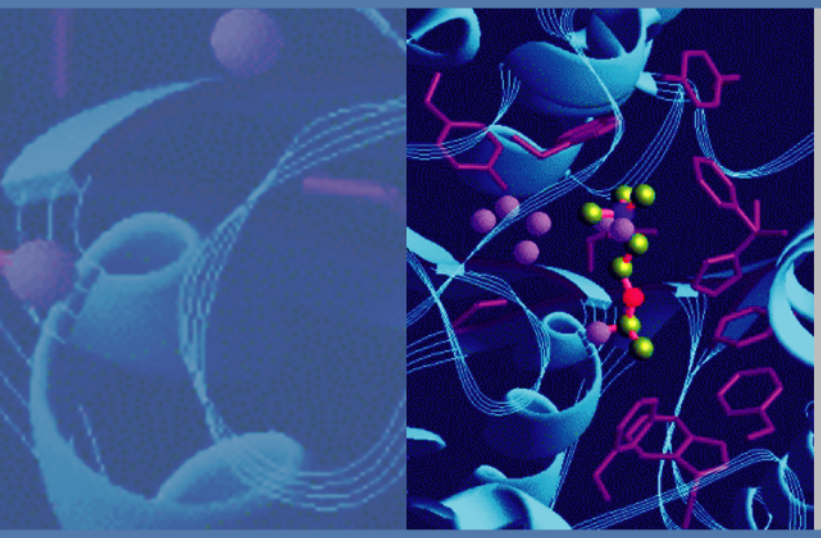

Ion Max

APPI Source

User Manual

97055-97021 Revision C February 2009

Thermo SCIENTIFIC

Release history: Revision C in February 2009. Revision B in June 2006. Revision A in September 2003.

Software version: Xcalibur 2.0

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Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations. For more information, refer to manual for your Thermo Scientific mass spectrometer.

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CAUTION Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

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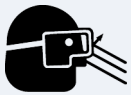
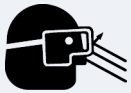
CAUTION Symbol	CAUTION	VORSICHT	ATTENTION	PRECAUCION	AVVERTENZA
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	**Elektroschock:** In diesem Gerät werden Hochspannungen verwendet, die Verletzungen verursachen können. Vor Wartungsarbeiten muß das Gerät abgeschaltet und vom Netz getrennt werden. Betreiben Sie Wartungsarbeiten nicht mit abgenommenem Deckel. Nehmen Sie die Schutzabdeckung von Leiterplatten nicht ab.	**Choc électrique:** L'instrument utilise des tensions capables d'infliger des blessures corprelles. L'instrument doit être arrêté et débranché de la source de courant avant tout intervention. Ne pas utiliser l'instrument sans son couvercle. Ne pas elensver les étuis protecteurs des cartes de circuits imprimés.	**Descarga eléctrica:** Este instrumento utiliza altas tensiones, capaces de producir lesiones personales. Antes de dar servicio de mantenimiento al instrumento, éste debera apagarse y desconectarse de la línea de alimentacion eléctrica. No opere el instrumento sin sus cubiertas exteriores quitadas. No remueva las cubiertas protectoras de las tarjetas de circuito impreso.	**Shock da folgorazione.** L'apparecchio è alimentato da corrente ad alta tensione che puo provocare lesioni fisiche. Prima di effettuare qualsiasi intervento di manutenzione occorre spegnere ed isolare l'apparecchio dalla linea elettrica. Non attivare lo strumento senza lo schermo superiore. Non togliere i coperchi a protezione dalle schede di circuito stampato (PCB).
	**Chemical:** This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	**Chemikalien:** Dieses Gerät kann gefährliche Chemikalien enthalten. Tragen Sie Schutzhandschuhe beim Umgang mit toxischen, karzinogenen, mutagenen oder ätzenden/reizenden Chemikalien. Entsorgen Sie verbrauchtes Öl entsprechend den Vorschriften in den vorgeschriebenen Behältern.	**Chimique:** Des produits chemiques dangereux peuven se trouver dans l'instrument. Proted dos gants pour manipuler tous produits chemiques toxiques, cancérigènes, mutagènes, ou corrosifs/irritants. Utiliser des récipients et des procédures homologuées pour se débarrasser des déchets d'huile.	**Química:** El instrumento puede contener productos quimicos peligrosos. Utilice guantes al manejar productos quimicos tóxicos, carcinogenos, mutagenos o corrosivos/irritantes. Utilice recipientes y procedimientos aprobados para deshacerse del aceite usado.	**Prodotti chimici.** Possibile presenza di sostanze chimiche pericolose nell'apparecchio. Indossare dei guanti per maneggiare prodotti chimici tossici, cancerogeni, mutageni, o corrosivi/irritanti. Utilizzare contenitori aprovo e seguire la procedura indicata per lo smaltimento dei residui di olio.
	**Heat:** Before servicing the instrument, allow any heated components to cool.	**Hitze:** Warten Sie erhitzte Komponenten erst nachdem diese sich abgekühlt haben.	**Haute Temperature:** Permettre aux composants chauffés de refroidir avant tout intervention.	**Altas temperaturas:** Permita que lop componentes se enfríen, ante de efectuar servicio de mantenimiento.	**Calore.** Attendere che i componenti riscaldati si raffreddino prima di effetturare l'intervento di manutenzione.
	**Fire:** Use care when operating the system in the presence of flammable gases.	**Feuer:** Beachten Sie die einschlägigen VorsichtsmaBnahmen, wenn Sie das System in Gegenwart von entzündbaren Gasen betreiben.	**Incendie:** Agir avec précaution lors de l'utilisation du système en présence de gaz inflammables.	**Fuego:** Tenga cuidado al operar el sistema en presencia de gases inflamables.	**Incendio.** Adottare le dovute precauzioni quando si usa il sistema in presenza di gas infiammabili.
	**Eye Hazard:** Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	**Verletzungsgefahr der Augen:** Verspritzte Chemikalien oder kleine Partikel können Augenverletzungen verursachen. Tragen Sie beim Umgang mit Chemikalien oder bei der Wartung des Gerätes eine Schutzbrille.	**Danger pour les yeux:** Dex projections chimiques, liquides, ou solides peuvent être dangereuses pour les yeux. Porter des lunettes de protection lors de toute manipulationde produit chimique ou pour toute intervention sur l'instrument.	**Peligro par los ojos:** Las salicaduras de productos químicos o particulas que salten bruscamente pueden causar lesiones en los ojos. Utilice anteojos protectores al mnipular productos químicos o al darle servicio de mantenimiento al instrumento.	**Pericolo per la vista.** Gli schizzi di prodotti chimici o delle particelle presenti nell'aria potrebbero causare danni alla vista. Indossare occhiali protettivi quando si maneggiano prodotti chimici o si effettuano interventi di manutenzione sull'apparecchio.
	**General Hazard:** A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	**Allgemeine Gefahr:** Es besteht eine weitere Gefahr, die nicht in den vorstehenden Kategorien beschrieben ist. Dieses Symbol wird im Handbuch auBerdem dazu verwendet, um den Benutzer auf Anweisungen hinzuweisen.	**Danger général:** Indique la présence d;un risque n'appartenant pas aux catégories citées plus haut. Ce symbole figure également sur l'instrument pour renvoyer l'utilisateur aux instructions du présent manuel.	**Peligro general:** Significa que existe un peligro no incluido en las categorias anteriores. Este simbolo también se utiliza en el instrumento par referir al usuario a las instrucciones contenidas en este manual.	**Pericolo generico.** Pericolo non compreso tra le precedenti categorie. Questo simbolo è utilizzato inoltre sull'apparecchio per segnalare all'utente di consultare le istruzioni descritte nel presente manuale.
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	Wenn Sie sich über die Sicherheit eines Verfahrens im unklaren sind, setzen Sie sich, bevor Sie fortfahren, mit Ihrer lokalen technischen Unterstützungsorganisation für Thermo Fisher Scientific San Jose Produkte in Verbindung.	Si la sûreté d'un procédure est incertaine, avant de continuer, contacter le plus proche Service Clientèle pour les produits de Thermo Fisher Scientific San Jose.	Cuando la certidumbre acerca de un procedimiento sea dudosa, antes de proseguir, pongase en contacto con la Oficina de Asistencia Tecnica local para los productos de Thermo Fisher Scientific San Jose.	Quando e in dubbio la misura di sicurezza per una procedura, prima di continuare, si prega di mettersi in contatto con il Servizio di Assistenza Tecnica locale per i prodotti di Thermo Fisher Scientific San Jose.

CAUTION Symbol	CAUTION	危険警告	危險警告
	**Electric Shock:** This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	**電撃：**この計測器は高電圧を使用し、人体に危害を与える可能性があります。保守・修理は、必ず操業を停止し、電源を切ってから実施して下さい。上部カバーを外したままで 計測器を使用しないで下さい。プリント配線板の保護カバーは外さないで下さい。	電擊：儀器設備使用會造成人身傷害的高伏電壓。在維修之前，必須先關儀器設備並切除電源。務必要在頂蓋蓋上的情況下操作儀器。請勿拆除PCB保護蓋。
	**Chemical:** This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	**化学物質：**危険な化学物質が計測器中に存在している可能性があります。毒性、発がん性、突然変異性、腐食・刺激性などのある薬品を取り扱う際は、手袋を着用して下さい。廃油の処分には、規定の容器と手順を使用して下さい。	化學品：儀器設備中可能存在有危險性的化學物品。接觸毒性致癌、誘變或腐蝕／刺激性化學品時，請配帶手套。處置廢油時，請使用經過許可的容器和程序。
	**Heat:** Before servicing the instrument, allow any heated components to cool.	**熱：**熱くなった部品は冷えるのを待ってから保守・修理を行って下さい。	高溫：請先等高溫零件冷卻之後再進行維修。
	**Fire:** Use care when operating the system in the presence of flammable gases.	**火災：**可燃性のガスが存在する場所でシステムを操作する場合は、充分な注意を払って下さい。	火災：在有易燃氣體的場地操作該系統時，請務必小心謹慎。
	**Eye Hazard:** Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	**眼に対する危険：**化学物質や微粒子が飛散して眼を傷つける危険性があります。化学物質の取り扱い、あるいは計測器の保守・修理に際しては防護眼鏡を着用して下さい。	眼睛傷害危險：飛濺的化學品或顆粒可能造成眼睛傷害。處理化學品或維儀器設備時請佩戴安全眼鏡。
	**General Hazard:** A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	**一般的な危険：**この標識は上記以外のタイプの危険が存在することを示します。また、計測器にこの標識がついている場合は、本マニュアル中の指示を参照して下さい。	一般性危險：說明未包括在上述類別中的其他危險。此外，儀器設備上使用這個標誌，以指示用戶本使用手冊中的說明。
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	**安全を確保する手順がよくわからない時は、作業を一時中止し、お近くのサーモエレクトロンサンローゼプロダクトのテクニカールサポートセンターごご連絡ください。**	如对安全程序有疑问，请在操作之前与当地的菲尼根技术服务中心联系。

Contents

Preface

This guide describes how to set up and install the PhotoMate® light source on the Ion Max ion source housing with an APCI probe. It also describes how to optimize the tune of your instrument after you have installed the Ion Max APPI source.

Safety and Special Notices

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

Safety and special notices include the following:

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

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

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ To contact Technical Support

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

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To copy manuals from the Internet

Go to mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

❖ To suggest changes to documentation or to Help

Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

Chapter 1 Introduction

The Ion Max™ ion source is a member of the Thermo Scientific family of mass spectrometer ion sources.

Your Ion Max APCI/APPI combination probe allows you to perform successful qualitative and quantitative analysis on a wide range of compounds, including steroids, peptides, basic drug entities, and pesticides. You can use APPI and APCI to study compounds that are not readily ionized by electrospray ionization (ESI). See Figure 1.

You can operate your APCI/APPI combination probe using the following ionization techniques:

- Atmospheric pressure chemical ionization (APCI)
- Atmospheric pressure photoionization (APPI)
- Combined APCI and APPI

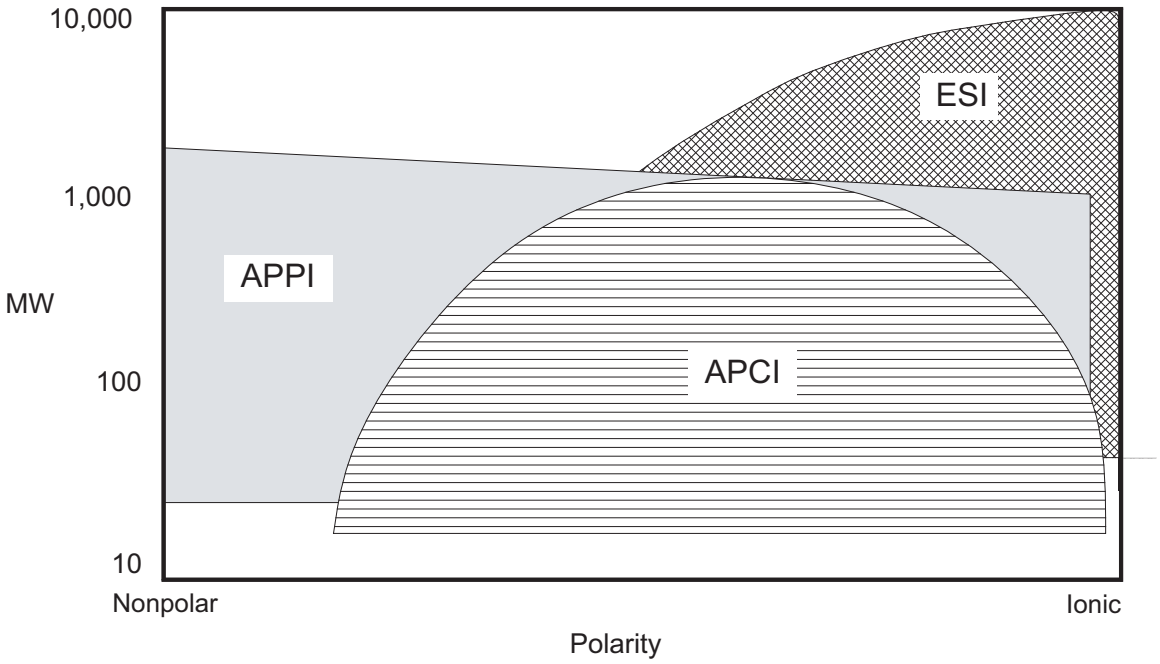


Figure 1. Ranges of applicability of APPI, APCI, and ESI

Atmospheric Pressure Chemical Ionization

Atmospheric pressure chemical ionization (APCI) is a soft ionization technique, but not as soft as ESI. APCI is used to analyze compounds of medium polarity that have some volatility.

In APCI, ions are produced and analyzed as follows:

1. The APCI nozzle sprays the sample solution into a fine mist of droplets.
2. The droplets are vaporized in a high temperature tube (the vaporizer).
3. A high voltage is applied to a needle located near the exit end of the tube. The high voltage creates a corona discharge that forms reagent ions through a series of chemical reactions with solvent molecules and nitrogen sheath gas.
4. The reagent ions react with sample molecules to form sample ions.
5. The sample ions enter the mass spectrometer and are analyzed.

Figure 1 shows the APCI process for positive adduct ion formation.

APCI is a gas phase ionization technique. Therefore, the gas phase acidities and basicities of the analyte and solvent vapor play an important role in the APCI process.

In the positive-ion mode, sample ionization occurs in a series of reactions that start with the electron-initiated cation formation. Typical examples of primary, secondary, and adduct ion formation are shown below:

Primary ion formation

$$e^- + N_2 \rightarrow N_2^{+\cdot} + 2e^-$$

Secondary ion formation

$$N_2^{+\cdot} + H_2O \rightarrow N_2 + H_2O^{+\cdot}$$

$$H_2O^{+\cdot} + H_2O \rightarrow H_3O^+ + HO\cdot$$

Proton transfer

$$H_3O^+ + M \rightarrow (M + H)^+ + H_2O$$

In negative-ion mode, $(M - H)^-$ is typically formed by the abstraction of a proton by OH^-.

APCI is typically used to analyze small molecules with molecular weights up to about 1500 u. APCI is a very robust ionization technique. It is not affected by minor changes in most variables, such as changes in buffers or buffer strength.

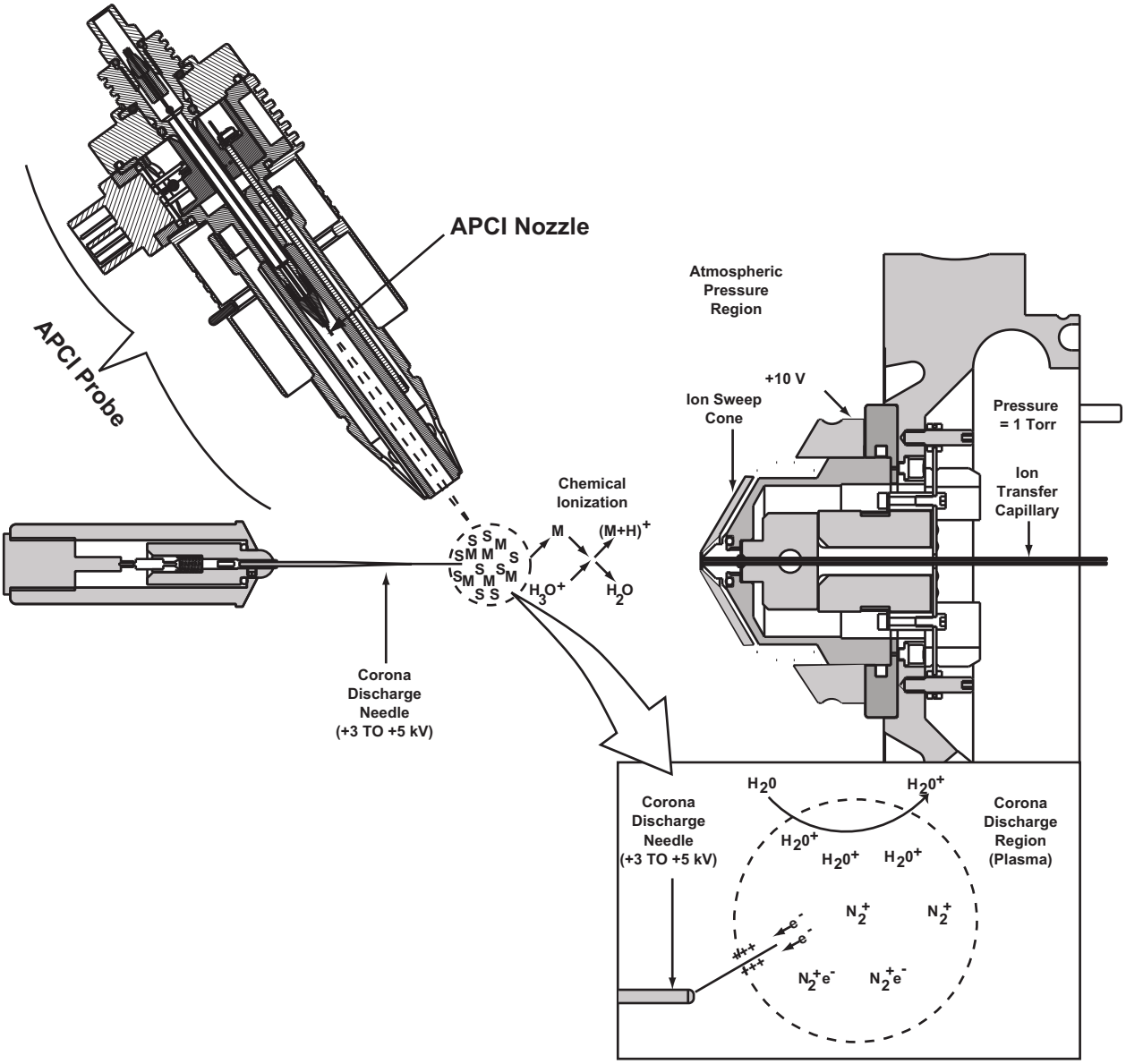


Figure 2. APCI process in the positive ion polarity mode

You can use APCI in positive or negative ion polarity mode. For most molecules, the positive-ion mode produces a stronger ion current. This is especially true for molecules with one or more basic nitrogen (or other basic) atoms. An exception to the general rule is that molecules with acidic sites, such as carboxylic acids and acid alcohols, produce more negative ions than positive ions.

Although, in general, fewer negative ions are produced than positive ions, negative ion polarity is sometimes the mode of choice. This is because the negative ion polarity mode sometimes generates less chemical noise than does the positive mode. Thus, selectivity might be better in the negative ion mode than in the positive ion mode.

Atmospheric Pressure Photoionization

Atmospheric pressure photoionization (APPI) is also a soft ionization technique. In APPI an ion is generated from a molecule when it interacts with a photon from a source of light such as the Syagen PhotoMate® light source. APPI generates molecular ions for molecules that have an ionization potential below the photon energy of the light being emitted by the light source.

In APPI, ions are produced and analyzed as follows:

1. The nozzle sprays the sample solution into a fine mist of droplets.
2. The droplets are vaporized in a high temperature tube (the vaporizer).
3. The analyte molecule interacts with the light from the PhotoMate light source. The analyte molecule M is ionized to a molecular ion M^+ if the ionization potential of the analyte is less than the photon energy $h\nu$:
4. $M + h\nu \rightarrow M^+ + e^-$
5. In the presence of protic solvents, the analyte ion may extract a hydrogen to form an $(M + H)^+$ ion:
6. $M^+ + S \rightarrow (M + H)^+ + (S - H)$
7. The analyte ions pass through the ion transfer capillary, enter the mass spectrometer, and are analyzed.

Molecules including steroids, basic-drug entities, and pesticides have ionization potentials below the threshold and protonated molecules are generated in the LC/MS experiment. APPI reduces fragmentation because only a small amount of energy is deposited in the molecule. Molecules such as the nitrogen sheath and auxiliary gas and the simple solvents used for LC/MS are not ionized because their ionization potentials are greater than the photon energy. The result is selective ionization of analyte versus background. See Figure 2 and Figure 3.

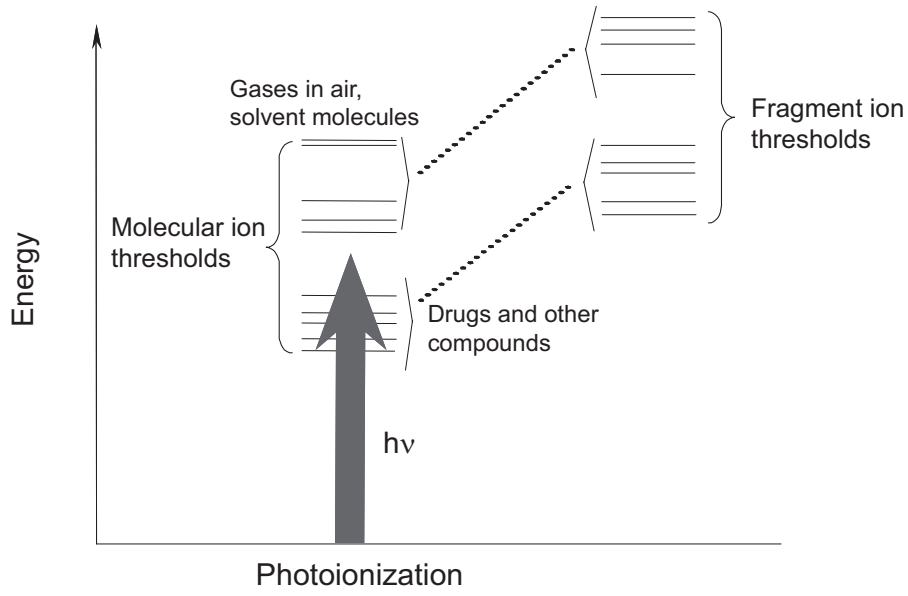


Figure 3. Energetics of photoionization

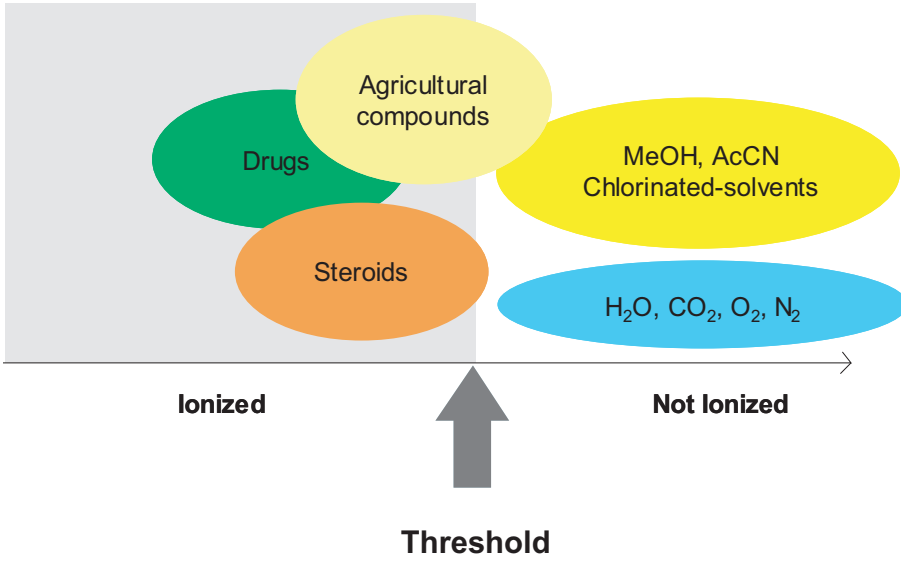


Figure 4. Illustration of selective photoionization

The PhotoMate light source uses a krypton lamp that emits photons with energies of 10.0 and 10.6 eV. Molecules with ionization potentials less than 10 eV ionize to form M^+ or $(M + H)^+$, while those with greater ionization potentials do not. Figure 4 shows ionization potentials of typical compounds and solvents.

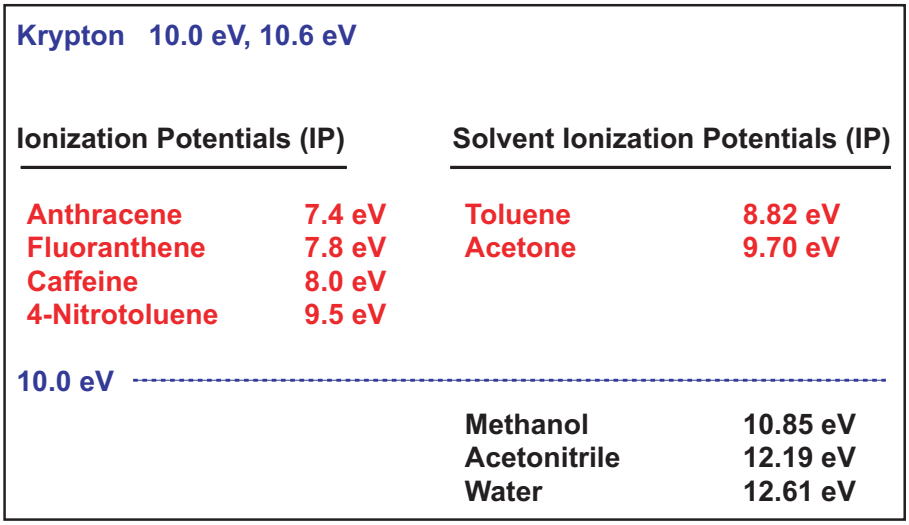


Figure 5. Ionization potentials of typical compounds and solvents

Combined APCI and APPI

You can easily change between APCI, APPI, and combined APCI and APPI techniques by turning on the corona discharge needle, the PhotoMate light source, or both. See Table 1. You turn the light source on and off by using the On/Off switch on the light source. You turn the corona discharge needle on and off by using the Xcalibur software. This allows you to quickly determine which technique yields the best ion signal.

Table 1. Light source and corona discharge needle status for APPI, APCI, and combined APCI/APPI modes

Ionization Technique	PhotoMate Light Source	Corona Discharge Needle
APPI	On	Off
APCI	Off	On
Combined APCI and APPI	On	On

What Types of Buffers Should I Use? What Types Should I Avoid?

Use volatile buffers, when possible, to obtain the highest performance for your assays. Many volatile buffer solutions are available that can be used instead of nonvolatile ones. Volatile buffer solutions can include the following:

- Acetic acid
- Ammonium acetate
- Ammonium formate
- Ammonium hydroxide
- Triethylamine (TEA)

Many LC applications use nonvolatile buffers such as phosphate and borate buffers. However, it's best to avoid the use of these nonvolatile buffers with the mass spectrometer. Nonvolatile buffers can block the capillary in the probe and can cause salt buildup in the nozzle, compromising the integrity of the spray.

How Should I Set Up the Mass Spectrometer for Various LC Flow Rates?

The APCI/APPI combination probe can generate ions from liquid flows[1] of 50 µL/min to 2.0 mL/min. This flow range allows you to use microbore LC, analytical LC, and semipreparative LC.

As you change the rate of flow of solvents entering the mass spectrometer, you need to adjust several parameters. For APCI and APPI, you need to adjust the ion transfer capillary temperature, the vaporizer temperature, and the gas flow rates for the sheath gas and auxiliary gas.

In general, an increase in the rate of liquid flowing into the mass spectrometer requires a higher temperature of the ion transfer capillary and vaporizer and a higher gas flow rate.

Table 2 provides starting guidelines for operating in the APPI, APCI, or combined APCI/APPI mode. **Chapter 4, 5 or 6** (depending upon your instrument) will help you to optimize these settings for your specific compound.

Table 2. Guidelines for setting operating parameters for APPI, APCI, and combined APCI/APPI modes *

LC Flow Rate (mL/min)	Capillary Temperature (°C)	Vaporizer Temperature (°C)	Sheath Gas (psi)	Auxiliary Gas (arbitrary units)
0.2 to 2.0	Typical setting: 150 to 225	Typical setting: 400 to 550	Required. Typical setting: 40 to 100	Not required, but usually helps to reduce solvent background ions Typical setting: 0 to 20

*For thermally labile compounds you might need to use a lower vaporizer temperature and a lower flow rate.

[1] Flows below 200 µL/min require more care to maintain a stable spray.

Chapter 2 Functional Description

This chapter describes the principal components of the APCI/APPI combination probe.

The APCI/APPI combination probe forms gas phase sample ions from sample molecules that are contained in solution. You can operate the APCI/APPI combination probe using either atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), or both.

The APCI/APPI combination probe includes:

- APCI Source
- PhotoMate Light Source

APCI Source

The APCI probe ionizes the sample by atmospheric pressure chemical ionization. The APCI probe accommodates liquid flows of 100 µL/min to 2 mL/min without splitting. See Figure 6. The APCI probe includes the APCI sample tube, nozzle, sheath gas and auxiliary gas plumbing, and vaporizer. See Figure 7 and Figure 8. Sample and solvent enter the APCI nozzle through the sample tube. The sample tube is a short section of 0.10 mm ID fused silica tubing that extends from the sample inlet to 1 mm past the end of the nozzle. The manifold houses the APCI nozzle and includes the sheath gas and auxiliary gas plumbing. The APCI nozzle sprays the sample solution into a fine mist. The sheath gas and auxiliary gas plumbing deliver dry nitrogen gas to the nozzle. The droplets in the mist then enter the vaporizer. The vaporizer flash vaporizes the droplets at temperatures up to 500 °C.

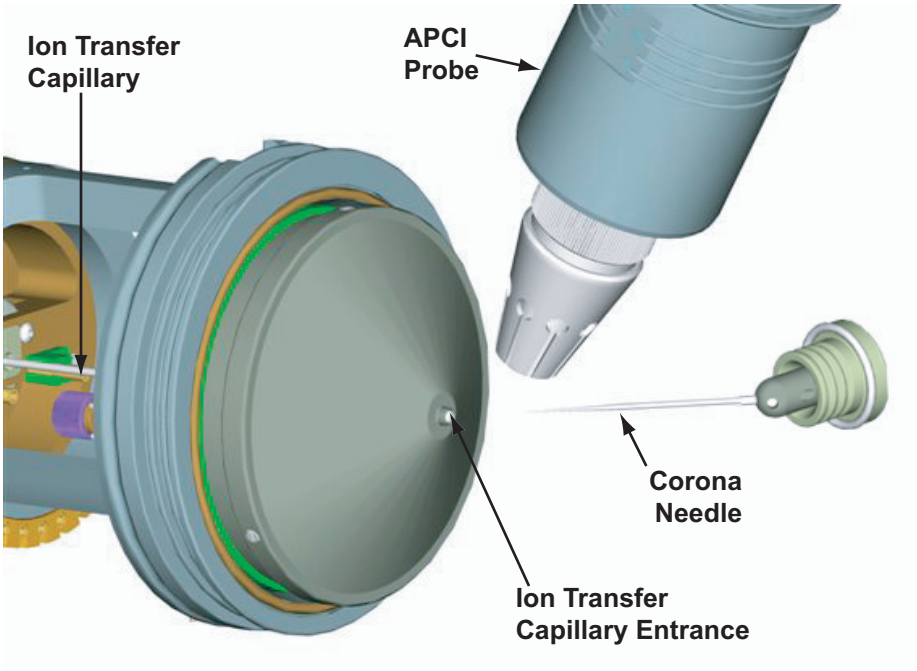


Figure 6. APCI probe, showing the corona discharge needle and ion transfer capillary

Typical vaporizer temperatures are 350 to 450 °C for flow rates of 0.1 to 2 mL/min. The sample vapor is swept toward the corona discharge needle by the flow of the sheath and auxiliary gasses.

The corona discharge needle assembly is mounted inside of the Ion Max API source housing. The tip of the corona discharge needle is positioned near the vaporizer. A high potential (typically ±3 to ±5 kV) is applied to the

corona discharge needle to produce a corona discharge current of up to 100 µA. (A typical value of the corona discharge current is 5 µA.) The corona discharge from the needle produces reagent ion plasma primarily from the solvent vapor. The sample vapor is ionized by ion-molecule reactions with the reagent ions in the plasma.

APCI requires a constant source of electrons for the ionization process. Thus, the corona discharge current is set to a specific value and regulated. The potential applied to the corona discharge needle varies, as needed, to provide the required current.

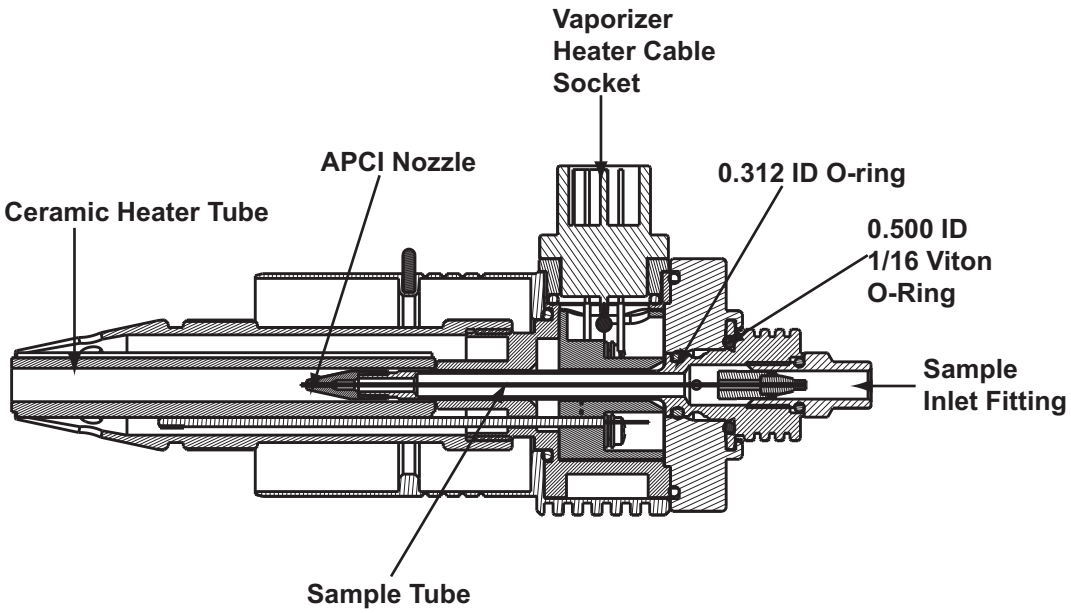


Figure 7. Cross sectional viewof the APCI probe

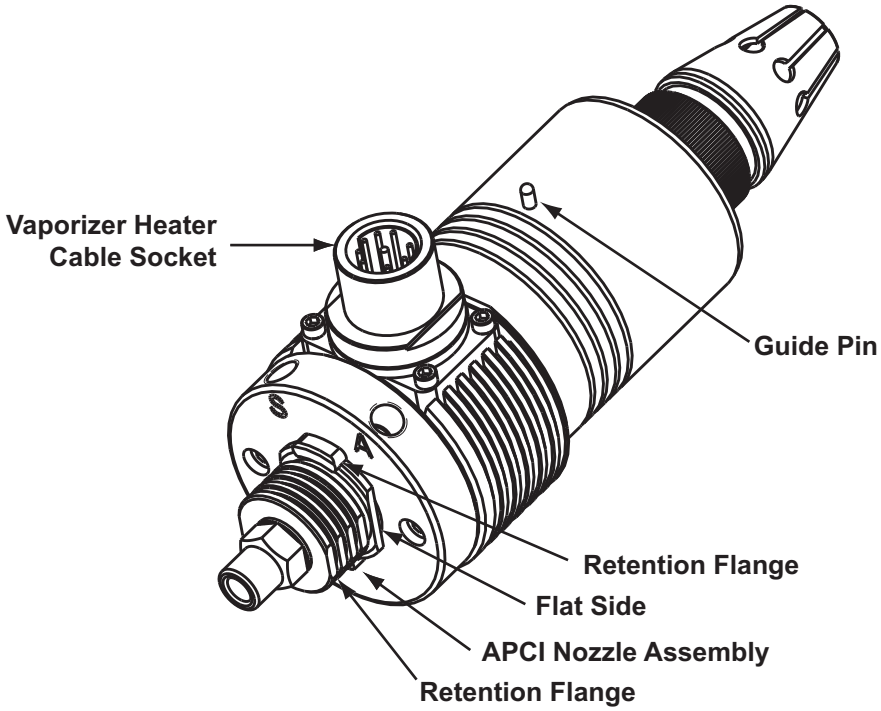


Figure 8. APCI probe exterior

PhotoMate Light Source

The light source is a Syagen PhotoMate vacuum ultraviolet (VUV) light source. The assembly is mounted on the left side of the Ion Max ion source housing using a specially designed adapter ring.

The PhotoMate light source includes the following components. See Figure 9 through Figure 11.

- Power supply
- Electronics PCB
- VUV lamp
- Lamp shield
- Box fan
- Lamp On/Off switch
- Safety interlocks
- Remote LEDs
- Window

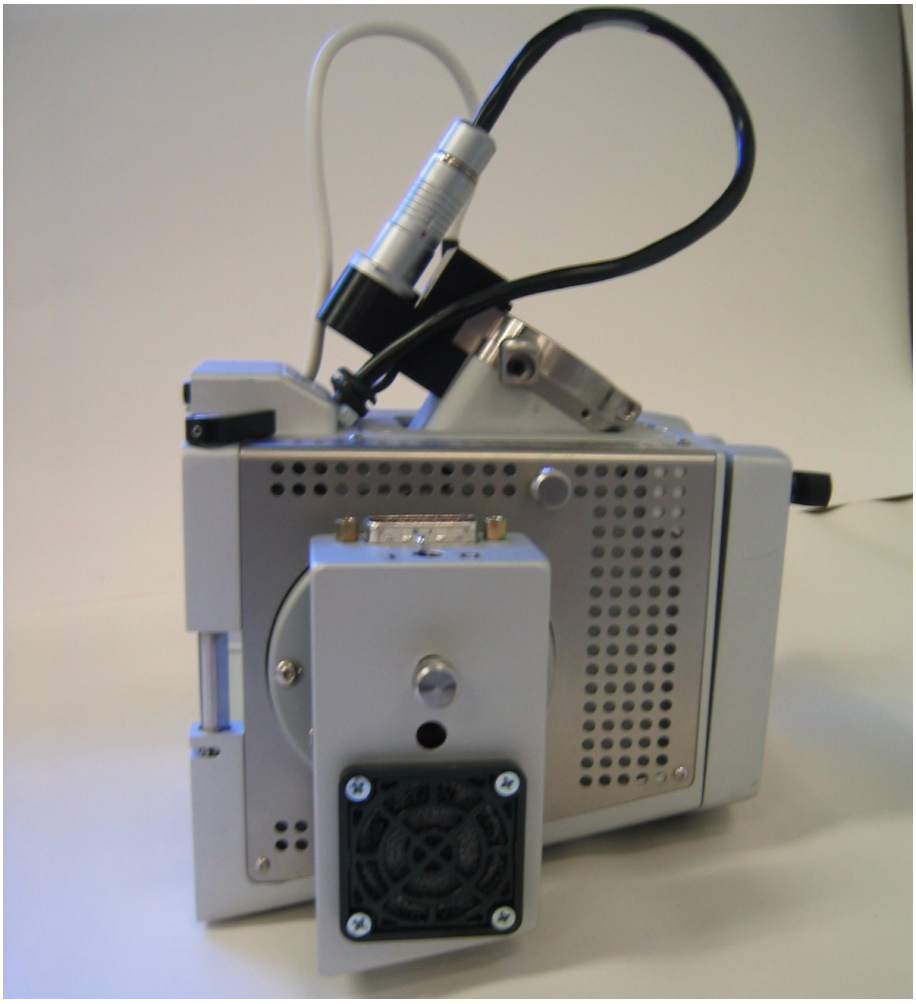


Figure 9. PhotoMate light source installed in the Ion Max ion source housing, side view

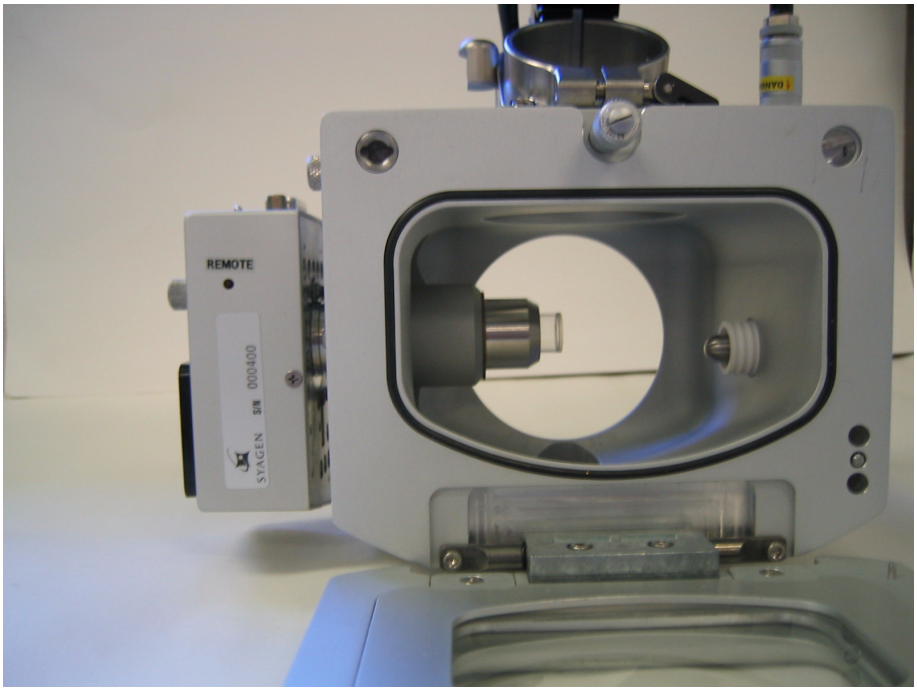


Figure 10. PhotoMate light source installed in the Ion Max ion source housing, front view

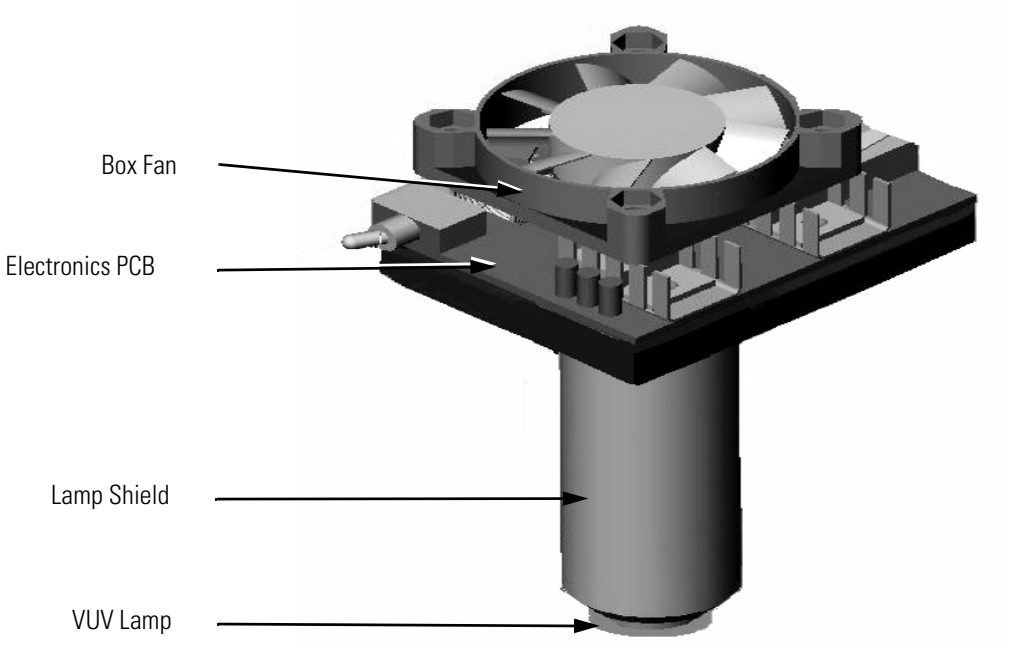


Figure 11. PhotoMate light source (internal components)

The power supply provides +48 V dc power for the electronics PCB and fan. The LTQ MS detector and TSQ Quantum Ultra mass spectrometer can both supply this power through a DB-15 cable connection. The LTQ MS detector is also able to control the lamp status through the connection. The LCQ Deca XP MAX requires that this power be supplied via an external power supply, which is also connected using the DB-15 connection.

The electronics PCB contains electronic circuitry for driving the VUV lamp, a photodiode for detecting light emitted by the VUV lamp, and a thermocouple for monitoring the temperature. The electronics PCB also powers the status LEDs.

The VUV lamp is an electrodeless, quartz cell with a magnesium fluoride window. (The window is transparent to VUV light, but the quartz is not.) The VUV lamp is filled with krypton gas. The lamp emits radiation at 10.0 eV and 10.6 eV.

Minimize the chance of breaking the VUV lamp by doing the following:

- Always wear clean gloves when you handle the VUV lamp. Fingerprints on the lamp can cause the lamp to fail when it is in use.
- Do not spray solvent on the VUV lamp while it is hot. The VUV lamp can crack.
- Do not leave the LC or other liquid delivery device on while the mass spectrometer is in Standby. The absence of sheath and auxiliary gas can cause the hot lamp to break upon contact with liquids.

The lamp shield protects the VUV lamp.

The box fan cools the electronics PCB and prevents heat from the vaporizer from damaging the electronics PCB. The fan draws cool air into the light source. The fan runs at all times – even if the lamp is off.

CAUTION Never disconnect the power cord from the PhotoMate light source or disconnect the PhotoMate power supply from line power while the vaporizer is hot. Without power, the fan will stop running and heat from the vaporizer could damage the electronics PCB.

The lamp On/Off switch turns on and off the power to the circuitry that powers the VUV lamp. The box fan remains on at all times and is not controlled by the lamp On/Off switch.

The safety interlock switch prevents the light source from operating when not attached to the Ion Max ion source housing.

The remote status light-emitting diode (LED) is located on the front of the light source. The LED is illuminated green if the APPI source is connected to an instrument that has remote control of the lamp status (the LTQ MS detector). When the Remote LED is illuminated, the switch on the light source is inactive and will not turn the lamp on and off. When the lamp is not illuminated, the instrument to which it is connected has no control of the lamp status and the lamp must be turned on and off with the switch on the source (TSQ Quantum Ultra mass spectrometer and LCQ Deca XP MAX MS detector).

The front window in the Ion Max ion source housing allows you to verify that the light source is working properly. You can see purple light if the light source is working properly. If the light is red (due to exposure to ambient nitrogen) then the lamp is broken.

Chapter 3 Setting Up the Ion Source for Acquiring Data in APCI/APPI/MS/MS Mode

This chapter provides information on setting up the ion source for acquiring data in the APCI/MS/MS mode.

This chapter contains the following sections:

Removing the ESI Probe

To remove the ESI source

1. Place the LC/MS system in Standby:

   a. Stop the flow of solvent to the ESI source.

Note The following procedures are intended for use with all systems. If you have questions regarding the operation of your MS system, please refer to the appropriate system-specific manuals. This might include the Getting Started and Hardware manuals for your system. For information on installation and maintenance of other Ion Max probes, please refer to the Ion Max API Source Hardware Manual.

Note The following procedures are intended for use with all systems. If you have questions regarding the operation of your MS system, please refer to the appropriate system-specific manuals. This might include the Getting Started and Hardware manuals for your system. For information on installation and maintenance of other Ion Max probes, please refer to the Ion Max API Source Hardware Manual.

   b. Turn off the ESI spray voltage.

   c. Click on the On/Standby button on the toolbar to place the mass spectrometer in Standby.

On Standby

Note If your ESI probe does not already have a sample tube (fused-silica capillary) and safety sleeve attached, you need to follow the procedure for installing a sample tube and PEEK safety sleeve that is outlined in the topic Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve in the **Ion Max API Source Hardware Manual**.

2. Disconnect the sample transfer tubing from the stainless steel ZDV fitting (grounding union). See Figure 12.

3. Remove the 8 kV cable from the ESI needle high voltage receptacle as follows: (See Figure 12.)

4. Unlock the cable by twisting the locking ring counter-clockwise.

5. Unplug the 8 kV cable from the ESI needle high voltage receptacle.

6. Disconnect the AUX Gas fitting (green) from the auxiliary gas inlet (A) on the probe manifold. (Figure 12)

7. Disconnect the Sheath Gas fitting (blue) from the sheath gas inlet (S) on the probe manifold.

8. Remove the stainless steel ZDV fitting (Grounding Union) from the grounding bar on the ion source housing.

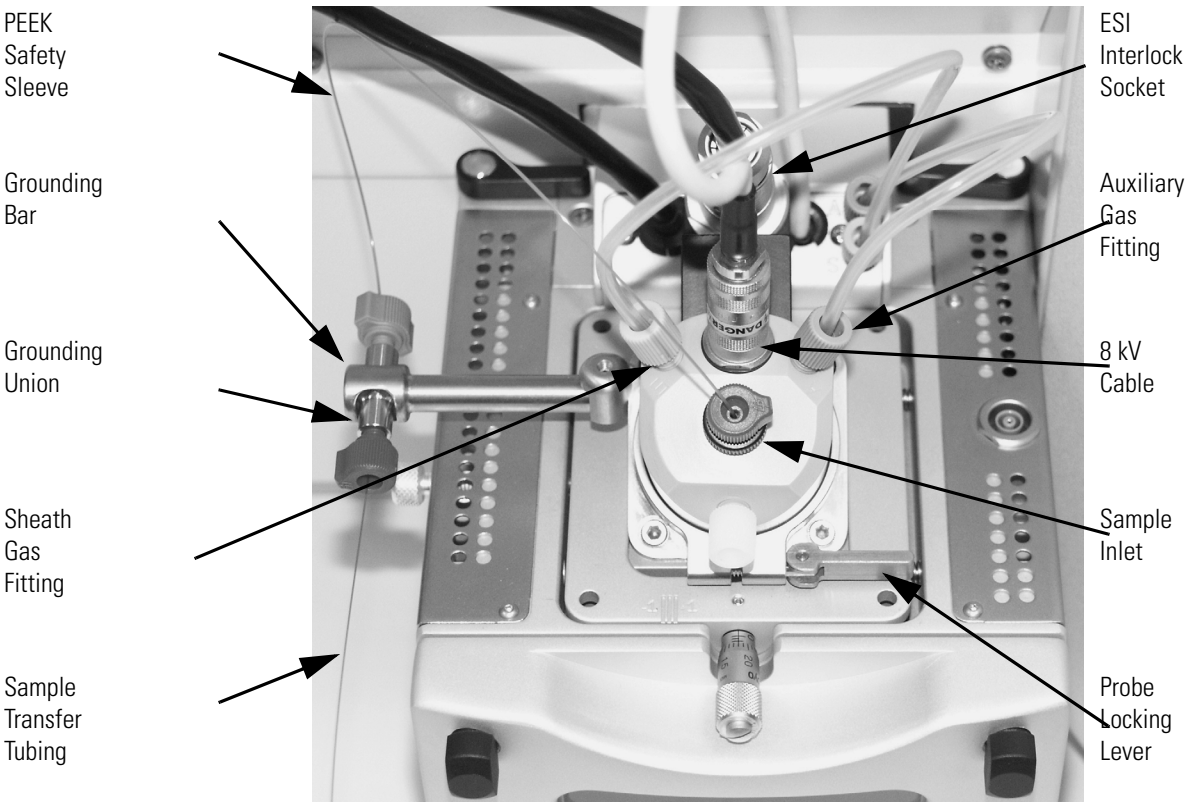


Figure 12. Ion Max ion source housing with ESI probe installed

9. Unlock the probe locking lever by twisting the lever open to its widest position.

10. Carefully pull the probe straight back in the port in the housing until it meets with the slot in the ESI interlock block. The guide pin on the probe manifold will prevent you from twisting the probe until the pin is aligned with the slot in the ESI interlock block.

11. After the probe is all the way back and aligned with the slot, turn the probe 45 degrees counter-clockwise to free the probe from the alignment notch. Be careful not to break the fused-silica sample tube or PEEK safety sleeve.

12. Pull the probe straight out to remove it from the ion source housing.

13. Store the ESI probe in its original shipping container.

Removing the Ion Max Ion Source Housing

Remove the Ion Max ion source housing to access the ion sweep cone.

Note Disconnect any external liquid lines connected to the ion source housing before removing the ion source housing.

To remove the ion source housing

1. Remove the drain tube from the ion source housing drain. See Figure 13.
2. Rotate the ion source housing locking levers 90 degrees to release the ion source housing from the ion source mount assembly.
3. Remove the ion source housing by it pulling straight off the ion source mount assembly.
4. Place the housing in a safe location for temporary storage.

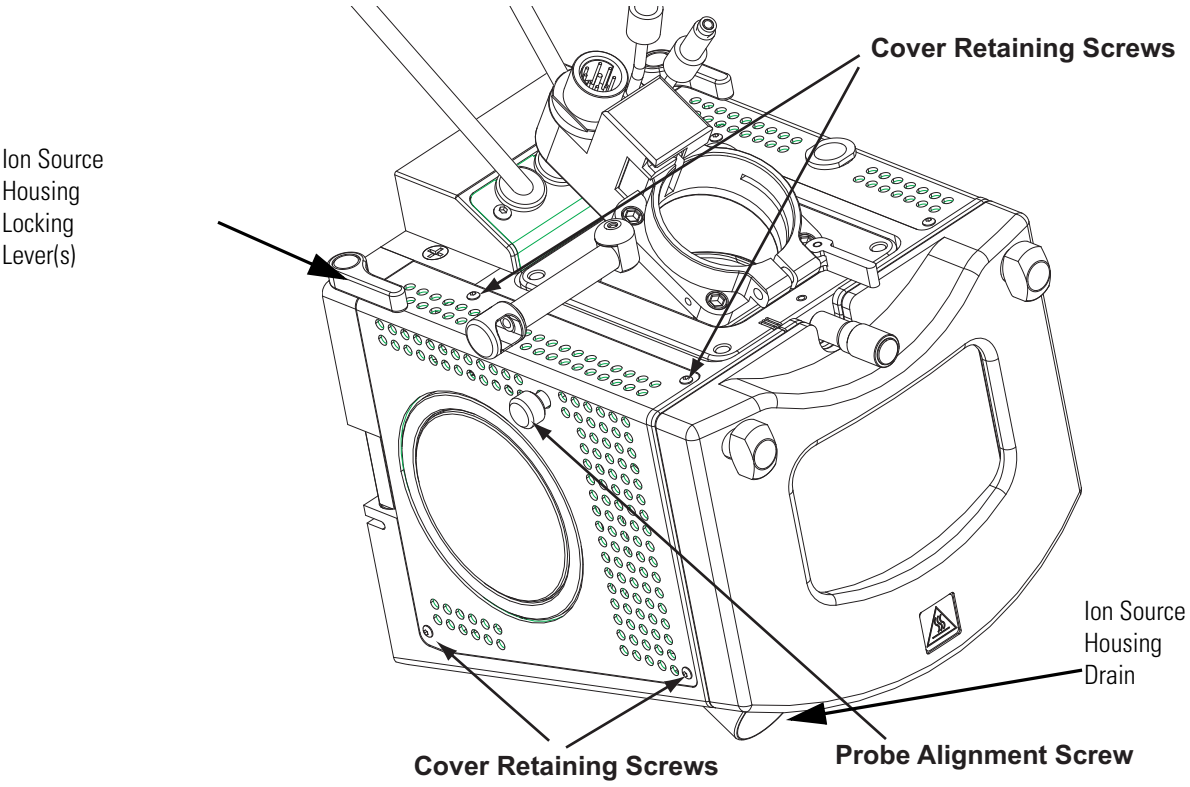


Figure 13. Ion Max ion source, showing details of components

Installing the APCI Probe

To install the APCI probe

1. Install the corona needle

CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin. Handle it with care.

   a. Unlock the ion source housing door by turning the locks 90 degrees so that the knobs are horizontal.

   b. Open the ion source housing door.

   c. Using pliers, grasp the needle by the gold plated contact and push the needle straight into the socket. See Figure 14.

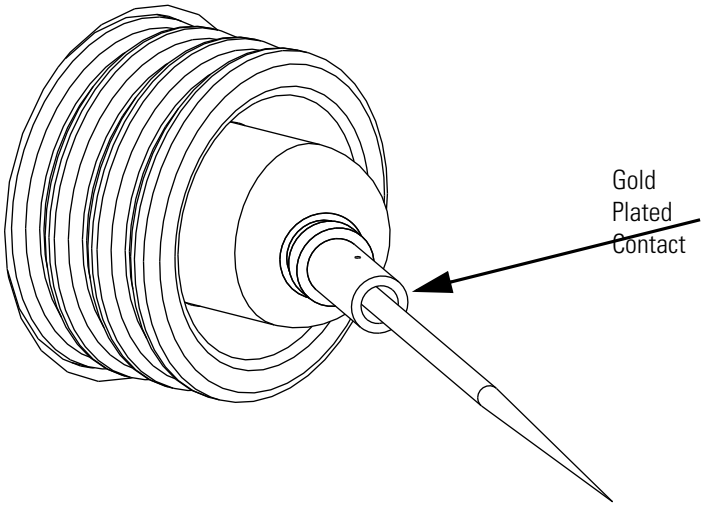


Figure 14. Corona needle, view from rear

   d. Make sure that the tip of the needle is aligned with the path of travel between the APCI probe and the ion source interface on the instrument.

   e. Close and lock the ion source housing door.

2. Connect the 8 kV cable to the corona needle high voltage receptacle:

   a. Plug the 8 kV cable into the corona needle high voltage receptacle on the right side of the top of the ion source housing.

   b. Lock the cable by twisting the locking ring clockwise.

3. Be sure to unlock the probe locking lever (widest open position) before attempting to install the probe.

4. Insert the APCI probe into the port in the ion source housing Align the guide pin on the probe body at a 45 degree angle from the ESI interlock block. See Figure 15.

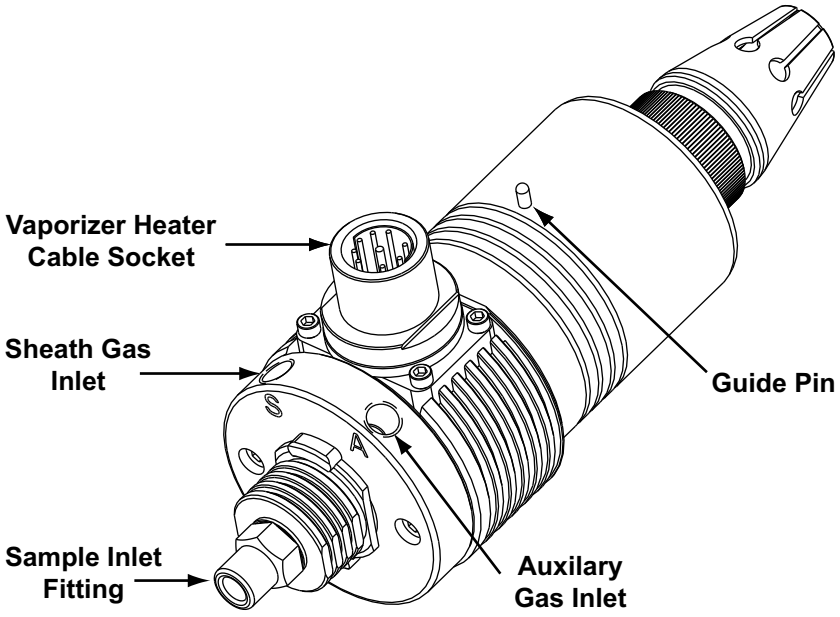


Figure 15. APCI probe

5. Push the probe into the port until the guide pin meets with the locking ring on the housing.

6. Turn the probe 45 degrees clockwise and align the guide pin with the slot in the ESI interlock block (you might need to pull the probe towards you slightly to properly align the pin with the notch).

7. After you have turned the probe far enough to align the pin with the alignment notch at the rear of the port, push the probe straight in until the guide pin stops at the bottom of the alignment notch.

8. Seat the probe all the way down into the alignment notch.

9. Lock the probe in place by twisting the probe locking lever towards the front of the housing. (Closing the probe locking lever towards the rear of the ion source housing may make it difficult to unlock.)

10. Unplug the vaporizer heater cable from the ESI interlock plug on the ion source housing.

11. Connect the vaporizer heater cable to the vaporizer heater cable socket on the APCI probe.

12. Connect the Auxiliary gas line (green colored fitting) to the inlet on the APCI probe marked *A*.

13. Connect the Sheath gas line (blue colored fitting) to the inlet on the APCI probe marked *S*.

14. Connect the sample transfer line to the APCI probe inlet.

CAUTION Prevent solvent waste from backing up into the ion source and mass spectrometer. Always ensure that liquid in the drain tube is able to drain to a waste container and that the outlet of the drain tube is above the level of liquid in the waste container.

The APCI source is now properly installed in the Ion Max ion source housing.

Installing the PhotoMate Light Source

If the APPI assembly is already installed

- If you have an LCQ Deca XP MAX MS Detector, follow the operating procedures in Chapter 4, "Optimizing the LCQ Deca XP MAX MS Detector with Your Analyte." .
- If you have an LTQ MS Detector, follow the operating procedures in Chapter 5, "Optimizing the LTQ MS Detector with Your Analyte." .
- If you have an TSQ Quantum mass spectrometer, follow the operating procedures in Chapter 6, "Optimizing the TSQ Quantum Ultra Mass Spectrometer with Your Analyte." .

If the APPI assembly is not installed

1. Remove the screws holding the window retainer ring

CAUTION If you have operated your APCI probe recently, be sure to allow it to cool completely before installing the PhotoMate light source. The APCI probe and corona needle may be hot

The corona needle is sharp. If you are installing the APPI assembly with the APCI probe installed, be careful not to poke yourself with the needle.

2. Carefully remove the retainer ring, while keeping your free hand under the side of the Ion Max ion source housing to be sure that the window does not fall out and break. If the window does not come out with the retainer ring, you might need to push gently on the window from inside the Ion Max ion source housing (Figure 16).

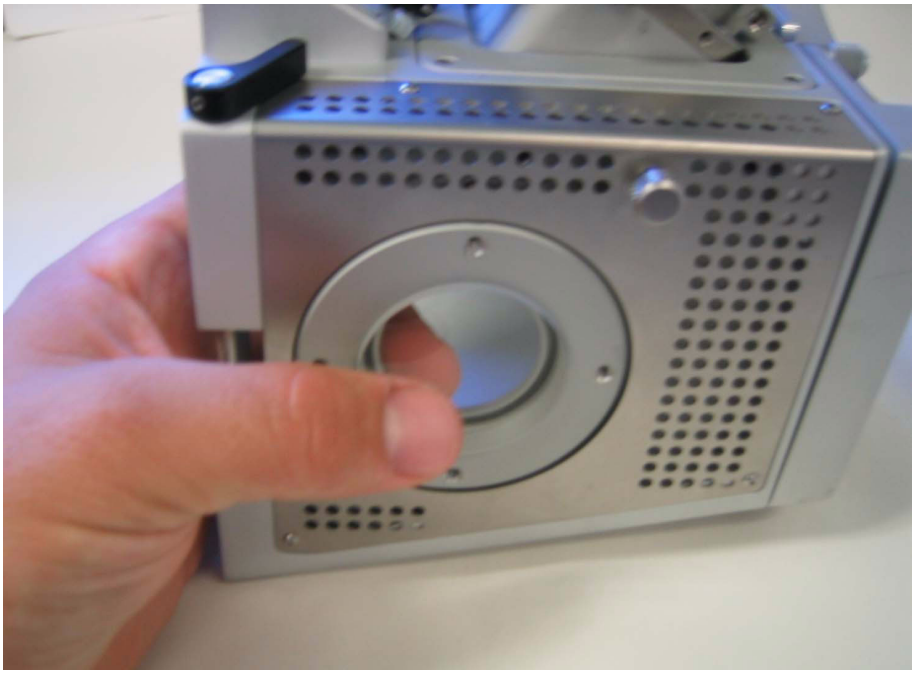


Figure 16. Removing the small viewport window

3. Place the window, retainer ring and screws in a secure place.

Note While inserting the PhotoMate light source into the Ion Max ion source housing, be sure to watch that the APPI lamp does not contact the corona discharge needle or the ceramic heater tube on the APCI probe.

4. Carefully align the PhotoMate light source so that the locking tab on the bottom of the APPI assembly aligns with the notch in the APPI adapter ring (Figure 17).

5. Secure the PhotoMate light source with two of the hex head screws.

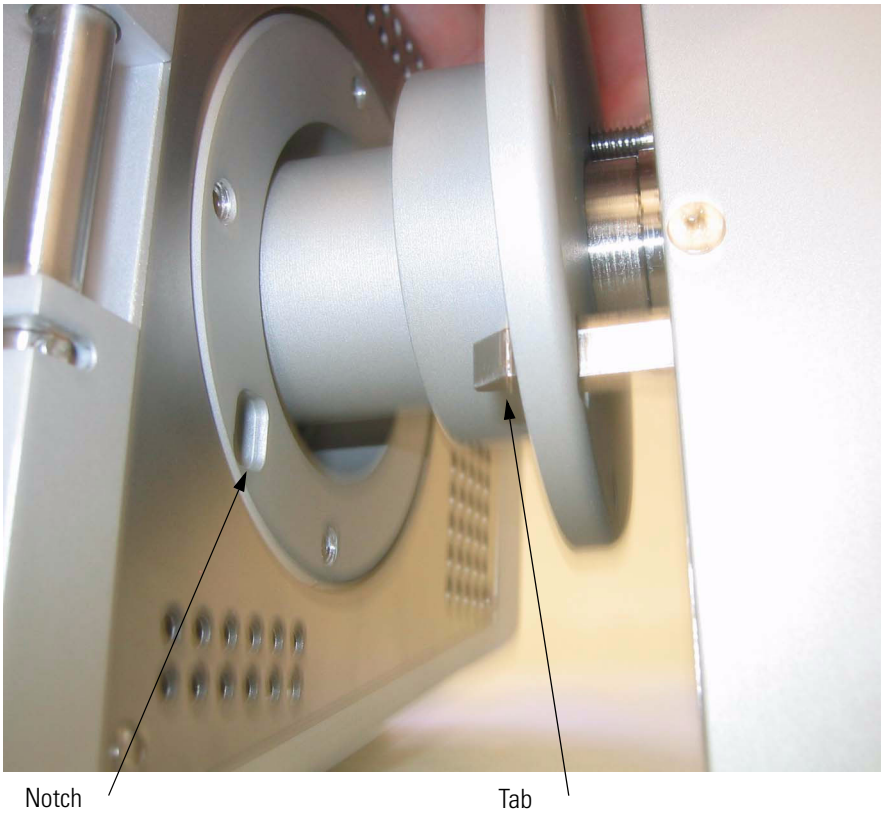


Figure 17. Aligning the tab on the PhotoMate light source with the adapter ring

Installing the Ion Max Ion Source Housing

To reinstall the Ion Max ion source housing

1. Carefully align the two guide pin holes on the rear of the ion source housing with the ion source housing guide pins on the mass spectrometer, and carefully press the ion source housing onto the ion source mount. See Figure 18 and Figure 19.

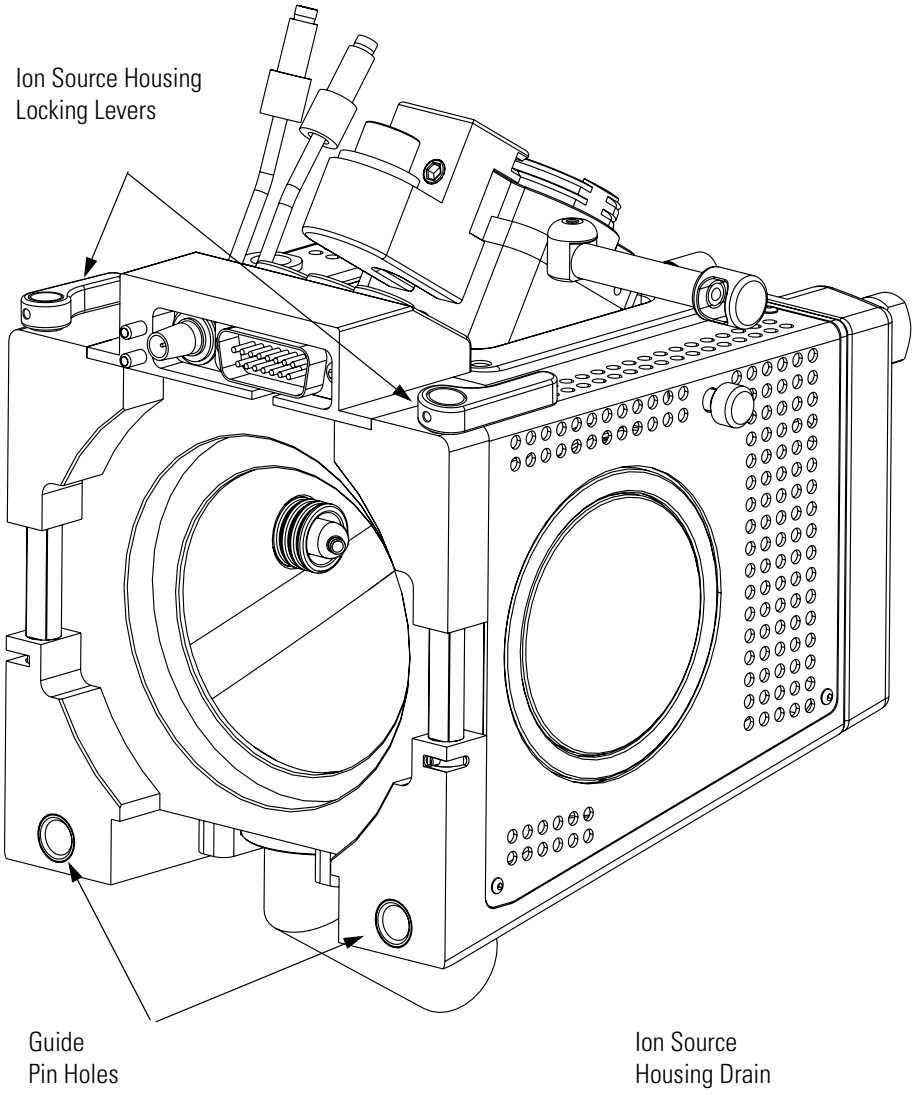


Figure 18. Rear view of the Ion Max ion source housing (PhotoMate light source not shown)

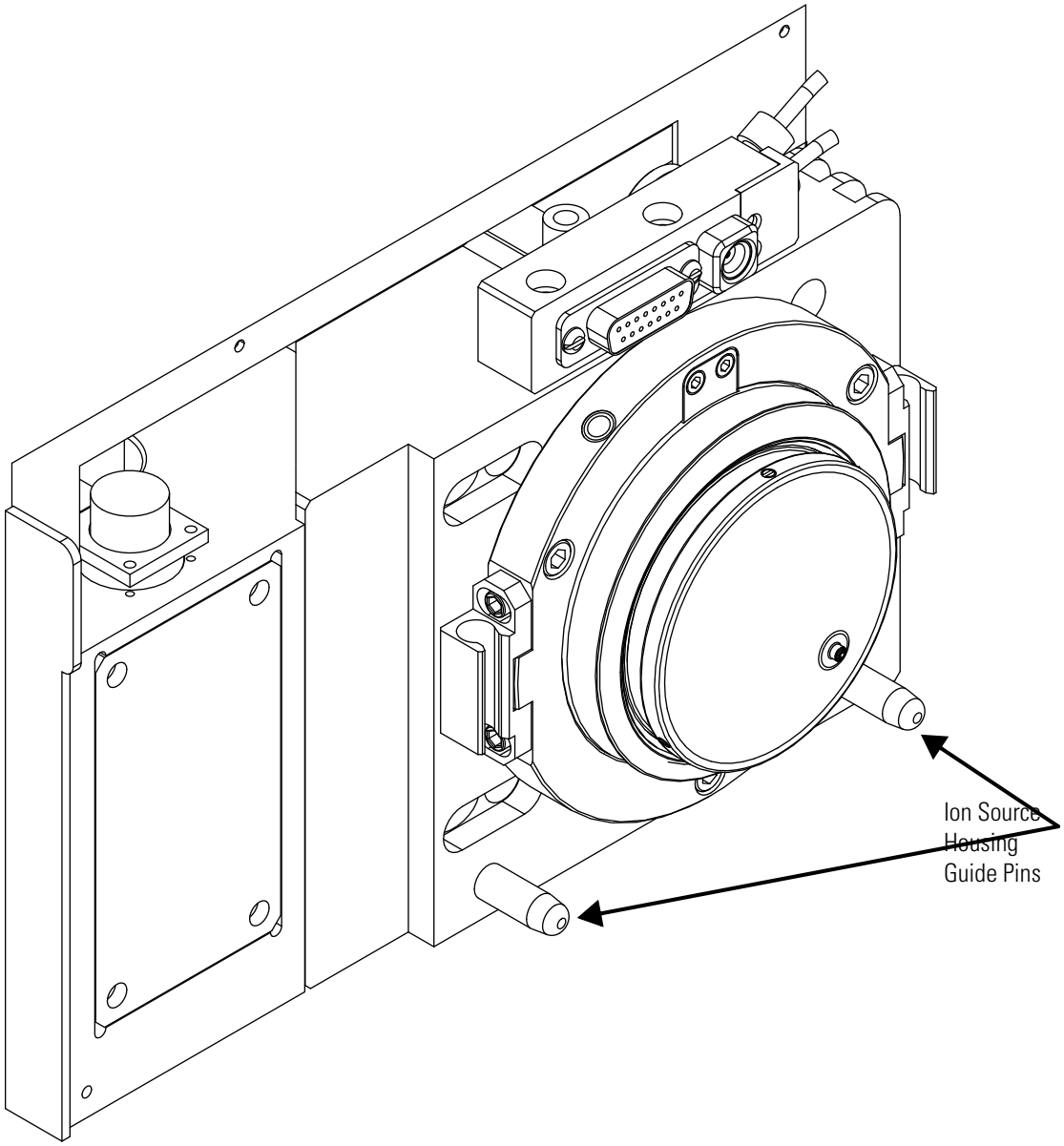


Figure 19. Ion source mount showing ion source housing guide pins

2. Rotate the ion source housing locking levers 90 degrees to lock the ion source housing onto the ion source mount assembly.

CAUTION Prevent solvent waste from backing up into the ion source and mass spectrometer. Always ensure that liquid in the drain tube is able to drain to a waste container and that the outlet of the drain tube is above the level of liquid in the waste container.

CAUTION Do not vent the API source drain tube (or any vent tubing connected to the waste container) to the same fume exhaust system to which you have connected the forepumps. The analyzer optics can become contaminated if the API source drain tube and the (blue) forepump exhaust tubing are connected to the same fume exhaust system.

Your laboratory must be equipped with at least two fume exhaust systems. Route the (blue) forepump exhaust tubing to a dedicated fume exhaust system. Route the drain tube from the API source to a waste container. Vent the waste container to a dedicated fume exhaust system.

3. Reinstall the ion source drain tube as follows:

   a. Connect the 1-in. ID Tygon tubing (P/N 00301-22922) to the ion source housing drain fitting.

   b. Attach the free end of the hose to a waste container. Ideally, the waste container should be vented to a fume exhaust system.

4. Complete the installation:

   - For LCQ Deca XP MAX MS detectors:
     Connect the external power supply of the PhotoMate light source to the DB-15 connector on the PhotoMate light source.

   - For LTQ MS detectors:
     Connect the female end of the communications cable to the DB-15 connector on the PhotoMate light source. Connect the other end of the cable to the female connector on the front of the LTQ MS detector, just to the right of where the Ion Max ion source housing mounts to the detector.

   - For TSQ Quantum Ultra mass spectrometers:
     Connect the female end of the communications cable to the DB-15 connector on the PhotoMate light source. Connect the other end of the cable to the female connector on the front of the mass spectrometer, just to the rigIon Max ion sourceIon Max ion sourceht of where the Ion Max ion source housing mounts to the detector. You also have the option to connect the DB-15 connector to the external power supply that is included with your APPI source instead of using the TSQ Quantum Ultra's power connector.

The APCI/APPI source is now properly installed on the mass spectrometer.

To optimize the tune of your mass spectrometer proceed to one of the following chapters:

- If you have an LCQ Deca XP MAX MS detector, refer toChapter 4, “Optimizing the LCQ Deca XP MAX MS Detector with Your Analyte.”
- If you have an LTQ MS detector, refer to Chapter 5, “Optimizing the LTQ MS Detector with Your Analyte.” .
- If you have an TSQ Quantum Ultra mass spectrometer, refer to Chapter 6, “Optimizing the TSQ Quantum Ultra Mass Spectrometer with Your Analyte.” .

Chapter 4 Optimizing the LCQ Deca XP MAX MS Detector with Your Analyte

This chapter provides information on optimizing the tune of your LCQ Deca XP MAX MS detector in the APCI/APPI/MS high flow mode. It is not necessary to recalibrate the MS detector when you switch to APCI/APPI/MS operation. You can use the calibration settings you obtained from the successful automatic calibration procedure you performed in the ESI/MS mode.

For APCI/APPI/MS operation you open a default tune method located in your *C:\Xcalibur\methods* folder, in this case *APCIhighflow.LCQTune*. From this starting point, you optimize automatically the tube lens offset voltage, capillary voltage, and ion transfer capillary temperature for your particular analyte.

Note The following procedures assume that you are familiar with your XP Plus instrument and the Tune Plus window. If you need information, refer to the LCQ Deca XP MAX online help, LCQ Deca XP MAX Getting Connected, or the LCQ Deca XP MAX Hardware Manual.

Ensure that you have completed the procedures in the sections that describe how to calibrate your instrument in ESI mode, and have properly set up your APCI/APPI source.

This chapter includes the following sections:

Setting Up the Inlet for Tuning Using High-Flow Infusion

The plumbing connections for the APCI/APPI/MS sample introduction from the syringe pump are shown in Figure 20.

To make the plumbing connections for APCI/APPI/MS sample introduction from the syringe pump into solvent flow from an LC

1. Connect a 4 cm (1.5 in) segment of Teflon tubing with a (brown) Fingertight fitting and a (brown) ferrule to the (black) LC union. (See Figure 21.)

Load a clean, 500-µL Unimetrics syringe (P/N 00301-19012) with 420 µL of a 1 ng/µL solution of reserpine or your analyte of interest. (Refer to Appendix B: **Reserpine Solution Formulations** for a procedure for making the reserpine tuning solution.)

2. Insert the needle of a syringe into the segment of Teflon tubing, and place the syringe in the syringe holder of the syringe pump.

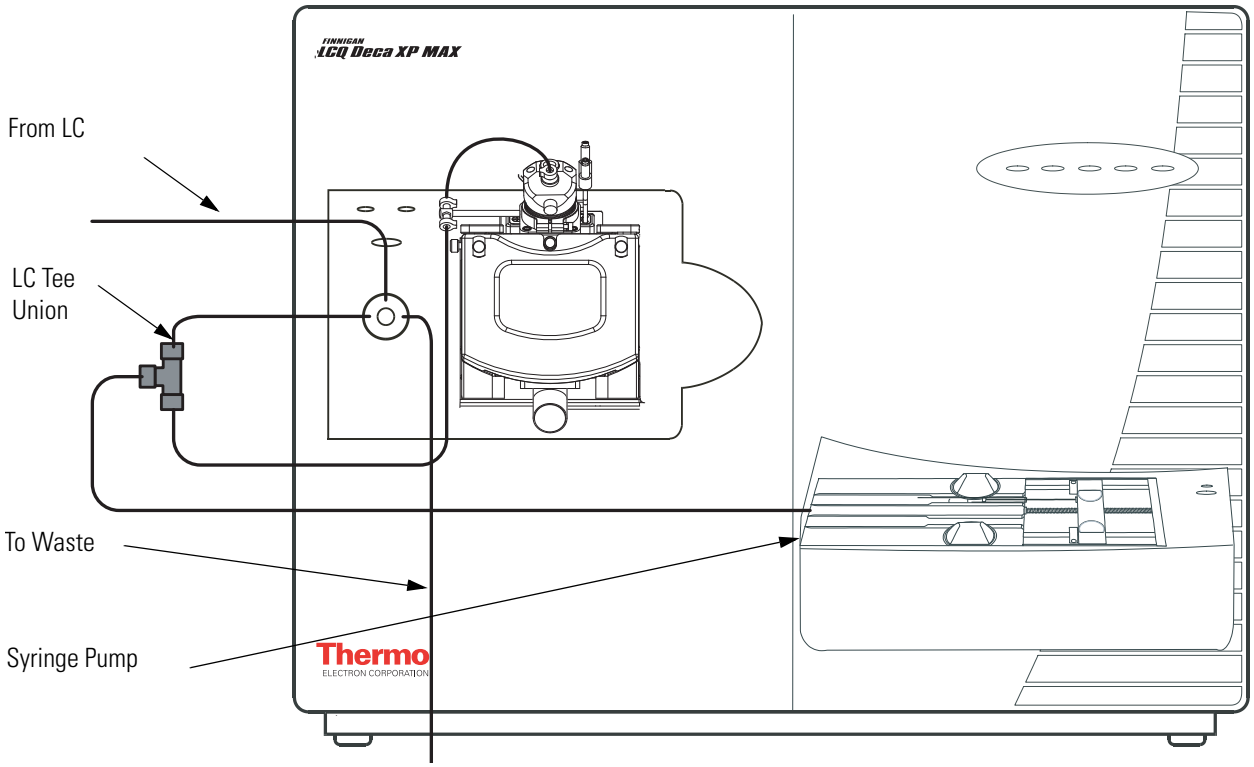


Figure 20. APCI/APPI/MS plumbing connections for sample introduction from the syringe pump into solvent flow from an LC

3. Connect a fused-silica infusion line from the (black) LC union to the (black) LC Tee union:

a. Connect the infusion line (P/N 00106-10504) with a (brown) Fingertight fitting and a (brown) ferrule to the free end of the LC union (Figure 20).

b. Connect the other end of the infusion line with a (red) Fingertight fitting and a (brown) ferrule to the side arm of the LC Tee union.

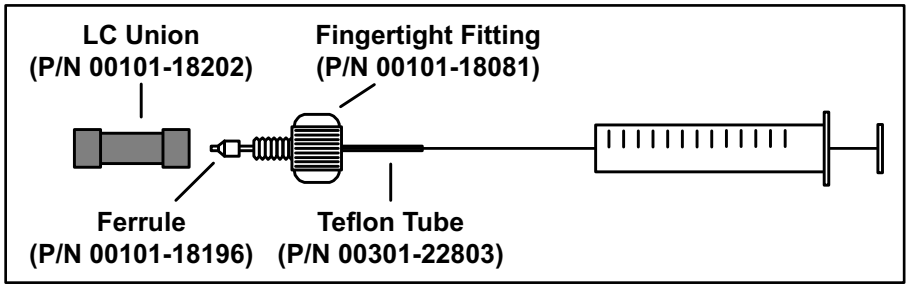


Figure 21. APCI/APPI/MS plumbing connections for the syringe pump

Note To cut the PEEK tubing used to connect your LC to the divert/inject valve and the divert/inject valve to the APCI source, use a PEEK tubing cutter. This ensures that the tubing is cut straight. In addition, make sure your LC fittings, ferrules, and PEEK tubing are installed properly. By using these precautions, you prevent void (dead) volumes. The exclusion of void volumes is critical to microbore LC. Also, void volumes affect the quality of the MS detector signal.

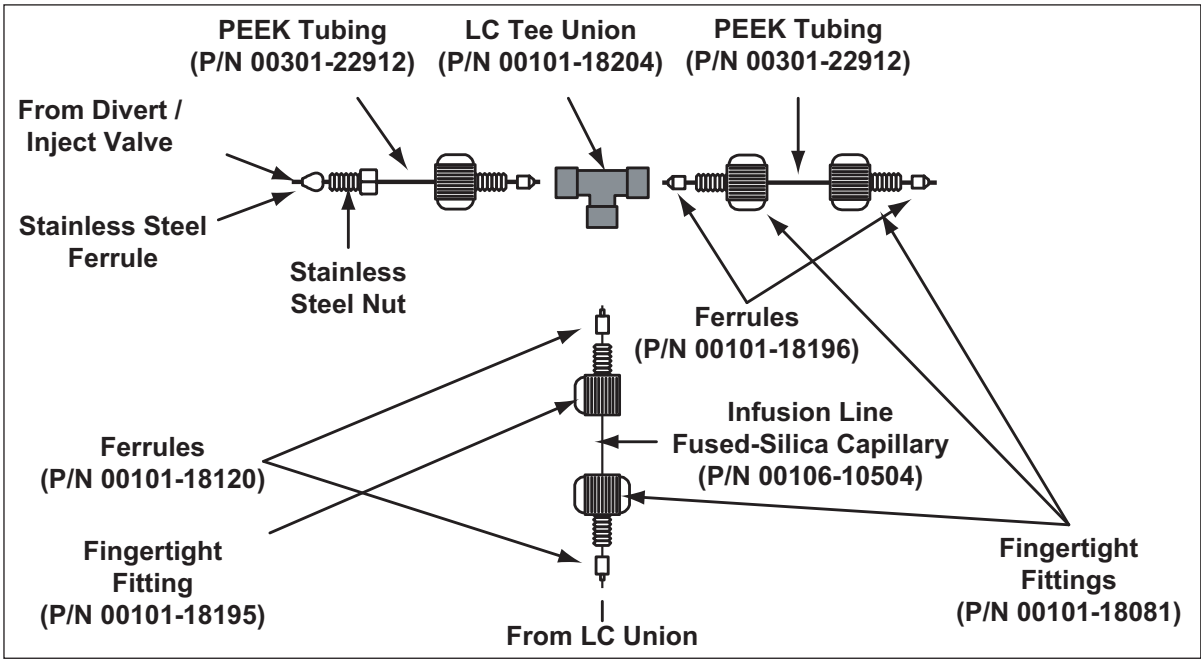


Figure 22. APCI/APPI/MS plumbing connections for the LC Tee union

4. Connect a segment of PEEK tubing from the (black) LC Tee union to the APCI LC inlet (Figure 22).

   a. Use a PEEK tubing cutter to cut a 4 cm (1.5 in.) length of the PEEK tubing.

   b. Connect the PEEK tubing with a (brown) Fingertight fitting and a (brown) ferrule to a free end of the (black) LC Tee union.

   c. Connect the other end of the PEEK tubing with a (red) Fingertight fitting and a (brown) ferrule to the LC inlet located on the APCI probe.

5. Connect an appropriate length of PEEK tubing (transfer line from the divert/inject valve) from the divert/inject valve to the LC Tee union: (Figure 22).

   a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 3 of the divert/inject valve.

   b. Connect the other end of the PEEK tubing with a (brown) Fingertight fitting and a (brown) ferrule to the free end of the LC Tee union.

6. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC:

   a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 2 of the divert/inject valve.

   b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.

7. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container:

   a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 1 of the divert/inject valve.

   b. Insert the other end of the PEEK tubing into a suitable waste container.

CAUTION Prevent solvent waste from backing up into the API ion source and MS detector. Always ensure that the PVC drain hose is above the level of liquid in the waste container.

8. Connect an appropriate length of 1-in. (2.5 cm) OD Tygon tubing hose to the Ion Max ion source housing drain fitting. Attach the other end of

the hose to a suitable waste container. Ideally, the waste container should be vented to a fume exhaust system.

Setting Up the MS Detector for APCI/APPI/MS Operation


On


Standby

To set up the MS detector for APCI/APPI/MS operation on the XP Plus

1. In Tune Plus, click on the On/Standby button to take the MS detector out of Standby mode and turn it on. The MS detector begins scanning, and applies the high voltage to the corona needle, and shows a real-time display in the Spectrum view.

2. Open the *APCITune.LCQTune* Tune Method, the Tune Method for high-flow APCI operation:

   a. Choose **File > Open** to display the Open dialog box.

   b. Scroll down until you see the folder *C:\Xcalibur\methods*. Then, select the file *APCITune.LCQTune*.

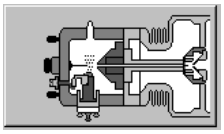
   c. Click on **OK** to open the file. XP Plus downloads the Tune Method parameters to the MS detector.

3. Verify that XP Plus opened the Tune Method, as follows:

   a. Choose **Setup > Change API Source Type** to specify the APCI source. Then, in the Change Source Type dialog box, which appears, select the APCI option button.

   b. Click on **OK** to return to the Tune Plus window.

   c. On the Instrument Setup toolbar, click on the API Source button to open the APCI Source dialog box (Figure 23).



   d. Verify that the settings in your dialog box are similar to those shown in Figure 23.

   - To operate in APPI mode only, set the discharge current to zero.
   - To operate in APCI/APPI or APCI mode only set the discharge current to 5 µA.

   e. Click on **OK** to close the dialog box.

4. If you are operating in APPI or APCI/APPI mode, turn the APPI lamp on. Verify that the lamp is operating by observing the lamp through the front window of the Ion Max ion source housing.

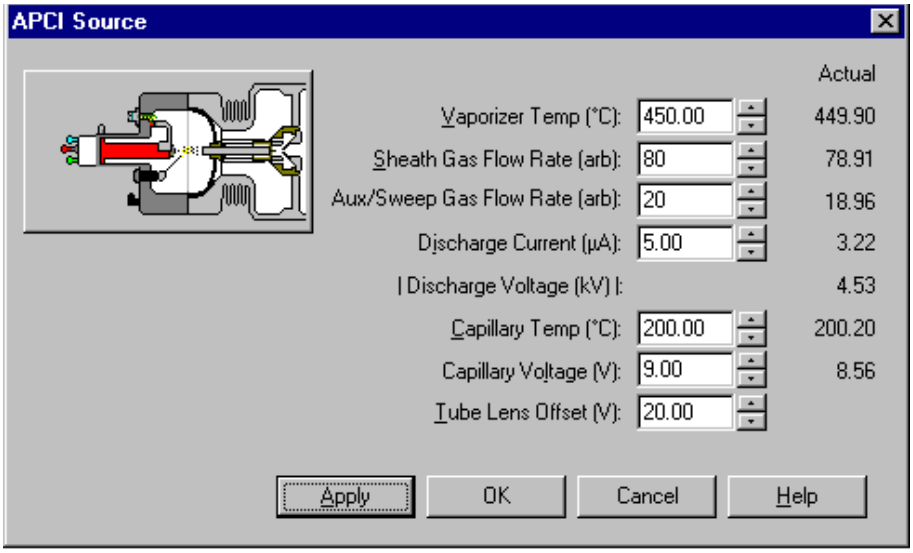


Figure 23. APCI Source dialog box, showing the proper settings for a typical high flow experiment

5. Define the scan parameters for tuning the MS detector in the APCI/APPI/MS mode:

   a. On the Control/Scan Mode toolbar, click on the Define Scan button to open the dialog box (Figure 24).

   b. In the Scan Description group box, select the Mass Range: Normal option button to specify a mass range of *m/z* 50 to 2000.

   c. Select the Scan Mode: MS option button to select the MS scan mode. Note that XP Plus sets the MS^n power to *1*.

   d. Click on the Scan Type: SIM option button to select the SIM data type. Note that XP Plus sets the Total Scan Ranges to *1*.

   e. In the Scan Time group box, in the Total Microscans spin box, enter **1** to set the total number of microscans to 1.

   f. In the Input Method group box, select the Center/Width option button to make available the Center Mass and Width text boxes in the Scan Ranges group box. (Refer to steps 4h and 4i, below.)

   g. In the [Ion] Source Fragmentation group box, confirm that the Turn On check box is not selected (☐) to specify that the ion source fragmentation option is turned off.

   h. In the Scan Ranges group box, in the Center Mass text box, enter **609.2** to set the center mass for the scan range to *m/z* 609.2.

   i. In the Width text box, enter **2** to set the width of the scan range to *m/z* 2.

j. Ensure that the settings in your Define Scan dialog box are the same as those shown in Figure 24.

k. Click on **OK** to save the MS detector scan parameters and return to the Tune Plus window.

6. If you are operating in APCI/APPI or APPI only modes, turn on the APPI source:

   a. Turn on the power to the APPI lamp by flipping the switch on the PhotoMate light source housing to the On position.

   b. Verify that the lamp is operating by looking through the front window of the Ion Max ion source housing.

7. On the Control/Scan Mode toolbar, click on the Centroid/Profile button to toggle the data type to centroid. (The picture on the button should be the same as that shown here).

8. Click on the Positive/Negative button to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here).

You have now completed setting up your MS detector for APCI/APPI/MS operation.

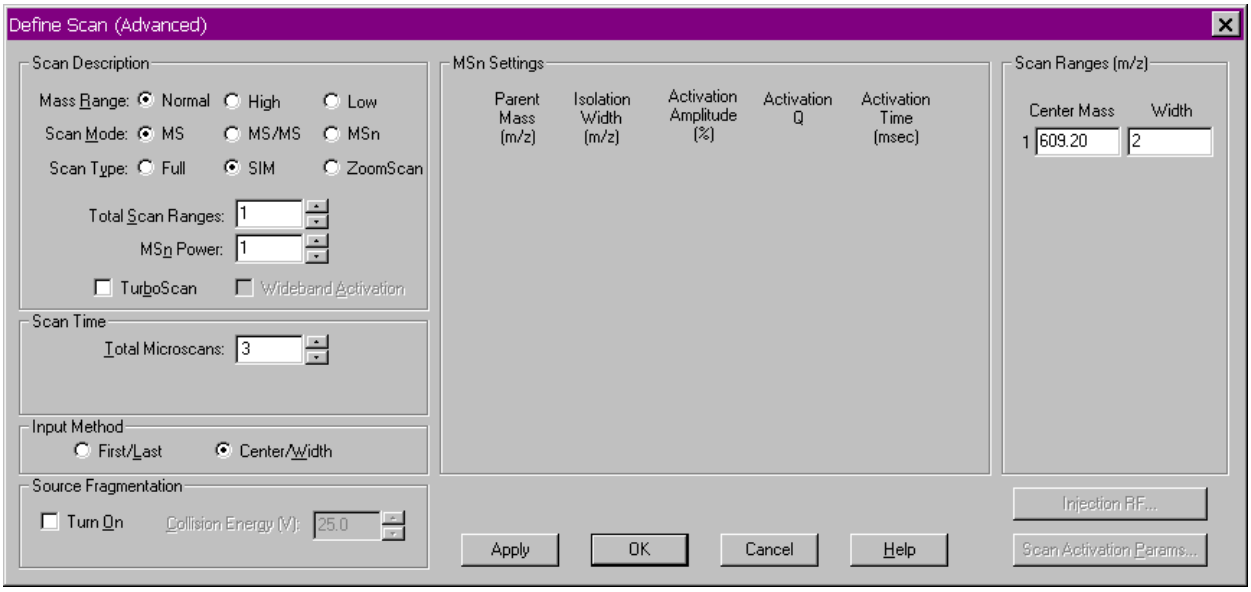


Figure 24. Define Scan dialog box, showing typical settings for APCI/APPI/MS operation

Optimizing the Tune of the MS Detector Automatically in APCI/APPI/MS Mode

You can optimize the tune of the MS detector automatically for APCI operation.

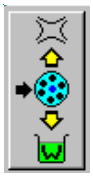
The most important parameters that affect the signal quality during APCI/APPI/MS operation are the vaporizer temperature, ion transfer capillary temperature, capillary voltage, tube lens offset voltage, gases, and solution flow rate. If any one of these parameters is changed, you need to re-optimize the MS detector parameters. (You can use the Semi-Automatic tune procedure to tune the MS detector on individual parameters.)

Use the following procedure to optimize the MS detector automatically on the reserpine peak at *m/z* 609.2 at your particular flow rate, for example, 1 mL/min. (Refer to Table 2 on page 1-11 for guidelines about flow rates and temperatures.)

To optimize the MS detector automatically on the reserpine peak at *m/z* 609.2 at your particular flow rate

1. On the Control/Scan Mode toolbar, click on the Tune button to display the Automatic tuning page in the Tune dialog box.

2. In the Mass spin box, enter 609.2 to specify that you want to tune on the peak at *m/z* 609.2.



3. Ensure that the Divert/Inject valve is in the Detector position:

   a. Click on the Divert/Inject Valve button to open the dialog box.

   b. Select the Detector option button, and then click **Close** to return to Tune Plus.

4. Start the automatic tuning procedure from the Tune dialog box:

   a. Click **Start**. A message box displays the following message:

   b. Please ensure that the 500 microliter syringe is full.

   c. Ensure the syringe pump contains at least 420 µL of the 1 ng/µL reserpine tuning solution.

   d. Click **OK** to close the message box and return to the Tune Plus window.

5. On the File/Display toolbar, click on the Graph View button to display the view.

6. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, LCQ Deca XP MAX displays various

tests in the Spectrum and Graph views in the Tune Plus window and displays various messages in the Status group box in the Tune dialog box. Your Tune Plus window should now look similar to the one shown in Figure 25.

You have now successfully tuned the MS detector in APCI/APPI/MS mode for the compound reserpine (or your analyte of interest). Leave the LC pumps on (with a flow rate of approximately 1 mL/min), leave the *APCIhighflow.LCQTune* file open in the Tune Plus window and go on to the next topic: Saving the APCI/APPI/MS Tune Method.

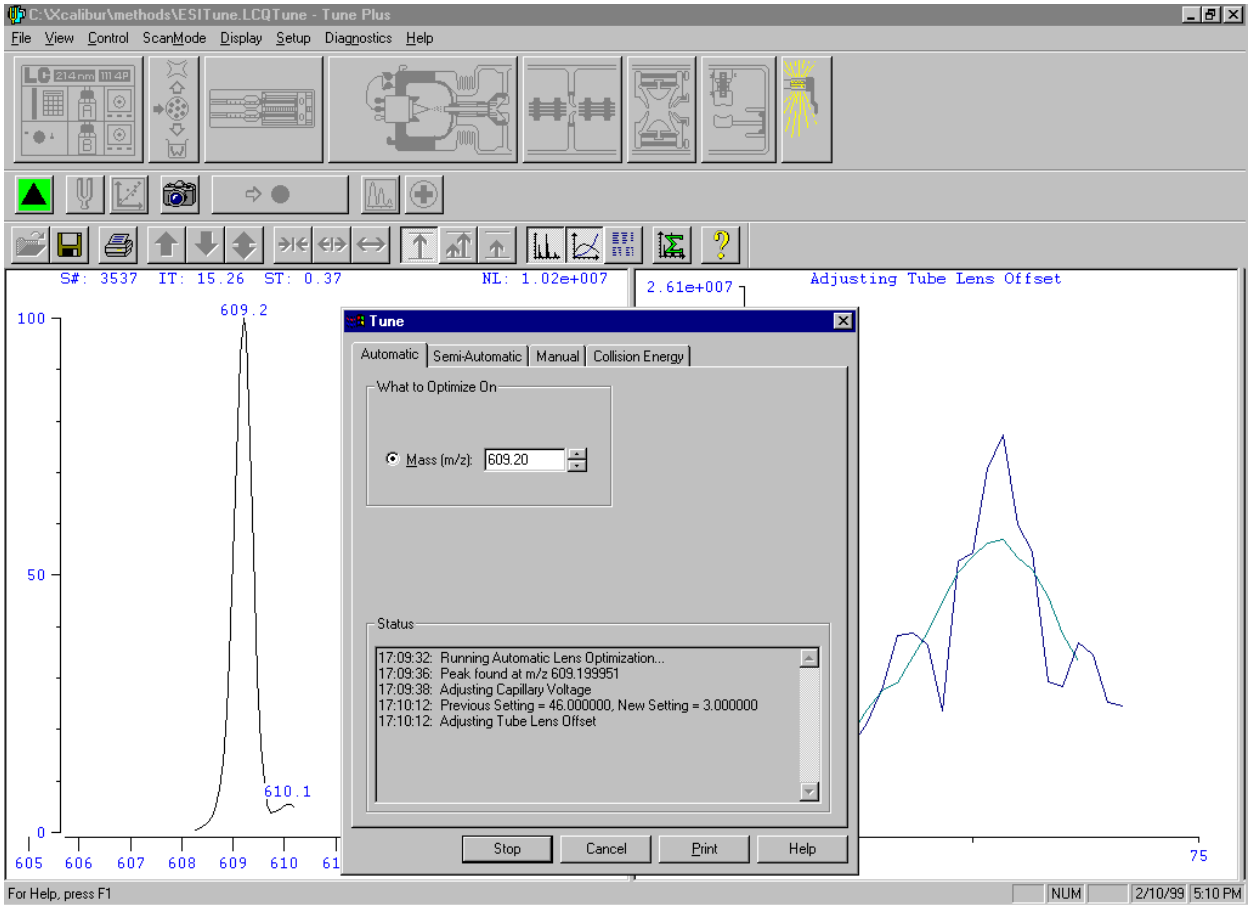


Figure 25. Tune Plus window with the Tune dialog box, showing the Automatic page

Saving the Tune Method

You can save the settings you just obtained in a tune method specific to your particular analyte and solvent flow rate. (In this case, you save settings obtained using reserpine.) You can recall the tune method and use it as a starting point for optimizing the MS detector on reserpine at a different flow rate.

Note Save the Tune Method while the MS detector is On.

To save the APCI/APPI/MS tune method

1. Choose **File > Save As** to display the Save As dialog box. See Figure 26.

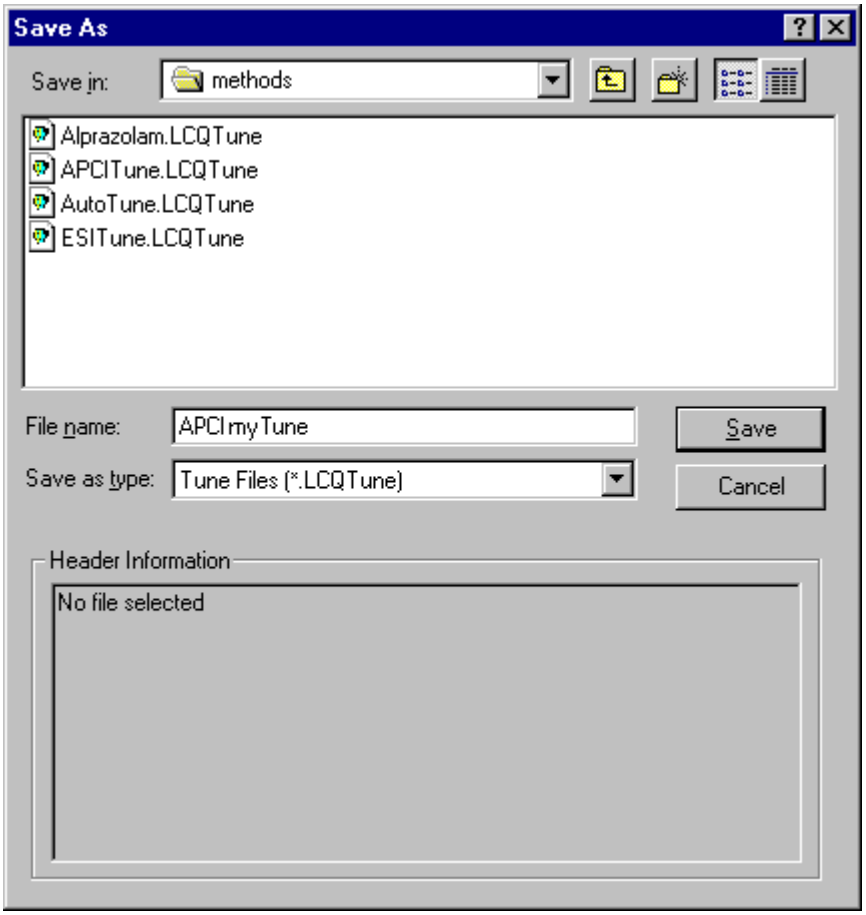


Figure 26. Save As dialog box, showing files in the folder *C:\Xcalibur\methods*

2. In the Save In list box, select the *C:\Xcalibur\methods* folder.

3. Click on the File Name text box, and then enter **APCImyTune** to name the Tune Method *APCImyTune.LCQTune*.

4. Click **Save** to save the Tune Method and return to the Tune Plus window. Note that the Tune Method is named *APCImyTune.LCQTune*.

Before you acquire data, go to the next topic: Cleaning the MS Detector after Tuning in APCI Mode.

Cleaning the MS Detector After Tuning in APCI Mode

OnStandby

To clean the MS detector after tuning on your analyte of interest

1. Click on the On/Standby button to put the MS detector in Standby mode. When the MS detector is in Standby, the LCQ Deca XP MAX MS detector turns off the vaporizer heater, corona discharge voltage, and syringe pump. The MS detector stops scanning and freezes the displays for the Spectrum and Graph views.

CAUTION Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is On could be unsafe. (The LCQ Deca XP MAX MS detector automatically turns off when you open the API source, however, it is best to take this added precaution.)

2. Remove the syringe from the syringe pump holder,:
   a. Squeeze the blue buttons, and pull back on the syringe pump handle to free the syringe.
   b. Remove the syringe from the holder.
   c. Disconnect the tip of the syringe needle from the Teflon tubing.
3. Clean the syringe thoroughly:
   a. Clean the syringe with a solution of 5% formic acid in water.
   b. Rinse the syringe with a solution of 50:50 methanol / water.
   c. Use acetone to rinse the syringe. Repeat this step several times.

CAUTION AVOID BURNS. The APCI vaporizer heater can reach temperatures of 600 ×C. Always allow the APCI probe to cool to ambient temperature, for approximately 20 min, before handling or removing the APCI probe from the APCI flange

CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin if you handle it without caution.

4. Remove the Ion Max ion source housing as described in the topic “Removing the Ion Max Ion Source Housing” on page 26.
5. Flush the sample transfer line, sample tube, and APCI probe thoroughly with a solution of 5% formic acid in water (or with another appropriate solvent):

Note The solvent that you use to flush the sample transfer line, sample tube, and APCI probe assembly depends on the solvent system you use to dissolve your samples. For example, if you are using a buffered solution of a high concentration, an acidic solution is appropriate.

a. Fill a clean, 250 µL Unimetrics syringe with an appropriate solvent.

b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the free end of the Teflon tube.

c. Flush the sample transfer line, sample tube, and APCI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the APCI probe on the inside of the probe assembly. Use a lint-free tissue to gently remove the excess solution as it exits the probe.

d. Remove the needle of the syringe from the Teflon tube.

6. Repeat step 5 with a solution of 50:50 methanol / water.

7. Reinstall the Ion Max ion source housing as described in topic "Installing the Ion Max Ion Source Housing" on page 33.

Your LCQ Deca XP MAX MS detector is now ready to perform experiments in APCI/APPI mode. For information regarding the maintenance of the Ion Max APPI source refer to Chapter 7, "Maintenance."

Chapter 5 Optimizing the LTQ MS Detector with Your Analyte

This chapter provides information on optimizing the tune of your LTQ MS detector in the APCI/APPI/MS high flow mode. It is not necessary to recalibrate the MS detector when you switch to APCI/APPI/MS operation. You can use the calibration settings you obtained from the successful automatic calibration procedure you performed in the ESI/MS mode.

For APCI/APPI/MS operation you simply open a default Tune Method located in your *C:\Xcalibur\methods* folder, in this case *APCIhighflow.LTQTune*. From this starting point, you optimize automatically the tube lens voltage for your particular analyte. The capillary voltage and ion transfer capillary temperature can then be optimized manually to enhance ion transmission.

Note The following procedures assume that you are familiar with your LTQ instrument and the Tune Plus window. If you need information, refer to the LTQ online Help, LTQ Getting Connected, and/or LTQ Hardware Manual.

Ensure that you have completed the procedures in the sections that describe how to calibrate your instrument in ESI mode, and have properly set up your APCI/APPI source.

This chapter includes the following sections:

Setting Up the Inlet for Tuning Using High-Flow Infusion

To make the plumbing connections for APCI/APPI/MS sample introduction from the syringe pump into solvent flow from an LC

1. Connect a 4 cm (1.5 in) segment of Teflon tubing with a (brown) FingerTight fitting and a (brown) ferrule to the (black) LC union (Figure 27).

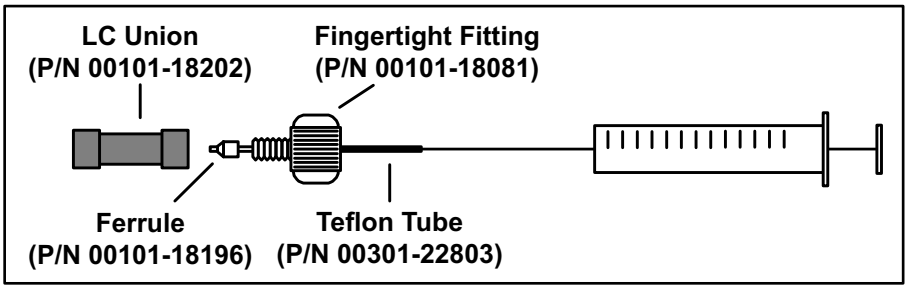


Figure 27. APCI/APPI/MS plumbing connections for the syringe pump

2. Load a clean, 500-µL Unimetrics syringe with 450 µL of a 125 fg/µL solution of reserpine or your analyte of interest. (Refer to Appendix B: Reserpine Solution Formulations for a procedure for making the reserpine tuning solution.)

3. Insert the needle of a syringe into the segment of Teflon tubing, and place the syringe in the syringe holder of the syringe pump.

4. Connect a fused-silica infusion line from the (black) LC union to the (black) LC Tee union:

   a. Connect the infusion line with a (brown) fingertight fitting and a (brown) ferrule to the free end of the LC union. See Figure 28.

   b. Connect the other end of the infusion line with a (red) fingertight fitting and a (brown) ferrule to the side arm of the LC Tee union.

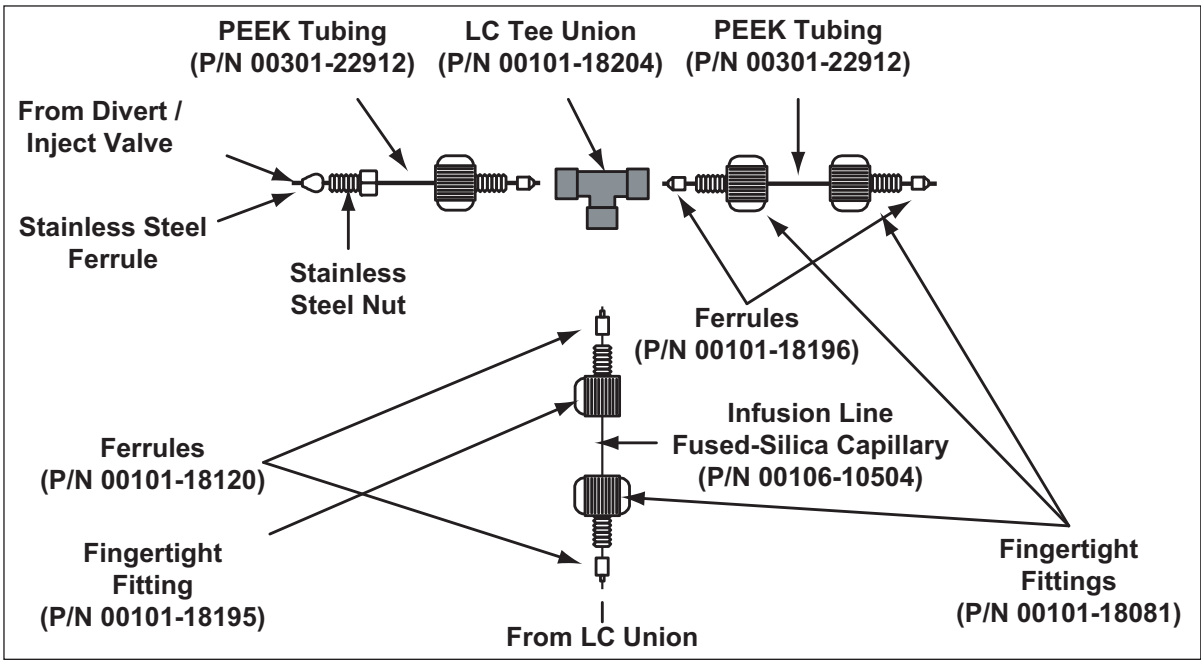


Figure 28. APCI/APPI/MS plumbing connections for the LC Tee union

Note To cut the PEEK tubing used to connect your LC to the divert/inject valve and the divert/inject valve to the APCI source, use a PEEK tubing cutter. This ensures that the tubing is cut straight. In addition, make sure your LC fittings, ferrules, and PEEK tubing are installed properly. By using these precautions, you prevent void (dead) volumes. The exclusion of void volumes is critical to microbore LC. Also, void volumes affect the quality of the MS detector signal.

5. Connect a segment of PEEK tubing from the (black) LC Tee union to the APCI LC inlet: (Figure 28.)

   a. Use a PEEK tubing cutter to cut a 4 cm (1.5 in.) length of the PEEK tubing.

   b. Connect the PEEK tubing with a (brown) fingertight fitting and a (brown) ferrule to a free end of the (black) LC Tee union.

   c. Connect the other end of the PEEK tubing with a (red) fingertight fitting and a (brown) ferrule to the LC inlet located on the APCI probe.

6. Connect an appropriate length of PEEK tubing (transfer line from the divert/inject valve) from the divert/inject valve to the LC Tee union: (Figure 28.)

a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 3 of the divert/inject valve.

b. Connect the other end of the PEEK tubing with a (brown) fingertight fitting and a (brown) ferrule to the free end of the LC Tee union.

7. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC:

   a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 2 of the divert/inject valve.

   b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.

8. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container:

   a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 1 of the divert/inject valve.

   b. Insert the other end of the PEEK tubing into a suitable waste container.

The LC plumbing connections are now properly made for APCI/APPI/MS sample introduction from the syringe pump into solvent flow from an LC.

Setting Up the MS Detector for APCI/APPI/MS Operation


OnStandby

To set up the MS detector for APCI/APPI/MS operation on the LTQ MS detector.

1. In Tune Plus, click on the On/Standby button to take the MS detector out of Standby mode and turn it on. The MS detector begins scanning and applies high voltage to the corona needle and shows a real-time display in the Spectrum view.

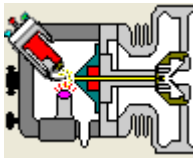
2. Open the *APCIhighflow.LTQTune* Tune Method, the Tune Method for high-flow APCI operation:

   a. Choose **File > Open** to display the Open dialog box.

   b. Scroll down until you see the folder *C:\Xcalibur\methods*. Then, select the file *APCIhighflow.LTQTune*.

   c. Click **OK** to open the file. The Tune Method parameters are uploaded to the MS detector.

3. Verify that the LTQ MS detector opened the Tune Method:



   a. On the Instrument Setup toolbar, click on the API Source button to open the APCI/APPI Source dialog box (Figure 29).

   b. Verify that the settings in your dialog box are similar to those shown in Figure 29. Set the APCI or APPI mode:

      - To operate in APPI mode only, set the discharge current to 0 and select the APPI Lamp On check box.

      - To operate in APCI/APPI mode, set the corona discharge to your desired value and select the APPI Lamp On check box (Figure 29).

      - To operate in APCI mode only, set the corona discharge to your desired value and make sure that the APCI Lamp on check box is not selected (Figure 29).

   c. Click **OK** to close the APCI/APPI Source dialog box.

   d. Verify that the lamp is operating by looking through the front window of the Ion Max housing. You can see purple light if the light source is working properly.

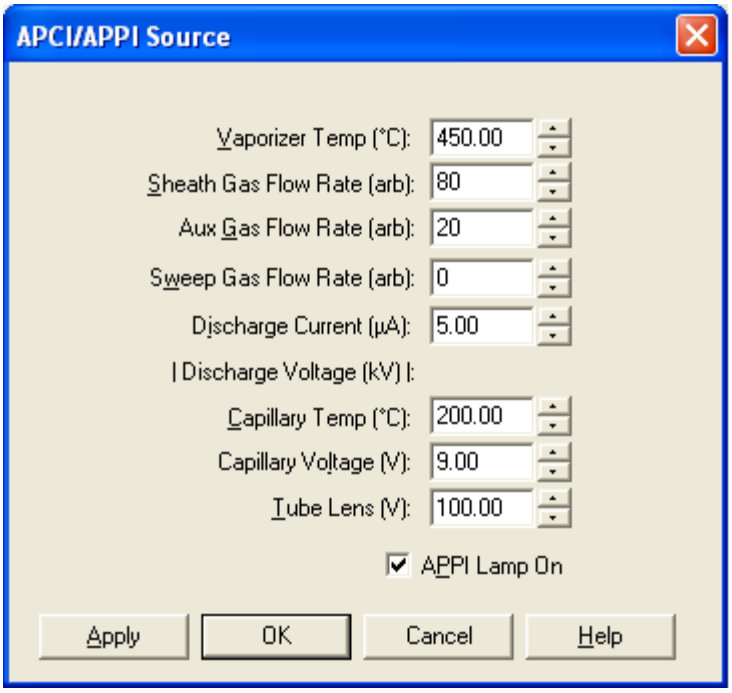


Figure 29. APCI/APPI Source dialog box, showing the proper settings for a typical high flow experiment

4. Define the scan parameters for tuning the MS detector in the APCI/APPI/MS mode,:

   a. On the Control/Scan Mode toolbar, click on the Define Scan button to open the Define Scan dialog box. See Figure 30. (If your dialog box appears different from the one shown in the figure, it is probably because the advanced settings are not displayed. You can turn on the advanced settings as follows: In Tune Plus, choose **ScanMode**, and then click **Advanced Scan Features** to select the option.)

   b. In the Scan Description group box, in the Mass Range list box, select *Normal* to allow for a selection of mass ranges between *m/z* 150 to 2000.

   c. In the Scan Rate list box, select *Normal* to specify a normal scan rate.

   d. In the Scan Type list box, select *SIM* to specify a selected ion monitoring scan.

   e. In the Scan Time group box, in the Microscans spin box, enter **1** to set the total number of microscans to 1.

   f. In the Max. Inject Time spin box, enter *200.000* to specify a 200 ms maximum injection time.

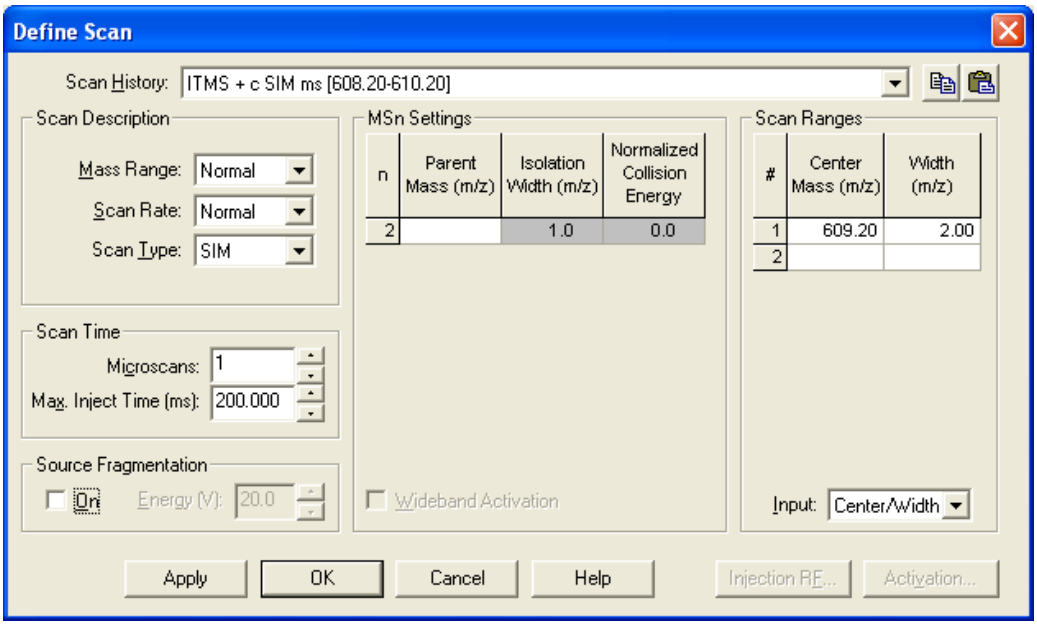


Figure 30. Define Scan dialog box, showing typical settings for APCI/APPI/MS operation

g. In the Source Fragmentation group box, confirm that the On check box is not selected (☐) to specify that the ion source fragmentation option is turned off.

h. In the Scan Ranges group box, in the Input list box, select *Center/Width* to make available the Center Mass and Width text boxes in the Scan Ranges table.

i. In the Scan Ranges table, in the Center Mass text box, enter **609.20** to set the center mass for the scan range to *m/z* 609.20.

j. In the Width text box, enter **2.00** to set the width of the scan range to *m/z* 2.00.

k. Ensure that the settings in your Define Scan dialog box are the same as those shown in Figure 30.

l. Click **OK** to apply the MS detector scan parameters and to close the Define Scan dialog box.

5. On the Control/Scan Mode toolbar, click **Centroid/Profile** to toggle the data type to centroid. The picture on the button should be the same as that shown here.

6. Click **Positive/Negative** to toggle the ion polarity mode to positive. The picture on the button should be the same as that shown here.

You have now completed setting up your MS detector for APCI/APPI/MS operation.

Optimizing the Tune of the MS Detector Automatically in APCI/APPI/MS Mode

You can optimize the tune of the MS detector automatically for APCI operation.

The most important parameters that affect the signal quality during APCI/APPI/MS operation are the vaporizer temperature, ion transfer tube temperature, API gas flows, and solution flow rate. If any one of these parameters is changed, you need to re-optimize MS detector parameters. (You can use the Semi-Automatic tune procedure to tune the MS detector on individual parameters.)

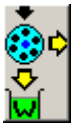
Use the following procedure to optimize the MS detector automatically on the reserpine peak at *m/z* 609.2 at your particular flow rate, for example, 400 µL/min. (Refer to Table 2 for guidelines about flow rates and temperatures.)

1. On the Control/Scan Mode toolbar, click on the Tune button to display the Automatic tuning page. See Figure 31.

2. In the What to Optimize On group box, select the Mass option button to make active the Mass spin box.

3. In the Mass spin box, enter 609.2 to specify that you want to tune on the peak at *m/z* 609.2.

4. Ensure that the Divert/Inject valve is in the Detector position:



   a. Click on the Divert/Inject Valve button to open the Divert/Inject Valve dialog box.

   b. Select the Detector option button, and then click **Close** to return to Tune Plus.

5. Start the automatic tuning procedure from the Tune dialog box:

   a. Click **Start**. A message box displays the following message:

   b. Please ensure that the 500 microliter syringe is full.

   c. Ensure the syringe pump contains at least 450 µL of the 125 fg/µL reserpine tuning solution.

   d. Click **OK** to close the message box and return to the Tune Plus window.

6. On the File/Display toolbar, click on the Graph View button to display the view.

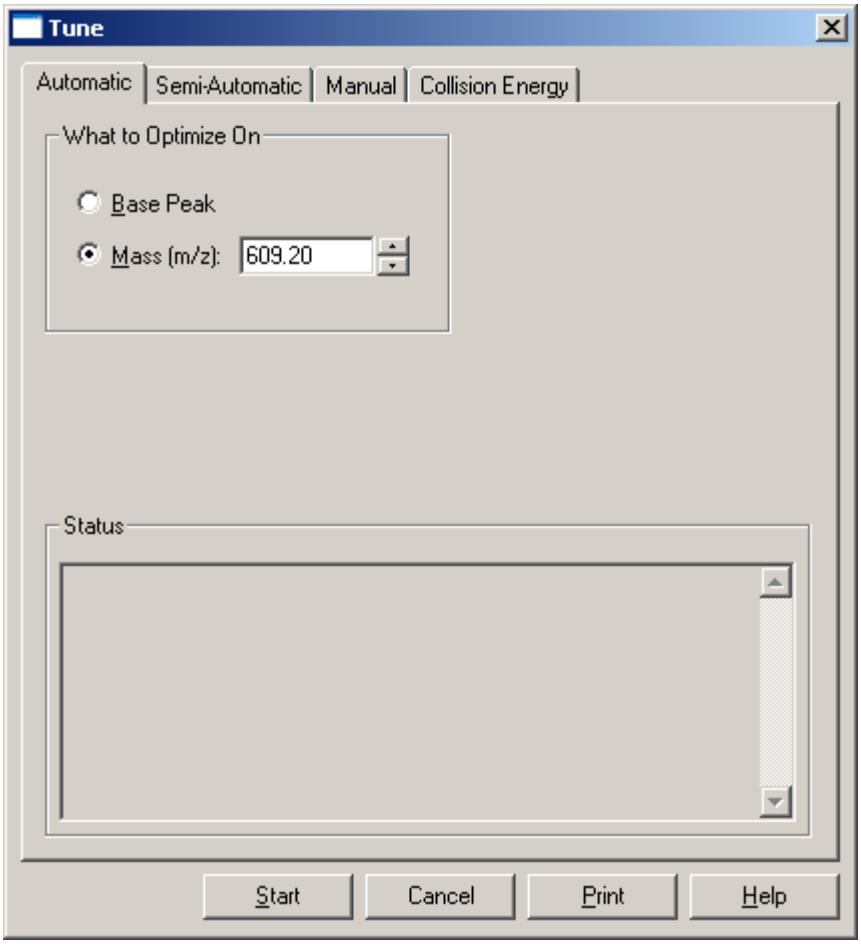


Figure 31. Tune dialog box, showing the Automatic tuning page

7. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, the LTQ MS detector displays various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages in the Status group box in the Tune dialog box. Your Tune Plus window should now look similar to the one shown in Figure 32.

You have now successfully tuned the MS detector in APCI/APPI/MS mode for the compound reserpine (or your analyte of interest). Leave the LC pumps on (with a flow rate of approximately 400 µL/min), leave the *APCIhighflow.LCQTune* file open in the Tune Plus window and go on to the next topic: Saving the APCI/APPI/MS Tune Method.

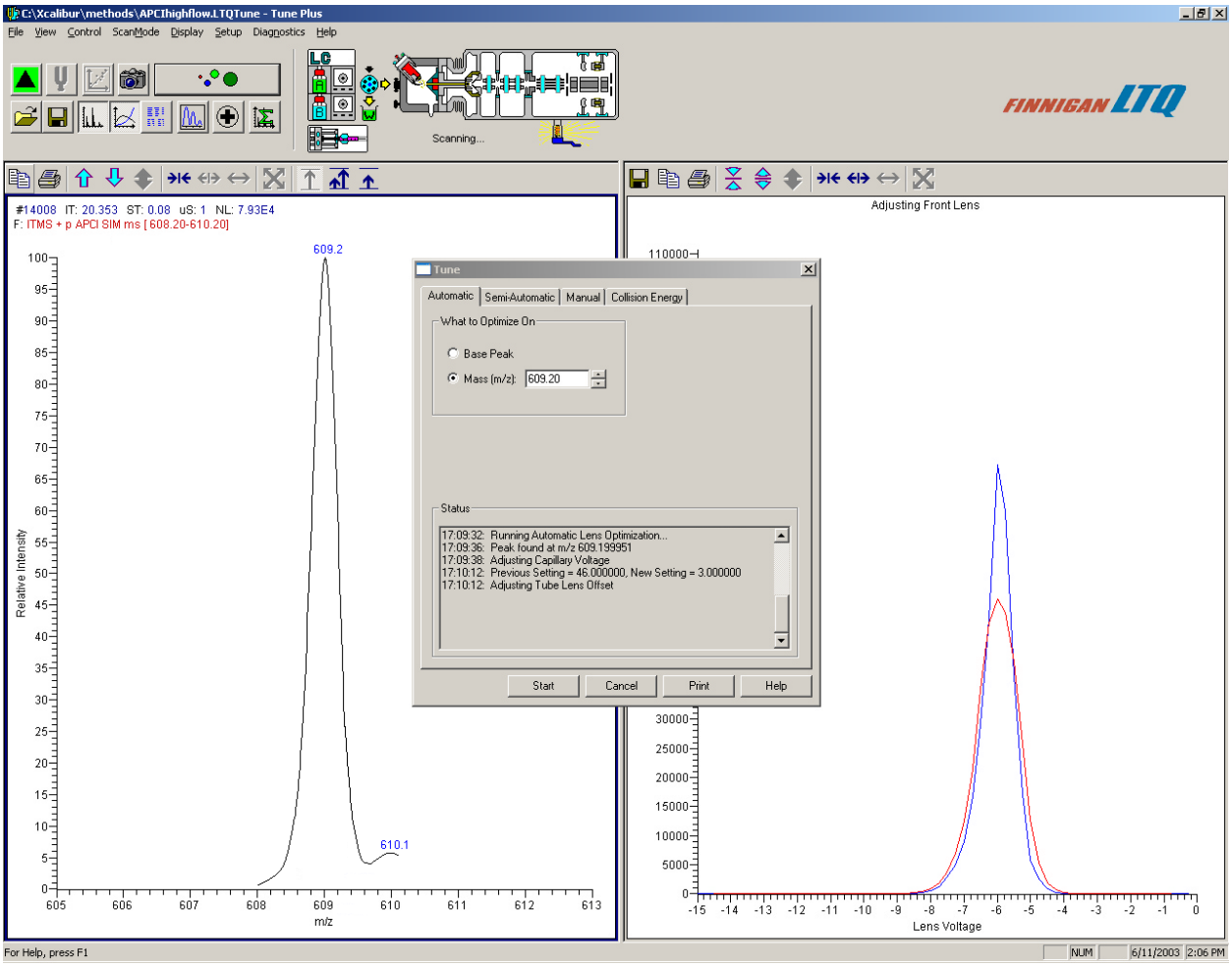


Figure 32. Tune Plus window with the Tune dialog box, showing the Automatic tuning page

Saving the APCI/APPI/MS Tune Method

You can save the settings you just obtained in a Tune Method specific to your particular analyte and solvent flow rate (in this case, the settings obtained using reserpine). You can recall the Tune Method and use it as a starting point for optimizing the MS detector on reserpine at a different flow rate.

Note Save the Tune Method while the MS detector is still On.

To save your APCI/APPI/MS Tune Method

1. Choose **File >Save As** to display the Save As dialog box. Figure 33.
2. In the Save In list box, select the *C:\Xcalibur\methods* folder.
3. Click on the File Name text box, and then enter **APPImyTune** to name the Tune Method *APPImyTune.LTQTune*.

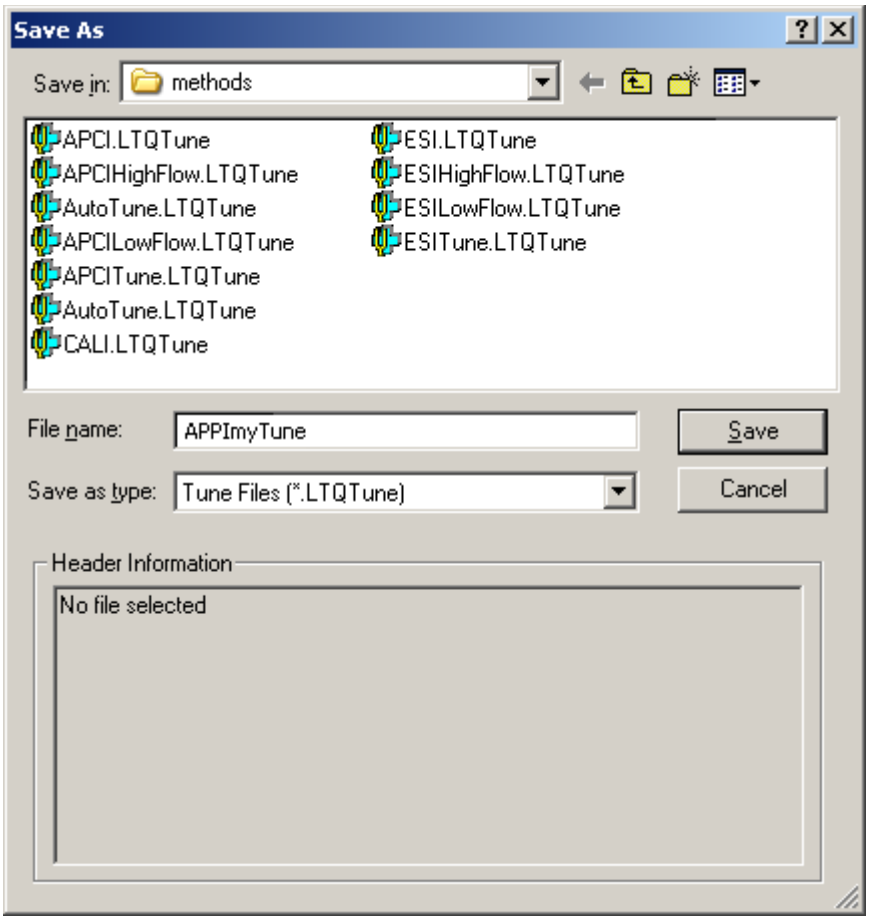


Figure 33. Save As dialog box, showing files in the folder *C:\Xcalibur\methods*

4. Click **Save** to save the Tune Method and close the Save As dialog box. Note that the Tune Method is named *APPImyTune.LTQTune.*

Before you acquire data, go to the next topic: Cleaning the MS Detector After Tuning in APCI Mode.

Cleaning the MS Detector After Tuning in APCI Mode


OnStandby

Use the following procedure to clean the MS detector after tuning on your analyte of interest.

1. Click on the On/Standby button to put the MS detector in Standby mode. When the MS detector is in Standby, the LTQ MS detector turns off the vaporizer heater, corona discharge voltage, and syringe pump. The MS detector stops scanning and freezes the displays for the Spectrum and Graph views.

CAUTION Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is On could be unsafe. The LTQ MS detector automatically turns off when you open the API source; however, it is best to take this added precaution.

2. Remove the syringe from the syringe pump holder, as follows:

   a. Squeeze the blue buttons and pull back on the syringe pump handle to free the syringe.

   b. Remove the syringe from the holder.

   c. Disconnect the tip of the syringe needle from the Teflon tubing.

3. Clean the syringe thoroughly:

   a. Clean the syringe with a solution of 5% formic acid in water.

   b. Rinse the syringe with a solution of 50:50 methanol / water.

   c. Use acetone to rinse the syringe. Repeat this step several times.

CAUTION AVOID BURNS. The APCI vaporizer heater can reach temperatures of 600 ×C. Always allow the APCI probe to cool to ambient temperature, for approximately 20 min, before handling or removing the APCI probe from the APCI flange.

CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin if you handle it without caution.

4. Remove the Ion Max ion source housing as described in the topic "Removing the Ion Max Ion Source Housing" on page 26.

5. Flush the sample transfer line, sample tube, and APCI probe thoroughly with a solution of 5% formic acid in water (or with another appropriate solvent), as follows:

Note The solvent that you use to flush the sample transfer line, sample tube, and APCI probe assembly depends on the solvent system you use to dissolve your samples. For example, if you are using a buffered solution of a high concentration, an acidic solution is appropriate.

   a. Fill a clean, 250 µL Unimetrics syringe with an appropriate solvent.
   b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the free end of the Teflon tube.
   c. Flush the sample transfer line, sample tube, and APCI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the APCI probe on the inside of the probe assembly. Use a lint-free tissue to gently remove the excess solution as it exits the probe.
   d. Remove the needle of the syringe from the Teflon tube.

6. Repeat step 5 with a solution of 50:50 methanol / water.

7. Reinstall the Ion Max ion source housing as described in topic "Installing the Ion Max Ion Source Housing" on page 33.

Your LTQ MS detector is now ready to perform experiments in APCI/APPI mode. Please refer to Chapter 7, "Maintenance." for information regarding the maintenance of the Ion Max APPI source.

Chapter 6 Optimizing the TSQ Quantum Ultra Mass Spectrometer with Your Analyte

This chapter provides information on fine tuning your TSQ Quantum Ultra in the APCI/APPI/MS/MS mode using your analyte as the tuning compound. You optimize the sensitivity of the mass spectrometer for your analyte with an automatic tuning procedure.

This chapter contains the following sections:

The Tune Methods that are provided with your TSQ Quantum Ultra are useful for a wide range of applications. They can often be used without further tuning of your mass spectrometer. However, for certain applications you might need to optimize several mass spectrometer parameters. For instance, the parameters that affect APCI performance and signal quality are as follows:

- Discharge current
- APCI vaporizer temperature
- Sheath gas pressure
- Auxiliary gas flow rate
- Capillary temperature
- Tube lens offset voltage

The optimum settings for these parameters depend on the solvent flow rate and on the structure of your analyte. In general, you need to fine tune the mass spectrometer parameters whenever you change the solvent flow rate conditions of your particular application. When you optimize the mass spectrometer parameters using the automatic tuning procedure, the procedure adjusts all the parameters listed above and the voltages applied to the ion optics until the ion transmission of your analyte is maximized.

Note Ensure that you have performed the TSQ Quantum Ultra tuning and calibration procedure within the previous three months before you optimize the tune for your compound. If you need to tune and calibrate the system, refer to your TSQ Quantum Ultra Getting Started manual and the procedure in Chapter 3: Tuning and Calibrating the Mass Spectrometer in ESI/MS/MS Mode.

To optimize the mass spectrometer for your compound in the APCI/APPI/MS/MS mode do the folowing:

- Set up the syringe pump and divert/injection valve for auto loop injection.
- Set up the mass spectrometer for your specific compound from the Tune Master.
- Run the automatic compound optimization procedure to fine tune the mass spectrometer parameters that are compound dependent.
- Save the new Tune Method.

Setting Up to Introduce Sample by Auto Loop Injection in APCI Mode

The following procedure describes how to introduce your compound by auto loop injection. The plumbing connections for APCI/MS sample introduction from the syringe pump into the solvent flow from an LC are shown in Figure 34.

Note You can use the reserpine sample solution described in Appendix B: Reserpine Solution Formulations, or you can use your compound of interest.

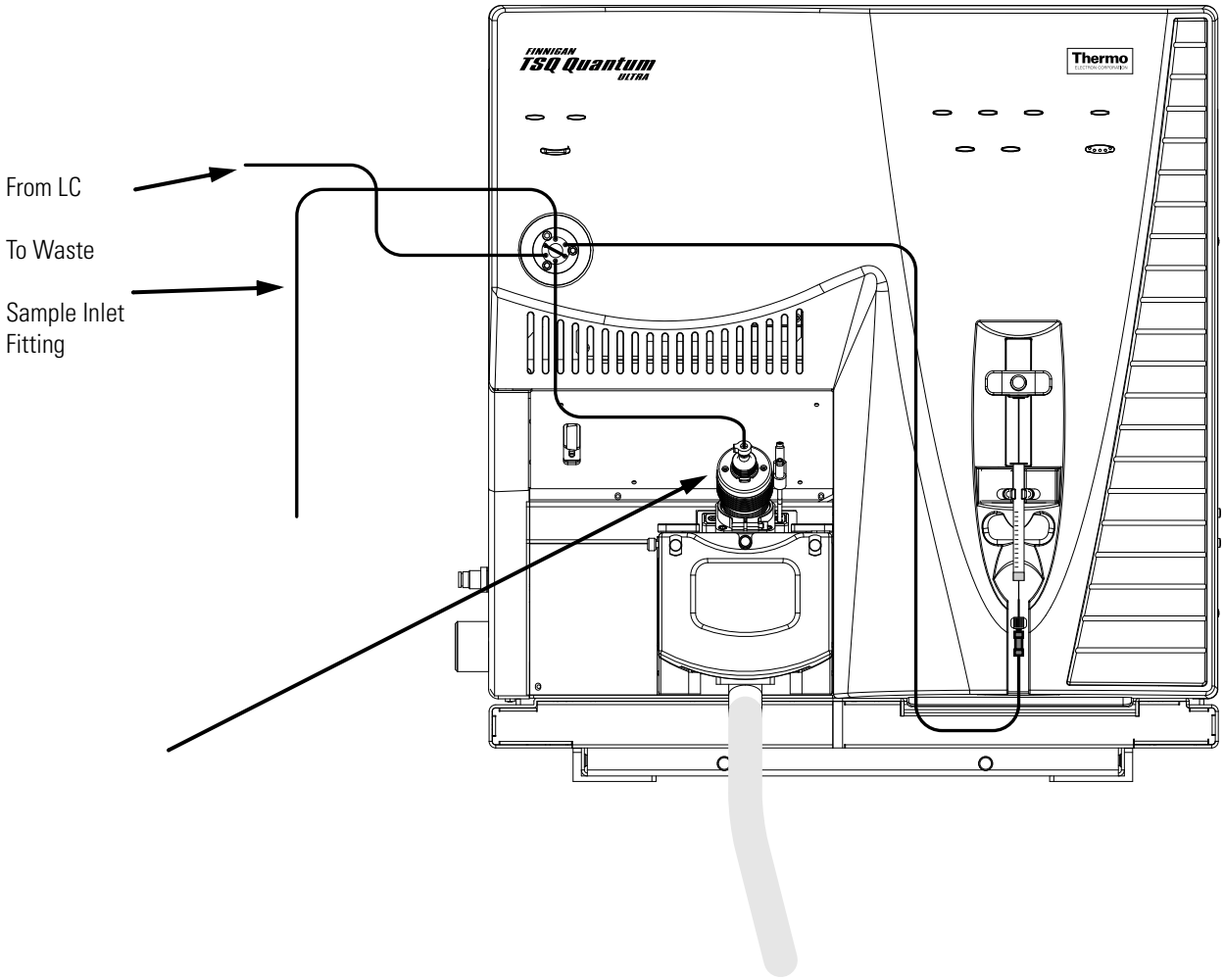


Figure 34. APCI/MS plumbing connections for sample introduction by auto loop injection into the solvent flow from an LC

To make the plumbing connections for APCI/MS sample introduction from the syringe pump into the solvent flow from an LC

1. Remove the syringe from the syringe pump holder:

a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

b. Remove the syringe.

c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly(Figure 35).

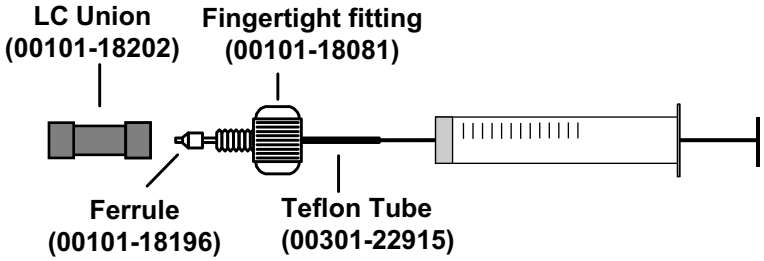


Figure 35. Syringe and syringe adapter assembly

2. Remove the sample transfer line installed between the syringe adapter assembly and the APCI probe.

3. Install a sample transfer line between the syringe adapter assembly and the divert/inject valve:

a. Connect an appropriate length of tubing to the LC union on the syringe adapter assembly.

b. Connect the other end of the tubing fitted with a nut and a ferrule to port 5 of the divert/inject valve (Figure 36).

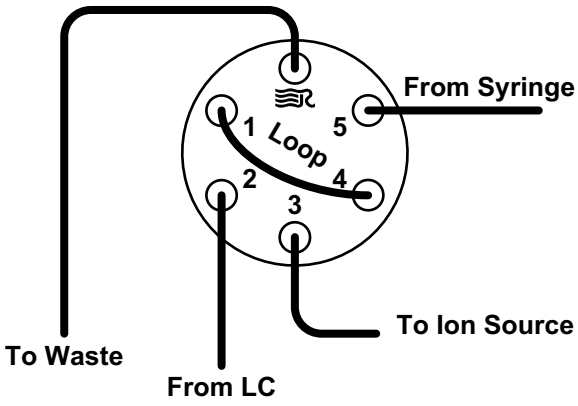


Figure 36. Divert/inject valve, showing plumbing for auto loop injection

Note To minimize the possibility of cross-contamination, use a different syringe and a different sample transfer line for your tuning and calibration solution than you do for your samples and compound optimization solution.

4. Load a clean, 500-µL Unimetrics syringe with 420 µL of the 2 pg/µL reserpine sample solution. (Refer to **Appendix B:** Reserpine Solution Formulations for the procedure for preparing the reserpine solution.)

Note Be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly to minimize the possibility of cross-contamination of the assembly.

5. While holding the plunger of the syringe in place, carefully reinsert the tip of the syringe needle into the end of the Teflon tube on the syringe adapter assembly (Figure 35).

6. Place the syringe into the syringe holders of the syringe pump.

7. While squeezing the black release button on the syringe pump handle, push the handle down until it just contacts the syringe plunger.

8. Install a sample transfer line between the divert/inject valve and the APCI probe:

   a. Gather the necessary fittings for installing a sample transfer line (Figure 37).

   b. Connect an appropriate length of tubing fitted with a nut and a ferrule to port 3 of the divert/inject valve (Figure 36)

   c. Connect the other end of the tubing with a FingerTight fitting and a ferrule to the sample inlet fitting (LC inlet) (Figure 34).

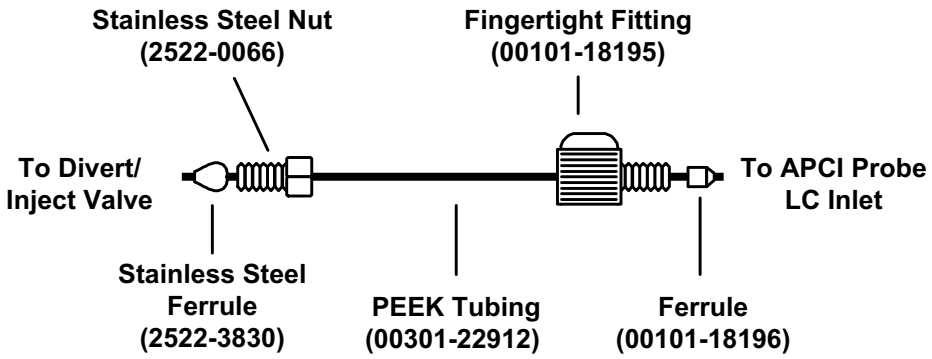


Figure 37. Sample transfer line, installed between the divert/inject valve and the APCI probe

9. Install a 5 µL sample loop with nuts and ferrules between ports 1 and 4 of the divert/inject valve.

10. Install a solvent line between the LC system and the divert/inject valve:

    a. Connect an appropriate length of tubing with a proper fitting and a ferrule to the outlet of the LC system.

    b. Connect the other end of the tubing with a nut and ferrule to port 2 of the divert/inject valve.

11. Install a waste line on the divert/inject valve and direct the outlet to a waste container:

    a. Connect an appropriate length of tubing with a nut and ferrule to port 6 of the divert/inject valve (port 6 is labeled with the Rheodyne logo).

    b. Insert the other end of the tubing into the waste container.

You have completed setting up to introduce your compound by auto loop injection. Go to the next section: **Setting Up to Optimize in APCI/APPI/MS/MS Mode with Your Compound**.

Setting Up to Optimize in APCI/APPI/MS/MS Mode with Your Compound



On Standby

To set up the mass spectrometer to optimize automatically on your compound in APCI/APPI/MS/MS mode

1. Click the **On/Standby** button on the Control/Scan Mode toolbar to turn on the mass spectrometer.

2. Tune Master must be placed in the APCI source mode before analyzing samples with the APCI source. Choose **Setup > Change Ion Source > APCI** to place Tune Master in the APCI source mode.

3. If desired, open an existing Tune Method:

   a. On the File / Display toolbar, click on the Open File button to display the Open dialog box.

   b.

   c. Confirm that the path is *C:\Xcalibur\methods* and then select the desired file.

   d. Click **Open** to open the file. Tune Master downloads the Tune Method parameters to the mass spectrometer.

4. Click the **Optimize Compound Dependent Devices** button on the Control / Scan Mode toolbar to display the Optimize Compound Dependent Devices view in the top right corner of the workspace (Figure 38).

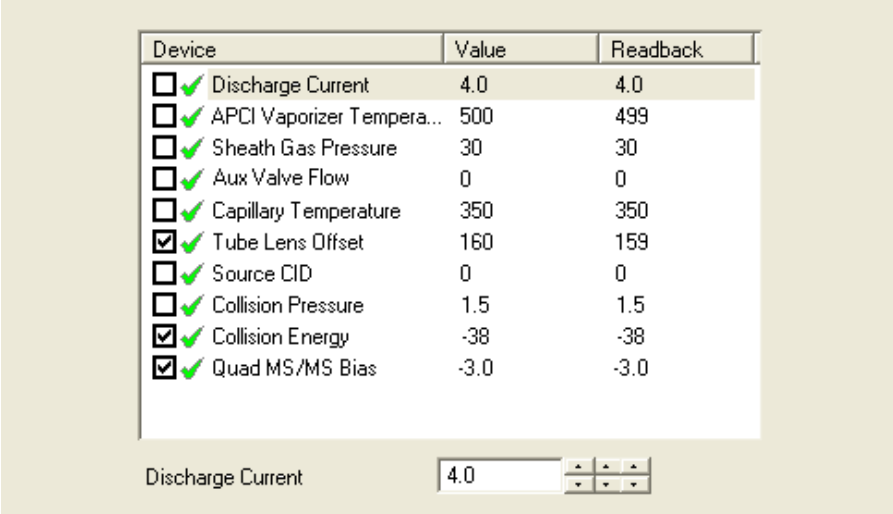


Figure 38. Optimize Compound Dependent Devices view, APCI settings

Note You might find that the presence of chemical contamination in the APCI vaporizer creates chemical noise in the mass spectrum. If this occurs, recondition the APCI vaporizer. To recondition the APCI vaporizer, you start LC solvent flow, elevate the temperature of the APCI vaporizer, and increase the sheath gas and auxiliary gas pressures for approximately 30 min to drive off the chemical contamination.

Typical values used for reconditioning the APCI vaporizer are as follows:

LC flow rate = 400 mL/min
Vaporizer temperature = 600 ×C
Sheath gas pressure = 80 psi
Auxiliary gas flow rate = 15 units

5. Set the values for the compound dependent devices:

   a. Ensure that Discharge Current is selected in the Device Display box.

   b. In the Optimize Compound Dependent Devices view, set the APCI or APPI mode:

      - To operate in APPI mode only, set the discharge current to 0 and select the APPI Lamp On check box.

      - To operate in APCI/APPI mode or APCI mode only, enter 4.0 in the Device spin box to set the discharge current to 4.0 µA.

   c. Set the temperature of the APCI vaporizer:

      i. In the Device Display box, click **APCI Vaporizer Temperature**. This changes the Device spin box label to *APCI Vaporizer Temperature* and enables you to set the APCI vaporizer temperature.

      ii. In the Device box, enter 500 to set the vaporizer temperature to 500 °C.

   d. Set the pressure of the sheath gas:

      i. Click **Sheath Gas Pressure** in the Device Display box.

      ii. Enter 30 in the Device box to set the sheath gas pressure to 30 psi.

   e. Set the flow rate of the auxiliary gas:

      i. Click **Aux Valve Flow** in the Device Display box.

      ii. Enter 0 in the Device box to set the auxiliary gas flow rate to 0 units.

f. Set the temperature of the ion transfer capillary:

   i. Click **Capillary Temperature** in the Device Display box.

   ii. Enter 350 in the Device spin box to set the capillary temperature to 350 °C.

g. Set the ion source fragmentation (CID) collision energy:

   i. Click **Source CID** in the Device Display box.

   ii. Enter 0 in the Device box to set the collision energy to 0 V.

h. Set the collision pressure:

   i. Click **Collision Pressure** in the Device Display box.

   ii. Enter 1.5 in the Device spin box to set the collision pressure to 1.5 mTorr.

i. Set the collision energy:

   i. Click **Collision Energy** in the Device Display box.

   ii. Enter -38 in the Device spin box to set the collision energy to -38 eV.

j. Set the Quad MS/MS bias voltage:

   i. Click **Quad MS/MS Bias** in the Device Display box.

   ii. Enter -3.0 in the Device box to set the bias voltage to -3.0 V.

Ensure that the readbacks in the Device Display box are approximately equal to the set values. You might need to wait for a few minutes for the capillary and vaporizer temperatures to stabilize at their set values.

6. If you are operating in APCI/APPI or APPI only modes, turn on the APPI source as follows:

   a. Turn on the power to the APPI lamp by flipping the switch on the PhotoMate light source housing to the On position.

   b. Verify that the lamp is operating by looking through the front window of the Ion Max ion source housing.

7. Configure the Syringe Pump to automatically inject the reserpine sample solution into the sample loop:

   a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop view in the top right corner of the workspace (Figure 39).

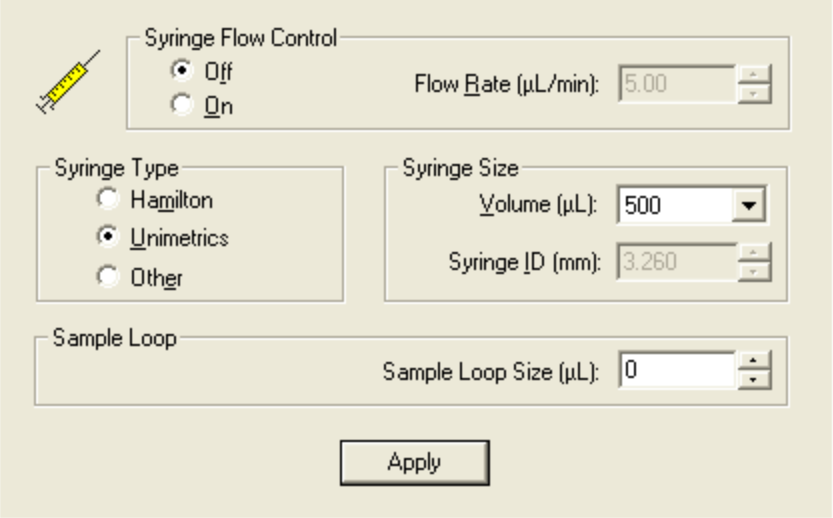


Figure 39. Syringe Pump and Sample Loop view, showing auto loop injection setup

b. Select the Off option button in the Syringe Flow Control group box to turn off the syringe pump.

- If you are using either a Unimetrics or Hamilton syringe, go to step 7.c.
- If you are not using either a Unimetrics or Hamilton syringe, go to step 7.e.

c. In the Syringe Type group box, select the Unimetrics (or Hamilton) option button to specify a Unimetrics (or Hamilton) syringe.

d. In the Syringe Size group box, select *500* (or the size of your syringe) from the Volume list box to specify that the volume of your syringe is 500 µL.

When you specify the syringe type and syringe volume, Tune Master automatically sets the proper syringe ID value. Go to step 7.f.

e. If you are using a make of syringe other than Unimetrics or Hamilton, you need to manually specify the syringe ID by doing the following:

i. Select the Other option button in the Syringe Type group box. This specifies that you are using a syringe other than Unimetrics or Hamilton syringe and enables the Syringe ID spin box.

ii. In the Syringe Size group box, select the volume of your syringe from the Volume list box.

iii. Enter the inner diameter of your syringe in the Syringe ID spin box.

f. In the Sample Loop box, enter **5** in the Sample Loop Size box to specify a loop size of 5 µL.

g. Click **Apply** to apply these settings. The syringe pump is now configured to fill the sample loop with the appropriate amount of sample.

8. Start the flow of solvent:

a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace (Figure 40).

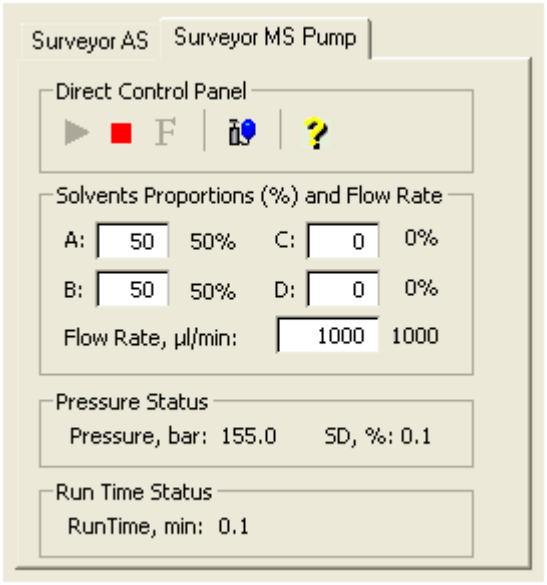


Figure 40. Inlet Direct Control view, showing high flow setup

Note The following procedure assumes that isopropyl alcohol and HPLC grade water are in the solvent bottles labeled A and B, respectively.

b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol / water at 1000 µL/min:

i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate group box, enter **50** in the text box labeled *A* to specify a delivery proportion of 50% solvent A.

ii. Enter 50 in the box labeled *B* to specify a delivery proportion of 50% solvent B.

iii. In the Flow Rate box, enter 1000 to set a flow rate of 1000 µL/min.

c. In the Direct Control Panel group box, click the **Start** button to start the Surveyor MS pump.

The system is now set up to automatically deliver reserpine to the ion source for optimizing the mass spectrometer with your compound.

Next you will optimize the compound dependent devices for your compound in APCI/APPI/MS/MS mode as discussed in the next section: Optimizing in APCI/APPI/MS/MS Mode Automatically with Your Compound.

Optimizing in APCI/APPI/MS/MS Mode Automatically with Your Compound

Optimize the mass spectrometer to maximize the ion transmission of your compound. Optimization is performed to fine tune compound dependent parameters such as discharge current, capillary temperature, and tube lens offset. It is recommended that you optimize the mass spectrometer after it has been successfully tuned and calibrated.

To automatically optimize the mass spectrometer in the APCI/APPI/MS/MS mode for the reserpine transition from *m/z* 609.281 to *m/z* 195.066

1. On the Control / Scan Mode toolbar, click **Compound Optimization Workspace** to display the Compound Optimization workspace (Figure 41).

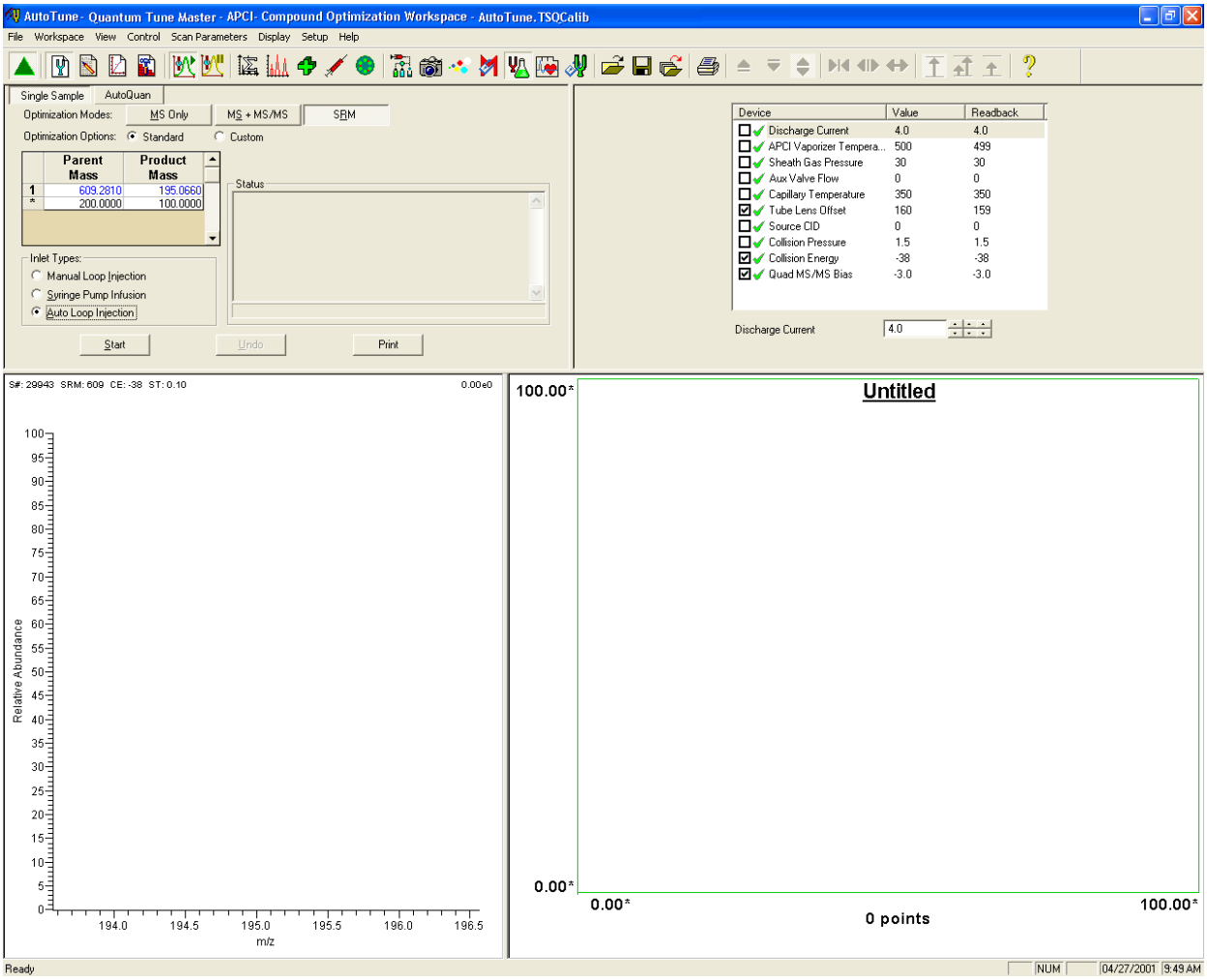


Figure 41. Compound Optimization workspace, APCI mode

2. In the Compound Optimization view in the top left corner of the workspace, ensure that the Single Sample page is displayed.

3. Set the optimization parameters for monitoring the reserpine transition from *m/z* 609.281 to *m/z* 195.066:

   a. Select **Optimization Modes: SRM** to optimize a selected reaction (Figure 42).

   b. Select **Optimization Options: Standard** to tune the default selection of devices. The tube lens offset and collision energy are the default compound sensitive devices that are optimized in this configuration.

   c. In the Optimization table, enter the parent mass 609.281 to set the parent mass of the SRM reaction to the ion at *m/z* 609.281.

   d. Enter the product mass 195.066 to set the product mass of the SRM reaction to the ion at *m/z* 195.066.

Note You need to select inlet type option button appropriate to the inlet mode you use to introduce your sample into the mass spectrometer. This procedure uses the Auto Loop Injection option.

   e. In the Inlet Types box, select the **Auto Loop Injection** option button to have the TSQ Quantum Ultra automatically inject the optimization solution.

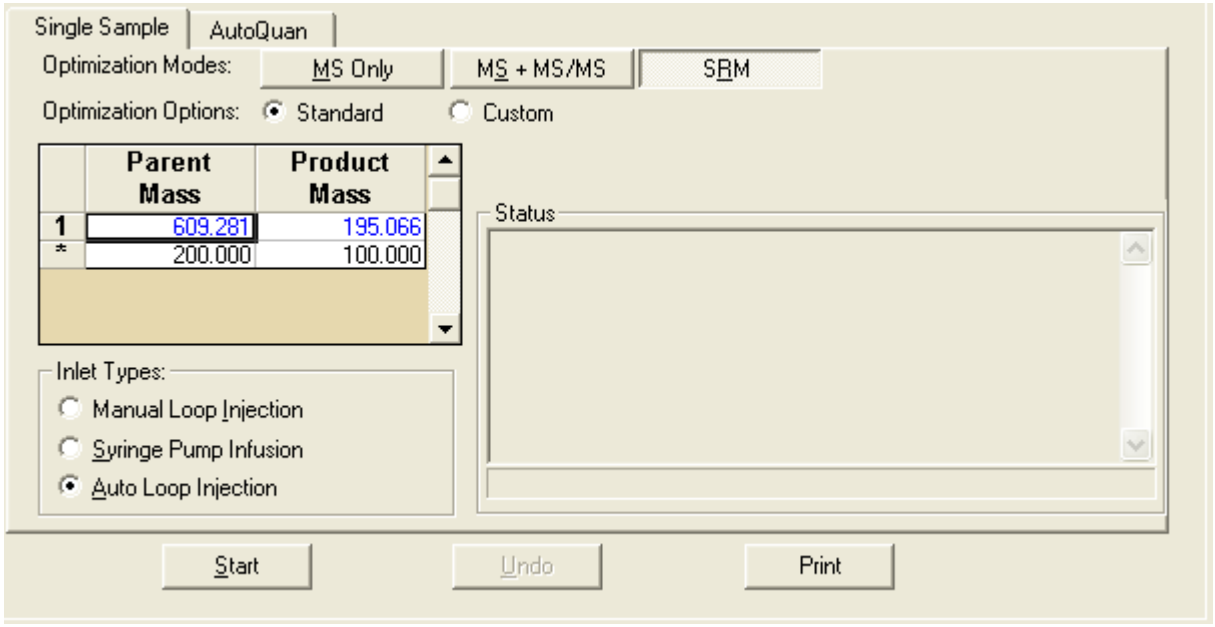


Figure 42. Compound Optimization view

4. Click **Start** to start the automatic tuning procedure.

Note If the syringe runs out of sample during the compound optimization procedure, the instrument pauses the automatic tuning and displays the message: Syringe out of sample, Reload and click OK. If this occurs, reload the syringe and click on OK to continue the optimization.

The message *Finish compound optimization* is displayed in the Status box in the Compound Optimization view when the compound optimization has completed successfully (Figure 43).

- If the compound optimization procedure finishes without errors and the breakdown curve of the 195.066 fragment is Gaussian-shaped (Figure 44) or is a smooth, positive-sloped curve, go to step 6.
- If errors occur during the compound optimization procedure or the breakdown curve of the 195.066 fragment oscillates, contains multiple peaks, or is excessively noisy, go to step 5.

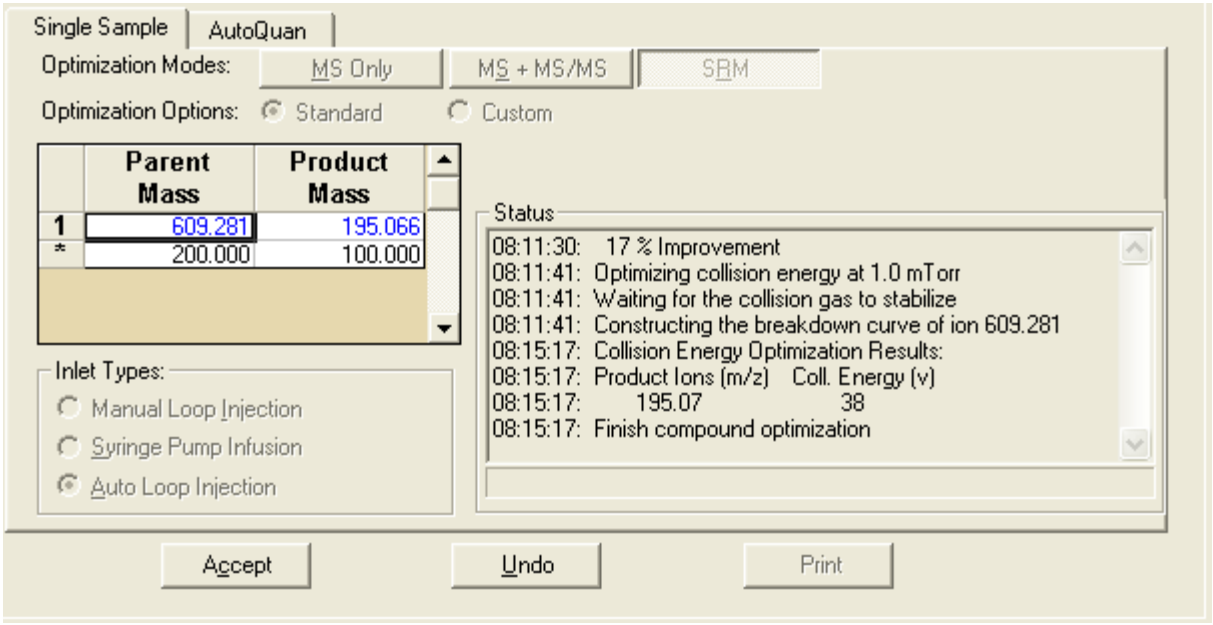


Figure 43. Compound Optimization workspace, showing the successful completion of compound optimization

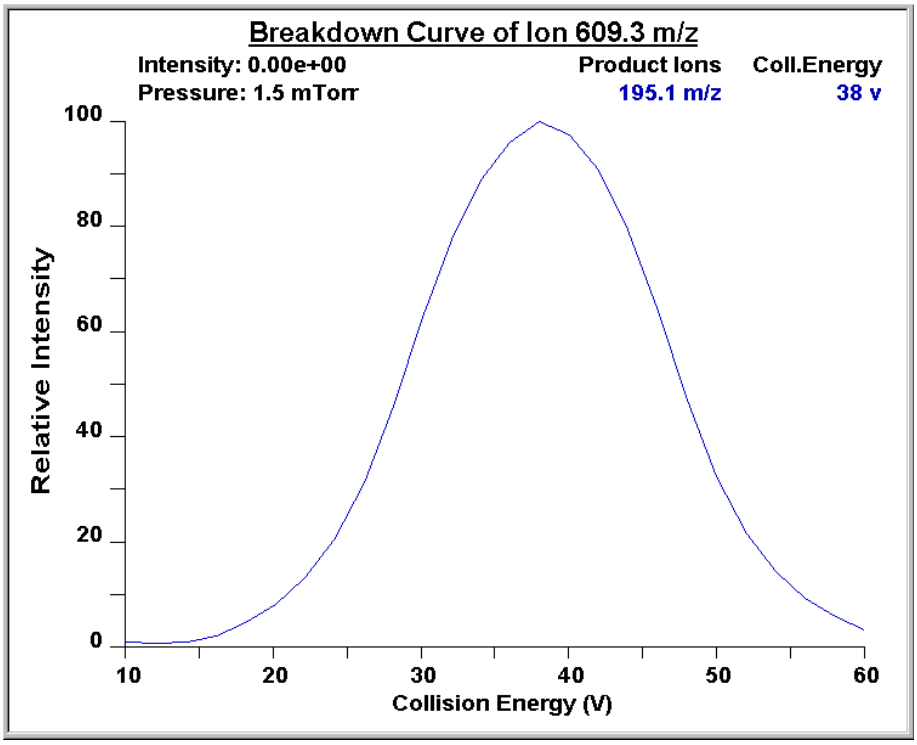


Figure 44. Breakdown curve of reserpine showing the relative intensity of the product ion at *m/z* 195.066 as a function of collision energy

5. If errors occurred during the compound optimization procedure, restore the previous mass spectrometer compound sensitive device settings by completing the following steps:

   a. Click **Undo** to restore the prior device settings.

   b. Click **Accept** to reload the prior device settings to the mass spectrometer.

   c. Troubleshoot and correct the situation that caused the optimization to fail.

   d. Go to step 4 of this procedure and restart the compound optimization procedure.

6. Click **Accept** to accept the results of the compound optimization.

Note Save the Tune Method while the mass spectrometer is On if any of the ion source parameters have been changed from their initial settings.

7. Save the Tune Method file by doing the following:

   a. Click **Save Tune As** to open the Save As dialog box.

b. Enter a file name (such as **APCI_reserpine**, or the name of your compound) for your Tune Method file in the File Name text box.

c. Click **Save** to save the Tune Method file.

If the file name that you entered in step 7.b is already in use, a message box appears and asks whether you want to replace the existing file.

- If you do want to overwrite the existing file, click **Yes**.
- If you do not want to overwrite the existing file, click **No**. Then, change the name in the File Name text box and click **Save**.

The mass spectrometer is now optimized in APCI/APPI/MS/MS mode for the compound reserpine (or for your compound).

Saving the Tune Method

You can save the settings obtained in a tune method specific to your particular analyte and solvent flow rate. These settings can be recalled to be used again. Using the example of the last section, the settings obtained using reserpine would be saved. This tune method can be recalled and used as a starting point for optimizing the mass spectrometer on reserpine at a different flow rate.

Note Save the Tune Method while the mass spectrometer is On.

To save your APCI/APPI/MS tune method

1. Choose **File > Save As** to display the Save As dialog box (Figure 45).

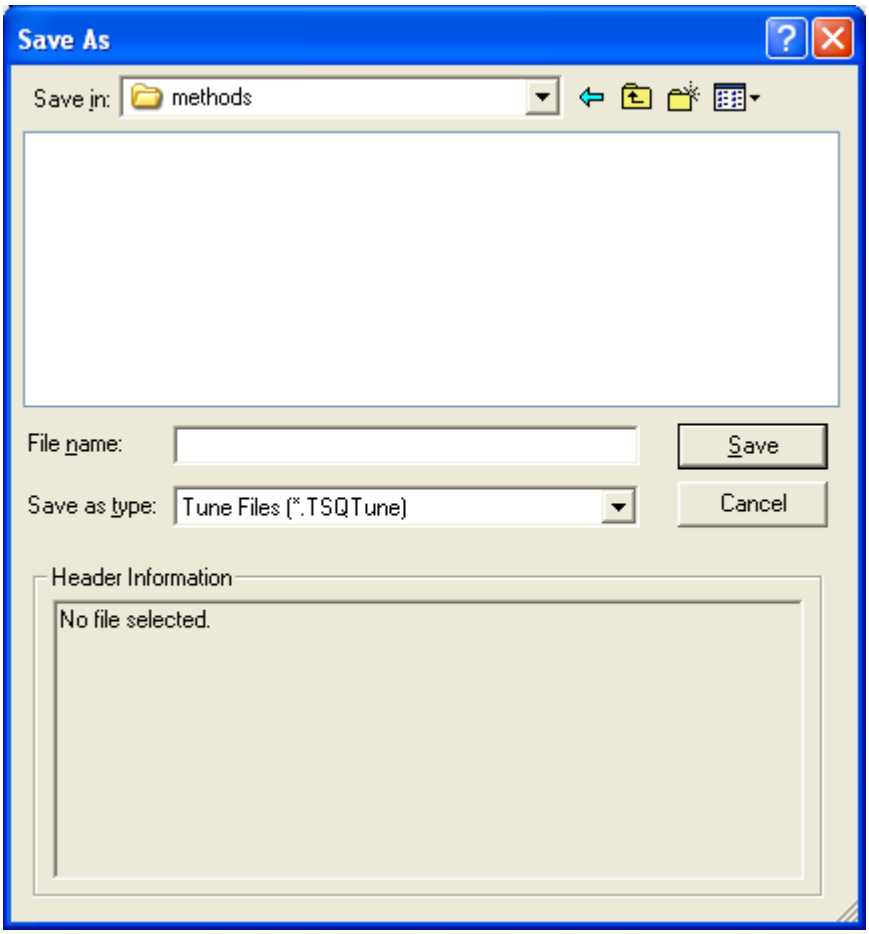


Figure 45. Save As dialog box, showing files in the folder *C:\Xcalibur\methods*

2. In the Save In list box, select the *C:\Xcalibur\methods* folder.

3. Click the File Name text box, and then enter **APCImyTune** to name the Tune Method *APCImyTune.TSQTune*.

4. Click **Save** to save the Tune Method, and return to the Tune Plus window. Note that the Tune Method is named *APCImyTune.TSQTune.*

Before you acquire data, go to the next topic: Cleaning the Mass Spectrometer After Tuning in APCI Mode.

Cleaning the Mass Spectrometer After Tuning in APCI Mode

OnStandby

Use the following procedure to clean the mass spectrometer after tuning on your analyte of interest.

1. Click on the On/Standby button to put the mass spectrometer in Standby mode. When the mass spectrometer is in Standby, the TSQ Quantum Ultra mass spectrometer turns off the vaporizer heater, corona discharge voltage, and syringe pump. The mass spectrometer stops scanning and freezes the displays for the Spectrum and Graph views.

CAUTION Always place the mass spectrometer in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the mass spectrometer is On could be unsafe. (The TSQ Quantum Ultra mass spectrometer automatically turns off when you open the API source; however, it is best to take this added precaution.)

2. Remove the syringe from the syringe pump holder:
   a. Squeeze the blue buttons, and pull back on the syringe pump handle to free the syringe.
   b. Remove the syringe from the holder.
   c. Disconnect the tip of the syringe needle from the Teflon tubing.
3. Clean the syringe thoroughly:
   a. Clean the syringe with a solution of 5% formic acid in water.
   b. Rinse the syringe with a solution of 50:50 methanol / water.
   c. Use acetone to rinse the syringe. Repeat this step several times.

CAUTION AVOID BURNS. The APCI vaporizer heater can reach temperatures of 600 ×C. Always allow the APCI probe to cool to ambient temperature, for approximately 20 min, before handling or removing the APCI probe from the APCI flange.

CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin if you handle it without caution.

4. Remove the Ion Max ion source housing as described in "Removing the Ion Max Ion Source Housing" on page 26.

5. Flush the sample transfer line, sample tube, and APCI probe thoroughly with a solution of 5% formic acid in water (or with another appropriate solvent):

Note The solvent that you use to flush the sample transfer line, sample tube, and APCI probe assembly depends on the solvent system you use to dissolve your samples. For example, if you are using a buffered solution of a high concentration, an acidic solution is appropriate.

   a. Fill a clean, 250 µL Unimetrics syringe with an appropriate solvent.
   b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the free end of the Teflon tube.
   c. Flush the sample transfer line, sample tube, and APCI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the APCI probe on the inside of the probe assembly. Use a lint-free tissue to gently remove the excess solution as it exits the probe.
   d. Remove the needle of the syringe from the Teflon tube.

6. Repeat step 5 with a solution of 50:50 methanol / water.

7. Reinstall the Ion Max ion source housing as described in "Installing the Ion Max Ion Source Housing" on page 33.

Your TSQ Quantum Ultra mass spectrometer is now ready to perform experiments in APCI/APPI mode. For information regarding the maintenance of the Ion Max APPI source please refer to Chapter 7, "Maintenance."

Chapter 7 Maintenance

This chapter describes routine APCI/APPI combination probe maintenance procedures that must be performed to ensure optimum performance of the probe. Most of the procedures involve cleaning. It is your responsibility to maintain the APCI/APPI combination probe by performing the routine maintenance procedures on a regular basis, as they are described in this chapter.

The APCI/APPI combination probe requires minimal maintenance. Periodically, you need to clean the components of the APCI/APPI combination probe to remove salts or other contaminants. The frequency of cleaning depends on the types and amounts of samples and solvents that are introduced into the system.

This chapter contains the following sections:

Maintaining the APCI Probe

The APCI probe requires a minimum of maintenance. The APCI sample tube (100-µm ID fused-silica tubing) is pre-loaded at the factory. However, if the sample tube becomes obstructed with salt precipitates or is broken, you need to replace it. Also, you might need to remove and clean the APCI nozzle.

Figure 7 and Figure 8 on Figure 9 show the major components of the APCI probe.

Note You should flush the APCI probe at the end of each working day by flowing a 50:50 methanol / water solution from the LC through the APCI source.

Wear clean gloves when you handle APCI probe components.

The following procedures are discussed in this section:

- Removing the APCI Nozzle
- Cleaning the APCI Probe Components
- Removing the APCI Sample Tube
- Installing a New APCI Sample Tube
- Reassembling the APCI Probe

Removing the APCI Nozzle

To remove the APCI nozzle from the APCI probe

CAUTION Do not break the APCI sample tube. In step 1, carefully pull the APCI nozzle straight back from the APCI probe to prevent the sample tube from touching the sides. If the sample tube hits the sides of the vaporizer, it can break.

CAUTION AVOID BURNS. At operating temperatures, the APCI vaporizer can severely burn you! The APCI vaporizer typically operates between 350 and 500 ×C. Always allow the heated vaporizer to cool to room temperature (for approximately 20 min) before you touch or remove these components.

See Figure 8 for the location of parts.

1. Place the instrument in Standby mode.

2. Hold onto the APCI probe body with one hand and grasp the head of the APCI nozzle assembly. Rotate the head of the nozzle assembly until the flat sides of the head are facing towards the retention flanges (See Figure 8). The nozzle assembly is now free of the probe.

3. Carefully pull the nozzle assembly straight out of the APCI probe.

4. Place the assembly on a clean, lint free tissue.

Cleaning the APCI Probe Components

To clean the APCI probe components

1. Remove the APCI nozzle from the probe body.

2. Check the condition of the O-rings on the APCI nozzle.

3. Clean the interior APCI components (excluding the ceramic heater) with a 50:50 solution of HPLC-grade methanol and distilled/deionized water and a lint-free swab. Dry the components with nitrogen gas and place them on a lint free tissue.

4. Reinstall any O-rings you have removed while cleaning.

If you do not want to replace the APCI sample tube, reinstall the APCI nozzle (P/N 97055-60089) as described in the topic "Reassembling the APCI Probe" on page 91.

Removing the APCI Sample Tube

To remove the APCI sample tube from the APCI manifold

1. With a 3/8-in. open-end wrench, remove the sample tube inlet fitting (P/N 70005-20250), 0.239-in. ID O-ring (P/N 00107-04000), and sample tube from the APCI manifold (Figure 46).

2. Remove the exit-end nut (P/N 70005-20220), 0.016-in. ID, PEEK ferrule (P/N 00101-18120), and sample tube from the sample tube inlet fitting.

3. Discard the old sample tube.

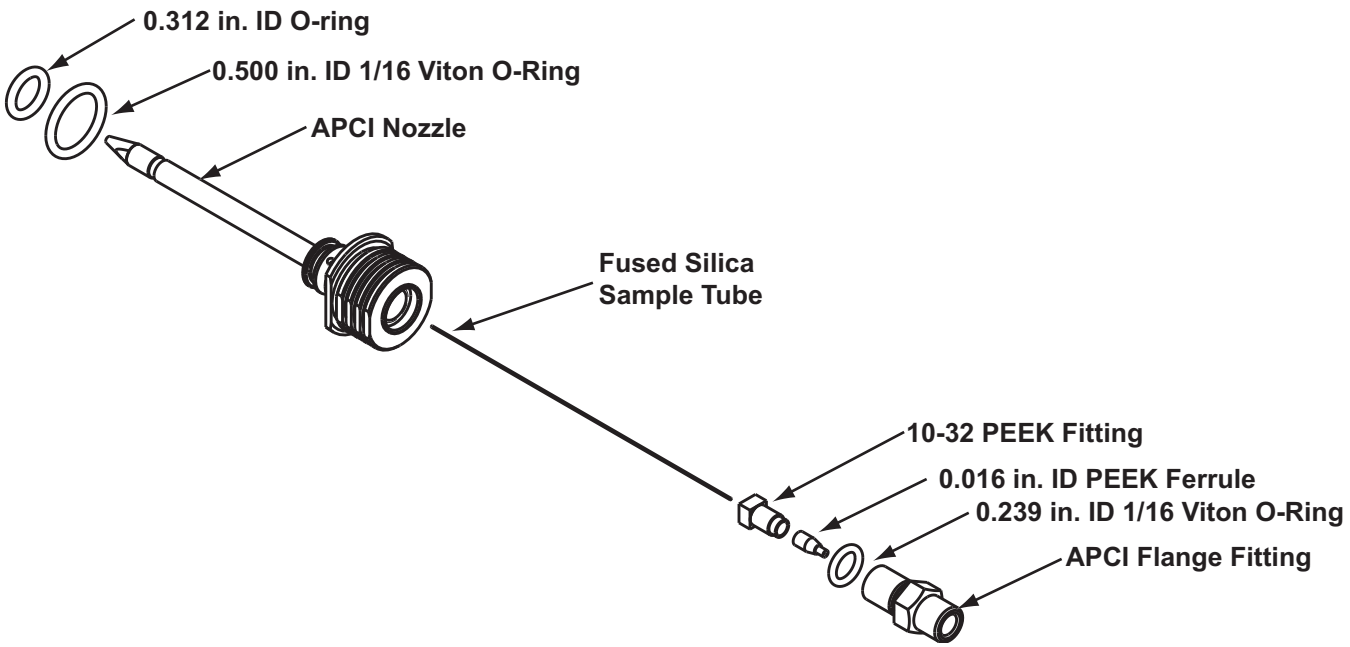


Figure 46. APCI sample tube connection

Installing a New APCI Sample Tube

To install a new APCI sample tube

1. Use a fused-silica cutting tool to cut a piece of 100 µm ID, 390 µm OD fused-silica tubing (P/N 00106-10498) to a length of approximately 15 cm (6 in.). Ensure that you squarely cut the ends of the fused-silica tubing.

2. Slide the PEEK fitting (P/N 70005-20220) and ferrule (P/N 00101-18120) onto the length of the fused-silica tubing. See Figure 46.

3. Check the condition of the 0.239-in. ID O-ring (P/N 00107-04000) on the sample tube inlet fitting. Replace it if necessary.

4. Insert the fused-silica tubing into the sample tube inlet fitting.

5. Slide the PEEK fitting and ferrule down the fused-silica tubing and into the sample tube inlet fitting.

6. Tighten the PEEK fitting to secure the new sample tube (fused-silica tubing).

7. Use a fused-silica cutting tool to cut the exit end of the sample tube so that approximately 1 mm protrudes past the tip of the APCI nozzle. See Figure 47.

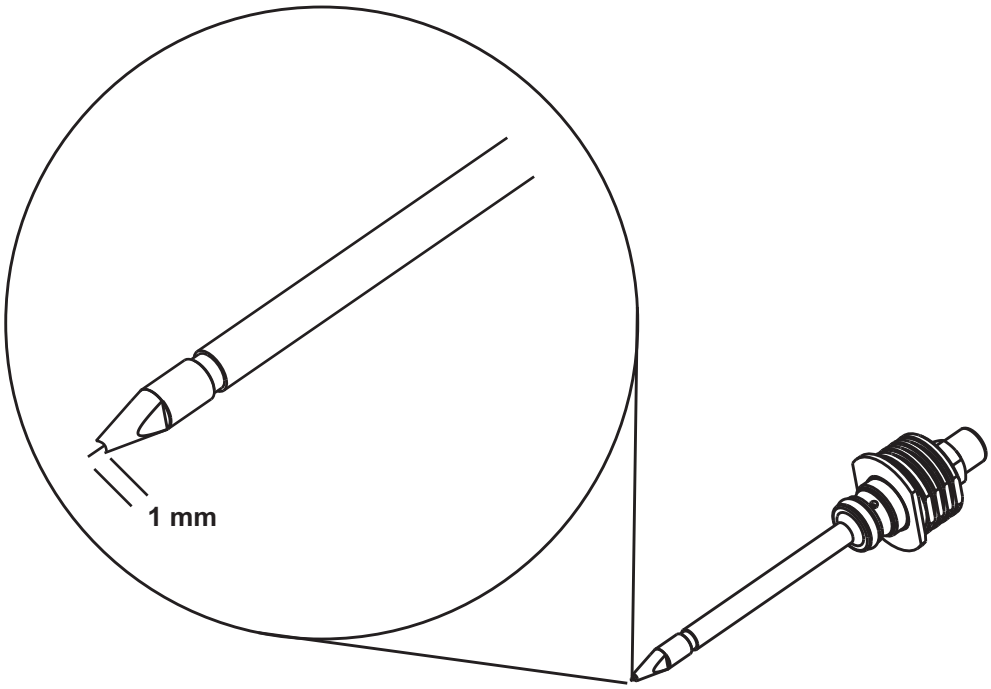


Figure 47. Proper position of the exit end of the APCI sample tube

Reassembling the APCI Probe

To reassemble the APCI probe

1. With one hand holding the APCI probe body to keep the probe from turning, carefully insert the APCI nozzle into the APCI probe.

2. With the flat sides of the APCI nozzle head facing upwards towards the retention flanges on the probe body, seat the nozzle head completely flat against the probe.

3. To secure the APCI nozzle in the probe, rotate the head of the nozzle 90 degrees to secure the round sides of the nozzle head in the retention flanges.

To reinstall the probe in the Ion Max API source housing, see "Installing the APCI Probe" on page 27.

Maintaining the PhotoMate Light Source

This section includes the following topics for cleaning and replacing components of the PhotoMate light source:

- Cleaning and Polishing the Window of the VUV Lamp
- Replacing the VUV Lamp

Cleaning and Polishing the Window of the VUV Lamp

Typically, flowing a 50:50 methanol / distilled water solution from the LC through the APCI/APPI combination probe at the end of each working day will clean the VUV lamp.

In some instances, the VUV lamp window might require additional cleaning or polishing. (A weak ion signal might indicate a build up of salts or other contaminants on the VUV lamp window.)

To clean or polish the VUV lamp window

CAUTION AVOID BURNS. The APCI vaporizer heater can reach temperatures of 600 ×C. Always allow the APCI/APPI combination probe to cool to ambient temperatures before handling or removing the APCI/APPI combination probe.

1. Ensure that the MS system is in Standby and the LC flow has been halted.
2. Unplug the power/communication cable from the PhotoMate light source.
3. Open the front door of the Ion Max ion source housing.

CAUTION Do not apply solvent to the VUV lamp while it is hot. The VUV lamp can crack.

AVOID INJURY. The corona discharge needle is very sharp. Use caution when cleaning the VUV lamp.

4. Use a cotton swab that is soaked with LC solvents to wash the window (end) of the VUV lamp. Ensure that no lint remains on the lamp.

5. Inspect the window of the VUV lamp:

- If no material remains on the window, close and secure the front door of the Ion Max ion source housing and proceed with your application.
- If you were unable to remove all material from the window, you need to remove the VUV lamp and polish the window. Continue with step 7.

CAUTION Always wear clean gloves when you are handling a functional VUV lamp. Fingerprints on the lamp can cause the lamp to fail when it is in use.

6. While wearing clean gloves, grasp the VUV lamp and pull the lamp out.
   - If you are able to remove the VUV lamp, go to step 10.
   - If you are having trouble removing the lamp, you need to push it out from the top. Go to step 7.
7. Remove the PhotoMate light source by removing the retaining screws that hold the assembly to the adapter ring, then pulling the assembly straight out until it is free from the Ion Max ion source housing.
8. Remove the box top from the PhotoMate light source:
   a. Remove the light source power cord from the light source.
   b. Remove the 4 Phillips screws that secure the box top to the light source.
   c. Remove the box top from the electronics PCB, but keep the fan power cable connected.
9. Insert an Allen key into the lamp hole in the electronics PCB. See Figure 48. Gently push out the VUV lamp with the Allen key.

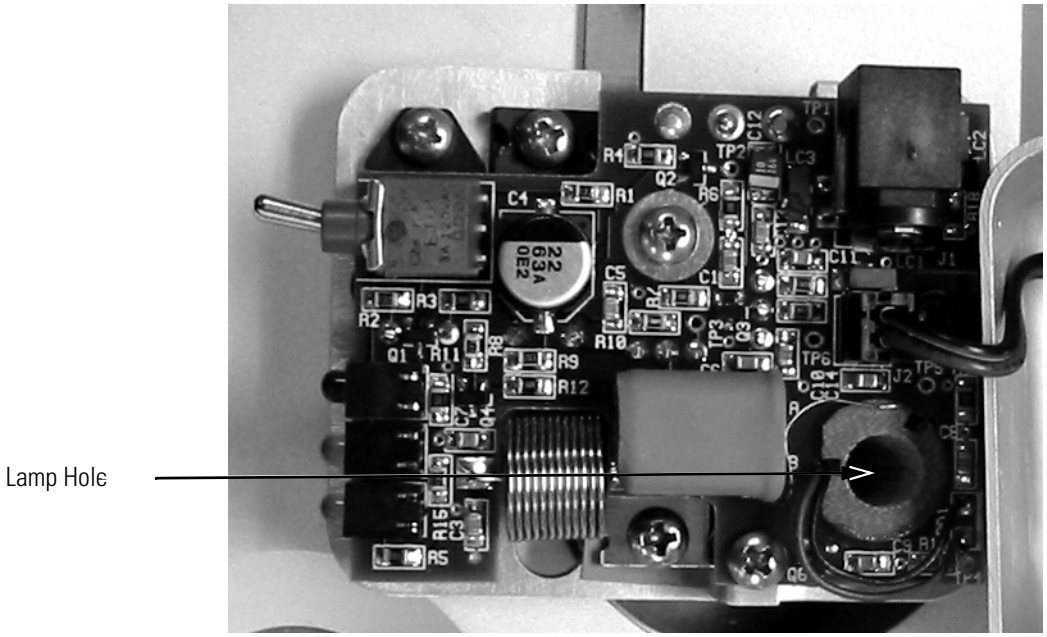


Figure 48. Light source with box top removed, showing the lamp hole

10. Dip a cotton swab in isopropyl alcohol, then dip the wet swab in aluminum oxide polishing compound (5 micron powder).

11. Polish the lamp window with the swab and polishing compound until the material is removed.

12. Use another cotton swab soaked in isopropyl alcohol to remove the polishing compound from the lamp window.

13. While wearing clean gloves, install the VUV lamp by pushing it all the way to the stopping position in the light source assembly.

14. If necessary, reinstall the box top:

    a. Tip the box top forward and make sure the lamp On/Off switch passes through the hole in the box top. Then push the box top down over the fan connector.

    b. Reinstall the four screws to secure the box top.

    c. Reconnect the light source power cord to the light source.

15. If necessary, reinstall the PhotoMate light source in the Ion Max ion source housing.

Replacing the VUV Lamp

You can minimize the chance of breaking the VUV lamp by doing the following:

- Always wear clean gloves when you are handling the VUV lamp. Fingerprints on the lamp can cause the lamp to fail when it is in use.
- Do not spray solvent on the VUV lamp while it is hot. The VUV lamp can crack.

If the VUV lamp beaks or fails, use the following procedures to replace it:

- Removing the Old VUV Lamp
- Installing the New VUV Lamp

Removing the Old VUV Lamp

To remove the old VUV lamp

1. Place the lamp On/Off switch in the Off (O) position.
2. Turn off the flow of liquid from the LC (or other sample introduction device) to the APCI/APPI combination probe:
3. Put the mass spectrometer in Standby mode

CAUTION AVOID BURNS. At operating temperatures, the APCI vaporizer, ion sweep cone, spray cone, and ion transfer capillary can severely burn you! The APCI vaporizer typically operates at 400 to 600 ×C and the ion sweep cone, spray cone, and ion transfer capillary typically operate at 100 to 300 ×C.

Allow the heated vaporizer, ion sweep cone, spray cone, and ion transfer capillary to cool to room temperature, for approximately 20 min, before you touch or remove these components.

4. Open the lid of the APCI/APPI combination probe.

CAUTION Always wear clean gloves when you are handling a functional VUV lamp. Fingerprints on the lamp can cause the lamp to fail when it is in use.

5. While wearing clean gloves, grasp the VUV lamp and pull the lamp out.
   - If you are able to remove the VUV lamp, go to the next section Installing the New VUV Lamp.

- If you are having trouble removing the lamp, you need to push it out from the top.

6. Remove the box top from the PhotoMate light source:

   a. Remove the light source power cord from the light source.

   b. Remove the 4 Phillips screws that secure the box top to the light source.

   c. Remove the box top from the electronics PCB, but keep the fan power cable connected.

7. Insert an Allen key into the lamp hole in the electronics PCB. See Figure 48 on page 94. Gently push out the VUV lamp with the Allen key.

Installing the New VUV Lamp

To install the new VUV lamp

CAUTION Always wear clean gloves when you are handling a functional VUV lamp. Fingerprints on the lamp can cause the lamp to fail when it is in use.

1. While wearing clean gloves, install a new VUV lamp by pushing it all the way to the stopping position in the light source assembly.

2. If necessary, reinstall the box top:

   a. Tip the box top forward and make sure the lamp On/Off switch passes through the hole in the box top. Then push the box top down over the fan connector.

   b. Reinstall the four screws to secure the box top.

   c. Reconnect the light source power cord to the light source.

3. Close and secure the lid of the APCI/APPI combination probe housing.

4. Place the lamp On/Off switch in the On position.

5. Check that the Lamp On and Drive On LEDs illuminate. Look through the Ion Max window and verify that the VUV lamp is on.

Note If the Drive On and Lamp On LEDs do not illuminate, verify that the front door of the Ion Max ion source housing is secured. The front door of the source housing must be closed and locked to engage the interlock.

Chapter 8 Replaceable Parts

This chapter contains part numbers for replaceable and consumable parts for the Ion Max APCI probe and Photomate light source. To ensure proper results in servicing the ion source, order only the parts listed or their equivalent. Please refer to the figures on the subsequent pages for the location of each part listed.

The replaceable parts are as follows:

APPI Lamp Kit OPTON-20026
- APPI circuit assembly & fan, Ion Max 00950-10010
- Cable, APPI, Ion Max 00950-10011
- APPI lamp 00950-10002
- APPI power supply 00950-10012

APCI Probe OPTON-20012

APCI Probe Nozzle Assembly 97055-60089
- Ferrule, 0.016 ID PEEK HPLC 00101-18120
- Tubing, fused silica 150 µm ID X 390 µm OD 00106-10498
- O-ring, 0.239 in. ID 1/16 in. Viton 00107-04000
- O-ring, 0.312 in. ID 1/16 in. 00107-04500
- O-ring, 0.500 in. ID 1/16 in.Viton 00107-05600
- Fitting, 10-32 male nut PEEK 70005-20220
- Fitting, APCI flange 70005-20250
- Nozzle, APCI probe 97055-20221

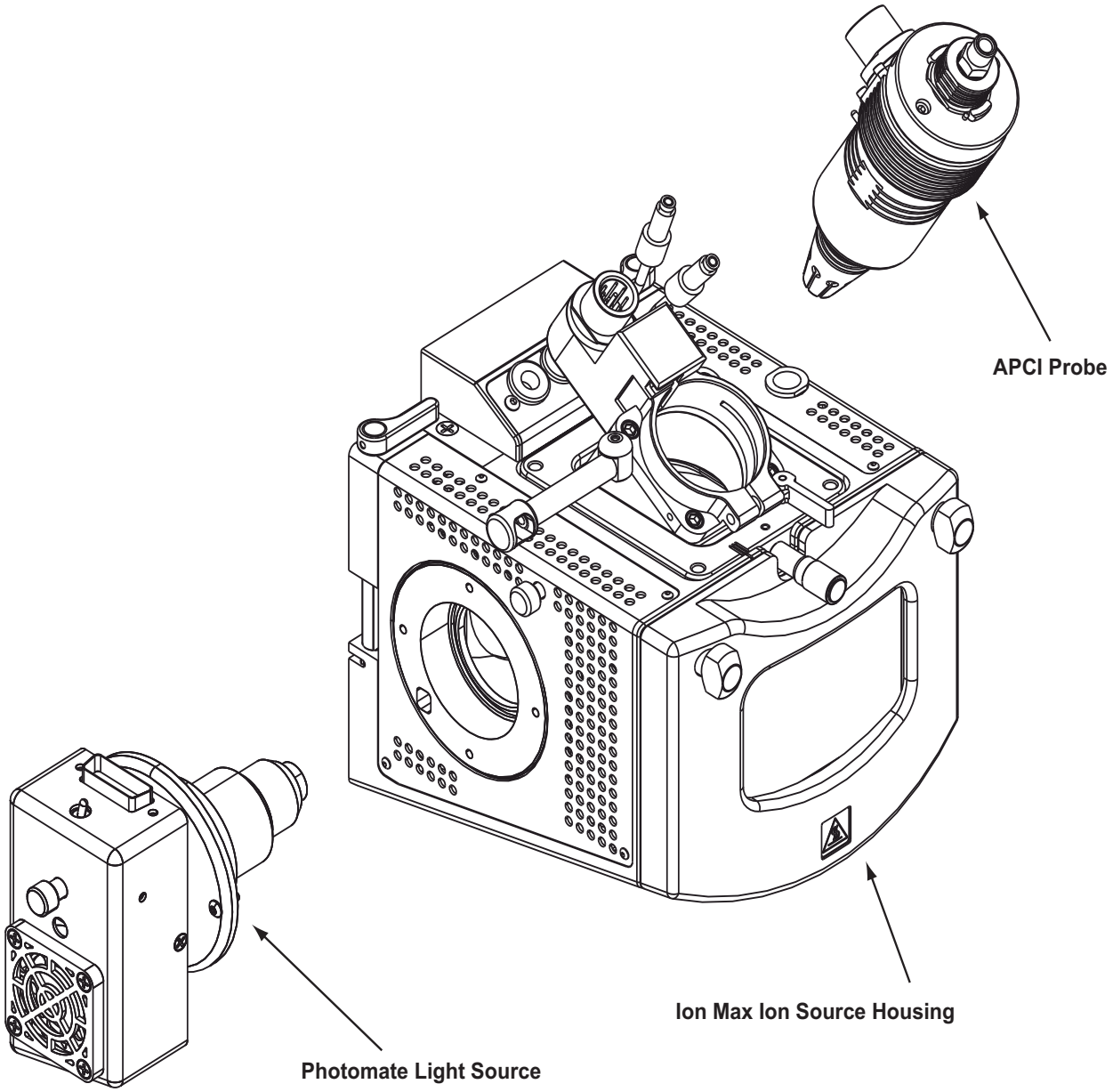


Figure 49. Ion Max ion source housing, showing the APCI probe and PhotoMate light source

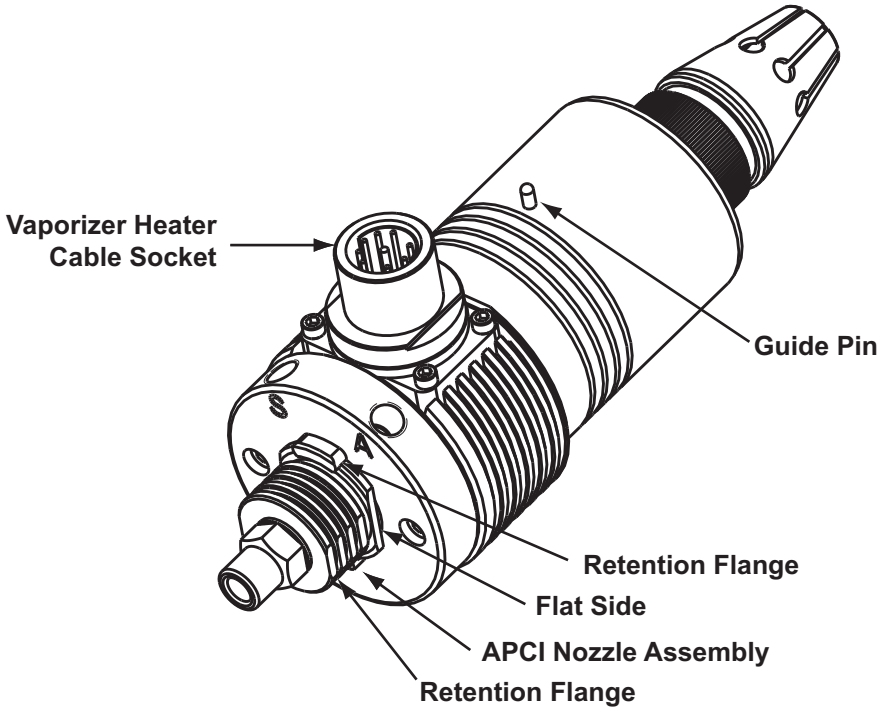


Figure 50. APCI Probe Assembly (OPTON-20012)

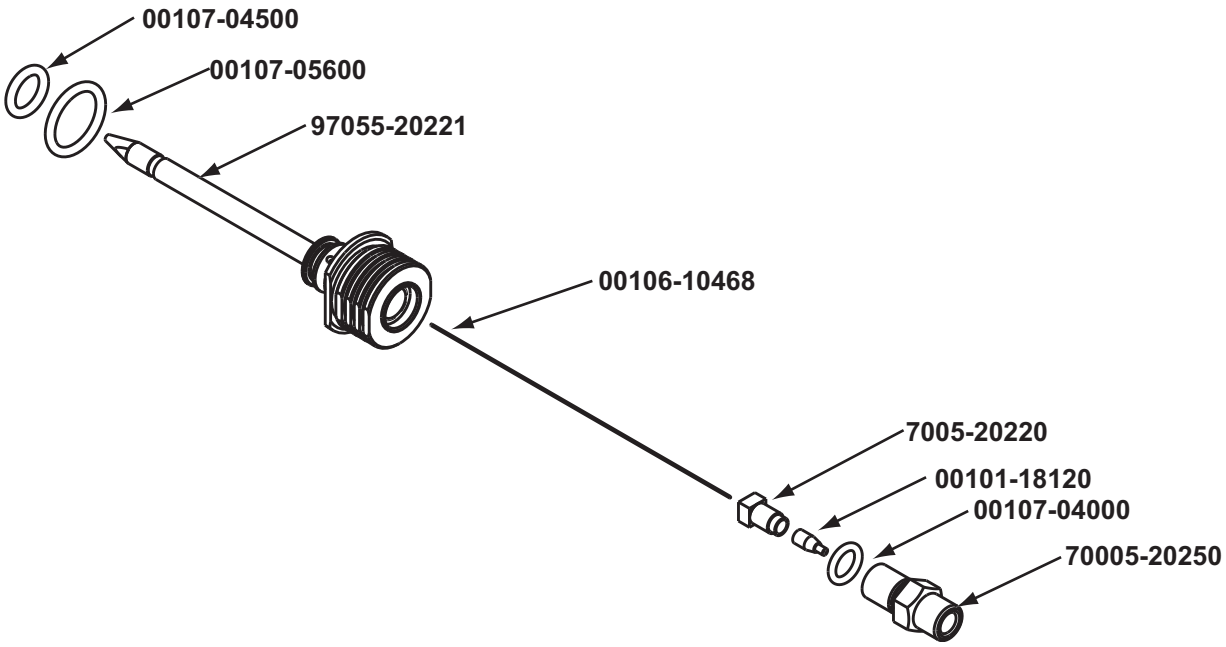


Figure 51. APCI probe nozzle assembly (P/N 97055-60089)

Appendix A Installing the PhotoMate Light Source Adapter Ring

This chapter provides information on installing the Photomate light source adapter ring in the Ion Max ion source housing. You only need to perform this procedure the first time you use your source. If you do not want to use the APPI lamp for a particular experiment, you can install the smaller viewport window that fits within the Photomate light source adapter ring. There is no need to remove the ring when not in APPI mode as long as the small viewport window is installed.

Install the APPI adapter ring as follows:

1. Stop the flow of sample solution from the LC.
2. Place the MS detector in standby.
3. Remove the currently installed API probe as described in the **Ion Max API Source Hardware Manual**.
4. Unscrew and remove the probe alignment screw on the left side of the Ion Max ion source housing. See Figure 52.
5. Using the special hex head wrench provided with your APPI source, remove the four retaining screws from the left hand side Ion Max ion source housing cover.
6. Pull the cover straight out and free from the Ion Max ion source housing.
7. Remove the two screws that hold the Ion Max viewport frame in place. Be careful not to allow the window to fall free and break. See Figure 53.
8. Align the APPI adapter ring so that the flat side fits against the rear of the Ion Max ion source housing and so that both holes in the ring align with the holes in the Ion Max ion source housing. Secure the ring with the two screws that you removed from the viewport frame. See Figure 54.

9. Replace the source housing cover and secure the cover with all four hex head screws.

10. Replace the probe alignment screw in the Ion Max ion source housing.

11. Complete the installation:

    - If you are going to proceed with an APPI experiment, install the APCI probe and PhotoMate light source as described in **Chapter 3, "Setting Up the Ion Source for Acquiring Data in APCI/APPI/MS/MS Mode"** .

    or

    - If you will not be performing an APPI experiment at this time, complete steps 12 to 14 before continuing with your experiments. Do not attempt to operate any Ion Max ion source without the viewport window installed.

12. Carefully install the small viewport window in the APPI adapter ring. See Figure 55.

13. Secure the retainer ring with four hex head screws. See Figure 56.

14. Reinstall your API probe in the Ion Max ion source housing.

You are now ready to perform ESI or APCI experiments or to install the PhotoMate light source.

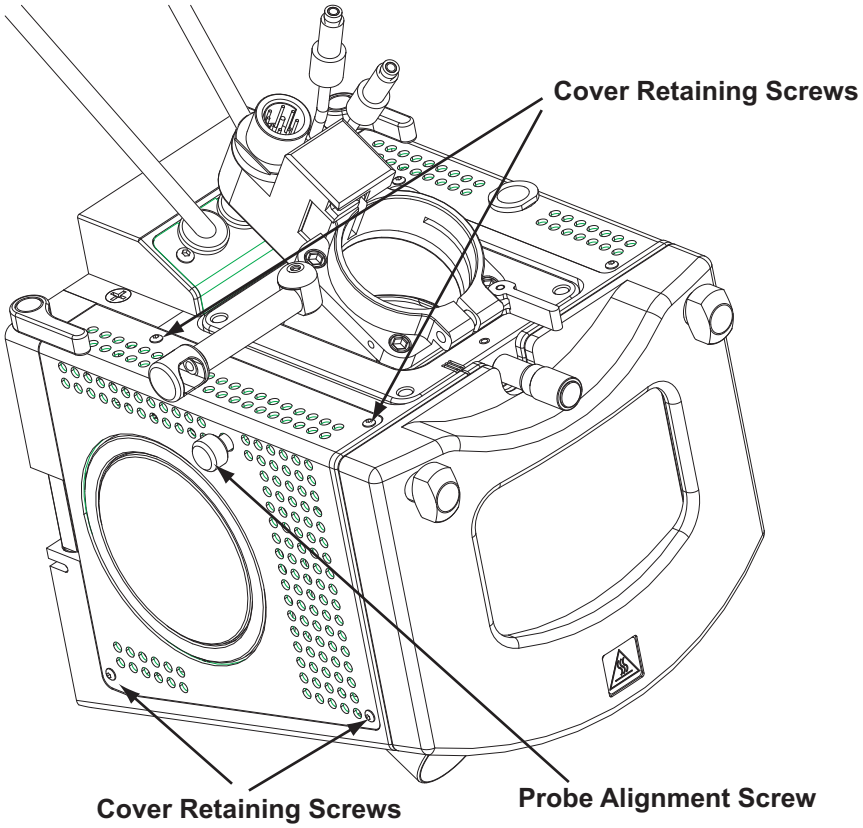


Figure 52. Left side of the Ion Max ion source housing, showing the probe alignment screw and cover retaining screws

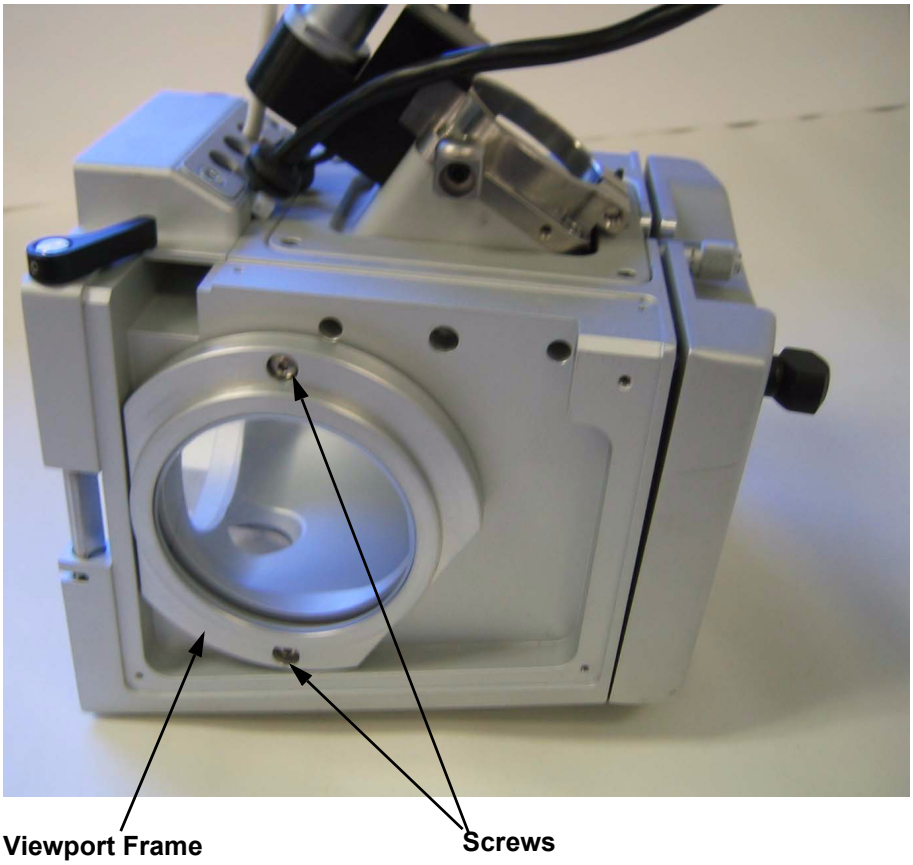


Figure 53. Ion Max ion source housing with left cover removed, showing the viewport frame and screws

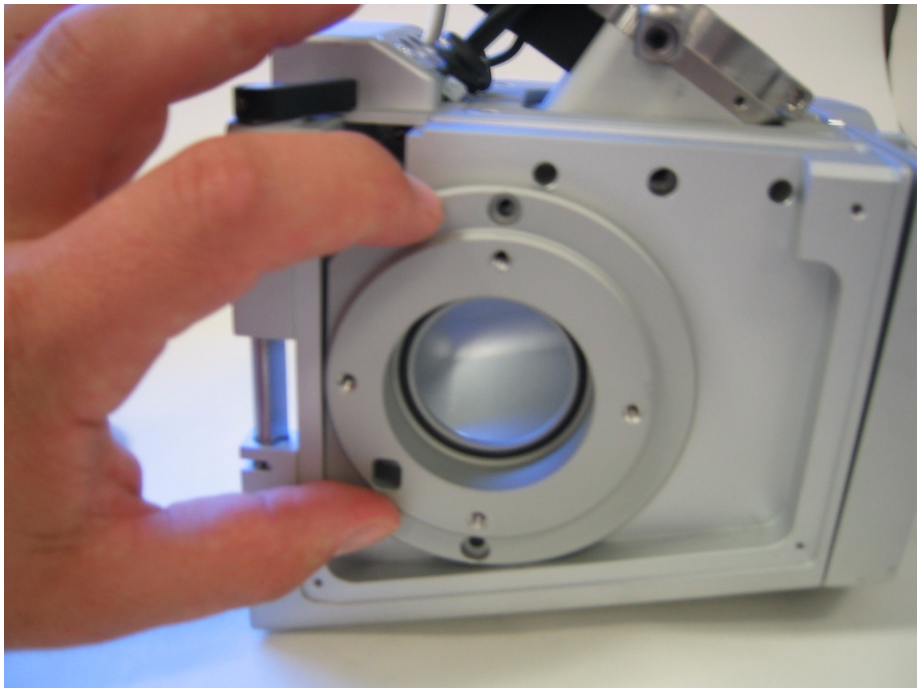


Figure 54. Aligning the APPI adapter ring

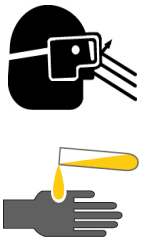
Figure 55. Inserting the small viewport window into the APPI adapter ring

Figure 56. Completed APPI adapter ring installation

Appendix B Reserpine Solution Formulations

This appendix provides instructions for preparing the tuning and calibration solution and the reserpine solution that is used to optimize the tune of the mass spectrometer.

The sections in this appendix are as follows:



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent.

Always take safety precautions when you handle chemicals and unknown samples. READ AND UNDERSTAND THE HAZARDS OF THE CHEMICALS USED IN THE FOLLOWING PREPARATIONS. Dispose of all laboratory reagents by the appropriate method for a specific reagent or solvent.

Material Safety Data Sheets (MSDS) provide summarized information on the hazards and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the MSDSs for each chemical you use. Examples of potentially hazardous chemicals used in procedures throughout this manual are as follows:

- Acetic acid
- Methanol
- Reserpine

Follow the directions given below to prepare a stock solution of reserpine. Then, use serial dilutions of the stock solution to make the compound optimization solution.

Reserpine Stock Solution

Prepare 10 mL of a 1 µg/µL Reserpine Stock Solution in 1% acetic acid

15. Carefully weigh 10 mg of Reserpine.
16. Transfer the 10 mg of reserpine into a 10 mL volumetric flask.
17. Add 1% acetic acid in methanol until the 10 mL mark is reached.
18. Mix the solution thoroughly.
19. Transfer the solution to a clean dry vial.
20. Label the vial *Reserpine Stock Solution (1 µg/µL)*.

LCQ Deca XP MAX Reserpine Sample Solution

Prepare 1 mL of the sample solution of 1 pg/µL (8.2 fmol/µL) in a solution of 1% acetic acid in methanol

1. Pipette 1 mL of reserpine stock solution (1 µg/µL) into a 10 mL volumetric flask and bring the volume up to the 10mL mark using methanol (1% acetic acid). Mix the resulting solution thoroughly and label it as Reserpine (100 ng/µL).

2. Place 75 mL of methanol (1% acetic acid) into a clean 100 mL volumetric flask.

3. Using the 10 µL syringe provided in the accessories kit or a calibrated pipette, add 5 µL of the Reserpine (100 ng/µL) solution to the 75 mL of methanol (1% acetic acid) in the 100 mL volumetric flask.

4. Rinse the pipette twice with the 75 mL of methanol (1% acetic acid) contained in the volumetric flask by dipping the pipette tip into the solution and rinsing it back into the flask.

5. Carefully bring up this volume with methanol (1% acetic acid) until the lower part of the meniscus is level with the 100 mL marking.

6. Mix this solution thoroughly. Label it as Reserpine (5 pg/µL)

7. Add 20 mL of the Reserpine (5 pg/µL) to another clean 100 mL volumetric flask, and carefully bring up this volume with methanol (1% acetic acid) until the lower part of the meniscus is level with the 100 mL marking.

8. Mix the solution thoroughly.

9. This is the LCQ Deca XP MAX Reserpine Sample Solution (1 pg/µL).

LTQ Reserpine Sample Solution

Prepare 1 mL of the sample solution of 125 fg/µL (205 amol/µL) in 1% acetic acid in 50:50 methanol / water

1. Pipette 1 mL of reserpine stock solution (1 µg/µL) into a 10 mL volumetric flask and bring the volume up to the 10 mL mark using methanol (1% acetic acid). Mix the resulting solution thoroughly and label it as Reserpine (100 ng/µL).

2. Place 75 mL of methanol (1% acetic acid) into a clean 100 mL volumetric flask.

3. Using the 10 µL syringe provided in the accessories kit or a calibrated pipette, add 5 µL of the Reserpine (100 ng/µL) solution to the 75 mL of methanol (1% acetic acid) in the 100 mL volumetric flask.

4. Rinse the pipette twice with the 75 mL of methanol (1% acetic acid) contained in the volumetric flask by dipping the pipette tip into the solution and rinsing it back into the flask.

5. Carefully bring up this volume with methanol (1% acetic acid) until the lower part of the meniscus is level with the 100 mL marking.

6. Mix this solution thoroughly. Label it as Reserpine (5 pg/µL)

7. Add 20 mL of the Reserpine (5 pg/µL) to another clean 100 mL volumetric flask, and carefully bring up this volume with methanol (1% acetic acid) until the lower part of the meniscus is level with the 100 mL marking.

8. Mix the solution thoroughly.

9. Label this Reserpine (1 pg/µL).

10. Transfer 100 µL of the 1 pg/µL solution into a clean polypropylene tube.

11. Add 700 µL of 1% acetic acid in 50:50 methanol / water to the tube.

12. Mix this solution (125 fg/µL) thoroughly.

13. Label the tube *LTQ Reserpine Sample Solution (125 fg/µL)* and store it in a refrigerator until it is needed.

TSQ Quantum Ultra Reserpine Sample Solution

Prepare 1 mL of the 200 fg/µL (0.329 fmol/µL) reserpine compound optimization solution in 1% acetic acid in methanol

1. Pipette 1 mL of reserpine stock solution (1 µg/µL) into a 10 mL volumetric flask and bring the volume up to the 10mL mark using methanol (1% acetic acid). Mix the resulting solution thoroughly and label it as Reserpine (100 ng/µL).

2. Place 75 mL of methanol (1% acetic acid) into a clean 100 mL volumetric flask.

3. Using the 10 µL syringe provided in the accessories kit or a calibrated pipette, add 5 µL of the Reserpine (100 ng/µL) solution to the 75 mL of methanol (1% acetic acid) in the 100 mL volumetric flask.

4. Rinse the pipette twice with the 75 mL of methanol (1% acetic acid) contained in the volumetric flask by dipping the pipette tip into the solution and rinsing it back into the flask.

5. Carefully bring up this volume with methanol (1% acetic acid) until the lower part of the meniscus is level with the 100 mL marking.

6. Mix this solution thoroughly. Label it as Reserpine (5 pg/µL)

7. Add 20 mL of the Reserpine (5 pg/µL) to another clean 100 mL volumetric flask, and carefully bring up this volume with methanol (1% acetic acid) until the lower part of the meniscus is level with the 100 mL marking.

8. Mix the solution thoroughly.

9. Label this Reserpine (1 pg/µL).

10. Transfer 200 µL of the 1 pg/µL solution into a clean polypropylene tube.

11. Add 800 µL of 1% acetic acid in methanol to the tube.

12. Label the vial *TSQ Quantum Ultra Reserpine Sample Solution (200 fg/µL)* and store it in a refrigerator until it is needed.

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