

HESI-II Probe

User Guide

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DOCUMENTATION
SURVEY

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Preface

This *HESI-II Probe User Guide* provides you with information on using the heated-electrospray ionization probe. It also provides procedures for installing and maintaining the HESI-II probe.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:



CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Precautions for Handling the HESI-II Probe

When operating the mass spectrometer in the heated-electrospray mode, do **not** touch the heated surfaces of the HESI-II probe or the Ion Max API source housing. Touching the heated surfaces of the probe or the source housing can cause burns when the probe is operating at typical temperatures of 350 to 450 °C.

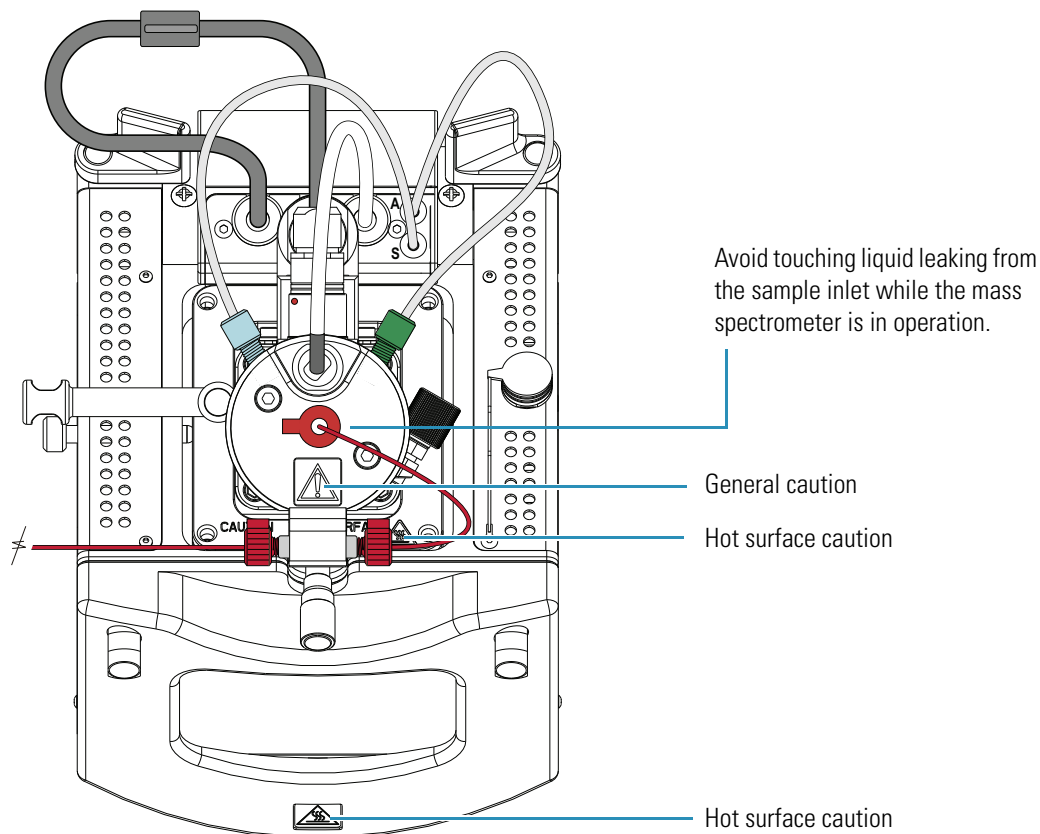


CAUTION AVOID BURNS. At typical operating temperatures (350 to 450 °C), the HESI-II probe can severely burn you. Before removing the probe from the API source housing, allow the probe to cool to room temperature (for approximately 20 minutes) before you touch it.

When operating the mass spectrometer in the heated-electrospray mode, if you observe liquid leaking from the sample inlet, place the mass spectrometer in Standby mode before you tighten the fitting to eliminate the leak.



CAUTION AVOID ELECTRIC SHOCK. Do not tighten the probe sample inlet fitting to eliminate a liquid leak while the mass spectrometer is in operation. If you touch liquid leaking from the probe sample inlet while the mass spectrometer is in operation, you might receive an electric shock.



Contacting Us

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|----------------|--|
| Phone | 800-532-4752 |
| Fax | 561-688-8736 |
| E-mail | us.techsupport.analyze@thermofisher.com |
| Knowledge base | www.thermokb.com |

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| | |
|----------|--|
| Phone | 800-532-4752 |
| Fax | 561-688-8731 |
| E-mail | us.customer-support.analyze@thermofisher.com |
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Introduction

Heated-electrospray ionization (H-ESI) transforms ions in solution into ions in the gas phase by using electrospray ionization (ESI) in combination with heated auxiliary gas. You can use H-ESI to analyze any polar compound that makes a preformed ion in solution.

Basic compounds (for example amines) can form a protonated molecule $[M + H]^+$, and acidic compounds (for example sulphonic acids) can form a deprotonated molecule $[M - H]^-$. In the positive ion polarity mode, the protonated molecule produces a peak at an m/z value of $M + 1$, where M equals the mass of the original molecule. In the negative ion polarity mode, the deprotonated molecule produces a peak at an m/z value of $M - 1$, where M equals the mass of the original molecule.

Because solution chemistry is the primary factor affecting mass spectra in ESI, other common adducts include sodium ions (Na^+), yielding an m/z value of $[M + 23]^+$; potassium ions (K^+), yielding an m/z value of $[M + 39]^+$; and ammonium ions (NH_4^+), yielding an m/z value of $[M + 18]^+$.

This chapter describes the principles of the heated-electrospray ionization technique through use of the HESI-II probe (see [Figure 1](#)) and a Thermo Scientific mass spectrometer.

Figure 1. HESI-II probe

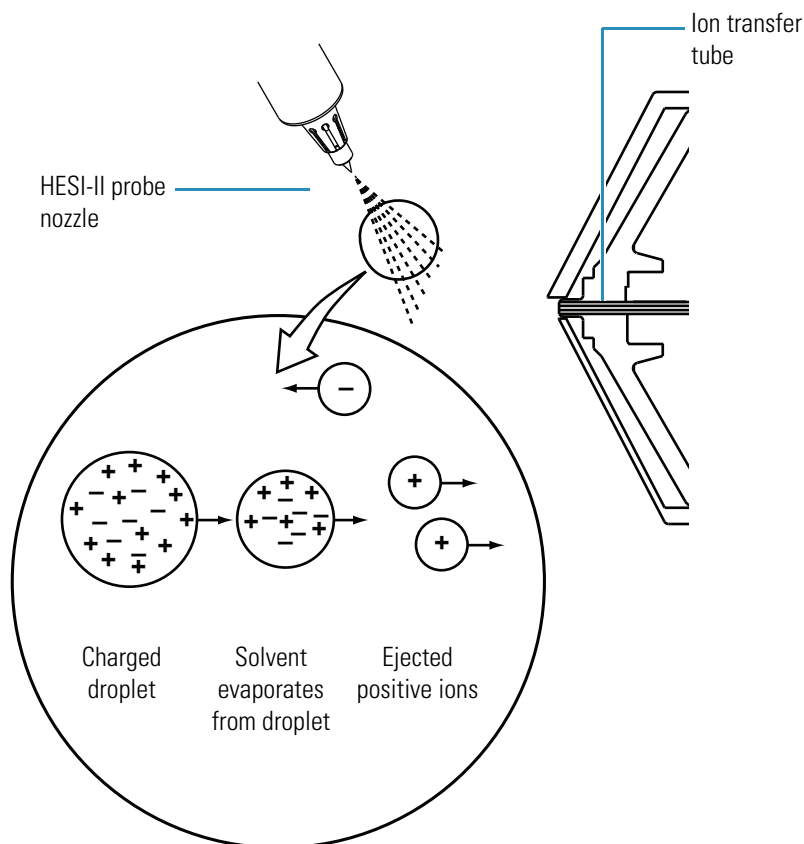


With H-ESI, a mass spectrometer can analyze a range of molecular weights greater than 100 000 u, due to multiple charging. H-ESI is especially useful for the mass analysis of polar compounds, which include biological polymers (for example, proteins, peptides, glycoproteins, and nucleotides); pharmaceuticals and their metabolites; and industrial polymers (for example, polyethylene glycols).

In H-ESI, ions are produced and introduced into the mass spectrometer as follows:

1. The sample solution enters the ESI needle, which receives a high voltage.
2. The ESI needle sprays the sample solution into a fine mist of droplets that are electrically charged at their surface.
3. The electrical charge density at the surface of the droplets increases as solvent evaporates from the droplets. In heated-electrospray ionization, heated auxiliary gas aids solvent evaporation.
4. The electrical charge density at the surface of the droplets increases to a critical point known as the Rayleigh stability limit. At this critical point, the droplets divide into smaller droplets because the electrostatic repulsion is greater than the surface tension. The process is repeated many times to form very small droplets.
5. Electrostatic repulsion ejects sample ions from the very small, highly charged droplets into the gas phase.
6. The sample ions enter the mass spectrometer through the ion transfer tube.

Figure 2 shows the steps in the formation of ions from highly charged droplets.

Figure 2. H-ESI process in the positive ion polarity mode

You can use the HESI-II probe in either positive or negative ion polarity mode. The ion polarity mode of choice is determined by the polarity of the preformed ions in solution: Acidic molecules form negative ions in solution, and basic molecules form positive ions. Because the ejection of sample ions from droplets is facilitated when the ionic charge and surface charge of the droplet have the same polarity, use the positive ion polarity mode to analyze positive ions and the negative ion polarity mode to analyze negative ions.

Sample ions can carry a single charge or multiple charges. The number of charges carried by the sample ion depends on the structure of the analyte of interest and the carrier solvent. (In H-ESI, the buffer and the buffer strength both have a noticeable effect on sensitivity, so it is important to choose these variables correctly.) In the case of higher molecular weight proteins or peptides, the resulting mass spectrum consists typically of a series of peaks corresponding to a distribution of multiply charged analyte ions.

Droplet size, surface charge, liquid surface tension, solvent volatility, and ion solvation strength are factors that affect the H-ESI process. Large droplets with high surface tension, low volatility, strong ion solvation, low surface charge, and high conductivity prevent good electrospray.

Organic solvents, such as methanol, acetonitrile, and isopropyl alcohol, are superior to water for H-ESI. Volatile acids and bases are good, but salts above 10 mM concentration and strong acids and bases are extremely detrimental.

Follow these rules for achieving a good electrospray:

- Keep salts out of the solvent system.
- Use organic/aqueous solvent systems and volatile acids and bases.
- Optimize the pH of the solvent system.

Table 1 shows initial heated electrospray settings for different liquid flow rates for a 50 percent aqueous solution. These initial settings provide a starting point for optimizing system performance. The optimal settings for your application depend on the compounds of interest, the solvent matrix, and the chromatographic conditions. For information on optimizing these settings, refer to the getting started guide for your mass spectrometer.

Tip For best results, avoid operating the HESI-II probe at elevated temperatures without solvent flow from the LC system or the syringe pump. Allowing the HESI-II probe to run dry at elevated temperatures can cause blockage of the replaceable metal needle (see “Replacing the Needle Insert” on page 20).

For an LTQ Series mass spectrometer, the allowable range for the auxiliary gas flow depends on the version of the instrument control software and the vaporizer temperature setting:

- For LTQ 2.5.0 or lower, the allowable range for the auxiliary gas flow is 5 to 60 units.
- For LTQ 2.5.5 or higher, the allowable range for the auxiliary gas flow is 0 to 60 units for vaporizer temperatures up to 100.00 °C. For vaporizer temperatures above 100.00 °C, the minimum auxiliary gas flow is 5 units.

For a TSQ Series mass spectrometer, you can set the auxiliary gas flow from 0 to 60 units.

Tip For best results, set the auxiliary gas flow to a minimum of 5 units for vaporizer temperatures above 100.00 °C.

Table 1. Initial heated electrospray settings

| Liquid flow rate (µL/min) | Ion transfer tube temperature (°C) | H-ESI vaporizer temperature (°C) | Sheath gas pressure (psi) | Auxiliary gas flow (arbitrary units) | Spray voltage (V) | Typical nitrogen gas consumption (L/min) |
|---------------------------|------------------------------------|----------------------------------|---------------------------|--------------------------------------|-------------------|--|
| 5 | 240 | Off to 50 | 5 | 0 | +3000 (-2500)* | <1 |
| 200 | 350 | 250 to 350 | 35 | 10 | +3000 (-2500) | 8 |
| 500 | 380 | 300 to 500 | 60 | 20 | +3000 (-2500) | 13 |
| 1000 | 400 | 500 | 75 | 20 | +3000 (-2500) | 17 |

* Negative ion mode

Functional Description

The HESI-II probe produces charged aerosol droplets that contain sample ions. The HESI-II probe accommodates liquid flows of 1 $\mu\text{L}/\text{min}$ to 1 mL/min without splitting.

The removable components of the HESI-II probe are the end cover that is secured to the probe body with socket head screws and the needle insert that screws into the probe body (see [Figure 3](#)). The end cover includes the high voltage feedthrough and the sample inlet port. The external components of the probe body include the grounding union holder, vaporizer cable connector socket, probe sleeve with depth markers, and probe nozzle (see [Figure 3](#) and [Figure 4](#)).

Figure 3. HESI-II probe removable components

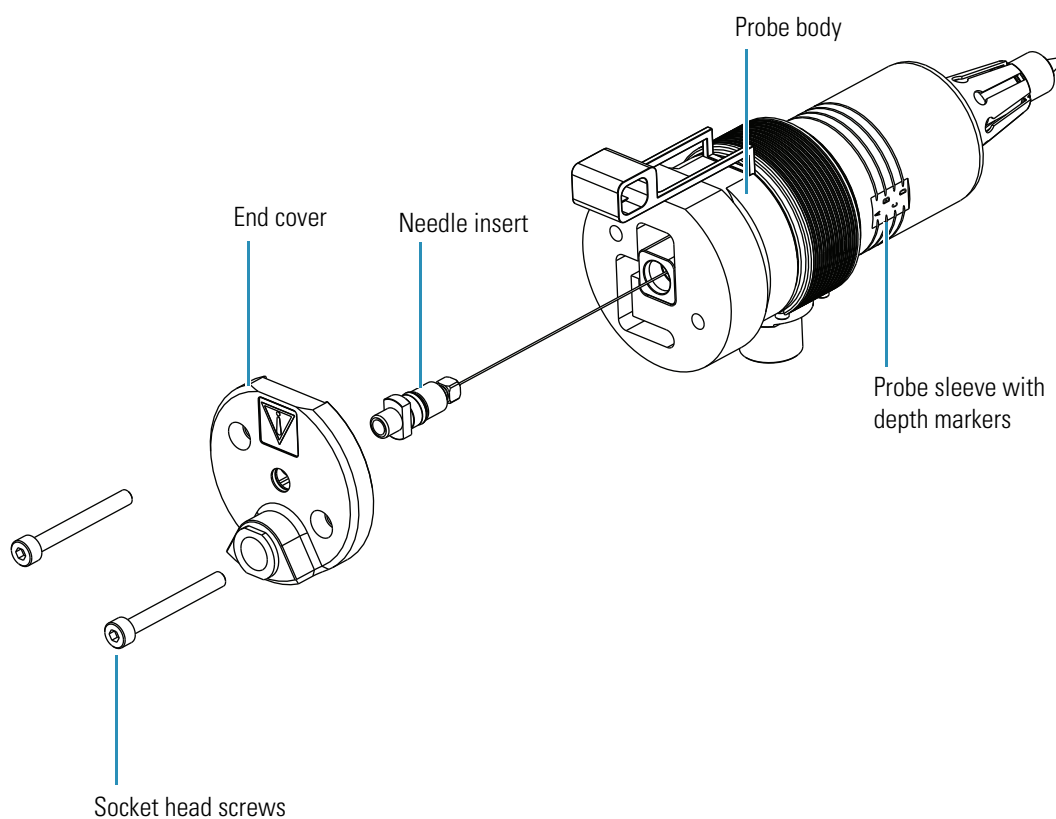
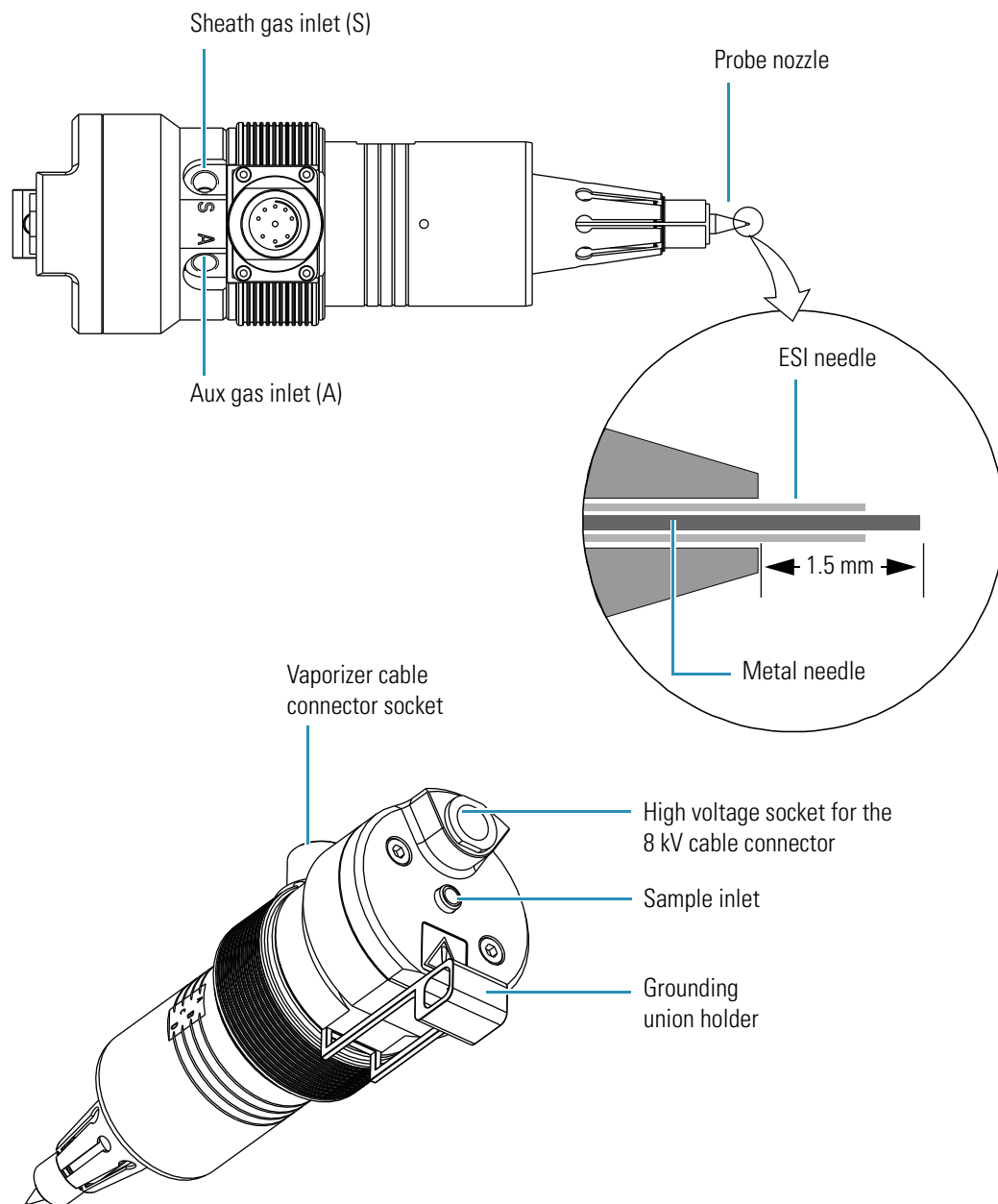


Figure 4. HESI-II probe views



Sample and solvent enter and exit the HESI-II probe through the needle insert, which protrudes from the sample inlet port at the back of the probe and from the nozzle at the front of the probe (see [Figure 4](#)). The needle insert (see [Figure 3](#) on [page 5](#)) includes an adjustable union, a needle guide fitting and ferrule, an ESI needle, and a metal needle. The adjustable union is used to adjust the protrusion of the needle insert tip from the probe nozzle (see [Figure 4](#)).

A large negative or positive voltage is applied to the ESI needle (typically ± 3 to ± 5 kV), which sprays the sample solution into a fine mist of charged droplets. The probe nozzle directs the flow of sheath gas and auxiliary gas at the droplets. The probe body houses the probe nozzle and needle insert, and includes the sheath gas and auxiliary gas plumbing.

The probe body has inlets for the sheath and auxiliary gases. The sheath gas is the inner coaxial nitrogen gas that sprays (nebulizes) the sample solution into a fine mist as it exits the metal needle sample tube (see Figure 5). The heated auxiliary gas is the outer coaxial nitrogen gas that assists the sheath gas in the desolvation of sample solutions (see Figure 6). The auxiliary gas heats as it passes through a vaporizer. The vaporizer is thermally insulated from the sample tube to prevent direct heating of the sample solution. You can control the vaporizer temperature from the Xcalibur™ data system. The temperature range is from ambient room temperature to 600 °C. For recommended operating temperatures and gas flow settings, see Table 1 on page 4.

Figure 5. Sheath gas plumbing

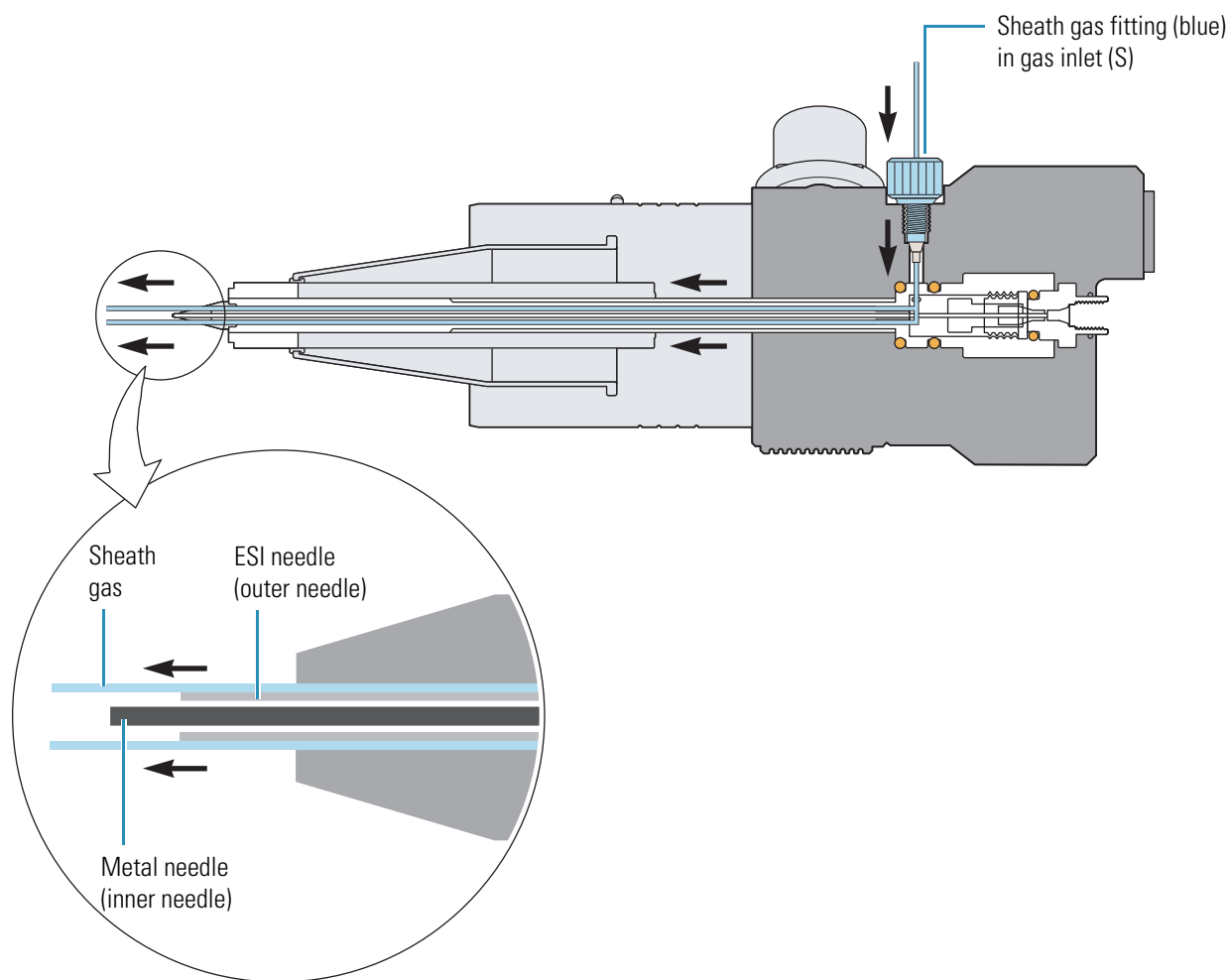
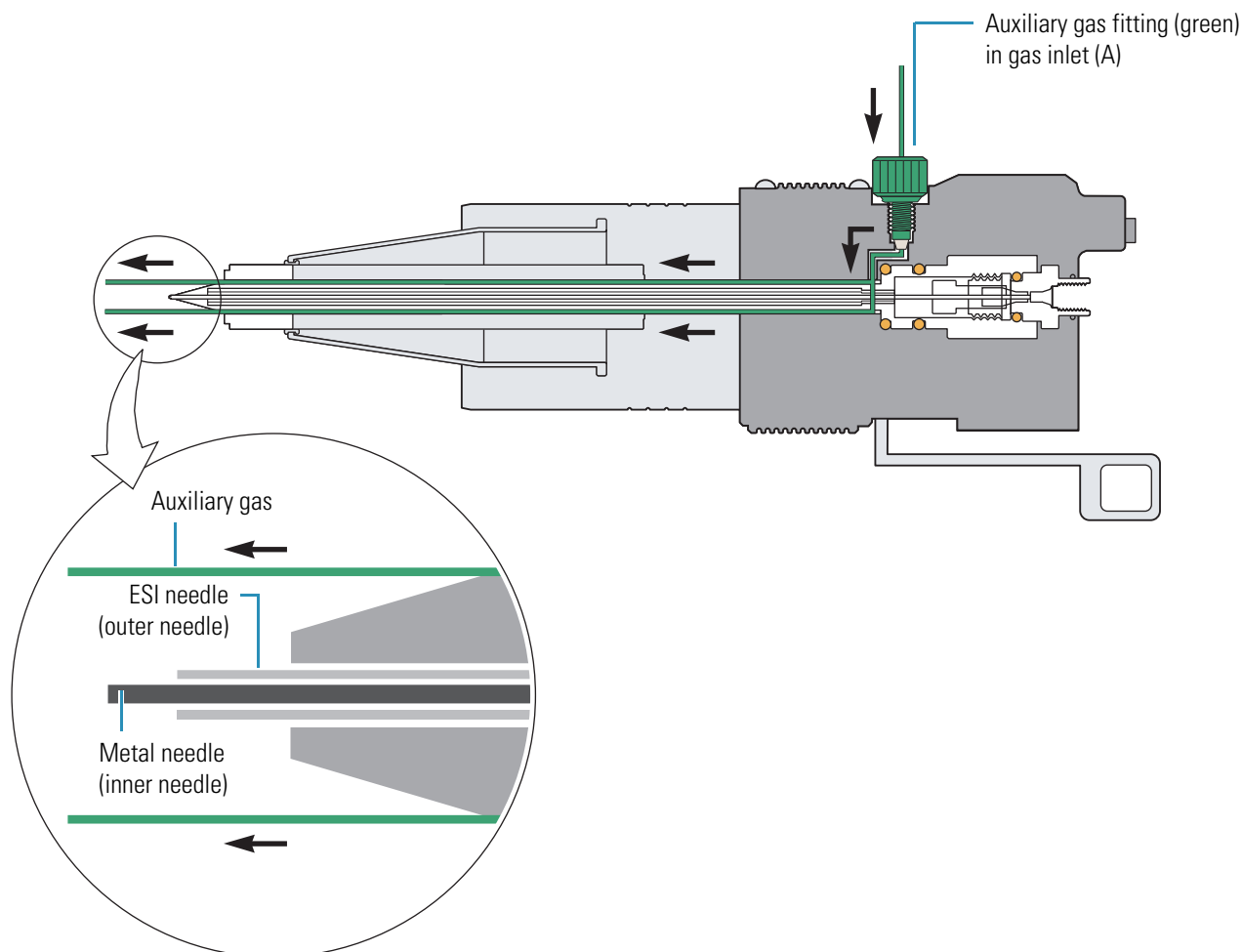


Figure 6. Auxiliary gas plumbing



The angle of the HESI-II probe is fixed at approximately 60 degrees. To help optimize the spray stability, use adjustment screws on the Ion Max source housing to make small changes to probe position. The fixed angle, off-axis spraying affords long-term signal stability (robustness) for most solutions that contain non-volatile matrix components, mobile phase buffers, or ion-pairing reagents. For information on adjusting the probe position, see [“Adjusting the Probe Position”](#) on page 17.

Removing and Installing the HESI-II Probe

This chapter describes how to set up the API source with the HESI-II probe. You must remove the HESI-II probe to perform maintenance on the probe.

Note To install the APCI probe, refer to the *Ion Max and Ion Max-S API Source Hardware Manual*.

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- [Removing the HESI-II Probe](#)
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Removing the HESI-II Probe



CAUTION AVOID BURNS. At typical operating temperatures (350 to 450 °C), the HESI-II probe can severely burn you. Before removing the probe from the API source housing, allow the probe to cool to room temperature (for approximately 20 minutes) before you touch it.

❖ To remove the HESI-II probe from the API source housing

1. Place the mass spectrometer in Standby mode, and allow the probe to cool to room temperature.

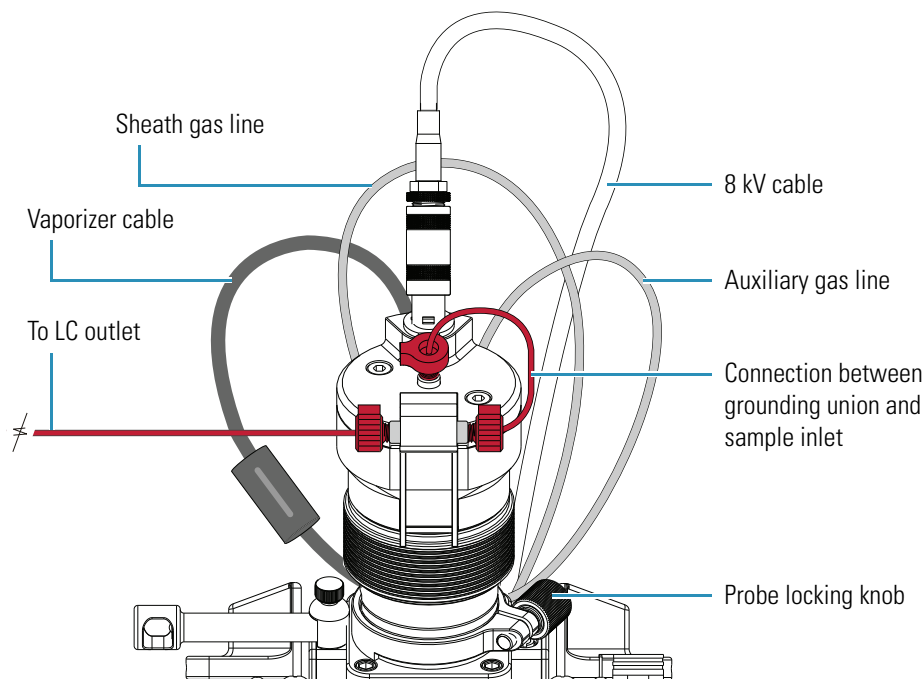
Wait approximately 20 minutes for the probe to reach room temperature when it has been operating at high temperature. If the mass spectrometer is connected to an LC system, leave the solvent flow from the LC pump on while the probe is cooling to room temperature.

2. If the mass spectrometer is connected to an LC system, turn off the solvent flow from the LC pump, and then disconnect the tubing from the left side of the HESI-II probe grounding union (see [Figure 7](#) on [page 10](#)).

3 Removing and Installing the HESI-II Probe

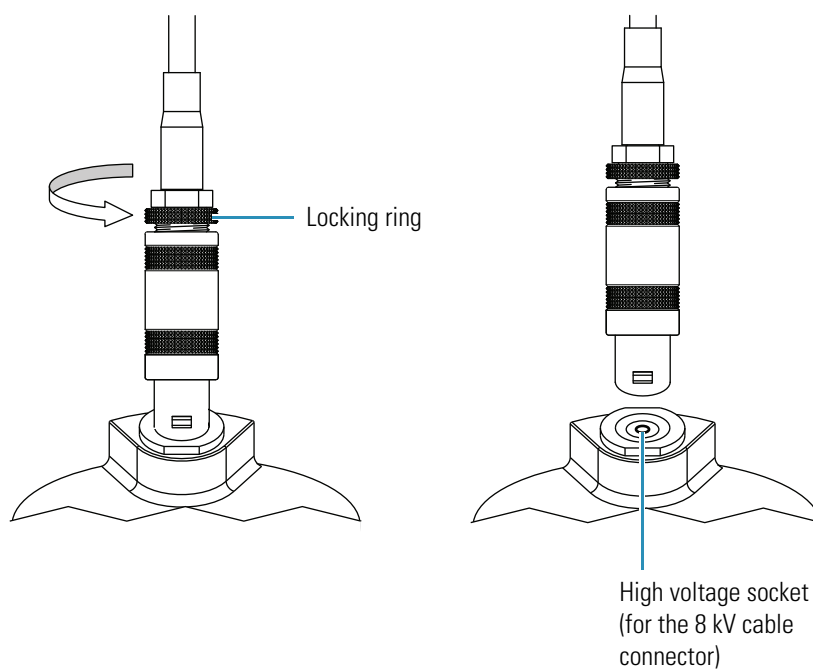
Removing the HESI-II Probe

Figure 7. Front view of the connections to the HESI-II probe



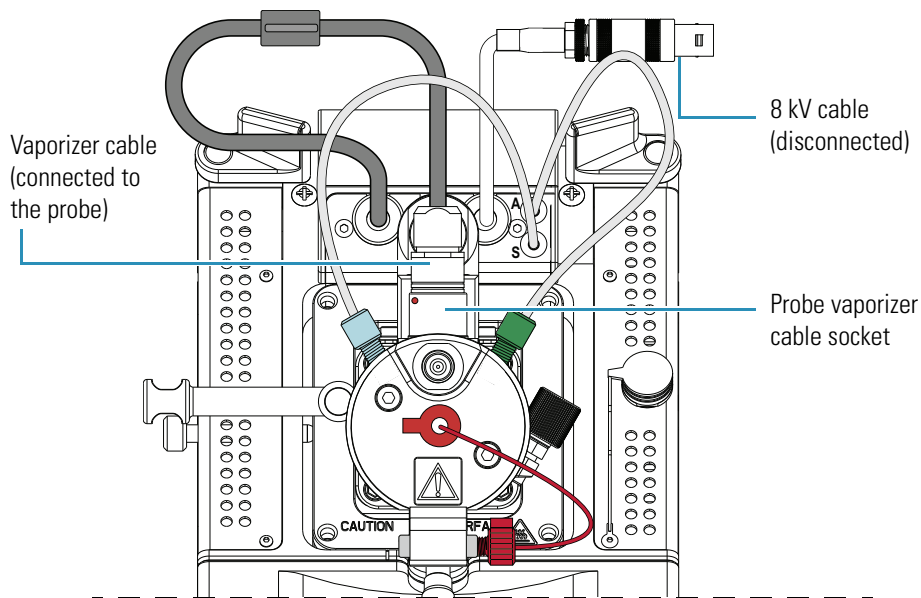
3. Disconnect the 8 kV cable from the HESI-II probe high voltage receptacle as follows (see [Figure 8](#) and [Figure 9](#)):
 - a. Unlock the cable by twisting the locking ring counterclockwise.
 - b. Unplug the 8 kV cable from the HESI-II probe high voltage socket.

Figure 8. Enlarged view of the 8 kV cable connector



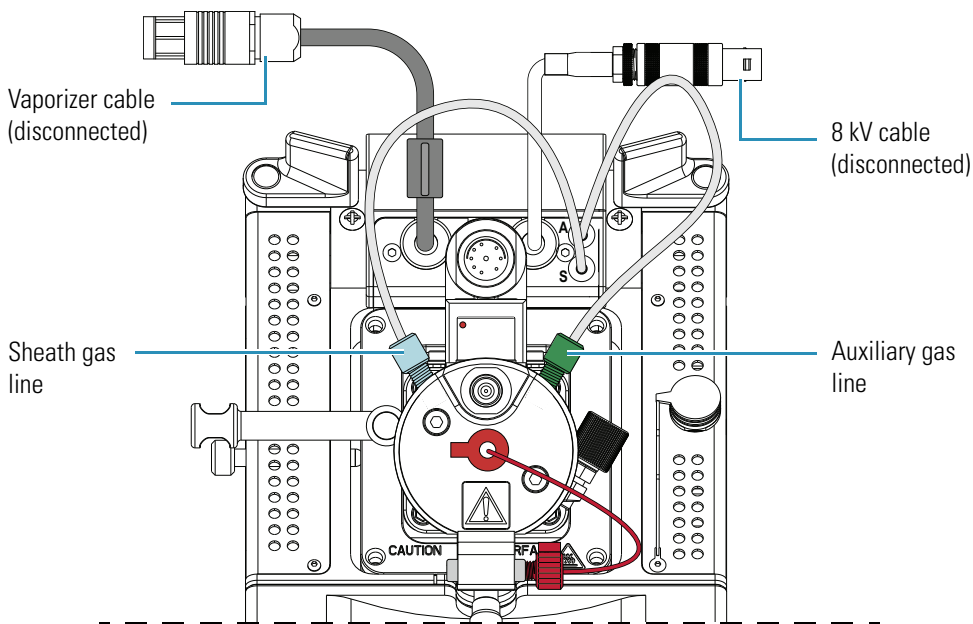
- Unplug the vaporizer cable from the HESI-II probe vaporizer cable socket (see [Figure 9](#) and [Figure 10](#)).

Figure 9. Top view of the API source housing and the HESI-II probe



- Disconnect the auxiliary gas fitting (green) from the auxiliary gas inlet (A) on the probe (see [Figure 10](#)).
- Disconnect the sheath gas fitting (blue) from the sheath gas inlet (S) on the probe (see [Figure 10](#)).

Figure 10. Top view showing the sheath and auxiliary gas lines connected to the probe

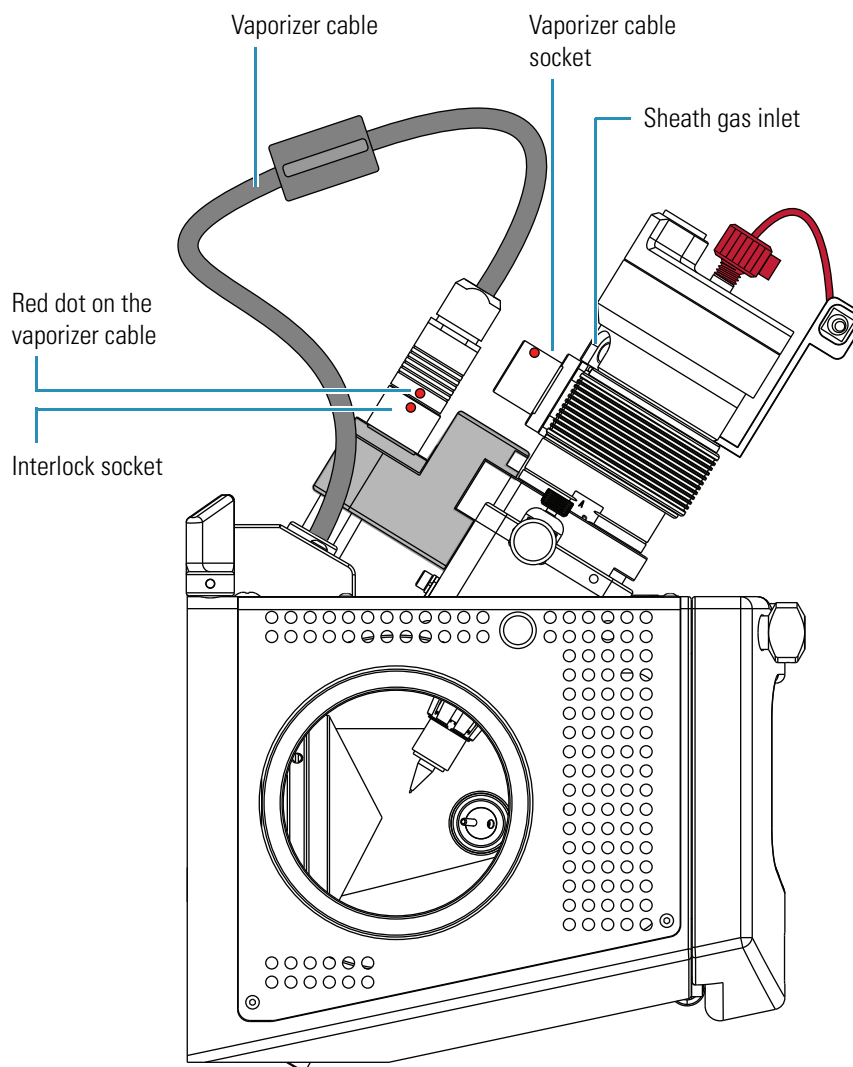


3 Removing and Installing the HESI-II Probe

Removing the HESI-II Probe

7. Connect the vaporizer cable to the interlock socket on the interlock block. To align the socket pins, align the red dot on the vaporizer cable connector with the red dot on the interlock socket (see [Figure 11](#)).

Figure 11. View of the left side of the API source housing



8. Unlock the probe locking ring by turning the probe locking knob (see [Figure 7](#) on [page 10](#)) counterclockwise.
9. Remove the probe from the port in the API source housing as follows:
 - a. Slowly pull the probe out of the port until you feel the resistance caused by the probe guide pin meeting the interlock block.
 - b. Turn the probe counterclockwise until the guide pin is free of the interlock block.
 - c. When the guide pin is free of the interlock block, pull the probe out of the port.
10. Store the HESI-II probe in its original shipping container.

Installing the HESI-II Probe

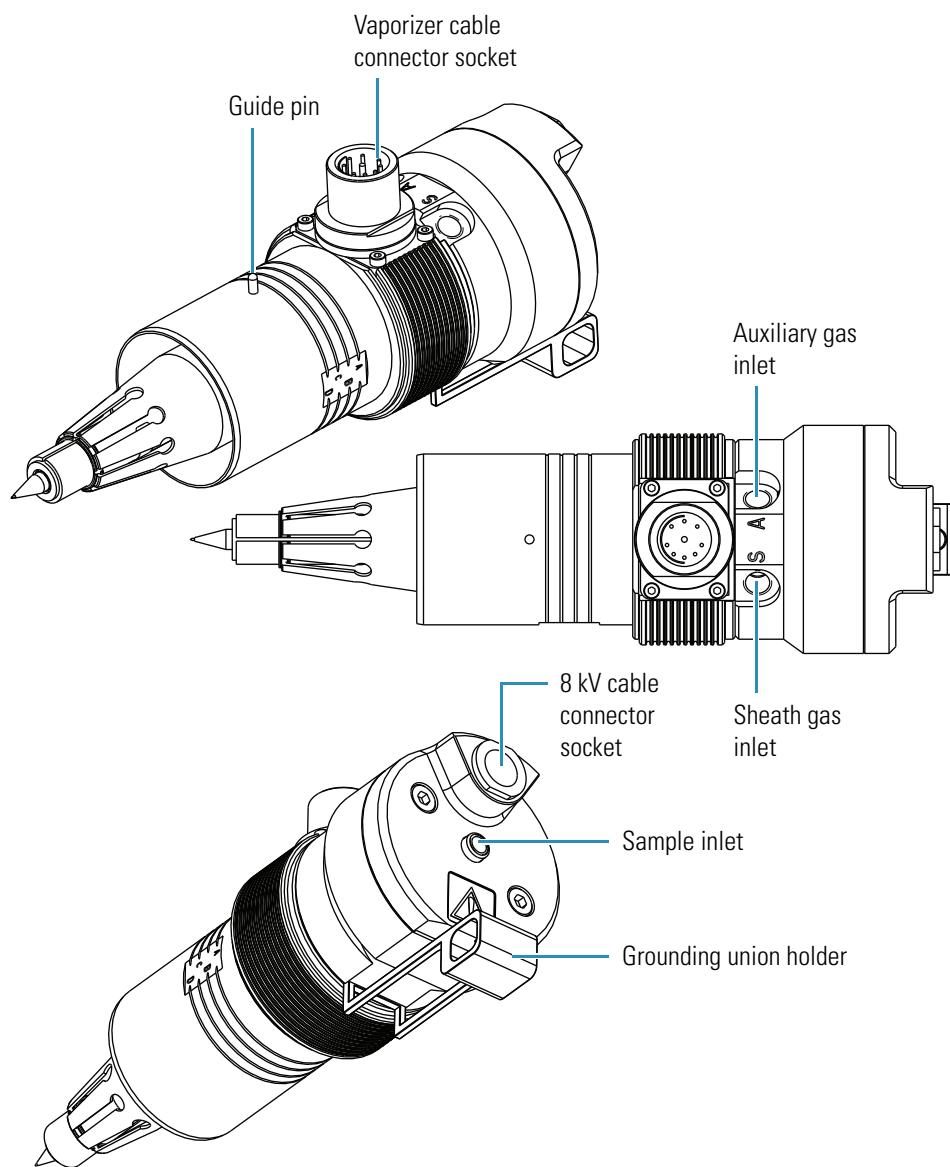
Note To remove an APCI or ESI probe, refer to the *Ion Max and Ion Max-S API Source Hardware Manual*.

❖ To install the HESI-II probe

1. Remove the HESI-II probe from its storage container. Inspect and clean it if necessary.

Figure 12 shows the locations of the probe guide pin, vaporizer cable connector socket, sheath and auxiliary gas inlets, 8 kV cable connector socket, sample inlet, and grounding union holder.

Figure 12. HESI-II probe views

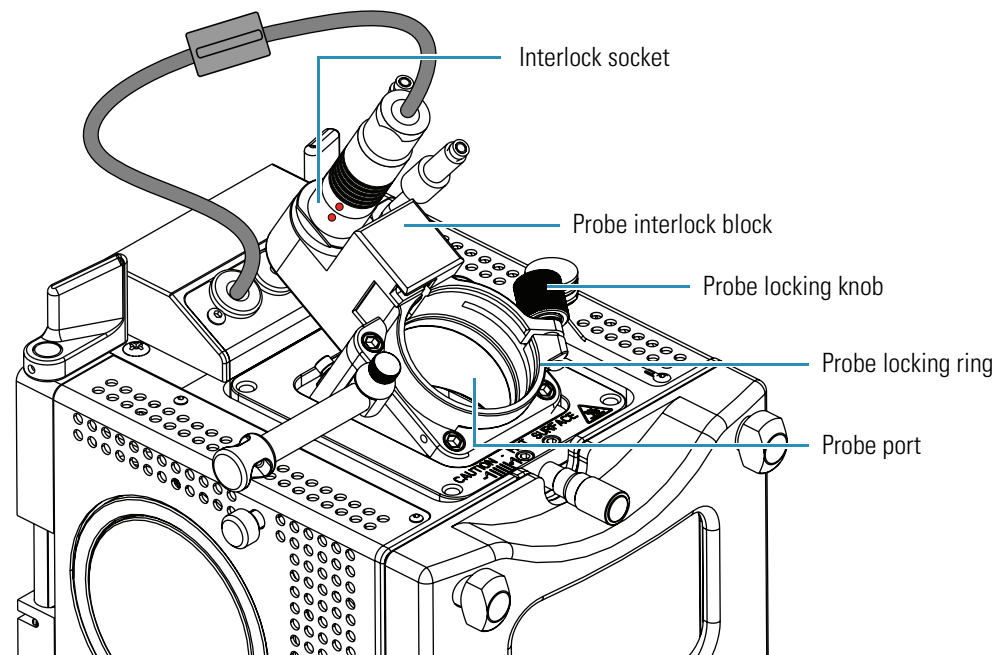


3 Removing and Installing the HESI-II Probe

Installing the HESI-II Probe

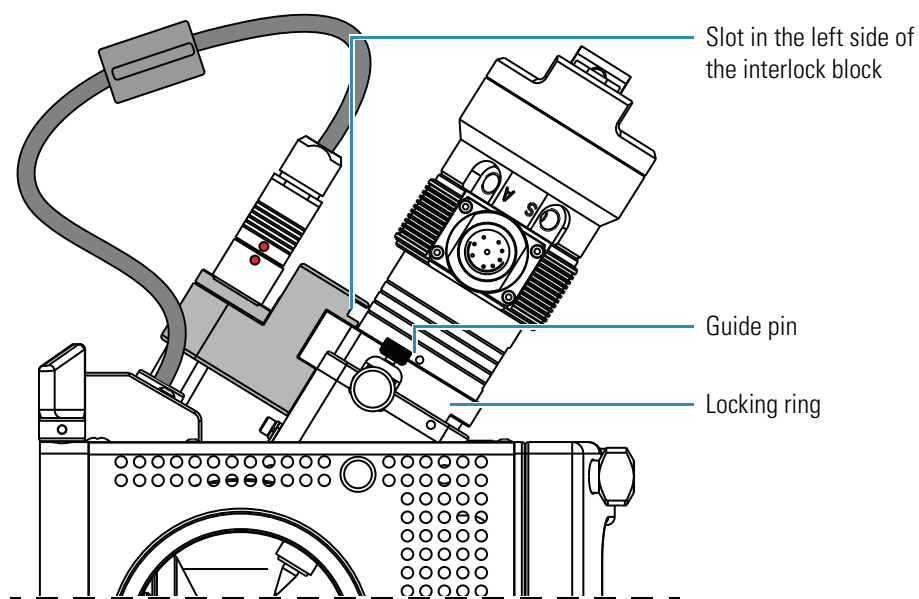
2. Turn the probe locking knob counterclockwise until the probe locking ring is opened to its widest position (see [Figure 13](#)).

Figure 13. Ion Max API source housing without a probe



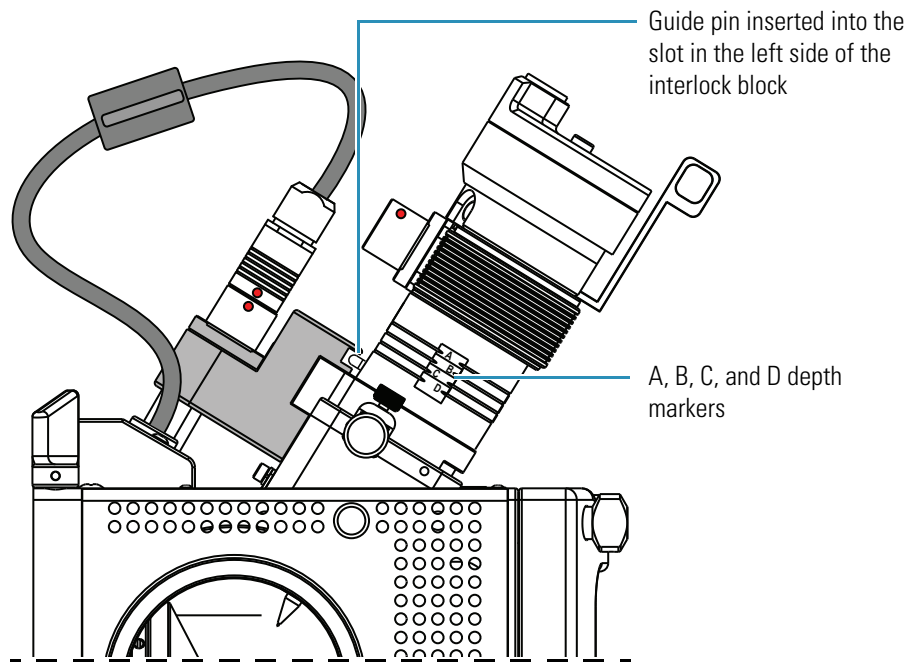
3. Seat the probe in the probe port in the API source housing as follows:
 - a. Holding the probe with the nozzle facing downward and the guide pin facing toward the left, slowly insert the probe into the port until the guide pin meets the locking ring on the API source housing (see [Figure 14](#)).

Figure 14. View of the guide pin touching the locking ring



- b. Pull the probe slightly upward until the guide pin is level with the slot on the left side of the interlock block. Then turn the probe clockwise until the guide pin meets resistance from the interlock block (see [Figure 15](#)).

Figure 15. View of the guide pin inserted into the slot



- c. Push the probe further downward into the port to the appropriate depth indicated by the A, B, C, and D depth markers on the probe.

In the H-ESI mode, insert the probe to a depth of B, C, or D. For high solvent flow rates, adjust the probe depth so that the nozzle is farther away from the ion interface (depth C or D). Conversely, for low solvent flow rates, adjust the probe depth so that the nozzle is closer to the ion interface (depth B or C).
4. Lock the probe in place by turning the probe locking knob (see [Figure 13](#) on [page 14](#)) clockwise until you feel resistance.
5. Connect the nitrogen gas lines to the HESI-II probe as follows (see [Figure 10](#) on [page 11](#)):
 - a. Connect the sheath gas fitting (blue) to the sheath gas inlet (S).
 - b. Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A).
6. Unplug the vaporizer cable connector from the interlock socket and connect it to the vaporizer cable connector socket on the HESI-II probe. To align the connector pins with the socket, align the red dot on the connector with the red dot on the socket. [Figure 11](#) on [page 12](#) shows the red alignment dots on the connector and the socket.
7. Connect the 8 kV cable connector to the 8 kV cable connector socket on the HESI-II probe. Tighten the locking ring (see [Figure 8](#) on [page 10](#)) on the 8 kV cable connector.

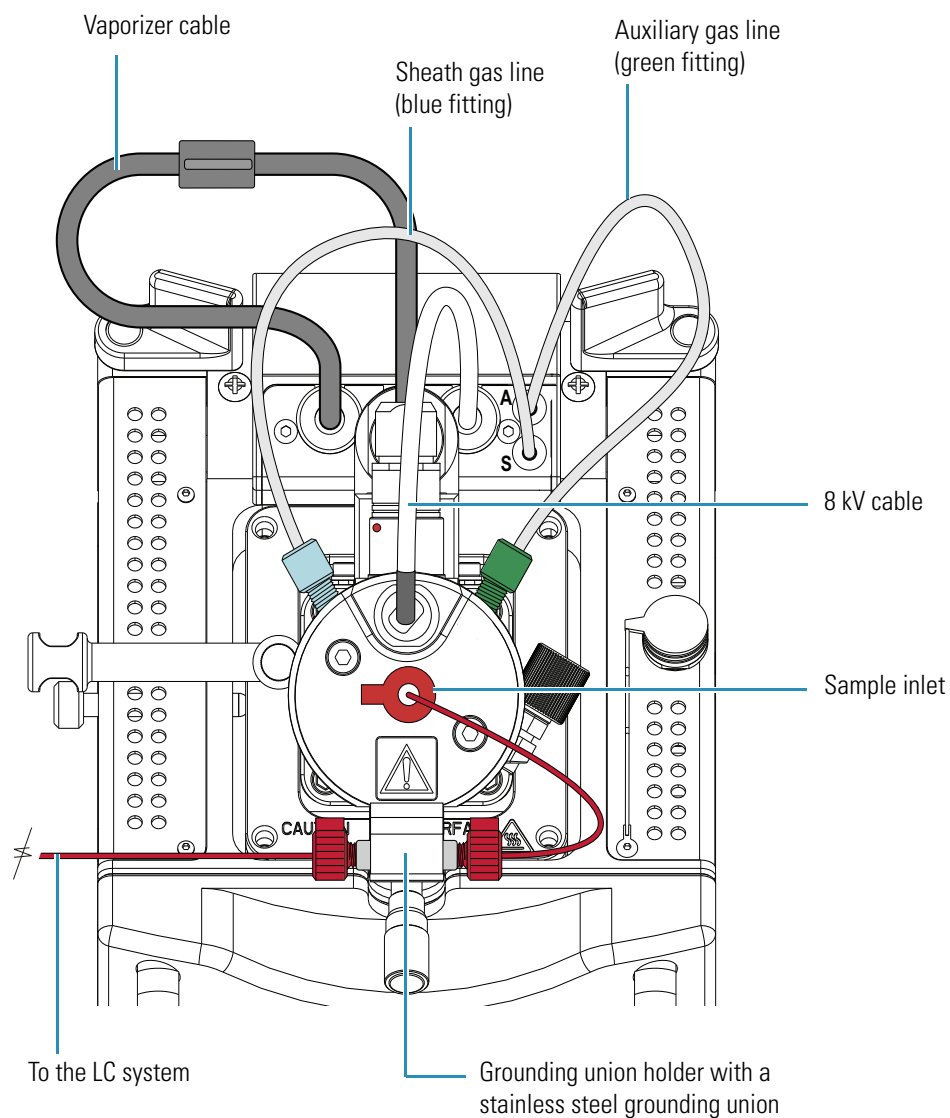
3 Removing and Installing the HESI-II Probe

Installing the HESI-II Probe

8. Ensure that the grounding union (stainless steel ZDV fitting) is seated in the grounding union holder on the HESI-II probe.
9. Using two fingertight fittings, connect a short length of red PEEK tubing to the right side of the grounding union and to the probe sample inlet.
10. Using two fingertight fittings, connect a length of red PEEK tubing to the LC outlet and to the left side of the grounding union.

Figure 16 shows the HESI-II probe installed in the Ion Max API source.

Figure 16. HESI-II probe installed in the Ion Max API source housing



Tip Before analyzing samples, change the ion source mode in TSQ EZ Tune by choosing **Setup > Change Ion Source > HESI**.

Adjusting the Probe Position

To maximize sensitivity, you can adjust the probe depth. If you have the Ion Max API source housing, you can also adjust the side-to-side and front-to-back probe position by a few millimeters.

For best results, follow the guidelines in [Table 2](#) as a starting point. These guidelines are based on the liquid flow rate of your application.

Table 2. Probe position guidelines

| Liquid flow rate range (µL/min) | Front-to-back position (micrometer setting) | Probe depth (probe depth line) | Side-to-side position (+1 to -1 marks) |
|---------------------------------|---|--------------------------------|--|
| 1 to 50 | 1.75* | B | 0 |
| 50 to 2000 | 1.75 | C | 0 |

* Fully turned to the right

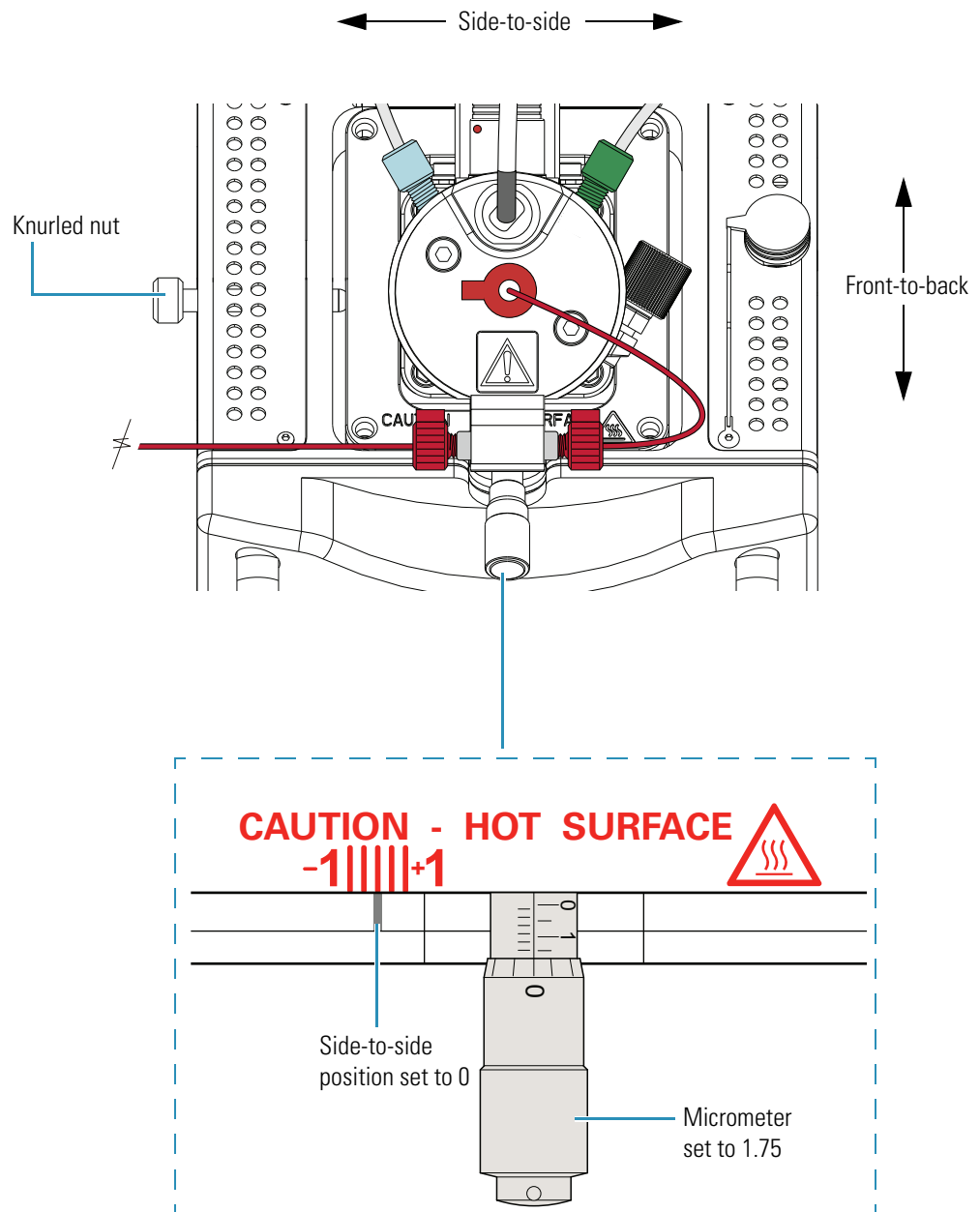
❖ To adjust the probe position

- Using the micrometer on the front of the Ion Max source housing, adjust the front-to-back probe position (see [Figure 17](#)).
- Using the knurled nut on the left side and the +1 to -1 markers on the top front of the Ion Max source housing, adjust the side-to-side probe position (see [Figure 17](#)).
- Using the A, B, C, and D markers on the probe as a guide, adjust the probe depth (see [Figure 15](#) on [page 15](#)).

3 Removing and Installing the HESI-II Probe

Adjusting the Probe Position

Figure 17. Top view of the Ion Max source housing with a HESI-II probe



Maintenance

The HESI-II probe requires minimum maintenance. If the metal needle sample tube is plugged, replace the needle insert. Replacing the needle insert requires a partial disassembly of the probe.

Tip For best results, flush the HESI-II probe at the end of each working day, using a 50:50 LCMS-grade methanol/distilled water solution from the LC pump through the HESI-II probe. To order LCMS grade solvents from Thermo Fisher Scientific, go to www.FisherLCMS.com

IMPORTANT For best results, avoid operating the HESI-II probe at elevated temperatures without solvent flow. Allowing the HESI-II probe to run dry at elevated temperatures can cause blockage of the replaceable metal needle.

IMPORTANT For best results, wear clean gloves when you handle HESI-II probe components.



CAUTION AVOID BURNS. At operating temperatures, the vaporizer can severely burn you. The vaporizer typically operates between 350 and 450 °C. Always allow the heated vaporizer to cool to room temperature (for approximately 20 min) before you remove or touch the HESI-II probe.

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- [Replacing the Needle Insert](#)

Flushing the Sample Transfer Line, Sample Tube, and HESI-II Probe

For best results, flush the sample transfer line, sample tube, and HESI-II probe for 15 minutes at the end of each working day (or more often if you suspect they are contaminated). Use a 50:50 methanol/distilled water solution from the LC system through the API source. After 15 minutes, turn off the flow of liquid from the LC to the API source, but keep the API source on (including the sheath gas and auxiliary gas) for an additional 5 minutes. Refer to the daily operations chapter in the hardware manual for your mass spectrometer.

Replacing the Needle Insert

If the metal needle is plugged, you can replace the needle insert. The following procedure describes how to replace the needle insert.

The needle insert is assembled at the factory and consists of an adjustable union, a needle guide fitting, a ferrule, an O-ring, an ESI needle, and a metal needle (see Figure 18). The ferrule is swaged onto the ESI needle. Factory adjusted, the metal needle protrudes 0.5 mm from the end of the ESI needle (see Figure 19).

IMPORTANT Because the protrusion of the metal needle from the ESI needle is factory-adjusted, do not disassemble the needle insert.

Figure 18. Exploded view of the needle insert

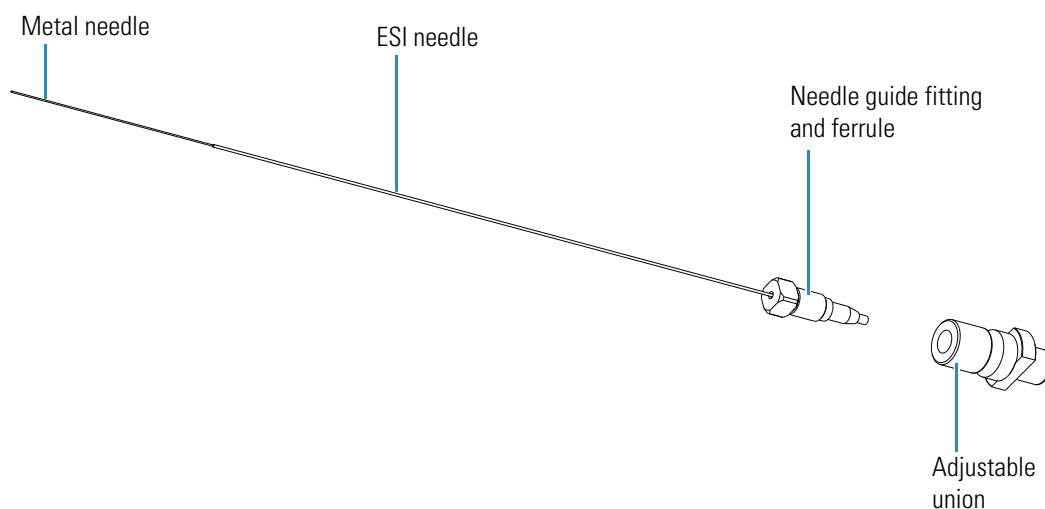
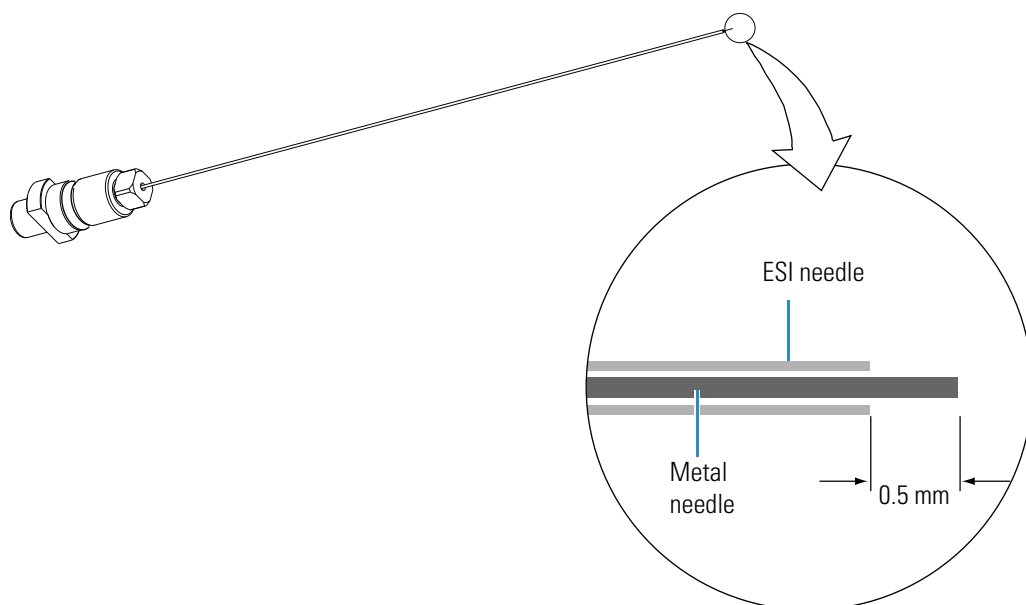


Figure 19. Metal needle insert assembly with an enlarged view of the stainless steel needle tip



To support flow rates from 1 to 2000 $\mu\text{L}/\text{min}$, Thermo Fisher Scientific provides two needle inserts for the HESI-II probe. The difference between the two inserts is the size of the metal needle and supporting ferrule (Table 3).

Table 3. Needle inserts

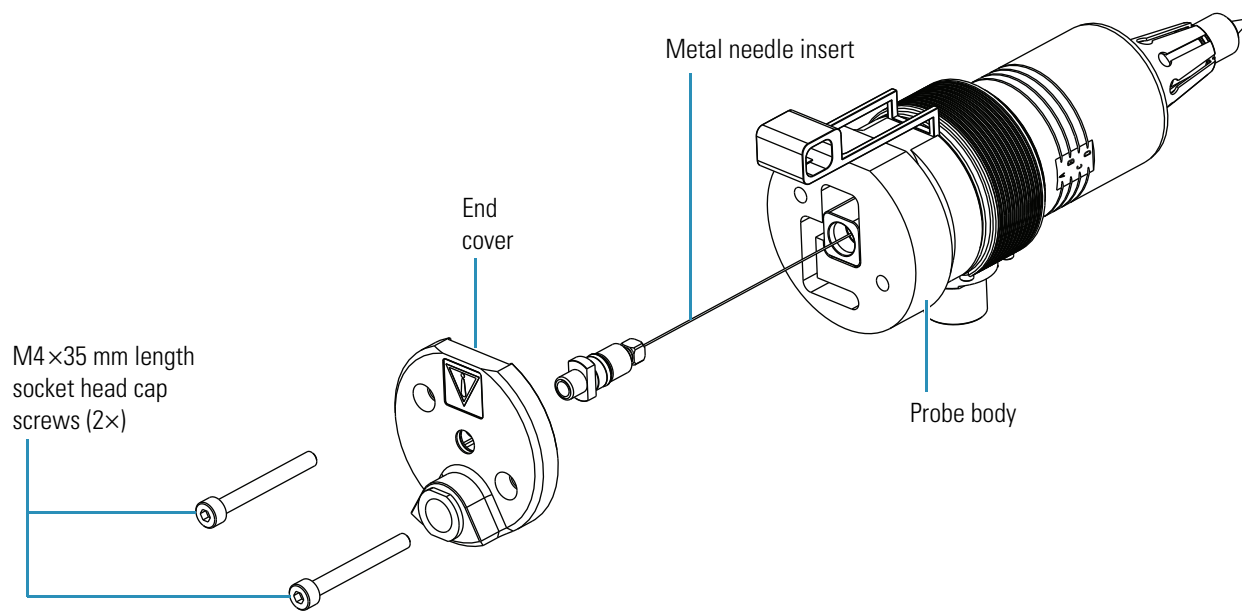
| Description | Metal needle | Ferrule | Flow rate range |
|---------------------------------------|------------------------------|------------------|------------------------------------|
| 32-gauge needle insert, HESI-II probe | 0.004 in. ID 0.009 in. OD | 0.4 mm thru-hole | 5 to 2000 $\mu\text{L}/\text{min}$ |
| 34-gauge needle insert, HESI-II probe | 0.003 in. ID 0.007 in. OD | 0.2 mm thru-hole | 1 to 10 $\mu\text{L}/\text{min}$ |

Replacing the needle insert requires a 3 mm (7/64 in.) hex wrench or ball driver.

❖ **To replace the needle insert**

1. Remove the HESI-II probe from the Ion Max API source (see “Removing the HESI-II Probe” on page 9).
2. Unscrew the fingertight fitting from the sample inlet port (see Figure 16 on page 16).
3. Remove the needle insert from the probe as follows (see Figure 20):
 - a. Using a 3 mm (7/64 in.) hex wrench or ball driver, remove the two M4×35 mm length, socket head cap screws.
 - b. Pull the end cover off of the probe.
 - c. Unscrew the needle insert, and then pull it out of the probe body.

Figure 20. Exploded view of the HESI-II probe

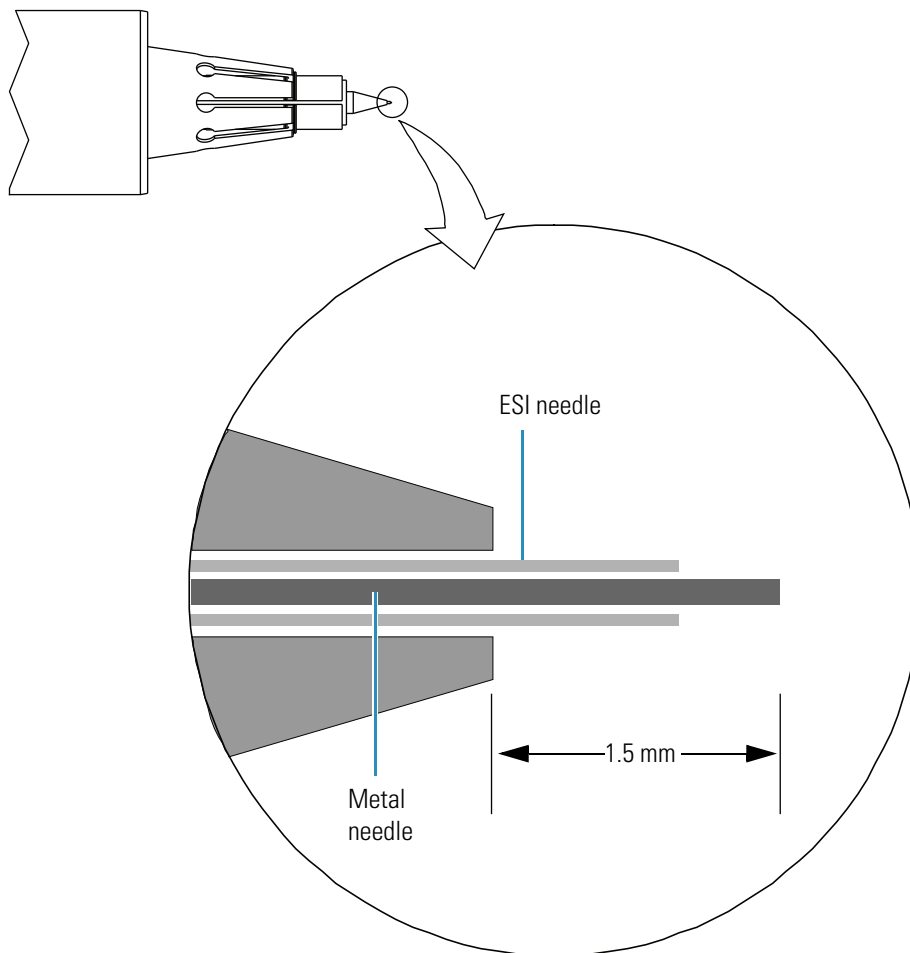


4 Maintenance

Replacing the Needle Insert

4. Insert a new needle insert into the probe body.
5. Hand tighten the adjustable union fitting (see [Figure 18](#) on [page 20](#)) until the needle insert tip protrudes from the probe nozzle by 1.5 mm (see [Figure 21](#)).

Figure 21. Enlarged view of the probe nozzle



6. Position the end cover on the probe body.
7. Insert the two M4×35 mm length, socket head cap screws into the end cover, and then tighten them with a 3 mm (7/64 in.) hex wrench or ball driver.
8. Reinstall the HESI-II probe (see [“Installing the HESI-II Probe”](#) on [page 13](#)).

Replaceable Parts

Use the following part numbers when you need to order the replaceable and consumable parts for the HESI-II probe.

You can order these parts directly from Thermo Fisher Scientific.

| | |
|---|--------------------|
| HESI-II Probe Kit | OPTON 20037 |
| High-flow needle insert assembly | OPTON-53010 |
| Low-flow needle insert assembly | OPTON-53011 |

The HESI-II Probe Kit contains the HESI-II probe with a factory-installed high-flow needle insert. The high-flow metal needle insert is suitable for flow rates equal to or greater than 5 $\mu\text{L}/\text{min}$. For low-flow rate applications, order the low-flow needle insert assembly. For more information on the needle inserts, see [Table 3](#) on [page 21](#).

You can order these parts from Upchurch Scientific; however, these part numbers are subject to change.

| | |
|--|-------|
| Fitting, fingertight, one-piece, for 1/16 in. OD high pressure tubing | F-120 |
| Fitting, fingertight with one wing, two-piece, red PEEK, for 1/16 in. OD high pressure tubing | F-200 |
| Union, stainless steel, for 1/16 in. OD high pressure tubing | U-435 |

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