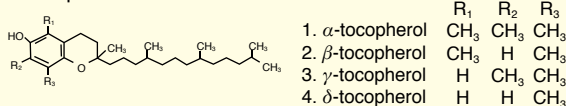


## Application data of CHP series

### Tocopherol



### Tocotrienol

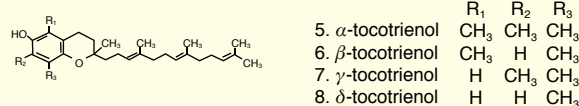


Fig. 5-41 Vitamin E in Rice Bran Oil

Conditions  
 Column : MCI GEL™ CMG20/C10  
 4.6mm I.D.×150mm  
 Eluent : Hexane-EtOH = 98/2 (vol.)  
 Flow rate : 0.5 ml/min  
 Detection : 295nm  
 Sample : Rice Bran Oil, 50 mg/ml  
 Injection : 10 $\mu$ L

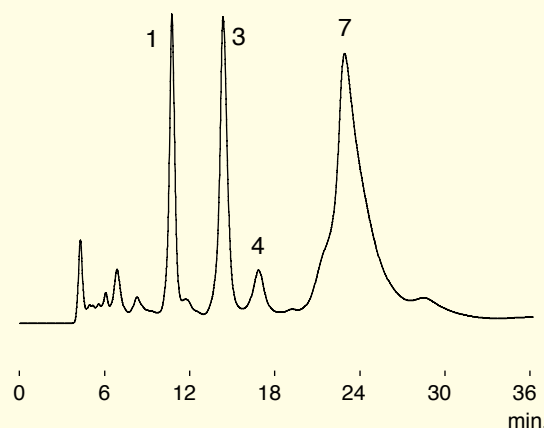


Fig. 5-42 Elution profile of Rice Bran Oil in preparative scale

Conditions  
 Column : MCI GEL™ CMG20/P30  
 20mm I.D.×500mm  
 Eluent : Hexane/C<sub>2</sub>H<sub>5</sub>OH = 98/2 (vol.)  
 Flow rate : 4.7 ml/min  
 Detection : 295 nm  
 Sample : Rice Bran Oil, 50 mg/ml  
 Injection : 1260 $\mu$ L

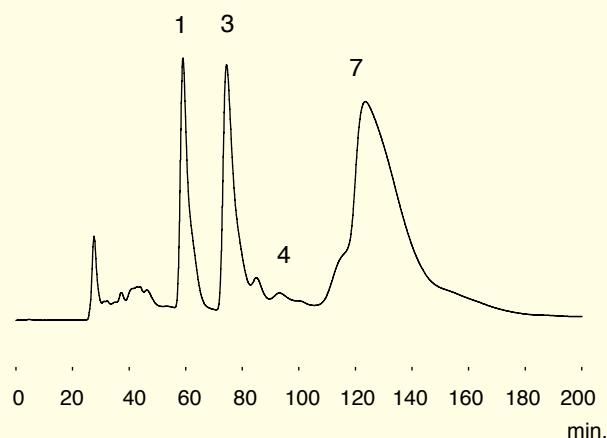


Fig. 5-43 Mixture of tocopherol and tocotrienol : Comparison with silica gel column

Conditions  
 Column : 1. Silica gel 5SIL, 4.6mm I.D.×250mm  
 2. MCI GEL™ CMG20/C04, 4.6mm I.D.×150mm  
 Eluent : 1. Hexane/EtOH = 99/1  
 2. Hexane/EtOH = 98/2  
 Flow rate : 1.0 ml/min  
 Column temp. : 25°C  
 Detection : UV 292nm  
 Sample : Mixture of tocopherol and tocotrienol  
 Injection : 10 $\mu$ L (1 mg/mL)

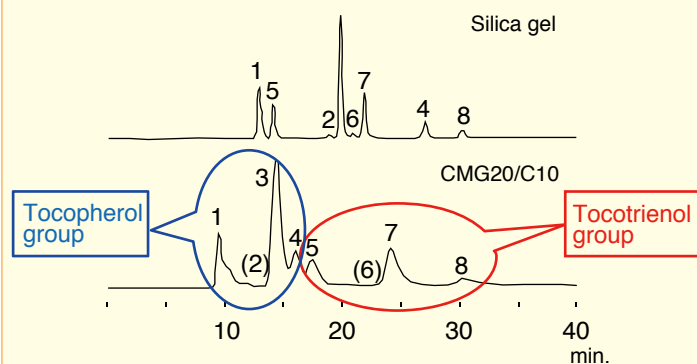
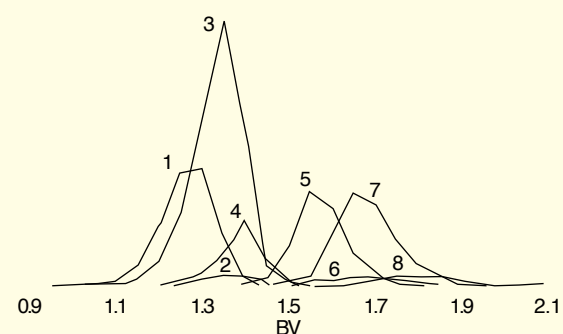


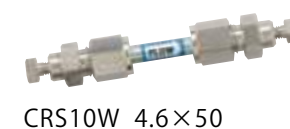
Fig. 5-44 Elution profile of tocopherol and tocotrienol in preparative scale

Conditions  
 Column : MCI GEL™ CMG20/P150, 41.2mm I.D.×550mm, ×4  
 Eluent : Hexane/EtOH = 90/10  
 Flow rate : 49.0 ml/min (SV=1.0)  
 Column temp. : 25°C  
 Detection : UV 292n  
 Sample : Mixture of tocopherol and tocotrienol  
 Injection : 150 mL (50g/L)



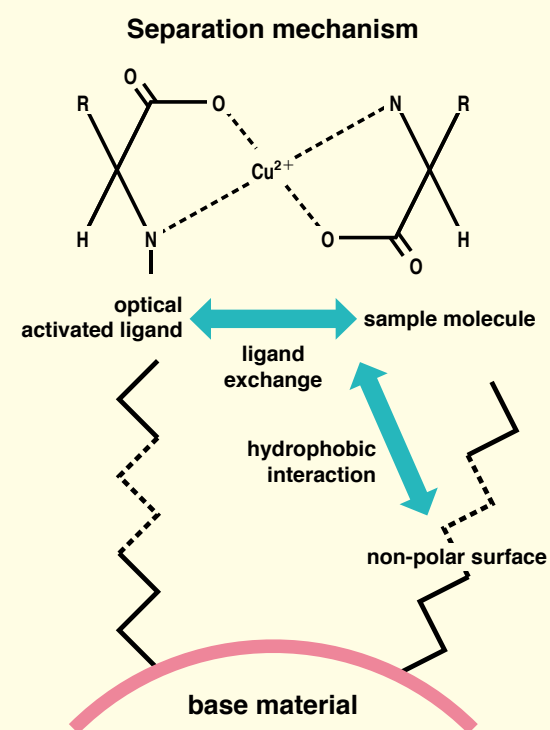
## 6 MCI GEL™ Chiral separation columns

### Chiral separation columns MCI GEL™ CRS10W (DLAA) MCI GEL™ CRS15W (LDAA)



MCI GEL™ column	Column dimensions	Particle size ( $\mu$ m)	USP
MCI GEL™ CRS10W	4.6×50mm	3	L32
MCI GEL™ CRS15W	4.6×50mm	3	L32

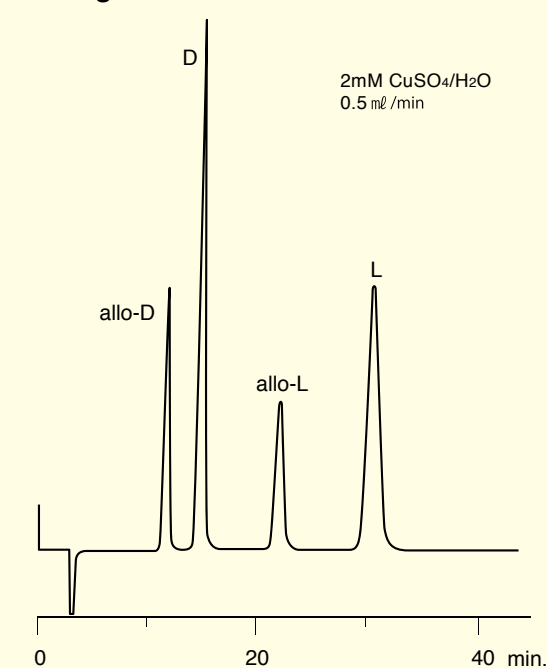
## Separation mechanism and performance of MCI GEL™ CRS series



### Separation mechanism

MCI GEL™ CRS10W and its companion product MCI GEL™ CRS15W (an optical isomer of CRS10W) are based on a 3 $\mu$ m with 10nm mean pore diameter of silica gel coated with N,N-Dioctyl-L(or D)-alanine which is a novel optical activated ligand. The chiral resolution mechanism is a combination of ligand exchange and hydrophobic interaction. A copper sulfate aqueous solution is used as an eluent. Elution samples are directly detected at wave length of 254 nm because complex compound, composed of sample molecule and copper in the eluent, are object of detection. With the CRS10W, D-isomers generally elute in front of L-isomers while L-isomers elute ahead of D-isomers on the CRS15W. The hydrophobic interaction mechanism allows hydrophilic samples to elute faster than hydrophobic molecules. Long alkyl chain or aromatic compounds will elute late or require an organic solvent (CH<sub>3</sub>CN or CH<sub>3</sub>OH, max. of 15v/v%) to prevent adsorption onto the stationary phase.

Application of CRS10W Fig. 6-1 DL-Isoleucine



### Separation performance

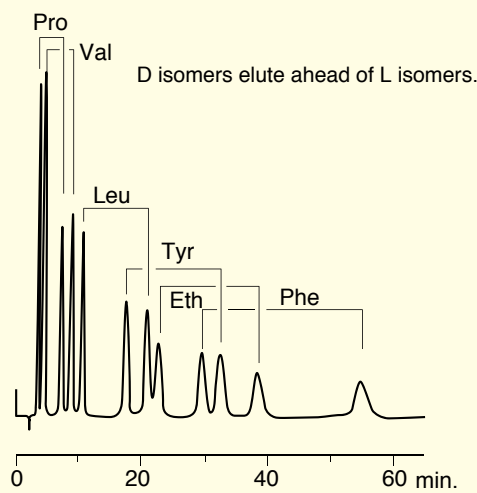
- The CRS series columns separate over 20 D,L- $\alpha$ -Amino acids by only single column. The columns separate not only  $\alpha$ -Amino acids but also  $\alpha$ -Hydroxy carboxylic acids and derivative amino acids such as Acetylated amino acids.
- The columns provide excellent resolution operated at room temperature.
- The columns show high durability.

### USP L32 column

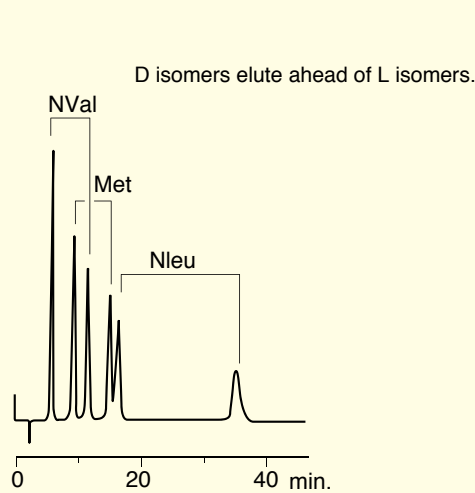
## Application data of CRS10W

For all chromatograms, column temperature is room temperature and wave length is 254nm.  
All eluents are CuSO<sub>4</sub> aqueous solution except for Fig. 6-9 and Fig. 6-10.

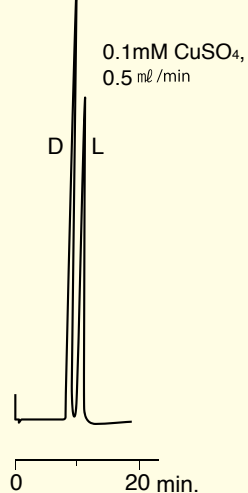
**Fig. 6-2 Separation of amino acids mixture**



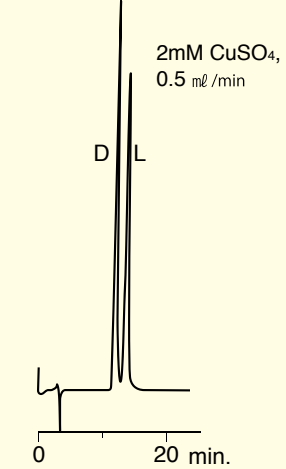
**Fig. 6-3 Separation of amino acids mixture**



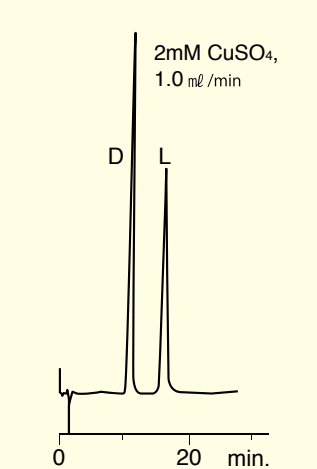
**Fig. 6-4 Separation of DL-Ser.**



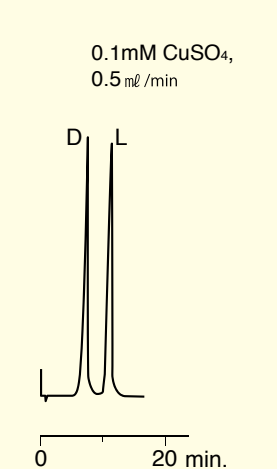
**Fig. 6-5 Separation of DL-aspartic acid**



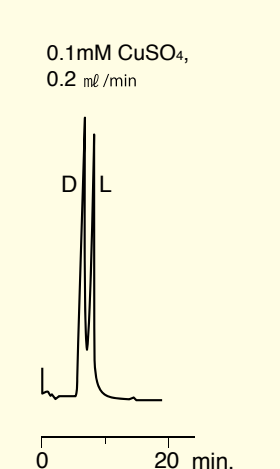
**Fig. 6-6 Separation of DL-glutamic acid**



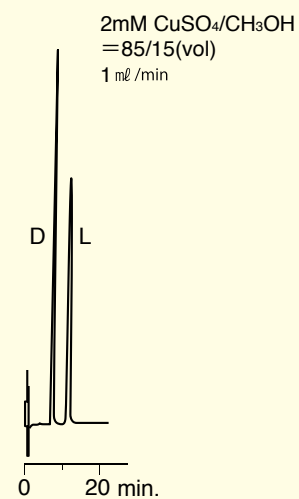
**Fig. 6-7 Separation of DL-histidine**



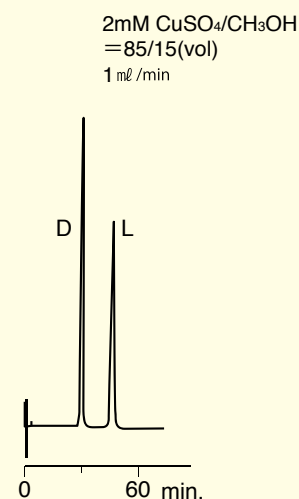
**Fig. 6-8 Separation of DL-lysine**



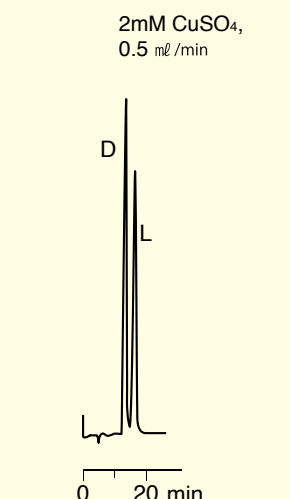
**Fig. 6-9 Separation of DL-phenylalanine**



**Fig. 6-10 Separation of DL-tryptophan**

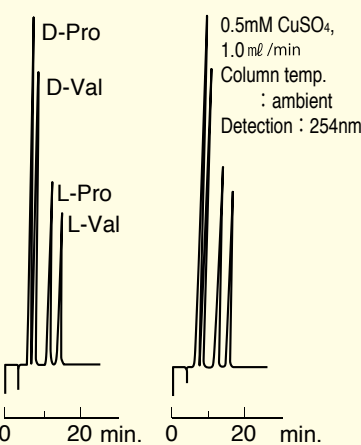


**Fig. 6-11 Separation of DL-lactic acid**



**Fig. 6-12 Durability test**

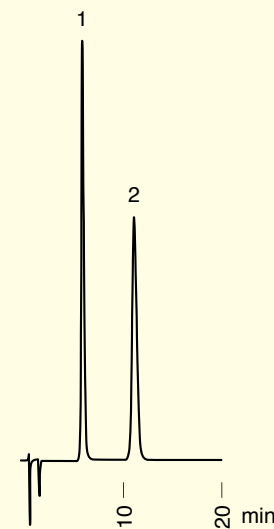
The sample was continuously injected 800 times for approximately 500 hrs. Changes of retention times and separation ability are not observed.



## Application data of CRS10W

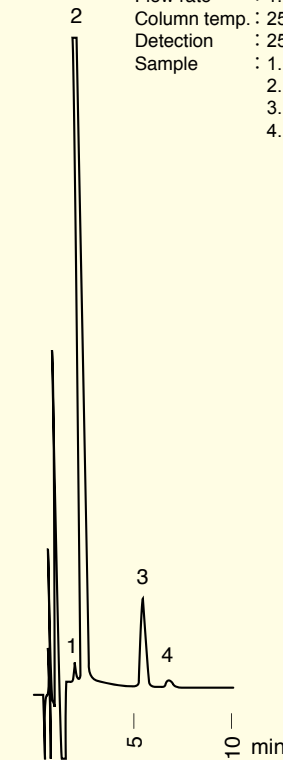
**Fig. 6-13 Separation of DL-α-Phenyglycine**

Conditions  
Column : MCI GEL™ CRS10W 4.6mm I.D.×50mm  
Eluent : 2mM CuSO<sub>4</sub>/CH<sub>3</sub>OH=85/15  
Flow rate : 1.0 ml/min  
Column temp. : 25°C  
Detection : 254nm  
Sample : 1. D-α-Phenyglycine  
2. L-α-Phenyglycine



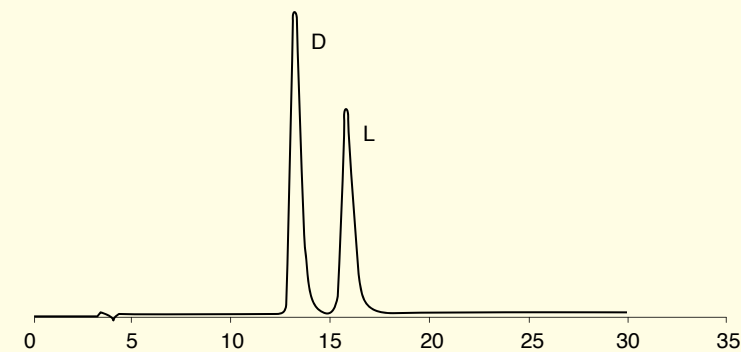
**Fig. 6-14 Separation of methionine and acetylmethionine**

Conditions  
Column : MCI GEL™ CRS10W 4.6mm I.D.×50mm  
Eluent : 2mM CuSO<sub>4</sub>/CH<sub>3</sub>CN=90/10  
Flow rate : 1.0 ml/min  
Column temp. : 25°C  
Detection : 254nm  
Sample : 1. D-Met  
2. L-Met  
3. Acetyl-D-Met  
4. Acetyl-L-Met



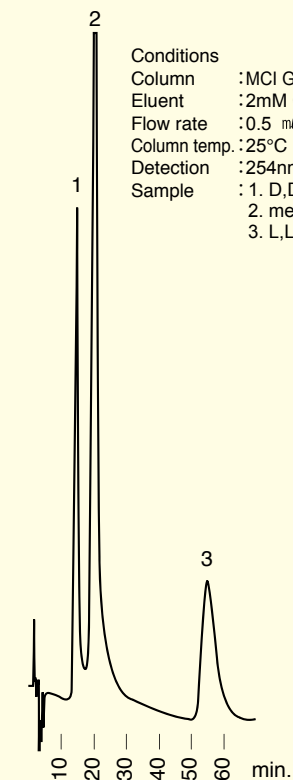
**Fig. 6-15 D/L-Aspartic acid**

Conditions  
Column : MCI GEL™ CRS10W 4.6mm I.D.×50mm  
Eluent : 0.4mM CuSO<sub>4</sub>  
Flow rate : 1.0 ml/min  
Temp. : ambient  
Detection : UV 254nm  
Sample : D/L Aspartic acid



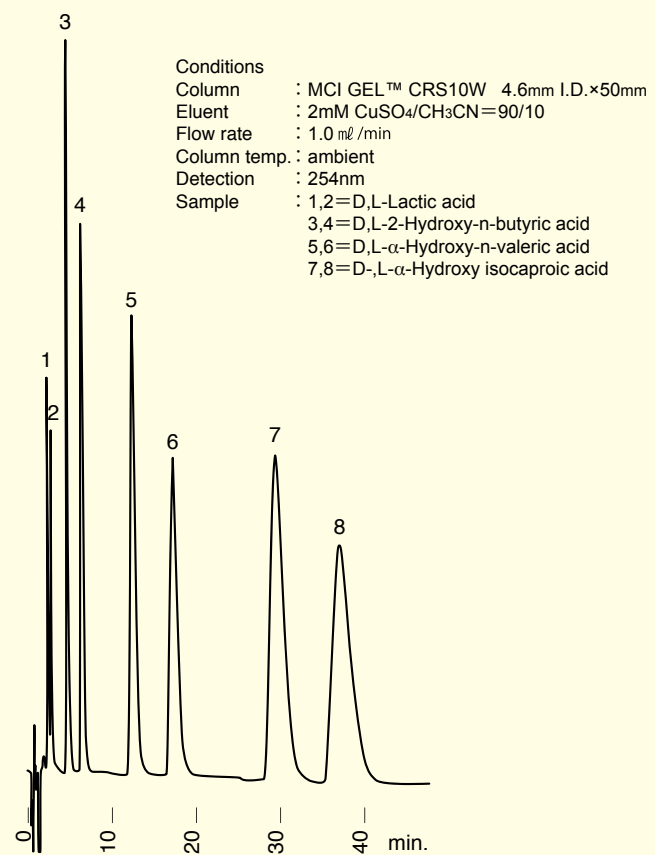
**Fig. 6-16 Separation of diaminopimelic acid**

Conditions  
Column : MCI GEL™ CRS10W 4.6mm I.D.×50mm  
Eluent : 2mM CuSO<sub>4</sub>  
Flow rate : 0.5 ml/min  
Column temp. : 25°C  
Detection : 254nm  
Sample : 1. D,D-2,6-Diaminopimelic acid  
2. meso-2,6-Diaminopimelic acid  
3. L,L-2,6-Diaminopimelic acid

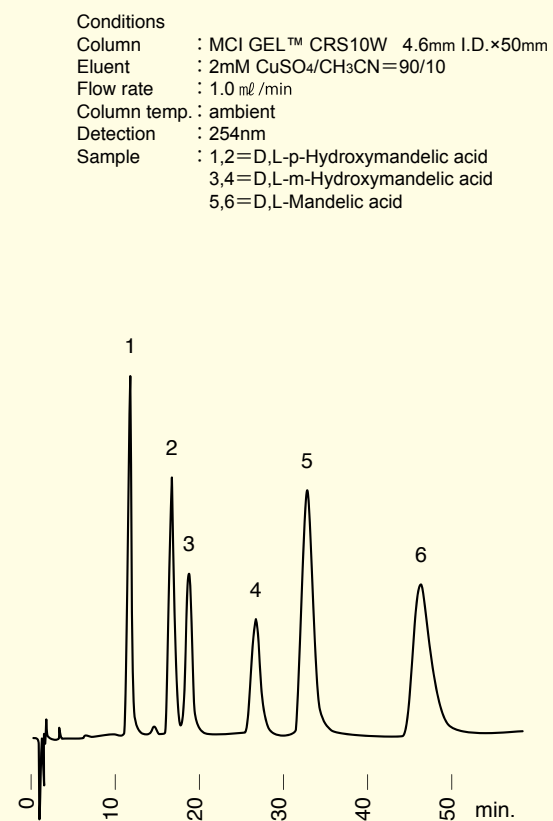


## Application data of CRS10W

**Fig. 6-17 Separation of 2-hydroxy carboxylic acids**

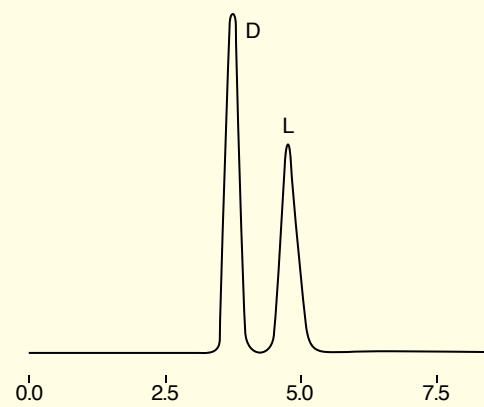


**Fig. 6-18 Separation of 2-hydroxy carboxylic acids**



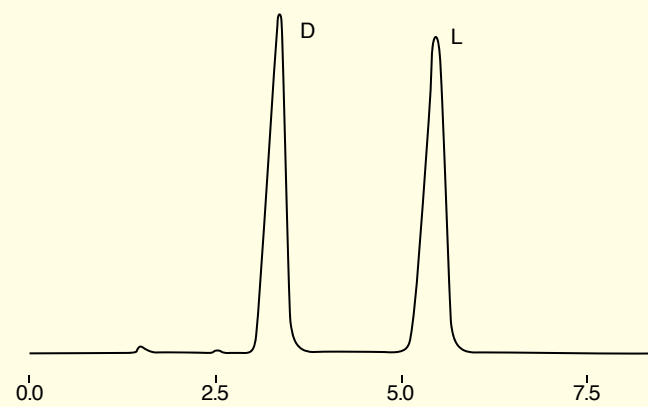
**Fig. 6-19 D/L Alanine**

Conditions  
 Column : MCI GEL™ CRS10W 4.6mm I.D.×50mm  
 Eluent : 0.1mM CuSO<sub>4</sub>  
 Flow rate : 1.0 ml/min  
 Temp. : 30°C  
 Detection : UV 254nm  
 Sample : D/L Alanine



**Fig. 6-20 DL-P-Hydroxyphenylglycine**

Conditions  
 Column : MCI GEL™ CRS10W 4.6mm I.D.×50mm  
 Eluent : 2mM CuSO<sub>4</sub>:MeOH=85:15  
 Flow rate : 1.0 ml/min  
 Temp. : 30°C  
 Detection : UV 254nm  
 Sample : DL-P-ydroxyphenylglycine

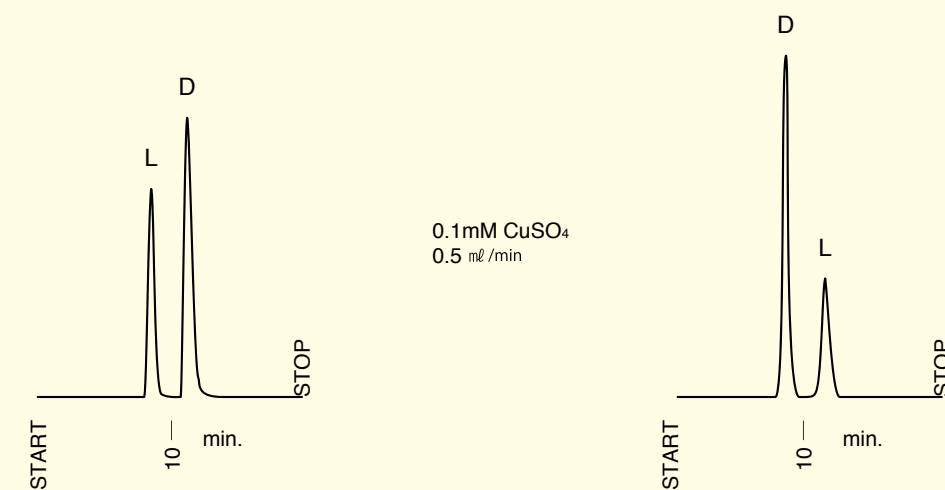


## Comparison data of CRS10W and CRS15W

**Fig. 6-21 Separation of DL-alanine**

CRS15W

CRS10W

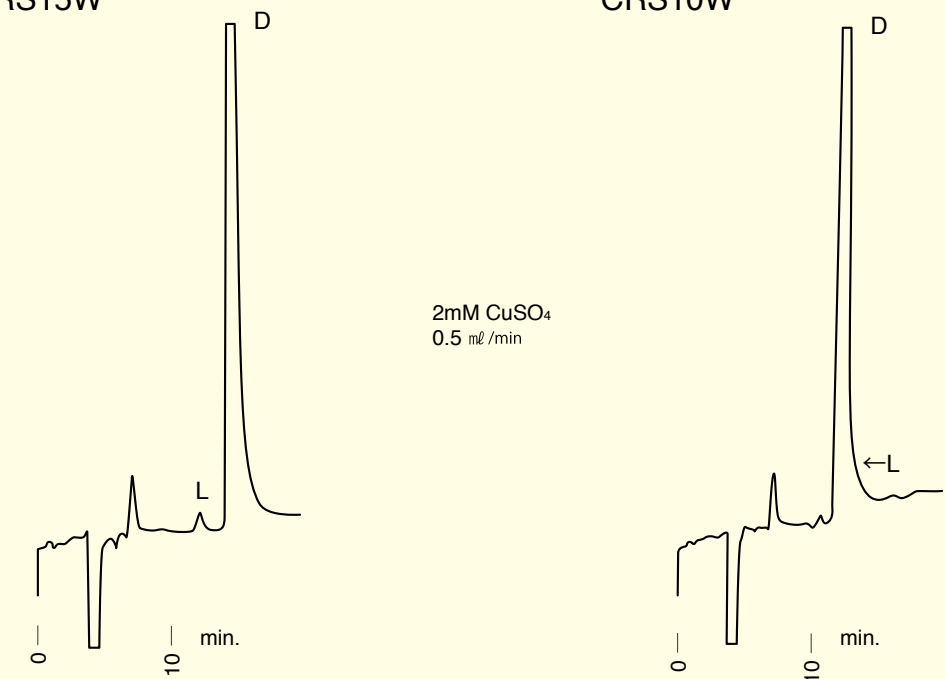


**Fig. 6-22 Analysis of a trace of L-lactic acid in 50 ppm D-lactic acid**

The CRS15W is recommended for analysis of a trace of L-isomer in a principal D-isomer when the CRS10W does not provide an adequate chromatogram.

CRS15W

CRS10W



## Examples of chromatographic conditions and datas

	Amino acids	CuSO <sub>4</sub> aq. soln. [mM]	Flow rate [ml/min]	Retention time; L-isomers [min]	Separation factor [α]	Separation rate [Rs]
1	Orn•HCl	0.1	0.2	6.8	1.26	<1
2	Lys•HCl	0.1	0.2	7.7	1.45	<1
3	Ala	0.1	0.5	11.0	1.39	1.4
4	His•HCl	0.1	0.5	10.5	1.63	1.7
5	Ser	0.1	0.5	10.1	1.25	1.0
6	Thr	0.1	0.5	11.3	1.29	1.3
7	Cit	0.5	0.5	10.4	1.75	2.3
8	Hyp	1.0	0.2	23.8	1.23	1.1
9	Pro	1.0	1.0	7.3	2.13	4.5
10	Val	1.0	1.0	8.9	2.04	5.0
11	Nval	1.0	1.0	11.5	2.07	4.7
12	Asp	2.0	0.5	13.2	1.18	0.8
13	Glu	2.0	1.0	16.2	1.54	2.3
14	Ileu(DL)	2.0	0.5	30.4	2.14	6.5
15	Ileu(allo)	2.0	0.5	21.9	1.97	6.0
16	Leu	2.0	1.0	14.6	1.97	4.6
17	Nleu	2.0	1.0	24.1	2.16	6.5
18	Met	2.0	1.0	10.3	1.64	2.6
19	Tyr	2.0	1.0	22.5	1.85	5.3
20	Eth	2.0	1.0	26.4	1.69	5.0
21	Phe	2.0	1.0	37.8	1.84	6.3

1. Column temperature; ambient Detection; 254nm
2. These are example data and do not guarantee the column specifications.
3. Improved resolution or appropriate chromatogram can be obtained by further investigating chromatographic conditions.
4. For each amino acid in the table, D-isomer elutes ahead of L-isomer except for Hydroxyproline.

## Notes

1. It will take hours for equilibrium between ligand of stationary phase and copper ion of eluent. Two to three hours of conditioning the column with the eluent is advised before sample injection or after changing concentration of CuSO<sub>4</sub> of eluent.
2. For acidic amino acids, higher CuSO<sub>4</sub> concentration of eluent provides better resolution.
3. For weakly retained hydrophilic amino acids, low flow rate (0.2-0.5 mL/min) yields better resolution.
4. Peak area may decrease with continuous injection of samples, when the concentration of amino acids in sample solution is much higher than that of CuSO<sub>4</sub> in the eluent.
5. Please be careful not to flow both water soluble organic solvents (CH<sub>3</sub>CN, CH<sub>3</sub>OH, etc) and non water soluble organic solvents (n-hexane, chloroform, etc) into the column. The column will be fatally damaged and will never separate optical isomers. Please be particularly careful if HPLC equipment is used together with RP mode and NP mode.
6. Please do not use acid or alkali solutions to adjust pH of eluent. And also do not use buffer solutions. These solutions may cause forming precipitation, hence cause of blockage of the column.
7. For strongly retained hydrophobic amino acids, addition of CH<sub>3</sub>CN or CH<sub>3</sub>OH in the eluent enables faster elution. The concentration of these organic solvents should be below 15 v/v%.
8. DOPA and other non-polar amino acids will be strongly adsorbed on the packing material and will cause contamination of the column.
9. Regeneration of contaminated column is difficult.

# 7

MCI GEL™

## SPE sorbent series

Solid phase extraction sorbents

For a pretreatment of analytical sample, we provide various SPE sorbents with various chemical structure, hydrophobicity, and micro-pore sizes. You can select our SPE sorbents depending on your molecule nature.

- CHP85/P120, CHP87/P120, CHPOD/P30: SPE sorbents with a controlled micro-pore size, high performance small molecule adsorption except large molecule mixture, like proteins.
- CSP800: SPE sorbents with high concentration ratio and high recovery, excellent for enrichment trace organic compounds and non-ionic substances such as trichloroethylene from environmental water. These SPE sorbents are to prepare samples for mutagenicity study or GC/MS analysis.
- SFP08/P25: SPE sorbents dedicated for small drug molecules extraction. Superior purity of this SPE extracts offers easier and faster sample preparation.
- CHL10P, CHL20P, CLB20P: SPE sorbents for rare earth metals that contains chelating functional group. CLB10P: SPE sorbents for borate, arsenic and selenium ions that contains glucamine groups on high porous ST/DVB matrix.

## Material list

### ● Synthetic adsorbents and reversed-phase materials

Name	Mean particle size [μm]	Pore size	Surface area [m <sup>2</sup> /g]	pH range	Typical Application
CHP85/P120	120	middle	880-940	full range	Small molecules extraction
CHP87/P120	120	small	820-910	full range	
CHPOD/P30	30	large	340-380	2~12	
CSP800	120	middle	790-920	full range	Enrichment of trace of organic compounds
SFP08/P25	25	middle	>1000	full range	Small molecules extraction

### ● Chelating type

Name	Functional group	Mean particle size [μm]	Ion exchange capacity [meq/ml]	Effective pH range	Typical Application
CHL10P	Iminodiacetic acid	120	>1.5	2-6	Metal Extraction
CHL20P	Polyamine	120	>1.8	2-6	Metal Extraction
CLB10P	Glucamine	120	>1.0	>3	Extraction Bron Removal