



Gubbs Mass Spec Utilities 2.x.x

GMSU 2.0.34 and earlier

Supported Platforms

Sciex Analyst 1.3.1 - 1.4.1

Users Manual

Gubbs, Inc.
265 Blue Spruce Circle
Alpharetta, GA 30005
P: 770-573-0169
F: 508-453-1338

gubbs@gubbsinc.com
www.gubbsinc.com



Table of Contents

<u>Section</u>	<u>Page Number</u>
1 INTRODUCTION	5
2 HIGH THROUGHPUT (HT) VS LOW THROUGHPUT (LT) ACQUISITION.....	5
3 CONSOLE.....	6
4 ACQUISITION METHOD GENERATOR UTILITIES	7
5 PI METHOD GENERATOR UTILITY.....	8
5.1 CHOOSE A SUBPROJECT	8
5.2 CHOOSE AN ASSAY REQUEST FILE.....	8
5.3 CLICK ON POPULATE FORM	8
5.4 ENTER APPROPRIATE INFORMATION IN THE ENTER NEW DEFAULT PARAMETERS BOX	9
5.5 CLICK ON GENERATE METHOD	9
6 MRM METHOD GENERATOR UTILITY	9
6.1 CHOOSE A SUBPROJECT	10
6.2 CHOOSE AN ASSAY REQUEST FILE.....	10
6.3 MODIFY THE ACCEPTANCE CRITERIA AS NEEDED	10
6.4 CLICK ON POPULATE FORM	10
6.5 INSPECT RESULTS	11
6.6 ENTER APPROPRIATE INFORMATION IN THE ENTER DEFAULT PARAMETERS BOX.....	11
6.7 CLICK ON GENERATE METHODS	11
7 MODIFY METHODS UTILITY	12
7.1 CHOOSE A 'POPULATE FORM' OPTION; EITHER 'ASSAY REQUEST FILE' OR 'ALL METHODS OF THE CHOSEN EXPT TYPE'.	12
7.2 CHOOSE AN EXPERIMENT TYPE OPTION FROM THE 'EXPT TYPE' FRAME.	12
7.3 CHOOSE SUBPROJECT	12
7.4 CHOOSE ASSAY REQUEST FILE FROM THE DROPBOX	12
7.5 ENTER VALUES FOR DESIRED VARIABLES	12
7.6 IF AN INTERNAL STANDARD IS TO BE ADDED, SELECT THE IS CHECKBOX.	12
7.7 CLICK ON POPULATE FORM TO PREVIEW DATA	13
7.8 CLICK THE 'MODIFY METHOD' BUTTON	14
8 AUTOMATON FILE CONVERTER UTILITY.....	14
8.1 CHOOSE AN AUTOMATON TEXT FILE.....	14
8.2 CHOOSE A SUBPROJECT	14
8.3 CLICK ON 'POPULATE FORM'	14
8.4 CLICK ON 'EXECUTE NAME CHANGE' BUTTON.....	16
9 BATCH QUEUE SUBMISSION UTILITY	17
9.1 CHOOSE AN ASSAY REQUEST FILE.....	17
9.2 CHOOSE A SUBPROJECT	17
9.3 CHOOSE A BATCH TYPE OPTION FROM THE BATCH TYPE FRAME.	17
9.4 ENTER SET NAME, IDENTIFIER #, BATCH OWNER, AND RAW DATA FILENAME (IF BATCH TYPE IS FINAL EXPT).....	17
9.5 CLICK ON THE 'POPULATE FORM' BUTTON	17
9.6 CLICK ON SUBMIT BATCH.....	17
10 HEPATIC CLEARANCE CALCULATOR - OVERVIEW	19



11	HEPATIC CLEARANCE CALCULATOR – INITIAL SETTINGS	20
11.1	CONSTANTS - SPECIES.....	20
11.2	TIME POINT SET	21
11.3	CHROM. METHOD	21
11.4	SAMPLE CHANGE WARNING BOXES	21
11.5	CHOOSE DATA ACCESS CONFIGURATION	21
11.6	CHOOSE DATA ACQUISITION MODE.....	21
11.7	CHOOSE A RAW DATA FILE OR RAW DATA FILE DIRECTORY.....	21
11.8	CHOOSE A SAMPLE.....	22
12	HEPATIC CLEARANCE CALCULATOR – CHROMATOGRAPHIC PROCESSING.....	23
12.1	DISPLAY CHROMATOGRAPHY.....	23
12.2	INTEGRATION PARAMETERS.....	25
12.3	MANUAL INTEGRATION	25
12.4	MANUAL PEAK SETTING	26
12.5	MANUAL PEAK RETENTION TIME ENTERING	26
12.6	CHROMATOGRAPHY REVIEW DROPDOWN BOX	26
12.7	SAVE CHROMATOGRAM	26
12.8	BATCH ACCEPT ALL CHROMATOGRAMS	26
13	HEPATIC CLEARANCE CALCULATOR – RESULTS.....	26
13.1	OVERVIEW	27
13.2	USE IS OPTION FRAME.....	28
13.3	PLOT OPTION FRAME	29
13.4	CONSTANTS DROPDOWN BOX.....	29
13.5	EXCLUDE DATA POINTS	30
13.6	RESTORE DATA POINTS	30
13.7	COMMENTS	30
13.8	REVIEW.....	30
13.9	SAVE RESULTS.....	31
14	HEPATIC CLEARANCE CALCULATOR – REPORTS.....	31
15	HEPATIC CLEARANCE CALCULATOR – ADDITIONAL FUNCTIONS	33
15.1	ACCEPT ALL CHROMATOGRAMS.....	33
15.2	CHANGE DATA FILE PATH	33
15.3	COPY PEAK INTEGRATIONS TO CLIPBOARD	33
15.4	GENERATE REPORTS	33
15.5	PEAK AREA TEXT FILE.....	33
15.6	MANUALLY ENTER PEAK RETENTION TIMES.....	34
15.7	PRINT	36
15.8	ENTER LT RETENTION TIME – ANALYTE	36
15.9	ENTER LT RETENTION TIME – INT STD.....	37
15.10	DISPLAY MULTIPLE RESULTS	37
16	PERMEABILITY CALCULATOR.....	37
17	REFERENCES	40

**Table of Figures**

<u>Figure</u>		<u>Page Number</u>
FIGURE 1	GMSU CONSOLE	6
FIGURE 2	ACQUISITION METHOD GENERATOR UTILITIES	7
FIGURE 3	PI METHOD GENERATOR STARTUP SCREEN	8
FIGURE 4	AFTER CLICKING THE POPULATE FORM BUTTON	9
FIGURE 5	MRM GENERATOR UTILITY	10
FIGURE 6	AFTER CLICKING POPULATE FORM BUTTON	11
FIGURE 7	MODIFY METHODS UTILITY	12
FIGURE 8	CHOOSE INTERNAL STANDARD METHOD FILE SOURCE	13
FIGURE 9	POPULATED MODIFY METHODS WINDOW	13
FIGURE 10	RESULTS OF 'MODIFY METHOD' BUTTON CLICK	14
FIGURE 11	AUTOMATON FILE CONVERTER UTILITY	15
FIGURE 12	CHANGED AUTOMATON FILENAMES	16
FIGURE 13	POPULATE FORM – PI/MRM EXPERIMENT	18
FIGURE 14	POPULATE FORM – FINAL EXPT EXPERIMENT	19
FIGURE 15	HEPATIC CLEARANCE CALCULATOR	20
FIGURE 16	LOADED RAW DATA FILE DATA	22
FIGURE 17	CLICK ON PEAK 1.40 OF ANALYTE RETENTION TIME/PEAK AREA TABLE GRID (WITH 'SYNCHRONIZE ANALYTE W/IS SELECTED	24
FIGURE 18	MANUAL INTEGRATION	25
FIGURE 19	HEPATIC CLEARANCE RESULTS PANE OVERVIEW	27
FIGURE 20	USE IS FRAME OPTIONS	28
FIGURE 21	PLOT FRAME OPTIONS	29
FIGURE 22	SPECIES CONSTANTS SETTINGS	29
FIGURE 23	EXCLUDING POINTS	30
FIGURE 24	GMSU CONSOLE	31
FIGURE 25	HEPATIC CLEARANCE CALCULATOR REPORTS MODULE	32
FIGURE 26	MENU ITEMS	33
FIGURE 27	MANUALLY ENTER PEAK RETENTION TIMES	34
FIGURE 28	USE PEAKS FROM AN EXISTING SAMPLE	35
FIGURE 29	USE PEAKS FROM AN EXISTING CHROMATOGRAPHIC METHOD	36
FIGURE 30	PERMEABILITY CALCULATOR – CHROMATOGRAPHY TAB	37
FIGURE 31	PERMEABILITY CALCULATOR – RESULTS TAB	38
FIGURE 32	PERMEABILITY CALCULATOR – CALIBRATION TAB	39



1 Introduction

Gubbs Mass Spec Utilities (GMSU) is a suite of utilities that enhance the ability of scientists to perform high volume actions in mass spectrometer data acquisition systems. This is useful in laboratories engaged in high-throughput studies.

GMSU uses either Microsoft® (MS) Access or MS SQL Server as a datastore. MS Access may be used to quickly setup a GMSU instance for user testing or production use. MS SQL Server may be used if a more secure environment is desired. The MS Access database can be directly upgraded to SQL Server without loss of data.

GMSU may be used in a multi-user environment. The GMSU database and acquired mass spectrometer raw data files may be stored in a common location accessible to others. Multiple installations of GMSU may be configured to use the database and access the raw data files located in the common location. This allows users in the analytical department to use GMSU to generate and optimize chromatographic integration acquired data. Once the chromatographic integration data is approved, users in the pharmacokinetics department may be notified and can immediately generate pharmacokinetic data.

GMSU data are stored in a database (either MS Access or MS SQL Server). This data may be accessed or generated from multiple installations of GMSU.

System requirements and installation procedures, please refer to the Gubbs Mass Spec Installation and Administration Manual.

2 High Throughput (HT) vs Low Throughput (LT) Acquisition

One of the salient features of GMSU 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' within a data file. This is referred to as LT acquisition in the GMSU system.

In HT acquisition, users open the 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 acquisition¹⁻⁴ 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.

3 Console

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

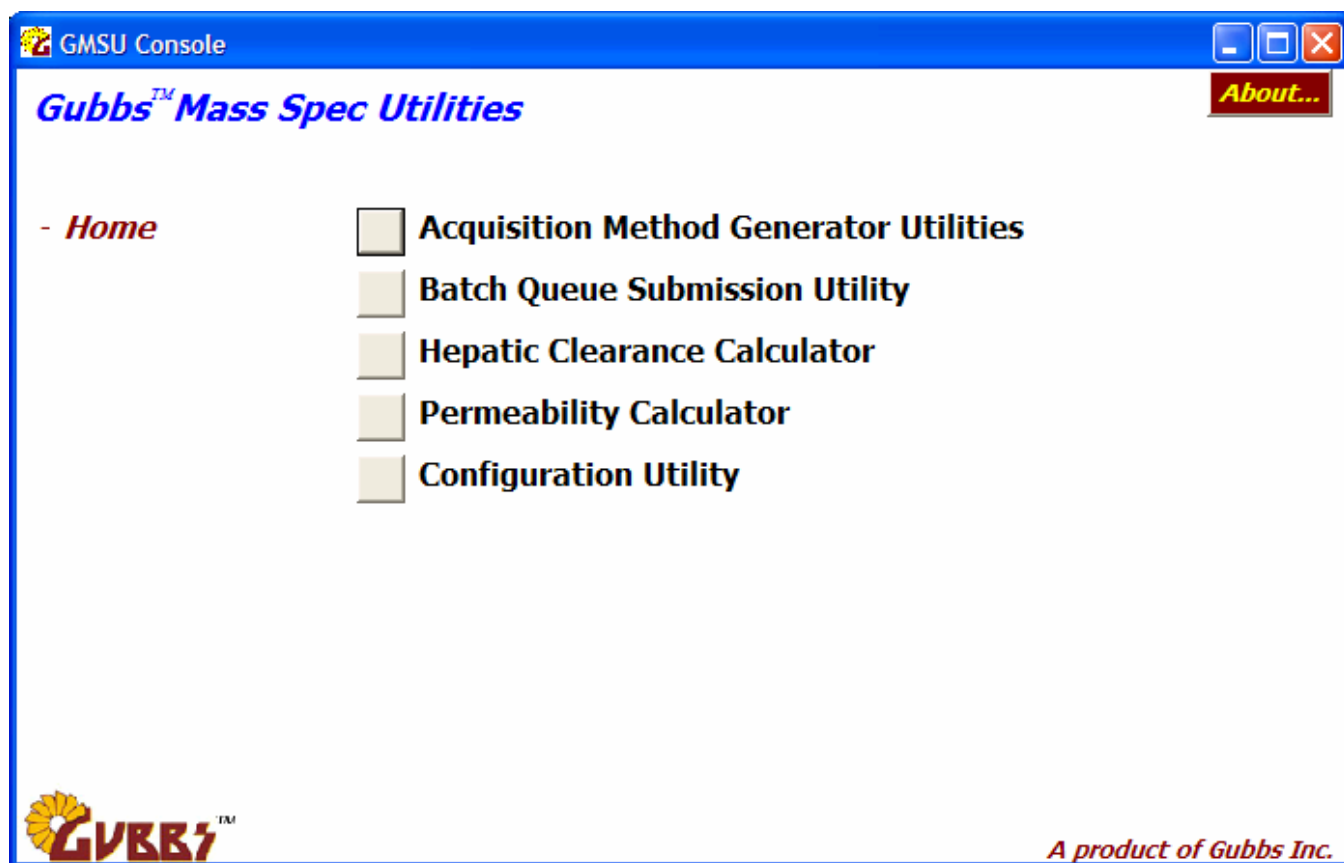


Figure 1 GMSU Console

Users click on the appropriate buttons either to open a utility or to navigate to an additional hierarchical level.

4 Acquisition Method Generator Utilities

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:

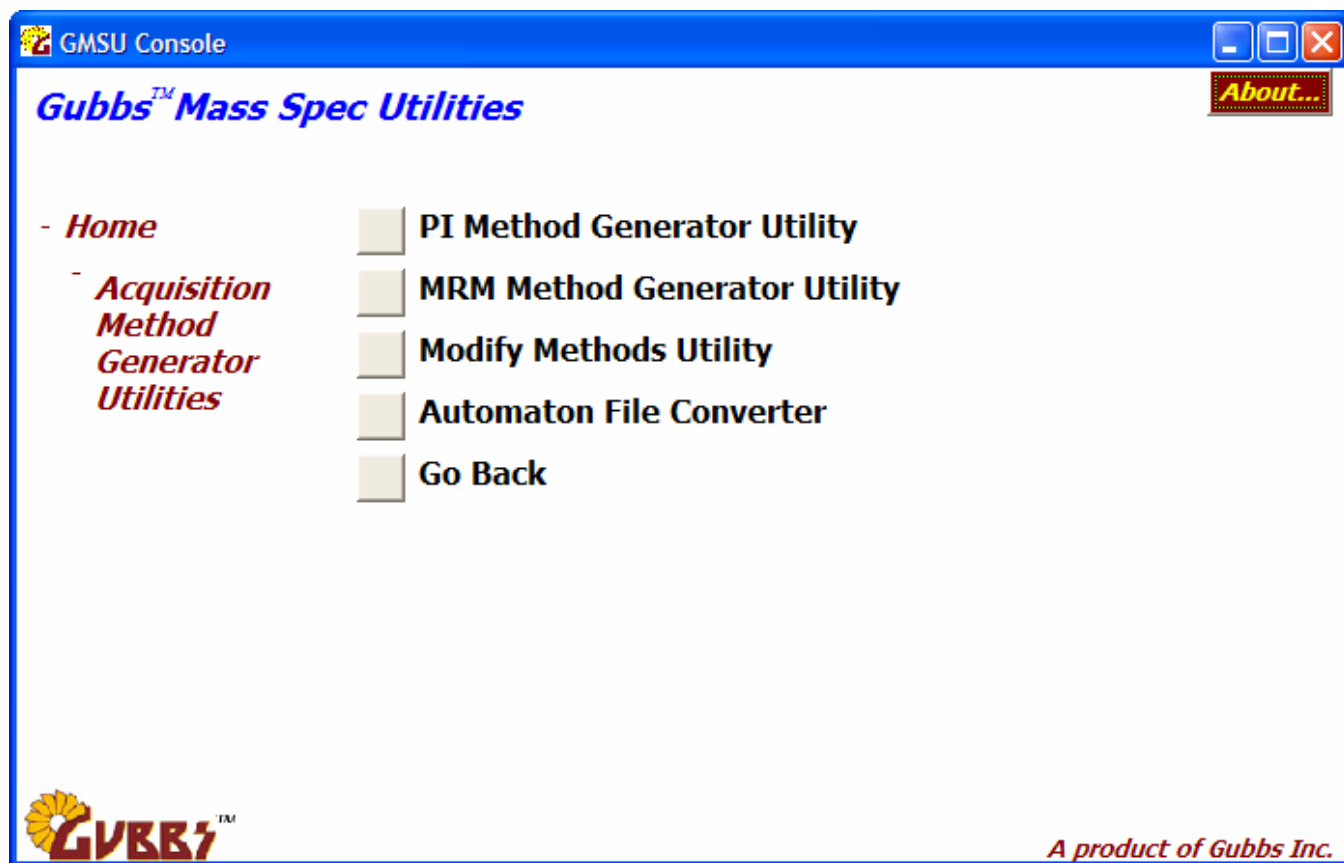
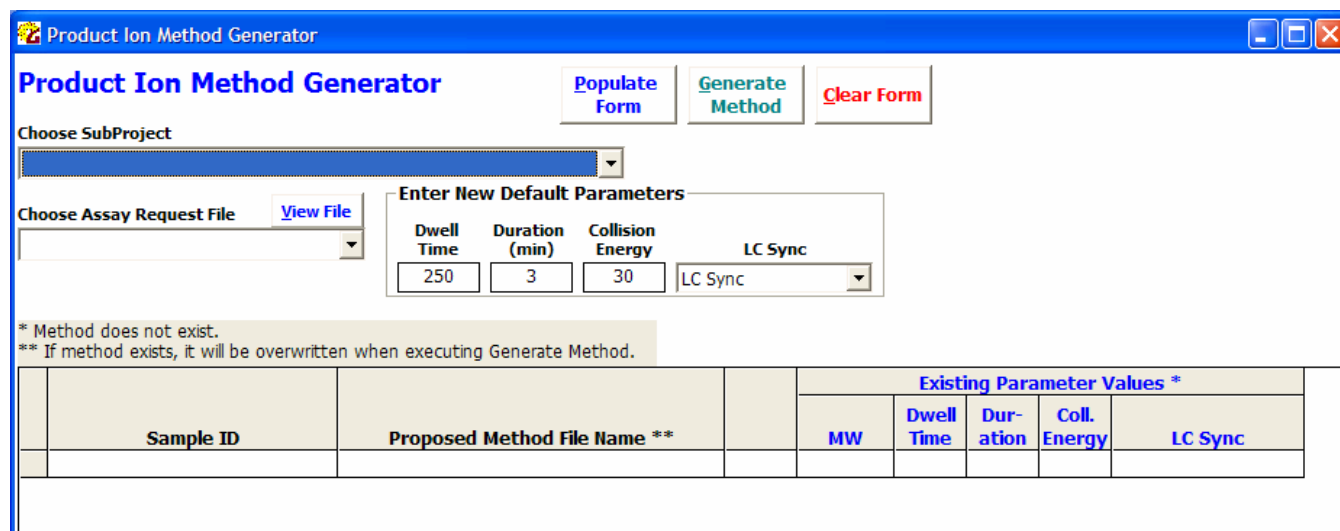


Figure 2 Acquisition Method Generator Utilities

5 PI Method Generator Utility

The PI Method Generator Utility generates Product Ion (PI) experiments configured with one positive ion (M+1) experiment and one negative ion (M-1) experiment. Methods are generated based on data contained in a selected Assay Request file that contains analyte name and its corresponding molecular weight. The following describes the procedure for using the PI Method Generator.



Sample ID	Proposed Method File Name **	Existing Parameter Values *				
		MW	Dwell Time	Duration	Coll. Energy	LC Sync

Figure 3 PI Method Generator startup screen

5.1 Choose a SubProject

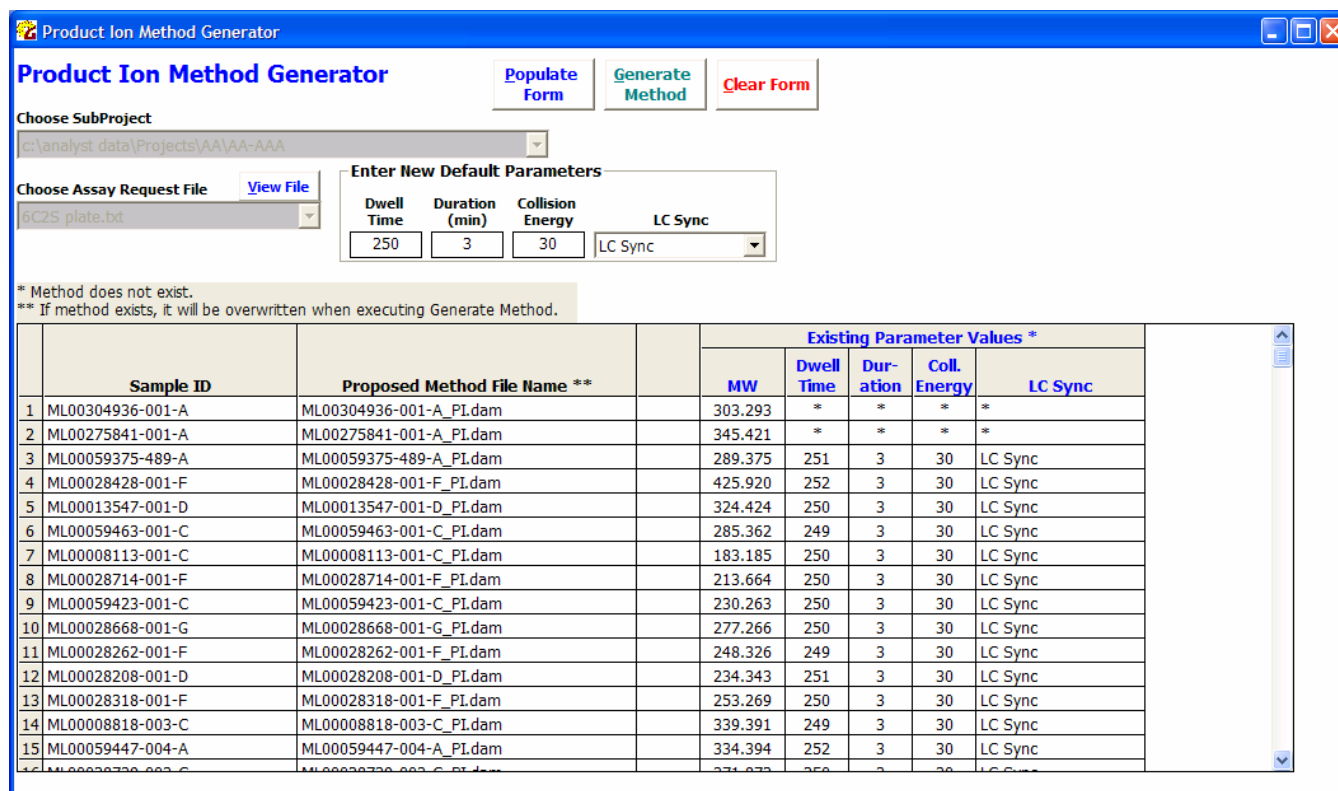
5.2 Choose an Assay Request File

Note: Users may view the Assay Request File by clicking the View File button

5.3 Click on Populate Form

The form is populated with information.

- If a PI acquisition method (.dam) file already exists, its parameter information will be displayed.
- If a PI .dam does not exist, the parameter information columns will contain asterisks
- If the user wishes to populate the form with information from a different Assay Request file, the Clear Form button must first be clicked.



	Sample ID	Proposed Method File Name **	Existing Parameter Values *				LC Sync
			MW	Dwell Time	Duration	Coll. Energy	
1	ML00304936-001-A	ML00304936-001-A.PI.dam	303.293	*	*	*	*
2	ML00275841-001-A	ML00275841-001-A.PI.dam	345.421	*	*	*	*
3	ML00059375-489-A	ML00059375-489-A.PI.dam	289.375	251	3	30	LC Sync
4	ML00028428-001-F	ML00028428-001-F.PI.dam	425.920	252	3	30	LC Sync
5	ML00013547-001-D	ML00013547-001-D.PI.dam	324.424	250	3	30	LC Sync
6	ML00059463-001-C	ML00059463-001-C.PI.dam	285.362	249	3	30	LC Sync
7	ML00008113-001-C	ML00008113-001-C.PI.dam	183.185	250	3	30	LC Sync
8	ML00028714-001-F	ML00028714-001-F.PI.dam	213.664	250	3	30	LC Sync
9	ML00059423-001-C	ML00059423-001-C.PI.dam	230.263	250	3	30	LC Sync
10	ML00028668-001-G	ML00028668-001-G.PI.dam	277.266	250	3	30	LC Sync
11	ML00028262-001-F	ML00028262-001-F.PI.dam	248.326	249	3	30	LC Sync
12	ML00028208-001-D	ML00028208-001-D.PI.dam	234.343	251	3	30	LC Sync
13	ML00028318-001-F	ML00028318-001-F.PI.dam	253.269	250	3	30	LC Sync
14	ML00008818-003-C	ML00008818-003-C.PI.dam	339.391	249	3	30	LC Sync
15	ML00059447-004-A	ML00059447-004-A.PI.dam	334.394	252	3	30	LC Sync

Figure 4 After clicking the Populate Form button

5.4 Enter appropriate information in the Enter New Default Parameters box

5.5 Click on Generate Method

PI acquisition methods with the Proposed Method File Name will be created that have parameters shown in the Default Parameters box.

If the method already exists, that method will be overwritten.

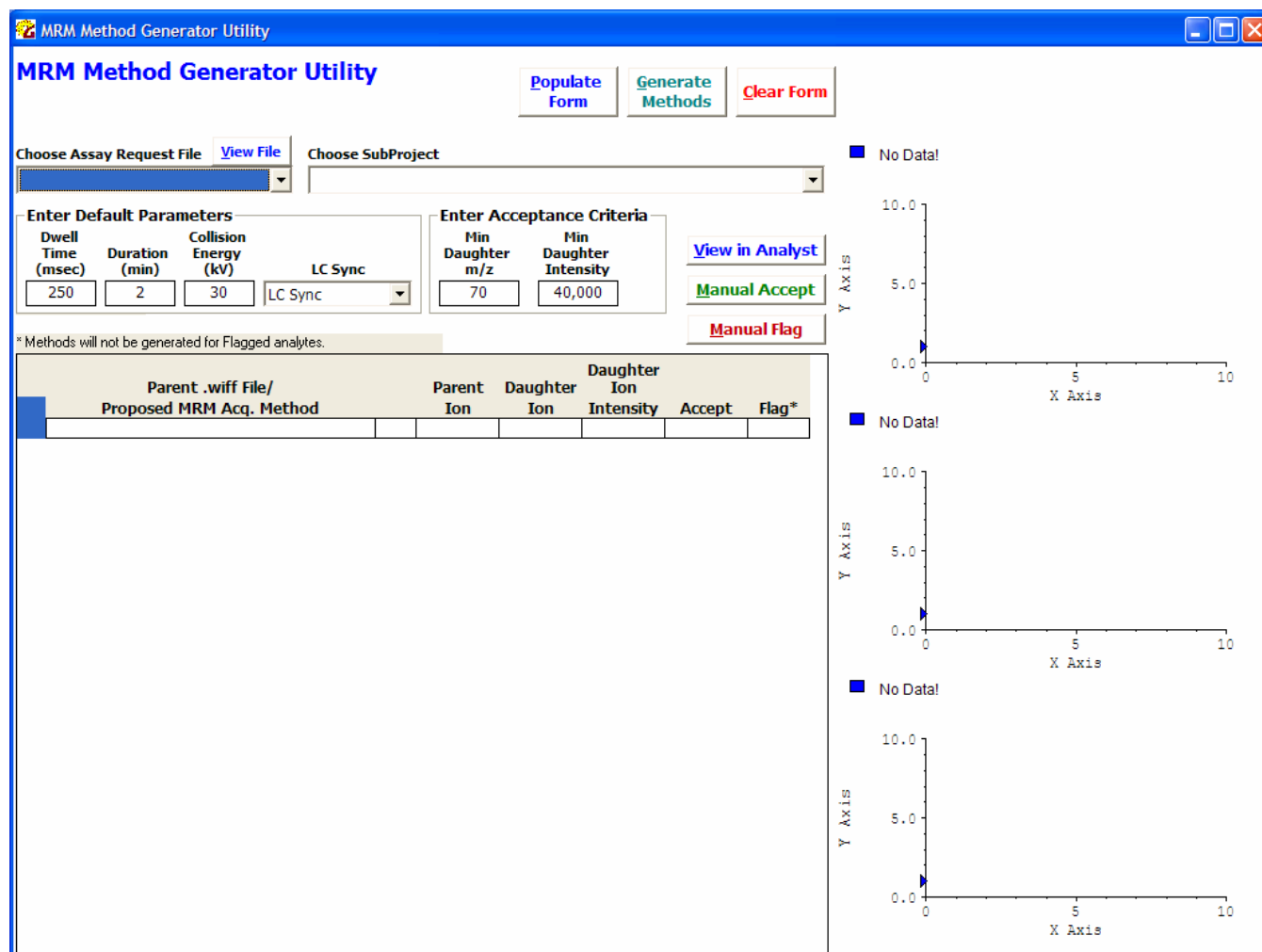
6 MRM Method Generator Utility

The Multiple Reaction Monitoring (MRM) Method Generator utility performs several functions:

- Allows users to quickly view Total Ion Current (TIC) and mass spectra contained in acquired .wiff files that contain an M+1 positive ion experiment and an M-1 negative ion PI experiment.
- Determines the most appropriate MRM experiment to generate based on user-configurable criteria.
- Flags .wiff files whose experiments do not meet criteria.
- Generates MRM methods for non-flagged experiments using parameters set by the user.

The utility will determine and record the most abundant daughter ions obtained from the M+1 positive ion experiment and M-1 negative ion PI experiment. The utility will then recommend the most appropriate MRM experiment to generate based on the daughter ion that is most abundant. Users may manually override any decision made by the utility.

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



MRM Method Generator Utility

Populate Form Generate Methods Clear Form

Choose Assay Request File View File Choose SubProject

Enter Default Parameters

Dwell Time (msec): 250 Duration (min): 2 Collision Energy (kV): 30 LC Sync: LC Sync

Enter Acceptance Criteria

Min Daughter m/z: 70 Min Daughter Intensity: 40,000

View in Analyst Manual Accept Manual Flag

* Methods will not be generated for Flagged analytes.

Parent .wiff File/ Proposed MRM Acq. Method	Parent Ion	Daughter Ion	Daughter Ion Intensity	Accept	Flag*

Y Axis X Axis

No Data!

Y Axis X Axis

No Data!

Y Axis X Axis

No Data!

Figure 5 MRM Generator Utility

6.1 Choose a SubProject

6.2 Choose an Assay Request File

Note: Users may view the Assay Request File by clicking the View File button

6.3 Modify the Acceptance Criteria as needed

- Min Daughter m/z: The minimum daughter ion m/z that will be considered when evaluating mass spectra
- Min Daughter Intensity: Ions must be greater than this value in order to be considered as acceptable

6.4 Click on Populate Form

If the user wishes to populate the form with information from a different Assay Request file, the Clear Form button must first be clicked.

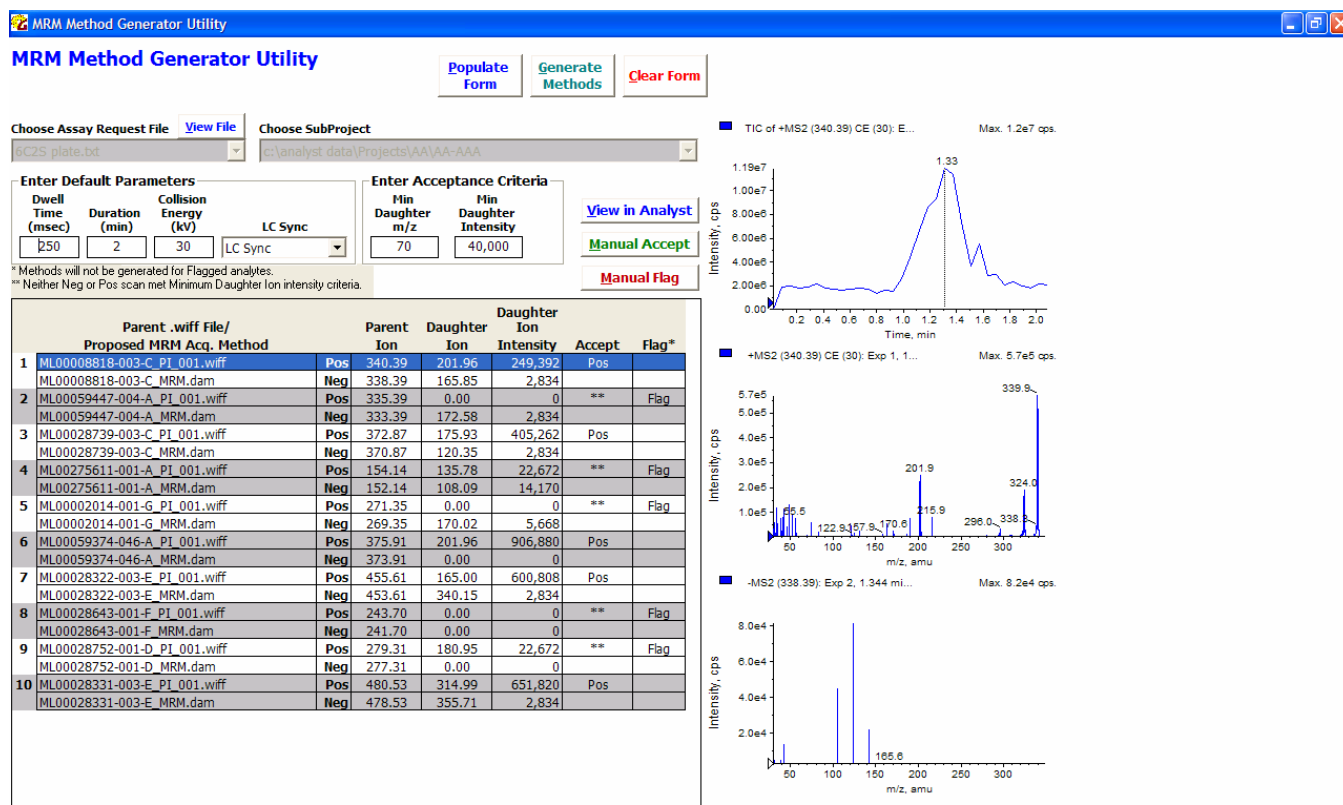


Figure 6 After clicking Populate Form button

6.5 Inspect results

Users should inspect the results by ensuring that the Accepted Pos or Neg ion is the desired daughter ion to be used when generating the MRM method. Users can view the mass spectra for each analyte by selecting it in the table.

Users have perform the following functions

- Manual Accept: Users may manually accept a Positive or Negative ion by selecting the appropriate row and clicking on the Manual Accept button.
- Manual Flag: Users may manually flag an analyte by selecting either the Positive or Negative row of an analyte and clicking on Manual Flag
- View in native data acquisition system: If desired, users may view the data in the native system by selecting the analyte and clicking on View.

6.6 Enter appropriate information in the Enter Default Parameters box

6.7 Click on Generate Methods

MRM acquisition methods with the Proposed Method File Name and the Accepted positive or negative transitions will be created.

If the method already exists, that method will be overwritten.

7 Modify Methods Utility

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.

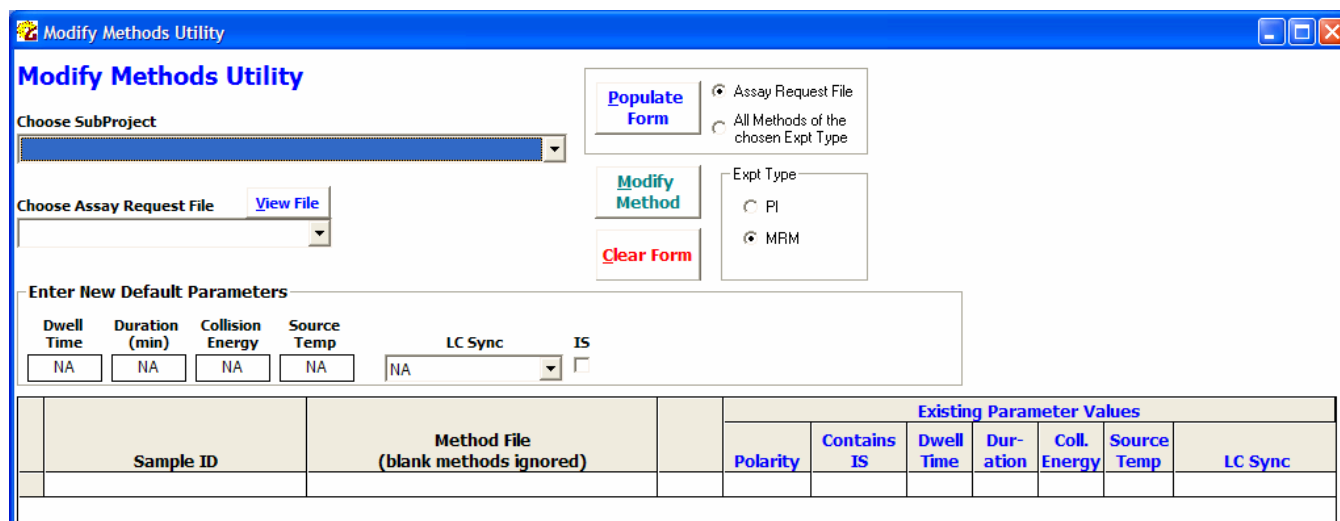
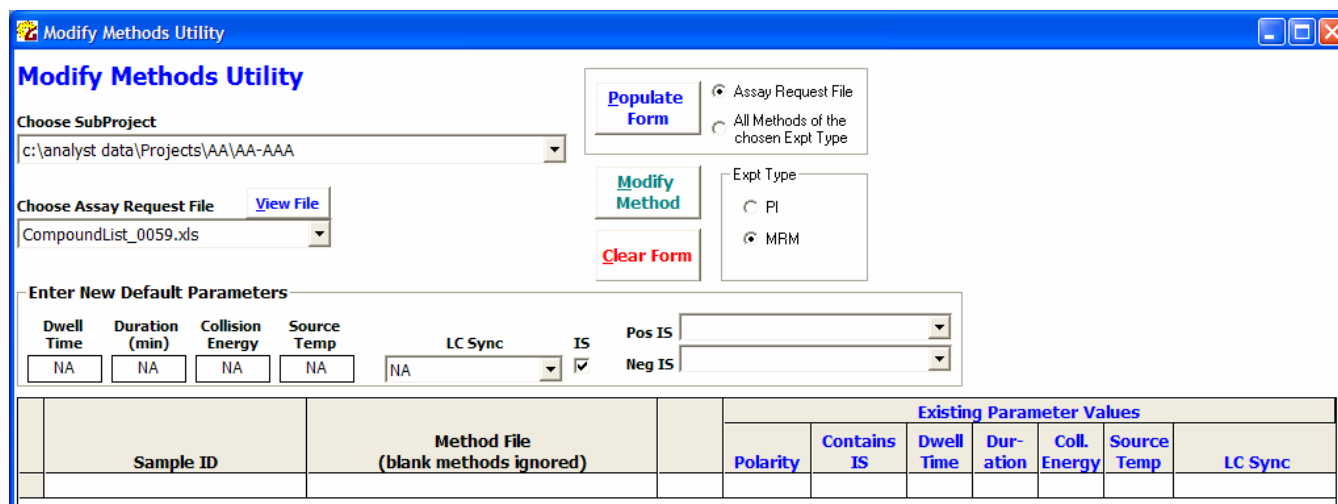


Figure 7 Modify Methods Utility

- 7.1 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' dropdown becomes disabled and is unneeded.
- 7.2 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.
- 7.3 Choose subproject
- 7.4 Choose Assay Request File from the dropdown
- 7.5 Enter values for desired variables
- 7.6 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 8).



Modify Methods Utility

Choose SubProject
c:\analyst data\Projects\AA\AA-AAA

Choose Assay Request File [View File](#)
CompoundList_0059.xls

[Populate Form](#) ☒ Assay Request File
☐ All Methods of the chosen Expt Type

[Modify Method](#) Expt Type
☐ PI
☒ MRM

[Clear Form](#)

Enter New Default Parameters

Dwell Time	Duration (min)	Collision Energy	Source Temp	LC Sync	IS	Pos IS	Neg IS
NA	NA	NA	NA	NA	☒		

Sample ID	Method File (blank methods ignored)	Existing Parameter Values						
		Polarity	Contains IS	Dwell Time	Duration	Coll. Energy	Source Temp	LC Sync

Figure 8 Choose Internal Standard Method File Source

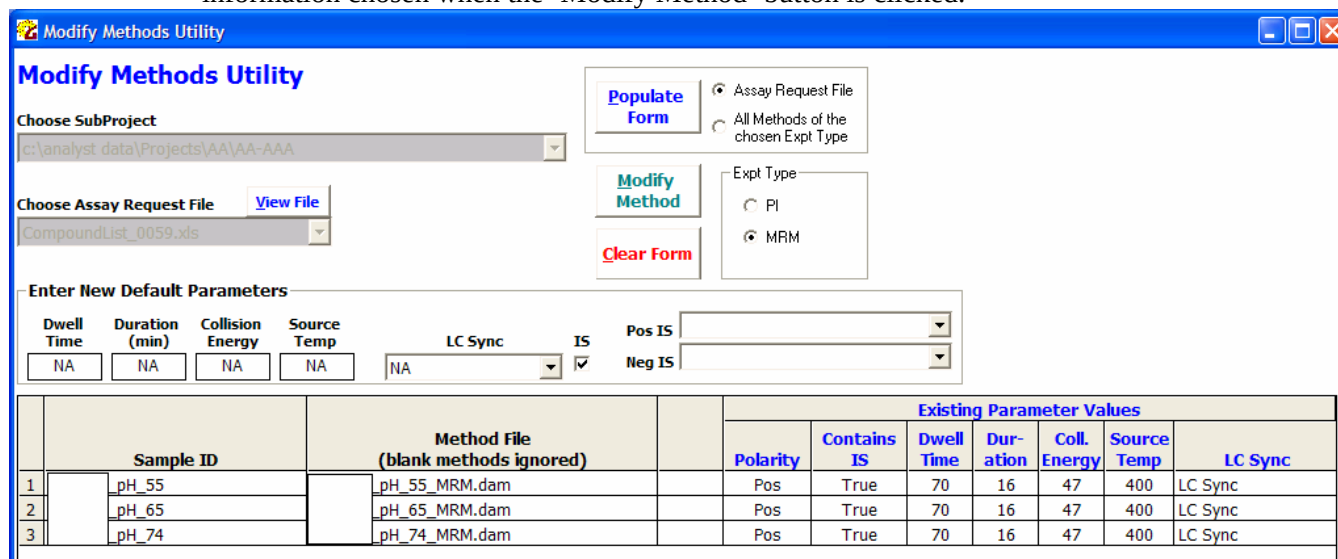
7.7 Click on Populate Form to preview data

A populated window is displayed (see Figure 9).

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.



Modify Methods Utility

Choose SubProject
c:\analyst data\Projects\AA\AA-AAA

Choose Assay Request File [View File](#)
CompoundList_0059.xls

[Populate Form](#) ☒ Assay Request File
☐ All Methods of the chosen Expt Type

[Modify Method](#) Expt Type
☐ PI
☒ MRM

[Clear Form](#)

Enter New Default Parameters

Dwell Time	Duration (min)	Collision Energy	Source Temp	LC Sync	IS	Pos IS	Neg IS
NA	NA	NA	NA	NA	☒		

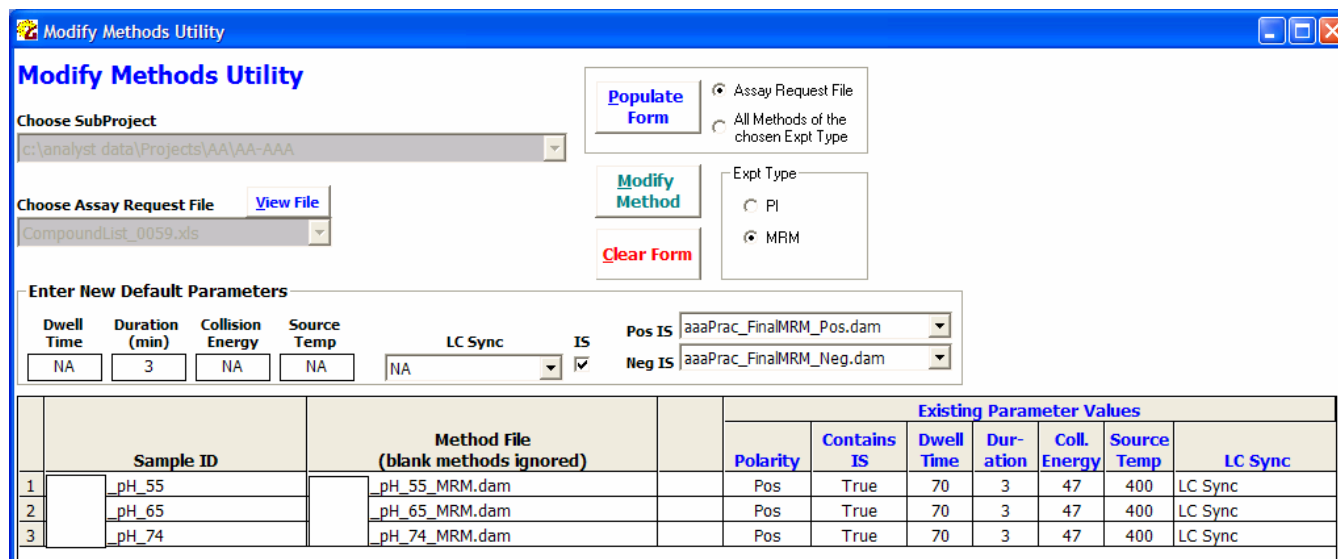
Sample ID	Method File (blank methods ignored)	Existing Parameter Values						
		Polarity	Contains IS	Dwell Time	Duration	Coll. Energy	Source Temp	LC Sync
1	pH_55_MRM.dam	Pos	True	70	16	47	400	LC Sync
2	pH_65_MRM.dam	Pos	True	70	16	47	400	LC Sync
3	pH_74_MRM.dam	Pos	True	70	16	47	400	LC Sync

Figure 9 Populated Modify Methods Window

7.8 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.



Sample ID		Method File (blank methods ignored)	Existing Parameter Values						
			Polarity	Contains IS	Dwell Time	Duration	Coll. Energy	Source Temp	LC Sync
1	pH_55	pH_55_MRM.dam	Pos	True	70	3	47	400	LC Sync
2	pH_65	pH_65_MRM.dam	Pos	True	70	3	47	400	LC Sync
3	pH_74	pH_74_MRM.dam	Pos	True	70	3	47	400	LC Sync

Figure 10 Results of 'Modify Method' button click

8 Automaton File Converter Utility

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.

8.1 Choose an Automaton Text File

8.2 Choose a SubProject

8.3 Click on 'Populate Form'

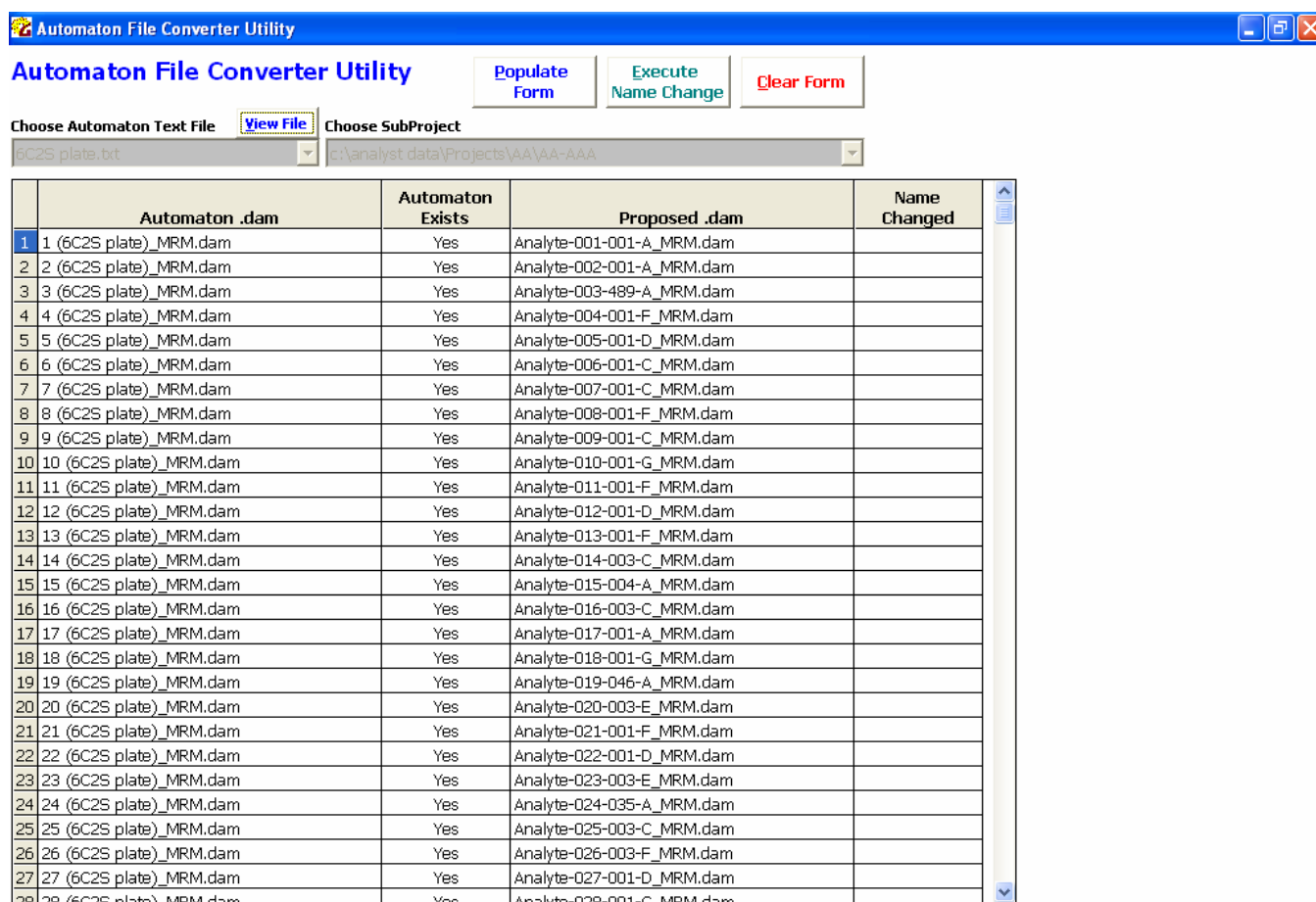
The form is populated according to the following rules (see Figure 11).

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	
28	28 (6C2S plate)_MRM.dam	Yes	Analyte-028-001-C_MRM.dam	

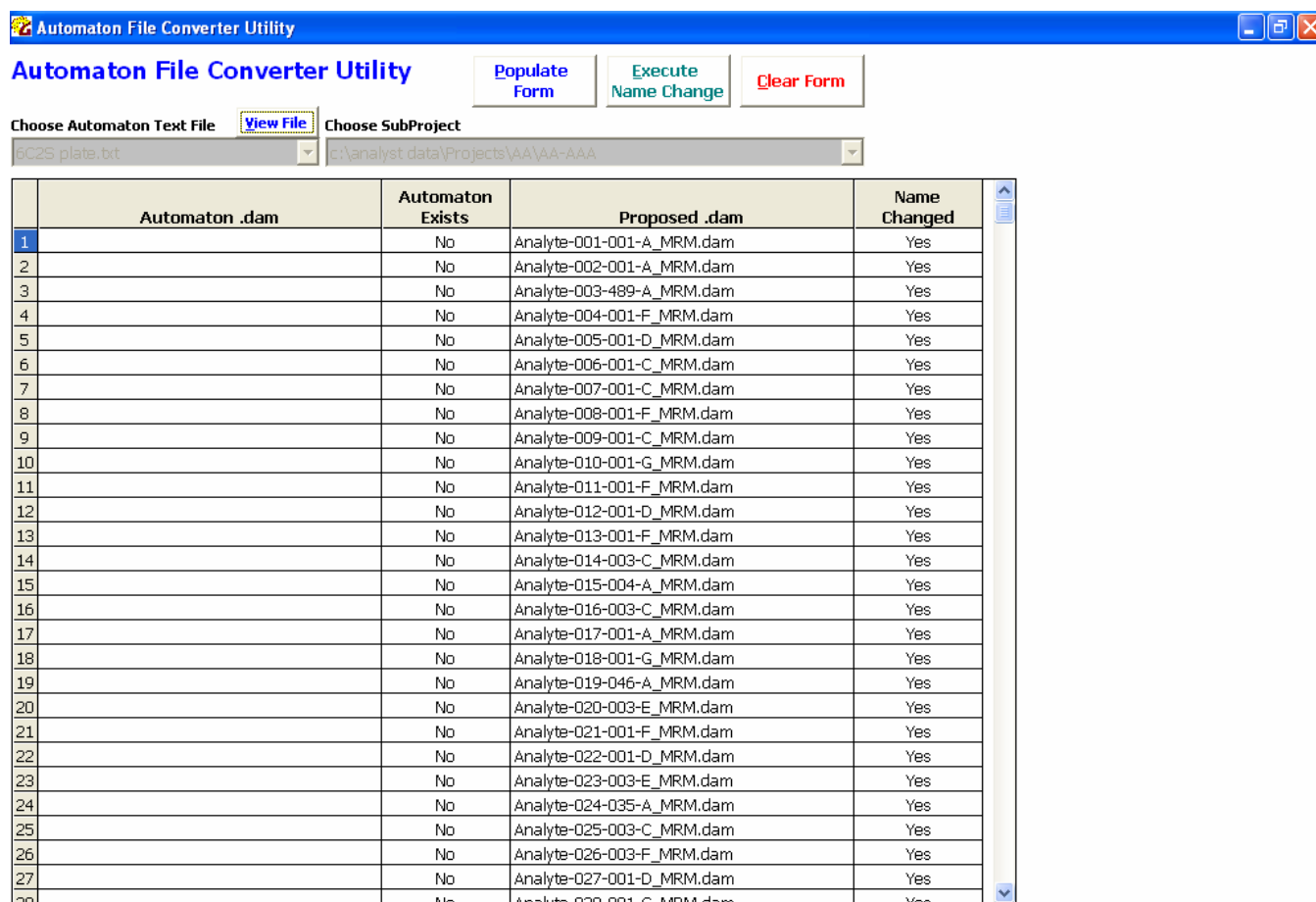
Figure 11 Automaton File Converter Utility

8.4 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 12).



	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 12 Changed Automaton Filenames

9 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.

9.1 Choose an Assay Request File

9.2 Choose a SubProject

9.3 Choose a Batch Type option from the Batch Type frame.

9.4 Enter Set Name, Identifier #, Batch Owner, and raw data filename (if Batch Type is Final Expt)

9.5 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 13). If the Batch Type was 'Final Expt', then the '.wiff File Name' column will be named according to the user entry described in Section 9.4 (see Figure 14).

9.6 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.



Batch Queue Submission Utility

Batch Queue Submission Utility

[Load a Saved Batch...](#) [Save the Batch...](#) [Populate Form](#) [Submit Batch](#) [Clear Form](#)

Choose Assay Request File [View File](#) Choose SubProject

Choose Assay Request File: Choose SubProject:

Enter Default Parameters

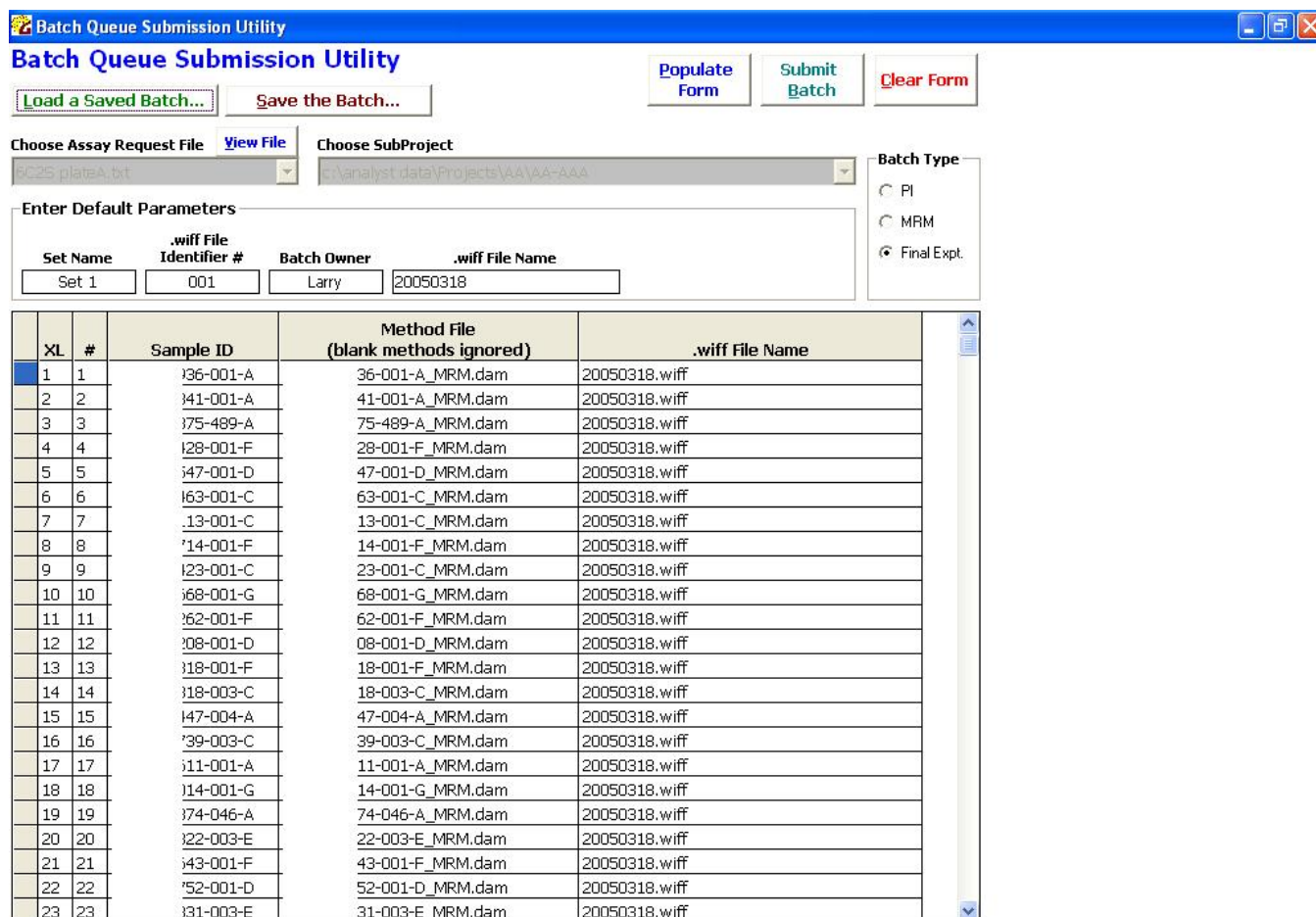
Set Name	.wiff File Identifier #	Batch Owner
Set 1	001	Larry

Batch Type

☒ PI
☐ MRM
☐ Final Expt.

XL	#	Sample ID	Method File (blank methods ignored)	.wiff File Name
1	1	36-001-A	36-001-A_P1.dam	36-001-A_P1_001.wiff
2	2	41-001-A	41-001-A_P1.dam	41-001-A_P1_001.wiff
3	3	75-489-A	75-489-A_P1.dam	75-489-A_P1_001.wiff
4	4	28-001-F	28-001-F_P1.dam	28-001-F_P1_001.wiff
5	5	47-001-D	47-001-D_P1.dam	47-001-D_P1_001.wiff
6	6	63-001-C	63-001-C_P1.dam	63-001-C_P1_001.wiff
7	7	13-001-C	13-001-C_P1.dam	13-001-C_P1_001.wiff
8	8	14-001-F	14-001-F_P1.dam	14-001-F_P1_001.wiff
9	9	23-001-C	23-001-C_P1.dam	23-001-C_P1_001.wiff
10	10	68-001-G	68-001-G_P1.dam	68-001-G_P1_001.wiff
11	11	62-001-F	62-001-F_P1.dam	62-001-F_P1_001.wiff
12	12	08-001-D	08-001-D_P1.dam	08-001-D_P1_001.wiff
13	13	18-001-F	18-001-F_P1.dam	18-001-F_P1_001.wiff
14	14	18-003-C	18-003-C_P1.dam	18-003-C_P1_001.wiff
15	15	47-004-A	47-004-A_P1.dam	47-004-A_P1_001.wiff
16	16	39-003-C	39-003-C_P1.dam	39-003-C_P1_001.wiff
17	17	11-001-A	11-001-A_P1.dam	11-001-A_P1_001.wiff
18	18	14-001-G	14-001-G_P1.dam	14-001-G_P1_001.wiff
19	19	74-046-A	74-046-A_P1.dam	74-046-A_P1_001.wiff
20	20	22-003-E	22-003-E_P1.dam	22-003-E_P1_001.wiff
21	21	43-001-F	43-001-F_P1.dam	43-001-F_P1_001.wiff
22	22	52-001-D	52-001-D_P1.dam	52-001-D_P1_001.wiff
23	23	31-003-E	31-003-E_P1.dam	31-003-E_P1_001.wiff

Figure 13 Populate Form – PI/MRM Experiment



XL	#	Sample ID	Method File (blank methods ignored)	.wiff File Name
1	1	36-001-A	36-001-A_MRM.dam	20050318.wiff
2	2	41-001-A	41-001-A_MRM.dam	20050318.wiff
3	3	75-489-A	75-489-A_MRM.dam	20050318.wiff
4	4	128-001-F	28-001-F_MRM.dam	20050318.wiff
5	5	147-001-D	47-001-D_MRM.dam	20050318.wiff
6	6	163-001-C	63-001-C_MRM.dam	20050318.wiff
7	7	113-001-C	13-001-C_MRM.dam	20050318.wiff
8	8	114-001-F	14-001-F_MRM.dam	20050318.wiff
9	9	123-001-C	23-001-C_MRM.dam	20050318.wiff
10	10	168-001-G	68-001-G_MRM.dam	20050318.wiff
11	11	162-001-F	62-001-F_MRM.dam	20050318.wiff
12	12	108-001-D	08-001-D_MRM.dam	20050318.wiff
13	13	118-001-F	18-001-F_MRM.dam	20050318.wiff
14	14	118-003-C	18-003-C_MRM.dam	20050318.wiff
15	15	147-004-A	47-004-A_MRM.dam	20050318.wiff
16	16	139-003-C	39-003-C_MRM.dam	20050318.wiff
17	17	111-001-A	11-001-A_MRM.dam	20050318.wiff
18	18	114-001-G	14-001-G_MRM.dam	20050318.wiff
19	19	174-046-A	74-046-A_MRM.dam	20050318.wiff
20	20	122-003-E	22-003-E_MRM.dam	20050318.wiff
21	21	143-001-F	43-001-F_MRM.dam	20050318.wiff
22	22	152-001-D	52-001-D_MRM.dam	20050318.wiff
23	23	131-003-E	31-003-E_MRM.dam	20050318.wiff

Figure 14 Populate Form – Final Expt Experiment

10 Hepatic Clearance Calculator - Overview

The Hepatic Clearance Calculator consists of three panes (see Figure Figure 15). Users use the top pane (Section 11) to choose data files, configure default constants, choose samples, and choose Warning settings. Users use the bottom left pane (Section 12) to review and optimize chromatographic integration. Users use the bottom right pane (Section 13) to process and optimize Hepatic Clearance results.

In addition, Hepatic Clearance Calculator allows users to generate reports (Section 0) and perform additional functions (Section 15, e.g. copy peak integration data to the clipboard).

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

11 Hepatic Clearance Calculator – Initial Settings

The initial state of the Hepatic Clearance Calculator is a screen lacking any data (Figure Figure 15). Users first must set default constants, then choose a data file for processing. The following describes the steps for performing these actions:

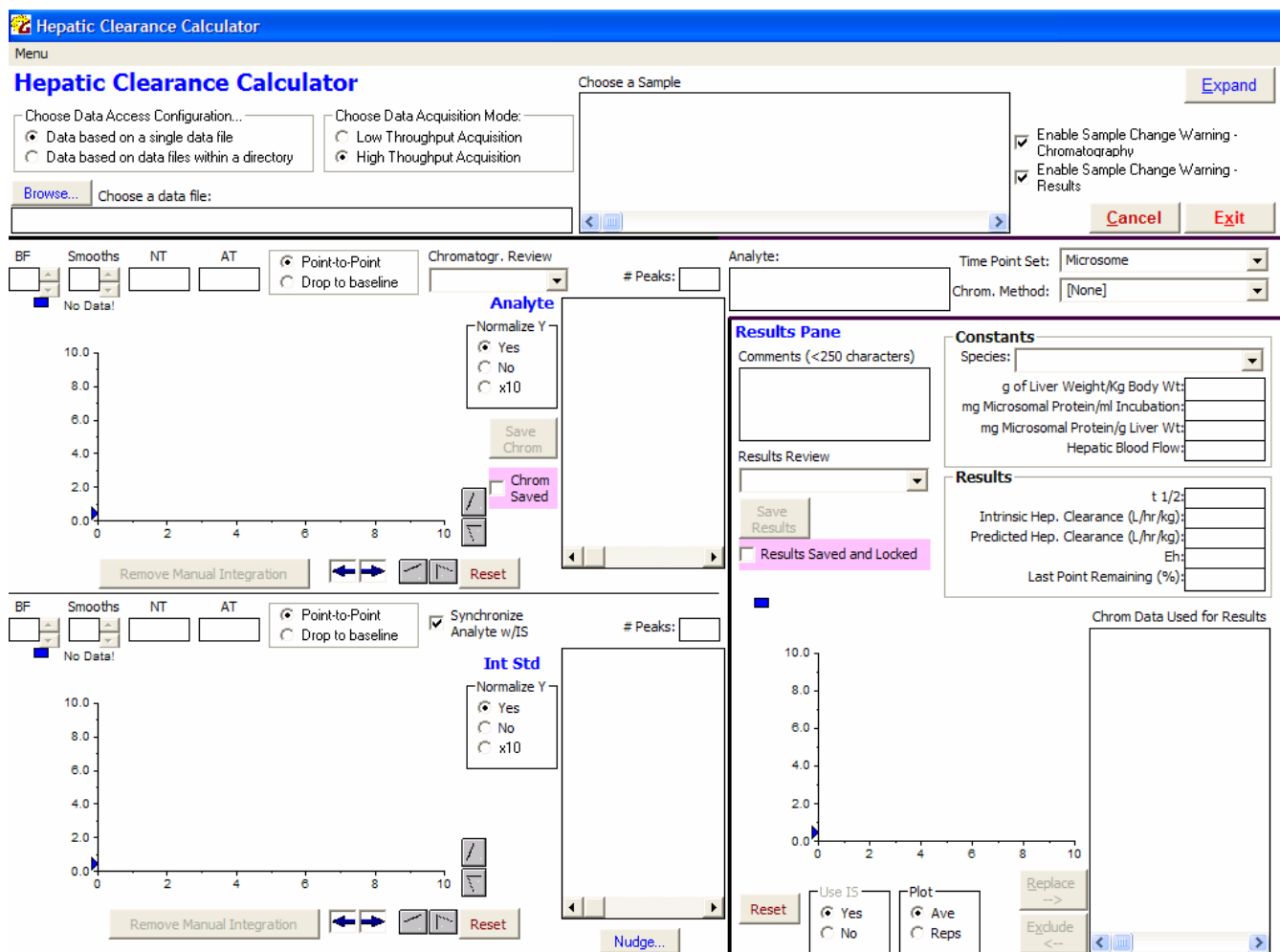


Figure 15 Hepatic Clearance Calculator

11.1 Constants - Species

Ensure the appropriate species-specific experiment constant set is displayed here. Constants Sets are created and configured in the Hepatic Clearance Calculator Configuration Settings tab of the Configuration Utility. The default constant is retrieved from the Hepatic Clearance Calculator Configuration Settings in the Configuration Utility.

Note: If a dataset contains mixed species experiments, the user later can change the assigned constants by changing the contents of the Species: dropdown box after the dataset has been loaded.



11.2 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 the Hepatic Clearance Calculator Configuration Settings tab of the Configuration Utility. The default Time Point Set is retrieved from the Hepatic Clearance Calculator Configuration Settings in the Configuration Utility.

11.3 Chrom. Method

Note: This field is optional for HT acquisition and not applicable to LT acquisition.

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 automatic peak identification algorithm is not successful (for, e.g., data files with low signal).

Ensure an appropriate Time Point Set 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).

11.4 Sample Change Warning boxes

If the user attempts to select a new sample, but hasn't saved the Chromatograms or Results, two warnings are 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.

11.5 Choose Data Access Configuration

Users may choose to load a single .wiff file or all .wiff files within a directory.

The default setting for this frame is configured in the Hepatic Clearance Calculator Configuration Settings in the Configuration Utility. If desired, an administrator can make this option frame invisible.

11.6 Choose Data Acquisition Mode

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.

The default setting for this frame is configured in the Hepatic Clearance Calculator Configuration Settings in the Configuration Utility. If desired, an administrator can make this option frame invisible.

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

11.7 Choose a raw data file or raw data file directory

Choose the Browse... button to browse to a raw data file or raw data file directory

Data will be loaded into the 'Choose a Sample' table grid and the first sample will be processed. The resulting window is shown in Figure 16.

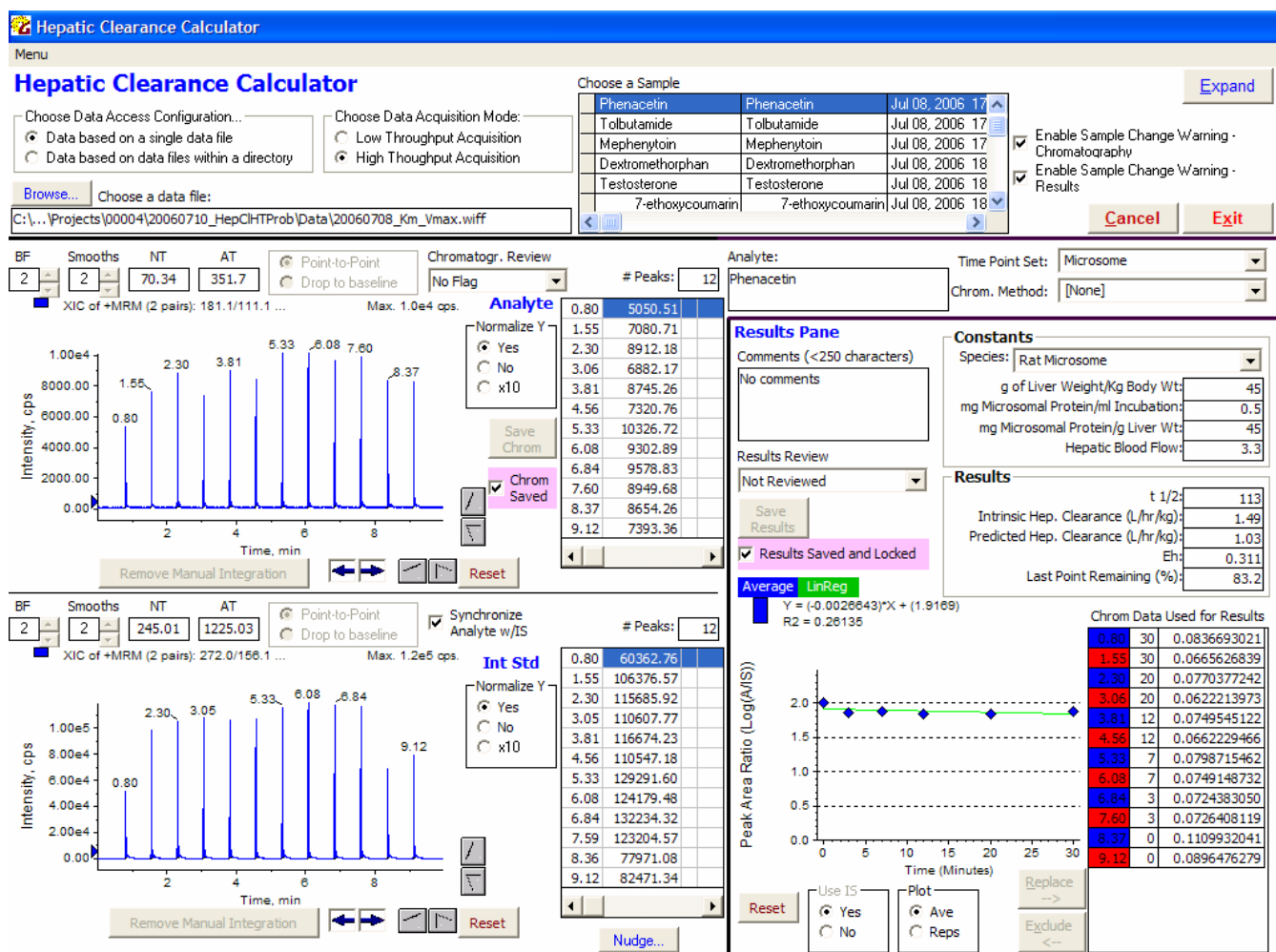


Figure 16 Loaded raw data file data

11.8 Choose a Sample

Users may choose a sample in the 'Choose a Sample' grid either by selecting with the mouse or using the arrow keys when the 'Choose a Sample' 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 by de-selecting the appropriate checkboxes located to the right of the 'Choose a Sample' grid (see Section 11.2).

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

12 Hepatic Clearance Calculator – Chromatographic Processing

The Chromatography bottom left pane allows users to perform several functions:

- Allows users to quickly view analytical runs generated in one or several raw data files and quickly identify possible problems in instrument operation or sample preparation.
- Automatically optimizes chromatographic integration parameters and picks peaks.
- Allows the user to further optimize chromatographic integration, including manual integration.
- View Hepatic Clearance Results on the fly as chromatography is being optimized.

12.1 Display chromatography

There are several ways in which chromatographic data may be displayed:

12.1.1 Retention Time/Peak Area table grids

If the user clicks on an entry in the 'Retention Time/Peak' Area table grid located to the right of the chromatogram, the corresponding chromatographic peak will be zoomed in the chromatogram.

For example, Figure 17 shows that, when peak retention time 1.40 in the 'Retention Time/Peak' Area table grid is selected, the corresponding peak obtains focus in the chromatogram. In the figure shown, the 'Synchronize Analyte w/IS' checkbox was selected, so the corresponding Internal Standard peak was also obtained focus.

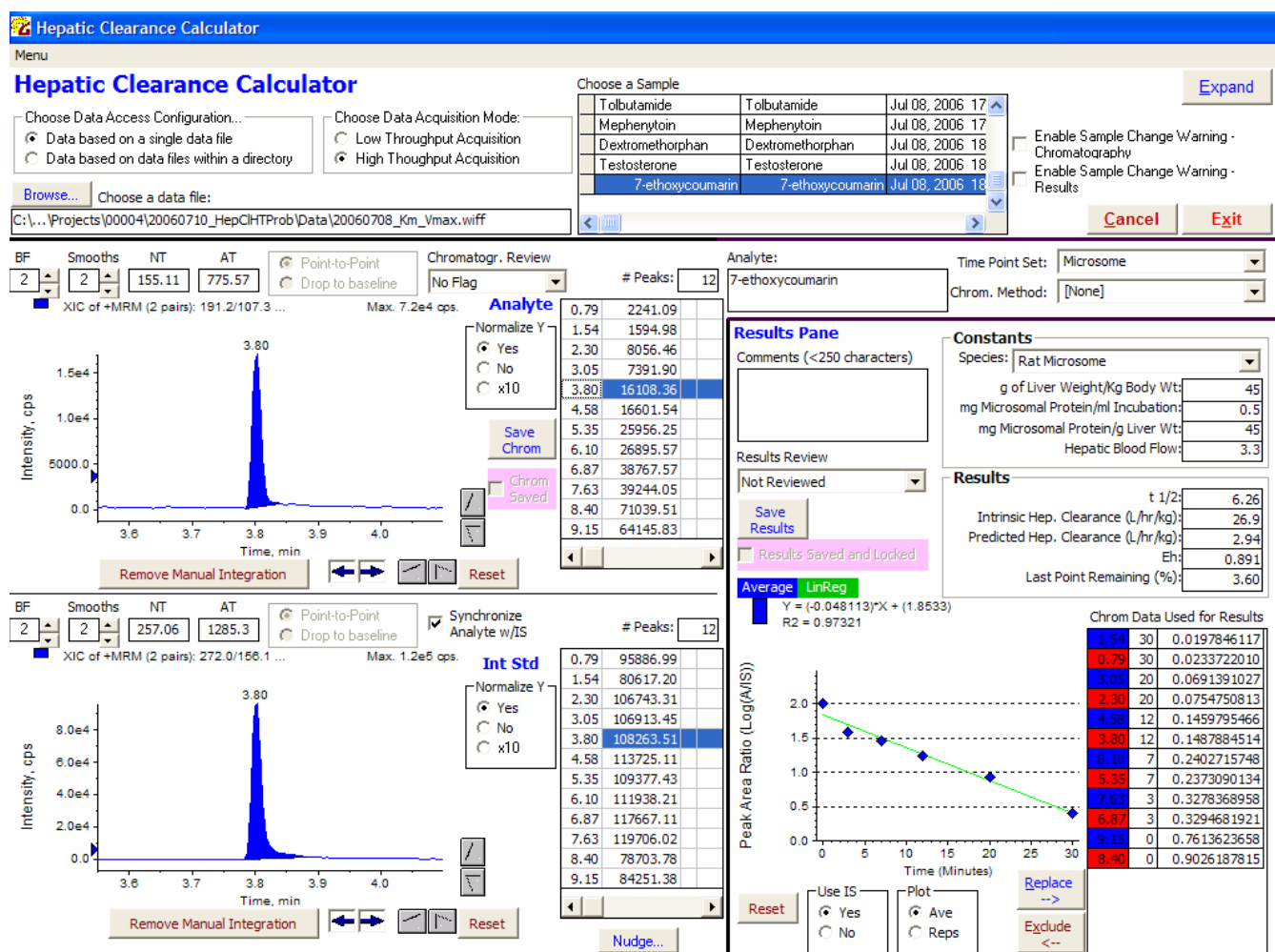


Figure 17 Click on Peak 1.40 of Analyte Retention Time/Peak Area table grid (with 'Synchronize Analyte w/IS' selected)

12.1.2 Synchronize Analyte w/IS

If the 'Synchronize Analyte w/IS' checkbox is selected, then a selection in either 'Analyte -' or 'Internal Standard Retention Time/Peak Area' table grid will result in the corresponding peak obtaining focus in both chromatograms.

12.1.3 Normalize Y

- If Normalize Y is set to Yes, then the Y-axis is automatically normalized to the peak height.
- If Normalize Y is set to No, then the Y-axis remains at its most recent setting
- If Normalize Y is set to x10, the Y-axis is automatically magnified by a factor of 10 with respect to the peak height of the selected peak

12.1.4 Y-Axis Scale buttons

The gray arrow buttons to the right of the chromatograms increase or decrease the scale of the Y-Axis

12.1.5 X-Axis Shift buttons

The gray arrow buttons to the bottom right of the chromatograms shift the X-axis to the left or right.

12.1.6 X-Axis Expansion buttons

The blue arrow buttons to the bottom of the chromatograms expand or contract the scale of the X-axis

12.2 Integration Parameters

Users may optimize integration with the following parameters:

- Bunching Factor (BF)
- Smoothing (Smooths)
- Noise Threshold (NT)
- Area Threshold (AT)

12.3 Manual Integration

Manual integration may be performed by clicking and dragging (Panel 1) with the left mouse button the peak desired to be integrated. The corresponding item in the Retention Time/Peak Area grid will be annotated with an “M” to signify that the peak has been integrated manually (Panel 2).

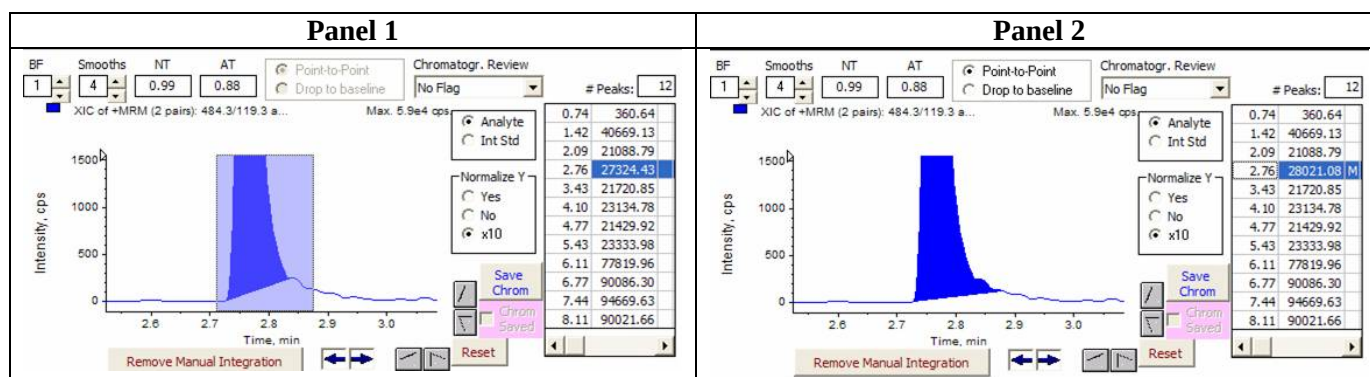


Figure 18 Manual Integration

The manual integration can be re-manually integrated if desired.

The manual integration may be removed by:

- ensuring that the correct peak is selected in the Retention Time/Peak Area grid
- clicking on the Remove Manual Integration button

12.4 Manual Peak Setting

In some instances (e.g. when peak heights are close to the signal-to-noise ratio or if integration parameters are not optimal), Hepatic Clearance Calculator will not identify the correct peak. The peak may be manually set by:

- ensuring the peak in question is selected in the appropriate Retention Time/Peak Area grid
- right-click on the desired peak

12.5 Manual Peak Retention Time Entering

In some instances, several peaks cannot automatically be picked or cannot be set with a right-mouse click. The user may manually enter retention times for the run by choosing Menu – Manually Enter Peak Retention Times. See Section 15.6 for further discussion

12.6 Chromatography Review Dropdown Box

The user may choose from a list of choices to denote an assessment of the chromatographic results. The contents of the ‘Chromatogr. Review’ dropdown box are configured by an administrator in the Configuration Utility.

12.7 Save Chromatogram

When the user is satisfied that the chromatography is acceptable, the user locks the chromatogram by clicking on ‘Save Chrom’. This disables all functions relating to chromatographic integration and the ‘Chrom Saved’ checkbox becomes selected. In addition, chromatographic data are stored in the GMSU data store.

If the user attempts to further process a Saved Chromatogram, an error message will be displayed. In order to further process a Saved Chromatogram, the user must deselect the ‘Chrom Saved’ checkbox.

12.8 Batch Accept All Chromatograms

In order to expedite the processing of a large number of samples, the user may batch process and save all samples by choosing from the menu Menu – Accept All Chromatograms. The samples listed in the ‘Choose a Sample’ grid will be processed sequentially and application-optimized chromatographic data will be stored in the GMSU data store. See Section 15.1 for further details.

13 Hepatic Clearance Calculator – Results

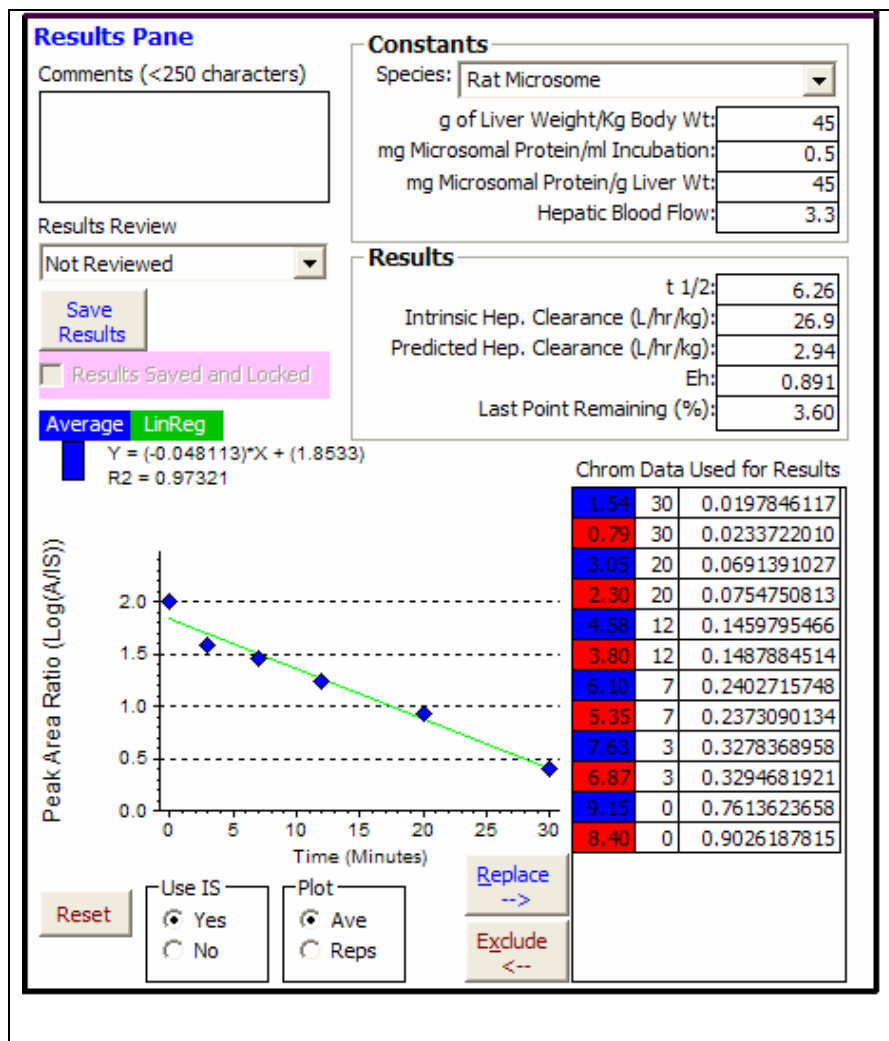
The Hepatic Clearance Calculator Results bottom right pane allows users to perform several functions:

- 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.

13.1 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 regression plot shows plotted data. Users may expand either axis by clicking/dragging the axis.
- The 'Chrom. Data Used for Results' shows the retention data, time point, and peak area or ratio data for each data point.
- 'Reset' button returns plot to full scale on both axes.
- '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 19 Hepatic Clearance Results Pane Overview

The following describes the functions available in the Hepatic Clearance Results pane.

13.2 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.

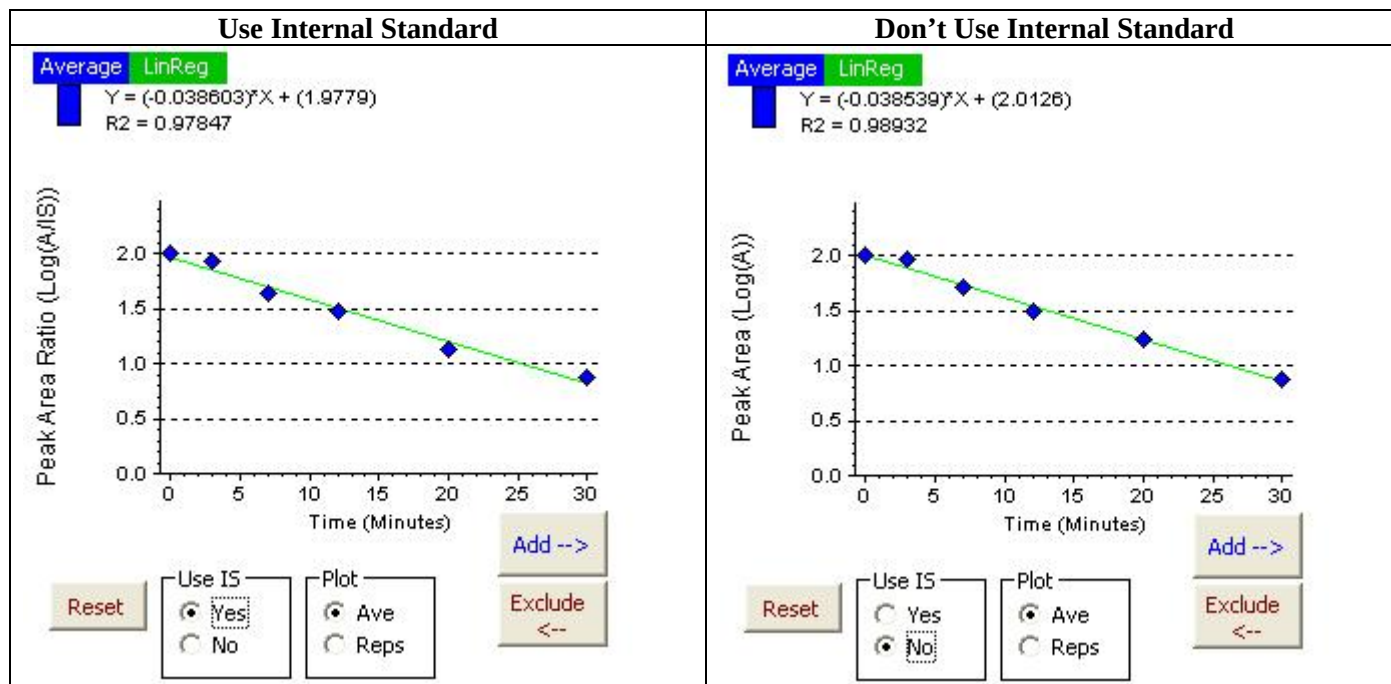


Figure 20 Use IS Frame Options

13.3 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.

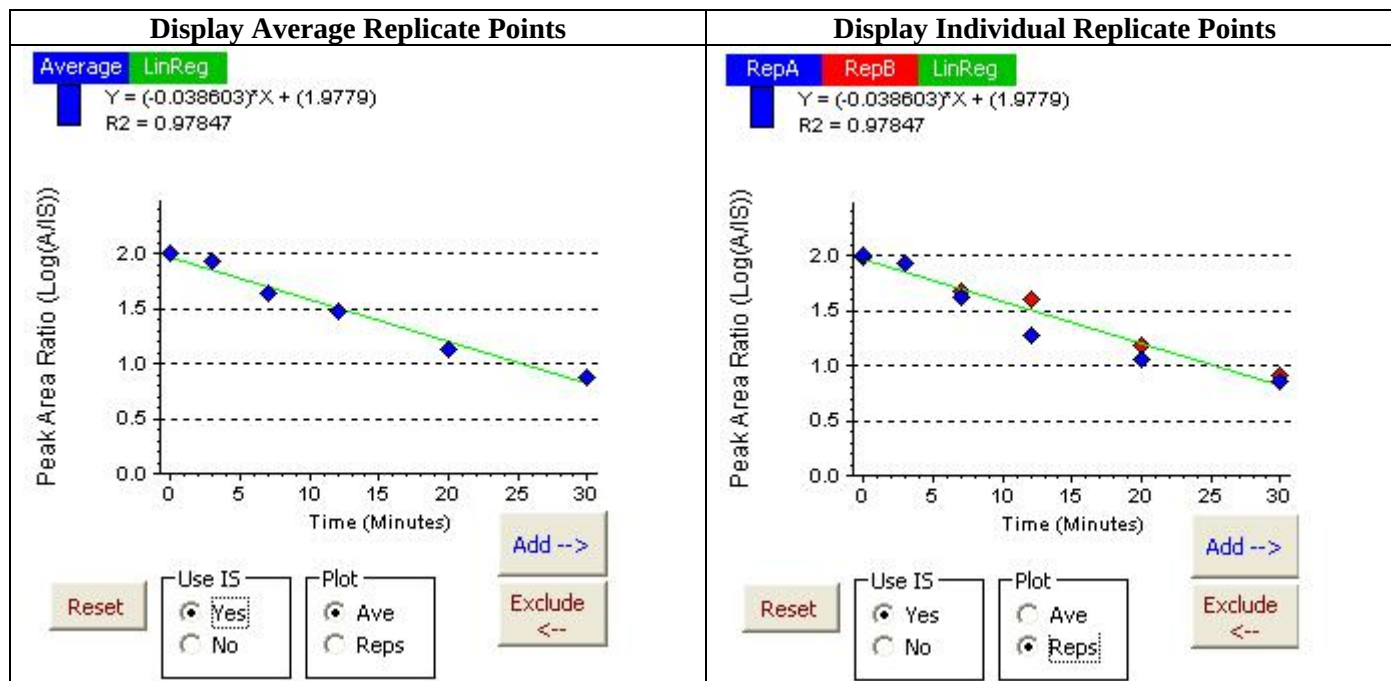


Figure 21 Plot Frame Options

13.4 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
Constants Species: Rat Microsome g of Liver Weight/Kg Body Wt: 45 mg Microsomal Protein/ml Incubation: 0.5 mg Microsomal Protein/g Liver Wt: 45 Hepatic Blood Flow: 3.3 Results t 1/2: 7.80 Intrinsic Hep. Clearance (L/hr/kg): 21.6 Predicted Hep. Clearance (L/hr/kg): 2.86 Eh: 0.867 Last Point Remaining (%): 6.95	Constants Species: Mouse Microsome g of Liver Weight/Kg Body Wt: 87.5 mg Microsomal Protein/ml Incubation: 0.5 mg Microsomal Protein/g Liver Wt: 45 Hepatic Blood Flow: 5.4 Results t 1/2: 7.80 Intrinsic Hep. Clearance (L/hr/kg): 42.0 Predicted Hep. Clearance (L/hr/kg): 4.78 Eh: 0.886 Last Point Remaining (%): 6.95

Figure 22 Species Constants Settings

13.5 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 30 minute replicate in Figure 23 is to be excluded, the user would select the red 30 minute time point in the 'Chrom. Data Used for Results' grid and click the Exclude button.

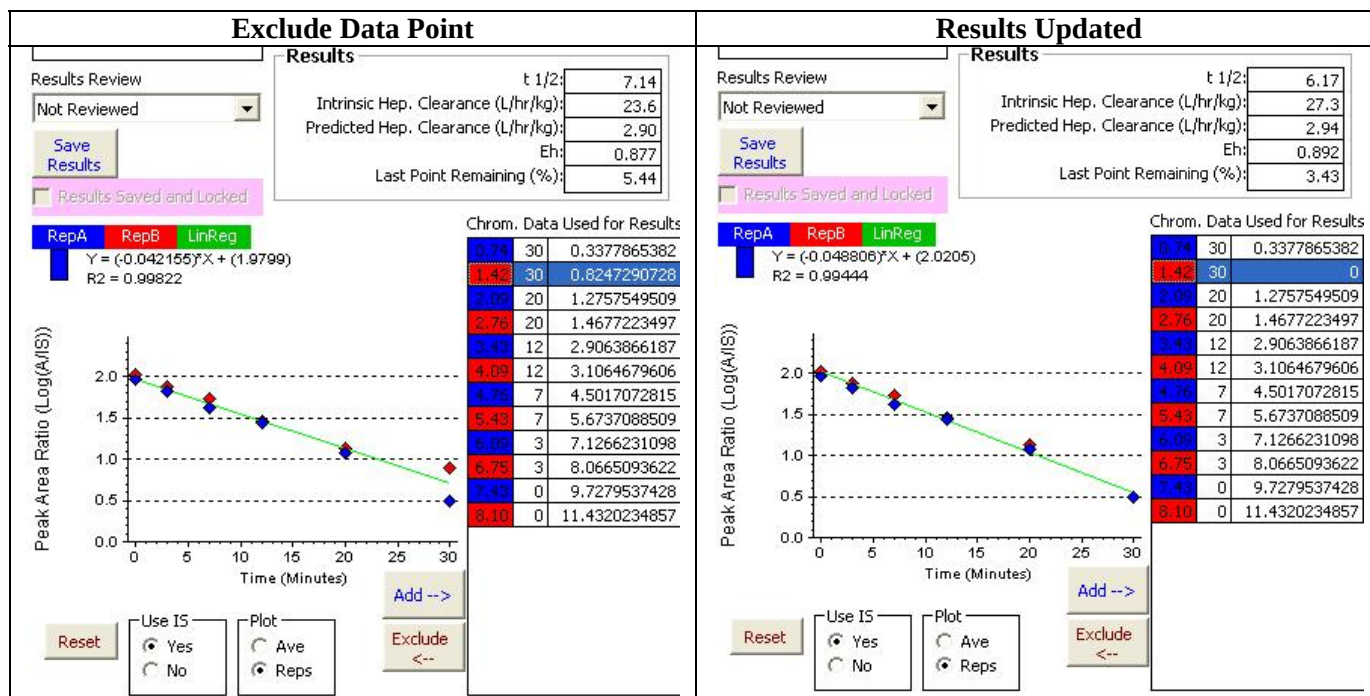


Figure 23 Excluding Points

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

13.6 Restore Data Points

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

13.7 Comments

Users may enter comments about the sample. The comments field may be selected for display in a user defined report (see Section 0).

13.8 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.

13.9 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 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.

14 Hepatic Clearance Calculator – Reports

Users may generate Microsoft® Word or Excel reports based on user-defined choices. The reports module may be activated from the GMSU Console (see Figure 24) or from the Hepatic Clearance Calculator menu

Menu – Generate Reports (see Section 15.1).

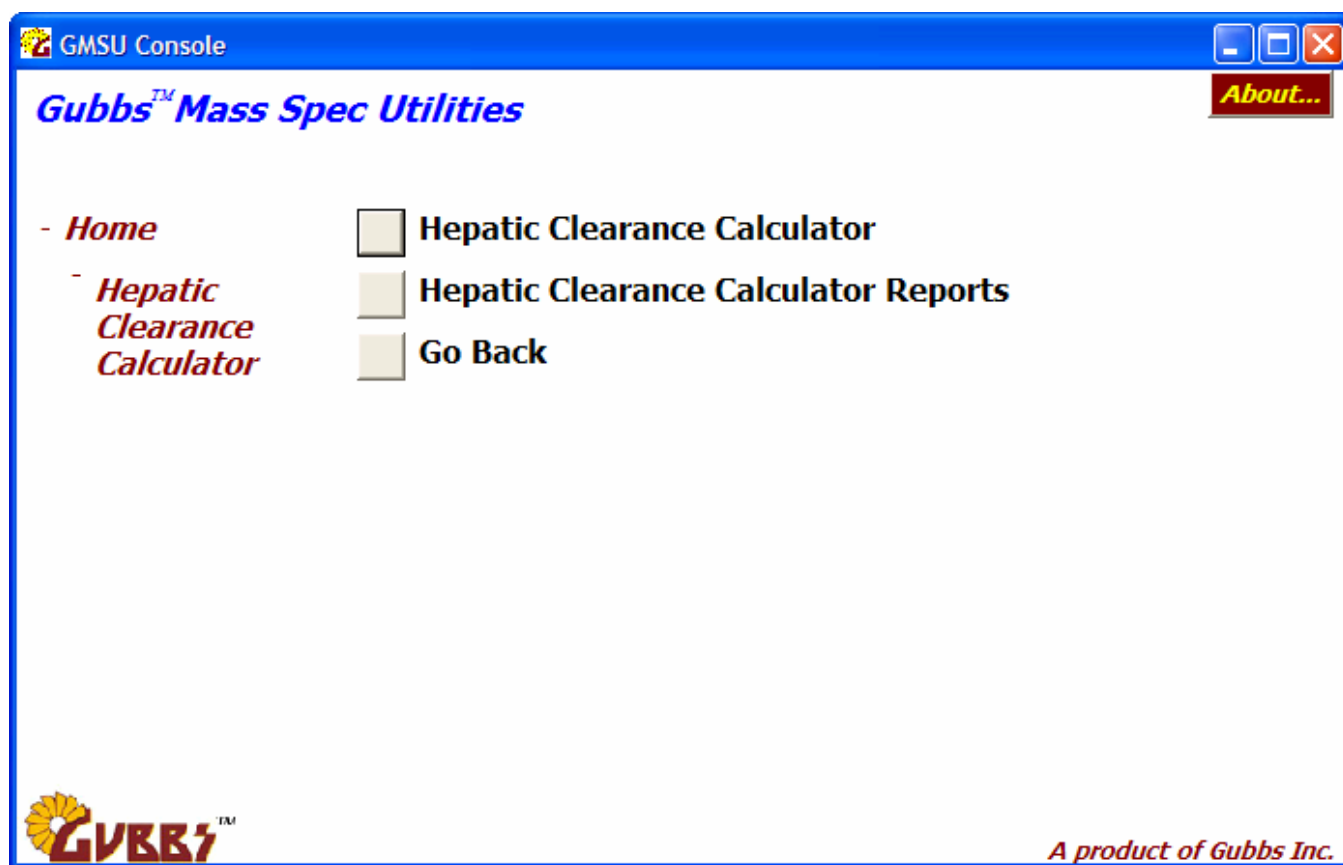


Figure 24 GMSU Console



The default field displayed in a Report include:

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

Additional fields that may be displayed in a Report include:

- Results Review (Acceptance Status)
- Comments
- Sample Number
- Species Experiment

To generate a report:

- User chooses one or more Analyte-specific items and clicks the Add button to add them to the print queue.
- Users may enter an optional Print Job Description.
- User chooses a Results Type filter and any additional report content.
- User chooses a Report Format.
- User clicks Generate Report button

Figure 25 Hepatic Clearance Calculator Reports Module

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 '<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 (see Section **Error! Reference source not found.**) of the Configuration Utility.

15 Hepatic Clearance Calculator – Additional Functions

Additional Hepatic Clearance Calculator functions are found under the menu Menu. The following sections describe these functions.

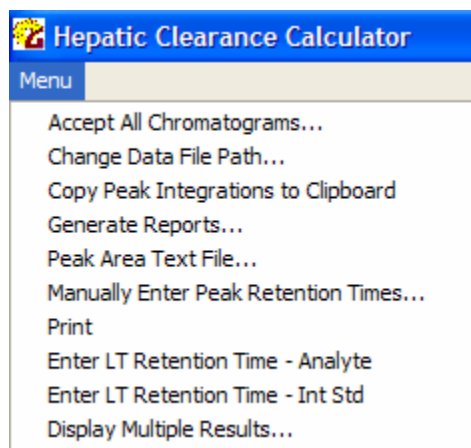


Figure 26 Menu Items

15.1 Accept All Chromatograms

The 'Accept All Chromatograms' function will batch process and save all Chromatographic and Hepatic Clearance Results for all samples displayed in the 'Choose a Sample' grid. This is a quicker way to review and accept data and is useful if the user knows that chromatography was most likely sufficient.

15.2 Change Data File Path

15.3 Copy Peak Integrations to Clipboard

15.4 Generate Reports

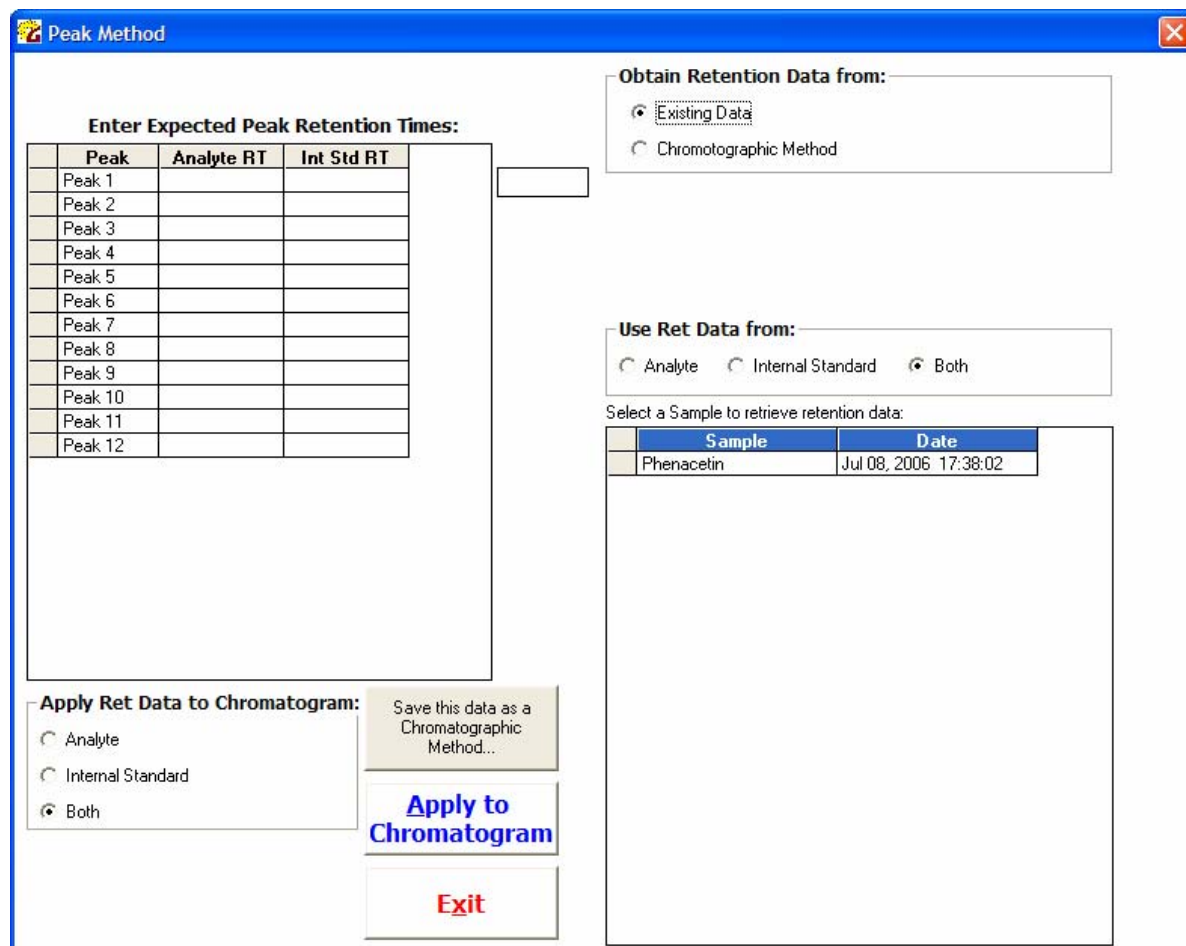
'Generate Reports' will display the Reports window shown in Figure 25.

15.5 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'.

15.6 Manually Enter Peak Retention Times

There are times when the automatic peak identification algorithm of GMSU will not identify peaks properly. This typically occurs when a peak or entire chromatographic signal is close to a signal/noise of approximately two. Users may use the 'Manually Enter Peak Retention Times' function to manually enter the expected peak retention times or generate and apply a new chromatographic method.



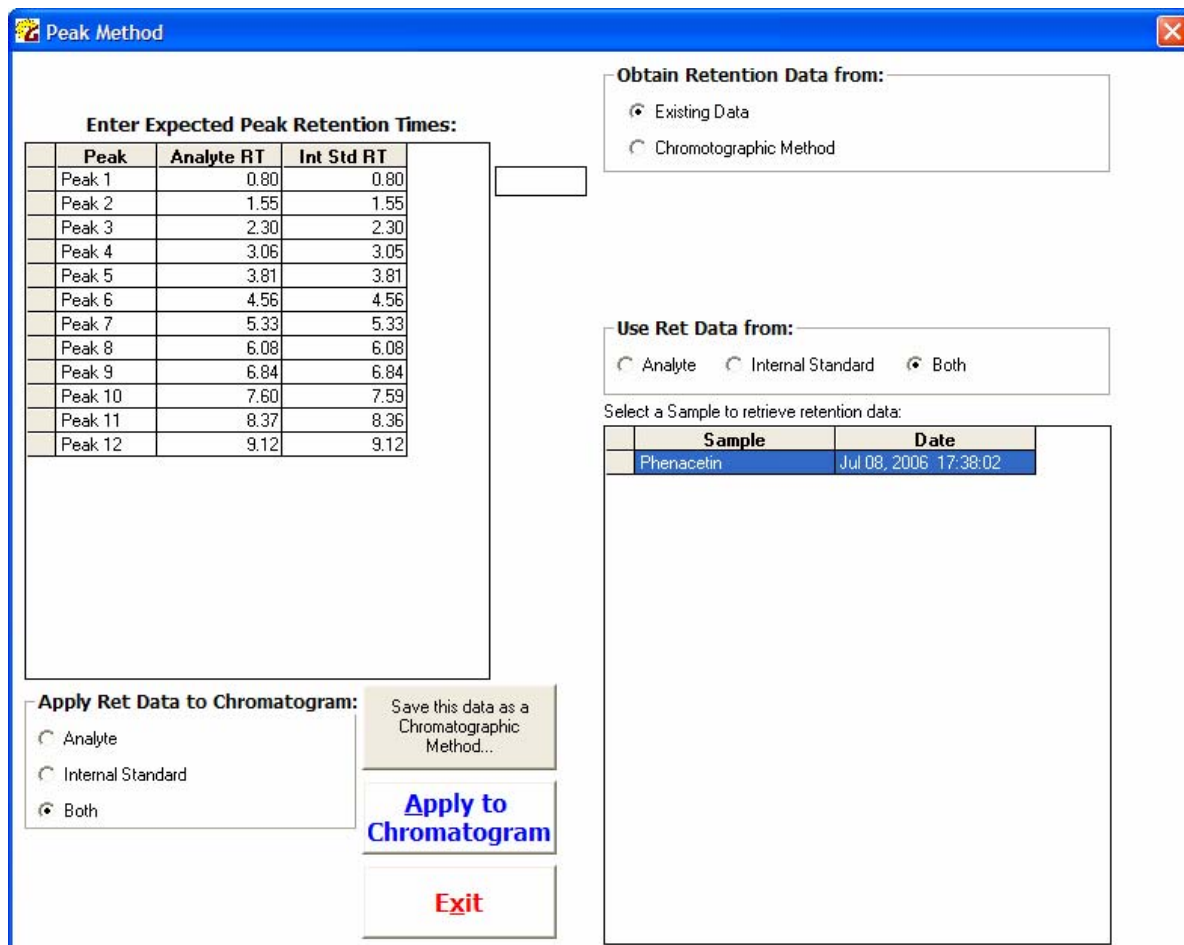
Peak	Analyte RT	Int Std RT
Peak 1		
Peak 2		
Peak 3		
Peak 4		
Peak 5		
Peak 6		
Peak 7		
Peak 8		
Peak 9		
Peak 10		
Peak 11		
Peak 12		

Sample	Date
Phenacetin	Jul 08, 2006 17:38:02

Figure 27 Manually Enter Peak Retention Times

Users may choose to enter retention time for Analyte, Internal Standard, or both, 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' check box located in the 'Obtain Retention Data from:' frame. If the user checks this box, 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 28).



Peak Method

Enter Expected Peak Retention Times:

Peak	Analyte RT	Int Std RT
Peak 1	0.80	0.80
Peak 2	1.55	1.55
Peak 3	2.30	2.30
Peak 4	3.06	3.05
Peak 5	3.81	3.81
Peak 6	4.56	4.56
Peak 7	5.33	5.33
Peak 8	6.08	6.08
Peak 9	6.84	6.84
Peak 10	7.60	7.59
Peak 11	8.37	8.36
Peak 12	9.12	9.12

Obtain Retention Data from:

☒ Existing Data
☐ Chromatographic Method

Use Ret Data from:

☐ Analyte ☐ Internal Standard ☒ Both

Select a Sample to retrieve retention data:

Sample	Date
Phenacetin	Jul 08, 2006 17:38:02

Apply Ret Data to Chromatogram:

☐ Analyte
☐ Internal Standard
☒ Both

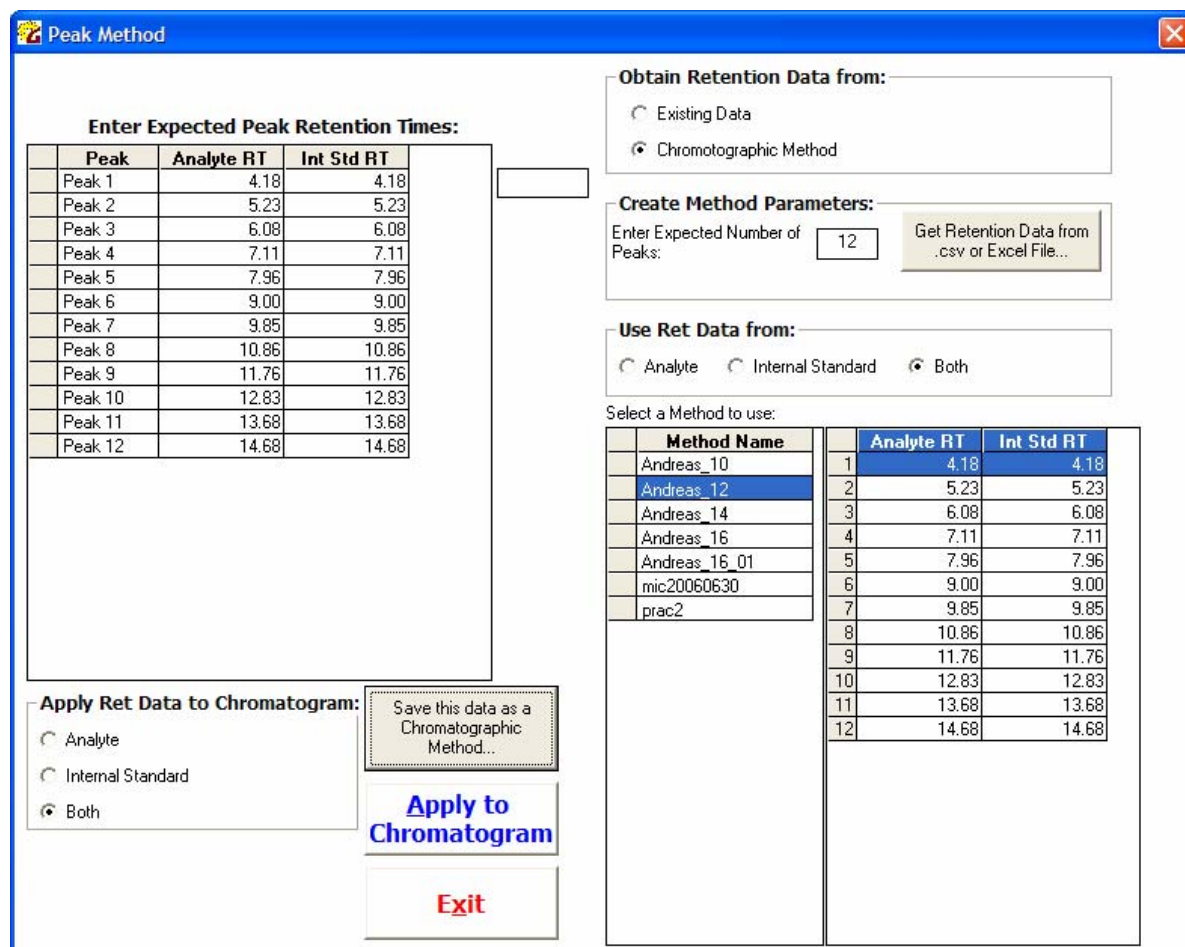
Save this data as a Chromatographic Method...

Apply to Chromatogram

Exit

Figure 28 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' check box located in the 'Obtain Retention Data from:' frame. If the user checks this box, 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 29).



Peak Method

Enter Expected Peak Retention Times:

Peak	Analyte RT	Int Std RT
Peak 1	4.18	4.18
Peak 2	5.23	5.23
Peak 3	6.08	6.08
Peak 4	7.11	7.11
Peak 5	7.96	7.96
Peak 6	9.00	9.00
Peak 7	9.85	9.85
Peak 8	10.86	10.86
Peak 9	11.76	11.76
Peak 10	12.83	12.83
Peak 11	13.68	13.68
Peak 12	14.68	14.68

Obtain Retention Data from:

☐ Existing Data

☒ Chromatographic Method

Create Method Parameters:

Enter Expected Number of Peaks:

Use Ret Data from:

☐ Analyte ☐ Internal Standard ☒ Both

Select a Method to use:

Method Name	Analyte RT	Int Std RT
Andreas_10	4.18	4.18
Andreas_12	5.23	5.23
Andreas_14	6.08	6.08
Andreas_16	7.11	7.11
Andreas_16_01	7.96	7.96
mic20060630	9.00	9.00
prac2	9.85	9.85
	10.86	10.86
	11.76	11.76
	12.83	12.83
	13.68	13.68
	14.68	14.68

Apply Ret Data to Chromatogram:

☐ Analyte

☐ Internal Standard

☒ Both

Figure 29 Use peaks from an existing chromatographic method

After retention data has been entered:

- the user clicks on the 'Apply to Chromatogram' button
- the retention data is entered into the appropriate chromatographic data grids
- the GMSU integration algorithm is called to calculate peak areas

15.7 Print

The print function will print the entire window as shown.
This function is not available at this time.

15.8 Enter LT Retention Time – Analyte

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

15.9 Enter LT Retention Time – Int Std

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

15.10 Display Multiple Results

This function will use MS Excel to plot and print regression results of several analytes on one regression plot.

This function is not available at this time

16 Permeability Calculator

The Permeability Calculator section is under construction. At this time, figures will be shown.

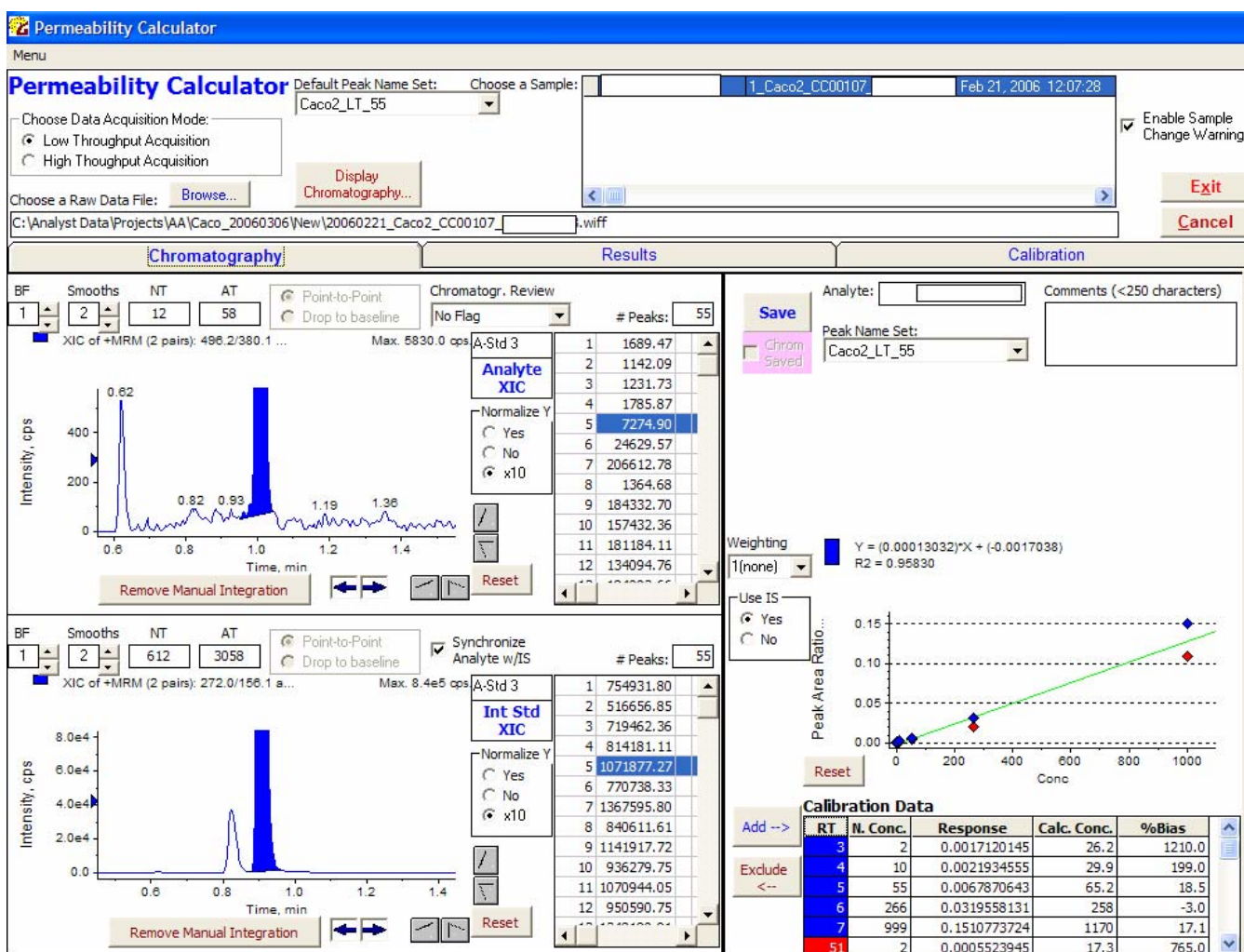


Figure 30 Permeability Calculator – Chromatography Tab

Permeability Calculator

Menu

Permeability Calculator Default Peak Name Set: Choose a Sample: Feb 21, 2006 12:07:28
Caco2_LT_55

Choose Data Acquisition Mode:
☒ Low Throughput Acquisition
☐ High Throughput Acquisition

Display Chromatography...

Choose a Raw Data File:

C:\Analyst Data\Projects\AA\Caco_20060306\New\20060221_Caco2_CC00107_ML00649843.wiff

Chromatography **Results** **Calibration**

Save

☐ Results Saved and Locked

Comments (<250 characters)

AQL=999
BQL=2

Select an Assay Time Point Set:
Default

Select a Papp Constant Set:
Default

Apical Dosing (mL)	0.2
Basal Dosing (mL)	0.12
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

A/B Assignment:
☐ DA -> RB ☐ DB -> RA ☒ Both

20060221_Caco2_CC00107

Raw Data	Conc.	pmole	RTime	Anal.
Blank	30.24632		1	1
Blank	30.03606		2	1
A-Std 1	26.21087		3	1
A-Std 2	29.90562		4	1
A-Std 3	65.15424		5	7
A-Std 4	258.2842		6	24
A-Std 5	1172.354		7	206
Blank	25.531		8	1
DA-0	1251.746	150.209E	9	184
DA-0	1303.334	156.400E	10	157
DA-0	1311.276	157.3531	11	181
DA-60	1095.523	131.4627	12	134
DA-60	1060.365	127.2437	13	184
DA-60	1066.7	128.004C	14	135
DB-0	1591.891	191.0265	15	218
DB-0	1615.146	193.8177	16	175

Donor Side Values

	DA	DB
Calc. Conc. Dx-0	1288.785	1580.217
Calc. Conc. Dx-60	1074.196	1835.502
Conc. (uM)	1.5384615	1.5384615
nmole	4	2.4
Calc. nmole Dx-0	3.350841	2.4651385
Calc. nmole Dx-60	2.7929096	2.8633831

Receiver Side: DA -> RB

Descr.	Ave	SEM	Ex1	Rep 1	Ex2	Rep 2	Ex3	Rep 3	Exclude Points
15 min	1.819122	7.182012		1.961045		1.767311		1.72900E	
30 min	4.101902	0.24840E		4.59852E		3.841712		3.86546E	
45 min	8.320687	0.93715C		10.18931		7.26022C		7.512523	
60 min	15.21964	1.356443		17.49843		15.3550E		12.80543	
Slope **	17.76814	1.770407		20.88117		17.6727C		14.75053	
R2	0.945313			0.960982		0.910307		0.964649	
Papp*	0.747817	7.45121C		0.878837		0.74380C		0.620813	
Slope-2pt	22.2354E								
Papp-2pt*	0.93583E								

Receiver Side: DB -> RA

Descr.	Ave	SEM	Ex1	Rep 1	Ex2	Rep 2	Ex3	Rep 3	Exclude Points
15 min	7.519797	0.716263		6.849754		8.95135C		6.758287	
30 min	21.75742	2.44543E		17.23523		25.6318E		22.4051E	
45 min	57.13944	8.144904		41.13671		62.5039E		67.77764	
60 min	78.5507E	7.040952		66.0044E		79.2860E		90.3617E	
Slope **	99.3899E	10.94921		80.54622		99.1504E		118.4731	
R2	0.970777			0.971112		0.97427C		0.96695C	
Papp*	4.18307E	0.46082E		3.389992		4.17300C		4.986243	
Slope-2pt	113.586E								
Papp-2pt*	4.780583								

Summary

	Papp (4-points) (x10 ⁻⁶ cm/s)	SEM (x10 ⁻⁶)	Papp (2-points) (x10 ⁻⁶ cm/s)	MB (%)	Sink Condition (%)
DA-to-RB	0.748	0.1	0.936	83.8	83.3
DB-to-RA	4.183	0.5	4.781	119.3	116.2
Ratio (DB/DA)	5.6		5.1		

Figure 31 Permeability Calculator – Results Tab



Permeability Calculator

Menu

Permeability Calculator Default Peak Name Set: Choose a Sample: 1_Caco2_CC00107 Feb 21, 2006 12:07:28

Choose Data Acquisition Mode:
☒ Low Throughput Acquisition
☐ High Throughput Acquisition

Display Chromatography...

Choose a Raw Data File: Browse... C:\Analyst Data\Projects\AA\Caco_20060306\New\20060221_Caco2_CC00107.wiff

Enable Sample Change Warning ☒

Exit Cancel

Chromatography **Results** **Calibration**

Chromatography Calibration Values

Apply to Chromatography

Enter Number of Calibration Points: 5

Concentrations

	Current
# Stds	5
Std 1	2
Std 2	10
Std 3	55
Std 4	266
Std 5	999

Caco Calibration Values

Time Point Set	Time Points
Default	15
Set_204060	30
306090120	45
Prac1	60

Caco Peak Name Sets

Peak Name Set	Peak Names
18A	1 RB-20 Set204060
18B	2 RB-20 Set204060
27B_5Calibr	3 RB-20 Set204060
27B_6Calibr	4 RB-40 Set204060
48AB_5Calibr	5 RB-40 Set204060
48AB_Prac01	6 RB-40 Set204060
52AB_5Calibr	7 RB-60 Set204060
54AB_2	8 RB-60 Set204060
54AB_5Calibr	9 RB-60 Set204060
aaaaaaa	10 Blank Set204060
aaaz	11 B-Std 1 Set204060
Caco2_HT_55	12 B-Std 2 Set204060
Caco2_LT_55	13 B-Std 3 Set204060
Test	14 Blank Set204060
Test1	15 DA-60 Set204060
zzz	16 DA-60 Set204060
zzzzzzzz	17 DA-0 Set204060
zzzzzzzzZ	18 Blank Set204060

Figure 32 Permeability Calculator – Calibration Tab



17 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.