Installation and Maintenance Instructions for 0.25mm and 0.32mm ID Fused Silica Capillary Columns

These instructions cover instrument preparation, column hanging, ferrule and column installation, leak checking, gas flow setting procedures, and maintenance requirements for 0.25mm and 0.32mm ID fused silica capillary systems. Along with your instrument's manual, this information will enable you to properly install and maintain these columns in your GC.

Key Words:
● fused silica capillary column ● injector ● detector ● ferrule

Instrument Preparation

Before installing your column, make certain the injector and detector liners (if present) are clean and free of sample residue or septum and capillary fragments. To prevent adsorption problems, the injector and detector liners should be silanized. Cleaning and silanizing procedures can be found under the Maintenance section of this Bulletin.

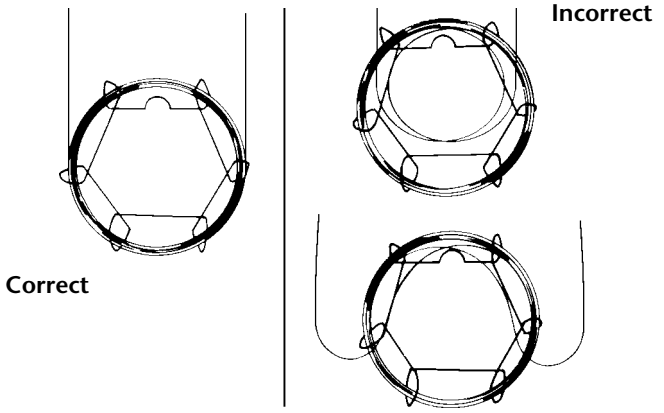
Once the system is clean, set the injector and detector temperatures according to the specifications provided with the test chromatogram. **Never** set these temperatures above the maximum limit of the stationary phase.

Oxygen and water, normally present in gas cylinders, must be removed from the carrier gas or column life will be shortened. This purified carrier gas is especially important for polar phases, such as SUPELCOWAX™ 10 and SP™-2330. A full line of gas purifiers is available from Supelco. Any carrier gas pressure regulator located downstream of the carrier gas purifier should contain a stainless steel diaphragm to prevent diffusion of oxygen into the carrier gas (Grob, K., HRC & CC, *3*, 173, 1978). Request Supelco Bulletin 848 for carrier gas purification information.

Column Hanging Procedure

Your capillary column must be suspended properly in the oven — supported by its metal cage, not by the fused silica tubing. Sharp bends in the tubing can weaken and eventually break the column. Avoid them by making sure column ends cross at the *bottom* of the cage, not at the top, when connecting the column to the injector and detector (Figure A).

Figure A. Proper Column Installation Prevents Stress on the Tubing



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Ferrule and Column Installation

Correct installation of the ferrule is critical to column performance. Fragments from an improperly installed ferrule can contaminate the column, causing it to adsorb active components. Use 0.4mm ID ferrules with 0.25mm ID fused silica columns, and 0.5mm ID ferrules with 0.32mm ID columns.

Some fused silica tubing has an oversized OD due to the thickness of the outside polyimide coating. Use a Pin Vise Drill Kit (Cat. No. 23820-U) or a Needle File (Cat. No. 23783) to enlarge the ferrule ID until it fits easily over the fused silica tubing.

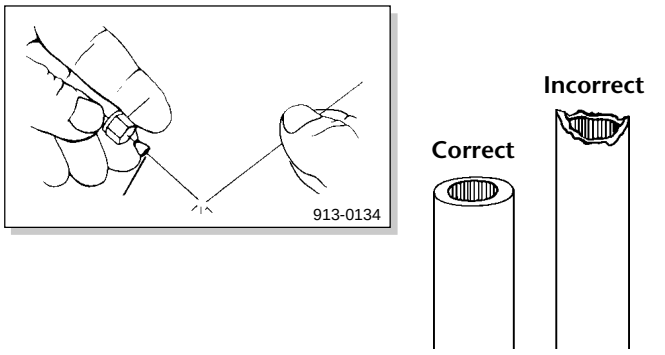
Both Supeltex™ M-2A ferrules (VESPEL® graphite, max. temp. 400°C) and Supeltex M-4 ferrules (graphite, max. temp. 450°C) are available from Supelco. See Bulletin 741, *The Supelco Guide to Leak-Free Connections,* for general installation tips and descriptions of ferrule types.

Once the ferrule is properly sized, squarely cut both sealed column ends with a Capillary Cleaving™ Tool (Cat. No. 23814 or 23740-U) or other scoring device. Install the nut and ferrule on the column according to your instrument instructions.

Always keep the column end pointed toward the floor while installing the nut and ferrule or when cutting the column end. Ferrule or column particles will then fall away from the column. After ferrule installation, remove stubborn particles that may remain in the column ends by cutting about one inch from each end of the column. Do this each time a ferrule is installed.

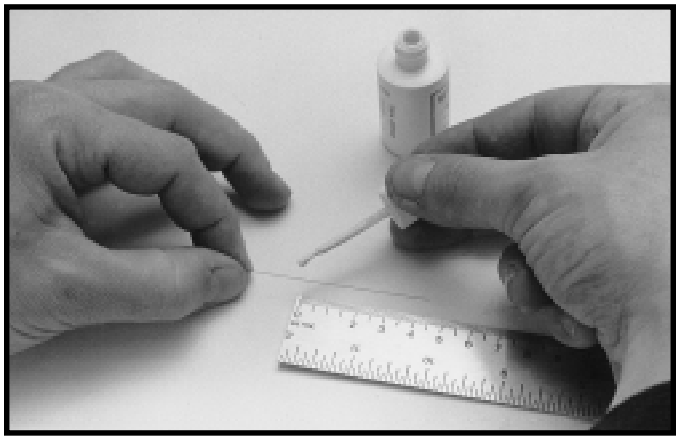
Examine the column ends closely with a magnifying glass to make sure they were cut squarely. Jagged edges from improperly cut tubing can seriously impede column performance (Figure B).

Figure B. Cutting Fused Silica Tubing



When installing a fused silica column in the injector port and detector, follow the instrument manufacturer's recommended insertion distances. Otherwise, column performance may be affected. Use typewriter correction fluid or a felt-tipped pen to mark the correct distances on the fused silica tubing (Figure C).

Figure C. Marking the Insertion Distance



Once these insertion distances are marked, install the column in the injection port. Before completing the hook-up to the detector, turn on the carrier gas flow to purge the column. Typical head pressures for various column IDs and lengths are given in Table 1. You will later fine-tune these tentative settings.

Table 1. Column Head Pressure

Column ID (mm)	Column Length (m)	Column Head Pressure (psig)
0.20	15	15
	30	30
	50	40
	60	50
0.25	15	10
	30	15
	60	30
0.32	15	5
	30	9
	60	18

When connecting the column to the flame ionization detector (FID), *make sure the flame is out*. Be careful not to push the column end through the FID jet orifice or beyond the radioactive foil in an electron capture detector (ECD). Improper installation in any detector will seriously impair chromatographic performance. After connecting the column to the detector, turn on the make-up gas.

Checking for Leaks

Once the column is connected to the instrument, turn on the carrier and make-up gases and check the fittings for leaks. **Do not use liquid leak detectors.** These liquids can be drawn into the column or column fittings and contaminate the system. The best way to leak-check a capillary system is with GOW-MAC® Gas Leak Detectors (Deluxe Model, Cat. No. 22409; Mini Model, Cat. No. 22807 or 22808). These detectors operate on the same principle as a thermal conductivity detector. They are highly sensitive to low concentrations of He, H_2, and N_2 and cannot contaminate the instrument or column.

If GOW-MAC Gas Leak Detectors are unavailable and you are using Supeltex™ M-2A or Supeltex M-4 ferrules, minimize the risk of leaks by tightening the ferrules until the tubing no longer moves in the fittings. (Be sure to readjust insertion distances.) One-fourth turn past fingertight is usually sufficient. But, be careful — oxygen entering a leaking connection could shorten the life of your column.

Gas Flow Setting Procedure

Once a leak-free system is established, gas flows can be set. There are three flows to adjust: (1) make-up gas flow, (2) splitter vent flow, and (3) column flow. The latter rate has already been tentatively set by back pressure (Table 1). These flows should be set in the above order at ambient temperature, until otherwise specified in these instructions.

1. The first and easiest flow to set is the make-up gas. Once set, it should not require altering. Refer to your instrument manual for instructions and specifications for setting this flow — typically 20-60cc/min.
2. The second flow to set, the splitter vent flow, determines the split ratio. Unlike the make-up gas flow, the splitter vent flow must be readjusted each time the starting oven temperature or carrier gas head pressure is altered. When testing the column with our test mix, we recommend setting the splitter vent flow according to Table 2, thus providing an approximate split ratio of 100:1. Other samples may require lower split ratios to improve detection or higher ratios to prevent column overload.

Table 2. Recommended Splitter Vent Flow

Column ID (mm)	Splitter Vent Flow* (for 100:1 split ratio) (cc/min)
0.20	45-55
0.25	65-70
0.32	100-110

*For helium, 20cm/sec

3. To prevent possible column damage, turn on your detector and check your capillary system before adjusting the oven temperature to operational level. This can be accomplished by injecting 25-50µL of a 1% methane in N_2 gas blend (Cat. No. 23443) onto the column and examining the resulting peak. Expect a very sharp, symmetrical peak. See the chromatogram supplied with your column for comparison. If tailing is evident, there could be dead volume in the system. If there is no peak at all, suspect a hook-up or detector problem. Before proceeding, corrections should be made.
4. To prevent oxidation once a symmetrical methane peak is obtained, purge the column with carrier gas for 30 to 60 minutes before heating. The column is then ready to be programmed up in temperature at about 2°C/min. A temperature of 20°C to 40°C below the column's maximum temperature should be held for 2 to 16 hours (see conditioning instructions with your column). Extensive conditioning should not be necessary.
5. If you wish to minimize bleed or baseline rise during a temperature programmed analysis, we recommend you program condition the column. Set the GC to repetitively cycle the oven temperature up and down overnight, following the same temperature program to be used for the analysis. Programmed conditioning stabilizes the baseline much faster than conditioning at a high isothermal temperature. Remember to heat and cool capillary column *slowly*. Use temperature programming rates of less than 25°C/min and allow the oven cooling mechanism to operate automatically. Thermal shocks could damage a capillary column by causing the phase to puddle. After conditioning the column, slowly drop the temperature to the level indicated on the test chromatogram specification sheet. Once the temperature has equilibrated, set the carrier gas flow rate by adjusting the average linear gas velocity ($\bar{u}$), the speed at which an unretained substance (typically methane) flows from the inlet to the outlet of the column. Calculate linear velocity by making a 20 to 50µL injection of 1% methane in N_2 gas blend (as used previously) onto your column and measure the methane peak retention time. If your are using an electrochemical detector, use an inert halogenated gas, such as Freon®22, to set the linear velocity. Use the following formula:

   Average Linear Gas Rate = $\bar{u} = L/t_m$

   L = length of column in cm

   t_m = retention time of methane in seconds

   Refer to Table 4 for optimum average linear gas rates. These retention times can be adjusted by varying the carrier gas head pressure (see your instrument manual for specific instructions). If you change the carrier gas head pressure or starting oven temperature, remember to reset the splitter vent flow.

Table 4 Recommended Methane Retention Times◆

Column Length (m)	Carrier Gas (min:sec) H_2	He	N_2
10	0:25	0:50	1:40
15	0:38	1:15	2:30
30	1:15	2:30	5:00
50	2:05	4:10	8:20
60	2:30	5:00	10:00
100	4:10	8:20	16:40

◆ There are different optimum average linear velocities for different carrier gases: i.e., H_2 — 40cm/sec, He — 20cm/sec, N_2 — 10cm/sec.

6. After all flows have been adjusted the system is ready for evaluation. Use the test mix shipped with your column to evaluate your system. For details on the use of this test mix and interpretation of the results, see the test mix instruction sheet and the QA test chromatogram.

Special Considerations for Nonbonded Phases

Chromatographically bonded and nonbonded phases provide the same resolution. They also perform similarly, with the following exceptions:

Sensitivity to thermal shock — Nonbonded phases are more sensitive to thermal shocks that can permanently damage a capillary column. Never heat or cool nonbonded columns at more than 25°C/min. Cool the column by allowing the oven door mechanism to operate automatically. Do not force the column to cool faster by opening the oven door wide or by using cryogenic cooling.

Choice of solvents for splitless or on-column injection — Choose a solvent that will minimize the temperature program rate needed to achieve good solvent focusing. We recommend a solvent with a boiling point 30°C to 40°C below the elution temperature for the first sample component of interest. This will produce the solvent effect at higher initial temperatures, reduce the programming rate needed for solvent focusing in a splitless analysis, and decrease the overall analysis time.

Precautions for on-column injections — A guard column (1 meter of deactivated fused silica tubing) should be used when performing on-column injections onto a nonbonded phase. A guard column is also recommended (but not required) for bonded phase columns. It will protect the nonbonded phase from being dissolved at the column inlet during injections. Guard columns also prevent nonvolatile and insoluble sample components from ruining your analytical column. Attach them to your analytical column with a Capillary Butt Connector. Refer to the Supelco catalog for more information.

Maintenance

Accurate qualitative and quantitative capillary chromatography requires a strict program of maintenance. Splitter liners and detector liners (if present) should be periodically cleaned and deactivated. Run the test mix weekly to verify consistent system performance, and routinely examine the column inlet end for particles and contaminants. Also, the column should be reversed (detector end of the column connected to the injection port) to help maintain a uniform film thickness, thereby increasing column life.

Injector/Detector Liners

Often only 1-5ng of a sample component passes through a column. Therefore, clean, inert liners are necessary to prevent adsorption of the sample components. The liners cover areas of direct sample contact which, if dirty, can diminish system performance. Most available liners are not deactivated, although they should be. Deactivated liners for most GCs can be found in the Supelco catalog.

Since injector sleeves can become contaminated with septum fragments and sample residue, examine the sleeves each time you change the septum. If dirty, rinse the sleeves with pentane, methylene chloride or acetone. These solvents do not affect the deactivated surface. If a harsher chemical clean-up is necessary, or if a water-soap solution is used, the surface may have to be redeactivated with Sylon™-CT (Cat. No. 33065-U). If a sleeve cannot be cleaned with organic solvents, we recommend discarding it and using a new, deactivated sleeve.

If samples are quite dirty, you may want to pack the splitter liner to catch sample residue. Simply pack about 1/2" of the splitter sleeve with an inert material such as SUPELCOPORT™ support. To prepare a pre-column, this material can be coated with the same phase as the capillary column. A discussion on this technique is on page 50 in *Introduction to Open Tubular Columns* by L.S. Ettre, Perkin-Elmer Corporation, 1974 (Cat. No. 23556).

Routine Column Maintenance

To ensure that your chromatographic system performs at optimum, make a weekly injection of the appropriate isothermal test mix and compare the results to the original test chromatogram. (Test mixes are available for all Supelco capillary columns.) If you do not obtain similar efficiency and adsorptivity results, you may have instrument or installation problems. If so, troubleshoot the system using these instructions and correct the problem. When you can duplicate the test results, your column will be performing at optimum. Interpretation of the test results is covered in the test mix instruction sheet.

Depending on use, capillary columns may eventually show tailing, broadening peaks, or retention changes. If your column shows tailing peaks and you have concluded that dirty liners are not the cause, then the problem could be septum fragments or sample residue contaminating the inlet end of the column. This can be quickly cured by cutting two loops (1/2 meter) from the inlet end of the column. If necessary, a bonded phase capillary column can be rinsed with a solvent to remove contaminants. See "Rinsing a Bonded Phase" (following) for details.

If you observe a gradual loss of column efficiency or decrease in retention times, two possible causes exist: (1) the column inlet is dirty or (2) phase has gradually bled from the inlet end of the column and recondensed farther down. This phase gradient results from having a continual one-directional flow. Avoid this by periodically (about every two weeks) reversing the inlet and detector ends of the column, and thus the direction of flow. This procedure is a preventative, not a cure.

Rinsing a Bonded Phase

You can rinse a Supelco bonded phase capillary column with certain solvents to remove soluble contaminants that cause adsorption and peak tailing. Rinsing, however, also removes polymer fragments formed by thermal degradation of the phase during analyses. Therefore, each time the column is rinsed and subsequently heated, analysis time decreases by approximately 5%.

When flushing contaminants from the inlet, solvent should always flow **to** the inlet **from** the outlet. You may find it easiest to attach the inlet end of the column to a vacuum source and pull the solvent through the column.

Alternatively, solvent may be pushed through the column from a pressurized reservoir at approximately 30psig. All Supelco bonded phase columns can be rinsed with pentane, methyl chloride or acetone. Other solvents have unpredictable results and therefore are not recommended. We suggest solvent volumes of 5-10mL.

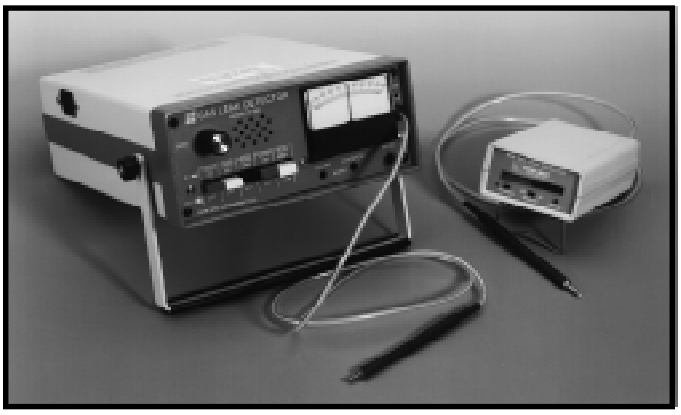
In some cases, contaminants polymerize in the column inlet and rinsing with solvents will not restore the column to acceptable performance. In such cases, cut off two loops (1/2 meter) from the column inlet. If samples are excessively dirty, attach a guard column to the column inlet, with a capillary butt connector to prevent contamination from reaching the analytical column. Refer to the Supelco catalog for more information regarding columns.

In summary, capillary columns are extremely sensitive to changing conditions. A routine injection of the test mix allows a week-to-week comparison of your system's condition, making it easy to pinpoint when conditions change. Injector and detector sleeves should also be examined frequently, and a periodic reversal of flow will prolong the useful life of your column.

Ordering Information:

Helpful Products To Simplify Column Installation

GOW-MAC Gas Leak Detectors for GC Fittings



995-0110

- Deluxe model with meter readout and audible leak alarm
- Smallest mini detector available, only one with a rechargeable battery.

GOW-MAC Gas Leak Detectors use thermal conductivity to accurately locate gas leaks. The units pinpoint leaks by detecting gases that have a thermal conductivity value different from that of air. This clean and efficient method of leak detection completely eliminates the risk of system contamination that can result from using soap solution.

Description	Cat. No.
Deluxe Model	**22409**
Mini Model	
115VAC/60Hz	**22807**
230VAC/50Hz	**22808**
Carrying case for mini model	**22809**

GlasSeal™ Capillary Column Connector

GlasSeal connectors immediately connect fused silica tubing of the same or different diameter — no tools, no leaks. Use to connect a guard column or transfer line, repair a broken column, or connect columns having the same or different phases. "Y" connectors split a sample to two columns or a column effluent to two detectors. Silanized for an inert inside surface. Choose borosilicate glass or fused silica. For use with our 0.25mm-0.53mm ID tubing.

Description	Cat. No.
GlasSeal Capillary Column Connectors	
Borosilicate, pk. of 12	**20479**
Fused Silica, pk. of 25	**23628**
"Y" GlasSeal Capillary Column Connectors	
Borosilicate, each	**20480**
Fused Silica, pk. of 3	**23632**

High Capacity Carrier Gas Purifier

994-0140

- Removes oxygen at high concentrations when disposable purifiers cannot
- Can triple the life of a capillary column
- Use with all carrier gas (except hydrogen) with flow rates as high as 1100cc/min Supelco's high capacity gas purifier prevents carrier gas with high concentrations of oxygen or water from destroying your capillary column. By ensuring that only pure gases enter the column, this purifier can extend the life of your column dramatically.

Power (VAC)	Fitting (inches)	Cat. No.
115	1/8	**23800-U**
115	1/4	**23802**
220	1/8	**23801**
220	1/4	**23803**

Capillary Cleaving™ Tool

912-0177

Make scalpel-like cuts in both polyimide and fused silica — no jagged edges to create problems. Industrial sapphire cutting edges remain sharp indefinitely. The spring-loaded retractable blade version reduces the chances of breakage if the tool is dropped.

Description	Cat. No.
Capillary Cleaving Tool with fixed blade	**23740-U**
Capillary Cleaving Tool with retractable blade	**23814**
Replacement Blade	**23815**

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The listing of documents available on ChromFax is updated every month. We encourage you to request a list of available ChromFax documents each time you use the ChromFax system.

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For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

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