P-PHAR37 (BPA)

Software-supported HPLC Method Development for Separating Four Regioisomers of a Sugammadex-related Impurity

Best Poster Award

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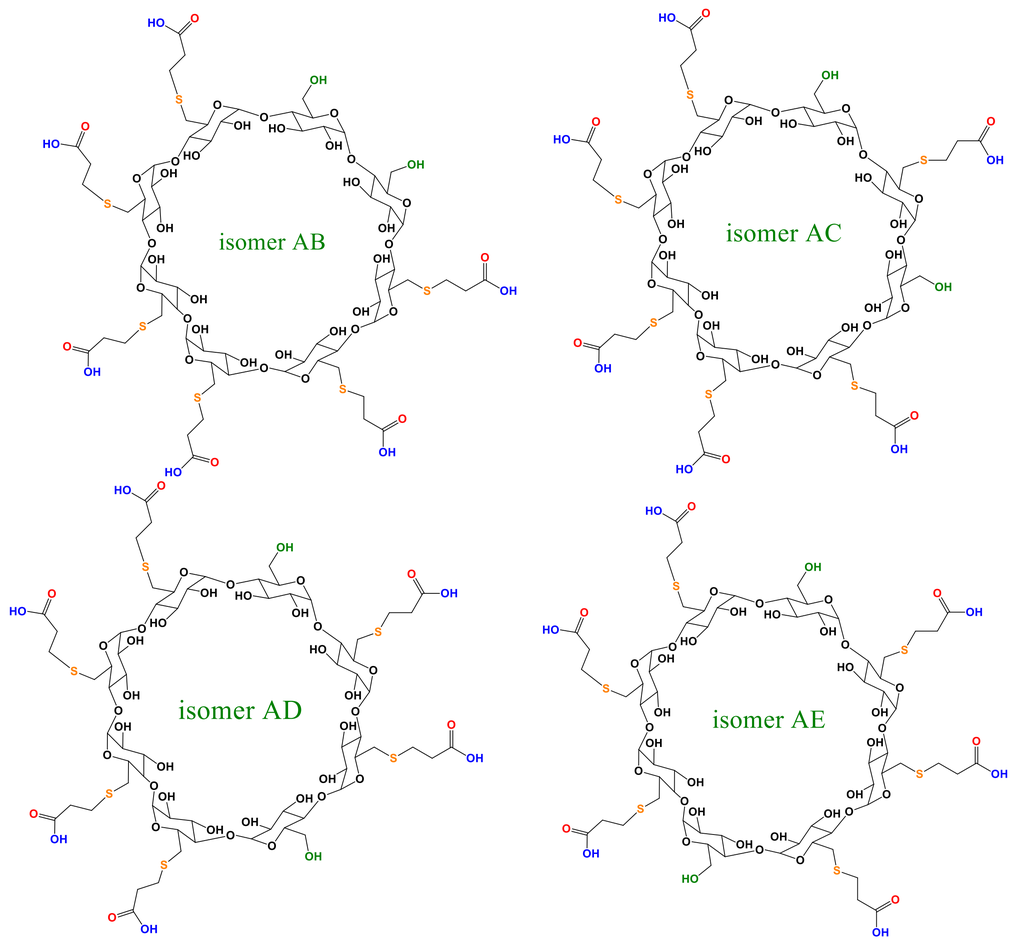
MOLNÁR-INSTITUTE for applied chromatography

CYCLOLAB PIONEERS IN CYCLODEXTRIN-BASED INNOVATION

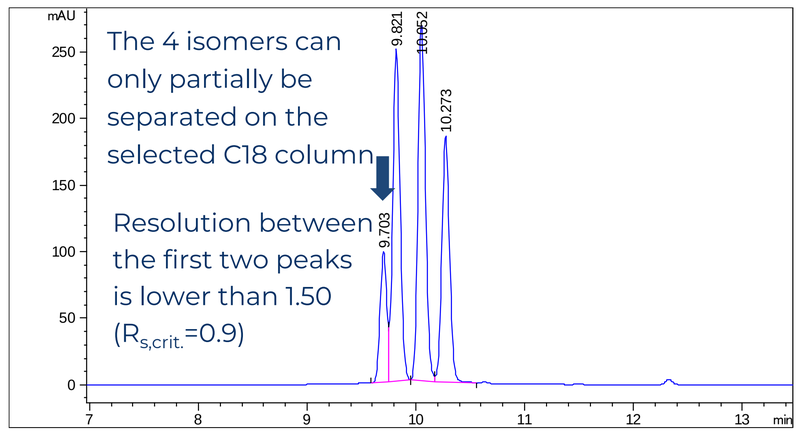
Introduction

This research focuses on a simple, relatively inexpensive reversed-phase high-pressure liquid chromatography (RP-HPLC) application in combination with UV-detection aiming for the separation of four relevant regioisomers (marked as AB, AC, AD, AE) of Di-OH-SGM (Hexakis(6-deoxy-6-(2-carboxyethyl)thio)-gamma-cyclodextrin). The presence of these regioisomers in the synthesized Di-OH-SGM reference material has already been proven by MS and NMR spectroscopy. Thus, the main goal of our project was to baseline-resolve these regioisomers—as this is required for the qualification of the synthetized Di-OH-SGM material. Although our preliminary results on the selected C18 stationary phase provided a promising initial separation, only $R_{s,crit.}$ = 0.9 of peaks could be realized. Hence, we decided to apply a systematic modeling approach (DryLab) to contextualize data and to try optimizing critical method parameters. We found that by lowering column temperature, better separation but still no baseline separation of peaks was possible—while the modeling software revealed some unique features of these molecules. The poster will present the current results of our software-supported development process, underlining the key benefits of modeling approaches in better understanding the complex nature and chromatographic behavior of regioisomers of Di-OH-SGM.

Structures of the four Di-OH-SGM Regioisomers



HPLC Chromatogram of Di-OH-SGM (Initial method)



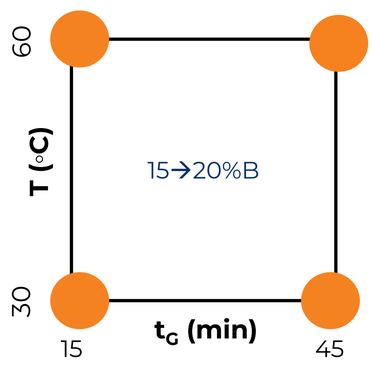
Initial HPLC method

Poroshell C_{18}, 150x4,6 mm, 2.7 µm, (Agilent)

Eluent A: 0.1 % H_3PO_4/Ammonium, pH= 2.6 , Eluent B: Acetonitrile, T = 40°C, F = 0.8 mL/min, stop 30 min

Grad. = 0-20 min, 15-25 %B; 20-25 min, 25-40 %B

Design of Experiments (DoE)

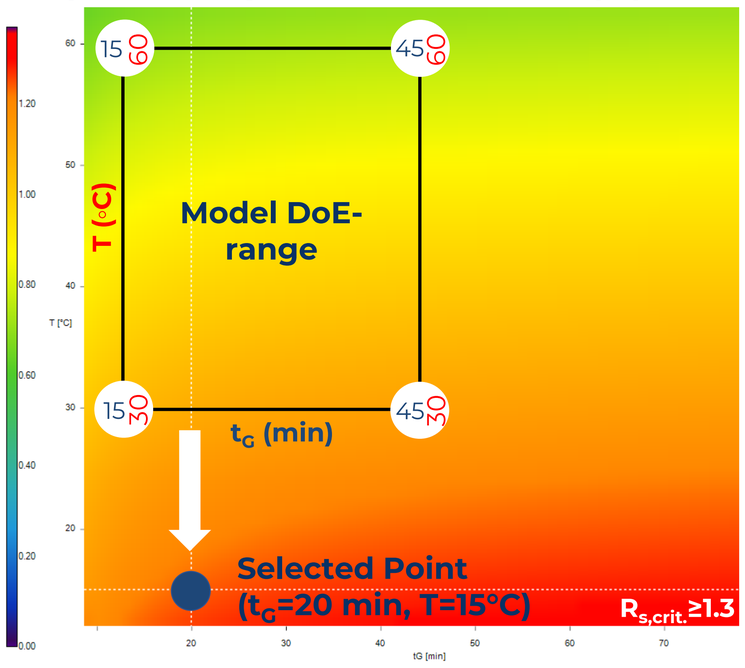


Gradient time (tG) and **temperature** (T) were systematically studied, whereas the gradient start-end%B was set constant (15→20%)

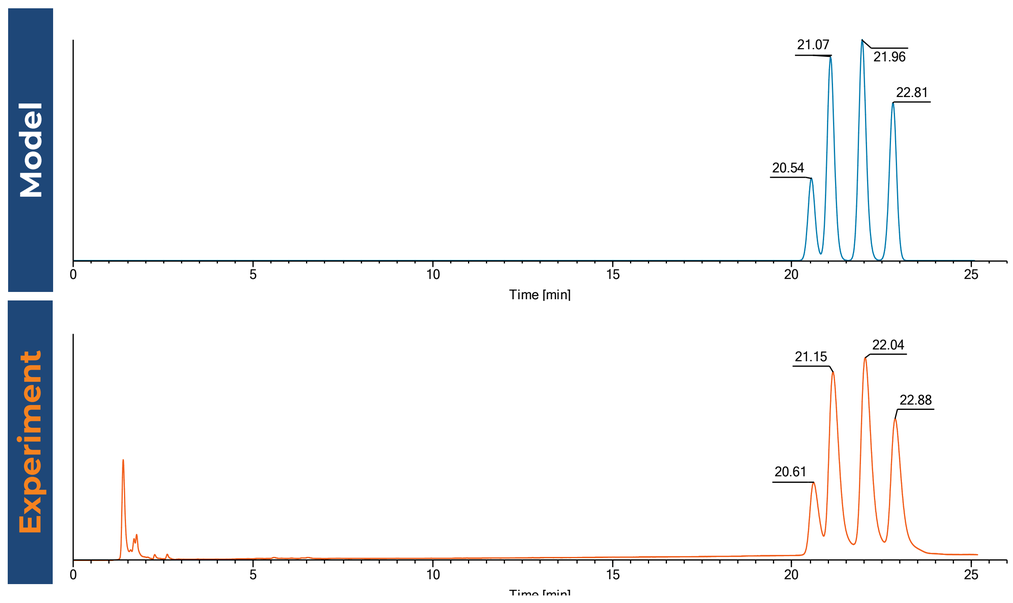
1. t_G= 15 min , **T**= 30 °C
2. t_G= 45 min , **T**= 30 °C
3. t_G= 15 min , **T**= 60 °C
4. t_G= 45 min , **T**= 60 °C

Results and Discussion

Updated DryLab Resolution Map (16-18%B)



Verification Experiment of the Selected Point



The updated **Resolution Map** (including optimized gradient conditions) explained the difficulties in achieving baseline separation. Working at lower temperatures might improve the initial results ($R_{s,crit.}$≥1.30 red area), however, Instrument settings allowed for a minimum temperature adjustment of 15°C, therefore a **working point** for confirming the modeling was selected and performed at the following conditions: t_G = 20 min (16-18 %B), T = 15 °C, F = 0.8 mL/min, stop time = 25 min.

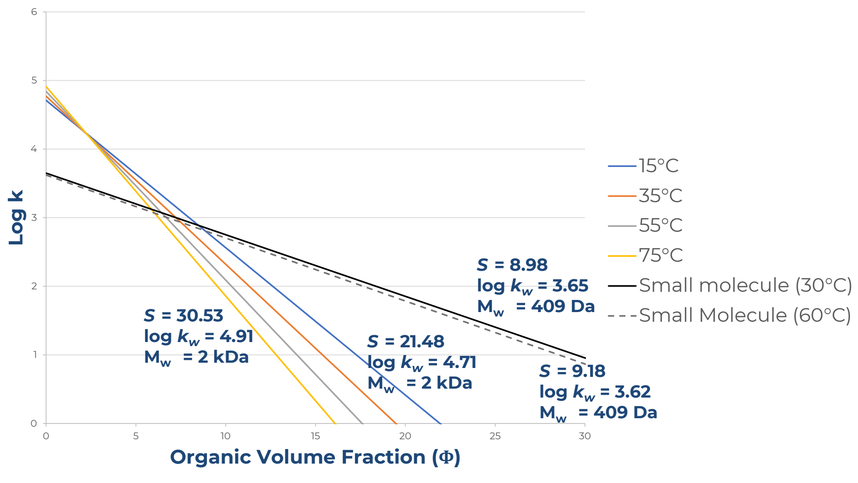
Despite the selected point was well outside the recommended modeling range, the experimental chromatogram was found in a **good agreement** with the modeled one.

Linear Solvent Strength Model

$$\log k = \log k_w - S \times \Phi$$

- k_w retention factor in weak eluent (water)
- S solute specific constant
- Φ volume fraction of the organic eluent

Calculated LSS-Parameters (Isomer AD)



Considering the small-medium molecule size (2kDa) of cyclodextrins, the model-calculated results indicated surprisingly **strong influence** of the **eluent strength** (Φ) on the calculated retention factors of peaks (ln *k*). Indeed, during the model gradient optimization process, it was found that 2-3 %B (start-end%B=16-18%) was just enough to completely elute all peaks in approx. 20-30 minutes time.

Furthermore, by scrutinizing the calculated LSSM-parameter values, we discovered intriguing **temperature-dependence** of the solute-specific coefficients (S-values).

These results show the **complex nature** of cyclodextrin molecules and suggests the use of software-supported development strategies.

Summary

- DryLab is commonly applied for pharmaceutical (U)HPLC development of small-, and large-molecules.
- This is the first time we used the software to study the complex separation of cyclodextrin isomers.
- The model as a «feasibility tool» showed no baseline separation was possible with the actual HPLC-setup.
- Modeling results revealed some interesting chromatographic behaviors of these compounds

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