Preparative SFC Separation of the Enantiomers of a Poorly Soluble Intermediate Using a Methyl-THF/MeOH Modifier Mixture

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Abstract

A preparative SFC separation method for resolving the enantiomers of a pharmaceutical development candidate is described. This method was used to prepare 2 kilograms of enantiopure intermediate that was used in the preparation of 2.2 kilograms of enantiopure API. SFC was chosen because of solvent saving relative to conventional HPLC.

SFC Screening

A crude mixture was screened on the analytical SFC to determine the best column for preparative separation. The sample was screened using several commercial available chiral stationary phases. The columns (5 μm, 250 x 4.6 mm) screened are as follows:

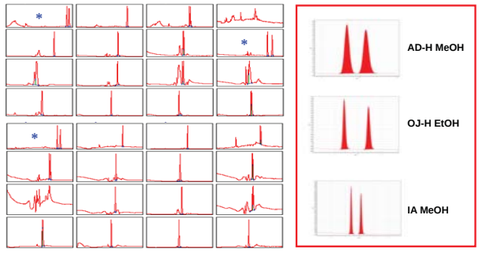
Coated phases: Chiralpak AD-H, Chiralpak AS-H, ChiralCel OJ-H, ChiralCel OD-H, Lux Cellulose-4

Immobilized phases: Chiralpak IA, Chiralpak IB, Chiralpak IC

Screening conditions:

4% modifier/CO_2 for 2 mins, ramp to 40% modifier/CO_2 at 4% modifier/min with a hold until the end of the run, 2.4 mL/min, 100 bar, 35°C, 220 nm, run time 15 minutes.

MeOH, EtOH, IPA, and MeCN as polar modifiers



Sample Preparation Issue

Sample solubility studies were performed at the outset. The choice of SFC diluent was limited due to the poor solubility of the intermediate in many organic solvents.

The sample was slightly soluble in MeCN, MeOH, DMF and DMSO. In addition, the sample was found to be soluble in pure CH_2Cl_2 following extended sonication. The feed was prepared by dissolving in CH_2Cl_2 to a concentration of 50 mg/mL.

In order to avoid the possibility of CH_2Cl_2 stripping of coated stationary phases, an immobilized stationary phase (Chiralpak IA) was chosen for this preparative separation.

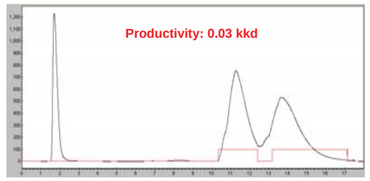
The separation was performed using a Multigram III SFC system. Conditions: Chiralpak IA (50 x 250 mm, 5 μm), 45% MeOH/ CO_2, 215 mL/mL, 100 bar, 35°C, 220 nm, 50 mg/mL in CH_2Cl_2, 1.5 mL/inj.

The sample precipitated in the sample loop and the pre-column frit became clogged after only two injections, suggesting that crystallization is occurring when the dichlormethane stream is mixed with CO_2.

Solution: THF showed superior solubility for the intermediate, and use of THF as the feed diluent at a concentration of 100 mg/mL showed no precipitation or insolubility issues in the separation.

Results

First Pass on Preparative SFC

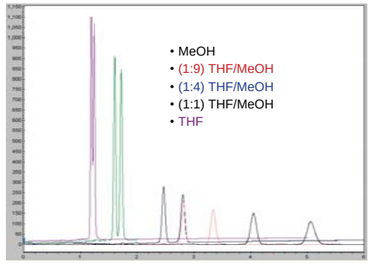


Chiralpak IA (51.6 x 250 mm,5 μm), 40% MeOH/CO_2, 230 mL/min, 35°C, 100 Bar, 220 nm, 100 mg/mL in THF, 1.5 mL inj, 450 second cycle time

Not feasible for a preparative scale separation because of the poor peak shapes

Impact of THF on SFC

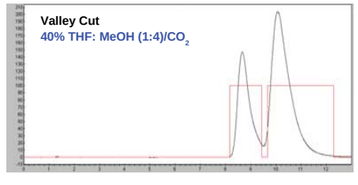
Broad peaks were observed when pure MeOH was used as modifier. Is sample precipitation and re-dissolution contributing to poor peak shape? Will the problem be minimized by using mixture of THF and methanol or even pure THF as the modifier?



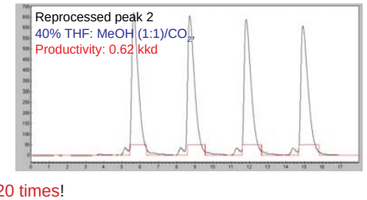
Chiralpak IA (250 x 4.6 mm), 40% co-solvent/CO_2, 5 mL/min, 100 Bar, 40°C, 300 nm

Second Pass on Preparative SFC Using THF/MeOH as Modifier

Chiralpak IA (51.6 x 250 mm, 5 μm), 230 mL/min, 35°C, 100 Bar, 300 nm, 100 mg/mL in THF, 1.5 mL inj, 270 second cycle time

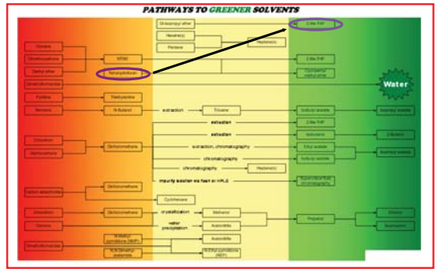


Chiralpak IA (51.6 x 250 mm, 5 μm), 230 mL/min, 35°C, 100 Bar, 300 nm, 100 mg/mL in THF, 4.5 mL inj, 175 second cycle time

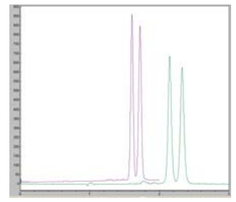


Productivity increased by 20 times!
Better peak shapes and shorter run time observed with modifier of 50% THF/MeOH

How About Greener Solvent 2-methyl-THF?

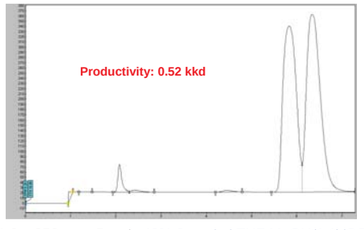


Greener Solvent 2-methyl-THF Has a Similar Eluting Power as THF



Chiralpak IA (250 x 4.6 mm), 40% co-solvent/CO_2, 5 mL/min, 100 Bar, 40°C, 300 nm

Optimized "Green" Preparative SFC Separation



Chiralpak IA (51.6 x 250 mm, 5 μm), 40% 2-methyl THF:MeOH(1:1)/CO_2, 200 mL/min, 35°C, 100 Bar, 300 nm, 150 mg/mL in THF, 3 mL inj, 120 second cycle time

Conclusions

- Sample solubility sometimes limits column selection on the preparative separation.
- Immobilized stationary phase column shows no deterioration on SFC when THF or 2-methyl THF was used as modifier.
- In this case, 2-methyl THF behaves similar to THF. Either of them can improve the productivity.
- The "greener" SFC preparative method was used for the 1st delivery of 2 kilograms enantiopure intermediate successfully. It will be considered as a possible route for the 2nd delivery of 15 kilograms enantiopure intermediate.