

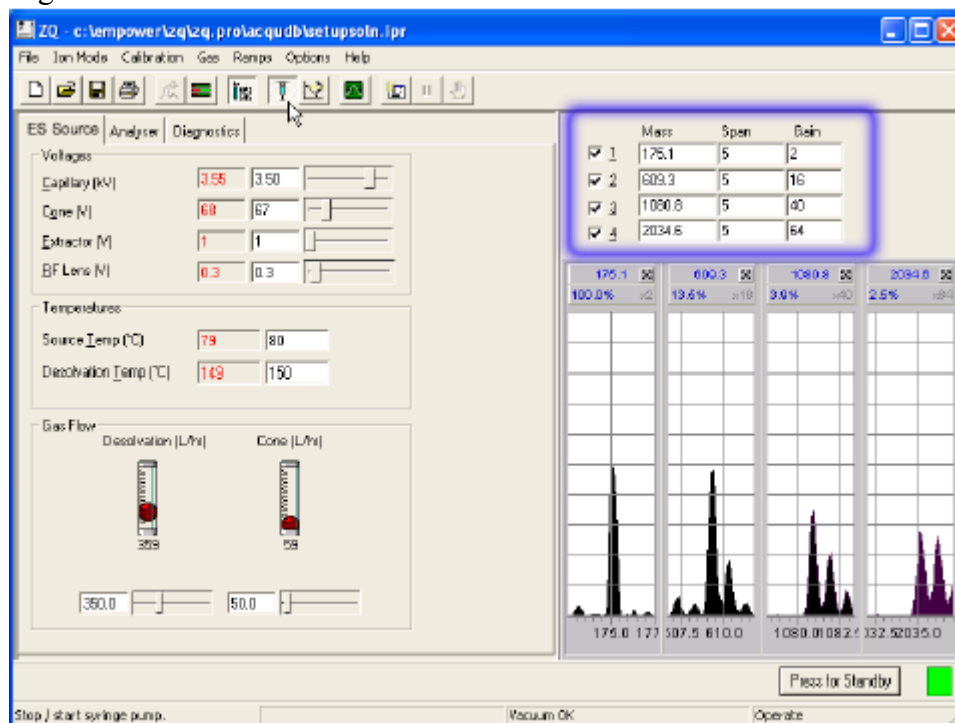
## Protocol LC-MS

### Preparation of HPLC

- Prepare solvents of HPLC from the most pure solvents.
- Prepare the HPLC ([according to the enclosed protocol <Waters\\_2690.html>](#)), make sure to have the injector washing solvents, Water, and MeOH or Acetonitrile, whatever is needed for the method. Wash, prepare, purge all system components as well as the injector.
- Run the mobile phase through the column for equilibration.

### Preparation of MS

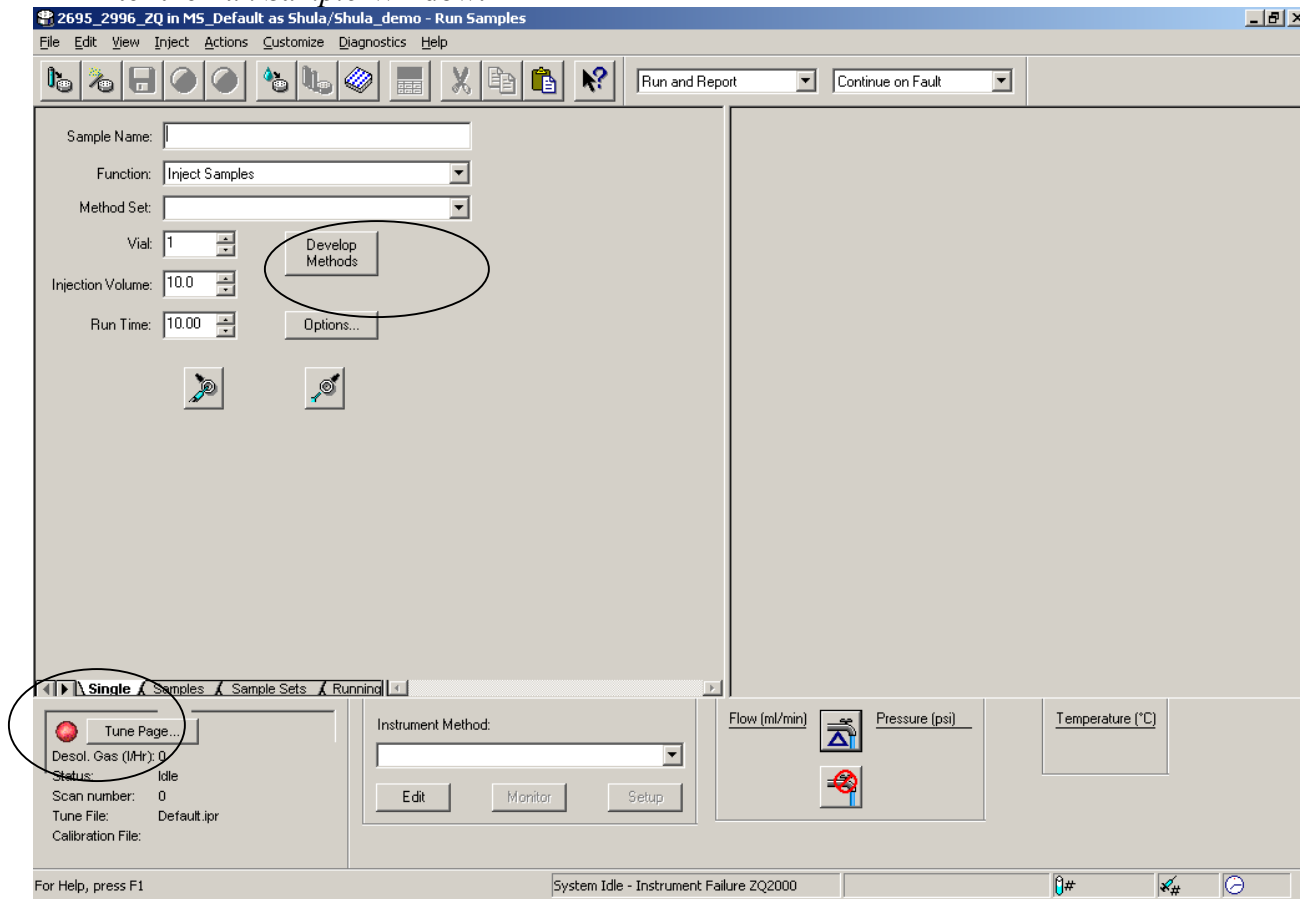
- Enter Millennium and *Run Sample* in the right project
- Press the *Tune Page* button, wait for the screen to appear.
- Open the right 'tune page' (*File -> Open -> filename.ipr*) and click the *API gas* icon and *Operate*. The red light will turn to green.



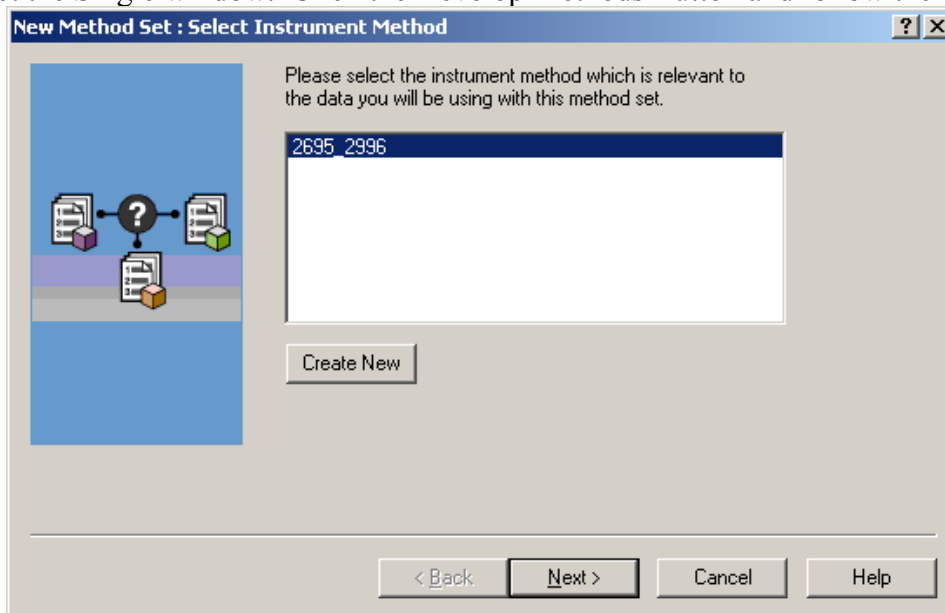
- Wash the MS with the syringe pump, fill with the wash solvent appropriate for the method (usually the diluent). Check that the ZQ functions OK and tune the MS to the best signal

# Preparation of Millennium

- Enter the *Run Sample* Window.

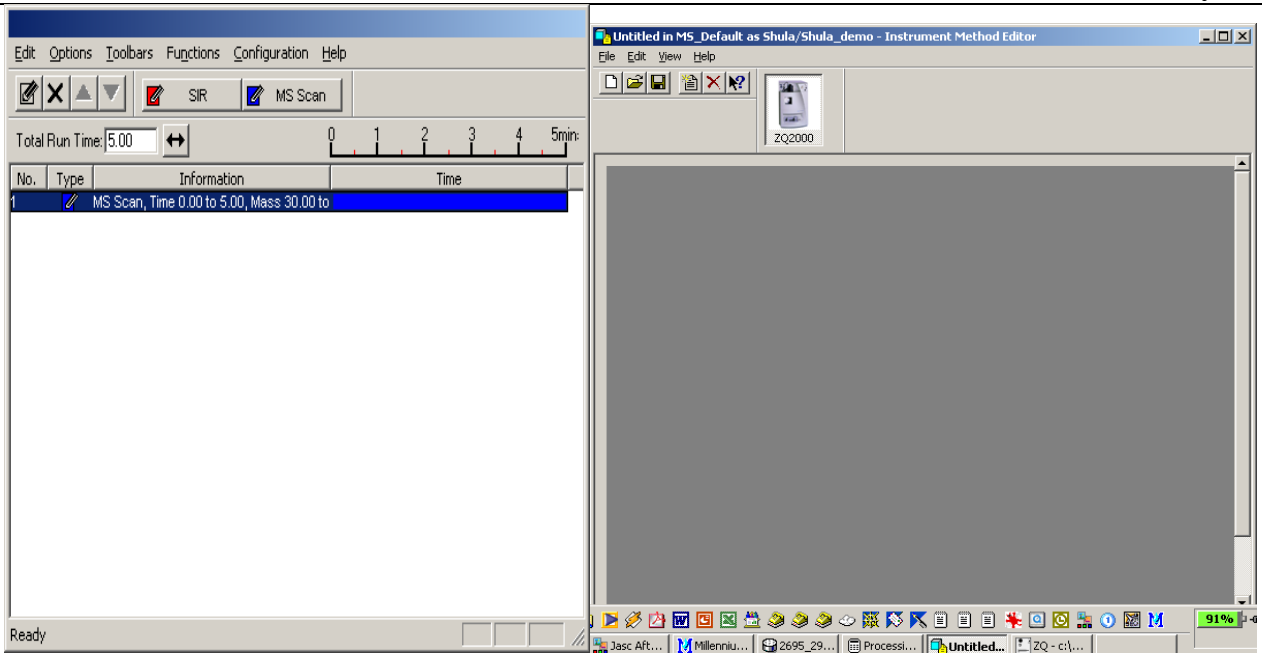


- Select the *Single* window. Click the *Develop Methods* Button and follow the wizard to create the Method

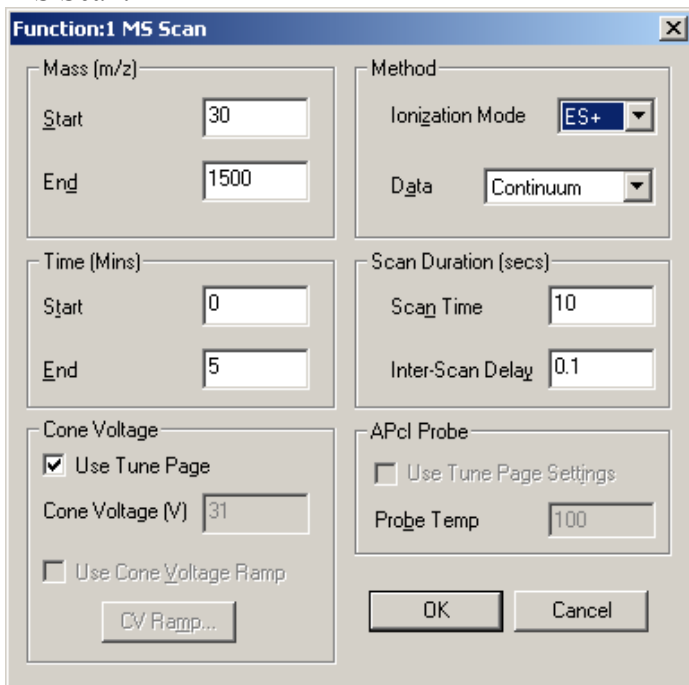


Set:

- Prepare two *Method Sets*: one with *MS only* Instrument Method:

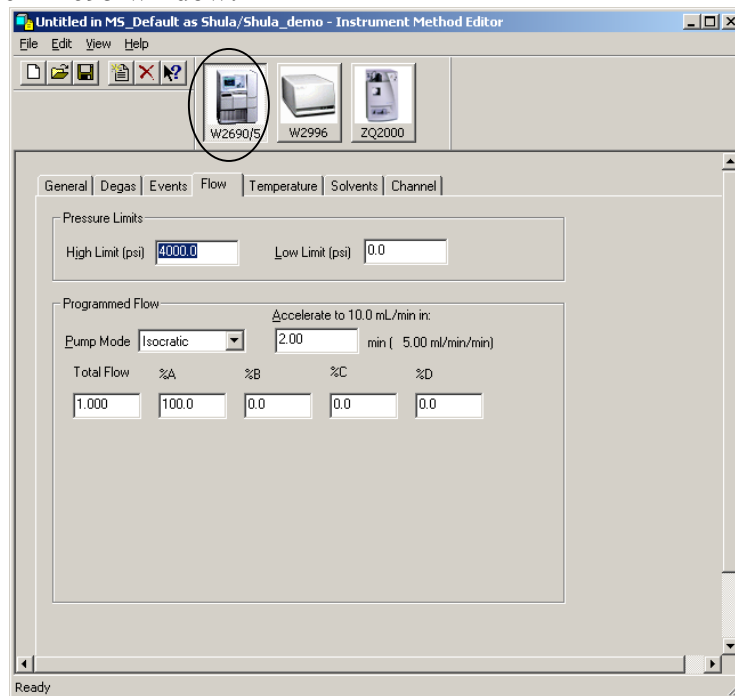


MS Scan:

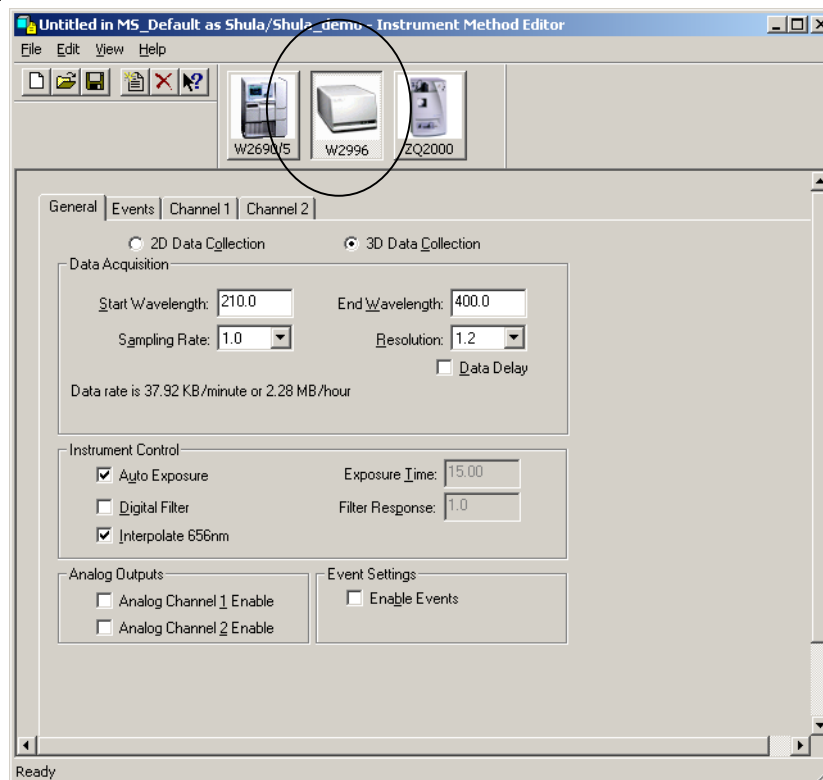


A second Method Set will include the HPLC system as well as the PDA and ZQ detectors for the LC-MS runs:

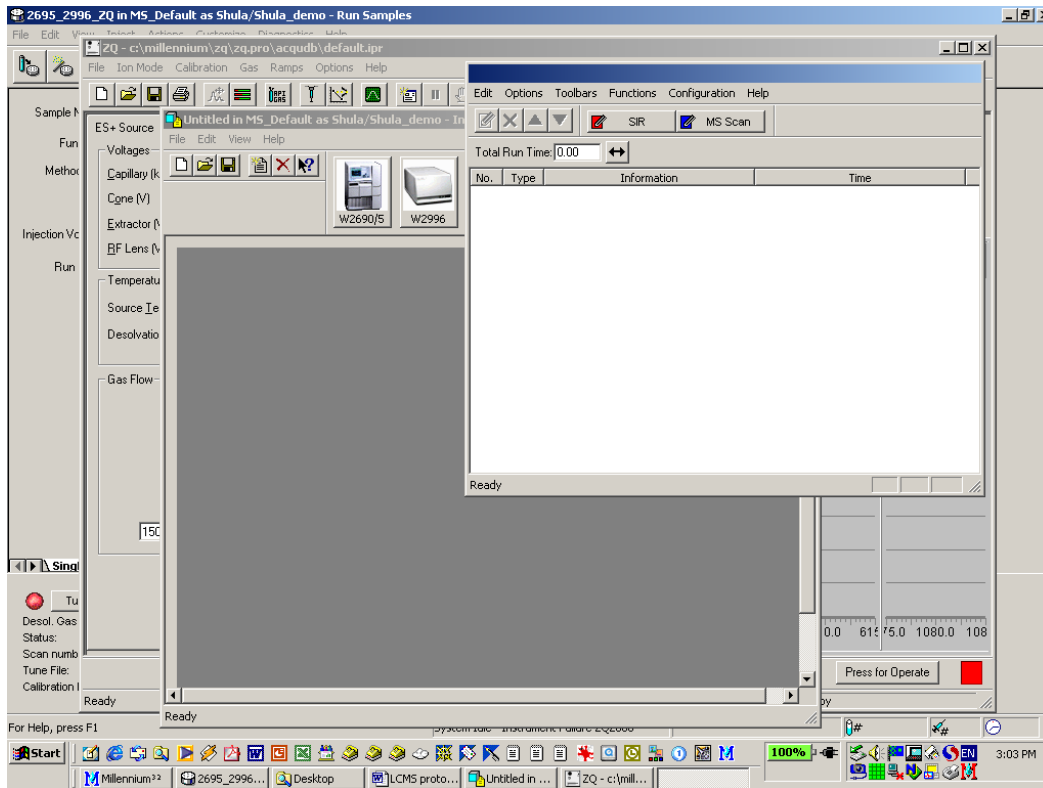
HPLC pump and Autosampler - 2695 window:



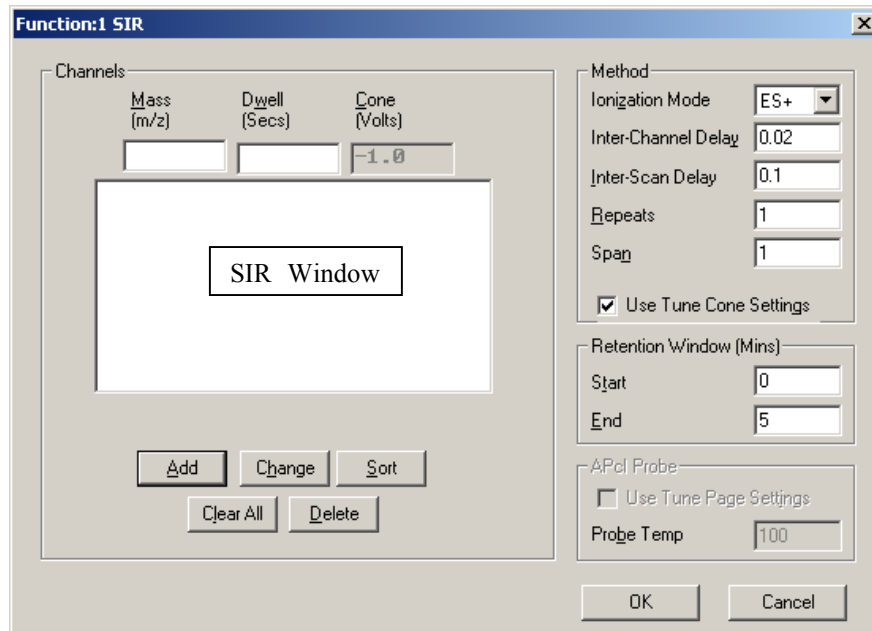
PDA Detector window:



ZQ Detector window:



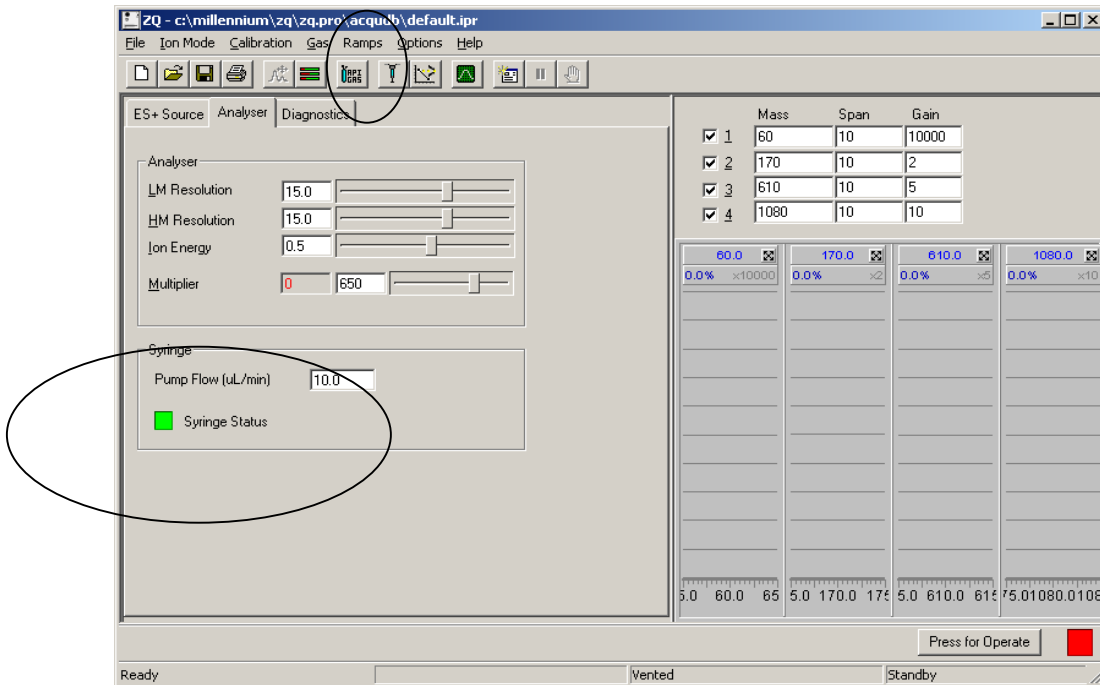
The ZQ can be used either with MS Scan or SIR:



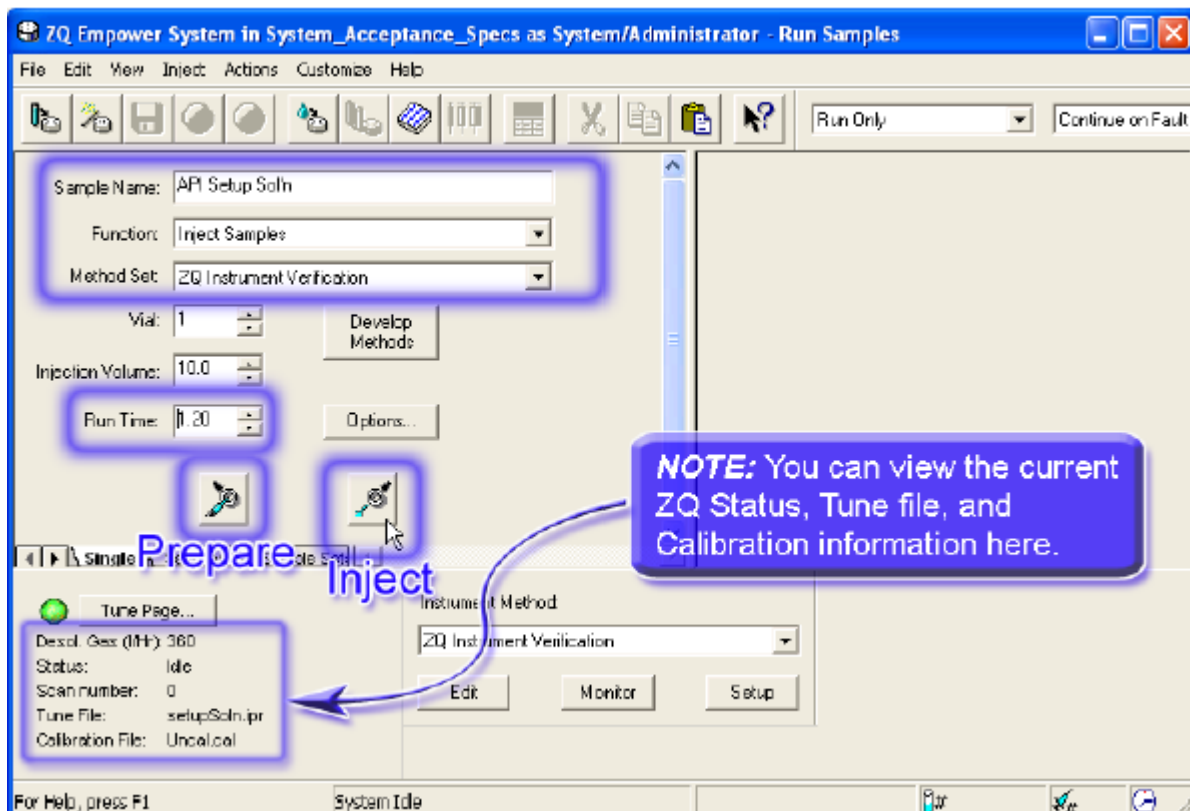
Collecting Spectra for print-out with the Infusion pump:

- Fill the syringe with the solution and go to the ZQ Tune page to click the infusion pump icon and set its

flow rate:



- Return to the *Run Samples* window and select the *MS Only* Method set. Click on the *Prepare* icon, then when the *Inject* icon is ready, click on it.

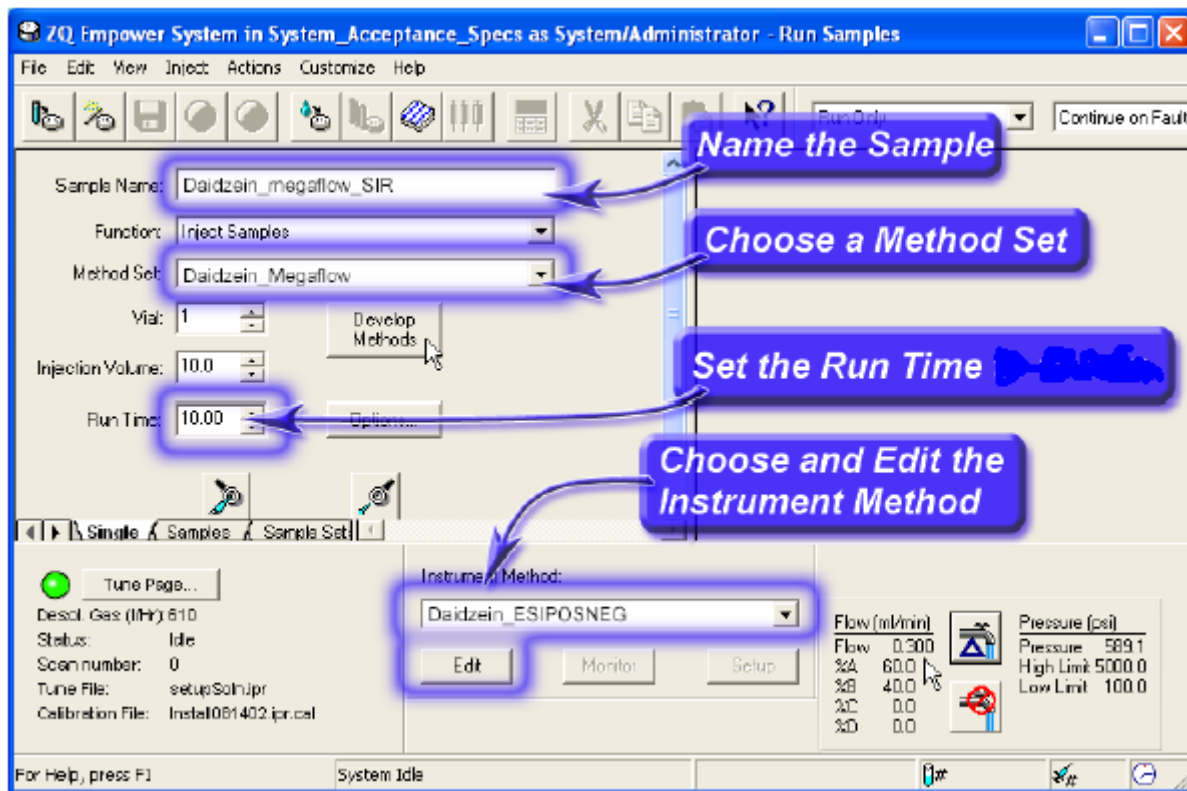


To see and print the spectrum go to *Tools* -> *Preview/Publish* and use the report method *MS Spectrum* (or

any report method that gives the MS spectrum after combining the TIC).

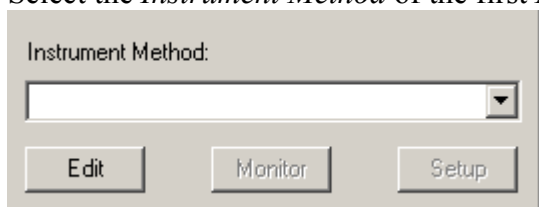
## Collecting LC-MS-UV data:

### A single Run:



## Collecting Sample Sets

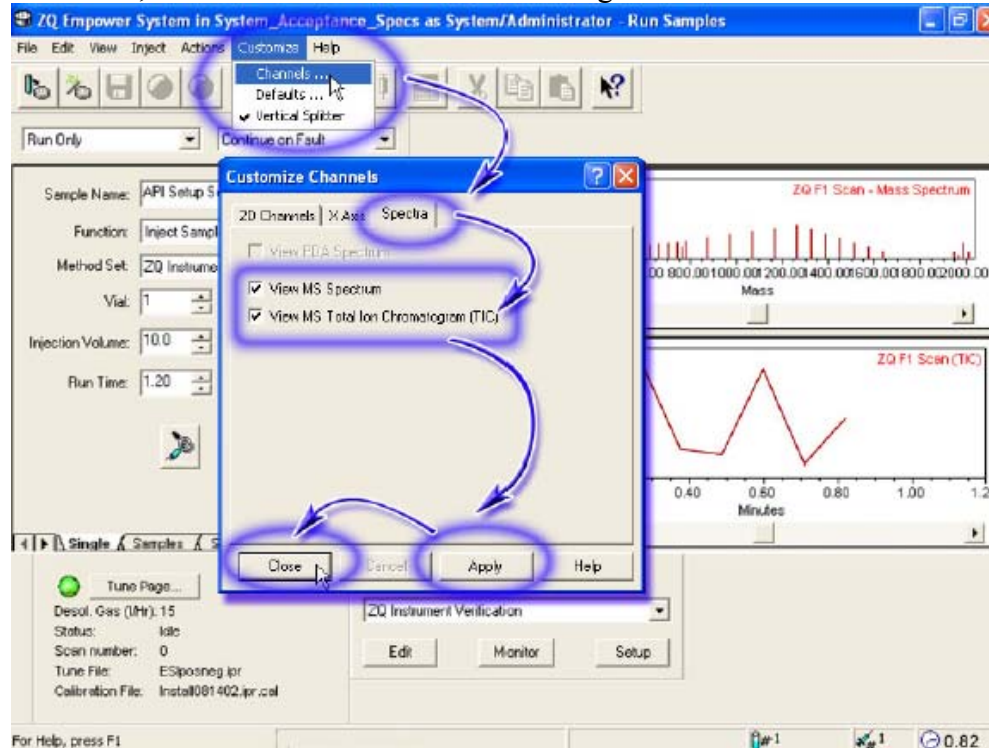
- Go to the *Run Samples* window and to the *Samples* window, prepare a sample set with the right parameters.
- Select the *Instrument Method* of the first *Method Set* in the *Sample set* in the following window:



and click *Setup*. Then click *Monitor* to check communication and monitor baseline.

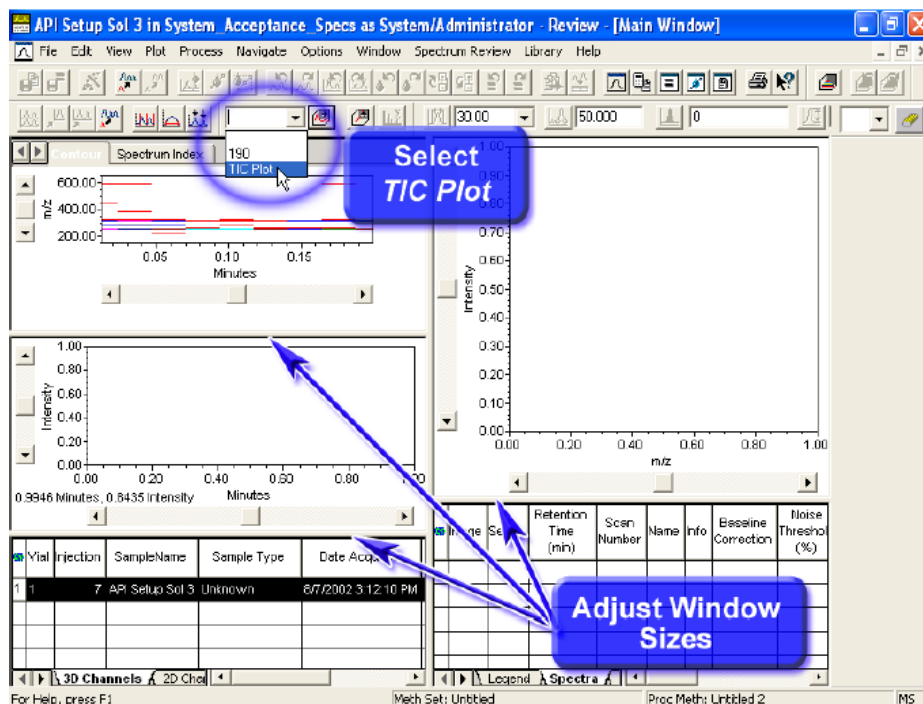
- Abort monitoring and run the sample set.

If you wish to change the display in the chromatogram view use *Customize Channels* and set up the MS spectrum and PDA spectrum if needed, and the 2996 and/or TIC chromatogram



## Reviewing the MS Data

Select the MS Scan channel and Review it. Make sure to be in the main window (Window -> Main Window) and in the 3D layout (View -> 3D layout).

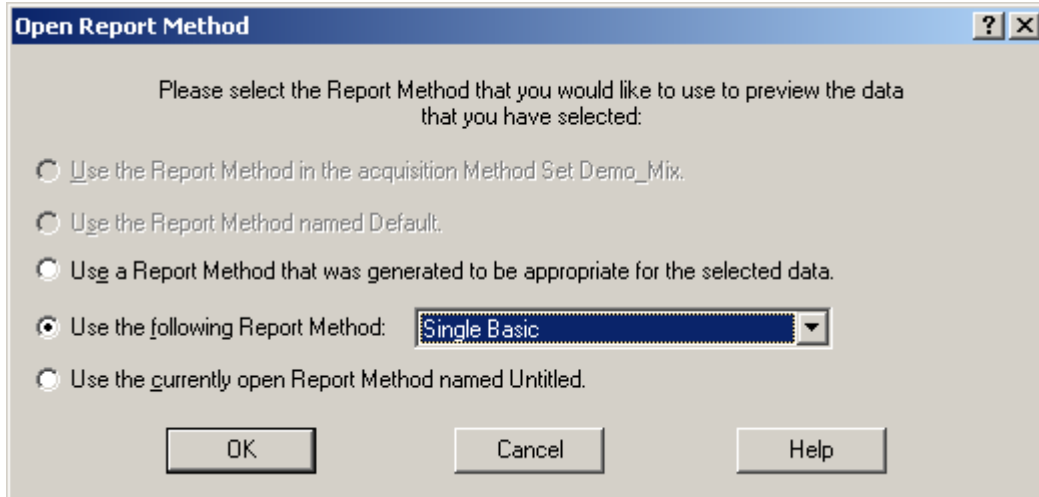


Open the MS *Processing Method*. Manually integrate the peaks on interest in the *TIC* chromatogram and select

Quantitate (*Process -> Quantitate*). Save the result (*File -> Save -> Result*).

## Reporting the Result

Go to the *Results View*, Select your result, and preview it (*Tools -> Preview/Publisher*), using the appropriate *Report Method*.



If you are satisfied, print it. If you need to revise the Report, click on *Close*.

Reported by User: Sr. Shulamit Levin (SHUL) Project Name: Training

**SAMPLE INFORMATION**

Sample Name:	mk: A.U70A.00 scan LO-MS	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	01 Dec 02 2:54:00 PM
Vial:	2	Acq. Method Set:	Demo_Mix
Injection #:	1	Date Processed:	02 Dec 02 8:17:13 PM
Injection Volume:	10.00 µl	Processing Method:	SHRM MS
Run Time:	10.03 Minutes	Channel Name:	216amu
Sample Set Name:	mk: subcondition	Proc. Opt. Descr.:	Z0 F1 Scan MS 216.16 m/z Peak

Spectrum Info: Ret  
VSH 1041 215 - 9.001

Intensity

Channel Description: 60.00-600.00 eV, Continuous, O-V=25; Channel Name: 216amu; Processed Channel Descr: Z0 F1 Scan MS 216.16 m/z Peak Separation: 1.0000

Peak Results

Name	RT	Area	Height
1	VSH 1041 215	62844481	382094100
2	MLC 1040 216		
3	MLC 1040 217		
4	VSH 1010 216		
5	VSH 1020 217		
6	OCG 1001 215		
7	MLC 1016 242		

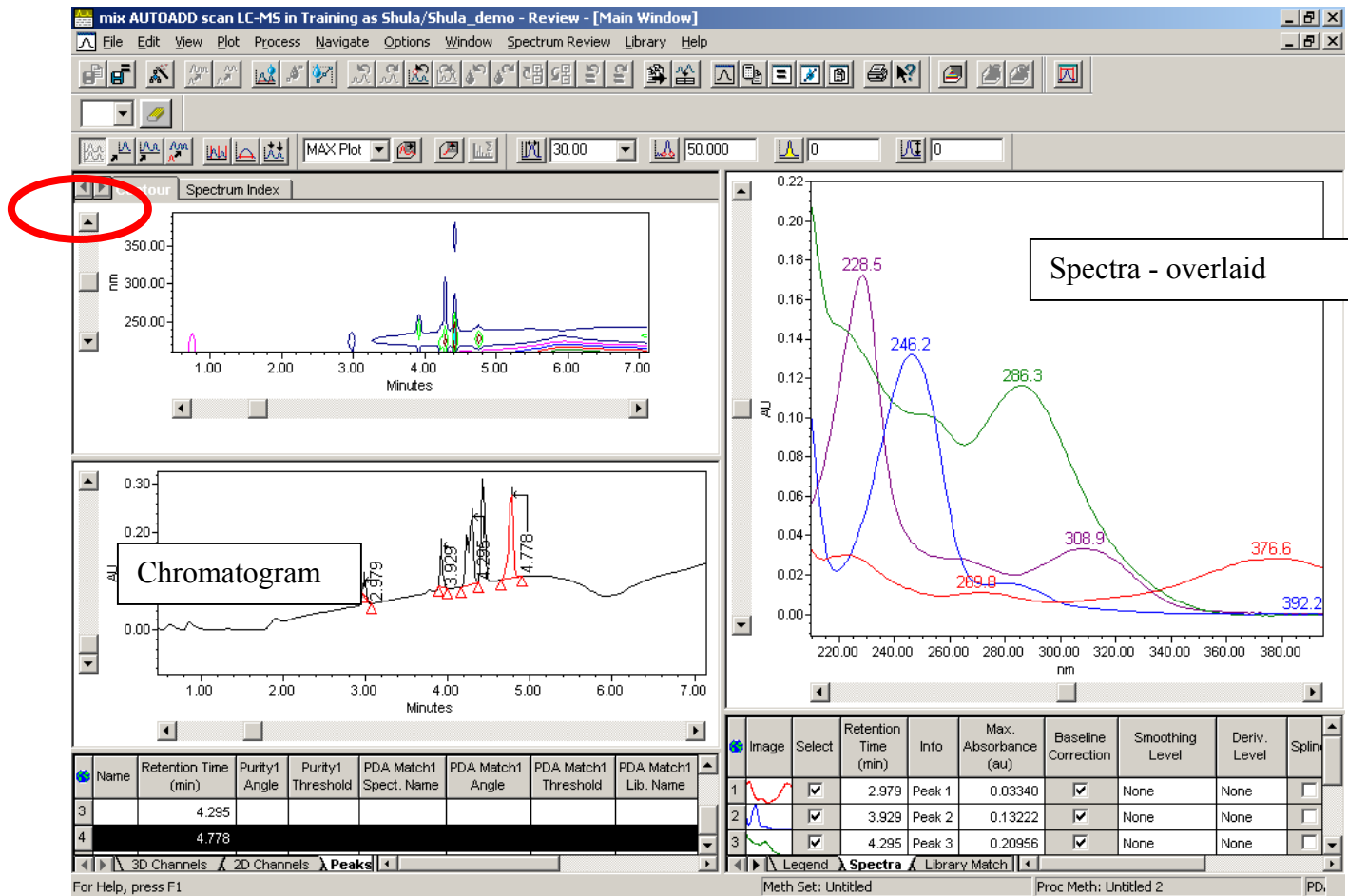
Page 1 of 2

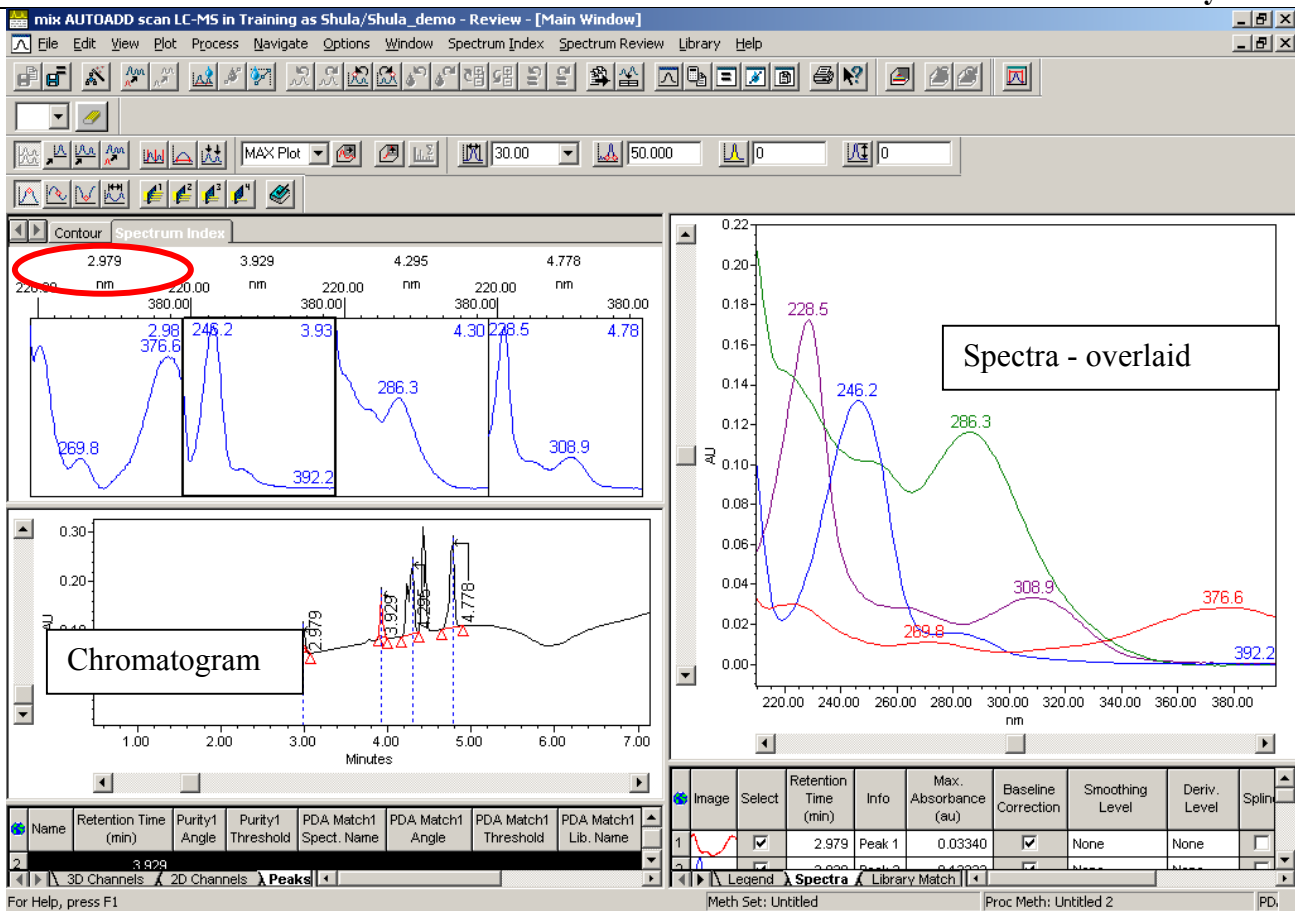
Project: Training Individual

Each element of the report can be edited by selecting it and using *Properties* in the right click of the mouse.

## Reviewing PDA Data

Select the PDA channel (w2996) and Review it. Make sure to be in the main window (Window -> Main Window) and in the 3D layout (View -> 3D layout). Select the MaxPlot or Extract any wavelength you prefer to get the chromatogram.





Open the *PDA Processing Method*. Manually integrate the peaks on interest in the chromatogram and select Quantitate (*Process -> Quantitate*). Save the result (*File -> Save -> Result*).

## Reporting the Result

Same as in the MS data.