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READ THIS FIRST

Welcome to **Getting Productive: Quantitative Analysis with LCQUAN.**

This manual gives you information on how to use your LCQ to acquire data specifically for an analyte of interest. You then quantitate the results and produce reports, as follows:

Chapter 1: Introduction describes an overview of this manual, and includes a checklist of tasks to complete before you proceed.

Chapter 2: Preparing Standards describes how to prepare calibration standards of an example compound, reserpine, and how to prepare solvent solution for LC flow.

Chapter 3: Creating a Tune Method for the Analyte of Interest describes how to tune the MS detector automatically in ESI mode for MS and MS/MS ions for an analyte of interest.

Chapter 4: Creating an MS Experiment Method describes how to set up LC parameters and MS detector parameters for your experiment.

Chapter 5: Optimizing the MS Experiment Method describes how to obtain data using a Sample List and then how to analyze the data to achieve optimum results on your LCQ system.

Chapter 6: Developing an MS/MS Experiment Method describes how to set up your LCQ for MS/MS quantitation.

Chapter 7: Acquiring Your Data Using an LCQ describes how to build a Sample List and acquire data for a 5-point calibration curve. This chapter includes information about blanks and quality control samples (QCs).

Chapter 8: Processing Your Data with LCQUAN describes how to quantitate your raw data and produce reports using the LCQUAN program.

Changes to the Manual

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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)
°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
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)
ID	inside diameter
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})
OD	outside diameter
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 or Enter 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 displayed 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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1

INTRODUCTION

This manual describes quantitative analysis of samples using a liquid chromatograph (LC) with an attached autosampler. The detector for the LC is an LCQ™, a high-sensitivity mass spectrometer (MS) detector.

In a typical analysis, a sample is introduced into the LC either by syringe or by autosampler injection. The LC separates the sample into its various components. Then, each component enters the MS detector where it is analyzed. The LCQ data system processes the data by using the LCQUAN™ quantitative analysis software program.

Quantitative analysis with the LCQ consists of the following series of procedures:

- Preparing standards
- Creating a Tune Method for the analyte of interest
- Creating an MS Experiment Method
- Optimizing the MS Experiment Method
- Developing an MS/MS Experiment Method
- Acquiring your data using an LCQ
- Processing your data with LCQUAN

This manual describes the procedures you can use to produce quantitative MS/MS data for the compound reserpine. These procedures can also be used to produce quantitative MS/MS data for any compound of interest. The quantitative analysis described here uses a 5-point calibration standard curve of reserpine, and this procedure can be extended for any number of points.

The LCQ obtains reserpine results by operating in the electrospray ionization (LC/ESI/MS/MS) mode. At atmospheric pressure, reserpine molecules in solution move into a fused silica capillary tube and exit an ESI needle. A co-axial flow of nitrogen sprays the solution into a fine mist of droplets. The needle applies an electrical charge to the droplets, creating ions on their surface. Electrostatic repulsion then ejects the charged sample ions into the gas phase. Finally, the sample (reserpine) ions pass through a heated metal capillary tube and enter the MS detector where they are analyzed.

To ensure that you are ready to start getting productive, check the following list before you proceed:

1. You are familiar with LCQ software and hardware, as outlined in the **LCQ Operator's Manual** and **LCQ MS Detector Hardware Manual**.

2. The instrument is calibrated and tuned in the ESI/MS mode.

3. The MS detector has been prepared for ESI/MS operation.

4. You are familiar with LCQ connections used for quantitation and have configured your instrument, as follows:

   - The autosampler is plumbed to the LC.
   - The LC has installed a reverse phase C-18 (or similar) column.
   - The outlet of the LC column is plumbed to the LCQ divert valve.
   - The divert valve is plumbed to switch flow to either the LCQ or to waste.
   - A T-connector is in line just before the ESI source.
   - There is LC flow into the LCQ consisting of the solvent of choice. For the reserpine experiment, you need an isocratic solvent mixture of 1% acetic acid in 80:20 methanol:water. (Refer to the next chapter: **Preparing Standards**.)
   - The LC pump is on at a flow rate of approximately 0.5 mL min^{-1}.

5. Methanol is available to wash syringes.

You are now ready to go to the next chapter: **Preparing Standards.**

Contents

2

PREPARING STANDARDS

This chapter describes the preparation of a stock solution of reserpine and the preparation of an LC solvent solution. It then goes on to describe how to use these two solutions to perform a series of dilutions of the stock solution. The dilutions are placed into vials and used as calibration standards. Finally, this chapter describes the order in which to load vials into an autosampler tray.

This chapter contains the following topics:

- Preparing reserpine stock solution A
- Mixing the solvent solution
- Formulating calibration standards and blanks
- Loading the autosampler

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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 a Material Safety Data Sheet (MSDS) for each compound. MSDSs provide summaries on the hazard and toxicity of specific chemical compounds. MSDSs describe the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks.

Read the MSDS for each chemical you use. The following potentially hazardous chemicals are used in procedures throughout this manual:

- Acetic Acid
- Acetonitrile
- Methanol
- Reserpine

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 Sheet (MSDS) for the proper handling of a particular solvent.

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2.1 Preparing Reserpine Stock Solution A

Prepare a stock solution of reserpine in acetonitrile, as follows:

> **Note.** Reserpine degrades over time. Store the reserpine vial in a refrigerator, and dispose of it on the expiration date.

1. Obtain a vial containing reserpine from a chemical supplier. Reserpine has a monoisotopic mass 608.27 u. The average molecular weight of reserpine is used for the experiment described in this manual. The calculated average molecular weight is 609.2 u and accounts for isotopes.

2. Weigh out 0.0050 g (5.0 mg) of reserpine into a 5.0-mL volumetric flask. Use an analytical balance to weigh the reserpine.

3. Add about 50 µL of HPLC-grade water to the flask. The water increases the solubility of reserpine in acetonitrile.

4. Add enough HPLC-grade acetonitrile to the vial to bring the volume up to exactly 5.0 mL.

5. Cap the flask tightly, then sonicate or vortex the sample at room temperature for about 1 min. This step ensures that the reserpine is thoroughly dissolved.

6. Obtain a clean 5-mL sample vial. Label the vial *Solution A, Reserpine*.

7. Transfer the contents of the volumetric flask into the labeled sample vial. The concentration of the stock reserpine solution is 1.0 µg μL^{-1}.

2.2 Mixing the Solvent Solution

Mix the LC solvent solution, as follows:

1. Combine 320 mL of HPLC-grade methanol and 76 mL HPLC-grade water in a clean 250-mL Erlenmeyer flask.

> **Note.** Use only glass pipets when measuring glacial acetic acid. Plastic pipet tips can cause contamination of acid stock solutions.

2. Add 4.0 mL of acetic acid to the flask, and mix the solution thoroughly.

3. Pour about 350 mL of the solution into a sealable glass or polyethylene container. Label the container *80:20 MeOH:H_2O, 1% HOAc*. Reserve the remaining solution for the preparation of standard, blank, and QC samples.

4. Place the sealable container in the LC system in the position for reservoir D. Prepare more solution as needed.

2.3 Formulating Calibration Standards and Blanks

This topic provides instructions for preparing calibration standards, blanks, and quality control samples (QCs). This topic includes the following topics:

- Performing serial dilutions to prepare calibration standards
- (Optional) preparing blanks and QCs

2.3.1 Performing Serial Dilutions to Prepare Calibration Standards

To prepare calibration standards, you perform a series of dilutions from the stock solution, as follows. See Table 2-1.

1. Obtain seven 2-mL sample vials. Label them *B* through *H*. Then, arrange them in a tray.
2. Prepare Solution B, as follows:
   a. Pipet 10 µL of stock Solution A into the vial labeled B.
   b. Add 990 µL of the solvent solution prepared in topic 2.2.
   c. Cap the vial, crimp it tightly, and mix the solution. The concentration of reserpine Solution B is 10 $\mu g\ mL^{-1}$ (equivalent to 10 $ng\ \mu L^{-1}$).
3. Prepare Solutions C through H, in order, in a similar manner to that described for Solution B. Refer to Table 2-1 for the volumes of solution. Mix each solution thoroughly before taking an aliquot to prepare the next dilution.
4. Crimp the vial caps tightly. When they are not in use, cap the vials with new crimp tops and refrigerate them.

Table 2-1. Preparation of Reserpine Calibration Standards, showing serial dilutions

To Make	Dissolve	To Produce a Solution Concentration of	Amount of Reserpine in a 10-µL Aliquot
Solution A	5 mg of reserpine in 5 mL solvent	1 mg mL^{-1} (µg $µL^{-1}$)	(Stock)
Solution B	10 µL Solution A plus 990 µL solvent	10 µg mL^{-1} (ng $µL^{-1}$)	100 ng
Solution C	100 µL Solution B plus 900 µL solvent	1 µg mL^{-1} (ng $µL^{-1}$)	10000 pg
Solution D	50 µL Solution B plus 950 µL solvent	0.5 µg mL^{-1} (ng $µL^{-1}$)	5000 pg
Solution E	100 µL Solution C plus 900 µL solvent	0.1 µg mL^{-1} (ng $µL^{-1}$)	1000 pg
Solution F	50 µL Solution C plus 950 µL solvent	50 ng mL^{-1} (pg $µL^{-1}$)	500 pg
Solution G	100 µL Solution E plus 900 µL solvent	10 ng mL^{-1} (pg $µL^{-1}$)	100 pg
Solution H	50 µL Solution E plus 950 µL solvent	5 ng mL^{-1} (pg $µL^{-1}$)	50 pg

2.3.2 (Optional) Preparing Blanks and QCs

To prepare optional blanks and QC samples, do the following. This procedure requires five clean 2-mL sample vials to prepare two blanks and three QC samples.

1. Prepare the blanks, as follows:

   a. Obtain two 2-mL vials. Label them *Blank A* and *Blank B*. Then, arrange them in a sample vial tray.

   b. Pipet 1 mL of solvent solution into each vial.

   c. Cap the vials, and crimp them tightly.

2. Prepare the QC samples, as follows:

   a. Obtain three 2-mL sample vials. Label them *QC1, QC2*, and *QC3*. Then, arrange them in the sample vial tray.

   b. Pipet 990 µL of solvent solution into each of the three vials.

   c. Pipet 10 µL of Solution B (10 ng $µL^{-1}$ reserpine) into each vial. The final volume of QC solution in each vial is 1 mL.

   d. Cap the vials, then crimp them tightly. The QC sample concentration is 100 ng mL^{-1} (equivalent to 100 pg $µL^{-1}$ or 0.1 ng $µL^{-1}$).

2.4 Loading the Autosampler

To load the autosampler, do the following. See Figure 2-1.

01	Blank A	02	Solution H	03	Solution G	04	Solution F	05	Solution E
06	Solution D	07	Solution C	08	Blank B	09	QC1	10	QC2
11	QC3	12		13		14		15	
16		17		18		19		20	
21		22		23		24		25	
26		27		28		29		30	
31		32		33		34		35	
36		37		38		39		40	
41		42		43		44		45	
46		47		48		49		50	

Figure 2-1. Chart, showing a typical sequence of vials for the standards, QCs, and blanks in an autosampler tray

1. Load the vials containing the reserpine standard solutions into the autosampler tray in order of increasing concentration, from low (Solution H) to high (Solution C).

2. Load the vial containing the (optional) blank and QC samples into the autosampler tray, as shown in Figure 2-1.

3. Check the placement of each vial and its identification.

You are now ready to go to the next chapter: **Creating a Tune Method for the Analyte of Interest.**

Contents

3

CREATING A TUNE METHOD FOR THE ANALYTE OF INTEREST

This chapter contains instructions on how to create a Tune Method for the analyte of interest. You optimize the MS detector sensitivity for reserpine by tuning automatically from the Tune Plus window. You then save the resulting Tune Method so that you can use it as part of the Experiment Method.

The MS detector is already calibrated using the standard calibration solution, as described in the **LCQ Operator's Manual**. The calibration is used for all analytical conditions. Tuning, however, is specific for your particular analyte and your particular conditions. Tuning, therefore, refines the analytical conditions and the MS detector for maximum sensitivity of the analyte of interest.

This chapter contains the following procedures:

- Setting up to tune the MS detector
- Tuning automatically with reserpine Solution B
- Saving the ESI/MS Tune Method
- Optimizing the relative collision energy

Note. The following procedures assume that you are familiar with your LCQ instrument and the Tune Plus window. If you need information, refer to the LCQ online Help and **LCQ Operator's Manual**. Refer to **Appendix A** of the **LCQ MS Detector Hardware Manual** for a detailed discussion on how to plumb the divert/inject valve.

Before you begin the procedures in this chapter, confirm that you have completed the checklist in Chapter 1.

3.1 Setting Up to Tune the MS Detector

In this topic, you set up to tune the MS detector for reserpine. Good laboratory practice requires that your analytical system is free of contamination that might invalidate the results obtained on it. In this topic, you first ensure that the connections through which sample solutions flow are clean. You then fill a syringe with reserpine solution and set up your LCQ conditions in preparation for tuning the MS detector.

This topic contains the following procedures:

- Preparing the sample transfer line and ESI probe
- Setting up parameters for automatic tuning

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, daβ 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, daβ Luftsauerstoff in die Ionenquelle eindringt. Sauerstoff in der Ionenquelle kann bei eingeschaltetem MS-Detektor eine Gefahr darstellen. (Auβerdem können beim 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).

3.1.1 Preparing the Sample Transfer Line and ESI Probe

To ensure your analytical system is as clean as possible before you begin any analyses, use the following procedure to flush the sample transfer line, sample tube, and ESI probe:

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 good practice 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 Vorsichtsmaβnahme 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).

1. Loosen completely the two ESI flange retainer bolts. For the location of these bolts, see Figure 2-7, ESI probe assembly, in the **LCQ MS Detector Hardware Manual**.

2. Slide the ESI flange out, away from the MS detector chassis, on the slide rails, so that the entrance end of the heated capillary tube is accessible.

WARNING. AVOID BURNS. DO NOT TOUCH THE HEATED CAPILLARY. The heated capillary can attain temperatures above 250°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 250°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 250°C. Eviter toujours soigneusement de toucher l'extrémité d'entrée du capillaire chauffé lorsqu'elle est exposée.

3. Be careful not to touch the heated capillary with your hand. Place a small Teflon®-coated septum (included in the LCQ accessory kit) carefully over the entrance end of the heated capillary to seal the vacuum chamber of the MS detector.

4. Remove the syringe containing calibration solution from the syringe holder on the syringe pump.

5. While holding the plunger of the syringe in place, disconnect the syringe needle from the Teflon tube leading to the MS detector. Put this syringe containing calibration solution aside.

Note. The solvent solution 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 use a buffered solution to dissolve your samples, use an acidic solution to clean component surfaces.

6. Fill a clean, 250-µL Unimetrics syringe with 200 µL of solvent solution (1% acetic acid in 80:20 methanol:water).

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

8. Slowly depress the syringe plunger to flush the sample transfer line, sample tube, and ESI probe with the solvent solution. Visually check that the solution exits 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.

9. Remove the syringe containing solvent solution from the Teflon tube and syringe holder, and put it aside.

10. Fill another 250-µL syringe with 200 µL reserpine Solution B (10 ng μL^{-1}). (Refer to Chapter 2.)

11. Install the syringe containing reserpine into the syringe holder, as follows:

    a. Squeeze the blue release levers on the pusher block to release the block to slide on its guide rails. Gently, pull the pusher block backward, away from the blue syringe holder. Then, let go of the levers.

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

    c. Put the syringe into either of the two blue syringe holders.

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

12. Remove the Teflon-coated septum from over the entrance end of the heated capillary, being careful not to touch the heated capillary with your hand. (You installed this septum in step 3.)

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

14. Secure the ESI flange by tightening the two flange retainer bolts.

15. On the MS detector front panel, ensure that the green light above the word *Detector* is illuminated. This light indicates the Divert/Inject valve allows flow into the MS detector. If the light is off, press the blue button to switch the valve to the Detector position. The light above the word *Detector* illuminates.

3.1.2 Setting Up Parameters for Automatic Tuning

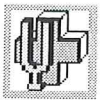
This topic describes how to set parameters in ESI/MS mode so that the LCQ tunes automatically using reserpine as the tuning compound.

WARNING. AVOID ELECTRICAL SHOCKS. ENSURE THAT YOUR SAMPLE TRANSFER LINE IS PROPERLY ATTACHED TO THE GROUNDED FITTING HOLDER ON THE ESI FLANGE. The 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! ELECTROSCHOCK VERMEIDEN! KONTROLLIEREN SIE, DASS DAS PROBENTRANSFERKABEL ENTSPRECHEND MIT DEM GEERDETEN FASSUNGSHALTER DES ESI-FLANCHES 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 RETUNUE 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.

Use the following procedure to set up LCQ for automatic tuning:



1. Open the Tune Plus window from the Navigator roadmap, as follows:
   Click on the Tune Plus button. See Figure 3-1.

2. Turn on the MS detector from the Tune Plus window, as follows:
   Click on the MS detector On/Standby/Off button in the Instrument Control toolbar. When the green triangle appears, the MS detector is On.

> **Note.** If the signals that pass between your LC and LCQ are contact closures only, ensure that your LC is on, and go to Step 3.

3. Start the LC pump, as follows:
   Choose **Setup | LC Pump**. Then, select the On option button.

4. Specify LC pump conditions, as follows:

   a. Specify an LC flow rate of 0.5 mL min^{-1}:
      Double-click on the Flow Rate spin box, and type **0.5.**

   b. This experiment uses only the solvent solution in the *D* solvent reservoir of the LC system. Specify a percentage of 100 in the Solvent D spin box:
      Double-click on the Solvent D spin box, and type **100.**

   c. Save the LC pump settings, close the dialog box, and return to the Tune Plus window:
      Click on **OK**.

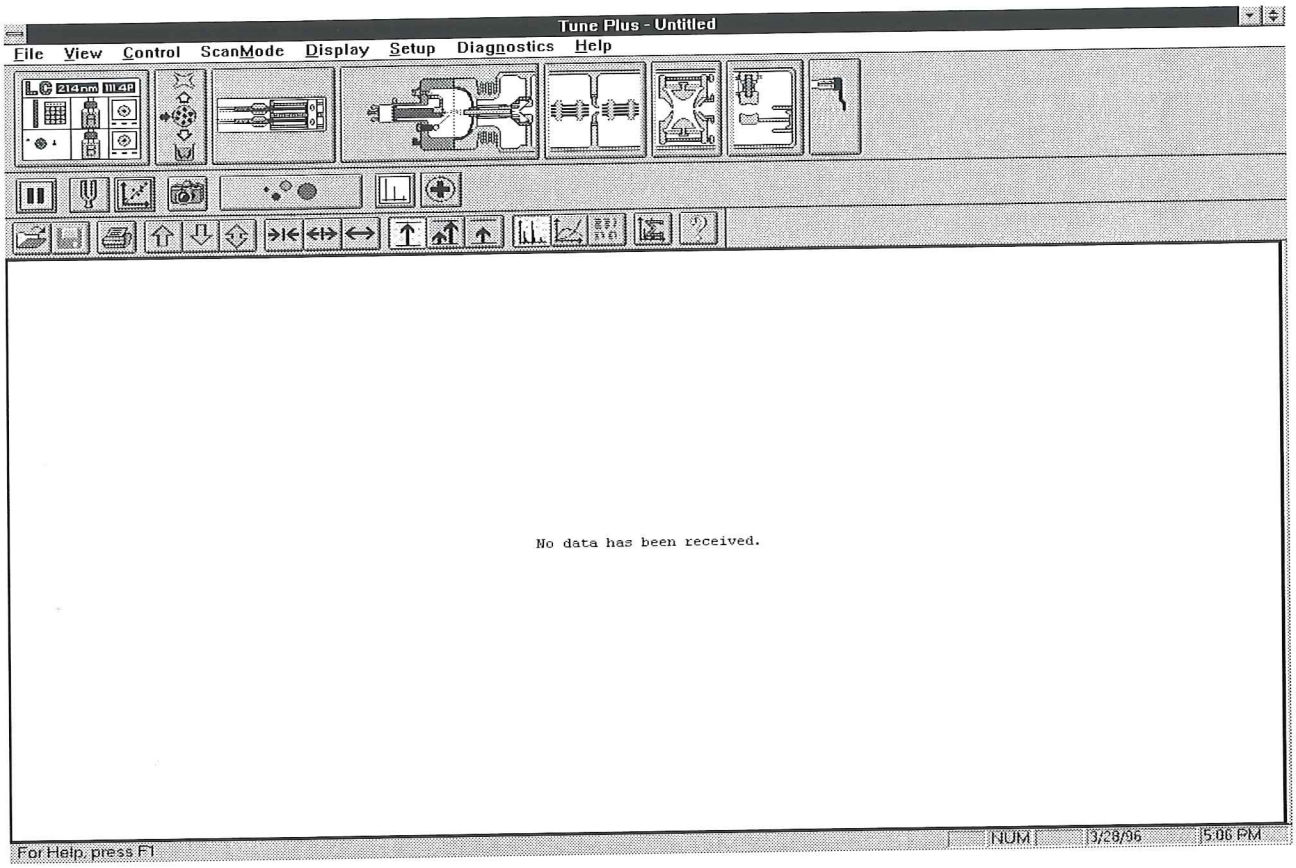


Figure 3-1. Tune Plus window, showing the MS detector in Standby mode

5. Confirm that the Navigator Status view indicates LC Ready status:
   Minimize the Tune Plus window to view the Navigator window. Observe the LC Status group box on the left side of the window. Confirm that the LC Status is Ready.

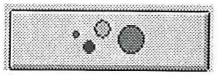
6. Redisplay the Tune Plus window:
   Click on the Tune Plus button on the taskbar at the bottom of your monitor display.

7. Display the Spectrum and Graph views in the Tune Plus window, as follows:

   a. Display the Spectrum view:
      If you have not already done so, click on the Spectrum button.

   b. Display the Graph view:
      Click on the Graph view button.

      The Tune Plus window splits to display two views.



8. Specify the scan parameters to detect reserpine in MS Full scan mode, as follows:

   a. Open the Define Scan dialog box:
   Click on the Define Scan button. See Figure 3-2.

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

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

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

   e. Set the maximum injection time to 200 ms:
   Double-click on the Maximum Inject Time spin box, and type **200.**

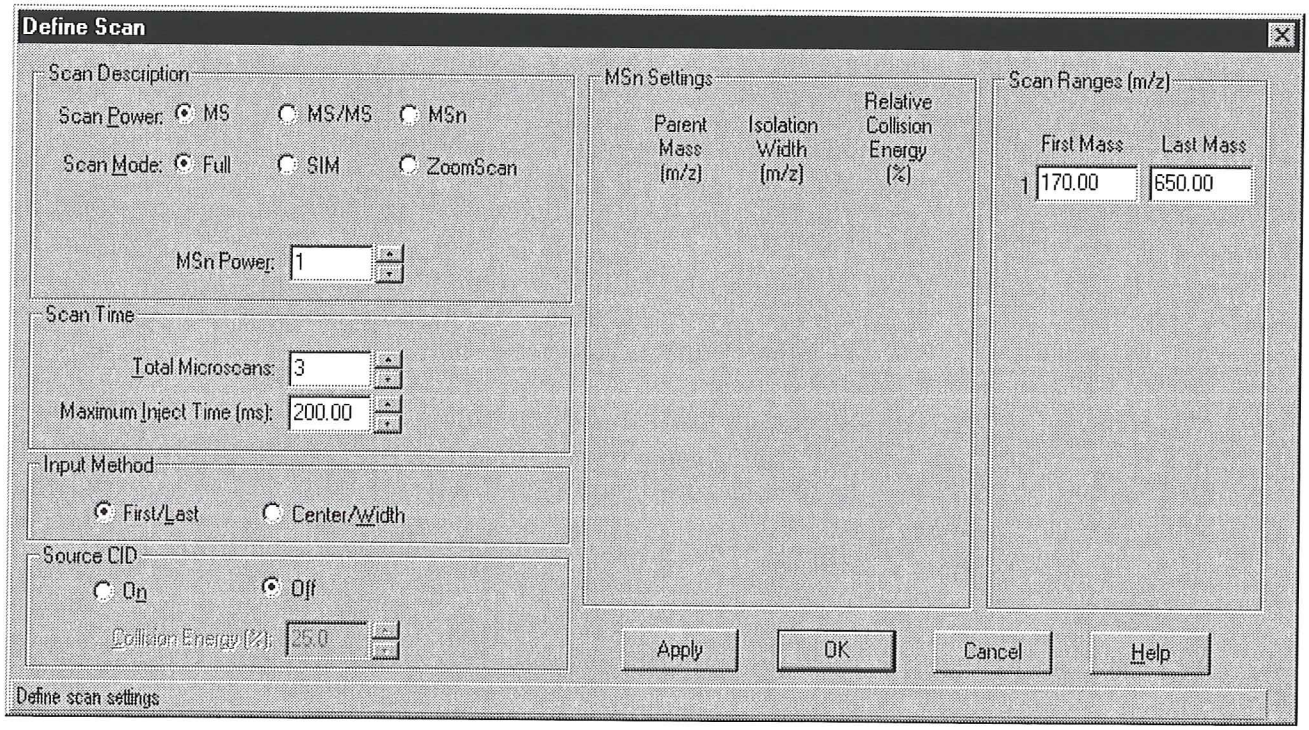


Figure 3-2. Define Scan dialog box, showing settings to perform automatic tuning with reserpine in MS Full scan mode

f. Select the first/last input method in the Input Method group box:
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 ion source CID off in the Source CID group box:
Click on the Off option button.

h. Set the first mass for the scan range to m/z 170 in the Scan Ranges group box:
Double-click on the First Mass text box, then type **170.**

i. Set the last mass for scan range to m/z 650:
Double-click on the Last Mass text box, then type **650.**

j. Confirm that your display resembles that shown in Figure 3-2.

k. Save the scan description, close the dialog box, and return to the Tune Plus window:
Click on **OK.**

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

10. 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.

11. Ensure the LC pump is On and the pressure is stabilized.

12. Set up the divert valve, as follows:

> **Note.** Use the divert valve to control the flow of sample into the ion source. You can set the divert valve to prohibit from flowing into the ion source that portion of your sample that might contaminate the ion source. Use the divert valve to keep the ion source clean longer.

a. Open the Divert/Inject dialog box:
Choose **Setup | Divert/Inject Valve.**

b. Select the option to direct LC flow into the MS detector:
Click on the Detector option button.

c. Close the dialog box, and return to the Tune Plus window:
Click on **Close.**

13. Specify the syringe pump parameters, and turn the syringe pump off, as follows:

a. Open the Syringe Pump dialog box:
Choose **Setup | Syringe Pump.** See Figure 3-3.

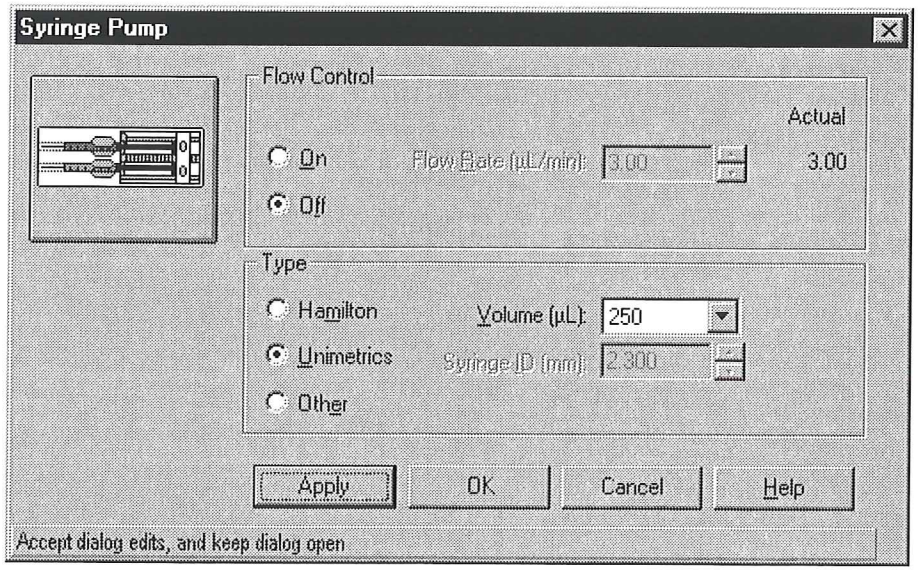


Figure 3-3. Syringe Pump dialog box, showing settings for reserpine

b. Specify the syringe pump off in the Flow Control group box:
Select the Off option button. (When automatic tuning is described in topic 3.2, you select the On option button.)

c. Specify a Unimetrics syringe type in the Type group box:
Select the Unimetrics option button.

d. Set the syringe volume to 250 µL:
Double-click on the Volume list box, then type **250.** Note that, if you specify a Unimetrics or Hamilton syringe, LCQ automatically sets the syringe ID to its proper value.

> **Note.** If you are not using a Unimetrics or Hamilton syringe, you need to set the syringe ID manually, as follows: Select the Other option button to activate the Syringe ID spin box. Then, double-click on the spin box, and type the inner diameter of your syringe.

e. Confirm that your settings are the same as those shown in Figure 3-3.

f. Save the syringe pump settings, leave the Syringe Pump dialog box open, and return to the Tune Plus window:
Click on **Apply.** Then, click on the Tune Plus window to make it active, and go to the next step.

14. Ensure the inlet is set up for ESI, not APCI, mode, as follows:

a. Open the ESI Source dialog box:
Choose **Setup | ESI Source**. See Figure 3-4. (If the command in Setup menu is APCI Source rather than ESI Source, choose **Setup | Change to ESI**. Then, choose **Setup | ESI Source**.)

> **Note.** The first three settings in the ESI Source dialog box can be changed only when the MS detector is in On mode.

b. Confirm that the settings approximate those shown in Figure 3-4.

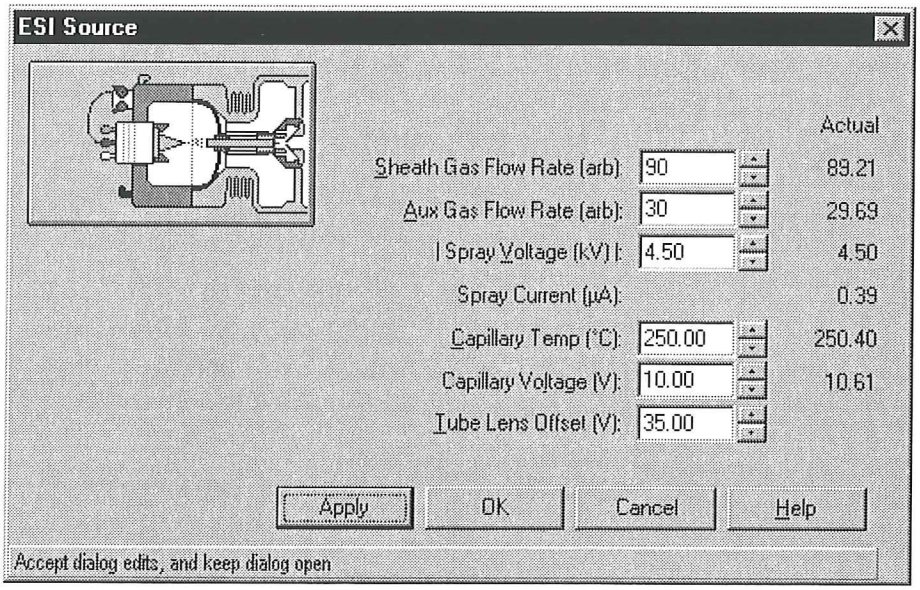


Figure 3-4. ESI Source dialog box, showing typical settings

c. Save the ESI source settings, close the dialog box, and return to the Tune Plus window:
Click on **OK.**

15. Ensure that the automatic gain control feature is on, as follows:

> **Note.** The settings in the Injection Control dialog box can be changed only when the AGC On option button is selected.

a. Open the Injection Control dialog box:
Choose **Setup | Injection Control**.

b. Turn on automatic gain control in the AGC group box:
Select the On option button. The spin boxes in the AGC On Settings group box become active.

c. Confirm that the value in the Full MS Target spin box is *2e+007*.

d. Save the settings, close the Injection Control dialog box, and return to the Tune Plus window:
Click on **OK.**

16. Ensure that the vacuum system pressures are correct, as follows:

    a. Open the Vacuum dialog box:
    Choose **Setup | Vacuum**. See Figure 3-5.

    b. Confirm the following:

    - Ion gauge: On
    - Ion gauge pressure: 1 to 2 ×10e5 Torr
    - Convectron™ gauge pressure: 1 to 2 Torr

    c. Save the vacuum settings, close the Vacuum dialog box, and return to the Tune Plus window:
    Click on **Close.**

You are now ready to start reserpine infusion. Go to the next topic: **Tuning Automatically with Reserpine Solution B**.

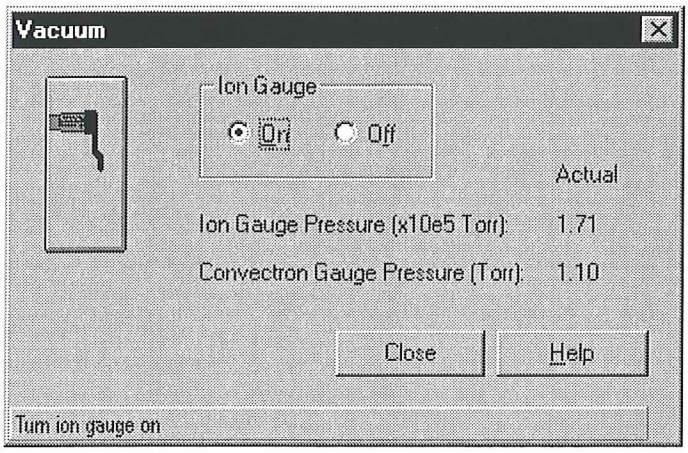


Figure 3-5. Vacuum dialog box, showing typical settings

3.2 Tuning Automatically with Reserpine Solution B

The LCQ automatic tuning program optimizes the MS detector for your specific analyte. Tune LCQ automatically with reserpine, as follows:

1. Turn on the MS detector from the Tune Plus window, as follows:
   Click on the MS detector On/Standby/Off button in the Instrument Control toolbar. The green triangle appears, showing that the MS detector is On. The blue Scan light on the LCQ front panel illuminates when the MS detector begins to scan. LCQ shows a real-time display in the Spectrum view of the Tune Plus window. The Start button in the Tune window becomes active to indicate automatic tuning is ready to start.
2. Turn on the syringe pump, and set the flow rate, as follows:
   a. Open the Syringe Pump dialog box:
      The dialog box should be open from step 13(f) of the previous topic. If it is open, click on the dialog box to make it active. If the dialog box is not open, choose **Setup | Syringe Pump** in the Tune Plus window. See Figure 3-6.

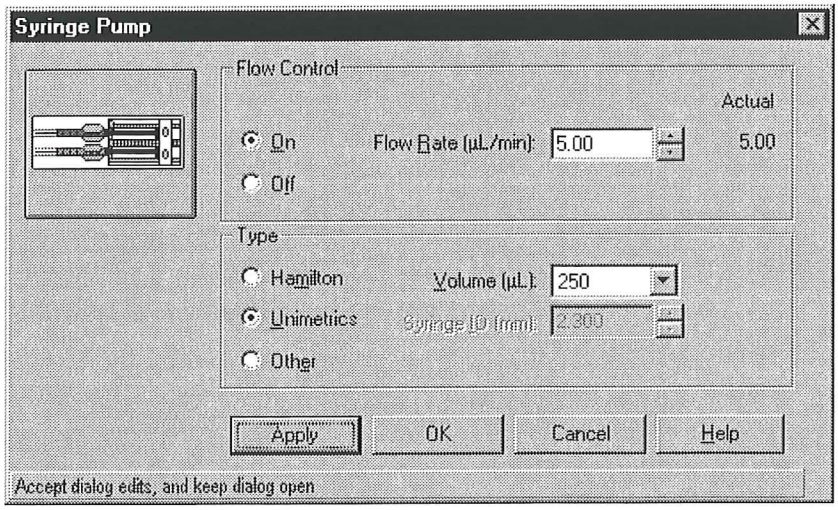


Figure 3-6. Syringe Pump dialog box, showing settings to infuse reserpine solution and syringe pump turned On

b. Turn on the syringe pump in the Flow Control group box:
Select the On option button. The Flow Rate spin box becomes active.

c. Specify an infusion flow rate of 5.0 μL min^{-1}:
Double-click on the Flow Rate spin box, then type **5.0**.

d. Start the syringe pump, close the dialog box, and return to the Tune Plus window:
Click on **OK**. LCQ saves the syringe pump settings. The green *Start / Stop* light on the LCQ front panel blinks and stays illuminated. The reserpine Solution B is now being infused into the ion source of the MS detector at a rate of 5.0 μL min^{-1}.

3. Specify the mass-to-charge ratio of the ion on which LCQ automatically tunes, as follows:

a. Open the Tune dialog box - Automatic page:
Click on the Tune button. Then, click on the Automatic tab. See Figure 3-7.

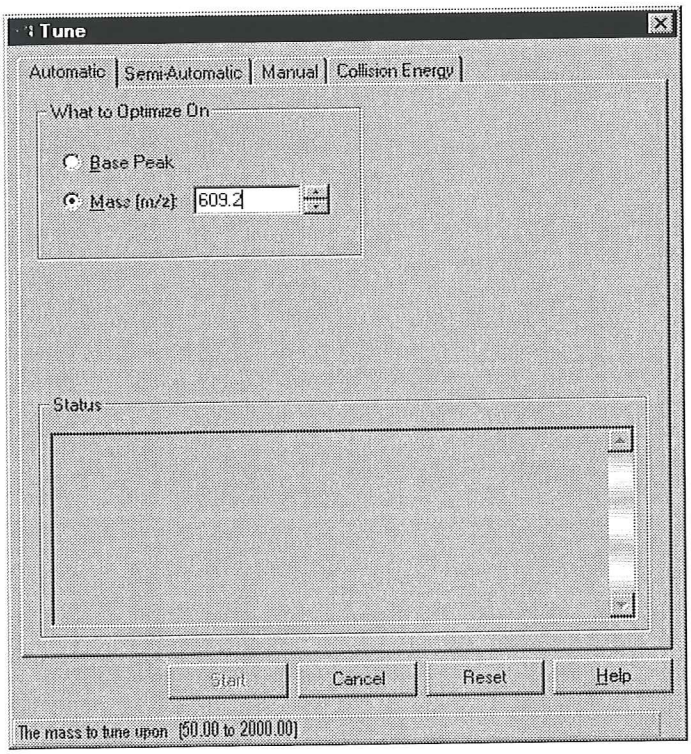


Figure 3-7. Tune dialog box, showing reserpine parent mass

b. Activate the Mass spin box in the What to Optimize On group box:
Select the Mass option button.

c. Set the mass-to-charge ratio to that of the parent ion for reserpine:
Double-click on the Mass spin box, then type **609.2**.

4. Start the automatic tuning procedure from the Tune dialog box. (The Tune Plus window and the Tune dialog box are open in your display at the same time.)

   a. Start automatic tuning:
   Click on **Start**. The following message appears: *Please ensure that the syringe pump is full*. See Figure 3-8.

   b. Close the message box, and return to the Tune dialog box:
   Click on **OK.**

> **Note**. Observe the Tune dialog box Status list box and the Tune Plus window. While automatic tuning is in progress, LCQ displays previous and new settings in the Status list box. At the same time, the Spectrum and Graph views in the Tune Plus window change as LCQ optimizes tune parameters.

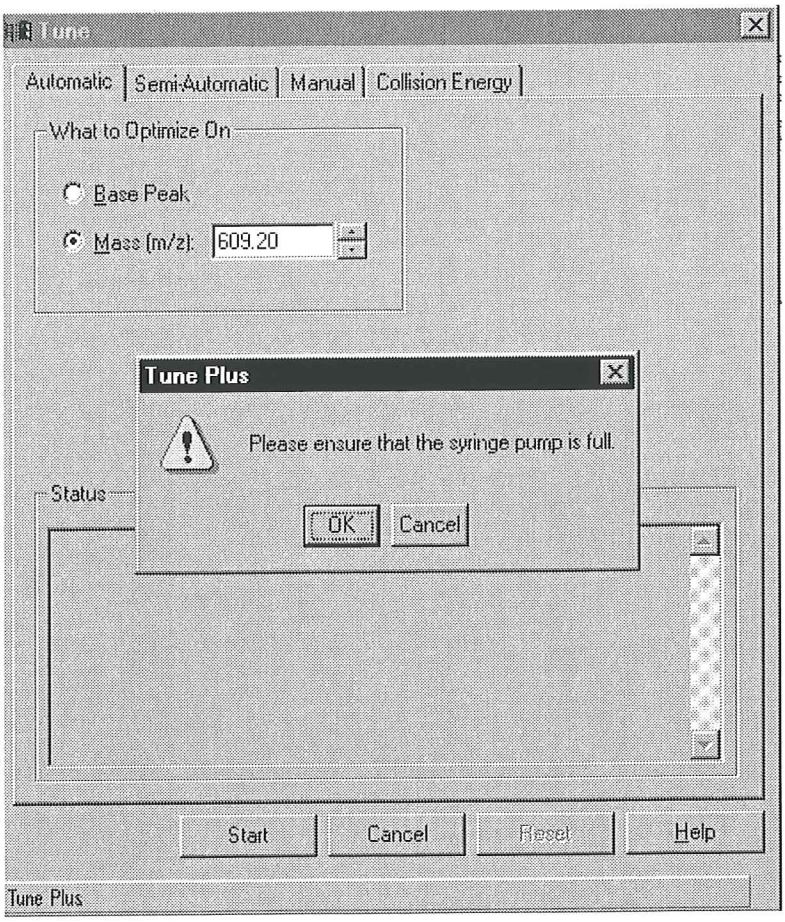


Figure 3-8. Tune dialog box, showing Automatic tuning message box

5. Observe the mass spectrum of reserpine while it infuses, as follows:

   a. Observe the Graph view in the Tune Plus window: LCQ displays real-time graph traces of the current optimization tests in the Graph view (right). The title of the current tune step appears at the top of the view as it progresses through the tuning procedure. The parameters for the current step are displayed on the X- and Y-axes. See Figure 3-9.

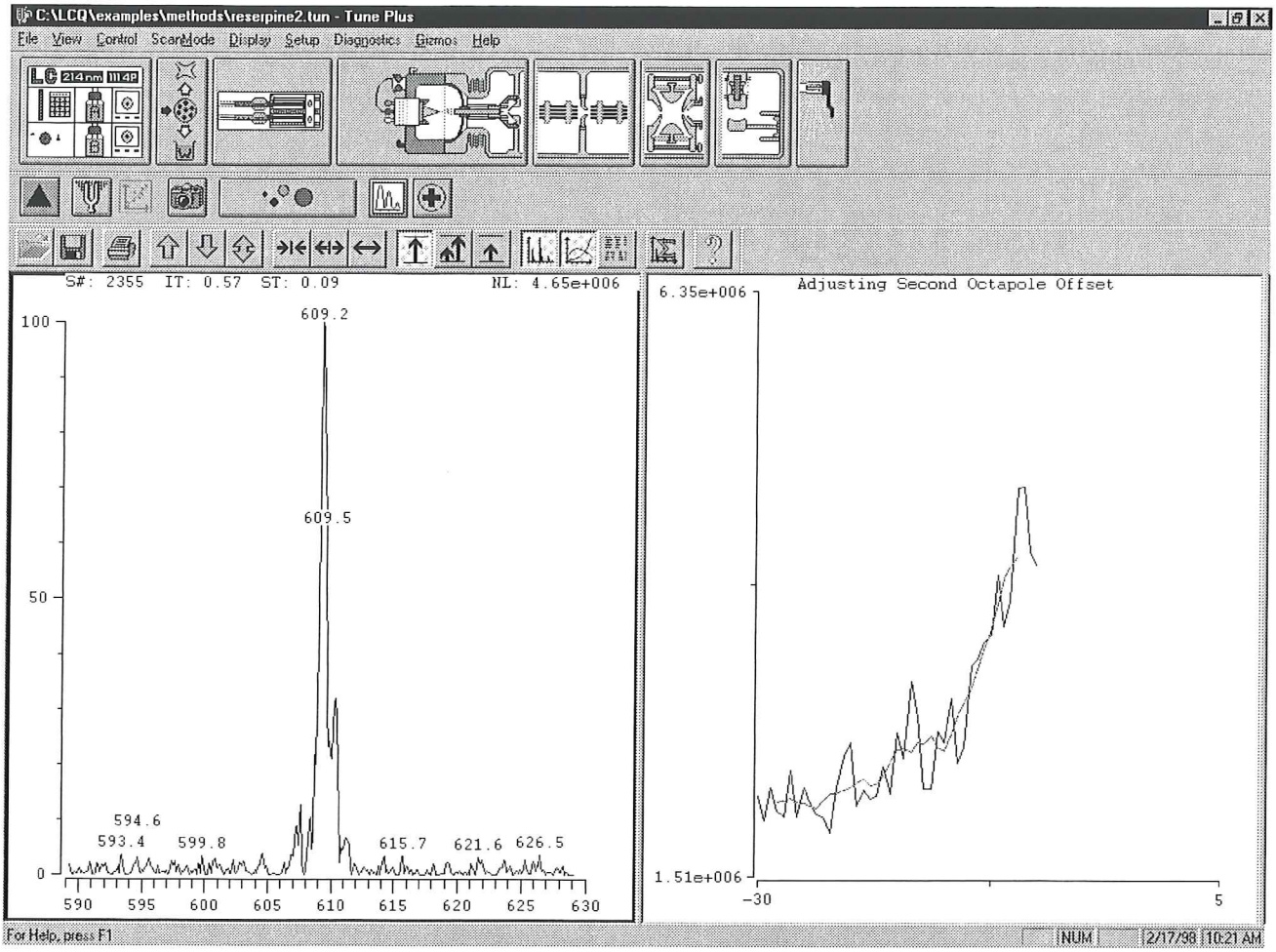


Figure 3-9. Tune Plus window, showing the Spectrum view (left) and Graph view during automatic tuning with the reserpine parent ion at *m/z* 609.2

b. Confirm that the reserpine parent ion at m/z 609.2 is visible in the Spectrum view. (During automatic tuning, LCQ changes from centroid scan mode to profile scan mode in the Tune Plus window.)

6. Automatic tuning is complete when the Tune dialog box resembles Figure 3-10. The following message is displayed at the end of the procedure:

   Optimization Complete -- change in signal = [value] %.

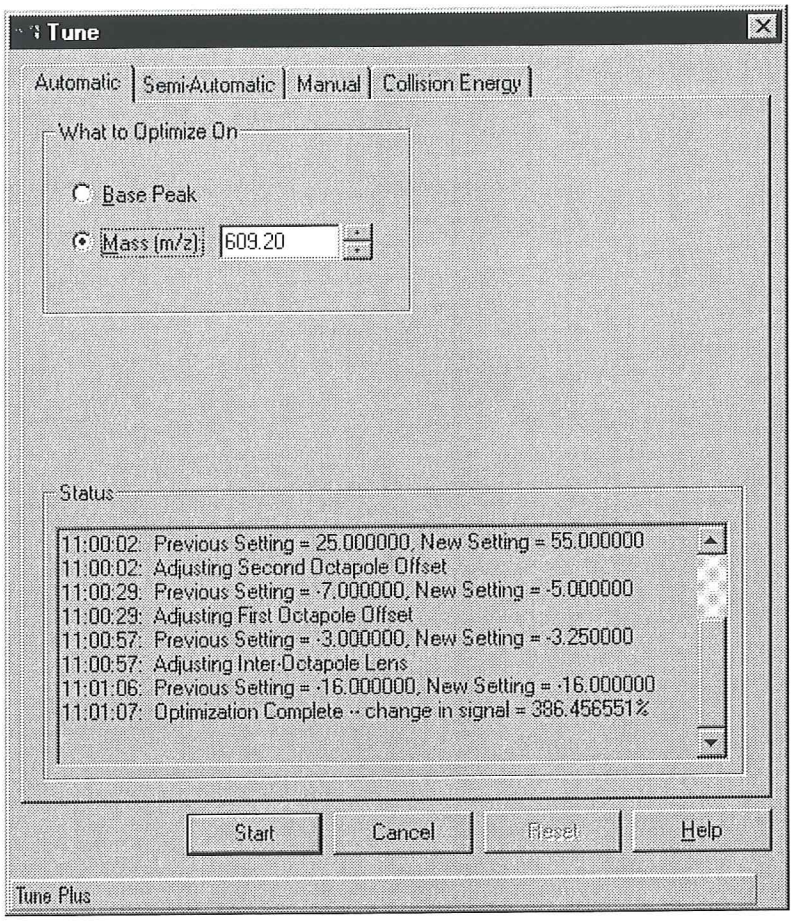


Figure 3-10. Tune dialog box, showing Optimization Complete for automatic tuning of the MS detector

7. Turn off the syringe pump, to stop the flow of reserpine solution into the ion source, as follows:
   Press the green *Start*/*Stop* switch on the syringe pump located on the door of the LCQ. When the syringe pump is off, the light above the switch goes out.

8. Divert LC flow to the waste container, away from the MS detector, as follows:
   Press the blue push-button switch on the front panel above the divert/inject valve. The button toggles the valve to direct LC flow to the waste container, and the light above the word *Waste* illuminates.

You are now ready to save the MS Tune Method. Leave the MS detector On, and go to the next topic:
Saving the ESI/MS Tune Method.

3.3 Saving the ESI/MS Tune Method

Save the tune parameters in a Tune Method file with extension *.tun* when automatic tuning is complete. (See Figure 3-10.)

Save the Tune Method while the MS detector is On, as follows. (Before you can save the method, you need to supply some information in the File Summary Information dialog box.)

1. Display the File Summary Information dialog box and then the Save As dialog box, as follows:

   a. Open the File Summary Information dialog box:
   Choose **File | Save As.** See Figure 3-11.

   b. Type a description of the Tune Method in the Description text box:
   For example, briefly describe the experiment conditions, scan parameters, and analyte of interest.

   c. Open the Save As dialog box:
   Click on **OK**.

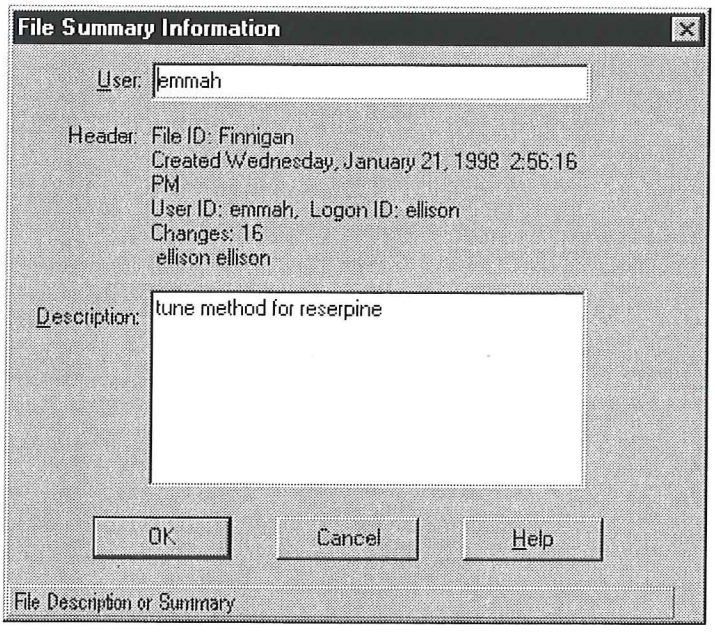


Figure 3-11. File Summary Information dialog box, showing a description of the saved file

2. Select the *C:\LCQ\examples\methods* directory, as follows: First, double-click on the *C:* directory. Scroll down in the *C:* directory until you find *LCQ*. Then, double-click on *LCQ*. Then, scroll down in the *LCQ* directory until you see the *examples* directory, and double-click on *examples*. Finally, scroll to the *methods* directory, and double-click on *methods*.

3. Name the Tune Method for the analyte of interest: Click on the File Name text box, and type **reserpine.tun**.

4. Save the Tune Method, close the dialog box, and return to the Tune Plus window, now named *reserpine.tun:* Click on **OK.**

You have now successfully tuned the MS detector in ESI/MS mode for the compound reserpine. Go on to the next topic: **Optimizing the Relative Collision Energy**.

3.4 Optimizing the Relative Collision Energy

The optimum relative collision energy for a particular analysis depends on the type of sample you analyze. In this procedure, you specify which product ion LCQ uses to optimize the relative collision energy for the dissociation of the reserpine product ion at m/z 609.2. The optimum relative collision energy is the one that produces the maximum product ion intensity.

Optimize the relative collision energy for the ESI/MS/MS analysis of reserpine, and save the Tune Method, as follows:

1. Specify the parameters to detect reserpine in the MS/MS Full scan mode, as follows:

    a. Open the Define Scan dialog box:
    Choose **Scan Mode | Define Scan**.

    b. Select the MS/MS scan power in the Scan Description group box:
    Click on the Scan Power: MS/MS option button. Note that LCQ sets the MSn Power to 2.

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

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

    e. Set the maximum injection time to 500 ms:
    Double-click on the Maximum Inject Time spin box, and type **500**.

    f. Specify parent ion settings in the MSn Settings group box:

        i. Specify a parent mass of m/z 609.2:
        Click on the Parent Mass text box, and type **609.2**.

        ii. Specify an isolation width of m/z 2.0:
        Double-click on the Isolation Width spin box and type **2**.

iii. Specify an initial relative collision energy of 25 % (with which LCQ can start the optimization):
Double-click on the Relative Collision Energy spin box, then type **25.0**. See Figure 3-12.

g. Save the MS detector settings, close the dialog box, and return to the Tune Plus window:
Click on **OK**.

2. Do the following before LCQ optimizes the relative collision energy:

   a. Ensure that the LC pump is on.

   b. Ensure that LC solvent solution is flowing into the MS detector.

   c. Ensure that the syringe in the syringe pump holder contains more than 50 µL of reserpine Solution B.

   d. Ensure that the syringe pump is on, and reserpine is infusing at a flow rate of 5 µL min^{-1}.

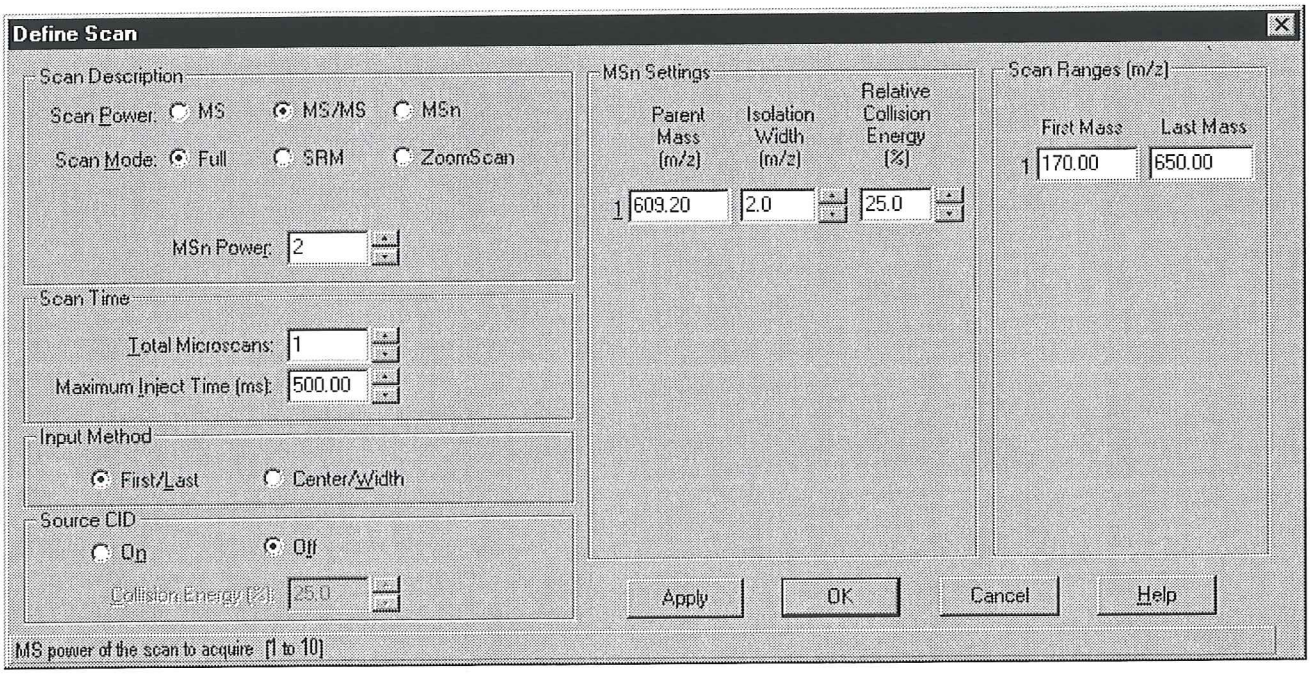


Figure 3-12. Define Scan dialog box, showing parameters to detect reserpine in MS/MS Full scan mode

e. Ensure that the monitor displays the Tune Plus window and the Tune dialog box.

f. Ensure that the centroid scan data type is selected. (The picture on the button should be the same as that shown here.)

g. Ensure that the MS detector is On.

> **Note.** At this time, you need to determine which product ion you want to specify. Increase the Relative Collision Energy in the MSn Settings group box by increments of 5.0% until you see spectral lines of product ion fragments in the Spectrum view (left) of the Tune Plus window. Choose the mass-to-charge ratio of the most abundant product ion.

3. Specify the mass-to-charge ratio of the reserpine product ion on which you want to optimize the collision energy, as follows:

   a. Open the Tune dialog box - Collision Energy page:
   Click on the Tune button. Then, click on the Collision Energy tab. See Figure 3-13.

   b. Activate the Product Ion Mass spin box in the What to Optimize On group box:
   Select the Product Ion Mass option button.

   c. Set the mass-to-charge ratio to that of the selected reserpine product ion:
   Double-click on the Product Ion Mass spin box, then type **397.2**.

4. Start the automatic procedure to optimize the relative collision energy from the Tune dialog box, as follows:

   a. Start optimizing:
   Click on **Start**. The following message appears: *Please ensure that the syringe pump is full.* (See Figure 3-8.)

   b. Close the message box, and return to the Tune dialog box:
   Click on **OK**.

   The Status list box in the Tune dialog box displays the message: *Optimizing Collision Energy....*

Observe the dynamic spectra in the Tune Plus window. Note the appearance of reserpine product ions as they are detected: m/z 397, 448, and 436.

5. Wait for LCQ to complete the optimization procedure. Optimization is complete when the Accept Optimized Value dialog box displays the following message:

   Collision energy optimization is done. The new value is [value] %. Accept it?

   LCQ presents three choices: *Accept*, *Reject*, or *Help*: Choose **Accept.** The Tune dialog box should resemble that shown in Figure 3-13.

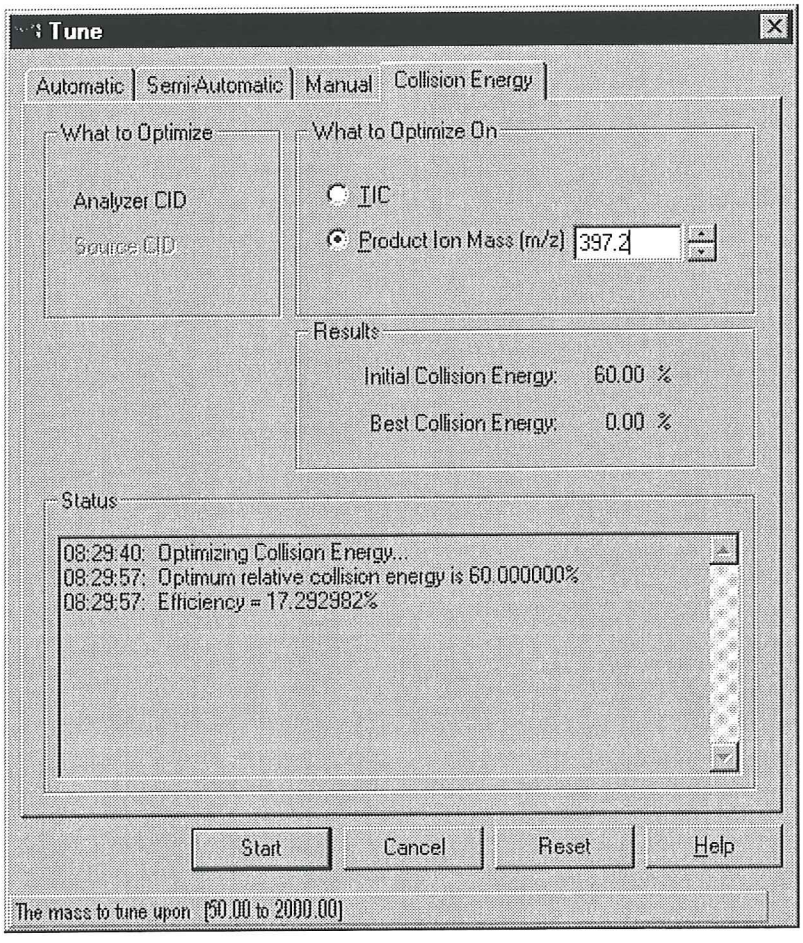


Figure 3-13. Tune dialog box, showing the Collision Energy page

> **Note.** When optimization is complete, make a written note of the value displayed next to the words *Optimum relative collision energy* in the Status list box of the Tune dialog box. You want to know this value for the MS/MS experiment, and it is not saved as part of the Tune Method file.

6. Close the dialog box, and return to the Tune Plus window:
   Click on **Cancel**.

7. Turn off the syringe pump, to stop the flow of reserpine solution into the ion source, as follows:
   Press the green Start/Stop switch on the syringe pump located on the door of the LCQ. When the syringe pump is Off, the light above the switch goes out.

8. Divert LC flow to the waste container, away from the MS detector, as follows:
   Press the blue push-button switch on the front panel above the divert/inject valve. The button toggles the valve to direct LC flow to the waste container, and the light above the word *Waste* illuminates.

9. Save the Tune Method while the MS detector is On, as follows. (Before you can save the method, you need to supply some information in the File Save -- Audit Trail dialog box.)

   a. Open the File Save -- Audit Trail dialog box:
      Choose **File | Save**. LCQ lists the parameters that you modified in the What Changed list box. See Figure 3-14.

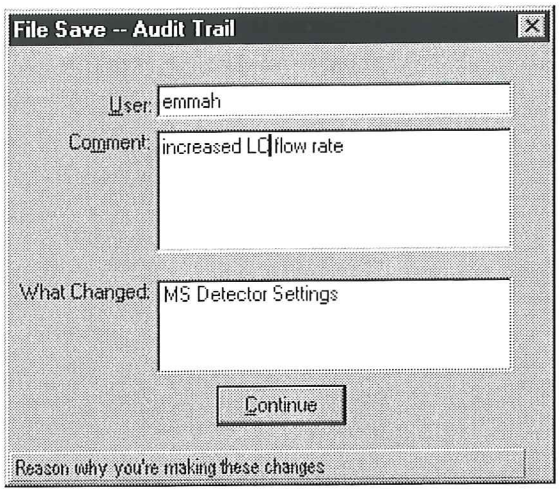


Figure 3-14. File Save -- Audit Trail dialog box, showing what parameters changed from the previously saved file

b. Enter a required comment in the Comment text box that describes the specific parameter(s) that changed and the reason for the change.

c. Close the dialog box, and return to the Tune Plus window, as follows:
Click on **Continue**. The previously saved Tune Method is overwritten.

10. Set the LCQ to Standby mode:

Click on the MS Detector On/Standby/Off button. When the Standby icon displays, the picture on the button should be the same as that shown here. The blue Scan light on the LCQ front panel goes out.

11. Click on **Cancel** to close the Tune Plus window, and return to the Navigator window.

> **Note.** Ensure that the Tune Plus window is closed. The Tune Plus window and the Navigator window cannot control the LCQ at the same time. Navigator waits for the Tune Plus window to close before it activates Start When Ready to run samples. Tune Plus has priority access to LCQ functions.

12. Rinse the flow lines that connect the LC to the LCQ with solvent solution after tuning with reserpine. It is good laboratory practice to clean tubing and all surfaces which have been in contact with chemical solutions. Use the following procedure to rinse the flow lines:

    a. While holding the plunger in the syringe, remove from the syringe holder the syringe containing reserpine Solution B. Disconnect the tip of the syringe needle from the Teflon tubing.

    b. Obtain the syringe containing solvent solution, and ensure that it contains at least 200 µL of solution. Connect the Teflon tubing to the syringe needle.

    c. Slowly depress the syringe plunger to flush the sample transfer line, sample tube, and ESI probe with the solvent solution. Use a lint-free paper to gently remove excess solution.

    d. Remove the syringe from the Teflon tube and syringe holder, and put it aside.

    e. Stop the LC pump in the Navigator window: Choose **Instrument | Stop LC Pump**.

    f. Disconnect the T-connector and the tubing that connects the syringe to the divert valve from the position between the LC and the divert valve. The syringe pump is not used in the following procedures.

    g. Install clean tubing to connect the LC directly to the divert valve.

Now go to the next chapter: **Creating an MS Experiment Method.**

Contents

4

CREATING AN MS EXPERIMENT METHOD

This chapter contains instructions on how to create an LC/MS Full scan Experiment Method. The LC/MS Experiment Method is the specific set of parameters used to operate the autosampler, LC, and MS detector. A text file with extension *.emd* stores the initial parameter settings until they are optimized in Chapter 5: **Optimizing the MS Experiment Method**.

You need an MS Experiment Method to ensure that most variables in your experiment system are considered and controlled before you start analyzing samples. The retention time of your analyte in your LCQ system is one of the variables you determine in this chapter. The experiment setup described here uses the reserpine standard samples made in Chapter 2: **Preparing Standards,** and the Tune Method created in Chapter 3: **Creating a Tune Method for the Analyte of Interest**.

The topics in this chapter are as follows:

- Specifying LC parameters
- Setting up the MS Detector
- Saving the MS Experiment Method

Note. If the signals that pass between your LC and the LCQ are contact closures only, disregard the LC setup description.

4.1 Specifying LC Parameters

Specify the LC parameters from the Experiment Method window, as follows:

> **Note.** Before you begin any of the procedures outlined in this chapter, ensure that your ESI/MS system is properly set up as described in Chapter 3: **Creating a Tune Method for the Analyte of Interest.**

1. Open the Experiment Method window from the Navigator roadmap, as follows:
   Click on the Experiment Method button.

2. Set up the autosampler parameters, as follows:

   a. Open the Autosampler dialog box:
   Choose **Setup | Autosampler Setup**. See Figure 4-1.

   b. Set the injection volume to 10 µL:
   Double-click on the Default Injection Volume text box, then type **10.0**.

   c. Close the dialog box, and return to the Experiment Method window:
   Click on **OK.**

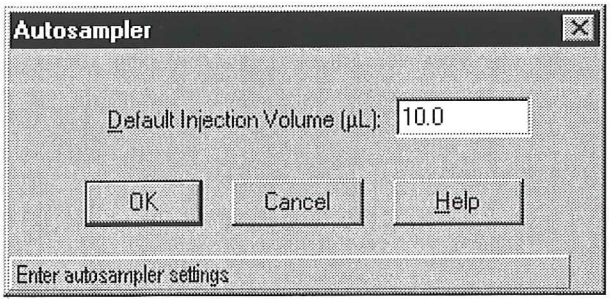


Figure 4-1. Autosampler dialog box, showing the default injection volume set to 10 µL

3. Specify the LC parameters, as follows:

> **Note.** The LC conditions for this Experiment Method specify a constant flow rate and constant solvent composition (isocratic conditions).

a. Open the Setup HPLC dialog box:
Choose **Setup | HPLC Setup**. See Figure 4-2.

b. Set the LC run time to 10.0 min:
First, leave the time in row 1 at its default value of **0.0** min. Then, type **10.0** in the Time text box in row 2.

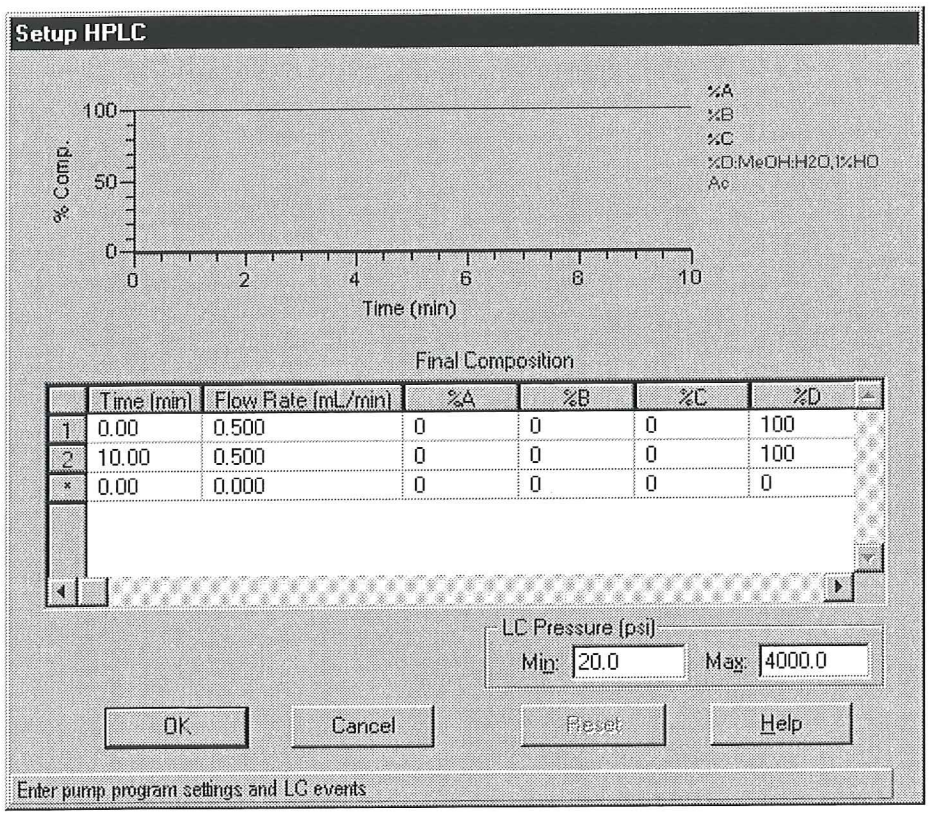


Figure 4-2. Setup HPLC dialog box, showing the settings for the reserpine experiment

c. Set a constant flow rate of 0.5 mL min^{-1} for the entire experiment:
Double-click in the Flow Rate text box in row 1, then type **0.5**. Repeat this step for row 2.

d. Set the composition of mobile phase to 100% solution from reservoir D for the entire experiment:
Click in the row 1 text box in the %D column, then type **100**. Repeat this step for row 2.

e. Leave the LC minimum pressure set to its default value of 20.0 psi in the LC Pressure group box.

f. Leave the LC maximum pressure set to its default value of 4000.0 psi.

Confirm that your display resembles that shown in Figure 4-2.

g. Save the LC settings, close the dialog box, and return to the Experiment Method window:
Click on **OK.**

4. Specify the LC column and solvent information according to your LC column and your solvent mixture, as follows:

a. Open the LC Column/Solvent Information dialog box:
Choose **Setup | LC Column/Solvent Setup.** See Figure 4-3.

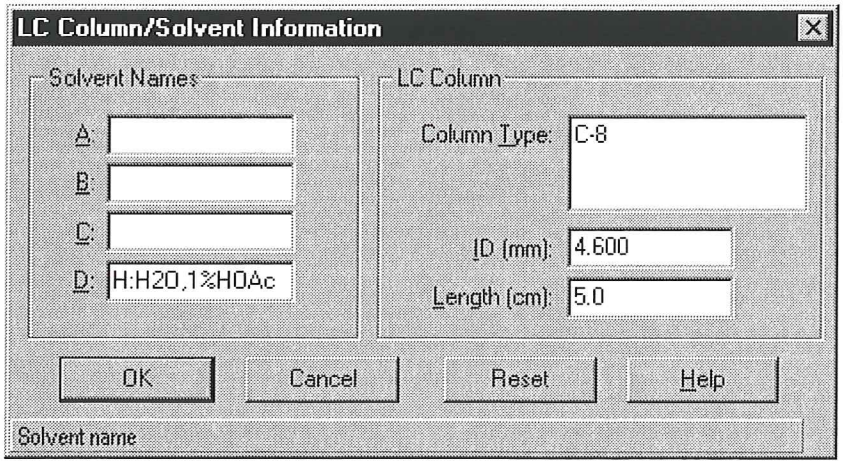


Figure 4-3. LC Column/Solvent Information dialog box, showing the solvents and column used in this experiment

b. Specify the LC solvent name as MeOH:H2O,1%HOAc:
Click on the *D* text box in the Solvent Names group box.
Then, type **MeOH:H2O,1%HOAc**.

c. Specify the type of column you are using:
Click in the Column Type text box in the LC Column group box, then type the name of your LC column. For example, type **C-8**.

d. Specify the inner diameter in millimeters of the column you are using:
Click on the ID text box. Then, type the inner diameter of your column. For this example, type **4.6**.

e. Specify the length in centimeters of the column you are using:
Click on the Length text box. Then, type the length of your column. For this example, type **5.0**.

f. Save the settings, close the dialog box, and return to the Experiment Method window:
Click on **OK.**

> **Note.** The divert valve is used to minimize contamination of the MS detector ion source from sample matrices. You specify the timing for the divert valve switches in the Divert Valve dialog box after you determine by experiment the retention time for the target analyte.

5. Specify the divert valve switch times, as follows:

   a. Open the Divert Valve dialog box:
   Choose **Setup | Divert Valve Setup**. See Figure 4-4.

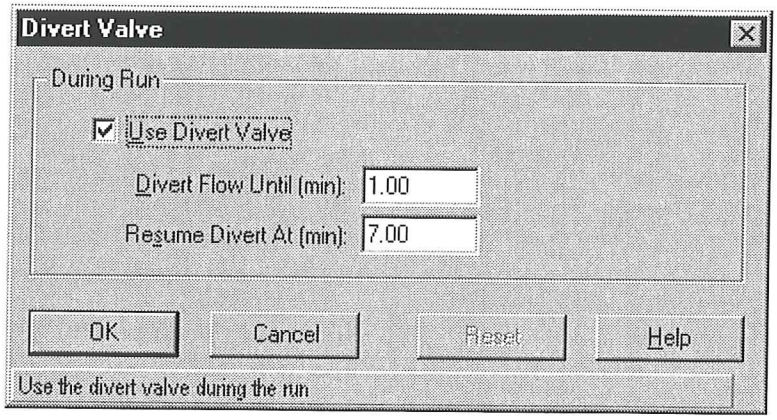


Figure 4-4. Divert Valve dialog box, showing the timing of the divert valve switches during the run

b. Activate the divert valve in the During Run group box:
Select (☑) the Use Divert Valve check box. The Divert Flow Until text box and the Resume Divert At text box become active.

c. Divert the LC flow away from the MS detector until 1 min after the run starts:
Enter **1** in the Divert Flow Until text box. LC flow begins to enter the MS detector 1 min into the run.

d. Again divert the LC flow away from the MS detector at 7 min after the run starts:
Enter **7** in the Resume Divert At text box.

LC flow enters the MS detector from 1 min into the run until 7 min into the run. At 7 min, LC flow is again diverted away from the MS detector.

e. Save the divert valve settings, close the dialog box, and return to the Experiment Method window:
Click on **OK.**

4.2 Setting Up the MS Detector

In the reserpine experiment, you perform an LC/MS analysis on reserpine standard solutions in which a single compound is analyzed in a clean matrix. The compound elutes without interference at a single retention time in a single time segment.

Analysis of other compounds might require detection of more than one analyte in a single run. For example, an experiment that uses an isotopically labeled internal standard might have two or more coeluting analytes.

Specify the MS detector parameters to acquire data in MS Full scan mode, as follows:

> **Note.** No raw file is required to create an Experiment Method. However, if an appropriate raw file exists, you can open it (choose **File I Open Raw File**) to help you build an Experiment Method.

1. Open the MS Detector Setup dialog box, as follows:
   Choose **Setup I MS Detector Setup**. See Figure 4-5.

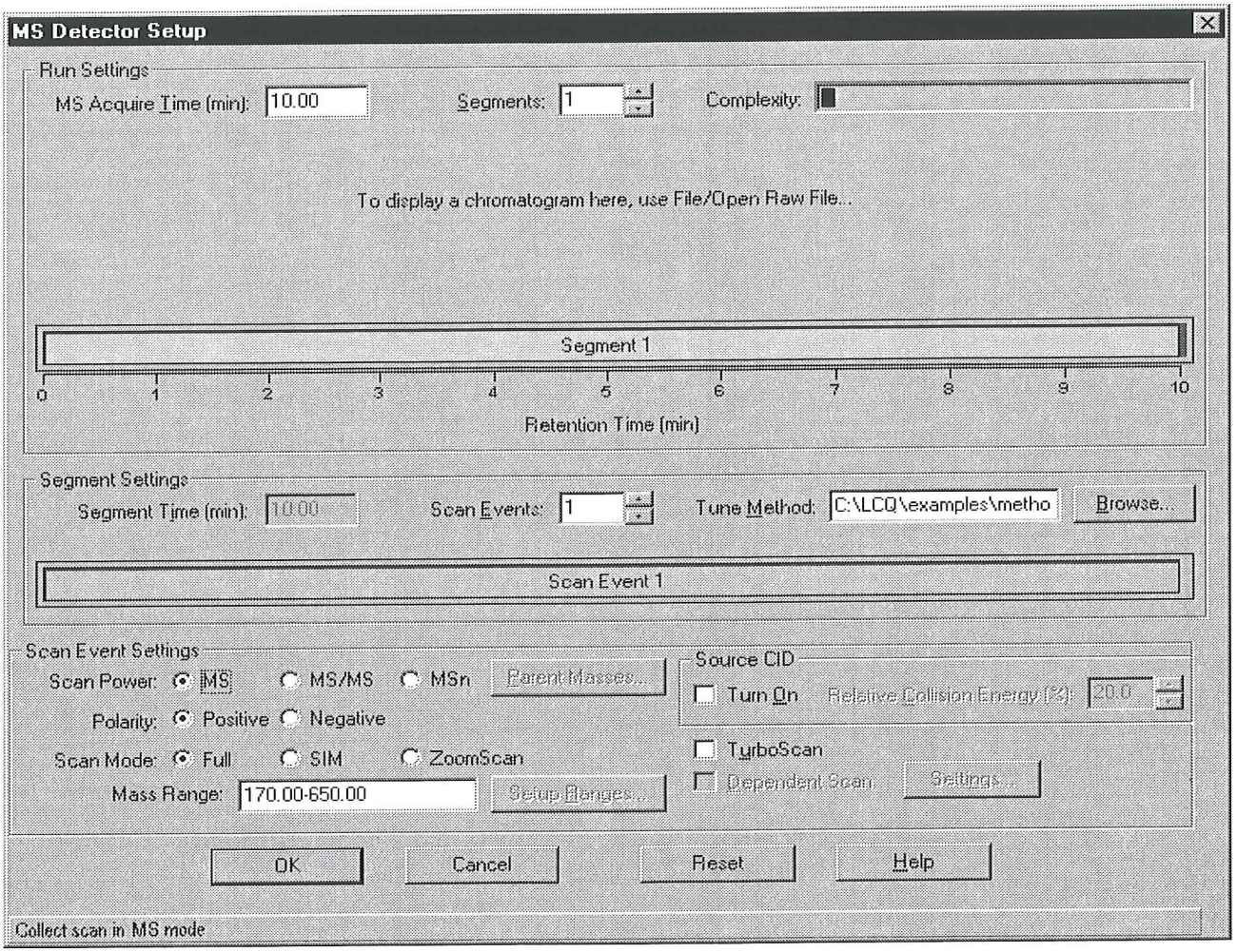


Figure 4-5. MS Detector Setup dialog box, showing MS Full scan settings to detect reserpine

2. Set the total acquisition time to 10 min in the Run Settings group box, as follows:
   Double-click on the MS Acquire Time text box, then, type **10**.

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

4. Find the Tune Method for the active segment, as follows:

   a. Display files of type *.tun* in the Open dialog box:
      Click on **Browse** in the Segment Settings group box.

   b. Select the directory path *C:\LCQ\examples\methods*.
      LCQ displays all of the tune files in the directory.

c. Select the file *reserpine.tun* in the directory:
Click on **reserpine.tun.** The file name is entered in the File Name text box.

d. Attach the Tune Method to the current Experiment Method, and return to the MS Detector Setup dialog box:
Click on **OK.**

LCQ displays *C:\LCQ\examples\methods\reserpine.tun* in the Tune Method text box.

5. Set the number of scan events for the time segment to 1, as follows:
Double-click on the Scan Events spin box, then, type **1**.

6. Activate the Scan Event 1 bar, as follows:
Click on the Scan Event 1 bar.

7. Specify the scan parameters for reserpine in the Scan Event Settings group box, as follows:

a. Select the MS scan power:
Click on the Scan Power: MS option button.

b. Select positive ion polarity:
Click on the Polarity: Positive option button.

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

d. Set the scan mass range to m/z 170 to 650:
Click on the Mass Range text box. Then, type **170-650**.

8. Ensure that ion source CID is Off in the Source CID group box:
Confirm that the Turn On check box is not selected (☐).

Compare your MS Detector Setup display to Figure 4-5.

9. Save the MS detector settings, close the dialog box, and return to the Experiment Method window, as follows:
Click on **OK**.

4.3 Saving the MS Experiment Method

Now that the MS experiment parameters are defined for reserpine, you save the Experiment Method.

> **Note.** You need to know where LCQ stores your saved files. Confirm the path names when you save the files so that you can find them later.

Save the Experiment Method as file name *reserpineMS1.emd*, as follows. (Before you can save the method, you need to supply some information in the File Summary Information dialog box.)

1. Open the File Summary Information dialog box:
   Choose **File | Save As**.

2. Type a description of the Experiment Method in the Description text box:
   For example, briefly describe the experiment conditions required to detect the analyte of interest.

3. Close the dialog box, and open the Save As dialog box:
   Click on **OK**. See Figure 4-6.

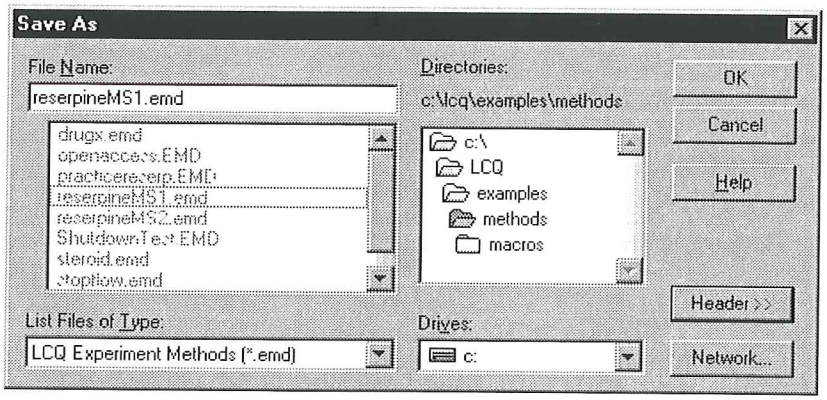


Figure 4-6. Save As dialog box, showing directory path for MS Experiment Method reserpineMS1

4. Select the path to the directory where you want to store your Experiment Method file of type *.emd*, as follows:
   For the reserpine experiment, select
   C:\LCQ\examples\methods.

5. Name the Experiment Method file for the reserpine experiment:
   Click on the File Name text box, and type **reserpineMS1.emd**.

6. Save the Experiment Method, close the dialog box, and return to the Experiment Method window, as follows:
   Click on **OK.**

Leave the Experiment Method window open, and go to the next chapter: **Optimizing the MS Experiment Method.**

Contents

5

OPTIMIZING THE MS EXPERIMENT METHOD

This chapter contains instructions on how to optimize the MS Experiment Method. First, you create a single-sample list to describe the analysis of a reserpine standard. Next, you monitor the data acquisition from the Navigator window, and LCQ creates a raw file to store the data. Then, you open the raw data file in the Explore program and decide which Experiment Method parameters to change to produce the best possible chromatography on your system. Finally, LCQ stores the parameters you specify as an optimized version of the MS Experiment Method.

This procedure is described in the topics of this chapter, as follows:

- Obtaining data for one sample with the Sample List window
- Analyzing the data in the Explore window
- Saving the optimized MS Experiment Method

Note. You can use the information in this chapter to develop the chromatography of samples containing a mixture of compounds you want to quantitate. If the chromatography in your experiment is good, and if the retention time of your analyte is optimized, then go to Chapter 6.

5.1 Obtaining Data for One Sample with the Sample List Window

You analyze one reserpine standard using the Sample List. In the Sample List, you associate the Tune Method and the Experiment Method with a new file name. You choose a file name in which to store the raw data from the analysis. After the analysis, the file name is assigned to the acquired data.

Analyze a sample of reserpine with LCQ, as follows:

- Create a Sample List
- Perform an analysis of one sample using the Sample List window

5.1.1 Creating a Sample List

Create a Sample List, as follows:

1. Open the Sample List window from the Navigator roadmap, as follows:
   Click on the Sample List button. See Figure 5-1.

> **Note.** If you want to add columns to or delete columns from the Sample List, choose **Change | Columns** and select (☑) the columns to be displayed. In this procedure, you use the following columns: *Sample Type, File Name, Sample ID, Path, Experiment Method,* and *Vial.*

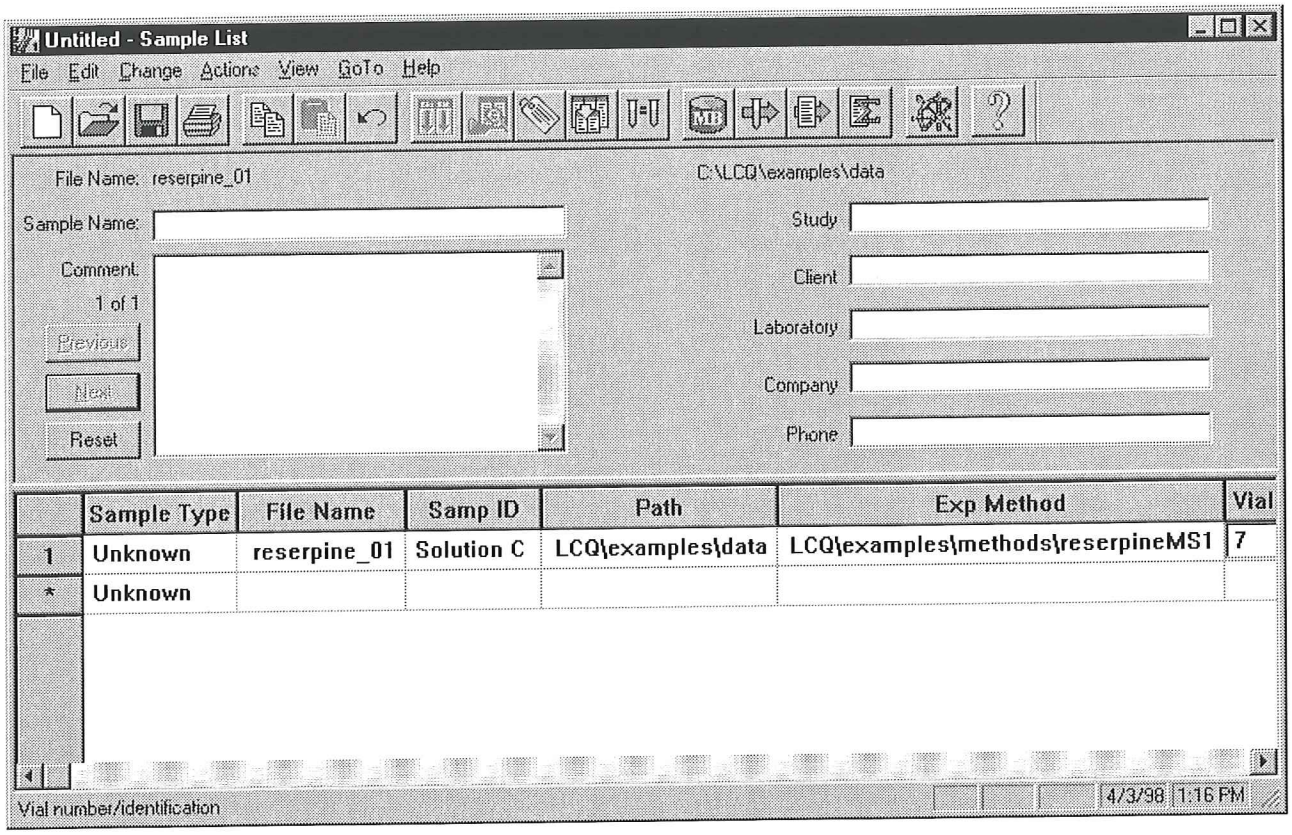


Figure 5-1. Sample List window, showing the entries for a single analysis of reserpine Solution C

2. Specify the sample type as Unknown in the top cell of the Sample Type column, as follows:

   a. Display the Sample Type list box down-arrow:
   Click on the word *Unknown* in the top cell.

   b. Set the sample type to Unknown:
   Click on the down-arrow to display the list of sample types, then click on *Unknown*.

3. Specify a file name in which to store the reserpine data, as follows:
   Click on the top cell of the File Name column, then type **reserpine_01**.

4. Specify a sample identification to distinguish this analysis from other analyses:
   Click on the top cell of the Samp ID column, then type **Solution C**.

5. Specify a directory path in which to store the file, as follows:

   a. Display the Select Directory dialog box:
   Click on the top cell of the Path column. Then, double-click on the cell. See Figure 5-2.

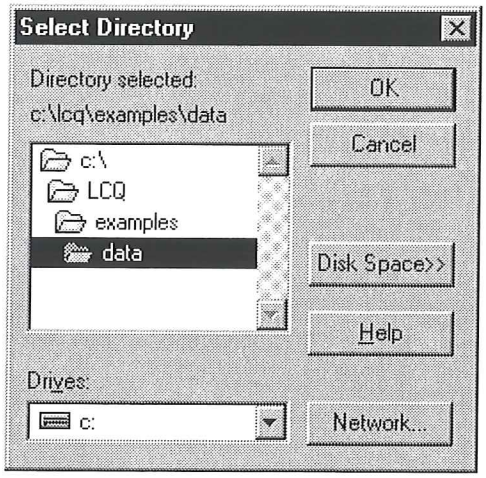


Figure 5-2. Select Directory dialog box, showing a selected directory

   b. Select the directory *C:\LCQ\examples\data*.

   c. Enter the directory path in the top cell of the Path column in Sample List.
   Click on **OK.**

6. Select the Experiment Method you want to associate with this analysis, as follows:

   a. Display the Select File Name dialog box:
   Click on the top cell of the Experiment Method column. Then, double-click on the cell. See Figure 5-3.

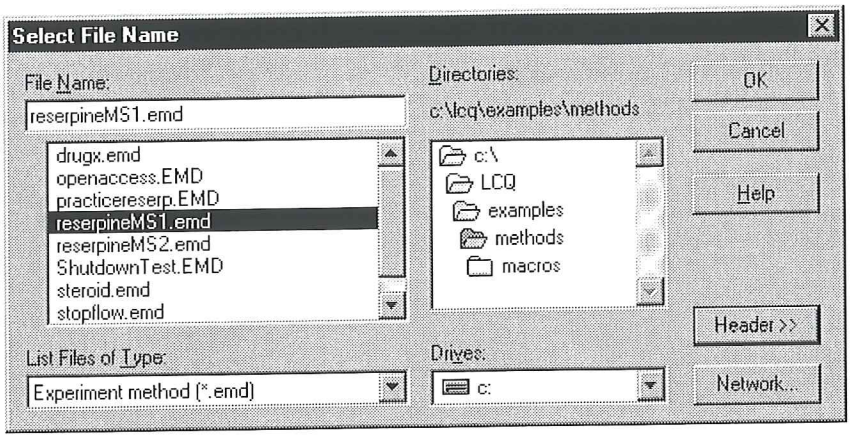


Figure 5-3. Select File Name dialog box, showing a selected directory path and file name

b. Choose the directory path where LCQ stores the Experiment Method (*.emd*) files:
Select the directory *C:\LCQ\examples\methods*.

c. Select the Experiment Method file *reserpineMS1.emd*. This is the file that you saved in Chapter 4. Confirm that your display resembles that shown in Figure 5-3.

d. Enter the Experiment Method in the top cell of the Exp Method column, and close the dialog box:
Click on **OK**. (See Figure 5-1.)

7. Specify the position in the autosampler of the vial containing Solution C, as follows:
The Solution C vial is in autosampler position 7. Click on the top cell of the Vial column. Then, type **7.**

8. Save the Sample List, as follows. (Before you can save the list, you need to supply some information in the File Summary Information dialog box.)

a. Open the File Summary Information dialog box:
Choose **File | Save As**.

b. Type a description of the Sample List in the Description text box:
For example, state the source of the sample.

c. Close the dialog box, and open the Save As dialog box:
Click on **OK.**

d. Select the directory path *C:\LCQ\examples\methods*.

e. Name the Sample List for the analysis of reserpine:
Click on the File Name text box, and type **reserpinesingle.sld**.

f. Save the Sample List, close the dialog box, and return to the Sample List window, now named *reserpinesingle.sld:*
Click on **OK.**

Go to the next topic: **Performing an Analysis of One Sample Using the Sample List Window.**

5.1.2 Performing an Analysis of One Sample 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 the data acquisition in the Navigator window.

Perform an analysis of the reserpine standard in the Sample List using the Sample List window, as follows:

1. Select the sample in the top row of the Sample List window:
Click on the number *1* at the far left of the Sample List. The entire row is highlighted in black when the row is selected.

2. Start the analysis, as follows:

   a. Open the Run This Sample dialog box:
   Choose **Actions | Run This Sample**. See Figure 5-4.

   b. Select automated start in the Start Options group box:
   Select the Automated option button. The Start When Ready check box becomes active.

   c. Select to start when ready:
   Select (☑) the Start when Ready check box.

   d. Start the data acquisition:
   Click on **OK**. LCQ displays the Navigator window.

Note. LCQ activates the Start When Ready check box only if your LCQ is configured to communicate with your autosampler via direct control (and only when you select the Automated option button). You select direct control in the Autosampler page of the Instrument Configuration dialog box. (Choose **Navigator | Options | Instrument Configuration** to display the dialog box).

The Start When Ready check box allows you to determine whether LCQ is in the Run mode or the Pause mode when the run listed after the current one is in the sample queue.

When you choose **OK** in the Run This Sample dialog box, LCQ downloads methods (refer to the following discussion, below) to the autosampler and LC. LCQ either starts the run or places the run in Pause mode.

- To have LCQ be in Run mode after it downloads methods (for continuous operation), select (☑) the Start When Ready check box.
- To put LCQ in Pause mode after it downloads methods but before it starts the run, ensure that the Start When Ready check box is clear (☐). Then, to resume the analysis from the Navigator window, choose **Samples | Start Analysis**.

Before the autosampler injection, while the Experiment Method, Tune Method, and LC parameters are downloading, your Navigator window displays the message:

Acquisition pending: No scans received yet.

Prior to the start of the first acquisition, the following occurs:

- Navigator downloads the Experiment and Tune Methods of the first sample in the Sample List to the MS detector.
- Navigator downloads the Experiment Method of the first sample in the Sample List to the LC and the autosampler.
- The analysis state in the Current group box changes from Idling to Waiting on LC.

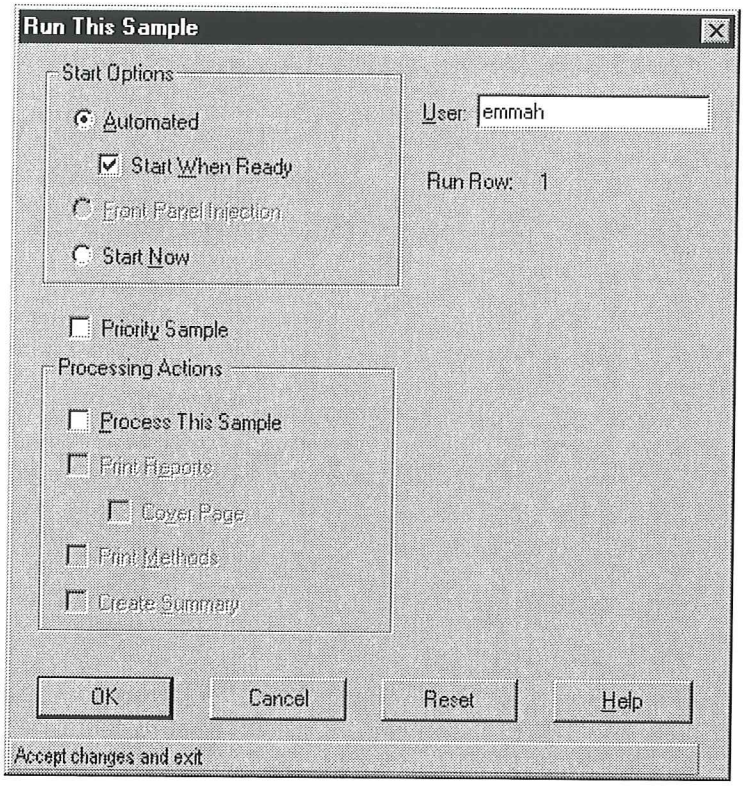


Figure 5-4. Run This Sample dialog box, showing Automated and Start When Ready options selected

Once LCQ downloads the methods to the MS detector and the LC, the autosampler begins its injection sequence. The run begins automatically when the autosampler injects the first sample in the Sample List into the LC stream. When this occurs, the analysis state goes to Acquiring. See Figure 5-5.

LCQ analyzes the sample according to the Experiment Method and creates a raw file named *reserpine_01.raw*.

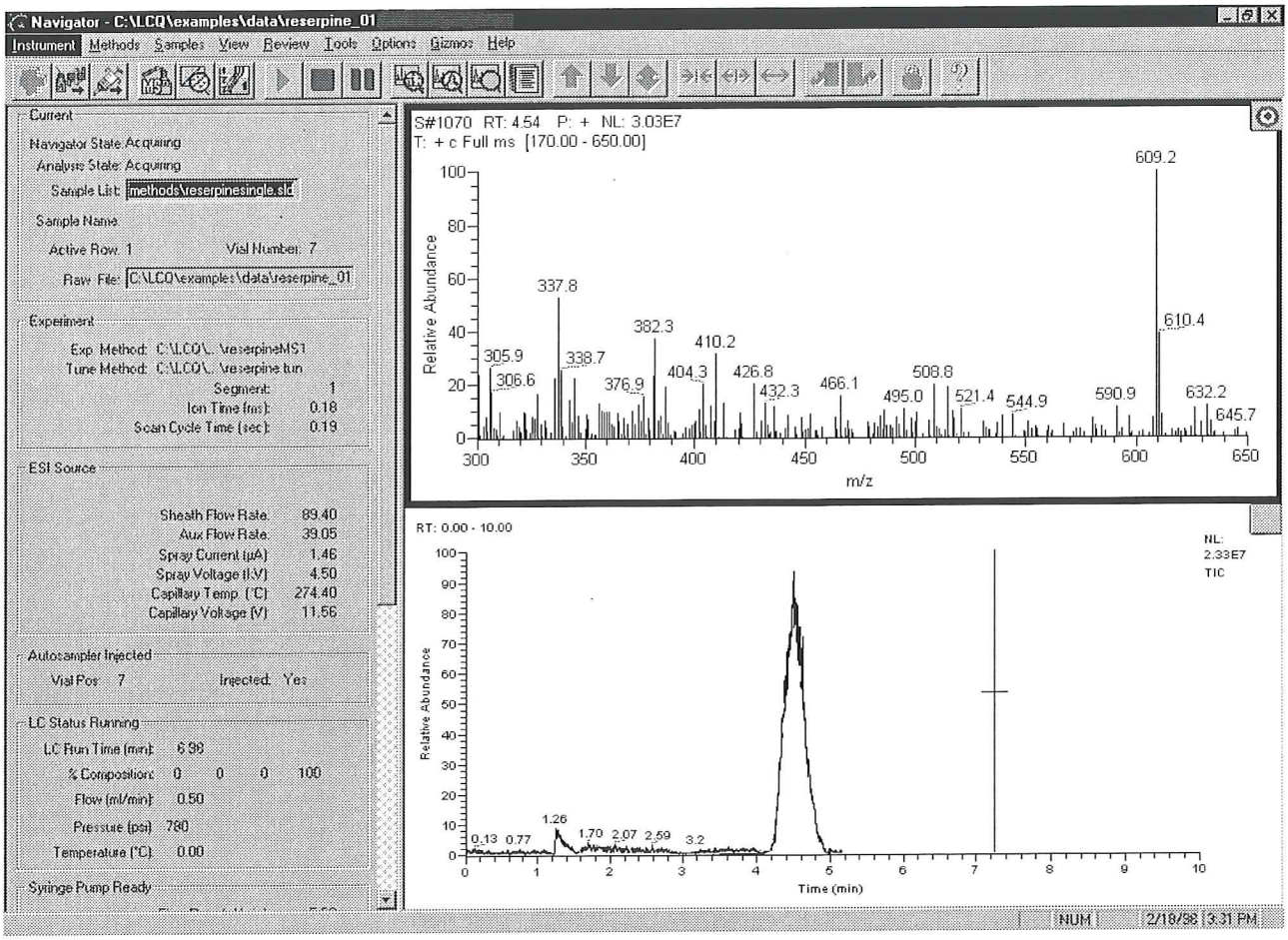


Figure 5-5. Navigator window, showing Status view (left) and the Data view (right). The Data view shows a real-time spectrum and chromatogram trace.

You can now monitor the real-time display of the mass spectrum and the chromatogram in the 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.

When the acquisition ends, the following occurs:

- The Navigator State returns to Ready to Download.
- The analysis state returns to Idling.

You are now ready to analyze the data in the Explore window. Go to the next topic: **Analyzing the Data in the Explore window**.

5.2 Analyzing the Data in the Explore Window

You now have a raw file containing data for one analysis of reserpine. In this topic you analyze that data in the Explore window.

To analyze the data in Explore, do the following:

1. Open the Explore window from the Navigator roadmap: Click on the Explore button. The default layout view (*Default.lyt)* appears. See Figure 5-6.

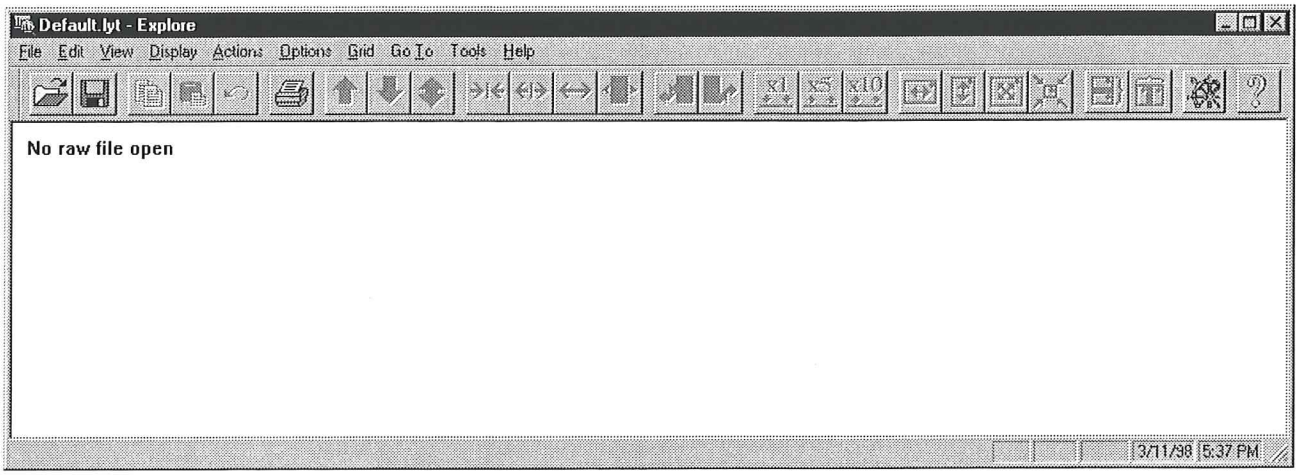


Figure 5-6. Explore window, showing the default layout view

2. Open the raw file, and display the total ion current (TIC) chromatogram in the Explore window, as follows:

   a. Display the Open Raw File dialog box:
   Click on the Open File icon. See Figure 5-7.

   b. Select the directory path and raw file *C:\LCQ\examples\data\reserpine_01.raw*, as shown in Figure 5-7.

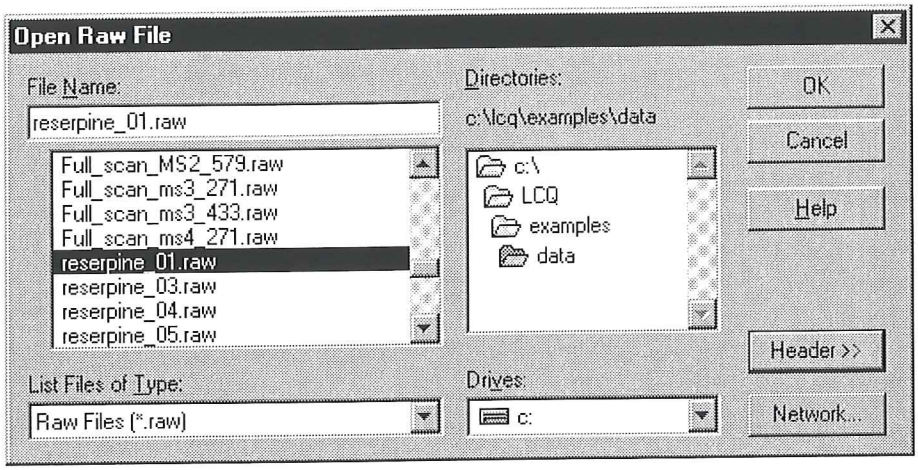


Figure 5-7. Open Raw File dialog box, showing the raw file to be opened in the Explore window

   c. Display the chromatogram in *reserpine_01.raw* in the Explore window:
   Click on **OK**. LCQ displays the total ion current (TIC) chromatogram for this file. See Figure 5-8.

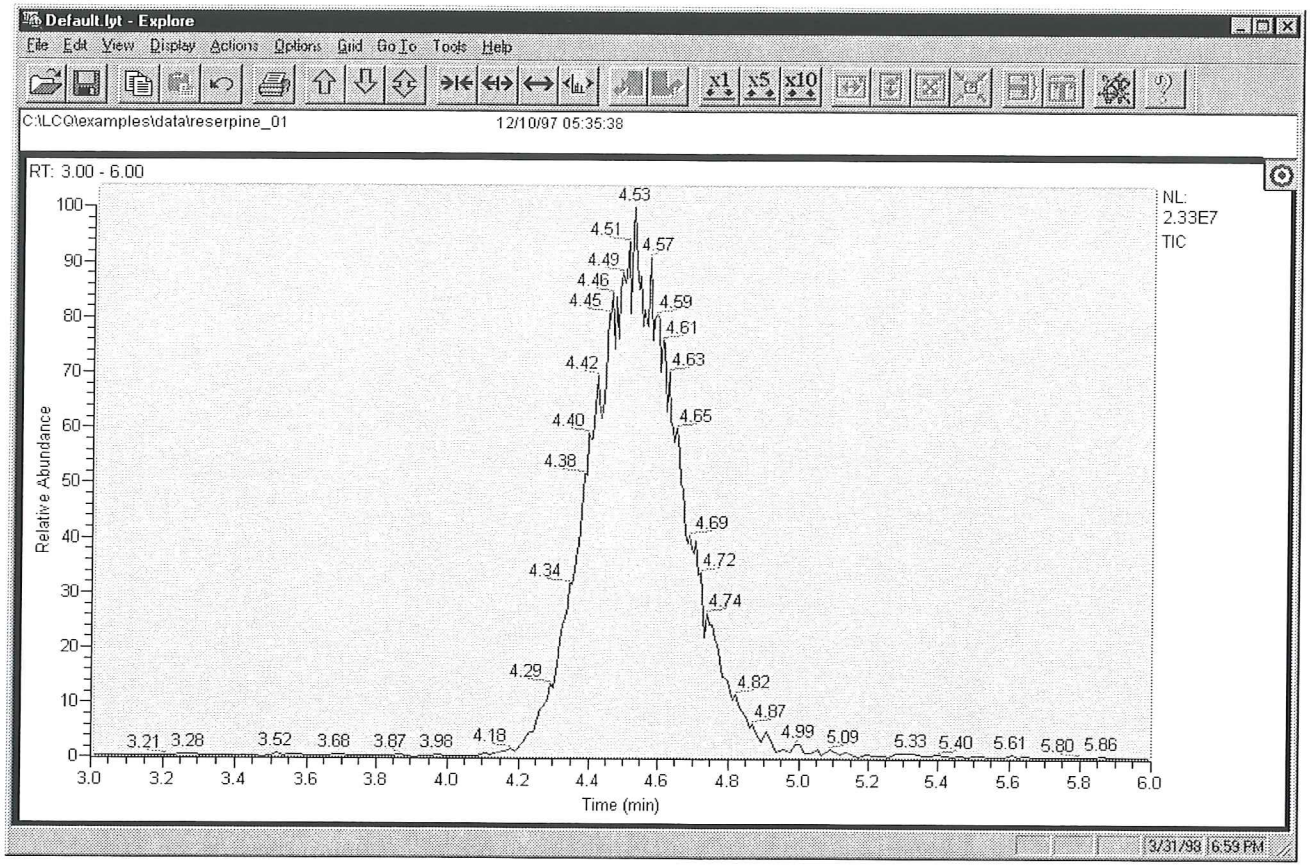


Figure 5-8. Explore window, showing the total ion current (TIC) chromatogram of *reserpine_01.raw*

3. Display the mass chromatogram for m/z 609.2 in the Explore window, as follows:

   a. Open the Chromatogram Ranges dialog box:
   Choose **Options | Ranges**. See Figure 5-9.

   b. Select (☑) the top row.

   c. Display the drop-down list box in the Type column:
   Click on the down-arrow in the Type list box.

   d. Select the mass range type:
   Select the *Mass Range* option from the list. The Mass Range(s) text box becomes active.

   e. Specify the parent ion for reserpine at m/z 609.2 in the text box at the top of the Mass Range(s) column:
   Double-click on the text box, then type **609.2**.

f. Select a mass filter in the Filter combo box:

   i. Display the drop-down list box in the Filter column:
   Click on the down-arrow in the Filter text box.

   ii. Select the filter + *c Full ms [170.00-650.00]*.

g. Save the mass range options, close the dialog box, and return to the Explore window:
Click on **OK.** LCQ displays the mass chromatogram for the peak at m/z 609.20.

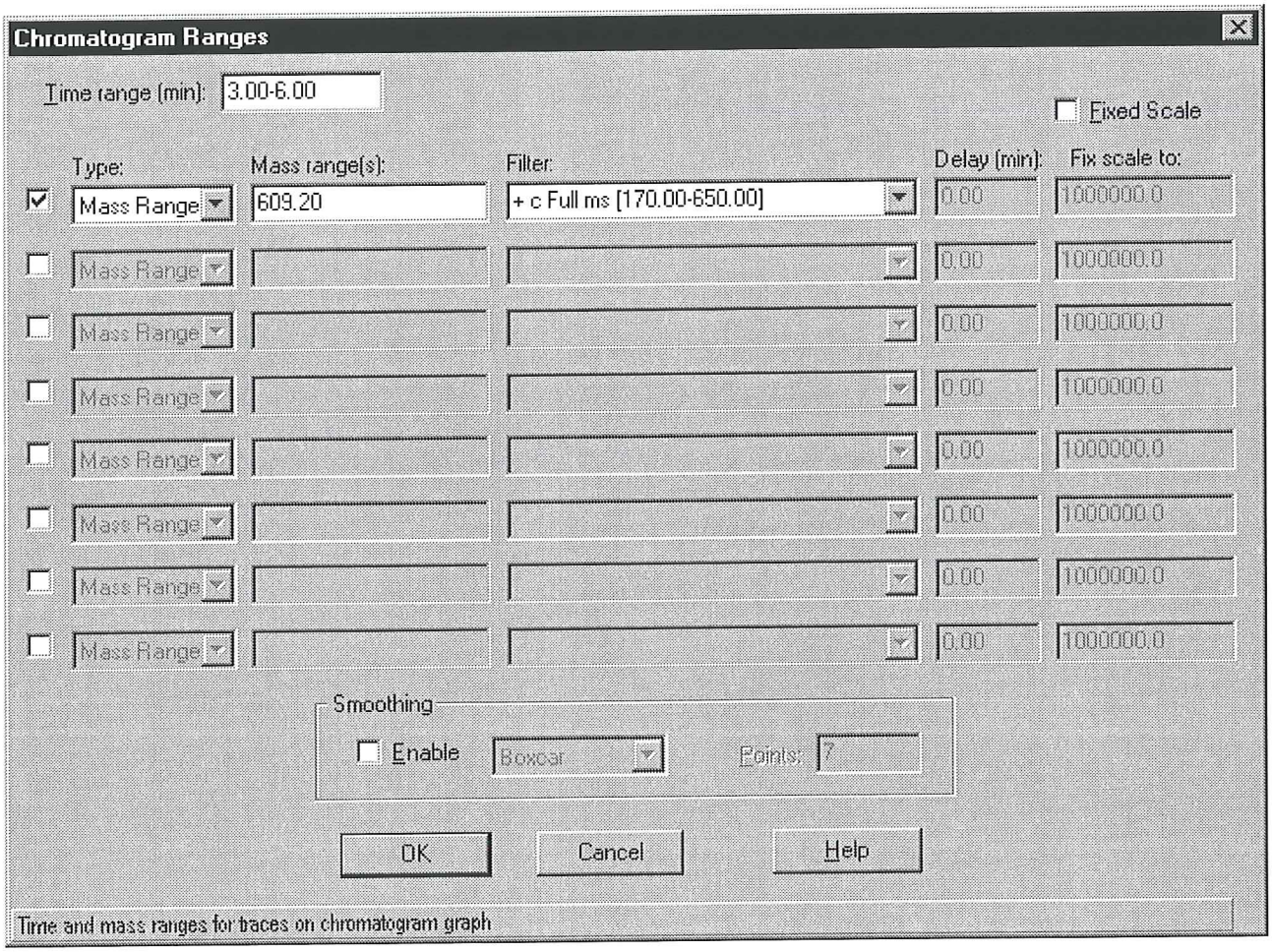


Figure 5-9. Chromatogram Ranges dialog box, showing the specifications to display a mass chromatogram of *reserpine_01.raw*

Look at the mass chromatogram in Explore. Consider if you would like to improve the appearance of the peak or the sensitivity of the experiment. The Experiment Method stores parameters that define an experiment. If you want the chromatogram to change, consider changing one or more parameters in the Experiment Method.

Consider how the following parameters affect the appearance of the mass chromatogram peak or the sensitivity of the experiment:

- LC flow rate
- divert valve timing
- solvent solution ratios
- sample volume injected
- column type
- retention time

You want to get the best results possible from your LCQ system. Make any changes in the Experiment Method that you think might improve the appearance of your chromatogram peak. Then, reanalyze the sample to acquire new data. Finally, analyze the new data in Explore to demonstrate how your changes affect the chromatogram.

You might need to perform these actions more than once to optimize the Experiment Method. When you are satisfied that the LCQ Experiment Method parameters are optimized, go to the next topic: **Saving the Optimized MS Experiment Method**.

5.3 Saving the Optimized MS Experiment Method

Save the optimized Experiment Method, as follows. (Before you can save the method, you need to supply some information in the File Save -- Audit Trail dialog box.)

1. Open the File Save -- Audit Trail dialog box:
   Choose **File | Save**. LCQ lists the parameters you modified in the What Changed list box.

2. Enter a required comment in the Comment text box describing the specific parameter(s) that changed and the reason for the change.

3. Close the dialog box, and return to the Experiment Method window, as follows:
   Click on **Continue**. The previously saved Experiment Method *reserpineMS1.emd* is overwritten.

Go to the next chapter: **Developing an MS/MS Experiment Method.**

Contents

6

DEVELOPING AN MS/MS EXPERIMENT METHOD

In this chapter, you develop an MS/MS Experiment Method by changing the scan event settings in the MS Detector Setup dialog box. This enables LCQ to identify and detect MS/MS product ions.

The following topics describe how to develop the MS/MS Experiment Method:

- Select MS detector settings for MS/MS scan events
- Save the MS/MS Experiment Method

6.1 Selecting MS Detector Settings for MS/MS Scan Events

In this topic, you select MS detector settings to describe MS/MS scan events. Change only the parameters specified in this procedure. Leave all other parameters as they are.

Specify the MS detector parameters for the ESI/MS/MS analysis of reserpine, as follows:

1. Open the Experiment Method window from the Navigator roadmap, as follows:
   Click on the Experiment Method button.

2. Open the Experiment Method file *reserpineMS1.emd* (that you created and optimized in Chapters 4 and 5), as follows:

   a. Display files of type *.emd* in the Open dialog box:
      Click on the Open File button in the toolbar.

   b. Select the directory path *C:\LCQ\examples\methods*.

   c. Enter the file *reserpineMS1.emd* in the File Name text box:
      Click on **reserpineMS1.emd**.

   d. Return to the Experiment Method window:
      Click on **OK**.

3. Open the MS Detector Setup dialog box:
   Choose **Setup | MS Detector Setup**. See Figure 6-1.

4. Set the total analysis run time to 7 min in the Run Settings group box, as follows:
   Double-click on the MS Acquire Time text box, then type **7**.
   (We determined that reserpine elutes at about 4 min in Chapter 5.)

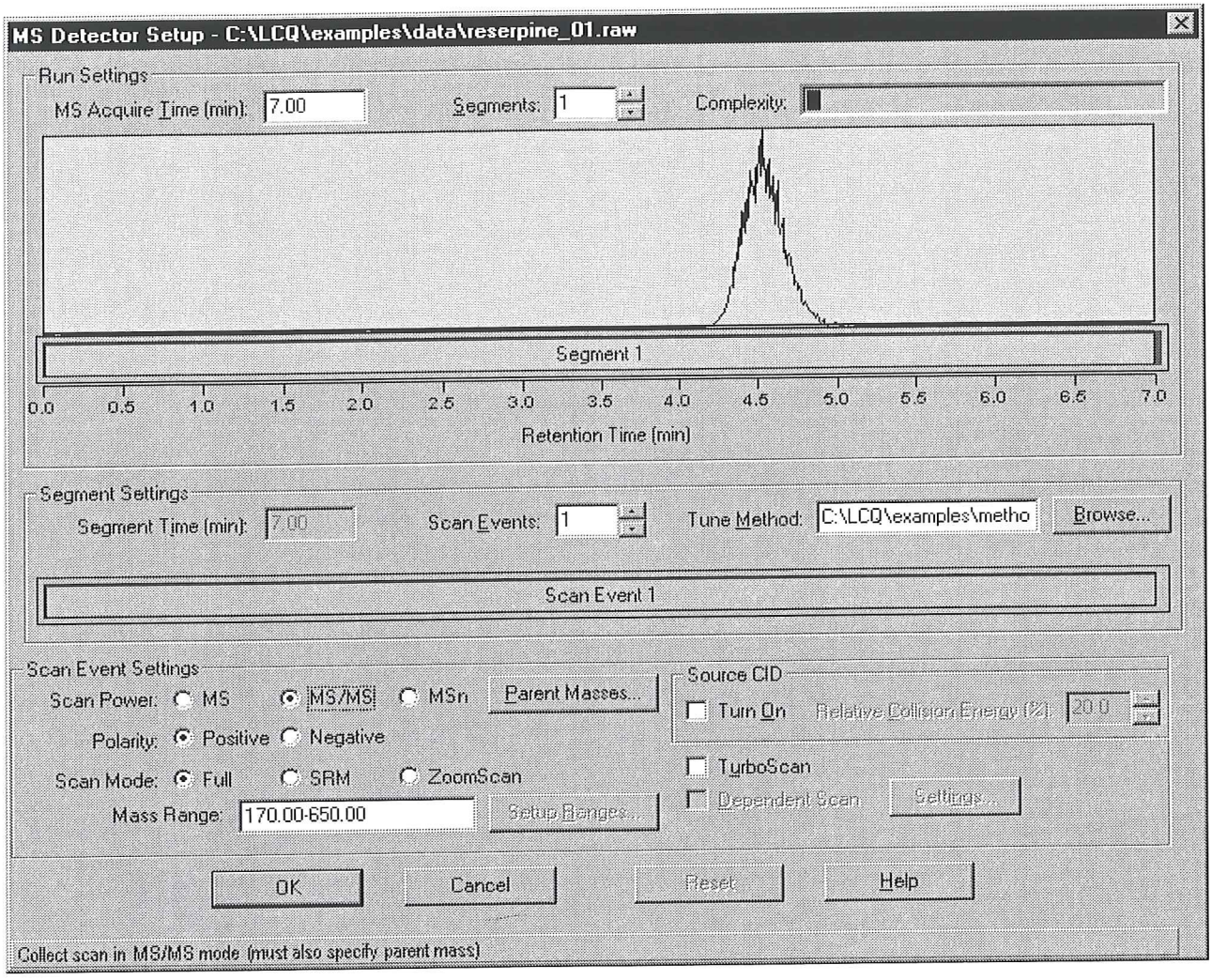


Figure 6-1. MS Detector Setup dialog box, showing the correct Acquire Time for the reserpine experiment

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

6. Specify the changes to the scan parameters in the Scan Event Settings group box, as follows:

   a. Select the MS/MS scan power:
      Click on the Scan Power: MS/MS option button. The Parent Masses button becomes active.

   b. Specify the parent ion parameters in the Set Parent Mass dialog box:

i. Display the Set Parent Mass dialog Box:
Click on **Parent Masses**. See Figure 6-2.

ii. Enter the mass-to-charge ratio of the parent ion as m/z 609.2:
Double-click on the Parent Mass text box, and type **609.2**.

iii. Specify the isolation width as m/z 2.0:
Double-click on the Isolation Width spin box, and type **2.0**.

iv. Specify the relative collision energy as determined in Chapter 3:
Double-click on the Relative Collision Energy spin box, and type the appropriate value.

v. Save the parent ion parameters, close the dialog box, and return to the MS Detector Setup dialog box:
Click on **OK**.

7. Save the MS detector settings, close the dialog box, and return to the Experiment Method window, as follows:
Click on **OK**.

Go to the next topic: **Saving the MS/MS Experiment Method.**

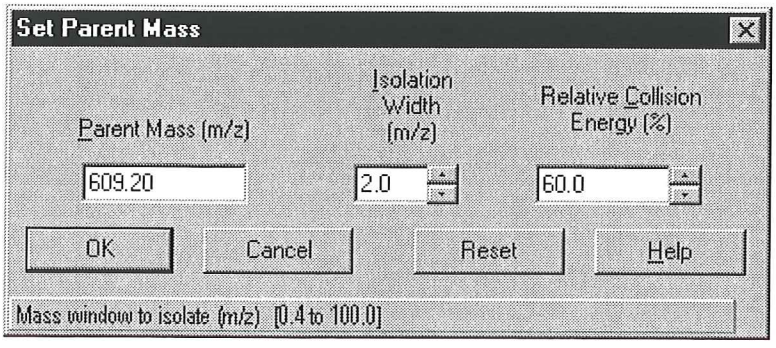


Figure 6-2. Set Parent Mass dialog box, showing the correct settings to detect reserpine product ions

6.2 Saving the MS/MS Experiment Method

Save the MS/MS Experiment Method, as follows: (Before you can save the method, you need to supply some information in the File Save -- Audit Trail dialog box.)

1. Open the File Save -- Audit Trail dialog box, as follows:
   Choose **File | Save**. LCQ lists the parameters that you modified in the What Changed list box.

2. Enter a required comment in the Comment text box describing the specific parameter(s) that changed and the reason for the change.

3. Close the dialog box, and return to the Experiment Method window, as follows:
   Click on **Continue**. The previously saved Experiment Method is overwritten.

Continue to display the Experiment Method window, and go to the next chapter: **Acquiring Your Data Using an LCQ.**

Contents

7

ACQUIRING YOUR DATA USING AN LCQ

This chapter describes how to acquire your data from the Sample List window using an LCQ. You create a sample list of five reserpine calibration standards. You then acquire the data, and LCQ stores the data for each sample in a separate raw file. An optional topic discusses the data acquisition of blank and quality control samples.

The final chapter in this manual: **Processing Your Data with LCQUAN**, uses the data produced in this chapter to demonstrate quantitation procedures and data reports.

This chapter describes the following topics:

- Building a Sample List
- Acquiring calibration standard data
- (Optional) Acquiring data for blanks and QCs

7.1 Building a Sample List

You build a multiple-entry sample list from the New File Template dialog box. You enter information about your analyses in the dialog box so that LCQ creates a Sample List automatically.

Build a Sample List, as follows:

1. Open the Sample List window from the Navigator roadmap, as follows:
   Click on the Sample List button.

2. Open the New File Template dialog box, as follows:
   Click on the New List button. See Figure 7-1. LCQ uses the information in this dialog box to build a sample list automatically.

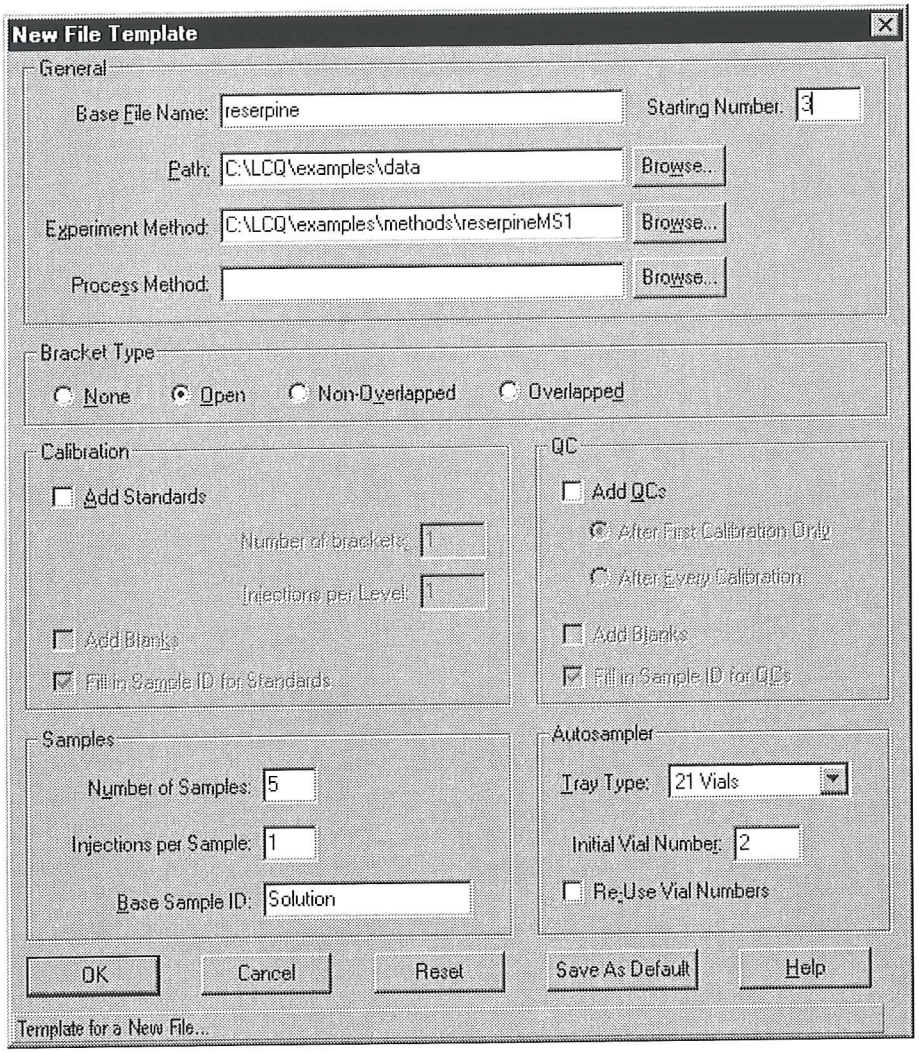


Figure 7-1. New File Template dialog box, showing the proper settings to create a Sample List for the reserpine calibration curve

3. Specify the parameters in the General group box, as follows:

   a. Specify the prefix (or base) of the raw data file names as *reserpine*:
   Double-click on the Base File Name text box, and type **reserpine.** The Base File Name is the file name prefix that is used to create all the raw file names in this list.

   b. Identify the suffix of the first sample in the sample list as 3:
   Double-click on the Starting Number text box, and type **3**.

c. Specify the path for the raw files as *C:\LCQ\examples\data*.

   i. Open the Select Directory dialog box:
   Click on the Path: Browse button.

   ii. Select the directory for the reserpine raw files:
   Choose *C:\LCQ\examples\data*.

   iii. Return to the New File Template dialog box:
   Click on **OK**. The specified directory appears in the Path text box.

d. Specify the Experiment Method as *reserpineMS1.emd*:

   i. Open the Select Experiment Method File dialog box:
   Click on the Experiment Method: Browse button.

   ii. Specify the path for and file name of the Experiment Method file:
   Choose
   C:\LCQ\examples\methods\reserpineMS1.emd

   iii. Return to the New File Template dialog box:
   Click on **OK.** The specified path and file name appear in the Experiment Method text box.

4. For this example, leave the parameters set to their default values in the Bracket Type, Calibration, and QC group boxes.

5. Specify the parameters in the Samples group box, as follows:

   a. Set the number of samples to 5:
   Double-click on the Number of Samples text box, then type **5**.

   b. Set the number of injections per sample to 1:
   Double-click on the Injections per Sample text box, then type **1**.

   c. Specify the prefix (or base) for sample identifications as Solution:
   Double-click on the Base Sample ID text box, then type **Solution**.

6. Specify the parameters in the Autosampler group box, as follows:

> **Note.** The values listed in the Tray Type list box correspond to the allowed values for your autosampler.

   a. Specify the tray type of your autosampler:
   Click on the down-arrow in the Tray Type list box, and choose a tray type. For example, choose *21 vials*.

   b. Set the number of the position in the tray as *2* where the first vial containing Solution H is located. (Refer to Chapter 2.)
   Double-click on the Initial Vial Number text box, then type **2**.

   c. Specify that you do not want to reuse vial numbers:
   Deselect (☐) the Re-Use Vial Numbers check box.

7. Build a Sample List according to the instructions you have provided in the New File Template dialog box, and close the dialog box, as follows:
   Click on **OK.** See Figure 7-2.

> **Note.** If you want to add columns to or delete columns from the Sample List, choose **Change | Columns,** and select (☑) the columns to be displayed. In this procedure, you use the following columns: *Sample Type, File Name, Sample ID, Path, Experiment Method, Vial*, and *Injection Volume*.

8. Specify the identification of the solutions in the Samp ID column, as follows:
   Double-click on the row 1 cell of the Samp ID column. Then, type **SolutionH**. Change each subsequent cell in the column similarly. Enter the identifications as SolutionG through SolutionD.

9. Ensure that the Sample List window displayed on your monitor resembles the one shown in Figure 7-2.

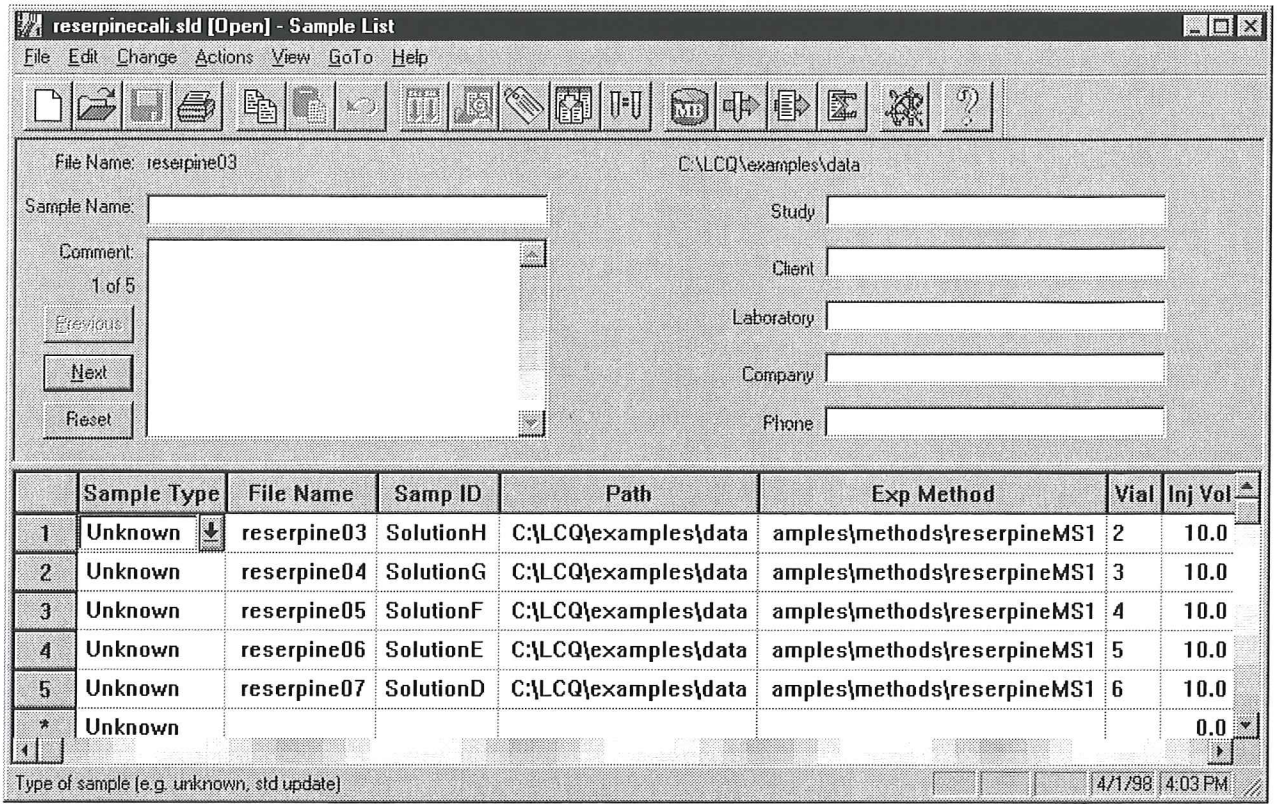


Figure 7-2. Sample List window, showing the Sample List with five calibration standards setup from the New File Template dialog box

> **Note.** LCQ requires that the cells in the Sample Type column contain the word *Unknown* prior to, and during, sample analysis. Chapter 8 describes how to associate each raw file with one of the possible Sample Types: *Standard, Blank, QC*, or *Unknown*.

10. Save the Sample List, as follows: (Before you can save the list, you need to supply some information in the File Summary Information dialog box.)

    a. Open the File Summary Information dialog box:
    Choose **File | Save As**.

    b. Type a description of the Experiment Method in the Description text box:
    For example, type **reserpine calibration**.

    c. Open the Save As dialog box:
    Click on **OK**.

d. Select the directory path as *C:\LCQ\examples\methods*:
Choose *C:\LCQ\examples\methods* for the reserpine experiment.

e. Name the sample list *reserpinecali.sld*:
Click on the File Name text box. Type **reserpinecali.sld** for the reserpine experiment.

f. Save the sample list, and return to the Sample List window, now named *reserpinecali.sld*:
Click on **OK**.

You are now ready to analyze the 5-point calibration standard curve for reserpine by LCQ-MS/MS. Go to the next topic: **Acquiring Calibration Standard Data**.

7.2 Acquiring Calibration Standard Data

Acquire calibration standard data by analyzing samples using the Sample List *reserpinecali.sld*. (See Figure 7-2.)

> **Note.** If the signals that pass between your LC and the LCQ are contact closures only, ensure that your LC is on and that solvent is flowing to the LCQ.

Start the analysis, as follows:

1. Open the Run Sample List dialog box, as follows:
   Choose **Actions | Run List**. See Figure 7-3.

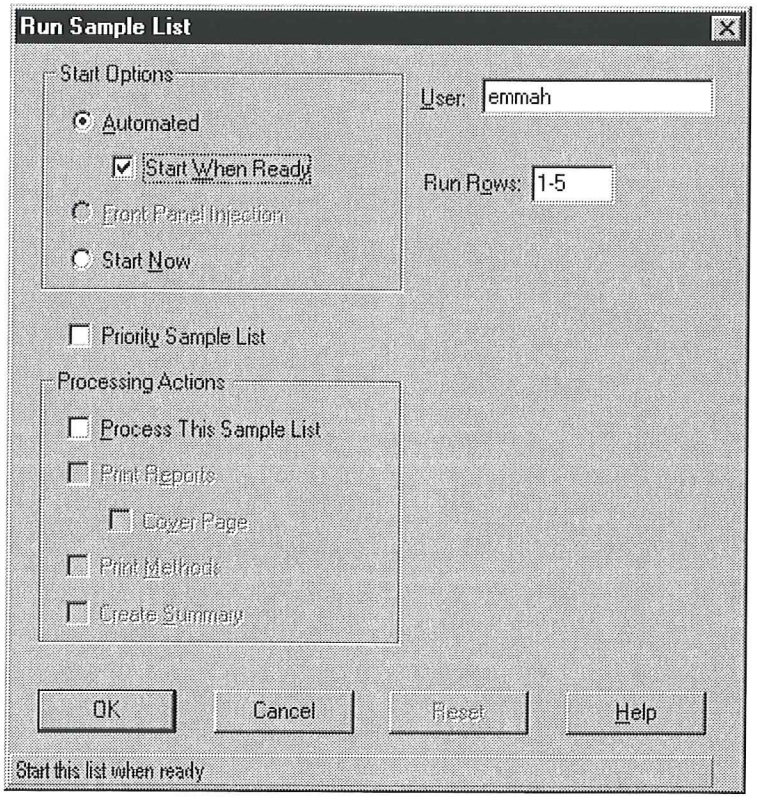


Figure 7-3. Run Sample List dialog box, showing the proper settings to start an analysis

2. Select automated start in the Start Options group box, as follows:
   Select the Automated option button.

3. Select to start when ready, as follows:
   Select (☑) the Start when Ready check box.

4. Start data acquisition, as follows:
   Click on **OK.** LCQ automatically opens the Navigator window. Refer to Chapter 5 for a description of the inject sequence events. LCQ creates a raw data file for each sample. See Figure 7-4.

You are ready to process and evaluate the data for the 5-point calibration curve acquired in this topic.

To include blank and QC samples in your Sample List, refer to the next topic: **Acquiring Data for Blanks and QCs**.

To analyze the data obtained in this chapter, go to Chapter 8: **Processing Your Data with LCQUAN**.

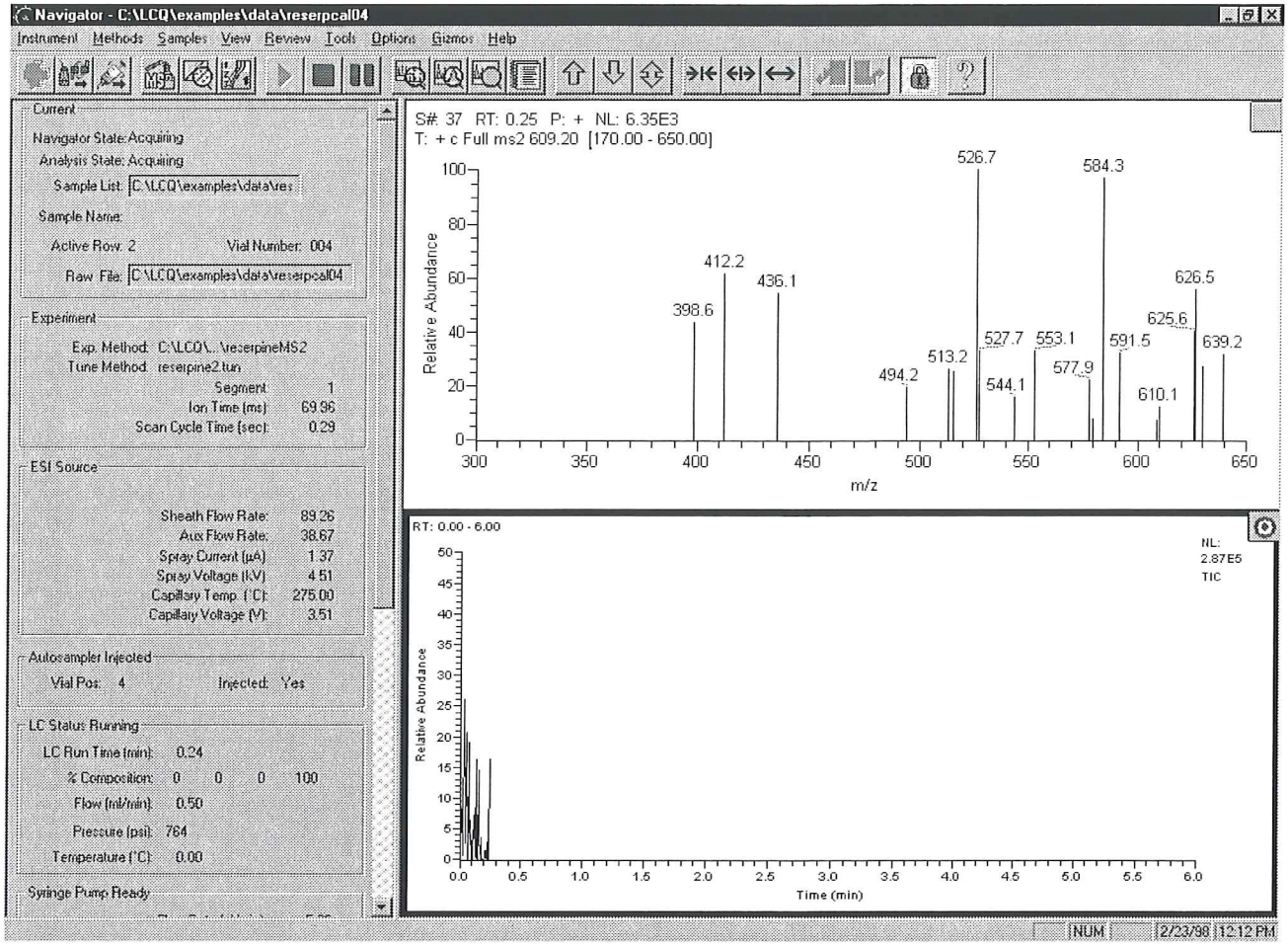


Figure 7-4. Navigator window, showing the Status view (left) and the Data view (right). The Status view shows the current settings of the instrument. The Data view shows the real-time data acquisition for the current sample in the Spectrum view (top) and Graph view.

7.3 (Optional) Acquiring Data for Blanks and QCs

Good Laboratory Practice requires the analysis of blanks and quality control samples to ensure that the various components of your analytical system are controlled for contamination and measurement errors.

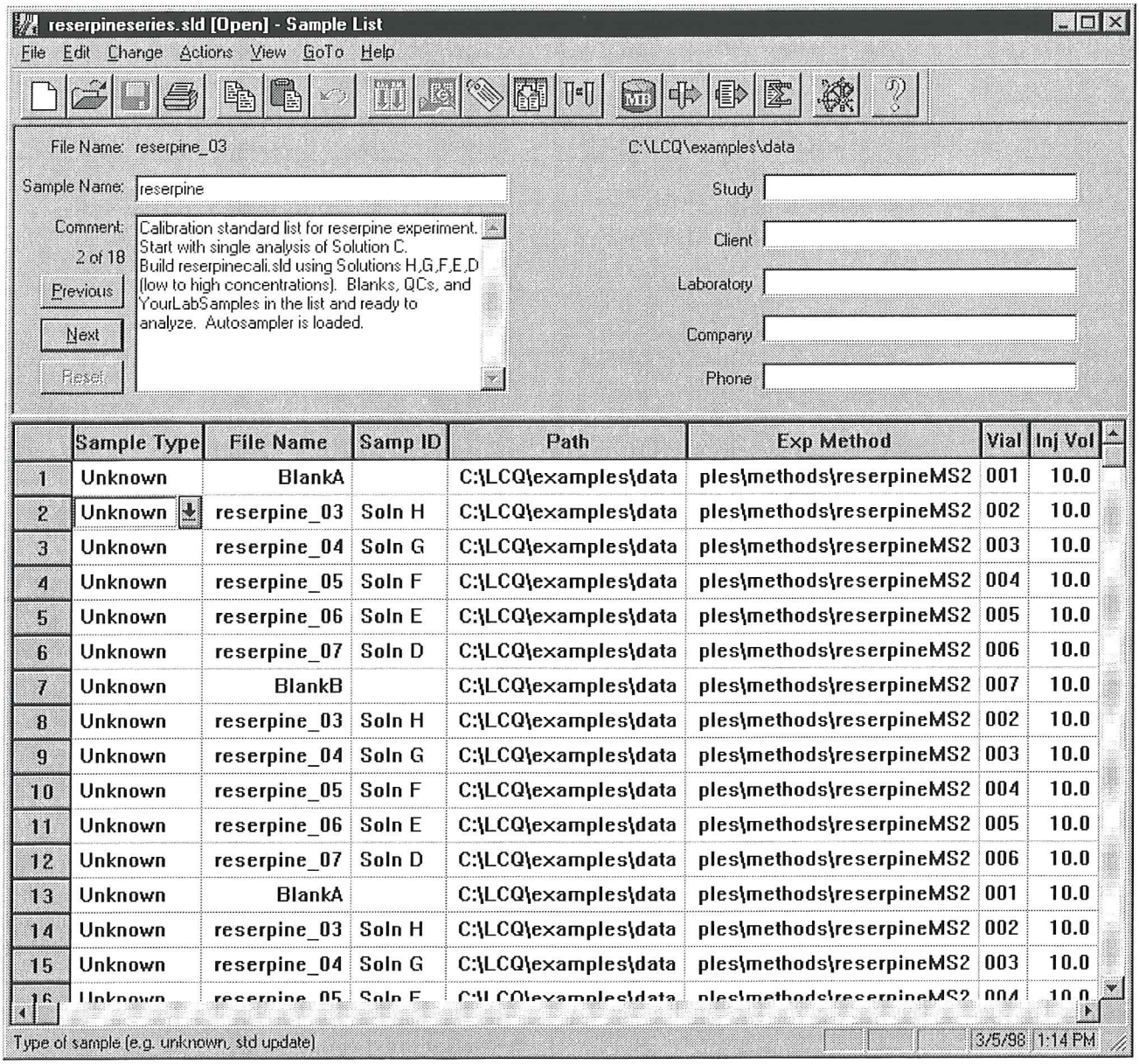
A multiple-entry sample list is required to analyze a 5-point calibration curve of reserpine. To demonstrate minimal carryover effect between samples, you analyze blanks and QCs along with the samples from the Sample List window, as follows:

In your autosampler, arrange one or two vials containing blank samples before and after a set of those containing standards, and before and after QCs. When you run real-world samples with possible matrix interference, you run blank samples intermittently to ensure a clean analytical system. See Figure 7-5, which shows a 5-point analytical sequence run in triplicate.

Create a Sample List according to the description in the topic: **Building a Sample List.** LCQ requires that, at this point in the process, you identify each of the samples as *Unknown* in the Sample Type column. (Refer to Note located after Figure 7-2.)

Analyze this list of samples, as described in the topic: **Acquiring Calibration Standard Data**. LCQ stores data from each run as a separate raw file. A file name is assigned to each raw data file from its corresponding entry in the Sample List.

In the next chapter: **Processing Your Data with LCQUAN**, you associate each raw file with one of the Sample Types: *Standard, Blank, QC*, or *Unknown.* LCQ then processes the data. You review the data, and finally, you specify the format LCQ uses to report your data.



	Sample Type	File Name	Samp ID	Path	Exp Method	Vial	Inj Vol
1	Unknown	BlankA		C:\LCQ\examples\data	ples\methods\reserpineMS2	001	10.0
2	Unknown	reserpine_03	Soln H	C:\LCQ\examples\data	ples\methods\reserpineMS2	002	10.0
3	Unknown	reserpine_04	Soln G	C:\LCQ\examples\data	ples\methods\reserpineMS2	003	10.0
4	Unknown	reserpine_05	Soln F	C:\LCQ\examples\data	ples\methods\reserpineMS2	004	10.0
5	Unknown	reserpine_06	Soln E	C:\LCQ\examples\data	ples\methods\reserpineMS2	005	10.0
6	Unknown	reserpine_07	Soln D	C:\LCQ\examples\data	ples\methods\reserpineMS2	006	10.0
7	Unknown	BlankB		C:\LCQ\examples\data	ples\methods\reserpineMS2	007	10.0
8	Unknown	reserpine_03	Soln H	C:\LCQ\examples\data	ples\methods\reserpineMS2	002	10.0
9	Unknown	reserpine_04	Soln G	C:\LCQ\examples\data	ples\methods\reserpineMS2	003	10.0
10	Unknown	reserpine_05	Soln F	C:\LCQ\examples\data	ples\methods\reserpineMS2	004	10.0
11	Unknown	reserpine_06	Soln E	C:\LCQ\examples\data	ples\methods\reserpineMS2	005	10.0
12	Unknown	reserpine_07	Soln D	C:\LCQ\examples\data	ples\methods\reserpineMS2	006	10.0
13	Unknown	BlankA		C:\LCQ\examples\data	ples\methods\reserpineMS2	001	10.0
14	Unknown	reserpine_03	Soln H	C:\LCQ\examples\data	ples\methods\reserpineMS2	002	10.0
15	Unknown	reserpine_04	Soln G	C:\LCQ\examples\data	ples\methods\reserpineMS2	003	10.0
16	Unknown	reserpine_05	Soln F	C:\LCQ\examples\data	ples\methods\reserpineMS2	004	10.0

Figure 7-5. Sample List window, showing a Sample List for analyzing standards and blanks

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8

PROCESSING YOUR DATA WITH LCQUAN

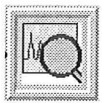
This chapter provides a procedure for processing the reserpine calibration (*.raw*) data you acquired in Chapter 7. You use the LCQUAN program to process your data. The LCQUAN program provides you with a flow chart that guides you through processing your data.

When you use LCQUAN to process quantitation data, you typically create a Sample List before you run your samples, and you create a Processing Method the first time you analyze your data. This procedure is the one you follow in this example.

Using LCQUAN to process your data involves the following:

- Displaying the LCQUAN Flow Chart
- Creating a Processing Method
- Opening an existing Sample List
- Processing the raw files
- Reviewing the calibration curve
- Reviewing all results
- Creating and reviewing reports
- Saving the LCQUAN method and exiting LCQUAN

8.1 Displaying the LCQUAN Flow Chart



Open the LCQUAN Flow Chart - Development Start dialog box from the Navigator roadmap, as follows:
Click on the LCQUAN button. See Figure 8-1.

To create a Processing Method, go to the next topic.

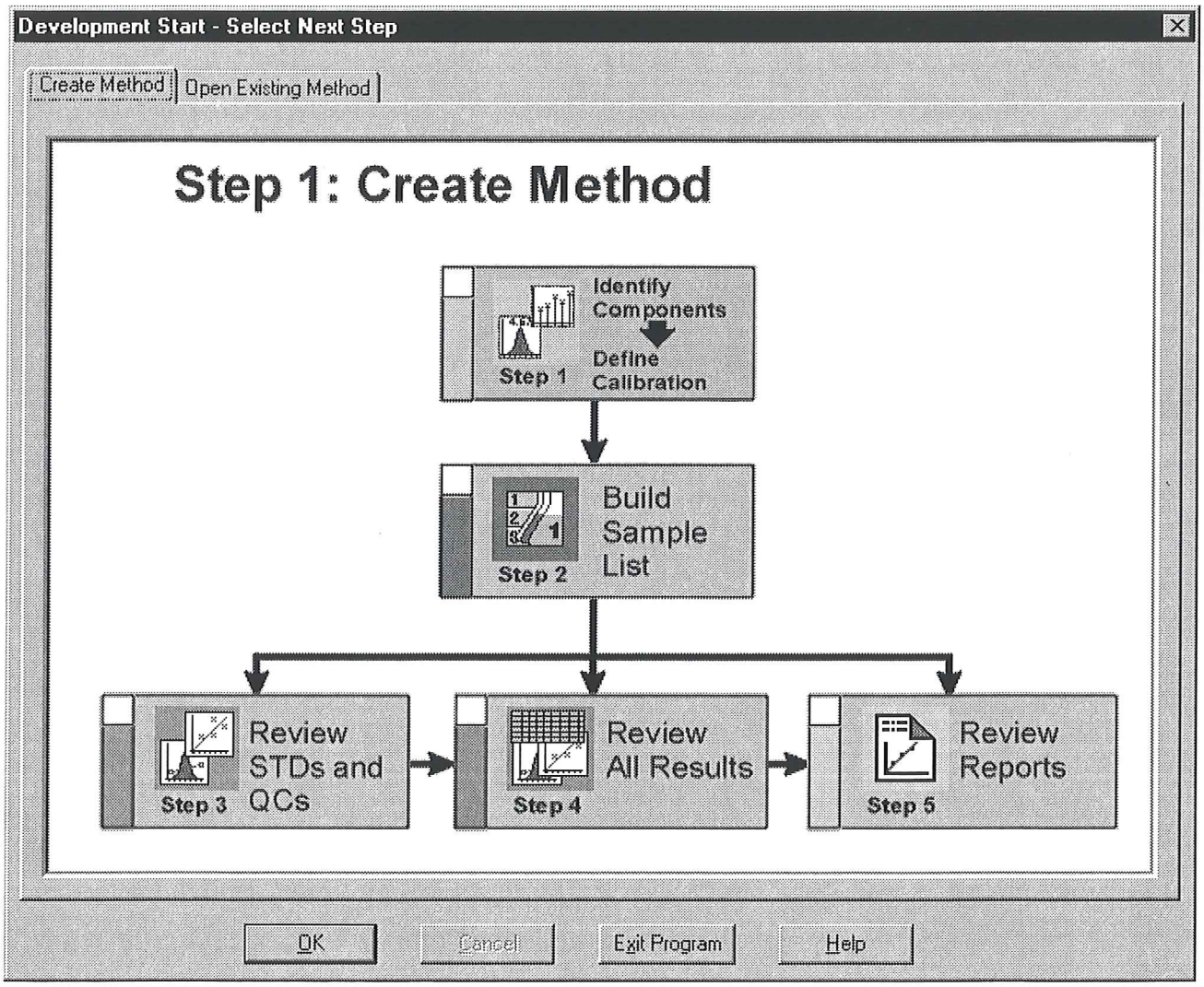


Figure 8-1. LCQUAN Flow Chart - Development Start dialog box

8.2 Step 1: Creating a Processing Method

In Step 1 of the LCQUAN procedure, you create a Processing Method. You initiate Step 1 from the LCQUAN Flow Chart - Development Start dialog box.

As you create a Processing Method, LCQUAN leads you through the following steps:

- Choose the Create Method tab. You need to choose the Create Method tab to begin to create a Processing Method.
- Open a raw data file. You need to open a raw file from your data set to test peak detection and integration parameters. LCQUAN also obtains scan filter information from the raw file.
- Specify calibrate by internal or external standard. LCQUAN needs to know which type of calibration you are using.
- Name the components in the sample. LCQUAN needs this information to associate components with chromatographic peaks.
- Match scan filters with components. LCQUAN filters the raw data to produce a mass chromatogram for each component.
- Enter peak identification settings and display the spectrum. You test peak detection settings using the data in the raw file you opened earlier. During batch processing, LCQUAN uses this information to detect all component peaks in all raw files in your data set.
- Enter peak integration settings. You also test peak integration parameters using the data in the raw file you opened earlier. During batch processing, LCQUAN uses this information to integrate all component peaks in all raw files in your data set.
- Enter component calibration parameters. LCQUAN uses these parameters to build the calibration curve and to quantitate (optional) quality control (QC) samples and unknowns.
- Save the Processing Method. This allows you to use the same Processing Method in the future.
- Accept the Processing Method. This tells LCQUAN that you are satisfied with the Processing Method parameters and are ready to move on to Step 2 of the LCQUAN procedure: create or open a Sample List.

For a flow diagram of how to create a Processing Method, see Figure 8-2.

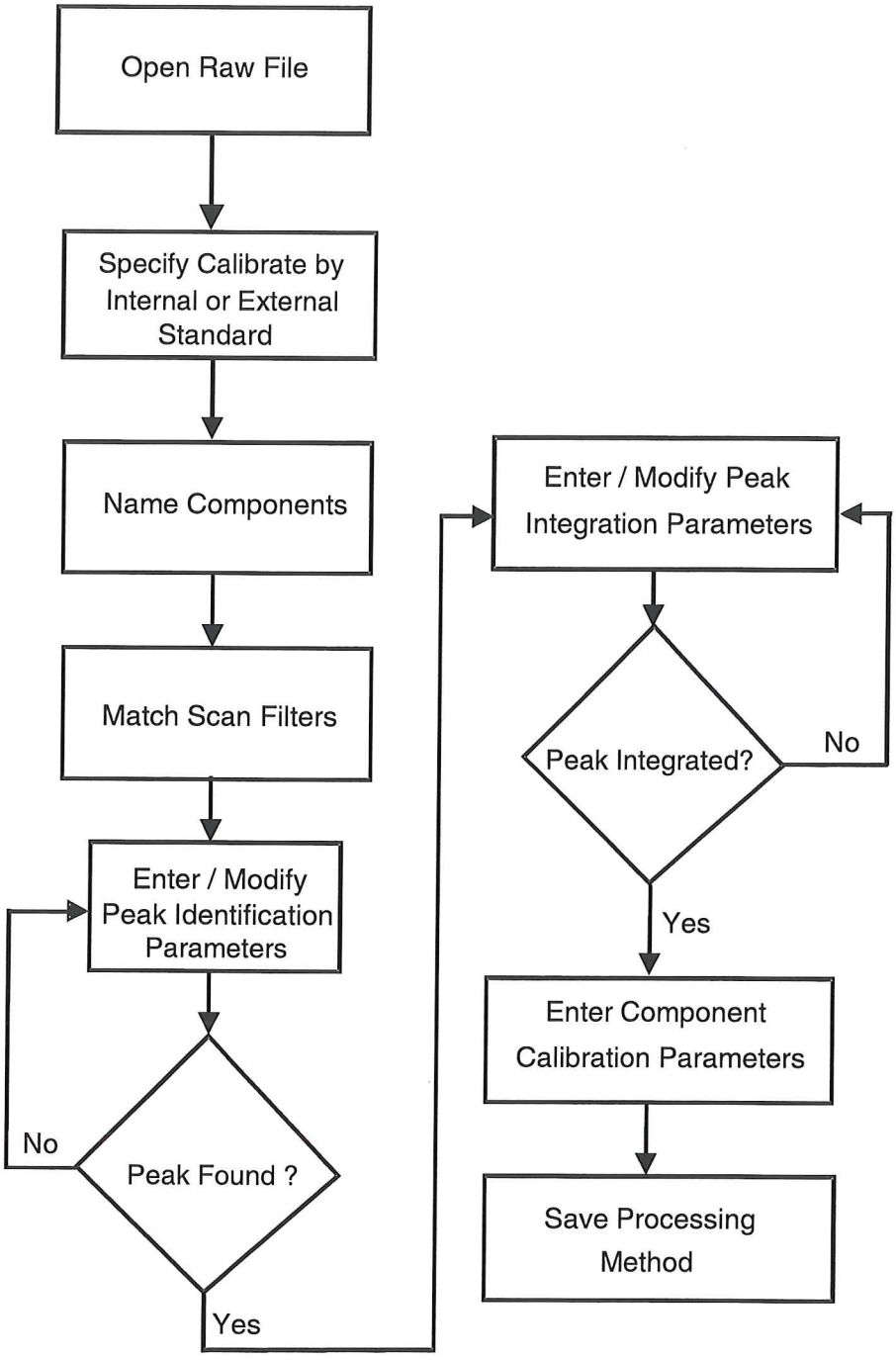
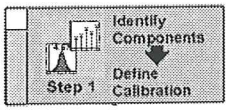


Figure 8-2. Flow diagram, showing how to create a Processing Method

8.2.1 Choosing the Create Method Tab

You need to choose the Create Method tab in the LCQUAN Flow Chart-Development Start dialog box to begin to create a Processing Method, as follows:



1. Display the Create Method page, as follows:
   Click on the Create Method tab. (See Figure 8-1.)
2. Open the Open Raw File dialog box, as follows:
   Click on the Step 1 box (or the **OK** button). See Figure 8-3.

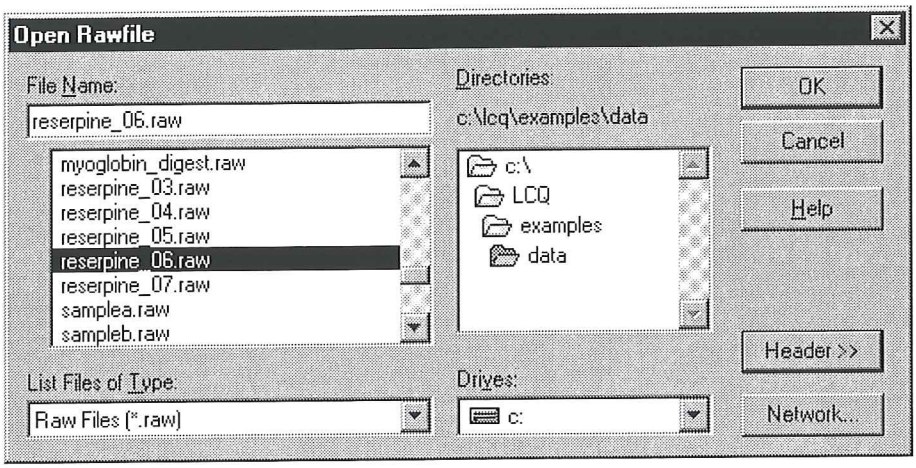


Figure 8-3. Open Raw File dialog box, showing the selected directory path and raw file

Go to the next topic: **Opening a Raw Data File.**

8.2.2 Opening a Raw Data File

You need to open a raw file from your data set to determine peak detection and integration parameters. In this example, you use the raw file named *reserpine_06.raw*. In general, you open a raw file corresponding to a low-concentration calibration standard.

To open *reserpine_06.raw* using the Open Raw File dialog box, proceed as follows:

1. Browse through the directories to find the file *C:\LCQ\examples\data\reserpine_06.raw.* (See Figure 8-3.)

2. Select the file name *reserpine_06.raw*
   Click on *reserpine_06.raw*. Then, click on **OK**. LCQUAN selects the *reserpine_06.raw* file and displays the Calibration Options dialog box. See Figure 8-4.

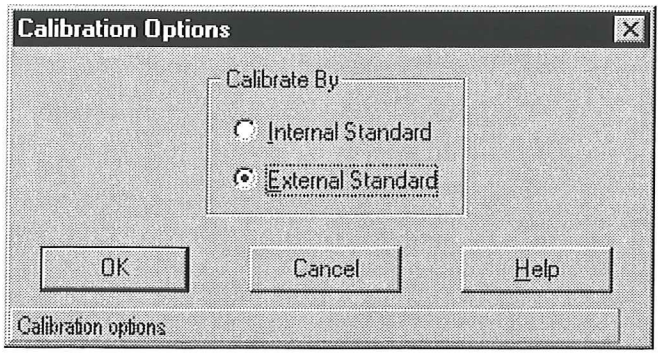


Figure 8-4. Calibration Options dialog box, showing calibrate by external standard

Go to the next topic: **Specifying Calibrate by Internal or External Standard**.

8.2.3 Specifying Calibrate by Internal or External Standard

You need to tell LCQUAN whether your experiment used internal or external standards. When you open a raw file (see above), LCQUAN displays the Calibration Options dialog box. (See Figure 8-4.) In this example, you use the Calibration Options dialog box to specify that you want to calibrate by external standard. Select the External Standard option button and then click on **OK**. LCQUAN displays the Create Method - Identify Components view. See Figure 8-5.

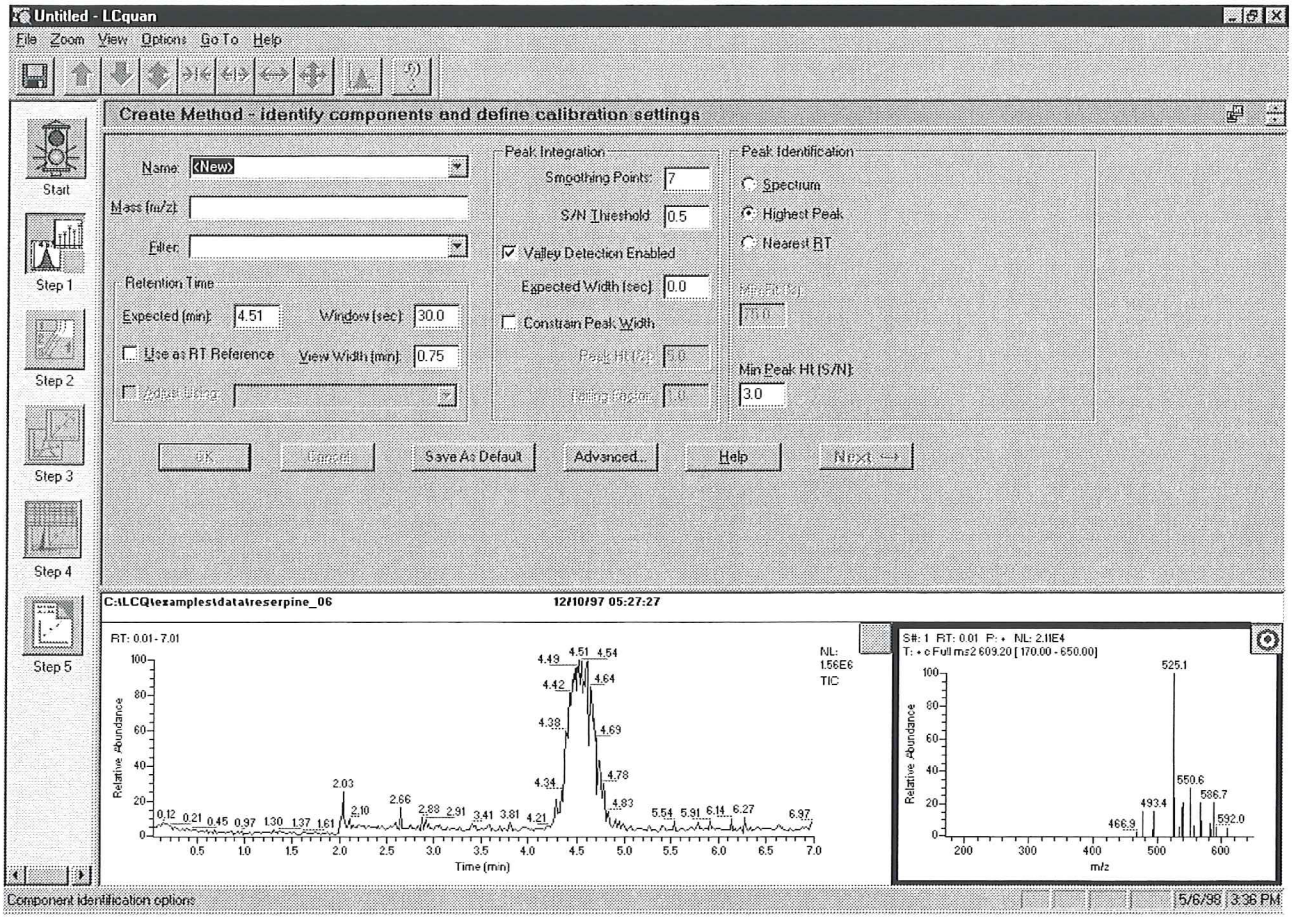


Figure 8-5. Create Method – Identify Components view, showing *reserpine_06.raw*

Go to the next topic: **Specifying Component Identification and Integration Settings for the Target Compound.**

8.2.4 Specifying Component Identification and Integration Settings for the Target Compound

When you specify calibrate by internal or external standard (see above), LCQUAN displays the Create Method - Identify Components view. (See Figure 8-5.) You use this view to specify component identification and integration settings.

The following topics describe how to specify the settings for the target compound, reserpine:

- Naming components
- Specifying product masses for quantitation
- Matching scan filters with components
- Entering peak identification information and displaying the spectrum
- Entering peak integration parameters

8.2.4.1 Naming Components

You use the Name combo box in the Create Method - Identify Components view to name the components of your sample. When you change settings, LCQUAN changes the settings for the named component only.

Enter the name of the target compound in the Name combo box, as follows:

1. Select **<New>** in the Name combo box.
2. Type **reserpine** in place of <New>.
3. Click on **OK**. LCQUAN changes the chromatogram and spectrum views based on naming the component. See Figure 8-6.

Notice that the chromatogram shows many peaks none of which are obvious for quantitation of reserpine. The total ion current (TIC) chromatogram from the MS/MS analysis displays all the ions in the range m/z 170.00 to 650.00. To demonstrate the presence of reserpine, you need only the reserpine product ion peaks. The following topic describes how to specify only selected MS/MS ions for quantitation.

Go to the next topic: **Specifying Product Masses for Quantitation**.

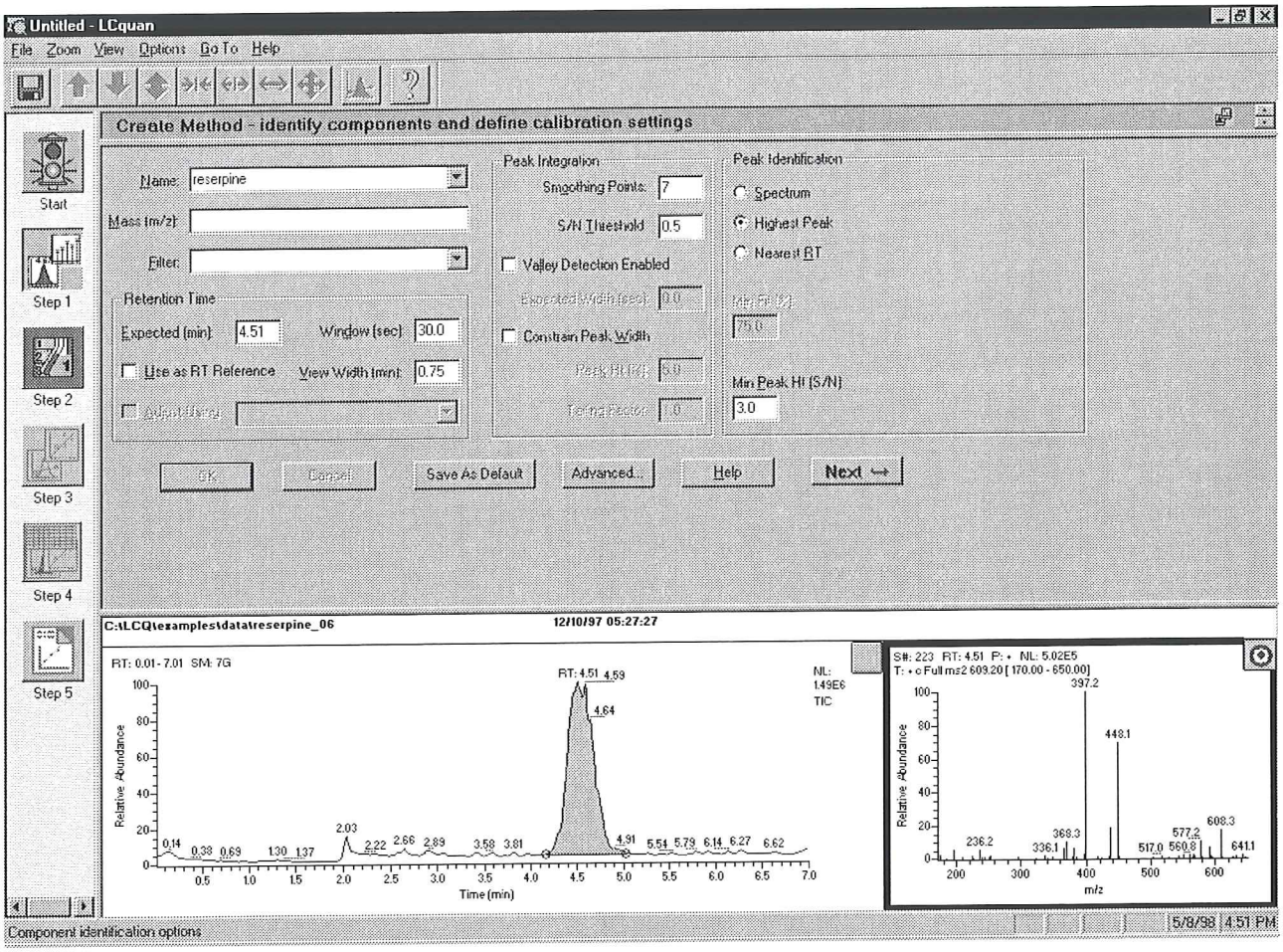


Figure 8-6. Create Method - Identify Components view, showing the total ion current (TIC) chromatogram of *reserpine_06.raw*

Note. Click on the **Help** button (near the center of the Create Method – Identify Components view) for a video demonstration on identifying components using this view. Then, in the Help window, click on the button next to the sentence: *Click here for a video demonstration of this view.*

8.2.4.2 Specifying Product Masses for Quantitation

At this time, the Create Method - Identify Components view shows the total ion current (TIC) chromatogram of the reserpine standard. To quantitate reserpine, you need to specify one or more of the product ions and display the mass chromatogram for those ion(s), as follows:

1. Specify two significant product ions of reserpine, as follows:
   Click on the Mass text box, then type **397.2, 448.2**.

2. Integrate the mass peaks, as follows:
   Click on **OK**. LCQUAN calculates the ion current contribution from *only* the two ions you specified. Then, LCQUAN displays a mass chromatogram (representing the *sum* of the areas of the two specified ions) corresponding to the target compound. See Figure 8-7.

Go to the next topic: **Matching Scan Filters with Components**.

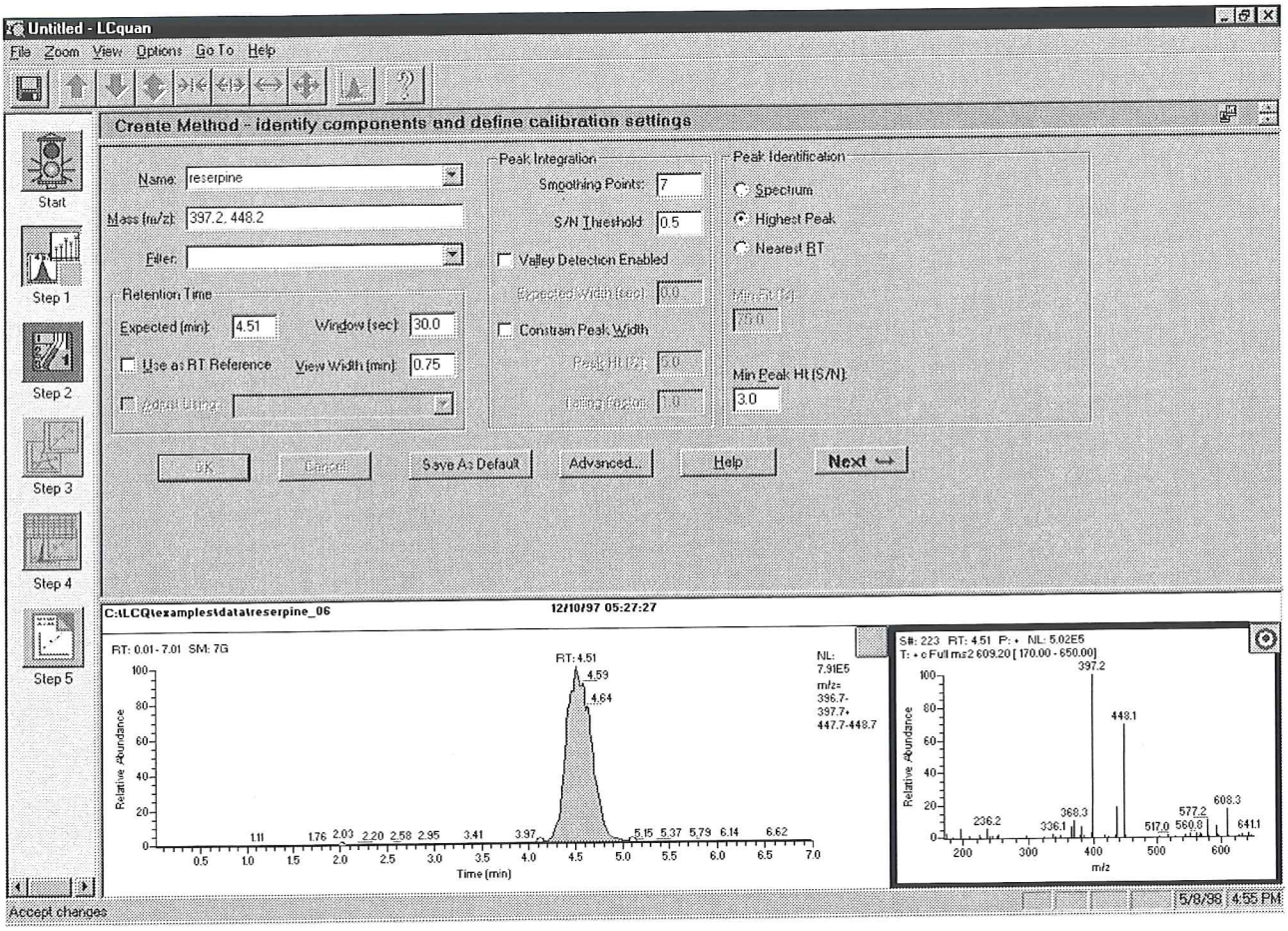


Figure 8-7. Create Method - Identify Components view, showing a mass chromatogram of *reserpine_06.raw*

8.2.4.3 Matching Scan Filters with Components

LCQ creates unique scan filters to acquire data according to the type of experiment you specify. In this example, MS/MS Full scan data were acquired in the mass range m/z 170.00 to 650.00. The ions are the results of fragmentation of the parent ion of reserpine m/z 609.2. It is necessary to match the scan filter with reserpine to calibrate and quantitate a TIC chromatogram.

Match reserpine with its scan filter, as follows:

1. Display the scan filters in the file *reserpine_06.raw*, as follows: Click on the down-arrow in the Filter combo box.

2. Select the scan filter for reserpine, as follows:
   Click on + *c Full ms2 609.20 [170.00 - 650.00].* Then, click on **OK**.

Go to the next topic: **Entering Peak Identification Information and Displaying the Spectrum**.

8.2.4.4 Entering Peak Identification Information and Displaying the Spectrum

To enter the peak identification information, display the mass spectrum, and automatically enter the retention time, do the following:

1. Select to identify the active component (currently reserpine), by use of the highest LC peak in the Peak Identification group box, as follows:
   Select the Highest Peak option button.

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 chromatogram view width of 2.5 min in the Retention Time group box:
      Type **2.5** in the View Width text box.

   b. Specify the default value of 3.0 in the Min Peak Ht (S/N) text box in the Peak Identification group box.

   c. Activate the Spectrum pane:
      Click on the Target button (currently grayed) in the upper right corner of the Spectrum pane.

   d. Display the mass spectrum:
      Click and drag the cursor in the Chromatogram pane from left to right across the chromatogram peak.

   e. Release the mouse button, and the following events occur automatically (see Figure 8-8):

      - LCQUAN enters the retention time corresponding to the selected scan in the Expected text box in the Retention Time group box.

- LCQUAN selects the retention time of the peak maximum and highlights the selected scan in the LC peak in red in the Chromatogram pane.
- LCQUAN displays the mass spectrum of the product ion(s) in the Spectrum pane.

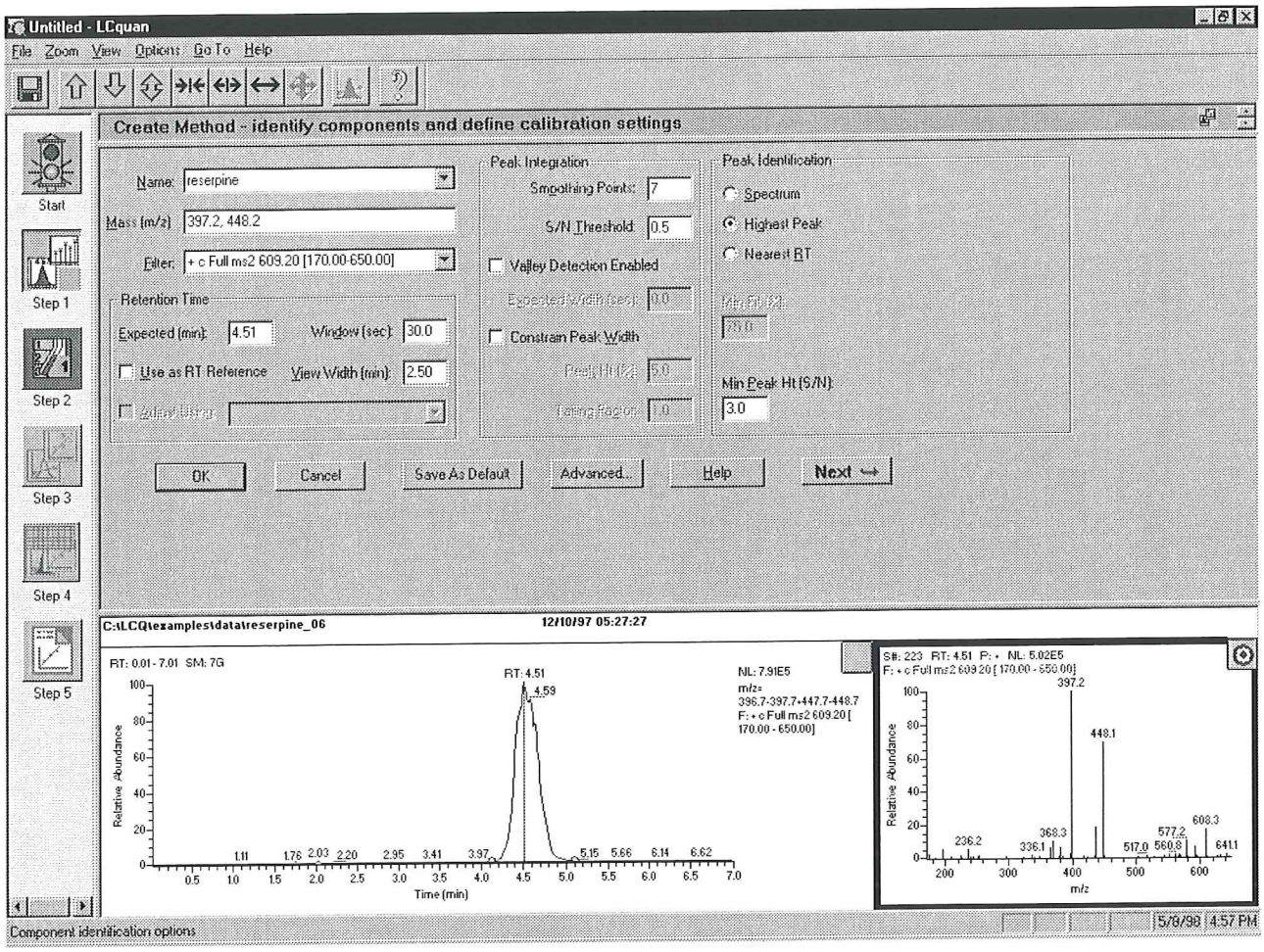


Figure 8-8. Create Method - Identify Components view, showing a marker (vertical line) indicating the retention time corresponding to the peak maximum

3. Save the component identification information, as follows: Click on **OK**. LCQUAN automatically shades the integrated portion of the peak gray and displays blue integration markers at the starting and ending points of the peak integration. The baseline is indicated by a blue line that connects the integration markers. See Figure 8-9.

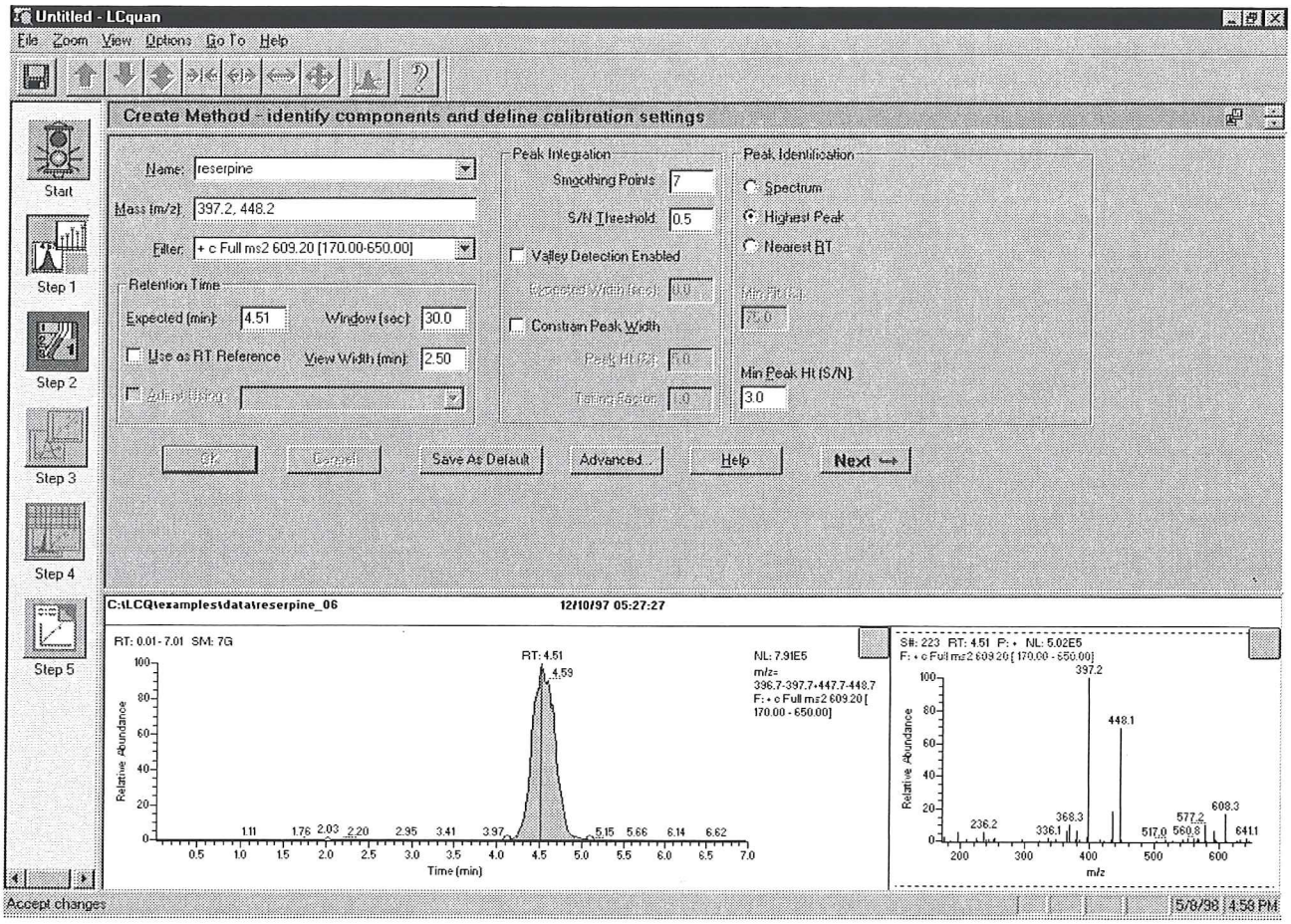


Figure 8-9. Create Method - Identify Components view, showing the area of peak integration (grayed) and the blue integration markers

4. Inspect the integrated peak and ensure the following:

   - Ensure that the retention time on the peak agrees with that in the Expected text box in the Retention Time group box.
   - Ensure that the entire chromatogram peak is displayed in the Chromatogram pane. If it is not, first activate the Chromatogram pane by clicking on the target button. 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.

If the peak has been identified properly, you are ready to specify how the peak is integrated. Go to the next topic: **Entering Peak Integration Parameters**.

If the peak has not been identified, return to step 2 of this topic and modify the peak identification parameters.

8.2.4.5 Entering Peak Integration Parameters

You enter the peak integration parameters to specify how LCQUAN determines the area of each peak in the chromatogram.

> **Note.** When you are entering many components with similar peak integration parameters, you first enter all of the identification parameters for one of the components. Then, you click on **Save As Default** in the Create Method - Identify Components view. These parameters then become the default values for new components.

Enter the peak integration parameters for reserpine, as follows:

1. Specify seven smoothing points in the Peak Integration group box, as follows:
   Type **7** in the Smoothing Points text box.

> **Note.** Integration S/N threshold is the number that controls how close you get 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.

2. Specify the default value of 0.5 in the S/N Threshold text box.

> **Note.** The Valley Detection Enabled check box allows you to use the LCQUAN approximation method of valley detection 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.

3. Leave the Valley Detection Enabled 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.

4. Leave the Constrain Peak Width check box empty (☐).

5. Save the peak integration parameters, as follows:
   Click on **OK**. Your display should resemble Figure 8-9.

You have finished entering component identification and integration settings for the target compound, reserpine.

- If your analytical process includes the use of internal standards, go to the next topic: **Specifying Component Identification and Integration Settings for the (Optional) Internal Standard.**
- If your analytical process uses external standards, skip to the topic: **Entering Component Calibration Parameters.**

8.2.5 Specifying Component Identification and Integration Settings for an (Optional) Internal Standard

You also use the Create Method - Identify Components view (see Figure 8-5) to specify component identification and integration settings for an internal standard. The settings for an internal standard are slightly different from those for a target compound.

Specify the settings for an internal standard, as follows:

1. Enter the name of the internal standard in the Name combo box.
2. Match the internal standard with its scan filter.
3. Select to identify the internal standard by the appropriate option.
4. Display the mass spectrum and automatically enter the retention time of the LC peak.
5. Integrate the peak and save the component identification information.
6. Enter the peak integration parameters.

When you have finished entering component detection and integration parameters for an internal standard, go to the next topic: **Entering Component Calibration Parameters**.

8.2.6 Entering Component Calibration Parameters

You use the Create Method - Define Calibration Settings view to enter component calibration parameters. In this example, you process calibration data using an external standard calibration.

For *each* raw data file, you first specify the component type for reserpine as a target compound. Then, you specify the concentration level of each calibration.

Note. Click on the **Help** button in the Create Method - Define Calibration Settings view for a video demonstration of entering component calibration parameters using this view. Then, in the Help window, click on the button next to the sentence: *Click here for a video demonstration of this view.*

LCQUAN can fit the calibration data to any of the following curve types:

- Linear
- Quadratic
- Linear log-log
- Quadratic log-log
- Average RF
- Point-to-point
- Cubic spline
- Locally weighted

When it is performing the least squares fit to the calibration data, LCQUAN can weight the calibration data with the following weighting functions:

- Equal
- 1/X
- $1/X^2$
- 1/Y
- $1/Y^2$
- $1/S^2$, where $S^2 = X^2 + Y^2$

In addition, you can have LCQUAN ignore the origin, use the origin as a data point, or force the calibration curve to include the origin.

You should already have the Create Method - Identify Components view displayed (see above).

Enter the component calibration parameters for reserpine, as follows:

1. Display the Create Method - Define Calibration Settings view from the Create Method - Identify Components view, as follows:
   Click on the Next button. See Figure 8-10.

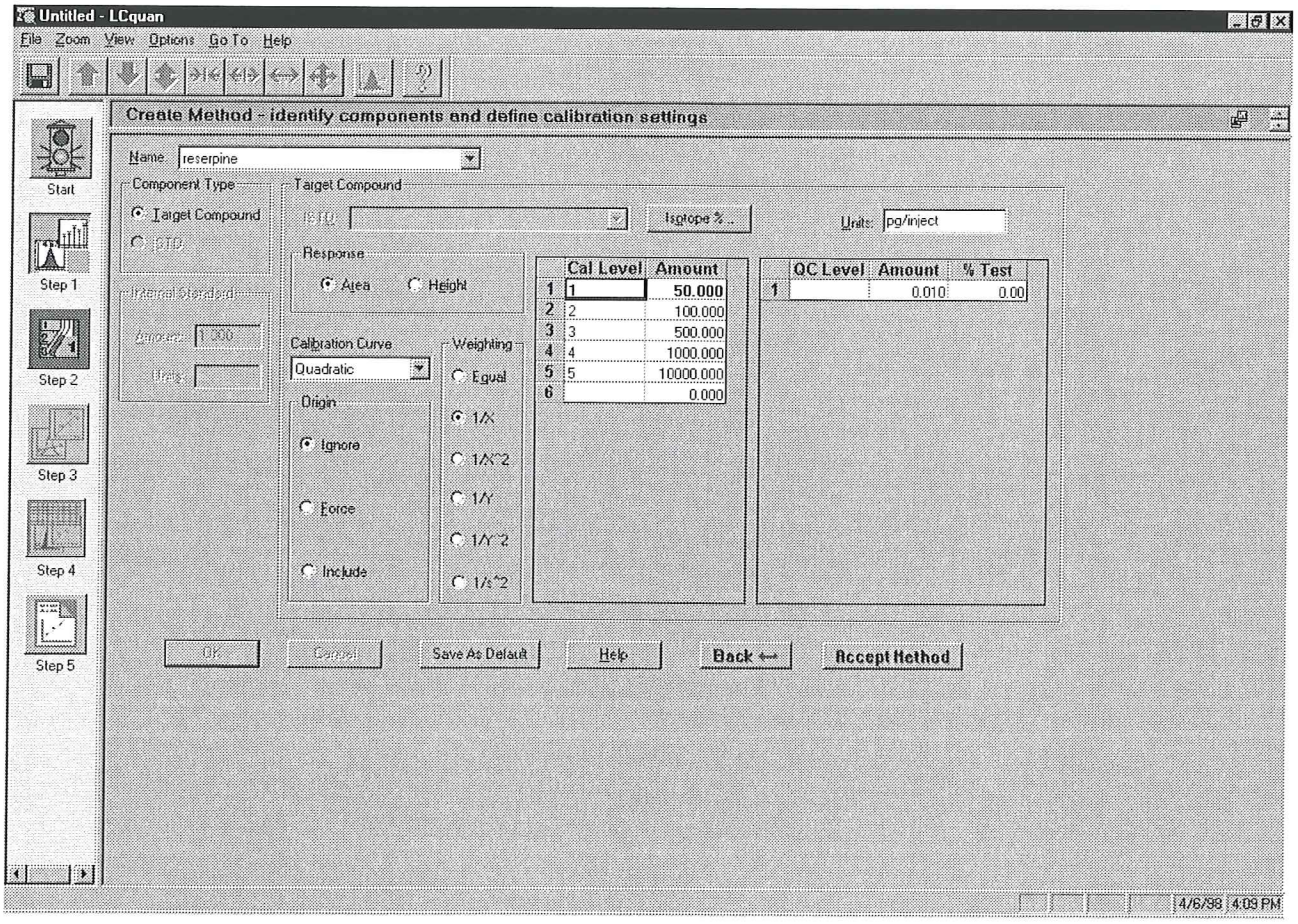


Figure 8-10. Create Method - Define Calibration Settings view, showing the correct settings for the calibration curve

2. Select the target compound in the Create Method - Define Calibration Settings view, as follows:
   Click on the down-arrow in the Name list box. Then, select *reserpine*.

3. Specify reserpine as a target compound in the Component Type group box, as follows:
   Click on the Target Compound option button.

4. Specify a quadratic calibration curve in the Target Compound group box, as follows:
   Click on the down-arrow in the Calibration Curve list box. Then, select *Quadratic*.

5. Leave the origin set to its default value of Ignore in the Origin group box.

6. Specify a weighting of 1/X in the Weighting group box, as follows:
   Click on the 1/X option button.

7. Leave the response set to its default setting of Area in the Response group box.

8. Specify the units of concentration as picograms per injection to appear on the calibration curve, as follows:
   Click on the Units text box and type **pg/inject**.

9. Enter five calibration levels in the Target Compound group box, as follows:
   First, click on the first Cal Level text box and type **1**. Next, press **<Tab>** and type **50** in the first Amount text box. Then, press **<Tab>** to start the second row.

   Repeat this procedure until you fill in the five calibration levels as shown in Table 8-1.

Table 8-1. Calibration Level Table, showing the amount of reserpine injected in 10 µL of the corresponding calibration solution

Calibration Level	Raw File Name	Amount (pg/injection)
1	reserpine_03	50
2	reserpine_04	100
3	reserpine_05	500
4	reserpine_06	1000
5	reserpine_07	10000

10. Accept the calibration parameters for reserpine, as follows:
    Click on **OK**. (See Figure 8-10.)

You have completed the procedure for creating a Processing Method for the reserpine example.

- If you want to save the Processing Method to the hard drive, go to the next topic: **Saving the Processing Method**.
- If you do not want to save the Processing Method to the hard drive, go to the topic: **Accepting the Processing Method**.

8.2.7 Saving the Processing Method

Normally, you save the Processing Method if you plan to perform a similar analysis in the future. Save the Processing Method as *practice.pmd*, as follows:

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

2. Find the *C:\LCQ\examples\methods* directory and name the Processing Method *practice.pmd*, as follows:

   a. Browse through the directory tree to find the *C:\LCQ\examples\methods* directory.

   b. Enter **practice.pmd** in the File Name text box.

3. Save the Processing Method and close the dialog box, as follows:
   Click on **OK**.

Go to the next topic: **Accepting the Processing Method**.

8.2.8 Accepting the Processing Method

Accept the Processing Method and display the LCQUAN Flow Chart - Sample List Creation dialog box (step 2), as follows:

Click on **Accept Method** in the Create Method - Define Calibration Settings view. See Figure 8-11. Note that the Step 1 button is checked.

You have now completed step 1 of the LCQUAN procedure. Go to the next topic: **Step 2: Opening an Existing Sample List.**

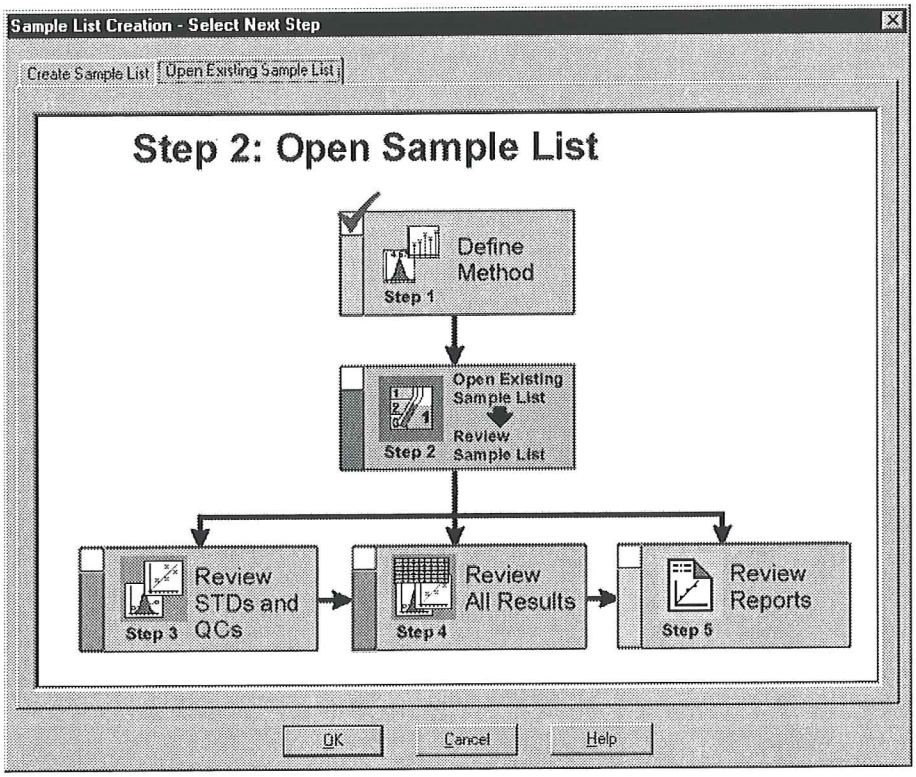


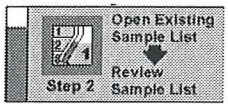
Figure 8-11. LCQUAN Flow Chart - Sample List Creation dialog box, showing that the next step is to open an existing Sample List

8.3 Step 2: Opening an Existing Sample List

In Step 2 of LCQUAN you open an existing Sample List. In the reserpine example, you open the Sample List, named *reserpinecali.sld,* that you created in Chapter 7.

> **Note.** A Sample List that you create with LCQUAN can be used to process raw files, but it cannot be used to run samples.

Open *reserpinecali.sld* from the LCQUAN Flow Chart - Sample List Creation dialog box, and display it in the Create Sample List-Review and Edit view, as follows:



1. Open the Open Sample File dialog box, as follows:
   Click on the Open Existing Sample List tab. Then, click on the Step 2 box (shown in the picture to the left), or click on **OK**. See Figure 8-12.

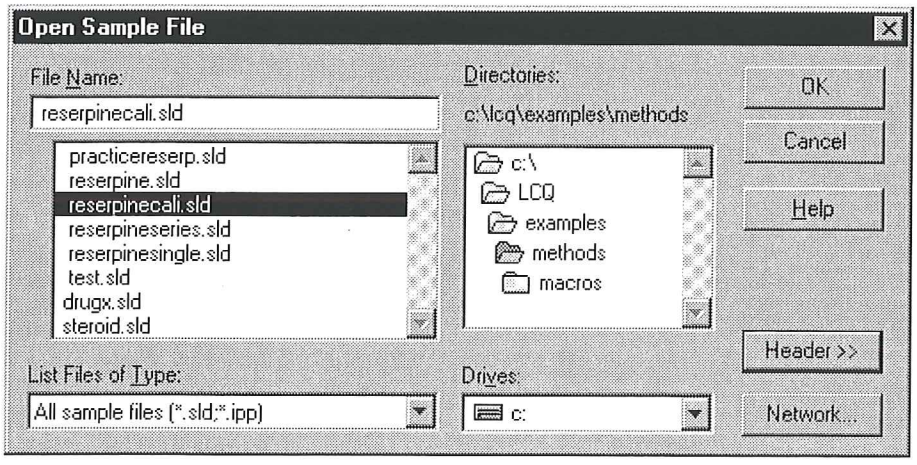


Figure 8-12. Open Sample File dialog box, showing *reserpinecali.sld* selected

2. Locate the *reserpinecali.sld* file, as follows:
   Browse through the directories to find the file *C:\LCQ\examples\methods\reserpinecali.sld.*

3. Select the *reserpinecali.sld* Sample List file, as follows:
   Click on *reserpinecali.sld.*

4. Close the dialog box and display the Create Sample List - Review and Edit view:
   Click on **OK**. See Figure 8-13.

> **Note.** Use the Create Sample List - Review and Edit view to modify the Sample List. Right-click the mouse in the Sample List to display a popup menu of commands.

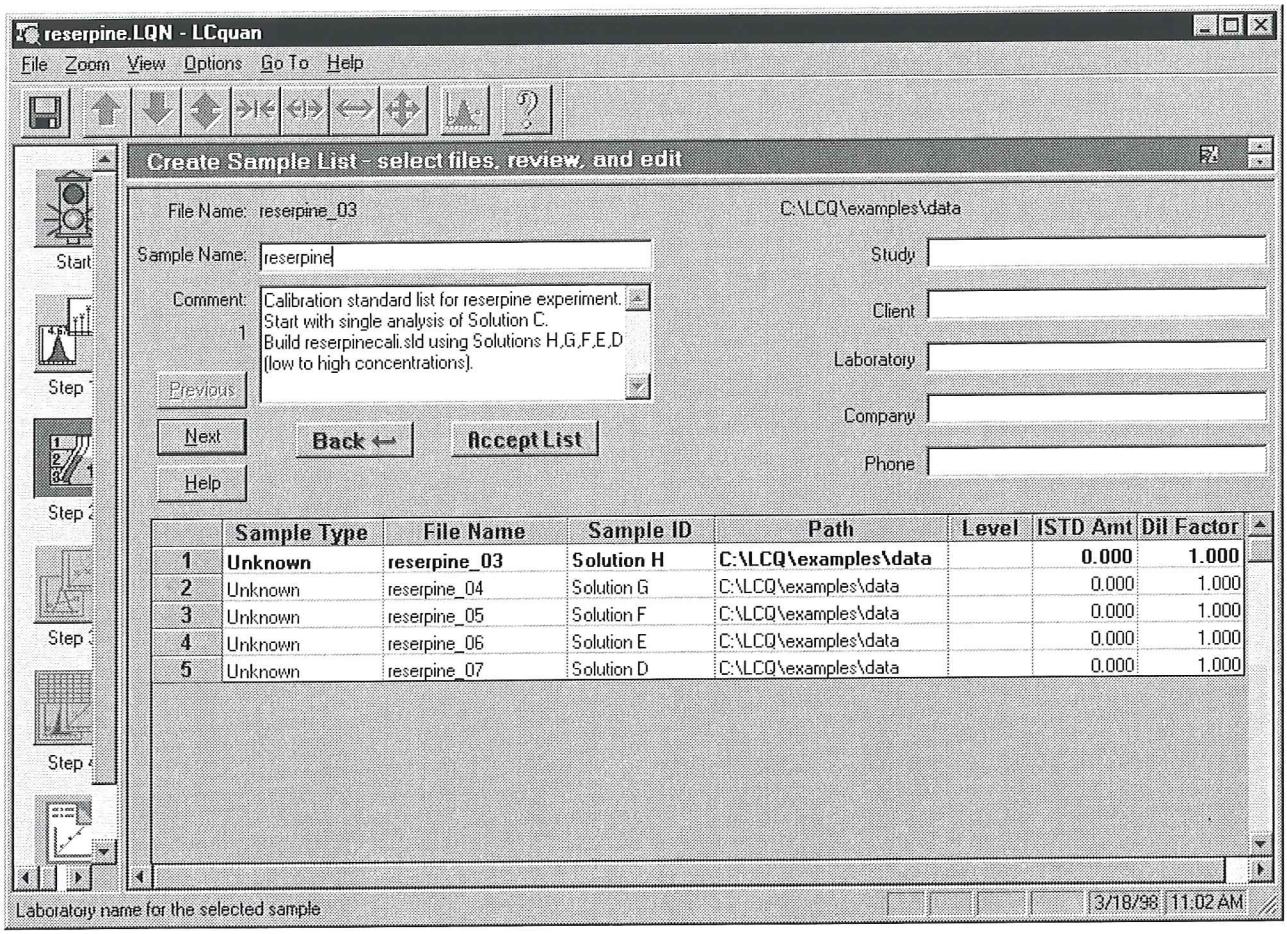


Figure 8-13. Create Sample List - Review and Edit view, showing the Sample List *reserpinecali.sld*

5. Specify the sample type for the first analysis as *Standard* in the Sample Type column, as follows:

   a. Display the down-arrow of the Sample Type list box in row 1:
   Click on the word *Unknown* in row 1.

   b. Display the Sample Type list box:
   Click on the down-arrow.

   c. Select Standard from the list box and open the Select Level dialog box:
   Scroll in the list box, and click on the word *Standard*.
   See Figure 8-14.

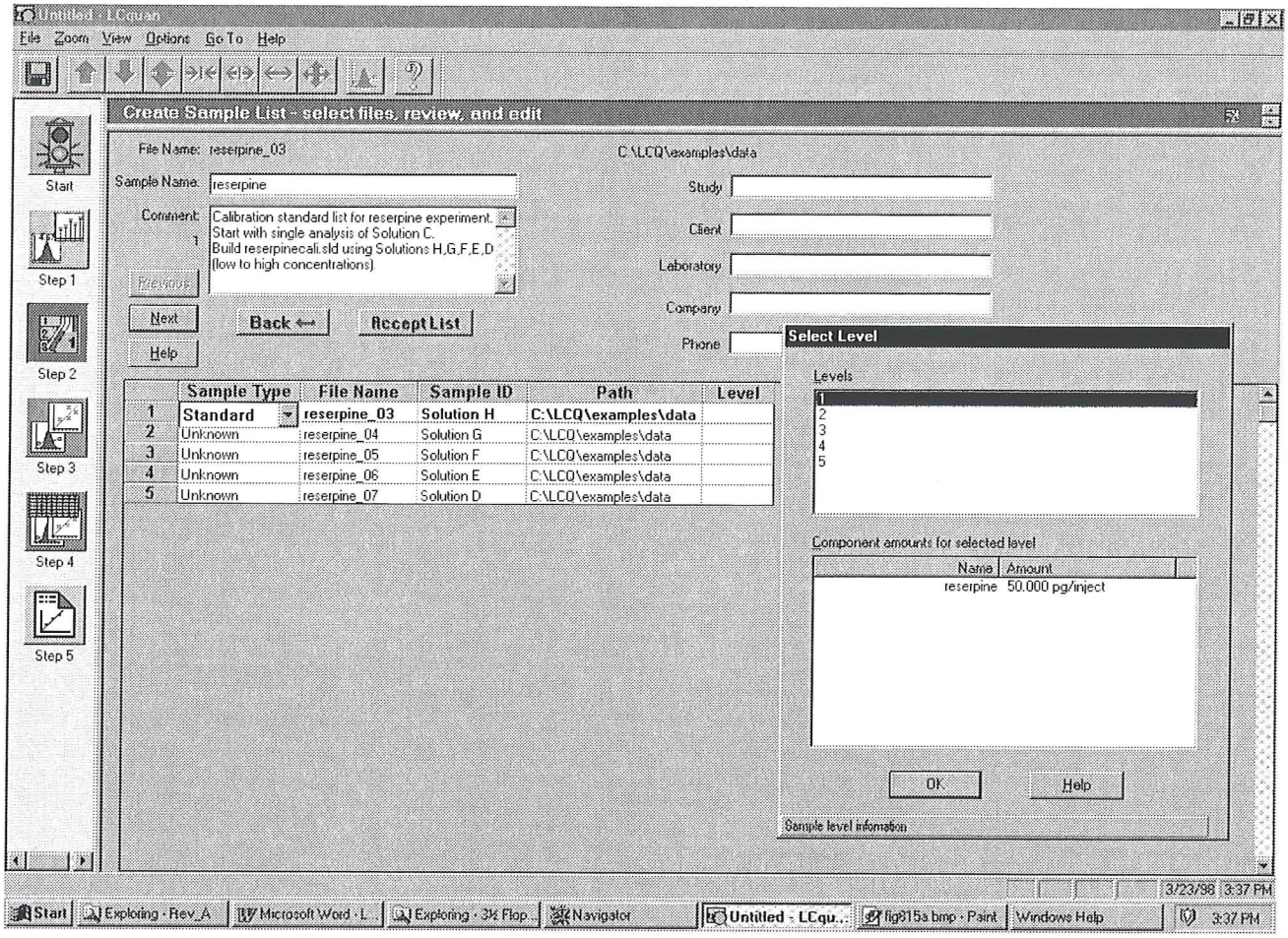


Figure 8-14. Create Sample List - Review and Edit view, showing the Select Level dialog box

d. Associate the standard level with the corresponding name and amount for the first analysis, as follows:

   i. Click on *1* in the Levels list box of the Select Level dialog box. Ensure that the component name and amount is correct for this level.

   ii. Close the dialog box, and return to the Create Sample List view:
   Click on **OK**.

6. Repeat the instructions in step 5 above for each row in the Create Sample List view. See Figure 8-15.

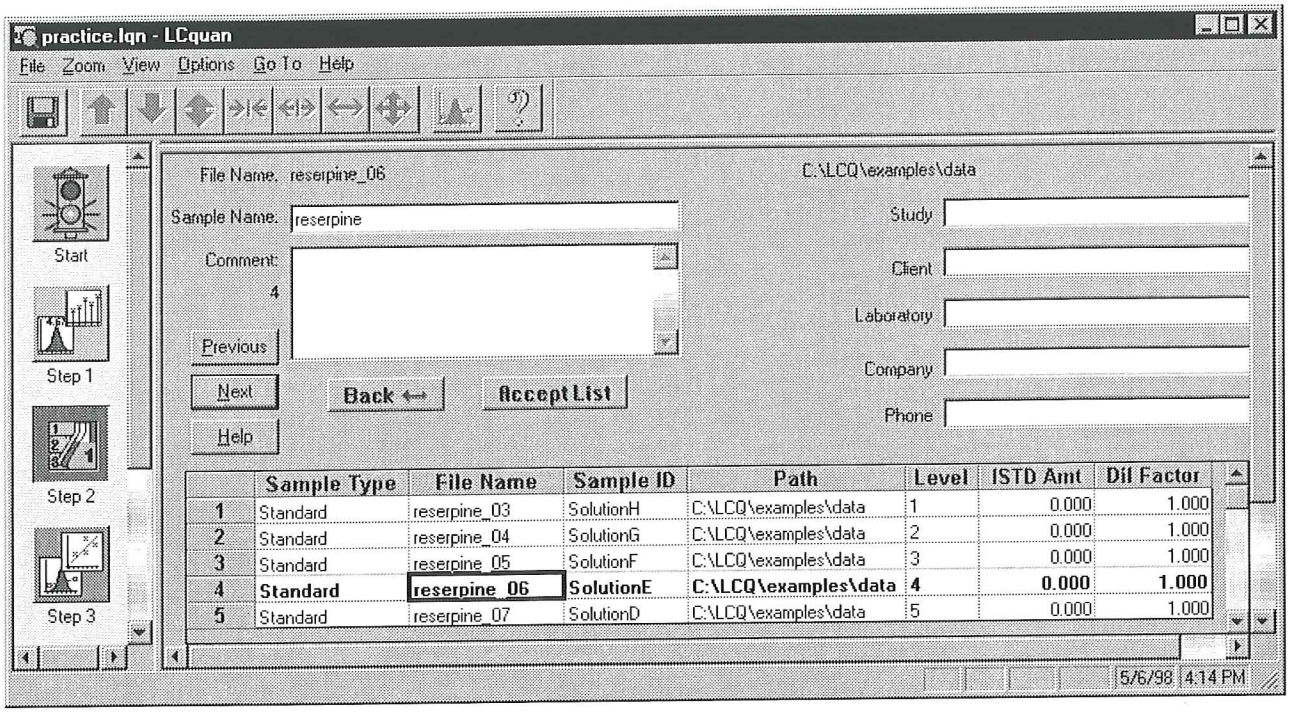


Figure 8-15. Create Sample List - Review and Edit view, showing associated calibration standard levels and raw files

7. LCQUAN Flow Chart – Build Results Options dialog box, as follows:
   Click on **Accept List**. See Figure 8-16.

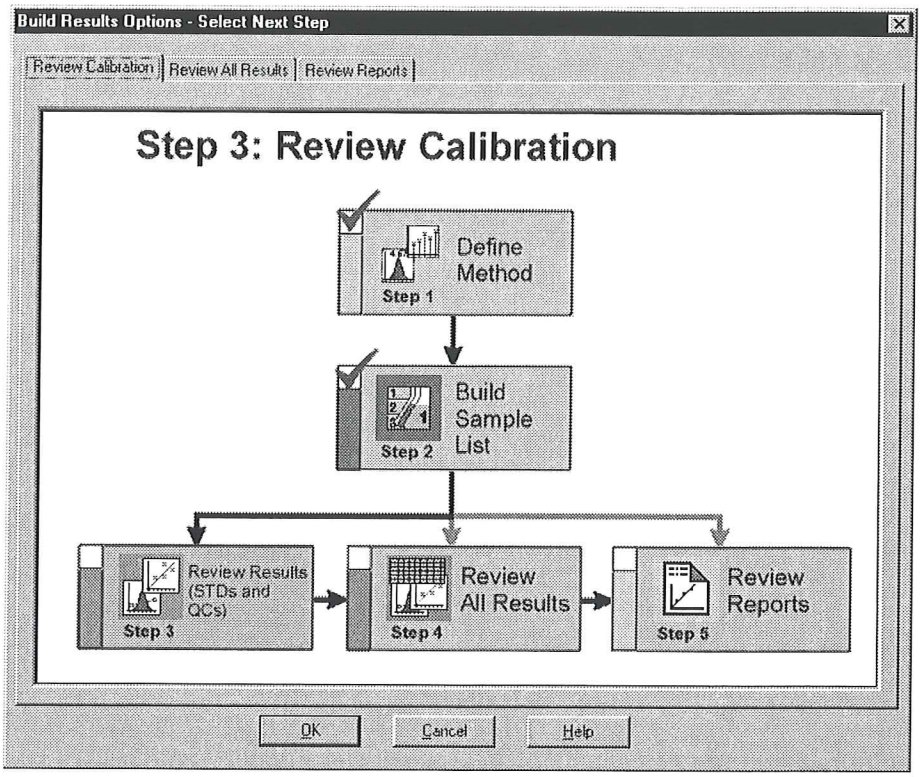


Figure 8-16. LCQUAN Flow Chart – Build Results Options dialog box, showing that the next step is to review the calibration results

You are now ready to review your analytical results and create reports. Go to the next topic: **Processing the Raw Files**.

8.4 Processing the Raw Files

LCQUAN processes the raw files in the Sample List when you initiate step 3, 4, or 5. LCQUAN uses the peak detection and integration parameters in the Processing Method to detect and integrate peaks for all components. LCQUAN then uses the calibration parameters in the Processing Method to build a calibration curve with which LCQUAN quantitates the QCs and unknowns.

When you are done reviewing the Sample List and you click on the Accept List button in the Create Sample List – Review and Edit view (refer to topic 8.3), LCQUAN displays the LCQUAN Flow Chart – Build Results Options dialog box. (See Figure 8-16.) You initiate step 3, 4, or 5 by selecting the appropriate tab in the LCQUAN Flow Chart – Build Result Options dialog box and then clicking on **OK**.

> **Note**. You need to complete step 1 (create or open a Processing Method) and step 2 (create or open a Sample List) before you can initiate step 3, 4, or 5 (review calibration, results, and reports).

To review the calibration curve, go on to the next topic:
Step 3: Reviewing the Calibration Curve.

8.5 Step 3: Reviewing the Calibration Curve

Although you can perform steps 3, 4, and 5 in any order, in this example you perform step 3 first. In step 3 of LCQUAN, you use the Review Calibration view to evaluate and rework calibration standards.

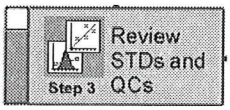
After you have processed the raw files, you review and rework the calibration standard data, as follows:

- Process the raw files and open the Review Calibration view
- Review calibration standards
- Modify the peak detection and integration settings
- Integrate peaks manually (optional)

8.5.1 Processing the Raw Files and Opening the Review Calibration View

To process the raw files and open the Review Calibration view from the LCQUAN Flow Chart - Build Results Options dialog box, do the following:

1. Select the page to review calibration data, as follows:
   Click on the Review Calibration tab.



2. Open the Review Calibration view, as follows:
   Click on the Step 3 box, shown in the picture to the left.
   See Figure 8-17.

The Review Calibration view includes the Result List, Chromatogram pane, and Calibration Curve pane. When you perform an action in the Result List, or Chromatogram pane, or Calibration Curve pane, LCQUAN instantly updates the other two.

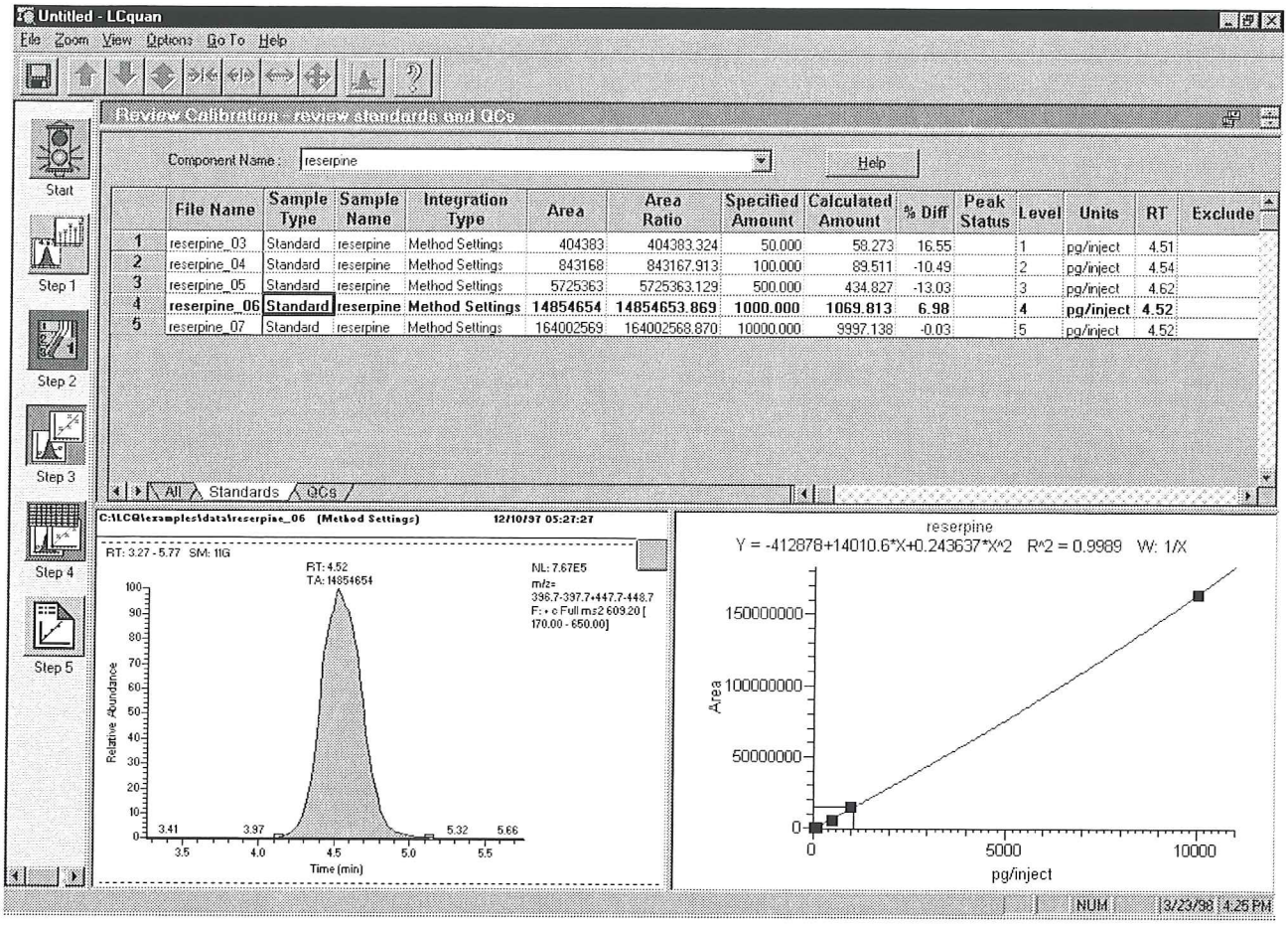


Figure 8-17. Review Calibration view, showing the Result List, Chromatogram pane, and Calibration Curve pane for standard level 4

8.5.2 Reviewing Calibration Standards

Review the calibration standards, as follows:

1. Select the target compound reserpine, as follows:
   Click on the down-arrow of the Component Name list box, and select *reserpine* from the component list. LCQUAN automatically updates the Result List, Chromatogram pane, and Calibration Curve pane.

2. Display calibration standard results, as follows:
   Click on the Standards tab located at the bottom of the Result List.

3. Inspect the curve in the Calibration Curve pane at all concentrations. When you process your own data, you evaluate the calibration curve according to the criteria used in your laboratory.

4. Make the low concentration portion of the calibration curve available for injection, as follows:
Click and drag the mouse diagonally across the low concentration data points in the Calibration Curve pane. Release the mouse button. LCQUAN displays only the selected, low-concentration data points. See Figure 8-18.

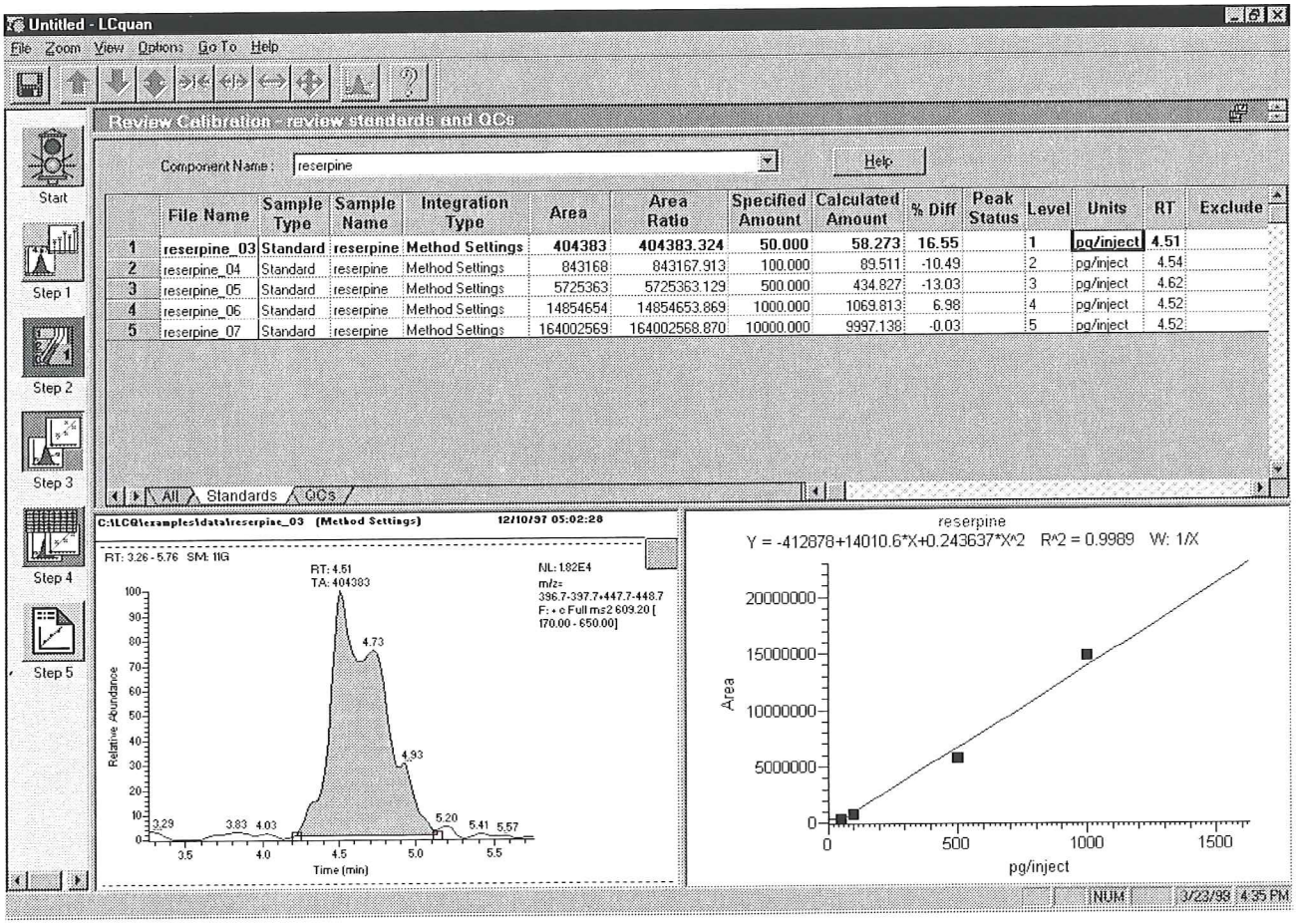


Figure 8-18. Review Calibration view, showing standard level 1 in the Chromatogram pane and standard levels 1 through 4 in the Calibration Curve pane

5. Reset the X-axis and the Y-axis ranges of the calibration curve as follows:

Click on the Reset Scaling button.

> **Note.** You can change the calibration curve parameters. For example, you can modify the curve type and weighting in the Calibration Settings dialog box. To open the Calibration Settings dialog box, right-click in the Calibration Curve pane and display the popup menu. Then, left-click on Calibration Settings in the popup menu to display a Calibration Settings dialog box.

6. Inspect the Result List, as follows:
Check the entries in the Result List for peak detection and integration problems. Make sure that the data files correspond to the correct levels and sample types. (You can right-click the mouse in the Result List to display a popup menu of commands for modifying the Result List.)

7. Select the first data file, as follows:
Click on the first row of the Result List.

8. Inspect in the Chromatogram pane the component peak, as follows:
Make sure that LCQUAN found the peak. LCQUAN shades the peaks it finds gray, and marks the starting and ending points with square integration markers. Make sure that the shaded area accurately represents the contribution of the component to the chromatogram.

   - If you need to modify the peak detection and integration settings that you set in the Create Method - Identify Components view, go to step 9.

   - If you need to integrate a peak manually, go to step 10.

9. Modify the peak detection and integration settings (optional), as follows:
Go to the topic: **Modifying the Peak Detection and Integration Settings**, below.

10. Perform manual integration (optional), as follows:
Go to the topic: **Integrating Peaks Manually**, below. You can manually change the starting and ending points, and the baseline of the peak.

11. Select the next data file, as follows:
    Click on the next row in the Result List.

    Repeat steps 8 through 10 for rows 2 through 5 in the Sample List.

When you are satisfied that LCQUAN has detected and integrated the peaks properly, you are ready to create a report of your results. Skip to the topic: **Step 5: Creating and Reviewing Reports**.

8.5.3 Modifying the Peak Detection and Integration Settings

Modify the peak detection and integration settings from the Review Calibration view (Step 3), as follows:

1. Display a pop-up menu, as follows:
   Right-click the mouse in the Chromatogram pane.

2. Open the User Identification Settings dialog box from the pop-up menu, as follows:
   Choose the User Peak Detection Settings command. See Figure 8-19.

3. Modify the peak detection settings, as follows:
   Select the Detection tab. Modify the settings, as needed.

4. Modify the peak integration settings, as follows:
   Select the Integration tab. Modify the settings, as needed, if you have problems with noise in the peak, unresolved peaks, or peak tailing.

5. Modify the settings in the Advanced page, as follows:
   Select the Advanced tab. Modify these settings if baseline noise is interfering with peak identification or integration.

6. Apply the modifications, and close the dialog box, as follows:
   Click on **OK**.

When you complete modifications in the User Identifications Settings dialog box, skip to the topic: **Step 5: Creating and Reviewing Reports.**

If you need to integrate peaks manually, go to the next topic: **Integrating Peaks Manually**.

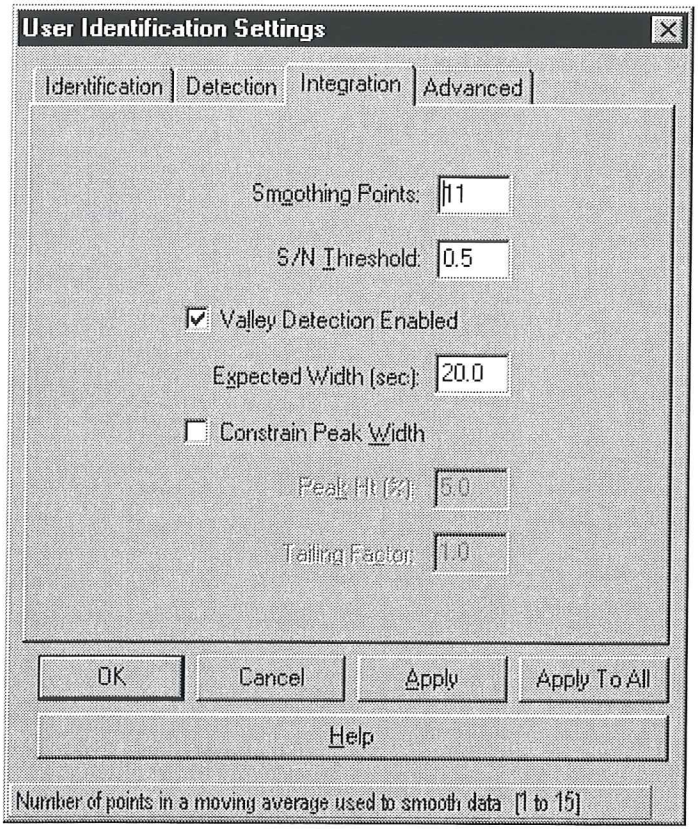


Figure 8-19. User Identification Settings dialog box, showing peak integration parameters

8.5.4 Integrating Peaks Manually

You can manually change the starting and ending points and the baseline of a peak, as follows:

1. Move the beginning point of the peak to a desired location, as follows:
   Position the cursor arrow over the (left) square integration markers. The symbol [+] displays over the square. Then, click and drag the square integration marker. Release the mouse.

2. Move the ending point of the peak to a desired location, as follows:
   Click and drag the (right) square integration marker. When you perform manual integration, LCQUAN changes the integration type to Manual Integration in the Integration Type list box in the Result List. See Figure 8-20.

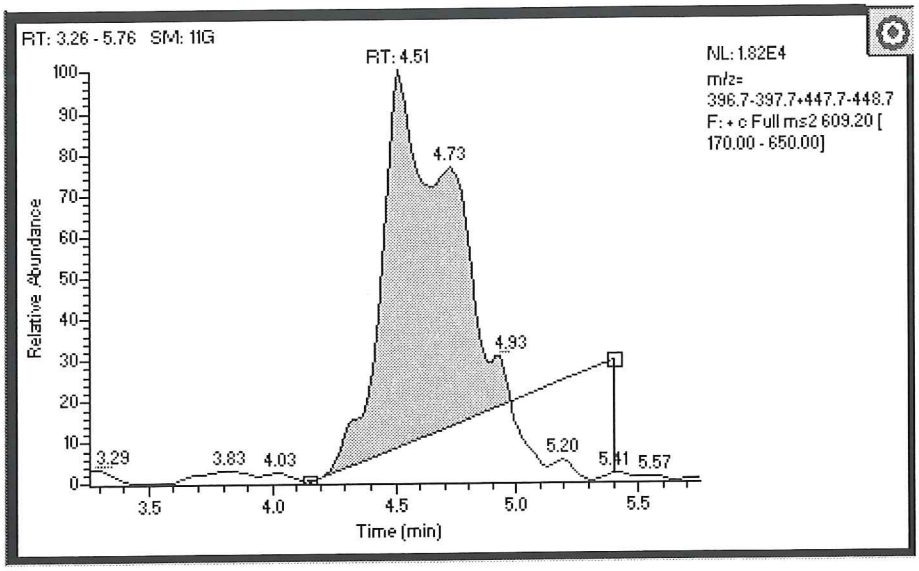


Figure 8-20. Review Calibration view, Chromatogram pane, showing how the integration marker can be adjusted to manually integrate a component peak

3. Undo manual integration, as follows:

   a. Select the appropriate cell in the Integration Type column of the Result List:
   Click on the cell, then click on the cell a second time to display the cell as a drop-down list box.

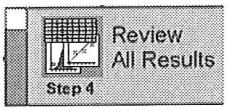
   b. Select the method settings from the drop-down list:
   Click on the down-arrow, and select *Method Settings*. The integration returns to its prior settings.

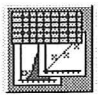
8.6 Step 4: Reviewing All Results

You can use the Review All Results view to review and rework (optional) unknowns and blanks.

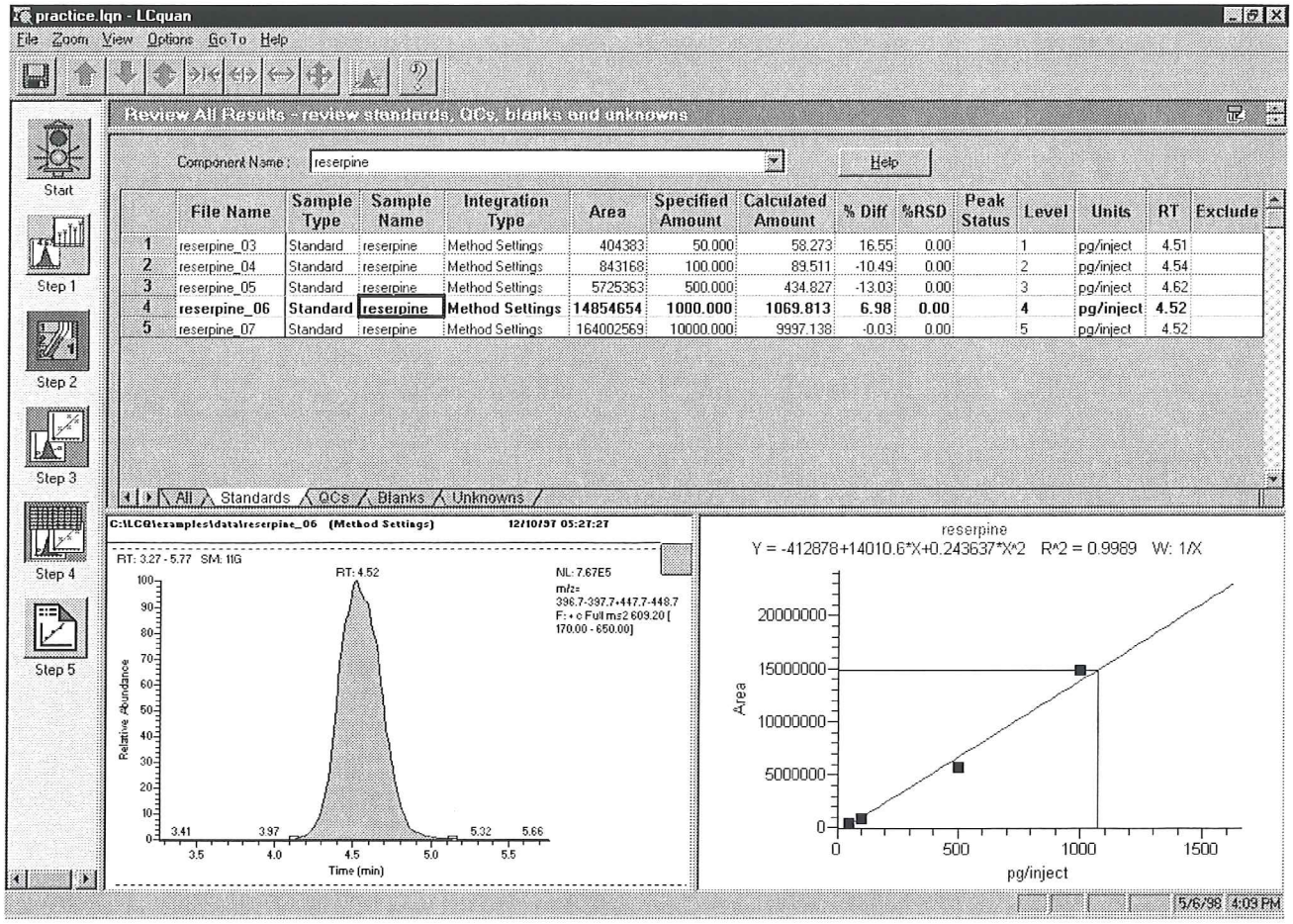
> **Note.** The reserpine data set does not contain raw files corresponding to unknowns or blanks.

Review unknowns and blanks, as follows:





1. Open the Review All Results view:
   Choose the Review All Results tab from the LCQUAN Flow Chart – Build Results Options dialog box. Then, click on the Step 4 box (or click on **OK**). Alternatively, click on the Step 4 button on the left side of the Review Calibration view. See Figure 8-21.



	File Name	Sample Type	Sample Name	Integration Type	Area	Specified Amount	Calculated Amount	% Diff	%RSD	Peak Status	Level	Units	RT	Exclude
1	reserpine_03	Standard	reserpine	Method Settings	404383	50.000	58.273	16.55	0.00		1	pg/inject	4.51	
2	reserpine_04	Standard	reserpine	Method Settings	843168	100.000	89.511	-10.49	0.00		2	pg/inject	4.54	
3	reserpine_05	Standard	reserpine	Method Settings	5725363	500.000	434.827	-13.03	0.00		3	pg/inject	4.62	
4	**reserpine_06**	**Standard**	**reserpine**	**Method Settings**	**14854654**	**1000.000**	**1069.813**	**6.98**	**0.00**		**4**	**pg/inject**	**4.52**	
5	reserpine_07	Standard	reserpine	Method Settings	164002569	10000.000	9997.138	-0.03	0.00		5	pg/inject	4.52	

Figure 8-21. Review All Results view, showing the Results list, Chromatogram pane, and Calibration Curve pane for Standard Level 4

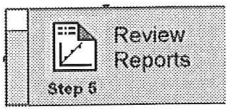
2. Review and rework unknowns and blanks, as follows:
   Perform steps 6 through 11 in the topic: **Reviewing Calibration Standards**.

Go to the next topic: **Step 5: Creating and Reviewing Reports**.

8.7 Step 5: Creating and Reviewing Reports

In Step 5 of LCQUAN, you use the Review Reports view to create and review reports.

Create and review reports, as follows:



1. Open the Review Reports view:
   Choose the Review Reports tab from the LCQUAN Flow Chart – Build Results Options dialog box. Then, click on the Step 5 box (or click on **OK**). Alternatively, click on the Step 5 button on the left side of the Review Calibration view or the Review All Results view. See Figure 8-22.

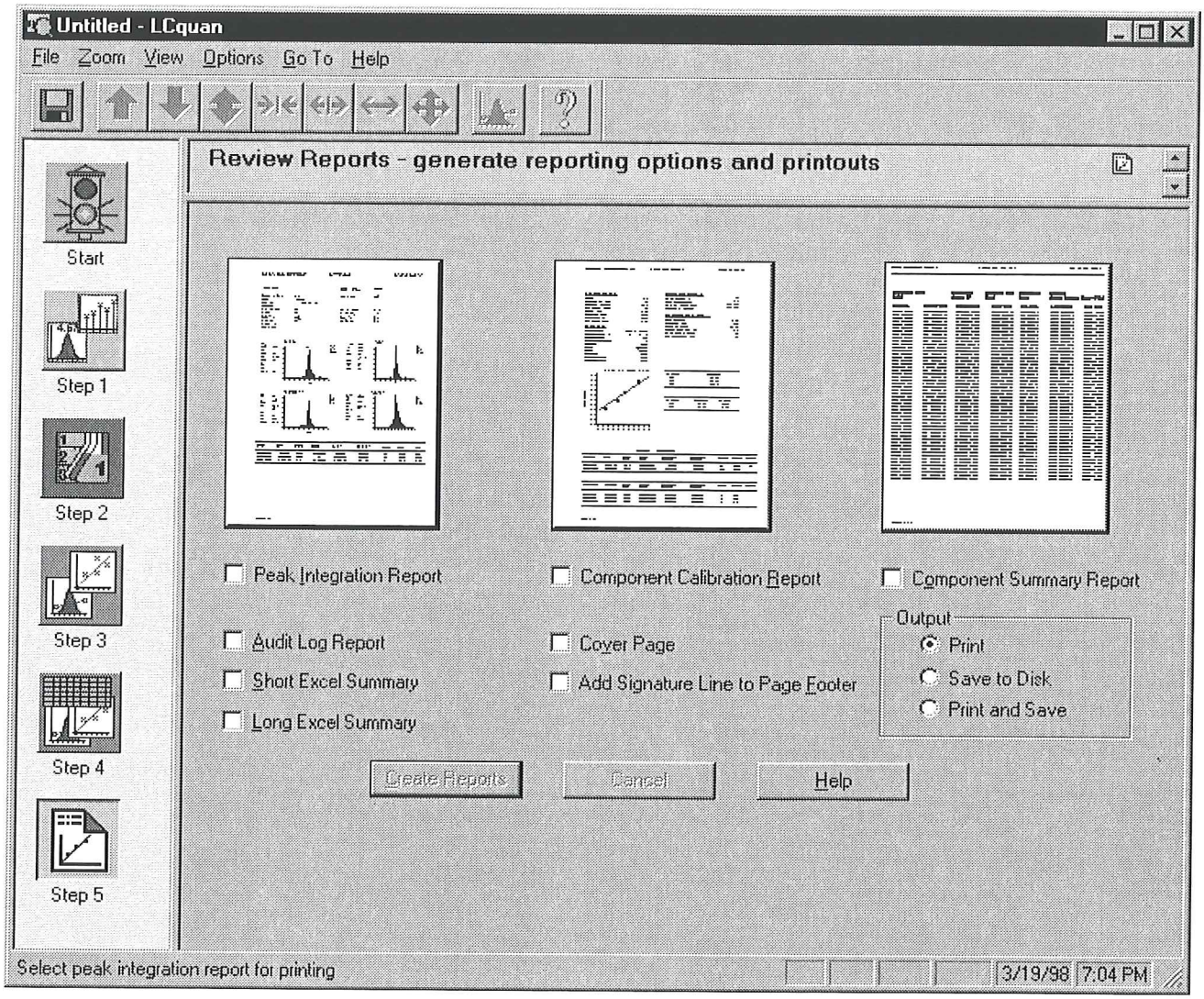


Figure 8-22. Review Reports view, showing the options for selecting report formats

2. Select (☑) one or more report options, as follows:

   - When you select Peak Integration Report, LCQUAN prints a report of the peak integration results. Each page of the peak integration report includes Sample List, Processing Method, and Result List information for all components in one sample. LCQUAN also prints plots of the chromatogram peaks for all components.

   - When you select Audit Log Report, LCQUAN prints a chronological listing of all changes made to method files and/or result files. Each log entry includes the logon name of the user who completed the log entry, the corresponding

user name, a description of what changed, the time when the log entry was completed, and optional comments made during the log entry.

- When you select Short Excel Summary, LCQUAN opens Microsoft® Excel® and displays the Result List in a spreadsheet (each component is located on a separate page/worksheet in the main workbook). The data in the spreadsheet can be reviewed, analyzed, and graphed using all of the functions and features of the Microsoft Excel application.

- When you select Long Excel Summary, LCQUAN opens Microsoft Excel and displays Result List, Sample List, and component identification and calibration information in a spreadsheet (each component is located on a separate page/worksheet in the main workbook). The data in the spreadsheet can be reviewed, analyzed, and graphed using all of the functions and features of the Microsoft Excel application.

- When you select Component Calibration Report, LCQUAN prints component identification and calibration parameters, calibration curves, calibration and QC levels and amounts, and Result List information (peak areas, ratios, calculated amounts, etc.) for all standards and QCs.

- When you select Cover Page, LCQUAN prints a cover page that lists the quantitation method (LCQUAN Method), dates of acquisition and processing, and the names of the data files.

- When you select Add Signature Line to Page Footer, LCQUAN prints signature and date lines at the bottom of the cover page.

- When you select Component Summary Report, LCQUAN prints the Result List (peak areas, ratios, calculated amounts, etc.) for all components.

3. Select the output option in the Output group box, as follows:

   - Click on the Print option button if you want LCQUAN to print the report on your printer.

   - Click on the Save to Disk option button if you want LCQUAN to save the report in a file on your hard drive.

- Click on the Print and Save option button if you want LCQUAN to print the report on your printer and save it in a file on your hard drive.

4. Create the reports, as follows:
   Click on the Create Reports button. LCQUAN prints reports similar to those shown in Appendix A.

Go on to the next topic: **Saving the LCQUAN Method and Exiting LCQUAN**.

8.8 Saving the LCQUAN Method and Exiting LCQUAN

You can save the LCQUAN parameters in an LCQUAN Method file during any step of the LCQUAN procedure. This allows you to exit LCQUAN in any step and reenter LCQUAN at the same place at a later time.

Save the LCQUAN Method as *practice.lqn,* and exit LCQUAN, as follows. (Before you can save the method, you need to supply some information in the File Summary Information dialog box.)

1. Open the File Summary Information dialog box, as follows:
   Choose **File | Save As**.

2. Type a description of the LCQUAN Method in the Description text box, as follows:
   For example, state the analyte and type of report format.

3. Close the dialog box, and open the Save As dialog box, as follows:
   Click on **OK**. See Figure 8-23.

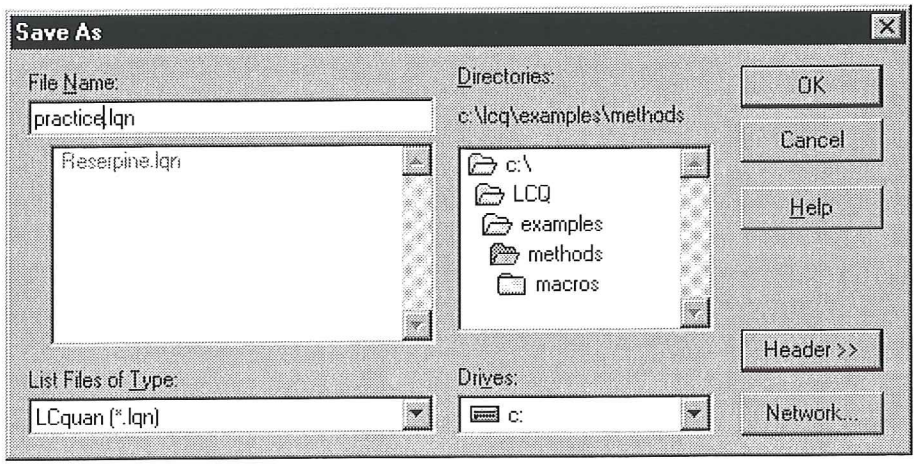


Figure 8-23. Save As dialog box, showing the directory path and file name where the LCQUAN file (*.lqn*) stores the quantitation parameters for reserpine

4. Select the directory path *C:\LCQ\examples\methods*.

5. Name the LCQUAN Method, as follows:
   Click on the File Name text box, and type **practice.lqn**.

6. Save the method, close the dialog box, and return to the LCQUAN window, as follows:
   Click on **OK**.

7. Exit LCQUAN, as follows:
   Choose **File | Exit**.

Contents

APPENDIX A: LCQUAN REPORTS

You create and review reports of your results in step 5 of LCQUAN. This Appendix contains sample reports that were created in the topic: **Step 5: Creating and Reviewing Reports** for information on creating reports with LCQUAN.

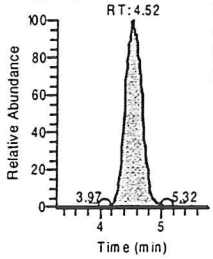
The following sample reports are included in this Appendix:

- Peak Integration report (reserpine_06 only)
- Component Calibration report
- Component Summary report

Sample Name:		Sample Type:	Standard
Sample ID:	06		
Acquisition Date:	12/10/97 05:27:27	Cal. Level:	4
Operator:			
Data Path:	C:\LCQ\examples\data		
Comments:			
Run Time (min):	6.99	Software Revision:	1.2
Vial:	006	Inj Vol (uL):	10.00
Samp Vol:	0.00	Samp Wt:	0.00
ISTD Amt:	0.000	Dil. Factor:	1.00
Exp Method:	C:\LCQ\methods\prt		
Proc Method:			

IT:	Method
ERT:	4.50
SW:	30.0
VW:	2.50
RTR:	no
ID:	Highest
MPH:	3.0
SM:	11
VD:	yes
EPW:	20.0
CP:	no

Component: reserpine
RT: 3.27 - 5.77 SM : 11G
RT: 4.52



NL: 7.67E5
m/z=
396.7-397.7+447.7-448.7
F: + c Full ms2 609.20 [
170.00 - 650.00]

Component Results

Name	Area	Area Ratio	Area ISTD	Specified Amount	Calculated Amount	%Diff	S/N	BL
reserpine	14854654	14854654	0	1000.000 pg/injection	1069.813 pg/injection	6.98	1021	BB

Identification

Mass Range:	397.2, 448.2
Filter:	+ c Full ms2 609.20 [170.00 - 650.00]
Expected RT (min):	4.50
Search window (sec):	30.00
View width (sec):	2.00
RT reference	no
Identify by:	Highest Peak
Min Peak Height(S/N):	3.00

Calibration

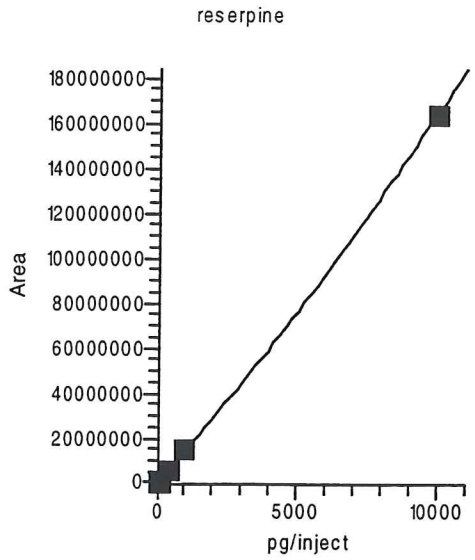
Component Type:	Target Component
Number of Cal. Levels:	5
Number of QC Levels:	0
Response:	Area
Curve:	Quadratic
Origin:	Ignore
Weighting:	1/X
Calibration Units:	pg/inject

Peak Integration

Smoothing points:	11
S/N Threshold:	0.50
Valley Detection:	Enabled
Expected Peak Width (sec):	20.00
Constrain Peak Width:	Disabled

Advanced Component Options

Rise Percentage:	10.0
Valley S/N:	2.0
Peak S/N Cutoff:	200.0
Baseline Noise Tol.(%):	10.0
Min Num Scans in Basline:	16
Num Background Scans:	2



Equation: Y = -412878+14010.6*X+0.243637*X^2 R^2 = 0.9989

Cal. Level	Amount
1	50.000 pg/inject
2	100.000 pg/inject
3	500.000 pg/inject
4	1000.000 pg/inject
5	10000.000 pg/inject

Standard Results

Sample	Area	Area Ratio	Area ISTD	Specified Amount	Calculated Amount	%Diff	%RSD	Excluded
reserpine_03	404383	404383	0	50.000 pg/inject	58.273 pg/inject	16.55	0.0	
reserpine_04	843168	843168	0	100.000 pg/inject	89.511 pg/inject	-10.49	0.0	
reserpine_05	5725363	5725363	0	500.000 pg/inject	434.827 pg/inject	-13.03	0.0	
reserpine_06	14854654	14854654	0	1000.000 pg/inject	1069.813 pg/inject	6.98	0.0	
reserpine_07	164002569	164002569	0	10000.000 pg/inject	9997.138 pg/inject	-0.03	0.0	

There are no QC results to report.

Component Name	Curve Index	Weighting Index	Origin Index	Equation
reserpine	Quadratic	1/X	Ignore	Y = -404452+13945.7*X+0.247927*X^2 R^2 = 0.998

Filename	Sample Type	Sample ID	Integ. Type	Area	ISTD Area	Area	Specified Amount	Calculated Amount	%Diff	%RSD	RT	Level	Units
reserpine_03	Std Bracket Sample	3	Method Settings	404148		404148	50.000	57.922	16%	0.0%	4.51	1	pg/inject
reserpine_04	Std Bracket Sample	4	Method Settings	854154		854154	100.000	90.106	-10%	0.0%	4.55	2	pg/inject
reserpine_05	Std Bracket Sample	5	Method Settings	5725992		5725992	500.000	436.211	-13%	0.0%	4.63	3	pg/inject
reserpine_06	Std Bracket Sample	6	Method Settings	14773704		14773704	1000.000	1068.094	7%	0.0%	4.51	4	pg/inject
reserpine_07	Std Bracket Sample	7	Method Settings	163792791		163792791	10000.000	9997.228	0%	0.0%	4.53	5	pg/inject

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