

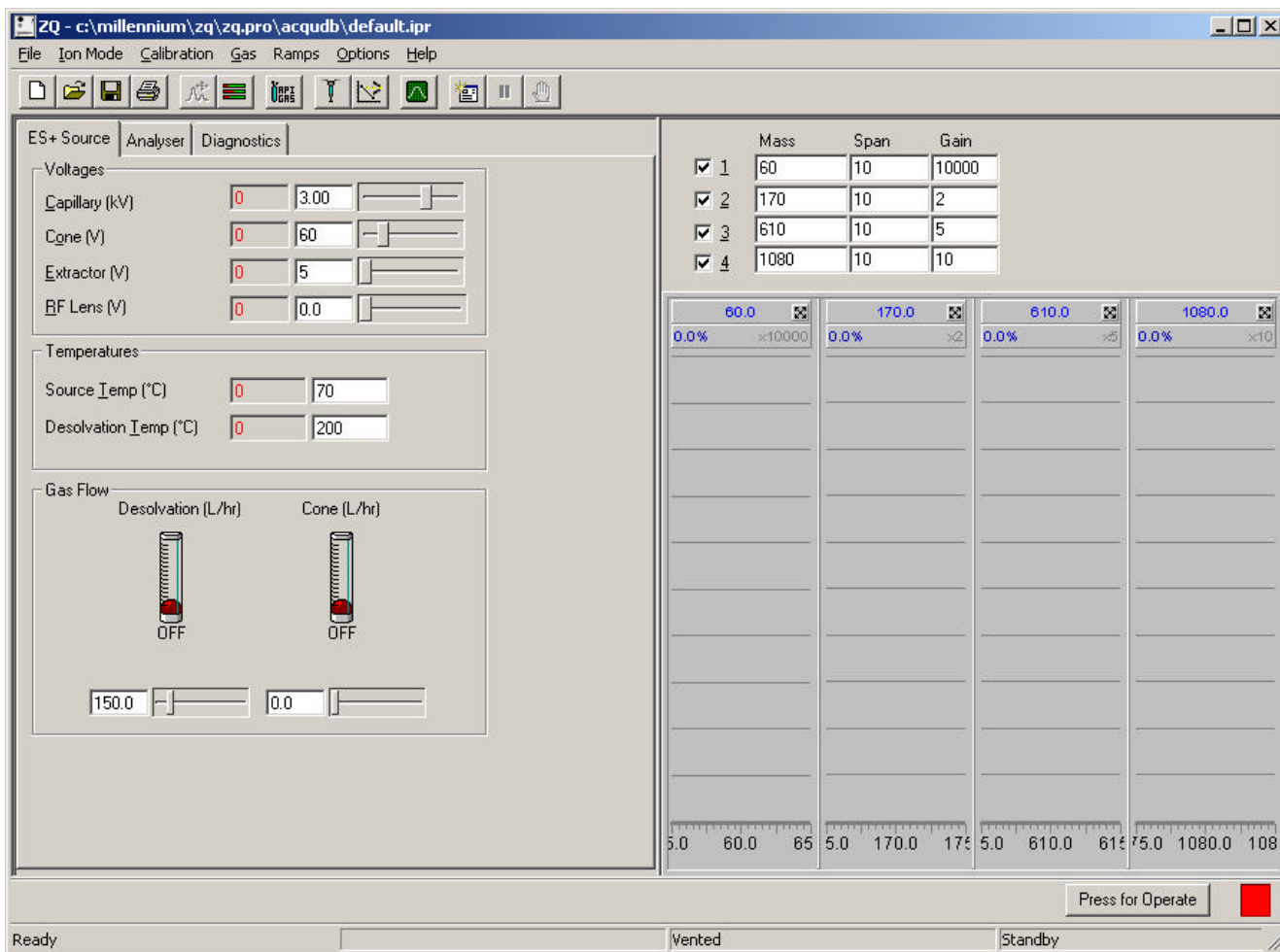
Protocol LC-MS

Preparation of the 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 the Mass Spectrometer

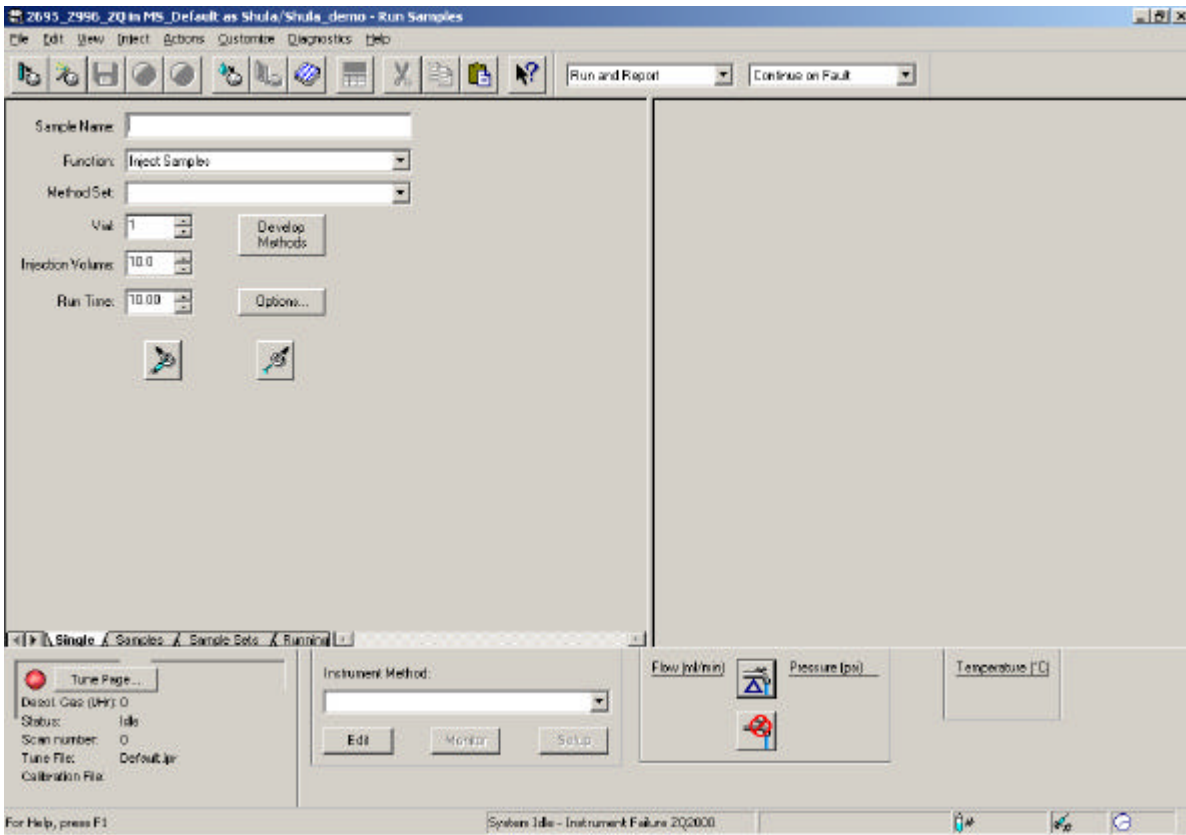
- Enter Empower and *Run Sample* in the right project
- Press the *Tune Page* button, wait for the screen to appear.
- Open the right 'tune page' (*File -> Open -> filename.ipr*) and click the *API gas* icon and *Operate*. The red light will turn to green.



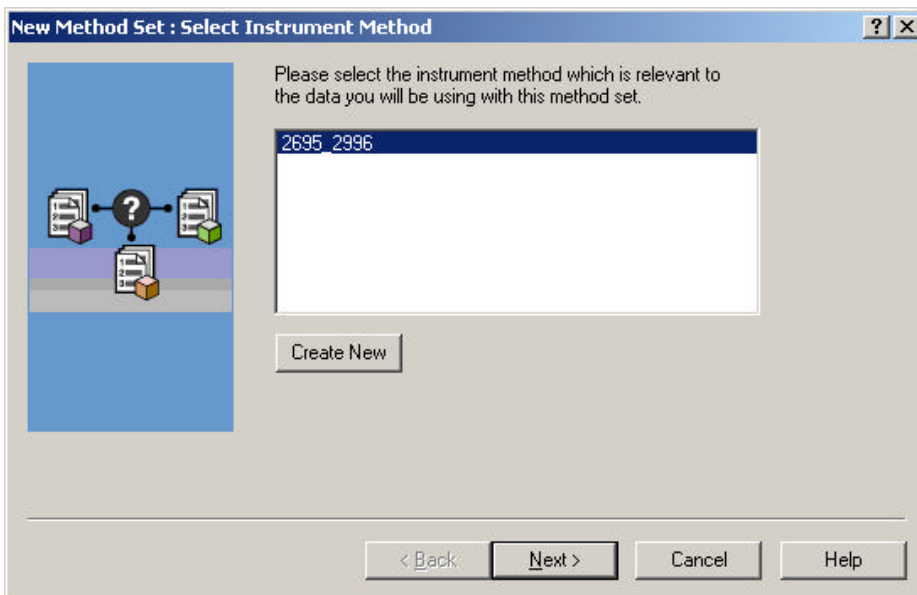
- Wash the MS by the syringe pump, using with the wash solvent appropriate for the method (usually the diluent). Check that the MS functions right and tune the MS to the best signal

Preparation of Empower

- Enter the *Run Sample* Window.

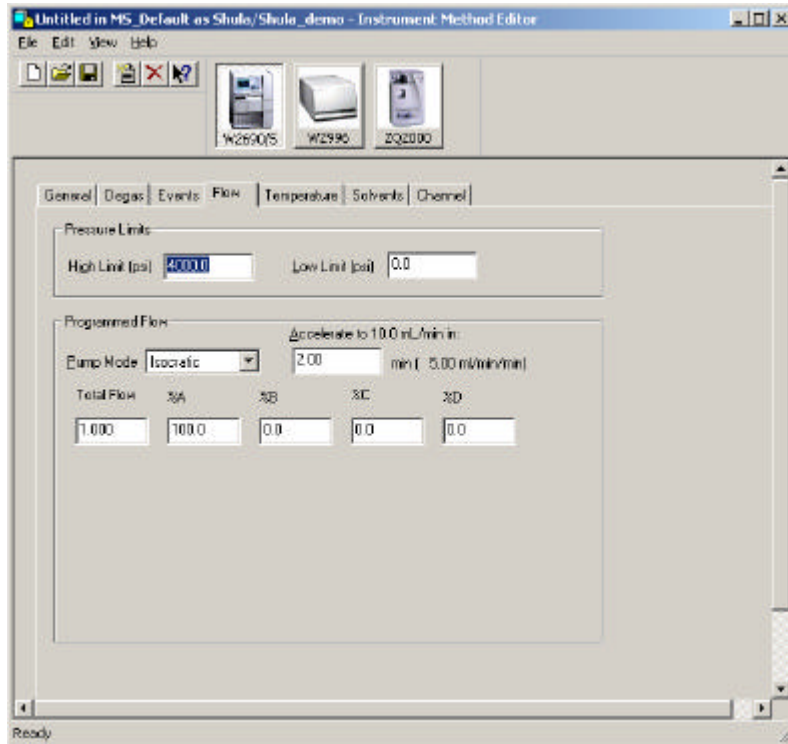


- Select the *Single* window. If you don't have a *Method Set* ready for work, click the *Develop Methods* Button and follow the wizard to create the *Method Set*:

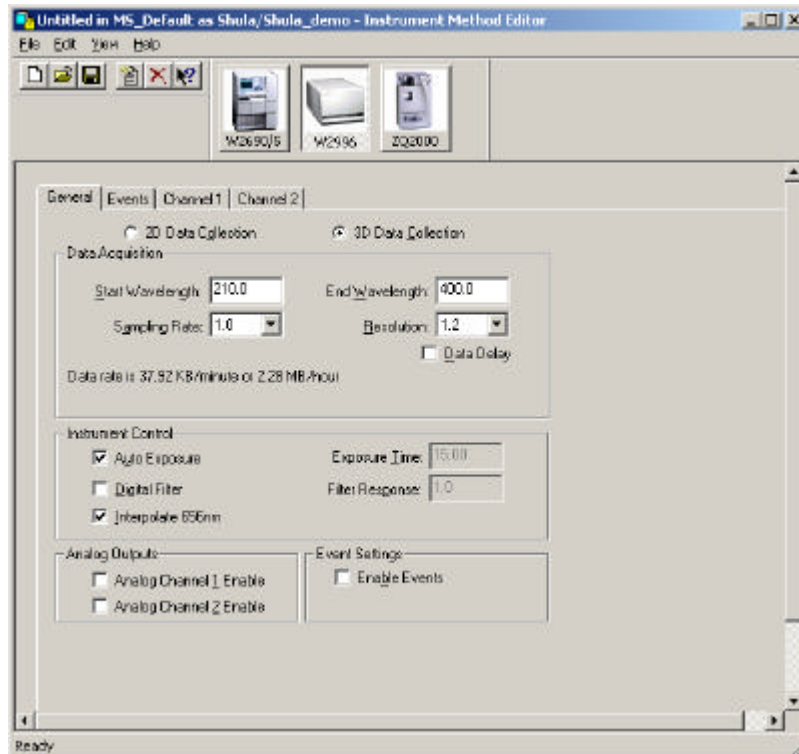


- Prepare a *Method Set* with the 2695 and ZQ with/without the 2996..

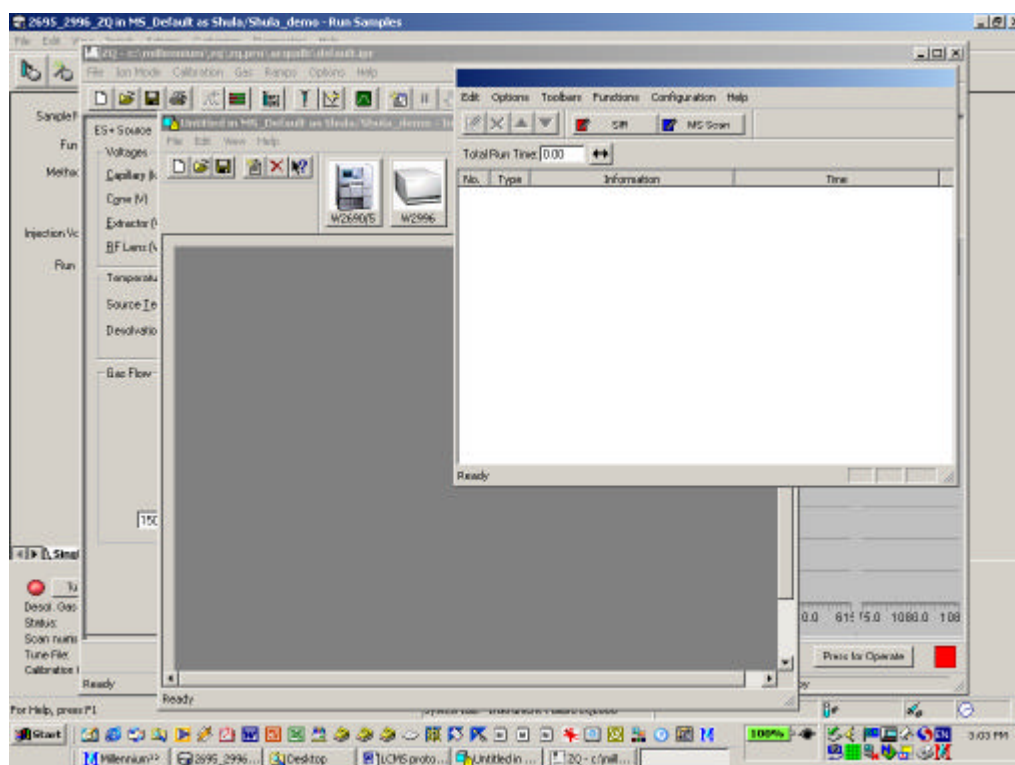
HPLC pump and Autosampler – the **2695 window**:



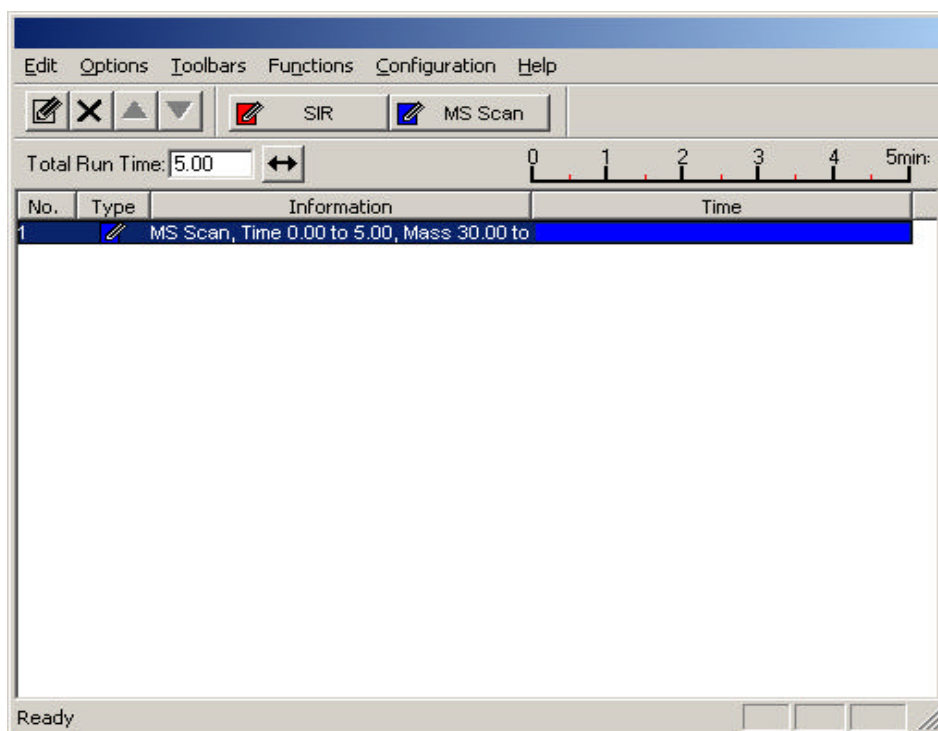
PDA Detector window (optional):



ZQ Detector window:



The ZQ can be used either with *MS Scan*:



MS Scan Window:

Function:1 MS Scan

Mass (m/z)

Start 30

End 1500

Method

Ionization Mode ES+

Data Continuum

Time (Mins)

Start 0

End 5

Scan Duration (secs)

Scan Time 10

Inter-Scan Delay 0.1

Cone Voltage

Use Tune Page

Cone Voltage (V) 31

Use Cone Voltage Ramp

CV Ramp...

APCl Probe

Use Tune Page Settings

Probe Temp 100

OK Cancel

or **SIR (Single Ion recording):**

Function:1 SIR

| Mass (m/z) | Dwell (Secs) | Cone (Volts) |
|------------|--------------|--------------|
| | | -1.0 |

Method

Ionization Mode ES+

Inter-Channel Delay 0.02

Inter-Scan Delay 0.1

Repeats 1

Span 1

Use Tune Cone Settings

Retention Window (Mins)

Start 0

End 5

APCl Probe

Use Tune Page Settings

Probe Temp 100

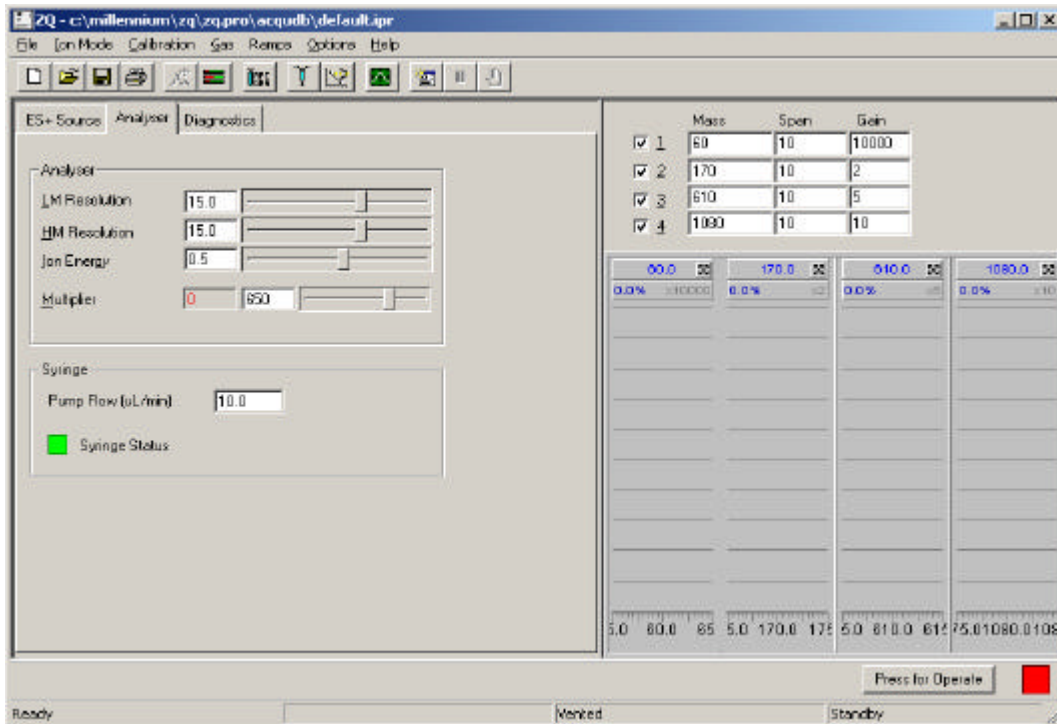
Add Change Sort

Clear All Delete

OK Cancel

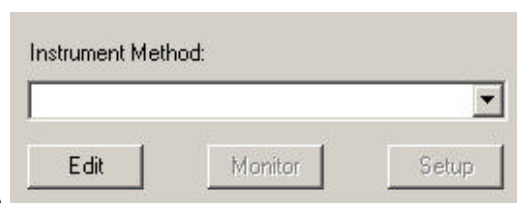
Collecting Spectra with the Infusion pump:

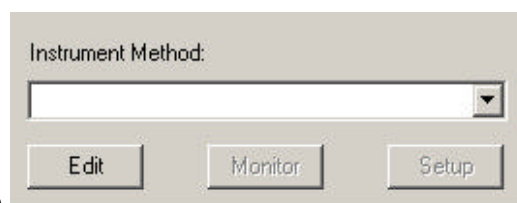
- Fill the syringe with the solution, connect it to the interface, and go to the ZQ Tune page to click the infusion pump icon and set its flow rate:



Please note: the LC system is disconnected now!

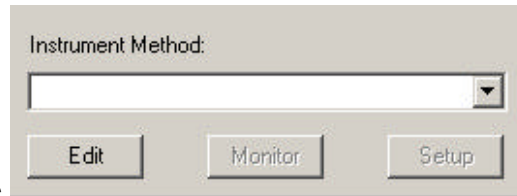
- Return to the *Run Samples* window and select the appropriate *Method Set*.



- Select the *Instrument Method* in the  and click *Setup*. Then click *Monitor* to check communication and monitor baseline.
- Abort monitoring.
- **Fill in the injection volume the value of 0.0**, so that nothing will be injected. Click on the *Inject* icon when it is ready.
- 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:

- Go to the *Run Samples* window and to the *Samples* window, prepare a sample set with the right parameters. Save the sample set method for your convenience.



- Select the *Instrument Method* in the 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