Development of an Improved Amylose-based Chiral Stationary Phase with Excellent Preparative Performance

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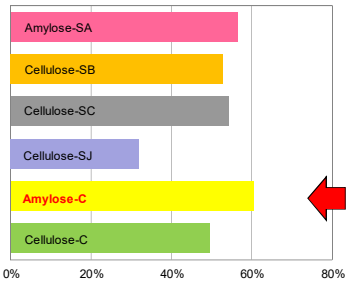
Introduction

Preparative chiral chromatography plays an increasingly important role in the pharmaceutical, fine chemicals, and food industries. Polysaccharide-based chiral stationary phases have been the most commonly applied stationary phases for prep-scale chiral chromatography. YMC has commercially produced 6 types of chiral stationary phases based on coated or immobilized 3, 5, 10 and 20 µm silica particles. These phases exhibit excellent chiral separation performance for a wide range of racemic compounds. Among them, amylose tris(3,5-dimethylphenylcarbamate) coated on silica particles (CHIRAL ART Amylose-C) was the most commonly used chiral stationary phase for our HPLC/SFC contract purification service.

Recently we succeeded at improving both the HPLC and SFC performance of our amylose tris(3,5-dimethylphenylcarbamate) coated phase by developing a new manufacturing process. This new phase (CHIRAL ART Amylose-C Neo) is the improved version of CHIRAL ART Amylose-C, and exhibits increased resolution (Rs) on separations of many racemic compounds, compared to CHIRAL ART Amylose-C as well as other conventional products. Additionally, CHIRAL ART Amylose-C Neo is expected to give better peak shape under high loading, resulting in excellent preparative performance.

In this poster, we present the improved preparative performance of CHIRAL ART Amylose-C Neo through various examples.

Hit ratio of chiral stationary phases for various compounds



- Hit criteria: Rs>1.5
- Database: Based on screening result of *ca.* 520 samples at our HPLC/SFC contract purification service

This work

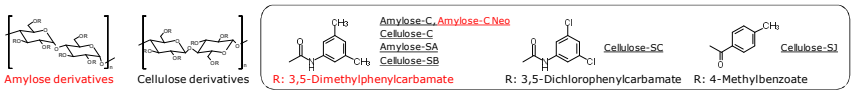
Aimed to improve of preparative performance of Amylose-C

- The 6 available CPSs can cover >90% of chiral separation of racemates
- Amylose-C scored the highest "hit ratio" and was the most commonly used CSP for our HPLC/SFC purification service.

Product lineup of 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	3 5 10 20	Amylose tris (3,5-dimethylphenylcarbamate)	Coated	–	4350 psi (30 MPa)
"New" CHIRAL ART Amylose-C Neo						
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 Cellulose-SJ *			Cellulose tris(4-methylbenzoate)			

* 10 and 20 µm particles of CHIRAL ART Cellulose-SJ will be available in the future.



- Excellent mechanical stability based on high strength super-wide pore silica gel
- Available in 3, 5, 10 and 20 µm - covering analytical to preparative applications
- Effective for cost reduction of analytical to preparative chiral separations

Comparison of chiral separation selectivity of a wide variety of racemic compounds

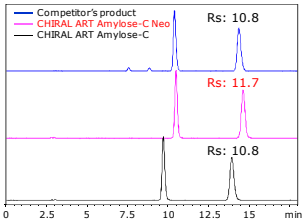
Compounds	Eluent	Separation factor (α)				
		Coated type			Immobilized type	
		Amylose-C Neo	Amylose-C	Competitor's Product	Amylose-SA	Competitor's Product
trans-Stilbene oxide	Hex/IPA (90/10)	3.2 ↑	2.9	3.0	2.7	2.8
	CO_2/MeOH (80/20)	2.0 ↑	1.8	1.9	1.2	1.2
Benzoin	Hex/IPA (90/10)	1.4 ↑	1.3	1.3	1.2	1.2
N-CBZ-DL-Alanine	Hex/IPA/TFA (80/20/0.1)	2.2 ↑	2.0	2.2	1.7	1.7
Ibuprofen	Hex/IPA/TFA (99/1/0.1)	1.1	1.1	1.1	1.1	1.1
Propranolol	Hex/IPA/DEA (80/20/0.1)	×	×	×	×	×
Verapamil	Hex/IPA/DEA (90/10/0.1)	1.3	1.3	1.3	1.2	1.2

Preparative performance of new amylose-based chiral stationary phase; Amylose-C Neo

Ex) Flavanone

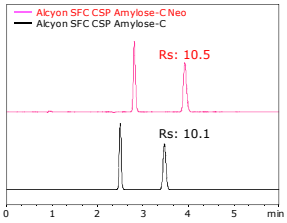
<Analytical HPLC condition>

Column	: 5 µm, 250 x 4.6 mm i.d.
Eluent	: Hex/EtOH (90/10)
Flow rate	: 1.0 ml/min
Detection	: UV at 254 nm
Temperature	: 25°C
Injection	: 10 µl (0.1 mg/ml)
System	: NexeraXR (Shimadzu)



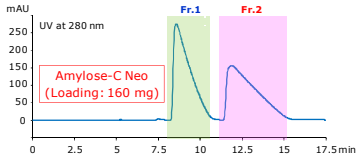
<Analytical SFC condition>

Column	: 5 µm, 250 x 4.6 mm i.d.
Eluent	: CO_2/EtOH (80/20)
Flow rate	: 3.0 ml/min
Detection	: UV at 220 nm
Back pressure	: 13.8 MPa (2,000 psi)
Temperature	: 35°C
Injection	: 5 µl (1 mg/ml)
System	: ACQUITY UPC² (Waters)



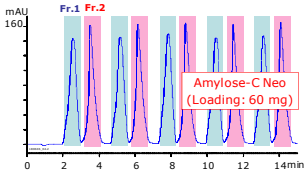
<Preparative HPLC condition>

Column	: 5 µm, 250 x 20 mm i.d.
Eluent	: Hex/EtOH (90/10)
Flow rate	: 20 ml/min
Detection	: UV
Temperature	: ambient

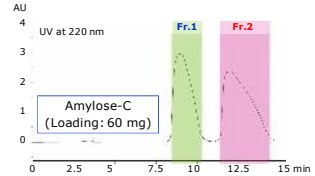


<Preparative SFC condition>

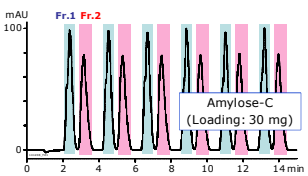
Column	: 5 µm, 250 x 20 mm i.d.
Eluent	: CO_2/EtOH (80/20)
Flow rate	: 60 ml/min
Detection	: UV at 280 nm
Back pressure	: 15 MPa (2,175 psi)
Temperature	: 30°C



- CHIRAL ART Amylose-C Neo exhibited improved peak shape under high loading compared to Amylose-C. This enabled higher loading.



- Even in SFC, the Alcyon SFC Amylose-C Neo column showed greater loadability than Alcyon SFC Amylose-C. It was suggested that the combination of SFC and Alcyon SFC Amylose-C Neo improved separation efficiency by 3.5 times compared to the combination of HPLC and Amylose C.

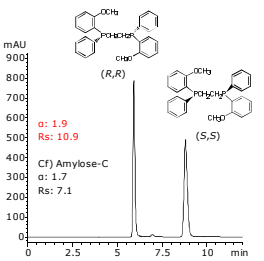


	HPLC				SFC			
	Amylose-C Neo		Amylose-C		Amylose-C Neo		Amylose-C	
	Fr. 1	Fr. 2	Fr. 1	Fr.2	Fr. 1	Fr. 2	Fr. 1	Fr. 2
Enantiomeric purity (%ee)	**>99.9**	**>99.9**	>99.9	99.7	**99.9**	**99.7**	>99.9	99.8
Yield (%)	**94.2**	**99.4**	95.7	93.7	**95.7**	**>99.9**	94.5	95.6
Productivity* (mg-product/hr)	**464**	**490** ← x 2.9	172	169	**595**	**650** ← x 1.8	340	344
Fractionated liquid volume (L-solvent/g-product)	**0.34**	**0.54**	1.15	2.88	**0.18**	**0.26**	0.39	0.57

* Injection intervals; [Amylose-C Neo] SFC: 2.7 min, HPLC: 9.0 min, [Amylose-C] SFC: 2.5 min, HPLC: 9.0 min.

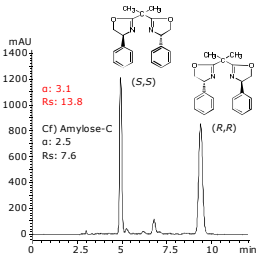
Applications of Amylose-C Neo

1,2-Bis[(2-methoxyphenyl)phenylphosphino]ethane



Column : 5 µm, 250 x 4.6 mm i.d.
Eluent : Hex/IPA (90/10)
Flow rate : 1.0 ml/min
Temperature : 25°C
Detection : UV at 285 nm
Injection : 10 µl (0.5 mg/ml)

2,2'-Isopropylidenebis(4-phenyl-2-oxazoline)



Column : 5 µm, 250 x 4.6 mm i.d.
Eluent : Hex/IPA (70/30)
Flow rate : 1.0 ml/min
Temperature : 25°C
Detection : UV at 210 nm
Injection : 10 µl (0.5 mg/ml)

- CHIRAL ART Amylose-C Neo showed enhanced resolution (Rs) on many racemic compounds compared to CHIRAL ART Amylose-C.

Conclusions

- **The new amylose-based chiral stationary phase (Amylose-C Neo) is an upgraded model of Amylose-C with enhanced resolution in both HPLC and SFC.**
- **It was suggested that Amylose-C Neo could show 2-3 times higher loadability than Amylose-C. This suggests that Amylose-C Neo could improve productivity per unit time by 2-3 times.**

List of solvent abbreviations Hex: *n*-Hexane, IPA: 2-Propanol, EtOH: Ethanol, MeOH: Methanol, DEA: Diethylamine, TFA: Trifluoroacetic acid