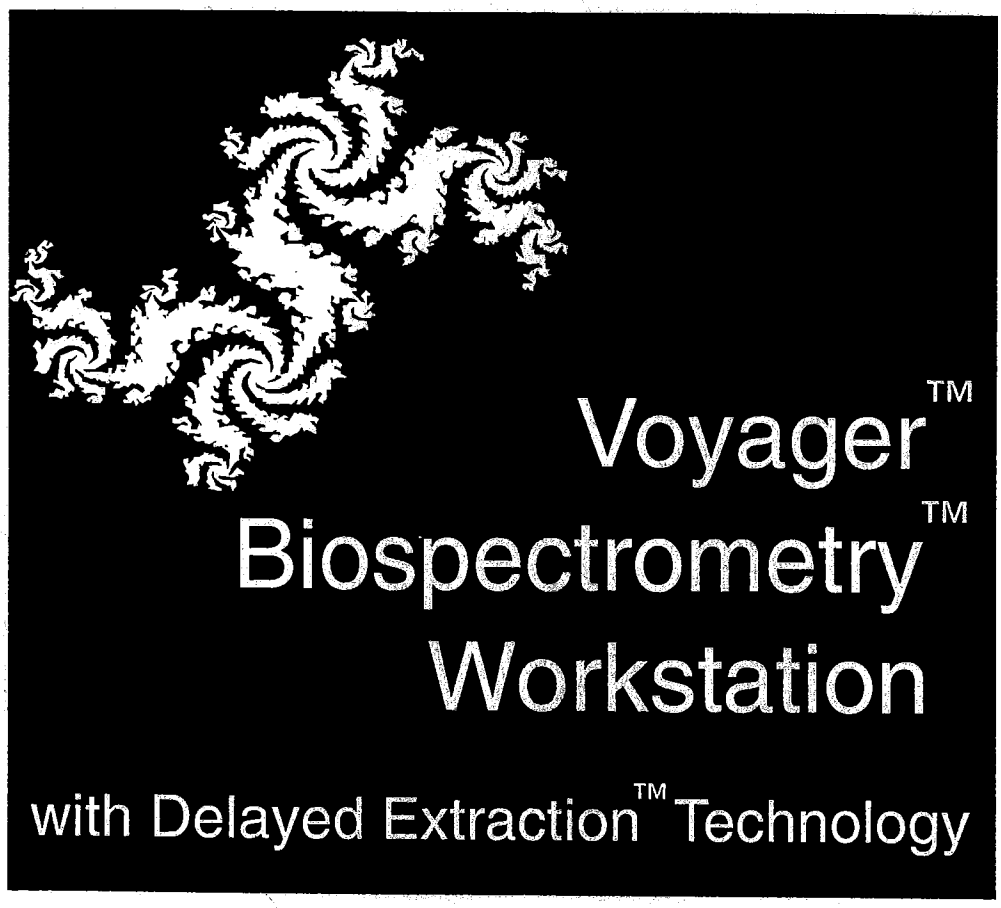

Getting Started Guide

Version 5 Series Software

PerSeptive Biosystems, Inc.
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Framingham, MA 01701 USA

A subsidiary of PE Corporation

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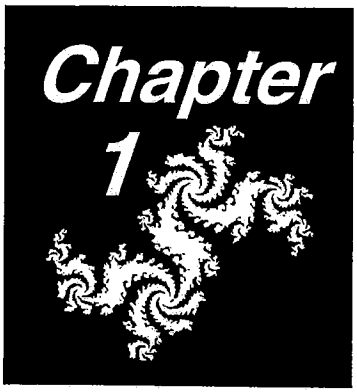


1 Before You Begin

This chapter contains the following sections:

1

1.1 Introduction to the System

Basics of the system

A PerSeptive Biosystems Technical Representative will give you a quick introduction to the following:

- General computer and software tasks
- Sample prep tasks
- Acquisition tasks
- Processing tasks

General computer and software tasks

- Start the computer, start the Voyager software, restart the computer by pressing Control+Alt+Delete, select Shut down, then select Shutdown and Restart.
- Use Windows NT basic techniques.
- Use the mouse, click, click-drag, double-click. Use the right mouse button.
- Use Window NT Explorer to create directories, copy files, delete files, display file information.
- Use the Open File dialog box, double-click to display lower level directories.
- Use Notepad to view text files.
- Resize windows.
- Change between tasks (press Alt+Tab or use the Windows Task Manager).
- Change between programs.
- Open and close programs.

Sample prep tasks

- Prepare peptide samples in α-cyano matrix.
- Prepare peptide samples in sinapinic acid matrix.
- Load a sample plate.
- Clean a sample plate.

Acquisition tasks

- Use the Instrument Control Panel.
- Use the Sequence Control Panel.
- Load, modify, and save instrument settings (.BIC) files.
- Use the software or control stick to change the x,y position of the laser on the sample.
- Acquire and save data.
- Use the Spectrum window, describe key features.
- Use the oscilloscope, describe key features.
- Use the LSA1000 LeCroy® digitizer, describe key features.

Processing tasks

- Manipulate a trace using all toolbar buttons.
- Calibrate a spectrum and apply the calibration to a .BIC file.

1

1.2 Running Your First Experiment

Purpose

The *Voyager™ Biospectrometry™ Workstation Getting Started Guide* is designed to help you quickly learn how to use the Voyager Biospectrometry Workstation. It provides step-by-step procedures for preparing and analyzing:

- A peptide standard containing Adrenocorticotropic hormone, clip 7–38 [ACTH (7–38)], Adrenocorticotropic hormone, clip 18–39 [ACTH (18–39)], and insulin
- A mock peptide sample containing the standard component ACTH (7–38)

Assumptions

***NOTE**: This guide assumes that your Voyager Workstation has been properly installed by a PerSeptive Biosystems Technical Representative and that the workstation vacuum pressure is less than 10^{-6} Torr on Voyager-DE and Voyager-DE PRO systems, or less than $5x10^{-7}$ on the Voyager-DE STR system.*

In this guide

This guide contains brief procedures. For more detailed procedures and reference information, refer to the *Voyager™ Biospectrometry™ Workstation User's Guide*.

In the following chapters, you will:

- Start up
- Prepare samples
- Acquire calibration standard spectra
- Create a calibration (.CAL) file
- Acquire sample spectra

What you need

To perform the experiment in this guide, you need:

- Sample plate with laser-etched sample positions or wells
- α-cyano-4-hydroxycinnamic acid (CHCA) matrix
- Adrenocorticotropic hormone, clip 7–38 [ACTH (7–38)], 50 pmol/µl in aqueous solution
- Adrenocorticotropic hormone, clip 18–39 [ACTH (18–39)], 50 pmol/µl in aqueous solution
- Bovine insulin, 50 pmol/µl in aqueous solution

NOTE: *You can use the Calibration Mix 2 standard from the Voyager Mass Standards Kit which contains all of the components listed above.*

See the *Voyager Biospectrometry User's Guide*, Appendix B, Warranty/Service Information, for standard ordering information.

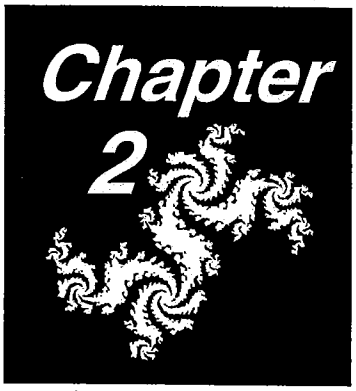
NOTE: *If you do not have a sample plate with laser-etched sample positions or wells, you can use a polished blank sample plate. If you use a polished blank sample plate, load the sample directly under the position number on the plate to ensure that the laser is centered on the sample when you move to a sample position.*

To prepare matrix and sample, you need:

- Analytical balance
- 1.5 ml microcentrifuge tubes
- Microcentrifuge (optional)
- Micropipettor and disposable tips
- Vortex mixer
- Deionized water
- Acetonitrile
- 3% Trifluoroacetic acid (TFA) in deionized water
- Finely-tapered pipette tips for dispensing matrix and sample solutions on sample plates

WARNING

CHEMICAL HAZARD. *Some of the solvents are hazardous. Refer to the Material Safety Data Sheet (MSDS) provided by the chemical manufacturer before handling any of the substances listed above.*



2 Starting Up

This chapter contains the following sections:

For more information, see the *Voyager Biospectrometry Workstation User's Guide*, Section 2.8, Startup and Shutdown.

2.1 Powering Up

The Voyager mass spectrometer is typically powered up at all times.

To power up the other components of the system:

1. Turn on the following system devices in this order:
   - Video monitor
   - External digitizer, if your system includes one
   - Oscilloscope, if your system includes one
   - Computer
   - Printer
2. If your system includes an oscilloscope, observe the oscilloscope and wait approximately one minute until it completes its initialization cycle. When the cycle is complete, a message stating that the power on self check passed is displayed, then the oscilloscope screen is displayed. See Appendix I, Using the Oscilloscope and Control Stick, for more information.

   If your system includes a LeCroy digitizer, wait approximately one minute until the digitizer completes its internal calibration before starting the Instrument Control Panel.
3. Press the **EMIS** button on the Vacuum Gauge Panel to turn on the gauges. Observe the vacuum gauge on the front panel (Figure 2-1).

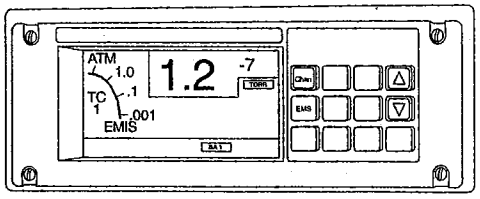


Figure 2-1 Vacuum Gauge Panel

CAUTION

Do not press any other buttons on the panel. Pressing buttons other than the Chan and EMIS buttons can recalibrate the pressure scale of the system.

Press **Chan** to cycle between BA1 and BA2.

Allow the pressure on BA1 and BA2 to reach:

Model	BA1 Pressure (Torr)	BA2 Pressure (Torr)
Voyager-DE and Voyager-DE PRO	Less than 10^{-6}	Less than 10^{-6}
Voyager-DE STR	Less than $5x10^{-7}$	Less than $5 x10^{-8}$

Readings from the vacuum gauges are displayed:

- On the Vacuum Gauge Panel.
- In the System Status control page in the Instrument Control Panel. See the *Voyager Biospectrometry Workstation User's Guide*, Section 2.9, Checking System Status, for more information.

4. Log on to the Voyager Workstation using your User Name and Password. See your system administrator for your User Name and Password.

2.2 Starting the Software

Starting Instrument Control Panel

To start the Voyager Instrument Control Panel from the Windows NT desktop, double-click the **Voyager Control** icon on the desktop.

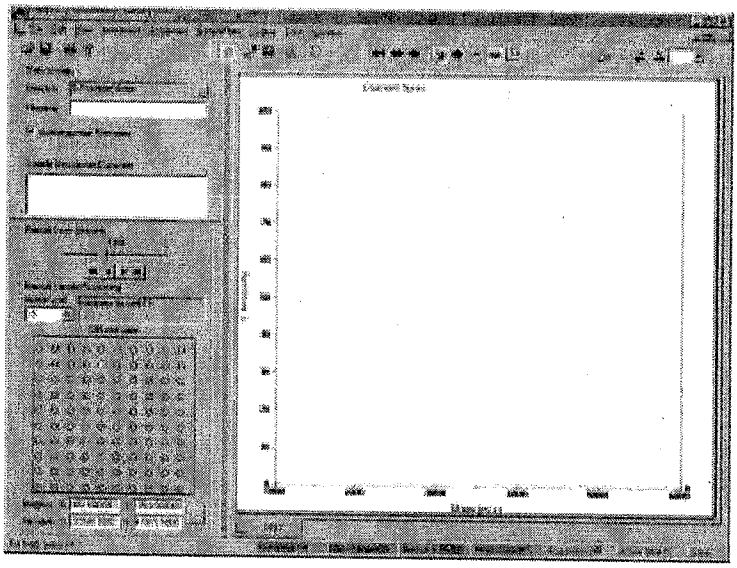


Figure 2-2 Instrument Control Panel

Initializing

The hardware is automatically initialized when you start the Instrument Control Panel.

Starting the Data Explorer software

To start the Data Explorer software from the Windows NT desktop, double-click the **Data Explorer** icon on the desktop.

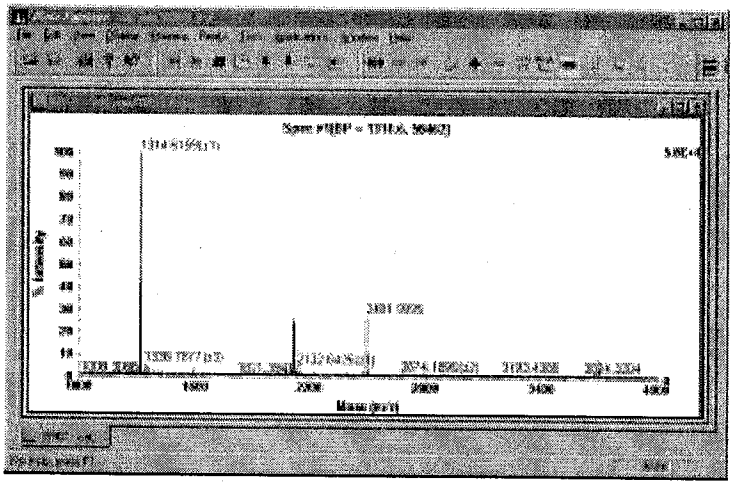
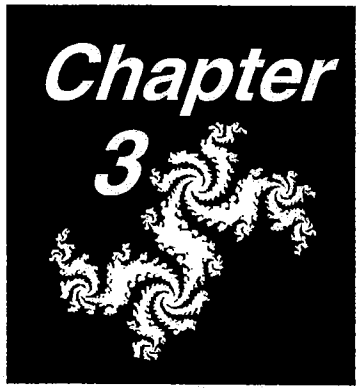


Figure 2-3 Data Explorer Window

2



3 Preparing Samples

This chapter contains the following sections:

For a detailed description of preparing samples and matrix, see the *Voyager Biospectrometry Workstation User's Guide*, Chapter 3, Preparing Samples.

3.1 Overview

Guidelines for successful sample preparation

To prepare samples, follow these guidelines:

- Prepare fresh matrix as needed. Some matrices require daily preparation. Other matrices can be stored for up to one week at room temperature.
- Determine the proper dilution of sample.
- Use clean sample plates.
- Properly apply sample and dry to allow good crystallization.

Matrix stability

Some matrices degrade upon exposure to light or humidity. Some matrices require daily preparation. Other matrices can be stored for up to one week at room temperature. Follow the matrix manufacturer's instructions for storage conditions of chemicals.

Additional information

Appendix C, Matrices, in the *Voyager Biospectrometry Workstation User's Guide*, contains additional information on matrix structure, appearance, and matrix solutions.

3.2 Preparing Matrix

Matrix function

In MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry), the matrix plays a key role in the ionization process. The well-developed crystals of matrix material assist in ionizing the biomolecules you are analyzing.

Selecting matrix

As a general guideline, the molecular weight of the peptide/protein you are analyzing determines the matrix to use:

- **> 10,000 Da**—Sinapinic acid (3,5-Dimethoxy-4-hydroxy cinnamic acid)
- **< 10,000 Da**—α-cyano-4-hydroxycinnamic acid

The compounds you are analyzing have masses less than 10,000 Da, so α-cyano-4-hydroxycinnamic acid is the appropriate matrix for this application.

Preparing

To prepare 10 mg/ml solution of α-cyano-4-hydroxycinnamic acid matrix:

1. Label a 1.5 ml microcentrifuge tube with the name of the matrix, the final concentration, and the date prepared.
2. Weigh approximately 10 mg of the dry matrix and add it to the tube.

NOTE: *Use a fresh tip each time you pipette a different substance.*

---

3. Add 400 µl deionized water, 100 µl 3% TFA, and 500 µl acetonitrile to the tube.
4. Cap the tube and vortex thoroughly for approximately one minute. You can shake the tube by hand if you do not have a vortex mixer.
5. Microcentrifuge the tube for 30 seconds at 2,000 to 5,000 rpm. Alternatively, allow the solution to settle for about 10 minutes. You may see a precipitate at the bottom of the tube.

   When you load matrix, use the supernatant, not the precipitate.

3.3 Preparing Samples

Preparing

Prepare a 50 pmol/µl solution of each of the following components:

- ACTH (7–38)
- ACTH (18–39)
- Bovine Insulin

From these solutions you will create:

- **Standard solution**—Containing all three components
- **Mock sample solution**—Containing ACTH (18–39)

NOTE: *Use preweighed vials of ACTH to ensure accurate final concentrations. See the Voyager Biospectrometry User's Guide, Appendix B, Warranty/Service Information, for standard ordering information.*

To prepare 50 pmol/µl solutions of each component:

1. Add 1.3 ml deionized water to a 250 µg vial of ACTH (7–38).
2. Add 4 ml deionized water to a 500 µg vial of ACTH (18–39).
3. Weigh out 1 mg Bovine Insulin. Add 3.5 ml 0.1% aqueous trifluoroacetic acid (TFA).

NOTE: *Alternatively, you can prepare the Calibration Mix 2 sample from the Voyager Mass Standards Kit. Follow the instructions listed in the kit to prepare a 50 pmol/µl solution of Calibration Mix 2.*

Storing

If you will not use the insulin within four hours, freeze it. If you will not use the other samples within one day, freeze them.

You can aliquot and freeze unused stock solution for future use.

3.4 Mixing Sample and Matrix

Preparing standard and sample solutions

Mix standard or sample and matrix before loading on the sample plate.

NOTE: *You can alternatively mix sample and matrix on the sample plate. However, when working with a concentrated sample, or when preparing many samples, premixing before loading on the sample plate is the preferred method.*

To mix sample and matrix:

1. Label two 1.5 ml microcentrifuge tubes with "Standard" and "Sample" and the date prepared.

Standard solution

2. To the "Standard" tube, add 22 µl of matrix solution. Add matrix before sample to prevent sample from adhering to the plastic tube.
3. To the "Standard" tube, add the following (initial concentration 50 pmol/µl for each component, for a final concentration of 2 pmol/µl of each component):
   - 1 µl ACTH (7–38)
   - 1 µl ACTH (18–39)
   - 1 µl Bovine Insulin

NOTE: *Alternatively, you can add 1 µl of the prepared 50 pmol/µl CalMix 2 standard solution.*

4. Vortex for 15 seconds.

Sample solution

5. To the "Sample" tube, add 24 µl of matrix solution.
6. To the "Sample" tube, add 1 µl ACTH (7–38) (initial concentration 50 pmol/µl, for a final concentration of 2 pmol/µl).
7. Vortex for 15 seconds.

3.5 Loading Samples on Sample Plates

Handling sample plates

To prevent contamination of your analysis:

- Start with a clean sample plate. See the *Voyager Biospectrometry Workstation User's Guide*, Section 3.3, Cleaning Sample Plates.
- Handle the sample plate by the edges.

Guidelines for good crystallization

To ensure good crystallization:

- Fill the entire well when spotting the sample plate, if possible. Surface tension and sample availability may determine whether you fill the well completely.
- When using α-cyano-4-hydroxycinnamic acid, do not touch the surface of the sample well with the pipette tip. It may cause uneven crystallization.

Guidelines

Load one sample position (or well) with the standard, and one with the sample.

NOTE: *For optimum mass accuracy, place standards in positions (or wells) adjacent to the samples for which you are calibrating, and do not use the outer sample positions (wells) on the sample plate.*

Create a log sheet listing sample position and sample name. Appendix D, Log Sheets, in the *Voyager Biospectrometry Workstation User's Guide,* contains master log sheets that you can copy and use.

Loading samples

To load samples:

1. Load 1 µl of standard/matrix and 1 µl of sample/matrix in adjacent sample positions on the plate.

***NOTE**: Use a fresh pipette tip each time you pick up a different substance.*

---

2. Allow the sample plate to dry for at least five minutes.
3. Visually examine the sample spots to make sure they are dry.
4. Place a protective cover over the plate to prevent contamination until you are ready to load the plate. Do not allow the cover to touch the surface of the plate.

3.6 Loading Sample Plates in the Mass Spectrometer

NOTE: *If you load the sample plate into the Voyager Biospectrometry Workstation before the plate is dry, the pressure in the sample chamber rises, and a "TC2 pressure too high" error code may be displayed in the Instrument Control Panel. Wait a few minutes for the chamber to reach pressure.*

Ejecting the sample holder

In the Voyager Instrument Control Panel (see Figure 2-2 on page 2-4), select **Eject** from the Sample Plate menu. The Load/Eject Sample Plate dialog box (Figure 3-1) is displayed.

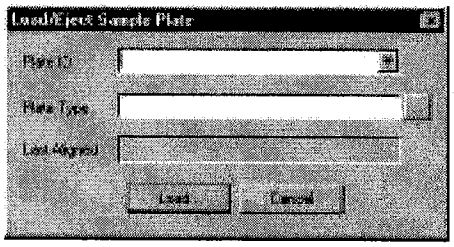


Figure 3-1 Load/Eject Sample Plate Dialog Box

After you select **Eject**, the following occurs:

- A Load Status dialog box is displayed during the ejection sequence which displays hardware status.
- High voltage is turned off.
- The video monitor displays the sample plate moving.
- The sample holder moves out of the main source chamber, out of the sample loading chamber, and is ejected from the instrument.

***NOTE**: Do not leave the sample holder in the Eject position for more than a few seconds. Minimize the time the instrument is exposed to the atmosphere to reduce the time needed to reach high vacuum when you insert a new plate.*

Voyager-DE and Voyager-DE PRO

To load sample plates:

1. Eject the sample holder as described in "Ejecting the sample holder" on page 3-9.
2. Hold the sample plate with the bottom of the numbers facing toward the analyzer (for standard 100-well plate) and with the slanted underside of the plate facing to the left (Figure 3-2).
3. Slide the sample plate into the holder from the right side until it snaps into place (Figure 3-2). The ball bearings on the holder snap into the plate.

CAUTION

If the sample plate does not snap into place, it may be inserted into the holder the wrong way, and it may jam inside the instrument. Remove the plate, slide it into the holder with the slanted underside of the plate facing to the left and toward the back of the instrument, and snap it into place.

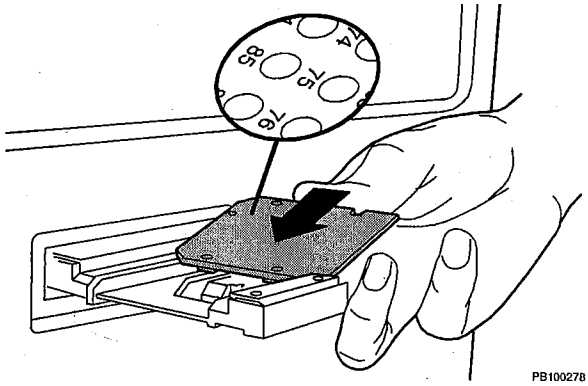


Figure 3-2 Loading the Sample Plate in a Voyager-DE or Voyager-DE PRO

WARNING

PHYSICAL INJURY HAZARD. *Fingers can get caught in the sample holder. To avoid injury, do not click* ***Load*** *to retract the sample holder when your fingers are near the sample holder.*

4. From the Sample Plate menu select **Load** to retract the sample plate and insert it into the main source chamber. The Load/Eject Sample Plate dialog box (Figure 3-3) is displayed.

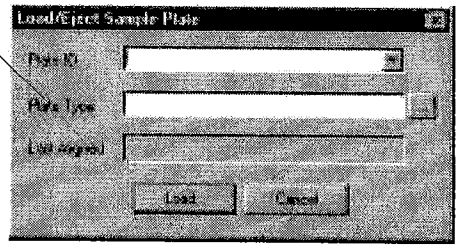


Figure 3-3 Load/Eject Sample Plate Dialog Box

NOTE: *A Select Sample Plate Type dialog box is also available from the Sample Plate menu in the Instrument Control Panel. This dialog box allows you to select a different plate type without ejecting the sample plate.*

Sample plate parameters

5. In the Plate Type field, select **100 well plate.PLT**. For more information on .PLT files, see the *Voyager Biospectrometry Workstation User's Guide*:
   - Section 3.5.2, Editable-Configuration Plate (.PLT) Types Provided with the System
   - Section 3.5.3, Guidelines for Defining Custom Plate Types
6. Click **OK**.

   The sample plate is aligned as needed. For more information, see the *Voyager Biospectrometry Workstation User's Guide*, "How the system aligns a plate" on page 2-40.

   It takes a minute or two for the sample plate to reach the correct position. While the sample plate is moving, the Load/Eject Status dialog box displays messages about the status of the hardware.

Voyager-DE STR

To load sample plates:

1. Eject the sample holder as described in "Ejecting the sample holder" on page 3-9.
2. Hold the sample plate vertically, with the sample surface facing to the right, and with the slanted underside of the plate facing toward the back of the instrument.
3. Slide the sample plate into the holder from the front until it snaps into place (Figure 3-4). The ball bearings on the holder snap into the plate.

CAUTION

If the sample plate does not snap into place, it may be inserted into the holder the wrong way, and it may jam inside the instrument. Remove the plate, slide it into the holder with the slanted underside of the plate facing to the left and toward the back of the instrument, and snap it into place.

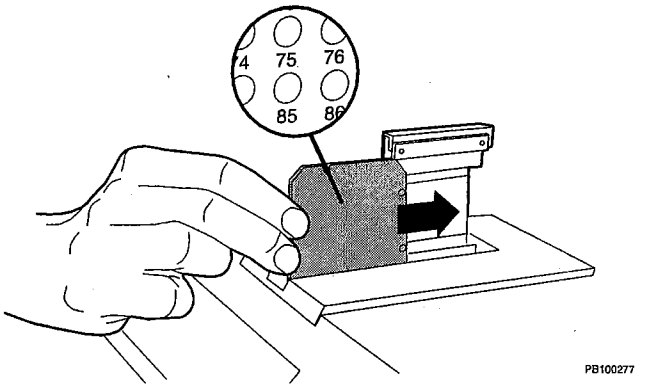


Figure 3-4 Loading the Sample Plate in a Voyager-DE STR

WARNING

PHYSICAL INJURY HAZARD. *Fingers can get caught in the sample holder. To avoid injury, do not click* ***Load*** *to retract the sample holder when your fingers are near the sample holder.*

4. From the Sample Plate menu, select **Load** to retract the sample plate and insert it into the main source chamber. The Load/Eject Sample Plate dialog box (Figure 3-5) is displayed.

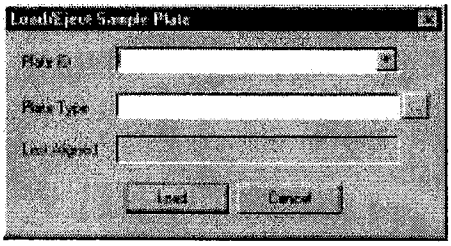


Figure 3-5 Load/Eject Sample Plate Dialog Box

NOTE: *A Select Sample Plate Type dialog box is also available from the Sample Plate menu in the Instrument Control Panel. This dialog box allows you to select a different plate types without ejecting the sample plate.*

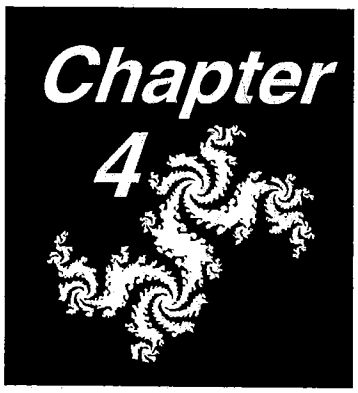
Sample plate parameters

5. In the Plate Type field, select **100 well plate.PLT**. For more information on .PLT files, see the *Voyager Biospectrometry Workstation User's Guide*:
   - Section 3.5.2, Editable-Configuration Plate (.PLT) Types Provided with the System
   - Section 3.5.3, Guidelines for Defining Custom Plate Types
6. Click **OK**.

   The sample plate is aligned as needed. For more information, see *the Voyager Biospectrometry Workstation User's Guide*, "How the system aligns a plate" on page 2-40.

   It takes a minute or two for the sample plate to reach the correct position. While the sample plate is moving, the Load/Eject Status dialog box displays messages about the status of the hardware.

4 Acquiring Calibration Standard Spectra



This chapter contains the following sections:

For a detailed description of acquiring spectra, see the *Voyager Biospectrometry Workstation User's Guide*, Chapter 6, Acquiring Mass Spectra.

4.1 Setting the Instrument Control Panel

To set the Instrument Control Panel for acquisition, you:

- Start the Instrument Control Panel
- Load an instrument settings (.BIC) file
- Set Data Storage parameters
- Select the sample position

4.1.1 Starting the Instrument Control Panel

When you double-click the Instrument Control Panel icon, the Instrument Control Panel opens (Figure 4-1).

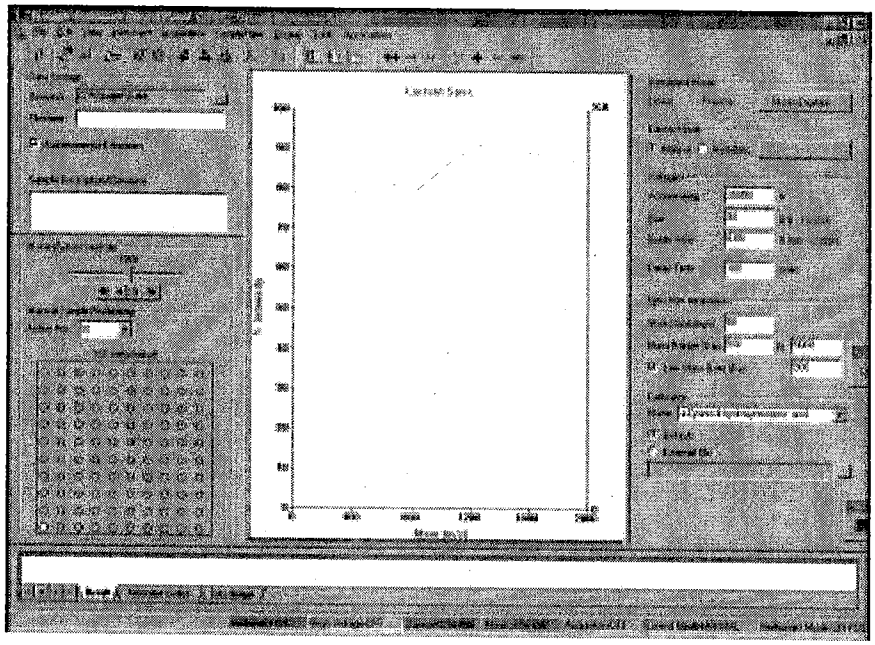


Figure 4-1 Instrument Control Panel

4.1.2 Loading an Instrument Settings (.BIC) File

Standard instrument settings (.BIC) files that have been optimized for your system are provided. You can use these files as a starting point to acquire data and optimize them as needed.

Loading an instrument settings (.BIC) file:

From the Instrument Control Panel:

1. Select **Open Instrument Settings** from the File menu. The Open dialog box is displayed (Figure 4-2).

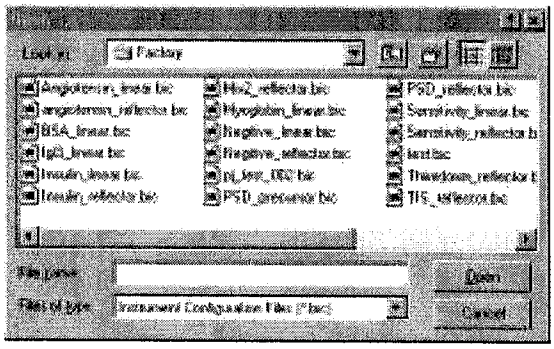


Figure 4-2 Open Dialog Box

Standard instrument setting (.BIC) files provided are located in the C:\VOYAGER\DATA\FACTORY directory.

2. Select the following instrument settings (.BIC) file:
   - **ACTH_Linear.BIC**—If you are running in Linear mode
   - **ACTH_Reflector.BIC**—If you are running in Reflector mode

---

***Hint:** If the .BIC file you need is not visible, click the scroll bar to view more instrument setting files.*

The .BIC file is loaded. The currently loaded .BIC file name is displayed in the title bar of the Instrument Control Panel (Figure 4-3).

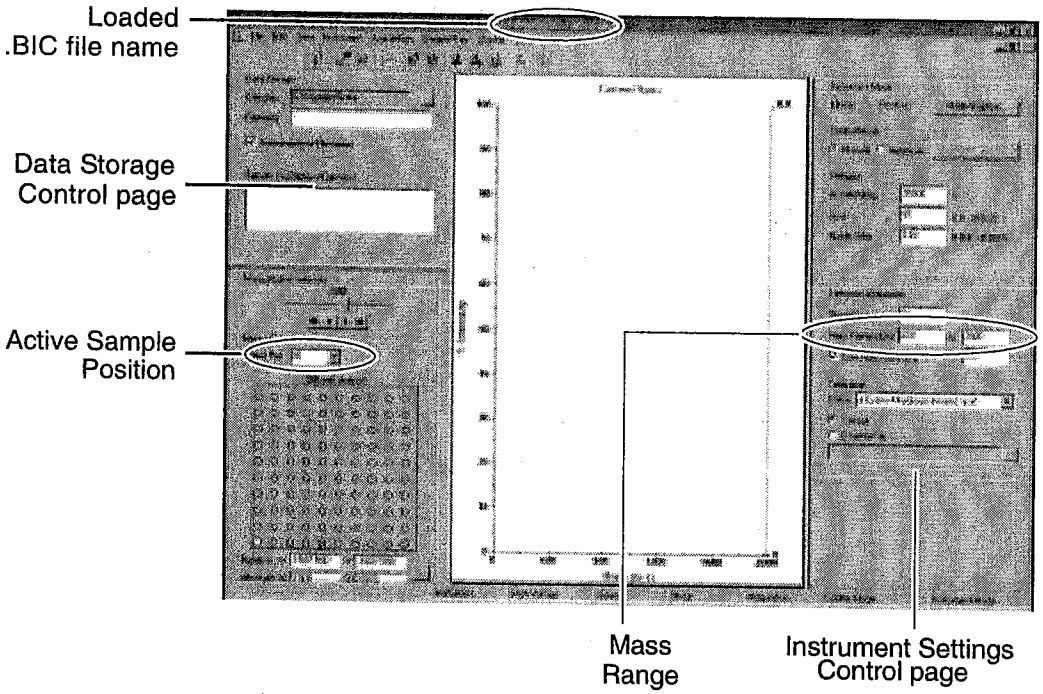


Figure 4-3 Instrument Control Panel with ACTH_Linear.BIC File Loaded

If the Instrument Settings control page is not displayed, select **Instrument Settings** from the View menu.

Changing instrument settings

Instrument settings in a standard .BIC file are optimized for the mass range shown on the Instrument Settings control page. It is not typically necessary to change settings.

If you find you need to optimize instrument setting parameters, see the *Voyager Biospectrometry Workstation User's Guide*, Section 5.4, Optimizing Instrument Settings Parameters.

Saving a new .BIC file If you change any of the optimized instrument settings parameters, do not overwrite the .BIC file loaded on your system. Select **Save As** from the File menu and type in a new file name.

4.1.3 Setting Data Storage Parameters

To set Data Storage parameters:

1. If the Data Storage control page is not displayed, select **Data Storage** from the View menu.

   The Data Storage control page is displayed (Figure 4-4).

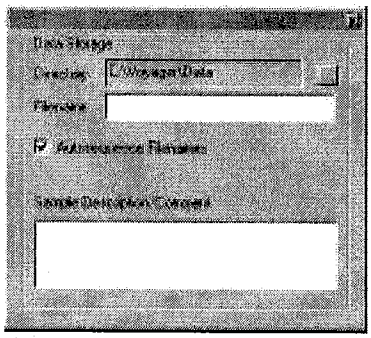


Figure 4-4 Data Storage Control Page

2. Click ... to select a directory in which all data files will be stored.

Hint: *To keep instrument settings and data organized, create a Getting Started directory. On the Windows desktop, double-click the* ***My Computer*** *icon. Double-click the C: drive, then double-click the Voyager directory. From the File menu, select* ***New Folder****, and type* ***Getting Started****.*

---

3. Type the file name **CalMix2**.
4. Select **Autosequence Filenames** if you want the software to determine the next available sequential file name.
5. In the Sample Description/Comment text box, enter text that will be saved with the data file. This step is optional.

   For more information on data storage, see the *Voyager Biospectrometry Workstation User's Guide*, "Setting Data Storage parameters" on page 6-14.

4.1.4 Selecting Sample Position

To select sample position, do the following:

1. If the Manual Laser/Sample Positioning control page is not displayed, select **Manual Laser/Sample Positioning** from the View menu.

   The Manual Laser/Sample Positioning control page is displayed (Figure 4-5) and reflects the .PLT file you selected when you loaded the sample plate.

2. Select the **standard** position to analyze by doing any of the following in the **Active Position** box:

   - Type a position number
   - Select a position number from the drop-down list
   - Single-click on a sample position

   You can hear the sample transport moving inside the mass spectrometer, and can see the sample plate moving on the video monitor. It takes a few seconds for the sample plate to move to the proper position.

NOTE: *You can also use the Control Stick to select the Active Position. See Figure 4-7 on page 4-10.*

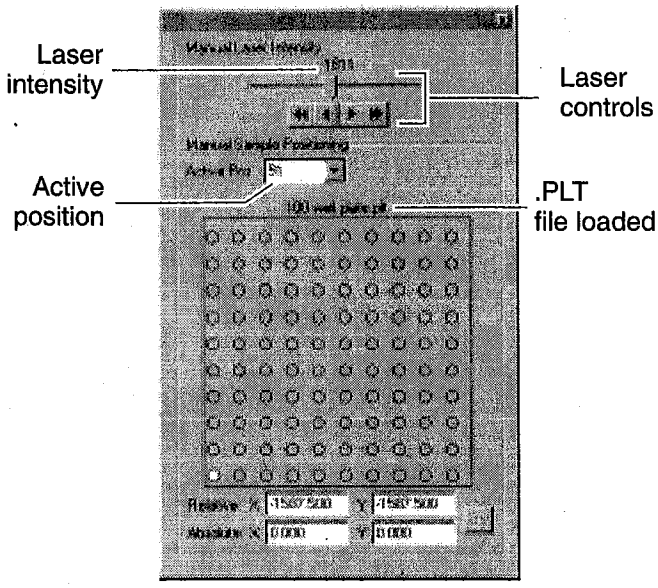


Figure 4-5 Manual Laser/Sample Positioning Control Page (Plate View) with a 100 Well Plate.PLT File Loaded

3. From the Sample Plate menu, select **Sample View** to display an expanded view of the selected sample position (Figure 4-6).

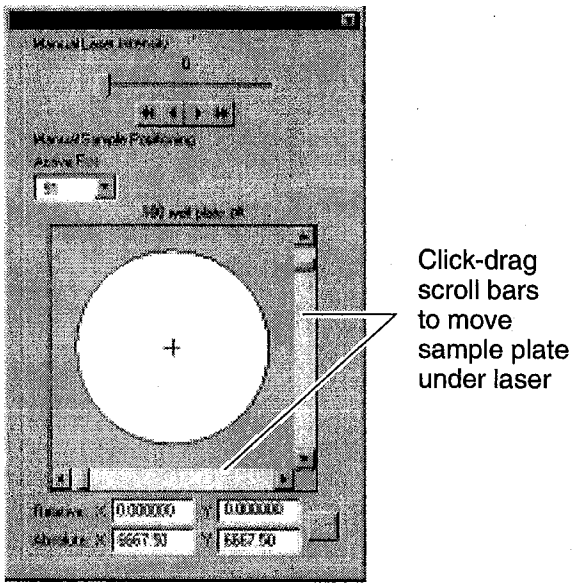


Figure 4-6 Manual Laser/Sample Positioning Control Page (Sample View)

4. You can fine-tune sample plate position under the laser by click-dragging the scroll bars or moving the control stick.

   For more information, see the *Voyager Biospectrometry Workstation User's Guide*, Section 4.5.2, Adjusting Laser Intensity and Selecting Sample Position.

4.2 Acquiring the Standard

In this section, you will:

- Adjust laser intensity
- Start acquisition
- Observe the signal
- Check for peaks of interest

4.2.1 Adjusting Laser Intensity

Determining the setting for your system

Optimized laser settings are stored along with the optimized instrument settings in the .BIC files provided with your system.

When you start the Instrument Control Panel, the laser intensity is set to 1,800.

When you load a .BIC file, the last laser setting saved in the .BIC file is loaded.

Adjusting laser intensity

To adjust laser intensity, click-drag the slider control or fine and coarse buttons on the Manual Laser/Sample Positioning control page (see Figure 4-5 on page 4-7). For more information, see *the Voyager Biospectrometry Workstation User's Guide*, Section 4.5.2, Adjusting Laser Intensity and Selecting Sample Position.

***Hint**: You can also set laser intensity by pressing **Ctrl+PgUp** and **Ctrl+PgDn** on the keyboard. Pressing these keys adjusts intensity in the same increments as the fine laser control buttons.*

Laser settings used in this guide

In this example, we will recommend certain laser intensities. However, the recommended laser intensities may not be appropriate for your system. Appropriate laser intensities vary from system to system. Use the appropriate setting for your system. For more information on laser settings, see the *Voyager Biospectrometry Workstation User's Guide*, Section 5.4.2, Determining the Laser Setting.

4.2.2 Starting Acquisition

High voltage warm-up for improved mass accuracy

For maximum mass accuracy, allow the high voltage power supplies to warm up for a short period of time before acquisition. Allowing the high voltage power supplies to warm up before acquisition reduces variability in accelerating voltages, and yields more reproducible ion flight times.

To turn on the high voltage power supplies, click  in the toolbar.

NOTE: *The high voltage power supplies are automatically turned off after 60 minutes if the mass spectrometer is not used. To change the Idle Time, see the Voyager Biospectrometry Workstation User's Guide, "High voltage configuration" on page 2-33.*

Starting acquisition

Start acquiring, which starts the laser, by doing one of the following:

- Click  in the toolbar.
- Select **Start Acquisition** from the Acquisition menu.
- Press the **left button** (the button farthest from the cable) on the base of the control stick (Figure 4-7).

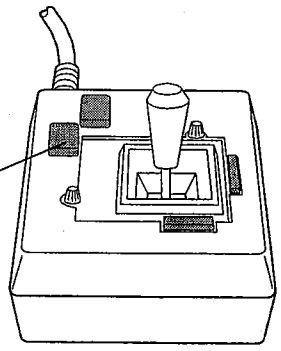


Figure 4-7 Control Stick

4.2.3 Observing the Signal

During acquisition, a Live trace is displayed and updated in the Spectrum window.

NOTE: *On systems with Tektronix oscilloscopes, no Live trace is displayed. A Current trace is displayed after acquisition is complete.*

When acquisition is complete, you should see a Current trace in the Spectrum window, similar to the figure shown in Figure 4-8, that includes the following peaks:

- **Angiotensin**—1296 Da
- **ACTH (18–39)**—2,466.71 Da
- **ACTH (7–38)**—3,660.17 Da
- **Insulin**—5734.54 Da

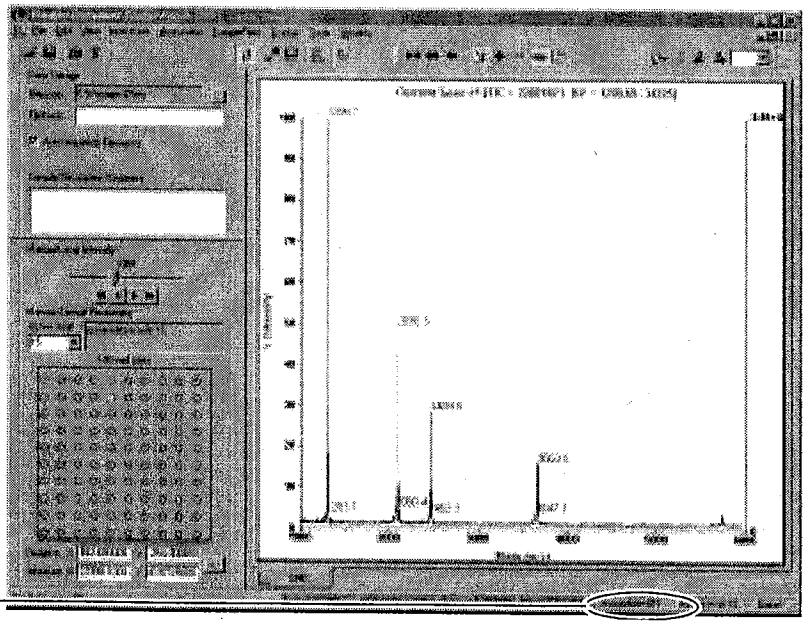


Figure 4-8 Current Trace in Spectrum Window

If you do not see signal

If you do not see any signal during acquisition, or if signal intensity is low, move the sample plate under the laser until you see a signal by doing one of the following:

- Click-drag scroll bars in the Sample View of Manual Laser/Sample Positioning control page (see Figure 4-6 on page 4-8)
- Move the control stick knob to the left and right, or up and down

When you find an acceptable signal, stop acquisition as described below, then restart acquisition. It is good practice to acquire actual data from one location in the same sample position.

Stopping acquisition

Acquisition continues until the number of shots specified in Shots/Spectrum in instrument settings are collected. Alternatively, you can stop acquisition when you observe an acceptable signal by:

- Clicking  in the toolbar.
- Selecting **Stop Acquisition** from the Acquisition menu.
- Pressing the **left button** (the button farthest from the cable) on the base of the control stick (see Figure 4-7 on page 4-10).

CAUTION

Check to see if acquisition has already stopped automatically before manually stopping. If acquisition has stopped, the Spectrum window stops updating, and the Instrument Control Panel status bar displays "Acquisition-OFF" (it displays "Acquisition-ON" during acquisition). See Figure 4-8 on page 4-11.

If acquisition has stopped and you restart acquisition with the software or control stick, you will begin a new acquisition and overwrite the Current spectrum.

The signal is acceptable when the peaks stabilize, and stop expanding upward.

4.2.4 Checking for Peaks of Interest

When acquisition is complete, check for peaks of interest:

- Zoom in and check peak shape
- Eliminating unwanted peaks
- Check masses
- Reacquire with lower laser intensity
- Save the data file

Zooming to check peak shape

To zoom in on the trace to examine peak shape:

1. Position the cursor above and to the left of the first peak of interest.
2. Hold down the **left mouse button** and click-drag a box around the area to zoom. Make sure to click-drag within the Spectrum window (Figure 4-9).

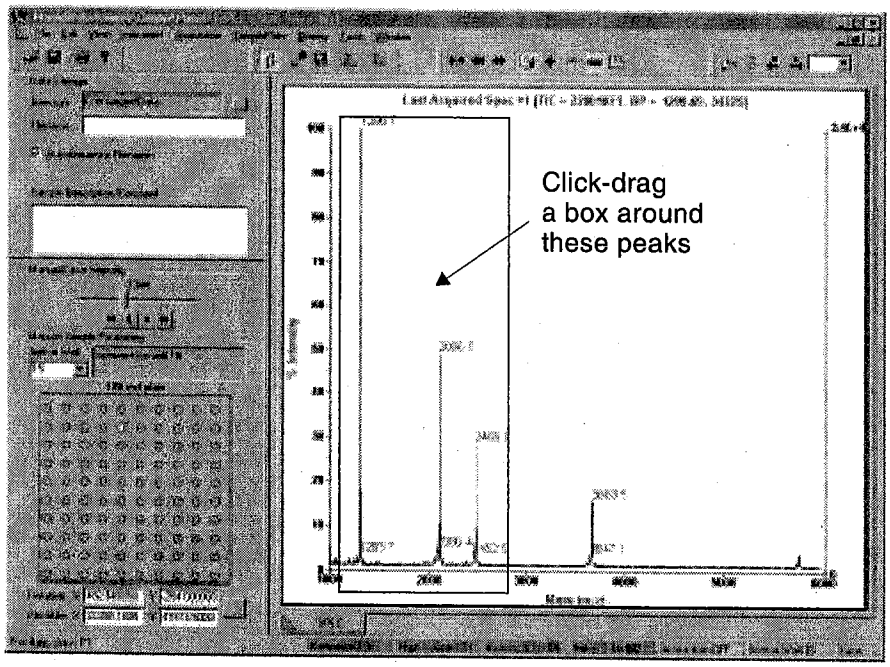


Figure 4-9 Zooming on Peaks

The selected area is magnified.

You can also use the following zoom toolbar buttons:

- Click  to Zoom in.
- Click  to Zoom out.
- Click  for Full Unzoom.

3. Examine the peaks and the right axis in the Current Spectrum trace to determine if signal is saturated (Figure 4-10).

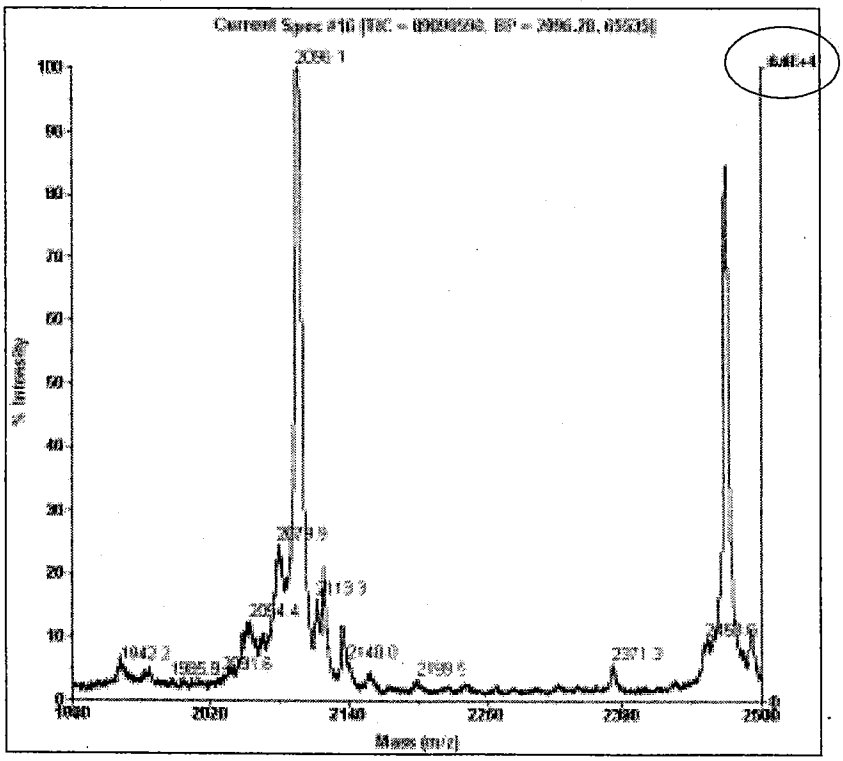


Figure 4-10 Evaluating the Spectrum–Saturated Signal and Broad Peaks

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NOTE: *By default, the right axis is set to Display Relative mode, which means it updates to reflect the intensity of the most intense peak in the currently displayed region of the trace. You can set the right axis to display an absolute value if desired. For more information, see Voyager Biospectrometry Workstation User's Guide, "Accessing graphic options" on page 4-22.*

Note the following about the example shown in Figure 4-10:

- Intensity of the large peak is near 66,000, indicating that the peak may be saturated.
- Peaks are broad. You can determine if peaks are broad by checking resolution as described on page 4-20.

These conditions indicate that the laser power is too high. Before collecting another spectrum at a lower laser setting, eliminate noise peaks and check masses to make sure the spectrum contains the peaks of interest.

Eliminating unwanted peaks

To eliminate unwanted peaks:

1. From the Tools menu, select **Peak Detection**.
2. In the Peak Detection dialog box, set the Minimum Intensity to **20** and click **OK**.

   Peaks with an intensity below 20 counts are no longer labeled (Figure 4-11).

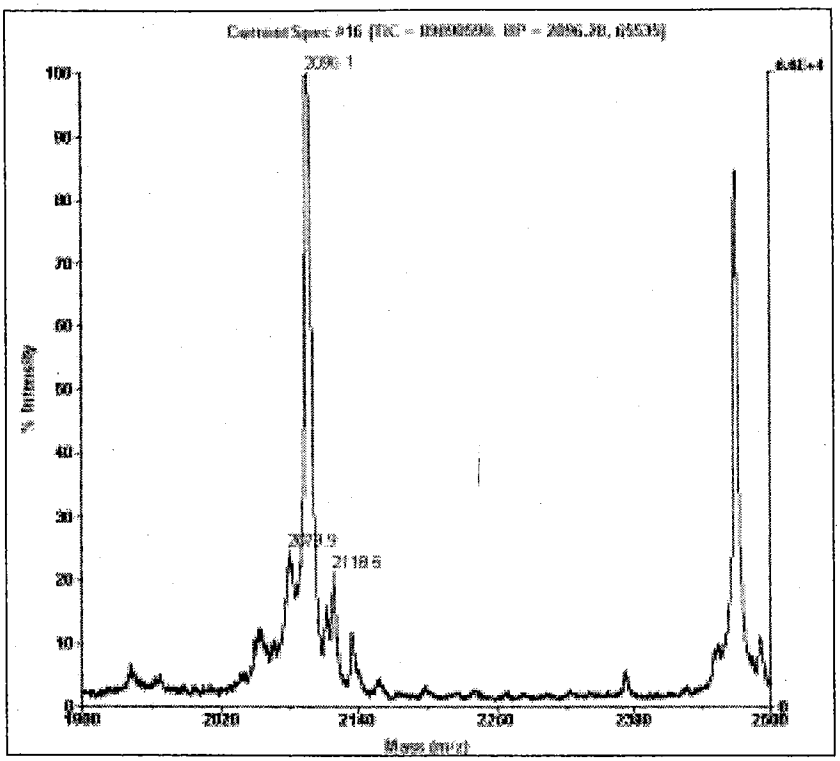


Figure 4-11 Spectrum with Noise Peaks Eliminated

4

Checking masses

With the noise peaks eliminated, you can more easily evaluate remaining masses. Masses are within the expected range for the standard, so you can reacquire using the same instrument settings, but a lower laser intensity.

Reacquiring with lower laser intensity

To reacquire with lower laser intensity:

1. Adjust laser intensity between **1,100** and **1,200** using the slider control on the Manual Laser Intensity/Sample Positioning Control page (see Figure 4-5 on page 4-7).
2. Start acquisition. See "Starting acquisition" on page 4-10 for information.
3. Observe the signal.

You should see a trace similar to the trace in Figure 4-12. At a lower laser intensity, signal is no longer saturated (examine the right axis) and peaks are narrow and well-resolved.

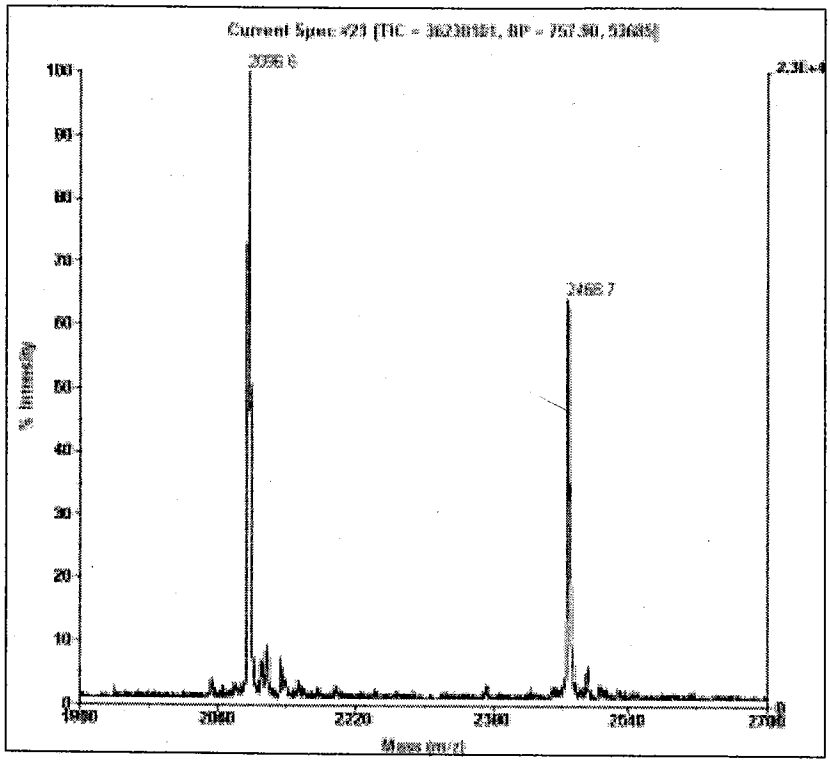


Figure 4-12 Spectrum Acquired at Lower Laser Intensity—Signal Not Saturated

Saving the data file

To save the data file:

1. Select (click on) the Current trace.
2. Click  in the toolbar to or select **Save Spectrum** from the Acquisition menu. The data is saved using the file name specified in the Data Storage control page. Information about the data file is displayed in the Data Storage tab in the Output window at the bottom of the Instrument Control Panel (Figure 4-13).

Figure 4-13 Data Storage Tab in Output Window

4.3 Evaluating Data

To evaluate data:

- Examine the spectrum
- Check resolution
- Calculate signal-to-noise

4.3.1 Examining the Spectrum

Examine the spectrum to check that:

- Peaks of interest are present
- Peaks are sharp
- Peaks are well-separated

Figure 4-14 show an acceptable signal in the Spectrum window.

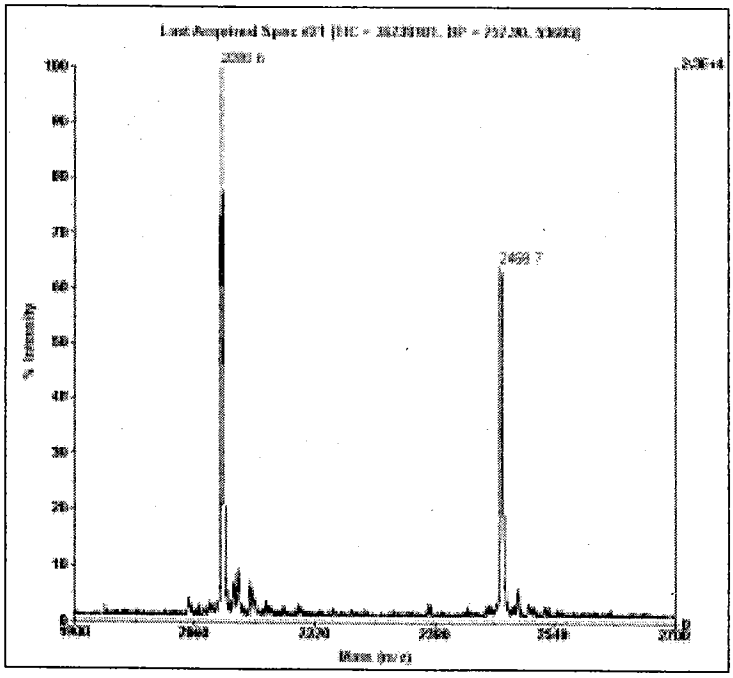


Figure 4-14 Acceptable Signal in Spectrum Window

4.3.2 Checking Resolution

You can calculate mass resolution for up to four peaks in the Current trace. The resolution values are displayed in the trace next to the mass value for the peak.

Calculating resolution

To calculate mass resolution:

1. Select the Current spectrum of interest.
2. From the Tools menu, select **Resolution Calculator**.
3. In the Resolution Calculator dialog box (Figure 4-15), set the percentage of **Peak Height** at which to calculate resolution. The default is 50%, which calculates the resolution at the full width/half maximum of the peak (FWHM).
4. Set **Minimum Peak Intensity**. Signals below this intensity are not included in the calculation.

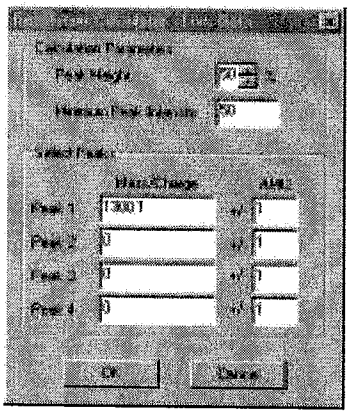


Figure 4-15 Resolution Calculator

5. Type in up to four **Mass/Charge** values for which to calculate resolution, or right-click-drag over peaks in a Current spectrum trace to select values.
6. For each Mass/Charge, enter the window for calculation (± AMU).

NOTE: *To label isotopes, set the ± AMU value low enough to prevent the calculation windows for each isotope peak from overlapping. If the calculation windows overlap, only the highest peak is labeled.*

7. Click **OK**.

   The peaks are labeled with (RXXXX) next to the peak mass, where XXXX is the resolution (Figure 4-16).

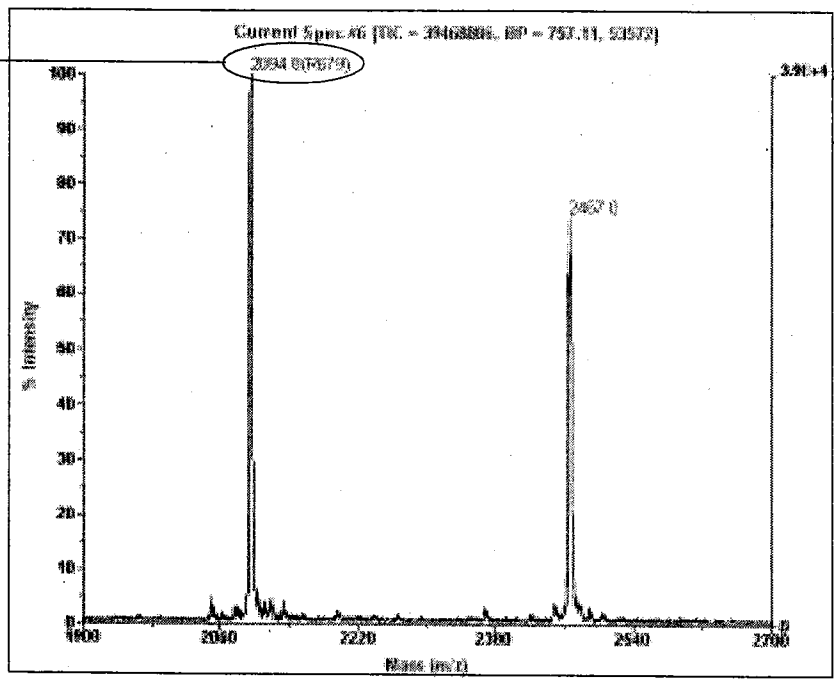


Figure 4-16 Resolution Calculator Results—Example of Good Resolution

Determining if resolution is acceptable

Compare the resolution you obtain to the following table (Table 4-1).

Table 4-1 Resolution Rating Scale

Compounds < 2,000 Da		Compounds 2,000 to 5,000 Da		Compounds 5,000 to 25,000 Da		Compounds > 25,000 Da	
Resolution	**Rating**	**Resolution**	**Rating**	**Resolution**	**Rating**	**Resolution**	**Rating**
500	Not acceptable	250	Not acceptable	500	Not acceptable	50	Acceptable
1,500	Acceptable	400	Acceptable	700	Acceptable	100	Good
2,000	Good	500	Good	900	Good	>200	Very good
2,500	Very good	>600	Very good	1,000	Very good	—	—

4.3.3 Calculating Signal-To-Noise

Calculating signal-to-RMS noise ratio

To calculate a signal-to-RMS noise ratio:

1. Select the Current spectrum of interest.
2. From the Tools menu, select **Signal-to-Noise Calculator**.
3. In the Signal to Noise Calculator dialog box (Figure 4-17), set the Baseline Region by doing one of the following:
   - Type in **From** and **To** values
   - In the Spectrum window, right-click-drag over the baseline area you want to use in calculating signal-to-noise ratio.
4. Type in up to four **Mass/Charge** values for which to calculate signal-to-noise ratio, or right-click-drag over peaks in a Current spectrum trace to select values.

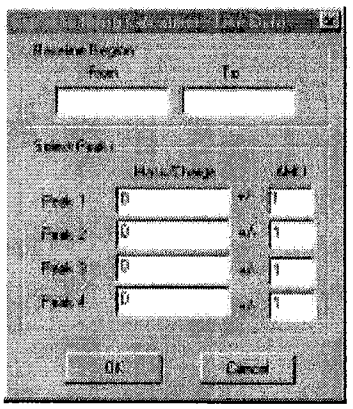


Figure 4-17 Signal to Noise Calculator

5. For each Mass/Charge, enter the window for calculation (± AMU).

***NOTE**: To label peaks, set the ± AMU value low enough to prevent the calculation windows for each peak from overlapping. If the calculation windows overlap, only the first peak is labeled.*

---

6. Click **OK**.

   The peaks are labeled with (SXXX) next to the peak mass, where XXX is the signal-to-noise ratio.

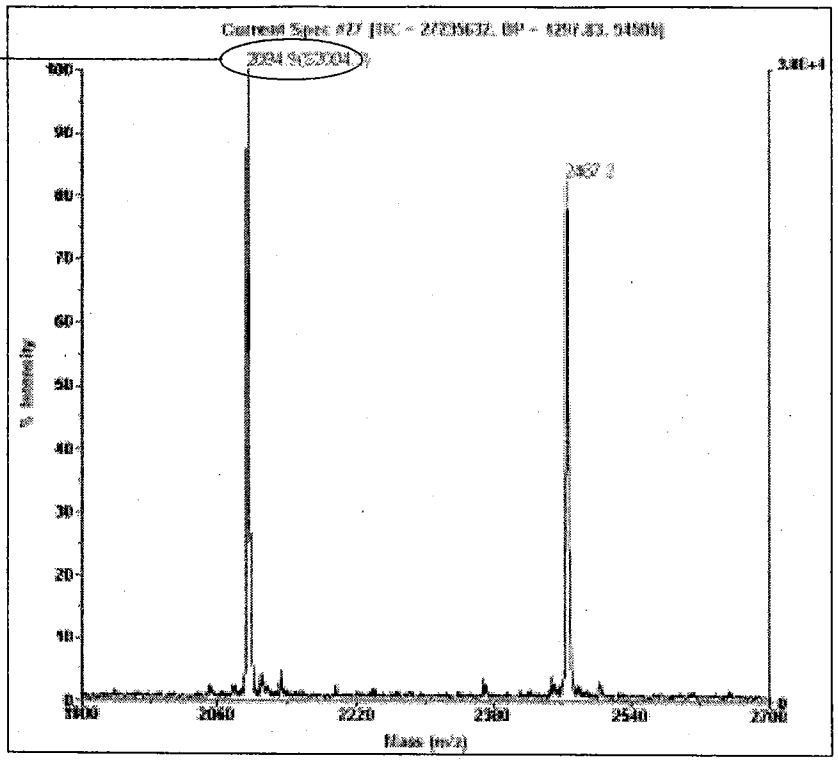
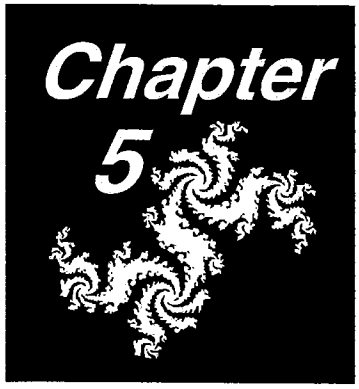


Figure 4-18 Example of Good Signal-To-Noise

After determining that the data is acceptable for the standard, create a calibration file as described in the next chapter that you will add to instrument settings and use to acquire the sample.

5 Creating a Calibration (.CAL) File



This chapter contains the following sections:

5.1 Overview

Before you analyze samples (described in Chapter 6, Acquiring Sample Spectra) generate a calibration (.CAL) file from the standard you acquired in the previous chapter. Before acquiring the sample, you will add this calibration (.CAL) file to your instrument settings.

The section describes the procedures necessary for you to calibrate data. In this chapter, you will:

- Open the data file in the Data Explorer software
- Perform procedures before calibrating to enhance mass accuracy
- Calibrate
- Export the calibration (.CAL) file

5.2 Opening the Data File in the Data Explorer Software

Last acquired file automatically opens

When acquisition is complete, the most recently acquired data file is automatically displayed in the Data Explorer window. If you are continuing from the previous chapter, skip to Section 5.3, Processing Before Calibrating.

Manually opening

If you are calibrating using a previously acquired data file, do the following in the Data Explorer window:

1. If the Data Explorer window is not visible, double-click on the icon in the Windows task bar at the bottom of the desktop.
2. From the File menu in the Data Explorer window, select **Open**.
3. Navigate to the **Getting Started** directory and select **CALMIX2.DAT**.

   The CALMIX2.DAT file is displayed in the Data Explorer window (Figure 5-1).

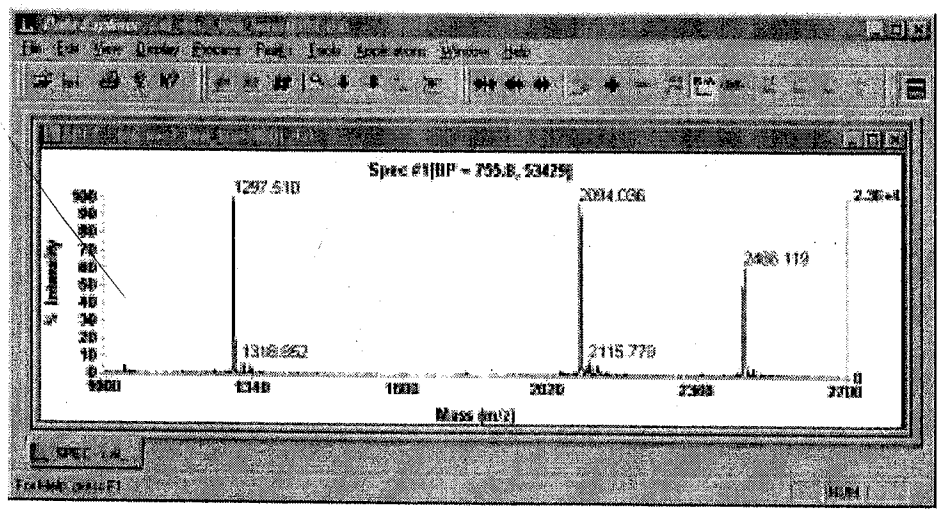


Figure 5-1 CALMIX2.DAT in Data Explorer

5

5.3 Processing Before Calibrating

To optimize mass accuracy, do the following before calibrating:

- Baseline correct
- Smooth
- Deisotope

To process:

1. In the Data Explorer window, click on the spectrum trace.

Baseline correcting

2. From the Process menu, select **Baseline Correction**.

   The spectrum is baseline corrected. For more information, see the *Data Explorer Software User's Guide*, Section 5.8.2, Using Baseline Correction.

Smoothing

3. From the Process menu, select **Noise Filter/Smooth**.

   The Noise Filter/Smooth dialog box (Figure 5-2) is displayed.

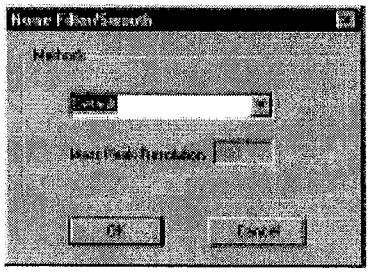


Figure 5-2 Noise Filter/Smooth Dialog Box

4. Select **Default** for the smoothing method and click **OK**.

   The spectrum is smoothed and displayed with an RSM trace label. For more information, see the *Data Explorer Software User's Guide*, Section 5.7, Noise Filtering/Smoothing.

Deisotoping

5. Make sure peaks are isotope-resolved (typical if you are analyzing Reflector mode data).

   If peaks are not isotope-resolved, skip to Section 5.4, Calibrating.

   If peaks are isotope-resolved, select **Peak Deisotoping** from the Peaks menu.

   The Deisotoping dialog box (Figure 5-3) is displayed.

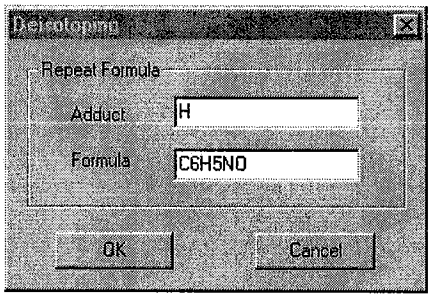


Figure 5-3 Deisotoping Dialog Box

6. For this example spectrum, specify **H** for Adduct and **C6H5NO** for Generic Formula.
7. Click **OK**.

   The spectrum is reduced to a monoisotopic centroided plot of the monoisotopic masses. For information, see the *Data Explorer Software User's Guide*, Section 3.4, Deisotoping a Spectrum.

5.4 Calibrating

In this example, you will calibrate using the ACTH (18–39) peak at 2,466.71 Da, and the Insulin peak at 5,734.50 Da.

To calibrate the deisotoped spectrum:

1. From the Peaks menu, select **Peak Label**, and select the **Mass Label Type** (peak apex or peak centroid) to use for calibration. Click **OK**.
2. From the Process menu, select **Mass Calibration** and then select **Manual Calibration**.

   The Manual Mass Calibration dialog box is displayed (Figure 5-4).

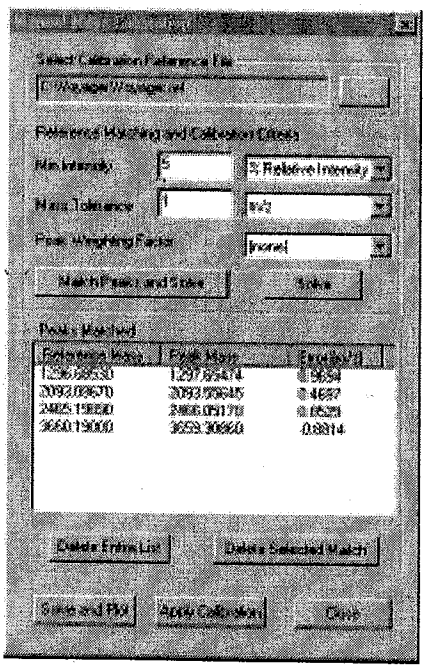


Figure 5-4 Manual Mass Calibration Dialog Box

3. Click [ ] and select the **VOYAGER.REF** calibration reference file.

4. Enter **Reference Matching and Calibration Criteria.**

NOTE: *For descriptions of calibration parameters, see the Data Explorer Software User's Guide, Section 5.3, Manual Calibration.*

Matching peaks

5. Click **Match Peaks and Solve.**

   The software compares observed masses in the spectrum to reference masses in the selected reference file, lists the matches in the Peak Matched list, calibrates the spectrum, and displays the calibration statistics in the Output window.

NOTE: *If you set Mass Tolerance too low, no peaks will match.*

You can also add peaks to the Peaks Matched list by right-click-dragging on a peak in the spectrum, then selecting the mass from the Reference Peak Information dialog box displayed. Click **Solve and Plot** after manually adding masses.

The spectrum is calibrated and displayed with an MC trace label. The calibration statistics are displayed in the Output window.

Applying

6. If the calibration statistics are acceptable, click **Apply Calibration** to save the calibration to the data file.

After calibration

Figure 5-5 shows the deisotoped spectrum after calibration. Note that the trace labels reflect the processing that has been performed on the data. For more information, see the *Data Explorer Software User's Guide,* Section 2.4.10, Viewing Trace Labels.

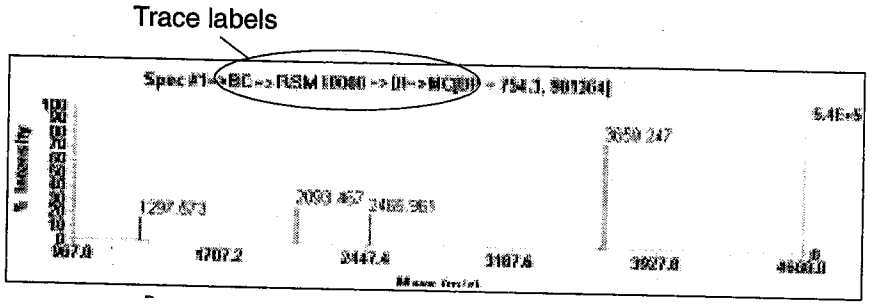


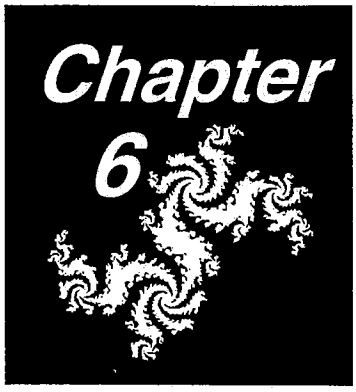
Figure 5-5 Deisotoped Spectrum After Calibration

For more information on creating an external .CAL file, see the *Data Explorer Software User's Guide*, Section 5.3, Manual Calibration.

5.5 Exporting the Calibration (.CAL) File

To export the calibration for use during acquisition:

1. From the File menu, select **Export**, then select **Calibration**. This function exports the last applied calibration constants to a stand-alone .CAL file.
2. In the Save As dialog box, type **ACTH_Linear_Cal** or **ACTH_Reflector_Cal** as the name for the exported .CAL file.
3. Click **Save**.



6 Acquiring Sample Spectra

This chapter contains the following sections:

6

6.1 Modifying Instrument Settings for Sample Acquisition

To modify instrument settings for sample acquisition, specify the calibration file you created in the previous chapter:

1. In the Instrument Settings control page, click **Manual**.
2. In the calibration section, select **External File** and the **ACTH_Linear_Cal** or **ACTH_Reflector_Cal** file that you created in Chapter 5, Creating a Calibration (.CAL) File.

Saving .BIC file

3. Save this new Instrument Settings (.BIC) file as **ACTH_Linear_Cal.BIC** or **ACTH_Reflector_Cal.BIC**.

6.2 Acquiring Sample

Setting Data Storage

Specify Data Storage location as described in Section 4.1.3, Setting Data Storage Parameters.

Selecting sample position

Select the sample position for the mock standard as described in Section 4.1.4, Selecting Sample Position.

Starting acquisition

To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .

Acquisition starts and continues until the number of Shots/Spectrum specified in Spectrum Acquisition on the Instrument Settings control page is collected, or until you select **Stop Acquisition** from the Acquisition menu or click  again.

Adjusting laser intensity

If necessary, increase or decrease the laser intensity as described in Section 4.2.1, Adjusting Laser Intensity, until you observe acceptable signal intensity.

During acquisition

During acquisition:

- The Live trace in the Spectrum window updates to display the spectrum that results from each laser shot.

NOTE: *If your system includes an oscilloscope, the Current Spectrum does not display a trace until acquisition is complete. The spectra that result from each laser shot are displayed on the oscilloscope screen.*

- The system averages all spectra acquired since you started acquisition.

When acquisition is complete, the software displays the data in a Current trace in the Spectrum window (see Figure 6-1).

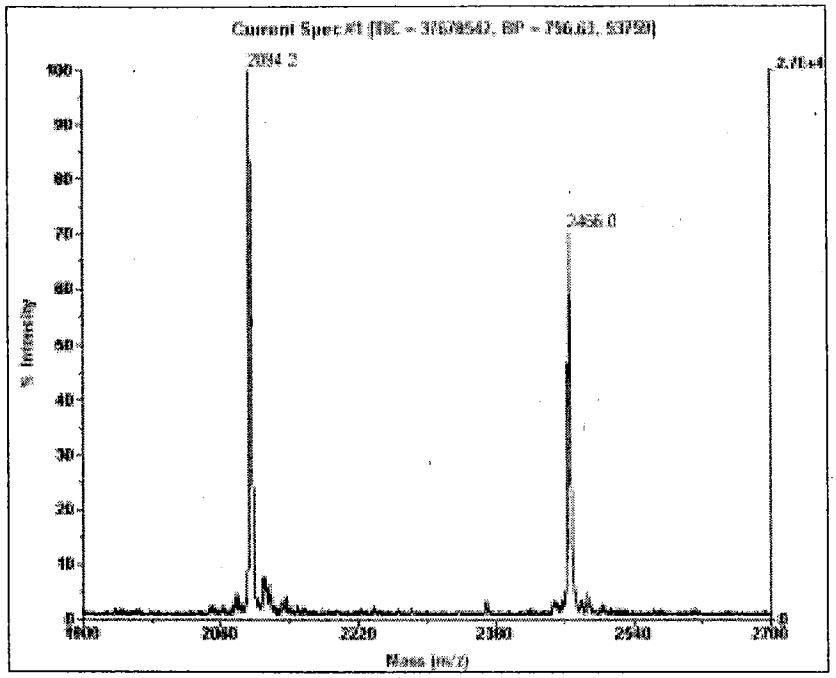


Figure 6-1 Sample Spectrum

Evaluating data

Evaluate the data as described in Section 4.3, Evaluating Data.

You can also examine the data in the Data Explorer software. See the *Data Explorer Software User's Guide* for information.

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