Agilent G3253A Dual Nanospray Ion Source

User's Guide

Agilent Technologies

Notices

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Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
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In This Guide

1 Basic Operation

This chapter describes basic operation for the dual nanospray ion source.

2 Troubleshooting

This chapter describes the steps that you follow when you have problems with the dual nanospray ion source.

3 Maintenance

This chapter describes maintenance for the dual nanospray ion source.

4 Installation

This chapter tells you how to install the dual nanospray ion source.

5 Reference

This chapter contains an overview of dual nanospray operation, safety precautions, technical specifications, and list of part numbers.

Contents

1
Basic Operation

This chapter describes the tasks that you need to operate the nanospray source.

Overview

This section provides basic information about the dual nanospray ion source, and what you need to do to get started using it.

For more information while you work with the TOF software, see the online help. Click on any tab or display window and press `F1`.

The dual nanospray ion source (DNS) lets users analyze compounds at nanoflow rates with a high degree of sensitivity and mass accuracy. The source utilizes two physically separated inlets: one for an analytical sprayer (typically the effluent from an LC or an infused offline sample), and the other for a reference sprayer to introduce reference mass standards. This combination can provide sensitivities on the order of 1-5 fm on-column for protein digests (PMF) analysis, and 20-50 fm for in-source CID TOFMS analysis. Mass accuracy is typically $\leq$ 3 ppm with internal reference mass (IRM) correction.

Figure 1 Dual nanospray ion source.

Sample solution enters the spray chamber through a grounded needle assembly (Figure 2). The liquid exiting the needle is subjected to a high voltage electrostatic field. The intense field at the needle's tip electrically charges the liquid, producing charged droplets and ions.

Heated nitrogen gas is introduced along the outside end of a glass capillary, directed towards the dual-cone assembly through a gas diffuser. The drying gas exiting the outer cone assists evaporation of these charged droplets. The electrostatic field gradient guides the ions towards the inlet orifice of the inner cone, which is connected to the glass capillary leading to the first stage vacuum region. The pressure differential between the spray chamber and the ion focus/transport region of the desolvation chamber pushes the ions through the capillary. Ions are subsequently transported, mass analyzed (TOF) and detected in a high vacuum region.

Figure 2 Needle Assembly with needle and transfer-line (or column).

The operator controls both the high voltage applied to the ion source electrodes, and the temperature and flow of the drying gas, using the TOF software interface. For nanospray operation, nebulizing gas is not used.

Getting started

Before you start using the dual nanospray ion source, you need to prepare the hardware and the tuning solutions. See the list below of necessary steps, and where to find information about them.

- Read and understand safety precautions: "Safety" on page 90
- Set up the syringe pump: "Step 2. Set up the KDS Model 100 syringe pump" on page 52.
- Prepare the syringe infusion line assemblies: "Step 3. Prepare the syringe infusion line assemblies" on page 53.

- Perform an initial tuning with the ESI source, even if you plan to use the dual nanospray source: "To tune the ESI source for subsequent DNS operation" on page 6.
- Switch from the ESI source (or the APCI source) to the DNS source: "To convert from ESI or APCI to dual nanospray" on page 67.
- Install the needle holder: "Step 4. Install the union holder" on page 60.
- Prepare a solution for DNS calibration and tuning: "To prepare an ES-TOF Tuning Mix, modified for DNS" on page 77.
- Calibrate the DNS source: "To calibrate or tune the dual nanospray source" on page 7.
- Prepare a solution of reference mass standards: "To prepare a reference mass standards solution" on page 80
- Optionally, prepare a solution of [Glu^1]-fibrinopeptide B: "To prepare a solution of [Glu^1]-fibrinopeptide B" on page 80
- Optionally, prepare a solution of BSA tryptic digest: "To prepare a BSA tryptic digest" on page 85.

Tune the instrument with ESI source before installing the dual nanospray source. The ESI tuning procedure automatically calibrates the mass axis; calibrate with the DNS source separately after tuning.

Safety

Some of the procedures in this manual require access to parts of the instrument and nanospray ion source while it is in Standby state or shortly after it is turned off. If you do not perform these procedures correctly, you are exposed to dangerous temperatures, voltages, and chemical hazards.

This section alerts you to the importance of safe practices. **Refer to the Reference chapter on page 75 for important safety details.**

Safety issues include, but are not limited to:

- **Needle hazard:** The needle in the needle holder is very sharp and can pierce your skin. Do not touch the tip, especially when you analyze toxic substances or when you use toxic solvents.

- **High temperatures:** Some parts in the nanospray chamber and desolvation interface reach temperatures high enough to cause serious burns. These parts include, but are not limited to the capillary, dual-cone endplate assembly, and lamp. *Do not touch these parts.*

  When converting from the APCI source, be particularly cautious; the surface temperature of the vaporizer might be extremely hot.
- **Hazardous voltages:** Whenever the instrument is not in Standby, hazardous voltages are present on one or more interior parts. Parts that use hazardous voltages include, but are not limited to, the dual-cone endplate assembly and back electrode assembly. Never open the DNS chamber while the instrument is in the **On** state or the HV voltage is turned on.
- **Biohazardous residue:** The exhaust from the mechanical pump can contain traces of sample and solvents. Vent all pump exhaust outside the building or into a fume hood. Comply with your local air quality regulations.

To tune the ESI source for subsequent DNS operation

Agilent recommends that you initially tune with your ESI source before switching to the dual nanospray source. Also, tune using the ESI source:

- at regular intervals (perhaps once a month)
- if tuning the dual nanospray source is unsuccessful

When you tune the ESI source, the source is automatically calibrated.

1. With the standard ESI source and spray chamber in place, load the **ESIautotune** method.
2. Tune and calibrate the instrument by running the Autotune procedure.
3. Following a successful Autotune, set TOF to Standby mode.

To install the dual nanospray source

For more detailed instructions, see "To convert from ESI or APCI to dual nanospray" on page 67.

1. In the TOF Software, check that the TOF has been set to **Standby** mode.
2. Open the standard ESI spray chamber, disconnect plumbing and separately cap off nebulizer gas line and reference nebulizer gas line using a ¼-28 cap.
3. Remove the spray chamber from hinges, cover and set aside.
4. Unscrew and remove the 1" diameter end cap ("spray shield"), capillary cap and the 2 1/4" diameter endplate (2 torx screws, torx driver 10).
5. Set these parts aside in a clean container. Install the dual nanospray source assembly and DNS spray chamber. Close the spray chamber door and identify source as **nanoESI** in the instrument control panel.
6. Load the **nanoESIautotune** method.

To set the instrument control software to nanospray mode

In this step, you set the instrument control software.

1 In the TOF Software, check that the control has been set to **Standby** mode.
2 Set **Ion Source** to **nanoESI**.
3 Check that the **Capillary voltage** is set to **1900 volts**.
4 Check that the **Drying gas flow** is **3.5-4.0 L/min** with a **Drying gas temperature** of **300°C**.

These source settings are only preliminary and should be adjusted for the application.

Keep the LC/MSD TOF in **Standby** mode until the nanospray is ready for use.

To calibrate or tune the dual nanospray source

This section assumes that you have performed initial tuning using the ESI source, before installing and using the DNS (see "To tune the ESI source for subsequent DNS operation" on page 6). The ESIautotune procedure automatically calibrates the mass axis. Calibrate the DNS source separately after the tuning.

This section tells you how to calibrate and tune the dual nanospray source for routine use.

Agilent recommends tuning the ESI source regularly (about once a month) even if you primarily use the DNS source for sample analysis. Consult your Agilent representative about how often to tune your ESI source for your particular needs.

To prepare for calibration or tuning

1 Close the DNS chamber and carefully adjust the needle.

When viewed through the front window port, the analytical needle tip should be aligned exactly *midway* between the score mark and the inner edge of the outer cone and centered with respect to the inner cone's orifice. See Figure 3 below.

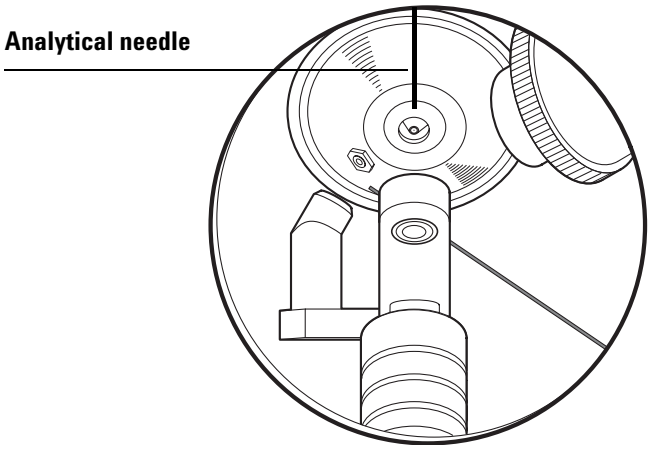

Figure 3 Alignment of PicoTip for tuning or calibration

2 Use the microscope to measure the Z direction distance from the back electrode.

The PicoTip is correctly positioned for calibration or tuning when it is midway between the score mark and the inner edge of the outer cone, and is 3.0–3.5 mm away from the back electrode. This position results in a strong stable response for DNS calibration and tuning when the proper TOF source parameters are applied.

a Check that the 250 µL syringe and transfer line assembly is loaded with ES-TOF Tuning mix (G1969-85000), modified for DNS use (see "To prepare an ES-TOF Tuning Mix, modified for DNS" on page 77). Set the infusion pump for a flow rate of 18 µl/hr (300 nl/min) but do not start the pump.

b Turn the TOF **On**.

c Load the method file nanoESIautotune.

d Change the TOF source parameters in the method to the following, as shown in Figure 4:

- Capillary voltage (Vcap): 2100 V
- Fragmentor (Frag): 215 V
- Drying gas temperature (DGT): 275°C
- Drying gas flow (DGF): 6 L/min

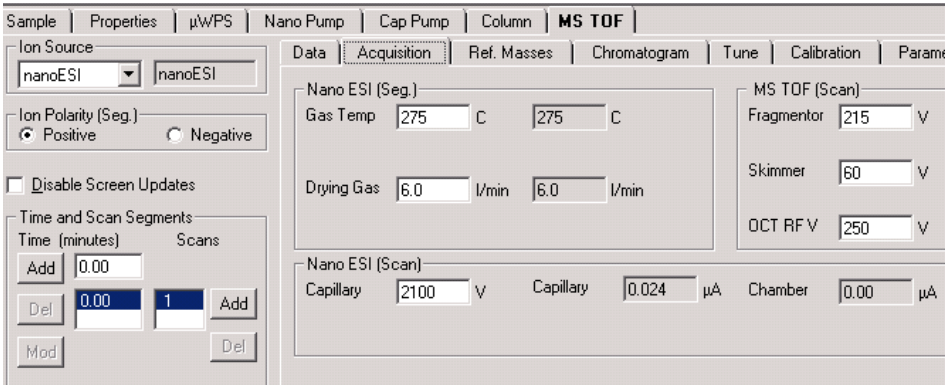

Figure 4 DNS TOF source parameters for calibration and tuning

e Click the **Calibration** tab.

f Mark the **Ignore CalB** checkbox. A checkmark here disables CDS calibrant flow.

g Save the changes to the method file (**nanoESIautotune.m**).

h Start the syringe pump flow.

i Observe the end of the needle. Within minutes there should be a stable nanospray plume. Check that there is good spectral response for the tune ions in the Agilent TOF Software.

To perform a mass axis calibration using the DNS source

1 Start calibrant flow on the syringe pump by completing the following steps.

a Make sure the method **nanoESIautotune.m** is loaded.

b Check that the 250 µl syringe is loaded with the ES-TOF Tuning Mix (G1969-85000), modified for DNS use, non-diluted, and infusing at 15-18 µl/hr.

The ES-TOF tuning mix, modified for DNS use, is introduced through the analytical needle holder port at the top of the DNS spray chamber.

c Start the syringe pump.

NOTE You do not need to introduce reference mass standards solution (via the reference sprayer) when calibrating or tuning.

2 Use the microscope to view the nanospray needle.

The nanospray plume should be vertically positioned, and not directed at either the inlet cone or the back electrode. You can rotate the needle holder clamp a bit to obtain the desired vertical spray configuration.

3 Monitor the capillary current, which should be about 25nA for the ES-TOF Tuning Mix (modified for DNS use).

4 Check that there is good spectral response for the tune ions in the Agilent TOF Software.

Maximum counts in the **MS Spectrum Plot** window should total less than 1.4 million.

- If the signal is too high, dilute the ES-TOF Tuning Mix (modified for DNS use) 1:10, with a 95:5 v/v acetonitrile/water mixture.
- If the signal is too low, lower the PicoTip another 0.5-1 mm (Y-axis position) and position the needle a bit closer (Z-direction) to the inlet orifice.
- Alternatively, the PMT voltage may be increased or decreased, depending upon signal response.

NOTE If you change the PMT voltage, you will need to recalibrate the mass axis. Changing the detector voltage will also affect linearity and dynamic range. Always analyze samples with the same TOF instrument parameters you used for calibration (with the exception of certain source parameters; for example, Drying Gas Flow, Drying Gas Temperature, and Capillary Voltage).

5 In the TOF software, click the **Calibration** tab. Set the parameters as shown below. Make sure to mark the **Ignore CalB** checkbox.

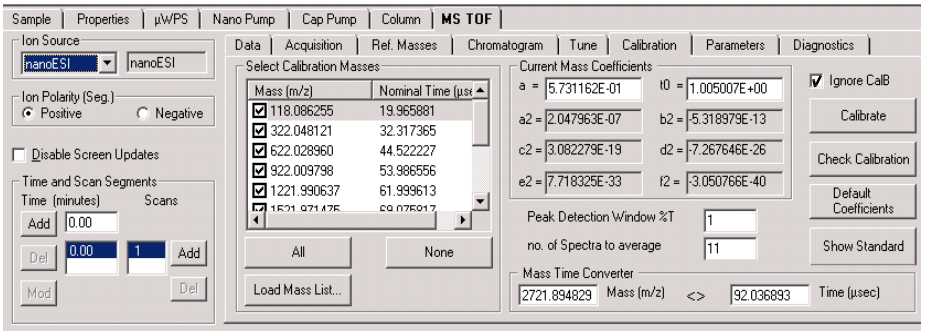

Figure 5 DNS TOF source parameters for mass calibration

6 Click the **Calibrate** button.

7 If the calibration is good, accept the calibration. Compare calibration results with the examples in Figure 7 and Figure 8 (positive mode calibration) or Figure 9 and Figure 10 (negative mode calibration).

The calibration is good when the *fully corrected* residual mass errors for all calibrant ions are ≤ 3 ppm. If calibration fails, see Chapter 2, “Troubleshooting”.

NOTE

After calibration, run a tune report (see “To create a simple Tune Report for mass axis calibration verification” on page 18),

The tune report provides important information about the operating state of the mass spectrometer. It includes a graphical profile of the low and high mass calibrant peaks and indicates their intensity, resolution and flight time. To a first approximation, flight times at 6.5 kV operation for the low mass calibrant ion (m/z 118) and high mass calibrant ion (m/z 2722) are 20 µsec and 92 µsec, respectively. The report also includes a mass spectrum, mass calibration table, tune parameters, and setpoint tables. The tune report lists the mass error (in ppm) for all the calibrant ions found. These errors should always be ≤ 3 ppm; otherwise, a new calibration or autotune is required.

NOTE Depending upon chemical performance requirements, it may be necessary to dilute the ES-TOF Tune Mix by a factor of ten (or more)— particularly in negative ion mode operation, or when a higher detector setting is required.

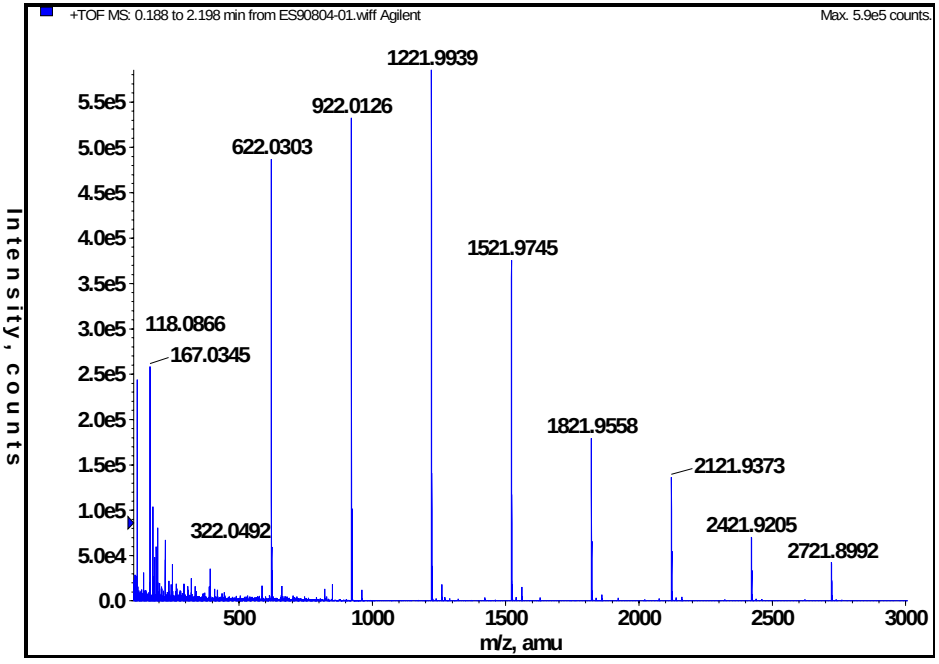

Figure 6 Sample positive ion mass spectrum of ES-TOF Tuning Mix

NOTE You can autotune or calibrate the instrument without having to switch back to standard ESI.

8 Following calibration, remove the needle holder clamp from the top (analytical) position.

CAUTION Do not let the needle dry out with sample inside it. Precipitation causes blockage. Before you store the needle for later use, use a clean solvent in which the sample is highly soluble to rinse the needle. For example, after you run the ES-TOF Tuning Mix, run a 90:10 v/v acetonitrile/water solution to flush needle.

CAUTION When sample delivery is off, separate the needle from drying gas heat by partially or fully withdrawing the needle holder assembly.

Positive ion mode DNS calibration examples

The following examples show representative results for positive ion mode DNS calibration. For information about calibrating the instrument, see "To calibrate or tune the dual nanospray source" on page 7.

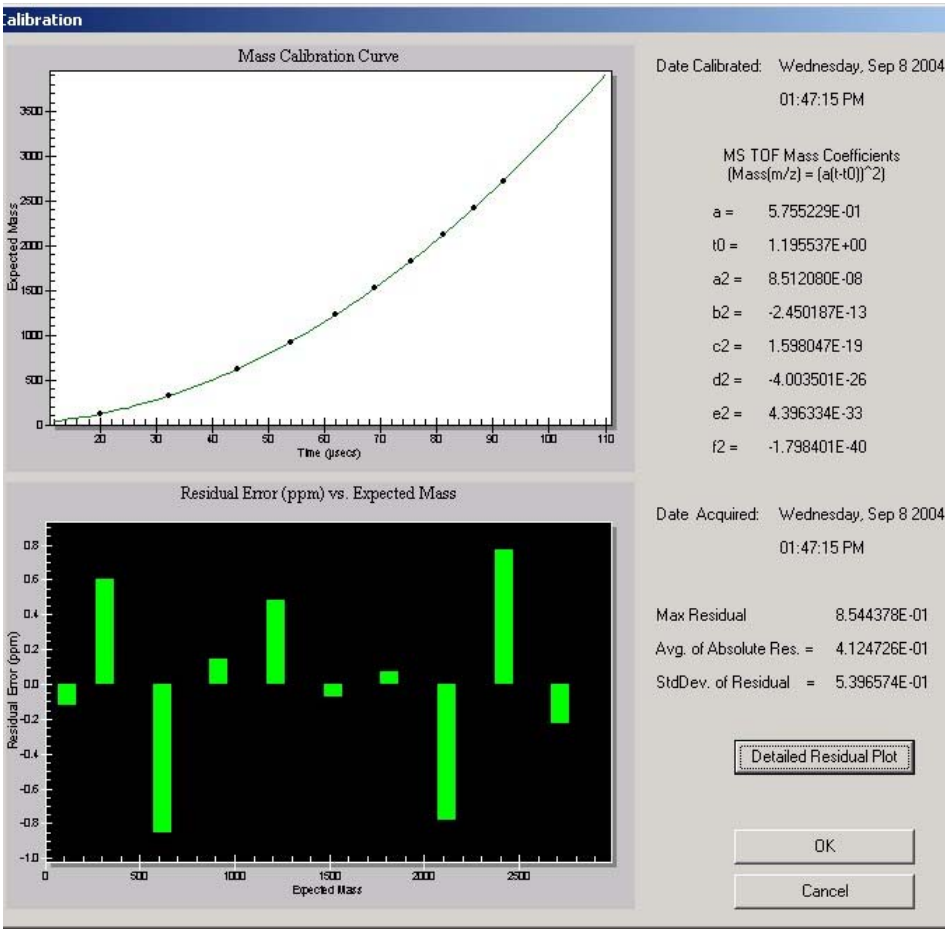

Figure 7 Calibration results (positive ion mode)

Click **Detailed residual plot** in the Calibration Results screen above to display the plot shown in Figure 8.

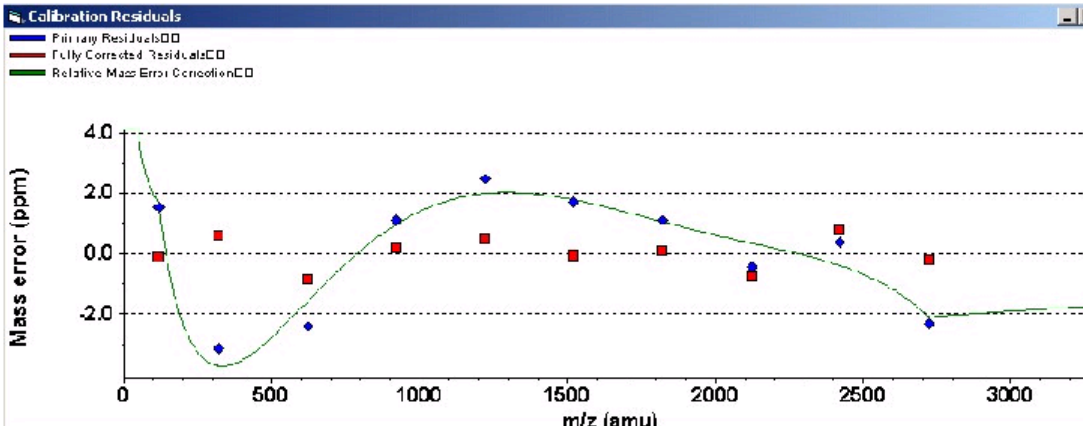

Figure 8 Detailed residual plot from positive ion calibration

Negative ion mode DNS calibration examples

1 Make sure your needle is aligned as described in step 2 under "To prepare for calibration or tuning" on page 7.

2 Follow the steps in "To perform a mass axis calibration using the DNS source" on page 9.

3 In the TOF Software, select **Negative Ion Polarity** in the MS TOF tab.

The following charts contain representative results for negative ion mode DNS calibration.

NOTE

After calibration, run a tune report (see "To create a simple Tune Report for mass axis calibration verification" on page 18) and compare it to the examples shown in this section,

The calibration is good when the *fully corrected* residual mass errors for all calibrant ions are $\leq$3 ppm. If calibration fails, see Chapter 2, "Troubleshooting".

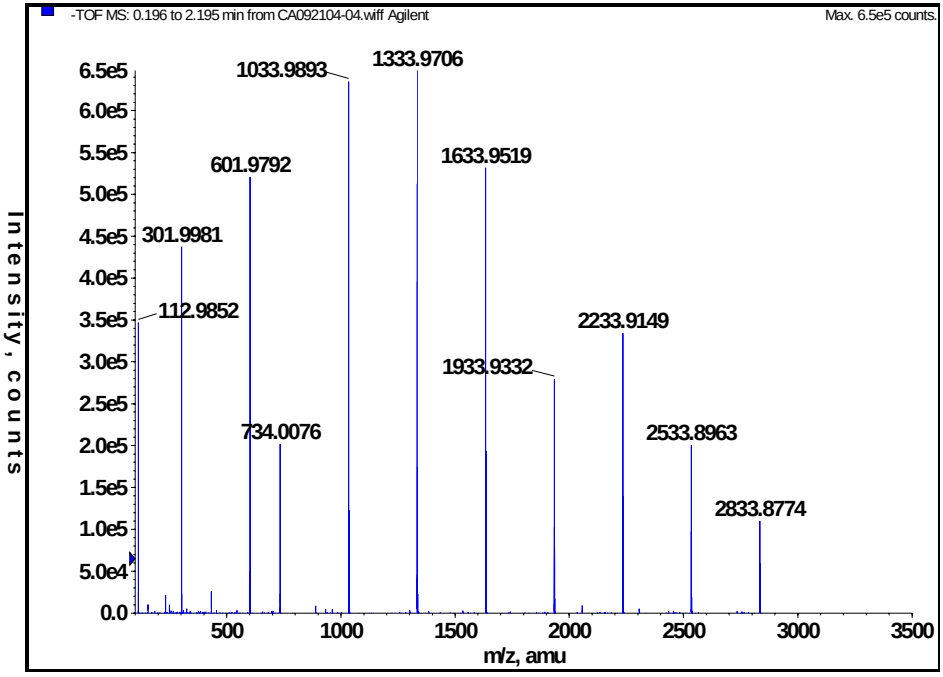

Figure 9 Sample negative ion mass spectrum of ES-TOF Tuning Mix.

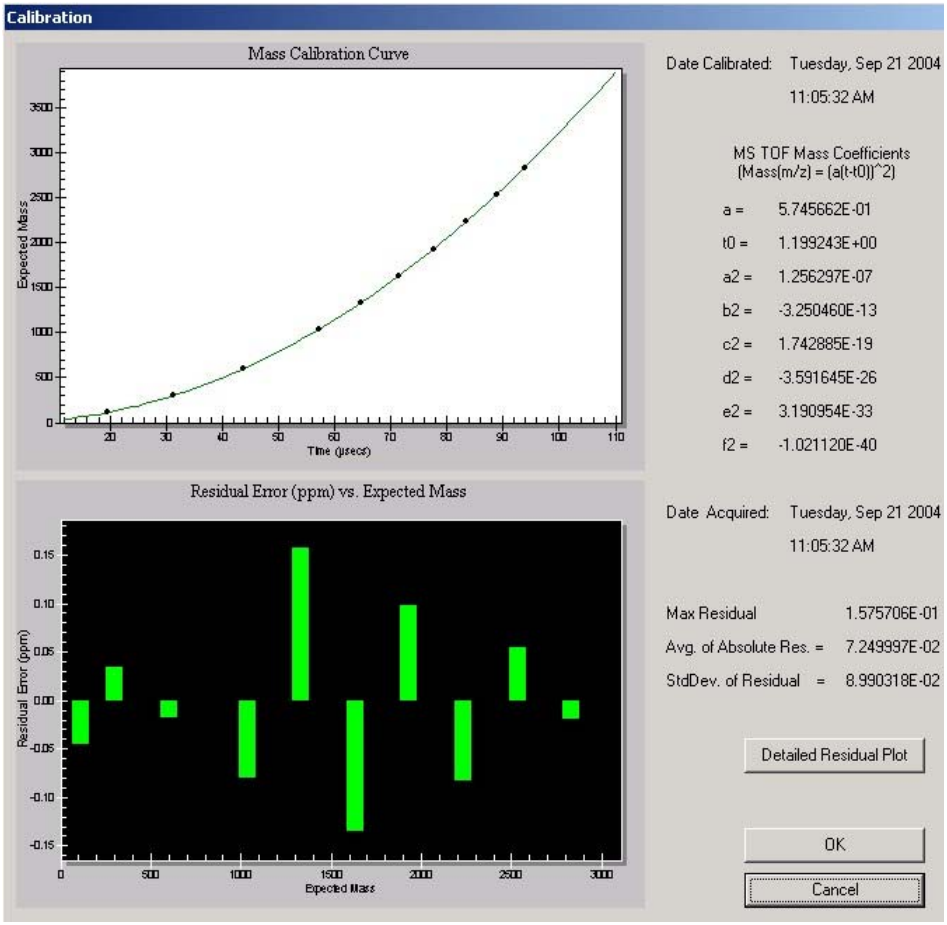

Figure 10 Calibration results (negative ion mode)

4 Click **Detailed Residual Plot** in the Calibration Results screen above to display the plot shown in Figure 11.

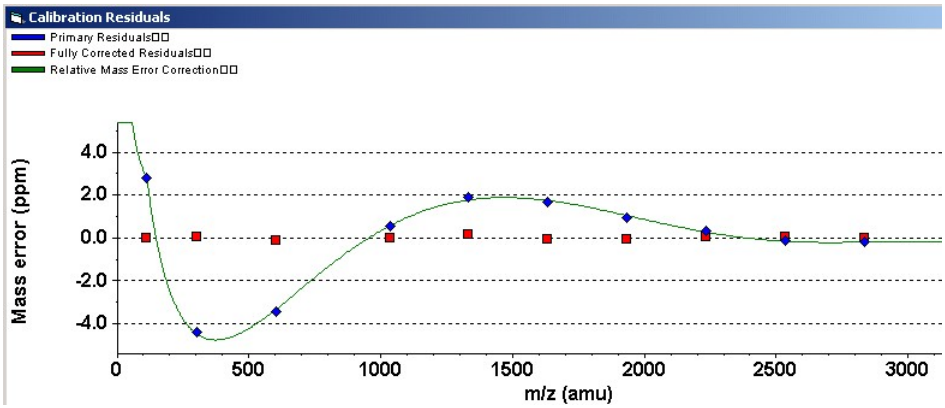

Figure 11 Detailed residual plot from negative ion calibration

To create a simple Tune Report for mass axis calibration verification

1. Load the **nanoESIautotune** method for performing the mass calibration.
2. Set the source parameters as follows:
   - Capillary voltage (Vcap): 2100 V
   - Fragmentor (Frag): 215 V
   - Drying gas temperature (DGT): 275°C
   - Drying gas flow (DGF): 6 L/min
3. Click the **Calibration** tab.
4. Click **Calibrate**.
5. If the calibration is good, accept the calibration.

   The calibration is good when the *fully corrected* residual mass errors for all calibrant ions are ≤ 3 ppm. If calibration fails, see Chapter 2, "Troubleshooting".
6. Click the **Tune** tab.
7. Click **Tune Report**.

   The tune report prints automatically. Tune reports are archived to **C:\Program Files\Agilent\TOF Software\data\TuneReport\ .**

   The system creates a subfolder for each report, using a date_time stamp as a naming convention.

To adjust the needle position

You need to adjust the needle separately for tuning and calibration, LC analysis, and offline sample infusion.

This section tells you how to adjust the needle for LC analysis.

- To adjust the needle for tuning and calibration, see "To prepare for calibration or tuning" on page 7.
- To adjust the needle for offline sample infusion (typically <100 nl/min), it may be necessary to lower the PicoTip (Y-axis position) and position the needle a bit closer (Z-direction) to the inlet orifice.

  Offline sample infusion generally requires lower drying gas temperatures and flow rates consistent with the sample medium and its delivery rate. Too much heat can result in analyte precipitation at the needle tip.

You can adjust the needle in two directions. The Y-axis control knob moves the needle in the vertical direction. The Z-axis control knob moves the needle in the horizontal direction, between the dual-cone assembly and the back electrode. See Figure 12.

CAUTION Be careful not to damage the tip of the needle while you adjust it.

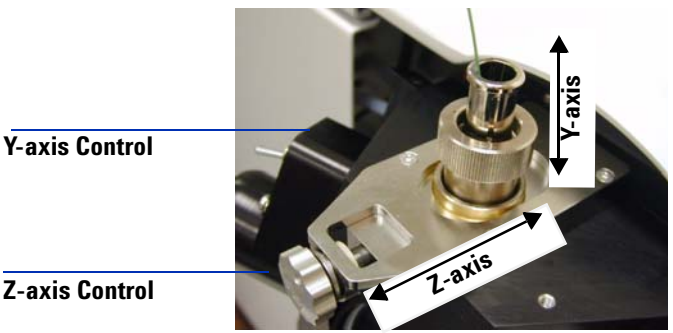

Figure 12 Adjustment of the needle position in the vertical direction uses the Y-axis control knob. Adjustment of the horizontal direction, between the back electrode and inlet capillary, uses the Z-axis control knob.

Follow the steps below to adjust the needle *for LC analysis*.

1. Mount the DNS on the LC/MSD TOF, close the spray chamber, and secure the latch.
2. Turn on the lamp.
3. Insert the needle holder assembly, with needle, into the analytical needle holder port.
4. With the source closed, look through the microscope to adjust the Z-axis control knob so that the needle tip is 3 mm from the back electrode.

   The easiest way to do this is to note that the microscope field of view is 3.5 mm. Therefore, with just part of the back electrode showing on one side and the tip just showing on the other, the separation will be 3 mm. Use the microscope to confirm; optionally, use the removable eyepiece scale insert provided with the microscope. See Figure 13 below.
5. Use the front window to observe the needle tip and direct it toward the axis connecting the back electrode to the dual-cone assembly. See Figure 13 on page 21.
6. Rotate the union holder within the needle holder mount to center the tip of the needle with the centering feature at the top of the outer cone.
7. Start the sample delivery system. Check for droplet formation at the needle tip.

NOTE Run liquid through the needle for several minutes at a reduced flow rate (~15 µl/hr) *before* turning the TOF on. This will help reduce the formation of air cavitations (which may cause partial blockage) within the needle tip when the HV is applied

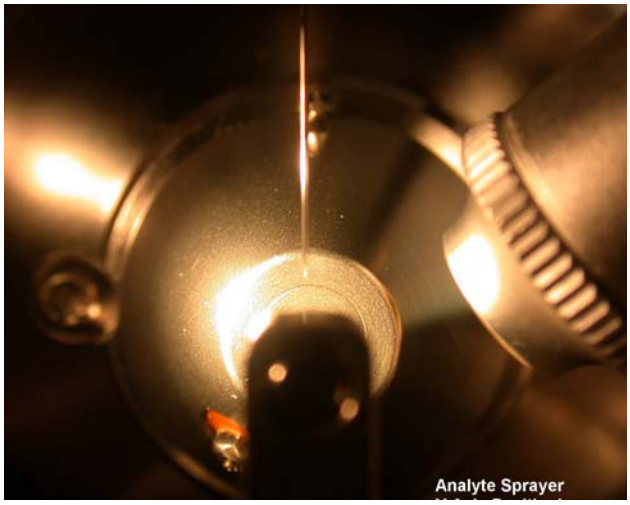

Figure 13 Needle positioned for LC analysis (front window view). Position the needle tip at the score mark between the dual-cone assembly and the back electrode.

8 Click the MS-TOF device image (Figure 14) and select **On** from the pop-up menu.

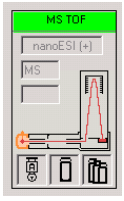

Figure 14 MS TOF device image

9 Set the appropriate TOF source parameters (see Figure 15 below).

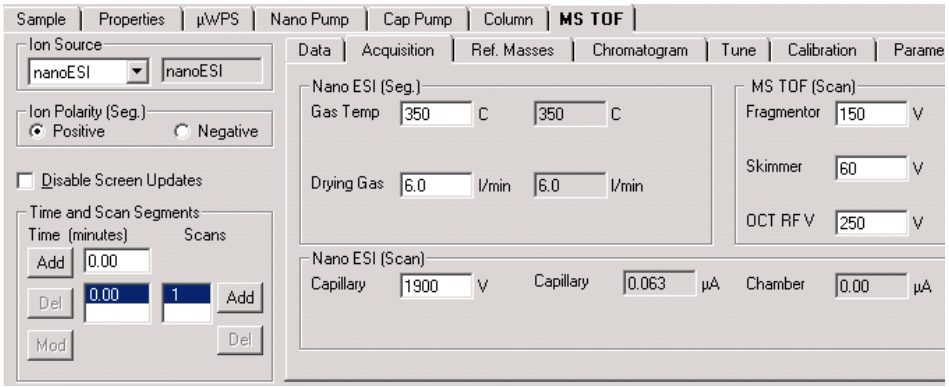

Figure 15 Example of nanoESI source parameters for LC analysis

10 Use the microscope view to monitor the needle spray position while adjusting the Z-axis.

The needle should just appear at one end of the field of view while the back electrode appears at the other. This constitutes a 3mm separation between the needle tip and the back electrode.

11 Finally, monitor the capillary current. The current should be about 25-100 nA or more, depending upon the specific solvent composition, analyte concentration and other factors. A capillary current of about 63nA is typical for a nanospray plume obtained with 85:15 H2O/ACN w/ 0.1% formic acid introduced at 300 nl/min.

To find the needle tip with the microscope, do *one* of the following:

- Look through the front window and align the microscope with the needle tip.
- Find the dual-cone assembly orifice and move the microscope view along the axis to the back electrode until the needle passes into view. See Figure 16.

12 Check that the stream or spray from the needle tip intercepts the axis that extends from the dual-cone assembly orifice to the back electrode.

If needed, rotate the union holder within the needle holder to point the needle towards the axis. Look through the front window to verify. See Figure 13 on page 21.

You may need to turn the needle union holder within the mount, to better align the needle tip to the dual-cone assembly/back electrode axis.

NOTE To fine-tune the needle position, monitor the ion signal as you vary the capillary voltage and the Z-axis control.

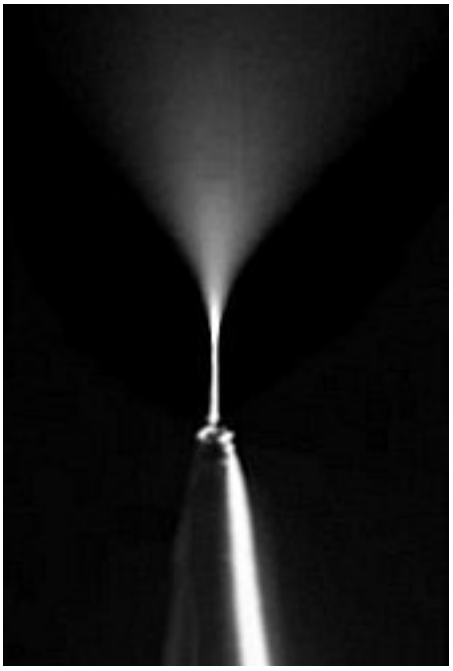

Figure 16 Nanoelectrospray plume as seen through microscope

13 Save the TOF source parameters to a *new* LC method file.

14 Shut off the lamp after adjusting the needle position.

NOTE Heat from the lamp may cause the sample to evaporate faster.

To prepare the dual nanospray for data acquisition

1 Prior to data acquisition, purge the G1376A NanoLC pump and its associated modules with:

A=100% water with 0.1% (v/v) formic acid

B=90:10 (v/v) acetonitrile:water with 0.1% (v/v) formic acid

2 It is best to condition a column overnight with a slow gradient and hold for several hours at 80-100% B.

NOTE Methods employed to analyze samples often use an injector program. It is important that the microwell plate sample loop (either 8 µl or 40 µl) is maintained at a fixed solvent composition (typically 95-98% aqueous for protein tryptic digest analyses); otherwise, sample injections will be problematic and retention times for the analytes will shift randomly, depending upon the actual solvent composition within the sample injection loop.

3 Perform the injections in highly aqueous solvent compositions with the injection valve in the mainpass position.

4 Several (6-10) minutes following a sample injection, switch the valve to the bypass position, and start the LC gradient.

5 The valve should remain in the bypass position through the end of the run and until the next injection takes place.

NOTE Make certain to allow sufficient equilibration time following an LC run's completion to ensure that the column has been fully equilibrated, and that the actual solvent composition in lines leading to the injection valve is equivalent to the initial setup composition specified in the method.

6 Prepare a separate needle holder clamp and ZDV assembly hardware for introducing the LC effluent. To do this, connect a PicoTip directly to the ZDV SS union end of the 50 mm LC checkout column (5065-9924) or the 150 mm LC analytical column (5065-9911). Both of these columns are Zorbax 300SB-C18, 3.5 µm, 0.075 mm i.d.).

7 Position the needle holder assembly containing the ZDV union with the LC column and PicoTip connected as described earlier for analytical analysis. Set Vcap = 1900V, Fragmentor = 150V, Drying Gas = 6 L/min, and Gas Temp= 350°C.

To adjust drying gas flow rate to ensure maximum ion generation

Drying gas flow rate and temperature influence ion generation.

For highly organic solvent compositions (in general, those with higher vapor pressures), lower drying gas temperatures are required (typically 275°C for the ES-TOF Tuning Mix when infused at 300 nl/min).

On the other hand, highly aqueous solvent compositions require higher drying gas temperatures (typically 325-350°C) for 85% or greater H_2O content at flow rates of 280-300 nl/min.

The actual drying gas flow required is somewhat dependent upon the nature of the analytes and is typically in the range of 4.5-7.5 L/min for flow rates of 280-320 nl/min. Too much heat can result in analyte precipitation at the needle tip.

To infuse reference mass standards

Biopolymer analysis reference mass standard solution (G1969-85003) is delivered to the DNS chamber in the same manner as the ES-TOF Tuning Mix, except that it is delivered via the reference needle port.

1 Use the same syringe pump to introduce either ES-TOF Tuning Mix (G1969-85000), modified for DNS use, or Biopolymer Analysis Reference Mass Standards (G1969-85003).

2 Use a separate syringe, adaptor, f.s. transfer capillary, ZDV SS union, PicoTip, and needle holder (clamshell) for introducing each solution. The reference mass standards solution is typically introduced at flow rates of 6-12 µl/hr (100-200 nl/min).

   a Refer to the specific instructions for preparing the Biopolymer Analysis Reference Mass Standards Solution from the G1969-85003 kit. These instructions were included with your sample kit.

   b Set the pump for 2.30 mm internal diameter and a flow rate of 6-12 µl/hr (100-200 nl/min).

   c Fast-forward the pump until you see liquid exiting the free end.

d Stop the pump flow and connect the free end of the G1375-87313 PEEK-clad f.s. capillary line to a ZDV SS union (5022-2184) using a round knurled head F·331 PEEK nut with 10/32” threads and F·113 PEEK ferrule for 1/32” O.D. tubing.

e Connect the nano needle nut/conductive ferrule holding a PicoTip fingertight to the ZDV union.

3 Insert the reference needle holder into the reference needle port.

4 Start the syringe pump to begin introducing the reference mass solution:

To ensure a stable nanospray, yielding about 2000-6000 counts for several ions (m/z 322, m/z 1222, m/z 2422), carefully position the reference PicoTip with respect to the reference inlet capillary and its back electrode (see Figure 17 on page 26).

To position the reference sprayer

1 With the spray chamber closed, visually position the reference needle about halfway between the reference back electrode and the flat surface of the reference inlet screw (not the reference inlet capillary). This is "Z-axis" positioning (refer to photo directly below).

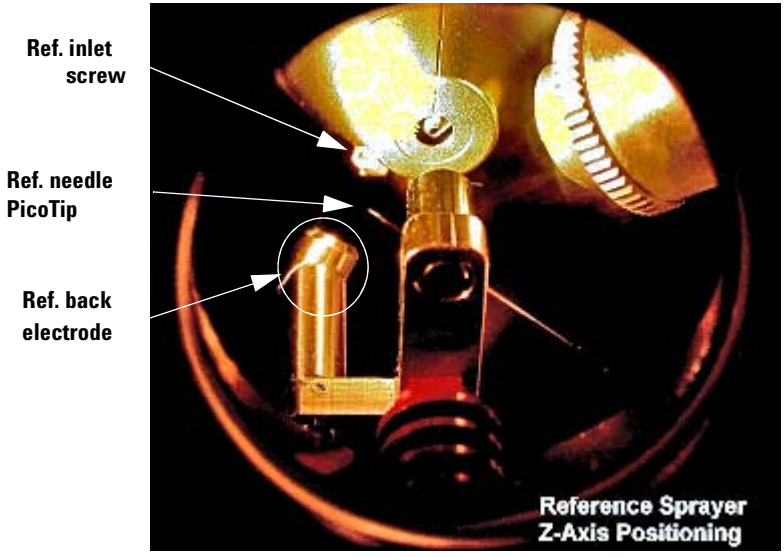

Figure 17 Reference sprayer Z-axis positioning

2 With the spray chamber open, position the reference needle so that it points towards the center of the reference back electrode. This is "Y-axis" positioning.

3 Adjust the position of the reference needle tip so that it is just inside the outer edge of the back electrode's flat surface. This is "X-axis" positioning. (Refer to the photo directly below illustrating X-Y axis positioning.)

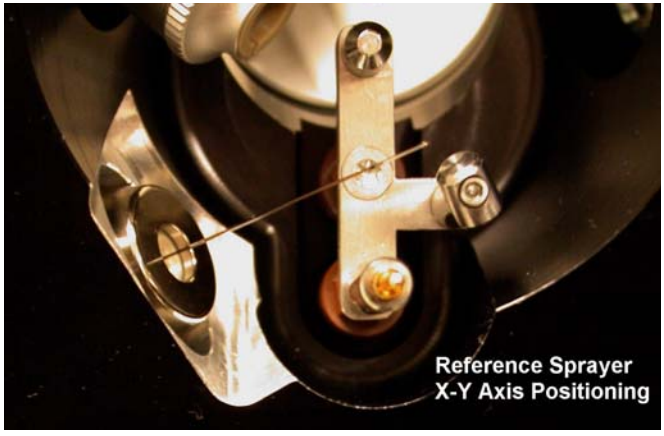

Figure 18 Reference sprayer X-Y axis positioning

4 Close the spray chamber, and observe the position of the reference sprayer through the front window with the spray chamber closed. It may appear that the tip of the needle is in a bit further with respect to the back electrode, and be about even with the outer edge of the flat surface of the reference inlet screw. This is normal.

NOTE

Typically, reference mass solution can be infused at 6-12 µl/hr. The best "X-axis" position is somewhat dependent upon the flow rate. Secure the needle holder sleeve (using the knurled needle holder lock) so that it can be moved slightly in or out ("X-axis" positioning) without change to its Y-Z axes position. From this initial position, it is not necessary to move the tip of the needle in more than half the radius (radius = 2.5 mm) of the back reference electrode's surface to optimize reference signal.

5 Click the **Ref Masses** tab.

a Select the specific reference mass standards for an analysis. (For positive ion analysis, load the **ESI_Pos_Default** mass list and select those ions corresponding to the prepared reference mass standards solution.)

b Select specific reference masses appropriate for your analysis.

c Mark the **Enable Reference Mass Correction** checkbox.

d Do not mark the **Use Bottle A** checkbox, and clear it if it is marked. The CDS is not used to deliver reference mass reference standards or MS calibrants in nanospray operation.

e Consider adjusting the **Auto Recalibration Parameters**. You can use the default settings; however, you might want to increase the **Reference Mass Minimum Height** parameter to `1000`. It is important to understand that during an analysis, one or more ions could interfere with a selected reference mass standard's mass measurement, particularly if the interfering ion and the reference mass ion are not completely resolved. The reference mass will be less resolved (in comparison to its resolution as indicated in the Tune Report) during the time of the interference. These errors usually occur at low mass (m/z) and can often be identified and corrected.

NOTE

You can view the separate nanospray needles individually by adjusting the microscope.

The microscope is intended for nanospray setup and occasional monitoring of the two sprays. Do not leave it in a position to view the reference spray (focused in), or the metal surfaces of the microscope may disturb the established electrostatic fields in the dual nanospray source, resulting in signal suppression.

To verify the performance

Verify the performance when the G1969A dual nanospray source is sold as an option with the ESI source.

NOTE

When you prepare the sample, infuse a 90:10% acetonitrile/water solution at 18 µl/hr to clean the sample delivery system. The ES-TOF Tuning Mix is soluble in acetonitrile.

To reduce the possibility of carryover, remove the tuning mix. If you do not remove the tuning mix, carryover can result in ion suppression of subsequent samples.

1 Prepare the [Glu[1]]-fibrinopeptide B sample. See "To prepare a [Glu[1]]-fibrinopeptide B solution" on page 80.

[Glu[1]]-fibrinopeptide B is a synthetic peptide consisting of 14 amino acids (EGVNDNEEGFFSAR). The molecular weight of the largest isotope is 1569.67. Therefore, the singly-, doubly- and triply-protonated molecular ions will have m/z values of (M+H)/1 = 1570.68, (M + 2H)/2 = 785.84, and (M + 3H)/3 = 524.23, respectively. (The exact mass values are 1570.676841, 785.842059, and 524.230465.)

Once infusion of this sample begins, look for the doubly- and triply-charged ions in the positive nanospray ionization mode.

2 Introduce the sample.

- **a** Fill the 250 µl syringe and place the syringe on the syringe pump. Be sure to adjust the syringe pump diameter setting to 2.3 mm.
- **b** Connect the syringe to the infusion assembly as discussed in "Step 3. Prepare the syringe infusion line assemblies" on page 53 and shown in Figure 32 on page 58.
- **c** Set the Rate of the syringe pump to 18 µl/hr (300 nl/min).
- **d** Observe the end of the nanospray needle.
- **e** Close the DNS chamber and close the latch.
- **f** Turn the TOF software to **On**.
- **g** Make small adjustments to the needle position and capillary voltage to achieve a fine spray from the needle (observe through microscope with lamp on).

The capillary voltage should not be set greater than 2200 volts in positive ion mode.

NOTE

If you see multiple streams, or the absence of a fine spray:

- The needle tip might be damaged or partly blocked. Replace the needle.
- The capillary voltage (Vcap) might be too high. Reduce the capillary voltage.

If the liquid wicks back up the shaft of the needle:

- The needle might be cracked or blocked.
- The high voltage might be insufficient, or turned off.

3 In TOF software, change the ion source to **nanoESI**.

4 Acquire spectra showing the doubly- and triply-charged molecular ions at m/z 785.84 and 524.23 m/z values, respectively.

You may need to adjust the needle position and the source settings.

Note that since the isotopic profile for *m/z* 785.84 represents doubly charged ions, the peaks are only half an amu apart.

CAUTION

When the nanospray infusion assembly is not in use, clean the needle. Pump a 50:50 H_2O/ACN or H_2O/MeOH solution at 18 µl/hr (300 nl/min), in nanoelectrospray mode, until the original sample is gone. If the leftover sample is allowed to remain in the needle, it might crystallize as the solvent evaporates and block the needle.

2 Troubleshooting

NOTE The TOF software provides the ability to display real-time signal plots of LC pump parameters (such as nanopump flow or nanopump pressure) and MS parameters (such as TIC or EIC). It is useful to display these parameters (as well as others) for monitoring LC/MS system performance and diagnosing problems.

If you get no current and no signal

If you see no signal in the mass spectrum window and no current in the Tune window, try these steps.

✔ Check the nanospray needle

- Check that the needle is not damaged. If either the tip of the needle is broken or the coating is damaged, replace the needle with a new one.
- Check that the sample is prepared correctly. Most molecules need acid for positive ion formation. Check the sample preparation.
- Check that the needle is not clogged by dust or residual sample (especially large molecules). If the needle is clogged, replace it with a new one.
- Check that no air bubbles are in the needle.
  - **a** Increase the flow and try to purge the line of bubbles. Wait until all dead volume in the transfer-line is purged. If bubbles return, increase the flow again.
  - **b** Check the drying gas temperature. If it is too high for the solvent or sample, decrease it. (The solvent can boil in the needle.)
  - **c** Check whether the sample or the solvent is not degassed. For LC-MS use the degasser. Degas the samples in an ultrasonic bath.
  - **d** Check that the needle is plugged or damaged. Replace it with a new one if it is.
- Check that the needle is grounded. The coating of the needle may be damaged. Measure the resistance from needle shaft to union holder. The measurement should between 5 to 50 kΩ. Replace the needle if necessary.

✔ Check the transfer-line

- Check that the transfer-line to the online nanospray source and to the needle (inside the source) are not clogged:
  - **a** Make sure the fused silica capillaries are not clogged. Replace the clogged capillary.
  - **b** Make sure that the fittings are not too tight on the union, which connects the needle to the fused silica capillary.

✔ Check the position of the needle.

Check that the needle is in the correct position: Put the needle in the direction of, and close to, the axis that extends from the dual-cone assembly orifice to the back electrode. Also, adjust the Z-axis control so that the needle is 3 mm or more from the back electrode.

✔ Check the flow.

Check that the flow is in the right range for the needle being used. If the flow is too low, the nanospray breaks down. Increase the flow.

✔ Check the voltage.

The voltage may be too low. In a previous analysis the voltage may have been set to a level that is too low for this sample. Increase the capillary voltage to get a signal.

✔ Check the MS-capillary.

Make sure the coating of the MS-capillary is not damaged. If it is damaged, replace it.

If you get a current but no signal

✔ Check the position of the needle.

Check that the needle is in the correct position: Put the needle in the direction of, and close to, the axis that extends from the dual-cone assembly orifice to the back electrode. Also, adjust the Z-axis so that the needle is 3 mm or more from the back electrode.

✔ Check the spray or stream from the needle.

Use a microscope to verify.

✔ Check the cleanliness of the cap.

Check that the cap is not dirty or clogged. Clean the cap and if necessary the capillary. Once cleaned, bake out for 30 minutes with dry gas temperature at 350°C and dry gas flow at 12 l/min.

If you get a current, but poor signal

✔ Check the nanospray needle.

- Check that the needle is not damaged. If either the tip of the needle is broken or the coating is damaged, replace the needle.
- Check that the needle is not clogged by dust or residual sample (especially large molecules). If the needle is clogged, replace it with a new one.
- Check that the sample is prepared correctly. Some molecules need acid to become protonated. Check the sample preparation.
- Check that no air bubbles are in the needle.

  a Increase the flow and try to purge the line of bubbles.Wait until all dead volume in the transfer line is purged. If bubbles return increase the flow again.

  b Check the drying gas temperature. If it is too high for the solvent or sample, decrease it. (The solvent can boil in the needle.)

  c The sample or the solvent is not degassed. For LC-MS use a degasser. Degas the samples in an ultrasonic bath.

  d The needle is plugged or damaged. Replace it with a new one.

✔ Check the transfer-line

- Check that the transfer-line to the online nanospray source and to the needle (inside the source) are not clogged:

  a Make sure the fused silica capillaries are not clogged. Replace the clogged capillary.

  b Check that the fittings are not too tight on the union, which connects the needle to the fused silica capillary. Replace the PEEK-coated fused silica capillary.

✔ Check the position of the needle.

Check that the needle is in the correct position: Put the needle in the direction of, and close to, the axis that extends from the dual-cone assembly orifice to the back electrode. Also, adjust the Z-axis so that the needle is 3 mm or more from the back electrode.

✔ Check the spray or stream from the needle.

Use the microscope to verify.

✔ Check the flow.

Check that the flow is not too high for the needle being used. (The needle works only in a defined flow range). Decrease the flow.

✔ Check the cleanliness of the cap.

Check that the cap is not dirty or partly clogged. Clean the cap and if necessary the capillary. Once cleaned, bake out for 30 minutes with dry gas temperature at 350°C and dry gas flow at 12 l/min.

If the lamp does not light

If the lamp does not light, do these steps in the order listed.

✔ Check that the main plug is in the wall outlet.

✔ Check that the wall outlet has power.

✔ Check that the 9V power plug is connected to the cover (see Figure 35 on page 62).

✔ Check the lamp

1 Turn the switch (see Figure 35 on page 62).

2 If the lamp does not illuminate measure the 9V output of the power supply.

- If no voltage is measured the power supply is defective and has to be replaced.
- If there is a voltage of 9 V or greater, reconnect the plug to the cover.

3 Replace the lamp.

Unscrew the cover screw (see Figure 35 on page 62), remove the cover and lamp (Figure 19 and Figure 20) from the online nanospray device and replace the lamp. Install the cover on the device and tighten the screw.

4 Turn the switch on and off several times. The new lamp will light.

Figure 19 Lamp and cover

Figure 20 Lamp detached from receptacle

If the transfer-line is clogged

Common symptoms of a plugged (or partially plugged) fused silica-capillary are absence of flow (or reduced flow), and therefore no signal or current (or poor signal and reduced current).

✔ Check if the entrance of the capillary is plugged.

1 Disconnect the transfer-line tube.

2 Allow the flow to continue.

3 Reverse the direction of flow temporarily.

✔ Check if the tube is broken

Slide your fingertips along the length of the tube. If you feel any sudden changes in direction, the fused silica may be broken.

The PEEK-clad fused silica cannot be cut and should be replaced if you cannot fix the problem any other way.

If the needle is clogged

If no droplets form, the stream splits into multiple streams, or the liquid wicks back up the outside of the needle, the needle tip is probably blocked, or the high voltage is insufficient.

NOTE To prevent the needle tip from clogging, see "To prevent nanospray needle clogging" on page 63.

✔ Check if the needle tip is blocked

1 Use the microscope to examine the needle tip. If small black particles are seen, then possibly:

- Some of the black conducting ferrule got caught inside as the blunt end was inserted through

- The blunt end wasn't cut, or the cut wasn't done well. See Figure 21 for example of needle tip clogged with ferrule material.

2 Replace the needle tip:

   a Insert the blunt end of the needle through the conductive ferrule.

   b Rinse again.

   Do not cut the needle.

3 If glass fragments appear to be trapped (see Figure 21), rinse out the exit end of the ZDV union.

   Remove residual solvent and particulates with a blast of clean air before you install another needle.

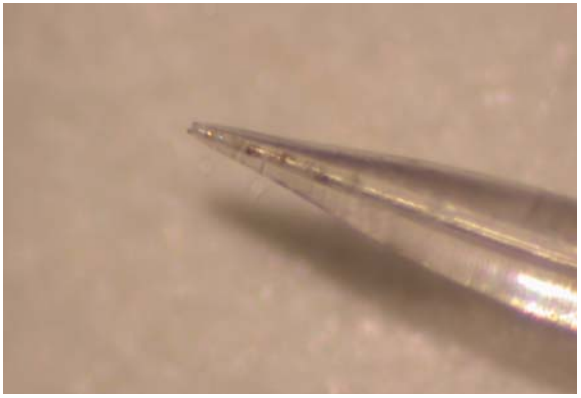

Figure 21 Needle tip clogged with metal flakes, or fragments of conductive ferrule, as well as glass fragments.

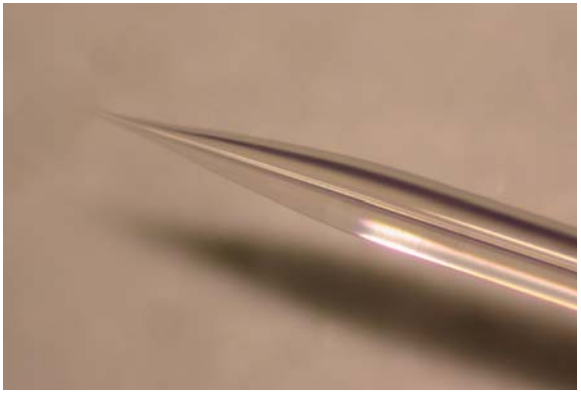

Figure 22 Clean needle tip

3 Maintenance

This chapter describes the tasks that you need to maintain the dual nanospray source.

To remove the needle holder

1 Remove the needle holder (Figure 23 on page 42) to access the needle and transfer-line. The nanospray chamber can remain closed

WARNING Never touch the needle tip, especially when you analyze toxic substances or when you use toxic solvents. The needle is very sharp and can pierce your skin.

CAUTION Do not touch the tip of the needle to any object (such as the dual-cone assembly, or assemblies within the dual nanospray chamber). The tip of the needle is very fragile. Replace the needle if it comes in contact with any object.

2 Check that the solvent delivery is turned off, or that the flow is reduced.

3 Remove the union holder from the needle holder mount on the spray chamber as follows.

Grasp the top of the union holder and pull it out of the needle holder (Figure 34 on page 61).

The contact springs provide some resistance.

The nanospray chamber can remain closed.

Figure 23 Union holder (‘clamshell’).

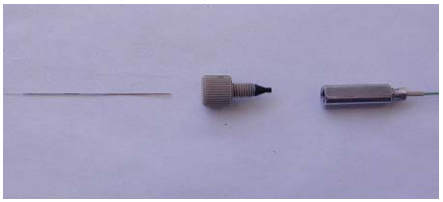

Figure 24 Needle, needle nut, ferrule and union.

4 Turn on the syringe pump. Make sure the syringe pump is set for a syringe diameter of 2.3 mm (250 µl syringe), and a flow rate of 6-12 µl/hr.

5 After 1 or 2 minutes, check that a small droplet emerges from the needle tip to ensure that the needle is clear. If the line is filled with liquid, droplets will emerge in about 10 to 20 seconds.

To change a needle

1 Stop the pump.

2 Remove the union holder (Figure 23 on page 42) containing the PicoTip emitter from the DNS chamber.

3 Open the clamshell and remove the infusion line union/PicoTip emitter assembly. Unscrew the nano needle nut holding the PicoTip emitter.

4 Pull out the PicoTip emitter and discard properly or retain it for later use if undamaged.

5 Take a new PicoTip emitter from the clear plastic box and carefully insert the blunt (distal) end completely through the bore in the nano needle nut and ferrule so that it protrudes from the other end.

6 Use a short blast of ‘clean air’ to dislodge any debris (ferrule or fused silica particulates) which may be present at the blunt end of the needle. Gently blow air through the needle tip to clear any residual debris from the bore.

7 Dip the blunt end of the needle in either methanol or acetonitrile to wet the needle’s inner surface. This helps to prevent small air bubbles from lodging within the needle’s cavity or tip which can result in reduced flow and/or erratic nanospray.

8 Pull the needle back so that the blunt end is positioned even with the tubular end of the ferrule.

9 Screw the nano needle nut holding the PicoTip emitter into the infusion line union so that it is *just* fingertight, and no tighter.

CAUTION Overtightening the nano needle nut/ZDV union fitting may damage the conductive ferrule and the PicoTip emitter, and may result in excessively high back-pressure. The conductive ferrule can be used for many months if not overtightened.

To clean the syringe infusion line assembly

1 Remove the infusion line union/PicoTip emitter from its union holder (clamshell).

2 Unscrew the nano needle nut holding the PicoTip emitter from the ZDV union and set aside.

3 Fill the syringe with a 95:5 v/v acetonitrile/water solution and connect it to the green (50 µm i.d.) infusion line via the PEEK microtight adapter and its F·125 nut.

4 Set the syringe pump flow rate to 3.0 ml/hr (50 µl/min) and start the pump flow.

5 Force liquid through the syringe/transfer line infusion assembly at this maximum flow rate setting for 5 minutes.

6 Stop the pump. Reset the flow rate to between 6-12 µl/hr, but do not restart the pump flow.

7 Remove any residual solvent from the ZDV exit union with a short blast of clean air.

8 Reconnect the nano needle nut holding the PicoTip emitter to the exit end of the union and secure it within the union holder.

To clean the dual-cone assembly

Clean the dual-cone assembly whenever you suspect contamination, or if you are experiencing low response.

Tools required

- Cloths, clean, lint free
- Isopropyl alcohol 99.5%, reagent grade or better
- Formic acid
- Water, reagent grade or better

WARNING **The dual-cone assembly may be very hot. Allow the parts to cool before handling them.**

1. Prepare a solution of 1:1 v/v isopropyl alcohol and water, with 1% formic acid, in a beaker large enough to accommodate the dual-cone endplate assembly.
2. Set the LC/MSD TOF Software to **Standby** mode.
3. Turn off the syringe pump.
4. Open the ion source.
5. Remove the dual-cone assembly:
   a. Remove the two screws (see Figure 41 on page 68.)
   b. Pull the assembly away from the capillary.
   c. Place the assembly in the beaker of alcohol/water solution from Step 1 above.
6. Do *one* of the following:
   a. Place the dual-cone assembly, in its beaker, into a sonicator.
   b. Lift the assembly out of the beaker onto a clean surface, dip a cotton swab into the beaker, and gently wipe the assembly surfaces with the swab, removing any mineral deposits or debris.
7. Rinse the dual-cone assembly with the water / isopropanol solution.
8. Reinstall the dual-cone assembly on the capillary.

NOTE When installing and removing the DNS source, handle the assembly with clean, lint-free gloves.

9 Wipe all other accessible surfaces (if necessary).

Pay special attention to the bottom of the spray chamber near the drain port and to areas that may be discolored.

10 Close the DNS chamber carefully, and secure the latch.

Extensive dual-cone cleaning procedure

Perform extensive cleaning on the dual-cone endplate assembly about every three months, depending on usage. Also perform extensive cleaning whenever you suspect contamination, or if you are experiencing low response, and a regular cleaning does not seem sufficient to resolve the problem.

Tools required

- Retaining ring pliers
- 3 mm hex driver
- Tweezers
- Abrasive mesh (4000 grit) or aluminum oxide (600 grit)
- Cotton swab
- Beaker large enough to hold the dual-cone assembly
- Sonicator
- DI water
- Methanol
- Hexane
- Acetonitrile
- Cloths, clean, lint-free
- Gloves, clean, lint-free

1 Place the dual nanospray source on a clean lint-free cloth.

2 From the back side of the dual-cone assembly, use the retaining ring pliers to remove the retaining clip.

3 Set the retaining clip, the wavy (spring) washer and endplate aside.

4 Remove the vespel flow ring (18x). If the flow ring fits snugly, you can carefully lift it upwards and out with the retaining ring pliers.

5 Loosen and remove the reference inlet screw and the underlying vespel insulator bushing from the dual-cone assembly, using the 3 mm hex driver. Set these parts aside.

6 Separate the inner cone from the outer cone and the front (16x) diffuser.

7 Clean the surfaces of the inner and outer cones, lightly rubbing their outer surfaces with the abrasive mesh (4000 grit).

CAUTION Do not overpolish these surfaces or attempt to abrasively clean the orifice of the inner cone. Overpolishing the outer cone may obscure the score ring used for positioning the analyte needle and will result in an increase in glare.

Another way to clean the cones is with a dilute slurry of 600 grit aluminum oxide in 50:50 methanol/water.

Use a cotton swab to apply the slurry to remove discoloration from the cone surfaces. Rinse the residue off using a clean cotton swab and flush repeatedly with DI water to remove all traces of aluminum oxide.

8 After abrasive cleaning is complete, place all the parts in a beaker, flush with methanol and decant.

9 Add clean hexane to the beaker so that it covers all the parts and sonicate for 30 minutes.

10 Decant and add acetonitrile and sonicate an additional 30 minutes. Remove the parts with clean tweezers and place on a lint-free cotton cloth to dry.

To reassemble the dual nanospray source

NOTE When installing and removing the DNS source, handle the assembly with clean, lint-free gloves.

1 Insert the vespel front diffuser (16X) onto the front of the inner cone such that the angle of its diagonal slit matches the cone.

The front diffuser is properly positioned when its opening is aligned with the tapped bore for the reference inlet screw within the inner cone.

2 Place the inner cone (with front diffuser) inside the outer cone so that the two are aligned with respect to the hole for the reference inlet screw assembly. See Figure 43 on page 69.

3 Insert the vespel insulator bushing in the outer cone, then insert the reference inlet screw.

4 Carefully tighten the reference screw with the 3 mm hex nut driver.

CAUTION

- Do not try to tighten the reference inlet screw without properly aligning it, or cross threading may occur.
- Do not overtighten the reference screw. Damage to the outer surface of the vespel insulator bushing may result.

5 Place the dual-cone assembly onto the endplate so that the alignment pin on the endplate engages the groove at the edge of the outer cone.

6 Orient the assembly so that it is resting on the flat surface of the outer cone.

7 Insert (press in) the vespel flow ring (18x) in the space between the inner and outer cones.

8 Place the wavy (spring) washer in a convex orientation (so that it acts as a spring) in the channel around the outer cone's back side.

9 Grasp the retainer clip with the retaining ring pliers and place it securely over the wavy washer.

The clean dual nanospray assembly is now ready to be reinstalled.

4 Installation

This chapter contains instructions for installing the G3253A DNS Chamber on the Agilent LC/MSD TOF instrument.

The instructions in this manual are supported with Agilent TOF Software A.01.01.

Step 1. Prepare to install

Before you install the DNS chamber, check that you have the appropriate parts and tools.

1 Check that you have these parts:

- KDS syringe pump
- Gas-tight syringe, 250 µl (9301-6418)
- DNS chamber (chassis, microscope, lamp, needle holder mount)
- Power adapter for the lamp
- Compatible adapter plug to connect the power adapter to a regionally configured outlet.
- Dual-cone assembly
- Needle union assembly (G1982-60032), includes protective sleeve and cap
- PicoTip, nanospray needles (5 cm x 8 µm id tip), box of 20 (9301-6378)
- 4mm open-end wrench (8710-1534)
- Microtight adapter (0100-2262)
- 50 µm id PEEK-clad fused silica tube, 70 cm length (G1375-87313)
- ZDV union (5022-2184)
- Conductive ferrules (0100-2262)
- Needle nut (G1982-60023)

2 Check that you have these tools, supplies and chemicals. The items in this list are not provided with your DNS chamber.

- Gloves and cloths, clean, lint-free
- Water and organic solvents, such as acetone, methanol, acetonitrile or isopropyl alcohol, all HPLC grade
- ¼” open-end wrench

NOTE Keep a can of 'clean air' handy to blow debris out of the DNS spray chamber and desolvation assembly interface. Keep any needle holder ports in the DNS spray chamber plugged if not in use, to prevent small debris from entering the DNS chamber.

3 Check that you have read and understand the information in:

- "Overview" on page 2
- "Safety" on page 90
- "Safety Symbols" on page 93

Step 2. Set up the KDS Model 100 syringe pump

To deliver MS calibrants and reference mass solutions to the DNS chamber, you need the following equipment:

Single-syringe pump (KDS Model 100 Syringe Pump, 3162-0178) set up for 115 VAC operation (US), with the syringe rest locked appropriately.

Figure 25 Views of KDS syringe pump

1. Use the hex key provided with the pump to loosen the locking screw on the adjustable syringe rest.
2. Move the pusher block down the guide rods to within 20-25 mm of the stationary syringe holder block by holding down its release button.
3. Reposition the syringe rest (or 'stop') so that it directly contacts the end of the pusher block.
4. Tighten the locking screw on the syringe rest to 'lock' it in place on the guide rod. Properly positioning and securing the syringe rest will limit the travel of the pusher block and prevent syringe breakage.

Some users may find that standing the pump on its side (upright) is a preferred orientation.

In addition to the steps above, refer to the figure and explanation on pages 7 and 8 of the KD Scientific Model 100 Series Users Manual.

Step 3. Prepare the syringe infusion line assemblies

To deliver MS calibrants and reference mass solutions to the DNS chamber, you need the following equipment:

- Single-syringe pump (KDS Model 100 Syringe Pump, 3162-0178) set up for 115 VAC operation (US), with the syringe rest locked appropriately.
- 250 µl Hamilton GasTight syringe (9301-6418). Table 8 on page 89 lists specifications of Hamilton-Microliter series GasTight syringes.
- ZDV microtight adaptor (0100-2435), a 'micro fingertight' nut (F·125), and green (50 µm x 70 cm) PEEK-clad fused silica transfer capillary (G1375-87313) to connect the 250 µl syringe for ES-TOF Tuning Mix delivery.

You need to build two syringe infusion line assemblies, one for calibration and one for reference.

1 Fill the 250 µl syringe with 100-250 µl of modified ES-TOF Tuning Mix, modified for DNS use, and expel any air pockets.

2 Connect the syringe needle to the inlet (6/32") end of the P·770 adaptor using the F·125 'micro fingertight' fitting. The syringe needle will fit tightly within the coned tip of the F·125 nut. The end of the needle should be flush with the end of the ferrule.

   The G1375-87313 comes with a pre-swaged SS nut, SS locking ring, PEEK sleeve and PEEK ferrule at one end and this should be connected to the outlet end (10/32") of the P·770 'ZDV microtight adaptor.' This setup will result in stable liquid delivery and will not leak.

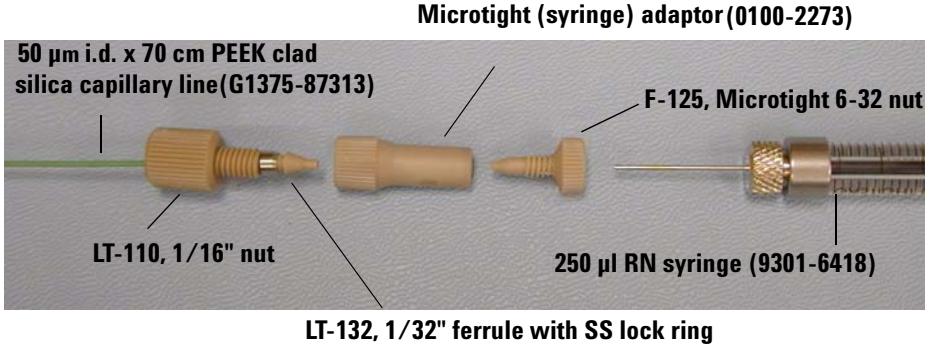

Figure 26 Components of syringe infusion assembly

3 Mount the syringe in the syringe pump.

4 Set the pump for 2.30 mm internal diameter and a flow rate of 15-18 µl/hr (250-300 nl/min).

5 Fast-forward the pump until you see liquid exiting the free end.

CAUTION

Do not fast-forward the syringe pump when a PicoTip emitter is attached to the syringe infusion line assembly. The needle tip may sustain damage.

For example, fast-forwarding the syringe pump with the 250 µl syringe installed would result in a potential flow rate of ~50,000 nl/min (more than 150 times the maximum specified flow rate for the 8 µm PicoTip emitter!) .

NOTE

Although an SS union and syringe adaptor with PTFE cone/ferrule insert is commonly used to infuse solutions in standard electrospray, **do not use this assembly for nanospray setups.** There is greater back-pressure from the 50 micron i.d. capillary line, small ZDV SS union and PicoTip. The increased back-pressure will cause upstream leaks (usually at the PTFE ferrule) and erratic (or no spray) if the solution delivery setup is incorrect.

6 Stop the pump flow and connect the free end of the green PEEK-clad f.s. capillary line to a ZDV SS union (5022-2184) using a round knurled head F·331 PEEK nut with 10/32” threads and F·113 PEEK ferrule for 1/32” O.D. tubing.

7 Push the capillary line completely into the ZDV union (until it can go no further), and maintain it in this position as you tighten the nut and ferrule. This ensures that the end of the capillary will be flush with the end of the ferrule inside the ZDV union. Use a ¼" wrench to hold the union securely when finger tightening the nut and ferrule.

Have a nano needle nut (G1982-60023) available for the next step.

CAUTION The spray tip is extremely fragile and should never be allowed to come in contact with any material that could damage it.

The square-cut back end of the PicoTip should be placed flush with the 'cone' end of the ferrule and tightened finger-tight within the ZDV SS union (5022-2184) while maintaining this position.

CAUTION Do not overtighten the nano needle nut or the conductive ferrule. Overtightening can break the needle's back end (and may damage the ferrule as well) and block the PicoTip. A symptom of this occurrence is an increase in nanopump pressure in order to sustain the nanopump flow rate.

8 Insert the syringe needle into the F·125 fitting end of the Microtight adapter, and tighten it.

9 Connect the infusion line to the ZDV union.

10 Carefully remove a PicoTip from its plastic case (9301-6378) and slip a nano needle nut followed by a conductive ferrule (0100-2262) over its square-cut back end (i.e. the non-tapered end).

See Figure 32 on page 58.

Prepare the needle and transfer-line

This task describes how to prepare the needle and transfer-line assembly. The assembly is used to introduce ES-TOF Tuning Mix (G1969-85000), modified for DNS use, to the DNS chamber for TOF calibration.

1 Assemble the union holder assembly:

a Use the 4 mm wrench (see Figure 27) to connect the green (50 µm I.D.) PEEK-clad fused silica capillary to the union.

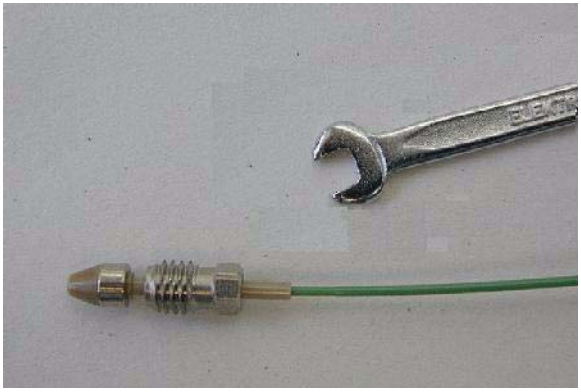

Figure 27 Capillary transfer-line and 4 mm wrench

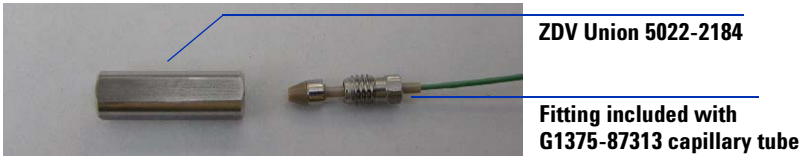

Figure 28 The union and capillary transfer-line.

CAUTION As you lower the PicoTip, it is critical that you do not allow it to contact any surface within the nanospray chamber. Damage may result.

b To prevent the needle from clogging, follow the procedure in "To prevent nanospray needle clogging" on page 63.

c Insert the blunt end of a 5 cm, 8 µm id tip, nanospray needle (9301-6378) into the nano needle nut and ferrule unit. Let the blunt end protrude through the ferrule. See Figure 29.

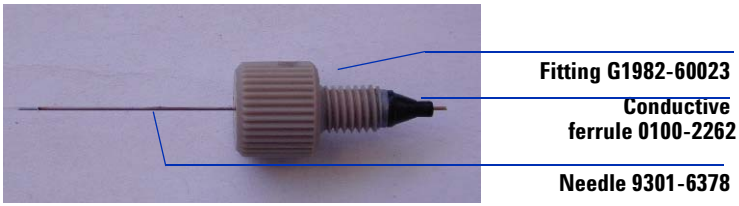

Figure 29 The needle/fitting/ferrule assembly.

d Rinse the outside of the needle with acetone or isopropanol to remove any bits of ferrule that may have come off onto the needle.

e Gently push the blunt end of the needle against the flat side of the union so that the needle's end is flush with the ferrule.

f Screw the fitting into the union. See Figure 30.

Figure 30 Needle and transfer-line connected to the union.

g Lay the needle/union/transfer-line assembly into one half of the union holder. See Figure 31.

Figure 31 The needle/union/transfer assembly seated into the union holder.

h Close the two halves of the union holder together, with the needle/union/transfer-line assembly inside.

CAUTION Make sure that the transfer line extends out the back of the union holder, and is not sandwiched between the two edges of the clamshell.

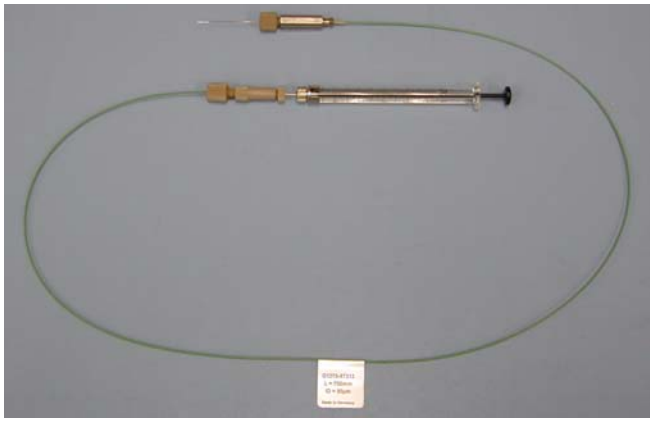

Figure 32 Completed infusion assembly with union holder (clamshell) removed for clarity.

2 Insert the union holder back into the top needle holder.

3 Close the source and check the Y-axis alignment of the needle tip. See "To adjust the needle position" on page 19.

4 Agilent recommends that you label the two syringe infusion line assemblies CAL and REF, respectively. See "To keep the pump flowing smoothly" below.

NOTE When the TOF infusion assembly is not in use, rinse with an appropriate solvent (e.g. acetonitrile for ES-TOF Tuning Mix), and protect the nanospray needle with the needle guard and protective cap (Figure 33). Both of these parts are included with the nanospray needle holder (G1982-60032).

Figure 33 Protective sleeve on needle assembly.

CAUTION Before turning off the capillary high voltage (Vcap), or switching ion source polarity (pos/neg), do the following steps:

1 Reduce the pump flow rate.

2 Remove or partially withdraw the needle holder(s) containing the PicoTip emitter(s) from the proximity of the nanospray dual-cone assembly (the high electric field region of the source).

These steps will prevent large droplets accumulating at the emitter's tip from being drawn into the capillary inlet, where arcing and instrument communication loss may result.

To keep the pump flowing smoothly

- Make sure the pump's flow rate (delivery pressure) is appropriate for the needle size.

  A pump delivers fluid to the needle. The high electric fields in the vicinity of the needle tip charge the liquid as it exits, resulting in the formation of a Taylor cone, a liquid jet of droplets, and a nanospray plume. If the pump's flow rate is set too high for a specific size needle, the needle tip can crack, resulting in no spray or erratic spray.

- Reduce the pump flow to 50 nl/min (3 µl/hr) or less prior to removing the needle holder assembly containing the PicoTip emitter from the dual nanospray chamber. If the pump remains on, at or near the maximum flow rate specified for a particular needle, the needle tip can crack.

Needle breakage can occur with either the syringe pump or the nanoLC pump delivering fluid. The flow rate range established by the manufacturer for nanoelectrospray operation with the 8-µm tip needles provided in the shipping kit is 50-300 nl/min. A flow rate guide for Hamilton Microliter Series GasTight Syringes is provided in Table 8 on page 89.

For more tips on avoiding needle damage, see "To prevent nanospray needle clogging" on page 63.

Step 4. Install the union holder

You install the union holder after you install a new needle or transfer line.

1. Lay the needle/union/transfer-line assembly into one half of the union holder. See Figure 31 on page 57.
2. Close the two halves of the union holder together, with the needle/union/transfer-line assembly inside.
3. Insert the union holder into the needle holder mount of the DNS chamber. See Figure 34 below. Push the union holder down until it stops. It is held in place with a contact spring.

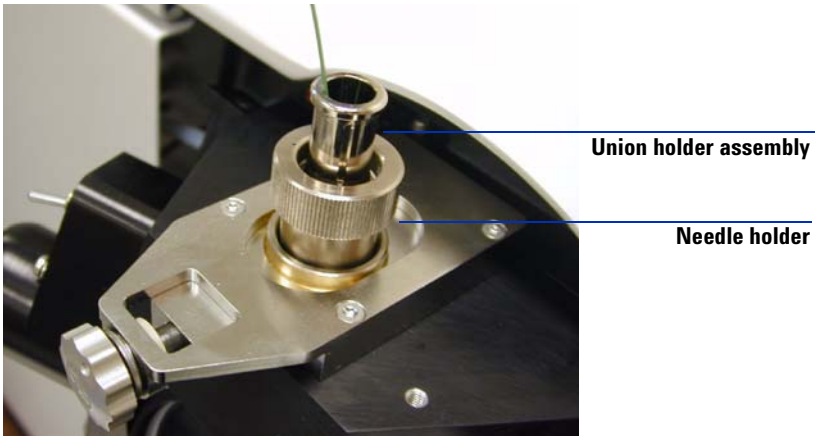

Figure 34 The union holder assembly inserted into the needle holder.

Step 5. Turn on the lamp

1. Connect the power plug to the lamp on the top of the DNS chamber (Figure 35 on page 62).
2. Plug the cord into the wall outlet. The power supply for the lamp can take 100 V to 240 V.
3. Turn on the lamp. If the lamp does not turn on, see "Troubleshooting" on page 31.

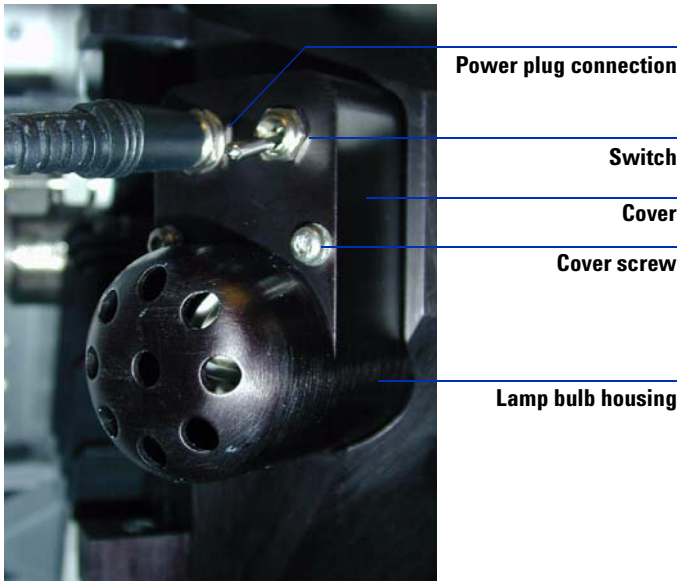

Figure 35 Lamp, switch, cover and cover screw

To prevent nanospray needle clogging

Agilent has created a technique to prevent the clogging of nanospray needles for customers who have the Agilent G3253A Dual Nanospray Source, the G1982A Nanoelectrospray Source, or the G1982B Orthogonal Nanospray Source.

Needle blockage, or clogging (Figure 36 below), can occur due to such factors as broken glass, pieces of the conductive ferrule, and pieces of the metal coating from the needle, which are then carried into the needle tip by the flow.

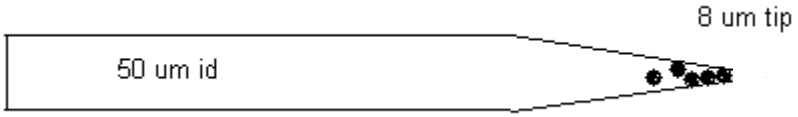

Figure 36 A blocked needle tip

You can prevent blockage by introducing 30um beads of C18 material into the needle before installation, as described in the procedure below.

Because the 30um particles are not large enough to fit in the tip of the needle, they can prevent the clogging material from making its way to the tip, while still allowing LC flow to become spray. The needle tip then looks something like that shown below.

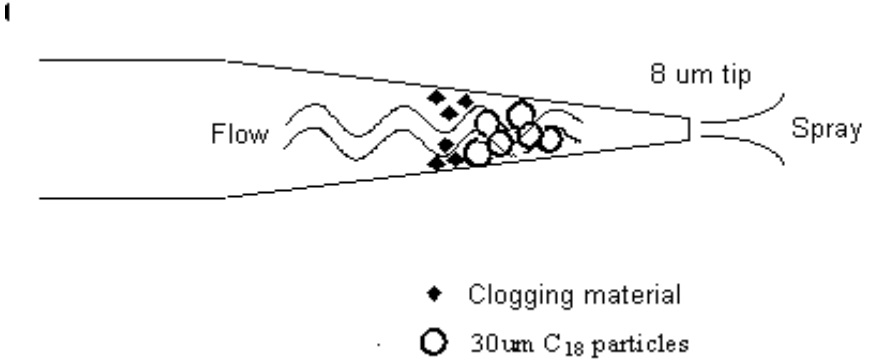

Figure 37 How C18 particles help prevent clogging

To prevent blockage, follow these steps:

1 Obtain the following materials:

- 30um C18 particles, available from Agilent as “Hypersil ODS, 30um, 5g bottle,” PN 79903-85031.
- 1 ml of organic solvent, preferably acetonitrile or methanol.
- Eppendorf micro-vial, 1.5ml capacity.

2 Take a very small amount (about 1mg) of the C18 beads and place them in the vial. You should barely be able to see them at the bottom of the vial. See the pictures below.

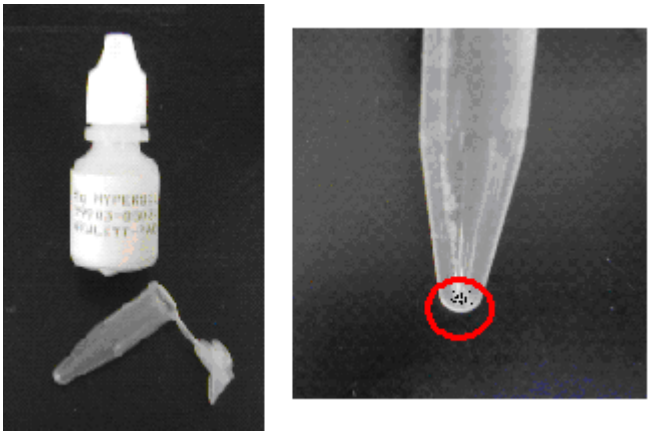

Figure 38 C18 beads barely visible at bottom of vial.

3 Add 1ml of the organic solvent, close the lid, and shake the vial rapidly for about 5 seconds.

4 Remove a needle from the box (9301-6378, or New Objective FS360-50-8-D), and dip the blunt end into the suspension of C18 particles for 1 second.

Do this step quickly, because it is easy to get too many of the C18 beads into the needle, which could result in undesired chromatography.

5 Re-cap the vial and store it.

6 Optionally, you can cut the needle at the blunt end using the CE column cutter (5183-4669).

7 Insert the blunt end of the needle through the nano needle nut and conductive ferrule.

8 Tighten the needle *very gently*. It should seem as if the needle is not tightened enough. However, over-tightening can lead to breaking off of glass particles at the blunt end.

9 Start flow through the needle and verify formation of a droplet at the needle tip.

NOTE Do not wipe this droplet away.

10 Insert the needle assembly into the DNS chamber.

11 Look through the microscope to observe the C18 aggregating just upstream of the needle tip opening. See the figure below.

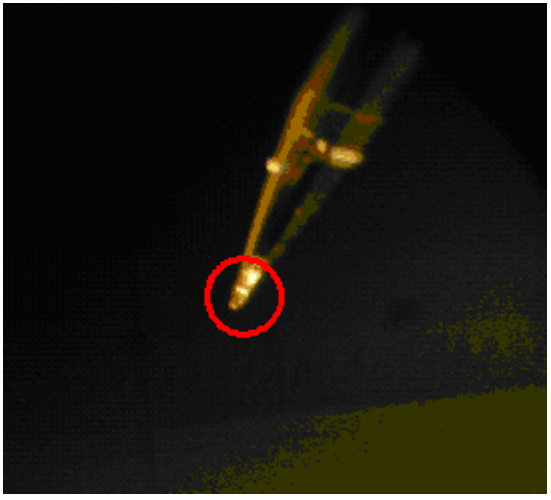

Figure 39 30 µm C18 particles aggregated just upstream of the needle tip opening. This photo actually shows too much C18 material, which is the result of dipping the needle into the suspension for 5 seconds. Only a barely discernible amount to is needed to prevent clogging.

CAUTION As a final step, start the spray with caution as described below.

12 Start the spray carefully, following these steps:

a Turn off the capillary voltage when first introducing the needle into the nanospray chamber.

b After you establish a coarse positioning of the needle tip and resume the flow, a droplet should continue to form at the tip. Adjust the Drying Gas settings properly for the flow rate.

c Turn the capillary voltage on (switch to **On**). Make sure that the starting voltage is no higher than 1900V for the G1969A source.

The droplet should evaporate within 10 to 20 seconds.

d Gradually increase the capillary voltage to achieve good spray. The typical capillary voltage for the G1969A is 1900V.

NOTE Increase the Vcap only to the point where stable spray (without spiking) is achieved, as the lifetime of the needle is usually longer at lower Vcap.

e Read the above note. Then, look for both a stable and reasonable MS spectrum as well as several minutes of stable chromatographic signal (no spiking). Then, save the method with the new Vcap setting.

f **You might need to do** fine adjustment of the needle tip position. See "To adjust the needle position" on page 19.

NOTE For more tips on preventing needle blockage or breakage, see "To keep the pump flowing smoothly" on page 59.

To convert from ESI or APCI to dual nanospray

Convert to dual nanospray when you want to analyze samples at low flow rates.

1 Put the instrument into **Standby** mode in the API-TOF Software.
   a Click on the MS TOF device image (Figure 40).

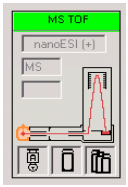

Figure 40 MS TOF device image

   b Select **Standby** from the pop-up menu.

2 Disconnect the inlet plumbing (the PEEK lines from the LC and the reference mass solution delivery).

3 Disconnect and cap off the nebulizing gas lines to the LC nebulizer and Reference nebulizer using the 1/4-28 threaded black caps.

4 Remove the spray chamber:
   a Loosen the clasp.
   b Open the spray chamber.
   c Lift the spray chamber off the hinge.

WARNING Do not touch the assemblies within the spray chamber, or the entrance to the desolvation chamber. They may be very hot. Let the parts cool before you handle them.

CAUTION Do not touch the tip of the needle to any object (such as the dual-cone assembly or nanospray chamber). The tip of the needle is very fragile. Replace the needle if it comes in contact with any object.

5 Attach the metal storage cover (provided with the TOF system) to the spray chamber, and set it aside.

6 Stuff a clean cloth or foil into the top of the source drain to prevent any parts from falling into the drain.

7 Unscrew and remove the standard spray shield and capillary cap. See Figure 41.

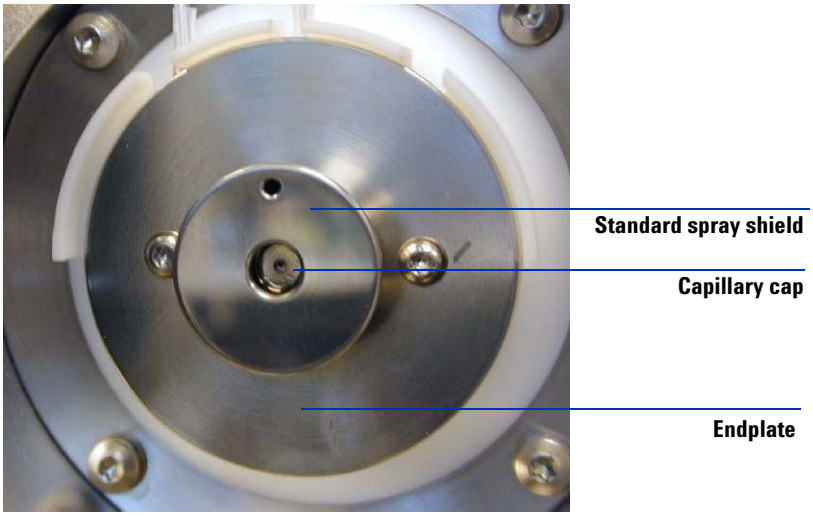

Figure 41 Standard spray shield and capillary cap for ESI or APCI

8 Remove the standard endplate shown in Figure 42.

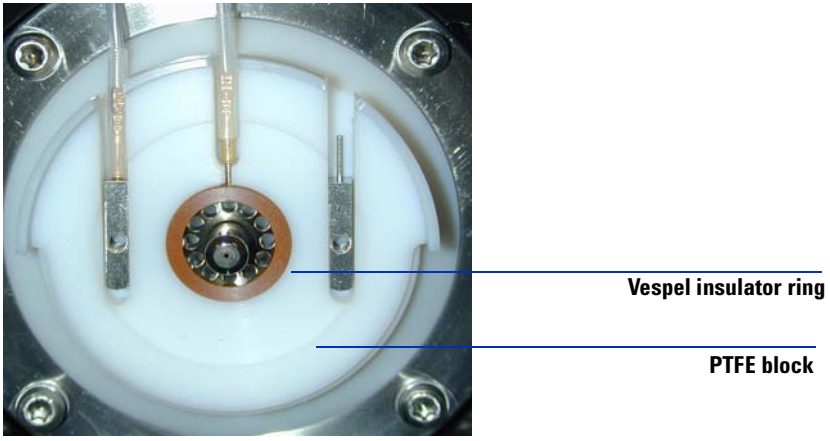

Figure 42 Spray shield, capillary cap, and endplate removed.

9 Check that the capillary is clean. See Figure 42.

If you need to clean the capillary, moisten a lint-free, clean cloth with isopropyl alcohol and gently wipe the capillary.

CAUTION Do not use any abrasive, such as abrasive mesh, to clean the capillary. Damage to the capillary plating would likely result.

10 Make sure that the vespel insulator ring remains installed as shown in Figure 42.

11 Insert the dual-cone endplate assembly over the end of the capillary.

NOTE When installing or removing the source, handle the assembly with clean, lint-free gloves.

12 Using two Torx T-10 screws, attach the nanospray dual cone assembly endplate to the PTFE block.

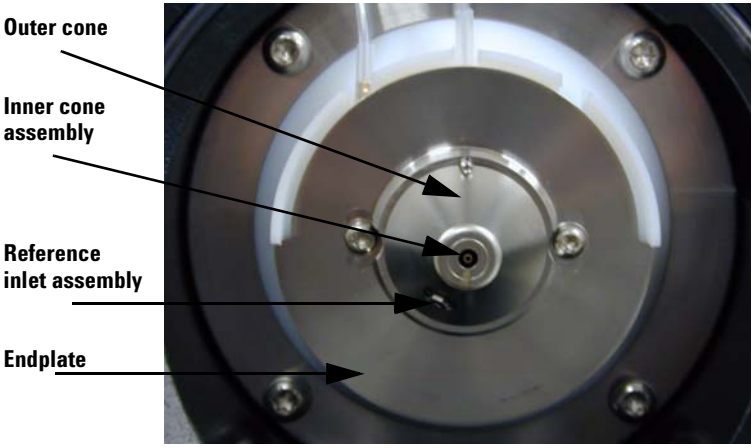

Figure 43 DNS installed

13 If needed, moisten a lint-free, clean cloth with isopropyl alcohol and gently wipe the front of the dual-cone assembly.

14 Close and latch the dual nanospray chamber.

The following warning applies when the DNS chamber is open.

WARNING Do not insert fingers or tools through the openings of the nanospray chamber. When in use, the capillary, dual-cone endplate assembly, and back electrode assembly are at high voltage (kilovolts).

15 In the TOF Software, click the **Method** tab and load the method **nanoESIautotune.m**.

16 In the TOF software, click the **MS TOF** tab and select **nanoESI** from the **Ion Source** pull-down menu.

The software displays **Source Unknown** until you turn on the source in the next step.

17 Click the MS TOF device image in the top part of the TOF window. and select **On** from the popup menu.

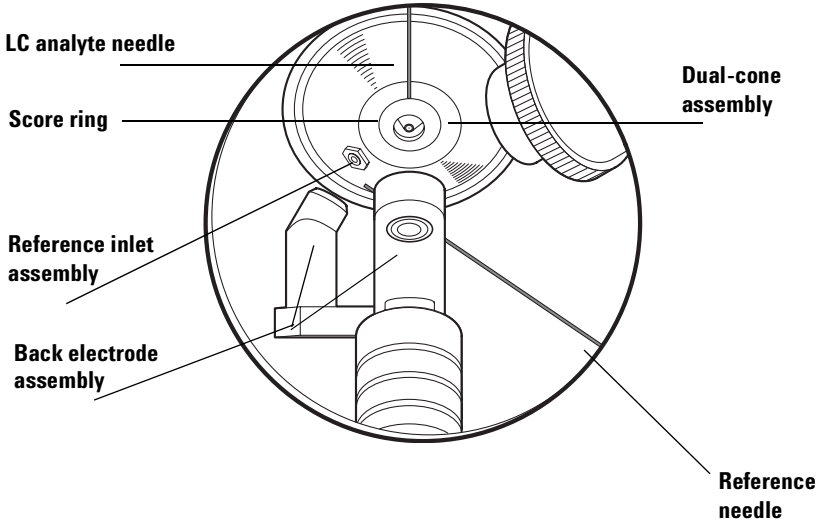

Figure 44 DNS assemblies and needle positions

To convert from dual nanospray to ESI

Convert to the ESI source when you want to use conventional flow rates.

1 Put the TOF Software in **Standby** mode.
2 Disconnect the inlet tubing.
3 Disconnect the power cable from the lamp.
4 Unlock the latch, then open and remove the DNS chamber.

WARNING **Do not touch the assemblies within the dual-nanospray chamber or the dual-cone endplate assembly. They may be very hot. Let the parts cool before you handle them.**

5 Stuff a clean cloth or foil into the top of the source drain to prevent parts from falling into the drain.
6 Remove the two screws that hold the dual-cone endplate assembly in place.
7 Remove the dual-cone endplate assembly.
8 Install the standard end-plate assembly, capillary cap, and spray shield. Do not fully tighten the screws until the small drying gas hole on the spray shield is at the top (12 o'clock) position; turn the knob to align it if necessary. Then tighten the screws.
9 Remove the cloth or aluminum foil from the top of the source drain.
10 Mount the electrospray chamber and close the latch.
11 Remove the black cap from the LC nebulizing gas line and connect it to the analytical (LC) nebulizer.
12 Remove the black cap from the Reference nebulizing gas line and connect it to the Reference nebulizer.
13 Connect the PEEK tubing from the selection valve to the analytical sprayer.
14 Connect the PEEK tubing from the calibrant delivery system to the reference sprayer.
15 In the TOF Software, click the **Method** tab and load the method **ESIautotune.m**.
16 In the left side of the **MS TOF** tab, select **ESI** from the **Ion Source** pull-down menu.

The software displays **Source Unknown** until you turn on the source in the next step.

17 Click the **MS TOF** device image in the top part of the TOF window, and select **On** from the popup menu.

To convert from dual nanospray to APCI

Convert to the APCI source when you want to use conventional flow rates.

1 Put the TOF Software in **Standby** mode.

2 Disconnect the inlet tubing.

3 Disconnect the power cable from the lamp.

4 Unlock the latch, then open and remove the DNS spray chamber.

WARNING **Do not touch the assemblies within the DNS spray chamber or the dual-cone endplate assembly. They may be very hot. Let the parts cool before you handle them.**

5 Stuff a clean cloth or foil into the top of the source drain to prevent any parts from falling into the drain.

6 Remove the two screws that hold the dual-cone endplate assembly in place.

7 Remove the dual-cone endplate assembly.

8 Install the standard end-plate assembly, capillary cap, and spray shield. Do not fully tighten the screws until the small drying gas hole on the spray shield is at the top (12 o'clock) position; turn the knob to align it if necessary. Then tighten the screws.

9 Remove the cloth or aluminum foil from the top of the source drain.

10 Mount the APCI chamber and close the latch.

11 Remove the black cap from the LC nebulizing gas line and set it aside.

12 Connect the LC ebulizing gas tubing to the APCI nebulizer.

13 Connect the PEEK tubing from the selection valve to the APCI nebulizer.

14 Connect the APCI heater cable to the APCI Heater connector.

15 Connect the APCI high voltage cable to the APCI High Voltage connector.

16 In the TOF Software, click the **Method** tab and load the method **APCIautotune.m**.

17 In the TOF software, click the **MS TOF** tab and select **APCI** from the **Ion Source** pull-down menu.

The software displays **Source Unknown** until you turn on the source in the next step.

18 Click the MS TOF device image in the top part of the TOF window. and select **On** from the popup menu.

5 Reference

This chapter contains reference information.

Chemicals

The following chemicals are used with the Agilent DNS system for tuning, calibration, reference, and monitoring system performance.

Chemical	Part Number
Biopolymer Analysis Reference Mass Standards Kit	G1969-85003
Bovine Serum Albumin (BSA) lyophilized tryptic digest, 500 pmole vial for monitoring instrument performance	G1990-85000
ES-TOF Tuning Mix	G1969-85000
[Glu^{1}]-fibrinopeptide B, 0.1 mg for monitoring instrument performance	G3253-85010
Insulin oxidized B chain (bovine), 1 mg (I6383, Sigma, St. Louis, MO) for monitoring instrument performance	

To prepare special-purpose solutions

This section tells you how to prepare solutions you need to perform various tasks, such as tuning.

To prepare an ES-TOF Tuning Mix, modified for DNS

Follow the steps below to prepare a modified ES-TOF Tuning Mix for calibration and tuning.

1 Add 25 ml of ES-TOF Tuning Mix (G1969-85000) to a clean, 30-ml Nalgene bottle.

2 Add 12.2 µl of 0.1 mM HP-0321 Reference Mass Standard solution to the bottle.

The 0.1 mM HP-0321 solution is contained in an individual ampoule in the G1969-85003 Biopolymer Analysis Reference Mass Standards Kit.

3 Cap the bottle and invert it several times to mix the solutions.

4 Label the bottle **Modified ES-TOF Tuning Mix for DNS.**

The calibrant solution is very stable; however, it should be held be at 4°C for long-term storage.

5 Transfer a portion of the solution to a small (about 4 ml) screw cap vial suitable for loading a syringe, and label as before.

6 Transfer the contents of the ampoule to a small PTFE-line screw cap vial, using a disposable pipette.

7 Transfer the ampoule's label to the vial and store it at 4°C, with the other mass reference standards in the G1969-85000 kit.

Table 1 MS calibrant ions (positive mode)

Mass	Chemical species	Exact m/z	Formula
118	HP-0117	118.086255	C5 H12 O2 N
322	HP-0321	322.048121	C6 H19 O6 N3 P3
622	HP-0621	622.028960	C12 H19 O6 N3 F12 P3
922	HP-0921	922.009798	C18 H19 O6 N3 F24 P3
1222	HP-1221	1221.990637	C24 H19 O6 N3 F36 P3
1522	HP-1521	1521.971475	C30 H19 O6 N3 F48 P3
1822	HP-1821	1821.952313	C36 H19 O6 N3 F60 P3
2122	HP-2121	2121.933152	C42 H19 O6 N3 F72 P3
2422	HP-2421	2421.913990	C48 H19 O6 N3 F84 P3
2722	HP-2721	2721.894829	C54 H19 O6 N3 F96 P3
MS calibrant dimers*			
2460	2(HP-1221) + H2O	2459.976736	C48 H38 O13 N6 F72 P6
2760	HP-1221 + HP-1521 + H2O	2759.957575	C54 H38 O13 N6 F84 P6
3060	2(HP-1521) + H2O	3059.938413	C60 H38 O13 N6 F96 P6
3360	HP-1521 + HP-1821+ H2O	3359.919251	C66 H38 O13 N6 F108 P6
3660	2(HP-1821) + H2O	3659.900090	C72 H38 O13 N6 F120 P6
3960	HP-1821 + HP-2121 + H2O	3959.880928	C78 H38 O13 N6 F132 P6
4260	HP-2121 + HP-2121 + H2O	4259.861767	C84 H38 O13 N6 F144 P6
4560	HP-2121 + HP-2421 + H2O	4559.842605	C90 H38 O13 N6 F156 P6
4860	2(HP-2421) + H2O	4859.823443	C96 H38 O13 N6 F168 P6
5160	HP-2421 + HP-2721 + H2O	5159.804282	C102 H38 O13 N6 F180 P6
5460	2(HP-2721) + H2O	5459.785120	C108 H38 O13 N6 F192 P6

* Dimer formation can be induced by reducing drying gas flow and temperature.

Table 2 MS calibrant ions (negative mode)

Mass	Chemical species	Exact m/z	Formula
113	HP-0114 (TFA)	112.985587	C2 O2 F3
302	HP-0285 + OH	301.998139	C6 H O N3 F9
602	HP-0585 + OH	601.978977	C12 H O N3 F21
1034	HP-0921 + TFA	1033.988109	C20 H18 O8 N3 F27 P3
1334	HP-1221 + TFA	1333.968947	C26 H18 O8 N3 F39 P3
1634	HP-1521 + TFA	1633.949786	C32 H18 O8 N3 F51 P3
1934	HP-1821 + TFA	1933.930624	C38 H18 O8 N3 F63 P3
2234	HP-2121 + TFA	2233.911463	C44 H18 O8 N3 F75 P3
2534	HP-2421 + TFA	2533.892301	C50 H18 O8 N3 F87 P3
2834	HP-2721 + TFA	2833.873139	C56 H18 O8 N3 F99 P3

Table 3 Immonium ions and tryptic Y1 fragment ions

Mass	Chemical species	Exact m/z	Formula
216	p-Y_{IM}	216.042021	C8 H11 O4 N P
175	R_{Y1}	175.118952	C6 H15 O2 N4
159	W_{IM}	159.091675	C10 H11 N2
147	K_{Y1}	147.112804	C6 H15 O2 N2
136	Y_{IM}	136.075690	C8 H10 O N
120	F_{IM}	120.080776	C8 H10 N
110	H_{IM}	110.071274	C5 H8 N3
86	L_{IM} / I_{IM}	86.096426	C5 H12 N
72	V_{IM}	72.080776	C4 H10 N
70	P_{IM}	70.065126	C4 H8 N

To prepare a reference mass standards solution

Refer to the specific instructions for preparing the Biopolymer Analysis Reference Mass Standards Solution from the G1969-85003 kit. These instructions were included with your sample kit.

Preparation of the reference mass standards solution is based upon the specific analysis.

- For protein tryptic digests (PMF) analysis, it is recommended to include HP-0321, HP-1221, and HP-1821 in the reference mass standards mix. Use a low fragmentor setting (typically 135–150V), and select a mass range of m/z 300-2000 (or slightly less). The DNS-TOF will detect singly- and multiply-charged peptide ions and will provide internal reference mass (IRM) correction.
- For in-source CID DNS-TOF analysis of protein tryptic digests, it is recommended to include HP-0120, HP-1221, and HP-2421 in the reference mass standards mix. Use two or more experiments (for example, 150V “low” fragmentor and 400V “high” fragmentor) and a mass range of m/z 70-3500. It is useful to specify an additional background reference mass at low m/z, since the HP-0120 will fragment above 250V (as will the HP-0321).

For updates to these instructions and MSDS, see http://whadmin.cos.agilent.com and search on product number G1969-85003.

To prepare a [Glu^1]-fibrinopeptide B solution

Use a [Glu^1]-fibrinopeptide B solution to verify performance when the G1969A DNS is sold as an option with the ESI source.

1. Remove the cap from the vial that contains 0.1 mg of [Glu^1]-fibrinopeptide B (G3253-85010), purity 91%.
2. Add 910 µl of H_2O and vortex to dissolve. (Refrigerate or freeze this stock solution. It is stable for up to two months in the refrigerator or freezer.)

   The resulting solution concentration is 100 ng/µl (63.7 µM). This stock solution is stable for up to two months when stored in a refrigerator or freezer.
3. Dilute the [Glu^1] fib B stock solution 1:50 with 1:1 MeOH/H2O containing 0.1% formic acid. The formic acid is a necessary component to ensure protonation of the [Glu^1]-fib B.

The resulting dilution is 2 ng/µl (1.274 µM or 1.274 pmoles/µl).

4 Inject ~200 fmoles (0.16 µl) on-column to compare with the mass spectra displayed below. Alternatively, the [Glu[1]] fibrinopeptide B dilution can be infused at 15-18 ul/min.

5 Dilute again at 1:1 MeOH/H_2O with 0.1% formic acid to a concentration of 0.01 mg/ml, or 10 ng/µl.

NOTE Use HPLC grade solvents to prepare the [Glu[1]]-fib B. Prepare sample using Nalgene containers. Discard the final dilution after performing the verification test.

[Glu[1]]-fibrinopeptide B is a synthetic peptide consisting of 14 amino acids (EGVNDNEEGFFSAR). The molecular weight of the largest isotope is 1569.67. Therefore, the singly-, doubly- and triply-protonated molecular ions will have

m/z values of (M+H)/1 = 1570.68, (M + 2H)/2 = 785.84, and (M + 3H)/3 = 524.23, respectively. (The exact mass values are 1570.676841, 785.842059, and 524.230465.)

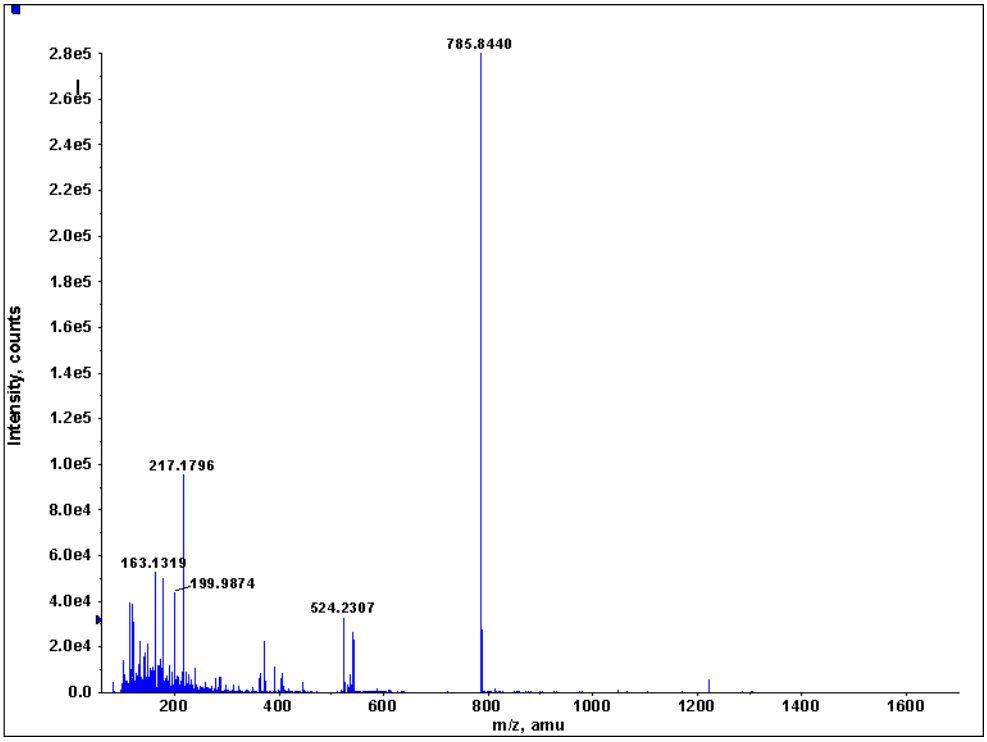

Figure 45 [Glu[1]]-fibrinopeptide B, 200 fm on-column, 150V fragmentor

Mass errors for the doubly- and triply-charged [Glu[1]]-fibrinopeptide B molecular ions:

- $(M+2H)^{2+}$ (m/z 785.8440) = 2.47 ppm
- $(M+3H)^{3+}$ (m/z 524.2307) = 0.45 ppm

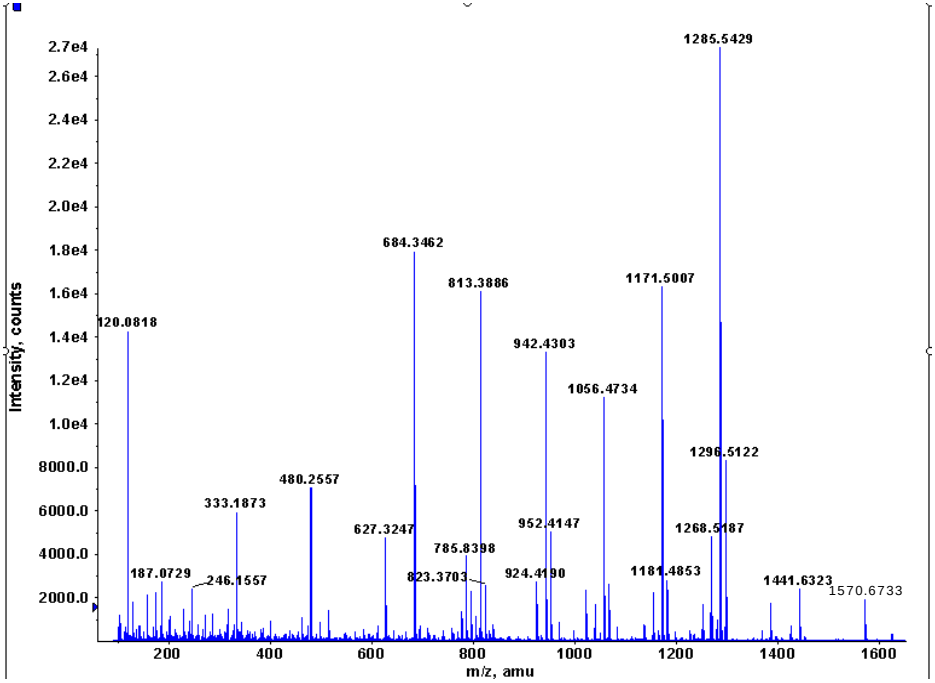

Figure 46 [Glu[1]]-fibrinopeptide B, 200 fm on-column, 400V fragmentor

Table 4 [Glu[1]]-fibrinopeptide B in-source CID fragments

Ion	Theoretical (m/z)	Experimental (m/z)	Mass Error (ppm)	Resolving Power	Intensity (cts)
M+H	1570.676841	1570.6733	-2.25	9969	1.89E+03
Y13	1441.634248	1441.6323	-1.35	10641	2.40E+03
Y12	1384.612784	1384.6101	-1.94	9755	1.74E+03
Y11	1285.544370	1285.5429	-1.14	10131	2.73E+04
Y10	1171.501443	1171.5007	-0.63	9878	1.63E+04
Y9	1056.474500	1056.4734	-1.04	9577	1.12E+04
Y8	942.431572	942.4303	-1.35	9552	1.33E+04
Y7	813.388979	813.3886	-0.47	8722	1.61E+04
Y6	684.346386	684.3462	-0.27	8163	1.79E+04
Y5	627.324922	627.3247	-0.35	8082	4.78E+03
Y4	480.256508	480.2557	-1.68	7072	7.08E+03
Y3	333.188094	333.1873	-2.38	7254	5.95E+03
Y2	246.156066	246.1557	-1.49	6321	2.41E+03
Y1	175.118952	175.1177	-7.15	4377	2.26E+03
F-im	120.080776	120.0818	8.53	4584	1.43E+04

To prepare a BSA tryptic digest

Michrom BSA digest sample for DNS-TOF, positive ion mode

1 Reconstitute 500 picomole BSA digest (G1990-85000) by adding 237.5 µl H2O + 12.5 µl ACN (95:5 v/v ratio, total volume = 250 µl).

2 Screw on the cap, but do not mix at this time. Label the vial with the concentration and the preparation date. Allow to stand in a refrigerator at 4°C for one hour.

3 Remove the reconstituted digest from the refrigerator.

4 Vortex for 30 seconds, then centrifuge for two minutes.

The resulting solution concentration is 2 picomoles/µl.

5 Return the solution to the refrigerator, and keep it there for 10 minutes.

6 Remove the vial from the refrigerator, and vortex the solution for 15 seconds.

7 If you plan to use the solution immediately, follow the instructions in "Dilution" below.

8 If you do not need the solution immediately, transfer the solution to a Nalgene vial, and label it with its contents, concentration, and preparation date. The solution can be stored for several months in a freezer at -20°C.

9 When you remove it from the freezer in preparation for using it, repeat step 4 above.

Dilution

1 Take a 20 µl aliquot of the 2 pmole/µl BSA tryptic digest solution prepared above and dilute to 200 µl in 95:5 H2O/ACN w/ 0.1% formic acid (~170 µl H2O, 10 µl ACN and 0.2 µl formic acid) in a Nalgene sample vial. Cap, mix and label the solution.

The resulting concentration is: 200 femtomole/µl

2 Inject 0.5 µl of this dilution for a total of 100 femtomoles on column **BSA tryptic digest**.

To prepare an insulin oxidized B chain sample for DNS-TOF, negative ion mode

1 Reconstitute 1.0 mg of insulin oxidized B chain sample contained in Sigma ampoule by adding 800 µl H2O plus 200 µl methanol.

The resulting stock solution is 286.04 micromolar.

2 Transfer the reconstituted stock solution to a Nalgene sample vial, and label it with its contents, concentration, and preparation date. The solution can be stored for several months in a freezer at -20°C.

Table 5 Bovine insulin oxidized B chain charge states

(-1)	(-2)	(-3)
3492.636220	1745.814472	1163.540556

First dilution

1 Take a 35.0 µl aliquot of the stock solution prepared above and dilute with 80:20 H2O/ACN + 0.1% formic acid in a 2 ml Nalgene sample vial to a final volume of 1.0 ml (add 771.5 µl H2O, 193.0 µl ACN, and 0.5 µl formic acid to the stock aliquot).

2 Mix and label.

The resulting concentration is 10 picomoles/µl in 80:20 H2O/ACN w/0.1% formic acid.

The solution can be stored in a refrigerator at 4°C for several weeks.

Second dilution

1 Take a 50 µl aliquot of the 10 picomole/µl dilution and dilute with 80:20 H2O/ACN + 0.1% formic acid to a final volume of 200 µl (add 120 µl H2O, 30 µl ACN, and 0.1 µl formic acid) in a Nalgene sample vial.

2 Mix and label.

The resulting concentration is: 2.5 picomole/µl.

The solution can be stored in a refrigerator at 4°C for up to a week.

3 Inject 0.1 µl of this dilution for a total of 250 femtomoles on column insulin oxidized B chain.

System background information

The following table lists ions associated with various solvents and materials often used with the DNS system.

Table 6 Common background ions (positive ion mode)

Theoretical	Formula	Chemical Species
149.023320	C8 H5 O3	phthalic anhydride
167.033885	C8 H7 O4	phthalic acid or isomer
331.190386	C20 H27 O4	dicyclohexyl phthalate
371.101233	C10 H31 O5 Si5	polydimethylcyclosiloxane, 5-mer
391.284286	C24 H39 O4	di-n-octylphthalate
413.266231	C24 H38 O4 Na	di-n-octylphthalate, Na adduct
445.120025	C12 H37 O6 Si6	polydimethylcyclosiloxane, 6-mer
519.138816	C14 H43 O7 Si7	polydimethylcyclosiloxane, 7-mer

(Acetonitrile)n - (H2O)x Clusters

Table 7 (Acetonitrile)n - (H2O)x Clusters

Ion	Species	Exact m/z
83	(CH3CN)2 + H	83.060375
101	(CH3CN)2 + H2O + H	101.070939
105	(CH3CN)2 + Na	105.042319
119	(CH3CN)2 + (H2O)2 + H	119.081504
142	(CH3CN)3 + H2O + H	142.097488
183	(CH3CN)4 + H2O + H	183.124038
201	(CH3CN)4 + (H2O)2 + H	201.134602
224	(CH3CN)5 + H2O + H	224.150587
283	(CH3CN)6 + (H2O)2 + H	283.187700

Syringe reference table

The following table lists specifications of Hamilton-Microliter series GasTight syringes.

Table 8 Hamilton-Microliter Series GasTight syringes

Size	Diameter	Flow rate	Conversion
0.5 µl	0.103 mm	50 nl/min	3 µl/hr
1 µl	0.146 mm	100 nl/min	6 µl/hr
2 µl	0.206 mm	150 nl/min	9 µl/hr
5 µl	0.326 mm	200 nl/min	12 µl/hr
10 µl	0.460 mm	250 nl/min	15 µl/hr
25 µl	0.729 mm	300 nl/min	18 µl/hr
50 µl	1.031 mm	500 nl/min	30 µl/hr
100 µl	1.46 mm	1 µl/min	60 µl/hr
250 µl	2.30 mm	2 µl/min	120 µl/hr
500 µl	3.26 mm	2.5 µl/min	150 µl/hr
1 ml	4.61 mm	5 µl/min	300 µl/hr
2.5 ml	7.28 mm	10 µl/min	600 µl/hr
5.0 ml	10.30 mm	20 µl/min	1200 µl/hr
10.0 ml	14.57 mm	50 µl/min	3000 µl/hr

Safety

Some of the procedures in this chapter require access to parts of the instrument and nanospray ion source while it is in Standby state or shortly after it is turned off. If you do not perform these procedures correctly, you are exposed to dangerous temperatures, voltages, and chemical hazards. This topic describes the potential dangers.

Needle Hazard

- The needle in the needle holder is very sharp and can pierce your skin. Do not touch the tip, especially when you analyze toxic substances or when you use toxic solvents.
- The tip of the needle is very fragile. Do not touch the tip to any objects, such as the dual-cone assembly or spray chamber. If you accidentally touch the needle tip, replace the needle.
- Use care when you adjust the needle. Do not damage the end of the needle.

High temperatures

Some parts in the nanospray chamber and desolvation interface reach temperatures high enough to cause serious burns. These parts include, but are not limited to the capillary, dual-cone endplate assembly, and lamp. *Do not touch these parts.*

- Certain parts remain hot for some time after the instrument is shut down or turned off. In particular the spray shield, endplate, and the vaporizer assembly could be very hot after working with APCI or ESI. Use extreme care when you work on an instrument that has recently been turned off.
- Do not touch the lamp on the top of the DNS chamber. It may be very hot.

Hazardous voltages

Whenever the instrument is not in Standby, hazardous voltages are present on one or more interior parts. Parts that use hazardous voltages include, but are not limited to, the dual-cone endplate assembly and back electrode.

These parts are usually covered or shielded. As long as the covers and shields are in place, you will not make contact with hazardous voltages.

- Do not insert fingers or tools through the openings on the DNS chamber. During operation the capillary and dual-cone endplate assembly are at high voltage up to 2.5 kV.
- If you connect the instrument to an ungrounded or improperly grounded power source, you create a shock hazard for the operator and can damage the instrument. Intentional interruption of instrument grounding is strictly prohibited.

Biohazardous residue

The exhaust from the pumps can contain traces of sample and solvents. Vent all pump exhaust outside the building or into a fume hood. Comply with your local air quality regulations.

- The exhaust fumes from the vacuum system and spray chamber contains trace amounts of the chemicals you analyze. Health hazards include chemical toxicity of solvents, samples, buffers, and pump fluid vapor, as well as potentially biohazardous aerosols of biological samples. Vent all exhausts out of the building where they cannot be recirculated by environmental control systems. Do not vent exhausts into your laboratory. See the warning labels on the instrument. Comply with your local air quality regulations.
- Fluid drained from the nanospray chamber is made of solvent and sample from your analyses. The fluid in the mechanical pump collects traces of samples and solvents. In addition, unnebulized solvent and sample collects at the bottom of the spray chamber. Connect the drain on the bottom of the spray chamber to a closed container, plumbed to a vent.
- Handle and dispose of all fluids using precautions appropriate for their biohazardous and biological content. Comply with local environmental regulations.
- Handle all used pump fluid as hazardous waste. Dispose of used pump fluid as specified by your local regulations.

Power supply information

This equipment must be installed in an environment of Category II installation as defined in IEC 664. Check that the supply voltage does not fluctuate more than +10% or -10% of rated voltage.

Cleanliness

Cleanliness and the prevention of accidental contamination during maintenance are very important. Contamination of the interior of the vacuum system or the sample path can affect the results of your analyses.

- Always wear clean, lint-free gloves when handling parts that come in contact with the sample path. Oil from your fingers is difficult to remove.
- When you set parts down, place them on clean, lint free cloths or clean aluminum foil, not directly on the laboratory bench.
- Keep parts covered so they do not get dirty.
- If possible, maintain a separate set of tools that have been thoroughly cleaned. Use these tools only when working on clean assemblies.
- With open ion sources, avoid dusty environments. Dust particles can be drawn into the instrument capillary and deposit on ion optics, causing loss of sensitivity

Safety Symbols

NOTE

This symbol is placed on the product where it is necessary for you to refer to the **manual** in order to understand a **hazar**d.

WARNING CAUTION

This symbol is placed on the product within the area where **hazardous voltage** is present or shock hazard can occur. Only trained service persons should perform work in this area.

WARNING

This symbol is placed on the product within the area where **hot parts and surfaces** are present. Allow the product to cool before performing work in this area.

WARNING

This symbol is placed on the product within the area where **biohazards** are present. Handle these areas with the respective care.

Technical Specifications

Size

Height	Length	Width
150 mm	130 mm	130 mm
5.9 in.	5.2 in.	5.2 in.

Weight 1.6 kg / 3.5 lbs.

Power source Power 65 VA

Primary

Voltage	100 V – 240 V
Current	0.6 A
Frequency	50 Hz / 60 Hz

Secondary

Voltage	9 V
Current	2.2 A

Operating Temperature 15°C – 35°C (59 °F – 95 °F) (Analytical specifications will be met only within a temperature range of 21°C ± 3°C (70°F ± 6°F).

Operating Humidity 15% – 95% (non condensing at 35°C)

Operating Altitude < 4572 m / < 15 000 ft

Note that the electrical settings are as follows (approximate values):

Table 9 Electrical Settings

	Positive ionization	Negative ionization
Capillary voltage	- 1500 V to - 2200 V	+ 1500 V to + 2200 V
End Plate Offset	+500 V	-500 V

Needle current: 15-300 nA (stability: +/- 5 nA, if no gradient is used).

Replacement Parts List

Agilent part numbers are listed unless otherwise noted.

Part	Part Number
Abrasive mesh, 4000 grit	8660-0827
Cap, black ETFE, 1/4-28 threads (P·755, UpChurch Scientific, Oak Harbor, WA)	
Conductive ferrule	0100-2262
Holder for 5mm trap cartridge	5065-9915
Lamp, MR-11, 20W	2140-0819
Magnifier	1000-1444
Nano needle nut	G1982-60023
Nut driver, 3mm	8710-2484
PEEK clad f.s. capillary, 50µm i.d. x 70cm (green), for syringe infusion assembly	G1375-87313
PEEK clad f.s. capillary, 25µm i.d. x 70cm (yellow), for LC analyte transfer to column	G1375-87324
PicoTip emitters, 8 µm tip, 5 cm length, Qty 20/box	9301-6378
Plug, threaded, 1/4-28	0100-1689
Plunger assembly replacement for 250 µl RN syringe (1162-03, Hamilton, Reno, NV)	
Reference inlet screw assembly	G3253-60304
Retaining ring pliers	8710-2487
Syringe, 250 µl, RN type, 0.75" needle point style 3	9301-6418
Wrench, 4mm open ends	8710-1534
Zorbax 300SB-C18, 3.5µm, 150 x 0.075mm PEEK-clad f.s. capillary column	5065-9911
Zorbax 300SB-C18, 3.5µm, 50 x 0.075mm PEEK-clad f.s. capillary column	5065-9924
Zorbax 300SB-C18, 5 um, 5 x 0.3mm trap cartridge (enrichment column), pkg. of 5	5065-9913

5 Reference

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In This Book

This book contains installation, operation, maintenance and troubleshooting instruction for the Dual Nanospray Ion Source.

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