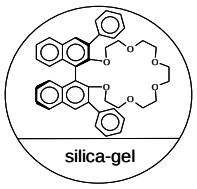
INSTRUCTION MANUAL FOR CROWNPAK® CR(+) / CR(-)

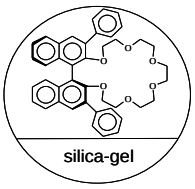
Please read this instruction sheet completely before using these columns

Column Description

Packing composition: Chiral Crown Ether coated on **5µm silica-gel**.



CROWNPAK® CR(-)



CROWNPAK® CR(+)

Shipping solvent: H_2O/MeOH 95:5 (v/v)

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

CAUTION

CROWNPAK® CR(+) / CR(-) require special care as limitations exist with solvents.
Exposing the column to inappropriate conditions will result in a rapid degradation of the stationary phase or to a loss in the column performance. Any traces of pure solvents must be removed.

BEFORE CONNECTING THE COLUMN, the entire HPLC system including the injector and the injection loop must be flushed with ethanol followed by 100% distilled water.

Chiral Recognition

Chiral recognition can be achieved with CROWNPAK® CR(+) / CR(-) columns when a complex is formed between the crown ether and an ammonium ion ($-NH_3^+$) derived from a sample, under acidic conditions.
These columns can resolve not only amino acids but also compounds bearing a primary amino group near the chiral center.

With CROWNPAK® CR(+), the D-form of amino acids always elutes in first position.
Using CROWNPAK® CR(-) will result in an inversion of the elution order.

Operating Restrictions

	150 x 4.0 mm i.d. Analytical column
Flow rate direction	As indicated on the column label
Typical Flow rate ①	~ 0.5mL/min Do not exceed 1.5mL/min
Pressure limitation ②	Should be maintained < 150 Bar (~2100 psi)③ for maximum column life Do not exceed 200 Bar (~2900 psi)
Temperature ④	-5°C to 50°C

①+② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition and temperature). Flow rate should be adjusted in accordance with pressure limitations.

Example	**25°C**	**0°C**
pH 2 $HClO_4$ 0.5mL/min	~ 50 Bar	~ 100 Bar

The back pressure value that should be taken into account is the one generated by the column itself.
This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

③ Ideal value for maximum column life, but stable up to 200 Bar.

④ Generally, the lower the temperature is, the better the resolution becomes, especially for hydrophilic samples. Note that some hydrophobic samples may be strongly retained on the stationary phase at low temperatures.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

This column should be operated under acidic condition as stated above.

	Aqueous solution of $HClO_4$❶	Aqueous solution of $HClO_4$❶ / Methanol❷
CROWNPAK® CR(+) / CR(-) 150 x 4.0 mm i.d.	100%	100/0 to 85/15 **(15% MeOH Max.)**

❶ ⇨ Typical pH range of the mobile phase: from pH 1 to pH 2 (column stable up to pH 9).
⇨ Lower pH will result in a good resolution but in a shorter column life. Choose the highest pH giving a satisfactory separation to prolong column life time.
⇨ Decreasing the temperature is also effective to increase the selectivity.
⇨ Other acids such as nitric acid and TFA can also be used. However, we recommend to use perchloric acid preferably which gives, in most cases, better resolutions and also for its low UV-absorption.

❷ ⇨ To shorten the retention time of hydrophobic compounds, the addition of methanol (15% max.) has been shown to be efficient.
Exceeding 15% of Methanol or using a different organic modifier is likely to damage the stationary phase contained in the column.

Examples of mobile phase preparation:

a) pH = 1.0
Weigh out 16.3 grams of commercially available perchloric acid (70%) and diluted to 1 L with distilled water.

b) pH = 2.0
100 mL of pH 1.0 solution is diluted to 1 L with distilled water.

c) pH = 1.5
316 mL of pH 1.0 solution is diluted to 1 L distilled water.

d) pH = 1.3
500 mL of pH 1.0 solution is diluted to 1 L distilled water.

Notes: *- Mobile phases should be completely degassed or thoroughly purged with helium.*

- Capacity factors on these columns depend on the hydrophobic nature of samples. Hydrophobic compounds are more retained compared to the hydrophilic ones. When your sample shows a little retention and a poor resolution the separation may be improved by decreasing the pH of the mobile phase (and column temperature).

- Although this column is not damaged by K^+ ions, the chiral recognition may be disturbed when present. The use of mobile phases containing K^+ ions should be avoided.

Column Care / Maintenance

- The use of a guard column is highly recommended for maximum column life.
- Samples should be dissolved in H_2O and filtered through a membrane filter of approximately 0.5µm porosity. Even for sample preparation, using a solution that contains more than 15% Methanol may cause irreversible damages.
- Injecting a too concentrated sample solution will lead to a low column efficiency.
  Injections of 10^{-8} to 10^{-7} mol of compound is usually enough to see a signal by UV.
- The column should be flushed with distilled water when your analysis is finished.
  For a long term storage (more than 1 week), keep the column end capped in the refrigerator (3-6°C) to avoid microbial contamination.
- If peaks start to split (after a long time of use), back-flushing the column with 100% H_2O may resolve the problem. Back-flushing could be a method to rescue the column but is usually not recommended.

Examples of Amino Acids

DL-Amino acid	$HClO_4$ (pH)	F.R. (mL/min)	Temp. (℃)	k_D'	k_L'	α	Rs
Alanine	1.5	0.4	25	0.38	0.70	1.86	3.17
Valine	1.5	0.4	0	1.09	1.64	1.51	3.47
Norvaline	2.0	0.8	25	0.69	1.17	1.69	2.74
Leucine	2.0	0.8	25	1.44	2.39	1.67	3.73
Norleucine	2.0	0.8	25	1.76	2.91	1.66	3.38
Isoleucine	2.0	0.4	0	1.76	2.79	1.58	4.29
tert-leucine	2.0	0.4	0	2.06	2.26	1.10	0.7
Phenylalanine	2.0	0.8	25	3.88	4.93	1.27	2.80
DOPA	2.0	0.8	25	2.88	3.67	1.28	2.47
Methionine	2.0	0.8	25	1.05	2.10	2.00	5.87
Ethionine	2.0	0.8	25	2.43	4.68	1.93	6.03
Phenylglycine	2.0	1.0	40	1.06	2.49	2.35	7.14
Serine	1.5	0.4	0	0.48	0.85	1.75	3.04
Threonine	2.0	0.4	0	0.39	1.00	2.58	4.20
Cysteine	1.5	0.4	25	0.44	0.74	1.67	3.31
Tyrosine	2.0	0.8	25	2.88	3.67	1.28	2.47
Asparagine	1.5	0.4	0	0.53	0.90	1.69	3.15
Glutamine	2.0	0.4	25	0.25	0.53	2.13	3.11
Aspartic acid	2.0	0.4	0	0.61	1.23	2.01	4.07
Glutamic acid	2.0	0.4	25	0.33	0.92	2.81	5.32
Ornithine	1.5	0.4	25	0.65	0.97	1.49	2.82
Lysine	1.5	0.4	25	1.18	1.50	1.26	2.20
Arginine	1.5	0.8	25	0.65	1.43	2.21	5.18
Citrulline	1.5	0.4	25	0.43	0.94	2.18	3.97
Proline	1.5	0.4	0	0.73	0.73	1.00	----
Histidine	1.5	0.4	0	0.90	1.64	1.82	5.28
Tryptophane	2.0	1.2	25	18.45	21.94	1.19	2.22

Sample Loda: 10^{-7}～10^{-8}mol Detection: UV200nm

These data are not guaranteed.

Analytical Conditions of Amino Acids (aq. $HClO_4$)

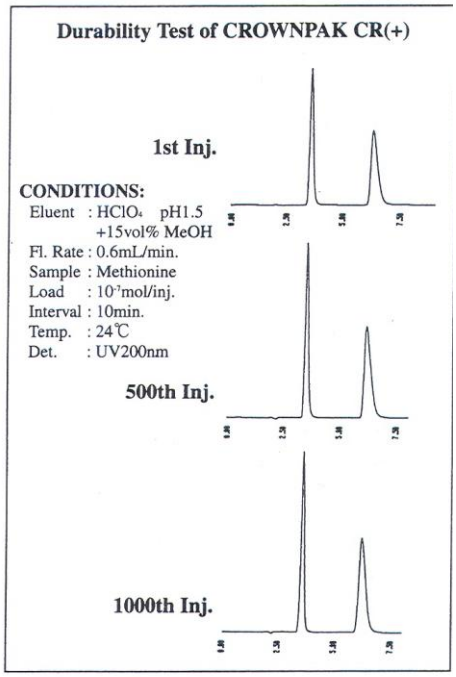
DL-Amino acid	pH2.0			pH1.5		pH1.0
	25℃	15℃	0℃	25℃	0℃	25℃
Alanine	P	C	C	C	C	
Valine	P	P	C	P	C	C
Norvaline	C	C	C	C	C	
Leucine	C	C	C	C	C	
Norleucine	C	C	C	C	C	
Isoleucine	A	C	C	C	C	
Phenylalanine	C	C	C	C	C	
DOPA	C	C	C	C	C	
Methionine	C	C	C	C	C	
Ethionine	C	C	C	C	C	
Phenylglycine	C	C	C	C	C	
Serine	U	U	A	U	C	
Threonine	P	A	C	A	C	
Cysteine	A	C	C	C	C	
Tyrosine	C	C	C	C	C	
Asparagine	U	U	P	P	C	A
Glutamine	C	C	C	C	C	
Aspartic acid	P	A	C	P	C	
Glutamic acid	C	C	C	C	C	
Ornithine	P	A	C	C	C	
Lysine	P	A	C	C	C	
Arginine	A	C	C	C	C	
Citrulline	A	C	C	C	C	
Proline	U	U	U	U	U	
Histidine	U	P	P	P	C	A
Tryptophane	C	C	C	C	C	

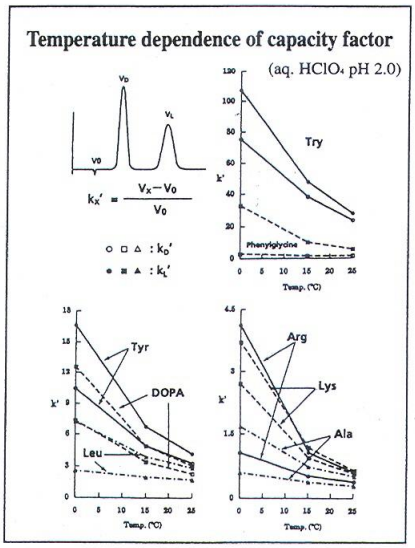
C : Completely resolved. ($2 \leqq Rs$)
A : Almost completely resolved. ($1.5 \leqq Rs < 2$)
P : Partially resolved. ($Rs < 1.5$)
U : Unresolved.

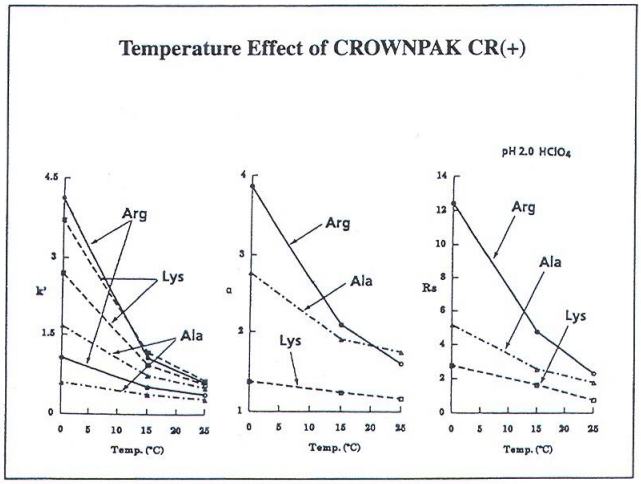
Examples of Amines and Aminoalcohols

Amines and Aminoalcohols	Eluent	Temp (℃)	Det.	k1'	α
	$HClO_4$ pH 1	1	OPA	2.57	1.10
	$HClO_4$ pH 1	1	OPA	1.84	1.15
	$HClO_4$ pH 2	25	UV	8.44	1.31
	$HClO_4$ pH 1.5	9	UV	21.8	1.17
	$HClO_4$ pH 1	1	OPA	0.67	1.58
	$HClO_4$ pH 1	1	OPA	1.67	1.42
	$HClO_4$ pH 1	1	OPA	1.54	1.30
	$HClO_4$ pH 1	1	OPA	4.19	1.43
	$HClO_4$ pH 2	10	UV	4.19	1.23
	$HClO_4$ pH 2	10	UV	9.07	1.18
	$HClO_4$ pH 1.5	40	UV	18.8	1.39
	$HClO_4$ pH 2	25	UV	0.54	1.83

Durability Test of CROWNPAK CR(+)







Operating this column in accordance with the guidelines outlined here will result in a long column life.

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

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