MS to 13C

Overview

Measurement data from the MS analysis of labelling experiments is used in the modelling of metabolic flux networks. Several steps are required to provide this data from chromatogram to flux optimization. In order to facilitate high throughput labelling experiments the majority of processing steps have been automated, providing a flow from performing the measurements through to a form usable by a variety of ^{13}C metabolic flux analysis software.

Where to Start

Step I: Create A New MassLynx Project

To keep your data organized *MassLynx* data is organized in a project folder (**`.PRO`**). This folder contains all of the necessary data and configuration files to process a given set of MS measurement data. It is for this reason that the first step is to create a project and not to acquire/convert the data. By creating a new *MassLynx* project we create the place where the data is to be stored.

You may be wondering why not put all data into one project, or how to decide which data should be grouped together into one project. The key feature in determining which data should be grouped together into a single project is based on how they are to be processed. The key variable in this is the ***retention time*** in the column used to generate the data. All sets of measurement data with the same retention time may be grouped together in the same project. Other variables such as replicates or amino acid groups may be naturally organised within *MassLynx*.

By default *MassLynx* will store these project folders in the *MassLynx* program folder. Instead I would suggest creating a folder specifically for these projects on your desktop.

> Throughout this set of instructions all *MassLynx* project data is stored in the folder "`GCMS Projects`" on the desktop.

1. Start the *MassLynx* program:

   To start the program you can double click on the desktop icon (`MassLynx v4.1`),

   or go to the program link through the *Windows Start menu*, on this system it is:

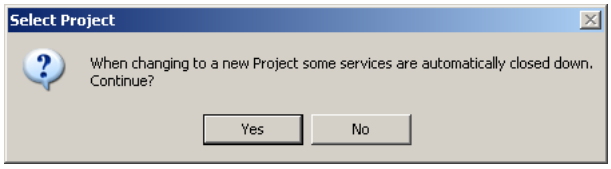
   `Start > Programs > MassLynx > MassLynx v4.1`

2. Start the *MassLynx* project wizard:

   To create a new project use the project wizard, under the *MassLynx* file menu,

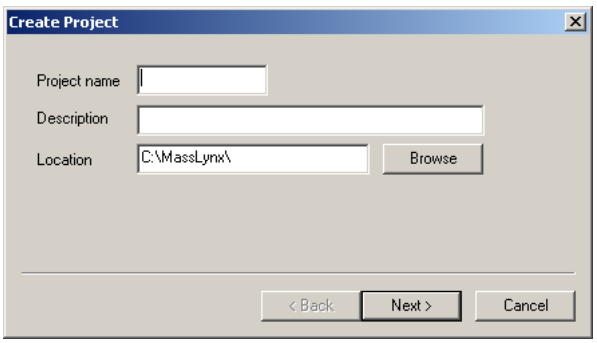
   `File > Project Wizard ...`

   Before starting the wizard a warning message will popup to make sure you want close the existing project:

   - Click the **`Yes`** button.

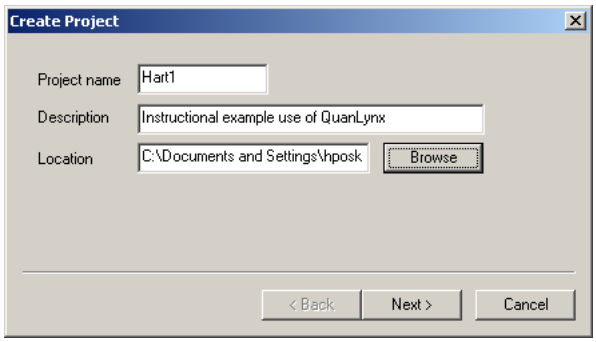
   Next the "Create Project" wizard will appear:

   - In the field next to "*Project name*" type a name for this project. This name will be used to create the project folder.
   - The "*Description*" field allows you to enter a more meaningful description for this set of experiments.
   - Finally the "*Location*" field allows you to specify where the project will be stored.

   Next to the "*Location*" field is the **`Browse`** button. Clicking this button opens a "*Browse for Folder*" dialog box. In most cases it is easier to select the location in this way, rather than typing the full path directly into the "*Location*" field.

3. Fill in the wizard:



For this example my project name is Hart1, located in:

`C:\Documents and Settings\user\Desktop\GCMS Projects`

- Enter a project name.
- Enter a project description.
- Click the **`Browse`** button, navigate to the desired folder to select the project location.

Click the **`Next`** button to continue.

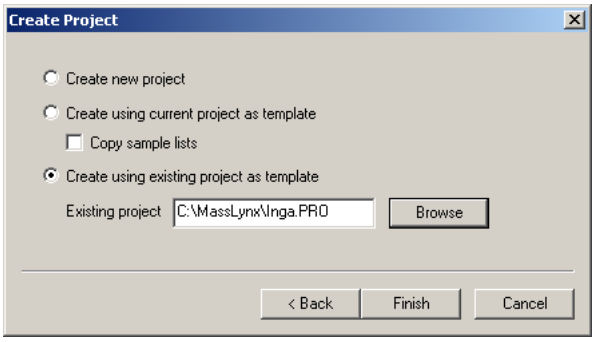
The second part of creating a new project allows you to select how you want to create it. The three options allow you to either create a completely empty project (*Create new project*), or to create one using some existing configuration files. In the latter case we can select to get these files from the ***current*** project (if there is a current one) or from any ***existing*** project.

- In this example we started with no current project, but it is useful to copy existing methods for processing the data, and editing it, rather than creating a new one.
- You may always delete any of these files if not wanted.

4. Select the option to create from an existing template:

Click the **`Browse`** button and navigate to the desired project. This example is based on an existing set of measurement data from `Inga.PRO`, so it is useful to use this project as the template:

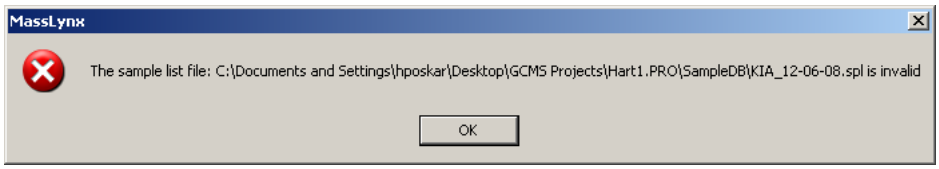
`C:\MassLynx\Inga.PRO`



- There is the option to copy the Sample Lists as well as other configuration files, typically this is not wanted, so deselect the option to “Copy sample lists” - if selected.

Click the **`Finish`** button to create the new Project.

As just mentioned, when you import configuration files from an existing project some information is no longer valid. Since we did not copy the sample lists, the one that was previously used by `Inga.PRO` cannot be opened causing the following message:



Click the **`OK`** button to continue.

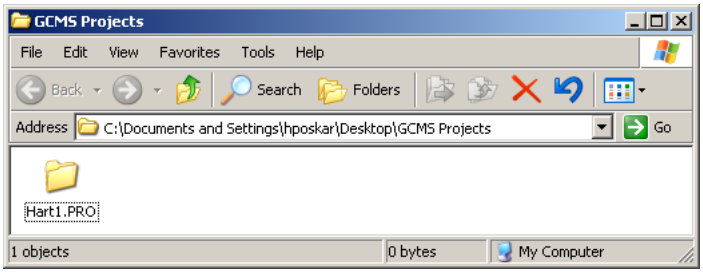
5. Minimize or exit *MassLynx*:

We are finished with MassLynx for the time being but will come back to it later.

The MassLynx Project

Before continuing to the next step, here is an overview of the files and folders in a *MassLynx* project.

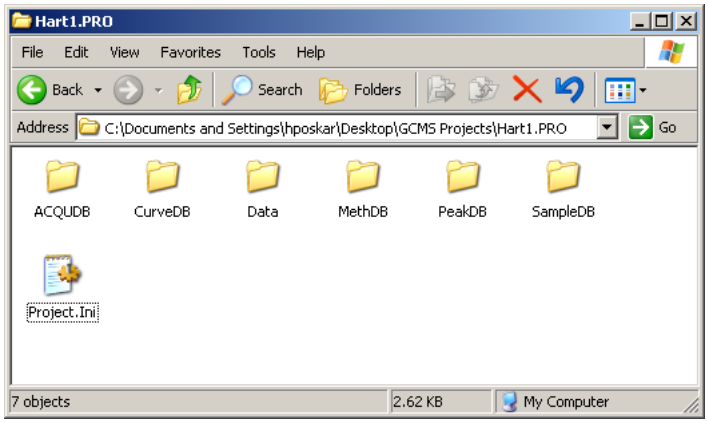
Opening up the GCMS Project folder on my desktop now reveals one project, `Hart1.PRO`:



If we look in this folder we see an additional six folders and one file:

- **ACQUDB** - Acquisition settings files.
- **CurveDB** - Calibration curves (file extension **`.CDB`**).
- **Data** - Measurement data files (file extension **`.RAW`**).
- **MethDB** - Methods for processing data (file extension **`.MDB`**).
- **PeakDB** - Peak lists.
- **SampleDB** - Sample Lists of data files (file extension **`.SPL`**).
- **Project.ini** - Project initialization settings file.

The *MassLynx* project may be saved anywhere on the computer. However all of the associated files for a project must be located in their appropriate folder relative to the project folder.



When a new project is created from an existing template the following files are automatically copied into the new project:

- the acquisition setting files,
- the calibration curves,
- the methods for processing samples, and
- the initialization file.

Now that we have created a new project, the next step is to get the data for the project and store it in the `Data` folder of this project.

Step II: Convert Measurement Data to Generic Format (CDF)

These instructions cover MS measurement data obtained from the following three sources, Agilent, Thermo and Waters, or previously obtained data stored in the ***ANDI/netCDF*** format.

1. Copy the MS data files to the Desktop:

   If the data is on some sort of portable media, start by coping the MS data files to a folder on your desktop.

   For measurement data already in Waters MassLynx format, copy the data into the `Data` folder of your project continue with "Create a MassLynx Sample List" on page 10.

   Otherwise you need to first convert data from it's native file format to an intermediate generic format for MS data, the ***ANDI/netCDF Mass Spectrometry Data Interchange*** format or ***CDF***.

   - For measurement data from Thermo continue where it says: "For Thermo" on page 5.
   - For measurement data from Agilent continue where it says: "For Agilent" on page 7.
   - For measurement data already in the CDF format continue where it says "Convert Generic CDF File to MassLynx" on page 9

   Once in this format Waters provides a program, *DataBridge*, to covert this data into the *MassLynx* format.

For Thermo

For this example the Thermo data files are in the folder: `DKA FEB 09`.

2. Create a folder on the desktop to receive the converted files.

   Right click on the Desktop. From the popup menu select `New` and `Folder`.

   - Name the folder `Thermo Out`.

3. Start the *Xcalibur* program by double clicking on the desktop icon, 

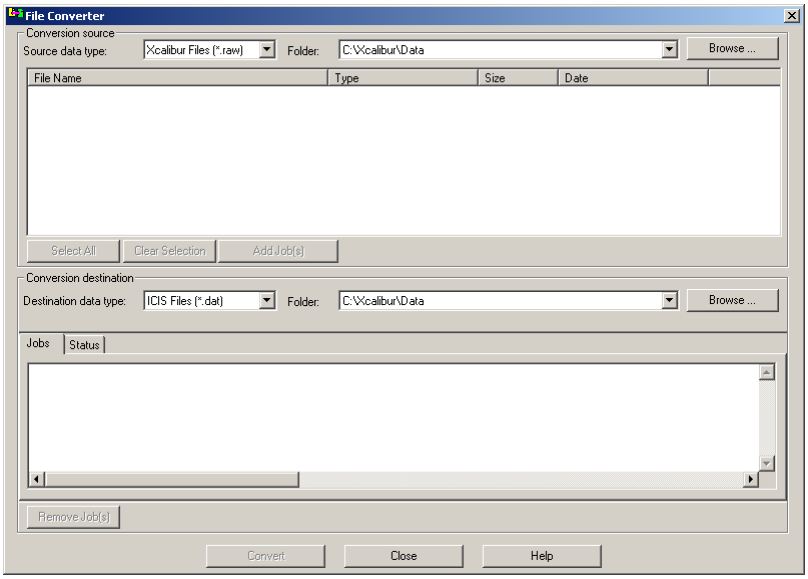
, or go to the program link through the *Windows Start menu*, on this system it is:

   `Start > Programs > Xcalibur > Xcalibur`

4. To export the MS data use the File Converter, under the *Tools* file menu,

   `Tools > File Converter`

   To open the *File Converter* window:

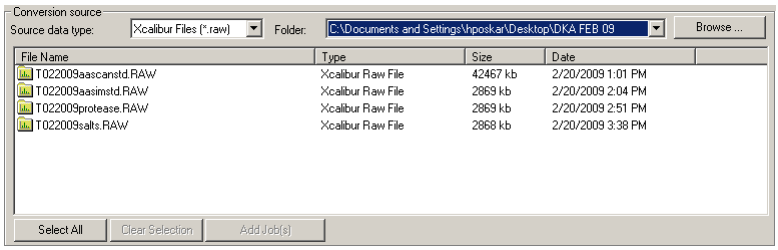


5. Select the data types:

   - In the upper (*Conversion source*) box the *Source Data Type* should already be set to: `Xcalibur Files (*.raw)`. If not select that.
   - In the lower (*Conversion destination*) box the *Destination Data Type* should be set to: `ANDI files (*.cdf)`. If not select that.

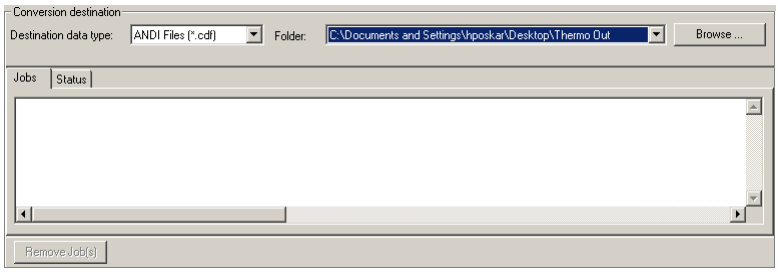
6. In the upper box click on the **Browse** button next to Folder, and select the path:

`C:\Document and Settings\`**`user`**`\Desktop\DKA FEB 09`

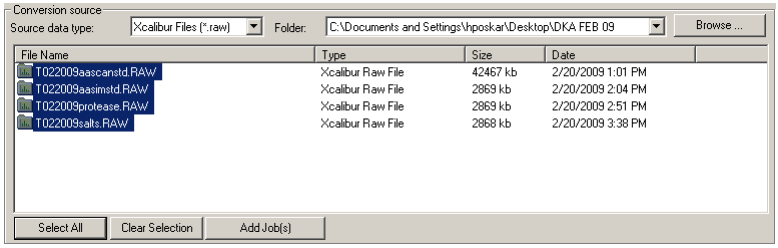


7. In the lower box click on the **Browse** button next to Folder, and select the path:

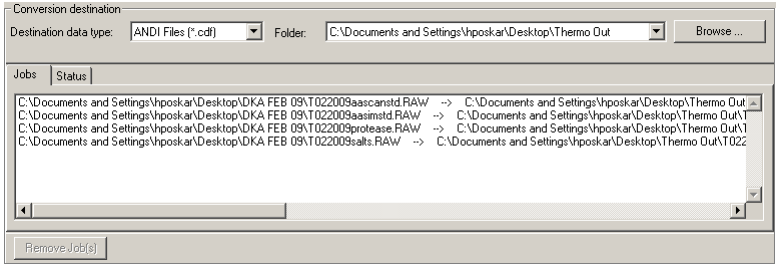
`C:\Document and Settings\`**`user`**`\Desktop\Thermo Out`



8. In the upper box click on the **Select All** button, to select all of the measurement files for conversion.



9. In the upper box click on the **Add Job(s)** button to add the selected measurement files to the lower box for conversion.



10. Click the **Convert** button to export these files.

11. Exit *Xcalibur*.

If you look in the export folder you will see that each dataset now has the **.CDF** extension.

- To finish converting the data continue with: “Convert Generic CDF File to MassLynx” on page 9.

For Agilent

For this example the Agilent data files are in the folder: `120608AAfullabrun2`.

2. Start the *Data Analysis* program by double clicking on the desktop icon, 

, or go to the program link through the *Windows Start menu*, on this system it is:

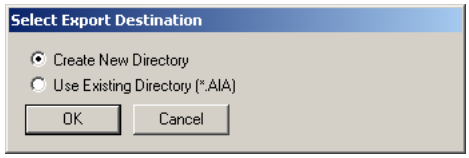
`Start > Programs > MSD ChemStation > Data Analysis`

3. To export the MS data use the Export Data to AIA format, under the *Data Analysis* file menu,

`File > Export Data to AIA format ...`

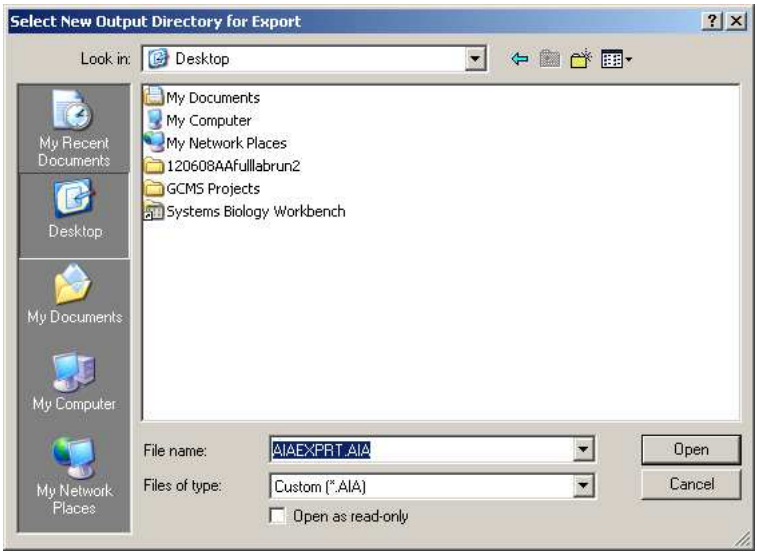
To open the "*Select Export Destination*" dialog box, asking where to save the exported files:

- Select "*Create New Directory*"
- click **OK**.

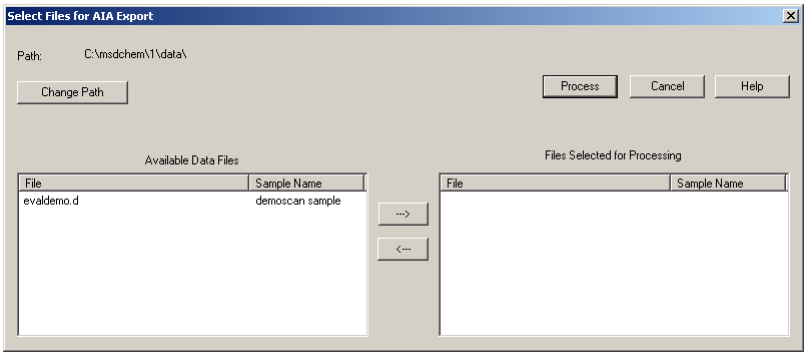


4. This opens the "*Select New Output Directory for Export*" window:

- Select the Desktop folder,
- Leave the default file name (`AIAEXPRT.AIA`), and
- click the **Open** button.

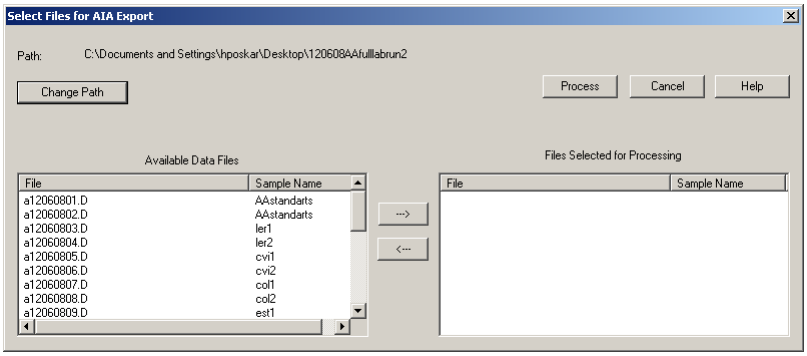


5. This opens the "*Select Files for AIA Export*" which allows you to select the Agilent data files that you want to export:

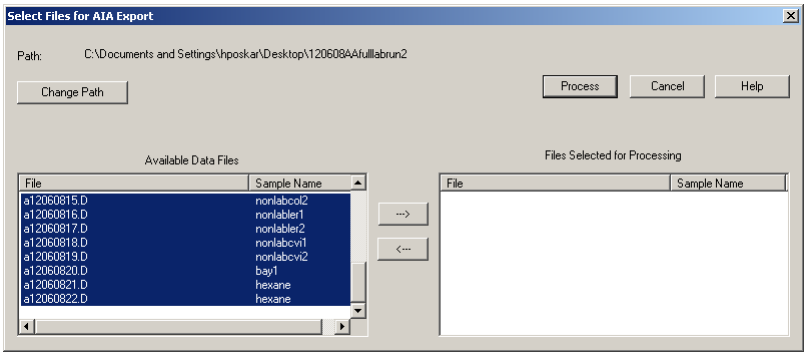


6. First you need to change the path to the folder containing your data. Click the **`Change Path`** button, and select the path:

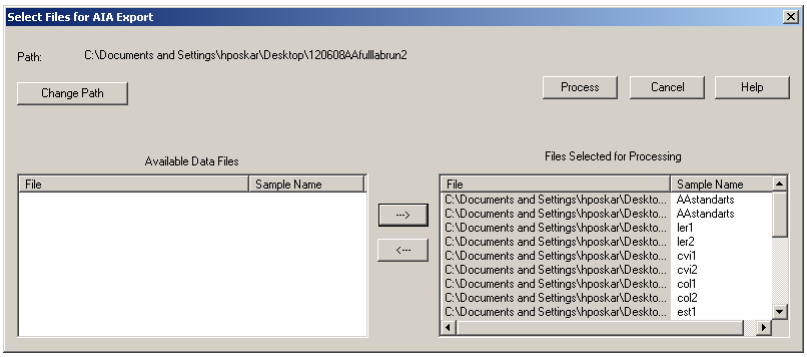
`C:\Document and Settings\`**`user`**`\Desktop\120608AAfullabrun2`



7. On the left hand side (LHS) are the available data files. To select them all, click on the first file in the list, then scroll down to the bottom and holding the Shift key click on the last file. All of the files should now be highlighted:



8. To select these files to be exported, click the arrow button pointing from the available files box to the selected files box (**`-->`**):



9. Click the **`Process`** button to export these files.

10. Exit *Data Analysis*.

If you look in the export folder you will see that each dataset now has the **`.CDF`** extension.

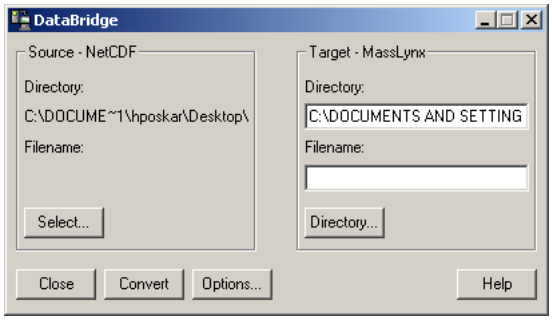
Step III: Convert Generic CDF File to MassLynx

Convert the CDF files for use with *MassLynx*:

1. Start the *DataBridge* program by double clicking on the desktop icon (*DataBridge*),

or go to the program link through the *Windows Start menu*, on this system it is:

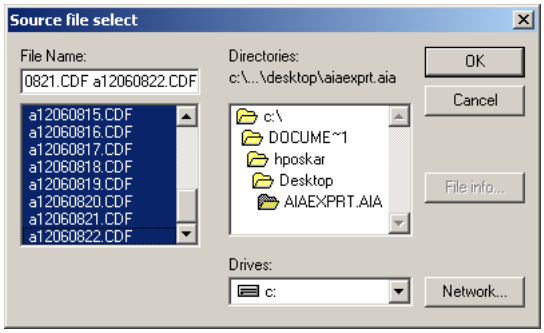
`Start > Programs > MassLynx > DataBridge`



2. To setup the conversion we start with the "Source - NetCDF" box on the LHS, where we specify which files are to be converted.

Click the **`Select...`** button to open the "*Source file select*" window.

- (RHS) Select the source directory, `AIAEXPRT.AIA` on the `Desktop`.
- (LHS) Select all files:
  - click on the first file,
  - scroll to the bottom, and
  - hold the shift key then click on the last file.
- Click the **`OK`** button.



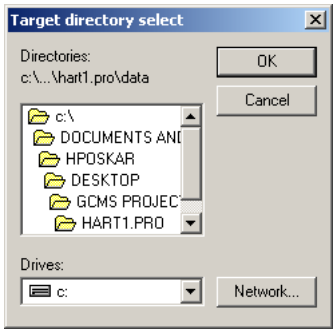
3. In the "Target - MassLynx" box on the RHS now setup the target directory - i.e. the data directory in the *MassLynx* project.

Click the **`Directory...`** button to open the "*Target directory select*" window.

- Select the path to the target folder:

`C:\Documents and Settings\`**`user`**`\Desktop\GCMS Projects\Hart1.PRO\Data`

- Click the **`OK`** button.

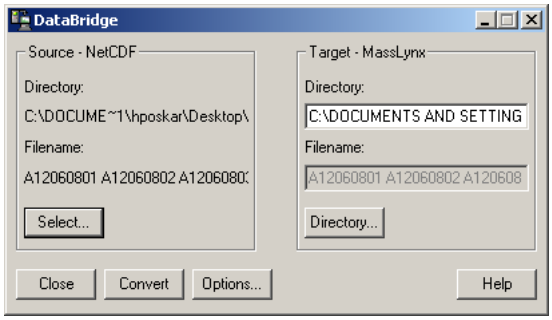


4. With *DataBridge* configured, you are now ready to convert the data:

- Click the **`Convert`** button.

A progress of the conversion of files is displayed. When done:

- Click the **`Close`** button.



Step IV: Create a MassLynx Sample List

Return to the *MassLynx* program. With the measurement data now available we can setup lists of these samples to process.

1. The sample list consists of one or more of the measurement data sets in this project. We will start with a new empty sample list.

   For a new sample list click on the **`New Sample List`** button  or go to the file menu and select new:

   `File > New`

2. Add the first sample file name:

   There is no nice way to add the first sample name to the list. You may cut (from Windows Explorer) and paste the name or you may simply type it in.

   - If your samples are sequentially numbered, the software can automatically generate a given number of new sample entries and name them with sequential numbering.
   - It is not necessary to enter the file extension (**`.RAW`**), but you may if you like.

   In this project there are twenty two sample files sequentially named from `A12060801.RAW` through `A12060822.RAW`. To enter these samples into the sample list start by entering the first file name:

   - Double click the empty field in the "*File Name*" column by sample #1 and type the first name:

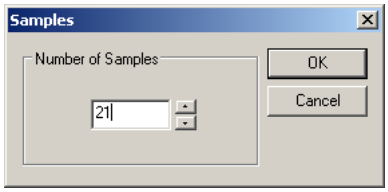
     `A12060801`

3. To add the remaining twenty one samples use the autofill:

   - Right click on the name that you just entered and select **`Add`** from the context menu.

   This opens the "*Samples*" dialog box and allows you to enter the number of samples you want to add.

   - Enter the "*Number of Samples*" - 21.
   - Click the **`OK`** button.



4. Save the sample list:

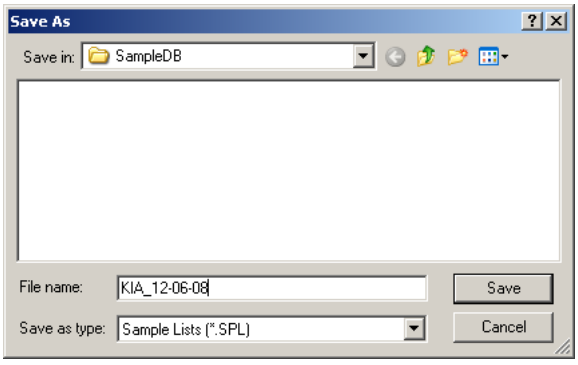
   To save this sample list click on the **`Save Sample List`** button  or go to the file menu and select save:

   `File > Save`

   Since it is a new sample list the "Save As" window opens, by default in the SampleDB folder for your project.

   - In the "*File name*" field enter: `KIA_12-06-08`.
   - Click the **`Save`** button.

   Notice: that after saving the sample list, the title bar now reflects the name of the sample list.



Step V: Edit a MassLynx Sample List

The reason for creating and saving sample lists is to group together data sets to be processed together in a given order. Different sample lists can be made to simply group together different subsets of the project data, or to re-organise the order of the samples.

The first sample list is fine for processing all of the samples in the same order they were generated on the MS. As it turns out these samples represent ten duplicate experiments and two single experiments. We want to specifically process the duplicate data sets to generate ten sets of aggregated measurement data for use with 13C metabolic flux analysis.

To do this we will create a second sample list, making simple changes to the first one in order to facilitate processing by the auto-correction software.

- The *iMS2Flux* software requires replicates of the same experiment to be presented in sequential order. Sample lists provide the mechanism to re-order, if necessary.

The following samples (identified by their sequential number) are duplicates (one standard and nine experimental):

`01&02, 03&04, 05&06, 07&08, 09&10, 12&13, 14&15, 16&17, 18&19, 20&11`

To create this sample list we need to move sample 11 after sample 20, and remove 21 and 22.

- To delete a sample, right click on the sample number and select **`Delete`** from the context menu.
- To edit a name, double click on the file name.

1. Edit the sample list to represent duplicates.

   To make this change, change sample 21 to be 11 and delete sample 22 and the original sample 11.

2. To save this modified sample go to the file menu and select save as:

   `File > Save As...`

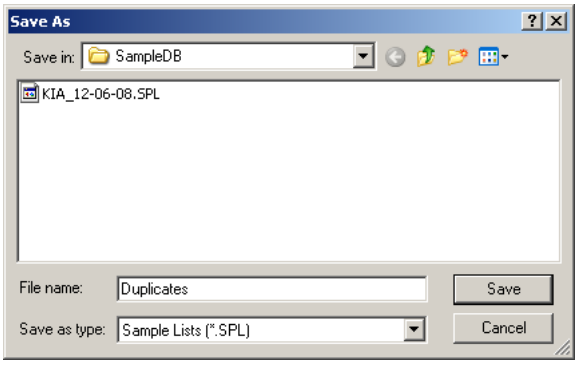
   - Do not select Save, as that will replace the existing sample list, instead of adding to it.

   The "Save As" window opens, by default in the SampleDB folder.

   - In the "*File name*" field enter:

     `Duplicates`

   - Click the **`Save`** button.



Step VI: Edit the Processing Method

Before processing the data we need to create a method to specify how they will be processed.

Setting up the processing method can be one of the most time consuming aspects of this entire process. Instead we have two generic methods (one targeting integrated area, and the other peak height) that encompasses all of the compounds (amino acids) that are generally of interest.

> When a set of samples is processed the output includes two integration values, area and response for each datum. By setting the method to give the Peak integration for the response value the method gives both a Peak and an Area value. Thus the method options for both setups use the Peak integration for the response value.

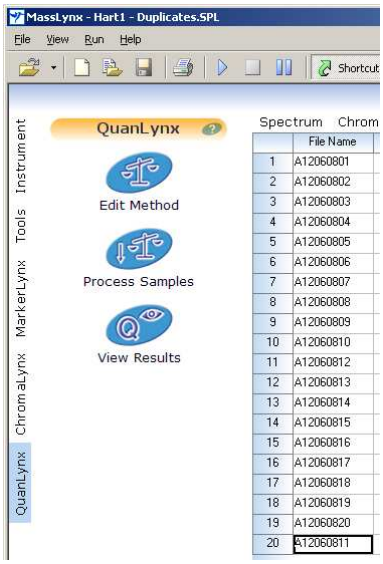
By utilizing these two generic methods, all that is required is to set the retention time for each compounds mass fragment(s). A secondary edit is the actual list of compounds, which may be added to or removed.

This section will concentrate on illustrating these edits, and not to go in depth into the large variety of method options. For more detailed information on the method options please see the Help documentation.

We will describe two methods for editing *QuanLynx* methods, the built in method editor and *Excel*.

First the QuanLynx Method Editor:

1. The Waters software incorporate several tools, make sure that the QuanLynx tool is selected.

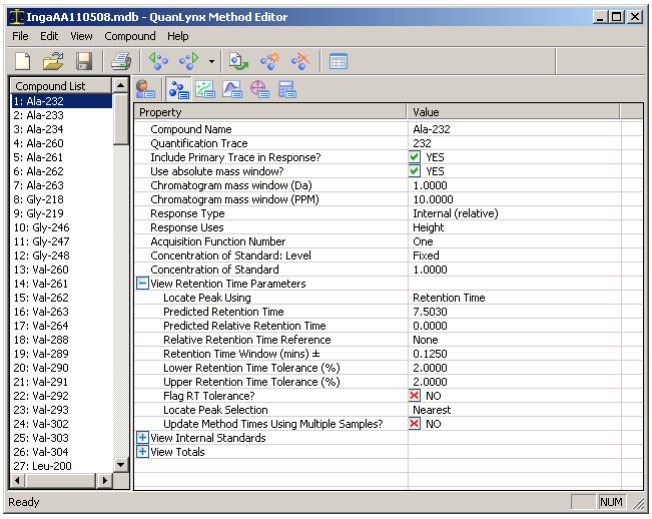


You will see the QuanLynx tool tab as shown on the right with the following three options:

- `Edit Method`,
- `Process Samples`, and
- `View Results`.

2. To start the method editor click on the **`Edit Method`** icon:

This opens the "*QuanLynx Method Editor*":



> The default view for the methods editor is the *Datasheet View*, which is comprised of two main parts - the compound list on the LHS and the compound properties on the RHS.
>
> - User-defined Properties
> - Compound Properties
> - Calibration Properties
> - Integration Properties
> - Targeting Properties
> - Calculation Factors

Changes made to the calibration properties and integration properties are propagated to all compounds. The user-defined properties simply summarize all five of the other properties, but adds no new properties itself.

3. Open the Method:

   to open a method click on the **Open Method** button or go to the file menu and select open:

   `File > Open`

   The "*open*" window opens, by default is in the MethDB folder for your project. Select the method you want to open, and click the **Open** button.

4. To change the retention time:

   In the "*Compound List*" box on the LHS, select the compound you want to edit.

   Select the **Compound Properties** .

   On the RHS, under the "*Property*" column go to the "*View Retention Time Parameters*" section.

   - If there is a "+" to the left of it, click on the "+" to expand the section.

   Next to the field "*Predicted Retention Time*" enter the retention time in the "*Value*" column.

5. To add a new compound:

   Click the **Add New Compound** button: , or go to the file menu and select add:

   `File > Add`

   This adds a new compound with all of the same settings as the other compounds. You only need to edit the compound name, mass and retention time. These can all be set under the Compound Properties:

   - The name is entered as the value for the "Compound Name" property.
   - The mass is entered as the value for the "Quantification Trace" property.
   - Follow step 1to set the retention time.

6. To delete a compound:

   In the "*Compound List*" box on the LHS, select the compound you want to delete, then click the **Delete Selected Compound** button: , or go to the file menu and select delete:

   `File > Delete`

7. To save the method:

   To save changes to an existing method click on the **Save Method** button or go to the file menu and select save:

   `File > Save`

   To save the modified method as a new method file go to the file menu and select save as:

   `File > Save As...`

   The "Save As" window opens, by default in the MethDB folder.

   - In the "*File name*" field enter the new name for this method.
   - Click the **Save** button.

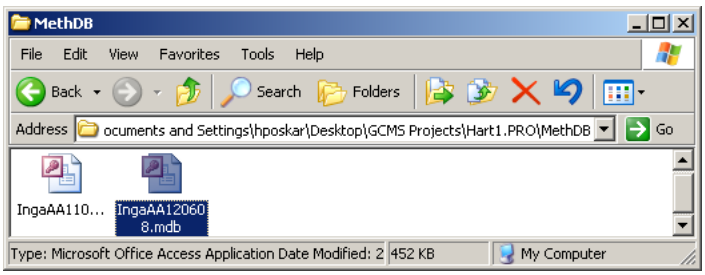
Second Using Excel to Edit the Method

The main reason for using excel to edit the method is that it is much quicker to perform large numbers of changes, such as entering a complete set of retention times for hundreds of compounds.

- The trade-off is that you must be more careful and work with the file manually.

1. Change the method file extension (**MDB**) to a text file (**TXT**):

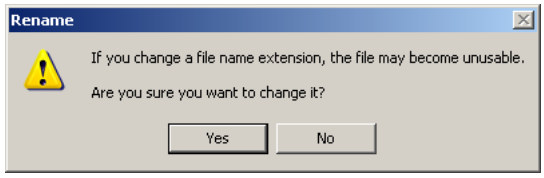
Open the `MethDB` folder for the project:



Change the file extension of the method `IngaAA120608:`

`from 'mdb' to 'txt'.`

- When you do this a popup appears asking if you are sure that you want to do this.
- Click the **`Yes`** button.
  The file should now appear as:



2. Open the method using *Excel*:

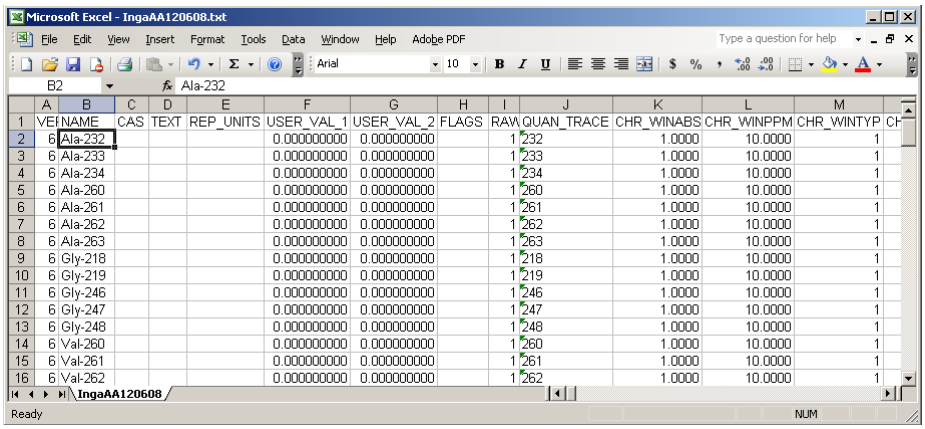
Right click on the re-named method and select:

`Open With > Microsoft Office Excel`

- If Excel is not listed, then select:

`Open With > Choose Program...`

to manually choose to open this file with the *Excel* program.



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	VEI	NAME	CAS	TEXT	REP_UNITS	USER_VAL_1	USER_VAL_2	FLAGS	RAW	QUAN_TRACE	CHR_WINABS	CHR_WINPPM	CHR_WINTYP
2	6	Ala-232				0.000000000	0.000000000		1	232	1.0000	10.0000	1
3	6	Ala-233				0.000000000	0.000000000		1	233	1.0000	10.0000	1
4	6	Ala-234				0.000000000	0.000000000		1	234	1.0000	10.0000	1
5	6	Ala-260				0.000000000	0.000000000		1	260	1.0000	10.0000	1
6	6	Ala-261				0.000000000	0.000000000		1	261	1.0000	10.0000	1
7	6	Ala-262				0.000000000	0.000000000		1	262	1.0000	10.0000	1
8	6	Ala-263				0.000000000	0.000000000		1	263	1.0000	10.0000	1
9	6	Gly-218				0.000000000	0.000000000		1	218	1.0000	10.0000	1
10	6	Gly-219				0.000000000	0.000000000		1	219	1.0000	10.0000	1
11	6	Gly-246				0.000000000	0.000000000		1	246	1.0000	10.0000	1
12	6	Gly-247				0.000000000	0.000000000		1	247	1.0000	10.0000	1
13	6	Gly-248				0.000000000	0.000000000		1	248	1.0000	10.0000	1
14	6	Val-260				0.000000000	0.000000000		1	260	1.0000	10.0000	1
15	6	Val-261				0.000000000	0.000000000		1	261	1.0000	10.0000	1
16	6	Val-262				0.000000000	0.000000000		1	262	1.0000	10.0000	1

3. Freeze the spreadsheet along the compound name:

It is helpful to freeze this column so that as you scroll to different columns (and there are many) you can easily reference the compound you are working with.

- Click on column C, the column after the one you want to freeze (B), or in cell C2.
- Select freeze panes under the windows menu:

`Window > Freeze Panes`

4. Edit the methods data:

   The actual format of the methods file is dBase IV, a database format that has some special characters and handles data in specific ways.

   - You can add or delete compounds by cut/copy/paste of rows, except the last row. You cannot add beyond the last row, nor copy/move it.
   - Some fields that appear to be numbers are actually numeric text characters, in particular notice column `J` in the proceeding figure (232). The green triangle in the upper right hand corner indicates that this number is being treated differently. To explicitly indicate to treat a number as a sequence of characters in Excel you need to type a single quote (`'`) before entering the number.

   The fields that you will want to edit are:

   - column `B` - the compound name,
   - column `J` - the compound mass (entered as character string),
   - column `HA` - repeat the compound name exactly as entered in column `B`, and
   - column `AM` - the retention time (entered as a number).

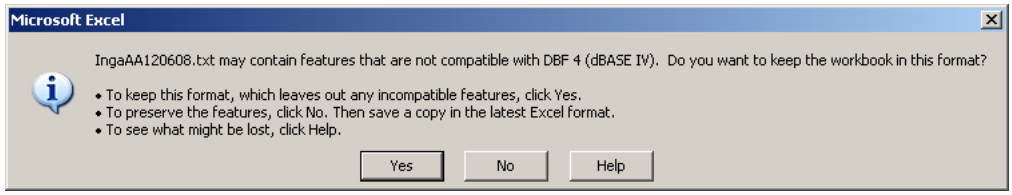
   Columns `B`, `J` and `HA` only need to be modified when you add new compounds.

5. Save the dBase file in Excel:

   To save the modified method click on the **`Save`** button  or go to the file menu and select save:

   `File > Save`

   A warning message appears to inform you that you are saving this as a dBase file and may lose any Excel specific formatting.

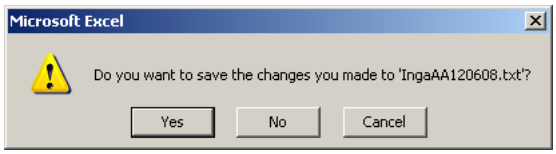


   - Click the **`Yes`** button.

6. Close, and do not save again.

   Exit Excel. You will be prompted to save this file again

   - Click the **`No`** button.



7. Change the method file extension back to (**`MDB`**) from (**`TXT`**).

   The method file should once again be: `IngaAA120608.mdb`.

Step VII: Process the Samples

You now have the required information to process the twenty data sets:

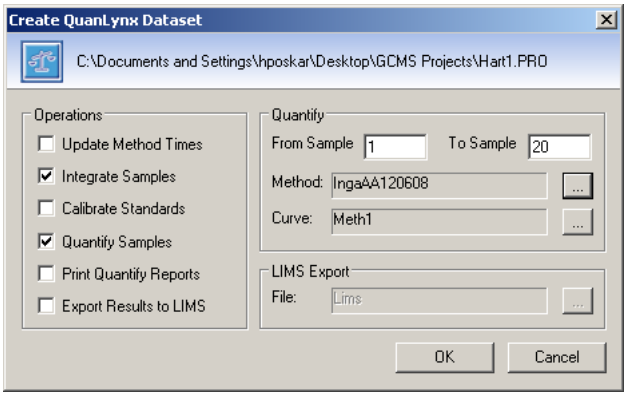
- the experimental data in MassLynx format,
- a sample list with the desired experimental data in the desired order, and
- a method with which to process the data.

1. Ensure that MassLynx (QuanLynx) is selected in the Waters software.

2. To start automatic processing click the **`Process Samples`** button:

To open the "Create *QuanLynx Dataset*" window:

- After the measurement data is processed by QuanLynx the result is called a QuanLynx Dataset.
- By default the open sample list is used, and all samples in the list are selected.
- Also by default the last method and the last curve file used in a processing step is chosen.



There are six processing operations that may be performed:

- **Update Method Times** - Updates the peak retention times of compounds in the Quantify method.
- **Integrate Samples** - Integrates each chromatogram defined in the method and assigns detected peaks to the method compounds.
- **Calibrate Standards** - Generates a calibration curve for each method compound based on samples identifies as calibration standards.
- **Quantify Samples** - Applies the integration results and calibration to the detected peak to produce a calculated concentration for each compound.
- **Print Quantify Reports** - Produces a print out of pre-defined reports for integration and quantitation.
- **Export Results to LIMS** - Produce a report file for use with an LIMS system.

3. Configure the processing:

Select the operations to be performed in the "*Operations*" box on the LHS:

- Integrate Samples, and
- Quantify Samples.

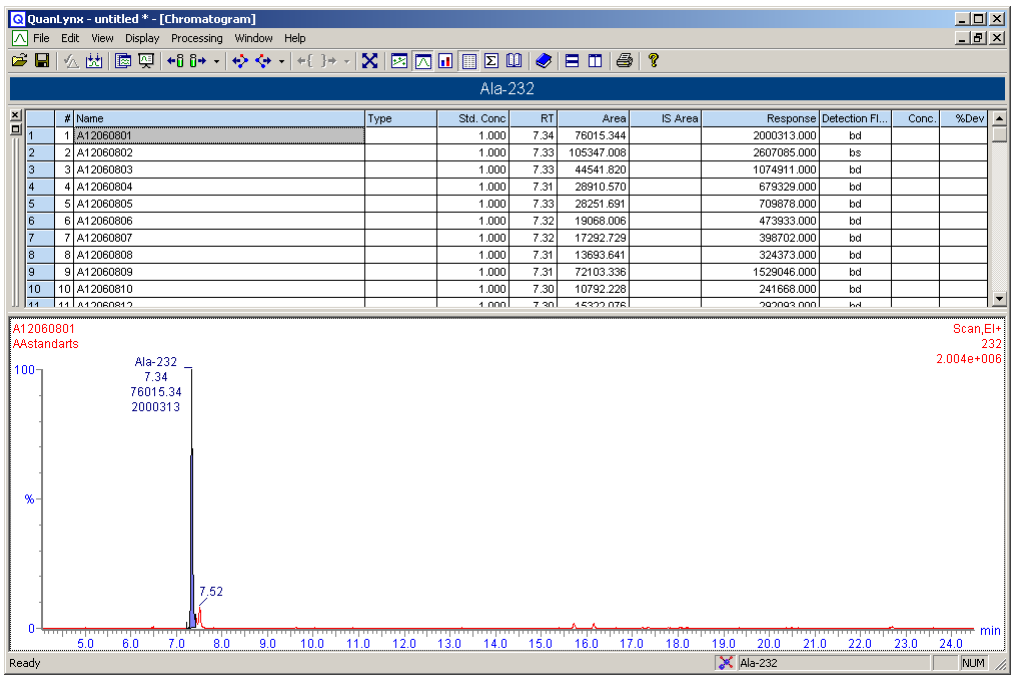
On the RHS in the "*Quantify*" box, leave the default selection for the sample range (i.e. all samples). If the method is not correct, click on ... and select the desired method.

4. Execute the process:

To execute the process click the **`OK`** button.

Step VIII: Review, Manually Edit and Export the Processed Data

When complete the processed data is displayed in tabulated form in the QuanLynx dataset viewer:



The viewer is broken up into two main regions. At the top is the table of processing values for a given compound. The compound, identified in the title bar, is `Ala-232`. Each row of the table lists processing information for this compound in each of the samples. The lower window is a chromatogram for the given compound and sample.

- If the chromatogram does not appear by default you can toggle it on by clicking the View chromatogram button ( ) or selecting chromatogram under the View menu:

  `View > chromatogram`

- In the chromatogram the area of integration is filled in from the peak down to a baseline calculated from the method that was used. If the chromatogram is not filled in you can toggle this option from the Display menu:

  `Display > Options > chromatogram Tab > Fill Detected Peaks`

The measurements are tabulated in the Area and Response columns.

- The area value comes from integration of the peak (within the highlighted area).
- The response value is either the area or the peak height as set in the method.
- The peak height, like the area is calculated relative to the baseline.

Before exporting the processed dataset you should first review the dataset. Each chromatogram may be manually edited by adjusting the baseline, or even adding and deleting peaks.

- Things to look for are the retention times (explicitly listed in the RT column),
- integration features; baseline, side peaks, ..., and
- missing data.

Missing data is perhaps the most important problem to correct, as it will lead to problems later on.

If you find yourself making the same type of correction to the integration area, you may want to make more detailed changes in your method and re-process the samples.

1. To manoeuvre through the compounds in your experiment,

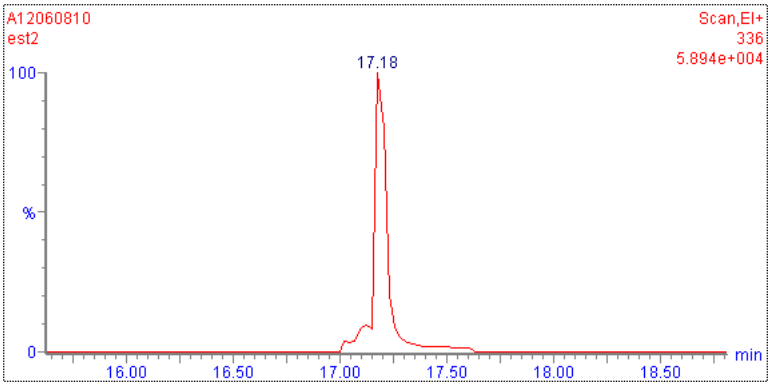
- to move between experiments click on the buttons:  to the right (next), left (previous), and down (choose from list), and
- to move between compounds click on the buttons:  to the right (next), left (previous), and down (choose from list).

2. To zoom in to a peak click and hold the left mouse button at one corner of the region you want to expand, then drag the mouse to a second corner (creating a rectangular region on-screen). Release the mouse button to expand the region. To zoom out click the  button.

- When editing a peak it is usually easier to start by zooming in on the peak or baseline.

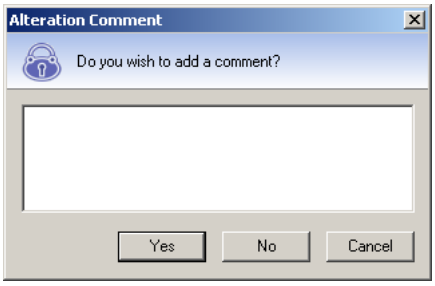
3. To add a peak:

When the software has not automatically selected a peak, you may add one manually.



- Right click inside the peak.
- From the popup menu select
  `Add Peak.`
- Right click in the chromatogram a second time.
- Select
  `Save Peak Modifications...`

This opens the "*Alteration Comment*" dialog box:



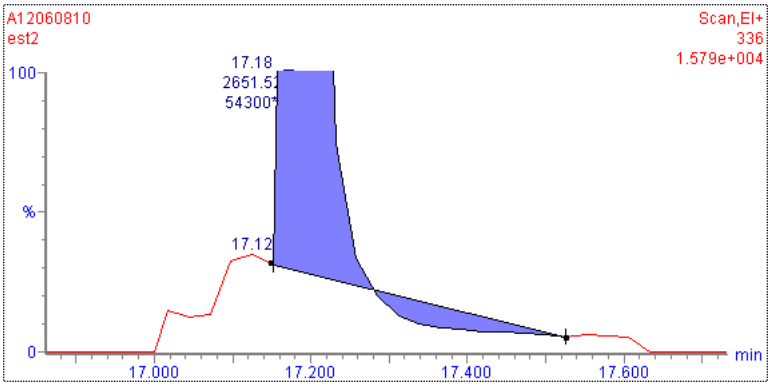
- You may enter a comment, and click the **`Yes`** button.
- Or just click the **`No`** button to continue without a comment.

If you are planning never to add comments you can disable off this popup from the Edit menu:

`Edit > Disable Comments`

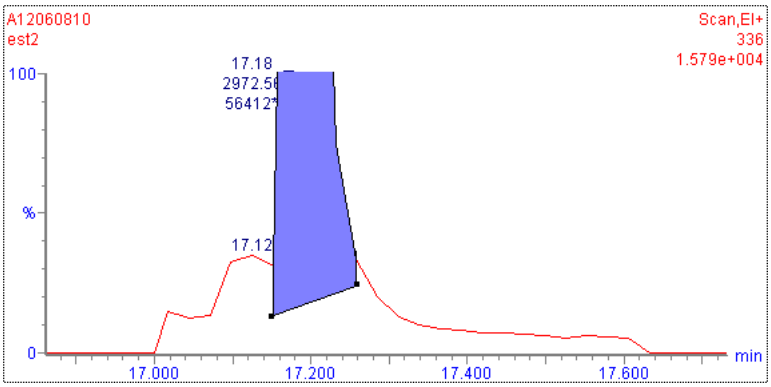
4. To edit a peak, first zoom in near the baseline:

This is the peak we just added. To fix this we can manipulate the baseline manually by dragging the two end points of the baseline.



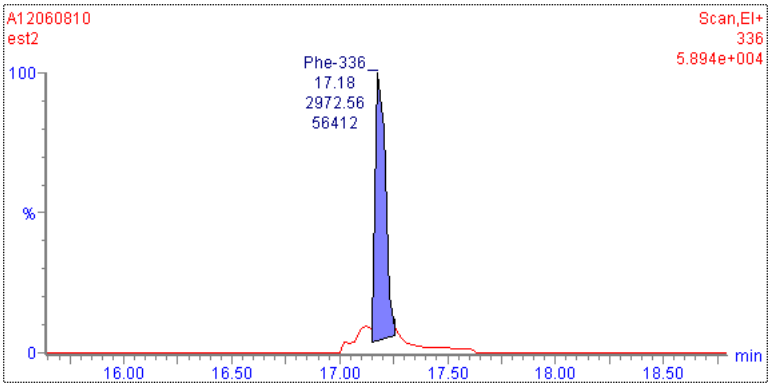
- Positioning the mouse over one end and the cursor changes to:

- Click and drag these two end points to their desired position - do not cross them as they are pre-defined as start and end points.
- Right click in the chromatogram and select:
  `Save Peak Modifications...`
- If you have not disabled comments, fill out and close the "*Alteration Comment*" dialog box.



After saving the modifications the chromatogram now shows the desired peak:

- The tabulated data is updated to reflect the change in the baseline.
- You can also check the retention time (the RT column).
- You can always find your manually edited peaks as the Detection flag column changes to `MM`.



5. To delete a peak:

- Right click inside the peak.
- From the popup menu select `Delete Peak`.
- Right click in the chromatogram a second time.
- Select `Save Peak Modifications...`

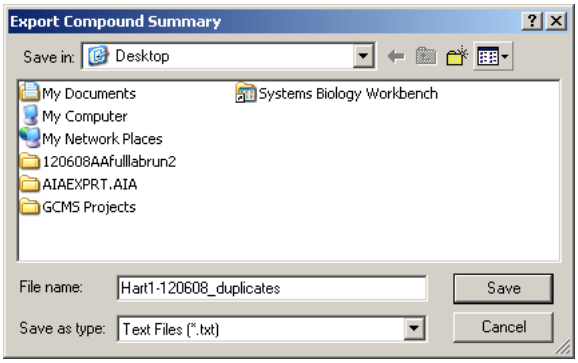
If you have not disabled comments this opens the "*Alteration Comment*" dialog box. You may enter a comment, and click the **`Yes`** button or click the **`No`** button to continue without a comment.

6. To export the processed compound data for use with the auto-correction software select complete summary from the export option under the file menu:

`File > Export > Complete Summary`

This opens the "*Export Compound Summary*" window.

- Save in: `Desktop`
- File name: `Hart1-120608_duplicates`
- Save as type: `Text Files (*.txt)`
- Click the **`Save`** button.



7. Close the QuanLynx viewer, you may be prompted to save the processed dataset, for future use. If you have made manual edits this is a good idea.

- QuanLynx datasets are saved in the root of the project folder.
- You can now close MassLynx as well.

Step IX: Process MS Data to 13C Measurement Data

8. This section takes you through the basic use of the auto-correction program, *iMS2Flux*, (see the accompanying user manual; *iMS2Flux-Manual.pdf*, for full details on this program and its use). This is a command line based perl script that:

- imports the data directly from QuanLynx and performs basic data checks on the processed data,
- optionally corrects for proton gain/loss, natural abundance, and original bio-mass,
- calculates averages and standard deviations for replicates, and
- generates output ready to be inserted into network models for a variety of ^{13}C-MFA software.

In addition this program can print out a variety of information, such as the retention times, intermediate data and failed data checks, in a spreadsheet friendly format. In step 4 this is used to help identify possible automatically processed data that needs manual investigation.

1. Setup a configuration file:

   In addition to the summary report file from QuanLynx, this program requires a configuration file. By default the configuration file is named **`config.txt`**, but may optionally be given a different (more meaningful) name. These two files must be in the same directory, but do not need to be located in the same directory as this program.

   - In this example the configuration file uses the default name and both of these files are located on the users Desktop.
   - Please see; *iMS2Flux - An Overview.pdf*, for full details on the configuration file.

2. Open the Command prompt:

   From the *Windows Start menu*:

   ```
   Start > Programs > Accessories > Command Prompt
   ```

3. Navigate to Desktop:

   The processed data was written to a file on the desktop. Therefore we want the command prompt to be in the desktop as well (i.e. set to be in the same directory as the data file). To do this we change directory (**`cd`**) to the desktop:

   ```
   cd Desktop <ENTER>
   ```

   - Throughout this step the term `<ENTER>` after a command means to press the Enter key on the keyboard.

4. Run the auto-correct program:

   ```
   iMS2Flux.pl  <ENTER>
   ```

   The program will either run to completion generating the desired output, or will terminate if there are problems with the data and/or configuration settings.

   Review and correct any problems flagged by the program, and re-run the program.

   - The only data that must be corrected to run this software is missing data.
     QuanLynx will sometimes give no data at all for a given compound in a given dataset. There is no clear reason for this. While it may occur where there really is no peak at the given retention time, it may also occur where there is a perfectly good peak at that time.
   - Other data checks are optional and may be fixed or disabled and used for information only.

5. The generated data files may be opened in Excel or any text editor.

Appendix A: Compatibility with the *iMS2Flux* software

1. Replicates:

Replicate experiments must be presented in a consecutive order in the sample list.

- *iMS2Flux* will automatically aggregate replicates assuming that they are already grouped in consecutive order.
- It is not required that each group have the same number of replicates.

2. Compound Names:

All compound names should be in the format: `AAA-###`

- `AAA` is the three letter abbreviation for the amino acid, and
- ### is the mass of the compound.

- With the following exceptions which use a four character compound abbreviation:

- Arg TBDMS5 is `arg5`
- Arg TBDMS3 is `arg3`
- His TBDMS3 is `his3`
- His TBDMS2 is `his2`

3. Mass Isotopomers:

For a given fragment, all isotopomer masses must be continuous, but do not need to be exhaustive.

- For example given a fragment of mass M with 3 carbons, it can have the following three mass isotopomers:
  - M+0
  - M+1
  - M+2
  - M+3
- You must start with the M+0 compound, and may include 1, 2 or all 3 of the mass isotopomer compounds.
- If you do not include the M+2 mass, you also cannot include the M+3 mass.
- You may however add the M+2 mass, and manually set it to zero.
- Additional measurements before M+0 or after M+3 may optionally be included. If so, the identical boundary measurements must be included with all measurements.

4. Filenames

When exporting data from QuanLynx for use by the auto-correction software, do not use spaces in the name of the file.

5. For more detailed information on installing and using *iMS2Flux* please see the accompanying user guides:

- iMS2Flux-Manual.pdf
- Getting Started with Windows.pdf
- Getting Started with Linux-Unix.pdf
- Getting Started with Mac OSX.pdf