

Chiral SFC in Support of Drug Discovery: Basic Research through Preclinical Development

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SFC Is Used Throughout Drug Discovery Process

Chirality plays an important role in drug action and much effort has been invested in developing tools to support its study in drug development. Supercritical fluid chromatography (SFC) has become an extremely useful technique for chiral analysis and purification due to its speed and environmentally friendly nature. This poster details our application of SFC techniques to aid drug discovery on a range of scales from basic research through pre-clinical development.

Why Supercritical Fluid Chromatography?

- "Green" technique
 - Low volume of solvents collected (minimize solvent use and waste as well as simplify)
 - Ability to recycle CO₂
- Speed
 - Lower viscosities/higher diffusivities allow for faster flow rates and shorter analysis times
- Selectivity
 - Complementary to HPLC
- Excellent mass recovery (routinely >90%)
- Scale-up
 - Methods translate well from Med. Chem. to Process scale (mg → g → kg)

Welch, Christopher J., et al. Preparative Chiral SFC as a Green Technology for Rapid Access to Enantiopurity in Pharmaceutical Process Research. LCGC, Vol. 23, No. 1, (2005)

SFC Application in Basic Research

Basic Research (Medicinal Chemistry, Discovery)

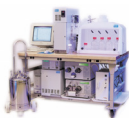
- Chiral entities isolated for initial biological screening
- Milligram-gram scale
- Multiple components routinely desired
- Focus on speed/throughput of many chemically diverse samples

Limited screening for first pass (i.e. keep it simple)



- 4 columns (AD, OD, OJ, AS)
- 3 solvents (MeOH, IPA, EtOH)
- Gradient (4-40% modifier) or isocratic (5, 15 or 30% modifier) screens
- Success rate for finding a suitable method to translate to preparative method is >90%

Semi-Preparative SFC in Basic Research



Berger Multigram II

- 50-70 ml/min, 2 cm columns
- good for smaller scale separations (mgs to tens of grams)
- fully automated sample injection and fraction collection

Berger Multigram III

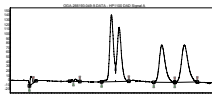
- 70-200+ ml/min, 2-5 cm columns
- good for mid-scale separations ~1-100+ grams
- fully automated sample injection and fraction collection



Small scale Separation of Multi-component mixture

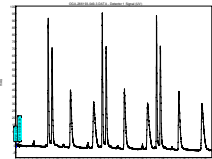
Analytical...

•40mg of diastereomeric mixture (4 components of interest)



...to Prep

•Screening results transferred to prep to isolate 4 components in 90% overall yield



SFC Application In Process Research

Process Research (Early Development)

- Chiral entities scaled up for advanced safety studies
- gram-kilogram scale
- Usually one enantiomer has been identified as target
- Focus on productivity of separation conditions as related to time/cost

More extensive screening

- 12 column gradient screens, tandem column screening focusing on selectivity, elution order.
- Loading studies to determine productivity or kkd (kg purified enantiomer/kg stationary phase/24 hr day)
- Routine comparison SFC vs. HPLC productivity

Preparative SFC in Process Research

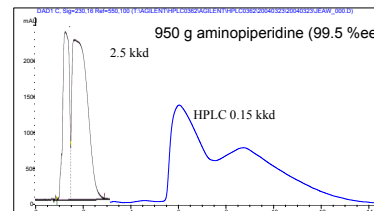


SuperPure 350
(350 g/min, 5 cm column)



SuperSep 50
(1 kg/min, 6 or 8 cm column)

Comparison of SFC/HPLC Chromatograms



Leonard, William, et al. Strategic Use of Preparative Chiral Chromatography for the Synthesis of a Preclinical Pharmaceutical Candidate. Chirality 19:693-700 (2007).

Preparation of 1 kg Enantiopure Aminopiperidine



SFC vs HPLC

16 x reduction in solvent utilization

| | HPLC | SFC |
|--------------------------|-------------------------------|--|
| Productivity (kkd) | 0.15 | 2.5 |
| Column Diameter | 30 cm | 6 cm |
| Instrument Time | 12 h | 20 h |
| Total Solvent Used | 4,800 L (10% EtOH/heptane) | 300 L (60% i-PrOH/CO ₂) |
| Total Solvent Evaporated | 800 L | 64 L |

SFC Application in Analytical Research

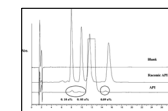
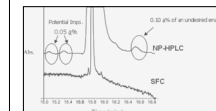
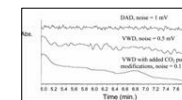
Analytical Research

- Chiral methods needed for release testing of active pharmaceutical ingredients (APIs)
- Focus on sensitivity/reproducibility of methods

•Use of SFC as a tool for chiral analysis in GMP release testing has been limited due to signal to noise issues

- Thermal, electronic, and mechanical sources of noise have been identified as key contributors to reduced sensitivity and several approaches have been taken to address these issues (i.e. pump, detector flow cell modifications)

Improve Sensitivity by Lowering Mechanical Noise



Assay results using standard pump configuration w/ variable wavelength detector (WVD)

Assay results using modified pump configuration w/ WVD (noise level reduced from 0.5mV to 0.1mV)

Helmy, R., et al. Improving Sensitivity in Chiral Supercritical Fluid Chromatography for Analysis of Active Pharmaceutical Ingredients. Chirality, published online (2007).

Conclusion

SFC has proven to be an extremely useful and flexible technique for chiral analysis and purification. As a result, this tool has been successfully implemented to accommodate the needs of a variety of research areas at Merck, from Basic Research through Preclinical Development.

Acknowledgments

| Merck Customers | Vendors |
|---------------------------------------|-----------------------------------|
| Merck Chemical Process Research | Mettler Toledo-Berger Instruments |
| Early Development Analytical Research | Thar Technologies |
| | Novasep |
| | Chiral Technologies |