

Astec CHIRALDEX® and Supelco DEX™ Column Care & Use

In addition to these guidelines, please follow the general care and use instructions for any capillary GC column. These are found on the column's Certificate of Analysis.

Choosing a Carrier Gas

The highest purity gases along with efficient moisture and oxygen removing purifiers installed in the carrier gas stream are essential to ensure continual optimal chromatographic performance. The three carrier gases most often used are nitrogen, helium, and hydrogen. All three have distinct advantages and disadvantages.

- Although we do not recommend nitrogen as a carrier gas, it does cost less and can provide the highest efficiency. The disadvantages are low optimum linear velocity (12 cm/sec), which results in long retention times, and a steep van Deemter curve above optimum (efficiency is rapidly lost with increasing linear velocity).
- Helium has a higher optimum linear velocity (20 cm/sec) and a flatter van Deemter curve above optimum (can be used at a linear velocity above optimum without a great loss of efficiency). However, helium may be the most expensive to use.
- Hydrogen has a very high optimum linear velocity (40 cm/sec) and the flattest van Deemter curve above optimum (can be used at a linear velocity above optimum without loss of efficiency). Hydrogen is also more expensive than nitrogen, and can form explosive mixtures with air. Therefore, it must be used with caution following the safety guidelines of your organization.

Carrier Gas Purifiers

Both moisture and oxygen adversely affect the selectivity and stability of GC phases, especially some chiral phases. Therefore, it is essential that efficient moisture and oxygen removing purifiers are installed in the carrier gas stream to ensure continual optimal chromatographic performance. Hydrocarbon traps are recommended, but not required, to remove impurities that can cause ghost peaks.

Maximum Allowable Operating Temperatures

All columns are temperature rated. The maximum temperatures for both isothermal and temperature programmed conditions are shown for each phase in Table 1.

Temperature Effects on Enantiomer Separations

Temperature plays a significant role in chiral selectivity and resolution for both GC and HPLC. If an analyte to be enantioresolved has a boiling point under the maximum temperature allowed on the GC column (elution temperatures are related to analyte vapor pressure, not boiling point) or can be derivatized to such a point, it is a candidate for separation by GC.

Enantiomeric selectivity decreases with increasing temperature, and lower temperatures should be used when possible. Indeed, enantioselectivity is rare above 200 °C. In general, the lower the temperature at which the compound can be eluted, the greater the opportunity for chiral separation. When conditions yield little or no separation of enantiomers, try reducing the analysis temperature. Selectivity can be dramatically enhanced with small temperature reductions at temperatures below 130 °C. Above this temperature, this temperature effect is less pronounced. Increasing carrier gas linear velocity (He or H₂, up to 50 cm/sec) is often more useful than increasing column temperature to affect retention and resolution. To this end, hydrogen and helium are the best carrier gas choices.

Table 1. Temperature Ranges

Phase	CD Type*	Derivative	Full Description	Min.Temp.	Max. Temp. (Isothermal)	Max. Temp. (Progr.)
α-DEX 120	α	Permethyl	20% permethylated α-cyclodextrin in SPB-35 poly(35% diphenyl/65% dimethylsiloxane)	30 °C	230 °C	230 °C
α-DEX 225	α	Diacetyl	25% 2,3-di-O-acetyl-6-O-TBDMS-α-cyclodextrin in SPB-20 poly(20% phenyl/80% dimethylsiloxane)	30 °C	230 °C	230 °C
α-DEX 325	α	Dimethyl	25% 2,3-di-O-methyl-6-O-TBDMS-α-cyclodextrin in SPB-20 poly(20% phenyl/80% dimethylsiloxane)	30 °C	230 °C	230 °C
β-DEX 110	β	Permethyl	10% permethylated β-cyclodextrin in SPB-35 poly(35% diphenyl/65% dimethylsiloxane)	30 °C	230 °C	230 °C
β-DEX 120	β	Permethyl	20% permethylated β-cyclodextrin in SPB-35 poly(35% phenyl/65% dimethylsiloxane)	30 °C	230 °C	230 °C
β-DEX 225	β	Diacetyl	25% 2,3-di-O-acetyl-6-O-TBDMS-β-cyclodextrin in SPB-20 poly(20% phenyl/80% dimethylsiloxane)	30 °C	230 °C	230 °C
β-DEX 325	β	Dimethyl	25% 2,3-di-O-methyl-6-O-TBDMS-β-cyclodextrin in SPB-20 poly(20% phenyl/80% dimethylsiloxane)	30 °C	230 °C	230 °C
γ-DEX 120	γ	Permethyl	20% permethylated γ-cyclodextrin in SPB-35 poly(35% phenyl/65% dimethylsiloxane)	30 °C	230 °C	230 °C
γ-DEX 225	γ	Diacetyl	25% 2,3-di-O-acetyl-6-O-TBDMS-γ-cyclodextrin in SPB-20 poly(20% phenyl/80% dimethylsiloxane)	30 °C	230 °C	230 °C
γ-DEX 325	γ	Dimethyl	25% 2,3-di-O-methyl-6-O-TBDMS-γ-cyclodextrin in SPB-20 poly(20% phenyl/80% dimethylsiloxane)	30 °C	230 °C	230 °C
CHIRALDEX A-DA	α	Dialkyl	2,6-di-O-pentyl-3-methoxy derivative of α-cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX A-TA	α	Trifluoroacetyl	2,6-di-O-pentyl-3-trifluoroacetyl derivative of α-cyclodextrin	-10 °C	180 °C	180 °C
CHIRALDEX B-DA	β	Dialkyl	2,6-di-O-pentyl-3-methoxy derivative of β-cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX B-DM	β	Dimethyl	2,3-di-O-methyl-6-t-butyl silyl derivative of β-cyclodextrin	-10 °C	200 °C	220 °C

Table 1. Temperature Ranges (contd.)

Phase	CD Type*	Derivative	Full Description	Min.Temp.	Max. Temp. (Isothermal)	Max. Temp. (Progr.)
CHIRALDEX B-DP	β	Dipropionyl	2,3-di-O-propionyl-6-t-butyl silyl derivative of β -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX B-PH	β	(S)-Hydroxypropyl	(S)-2-hydroxy propyl methyl ether derivative of β -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX B-PM	β	Permethyl	2,3,6-tri-O-methyl derivative of β -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX B-TA	β	Trifluoroacetyl	2,6-di-O-pentyl-3-trifluoroacetyl derivative of β -cyclodextrin	-10 °C	180 °C	180 °C
CHIRALDEX G-BP	γ	Butyryl	2,6-di-O-pentyl-3-butyryl derivative of γ -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX G-DA	γ	Dialkyl	2,6-di-O-pentyl-3-methoxy derivative of γ -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX G-DM	γ	Dimethyl	2,3-di-O-methyl-6-t-butyl silyl derivative of γ -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX G-TA	γ	Trifluoroacetyl	2,6-di-O-pentyl-3-trifluoroacetyl derivative of γ -cyclodextrin	-10 °C	180 °C	180 °C
CHIRALDEX G-DP	γ	Dipropionyl	2,3-di-O-propionyl-6-t-butyl silyl derivative of γ -cyclodextrin	-10 °C	200 °C	220 °C
CHIRALDEX G-PN	γ	Propionyl	2,6-di-O-pentyl-3-propionyl derivative of γ -cyclodextrin	-10 °C	200 °C	220 °C

* α (alpha) CD = 6 glucose units, β (beta) CD = 7 glucose units, γ (gamma) CD = 8 glucose units

Oven Ramp Rates

CHIRALDEX stationary phases are sensitive to thermal shock. Never heat or cool the column at more than 15 °C/min. For separations below 130 °C, use temperature ramp rates of 1-5 °C/min. Over 130 °C, use temperature ramp rates of 5-10 °C. Supelco DEX columns do not exhibit this sensitivity.

Dedicated Columns Based on Oven Temperature Used

Columns used at high temperatures (>180 °C) for extended periods of time may lose their efficiency when then used at low temperatures, even though their performance remains constant at the high temperature. Therefore, we recommend that columns be dedicated for use at either low or high temperatures. This is more true with Astec CHIRALDEX than with the Supelco DEX phases.

Sample Preparation & Moisture Removal from Sample

The sample capacity of chiral capillary columns is lower than conventional columns, so samples should be sufficiently pure to protect the stationary phase. For the CHIRALDEX TA series, it is especially important to have the sample free of moisture. Methylene chloride extracts of aqueous samples contain >100 ppm water, sufficient to cause the hydrolysis of the TA. Evaporation to near dryness in the presence of dimethoxypropane or passing the extract through a bed of anhydrous sodium sulfate will adequately dry the sample. An additional step for routine analysis that can protect the TA columns would be to use a retention gap (methylphenyl) as a guard column. In addition to picking up residual moisture, the retention gap will protect the TA column from the high temperature of the injector. Typically 5 meters is used. If the TA column has been hydrolyzed, it may be regenerated using the regeneration procedures. The instructions can be obtained by contacting Supelco Technical Service at techservice@sial.com.

Sample Derivatization

Enantiomeric separation data for a variety of derivatized analytes indicates there is an enantioselectivity dependency on the type of derivatization reagent used. In many cases, changing the derivatization reagent provides resolution. The optimal derivative on one phase may not be the optimal derivative on another phase. Therefore, it is prudent to prepare a variety of derivatives for compounds that require derivatization.

It is sometimes desirable to derivatize analytes for the purpose of:

- Increasing volatility
 - Expands the range of compounds that can be analyzed using GC
 - Reduces analysis time
- Improving selectivity
 - Changing the interactions between the analyte and the chiral stationary phase.
 - May result in reversal of elution order.
- Increasing efficiency
 - Less tailing of basic and acidic compounds.

The first consideration when contemplating derivatization is that the derivatization reaction does not cause racemization of the analytes. Second, the possibility that the derivatization reaction may produce by-products that interfere with the analysis must be taken into account. Third, analytes with multiple function groups may require longer time for the reaction to go to completion. Common derivatization reagents are listed for specific compound classes. Sigma-Aldrich carries a complete line of derivatization reagents for chromatography.

- For alcohols and amines:
 - Trifluoroacetic anhydride makes the trifluoroacetyl derivative via acylation
 - Acetic anhydride makes the acetate via acylation
- For acids:
 - Methanolic HCl (2M) makes the methyl esters via alkylation
 - BF_3 -methanol makes the methyl esters via alkylation
 - BSTFA make the trimethyl silyl esters via silylation

Guard Columns (Methyl Phenyl Deactivated)

Use a methyl phenyl deactivated guard column to:

- Protect the column from non-volatile impurities.
- Protect the column from the high temperature of the injector and/or detector.
- Allow for the injection of sample volumes up to 7 μL on-column.

Typically, a 5-10 m long guard column is used. This can be connected via a GlasSeal™ Capillary Column Connector (or other column connector). To couple to a mass spectrometer, use a 1 m length as a transfer line.

Injection Technique

Astec CHIRALDEX stationary phases are not bonded and can only be run in a split mode unless a retention gap is used. Make sure the splitter flow is on and the split ratio is >30:1 before injection. Splitless or on-column injections will permanently damage Astec CHIRALDEX capillary columns (without the use of a retention gap). Although Supelco DEX phases are non-bonded, they can accommodate split or splitless injection without a retention gap.

Injection Solvents

Solvents routinely used to dissolve or dilute samples include ethyl ether, hexane, methylene chloride, and ethanol. Ethanol is not suitable for injection onto the Astec CHIRALDEX TA phases or for extracts of TFA derivatized alcohols. Select a solvent that volatilizes at least 40 °C below the elution temperature of the first component of interest. Use only the highest purity anhydrous solvents that contain no water. For the CHIRALDEX TA phases, it is especially important to have the extract free from moisture. Because the Supelco DEX phases are not bonded, they should not be rinsed with organic solvents.

Injection Volume

Separation performance is sometimes affected by the amount of sample injected, so it is best to routinely work with minimum sample and highest instrument sensitivity (such as 1.0 μL of a 5 mg/mL solution with a split ratio of 100:1). It is possible to use larger injection volumes when using a guard column. However, care must be taken to properly volatilize the solvent so as not to adversely affect film integrity. The procedure for large volume injections while using a guard column is:

- The large volume (1-7 μL) is slowly injected over 1-2 minutes at a temperature above the boiling point of the solvent but below the vaporization temperature of the analytes. For this to work, an elution temperature difference of at least 40 $^{\circ}\text{C}$ is necessary between solvent and analytes.
- After completion of the injection, the oven temperature is programmed at 5 $^{\circ}\text{C}/\text{min.}$ to the normal operating temperature. Minimal loss in efficiency (~3%) is involved, however, lower limits of detection are generally obtained.

Injector & Detector Temperature Settings

Keep the injector and detector temperatures (usually 200-250 $^{\circ}\text{C}$) subsequently higher than the column temperature. If necessary, use 1 m lengths of methyl phenyl deactivated tubing as transfer lines between the injector and the column and also between the column and the detector to protect it from the high temperatures.

Detection and Optimizing Sensitivity

All types of GC detectors, including MS, have been used with these phases. To obtain the highest column efficiency and chiral selectivity it is important to avoid overload conditions. Set the detector at the highest usable sensitivity and inject the lowest amount of sample that is detectable.

Storage

We recommend sealing all chiral capillary columns with a flame or by inserting the ends into a septum to maximize column lifetime. However, proper storage conditions are essential to maintain the Astec CHIRALDEX TA (trifluoroacetyl, A-TA, B-TA, G-TA) columns. The procedure we recommend for the TA series is as follows: With the carrier gas on, heat the column in a GC oven to 150 $^{\circ}\text{C}$ for 30-60 minutes. This will remove residual moisture from the column. Allow the column to cool in the oven with carrier gas flow. When cool, flame seal one end, pull a vacuum on the other end for 10 minutes then flame seal.

Regeneration Procedure for Astec CHIRALDEX A-TA, B-TA, and G-TA

The trifluoroacetic anhydride (TFA) derivative of the cyclodextrins used in Astec CHIRALDEX TA phases will hydrolyze in the presence of moisture at room temperature or above. Sources of moisture include the carrier gas, sample extracts, injection solvents, and air during unsealed storage. To ensure long column life, be sure the carrier gas has an efficient and functional moisture trap, all extracts are free of moisture before injecting, the injection solvent is anhydrous, and that the column is stored properly when not in use. The use of a guard column will also help protect the column from the damaging effects of moisture. The regeneration instructions can be obtained by contacting Supelco Technical Service at techservice@sial.com.

Special Considerations:

Optimizing Enantiomer Separation

GC method development on chiral stationary phases differs from traditional (achiral) methods in achieving and optimizing the selectivity. In chiral GC, the highest enantiomeric selectivity is achieved by maximizing the energy differences in the diastereomeric association complexes formed between each enantiomer and the chiral stationary phase. These energy differences become smaller with increasing temperature. Therefore, to optimize a chiral separation, low elution temperature in conjunction with relatively high carrier gas linear velocities are generally best.

Reversing Elution Order (Enantio reversal)

One of the most interesting and useful phenomena observed with chiral stationary phases is the ability to reverse elution order. The significance of this reversal is the reliable quantitation of trace enantiomers. It is always desirable to have the trace enantiomer elute first to avoid interference from tailing of the larger component. This reversal has been accomplished in four ways, listed in order of probability:

- Changing the cyclodextrin (such as from β -cyclodextrin to γ -cyclodextrin).
- Changing the phase type (such as from B-DA to B-PH).
- Changing the derivatization reagent (if analytes are derivatized).
- Operating below ambient temperature.

Current data indicate that molecules with limited inclusion complexing strength can reverse their elution order by any one of the first three methods. The reversal at sub-ambient condition is only possible for molecules eluted below 100 $^{\circ}\text{C}$ under normal operating conditions.

Test Mixes for Chiral Columns

To retest your column to ensure it is performing efficiently, refer to your column's Certificate of Analysis for test conditions. Or, contact Supelco Technical Service group at 814-359-3041 or techservice@sial.com. The table below lists our chiral GC test mixes.

Description	For Use with Phases	Cat. No.
α -DEX™ 120 Column Test Mix analytical standard, 500 $\mu\text{g}/\text{mL}$ each component in methylene chloride	Supelco α -DEX 120	48013
β -DEX™ 120 Column Test Mix analytical standard, 500 $\mu\text{g}/\text{mL}$ each component in methylene chloride	Supelco β -DEX 120	48028
Tetrahydro-2-(2-propynyloxy)-2H-pyran analytical standard, ampul of 1 mL, 5000 $\mu\text{g}/\text{mL}$ in ethanol: isopropanol (95:5)	CHIRALDEX A-DA	90001AST
1-(N-TFA)-2-Methylpiperidine analytical standard, ampul of 1 mL, 5000 $\mu\text{g}/\text{mL}$ in ethanol: isopropanol (95:5)	CHIRALDEX G-TA	90002AST
2-(N-TFA)aminoheptane analytical standard, ampul of 1 mL, 5000 $\mu\text{g}/\text{mL}$	CHIRALDEX B-PH, A-TA, G-DM and G-DP	90003AST
1-(N-TFA)aminoindan analytical standard, ampul of 1 mL, 5000 $\mu\text{g}/\text{mL}$ in ethanol: isopropanol (95:5)	CHIRALDEX B-DA and G-DA	90004AST
2-(Bromomethyl)tetra-2H-pyran analytical standard, ampul of 1 mL	CHIRALDEX B-TA and B-DP	90005AST
3,4-Dihydro-2-ethoxy-2H-pyran ampul of 1 mL	CHIRALDEX A-PH and G-PH (customs)	90006AST
1-Phenyl-1-ethanol analytical standard, ampul of 1 mL, 5000 $\mu\text{g}/\text{mL}$ in ethanol: isopropanol (95:5)	CHIRALDEX B-PM and B-DM	90007AST

Related Products

We carry chiral HPLC columns, derivatization reagents (chiral and achiral), test mixes, carrier gas purifiers, and all GC accessories. Please visit our website sigma-aldrich.com/chiral, our corporate chiral web portal, where you can view our other products for chiral chemistry, like chiral catalysts, building blocks, mobile phase additives, derivatization reagents, services, and more.

Supelco Chiral Services: Column Screening and Small-Scale Purification

Consult Supelco to obtain a quotation for our expert services for chiral method development column screening (HPLC and GC) and optimization, as well as isolation of up to 10 grams of purified enantiomer.

Trademarks

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