Mariner™ API-TOF Workstation

Nanospray™ Module

Supplement

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

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Instrument Safety

In this section

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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 and appears as:

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

A Hint provides helpful suggestions not essential to the use of the system and appears as:

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 and appears as:

CAUTION

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

A Warning provides specific information essential to the safety of the operator and appears as:

WARNING

CHEMICAL HAZARD. *Familiarize yourself with the MSDSs before using reagents or solvents.*

Remarques, recommandations et avertissements

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

WARNING

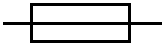
RISQUE CHIMIQUE. *Il convient de se familiariser avec la MSDS (feuille de données concernant la sécurité des matériaux) avant d'utiliser des réactifs ou des solvants.*

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.

	This symbol indicates the **on** position of the main power switch.
	This symbol indicates the **off** position of the main power switch.
	This symbol indicates the **on/off** position of a push-push main power switch.
	This symbol indicates that a terminal may be connected to another instrument's signal ground reference. This is not a protected ground terminal.
	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.
	A terminal marked with this symbol either receives or delivers alternating current or voltage.
	A terminal marked with this symbol can receive or supply an alternating and a direct current or voltage.
	This symbol appears next to the values of the fuses required by the system.

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

Non-electrical Symbols

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

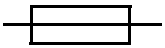
	**WARNING**: This symbol illustrates a heater hazard. Proceed with caution when working around these areas to avoid being burned by hot components.
	This symbol indicates that a laser is present inside the instrument.

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 *MARCHE* de l'interrupteur d'alimentation principal.
	Position *ARRÊT* de l'interrupteur d'alimentation principal.
	Positions MARCHE-ARRÊT 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 the 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. *Familiarize yourself with the MSDSs before using reagents or solvents.*

WARNING

RISQUE CHIMIQUE. *Il convient de se familiariser avec la MSDS (feuille de données concernant la sécurité des matériaux) avant d'utiliser des réactifs ou des solvants.*

General Warnings

WARNING

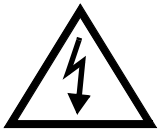
FIRE HAZARD. *Using a fuse of the wrong type or rating can cause a fire. Replace fuses with those of the same type and rating.*

WARNING

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

ELECTRICAL SHOCK HAZARD. *Severe electrical shock can result by operating the instrument without the front or side panels. 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.*

WARNING

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 system only as specified in this document. Using this system in a manner not specified may result in injury or damage to the system.*

WARNING

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

WARNING

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

WARNING

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.*

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 materiel brouilleur du Canada.

European Safety and EMC Standards

CE

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”.

1 Overview of the Nanospray Module

The Mariner™ Nanospray Module (Figure 1-1) is an optional spray chamber and associated parts that allow low-flow and low-volume operation to conserve sample.

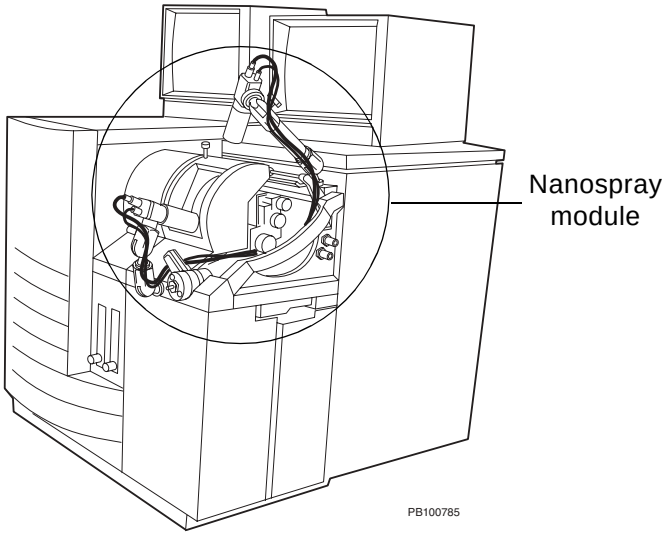


Figure 1-1 Mariner Workstation with Nanospray Module Installed

Hardware requirements

The nanospray module requires a curtain gas plate located in front of the nozzle and internal curtain gas tubing. If your Mariner Workstation serial number is:

- **5058 or later**—Curtain gas plate and plumbing is installed before your system is shipped to you.
- **5057 or earlier**—Curtain gas plate and plumbing must be installed by an Applied Biosystems Technical Representative. Contact Applied Biosystems Technical Support at a number listed at the end of the User's Guide.

Parts of the nanospray module

The nanospray module includes:

- Nanospray spray chamber with 0.5 µl injection valve
- Two cameras that you mount on the spray chamber
- Two video monitors that display spray tip position
- Nanospray kit, including nanospray two types of spray tips
- Microspray kit, including microspray and low-flow electrospray spray tips
- Syringe kit, including tweezers and carbide blades

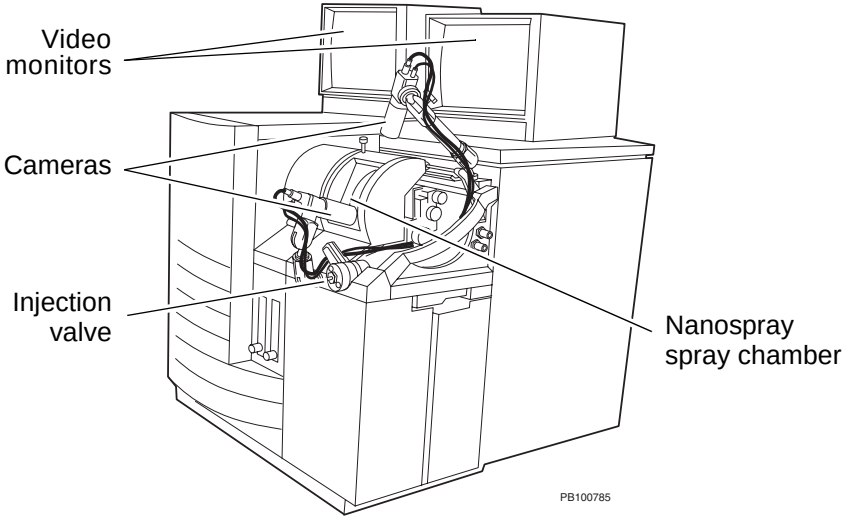


Figure 1-2 Parts of the Nanospray Module

Modes of operation

You can use the nanospray module in three modes of operation:

Mode	Flow Rates	Comments
Nanospray	• 1 to 100 nanoliters per minute • Single-sample, single-tip operation	Accommodates sample volumes of up to 15 µl, with flow rates of 60 to 70 nl/min when using the standard open-end spray tips provided and a 50:50 acetonitrile:water solvent.
Microspray	200 to 500 nanoliters per minute (with the tips provided) ***NOTE**: You can run at lower (100 nl/min) or higher (1 µl/min) flow rates if you use spray tips with smaller or larger bores than the spray tips provided.*	Allows continuous-flow loop injections using the integrated low-volume injection valve. Microspray mode provides two modes of sample introduction: • Continuous-flow loop injection using the integrated low-volume injection valve or an LC system • Infusion using the integrated syringe pump ***NOTE**: As tips wear, the opening enlarges, and you may see stable spray at higher flow rates but not at lower flow rates. If you flow at >500 nl/min, even for a short time, the opening on the tip may be permanently enlarged, and may not yield acceptable performance at lower flow rates. For more information, see "When to replace the spray tip" on page 76.*
Low-flow electrospray	1 to 5 µl per minute	See Microspray mode comments.

2 Installing the Nanospray Module

Installation includes:

- Installing the cameras and video monitors
- Connecting the curtain gas supply

2.1 Installing the Cameras and Video Monitors

Installing

To install the cameras and video monitors:

1. Place the nanospray spray chamber on a bench next to the Mariner Workstation.
2. Screw the cameras onto the mounting arms on the front and top of the nanospray spray chamber (see Figure 2-1 on page 7).

***NOTE**: Make sure the wing nut holding the front camera mount is installed on the left side of the mount. If it is installed on the right side of the mount, it interferes with the injection valve lever.*

---

3. Install the nanospray spray chamber. See Section 3.2, Installing and Removing the Nanospray Spray Chamber.
4. Place the video monitors on the top left of the Mariner Workstation (see Figure 1-2 on page 2).
5. Plug the video monitor power cords into an electrical outlet.
6. Connect the RCA-end of the video cable to the Video Out connector on the camera.

7. Connect the video cable from round BNC connector, under the source handle, to the back of the video monitor.
8. Connect the end of the power cable to the Power connector on the camera.
9. Power up the video monitors.

   Aim the cameras in the direction of the nozzle and spray tip. Figure 2-1 shows approximate positions of the cameras.

Focusing

After installing a spray tip as described in Section 4.4, Preparing the Nanospray Spray Tip, or Section 6.3, Replacing the Microspray/ Low-Flow Electrospray Fused Silica Spray Tip and Capillary, focus the cameras:

1. Use the adjustment knobs in the spray chamber (see Figure 3-3 on page 18) to position the spray tip about 1 cm from the opening in the curtain gas plate.

   Figure 2-1 shows approximate positions of the cameras.

   Figure 2-2 shows the view through the camera when the spray tip is positioned properly.

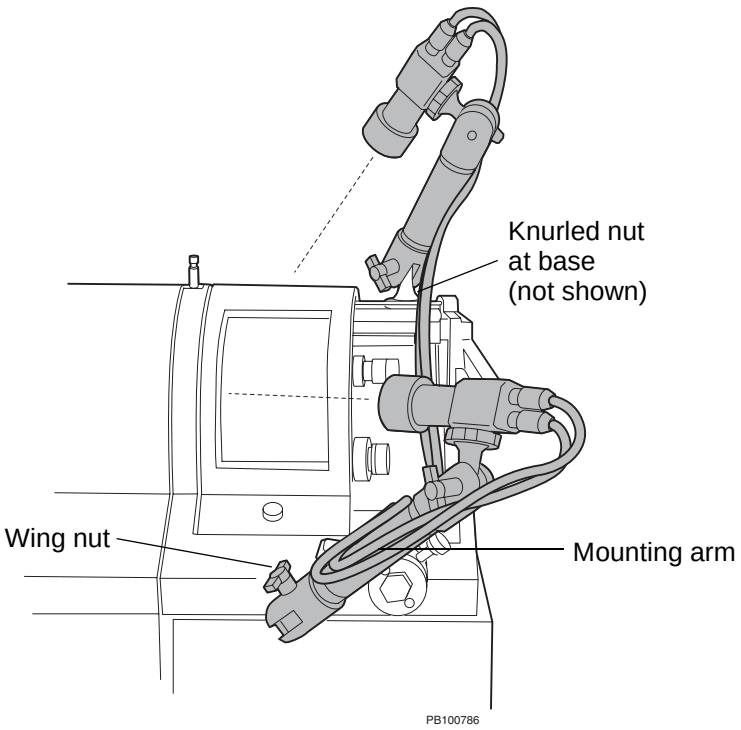


Figure 2-1 Approximate Positions of Cameras

***NOTE**: Do not aim the front camera straight into the spray chamber. If you do so, the camera will point at the lamp at the back of the spray chamber and create glare on the video monitor. Instead, angle the camera to the right and aim it toward the nozzle.*

***Hint**: You can move the top camera out of the way when necessary to open the cover without altering the relative position of the lens. Loosen the knurled nut at the base of the mounting arm on the top of the spray chamber. Do not loosen the wing nuts holding the mounting arms in position. Rotate the camera assembly to the right.*

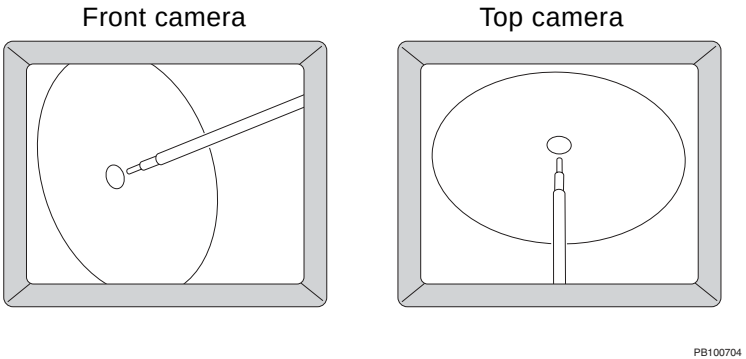


Figure 2-2 View Through Camera When Spray Tip is Properly Positioned

2. Focus the cameras on the spray tip and nozzle opening by grasping the mounting arms and moving the cameras closer to or farther from the tip. You can make fine adjustments in the focus by loosening the locking screw on the lens and extending or retracting the lens of the camera. Tighten the locking screw after focusing.

2.2 Connecting and Configuring the Curtain Gas Supply

This section includes:

- Connecting the curtain gas
- Configuring the curtain gas
- Adjusting the curtain gas

Using one gas supply

If you use nitrogen for the nebulizer (electrospray applications only) and curtain gases, you can connect one line from the regulated nitrogen supply and install a quick-connect tee (provided in the Mariner Workstation startup kit) just before the Gas 1 and Gas 2 connections on the right side panel of the workstation.

Curtain gas requirements

Use one of the following for curtain gas:

- Compressed dry nitrogen, grade 4.8 or better
- Liquid nitrogen in a dewar (requires drying if you see water clusters or water adducts on proteins)

2.2.1 Connecting Curtain Gas

For more information on this procedure, please see the *Mariner API-TOF Workstation User's Guide* formerly called *Mariner Biospectrometry™ Workstation User's Guide.*

To connect the curtain gas supply line:

Using Teflon® tubing (1/16-inch ID, 1/8-inch OD), connect the gas supply to the **Gas 2** fitting on the right side of the mass spectrometer.

Push the tubing on to the push-on fittings on the mass spectrometer.

2.2.2 Configuring the Curtain Gas

To configure the curtain gas:

1. In the Instrument Control Panel, select **System Settings** from the Instrument menu, then click the **Gas Flow** tab to display the Gas Flow page (Figure 2-3).

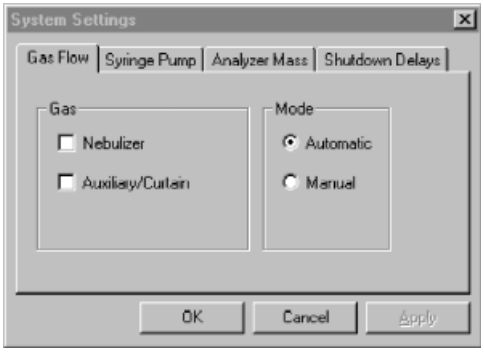


Figure 2-3 Gas Flow Page

2. Select **Auxiliary/Curtain**.

***NOTE**: Nebulizer gas is not used for nanospray or microspray applications.*

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3. Select the gas mode:

   - **Automatic**—Gas flow starts automatically when instrument state is set to On.

     ***NOTE**: You can override automatic control mode by clicking*  *in the toolbar during operation.*

   - **Manual**—Gas flow does not start automatically when instrument state is set to On. Click  in the toolbar to start gas flow during operation.

2.2.3 Adjusting the Curtain Gas

To adjust the curtain gas:

1. Regulate the gases at the supply to **80** to **100** psi.

CAUTION

Do not regulate gas above 100 psi. Doing so can damage the gas seals in the Mariner Workstation. Regulating the gas below 80 psi may not generate high enough gas pressure at the flow meters and may not engage the gas seals.

2. Turn gas on using toolbar buttons in the Instrument Control Panel:
   - If the gas flow is in automatic mode, click  in the toolbar to turn on.
   - If the gas flow is in manual mode, click  in the toolbar to turn on.
3. Adjust the curtain gas flow rate using the left flow meter on the front panel of the mass spectrometer. Adjust to:

Mode	Flow Rate
Nanospray	0.1 to 0.2 liter/min
Microspray	0.5 to 0.8 liter/min
Low-flow electrospray	0.5 to 0.8 liter/min

***NOTE**: It can be difficult to adjust gas flow exactly to the low flow rates used for nanospray, microspray, and low-flow electrospray applications. Note that if the curtain gas flow rate is too high, you will see unstable spray; if it is too low, desolvation will be incomplete.*

***NOTE**: If you adjust the curtain gas above 0.5 liter/min, examine the tip to ensure that the higher gas flow rate is not causing the tip to oscillate.*

4. Turn gas off as needed using toolbar buttons in the Instrument Control Panel:

   - If the gas flow is in automatic mode, click  in the toolbar to turn off.
   - If the gas flow is in manual mode, click 

in the toolbar to turn off.

CAUTION

Do not turn gas off using the flow meter on the front panel of the mass spectrometer. Doing so will damage the valve in the flow meter. Turn off gas using toolbar buttons or at the gas supply.

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3 Overview of the Spray Chamber

This section describes:

- Safety information
- Installing and removing the nanospray spray chamber
- Using spray tip adjustment knobs
- Factors affecting signal quality
- Handling spray tips

3.1 Safety Information

Safety interlocks

The Mariner Workstation includes safety interlocks that prevent you from turning on high voltages if:

- Spray chamber is not installed
- Spray chamber is open
- Mass spectrometer top cover panel is not in place

If any of the above conditions are true, interlocks are considered “open”, and:

- Interlocks LED on the front panel is yellow and blinking. See Figure 1-10 IN the *Mariner API-TOF Workstation User’s Guide*.
- Interlocks indicator on the System Status page in the Instrument Control Panel is yellow. See the relevant section for system status in the *Mariner API-TOF Workstation User’s Guide*.

To reset the safety interlocks, install or close the spray chamber and replace the top cover panel. If the fault does not clear, press the Reset button on the right side panel of the system near the back. See Figure 2-1 in the *Mariner API-TOF Workstation User’s Guide*.

Spray chamber precautions

Read the following warnings before handling the spray chamber.

WARNING

***BURN HAZARD**. When the spray chamber is removed, the curtain gas plate is exposed and may be hot. Do not touch.*

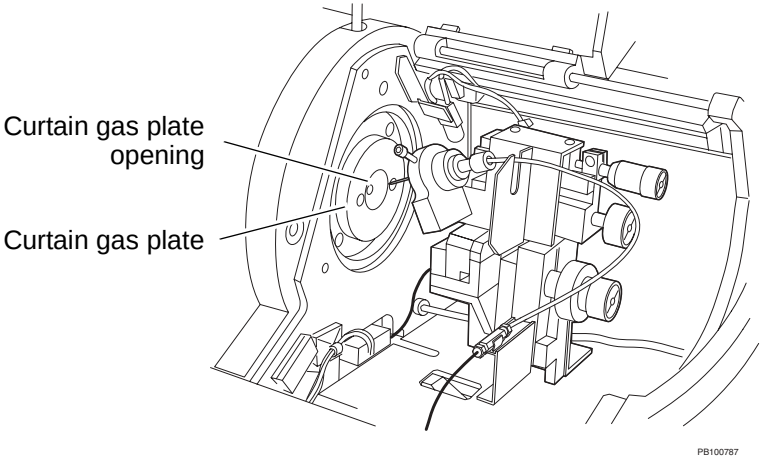
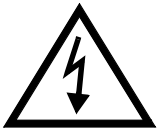


Figure 3-1 Spray Chamber with Cover Open—Curtain Gas Plate Location (Nanospray Spray Head Installed)



WARNING

***ELECTRICAL SHOCK HAZARD**. High voltage safety interlocks automatically turn off high voltages when you open the spray chamber cover. If interlocks fail or are disabled, high voltages are present when you open the spray chamber cover.*

3.2 Installing and Removing the Nanospray Spray Chamber

Installing the spray chamber

To install the nanospray spray chamber:

1. Read “Spray chamber precautions” on page 14 before proceeding.
2. Make sure the spray chamber lever is pulled out (see Figure 3-2 on page 17).
3. Hold the spray chamber at the top front right corner of the workstation with the position knobs facing toward you.
4. Push the spray chamber to the back wall of the workstation and slide the spray chamber to the left into place.

   Watch to make sure that the electrical and gas connectors on the spray chamber and the workstation are aligned.
5. Press the spray chamber firmly into place and push in the spray chamber lever so that it is flush with the side of the mass spectrometer.

   When the spray chamber is properly installed, the lamp in the spray chamber turns on, and the Interlock LED turns green.
6. If you are setting up for a microspray application, connect the fused silica capillary to the injection valve or to the syringe. See Section 5.3, Connecting Injection Devices.
7. Connect the video and power connectors to both cameras.

Removing the spray chamber

To remove the nanospray spray chamber:

1. Read “Spray chamber precautions” on page 14 before proceeding.
2. Stop solvent flow (microspray only).

CAUTION

Always turn off solvent flow before turning off high voltages to prevent liquid from accumulating in the spray chamber. Accumulated liquid can cause degradation of gas seals.

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3. In the Instrument Control Panel, select **Instrument-Off** from the Instrument menu.

CAUTION

Removing the spray chamber when the instrument is in Standby or On state (and gas and internal heaters are on) can damage the gas seals and cause fuses to blow.

***NOTE**: Do not leave the instrument state set to Off for any longer than necessary. Setting the state to Off turns off instrument heaters. Internal elements of the system can take up to an hour and a half to equilibrate.*

High voltages, integrated syringe pump, and gases are turned off. The gas seals release when the gases are turned off.

4. In the Instrument Control Panel, check the status bar to make sure the High Voltage status is Off.

5. Disconnect the inlet tubing from the integrated injection valve, if it is connected, or from the syringe (Figure 3-2).

***NOTE**: Disconnect the inlet tubing at the syringe pump or injection valve, instead of at the microspray head. The fused silica capillary fitting connection on the microspray head end of the inlet tubing requires careful adjustment of the capillary in the fitting.*

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6. Remove the video and power connectors from both cameras (Figure 3-2).

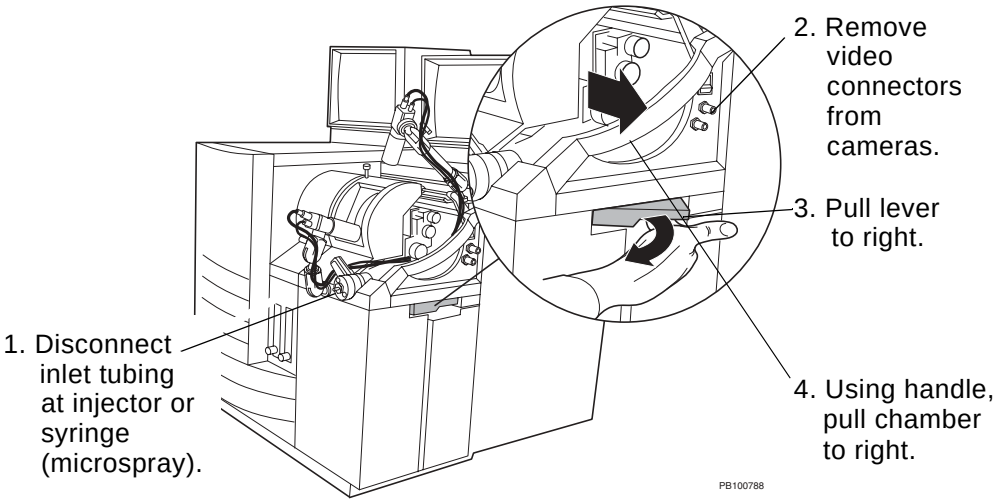


Figure 3-2 Removing the Nanospray Spray Chamber

7. Pull the spray chamber lever to the right (Figure 3-2).

8. Remove the spray chamber by pulling it to the right, away from the workstation (Figure 3-2), and lifting it out of the way.

3.3 Using Spray Tip Adjustment Knobs

Use the adjustment knobs in the spray chamber to adjust the position of the spray tip (Figure 3-3).

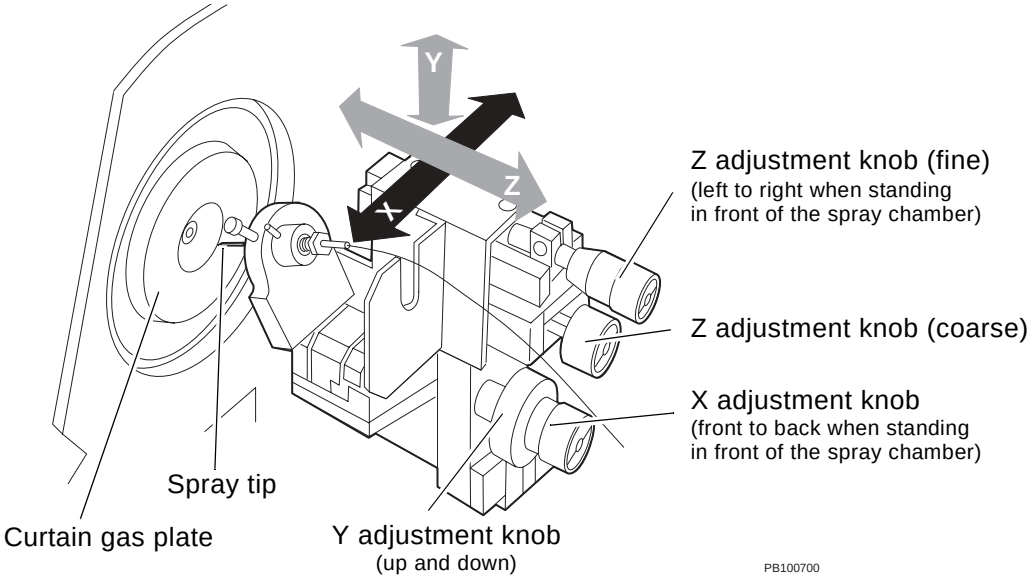


Figure 3-3 Spray Tip Adjustment Knobs

3.4 Factors Affecting Signal Quality

This section describes the factors that have a strong effect on signal quality.

- Spray tip position
- Spray Tip Potential
- Curtain gas

3.4.1 Spray Tip Position

This section includes:

- Angle of the spray tip
- Distance from nozzle opening (Z adjustment)
- Relationship of flow rate, spray tip position, and Spray Tip Potential

For more information, see:

- Section 4.5, Optimizing Signal in Nanospray Mode
- Section 5.6, Optimizing Signal in Microspray/ Low-Flow Electrospray Mode

Angle of the spray tip

Figure 3-4 is a side view of the nanospray spray tip that illustrates the position of the spray tip relative to the nozzle opening.

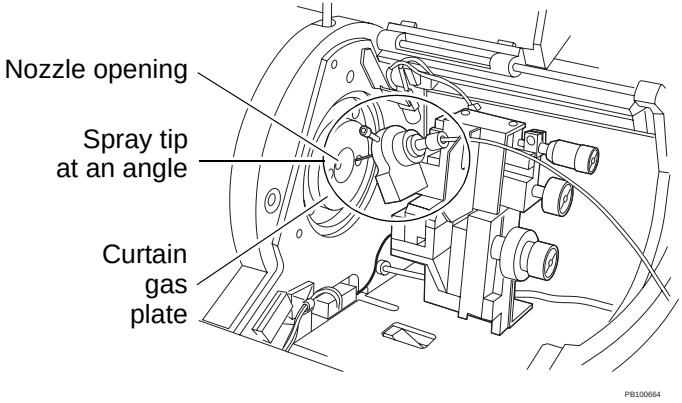


Figure 3-4 Nanospray Spray Tip Position (Side View)

The spray chamber is designed with the nanospray spray tip at an angle to the nozzle opening to prevent sample from flowing out the back end of the spray tip.

***NOTE**: The microspray spray tip is also positioned at an angle because it uses the same mounting bracket as the nanospray spray tip.*

Distance from nozzle opening (Z adjustment)

The distance that you adjust between the spray tip and the nozzle opening is critical in the electrospray process. The exact position you set for the spray tip depends on sample, solvent, and ID of the spray tip. Note the following general guidelines:

- **In nanospray applications**—Optimum spray tip position is typically 2 to 5 mm from the nozzle opening, or just inside the curtain gas opening during operation. See Figure 4-8 on page 42.
- **In microspray applications**—Optimum spray tip position is typically 0 to 5 mm from the nozzle opening during operation. See Figure 5-5 on page 67.
- **In low-flow electrospray applications**—Optimum spray tip position is typically 2 to 10 mm from the nozzle opening during operation. See Figure 5-6 on page 67.
- **If the tip is too far from the nozzle opening**—Field is not strong enough. Charged droplets do not form. You may still see droplets, but they are not charged.
- **If the tip is too close to the nozzle opening**—Electrical discharge (arcing) occurs between needle and nozzle.

***NOTE**: The spray tip must be also centered on the nozzle opening (X and Y adjustment).*

Effect of flow rate on spray tip position

The flow rate you use will determine the spray tip position and Spray Tip Potential needed to obtain optimum sensitivity.

Higher flow rates require larger distances between the spray tip and the nozzle opening.

Flow rate is dependent on sample and solvent viscosity and the internal diameter of the spray tip.

3.4.2 Spray Tip Potential

Spray Tip Potential affects the formation of a stable spray:

- **Increasing the Spray Tip Potential**—Helps nebulize more polar solvents with higher surface tension.
- **If Spray Tip Potential is too high**—Causes electrical breakdown with corona discharge, no electrospray ionization.
- **Decreasing the Spray Tip Potential**—Minimizes electrochemical reaction of sample, for example, oxidation of cysteine in a peptide.
- **If Spray Tip Potential is too low**—Charged droplets do not form.

Effect of flow rate on Spray Tip Potential

Higher Spray Tip Potential is required with higher flow rates to allow desolvation.

You set the Spray Tip Potential on the Spray Chamber control page in the Instrument Control Panel. For more information, see *Mariner API-TOF Workstation User's Guide*.

3.4.3 Curtain Gas

Curtain gas affects ionization and desolvation. Curtain gas is applied between the curtain gas plate and the nozzle. Typical flow rates for curtain gas are:

Mode	Flow Rate
Nanospray	0.1 to 0.2 liter/min
Microspray	0.5 to 0.8 liter/min
Low-flow electrospray	0.5 to 0.8 liter/min

Use lower curtain gas flow for lower solvent flow rates.

***NOTE**: If you adjust the curtain gas above 0.5 liter/min, examine the tip to ensure that the higher gas flow rate is not causing the tip to oscillate.*

3.5 Handling Spray Tips

WARNING

PHYSICAL INJURY HAZARD*. Handle glass and fused silica spray tips with care. Spray tips can break and cause injury.*

Storage

After opening the spray tip shipping bag, store spray tips in a dust-free environment, for example, in a desiccator or resealable plastic bag.

When retrieving tips from the plastic shipping case, do not leave the case open any longer than necessary.

Handling

Handle glass nanospray tips with tweezers.

Do not touch the tapered ends of nanospray or microspray tips with bare fingers.

Handle fused silica microspray tips with gloves.

4 Nanospray Operation

This section includes:

- Overview
- Sample, solvent, and LC column guidelines
- Spray tip specifications
- Preparing the nanospray spray tip
- Optimizing signal in nanospray mode

4.1 Overview

Sample volume and flow rate

Nanospray operation allows analysis of very low sample volumes (up to 15 µl) at flow rates of 1 to 100 nl/min. Each analysis requires a new sample tip. A combination of gravity and voltage induce sample flow. Viscous samples or samples with high surface tension may require the application of gentle air pressure to initiate flow from the nanospray spray tip.

Spray tips

Two types of nanospray spray tips are provided:

- **Open-end tips with preformed opening**—Provided for ease-of-use and better reproducibility
- **Closed-end tips without preformed opening**—Provided as a lower-cost option

For more information, see Section 4.3, Spray Tip Specifications.

Parts of the spray chamber in nanospray mode

Figure 4-1 illustrates the parts of the nanospray spray chamber with a nanospray spray tip installed.

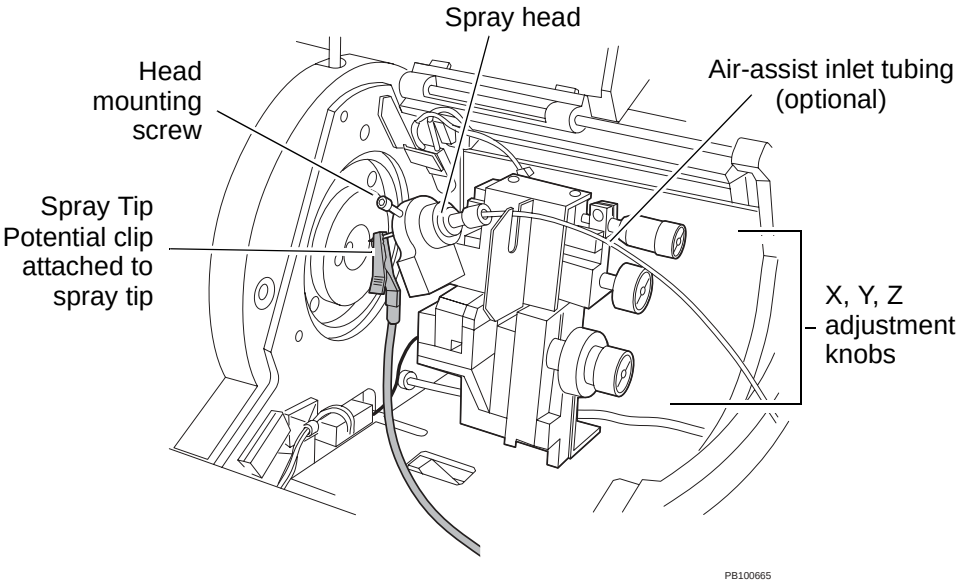


Figure 4-1 Parts of the Nanospray Spray Chamber

Overview of nanospray operation

Nanospray operation involves:

- Breaking off the fine tip to open the tip (if you are using closed-end tips)
- Filling a disposable tip with sample
- Installing the tip
- Positioning the spray tip to optimize signal intensity

The steps above are described in detail in:

- Section 4.4, Preparing the Nanospray Spray Tip
- Section 4.5, Optimizing Signal in Nanospray Mode

After performing the steps above, operation is identical to standard electrospray operation. Refer to the relevant sections of the *Mariner API-TOF Workstation User's Guide* for information on performing the following tasks:

- tuning and calibrating the mass analyzer
- acquiring data

Refer to the *Data Explorer User's Guide,* formerly called *Biospectrometry™ Data Explorer User's Guide*, to learn how to use the Data Explorer software to process and analyze data.

For additional information, refer to the following sections in this document:

- Section 6, Maintenance
- Section 7, Troubleshooting

4.2 Sample, Solvent, and LC Column Guidelines

For information on sample, solvent and LC column guidelines, and sample cleanup, refer to the relevant sections of the *Mariner API-TOF Workstation User's Guide*.

4.3 Spray Tip Specifications

Open-end and closed-end nanospray tips are provided. The nanospray spray tips have the following specifications:

Spray Tip/Use	Specifications
Open-end tips with preformed opening (Easier to use because it is not necessary to break off the fine tip to open the tip; better reproducibility)	• Metal-coated borosilicate glass, with open tapered end • 4 μm opening • 1.2 mm OD • 5 cm uncut length
Closed-end tips without preformed opening (Lower cost, but require you to break off the fine tip to open the tip) Three variations: • **Normal (N)**—For typical applications. High taper, moderate fine tip. • **Long (L)**—For low-flow, low-viscosity samples free of particulate matter. Lower taper, longer fine tip. • **Short (S)**—For higher viscosity samples. High taper, very short fine tip. Difficult to break off at correct point to yield low flow rate.	• Metal-coated glass, with closed tapered end • Size of opening determined by how you break off end during optimization (see Section 4.5.1, Preparing the Workstation) • 1.2 mm OD • 5 cm uncut length

Reusing spray tips

You can reuse spray tips several times when introducing standards, if desired. Tips will eventually become clogged. If you see decreased performance with a previously-used tip, use a new tip.

4.4 Preparing the Nanospray Spray Tip

This section includes:

- Removing the nanospray head
- Removing the old spray tip
- Breaking off the fine tip (if you are using closed-end tips)
- Filling the nanospray spray tip
- Inserting a filled spray tip
- Reinstalling the nanospray head

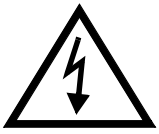
4.4.1 Removing the Nanospray Head

WARNING

***PHYSICAL INJURY HAZARD**. To prevent eye injury, wear safety glasses when performing this procedure.*

To remove the nanospray head:

1. In the Instrument Control Panel, select **Instrument-Off** from the Instrument menu.
2. Move the top camera out of the way and open the spray chamber cover.



WARNING

***ELECTRICAL SHOCK HAZARD**. High voltage safety interlocks automatically turn off high voltages when you open the spray chamber cover. If interlocks fail or are disabled, high voltages are present when you open the spray chamber cover.*

***Hint**: You can move the top camera out of the way when necessary without altering the relative position of the lens. Loosen the knurled nut at the base of the mounting arm on the top of the spray chamber. Do not loosen the wing nuts holding the mounting arms in position. Rotate the camera assembly to the right.*

3. Turn the coarse Z adjustment knob (see Figure 3-3 on page 18) fully counterclockwise to move the spray tip as far away from the curtain gas plate as possible. Turn the fine Z adjustment knob counterclockwise until the spray tip mounting block (Figure 4-2) stops moving. The fine adjustment knob will fall off if you turn it too far.

***NOTE**: The spray tip mounting block is spring-loaded. Always retract it fully before handling the spray tip to avoid accidentally hitting the tip against the curtain gas plate.*

---

4. Remove flow-assist air inlet tubing from the back of the nanospray head if it is connected (Figure 4-2).

5. Remove the Spray Tip Potential clip from the spray tip if it is installed.

6. Loosen the head mounting screw and remove the head from the chamber.

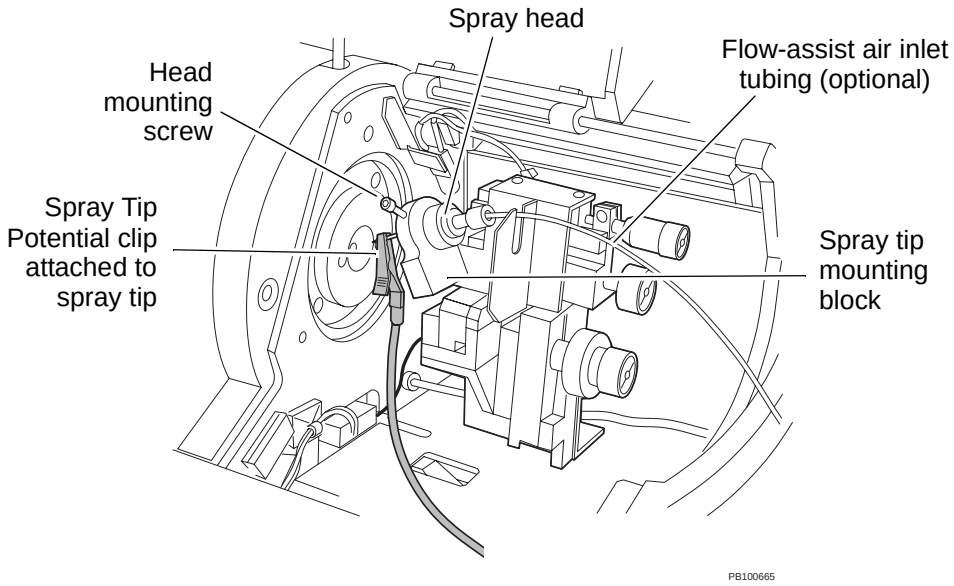


Figure 4-2 Removing the Nanospray Head

4.4.2 Removing the Old Spray Tip

WARNING

***PHYSICAL INJURY HAZARD**. To prevent eye injury, wear safety glasses when performing this procedure.*

To remove the old nanospray spray tip:

1. Carefully grasp the middle of the spray tip with tweezers and pull straight out through the front of the head (Figure 4-3).

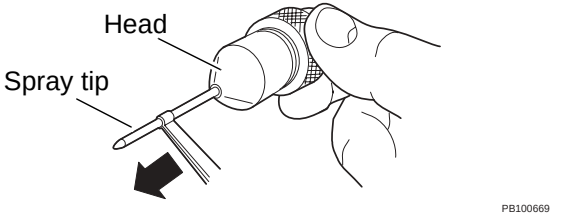


Figure 4-3 Removing the Old Spray Tip

WARNING

***PHYSICAL INJURY HAZARD**. Handle glass and fused silica spray tips with care. Spray tips can break and cause injury.*

2. Discard the old spray tip in a container appropriate for needles and other sharp objects.

4.4.3 Breaking Off the Fine Tip (Closed-End Tips Only)

WARNING

PHYSICAL INJURY HAZARD*. To prevent eye injury, wear safety glasses when performing this procedure.*

NOTE*: Wear powder-free gloves when handling new spray tips. Finger oil on the spray tip can affect performance.*

If you are using a closed-end tip (described on page 28), break off the fine tip to open the tip before filling:

1. Place a pad of paper (or other flat surface that is approximately 1/4-inch high) under a 10 to 20X magnifying source.

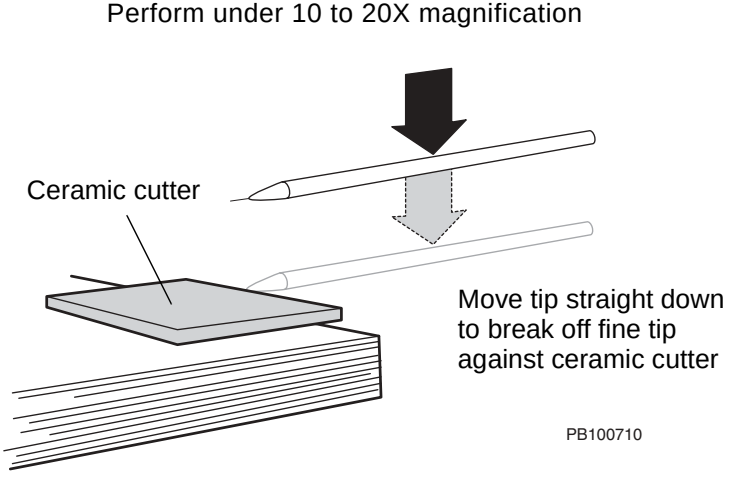


Figure 4-4 Breaking Off the Fine Tip of a Closed-End Tip

2. Hold a ceramic cutter flat on the pad so that the edge of the cutter extends beyond the edge of the pad (Figure 4-4).

3. Hold a tip over the ceramic cutter and move the tip straight down until the fine tip (hair-like projection on the taper) touches the ceramic and breaks off.

***NOTE**: Break off the fine tip protruding from the taper, not the taper itself (Figure 4-5). If you break the taper, the opening will be too large and cause poor performance.*

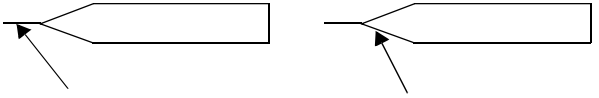


Figure 4-5 Breaking Open a Closed-End Spray Tip

4.4.4 Filling the Nanospray Spray Tip

WARNING

PHYSICAL INJURY HAZARD*.To prevent eye injury, wear safety glasses when performing this procedure.*

NOTE*: Wear powder-free gloves when handling new spray tips. Finger oil on the spray tip can affect performance.*

Breaking open closed-end tips

If you are using a closed-end nanospray spray tip, break open the tip before filling. See Section 4.4.3, Breaking Off the Fine Tip (Closed-End Tips Only).

Filling open-end or closed-end tips

To fill open-end or closed-end nanospray spray tips with sample:

1. Using the tweezer provided in the nanospray kit, pick up a nanospray spray tip.
2. Connect a gel-loader pipet tip (provided in the nanospray kit) to a 10 µl pipettor.
3. Aspirate sample. Nanospray tip maximum volume is 15 µl.
4. Insert the pipet tip into the non-tapered end of the spray tip, as far down into spray tip as possible.
5. Holding the spray tip at a 45° angle with the tapered end down, ***slowly*** dispense the sample into the spray tip.
6. Visually examine the spray tip for air bubbles. Air bubbles will prevent sample from flowing out of the sample tip.
7. If you see bubbles in the tip, hold the tip vertically with the tapered end down. If bubbles are not displaced by gravity, shake the tip, tapered end down, with a snapping motion to displace the bubbles.

CAUTION

Be extremely careful when handling or shaking the spray tip. Any contact with other objects can damage the spray tip.

8. If you cannot remove air bubbles by shaking the tip, remove sample using the 10 µl pipettor and gel-loader pipet tip, and redispense into the spray tip.
9. To prevent sample evaporation, you can wrap the non-tapered end of the tip with a small piece of parafilm.

***NOTE**: Samples with high organic solvent content can extract compounds such as phthalate from the parafilm.*

4.4.5 Inserting a Filled Spray Tip

WARNING

***PHYSICAL INJURY HAZARD**. To prevent eye injury, wear safety glasses when performing this procedure.*

***NOTE**: If you are using a closed-end tip, make sure the tip is open before inserting the tip. For information, see Section 4.4.3, Breaking Off the Fine Tip (Closed-End Tips Only).*

To insert a new filled tip into the spray head:

1. With tweezers, hold the filled spray tip at the center of the tip, tapered end down.
2. Insert the non-tapered end into the front of the nanospray head until 2.5 to 3 cm of the tapered end extends from the front of the spray head (Figure 4-6).

WARNING

***PHYSICAL INJURY HAZARD**. Handle glass and fused silica spray tips with care. Spray tips can break and cause injury.*

CAUTION

Do not handle the spray tip near the tapered end. You can damage the metal coating and break the tip.

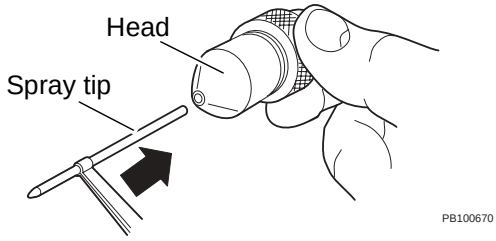


Figure 4-6 Inserting a New Spray Tip

You should feel firm resistance from the O-ring in the head when you insert the filled spray tip:

- If you cannot insert the spray tip without excessive force, loosen the set screw inside the head. See Figure 6-1 on page 73.
- If you cannot insert the spray tip after loosening the set screw inside the head, remove the O-ring from the head and slide the tip through the O-ring several times. Reassemble the head, and insert the tip. For information, see Section 6.1, Replacing the Nanospray Head O-Ring.
- If the spray tip slips through the head, tighten the set screw inside the head. See Figure 6-1 on page 73.
- If tightening the set screw does not secure the spray tip, replace the head O-ring. See Section 6.1, Replacing the Nanospray Head O-Ring.

4.4.6 Reinstalling the Nanospray Head

***NOTE**: If you are using a closed-end tip, make sure the tip is open before reinstalling the nanospray head. For information, see Section 4.4.3, Breaking Off the Fine Tip (Closed-End Tips Only).*

To reinstall the nanospray head:

1. Make sure the mounting block in the spray chamber is retracted as far away from the curtain gas plate as possible. Turn the coarse Z adjustment knob (see Figure 3-3 on page 18) completely counterclockwise. Turn the fine Z adjustment knob counterclockwise until the mounting block stops moving.
2. Install the head in the spray tip mounting block and tighten the mounting screw (see Figure 4-2 on page 31).
3. Perform the following procedures in Section 4.5, Optimizing Signal in Nanospray Mode:
   - Section 4.5.1, Preparing the Workstation
   - “Applying voltages and gas and observing initial signal” on page 43

   If flow does not start, apply gentle air pressure. See Section 4.5.3, Installing the Flow-Assist Air Syringe.

4.5 Optimizing Signal in Nanospray Mode

This section includes:

- Preparing the workstation
- Optimizing spray tip position and Spray Tip Potential
- Installing the flow-assist air syringe

4.5.1 Preparing the Workstation

WARNING

PHYSICAL INJURY HAZARD*. To prevent eye injury, wear safety glasses when performing this procedure.*

To prepare the workstation before optimizing signal:

1. Read “Spray chamber precautions” on page 14 before proceeding.
2. Be familiar with the information in Section 3.4, Factors Affecting Signal Quality.
3. Load an instrument settings file. See the *Mariner API-TOF Workstation User’s Guide* for additional information.
4. In the Instrument Control Panel, select **Instrument-Standby** from the Instrument menu.

5. Attach the Spray Tip Potential clip to the spray tip, close to the tapered end, without blocking your view of the tip on the video monitors (Figure 4-7). The clip must be attached near the end of tip (but not at the taper) to ensure that it is in contact with the metal coating.

WARNING

***PHYSICAL INJURY HAZARD**. Handle glass and fused silica spray tips with care. Spray tips can break and cause injury.*

***Hint**: Attach the clip horizontally, then gently push down to a vertical position to move it out of the way of the camera view.*

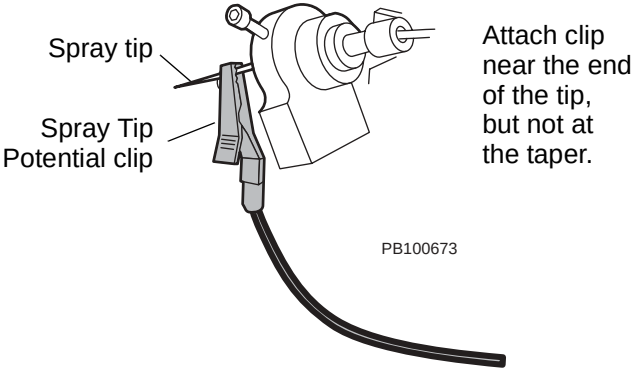


Figure 4-7 Attaching the Spray Tip Potential Clip

6. In the Instrument Control Panel, set:

   - Spray Tip Potential to **1,700 V**
   - Nozzle Temperature to **100°C**

7. Adjust the initial position of the spray tip using the adjustment knobs in the spray chamber (shown in Figure 3-3 on page 18) so that it is:

   - **1.5 cm** away from the nozzle opening (use coarse Z adjustment knob)
   - Visually centered on the nozzle opening (use X and Y adjustment knobs)

CAUTION

If the spray tip is too close to the nozzle opening when you apply voltages, arcing can occur and damage the spray tip.

***NOTE**: When the spray tip is 1.5 cm away from the nozzle opening, it may not be displayed on the video monitors. Observe the spray tip and nozzle opening directly to position the spray tip.*

4.5.2 Optimizing Spray Tip Position and Spray Tip Potential

This section includes:

- Spray tip position
- Applying voltages and gas and observing initial signal
- If you do not see signal
- When you see valid peaks
- Example TIC data

Spray tip position

The ideal position for the nanospray spray tip is 2 to 5 mm from the nozzle opening (Figure 4-8).

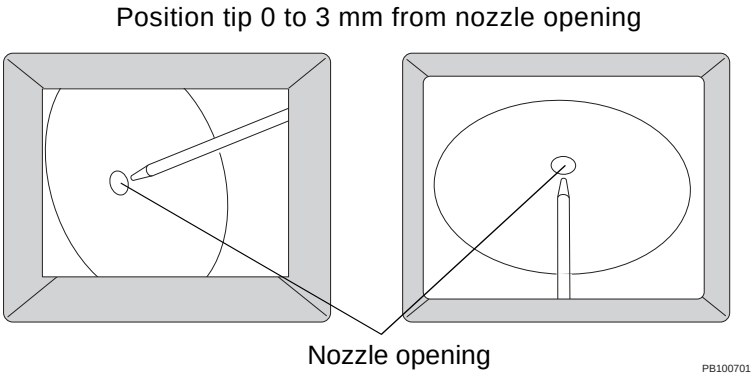


Figure 4-8 Optimum Nanospray Spray Tip Position (Camera View)

CAUTION

Touching the spray tip to the curtain gas plate, or positioning the tip less than 2 mm from nozzle opening when voltages are on, causes arcing and damages the metal coating on the spray tip. If you touch the nanospray spray tip to the curtain gas plate, discard the tip and use a new tip.

Applying voltages and gas and observing initial signal

***NOTE**: This procedure starts with the spray tip 1.5 cm away from the nozzle opening to minimize the risk of arcing. If you are familiar with the Spray Tip Potential setting and spray tip distance that yield acceptable signal without arcing, you can start optimization with the spray tip closer than 1.5 cm to the nozzle opening. If the spray tip is within 0.5 cm of the nozzle opening, the Spray Tip Potential must be set to 1,200 V or lower to avoid arcing.*

After performing the steps in Section 4.5.1, Preparing the Workstation, apply voltages and observe initial signal:

1. In the Instrument Control Panel, select **Instrument-On** from the Instrument menu.

   When you set the instrument state to On, voltages are applied, and flow should start.

2. If curtain gas is in manual mode click 

in the toolbar to turn on.

3. Adjust the curtain gas to **0.1** to **0.2 liter/min**. For more information, see Section 2.2.3, Adjusting the Curtain Gas.

4. Open the shutter.

5. Observe signal intensity in the Instrument Control Panel.

   It may take up to a minute before you see signal because of the low flow rates used in nanospray. It may take 3 to 4 minutes before you see stable signal.

***NOTE**: Depending on sample volume and flow rate, signal should last for at least several minutes. For example, with 600 nl sample volume at a flow rate of 60 nl/min, signal should last about 10 minutes.*

If signal does not last for more than one minute, the spray tip may be blocked. Make sure that sample is clean and solvents are fresh.

6. If you do not see signal, proceed to “If you do not see signal” on page 44.

   If you see signal, proceed to “When you see valid peaks” on page 45.

If you do not see signal

If you do not see signal after a minute:

1. Move the spray tip to within 1.0 cm of the curtain gas opening and wait about 20 seconds to observe signal.
2. If you still do not see signal, check:
   - Voltages are on
   - Spray Tip Potential clip is attached
   - Shutter is open
3. If you do not see signal when the spray tip is within 1.0 cm of the nozzle opening, flow has not started, and may be caused by:
   - **High sample viscosity or surface tension**—Requires the application of air pressure. Connect the syringe as described in “Installing the flow-assist air syringe” on page 48.
   - **Blocked tip**—May be caused by an air bubble or a dirty sample. Remove the bubble, clean up sample, or reload sample into a new tip.
   - **Closed-end tip not open**—Open the tip as described in Section 4.4.3, Breaking Off the Fine Tip (Closed-End Tips Only).

When you see valid peaks

When you see valid peaks:

***NOTE**: If you observe peaks that are 18 Da apart, it indicates that water clusters are forming. Increase the curtain gas flow rate by 0.1 liter/min.*

1. Turn the coarse Z adjustment knob in the spray chamber (Figure 3-3 on page 18) to move the tip **1 cm** away from the nozzle opening.
2. ***Slowly*** turn the fine Z adjustment knob in the spray chamber (Figure 3-3 on page 18) to move the spray tip about **1 mm** closer to the nozzle opening.
3. Observe signal intensity.
4. Decrease the Spray Tip Potential by **50** to **100 V**.
5. Observe signal intensity.
6. Continue repeating step 2 through step 5 to maximize signal intensity and stabilize the TIC (Figure 4-9). Note the following guidelines:
    - When the spray tip is within 5 mm of the nozzle opening, set the Spray Tip Potential to **1,200 V** or **lower** to avoid arcing.
    - Use the **fine** Z adjustment knob when the spray tip is within 5 mm of the nozzle opening. Be careful not to crash the tip into the curtain gas plate.
    - Optimum spray tip position is 2 to 5 mm from the nozzle opening. See Figure 4-8 on page 42.
    - Make initial Spray Tip Potential adjustments in increments of **100 V**, then decrease in increments of **50 V**.
    - If you set the Spray Tip Potential too low, flow will stop. Sample with high salt and buffer content can evaporate and block the spray tip.
    - Final optimum Spray Tip Potential is **900 V** to **1,000 V** (may vary depending on solvent).

7. Fine-tune the Spray Tip Potential within 100 V of the current setting by adjusting it in **25 V** increments.

8. Fine-tune the X and Y positions to better position the spray and direct ions into the nozzle opening.

Example TIC data

Figure 4-9 represents typical TIC data for nanospray applications.

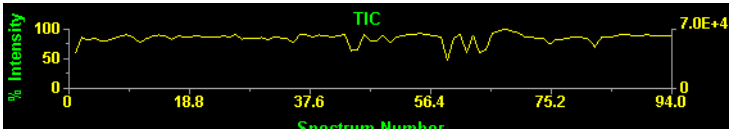


Figure 4-9 Example Nanospray TIC Data

4.5.3 Installing the Flow-Assist Air Syringe

Install a flow-assist air syringe if sample does not flow when you set the instrument state to On (applies voltages to the spray tip).

This section includes:

- Before connecting the syringe
- Connecting the flow-assist air syringe
- Determining if continuous air pressure is needed
- Installing the syringe in the clamp
- Determining if air pressure is adequate

Before installing the syringe

Before installing the syringe:

1. Turn the coarse Z adjustment knob (see Figure 3-3 on page 18) fully counterclockwise to move the spray tip as far away from the curtain gas plate as possible. Turn the fine Z adjustment knob counterclockwise until the mounting block stops moving. The fine adjustment knob will fall off if you turn it too far.

   ***NOTE**: The spray tip mounting block is spring-loaded. Always fully retract it before handling the spray tip to avoid accidentally hitting the tip against the curtain gas plate.*

   ---

2. Close the shutter.
3. In the Instrument Control Panel, select **Instrument-Standby** from the Instrument menu.
4. If curtain gas is in manual mode click 

in the toolbar to turn off.
5. Proceed to “Installing the flow-assist air syringe” on page 48.

Installing the flow-assist air syringe

If sample flow does not start when you apply voltage to the spray tip, you may need to initiate flow by the application of air pressure.

To install a flow-assist air syringe:

1. Retract the plunger from the glass syringe provided in the syringe kit, to fill the syringe with air.
2. Connect the Luer end fitting of the flow-assist air inlet tubing provided in the syringe kit to the syringe.
3. Connect the other end of the tubing to the back end of the nanospray head (see Figure 4-2 on page 31).

***NOTE**: The spray tip mounting block that holds the nanospray head is spring-loaded. Always retract it fully before handling the spray tip to avoid accidentally hitting the tip against the curtain gas plate.*

---

4. Turn the coarse Z adjustment knob (see Figure 3-3 on page 18) until the spray tip is displayed on the video monitors.
5. To initiate flow, gently but firmly press on the syringe plunger as you observe the video monitor. Watch for the formation of a droplet on the spray tip.
6. When you see a droplet on the spray tip, stop pressing on the syringe plunger. The plunger may push back out of the syringe slightly.

   If no droplet forms when you apply air pressure, one of the following may be true:

   - **Tip is blocked**—Clean up the sample, then prepare a new tip.
   - **Closed-end tip is not open**—Open the tip as described in Section 4.4.3, Breaking Off the Fine Tip (Closed-End Tips Only).
7. Remove the tubing from the back end of the nanospray head and place the syringe and tubing out of the way.
8. Perform the steps in “Determining if continuous air pressure is needed” on page 49.

Determining if continuous air pressure is needed

To determine if continuous air pressure is needed to keep sample flowing:

1. Make sure the spray tip is no closer than 1.5 cm to the nozzle opening.
2. Set the Spray Tip Potential to **1,700 V**.
3. In the Instrument Control Panel, select **Instrument-On** from the Instrument menu.
4. Observe the spray tip on the video monitors, and adjust the distance of the spray tip from the nozzle opening and the Spray Tip Potential as described below until the droplet disperses:
   - 1.5 cm, maintain Spray Tip Potential at 1,700V
   - 1.0 cm, maintain Spray Tip Potential at 1,700V
   - 0.5 cm, decrease Spray Tip Potential by 500V
5. When the droplet disperses:
   - Adjust the tip so that is **1 cm** away from the nozzle opening.
   - Set the Spray Tip Potential to **1,700 V**.

   If the droplet does not disperse, make sure:
   - Spray Tip Potential clip is installed. See Section 4.5.1, Preparing the Workstation.
   - Instrument state is set to **On**.
6. Open the shutter.
7. If curtain gas is in manual mode click 

in the toolbar to turn on.
8. Adjust the curtain gas to **0.1** to **0.2 liter/min**. For more information, see Section 2.2.3, Adjusting the Curtain Gas.

9. If you observe stable signal within 3 or 4 minutes in the Instrument Control Panel, continuous pressure is not needed to maintain flow. Proceed to Section 4.5, Optimizing Signal in Nanospray Mode.

   If you do not observe signal, or if signal increases then decreases to 10 percent or less of the original intensity, continuous air pressure is needed to maintain flow. Proceed to “Installing the syringe in the clamp” on page 50.

Installing the syringe in the clamp

If flow is not maintained by an initial application of air pressure, install the flow-assist air syringe in the syringe clamp provided to apply constant pressure for sample flow:

1. Perform step 1 through step 5 in “Installing the flow-assist air syringe” on page 48.
2. When you see the droplet form, install the syringe in the clamp provided (Figure 4-10). You may need to insert or retract the plunger slightly to align with the slots in the clamp.

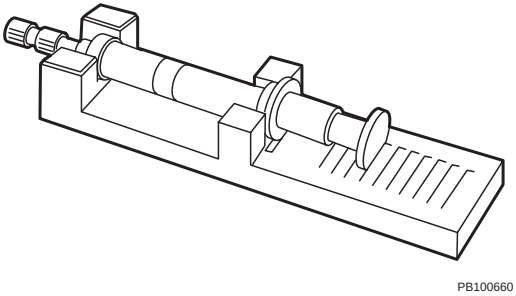


Figure 4-10 Installing the Air-Assist Syringe in the Clamp

3. Determine if air pressure is adequate as described on page 51.

Determining if air pressure is adequate

You may need to adjust the air pressure. To determine if pressure is adequate:

1. Adjust the tip so that is **1 cm** away from the nozzle opening.
2. Set the Spray Tip Potential to **1,700 V**.
3. Turn on the curtain gas and adjust to **0.1** to **0.2 liter/min**.
4. In the Instrument Control Panel, select **Instrument-On** from the Instrument menu.
5. Open the shutter.
6. Observe the spray tip and signal intensity and determine if air pressure is adequate:

If pressure is...	**You see the following...**
Too low	• Droplet forms on spray tip, and then disperses • Flow stops • No signal
Too high	• Stable spray does not form • Droplet forms on spray tip • If you move the spray tip off-center from the curtain gas opening and observe the nozzle opening in the video monitor, the plate appears wet. The tip is emitting a constant jet of liquid. • No signal

7. Adjust the air pressure if needed by adjusting the position of the syringe plunger.

5 Microspray and Low-Flow Electrospray Operation

This section includes:

- Overview
- Sample, solvent, and LC column guidelines
- Connecting injection devices
- Preparing samples
- Preparing the microspray/low-flow electrospray spray tip
- Optimizing signal in microspray/low-flow electrospray mode

5.1 Overview

Flow rates

Microspray and low-flow electrospray operation allow analysis of low-volume sample at low flow rates:

- **Microspray**—200 nl/min to 500 nl/min with the tips provided

  ***NOTE**: As tips wear, the opening enlarges, and you may see stable spray at higher flow rates but not at lower flow rates. If you flow at >500 nl/min, even for a short time, the opening on the tip may be permanently enlarged, and may not yield acceptable performance at lower flow rates. For more information, see "When to replace the spray tip" on page 76.*

- **Low-flow electrospray**—1 µl/min to 5 µl/min with the tips provided

Sample introduction

Two modes of sample introduction are available:

- **Continuous-flow loop injection**—Using the integrated low-volume injection valve or an LC system
- **Infusion**—Using the integrated syringe pump

Parts of the spray chamber

Figure 5-1 illustrates the parts of the nanospray spray chamber with a microspray or low-flow electrospray spray tip installed.

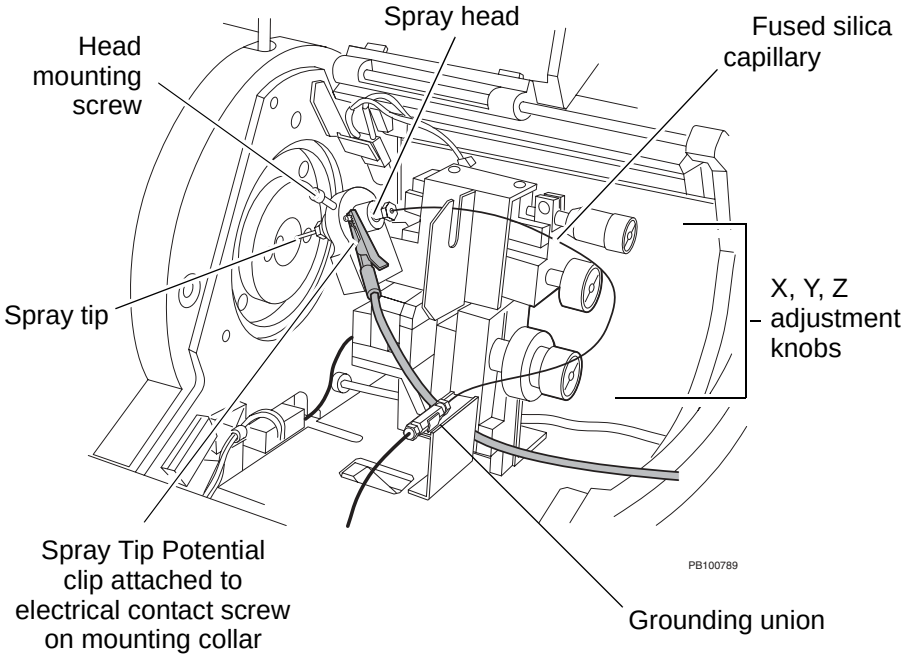


Figure 5-1 Parts of the Microspray/Low-Flow Electrospray Spray Chamber

Overview of microspray/ low-flow electrospray operation

Microspray operation involves:

- Connecting the microspray/low-flow electrospray injection device as needed
- Preparing samples
- Preparing the microspray/low-flow electrospray spray tip
- Infusing or injecting sample
- Positioning the spray tip to optimize signal intensity

The steps above are described in detail in:

- Section 5.3, Connecting Injection Devices
- Section 5.4, Preparing Samples
- Section 5.5, Preparing the Microspray/ Low-Flow Electrospray Spray Tip
- Section 5.6, Optimizing Signal in Microspray/ Low-Flow Electrospray Mode

After performing the steps above, operation is identical to standard electrospray operation. Refer to the relevant sections of the *Mariner API-TOF Workstation User's Guide* to perform the following tasks:

- tune and calibrating the mass analyzer
- acquiring data

Refer to the *Data Explorer's User's Guide* to learn how to use the Data Explorer software to process and analyze data.

For additional information, refer to the following sections in this document:

- Section 6, Maintenance
- Section 7, Troubleshooting

5.2 Sample, Solvent, and LC Column Guidelines

For information, refer to the *Mariner API-TOF Workstation User's Guide*.

5.3 Connecting Injection Devices

This section describes connecting the following for microspray/low-flow electrospray applications:

- 0.5 µl injection valve
- Integrated syringe pump
- Inline filter

0.5 µl injection valve

Plumb the integrated 0.5 µl injection valve for microspray/low-flow electrospray applications. The integrated injection valve has an internal 0.5 µl sample loop.

1. Connect the outlet of a syringe pump or LC system to the side connector on the top plate of the injection valve (Figure 5-2).

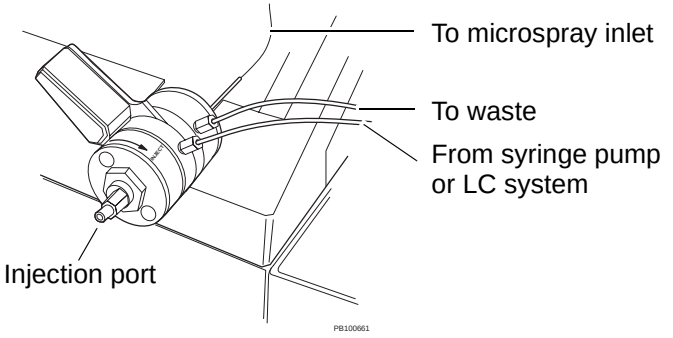


Figure 5-2 Integrated 0.5 µl Injection Valve

2. Connect a length of waste tubing to the side connector on the bottom plate of the injection valve. Place the waste tubing in a waste container.

3. Follow the procedure described in Section 6.3.6, Installing the Fused Silica Capillary to connect the rear port of the valve to the fused silica capillary leading to the microspray/low-flow electrospray head. Note the following:

   - Use a Rheodyne stainless steel ferrule (Figure 5-3) in step 1 on page 86, instead of the Valco stainless steel fitting used at the spray tip inlet fitting.
   - Make sure the fused silica is flush with the yellow PEEK sleeve before installing in the valve.

CAUTION

If the fused silica is not flush with the sleeve, it creates a dead volume that affects performance.

Rheodyne ferrule
Use for injection valve

Valco ferrule
Use for microspray head

Figure 5-3 Rheodyne and Valco Stainless Steel Ferrules

Integrated syringe pump

For further information on this procedure, please refer to the *Mariner API-TOF Workstation User's Guide*.

To plumb the integrated syringe pump:

1. Install a 100 µl or smaller gas-tight syringe provided in the startup kit on the integrated syringe pump.

   Plumb the outlet of the syringe pump as needed using the tubing indicated:

   - **Inlet of the integrated 0.5 µl injection valve on the spray chamber** (as illustrated in Figure 5-2 on page 56)—0.005-inch ID PEEK tubing to minimize the volume between the syringe and the spray chamber.
   - **Directly to the microspray/low-flow electrospray head**—Fused silica capillary. For information, see "Installing the Fused Silica Capillary" on page 86.

2. Use plastic fittings for connections to the syringe.

***NOTE**: The fused silica capillary connection to the microspray/low-flow electrospray head requires careful adjustment of the capillary in the fitting. When you remove the spray chamber from the Mariner Workstation, disconnect the capillary connector at the syringe pump, instead of at the microspray/low-flow electrospray head, to allow for easier reattachment.*

Inline filter

To reduce the occurrence of tip blockage, you can install a ***low-volume*** inline filter (less than 20 nl swept volume). Install the inline filter in the location appropriate for your injection device:

- **Loop injection**—Install the inline filter between the injector and the microspray/low-flow electrospray head
- **Infusion**—Install the inline filter between the syringe and the microspray/low-flow electrospray head

However, an inline filter will cause some band-broadening.

5.4 Preparing Samples

To prevent blockage to the microspray/low-flow electrospray spray tip, samples must be as clean as possible. The sample preparation method of choice depends on your sample and sample volume.

This section provides information on four types of sample preparation:

- Filtering
- Drop dialysis
- Desalting
- LC cleanup

When to perform

Filter samples as routine practice if you are not using an inline filter. Tip blockage is most commonly caused by sample particulates.

Perform drop dialysis, desalting, or LC cleanup if samples:

- Are prepared in phosphate or sulfate buffers. Nitrogen-containing buffers do not usually cause a problem.
- Contain salt, for example, from cation or anion exchange purification.
- Are contaminated with detergent.

The method of choice depends on your application.

5.4.1 Filtering

The most common cause of tip blockage is particulate matter in samples. If you are not using an inline filter, use any of the following to routinely filter samples before microspray or low-flow electrospray analysis:

- Centrifuge filters, 2 µm
- Syringe filters, 2 µm, nylon (not appropriate for certain organic solvents)

***NOTE**: Rinse filters with appropriate solvents before use.*

5.4.2 Drop Dialysis

When to use

Use this technique on polar compounds when you know contaminants are of low molecular weight. This technique works well for DNA and polar proteins.

***NOTE**: Use this technique for larger peptides and proteins. Smaller peptides may be lost using this technique.*

What you need

For drop dialysis, you need a membrane:

- With a pore size of 0.025 µm or smaller
- That does not adhere to your sample

Procedure

To perform drop dialysis:

1. Fill a small container (for example, a pipet tip box) with about an inch of deionized water.
2. Place the container on a stable surface.

3. Use forceps to place the membrane in the water with the appropriate side up (refer to manufacturer's information). Do not use your fingers because you can contaminate the membrane with oil and salt.
4. Place about 10 parts of sample (for example, 10 µl) in the center of the membrane.
5. Place 1 part of pure acetonitrile (for example, 1 µl) on top of the sample spot. Do not exceed a 10 percent concentration of organic. It may dissolve the membrane.
6. Cover the container to prevent drying and allow it to sit for 45 minutes.
7. Note the size of the sample/organic spot, then let it sit for 15 more minutes.
8. Observe the size of the sample/organic spot. If the size of the spot is larger than the first time you checked it, allow the sample to sit for 15 more minutes. Observe the size of the sample/organic spot again.

***NOTE**: Sample also passes through the membrane during dialysis, particularly low mass samples. Dialyze small molecules for a shorter time than larger molecules. In general, do not dialyze for more than 2 hours.*

---

9. When the size of the sample spot stabilizes, remove the sample and place it in a microcentrifuge tube.

***NOTE**: The size of the sample spot can increase by a factor of 10 when salt concentration is high.*

5.4.3 Desalting

To desalt samples, you can use Microcon microconcentrators. Use the pore size appropriate for the sample type you are desalting.

5.4.4 LC Cleanup

When to use Use this technique on polar compounds when you know contaminants are of low molecular weight. This technique works well for DNA and polar proteins.

What you need For LC cleanup, you need:

- C_{18} column for low-mass proteins
- C_4 column for low-mass proteins

Procedure Inject or flow sample over the column.

5.5 Preparing the Microspray/ Low-Flow Electrospray Spray Tip

Overview

This section describes the occasional preparation required by microspray and low-flow electrospray spray tips. The microspray and low-flow electrospray spray tips do not require daily preparation as nanospray tips do.

Spray tips for microspray and low-flow electrospray applications are very similar. The differences are:

- **Microspray tip**—Uses a tapered fused silica tip and 0.016-inch ID orange PEEK tubing sleeve (Figure 6-3 on page 82)
- **Low-flow electrospray tip**—Uses a blunt fused silica tip and 0.005-inch ID red PEEK tubing sleeve (Figure 6-4 on page 82)

For more information on spray tips, see:

- Section 6.3.1, Microspray Spray Tip Specifications and Overview
- Section 6.3.2, Low-Flow Electrospray Spray Tip Specifications and Overview

Although microspray and low-flow electrospray applications use different spray tips, operation is very similar, with different flow rates and voltages as noted in Section 5.6, Optimizing Signal in Microspray/ Low-Flow Electrospray Mode.

Installing a spray tip

To install the a spray tip, perform the procedures in the following sections:

- Section 6.3.5, Cutting Tubing
- Section 6.3.6, Installing the Fused Silica Capillary
- Section 6.3.7, Installing the Microspray Fused Silica Tip, or Section 6.3.8, Installing the Low-Flow Electrospray Fused Silica Tip
- Section 6.3.9, Reinstalling the Spray Head

5.6 Optimizing Signal in Microspray/ Low-Flow Electrospray Mode

This section includes:

- Preparing the workstation
- Optimizing spray tip position and Spray Tip Potential

5.6.1 Preparing the Workstation

To prepare the workstation before optimizing signal:

1. Read “Spray chamber precautions” on page 14 before proceeding.
2. Be familiar with the information in Section 3.4, Factors Affecting Signal Quality.
3. Filter samples. See Section 5.4.1, Filtering.
4. Load an instrument settings file. For more information, see the *Mariner API-TOF Workstation User’s Guide*.
5. Connect the injection device as described in Section 5.3, Connecting Injection Devices.
6. In the Instrument Control Panel, select **Instrument-Standby** from the Instrument menu.
7. Attach the Spray Tip Potential clip to the electrical contact screw on the spray tip mounting collar (Figure 5-4).

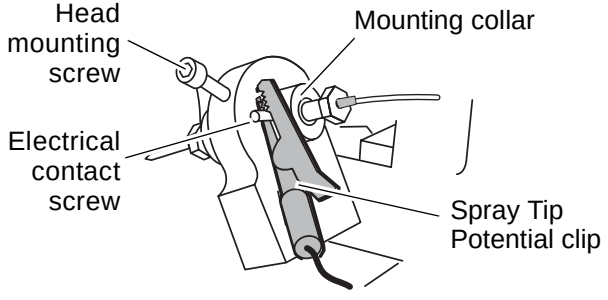


Figure 5-4 Attaching the Spray Tip Potential Clip

Note the following when attaching the clip:

- Make sure to attach the clip to the electrical contact screw, not the mounting screw.
- Attach the middle of the clip to the screw, not the tip of the clip.
- If you loosen the head mounting screw and pull the head back out of the mounting collar a little, you can attach the clip more easily. Then push the mounting collar back in, so that the clip is held in place between the mount and the screw, with the wire positioned down. Tighten the mounting collar screw.

8. In the Instrument Control Panel, set Spray Tip Potential to:

Mode	Setting
Microspray	2,500 V
Low-flow electrospray	3,500 V

***NOTE**: The voltages above are useful for aqueous (>90% water) solvent systems. If you are using a high organic solvent system, you may need to start with lower voltages (for example, 1,800 V for Microspray and 2,500 V for Low-flow electrospray) to obtain signal.*

---

9. In the Instrument Control Panel, set Nozzle Temp (for either operation mode) to **100** to **120°C**.

10. Adjust the initial position of the spray tip using the adjustment knobs in the spray chamber (shown in Figure 3-3 on page 18) so that it is:

    - **1.5 cm** away from the nozzle opening (use coarse Z adjustment knob)
    - Visually centered on the nozzle opening (use X and Y adjustment knobs)

CAUTION

If the spray tip is too close to the curtain gas plate when you apply voltages, a corona discharge can occur. A corona discharge prevents proper formation of spray.

***NOTE**: When the spray tip is 1.5 cm away from the nozzle opening, it may not be displayed on the video monitors. Observe the spray tip and nozzle opening directly to position the spray tip.*

5.6.2 Optimizing Spray Tip Position and Spray Tip Potential

This section includes:

- Spray tip position
- Applying voltages and gas and observing initial signal
- If you do not see signal
- When you see valid peaks
- Example TIC data
- Flushing the microspray/low-flow electrospray spray tip

Spray tip position

The ideal position for the spray tips are:

Mode	Ideal Position
Microspray	0 to 5 mm from the nozzle opening (Figure 5-5)
Low-flow electrospray	2 to 10 mm from the nozzle opening (Figure 5-6)

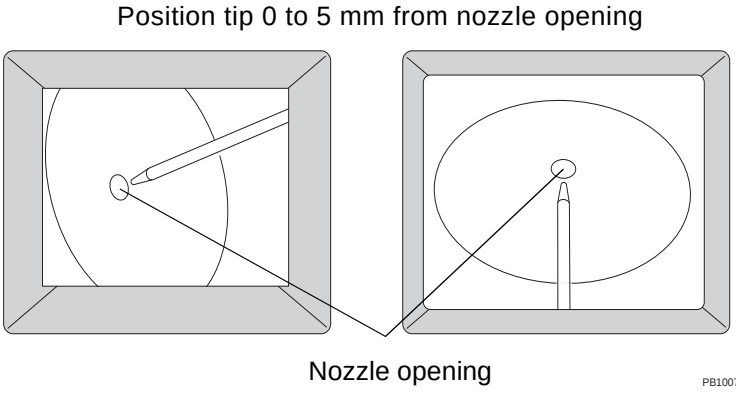


Figure 5-5 Optimum Microspray Spray Tip Position (Camera View)

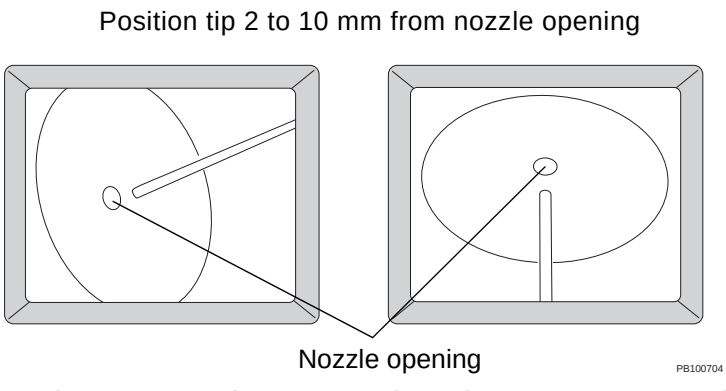


Figure 5-6 Optimum Low-Flow Electrospray Spray Tip Position (Camera View)

Applying voltages and gas and observing initial signal

After performing the steps in Section 5.6.1, Preparing the Workstation, apply voltages and gas, and observe initial signal:

1. In the Instrument Control Panel, select **Instrument-On** from the Instrument menu.
2. If curtain gas is in manual mode click  in the toolbar to turn on.
3. Adjust the curtain gas to **0.5 to 0.8 liter/min**. See Section 2.2.3, Adjusting the Curtain Gas.
4. Start solvent flow:

Mode	Flow Rate
Microspray	Up to 500 nl/min
Low-flow electrospray	Up to 5 µl/min

5. Inject sample or start the LC system as needed.
6. Open the shutter.
7. Observe signal intensity in the Instrument Control Panel.
8. If you do not see signal immediately, move the spray tip to within 1.0 cm of the curtain gas opening.
9. If you still do not see signal, move the spray tip to within 0.5 cm of the curtain gas opening and decrease the Spray Tip Potential to:

Mode	Setting
Microspray	1,800 V
Low-flow electrospray	2,500 V

CAUTION

If you do not decrease the Spray Tip Potential below 1,800 V (microspray) or 2,500 (low-flow electrospray) when the spray tip is within 0.5 cm of the curtain gas opening, a corona discharge can occur. A corona discharge prevents proper formation of spray.

10. If you do not see signal, proceed to “If you do not see signal” on page 69.

    If you see signal, proceed to “When you see valid peaks” on page 70.

If you do not see signal

If you still do not see signal:

1. Check that:

   - Voltages are on
   - Spray Tip Potential clip is attached
   - Solvent is flowing
   - Shutter is open
   - There are no leaks in the solvent stream or spray head

***NOTE**: Make sure the Spray Tip Potential clip is attached to the electrical contact screw, not the mounting screw. See Figure 5-4 on page 64.*

---

2. If you still do not see signal, make sure solvent is flowing through the tip:

   - Close the shutter
   - Set the instrument state to Standby
   - Turn off the curtain gas
   - Observe the tip for the formation of a droplet
   - If no droplet forms, the tip is blocked. Stop flow, and replace the tip. See Section 6.3, Replacing the Microspray/ Low-Flow Electrospray Fused Silica Spray Tip and Capillary.

***NOTE**: The most common cause of tip blockage is particulate matter in samples. Filter samples before reintroducing. For more information, see Section 5.4.1, Filtering.*

When you see valid peaks

When you see valid peaks and the TIC is stable, perform the following steps. If the capillary contains air, it may take several minutes for the TIC to stabilize.

1. Adjust the tip so that it is **1 cm** away from the nozzle opening.
2. ***Slowly*** turn the fine Z adjustment knob in the spray chamber (Figure 3-3 on page 18) to move the spray tip about 1 mm closer to the nozzle opening.
3. Observe signal intensity.
4. Decrease the Spray Tip Potential by **100 V**.
5. Observe signal intensity.
6. Continue repeating step 2 through step 5 to maximize signal intensity and stabilize the TIC (Figure 5-7 and Figure 5-8). Note the following guidelines:

    - When the spray tip is within 0.5 cm of the nozzle opening, set the Spray Tip Potential to the following to avoid corona discharge:

Mode	Setting
Microspray	1,700 V or lower
Low-flow electrospray	2,300 V or lower

    - Use the **fine** Z adjustment knob when the spray tip is within 0.5 cm of the nozzle opening. Be careful not to crash the tip into the curtain gas plate.

- Optimum spray tip position is:

Mode	Ideal Position
Microspray	0 to 5 mm from the nozzle opening. See Figure 5-5 on page 67.
Low-flow electrospray	2 to 10 mm from the nozzle opening. See Figure 5-6 on page 67.

- Optimum Spray Tip Potential depends on precise distance from the nozzle opening and the conditions of your application.

7. Fine-tune the Spray Tip Potential within 100 V of the current setting by adjusting it in 25 to 50 V increments.

8. Fine-tune the X and Y positions to better position the spray and direct ions into the nozzle opening.

Example TIC data

Figure 5-7 and Figure 5-8 represent typical TIC data for microspray and low-flow electrospray applications.

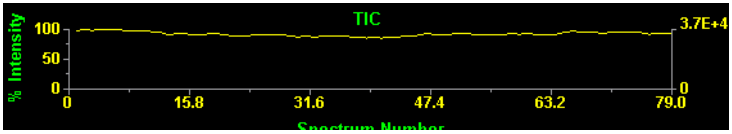


Figure 5-7 Example Microspray or Low-Flow Electrospray Infusion TIC Data

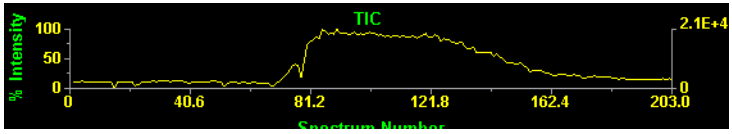


Figure 5-8 Example Microspray or Low-Flow Electrospray Loop Injection TIC Data

Flushing the microspray/ low-flow electrospray spray tip

Flush the microspray/low-flow electrospray spray tip at the end of the day with deionized water then isopropanol, to minimize corrosion in the union.

***NOTE**: If you flow solvent at flow rates above 1 µl/min, close the shutter before flushing.*

If you are using an LC system, you can alternatively flush by running isopropanol or acetonitrile:water overnight at:

Mode	Flow Rate
Microspray	100 to 200 nl/min
Low-flow electrospray	Up to 5 µl/min

6 Maintenance

This section includes:

- Replacing the nanospray head O-ring
- Flushing the microspray/low-flow electrospray tip
- Replacing the microspray/low-flow electrospray fused silica spray tip and capillary

6.1 Replacing the Nanospray Head O-Ring

When to replace

Replace the nanospray head O-ring if you do not feel firm resistance from the O-ring in the head when you insert the new spray tip, and when tightening the set screw does not secure the spray tip.

Replacing

To replace the O-ring:

1. Remove the nanospray head as described in Section 4.4.1, Removing the Nanospray Head.
2. Remove the spray tip as described in Section 4.4.2, Removing the Old Spray Tip.
3. Remove the cap from the head and unscrew the retaining screw (Figure 6-1 on page 73).
4. Shake the plunger and O-ring out of the housing.

Figure 6-1 illustrates the parts of the nanospray head.

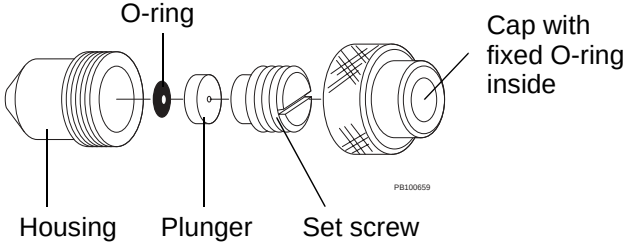


Figure 6-1 Parts of the Nanospray Head

5. Insert the new O-ring into the housing. Make sure it is seated.
6. Insert the Teflon plunger into the housing. Make sure it is seated.
7. Screw in the set screw until finger tight. Do not overtighten.
8. Insert the spray tip as described in Section 4.4.5, Inserting a Filled Spray Tip.

   If you cannot insert the spray tip, loosen the set screw inside the head.

   If you cannot insert the spray tip after loosening the set screw, remove the O-ring from the head and slide the tip through the O-ring several times, reassemble the head, and insert the tip.

   If you do not feel firm resistance, tighten the retaining screw.
9. Replace the cap.
10. Reinstall the nanospray head as described in Section 4.4.6, Reinstalling the Nanospray Head.

6.2 Flushing the Microspray/ Low-Flow Electrospray Tip

Flush the microspray spray tip at the end of the day with deionized water then isopropanol, to minimize corrosion in the union.

***NOTE**: If you flow solvent at flow rates above 1 µl/min, close the shutter before flushing.*

If you are using an LC system, you can alternatively flush by running isopropanol or acetonitrile:water overnight at

Mode	Flow Rate
Microspray	100 to 200 nl/min
Low-flow electrospray	Up to 5 µl/min

6.3 Replacing the Microspray/ Low-Flow Electrospray Fused Silica Spray Tip and Capillary

This section includes:

- Microspray spray tip specifications and overview
- Low-flow electrospray tip specifications and overview
- Removing the spray head
- Disassembling and inspecting the spray tip
- Cutting tubing
- Installing the fused silica capillary
- Installing the microspray fused silica tip
- Installing the low-flow electrospray fused silica tip
- Reinstalling the spray head

6.3.1 Microspray Spray Tip Specifications and Overview

Spray tip specifications

The microspray spray tip has the following specifications:

- Uncoated fused silica, with tapered end
- 15 μ opening
- 360 μm OD, 75 μm ID
- 5 cm uncut length

Parts of the spray tip and capillary are shown in Figure 6-3 on page 82.

When to replace the spray tip

The fused silica tip wears with use, and the opening enlarges. As the opening of the tip gets larger, higher voltages will be required to obtain a stable spray. Frequency of replacement depends on the solvents used. Highly-basic solvents degrade the tip more quickly.

Replace the spray tip when you cannot obtain a stable signal at low flow rates and low Spray Tip Potentials, or when the tip becomes plugged.

You should be able to see a stable signal with a peptide sample under the following conditions:

- **Solvent**—50% acetonitrile and water with 1% acetic acid
- **Flow rate**—100 to 500 nl/min
- **Spray Tip Potential**—1,500 to 1,700 V
- **Curtain gas flow rate**—0.5 to 0.8 liter/min

When to replace the capillary

Replace the fused silica capillary if it becomes plugged or contaminated.

When to replace the grounding union

The grounding union is plumbed inline if you are introducing sample and solvent using the integrated syringe pump. It is not needed if you are using the integrated injection valve, which is grounded.

Replace the grounding union if:

- Union becomes plugged or contaminated
- You see signal instability, particularly in the UV signal, which may indicate that the union is dirty and is not grounding properly

Where to assemble the spray tip

Remove the spray chamber as described in Section 3.2, Installing and Removing the Nanospray Spray Chamber, and assemble and connect the spray tip while sitting at a lab bench.

6.3.2 Low-Flow Electrospray Spray Tip Specifications and Overview

Spray tip specifications

The low-flow electrospray spray tip has the following specifications:

- Fused silica
- 20 μm opening
- 90 μm OD, 20 μm ID
- 4 cm length (provided in 2 m coils)

Parts of the spray tip and capillary are shown in Figure 6-4 on page 82.

When to replace the spray tip

Replace the low-flow electrospray spray tip when the tip becomes plugged or damaged.

When to replace the capillary

Replace the fused silica capillary if it becomes plugged or contaminated.

Where to assemble the spray tip

Remove the spray chamber as described in Section 3.2, Installing and Removing the Nanospray Spray Chamber, and assemble and connect the low-flow electrospray spray tip while sitting at a lab bench.

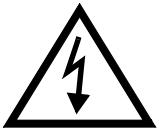
6.3.3 Removing the Spray Head

WARNING

***PHYSICAL INJURY HAZARD**. To prevent eye injury, wear safety glasses when performing this procedure.*

To remove the spray head when replacing a microspray or low-flow electrospray capillary and tip:

1. In the Instrument Control Panel, select **Instrument-Off** from the Instrument menu.
2. Remove the spray chamber as described in “Removing the spray chamber” on page 16. Place the spray chamber on the bench.
3. Move the front camera out of the way and open the spray chamber cover.



WARNING

***ELECTRICAL SHOCK HAZARD**. High voltage safety interlocks automatically turn off high voltages when you open the spray chamber cover. If interlocks fail or are disabled, high voltages are present when you open the spray chamber cover.*

***NOTE**: To make repositioning easier, loosen just the joint at the base of the camera mount and move the camera.*

4. Turn the coarse Z adjustment knob (see Figure 3-3 on page 18) fully counterclockwise to move the spray tip as far away from the curtain gas plate as possible.
5. Turn the fine Z adjustment knob counterclockwise until the mounting block stops moving.
6. Disconnect the capillary from the back of the injection valve or from the solvent source.
7. If a grounding union is installed, remove it from the bracket in the base of the spray chamber.
8. Disconnect the Spray Tip Potential clip from the electrical contact screw.
9. Loosen the head mounting screw and remove the head from the chamber (Figure 6-2).

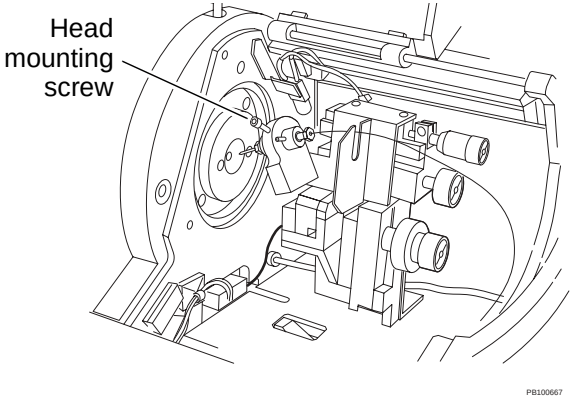


Figure 6-2 Removing the Spray Head

6.3.4 Disassembling and Inspecting the Spray Tip

Spray tips for microspray and low-flow electrospray applications are very similar. The differences are:

- **Microspray tip**—Uses a tapered fused silica tip and 0.016-inch ID orange PEEK tubing sleeve (Figure 6-3)
- **Low-flow electrospray tip**—Uses a blunt fused silica tip and 0.005-inch ID red PEEK tubing sleeve (Figure 6-4)

***NOTE**: The capillary is identical in both spray tip assemblies.*

Disassembling

To disassemble the spray tip:

1. Hold the union with a wrench, and use another wrench to loosen and remove the capillary fitting from the back of the union. See Figure 6-3 and Figure 6-4 on page 82.
2. Remove the mounting collar from the union:
   - Use the hex key provided in the microspray kit to loosen the electrical contact screw and the set screw in the mounting collar
   - Slip the mounting collar off the union
3. Use a wrench to remove the tip and fitting from the union.

   If you are replacing the tip, remove the sleeve, ferrule, and tip from the nut and discard.

   If you are reusing the tip, set it aside while you replace the capillary.
4. Remove the sleeve, ferrule, and fused silica capillary from the other nut.
5. Discard the sleeves, ferrules, and fused silica.
6. Use a flashlight to examine the inside of the union.

7. Replace the union if you see discoloration, corrosion, or pitting.

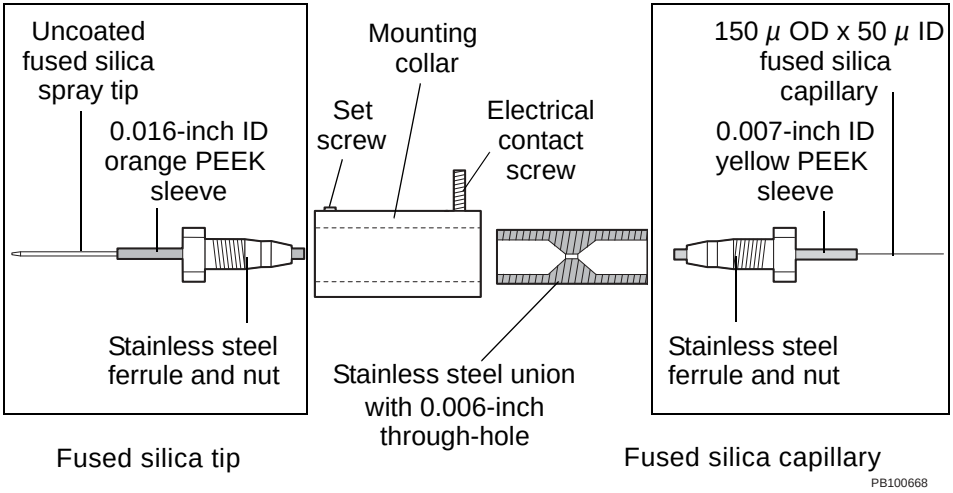


Figure 6-3 Microspray Spray Tip

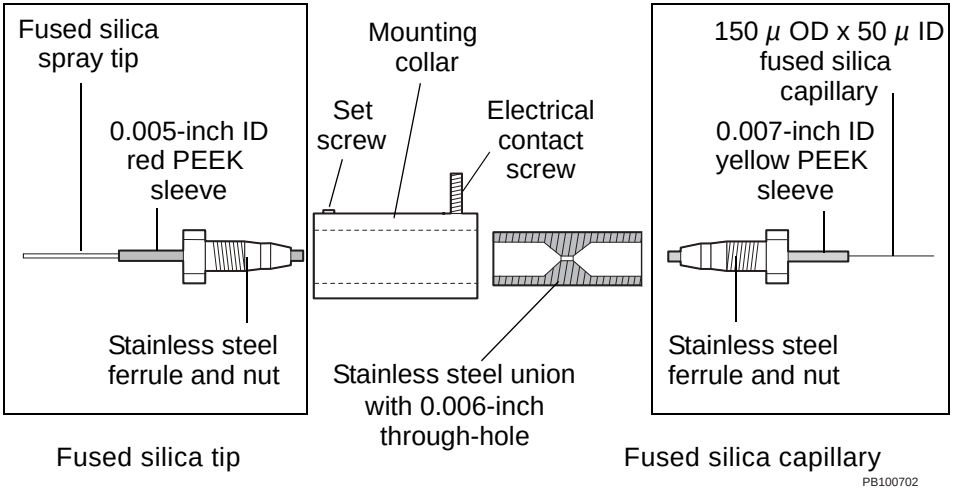


Figure 6-4 Low-Flow Electrospray Spray Tip

6.3.5 Cutting Tubing

Cut the following lengths of tubing:

Part of Spray Tip Assembly	Type of Tubing	Length
Spray tip (microspray)	Uncoated fused silica spray tip (360 µm OD, 75 µm ID)	1-1/4 to 1-1/2 inches
	Orange PEEK sleeve (0.016-inch ID)	3/4 inches
Spray tip (low-flow electrospray)	Fused silica capillary (90 µm OD, 20 µm ID)	4 cm (provided in 2 m coil)
	Red PEEK sleeve (0.005-inch ID) ***NOTE**: Cut this sleeve perfectly squarely. If you do not, you will not be able to seat the tip properly in the through-hole in the union.*	3/4 inches
Capillary (microspray)	Fused silica capillary (150 µm OD, 50 µm ID) ***NOTE**: A longer fused silica capillary causes longer delay time.*	One of the following: • 8 inches to connect the 0.5 µl injection valve to the spray tip • 6 inches to connect to the grounding union, then 11 inches to connect to the integrated syringe pump • 6 inches to connect to the grounding union, then length needed to reach your LC system
	Yellow PEEK sleeve (0.007-inch ID)	3/4 inches (three sleeves needed if grounding union used)

Part of Spray Tip Assembly	Type of Tubing	Length
Capillary (low-flow electrospray)	Fused silica capillary (150 µm OD, 50 µm ID) ***NOTE**: A longer fused silica capillary causes longer delay time.*	One of the following: • 8 inches to connect the 0.5 µl injection valve to the spray tip • 15 inches to connect to the integrated syringe pump • Length needed to reach your LC system
	Yellow PEEK sleeve (0.007-inch ID)	3/4 inches

Fused silica considerations

When cutting the fused silica tip or fused silica capillary:

1. Hold the fused silica tip or capillary on the bench. Do not touch the end of the fused silica tip with bare fingers.
2. Draw the ceramic tubing cutter (provided in the startup kit) squarely across the fused silica tip or capillary to put a scratch in the tubing. Do not cut through the tubing.
3. Snap off the fused silica tip or capillary at the cut. Check under 10 to 20X magnification to ensure that edges are smooth. Cut a new piece if the edges are not smooth.

***NOTE**: A square cut on the end of the fused silica tip or capillary is necessary for optimum performance.*

PEEK tubing considerations

When cutting PEEK tubing, make sure the end of the tubing you will insert into the union is cut squarely. Uneven cuts can cause a void volume in the union and affect performance.

CAUTION

PEEK tubing must be cut squarely. If it is not, you may see leaking or increased dead volumes.

Cutting PEEK tubing

To cut PEEK tubing squarely:

1. Insert the PEEK tubing into the smaller hole (1/16-inch OD) in the PEEK cutter provided in the startup kit. Do not squeeze cutter onto tubing.

   ***NOTE**: If you insert the tubing into the larger hole, the cut may not be square.*

   ***NOTE**: If necessary, replace the blade in the PEEK cutter.*

2. Rotate the cutter around the tubing to score the tubing.
3. Squeeze the cutter, making sure the cutter is in the score, to cut the tubing.

Checking the PEEK sleeves

The ID of PEEK tubing and OD of fused silica and capillaries can vary slightly. After you cut the orange 0.016-inch PEEK sleeve and red 0.005-inch PEEK sleeve, insert the appropriate tip or capillary into the sleeve. If the tip or capillary does not fit, cut a new sleeve.

6.3.6 Installing the Fused Silica Capillary

***NOTE**: If you are replacing the tip only, proceed to Section 6.3.7, Installing the Microspray Fused Silica Tip, or Section 6.3.8, Installing the Low-Flow Electrospray Fused Silica Tip.*

Installing the capillary

To install the fused silica capillary for microspray or low-flow electrospray applications:

1. Place a Valco ferrule (see Figure 5-3 on page 57) and nut on the square-cut end of the 3/4-inch length of yellow PEEK sleeve (Figure 6-5).

   ***NOTE**: The end of the PEEK tubing you insert into the union must be squarely cut to avoid dead volume.*

2. Bottom the nut, ferrule, and sleeve in the union. Tighten the nut by hand until finger-tight, then turn 1/4 to 1/2 turn with a wrench.

   This action seats the ferrule on the sleeve at the proper depth.

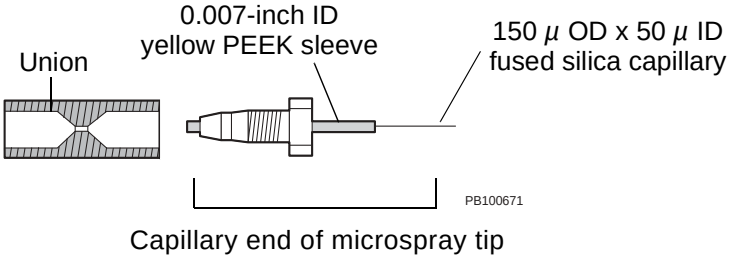


Figure 6-5 Installing Fused Silica Capillary in the Microspray/Low-Flow Electrospray Spray Tip

3. Remove the nut, ferrule, and yellow PEEK sleeve from the union.
4. Slide the fused silica capillary through the nut, ferrule, and yellow PEEK sleeve until about 2 inches extend from the ferrule/sleeve, to make sure there are no obstructions.
5. Wipe the extended 2 inches of fused silica capillary with a lint-free tissue.
6. Withdraw the fused silica capillary back into the ferrule/sleeve. Bottom the fused silica capillary in the ferrule/sleeve by holding a clean finger over the end of the ferrule/sleeve and pushing the fused silica capillary until it stops.
7. Hold the nut, ferrule, sleeve, and fused silica capillary steady, and screw the union onto the nut until finger-tight.

***NOTE**: Make sure the capillary does not shift. It must be flush with the end of the fitting. The narrow-bore capillary is small enough to fit through the 0.006-inch through-hole in the union.*

---

8. Tighten the nut 1/4 turn with a wrench.
9. Remove the nut, ferrule, sleeve, and fused silica capillary from the union.

   The ferrule should hold the capillary snugly in position.

   Verify that the capillary is still flush with the end of the ferrule by placing a clean finger over the end of the ferrule and pushing the capillary toward the ferrule.
10. Reinstall the nut, ferrule, sleeve, and fused silica capillary by screwing the union onto the nut until finger tight.
11. Tighten the nut 1/2 turn with a wrench.
12. Gently pull on the capillary to make sure it is firmly held in place.

13. If the capillary moves, repeat step 9 through step 12 until the capillary is seated.
14. Tighten the nut a final 1/16 turn with a wrench.

***NOTE**: Do not overtighten. Overtightening can crush the fused silica capillary.*

---

15. If you are installing a microspray spray tip, remove the nut and capillary and place it out of the way to avoid damage.

    If you are installing a low-flow electrospray spray tip, leave the nut and capillary installed.
16. If you are installing a microspray tip and are not using the integrated injection valve, install a grounded union to the outlet of the capillary as described page 89.
17. If you are not replacing the fused silica tip:
    - Re-attach the tip you set aside when disassembling the spray tip (step 3 on page 81).
    - Slide the mounting collar (Figure 6-3 on page 82) on to the capillary-end of union until it is aligned with the front of the union.
    - Tighten the set screw and electrical contact screw (Figure 6-3 or Figure 6-4 on page 82).
    - Replace the fitting and capillary if you removed it in step 15.
    - Reinstall the head as described in Section 6.3.9, Reinstalling the Spray Head.

CAUTION

Do not slide the mounting collar past the front of the union. Doing so can damage the fused silica tip.

Installing the grounding union

A grounding union is required if you are introducing sample and solvent using:

- The integrated syringe pump
- An LC system with UV detector

It is not needed if you are using the integrated injection valve, which is grounded.

To install the grounding union (Figure 5-1 on page 54):

- Use Valco ferrules
- Follow the procedure in “Installing the capillary” on page 86, step 1 through step 14, to connect capillary to the inlet and outlet of the grounding union.

6.3.7 Installing the Microspray Fused Silica Tip

Cutting the tip Using the ceramic cutter provided, score the tip about 1 cm from the non-tapered end. Snap off the 1 cm end.

Installing To install the fused silica tip:

NOTE*: For ease of handling, the capillary end of the union should not be connected at this point.*

1. Place a ferrule and nut on the square-cut end of the 3/4-inch length of orange PEEK sleeve (Figure 6-6).

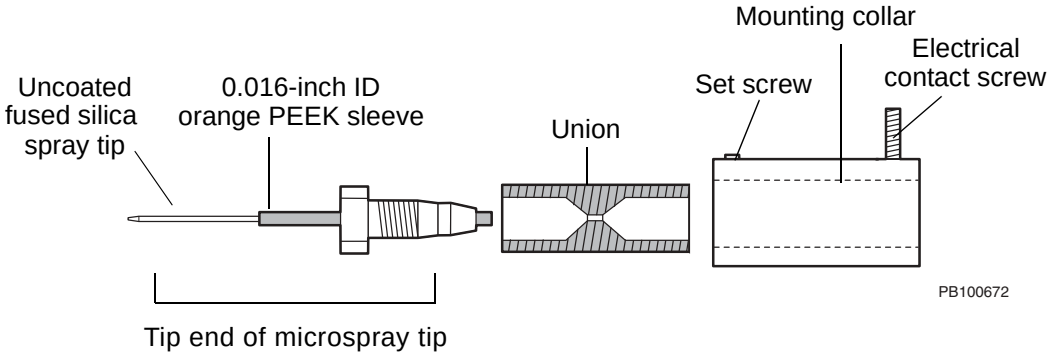


Figure 6-6 Installing Fused Silica Tip in the Microspray Spray Tip

2. Bottom the nut, ferrule, and sleeve in the union. Tighten the nut by hand until finger-tight, then turn 1/4 to 1/2 turn with a wrench.

   This action seats the ferrule on the sleeve at the proper depth.

3. Remove the nut, ferrule, and orange PEEK sleeve from the union.

4. Hold the 1-1/4 to 1-1/2-inch length of fused silica tip with your fingers or with tweezers, and slide the cut end (not the tapered end) through the nut, ferrule, and orange PEEK sleeve until the cut end slightly extends from the ferrule/sleeve, to make sure there are no obstructions.
5. Remove the fused silica tip from the fitting and set aside.
6. Bottom the nut, ferrule, and sleeve in the union. Finger tighten.
7. Pick up the fused silica tip with your fingers or with tweezers, and gently slide the non-tapered end into the sleeve. Bottom in the union.

***NOTE**: The diameter of the fused silica tip is too large to fit through the 0.006-inch through-hole in the union. When you feel resistance, the tip is bottomed.*

---

8. Hold the union with a 1/4-inch wrench and tighten the nut 1/4 turn with a wrench.
9. With tweezers, gently pull on the fused silica capillary to make sure it is firmly held in place.
10. If the tip moves, bottom it in the fitting again and continue tightening 1/4 turn and gently pulling with tweezers until it is seated.
11. Tighten the nut a final 1/16 turn with a wrench.

***NOTE**: Do not overtighten the nut. Overtightening can crush the fused silica tip.*

---

12. To check the integrity of the tip, connect a syringe with solvent to the union, and gently push solvent through the tip. Observe the tip to make sure you see a spray. If you do not, install a new tip.

13. Slide the mounting collar on to the capillary-end of the union until it is aligned with the front of the union. Tighten the set screw and electrical contact screw.

CAUTION

Do not slide the mounting collar past the front of the union. Doing so can damage the fused silica tip.

14. Replace the fitting and capillary removed in step 5.
15. Reinstall the head as described in Section 6.3.9, Reinstalling the Spray Head.

6.3.8 Installing the Low-Flow Electrospray Fused Silica Tip

***NOTE**: The capillary must be installed in the union before you install the fused silica tip in the union.*

Installing

To install the fused silica tip:

1. Place a ferrule and nut on the square-cut end of the 3/4-inch length red PEEK sleeve (Figure 6-7).

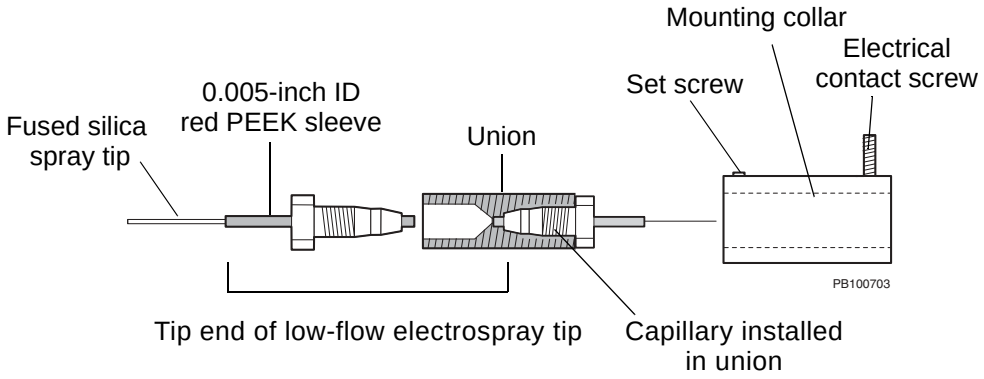


Figure 6-7 Installing Fused Silica Tip in the Low-Flow Electrospray Spray Tip

2. Bottom the nut, ferrule, and sleeve in the union. Tighten the nut by hand until finger-tight, then turn 1/4 to 1/2 turn with a wrench to seat the ferrule on the sleeve.

3. Remove the nut, ferrule, and red PEEK sleeve from the union.

4. Hold the 1-1/4 to 1-1/2-inch length of fused silica tip with your fingers or with tweezers, and slide the end through the nut, ferrule, and red PEEK sleeve until the end slightly extends from the ferrule/sleeve, to make sure there are no obstructions.
5. Remove the fused silica tip from the fitting and set aside.
6. Bottom the nut, ferrule, and sleeve in the union. Finger tighten.
7. Pick up the fused silica tip with your fingers or with tweezers, and gently slide the end into the sleeve. Bottom the tip in the through-hole in the union.

CAUTION

Do not remove the fitting and tip to check that the tip extends beyond the end of the fitting. You will break the fused silica tip when you reinsert the fitting and tip into the union.

***NOTE**: The capillary must be installed in the union before you install the fused silica tip in the union.*

***Hint**: Twirl the tip between your fingers until it slides into the through-hole. If the tip is not seated against the capillary at the other end of the through-hole in the union, you will see band broadening in chromatograms.*

8. Hold the union with a 1/4-inch wrench and tighten the nut 1/4 turn with a wrench.
9. With tweezers, gently pull on the capillary to make sure it is firmly held in place.
10. If the tip moves, bottom it in the fitting again and continue tightening 1/4 turn and gently pulling with tweezers until it is seated.

11. Tighten the nut a final 1/16 turn with a wrench.

***NOTE**: Do not overtighten the nut. Overtightening can crush the fused silica tip.*

---

12. To check the integrity of the tip, connect a syringe with solvent to the union, and gently push solvent through the tip. Observe the tip to make sure you see a spray. If you do not, install a new tip.

13. Slide the mounting collar on to the capillary-end of the union until it is aligned with the front of the union. Tighten the set screw and electrical contact screw.

CAUTION

Do not slide the mounting collar past the front of the union. Doing so can damage the fused silica tip.

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14. Reinstall the head as described in Section 6.3.9, Reinstalling the Spray Head.

6.3.9 Reinstalling the Spray Head

To reinstall the spray head after replacing microspray or low-flow electrospray capillary and tip:

1. Make sure the mounting block in the spray chamber is retracted as far away from the curtain gas plate as possible. Turn the coarse Z adjustment knob (see Figure 3-3 on page 18) completely counterclockwise. Turn the fine Z adjustment knob counterclockwise until the mounting block stops moving.
2. Place the spray head in mounting bracket and secure with mounting screw (see Figure 4-1 on page 26).
3. Reconnect the capillary from the back of the injection valve or from the solvent source.
4. Snap the grounding union, if installed, into the bracket in the base of the spray chamber.
5. Attach the Spray Tip Potential clip to the electrical contact screw.

***NOTE**: Make sure to attach the clip to the electrical contact screw, not the mounting screw.*

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7 Troubleshooting

This section includes troubleshooting information for the nanospray module. For additional troubleshooting information, refer to the *Mariner API-TOF Workstation User's Guide*.

Troubleshooting information is organized according to likelihood of possible cause, from most likely to least likely possible cause. If you are unable to solve your problem using the information in the following tables, call Applied Biosystems Technical Support. To reach Applied Biosystems Technical Support, refer to the Technical Support section of the User's Guide.

This section includes:

- Nanospray troubleshooting
- Microspray/low-flow electrospray troubleshooting

7.1 Nanospray Troubleshooting

Table 7-1 Nanospray Troubleshooting

Symptom	Possible Cause	Action
Background interference in low mass range	Corona discharge, Spray Tip Potential too high	Decrease Spray Tip Potential. ***NOTE**: A high Spray Tip Potential can damage the spray tip. If decreasing the Spray Tip Potential does not minimize background interference, replace the spray tip. See Section 4.4, Preparing the Nanospray Spray Tip.*
	Spray Tip Potential is set higher than 1,700 V	Decrease Spray Tip Potential.
	Spray Tip Potential too high for spray tip position	Move spray tip away from nozzle opening or decrease Spray Tip Potential.
No signal	Spray Tip Potential clip not contacting metal coating	Adjust position of clip.
	Metal coating on nanospray spray tip is damaged	Replace tip. See Section 4.4, Preparing the Nanospray Spray Tip.
	No flow, Spray Tip Potential too low	Increase Spray Tip Potential. ***NOTE**: Do not increase above 1,700 V.*

Continued

Table 7-1 Nanospray Troubleshooting (Continued)

Symptom	Possible Cause	Action
No signal (continued)	No flow, Spray Tip Potential decreased in increments greater than 50 V	Restart flow. Decrease Spray Tip Potential in 50 V increments.
	Spray tip blocked, sample is contaminated	Filter or centrifuge sample. Prepare and install new spray tip. See Section 4.4, Preparing the Nanospray Spray Tip.
	Spray tip blocked, sample precipitated	Prepare and install new spray tip. See Section 4.4, Preparing the Nanospray Spray Tip. Change condition that caused sample precipitation.
	Spray tip blocked by dust	Prepare and install new spray tip. See Section 4.4, Preparing the Nanospray Spray Tip. Store unused tips in dust-free environment.
Unstable signal	Spray Tip Potential not optimized	Optimize. See Section 4.5, Optimizing Signal in Nanospray Mode.
	Spray tip position not optimized	Optimize. See Section 4.5, Optimizing Signal in Nanospray Mode.

Continued

Table 7-1 Nanospray Troubleshooting (Continued)

Symptom	Possible Cause	Action
Unstable signal (continued)	Curtain gas flow too high	Decrease curtain gas flow rate. See Section 2.2.3, Adjusting the Curtain Gas.
	Sample flow not stable	See “Sample flow not stable” below.
Sample flow not stable	Sample viscosity or surface tension is high	Apply air pressure. See Section 4.5.3, Installing the Flow-Assist Air Syringe, and apply continuous pressure.
	Tip partially blocked	Replace. See Section 4.4, Preparing the Nanospray Spray Tip.
	Nozzle Temperature too high	Decrease to 100°C, or lower for volatile solvents.
Peaks ±18 m/z	Water clusters, curtain gas flow rate too low	Increase curtain gas flow rate. See Section 2.2.3, Adjusting the Curtain Gas.
Sample carryover	Tip previously used	Replace tip. See Section 4.4, Preparing the Nanospray Spray Tip.

7.2 Microspray/Low-Flow Electrospray Troubleshooting

Table 7-2 Microspray/Low-Flow Electrospray Troubleshooting

Symptom	Possible Cause	Action
Sample tailing, band broadening, poor chromatographic resolution with LC/MS	Microspray fused silica tip is not bottomed in union	Reassemble microspray spray tip. Make sure fused silica tip is bottomed in union. See Section 6.3.7, Installing the Microspray Fused Silica Tip.
	Low-flow electrospray fused silica tip is not inserted in through-hole in union and seated against capillary	Reassemble low-flow electrospray spray tip. Make sure fused silica tip is bottomed in union. See Section 6.3.8, Installing the Low-Flow Electrospray Fused Silica Tip.
No flow	Spray tip blocked	Replace tip. See: • Section 6.3.7, Installing the Microspray Fused Silica Tip • Section 6.3.8, Installing the Low-Flow Electrospray Fused Silica Tip Filter or clean sample and install inline filter. See “Inline filter” on page 58, and Section 5.4, Preparing Samples.

Continued

Table 7-2 Microspray/Low-Flow Electrospray Troubleshooting (Continued)

Symptom	Possible Cause	Action
Unstable spray (examine the spray tip closely to observe gas bubbles in the clear part of the tip)	Spray Tip Potential too high, electrolysis occurring in union	Decrease Spray Tip Potential.
	Leak in fitting in microspray head, or anywhere upstream of microspray head	Check and tighten all fittings.
	Solvent not degassed	Degas solvent.
	Nozzle Temperature too high	Decrease to 100 to 120°C, or lower for volatile solvents.
	Tip damaged or worn out	Replace. See: • Section 6.3.7, Installing the Microspray Fused Silica Tip • Section 6.3.8, Installing the Low-Flow Electrospray Fused Silica Tip
Pulsing in signal	Pulsing in sample flow	Use pump capable of delivering pulse-free flow at 100 nl/min to 1 µl/min flow rates.
	Syringe installed on integrated syringe pump is too large for flow rate	Use 100 µl syringe or smaller.
Unstable signal	Union in spray head dirty	Clean or replace. See Section 6.3.4, Disassembling and Inspecting the Spray Tip.
	Grounding union dirty	Clean or replace. See “Installing the grounding union” on page 89.

Table 7-2 Microspray/Low-Flow Electrospray Troubleshooting (Continued)

Symptom	Possible Cause	Action
Unstable UV signal	Union in spray head dirty	Clean or replace. See Section 6.3.4, Disassembling and Inspecting the Spray Tip.
	Grounding union dirty	Clean or replace. See “Installing the grounding union” on page 89.
No peaks observed above 300 m/z. Corona discharge observed at spray tip.	Spray Tip Potential too high, higher m/z compounds breaking down	Decrease Spray Tip Potential.
No peaks observed above 300 m/z. No corona discharge observed at spray tip.	Solvent, sample, or tubing contaminated, contaminants ionizing	Use fresh solvent, clean up sample, change tubing.

A Spare Parts

Table A-1 Mariner Microspray Kit

Item	Where used	Quantity	Part Number
Mariner Microspray Kit, includes:		1 kit	V700655
Capillary, fused silica 50 μm ID, 150 μm OD	Capillary between spray head and solvent source	5 ft	——
Capillary, fused silica 20 μm ID, 90 μm OD	Low-flow electrospray tip	10 ft	——
Capillary cutter, fused silica	Cutting fused silica	1	——
Collar, mounting	Holds spray head in mount	1	——
Ferrule, 1/16", stainless steel	Tip and capillary connection to union	Pkg of 10	——
Loupe, 10X	Magnification	1	——
Nut, 1/16", stainless steel	Tip and capillary connection to union	Pkg of 10	——
Scale (ruler), 6"	Measuring tubing	1	——
Spray tip, uncoated silica	Microspray spray tip	Pkg of 5	——
Tubing, PEEK, orange, 0.016-inch ID	Sleeve for microspray tip connection to union	5 feet	——
Tubing, PEEK, red, 0.005-inch ID	Sleeve for low-flow electrospray tip connection to union	5 feet	——
Tubing, PEEK, yellow, 0.007-inch ID	Sleeve for capillary connection to union	5 feet	——

Table A-1 Mariner Microspray Kit (Continued)

Item	Where used	Quantity	Part Number
Tubing Cutter, PEEK	Cutting PEEK tubing	1	——
Union, 1/16" OD ports with 0.006" ID through-hole, stainless steel	Body of spray head	2	——
Wrench, 1/4"	Tip and capillary connection to union	2	——
Wrench, Allen (hex) 1/16"	Tightening set screw in mounting collar	1	——
Operating Instructions, Microspray Kit	Assembling microspray head	1	——

Table A-2 Mariner Microspray Consumables Kit

Item	Where used	Quantity	Part Number
Microspray spray head consumables kit, includes:		1 kit	V340066
Ferrule, 1/16", stainless steel	Tip and capillary connection to union	Pkg of 20	——
Capillary, fused silica 50 μm ID, 150 μm OD	Microspray capillary between spray head and solvent source	10 ft	——
Capillary, fused silica 20 μm ID, 90 μm OD	Low-flow electrospray tip	10 ft	——
Nut, 1/16", stainless steel	Tip and capillary connection to union	Pkg of 20	——
Tubing, PEEK, orange, 0.016-inch ID	Sleeve for microspray tip connection to union	10 feet	——
Tubing, PEEK, red, 0.005-inch ID	Sleeve for low-flow electrospray tip connection to union	10 feet	——
Tubing, PEEK, yellow, 0.007-inch ID	Sleeve for capillary connection to union	10 feet	——
Union, 1/16" OD ports with 0.006” ID through-hole, stainless steel	Body of spray head	2	——

Table A-3 Mariner Miscellaneous Spare Parts

Item	Where used	Quantity	Part Number
O-ring, nanospray head	Nanospray head	Pkg of 10	V340065
Spray tip, nanospray, metal-coated borosilicate glass, open-end	Nanospray head	Pkg of 10	V207015
Spray tip, microspray, fused silica, uncoated	Microspray head	Pkg of 5	V207014
Tip, pipet, gel-loader	Nanospray tip sample loading	Box of 200	V340064

B Warranty/Service Information

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers, installing unauthorized hardware or software may void the Warranty or Service Plan.

B.1 Limited Product Warranty

Limited warranty

Applied Biosystems warrants that all standard components of the Mariner™ API-TOF Workstation Nanospray™ Module will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its Mariner™ API-TOF Workstation Nanospray™ Module, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or

replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on the instrument are themselves warranted to be free of defects in materials and workmanship for ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date of installation, the software designated for use with the product will perform substantially in accordance with the function and features described in its accompanying documentation when properly installed on the product. Applied Biosystems does not warrant that the operation of the instrument or software will be uninterrupted or error free. Applied Biosystems will provide any software corrections or “bug-fixes” if and when they become available, for a period of ninety (90) days after installation.

Warranty period effective date

Any applicable warranty period under these sections will begin on the date of installation for hardware and software installed by Applied Biosystems personnel, unless that date has been delayed at the buyer's request. In that case, and for all hardware and software installed by the buyer, and for all other products, the applicable warranty period begins the date the component is received by the buyer.

Warranty exceptions

The above warranties shall not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specification instructions for the product or accessories; performance of improper or inadequate maintenance by the user; installation of software or interfacing not supplied by Applied Biosystems; and modification or repair of the product or the software not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed. Such limited warranty is given only to buyer or any third party in the event of use of products furnished hereunder by any third party.

Warranty limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, in tort, warranty, or under any statute (including without limitation, any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damage, including without limitation, damage arising from or related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement. Any such affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

B.2 Damages, Claims, Returns

Damages If shipping damage to the instrument is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims After a damage inspection report is secured, Applied Biosystems will supply the replacements and process claims that are initiated by either party.

Returns Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the appropriate address.

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