Fast Method Screening for Separation of Enantiomers in HPLC and SFC Utilising 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 the pharmaceutical industry. It is known that some enantiomers of racemic drugs show great differences in biological activity including their pharmacology, toxicology, pharmacokinetics, and metabolism. With many single-enantiomer drugs being marketed, the demand for analytical quantitation and preparative separation is increasing.

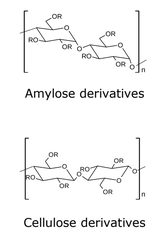
The mechanisms involved in chiral separation by liquid chromatography are very complex, and the separation are achieved by combinations of the various interactions, which include hydrophobic, hydrogen bonding, dipole-dipole, and π-π interactions. This makes method development for chiral separation more challenging. Column screening is essential as the first stage of any chiral method development and fast column screening is a pre-requiste for the rapid development of separation method.

Recently, YMC has developed chiral stationary phases (CSPs) consisting of derivatives of polysaccharides immobilised on 3 µm silica particles. These materials are ideal for fast method screening due to high column efficiency over a wide range of flow rates.

In this poster, we will present some examples of fast method screening for separation of enantiomers utilising short columns packed with 3 µm immobilised CSP and various mobile phase conditions. We will also show the potential of such columns for fast method screening using supercritical fluid chromatography (SFC).

Characterisation of new immobilised 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)				

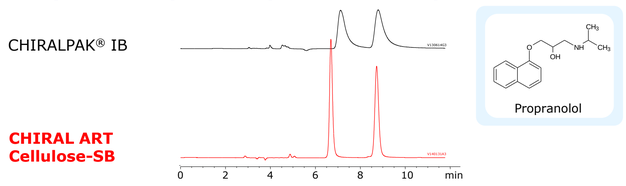


*1 CHIRAL ART Amylose-SE will be available in the near future. *2 Please contact your local YMC subsidiary for larger particles than 20 µm.

- CHIRAL ART columns are available, packed with four different types of immobilised polysaccharide CSP based on high strength super-wide pore silica particles having 20, 10, 5 or 3 µm in diameter. Consistent retention and selectivity parameters within the same chiral selector are obtained across particle sizes.
- CHIRAL ART immobilised chiral columns have excellent chiral recognition ability and high solvent versatility. Initial screening with these four columns, which have different selectivities, using various mobile phase compositions can provide rapid method optimization for enantioseparation of a wide range of racemic compounds.
- Alcyon SFC CSP columns, which are specifically packed with the same packing materials but in SFC compatible hardware, are also available. These offer superior resolution and certain advantages over HPLC under a SFC condition.

Characterisation of new immobilised polysaccharide chiral stationary phases

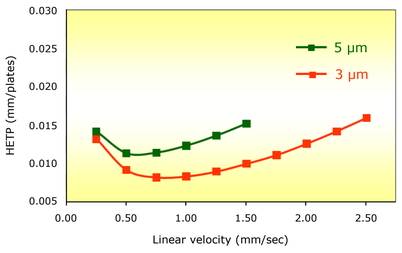
Comparison of peak shape of ionic compounds



Column	: 5 µm, 250 x 4.6 mm ID
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.

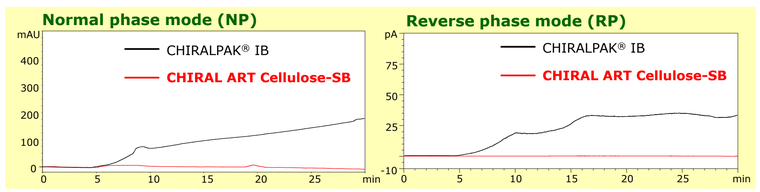
Relationship between column efficiency and linear velocity of 5 µm and 3 µm particles



Column	: CHIRAL ART Cellulose-SB, 250 x 4.6 mm ID
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 particles show higher efficiency over a wider range of flow rates compared to 5 µm particles.
- Fast analysis can be achieved by using a shorter length column packed with 3 µm particle and increasing flow rate.

Comparison of column bleeding



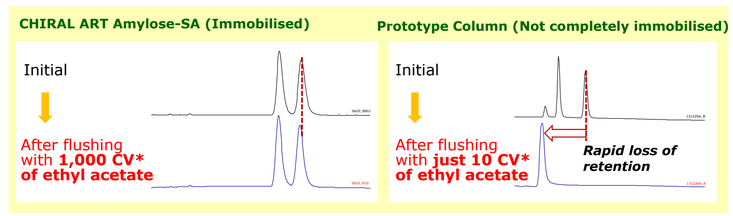
Gradient test	
Column	: 5 µm, 250 x 4.6 mm ID
Eluent	: A) Hex, B) EtOH 2-80%B (0-30 min)
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 230 nm

Gradient test	
Column	: 5 µm, 250 x 4.6 mm ID
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 an extremely low background signal under the typical gradient conditions of both NP and RP elution systems.
- This low column bleeding provides stable baselines and improved sensitivity even when analysing using high-sensitivity detectors such as a Corona* charged aerosol detector or mass spectrometer (MS).

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

Solvent resistance for various organic solvents



*CV = Column Volume

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

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

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

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

Suggested screening protocol and experimental results for rapid HPLC method development

Screening protocol

Column	Mode	Eluent A	Eluent B	Analytes	Additive*2
CHIRAL ART Amylose-SA (SA)	NP	Hex	IPA EtOH	Acidic compounds	Trifluoroacetic acid, acetic acid, formic acid, etc.
CHIRAL ART Cellulose-SB (SB)		MTBE		Basic compounds	DEA, butylamine, ethanolamine, etc.
CHIRAL ART Cellulose-SC (SC)		EtOAc		Zwitterionic compounds	Both or either of acidic and basic additive
CHIRAL ART Amylose-SE (SE)	PO*1	ACN MeOH	EtOH	Nonionic compounds	None

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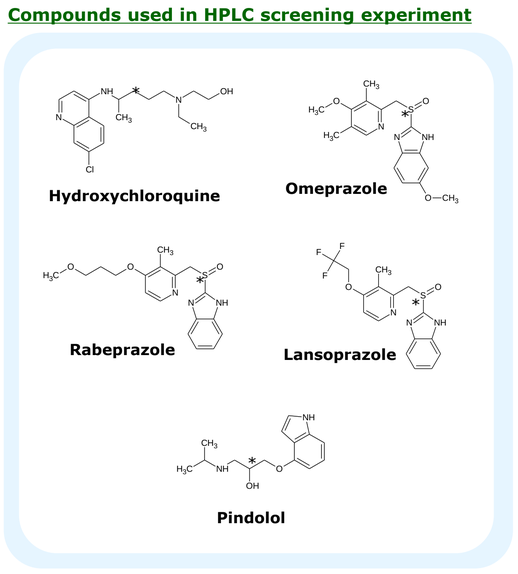
*1 polar organic mode *2 usually 0.1% (upper limit 0.5%)

HPLC conditions for screening

Column	: 3 µm, 50 x 3.0 mm ID
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 on the left. The combination of short columns packed with four types of 3 µm immobilised CSP and rapid gradient elution with 8 types of normal phase (NP) or polar organic (PO) mobile phases can be employed for chiral phase method screening for pharmaceutical compounds.

Compounds used in HPLC screening experiment



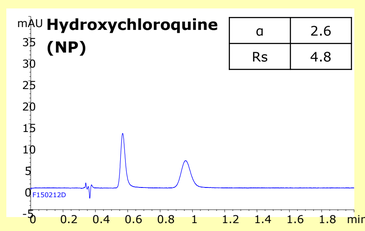
All chromatograms obtained from initial screening of hydroxychloroquine separation

mode	NP						PO	
Eluent A	Hex		MTBE		EtOAc		ACN	MeOH
Eluent B	IPA	EtOH	IPA	EtOH	IPA	EtOH	EtOH	EtOH
Additive	0.1% DEA							
Column SA				Select				
Column SB								
Column SC *3								
Column SE *3								

Shown with red circle: Rs > 1.5

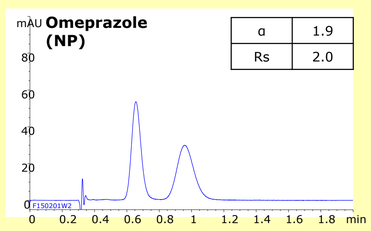
*3 Prototype columns of CHIRAL ART Amylose-SE were used in this experiment

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



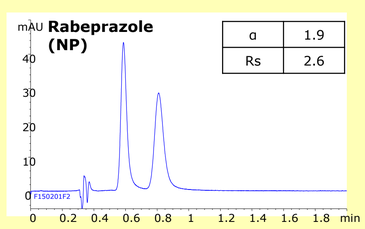
α	2.6
Rs	4.8

Column	: CHIRAL ART Amylose-SA 3 µm, 50 x 3.0 mm ID
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)



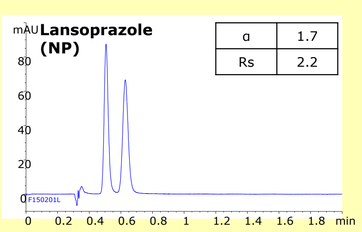
α	1.9
Rs	2.0

Column	: CHIRAL ART Amylose-SE*3 3 µm, 50 x 3.0 mm ID
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)



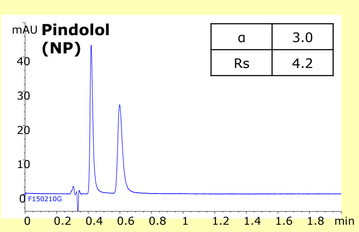
α	1.9
Rs	2.6

Column	: CHIRAL ART Cellulose-SC*3 3 µm, 50 x 3.0 mm ID
Eluent	: A) AcOEt/DEA (100/0.1) B) IPA/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)



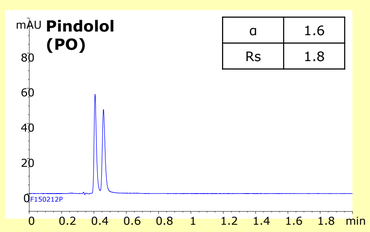
α	1.7
Rs	2.2

Column	: CHIRAL ART Amylose-SA 3 µm, 50 x 3.0 mm ID
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)



α	3.0
Rs	4.2

Column	: CHIRAL ART Cellulose-SB 3 µm, 50 x 3.0 mm ID
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)



α	1.6
Rs	1.8

Column	: CHIRAL ART Cellulose-SB 3 µm, 50 x 3.0 mm ID
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)

- Baseline resolution is achieved under four conditions in the initial screening of hydroxychloroquine as shown above. The combination of CHIRAL ART Amylose-SA phase and MTBE/EtOH containing 0.1% DEA was selected as the most favourable elution condition based on retention and resolution.
- As shown in the chromatograms on the right, the selected conditions from screening with gradient elution for each compound were transferred to isocratic elution and optimized for a fast separation method of less than 2 minutes. The results for Omeprazole, Rabeprazole, and Lansoprazole indicate that structural similarity would not necessarily lead to similar separation behaviours on chiral phases. The fast method development for racemic compounds resulted from the initial screening process.

Suggested screening protocol and experimental results for rapid SFC method development

Screening protocol

Column	Mobile phase	
	Eluent A	Eluent B (modifier)
Alcyon SFC CSP Amylose-SA	supercritical carbon dioxide (CO_2)	MeOH EtOH IPA
Alcyon SFC CSP Cellulose-SB		
Alcyon SFC CSP Cellulose-SC		
Alcyon SFC CSP Amylose-SE		

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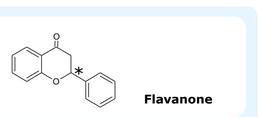
SFC conditions for screening

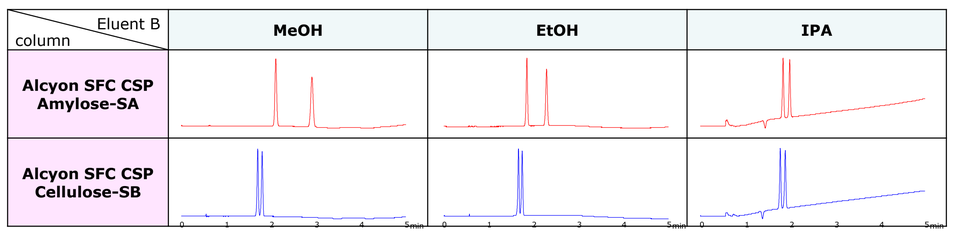
Column	: 5 µm, 150 x 4.6 mm ID
Flow rate	: 3.0 ml/min
Eluent	: shown in figure on left
Gradient	: 10-50%B (0-5 min)
Temperature	: 40℃
Detection	: UV at 220 nm
Injection	: 5 µL (100 µg/ml)
Back pressure	: 2,000 psi (14.0 MPa)

Compound used in SFC screening



- The suggested screening protocol and conditions for chiral SFC using Alcyon SFC CSP immobilised chiral columns are shown above. This can offer advantages over HPLC in terms of resolution and reduction of method development time. The low viscosity of supercritical carbon dioxide can allow use of longer columns with higher efficiency at higher flow rates. In addition it can significantly reduce organic solvent consumption.

Major results from initial screening of Flavanone separation



- The acceptable resolution of Flavanone enantiomers was achieved within a short run time using Alcyon SFC CSP columns under all of the SFC screening conditions shown in the above chromatograms. The combination of Alcyon SFC CSP Amylose-SA phase and mobile phases consisting of CO_2/MeOH or CO_2/EtOH proved to give superior resolution.

Conclusions

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

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