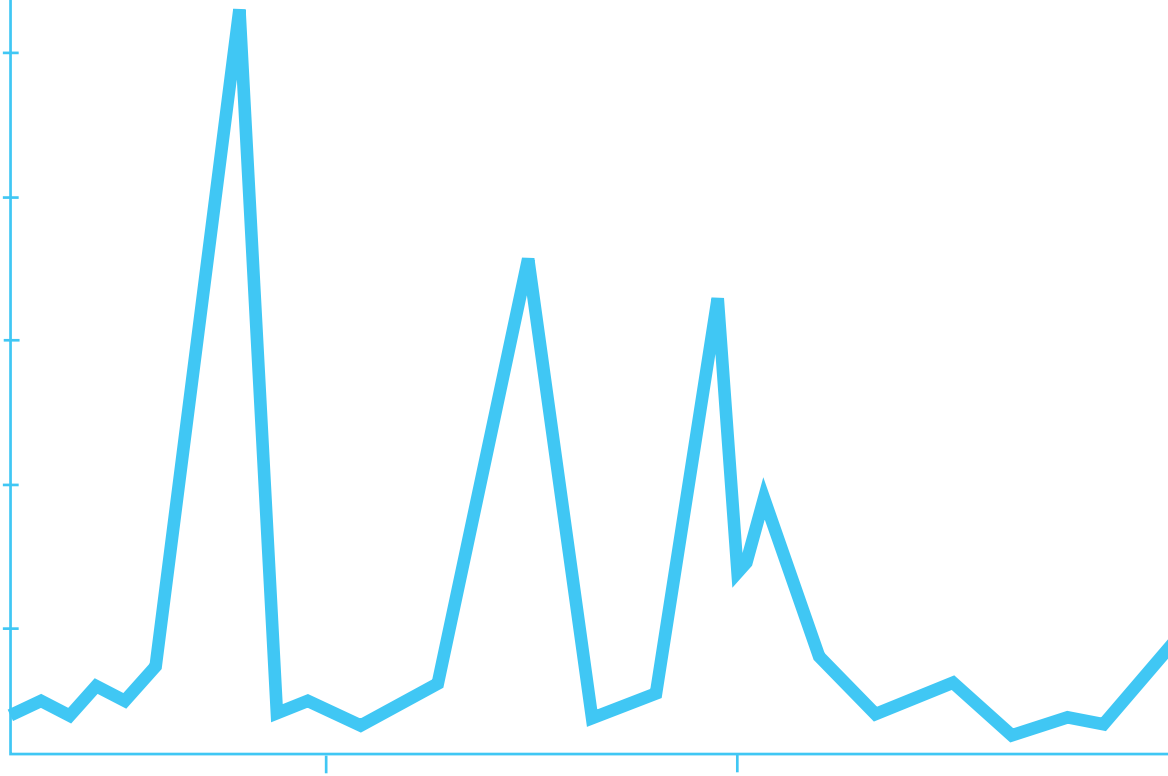
API 2000 and Q Trap LC/MS/MS

APCI Ion Source Manual

P/N: 1004466B
June 2002



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Equipment built by MDS SCIEX, a division of MDS Inc.,
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Printed in Canada.

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About This Manual

This manual contains the instructions required to operate the atmospheric pressure chemical ionization (APCI) ion source (also known as the Heated Nebulizer source) for the Q Trap and API 2000 LC/MS/MS systems.

Conventions

Within this manual, the following conventions are used:

WARNING! This symbol indicates an operation that may cause personal injury if precautions are not followed.

WARNING! This symbol indicates a warning of electrical shock hazard. You should read the warning before attempting any procedure described in this manual. Failure to do so can result in serious injury.

WARNING! This symbol indicates an operation that may cause injury if precautions are not followed. Handle after allowing the unit time to cool, or use appropriate insulating gloves to avoid burns.

WARNING! Standard laboratory rules should apply when using or handling flammable compounds with APCI source. Since this source includes a heating element with an operating temperature above the flammability point of some solvents, it is important to maintain and verify the API instrument before each use of the APCI source.

CAUTION! Indicates an operation that may cause damage to the instrument if precautions are not followed.

NOTE: Emphasizes significant information in a procedure or description.

Introduction

The atmospheric pressure chemical ionization (APCI) source offers an alternative method of introducing samples to the Q Trap and API 2000 mass spectrometers. The APCI, much like the standard IonSpray source, generates ions representative of the molecular composition of the sample. Where the IonSpray source produces ions by the process of ion evaporation, the APCI source vaporizes the sample prior to inducing ionization by the process of atmospheric pressure chemical ionization.

The APCI source produces ions by nebulizing the sample in a heated tube and vaporizing the finely dispersed sample drops. This process leaves the molecular constituents of the sample intact. These molecules are ionized by the APCI process, induced by a corona discharge needle, as they pass through the ion source chamber and into the interface region.

Features

The following list outlines the features of the APCI source:

- Able to function with flow rates up to 1.5 mL/min, and can handle the entire flow from a wide bore column without splitting.
- Able to vaporize a 100% aqueous mobile phase.
- Able to handle volatile mobile phase buffers.
- Able to vaporize volatile and labile compounds with minimal thermal decomposition.
- The simple spectrum of ions generated by APCI is ideal for MS/MS analysis.
- Capable of being used for rapid sample introduction by flow injection with or without an liquid chromatography (LC) column.

APCI Components

The inlet requires that the source exhaust system of the mass spectrometer be on and operating to specification. If the source exhaust system is not working properly, the instrument power supplies are disabled.

The APCI ion source consists of:

- Nebulizer vaporization chamber with replaceable quartz tube.
- Heater with computerized temperature control and control circuit board.

Specifications

Ion Source Temperature Range

- Probe temperature from 50–500 °C

Liquid Chromatography

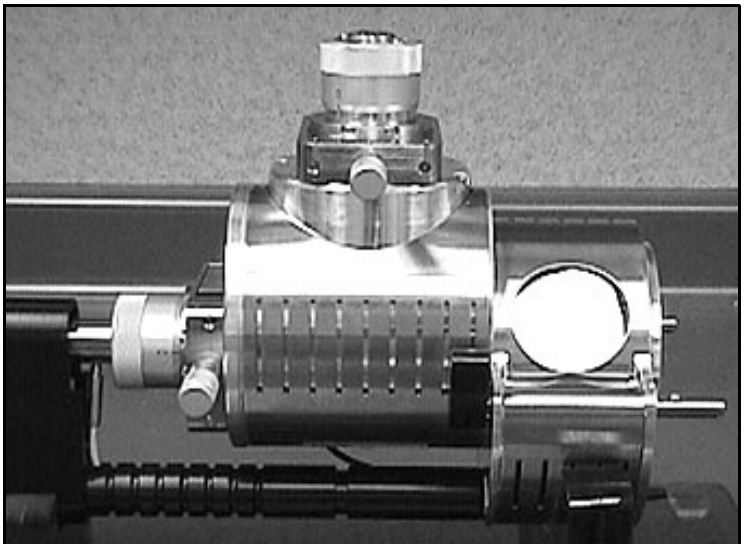
- Interfaces to any liquid chromatography system

Nebulizer Gas (Gas 1)

- Zero grade air regulated at 100 psi

Auxiliary Gas (Gas 2)

- Zero grade air regulated to 100 psi



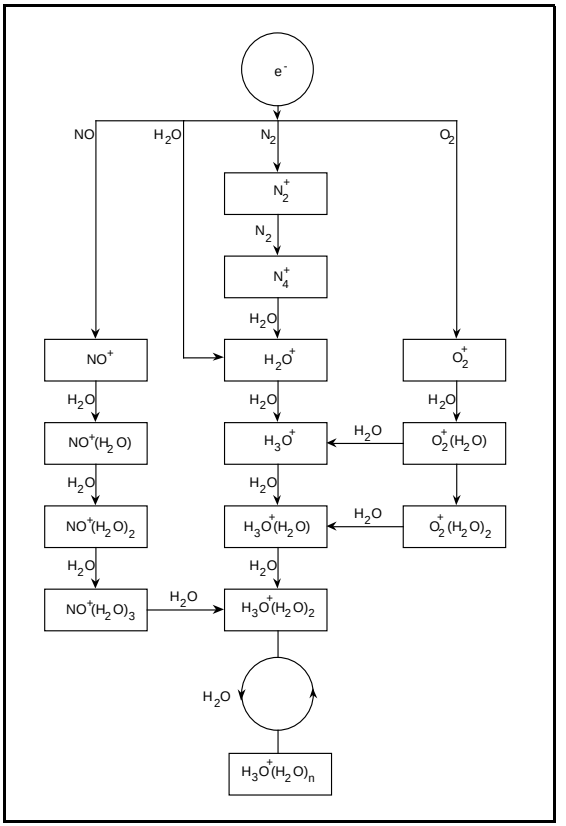
APCI ion source

Ionization Process

The basis for past incompatibilities of linking liquid chromatography with mass spectrometry arises from difficulties converting relatively involatile molecules solvated in a liquid into a molecular gas, without inducing excessive decomposition. The APCI process of gently nebulizing the sample into finely dispersed small droplets in a heated tube ensures rapid vaporization of the sample so that the sample molecules are not decomposed.

The following figure shows the reaction pathway of the APCI process for reactant positive ions (the protonated water cluster ions, $H_3O^+[H_2O]_n$). This sequence is derived from experimental results summarized by Huertas and Fontan[1]. The major primary ions N_2^+, O_2^+, H_2O^+, and NO^+ are formed by electron impact of corona-created electrons on the major neutral components of air. Although NO is normally not a major constituent of clean ambient air, the concentration of this species in the source is enhanced due to neutral reactions initiated by the corona discharge.

1. Huertas, M.L. and J. Fontan. “Evolution Times of Tropospheric Positive Ions.” *Atmospheric Environ*, 1975, 9, 1018.

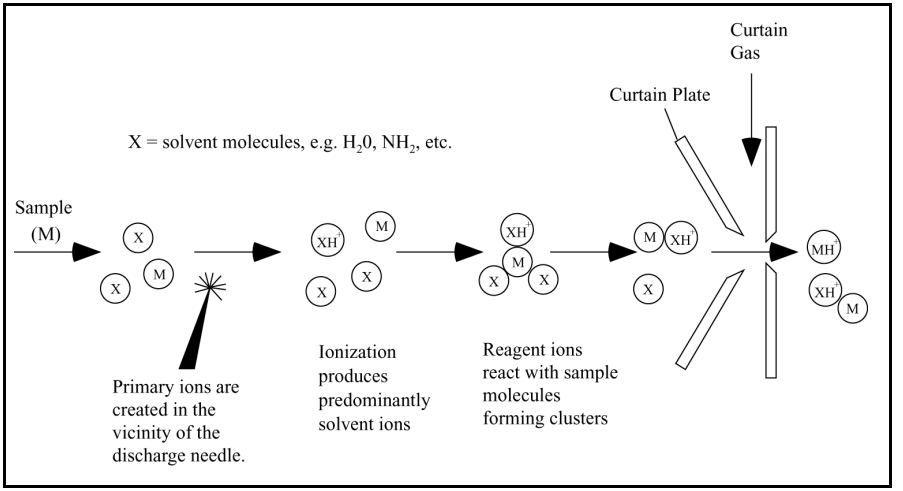


APCI reaction flow diagram

Samples introduced through the APCI are sprayed into a heated probe with the aid of a nebulizing gas. Within the probe, the finely dispersed droplets of sample and solvent undergo a rapid vaporization with minimal thermal decomposition. The gentle vaporization preserves the molecular identity of the sample.

The gaseous sample and solvent molecules are swept from the probe by a second gas flow (auxiliary gas) into the ion source where the ionization by APCI is induced by a corona discharge needle. The sample molecules are ionized by collision with the reagent ions created by the ionization of mobile phase solvent molecules. The vaporized solvent molecules ionize to produce the reagent ions $(X+H)^+$ in the positive mode and $(X-H)^-$ in the negative mode. These reagent ions collide with the sample molecules to produce stable sample ions. Refer to the following APCI figure.

The sample molecules are ionized by a process of proton transfer in the positive mode, and by either electron transfer or proton transfer in the negative mode. The energy for the APCI process is collision dominated because of the “high” pressure of the APCI source.



Atmospheric pressure chemical ionization (APCI)

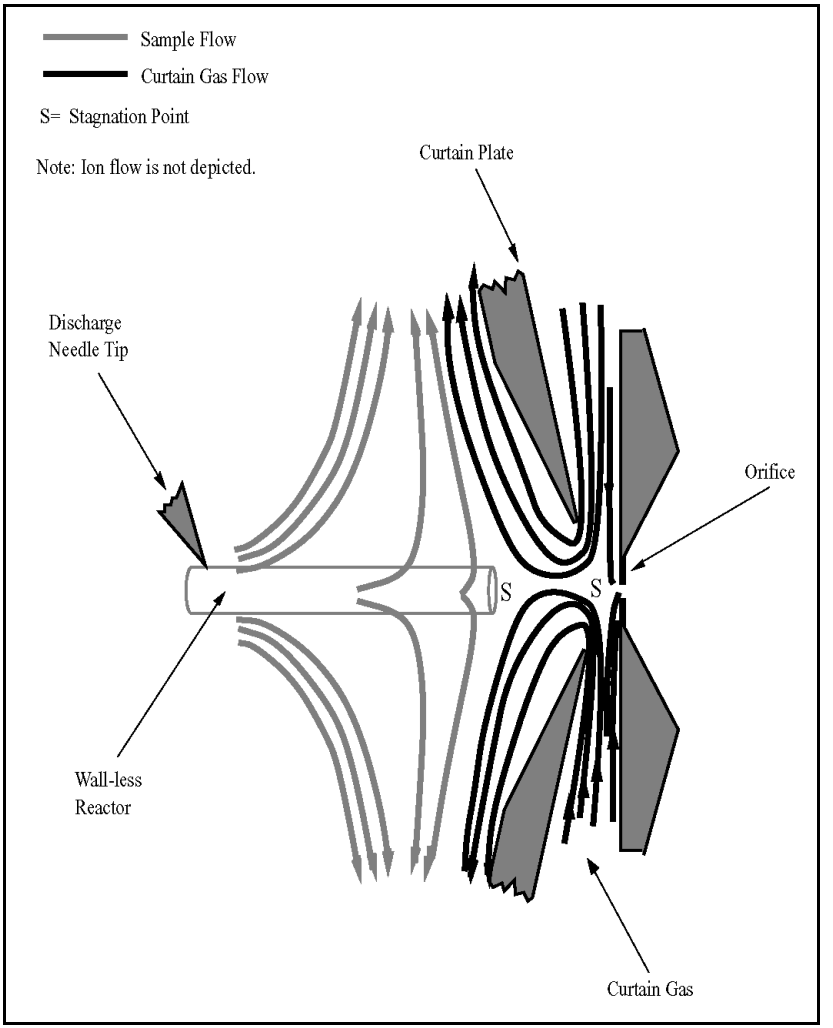
NOTE: For reverse phase applications, the reagent ions consist of protonated solvent molecules in the positive mode, and solvated oxygen ions in the negative mode. With favorable thermodynamics, the addition of modifiers changes the reagent ion composition. For example, the addition of acetate buffers or modifiers can make the acetate ion, $(CH_3COO)^-$, the primary reagent in the negative mode. Ammonium modifiers may make protonated ammonia, $(NH_4)^+$, the primary reagent in the positive mode.

Through collisions, an equilibrium distribution of certain ions (for example, protonated water cluster ions) is maintained. The likelihood of premature fragmentation of the sample ions in the ion source is reduced given the moderating influence of solvent clusters on the reagent ions, and the relatively high gas pressure in the source. As a result, the ionization process yields primarily molecular ions for mass analysis in the mass spectrometer.

Ionization Region

The general location of the ion-molecule reactor of the APCI source is indicated by the dotted cylinder (as shown in the following figure) that

constitutes a wall-less reactor. A self-starting corona discharge ion current, in the microamp range, is created as a result of the electric field between the discharge needle and the curtain plate. Primary ions, for example N_2^+ and O_2^+, are created by the loss of electrons that originate in the plasma in the immediate vicinity of the needle tip. The energy of these electrons is moderated by a number of collisions with gas molecules before attaining an energy where their effective ionization cross-section allows them to ionize neutral molecules efficiently.



APCI ion source—source flow streamlines

The primary ions, in turn, generate intermediate ions that finally lead to the formation of sample ions. Ions of the chosen polarity drift under the influence of the electric field in the direction of the curtain plate and through the gas curtain into the mass analyzer. The whole ion formation process is collision dominated because of the “high” pressure of the APCI source. Except in the immediate vicinity of the needle tip, where the electric field strength is greatest, the energy imparted to an ion by the electric field is small in comparison with its thermal energy.

Through collisions, an equilibrium distribution of certain ions, for example, the protonated water cluster ions, $H_3O^+(H_2O)_n$, is maintained. Any excess energy that an ion may acquire in the ion-molecule reaction process is thermalized through the process known as collisional stabilization. Both product ion and reactant ion formation are governed by equilibrium conditions at 760 torr operating pressure.

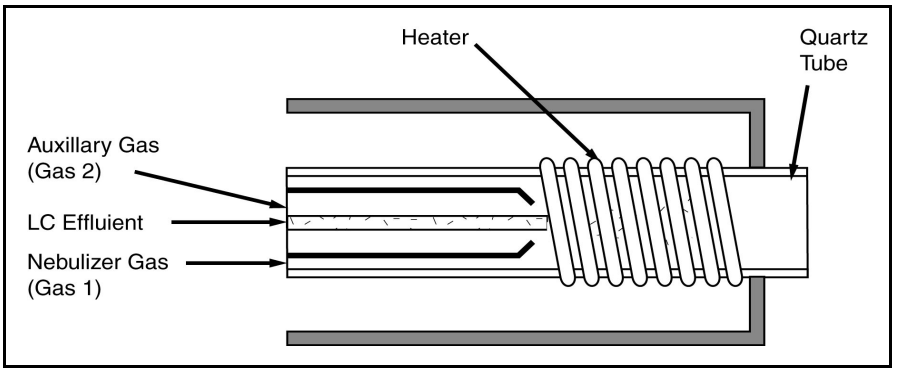
NOTE: The ion source functions as a wall-less reactor since the ions pass from the source to the vacuum chamber and eventually to the detector without experiencing collisions with a wall. Ions formed outside the designated ion source are neutralized by interacting with a wall surface and are not detected.

The temperature of the probe is an important factor for APCI operation. In essence, the temperature must be set high enough to ensure a rapid evaporation. At a sufficiently high operating temperature, the droplets are vaporized quickly so that organic molecules are desorbed from the droplets with minimal thermal degradation. If the temperature is set too low, the evaporation process is slower and pyrolysis, or decomposition, may occur before vaporization is complete. To preserve the molecular identity, the temperature of the probe must be set to ensure rapid evaporation. Operating the APCI at temperatures above the optimal temperature may cause thermal decomposition of the sample.

Inlet Description

The sprayer probe consists of 100 µm (0.004") ID stainless steel tubing surrounded by a flow of nebulizer gas. The liquid sample flow is pumped through the sprayer where it is nebulized into a quartz tube surrounded by a heater. The inner wall of the quartz tube is maintained at a temperature of about 100–250 °C. When the liquid sample is pumped into the quartz tube, the sample and solvent are vaporized. A flow of auxiliary gas (Gas 2) surrounds the sprayer carrying the vaporized sample through the quartz tube into the ionization region in the ion source.

The liquid sample is introduced through a zero volume LC fitting on the probe handle, from where it flows by stainless steel tubing (0.004" ID) to the tip of the sprayer. A high velocity jet of nebulizer gas (Gas 1) flows coaxially over the sprayer to disperse the sample as a mist of fine particles. A flow of auxiliary gas (Gas 2) sweeps the sample mist through the quartz vaporization tube into the reaction region of the ion source past the corona discharge needle where the sample molecules are ionized.



APCI probe close-up

The probe temperature is maintained by a heater coil wrapped around the outside of the quartz tube. The power to the heater, and as a direct result, the heater temperature, is controlled by the temperature control board (TCB) mounted inside the instrument. The TCB adjusts the flow of power to the heater element as a function of the difference between the actual heater temperature and the temperature setting at the applications computer. The probe temperature is monitored by a sensor connected directly to the heater element.

NOTE: The temperature is controlled by monitoring the output of a sensor connected to the heater surrounding the quartz tube. At the temperature control board the sensor output is compared with the temperature setting; the difference determines the power flow to the heater.

Corona Discharge Characteristics

The corona discharge in the APCI source is formed by three major electric fields and fluid flow elements:

1. Corona discharge needle
2. Curtain plate
3. Orifice lens

The purpose of the corona discharge is to produce ionization of the trace species, or sample gas. Primary ions, which are formed as a result of the discharge, are converted by collisional processes to final ion-molecule reaction products.

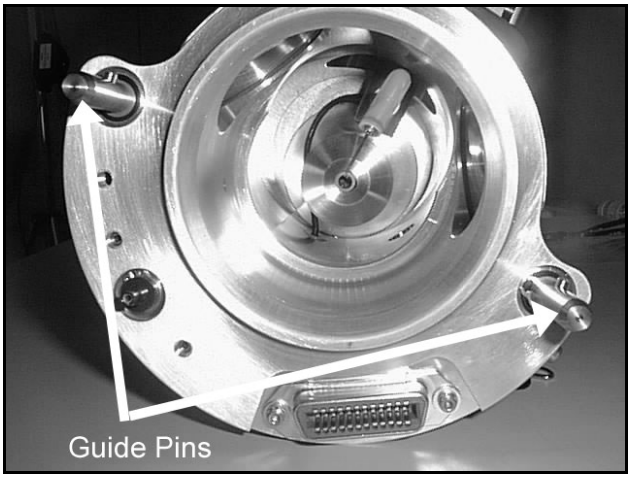
The operator has the ability to set the corona discharge setting at the applications computer by adjusting the value of needle current (NC), which is normally set to 2 in Analyst.

Installation

The APCI source, like the TurboIonSpray source, connects to the vacuum interface housing. Two latches mounted on the source housing secure the APCI against the vacuum interface.

WARNING! Some surfaces on the APCI source become hot during operation. Use caution when installing or removing the source or the heated probe.

To install the APCI on your instrument



Guide pin alignment

1. Ensure that the two source latches are in the unlocked (up) position.
2. Align the two guide pins on the source with the vacuum interface receptacles.
3. Slide the source along the guide pins toward the interface as far as it can go without using excessive force.
4. Turn the two source latches to the locked (down) position.

   The nebulizer and auxiliary gases (Gas 1 and 2), the discharge needle, high voltage, and heater connectors are automatically engaged once the source is locked in position.

5. Connect the liquid sample tubing to the fitting on the inlet end of the sprayer probe. Ensure that all fittings are properly seated in order to minimize dead volumes.

NOTE: In order to reduce the band broadening of sample with solvent, thus maximizing sensitivity, the use of 127 µm (0.005") ID peek, or fused silica tubing between the source and the injector/column is recommended.

To remove the APCI from the instrument

1. Stop all scans and place the instrument in **Standby**.
2. Turn off the liquid flow entering the ion source.
3. Disconnect the liquid sample tubing from the probe fitting.
4. Turn the two source latches to the unlocked (up) position.

   The nebulizer and auxiliary gases (Gas 1 and 2), the discharge needle, high voltage, and heater connectors are automatically disengaged once the source is unlocked from its position.

WARNING! The APCI ion source may be hot for several minutes after it is removed from the instrument.

5. Slide the source along the guide pins away from the interface to remove it.

Source Exhaust Pump

WARNING! The APCI source exhaust system is a safety measure and must be kept operational to ensure continued safe operation.

The APCI source requires that the source exhaust system is properly connected and functioning. A filtered air gas supply (free from pump oil) is delivered to the source exhaust pump at 60 psi pressure at a flow of at least 4 to 8 L per minute. The source exhaust pump is used to vent solvent vapors that develop in the ion source plenum. It is highly recommended that these vapors be passed through a trap, and then vented to a fume hood or outside port.

The source exhaust system is interlocked to the system electronics, such that if the source exhaust pump is not operating to specification, the instrument electronics are disabled.

The exhaust system lowers the pressure in the source slightly below atmospheric pressure. If the pressure in the source rises beyond a pressure sensor trip point, the instrument high voltage power supply is disabled.

WARNING! The source exhaust pump must be vented to either an external fume hood or external exhaust source.

WARNING! Standard laboratory rules should apply when using or handling flammable compounds with APCI source. Since this source includes a heating element with an operating temperature above the flammability point of some solvents, it is important to maintain and verify the API instrument before each use of the APCI source.

Before each use, test the pressure switch for the exhaust line by shutting off the source exhaust gas supply. If the hose is connected to a forced ventilation system, disconnect the hose from the drain bottle. Fault messages will be displayed on the monitor indicating the source exhaust gas is off, which verifies that the pressure switch is working. If the fault messages are not displayed the pressure switch is defective and the APCI source must not be used. A service call is mandatory. If the above procedure is not followed, the ion source pressure sensor may inadvertently allow the system to operate when the ion source is not being properly exhausted. If the source is not properly exhausted, vapor can escape through the heated nebulizer probe and condense within the probe's electrical wiring. This could cause a short circuit and the possibility of a fire if flammable solvents are used.

Optimizing the APCI Setup

CAUTION! If the source is to be left unattended while in operation, ensure that an LC shutoff is in use to prevent flooding of the plenum chamber.

The following section outlines the practical considerations which must be considered when optimizing the APCI performance. It is intended to provide the qualitative information necessary to aid you in quantifying the separate operating parameters.

Several parameters impact the performance of the APCI. To optimize the performance, inject by flow injection a known compound (reserpine is recommended) and monitor the signal of the known ion. Adjust the following parameters to maximize the signal-to-noise ratio:

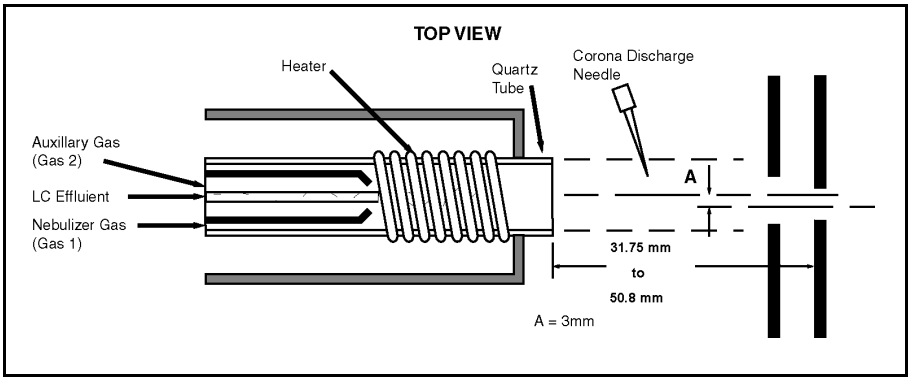
Parameter optimization for APCI

Parameter	Nominal Value	Normal Range
LC flow (mL/min)	1	0.2 to 2
NC (µA)	2	1 to 5
Gas 1 (psi)	60	40 to 80
Gas 2 (psi)	15	10 to 25
Temperature (°C)	450	300 to 500
DP (V)	20	5 to 80
Curtain gas (psi)	40	25 to 50
Probe lateral position	Scale 5 mm	Scale 3 to 10

Probe Position

The position of the probe relative to the orifice and the corona discharge is an important factor in optimizing the APCI performance. The probe has a built-in minimum 3 mm offset with respect to the center of the orifice. The distance of the probe from the orifice plane is not as critical; it is typically as close to the orifice plate as possible. The corona discharge needle should be

on the same plane as the quartz tube, such that if the quartz tube were projected to the interface, the tip of the needle should touch the top of the virtual quartz tube.



Probe and corona discharge needle position

Initial Warm-Up of the APCI

Setup of the APCI should begin with a warm-up stage to allow the probe to heat prior to initiating the liquid sample flow. A two minute warm-up will eliminate the possibility that solvent vapors may condense in a cold probe.

To warm up the APCI

1. In Analyst, set the value for the curtain gas to 35.

NOTE: It is suggested that you operate the APCI with curtain gas settings adjusted to the highest flow rate possible without signal loss.

2. Turn on the nebulizer gas (Gas 1) to 60 psi.
3. Set the auxiliary gas (Gas 2) to 15.
4. Set the heater temperature (TEM) to 450 °C, or the optimal temperature for the samples to be run.
5. Let the APCI warm-up for two minutes.
6. Connect the LC solvent line from the injector or autosampler to the LC connection on the probe.
7. Start the flow.

Temperature (TEM)

The liquid flow rate, composition, and type of sample determine the optimal APCI temperature. At higher flow rates, the optimal temperature increases. A more significant factor is the composition of the solvent. As the organic content of the solvent increases, the optimal probe temperature should decrease. With solvents consisting of 100% methanol or acetonitrile, the probe performance may optimize as low as 300 °C. Aqueous solvents consisting of 100% water at flows of approximately 1 mL/min require a minimum probe temperature of 450 °C. Normal optimization is usually performed in increments of 25 °C.

The APCI is normally used with sample flow rates of 1 mL/min but has been used with flows from 200 µL/min to 2 mL/min. The heat is used to vaporize the sample and solvent sprayed into the ion source chamber. If the temperature is set too low the vaporization is incomplete and visible large droplets are expelled into the plenum. However, setting the temperature too high can induce thermal degradation of the sample. The optimal temperature is the lowest setting that ensures the complete vaporization of the sample.

CAUTION! Do not operate the APCI with probe temperatures greater than 500 °C.

Declustering Potential (DP)

Optimal declustering potential voltage should be set high enough to reduce the chemical noise, but low enough to avoid fragmentation. Start with the declustering potential (DP) at 20 V.

NOTE: The fragmentation energy of a compound is a function of its structure and molecular weight. Generally, lower molecular weight compounds require less energy — lower declustering potential voltage — to induce fragmentation.

In general terms, the higher the declustering potential voltage the greater the energy imparted to the ions entering the analyzing region of the mass spectrometer. The energy helps to decluster the ions and to reduce the chemical noise in the spectrum, resulting in an increase in signal-to-noise, or sensitivity. Increasing the voltage beyond optimal conditions can induce fragmentation before the ions enter the mass filters, resulting in a decrease in sensitivity. In some instances, fragmentation is a valuable tool that provides additional structural information.

Curtain Gas (CUR)

The curtain gas ensures a stable, clean environment for the sample ions entering the mass spectrometer. The gas curtain prevents air or solvent from entering the analyzer region of the instrument while permitting the sample ions to be directed into the vacuum chamber by the electrical fields generated between the vacuum interface and the corona discharge needle. The presence of the solvent vapor or moisture in the analyzer region of the mass spectrometer contaminates the Q0 rod set causing a reduction in Q0 resolution, stability and sensitivity, and an increase in chemical background noise.

In order to prevent instrument contamination the curtain gas flow **should be optimized at the highest possible setting (never below 11 psi)** that does not result in a significant reduction in signal intensity.

Solvent Composition

Commonly used solvents and modifiers are acetonitrile, methanol, propanol, water, acetic acid, formic acid, ammonium formate, and ammonium acetate. The modifiers such as triethyl amine (TEA), sodium phosphate, trifluoroacetic acid (TFA), and sodium dodecyl sulfate are not commonly used because they complicate the spectrum with their ion mixtures and cluster combinations. They may also suppress the strength of the target compound ion signal. The standard concentration of ammonium formate or ammonium acetate is from 2 to 10 millimole per liter for positive ions and 2 to 100 millimole per liter for negative ions. The concentration of the organic acids is 0.1% to 2.0% by volume.

Changing the Quartz Tube

The following procedure should be used to exchange the quartz tube in the APCI when it becomes dirty and performance begins to degrade (for example, loss in sensitivity, or excessive peak tailing).

To change the quartz tube in the APCI

1. Allow the source to cool to room temperature.
2. Remove the ion source and set on its side.

WARNING! Some surfaces on the APCI source become hot during operation. Use caution when installing or removing the source or the heated probe.

3. Remove the corona discharge needle from its holder.
4. Make note of the needle position settings, and then move the needle holder arm up away from the heater probe so there is enough clearance for the heater probe shell to be removed.
5. Ensure that the heater probe has cooled down. Grasp the end of the heater probe and turn clockwise. The probe shell will come loose and can be pulled away from the source, exposing the quartz tube assembly.
6. Grasp the end of the quartz tube and pull it out of the heater coil.
7. Place a new quartz tube into the heater coil.
8. Slide the probe shell over the quartz tube assembly and turn it counter clockwise until it locks in place.
9. Move the needle holder back into its original position using the settings noted in step 4.
10. Place the discharge needle back into the needle holder.

The source is now ready for use.

Changing the Stainless Steel Sprayer Tube

The standard APCI setup uses a 100 µm (0.004") ID metal sprayer tube. The following procedure should be used to exchange the metal tube in the APCI in the event of blockage.

To change a blocked sprayer tube

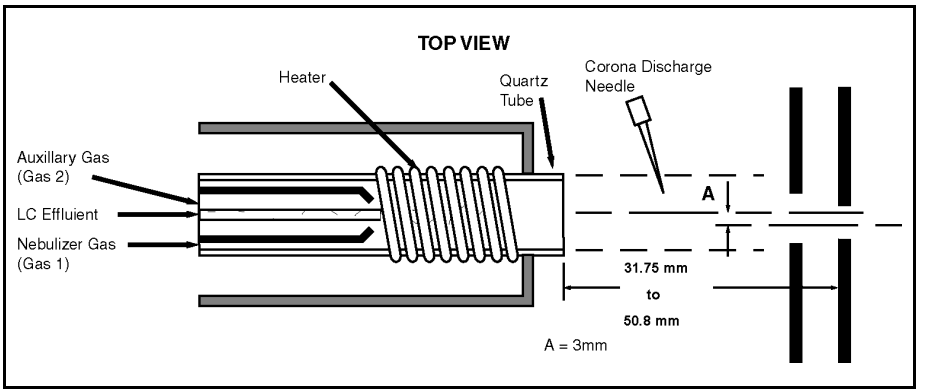
WARNING! The APCI source may be hot for several minutes after it is removed from the instrument. Allow the source to cool prior to working on it.

1. Remove the ion source and set on its side.
2. Unscrew and remove the metal inlet probe fitting. The union and the metal sprayer tube can now be removed from the probe.
3. Unscrew the fitting holding the metal tube to the union. Remove the tube from the fitting. Remove the O-ring from the steel tube. Do not discard the fittings or the O-ring.
4. Place a new sprayer tube and ferrule in the fitting and screw into the union. Ensure that the sprayer tube is placed as far as it can go into the union to guarantee a leak-proof seal (two wrenches should also be used to tighten the fittings).
5. Place an O-ring over the steel tube so that it sits atop the metal nut that holds the tube in the union (use the O-ring removed earlier if it is not damaged, or use a new one provided in the Consumables Kit).
6. Place the metal tube and union back into the probe.
7. Place the metal inlet probe fitting over the union and tighten. This fitting must be tightened by hand as far as it will go to ensure the proper protrusion of the metal sprayer tube tip at the end of the probe.

The source is now ready for use again.

To change the corona discharge needle

1. Install the corona discharge needle into the needle chuck (friction fit).
2. Align the corona discharge needle.



Appendix A: Consumable Parts

The following is a list of parts included in the kit of consumable parts (part number 022568) supplied with the APCI assembly.

Item	Part No.	Description	Quantity
1	015656	Needle - Ionizer	4
2	018722	Fitting - PEEK Blk Union	1
3	017765	Tube - Quartz Modified	2
4	017773	Line - Sample	1
5	022554	O-ring - 0.042" ID x 0.05" W Vitron	2
6	022542	Ferrule - 1/16" PEEK	2
7	022553	Nut - 1/16" Tube 10-32 SS	1

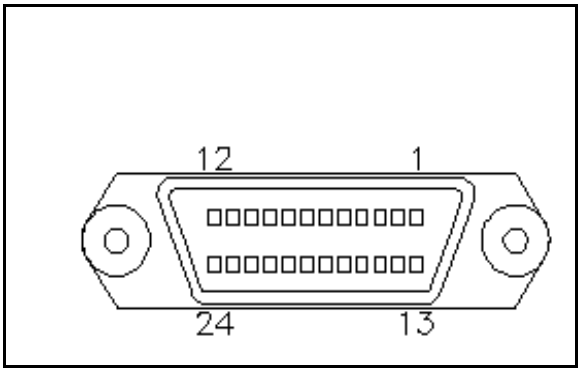
Appendix B: Troubleshooting

Introduction

This troubleshooting section is suitable for use by operators who have access to an ohm meter and are familiar with its use. Diagnosis does not require removal of the instrument covers and does not expose the operator to hazardous voltages.

The heater control circuits are protected by a fuse that can be reset. This device may be tripped by excessive heater current, or by failure to approach the desired operating temperature within about 5 minutes. When tripped, the device will remove power from the heater. To reset the protective device, power off the system for about one minute or remove the ionizer from the system for about one minute.

Diagnosis of the nature of the fault is done by means of ohm meter readings taken across the pins of the 24-pin connector on the ionizer shown in the following figure.



Ionizer 24-pin connector

Failure Modes Heated Ionizer Table

Cause	Symptoms	Diagnosis	Cure
Short circuit in heater.	No heat.	Heater resistance measured across pins 7 and 19 is less than 15 ohms.	Call Service to replace heater.
Open circuit in heater.	No heat.	Heater resistance measured across pins 7 and 19 is greater than 25 ohms.	Call Service to replace heater.
Heater grounded.	No heat.	Heater resistance from pin 9 to ionizer frame is less than 100,000 ohms.	Call Service to replace heater.
RTD short circuit.	No heat.	RTD resistance measured across pins 9 and 10 is less than 100 ohms.	Call Service to replace RTD.
RTD open circuit.	No heat.	RTD resistance measured across pins 9 and 10 is greater than 300 ohms.	Call Service to replace RTD.
RTD grounded.	No heat.	RTD resistance from pin 10 to ionizer frame is less than 100,000 ohms.	Call Service to replace RTD.
Poor thermal contact between the RTD and the heater.	When starting the heater with the temperature set to greater than 300 °C, the heat comes on for about 5 minutes and then goes off.	The heater works normally when the temperature is set to 150 °C.	Call Service to correct clips attaching the RTD to the heater.