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READ THIS FIRST

Welcome to the Finnigan LCQ™ LC/MS^n System!

The **LCQ Operator's Manual** gives you information on how to use your instrument to perform a variety of applications to analyze various samples. The following chapters are included in this manual:

Chapter 1: Introduction describes the LCQ MS detector attributes, operational modes, and levels of specificity. It also provides a very brief description of the API source.

Chapter 2: Calibrating and Tuning in the ESI/MS Mode describes how to calibrate and tune the LCQ MS detector for operation in the ESI/MS mode.

Chapter 3: Analyzing Samples in the LC/ESI/MS Mode describes how to analyze samples by flow injection analysis in the LC/ESI/MS mode, describes how to open a raw file in the Explore window, and gives guidelines for LC/ESI/MS operation.

Chapter 4: Tuning in the APCI/MS Mode describes how to set up and tune the MS detector for APCI/MS operation.

Chapter 5: Developing Methods for LC/MS/MS Analysis of Testosterone in the APCI Mode describes procedures for developing methods for LC/MS/MS analysis of testosterone or any target analyte of interest.

Chapter 6: LC/MS/MS Analysis of Samples in a Mixture with Autosampler Injection describes a procedure for performing alternate product ion scans on a target-analyte / internal-standard combination of interest using an Experiment Method and a Sample List and monitoring data acquisition in the Navigator window.

Chapter 7: Quantitative Analysis of Data describes a procedure for performing quantitative analysis of data including creating a Processing Method and a Sample List; processing quantitation data; and creating a Summary Sheet to review quantitation data.

Appendix A: Sample Formulations describes how to prepare the solutions needed for calibrating and tuning the LCQ MS detector and for performing the applications described in this manual.

Changes to the Manual and Online Help

To suggest changes to this manual or the online Help, please send your comments to:

Editor, Technical Publications
Finnigan Corporation
355 River Oaks Parkway
San Jose, CA 95134-1991

You are encouraged to report errors or omissions in the text or index. Thank you.

Abbreviations

The following abbreviations are used in this and other LCQ manuals and in the online Help.

A	ampere
ac	alternating current
ADC	analog-to-digital converter
AP	acquisition processor
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
b	bit
B	byte (8 b)
baud rate	data transmission speed in events per second
°C	degrees Celsius
cfm	cubic feet per minute
CI	chemical ionization
CIP	Carriage and Insurance Paid To
cm	centimeter
cm^3	cubic centimeter
CPU	central processing unit (of a computer)
CRM	consecutive reaction monitoring
<Ctrl>	control key on the terminal keyboard
d	depth
DAC	digital-to-analog converter
dc	direct current
DDS	direct digital synthesizer
DS	data system
DSP	digital signal processor
ESD	electrostatic discharge
ESI	electrospray ionization
eV	electron volt
f	femto (10^{-15})
°F	degrees Fahrenheit
FOB	Free on Board
ft	foot
g	gram
G	giga (10^9)
GND	electrical ground
GPIB	general-purpose interface bus

h	height
h	hour
HPLC	high performance liquid chromatograph
HV	high voltage
Hz	hertz (cycles per second)
IEC	International Electrotechnical Commission
IEEE	Institute of Electrical and Electronics Engineers
in.	inch
I/O	input/output
k	kilo (10^3, 1000)
K	kilo (2^{10}, 1024)
kg	kilogram
l	length
L	liter
LAN	local area network
lb	pound
LC	liquid chromatograph
LC/MS	liquid chromatograph / mass spectrometer
LED	light-emitting diode
m	meter
m	milli (10^{-3})
M	mega (10^6)
M^+	molecular ion
µ	micro (10^{-6})
min	minute
mL	milliliter
mm	millimeter
MS	scan power: MS^1
MS/MS	scan power: MS^2
MS^n	scan power: MS^n, n = 1 through 10
m/z	mass-to-charge ratio
n	nano (10^{-9})
Ω	ohm
p	pico (10^{-12})
Pa	pascal
PCB	printed circuit board
PID	proportional / integral / differential
P/N	part number
P/P	peak-to-peak voltage
ppm	parts per million

psig	pounds per square inch, gauge
RAM	random access memory
<Return>	<Return> key on the terminal keyboard
RF	radio frequency
RMS	root mean square
ROM	read-only memory
RS232	industry standard for serial communications
s	second
SIM	selected ion monitoring
solids probe	direct insertion probe
SRM	selected reaction monitoring
TIC	total ion current
Torr	torr
u	atomic mass unit
V	volt
V ac	volts alternating current
V dc	volts direct current
VGA	Video Graphics Array
w	width

Note: The symbol for a compound unit that is a quotient (e.g., degrees Celsius per minute or grams per liter) is written with a negative exponent to indicate the denominator. In the corresponding online Help, these symbols are written with a slash (/) because of design constraints in the online Help. For example:

°C min^{-1} (in this manual)	°C/min (in the online Help)
g L^{-1} (in this manual)	g/L (in the online Help)

Exponents are written as superscripts. In the corresponding online Help, exponents are written with a caret (^) or with *e* notation, again, because of design constraints in the online Help. For example:

MS^n (in this manual)	MS^n (in the online Help)
10^5 (in this manual)	10e5 (in the online Help)

Typographical Conventions

Typographical conventions have been established for Finnigan manuals for the following:

- Data input
- Notes, Cautions, and WARNINGS
- Topic headings

Data Input

Throughout this manual, the following conventions indicate data input and output via the computer:

- Prompts and messages displayed on the screen are represented in this manual by capitalizing the initial letter of each word and italicizing each word.
- Input that is to be entered by keyboard or buttons that are to be clicked on by the mouse is represented in **bold face letters**. (Titles of topics, chapters, and manuals also appear in bold face letters.)
- For brevity, expressions such as "choose **File | Directories**" are used rather than "pull down the File menu and choose Directories."
- Any command enclosed in angle brackets < > represents a single keystroke. For example, "press **<F1>**" means press the key labeled F1.
- Any command that requires pressing two or more keys simultaneously is shown with a hyphen connecting the keys. For example, "press **<Shift>-<F1>**" means depress and hold the <Shift> key and then press the <F1> key.

Notes, Cautions, and Warnings

Notes, cautions, and WARNINGS are dispayed in boxes such as the one below.

> **Note:** Boxes such as this are used to display notes, cautions, and WARNINGS.

A ***note*** contains information that can affect the quality of your data. In addition, notes often contain information that you may need if you are having trouble.

A ***caution*** contains information necessary to protect your instrument from damage.

A ***WARNING*** describes hazards to human beings.

Topic Headings

The following headings are used to show the organization of topics within a chapter:

CHAPTER NAME

1.1 First Level Topics

1.1.1 Second Level Topics

1.1.1.1 Third Level Topics

Fourth Level Topics

Fifth Level Topics

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INTRODUCTION

The LCQ is an analytical instrument that includes a syringe pump, a divert/inject valve, an API ion source, a mass spectrometer (MS) detector, and a data system. In a typical analysis, a sample can be introduced in any of the following ways:

- Using a syringe pump via direct infusion
- Using the divert/inject valve fitted with a loop and an LC via flow injection analysis (loop injections)
- Using a divert/inject valve and an LC via LC/MS

In analysis by LC/MS, a sample is introduced onto an LC column. The sample is then separated into its various components. The components elute from the LC column and pass into the MS detector where they are analyzed. Analysis by direct infusion or flow injection provide no chromatographic separation of components in the sample before it passes into the MS detector. The data from the MS detector are then stored and processed by the data system.

The LCQ solves a wide variety of analytical problems. The LCQ MS detector can be used in a variety of operational modes; it exhibits unique attributes, as well as attributes typical of other LC detectors; and it provides many levels of analytical specificity.

This chapter contains the following topics:

- LCQ MS detector attributes
- LCQ MS detector operational modes
- LCQ MS detector levels of specificity
- API source

1.1 LCQ MS Detector Attributes

The LCQ MS detector is a universal, selective and specific LC detector.

A ***universal LC detector*** is one that detects compounds of all (or most) classes with moderate to good sensitivity. A universal LC detector tells you that a compound is present at a given retention time, but it provides no information on the identity of the compound.

A ***selective LC detector*** is one that detects compounds of one (or more) classes with excellent sensitivity. Selectivity is related to compound class. A selective LC detector tells you that a compound of a certain class is present at a given retention time, but once again, it provides no information, other than the class, on the identity of the compound.

A ***specific LC detector*** is one that allows for the positive identification of unknown or target compounds. Specificity is related to compound structure. A specific LC detector tells you not only that a compound is present at a given retention time, it also provides information on the structure of the compound. (For a discussion of the levels of specificity of the LCQ MS detector, refer to the next topic.)

The LCQ MS detector is, by its nature, a specific LC detector: mass data are always obtained regardless of the mode of operation. However, it also has attributes of universal and selective detectors.

1.2 LCQ MS Detector Operational Modes

The LCQ MS detector consists of an ion source, ion optics, a quadrupole ion trap mass analyzer, and an ion detection system.

A sample can be ionized in either of two atmospheric pressure (API) ionization modes: electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The two techniques are complimentary in that generally acidic or basic compounds or compounds that are ionized in solution are best analyzed by ESI. Neutral and less polar compounds tend to be better analyzed in the APCI mode.

Both atmospheric pressure ionization modes are extremely soft. Virtually all compounds ionizable by ESI or APCI produce a molecular weight associated ion such as MH^+, MNa^+, or $(M-H)^-$.

- ***ESI***: Sample molecules contained in solution move through a high-voltage gradient which forms a fine mist of electrically charged droplets. The sample ions are then transferred from solution into the gas phase via solvent evaporation. ESI is the softest ionization technique, thus little or no fragmentation of sample molecules occurs. Because electrospray ionization allows production of molecular ions directly from solution, it is strongly dependent on solution-phase chemistry.

- ***APCI***: A gas phase ionization process in which analyte molecules react with reagent ions to form analyte ions at atmospheric pressure. Chemical ionization (CI) reagent ions are formed via solvent vaporization and subsequent interaction with high voltage to create a corona discharge. Reagent ions are formed through a series of chemical reactions. APCI is a soft ionization technique, thus little or no fragmentation of sample molecules occurs. APCI provides is a powerful technique for the analysis of thermally stable compounds of widely differing polarity.

For an in depth discussion of the theory and mechanism involved in the ESI process, refer to the following scientific articles:

- Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M. *Mass Spectrom. Rev.*, **1990**, ***9***, 37

- Smith, R.D.; Loo, J.A.; Edmonds, C.G.; Barinaga, C.J.; Udseth, H.R. *Anal. Chem.*, **1990**, ***62***, 882

- Ikonomou, M.G.; Blades, A.T.; Kebarle, P. *Anal. Chem.*, **1991**, *63*, 1989
- Kebarle, P.; Tang, L. *Anal. Chem.*, **1993**, *65*, 972
- Dole, M.; Mack, L,L.; Hines, R.L.; Mobley, R.C.; Ferguson, L.D.; Alice, M.B. *J. Chem. Phys.*, **1968**, *49*, 2240
- Mack, L.L.; Kralik, A.; Rheude, A.; Dole, M. *J. Chem. Phys.*, **1970**, *52*, 4977
- Iribane, J.V.; Thompson, B.A. *J. Chem. Phys.*, **1976**, *64*, 2278
- Iribane, J.V.; Thompson, B.A. *J. Chem. Phys.*, **1979**, *71*, 4451
- Fenn, J.B. *J. Am. Soc. Mass Spectrom.*, **1993**, *4*, 524

The LCQ MS detector can be operated in any of nine scan mode / scan power combinations:

- ***Full scan MS***: The full scan MS mode provides a full mass spectrum of analytes introduced into the ion source. Molecular weight information on pure compounds and compounds in a mixture can be determined. Therefore, this scan mode provides the maximum amount of information available from a single stage mass spectrometry experiment.
- ***SIM***: A technique in which a particular ion or set of ions is monitored. Because only a few ions are monitored, SIM can provide lower detection limits and greater speed than a full scan MS analysis. The detection limit in a SIM experiment is defined by the chemical noise in the system. For instance, a SIM/MS experiment using the mass chromatogram of a molecular ion is more specific than a UV detector for quantitation of a particular component. However, SIM cannot be used to quantitate if there is an interfering ion at the same mass as the ion of interest.
- ***Full scan MS/MS***: In the full scan MS/MS scan mode the ion of interest (the parent ion) is selected and stored in the analyzer. The parent ion is then dissociated through collisionally induced dissociation (CID) to produce fragment ions (product ions). The analyzer is then scanned to produce a full mass spectrum of the product ions produced by the dissociation of the selected parent ion. Therefore, this scan mode provides information on the molecular structure of one

particular ion. Structural information can also be obtained for compounds in a mixture.

- ***SRM***: A technique in which a particular CID transition is monitored. In SRM, a parent ion is selected and stored in the analyzer. The parent ion is then dissociated to produce product ions. One product ion is then selected and stored. Finally, the product ion is scanned out of the analyzer. Therefore, ion current signal is obtained only if a specific parent ion fragments to produce a specific product ion. Quantitation by SRM is more specific than a SIM experiment because it uses the mass chromatogram of a specific product ion of a specific parent ion.

- ***Full Scan MS^n***: A technique in which a parent ion is selected and stored in the analyzer. The parent ion is then dissociated through CID to produce product ions. A product ion is then stored in the ion trap and becomes the parent ion for the next stage of MS. The ion is dissociated through CID and the product ions are scanned out of the analyzer to produce a full scan product ion spectrum.

- ***CRM*** (Continuous Reaction Monitoring): A technique in which multiple reactions are monitored, employing multiple stages of mass analysis. In CRM, a parent ion is selected and stored in the analyzer. The parent ion is then dissociated to produce product ions. One product ion is then selected and stored in the mass analyzer (second stage). This product ion then becomes the parent ion for the next MS stage, and is dissociated to produce new product ions. One product ion is then selected and stored in the mass analyzer (third stage). Finally, the product ion is scanned out of the analyzer. CRM provides highly specific quantitation information because it uses the mass chromatogram of a specific final product ion which results from a specific multi-stage reaction path.

- ***ZoomScan MS***: A higher resolution technique in which information about the charge state of one or more ions of interest is obtained for molecular weight determination. In ZoomScan, ions formed in the source are stored in the analyzer. Ions in one or more 10 u window(s) are selected and all other ions are ejected from the analyzer. Finally, the ions in the 10 u window(s) are scanned out of the analyzer at higher-than-normal mass resolution to produce a ZoomScan mass spectrum. Charge states up to ±4 can be determined for peptides and organometallics.

- ***ZoomScan MS/MS***: A technique in which the charge state of a product ion up to ±4 can be elucidated.
- ***ZoomScan MS^n***: A technique in which the charge state of a final product ion up to ±4 can be elucidated.

1.3 LCQ MS Detector Levels of Specificity

The attribute that sets the LCQ MS detector apart from other LC detectors is the high level of analytical specificity that it provides. The LCQ can provide five levels of analysis. Each level of analysis adds a new dimension of specificity for positive compound identification. The five levels of analysis, which are summarized in Table 1-1, are as follows:

- Chromatographic separation and compound detection (Retention time)
- Mass analysis (Parent ion mass-to-charge ratio)
- Two-stage mass analysis (Product ion mass-to-charge ratio)
- Multi-stage mass analysis (Final product ion mass-to-charge ratio)
- ZoomScan analysis (Parent/Product charge state)

Chromatographic separation and compound detection are obtained by all LC detectors. Retention time alone, however, does not positively identify a compound because many compounds can have the same retention time under the same experimental conditions. In addition, even if a compound is identified correctly by retention time, quantitation results can be in error because other compounds in the sample might coelute with the compound of interest.

Level	Analysis Technique	Information Obtained
Level 1	Chromatographic separation and compound detection	retention time
Level 2	Mass analysis (full scan MS and SIM)	multiply charged ions (ESI) & molecular weight information (ESI, APCI)
Level 3	Two-stage mass analysis (full scan MS/MS and SRM)	reaction path: one specific ion (parent ion) dissociates to produce one specific product ion (SRM) or full scan product ions. Product ions then become parent ions for the next MS stage and are dissociated to produce one or more new product ions. Full scan MS/MS: metabolite identification, molecular structure determination by using the full scan product ion mass spectrum SRM: highly specific quantitation information by using the mass chromatogram of a specific product ion of a specific parent ion
Level 4	Multi-stage mass analysis (full scan / MS^n and CRM)	reaction path: a specific parent ion is dissociated to produce product ions. A product ion is then stored in the ion trap and becomes the parent ion for the next stage of MS. The ion is dissociated and the product ions are scanned out of the analyzer. full scan/MS^n: metabolite identification, structural determination of sugars, natural products
Level 5	ZoomScan analysis (ZoomScan / MS, ZoomScan / MS/MS, ZoomScan / MS^n)	reaction path: one or more ions are monitored in 10 u window(s) to determine the charge state of up to 10 ions. Resolves isotopic envelopes of multiply-charged ions by using slower scans with higher resolution than normal scans; charge state information, molecular weight determination

Table 1-1. LCQ MS detector levels of analytical specificity

Figure 1-1 shows the effect of each stage of MS analysis on ion signal, noise, and signal-to-noise ratio.

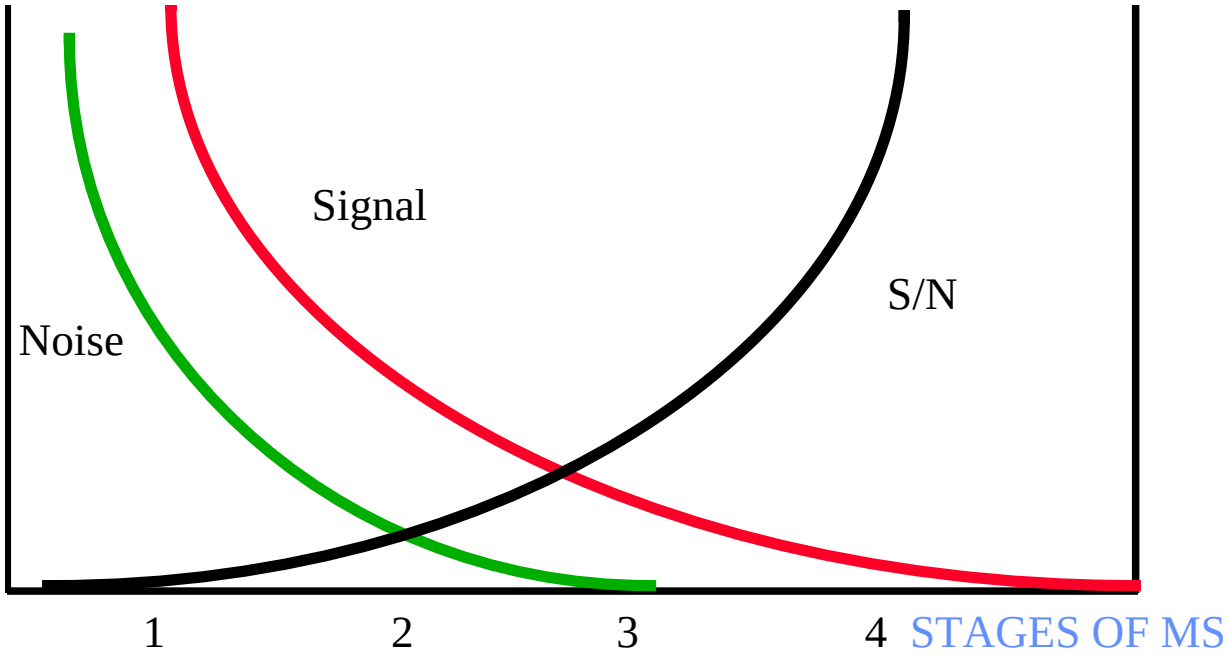


Figure 1-1. Effect of stages of MS on ion signal, noise, and signal-to-noise ratio (S/N)

Mass analysis allows for the identification of analytes of interest. Full scan / MS / ESI / positive ion mode typically produces mass spectra consisting of a multiply charged molecular ions depending on the structure of the analyte and the solvent. In the case of higher molecular weight proteins or peptides, the resulting mass spectrum typically consists of a distribution of multiply charged analyte ions. The resulting mass spectrum can be mathematically manipulated to determine the molecular weight of the sample. Full scan / MS / APCI / positive or negative ion mode provides molecular weight information for relatively non-polar small molecules, such as steroids.

Figure 1-2 shows the full scan / APCI mass spectrum of a mixture of caffeine, the peptide MRFA, and the perfluoronated compound Ultramark 1621.

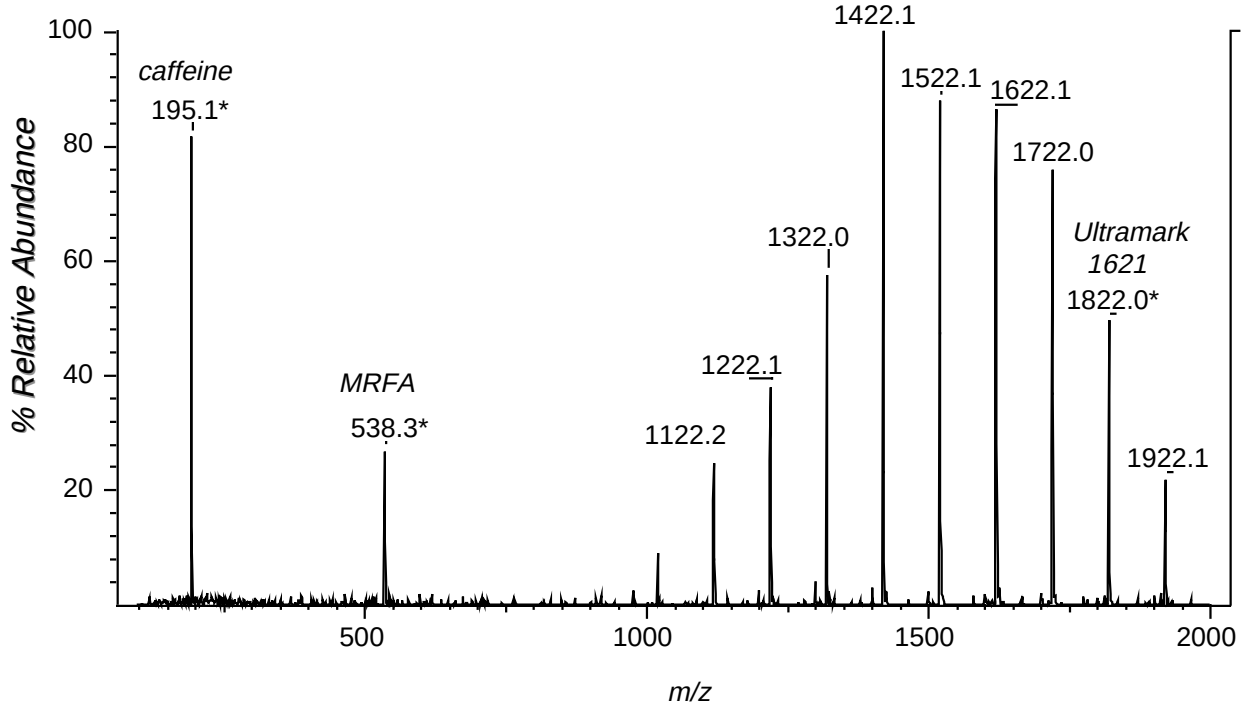


Figure 1-2. Full scan / MS / APCI mass spectrum of caffeine, MRFA, and Ultramark 1621

Two-stage mass analysis allows for even more positive compound identification. MS/MS analysis monitors a reaction path: the production of a specific product ion from a specific parent ion (SRM). Using SRM analysis, for example, you can easily quantitate target analytes in complex matrices such as plant or animal tissue, plasma, urine, groundwater, and soil. Because of the specificity of MS measurements and the ability to eliminate interferences by an initial mass filtering stage, quantitative target compound analysis is easily accomplished using the LCQ MS detector. Figure 1-3 illustrates the increase in the signal-to-noise ratio of an LC peak on going from a SIM (MS) experiment to an SRM (MS/MS) experiment.

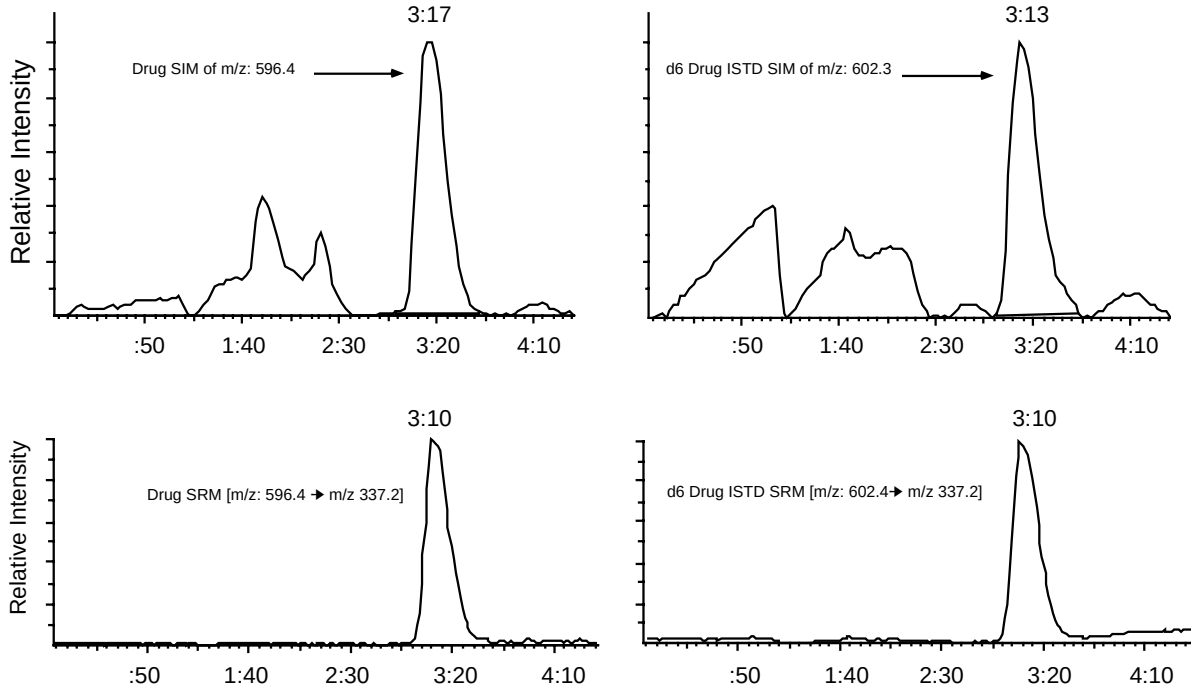


Figure 1-3. Chromatograms of a drug and an isotopically labeled internal standard in human plasma run in the SIM scan mode (top) and the SRM scan mode (bottom)

Multi-stage mass analysis provides a unique capability to obtain structural information which can be useful in molecular structure analyses of metabolites, natural products, and sugars. MS^n techniques on the LCQ allows for stepwise fragmentation pathways, making interpretation of MS^n spectra relatively straightforward.

ZoomScan analysis provides information about the charge state of one or more ions of interest. ZoomScan data is collected by using slower scans in a narrow range at higher resolution. This makes possible the resolution of the C-12 / C-13 isotopes of the analyte ion, which allows unambiguous determination of charge state, which in turn allows for the correct determination of molecular weight. Figure 1-4 shows a ZoomScan around the $[M+4H]^{4+}$ ion at *m/z* 1962.

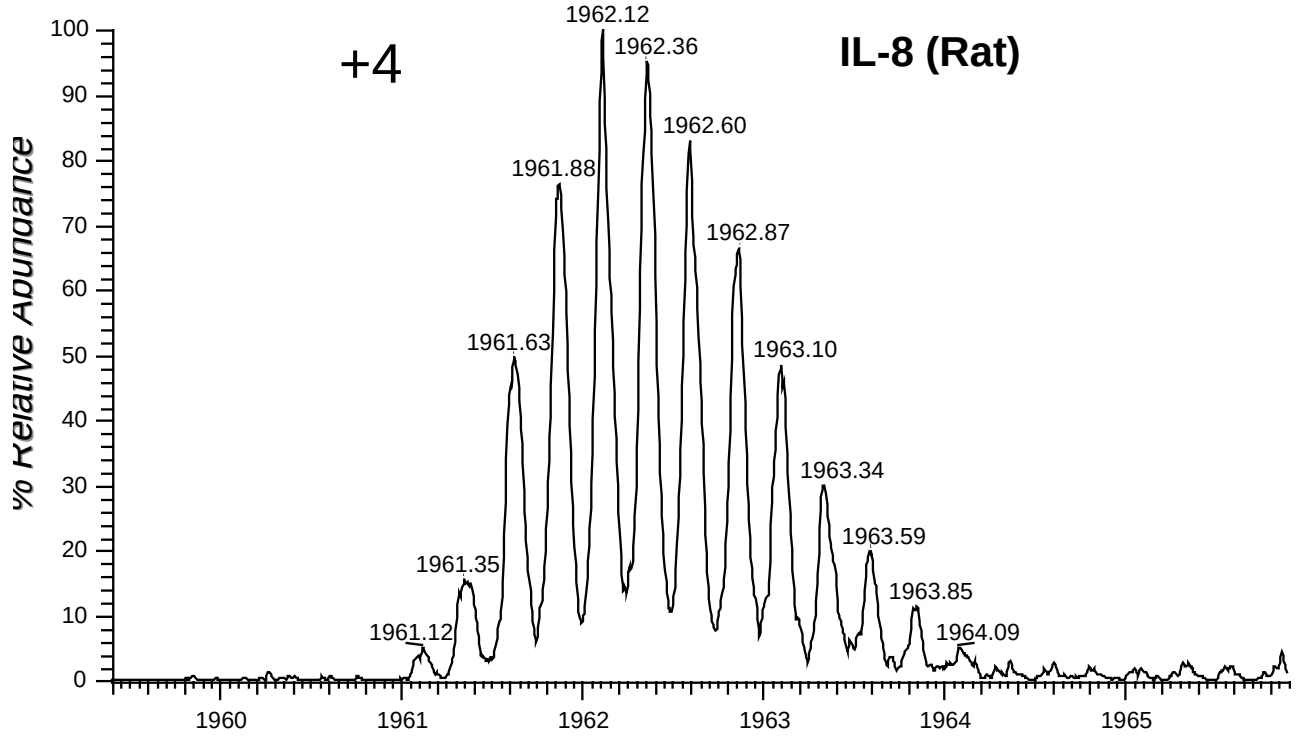


Figure 1-4. ZoomScan spectrum of rat interleukin-8, showing the isotope envelope around the $[M+4H]^{4+}$ ion at *m/z* 1962

1.4 API Source

The API source consists of the following components:

- Flange (APCI or ESI)
- Probe (APCI or ESI)
- Heated capillary
- Tube lens
- Skimmer

Detection of the sample occurs as follows. First the sample is ionized. Next, the ions pass through the lenses and enter the mass analyzer. Then, the ions are stored in the analyzer, and are scanned out of the analyzer into the ion detection system. Figure 1-5 shows an overall schematic diagram of the API source configured in the ESI mode.

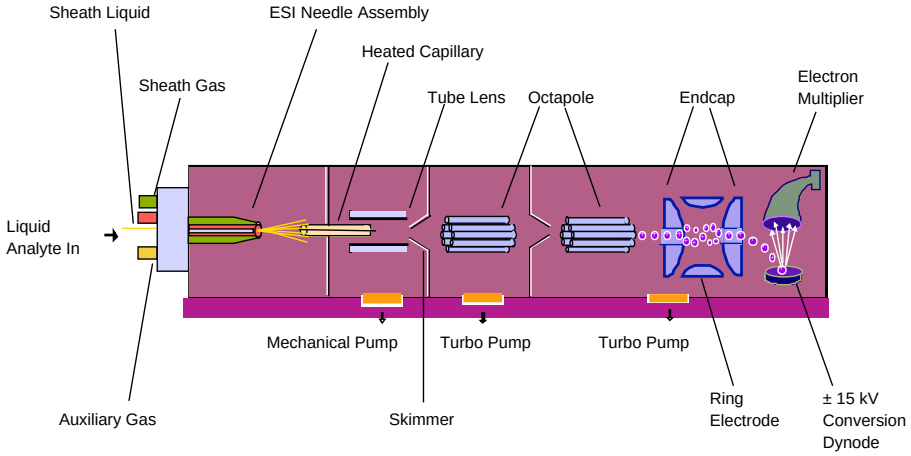


Figure 1-5. Schematic diagram of the LCQ API source, ion optics, mass analyzer, and ion detection system

The liquid analyte enters the atmospheric pressure region along with nebulizing sheath and/or auxiliary gas and exits the ESI needle assemble at a voltage difference of typically ±3 to 5 kV, relative to a heated metal capillary held at ground potential. Dry nitrogen gas is commonly used as the sheath gas and auxiliary gas. The operating temperature for the heated capillary is typically between 150 and 250 °C.

The nebulization gases sweep the liquid effluent and help focus the aerosol into the entrance of the heated capillary. The auxiliary gas typically is used for solvent flow rates above 50 µL min^{-1} but is not required normally at very low flow rates (2 to 5 µL min^{-1}). The auxiliary gas assists in desolvation when low-volatility solvents are used. Following sample desolvation, the ions are accelerated through a differentially pumped vacuum system from atmospheric pressure in the needle assembly region, to 0.5 to 1.0 Torr in the capillary/skimmer region, then to approximately 10^{-5} Torr in the analyzer region with 10^{-3} Torr of helium bath gas.

2.1 Contents

List of Illustrations

CALIBRATING AND TUNING IN THE ESI/MS MODE

This chapter provides information on how to calibrate and tune the LCQ MS detector in the ESI/MS (or ESI/MS/MS) mode. For most applications, you calibrate and tune in the ESI mode of operation through two automatic procedures.

To calibrate and tune your MS detector in the ESI mode, you do the following:

- Open the Tune Plus window.
- Set up the MS detector for ESI/MS operation.
- Set up the inlet, in this case a syringe infusion pump.
- Set up the ESI source for ESI/MS operation.
- Test the operation of the MS detector in the ESI/MS mode.
- Calibrate the MS detector in the ESI/MS mode.
- Tune the MS detector in the ESI/MS mode.
- Prepare the MS detector for normal LC/MS operation.

You set up the MS detector from the Tune Plus window.

You use a syringe infusion pump to infuse a tuning solution of a mixture containing caffeine, MRFA, and Ultramark 1621 into the ESI source.

You test the operation of the ESI source by observing the singly-charged, positive ions for caffeine, MRFA, and Ultramark 1621 in the Tune Plus window.

You calibrate and tune the MS detector from the Tune Plus window.

The automatic calibration and tuning procedures properly calibrate and tune the MS detector for ESI operation for the entire mass range of the MS detector. The procedures use the tuning solution.

In general, once you have calibrated and tuned the MS detector, you do not need to repeat these procedures very often. However, if your particular analyses require exact molecular weight determination, you will need to check the MS detector calibration periodically.

3.1 Opening the Tune Plus Window

Use the following procedure to open the Tune Plus window.

1. Open the LCQ data system, as follows:
   Double-click on the LCQ Navigator icon in the LCQ program group in the Program Manager.

1. Open the Tune Plus window, as follows:
   Click on the Tune Plus button in the Navigator toolbar.

   Ensure that LCQ displays a Tune Plus window similar to the one shown in Figure 3-1.

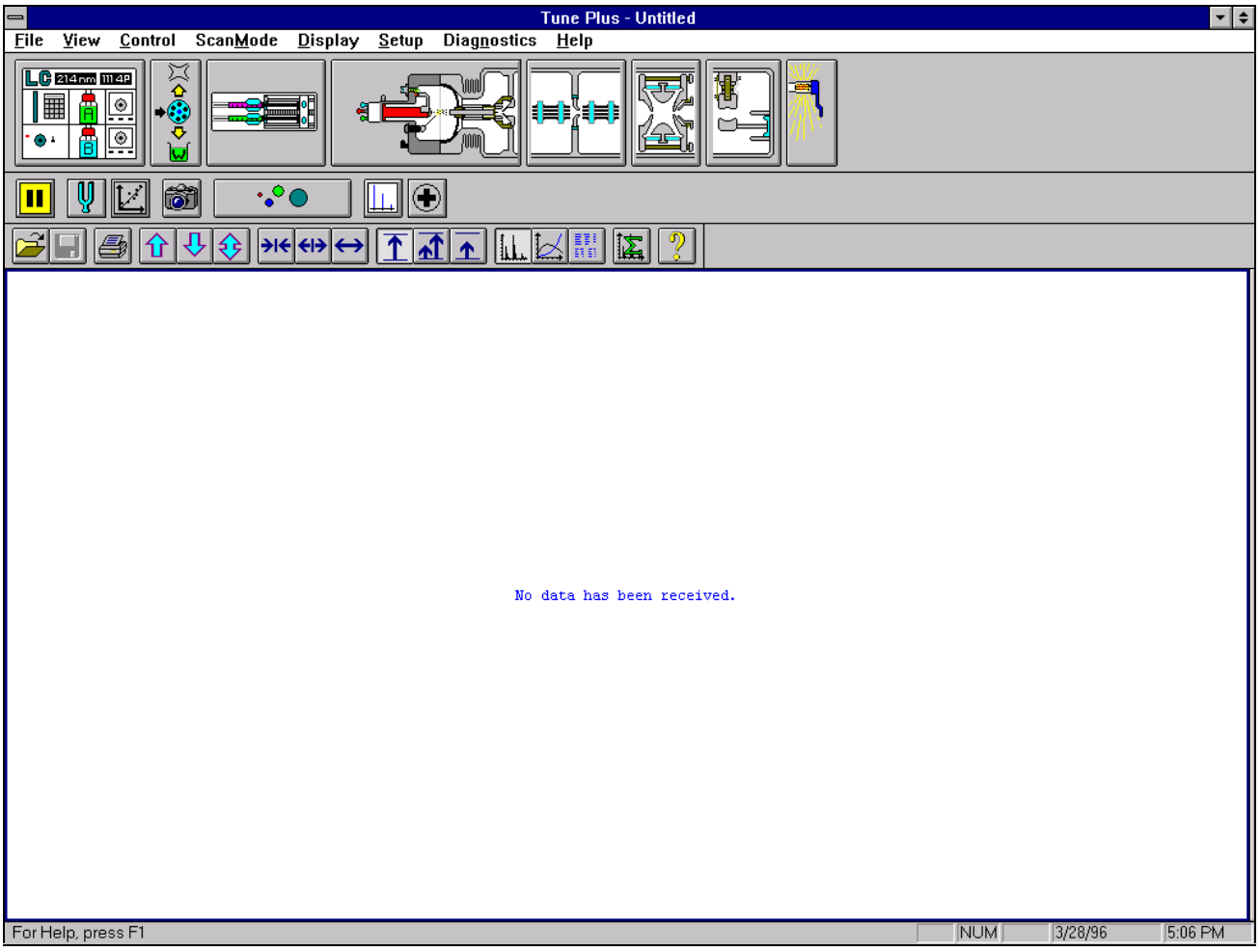


Figure 3-1. Tune Plus window, showing the MS detector in the Standby mode

3.2 Setting Up the MS Detector for ESI/MS Operation

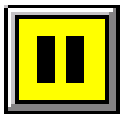
Use the following procedure to set up the MS detector for ESI/MS operation.

Note. The following procedures assume that you are familiar with your LCQ instrument and the Tune Plus window. If you need assistance, refer to: LCQ online Help (or the **LCQ Software Manual**) and/or the **LCQ MS Detector Hardware Manual**.

WARNING. Before you begin normal operation each day, ensure that you have sufficient nitrogen for your API source. If you run out of nitrogen, LCQ automatically turns the MS detector Off to prevent the possibility of atmospheric oxygen from entering the ion source. The presence of oxygen in the ion source when the MS detector is ON could be unsafe. (In addition, if LCQ turns Off the MS detector during an analytical run, you could lose data.) .

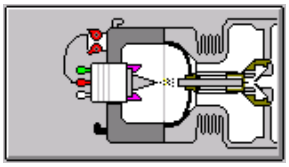
WARNUNG. Stellen Sie täglich vor Beginn des normalen Betriebs sicher, daß Sie genügend Stickstoff für Ihre Atmosphärendruck-Ionisationsquelle (API) haben. Wenn der Stickstoff nicht ausreicht, schaltet das LCQ den MS-Detektor automatisch auf AUS, um zu verhindern, daß Luftsauerstoff in die Ionenquelle eindringt. Sauerstoff in der Ionenquelle kann bei eingeschaltetem MS-Detektor eine Gefahr darstellen. (Außerdem können biem Abschalten des MS-Detektors während einer Analyse Daten verloren gehen.)

AVERTISSEMENT. Tous les jours avant d'utiliser l'appareil, vérifiez le niveau d'azote de la source d'ionisation à la pression atmosphérique. En effet, si vous tombez à court d'azote, LCQ désactive automatiquement le détecteur de SM pour empêcher que la source d'ions ne soit contaminée par l'oxygène présent dans l'air. Toute présence d'oxygène dans la source d'ions lorsque le détecteur de SM est activé peut comporter des risques. (En outre, si LCQ désactivait le détecteur de SM pendant une série d'analyses, vous pourriez perdre des données).



1. In Tune Plus, take the MS detector out of Standby mode and turn it On, as follows:
   Click on the On/Standby button in the Instrument Control toolbar. The MS detector begins scanning, LCQ applies high voltage to the ESI probe, and LCQ shows a real-time display in the Spectrum view.
2. Open the *esilowflow.tun* Tune Method, the Tune Method for low-flow ESI operation, as follows:
   a. Display the Open dialog box, as follows:
      Choose **File | Open**.
   b. Select the file *esilowflow.tun* in the directory *C:\LCQ\Methods*, as follows:
      Scroll down in the File Name combo box until you see *esilowflow.tun*. Then, click on the file name.
   c. Open the file, and close the dialog box, as follows:
      Click on **OK**. LCQ downloads the Tune Method parameters to the MS detector.

1. Verify that LCQ opened the Tune Method, as follows:



   a. Open the ESI Source dialog box, as follows:
      Click on the ESI Source button in the Instrument Setup toolbar. See Figure 3-2.
   b. Verify that the settings in your dialog box are the same as those shown in Figure 3-2.
   c. Close the dialog box, as follows:
      Click on **OK**.

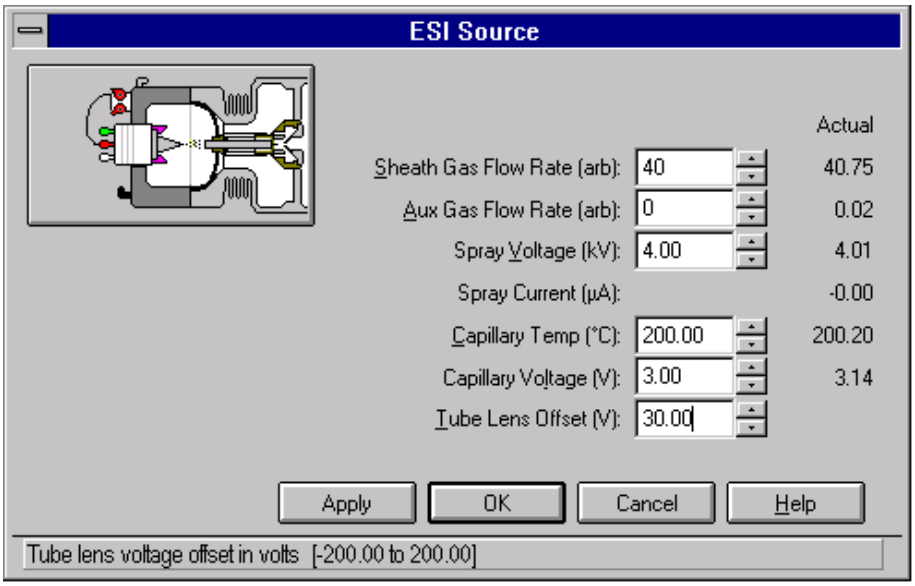
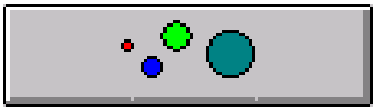


Figure 3-2. ESI Source dialog box, showing the proper settings for a typical low flow experiment

4. Define the scan parameters for calibrating and tuning, as follows:



   a. Open the Define Scan dialog box, as follows:
   Click on the Define Scan button in the Instrument Control toolbar. See Figure 3-3.

   b. Select the MS scan power in the Scan Description group box, as follows:
   Click on the Scan Power: MS option button. Note that LCQ sets the MS^n power to *1*.

   c. Select the Full scan mode, as follows:
   Click on the Scan Mode: Full option button.

   d. Set the total number of microscans to 2 in the Scan Time group box, as follows:
   Double-click in the Total Microscans spin box, then type **2**.

   e. For this example, leave the maximum injection time set to its default value of 200.00 ms.

   f. Select the first / last input method in the Input Method group box, as follows:
   Click on the First / Last option button. Note that LCQ

displays the First Mass and Last Mass text boxes in the Scan Ranges group box.

g. Select source CID Off in the Source CID group box, as follows:
Click on the Off option button.

h. Set the first mass for scan range to *m/z* 150 in the Scan Ranges group box, as follows:
Double-click in the First Mass text box, then type **150**.

i. Set the last mass for scan range to *m/z* 1850, as follows:
Double-click in the Last Mass text box, then type **1850**.

j. Save the MS detector scan parameters and close the Define Scan dialog box, as follows:
Click on **OK**.

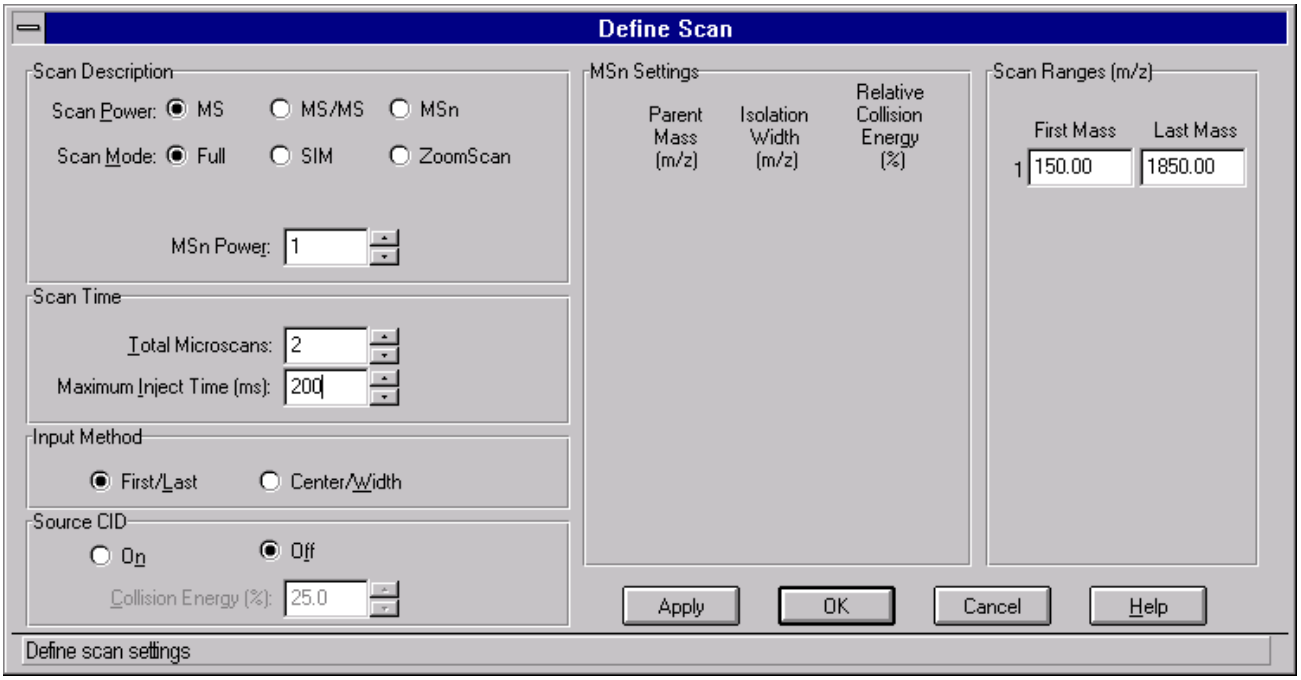
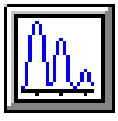


Figure 3-3. Define Scan dialog box, showing typical settings for ESI/MS operation



5. Select the profile scan data type, as follows:
Click on the Centroid/Profile button in the Instrument Control toolbar to toggle the scan data type to profile. (The picture on the button should be the same as that shown here).

5. Select the positive ion polarity mode, as follows:
   Click on the Positive/Negative button in the Instrument Control toolbar to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here).

You have now completed setting up your MS detector for ESI/MS operation. Go to the next topic: **Setting Up the Inlet: Syringe Pump**.

3.3 Setting Up the Inlet: Syringe Pump

The inlet that you use for ESI calibrating and tuning is a syringe pump. A syringe pump allows you to infuse a sample solution into the ESI source for extended periods of time.

The syringe pump and syringe are located on the front panel of your LCQ MS detector. To infuse a solution, you install on the pump a syringe containing the sample solution. Then you connect the tip of the syringe needle to a Teflon® tube. When you turn the pump On, sample solution flows from the syringe, through the Teflon tube, through a connector, through a fused-silica transfer line, through a grounded holder, through a fused-silica sample tube, through the ESI probe, and into the ion source via the spray needle of the ESI probe.

Use the following procedure to set up the syringe pump for infusion. See Figure 3-4.

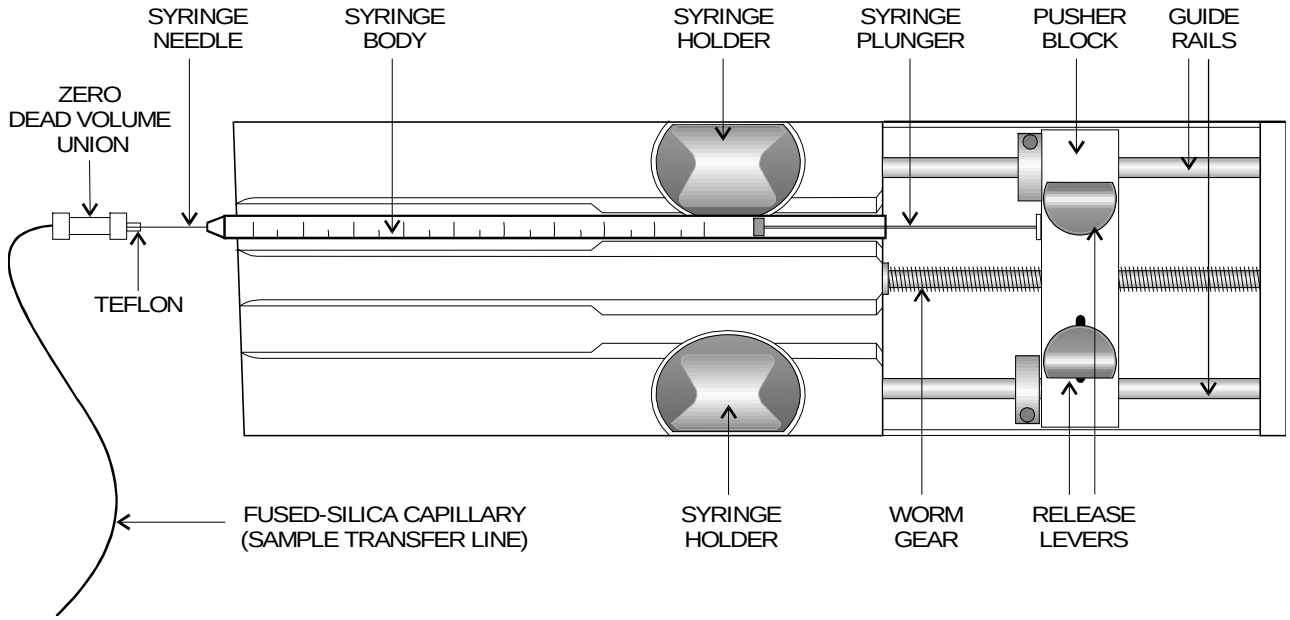


Figure 3-4. Syringe pump, showing the proper setup for direct infusion

1. Remove the syringe from the syringe holder as follows:

   a. Pull up and rotate the blue syringe-holder finger to free the syringe.

   b. Remove the syringe from the holder.

2. Disconnect the tip of the syringe needle from the (1/16-in. OD) Teflon tubing (P/N 00301-22915), if the tubing is attached.

3. Ensure that the syringe is a clean, 250 µL (2.30 mm ID) Unimetrics syringe (P/N 00301-19012). If necessary, replace the syringe.

> **Note.** The Unimetrics syringe needle provided with your LCQ system has a square tip. If you use a different kind of syringe, make sure that its needle also has a square tip.

4. Check that the needle tip of the syringe fits readily into the opening in the free end of the Teflon tubing. If necessary, you can enlarge the opening in the end of the tubing slightly. Do not connect the tubing to the syringe needle yet.

5. Squeeze the blue release levers on the pusher block (on the right-hand side of the front door panel). Pull the pusher block backward, away from the blue syringe holders, on the guide rails.

6. Load the 250 µL Unimetrics syringe with 200 µL of the tuning solution. (Refer to **Appendix A: Sample Formulations** for a procedure for making the tuning solution.)

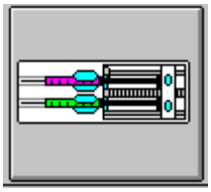
7. Replace the syringe into one of the blue syringe holders to secure it in place.

8. While squeezing the blue release levers on the pusher block, push the pusher block forward until the pusher block just contacts the syringe plunger.

Note. For most applications, the sample solution is infused at a rate of 1 to 5 µL min^{-1}. You set this flow rate in the Syringe Pump dialog box by entering the flow rate value and the inner diameter of the syringe. From this information, the pump calibrates the flow rate for the syringe.

Note. The Operating and Maintenance Instructions for your LCQ syringe pump gives the ID of several syringes. If you are not using a Unimetrics syringe, consult this manual for the proper syringe diameter.

9. Enter syringe pump parameters, as follows:



   a. Open the Syringe Pump dialog box, as follows:
   Click on the Syringe Pump button in the Instrument Setup toolbar. See Figure 3-5.

   b. Set an infusion flow rate of 3 µL min^{-1}, as follows:
   Double-click in the Flow Rate spin box, then type **3.00**.

   c. Specify a Unimetrics syringe type in the Type group box, as follows:
   Click on the Unimetrics option button.

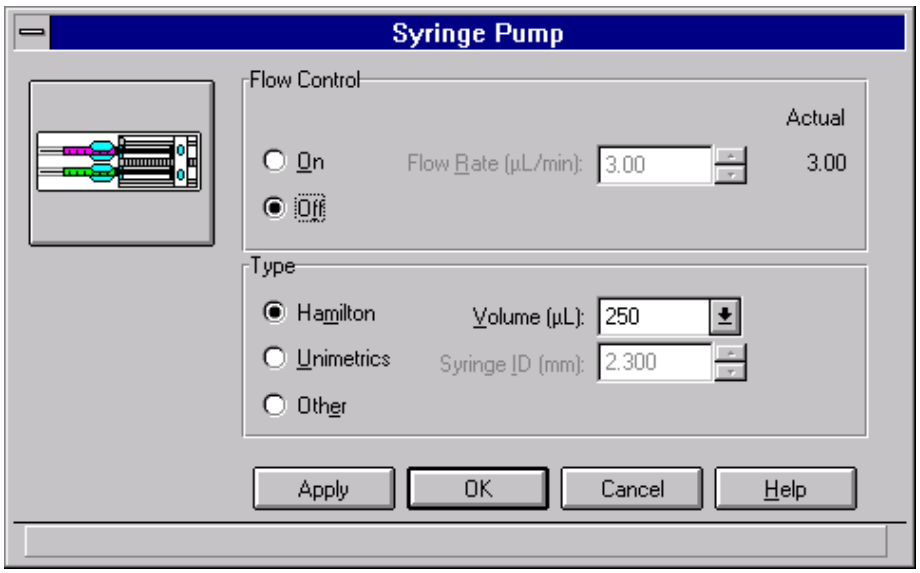


Figure 3-5. Syringe Pump dialog box, showing typical settings for infusion of tuning solution

> **Note.** This procedure assumes that you are using the Unimetrics syringe that is provided with your LCQ system. If you are using another type of syringe, select the option button corresponding to your syringe.

d. Set the syringe volume to 250 µL, as follows:
Click on the Volume list box arrow to display the list of available volumes, then select **250** from the list. Note that, if you are using a Unimetrics syringe, LCQ automatically sets the syringe ID to its proper value of 2.30 mm.

e. If you are not using a Unimetrics (or Hamilton) syringe, you need to set the syringe ID manually, as follows:
Double-click in the Syringe ID spin box, then type the inner diameter of your syringe.

Leave the Syringe Pump dialog box open. You will return to it later.

11. Connect the Teflon tube to the syringe needle by placing an Upchurch Fingertight fitting and ferrule and over the Teflon tube, and then pushing the tip of the needle through the tube. While pushing the syringe tip as far forward as possible, screw the fitting into one end of an Upchurch union and tighten until finger-tight.

12. Connect the fused-silica transfer line to the Upchuch union by inserting the fused-silica through an Upchurch Fingertight fitting and ferrule. While pushing the end of the fused-silica as far forward as possible, screw the fitting into the other end of the Upchurch union until finger-tight.

WARNING: AVOID ELECTRICAL SHOCKS. ENSURE THAT YOUR SAMPLE TRANSFER LINE IS PROPERLY ATTACHED TO THE GROUNDED FITTING HOLDER ON THE ESI FLANGE. Your ESI probe is designed to electrospray highly conductive solutions. However, you must isolate high-voltage current by properly grounding the sample transfer line and sample tube. .

WARNUNG! ELEKTROSCHOCK VERMEIDEN! KONTROLLIEREN, DASS DAS PROBENTRANSFERKABEL ENTSPRECHEND MIT DEM GEERDETEN FASSUNGSHALTER DES ESI-FLANSCHES VERBUNDEN IST! Die ESI-Sonde dient dem elektronischen Versprühen hochleitender Lösungsmittel. Hochspannung muß aber durch entsprechendes Erden des Probentransferkabels und der Probenleitung isoliert werden.

AVERTISSEMENT. EVITER LES CHOCS ELECTRIQUES. S'ASSURER QUE LA LIGNE DE TRANSFERT D'ECHANTILLONS EST CORRECTEMENT FIXEE A LA RETENUE DE RACCORD MISE A LA TERRE, SITUEE SUR LA BRIDE ESI. La sonde ESI est conçue pour l'électrovaporisation de solutions à haute conductivité ; cependant, le courant haute tension doit être isolé par une mise à la terre correcte de la ligne de transfert et du tube d'échantillon.

12. Connect the sample transfer line (fused-silica capillary) from the Upchurch connector to the grounded fitting connector on the ESI flange. **BE SURE TO SECURE THE FITTING PROPERLY IN THE GROUNDED FITTING HOLDER**. See Figure 3-6.

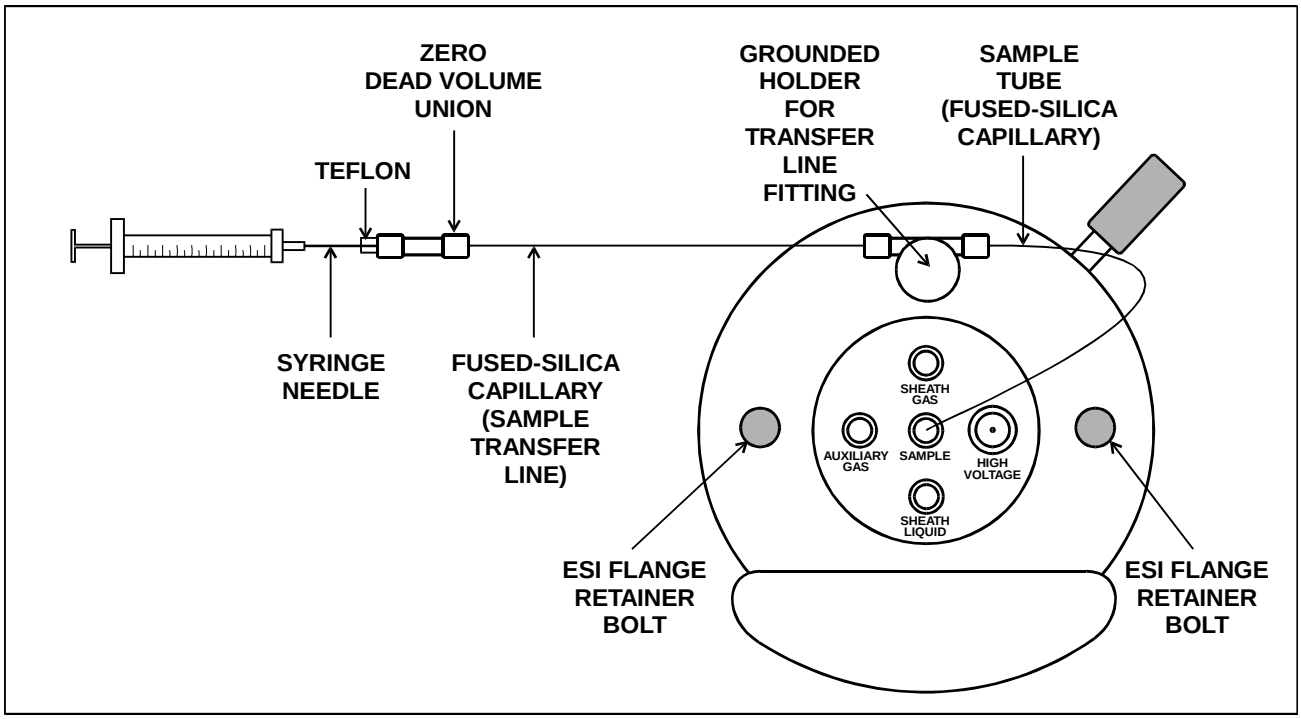


Figure 3-6. ESI probe assembly, showing the connections for direct infusion

13. Plug the Sheath Liquid inlet on the ESI probe assembly with an Upchurch 1/4-in × 28 Tefzel® plug. Always use a plug fitting on the Sheath Liquid inlet when you are not using a sheath liquid. The plug prevents solvent from entering the ESI probe assembly and short circuiting the ESI needle.

You have now completed setting up your syringe pump. Go to the next topic: **Setting Up the ESI Source for ESI/MS Operation**.

3.4 Setting Up the ESI Source for ESI/MS Operation

To set up your ESI source for ESI/MS operation, you do the following:

- Flush the sample transfer line, sample tube, and ESI probe.
- Infuse the tuning solution of caffeine, MRFA, and Ultramark 1621.

3.4.1 Flushing the Sample Transfer Line, Sample Tube, and ESI Probe

Use the following procedure to flush the sample transfer line, sample tube, and ESI probe:

1. Loosen completely the two ESI flange retainer bolts.

WARNING. Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is ON could be unsafe. (LCQ automatically turns the MS detector Off when you open the API source, however, it is best to take this added precaution.) .

WARNUNG. Stellen Sie den MS-Detektor auf Bereitschaft (oder AUS), bevor Sie die Atmosphärendruck-Ionisationsquelle (API) öffnen. Luftsauerstoff in der API-Quelle kann bei eingeschaltetem MS-Detektor eine Gefahr darstellen. (Das LCQ schaltet den MS-Detektor automatisch AUS, wenn Sie die API-Quelle öffnen. Es wird jedoch empfohlen, diese zusätzliche Vorsichtsmaßnahme zu ergreifen.)

AVERTISSEMENT. Mettez le détecteur de SM en veilleuse (ou hors tension) avant d'ouvrir la source d'ionisation à la pression atmosphérique. Toute présence d'oxygène dans la source d'ionisation lorsque le détecteur de SM est activé peut comporter des risques. (Bien que LCQ désactive automatiquement le détecteur de SM lorsque vous ouvrez la source d'ionisation à la pression atmosphérique, il est préférable de prendre cette précaution supplémentaire).

WARNING. AVOID BURNS DO NOT TOUCH THE HEATED CAPILLARY. The heated capillary can attain temperatures above 350 °C. Always be careful not to touch the entrance end of the heated capillary when it is exposed . .

WARNUNG! VERBRENNUNGEN VERMEIDEN! BEHEIZTE KAPILLAREN NICHT BERÜHREN! Die beheizten Kapillaren können Temperaturen von über 350°C erreichen. Das Eingangsende der beheizten Kapillaren niemals berühren, wenn es offen liegt.

AVERTISSEMENT. EVITER LES BRULURES. NE PAS TOUCHER AU CAPILLAIRE CHAUFFE. Le capillaire chauffé peut atteindre une température supérieure à 350 °C. Eviter toujours soigneusement de toucher l'extrémité d'entrée du capillaire chauffé lorsqu'elle est exposée.

2. Slide the ESI flange out, away from the MS detector chassis, on the slide rails.

3. Being careful not to touch the heated capillary with your hand, place a small septum over the entrance end of the

heated capillary to seal the vacuum chamber of the MS detector.

4. Fill a clean, 250 µL Unimetrics syringe with a solution of 1% acetic acid in 50:50 methanol:water or with another appropriate solvent.

> **Note.** The solvent that you use to flush the sample transfer line, sample tube, and ESI probe assembly depends on the solvent system you use to dissolve your samples. For example, if you are using a buffered solution of a high concentration, an acidic solution is appropriate.

5. Disconnect the syringe containing the tuning solution from the Teflon tube.

6. While holding the plunger of the second syringe in place, carefully insert the syringe needle containing 1% acetic acid in 50:50 methanol:water into the free end of the Teflon tube.

7. Flush the sample transfer line, sample tube, and ESI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the ESI probe on the inside of the probe assembly. Use a lint-free paper to gently remove the excess solution as it exits the probe.

8. Remove the syringe containing methanol:water from the Teflon tube.

9. Reconnect the syringe containing the tuning solution to the Teflon tube.

3.4.2 Infusing the Tuning Solution

Use the following procedure to infuse the tuning solution into the ESI source:

1. If you have not already done so, set up the syringe infusion pump and load the syringe with tuning solution as described in the topic **Setting Up the Inlet: Syringe Infusion Pump**.

2. Start the syringe pump, as follows:
   Click on **OK** in the Syringe Pump dialog box. LCQ enters the syringe pump parameters, and turns on the syringe pump. The tuning solution should now be flowing at a rate of 3 µL min^{-1}.

3. Ensure that tuning solution is exiting the from the ESI probe, inside the probe assembly.

4. Ensure that the fused-silica sample tube is connected properly in the ESI probe, as follows:

   a. Loosen the sample tube fitting on the ESI flange.

   b. Carefully push the sample tube into the ESI flange until you can see the tip of the tube.

   c. Withdraw the sample tube so that the end of the tube is slightly recessed in the ESI needle.

   d. Gently, retighten the sample tube to secure the sample tube. Do not overtighten the fitting.

WARNING. Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is ON could be unsafe. (LCQ automatically turns the MS detector Off when you open the API source, however, it is best to take this added precaution.) .

WARNUNG. Stellen Sie den MS-Detektor auf Bereitschaft (oder AUS), bevor Sie die Atmosphärendruck-Ionisationsquelle (API) öffnen. Luftsauerstoff in der API-Quelle kann bei eingeschaltetem MS-Detektor eine Gefahr darstellen. (Das LCQ schaltet den MS-Detektor automatisch AUS, wenn Sie die API-Quelle öffnen. Es wird jedoch empfohlen, diese zusätzliche Vorsichtsmaßnahme zu ergreifen.)

AVERTISSEMENT. Mettez le détecteur de SM en veilleuse (ou hors tension) avant d'ouvrir la source d'ionisation à la pression atmosphérique. Toute présence d'oxygène dans la source d'ionisation lorsque le détecteur de SM est activé peut comporter des risques. (Bien que LCQ désactive automatiquement le détecteur de SM lorsque vous ouvrez la source d'ionisation à la pression atmosphérique, il est préférable de prendre cette précaution supplémentaire).

WARNING. AVOID BURNS DO NOT TOUCH THE HEATED CAPILLARY. The heated capillary can attain temperatures above 350 °C. Always be careful not to touch the entrance end of the heated capillary when it is exposed . .

WARNUNG! VERBRENNUNGEN VERMEIDEN! BEHEIZTE KAPILLAREN NICHT BERÜHREN! Die beheizten Kapillaren können Temperaturen von über 350°C erreichen. Das Eingangsende der beheizten Kapillaren niemals berühren, wenn es offen liegt.

AVERTISSEMENT. EVITER LES BRULURES. NE PAS TOUCHER AU CAPILLAIRE CHAUFFE. Le capillaire chauffé peut atteindre une température supérieure à 350 °C. Eviter toujours soigneusement de toucher l'extrémité d'entrée du capillaire chauffé lorsqu'elle est exposée.

5. Being careful not to touch the heated capillary with your hand, remove the septum from the entrance end of the heated capillary.

6. Slide the ESI flange forward along the slide rails and into the ion source housing.

7. Secure the ESI flange with the two flange retainer bolts.

8. Close the Syringe Pump dialog box, as follows:
   Click on **OK**.

You are now ready to test whether your MS detector is operating properly. Go to next topic: **Testing the Operation of the MS Detector in the ESI/MS Mode**.

3.5 Testing the Operation of the MS Detector in the ESI/MS Mode

You are now ready to test if your MS detector is operating properly. To test for proper operation, you infuse the tuning solution into the ESI source, and then you monitor the real-time display of the mass spectrum of tuning solution.

Observe the singly-charged ions of tuning solution in the Spectrum view of the Tune Plus window. The ions are as follows. See Figure 3-7.

- Caffeine: m/z 195
- MRFA: m/z 525
- Ultramark 1621: m/z 1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, 1822

While you observe the Spectrum view, ask yourself the following questions:

- Is the ion current signal present?
- Is the signal stable, varying by less than ~15% from scan to scan?
- Is there good resolution between singly-charged ions and their isotopes?

If you answer "yes" to all of the questions, your MS detector is operating properly. Well Done!

If you answered no to one or more of these questions try the following troubleshooting measures:

- Ensure the fused-silica sample tube does not extend beyond the tip of the ESI needle.
- Ensure the entrance to the heated capillary is clean, and isn't covered with a piece of septum.
- Ensure that the automatic gain control feature is **On**, and the Full MS Target is set to 5e+007 (5×10^7). Verify this by choosing **Setup | Injection Control** from the Tune Plus window.

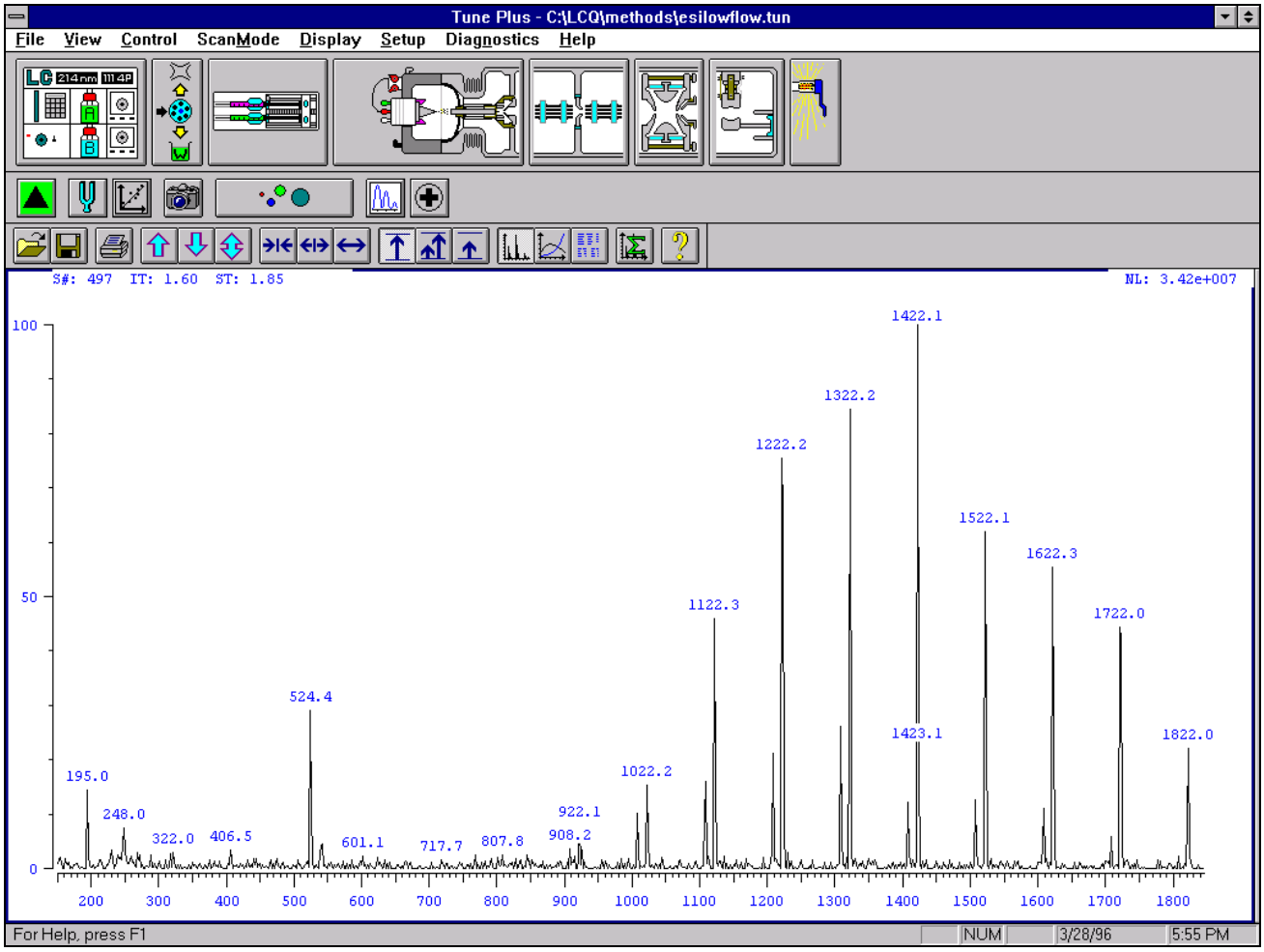
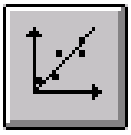


Figure 3-7. Spectrum view of the Tune Plus window, showing a real-time display of tuning solution

Congratulations! You have demonstrated that your MS detector is operating properly in the ESI mode. You are now ready to calibrate and tune the MS detector. Go to the next topic: **Calibrating the MS Detector in the ESI/MS Mode**.

3.6 Calibrating the MS Detector in the ESI/MS Mode

Use the following procedure to calibrate the MS detector automatically:



1. Open the Calibrate dialog box - Semi-Automatic tab, as follows:
   First, click on the Calibrate button in the Instrument Control toolbar. Then, click on the Semi-Automatic tab. See Figure 3-8.

2. Start the automatic calibration procedure, as follows:

   a. To calibrate all parameters automatically, do the following:
      Select [x] the Select All check box.

   b. Start the procedure, as follows:
      Click on **Start**.

3. Observe the Tune Plus window and the Calibrate dialog box. While the automatic calibration is in progress, LCQ displays various tests in the Spectrum and Graph views in Tune Plus and displays various messages in the Status group box in the Calibrate dialog box. See Figure 3-9 for a typical display.

When LCQ completes the calibration procedure, LCQ restores the full scan ESI mass spectrum in the Spectrum view, and the Instrument Messages box indicates whether or not the calibration procedure was successful. If it was successful, LCQ saves the new calibration parameters automatically to the hard disk.

If the calibration procedure failed, you can try it again after you have performed the automatic tuning procedure, below. In that procedure, LCQ optimizes several MS detector parameters to increase the intensity of the signal and improve the overall quality of the mass spectrum.

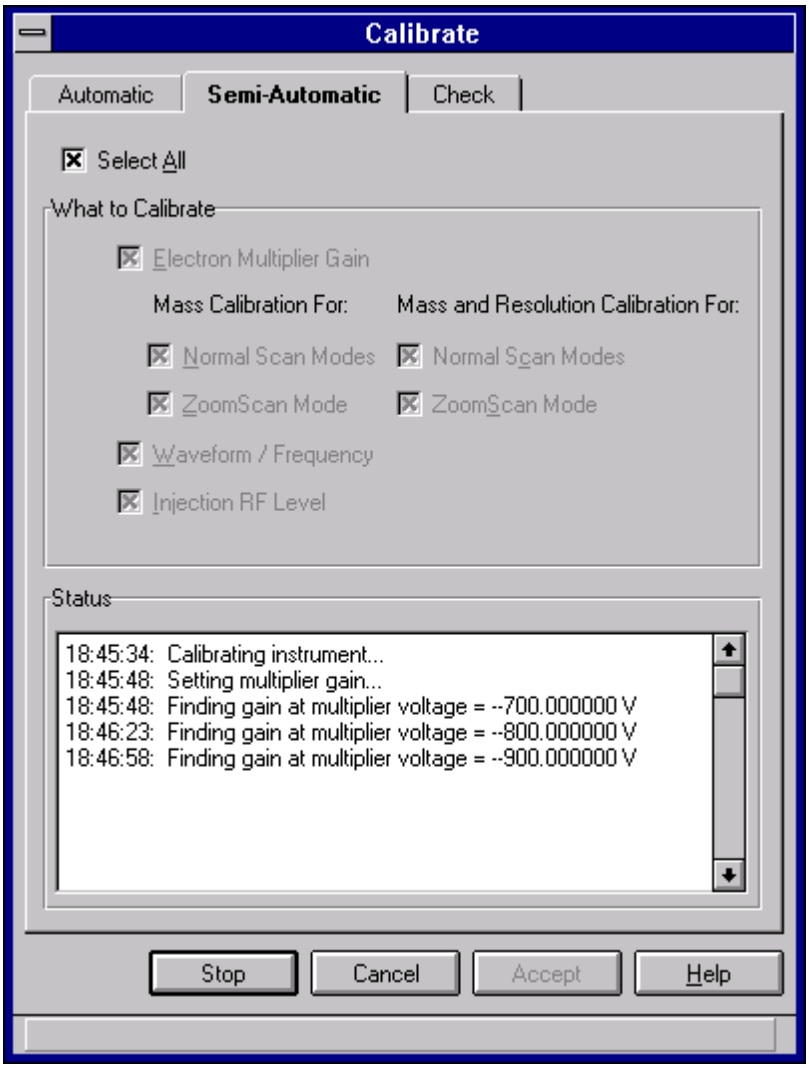


Figure 3-8. Calibrate dialog box, showing the Semi-Automatic tab ready to perform an automatic calibration

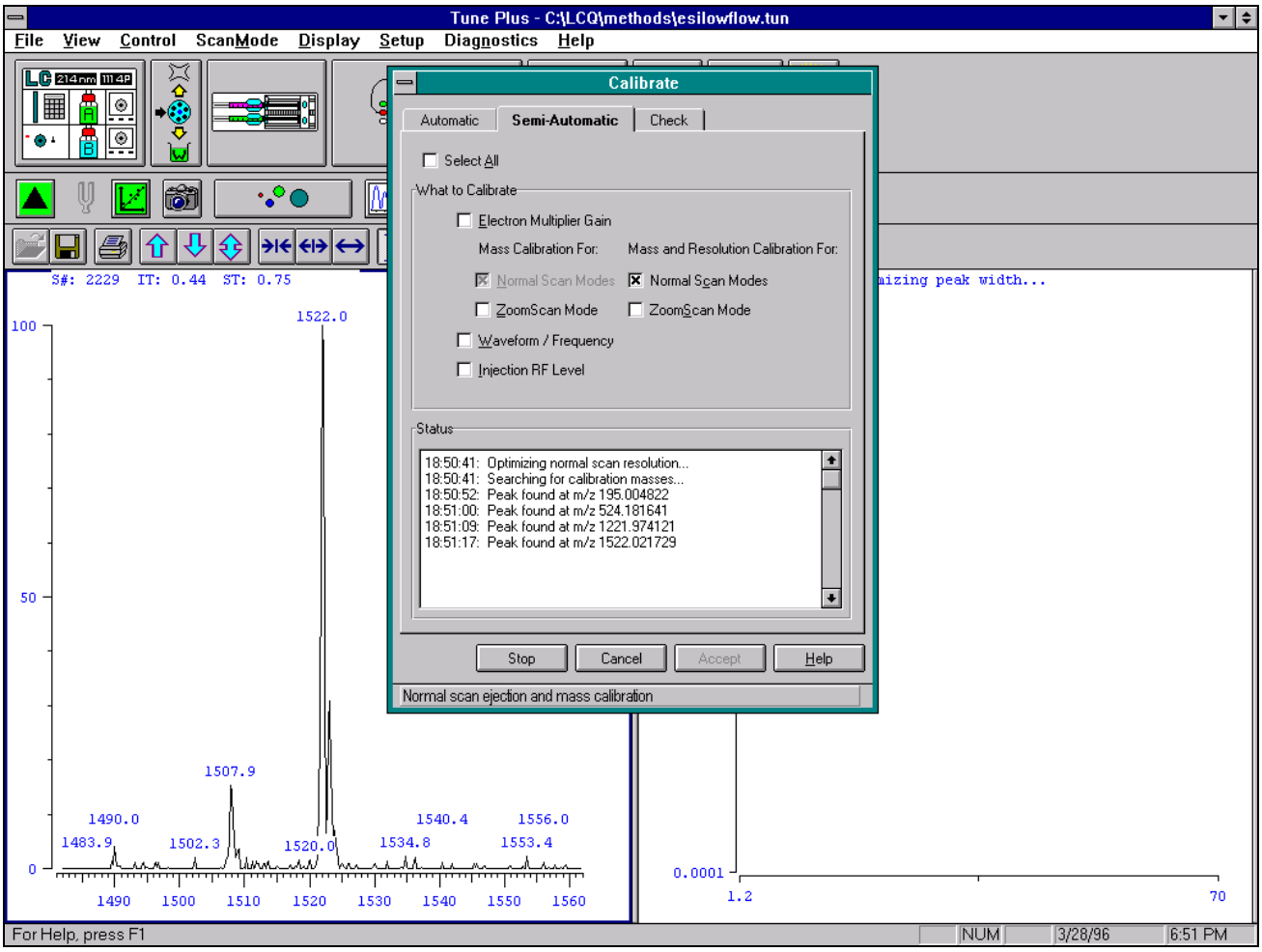
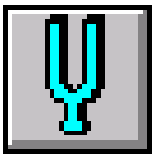


Figure 3-9. Tune Plus window, showing the results of a typical automatic calibration procedure

Your MS detector is now properly calibrated. Go to the next topic: **Tuning the MS Detector for ESI/MS Operation**.

3.7 Tuning the MS Detector in the ESI/MS Mode

Use the following procedure to tune the MS detector automatically:



1. Open the Tune dialog box - Automatic tab, as follows:
   First, click on the Tune button in the Instrument Control toolbar. Then, click on the Automatic tab. See Figure 3-10.

2. Start the automatic tuning procedure, as follows:

   a. To tune optimize on a particular mass peak, do the following:
   Select the Mass option button in the What to Optimize On group box.

   b. Set the mass peak to optimize on to the caffeine peak at *m/z* 195, as follows:
   Double-click on the Mass spin box, then type **195**.

> **Note.** In this example, you use the mass peak at *m/z* 195 to optimize the tune. However, you can optimize the tune on any of the mass peaks of the tuning solution. Choose the mass peak of the tuning solution that most closely matches the mass peak of interest in your analytical sample.

   c. Start the procedure, as follows:
   Click on **Start**.

4. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, LCQ displays various tests in the Spectrum and Graph views in Tune Plus and displays various messages in the Status group box in the Tune dialog box. See Figure 3-10 for a typical display.

Note. If your MS detector failed the automatic calibration procedure, you can now return to the topic Calibrating the MS Detector for ESI/MS Mode and repeat the procedure. Now that you have tuned the MS detector, the calibration procedure will probably run successfully.

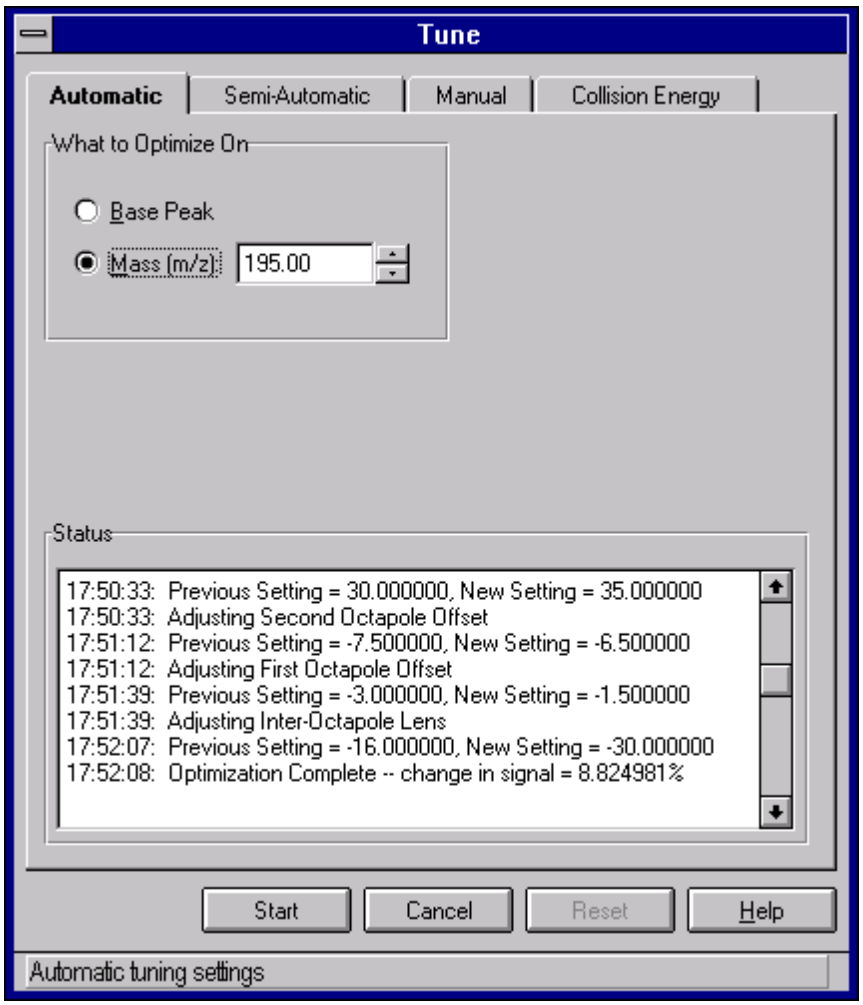


Figure 3-10. Tune dialog box, showing the proper settings for automatic tuning using the caffeine mass peak at *m/z* 195

Your Tune Plus window should now look like the one shown in Figure 3-11.

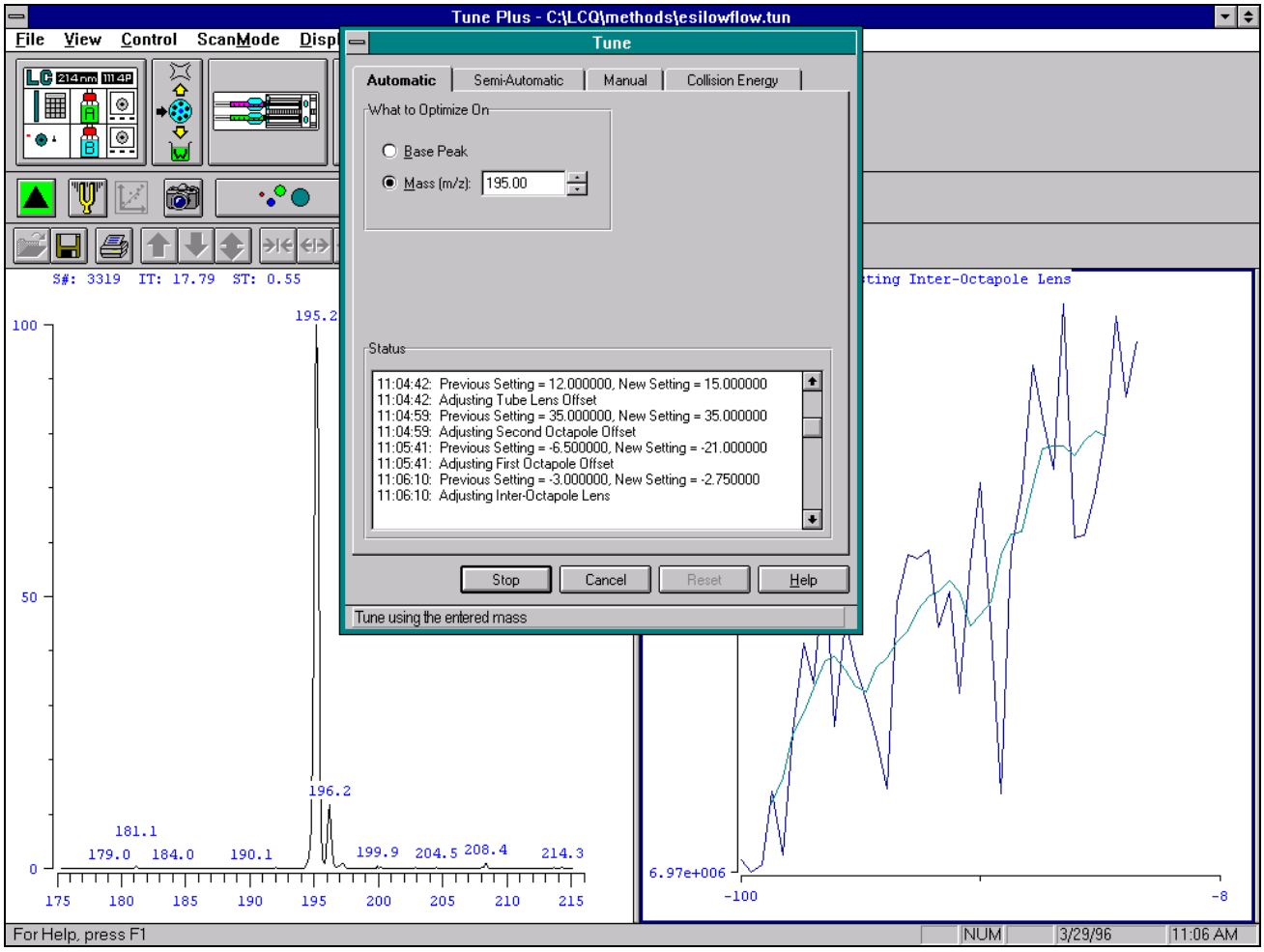
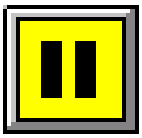


Figure 3-11. Tune Plus window, showing the results of a typical automatic tune procedure

You have successfully calibrated and tuned your MS detector. Go on to the next topic **Preparing for the MS Detector for Normal Operation**.

3.8 Preparing the MS Detector for Normal Operation

Use the following procedure to prepare the MS detector for normal operation. This procedure describes how to clean your MS detector in preparation for running analytical samples.



1. Put the MS detector in Standby, as follows:
   Click on the On/Standby button. When the MS detector is in Standby, LCQ turns Off the sheath gas, auxiliary gas, and ESI high voltage. The MS detector stops scanning, and LCQ freezes the displays for the Spectrum and Graph view.

2. Turn Off the syringe pump, to stop the flow of tuning solution into the ion source, as follows:
   Press the Start/Stop switch on the syringe pump located on the door of the MS detector. When the pump is Off, the light above the switch goes out.

3. Remove the syringe from the syringe holder as follows (See Figure 3-4):

   a. Pull up and rotate the blue syringe-holder finger to free the syringe.

   b. Remove the syringe from the holder.

   c. Disconnect the tip of the syringe needle from the Teflon tubing.

   d. Clean the syringe thoroughly with a solution of 5% acetic acid in water.

   e. Repeat step 3d with a solution of 50:50 methanol:water.

4. Loosen completely the two ESI flange retainer bolts.

WARNING. Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is ON could be unsafe. (LCQ automatically turns the MS detector Off when you open the API source, however, it is best to take this added precaution.) .

WARNUNG. Stellen Sie den MS-Detektor auf Bereitschaft (oder AUS), bevor Sie die Atmosphärendruck-Ionisationsquelle (API) öffnen. Luftsauerstoff in der API-Quelle kann bei eingeschaltetem MS-Detektor eine Gefahr darstellen. (Das LCQ schaltet den MS-Detektor automatisch AUS, wenn Sie die API-Quelle öffnen. Es wird jedoch empfohlen, diese zusätzliche Vorsichtsmaßnahme zu ergreifen.)

AVERTISSEMENT. Mettez le détecteur de SM en veilleuse (ou hors tension) avant d'ouvrir la source d'ionisation à la pression atmosphérique. Toute présence d'oxygène dans la source d'ionisation lorsque le détecteur de SM est activé peut comporter des risques. (Bien que LCQ désactive automatiquement le détecteur de SM lorsque vous ouvrez la source d'ionisation à la pression atmosphérique, il est préférable de prendre cette précaution supplémentaire).

WARNING. AVOID BURNS DO NOT TOUCH THE HEATED CAPILLARY. The heated capillary can attain temperatures above 350 °C. Always be careful not to touch the entrance end of the heated capillary when it is exposed . .

WARNUNG! VERBRENNUNGEN VERMEIDEN! BEHEIZTE KAPILLAREN NICHT BERÜHREN! Die beheizten Kapillaren können Temperaturen von über 350°C erreichen. Das Eingangsende der beheizten Kapillaren niemals berühren, wenn es offen liegt.

AVERTISSEMENT. EVITER LES BRULURES. NE PAS TOUCHER AU CAPILLAIRE CHAUFFE. Le capillaire chauffé peut atteindre une température supérieure à 350 °C. Eviter toujours soigneusement de toucher l'extrémité d'entrée du capillaire chauffé lorsqu'elle est exposée.

5. Slide the ESI flange out, away from the MS detector chassis, on the slide rails.

6. Being careful not to touch the heated capillary with your hand, place a small septum over the entrance end of the heated capillary to seal the vacuum chamber of the MS detector.

7. Flush the sample transfer line, sample tube, and ESI probe thoroughly with a solution of 5% acetic acid in water as follows:

   a. Fill a clean, 250 µL Unimetrics syringe with a solution of 5% acetic acid in water (or with another appropriate solvent).

Note. The solvent that you use to flush the sample transfer line, sample tube, and ESI probe assembly depends on the solvent system you use to dissolve your samples. For example, if you are using a buffered solution of a high concentration, an acidic solution is appropriate.

   b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the free end of the Teflon tube.

   c. Flush the sample transfer line, sample tube, and ESI probe with the solution by slowly depressing the syringe

plunger. Visually check that the solution is exiting the tip of the ESI probe on the inside of the probe assembly. Use a lint-free paper to gently remove the excess solution as it exits the probe.

d. Remove the needle of the syringe from the Teflon tube.

2. Repeat step 7 with a solution of 50:50 methanol:water.

WARNING. AVOID BURNS DO NOT TOUCH THE HEATED CAPILLARY. The heated capillary can attain temperatures above 350 °C. Always be careful not to touch the entrance end of the heated capillary when it is exposed.

WARNUNG! VERBRENNUNGEN VERMEIDEN! BEHEIZTE KAPILLAREN NICHT BERÜHREN! Die beheizten Kapillaren können Temperaturen von über 350°C erreichen. Das Eingangsende der beheizten Kapillaren niemals berühren, wenn es offen liegt.

AVERTISSEMENT. EVITER LES BRULURES. NE PAS TOUCHER AU CAPILLAIRE CHAUFFE. Le capillaire chauffé peut atteindre une température supérieure à 350 °C. Eviter toujours soigneusement de toucher l'extrémité d'entrée du capillaire chauffé lorsqu'elle est exposée.

9. Being careful not to touch the heated capillary with your hand, remove the septum from the entrance end of the heated capillary.

10. Slide the ESI flange forward along the slide rails and into the ion source housing.

11. Secure the ESI flange with the two flange retainer bolts.

Once your LCQ MS detector has successfully passed the calibration procedure and tuning procedure and you have cleaned it, the MS detector is now ready to perform experiments in all scan modes and scan powers. Go to the next chapter: **LC/MS Operation in the ESI Mode**.

Contents

List of Illustrations

List of Tables

ANALYZING SAMPLES IN THE LC/ESI/MS MODE

This chapter contains information on analyzing samples with your LCQ MS detector in the LC/ESI/MS mode. The major topics are as follows:

- Analyzing a sample by flow injection analysis in the LC/ESI/MS mode
- Opening a Raw File in the Explore Window
- Guidelines for LC/ESI/MS operation

Note. Before you begin any of the procedures in this chapter, ensure that you have set up, calibrated, and tuned your MS detector as is described in the chapter: **Calibrating and Tuning in the ESI/MS Mode**.

3.1 Analyzing a Sample by Flow Injection Analysis in the LC/ESI/MS Mode

Flow injection analysis (also called loop injection analysis) is a technique in which you introduce a sample directly into the ESI source with liquid chromatography (LC) pumps. No chromatographic separation occurs. You load the sample solution into a sample loop, and then flush it into the ESI source with a solvent from an LC.

This topic provides instructions for setting up and using your MS detector for flow injection analysis in the LC/ESI/MS mode. To analyze a sample by flow injection analysis, you do the following:

- Open the Tune Plus window.
- Set up the MS detector for LC/ESI/MS operation.
- Set up the inlet, in this case an LC.
- Fine tune and optimize for LC/ESI/MS operation.
- Acquire data in the SIM scan mode.

3.1.1 Opening the Tune Plus Window

Use the following procedure to open the Tune Plus window.

1. Open the LCQ data system, as follows:
   Double-click on the LCQ Navigator icon in the LCQ program group in the Program Manager.

1. Open the Tune Plus window, as follows:
   Click on the Tune Plus button in the Navigator toolbar.

2. Ensure that LCQ displays a Tune Plus window similar to the one shown in Figure 3-1.

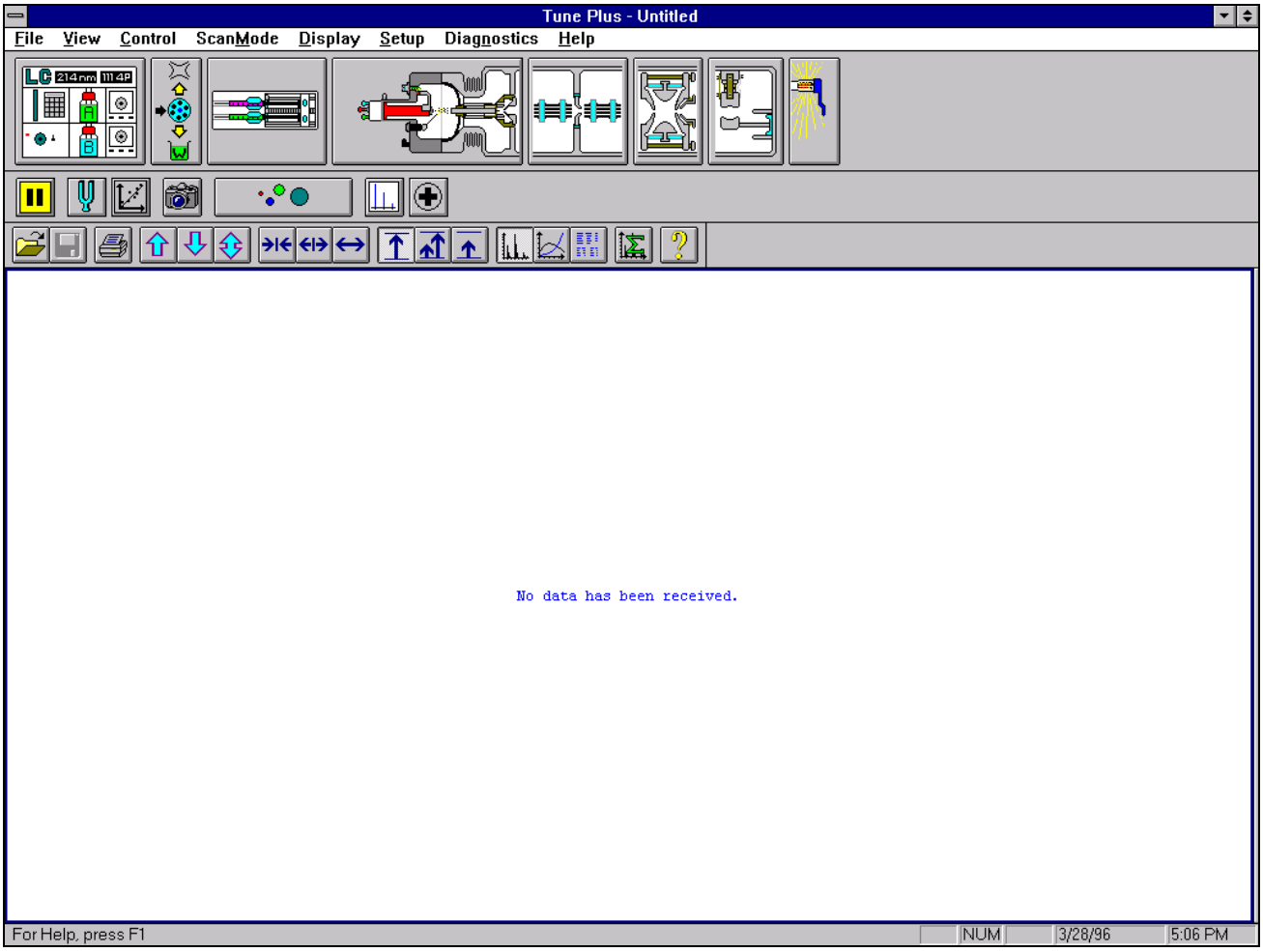
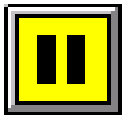


Figure 3-1. Tune Plus window, showing MS detector in standby mode

3.1.2 Setting Up the MS Detector for LC/ESI/MS Operation

Use the following procedure to set up the MS detector for LC/ESI/MS operation.

> **Note**. The following procedures assume that you are familiar with your LCQ instrument and the Tune Plus window. If you need assistance, refer to the **LCQ Software Manual**, **LCQ MS Detector Hardware Manual**, and LCQ online Help.



1. In Tune Plus, take the MS detector out of Standby mode and turn it On, as follows:
   Click on the On/Standby button in the Instrument Control toolbar. The MS detector begins scanning, LCQ applies high voltage to the ESI probe, and LCQ shows a real-time display in the Spectrum view.

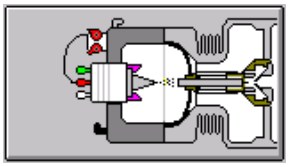
> **Note.** Your LCQ data system contains customized Tune Methods for a variety of applications, including those for ESI high-flow, ESI low-flow, APCI high-flow, and APCI low-flow LC/MS experiments. These Tune Methods contain the optimum ESI or APCI source parameters for their respective applications. For instance, in this procedure you use the ESI source with a comparatively high LC flow rate (approximately 200 µL min^{-1}). Therefore, you are asked to select the *esihighflow.tun* Tune Method. By downloading the *esihighflow.tun* Tune Method in the Tune Plus window, the optimum ESI source parameters are automatically set. For most applications this eliminates the need for further tuning and optimizing of your LCQ MS detector.

2. Open the *esihighflow.tun* Tune Method, the Tune Method for high-flow ESI operation, as follows:

   a. Display the Open dialog box, as follows:
      Choose **File | Open**.

   b. Select the file *esihighflow.tun* in the directory *C:\LCQ\Methods*, as follows:
      Scroll down in the File Name combo box until you see *esihighflow.tun*. Then, click on the file name.

c. Open the file, and close the dialog box, as follows:
Click on **OK**. LCQ downloads the Tune Method parameters to the MS detector.



3. Verify that LCQ opened the Tune Method, as follows:

a. Open the ESI Source dialog box, as follows:
Click on the ESI Source button in the Instrument Setup toolbar. See Figure 3-2.

b. Verify that the settings in your dialog box are similar to those shown in Figure 3-2.

c. Close the dialog box, as follows:
Click on **OK**.

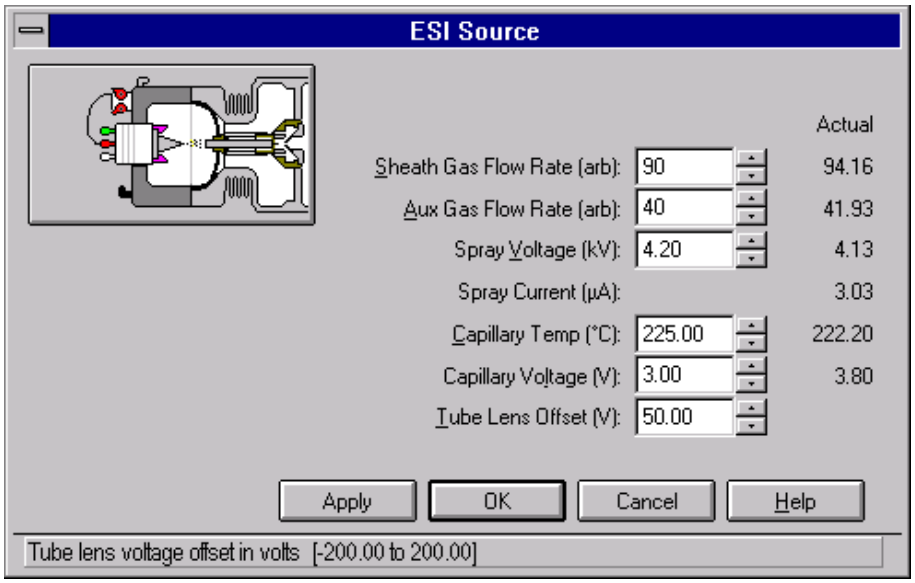
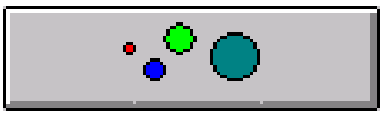


Figure 3-2. ESI Source dialog box, showing proper settings for a typical high flow experiment



4. Define the scan parameters for calibrating and tuning, as follows:

   a. Open the Define Scan dialog box, as follows:
   Click on the Define Scan button in the Instrument Control toolbar. See Figure 3-3.

   b. Select the MS scan power in the Scan Description group box, as follows:
   Click on the Scan Power: MS option button. Note that LCQ sets the MS^n power to *1*.

   c. Select the SIM (selected ion monitoring) scan mode, as follows:
   Click on the Scan Mode: SIM option button. Note that LCQ sets the Total Scan Ranges to *1*.

   d. Set the total number of microscans to 3 in the Scan Time group box, as follows:
   Double-click in the Total Microscans spin box, then type **3**.

   e. For this example, leave the maximum injection time set to its default value of 200.00 ms.

   f. Select the center/width input method in the Input Method group box, as follows:
   Click on the Center / Width option button. Note that LCQ displays the Center Mass and Width text boxes in the Scan Ranges group box.

   g. Select source CID Off in the Source CID group box, as follows:
   Click on the Off option button.

   h. Set the center mass for the scan range to *m/z* 609.20 in the Scan Ranges group box, as follows:
   Double-click in the Center Mass text box, then type **609.2**.

   i. Set the width of the scan range to *m/z* 2, as follows:
   Double-click in the Width text box, then type **2**.

   j. Save the MS detector scan parameters and close the Define Scan dialog box, as follows:
   Click on **OK**.

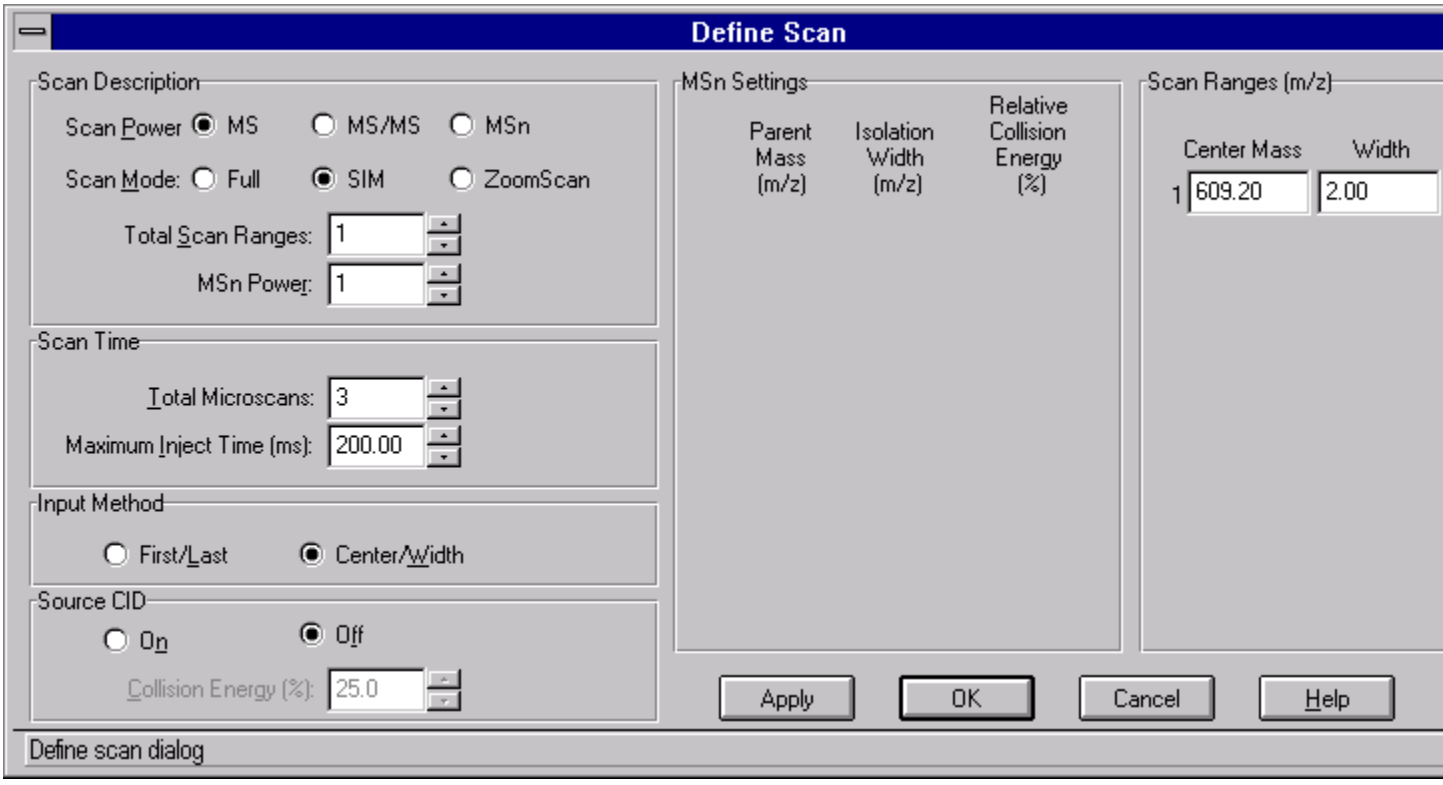
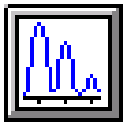


Figure 3-3. Define Scan dialog box, showing typical settings for the SIM scan mode



5. Select the profile scan data type, as follows:
   Click on the Centroid/Profile button in the Instrument Control toolbar to toggle the scan data type to profile. (The picture on the button should be the same as that shown here.)

5. Select the positive ion polarity mode, as follows:
   Click on the Positive/Negative button in the Instrument Control toolbar to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here.)

The Spectrum view of your Tune Plus window should now look like the one shown in Figure 3-4 (on the left side of the Figure).

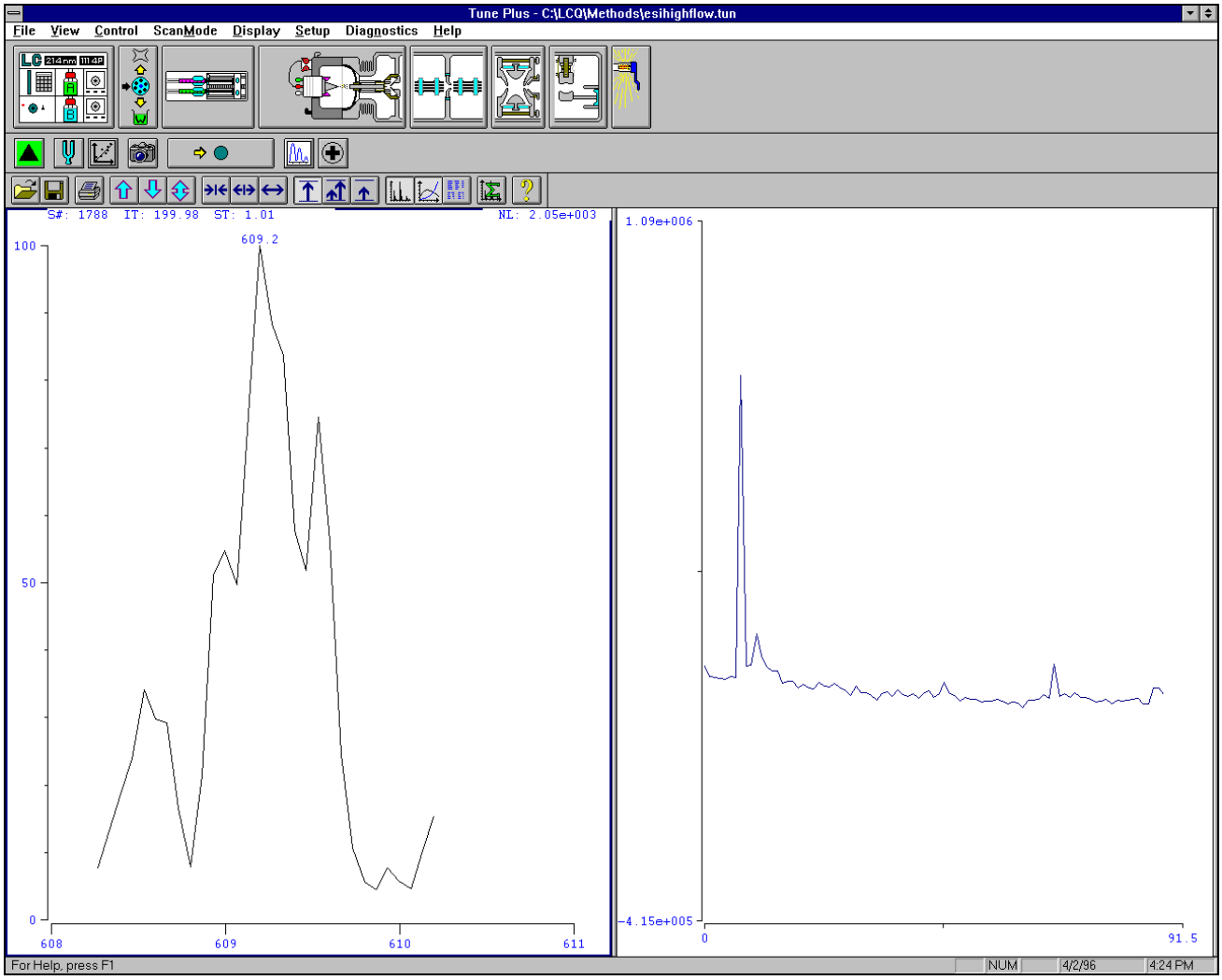


Figure 3-4. Tune Plus window, showing a SIM scan window for the *m/z* 609.2 ion for reserpine in the Spectrum view (on the left side of the display)

You have now completed setting up your MS detector for LC/ESI/MS operation. Go to the next topic: **Setting Up the Inlet: LC**.

3.1.3 Setting Up the Inlet: LC

Use the following procedure to set up the LC for LC/ESI/MS operation. See Figure 3-5 and Figure 3-6.

> **Note**. To cut the PEEK tubing used to connect your LC to the divert/inject valve and the divert/inject valve to the ESI probe, use a PEEK tubing cutter. This ensures that the tubing is cut straight. In addition, make sure your LC fittings, ferrules, and PEEK tubing are installed properly. By using these precautions, you prevent void (dead) volumes. The exclusion of void volumes is critical to microbore LC. Also, void volumes affect the quality of the MS detector signal.

1. Connect your LC to the divert/inject (Valco injector) valve as follows.

   a. Connect one end of a piece of (0.005-in ID) PEEK tubing of appropriate length to the outlet of the solvent delivery system of your LC. Use the proper fitting and ferrule for you LC.

   b. Connect the other of the PEEK tubing to port 2 of the divert/inject valve. Use a Valco bushing (P/N 00110-16008) and ferrule (P/N 00101-18122).

2. Connect the divert/inject valve to the ESI source as follows:

   a. Connect one end of another piece of PEEK tubing (the sample transfer line) of appropriate length to port 3 of the divert/inject valve. Use a Valco bushing and ferrule.

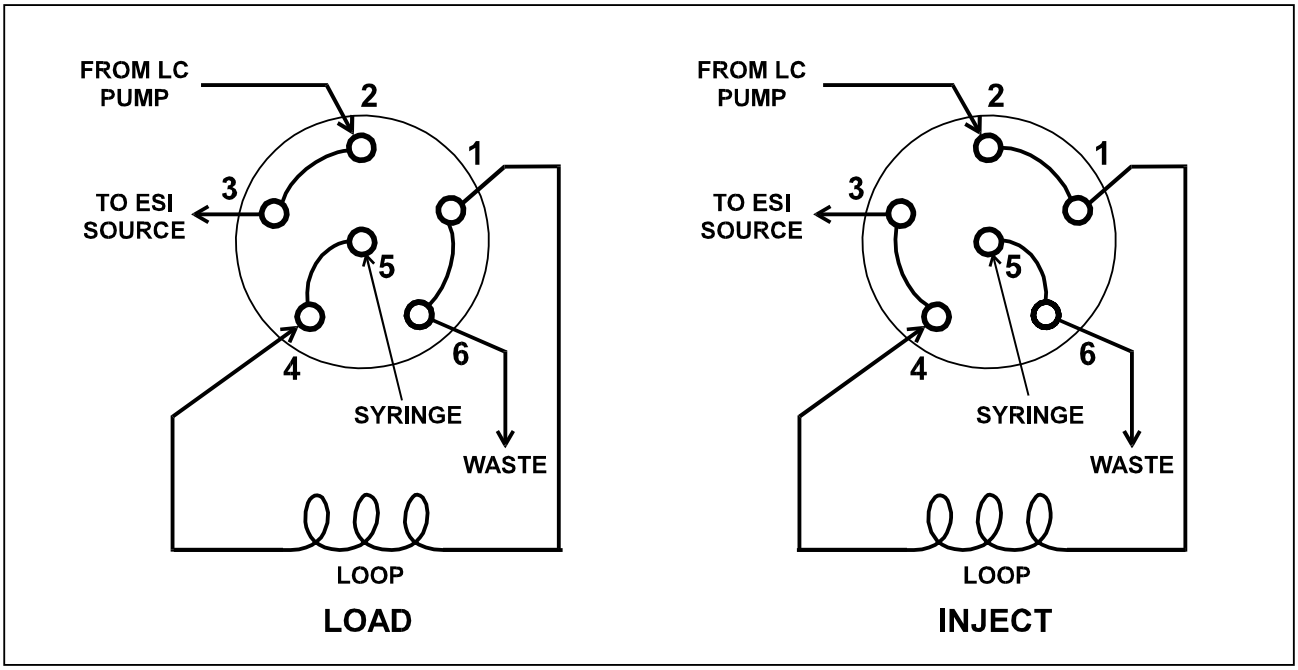


Figure 3-5. Divert/inject (Valco injector) valve, showing the correct set up for flow injection analysis and showing the flow of liquid through the valve in the LOAD and INJECT positions

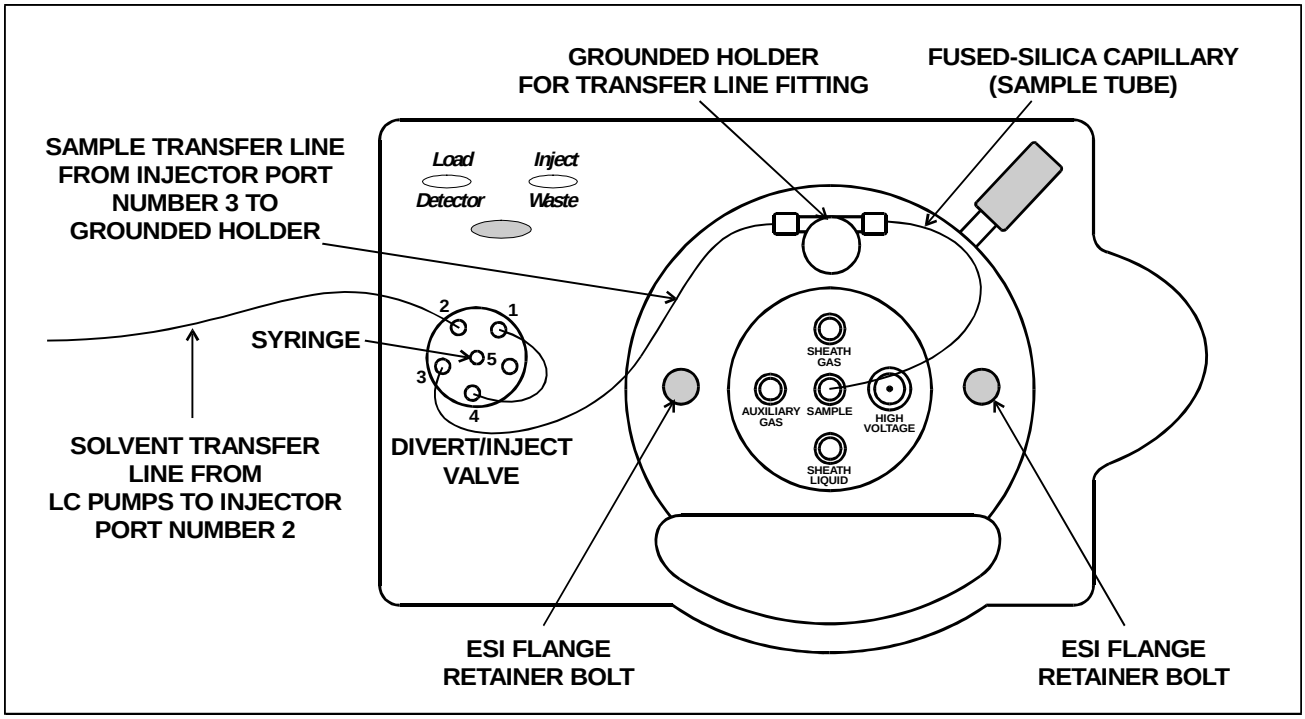


Figure 3-6. MS detector console, showing the proper connections for loop injections

WARNING. Before you begin normal operation each day, ensure that you have sufficient nitrogen for your API source. If you run out of nitrogen, LCQ automatically turns the MS detector Off to prevent the possibility of atmospheric oxygen from entering the ion source. The presence of oxygen in the ion source when the MS detector is ON could be unsafe. (In addition, if LCQ turns Off the MS detector during an analytical run, you could lose data.) .

WARNUNG. Stellen Sie täglich vor Beginn des normalen Betriebs sicher, daß Sie genügend Stickstoff für Ihre Atmosphärendruck-Ionisationsquelle (API) haben. Wenn der Stickstoff nicht ausreicht, schaltet das LCQ den MS-Detektor automatisch auf AUS, um zu verhindern, daß Luftsauerstoff in die Ionenquelle eindringt. Sauerstoff in der Ionenquelle kann bei eingeschaltetem MS-Detektor eine Gefahr darstellen. (Außerdem können biem Abschalten des MS-Detektors während einer Analyse Daten verloren gehen.)

AVERTISSEMENT. Tous les jours avant d'utiliser l'appareil, vérifiez le niveau d'azote de la source d'ionisation à la pression atmosphérique. En effet, si vous tombez à court d'azote, LCQ désactive automatiquement le détecteur de SM pour empêcher que la source d'ions ne soit contaminée par l'oxygène présent dans l'air. Toute présence d'oxygène dans la source d'ions lorsque le détecteur de SM est activé peut comporter des risques. (En outre, si LCQ désactivait le détecteur de SM pendant une série d'analyses, vous pourriez perdre des données).

WARNING: AVOID ELECTRICAL SHOCKS. ENSURE THAT YOUR SAMPLE TRANSFER LINE IS PROPERLY ATTACHED TO THE GROUNDED FITTING HOLDER ON THE ESI FLANGE. Your ESI probe is designed to electrospray highly conductive solutions, however, you must isolate high-voltage current by properly grounding the sample transfer line and sample tube.

WARNUNG! ELEKTROSCHOCK VERMEIDEN! KONTROLLIEREN, DASS DAS PROBENTRANSFERKABEL ENTSPRECHEND MIT DEM GEERDETEN FASSUNGSHALTER DES ESI-FLANSCHES VERBUNDEN IST! Die ESI-Sonde dient dem elektronischen Versprühen hochleitender Lösungsmittel. Hochspannung muß aber durch entsprechendes Erden des Probentransferkabels und der Probenleitung isoliert werden.

AVERTISSEMENT. EVITER LES CHOCS ELECTRIQUES. S'ASSURER QUE LA LIGNE DE TRANSFERT D'ECHANTILLONS EST CORRECTEMENT FIXEE A LA RETENUE DE RACCORD MISE A LA TERRE, SITUEE SUR LA BRIDE ESI. La sonde ESI est conçue pour l'électrovaporisation de solutions à haute conductivité ; cependant, le courant haute tension doit être isolé par une mise à la terre correcte de la ligne de transfert et du tube d'échantillon.

b. Connect the other end of the PEEK tubing to the 1/16-in. zero dead volume union and place it in the grounded fitting holder on the ESI flange. Use an Upchurch Fingertight fitting (P/N 00101-18195) and ferrule (P/N 00101-18196). **BE SURE TO SECURE THE FITTING PROPERLY IN THE GROUNDED FITTING HOLDER**. See Figure 3-6.

2. Ensure your divert/inject valve is fitted with a 5 µL loop between port 1 and port 4.

3. Fill the a solvent reservoir of your LC with a solution 1% acetic acid in 50:50 methanol:water. [Or fill two solvent reservoirs, one reservoir (A) for 1% acetic acid in water and a second reservoir (B) for 1% acetic acid in methanol. Then, meter the reservoirs at 50% A and 50% B.]

4. Degas the solvent(s) either by sparging or by vacuum degassing.

5. Turn on the LC pumps to flush solvent (1% acetic acid in 50:50 methanol:water) into the ESI source at a flow rate of approximately 200 µL min^{-1}.

Note. You can turn your LC pump on now or wait to turn it on until shortly before you inject a sample.

Note. Ensure that the fused silica sample tube is connected properly in the ESI probe. Refer to the topic **Setting up the ESI Source for LC/ESI/MS Operation** for a procedure for installing the sample tube.

Your LC is now set up for LC/ESI/MS operation. Go the next topic: **Fine Tuning and Optimizing for LC/ESI/MS Operation**.

3.1.4 Fine Tuning and Optimizing the MS Detector for LC/ESI/MS Operation

The customized Tune Methods contained in your LCQ data system are optimized for a wide range of applications, and they can often be used without further tuning of your MS detector. However, for certain applications you may need to fine tune and optimize several MS detector parameters. For instance, the most important parameters that interact with the ESI interface and signal quality, are the tube lens offset voltage, heated capillary temperature, and capillary voltage. The settings for these parameters depend on the solvent flow rate and target analyte composition. In general, you should fine tune your MS detector whenever you change the solvent flow rate conditions of your

particular application. You use the ESI high-flow Tune Method, *esihighflow.tun,* as a starting point, and then further optimize the MS detector.

Use the following procedure to optimize the tube lens offset voltage and heated capillary temperature for LC/MS solvent flow conditions. You can use the same procedure to optimize the capillary voltage and the sheath gas for the flow rate of your particular application. This procedure allows you to maximize the ion transmission of a particular target analyte at the relatively higher-flow conditions of a typical LC/MS experiment. It is recommended that you begin fine tuning and optimizing after you have successfully passed an automatic calibration procedure and an automatic tuning procedure with the tuning solution infused at 3 $\mu L\ min^{-1}$.

In this procedure, you optimize the voltage applied to the tube lens and heated capillary, and the temperature of the heated capillary by monitoring loop injections of reserpine. You can follow the same procedure using your analyte of interest. To optimize the tube lens offset voltage, capillary voltage, and heated capillary temperature, do the following:

1. Prepare a solution of 10 pg μL^{-1} of reserpine. Refer to **Appendix A: Sample Formulations** for instructions on how to prepare the solution.

2. Turn on the LC pumps to flush solvent (1% acetic acid in 50:50 methanol:water) into the ESI source at a flow rate of approximately 200 $\mu L\ min^{-1}$.

3. If you have not already done so, open the *esihighflow.tun* Tune Method, as follows:

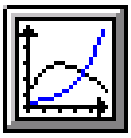
   a. Display the Open dialog box, as follows:
   Choose **File | Open**.

   b. Select the file *esihighflow.tun* in the directory *C:\LCQ\Methods*, as follows:
   Scroll down in the File Name combo box until you see *esihighflow.tun*. Then, click on the file name.

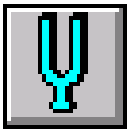
   c. Open the file, and close the dialog box, as follows:
   Click on **OK**. LCQ downloads the Tune Method parameters to the MS detector, and the title bar in the Tune Plus window should read as follows:

   Tune Plus - C:\LCQ\Methods\esihighflow.tun

4. Monitor in the Graph view a plot of intensity versus time for the mass peak that you want to optimize, as follows:



a. If necessary, open the Graph view, as follows
Click on the Display Graph View button in the Data Display toolbar.



a. Open the Tune dialog box - Manual tab, as follows:
First, click on the Tune button in the Instrument Control toolbar. Then, click on the Manual tab. See Figure 3-7.

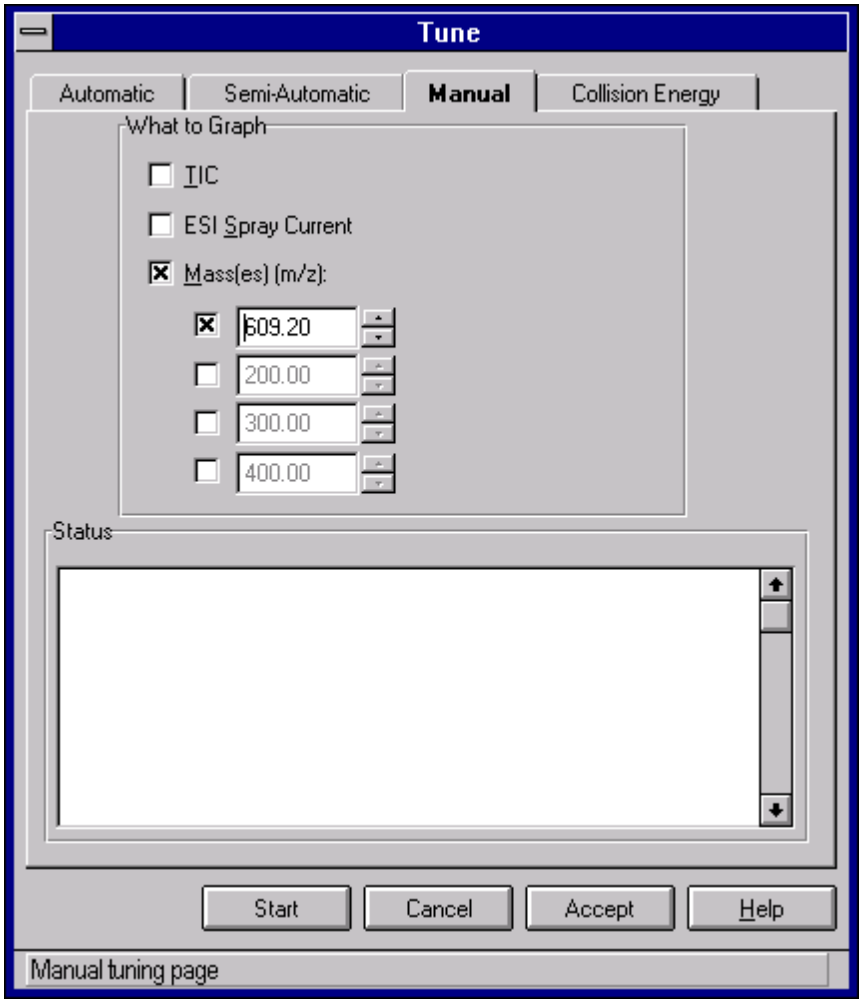


Figure 3-7. Tune dialog box, showing the Manual tab with the proper settings for graphing the *m/z* 609.2 ion current of reserpine

c. Specify the peak at *m/z* 609.2 as the peak to graph, as follows:
First, select [x] the Mass(es) check box. Next, select [x] the first Mass(es) spin box. Finally, double-click in the first Mass(es) spin box, then type **609.2**.

d. Click on **Start** to begin graphing the intensity of the *m/z* 609.2 ion current.

2. Load the reserpine solution, or your particular analyte, into the sample loop as follows:

a. On the MS detector front panel, ensure the green light below the word *Load* is illuminated (which indicates the divert/inject valve is in the Load position). If it is not, press the blue Load/Inject button to switch the valve to the Load position. See Figure 3-6.

b. Load a Unimetrics syringe (P/N 00301-19012) with a 10 pg μL^{-1} reserpine solution, or a solution of your particular analyte.

> **Note.** The Unimetrics syringe needle has a square tip. If you use a different kind of syringe, make sure that its needle also has a square tip.

c. Inject approximately 10 µL of the testosterone solution into the divert/inject valve to overfill the injector loop. The excess solution goes to the waste container.

> **Note**. By overfilling the 5 µL injector loop in step 5c, you ensure that you are reliably delivering 5 µL of the sample solution to the ESI source.

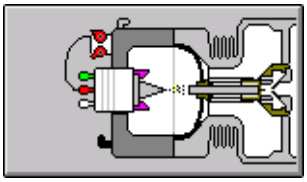
6. Inject the solution into the ESI source, as follows:
Press the blue Load/Inject button. Solvent now flows through the loop, and the reserpine solution is pushed into the ESI source.

7. Return the divert/inject valve to the Load position, as follows:
Press the blue Load/Inject button.

8. Repeat steps 5 through 7.

   LCQ displays the trace of the loop injections in the Graph view.

9. Vary the tube lens offset voltage and observe how the change affects the intensity of the ion current in the Graph view, as follows:



   a. Open the ESI Source dialog box, as follows:
   Click on the ESI Source button in the Instrument Setup toolbar. (See Figure 3-2).

   b. Increase the tube lens offset voltage by 5 V, as follows:
   Double-click on the Tube Lens Offset spin box, then type **55** (to add 5 V to the default value of 50 V).

   c. Repeat step 8.

   Observe how the increase in tube lens offset voltage affects the maximum intensity of the loop injections as compared to the initial tube lens offset voltage.

9. Perform the process in step 9 repeatedly until you find the offset voltage that provides the maximum ion current intensity. See Figure 3-8.

Your Tune Plus window should now look similar to the one shown in Figure 3-8.

You can repeat the above procedure to optimize the temperature of the heated capillary.

Refer to Table 3-1 at the end of this chapter for additional guidelines for optimizing the MS detector for LC/ESI/MS analysis.

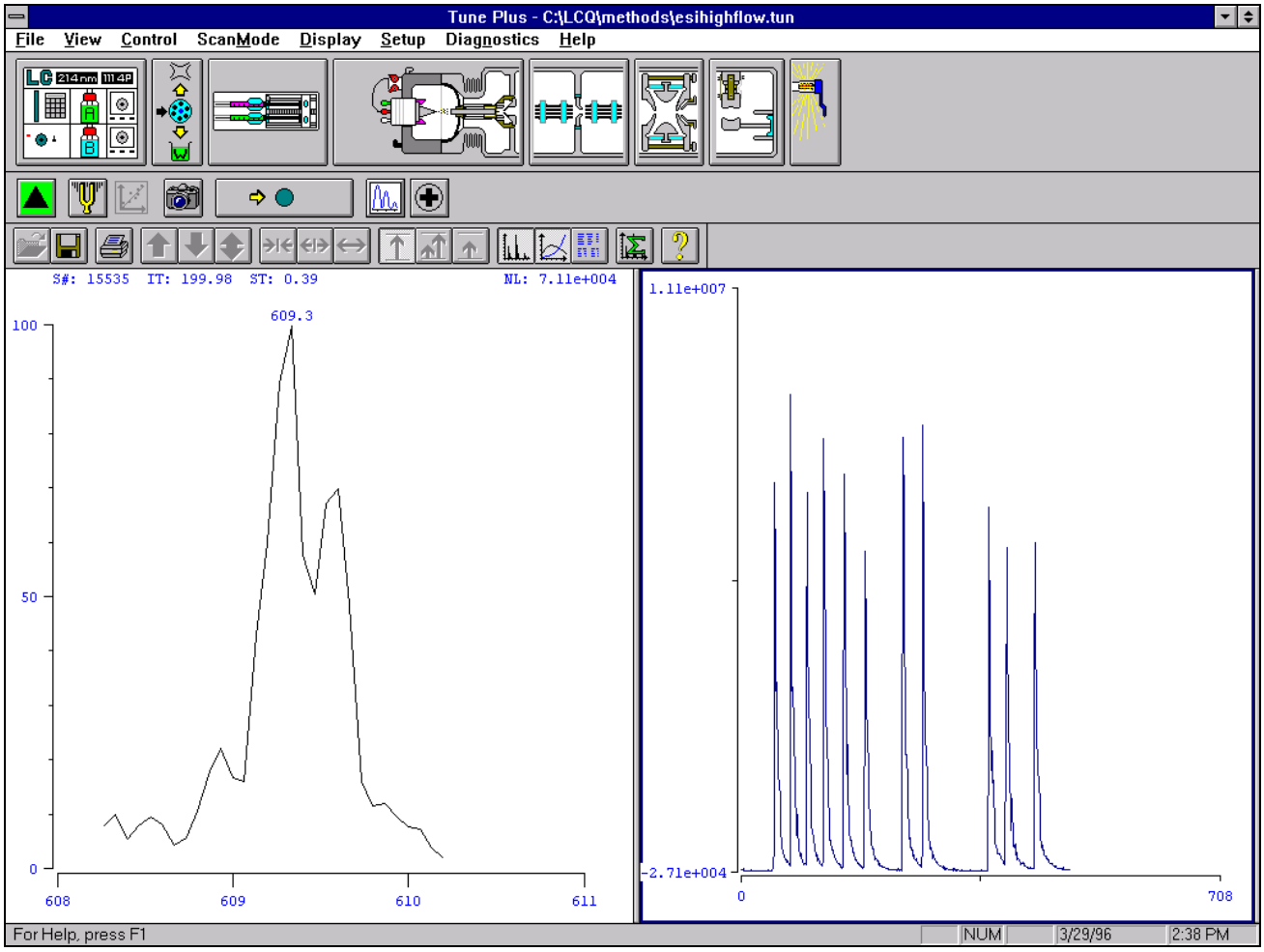


Figure 3-8. Tune Plus window, showing the effect on signal intensities for changes in tube lens offset voltage for loop injections of reserpine

3.1.5 Saving a Tune Method

You can save the tune parameters you just set by saving them in a tune method. You name the tune method for the target analyte you used to optimize the MS detector. The tune settings stored in a tune method are specific to the particular target analyte and solvent flow rate. If you use the same solvent flow rate, the tune method can be recalled for future analyses of a particular target analyte with no further optimization of the MS detector. However, if you change the solvent flow rate, you may need to reoptimize the MS detector using the procedure outlined in the previous topic: **Fine Tuning and Optimizing the MS Detector for LC/ESI/MS Operation**.

Save the MS detector tune parameters in a tune method, as follows:

1. Display the Save As dialog box, as follows:
   Choose **File | Save As**.

2. Select the *C:\LCQ\Methods directory*, as follows:
   First, double-click on the *C:* directory. Next, Scroll down in the Directories combo box until you see LCQ. Then, double-click on the LCQ directory. Then, scroll down in the Directories combo box until you see Methods. Finally, double-click on the Methods directory.

3. Name the tune method for the analyte you optimized the MS detector for. If you optimized the MS detector for reserpine, specify the name of the tune method as *reserpine.tun* in the *C:\LCQ\Methods* directory, as follows:
   Double-click in the File Name combo box, then type **reserpine.tun**.

4. Save the tune method and close the dialog box, as follows:
   Click on **OK**.

3.1.6 Acquiring Data in the SIM Scan Mode

This topic provides a procedure for you to set up for data acquisition in the single ion monitoring (SIM) mode. LCQ automatically saves the data you acquire on your hard disk.

Use the following procedure to acquire a data file for several loop injections of reserpine, or your particular analyte:

1. Open the Acquire Data dialog box, as follows:
   Click on the Acquire Data button in the Instrument Control toolbar. See Figure 3-9.

2. Define acquisition parameters as follows:

   a. Specify a filename, as follows:
   Double-click in the File Name text box, then type **esisimres.**

   b. Specify the sample identity, as follows:
   Double-click in the Sample Name text box, then type **reserpine**. If you are not using reserpine, type the name of your particular analyte.

   c. Specify a comment, such as the scan mode, ionization method, sample amount, and method of sample introduction, as follows:
   Double-click in the Comment text box, then type **SIM, ESI, 50 pg loop inj**. LCQ includes the comment information in hard copies of the data.

   d. Select to acquire data continuously (until you stop the acquisition) in the Acquire Time group box, as follows:
   Select the Continuously option button (formerly called the Forever option button).

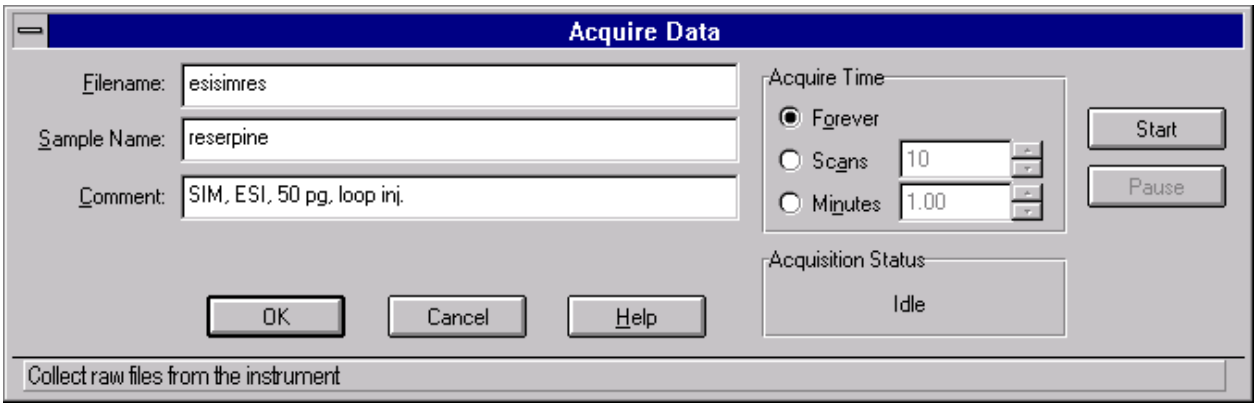


Figure 3-9. Acquire Data dialog box, showing typical sample information for data acquisition

3. Leave the Acquire Data dialog box open during data acquisition, but move it to a corner of the Tune Plus window to get it out of the way.

4. With the divert/inject valve in the Load position, overfill the injector loop with a 10 pg μL^{-1} reserpine solution, or a solution of your analyte of interest.

5. Click on **Start** in the Acquire Data dialog box to begin acquiring data to the file *esisimres.raw*. The Acquisition Status group box displays the following message:

   Acquisition in Progress

6. Inject the reserpine solution into the ESI source.

7. Observe the reserpine peak (*m/z* 609.2) or your paricular analyte of interest come up in the Spectrum view, and observe the chromatogram peak appear in the Graph view.

8. Repeat steps 4 and 6 several times to obtain several consecutive loop injections of reserpine in the SIM scan mode.

9. Click on **Stop** in the Acquire Data dialog box to end data acquisition.

10. Close the Acquire Data dialog box, as follows:
    Click on **Cancel**.

To review the mass spectrum and chromatogram in the raw file you just collected go to the next topic: **Opening a Raw File in the Explore Window**.

3.2 Opening a Raw File in the Explore Window

Use the following procedure to open a raw file in the Explore window:

1. Return to the Navigator window, as follows:
   First, hold down the **<Alt>** key. Next, press the **<Tab>** key until the Navigator button appears. Finally, let go of the **<Alt>** key.

1. Open the Explore window, as follows:
   Click on the Explore button in the Navigator toolbar.

2. Open the *esisimres.raw* raw file, as follows:

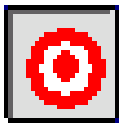
   a. Display the Open Layout or Open Raw File dialog box, as follows:
      Choose **File | Open**.

   b. Select the file *esisimres.raw* in the directory *C:\LCQ\Data*, as follows:
      Scroll down in the File Name combo box until you see *esisimres.raw*. Then, click on the file name.

   c. Open the file, and close the dialog box, as follows:
      Click on **OK**. LCQ opens the raw file in the active cell of the Explore window.

> **Note**. The active cell is the cell containing the highlighted target button, and has a dark blue border around it.

4. Insert a duplicate cell to the right of the active window, as follows:
   Choose **Grid | Insert Cells | Right**.



4. Activate the right cell, as follows:
   Click on the Target button in the right cell.

5. Display the spectrum in the active cell, as follows:
   Choose **Display | Spectrum**.

7. Display the mass spectrum of reserpine or your paricular target analyte in the Target active cell, as follows:
First, move the cursor to the left, inactive cell (chromatogram display). Then, click on the peak maximum.

Your Explore window should now look similar to the one shown in Figure 3-10.

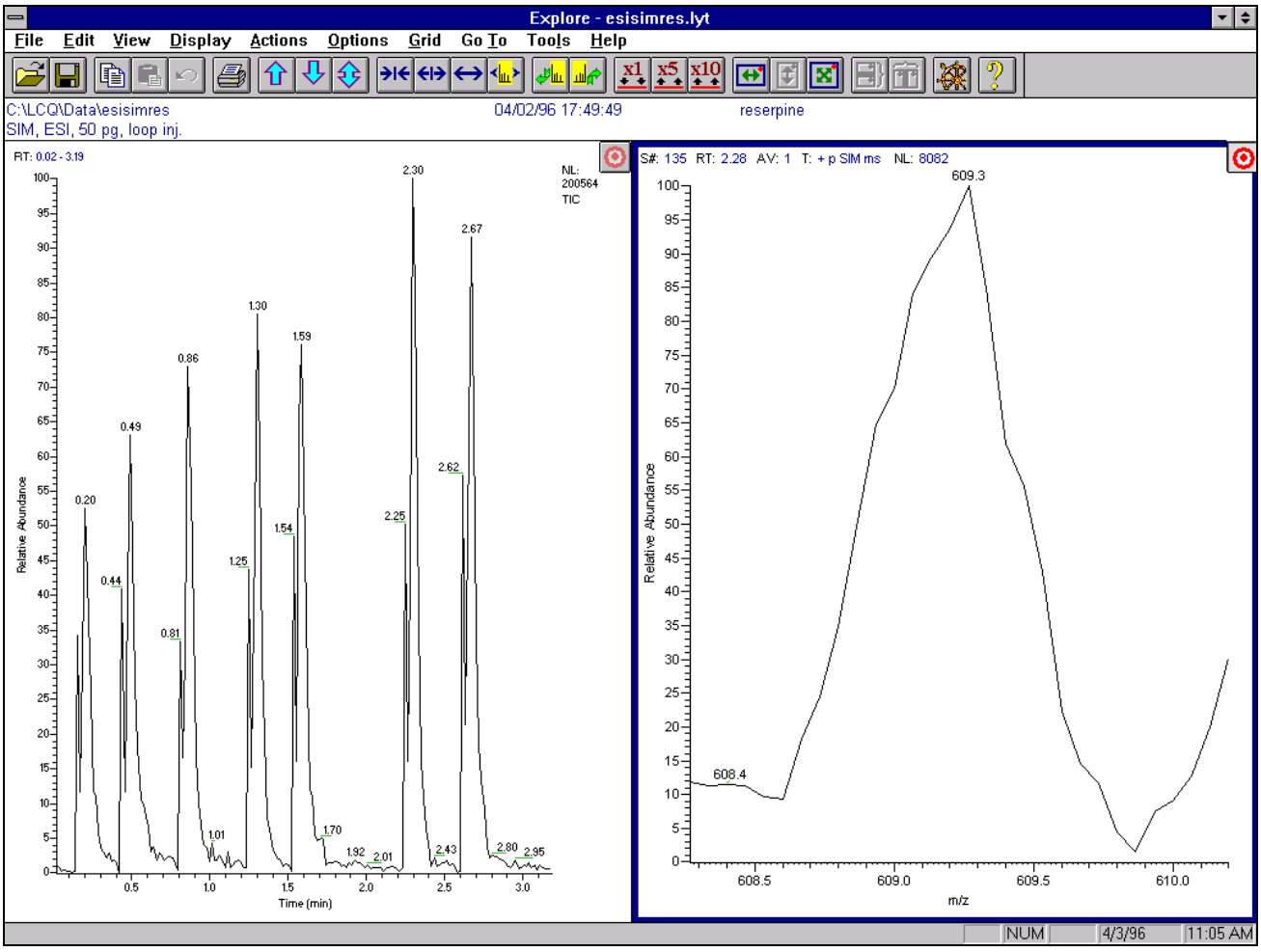


Figure 3-10. Explore window, showing the total ion chromatogram for loop injections of reserpine (left) and the SIM mass spectrum of reserpine (right)

3.3 Guidelines for LC/ESI/MS Operation

Table 3-1 provides guidelines for heated capillary temperatures and gas flow rates for various solvent flow rates.

Table 3-1. Guidelines for LC/ESI/MS Operation

Application	Typical Capillary Temperature	Sheath Gas	Auxiliary Gas
Infusion or LC at flow rates of 10 µL min^{-1}	150 to 200 °C	Required	Not required
LC at flow rates of 50 µL min^{-1}	200 °C	Required	Not required but may help depending on conditions
LC at flow rates of 200 µL min^{-1}	250 °C	Required	Usually helps to reduce solvent background ions
LC at flow rates of 1 mL min^{-1}	250 °C	Required	Required

Contents

List of Illustrations

TUNING IN THE APCI/MS MODE

This chapter provides information on how to tune the LCQ MS detector in the APCI/MS (or APCI/MS/MS) mode. It is not necessary to recalibrate the MS detector for APCI/MS operation. You can use the calibration settings you obtained from the successful automatic calibration procedure you performed in the ESI/MS mode. For APCI/MS operation you simply open a default tune method located in your *C:\LCQ\Methods* directory, in this case *apcihighflow.tun*. You then optimize the tube lens offset voltage, capillary voltage, and heated capillary temperature for your particular analyte.

To set up for tuning your MS detector in the APCI/MS mode, do the following:

- Open the Tune Plus window.
- Set up the MS detector for APCI/MS operation.
- Set up the inlet, in this case an LC.
- Bake out the APCI source.
- Test the operation of the MS detector in the APCI/MS mode.
- Optimize the tube lens offset voltage and heated capillary temperature.

You set up the MS detector from the Tune Plus window.

You use an LC to flush the sample solution of 50 pg μL^{-1} reserpine in 1% acetic acid in 50:50 methanol:water from a sample loop into the APCI source.

You test the operation of the APCI source by observing loop injections of reserpine in the Tune Plus window.

You optimize the tube lens offset voltage and heated capillary temperature by observing loop injections of reserpine in the Tune Plus window.

4.1 Opening the Tune Plus Window

Use the following procedure to open the Tune Plus window.

1. Open the LCQ data system, as follows:
   Double-click on the LCQ Navigator icon in the LCQ program group in the Program Manager.

1. Open the Tune Plus window, as follows:
   Click on the Tune Plus button in the Navigator toolbar.

2. Ensure that LCQ displays a Tune Plus window similar to the one shown in Figure 4-1.

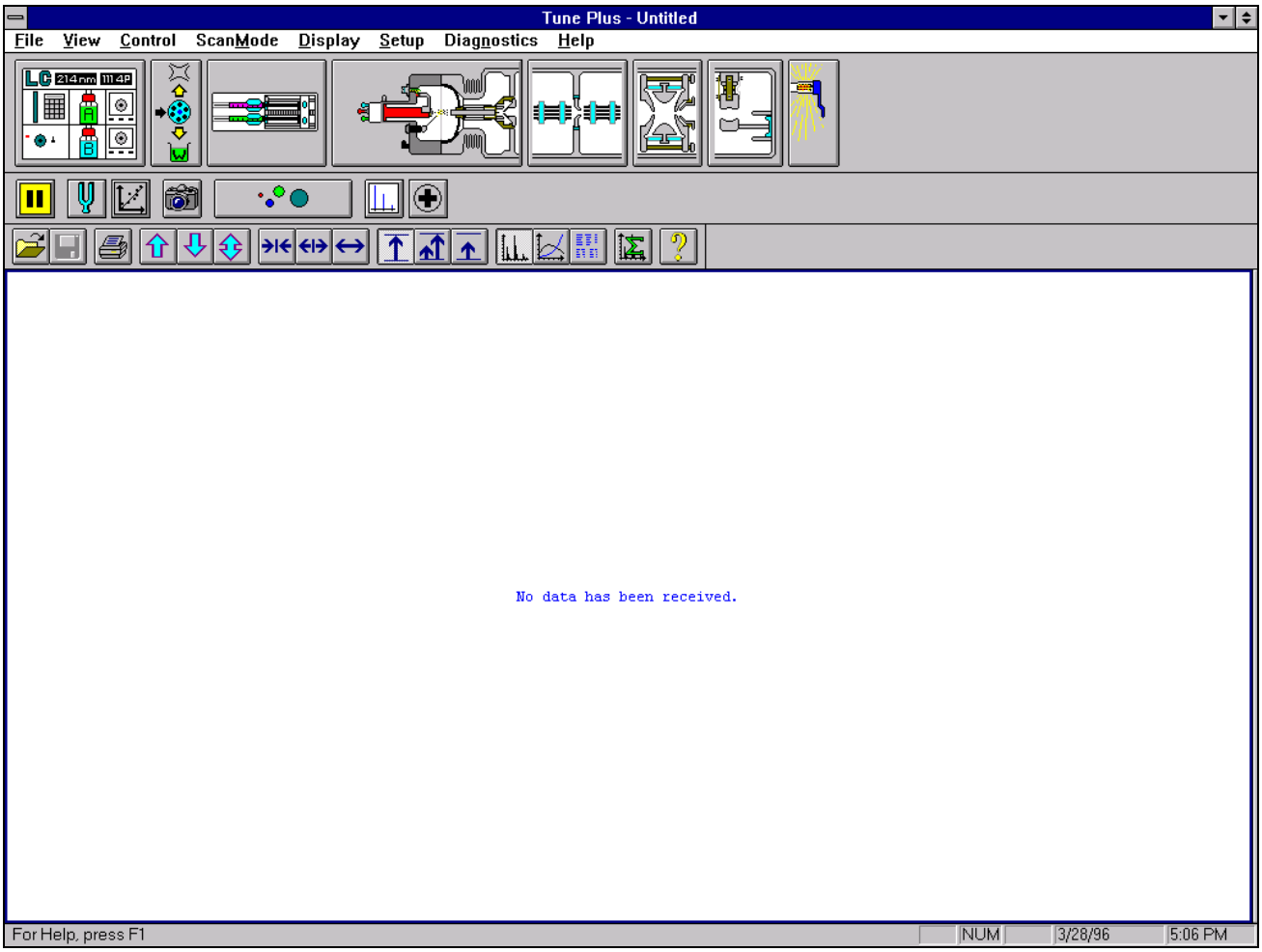
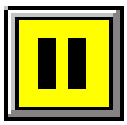


Figure 4-1. Tune Plus window, showing the MS detector in the Standby mode

4.2 Setting Up the MS Detector for APCI/MS Operation

Use the following procedure to set up the MS detector for APCI/MS operation.

> **Note**. The following procedures assume that you are familiar with your LCQ instrument and the Tune Plus window. If you need assistance, refer to: LCQ online Help (or the **LCQ Software Manual**) and/or the **LCQ MS Detector Hardware Manual**.



1. In Tune Plus, take the MS detector out of Standby mode and turn it On, as follows:
   Click on the On/Standby button in the Instrument Control toolbar. The MS detector begins scanning, LCQ applies high voltage to corona needle, and LCQ shows a real-time display in the Spectrum view.

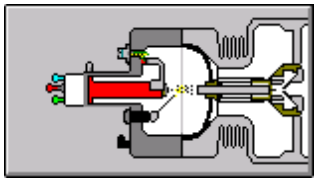
2. Open the *apcihighflow.tun* Tune Method, the Tune Method for high-flow APCI operation, as follows:

   a. Display the Open dialog box, as follows:
      Choose **File | Open**.

   b. Select the file *apcihighflow.tun* in the directory *C:\LCQ\Methods*, as follows:
      Scroll down in the File Name combo box until you see *apcihighflow.tun*. Then, click on the file name.

   c. Open the file, and close the dialog box, as follows:
      Click on **OK**. LCQ downloads the Tune Method parameters to the MS detector.

1. Verify that LCQ opened the Tune Method, as follows:



   a. Open the APCI Source dialog box, as follows:
      Click on the APCI Source button in the Instrument Setup toolbar. See Figure 4-2.

   b. Verify that the settings in your dialog box are similar to those shown in Figure 4-2.

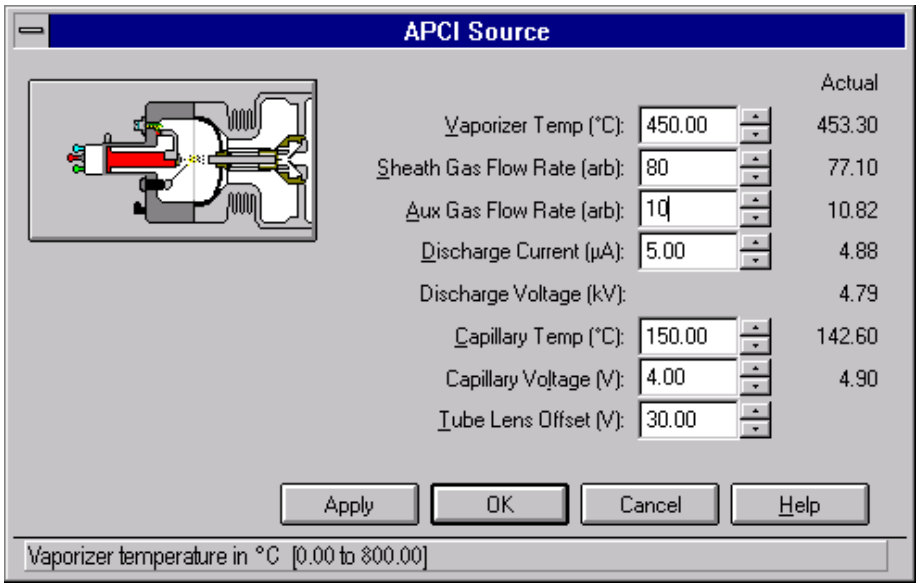
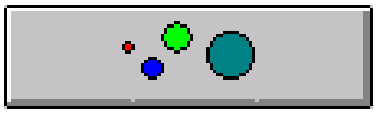


Figure 4-2. APCI Source dialog box, showing the proper settings for a typical high flow experiment

c. Close the dialog box, as follows:
Click on **OK**.



3. Define the scan parameters for tuning the MS detector in the APCI/MS mode, as follows:

a. Open the Define Scan dialog box, as follows:
Click on the Define Scan button in the Instrument Control toolbar. See Figure 4-3.

b. Select the MS scan power in the Scan Description group box, as follows:
Click on the Scan Power: MS option button. Note that LCQ sets the MS^n power to *1*.

c. Select the SIM scan mode, as follows:
Click on the Scan Mode: SIM option button. Note that LCQ sets the Total Scan Ranges to *1*.

d. Set the total number of microscans to 3 in the Scan Time group box, as follows:
Double-click in the Total Microscans spin box, then type **3**.

e. For this example, leave the maximum injection time set to its default value of 200.00 ms.

f. Select the center / width input method in the Input Method group box, as follows:
Click on the Center / Width option button. Note that LCQ displays the Center Mass and Width text boxes in the Scan Ranges group box.

g. Select source CID Off in the Source CID group box, as follows:
Click on the Off option button.

h. Set the center mass for the scan range to *m/z* 609.2 in the Scan Ranges group box, as follows:
Double-click in the Center Mass text box, then type **609.2**.

i. Set the width of the scan range to *m/z* 2, as follows:
Double-click in the Width text box, then type **2**.

j. Save the MS detector scan parameters and close the Define Scan dialog box, as follows:
Click on **OK**.

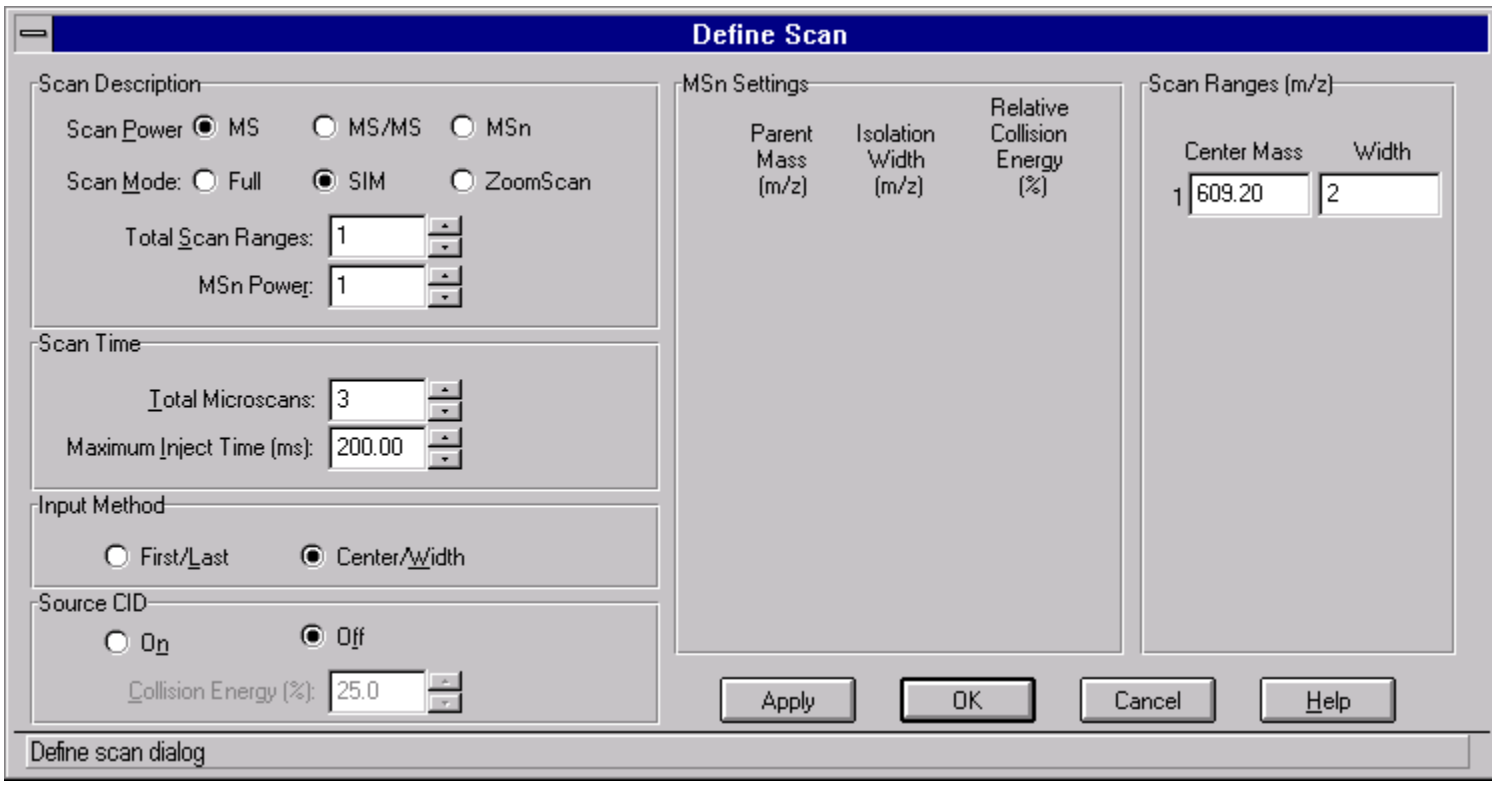
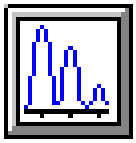
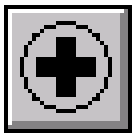


Figure 4-3. Define Scan dialog box, showing typical settings for APCI/MS operation



5. Select the profile scan data type, as follows:
   Click on the Centroid/Profile button in the Instrument Control toolbar to toggle the scan data type to profile. (The picture on the button should be the same as that shown here).



5. Select the positive ion polarity mode, as follows:
   Click on the Positive/Negative button in the Instrument Control toolbar to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here).

You have now completed setting up your MS detector for APCI/MS operation. Go to the next topic: **Setting Up the Inlet: LC**.

4.3 Setting Up the Inlet: LC

Use the following procedure to set up the LC for LC/APCI/MS operation. See Figure 4-4.

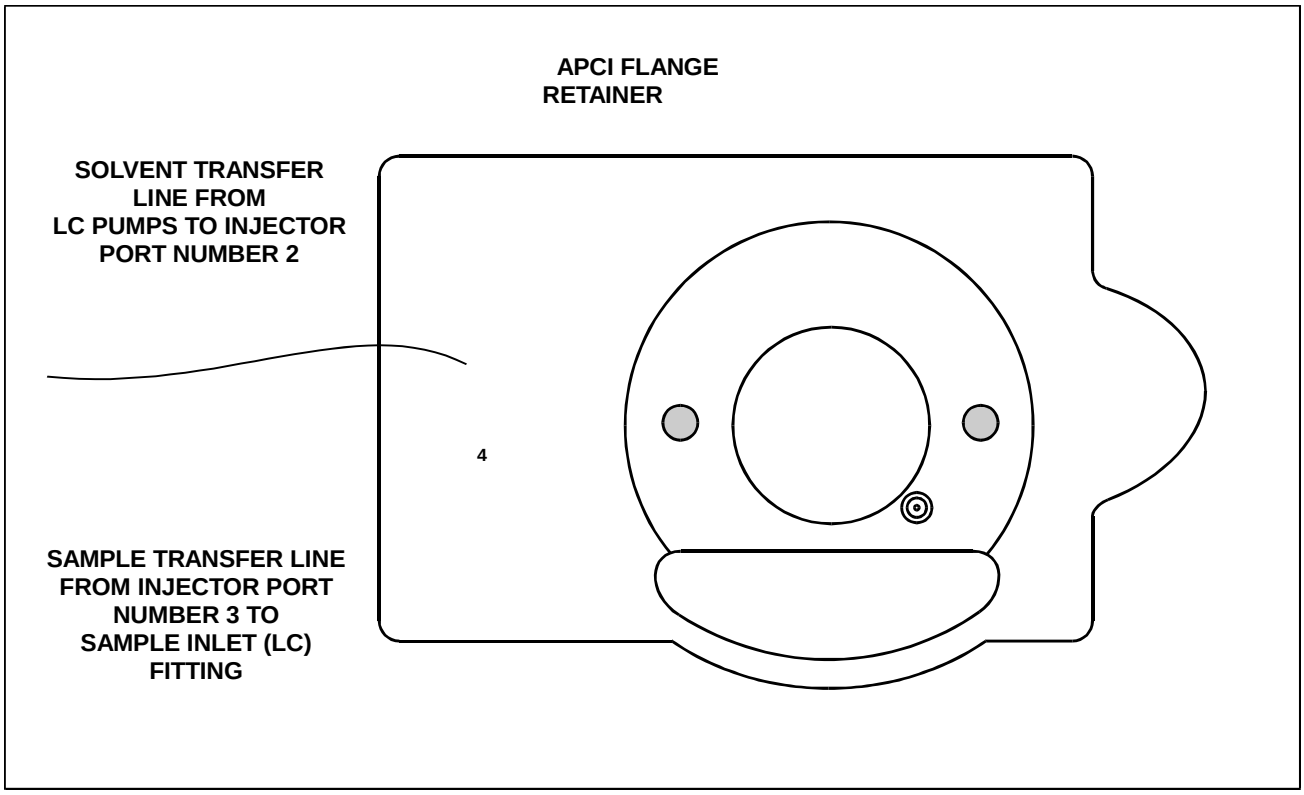


Figure 4-4. MS detector console, showing the proper connections for loop injections

> **Note**. To cut the PEEK tubing used to connect your LC to the divert/inject valve and the divert/inject valve to the APCI source, use a PEEK tubing cutter. This ensures that the tubing is cut straight. In addition, make sure your LC fittings, ferrules, and PEEK tubing are installed properly. By using these precautions, you prevent void (dead) volumes. The exclusion of void volumes is critical to microbore LC. Also, void volumes affect the quality of the MS detector signal.

1. Connect your LC to the divert/inject (Valco injector) as follows:

   a. Connect one end of a piece of (0.005-in ID) PEEK tubing of appropriate length to the outlet of the solvent delivery

system of your LC. Use the proper fitting and ferrule for your LC.

b. Connect the other end of the PEEK tubing to port 2 of the divert/inject valve. Use a Valco bushing (P/N 00110-16008) and ferrule (P/N 00101-18122). See Figure 4-5.

2. Connect the divert/inject valve to the APCI source as follows:

   a. Connect one end of another piece of PEEK tubing (the sample transfer line) of appropriate length to port 3 of the divert/inject valve. Use a Valco bushing and ferrule.

   b. Connect the other end of the PEEK tubing to the sample inlet fitting on the APCI source. Use an Upchurch Fingertight fitting (P/N 00101-18195) and ferrule (P/N 00101-18196). See Figure 4-4.

3. Ensure your divert/inject valve is fitted with a 5 µL loop between port 1 and port 4. See Figure 4-5.

4. Fill the a solvent reservoir of your LC with a solution of 50:50 methanol:water. [Or fill two solvent reservoirs, one reservoir (A) for water and a second reservoir (B) for methanol. Then, meter the reservoirs at 50% A and 50% B.]

5. Degas the solvent(s) either by sparging or by vacuum degassing.

6. Turn on the LC pumps to flush solvent (50:50 methanol:water) into the APCI source at a flow rate of approximately 1 mL min^{-1}.

Note. You can turn your LC pump on now or wait to turn it on until shortly before you inject a sample.

Note. For information on how to plumb the divert/inject valve to divert flow to waste, see Figure 4-6.

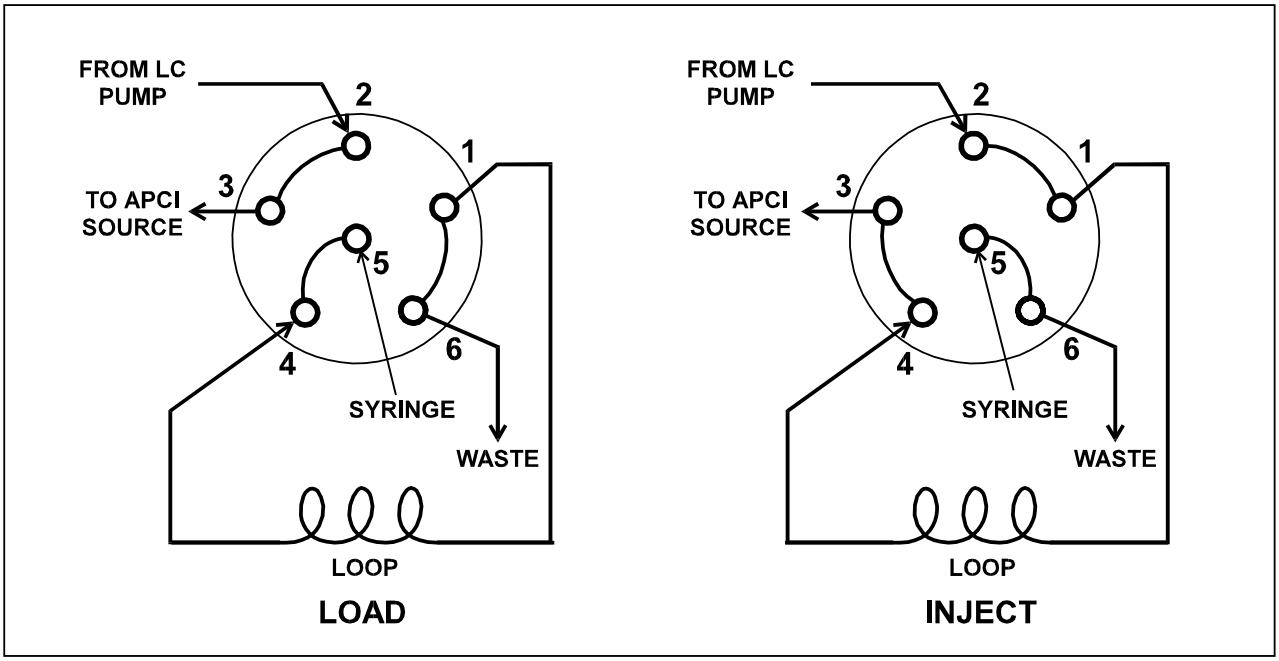


Figure 4-5. Divert/inject (Valco injector) valve, showing the correct set up for flow injection analysis and showing the flow of liquid through the valve in the LOAD and INJECT positions

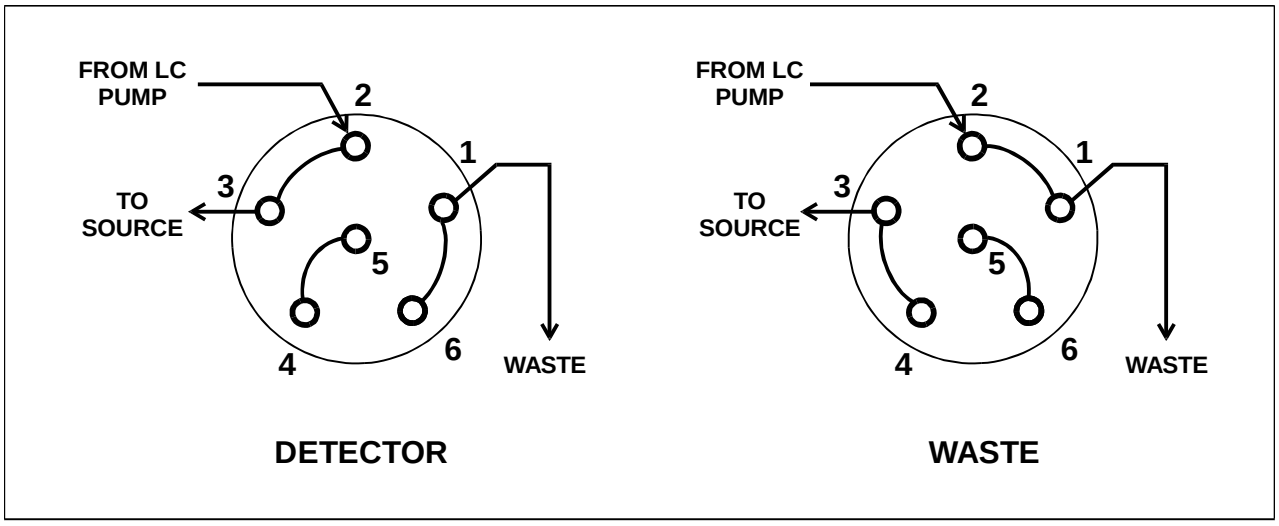
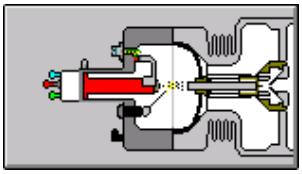


Figure 4-6. Divert/inject (Valco injector) valve, showing the correct set up for diverting flow to waste and showing the flow of liquid through the valve in the DETECTOR and WASTE positions

4.4 Baking Out the APCI Source

To set up your APCI source for APCI/MS operation, you bake out the APCI source to remove any residual contaminants. This may be necessary after switching from the ESI mode to the APCI mode or in normal APCI operation when background noise is excessive.

Use the following procedure to bake out the APCI source:



1. Open the APCI Source dialog box, as follows:
   Click on the APCI Source button in the Instrument Setup toolbar. See Figure 4-2.

2. Verify that the settings in your dialog box are similar to those shown in Figure 4-2.

3. Set the vaporizer temperature to 550 °C to bake out the APCI source, as follows:
   Double-click in the Vaporizer Temp spin box, then type **550**.

4. Apply the APCI source parameters and keep the dialog box open, as follows:
   Click on **Apply**.

5. Observe the actual vaporizer temperature increase to 550 °C in the APCI Source dialog box.

6. Turn on the LC pumps to introduce solvent (50:50 methanol:water) into the APCI source at a flow rate of approximately 1 mL min^{-1}.

7. Wait 15 minutes for the APCI source to bake out.

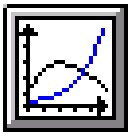
8. Reset the vaporizer temperature to 450 °C for normal APCI/MS operation, as follows:
   Double-click in the Vaporizer Temp spin box, then type **450**.

9. Apply the APCI source parameters and close the dialog box as follows:
   Click on **OK**.

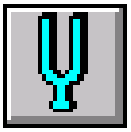
4.5 Testing the Operation of the MS Detector in the APCI/MS Mode

You are now ready to test if your MS detector is operating properly. To test for proper operation, you introduce 5 µL of a 10 pg μL^{-1} reserpine solution, or your particular analyte of interest, from a sample loop into the APCI source. You then monitor the real-time display of the mass spectrum of reserpine or your analyte of interest in the Spectrum view. You also monitor the intensity of each loop injection in the Graph view.

Observe in the Graph view a plot of intensity versus time for the mass peak that you want to monitor, as follows:



1. If necessary, open the Graph view, as follows:
   Click on the Display Graph View button in the Data Display toolbar.



2. Open the Tune dialog box - Manual tab, as follows:
   First, click on the Tune button in the Instrument Control toolbar. Then, click on the Manual tab. See Figure 4-7.

3. Specify the reserpine peak at *m/z* 609.2 as the peak to graph, or choose the parent ion of your analyte of interest, as follows:
   First, select [x] the Mass(es) check box. Next, select [x] the first Mass(es) spin box. Finally, double-click in the first Mass(es) spin box, then type **609.2**.

4. Click on **Start** to begin graphing the intensity of the *m/z* 609.2 ion current.

5. Close the Tune dialog box, as follows:
   Click on **Cancel**.

6. Load the reserpine solution, or a solution of your analyte of interest, into the sample loop as follows:

   a. On the MS detector front panel, ensure the green light below the word *Load* is illuminated (which indicates the divert/inject valve is in the Load position). If it is not, press the blue Load/Inject button to switch the valve to the Load position. See Figure 4-4.

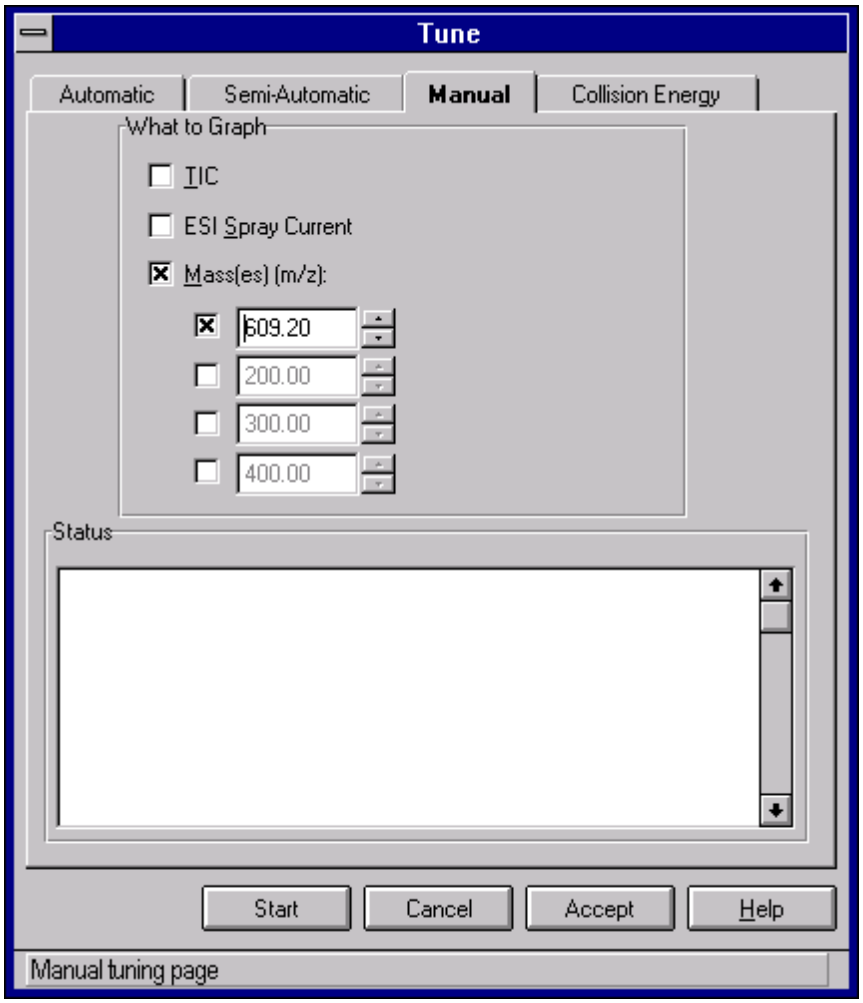


Figure 4-7. Tune dialog box, showing the Manual tab with the proper settings for graphing the *m/z* 609.2 ion current of reserpine

b. Load a Unimetrics syringe (P/N 00301-19012) with a 10 pg μL^{-1} reserpine solution, or a solution of your analyte of interest.

Note. The Unimetrics syringe needle has a square tip. If you use a different kind of syringe, make sure that its needle also has a square tip.

c. Inject approximately 10 µL of the solution into the divert/inject valve to overfill the injector loop. The excess solution goes to the waste container.

> **Note**. By overfilling the 5 µL injector loop in step 5c, you ensure that you are reliably delivering 5 µL of the sample solution to the APCI source.

7. Inject the solution into the APCI source, as follows:
   Press the blue Load/Inject button. Solvent now flows through the loop, and the solution is pushed into the APCI source.

8. Return the divert/inject valve to the Load position, as follows:
   Press the blue Load/Inject button.

9. Repeat steps 6 through 8 several times to obtain several consecutive loop injections of reserpine.

   LCQ displays the trace of the loop injections in the Graph view.

While you observe the Graph and Spectrum views, ask yourself the following questions:

- Do peaks appear to increase in intensity in both views following loop injection?

- Are the peaks in the Graph view reproducible, varying by less than 15% from one injection to another?

If you answer "yes" to these questions, your MS detector is operating properly. Well done!

If you answered "no" to one of these questions, do the following:

- Ensure that the actual vaporizer temperature, as displayed in the APCI Source dialog box, is at approximately 450 °C.

- Ensure that the divert/inject (Valco injector) valve is plumbed correctly, and the sample loop is properly installed as described in the topic **Setting Up the Inlet: LC.**

- Ensure that your LC fittings, ferrules, and PEEK tubing are installed properly to prevent dead volumes, which can affect your signal quality.

- Ensure that the divert/inject valve is in the Load position when you complete steps 6 a through c.

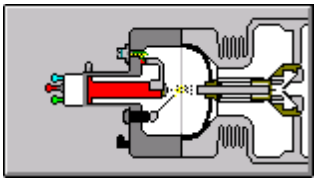
Leave the LC pumps on (with a flow rate of approximately 1 mL min^{-1}) and keep the Tune Plus window open to complete the next topic: **Optimizing the Tube Lens Offset Voltage and Heated Capillary Temperature.**

4.6 Optimizing the Tube Lens Offset Voltage and Heated Capillary Temperature

You optimize the tube lens offset voltage and heated capillary temperature independently for APCI operation. In this procedure, you adjust the tube lens offset voltage until the ion transmission of reserpine is maximized. You then repeat the procedure to optimize the heated capillary temperature. The capillary is heated to maximize the ion transmission to the MS detector. You can optimize other source parameters, such as the capillary voltage and the sheath gas using the same procedure.

> **Note**. The most important parameters that affect the signal quality during APCI/MS operation are the vaporizer temperature, heated capillary temperature, capillary voltage, tube lens offset voltage, and the solution flow rate. If any one of these parameters is changed, you need to reoptimize the tube lens offset voltage and/or heated capillary temperature as described here.

To optimize the tube lens offset voltage and heated capillary temperature, do the following:



1. Vary the tube lens offset voltage and observe how the change affects the intensity of the ion current in the Graph view, as follows:

   a. Open the APCI Source dialog box, as follows:
   Click on the APCI Source button in the Instrument Setup toolbar.

   b. Increase the tube lens offset voltage by 5 V, as follows:
   Double-click on the Tube Lens Offset spin box, then type **35** (to add 5 V to the default value of 30 V).

   c. Apply the new tube lens offset voltage and close the dialog box, as follows:
   Click on **OK**.

2. With the divert/inject valve in the Load position, overfill the loop with the reserpine solution, or a solution of your analyte of interest.

3. Inject the solution into the APCI source.

4. Return the divert/inject valve to the Load position.

5. Repeat steps 1 through 4, varying the tube lens offset voltage by 5 V each time.

   LCQ displays the trace of the loop injections in the Graph view.

6. Repeat step 5 several times to obtain several consecutive loop injections of reserpine, or your particular analyte of interest.

   Observe how the increase in tube lens offset voltage affects the maximum intensity of the loop injections as compared to the initial tube lens offset voltage.

7. Perform the process in steps 1 through 4 repeatedly, varying the offset voltage each time, until you find the offset voltage that provides the maximum ion current intensity.

Your Tune Plus window should now look similar to the one shown in Figure 4-8.

You can repeat the above procedure to optimize the heated capillary temperature, and the capillary voltage.

Once you have found the optimum tube lens offset voltage and heated capillary temperature, you are now ready to perform APCI/MS experiments in all scan modes and scan powers.

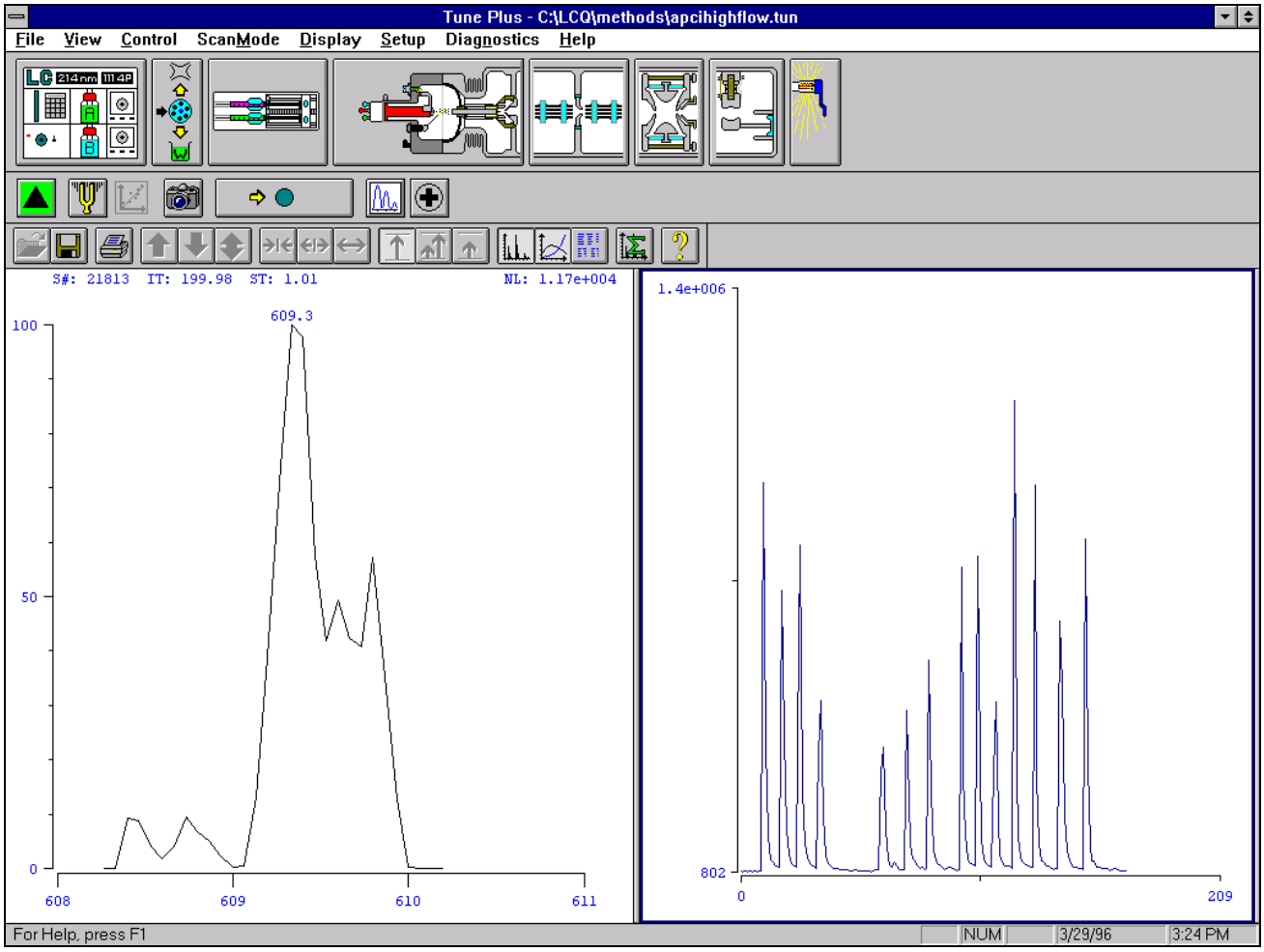


Figure 4-8. Tune Plus window, showing the effect on signal intensities for changes in tube lens offset voltage for loop injections of reserpine

Leave the LC pumps on (with a flow rate of approximately 1 mL min^{-1}), and leave the Tune Plus window with the *apcihighflow.tun* tune method open to complete the next chapter: **Developing Methods for LC/MS/MS Analysis of Testosterone in the APCI Mode**.

Contents

List of Illustrations

DEVELOPING METHODS FOR LC/MS/MS ANALYSIS OF TESTOSTERONE IN THE APCI MODE

This chapter contains information on LC/MS/MS analysis of testosterone in the APCI mode. In this example you optimize the MS detector sensitivity for testosterone, and develop an LC/MS/MS method for analysis of testosterone. The major topics are as follows:

- Optimizing the MS detector sensitivity in Tune Plus
- Setting up the MS detector for APCI/MS/MS operation
- Developing a method for LC/MS/MS analysis of testosterone

> **Note**. Before you begin any of the procedures outlined in this chapter, ensure the following: Your APCI/MS system is properly set up as described in the **Setting Up the MS Detector for APCI/MS Operation** topic in the **Tuning in the APCI/MS Mode** chapter.

You optimize the MS detector sensitivity for testosterone in the Tune Plus window. You then save the Tune Method and recall the tune settings in an Experiment Method in the next chapter: **LC/MS/MS Analysis of Samples in a Mixture with Autosampler Injection**.

You develop an LC/MS/MS method for analysis of testosterone using an LC analytical column installed in-line between the autosampler of your LC and your LCQ MS detector. Typically a reverse-phase C8 or C18 column works well for analysis of testosterone. However, you can use any reverse-phase column you have in your laboratory.

The method you develop here is the one you use to collect data for quantitative analysis of testosterone in the chapter: **LC/MS/MS Analysis of Samples in a Mixture with Autosampler Injection**. You can then process the data and build a calibration curve as described in the chapter: **Quantitative Analysis of Data**.

5.1 Optimizing the MS Detector Sensitivity in the Tune Plus Window

You optimize the MS detector sensitivity for testosterone before installing your LC column in-line. In this procedure, you define the MS detector scan parameters for analysis of testosterone. You then optimize the tube lens offset voltage and heated capillary temperature. You use the same procedure you used in the topic **Optimizing the Tube Lens Offset Voltage and Heated Capillary Temperature** in the **Tuning in the APCI/MS Mode** chapter. Instructions are provided for the following:

- Defining the MS detector scan parameters
- Optimizing the tube lens offset voltage and heated capillary temperature
- Saving a tune method

5.1.1 Defining the MS Detector Scan Parameters

Define the MS detector scan parameters for analysis of testosterone in the SIM scan mode, as follows:

1. Open the Define Scan dialog box, as follows:
   Click on the Define Scan button in the Instrument Control toolbar. See Figure 5-1.

2. Select the MS scan power in the Scan Description group box, as follows:
   Click on the Scan Power: MS option button. Note that LCQ sets the MS^n power to *1*.

3. Select the SIM scan mode, as follows:
   Click on the Scan Mode: SIM option button. Note that LCQ sets the Total Scan Ranges to *1*.

4. Set the total number of microscans to 1 in the Scan Time group box, as follows:
   Double-click in the Total Microscans spin box, then type **1**.

5. Set the maximum inject time to 100 ms in the Scan Time group box, as follows:

Double-click in the Maximum Inject Time spin box, then type **100**.

6. Select the center / width input method in the Input Method group box, as follows:
   Click on the Center / Width option button. Note that LCQ displays the Center Mass and Width text boxes in the Scan Ranges group box.

7. Select source CID Off in the Source CID group box, as follows:
   Click on the Off option button.

8. Set the center mass for the scan range to *m/z* 289 in the Scan Ranges group box, as follows:
   Double-click in the Center Mass text box, then type **289**.

9. Set the width of the scan range to *m/z* 5, as follows:
   Double-click in the Width text box, then type **5**.

10. Save the MS detector scan parameters and close the Define Scan dialog box, as follows:
    Click on **OK**.

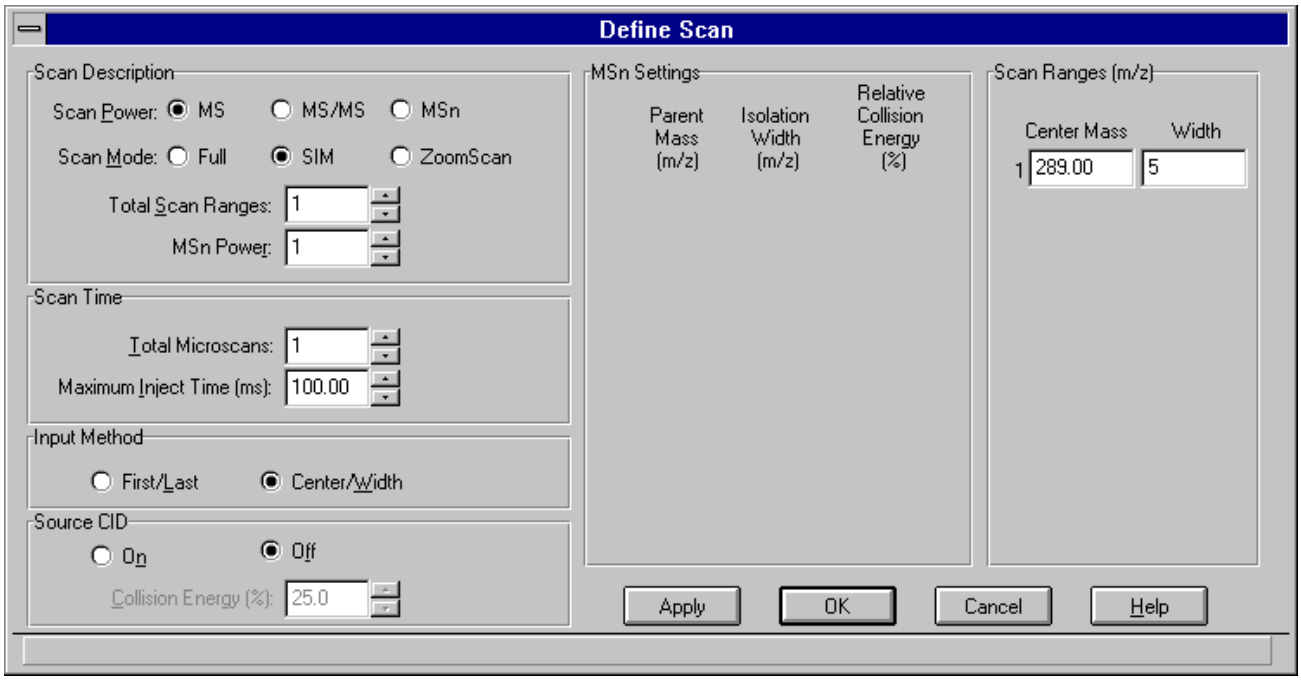
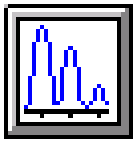
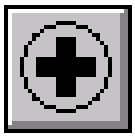


Figure 5-1. Define Scan dialog box, showing typical settings for a SIM experiment on testosterone



11. Select the profile scan data type, as follows:
    Click on the Centroid/Profile button in the Instrument Control toolbar to toggle the scan data type to profile. (The picture on the button should be the same as that shown here).



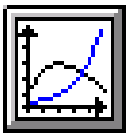
11. Select the positive ion polarity mode, as follows:
    Click on the Positive/Negative button in the Instrument Control toolbar to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here).

5.1.2 Optimizing the Tube Lens Offset Voltage and Heated Capillary Temperature

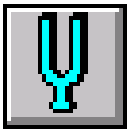
To optimize the tube lens offset voltage and heated capillary temperature to maximize the ion transmission of testosterone to the MS detector, you do the following:

- Introduce 5 µL of a 100 pg μL^{-1} testosterone solution from a sample loop into the APCI source. Refer to **Appendix A: Sample Formulations**.
- Then monitor the intensity of each loop injection in the Graph view of the Tune Plus window.

You observe in the Graph view a plot of intensity versus time for the testosterone parent ion mass peak. To optimize the tube lens offset voltage while monitoring loop injections of testosterone, do the following:



1. If necessary, open the Graph view, as follows:
   Click on the Display Graph View button in the Data Display toolbar.



2. Open the Tune dialog box - Manual tab, as follows:
   First, click on the Tune button in the Instrument Control toolbar. Then, click on the Manual tab. See Figure 5-2.

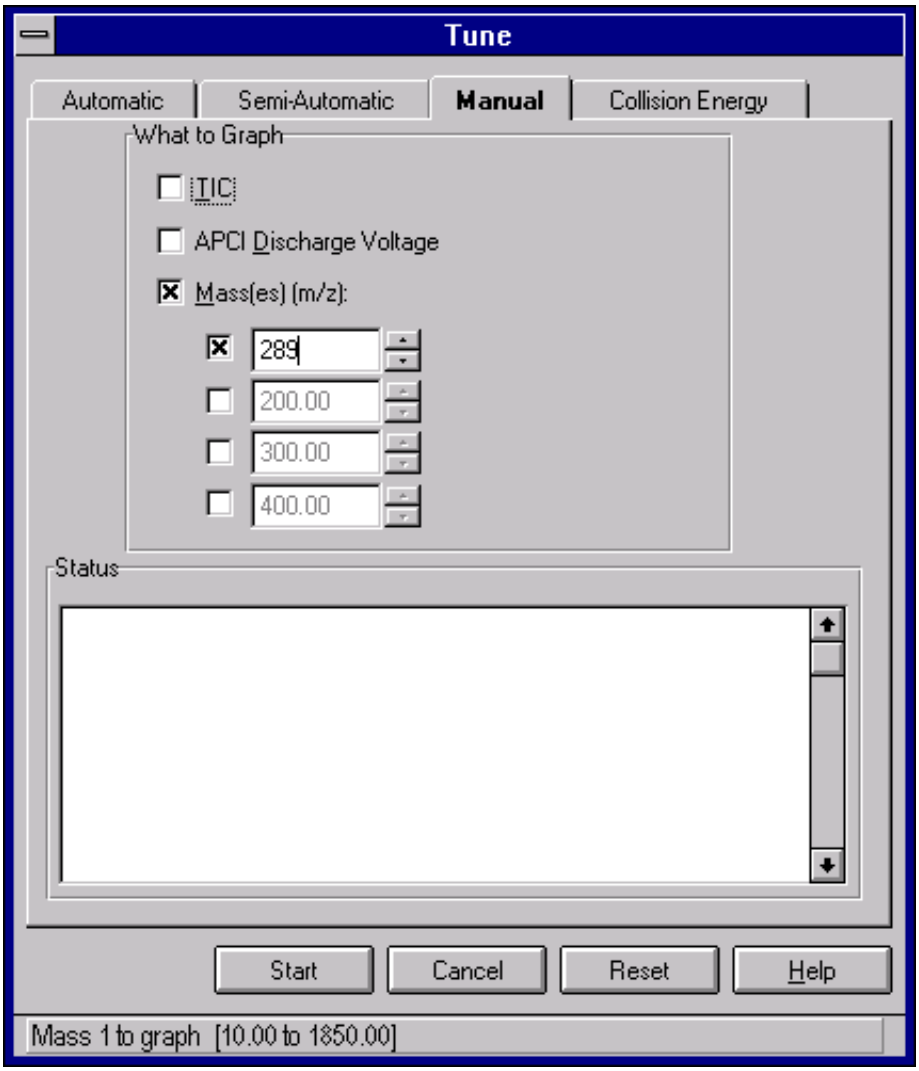


Figure 5-2. Tune dialog box, showing the Manual tab with the proper settings for graphing the *m/z* 289 ion current of testosterone

3. Specify the peak at *m/z* 289 as the peak to graph, as follows:
   First, select [x] the Mass(es) check box. Next, select [x] the first Mass(es) spin box. Finally, double-click in the first Mass(es) spin box, then type **289**.

4. Begin graphing the intensity of the *m/z* 289 ion current, as follows:
   Click on **Start**.

5. Close the Tune dialog box as follows:
   Click on **Cancel**.

6. Load the testosterone solution into the sample loop as follows:

   a. On the MS detector front panel, ensure the green light below the word *Load* is illuminated (which indicates the divert/inject valve is in the Load position). If it is not, press the blue Load/Inject button to switch the valve to the Load position. See Figure 5-3.

   b. Load a Unimetrics syringe (P/N 00301-19012) with a 100 pg μL^{-1} testosterone solution.

> **Note.** The Unimetrics syringe needle has a square tip. If you use a different kind of syringe, make sure that its needle also has a square tip.

   c. Inject approximately 10 µL of the testosterone solution into the divert/inject valve to overfill the injector loop. The excess solution goes to the waste container.

> **Note**. By overfilling the 5 µL injector loop in step 5c, you ensure that you are reliably delivering 5 µL of the sample solution to the APCI source.

7. Inject the testosterone solution into the APCI source, as follows:
   Press the blue Load/Inject button. Solvent now flows through the loop, and the testosterone solution is pushed into the APCI source.

8. Return the divert/inject valve to the Load position, as follows:
   Press the blue Load/Inject button.

9. Repeat steps 6 through 8 several times to obtain consecutive loop injections of testosterone.

   LCQ displays the trace of the loop injections in the Graph view.

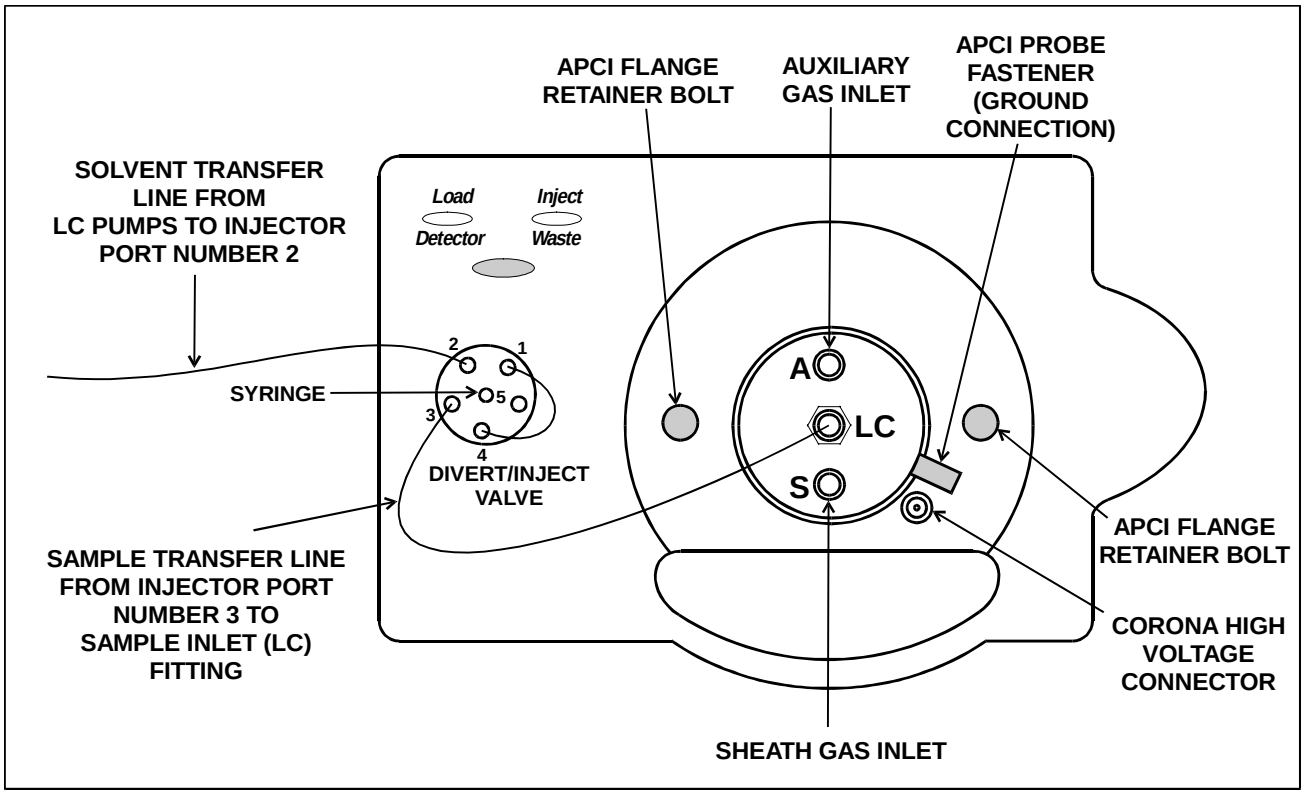
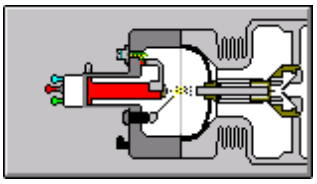


Figure 5-3. Diagram depicting the MS detector console, showing the proper connections for loop injections using an injector loop

10. Vary the tube lens offset voltage and observe how the change affects the intensity of the ion current in the Graph view, as follows:



a. Open the APCI Source dialog box, as follows:
Click on the APCI Source button in the Instrument Setup toolbar.

b. Increase the tube lens offset voltage by 5 V, as follows:
Double-click on the Tube Lens Offset spin box, then add 5 V to the current value.

c. Apply the new tube lens offset voltage and close the dialog box, as follows:
Click on **OK**.

2. Repeat steps 9 and 10 several times, varying the tube lens offset voltage by 5 V each time, to obtain consecutive loop injections of testosterone.

Observe how the increase in tube lens offset voltage affects the maximum intensity of the loop injections as compared to the initial tube lens offset voltage.

3. Perform the process in steps 9 and 10 repeatedly, varying the offset voltage each time, until you find the offset voltage that provides the maximum ion current intensity.

Your Tune Plus window should now look similar to the one shown in Figure 5-4.

You can repeat the above procedure to optimize the temperature of the heated capillary.

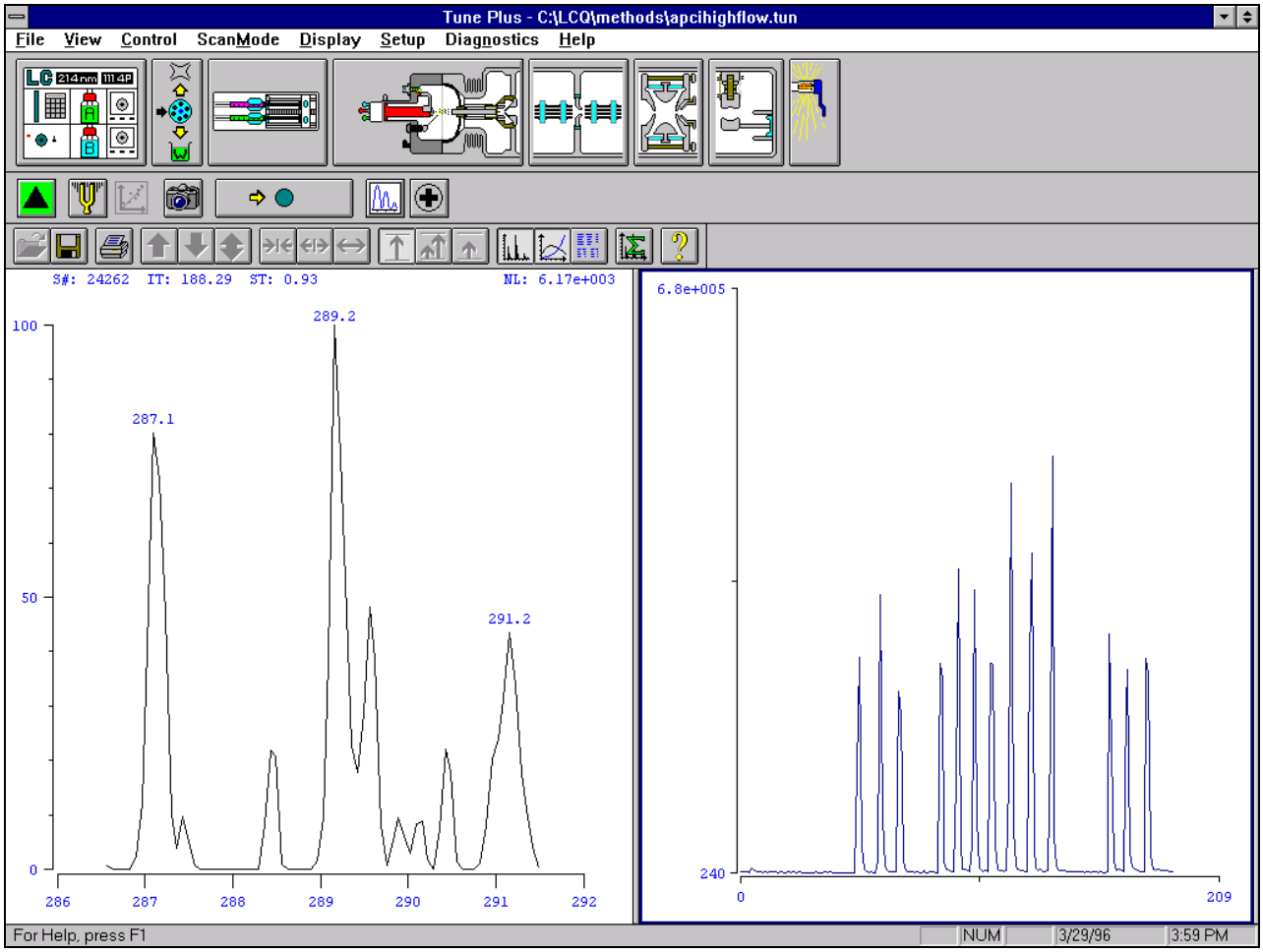


Figure 5-4. Loop injections of testosterone, showing the effect on signal intensities for changes in tube lens offset

5.1.3 Saving a Tune Method

You can save and recall the tune parameters you just set, including tube lens offset voltage, heated capillary temperature, total number of microscans, and maximum inject time by saving them in a Tune Method. You recall these tune settings in an Experiment Method in the next chapter: **LC/MS Analysis of Samples in a Mixture with Autosampler Injection**.

Save the MS detector tune parameters in a Tune Method, as follows:

1. Display the Save As dialog box, as follows:
   Choose **File | Save As**.

2. Select the *C:\LCQ\Methods directory*, as follows:
   First, double-click on the *C:* directory. Next, Scroll down in the Directories combo box until you see *LCQ*. Then, double-click on the *LCQ* directory. Then, scroll down in the Directories combo box until you see *Methods*. Finally, double-click on the *Methods* directory.

3. Specify the name of the Tune Method as *testosterone.tun* in the *C:\LCQ\Methods* directory, as follows:
   Double-click in the File Name combo box, then type **testosterone.tun**.

4. Save the *testosterone.tun* Tune Method and close the dialog box, as follows:
   Click on **OK**.

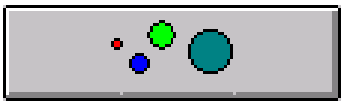
5.2 Setting Up the MS Detector for APCI/MS/MS Operation

To set up the MS detector for APCI/MS/MS operation, do the following:

- Define the MS detector scan parameters.
- Determine the relative collision energy for an MS/MS experiment.

5.2.1 Defining the MS Detector Scan Parameters

Define the MS detector scan parameters for analysis of testosterone in the full scan MS/MS mode, as follows:



1. Open the Define Scan dialog box, as follows:
   Click on the Define Scan button in the Instrument Control toolbar. See Figure 5-5.

2. Select the MS/MS scan power in the Scan Description group box, as follows:
   Click on the Scan Power: MS/MS option button. Note that LCQ sets the MS^n power to *2*. LCQ also displays the Parent Mass text box, Isolation Width spin box, and Relative Collision Energy spin box in the MS^n Settings group box.

3. Select the Full scan mode, as follows:
   Click on the Scan Mode: Full option button.

4. Set the total number of microscans to 1 in the Scan Time group box, as follows:
   Double-click in the Total Microscans spin box, then type **1**.

5. Set the maximum inject time to 100 ms in the Scan Time group box, as follows:
   Double-click in the Maximum Inject Time spin box, then type **100**.

6. Select the first / last input method in the Input Method group box, as follows:
   Click on the First / Last option button. Note that LCQ displays the First Mass and Last Mass text boxes in the Scan Ranges group box.

7. Select source CID Off in the Source CID group box, as follows:
   Click on the Off option button.

8. Set the parent mass for testosterone to *m/z* 289.1 in the MS^n Settings group box, as follows:
   Double-click in the Parent Mass text box, then type **289.1**.

9. Set the isolation width for testosterone to *m/z* 2 in the MS^n Settings group box, as follows:
   Double-click in the Isolation Width spin box, then type **2**.

10. Set the relative collision energy for testosterone to 0.1% in the MS^n group box, as follows:
    Double-click in the Relative Collision Energy spin box, then type **0.1**.

11. Set the first mass for the scan range to *m/z* 90 in the Scan Ranges group box, as follows:
    Double-click in the First Mass text box, then type **90**.

12. Set the last mass for the scan range to *m/z* 300, as follows:
    Double-click in the Last Mass text box, then type **300**.

13. Save the MS detector scan parameters and close the Define Scan dialog box, as follows:
    Click on **OK**.

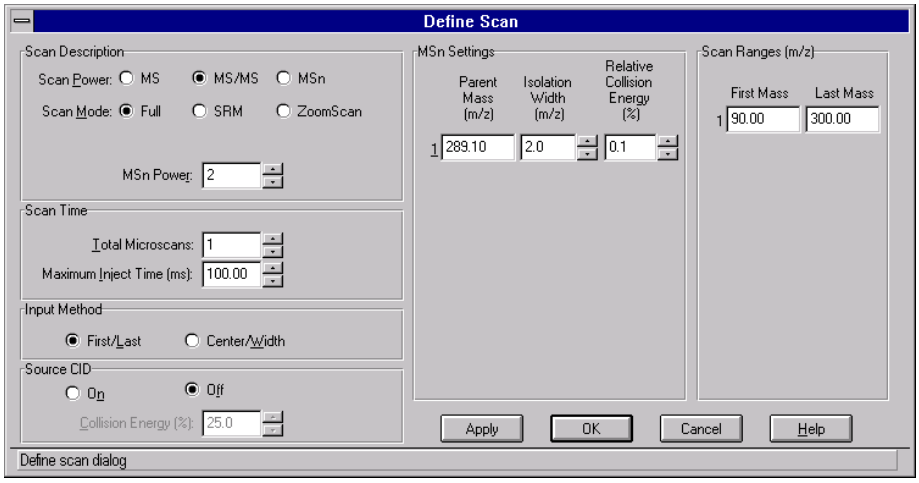
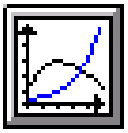


Figure 5-5. Define Scan dialog box, showing the proper settings to isolate the parent ion of testosterone

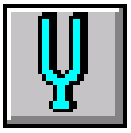
5.2.2 Optimizing the Relative Collision Energy for APCI/MS/MS Analysis of Testosterone

The relative collision energy for a particular analysis depends on the type of sample you are analyzing. In this procedure, you first isolate the parent ion of testosterone at 0.1% relative collision energy. You then increase the relative collision energy while monitoring the product ion(s) in consecutive loop injections of testosterone in the Graph view. You use an iterative process of adjusting the MS detector parameters and then monitoring loop injections. The optimum relative collision energy is the one that produces the maximum product ion intensity.

Optimize the relative collision energy for APCI/MS/MS analysis of testosterone while monitoring loop injections, as follows:



1. If necessary, open the Graph view, as follows:
   Click on the Display Graph View button in the Data Display toolbar.



2. Open the Tune dialog box - Manual tab, as follows:
   First, click on the Tune button in the Instrument Control toolbar.
   Then, click on the Manual tab. See Figure 5-6.

3. Specify the peak at *m/z* 289 as the peak to graph, as follows:
   First, select [x] the Mass(es) check box. Next, select [x] the first Mass(es) spin box. Finally, double-click in the first Mass(es) spin box, then type **289**.

4. Begin graphing the intensity of the *m/z* 289 ion current, as follows:
   Click on **Start**.

5. Close the Tune dialog box, as follows:
   Click on **Cancel**.

6. Load the testosterone solution into the sample loop with the divert/inject valve in the Load position.

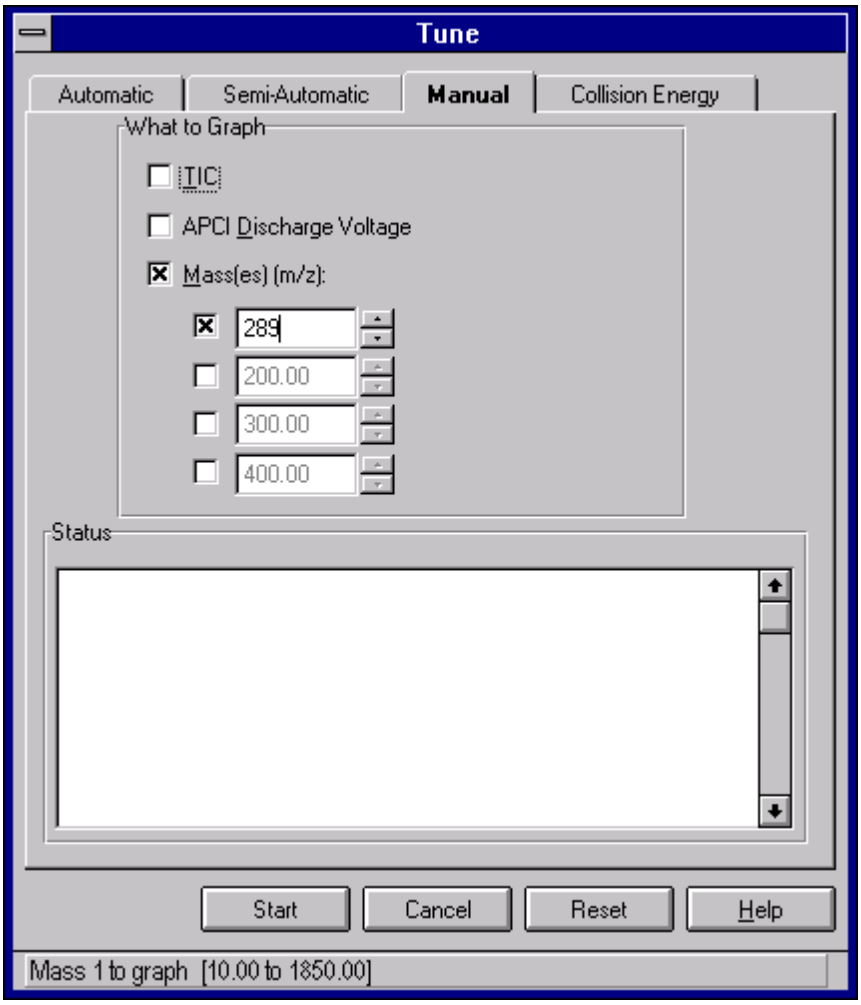


Figure 5-6. Tune dialog box, showing the Manual tab with the proper settings for graphing the *m/z* 289 ion current of testosterone

7. Acquire full scan MS/MS data for loop injections of testosterone, as follows:

   a. Open the Acquire Data dialog box, as follows:
   Click on the Acquire Data button in the Instrument Control toolbar. See Figure 5-7.

   b. Specify the name of the raw file as *isolate289.raw*, as follows:
   Double-click in the Filename text box, then type: **isolate289**.

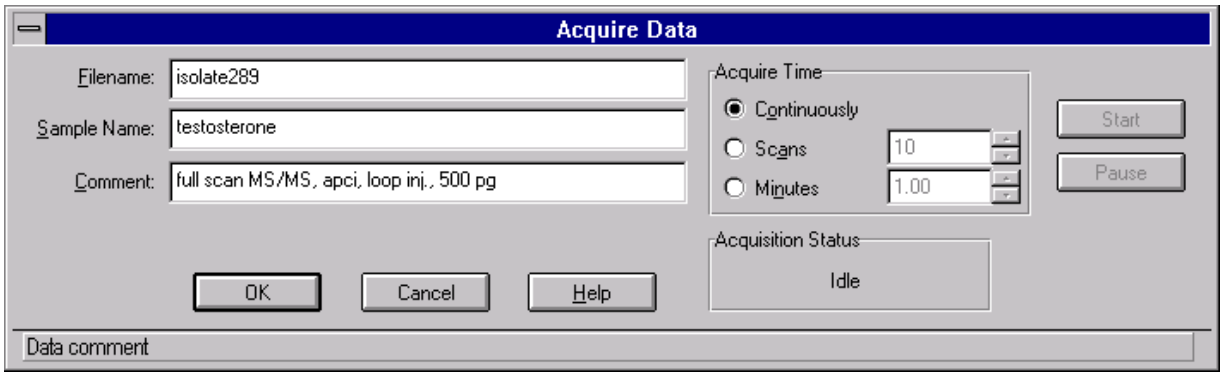


Figure 5-7. Acquire Data dialog box, showing sample information for data acquisition

c. Specify the sample name as testosterone, as follows:
Double-click in the Sample Name text box, then type **testosterone**.

d. Specify the information you want to appear on a hardcopy of the data, such as the scan mode, ionization method, sample amount, and method of sample introduction, as follows:
Double-click in the Comment text box, then type **full scan MS/MS, apci, loop inj., 500 pg**.

e. Select to acquire data continuously in the Acquire Time group box, as follows:
Click on the Continuously option button.

LCQ displays the current acquisition status, *Idle*, in the Acquisition Status group box.

Leave the Acquire Data dialog box open during data acquisition, but move it to the upper right hand corner of the Tune Plus window to get it out of the way.

f. Begin acquiring data, as follows:
Click on **Start**.

LCQ displays the current acquisition status, Acquisition in Progress, in the Acquisition Status group box.

8. Inject the testosterone solution into the APCI source.

LCQ displays in the Graph view the trace of the loop injections as well as the directory and file name *C:\LCQ\Data\isolate289*.

9. Return the divert/inject valve to the Load position.

10. Once the trace of the loop injection appears in the Graph view, stop the data acquisition in the Acquire Data group box, as follows:
    Click on **Stop**.

Observe the trace of the loop injection of testosterone in the Graph view. Your Tune Plus window should now look like the one shown in Figure 5-8.

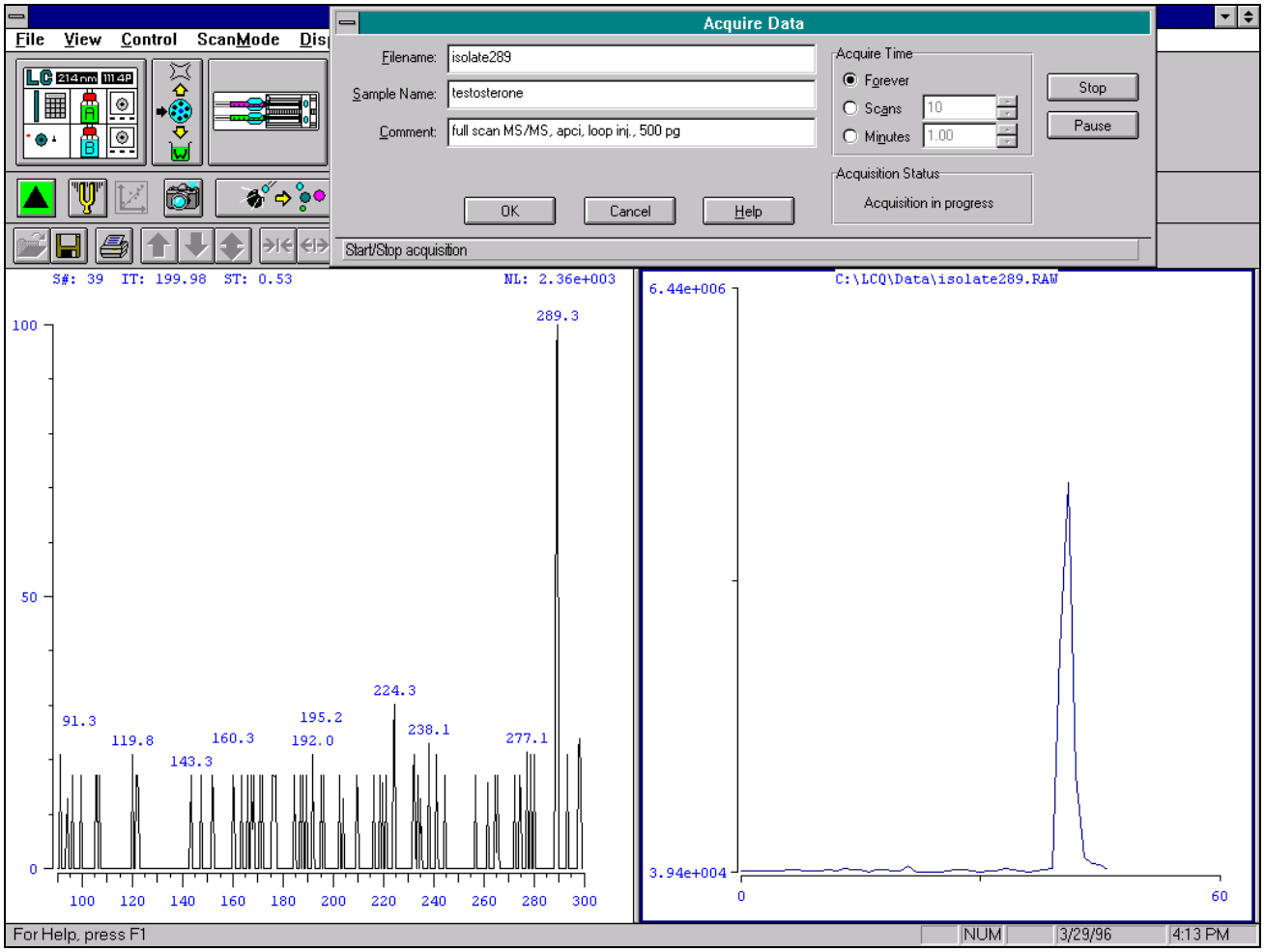
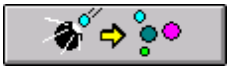


Figure 5-8. Tune Plus window, showing a loop injection of testosterone in the Graph view and the spectrum of the isolated parent ion of testosterone at *m/z* 289 in the Spectrum view

5.2.3 Adjusting the Relative Collision Energy and Monitoring Product Ion Intensity in Loop Injections of Testosterone

Vary the relative collision energy and observe how the change affects the intensity of the *m/z* 253 product ion current in the Graph view, as follows:



1. Open the Define Scan dialog box, as follows:
   Click on the Define Scan button in the Instrument Control toolbar. See Figure 5-9.

2. Set the relative collision energy for testosterone to 10% in the MS^n Settings group box, as follows:
   Double-click in the Relative Collision Energy spin box, then type **10**.

3. Apply the MS detector scan parameters, as follows:
   Click on **Apply**. (Leave the Define Scan dialog box open.)

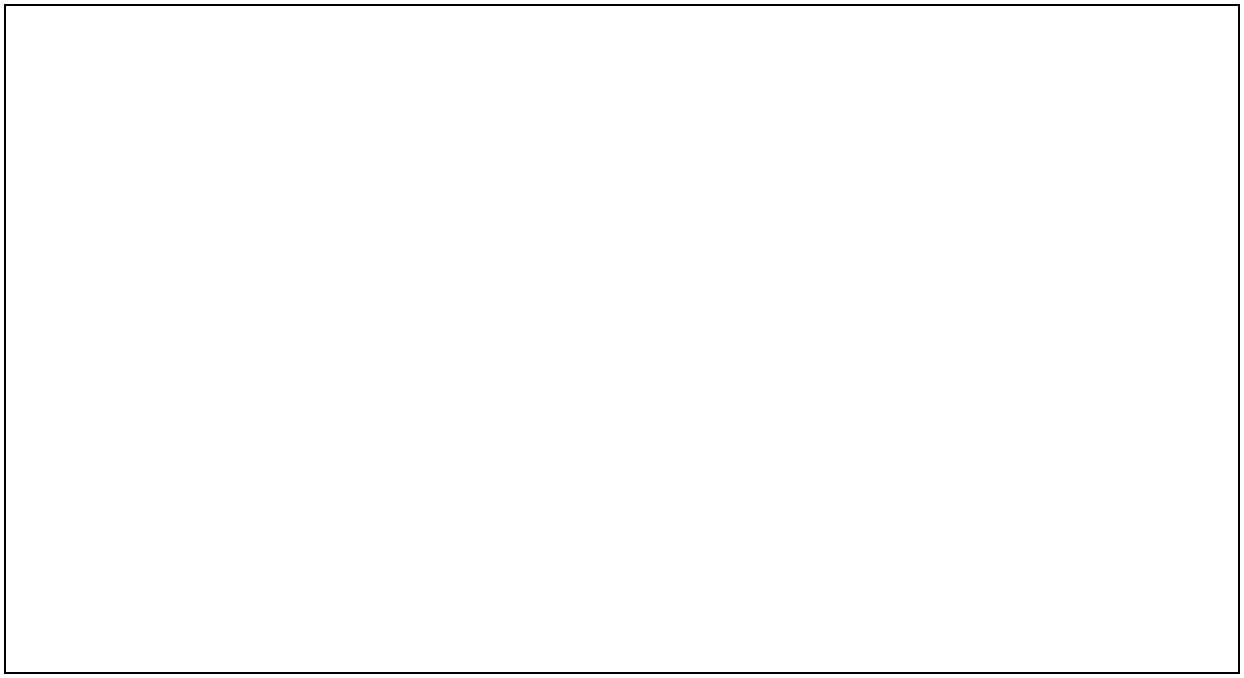


Figure 5-9. Define Scan dialog box, showing 10% relative collision energy imparted to parent mass *m/z* 289.1 of testosterone in the full scan MS/MS mode

4. If you have not already done so, open the Graph view, as follows:
   Click on the Display Graph View button in the Data Display toolbar.

4. If you have not already done so, open the Tune dialog box - Manual tab, as follows:
   First, click on the Tune button in the Instrument Control toolbar. Then, click on the Manual tab.

5. Specify the product ion of testosterone at *m/z* 253 as the peak to graph, as follows:
   First, select [x] the Mass(es) check box. Next, select [x] the first Mass(es) spin box. Finally, double-click in the first Mass(es) spin box, then type **253**.

6. Begin graphing the intensity of the *m/z* 253 ion current, as follows:
   Click on **Start** in the Tune dialog box - Manual tab.

7. Load the testosterone solution into the sample loop with the divert/inject valve in the Load position.

8. Acquire full scan MS/MS data for loop injections of testosterone, as follows:

   a. Open the Acquire Data dialog box, as follows:
      Click on the Acquire Data button in the Instrument Control toolbar. See Figure 5-10.

   b. Specify the name of the raw file as *10%coll*, as follows:
      Double-click in the Filename text box, then type **10%coll**.

   c. Specify the sample name as testosterone, as follows:
      Double-click in the Sample Name text box, then type **testosterone**.

   d. Specify the information you want to appear on a hardcopy of the data, such as the scan mode, ionization method, sample amount, and method of sample introduction, as follows:
      Double-click in the Comment text box, then type **full scan MS/MS, apci, loop inj., 500 pg**.

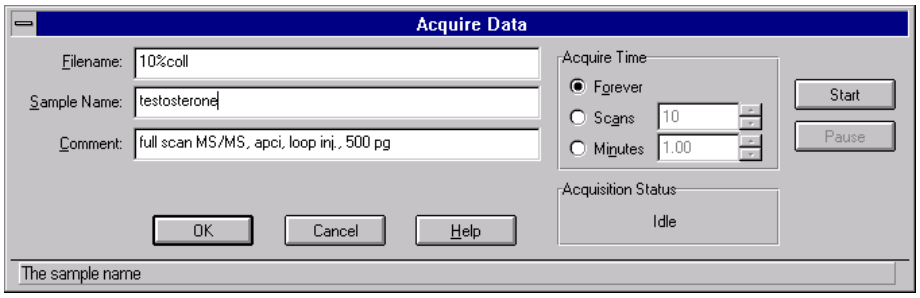


Figure 5-10. Acquire Data dialog box showing sample information for data acquisition

e. Select to acquire data continuously in the Acquire Time group box, as follows:
Click on the Continuously option button.

LCQ displays the current acquisition status, *Idle*, in the Acquisition Status group box.

f. Begin acquiring data, as follows:
Click on **Start** in Acquire Data dialog box. LCQ displays the current acquisition status, *Acquisition in Progress*, in the Acquisition Status group box.

13. Inject the testosterone solution into the APCI source.

14. Return the divert/inject valve to the Load position.

LCQ displays in the Graph view the trace of the loop injections as well as the file name and directory *C:\LCQ\Data\10%coll.raw*.

15. Observe the trace of the loop injection of testosterone in the Graph view.

16. Repeat steps 2, 3, 8, and 10 through 12, varying the relative collision energy by 1% each time.

LCQ displays the trace of the loop injections in the Graph view.

17. Repeat step 13 repeatedly to obtain several consecutive loop injections of testosterone for each change in the relative collision energy.

Vary the relative collision energy and observe how the increase in the relative collision energy imparted to the *m/z* 289 parent ion of testosterone affects the maximum intensity of the *m/z* 253 product ions in each loop injection.

Your Tune Plus window should now look similar to the one shown in Figure 5-11.

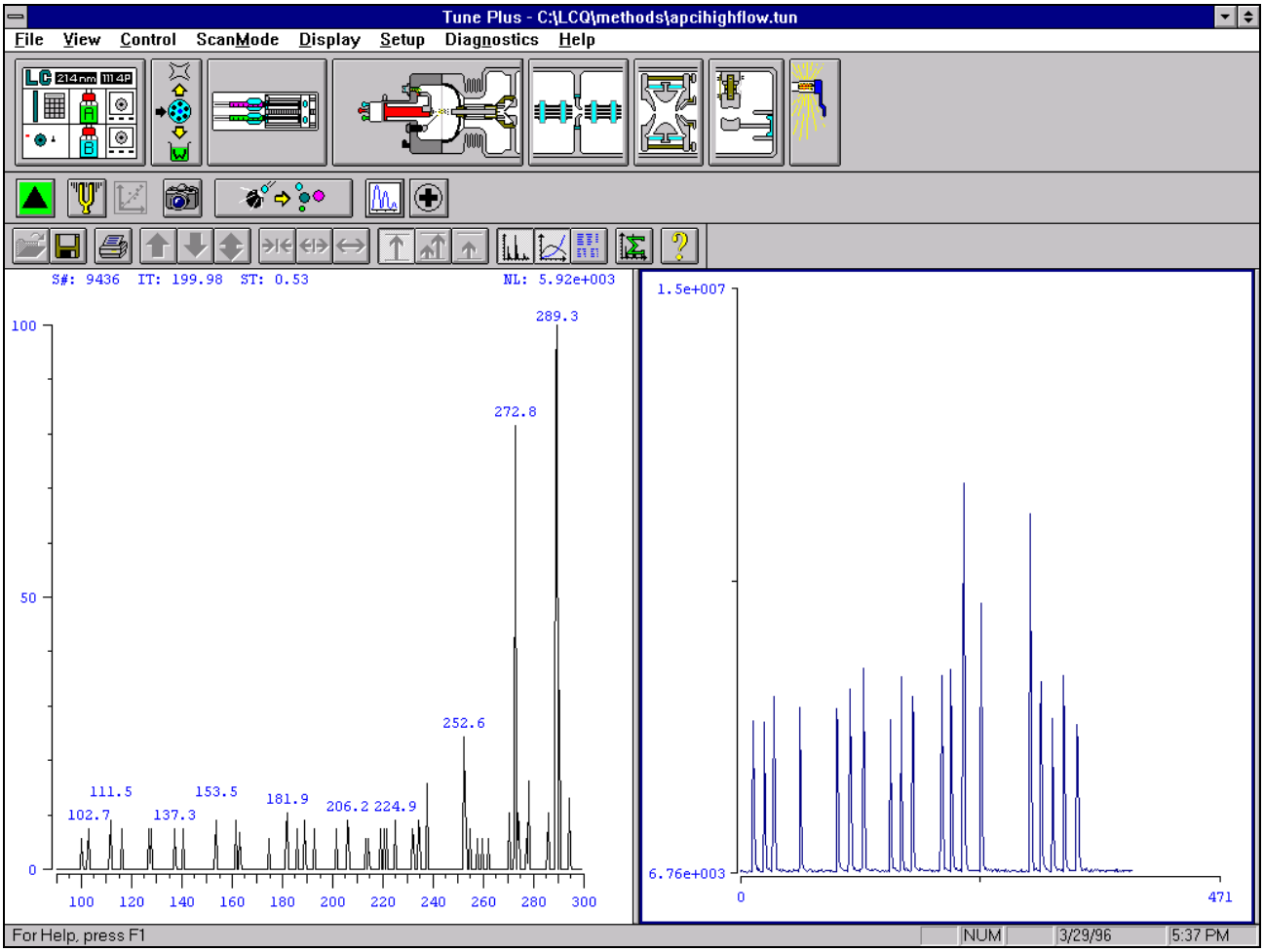


Figure 5-11. Tune Plus window, showing the results of three consecutive loop injections of testosterone at six different relative collision energies: 10, 11, 12, 13, 14 and 15% respectively

15. When you have found the optimum relative collision energy, stop the data acquisition in the Acquire Data dialog box, as follows:
    Click on **Stop**.

16. Close the Define Scan dialog box, Tune Plus dialog box - Manual tab, and Acquire Data dialog box, as follows:
    Click on **Cancel** in each dialog box.

In this case, Figure 5-11 shows that the maximum intensity product ions (monitored in the Graph view on consecutive loop injections) of testosterone under these conditions is produced at a relative collision energy of 14%. Your LCQ MS detector may have a different optimum relative collision energy.

Leave the Tune Plus window open to complete the next topic: **Developing a Method for LC/MS Analysis of Testosterone**.

5.3 Developing a Method for LC/MS Analysis of Testosterone

In this procedure you develop an LC/MS method for analysis of testosterone. An LC/MS method is the set of conditions (including solvent composition and solvent flow rate) used to separate and elute individual components from a chromatographic column and to detect them with an MS detector. In this example, LC/MS analysis of testosterone is carried out under isocratic conditions. Isocratic conditions are a set of operating conditions in which the solvent composition and the flow rate are held constant during the course of the chromatographic run.

To carry out this procedure, your LC system should be equipped with an autosampler and with at least a dual-channel solvent delivery system.

To develop an LC/MS method for analysis of testosterone, do the following:

- Measure the void volume of the LC column
- Adjust the retention time (RT) of the LC peak

5.3.1 Measuring the Void Volume of the LC column

You measure the void volume of your LC column by measuring the retention time (RT) for an unretained compound t_m. In this example you measure the void volume by injecting testosterone on-column under conditions such as a high organic solvent composition (90:10% methanol:H_2O), in which it is expected to be unretained. Under constant flow conditions during LC, the void volume is directly related to time. The center of the unretained testosterone peak is the void volume peak.
See Figure 5-12.

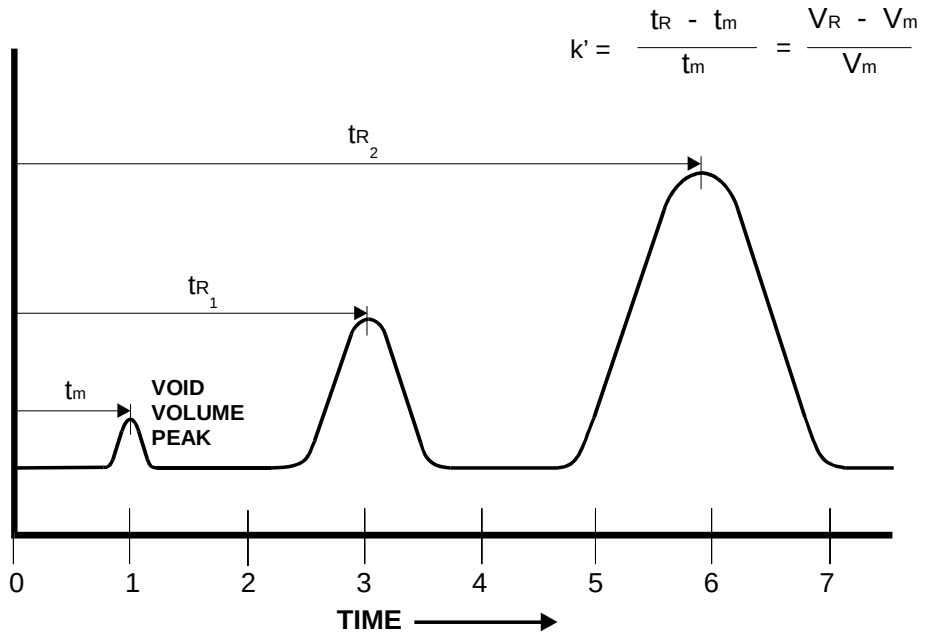
It is good practice to measure the void volume of your column because it allows for a correction in the separation parameters for variations in column lengths. It also allows for a calculation of the retention factor (k′). The retention factor is a measure of the relative retention of each peak on the column, according to the following equation:

$$k' = \frac{t_R - t_m}{t_m} = \frac{V_R - V_m}{V_m}$$

k′ is expressed in multiples of the void volume. Peak elution is thus expressed in terms of k′. See Figure 5-12 for definition of the terms. For most chromatographic separations, it is desirable to have components elute within a k′ of 2 to 6. This assures the component is retained on the LC column and does not elute in the void volume. It also minimizes separation time and cuts down on the amount of solvent used for a separation.

To measure the void volume of your LC column, do the following:

- Measure the RT for unretained testosterone.
- Analyze the chromatogram in Explore.



t_m = retention time for unretained solute

t_R = retention time for retained solutes

V_R = retention volume

V_m = volume of mobile phase

Figure 5-12. Typical chromatogram, showing the void volume peak and relative retention times for retained solutes

5.3.1.1 Measuring the RT for Unretained Testosterone and Acquiring LC/MS/MS Data

Using the same MS detector parameters you set in the topic: **Setting Up the MS Detector for APCI/MS/MS Operation**, use the following procedure to measure the RT for unretained testosterone:

1. Connect your LC column to the divert/inject (Valco injector) as follows:

    a. Connect one end of a piece of (0.005-in ID) PEEK tubing of appropriate length to port 3 of the divert/inject valve. Use a Valco bushing (P/N 00110-16008) and ferrule (P/N 00101-18122).

    b. Connect the other end of the PEEK tubing to your LC column. Use the appropriate fittings for your particular column.

    c. Connect one end of another piece of (0.005-in ID) PEEK tubing of appropriate length to the other end of your LC column. Use the appropriate fittings for your particular column.

    d. Connect the other end of the PEEK tubing to the sample inlet fitting on the APCI source. Use an Upchurch Fingertight fitting (P/N 00101-18195) and ferrule (P/N 00101-18196).

2. Ensure your divert/inject valve is fitted with a 5 µL loop between port 1 and port 4.

3. Fill one solvent reservoir of your LC with 100% methanol and a second solvent reservoir with 100% H_2O. This allows you to meter the reservoirs to quickly change solvent composition as you develop your LC method.

4. Degas the solvents either by sparging or by vacuum degassing.

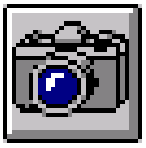
5. Set a gradient on the LC you are using to gradually bring the solvent composition to 90% methanol:10% H_2O over a 5 min period.

6. Turn on the LC pumps to flush the methanol:H_2O solution into the APCI source at a flow rate of approximately 1 mL min^{-1}.

7. Once the LC reaches the desired solvent composition of 90% methanol:10% H_2O, allow your column to equilibrate for 10 min at this solvent composition.

8. Load the testosterone solution into the sample loop with the divert/inject valve in the Load position.

9. Acquire full scan MS/MS data for loop injections of testosterone, as follows:



   a. Open the Acquire Data dialog box, as follows:
   Click on the Acquire Data button in the Instrument Control toolbar. See Figure 5-13.

   b. Specify the name of the raw file as *void1*, as follows:
   Double-click in the Filename text box, then type: **void1**.

   c. Specify the sample name as testosterone, as follows:
   Double-click in the Sample Name text box, then type **testosterone, 90% methanol:10% H_2O**.

   d. Specify the information you want to appear on a hardcopy of the data, such as the scan mode, ionization method, sample amount, and method of sample introduction, as follows:
   Double-click in the Comment text box, then type **LC/MS, full scan MS/MS, apci, loop injection, 500 pg**.

   e. Select to acquire data continuously in the Acquire Time group box, as follows:
   Click on the Continuously option button.

   LCQ displays the current acquisition status, *Idle*, in the Acquisition Status group box.

   Leave the Acquire Data dialog box open during data acquisition, but move it to a corner of the Tune Plus window to get it out of the way.

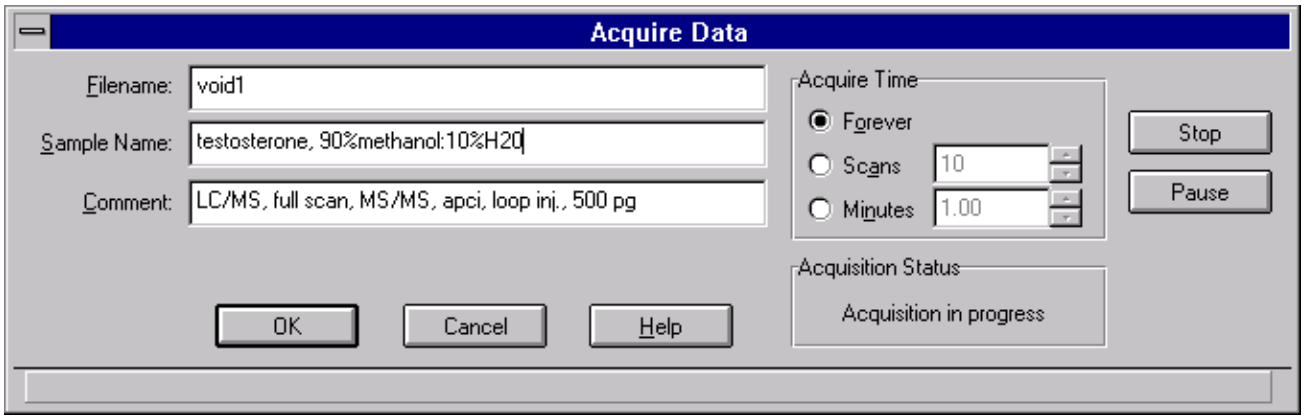


Figure 5-13. Acquire Data dialog box showing sample information for measuring the LC column void volume

f. Begin acquiring data, as follows:
Click on **Start**. LCQ displays the current acquisition status, *Acquisition in Progress*, in the Acquisition Status group box.

10. Inject the testosterone solution into the APCI source.

11. Return the divert/inject valve to the Load position.

LCQ displays the trace of the loop injections in the Graph view as well as the file name and directory *C:\LCQ\Data\void1.raw*

Observe the trace of the loop injection of testosterone in the Graph view. Your Tune Plus window should now look like the one shown in Figure 5-14.

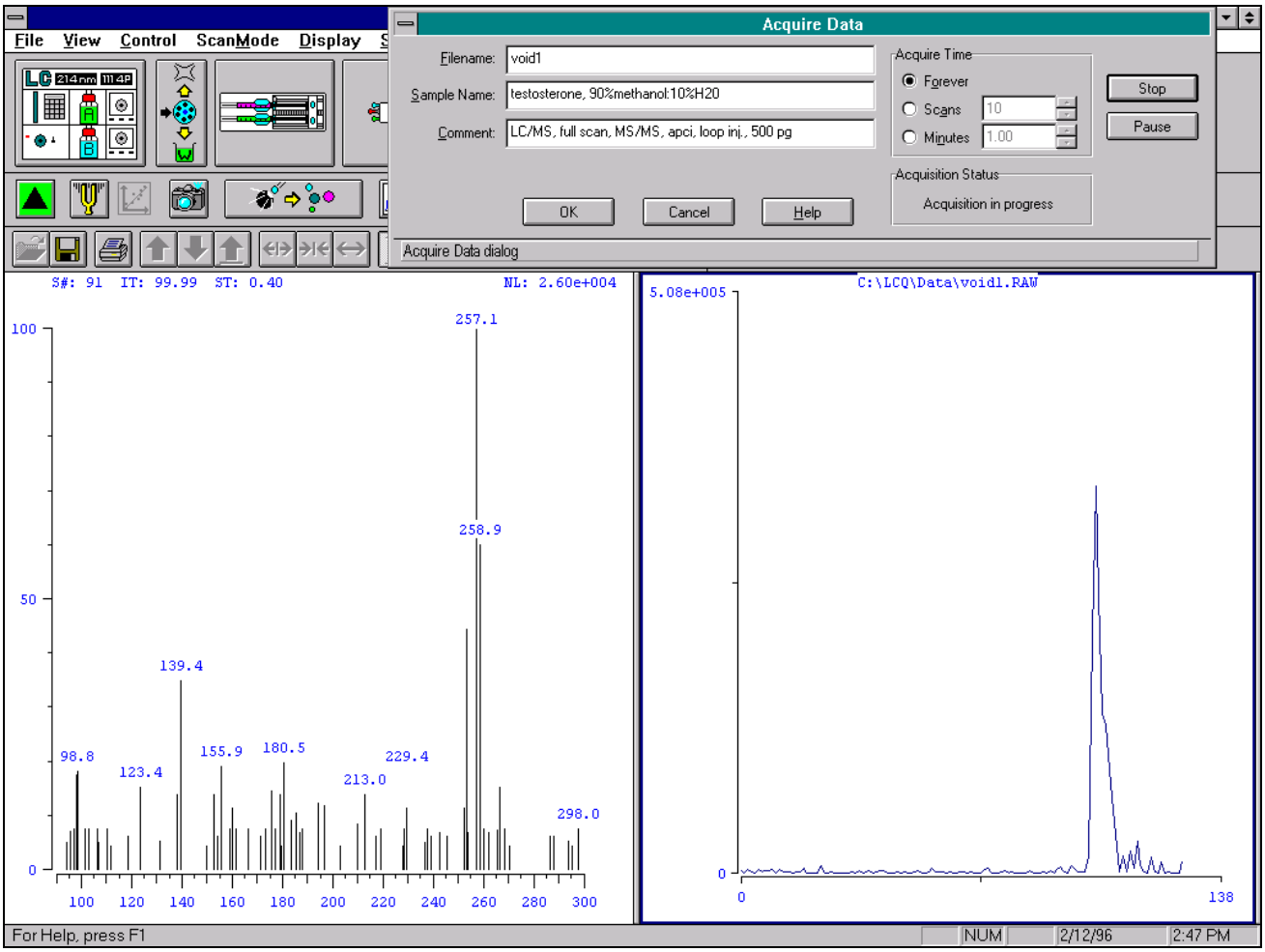


Figure 5-14. Tune Plus window showing, column void volume measurement by LC/MS of testosterone in 90% methanol:10% H_2O

12. When the peak is finished eluting from the column, stop the data acquisition in the Acquire Data group box, as follows: Click on **Stop**.

5.3.1.2 Analyzing the Chromatogram in Explore

Use the following procedure to open a raw file and analyze the chromatogram and mass spectrum in the Explore window:

1. Return to the Navigator window, as follows
   First, hold down the **<Alt>** key. Next, press the **<Tab>** key until the Navigator button appears. Finally, let go of the key.

1. Open the Explore window, as follows:
   Click on the Explore button in the Navigator toolbar.

2. Open the *void1.raw* raw file, as follows:

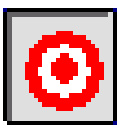
   a. Display the Open Layout or Open Raw File dialog box, as follows:
      Choose **File | Open**.

   b. Select the file *void1.raw* in the directory *C:\LCQ\Data*, as follows:
      Scroll down in the File Name combo box until you see *void1.raw*. Then, click on the file name.

   c. Open the file, and close the dialog box, as follows:
      Click on **OK**. LCQ opens the raw file in the active cell of the Explore window.

> **Note**. The active cell is the cell containing the highlighted target button, which has a dark blue border around it.

4. If you have not already done so, insert a duplicate cell to the right of the active window, as follows:
   Choose **Grid | Insert Cells | Right**.



4. Activate the right cell, as follows:
   Click on the Target button in the right cell.

5. Display the spectrum in the active cell, as follows:
   Choose **Display | Spectrum**.

6. Display the mass spectrum of reserpine in the Target active cell, as follows:
   First, move the cursor to the left, inactive cell (chromatogram display). Then, click on the peak maximum.

7. Display a mass chromatogram for the *m/z* 253 product ion, as follows:

   a. Activate the left-hand cell as follows:
   Click on the target button on the left-hand side of the display. LCQ highlights the left cell with a blue border.

   b. Open the Chromatogram Ranges dialog box, as follows:
   Choose **Options | Ranges**. See Figure 5-15.

   c. Enter a 1 u mass range around the *m/z* 253 product ion of testosterone, as follows:
   Double-click in the Mass Range(s) text box, then type **253**.
   LCQ automatically sets the mass range to 252.5-253.5.

   d. Close the dialog box and enter the mass range parameters as follows:
   Click on **OK**.

8. Save the Explore window layout, as follows:

   a. Open the Save Layout File As dialog box, as follows:
   Choose **File | Save Layout**.

   b. Specify the layout file name as *LC1.lyt*, as follows:
   Double-click in the File Name combo box, then type **LC1.lyt**.

   c. Save the layout file and close the dialog box as follows:
   Click on **OK**.

   Your Explore window should now look similar to the one shown in Figure 5-16.

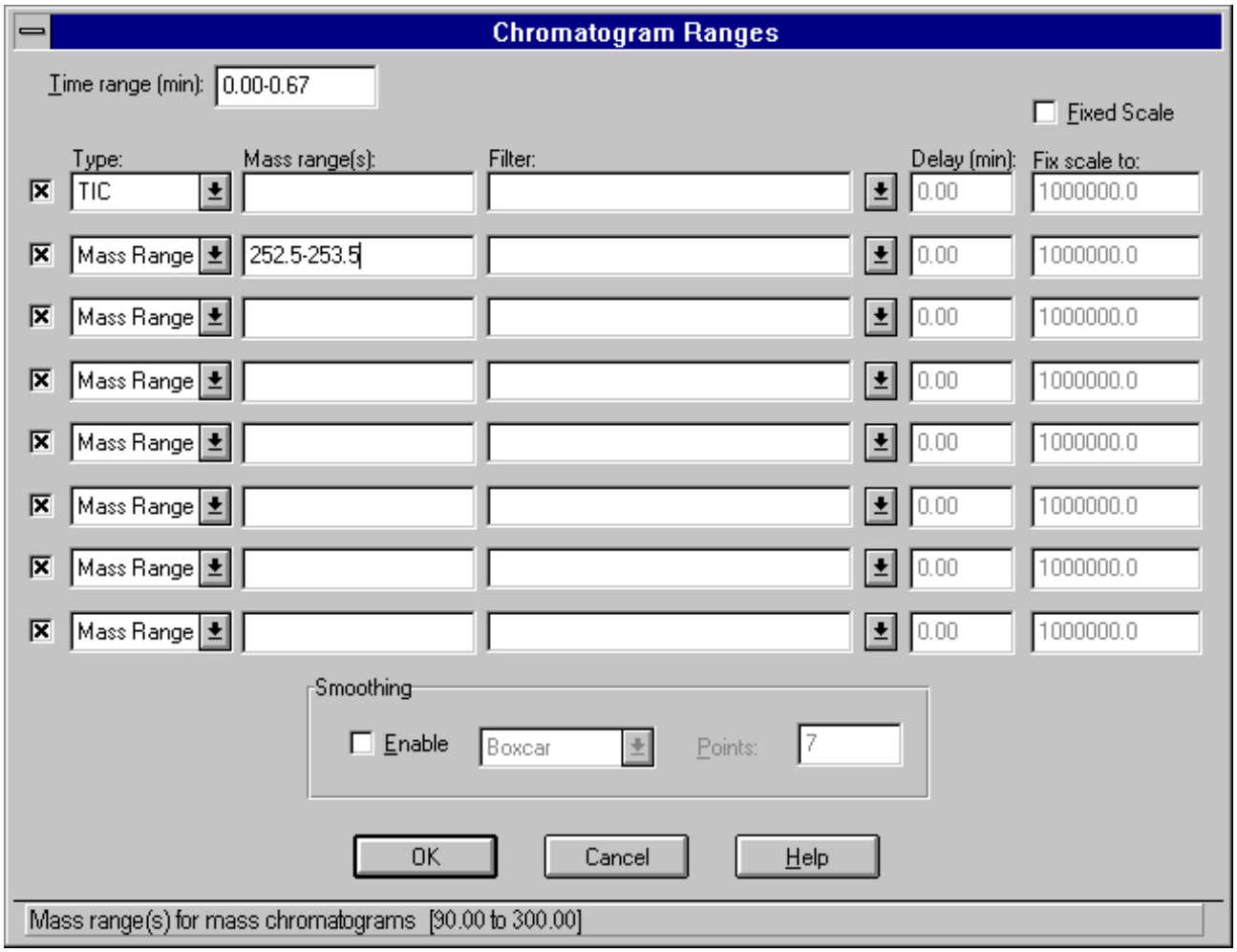


Figure 5-15. Chromatogram Ranges dialog box, showing the proper settings to display a *m/z* 253.1 mass chromatogram

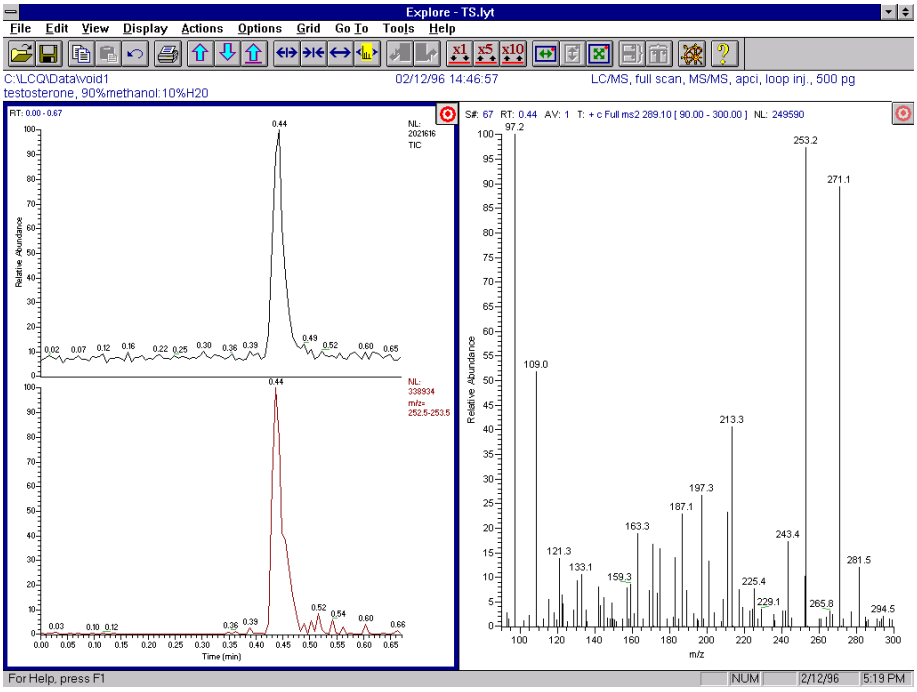


Figure 5-16. Explore window showing the TIC (upper-left) and *m/z* 253.1 mass chromatogram (lower-left) in the active cell and the full scan MS/MS spectrum of testosterone in the inactive cell (right)

You can see from Figure 5-16 that the unretained testosterone peak elutes from the LC column at approximately 0.44 min in a 90% methanol:10% H_2O solvent system at a flow rate of 1 mL min^{-1}. Therefore, in this example the column void volume is approximately 0.44 min.

10. Restore the Tune Plus window as follows:
First, reopen the Navigator window by choosing **Go To | Navigator** in Explore. Then, click on the Tune Plus button on the toolbar in Navigator.

5.3.2 Adjusting the Retention Time (RT) of the LC Peak

In this example, you adjust the RT of the LC peak for testosterone by varying the organic:aqueous solvent ratio. In this case, the goal is to find the methanol:H_2O ratio that will retain the target analyte on the LC column for approximately 1.5 to 2 min.

Use the following procedure to adjust the retention time of testosterone on your LC column:

1. Set a gradient on the LC you are using to gradually bring the solvent composition to 50% methanol:50% H_2O over a 5 min period.

2. If you have not already done so, turn on the LC pumps to flush the methanol:H_2O solution into the APCI source at a flow rate of approximately 1 mL min^{-1}.

3. Once the LC reaches the desired solvent composition of 50% methanol:50% H_2O, allow your column to equilibrate for 10 min at this solvent composition.

4. Load the testosterone solution into the sample loop with the divert/inject valve in the Load position.

5. Acquire full scan MS/MS data for loop injections of testosterone, as follows:

   a. Open the Acquire Data dialog box, as follows:
   Click on the Acquire Data button in the Instrument Control toolbar in the Tune Plus window. See Figure 5-17.

   b. Specify the name of the raw file as *LC1* in the Acquire Data dialog box, as follows:
   Double-click in the Filename text box, then type: **LC1**.

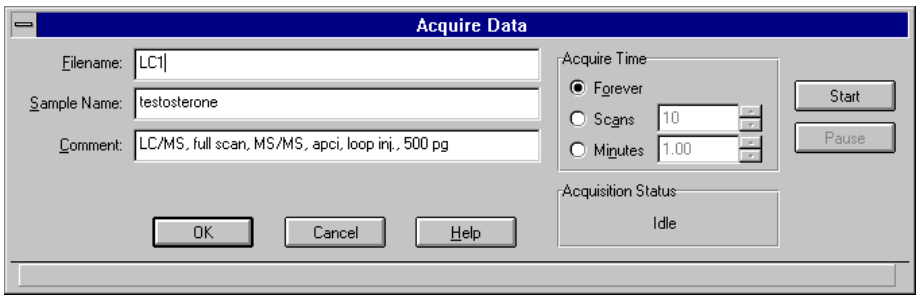


Figure 5-17. Acquire Data dialog box showing sample information for LC/MS data acquisition

   c. Specify the sample name as testosterone, as follows:
   Double-click in the Sample Name text box, then type **testosterone**.

d. Specify the information you want to appear on a hardcopy of the data, such as the scan mode, ionization method, sample amount, and method of sample introduction, as follows:
Double-click in the Comment text box, then type **LC/MS, full scan MS/MS, apci, loop inj., 500 pg**.

e. Select to acquire data continuously in the Acquire Time group box, as follows:
Click on the Continuously option button.

LCQ displays the current acquisition status, *Idle*, in the Acquisition Status group box.

f. Begin acquiring data, as follows:
Click on **Start**. LCQ displays the current acquisition status, *Acquisition in Progress*, in the Acquisition Status group box.

6. Inject the testosterone solution into the APCI source.

7. Return the divert/inject valve to the Load position.

LCQ displays the trace of the loop injections in the Graph view as well as the file name and directory *C:\LCQ\Data\LC1.raw*.

Observe the trace of the testosterone peak in the Graph view. Your Tune Plus window should now look like the one shown in Figure 5-18.

8. When the peak is finished eluting from the column, stop the data acquisition in the Acquire Data group box, as follows:
Click on **Stop**.

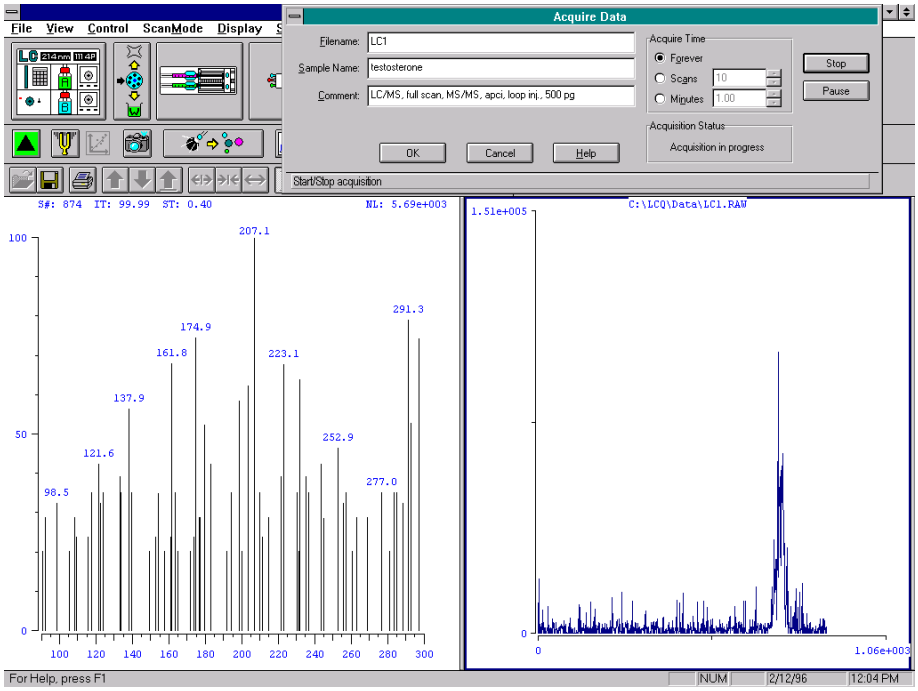


Figure 5-18. Tune Plus window, showing LC/MS analysis of testosterone in real-time

5.3.2.1 Analyzing the Chromatogram in Explore

1. Return to the Explore window as follows:
   First, hold down the **<Alt>** key. Next, press the **<Tab>** key until the Explore button appears. Finally, let go of the **<Alt>** key.

2. Open the *LC1.raw* raw file, as follows:

   a. Display the Open Layout or Open Raw File dialog box, as follows:
      Choose **File | Open**.

   b. Select the file *LC1.raw* in the directory *C:\LCQ\Data*, as follows:
      Scroll down in the File Name combo box until you see *LC1.raw*. Then, click on the file name.

   c. Open the file, and close the dialog box, as follows:
      Click on **OK**. LCQ opens the raw file in the active cell of the Explore window.

The open layout file, *LC1.lyt*, automatically displays the raw file in the previously saved cell layout and automatically updates chromatogram and spectrum ranges and any other options stored in the layout file.

Your Explore window should now look similar to the one shown in Figure 5-19.

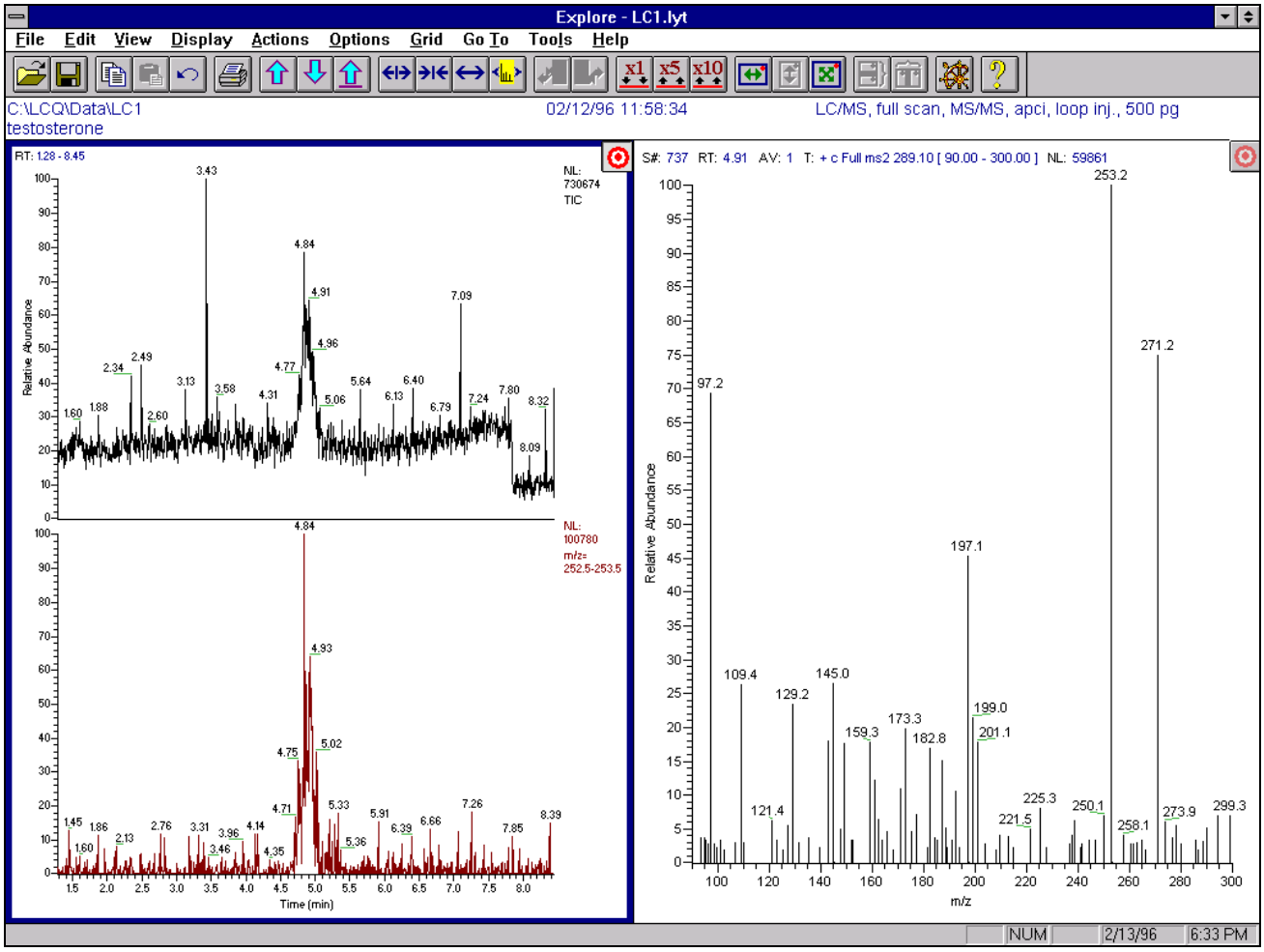


Figure 5-19. Explore window showing the TIC (upper-left) and *m/z* 253.1 mass chromatogram (lower-left) in the active cell and the full scan MS/MS spectrum of testosterone in the inactive cell (right)

You can see from that the testosterone peak elutes from the LC column at approximately 4.9 min in a 50% methanol:50% H_2O solvent system at a flow rate of 1 mL min^{-1}. It is therefore necessary to decrease the retention of the target analyte on the LC column by increasing the organic ratio in the solvent system and repeating the same procedure to determine the observed RT using the new solvent system.

Try increasing the organic ratio to 55% methanol:45% H_2O and repeat the above procedure. Continue increasing the amount of methanol in the solvent until the testosterone LC peak elutes at approximately 1.5 min.

Contents

List of Illustrations

LC/MS/MS ANALYSIS OF SAMPLES IN A MIXTURE WITH AUTOSAMPLER INJECTION

This chapter provides instructions for the LC/MS/MS analysis of samples in a mixture with autosampler injection. In this example you identify and quantitate testosterone and a deuterium-labeled internal standard in the full scan APCI/MS/MS mode. Alternatively, you can analyze any target analyte and internal standard combination for your particular application using the same procedure.

In this example, testosterone and d3-testosterone coelute from an LC analytical column. It is therefore necessary to perform alternating product ion scans to collect data in a single acquisition. To acquire data using alternating product ion scans with autosampler injection, you need to use an Experiment Method and run in the Navigator window.

To set up an Experiment Method and acquire data in the Navigator window, you do the following:

- Create an Experiment Method in the Experiment Method window.
- Run a Sample List using the Sample List window.
- Monitor acquisition of raw files in the Navigator window.

Note. Before you begin any of the procedures outlined in this chapter, ensure the following: Your APCI/MS system is properly set up as described in the chapter: **Tuning in the APCI/MS Mode**.

6.1 Creating an Experiment Method in the Experiment Method Window

In this example you create an Experiment Method, and name it *tcurve.emd*. This is the Experiment Method you use for LC/MS analysis of a mixture of testosterone and d3-testosterone. The Experiment Method is the specific set of parameters used to operate the autosampler, LC pumps, and MS detector during an analysis. Instructions are provided for the following:

- Opening the Experiment Method window
- Setting the autosampler parameters
- Setting the LC parameters
- Setting the LC column/solvent information
- Setting the MS detector parameters

6.1.1 Opening the Experiment Method Window

Use the following procedure to open the Experiment Method window:

1. Open the LCQ data system, as follows:
   Double-click on the LCQ Navigator icon in the LCQ program group in the Program Manager.

1. Open the Experiment Method window, as follows:
   Click on the Experiment Method button in the Navigator toolbar.

2. Ensure that LCQ displays an Experiment Method window similar to the one shown in Figure 6-1.

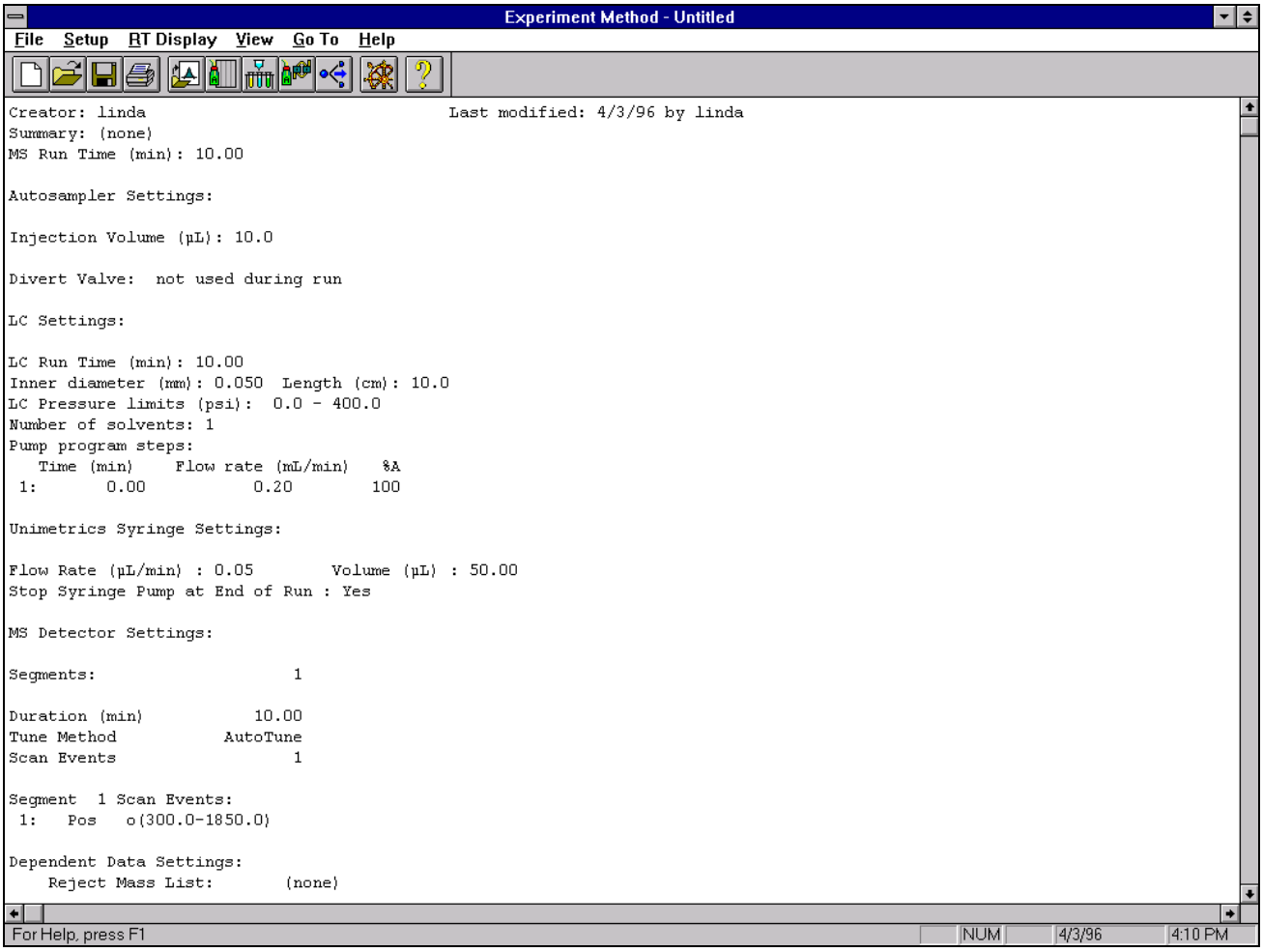
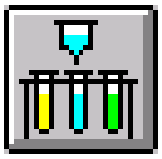


Figure 6-1. Experiment Method window, showing the method summary for an untitled Experiment Method

6.1.2 Setting the Autosampler Parameters

Use the following procedure to set the autosampler parameters:



1. Open the Autosampler dialog box, as follows:
   Click on the Setup Autosampler button in the toolbar.
   See Figure 6-2.

2. For this example, leave the default injection volume set to its default value of 10.0 µL.

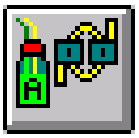
Figure 6-2. Autosampler dialog box, showing the default injection volume set to 10 μL

3. Enter the autosampler settings and close the dialog box, as follows:
   Click on **OK**.

6.1.3 Setting the LC Parameters

In this example, you set the following LC parameters for LC/MS analysis of testosterone and d3-testosterone using the results you obtained in the topic: **Developing a Method for LC/MS Analysis of Testosterone**, in the chapter**: Developing Methods for LC/MS/MS Analysis of Testosterone in the APCI Mode.**

Set the LC parameters as follows:



1. Open the Setup HPLC dialog box, as follows:
   Click on the Setup HPLC button in the toolbar. See Figure 6-3.

2. Set an LC run time of 3 min, as follows:
   First, leave the time in row 1 set to its default value of 0.0 min. Next, double-click in the Time text box in row 2, then type **3.0**.

3. Set a constant flow rate of 1.0 mL min^{-1} as follows:
   First, double-click in the Flow Rate text box in row 1, then type **1.0**. Next, double-click in the Flow Rate text box in row 2, then type **1.0**.

4. Set a constant composition of mobile phase (isocratic conditions) during the LC run using the aqueous:organic ratio you determined before, as follows:
   First, double-click in the %B text box in row 1, then type the percentage of B (CH_3OH) you want in the solvent mixture.

5. Next, press the **<Enter>** or **<Tab>** key. LCQ automatically sets the %A (H_2O) in the solvent mixture.

> **Note**. In the Setup HPLC dialog box solvent A automatically changes when you set the percentage(s) of other solvent(s) in the mixture, so that the final composition is always equal to 100%.

6. For this example, leave the minimum LC pressure set to its default value of 0.0 psi.

7. Set a maximum LC pressure of 4000 psi in the LC Pressure group box, as follows:
   Double-click In the Max text box, then type **4000**.

   Your Setup HPLC dialog box should look similar to the one shown in Figure 6-3.

8. Enter the LC settings and close the dialog box, as follows:
   Click on **OK**.

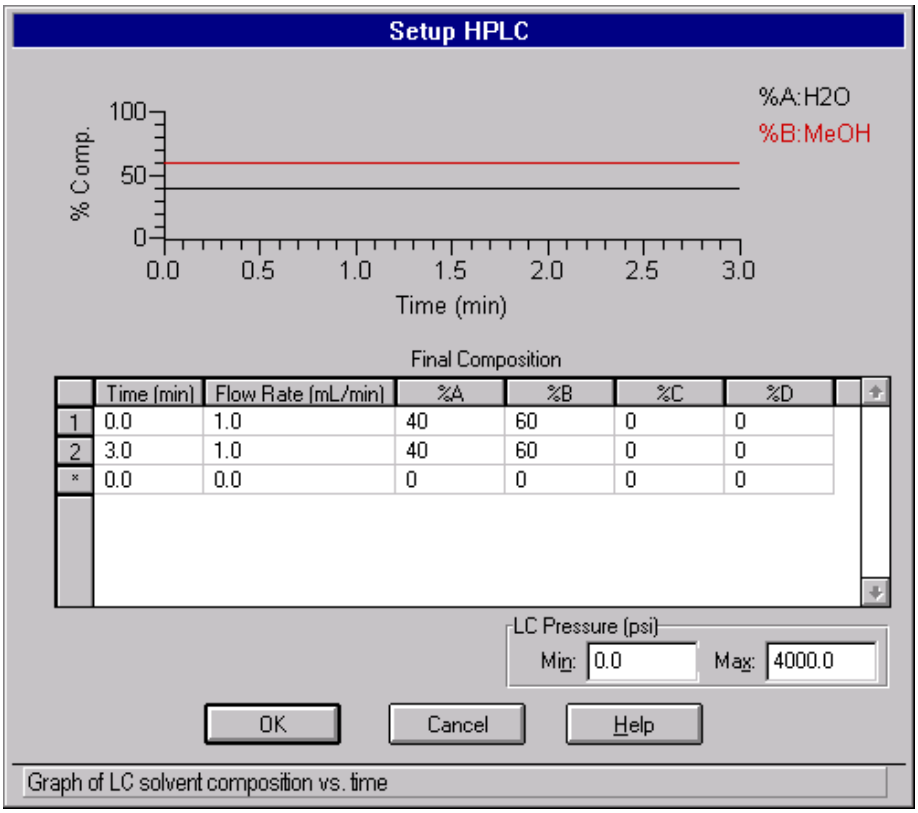
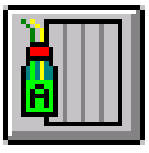


Figure 6-3. Setup HPLC dialog box, showing the proper settings for LC/MS analysis of testosterone

6.1.4 Entering the LC Column and Solvent Information

Enter the LC column and solvent information according to your particular LC column and the solvents you are using, as follows:



1. Open the LC Column/Solvent Information dialog box, as follows:
   Click on the Setup Column Solvent Info button in the toolbar. See Figure 6-4.

2. Enter the solvent names in the Solvent Names group box, as follows:
   First, click in text box A, then type **H2O**. Next, click in text box B, then type **CH3OH**.

3. Enter the type of column you are using in the LC Column group box, as follows:

Click in the Column Type text box, then type the name of your particular LC column.

4. Enter the inner diameter of the column you are using, as follows:
   Click in the ID text box in the LC Column group box, then type the inner diameter of your particular column.

5. Enter the length of the column you are using, as follows:
   Click in the Length text box in the LC Column group box, then type the length of your particular column.

6. Enter the LC column and solvent information and close the dialog box, as follows:
   Click on **OK**.

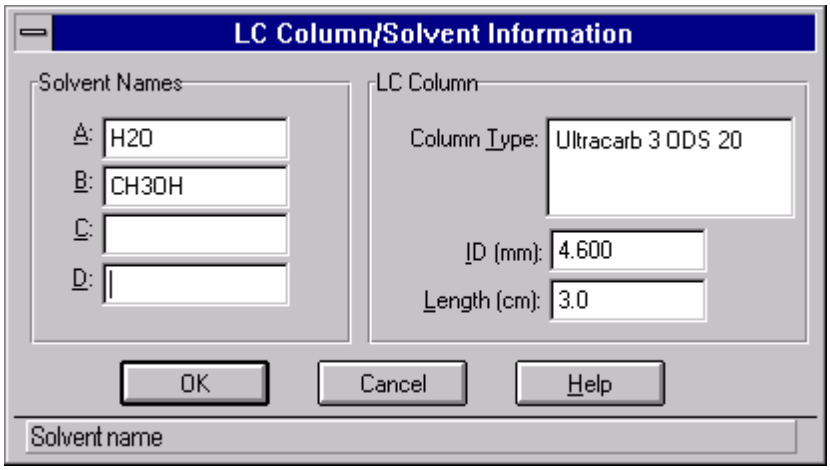


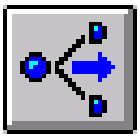
Figure 6-4. LC Column/Solvent Information dialog box, showing typical column and solvents used for LC/MS analysis of testosterone

6.1.5 Setting the MS Detector Parameters

In this example you perform LC/MS analysis on a mixture of testosterone and an isotopically labeled internal standard, d3-testosterone. Alternatively, you can analyze any target analyte and internal standard combination for your particular application using the same procedure.

Because in this example, the target analyte (testosterone) and the internal standard (d3-testosterone) are structurally identical, and differ only in their molecular weights, they co-elute from an LC column. To detect both the target analyte and internal standard in the same acquisition, you set up the MS detector to have one scan segment containing 2 scan events. A scan segment refers to a time period during the chromatographic run. Since both compounds elute at the same retention time, only one scan segment is necessary. Scan event 1 contains scan settings for detection of the target analyte, and scan event 2 contains settings for detection of the internal standard. During an acquisition, the MS detector continuously alternates the scan events such that it detects the target analyte product ions 50% of the time, and the internal standard product ions 50% of the time.

Use the following procedure to set the MS Detector parameters:



1. Open the MS Detector Setup dialog box, as follows:
   Click on the Setup MS Detector button in the toolbar.
   See Figure 6-5.

2. Set the total acquisition time to 3 min in the Run Settings group box, as follows:
   Double-click in the MS Acquire Time text box, then type **3**.

3. Set the total number of segments to 1, as follows:
   Double-click in the Segments spin box, then type **1**.

4. Select the *testosterone.tun* Tune Method for the active segment, as follows:

   a. Display the Open dialog box, as follows:
      Click on the Browse button in the Segment Settings group box.

   b. Select the file *testosterone.tun* in the directory *C:\LCQ\Methods*, as follows:
      Scroll down in the File Name combo box until you see *testosterone.tun*. Then, click on the file name.

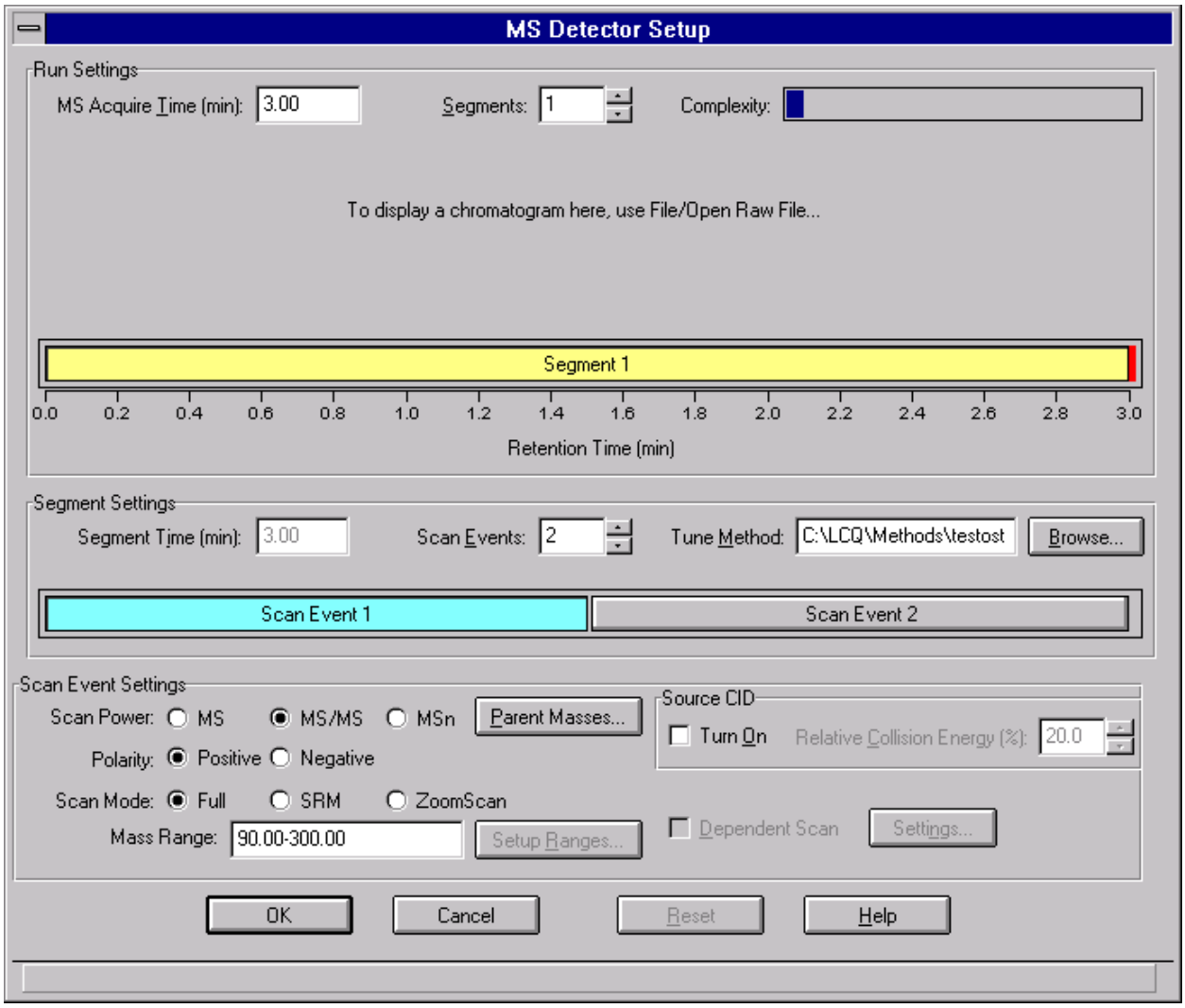


Figure 6-5. MS Detector Setup dialog box, showing the proper settings for alternating product ion scans for target analyte and internal standard

c. Enter the file, and close the dialog box, as follows:
Click on **OK**. LCQ displays
C:\LCQ\Methods\testosterone.tun in the Tune Method text box.

5. Set the number of scan events for the time segment to 2 in the Segment Settings group box, as follows:
Double-click in the Scan Events spin box, then type **2**.

6. Set the scan parameters for testosterone in the Scan Event Settings group box, as follows:

a. Activate the Scan Event 1 bar in the Segment Settings group box, as follows:
Click on the Scan Event 1 bar.

b. Select the MS/MS scan power in the Scan Event Settings group box, as follows:
Click on the Scan Power: MS/MS option button.

c. Select the Positive ion polarity, as follows:
Click on the Polarity: Positive option button.

d. Select the full scan mode, as follows:
Click on the Full option button.

e. Set the mass range to *m/z* 90 to 300, as follows:
Double-click in the Mass Range text box, then type **90-300**.

f. For this example, leave the source CID set to its default value of Off.

7. Set the parent mass, isolation width and relative collision energy to the value determined in the topic: **Determining the Relative Collision Energy for an MS/MS Experiment**, in the chapter: **Developing Methods for LC/MS/MS Analysis of Testosterone in the APCI Mode**, as follows:

   a. Display the Set Parent Mass dialog box, as follows:
   Click on the Parent Masses button in the Scan Event Settings group box. See Figure 6-6.

   b. Set the parent mass to the parent mass of testosterone, *m/z* 289.1, as follows:
   Double-click in the Parent Mass text box, then type **289.1**.

   c. Set the isolation width to *m/z* 2.0, as follows: Double-click in the Isolation Width text box, then type **2.0**.

   d. Set the Relative Collision Energy to 14%, as follows:
   Double-click in the Relative Collision Energy spin box, then type **14**.

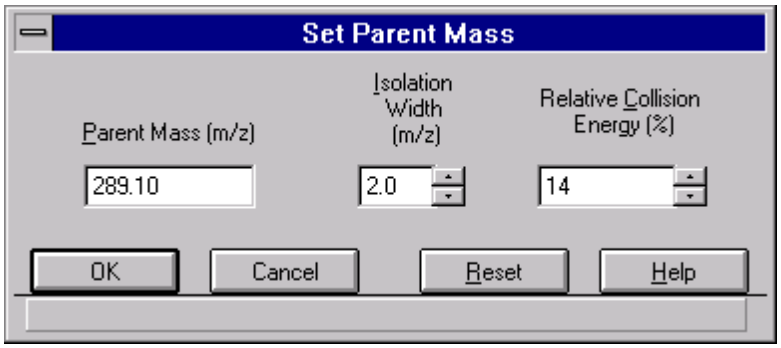


Figure 6-6. Set Parent Mass dialog box, showing the proper settings for detection of the testosterone product ions

e. Enter the parameters and close the dialog box, as follows:
Click on **OK**.

2. Set the scan parameters for d3-testosterone in the Scan Event Settings group box, as follows:

c. Activate the Scan Event 2 bar in the Segment Settings group box, as follows:
Click on the Scan Event 2 bar.

d. Select the MS/MS scan power in the Scan Event Settings group box, as follows:
Click on the Scan Power: MS/MS option button.

e. Select the Positive ion polarity, as follows:
Click on the Polarity: Positive option button.

f. Select the full scan mode, as follows:
Click on the Scan Mode: Full option button.

g. Set the mass range to *m/z* 90 to 300, as follows:
Double-click in the Mass Range text box, then type **90-300**.

h. For this example, leave source CID set to its default value of Off.

3. Set the parent mass, isolation width and relative collision energy to the values determined in the topic: **Determining the Relative Collision Energy for an MS/MS Experiment**, in the chapter: **Developing Methods for LC/MS/MS Analysis of Testosterone in the APCI Mode**, as follows:

a. Display the Set Parent Mass dialog box, as follows: Click on the Parent Masses button in the Scan Event Settings group box.

b. Set the parent mass to the parent mass of d3-testosterone, *m/z* 292.1, as follows: Double-click in the Parent Mass text box, then type **292.1**.

c. Set the isolation width to *m/z* 2.0, as follows: Double-click in the Isolation Width text box, then type **2.0**.

d. Set the Relative Collision Energy to 14%, as follows: Double-click in the Relative Collision Energy spin box , then type **14**.

e. Enter the parameters and close the dialog box, as follows: Click on **OK**.

Your MS Detector Setup dialog box should look similar to the one shown in Figure 6-5.

6.2 Running a Sample List Using the Sample List Window

To start an acquisition, you run a Sample List. The Sample List contains all of the methods needed to run your particular analysis. You run the Sample List and then monitor data acquisition in the Navigator window. You analyze the calibration standards you prepare in **Appendix A: Sample Formulations**. The LCQ data system contains a customized Sample List, *tcurve.sld*, which you can use to perform an LC/MS/MS analysis of testosterone and d3-testosterone.

To run a Sample List, do the following:

1. Open the Sample List window from the Experiment Method window, as follows:
   Choose **Go To | Sample List.** See Figure 6-7**.**

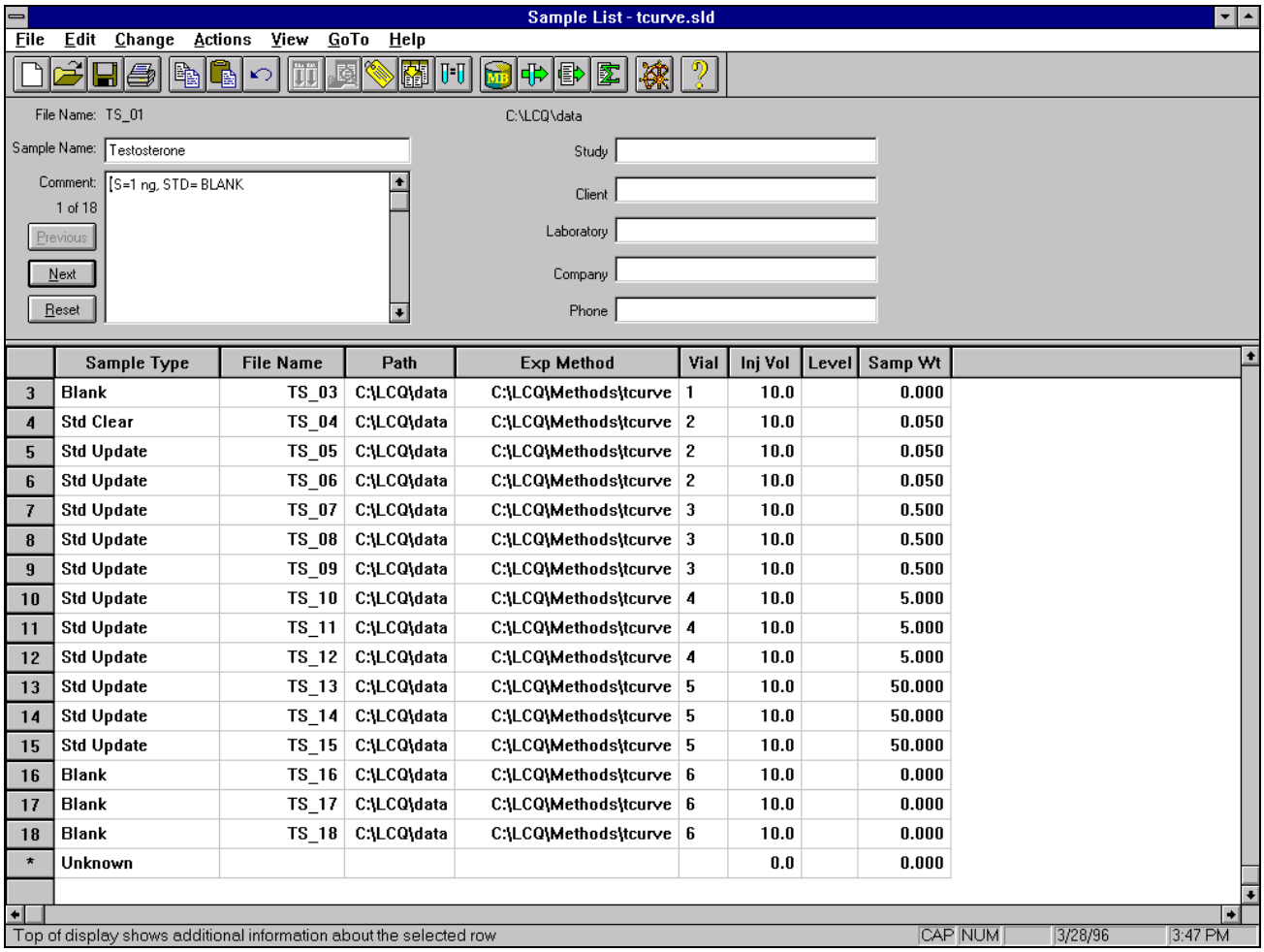
2. Open the *tcurve.sld* Sample List, the Sample List you use to analyze the testosterone and d3-testosterone calibration levels, as follows:

   a. Display the Open dialog box, as follows:
      Choose **File | Open**.

   b. Select the file *tcurve.sld* in the directory *C:\LCQ\Methods*, as follows:
      Scroll down in the File Name combo box until you see *tcurve.sld*. Then, click on the file name.

   c. Open the file, and close the dialog box, as follows:
      Click on **OK**.

      Your Sample List window should look like the one shown in Figure 6-7.



	Sample Type	File Name	Path	Exp Method	Vial	Inj Vol	Level	Samp Wt
3	Blank	TS_03	C:\LCQ\data	C:\LCQ\Methods\tcurve	1	10.0		0.000
4	Std Clear	TS_04	C:\LCQ\data	C:\LCQ\Methods\tcurve	2	10.0		0.050
5	Std Update	TS_05	C:\LCQ\data	C:\LCQ\Methods\tcurve	2	10.0		0.050
6	Std Update	TS_06	C:\LCQ\data	C:\LCQ\Methods\tcurve	2	10.0		0.050
7	Std Update	TS_07	C:\LCQ\data	C:\LCQ\Methods\tcurve	3	10.0		0.500
8	Std Update	TS_08	C:\LCQ\data	C:\LCQ\Methods\tcurve	3	10.0		0.500
9	Std Update	TS_09	C:\LCQ\data	C:\LCQ\Methods\tcurve	3	10.0		0.500
10	Std Update	TS_10	C:\LCQ\data	C:\LCQ\Methods\tcurve	4	10.0		5.000
11	Std Update	TS_11	C:\LCQ\data	C:\LCQ\Methods\tcurve	4	10.0		5.000
12	Std Update	TS_12	C:\LCQ\data	C:\LCQ\Methods\tcurve	4	10.0		5.000
13	Std Update	TS_13	C:\LCQ\data	C:\LCQ\Methods\tcurve	5	10.0		50.000
14	Std Update	TS_14	C:\LCQ\data	C:\LCQ\Methods\tcurve	5	10.0		50.000
15	Std Update	TS_15	C:\LCQ\data	C:\LCQ\Methods\tcurve	5	10.0		50.000
16	Blank	TS_16	C:\LCQ\data	C:\LCQ\Methods\tcurve	6	10.0		0.000
17	Blank	TS_17	C:\LCQ\data	C:\LCQ\Methods\tcurve	6	10.0		0.000
18	Blank	TS_18	C:\LCQ\data	C:\LCQ\Methods\tcurve	6	10.0		0.000
*	Unknown					0.0		0.000

Figure 6-7. Sample List window, showing the Sample List method *tcurve.sld*

3. To run the Sample List and start the analysis, do the following:

   a. Display the Run Sample List dialog box, as follows:
   Choose **Actions | Run List**. See Figure 6-8.

   b. Select automated start in the Start Options group box, as follows:
   Click on the Automated option button.

   c. Select to start when ready in the Start Options group box, as follows:
   Select [x] the Start When Ready check box.

d. Run the Sample List and start the data acquisition, as follows:
Click on **OK**. LCQ displays the Navigator window.

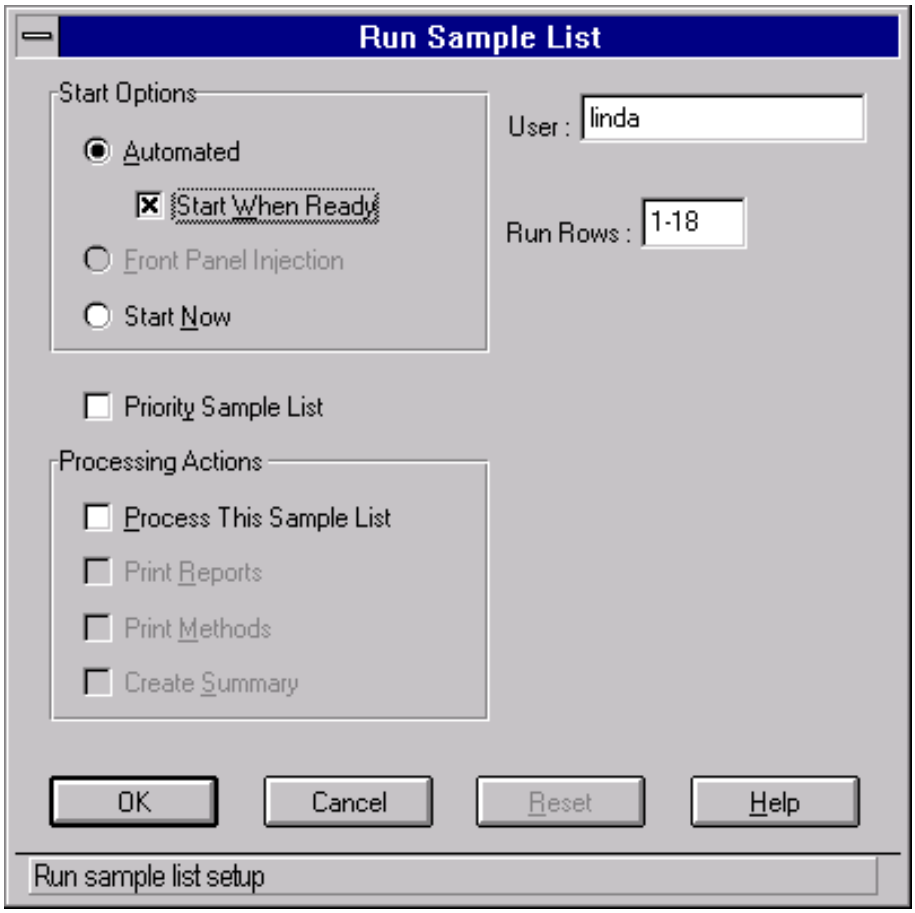


Figure 6-8. Run Sample List dialog box, showing the proper settings to start an analysis

6.3 Monitoring Acquisition of Raw Files in the Navigator Window

You can now monitor the real-time display of the mass spectrum and the chromatogram in Data view in the Navigator window. The Data View consists of a Spectrum view, shown at the top of the display, and a Chromatogram view, shown at the bottom of the display. You can also monitor information about the LCQ system in the Status view in the Navigator window.

Before the autosampler injection, while the Experiment Method, Tune Method, and LC parameters are downloading, your Navigator window should look similar to the one shown in Figure 6-9.

Prior to the start of the first acquisition, the following occurs:

- The Navigator downloads the Experiment and Tune Methods of the first sample in the Sample List to the MS detector.
- The Navigator downloads the Experiment Method of the first sample in the Sample List to the LC.
- The analysis state goes from Idling to Waiting on LC.
- The autosampler then injects the first sample into the LC stream.
- The analysis state goes to Acquiring.

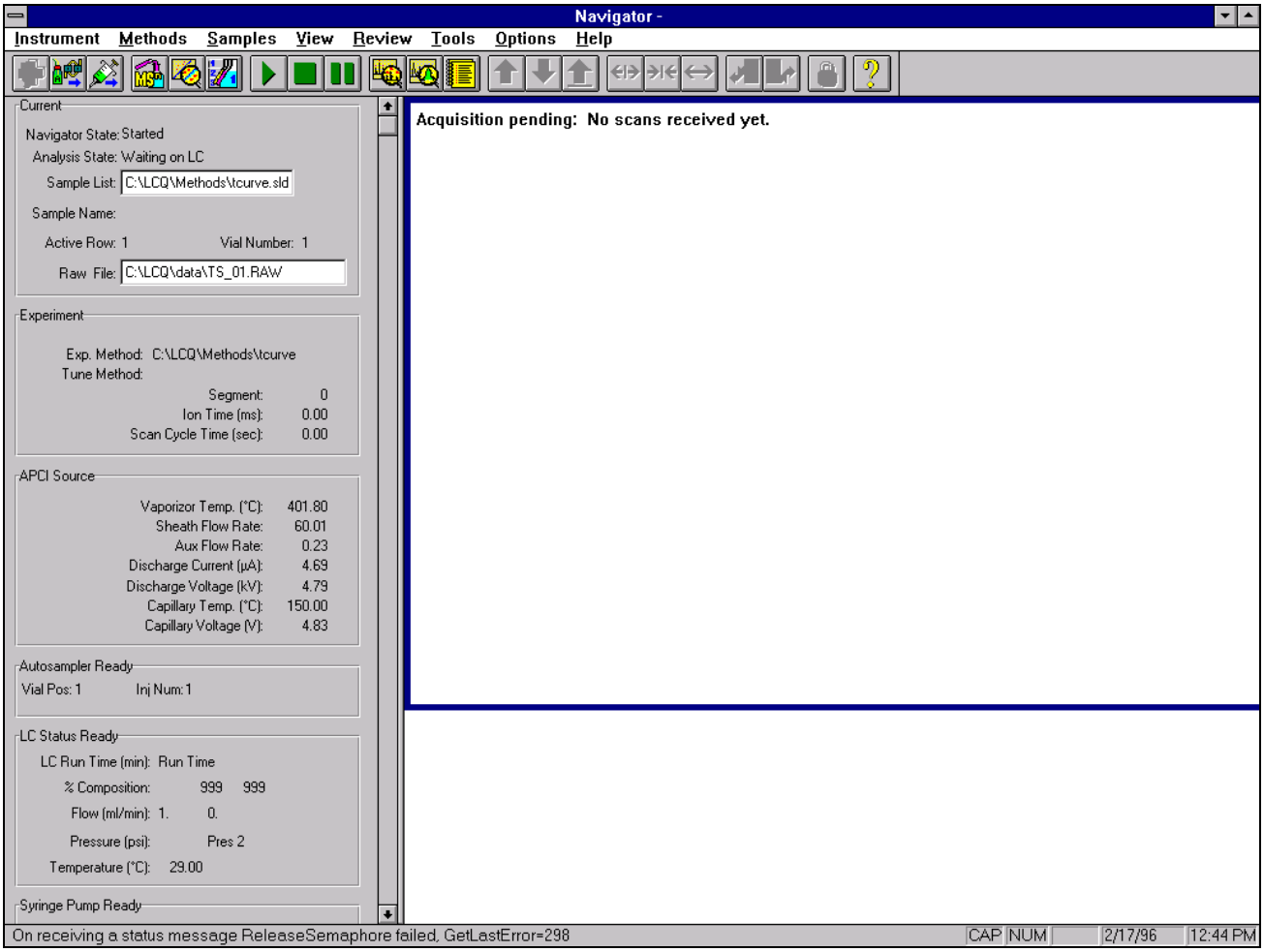


Figure 6-9. Status view (left) and Data view (right) of the Navigator window, showing a real-time display before autosampler injection

Once LCQ downloads the methods to the MS detector and the LC, the autosampler begins its inject sequence. The run begins automatically when the autosampler injects the first sample in the Sample List into the LC stream.

Observe the real-time alternating product ion scans for testosterone and d3-testosterone in the Spectrum view at the top right of the Navigator window. Also monitor the real-time total ion chromatogram displayed below.

Your Navigator window should look similar to the one shown in Figure 6-10.

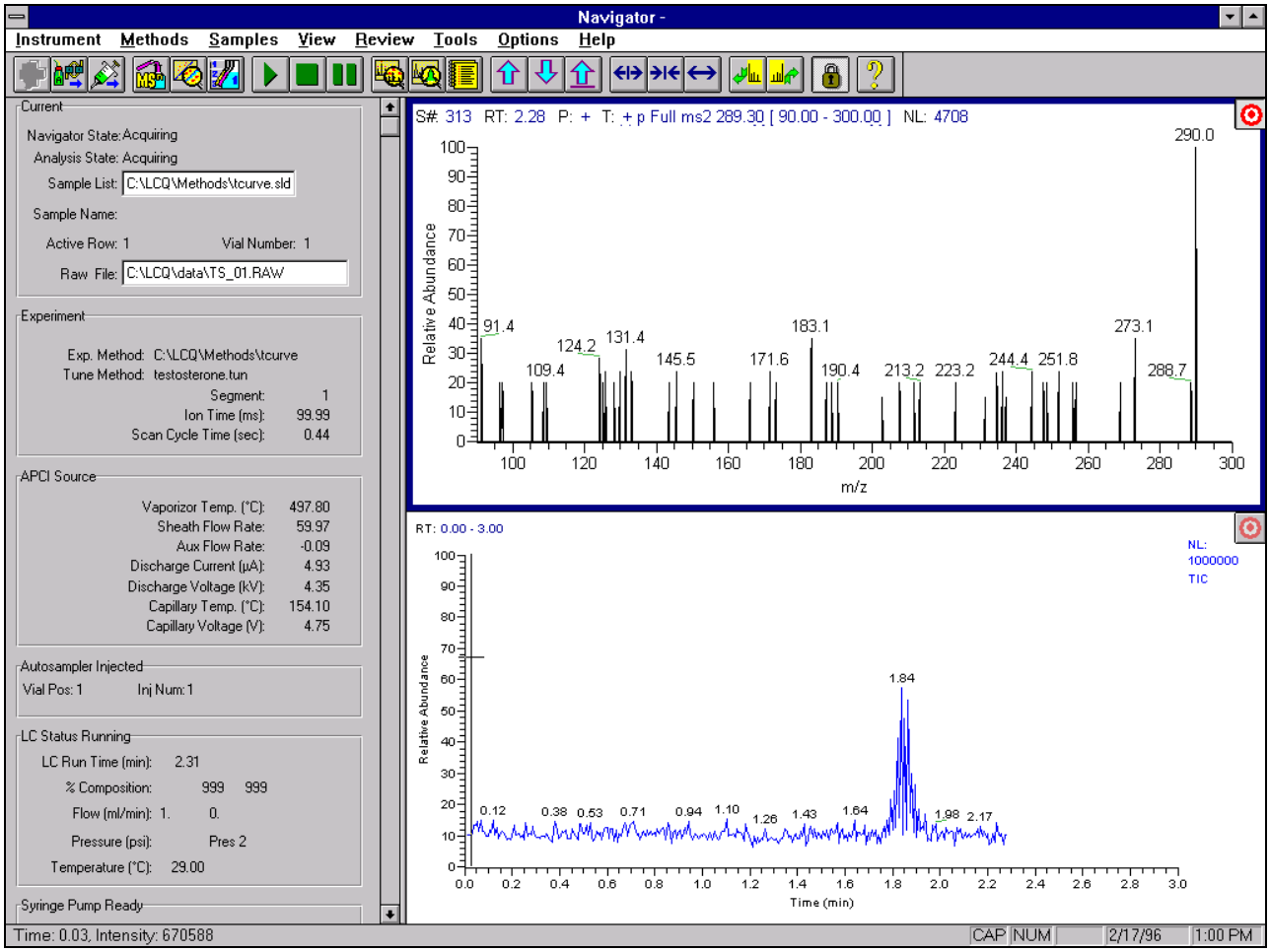


Figure 6-10. Navigator window, showing the Status view (left) and Data view (right). The Data view shows a real-time spectrum and chromatogram trace.

At the conclusion of the first acquisition, the following occurs:

- The Navigator downloads the Experiment and Tune Methods of the second sample in the Sample List to the MS detector.
- The Navigator downloads the Experiment Method of the second sample in the Sample List to the LC.
- The analysis state goes from Pending to Waiting on LC.
- The autosampler then injects the second sample into the LC stream.
- The analysis state goes to Acquiring.

At the conclusion of the acquisition sequence (all ten acquisitions), the following occurs:

- The Navigator state returns to Ready to Download.
- The analysis state returns to Idling.
- The LC pump stays on in preparation to run the next Sample List.

You are now ready to analyze your data. Go to the next chapter: **Quantitative Analysis of Data**.

Contents

List of Illustrations

QUANTITATIVE ANALYSIS OF DATA

This chapter gives a stepwise procedure for performing quantitative analysis on an example data set provided in the *C:\LCQ\examples\data* directory of your LCQ data system. In this example you use data that was collected on a proprietary pharmaceutical product in the applications laboratory at Finnigan using an LCQ MS detector. The pharmaceutical is a tertiary amine complex and was analyzed using LC/MS/MS techniques in the electrospray ionization mode.

The pharmaceutical has the code name *drugx*. An isotopically labeled internal standard (ISTD) was used to quantitate the pharmaceutical. The internal standard is a deuterated analogue of drugx and has four deuterium atoms exchanged for protons in the compound. In this example, the internal standard has the code name *D4*. The concentration of the internal standard in the calibration standards and quality control (QC) samples in this example is 100 pg mL^{-1}.

Calibration standards were prepared by spiking human plasma with drugx to give nine calibration levels with concentrations of 10, 25, 50, 100, 200, 400, 600, 800, and 1000 pg mL^{-1}. Triplicate samples were run at the high (1000 pg mL^{-1}) and low (10 pg mL^{-1}) ends of the curve with single samples run in between. The calibration curve included two plasma blanks, one with internal standard and one without internal standard.

QC samples were prepared similarly by spiking human plasma with drugx to give three QC levels with concentrations of 10, 400, and 1000 pg mL^{-1}. Six replicates per QC level were run.

You use the data files provided in the *C:\LCQ\examples\data* directory, and you use the methods provided in the *C:\LCQ\examples\Methods* directory. We are providing all of the methods used to process and collect the data originally. These include the following:

- Sample List: *drugx.sld*
- Experiment Method: *drugx.emd*
- Processing Method: *drugx.pmd*

In addition, instructions are also provided in this chapter to create your own Sample List and Processing Method. Instructions for preparing an Experiment Method are provided in the chapter: **LC/MS/MS Analysis of Samples in a Mixture with Autosampler Injection**.

You use either the Processing Method we provide you (*drugx.pmd*) or the one you create in the topic: **Creating a Processing Method Using the Processing Method Window**. LCQ uses the Processing Method to automatically determine the concentration (amount) of the sample being analyzed. How does LCQ do this? A Processing Method is created to measure the response of the LC/MS system to the compounds in your sample. The response measurement is taken from the area under each peak and is determined by an integration calculation. This measurement, known as the response factor (RF), is calculated as follows:

$$RF = \frac{\text{area of analyte}}{\text{area of ISTD}} \times \frac{\text{amount of ISTD}}{\text{amount of analyte}}$$

The goal of a calibration curve is to achieve a constant response factor by the LC/MS system to varying amounts of analyte injected. A constant response factor is indicated by linearly increasing peak area measurements with increasing amounts of analyte injected. For instance the peak area measured for a 100 pg mL^{-1} calibration standard should be twice that measured for a 50 pg mL^{-1} calibration standard. The response factor is expected to be constant with increasing amounts of analyte (because analyte area is divided by the amount of analyte injected) according to the above equation, as shown in Figure 7-1.

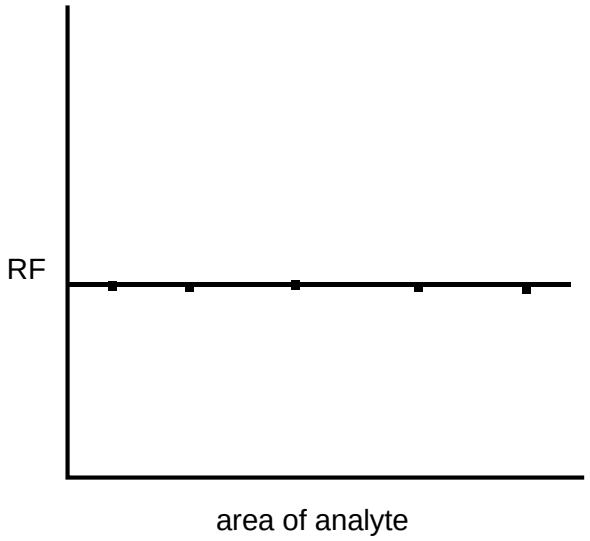


Figure 7-1. Plot of response factor (RF) vs. area of analyte

Once you have established that a particular compound has a constant response factor, amounts of unknown samples can be determined according to the same equation (in a rearranged form):

$$\text{amount of analyte} = \frac{\text{area of analyte}}{\text{area of ISTD}} \times \frac{\text{amount of ISTD}}{\text{RF}}$$

When peak areas of several known standards are measured, a plot of amount vs. area can be drawn, as shown in Figure 7-2. The LCQ data system accomplishes quantitation by comparing the integrated area of a sample peak to the standard curve and reading the amount of compound (according to the curve) that gives rise to a peak of that area. This is the calculated amount.

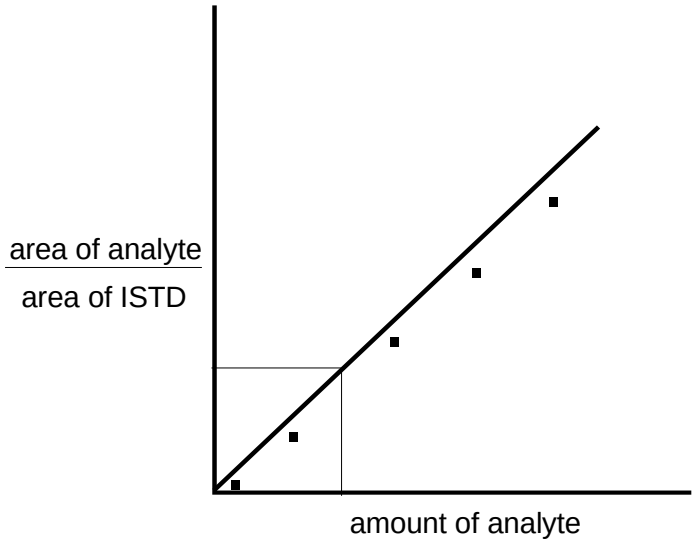


Figure 7-2. Plot of amount of analyte injected vs. area of analyte divided by area of internal standard

For a flow diagram of how to perform quantitative analysis of data with an LCQ, see Figure 7-3.

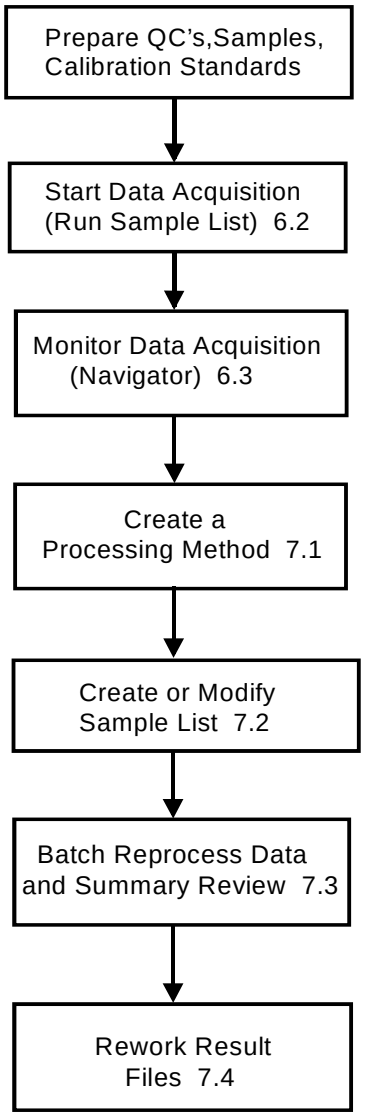


Figure 7-3. Flow diagram for quantitative analysis of data

To perform quantitative analysis on the example data in this chapter, instructions are provided for the following:

- Creating a processing method using the Processing Method window
- Creating a Sample List in the Sample List window
- Processing calibration data using the Sample List window
- Reviewing and editing the calibration data in Rework

7.1 Creating a Processing Method in the Processing Method Window

To create a Processing Method for this example, do the following:

- Start a new Processing Method in the Processing Method window.
- Open a raw file in the Processing Method window.
- Name components in the Component Identification view.
- Match scan filters with components.
- Enter peak identification information and display the spectrum.
- Enter peak integration parameters.
- Enter component calibration parameters.
- Save the Processing Method.
- Close the Processing Method.

For a flow diagram of how to create a Processing Method, see Figure 7-4.

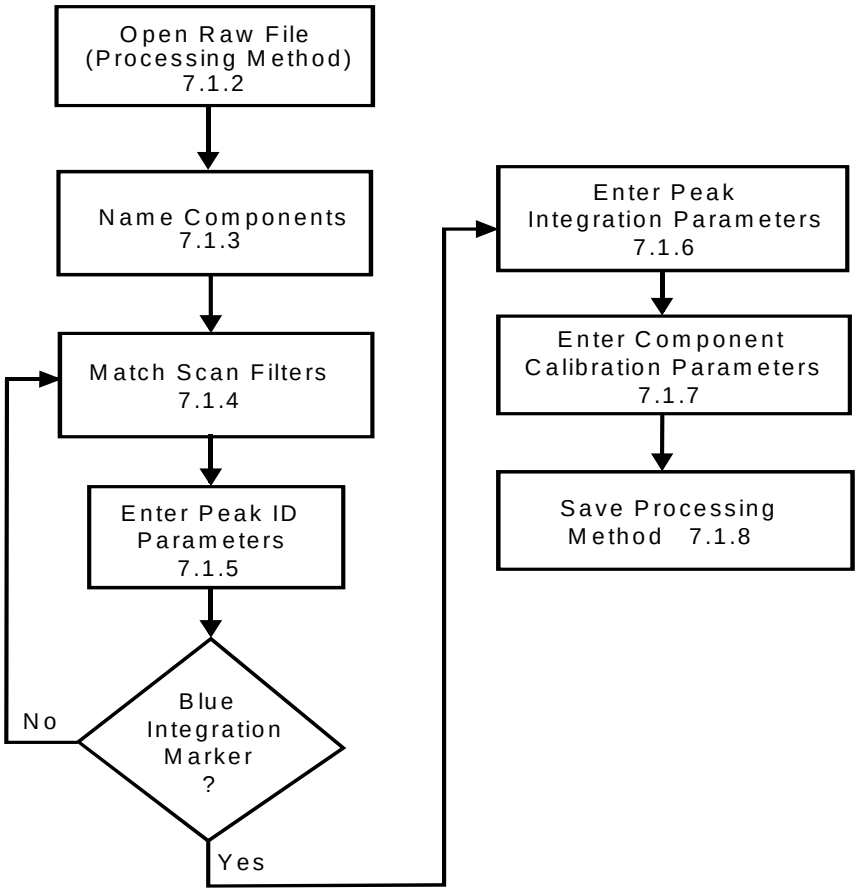
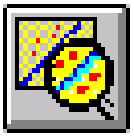


Figure 7-4. Flow diagram for creating a Processing Method

7.1.1 Starting a New Processing Method in the Processing Method Window

Start a new Processing Method in the Processing Method window, as follows:



1. Open the Processing Method window from the Navigator window, as follows:
   Click on the Processing Method button in the Navigator window.

2. Start a new Processing Method and display the Calibration Options dialog box, as follows:
   Choose **File | New**. See Figure 7-5.

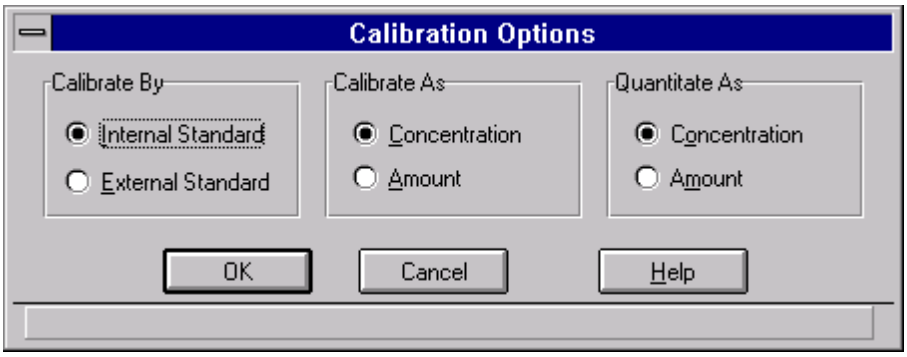


Figure 7-5. Calibration Options dialog box, showing the proper settings for calibration and quantitation of drugx using an internal standard

3. Leave the default setting of Calibrate By: Internal Standard selected to calibrate using an internal standard.

4. Leave the default setting of Calibrate As: Concentration selected to calibrate by concentration.

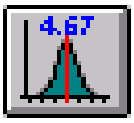
5. Leave the default setting of Quantitate As: Concentration to quantitate by concentration.

6. Start a new Processing Method and close the dialog box, as follows:
   Click on **OK**.

7.1.2 Opening a Raw File in the Processing Method Window

You create a processing method by opening a raw file in the Processing Method window and using the data contained in the file to specify peak identification parameters such as retention time and scan filters. Once the peak identification parameters are saved, you verify in the Chromatogram view of the Processing Method window that LCQ found the LC peak in your chromatogram. This is indicated by a blue integration marker.

Open a raw file contained in the *C:\LCQ\examples\data* directory in the Processing Method window, as follows:



1. Display the Component Identification view, as follows:
   Click on the Component Identification button in the Processing Method window. See Figure 7-6.

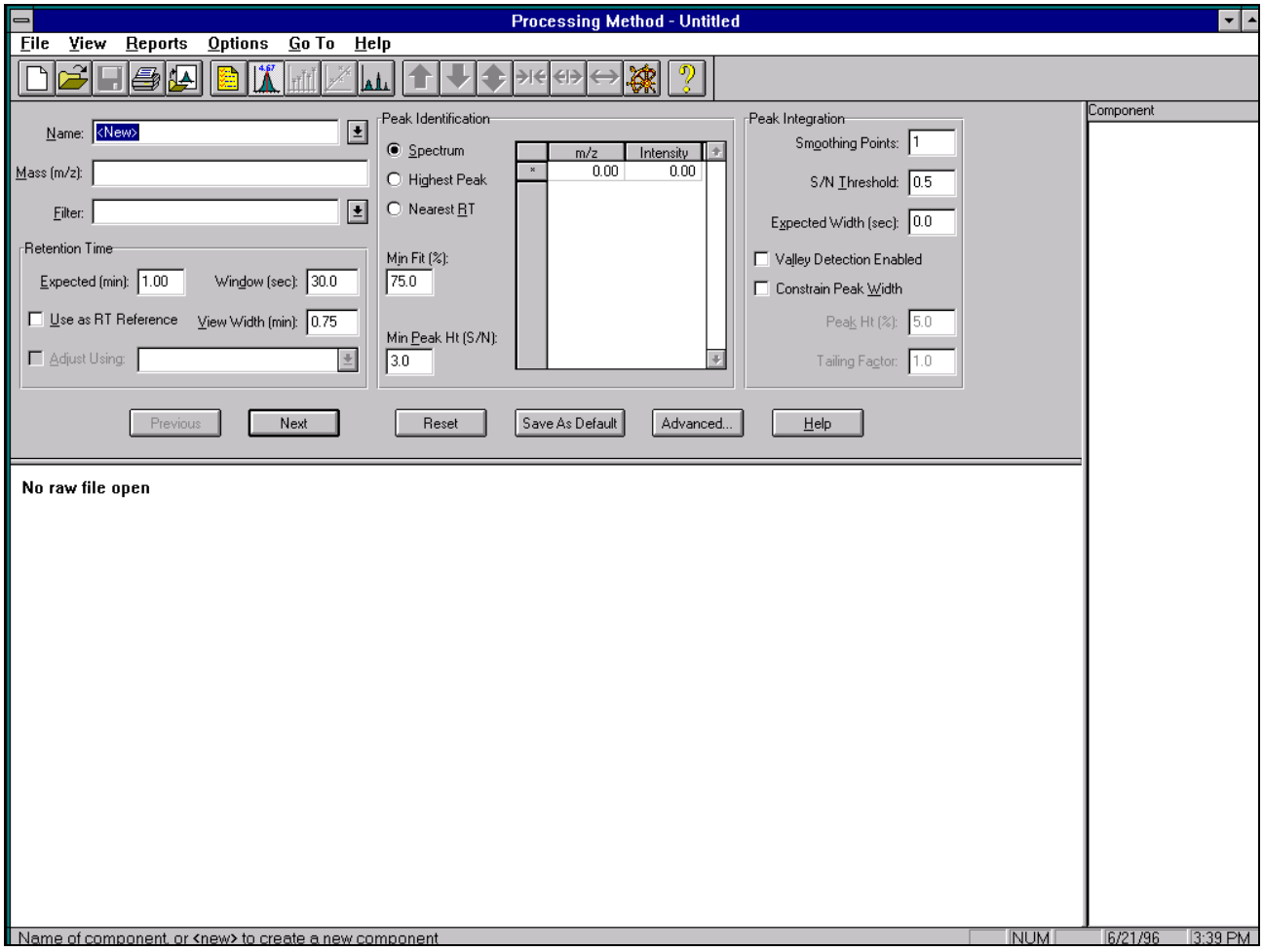


Figure 7-6. Processing Method window, showing a new untitled Processing Method

2. Display the Open Raw File dialog box, as follows:
   Click on the Open Raw button in the Processing Method window. See Figure 7-7.

3. Change to the *C:\LCQ\examples\data* directory and select the file *drugx_10.RAW*, as follows:
   Scroll down in the File Name combo box until you see *drugx_10.RAW*. Then, click on the file name.

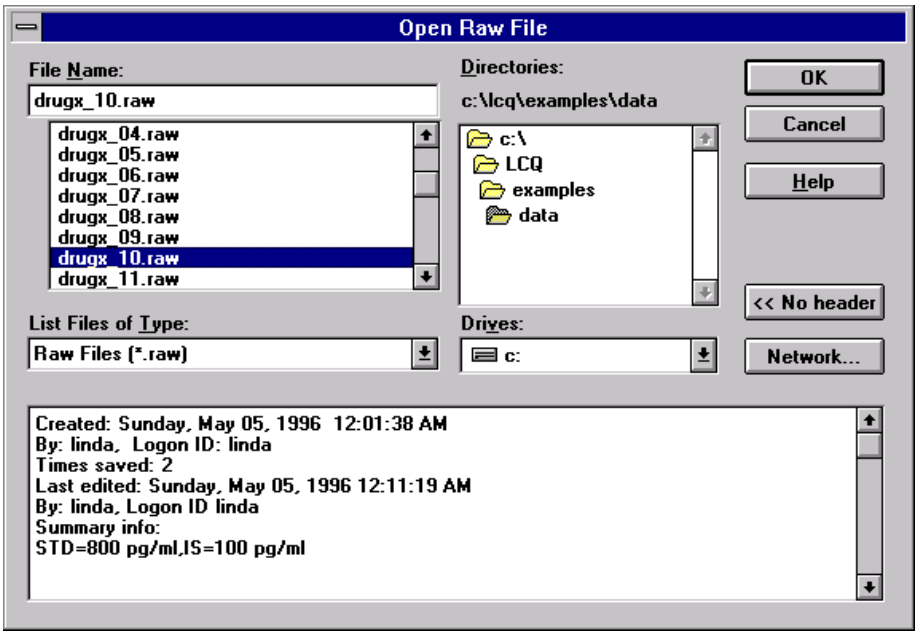


Figure 7-7. Open Raw File dialog box, showing raw files in the *C:\LCQ\examples\data* directory

4. Open the file, and close the dialog box, as follows:
   Click on **OK**. LCQ displays the total ion chromatogram on the left-hand side of the Component Identification view in the Processing Method window. See Figure 7-8.

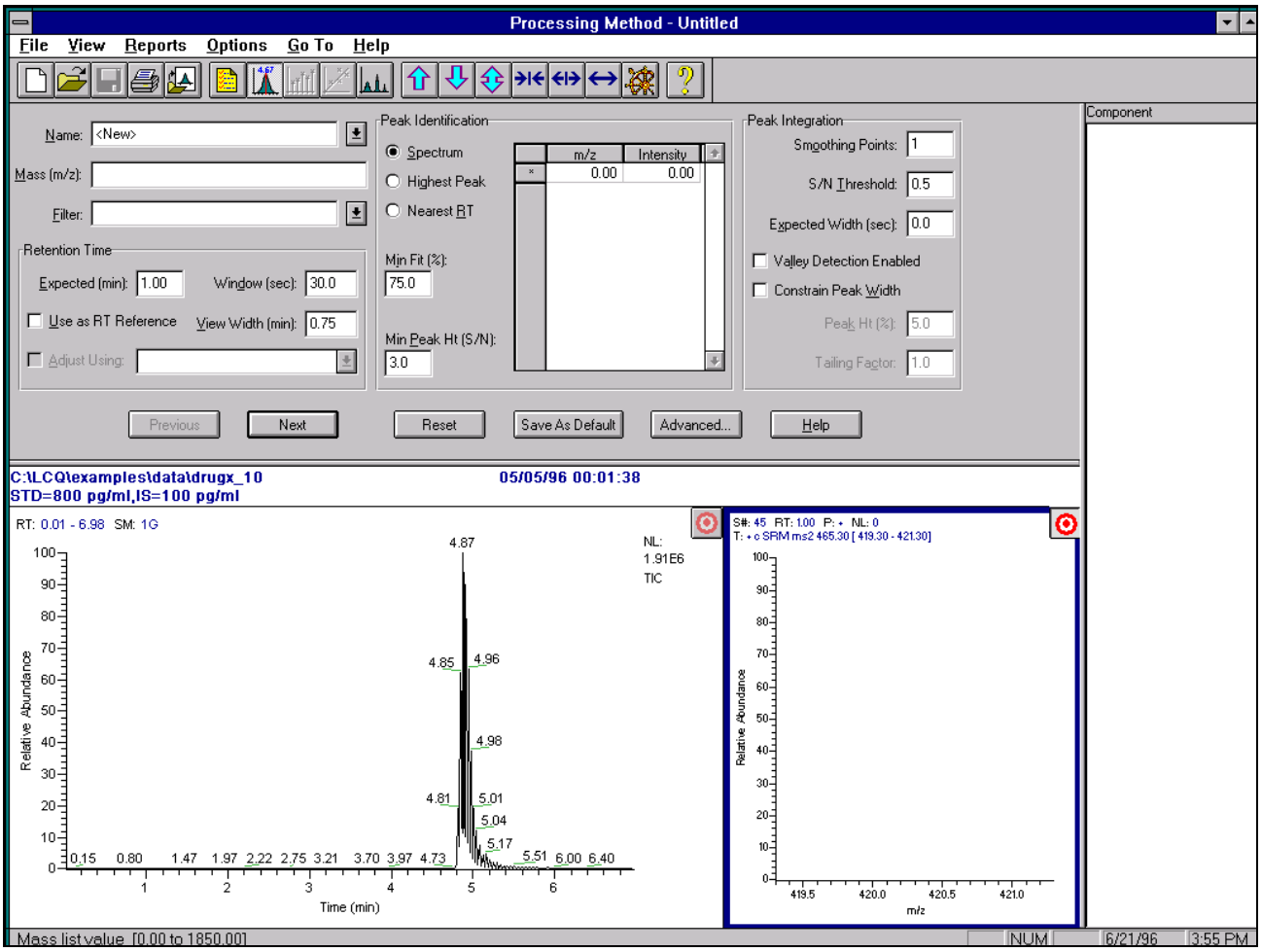


Figure 7-8. Processing Method window, showing an unfiltered total ion chromatogram in the Component Identification view

Note. The Processing Method default settings may be changed by pressing the **Save As Default** button in the Processing Method window. If the default settings in your Processing Method have been changed and are different than the ones in this example, use the parameters specified in the procedure.

7.1.3 Naming Components in the Component Identification View

Name components in the Component Identification view, as follows:

1. Enter the name of the first component as drugx (the target analyte) in the Name combo box, as follows:
   Type **drugx** then click on **OK**. See Figure 7-9.

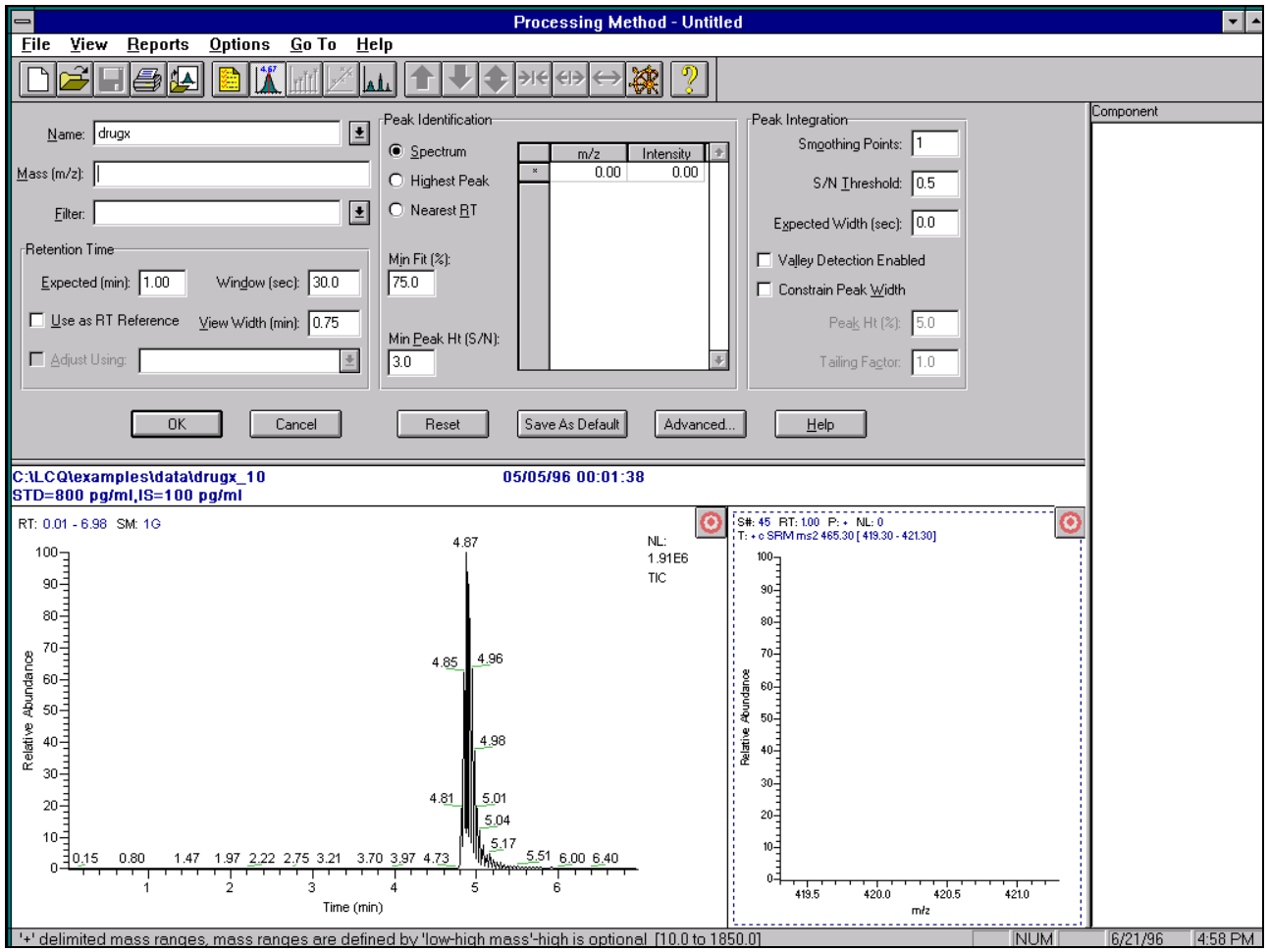


Figure 7-9. Component Identification view of the Processing Method window, showing the component drugx

> **Note**. When you click on the **OK** or **Cancel** button, LCQ changes the buttons labeled **OK** and **Cancel** to **Previous** and **Next,** respectively. Each time you edit a parameter the buttons toggle back to **OK** and **Cancel** to remind you that you need to save a change. The **Previous** and **Next** buttons indicate that you are moving between components in the Component list and no changes have been made.

2. Enter the name of the second component as D4 (the internal standard) in the Name combo box, as follows:
   First, click on **Next**. Then, double-click in the Name combo box and type **D4**. Finally, click on **OK**.

7.1.4 Matching Scan Filters with Components

LCQ creates unique scan filters to acquire data according to the type of experiment you specify. In this example, SRM data were acquired on a proprietary drug of molecular weight 465 u and a deuterated internal standard of molecular weight 469 u (drugx and D4, respectively) using alternating product ion scans. To calibrate and quantitate drugx it is necessary to filter the total ion chromatogram and match each scan filter to its corresponding component in the Processing Method.

To match each scan filter to the appropriate component in the Processing Method, do the following:

1. Display the scan filters in the file *drugx_10.RAW*, as follows:
   Click on the drop-down menu in the Filter combo box.

> **Note**. LCQ highlights D4 in the Component list in the far right side of the Processing Method window to identify it as the active component.

2. Select the filter that matches the active component, D4, as follows:
   Click on + *c SRM ms2 469.40 [423.30-425.30]*, then click on **OK**. LCQ automatically displays the filtered total ion chromatogram corresponding to the internal standard (D4). See Figure 7-10.

3. Display the target analyte component information, as follows:
   Click on **Next**. LCQ highlights the name of the active component on the right side of the window. The active component should now be the analyte, drugx.

4. Repeat step 1.

5. Select the filter that matches the target analyte (drugx), as follows:
   Click on *+ c SRM ms2 465.30 [419.30-421.30]*, then click on **OK**. LCQ automatically displays the filtered total ion chromatogram corresponding to the target analyte (drugx).

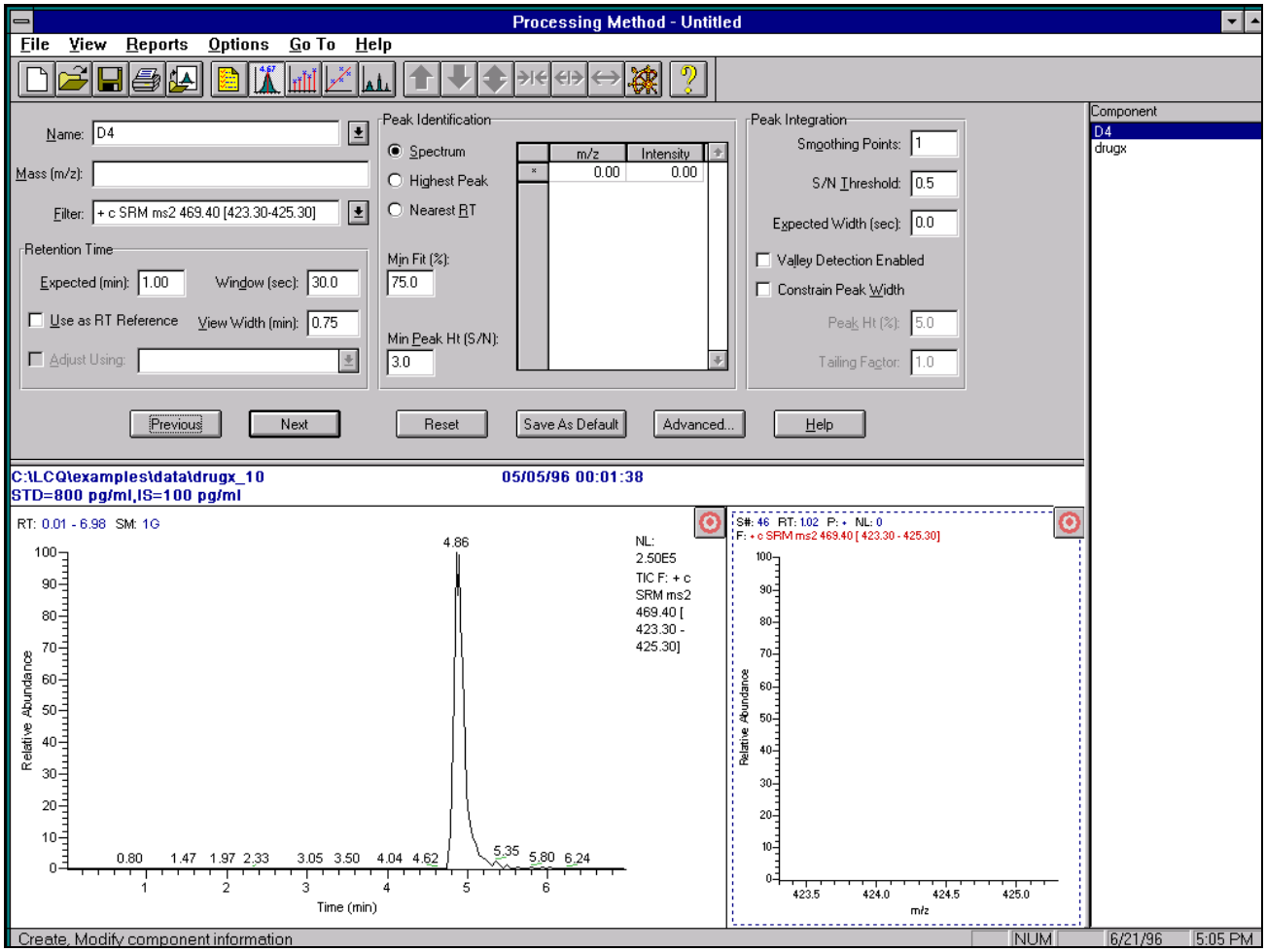


Figure 7-10. Processing Method window, showing a filtered total ion chromatogram in the Component Identification view

7.1.5 Entering Peak Identification Information and Displaying the Spectrum

To enter peak identification information, display the mass spectrum, and automatically enter the peak retention time for each component in the Processing Method, do the following:

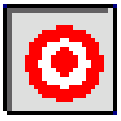
1. Select to identify drugx (currently the active component), by nearest retention time of the LC peak in the Peak Identification group box, as follows:
   Click on the Nearest RT option button.

> **Note**. LCQ automatically hides the spectrum identification mass/intensity list in the Peak Identification group box.

2. Display the mass spectrum of the currently active component and automatically enter the retention time of the LC peak, as follows:

   a. Specify a 2 min Chromatogram view width in the Retention Time group box, as follows:
   Double-click in the View Width text box and type: **2**.

   b. Leave the minimum peak height S/N set to its default value of 3.0 in the Min Peak Ht (S/N) text box in the Peak Identification group box.



   a. Activate the Spectrum view, as follows:
   Click on the Target button in the right cell in the Processing Method window.

   b. Display the mass spectrum, as follows:
   First, click and hold down the cursor just to the left side of the LC peak. Then, drag the cursor across the peak just to the right side of the peak. Finally, let go of the cursor. See Figure 7-11.

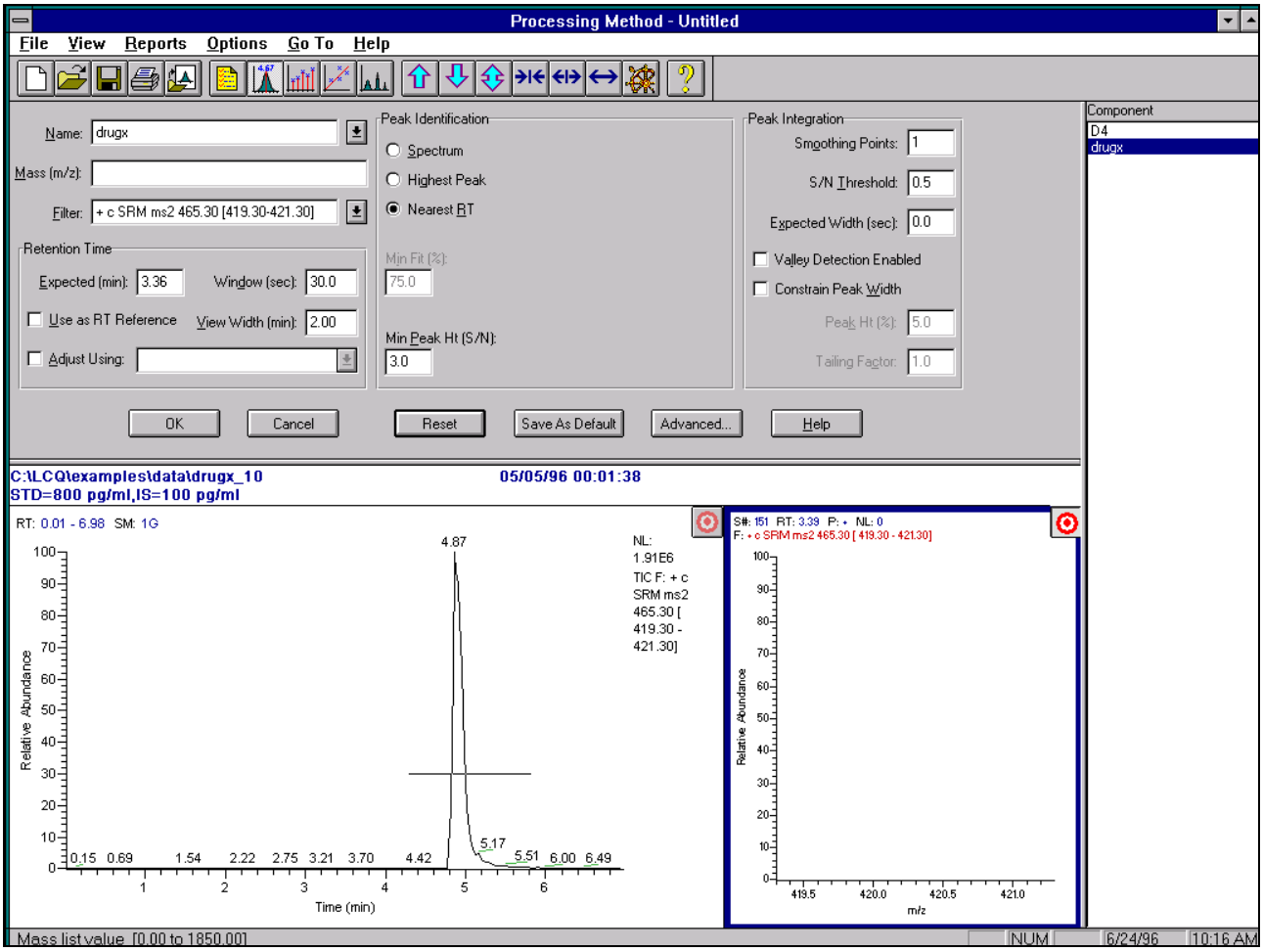


Figure 7-11. Processing Method window, showing the click-and-drag procedure for selecting the scan corresponding to the LC peak maximum in the Chromatogram view

Note the following occurs automatically:

- LCQ selects the peak maximum and highlights the selected scan in the LC peak in red in the Chromatogram view.
- LCQ displays the mass spectrum in the Spectrum view.
- LCQ enters the retention time corresponding to the selected scan in the Expected text box in the Retention Time group box.

e. Enter the peak identification information, as follows: Click on **OK**. LCQ automatically displays a blue marker under the LC peak and adjusts the Chromatogram view width to 2 min. See Figure 7-12.

3. Enter the peak identification information, as follows: Click on **OK**. LCQ automatically displays a blue marker under the LC peak and adjusts the Chromatogram view width to 2 min. See Figure 7-12.

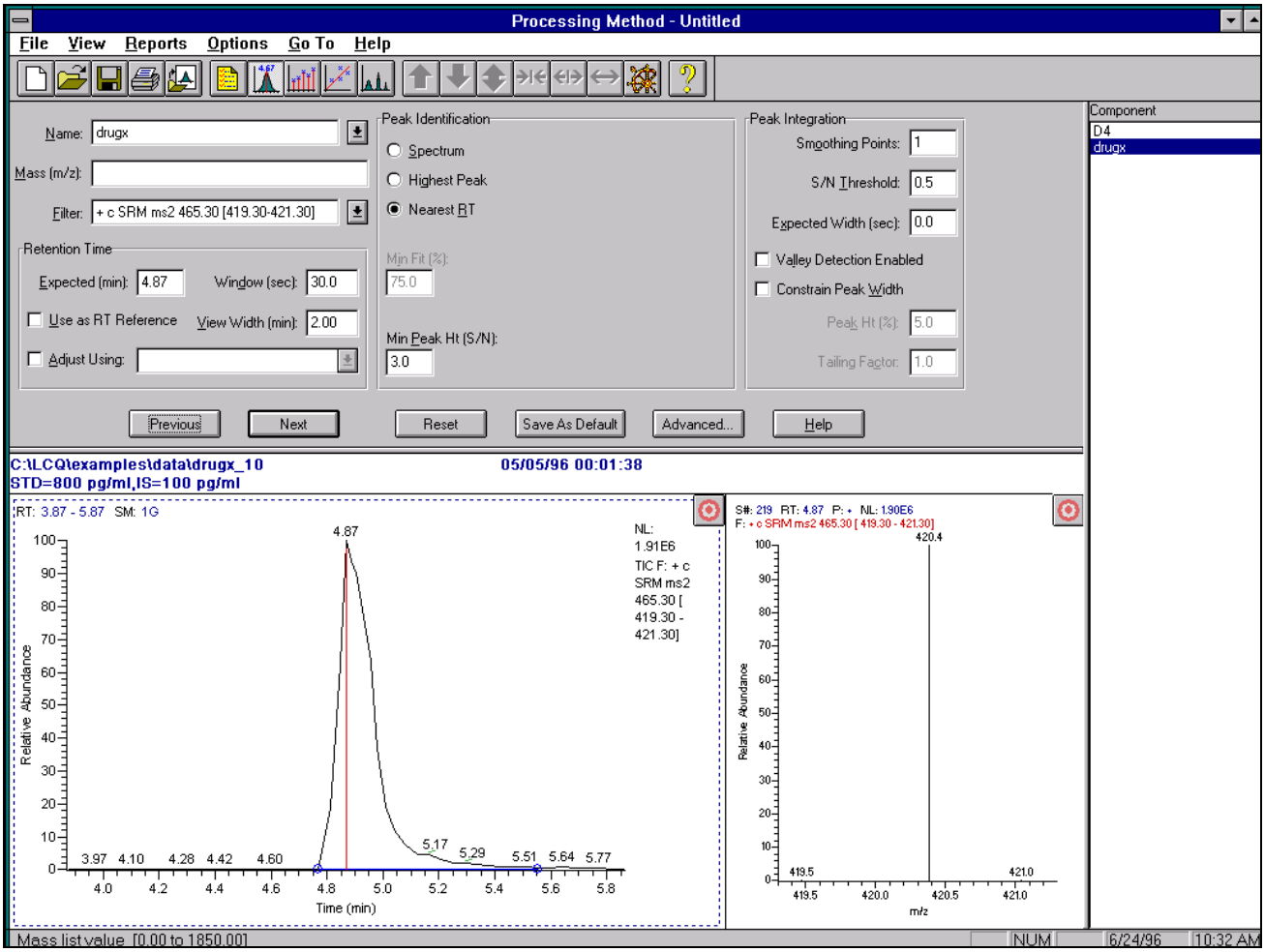
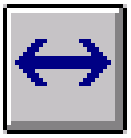


Figure 7-12. Processing Method window, showing a marker under the LC peak in the Chromatogram view



- Ensure that in the Retention Time group box, the value of approximately 4.9 min is set in the Expected text box.
- Ensure that the entire chromatogram is displayed in the Chromatogram View. If it is not, first activate the Chromatogram view by clicking on the target button and then click on the Display All button.
- Ensure that the scan filter in the Filter text box is matched to the correct component in the component list

4. Select to identify D4 by nearest retention time of LC peak in the Peak Identification group box, as follows:
   First, click on **Previous** to display information for the previous component in the component list. Then, click on the Nearest RT option button.

5. Select to use D4 as the retention time reference, as follows:
   Click on the Use as RT Reference check box.

6. Display the mass spectrum of D4 (the currently active component) and automatically enter the retention time of the LC peak, as follows:
   Repeat steps 2 and 3.

7.1.6 Entering Peak Integration Parameters

Peak integration parameters allow you to specify how LCQ determines the area of each peak in the chromatogram.

To enter the peak integration parameters for the currently active component in the Processing Method (D4), do the following:

1. Specify five smoothing points to be used for peak integration in the Peak Integration dialog box, as follows:
   Double-click in the Smoothing Points text box and type: **5**.

> **Note**. LCQ uses two different chromatogram smoothing algorithms; Boxcar and Gaussian. The Processing Method window uses Gaussian as its default smoothing algorithm.

2. Leave the default value of 0.5 in the S/N Threshold text box.

> **Note**. Integration S/N threshold is the number that controls how close you have to come to the calculated baseline to have the peak edges "touch" the baseline. In this example, the right and left peak edges are considered to be touching the baseline if the height above the baseline gets below 0.5 times the calculated noise.

3. Specify an expected peak width of 20 s in the peak integration group box, as follows:
   Double-click in the Expected Peak Width text box, and type: **20**.

4. Select [x] to enable valley detection of chromatographic peaks, as follows:
   Click on the Valley Detection Enabled check box to place an [x] there.

> **Note**. The Valley Detection Enabled check box allows you to use the LCQ valley detection approximation method to integrate unresolved peaks. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.

5. Leave the Constrain Peak Width check box empty.

> **Note**. The Constrain Peak Width check box allows you to constrain the width of a component during peak integration of a chromatogram. With this box checked, you adjust peak tailing by setting a factor that limits tailing as a percentage of the peak height.

6. Save the peak integration parameters, as follows:
   Click on **OK**. See Figure 7-13.

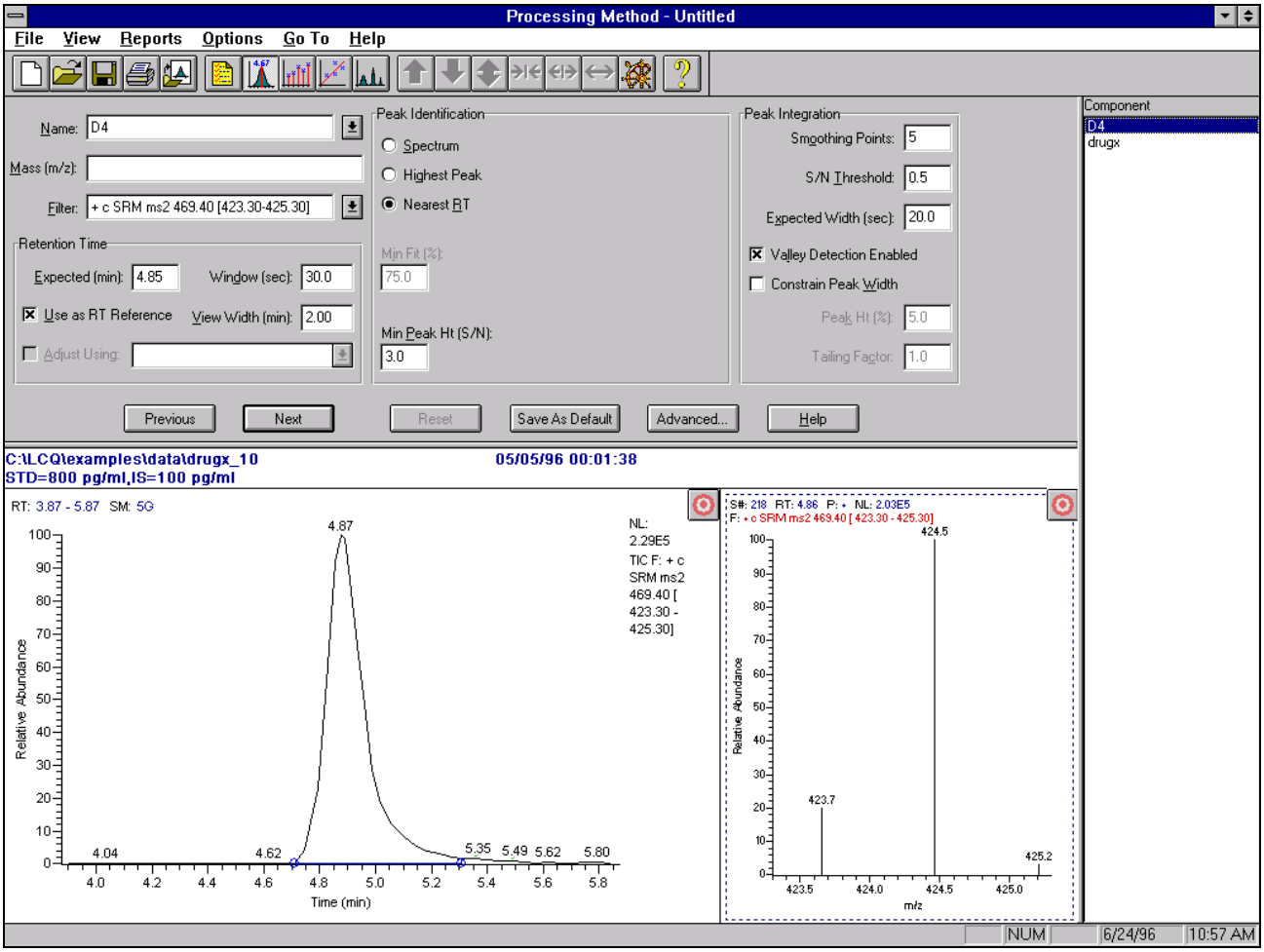


Figure 7-13. Component Identification view of the Processing Method window, showing the peak integration parameters for the component D4

7. Display the component identification parameters for drugx, as follows:
   Click on **Next**. Note that LCQ highlights the currently active component.

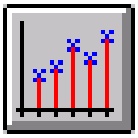
> **Note**. When entering many components with similar peak integration parameters you can enter all of the identification parameters for one of the components and then press the **Save As Default** button in the Component Identification view of the Processing Method window. These parameters then become the default values for new components.

8. Enter the peak integration parameters for drugx, as follows:
   Repeat steps 1 through 6.

7.1.7 Entering Component Calibration Parameters

In this example, you process calibration data using an internal standard calibration. In the Component Calibration view you first specify which components are internal standards. Then for each additional component in the Component list, you specify the component type as either a target compound or undefined; specify an internal standard and the amount of internal standard; and specify the concentration of each calibration and QC level for target compounds.

To enter component calibration parameters for this example, do the following:



1. Display the Component Calibration view, as follows:
   Click on the Component Calibration button in the Processing Method toolbar. See Figure 7-14.

2. Display the component identification parameters for the internal standard, D4, as follows:
   Click on the **Previous** button.

3. Specify D4 as an internal standard in the Component Type group box, as follows:
   Click on the ISTD option button. See Figure 7-15.

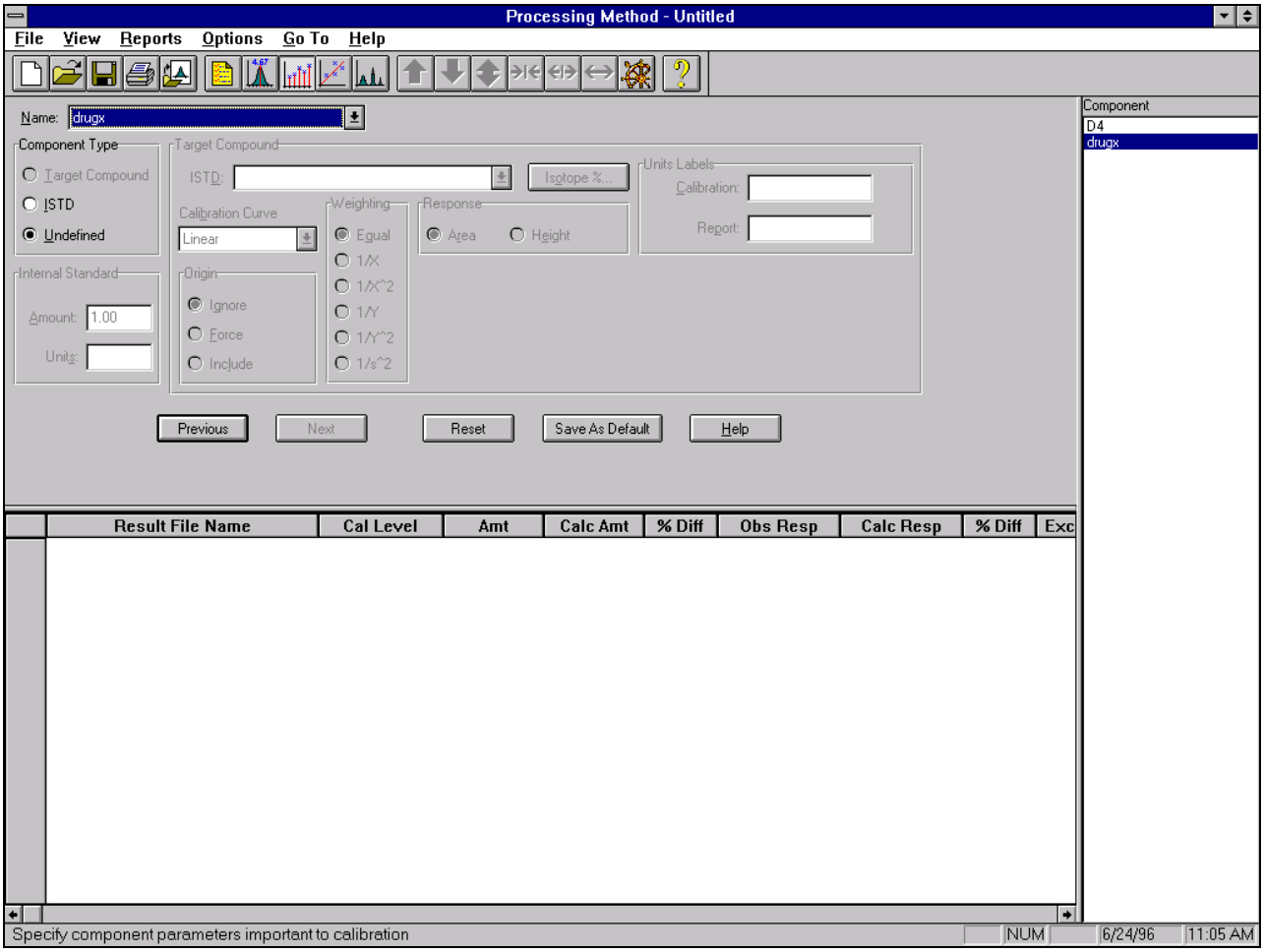


Figure 7-14. Component Calibration view of the Processing Method window

4. Enter the internal standard amount in the Internal Standard group box, as follows:
   Double-click in the Amount text box and type: **100**.

5. Enter the internal standard units in the Internal Standard group box, as follows:
   Click in the Units text box and type: **pg/ml**.

6. Accept the calibration parameters, as follows:
   Click on **OK**.

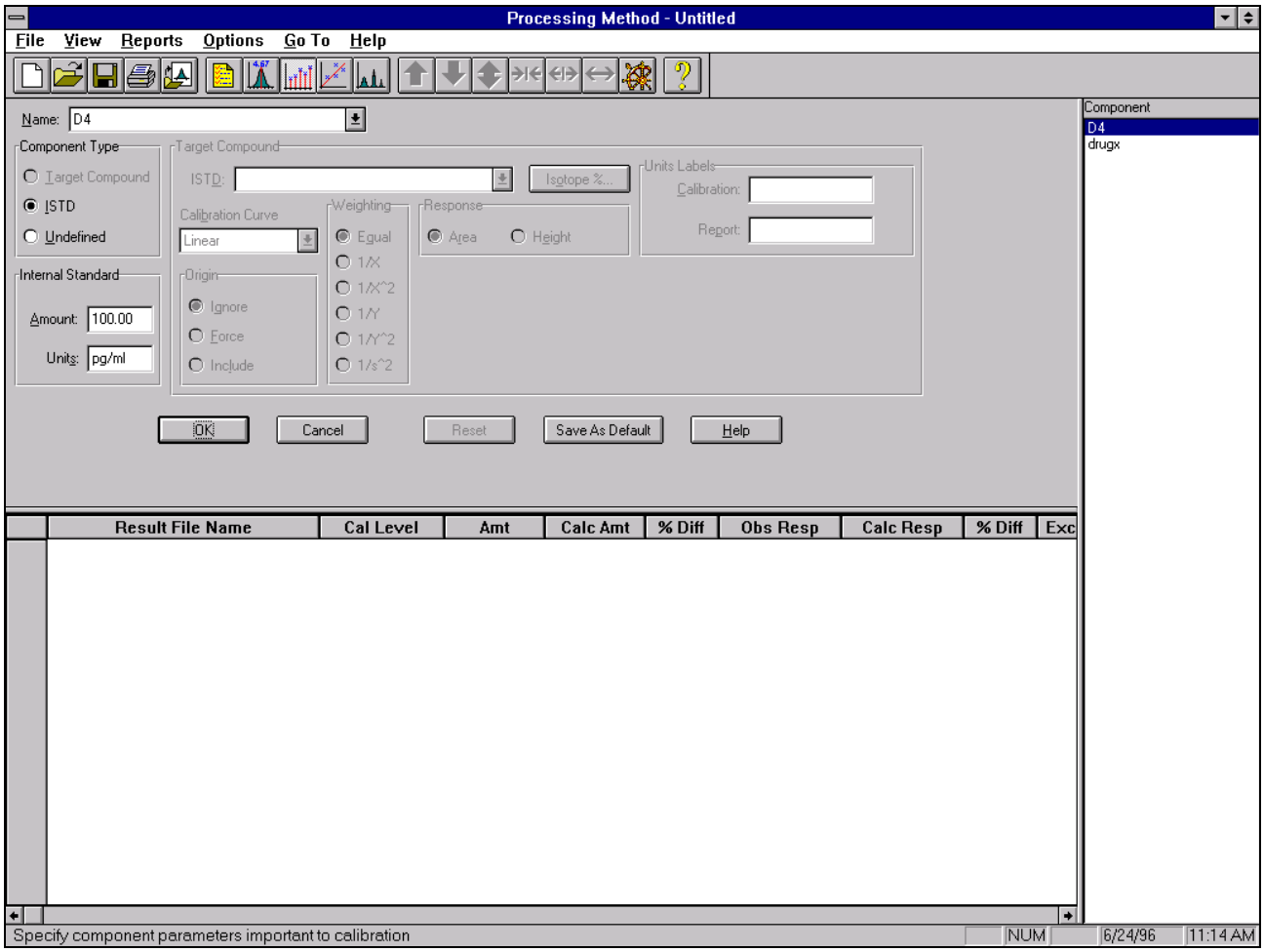


Figure 7-15. Component Calibration view, showing the correct internal standard information

7. Display the component calibration parameters for the next component in the Processing Method, as follows:
   Click on **Next**. Note that LCQ highlights the active component, drugx.

8. Specify drugx as a target compound in the Component Type group box, as follows:
   Click on the Target Compound option button.

9. Leave the internal standard set to its default value of D4 in the ISTD text box in the Target Compound group box.

> **Note**. D4 is the only internal standard used in this example.

10. Specify a quadratic calibration curve in the Target Compound group box, as follows:
    First, click on the drop-down menu in the Calibration Curve combo box. Then, click on Quadratic.

11. Leave the origin set to its default value of Ignore in the Origin group box.

12. Specify a $1/X^2$ weighting in the Weighting group box, as follows:
    Click on the $1/X^2$ option button.

13. Leave the response set to its default setting of Area in the Response group box.

14. Specify the unit of concentration to appear on the calibration curve in the Units Labels group box, as follows:
    Click in the Calibration text box and type: **pg/ml**.

15. Specify the unit of concentration to appear in the quantitation report in the Units Labels group box, as follows:
    Click in the Report text box and type: **pg/ml**.

16. Enter nine calibration levels in the Target Compound group box, as follows:
    First, click in the first Cal Level text box and type: **cal 1**. Next, press the **uantitiesDown Arrow>** key and in the second Cal Level text box type: **cal 2**. Then, press the **<Down Arrow>** key and in the third Cal Level text box type: **cal 3**. Repeat this procedure, incrementally increasing each Cal Level until you reach nine calibration levels.
    See Table 7-1 and Figure 7-16.

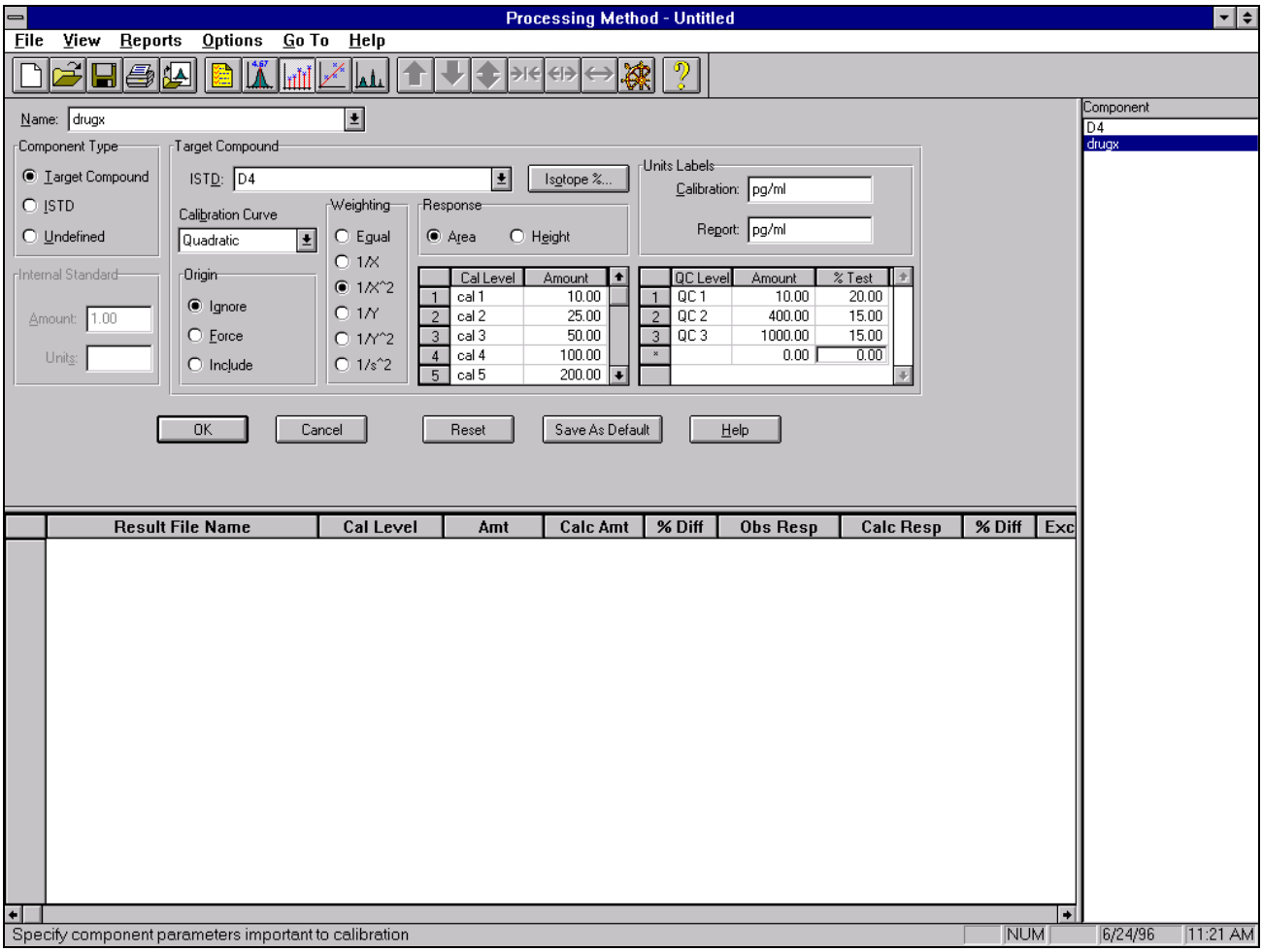


Figure 7-16. Component Calibration view, showing the Cal Level and QC Level parameters for drugx

17. Enter the amount for each calibration level, as follows: First, click in the first Amount text box and type: **10**. Next, press the **<Down Arrow>** key and in the second Amount text box type: **25**. Then, press the **<Down Arrow>** key and in the third text box type: **50**. Repeat this procedure using the appropriate amounts for each Cal Level listed in Table 7-1.

Cal Level	Amount (pg/ml)
cal 1	10
cal 2	25
cal 3	50
cal 4	100
cal 5	200
cal 6	400
cal 7	600
cal 8	800
cal 9	1000

Table 7-1. Calibration levels and corresponding amounts for drugx

18. Enter three QC levels in the Target Compound group box, as follows:
    First, click in the first QC Level text box and type: **QC 1**. Next, press the **<Down Arrow>** key and in the second QC Level text box type: **QC 2**. Then, press the **<Down Arrow>** key and in the third QC Level text box type: **QC 3**. See Table 7-2 and Figure 7-16.

19. Enter the amount for each QC Level, as follows:
    First, click in the first QC Level Amount text box and type: **10**. Next, press the **<Down Arrow>** key and in the second QC Level Amount text box type: **400**. Then, press the **<Down Arrow>** key and in the third QC Level Amount text box type: **1000**.

QC Level	Amount (pg/ml)	% Test
QC 1	10	20
QC 2	400	15
QC 3	1000	15

Table 7-2. QC levels and corresponding amounts for drugx

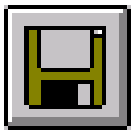
20. Set the % Test value for the QC Level 1 to 20% and the % Test values for QC Levels 2 and 3 to 15%, as follows:
    First, click in the first % Test text box and type: **20**. Next,

press **<Down Arrow>** and in the second % Test text box type: **15**. Then, press **<Down Arrow>** and in the third % Test level text box type: **15**.

> **Note**. The % Test values for QC's in this example are shown in Table 7-2. These are the criteria used in this example to determine whether QC's pass. You can use any % Test parameters that are appropriate for your particular application.

21. Save the component calibration parameters, as follows:
    Click on **OK**.

7.1.8 Saving the Processing Method



Save the Processing Method as *practice.pmd*, as follows:

1. Display the File Save - Audit Trail dialog box, as follows:
   Click on the Save button in the Processing Method toolbar.
   See Figure 7-17.

2. Specify the comment information in the File Save - Audit Trail dialog box, as follows:
   Click in the Comment text box, and type: **drugx processing method**.

> **Note**. LCQ automatically displays what changes you made to the Processing Method in the What Changed text box. In this example, only the Component Identification view has changed so far.

3. Display the Save As dialog box, as follows:
   Click on **Continue**. See Figure 7-18.

Figure 7-17. File Save - Audit Trail dialog box, showing representative comment information

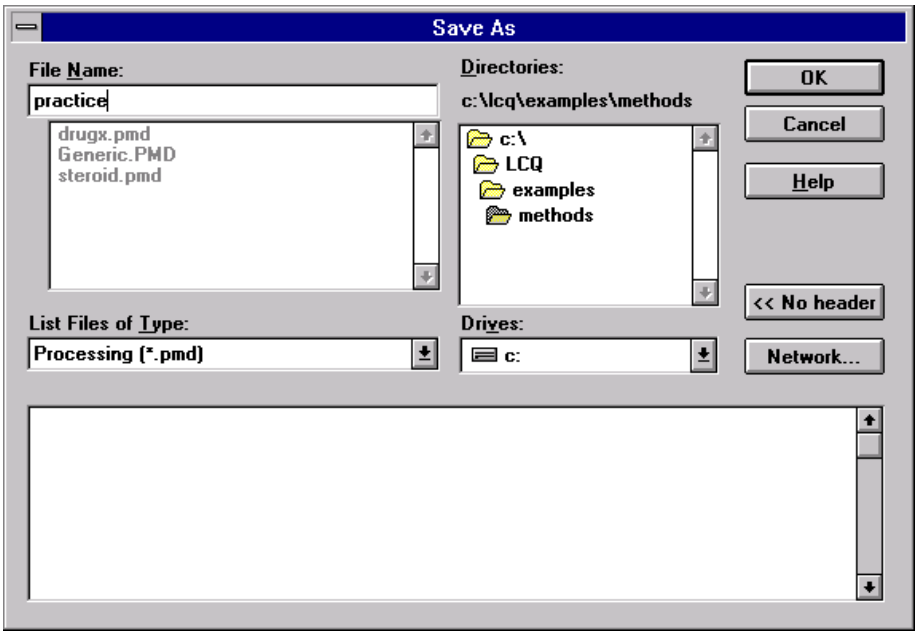


Figure 7-18. Save As dialog box, showing the correct file name and directory *C:\LCQ\examples\Methods* for the *practice.pmd* Processing Method

4. Name the Processing Method *practice.pmd* and change to the *C:\LCQ\examples\Methods* directory, as follows:
   Double-click in the File Name text box, and type: **practice**.

5. Save the Processing Method and close the dialog box, as follows:
   Click on **OK**.

7.1.9 Closing the Processing Method

Close the Processing Method window, as follows:
Choose **File | Exit**.

7.2 Creating a Sample List in the Sample List Window

To create a Sample List in the Sample List window, do the following:

- Use the new file template to start a new Sample List.
- Save the Sample List.

7.2.1 Using the New File Template to Start a New Sample List

To start a new Sample List using the New File Template dialog box, do the following:

1. Open the Sample List window from the Navigator window, as follows:
   Click on the Sample List button in the Navigator window.

2. Start a new Sample List and display the New File Template dialog box, as follows:
   Choose **File | New**. See Figure 7-19.

3. Enter the base raw file name in the General group box, as follows:
   Click in the Base File Name text box and type **drugx**_.

4. Leave the starting number set to its default value of 1 in the Starting Number text box.

5. Enter the path where the raw files are saved, as follows:

   a. Display the Select Directory dialog box, as follows:
      Click on the Path Browse button [in the New File Template dialog box (Fig. 7-19)]. See Figure 7-20.

   b. Select the *C:\LCQ\examples\data* directory in the Select Directory dialog box, as follows:
      First, double-click on the *C:* directory in the Directories Selected combo box. Next, scroll down in the Directories Selected combo box and double-click on the *LCQ* directory. Then, double-click on the *examples* directory. Finally, double-click on the *data* directory.

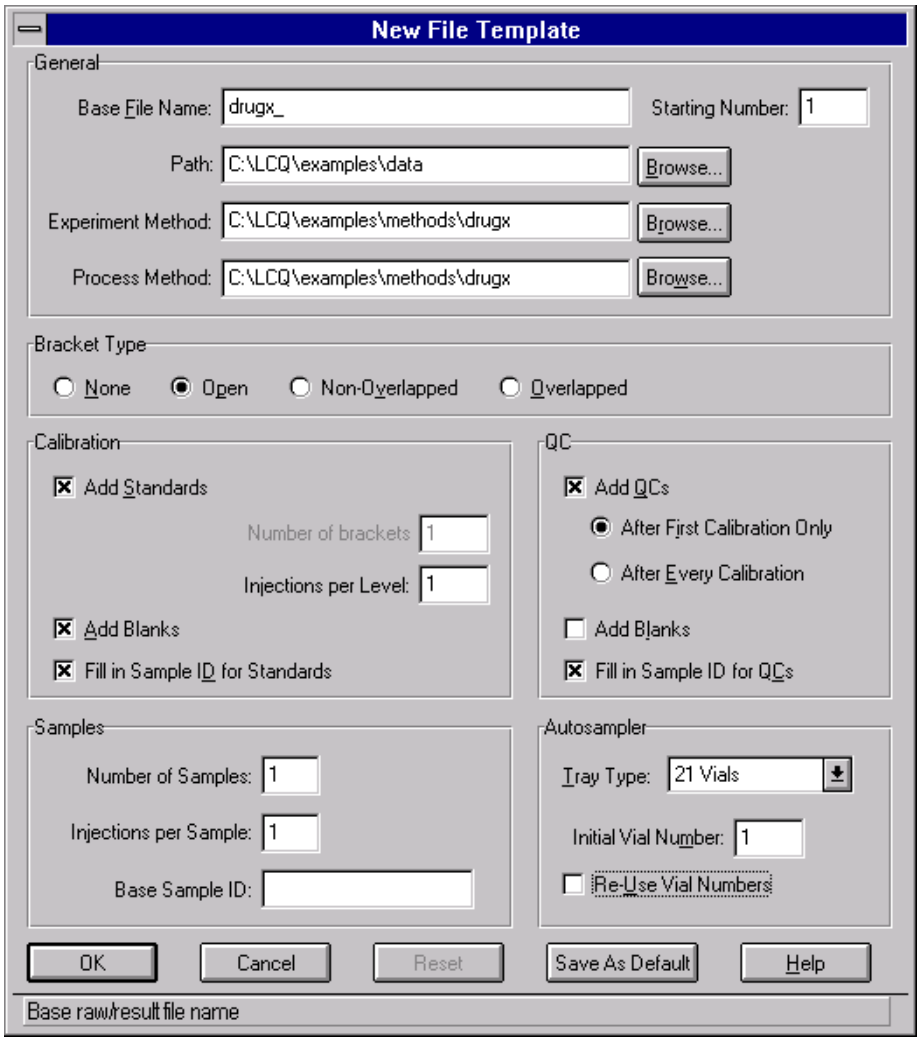


Figure 7-19. New File Template dialog box, showing the proper settings to create a Sample List for this example

c. Enter the raw file path and close the dialog box, as follows:
Click on **OK**. LCQ automatically enters the correct path in the Path text box in the New File Template dialog box.

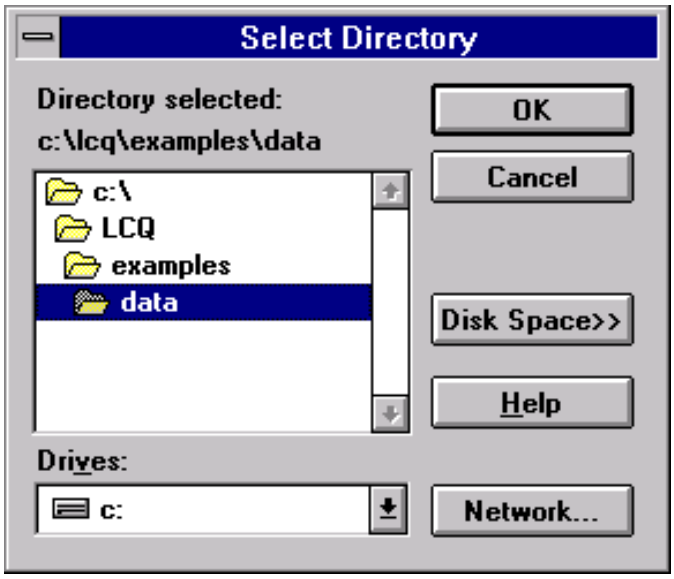


Figure 7-20. Select Directory dialog box, showing the *C:\LCQ\examples\data* path used for raw files

6. Enter the *drugx.emd* Experiment Method, which is the original Experiment Method used to collect the data in this example, as follows:

   a. Display the Select Experiment Method File dialog box, as follows:
   Click on the Experiment Method Browse button [in the New File Template dialog box (Fig. 7-19)]. See Figure 7-21.

   b. Select the *drugx.emd* experiment method in the *C:\LCQ\examples\Methods* directory, as follows:
   Scroll down in the File Name combo box until you see *drugx.emd*. Then, click on the file name.

   c. Enter the Experiment Method and close the dialog box, as follows:
   Click on **OK**. LCQ automatically enters the method in the Experiment Method text box in the New File Template dialog box.

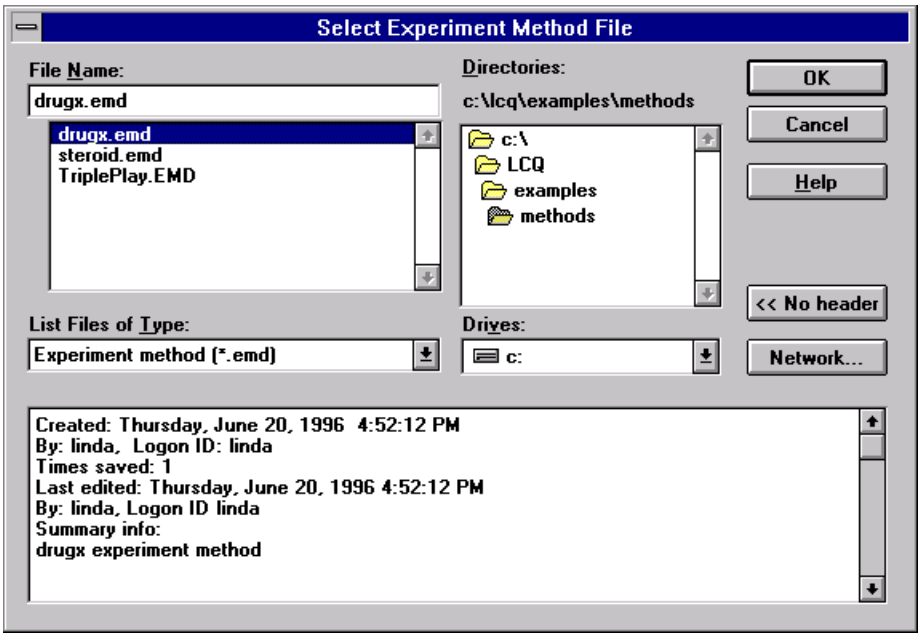


Figure 7-21. Select Experiment Method dialog box, showing Experiment Methods saved in the *C:\LCQ\examples\Methods* directory

7. Enter the *practice.pmd* Processing Method in the General group box, as follows:

   a. Display the Select Process Method File dialog box, as follows:
   Click on the Process Method Browse button [in the New File Template dialog box (Fig. 7-19)]. See Figure 7-22.

   b. Select the *practice.pmd* Processing Method in the *C:\LCQ\examples\Methods* directory, as follows:
   Scroll down in the File Name combo box until you see *practice.pmd*. Then, click on the file name.

   c. Enter the Process Method and close the dialog box, as follows:
   Click on **OK**. LCQ automatically enters the method in the Process Method text box in the New File Template dialog box.

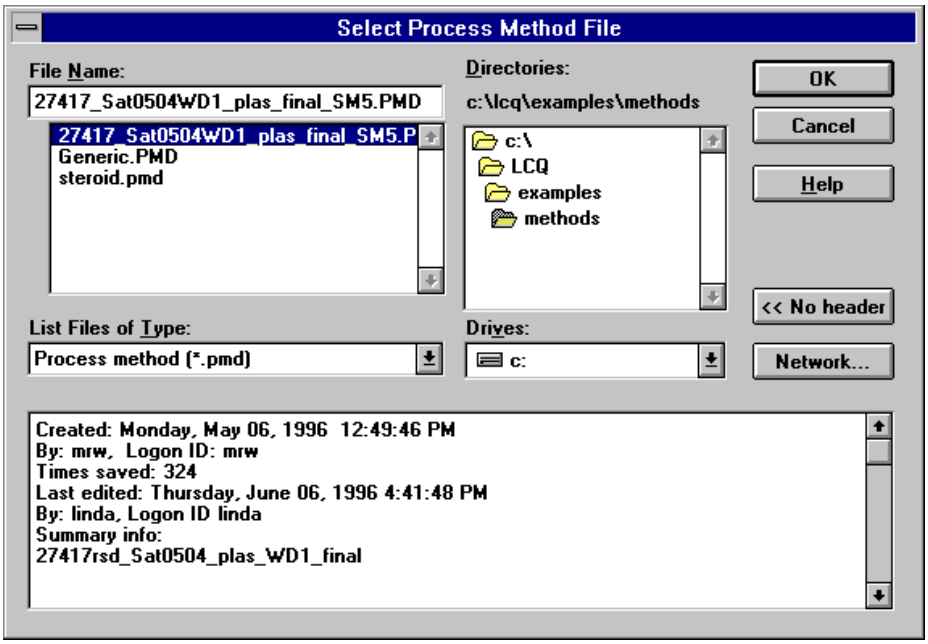


Figure 7-22. Select Process Method dialog box, showing Processing Methods in the *C:\LCQ\examples\Methods* directory

8. Select to process the data using open brackets in the Bracket Type group box, as follows:
   Click on the Open option button.

9. Set the parameters in the Calibration group box, as follows:

   a. Select [x] to add calibration standards, as follows:
      Click on the Add Standards check box. Note that LCQ activates the Calibration group parameters.

   b. Leave the injections per level set to its default value of 1.

   c. Select [x] to add blanks in the Calibration group box, as follows:
      Click on the Add Blanks check box to place an x there.

   d. Leave the default setting to fill in sample ID for standards.

10. Set the parameters in the QC group box, as follows:

    a. Select [x] to add QC's , as follows:
    Click on the QC check box to place an x there. Note that LCQ activates the QC group parameters.

    b. Leave the After First Calibration Only option button selected.

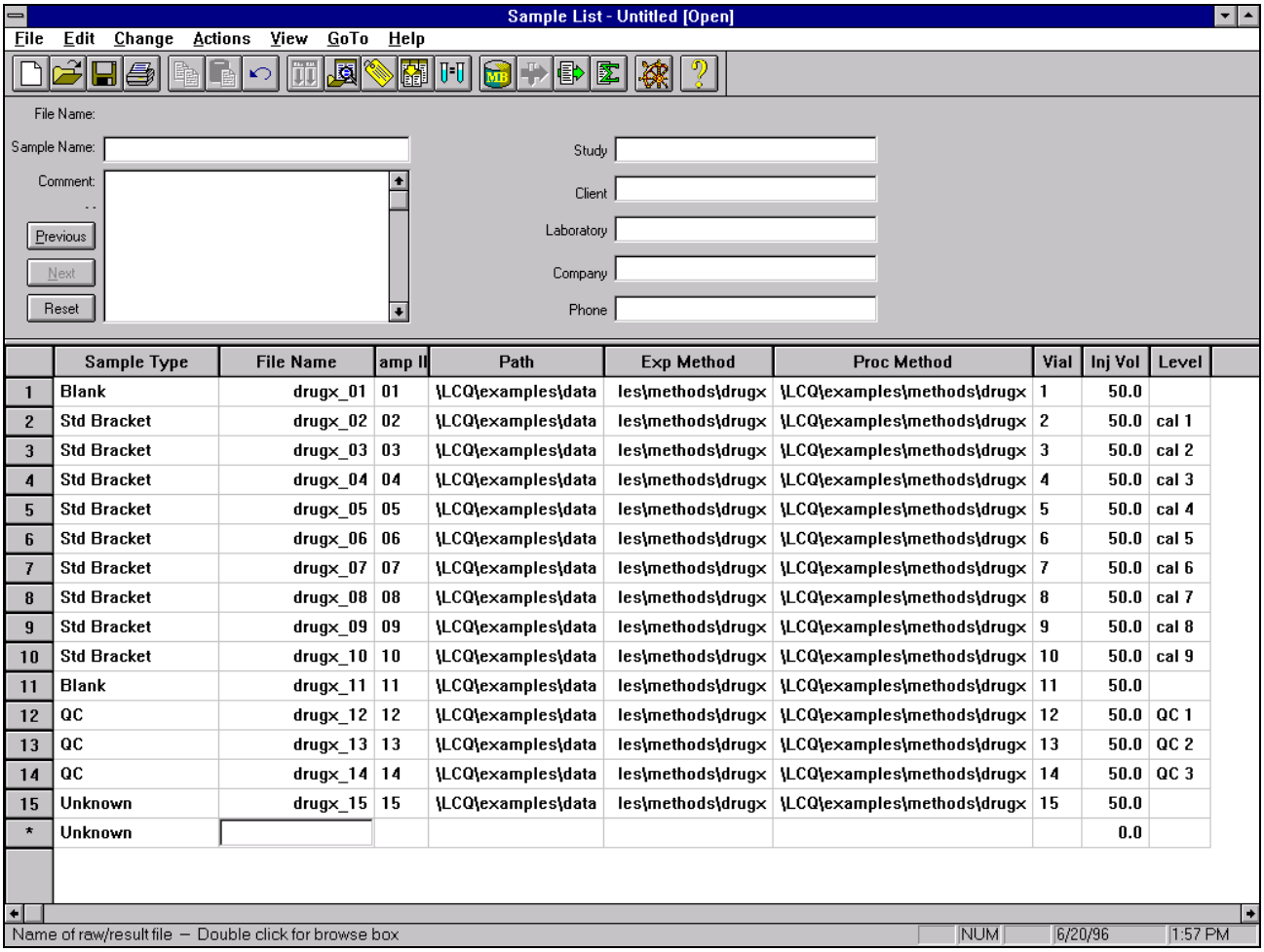
    c. Leave the Add Blanks check box empty.

    d. Leave the Fill in Sample ID for QC's check box selected.

11. For this example, use the default settings specified in the Samples group boxes.

12. Set the parameters in the Autosampler group box, as follows:
    Select to not re-use vial numbers, as follows:
    Click on the Re-Use Vial Numbers check box to remove the x.

13. Open the Sample List and close the New File Template dialog box, as follows:
    Click on **OK**. LCQ automatically generates a Sample List and correctly enters the calibration level and injection volume for each calibration standard in the Experiment Method.
    See Figure 7-23.



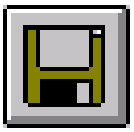
	Sample Type	File Name	amp ll	Path	Exp Method	Proc Method	Vial	Inj Vol	Level
1	Blank	drugx_01	01	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	1	50.0	
2	Std Bracket	drugx_02	02	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	2	50.0	cal 1
3	Std Bracket	drugx_03	03	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	3	50.0	cal 2
4	Std Bracket	drugx_04	04	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	4	50.0	cal 3
5	Std Bracket	drugx_05	05	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	5	50.0	cal 4
6	Std Bracket	drugx_06	06	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	6	50.0	cal 5
7	Std Bracket	drugx_07	07	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	7	50.0	cal 6
8	Std Bracket	drugx_08	08	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	8	50.0	cal 7
9	Std Bracket	drugx_09	09	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	9	50.0	cal 8
10	Std Bracket	drugx_10	10	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	10	50.0	cal 9
11	Blank	drugx_11	11	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	11	50.0	
12	QC	drugx_12	12	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	12	50.0	QC 1
13	QC	drugx_13	13	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	13	50.0	QC 2
14	QC	drugx_14	14	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	14	50.0	QC 3
15	Unknown	drugx_15	15	\LCQ\examples\data	les\methods\drugx	\LCQ\examples\methods\drugx	15	50.0	
*	Unknown							0.0	

Figure 7-23. Sample List window, showing the proper settings for batch reprocessing data files in this example

The Sample List generates an entry for each of the nine calibration and three QC level specified in the Processing Method. In this example, you specify one unknown sample, one injection per calibration level and do not re-use vial numbers. Blanks are entered at each end of the calibration set to give a total of fifteen entries in the Sample List.

7.2.2 Saving the Sample List

Save the Sample List as *practice.sld*, as follows:



1. Display the Save As dialog box, as follows:
   Click on the Save button in the Sample List toolbar.
   See Figure 7-24.

2. Name the Sample List *practice.sld* and save it in the *C:\LCQ\examples\Methods* directory as follows:
   Double-click in the File Name text box, and type: **practice**.

3. Save the Sample List and close the dialog box, as follows:
   Click on **OK**.

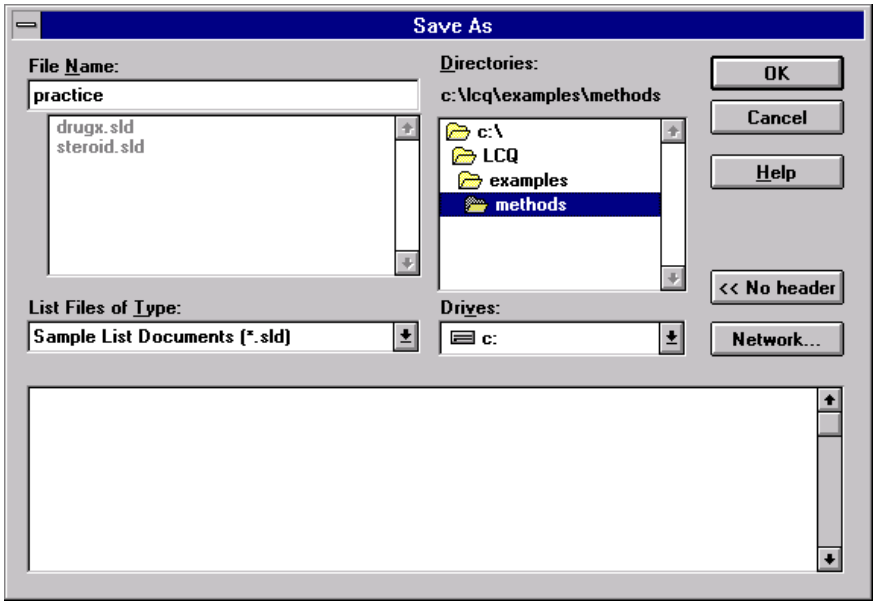


Figure 7-24. Save As dialog box, showing the proper settings to save the Sample List as *practice.sld* to the *C:\LCQ\examples\Methods* directory

7.3 Processing Calibration Data Using the Sample List Window

In this example you use the Sample List *drugx.sld* contained in the *C:\LCQ\examples\Methods* directory and raw files provided in the *C:\LCQ\examples\data* directory. This is the same Sample List that was used to collect the data originally. The Processing Method, *drugx.pmd* is also provided in the *C:\LCQ\examples\Methods* directory. You can use either this Processing Method or the one you created (*practice.pmd*) in the topic: **Creating a Processing Method using the Processing Method Editor**. To process the calibration data in this example, do the following:

- Open a Sample List in the Sample List window
- Batch reprocess the calibration data

For a flow diagram of how to batch reprocess calibration data using the Sample List window, see Figure 7-25.

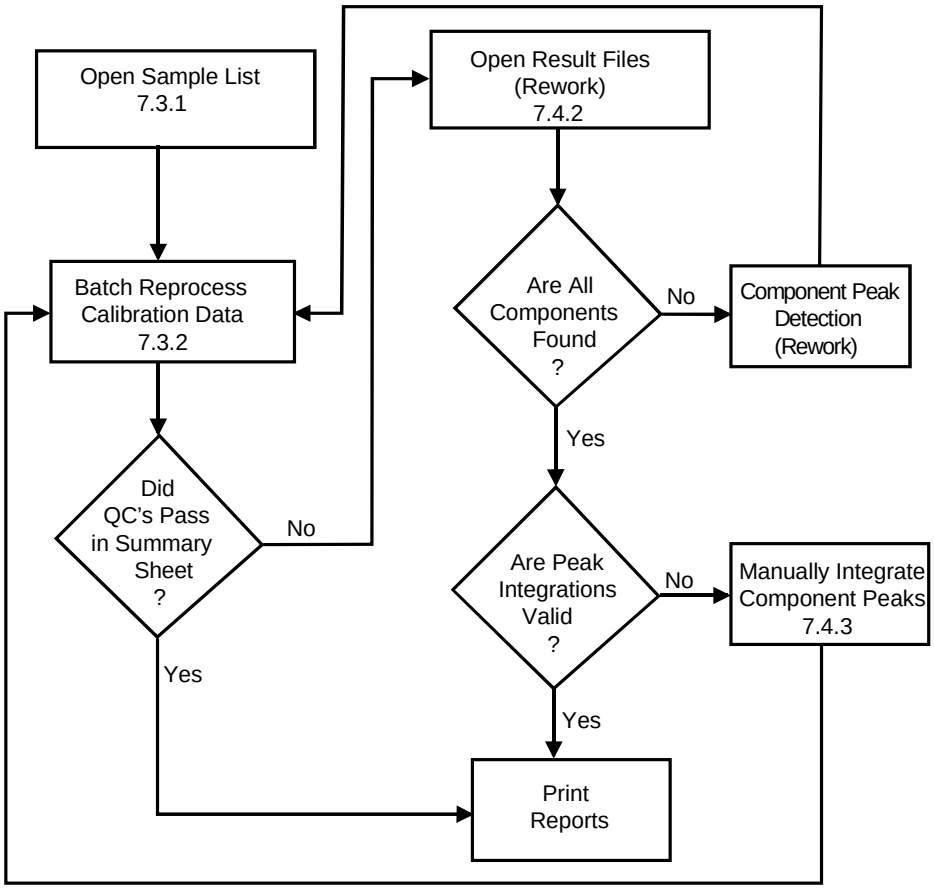


Figure 7-25. Flow diagram for batch reprocessing and reviewing calibration data

7.3.1 Opening a Sample List in the Sample List Window

Open the *drugx.sld* Sample List, as follows:

1. Display the Open dialog box, as follows:
   Click on the Open button in the Sample List toolbar.
   See Figure 7-26.

2. Select the *drugx.sld* Sample List in the *C:\LCQ\examples\Methods* directory, as follows:
   Scroll down in the File Name combo box until you see *drugx.sld*. Then, click on the file name.

3. Open the Sample List and close the dialog box, as follows: Click on **OK**. See Figure 7-27.

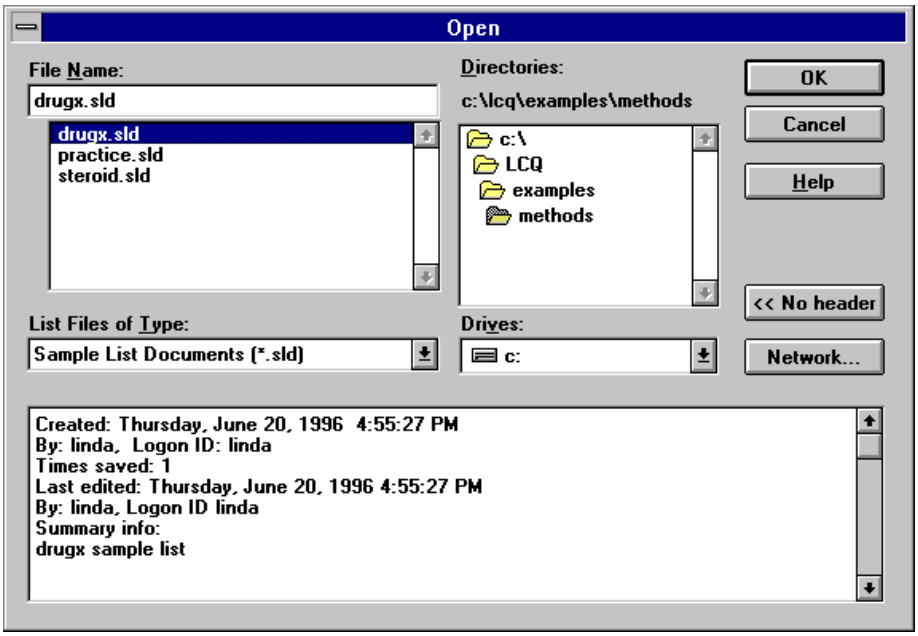
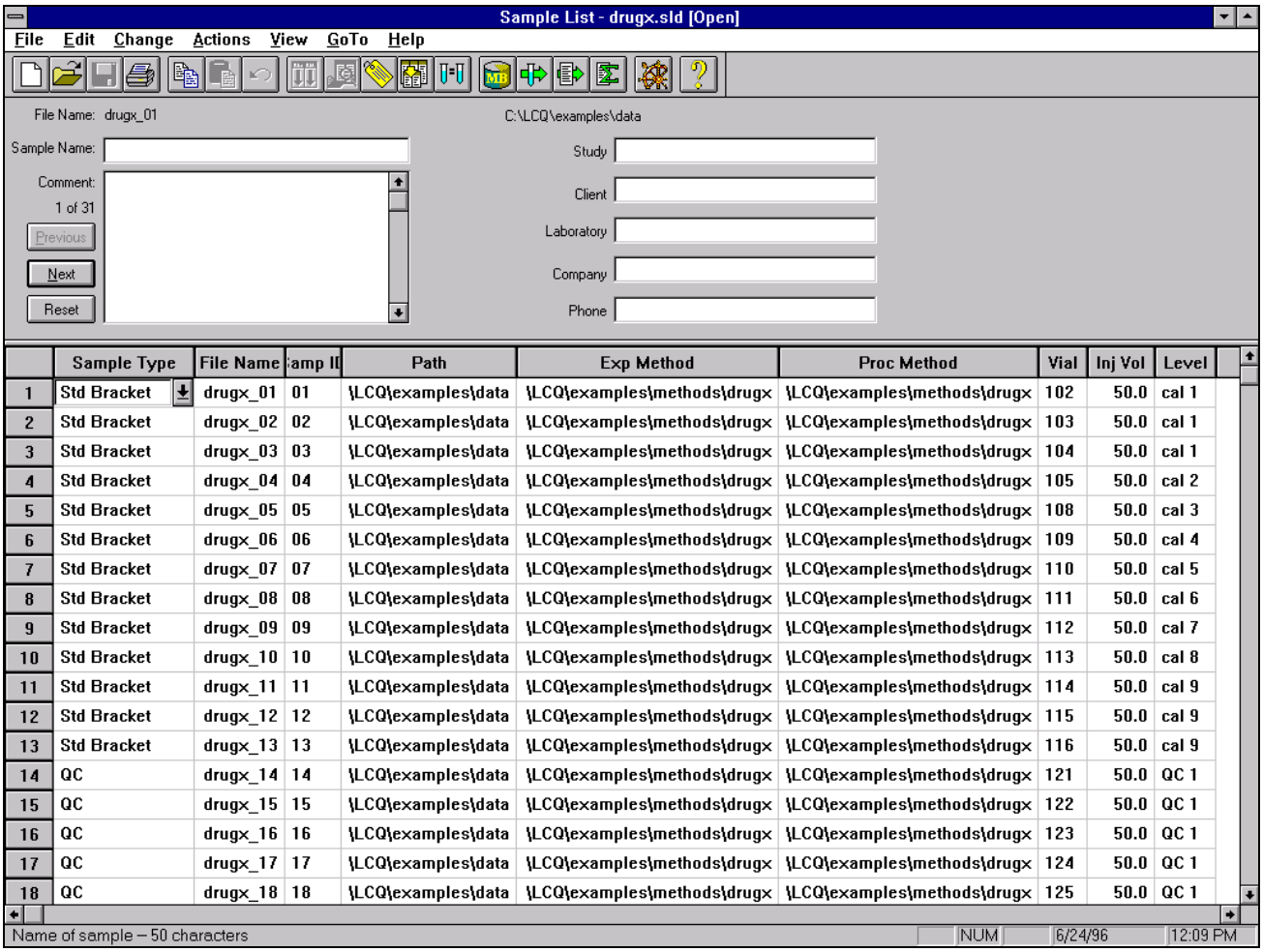


Figure 7-26. Open dialog box, showing the *drugx.sld* Sample List selected

Note. LCQ creates data files containing either .raw or .rst extensions for raw files and result files. The Sample List window recognizes both extensions. For instance, if you are running a Sample List, LCQ creates raw files with the file name you specify. If you are batch reprocessing data, LCQ uses raw files when you choose the peak detection & integration processing action, and it uses result files when you choose the calibration or quantitation processing actions.



	Sample Type	File Name	amp ID	Path	Exp Method	Proc Method	Vial	Inj Vol	Level
1	Std Bracket	drugx_01	01	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	102	50.0	cal 1
2	Std Bracket	drugx_02	02	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	103	50.0	cal 1
3	Std Bracket	drugx_03	03	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	104	50.0	cal 1
4	Std Bracket	drugx_04	04	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	105	50.0	cal 2
5	Std Bracket	drugx_05	05	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	108	50.0	cal 3
6	Std Bracket	drugx_06	06	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	109	50.0	cal 4
7	Std Bracket	drugx_07	07	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	110	50.0	cal 5
8	Std Bracket	drugx_08	08	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	111	50.0	cal 6
9	Std Bracket	drugx_09	09	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	112	50.0	cal 7
10	Std Bracket	drugx_10	10	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	113	50.0	cal 8
11	Std Bracket	drugx_11	11	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	114	50.0	cal 9
12	Std Bracket	drugx_12	12	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	115	50.0	cal 9
13	Std Bracket	drugx_13	13	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	116	50.0	cal 9
14	QC	drugx_14	14	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	121	50.0	QC 1
15	QC	drugx_15	15	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	122	50.0	QC 1
16	QC	drugx_16	16	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	123	50.0	QC 1
17	QC	drugx_17	17	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	124	50.0	QC 1
18	QC	drugx_18	18	\LCQ\examples\data	\LCQ\examples\methods\drugx	\LCQ\examples\methods\drugx	125	50.0	QC 1

Figure 7-27. Sample List window, showing the *drugx.sld* Sample List

7.3.2 Batch Reprocessing the Calibration Data

In this example, you batch reprocess raw files contained in the *C:\LCQ\examples\data* directory and evaluate the calibration curve with the QC's. LCQ batch reprocesses data according to the processing actions you choose in the Batch Reprocess Setup dialog box and the way the calibration standards, QC's, and unknowns are bracketed in the Sample List.

To batch reprocess the calibration data using open brackets in the *drugx.sld* Sample List, do the following:

1. Display the Batch Reprocess Setup dialog box, as follows:
   Click on the Batch Reprocess button in the Sample List toolbar. See Figure 7-28.

2. Select [x] to detect and integrate peaks in the raw files in the Processing Actions dialog box, as follows:
   Click on the Peak Detection & Integration check box.

3. Select [x] to implement calibration on the result files in the Processing Actions dialog box, as follows:
   Click on the Calibration check box.

4. Select [x] to implement quantitation on the result files in the Processing Actions dialog box, as follows:
   Click on the Quantitation check box.

5. Select [x] to create a summary report in the Processing Actions dialog box, as follows:
   Click on the Create Summary check box.

6. Begin processing the data and close the dialog box, as follows:
   Click on **OK**.

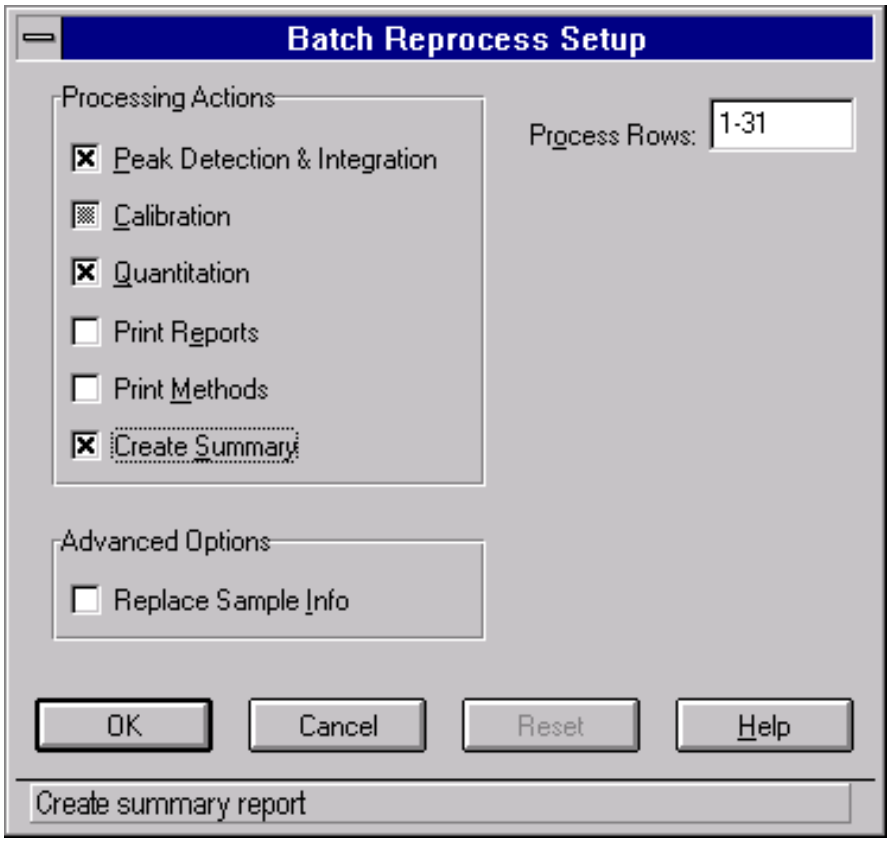


Figure 7-28. Batch Reprocess dialog box, showing the proper settings for processing the calibration data in this example

Note the following occurs automatically:

- LCQ begins processing the data.
- The Batch Reprocess Setup dialog box disappears and the Queue Manager icon appears in the Sample List window. The text below the icon updates the progress of data processing.
- The Excel Export dialog box appears. See Figure 7-29.
- Microsoft® Excel opens and the summary sheet appears. See Figure 7-30.

Note. To display the summary information for the target compound click on the drugx tab in the lower left corner of the Summary Sheet in the Excel window.

Figure 7-29. Excel Export dialog box

Microsoft Excel - DRUGX_drugx.XLS

File Edit View Insert Format Tools Data Window Help

Component Name	Curve Index	Weighting Index	Origin Index	Equation
drugx	Quadratic	1/x^2	Ignore	Y = 0.00572482+0.0090253*X-1.29147e-007*X^2 R^2 = 0.9960

Filename	Sample Type	Sample Name	Sample ID	Exp Conc	Calc Conc	Units	%Diff	Level	%RSD	Peak Status	Response
drugx_01	Std Bracket Sample		2	10.00	9.42	pg/ml	-6%	cal 1	9.6%		
drugx_02	Std Bracket Sample		3	10.00	11.19	pg/ml	12%	cal 1	9.6%		
drugx_03	Std Bracket Sample		4	10.00	9.61	pg/ml	-4%	cal 1	9.6%		
drugx_04	Std Bracket Sample		5	25.00	23.98	pg/ml	-4%	cal 2			
drugx_05	Std Bracket Sample		8	50.00	47.50	pg/ml	-5%	cal 3			
drugx_06	Std Bracket Sample		9	100.00	101.63	pg/ml	2%	cal 4			
drugx_07	Std Bracket Sample		10	200.00	203.31	pg/ml	2%	cal 5			
drugx_08	Std Bracket Sample		11	400.00	409.72	pg/ml	2%	cal 6			
drugx_09	Std Bracket Sample		12	600.00	610.34	pg/ml	2%	cal 7			
drugx_10	Std Bracket Sample		13	800.00	864.21	pg/ml	8%	cal 8			
drugx_11	Std Bracket Sample		14	1000.00	965.13	pg/ml	-3%	cal 9	1.8%		
drugx_12	Std Bracket Sample		15	1000.00	957.14	pg/ml	-4%	cal 9	1.8%		
drugx_13	Std Bracket Sample		16	1000.00	991.23	pg/ml	-1%	cal 9	1.8%		
drugx_14	QC Sample		21	10.00	14.63	pg/ml	46%	QC 1	11.3%	QC Failed	
drugx_15	QC Sample		22	10.00	12.26	pg/ml	23%	QC 1	11.3%	QC Failed	
drugx_16	QC Sample		23	10.00	11.37	pg/ml	14%	QC 1	11.3%		
drugx_17	QC Sample		24	10.00	11.23	pg/ml	12%	QC 1	11.3%		
drugx_18	QC Sample		25	10.00	10.83	pg/ml	8%	QC 1	11.3%		
drugx_19	QC Sample		26	10.00	12.50	pg/ml	25%	QC 1	11.3%	QC Failed	
drugx_20	QC Sample		33	400.00	425.32	pg/ml	6%	QC 2	4.4%		
drugx_21	QC Sample		34	400.00	430.97	pg/ml	8%	QC 2	4.4%		
drugx_22	QC Sample		35	400.00	445.23	pg/ml	11%	QC 2	4.4%		
drugx_23	QC Sample		36	400.00	405.31	pg/ml	1%	QC 2	4.4%		
drugx_24	QC Sample		37	400.00	398.82	pg/ml	0%	QC 2	4.4%		
drugx_25	QC Sample		38	400.00	404.46	pg/ml	1%	QC 2	4.4%		
drugx_26	QC Sample		39	1000.00	1040.14	pg/ml	4%	QC 3	4.3%		
drugx_27	QC Sample		40	1000.00	1001.76	pg/ml	0%	QC 3	4.3%		
drugx_28	QC Sample		41	1000.00	919.67	pg/ml	-8%	QC 3	4.3%		
drugx_29	QC Sample		42	1000.00	971.79	pg/ml	-3%	QC 3	4.3%		
drugx_30	QC Sample		43	1000.00	1006.81	pg/ml	1%	QC 3	4.3%		
drugx_31	QC Sample		44	1000.00	1023.57	pg/ml	2%	QC 3	4.3%		

Ready Sum=0 NUM

Figure 7-30. Excel Summary Sheet, showing calibration information for the data in this example

You evaluate the results in the Summary Sheet (Figure 7-30) to determine if the calibration is good. To ensure that the QC's for every level passed, you compare the accuracy, expressed in terms of %Diff, to the % Test criteria you specified in the Processing Method. For instance, replicates at the lowest QC level (QC 1) must have %Diff values of <20% to be considered passing. In this example QC 1 (with an expected concentration of 10 pg mL^{-1}) has three replicates that failed with %Diff values of 46%, 23%, and 25%, respectively. Replicates in the QC 2 and QC 3 levels should have %Diff values of <15% to be considered passing. In this case, all of the replicates in the QC 2 and QC 3 levels, at expected concentrations of 400 and 1000 pg mL^{-1} are within the 15% Diff criteria so they all pass.

You can also see from Figure 7-30 that the precision of the calibration data is good. Precision in the Summary Sheet, expressed in terms of %RSD, is 11.3% for the lowest QC level (10 pg mL^{-1}) and 4.4% and 4.3%, respectively at the QC 2 and QC 3 levels.

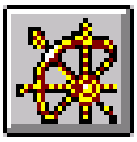
7.4 Reviewing and Editing the Calibration Data in the Rework Window

To review the calibration data in the Rework window, do the following:

- Open the Rework window.
- Open a Sample List in the Rework window and review the result files.
- Manually integrate a component.

7.4.1 Opening the Rework Window

To open the Rework window, do the following:



1. Return to the Navigator window as follows:
   First, hold down the **<Alt>** key and press the **<Tab>** key until you see the Navigator button. Then let go of the **<Alt>** key.

2. Open the Rework window, as follows:
   Click on the Rework button in the Navigator toolbar.

   If LCQ displays the Component Review view (Figure 7-35), go to the next topic: **Opening a Sample List in the Rework window and Reviewing the Result Files**.

   If LCQ does not display the Component Review view in the Rework window, you may see a message box. See Figure 7-31.

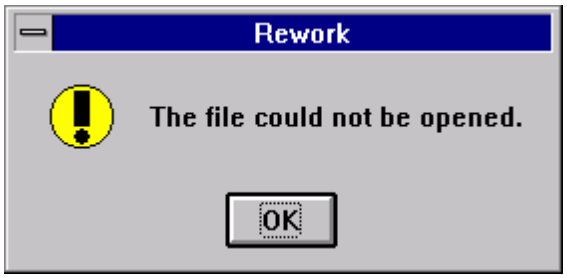


Figure 7-31. Message box in the Rework window

If the message: *The file could not be opened* appears, that indicates that the file that was last open in the Rework window can no longer be opened. To continue, do the following:

3. Display the New dialog box, as follows:
   Click on **OK**. See Figure 7-32.

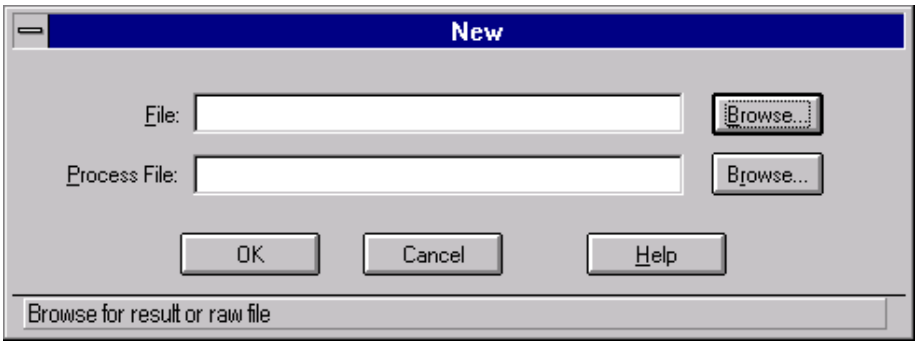


Figure 7-32. New dialog box in the Rework window

4. Open the result file *drugx_10.rst*, as follows:

   a. Display the Open dialog box, as follows:
      Click on the File Browse button. See Figure 7-33.

   b. Change to the *C:\LCQ\examples\data* directory and select the *drugx_10.rst* result file, as follows:
      Scroll down in the File Name combo box until you see *drugx_10.rst*. Then, click on the file name.

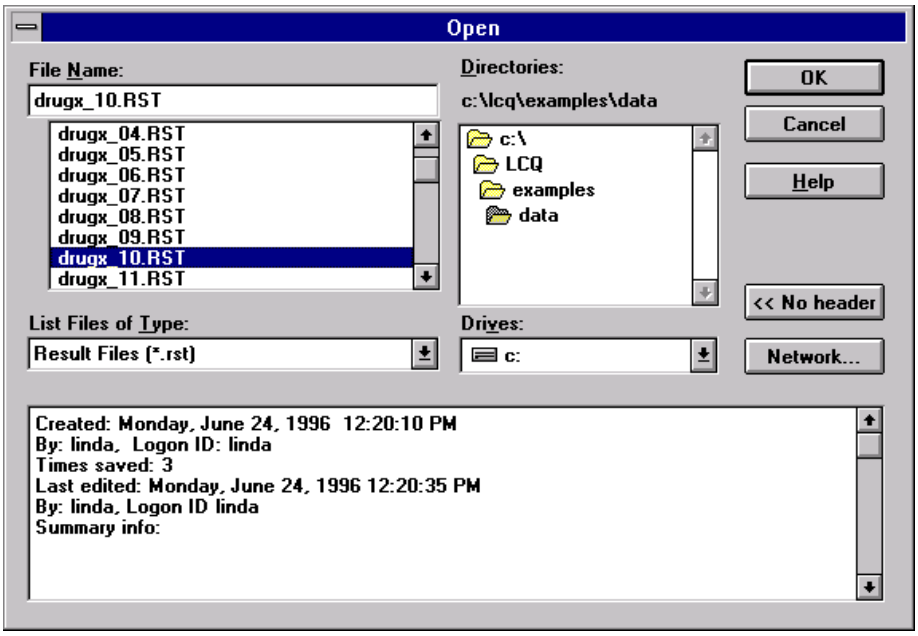


Figure 7-33. Open dialog box in the Rework window, showing the *drugx_10.rst* file selected

c. Enter the result file and close the dialog box, as follows: Click on **OK**. LCQ automatically enters the *C:\LCQ\examples\data\drugx_10.rst* directory in the File text box. See Figure 7-34.

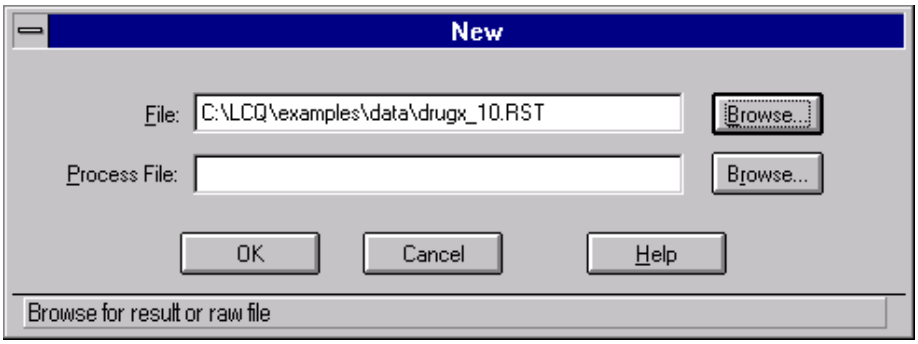


Figure 7-34. New dialog box, showing the *C:\LCQ\examples\data\drugx_10.rst* directory

d. Open the result file and close the dialog box, as follows: Click on **OK**. LCQ automatically displays drugx_10.rst in the Component Review view of the Rework window. See Figure 7-35.

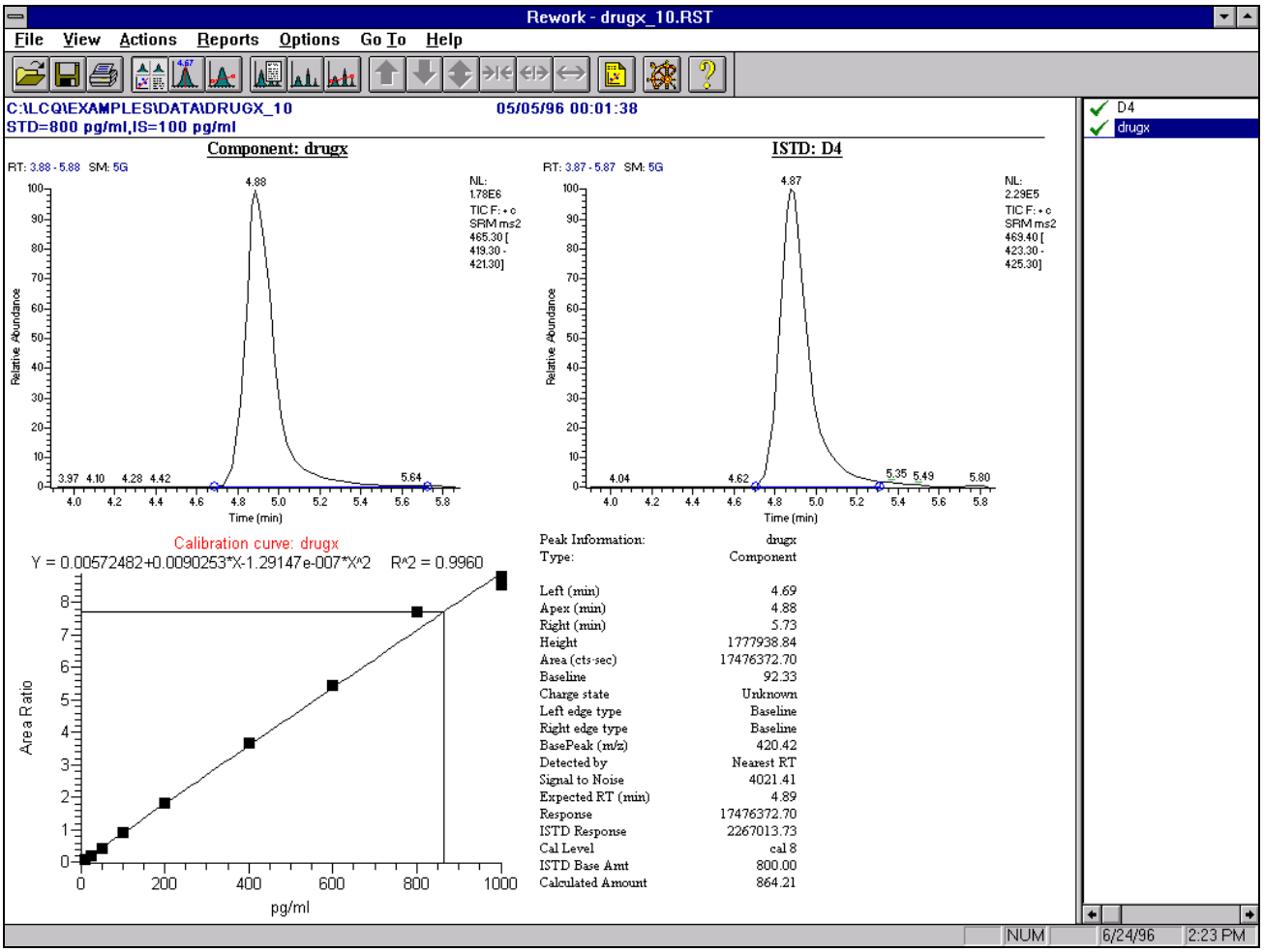


Figure 7-35. Component Review view of the Rework window, showing drugx as the active component

> **Note**. If drugx is not the active component, click on the name of the component in the Component List at the extreme right of the Rework window to activate it.

7.4.2 Opening a Sample List in the Rework Window and Reviewing the Result Files

You open the same Sample List you used to perform the batch reprocessing of data and then review the result files in the Rework window. This allows you to easily scroll through result files created in the same Sample List without having to separately open each result file in Rework.

To open a Sample List in the Rework window, do the following:

1. Display the Sample List dialog box, as follows:
   Choose **File | Open Sample List** in the Rework window.
   See Figure 7-36.

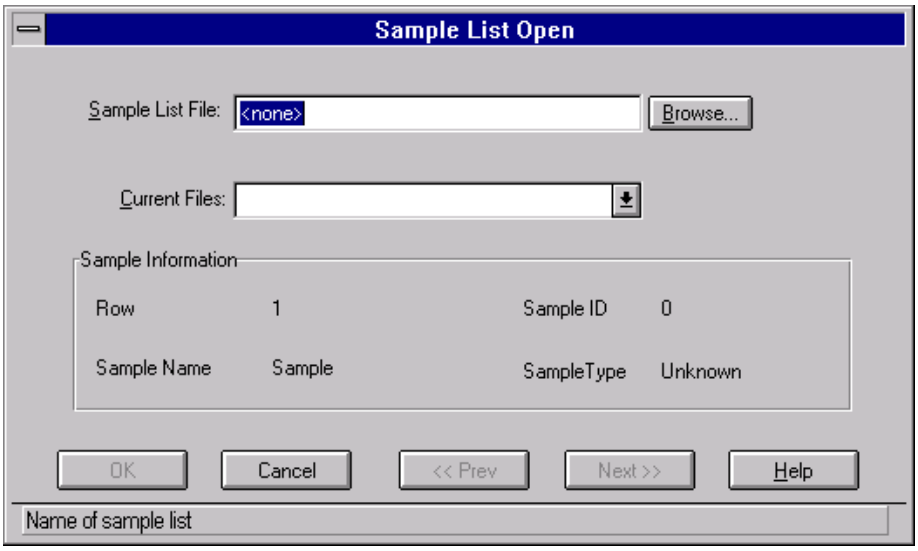


Figure 7-36. Sample List Open dialog box

2. Enter the *drugx.sld* Sample List in the Sample List File text box, as follows:

   a. Display the Open dialog box, as follows:
      Click on the Sample List File Browse button.
      See Figure 7-37.

   b. Change to the *C:\LCQ\examples\Methods* and select the *drugx.sld* Sample List, as follows:
      Scroll down in the File Name combo box until you see *drugx.sld*. Then, click on the file name.

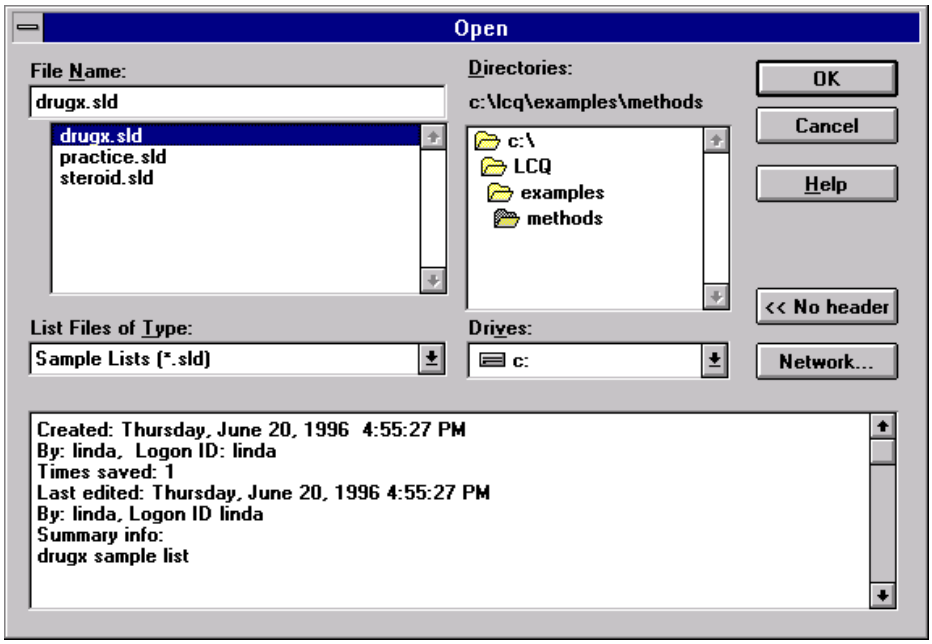


Figure 7-37. Open dialog box in the Rework window, showing the *drugx.sld* selected in the C:\LCQ\examples\methods directory

c. Enter the Sample List and close the Open dialog box, as follows:
Click on **OK**. LCQ automatically enters the method in the Sample List File text box. See Figure 7-38.

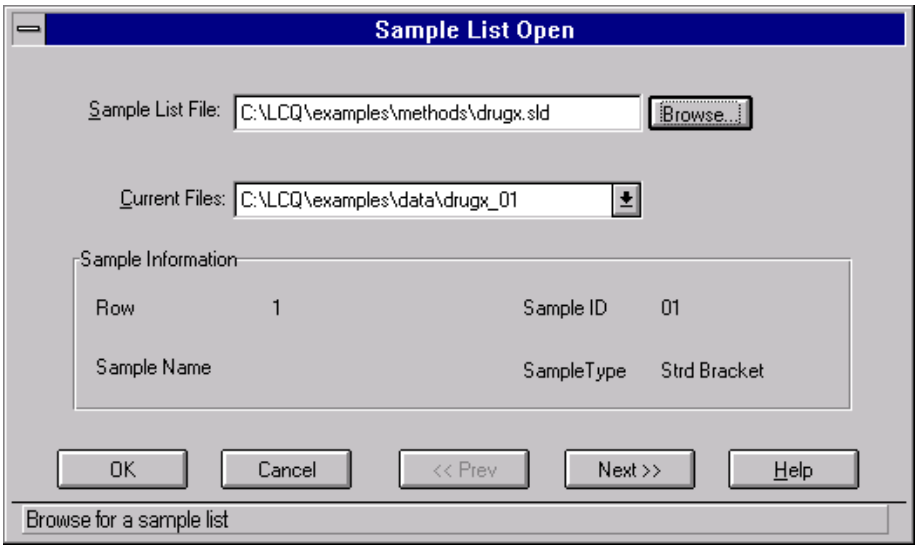


Figure 7-38. Sample List Open dialog box, showing the *drugx.sld* Sample List file containing result files

3. Open the Sample List file and close the Sample List Open dialog box, as follows:
   Click on **OK**.

> **Note**. LCQ displays a message box asking if you want to save changes to the currently open result file. See Figure 7-39. Because no changes have been made, answer no.

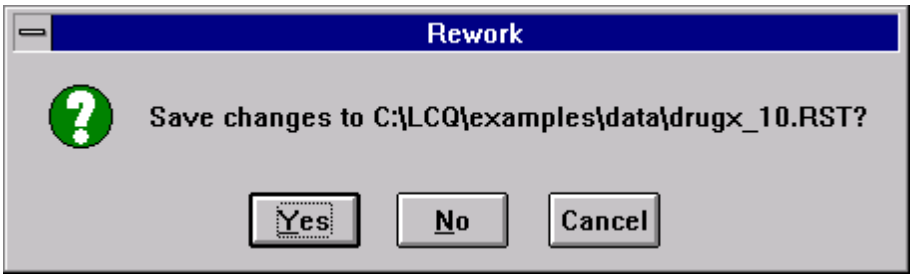


Figure 7-39. Rework message box

LCQ automatically opens the Sample List file and displays the current Sample List file name in the Rework window title bar. See Figure 7-40.

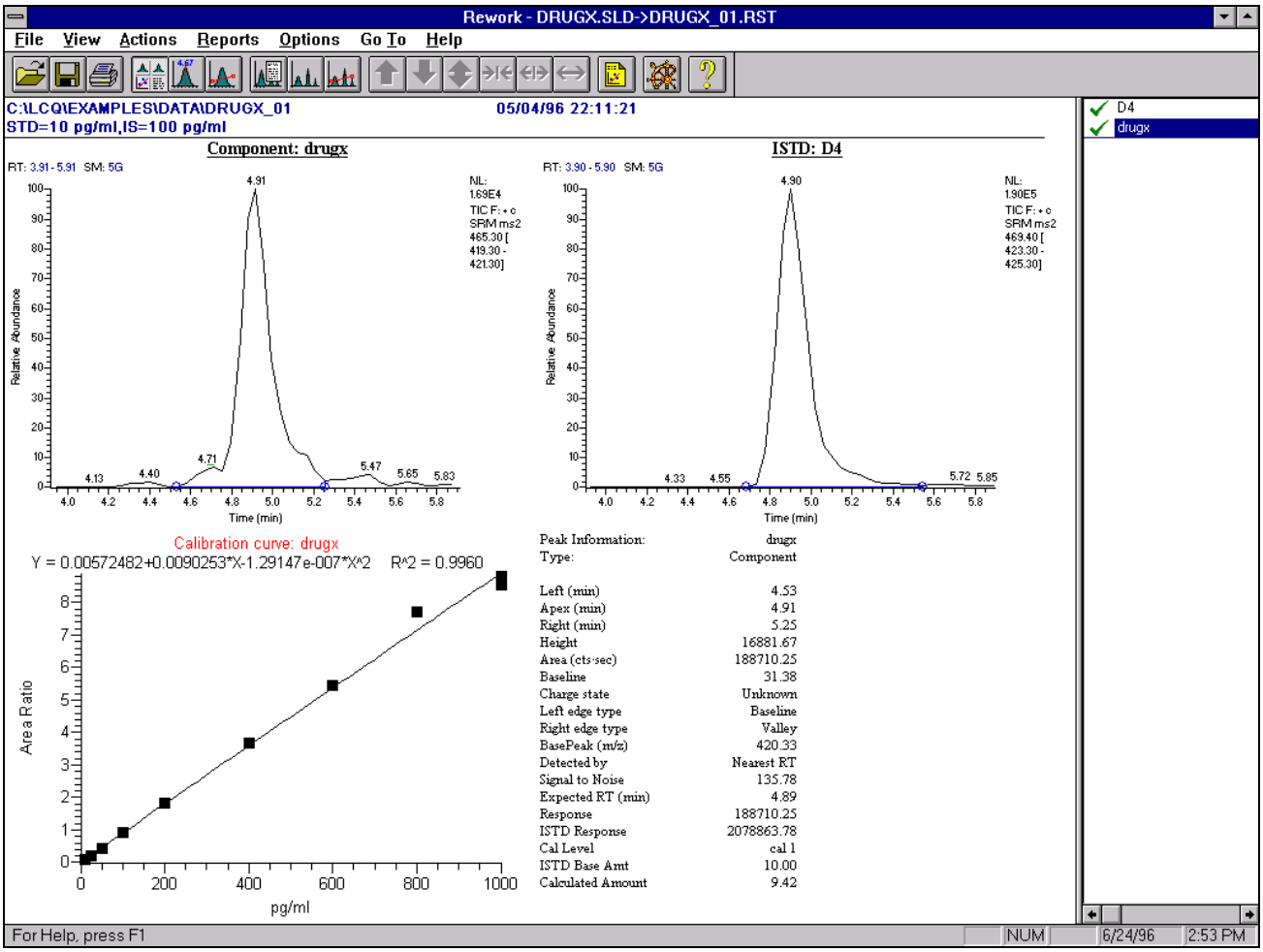


Figure 7-40. Component Review view of the Rework window, showing the open Sample List *drugx.sld* displayed in the title bar across the top of the window

4. Display the next result file in the Sample List file, as follows: Choose **File | Next File**. Or alternatively, you can hold the **<Ctrl>** key and press the **<Page Down>** key.

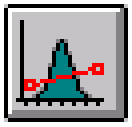
   Scroll through the result files in the Sample List file and do the following:

   - Evaluate the calibration curve according to the criteria used in your laboratory.
   - Check the peak areas for all components and ensure they match those listed in the Summary Sheet.

- Verify that the integration markers for LC peaks are correct.

7.4.3 Manually Integrating a Component Peak

You can manually integrate a component peak by changing its integration marker in the Manual Component view in the Rework window. To manually integrate a component peak, do the following:



1. Display the Manual Component Integration view, as follows: Click on the Manual Component Integration button in the Rework window. See Figure 7-41.

2. To change the integration marker in one of the result files in this example, do the following:

   a. Click on the left or right square editable handle on the blue baseline of the LC peak. LCQ automatically changes the cursor to a cross-hair. See Figure 7-42.

   b. Drag the handle to define a new location of the left or right peak limit. Repeat this procedure for the opposite side of the peak. LCQ automatically updates the parameters in the Peak Results and the Peak Limits group boxes.

   c. Enter the new peak limits and peak area, as follows: Click on **OK**.

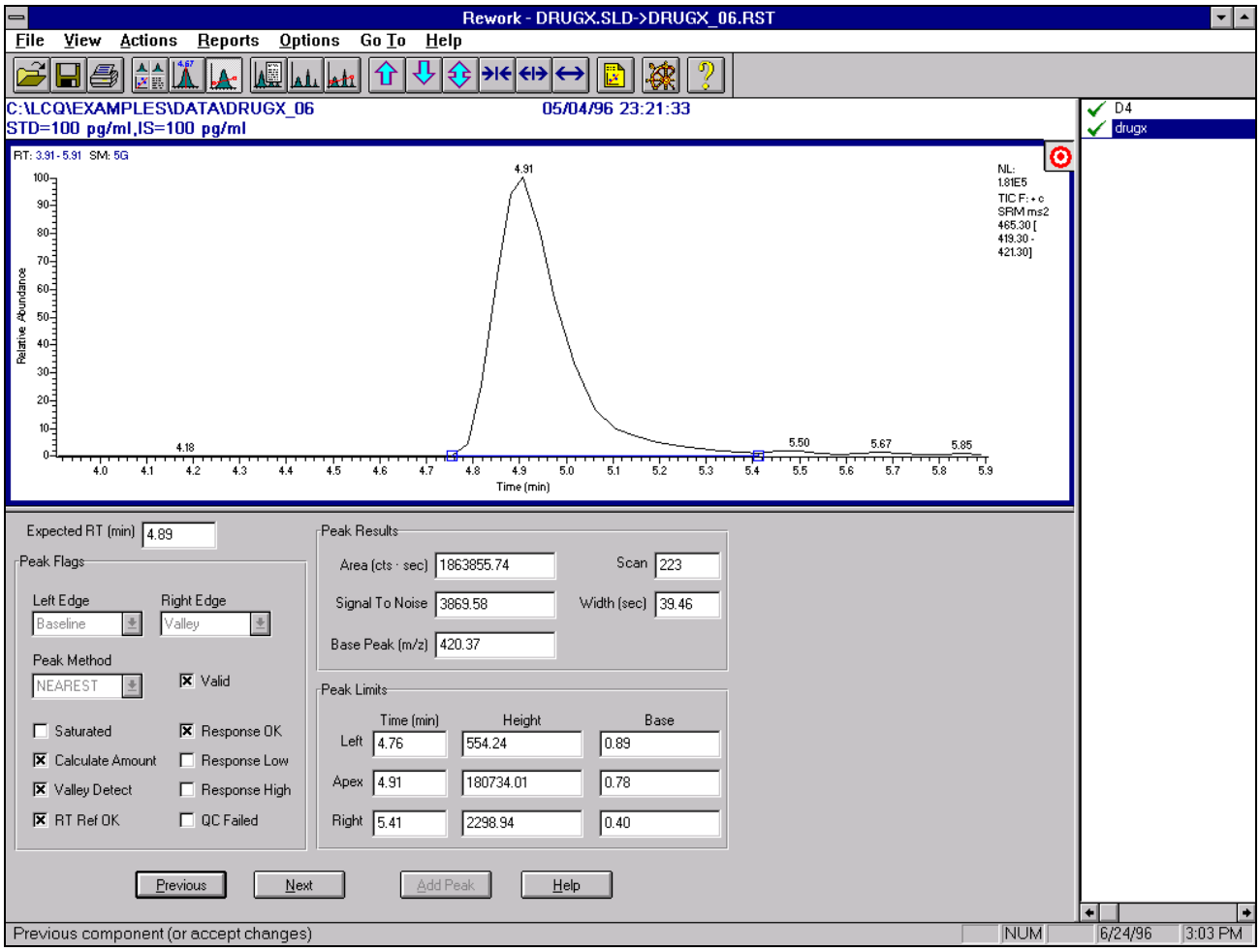


Figure 7-41. Manual Component view, showing the integration marker and peak integration parameters for a result file in this example

> **Note**. In this example, it is probably not necessary to save the new peak limits and peak area. By clicking on **OK** in step 2c you merely enter the new parameters into the result file. To save them in the result file you must overwrite the existing .rst file by displaying the Rework Save Changes message box and answering Yes. See Figure 7-43.

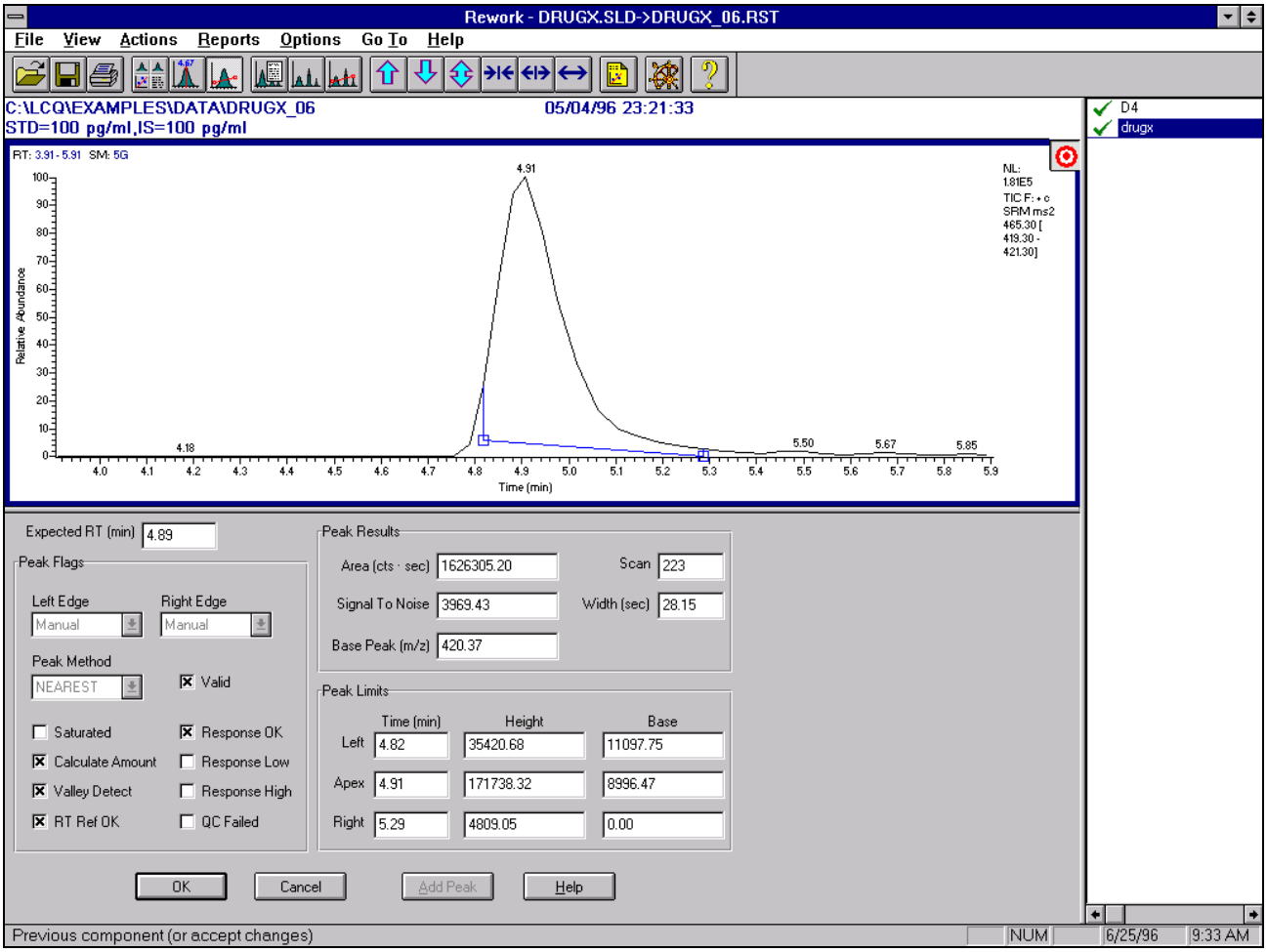


Figure 7-42. Manual Component view, showing how the integration marker can be adjusted to manually integrate a component peak

3. To save the changes and overwrite the current result file, do the following:

   a. Display the Rework Save Changes message box, as follows:
   Advance to the next result file in the Sample List file by holding the **<Ctrl>** key and pressing the **<Page Down>** key. See Figure 7-43.

   b. Save the peak results and peak limits, as follows:
   Click on **Yes**.

Or cancel the changes and restore the original result file, as follows:
Click on **No**.

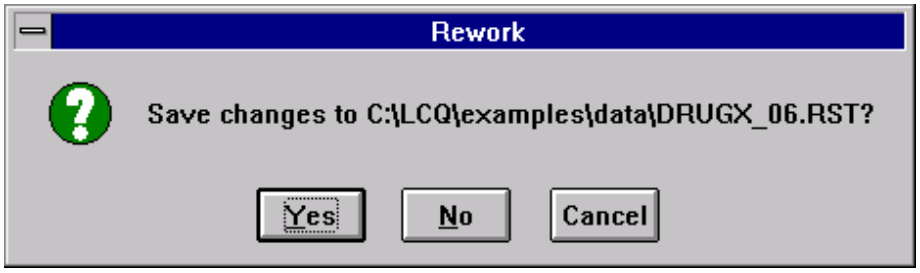


Figure 7-43. Rework Save Changes message box

Contents

APPENDIX A: SAMPLE FORMULATIONS

This Appendix provides instructions for the preparation of several stock solutions. These solutions are used for tuning, calibrating, and demonstrating applications of the APCI / ESI system. Formulations for sample solutions in this appendix are as follows:

- Caffeine, MRFA, and Ultramark 1621 stock solutions
- ESI Tuning Solution: Caffeine, MRFA, Ultramark 1621
- Reserpine
- Testosterone and d3-Testosterone

Always take safety precautions when you handle chemicals and unknown samples. **ENSURE THAT YOU READ AND UNDERSTAND THE HAZARDS OF THE CHEMICALS USED IN THE FOLLOWING PREPARATIONS.** Dispose of all laboratory reagents by the appropriate method for a specific reagent or solvent.

Material Safety Data Sheets (MSDS) provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provides information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the material safety data sheets for each chemical you use. Examples of potentially hazardous chemicals used in procedures throughout this manual are as follows:

- acetic acid
- acetonitrile
- methyl alcohol (methanol)
- reserpine
- testosterone

A.1 Caffeine, MRFA, and Ultramark 1621 Stock Solutions

For tuning and calibrating the ESI system, you use a solution of caffeine, MRFA, and Ultramark 1621 in an acetonitrile:methanol:water solution containing 1% acetic acid. You prepare the tuning solution from each of the following:

- caffeine stock solution
- MRFA stock solution
- Ultramark 1621 stock solution

Note. Vials of caffeine, MRFA, and Ultramark 1621 are included in the API accessory kit. To order more of these compounds or to order testosterone or d3-testosterone, write or call:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
(800) 325-3010 (in the USA or Canada)
(314) 771-3750 (outside the USA or Canada)

WARNING. AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheets (MSDS) for the proper handing of a particular solvent.

WARNUNG! KONTAKT MIT POTENTIELL GEFÄHRLICHEN SUBSTANZEN VERMEIDEN! Bei der Verwendung von Lösungs- oder Korrosionsmitteln sind stets Schutzhandschuhe und Schutzbrille zu tragen. Ablußstoffe sind aufzufangen, und gute Belüftung muss vorhanden sein. Nähere Information über den Umgang mit bestimmten Lösungsmitteln können Sie den Sicherheitsdatenblättern vom Lieferanten der jeweiligen Substanz entnehmen.

AVERTISSEMENT. EVITER D'ETRE EXPOSE AUX PRODUITS POTENTIELLEMENT DANGEREUX. Porter toujours des gants de protection et des lunettes de sécurité pour utiliser des solvants ou des matières corrosives. En outre, renfermer les écoulements de déchets et maintenir une ventilation appropriée. Consulter les fiches toxicologiques (MSDS) du fournisseur pour le mode d'emploi d'un solvant particulier.

A.1.1 Stock Solution: Caffeine

The 1 mg mL^{-1} stock solution of caffeine in 100% methanol is provided with your LCQ system.

A.1.2 Stock Solution: MRFA

Prepare a 1 mL stock solution of 5 nmol μL^{-1} MRFA in 50:50 methanol:water as follows:

1. Obtain the vial of L-methionyl-arginyl-phenylalanyl-alanine acetate•H_20 (MRFA) in your accessory kit. In this form, the MRFA sample has a molecular weight of 607.7 u. Carefully weigh 3.0 mg of the MRFA sample.

2. Dissolve the MRFA sample in a total volume of 1 mL of 50:50 methanol:water. Mix the solution thoroughly.

3. Label the vial MRFA stock solution.

A.1.3 Stock Solution: Ultramark 1621

Prepare a 10 mL stock solution of 0.1% Ultramark 1621 in acetonitrile as follows:

1. Obtain the vial of Ultramark 1621 in your accessory kit.

2. Using a syringe, measure out 10 µL of Ultramark 1621 and dissolve it in 10 mL of acetonitrile. Mix the solution thoroughly.

3. Label the vial Ultramark 1621 stock solution.

Go to the next topic: **ESI Tuning Solution: Caffeine, MRFA, Ultramark 1621**.

A.2 ESI Tuning Solution: Caffeine, MRFA, Ultramark 1621

Prepare 5 mL of the tuning solution as follows:

1. Pipet 100 µL of the stock solution of caffeine into a clean, dry vial.
2. Pipet 15 µL of the stock solution of MRFA into the vial.
3. Pipet 2.5 mL of the stock solution of Ultramark 1621 into the vial.

> **Note**. Use only glass pipets or stainless steel syringes when measuring glacial acetic acid. Use of plastic pipet tips causes contamination of acid stock solutions which can introduce contaminants in the tuning solution.

4. Pipet 50 µL of glacial acetic acid into the vial.
5. Pipet 2.34 mL of 50:50 methanol:water into the vial, and then mix the solution thoroughly.
6. Label the vial and store it in a refrigerator until it is needed.

A.3 Reserpine

Follow the directions given below to prepare a stock solution of reserpine. Then use serial dilutions of the stock solution to make either an APCI or ESI sample solution as described in: **ESI / APCI Sample Solution: Reserpine**.

A.3.1 Stock Solution: Reserpine

Prepare a stock solution of 1µg μL^{-1} reserpine in 1% acetic acid in 50:50 methanol:water:

1. Obtain the 1 gram vial of reserpine in your accessory kit. (The molecular weight of resperine is 608.7 u. Weigh out 1 mg of reserpine and transfer the sample to a polypropylene microcentrifuge tube.

2. Dissolve the reserpine sample in a total volume of 1 mL of 1% acetic acid in 50:50 methanol:water.

3. Ensure the sample is thoroughly dissolved in solution.

A.3.2 ESI / APCI Sample Solution: Reserpine

Prepare 1 mL of the sample solution of 50 pg μL^{-1} (82 fmol μL^{-1}) in 1% acetic acid in 50:50 methanol:water as follows:

1. Pipet 100 µL of the stock solution (1µg μL^{-1}) of reserpine into a clean polypropylene vial.

2. Add 900 µL of 1% acetic acid in 50:50 methanol:water to the vial.

3. Mix this solution (100 ng μL^{-1}) thoroughly.

4. Transfer 10 µL of the 100 ng μL^{-1} solution into a clean polypropylene vial.

5. Add 990 µL of 1% acetic acid in 50:50 methanol:water to the vial.

6. Mix this solution (1 ng μL^{-1}) thoroughly.

7. Transfer 50 µL of the 1 ng μL^{-1} solution into a clean polypropylene vial.

8. Add 950 µL of 1% acetic acid in 50:50 methanol:water to the vial.

9. Mix this solution (50 pg μL^{-1}) thoroughly.

10. Store the ESI / APCI sample solution (50 pg μL^{-1}) in a refrigerator until it is needed.

A.4 Testosterone and d3-Testosterone

Follow the directions given below to prepare a stock solution of testosterone. Then use serial dilutions of the stock solution to prepare the calibration levels.

A.4.1 Stock Solution: Testosterone

Prepare a stock solution of 100 ng μL^{-1} testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

1. Weigh out 1 mg of testosterone (or your analyte of interest) and transfer the sample to a polypropylene microcentrifuge tube.

2. Dissolve the sample in a total volume of 10 mL 50:50 methanol:water.

3. Ensure the sample is thoroughly dissolved in solution. Label the tube.

4. Prepare a 10 ng μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

    a. Pipet 100 µL of the 100 ng μL^{-1} stock solution of testosterone into a clean autosampler vial.

    b. Add 900 µL of 50:50 methanol:water to the vial. (10 ng μL^{-1})

    c. Mix the resulting (10 ng μL^{-1}) solution thoroughly. Label the vial.

5. Prepare a 100 pg μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

    a. Pipet 10 µL of the 10 ng μL^{-1} of testosterone solution into a clean autosampler vial.

    b. Add 990 µL of 50:50 methanol:water to the vial. (100 pg μL^{-1})

    c. Mix the resulting (100 pg μL^{-1}) solution thoroughly. Label the vial.

A.4.2 Stock Solution: d3-Testosterone

Prepare a stock solution of 100 ng μL^{-1} d3-testosterone (or your internal standard of interest) in 50:50 methanol:water as follows:

1. Weigh out 1 mg of d3-testosterone (or your internal standard of interest) and transfer the sample to a polypropylene microcentrifuge tube.

2. Dissolve the sample in a total volume of 10 mL 50:50 methanol:water.

3. Ensure the sample is thoroughly dissolved in solution. Label the tube.

A.4.3 Preparing the Calibration Levels

1. Prepare a 50 ng μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

   a. Pipet 500 µL of the 100 ng μL^{-1} stock solution of testosterone into a clean autosampler vial.

   b. Add 500 µL of 50:50 methanol:water to the vial. (50 ng μL^{-1})

   c. Mix the resulting (50 ng μL^{-1}) solution thoroughly. Label the vial.

2. Prepare a 5 ng μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

   a. Pipet 100 µL of the 50 ng μL^{-1} solution of testosterone into a clean autosampler vial.

   b. Add 900 µL of 50:50 methanol:water to the vial. (5 ng μL^{-1})

   c. Mix the resulting (5 ng μL^{-1}) solution thoroughly. Label the vial.

3. Prepare a 500 pg μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

   a. Pipet 100 µL of the 5 ng μL^{-1}solution of testosterone into a clean polypropylene vial.

b. Add 900 µL of 50:50 methanol:water to the vial. (500 pg μL^{-1})

c. Mix the resulting (500 pg μL^{-1}) solution thoroughly. Label the vial.

4. Prepare a 50 pg μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

   a. Pipet 100 µL of the 500 pg μL^{-1} solution of testosterone into a clean polypropylene vial.

   b. Add 900 µL of 50:50 methanol:water to the vial. (50 pg μL^{-1})

   c. Mix the resulting (50 pg μL^{-1}) solution thoroughly. Label the vial.

5. Prepare a 5 pg μL^{-1} calibration level of testosterone (or your analyte of interest) in 50:50 methanol:water as follows:

   a. Pipet 100 µL of the 50 pg μL^{-1} solution of testosterone into a clean polypropylene vial.

   b. Add 900 µL of 50:50 methanol:water to the vial. (5 pg μL^{-1})

   c. Mix the resulting (5 pg μL^{-1}) solution thoroughly. Label the vial.

6. Into two separate autosampler vials, place 1 mL each of 50:50 methanol:water solvent to analyze as blanks. Label each vial.

7. Add internal standard to each of the calibration levels and to the blank samples as follows:

   a. Using a clean pipet tip for each vial, pipet 1 µL of d3-testosterone (or your internal standard of interest) into each of the six autosampler vials.

   b. Cap the vials immediately.

8. Store the calibration levels in a refrigerator until they are needed.

Index

Notes: This index is sorted word-by-word, not letter-by-letter. Spaces between words and punctuation marks are treated as special characters. Dialog box is abbreviated d.b.

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