

ChiroSil® Care & Use Guide



OPERATING INSTRUCTIONS FOR ChiroSil[®] STATIONARY PHASES

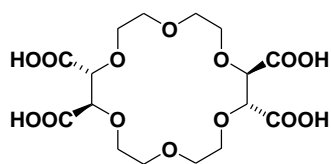
1. ChiroSil[®] description

◆ Application Range ◆

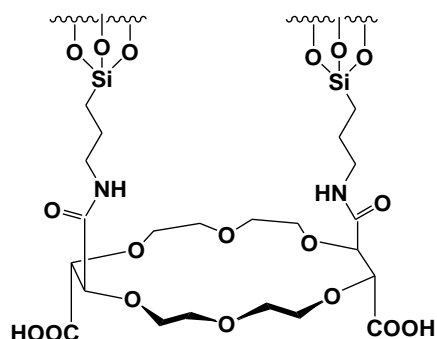
ChiroSil[®] columns are very effective for enantiomer separation of various natural and unnatural α -amino acids, α -amino acids derivatives, β -amino acids and primary amines. Other Racemic compounds, such as amino alcohols and secondary amines can also be resolved on ChiroSil[®] columns.

◆ Chiral Stationary Phase ◆

The chiral stationary phase for ChiroSil[®] RCA(+) and SCA(-) is prepared by a covalent trifunctional bonding of (+) or (-)-(18-Crown-6)-tetracarboxylic acid as the chiral selector to aminopropyl silica gel.



(+) and (-)- (18-Crown-6)-tetracarboxylic acid



◆ Separation Mechanism ◆

The mechanism of ChiroSil[®] based on chiral crown ether might originate from two different mechanisms.

- One mechanism is the complexation of the primary ammonium group (R-NH₃⁺) formed by protonation α -amino acids and primary amines under acidic condition inside the cavity of the 18-crown-6 ring of the ChiroSil[®] CSP.
- The other mechanism is the side two carboxylic acid groups of ChiroSil[®] CSP can act as steric barrier groups or as hydrogen bonding donor or acceptor groups.

2. General Operating Conditions

◆ Equilibration ◆

**NOTE - For stable retention factors ChiroSil[®] needs
Extended Equilibration Time.**

Prior to use be sure to precondition the column according to a procedure below.

Acidic Modifier change		Equilibration time	Flow rate	Temperature
Before Condition	After Condition			
100% MeOH (Virgin Column)	84%MeOH in H ₂ O + Perchloric acid (HClO ₄) 5mM	7 hr	1ml	20°C
	84%MeOH in H ₂ O + Sulfuric acid (H ₂ SO ₄) 10mM			
84%MeOH in H ₂ O + Sulfuric acid (H ₂ SO ₄) 10mM	84%MeOH in H ₂ O + Acetic acid (AcOH) 10mM	3hr	1ml	20°C
84%MeOH in H ₂ O + Acetic acid (AcOH) 10mM	84%MeOH in H ₂ O + Perchloric acid (HClO ₄) 5mM	2hr	1ml	20°C
84%MeOH in H ₂ O + Perchloric acid (HClO ₄) 5mM	84%MeOH in H ₂ O + Sulfuric acid (H ₂ SO ₄) 10mM	2hr	1ml	20°C

pH range

ChiroSil[®] can be used in the pH range 1.5 - 7.5.

Pressure

Operating pressure for ChiroSil[®] Columns is generally in range of 1000 psi to 5000 psi.

Temperature

The temperature that can be safely employed is from -5°C to 50°C in all solvent modes.

In many cases, lower temperature shows better resolution of analytes

◆ Storage and Cleaning ◆

1. Wash the column with 20ml of distilled water – first at a flow-rate of 1ml/min then gradually increasing the amount of methanol.
 2. Finally, wash it with 20ml of methanol at a flow-rate of 1ml/min.
 3. ChiroSil[®] is recommended to be filled with methanol 100% after washing.
- **Never store ChiroSil[®] with acidic components.**

ChiroSil[®] columns are shipped in 100% methanol.

3. Method Development

Aqueous acidic mobile phases are recommended for separation of α -amino acids, primary amines and amino alcohols.

◆ Effect of organic modifier ◆

The complexation of analytes inside the cavity of the 18-crown-6-ring of the CSP is expected to improve as the organic modifier content in the mobile phase increases. Higher organic modifier concentrations decrease the polarity of the mobile phase which drives the protonated amines into the less hydrophobic cavity of the 18-crown-6 ring of the ChiroSil[®] CSP, where the ionic moiety of the analytes can favorably interact with the lone-pair electrons of the oxygen atoms of the crown ether.

The capacity factors (k') generally decrease as the content of organic modifier increases and the separation factors (α) and the resolution factors (R_s) generally increase as the concentration of organic modifier in the aqueous mobile phase increases.

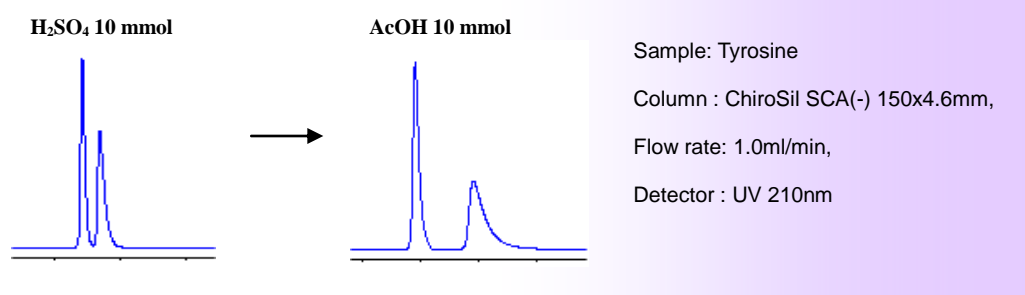
◆ Effect of acidic modifier and acid concentration ◆

Acidic modifier in the mobile phase plays an important role in protonating α -amino acids and enhancing the diastereomeric complex formation of α -amino acids inside the cavity of the chiral selector of the ChiroSil[®] CSP.

Acid Modifiers

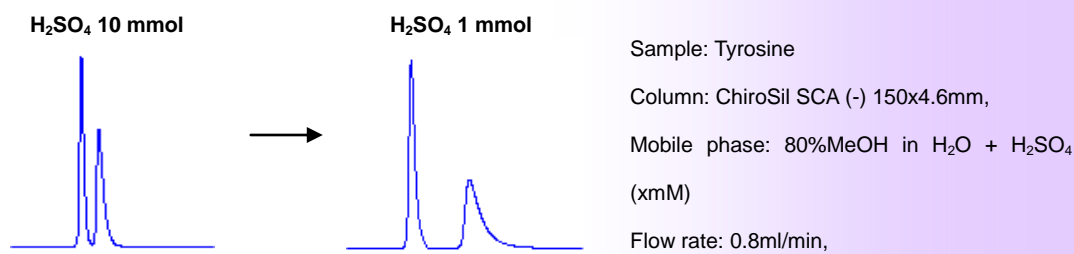
The enantioselectivity of enabled by different acids vary so it is recommended that you find the proper acid by screening.

Acids such as acetic acid, perchloric acid, sulfuric acid, phosphoric acid and trifluoroacetic acid can be used with ChiroSil[®]



Acid Concentration

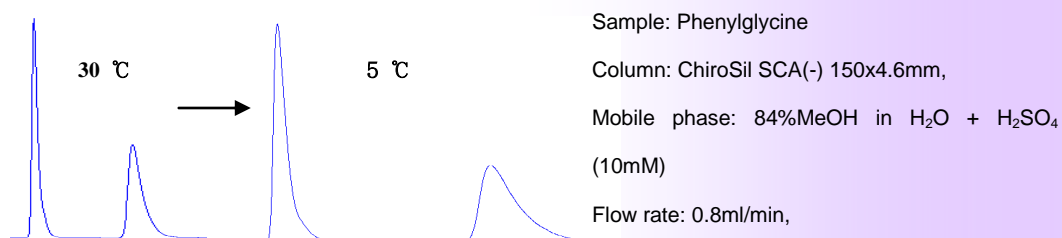
Generally the capacity factors (k') increase as the concentration of acidic modifier in the mobile phase increases. However some analytes separate better under low acid concentrations so we recommend testing under both high and low acid conditions.



◆ Effect of temperature ◆

At lower temperature, the formation of the two diastereomeric complexes formed by the two enantiomers of racemic compounds inside the cavity of the crown ether ring of CSP is expected to be much more favorable than that of the less stable diastereomeric complex. The difference in the stability of the two diastereomeric complexes increases as the temperature of the column is lowered.

The capacity factors (k'), the separation factors (α) and the resolution factors (R_s) are improved as the temperature is lowered.



4. Advantages of ChiroSil[®]

◆ Universal Solvent Capability ◆

Because the chiral selector of ChiroSil[®] is bonded covalently to silica gel and important advantage of over other commercial crown ether-based columns is that it can be used with various mobile phases, without any deterioration in its chiral recognition ability,

ChiroSil[®] Chiral Stationary Phases can be used in both normal and reversed-phased solvents.

For example, even 100% methanol can be used as a mobile phase for the resolution of racemic compound on ChiroSil[®]

◆ Ability to Invert Elution Order ◆

ChiroSil[®] has an ability to invert the elution order of enantiomers by switching columns.

In case of Amino acid, most **L-enantiomers elute first on the ChiroSil[®] RCA(+)** and **D-enantiomers elute first on the ChiroSil[®] SCA(-)** column.

◆ Excellent Column Durability ◆

ChiroSil[®] stability was tested under highly acidic conditions.

After 300 hours of continuous operation, there was no observable change in α and k' .

Test conditions:

Column: ChiroSil RCA 150mm X 4.6mm

Mobile phase: MeOH/H₂O = 84/16 in 0.5ml Perchloric Acid/1000ml, pH 2.09

Flow rate: 1ml/min

Detector: 210nm

Injection: 5ul (1-Aminoindan),

Press: 86–83bar