Electron Transfer Dissociation (ETD)

User Guide

Agilent Technologies

Notices

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In This Guide

This guide provides information about your ETD source.

Chapter 1 Basic Operation and Maintenance

This chapter describes basic operation and maintenance of the ETD source.

Chapter 2 Installation

This chapter tells you how to install and maintain components related to the ETD source. It also tells you how to verify the installation.

Chapter 3 Troubleshooting

This chapter describes the steps that you follow when you suspect that you have a problem with the ETD source.

Chapter 4 Concepts

This chapter contains an overview of the concepts regarding the ETD source.

Chapter 5 Reference

This chapter contains references to additional information about the ETD source.

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1 Basic Operation and Maintenance

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This chapter will familiarize you with the basic operation and maintenance of the Electron Transfer Disassociation (ETD) Source, which is part of the Agilent 6340 Ion Trap LC/MS.

Refer to the other documentation shipped with the 6340 for information about the other components.

Basic Operation

To access the ETD dialog box

1 Click the **MS(n)** tab in the 6300 Trap Control window.

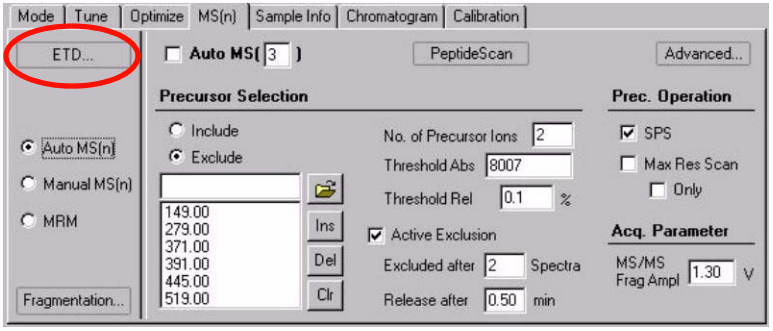

Figure 1 ETD button in the MS(n) tab

2 Click **ETD**.

The ETD dialog box appears.

The buttons at the top of the ETD dialog box allow the source to be turned on or off. In addition, there is a flush option which can be used to flush the methane lines after a tank has been changed.

You can set the ETD Mode in this dialog box. Options include doing manual or automatic ETD experiments, as well as for setting up CID fragmentation. A Reactant-only mode can be used to monitor and optimize the fluoranthene reactant ions in the source.

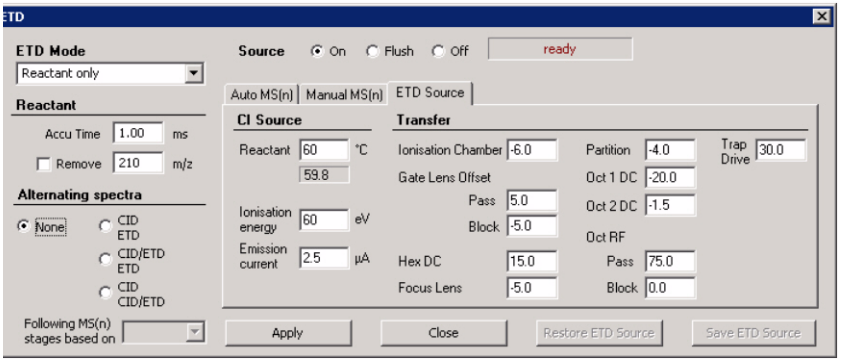

Figure 2 ETD dialog box

To optimize for ETD fragmentation

ETD fragmentation works best when the peptides are triply and more charged.

1 Click the **MS(n)** tab in the 6300 Trap Control window.

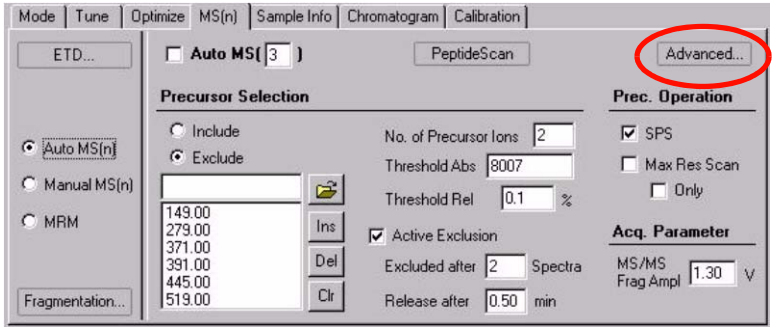

Figure 3 Advanced button in the MS(n) tab

2 Click **Advanced**.

3 Click the **Precursor Selection** tab.

4 Check that **> Double** is selected, then click **Close**.

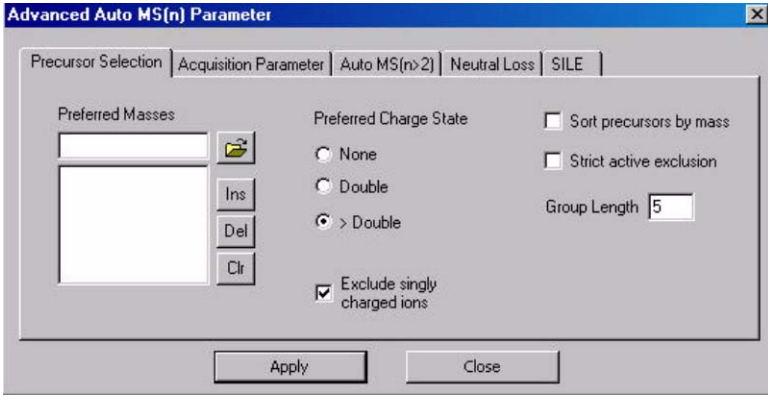

Figure 4 Advanced Auto MS(n) Parameter dialog box - Precursor Selection tab

To set up an ETD experiment

Electron Transfer Dissociation (ETD) occurs only when proper formation of the fluoranthene anion reagent is achieved. The objective of optimizing the fluoranthene anion is to maximize the abundance of *m/z* 202 while maintaining adequate resolution of *m/z* 203. See Figure 6.

Method ETD Demo.ms is provided with the software and contains good starting values for optimizing the Fluoranthene reagent.

Parameters which can affect the reagent ion formation are accessible by clicking on the ETD Source tab in the ETD panel

1 In the ETD dialog box, select **Reactant only** from the ETD Mode list to view the fluoranthene reagent ions.

2 Turn on the ETD source.

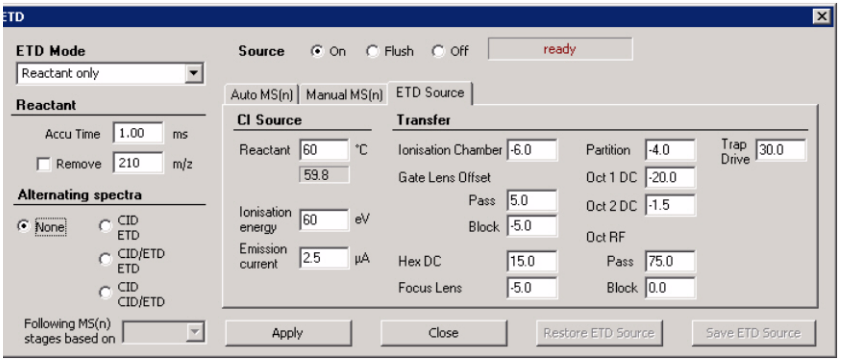

Figure 5 "Reactant only" selected for the ETD Mode

3 Set the appropriate values for the ETD source.

The recommended values for the ETD Source parameters are listed in Table 1 on page 12

Table 1 ETD Source Parameters

Parameter	Recommended Value
Reactant Temp	70° C
Ionization energy	60 to 80eV
Emission Current	2 to 5 uA
Ionization chamber	-2V to -8V
Gate Lens offset pass	2V to 10V
Gate Lens offset block	-2V to -10V
Hexapole DC offset	10V to 20V
Focus Lens	-3V to -5V
Partition	-2V to -4V
Oct 1-DC	-2.2V
Oct 2-DC	-1.2V
Pass	75V
Block	0V

Slight changes to the Ionization chamber and the Gate lens offset parameters may fine tune the relative ratio and resolution of the reactant ions. To maximize the resolution of the reactant ion, the accumulation time may have to be lowered from 1.0 ms to 0.6 ms or 0.7 ms.

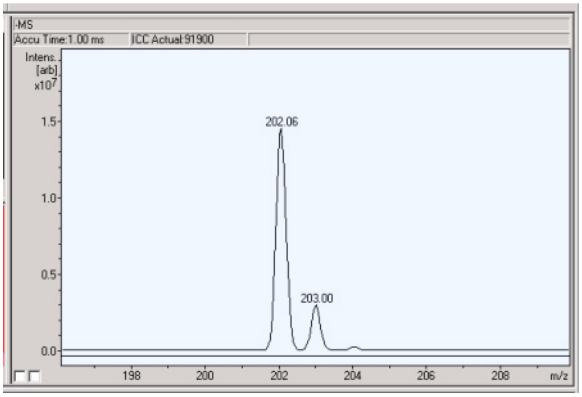

Figure 6 Proper ratio of *m/z*=202 to *m/z* =203 fluoranthene reactant anion

4 Optimize the methane gas flow.

Generally a methane tank pressure of 1 to 2 bar results in adequate production of the fluoranthene anion. To more accurately determine the production rate, monitor the fluoranthene reactant ion:

- Begin with a value of 1 bar.
- Slowly increase the tank pressure while you monitor the increase in the reactant ion. A maximum abundance is usually observed somewhere in the 1 to 2 bar range.
- Choose a tank pressure that results in the most abundant production of the reactant ion.

5 Select the ETD mode.

Figure 7 shows the AutoMS(n) parameter tab.

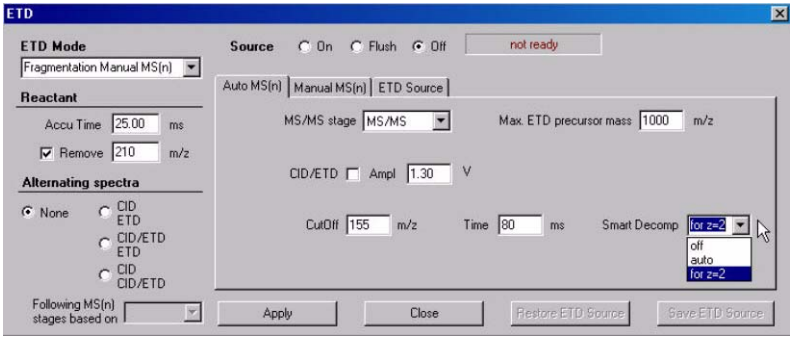

Figure 7 AutoMS(n) choices

Figure 8 shows the settings for manual fragmentation. When you set up for manual fragmentation, specify the precursor ions and the number of MS/MS stages to be done.

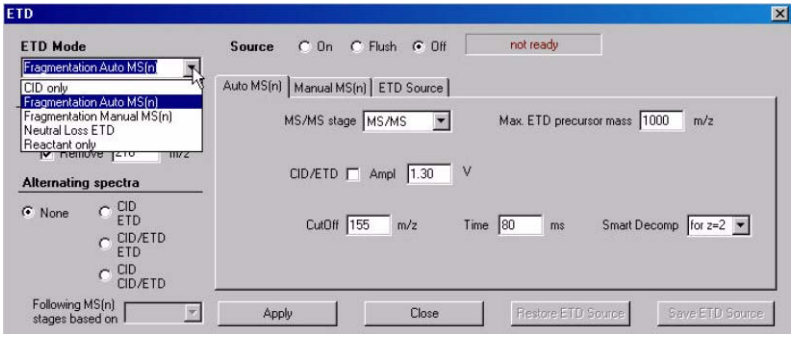

Figure 8 ETD Modes

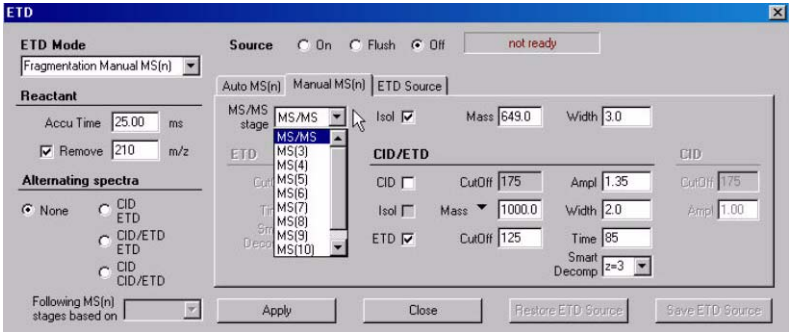

Figure 9 Specifying multiple MS/MS stages

Note that information specified in the ETD manual fragmentation panel is linked to the MS(n) screen in the trap control screen.

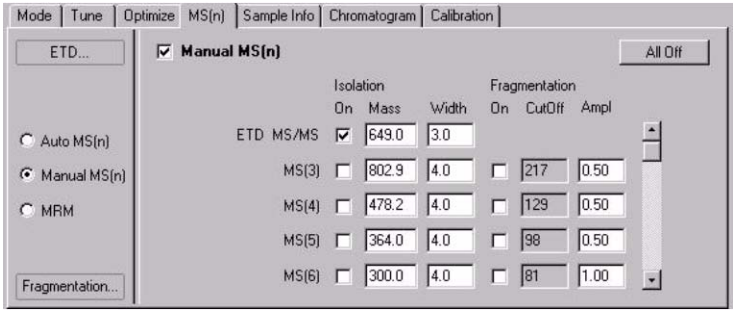

Figure 10 MS(n) tab - for manual fragmentation

ETD Source Maintenance

For information about maintaining the methane gas supply, and the fluoranthene supply see the appropriate sections in “Installation” on page 27.

The following figure shows the ETD:

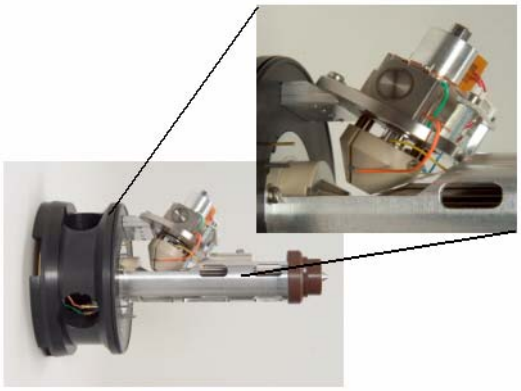

Figure 11 ETD

Needed Tools

The following tools are required to perform the operations described in this chapter:

- wrench 8 mm
- wrench 12 mm
- gloves, clean, lint free
- chemical safety goggles
- torx 10
- torx 20
- slit screw driver (about 2 mm)
- slit screw driver (about 4 mm)
- ohmmeter

To mount and adjust the cooling plate

Tools needed

- torx 10
- torx 20

1 Turn the heater device so that the “nose” (the part to which the cooling plate attaches) is in a line with the octopole (pre-adjustment).

2 Mount the cooling plate, and fine adjust the clamp direction and the angle of the nCI source with octopole assembly, so that the cooling plate fits to the thread without a gap between the plate and clamp.

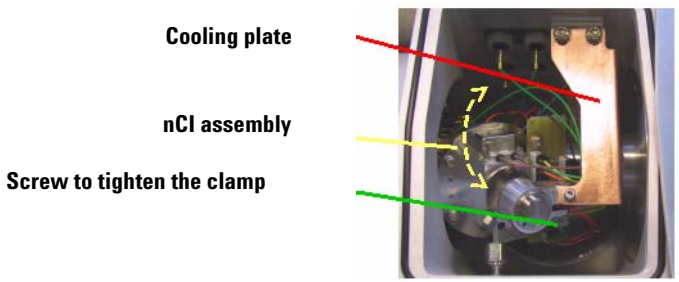

Figure 12 Cooling plate and nCI assembly

3 Uninstall the plate carefully so that the position of the clamp is not shifted.

4 Push back the skimmer spacer in the same way as when removing the Ion optics assembly. This allow the Ion optics assembly to turn freely for positioning.

5 Turn the nCI assembly so that the clamp can be tightened by screwing in the screw.

6 Turn back the nCI assay and remount the cooling plate.

7 Remount the desolvation assembly.

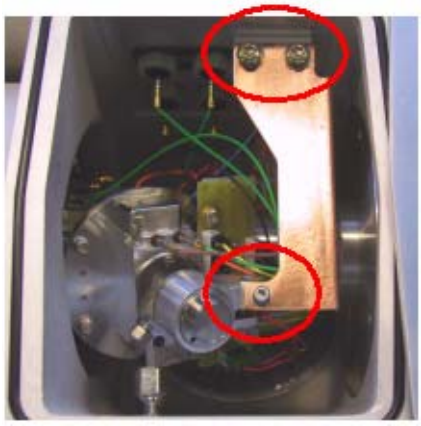

Figure 13 Cooling plate and mounting screws

The cooling plate is mounted on the mounting angle with two screws, and to the nCI source with one screw.

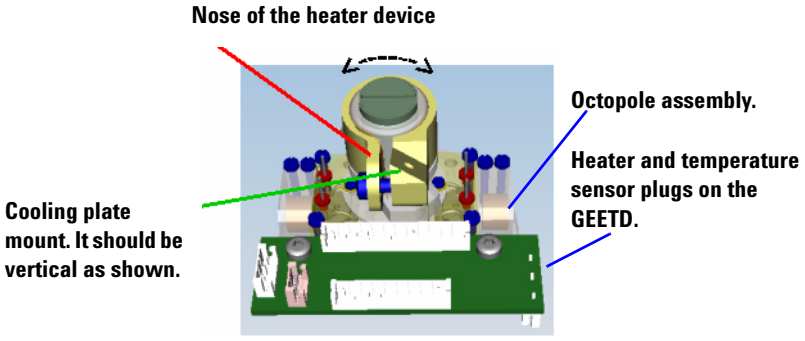

Figure 14 Heater device and cooling plate mount without the octopole

The whole circular brass colored ring in Figure 14 depicts the heater. It is also referred to as the clamp. The heater consists of a heater coil that fits into the clamp, which binds closely to the crucible to heat the fluoranthene inside the crucible.

The octopole assembly is partially visible is Figure 14. It is located behind the lower single screw on the cooling plate. It looks like a silver tube running horizontally, and the “nose” runs in line with the octopole.

To mount and adjust the heater foil

Tools needed:

- torx 10
- torx 20

To dismount the cooling plate

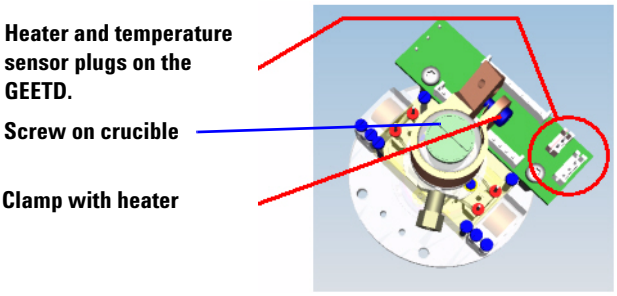

Figure 15 Heater, temperature sensor plugs, and clamp

1 Disconnect the heater and the temperature sensor plugs from the little green circuit board (GEETD).

2 Pull the temperature sensor out.

3 Unscrew the screw and carefully drag the clamp with the heater from the nCI source.

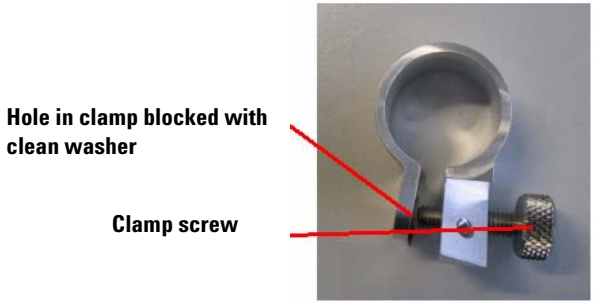

Figure 16 Clamp

4 Spread the clamp:
   - Screw the screw into the thread. The screw shown in Figure 15 on page 19 is the same screw that holds the top cover of the instrument. You can use the screw from the cover of the top manifold if necessary.
   - Block the hole at the opposite side (e.g., with a clean washer).
   - Be sure to use a clean screw and washer for this procedure.
5 Screw the screw into the thread until it reaches the blocked hole.
6 Spread the clamp by turning the screw a maximum of 2½ turns.

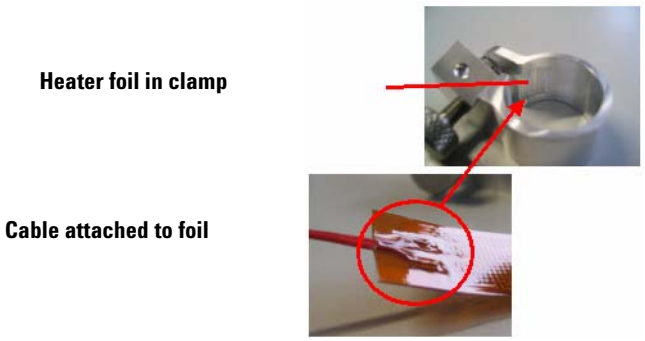

Figure 17 Heater foil, cable, and clamp

To insert the heater foil

1 Place the heater foil into the clamp (with the cable attached to the countersink). The counter sink is on the opposite side from that on which the screw is being screwing. (See Figure 18 on page 20.)

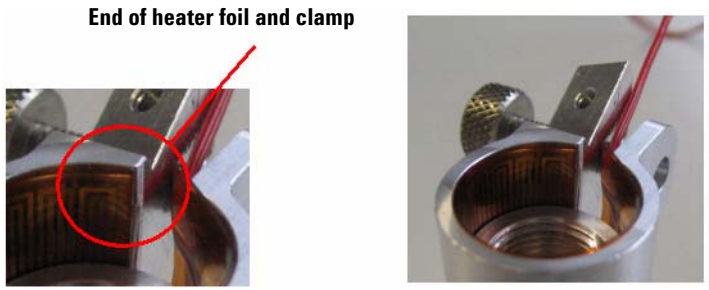

Figure 18 Heater foil, clamp and cable

The picture on the right shows the cable position relative to the opposite side of the heater band. Notice that the heater fits into the slightly recessed area in the clamp.

2 The end of the heater foil must end with the clamp and not overlap it.

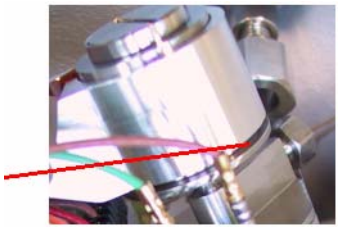

Figure 19 Gap between heater device and crucible

3 Carefully slide the device over the crucible retainer.

4 Do not damage the heater foil.

5 Do not slide the heater device to the bottom of the crucible retainer. Leave a gap of about 1 mm between them.

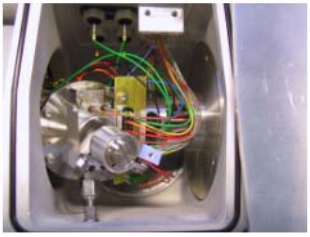

Figure 20 Heater foil cables and nCI body

6 Check with an ohmmeter to verify that there is not an electrical short between:

- the heater foil cables and the ground, or
- the heater foil cables and the nCI body.

7 If you detect an electrical short, then check that the following are installed correctly:

- the heater foil and clamp position,
- the heater foil itself.

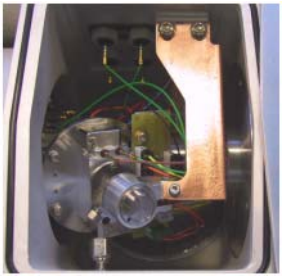

Figure 21 Finished assembly

8 Next, follow the procedure: "To mount and adjust the cooling plate" on page 17.

To replace the filament

Tools needed:

- torx 10
- torx 20
- small screw driver
- wrench 8 mm

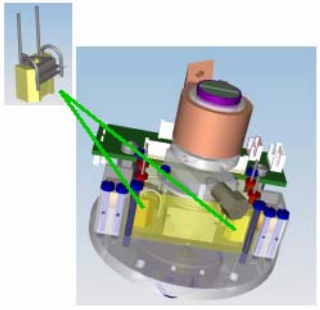

Figure 22 Filaments in the nCI source

Two filaments are installed in the nCI source. Only one filament is connected. If one of the filaments is burnt through, then you can connect the other one without impact on the functionality of the ETD source.

1. Dismount the cooling plate.
2. Disconnect the fused silica capillary.
3. Disconnect the heater and the temperature sensor plugs from the GEETD board.

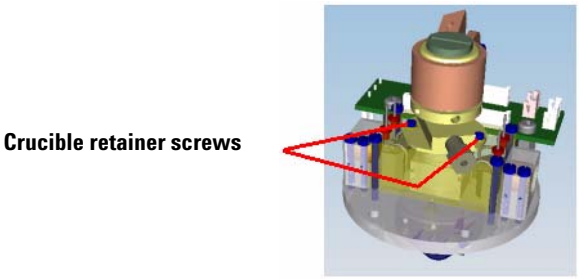

Figure 23 Crucible retainer screws

4 Unscrew the two screws of the crucible retainer and pull it out.

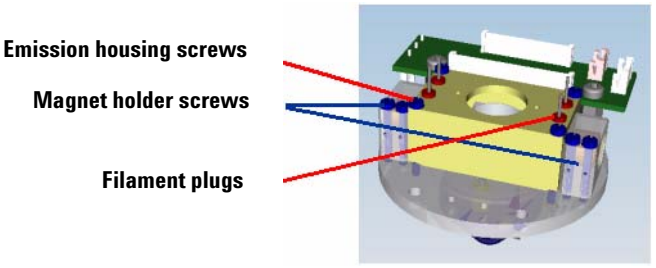

Figure 24 Emission housing

5 Unscrew the four screws of the emission housing.

6 It may be necessary to loosen the screws of the two magnet holders.

7 Carefully pull the emission housing out, but do not to loosen the four plugs of the filaments.

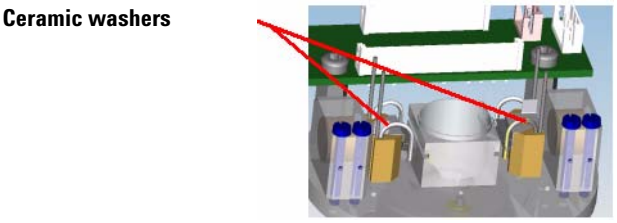

Figure 25 Ceramic washers

8 Unscrew the screws holding the filaments to the ceramic block. Do not damage the ceramic washers.

9 Replace the filaments and tighten the screws.

10 Reinstall the emission housing with the four plugs for the filaments.

11 Tighten the four screws.

12 Tighten the screws of the magnet holder, if they were loosened previously.

13 Reinstall the crucible retainer with the two screws and tighten them.

14 Reinstall the temperature sensor.

15 Reconnect the plugs of the heater and the temperature sensor.

16 Reconnect the fused silica capillary.

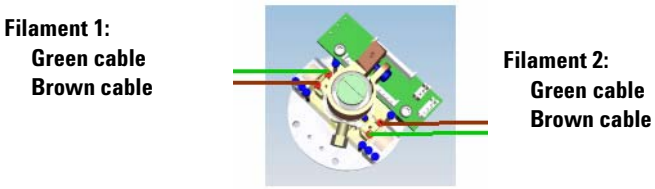

Figure 26 Filaments and cable connections

17 Connect the green cable with one of the filaments.

18 Connect the brown cable with the same filament.

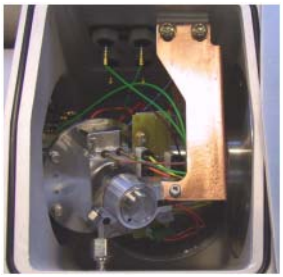

Figure 27 Finished assembly

19 Continue at "To mount and adjust the cooling plate" on page 17.

2 Installation

Step 1. Install or replace the methane bottle 29
Step 2. Vent the methane pressure regulator 30
Step 3. Do a leak test for methane 32
Step 4. Fill the fluoranthene crucible 36
Step 5. Verify performance of the ETD with Angiotensin 1 39
Step 6. Verify ETD performance with the phosphopeptide standard mix 42

This chapter contains instructions for installing and maintaining the methane and fluoranthene used by the ETD source on the Agilent 6340 Ion Trap.

Before using your system, you should install the 6340 and verify its performance according to the procedures specified in its Installation guide. Then, perform the procedures described in this chapter.

NOTE These procedures and verification methods are to be used only for bundled instruments shipped with an ETD source.

Needed Tools

Obtain the following tools, which are required to perform the operations described in this chapter:

- wrench 8 mm
- wrench 12 mm
- gloves, clean, lint free
- chemical safety goggles
- torx 10
- torx 20
- slit screw driver (about 2 mm)
- slit screw driver (about 4 mm)
- ohmmeter

Step 1. Install or replace the methane bottle

Do this step only if pressurized methane containers or gas bottles are used to supply methane.

1 Make sure you understand the safety issues described in "Methane Safety" on page 62.

WARNING **Methane is highly flammable. You must understand the safety issues described in "Methane Safety" on page 62 before you proceed.**

2 Close the methane outlet shut-off valve.

3 Close the main valve of the methane gas bottle.

4 Adjust the pressure regulator to the lowest possible value to separate the low-pressure stage from the high-pressure stage.

5 Remove the old methane gas bottle.

6 Mount the new methane gas bottle.

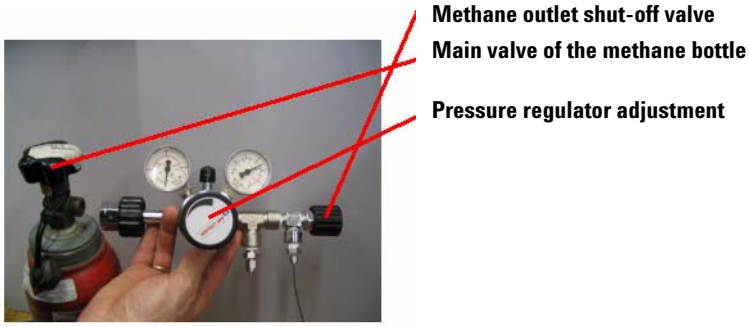

Figure 28 Methane gas bottle and regulator

Step 2. Vent the methane pressure regulator

The following venting steps prevent ambient air from diffusing back into the methane gas bottle. Any unnecessary opening of the methane gas bottle before complete venting of the pressure regulator must be avoided.

Figure 29 Methane outlet shut-off valve

1. Open the main valve of the methane bottle for a short time, and only as much as necessary to fully pressurize the inlet stage of the pressure regulator.
2. Close the main valve of the methane gas bottle immediately afterwards.

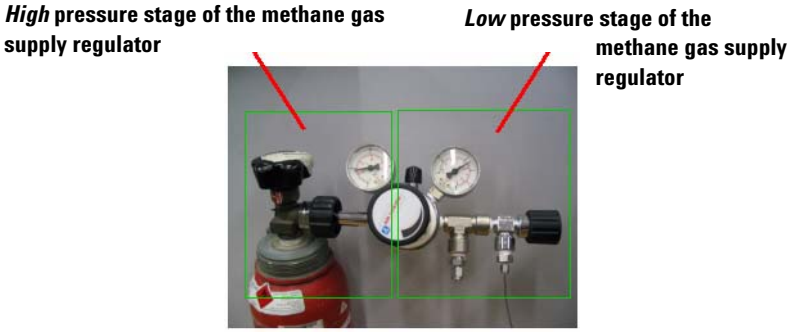

Figure 30 High and low pressure stages of the methane gas supply

3. In the high pressure stage of the methane supply, as indicated by the pressure gauge, the pressure should not drop recognizably. If it does, then there is a leak between the methane bottle and the regulator, which must be fixed.

4 The pressure in the low pressure stage of the methane supply, as indicated by the pressure gauge, should not drop recognizably. If it does, then there is a leak between the regulator and the shut-off valve, which must be fixed.

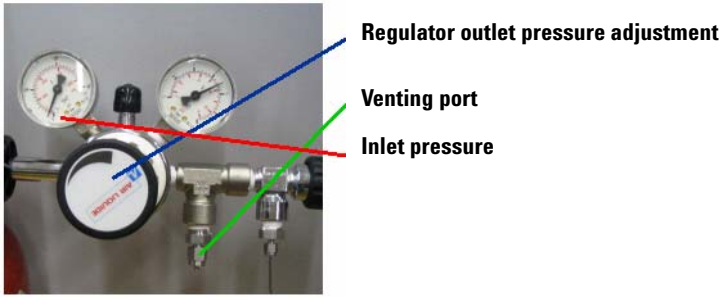

Figure 31 Venting Port and regulator

5 Adjust the outlet pressure of the regulator to about 2.5 bar.

6 Slightly open the venting port to allow methane to exhaust.

7 Close the venting port **before** the inlet pressure drops **below 10 bar**.

8 Repeat steps 1 to 7 a second time.

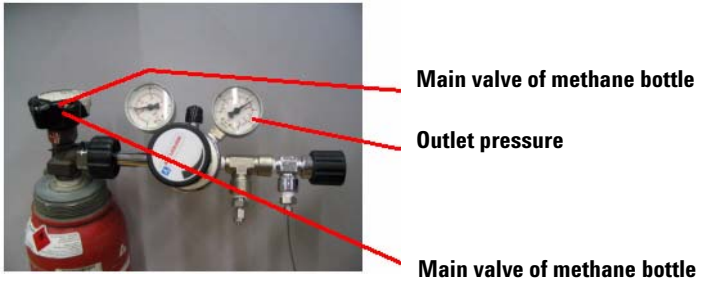

Figure 32 Main valve and outlet pressure gauge

9 Open the main valve of the methane gas bottle.

10 Adjust the outlet pressure of the pressure regulator to your ETD operating pressure of 2.5 bar.

11 Close the main valve of the methane bottle.

Step 3. Do a leak test for methane

In this step, you do two separate leak tests to make sure that methane is not leaking from either connections.

Leak Test I

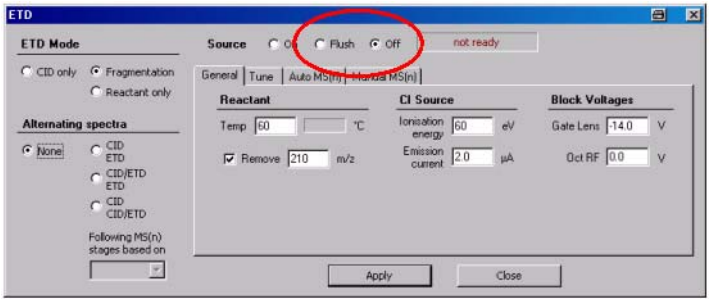

Figure 33 ETD dialog box

1 Make sure that the ETD nCI-source is switched off from the instrument-control software and that the source flush option (for venting the methane line) is not activated.

2 Avoid excessive opening of the main valve of the methane bottle before a leak test has been performed.

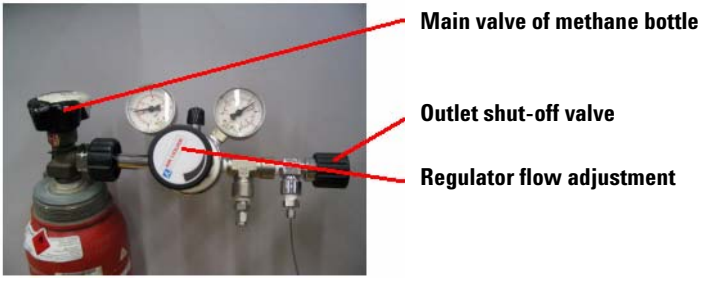

Figure 34 Main valve,

3 Close the main valve of the methane bottle.

4 Close the methane supply outlet shut-off valve.

5 Close the flow into the low-pressure stage of the methane supply outlet.

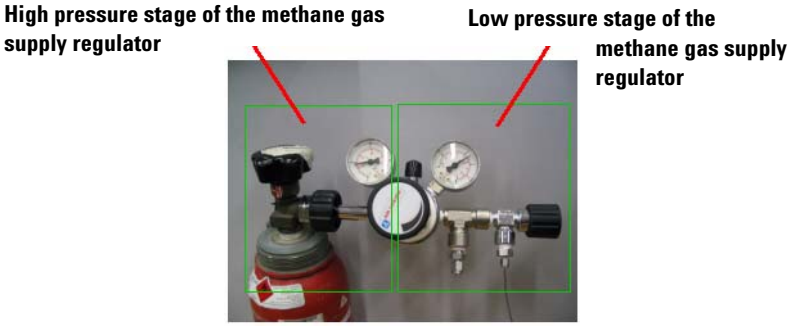

Figure 35 High and low pressure stages of the methane gas supply

6 If the pressure in the high pressure stage of the methane supply, as indicated by the pressure gauge, drops recognizably, fix the leak between the methane bottle and the regulator.

7 If the pressure in the low pressure stage of the methane supply, as indicated by the pressure gauge, drops recognizably, fix the leak between the regulator and the shut-off valve.

Leak Test II

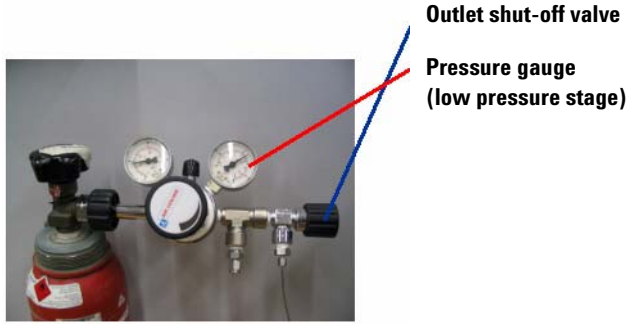

Figure 36 Outlet shut-off valve and Pressure gauge (low pressure stage)

1 Open the methane supply outlet shut-off valve.

2 If the pressure indicated by the pressure gauge continues to drop after a slight drop-off while opening the shut-off valve, fix the leak in the transfer line between the methane supply and the methane inlet valve inside the instrument.

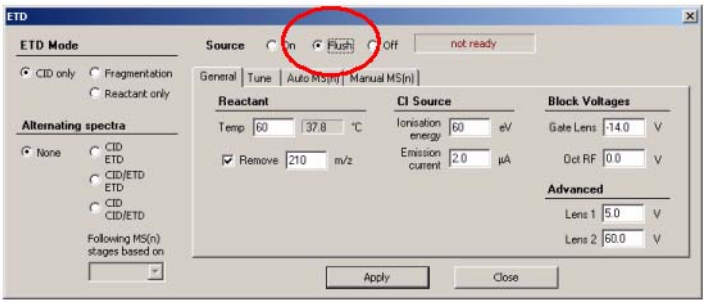

Figure 37 ETD source Flush option (ETD dialog box)

3 Activate the source flush option (for venting the methane line) from the instrument-control software (ETD dialog box).

The pressure indicated by the pressure gauge (low pressure stage) should drop slowly.

- If a gas bottle with a pressure regulator is used, then adjust the low pressure to about 2.5 bar.
- The pressure in the high-pressure stage must drop before the pressure in the low-pressure stage starts dropping. This part of the test may take several hours.

4 If the pressure indicated by the pressure gauge (low pressure stage) does not drop at all, then check the following:

- If you cannot hear a sound when activating the flush valve, this may indicate a failure of the methane valve. If so, then determine if the electrical connection on the valve is good, and determine if the correct voltage is being supplied to the valve.
- Is the main valve of the methane bottle is completely closed? If not, then close it.
- Is the stainless steel methane capillary plugged? If so, then replace it.

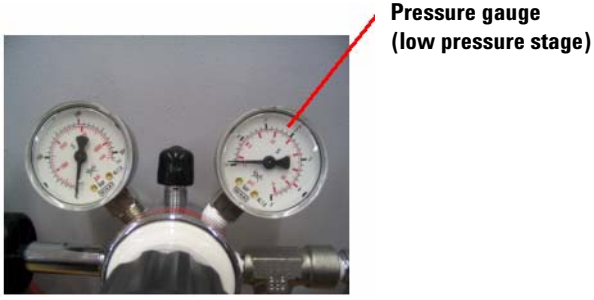

Figure 38 Pressure gauge (low pressure stage)

The pressure indicated by the pressure gauge (low pressure stage) must drop below zero (below ambient).

5 If the gauge is able to indicate pressure values below ambient, but the indicated pressure sticks at ambient pressure; then fix the leak between either the methane gas bottle and the pressure regulator, or the stainless steel methane capillary to the instrument.

Figure 39 Pressure gauge (low pressure stage) indicating about -0.8 bar (below ambient)

6 If there is no leak, then the pressure indicated by the pressure gauge (low pressure stage) should drop below -0.5 bar (below ambient).

Depending upon the volume attached to the low-pressure stage of the methane supply outlet, this part of the test may take several hours.

Step 4. Fill the fluoranthene crucible

Tools needed:

- Gloves, clean, lint free (# 01213)
- Chemical safety goggles (# 225687)
- Slit screw driver (# 70119)
- Wrench 12 mm (# 27376)

Parts required:

- Fluoranthene (99%) (Sigma Aldrich 423947)

Note that new instruments arrive with the crucible filled with fluoranthene.

Refill

Fluoranthene is used as a solid compound inside the crucible of the nCI source. The fluoranthene evaporates from the crucible during the ETD operation. The crucible must be refilled when it is empty.

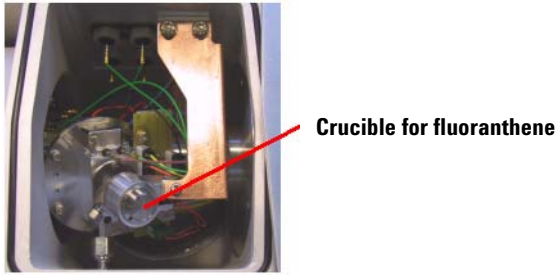

Figure 40 Crucible for fluoranthene

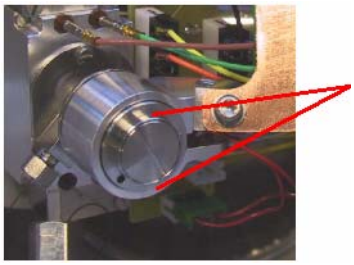

Figure 41 Crucible

1 Understand the safety issues related to using fluroanthene (see "Fluoranthene Safety" on page 63).

WARNING **fluoranthene is highly toxic. You must understand the safety issues described in "Fluoranthene Safety" on page 63 before you continue.**

2 Unscrew the crucible using a 12 mm wrench.

3 Do not open the lid of the crucible outside a fume hood.

WARNING **For personal protection the following steps must be performed under a fume hood according to the "Fluoranthene Safety" on page 63, and the Material Safety Data Sheet from your fluoranthene supplier.**

Figure 42 Crucible and lid (top) and Fluoranthene (bottom)

4 Open the lid of the crucible using a wrench and screw driver.

5 Check that the quartz wool properly covers the outlet hole at the bottom of the crucible.

6 Fill the crucible with fluoranthene (approximately 150 mg).

7 Close the crucible by remounting the lid finger-tight.

8 Remount the crucible into the ETD nCI source.

Step 5. Verify performance of the ETD with Angiotensin 1

Angiotensin 1 (G1982-85000) can be infused into the ion trap to observe the performance of ETD and CID fragmentation. Angiotensin 1 displays a singly charged ion at *m/z* 1295.7, a doubly charged ion at 648.8, and a triply charged ion at *m/z* 432.9.

Sample Preparation

1. Remove the cap from the 1mg Angiotensin 1 vial (G1982-85000) and add 1 ml of water.
2. Further dilute the starting solution in water to a concentration of 0.1 mg/ml.
3. Dilute again with 1:1 methanol/water with 0.1% acetic acid to a final concentration of 0.01 mg/ml.

Infuse Angiotensin 1

1. Set up the syringe pump to infuse the 0.01mg/mL Angiotensin 1 at:
   - 18 µL/hr with a nanospray source or an HPLC-Chip Cube MS interface
   - 300 µL/hr with an ESI source

   Allow sufficient time for the Angiotensin 1 to make its way through the tubing into the ion trap.
2. From the Agilent 6300 Trap Control screen, load Method Def_LCMS.ms.
3. Click on the **Operate** tab to turn on the 6300 ion trap.
4. Observe that the Angiotensin 1 ions are present in Figure 43 on page 40.

   The singly charged ion at *m/z* 1295 may not be seen, or may give a weak signal. (If Ions are not detected in this scan mode, refer to *6300 Series Ion Trap LC/MS User Guide* for help.
5. After seeing the ions with the Def_LCMS.ms method, switch methods and load ETD Demo.ms.

   This method is optimized for demonstrating the fragmentation operation of both CID and ETD.
6. To access the ETD software control parameters, select the Ms(n) tab, then click on the ETD button.

In the ETD dialog box, click **Source on** to turn on the ETD source.

This action begins the process of turning on the ETD source. You may need to wait a few minutes for the ETD source to turn on. This ETD Demo method is made up of three segments. The first segment is optimized for viewing only the fluoranthene reaction ions at *m/z* 202 and *m/z* 203. The second segment will do CID fragmentation on the doubly charged 648.9 ion. And the third segment will do ETD fragmentation on the 432.9 ion.

7 To acquire a data file from this infused sample, click on the **Sample Info** tab in the 6300 ion trap control screen.

8 Supply a data file name and specify a subdirectory for the data file.

9 Click on the acquisition menu item at the top of the screen.

10 Select run method.

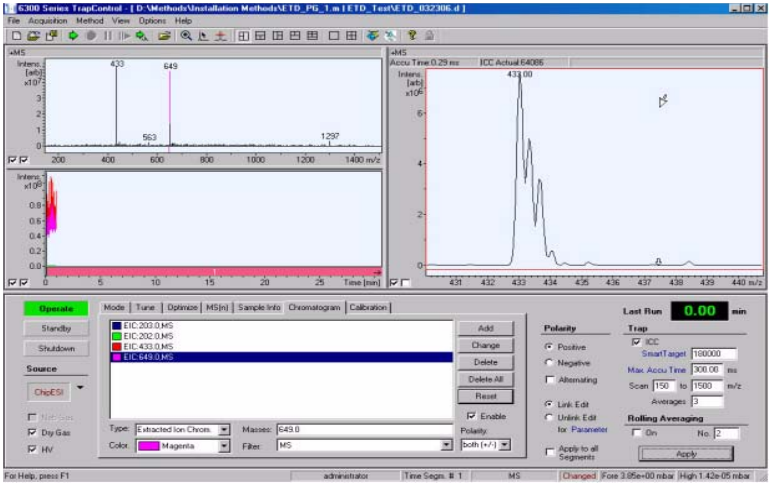

Figure 43 Angiotensin 1 triply charged 433 ion infusion at 18 µL/hr. The 6300 series trap control screen during the infusion of Angiotensin 1.

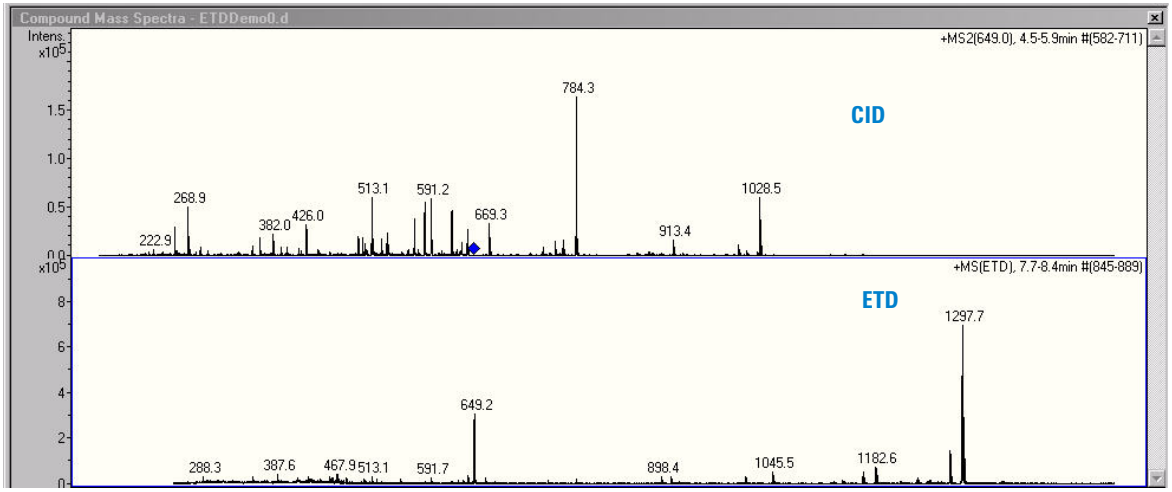

Figure 44 Comparing ETD and CID of triply-charged angiotensin I (*m/z* = 432.9). Triply-charged angiotensin I gave more ETD fragments.

Step 6. Verify ETD performance with the phosphopeptide standard mix

The Phosphopeptide mix (G2474-85001) is used to demonstrate a full chromatographic data acquisition of ETD fragmentation.

Sample preparation

1 Add 400 µL of 85%/15% Water/ Acetonitrile to the Phosphopeptide standard mix.

2 Vortex thoroughly for 15 seconds to mix

Setup and acquire data

1 Load method ETD_Phos. This method is supplied with the software and is optimized for analyzing the Phosphopeptide mix.

2 If you are using a nanospray source, configure the nanospray for direct or 1D mode.

This method was developed for use with the Agilent G4240A HP-LC Chip Cube MS interface.

The resulting data file should appear similar to the figure below.

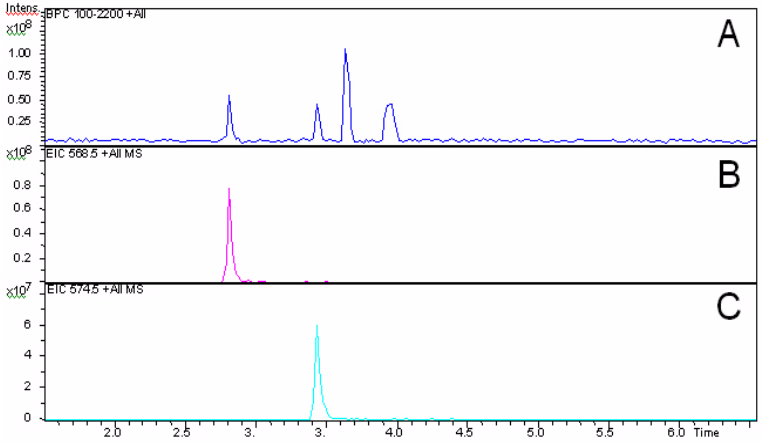

Figure 45 The phosphopeptides mixture is analyzed by an HPLC-Chip Ion Trap with ETD.

The first peak in the chromatogram is a phosphorylated peptide with the spectrum shown below.

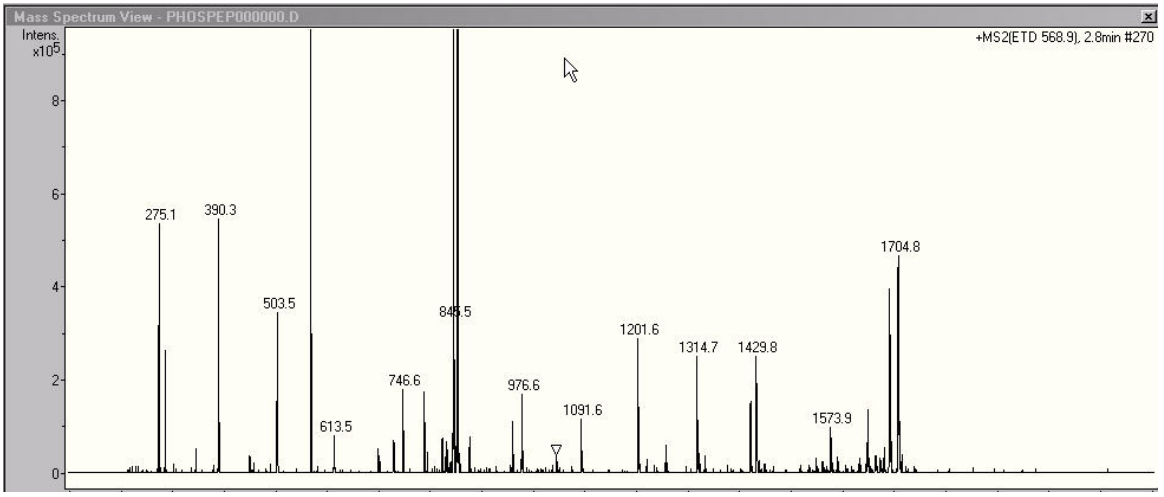

Figure 46 Phosphorylated peptide spectrum

3
Troubleshooting

To verify correct anion reactant signal 46
If no reactant Ion 202 is found 48
If there are diagnostics failures 49

This chapter describes how to troubleshoot the ETD source. Refer to the other documentation shipped with the 6340 for information about the other components.

To verify correct anion reactant signal

An important step in verifying the performance of ETD fragmentation is to confirm that the fluoranthene reactant ions are being formed. Without these reactant ions the ETD process will not occur. Load the Method ETD Demo.ms as a starting point for observing the reactant ions.

1 From the 6300 Trap control Screen select the MS(n) tab. Then select the ETD dialog box.

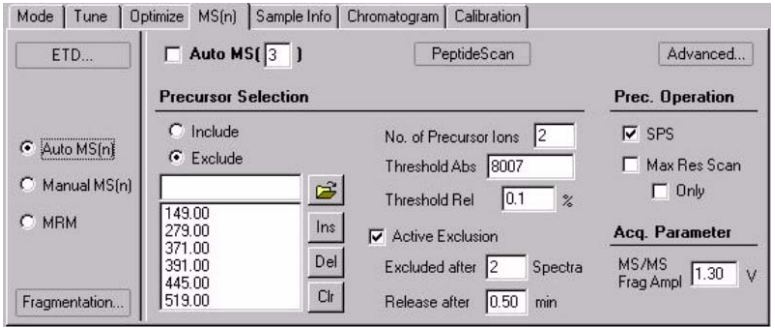

Figure 47 MS(n) tab

2 From the ETD control dialog box, select **Reactant only** as the **ETD mode**, and then turn on the source.

The ETD source can take several minutes to turn on.

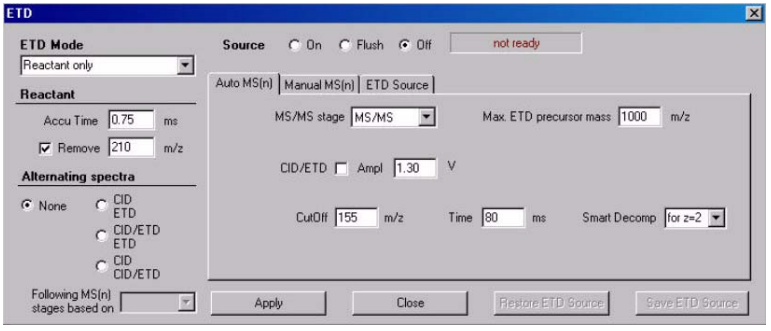

Figure 48 Select **Reactant only** form the ETD Mode menu

The reactant ions at m/z = 202, and m/z = 203 should now be visible. The ratio of the 203/203 should be as shown (with 203 being approximately 17-20% of the 202 peak). The 203 peak should be baseline resolved from the 202 peak.

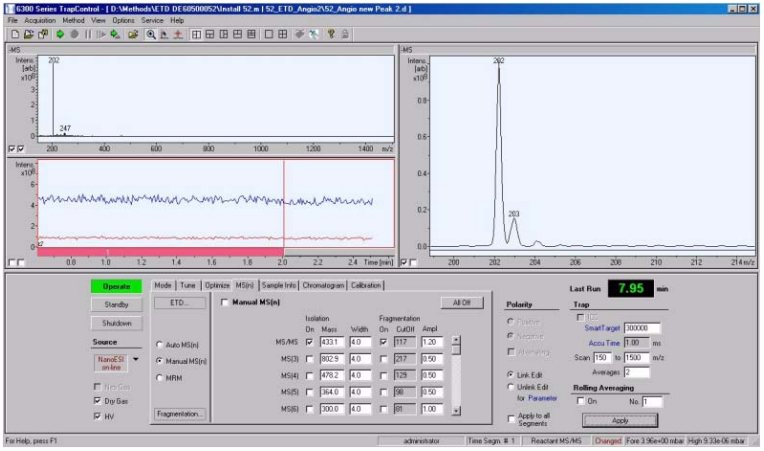

Figure 49 Proper appearance of the 202/203 reactant ions.

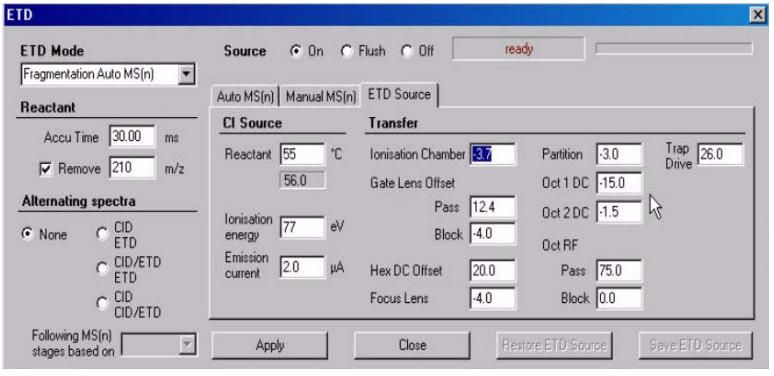

Figure 50 ETD source settings for proper ETD reactant ion formation.

3 Slightly adjust the Ionization chamber, and the Gate Lens offset parameters to fine-tune the appearance of the reactant ion in the display.

An error message is generated if the ETD filament cannot be turned on.

If no reactant Ion 202 is found

- ✔ Check that the methane gas is turned on and the methane bottle is not empty.
- ✔ Check that the methane pressure is optimized, usually between 1 and 2 bar.
- ✔ Check (from the ETD panel) that the ETD source is on. If the ETD source cannot be turned on, or if it generates an error message, run the ETD diagnostics from the diagnostics program.
- ✔ Check that the helium is turned on and set correctly for the Ion Trap.
- ✔ Check that CID is working.
- ✔ Check that you can hear the valve for the methane gas valve click when the source is flushed.
- ✔ Check that there is a slight rise in the high vacuum pressure of at least 0.1 mbar.

If there are diagnostics failures

✔ Type `http:\192.168.254.10` into your browser (Internet Explorer). The ETD diagnostic's application appears.

Figure 51 Diagnostic Menu

✔ Select ETD on the Diagnostic menu.

✔ Check that all ETD diagnostics pass.

✔ If any ETD diagnostic fails, then run the individual test for the failing diagnostic.

Test ETD: PASSED

Result	Value	Range	Comment
passed	4.94	0.00: 10.00	VacOK AI54
passed	1	1	Cover Interlock DI0
passed	1.25	0.00: 10.00	GelvId AI79
passed	115.6	110.0: 125.0	115V pos AI44
passed	-116.9	-125.0:-110.0	115V neg AI45
passed	401.3	380.0: 430.0	400V pos AI42
passed	-397.6	-430.0:-380.0	400V neg AI43
passed	-301.8	-315.0:-285.0	[V] Filament Voltage (VO17) AI64
passed	-151.4	-160.0:-140.0	
passed	-0.55	-1.00: 1.00	
passed	-99.9	-105.0:-95.0	[V] Ionization Chamber Voltage (VO18) AI65
passed	-50.17	-53.00:-47.00	
passed	-0.24	-0.50: 0.50	
passed	49.62	47.00: 53.00	
passed	99.6	95.0: 105.0	
passed	-100.2	-105.0:-95.0	[V] Gateg Block Voltage (VO19) AI66
passed	-50.17	-53.00:-47.00	
passed	-0.06	-0.50: 0.50	
passed	49.99	47.00: 53.00	
passed	100.4	95.0: 105.0	

Figure 52 Details of the ETD diagnostic screen

4 Concepts

CID Fragmentation 52
ETD Fragmentation 53

This chapter discusses concepts relating to the ETD Source used with the Agilent 6340 Ion Trap.

Refer to the other documentation shipped with the 6340 for information about the other components.

Ion trap mass spectrometers are ideally suited for determining the sequences of peptides and proteins. Typically, peptide mixtures are separated by chromatographic processes, and introduced into the ion trap. Multiply charged peptides are preferentially chosen for isolation and fragmentation. The resulting fragments can then be compared with protein data bases to confirm identification.

CID Fragmentation

The fragmentation process is achieved by causing the isolated peptides to undergo repeated collisions with the helium cooling gas that is present within the trap. This process, called Collision-Induced Dissociation (CID), adds internal energy to the peptides and induces random fragmentation along the peptide backbone. Fragments produced by CID are termed B and Y ions, as shown in Figure 53. While much useful information can be gathered by this process, a serious limitation is the inability to study many post-translational modification of proteins. This is due to the fact that the bonds which attach many modifications tend to be weaker and cleave before the amino acid bonds.

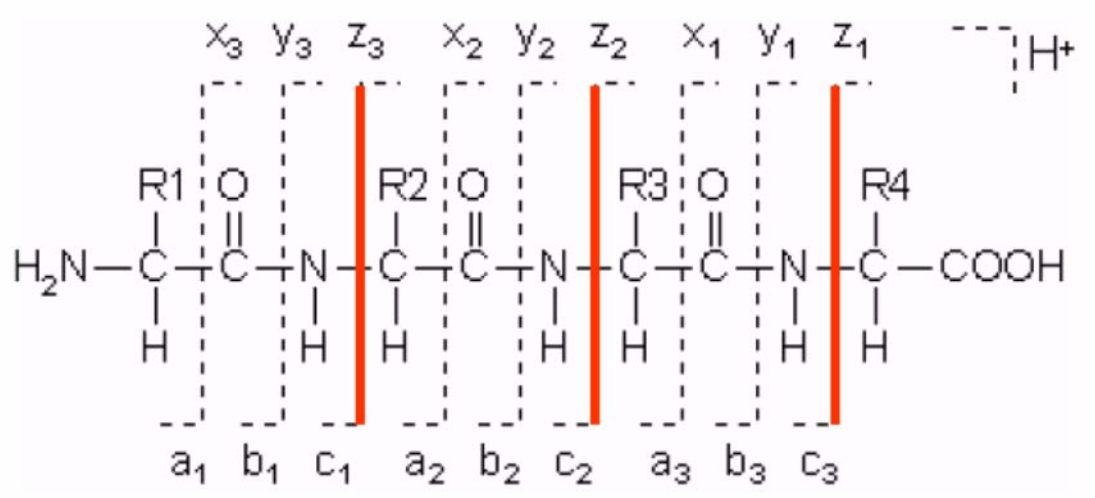

Figure 53 Fragments produced by CID and by ETD

ETD Fragmentation

Electron Transfer Dissociation (ETD) is a new and complementary way of sequencing peptides. Since ETD uses lower energy levels, many post-translational modifications remain attached to the amino acids. In addition, the fragmentation patterns produced by ETD tend to result in the formation of c and z ions, as shown in Figure 53. This complementary fragmentation pattern provides greater confidence in identifying peptides by data base searches.

ETD is a fragmentation method that works by reacting positively charged peptides with negatively charged anions to induce fragmentation. The Agilent 6340 ion trap uses fluoranthene as the reagent ion. Positively charged peptides enter the ion trap. Multiply charged precursor ions are selected for fragmentation. This fragmentation is achieved by gating the fluoranthene ions into the trap. The resulting reaction transfers an electron to the positive ions which then become unstable and undergo fragmentation. The reactions occur on the order of 40 to 100 ms.

With the Agilent 6340 ETD ion trap, it is possible to do either CID or ETD, and to alternate between modes within a single sample analysis. Thus, a single instrument run can generate both CID and ETD spectra.

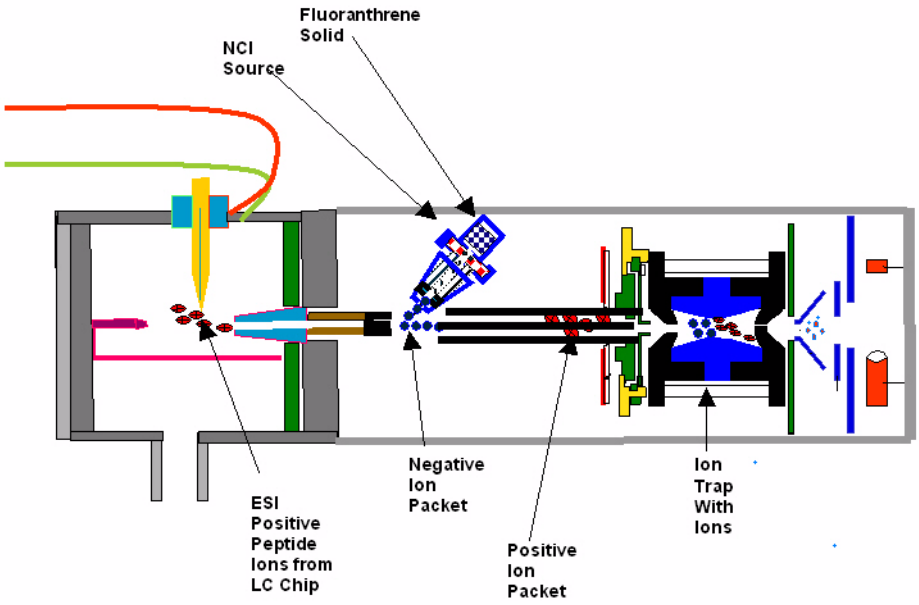

Figure 54 Agilent 6340 ETD Ion Trap

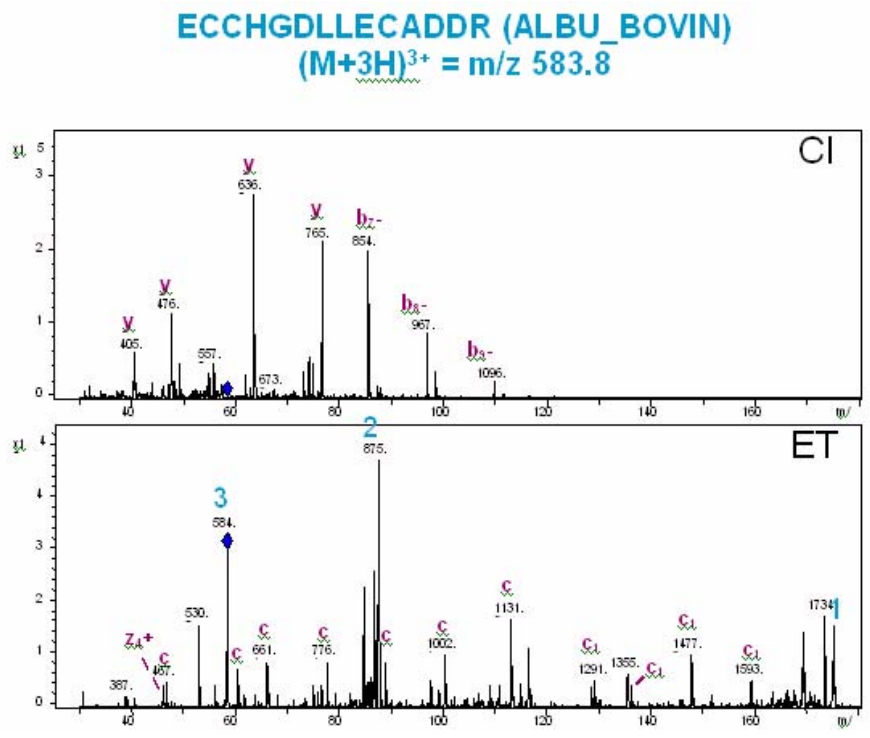

Figure 55 Comparison of ETD and CID Spectra

5
Reference

Safety 58
Safety Symbols 61
Methane Safety 62
Fluoranthene Safety 63
Parts List 64

Safety

Some of the procedures in this chapter require access to parts of the instrument and ETD source while it is in the Shutdown 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 describes the potential dangers.

Nebulizer Needle Hazard

- The nebulizer needle tip is very fragile. Do not touch the tip to any objects, such as the capillary cap or spray chamber. If you accidentally touch the nebulizer needle tip, replace the needle.
- The vaporizer temperature sensor 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.
- Use care when you adjust the nebulizer needle. Do not damage the end of the needle.

High temperatures

Most parts in the ETD source operate at or reach temperatures high enough to cause serious burns. These parts include, but are not limited to the capillary, capillary cap, spray shield, vaporizer temperature sensor, APCI corona needle, counter electrode, source hinge and spray chamber.

Do not touch these parts.

- Certain parts remain hot for many minutes after the instrument is shut down or turned off. In particular the spray shield and the capillary cap could be very hot after working with the ETD. Use extreme care when you work on an instrument that has recently been turned off.
- Do not touch any surfaces in the source spray chamber. The spray chamber is usually 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 capillary cap and counter 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.

- Never open the ETD spray chamber while the instrument is in operation or when the HV voltages are turned on.
- Do not insert fingers or tools through the openings on the ETD spray chamber. During operation the capillary and capillary cap are at a high voltage, up to 4 kV.
- A proper earth ground is required for instrument operation. Any interruption of the earth ground could create a shock hazard for the operator.
- Whenever you need to access internal components of the source, disconnect the instrument from the power source.

Biohazardous residue

The ETD interface does not ionize all of the sample and solvent. The vacuum pumps of the instrument removes the sample that is not ionized and solvent. The exhaust from these 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. Comply with your local air quality regulations.
- Fluid drained from the ETD chamber is made up of both 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.
- 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.

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 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 that 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, such as API, avoid dusty and fibrous environments. Dust particles can enter MS ion source and deposit on ion optics, causing sensitivity loss.

Safety Symbols

NOTE

This symbol is placed on the product when 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 personnel should perform work in this area.

WARNING HOT

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 BIOHAZARDS

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

Methane Safety

The following symbol is placed on the product within the area where extremely flammable substances (methane) are present.

Figure 56 Warning label - flammable substances are present

- Handle flammable substances with appropriate care.
- Methane can form explosive mixtures with air. Therefore, ignition sources must be kept away from the methane source and the 6340. Take the appropriate precautionary measures to eliminate the chance of an electrostatic discharge near the methane source and the 6340.
- For safety reasons, the size of the room, in which the instrument is installed, must have at least 20 square meters. The height should not be less than 2.5 m.
- An air exchange rate greater than 25 m^3/m^2h is recommended. It must not fall below 10 m^3/m^2h while the methane supply is open.

WARNING **The following procedures in this guide must be done according to your local regulations. Consult the safety representative of your local facility for advice about replacing gas bottles for pressurized methane. Refer to the Material Safety Data Sheet (MSDS) from your methane gas bottle supplier. If you have any questions or doubts about the appropriate safety measures when working with methane, then refer to your local regulations and to the requirements of the safety representative of your local facility.**

Fluoranthene Safety

- Fluoranthene is harmful when swallowed.
- Fluoranthene is used as a solid compound inside the crucible of the nCI source.
- Handle these areas with appropriate care and follow the precautions listed below.

Fluoranthene Precautions

WARNING

- **Do not breathe the fluoranthene dust and avoid contact with your eyes, skin, and clothing.**
- **Avoid prolonged or repeated exposure to Fluoranthene.**
- **Use government approved respirators for respiratory protection when handling fluoranthene.**
- **Wear chemical-resistant gloves for hand protection when handling fluoranthene.**
- **Wear chemical safety goggles for eye protection when handling fluoranthene.**
- **Refer to the fluoranthene MSDS regarding safety measures when working with fluoranthene**

Parts List

NOTE This parts list is subject to change between revisions of this document. Contact your customer service representative for current information.

Table 2 ETD Parts List

Part Number	Description
BDAL-235320	GELVX-1 Board
BDAL-232976	GEICX-1 Board
BDAL-237584	GELV-2
BDAL-239219	Transfer Unit
BDAL-238787	NCI Source
BDAL-243458	Filament Set
BDAL-239609	Temperature Sensor
BDAL-239608	Heater
BDAL-238155	GEETD-2
BDAL-238121	Methane Valve
BDAL-244093	Methane Inlet Pipe
BDAL-238122	Methane Pipe
BDAL-247000	ETD Cable Set
BDAL-243502	ETD Lens Voltage Package
G2440-60350	ETD Gas Module
BDAL-237603	GELV-5 Board
G1982-85000	Angiotensin 1
G4533-80001	GEPS-2 Board
G2440-60019	Octopole PCA
G1375-87300	PEEK Tubing 50 µm, 150 mm (Green)

Table 2 ETD Parts List

Part Number	Description
G1375-87316	PEEK Tubing 75 µm, 150 mm (Blue)
5042-8933	PEEK Sil Tubing 75 µm,150 mm
0100-2430	Ferrule 1/32-inch Graphite/Vespel
BDAL-217357	High Vac Gauge

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In This Book

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