

SFC-based Chiral Separations: The Selectivity Power of Dual Co-Solvent Systems

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Abstract

Supercritical fluid chromatography has emerged as the separation method of choice for the resolution of enantiomers in the pharmaceutical industry. The separation efficiency and loading capacity are both directly related to the identification of a scalable analytical method and the proper use of a polar mobile phase. We report in this presentation, case studies which highlight the enantioselectivity enhancements obtained through increasing the solvating strength of the mobile phase with dual co-solvent systems as applied to our most challenging separations of racemic mixtures. The effects of modifiers on retention and separation factors as well as column efficiency will also be discussed.

Purpose

To identify a method to separate a few hundred milligrams to a few grams of racemic mixtures with Thar Prep30 SFC-SFC-MS, without the implementation of stacked injection.

Experimental

Instrumentation

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



Experimental Conditions

- Rapid SFC analytical screening was performed to determine suitable enantiomeric resolution with good ionization patterns. Polysaccharide-based CSPs, 5 μ m, 4.6 \times 100 mm columns (Chiral Technologies, Inc.), with the following columns and 30% co-solvent sequences were screened: IA > IB > IC and MeOH > IPA > ACN, based on our previously reported optimized workflow (ref).
- Only Chiralpack 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.
- Analytical methods were directly scalable or were slightly modified to afford optimal preparative separation.

Results and Discussion

1. Primary Screening: Selection of a Stationary Phase

- A typical example is given, where analytical SFC screening identified Chiralpack IA as the choice of stationary phase (Fig. 1) for resolving the enantiomeric mixture (performed at 2 min runs).

Fig. 1. Primary analytical screening on Chiralpack IA, IB, IC CSPs, 4.6 \times 100 mm, 4 mL/min, 30% MeOH, 150 bar

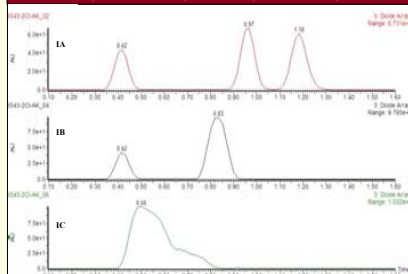
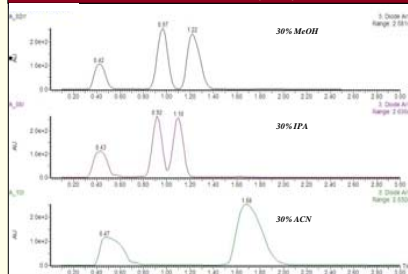


Fig. 2. Secondary analytical screening on Chiralpack IA with 30% co-solvent: MeOH, IPA, ACN



2. Secondary Screening: Identification of an Optimized Co-solvent System

- Initial secondary screening of 30% co-solvent systems on Chiralpack IA identified MeOH followed by IPA as the choice of single co-solvent mobile phase for further optimization (Fig. 2).
- Different combinations of MeOH with IPA were then screened to identify the dual co-solvent system with the highest separation factor and load capacity feasible for the preparatory separation (Fig. 3).
- The selectivity power of dual co-solvent systems on retention factor (k) and separation factors (α) is summarized in Table 1.
- Proof of concept was established on the semi-prep level (Fig. 4 & Table 2)
- Simulated stacked injections led to maximum purification output (Fig. 5).

Fig. 3. Secondary analytical screening on Chiralpack IA with 10% co-solvent, MeOH and different ratios of MeOH/IPA mixtures

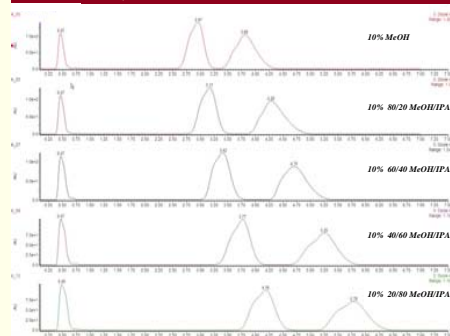


Table 1. Comparison of selectivity power at analytical level

Co-Solvent	%	k ₁	k ₂	α
MeOH		4.94	6.60	1.33
MeOH/IPA	80/20	5.34	7.56	1.41
	60/40	5.84	8.40	1.44
	40/60	6.54	9.50	1.45
	20/80	7.36	10.81	1.47

Table 2. The effect of co-solvent composition on the separation factor at the semi-prep level

Co-solvent	%	k ₁	k ₂	α
MeOH		2.88	3.19	1.1*
MeOH/IPA	20/80	16.99	21.19	1.32

*marginal separation

Fig. 4. Preparative profile on IA, 21 \times 250 mm column, 30 mL/min, with 25% co-solvent: 20/80 MeOH/IPA and MeOH, 150 bar

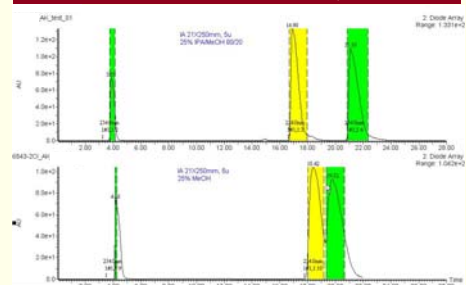
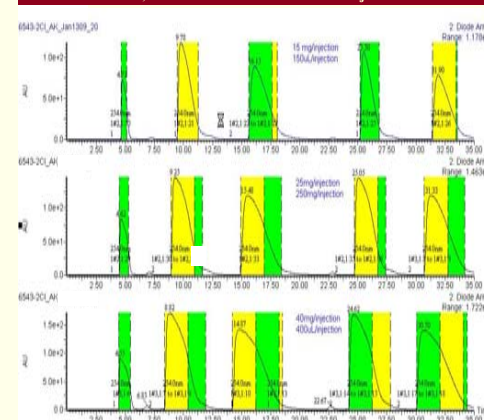


Fig. 5. Preparative SFC-MS on IA, 21 \times 250 mm, 30 mL/min, 25% 80/20 MeOH/IPA, 150 bar with simulated stacked injections



Conclusions

- The dual co-solvent system was superior to a single co-solvent system and offered better separation efficiency.
- The Thar Prep30 SFC-MS system provided excellent reproducibility and implementation of the dual co-solvent system and simulated stacked injections enabled preparative separation of up to gram scale of racemates with a significant reduction of the overall purification time.
- Excellent separation performance was usually observed with high recovery (> 80%) and purity of the resolved enantiomers (ee \geq 98.5%).
- The dual co-solvent system has been successfully applied to other challenging separations of racemic mixtures.

Reference

Vivi Lazarescu, Yingjie Li, Mark J. Mulvihill and Lifu Ma: poster presented at the 2nd International Conference on Packed-Column SFC in Zurich, Switzerland, October 1-2, 2008.

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