

Introduction

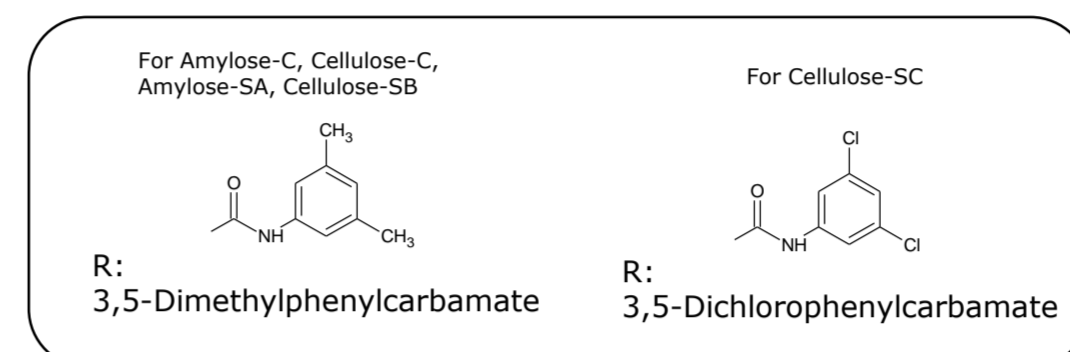
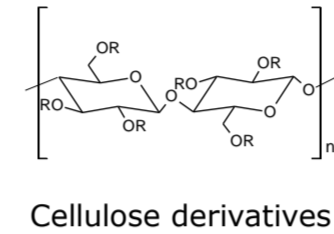
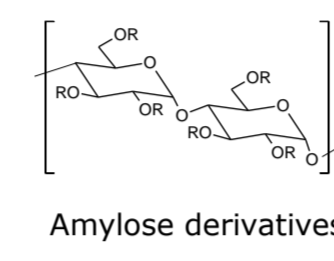
The role of enantioseparations is becoming more and more important especially in the pharmaceutical industry. It is known that some enantiomers of racemic drugs show great differences in biological activities such as pharmacology, toxicology, pharmacokinetics and metabolism. Nowadays, many single-enantiomer drugs are marketed, and the demand for determinations of enantiopurity and enantiopurifications is increasing. The mechanism of chiral separations in liquid chromatography is very complicated, and the separation is made by a complex combination of various interactions, including hydrophobic interactions, hydrogen bonding, dipole-dipole and π - π interactions. This makes method development for chiral separations difficult. Therefore, method development is commonly recognized as the rate-determining stage in the whole optical resolution process. To shorten the time for column screening is the key driver for the rapid establishment of any separation method.

Recently, we developed chiral stationary phases (CSPs) consisting of polysaccharides derivatives coated or immobilised on 3 μ m silica particles. These new materials are ideal for fast method screening due to their high column efficiency across a wide range of flowrates. Moreover, they show identical separation selectivity to the conventional materials based on 5, 10 and 20 μ m particle sizes. This feature enables predictable method transfer from 3 μ m ultrafast separation methods to a 5 μ m conventional method, and even to a 10 or 20 μ m preparative method.

In this poster, we will present a fast method screening for chiral separations in supercritical fluid chromatography (SFC) utilizing 3 μ m chiral columns. Also, we will show a specific example of a scale-up for preparative optical resolution.

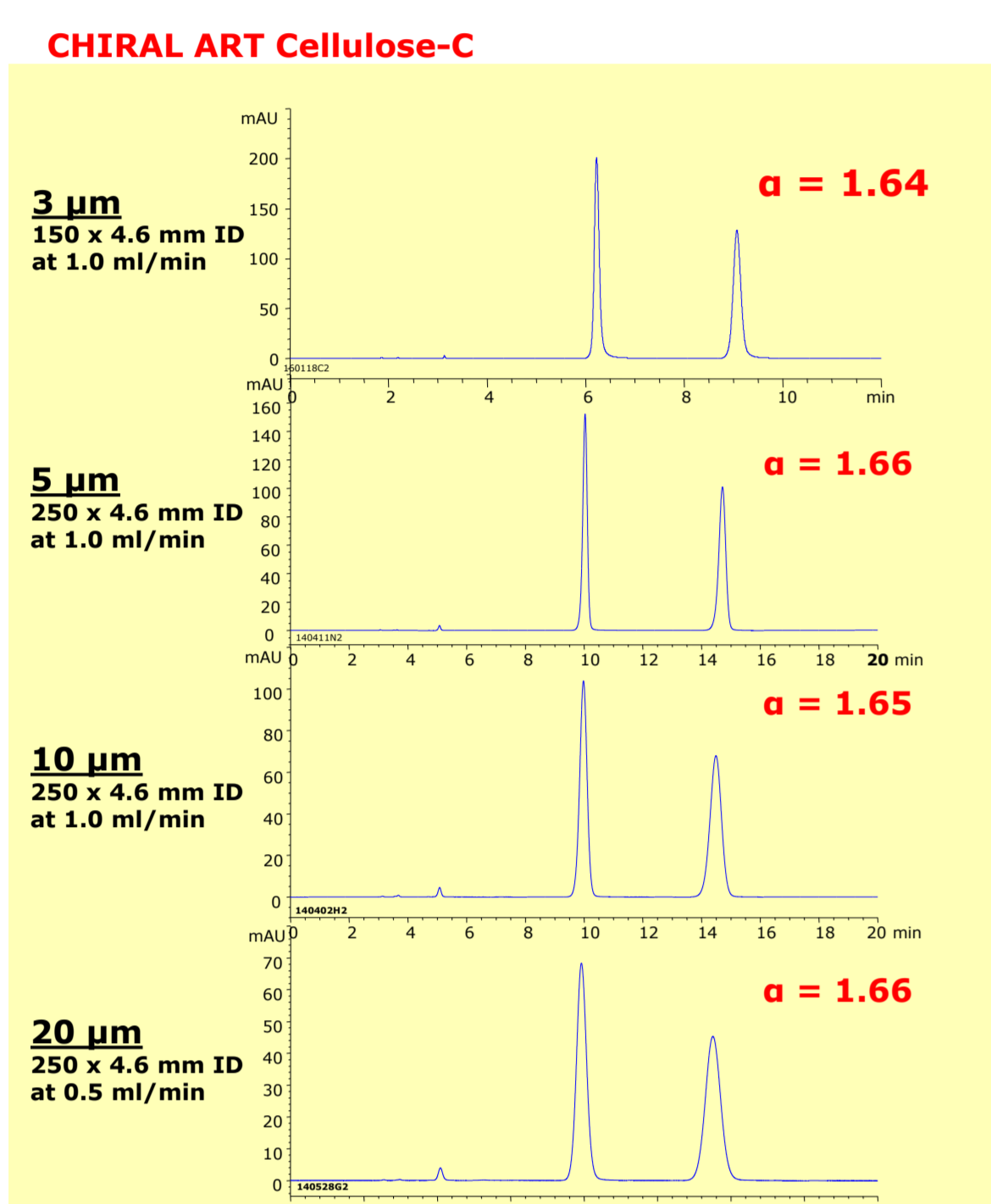
Characterisation of Coated/Immobilised Polysaccharide Chiral Stationary Phases

Type	Product name	Base material	Particle size (μ m)	Chiral selector	Usable pH range	Pressure limit
Coated type	CHIRAL ART Amylose-C	Porous silica	3, 5, 10, 20	Amylose tris (3,5-dimethylphenylcarbamate)	-	4,350 psi (30 MPa)
	CHIRAL ART Cellulose-C			Cellulose tris (3,5-dimethylphenylcarbamate)		
Immobilised type	CHIRAL ART Amylose-SA	Porous silica	3, 5, 10, 20	Amylose tris (3,5-dimethylphenylcarbamate)	2.0 - 9.0	4,350 psi (30 MPa)
	CHIRAL ART Cellulose-SB			Cellulose tris (3,5-dimethylphenylcarbamate)		
	CHIRAL ART Cellulose-SC			Cellulose tris (3,5-dichlorophenylcarbamate)		

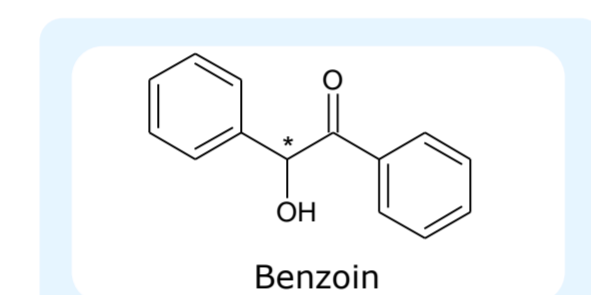


- CHIRAL ART columns are based on high strength super-pore silica particles with 20, 10, 5 and 3 μ m diameters. The consistent retention and selectivity within the same chiral selector are obtained across particle sizes.
- Alcyon SFC CSP columns, specifically packed in SFC compatible hardware with the same packing materials used for HPLC columns, are also available.

Comparison of Selectivity Among Various Particle Sizes

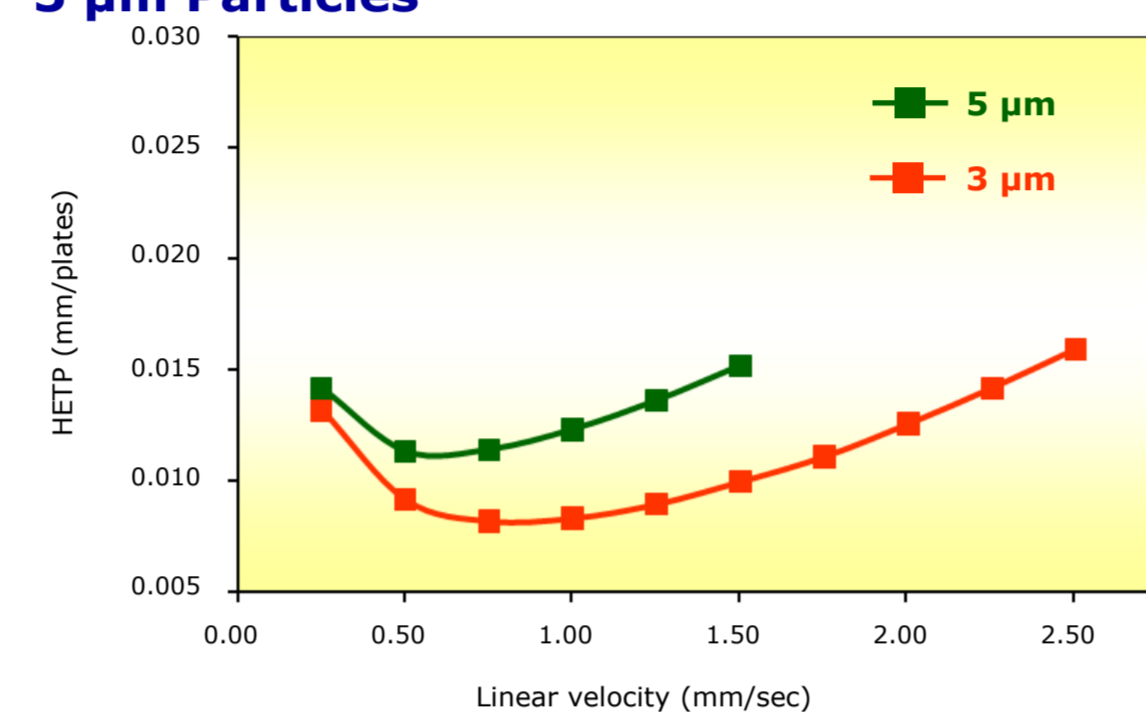


- CHIRAL ART columns shows identical α -values and retention times for all the different particle sizes.
- Identical selectivity allows predictable scale-up from 3 μ m ultrafast methods to 5 μ m conventional methods, and even to 10 or 20 μ m preparative methods.



Eluent : Hex/IPA (90/10)
 Flow rate : 1.0 ml/min (for 3, 5, 10 μ m)
 0.5 ml/min (for 20 μ m)
 Temperature : 25°C
 Detection : UV at 254 nm
 Sample : Benzoin
 Injection : 10 μ l (0.1 mg/ml)

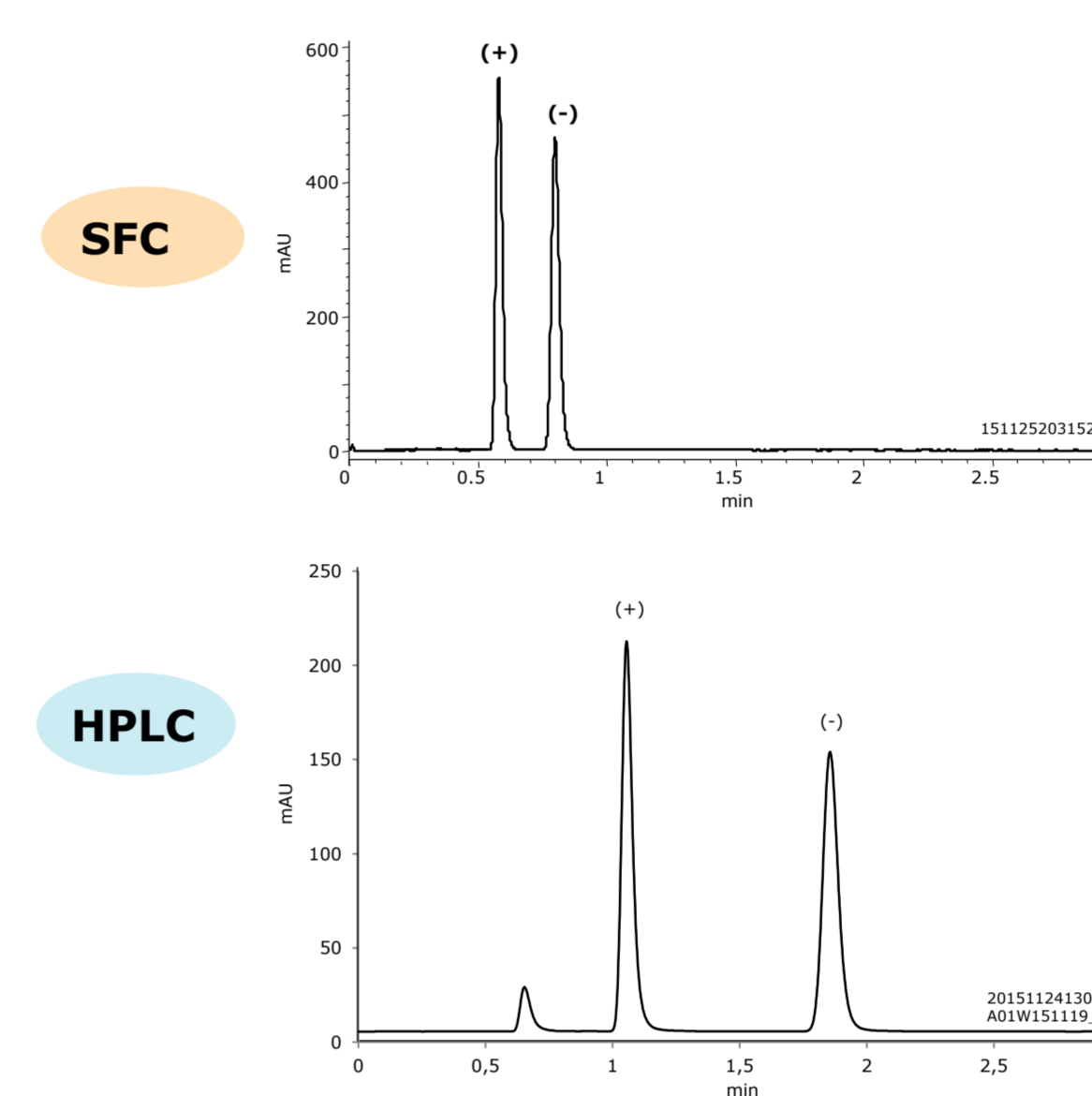
Comparison of Column Efficiency of 5 μ m and 3 μ m Particles



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 μ m
 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 ml/min for 3 μ m
 Temperature : 25°C
 Detection : UV at 230 nm
 Sample : trans-Stilbene oxide

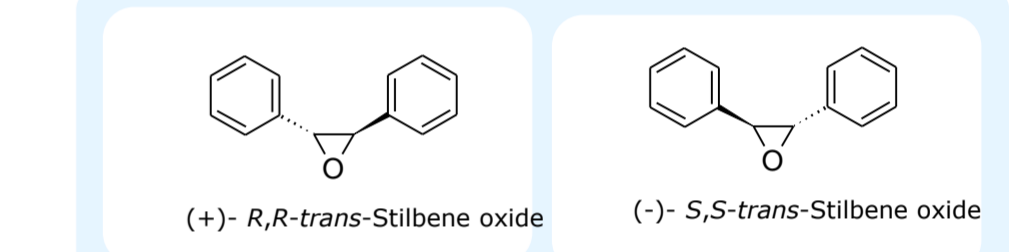
- 3 μ m particles show higher efficiency over a wider range of flow rate compared with 5 μ m particles.
- Faster analysis was achieved using a shorter length column packed with 3 μ m particles and increasing the flowrate.

Comparison of the Separation in SFC with HPLC



Column : Alcyon SFC CSP Amylose-C 3 μ m,
 50 x 2.1 mm ID
 Eluent : CO₂/MeOH (70/30)
 Flow rate : 0.6 ml/min
 Temperature : 40°C
 Detection : UV at 230 nm
 Back pressure : 17.2 MPa (2,500 psi)

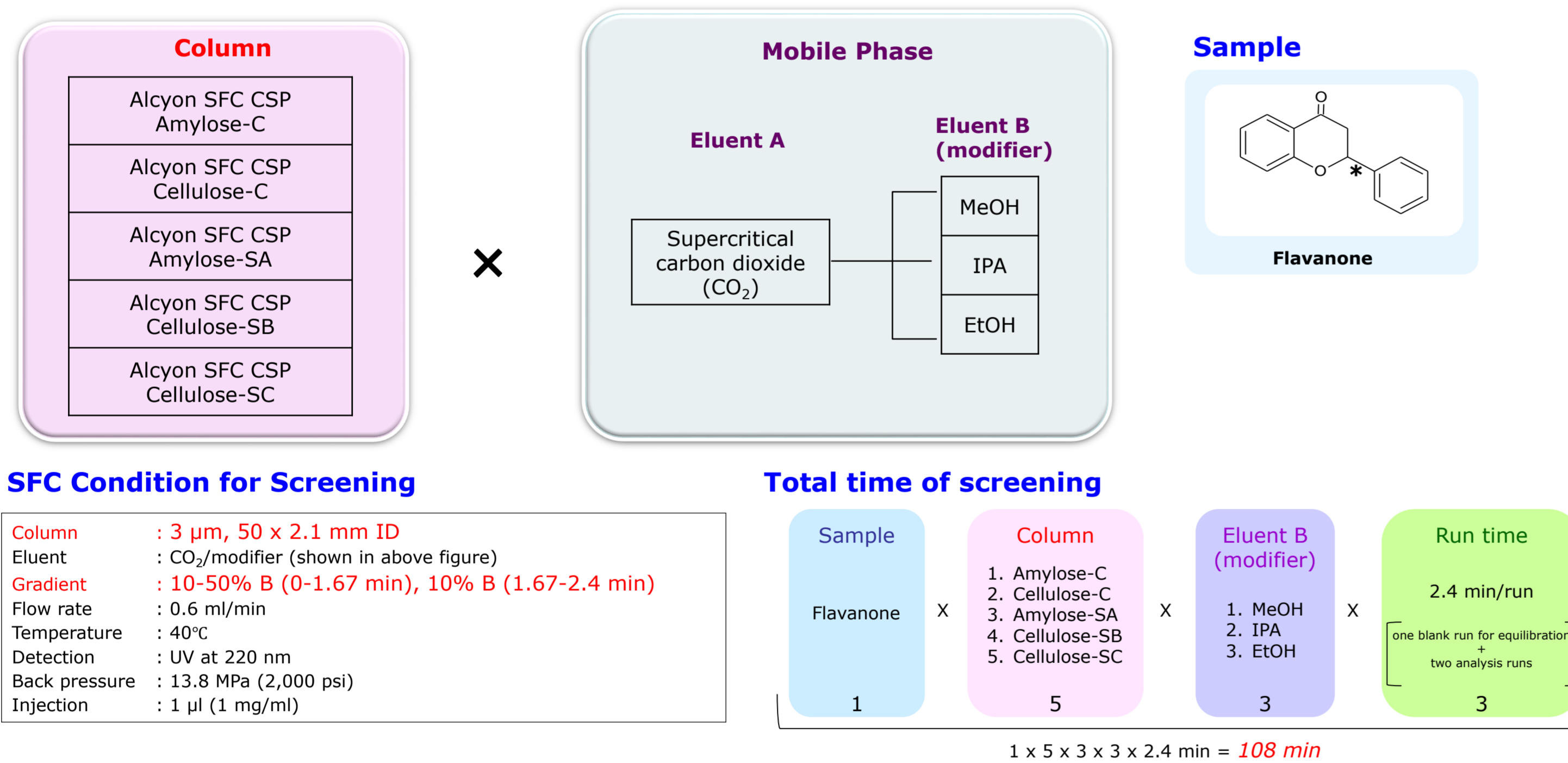
Column : CHIRAL ART Amylose-C 3 μ m,
 50 x 2.1 mm ID
 Eluent : Hex/IPA (90/10)
 Flow rate : 0.2 ml/min
 Temperature : ambient
 Detection : UV at 230 nm



- Faster chiral separation of trans-stilbene oxide is achieved using SFC compared with HPLC separation.
- CHIRAL ART shows good performance even under SFC conditions as well as under HPLC conditions.

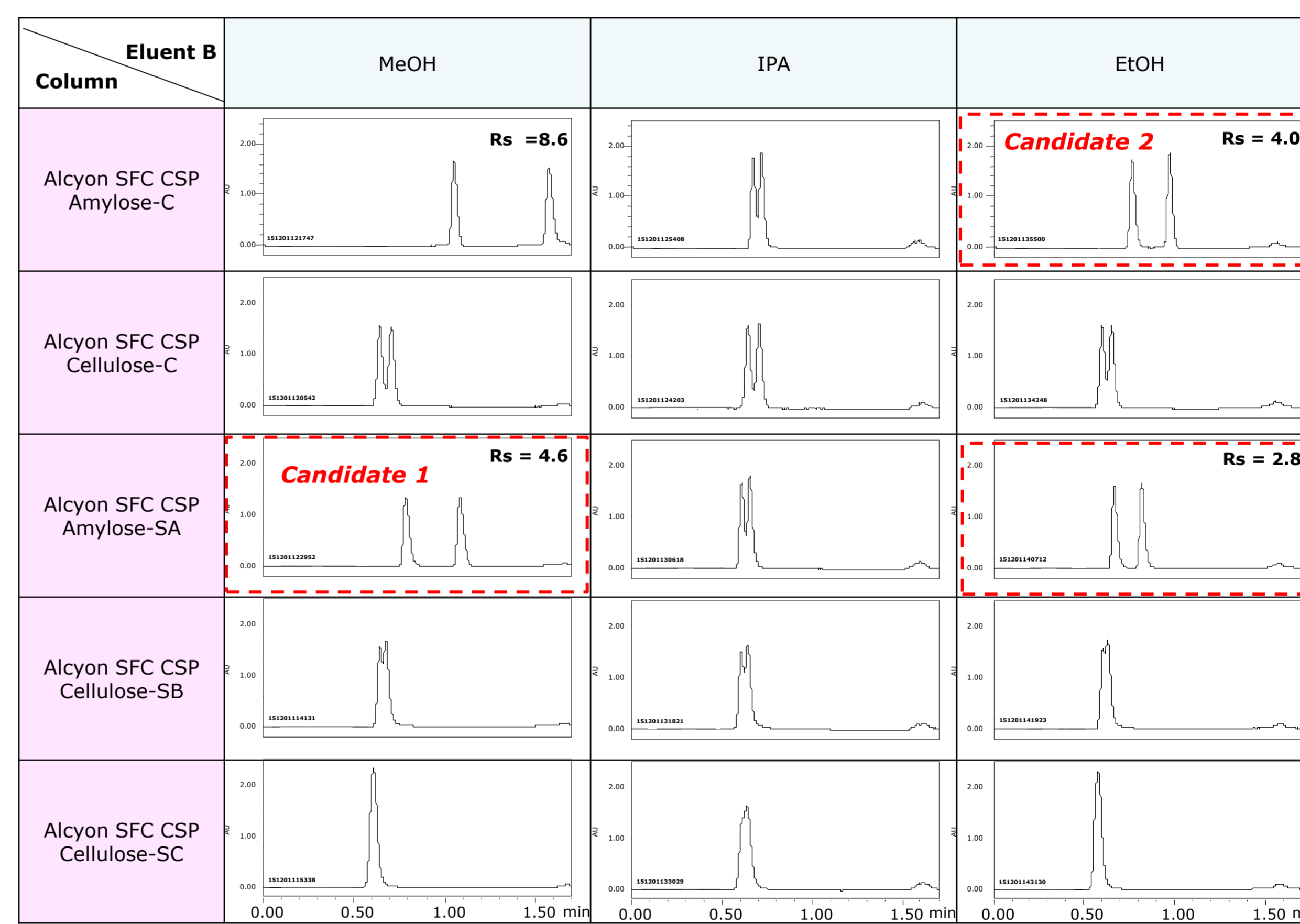
Efficient Method Development Strategy from Column Screening to Preparative Separation in SFC

SFC Screening Protocol



- The suggested screening protocol and conditions used for the chiral SFC are shown above. The combination of the short columns packed with five types of 3 μ m CSPs and rapid gradient elution with three different modifiers was used for the initial screening.
- By using 3 μ m short columns, the time required for the initial screening is approximately 108 min (1.8 hr). This is about one-third that with a conventional column such as 150 mm length packed with 5 μ m particles.

Overview of the Column Screening Results for Flavanone

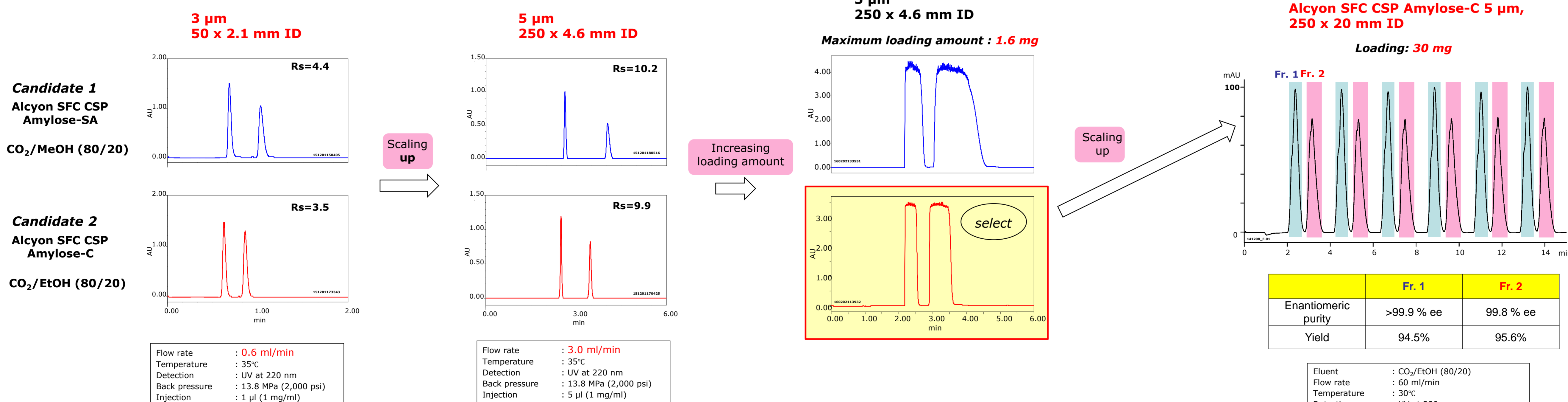


- For efficient preparative work, conditions that met $R_s > 1.5$ and analysis time < 1.7 min were selected.
- Three conditions met these criteria, as shown above. From these, two candidate conditions were chosen for the next step.

Method Optimization

Theoretical Scale-up and Loadability Study

Preparative Optical Separation



- The gradient conditions from the screening were transferred to the isocratic conditions.
- The 3 μ m short columns provide an ultrafast separation of flavanone within 2 minutes under both conditions.
- Theoretical scale-up was achieved from the 3 μ m particle 50 x 2.1 mm ID column to the 5 μ m particle 250 x 4.6 mm ID column.
- The combination of Amylose-C phase and CO₂/ethanol was finally selected due to the lower fraction volume and rapid cycle time. The maximum loading amount to obtain the fractions with high purity in the preparative separation was estimated to be 1.6 mg.
- The predicted loading amount could be applied for the scale-up (4.6 mm ID \rightarrow 20 mm ID).
- Highly pure optical isomers were obtained with high recovery.

Conclusions

- CHIRAL ART phases show identical selectivity across all available particle sizes. This allows predictable scale-up from short column screening with 3 μ m particles to conventional analysis with 5 μ m particles and even to preparative purification using 10 or 20 μ m particles.
- Efficient method development for the optical resolution in SFC was demonstrated using Flavanone. By using 50 mm columns packed with 3 μ m particles, the time required for the initial screening was reduced of one-third that required when using 150 mm columns packed with 5 μ m particles.