Development of a Novel Immobilized Type Polysaccharide Chiral Stationary Phase for Enantiomeric Separations

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Introduction

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

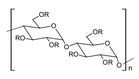
Silica gel coated and immobilized with polysaccharides are known to be useful for the separation of a wide range of racemic compounds. Among these 2 types, immobilized chiral stationary phase (CSP) columns are now common for measuring optical purity, and offer distinct advantages when analyzing and isolating chiral materials. We developed a novel immobilized type CSP (named "CHIRAL ART Cellulose-SJ"), consisting of cellulose tris(4-methylbenzoate) immobilized on silica particles.

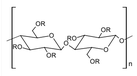
In this poster, we will show characteristics of this new CSP column, and some examples of efficient method screening for the separation of racemic compounds.

Characterization of new immobilized polysaccharide chiral stationary phase

Product lineup of immobilized type CSPs

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			Cellulose tris (3,5-dichlorophenylcarbamate)	R: 3,5-Dichlorophenylcarbamate			
CHIRAL ART Cellulose-SJ			Cellulose tris (4-methylbenzoate)	R: 4-Methylbenzoate			


Amylose derivatives

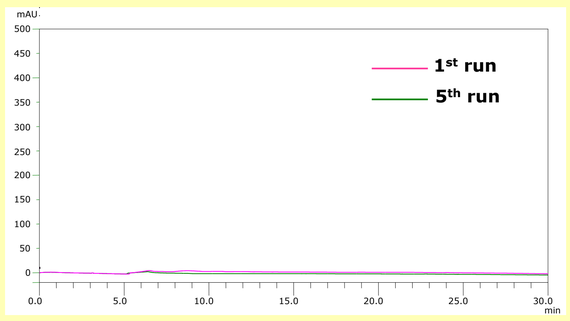

Cellulose derivatives

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

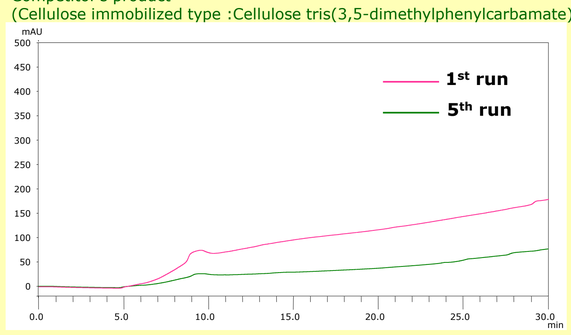
- CHIRAL ART columns include four different types of immobilized polysaccharide CSPs based on high strength super-wide pore silica with 20, 10, 5 and 3 µm particle size. Consistent retention and selectivity within the same chiral selector are obtained across particle sizes.
- CHIRAL ART immobilized type 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.

Comparison of column bleeding

CHIRAL ART Cellulose-SJ



Competitor's product
(Cellulose immolibized type :Cellulose tris(3,5-dimethylphenylcarbamate))



Gradient test

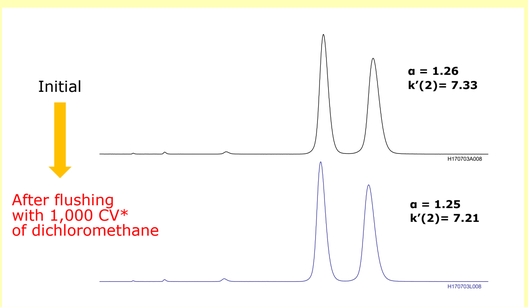
Column	: 5 µm, 250 X 4.6 mm i. d.
Eluent	: A) *n*-hexane, B) ethanol 2-80%B (0-30 min)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 230 nm

- CHIRAL ART Cellulose-SJ shows remarkably reduced background signal under typical gradient conditions.
- Low column bleeding can provide a stable baseline and improved sensitivity even in an analysis using a high-sensitivity detector such as Corona* charged aerosol detector or mass spectrometer (MS).

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

Durability with various organic solvents

CHIRAL ART Cellulose-SJ (Immobilized type)



*CV = column volume

Test conditions of column performance

Eluent	: *n*-hexane/2-propanol (95/5)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Sample	: Benzoin

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

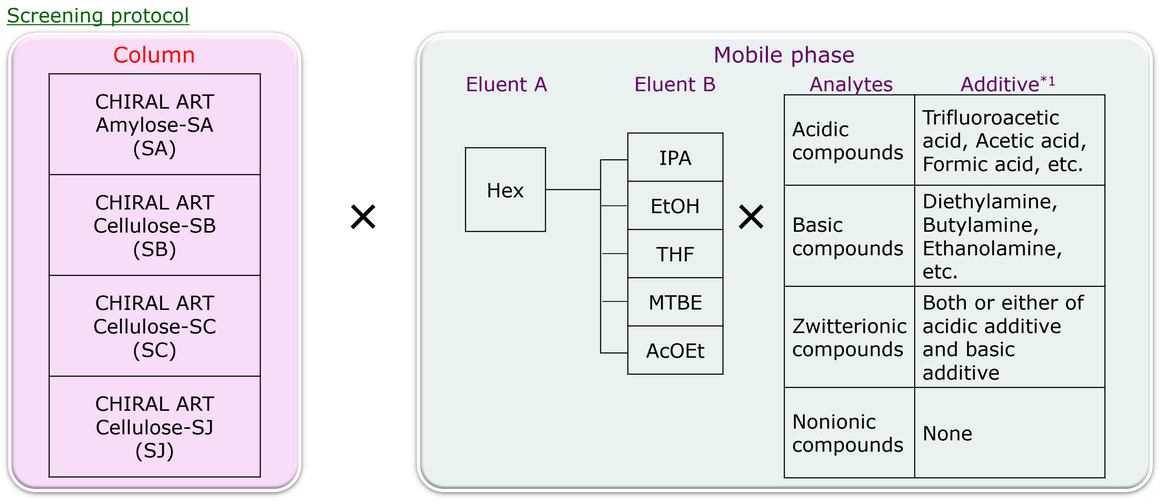
solvent	α	k'(2)
Ethyl acetate	99.3%	99.0%
Tetrahydrofuran	99.2%	99.7%
Dichloromethane	99.4%	98.4%
t-Butyl methyl ether	99.9%	101.9%

- On CHIRAL ART Cellulose-SJ, the change in column performance after 1,000 CV flushing with various solvents was less than 2%.
- Cellulose-SJ, having high solvent versatility, can be used in any mobile phase condition 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

Screening protocol and experimental results for rapid HPLC method development

Screening protocol

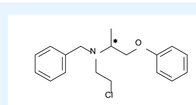


*1 usually 0.1% (upper limit 0.5%)

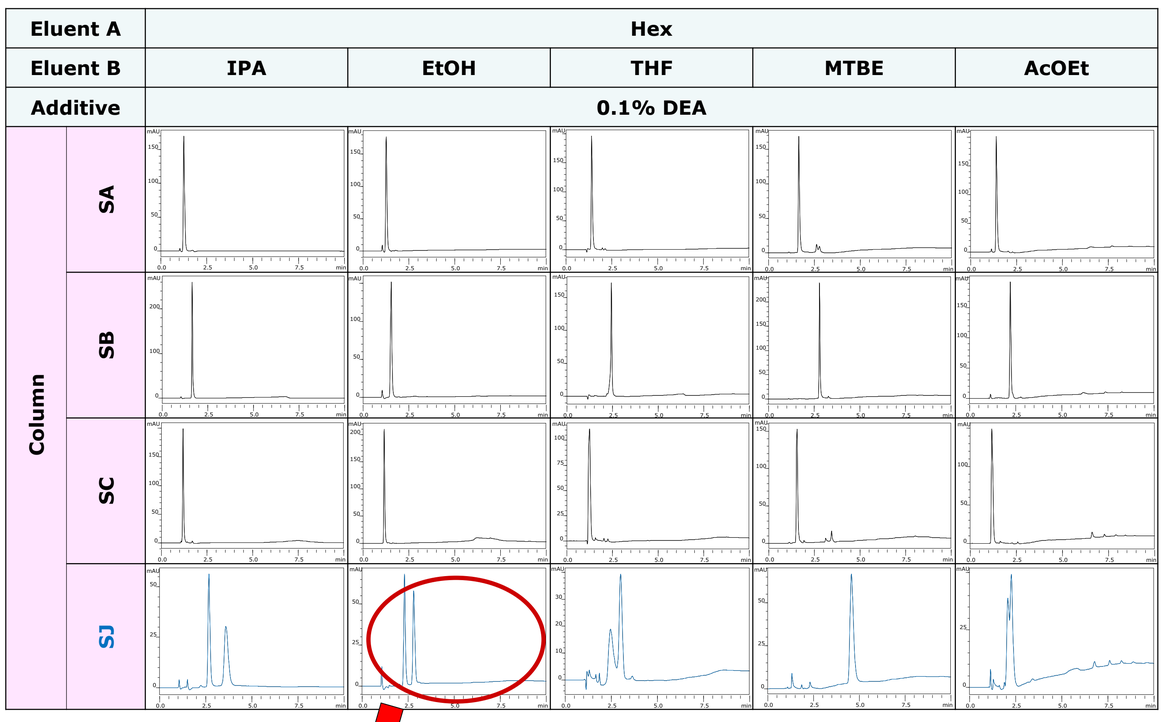
HPLC conditions for screening

Column	: 5 µm, 75 X 3.0 mm i. d. for SJ 3 µm, 75 X 3.0 mm i. d. for SA, SB, SC
Flow rate	: 0.425 ml/min
Eluent	: shown in below figure
Gradient	: 5-45%B (0-6 min), 45%B (6-10 min), 5%B (10-15 min)
Temperature	: 25℃
Detection	: UV at 270 nm
Injection	: 3 µl (0.5 mg/ml)

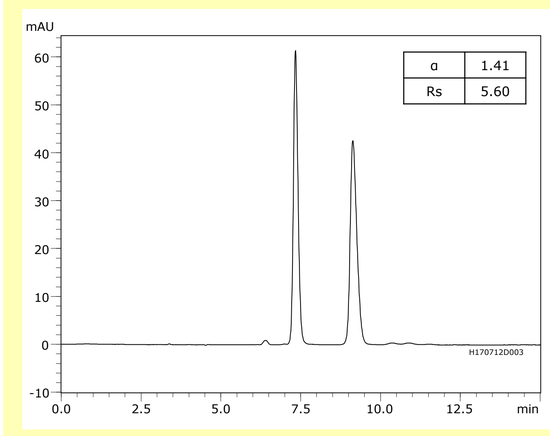
- The screening protocol and conditions in chiral HPLC are shown. The combination of short columns packed with four types of immobilized CSP and rapid gradient elution of five types of mobile phases are employed.


Phenoxybenzamine

All chromatograms obtained from screening of phenoxybenzamine



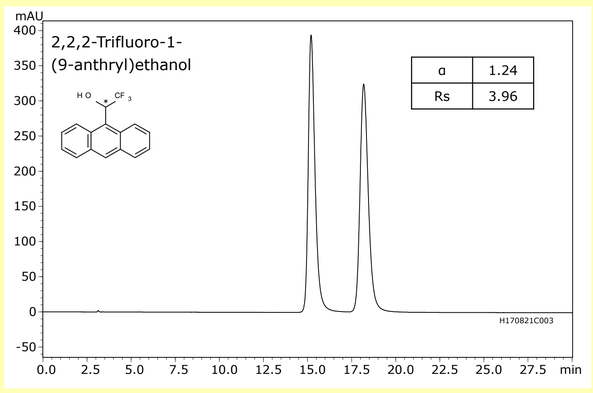
Optimization



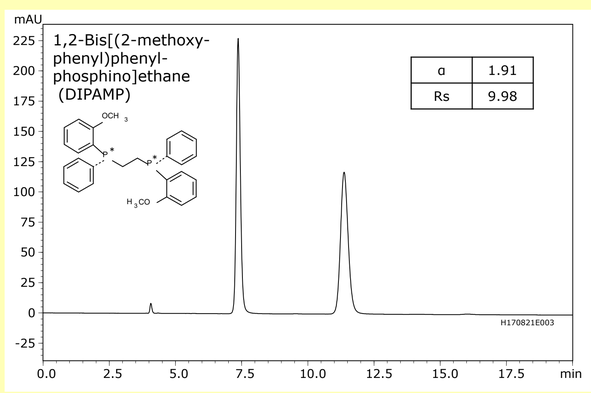
Column	: CHIRAL ART Cellulose-SJ 5 µm, 250 X 4.6 mm i. d.
Eluent	: *n*-hexane/ethanol/diethylamine (95/5/0.1)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 270 nm
Injection	: 5 µl (1 mg/ml)

- As a result of this screening process, baseline separation of phenoxybenzamine was not obtained on SA, SB and SC columns. This compound only separated on the Cellulose - SJ column, due to SJ having a featured ester linkage.
- The combination of SJ and Hex/EtOH containing 0.1% DEA was selected to be optimized in consideration of retention and resolution.

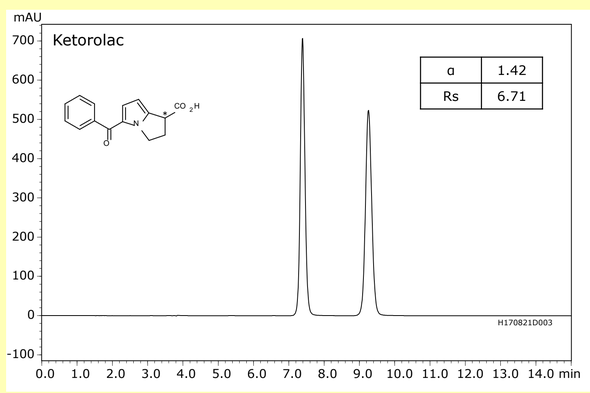
Applications of chiral separation using CHIRAL ART Cellulose-SJ



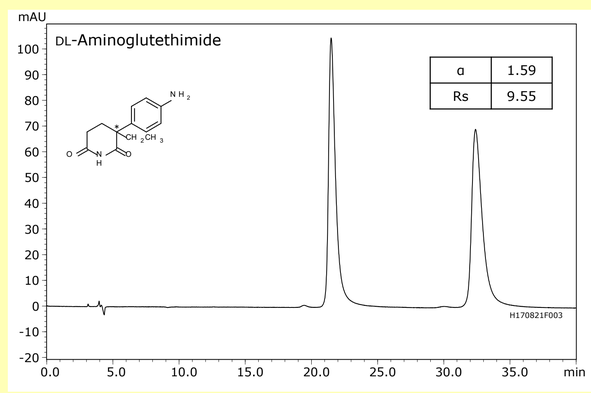
Column	: CHIRAL ART Cellulose-SJ 5 µm, 250 X 4.6 mm i. d.
Eluent	: *n*-hexane/tetrahydrofuran (90/10)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 254 nm
Injection	: 10 µl (0.1 mg/ml)



Column	: CHIRAL ART Cellulose-SJ 5 µm, 250 X 4.6 mm i. d.
Eluent	: *n*-hexane/chloroform (80/20)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 290 nm
Injection	: 5 µl (1 mg/ml)



Column	: CHIRAL ART Cellulose-SJ 5 µm, 250 X 4.6mm i. d.
Eluent	: *n*-hexane/tetrahydrofuran/TFA (70/30/0.1)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 305 nm
Injection	: 5 µl (1 mg/ml)



Column	: CHIRAL ART Cellulose-SJ 5 µm, 250 X 4.6 mm i. d.
Eluent	: *n*-hexane/ethyl acetate/DEA (70/30/0.1)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 254 nm
Injection	: 5 µl (1 mg/ml)

Conclusions

- CHIRAL ART Cellulose-SJ, having a featured ester linkage, leads to different separation characteristics in comparison with other conventional carbamate-linked polysaccharide columns.
- CHIRAL ART Cellulose-SJ exhibits excellent durability against various solvents, and provides extended flexibility for method development of enantioseparations.

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