

Enhancing Achiral Purification Workflows in Drug Discovery with Open-Access SFC-MS Purification Platform

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1. Introduction

In drug discovery and development, the synthesis, screening, and purification of target compounds are essential processes. Supercritical fluid chromatography (SFC) is commonly used for purification, but there is a need to enhance its efficiency. SFC offers significant advantages, involving improved separation through optimal column selection, resulting in high-purity products. Additionally, SFC can reduce solvent consumption and increase productivity.

This study explores the feasibility of an automated scale-up platform using open-access SFC-MS. The platform features an automated function that recommends the optimal purification column and generates ideal preparative conditions. Three types of columns with different retention selectivities were used for screening, specifically chosen for the separation of low molecular weight pharmaceuticals. This approach enables effective separation and purification of a wide range of compounds.

Additionally, this workflow is controlled by open-access software, ensuring consistent quality regardless of the operator's expertise.

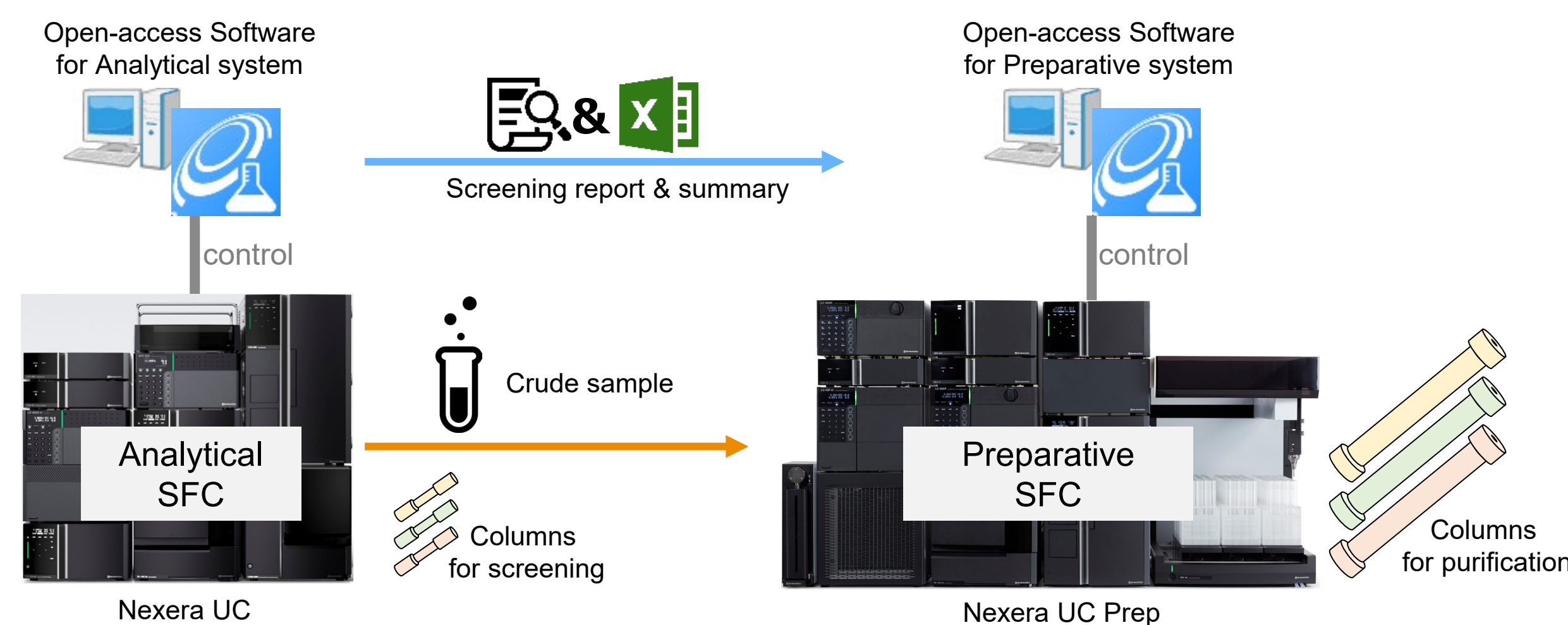


Fig. 1 Overview of open-access SFC-MS purification platform

2. Methods

2.1. Purification Workflows with Open-Access SFC-MS

The purification workflows utilizing the open-access SFC-MS platform are designed to streamline the process of compound purification in drug discovery. The workflow mainly consists of two steps, which are outlined as follows:

1. **Screening with Various Columns:** Screening using some columns with different selectivities.

2. **Scale-Up with the Best Column:** Scaling up using the column that provides the best separation in Step 1.

In this process, columns with the same stationary phase are prepared for both screening and purification. This approach not only provides a systematic screening and purification process but also maximizes the benefits of using SFC.

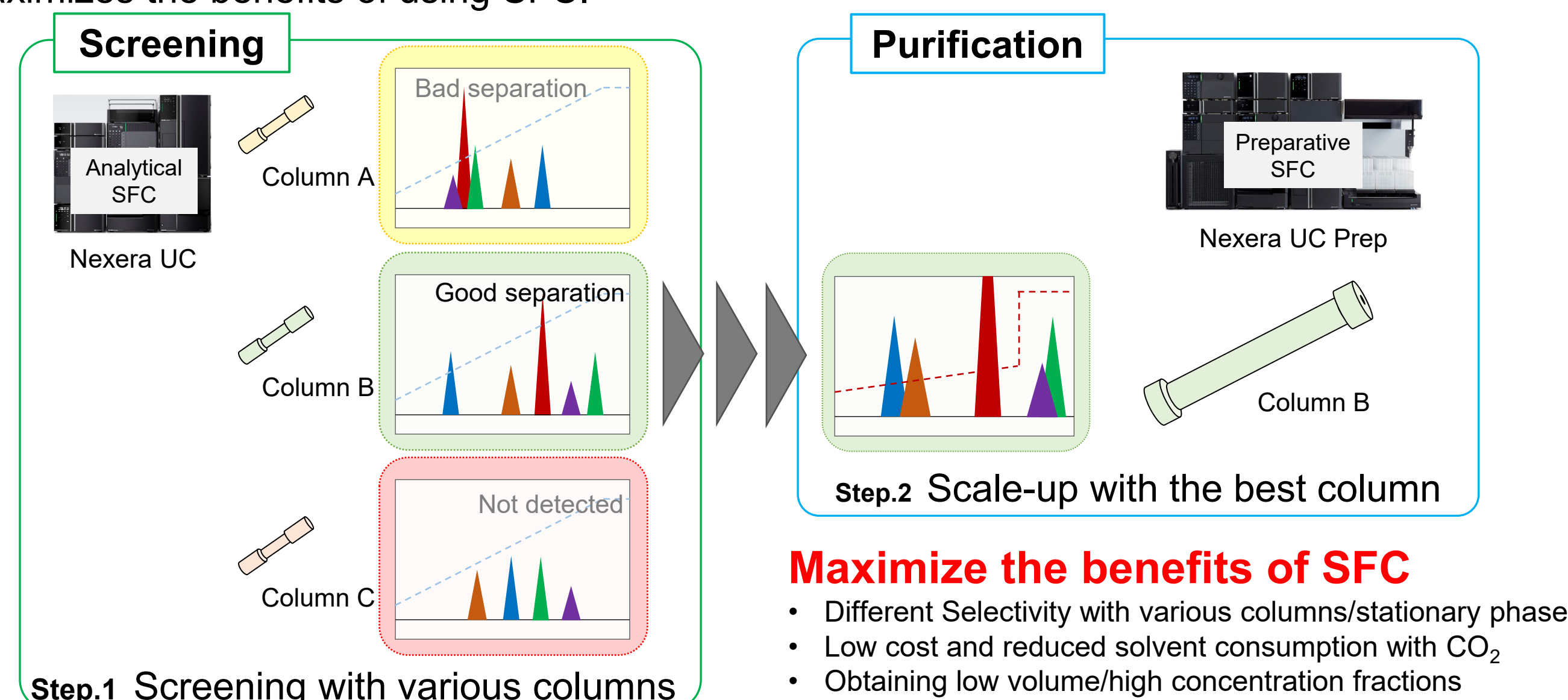


Fig. 2 Workflows of open-access SFC-MS purification platform

2.2. Scale-Up Algorithm from Screening to Purification

The scale-up algorithm is designed to automatically generate optimal focused gradient profiles for purification. The Initial concentration of solvent B (Ini.B.conc.) in the preparative scale was determined based on the screening retention time in the analytical SFC-MS. The gradient profile, involving the duration of the gradient and the rate of change in the B.conc. was fixed to ensure consistency across all runs. Notably, the target peaks were expected to elute at approximately the same time, around 4.5 minutes.

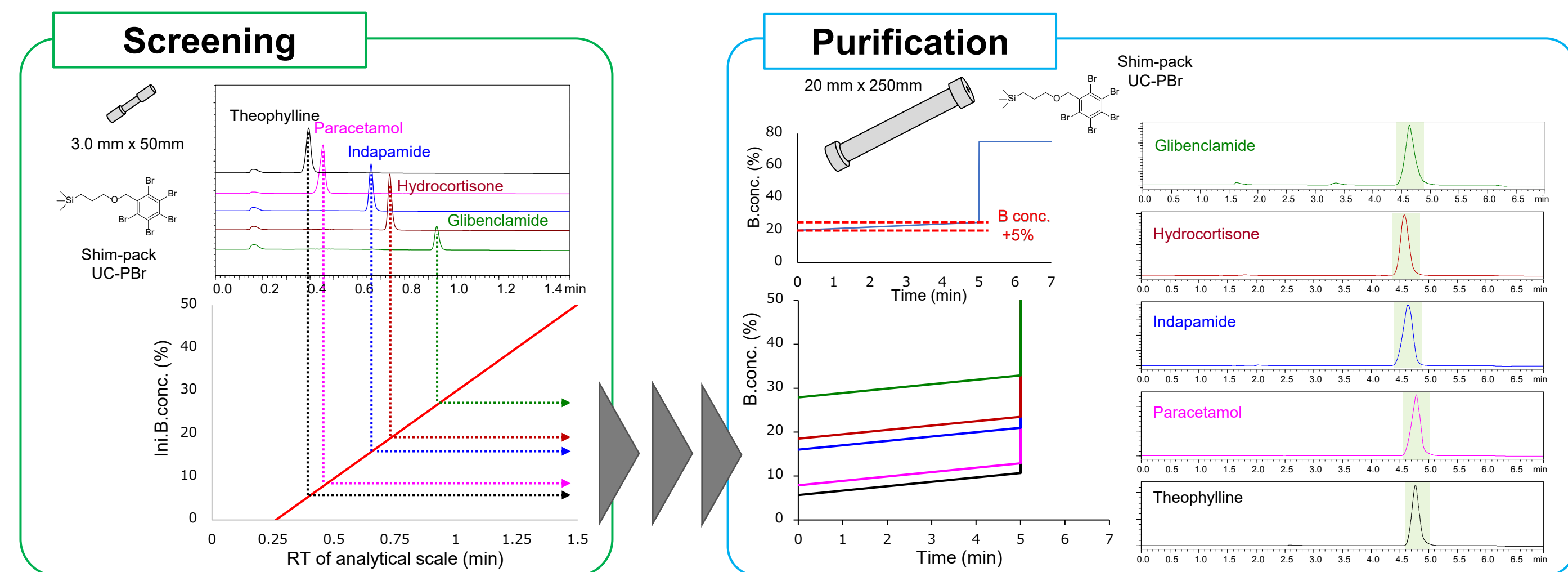


Fig. 3 Overview of the scale-up algorithm from screening to purification

Table 1 Screening conditions

SFC Conditions	MS make-up solution	MS conditions
Column	: Shim-pack UC-PolyVP ¹ , PBr ² , Diol II ³ (50 mm x 3.0 mm I.D., 3 μm for each column)	Flow rate (MS make-up)
Mobile phase	: (A) CO ₂ (B) 0.1 w/v% ammonium formate in methanol	Ionization
Flow rate (Mobile phase)	: 3.0 mL/min	Nebulizing Gas Flow
Gradient profile	: B Conc. 5%(0 min)→75%(1.0-1.5 min) →5%(1.5-2.0 min)	Drying Gas Flow
Column temp.	: 25 °C	Heating Gas Flow
BPR press.	: 10 MPa	DL Temp.
Detection (PDA)	: 254 nm (SPD-M40, conventional cell)	Desolvation Temp.
Sample concentration	: 1 mg/mL in DMSO	Interface Voltage
Injection volume	: 2 μL	

*1 P/N: 227-32507-11 *2 P/N: 227-32602-04 *3 P/N: 227-32606-07

Table 2 Purification conditions

SFC Conditions	Column temp.	BPR press.	Detection (PDA)	Sample concentration	Injection volume
Column	: Shim-pack UC-PolyVP ⁴ , PBr ⁵ , Diol II ⁶ (250 mm x 20 mm I.D., 5 μm for each column)	: 25 °C	: 254 nm (SPD-M40, high pressure cell)	: 10 mg/mL in DMSO	: 200 μL
Mobile phase	: (A) CO ₂ (B) 0.1 w/v% ammonium formate in methanol				
Flow rate (Mobile phase)	: 3.0 mL/min				
Gradient profile	: B Conc. x% (0 min)→(x+5)%(5 min) →75%(5.01-7.0 min)				

*4 P/N: 227-32511-11 *5 P/N: 227-32602-04 *6 P/N: 227-32606-04

*7 Values calculated by the scale-up algorithm

3. Results and discussion

3.1. Scale-Up Algorithm from Screening to Purification

The scale-up algorithms of PBr column was represented by a single linear function, while PolyVP and Diol II columns were depicted with the combination of two linear functions.

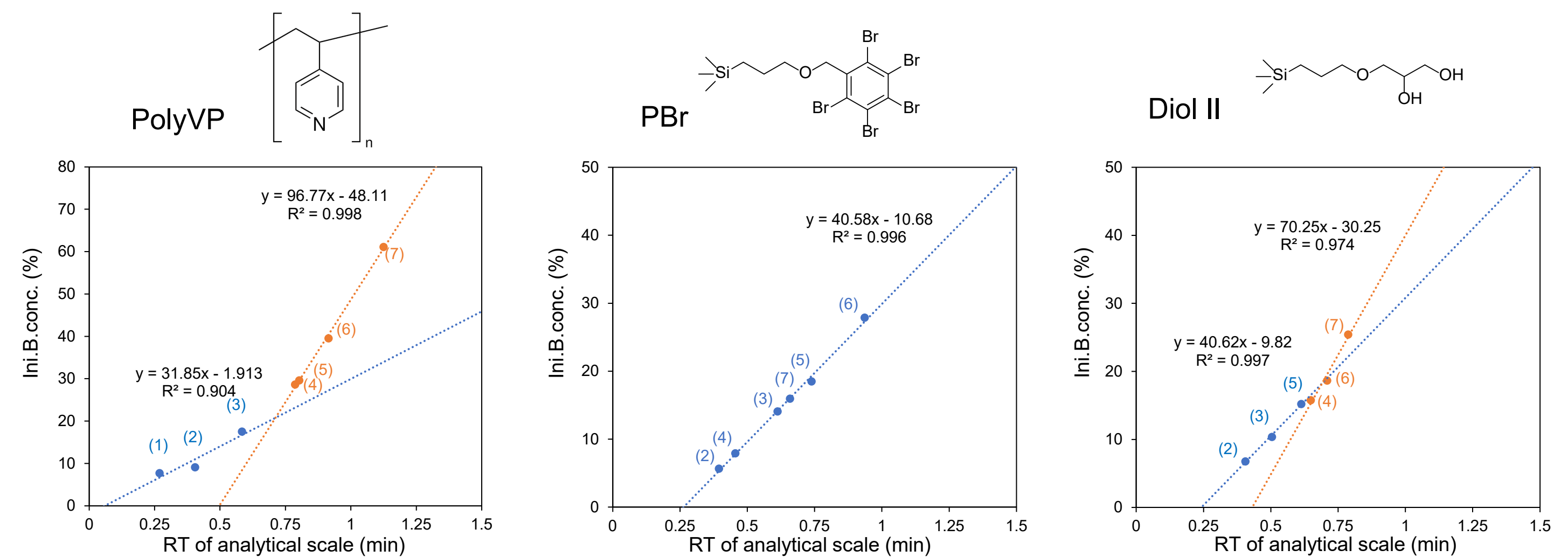


Fig. 4 The scale-up algorithms created based on the results the following seven compounds; (1) Nitronaphthalene, (2) Theophylline, (3) Nifedipine, (4) Paracetamol, (5) Hydrocortisone, (6) Glibenclamide, (7) Indapamide

3.2. Practical examples with mixture compounds

Model samples of low molecular weight pharmaceutical mixtures were used to adapt the purification workflow. Scaling up with the column that provided the best separation in screening resulted in effective purification, achieving high purity for the target compounds.

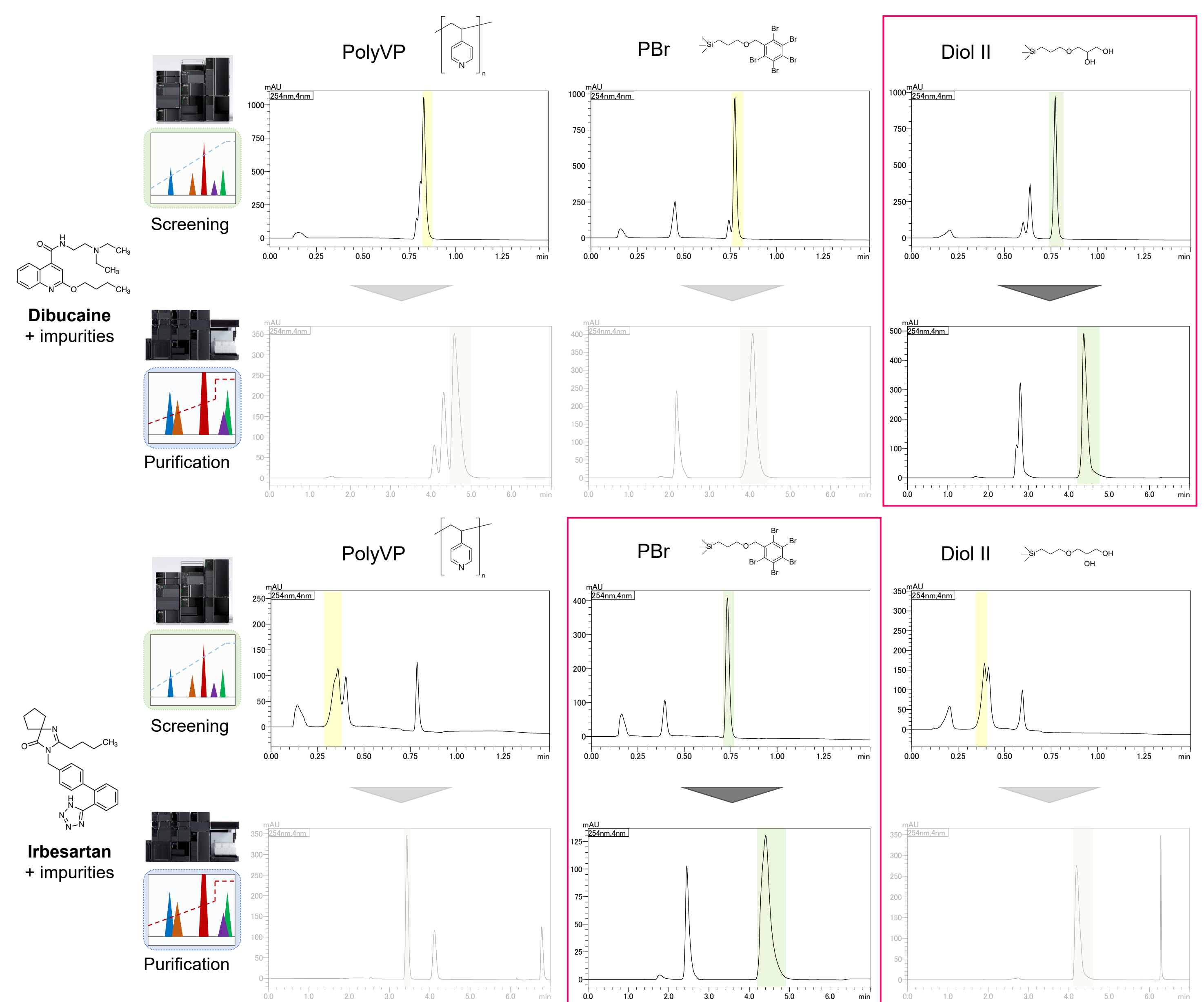


Fig. 5 Examples of adapting model samples to the workflow. The red boxes highlight the scale-up results with the best conditions.

3.3. "On-Column Dilution" Injection for Preparative SFC

In the preparative SFC, the "On-Column Dilution" injection method was used (Fig. 6). Although separation depends on the compounds, good separation was achieved in purification even when the peaks had very close retention times in screening. Additionally, increasing the injection volume did not compromise the separation or peak shape (Fig. 7).

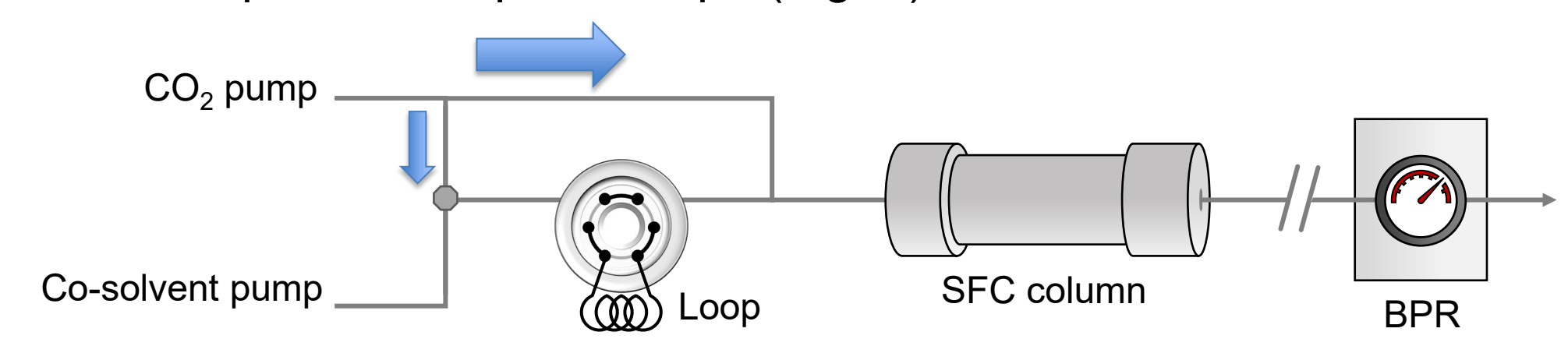


Fig. 6 Flow path of "on-column dilution" injection

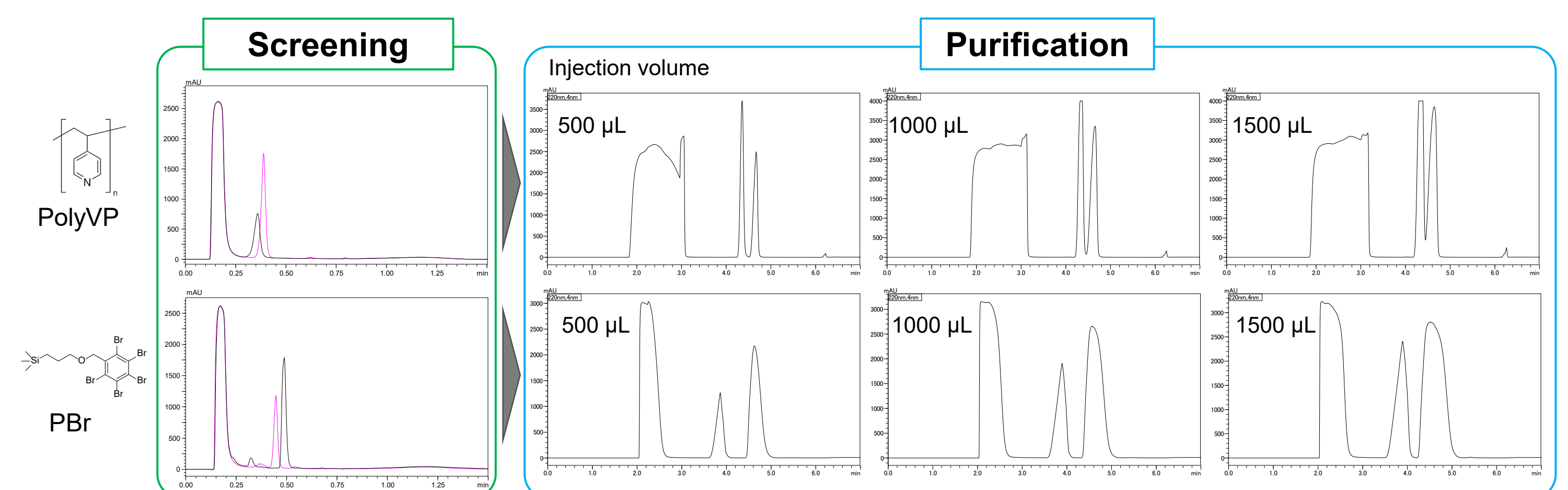


Fig. 7 Comparison of chromatograms obtained with different injection volumes in purification

4. Conclusion

This study highlights the effectiveness of the automated scale-up platform using open-access SFC-MS for purifying low molecular weight pharmaceuticals. The combination of systematic column screening and the on-column dilution method enhances purification efficiency, achieving high-purity target compounds even with closely eluting peaks. This platform streamlines the workflow and makes it accessible to researchers of different expertise, supporting more efficient drug discovery.