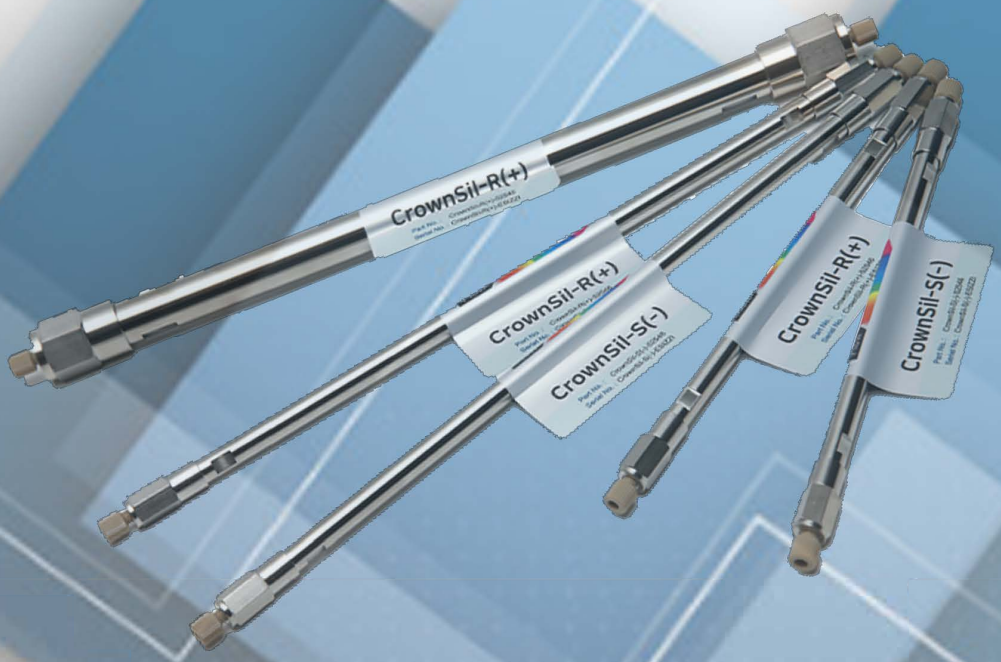
CELLACHROM®

CrownSil™ Care and Use Guide

Specialized Columns for the separation of Amino acids and Amines



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CELLION BIOMED

1. CrownSil

1-1. Introduction

Application Range

CrownSil columns are very effective for enantiomer separation of various natural and unnatural α-amino acids and primary amines.

Other racemic compounds, such as amino alcohols (β-blockers), secondary amines, drugs containing primary amines and secondary amines are also expected to be resolved on CrownSil columns.

The structure of CrownSil Stationary phase

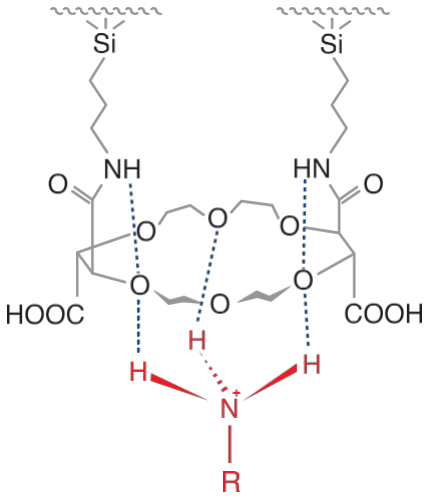
The chiral stationary phase for CrownSil R(+) and S(-) is prepared by a covalent trifunctional bonding of (+) or (-)-(18-Crown-6)-tetracarboxylic acid as the chiral selector to aminopropyl silica gel.

Separation Mechanism

The mechanism of CrownSil 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 CrownSil CSP.

The other mechanism is the side two carboxylic acid groups of CrownSil CSP can act as steric barrier groups or as hydrogen bonding donor or accepter groups.



1-2. Advantages of CrownSil

Universal Solvent Capability

An important advantage of CrownSil 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, because the chiral selector of CrownSil is bonded to silica gel covalently.

CrownSil Chiral Stationary Phases can be used in both normal and reversed-phased solvent. For example, even 100 % methanol can be used as a mobile phase for the resolution of racemic compound on CrownSil.

Ability to Invert Elution Order

CrownSil 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 CrownSil R(+) and D-enantiomers elute first on the CrownSil S(-) column.

Excellent Column Durabiltiy

CrownSil stability was tested under highly acidic condition. After 300 ours of continuous operatioin, there was no observable change in α and k'.

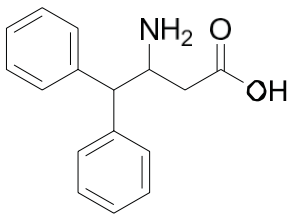
1-3. Method Development

CrownSil should be operated under an aqueous acidic condition for the separation

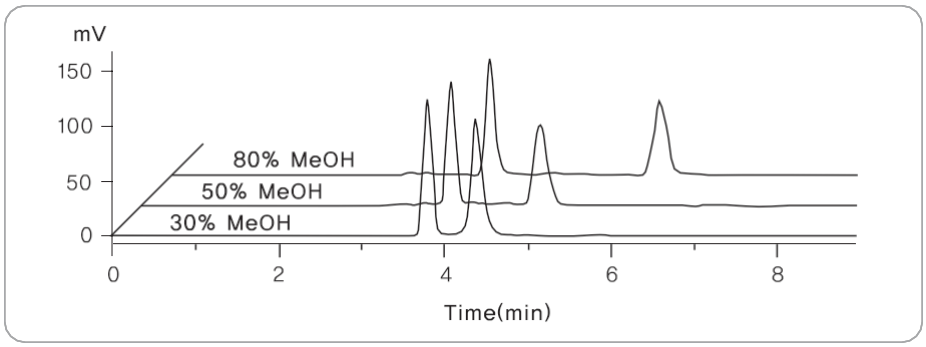
Effect of organic modifier

As the content of organic modifier increases, the aqueous mobile phase becomes less polar and more hydrophobic. In this instance, the hydrophilic interaction between polar-protonated analytes and the mobile phase decreases and consequently, the retention is expected to increase as the content of organic modifier in aqueous mobile phase increases.

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



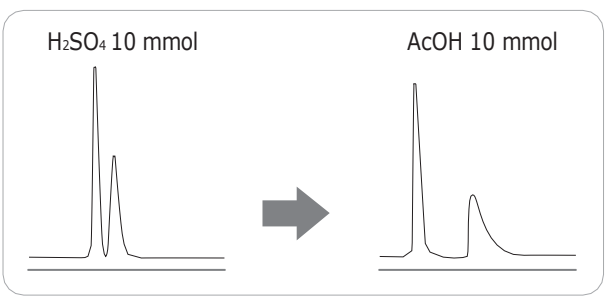
Mobile phase: Methanol in H_2O+ sulfuric acid (10mM)
Column: CrownSil R(+)type
Flow rate: 0.5ml/min
Detector: UV 210nm
Sample: 3-amino-4, 4-diphenylbutyric acid

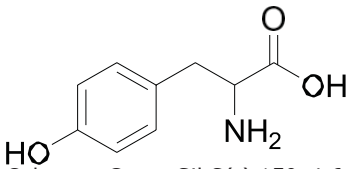


Effect of acidic modifier and acid concentration

*Acidic modifier
Various kinds of acids such as acetic acid, perchloric acid, sulfuric acid, phosphoric acid and trifluoroacetic acid can be used in CrownSil®

As the enatioselectivity of each acid is different so it is recommended that you find the proper acid for getting a good resolution by the trial and error method.

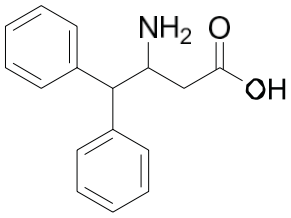




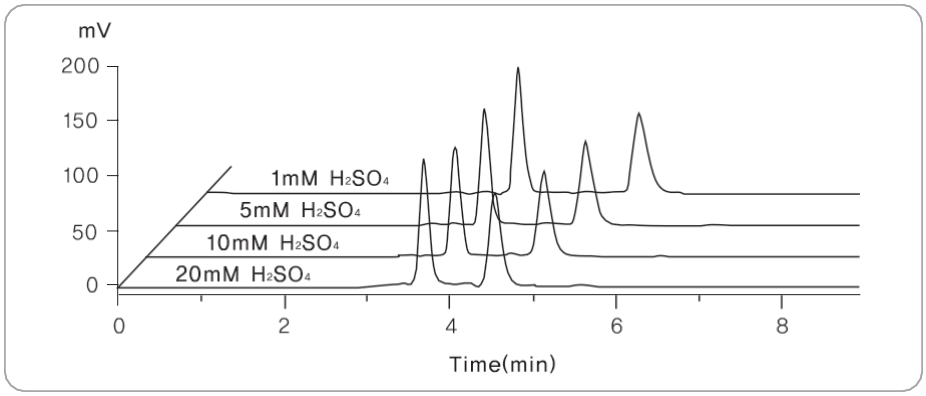
Column : CrownSil S(-) 150x4.6mm
Flow rate: 1.0ml/min
Detector : UV 210nm
Sample: Tyrosine

*Acid Concentration
As the content of acidic modifier in aqueous mobile phase increases, the ionic strength of mobile phase increases and consequently, the hydration or the dissolution of polar-protonated analytes by mobile phase is expected to increase. In this instance, polar-protonated analytes are eluted faster and faster as the content of acidic modifier increases.

Generally the capacity factors (k') decease as the concentration of acidic modifier in the mobile phase increases but we recommend trying an analysis for new analytes under low acid concentration because higher acid concentration is not always performing better resolutions.

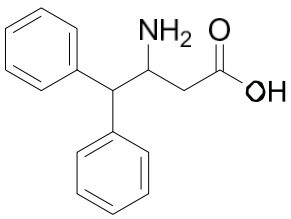


Mobile phase: 50% Methanol in H_2O+ sulfuric acid (10mM)
Column: CrownSil R(+) type
Flow rate: 0.5ml/min
Detector: UV 210nm
Sample: 3-amino-4, 4-diphenylbutyric acid

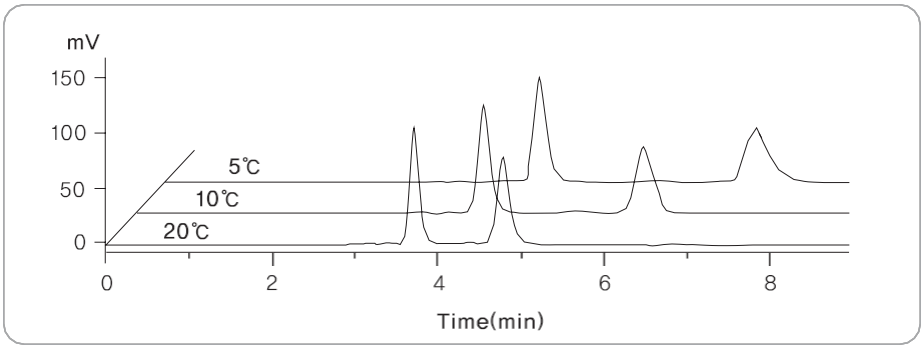


Effect of temperature

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 (Rs) are improved as the temperature is lowered.



Mobile phase: 50% Methanol in H_2O+ sulfuric acid (10mM)
Column: CrownSil R(+) type
Flow rate: 0.5ml/min
Detector: UV 210nm
Sample: 3-amino-4, 4-diphenylbutyric acid



1-4. General Operation Conditions

Storage

CrownSil columns are shipped in methanol only.

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.

pH range

CrownSil can used in the pH range 1.5 ~ 7.5.

Pressure

Operating pressure for CrownSil columns is generally in range of 1000 psi of 5000psi

Cleaning of the Column

After using CrownSil under acidic conditions, never store with acidic components

When analysis is complete, wash the column with 20mL of distilled water - first at a flow rate of 1mL/min then gradually increasing the amount of methanol

Finally, wash it with 20mL of methanol at a flow-rate of 1.0L/min.

CrownSil is recommended to be filled with methanol 100% after washing

Equilibration Time

CrownSil needs enough equilibration time to develop stable retention factors. (See the below table)

During mobile phase equilibration, enantioselective separations are obtained for all analytes, but retention factors are slowly decreased until stable retention factors are obtained

Mode	Before condition	After condition	Flow rate (mL/min)	Temp. (°C)	Equilibration Time(min)
RP	100% MeOH	Organic solvent in water	1.0mL/min	20°C	7hr
	Organic solvent in water + x mM Acid	Organic solvent in water + x mM Acid	1.0mL/min	20°C	2hr
NP	100% MeOH	EtOH or IPA 30min → Organic solvent in EtOH + x mM Acid	1.0mL/min	20°C	7hr
	Organic solvent in EtOH + x mM Acid	Organic solvent in EtOH + x mM Acid	1.0mL/min	20°C	2hr