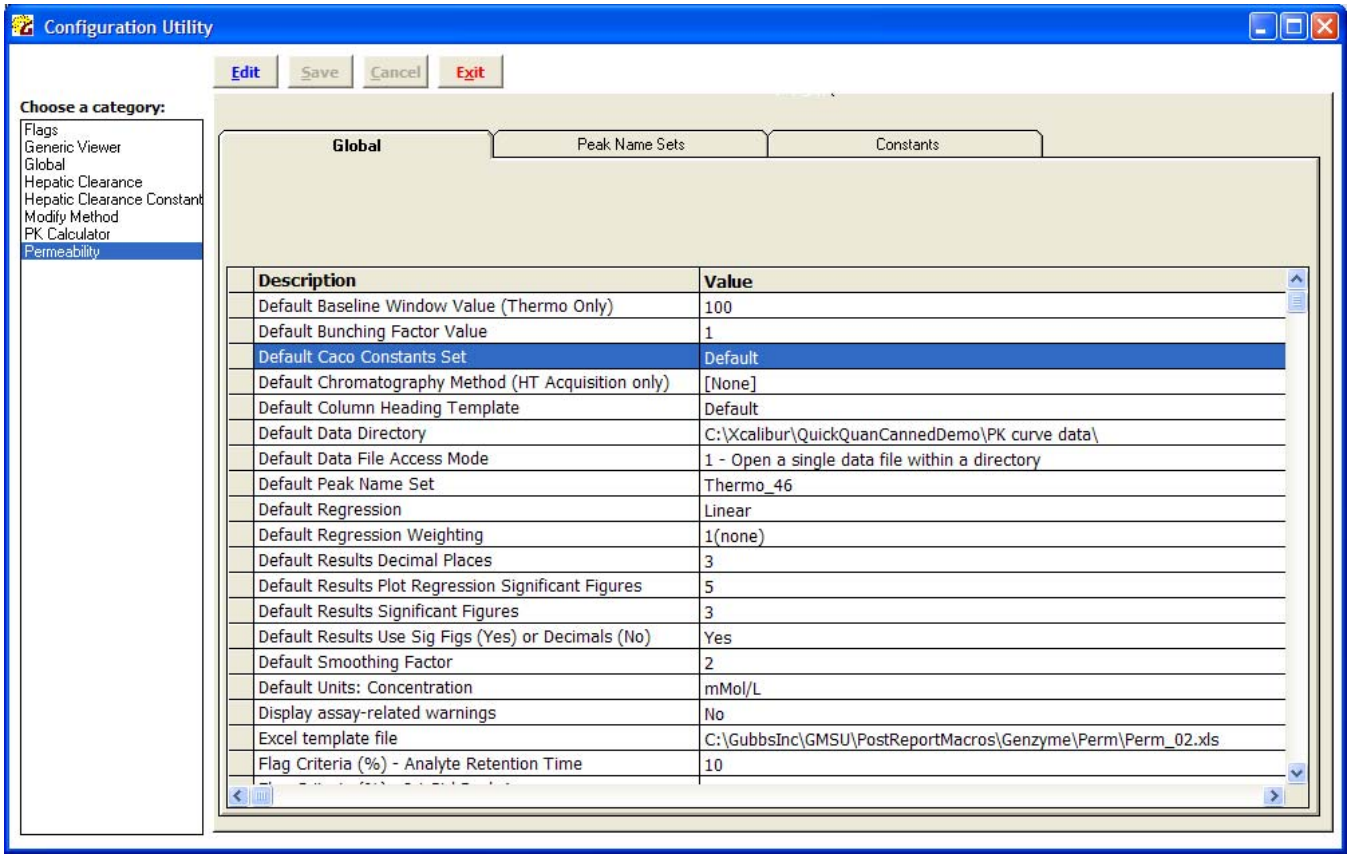
25.2.1 Apply a Peak Name set

Users apply a Peak Name set by choosing a Peak Name Set from the Configuration Actions tab Peak Name Set table (see Section 25.1 for further information).

25.2.2 Select a P_{app} Constant Set

Users must select a Constant Set whose values will be used by GMSU/QC to generate P_{app}, Mass Balance, and Sink Condition.

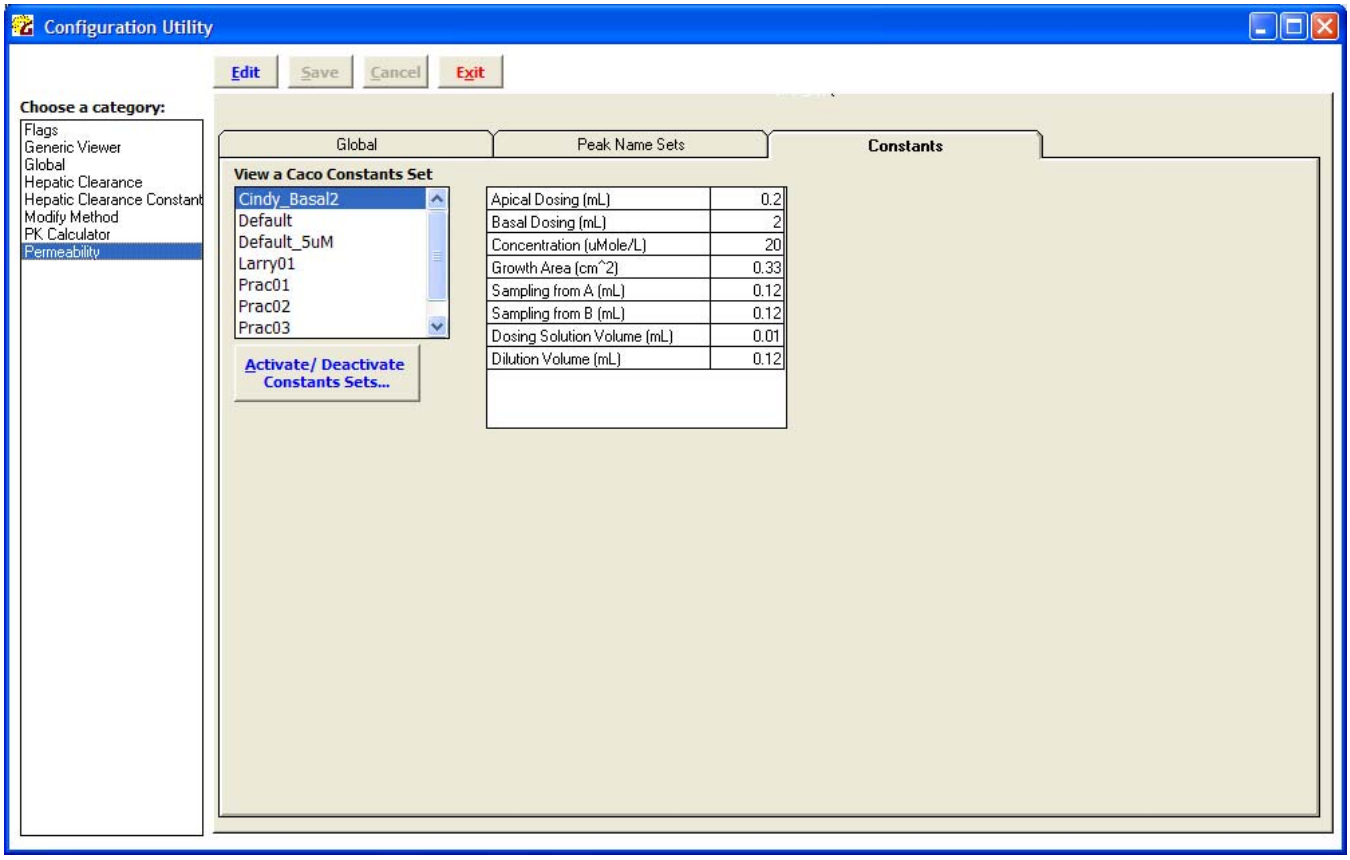
Alternatively, a default Constant Set may be configured in the Configuration Utility – Permeability – Global – Default Caco Constant Set entry. This Constant Set will be applied to new or unsaved data when opened in the Permeability Calculator.



25.2.2.1 Create a new Constant Set

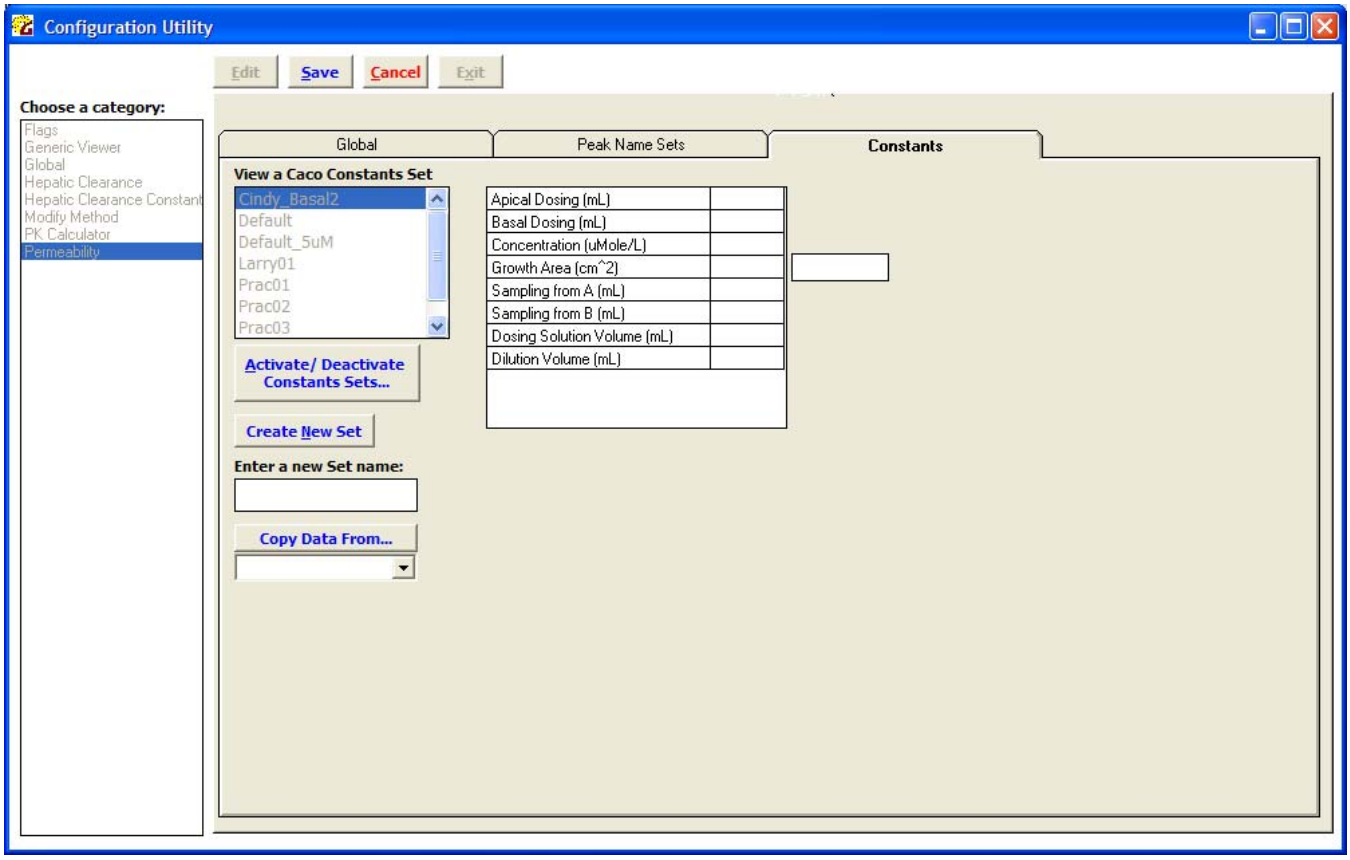
Please note that the values of existing Constant Sets cannot be modified.

P_{app} Constant Sets are created in the Configuration Utility – Permeability – Constants tab.



To create a new Constant Set, perform the following actions:

- Click the Edit button
- Click the Create New Set button
- Enter a Constant Set name in the field
- Enter values for each field in the Constant values table to the right
- Click the Save button to save the new Constant Set

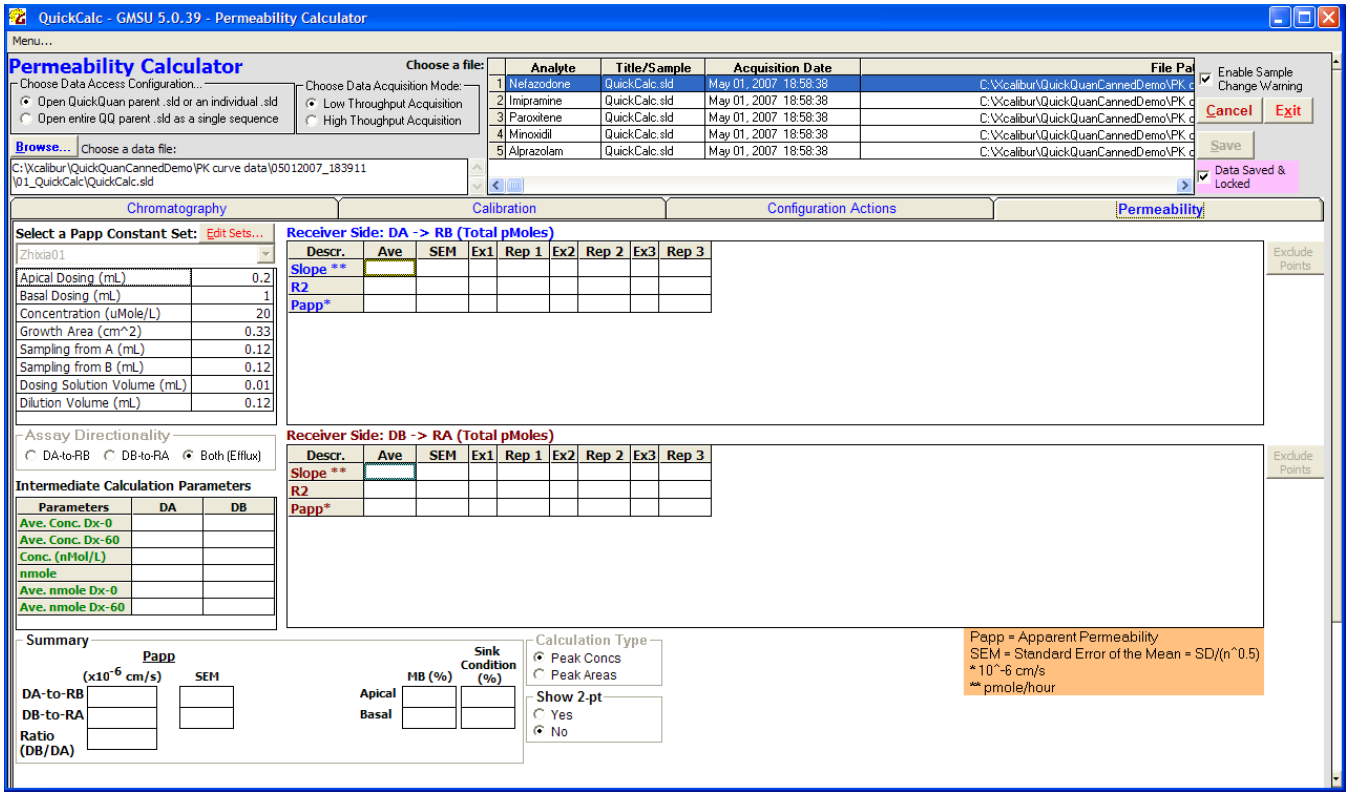


25.2.3 Exclude Points

Users may exclude points by selecting one or more Rep X values and clicking the Exclude Points button.

Users may include points by selecting one or more Rep X values and clicking the Exclude Points button. The ExX column will be populated with an X and the cell background will be changed to yellow.

If the selected Rep X value has been excluded, the button title changes to Include Points. If the selected Rep X value has not been excluded, the button title changes to Exclude Points.



25.2.4 Calculation Types

Users may choose two types of calculation methods.

25.2.4.1 Peak Concs

Peak concentrations calculated from a calibration curve are used to generate permeability values.

25.2.4.2 Peak Areas

Peak areas of samples are used to generate permeability values. A calibration curve is not needed in this case.

25.2.5 Show 2-pt

Function for Millennium Pharmaceuticals process.

25.3 Permeability Calculations

The Permeability Calculator uses the following the calculations to generate permeability values. This discussion makes the following assumptions:

- Sample time points are performed in triplicate
- Donor Side time points are 0 and 60 minutes
- Receiver Side time points are 15, 30, 45, and 60 minutes (0.25, 0.50, 0.75, and 1.0 hours)
- Reported concentrations are nM (nMole/L)

In order to calculate permeability values, the following constants must be known:

Constants	Value [1]	Comments
Drug Concentration (uMole/L):	20.0000	Used in calculation of P_{app}
GrowthArea (cm^2):	0.3300	Used in calculation of P_{app}
DA ApicalDosing (mL):	0.2000	Used in calculation of 'Corrected Calc Amt' and TpMole
RB BasalDosing (mL):	0.1200	Used in calculation of 'Corrected Calc Amt' and TpMole
Apical Sampling (mL):	0.1200	Used in calculation of pMole and TpMole
Basal Sampling (mL):	0.1200	Used in calculation of pMole and TpMole
DosingSolutionVolume (mL):	0.0100	Used in calculation of DonorConcFactor
DilutionVolume (mL):	0.1200	Used in calculation of DonorConcFactor
DonorConcFactor:	13.0000	=> (DilutionVolume+DosingSolutionVol)/DosingSolutionVol, used in calculation of MB Calculation 'Corrected Amt'

[1] Values used for example purposes

25.3.1 Donor Side Calculations

Sink Condition and Mass Balance
This example is for Apical values (DA). The same calculations are used for Basal values (DB).

Samples called DA are analyzed in replicate at time points 0 (DA-0) and 60 (DA-60) minutes. The average of the replicate concentrations are used for Sink Condition calculations, while the average amount (corrected for DonorConcFactor) is used for Mass Balance calculations.

Apical	Concentration (nMole/L)	
	0 min	**60 min**
DA1	1350.00	980.0000
DA2	1590.00	1150.0000
DA3	1500.00	1240.0000
For Sink Condition Calcs	Conc0A	Conc60A
Average Conc (nMole/L)	1480.0	1123.333
For MB Calculations	Amt0A	Amt60A
Corrected Amt (nMole)	3.8480	2.9207

$$CorrectedAmt = \frac{Conc0\ nMole}{L} * \frac{DonorConcFactor}{} * \frac{ApicalDosing\ mL}{} * \frac{L}{1000\ mL}$$

25.3.1.1 Mass Balance

The formula for Mass Balance is:

$$\left(\frac{Amt60\ nMole}{} + \left(\frac{AveAmt\ pMole}{} * \frac{nMole}{1000\ pMole} \right) \right) * \frac{}{0Amt\ nMole}$$

where AveAmt is described in Section 25.3.2, linear regression slope table

Using the Apical data above, the reported Mass Balance = 78.1%

25.3.1.2 Sink Condition

The formula for Sink Condition is:

Conc0 / Conc60

Using the Apical data above, the reported Sink Condition = 75.9%

25.3.2 Receiver Side Calculations

This example is for Donor Apical-to- Receiver Basal values (DA-to-RB). The same calculations are used for Donor Basal-to-Receiver Apical values (DB-to-RA).

Samples called RB are analyzed in replicate at times points 15, 30, 45, and 60 minutes (labeled as RB-15, RB-30, RB-45, and RB-60, respectively). The number of pMoles of each sample are calculated, then corrected (TpMole = total pMoles) for loss of sample occurring during experiment execution.

	15 min (0.25 hr)			30 min (0.50 hr)		
Rep	Conc (nMole/L)	pMole	TpMole	Conc (nMole/L)	pMole	CTpMole
1	6.84	0.8208	0.8208	82.20	9.8640	10.6848
2	12.40	1.4880	1.4880	51.40	6.1680	7.6560
3	6.15	0.7380	0.7380	70.20	8.4240	9.1620

	45 min (0.75 hr)			60 min (1.0 hr)		
Rep	Conc (nMole/L)	pMole	CTpMole	Conc (nMole/L)	pMole	CTpMole
1	276.00	33.1200	43.8048	391.00	46.9200	90.7248
2	210.00	25.2000	32.8560	382.00	45.8400	78.6960
3	203.00	24.3600	33.5220	395.00	47.4000	80.9220

Legend	
pMole:	Conc * SamplingA
TpMole (Total pMole):	pMole * BasalDosing / SamplingB
CTpMole:	$TpMole_{(n)} + pMole_{(n-1)} + \ldots + pMole_{(1)}$ where n = Time Point

The linear regression slope of number of pMole (TpMole and CTpMole) vs time (hour) is used to calculate permeability.

Replicate:	1	2	3	Average	SEM
time point (hr)					
0.25	0.8208	1.4880	0.7380	1.0156	0.24
0.50	10.6848	7.6560	9.1620	9.1676	0.87
0.75	43.8048	32.8560	33.5220	36.7276	3.54
1.00	90.7248	78.6960	80.9220	[1] 83.4476	3.69
Slope (pMole/hr)	121.13	102.73	105.96	109.94	5.67
R-Squared	0.92951	0.89338	0.90175	0.90821	
Papp (10^{-6} cm/s)	5.10	4.32	4.46	4.63	0.24

[1] 60 min Average used in MB Calculation (AveAmtA)

Finally, the ratio of DB-to-RA and DA-to-RB permeability values (Ratio B/A) is reported.

26 Permeability Report

The functions in the Permeability Report window are straightforward and not all features will be described. In addition, the user is referred to Section 10 for a discussion of features conserved between all module Report windows. Figure 69 shows the Reports window. Figure 70 and Figure 71 show examples of a PK Calculator Report.

Please note that the report content itself is highly configurable. PK Calculator Reports support post-report macros in Excel. Users may create a blank Excel file that has embedded a macro named "DoGMSU". If this Excel file is configured in Configuration Utility – PK Calculator – Excel Template File, then PK Calculator Report will use this file as to generate the report, then execute the macro upon completion. In this manner, users can do such things as modify the format and/or content of the report, or send report information to an internal LIMS system.

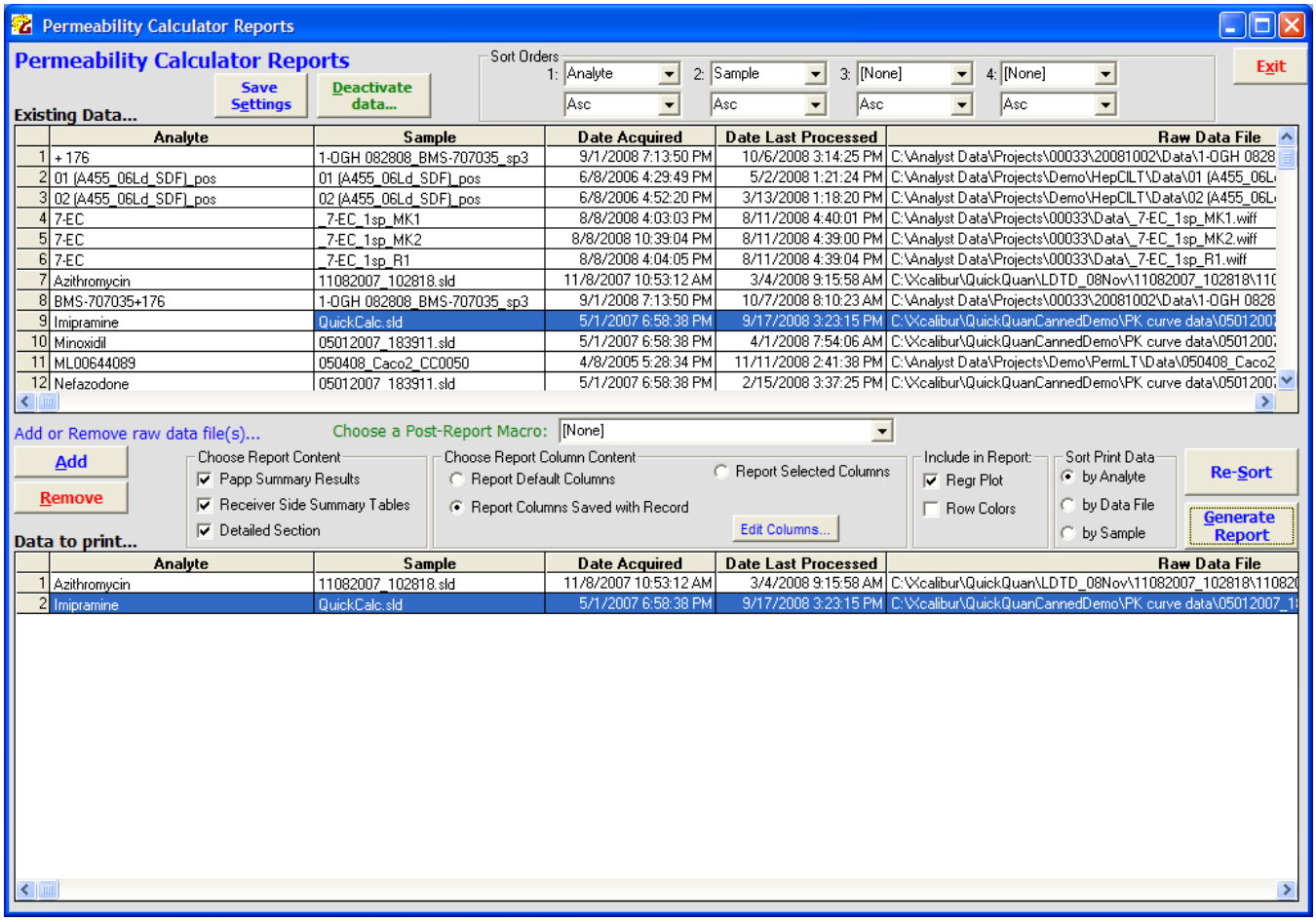


Figure 50 Permeability Report

26.1 Summary page

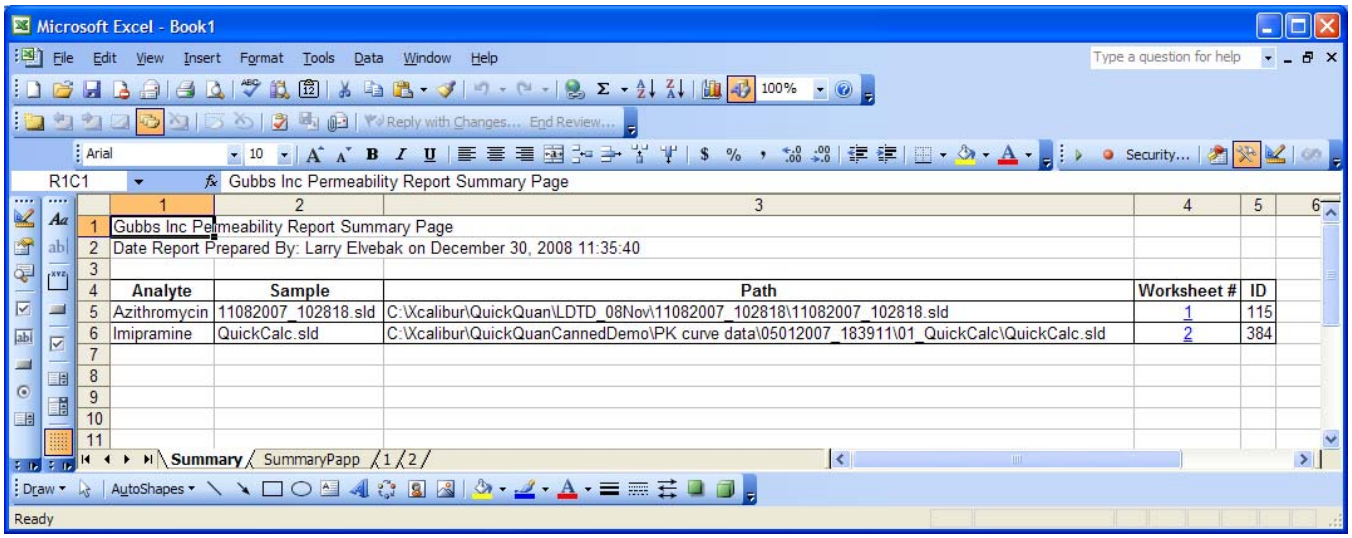
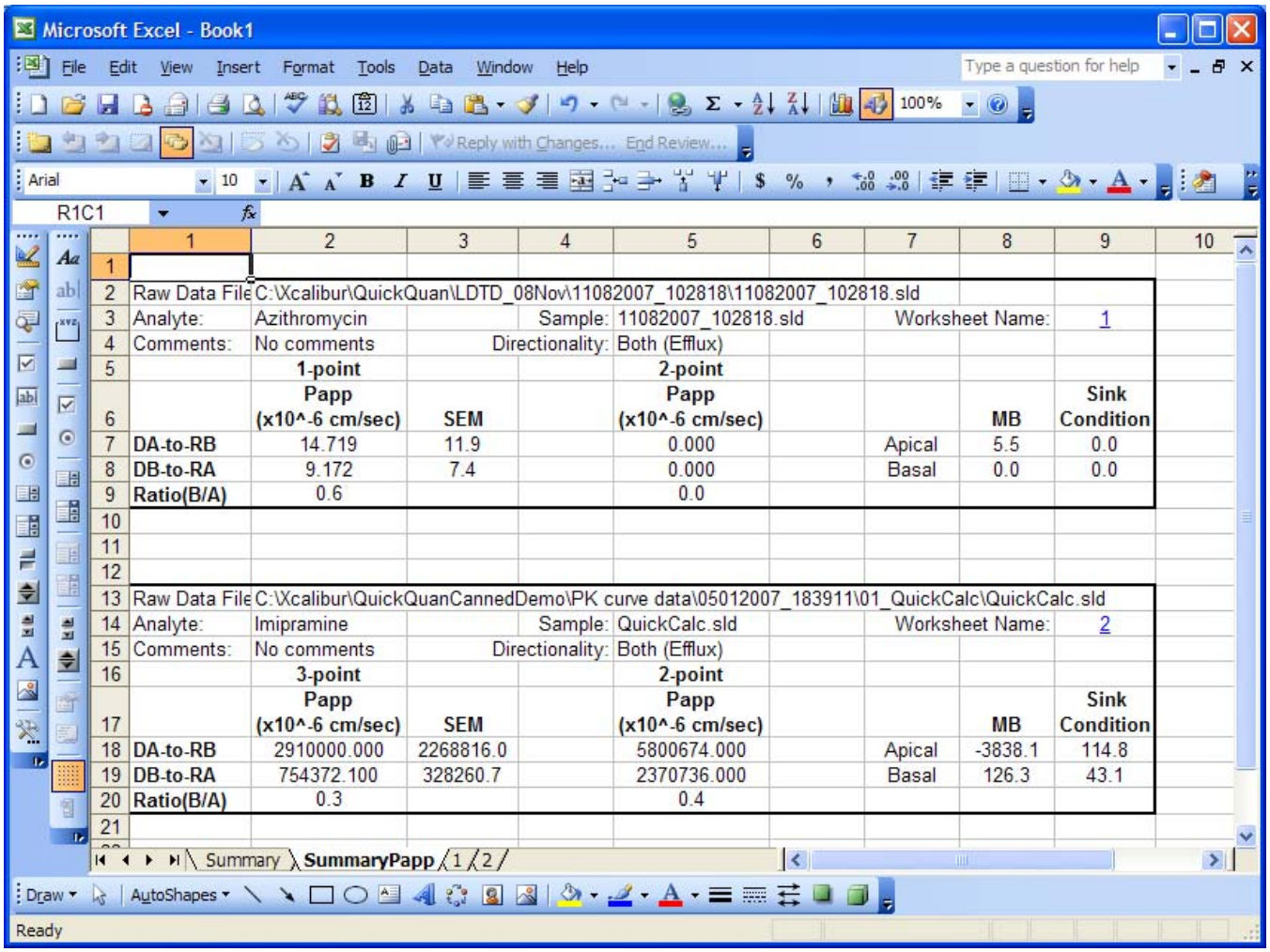


Figure 51 Permeability Report – Summary Results page

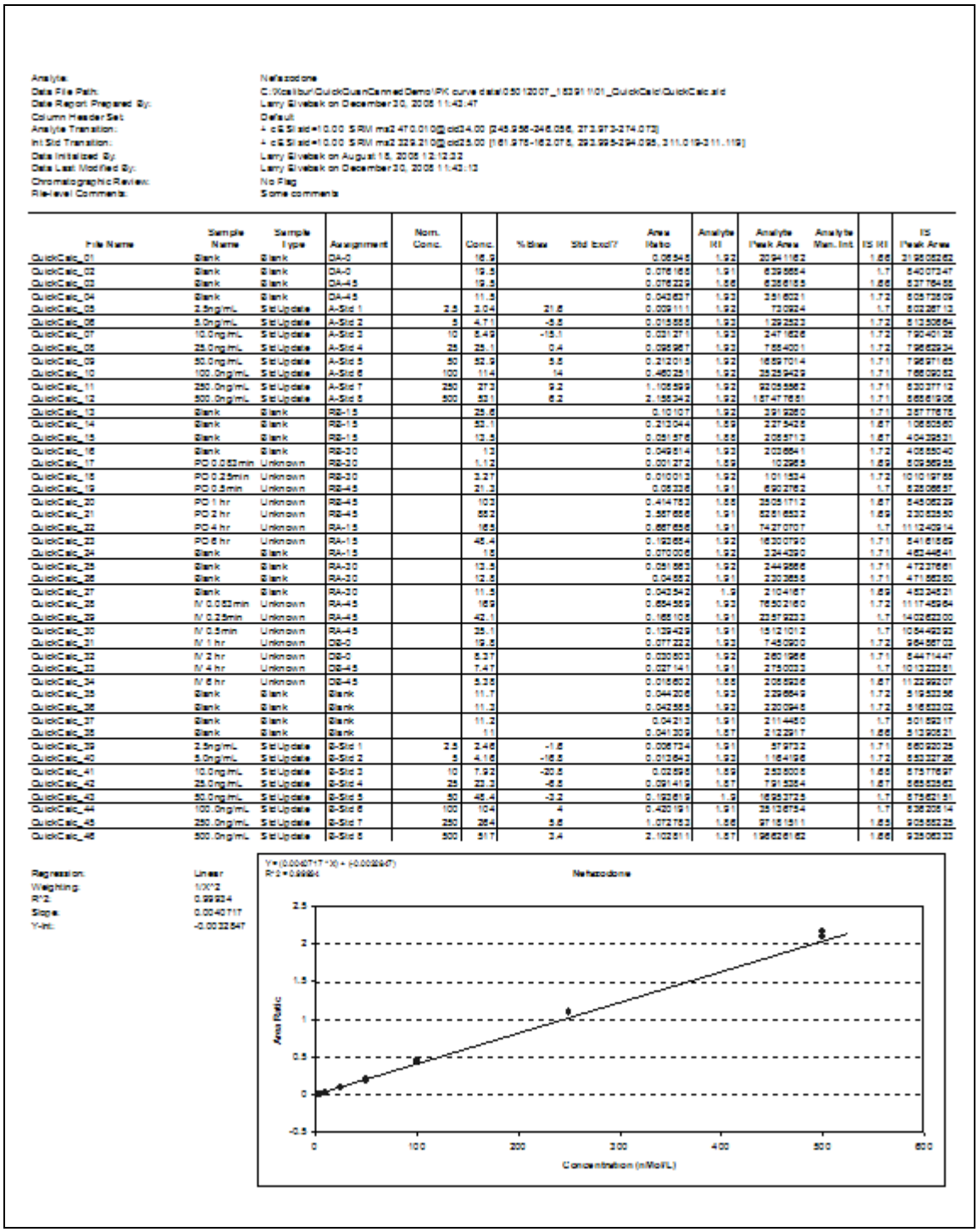
26.2 Summary Papp page



1									
2	Raw Data File	C:\Xcalibur\QuickQuan\LDTD_08Nov\11082007_102818\11082007_102818.sld							
3	Analyte:	Azithromycin		Sample:	11082007_102818.sld		Worksheet Name:	1	
4	Comments:	No comments		Directionality:	Both (Efflux)				
5		**1-point**			**2-point**				
6		**Papp (x10^-6 cm/sec)**	**SEM**		**Papp (x10^-6 cm/sec)**			**MB**	**Sink Condition**
7	**DA-to-RB**	14.719	11.9		0.000		Apical	5.5	0.0
8	**DB-to-RA**	9.172	7.4		0.000		Basal	0.0	0.0
9	**Ratio(B/A)**	0.6			0.0				
10									
11									
12									
13	Raw Data File	C:\Xcalibur\QuickQuanCannedDemo\PK curve data\05012007_183911\01_QuickCalc\QuickCalc.sld							
14	Analyte:	Imipramine		Sample:	QuickCalc.sld		Worksheet Name:	2	
15	Comments:	No comments		Directionality:	Both (Efflux)				
16		**3-point**			**2-point**				
17		**Papp (x10^-6 cm/sec)**	**SEM**		**Papp (x10^-6 cm/sec)**			**MB**	**Sink Condition**
18	**DA-to-RB**	2910000.000	2268816.0		5800674.000		Apical	-3838.1	114.8
19	**DB-to-RA**	754372.100	328260.7		2370736.000		Basal	126.3	43.1
20	**Ratio(B/A)**	0.3			0.4				
21									

Figure 52 Permeability Report – Summary Papp page

26.3 Detailed Report section of the results page



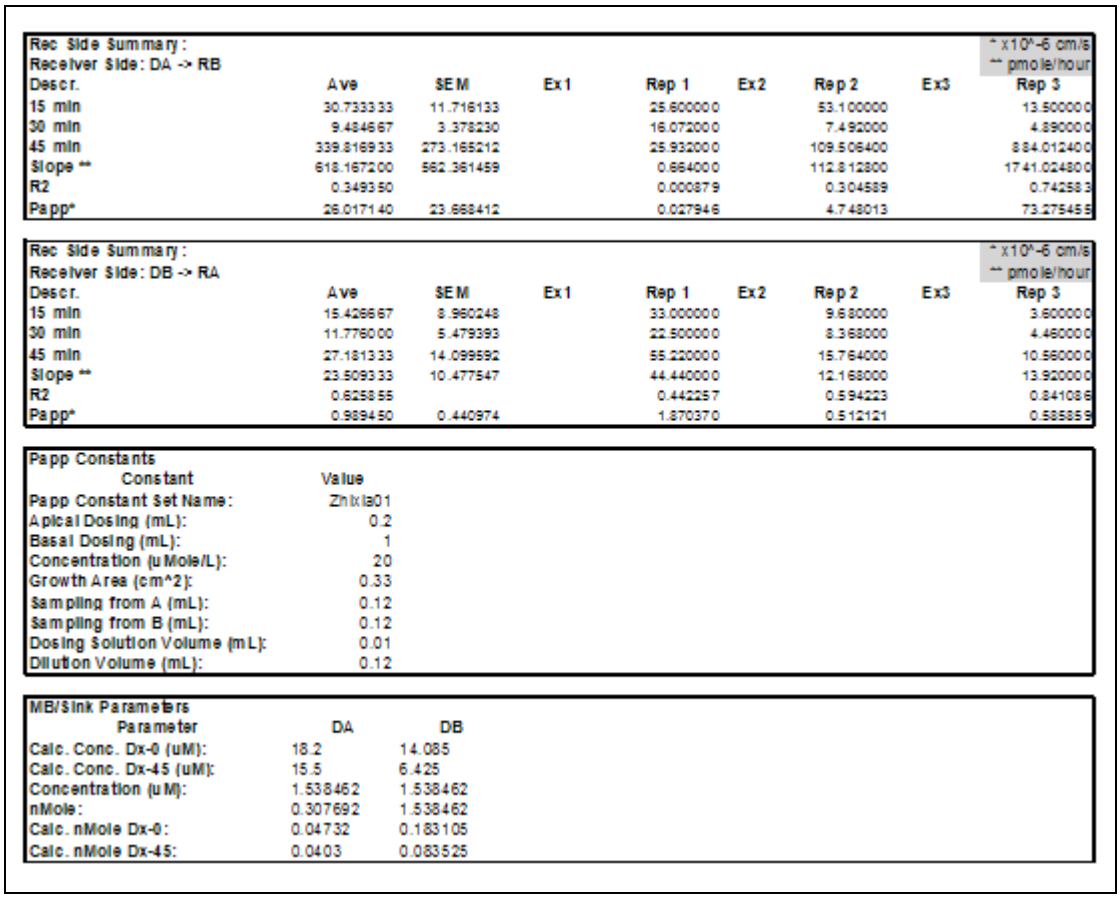
Analyte: Nefazodone
Data File Path: C:\Xcalibur\QuickQuanCannedDemo\PK curve data\050120071_153911\01_QuickCalc\QuickCalc.xld
Data Report Prepared By: Larry Silverbek on December 30, 2005 11:43:47
Column Header Set: Default
Analyte Transition: + c ESI sid=10.00 SRM ms2 470.010 [cid24.00 [245.956-246.056, 273.973-274.073]
Int Std Transition: + c ESI sid=10.00 SRM ms2 329.210 [cid25.00 [161.975-162.075, 293.995-294.095, 311.019-311.119]
Data Initialized By: Larry Silverbek on August 15, 2005 12:12:32
Data Last Modified By: Larry Silverbek on December 30, 2005 11:43:13
Chromatographic Review: No Flag
File-level Comments: Some comments

File Name	Sample Name	Sample Type	Assignment	Nom. Conc.	Conc.	%Bias	Std Excl?	Area Ratio	Analyte RT	Analyte Peak Area	Analyte Man. Int.	IS RT	IS Peak Area
QuickCalc_01	Blank	Blank	DA-0		16.9			0.06545	1.92	2094162		1.66	31952562
QuickCalc_02	Blank	Blank	DA-0		19.5			0.076165	1.91	6396654		1.7	84007247
QuickCalc_03	Blank	Blank	DA-45		19.5			0.076229	1.66	6356155		1.66	83778455
QuickCalc_04	Blank	Blank	DA-45		11.5			0.043637	1.93	3516021		1.72	80573609
QuickCalc_05	2.5ng/mL	Std Update	A-Std 1	2.5	3.04	21.6		0.009111	1.92	730924		1.7	80228712
QuickCalc_06	5.0ng/mL	Std Update	A-Std 2	5	4.71	-5.8		0.015555	1.93	1292523		1.72	83150864
QuickCalc_07	10.0ng/mL	Std Update	A-Std 3	10	8.49	-15.1		0.031271	1.93	2471626		1.72	79040128
QuickCalc_08	25.0ng/mL	Std Update	A-Std 4	25	25.1	0.4		0.096967	1.93	7854001		1.72	79562934
QuickCalc_09	50.0ng/mL	Std Update	A-Std 5	50	52.9	5.8		0.212015	1.93	16597014		1.71	79597165
QuickCalc_10	100.0ng/mL	Std Update	A-Std 6	100	114	14		0.460251	1.92	35253429		1.71	76609033
QuickCalc_11	250.0ng/mL	Std Update	A-Std 7	250	272	9.2		1.106599	1.92	92059562		1.71	83037712
QuickCalc_12	500.0ng/mL	Std Update	A-Std 8	500	531	6.2		2.166342	1.92	167471661		1.71	86561906
QuickCalc_13	Blank	Blank	RB-15		25.6			0.10107	1.92	3919280		1.71	38778175
QuickCalc_14	Blank	Blank	RB-15		53.1			0.213044	1.89	8275428		1.67	38850560
QuickCalc_15	Blank	Blank	RB-15		13.5			0.051576	1.65	2055713		1.67	40429831
QuickCalc_16	Blank	Blank	RB-30		1.3			0.049514	1.93	203664 1		1.72	4056540
QuickCalc_17	PD 0.083min	Unknown	RB-30		1.12			0.001272	1.89	10296 5		1.69	5095955
QuickCalc_18	PD 0.25min	Unknown	RB-30		3.27			0.010013	1.92	101152 4		1.72	10101978
QuickCalc_19	PD 0.5min	Unknown	RB-45		21.2			0.05336	1.91	690276 2		1.7	5285865
QuickCalc_20	PD 1 hr	Unknown	RB-45		103			0.414753	1.66	3505171 2		1.67	5458629
QuickCalc_21	PD 2 hr	Unknown	RB-45		552			3.587656	1.91	5251653 2		1.69	2303535
QuickCalc_22	PD 4 hr	Unknown	RA-15		165			0.667656	1.91	7427070 7		1.7	1112409 14
QuickCalc_23	PD 6 hr	Unknown	RA-15		45.4			0.193654	1.92	1630 0790		1.71	8416569
QuickCalc_24	Blank	Blank	RA-15		15			0.070006	1.92	234 4290		1.71	4834481
QuickCalc_25	Blank	Blank	RA-30		12.5			0.051663	1.92	244 9866		1.71	4723766
QuickCalc_26	Blank	Blank	RA-30		12.5			0.04552	1.91	230 3656		1.71	4716650
QuickCalc_27	Blank	Blank	RA-30		11.5			0.043542	1.9	210 4167		1.69	4833452
QuickCalc_28	IV 0.083min	Unknown	RA-45		169			0.654559	1.93	7650 2160		1.72	1117 45564
QuickCalc_29	IV 0.25min	Unknown	RA-45		42.1			0.165108	1.91	2357 9223		1.7	1402 62200
QuickCalc_30	IV 0.5min	Unknown	RA-45		25.1			0.139429	1.91	1512 1012		1.7	1084 49292
QuickCalc_31	IV 1 hr	Unknown	DB-0		19.5			0.077222	1.93	745 0900		1.72	9645870
QuickCalc_32	IV 2 hr	Unknown	DB-0		8.37			0.030503	1.93	260 1966		1.71	8447144
QuickCalc_33	IV 4 hr	Unknown	DB-45		7.47			0.027141	1.91	275 0033		1.7	1013 23351
QuickCalc_34	IV 6 hr	Unknown	DB-45		5.35			0.018503	1.65	205 5926		1.67	1122 99207
QuickCalc_35	Blank	Blank	Blank		11.7			0.044206	1.93	229 6549		1.72	5193 3356
QuickCalc_36	Blank	Blank	Blank		11.3			0.042565	1.93	220 0945		1.72	5165 3302
QuickCalc_37	Blank	Blank	Blank		11.2			0.04213	1.91	211 4450		1.7	5015 2217
QuickCalc_38	Blank	Blank	Blank		11			0.041309	1.67	212 2917		1.66	5129 5521
QuickCalc_39	2.5ng/mL	Std Update	B-Std 1	2.5	2.46	-1.6		0.006734	1.91	57 9732		1.71	8609 2025
QuickCalc_40	5.0ng/mL	Std Update	B-Std 2	5	4.16	-16.8		0.012643	1.93	116 4196		1.72	9232 7226
QuickCalc_41	10.0ng/mL	Std Update	B-Std 3	10	7.92	-20.8		0.02595	1.89	253 6005		1.66	9757 7697
QuickCalc_42	25.0ng/mL	Std Update	B-Std 4	25	23.3	-6.8		0.091419	1.67	791 9364		1.67	8658 3563
QuickCalc_43	50.0ng/mL	Std Update	B-Std 5	50	48.4	-3.2		0.193619	1.9	1695 3725		1.7	8756 2151
QuickCalc_44	100.0ng/mL	Std Update	B-Std 6	100	104	4		0.420191	1.91	3513 6754		1.7	8363 0914
QuickCalc_45	250.0ng/mL	Std Update	B-Std 7	250	264	5.6		1.072753	1.66	9715 1511		1.65	9058 5225
QuickCalc_46	500.0ng/mL	Std Update	B-Std 8	500	511	3.4		2.102511	1.67	19663 6162		1.66	9350 8333

Regression: Linear
Weighting: 1/X^2
R^2: 0.99924
Slope: 0.0040717
Y-Int: -0.0033547

Figure 53 Permeability Report – Detailed Report section of Results page

26.4 Receiver Side Tables section of the Results page



Rec Side Summary: (* x10^-6 cm/s, ** pmole/hour)
Receiver Side: DA -> RB

Descr.	Ave	SEM	Ex1	Rep 1	Ex2	Rep 2	Ex3	Rep 3
15 min	30.733333	11.716133		25.600000		53.100000		13.500000
30 min	9.484667	3.378230		16.072000		7.492000		4.890000
45 min	339.816933	273.165212		25.932000		109.506400		884.012400
Slope **	618.167200	562.361459		0.864000		112.812800		1741.024800
R2	0.349350			0.000879		0.304589		0.742583
Papp*	26.017140	23.668412		0.027946		4.748013		73.275455

Rec Side Summary: (* x10^-6 cm/s, ** pmole/hour)
Receiver Side: DB -> RA

Descr.	Ave	SEM	Ex1	Rep 1	Ex2	Rep 2	Ex3	Rep 3
15 min	15.426667	8.960248		33.000000		9.680000		3.600000
30 min	11.776000	5.479393		22.500000		8.368000		4.460000
45 min	27.181333	14.099592		55.220000		15.764000		10.560000
Slope **	23.509333	10.477547		44.440000		12.168000		13.920000
R2	0.625855			0.442257		0.594223		0.841086
Papp*	0.989450	0.440974		1.870370		0.512121		0.585859

Papp Constants

Constant	Value
Papp Constant Set Name:	Zhixia01
Apical Dosing (mL):	0.2
Basal Dosing (mL):	1
Concentration (uMole/L):	20
Growth Area (cm^2):	0.33
Sampling from A (mL):	0.12
Sampling from B (mL):	0.12
Dosing Solution Volume (mL):	0.01
Dilution Volume (mL):	0.12

MB/Sink Parameters

Parameter	DA	DB
Calc. Conc. Dx-0 (uM):	18.2	14.085
Calc. Conc. Dx-45 (uM):	15.5	6.425
Concentration (uM):	1.538462	1.538462
nMole:	0.307692	1.538462
Calc. nMole Dx-0:	0.04732	0.183105
Calc. nMole Dx-45:	0.0403	0.083525

Figure 54 Permeability Report – Receiver Side Tables section of Results page

27 Generic Chromatographic Viewer (GCV)

The salient feature of GCV is that it can view in two ways Thermo QuickQuan (QQ) generated data:

- By selecting the Choose Data Access Configuration option 'Open QuickQuan parent .sld or an individual .sld' to load data, GMSU/QC will parse and list separately the different analytes acquired during the data acquisition event (see Figure 55)
- By selecting the Choose Data Access Configuration option 'Open entire QQ parent .sld as a single sequence' to load data, GMSU/QC will not parse the different analytes acquired during the data acquisition event and list them in the sample list as they were acquired (See Figure 56).

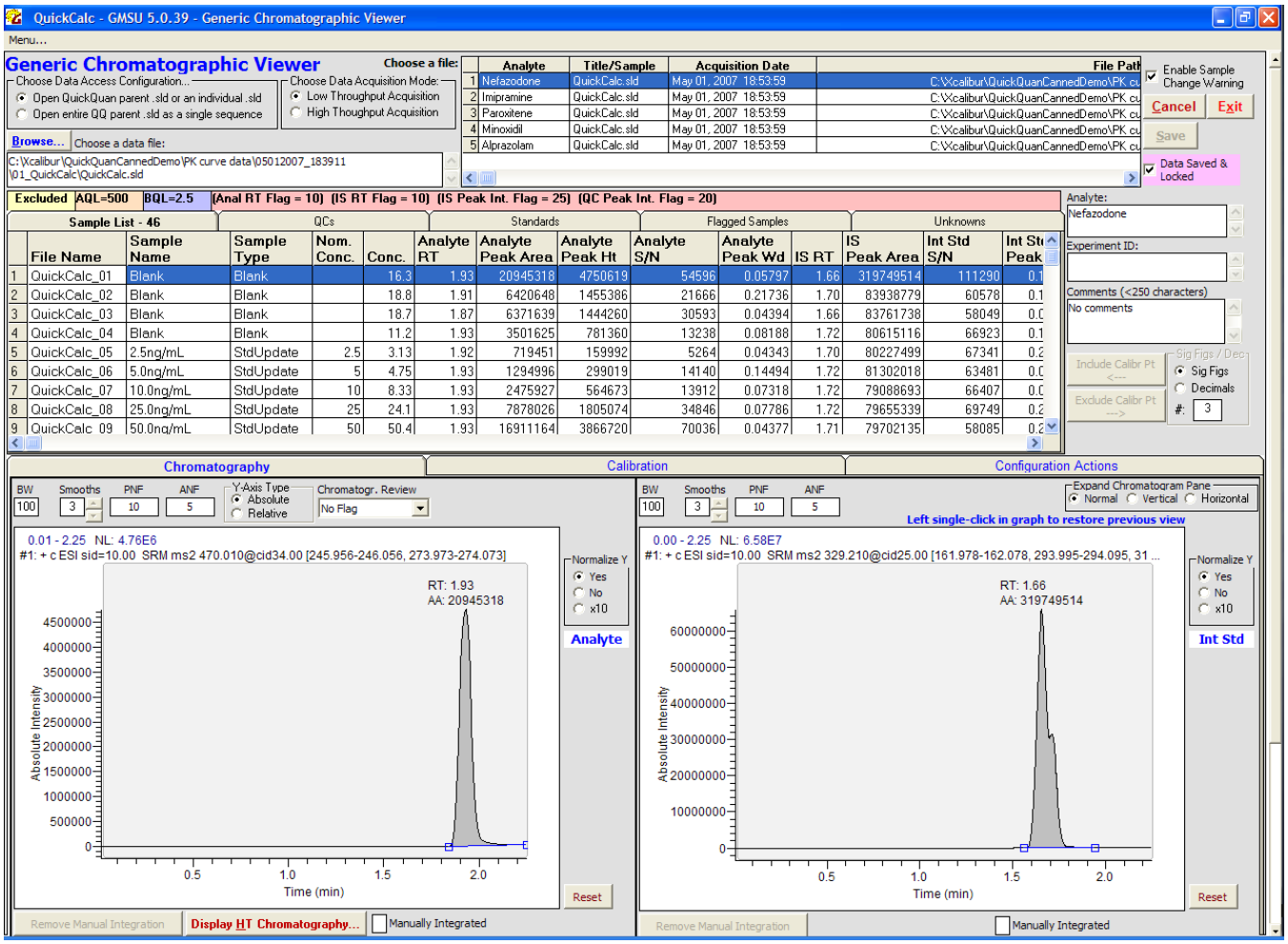


Figure 55 QuickQuan data – Open…a QuickQuan parent .sld

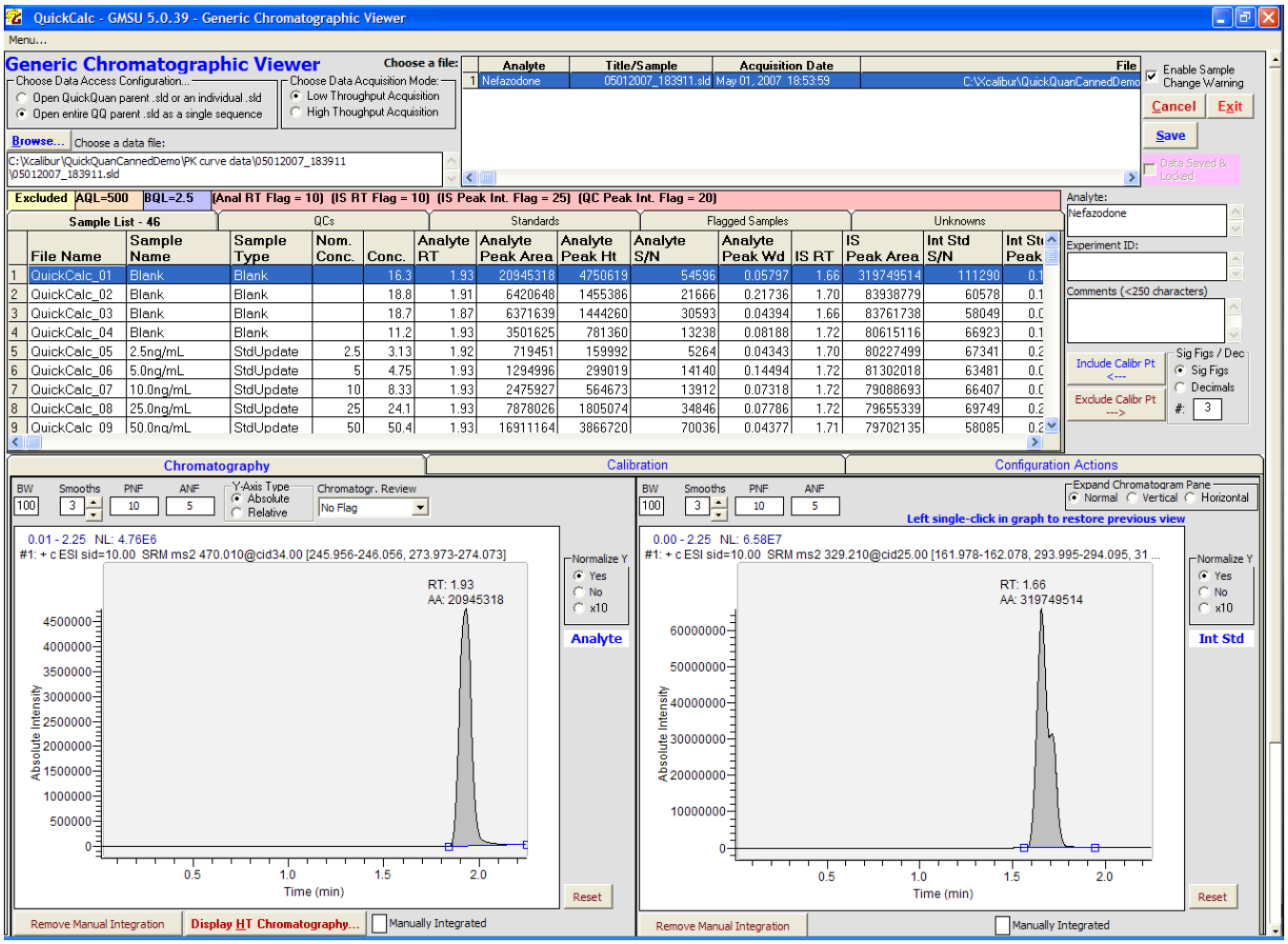


Figure 56 QuickQuan data – Open all data files within a directory

GCV doesn't calculate any pharmacokinetic parameters itself – it's only purpose is to review data. All GCV functionality is described in Sections 9 - 10.

27.1 Generic Chromatographic Viewer Reports

The functions in the GCV Report window are straightforward and not all features will be described. Figure 57 shows the Reports window. Figure 58 shows an example of a GCV Report.

Please note that the report content itself is highly configurable. GCV Reports support post-report macros in Excel. Users must create a blank Excel file that has embedded a macro named "DoGMSU". If this Excel file is configured in Configuration Utility – Generic Viewer – Excel Template File, then GCV Report will use this file as to generate the report, then execute the macro upon completion. In this manner, users can do such things as modify the format and/or content of the report, or send report information to an internal LIMS system.

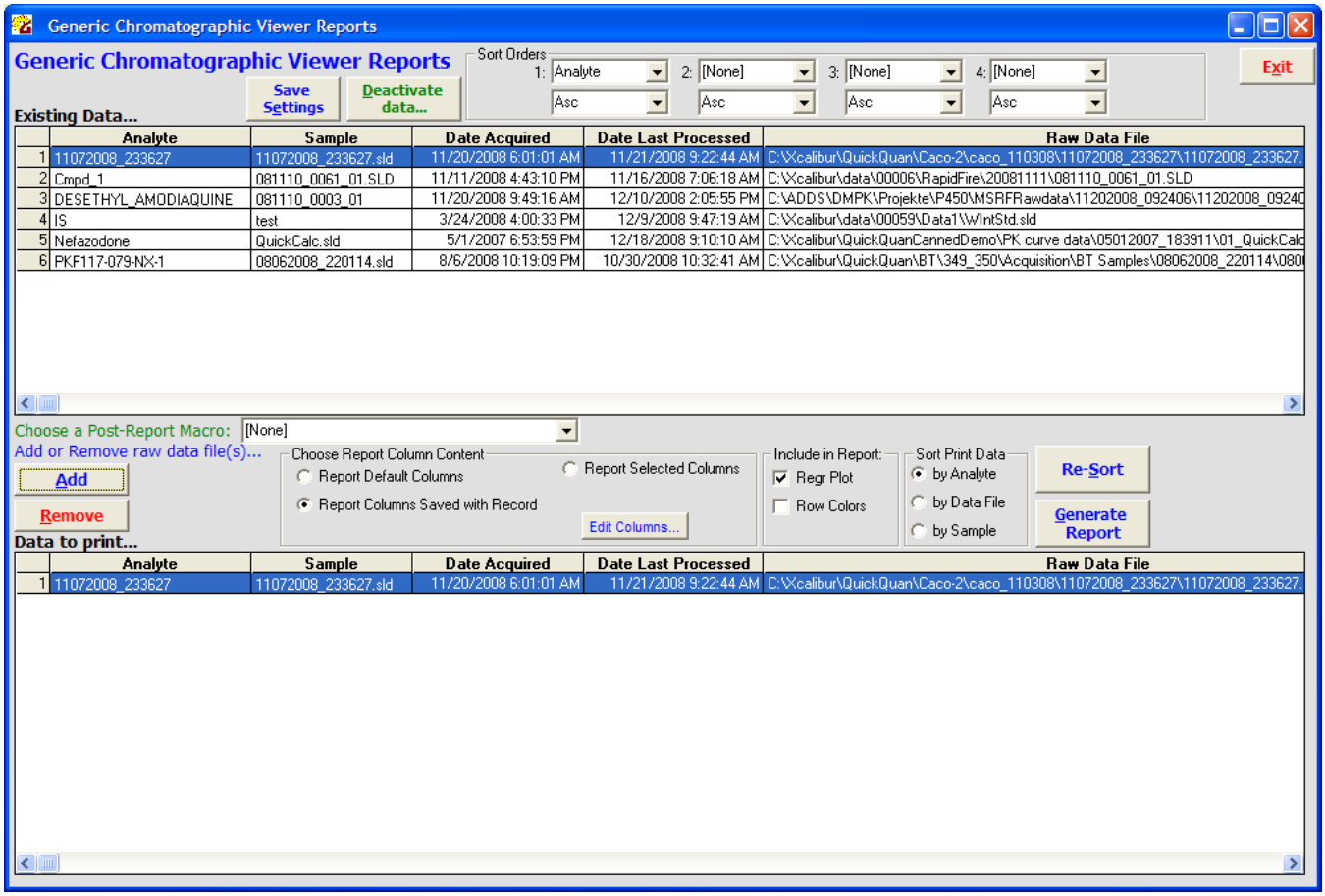


Figure 57 Generic Chromatographic Viewer Reports

27.1.1 Choose Report Column Content

27.1.1.1 Report Default Columns

This option will have the report sample list columns be those configured in the Default Column Set Name (see Section 10).

27.1.1.2 Report Columns Saved with Record

This option will have the report sample list columns be those configured in the Column Set Name saved with the record.

27.1.1.3 Report Selected Columns

This option will allow the user to choose a different Column Set Name with which to build the report. Users may also click on the Edit Columns button to bring up the Design Sample Table window to edit Column Name Set column content.

27.1.2 Include in Report

The following items may be selected to be included in the report

27.1.2.1 Regr Plot

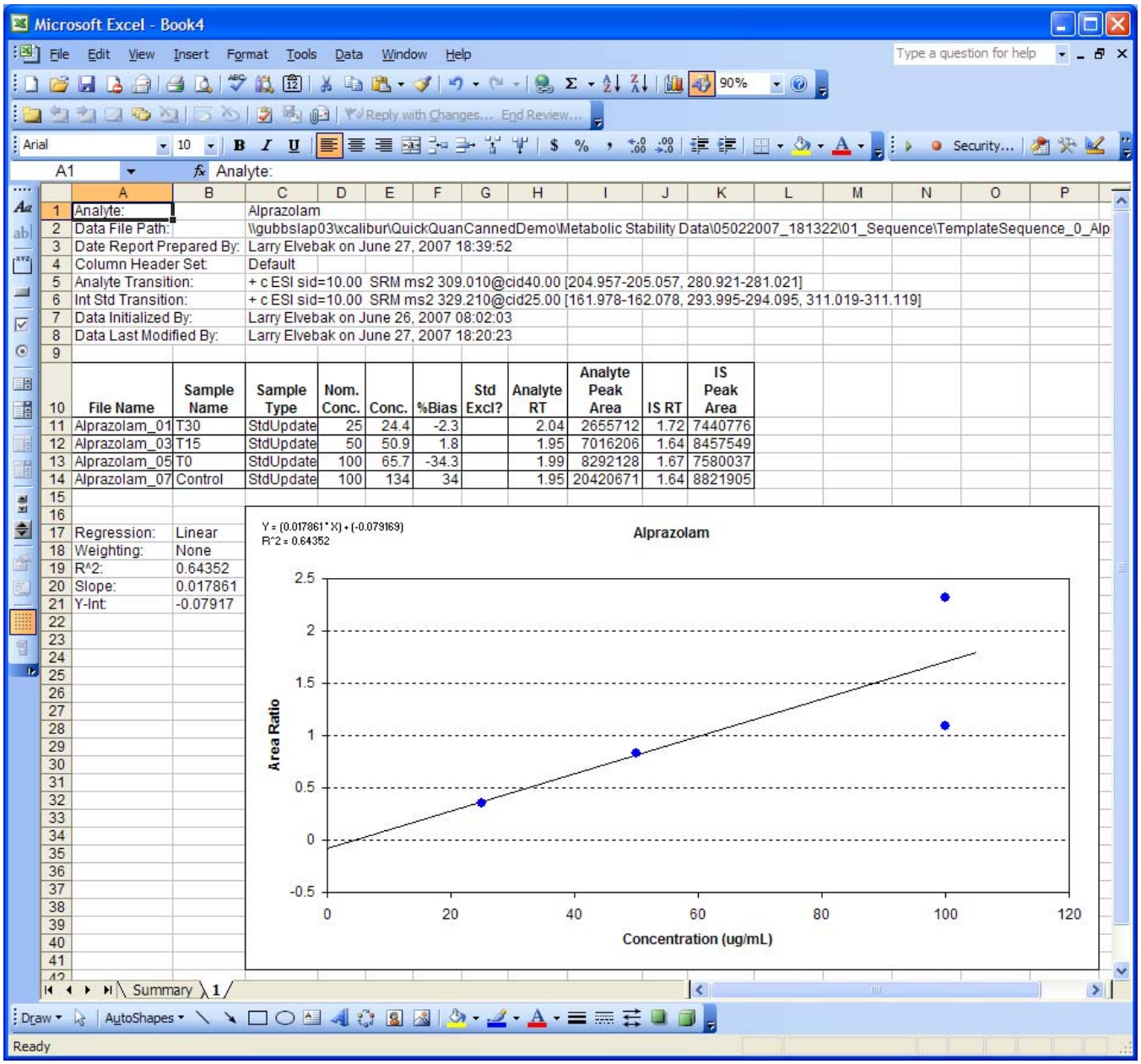
If a regression plot is included in the data, then it will be included in the report. Please note that, in order for the regression plot to be included, the Column Set Name must include the following columns:

- Standard Nominal Concentration
- Sample Concentration
- Standard Excluded?
- Sample Type

In addition, the dataset must have a slope.

27.1.2.2 Row Colors

If there have been any Flags configured in the report, this selection will color the flagged rows.



	A	B	C	D	E	F	G	H	I	J	K
1	Analyte:		Alprazolam								
2	Data File Path:		\\gubbslap03\xcalibur\QuickQuanCannedDemo\Metabolic Stability Data\05022007_181322\01_Sequence\TemplateSequence_0_Alp								
3	Date Report Prepared By:		Larry Elvebak on June 27, 2007 18:39:52								
4	Column Header Set:		Default								
5	Analyte Transition:		+ c ESI sid=10.00 SRM ms2 309.010@cid40.00 [204.957-205.057, 280.921-281.021]								
6	Int Std Transition:		+ c ESI sid=10.00 SRM ms2 329.210@cid25.00 [161.978-162.078, 293.995-294.095, 311.019-311.119]								
7	Data Initialized By:		Larry Elvebak on June 26, 2007 08:02:03								
8	Data Last Modified By:		Larry Elvebak on June 27, 2007 18:20:23								
9											
10	File Name	Sample Name	Sample Type	Nom. Conc.	Conc.	%Bias	Std Excl?	Analyte RT	Analyte Peak Area	IS RT	IS Peak Area
11	Alprazolam_01	T30	StdUpdate	25	24.4	-2.3		2.04	2655712	1.72	7440776
12	Alprazolam_03	T15	StdUpdate	50	50.9	1.8		1.95	7016206	1.64	8457549
13	Alprazolam_05	T0	StdUpdate	100	65.7	-34.3		1.99	8292128	1.67	7580037
14	Alprazolam_07	Control	StdUpdate	100	134	34		1.95	20420671	1.64	8821905
15											
16											
17	Regression:	Linear									
18	Weighting:	None									
19	R^2:	0.64352									
20	Slope:	0.017861									
21	Y-Int:	-0.07917									

Figure 58 A Generic Chromatographic Viewer Report

28 PK Calculator

The Chromatography, Calibration, and Configuration Actions tabs are exactly the same as the Generic Chromatographic Viewer (GVC). The user is referred to the GVC section (Section 27) and the section on Conserved Features (Section 9) for a description on the use of these tabs and features.

The PK Calculator is used to generate the following PK parameters:

- Half life (t ½)
- Concentration Maximum (Cmax)
- Area Under the Curve (AUC)
- Clearance

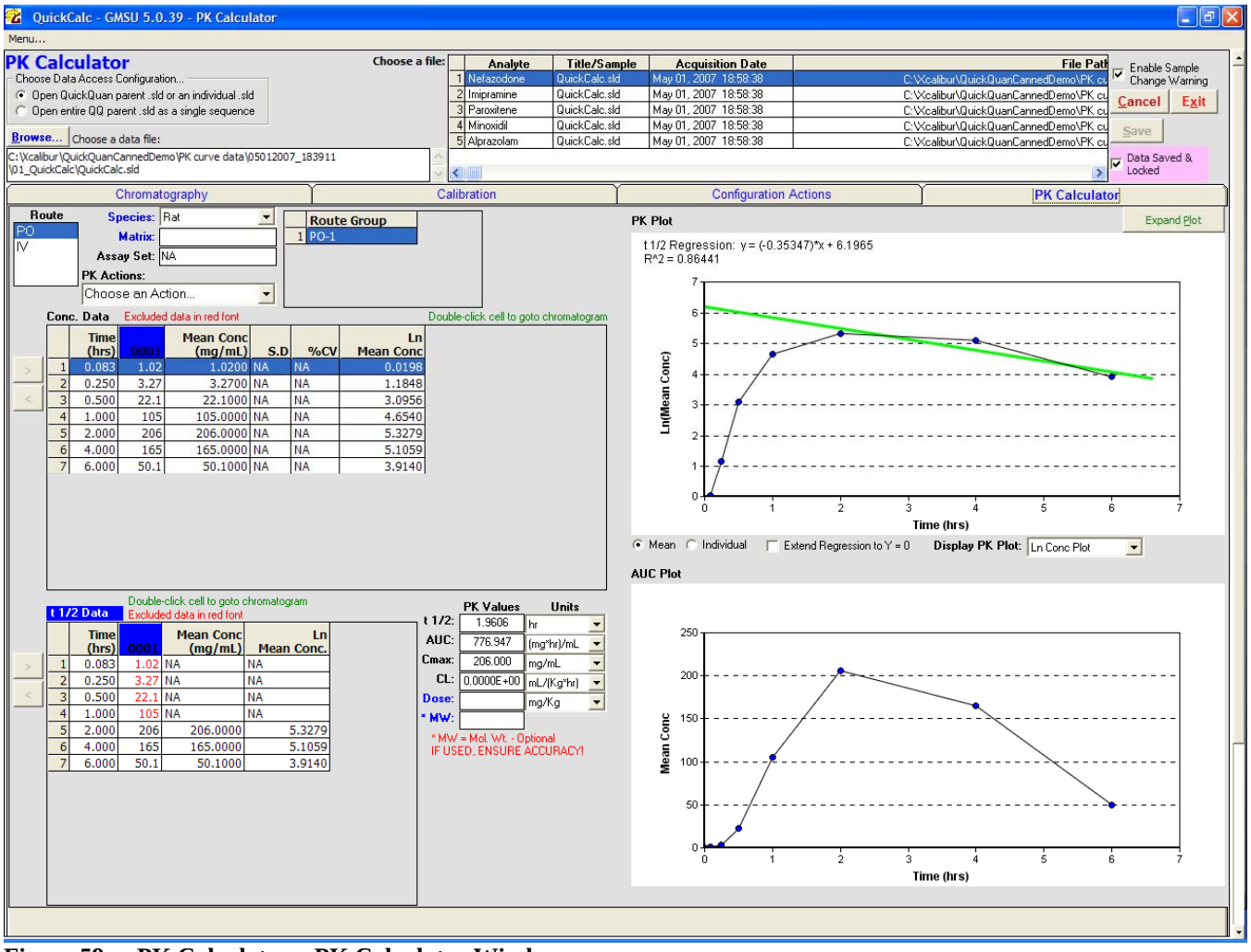


Figure 59 PK Calculator – PK Calculator Window

The Chromatography, Calibration, and Configuration Actions tabs are identical to that of the Generic Chromatographic Viewer. The functions of all but the PK Calculator tab are described in Sections 9 - 10.

PK data are automatically generated when data is loaded if the assay is identical to the Assay Set Name configured in Configuration Utility – PK Calculator – Default Assay Set Name. If the configured Assay Set Name is blank or if the loaded dataset sample list doesn't have the same number of samples as the configured Assay Set Name, then the user must manually configure the PK window according to the procedures following in this section.

28.1 Description of functions

28.1.1 Species dropdown box

Users may assign Species with this box. The content of the Species dropdown box is configured in Configuration Utility – PK Calculator – Species.

28.1.2 Matrix

Users may wish to enter a matrix used in the experiment.

28.1.3 PK Actions

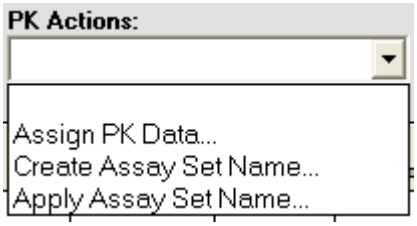


Figure 60 PK Actions dropdown box

28.1.3.1 Assign PK Data

If the PK Calculator tab data are blank, users must execute 'Assign PK Data.' See Section 28.2 for further details.

28.1.3.2 Create Assay Set Name

If a user wishes to create a new Assay Name Set based on the current PK data, then the user would execute 'Create Assay Set Name.' See Section 28.4.3 for further discussion. Please note that this feature can also be activated through the Assign PK Data window.

28.1.3.3 Apply Assay Set Name

If a user wishes to apply and existing Assay Set Name, then the user would execute 'Apply Assay Set Name'. See Section 28.4.3 for further details. Please note that this feature can also be activated through the Assign PK Data window.

28.1.4 Excluding/Including concentration data points

The Conc. Data table includes concentration values used in the generation of PK parameters.

To exclude data points from the data set, choose one or more concentration values within the 'Conc. Data' table and click on the Exclude (<) button. The font color of excluded data points is changed to red.

To include data points that have been excluded, choose one or more excluded data points and click the Include (>) button. The font color of included data points is changed to black.

28.1.5 Excluding/Including t ½ data points

The 't ½ Data' table includes concentration values used in the generation of the slope used to determine t ½.

To exclude data points from the data set, choose one or more concentration values within the 't ½ Data' table and click on the Exclude (<) button. The font color of excluded data points is changed to red.

To include data points that have been excluded, choose one or more excluded data points and click the Include (>) button. The font color of included data points is changed to black.

28.1.6 PK value units

Calculated PK values are dependent on the desired units of the underlying values. It is not uncommon that the units of an underlying variable used in the equation may be different than the units wished to be displayed for the value. For example, calibration curve units (shown in the Calibration tab) may be displayed as nMol/L, while it may be desired to display AUC as (mg*hr)/mL. GMSU/QC will automatically convert units of underlying variables to the units selected in the respective Units dropdown box.

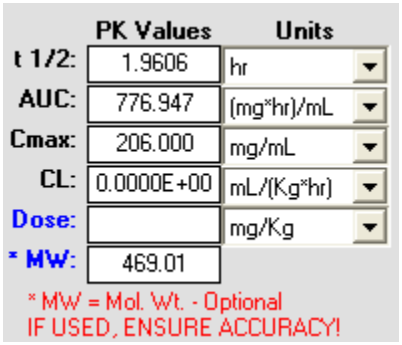


Figure 61 PK Value Units

28.1.7 Clearance

In order for Clearance to be calculate, Dose must be entered manually by the user. The calculation used by GMUS for Clearance is:

$$CL = Dose/AUC$$

28.2 Automatically Assign PK Data

If the Sequence Sample ID conforms to the following naming syntax, then PK information is automatically assigned to PK samples and PK plots are automatically generated.

The GMSU/QC convention is as follows:

PK Information	Convention
Species	s_[species]_s
Animal #	a_[animal #]_a
Route	r_[Route]_r
Route Group	g_[Group]_g
Time Point	h_[time point]_h
Matrix	m_[matrix]_m

For example, the following sample name:

s_Rat_sa_0001_ar_PO_rg_1_gh_0.083_hm_Plasma_m

would result in the following PK information:

PK Information	Results
Species	Rat
Animal #	0001
Route	PO
Route Group	1
Time Point	0.083
Matrix	Plasma

Notes:

- Time Point must be in hours
- Route values are restricted to PO, IV, SC, and IP
- The order in which the parameters is listed in the sample name is not important
- In order for GMSU/QC to automatically generate PK plots, the minimum parameters contained in the Sample Name are:
  - Animal#
  - Route
  - Route Group
  - Time Point

28.3 Assign PK Data

The Assign PK Data window is used to assign PK information to sample list samples. Please see Section 28.4 for a description of automated features designed to make more efficient this process.

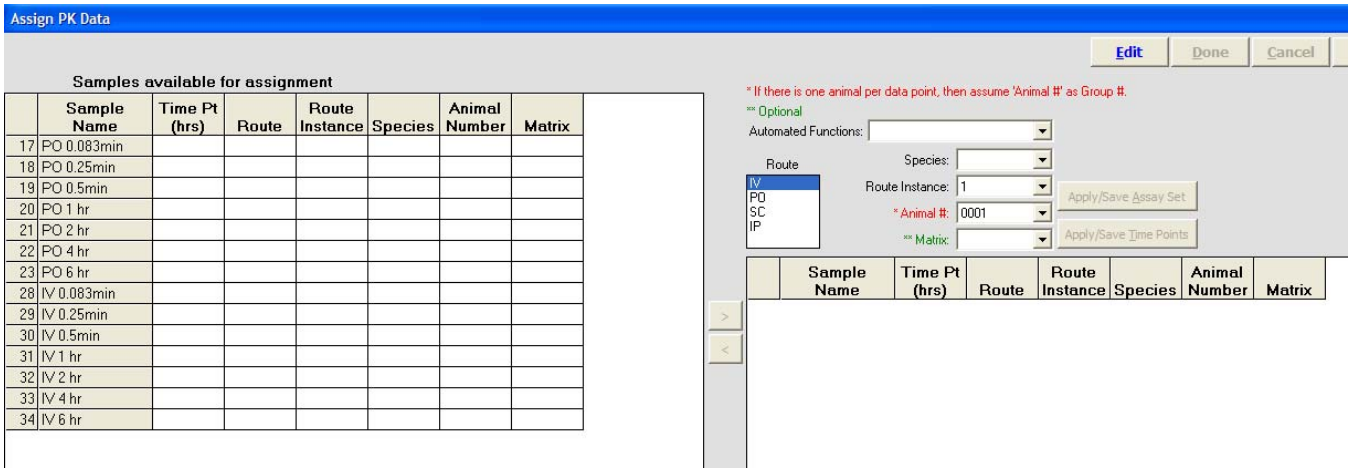


Figure 62 Assign PK Data – Initial View

To assign PK information to samples, the user performs the following steps:

- Click on the Edit button
- Select the appropriate Route item in the Route index list
- Make all the desired selections in the dropdown boxes (Species, Route Instance, Animal #, Matrix (optional))
- Choose the samples in the 'Samples available for assignment' table that this information is to be assigned (see Figure 63)
- Click the Apply (>) button (see Figure 64)
- Enter by hand the time points for the samples in the right hand table. See Section 28.4 for alternative methods for entering time points.
- Repeat bullets 2 – 6 for additional samples
- When complete, click the Done button
- If the user wishes to discard all actions, click on the Cancel button
- If the user wishes to save any of these settings as a Time Point Set or an Assay Set, see Section 28.4.
- When completely finished, click on Exit and the PK information will be applied to the data set in the PK Calculator window.

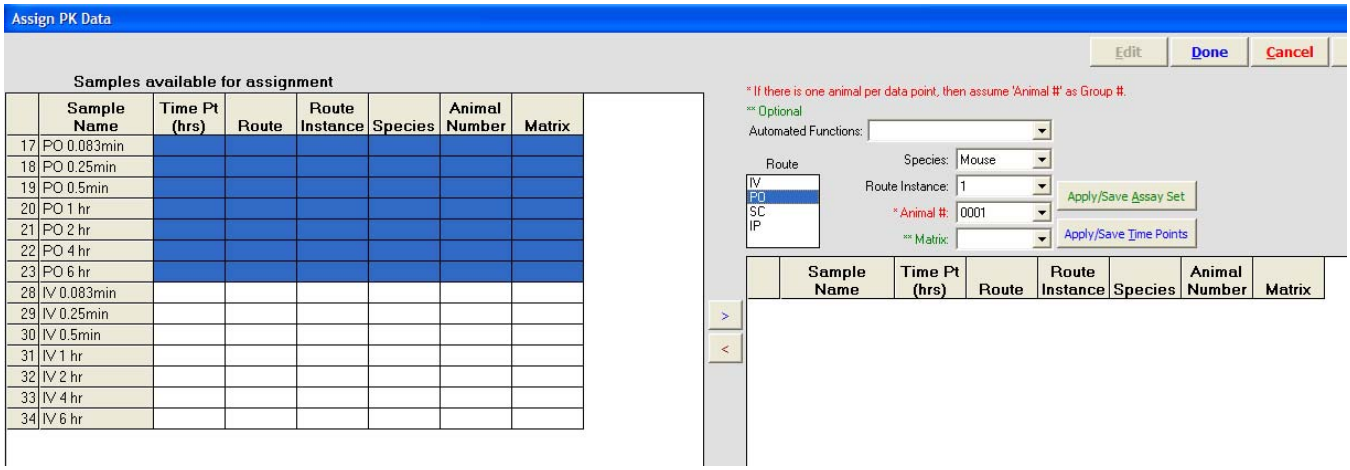


Figure 63 Assign PK Data - Select Samples

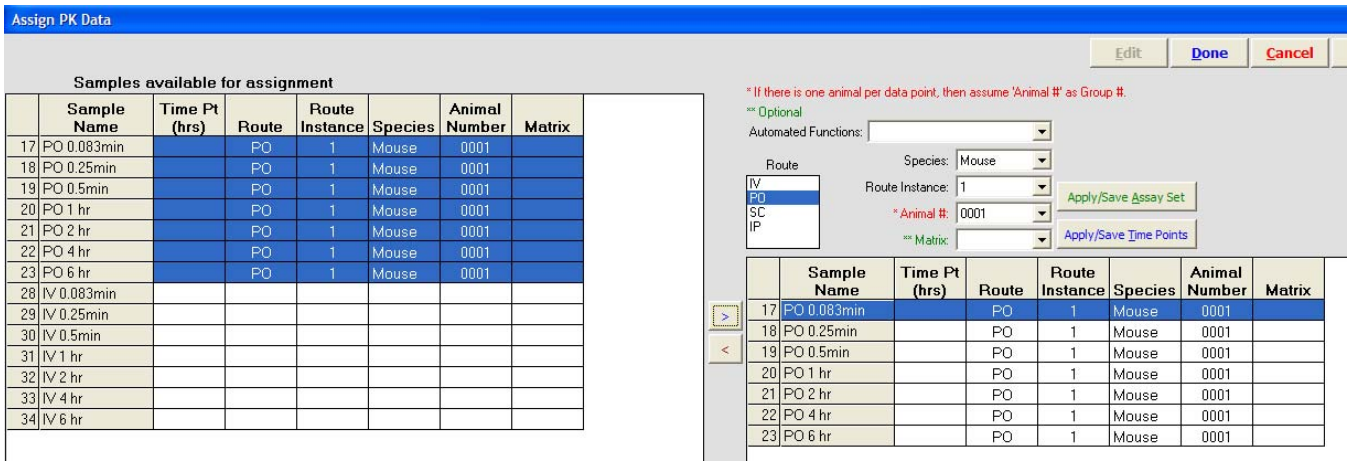


Figure 64 Assign PK Data - Apply Samples

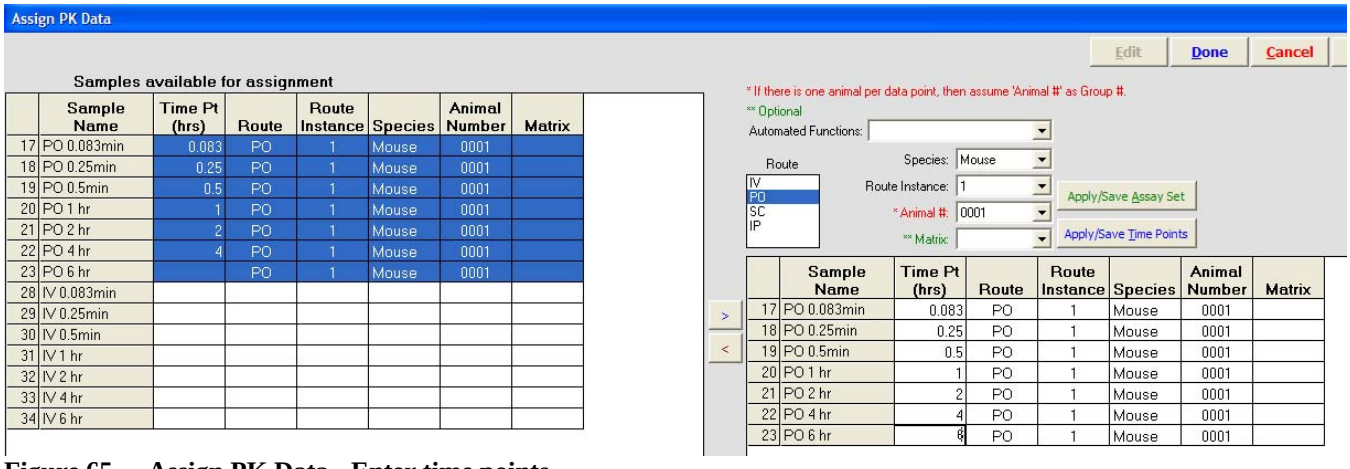


Figure 65 Assign PK Data - Enter time points

28.4 Additional Features

The Assign PK Data window contains two features designed to automate the process of assigning PK data

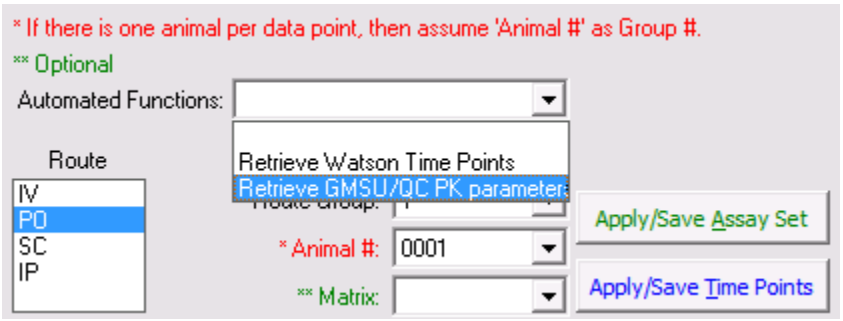


Figure 66 PK Calculator – PK tab – Additional Features

28.4.1 Automated Functions

The 'Automated Functions' feature contains functions that will retrieve PK information from the Sample Name.

28.4.1.1 Retrieve Watson Time Points

If the Sample Name originates from ThermoO® Watson®, then the time point may be pulled out automatically. Choosing this option will automatically populate the Time Point column of the Samples Available for Assignment table (see Figure 65).

28.4.1.2 Retrieve GMSU/QC PK parameters

If the Sample Name conforms to the GMSU/QC convention, PK information will be retrieved from it and placed in the appropriate table column. The GMSU/QC convention is as follows:

PK Information	Convention
Species	s_[species]_s
Animal #	a_[animal #]_a
Route	r_[Route]_r
Route Group	g_[Group]_g
Time Point	h_[time point]_h
Matrix	m_[matrix]_m

For example, the following sample name:

s_Rat_sa_0001_ar_PO_rg_1_gh_0.083_hm_Plasma_m

would result in the following PK information:

PK Information	Results
Species	Rat
Animal #	0001
Route	PO
Route Group	1
Time Point	0.083
Matrix	Plasma

Notes:

- Time Points must be in hours
- Route values are restricted to PO, IV, SC, and IP
- The order in which the parameters is listed in the sample name is not important
- In order for GMSU/QC to automatically generate PK plots, the minimum parameters contained in the Sample Name are:
  - Animal#
  - Route
  - Route Group
  - Time Point

28.4.2 Apply/Save Time Points

It is understood that entering Time Point values for each Route set is repetitive. To enhance the efficiency of the process, users may configure a new Time Point Set or apply an existing Time Point Set by clicking the Apply/Save Time Points button.

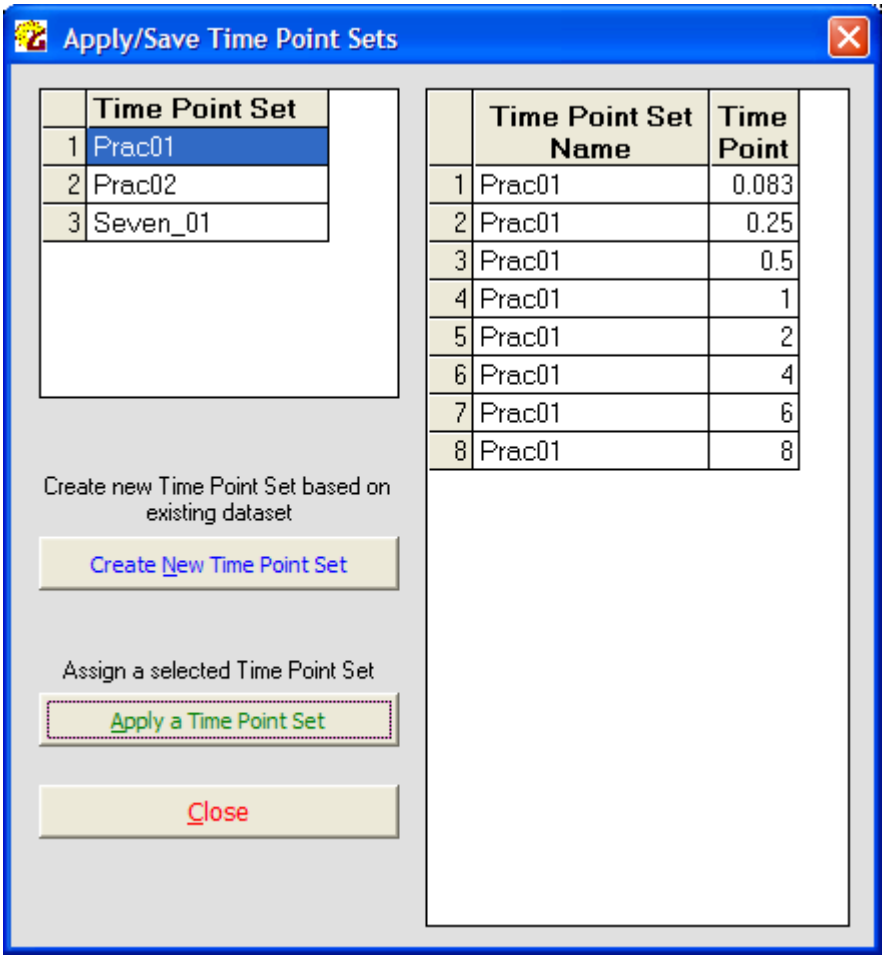


Figure 67 Apply/Save Time Points

Users may create a new time point set by clicking the 'Create New Time Point Set' button. Users will be prompted to enter a Time Point Set name. A new time point set will be created based on the time points in the underlying data set.

Users may apply a time point set by choosing an existing time point set and clicking the 'Apply a Time Point Set' button.

28.4.3 Apply/Save Assay Set

Once a PK data set has been fully assigned, users may save this configuration by clicking the 'Apply/Save Assay Set' button. Once saved, the assay set may be applied to future data sets that have identical sequence structures.

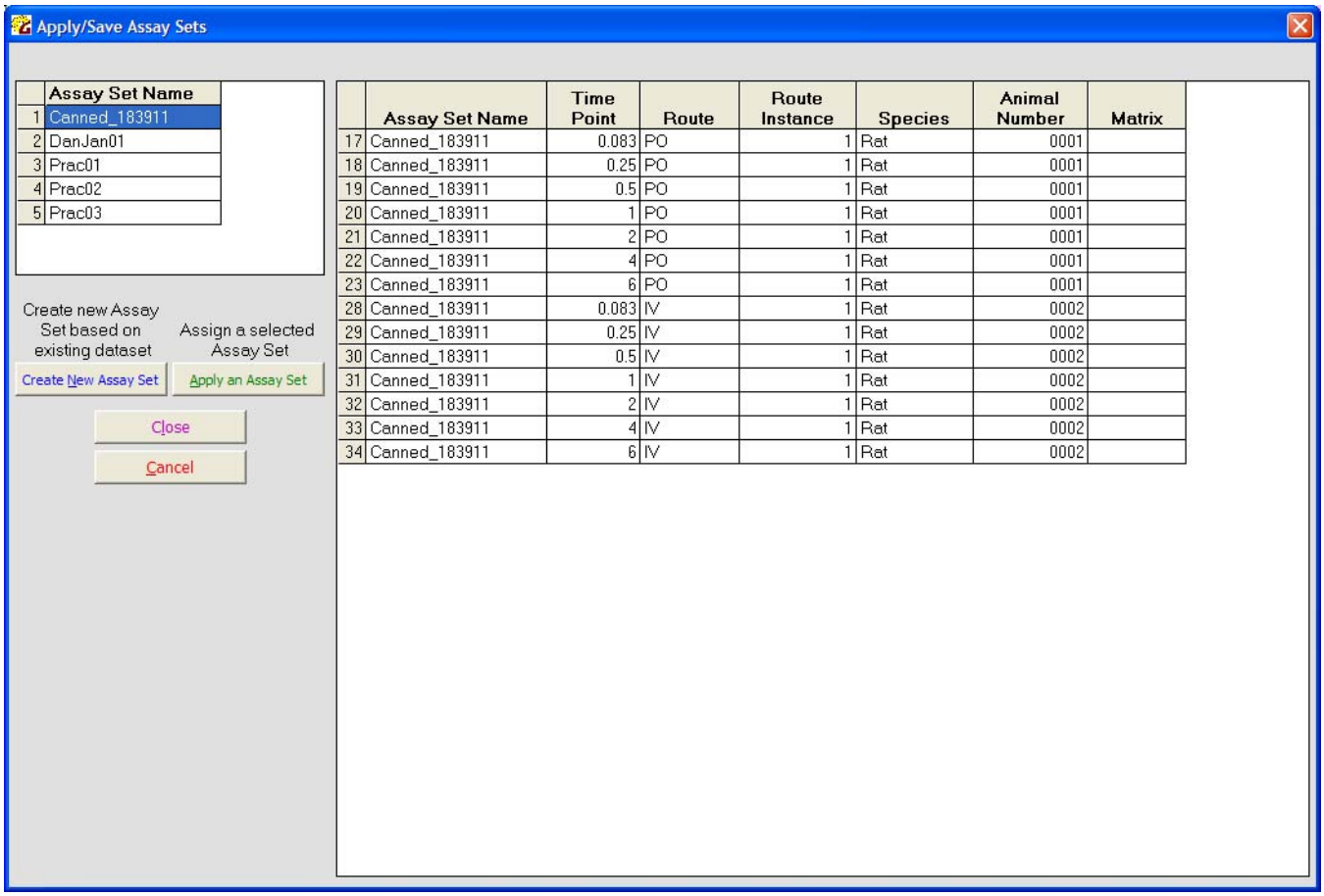


Figure 68 Apply/Save Assay Sets

Users may create a new assay set by clicking the 'Create New Assay Set' button. Users will be prompted to enter an assay set name. A new assay set will be created based on the PK information in the underlying data set.

Users may apply an assay set by choosing an existing assay set and clicking the 'Apply an Assay Set' button.

29 PK Calculator Reports

The functions in the PK Calculator Report window are straightforward and not all features will be described. In addition, the user is referred to Section 10 for a discussion of features conserved between all module Report windows. Figure 69 shows the Reports window. Figure 70 and Figure 71 show examples of a PK Calculator Report.

Please note that the report content itself is highly configurable. PK Calculator Reports support post-report macros in Excel. Users may create a blank Excel file that has embedded a macro named "DoGMSU". If this Excel file is configured in Configuration Utility – PK Calculator – Excel Template File, then PK Calculator Report will use this file as to generate the report, then execute the macro upon completion. In

this manner, users can do such things as modify the format and/or content of the report, or send report information to an internal LIMS system.

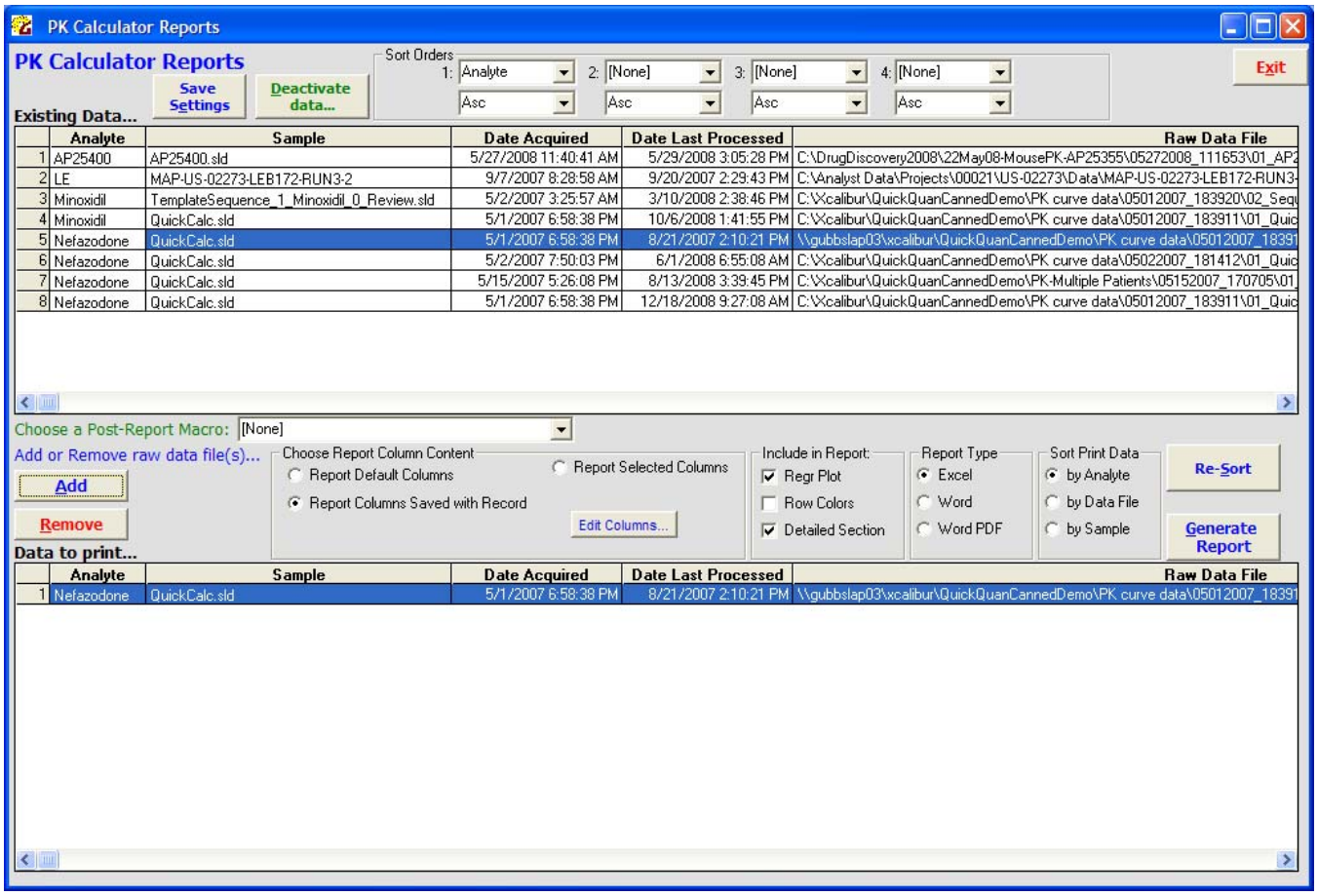


Figure 69 PK Calculator Reports

29.1 Normal Report

A Normal report (see Figure 70) contains a single page containing the following fields (plus Units):

- Route
- Species
- Matrix
- t ½
- AUC
- Cmax
- Dose
- Clearance

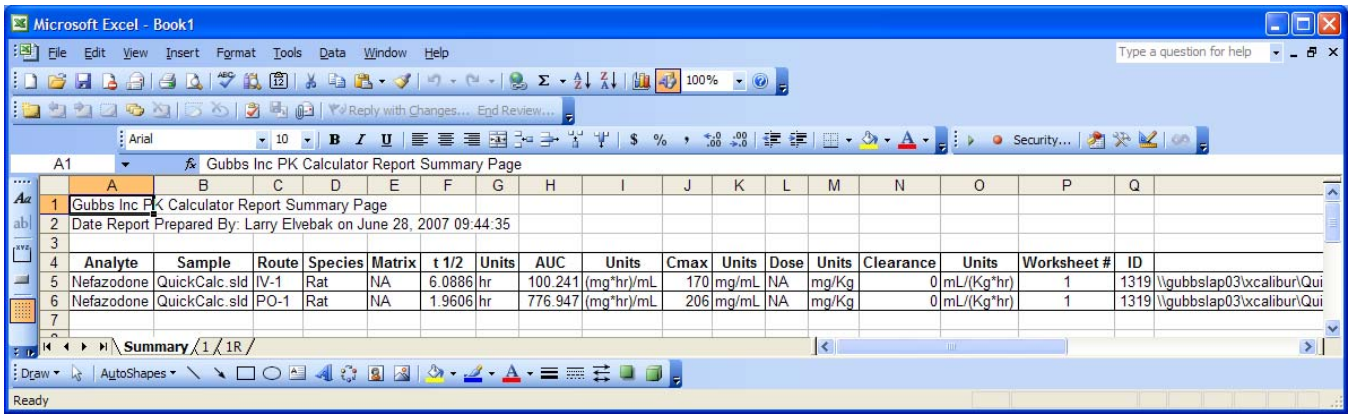


Figure 70 PK Calculator Report – Report Page

29.2 Detailed Report

A Detailed Section Report will contain the normal report page, plus two extra worksheets for each Analyte set in the print queue.
The first detailed page contains the sample list as well as the calibration curve plot (see Figure 71).
The second detailed page contains all the information used to generate PK values (see Figure 72).

29.3 Additional Features

29.3.1 Choose Report Column Content

29.3.1.1 Report Default Columns

This option will have the report sample list columns be those configured in the Default Column Set Name (see Section 10).

29.3.1.2 Report Columns Saved with Record

This option will have the report sample list columns be those configured in the Column Set Name saved with the record.

29.3.1.3 Report Selected Columns

This option will allow the user to choose a different Column Set Name with which to build the report. Users may also click on the Edit Columns button to bring up the Design Sample Table window to edit Column Name Set column content.

29.3.2 Include in Report

The following items may be selected to be included in the report

29.3.2.1 Regr Plot

If a regression plot is included in the data, then it will be included in the report. Please note that, in order for the regression plot to be included, the Column Set Name must include the following columns:

- Standard Nominal Concentration
- Standard Excluded?

- Sample Concentration
- Sample Type

In addition, the dataset must have a slope.

29.3.2.2 Row Colors

If there have been any Flags configured in the report, this selection will color the flagged rows.

29.3.2.3 Detailed Section

See Section 29.2.

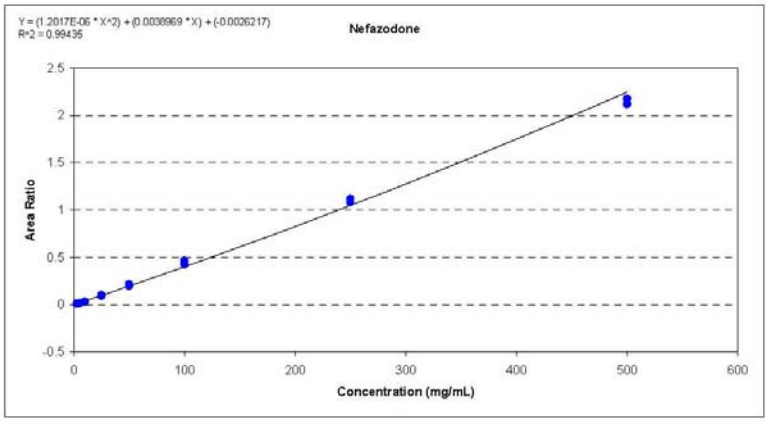
29.3.3 Report Type

Reports may be generated as Excel workbook files, Word documents, or PDF

Analyte: Nefazodone
Data File Path: \\gubbslap03\xcalibur\QuickQuanCannedDemo\PK curve data\05012007_183911\01_QuickCalc\QuickCalc.sld
Date Report Prepared By: Larry Elvebak on June 28, 2007 09:44:35
Column Header Set: Prac01
Analyte Transition: + c ESI sid=10.00 SRM ms2 470.010@cid34.00 [245.956-246.056, 273.973-274.073]
Int Std Transition: + c ESI sid=10.00 SRM ms2 329.210@cid25.00 [161.978-162.078, 293.995-294.095, 311.019-311.119]
Data Initialized By: Larry Elvebak on June 26, 2007 07:50:52
Data Last Modified By: Larry Elvebak on June 28, 2007 07:58:27

Sample Name	File Name	Sample Type	Nom. Conc.	Conc.	%Bias	Analyte RT	Analyte Peak Area	IS RT	IS Peak Area	Std Excl?	Area Ratio	Dose Form	DF	Instance	Species	Animal#	Time Point	HL1?	t 1/2
Blank	QuickCalc_01	Blank		17.6		1.93	20945318	1.66	315857929		0.06631247		0					FALSE	
Blank	QuickCalc_02	Blank		20.3		1.91	6419944	1.7	83395653		0.07698176		0					FALSE	
Blank	QuickCalc_03	Blank		20.3		1.87	6371639	1.66	82843803		0.07691147		0					FALSE	
Blank	QuickCalc_04	Blank		11.9		1.93	3501626	1.72	79972536		0.04378536		0					FALSE	
2.5ng/mL	QuickCalc_05	StdUpdate	2.5	3	19.8	1.92	719451	1.7	79393388		0.00906185		0					FALSE	
5.0ng/mL	QuickCalc_06	StdUpdate	5	4.79	-4.2	1.93	1294996	1.72	80533121		0.01608029		0					FALSE	
10.0ng/mL	QuickCalc_07	StdUpdate	10	8.76	-12.4	1.93	2475613	1.72	78339048		0.03160126		0					FALSE	
25.0ng/mL	QuickCalc_08	StdUpdate	25	26.1	4.3	1.93	7878026	1.72	78917873		0.09982563		0					FALSE	
50.0ng/mL	QuickCalc_09	StdUpdate	50	54.7	9.3	1.93	16911164	1.71	79029293		0.213986		0					FALSE	
100.0ng/mL	QuickCalc_10	StdUpdate	100	116	15.9	1.92	35320868	1.71	75907641		0.4653137		0					FALSE	
250.0ng/mL	QuickCalc_11	StdUpdate	250	266	6.2	1.92	91892840	1.71	82270264		1.116963		0					FALSE	
500.0ng/mL	QuickCalc_12	StdUpdate	500	486	-2.8	1.92	187654669	1.71	86308031		2.174243		0					FALSE	
Blank	QuickCalc_13	Blank		26.5		1.92	3913101	1.71	38549340		0.1015089		0					FALSE	
Blank	QuickCalc_14	Blank		15.7		1.89	2269147	1.68	38634296		0.05873401		0					FALSE	
Blank	QuickCalc_15	Blank		13.9		1.88	2081086	1.67	40149375		0.05183358		0					FALSE	
Blank	QuickCalc_16	Blank		13.5		1.93	2037515	1.72	40598237		0.05018728		0					FALSE	
PO 0.083min	QuickCalc_17	Unknown		1.02		1.89	108913	1.69	80208934		0.00135787	PO	1		Rat	1	0.083	FALSE	1.9606
PO 0.25min	QuickCalc_18	Unknown		3.27		1.92	1011737	1.72	99993835		0.01011799	PO	1		Rat	1	0.25	FALSE	1.9606
PO 0.5min	QuickCalc_19	Unknown		22.1		1.91	6889352	1.7	82005714		0.08401063	PO	1		Rat	1	0.5	FALSE	1.9606
PO 1 hr	QuickCalc_20	Unknown		105		1.88	35112363	1.67	83745545		0.4192744	PO	1		Rat	1	1	FALSE	1.9606
PO 2 hr	QuickCalc_21	Unknown		206		1.91	82741417	1.7	97262414		0.8507029	PO	1		Rat	1	2	TRUE	1.9606
PO 4 hr	QuickCalc_22	Unknown		165		1.91	74321161	1.7	110139805		0.6747893	PO	1		Rat	1	4	TRUE	1.9606
PO 6 hr	QuickCalc_23	Unknown		50.1		1.92	16299835	1.72	83272671		0.1957405	PO	1		Rat	1	6	TRUE	1.9606
Blank	QuickCalc_24	Blank		18.7		1.93	3244339	1.72	45930513		0.07063581		0					FALSE	
Blank	QuickCalc_25	Blank		14		1.92	2448003	1.71	46994886		0.05209084		0					FALSE	
Blank	QuickCalc_26	Blank		13.2		1.92	2300332	1.71	46752217		0.04920263		0					FALSE	
Blank	QuickCalc_27	Blank		11.9		1.9	2106326	1.69	48052539		0.04383381		0					FALSE	
IV 0.083min	QuickCalc_28	Unknown		170		1.93	76694949	1.72	110714108		0.6927297	IV	1		Rat	2	0.083	FALSE	6.0886
IV 0.25min	QuickCalc_29	Unknown		43.6		1.91	23542067	1.7	138725698		0.1697023	IV	1		Rat	2	0.25	FALSE	6.0886
IV 0.5min	QuickCalc_30	Unknown		36.4		1.91	15121064	1.7	107272398		0.1409595	IV	1		Rat	2	0.5	FALSE	6.0886
IV 1 hr	QuickCalc_31	Unknown		20.5		1.93	7448954	1.72	95571865		0.07794087	IV	1		Rat	2	1	FALSE	6.0886
IV 2 hr	QuickCalc_32	Unknown		8.64		1.92	2603544	1.71	83637935		0.03112875	IV	1		Rat	2	2	TRUE	6.0886
IV 4 hr	QuickCalc_33	Unknown		7.69		1.91	2748910	1.7	100219492		0.0274289	IV	1		Rat	2	4	TRUE	6.0886
IV 6 hr	QuickCalc_34	Unknown		5.48		1.88	2086510	1.67	111156231		0.01877097	IV	1		Rat	2	6	TRUE	6.0886
Blank	QuickCalc_35	Blank		12.1		1.93	2296952	1.72	51431115		0.04466075		0					FALSE	
Blank	QuickCalc_36	Blank		11.7		1.93	2198717	1.72	51190231		0.04295189		0					FALSE	
Blank	QuickCalc_37	Blank		11.5		1.91	2110414	1.7	49798071		0.04237943		0					FALSE	
Blank	QuickCalc_38	Blank		11.3		1.87	2123564	1.66	50917016		0.04170637		0					FALSE	
2.5ng/mL	QuickCalc_39	StdUpdate	2.5	2.42	-3.3	1.91	580048	1.71	85262266		0.0068031		0					FALSE	
5.0ng/mL	QuickCalc_40	StdUpdate	5	4.21	-15.8	1.93	1164765	1.72	84349481		0.0138088		0					FALSE	
10.0ng/mL	QuickCalc_41	StdUpdate	10	8.17	-18.3	1.89	2537070	1.69	86633767		0.029285		0					FALSE	
25.0ng/mL	QuickCalc_42	StdUpdate	25	24.1	-3.5	1.87	7898317	1.67	85723701		0.09213691		0					FALSE	
50.0ng/mL	QuickCalc_43	StdUpdate	50	50	0.1	1.9	16918591	1.7	86598771		0.1953676		0					FALSE	
100.0ng/mL	QuickCalc_44	StdUpdate	100	106	6.1	1.91	35064795	1.7	82642301		0.424296		0					FALSE	
250.0ng/mL	QuickCalc_45	StdUpdate	250	257	3	1.86	96939076	1.65	89738829		1.080236		0					FALSE	
500.0ng/mL	QuickCalc_46	StdUpdate	500	475	-5.1	1.87	196164175	1.66	92635747		2.117586		0					FALSE	

Regression: Quadratic
Weighting: 1/X^2
R^2: 0.99435
a: 1.2017E-06
b: 0.0038969
c: -0.0026217



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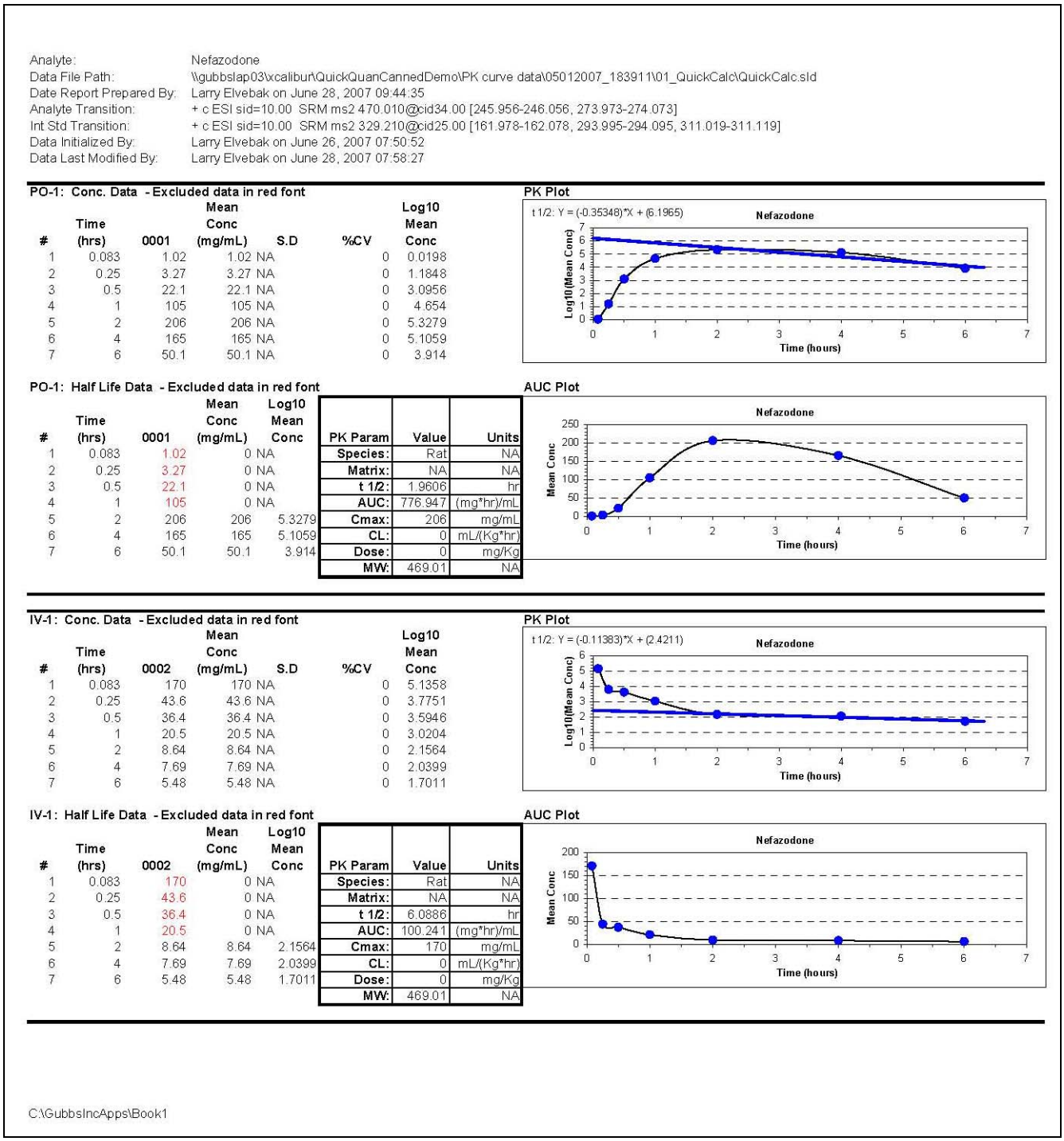
Page 1 of 1

Figure 71 PK Calculator Report – Detailed Section – Calibration Page



Analyte: Nefazodone
Data File Path: \\gubbslap03\xcalibur\QuickQuanCannedDemo\PK curve data\05012007_183911\01_QuickCalc\QuickCalc.sld
Date Report Prepared By: Larry Elvebak on June 28, 2007 09:44:35
Analyte Transition: + c ESI sid=10.00 SRM ms2 470.010@cid34.00 [245.956-246.056, 273.973-274.073]
Int Std Transition: + c ESI sid=10.00 SRM ms2 329.210@cid25.00 [161.978-162.078, 293.995-294.095, 311.019-311.119]
Data Initialized By: Larry Elvebak on June 26, 2007 07:50:52
Data Last Modified By: Larry Elvebak on June 28, 2007 07:58:27

PO-1: Conc. Data - Excluded data in red font

#	Time (hrs)	0001	Mean Conc (mg/mL)	S.D	%CV	Log10 Mean Conc
1	0.083	1.02	1.02	NA	0	0.0198
2	0.25	3.27	3.27	NA	0	1.1848
3	0.5	22.1	22.1	NA	0	3.0956
4	1	105	105	NA	0	4.654
5	2	206	206	NA	0	5.3279
6	4	165	165	NA	0	5.1059
7	6	50.1	50.1	NA	0	3.914

PK Plot

PO-1: Half Life Data - Excluded data in red font

#	Time (hrs)	0001	Mean Conc (mg/mL)	Log10 Mean Conc
1	0.083	1.02	0	NA
2	0.25	3.27	0	NA
3	0.5	22.1	0	NA
4	1	105	0	NA
5	2	206	206	5.3279
6	4	165	165	5.1059
7	6	50.1	50.1	3.914

PK Param	Value	Units
Species:	Rat	NA
Matrix:	NA	NA
t 1/2:	1.9606	hr
AUC:	776.947	(mg*hr)/mL
Cmax:	206	mg/mL
CL:	0	mL/(Kg*hr)
Dose:	0	mg/Kg
MW:	469.01	NA

AUC Plot

IV-1: Conc. Data - Excluded data in red font

#	Time (hrs)	0002	Mean Conc (mg/mL)	S.D	%CV	Log10 Mean Conc
1	0.083	170	170	NA	0	5.1358
2	0.25	43.6	43.6	NA	0	3.7751
3	0.5	36.4	36.4	NA	0	3.5946
4	1	20.5	20.5	NA	0	3.0204
5	2	8.64	8.64	NA	0	2.1564
6	4	7.69	7.69	NA	0	2.0399
7	6	5.48	5.48	NA	0	1.7011

PK Plot

IV-1: Half Life Data - Excluded data in red font

#	Time (hrs)	0002	Mean Conc (mg/mL)	Log10 Mean Conc
1	0.083	170	0	NA
2	0.25	43.6	0	NA
3	0.5	36.4	0	NA
4	1	20.5	0	NA
5	2	8.64	8.64	2.1564
6	4	7.69	7.69	2.0399
7	6	5.48	5.48	1.7011

PK Param	Value	Units
Species:	Rat	NA
Matrix:	NA	NA
t 1/2:	6.0886	hr
AUC:	100.241	(mg*hr)/mL
Cmax:	170	mg/mL
CL:	0	mL/(Kg*hr)
Dose:	0	mg/Kg
MW:	469.01	NA

AUC Plot

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Figure 72 PK Calculator Report – Detailed Section – PK Page

30 References

[1] R. O. Cole. Quantitation of Biofluids with High Speed HPLC/MS/MS: Early Discovery Applications. Invited Presentation, at "Early ADME and Toxicology in Drug Discovery: Techniques for accelerating and Optimizing Drug Candidate Selection", Berkley, CA, October 1998.

[2] Adam H. Brockman, Donna L. Hiller, and Roderic O. Cole. High Speed HPLC/MS/MS Analysis of Biological Fluids in Support of ADME Screens: A Practical Review. Current Opinion in Drug Discovery, 4(3), (2000), 432.

[3] Janiszewski JS, Rogers KJ, Whalen KM, Cole MJ, Liston TE, Duchoslav E, et al: A high capacity LC/MS system for the bioanalysis of samples generated from plate-based metabolic screening. Anal Chem 2001; 73:1495-1501.

[4] Z. Yan, J. Wu, L.E. Elvebak, A. Brockman: Validation of a Totally Commercially Available High Throughput -ADME System and Results for 60 Literature Compounds. Rapid Communications in Mass Spectrometry, Rapid Commun. Mass Spectrom. 2005; 19: 1191–1199.