

Finnigan LCQ Advantage-Training Manual

9/2/2006 K.H.

Location: 1238 Hach Hall

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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.



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1. Introduction:

The Finnigan LCQ Advantage is an ion trap mass spectrometer. The instrument is capable of performing single stage LC/MS and two-stage LC/MS/MS experiments. Sample solutions could be introduced to the mass spectrometer via a loop injection or directly infused by a syringe pump. The LCQ components are shown in Figure 1. The instrument is equipped with an atmospheric Pressure ionization (API) interface, LC pump, Syringe pump, PDA detector, and Surveyor auto-sampler. Ions are generated in the Electrospray or APCI ion sources. The instrument can be operated in positive ion mode or negative ion mode.

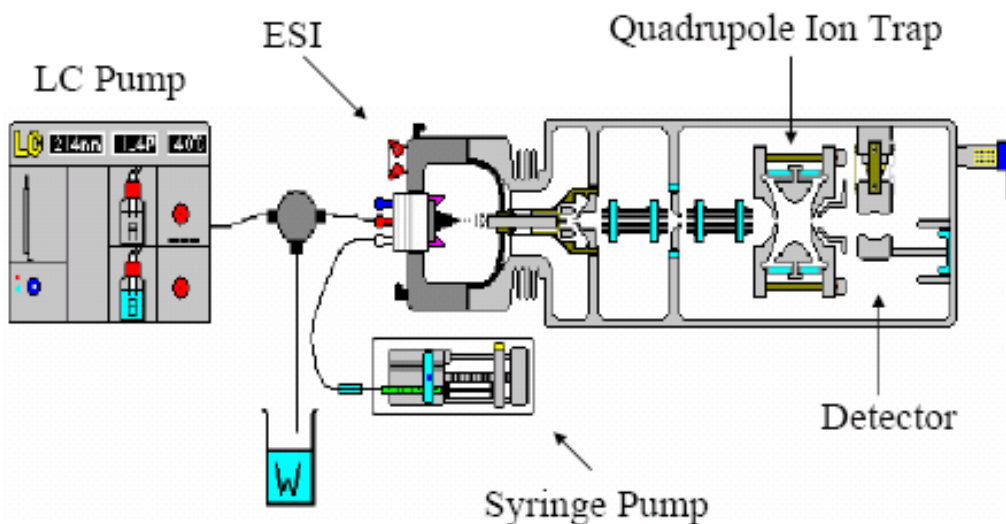


Figure 1: LCQ Advantage basic components

The instrument will always be tuned and calibrated by the mass spec personnel. A S/N of 10:1 m/z 609 from 5 μ l of 10 ng/ μ l of Reserpine is used for routine sensitivity test (positive ion mode ESI).

The tuning mixture consists of Caffeine, MRFA, and Ultramark.

ESI probe:

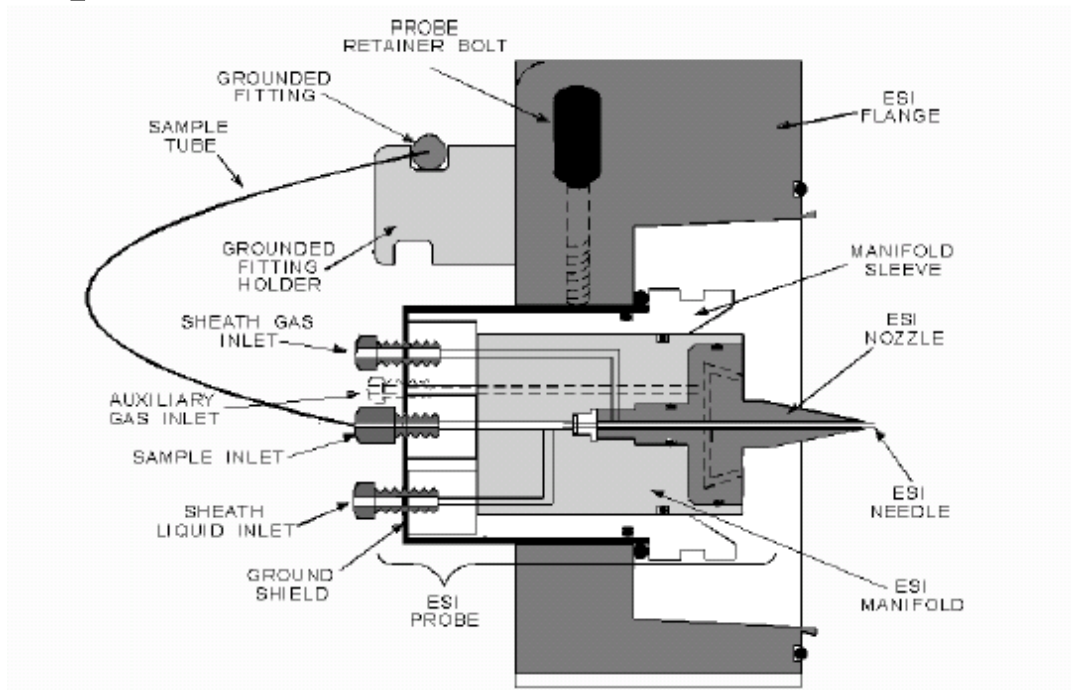


Figure 2: Schematic representation of the LCQ ESI probe.

2. Ionization Techniques:

2.1 Electrospray Ionization

Among the most used spray ionization techniques is Electrospray Ionization (ESI). This technique has been and still is the method of choice for analyzing thermolabile chemicals. Its capabilities are well documented. It uses an electrical stress between the ESI probe exit (e.g. capillary) and the counter electrode, which is located a few millimeters from the probe. The process results in the generation of highly charged droplets directly from the infused solution. Multiply and/or singly charged analyte molecules desorb from the sprayed droplets and are sampled through the rest of the mass spectrometer for mass analysis. ESI has been distinguished for its ability to produce multiply charged molecular ions from a large variety of polymers such as protein and DNA fragments; it also allows sensitive detection of singly charged low molecular weight polar species such as drugs and drug metabolites.

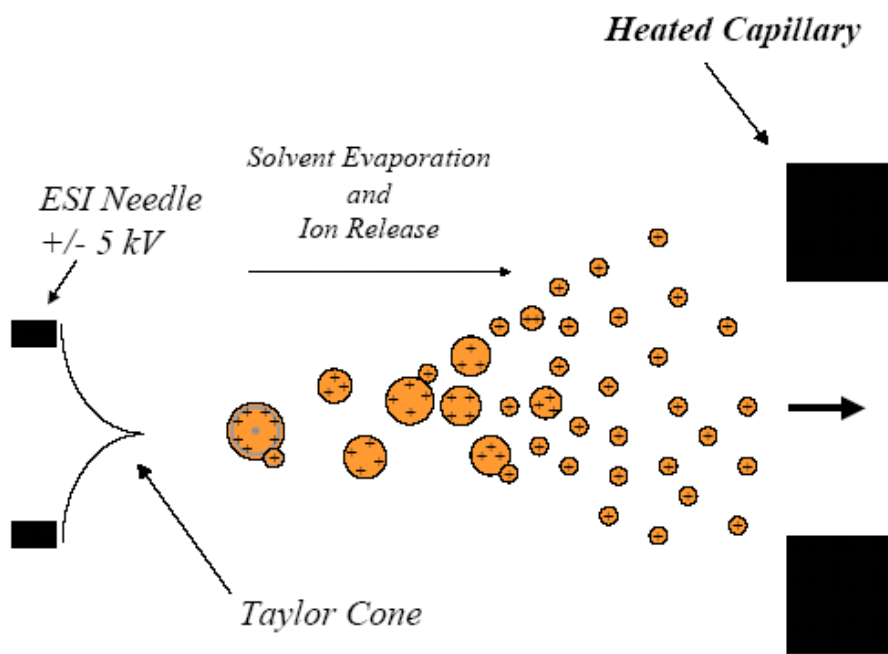


Figure 3: Typical electrospray ion source and ESI process

The formation of positive or negative ions (depending on the sign of the applied electrical field) occurs in high yield. In the positive ion mode protonated and/or alkali adduct analyte molecules are generally observed in the mass spectra. In the negative ion mode operation peaks corresponding to deprotonated analyte molecules are observed. ESI is described as a very "soft" ionization technique where the surrounding bath gas has a moderating effect on the internal and translational energies of desorbed ions.

Advantages of ESI:

- Soft ionization process so intact molecular ions are observed
- ESI allows production of multiply charged ions. This results in the ability of analyzing very high molecular weight species using the most available mass analyzers (e.g. quadrupoles).
- ESI is an atmospheric pressure process. This makes it easy to use and easy to interface with HPLC and CE separation techniques.

2.2 Atmospheric Pressure Chemical Ionization (APCI)

APCI uses a corona discharge technique to produce ions. This ionization technique is used mostly for low molecular samples that are more or less polar. It

is an excellent LC/MS interface that it's easy to use and can accommodate high flow rates.

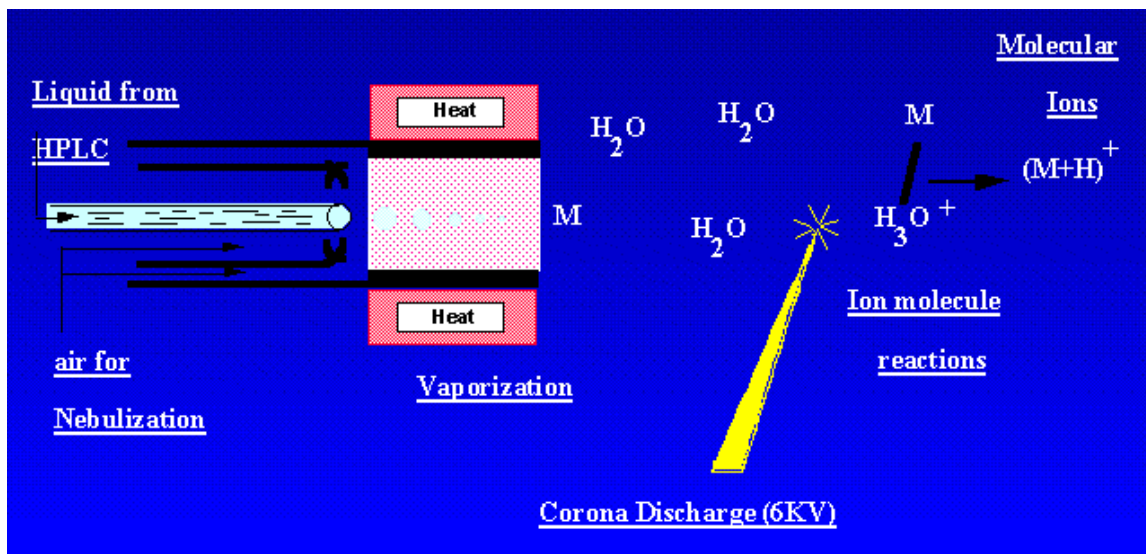


Figure 4: Atmospheric pressure chemical ionization process

3. LCQ Scan Types:



The LCQ mass spectrometer can be operated in the following scan types:

- 1- **Single Stage Full Scan:** This is a single stage scan type that provides a full mass spectrum of each analyte. The mass analyzer is scanned from the low mass to the high mass of the user's defined mass range (up to 2000 daltons).
- 2- **Two Stage MS/MS Full Scan:** This type of analysis involves the selection of ions of one mass-to-charge ratio (the parent ions). Then, those ions are subjected to collisions with the molecules of the helium gas in the trap. The helium gas is present at a pressure of about 10^{-3} torr. The collisions of the parent ions cause them to fragment and produce product ions.
- 3- **Selected Ion Monitoring SIM:** This is a single stage technique in which a desired ion or set of ions is monitored. In the SIM scan type, the ions formed in the ion source are stored in the trap. Ions of one mass-to-charge ratios are selected and all other ions are ejected from the mass analyzer. Then, the selected ions are sequentially scanned out of the mass analyzer yielding a SIM spectrum.

- 4- **Selected Reaction Monitoring SRM:** This is a two stage (MS/MS) technique in which parent ion and product ion pairs are monitored. In this scan type, ions formed in the ion source are stored in the mass analyzer. Ions of one mass-to-charge ratio (the parent ions) are being selected and all other ions are ejected. Then the parent ions are excited so they collide with the background gas (He). Those collisions allow the parent ions to fragments. Only selected product ions are selected and all other ions are ejected. The desired product ions are then scanned out of the analyzer thus yielding a SRM mass spectrum.
- 5- **Zoom Scan:** The zoom scan is a scan type that yields higher resolution data. This is especially important in the determination of the charge state of a given peak. If the isotopic peaks are 0.33 unit apart, the ion has a charge state of +/-3. Up to 10 ions mass-to-charge ratios can be specified in the zoom scan experiment.

4. LCQ training:

4.1 HPLC and MS theory [8 hours-software training]

A potential user will be given access to HPLC/MS training software entitled “Introduction to LCMS”. This software will require from 4 to 8 hours to complete. The user is expected to understand and assimilate all the material contained in the program. The user must successfully complete all of the quizzes in the software and provide the mass spec personnel with a final “Progress Page” report verifying a 100% score. See Appendix A for more details.

4.2 Good Laboratory Practice [1 hour – staff training]

We will discuss rules and regulations regarding the use of our laboratory and all the items used for the LCQ. This will include methods and protocols for making and storing buffer solutions, filtering and degassing solvents, et cetera. The user will have access to our wet lab for all tasks pertaining to solvent preparation, weighting sample, making standard solutions, and filtering solvents and samples. See Appendix B for more details.

4.3 Basic operation of the LCQ [4 hours – staff training]

First, you have to fill the solvent reservoirs with the appropriate solvents. The Surveyor LC pump is a quaternary pump so up to four solvent systems could be used in a given experiment. We prefer to have reservoir A used only for aqueous mobile phases. All the solvents should be filtered. Make sure that all the tubing are set and connected properly. It is expected from the user to know or have a good idea on the LC method to be used (in case of LC/MS experiments).

Make sure that all the lights on the front panel are on and indicate the proper color (e.g. green for Communication) as shown in Figure 5.

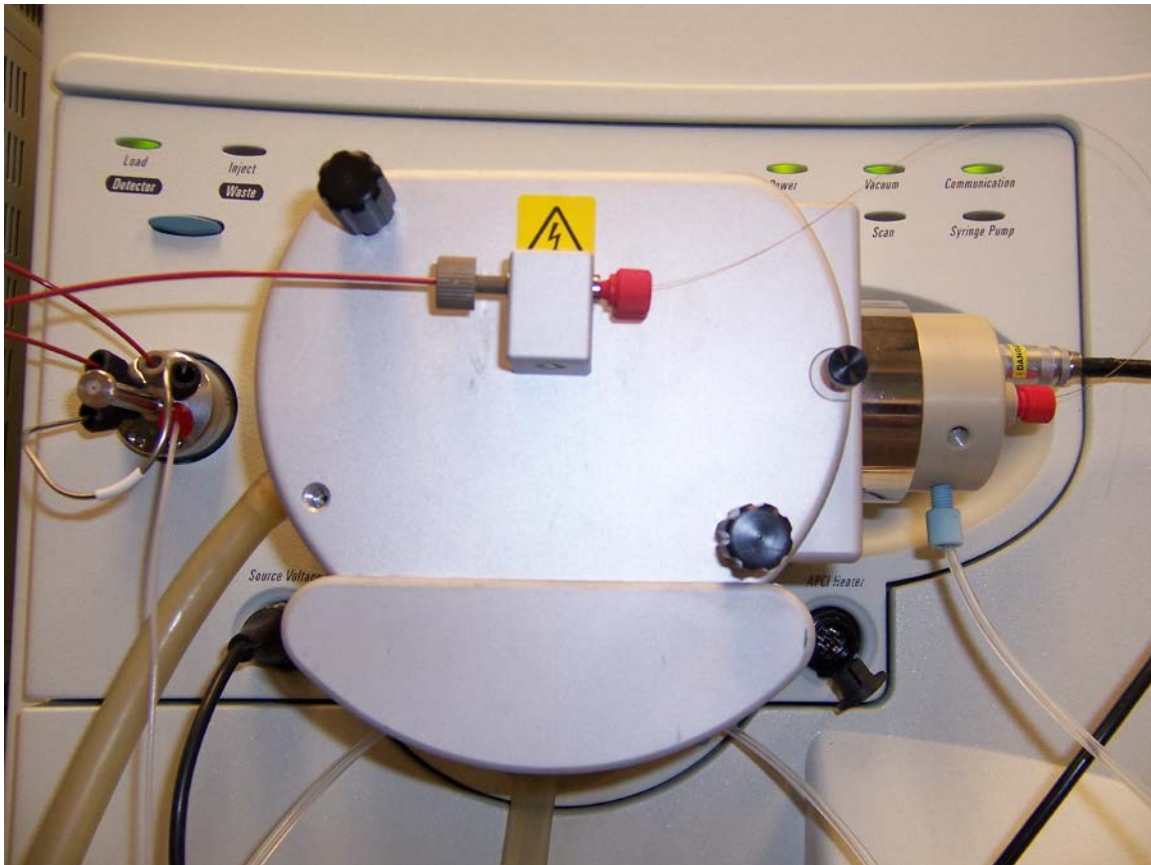


Figure 5: Front panel of the LCQ showing the status light indicators

The instrument is under total computer control and will already be tuned and calibrated by the mass spec lab personnel.

The operating system is windows XP. Some familiarity with the XP platform is desired.

You will have to deal mainly with *Xcalibur* and *LCQ tune* windows. Open Xcalibur first. Note that the mass spec, the LC, and the auto sampler are ready.

The Xcalibur window, also called *Roadmap-homepage*, should look like the following:

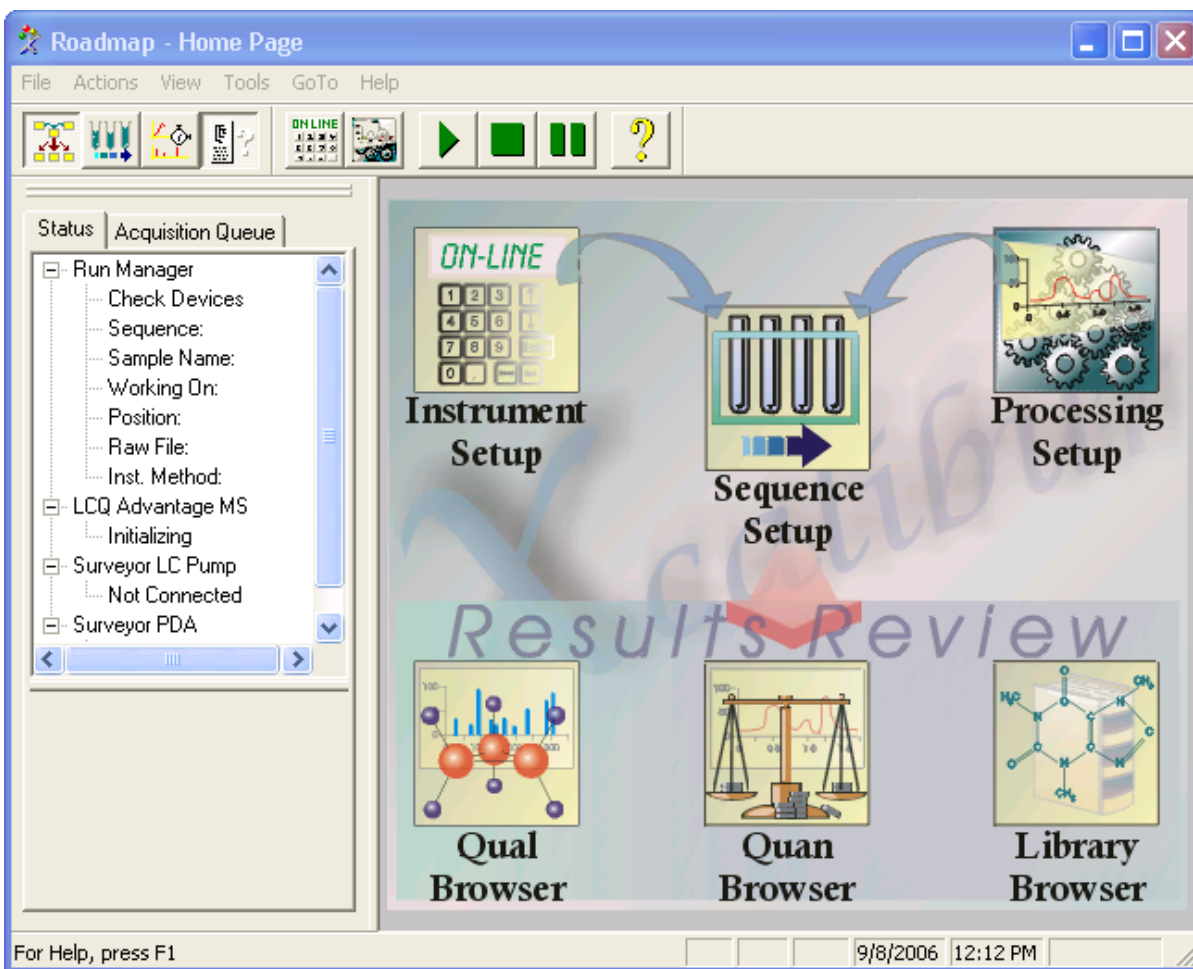


Figure 6: Roadmap “Homepage” for Xcalibur Software

The *Roadmap-Homepage* window allows you to open data files for processing and printing the results.

For data acquisition, your first step from the *Roadmap-Homepage* should be to specify the location of your data files using the pull down menu:

Tools > Configuration > Folders. Specify the location you have been assigned for your acquired data files.

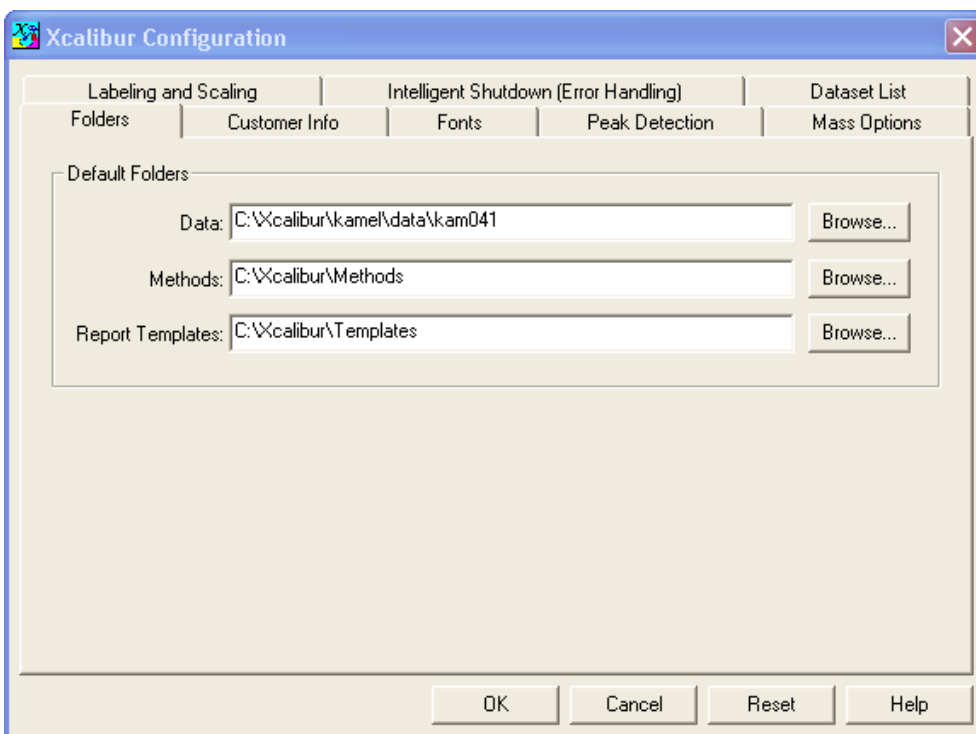


Figure 7: Setting data file locations, methods and templates

Also, from this window, you can set your LC gradient, by clicking on the **Instrument Setup** button. Then, click on the **Surveyor LC pump**, then **Gradient Program**.

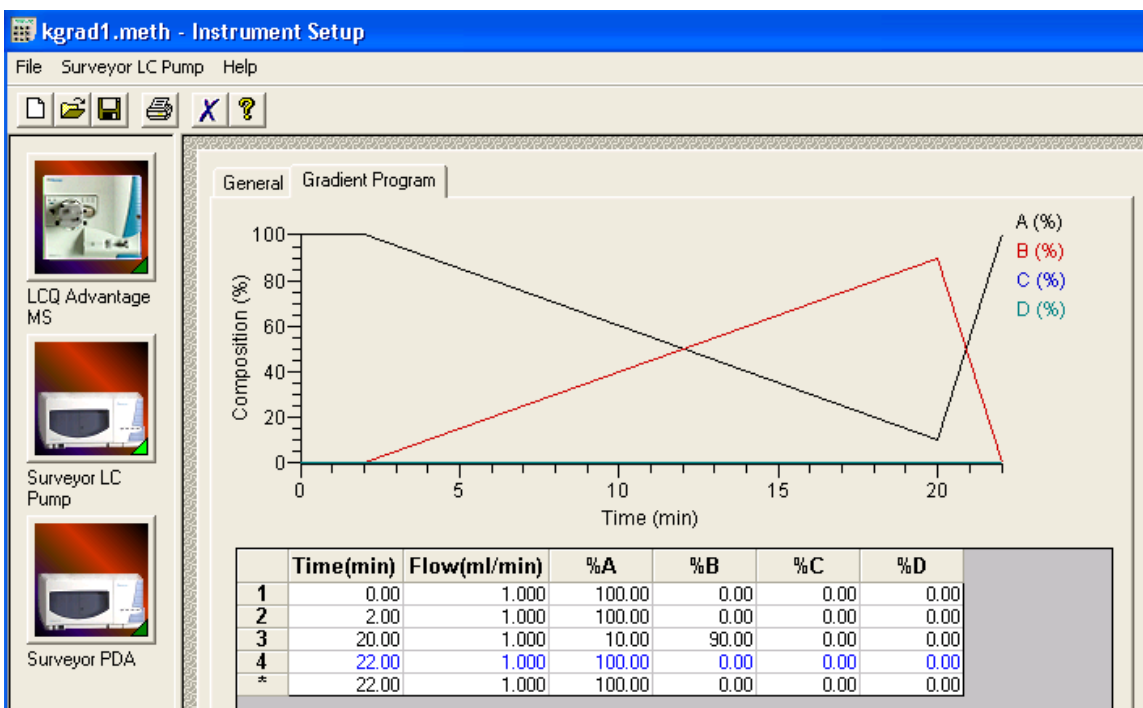


Figure 8: LC gradient program setup page

At this stage you can double click and open the LCQ tune windows. This window allows data acquisition.

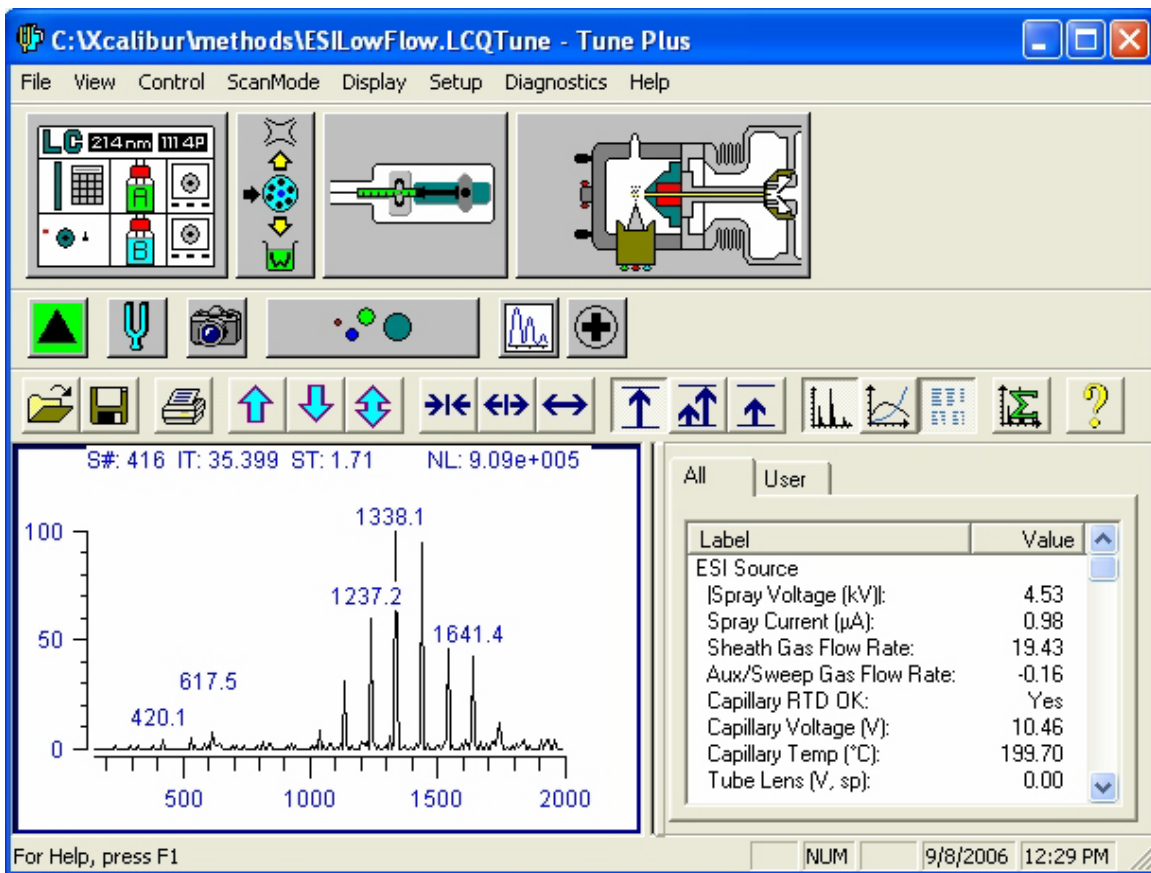


Figure 9: LCQ Tune and Acquisition window

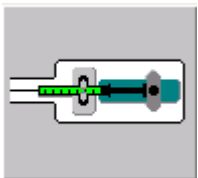
The following are the necessary menu actions and button function descriptions:

Setup > change API source type : to specify ESI or APCI ionization methods.

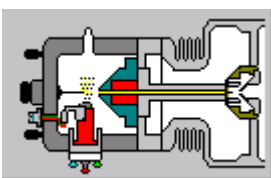
File > open : allows the opening of a method file.





Direct control of LC pump. For isocratic experiments





Direct control of the syringe pump



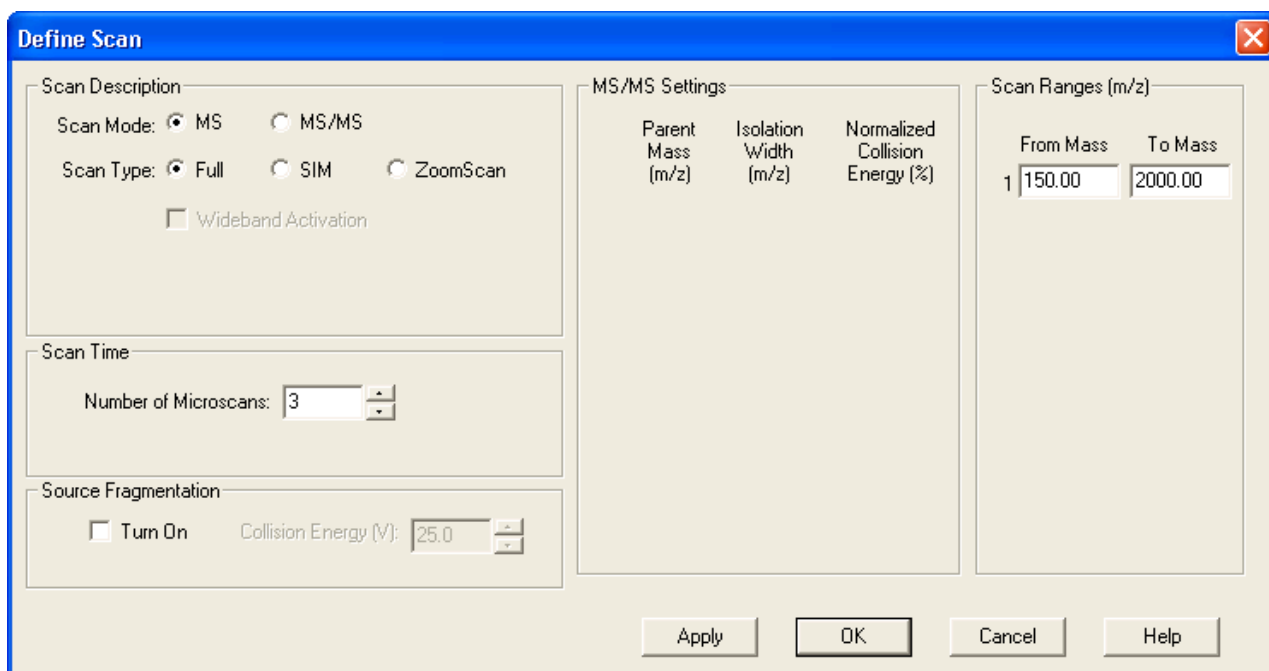
Ion source parameters

  toggle between positive and negative ion mode.

 Turns on the detector and the source voltages

 Source and optics voltages are on. When clicked, it turned the system to standby

 Enter experiment condition, e.g. mass range and scan type:

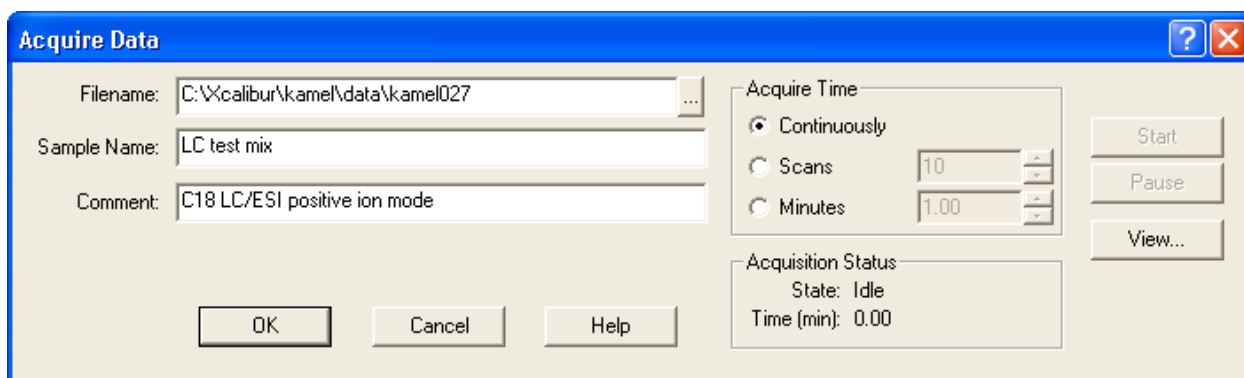


The 'Define Scan' dialog box is used to configure scan parameters. It includes sections for Scan Description, MS/MS Settings, Scan Ranges, Scan Time, and Source Fragmentation. The Scan Description section has radio buttons for Scan Mode (MS, MS/MS) and Scan Type (Full, SIM, ZoomScan), along with a checkbox for Wideband Activation. The MS/MS Settings section has columns for Parent Mass (m/z), Isolation Width (m/z), and Normalized Collision Energy (%). The Scan Ranges section has a table with columns for From Mass and To Mass. The Scan Time section has a spinner for Number of Microscans. The Source Fragmentation section has a checkbox for Turn On and a spinner for Collision Energy (V).

Scan Ranges (m/z)	From Mass	To Mass
1	150.00	2000.00

Figure 10: Define the mass range and scan type

 Acquire data:



The 'Acquire Data' dialog box is used to set up data acquisition. It includes fields for Filename, Sample Name, and Comment. The Acquire Time section has radio buttons for Continuously, Scans, and Minutes, with spinners for Scans and Minutes. The Acquisition Status section shows State (Idle) and Time (min) (0.00). Buttons for Start, Pause, View..., OK, Cancel, and Help are also present.

Figure 14: Acquire data window

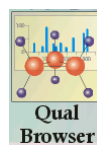
Specify number of scans, or run time. If **Continuously** is checked, the experiment needs to be stopped manually. Click *Start* to start the acquisition (The *Stop* button will terminate the experiment).



Toggles between profile and centroid data

View > Display Graph View : displays the total ion chromatogram.

The data can be processed after or during data acquisition.



From the *Roadmap homepage* window click on **Qual Browser** to open the data processing window:

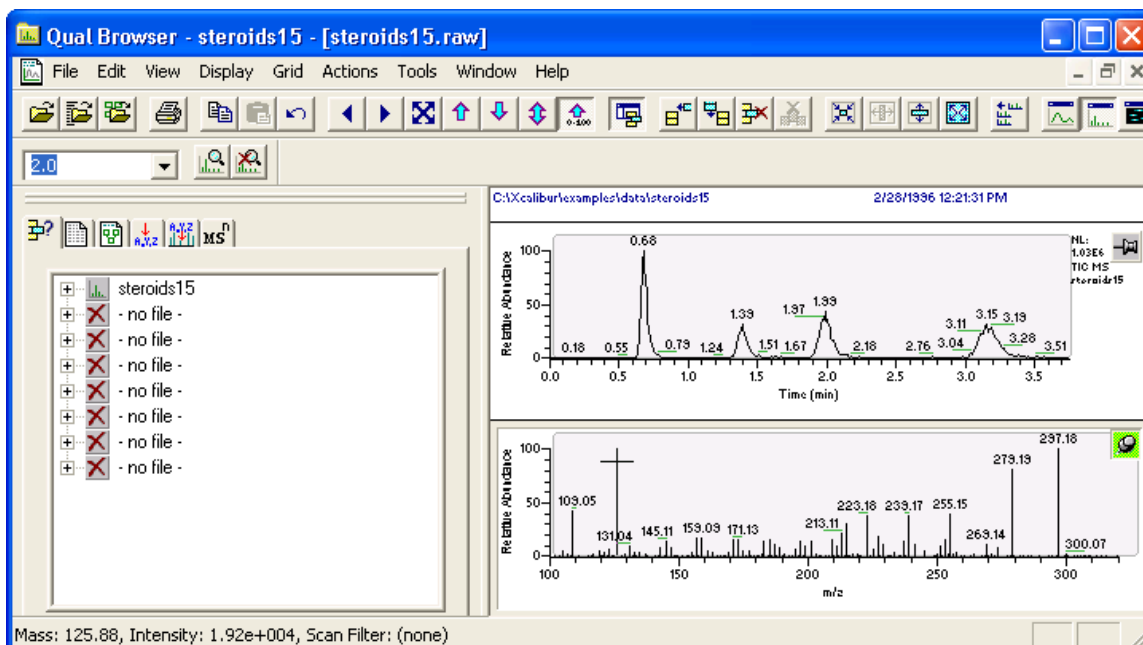


Figure 15: Data processing window

The user will be given 1-2 hours training for processing data using the Xcalibur Qual browser.

5. Additive and buffer summary:

For positive ion mode ESI use proton donor, e.g. Acetic Acid or Formic acid.

For negative ion mode ESI, use proton acceptor, e.g. TEA

Trifluoroacetic acid (<0.1 % v/v) is often used to assist the chromatographic separation.

Ammonium Acetate and Ammonium Formate are often used as buffer systems.

Additives considerations:

1- Acids

- a- Do not use inorganic acids (may cause source corrosion).
- b- Two possible choices are:
 - 0.1 % Formic acid
 - 0.1 % Acetic acid with 0,02 % TFA
- c- Best results arise from acids stored in glass
- d- Excellent results have been found with JT Baker acids

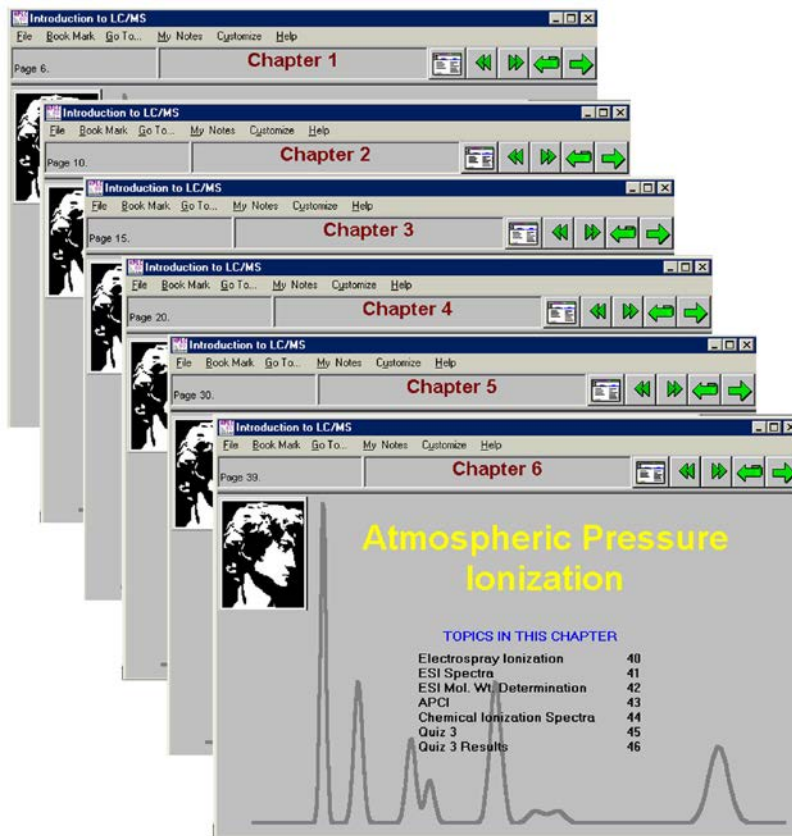
2- Surface Active Agents

Detergents and other surface active agents may suppress ionization.

Appendix A: Computer Based Training

A.1 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, 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. Harrata. 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.

Appendix B: Common Lab Procedures

B.1 Scheduling and Access to the Lab

You may apply on-line at www.cif.iastate.edu for a Marlok key to allow you access to the lab at any time. (*Note: As of 9/12/06, we are still waiting for ISU Facilities to install the Marlock hardware.*) If you already have a Marlok key, we can add access permission to your existing key.

An “on-line” reservation system may be used in the future. For the moment, written requests for time must be given or e-mailed to Dr. Harrata, who will then assign you time. At the moment there is ample time available. As instrument use increases, procedures used for assigning fair access will be more formal.

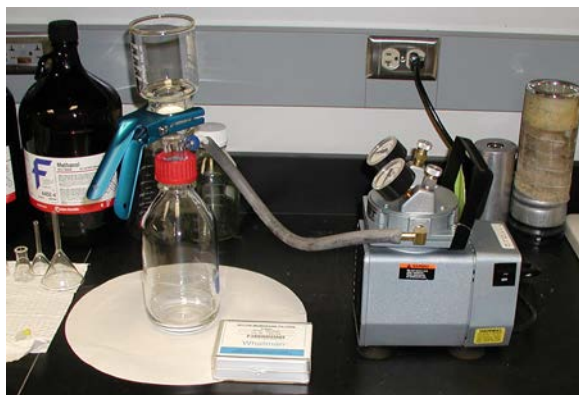
B.2 Solvents

B.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.

B.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. Harrata will show you how to use the filtering apparatus located in our wet lab.



B.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. Harrata will demonstrate the procedure for you.



B.3 Samples

B.3.1 Preparation

If possible, 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 do have a supply of nanopure water available. This can be used for final rinse or sample dilutions. 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.

B.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.

B.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). You WILL be charged for damage to rotor seals if you use sharp-tip syringes.

B.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. (You may decide to simply use our default instrument performance protocols, or you may wish to devise protocols more representative of your experiments.) If you ignore this requirement you will no longer be allowed to use the instrument. Dr. Harrata will assist you in verifying that the test protocol for your project is sufficient.