Fast Method Screening for Separation of Enantiomers in HPLC and SFC Utilizing Novel Polysaccharides Type Chiral Stationary Phases Based on Small Particles

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Introduction

The role of enantioseparation is becoming more and more important especially in pharmaceutical industry. It is known that some enantiomers of racemic drugs show great differences in biological activities such as pharmacology, toxicology, pharmacokinetics, and metabolism. Nowadays, many single-enantiomer drugs are marketed, and the demand for determinations of enantiopurity and enatiopurifications are increasing.

The mechanism of chiral separation on liquid chromatography is very complicated, and the separation is made by complex combination of various interactions, such as hydrophobic, hydrogen bonding, dipole-dipole, and n-n. This makes method development of chiral separation difficult. Therefore, the column screening is commonly recognized as the first stage of separation method development. The fast column screening is the key driver for the rapid establishment of separation method.

Recently, we developed the chiral stationary phases (CSPs) consisting of polysaccharides derivatives immobilized on 3 µm silica particles. The new materials are ideal for fast method screening due to the high column efficiency across a wide range of flow rate.

In this poster, we will present some examples of fast method screening for separation of enantiomers utilizing the short columns packed with 3 µm immobilized CSP and various mobile phase conditions. We will also show the possibility of further reduction of method screening period by a combination of these columns and supercritical fluid chromatography (SFC).

Characterization of new immobilized polysaccharide chiral stationary phases

Product name	Base material	Particle size*1, 2 (µm)	Chiral selector		Usable pH range	Pressure limit	Usable organic solvent
CHIRAL ART Amylose-SA	Porous silica	3 5 10 20	Amylose tris (3,5-dimethylphenylcarbamate)	R: 3,5-Dimethylphenylcarbamate	2.0 – 9.0	4,350 psi (30 MPa)	*n*-Hexane *n*-Heptane Chloroform Dichloromethane *t*-Butyl methyl ether Ethyl acetate Tetrahydrofuran Alcohol Acetonitrile etc.
CHIRAL ART Cellulose-SB			Cellulose tris (3,5-dimethylphenylcarbamate)				
CHIRAL ART Cellulose-SC	Porous silica	3 5 10 20	Cellulose tris (3,5-dichlorophenylcarbamate)	R: 3,5-Dichlorophenylcarbamate			
CHIRAL ART Amylose-SE			Amylose tris (3,5-dichlorophenylcarbamate)				

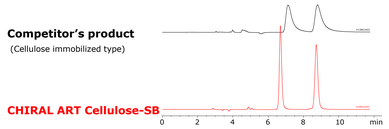

Amylose derivatives

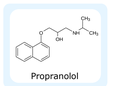

Cellulose derivatives

*1 3, 10, 20 µm particles of SC/SE will be available in the near future. *2 Please contact your local YMC subsidiary for larger particles than 20 µm.

- CHIRAL ART columns include four different types of immobilized polysaccharide CSPs based on high strength super-wide pore silica particles with 20, 10, 5 and 3 µm in diameter. The consistent retention and selectivity within the same chiral selector are obtained across particle sizes.
- CHIRAL ART immobilized type columns have excellent chiral recognition ability and high solvent versatility. The initial screening of these four columns with different selectivity and various mobile phase conditions can provide rapid method optimization for enantioseparation of a wide range of racemic compounds.
- Alcyon SFC CSP columns, which are specifically packed the same packing materials in a SFC compatible hardware, are also available. They would offer superior resolution and some advantages over HPLC under a SFC condition.

Comparison of peak shape of ionic compounds

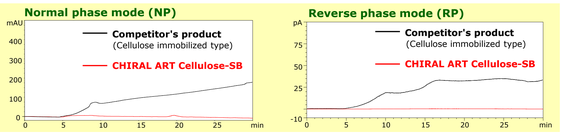



Propranolol

Column : 5 µm, 250 X 4.6 mm i. d.
Eluent : Hex/IPA/DEA (80/20/0.1)
Flow rate : 1.0 ml/min
Temperature : 25℃
Detection : UV at 230 nm
Injection : 10 µl (0.1 mg/ml)

- CHIRAL ART columns provide good peak shapes of ionic and metal coordination compounds.

Comparison of column bleeding



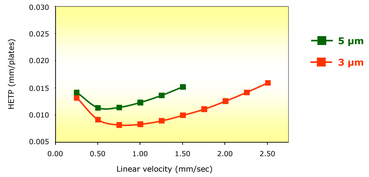
Gradient test
Column : 5 µm, 250 X 4.6 mm i. d.
Eluent : **A) Hex, B) EtOH**
2-80%B (0-30 min)
Flow rate : 1.0 ml/min
Temperature : 25℃
Detection : UV at 230 nm

Gradient test
Column : 5 µm, 250 X 4.6 mm i. d.
Eluent : **A) water, B) ACN**
10-100%B (0-30 min)
Flow rate : 1.0 ml/min
Temperature : 25℃
Detection : Corona* CAD*

- CHIRAL ART Cellulose-SB shows remarkably reduced background signal under the typical gradient conditions of both NP and RP mode.
- The low column bleeding can provide stable baseline and improved sensitivity even in a analysis using high-sensitivity detector such as Corona* charged aerosol detector or mass spectrometer (MS).

* Corona and CAD are registered trademarks of Thermo Fisher Scientific.

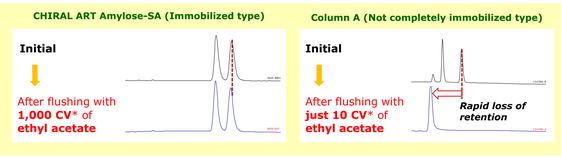
Relationship between column efficiency and linear velocity of 5 µm and 3 µm particles



Column : CHIRAL ART Cellulose-SB, 250 X 4.6 mm i. d.
Eluent : Hex/IPA(90/10)
Flow rate : 0.25, 0.50, 0.75, 1.0, 1.25, 1.50 ml/min for 5 µm
0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 ml/min for 3 µm
Temperature : 25℃
Detection : UV at 230 nm
Sample : *trans*-Stilbene oxide

- 3 µm particle shows higher efficiency over a wide range of flow rate compared to 5 µm particle.
- Fast analysis would be achieved by using a shorter length column packed with 3 µm particle and increasing flow rate.

Solvent resistance for various organic solvents



% change in selectivity (α) and capacity factor (k') of CHIRAL ART Amylose-SA after flushing with 1,000CV* of each solvent at 40℃

*CV = Column Volume

solvent	α	k'(2)
Ethyl acetate	100.3%	101.2%
Tetrahydrofuran	100.0%	100.0%
Dichloromethane	100.3%	100.6%
t-Butyl methyl ether	100.9%	98.8%

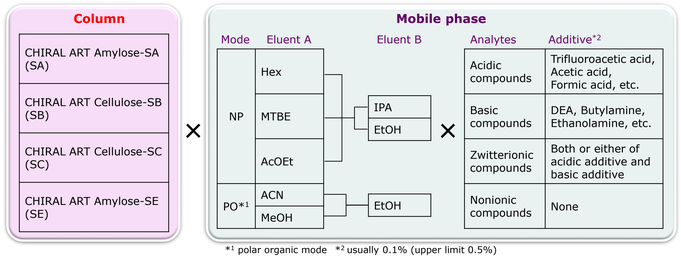
Test conditions of column performance
Eluent : Hex/IPA (95/5)
Flow rate : 1.0 ml/min
Temperature : 25℃
Sample : Benzoin

- On CHIRAL ART Amylose-SA, the change in column performance after 1,000 CV flushing with each solvent was less than 2%.
- CHIRAL ART immobilized type columns having high solvent versatility make it possible to choose the most suitable mobile phase by considering the solubility, resolution, and loadability of target compounds based on the purpose of separation.

Efficient approach for method screening and optimization of chiral separation in HPLC and SFC

Suggested screening protocol and experimental results for rapid HPLC method development

Screening protocol



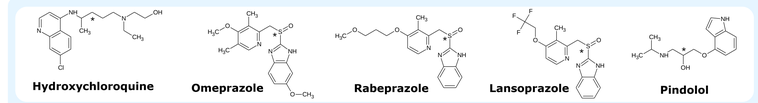
*1 polar organic mode *2 usually 0.1% (upper limit 0.5%)

HPLC conditions for screening

Column : **3 µm, 50 X 3.0 mm i. d.**
Flow rate : **0.85 ml/min**
Eluent : shown in left figure
Gradient : 5%B (0-0.5 min), 5-50%B (0.5-1.5 min), 50%B (1.5-2.0 min) for NP mode
0%B (0-0.5 min), 0-20%B (0.5-1.5 min), 20%B (1.5-2.0 min) for PO mode
Temperature : 25℃
Detection : UV at 265, 290, 334 nm
Injection : 2 µl (100 µg/ml)

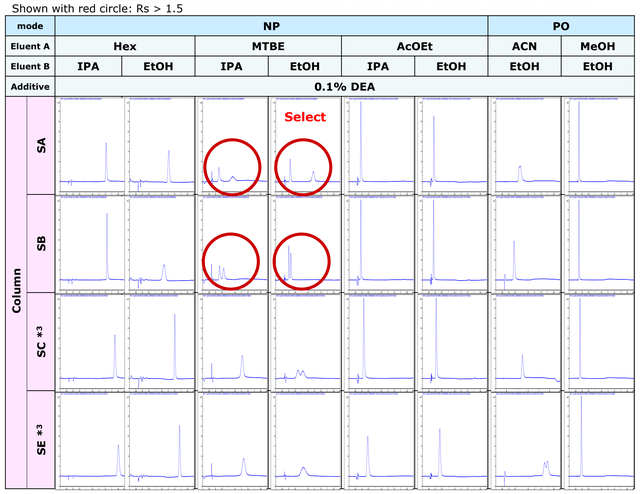
- The suggested initial screening protocol and conditions in chiral HPLC are shown in left. The combination of the short columns packed with four types of 3 µm immobilized CSP and the rapid gradient elution of eight types of Normal Phase (NP) and Polar Organic (PO) mobile phase are employed for separation method screening of pharmaceutical compounds below.

Compounds used in HPLC screening experiment



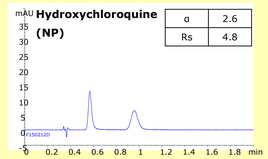
All chromatograms obtained from initial screening of hydroxychloroquine separation

Shown with red circle: Rs > 1.5

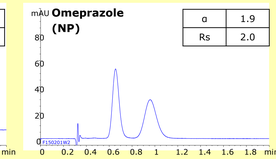


- The baseline resolution is achieved under four conditions in the initial screening for hydroxychloroquine as shown in above. The combination of SA phase and MTBE/EtOH containing 0.1% DEA is selected as the most favorable condition in consideration of retention and resolution.
- As shown in right chromatograms, the selected conditions from screening with gradient elution for each compound are transferred to the isocratic elution and optimized as the fast separation method within 2 minutes. The results of Omeprazole, Rabeprazole, and Lansoprazole indicate that structural similarity would not lead directly to similar separation behavior on chiral phases. The fast method development for racemic compounds is allowed through the initial screening process.

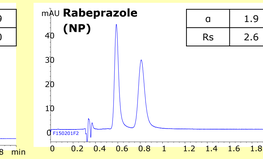
Separation results under simple isocratic conditions optimized through screening of each compound



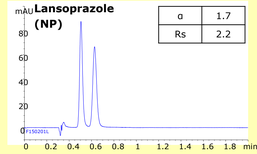
Column : CHIRAL ART Amylose-SA
3 µm, 50 X 3.0 mm i. d.
Eluent : A) MTBE/DEA (100/0.1)
B) EtOH/DEA (100/0.1)
10%B
Flow rate : 0.85 ml/min
Temperature : 25℃
Detection : UV at 344 nm
Injection : 2 µl (100 µg/ml)



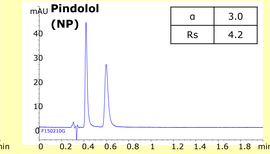
Column : CHIRAL ART Amylose-SE*3
3 µm, 50 X 3.0 mm i. d.
Eluent : A) AcOEt/DEA (100/0.1)
B) EtOH/DEA (100/0.1)
20%B
Flow rate : 0.85 ml/min
Temperature : 25℃
Detection : UV at 290 nm
Injection : 2 µl (100 µg/ml)



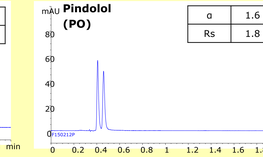
Column : CHIRAL ART Cellulose-SC*3
3 µm, 50 X 3.0 mm i. d.
Eluent : A) AcOEt/DEA (100/0.1)
B) EtOH/DEA (100/0.1)
5%B
Flow rate : 0.85 ml/min
Temperature : 25℃
Detection : UV at 290 nm
Injection : 2 µl (100 µg/ml)



Column : CHIRAL ART Amylose-SA
3 µm, 50 X 3.0 mm i. d.
Eluent : A) AcOEt/DEA (100/0.1)
B) EtOH/DEA (100/0.1)
5%B
Flow rate : 0.85 ml/min
Temperature : 25℃
Detection : UV at 290 nm
Injection : 2 µl (100 µg/ml)



Column : CHIRAL ART Cellulose-SB
3 µm, 50 X 3.0 mm i. d.
Eluent : A) Hex/DEA (100/0.1)
B) EtOH/DEA (100/0.1)
60%B
Flow rate : 0.85 ml/min
Temperature : 25℃
Detection : UV at 265 nm
Injection : 2 µl (100 µg/ml)

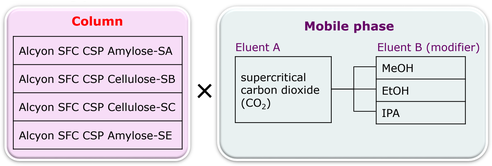


Column : CHIRAL ART Cellulose-SB
3 µm, 50 X 3.0 mm i. d.
Eluent : MeOH/DEA (100/0.1)
Flow rate : 0.85 ml/min
Temperature : 25℃
Detection : UV at 265 nm
Injection : 2 µl (100 µg/ml)

*3 The prototype columns of SC/SE were used in this experiment

Suggested screening protocol and experimental results for rapid SFC method development

Screening protocol

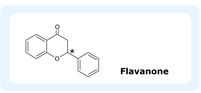


- The suggested screening protocol and conditions for chiral SFC using Alcyon SFC CSP immobilized type columns are shown in above. They sometimes offer advantages over HPLC in a resolution and reduction of method development. The low viscosity of supercritical carbon dioxide can allow use of longer column with higher efficiency at higher flow rate, and also it enables great reduction of organic solvent consumption.

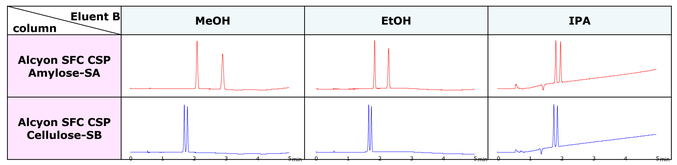
SFC conditions for screening

Column : **5 µm, 150 X 4.6 mm i. d.**
Flow rate : **3.0 ml/min**
Eluent : shown in left figure
Gradient : 10-50%B (0-5 min)
Temperature : 40℃
Detection : UV at 220 nm
Injection : 5 µL (100 µg/ml)
Back pressure : **2,000 psi (14.0 MPa)**

Compound used in SFC screening



Major results from initial screening of Flavanone separation



- The acceptable resolution of Flavanone enantiomers is achieved within a short time by Alcyon SFC CSP columns under all of the SFC screening conditions shown in above chromatograms, and especially the combinations of SA phase and mobile phase of CO_2/MeOH or CO_2/EtOH provide superior resolution.

Conclusions

- The excellent separation of various racemic compounds was achieved through HPLC screening utilizing the short columns packed with four different types of 3 µm immobilized CSPs and the rapid gradient elution of eight types of NP and PO mobile phase. The initial screening process led to develop rapidly a robust and simple method of enantiosepartion.
- The example of chiral SFC screening with these four CSPs and three different alcohols as mobile phase modifiers showed advantages in higher resolution and reduction of time for method development.

List of solvent abbreviations
Hex: *n*-Hexane, MTBE: *t*-Butyl methyl ether, AcOEt: Ethyl acetate, IPA: 2-Propanol, EtOH: Ethanol, MeOH: Methanol, ACN: Acetonitrile, DEA: Diethylamine