Highly efficient purification of enantiomers using polysaccharide type chiral stationary phases and continuous purification technology

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Introduction

The role of chiral separation is becoming more and more important especially in pharmaceutical industry, and the demand for isolating an enantiomer with high purity is increasing. However, there are two hurdles to be overcome; difficulty in method development and cost effectiveness of purification.

We have recently developed various chiral stationary phases coated/immobilized with polysaccharide derivatives. The coated phases give great resolution and the immobilized phases offer wide range of solvent compatibility. These phases greatly contribute to reduction of method screening period by combining column screening and mobile phase screening.

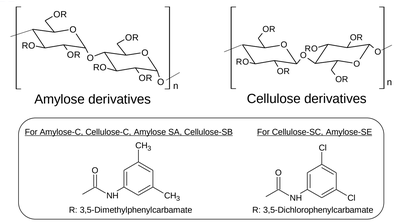
The separation method developed at analytical scale can be easily and linearly scaled up to purification from milligrams to kilograms process by using preparative scale column and LC-Forte/R preparation LC system. In addition, the efficacy of purification is improved by applying recycling preparative method of LC-Forte/R. This recycling method is also applicable to cases where ideal resolution is not achieved at method screening stage.

In this poster, we will show an example of method development including column and solvent screening at analytical scale, and then method transferring to purification scale. We will also estimate the purification efficacy of recycling LC method.

Specifications of new chiral stationary phases consisting of polysaccharide derivatives

Product name	Base material	Particle size* (µm)	Chiral selector	Type	Usable pH range	Pressure limit
CHIRAL ART Amylose-C	Porous silica	5, 10, 20	Amylose tris(3,5-dimethylphenylcarbamate)	Coated	-	4350 psi (30 MPa)
CHIRAL ART Cellulose-C			Cellulose tris(3,5-dimethylphenylcarbamate)			
CHIRAL ART Amylose-SA	Porous silica	3, 5, 10, 20	Amylose tris(3,5-dimethylphenylcarbamate)	Immobilized	2.0 – 9.0	4350 psi (30 MPa)
CHIRAL ART Cellulose-SB			Cellulose tris(3,5-dimethylphenylcarbamate)			
CHIRAL ART Cellulose-SC			Cellulose tris(3,5-dichlorophenylcarbamate)			
CHIRAL ART Amylose-SE			Amylose tris(3,5-dichlorophenylcarbamate)			

* SC and SE phases in 3, 10, and 20 µm will be available shortly.

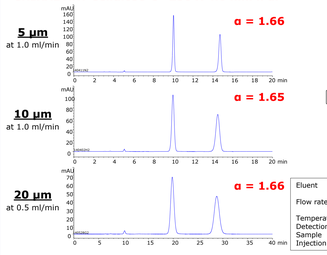


Advantages of CHIRAL ART on purification

- Superior selectivity and peak shape on enantioseparation of various chiral compounds
- Excellent mechanical stability
- High solvent compatibility and resistance (Immobilized type)
- Low bleeding from column
- Available in 3, 5, 10 and 20 µm that cover from analytical to preparative applications

Identical separation selectivity among particle sizes

CHIRAL ART Cellulose-C 250 X 4.6 mm i. d.

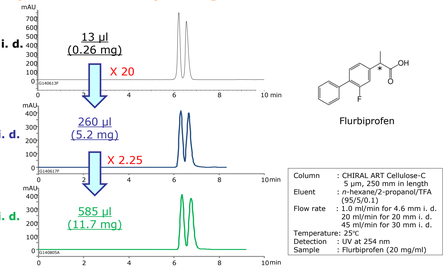


Benzoin

Eluent	: *n*-hexane/2-propanol (90/10)
Flow rate	: 1.0 ml/min (for 5, 10 µm) 0.5 ml/min (for 20 µm)
Temperature	: 25℃
Detection	: UV at 254 nm
Sample	: Benzoin
Injection	: 10 µl (0.1 mg/ml)

- The same selectivity among particle sizes provides linear and seamless scale up.

Same separation efficiency among column inner diameters



Flurbiprofen

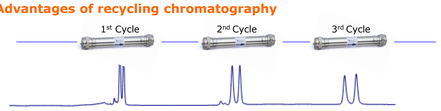
Column	: CHIRAL ART Cellulose-C 5 µm, 250 mm in length
Eluent	: *n*-hexane/2-propanol/TFA (95/5/0.1)
Flow rate	: 1.0 ml/min for 4.6 mm i. d. 20 ml/min for 20 mm i. d. 45 ml/min for 30 mm i. d.
Temperature	: 25℃
Detection	: UV at 254 nm
Sample	: Flurbiprofen (20 mg/ml)

- High quality and uniform packing for all column inner diameters enables predictable scale up.

Combination of CHIRAL ART and Recycling LC (LC-Forte/R) offers cost-effective and easy chiral purification

Usefulness of recycling HPLC method on semi-preparative purification

Advantages of recycling chromatography



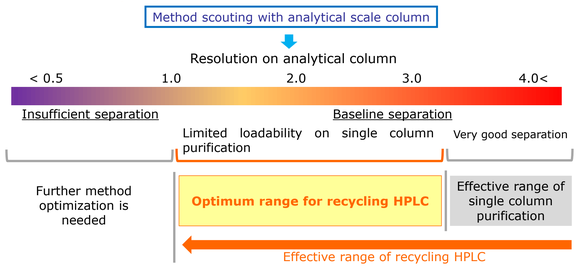
- In recycling HPLC, the sample flows through the same column repeatedly and it improves the resolution of compounds with similar retention characteristics, such as enantiomers, diastereomers, and positional isomers under isocratic condition.
- Another advantage of the recycling HPLC is that no fresh solvent is required in the recycling period and it is effective for cost reduction and for environmental conservation.

Features of LC-Forte/R system

- Preparative system compatible with both MPLC and HPLC in from milligram to gram scale purification
- All functions necessary for preparative chromatography (gradient mode for MPLC, recycling, auto replay, auto injection, stacking injection, and auto flushing)
- Compact body and easy operation

Multiple preparative HPLC
LC-Forte/R

The case where recycling HPLC is suitable



- Insufficient resolution can be often observed in a separation of structural similar compounds such as enantiomers. In such a case, recycling LC provides the same effect as using a longer column.
- Recycling purification is especially suitable or the case where resolution is between 1.0 and 3.5.

Cost-effective semi-preparative separation of racemic drug using recycle HPLC

Method screening and loadability study on analytical column

[STEP 1] <Method screening>

CHIRAL ART Columns
CHIRAL ART Cellulose-C
CHIRAL ART Cellulose-SB

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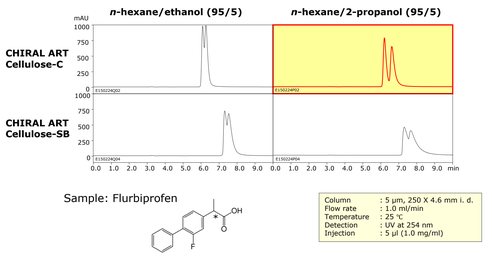
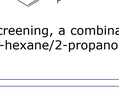
Mobile phases
n-hexane/ethanol (95/5)
n-hexane/2-propanol (95/5)



Sample: Flurbiprofen

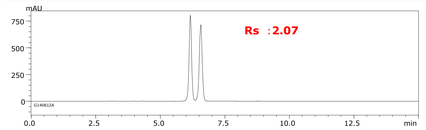


Column	: 5 µm, 250 X 4.6 mm i. d.
Flow rate	: 1.0 ml/min
Temperature	: 25 ℃
Detection	: UV at 254 nm
Injection	: 5 µl (1.0 mg/ml)

- On this method screening, a combination of CHIRAL ART Cellulose-C and mobile phase of *n*-hexane/2-propanol mixture showed higher resolution.

[STEP 2] <Method optimization>

- Additive to mobile phase
- Flow rate
- Column temperature
- Injection volume

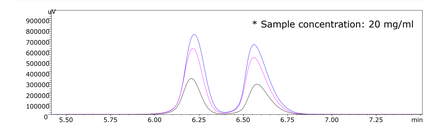


Final conditions	
Column	: **CHIRAL ART Cellulose-C 5 µm, 250 X 4.6 mm i. d.**
Eluent	: ***n*-hexane/2-propanol/TFA (95/5/0.1)**
Flow rate	: 1.0 ml/min
Temperature	: 25℃
Detection	: UV at 254 nm
Injection	: 5 µl (1.0 mg/ml)

- Baseline separation was achieved by adding trifluoroacetic acid (TFA) in the mobile phase.
- For acidic compounds, it is sometimes effective to add acidic additives such as TFA, acetic acid or formic acid in mobile phase for improving resolution.
- For basic compounds, basic additives such as diethylamine, butylamine or ethanolamine are effective.

[STEP 3] <Loadability study>

- Enantiomeric purity
- Sample solubility
- Recovery



Loading (mg)	Resolution (Rs)
0.1	1.80
0.2	1.61
0.26	**1.53**

Maximum loading amount = 0.26 mg

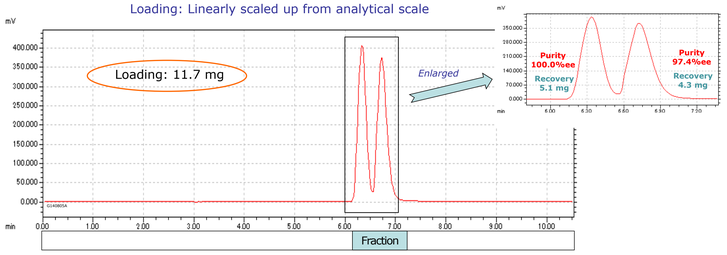
- Maximum loading amount was determined based on the enantiomeric purity and recovery, and found to be 0.26 mg per 250 X 4.6 mm i. d. column.

Scaling up to 250 X 30 mm i. d. column

Comparison of productivity and solvent consumption between single column purification and recycling chromatography

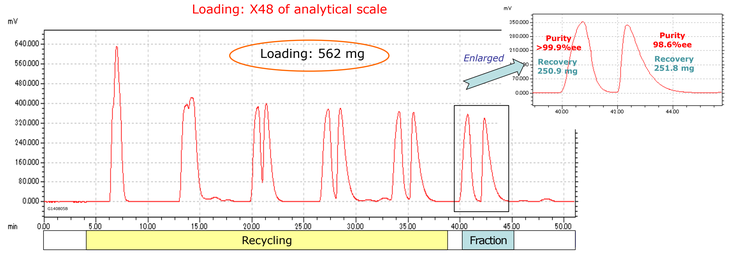
Single column purification method

Loading: Linearly scaled up from analytical scale



Recycling chromatography method

Loading: X48 of analytical scale



		Single column*		Recycling	
		1st peak	2nd peak	1st peak	2nd peak
Purity	(%ee)	100.0	97.4	>99.9	98.6
Recovery rate	(%)	87.2	73.5	89.3	89.6
Productivity	(mg product/hr)	122.4	103.2	334.5	335.7
Solvent consumption	(l solvent/g product)	22.1	26.2	2.0	2.0

* Calculated based on the injection every 2.5 minutes.

Column	: 5 µm, 250 X 30 mm i. d.
Eluent	: *n*-hexane/2-propanol/TFA (95/5/0.1)
Flow rate	: 45 ml/min
Detection	: UV at 280 nm
Sample	: Flurbiprofen (20 mg/ml)

- On the recycling method, the productivity per hour of each enantiomer was increased up to three times, and the solvent consumption was reduced by 90%, compared to the single column purification.
- The combination of CHIRAL ART semi-preparative columns and recycling separation with LC-Forte/R is a powerful tool for improving resolution and for reducing the cost of purification.

Conclusions

- Full scalability over particle sizes and column dimensions of CHIRAL ART columns is ideal for from analytical to preparative chiral separation.
- Recycling chromatography offers three times or more productivity compared to single column purification. Further to that, it offers “greener” separation by suppressing solvent consumption.
- Combination of CHIRAL ART and recycling chromatography is the fastest way to obtain an enantiomerically pure compound at a desired quantity.