

# **Supercritical Fluid Chromatography - A Phase Appropriate Technology for Pharmaceutical Development**

---

**Tony Q. Yan, Fang Xia, J. Preston**

**Process Analytics and Separation, Chemical Process  
Research and Development, Amgen, Inc. Amgen One  
Center Dr. Thousand Oaks, CA 91320**

# Agenda

---

- Introduction
  - Case Studies of SFC Purifications for Small Molecule Pharmaceutical Development at Amgen
  - Comparisons of SFC with HPLC Processes
  - Conclusions
-

# What is Phase Appropriate Technology for Pharmaceutical Development?

---

- The ability to develop the method rapidly and the subsequent ability to scale up the method to turn around the samples quickly
  - The phase appropriate technologies are necessary in order to shorten the drug development timeline:
    - Fast to move the candidates to the clinic trails
    - Fast to kill the candidates since the surviving rate is low at the early stage of the development
-

# Advantages of Chromatography

---

- Fast:**
    - Able to turn around the samples quickly to meet the tough time-lines for various tox. batches
  - High purity:**
    - Able to meet the purity requirement, for most tox. supplies, purity >97% and no single impurity >1%
  - High yield:**
    - Able to collect as much of the desired component as possible
-

# Phase Appropriate Technologies

---

The following chromatographic technologies can turn the samples around quickly:

- Supercritical fluid chromatography (SFC)
- Batch HPLC
- Steady state recycling (SSR)

*SFC should be the first thing to try because of its rapid processing time and less solvent usage (Green chemistry).*

---

# **Supercritical Fluid Chromatography (SFC) (Advantages)**

---

- **Due to lower viscosities and higher temperature conditions, it allows for running chromatography at faster flow rates**
  - **Opportunity to use less solvent in final fraction**
    - **Depending on the conditions also overall less solvent usage**
  - **Allows use of small particle diameter columns (typically 5 micron prep)**
  - **Transfer methods to and from LC easily**
    - **Sometimes the only way to scale the separation of chiral compounds**
-

# Supercritical Fluid Chromatography (SFC) (Disadvantages)

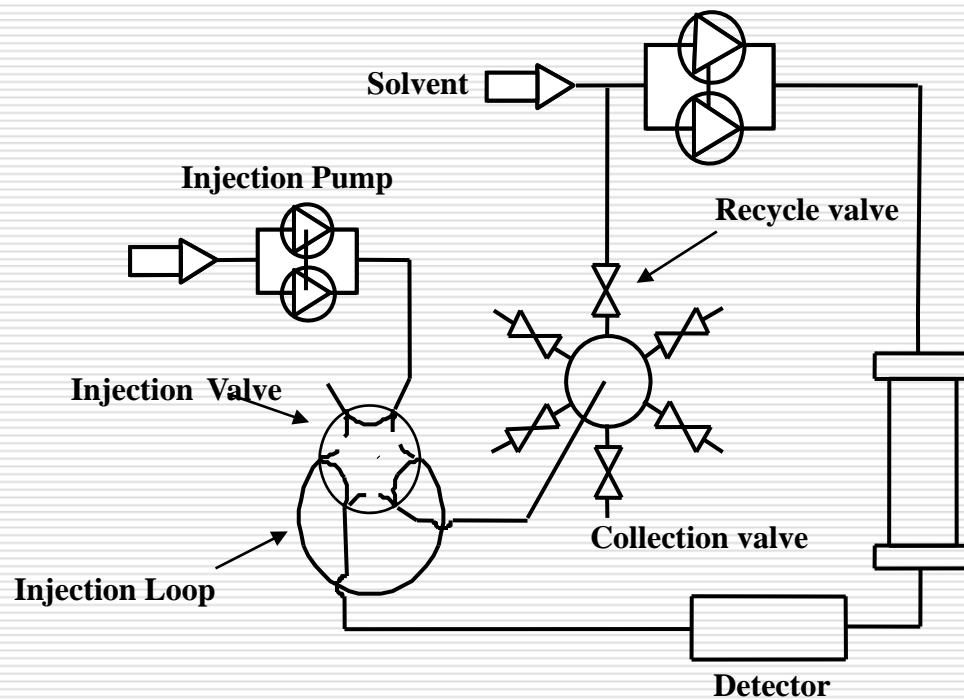
---

- Capital cost is relatively high
    - Kilo per minute SFC system:  
>\$500,000
  - System reliability is less than HPLC
    - We observe that SFC has more down time than HPLC
  - Sample solubility
    - Limited sample scope
  - Batch mode separation
    - SFC-SMB
-

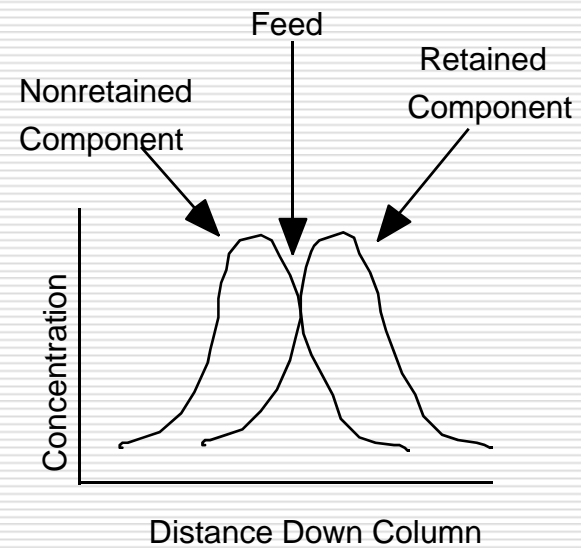
# Steady State Recycling (SSR)

---

## SSR system



## SSR profile



# Preparative Chromatography Systems in Amgen (PD at Thousand Oaks Site)

---

- ❑ SFC system from Novasep with ~275g CO<sub>2</sub>/min pump was frequently used for chiral purifications (g to kg) on 1" and 2" chiral columns
  - ❑ SSR systems from Hitachi with 800 ml/min pump were routinely used for large scale binary separations (~100g to kg scale) on 4" and 2" chiral columns
  - ❑ GMP purification suite (6" load and lock column and 2.5 liter per minute HPLC system with SSR capability)
  - ❑ Adding a Kilo per minute SFC system next year
-

# Example#1: CRCX3 Intermediate

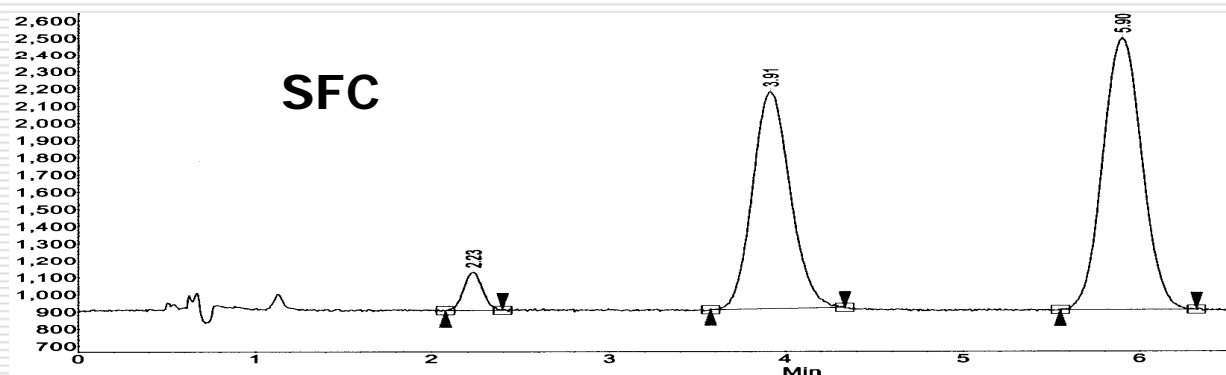
---

- Intermediate of early development candidate, ~70g diastereomer
  - Peak-2 is the desired isomer
  - Timeline: two weeks
  - What is best chromatographic approach for resolution?
-

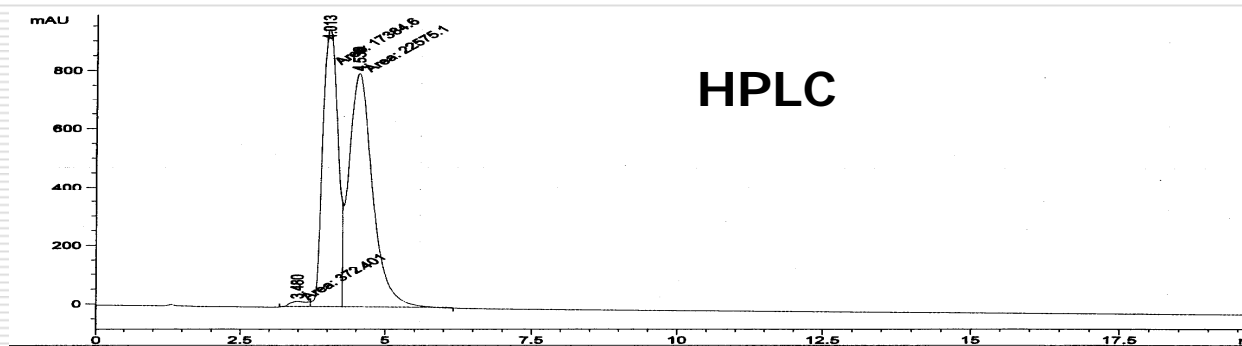
# SFC and HPLC Purifications

## Analytical Method Development

---



Chiralcel-OD-H, 5 $\mu$ , 4.6x250mm, 20% MeOH as co-solvent



Chiralcel-OD, 10 $\mu$ , 4.6x250mm, 1.0ml/min, 100% ACN

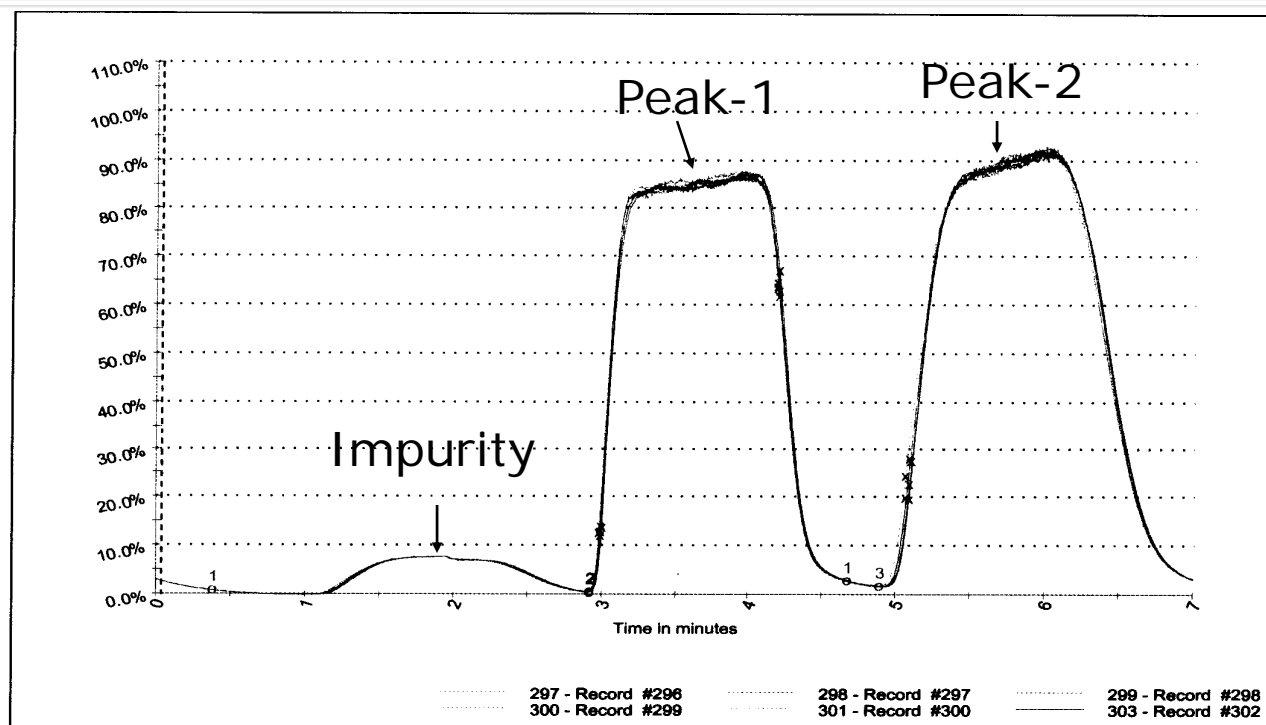
---

# SFC Purification Conditions

---

- ❑ SFC system: Novasep (Supersep 30/50)
  - ❑ Column: Chiralcel-OD-H, 5u.
  - ❑ Size: 3cmx25cm, Chiral Technology
  - ❑ Flow rate: 160g CO<sub>2</sub> with 20% methanol as co-solvent
  - ❑ Temperature: 40°C
  - ❑ Pressure: 100 bar
-

# Preparative SFC Chromatogram



**70mg per cycle; Cycle time: 7 minutes; Cycles: ~1000,  
Chiralcel-OD-H, 3cmx25cm, 160g CO<sub>2</sub>/min, 40°C, 100bar**

## Example#2: PKB Intermediate

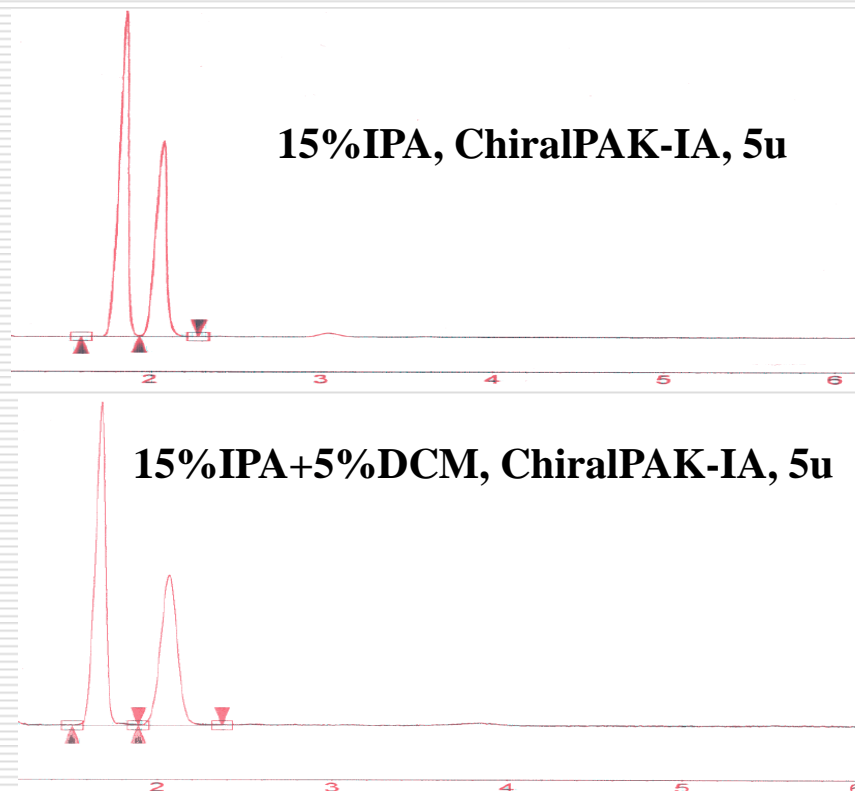
---

- Intermediate for an early tox. study, ~300g racemate
  - Peak-2 is the desired enantiomer
  - Timeline: very urgent
  - SFC was used to process the sample
  - How to improve the SFC separation on ChiralPAK-IA column?
-

# SFC Purification

## Analytical Method Development

---

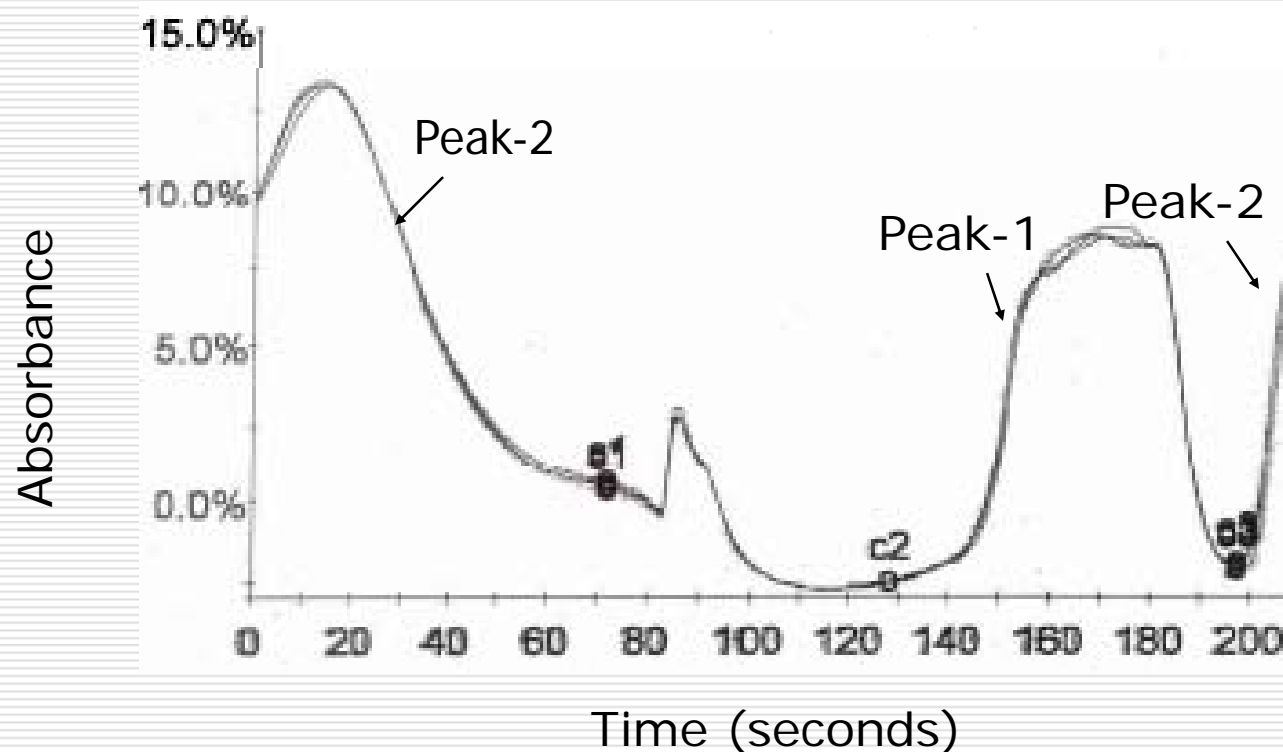


**Flow rate: 3g CO<sub>2</sub>/min, 40°C, 100bar, column size: 4.6x250mm**

---

# Preparative SFC Chromatogram

---



**160g CO<sub>2</sub>/minute, 20% co-solvent (IPA:DCM:3:1), 40°C, 100bar**

**5x25cm, 5 $\mu$ , ChiralPAK-IA, ~205mg/cycle, ~300g in ~2days**

---

# Example#3: 11 $\beta$ HSD Intermediate

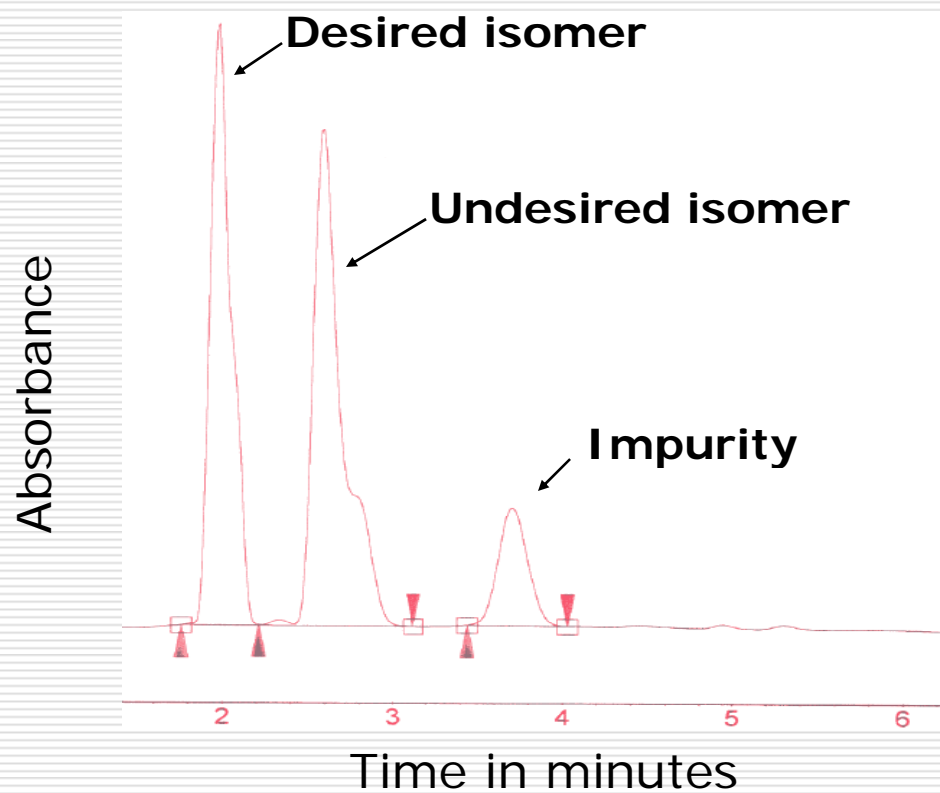
---

- Intermediate for a GLP tox. study, ~590g distereomers
  - Peak-1 is the desired isomer
  - Sample also contains an impurity at ~20%
  - Timeline: two weeks
  - SFC was used to process the sample
  - SFC results were compared to HPLC results
-

# SFC Purification

## Analytical Method Development

---

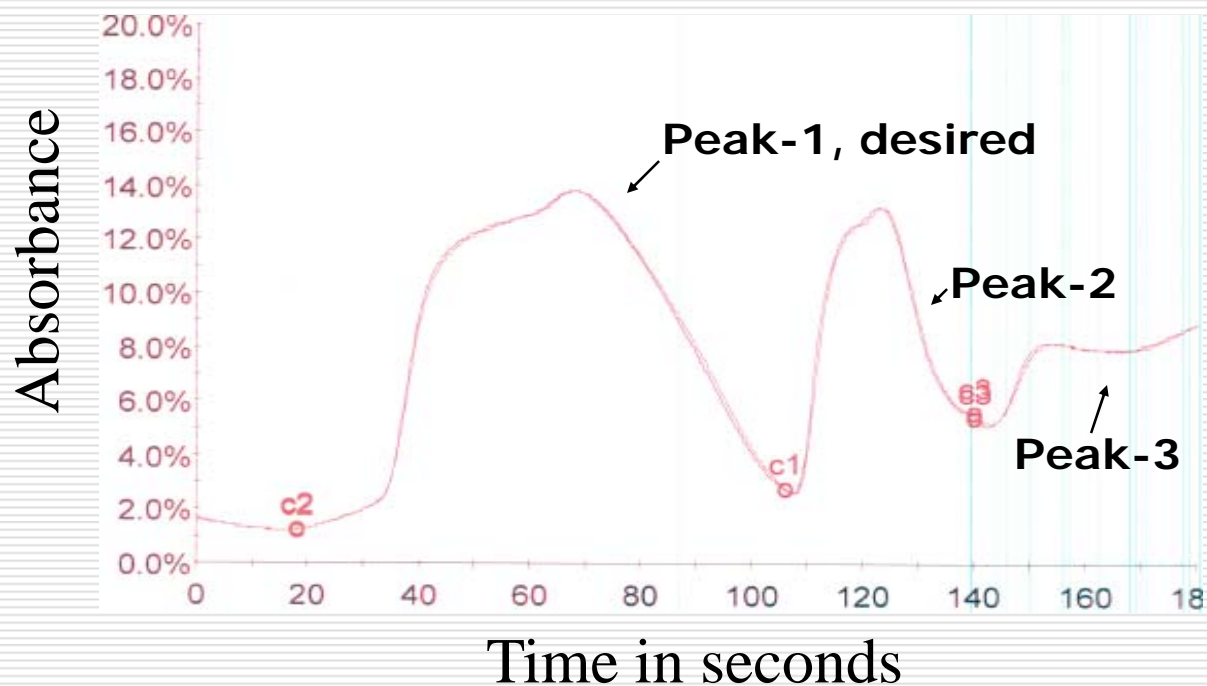


**ChiralPAK-AD-H (4.6x250mm), 5 $\mu$ , 3g CO<sub>2</sub>/min, 20%MeOH+0.1%IPAmine**

---

# Preparative SFC Chromatogram

---



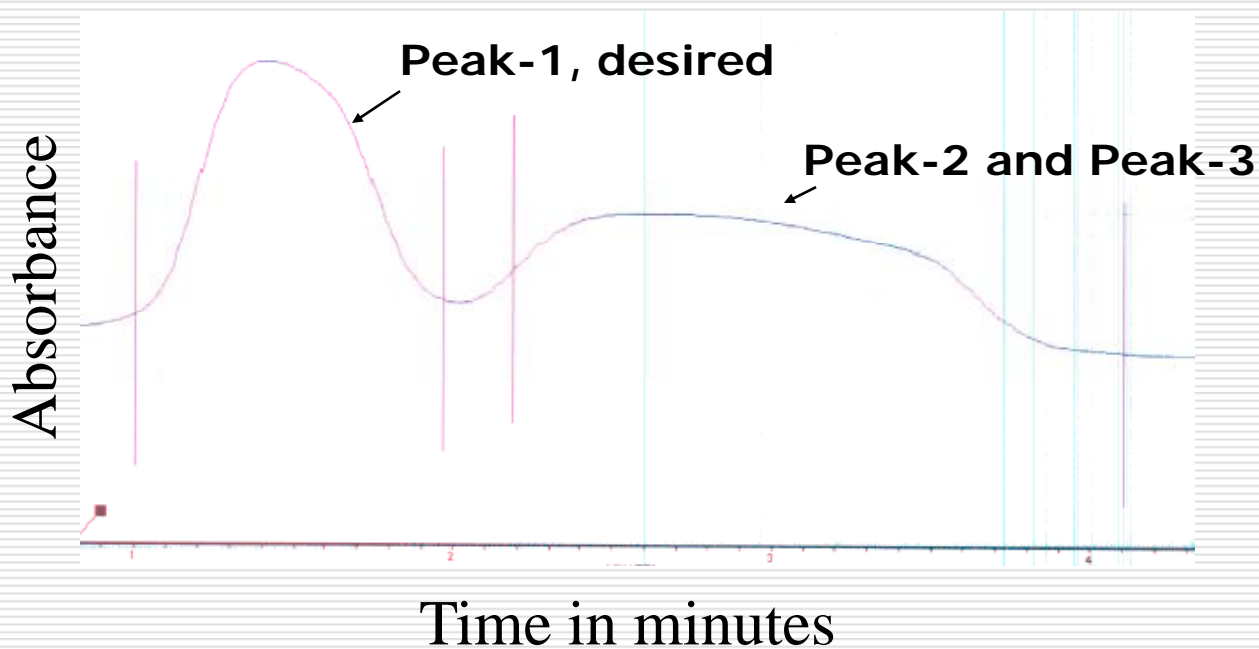
**170g CO<sub>2</sub>/minute, 25% co-solvent (methanol)+0.1%IPAmine, 40°C, 100bar**

**5x25cm, 5u, ChiralPAK-AD-H, ~149mg/cycle, ~590g in ~3days**

---

# Preparative Batch HPLC Chromatogram

---



**400ml/minute, 100% methanol as mobile phase, 30°C**

**10x35cm, 20u, ChiralPAK-AD, ~500mg/cycle, ~60cycles**

---

# Example#4: CRTH2 Intermediate

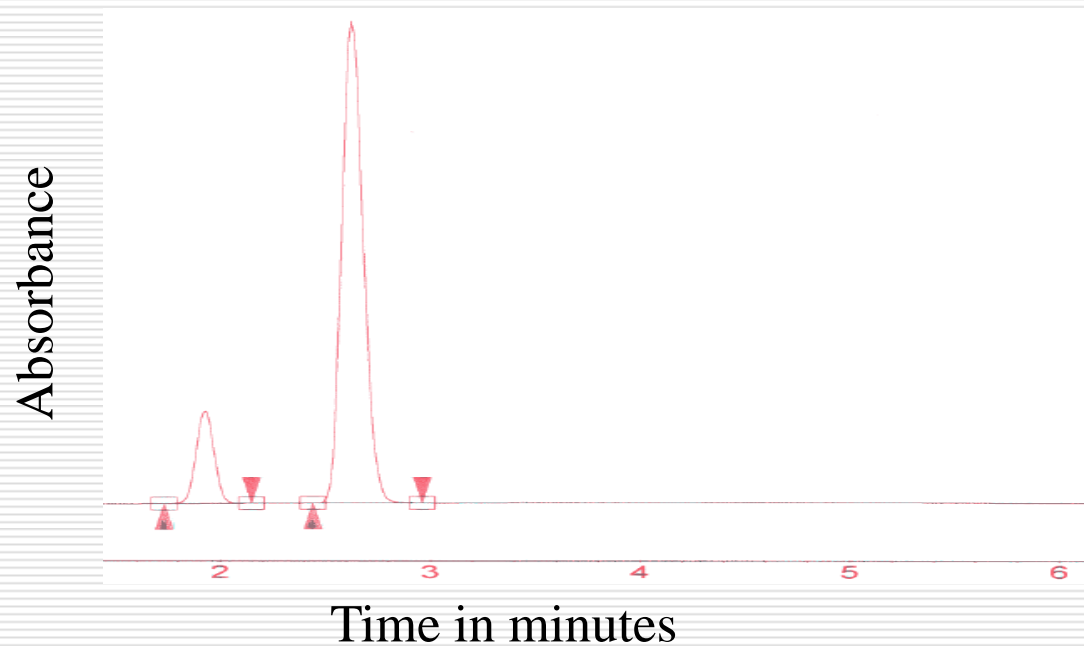
---

- ❑ Intermediate for a GMP tox. lot, ~2000g material
  - ❑ Sample contains a 5% impurity by weight analysis
  - ❑ This impurity was very difficult to be removed by classic resolution and conventional chromatography
  - ❑ Timeline: very urgent
  - ❑ SFC and SSR were used to process the sample
-

# SFC Purification

## Analytical Method Development

---

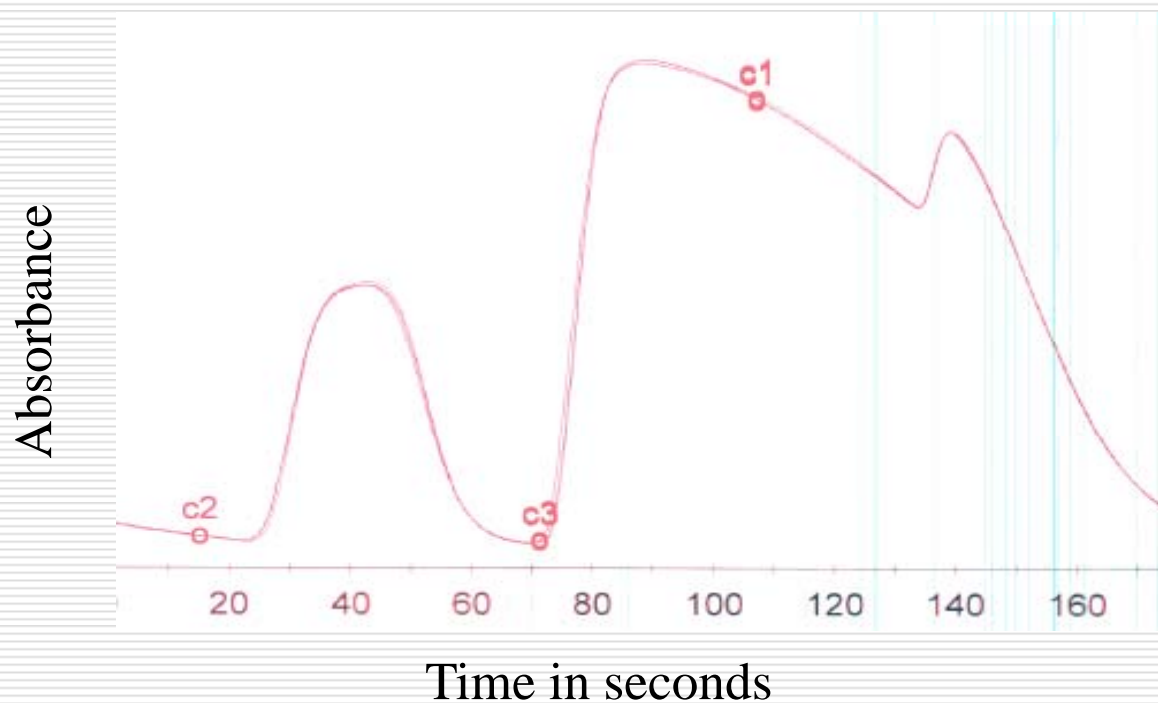


**ChiralPAK-AS-H, 5u, 4.6x250mm, 20% methanol and  
0.1%IPAmine as co-solvents, 3g CO<sub>2</sub>/minute, 40°C, 100bar**

---

# Preparative SFC Chromatogram

---

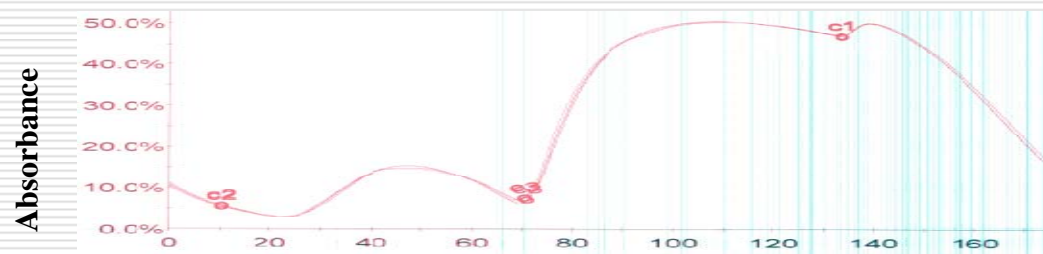


**180g CO<sub>2</sub>/minute, 25%MeOH as co-solvent+0.1%IPAmine, 40°C, 100bar**

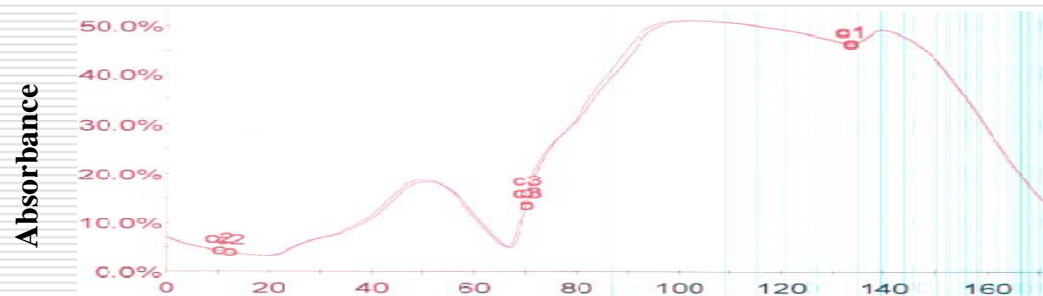
**5x25cm,5u, ChiralPAK-AS-H, ~215mg per cycle, ~1.3kg in ~one week**

---

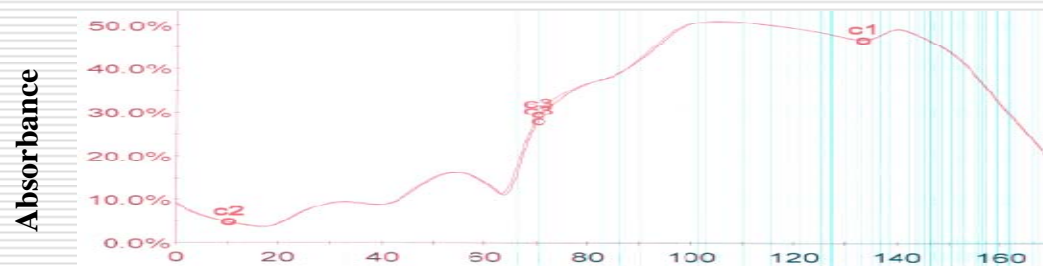
# Preparative SFC Chromatogram



After ~24 hrs



After ~30 hrs



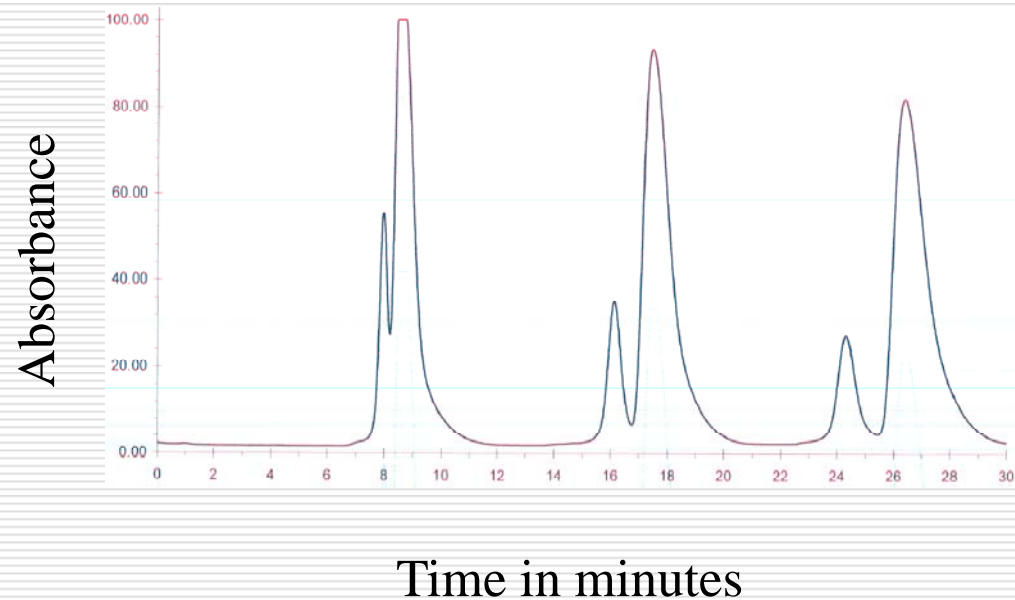
After ~36 hrs

Time in seconds

# SSR Purification

## SSR Method Development: 3 Conventional Cycles

---



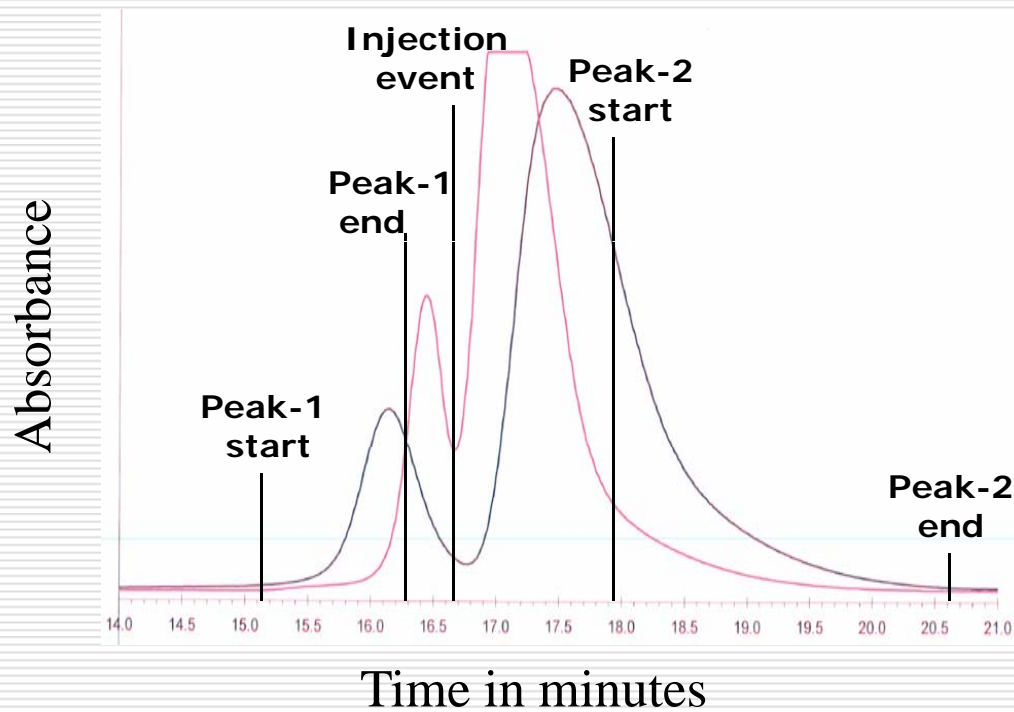
**ChiralPAK-AS, 20u, MODCOL spring load column, 10x35cm,  
100% methanol and 0.1%DEA as mobile phase, 400ml/min, 30°C**

---

# SSR Purification

## SSR Method Development: Overlay Profile

---

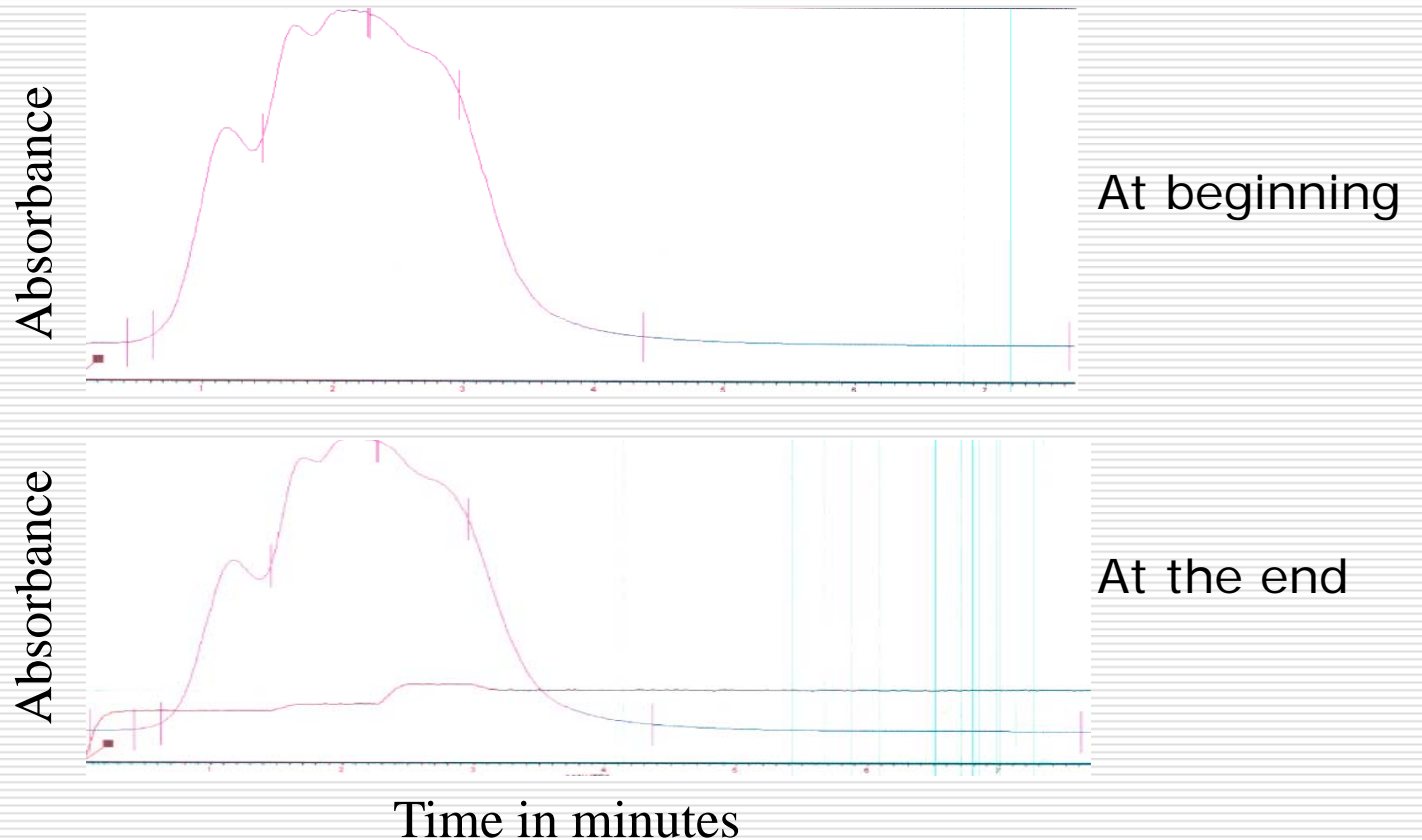


**Overlay the first cycle into the second cycle in Excel**

---

# SSR Purification Steady State Cycle

---



**~2.5g per cycle at ~8 minutes cycle time, ~1.3kg in <50hrs**

---

## Results of Example#4 Purification

---

- Purified ~1.30kg of crude material and generated ~1.15kg of the desired product at a purity of ~99% with the undesired impurity at 0.12%
  - With combined SFC and SSR efforts, we are able to turn around the sample from the impurity identification to isolation in ~ two weeks.
-

# Conclusions

---

- **The use of a phase appropriate technology such as SFC and SSR can accelerate the development of a drug candidate significantly**
  - **SFC is a technology that can be used in the lab (kg scale)**
    - **Improving resolution**
    - **Reduce the solvent usage**
    - **Increase in productivity**
    - **Green Chemistry**
  - **The integration of separations input early in the drug development stage is essential for rapid development and delivery of material**
-