

# Case Studies on Compound-Specific Achiral SFC Purification

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## Abstract

Recently, supercritical fluid chromatography (SFC) has gained wide acceptance for achiral purifications in drug discovery research, especially for generating small quantities of pure materials to enable the early stage of pharmaceutical discovery and development. We report in this presentation the implementation of compound-specific gradient methods, a strategy that is commonly used in reversed-phase liquid chromatography, to the preparative SFC purification of our challenging achiral separations, including crude isomeric and complex reaction mixtures. Also included are several case studies which highlight the effectiveness of using chiral stationary phases to maximize the separation efficiency of difficult achiral separations. This approach has facilitated the SFC purification workflow by reducing method development and purification time.

## Purpose

To explore the feasibility of using SFC chiral or achiral stationary phases for preparative purification and separation of complex samples, including structurally similar diastereomeric and regioisomeric mixtures that are difficult to resolve via reversed phase HPLC.

## Experimental

### Instrumentation

- Analytical SFC-MS and Prep30 SFC-MS systems were from Thar Technologies equipped with Waters ZQ and 3100 SQD mass spectrometers.
- Prep30 SFC-MS was equipped with stream injection and a 2757 open beds fraction collector (shown).



### Experimental Conditions

- Rapid SFC analytical screening was performed primarily on a number of chiral stationary phases and occasionally on achiral stationary phases only when chiral columns failed to achieve resolution of the analytes. The rationale behind this approach was based on the fact that CSPs through their chiral recognition nature can also be used to isolate structurally similar compounds.
- Polysaccharide-based CSPs, 5  $\mu$ m, 4.6  $\times$  100 mm columns (Chiral Technologies, Inc.), and achiral alkyl bonded stationary phases with the following sequences were screened: IA > IB > IC and 4-Ethylpyridine > Chromagabond Pyridine/Amide > ZymorSPHER-Pyridine/Diol > CN.
- Only Chiralpak IA, IB and IC columns were screened because of their high compatibility with a broad range of sample solubilizing solvents (i.e. DMSO, DMF) unlike the Daicel polysaccharide-coated CSPs.<sup>2</sup>
- The analytical screening was performed under gradient conditions with 0.2% IPAmine in methanol as modifier. In our hands, the use of 0.2% IPAmine helps achieve improved resolution of basic and/or halogenated mixtures, providing both good ionization patterns and enhanced loading capacity.
- A unique compound-specific preparative SFC gradient was derived from retention time of the compound and the elution profile in the analytical run, using an Accelerated Retention Window (ARW) approach.

## Results and Discussion

### Case Study #1 Separation and Purification of a Diastereomeric Mixture on a Chiral Stationary Phase

- Analytical SFC screening identified Chiralpak IA as the choice of stationary phase (Fig. 1) for resolving the mixture, which is fully un-resolvable on achiral SFC stationary phases and reversed-phase HPLC (data not shown).
- Compound-specific preparative SFC gradient was applied to successfully separate the mixture with results shown in Fig. 2.

Fig. 1. Analytical screening on Chiralpak IA, IB, IC vs achiral 4-EP

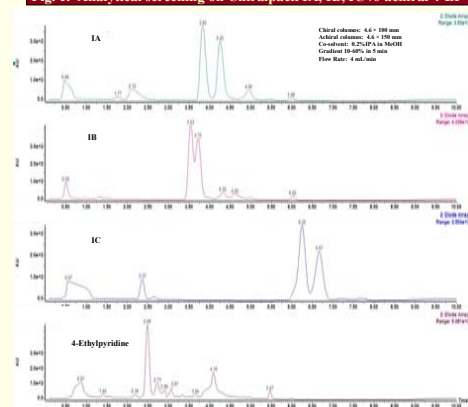
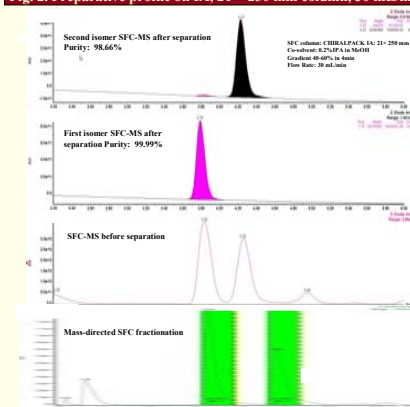


Fig. 2. Preparative profile on IA, 21  $\times$  250 mm column, 30 mL/min



### Case Study #2: Separation and Purification of a Challenging Regioisomeric Mixture on a Chiral Stationary Phase

- A complex mixture of regioisomeric pairs with their methylated impurities (+14 Da) was hardly resolvable on LC/MS but analytical SFC screening identified CHIRALPAK IC as the best choice of stationary phase to separate the challenging mixture (Fig. 3).
- Mass-directed preparative SFC purification of the mixture resulted in the isolation of the purified isomers as well as their methylated byproducts (Fig. 4).

Fig. 3. LC/MS and SFC-MS Analytical Profile of the Mixture

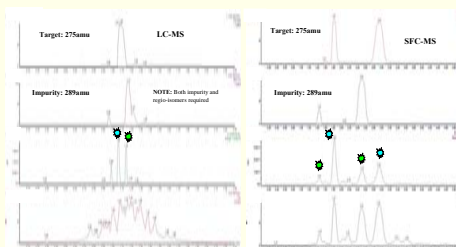
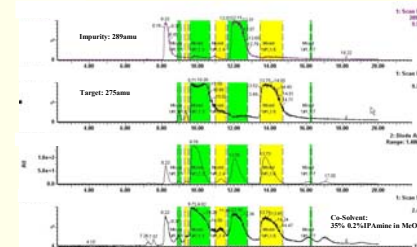


Fig. 4. Preparative profile on IC, 21  $\times$  250 mm, 30 mL/min



### Case Study #3: Separation and Purification of a cis/trans-Isomeric Mixture on an Achiral Stationary Phase

- Analytical SFC screening identified Chromagabond Pyridyl/Amide as the choice of stationary phase (Fig. 5 & Table 1) for resolving the mixture.
- Performance of preparative separation results is shown in Fig. 6. The isolated trans-isomer had a purity of ~99.99%, while the cis-isomer had a purity of 94.16%.

Fig. 5. LC-MS vs. SFC-MS after SFC analytical screening

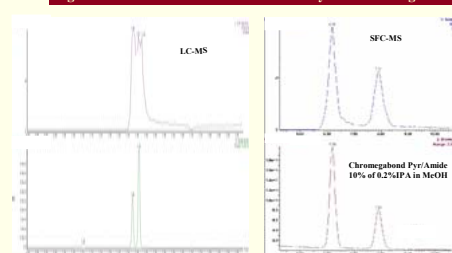


Fig. 6. Preparative profile on Chromagabond Pyr/Amide

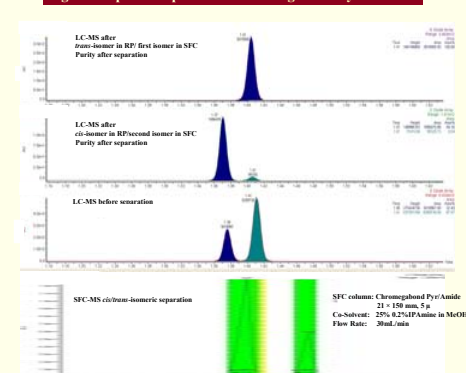


Table 1. Selectivity power at analytical level

LC-MS		SFC-MS							
RT <sub>1</sub>	RT <sub>2</sub>	k <sub>1</sub>	k <sub>2</sub>	$\alpha$	RT <sub>1</sub>	RT <sub>2</sub>	k <sub>1</sub>	k <sub>2</sub>	$\alpha$
1.38	1.41	4.75	4.87	1.03*	5.63	7.15	1.68	2.37	1.41

\*marginal separation; RT unit in minute.

NOTE: Reversed elution order of cis/trans-isomers on achiral columns in SFC was observed vs. in reversed phase HPLC and the same elution order (data not shown) on chiral columns in SFC.

## Conclusions

- An effective workflow using chiral stationary phases, in combination with compound-specific gradient methods for fast mass-directed SFC purification of complex mixtures that have little or no separation on reversed phase HPLC, has been established and validated.
- Superior performance, especially in the challenging separation of cis/trans-, stereo- and regio-isomers has also been demonstrated, with excellent performance in recovery (> 90%) and purity ( $\geq$  95%) for the resolved isomers.
- The chiral columns greatly outperformed the achiral ones in isomeric purification in terms of efficiency and separation power, offsetting the higher costs associated with the chiral columns.
- This work highlights the SFC as a complementary chromatographic tool to the commonly used reversed phase HPLC.

## References

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