

# Chiral Stationary Phase Optimized Selectivity Supercritical Fluid Chromatography (SOS-SFC): a novel approach for optimizing the separation of enantiomers

## INTRODUCTION

Stationary phase Optimized Selectivity Liquid Chromatography (SOS-LC) has been successfully further developed and increasingly used in the last decade as a novel tool for the separation of solutes in a predictable way on combined stationary phases<sup>1,2</sup>. Progress has been made in the extension of the approach to allow for gradient analysis and the model also proves applicable on the compressible phases used in supercritical fluid chromatography (SFC)<sup>3</sup>. Thus far the potential of the approach to facilitate the separation and purification of stereoisomers via supercritical fluid chromatography (SFC) has not been investigated, although especially in the latter case SOS-SFC could offer significant benefits to speed up the purification process or to obtain improved chiral screening of complex mixtures. In this work phase the possibilities of isocratic chiral SOS-SFC are therefore explored. The approach combines different chiral column segments with divergent stationary phases based on the predictions made through the PRISMA model<sup>4,5</sup> such that the optimal separation can be obtained in this way. An optimized column segment combination, giving the highest separation selectivity for all the enantiomers in a mixture within shortest analysis time is predicted.

## EXPERIMENTAL

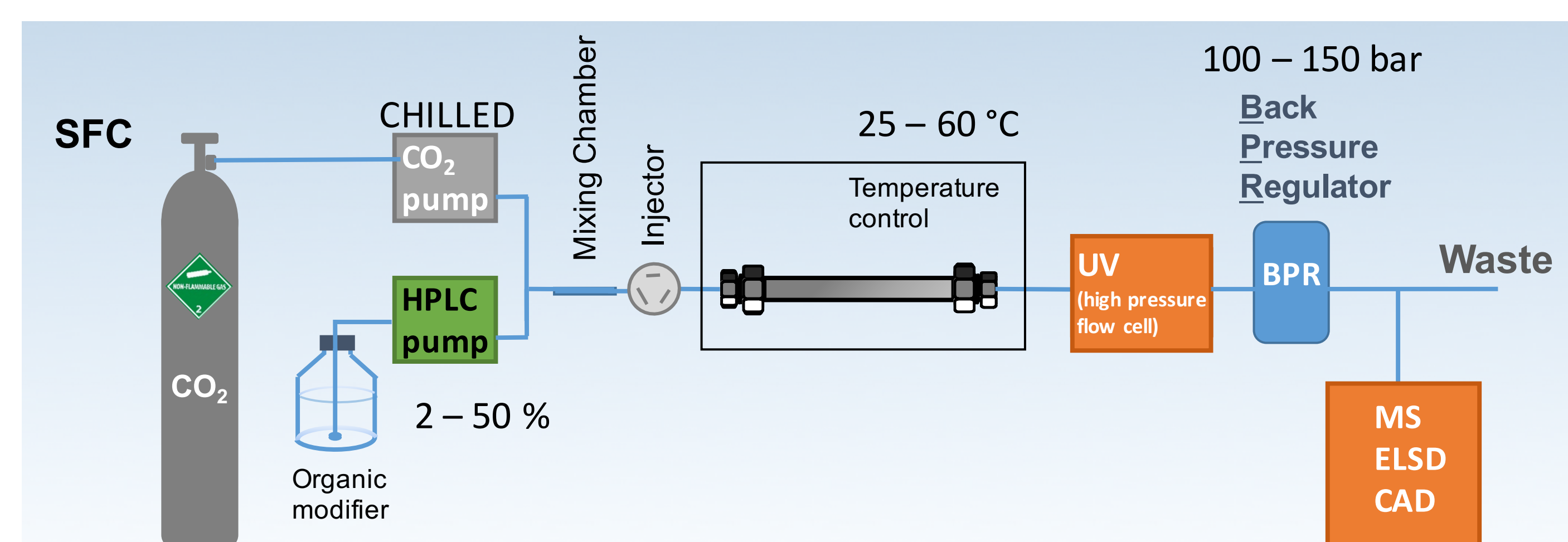
### 1. Chemicals and reagents

4.8 grade CO<sub>2</sub>, HPLC grade methanol, trans-Stilbene oxide, chiral mixture (1,2,3,4 Tetrahydro-1-naphthol and 4-phenyl-1,3-dioxane)

### 2. Chromatographic conditions

a. Instrument: Jasco 2080 SFC system; b. Mobile Phase: A. 4.8 grade CO<sub>2</sub>, B. methanol, c. Flow: 2.0 ml/min; d. Detector Wavelength: 210 nm; e. Column Oven: 50°C, f. Inj. Vol. : 10µL; g. Lux 3u Columns (50 mm x 4.6 mm, 3µm): Amylose 2, Cellulose 1, Cellulose 2, Cellulose 3 and Cellulose 4 (Phenomenax, USA) with Lux Amylose 2 (4 x 3.0 mm) pre-column; h. Software: ChromNav (Jasco) and POPLC optimizer v 1.04.03 (Bischoff Chromatography)

### 3. SFC Instrumentation



### 4. Optimization of the stationary phase

Baseline separation of the chiral compounds is a challenging task, which has been successfully accomplished by optimizing the composition of coupled chiral stationary phase approach. The equation 1 below provides an effective chiral stationary phase approach. The equation 1 provides an effective chiral stationary phase approach, which helps to predict the retention time of individual analytes in a mixture.

$$k_{ABCDE} = \frac{k_A\phi_A + k_B\phi_B + k_C\phi_C + k_D\phi_D + k_E\phi_E}{\phi_A + \phi_B + \phi_C + \phi_D + \phi_E} \quad \text{Equation 1}$$

Thereby  $k_A$ ,  $k_B$ ,  $k_C$ ,  $k_D$  and  $k_E$  correspond to the retention factors of a compound on five (A-E) stationary phases.  $\phi_A$ ,  $\phi_B$ ,  $\phi_C$ ,  $\phi_D$  and  $\phi_E$  represent the lengths of the five (A-E) segments in a combined column. Use of POPLC optimizer software v 1.04.03 (Bischoff Chromatography) also gives similar data with predicted chromatogram which is also based on same equation.

## RESULTS & DISCUSSION

### 1. Separation of a chiral mixture on pure chiral phases

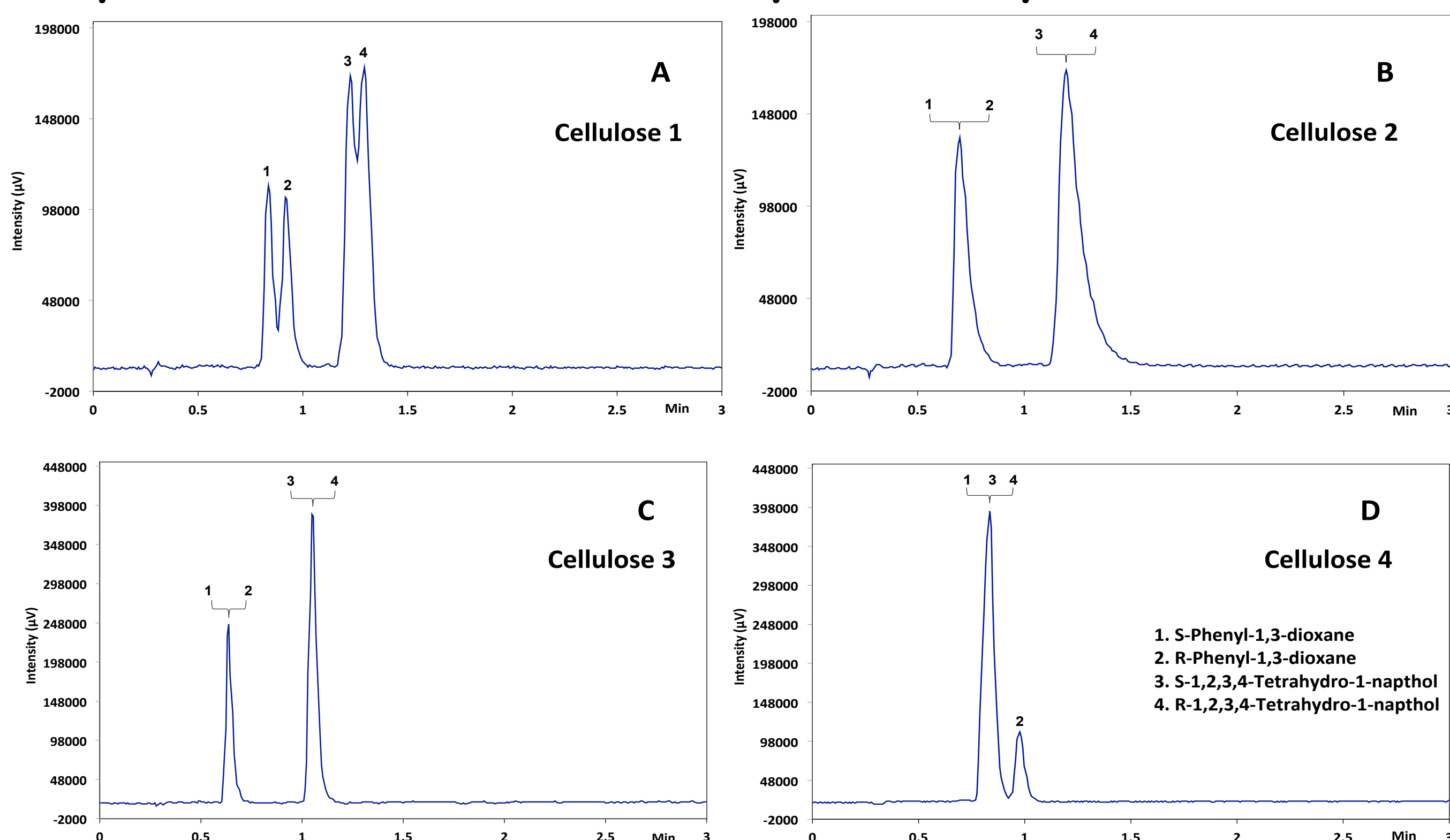


Figure 1 : Chromatogram of a chiral mixture on Lux 3u Cellulose 1, 2, 3, and 4 (for annotations Refer Table Figure 1D)

On none of the individual stationary phases, baseline separation could be obtained for the chiral mixture. Figure 1 represents poor separation of a chiral mixture with the used mobile phase conditions.

### 2. Retention time prediction in SFC

As a first practical step, void time, retention time and retention factor were measured for the individual enantiomers from chiral mixture on each column segments by allowing the identical chromatographic conditions. The measured retention factors on the individual column segments are then used to predict the retention time on a possible linear column combination of the segments, which is based on Equation 1. The prediction of retention time for enantiomers on any set of column combinations can be possible through equation 1. e.g. Figure 2 demonstrates good phase for the separation of trans-stilbene oxide enantiomers. (For annotations refer Figure 1, D)

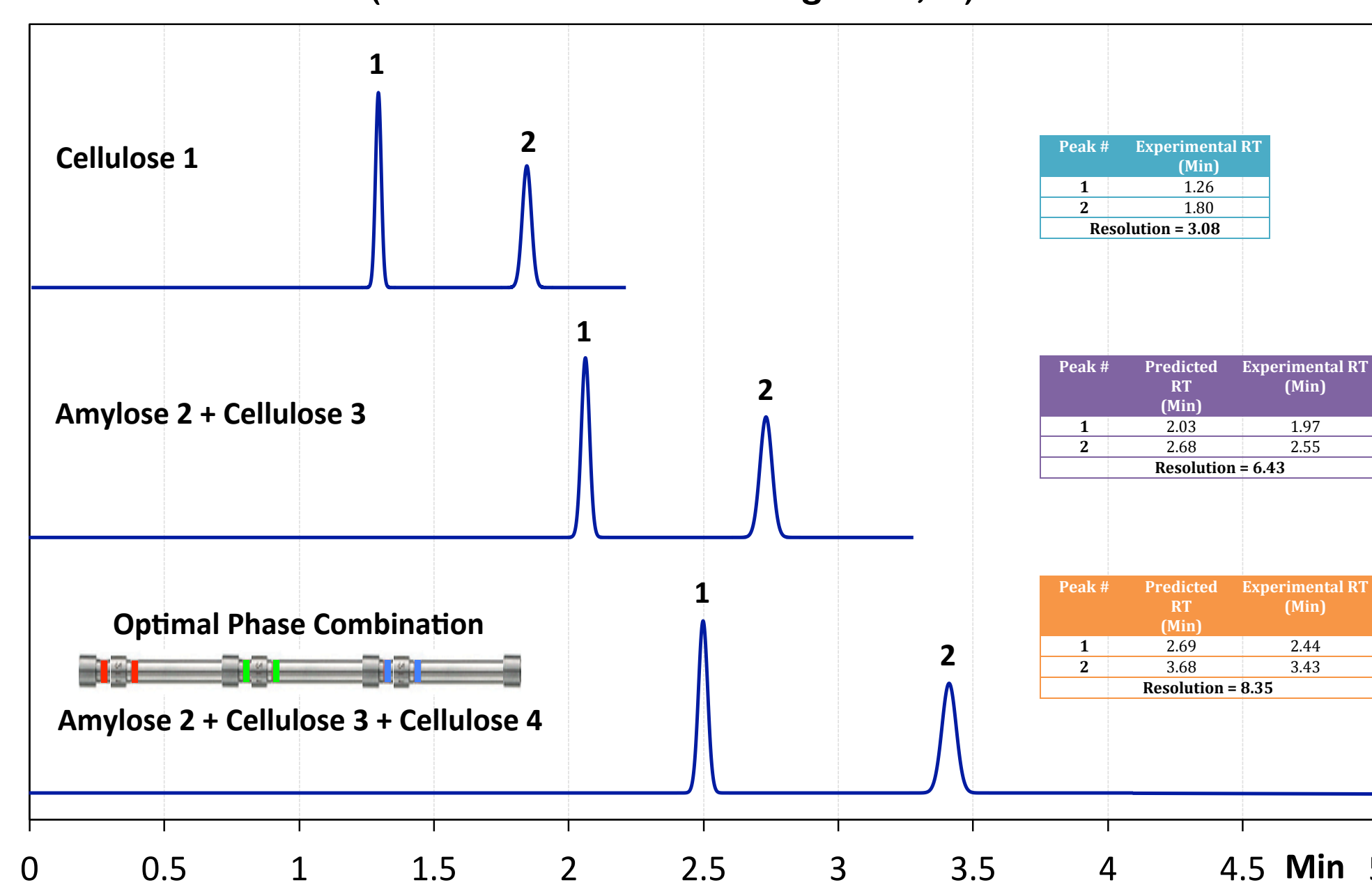


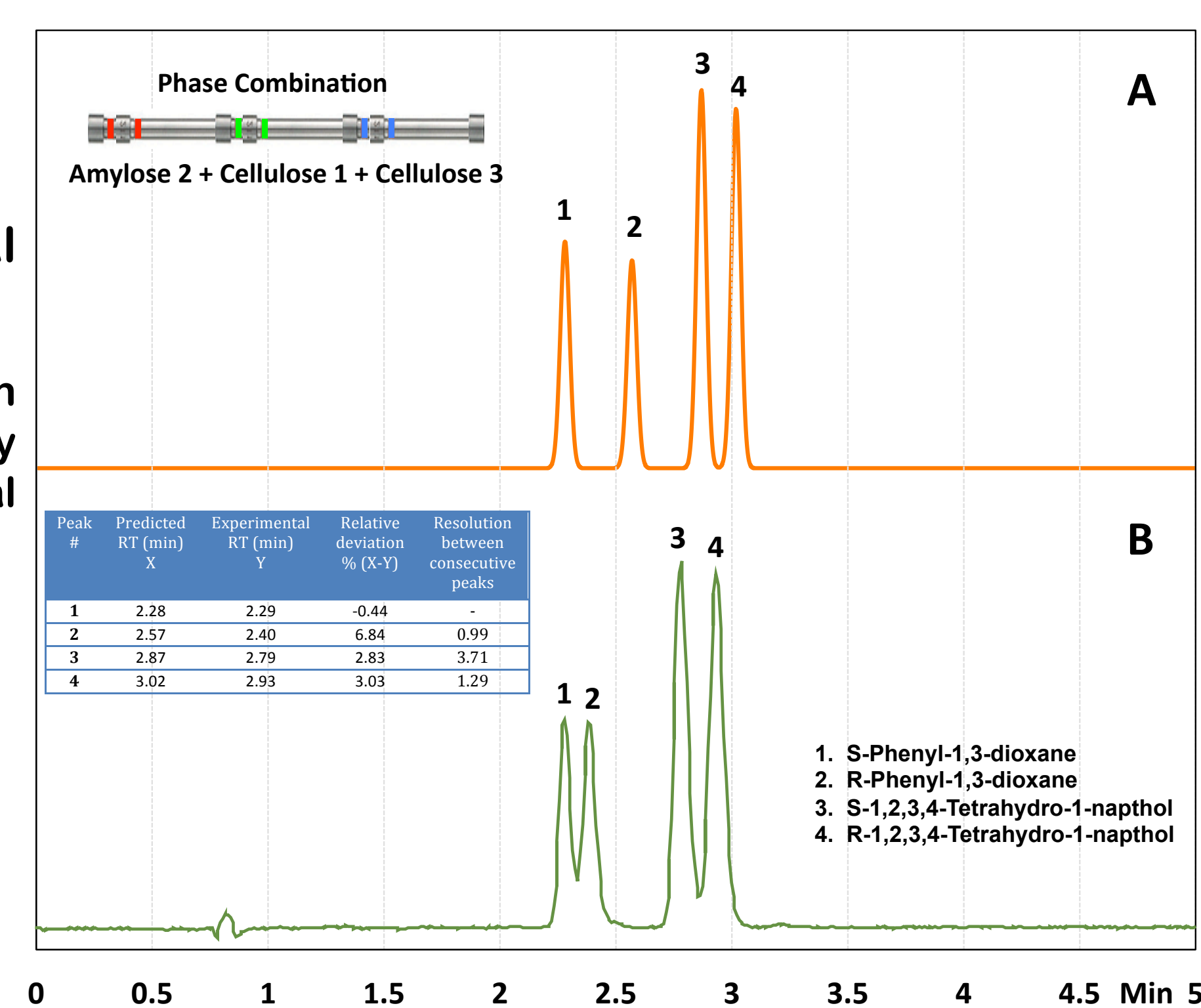
Figure 2: Phase Optimization for enantiomers of trans stilbene oxide

This proves that SOS-SFC approach can be successfully applied to resolve mixtures of chiral pairs. The same proof of principle has been demonstrated to resolve chiral mixture in a efficient way by coupling 3 different column segment based on retention factor prediction, via equation 1.

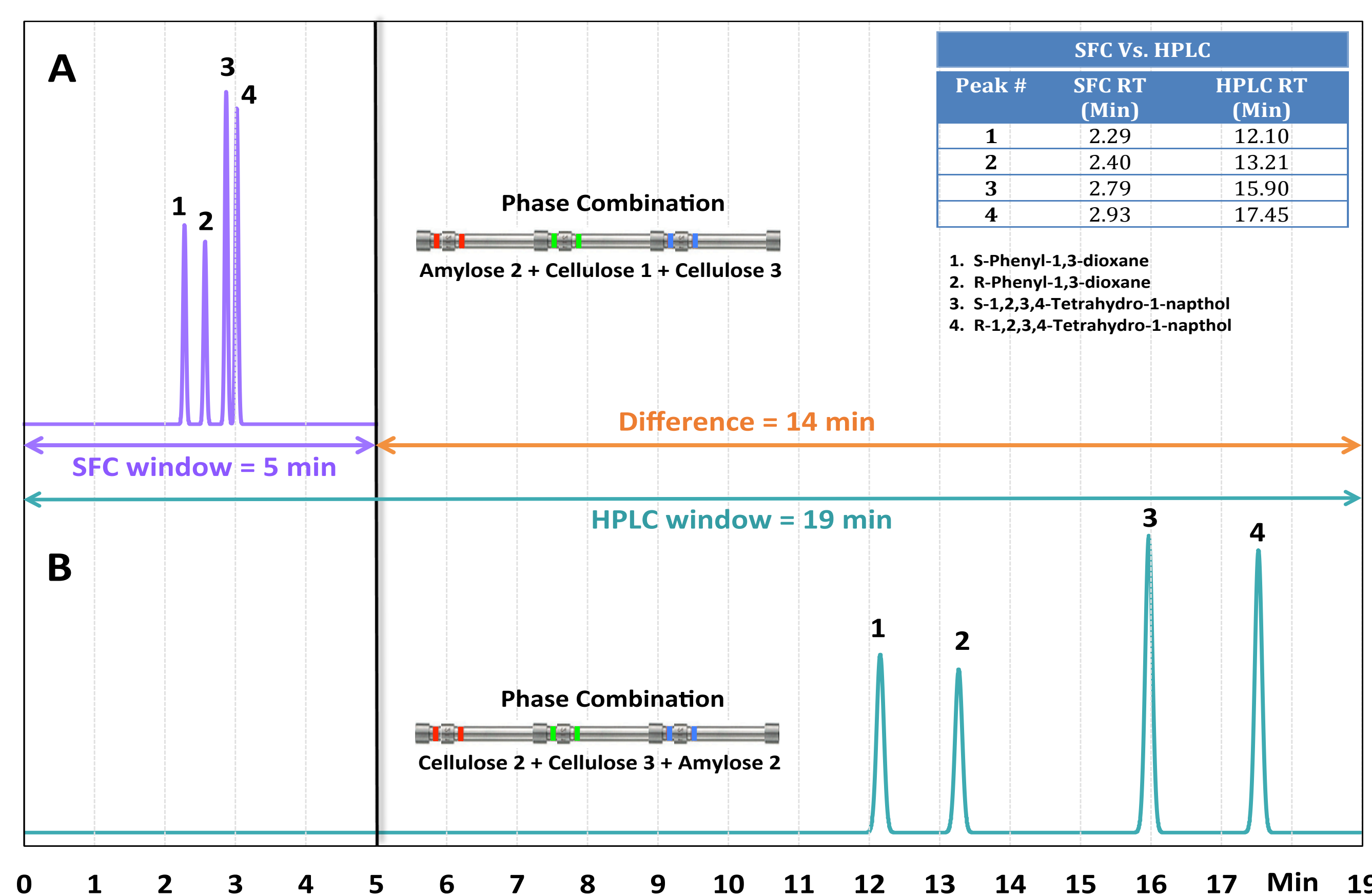
### 3. Proof of principle of chiral SOS-SFC approach

From all possible column combinations, the satisfactory phase to resolve the 2 chiral pairs is shown in figure 3.

Figure 3: Predicted (A) & experimental (B) chromatogram of the chiral mixture on 3 combined column segments (Amylose 2 + Cellulose 1 + Cellulose 3)



### 4. Illustration of the gain in separation efficiency for coupled chiral column SFC (A) Vs. HPLC (B)



## CONCLUSION

- Stationary Phase Optimized SFC approach has been successfully applied for the separation of chiral pairs.
- Manual prediction of retention time is possible for any set of column combination.
- Stationary Phase optimized chiral SFC provides improved solution for the separation and purification of enantiomers
- Further investigations need to be done to check the impact of isopycnic approach, which may help to resolve more complex mixtures of enantiomers.

## REFERENCES

- M. De Beer, F. Lynen, K. Chen, P. Ferguson, M. Hanna-Brown, P. Sandra, Stationary-phase optimized selectivity liquid chromatography: Development of a linear gradient prediction algorithm, Anal. Chem. 82 (2010) 1733–1743.
- S. Delen, K. Lynen, Frederic, De Beer, Maarten; et al. J. Chromatogr. A (2010) Volume 1217 Issue: 46 Pages 7222-7230.
- S. Chelahaye, F. Lynen, Implementing stationary-phase optimized selectivity in supercritical fluid chromatography, Anal. Chem. 86 (2014) 12220–12228.
- P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, L. De Galan, Gradient selection in reversed-phase liquid chromatography, J. Chromatogr. 149 (1978).
- S. Nyireddy, Z. Szucs, L. Szepegy, Stationary phase optimized selectivity liquid chromatography: Basic possibilities of serially connected columns using the "PRISMA" principle, J. Chromatogr. A. 1157 (2007) 122–130.