Exploring the Use of Packed Column Preparative SFC for Pre-clinical Research at Merck

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Introduction

SFC (supercritical fluid chromatography) is a very useful and environmentally "greener" separation technology. Compared with traditional HPLC, SFC has many advantages such as solvent cost saving, better peak shape, and shorter retention time. For several years, SFC with packed-columns has been a preferred replacement for HPLC for the large number of separations/purifications required to accelerate our pre-clinical research at Merck. In this poster we will explore the applications of preparative SFC for chiral resolution and purity upgrading of APIs (active pharmaceutical ingredients) or drug intermediates, and for the use in impurity isolation. Recent experience in the choice of columns, the use of different modifier solvents, and the impact of modifier additives for the preparative SFC separation/purification in our research will be presented and illustrated with several case studies.

Experimental

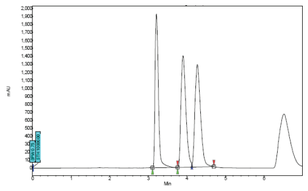
Preparative SFC instrument:	Berger MultiGram III Berger MultiGram II
Analytical SFC instrument:	Mettler Toledo – Berger MiniGram, Berger Analytical SFC
Column for SFC separation:	ChiralPak AD, ChiralPak AS, Chiralcel OD, Chiralcel OJ, ChiralPak IA, ChiralPak IC from Chiral Technologies Inc. Cyano 60A 5u from Princeton Chromatography Inc. Ethylpyridine from Princeton Chromatography Inc.
For analytical SFC:	4.6 X 250 mm, 5µ columns
For preparative SFC:	21 X 250 mm, 5µ 30 X 250 mm, 5µ 50 X 250 mm, 5µ (or 20µ)

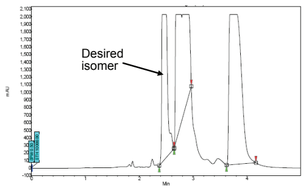
Explore the Application of SFC

Chiral Resolution

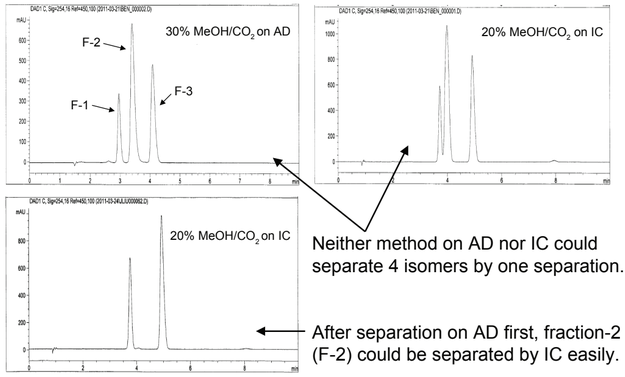
The most SFC separation work we do is chiral resolution, which can save a lot of asymmetrical synthesis work for early drug discovery research. Because of its advantages over traditional HPLC, SFC replaces HPLC as major technology for thousands of chiral resolution at Merck to significantly reduce time, manpower, and solvent cost. For some API, the desired isomer could be identified after the first resolution of a small sample. Then the separation can be quickly scaled up to provide large amount of API sample for continuing research.

First sample: 0.41 g, 4 isomers were collected	Second sample: 3.7 g, 1st isomer is desired
30 inj, 4 min interval time, total 70 min	60 inj, 2 min interval time, total 140 min (vs. 632 min)





SFC is also excellent for the challenging chiral resolution to a sample with two chiral centers. For these samples two separations on two different columns usually are better and faster than one difficult separation with one column.



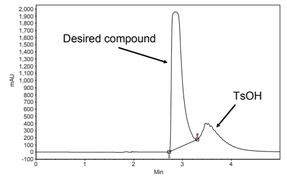
Neither method on AD nor IC could separate 4 isomers by one separation.

After separation on AD first, fraction-2 (F-2) could be separated by IC easily.

Explore the Application of SFC (cont'd)

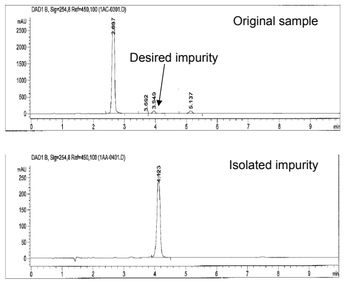
Purity Upgrading

More and more purity upgrading job is being completed by preparative SFC, because SFC uses much less solvent, affords better peak shape, and can easily run stacked injections. One interesting case in our work is the salt breaking of an API sample. The sample is a partial toluenesulfonic acid (TsOH) salt from unsuccessful salt breaking via neutralization. Purification was needed to get pure compound for crystallization investigation. SFC purification (50% MeOH (0.2% DEA)/CO_2 on OD column) resulted in complete salt breaking and the first crystalline solid sample of the compound.

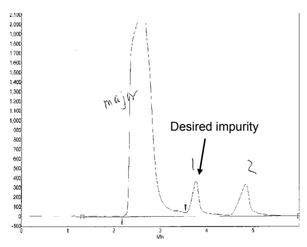


Impurity Isolation

The isolation of impurities is important for understanding reaction mechanism, ensuring the quality of API, and increasing the purity and/or yield of desired product. Because HPLC is a very popular tool in pre-clinical research, the request of impurity isolation via reverse phase LC (RPLC) often be submitted by our chemists. Sometimes SFC is a "greener" and faster alternative way to meet these requests. Following example shows our recent success for the isolation of 50 mg impurity from a 10 g sample in few hours.



Preparative SFC isolation:
20% 2:1 MeOH:MeCN/CO_2 on Ethylpyridine



Choice of Packed-columns and Modifiers

Our experience based on our thousands of preparative SFC separation/purification:

- 99% of chiral resolution and/or purity upgrading job could be completed on ChrialPak AD, AS, IC, IA, Chrialcel OJ, OD columns, or their combination. Most of our achiral purification and/or impurity isolation were completed on Cyano, and Ethylpyridine columns.
- If possible, the method developed by using column IA and IC should be the first choice because these immobilized columns can tolerate almost all organic solvent as modifier. Very common solubility issue in the separation/purification could be solved by adding DCM, DMF,THF, Methyl-THF (see the poster of our colleagues) or other organic solvent into alcoholic modifier on these columns.
- Methanol is our primary alcoholic modifier for preparative SFC separation because of its relatively good solubility, low costing and good peak shape.
- Hexane or heptane is usually added to modifier for the separation of some very non-polar samples to increase modifier percentage and keep sample in solution.
- The mixture of alcohols and acetonitrile is often a "magic" modifier to separate some sample with solubility issue.
- Sometimes modifier change may switch peak order. Feeding solvent change may increase productivity of some separation.

Conclusions

- SFC as a very useful and environmentally "greener" separation technology could find and accelerate many applications in pre-clinical research.
- Chiral resolution, purity upgrading, and impurity isolation can be well done by SFC with a few packed columns instead of traditional HPLC.
- The correct or better choice of modifier for preparative SFC separation/purification is important to ensure the successful and productive results.
- The addition of acid or base in the modifier may significant improve the separation result.

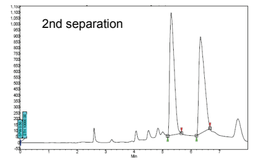
Experience in Recent Research

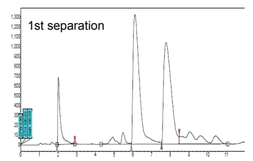
Impact of Modifier Solvent

An excellent analytical method may not ensure the success of preparative separation. We had some unsuccessful separation due to the sudden clog of column by sample crystallization. In the first SFC separation of a sample when MeOH (0.2% DEA) was used as modifier, high pressure and significant sample loss were observed. After we changed modifier to 2:1 MeOH:MeCN for the second separation of the same sample, separation ran well without any issue and productivity (KKD) increased to 0.266 from 0.0473 (5.6 times higher).

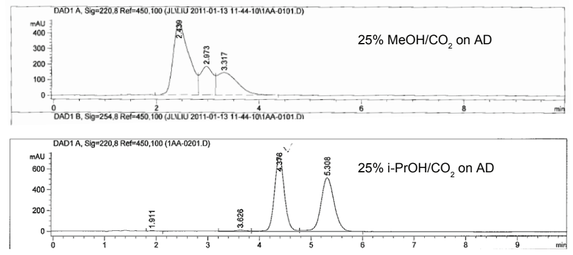
KKD = Kg of enantiomer/Kg of chiral stationary phase/Day

30% 2:1 MeOH:MeCN/CO2 on AD (21 X 250 mm), 70 ml/min,220 nm, 48 mg/ml in MeCN/DCM, 1.8 ml/inj	15% MeOH (0.2% DEA)/CO2 on AD (21 X 250 mm), 70 ml/min, 220 nm, 10 mg/ml in MeOH, 1.0 ml/inj





Beside solubility, the stability of separation sample in modifier solvent(s) may impact SFC preparative separation too. Recently we run method screening of a sample with MeOH as modifier and had very confusing results. It looked like the sample was very impure, but our customer chemist strongly disagreed with our conclusion. When we changed modifier solvent to i-PrOH, the separation method for the sample was well developed.

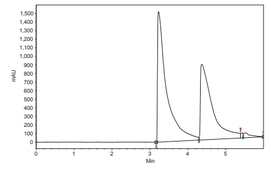


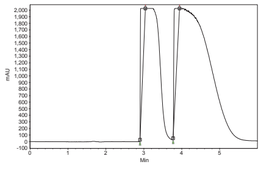
Impact of Basic Additive

Broad and tailing peaks are frequently observed in the SFC separation of the sample with basic function group (such as amine or pyridine). Basic additive, such as i-butylamine (IBA) or diethylamine (DEA), in the modifier may improve peak shape and increase productivity. The following example clearly shows the impact of basic additive to SFC separation. After addition of DEA in modifier, the separation was completed with better quality and productivity (KKD 0.462 vs. 0.077).

KKD = Kg of enantiomer/Kg of chiral stationary phase/Day

30% MeOH/CO_2 on AD (30 X 250 mm), 70 ml/min, 235 nm, 100 bar, 35°C, 40 mg/inj	30% MeOH (0.2% DEA)/CO_2 on AD (30 X 250 mm), 70 ml/min, 235 nm, 100 bar, 35°C, 239 mg/inj





Impact of Acidic Additive

For the sample with acid group the addition of acid in the modifier may significantly improve peak shape, which makes the separation possible and easier. We could not find a practically useable method for the SFC separation of following sample under neutral conditions. The sample was successfully separated after trifluoroacetic acid was added into modifier.