

## **Shimadzu LCMS-2010 Training Manual**

*02/13/2013 S.V.*

**Location:** 1238 Hach Hall  
**Contact:** Kamel Harrata, 1236 Hach Hall

### **Safety**

All researchers working in 1238 Hach Hall must complete the EH&S courses: “*Fire Safety and Extinguisher Training*”, and “*Lab Safety: Compressed Gas Cylinders*”. When preparing samples in 1238A, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available.

Properly dispose of waste solvents and glass pipettes in the containers provided in 1238A. All of the data processing computers and many of the data acquisition computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.



# Shimadzu LCMS - Training Manual

1/24/02

Welcome! Proper operation of the Shimadzu LCMS system requires detailed knowledge in several areas. The extent of the training and the time necessary to acquire the knowledge will depend upon the background and experience of the user, and the commitment made to the learning process. Dr. Shu Xu will normally provide this training. Only users with approved projects will be trained on this instrument. We have prepared condensed training documents to supplement the various instrument operation manuals. This material has been taken directly from the manuals, and we thank Shimadzu for their permission.

In order to insure proper operation of the instrument for everyone, *users will not be authorized to use the instrument until all aspects of the required training have been accomplished and verified.*

## **Step One - HPLC and Mass Spectrometry Theory** [8 hours - self learning]

You will be given access to generic LCMS training software entitled "Introduction to LCMS". This software will require from 4 to 8 hours to complete, depending upon your background. We expect you to understand all the material contained in all of the chapters except chapter 7. When you are finished, you must remove the software from your computer and return the disks to us. The software must not be duplicated or shared with other parties.

*Verification: You must successfully complete all of the quizzes in the software and provide us with a final "Progress Page" report verifying a 100% score.*

## **Step Two - Learning Good Laboratory Practice in our Lab** [2 hours - staff training]

We will discuss rules and regulations regarding your use of our lab facilities and those items specific to the operation of the Shimadzu LCMS. This will include methods and protocols for creating and storing buffer solutions, filtering and degassing solvents, filtering samples immediately prior to analysis, et cetera.

*Verification: under the supervision of staff, you must demonstrate all procedures.*

## **Step Three - Learning the Instrumentation Hardware** [ 4 hours - staff training]

Using sections of the Shimadzu LCMS2010 Users guide and summary materials prepared by us, you will be given instruction in all aspects of the system hardware and capabilities pertinent to your project needs, including a discussion of practical ESI and APCI capabilities. The discussion will also include critical aspects of the LC equipment and screen menus, installing your column, proper routing and connection of the low pressure solvent lines and high pressure flow lines, proper injection technique, methods for purging lines, recognizing flow problems, et cetera.

*Verification: under the supervision of staff, you must install your column, establish proper leak free flows, start the nebulizer, and demonstrate proper sample introduction.*

#### **Step Four - Learning the Instrumentation Software** [2 hours - staff; 8 hours - self]

Using sections of the Shimadzu LCMS2010 Users guide and summary materials prepared by us, the following tasks will be learned and/or accomplished:

- 1) Setting up your user account and file structure
- 2) How to set up an experimental method / setting up *your* experimental method
- 3) Verification of instrument performance, including background and standards
- 4) Acquiring and viewing data
- 5) Processing data
- 6) Record keeping and archiving data files.

*Verification: under the supervision of staff, you must set up an experimental method from beginning to end, acquire data, and process data.*

#### **Step Five - Application Assistance** [2 hours - staff]

All of the training requirements listed above should be accomplished using standard test samples of known purity, concentration, and chromatographic performance, which you must provide. These samples should be similar in nature to the compounds you will be analyzing. Only when experiments using your standards have been completely successful will we allow you to use the instrument to analyze your research samples. At this point, Dr. Harrata will be available for consultation and assistance.

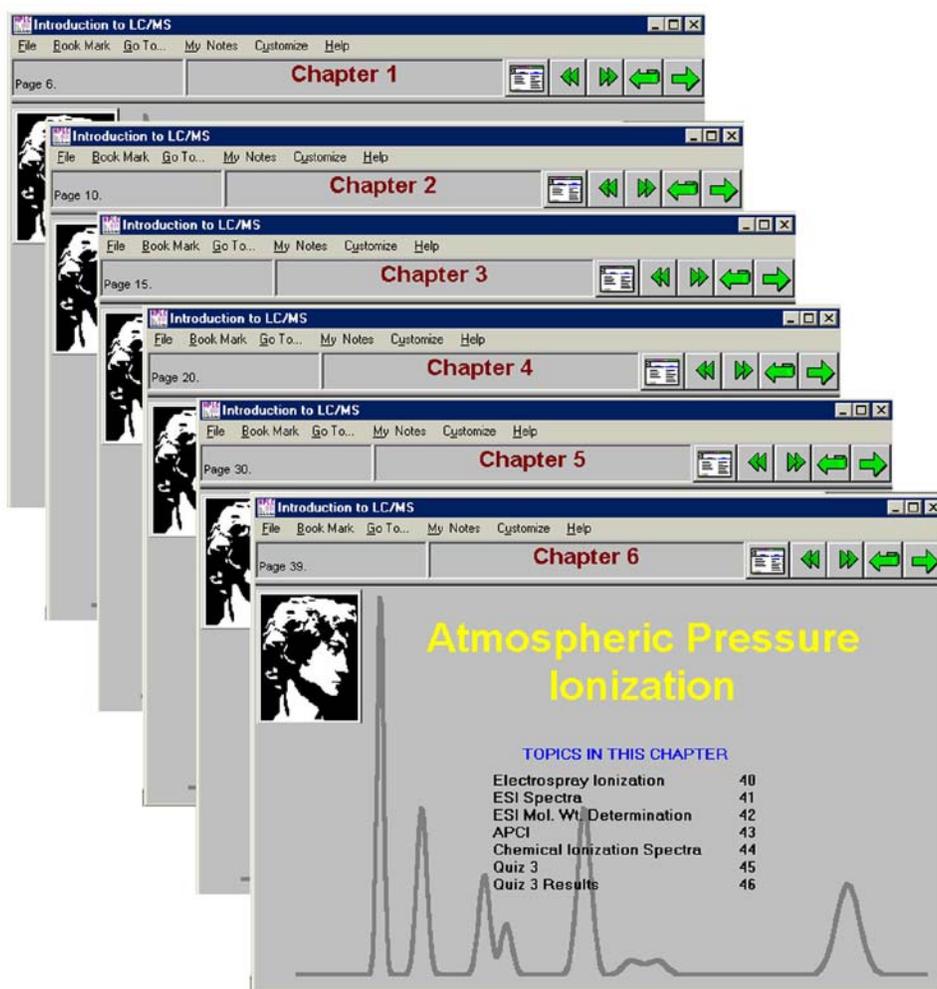


Corbin Zea Uses the Shimadzu LCMS-2010

# Step One - HPLC and Mass Spectrometry Theory

## 1.0 Savant Computer-Based Training

The first step in your training involves a review of key chromatographic and mass spectrometry concepts that you must understand in order to use the LCMS effectively, independently, and with the greatest chance of success. The Savant computer-based training module will give you the fundamental background you will need. Depending on your background, it may take as little as five minutes to go through each chapter, or it might take considerably longer. After each two chapters there is a short quiz. At the end of the program there is a review and a final test. When you are certain you understand all of the material in the module (ignore chapter 7 if you wish), go through the program one more time in "progress tracking" mode. At the end of the program, print the Summary View of the Progress Page. This will verify your score on the tests.



When you are finished, return the program disks and the Progress Report sheet to Dr.Xu. Delete any copies of the program you may have loaded in your lab. The software may not be duplicated or distributed to anyone outside the LCMS user group.

# Step Two - Lab Procedures

revised: 1/28/02

**Note:** This section is subject to change!! Please keep this section current in your binder!!

## 2.1 Scheduling and Access to the Lab

You may apply on-line at [www.cif.iastate.edu](http://www.cif.iastate.edu) for a Marlok key to allow you access to the lab at any time. If you already have a Marlok key, we can add access permission to your existing key. Students of Professor Pohl's research group have scheduling priority for the LCMS. These students are allowed to use the on-line reservation program at [www.cif.iastate.edu/MassSpec](http://www.cif.iastate.edu/MassSpec). You may use the program to see which times are open in the near future, but requests for time must be given to Dr. Xu, who will then use the reservation program to assign you time.

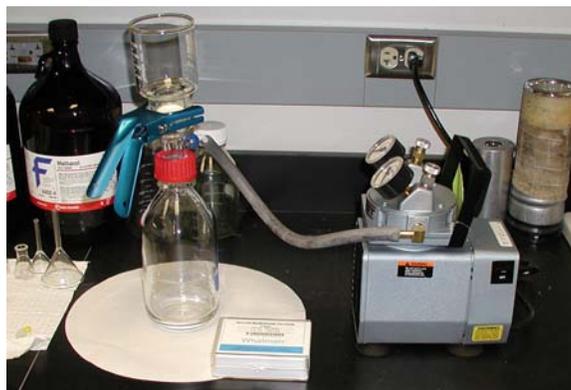
## 2.2 Solvents

### 2.2.1 Solvent Bottles

You must use your own solvents in your own bottles, or bottles that have been assigned to you. You may *never* use solvents prepared for someone else, or solvent containers that are not yours. We have a supply of 500 mL and 1000 mL bottles especially designed for use with the LCMS. These can be loaned to you for the duration of your project. The content and the owners name must be CLEARLY visible on all containers in our lab. Unlabelled containers will be discarded without hesitation.

### 2.2.2 Solvent Filtering

Pure organic solvents do not need to be filtered prior to use. If any modifiers have been added to the organic phase, then the mixture should be filtered. The aqueous phase, regardless of the presence or absence of modifiers, should be filtered immediately prior to use. Bacterial growth, especially in aqueous solutions, can lead to clogs in the HPLC mixer, the high-pressure stainless steel tubing, or the capillary transfer tubing, as well as high background and extraneous mass peaks in the mass spectrum. As part of your training, Dr. Xu will show you how to use the filtering apparatus located in our wet lab.



### 2.2.3 Solvent Sparging

Solvents, both organic and aqueous, should be sparged prior to use to remove dissolved gases (nitrogen and oxygen) that have the potential to come out of solution during the LC run and cause clogs and/or instability in the flow. The procedure is quick and simple, and involves passing helium gas through the solvents through a diffuser for a period of several minutes. Each LCMS user will be given a personal sparge line and diffuser for the duration of their project. Never use someone else's sparge line. Dr. Xu will demonstrate the procedure for you.



## 2.3 Samples

### 2.3.1 Preparation

Samples should be prepared in your own lab. In emergency situations you may use the equipment in our wet lab, including the balance, centrifuge, acid-washed glassware, and sample filtration cartridges. We have a limited supply of nanopure water available, which we obtain from another lab. This can be used for final rinse or sample dilutions. We hope to have our own supply in the near future. If you use any of our glassware, *do not* return it to the glassware rack or shelf. Put it in the sink and we will eventually clean and acid wash it. At no time should phosphate detergents be brought into or used in our lab.

### 2.3.2 Sample Filtration

All samples should be filtered through a 0.45 micron filter just prior to analysis. No exceptions. Ignoring this rule will be cause for suspension of privileges. We have a limited supply of these filters for use in cases of emergency. You should order and use your own.

### 2.3.2 LC Columns and Syringes

You must provide your own LC column, as appropriate for your project. You must provide your own syringes (blunt-tip; especially designed for LC use).

## 2.4 Instrument Configuration and Testing

As part of your project, you must define specific test samples and protocols you will use to verify instrument performance at the beginning of each session, and at the end of each session. If you ignore this requirement you will no longer be allowed to use the instrument. Dr. Xu or Dr. Harrata will assist you in verifying that the test protocol for your project is sufficient.

### **2.4.1 Loop Injection, Standard Sample**

The mass spectrometer side of the instrument will always be tuned for optimum operation. Before starting the LCMS run, always acquire data with a 5ul loop injection of your standard test compound. Keep a record of the day-to-day loop injection performance on your test sample. If you see significant changes in instrument performance, consult with facility staff before continuing.

### **2.4.2 Loop Injection, Research Sample**

Whenever possible, try a loop injection of your research sample in order to test the performance of the instrument *viv-a-vis* your sample. Only when you are satisfied that sample ionization is occurring and sample concentration is adequate, should you proceed with LC introduction of your sample.

### **2.4.3 Ending Your Session**

At the end of use, the LC system and the tubing lines to the ESI source should always be flushed with DI water (A) and isopropanol (B) 10/90.

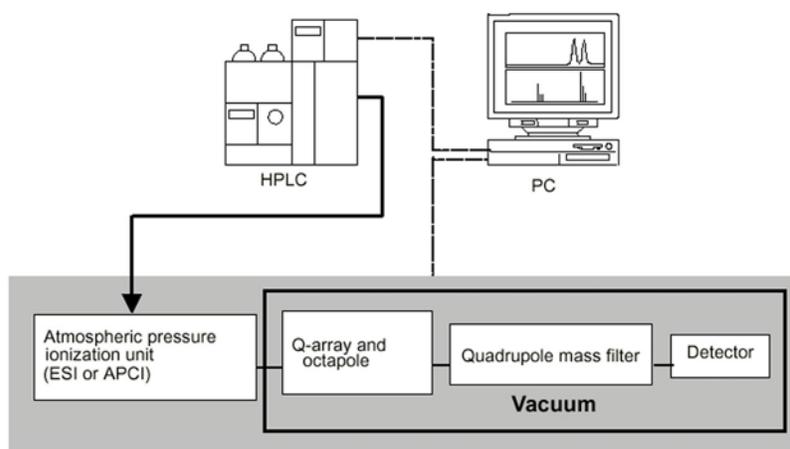
- 1) Turn off pumps and nebulizer gas
- 2) Place DI (A) and isopropanol (B) into lines A and B respectively
- 3) Purge pumps A and B (will be included in the training)
- 4) Turn on the pumps from the LC/MS solution program and run (10 min) a flow of 200 uL per minute with 10/90 A/B.
- 5) Stop pumps and nitrogen
- 6) Log off.
- 7) Turn off power of all LC components including LC pumps, degazer, UV detector, auto-injector. Exit from the **LCMSsolutions** and **LCMS Post Run** software programs.

**!!! NEVER turn off the mass spectrometer !!!**

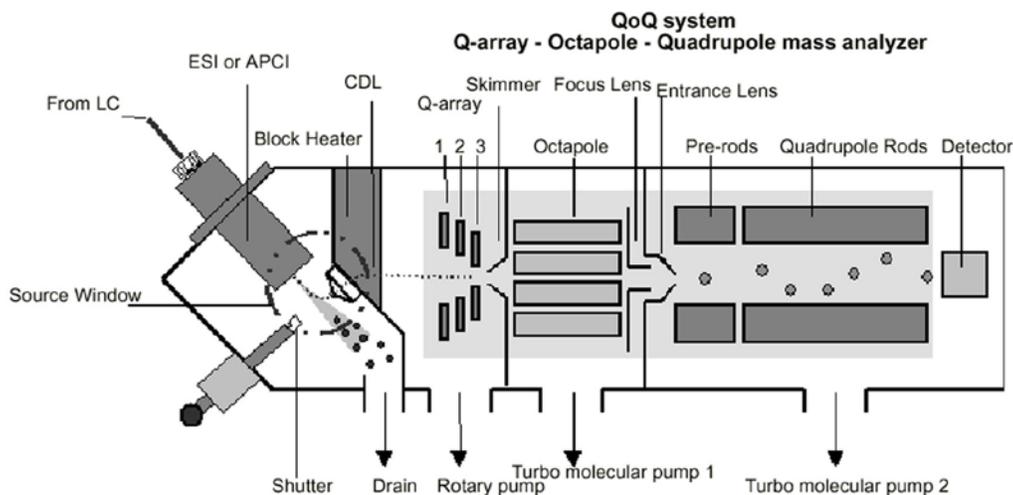
# Step Three - LCMS-2010 Instrument Overview

## 3.0 Overview

The LCMS-2010 has been designed for liquid chromatographic detection using Atmospheric Pressure Ionization (API). The single stage quadrupole mass analyzer, coupled with electrospray (ESI) and atmospheric pressure chemical ionization method (APCI) provides the most sensitive and selective detection of organic molecules. To generate sample ions, sample molecules are ionized at atmospheric pressure from a highly charged aerosol. Once in the gas phase, ions are electrostatically extracted from the charged spray using an orthogonal source geometry. Ion transmission to the quadrupole mass analyzer is enhanced using a Q-array and octapole configuration. Sample ions are then separated according to the mass-to-charge ratio ( $m/z$ ) and detected with the secondary electron multiplier detector. The detected signal is amplified and then processed by the data processing software, LCMSsolution.

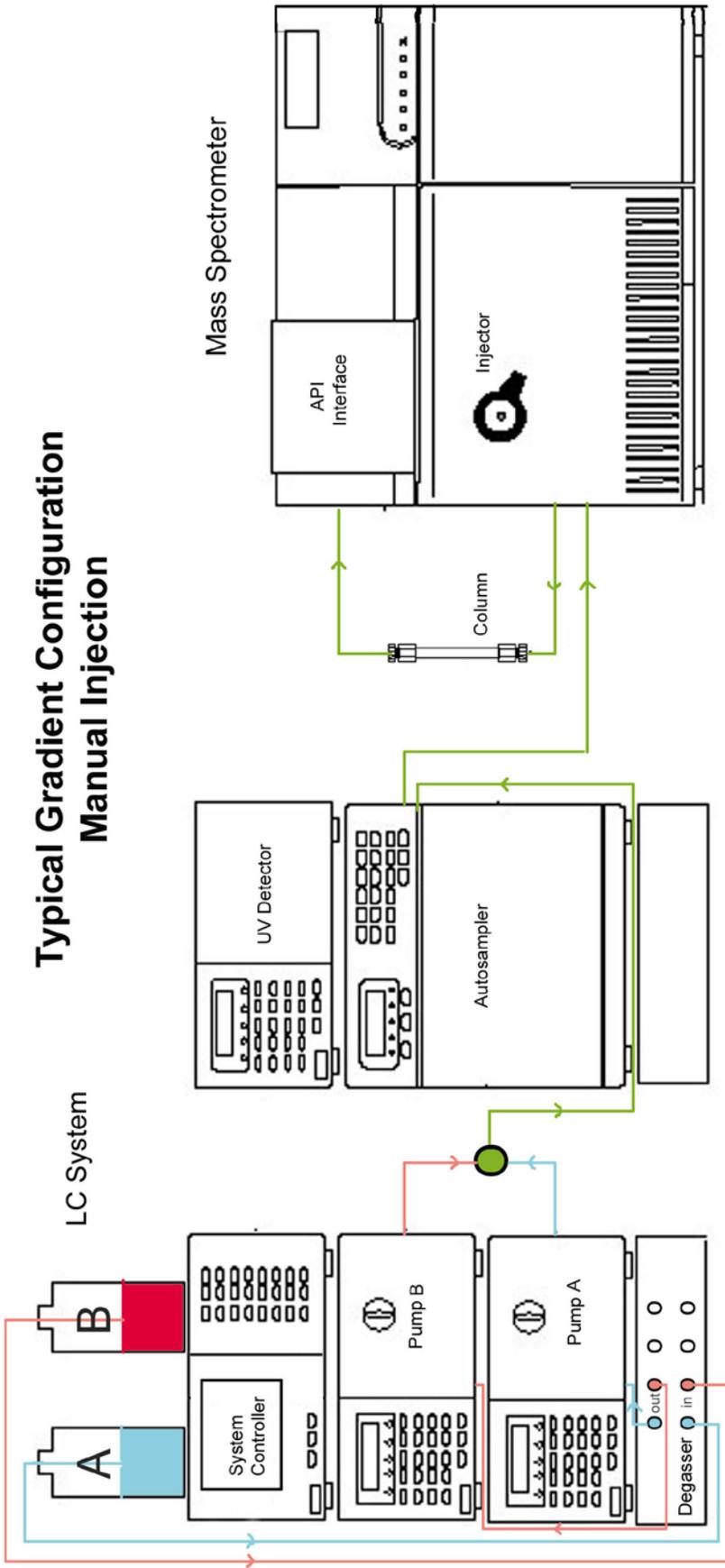


LCMS-2010 System Configuration



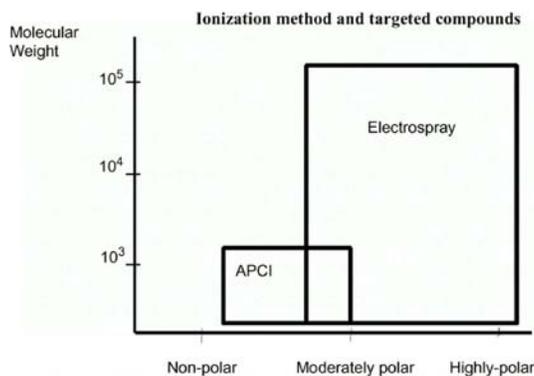
Schematic diagram of the LCMS-2010

# Typical Gradient Configuration Manual Injection



### 3.1 Atmospheric Pressure Ionization

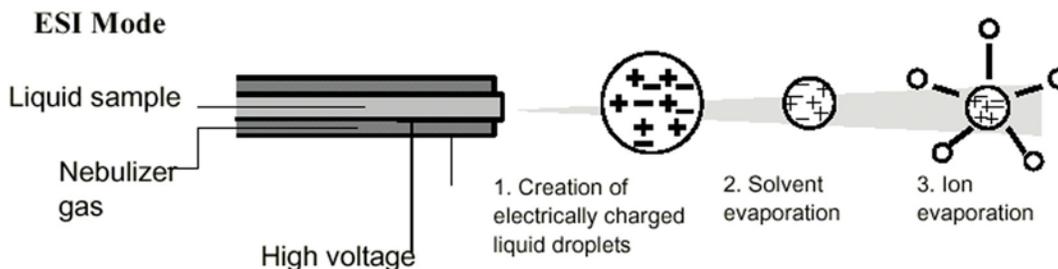
In atmospheric pressure ionization (API) mass spectrometry, sample ionization take place outside the vacuum system of the MS at atmospheric pressure. Sample molecules enter the ion source directly from the LC and become ionized to produce an ion beam of pseudomolecular ions using either **atmospheric pressure chemical ionization** or **electrospray**. The two techniques differ in the mechanism for ion generation, consequently affecting the response to different sample molecules.



### 3.2 Electrospray - Overview

Ion generation in electrospray involves placing a high electric charge, typically 3 – 5kV, to the droplets emerging from the ESI capillary tubing. The droplets are dispersed into an electrically charged aerosol using a high-velocity nitrogen gas flow that acts as a concentric pneumatic nebulizer. Once the charged aerosol is formed, solvent evaporation takes place, decreasing the size of the droplets and increasing the density of charge at the surface. The surface charge increases to a point where the coulombic repulsion forces are sufficient to overcome the forces of surface tension. At this point, the droplets explode into smaller droplets as the Rayleigh stability limit is exceeded. Further solvent evaporation takes place, reducing the size of the droplets and increasing the surface charge density. Once again, the Rayleigh limit is reached and the droplets explode into even smaller droplets. At some stage, the electric field at the surface of the very small droplets is so high that the solute ions enter the vapor phase directly.

One model for ion evaporation proposes that ions are ejected from the droplet surface into the gas phase. This process takes place as the electrostatic repulsion between ions forces ions from the liquid phase. An alternative model proposes that ions enter the vapor phase when most of the solvent molecules are stripped from the sample molecules by solvent evaporation. This process continues until most of the solvent is evaporated from a droplet and a single ion remains.



The sprayed sample solution transforms into electrically charged droplets because of the high voltage. Solvent evaporates from the droplets and then the sample is ionized by ion evaporation.

### 3.2.1 ESI - Products of Ionization

With ESI, monovalent ions are typically produced by the addition or loss of a proton (H) ( $[M+H]^+$  in the positive ion mode:  $m/z M+1$ ,  $[M-H]^-$  in the negative ion mode:  $m/z M-1$ ). The addition of ammonium,  $NH_4^+$   $[M+18]^+$ , sodium,  $Na^+$   $[M+23]^+$ , potassium,  $K^+$   $[M+39]^+$  may also be observed. If the voltage at Q-arrays 1, 2, and 3 is increased, fragmentation is observed. The structural analysis of the sample can be made from these fragment ions.

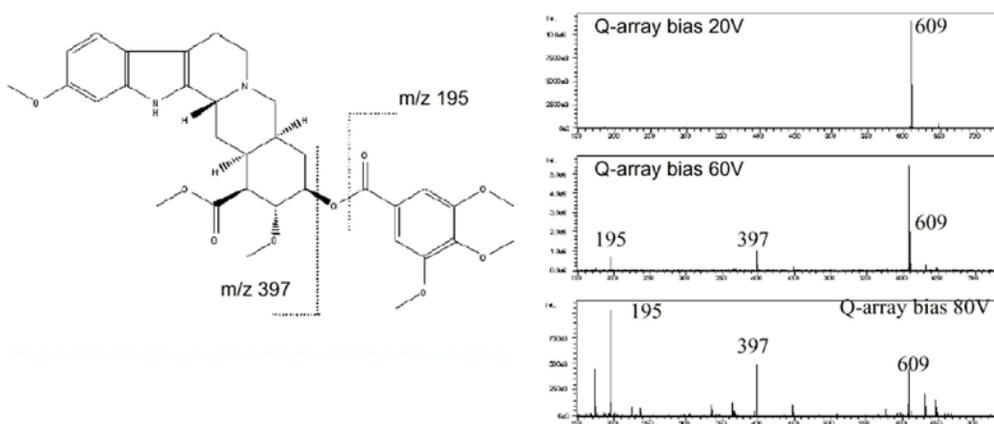
### 3.2.2 ESI - Flows

For the ESI, the flow rate ranges from 1  $\mu L/min$  to 1  $mL/min$ . Normally, the optimal flow rate is approximately 0.2  $mL/min$ . To optimize the signal response to different LC flow rates, changing the nebulizer gas flow rate is recommended. The standard values are given below:

LC pump flow rate	10 $\mu L / min$	0.2 $mL / min$
Nebulizer gas flow rate	1 - 2 $L / min$	3 - 5 $L / min$

### 3.2.3 ESI Fragmentation

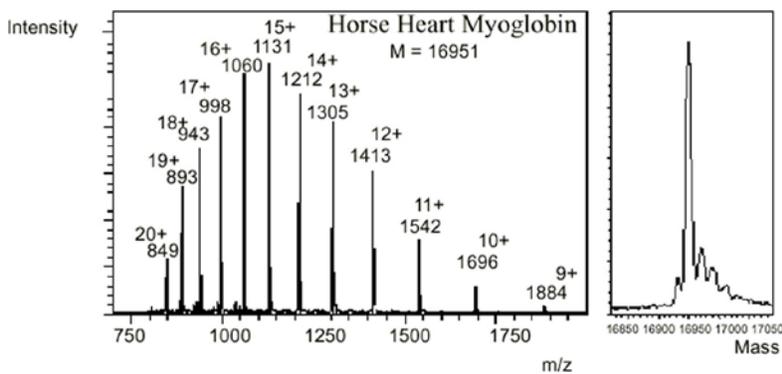
When the energy acquired by the molecule exceeds the ionization potential, the excess energy is distributed inside the molecule; when the energy exceeds the dissociation energy for a particular chemical bond, fragmentation occurs. By increasing the voltage at Q-arrays 1, 2 and 3, the weaker bonds in the molecule fragment. For reserpine, fragment ions appear at  $m/z$  397 and 195. Normally, fragmentation occurs at weaker bonds, such as C-N and C-O.



Fragment ions are generated between the heated capillary (CDL) and the Q-array. In this area, the pressure is 10<sup>-2</sup> Pa and has a large amount of neutral molecules present, such as solvent molecules and nitrogen gas. By applying an accelerating potential on the Q-array, ions and neutral molecules collide with each other, resulting in fragment ions.

### 3.2.4 Multiply-charged ions

The electrospray process generates multiply-charged ions for certain molecules, effectively increasing the mass range of the instrument. This property enables proteins and peptides to be determined; the example below shows myoglobin (molecular weight 16951).



### 3.2.5 Deconvolution Concepts

The charge envelope can be simply deconvoluted to provide the molecular weight of the protein. In this example, multiple charges were observed with the highest intensity at  $m/z$  1131, corresponding to 15 charges (i.e.,  $1131 = m/z = 16951/15$ ).

Assume that a proton is added (or Na, K,  $NH_4^+$ , or similar adducts are added). Also assume that the  $m/z$  value is  $m_i$  when molecular weight is  $M$  and the number of valences is  $i$ .

When the mass of proton is assumed as  $M_H$ , valent number,  $i$  and  $(i + 1)$  lead to:

$$m_i = [M + iM_H] / i$$

$$m_{i+1} = [M + (i + 1)M_H] / (i + 1)$$

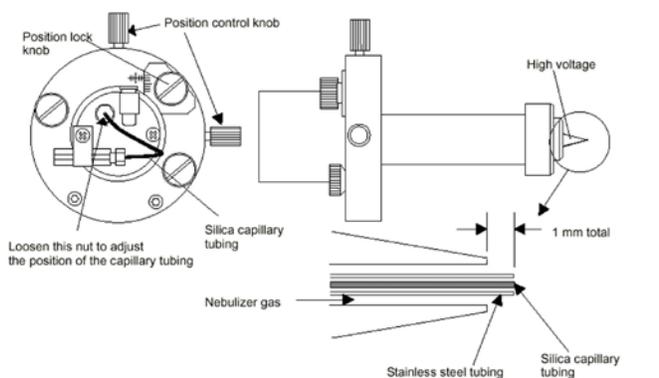
When  $M_H = 1$ , the above two equations lead to:

$$i = (m_{i+1} - 1) / (m_i - m_{i+1})$$

Now the number of valences for a peak as observed using two neighboring peaks is obtained, allowing you to calculate the molecular weight.

### 3.2.6 Adjusting the ESI Probe

With ESI, the condition of the silica capillary tubing tip has a significant impact on the intensity and stability of ionization. In order to ensure that the spray is stable, check the following points:



Adjust the position of the silica capillary tubing so that the outlet of the tubing is aligned with the end of the stainless steel tubing. If the capillary tubing protrudes too far, no ions will be detected.

If the end of the capillary tubing loses its polyamide coating, it becomes fragile and cracked. Ions may not be detected, and the baseline becomes unstable. Clip the end of the tubing or replace the tubing if this occurs.



Normally, the end of the ESI probe should be aligned with the end of the CDL. Loosen the position control knob to make the adjustment, then lock the tubing in position with the position lock knob.

### 3.2.7 Precautions for Operating in ESI Mode

**Influence of Mobile Phase Acidic Modifiers** - In positive ionization mode, the sensitivity can be improved by adding a volatile buffer such as acetic or formic acid of approximately 1% to the mobile phase. If acid is added in the negative ionization mode, the interface current increases and unstable electric discharge occurs. This can make the ionization unstable. Avoid adding acid wherever practical in negative ionization use. To suppress electric discharge in the negative ionization mode, decrease the high voltage applied to the probe (down to -3 or -2.5 kV while the high voltage is typically -3.5 kV), or change the nebulizer gas from nitrogen to compressed air. (Do not use compressed air with flammable solvent mixtures).

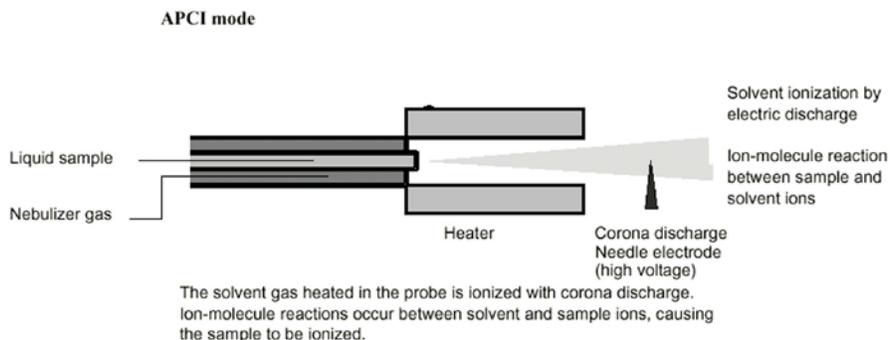
**Influence of High Water Content Mobile Phases** - If the ratio of water in the mobile phase is high or if a sample is analyzed with a high flow rate, ion evaporation may be limited and large liquid droplets may form. In this case, set the temperature of the block heater between 250 and 300 ° C (normally, it is set to 200 ° C) If the temperature of the CDL or heater block is lowered, cluster ions resulting from the mobile phase may form.

**Note:** Cluster ions may cause isobaric interference with target ions. If this occurs, change the mobile phase solvent or increase the heater temperature.

**Organic Solvent Precautions** - Parts of the ESI probe contain polymers such as PEEK and polyimide. Ensure that the solvent choice is compatible with these polymers. PEEK and polyimide may swell or dissolve in organic solvents such as dichloromethane or DMF.

### 3.3 APCI (Atmospheric Pressure Chemical Ionization)

Ion generation in APCI involves a multiple step reaction sequence: in positive ion mode, the first step involves the creation of proton hydrates,  $\text{H}_3\text{O}^+ [\text{H}_2\text{O}^+]_n$ , by a cascade process involving the major primary ions, such as  $\text{N}_2^+$ ,  $\text{O}_2^+$ ,  $\text{H}_2\text{O}^+$  and  $\text{NO}^+$ . The primary ions are formed by nebulizer gas and solvent molecules passing through the corona discharge region. This cascade reaction process is rapid, approaching 1  $\mu\text{s}$  to form a terminal equilibrium set of proton hydrates. The proton hydrates are highly charged ions which react with the sample molecules by charge transfer and proton transfer to create sample ions. In negative ionization mode, the mechanism of ion generation involves a different process. The corona discharge produces electrons which are then captured by electronegative species such as  $\text{O}_2$  to form  $\text{O}_2^-$  and  $\text{O}^-$ . The major source of negative ions in APCI is the superoxide anion ( $\text{O}_2^-$ ) and the corresponding hydrates and clusters ( $\text{O}_2^- [\text{H}_2\text{O}]_n$  and  $\text{O}_2^- [\text{O}_2]_n$ , respectively). Once the primary ions have been generated, either as proton hydrates or the superoxide anions, the sample molecule can be ionized either by charge transfer or proton transfer.



Like ESI, APCI typically produces singly-charged ions with the addition or loss of a proton (in the positive ion mode,  $[M+H]^+$  :  $m/z$   $M+1$ ; in the negative ion mode,  $[M-H]^-$  :  $m/z$   $M-1$ ). In addition, the adducts of ammonium,  $NH_4^+$   $[M+18]^+$ , sodium,  $Na^+$   $[M+23]^+$ , potassium,  $K^+$   $[M+39]^+$  may be observed, but are usually less prevalent than with ESI.

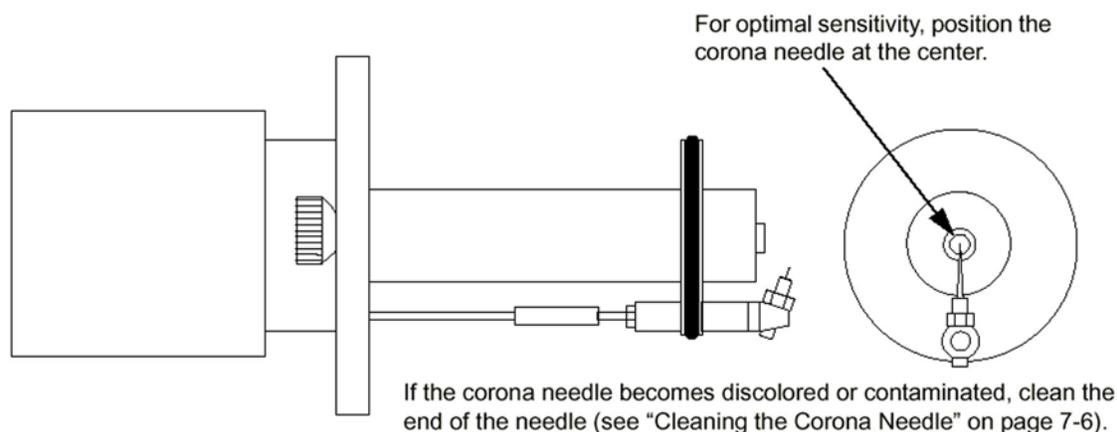
Unlike ESI, APCI only produces singly-charged ions.

As with ESI, fragmentation occurs when the voltage at Q-arrays 1, 2, and 3 is increased.

Normally with APCI, the HPLC flow rate is 0.05 to 2 mL/min. The optimal flow rate is approximately 0.5 to 1.0 mL/min. The typical nebulizer gas flow rate is between 2 and 2.5 L/min.

### 3.3.1 Adjusting the APCI Probe

In the APCI mode, the position of the corona needle is important. Check the following items:



### 3.3.2 APCI Precautions

**Influence of Acidic Modifiers To The Mobile Phase** - Acidic mobile phases can result in suppressed ionization in negative ion mode. This can also result in an increased interface current and possible electrical discharge. Avoiding the addition of acid is recommended if possible. To suppress electric discharge in negative ionization mode, decrease the high voltage applied to the probe (down to -2.5 or 2.0 kV from the typical voltage of -3.0 kV), or switch the nebulizer gas from nitrogen to compressed air.

**APCI Parameters** - If a sample at a low flow rate is analyzed, the temperature of the APCI heater must be decreased. The standard values are given below:

0.05 – 0.1mL/min	300° C
0.1 – 0.2 mL/min	350° C
0.2 – 2 mL/min	400° C – 450° C

When the temperature of the CDL, block, or APCI heater is decreased, cluster ions resulting from the mobile phase may be observed. Note that cluster ions can increase background noise. If this occurs, change the mobile phase or decrease the heater temperature.

## Step Four - LCMS-2010 Data Acquisition and Processing Software

This summary describes the basic LCMS-2010 operations. It covers the analysis steps, from sample injection to data acquisition, as well as data analysis and report preparation. This information is contained in the [System Users Guide Manual](#). For detailed software explanations, also refer to the [LCMS Solution Software Reference Guide](#). A summary of the operations is shown below.

Create an analysis method file	Specify the LC and MS analytical parameters. Save the parameters in a method file.
Create a schedule file	Indicate the sample(s) to run, the method(s) to use for the analyses, and the data file names for saving the results. The schedule can consist of a batch of multiple samples or a single sample.
Acquire data	Inject the sample and start data acquisition. The sample data is saved in a data file.
Analyze Data	Open the data file to process and analyze the data. Analyze the data using postrun or profile postrun mode (for profile data).
Print Results	Print the results with the report function.

### 4.0 Preparation for Analysis

#### 4.1 Creating a Method File

Specify the LC and MS analytical conditions.

## LC parameters

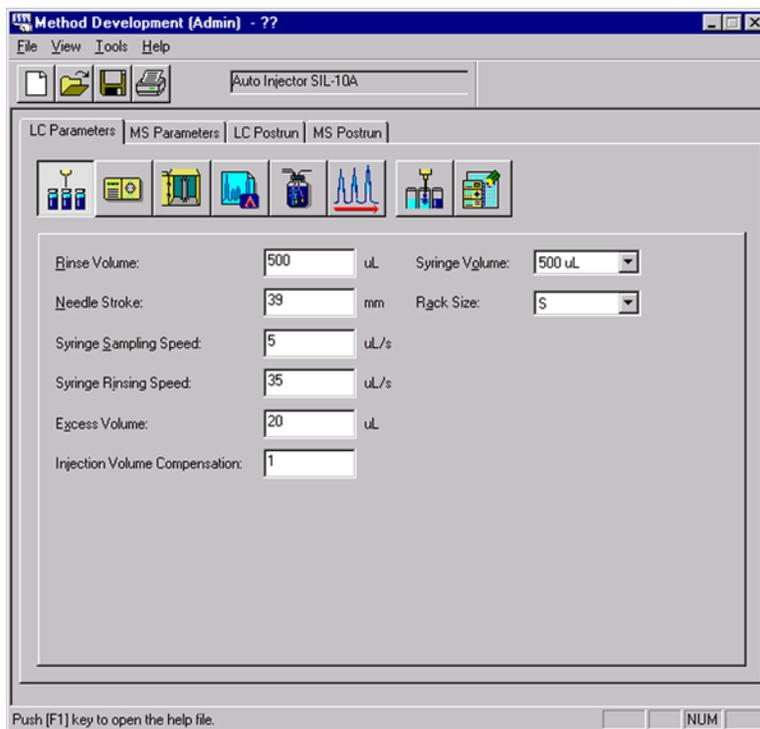


## MS parameters



The LC parameter settings depend on the component configuration.

Select **Tools > Method Editor** from the LCMS Solution main window to open the Method Development window.



Select **Tools > LCMS Configuration**, then click on the **Load Real Configuration** button.

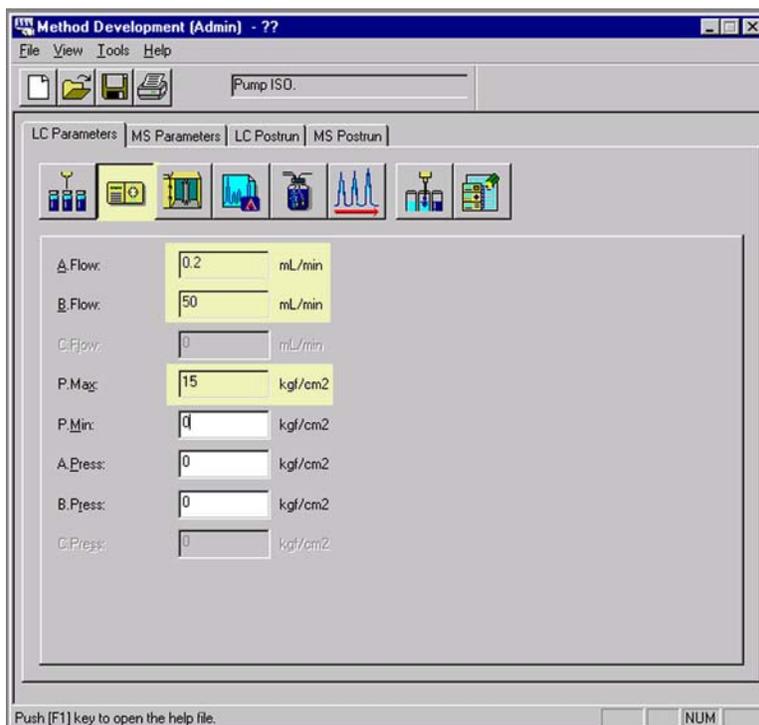


The currently connected LC and MS system configuration is displayed. Double click on each unit icon to verify that the unit connection is detected. Then click **Close** to exit the window.

#### 4.1.1 Setting the LC Parameters

##### 1. Pump parameters

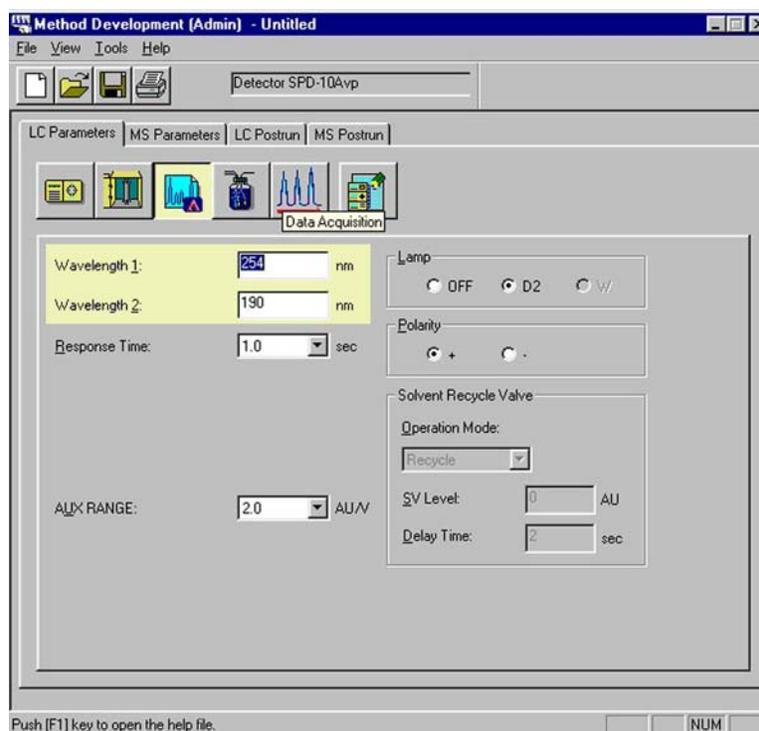
Select the solvent flow mode.



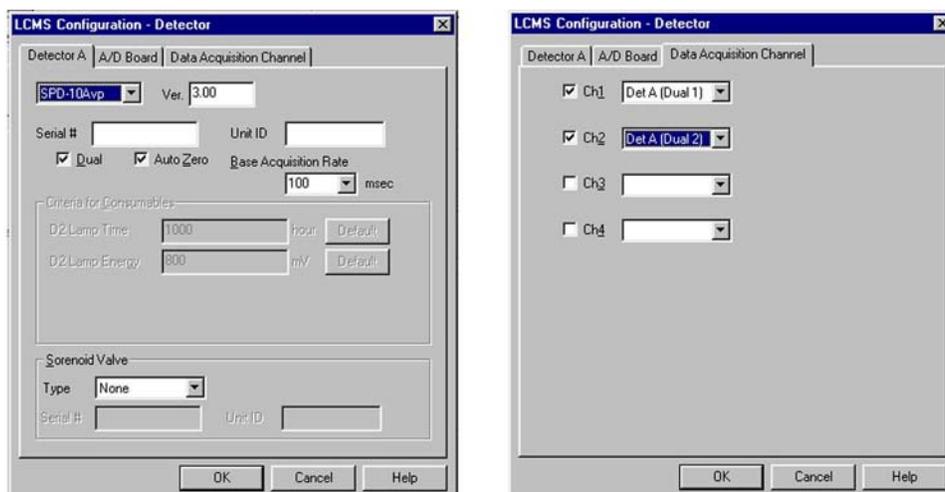
Enter the total flow rate (T.Flow), initial concentration of solvent B (B.Conc), and maximum pressure.

##### 2. UV detector A parameters

For dual wavelength measurement, specify the wavelengths for the analysis (Wavelengths 1 and 2)



To switch between single and dual wavelength measurement, select **Tools > LCMS Configuration** from the Method window, and then double-click on **LC System > Detector**.



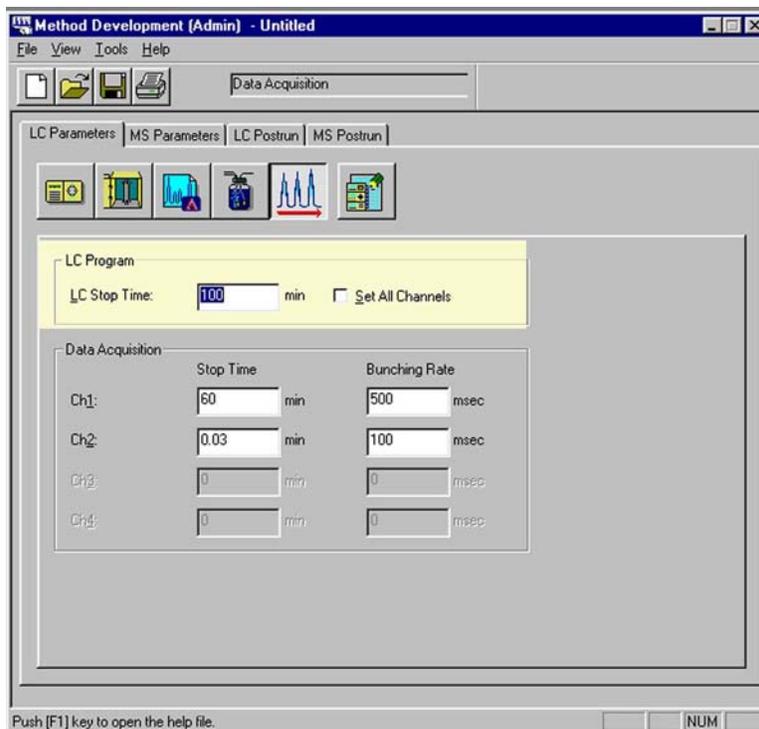
**For single wavelength measurement:** Deselect **Dual Mode** on the Detector tab. In the Data Acquisition Channel tab, select **Ch1** and choose Detector A from the drop-down menu.

**For dual wavelength measurement:** Select **Dual Mode** on the Detector tab. In the Data Acquisition Channel tab, select **Ch1** and choose Detector A (Dual 1), and then select **Ch2** and choose Detector A (Dual 2).

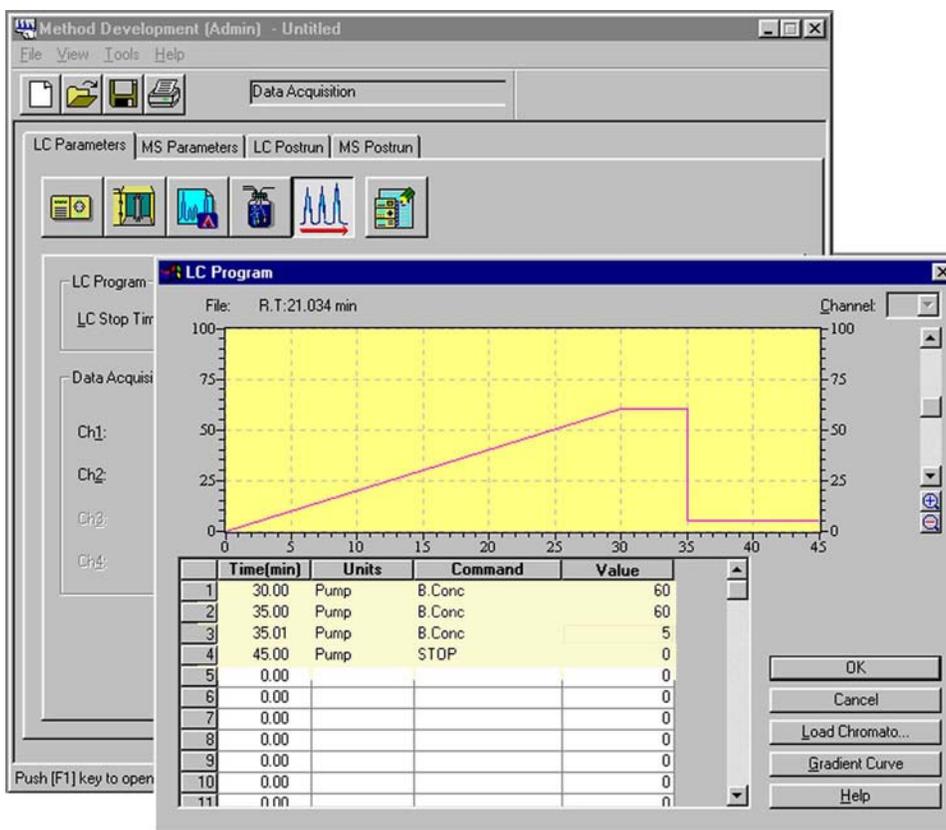
After completing the settings, click **OK** to exit the System Configuration window.

3. Data acquisition time

Enter the LC Stop Time and select the **Select All Channels** check box.



4. LC program
  1. Create the solvent gradient program.
  2. Enter the desired **Time, Unit, Command** and, for some parameters, **Value**. Click **Gradient Curve** to draw the set gradient curve on the screen.



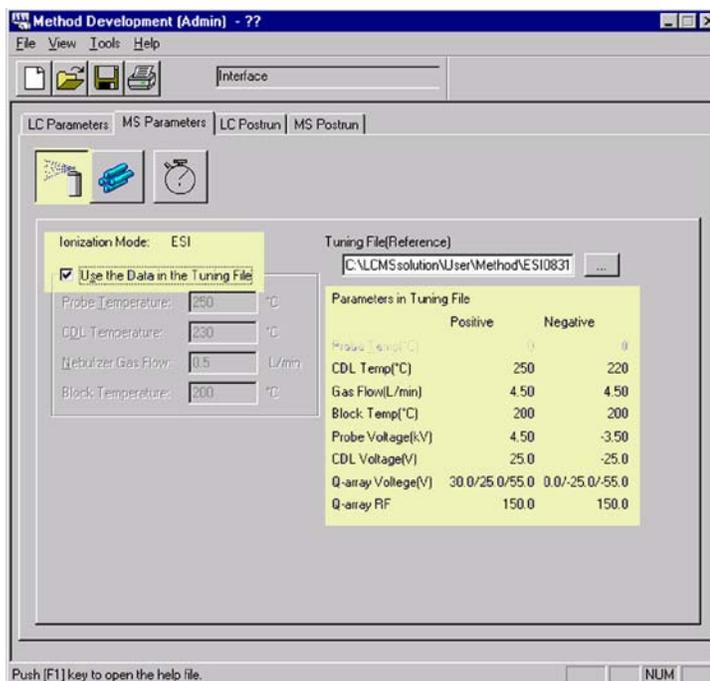
When the program is set, click on the **OK** button.

The LC analytical conditions are complete. Next, specify the MS parameters.

## 4.1.2 Setting the MS Parameters

### 1. Interface parameters

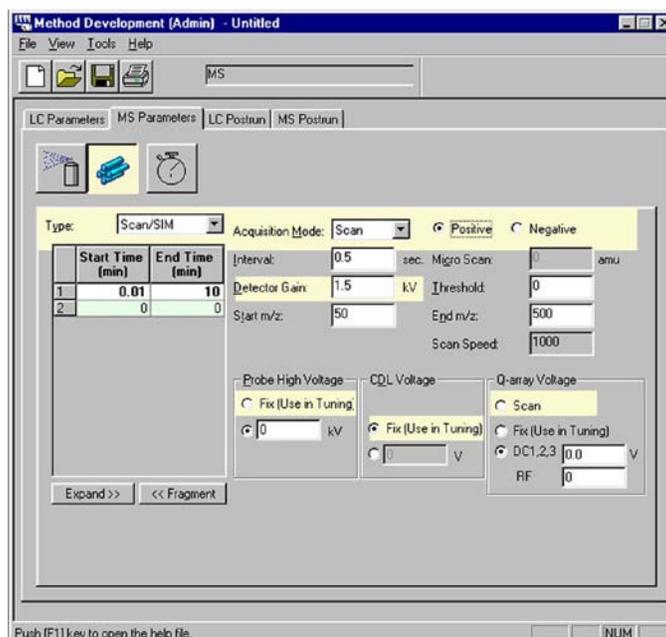
Select the **Use the Data in the Tuning File** check box. This will nearly always be the conditions for analysis. It may be useful to change the temperatures when analyzing thermally liable compounds or to minimize adduct formation.



The ionization mode is set according to the currently installed ionization probe. To change the interface, select **Tools> LCMS Configuration > MS**.

The most recent tuning file is displayed in Tuning File(Reference), which contains the lens voltage values and other tuning parameters

### 2. MS Parameters



**Type:** Select whether data is to be obtained in either Scan/SIM or profile mode.

For more information on the data obtained in scan, SIM, or profile mode see “Description of MS Analysis Mode” on page 9-11 of the System Users Guide.

**Acquisition Mode:** Select Scan or SIM (this parameter is not required for profile measurement).

**Ionization Polarity:** Select positive or negative ionization.

**Detector Gain:** Normally, enter the auto-tuning value (approximately 1.3 - 1.6 kV).

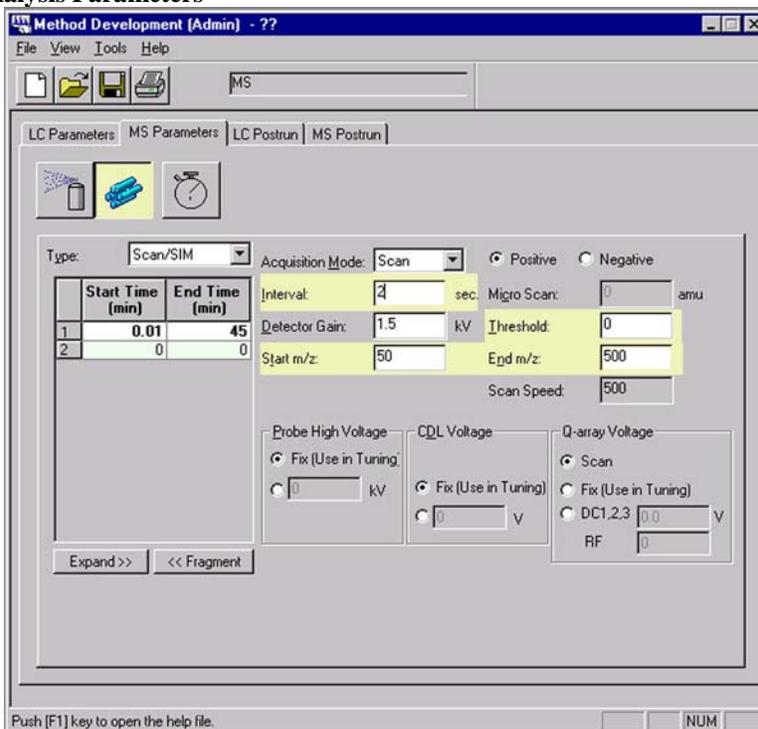
If there are any peaks with a signal intensity exceeding 8,000,000 (which can occur when a high-concentration sample is analyzed), data cannot be obtained. To avoid damaging the detector, the voltage will be turned off. If this occurs, decrease the detector voltage according to the intensity. If the detector voltage is decreased by 0.1 kV, the peak intensity increases by a factor of 1.5 to 2.0. Reduce the detector voltage until the largest peak does not exceed 8,000,000 counts.

**Probe High Voltage:** Select Fix (This selects the voltage obtained from the tune file).

**CDL Voltage:** Select Fix (This selects the voltage obtained from the tune file).

**Q-array Voltage:** Select Scan

### Scan Analysis Parameters



**Interval:** Set this parameter between 2.0 -3.0 seconds. The combination of interval and scan range determines the scan speed.

$[(\text{measurement end m/z}) - (\text{measurement start m/z}) / \text{sampling rate (sec)}]$  is 6000 (amu/sec) maximum. As this value (scan speed amu/sec) decreases, more data points are obtained. Specify a speed so that more than 4 points are sampled across each of the chromatogram peaks.

Typically, scan speeds of 1000 amu/sec or lower are used. This minimizes data file size and eliminates oversampling. As an example,  $(500-50) \text{ amu} / 2 \text{ sec} = 225 \text{ amu/sec}$ .

**Scan Range:** Set the Start m/z and End m/z. Enter a value between 10 and 2000, depending on the system mass range.

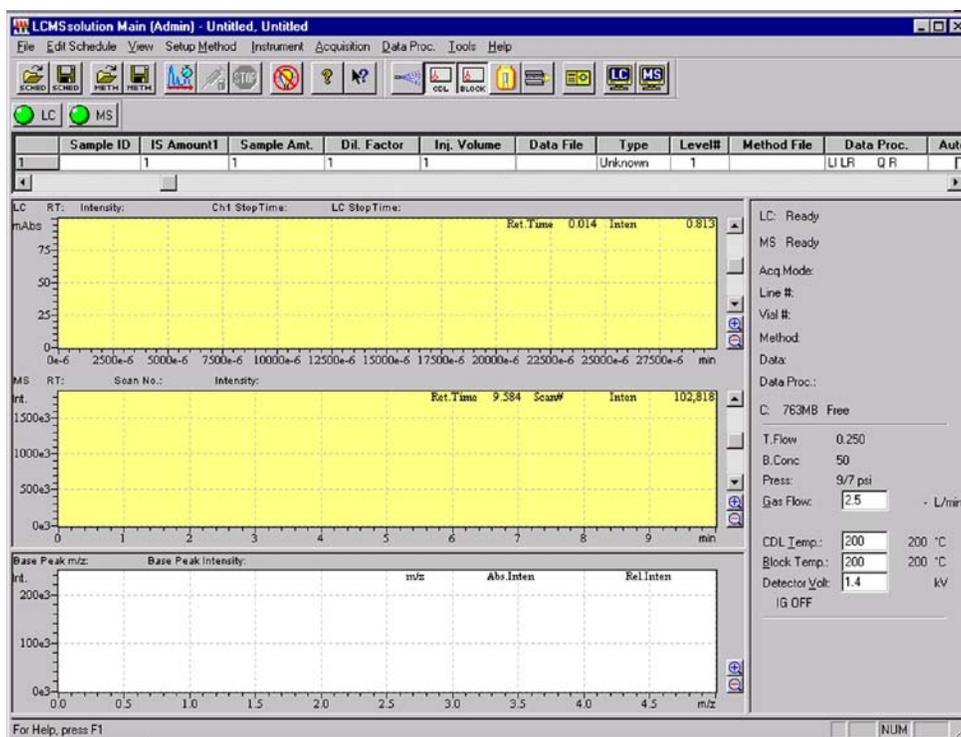
**Threshold :** Enter the borderline value between signal and noise. An appropriate threshold value can improve data quality. A value of 0 indicates that all the signals are detected.

If there is considerable background noise, enter a value higher than the background level of data or a value lower than the signal intensity of the sample. This can improve the data quality.

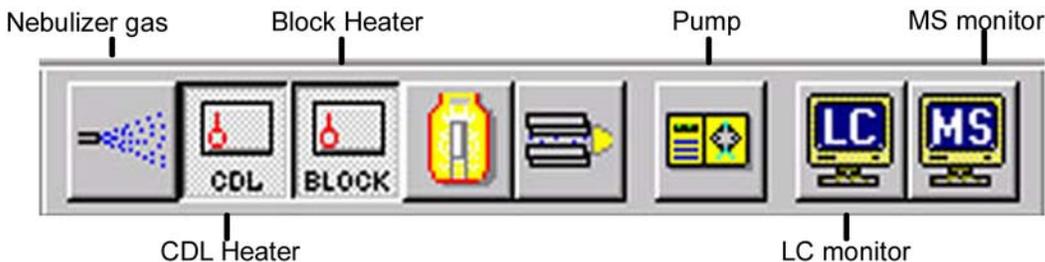
**Start Time:** 0 min.

**End Time:** Enter the measurement end time (generally the same as the LC end time).

## 4.2 Starting an Analysis



1. Turn on the CDL and block heater.
2. Turn on the nebulizer gas.
3. Turn ON the pump and column oven.
4. In APCI mode, turn ON the APCI heater.

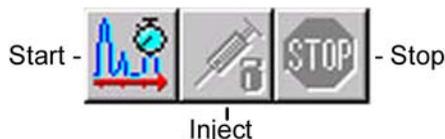


Wait until the LCMS-2010 has stabilized. (For reproducible results studies, allow the LCMS-2010 to stabilize for approximately 20 minutes once the heater temperatures have been reached.)

The status of each LCMS-2010 parameter can be verified through the LC or MS monitor. Click on the relevant button.



Once the LCMS-2010 becomes stable, start the analysis.

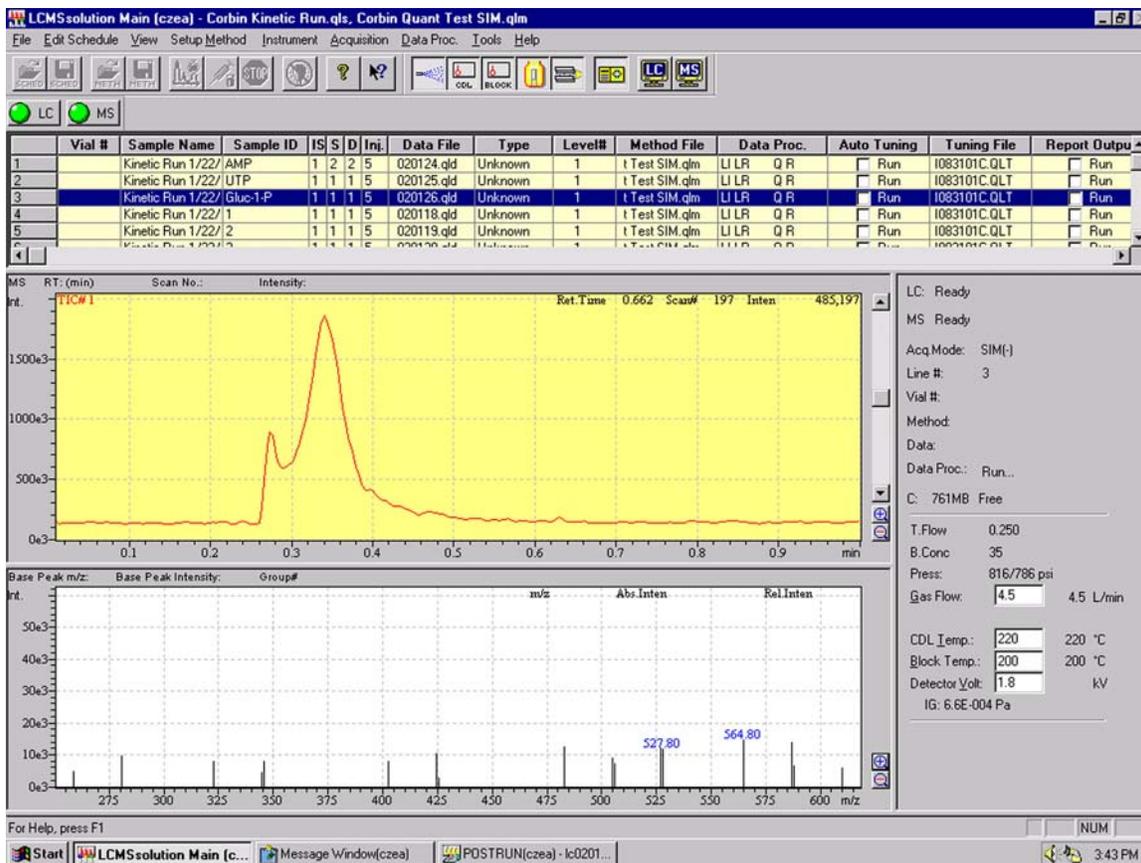


Click on the **Start** button to begin the analysis.

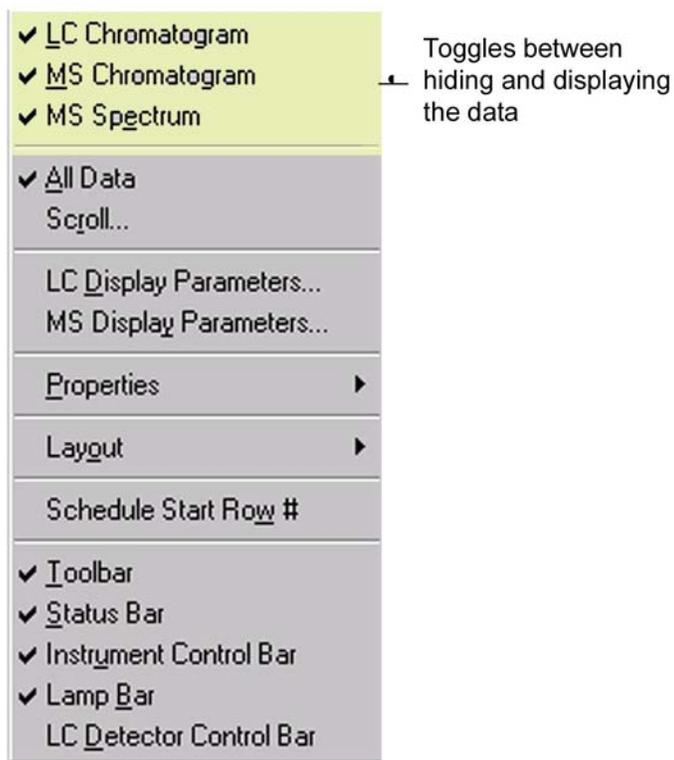
Autosampler analyses start automatically when all the heated zones are ready.

If the manual injector is used, inject the sample once the **Inject** button is available. The analysis is started when the manual injector switches from the Load position to the Inject position. Alternatively, start the analysis by clicking on the **Inject** button.

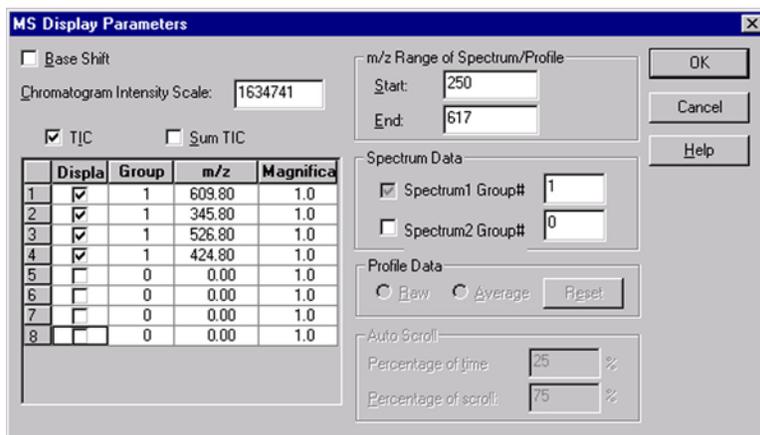
To stop the analysis, click on the **Stop** button.



To specify the data displayed during the analysis, click on the Data Display window with the right mouse button and select the Display Parameters.



## MS Display Parameters



Enter the m/z to display in real time as a MC (mass chromatogram) value.

To display each m/z chromatogram using the base shift function, select the Base Shift check box.

Select the **Sum TIC** check box to display the total signals of all the mass chromatograms measured in multi-sequence mode (a chromatogram with the intensities of negative and positive ions added).

### 4.3 Postrun Analysis (data processing)

Open the Postrun program by double-clicking on the postrun icon from the Desktop.



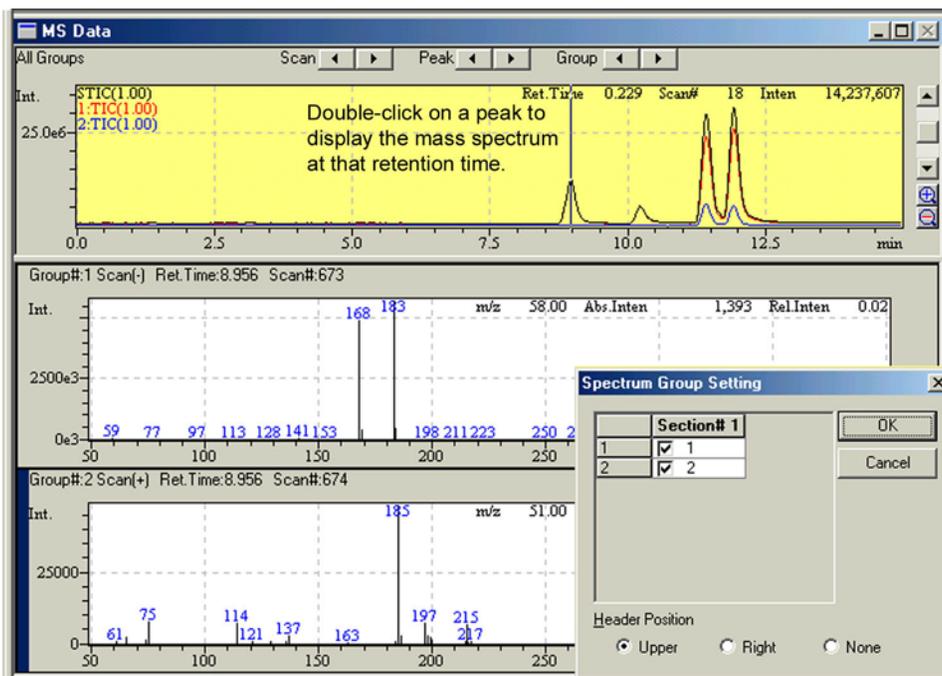
Enter your user name and password. The postrun program opens.

Select **File > Open Data File**, and specify the data file to open.

The examples in this section are Scan data. However, the same operations are performed for SIM data.

#### 4.3.1 Displaying a Mass Spectrum

Double-click on a chromatogram to display the mass spectrum at that retention time. (The mass spectrum can also be obtained by double-clicking on the UV chromatogram.)



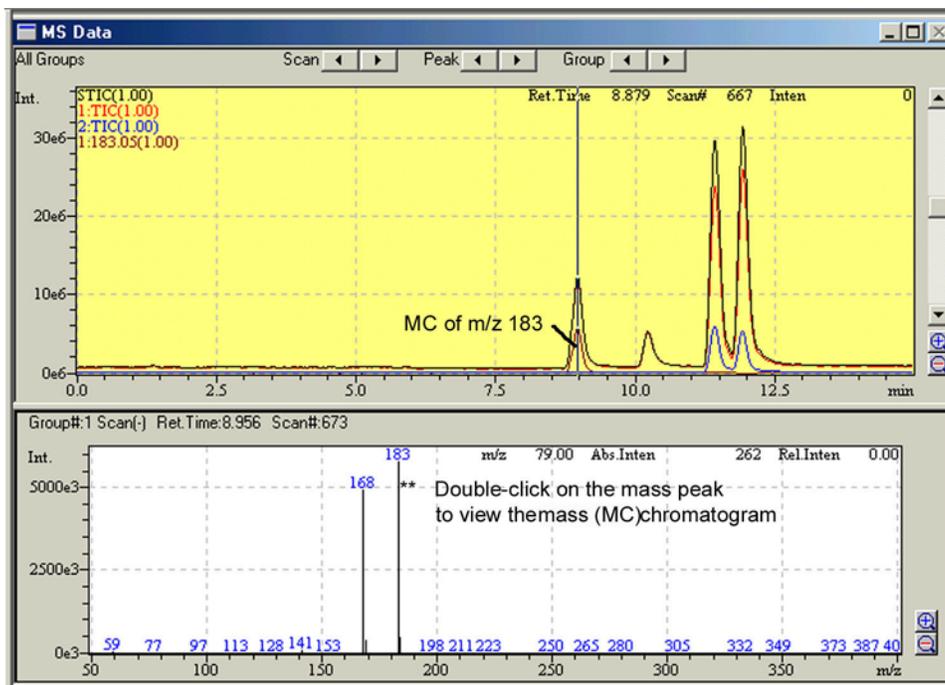
In multi-sequence mode, multiple spectra are displayed.

In normal analysis mode, a single spectrum is displayed. (Spectra for positive and negative ionization are displayed above.)

To hide these spectra, click on the spectrum display window with the right mouse button, select **Select Spectrum Group**, and deselect the check box to hide the spectra.

### 4.3.2 Displaying a Mass Chromatogram (MC)

Double-click on a mass peak in the spectrum window to display the corresponding m/z chromatogram. A mass chromatogram helps both to determine whether the peak originates from the sample, and to extract a targeted component from miscellaneous components.



- Each window can be magnified (zoomed) by dragging to specify the display range. Magnify a spectrum and select a mass number to accurately select the MC.
- To return to the previous level of magnification, click on the window with the right mouse button, and select Redo Zoom. Select Initialize Zoom to restore the original zoom level.



- To manually specify the MC, click on the  button. The Fragment Table window opens. Enter the mass number to display on the MC. Enter the magnification Factor, to zoom the MC display for that mass.
- For an analysis displaying multiple sections, the MC must be set up for each section.

**Fragment Table**

Section# 1

Group: 1 - 2

IIC  
 MIC  
 None

Base Shift  Sum TIC

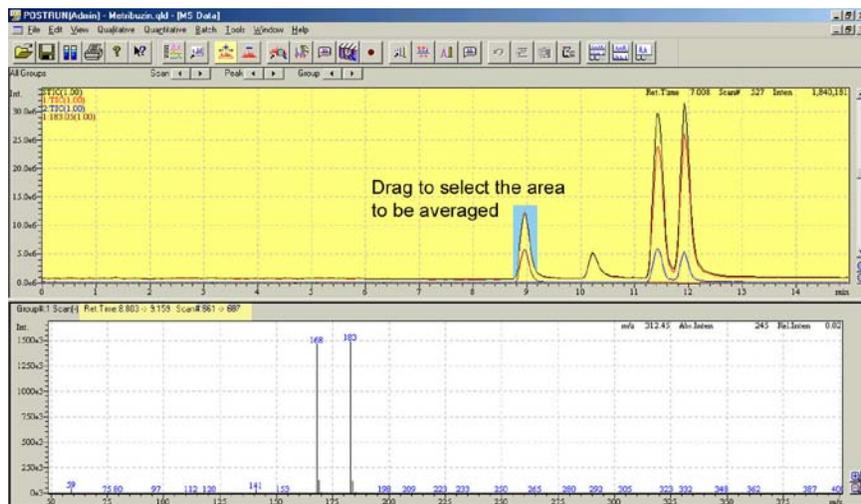
Disp	Gr#	Mass	Factor
1	<input checked="" type="checkbox"/>	183.05	1.00
2	<input type="checkbox"/>	0.00	0.00

### 4.3.3 Averaging Mass Spectra

In order to average the mass spectrum data and then subtract the background noise component from it, average the spectrum data first and then subtract the background.

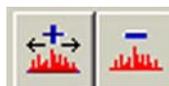


To average the mass spectrum data, click on the  button and drag on the chromatogram to indicate the averaging range.



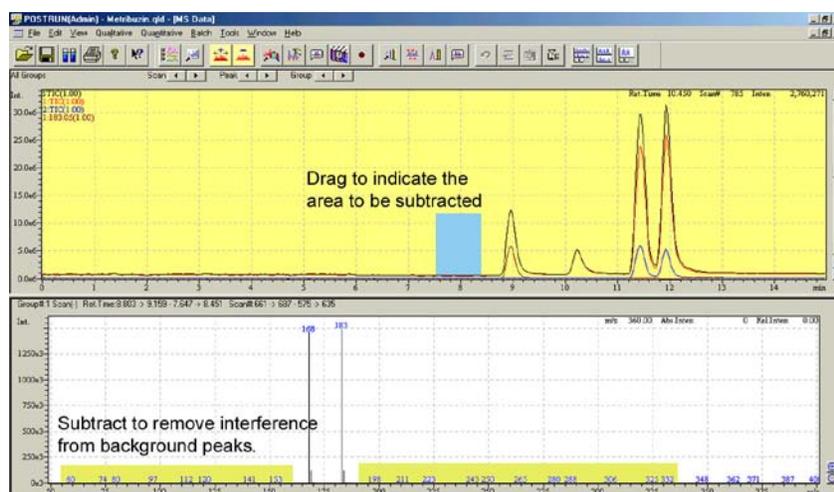
The averaging information is displayed on the mass spectrum as follows:

Ret. time [A->B] (Scan # [A' -> B'])



To subtract the background from this spectrum, click on both the   buttons (average and subtract). Drag on the chromatogram where the background to be subtracted is located. The background is automatically subtracted from the averaged spectrum. The background/averaging information is displayed on the mass spectrum as follows:

Ret. time [A->B] - [C -> D] (Scan # [A' -> B'] - [C' -> D'])



This information shows that the spectrum has been obtained by averaging A to B and subtracting C to D.

## 4.4 Printing Reports

There is no simple way to print the spectra and chromatograms displayed on the screen. The software has very powerful formatting and printing procedures, but it will take some effort on your part to learn how they work. To begin with, you must learn the relationship between "templates", "formatted reports", "registered spectra", and the "Spectrum Process Table".



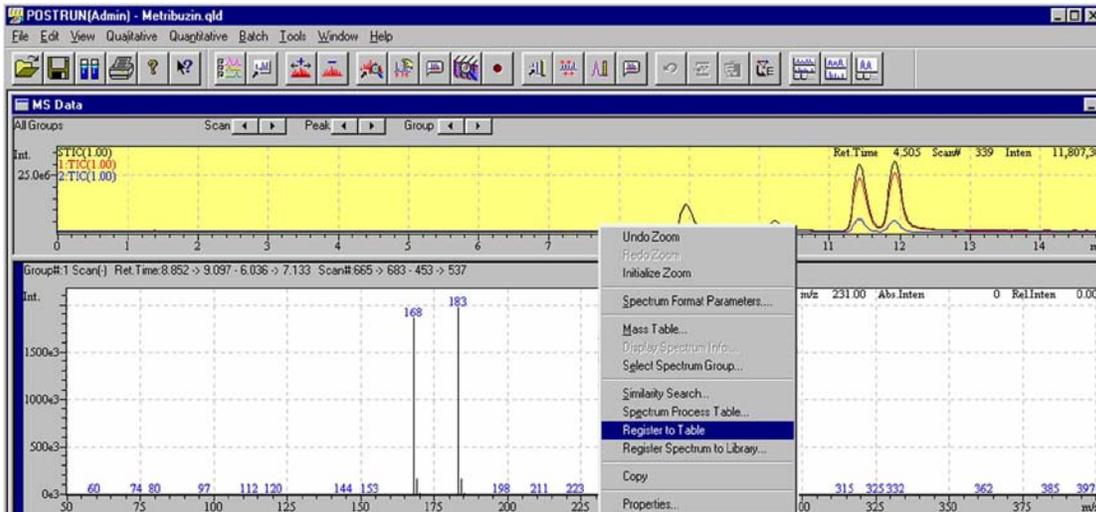
There are two ways to print. The first uses the **File -> Report...** command, and the second uses the **File -> Report Graph Image** command. Both methods require the generation of a formatted report from a template. There are both system default templates, and user-generated templates. There are templates linked to specific data files, and templates that are independent of the data file being processed.

If you just want to print the views displayed on the screen, you will use the **File -> Report Graph Image** command.

If you plan to print multiple spectra from an LC run (e.g. spectra representative of the various components in the mixture), you will need to first register each spectrum to the Spectrum Process Table. You will then format and print using the **File -> Report...** command.

### 4.4.1 Registering Mass Spectra

Spectra can be registered to a table to facilitate printing and further processing. To register a mass spectrum, right-mouse click on the spectrum and select **Register to Table**.



To view the list of registered spectra, right-mouse click on the spectrum and select **Spectrum Process Table**.

Spectrum Process Table									
Edit Unit Draw Search									
	Spectrum			Background			Group	Search	Report
	#(Scan)	Start#	End#	#(Scan)	Start#	End#			
1	895	...	...	...	...	...	1		<input checked="" type="checkbox"/>
2	...	851	865	...	...	...	1		<input checked="" type="checkbox"/>
3	...	665	681	...	551	643	1		<input checked="" type="checkbox"/>
4	767	...	...	...	...	...	1		<input checked="" type="checkbox"/>

#### 4.4.2 Attributes of: "File -> Report Graph Image" Method of Printing

- Formats the *currently viewed* chromatogram and spectra.
- Formatting is based upon a *system default template*.
  - You **cannot** load a different template
  - You **can** modify the format (views, size, header info...)
  - You **cannot** save the formatting changes
  - You **do not** need to register the spectrum
  - You **cannot** (easily) print spectra contained in the Spectrum Process Table

#### 4.4.3 Attributes of: "File -> Report... " Method of Printing

- Formats the chromatograms and other data and the enabled spectra in the SPT.
- Formatting defaults to the *last report template saved with the data file*.
  - You **can** load a different report template from your user directory
  - You **can** modify the format (views, sizes, header info...)
  - You **can** link the modified format to the data file (e.g. "close -> save changes - YES")
  - You **can** save the modified format as a new report template (e.g. "File -> Export -> ....\username\report1.qlr")
  - You **can** print all the spectra enabled in the SPT. Typically the sample info, chromatogram, and first spectra frames can be seen on the first page.

-The remaining spectra to be printed **can** be viewed using the  button.

#### 4.4.4 Formatting the Report

Upon entering the Report Program (**File > Report Graph Image** or **File > Report**) you will be able to use the report template "as is" if it meets your needs, or modify the report format. "Left-mouse-click" on a view to select it. Use the mouse to resize or relocate the view on the page; or use the "delete" key to remove the view from the page. Additional views can be selected from the button bar, inserted, and sized on the page as necessary.



For example, to display a MS or LC chromatogram, select one of the icons shown below and drag the cursor to specify the display area. (Alternatively, select an item from the item menu.)



Mass chromatogram

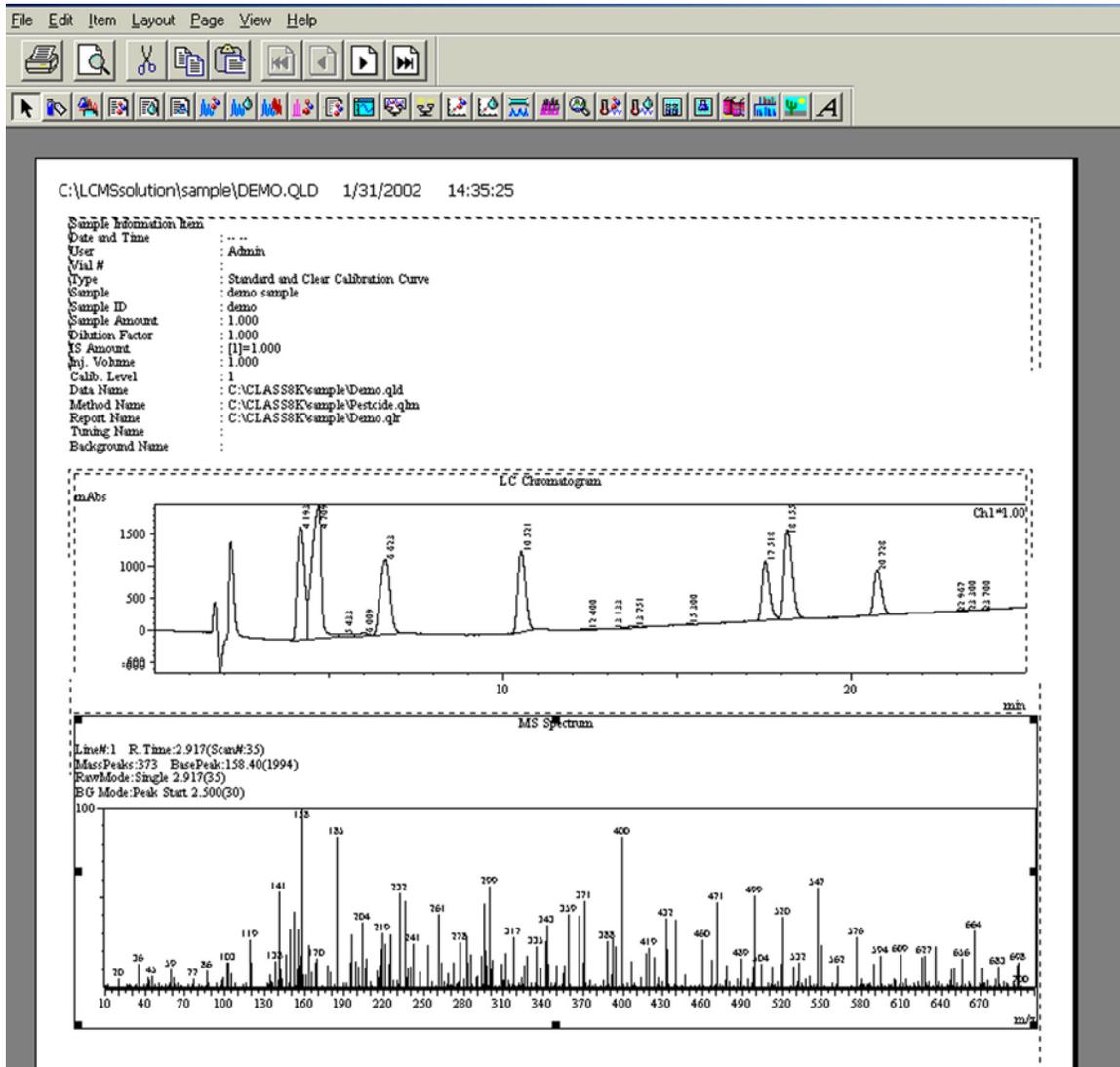


LC chromatogram.

When the report format is correct, use the  button to preview the pages that will be printed.

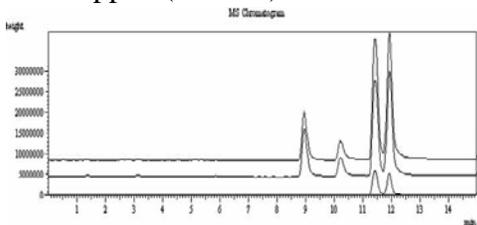
Use **File > Print** or  to print the report.

A typical formatted page:

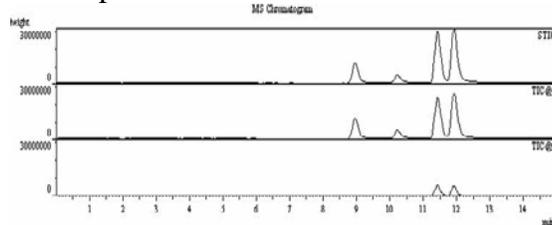


The Report Program contains a number of advanced features that will not be discussed in detail here. To access these features, double-click on any view to select or modify its properties. For example, mass chromatograms can be displayed as stacked plots or separate plots:

Overlapped (stacked)



Separated



Many of the tasks you might think should be done from the Post Run Page can be more efficiently accomplished from the Report Page. **Take the time to explore the capabilities of this program.**