

Voyager™ Biospectrometry™ Workstation

With Delayed Extraction® Technology

Getting Started Guide

Version 5.1 Series Software

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Safety and Compliance Information

In this section This section includes:

- Instrument safety
- Safety and EMC standards
- Laser safety

Instrument Safety

In this section This section includes:

- Notes, Hints, Cautions, and Warnings
- Safety symbols
- Before operating this instrument
- Material Safety Data Sheets (MSDSs)
- General Warnings

Notes, Hints, Cautions, and Warnings

Notes, Hints, Cautions, and Warnings are used in this document as follows.

A Note provides important information to the operator. For example:

NOTE: *If you are prompted to insert the boot diskette into the drive, insert it, then press any key.*

A Hint provides helpful suggestions not essential to the use of the system. For example:

Hint: *To avoid complicated file naming, use Save First to Pass or Save Best Only modes.*

A Caution provides information to avoid damage to the system or loss of data. For example:

CAUTION

Do not touch the lamp. This may damage the lamp.

A Warning provides specific information essential to the safety of the operator. For example:

WARNING

CHEMICAL HAZARD. *Wear appropriate personal protection and always observe safe laboratory practices when operating your system.*

*Remarques,
recommandations
et avertissements*

Une remarque fournit une information importante à l'opérateur et se présente ainsi:

REMARQUE: *Si on vous demande d'insérer la disquette de démarrage dans le lecteur, insérez-la puis appuyez sur n'importe quelle touche.*

Une recommandation fournit une information destinée à éviter des détériorations du système ou la perte de données:

RECOMMANDATION

La lampe peut être endommagée. N'y touchez pas.








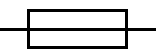
Un avertissement fournit une information indispensable à la sécurité de l'opérateur et se présente ainsi:



AVERTISSEMENT

Conformez-vous toujours aux règlements du laboratoire quand vous utilisez votre système.



Safety symbols The following symbols may be displayed on the system. These symbols may also appear next to associated warnings in this document.

Electrical Symbols The following chart is an illustrated glossary of electrical symbols that may be displayed on your instrument. Whenever such symbols appear on instruments, please observe appropriate safety procedures.

	<p>This symbol indicates the on position of the main power switch.</p>
	<p>This symbol indicates the off position of the main power switch.</p>
	<p>This symbol indicates the on/off position of a push-push main power switch.</p>
	<p>This symbol indicates that a terminal may be connected to another instrument's signal ground reference. This is not a protected ground terminal.</p>
	<p>This symbol indicates that this is a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.</p>
	<p>A terminal marked with this symbol either receives or delivers alternating current or voltage.</p>
	<p>A terminal marked with this symbol can receive or supply an alternating and a direct current or voltage.</p>
	<p>This symbol appears next to the values of the fuses required by the system.</p>

	<p>WARNING: This symbol indicates the presence of high voltage and warns the user to proceed with caution.</p>
	<p>WARNING: This symbol alerts you to consult the manual for further information and to proceed with caution.</p>

Non-electrical Symbols The following is an illustrated glossary of non-electrical safety alert symbols that may be displayed on your instrument.








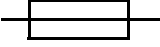
	<p>WARNING: This symbol illustrates a heater hazard. Proceed with caution when working around these areas to avoid being burned by hot components.</p>
	<p>This symbol indicates that a laser is present inside the instrument.</p>



Symboles des alertes de sécurité

Les symboles suivants peuvent être affichés sur le système. Dans ce document, ces symboles peuvent aussi apparaître à côté des avertissements auxquels ils s'associent.

Symboles électriques



Le tableau suivant donne la signification de tous les symboles électriques qui figurent sur les appareils. En présence de l'un de ces symboles, il est impératif de se conformer aux consignes de sécurité appropriées.

	Position <i>MARCHE</i> de l'interrupteur d'alimentation principal.
	Position <i>ARRÊT</i> de l'interrupteur d'alimentation principal.
	Positions <i>MARCHE-ARRÊT</i> de l'interrupteur d'alimentation principal à bouton poussoir.
	Borne pouvant être reliée à la mise à la terre d'un autre appareil. Ce n'est pas une borne de mise à la terre protégée.
	Borne de mise à la terre de protection devant être reliée à la terre avant d'effectuer tout autre raccordement électrique à l'appareil.
	Borne recevant ou fournissant une tension ou un courant de type alternatif.
	Borne pouvant recevoir ou fournir une tension ou un courant de types alternatif et continu.
	Ce symbole apparaît à côté des valeurs des fusibles requis par le système.

	AVERTISSEMENT: Indique la présence d'une haute tension et avertit l'utilisateur de procéder avec précaution.
	AVERTISSEMENT: Avertit l'utilisateur de la nécessité de consulter le manuel pour obtenir davantage d'informations et de procéder avec précaution.

Symboles non électriques

Le tableau suivant donne la signification des symboles d'alertes de sécurité non électriques qui figurent sur les appareils.

	AVERTISSEMENT: Danger associé à la présence d'un appareil de chauffage. Procéder avec précaution pour éviter de se brûler au contact de pièces ou d'éléments chauds.
	Indique que l'appareil renferme un laser.

**Before operating
this instrument**

Ensure that anyone involved with the operation of the instrument is instructed in both general safety practices for laboratories and specific safety practices for the instrument. Make sure you have read and understood all related Material Safety Data Sheets.

**Material Safety
Data Sheets
(MSDSs)**

Some of the chemicals that may be used with your system are listed as hazardous by their manufacturer. When hazards exist, they are prominently displayed on the labels of all chemicals. In addition, MSDSs supplied by the chemical manufacturer provide information about:

- Physical characteristics
- Safety precautions
- Health hazards
- First-aid
- Spill clean-up
- Disposal procedures

WARNING

CHEMICAL HAZARD. *Before handling any chemicals, refer to the Material Safety Data Sheet provided by the manufacturer, and observe all relevant precautions.*

AVERTISSEMENT

RISQUE CHIMIQUE. *Avant de manipuler des produits chimiques, veuillez consulter la fiche de sécurité du matériel fournie par le fabricant, et observer les mesures de précaution qui s'imposent.*

To order MSDSs...	Then...
Over the Internet	<p>Go to our Web site at www.pebio.com/techsupport.</p> <p>Click MSDSs.</p> <p>Enter keywords (or partial words), or a part number, or the MSDSs Documents on Demand index number.</p> <p>Click Search.</p> <p>Click the Adobe Acrobat symbol to view, print, or download the document, or check the box of the desired document and delivery method (fax or e-mail).</p>

General Warnings

WARNING

FIRE HAZARD. Using a fuse of the wrong type or rating can cause a fire. Replace fuses with those of the same type and rating.

AVERTISSEMENT

DANGER D'INCENDIE. L'usage d'un fusible de type ou de valeur nominale différents risque de provoquer un incendie. Il convient donc de remplacer les fusibles usagés par des fusibles du même type et de la même valeur nominale.

WARNING

LASER HAZARD. The laser emits ultraviolet radiation. Lasers can burn the retina and leave permanent blind spots. Do not remove any instrument panels or look directly into the laser beam or allow a reflection of the beam to enter your eyes. Wear proper eye protection if any panels are removed for service.

AVERTISSEMENT

DANGER LASER. Le laser émet des radiations ultraviolettes. Les lasers peuvent brûler la rétine et laisser des points aveugles permanents. Il convient de ne pas retirer le panneau avant ou les panneaux latéraux de l'appareil et de ne pas regarder directement dans le faisceau laser ou laisser une réflexion du faisceau entrer dans les yeux. Portez des protections adéquates pour les yeux si le panneau avant ou les panneaux latéraux ont été retirés afin d'effectuer l'entretien.



WARNING

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result by operating the instrument without the panels in place. Do not remove instrument front or side panels. High voltage contacts are exposed with front or side panels removed.



AVERTISSEMENT

RISQUE DE DÉCHARGE ÉLECTRIQUE. Des décharges électriques sérieuses peuvent résulter du fonctionnement de l'appareil lorsque le panneau avant et les panneaux latéraux sont retirés. Ne pas retirer le panneau avant ou les panneaux latéraux. Des contacts haute tension sont exposés lorsque les panneaux sont retirés.

WARNING

CHEMICAL HAZARD. To prevent eye injury, always wear eye protection when working with solvents.

AVERTISSEMENT

RISQUE CHIMIQUE. Pour éviter les blessures aux yeux, porter toujours des protections pour les yeux lorsque vous manipulez des solvants.

WARNING

PHYSICAL INJURY HAZARD. Use the Voyager Biospectrometry Workstation only as specified in this document. Using this system in a manner not specified may result in injury or damage to the system.

AVERTISSEMENT

DANGER DE BLESSURES CORPORELLES. Veuillez suivre avec attention les indications figurant dans ce document lorsque vous utilisez la Station de Travail de Biospectrométrie Voyager. Un usage différent de la station pourrait causer un accident ou endommager le système.

Safety and EMC (Electromagnetic Compliance) Standards

US Safety and EMC Standards

Safety

This instrument has been tested to and complies with standard ANSI/UL 3101-1, "Electrical Equipment for Laboratory Use; Part 1: General Requirements", 1st Edition. It is an ETL Testing Laboratories listed product.

EMC

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

WARNING

Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTE: *This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.*

NOTE: *Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.*

**Canadian Safety
and EMC
Standards**

Safety

This instrument has been tested to and complies with standard CSA 1010, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements”. It is an ETL Testing Laboratories listed product.

Sécurité

Cet instrument a été vérifié avec la norme CSA 1010, «Spécifications de sécurité du matériel électrique utilisé pour les mesures, les contrôles et dans les laboratoires ; Partie 1 : Spécifications générales», et il est conforme à cette norme. C’est un produit homologué par les ETL Testing Laboratories.

EMC

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.

**European Safety
and EMC
Standards**



Safety

This instrument meets European requirements for safety (EMC Directive 73/23/EEC). This instrument has been tested to and complies with standard EN61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use”.

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 98/336/EEC). This product has been evaluated to the EN55011:1992, Group 1, Class B “Radiated Emissions”, and EN50082-1:1992, “Generic Immunity”.

Laser Safety

Laser classification

The Voyager™ Biospectrometry™ Workstation uses a standard nitrogen laser and an optional Nd:YAG laser. Under normal operating conditions, the instrument laser is categorized as a Class I laser. Under certain conditions during servicing, when interlocks have been circumvented, the lasers fall into the following categories (can cause permanent eye damage):

- Nitrogen—Class IIIb
- Nd:YAG—Class IV

The Voyager Biospectrometry Workstation complies with Title 21, U.S. Government DHEW/BRH Performance Standards, Chapter 1, Subchapter J, Section 1040, as applicable.

Laser safety features

The following safety features are included on the Voyager Biospectrometry Workstation:

- Cabinet is designed to prevent access to collateral laser radiation exceeding the accessible emission limits in Performance Standards for Laser Products, 21 CFR 1040.10.
- Front and side panels have interlock switches that disable the laser when panels are removed.
- Safety labels for Class I standards are affixed to the unit.

Laser safety requirements

To ensure safe laser operation, note the following:

- The system must be installed and maintained by an Applied Biosystems Technical Representative.
- All panels must be installed during operation. When all panels are installed, there should be no detectable radiation present. If any panels are removed when the laser is operational, you may be exposed to laser emissions in excess of Class 1 rating.
- Do not remove labels or disable safety interlocks.

Additional safety information

Refer to the users manual provided with the laser for additional information on government and industry safety regulations.

1 Before You Begin



This chapter contains the following sections:

- 1.1 Introduction to the System 1-2
- 1.2 Running Your First Experiment 1-4

1.1 Introduction to the System

Basics of the system

An Applied 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.
- Use the Acqiris® digitizer, describe key features.

Processing tasks

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

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 Adrenocorticotrophic hormone, clip 7–38 [ACTH (7–38)], Adrenocorticotrophic 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 an Applied 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 5×10^{-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

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.*

- α -cyano-4-hydroxycinnamic acid (CHCA) matrix
- Adrenocorticotrophic hormone, clip 7–38 [ACTH (7–38)], 50 pmol/ μ l in aqueous solution
- Adrenocorticotrophic hormone, clip 18–39 [ACTH (18–39)], 50 pmol/ μ l in aqueous solution
- Bovine insulin, 50 pmol/ μ l in aqueous solution

WARNING

CHEMICAL HAZARD. *Alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix may cause eye, skin, and respiratory tract irritation. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING

CHEMICAL HAZARD. *ACTH and Insulin may cause an allergic skin and respiratory reaction. Exposure may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

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

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

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

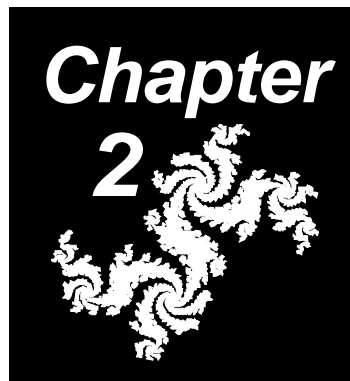
WARNING

CHEMICAL HAZARD. Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Acetonitrile is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.

Trifluoroacetic acid causes severe burns to the eyes, skin, and respiratory tract.

2 Starting Up



This chapter contains the following sections:

- 2.1 Powering Up 2-2
- 2.2 Starting the Software 2-3

For more information, see the *Voyager Biospectrometry Workstation User's Guide*, Section 2.10, 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, wait approximately one minute for its initialization cycle to finish. A message indicates that the power on self check passed, then the oscilloscope screen is displayed. See the *Voyager Biospectrometry Workstation User's Guide*, 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. 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 (Figure 2-1) from the Windows NT desktop, double-click the **Voyager Control Panel** icon on the desktop.

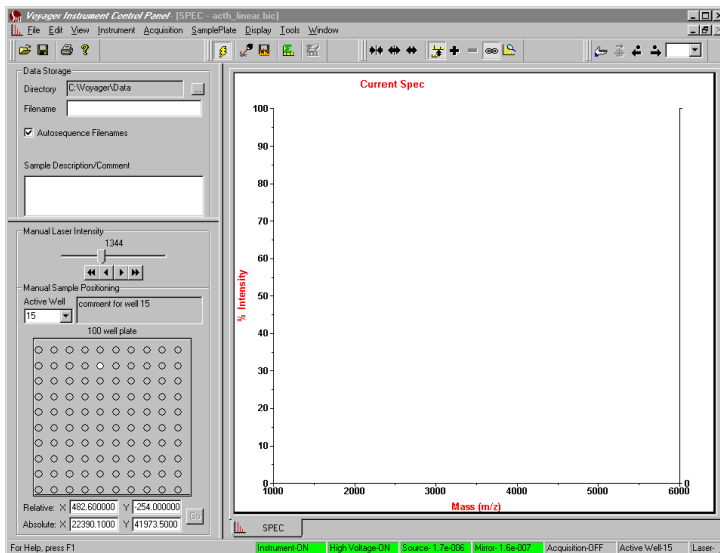


Figure 2-1 Instrument Control Panel

Initializing

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

2

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.

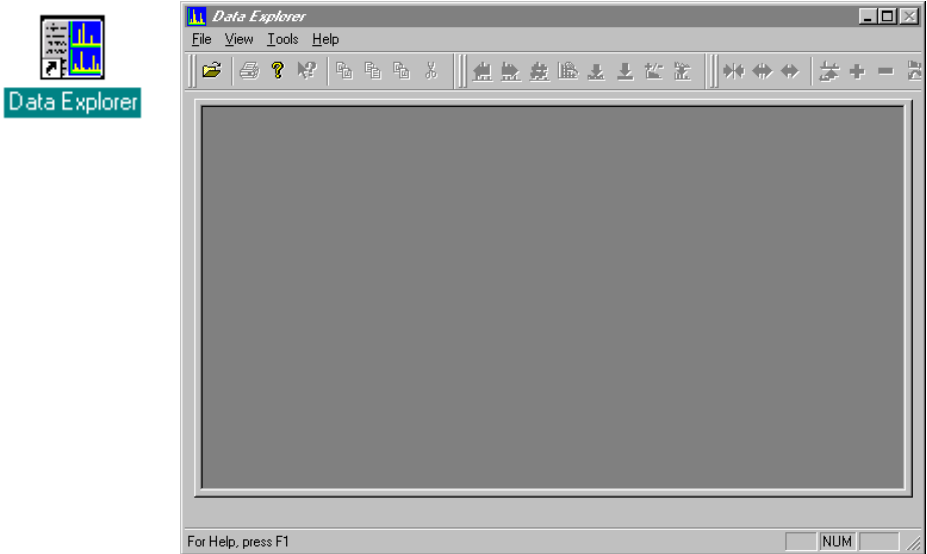
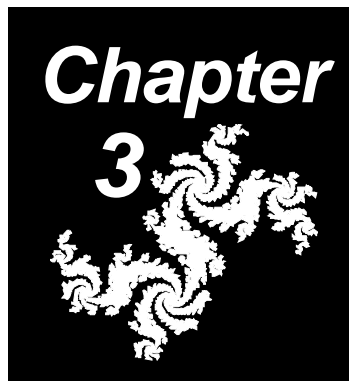


Figure 2-2 Data Explorer Window with No Data

Menus are not displayed and toolbar buttons are not active until you open a data file.

2

3 Preparing Samples



This chapter contains the following sections:

3.1	Overview	3-2
3.2	Preparing Matrix	3-3
3.3	Preparing Samples	3-5
3.4	Mixing Sample and Matrix	3-7
3.5	Loading Samples on Sample Plates.....	3-8
3.6	Loading Sample Plates in the Mass Spectrometer.....	3-10

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, Matrixes, 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

Because the compounds you are analyzing have masses less than 10,000 Da, 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 (CHCA) 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 CHCA matrix and add it to the tube.

WARNING

CHEMICAL HAZARD. *Alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix may cause eye, skin, and respiratory tract irritation. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

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

3. Add 400 μ l deionized water, 100 μ l 3% TFA, and 500 μ l acetonitrile to the tube.

WARNING

CHEMICAL HAZARD. Please read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Acetonitrile is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage.

Trifluoroacetic acid causes severe burns to the eyes, skin, and respiratory tract.

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

WARNING

CHEMICAL HAZARD. *ACTH and Insulin may cause an allergic skin and respiratory reaction. Exposure may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

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 Workstation 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).

WARNING

CHEMICAL HAZARD. *Trifluoroacetic acid causes burns to the eyes, skin, and respiratory tract. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

NOTE: *Alternatively, you can prepare the Calibration Mix 2 sample from the Sequazyme 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:

Standard solution

1. Label two 1.5 ml microcentrifuge tubes with “Standard” and “Sample” and the date prepared.
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.*

Sample solution

4. Vortex for 15 seconds.
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

WARNING

CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet provided by the manufacturer, and observe all relevant precautions.

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 2-1 on page 2-3), select **Eject** from the Sample Plate menu. The Load/Eject Sample Plate dialog box (Figure 3-1) is displayed.

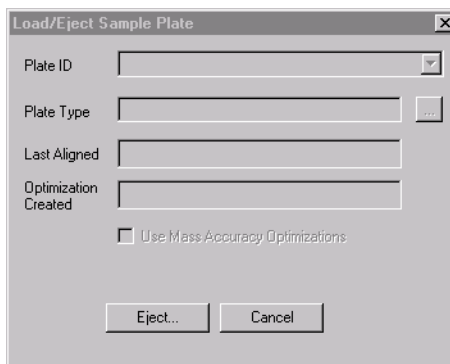


Figure 3-1 Load/Eject Sample Plate Dialog Box

Click **Eject**. The following occurs:

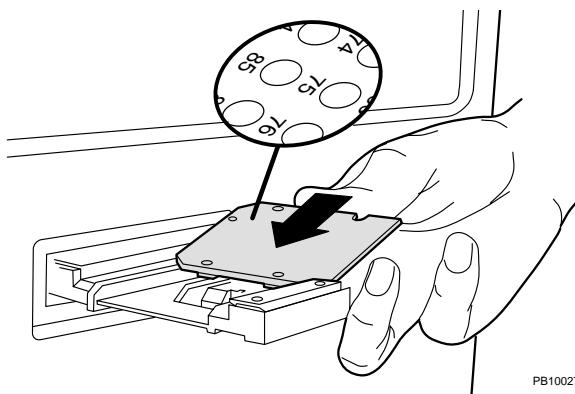
- A Load/Eject Cycle Status dialog box is displayed during the ejection sequence which displays hardware status.
- High voltage is turned off.
- The video monitor displays the sample plate moving.
- The sample holder moves out of the main source chamber, out of the sample loading chamber, and is ejected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

NOTE: Do not leave the sample holder in the Eject position for more than a few minutes. 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 3-10.
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).



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

- 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.



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.*

- 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.

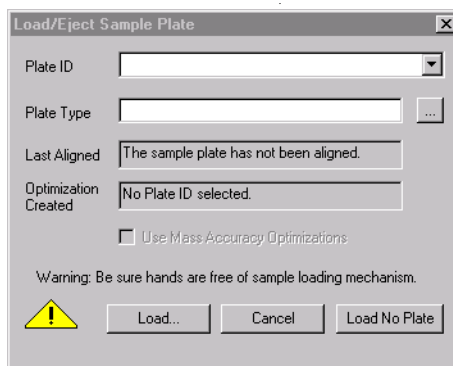



Figure 3-3 Load/Eject Sample Plate Dialog Box

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 ID field, type **100 well**.
6. In the Plate Type field, click  , then 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

For more information on Plate IDs, see the *Voyager Biospectrometry Workstation User's Guide*, "Assigning Plate IDs" on page 3-50.

7. Select **Use Mass Accuracy Optimizations**, if desired, to apply calibration corrections to the data acquired from the plate. For information, see the *Voyager Biospectrometry Workstation User's Guide*, Section 3.4.2, Using the Mass Accuracy Optimization Option.
8. 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 2-47.

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.

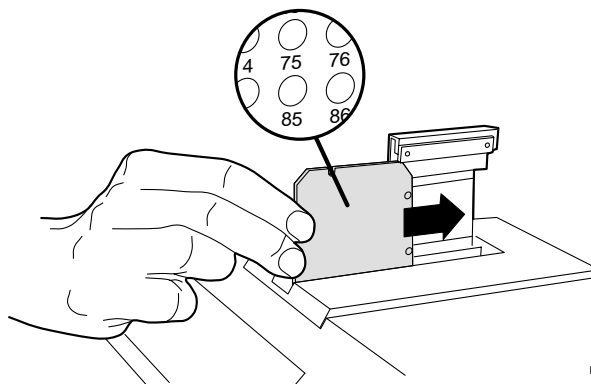


Voyager-DE STR To load sample plates:

1. Eject the sample holder as described in “Ejecting the sample holder” on page 3-10.
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.



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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.

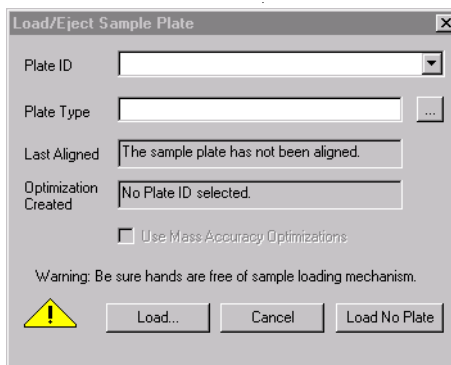



Figure 3-5 Load/Eject Sample Plate Dialog Box

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 ID field, type **100 well**.
6. In the Plate Type field, click  , then 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

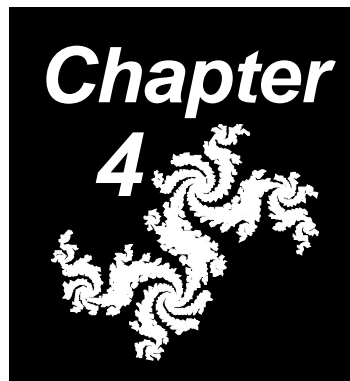
For more information on Plate IDs, see the *Voyager Biospectrometry Workstation User's Guide*, "Assigning Plate IDs" on page 3-50.

7. Select **Use Mass Accuracy Optimizations**, if desired, to apply calibration corrections to the data acquired from the plate. For information, see the *Voyager Biospectrometry Workstation User's Guide*, Section 3.4.2, Using the Mass Accuracy Optimization Option.
8. 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 2-47.

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:

- 4.1 Setting the Instrument Control Panel 4-2
- 4.2 Acquiring the Standard 4-10
- 4.3 Evaluating Data 4-20

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

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

Double-click the Instrument Control Panel icon to start the Instrument Control Panel (Figure 4-1).

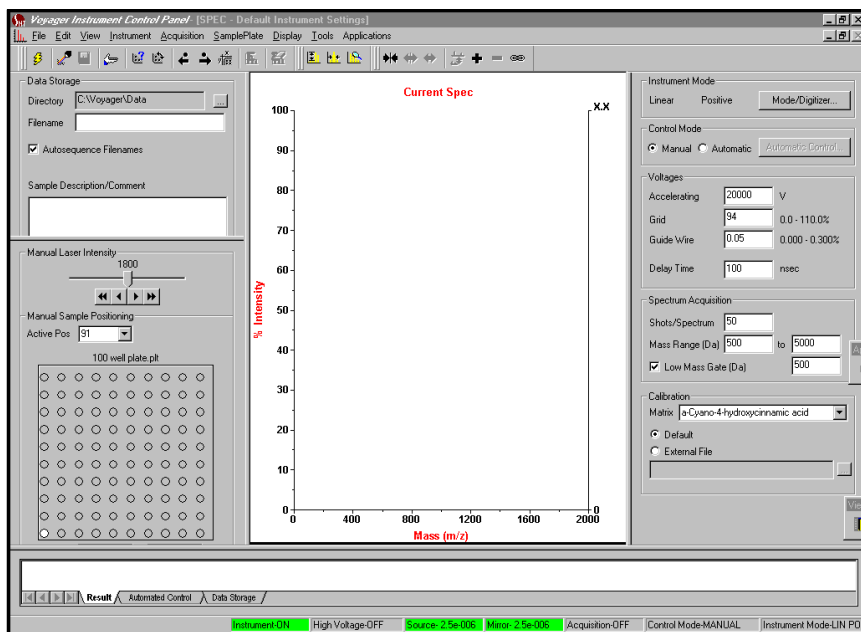


Figure 4-1 Instrument Control Panel

Checking system pressure From the View menu, select **System Status**.

Before acquiring data, allow the pressure in the Source Chamber (BA1) and Mirror Chamber (BA2) to reach:

Model	Source Chamber (BA1) Pressure (Torr)	Mirror Chamber (BA2) Pressure (Torr)
Voyager-DE and Voyager-DE PRO	Less than 10^{-6}	Less than 10^{-6}
Voyager-DE STR	Less than 5×10^{-7}	Less than 5×10^{-8}

Readings from the vacuum gauges are displayed in the System Status control page in the Instrument Control Panel. See the *Voyager Biospectrometry Workstation User's Guide*, Section 2.11, Checking System Status and Pressures, for more information.

4.1.2 Loading an Instrument Settings (.BIC) File

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

Loading an instrument settings (.BIC) file

To load a .BIC file:

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

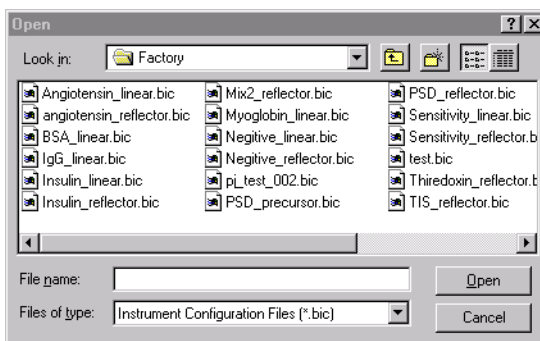


Figure 4-2 Open Dialog Box

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).

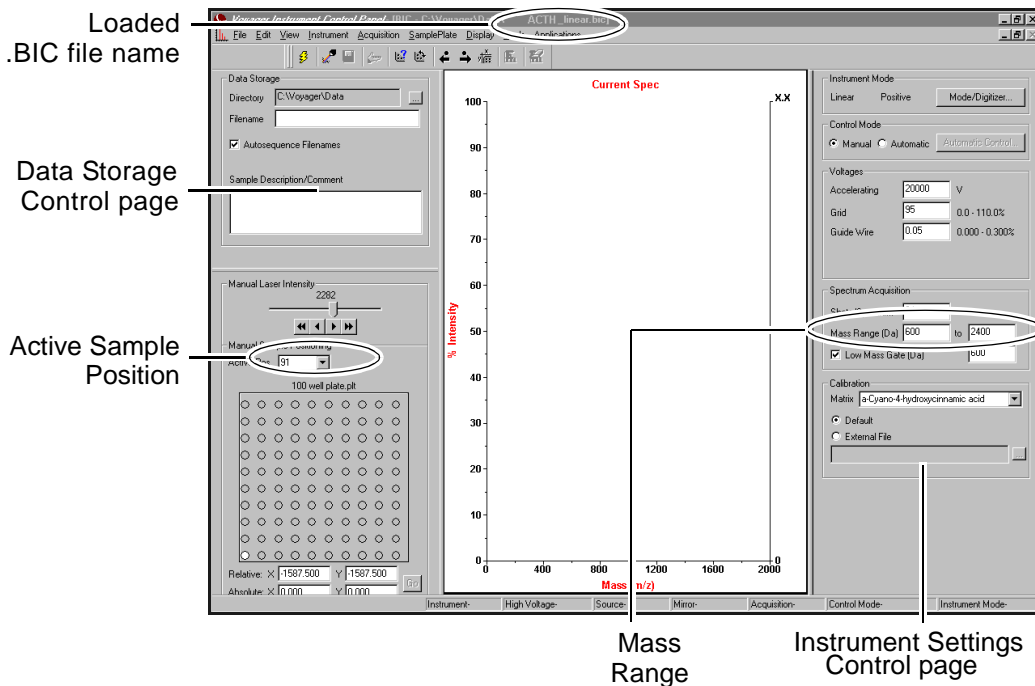


Figure 4-3 Instrument Control Panel with ACTH_Linear.BIC File Loaded

Changing instrument settings

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

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 Instrument Settings** 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).

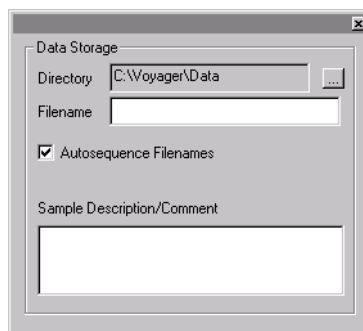


Figure 4-4 Data Storage Control Page

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.

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 4-11.

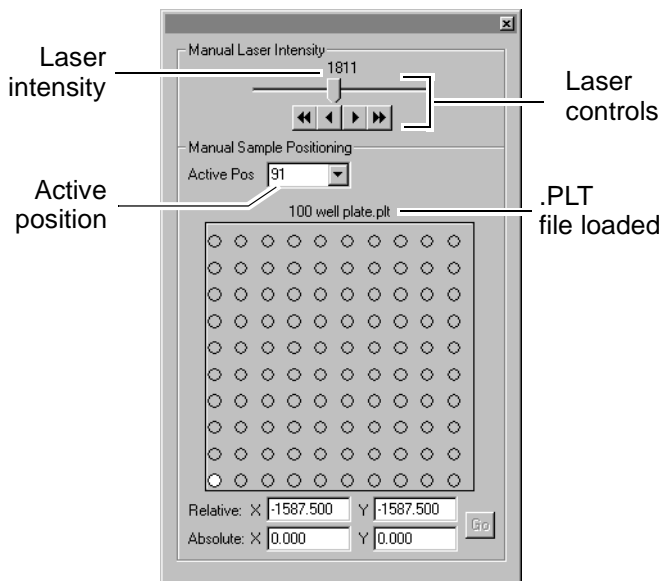


Figure 4-5 Manual Laser/Sample Positioning Control Page (Plate View) with a 100 Well Plate.PLT File Loaded

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

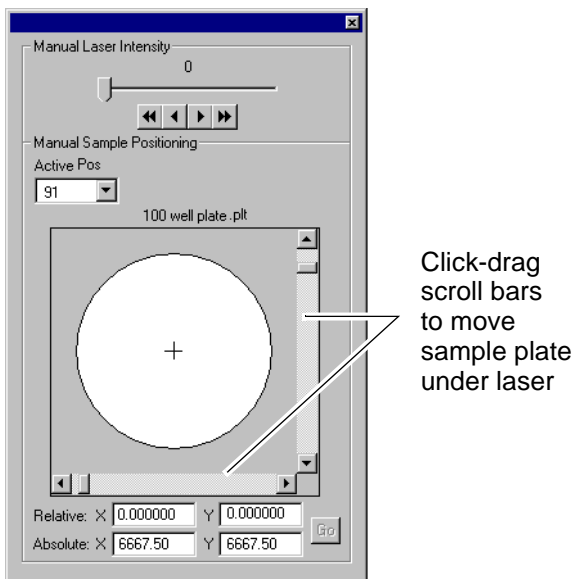


Figure 4-6 Manual Laser/Sample Positioning Control Page (Sample View)

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 laser setting saved in the .BIC file is loaded.

Adjusting laser intensity

To adjust laser intensity, click-drag the slider control or click the fine and coarse buttons on the Manual Laser/Sample Positioning control page (see Figure 4-5 on page 4-8). 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.

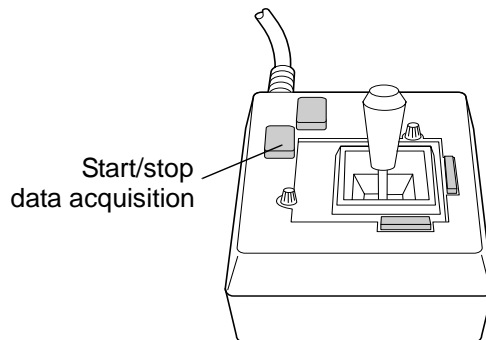
To turn on the high voltage power supplies, click  in the toolbar.

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 2-38.

Starting acquisition

Start acquisition, 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).



Move stick up and down, left and right to adjust sample position under laser beam

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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 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

4

Status bar shows acquisition status

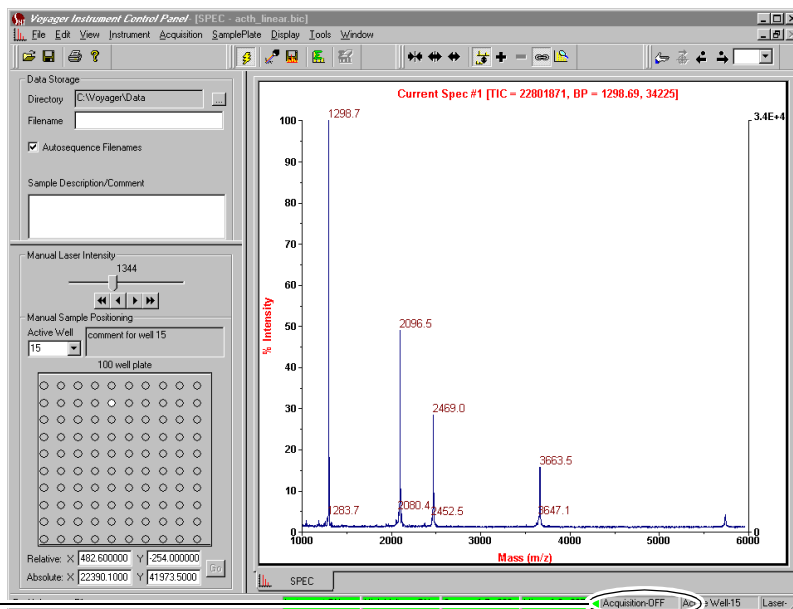


Figure 4-8 Current Trace in Spectrum Window

**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 4-9)
- 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 doing any of the following:

- 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 4-11).

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 4-12.

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).

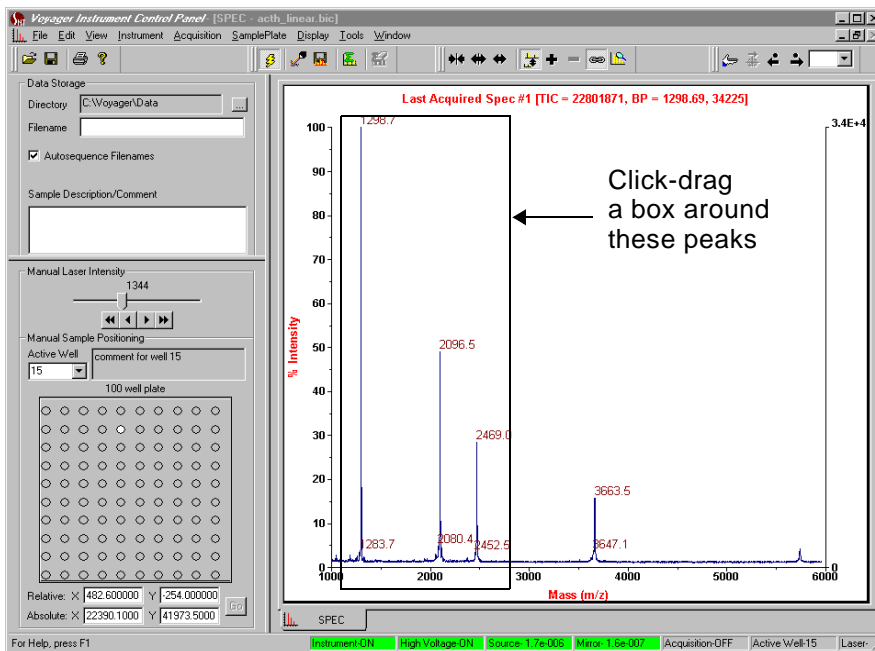



Figure 4-9 Zooming on Peaks

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).

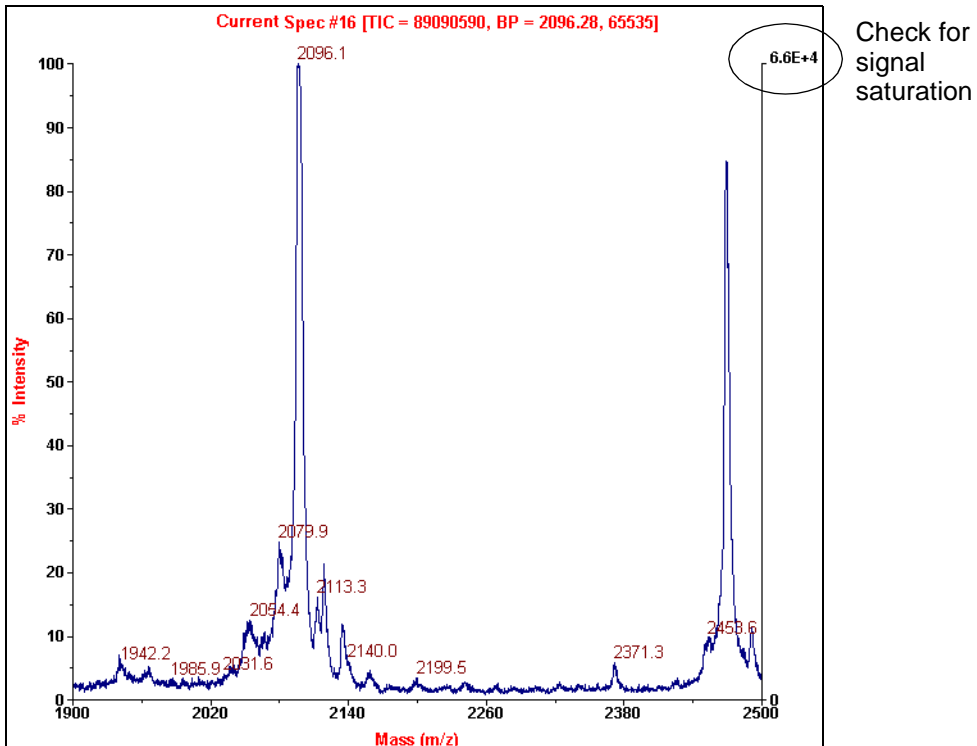


Figure 4-10 Evaluating the Spectrum—Saturated Signal and Broad Peaks

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 calculating resolution as described on page 4-21.

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**, then click **OK**.

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

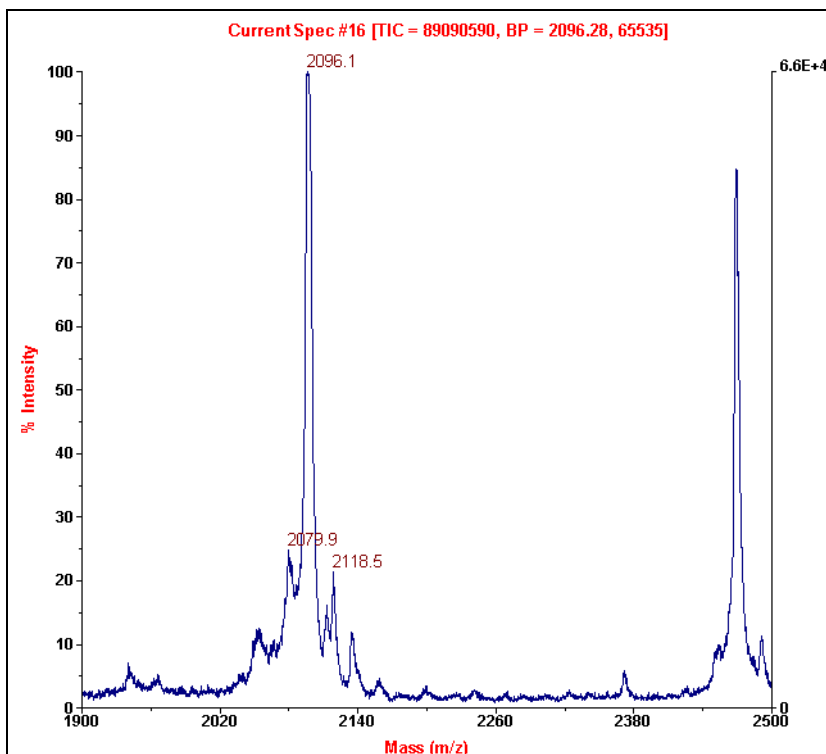


Figure 4-11 Spectrum with Noise Peaks Eliminated

Checking masses

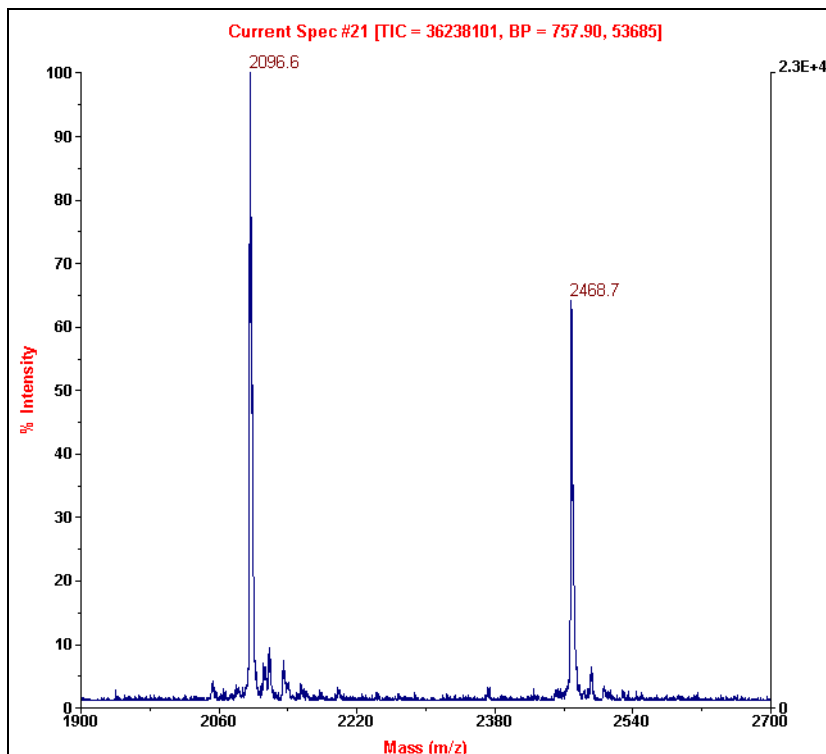
With the noise peaks eliminated, you can more easily evaluate remaining masses. Masses are within the expected range for the standard, so you can reacquire using the same instrument settings, but a lower laser intensity.

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 4-8).
2. Start acquisition. See “Starting acquisition” on page 4-11 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.




4

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

Saving the data file

To save the data file:

1. Click the Current trace.
2. Click  in the toolbar 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).

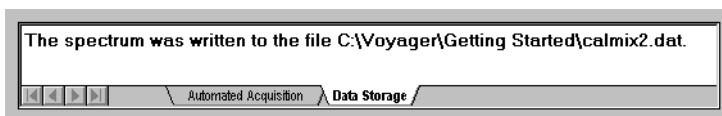


Figure 4-13 Data Storage Tab in Output Window

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.

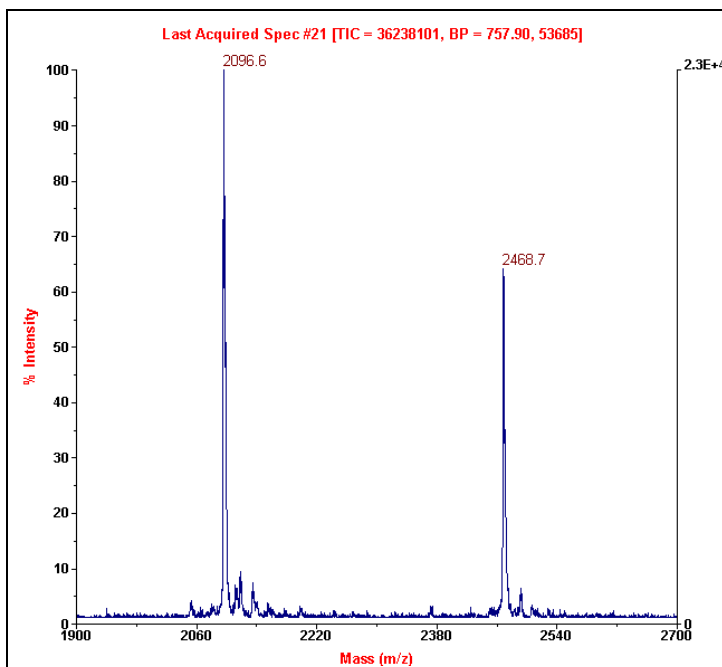


Figure 4-14 Acceptable Signal in Spectrum Window in Instrument Control Panel

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.

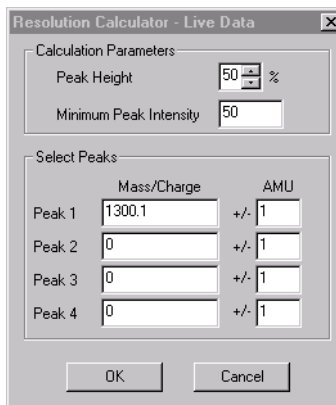


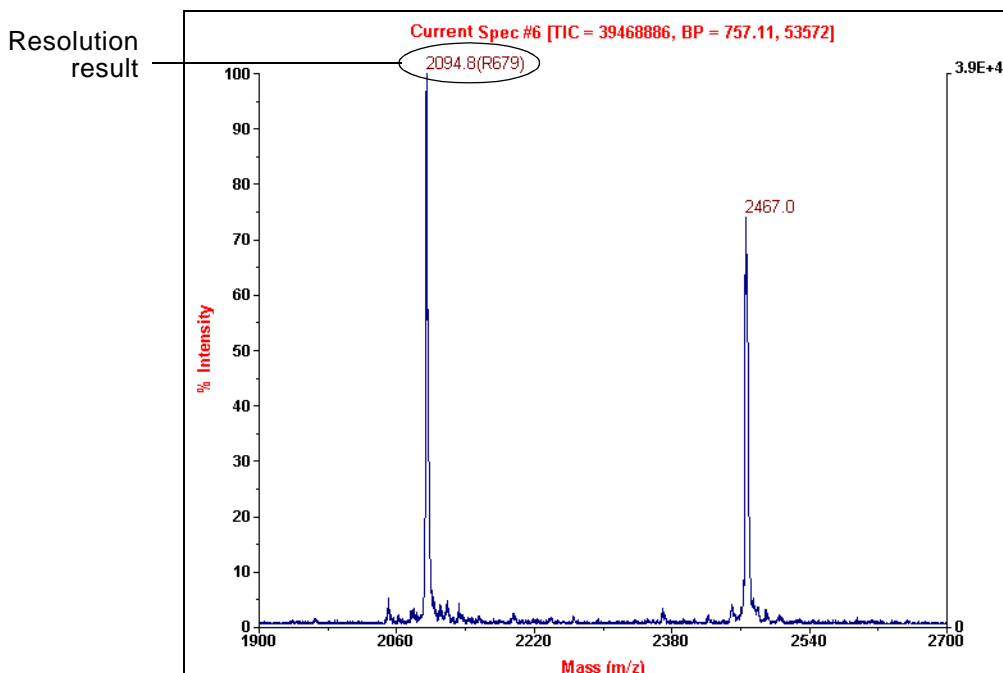
Figure 4-15 Resolution Calculator

5. Type in up to four Mass/Charge values for which you want 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 (\pm AMU).

NOTE: To label isotopes, set the \pm 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 in Instrument Control Panel—
Example of Good Resolution**

4

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 **S/N 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 you want to calculate signal-to-noise ratio, or right-click-drag over peaks in a Current spectrum trace to select values.

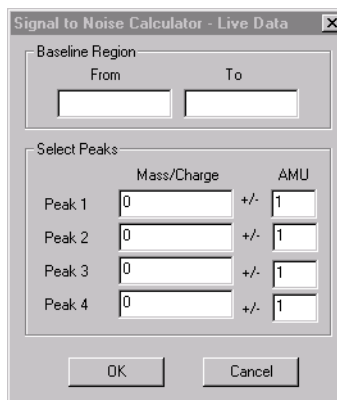


Figure 4-17 Signal to Noise Calculator

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

NOTE: To label peaks, set the \pm 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.

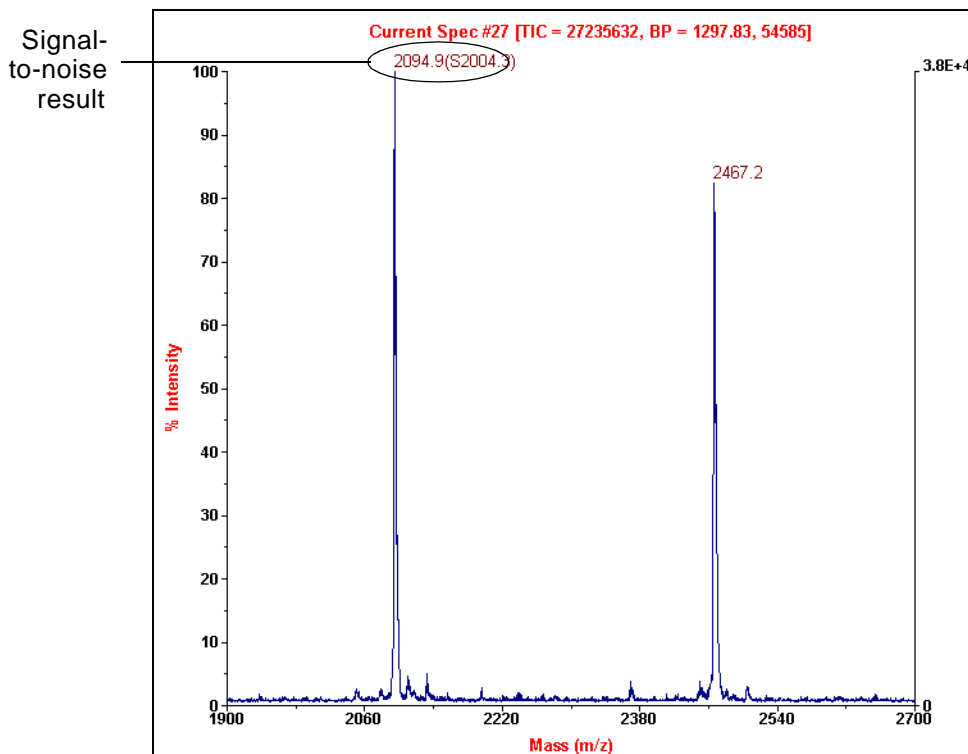
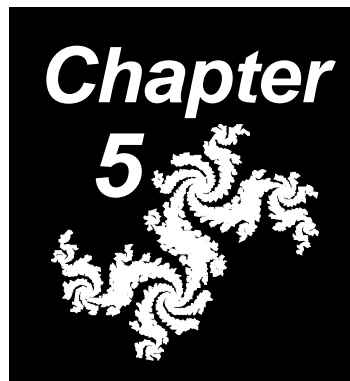


Figure 4-18 Example of Good Signal-To-Noise

After determining that the data is acceptable for the standard, create a calibration file (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	5-2
5.2	Opening the Data File in Data Explorer	5-3
5.3	Processing Before Calibrating.....	5-4
5.4	Calibrating	5-6
5.5	Exporting the Calibration (.CAL) File.....	5-10

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

Opening from Instrument Control Panel

When acquisition is complete, you can open the most recently acquired data file by clicking  in the Instrument Control Panel toolbar.

The data file opens in the Data Explorer software.

Opening from Data Explorer software

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).

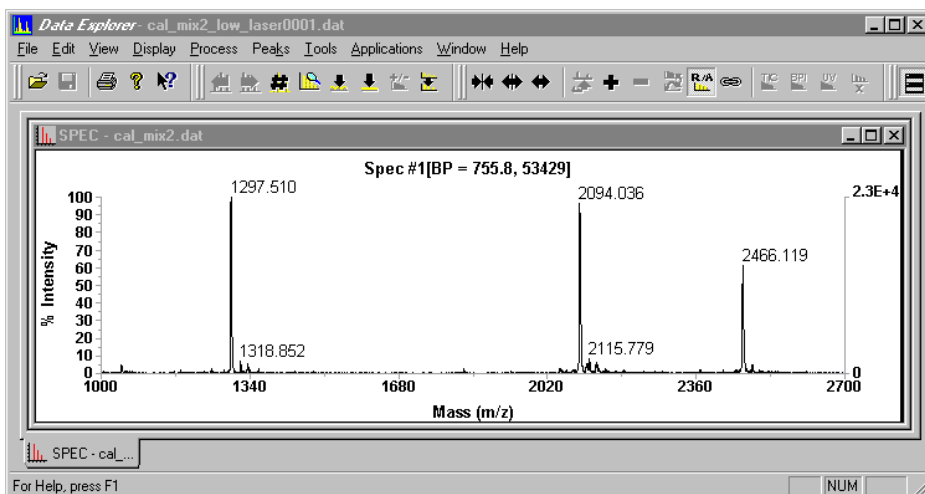


Figure 5-1 CALMIX2.DAT in the Data Explorer Window

5.3 Processing Before Calibrating

To optimize mass accuracy, do the following before calibrating:

- Baseline-correct
- Smooth
- Deisotope

Creating macros to combine processing functions

You can create macros that perform the multiple functions listed above, and then start the macro with one mouse click.

For information, see the *Data Explorer Software User's Guide*, Section 6.7, Using the Macro Recorder.

Processing

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.

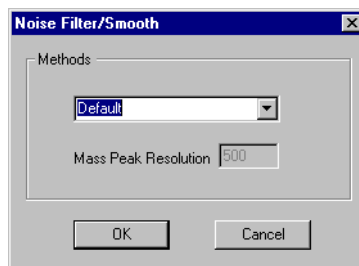


Figure 5-2 Noise Filter/Smooth Dialog Box

4. Select **Default** for the smoothing method, then 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.

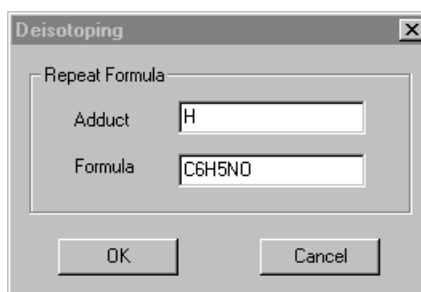


Figure 5-3 Deisotoping Dialog Box

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 Process menu, select **Mass Calibration** and then select **Manual Calibration**.

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

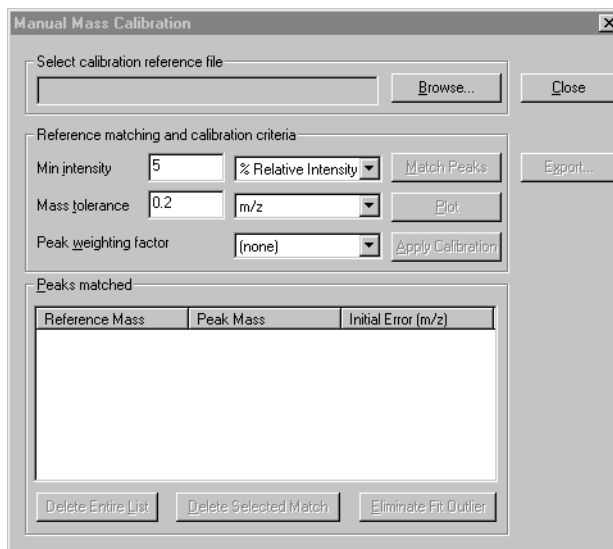


Figure 5-4 Manual Mass Calibration Dialog Box

2. Click and select the **VOYAGER.REF** calibration reference file.

3. 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*.

Manually matching peaks

4. To manually select the reference mass for a peak, right-click-drag over the peak of interest.

The Select or Create Reference Peak Information dialog box (Figure 5-5) is displayed and lists all masses in the selected reference file. The entry highlighted is the nearest match in the calibration reference file to the selected peak.

The dialog box contains the following fields and controls:

- Calibration reference file: C:\VOYAGER\Voyager.ref
- Name: Glu1-Fibrinopeptide B
- Theoretical Mass: 1570.6774
- Charge: 1
- Elemental Composition: (empty)
- Buttons: Browse..., OK, Cancel, Save, Insert, Delete, Update, Save As...
- Radio buttons: Resolved Isotope Mass (selected), Average Mass

Reference Mass	Type	Name	Charge	Elemental Comp
1061.240000	Average	Bradykinin	1	
1182.580800	Resolved	LH-RH	1	
1183.320000	Average	LH-RH	1	
1296.685300	Resolved	Angiotensin I	1	
1297.510000	Average	Angiotensin I	1	
1347.736000	Resolved	Substance P-amide	1	
1348.660000	Average	Substance P-amide	1	
1363.730900	Resolved	Tyr8-Substance P	1	
1364.660000	Average	Tyr8-Substance P	1	
1388.730000	Average	Pro9-Substance P	1	
1570.677400	Resolved	Glu1-Fibrinopeptide B	1	
1571.610000	Average	Glu1-Fibrinopeptide B	1	

Figure 5-5 Select or Create Reference Peak Information Dialog Box

5. Check that the select mass is correct for the selected peak.

If it is not correct, select the correct mass, then click **OK** to accept the highlighted reference mass for matching, and add it to the Peaks Matched list.

The Manual Mass Calibration dialog box is displayed again (see Figure 5-4 on page 5-6) with the observed mass and the reference mass you selected displayed in the Peaks Matched list.

Hint: You can sort the list of matches by clicking on the column header buttons. You can display complete information about a reference mass by double-clicking on the mass.

6. Repeat step 4 through step 5 until the desired peaks are in the matched list.

Eliminating data points

7. To eliminate unacceptable data points from the calibration, do either of the following:
- Select the data point (mass) in the Peaks matched list, then click **Delete Selected Match**.
 - Click **Eliminate Fit Outlier**. The software deletes the data point from the calibration with the largest calibrated Fit Error (difference between the calibrated mass and the reference mass) as reported in the Output Window (Figure 5-4).

To clear the entire list, click **Delete Entire List**.

Plotting

8. To apply the calibration constants to the spectrum displayed, click **Plot**.

The spectrum is calibrated and displayed with an MC trace label. The calibration statistics are displayed in the Result tab of the Output window (Figure 5-5).

Applying new constants to the data file

To apply the calibration constants to the data, click **Apply Calibration**. The current spectrum is calibrated and displayed with an MC trace label. The calibration constants are saved with the spectrum.

After calibration

Figure 5-6 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.

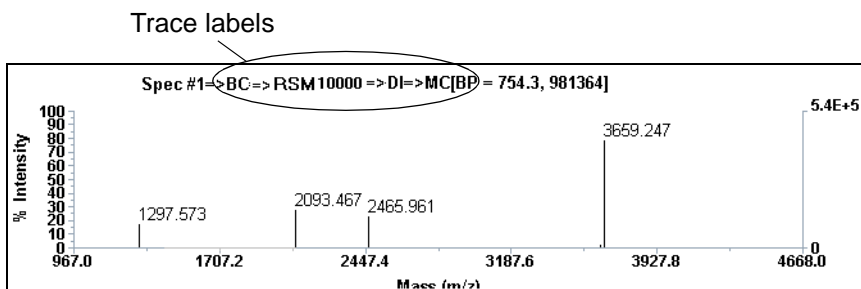


Figure 5-6 Deisotoped Spectrum After Calibration

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

5.5 Saving (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:

- 6.1 Modifying Instrument Settings for Sample Acquisition 6-2
- 6.2 Acquiring Sample 6-3

6.1 Modifying Instrument Settings for Sample Acquisition

To modify instrument settings for sample acquisition:

1. In the Instrument Settings control page, click **Manual**.
2. In the calibration section, select **External File**, then select 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).

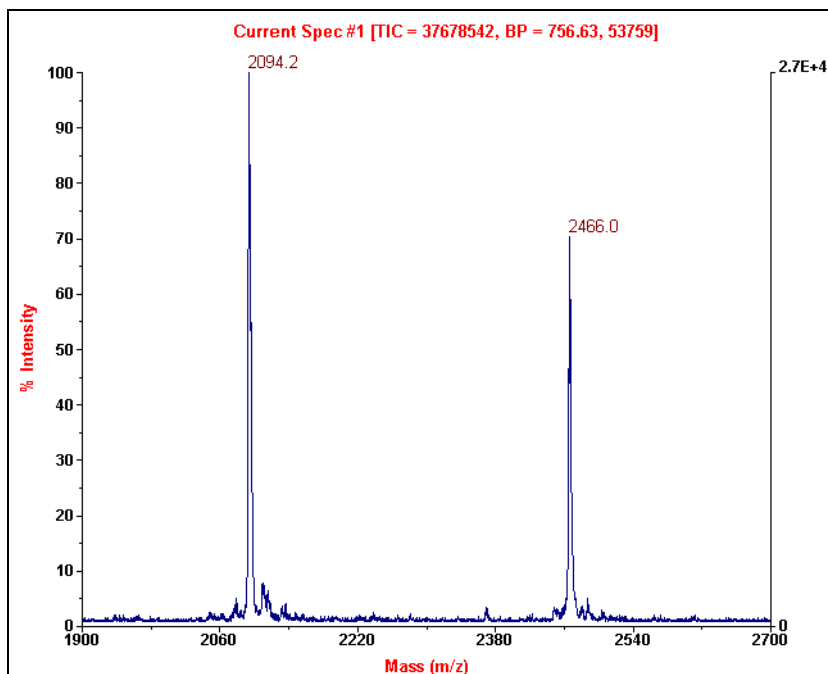


Figure 6-1 Sample Spectrum

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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