

## RStech Corporation

RStech covers various technologies from synthesis of chiral intermediates to chiral separation & analysis



Venture town #304, Sinil-dong 1688-5,  
Daedeok-gu, Daejeon 306-230, Republic of Korea  
Tel : +82-42-936-0131~3  
Fax : +82-42-936-0134  
e-mail : [rstech@rstechcorp.com](mailto:rstech@rstechcorp.com)  
[www.rstechcorp.com](http://www.rstechcorp.com)

## HPLC Column CHIROSIL

RStech covers various technologies from synthesis of chiral intermediates to chiral separation & analysis



**RStech Corporation**  
[www.rstechcorp.com](http://www.rstechcorp.com)

# CONTENTS

- 1. Introduction / 03
- 2. Advantages of ChiroSil / 04
- 3. Method Development / 06
- 4. General Operating Conditions / 08
- 5. ChiroSil Application / 09
- 6. References / 25
- 7. Product list / 27

## 1. Introduction

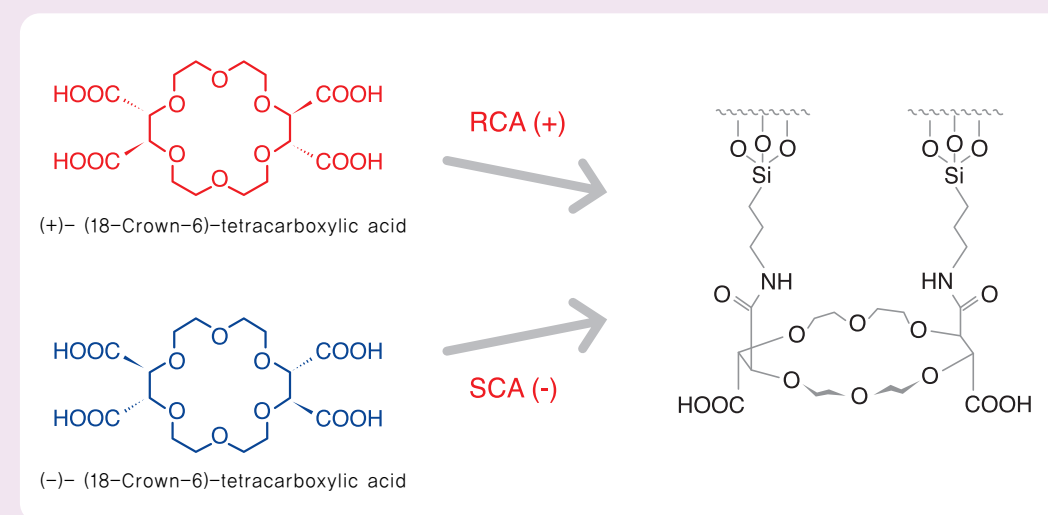
### Application Range

ChiroSil® columns are very effective for enantiomer separation of various natural and unnatural  $\alpha$ -amino acids,  $\alpha$ -amino acids derivatives,  $\alpha$ -amino acids and primary amines.

Other Racemic compounds, such as amino alcohols ( $\beta$ -blockers), secondary amines, drugs containing primary amines and secondary amines are also expected to be resolved on ChiroSil® columns.

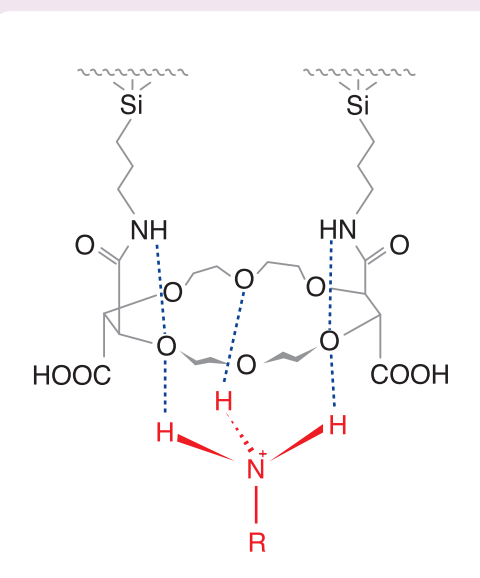
### The structure of ChiroSil® Stationary phase

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.



### 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 ( $R-NH_3^+$ ) formed by protonation  $\alpha$ -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. Advantages of ChiroSil®

### High selectivity:

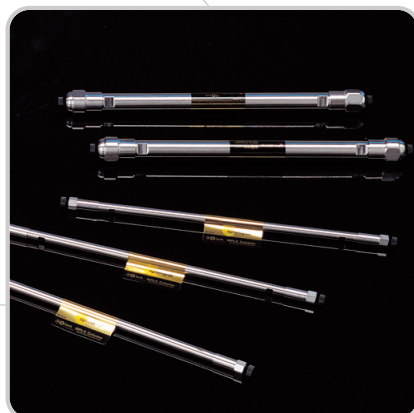
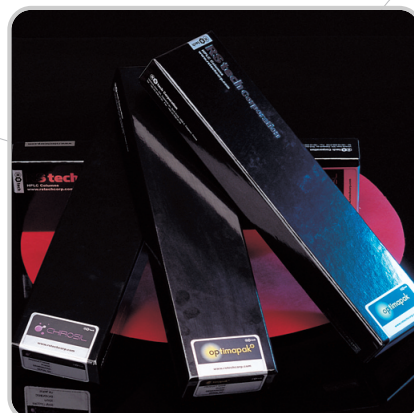
- $\alpha$ -Amino Acids
- $\alpha$ -Amino Amides and Esters
- Amines
- Amino Alcohols
- $\beta$ -Blockers
- $\beta$ -Amino Acids
- Aryl  $\alpha$ -Amino Ketones
- Tocainide's Analogues
- Gemifloxacin
- *N*-(3, 5-dinitrobenzoyl)- $\alpha$ -Amino Acids
- *N*-(3-dinitrobenzoyl)- $\alpha$ -Amino Acids
- *N*-benzyl- $\alpha$ -Amino Acids



### Universal Solvent Capability

### Ability to Invert Elution Order

### Excellent Column Durability



### High selectivity

ChiroSil® column have a high selectivity for enantiomer separation of various natural and unnatural  $\alpha$ -amino acids,  $\alpha$ -amino acids derivatives,  $\beta$ -amino acids and primary amines.

Other Racemic compounds, such as amino alcohols ( $\beta$ -blockers), secondary amines, drugs containing primary amines and secondary amines are also expected to be resolved on ChiroSil® columns.

### Universal Solvent Capability

An important advantage of ChiroSil® 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 ChiroSil® is bonded to silica gel covalently.

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.

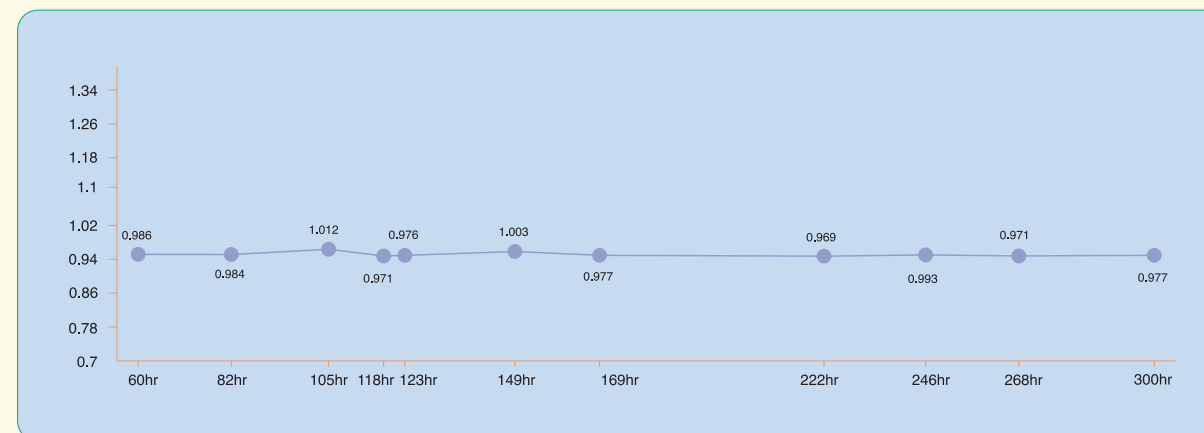
In case of Amino acid, most L-enantiomers elute first on the ChiroSil® RCA(+) and D-enantimoers elute first on the ChiroSil® SCA(-) column.

### Excellent Column Durability

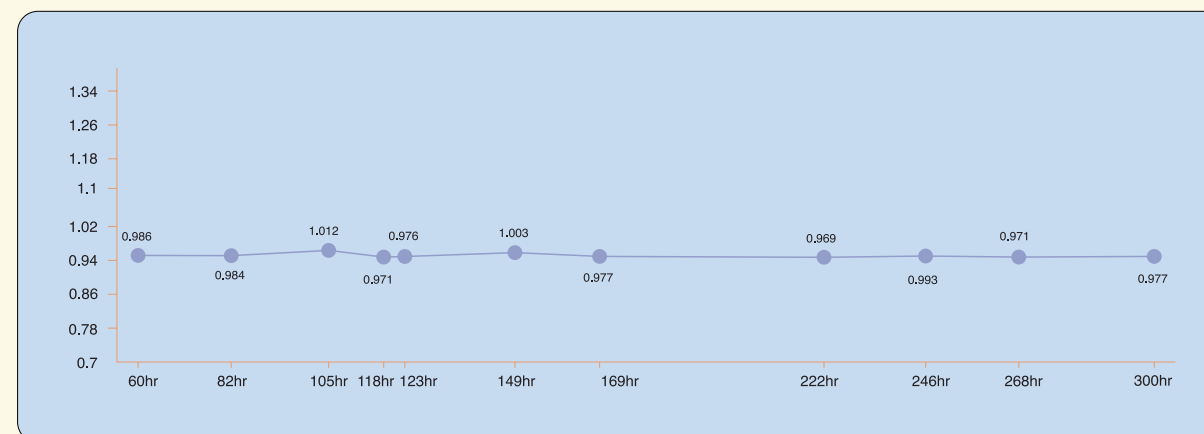
ChiroSil® stability was tested under highly acidic conditions.

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

<ChiroSil Duration  $K_1$ >



<ChiroSil Duration  $\alpha$ >



### conditions:

Column: ChiroSil RCA 150mm X 4.6mm

Mobile phase: MeOH/H<sub>2</sub>O = 84/16 in 0.5ml Perchloric Acid/1000ml, pH 2.09

Flow rate: 1ml/min

Detector: 210nm

Injection: 5  $\mu$ l (1-Aminoindan)

Press: 86~83bar

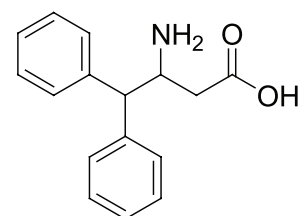
### 3. Method Development

ChiroSil® should be operated under an aqueous acidic condition for the separation

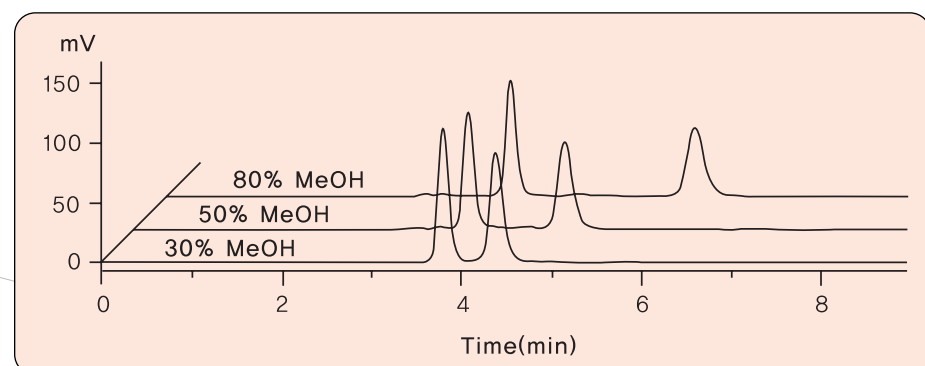
#### Effect of organic modifier

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 ( $\alpha$ ) and the resolution factors ( $R_s$ ), in general, increase as the content of organic modifier in the aqueous mobile phase increases.



Sample: 3-amino-4, 4-diphenylbutyric acid  
Mobile phase: Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
Column: ChiroSil RCA type  
Flow rate: 0.5ml/min  
Detector: UV 210nm

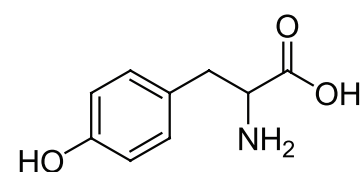
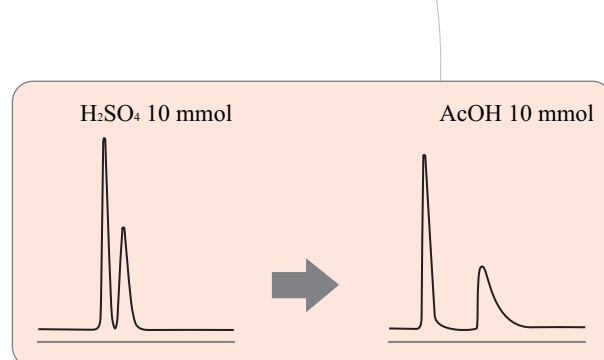


#### Effect of acidic modifier and acid concentration

##### \*Acidic modifier

Various kinds of acids such as acetic acid, perchloric acid, sulfuric acid, phosphoric acid and trifluoroacetic acid can be used in ChiroSil®

As the enantioselectivity 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.

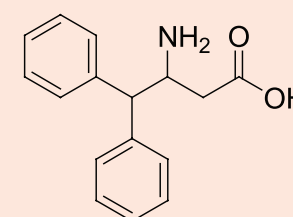


Sample: Tyrosine  
Column : ChiroSil SCA(-) 150x4.6mm  
Flow rate: 1.0ml/min  
Detector : UV 210nm

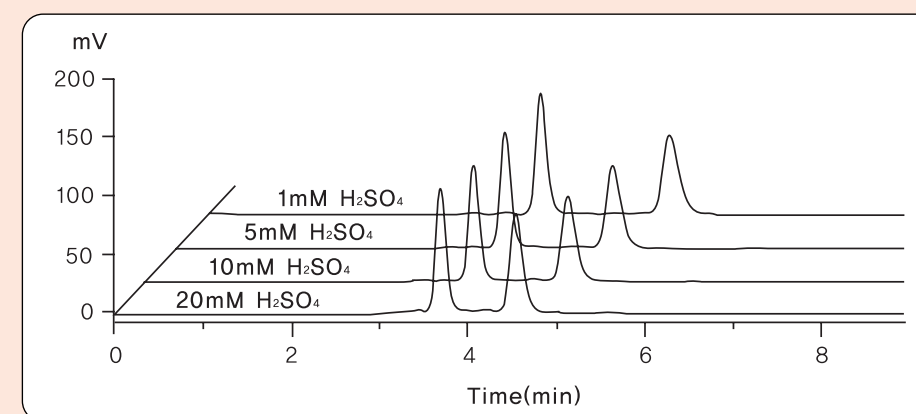
##### \*Acid Concentration

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 ( $k'$ ) decrease 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.



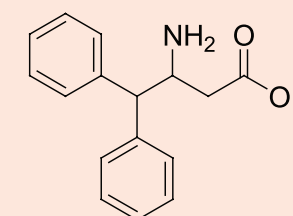
Sample: 3-amino-4, 4-diphenylbutyric acid  
Mobile phase: 50% Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
Column: ChiroSil RCA type  
Flow rate: 0.5ml/min  
Detector: UV 210nm



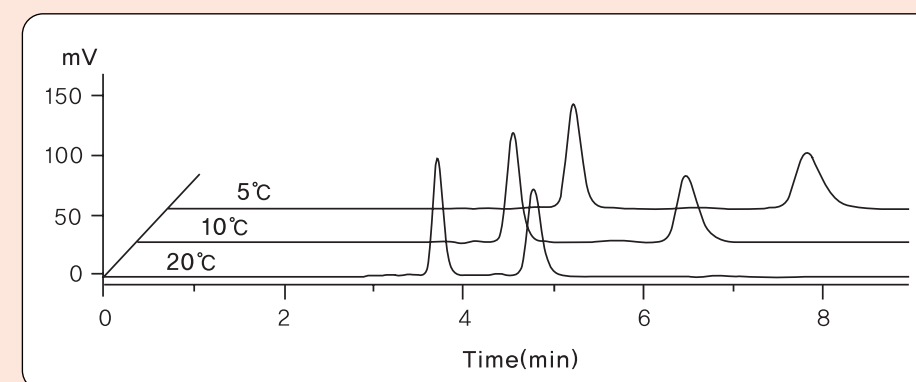
#### Effect of temperature

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 ( $\alpha$ ) and the resolution factors ( $R_s$ ) are improved as the temperature is lowered.



Sample: 3-amino-4, 4-diphenylbutyric acid  
Mobile phase: 50% Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
Column: ChiroSil RCA type  
Flow rate: 0.5ml/min  
Detector: UV 210nm



## 4. General Operating Conditions

### Storage

ChiroSil® 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

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

### Cleaning of the column

After using ChiroSil® 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 1ml/min.

ChiroSil® is recommended to be filled with methanol 100% after washing.

### Equilibration Time

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

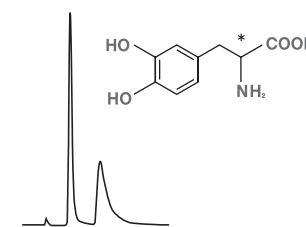
During mobile phase equilibration, enantioselective separations are obtained for all analytes, but retention factors are slowly decreased until stable retention factors are obtained.

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

## 5. ChiroSil® Application

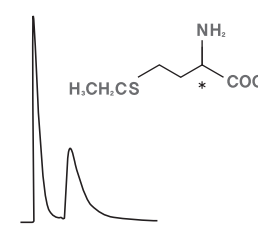
### α-Amino Acids

#### DOPA



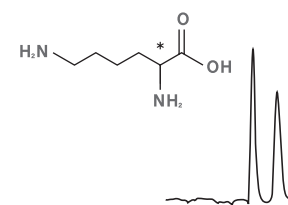
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 0.01%  
Phosphoric acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 5.5 min  
 $k_1$  : 0.97  $\alpha$  : 2.30

#### Ethionine



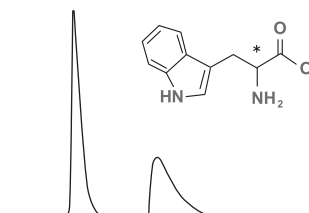
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (75/25) CH<sub>3</sub>OH/H<sub>2</sub>O + 0.02%  
Acetic acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 6.2 min  
 $k_1$  : 1.29  $\alpha$  : 2.07

#### Lysine



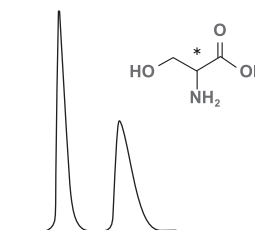
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 0.01%  
Phosphoric acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 5.3 min  
 $k_1$  : 1.44  $\alpha$  : 1.48

#### Tryptophan



Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.5 ml/min  
Detection : UV 210nm  
Run time : 11.0 min  
 $k_1$  : 4.06  $\alpha$  : 2.15

#### Serine



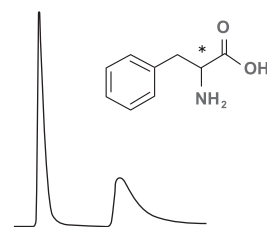
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (84/16) CH<sub>3</sub>OH/H<sub>2</sub>O + 5mM  
HClO<sub>4</sub>  
Flow Rate : 0.8 ml/min  
Detection : UV 210nm  
Run time : 6.0 min  
 $k_1$  : 1.37  $\alpha$  : 1.99

#### Phenylglycine



Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
H<sub>2</sub>SO<sub>4</sub> and 0.1% TEA  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 13.1 min  
 $k_1$  : 3.14  $\alpha$  : 2.60

### Phenylalanine



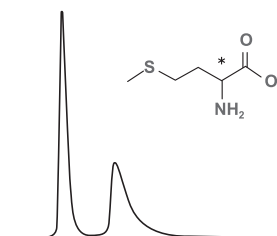
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.5 ml/min  
Detection : UV 210nm  
Run time : 8.9 min  
 $k_f$  : 2.66  $\alpha$  : 2.57

### Norvaline



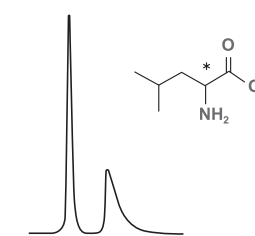
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (45/55) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 5.3 min  
 $k_f$  : 1.15  $\alpha$  : 1.79

### Methionine



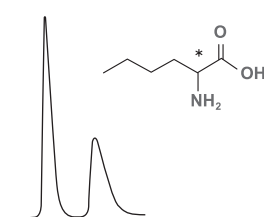
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (45/55) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 7.5 min  
 $k_f$  : 1.64  $\alpha$  : 2.04

### Leucine



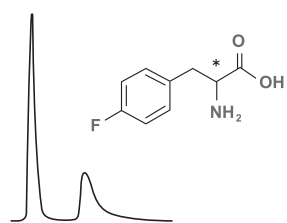
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (45/55) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 5.5 min  
 $k_f$  : 1.03  $\alpha$  : 2.14

### Norleucine



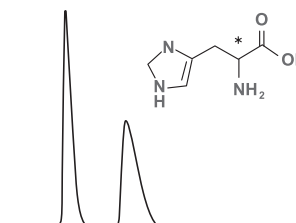
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (45/55) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 5.6 min  
 $k_f$  : 1.28  $\alpha$  : 1.75

### 4-Fluorophenylalanine



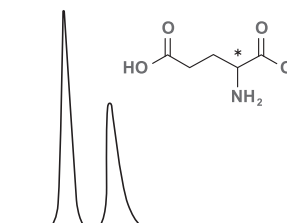
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.5 ml/min  
Detection : UV 210nm  
Run time : 9.6 min  
 $k_f$  : 2.92  $\alpha$  : 2.56

### Histidine



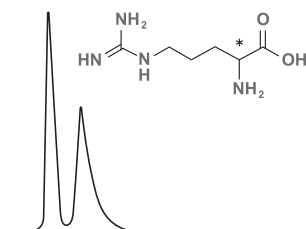
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (45/55) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 26.0 min  
 $k_f$  : 10.96  $\alpha$  : 1.27

### Glutamic Acid



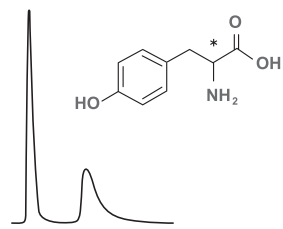
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (65/35) CH<sub>3</sub>OH/H<sub>2</sub>O + 0.05%  
Phosphoric acid  
Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 4.5 min  
 $k_f$  : 0.71  $\alpha$  : 2.27

### Arginine



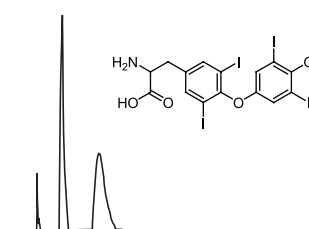
Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (84/16) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
H<sub>2</sub>SO<sub>4</sub>  
Flow Rate : 0.8 ml/min  
Detection : UV 210nm  
Run time : 4.9 min  
 $k_f$  : 1.21  $\alpha$  : 1.64

### Tyrosine

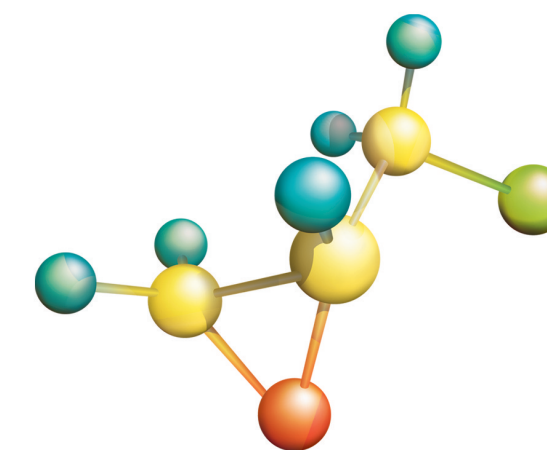


Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (70/30) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
Acetic acid  
Flow Rate : 1.5 ml/min  
Detection : UV 210nm  
Run time : 9.1 min  
 $k_f$  : 2.95  $\alpha$  : 2.38

### Thyroxine<sup>[8]</sup>



Column: ChiroSil Type  
Mobile phase: 80 % methanol in water +  
sulfuric acid (5 mM)  
Flow rate: 0.5 ml/min  
Detection: 210 nm UV  
Temperature: 20°C



### Other $\alpha$ -Amino Acids<sup>[1]</sup>

$\alpha$ -Amino Acids	Structure	$k_f$	$\alpha$	$R_S$
Alanine		1.37	1.28	1.33
Asparagine		1.31	1.10	0.63
Aspartic acid		1.51	1.22	1.25
Cysteine		1.32	1.10	0.30
Glutamine		1.31	1.32	1.72
Threonine		0.24	1.42	1.30
Valine		0.40	1.31	1.14

#### Condition

Column: ChiroSil Type  
 Mobile phase: 80% Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20 °C

### $\alpha$ -Amino amides and esters<sup>[1]</sup>

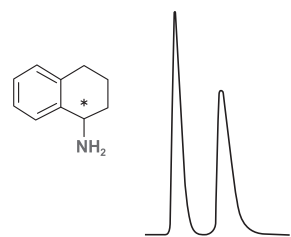
		$k_f$	$\alpha$	$R_S$
R	Y			
CH <sub>3</sub>	NH(CH <sub>3</sub> )-CH <sub>3</sub>	1.60	1.41	2.34
	NHC(CH <sub>3</sub> ) <sub>2</sub>	1.39	1.42	2.32
	NHCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	2.58	1.38	2.33
CH(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	1.36	1.10	0.48
	NH(CH <sub>3</sub> )-CH <sub>3</sub>	0.28	1.64	1.32
	NHC(CH <sub>3</sub> ) <sub>2</sub>	0.25	1.59	1.11
	NHCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	0.46	1.48	1.50
CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>2</sub> CH <sub>3</sub>	0.39	1.33	0.80
	NH(CH <sub>3</sub> ) <sub>2</sub> -CH <sub>3</sub>	1.07	2.48	8.15
	NH(CH <sub>3</sub> )-CH <sub>3</sub>	1.03	2.71	8.30
CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	0.42	1.24	0.94
	NH(CH <sub>3</sub> ) <sub>2</sub> -CH <sub>3</sub>	1.94	2.45	6.99
	NHC(CH <sub>3</sub> ) <sub>2</sub>	2.06	2.28	7.36
C <sub>6</sub> H <sub>5</sub>	NH(CH <sub>3</sub> ) <sub>2</sub> -CH <sub>3</sub>	1.55	2.46	7.27
	NHC(CH <sub>3</sub> ) <sub>2</sub>	1.28	2.67	6.32
	N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	1.40	3.15	9.77
	OCH <sub>3</sub>	2.10	2.09	6.85
4-CH <sub>3</sub> O-C <sub>6</sub> H <sub>4</sub>	NHCH <sub>3</sub>	1.73	2.39	8.63
	NH(CH <sub>3</sub> ) <sub>2</sub> -CH <sub>3</sub>	1.59	2.43	7.27
	NHC(CH <sub>3</sub> ) <sub>2</sub>	1.35	2.62	7.47

#### Condition

Column: ChiroSil Type  
 Mobile phase: 80% Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20 °C

## Amines<sup>[2]</sup> [10]

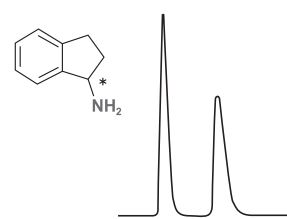
### 1,2,3,4-Tetrahydro-1-naphthylamine



Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (84/16) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
H<sub>2</sub>SO<sub>4</sub> and 0.1% TEA

Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 3.5 min  
 $k_1$  : 0.82  $\alpha$  : 1.76

### 1-Aminoindan



Column : ChiroSil RCA(+) or SCA(-)  
15cm × 4.6mm  
Mobile Phase : (84/16) CH<sub>3</sub>OH/H<sub>2</sub>O + 10mM  
HClO<sub>4</sub> and 0.1% TEA

Flow Rate : 1.0 ml/min  
Detection : UV 210nm  
Run time : 4.8 min  
 $k_1$  : 1.44  $\alpha$  : 1.91

Amines	$k_1$	$\alpha$	R <sub>S</sub>	Condition
	2.45	1.10	0.80	A
	1.90	1.28	2.57	A
	1.38	1.84	5.23	A
	2.86	1.11	1.05	A
	1.40	1.11	1.02	A
	0.42	1.22	0.82	B
	0.41	1.11	0.38	B
	0.51	1.39	1.69	A
	5.21	3.46	12.00	A

#### Condition A

Column: ChiroSil Type  
Mobile phase: 80% Methanol in H<sub>2</sub>O+  
sulfuric acid (10mM)

Flow rate: 0.5 ml/min  
Detection: 210 nm UV  
Temperature: 20 °C

#### Condition B

Column: ChiroSil Type  
Mobile phase: 50% Methanol in H<sub>2</sub>O+  
sulfuric acid (10mM)

Flow rate: 0.5 ml/min  
Detection: 210 nm UV  
Temperature: 20 °C

## Amino Alcohols<sup>[2]</sup> [10]

Amino Alcohols	Structure	$k_1$	$\alpha$	R <sub>S</sub>	Condition
2-amino-1-phenylethanol		1.10	1.40	1.52	B
4-(2-amino-1-hydroxyethyl)phenol		0.92	1.19	1.41	B
4-(2-amino-1-hydroxyethyl)benzene-1,2-diol		0.90	1.15	1.00	B
4-(2-amino-1-hydroxyethyl)-2-methoxyphenol		1.25	1.18	1.23	B
2-amino-2-phenylethanol		1.44 (S)	1.35	2.18	A
2-amino-2,3-dihydro-1H-inden-1-ol		1.98 (1R, 2S)	1.78	0.80	A
2-amino-1,2-diphenylethanol		0.29 (1S, 2R)	1.53	1.48	C

#### Condition A

Column: ChiroSil Type  
Mobile phase: 100% H<sub>2</sub>O+ sulfuric acid (10mM)  
Flow rate: 0.5 ml/min  
Detection: 210 nm UV  
Temperature: 20 °C

#### Condition B

Column: ChiroSil Type  
Mobile phase: 80% Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
Flow rate: 0.5 ml/min  
Detection: 210 nm UV  
Temperature: 20 °C

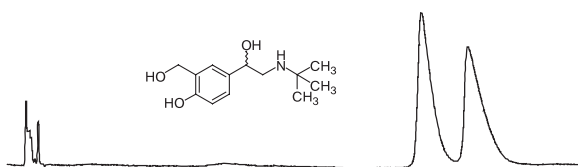
#### Condition C

Column: ChiroSil Type  
Mobile phase: 50% Methanol in H<sub>2</sub>O+ sulfuric acid (10mM)  
Flow rate: 0.5 ml/min  
Detection: 210 nm UV  
Temperature: 20 °C



## β-Blockers<sup>[9]</sup>

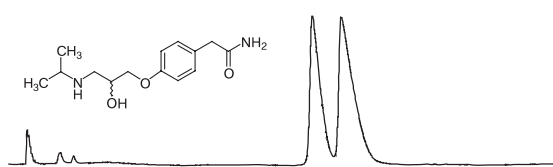
### Albuterol



Column: ChiroSil Type  
Mobile phase: 0.1 / 0.1 / 50/50 acetic acid-triethylamine-methanol-acetonitrile (v/v/v/v)

Flow rate: 1 ml/min  
Detection: 260 nm UV  
Temperature: 20°C  
 $k_1$ : 21.34  $\alpha$ : 1.2

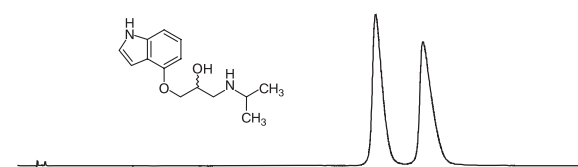
### Atenolol



Column: ChiroSil Type  
Mobile phase: 0.1 / 0.1 / 50/50 acetic acid-triethylamine-methanol-acetonitrile (v/v/v/v)

Flow rate: 1 ml/min  
Detection: 260 nm UV  
Temperature: 20°C  
 $k_1$ : 15.86  $\alpha$ : 1.10

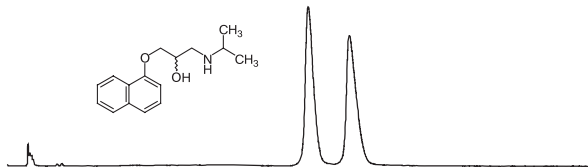
### Pindolol



Column: ChiroSil Type  
Mobile phase: 0.1 / 0.1 / 50/50 acetic acid-triethylamine-methanol-acetonitrile (v/v/v/v)

Flow rate: 1 ml/min  
Detection: 260 nm UV  
Temperature: 20°C  
 $k_1$ : 18.99  $\alpha$ : 1.14

### Propranolol



Column: ChiroSil Type  
Mobile phase: 0.1 / 0.1 / 50/50 acetic acid-triethylamine-methanol-acetonitrile (v/v/v/v)

Flow rate: 1 ml/min  
Detection: 260 nm UV  
Temperature: 20°C  
 $k_1$ : 15.16  $\alpha$ : 1.15

## Other β-Blockers<sup>[19]</sup>

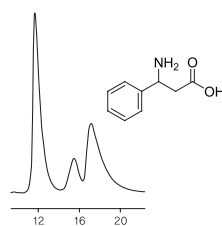
β-Blockers	Structure	$k_1$	$\alpha$	$R_S$
Alprenolol		29.35	1.26	2.12
Oxprenolol		24.61	1.22	2.29
Acebutolol		45.60	1.29	2.90
Bambuterol		22.52	1.85	4.21
Clenbuterol		53.61	1.59	4.37
Clenpropol		48.61	1.13	1.58
Fumoterol		98.08	1.23	1.36
Mabuterol		43.07	1.64	5.79

### Condition

Column: ChiroSil Type  
Mobile phase: 0.1 / 0.5 / 20/80 trifluoroacetic acid-triethylamine-ethanol-acetonitrile (v/v/v/v)  
Flow rate: 1 ml/min  
Detection: 260 nm UV  
Temperature: 20 °C

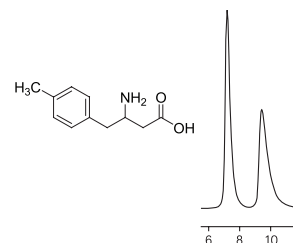
## $\beta$ -Amino Acids<sup>[6]</sup>

### 3-amino-3-phenylpropanoic acid



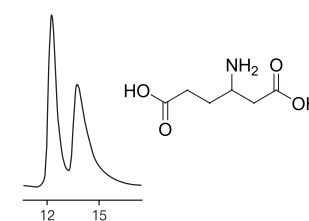
Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 3.60  $\alpha$  : 102

### 3-amino-4-(4-methylphenyl)butanoic acid



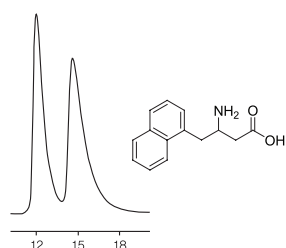
Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 1.26  $\alpha$  : 1.40

### 3-aminoadipic acid



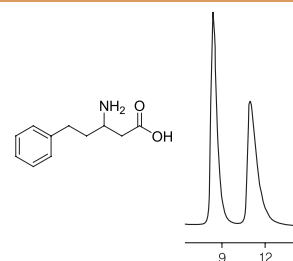
Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 3.83  $\alpha$  : 1.16

### 3-amino-4-(1-naphthyl)butanoic acid



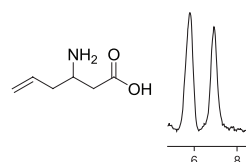
Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 3.72  $\alpha$  : 1.28

### 3-amino-5-phenylpentanoic acid



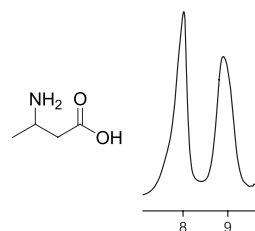
Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 2.30  $\alpha$  : 1.44

### 3-amino-5-hexenoic acid



Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 1.02  $\alpha$  : 1.37

### 3-aminobutyric acid



Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$  : 2.16  $\alpha$  : 1.16

### Other $\beta$ -Amino Acids<sup>[11]</sup>

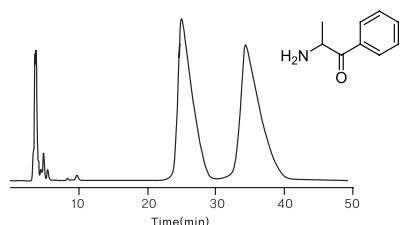
$\beta$ -Blockers	Structure	$k_f$	$\alpha$	$R_S$
3-amino-4-(2-furyl)butyric acid		1.33	1.33	1.66
3-amino-4-(2-naphthyl)butyric acid		2.38	1.53	2.07
3-amino-4,4-diphenylbutyric acid		0.67	1.34	1.38

#### Condition

Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water + acetic acid (10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20 °C

## Aryl $\alpha$ -Amino Ketones<sup>[15]</sup>

### Cathinone



Column: ChiroSil Type  
 Mobile phase: 50 % methanol in water +  
 acetic acid(10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C  
 $k_f$ : 3.83  $\alpha$ : 1.16

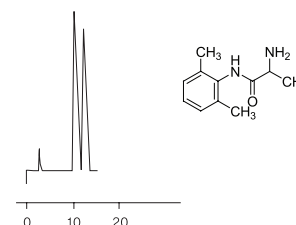
		$k_f$	$\alpha$	$R_S$
Ar	R			
C <sub>6</sub> H <sub>5</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>	0.11	2.12	2.13
C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	0.34	1.95	3.11
C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> CH <sub>2</sub> SCH <sub>3</sub>	0.84	1.57	2.29
C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	1.03	1.55	3.55
4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>3</sub>	1.22	1.55	2.80
4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>	0.16	2.08	1.89
4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	0.31	1.99	2.88
4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>2</sub> CH <sub>2</sub> SCH <sub>3</sub>	0.78	1.65	2.98
4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	0.86	1.58	3.09
1-Naphthyl	CH(CH <sub>3</sub> ) <sub>2</sub>	0.25	2.20	3.87
2-Naphthyl	CH(CH <sub>3</sub> ) <sub>2</sub>	0.26	2.19	3.77

#### Condition

Column: ChiroSil Type  
 Mobile phase: 80 % ethanol in water + Sulfuric acid (10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20 °C

## Tocainide's Analogues

### Tocainide



Column: ChiroSil Type  
 Mobile phase: 80 % methanol in water +  
 sulfuric acid (5 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20°C

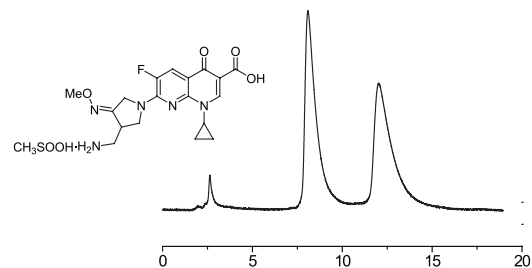
Tocainide's Analogue	Structure	$k_f$	$\alpha$	$R_S$
2-amino-N-phenylpropanamide		1.82	1.73	2.52
2-amino-N-benzylpropanamide		1.38	1.44	2.10
2-amino-3-methyl-N-phenylbutanamide		0.34	2.10	2.56
2-amino-N-benzyl-3-methylbutanamide		0.30	1.42	1.00
2-amino-4-methyl-N-(2,6-dimethylphenyl)pentanamide		0.25	1.17	0.25
2-amino-4-methyl-N-phenylpentanamide		1.35	5.00	4.00
2-amino-N-benzyl-4-methylpentanamide		1.07	2.39	5.50
2-amino-N,3-diphenylpropanamide		2.29	3.72	5.33
2-amino-N-benzyl-3-phenylpropanamide		2.08	2.19	3.29
2-amino-N-(2,6-dimethylphenyl)-2-phenylacetamide		1.49	2.05	3.52
2-amino-N,2-diphenylacetamide		1.55	3.50	5.50
2-amino-N-benzyl-2-phenylacetamide		1.60	2.58	4.89

#### Condition

Column: ChiroSil Type  
 Mobile phase: 80 % methanol in water + sulfuric acid (10 mM).  
 Flow rate: 0.5 ml/min  
 Detection: 210 nm UV  
 Temperature: 20 °C

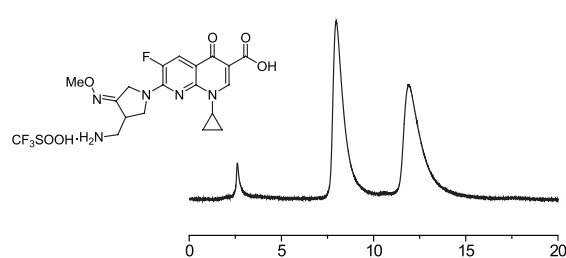
## Gemifloxacin<sup>[7]</sup>

### Gemifloxacin mesylate



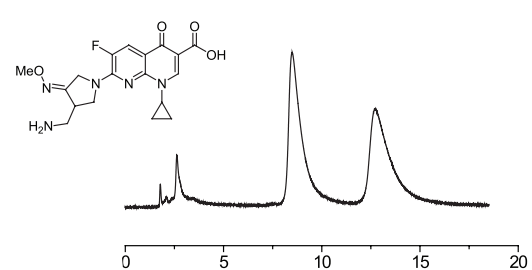
Column: ChiroSil Type  
 Mobile phase: 80 % methanol in water + perchloric acid (10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm UV  
 Temperature: 20°C  
 $k_1$  : 3.55  $\alpha$  : 1.62

### Gemifloxacin triflate



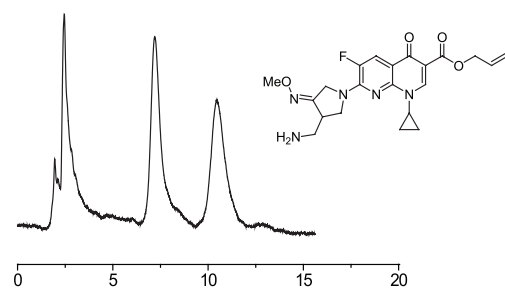
Column: ChiroSil Type  
 Mobile phase: 80 % methanol in water + perchloric acid (10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm UV  
 Temperature: 20°C  
 $k_1$  : 3.49  $\alpha$  : 1.63

### Free form of Gemifloxacin



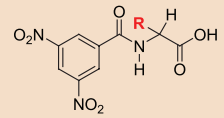
Column: ChiroSil Type  
 Mobile phase: 80 % methanol in water + perchloric acid (10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm UV  
 Temperature: 20°C  
 $k_1$  : 3.78  $\alpha$  : 1.63

### Allyl ester of Gemifloxacin



Column: ChiroSil Type  
 Mobile phase: 80 % methanol in water + perchloric acid (10 mM)  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm UV  
 Temperature: 20°C  
 $k_1$  : 3.05  $\alpha$  : 1.60

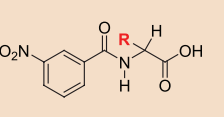
## N-(3, 5-dinitrobenzoyl)- $\alpha$ -Amino Acid<sup>[18]</sup>

	$k_1$	$\alpha$	$R_S$
<b>R</b>			
CH <sub>3</sub>	6.81	1.20	0.76
(CH <sub>3</sub> ) <sub>2</sub> CH	3.22	1.81	2.81
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	4.56	1.57	2.01
C <sub>6</sub> H <sub>5</sub>	3.56	1.47	1.49
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	5.11	1.61	1.78
HOCH <sub>2</sub>	21.25	1.06	0.54
CH <sub>2</sub> (OH)CH	8.85	1.23	0.74
4-OH-C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	36.20	1.27	0.73

### Condition

Column: ChiroSil Type  
 Mobile phase: 0.05 / 0.25 / 100 acetic acid-triethylamine-ethanol-acetonitrile (v/v/v)  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm UV  
 Temperature: 20 °C

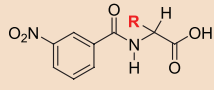
## N-(3-dinitrobenzoyl)- $\alpha$ -Amino Acids<sup>[18]</sup>

	$k_1$	$\alpha$	$R_S$
<b>R</b>			
CH <sub>3</sub>	5.71	1.14	0.60
(CH <sub>3</sub> ) <sub>2</sub> CH	3.06	1.46	1.40
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	4.26	1.45	1.68
C <sub>6</sub> H <sub>5</sub>	2.86	1.25	0.97

### Condition

Column: ChiroSil Type  
 Mobile phase: 0.05 / 0.25 / 100 acetic acid-triethylamine-ethanol-acetonitrile (v/v/v)  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm UV  
 Temperature: 20 °C

## N-benzyl- $\alpha$ -Amino Acids<sup>[18]</sup>

	$k_I$	$\alpha$	$R_S$
R			
(CH <sub>3</sub> ) <sub>2</sub> CH	3.35	1.11	0.41
(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	3.68	1.14	0.55

### Condition

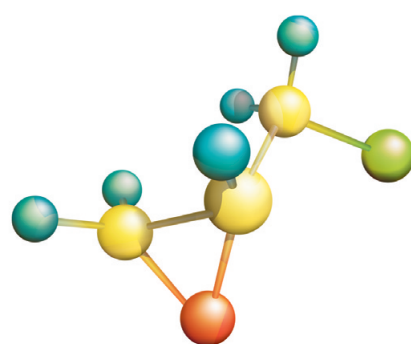
Column: ChiroSil Type

Mobile phase: 0.05 / 0.25 / 100 acetic acid-triethylamine-ethanol-acetonitrile (v/v/v)

Flow rate: 0.5 ml/min

Detection: 254 nm UV

Temperature: 20 °C



RStech covers various technologies from synthesis of chiral intermediates to chiral separation & analysis

## 6. References

1. Myung Ho Hyun, Jong Sung Jin and Wonjae Lee, "Liquid Chromatographic Resolution of Racemic Amino Acids and Their Derivatives on a New Chiral Stationary Phase Based on Crown Ether", *J. Chromatogr. A*, 822(1), 155-161 (1998)
2. Myung Ho Hyun, Jong Sung Jin, Hye Jin Koo and Wonjae Lee, "Liquid Chromatographic Resolution of Racemic Amines and Amino Alcohols on a Chiral Stationary Phase Derived from Crown Ether", *J. Chromatogr. A*, 837, 75-72 (1999)
3. Myung Ho Hyun, Sang Cheol Han, Jong Sung Jin and Wonjae Lee, "Separation of the Stereoisomers of Racemic Fluoroquinolone Antibacterials on an HPLC Chiral Stationary Phase Based on Chiral Crown Ether", *Chromatographia*, 52(7-8), 473-476, 2000.
4. Jong Sung Jin, Apryll M. Stalcup, Myung Ho Hyun, "The Impact of Triethylamine as a Mobile Phase Additive on the Resolution of Racemic Amino Acids on an (+)-18-Crown-6-tetracarboxylic Acid-derived Chiral Stationary Phase", *J. Chromatogr. A*, 933(1-2), 83-90, 2001.
5. Myung Ho Hyun and Jong Sung Jin, Sang Cheol Han and Yoon Jae Cho, "The Effect of Analyte Lipophilicity on the Resolution of  $\alpha$ -Amino Acids on an HPLC Chiral Stationary Phase Based on Crown Ether", *Microchemical Journal*, 70, 205-209, 2001.
6. Myung Ho Hyun, Yoon Jae Cho and Jong Sung Jin, "Liquid Chromatographic Direct Resolution of  $\beta$ -Amino Acids on a Chiral Crown Ether Stationary Phase", *J. Separation Science* 25 (10-11), 648-652, 2002.
7. Myung Ho Hyun, Sang Cheol Han, Yoon Jae Cho and Jong Sung Jin, Wonjae Lee, "Liquid Chromatographic Resolution of Gemifloxacin Mesylate on a Chiral Stationary Phase Derived from Crown Ether", *Biomed. Chromatogr.* 16, 356-360, 2002.
8. Hassan Y. Aboul-Enein, Imran Ali, Myung Ho, Yoon Jae Cho and Jong Sung Jin, "Effect of acidity on the enantiomeric resolution of thyroxine and tocinide by HPLC on a (+)-(18-crown-6)-2, 3, 11, 12-tetracarboxylic acid column", *J. Biochem. Biophys. Meds.* 54(1-3), 407-413, 2002.
9. Robert J. Steffek, Yury Zelechok and Kyung H. Gahm, "Enantioselective separation of racemic secondary amines on a chiral crown ether-based liquid chromatography stationary phase", *J. Chromatogr. A* 947(2), 301-305, 2002 · SHORT COMMUNICATION
10. Myung Ho Hyun, Yoon Jae Cho, Jin Ah Kim and Jong Sung Jin, "Preparation and Application of a New Modified Liquid Chromatographic Chiral Stationary Phase Based on (+)-(18-Crown-6)-2, 3, 11, 12-tetracarboxylic Acid", *J. Chromatogr. A*, 984(2), 163-171, 2003.
11. Myung Ho Hyun, Yoon Jae Cho, Jin Ah Kim and Jong Sung Jin, "Effect of the Tethering Groups of Chiral Stationary Phases Based on (+)-(18-Crown-6)-2, 3, 11, 12-tetracarboxylic Acid on the Liquid Chromatographic Resolution of  $\beta$ -Amino Acids", *J. Liq. Chromatogr. & Rel. Tech.* 26 (7), 1081-1092, 2003.

**12. Myung Ho Hyun, Hye Jung Min and Yoon Jae Cho,**

"Resolution of Tocainide and Its Analogues on Liquid Chromatographic Chiral Stationary Phases Based on (+)-(18-Crown-6)-2, 3, 11, 12-tetracarboxylic Acid", *Bull. Kor. Chem. Soc.* 24, 911-915, 2003.

**13. Myung Ho Hyun, Young Hwa Kim and Yoon Jae Cho,**

"Effect off the Protection of the Residual Aminopropyl Groups of a Chiral Stationary Phase Based on (+)-(18-Crown-6)-2, 3, 11, 12-tetracarboxylic Acid on the Chiral Resolution Behaviors", *Bull. .25*, pp 400 -402 (2004)

**14. Myung Ho Hyun and Do Hun Kim,**

"Spacer Length Effect of a Chiral Stationary Phase Based on (+)-(18-Crown-6)-2, 3, 11, 12-tetracarboxylic acid", *Chirality* 16, pp294-301 (2004)

**15. Myung Ho Hyun, Guanghui Tan and Yoon Jae Cho,**

"Liquid Chromatographic Resolution of Aryl  $\alpha$ -Amino Ketones on Chiral Stationary Phases Based on (+)-(18-Crown-6)-2, 3, 11, 12-tetracarboxylic acid", *J. Liq. Chromatogr. Rel. Tech.* 27(11), pp1671-1690 (2004)

**16. Myung Ho Hyun and Yoon Jae Cho,**

"Preparation and application of a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid without extra free aminopropyl groups on silica surface", *J. Separation Science.* 28(1), 31-38 (2005)

**17. Uwe Conrad, Bezhn Chankvetadze, Gerhard K. E. Scriba,**

"High performance liquid chromatographic separation of dipeptide and tripeptide enantiomers using a chiral crown ether stationary phase", *J. Separation Science.* 28, 2275-2281 (2005)

**18. Myung Ho Hyun, Guanghui Tan and Jin Ying Xue,**

"Unusual resolution of N-(3, 5-dinitrobenzoyl)- $\alpha$ -amino acids on a chiral stationary phase based on (+)-(18-crown-6)-2, 3, 11, 12-tetracarboxylic acid", *Journal of Chromatography A, In Press, Corrected Proof, Available online 2 November 2005*, · SHORT COMMUNICATION

**19. Dandan Zhang, Famei Li, Do Hun Kim, Hee Jung Choi and Myung Ho Hyun,**

"Resolution of  $\beta$ -blockers on a chiral stationary phase based on (+)-(18-crown-6)-2, 3, 11, 12-tetracarboxylic acid: Unusual temperature effect", *J. Chromatogr. A*, 1083(1-2), 89-95, 2005 · ARTICLE

## 7. Product list

Product Name	CSPs, Particle size	dimension
CH RCA(+)-51001021	Chirosil RCA(+), 5micron, 100	100mm x 2.1mm
CH RCA(+)-51001521	Chirosil RCA(+), 5micron, 100	150mm x 2.1mm
CH RCA(+)-51001546	Chirosil RCA(+), 5micron, 100	150mm x 4.6mm
CH RCA(+)-51002546	Chirosil RCA(+), 5micron, 100	250mm x 4.6mm
CH RCA(+)-51002510	Chirosil RCA(+), 5micron, 100	250mm x 10.0mm
CH RCA(+)-51002520	Chirosil RCA(+), 5micron, 100	250mm x 21.1mm
CH RCA(+)-101001546	Chirosil RCA(+), 10micron, 100	150mm x 4.6mm
CH RCA(+)-101002546	Chirosil RCA(+), 10micron, 100	250mm x 4.6mm
CH RCA(+)-101002510	Chirosil RCA(+), 10micron, 100	250mm x 10.0mm
CH RCA(+)-101002520	Chirosil RCA(+), 10micron, 100	250mm x 21.1mm
CH SCA(-)-51001021	Chirosil SCA(-), 5micron, 100	100mm x 2.1mm
CH SCA(-)-51001521	Chirosil SCA(-), 5micron, 100	150mm x 2.1mm
CH SCA(-)-51001546	Chirosil SCA(-), 5micron, 100	150mm x 4.6mm
CH SCA(-)-51002546	Chirosil SCA(-), 5micron, 100	250mm x 4.6mm
CH SCA(-)-51002510	Chirosil SCA(-), 5micron, 100	250mm x 10.0mm
CH SCA(-)-51002520	Chirosil SCA(-), 5micron, 100	250mm x 21.1mm
CH SCA(-)-101001546	Chirosil SCA(-), 10micron, 100	150mm x 4.6mm
CH SCA(-)-101002546	Chirosil SCA(-), 10micron, 100	250mm x 4.6mm
CH SCA(-)-101002510	Chirosil SCA(-), 10micron, 100	250mm x 10.0mm
CH SCA(-)-101002520	Chirosil SCA(-), 10micron, 100	250mm x 21.1mm
CH RCA(+)-Guard	Chirosil RCA(+), 5micron, 100	10.0mm x 4.0mm
CH SCA(-)-Guard	Chirosil SCA(-), 5micron, 100	10.0mm x 4.0mm

\*Guard holder