Handbook of chiral MPLC column

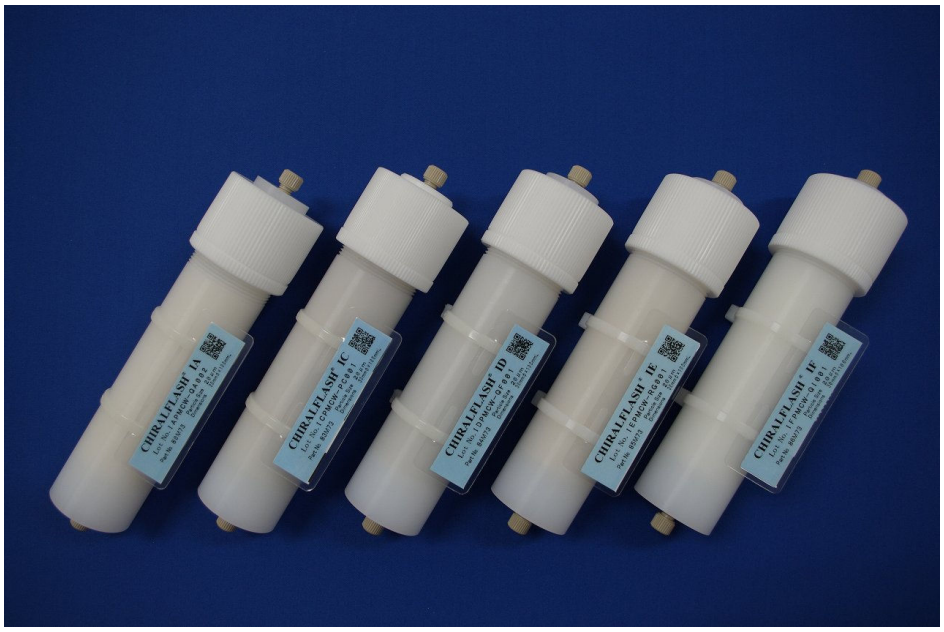
DAICEL CORPORATION

CPI Company

Contents

Chiral MPLC column

CHIRALFLASH® IA　　CHIRALFLASH® IE
CHIRALFLASH® IC　　CHIRALFLASH® IF
CHIRALFLASH® ID



✓CHIRALFLASH® is chiral columns for medium pressure liquid chromatography(MPLC), while filling up the solvent resistant semi-transparent fluoroplastic column with the immobilized-type polysaccharide-derived chiral stationary phase (CSP).

✓Immobilized type CSP is fixed chiral selector in the silica gel substrates, and use all the solvents that can be used for silica gel based column as a mobile phase.
(not only n-Hexane, alcohol but also ethyl acetate, tetrahydrofuran, chloroform etc.)

✓It's joint size is 1/4-28UNF. So it is easy to equip inside MPLC equipment with column.

【 Features 】

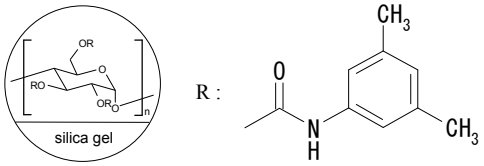
- **Load amount is about 50~100 mg per injection.**
- **Wide variety of organic solvents can be used as a mobile phases.**
- **It is possible to be reverse cleaning and reuse.**
- **Column inside is visible. (Translucent column tube)**

1. Specifications

Structural formula of chiral selector

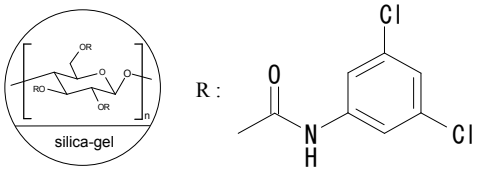
CHIRALFLASH® IA

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3,5-dimethylphenylcarbamate)
Particle size : 20μm



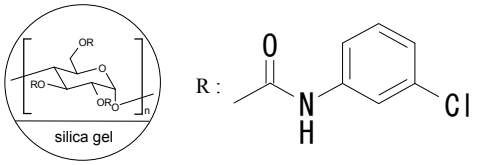
CHIRALFLASH® IC

Column fitting : 1/4-28 UNF
Packing composition : Cellulose tris(3,5-dichlorophenylcarbamate)
Particle size : 20μm



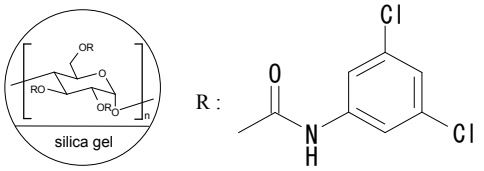
CHIRALFLASH® ID

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3-chlorophenylcarbamate)
Particle size : 20μm



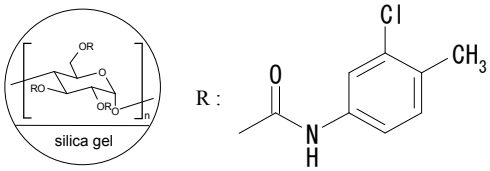
CHIRALFLASH® IE

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3,5-dichlorophenylcarbamate)
Particle size : 20μm



CHIRALFLASH® IF

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3-chloro-4-methylphenylcarbamate)
Particle size : 20μm



Column size	Packing size Tube size	30 mm I.D. x 100 mmL 38 mm O.D. x 150 mmL
Column material		Fluoroplastic
CSP weight	g	ca. 40
Bed volume	mL	50
Pressure limitation	MPa	Should be maintained < 1.5 MPa (218 psi) for maximum column life
Temperature	℃	0 ~ 40
Typical flow rate	mL/min.	12
Sample loading	mg/inj.	50 ~ 100

<Important reminder>
・Do not give strong shocks to the column, or disassemble it. It may result in damage to the column and result in poor separation performance.
・When using a column, it is highly recommended to discard at least the first 300mL ～ 600mL of eluent at the beginning of a preparative work.
・When back flushing it is highly recommended to keep the flow rate below the value recommended in the operating instructions.

2. Usable solvents

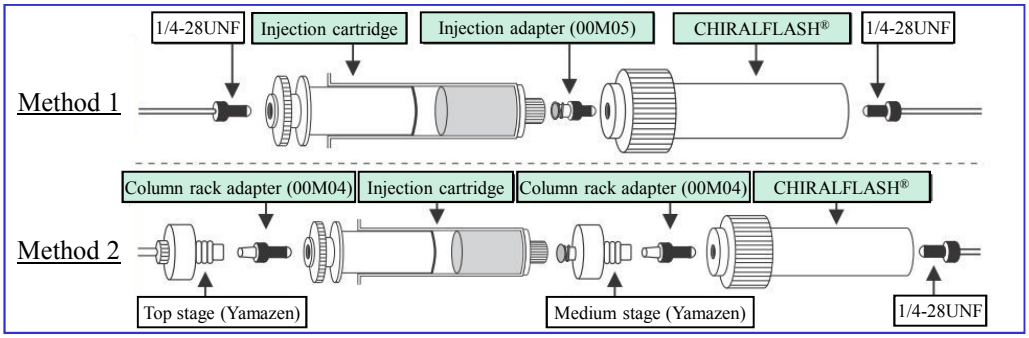
CHIRALFLASH® can use wide variety of organic solvents for a mobile phase or a sample solution.

・Alkane (n-Hexane, n-Heptane)
・Alcohol (Methanol, Ethanol, 2-Propanol)
・t-Butyl methyl ether（MTBE）
・Dichloromethane
・Chloroform
・Ethyl acetate
・Tetrahydrofuran
・Acetonitrile
・Acetone
・Toluene
・1,4-dioxane
・Other solvent can be used for silica gel based column as a mobile phase

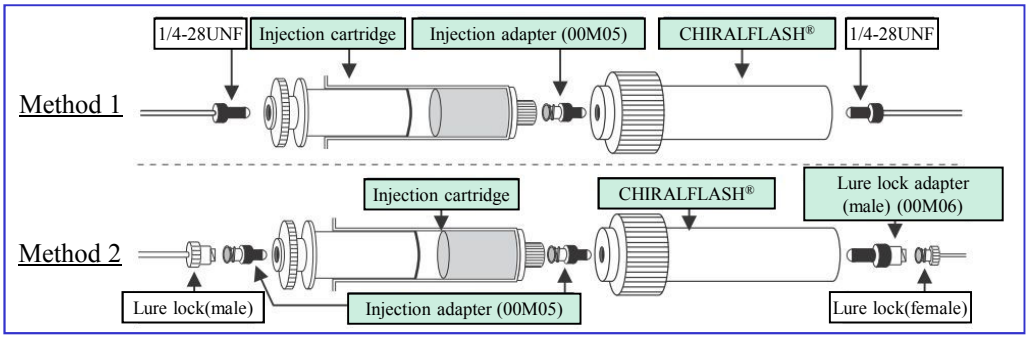
3. Installation to MPLC

CHIRALFLASH® can install each company MPLCs using following joints.
Recommended to use the injection cartridge （ The details of the injection cartridge should look at p.4. ）

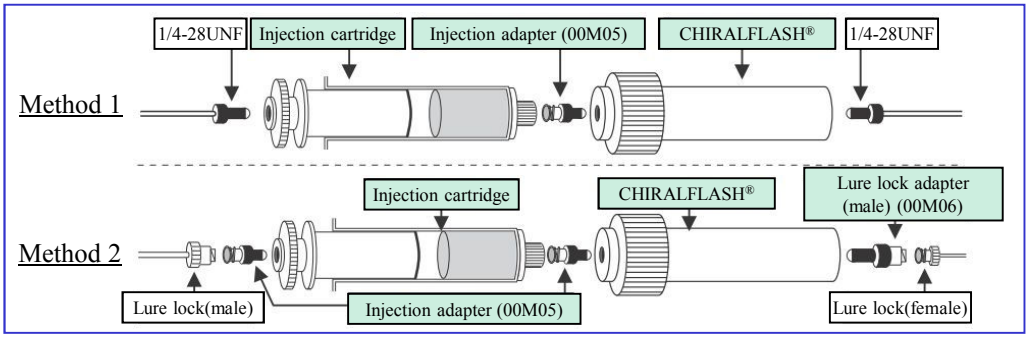
Yamazen



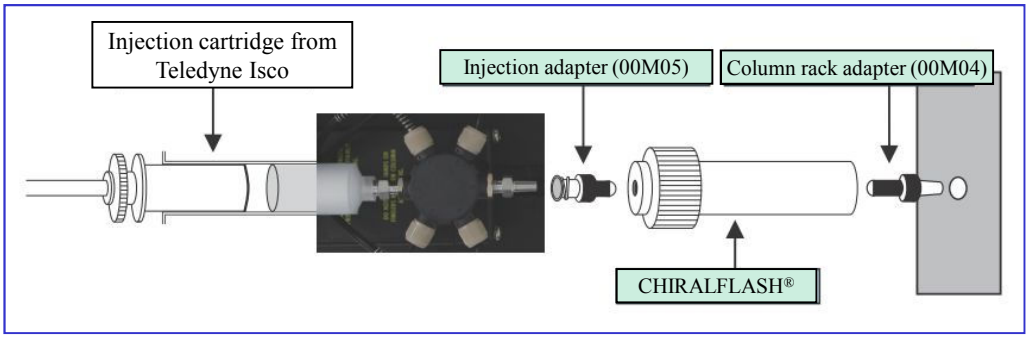
Biotage



SHOKO scientific



Teledyne Isco

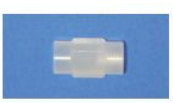


Lineup of Adapters

Column rack adapter (00M04)

Injection adapter (00M05)

Lure lock adapter (male) (00M06)



Syringe union (00M07)

LL adapter [INTRM Connector - female] (00M08)

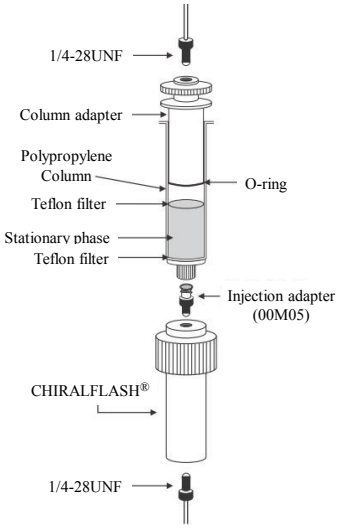
LL adapter [INTRM Connector - male] (00M09)

4. Accessories (Injection cartridge, Fitting)

Accessories fit in CHIRALFLASH® are provided such as injection cartridges and fittings.

4-1. Injection cartridge

We're preparing Injection cartridges as the guard column for preventing contamination of CHIRALFLASH®, and as the injection column for loading samples.
It is filled up with modified silica gel (C1), and acidity and any basic and neutral compound can be used.
3 Sizes are preparing. (S, M, and L)



←00M03 Injection cartridge L

←00M02 Injection cartridge M

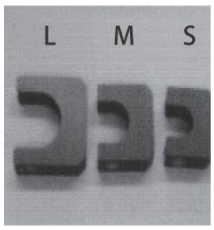
←00M01 Injection cartridge S

Size		Size S	Size M	Size L
Column Size (mm)	Column Length	φ15×44	φ20×75	φ26×80
	Tube Length	φ15×85	φ20×95	φ26×135
Packing Weight (g)		4.5	13	25
Max. Injection Volume (mL)		4.5	13	25

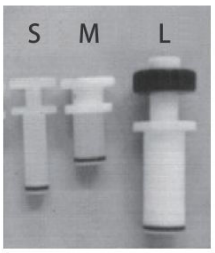
4-2. Fitting

In order to use Injection cartridge, corresponding to the size (S, M, L) of the column adapter and the column holder are required.

00M13 Column holder S
00M14 Column holder M
00M15 Column holder L



00M10 Column adapter S
00M11 Column adapter M
00M12 Column adapter L



5. Method development on CHIRALFLASH®

5-1. Using HPLC analytical column for method development of CHIRALFLASH®

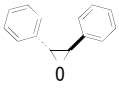
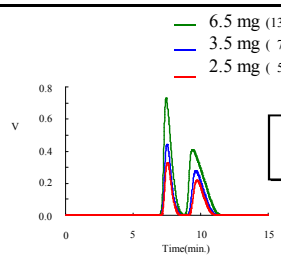
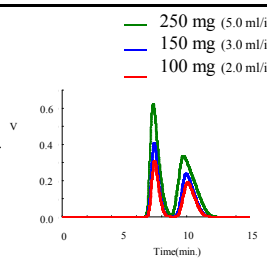
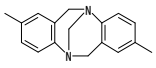
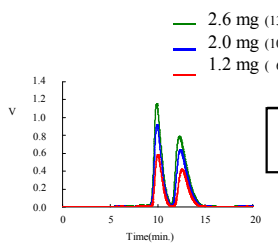
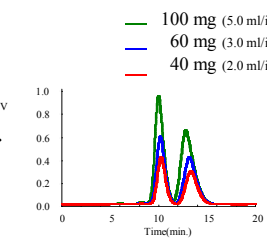
1) The optimization of separation condition and 2) the estimation of the sample loading quantity on CHIRALFLASH® are possible by using HPLC analytical columns for method development of CHIRALFLASH®.
There are 5 products lineup of CHIRALPAK® (IA, IC, ID, IE and IF : the particle size is 20μm) corresponding to each types of CHIRALFLASH®.

The conditions established on CHIRALPAK® IA, IC, ID, IE and IF (20 μm) can be scaled up to CHIRALFLASH® IA, IC, ID, IE and IF directly on the basis of column dimensions, as the same stationary phases are used in both columns. For example, approximately 40-fold sample quantity compared with 4.6 mm I.D. x 100 mm L CHIRALPAK® IA, IC, ID, IE and IF (20 μm) will be applicable on 30 mm I.D. x 100 mm L CHIRALFLASH® IA, IC, ID, IE and IF.

The quantity of sample load of *CHIRALFLASH* : $Y(mg) = X(mg) \times \dfrac{\left(\frac{30}{2}\right)^2 \times \pi}{\left(\frac{4.6}{2}\right)^2 \times \pi} \fallingdotseq X(mg) \times 40 times$

X : The quantity of sample load of CHIRALPAK® (20 μm)

In the optimization study, all of the conditions, such as the column temperature, the sample concentration, the eluent composition, the linear flow velocity, the additives, the peak detection conditions, and so on, should preferably be representative of a preparative column.

Column size (I.D. x Length)	<CHIRALPAK® IC(20 μm)> **4.6 x 100 mm**	<CHIRALFLASH® IC> **30 x 100 mm**
Flow rate	0.28 mL/min.	12.0 mL/min.
trans-Stilbene Oxide (t-SO)  α=1.7	Sample conc. : 50 g/L in Eluent  About 40 times 	
Tröger's-Base (TB)  α=1.4	Sample conc. : 20 g/L in Eluent  About 40 times 	

Mobile phase : n-Hexane / 2-Propanol = 90 / 10 (v/v)
Temp. : R.T.
Detect : UV 254 nm

5-2. Recommended mobile phases and additives

We recommend that the conditions shown in Table 1 are used as the basis for initial method development for CHIRALFLASH® IA. After the initial evaluation the most promising methods can be optimized using the suggested ranges below. MTBE and chlorinated solvents may also be used in their pure form as the mobile phase. Moreover, in the case of solvents with strong elution intensity, such as THF and ethyl acetate, it is advised to mix them with a hydrocarbon solvent (e.g. hexane or heptane) to modulate retention and selectivity.

<The procedure of mobile phase selection>

1. For acidic or basic samples, it is necessary to incorporate an additive into the mobile phase in order to optimize the chiral separation. First choice is Acetic acid for acidic samples and Diethylamine for basic samples.

2. Please try "Typical starting condition" indicated about Table 1. sequentially from the left sides on HPLC analytical column for method development.

3. If you get separation (baseline or partial), Please optimize mobile phase composition in reference to "Advised optimization range" indicated about Table 1.

Table 1. Recommended organic miscible solvents

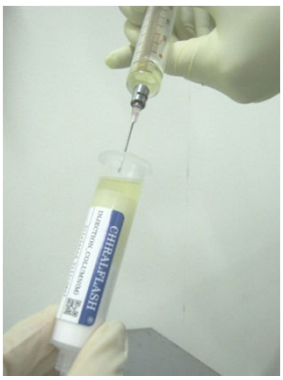
	Alkane❶/ Alcohol❷	Alkane❶/ EtOAc	Alkane❶/ $CHCl_3$	Alkane❶/ THF	MTBE / EtOH
Typical starting conditions	90:10	90:10	70:30	90:10	100:0
Advised optimization range	95:5 ~ 0:100❸	95:5 ~ 0:100	95:5 ~ 0:100	95:5 ~ 0:100	100:0 ~ 40:60

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ Methanol, ethanol, and 2-propanol are raised as typical alcohol.
Moreover, as alcohol other than the above, 1-propanol, 1-butanol, 2-butanol, etc. can be used.
Depending on a sample, separation may change greatly with kinds of alcohol.
❸As for the mixed solvent of alcohol, viscosity may become high with composition.
Please adjust the flow velocity if needed not to exceed the maximum working pressure range of a column.
Usually, retention time becomes short so that composition of alcohol becomes high, In not less than 50% of domain of alcohol, a prominent effect may not no longer be seen.
Although operating composition of methanol does not have restriction, it recommends being used mixing with the above ethanol or 2-propanol in equivalent amount with composition of methanol for compatibility with alkane.
When ethanol or 2-propanol is not mixed, if not less than 5% of methanol is used, a possibility of dissociating two layers will increase.
Moreover, even when you use it at 5% or less, if adjusted by the methanol independent, it is recommended to agitate a mobile phase continuously.

6. Methods of sample injection

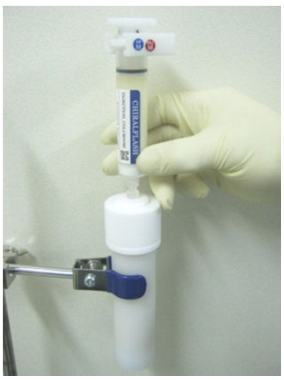
There're 2 methods of sample injection.

6-1. Using injection cartridge



1. The sample solution is poured into injection cartridge.

2. The sample solution is permeated by the injection cartridge.



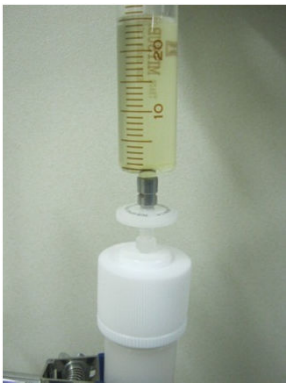
3. Fix the column adapter and the column holder to the injection column, and connect with CHIRALFLASH®.

6-2. Direct injection

1. Injection adapter is connected to the inlet of CHIRALFLASH®.

2. The sample solution is syringed.

3. Attach the membrane filter (0.5µm) to the tip of a syringe.

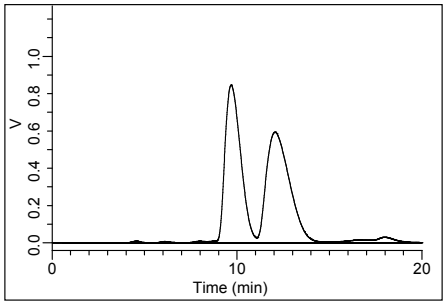


4. The sample is poured into CHIRALFLASH®.

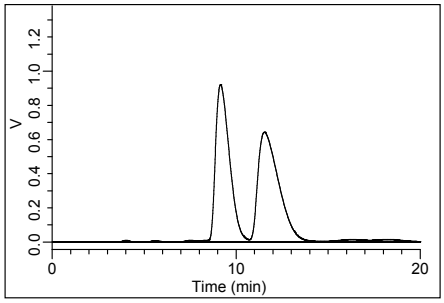
- In case of pouring in dilute concentration of the sample in large quantities, the separation might get worse because of diffusion within injection cartridge. In such a case, please try the method by "Direct injection".
- For maximum column life, the sample solution should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.

Column : CHIRALFLASH® IC
Sample : Tröger's-Base, 20g/L in Eluent × 4.5mL/inj.
Mobile Phase : 90/10 = n-Hexane/2-Propanol
Flow-rate : 12.0mL/min
Detection : UV 254nm

Using injection cartridge (Size-S)



Direct injection



7. Column care

7-1. Column cleaning

When acidic or basic additives are used, remove them by flushing the column with the mobile phase without the additive.
Moreover, Column cleaning (flash with ethanol at 6 mL/min for more than 30 minutes) is recommended after use of column.

7-2. Regeneration procedures

The separation characteristic of the column for polysaccharide optical resolution is dependent on the high order structure of polysaccharide. This high order structure may change depending on a mobile phase or temperature conditions, following extensive use of the column in multiple solvents for a long time, there may be a change in separation reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in chiral recognition due to the history of the column (mobile phases, additives…). Moreover, it is able to use the following procedure also as washing conditions for a column. (However, when the solubility of the sample to the following solvent or impurities is low, please carry out the following procedure after through a mobile phase with those high solubility solvents among several hours～about ten hours.

＜Regeneration procedures of CHIRALFLASH® IA, ID, IE, IF＞

1. Flush with ethanol at 6 mL/min for 120 min.
2. Flush with N,N-dimethylformamide (DMF) at 6 mL/min for 120 min.
3. Flush with ethanol at 6 mL/min for 60 min.
4. IA: Equilibrate with n-hexane/ethanol = 90/10 (v/v) at 12 mL/min for 60 min, prior to retesting the column.
   ID, IE, IF: Equilibrate with n-hexane/ethanol = 90/10 (v/v) at 12 mL/min for 60 min, prior to retesting the column.

＜Regeneration procedure of CHIRALFLASH® IC＞

1. Flush with ethanol at 6 mL/min for 120 min.
2. Flush with ethyl acetate at 6 mL/min for 120 min.
3. Store the column at room temperature for 2 days or longer.
4. Flush with ethanol at 6 mL/min for 60 min.
5. Equilibrate with n-hexane/IPA = 90/10 (v/v) at 12 mL/min for 60 min, prior to retesting the column.

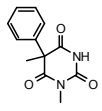
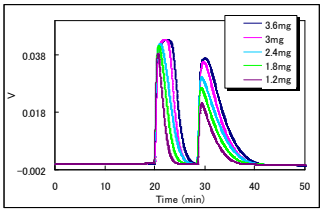
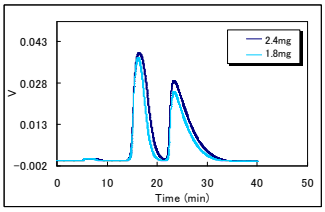
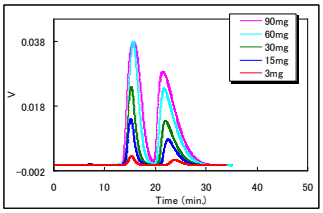
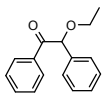
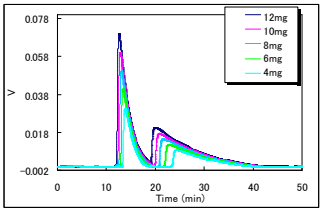
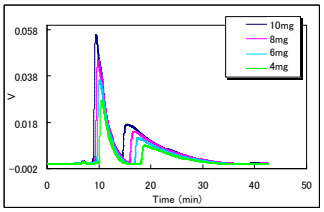
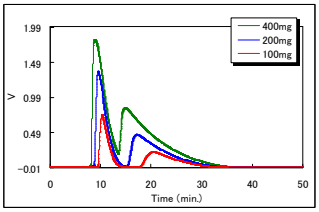
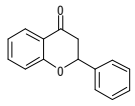
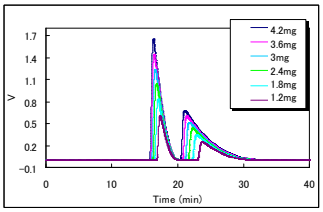
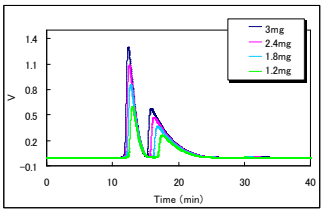
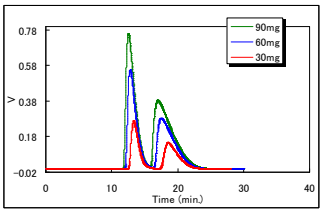
If this is not successful, then try this procedure again. However, a number of columns is affected by equipment and the column wearing method in use. Moreover, it may be subject to the influence of a temporal change of a filling state by repetition use of a column.

7-3. Column storage

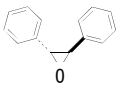
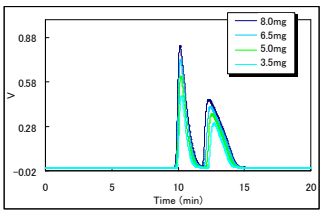
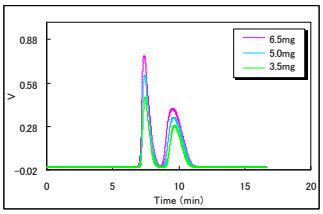
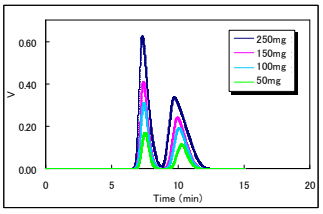
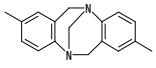
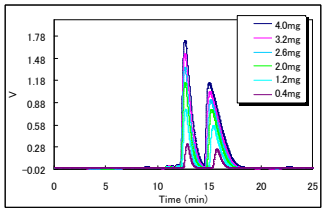
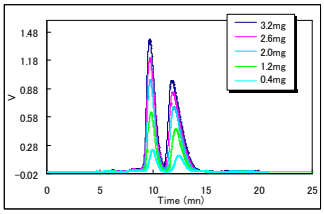
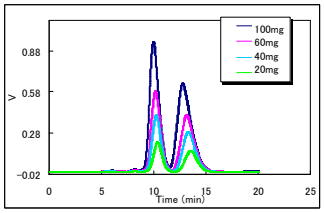
・For column storage, remove the acidic or basic additives by flushing the column with the same mobile phase without the additive. Columns can be stored end capped with additive-free mobile phases.
・Ethanol is recommended for longer column storage (longer than one week).

8. Application data

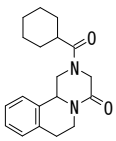
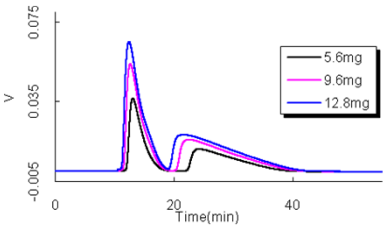
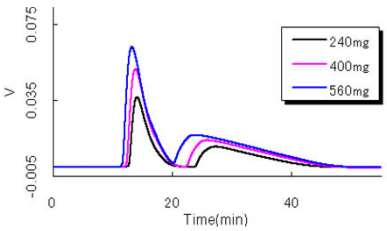
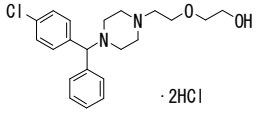
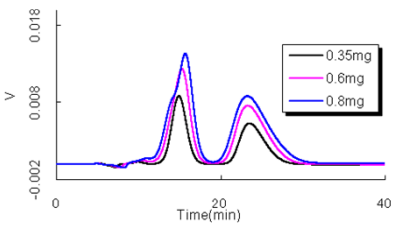
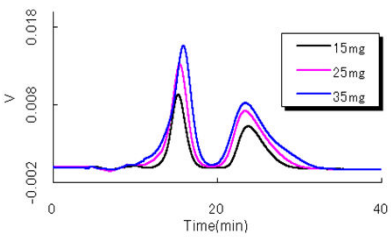
8-1. CHIRALFLASH® IA

	HPLC Analytical column (CHIRALPAK® IA)		CHIRALFLASH® IA
	dp=5μm 4.6mm ID × 150mmL	dp=20μm 4.6mm ID × 100mmL	dp=20μm 30mm ID × 100mmL
1) Hexobarbital  n-Hexane/2-Propanol = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 3g/L, ～1200μL/inj. (= **～3.6mg**) Analytical Data : k'1=2.1, k'2=3.9, α=1.9	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 3g/L, ～800μL/inj. (= **～2.4mg**) Analytical Data : k'1=2.5, k'2=5.1, α=2.0	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 3g/L, ～30mL/inj. (= **～90mg**) Analytical Data : k'1=2.5, k'2=5.0, α=2.0
2) Benzoin ethyl ether  n-Hexane/EtOAc = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 100g/L, ～120μL/inj. (= **～12mg**) Analytical Data : k'1=1.4, k'2=4.4, α=3.1	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 100g/L, ～100μL/inj. (= **～10mg**) Analytical Data : k'1=1.5, k'2=4.9, α=3.3	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 100g/L, ～4mL/inj. (= **～400mg**) Analytical Data : k'1=1.6, k'2=4.9, α=3.3
3) Flavanone  n-Hexane/EtOH = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 30g/L, ～140μL/inj. (= **～4.2mg**) Analytical Data : k'1=1.8, k'2=3.3, α=1.8	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 30g/L, ～100μL/inj. (= **～3.0mg**) Analytical Data : k'1=2.1, k'2=3.8, α=1.8	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 30g/L, ～100μL/inj. (= **～90mg**) Analytical Data : k'1=2.1, k'2=3.9, α=1.9

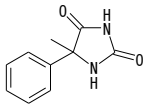
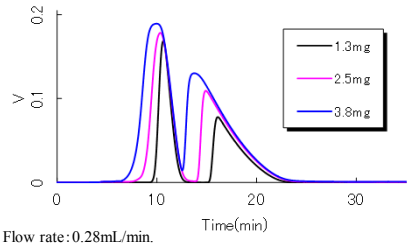
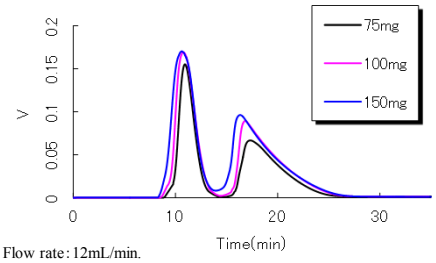
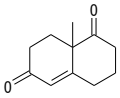
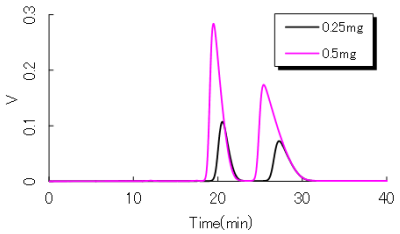
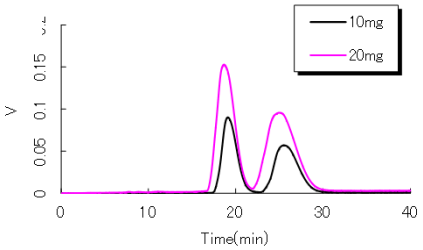
8-2. CHIRALFLASH® IC

	HPLC Analytical column (CHIRALPAK® IC)		CHIRALFLASH® IC
	dp=5μm 4.6mm ID × 150mmL	dp=20μm 4.6mm ID × 100mmL	dp=20μm 30mm ID × 100mmL
1) trans-Stilbene oxide  n-Hexane/2-Propanol = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 50g/L, ～160μL/inj. (= **～8.0mg**) Analytical Data : k'1=0.5, k'2=0.9, α=1.8	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 50g/L, ～160μL/inj. (= **～6.5mg**) Analytical Data : k'1=0.7, k'2=1.3, α=1.7	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 50g/L, ～5mL/inj. (= **～250mg**) Analytical Data : k'1=0.8, k'2=1.4, α=1.8
2) Tröger's-Base  n-Hexane/2-Propanol = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 20g/L, ～200μL/inj. (= **～4mg**) Analytical Data : k'1=0.9, k'2=1.3, α=1.5	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 20g/L, ～160μL/inj. (= **～3.2mg**) Analytical Data : k'1=1.4, k'2=1.9, α=1.4	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 20g/L, ～5mL/inj. (= **～100mg**) Analytical Data : k'1=1.4, k'2=1.9, α=1.4

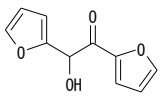
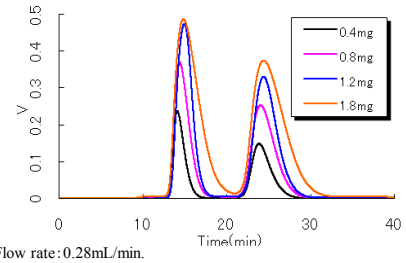
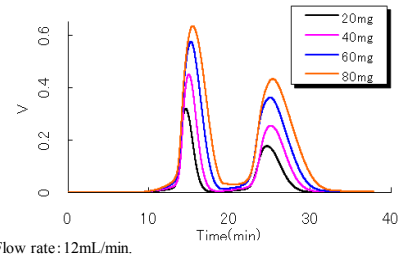
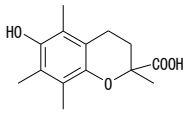
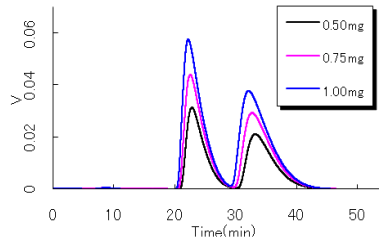
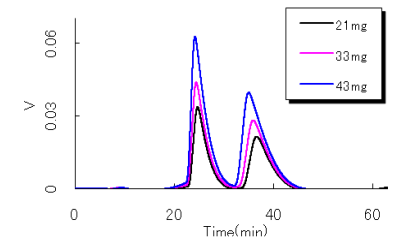
8-3. CHIRALFLASH® ID

	HPLC Analytical column (CHIRALPAK® ID) dp=20μm 4.6mm ID × 100mmL	CHIRALFLASH® ID dp=20μm 30mm ID × 100mmL
1) Praziquantel  EtOH = 100 vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 80g/L, ～160μL/inj. (= **～12.8mg**) Analytical Data : k'1=2.2, k'2=5.5, α=2.5	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 80g/L, ～7mL/inj. (= **～560mg**) Analytical Data : k'1=2.0, k'2=5.2, α=2.6
2) Hydroxyzine dihydrochloride  n-Hexane/2-Propanol/DEA = 80/20/0.1 vol/vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 5g/L, ～160μL/inj. (= **～0.8mg**) Analytical Data : k'1=2.3, k'2=4.1, α=1.8	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 5g/L, ～7mL/inj. (= **～35mg**) Analytical Data : k'1=2.4, k'2=4.6, α=1.9

8-4. CHIRALFLASH® IE

	HPLC Analytical column (CHIRALPAK® IE) dp=20μm 4.6mm ID × 100mmL	CHIRALFLASH® IE dp=20μm 30mm ID × 100mmL
1) 5-methyl-5-phenylhydantoin  n-Hexane/EtOH = 70/30 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV230nm Sample : 25g/L, ～150μL/inj. (= **～3.8mg**) Analytical Data : k'1=1.6, k'2=3.8, α=2.4	 Flow rate : 12mL/min. Temp : R.T. Detection : UV230nm Sample : 25g/L, ～6mL/inj. (= **～150mg**) Analytical Data : k'1=1.6, k'2=3.8, α=2.4
2) Wieland–Miescher ketone  n-Hexane/EtOH = 60/40 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV230nm Sample : 100g/L, ～5μL/inj. (= **～0.5mg**) Analytical Data : k'1=3.7, k'2=5.2, α=1.4	 Flow rate : 12mL/min. Temp : R.T. Detection : UV230nm Sample : 100g/L, ～0.2mL/inj. (= **～20mg**) Analytical Data : k'1=3.7, k'2=5.2, α=1.4

8-5. CHIRALFLASH® IF

	HPLC Analytical column (CHIRALPAK® IF) dp=20μm 4.6mm ID × 100mmL	CHIRALFLASH® IF dp=20μm 30mm ID × 100mmL
1) Furoin  n-Hexane/EtOH = 70/30 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV273nm Sample : 2g/L, ～900μL/inj. (= **～1.8mg**) Analytical Data : k'1=2.4, k'2=5.3, α=2.2	 Flow rate : 12mL/min. Temp : R.T. Detection : UV273nm Sample : 2g/L, ～40mL/inj. (= **～80mg**) Analytical Data : k'1=2.4, k'2=5.3, α=2.2
2) 6-Hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid  n-Hexane/Chloroform/AcOH = 40/60/0.1 vol/vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV290nm Sample : 2.5g/L, ～400μL/inj. (= **～1.0mg**) Analytical Data : k'1=4.7, k'2=8.0, α=1.7	 Flow rate : 12mL/min. Temp : R.T. Detection : UV290nm Sample : 2.5g/L, ～17mL/inj. (= **～43mg**) Analytical Data : k'1=4.7, k'2=8.0, α=1.7