Column Care and Use Instructions

GRACE

GC Capillary Columns

M171c

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Important – Please Read!

Warning!

- To avoid leaks or pressure related failures, please ensure all fittings and connections are tight and secure before operating the column.
- Do not operate the column outside of the temperature limits listed on the certificate of analysis.
- Use appropriate safety measures when handling compressed gases.
- Hydrogen is a flammable gas. If hydrogen or any other flammable gas is used, periodic leak tests should be performed on column connections. Be sure that the gas supply is off until all connections are made, and insure that the inlet fittings are either connected to the column or capped at all times when flammable gas is being used.
- Do not use hydrogen as the carrier for conditioning! It could vent into the GC oven and present an explosion hazard.
- Always use appropriate personal protective equipment. The GC oven may be hot enough to cause burns. Wear heat resistant gloves and use caution when removing or installing the column. Wear safety glasses to protect your eyes from flying particles while handling, cutting, or installing capillary columns. Use care in handling columns to prevent puncture wounds.
- Refer to the certificate of analysis for your specific column for additional information.

Alltech® Capillary Instruction Manual

Alltech® fused silica capillary columns are supplied on low-mass cages. Inherently straight and flexible, fused silica capillaries are easy to install. However, proper installation and operation of your Alltech® capillary column requires attention to the following topics.

1.0 GC Preparation

Before installing your column, the gas chromatograph needs to be inspected to ensure accurate results and to prolong the life of your column.

1.1 Gas Purification

It is extremely important to use clean, oxygen-free carrier gas for capillary work. Stationary phases of capillary columns are susceptible to oxidation at elevated temperatures. We strongly suggest using both a high capacity non-indicating oxygen trap and an indicating oxygen trap. A moisture trap is also suggested.

We also suggest that all Flame Ionization Detector (FID) air and make-up gas lines contain moisture and hydrocarbon traps. Electron Capture Detector (ECD) make-up gas lines should contain moisture traps. Contamination of these gases can result in noisy baselines.

1.2 Injection Port

The injection port is a common place for contamination. A clean, deactivated liner is essential for optimum column performance. Before installing a column, check that your liner is free of sample residue as well as septa and ferrule particulates. The liner should also be deactivated to reduce adsorption and decomposition of sensitive compounds.

The septum is another source of contamination and leaks. With use, septa tend to “core” or develop a hole in the center. This can cause leaks and loss of sample as well as septa fragmentation and liner contamination. Replace the septum often, preferably with high temperature, low bleed septa. Condition a newly installed septum overnight to reduce septa bleed.

1.3 Detectors

Make sure that your detector is in good working order. FIDs, in particular, require periodic cleaning of the flame jet. The detector gas flow rates should be checked for optimum column performance. Contamination of the detector or improper detector gas flow rates can reduce sensitivity and efficiency.

1.4 Ferrule Selection

Choosing a ferrule for column connections can be confusing. There are pros and cons associated with all ferrule materials. Use the following summary to determine the ferrule material right for your application.

Graphite — This material deforms easily, giving it excellent resealing properties. However, its reusability, or lifetime, is limited because of its pliability. Graphite has an upper temperature limit of 450°C.

Vespel® — Vespel® is very rugged with respect to reusability but its resealing properties suffer from its stiffness. Vespel® commonly sticks to glass making it hard to remove. Vespel® has an upper temperature limit of 350°C.

Vespel® Graphite 1 (VG1) — This material is 85% Vespel® and 15% graphite. This combination reduces the sticking property and raises the temperature limit of Vespel® while improving the reusability of the ferrule. The upper temperature limit of VG1 is 400°C.

Vespel® Graphite 2 (VG2) — This material is very similar to VG1 except the material contains 60% Vespel® and 40% graphite. This material has an upper temperature limit of 400°C.

Ferrules also come in different sizes. **Table 1** shows the proper ferrule i.d. for capillary columns of various internal diameters.

Table 1 — Ferrule Selection

Column i.d.	Ferrule i.d.
0.10mm	0.4mm
0.18mm	0.4mm
0.25mm	0.4mm
0.32mm	0.5mm
0.45mm	0.8mm
0.53mm	0.8mm

2.0 Preparing the GC Oven

The first step when working inside the GC oven is to cool down the injector and detector. This allows you to install your column without burning yourself.

Some GC ovens have built-in capillary hangers. You can also make a hanger from soft, smooth wire by attaching one end to the top of the oven and the other end to the capillary column cage. DO NOT allow the wire or the hanger to contact the capillary tubing, as this may scratch and break the column. Secure the capillary cage on the hanger while you perform the rest of the installation steps.

3.0 Preparing the Capillary Column

Slip the nut and then the ferrule over the injector end of the capillary column and slide them 6 inches down the column. Then cut off a two inch section of column from the injector end. This eliminates the possibility that ferrule fragments have entered the column.

The ends must be cut square, with none of the polyimide coating projecting beyond the ends. Inspect the ends with a 20X magnifier to make sure the cut is square. If the end is not square, make a small scratch about 1-inch from the end with a diamond-tipped or ceramic scriber. Hold the column between the thumb and forefinger, one hand on either side of the scratch. Place the scratch away from you and apply slight pressure with your thumbs. The column should snap easily and cleanly. Avoid making any scratches elsewhere on the column as this can cause your column to break.

4.0 Connecting to the Injector

Insert the column into the injection port as recommended by the instrument manufacturer (**see Figure 1**). This depth will vary depending upon whether you are using split, splitless, on-column or a direct injector. Tighten the nut until it is finger-tight. With an appropriate sized wrench, carefully tighten the nut 1/2 turn more. The nut and ferrule should be tight enough that the capillary column does not move when gently pulled. Check all connections for leaks. To prevent damage to your equipment, we recommend using a thermal conductivity type leak detector over bubble or soap containing leak detectors.
The nut should be retightened after several thermal cycles. Vespel® ferrules and composites of graphite and Vespel® tend to shrink when initially heated. This shrinkage may cause a leak.

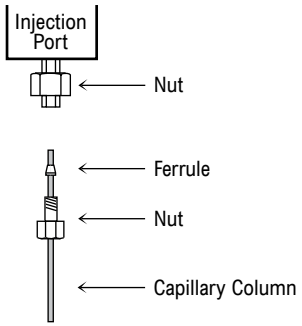

Figure 1 — Connecting to the Injector

5.0 Connecting to the Detector

Please consult your detector manufacturer's instruction manual for the correct insertion distance. Insert the column into the detector and tighten the nut until it is finger-tight (**see Figure 2**). With an appropriate sized wrench, carefully tighten the nut a 1/2 turn more. The nut and ferrule should be tight enough that the capillary column does not move when gently pulled. Check all connections for leaks. To prevent damage to your equipment, we recommends using a thermal conductivity type leak detector over bubble or soap containing leak detectors.

For an FID, the column is usually inserted all the way to the tip of the detector jet and then pulled back about 2mm. This distance is close enough to prevent dead volume, but far enough away from the jet that it prevents the column from being crushed against the jet when the nut is tightened.

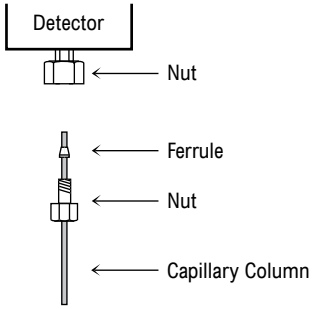

Figure 2 — Connecting to the Detector

6.0 Column Conditioning

Capillary columns can be exposed to volatile compounds during handling, storage, and installation. Proper conditioning eliminates the baseline interference that these compounds may cause.

6.1 When to Condition

After installation, all capillary columns should be conditioned by heating, at least once, prior to analysis. For high temperature analysis and for operations at high sensitivities, you should condition the column by putting it through the conditioning cycle several times.

6.2 Conditioning Instructions

After installing the capillary column, begin the carrier gas flow and allow the column to purge at ambient temperature for 15 minutes. Then heat the column from ambient to 25°C above the maximum anticipated operating temperature or up to the upper temperature limit for the column, whichever is less, at 5°C/minute and hold there for 45 minutes. If you are working at high sensitivities or with thick-film columns (>1µm), the conditioning cycle should be repeated several times. Keep the injector and detector temperatures below the upper temperature limit to ensure column stability.

Table 2 is a list of upper temperature limits for Alltech® columns. Note that thicker films have lower temperature limits.

6.3 Alltech® Capillary Upper Temperature Limits

Table 2 — Upper Temperature Limit

Phase	Film Thickness	Temperature
AT™-1, EC™-1	0.10-1.20µm	350ºC
	1.50-2.50µm	330ºC
	2.65-5.00µm	300ºC
AT™-1ht	0.10µm	380ºC
AT™-1ms	0.10-1.00µm	350ºC
AT™-2887	1.20µm	350ºC
AT™-3710	5.00µm	300ºC
AT™-Petro	0.50µm	350ºC
AT™-Sulfur	4.00µm	350ºC
AT™-5, EC™-5	0.10-1.20µm	350ºC
	1.50-2.50µm	330ºC
	2.65-5.00µm	300ºC
AT™-5ms	0.10-1.00µm	350ºC
AT™-20, EC™-20	0.25-0.75µm	320ºC
	1.00-1.20µm	300ºC

Table 2 — Upper Temperature Limit (continued)

Phase	Film Thickness	Temperature
AT™-35, AT™-35ms	0.10µm	350ºC
	0.25-0.50µm	320ºC
	1.00-1.20µm	300ºC
	2.00-2.65µm	280ºC
AT™-50	0.25µm	325ºC
	0.50µm	300ºC
	1.00µm	280ºC
AT™-210	0.15-0.50µm	260ºC
	1.00-1.20µm	240ºC
AT™-225	0.15-0.30µm	240ºC
	0.50-1.20µm	220ºC
AT™-WAX, EC™-WAX,	0.10-0.40µm	280ºC
AT™-WAXms	0.50-2.00µm	260ºC
	2.50-5.00µm	250ºC
AT™-AquaWax™	0.25µm	260ºC
AT™-AquaWax™-DA	0.25µm	250ºC
AT™-1000, EC™-1000	0.20-1.20µm	250ºC
AT™-FAME	0.25µm	280ºC
AT™-1301	0.25µm	280ºC
	1.00µm	260ºC
AT™-1701	0.20-1.20µm	280ºC
AT™-Pesticide	0.25-0.60µm	240ºC
AT™-502.2	1.40-3.00µm	270ºC
AT™-624	1.40-3.00µm	260ºC
Drug 1,2,& 3	0.25-1.20µm	330ºC
AT™-Silar™ 90	0.25µm	250ºC
AT™-Silar™ 100	0.25µm	250ºC
AT™-Amine	0.25µm	200ºC
AT™-Amino Acid	1.20µm	350ºC
AT™-CAM	0.25-1.00µm	250ºC

** Porous Layer Open Tubular (PLOT) columns.*

7.0 Testing the Column

Testing ensures that the capillary column has been installed correctly and aids in establishing the correct linear velocity of the carrier gas. See **Table 3** for recommendations on detector/compound combinations.

With the column installed, set the operating temperatures. The oven temperature used is determined by the compounds being analyzed. Adjust the oven temperature to the isothermal analysis temperature or the midpoint of your temperature program. Injector and detector temperatures are normally set 25°C above your highest oven temperature.

Table 3 — Recommended Compounds ($t_{Ø}$)

Detector	Compound
FID/TCD	methane
NPD	acetonitrile vapors
ECD	methylene chloride vapors
ELCD	dichlorodifluoromethane vapors
MS	air
PID	ethylene

Inject 1µL of the unretained compound recommended for your detector in **Table 3**. The peak should be very sharp and symmetrical. See **Figure 3a**. If tailing is observed, **Figure 3b**, this is an indication that there is some “dead” or unswept volume present in the system. This could be caused by improper installation of the column into either the injector or detector, ferrule fragments in the column, lack of make-up gas, or a badly contaminated injector or detector.

Inject 1µL of the sample solvent and check its symmetry. If it tails, the problem is most likely in the injector. Check to see if the column end still has a square cut and that its insertion distance was correct. Also check the injection port itself to see if the liner is cracked or contains debris. When a symmetrical peak is obtained, the linear velocity and hold-up time (see next page) can be determined.

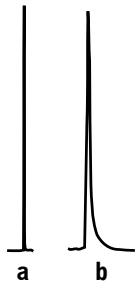

Figure 3 — Peak Shapes

Measure the retention time of the peak using the appropriate compound as shown in **Table 3**. This is the hold-up time (tØ) in seconds. The average linear velocity ($\bar{u}$) is calculated by the following equation, where L is the length of the column in cm.

$$\bar{u} = L/t_{Ø}$$

The linear velocity should be adjusted to

20-25cm/sec for helium carrier gas by increasing or decreasing the carrier gas head pressure. See **Table 4** for approximate head pressures for various lengths and internal diameters using helium or hydrogen as the carrier gas.

Table 4 — Approximate Head Pressures

Length	Column i.d.					
	0.10mm	0.18mm	0.25mm	0.32mm	0.45mm	0.53mm
10m	27psig	7.3psig	3.8psig	2.3psig	1.2psig	0.8psig
15m	—	—	5.7psig	3.4psig	1.7psig	1.3psig
20m	54psig	15psig	—	—	—	—
25m	—	—	10psig	5.8psig	2.9psig	2.1psig
30m	—	—	12psig	6.9psig	3.5psig	3.5psig
40m	—	32psig	—	—	—	—
50m	—	—	21psig	12psig	5.8psig	4.2psig
60m	—	—	25psig	15psig	7.0psig	5.0psig
100m	—	—	42psig	25psig	12psig	8.6psig

All values shown are for Helium or Hydrogen

8.0 Test Mixes

Once it has been determined that the column has been installed properly and the optimum linear velocity has been set, a test probe mixture should be run to evaluate the performance of the column and the system as a whole. This test should be repeated periodically and a record kept to monitor column performance. **Table 5** contains a complete list of test mixes for Alltech® capillary columns.

A variety of probes and mixtures are used to test capillary columns. Alcohols and diols are commonly used to detect the presence of active silanol groups in the capillary column or the injection system. Ketones and aldehydes will show the presence of sites that interact with Lewis acids. Naphthalene is sometimes used to indicate metal adsorptive sites. An assortment of acidic or basic compounds is frequently used to determine how "neutral" a column is. Esters and n-paraffins are used to determine column efficiencies and as retention markers for calculating McReynolds constants.

Table 5 — Test Mix Selection

Description	Part No.
Grob Mix	**41761**
For AT™-1, EC™-1	**441700**
For AT™-5, AT™-1701, EC™-5, AT™-20, EC™-20, AT™-502.2	**441701**
For AT™-1000, EC™-1000, AT™-AquaWax™-DA	**441704**
For AT™-WAX, EC™-WAX, AT™-AquaWax™	**441716**
For AT™-225	**441705**
For AT™-624	**441707**
For AT™-50	**441708**
For AT™-35	**441709**
For AT™-35 (0.53)	**441710**

9.0 Stationary Phase Polarity

Stationary phase polarity is related to the amount and polarity of the stationary phase functional groups. Polarity is a useful tool because it influences component separation. A pair of components that co-elute on a non-polar column might separate on a polar column, or vice versa. **Figure 5** lists the polarities of some common stationary phases. Each bar represents the sum of the McReynolds constants for that stationary phase.

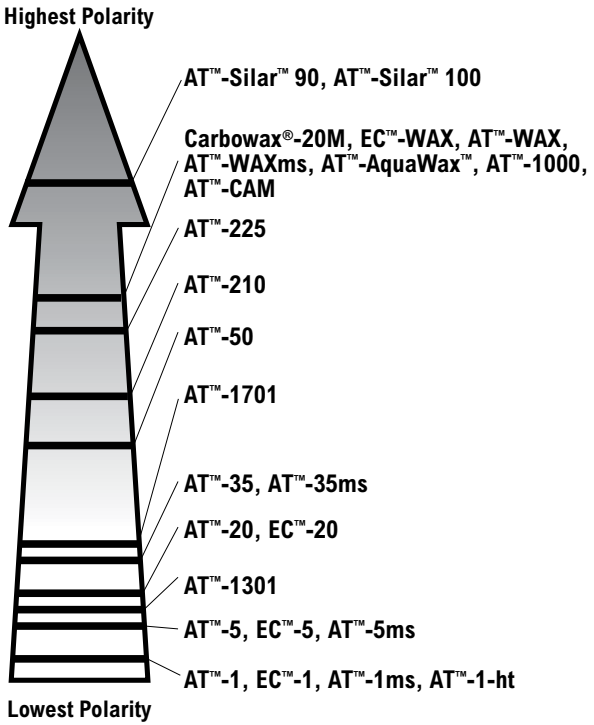

Figure 5 — Relative Stationery Phase Polarity

10.0 Sample Capacity

Sample capacity is, by one definition, the maximum amount (in nanograms) of a single analyte that can be introduced onto a column without the peak being distorted (peak fronting). **Figure 6** shows an example of a fronting peak. This fronting widens the peak, causing possible integration and co-elution problems.

The sample capacity of a column is related to the amount of stationary phase present in any given length of column. An increase in phase amount equals an increase in capacity. The sample capacity may also be increased by using a larger bore column. The absolute quantity of stationary phase per unit length is larger with a larger diameter column. **Table 6** shows some common sample capacities for capillary columns.

Figure 6 — Fronting Peak

Table 6 — Approximate Sample Capacity

Column i.d.	Film Thickness			
	0.1µm	0.25µm	0.5µm	1.0µm
0.10mm	10ng	30-40ng	50-70ng	100-200ng
0.18mm	20-30ng	60-80ng	100-150ng	250-350ng
0.25mm	30-40ng	125-175ng	175-250ng	400-500ng
0.32mm	50-70ng	200-250ng	250-350ng	600-800ng
0.45mm	80-100ng	300-400ng	400-500ng	800-1000ng
0.53mm	100-120ng	400-500ng	500-700ng	1000-1500ng

11.0 Column Contamination

Column contamination can cause a myriad of problems. Most frequently they appear as peak shape anomalies, baseline disturbances, retention time changes, or even adsorption. The following sections explain how to prevent and fix contamination problems.

11.1 Preventing Column Contamination

The first line of defense against column contamination is the injection port liner. An injection port liner that is packed with glass wool provides a surface for high molecular weight and inorganic compounds to collect upon. This limits the possibility of contamination reaching the column. When using “dirty” samples, change the injection port liner frequently.

Even when using an injection port liner, it is possible for contaminants to reach the column. These contaminants can condense, pyrolyze, or salt out on the front of the capillary column and reduce the sensitivity (due to adsorption) and efficiency of later runs. Using a guard column greatly reduces the effects of contamination on your analysis.

A guard column is a 1-5 meter piece of uncoated, deactivated fused silica connected between the injector and the front of the analytical column. It collects residue that would otherwise be retained on the analytical column. The stationary phase on a standard column slows down the sample and allows it to interact with the already trapped contamination for a longer period of time. An uncoated guard column, on the other hand, condenses or traps the contamination but does not slow down the sample, thus reducing any interaction time. Grace offers 5-meter guard columns.

11.2 Restoring a Contaminated Column

The easiest way to restore a contaminated column’s performance is to remove 1-2 coils of the column on the inlet end. Since most of the contamination collects on the first meter of the column, removing these coils will usually remove the contamination.

Another alternative involves rinsing the column with a solvent. This is a more time consuming method that might damage the stationary phase if the wrong solvent is chosen. Please contact Grace’s Technical Support Department before attempting to rinse a column.

12.0 Making Connections

A wide variety of devices have become available for connecting capillary columns with guard columns, other capillary columns, and fused silica tubing. The following sections cover some of the popular types.

12.1 Metal Unions

Various types of unions are available to connect two or more capillary columns to each other. By changing the ferrules, two differently sized pieces of tubing can be joined. However, metal unions typically contain an internal metal pathway which could adsorb sensitive compounds. Zero dead-volume unions, which butt the capillary columns up against one another, reduce potential activity by eliminating the internal metal pathway, and are the best choice in metal unions.

12.2 Fused Silica Connectors

Universal Capillary Connectors (**Figure 7**), available both straight and angled, provide a convenient method to connect two or more capillary columns together without any introduction of dead volume or active sites. A leak-tight connection is formed between the polyimide coating and the fused silica connector. Note that a curing process is required to make a good seal.

Figure 7 - Straight Universal Capillary Connectors

13.0 Storage

Always place the column on a clean, dirt-free surface, preferably in the original shipping container, when not in use. All columns, especially Carbowax® and cyano-containing stationary phases, should have their ends sealed to prevent oxidation. Oxidation can show up as a noisy or rising baseline.

14.0 Troubleshooting Guide

Missing Peaks	
Possible Cause:	**Possible Solution:**
Defective syringe	Verify with new syringe.
Detector off or not functioning	Check settings.
Injection port temperature too low	Check temperatures, adjust as needed.
Oven temperature too low	Check temperatures, adjust as needed.
No carrier flow	Check pressure regulators. Also check for leaks. Verify flow from column exit.
Column broken	Column can be salvaged if broken at inlet or detector end. Cut column and reinstall.
Fronting Peaks	
Possible Cause:	**Possible Solution:**
Column overloaded	Decrease sample size.
Two compounds co-eluting	Increase sensitivity and reduce sample size. Lower temperature 10-20°C to resolve peaks.
Sample condensation	Check injection port and column temperatures. Increase if necessary.
Sample decomposition	Use deactivated injection port liners, or lower injection port temperature.
Tailing Peaks	
Possible Cause:	**Possible Solution:**
Active sample adsorbing on injection port liner or column	Replace liner. If that does not solve the problem, remove 1-2 coils from the inlet end of the column and reinstall.
Column or injection port temperature too low	Increase temperature (do not exceed upper temperature limit for column). Injection port should be 25°C greater than the highest boiling point in sample.
Two compounds co-eluting	Increase sensitivity and reduce sample size. Lower temperature 10-20°C to resolve peaks.
Column deteriorating	Replace column.
Column contaminated	Remove 1-2 coils from the inlet end of the column and reinstall.
Ghost Peaks	
Possible Cause:	**Possible Solution:**
Column adsorption and subsequent desorption of sample	Replace liner. If that does not solve the problem remove 1-2 coils from the inlet end of the column and reinstall.
Contaminated syringe	Try new syringe with clean solvent. If ghost peaks disappear, clean syringes more thoroughly.
Sample too large	Lower injection amount.
Poor injection technique (too slow)	Use rapid, smooth injection technique.
Solvent Peak Only	
Possible Cause:	**Possible Solution:**
Defective syringe	Verify with new syringe.
Incorrect carrier gas flow (too low)	Check flow and adjust if necessary.
Sample too dilute	Inject a sample known to give good results. If results are good, raise sensitivity or injection amount.
Oven temperature too hot	Check temperature and adjust as needed.
Column cannot resolve components from solvent peak	Change column to a thicker film or different polarity. Change solvent.
Carrier gas leak	Check for leaks.
Sample being adsorbed by column or injection port liner	Replace liner. If that does not solve the problem, remove 1-2 coils from the inlet end of the column and reinstall.
Split flow too high	Adjust flow.

Broad Solvent Peaks	
Possible Cause:	**Possible Solution:**
Dead volume in injection port due to improper column installation	Reinstall column.
Poor injection technique (too slow)	Use rapid, smooth injection techniques.
Injection port temperature too low	Increase injection port temperature.
Sample solvent interacts with the detector (i.e. Methylene Chloride/ECD)	Change sample solvent.
Sample solvent retained by column	Change sample solvent.
Septum purge not working properly	Adjust or fix septum purge.
Incorrect split ratio (insufficient split vent flow)	Adjust flow.
Unresolved Peaks on a Column Which Worked Well in the Past	
Possible Cause:	**Possible Solution:**
Wrong column temperature	Check and adjust temperature.
Wrong carrier gas flow rate	Check and adjust flow rate.
Sample size too large	Reduce sample size.
Poor injection technique (too slow)	Use a rapid, smooth injection technique.
Column or liner contaminated	Replace liner. If that does not solve the problem remove 1-2 coils from the inlet end of the column and reinstall.
Irregular or Unstable Baseline	
Possible Cause:	**Possible Solution:**
Column bleed or contamination	Replace liner. If that does not solve the problem, remove 1-2 coils from the inlet end of the column and reinstall.
Contaminated detector or injection port	Clean detector and/or injection port.
Carrier gas leak	Change septum and check for column leaks.
Inconsistent carrier gas regulation	Check carrier gas supply for sufficient pressure. Replace tank if ≤500 psig pressure.
Gas impurities or contaminated gas line	Change gas tank, use gas purifiers, and clean metal tubing.
Gas flows not within minimum/maximum limits of the instrumentation (including hydrogen and air on FID), or poorly regulated flow	Measure flows and verify against manual specifications.
Defective detector	Consult instrument manual.
Septum bleed	Condition or replace septum.
Retention Times Longer (or Shorter) on Same Column	
Possible Cause:	**Possible Solution:**
Column temperature too low or too high	Check and adjust temperature.
Carrier gas flow rate too low or too high	Measure flow rate with a properly calibrated source of measurement at column exit and adjust.
Septum or column leak	Check and correct if needed.
Column contaminated or deteriorated	Recondition or replace column.
Sample overload	Reduce sample size.
Recorder defect	Check recorder.
Inconsistent carrier gas regulation	Check carrier gas supply for sufficient pressure. Replace tank if ≤500 psig pressure.

Alltech® Heliflex® and Econo-Cap™ Columns

Grace offers two capillary column lines. **Alltech® Heliflex® columns** are Grace's premium full line of individually tested capillary columns. **Alltech® Econo-Cap™ columns** are available in popular phases and are batch tested to dramatically reduce the price without sacrificing quality. Both lines are manufactured under identical conditions using the same high quality polyimide coated, fused silica tubing, and immobilized stationary phases. Every Alltech® capillary column is mounted on a rugged 7" cage.

	Alltech® Econo-Cap™ Columns	Alltech® Heliflex® Columns
Advantages:	Save Considerably Over Competitors' Columns	Wide Selection of Phases and Column Dimensions
	Identical Manufacturing to Heliflex and Other Manufacturers' Premium Lines	Custom Columns Available for Specialty Applications
Differences:	Batch Tested	Individually Tested
	5 Phases Available	28 Phases Available
Construction:	Polyimide Coated Fused Silica Tubing	Polyimide Coated Fused Silica Tubing
	Crosslinked and Bonded Stationary Phases	Crosslinked and Bonded Stationary Phases
Recommend For:	Aggressive Applications Where Column Replacement is Frequent	Any Capillary Column Application
	Cost Conscious Customers	Unique Separation that Requires Custom Phase or Dimensions
	Training Laboratories	Exact Equivalent to Other Manufacturers' Columns

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