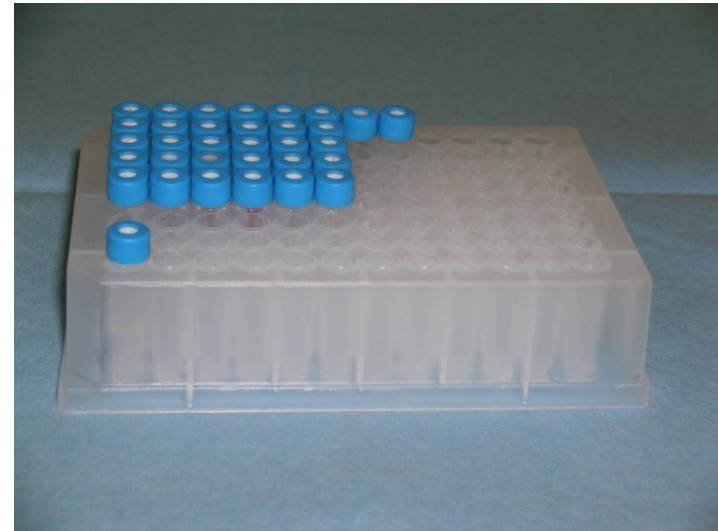# How to Make an HPLC Sequence File

The HPLC software needs to 'know' where to find your samples in the autosampler tray. This is accomplished by creating a *sequence file* which associates a given vial in the autosampler tray with a sample description, amount injected, data file name, and HPLC conditions for the analysis.

How to Make an HPLC Sequence File

- **After you have placed your samples in vials, a TA will assist you to place them in an autosampler tray.**

- **A representation of the tray is shown on the right. Notice the markings 1-12 on the top and A-H on the left side.**

- **Place your samples in a row in the tray (keep them together as a group). If you have more samples than the row will contain just keep going into the next row starting in column 1.**

- **Note the grid location of your first sample and write it down. In this case it is D1 (row D, column 1). You will use the location of your first sample when you create the sequence file.**
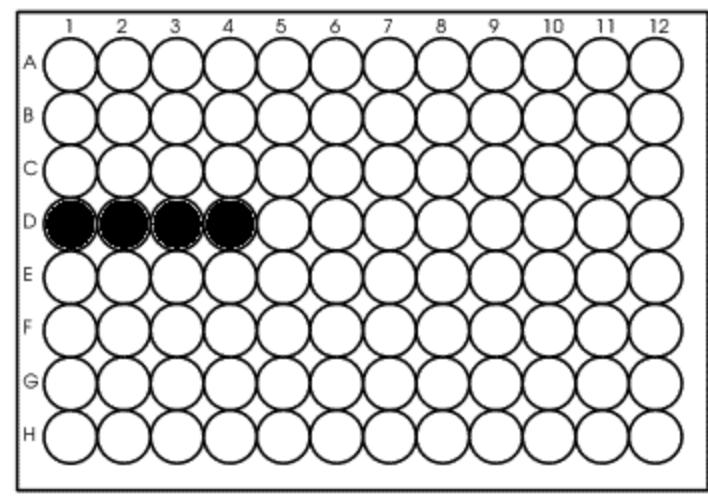
Load the CASPiE Instrument Site

- **To open the the HPLC software launch your web browser and navigate to the CASPiE instrument website at https://instruments.caspie.org.**
- **Enter your account information to login.**

The HPLC Data Processing Software

You will use a copy of the HPLC software that *is <u>not</u> attached to the instrument* to produce the sequence file. The version of the software not attached to an instrument is called the 'HPLC Data Processing'.

You will always have access to the HPLC data processing software during a CASPiE module. You will only have access to the instrument for remote control during your scheduled hours.

You will need the Citrix client installed on the computer you are using to proceed. If you are using a lab computer it will already be installed.

Launch HPLC Data Processing Software

- **You should see one icon in the 'Applications" box.**
- **Launch the HPLC data processing software by clicking once on the icon.**
- **If the 'HPLC Data Processing' icon is not present ask your TA for help.**

Open Software 'Offline'

- **Because this software is not attached to an instrument it must be opened 'offline'.**

- **To do this right click on the icon and select 'Open Offline'. If you are using a Macintosh select File|Open Offline…**
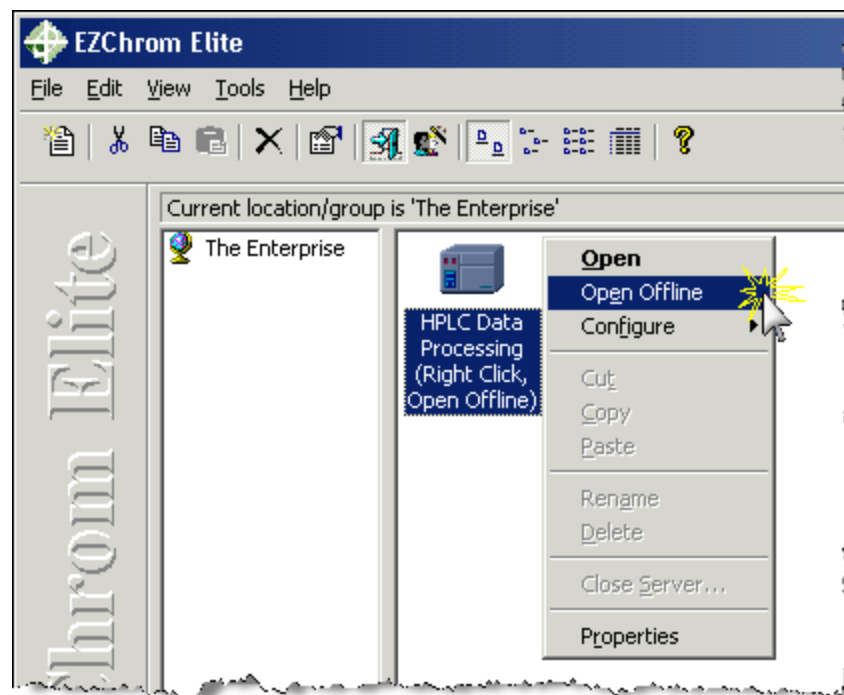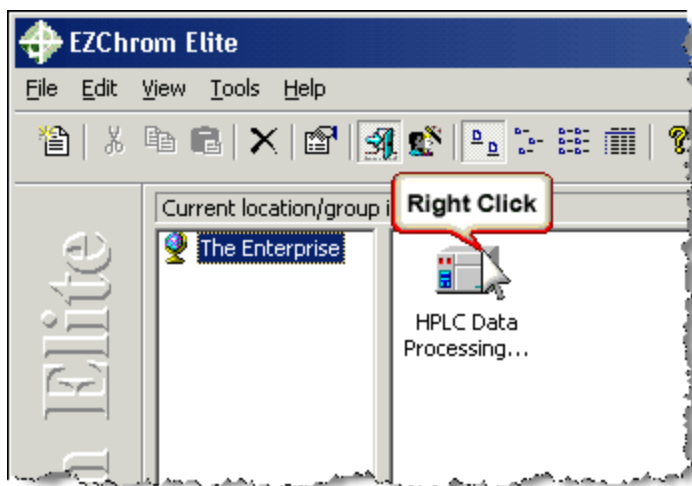
Instrument Wizard – Create a sequence

- **The next screen will have an 'Instrument Wizard' window.**

- **Click on the 'Create a sequence' icon to launch the sequence wizard.**

- **If the Instrument Wizard window is not there select File|Sequence|Wizard.**
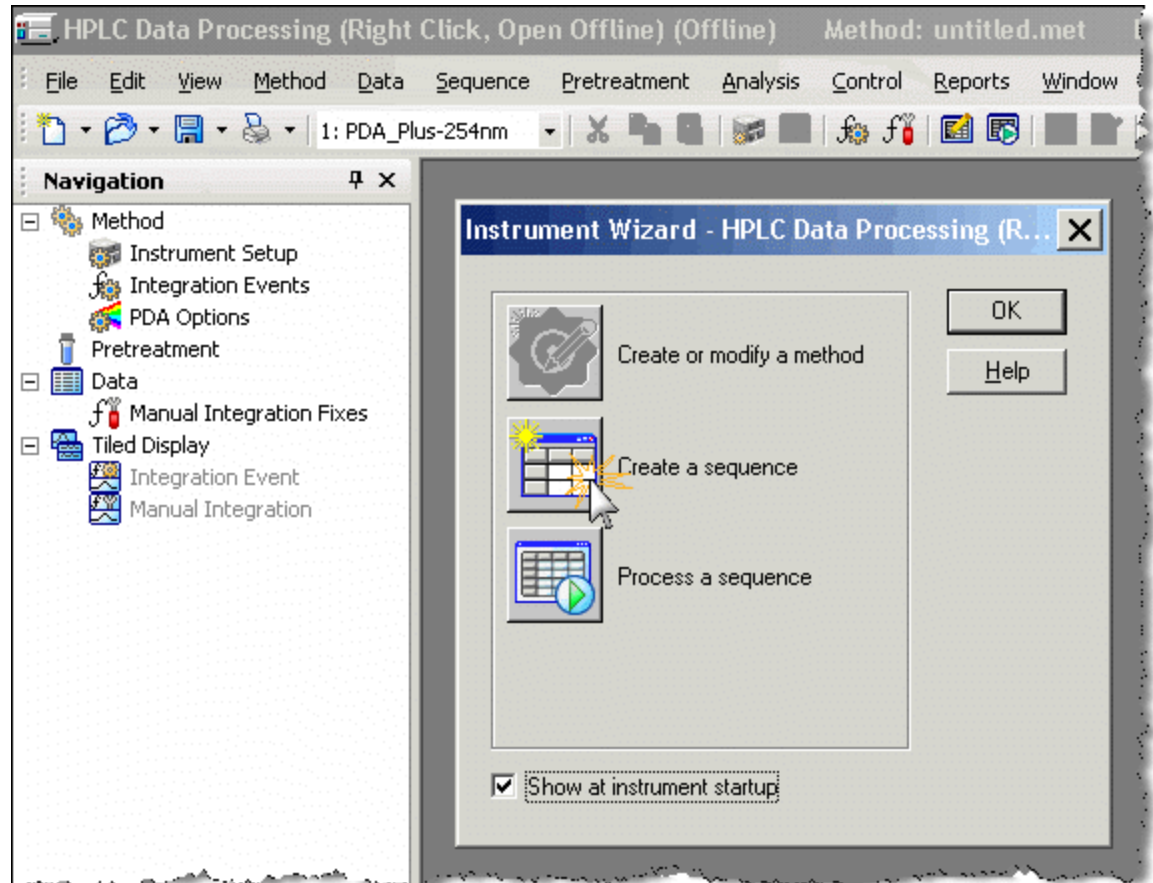
Sequence Wizard - Select a Method

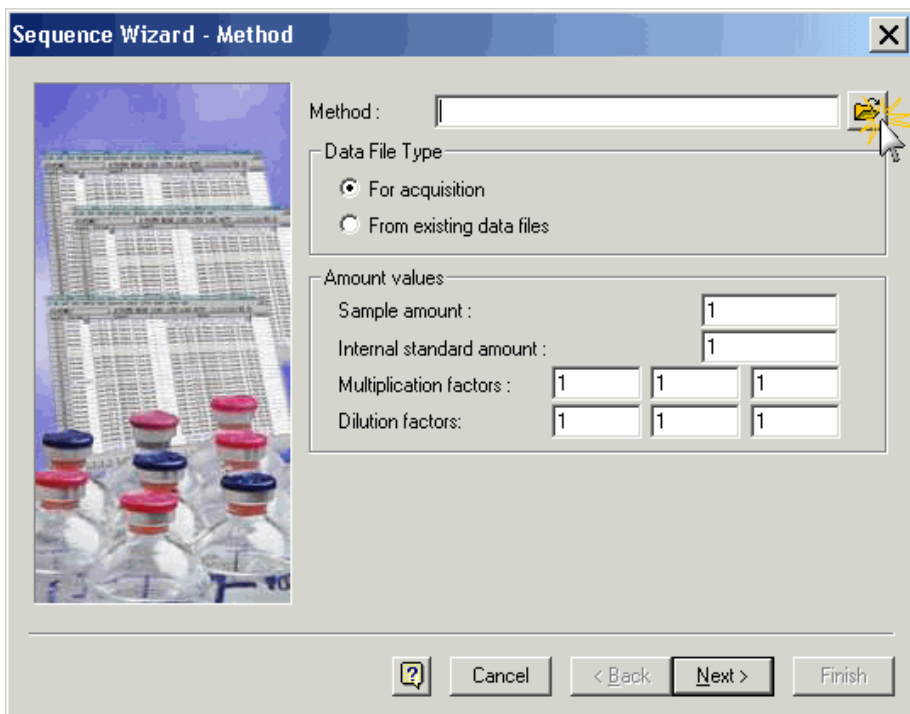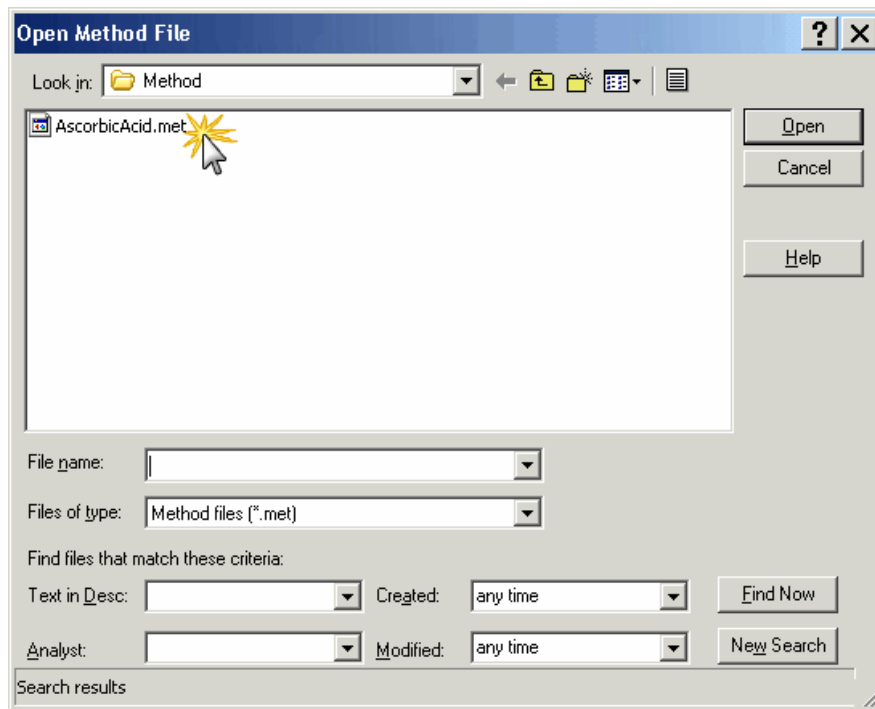- **In the Sequence Wizard – Method window (a) click on the Method open icon this will open another window (b).**
  - **Select AscorbicAcid.met from the Open Method File window and click Open.**
  - **This file contains all the information needed to run the instrument such as mobile phase flow rates and detector wavelength.**
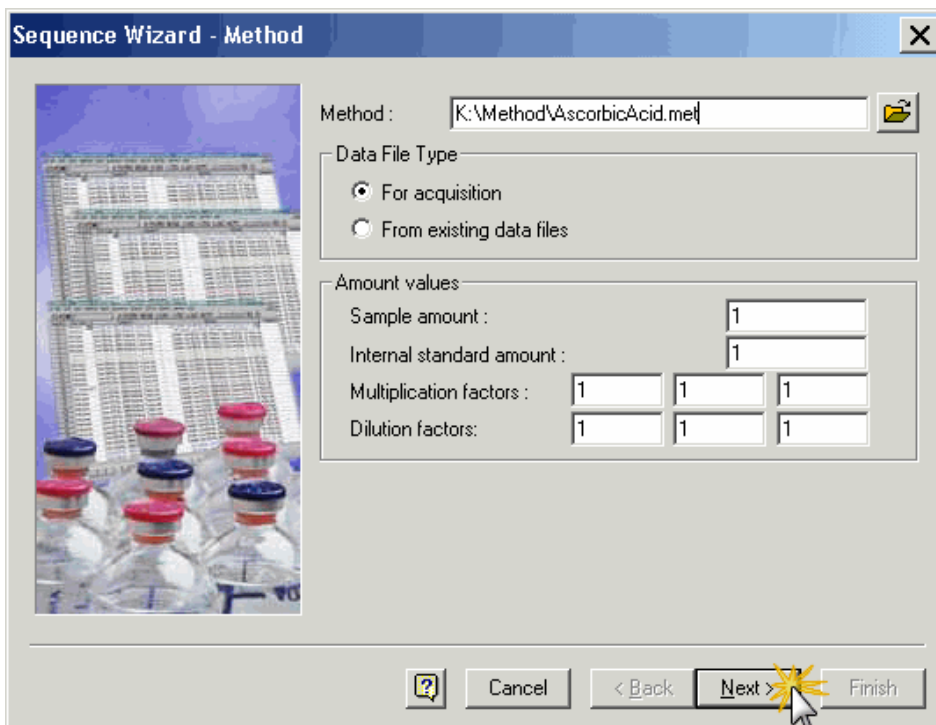
(a)   (b)

Sequence Wizard - Unknowns

- **After the method is selected, click Next at the bottom of the window (a).**
- **In the next window (b) enter a base name for all your samples in the Sample ID box.**
- **The base name will be the same for all your sample IDs. Next, add a number at the end of the base name for each subsequent sample. To do this click on the blue arrow to the right of the Sample ID box.**

(a)

(b)
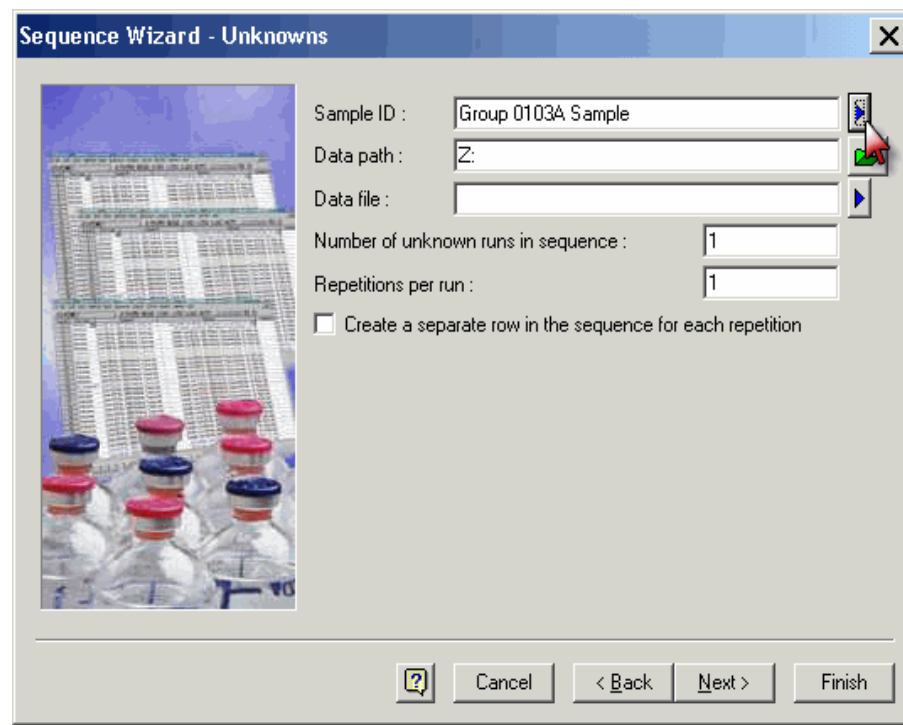
Sequence Wizard – Unknowns Sample ID

- **After clicking on the blue arrow you will see a menu to the right.**

- **Select "Increment Number." This will add a numerical code to the Sample ID base name.**
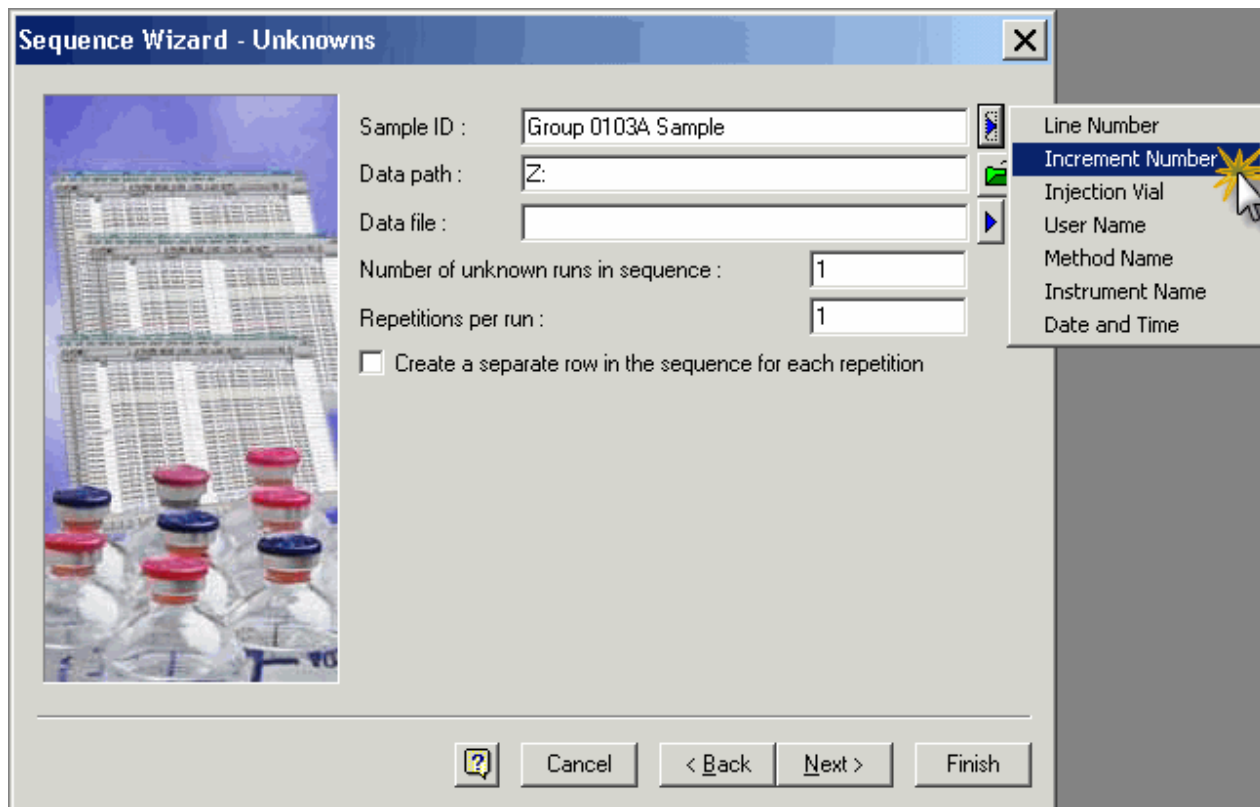
Sequence Wizard – Unknowns File Name

- **In the box to the right of "Data file" enter a base name for the data file names for your samples. The base file name will be the same for all your files.**

- **Add a unique number to the base name by clicking on the blue arrow. Then select "Increment Number."**
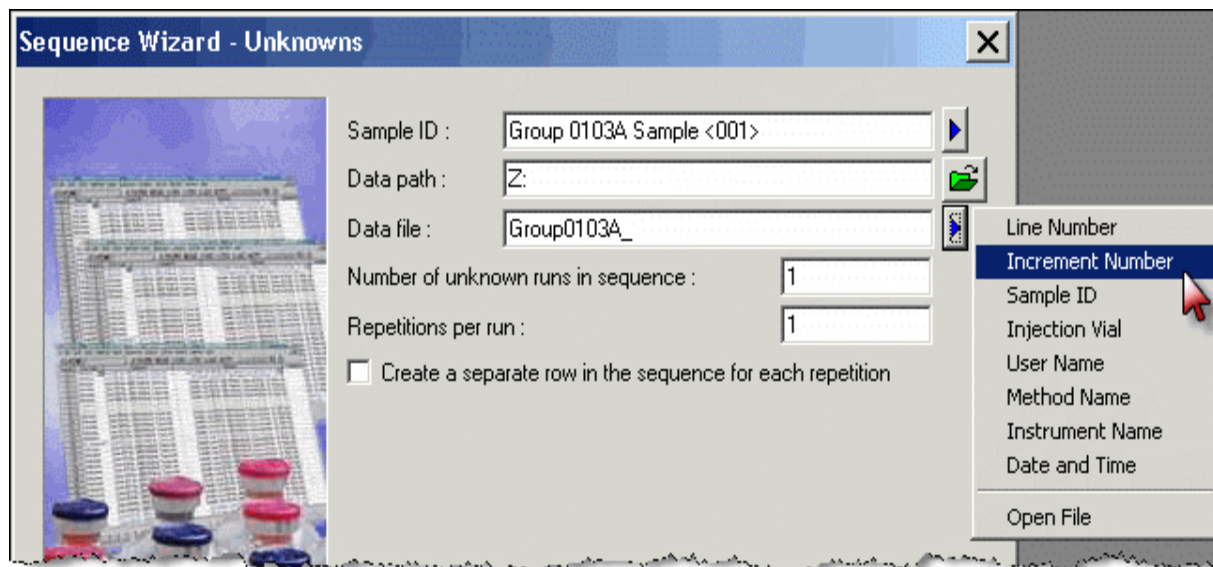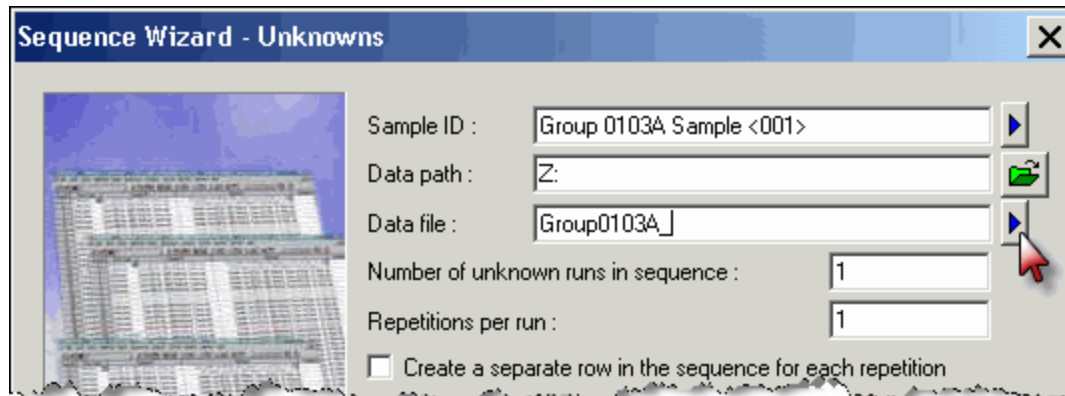
Sequence Wizard–Unknowns No. of Samples

- **In the box to the right of "Number of unknown runs in sequence"** <u>enter the number of samples you placed in the autosampler tray</u>.

- **Click "Next" to proceed to the next window.**
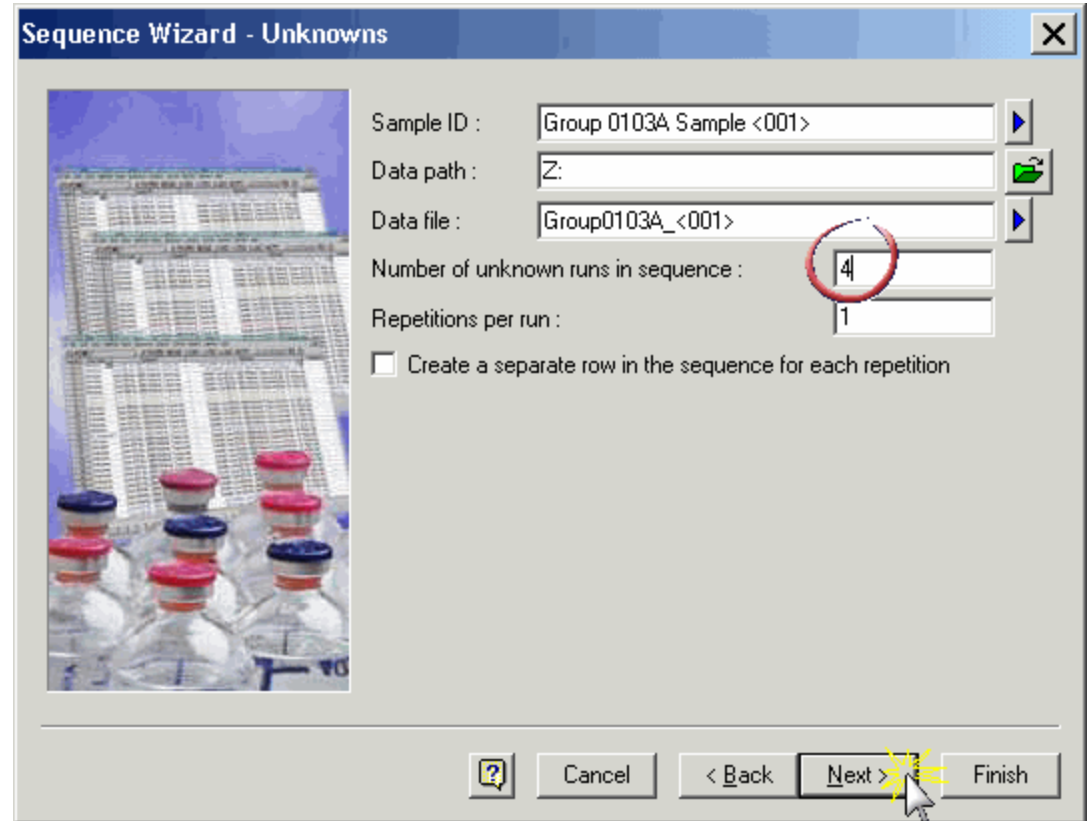
Sequence Wizard – Autosampler

- **In the box to the right of "First vial" enter the location in the autosampler tray where you placed your <u>first</u> sample. In this example the first vial was in row D column 1.**

- **The grid location <u>must</u> be preceded by the letter A and a colon(;). This is important as this tells the autosampler which of 3 tray slots the tray is located.**

- **Nothing else need to be modified in this window so click "Next" to proceed to the next window.**
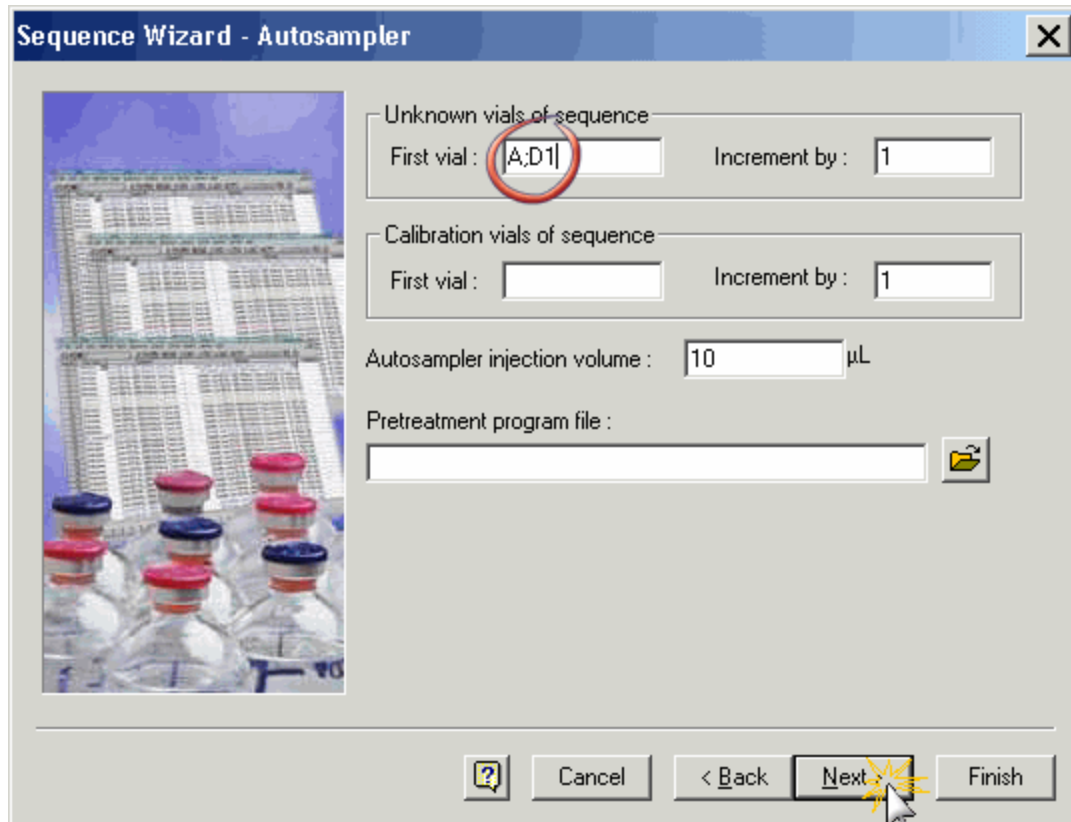
Sequence Wizard – Calibration, Reports

Do not change anything in these next two windows; just click "Next" in the Calibration window and "Finish" in the Reports window.

Sequence Wizard – Finished Sequence

- **The finished sequence will look something like this.**
- **Proof read the information in the Sequence window and make any necessary corrections by clicking on the cell and typing the correction.**

Sequence: untitled.seq

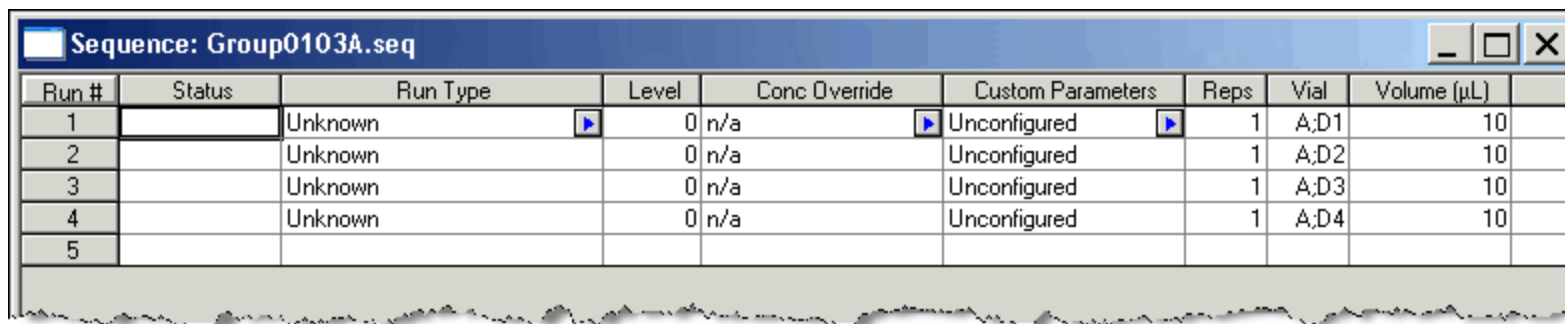| Run #	Reps	Vial	Volume (µL)	Pretreatment	Sample ID	Method	Filename	Sample Am
1	1	A;D1	10		Group 0103A Sample001	AscorbicAcid.met	Group0103A_001.dat	
2	1	A;D2	10		Group 0103A Sample002	AscorbicAcid.met	Group0103A_002.dat	
3	1	A;D3	10		Group 0103A Sample003	AscorbicAcid.met	Group0103A_003.dat	
4	1	A;D4	10		Group 0103A Sample004	AscorbicAcid.met	Group0103A_004.dat	
5								

Sequence Wizard – Save Sequence File

- **Save the sequence file by selecting File| Sequence| Save As…**
- **In the next window type a sequence file name in the box to the right of "File name" then click "Save".**
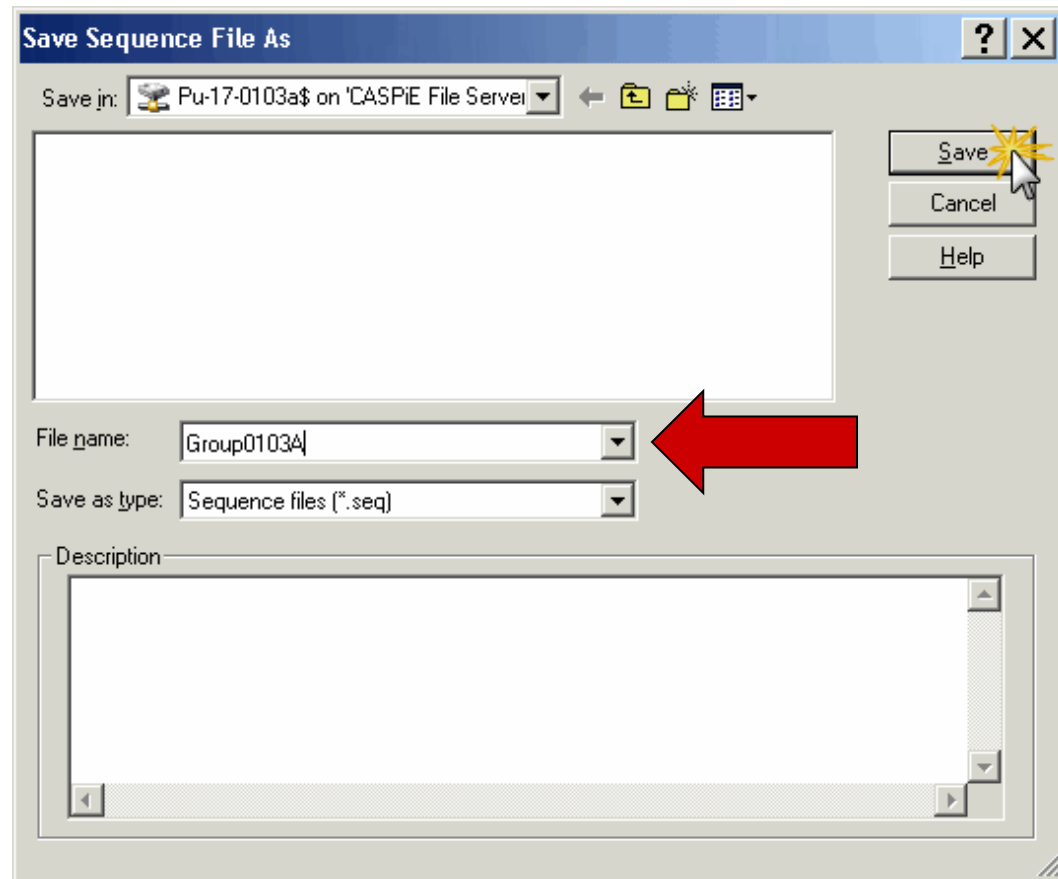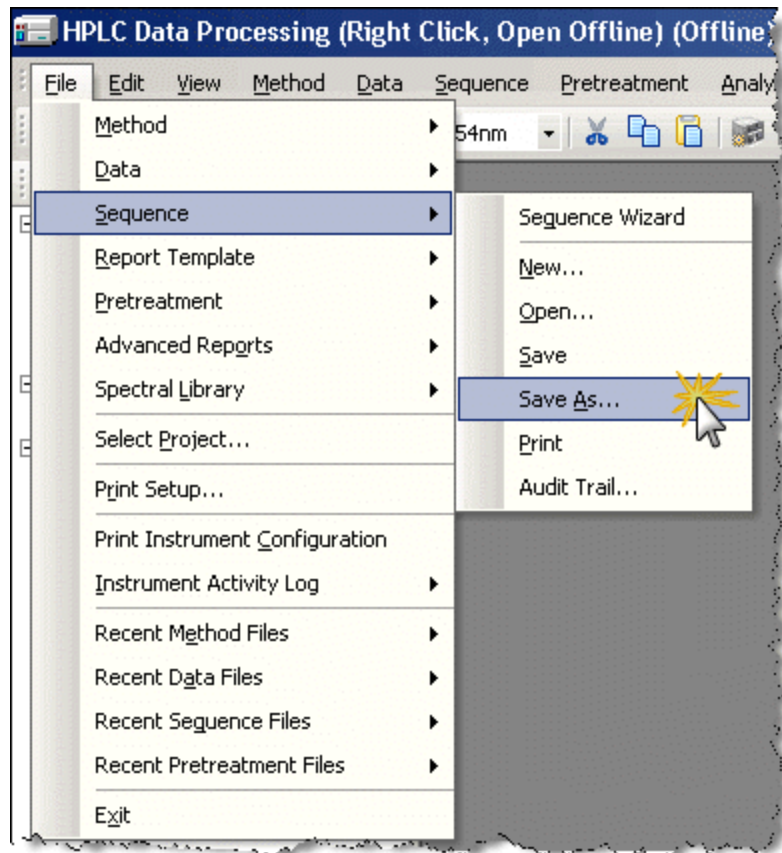
Sequence Wizard – Saved Sequence

- **After the sequence is saved the file name will appear at the top of the Sequence window.**

- **You are now finished; exit the program.**

- **When you are ready to remotely control the HPLC you will retrieve your sequence file and initiate a 'sequence run' to collect data for your samples.**

- **Instructions for running the HPLC are given in another tutorial.**

Sequence: Group0103A.seq

Run #	Status	Run Type	Level	Conc Override	Custom Parameters	Reps	Vial	Volume (µL)	
1		Unknown ▶	0	n/a ▶	Unconfigured ▶	1	A;D1	10	
2		Unknown	0	n/a	Unconfigured	1	A;D2	10	
3		Unknown	0	n/a	Unconfigured	1	A;D3	10	
4		Unknown	0	n/a	Unconfigured	1	A;D4	10	
5									

Close Windows

When you have saved the sequence file exit all EZChrom Elite HPLC software windows.