

Preparative Chiral SFC in Drug Discovery: Promises and challenges

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Scope

- Introduction
- Chiral SFC in Discovery Phase
- Benefits of SFC in Chiral Separations
- Preparative Chiral SFC
- Conclusions



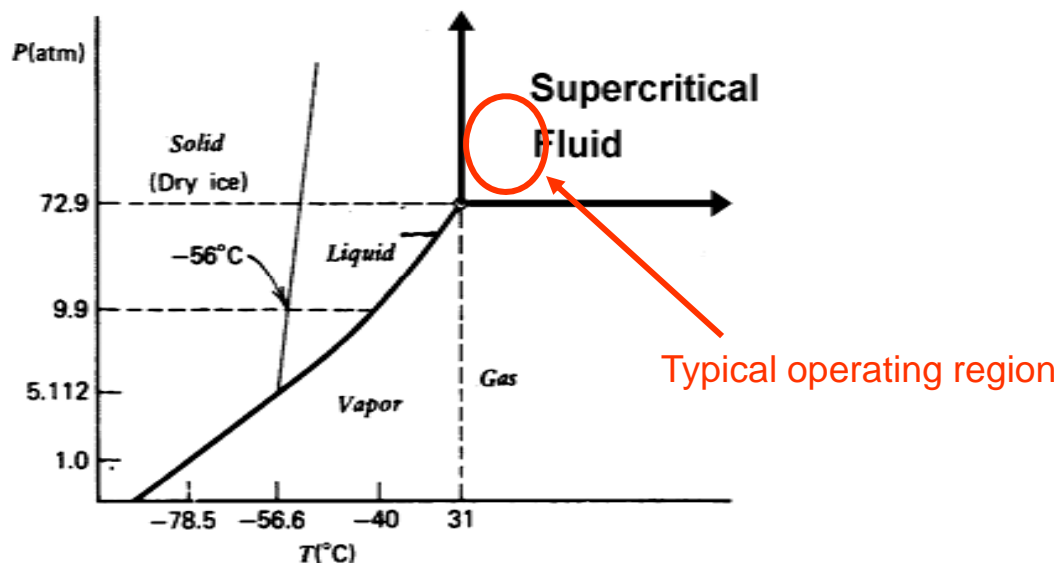
Introduction

- Significance of optically pure compounds
 - ✓ Meet FDA regulations
 - ✓ Eliminate side effects
 - ✓ Reduce dosage
- Chiral resolution of racemic mixtures
 - ✓ Crystallization
 - ✓ Chromatographic methods (e.g. HPLC, SFC, SMB)
 - ✓ Enzymatic routes

Introduction

Supercritical Fluid Chromatography (SFC) is a separation technique that uses super/subcritical fluid CO₂ and polar organic modifiers such as alcohols as mobile phases. In general, SFC can potentially separate any compound soluble in methanol or a less polar solvent.

Phase Diagram of CO₂ and Principle of SFC





Chiral SFC in Discovery Phase

- Efficiently provide chirally pure materials to support
 - Scale-up synthesis
 - In-vitro and in-vivo toxicological studies (>99%ee)
- Process *several hundreds* of requests per year from milligrams to multigrams
 - >95% preparative chiral separations by SFC
- Determine enantiomeric excess (ee) of early lead compounds

Typical SFC Systems



SFC-350



MGIII



Benefits of SFC in Chiral Separations

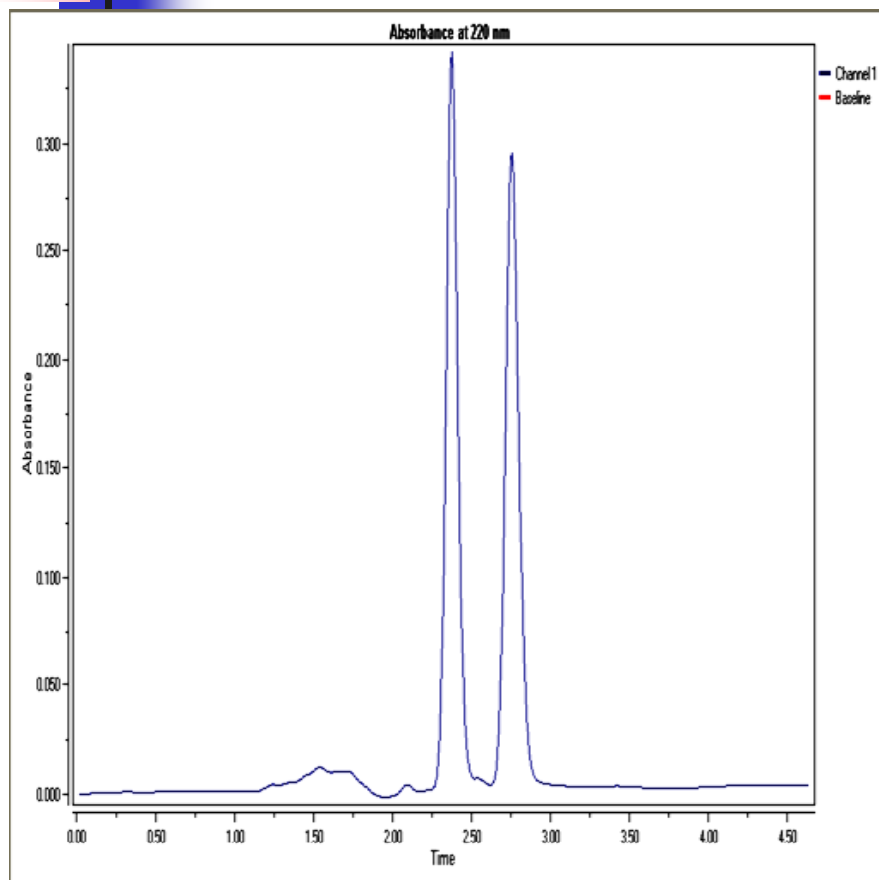
- No acid or base additives needed for **most** acidic and basic compounds except primary amines
- Little memory effect from acid and base additives
- CO₂ enhances CSP compatibility to exotic solvents, prolonging CSP life time
- Water can replace TFA if needed



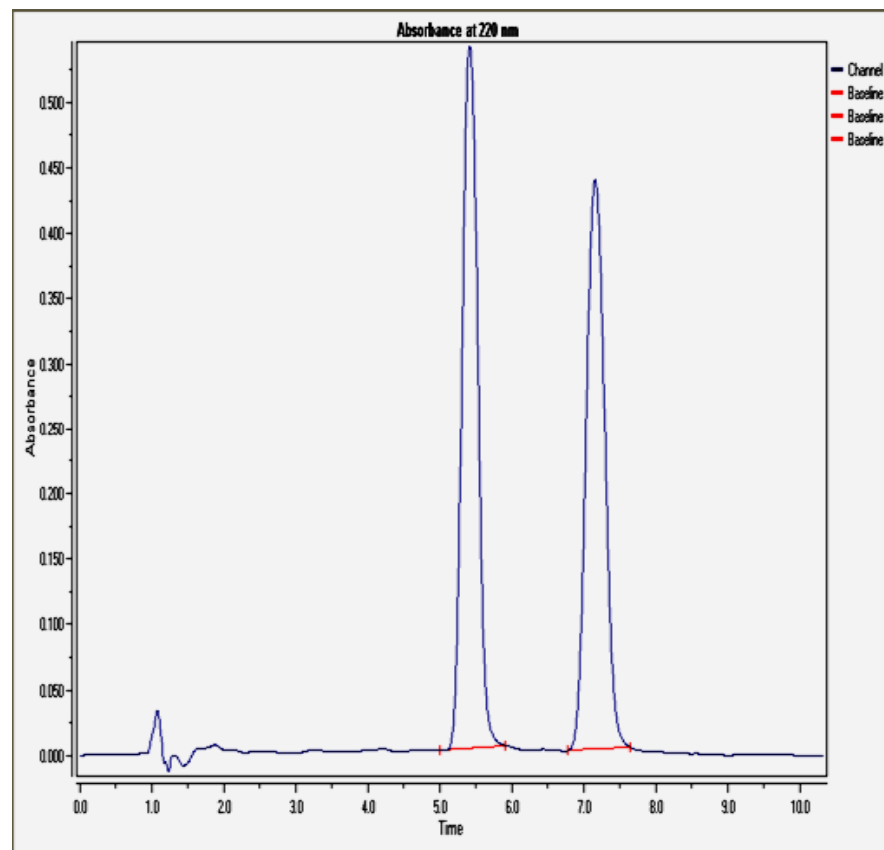
Benefits of SFC in Chiral Separations- Continued

- Shorter cycle time
- More flexible in solvent selection
improving selectivity and solubility
- Having polar modifiers direct mixing
with CO₂

Shorter Cycle Time and Improved Selectivity with Polar Co-solvents

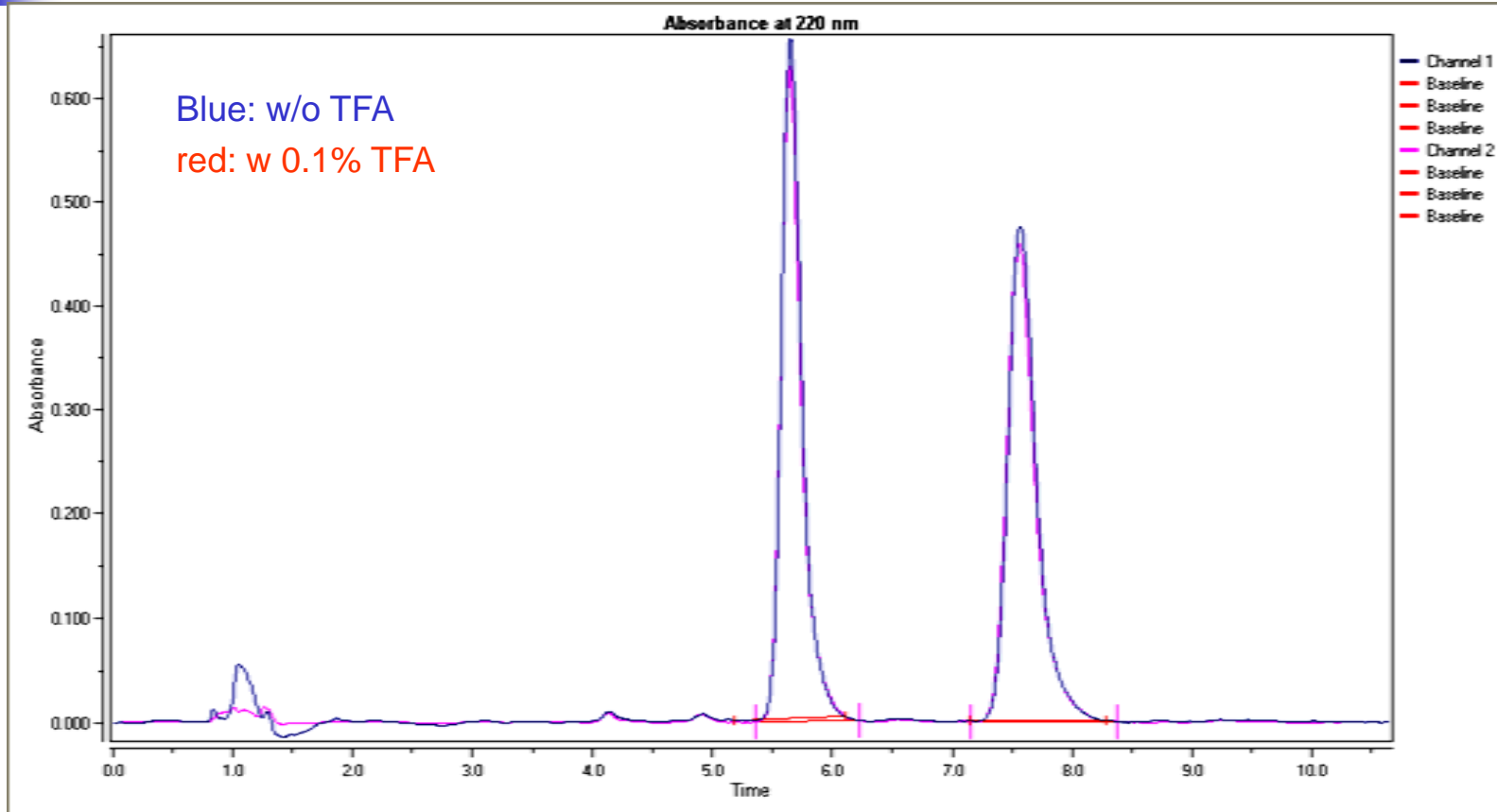


- Column: OJ-H (.46x25cm)
- Mobile phase: 30%MeOH in CO₂ @40C



- Column: OJ-H (.46x25cm)
- Mobile phase: 15%(ACN/TFA) in CO₂ @35C

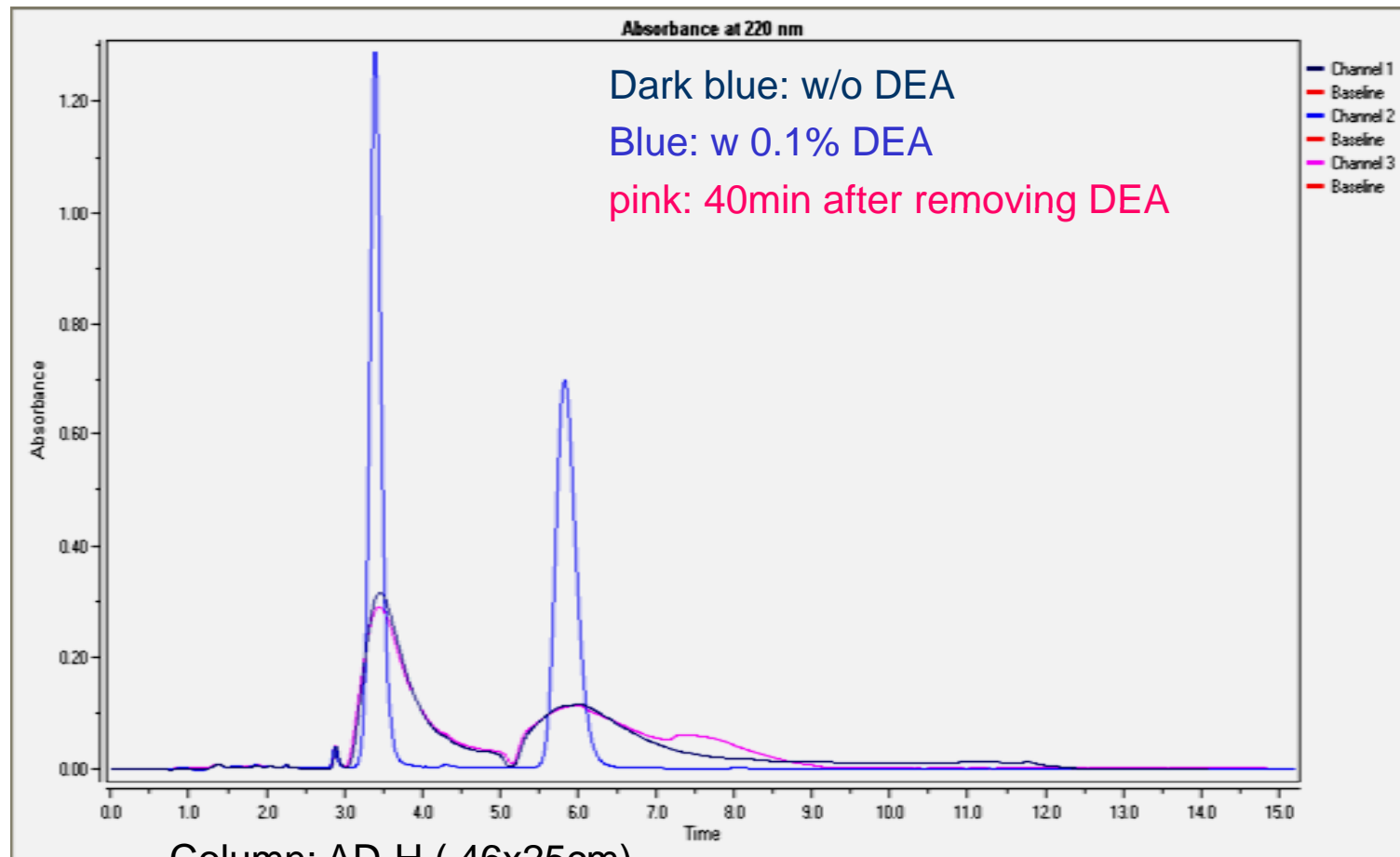
No TFA is needed in SFC for most acidic compounds



■ Column: AD-H (.46x25cm)

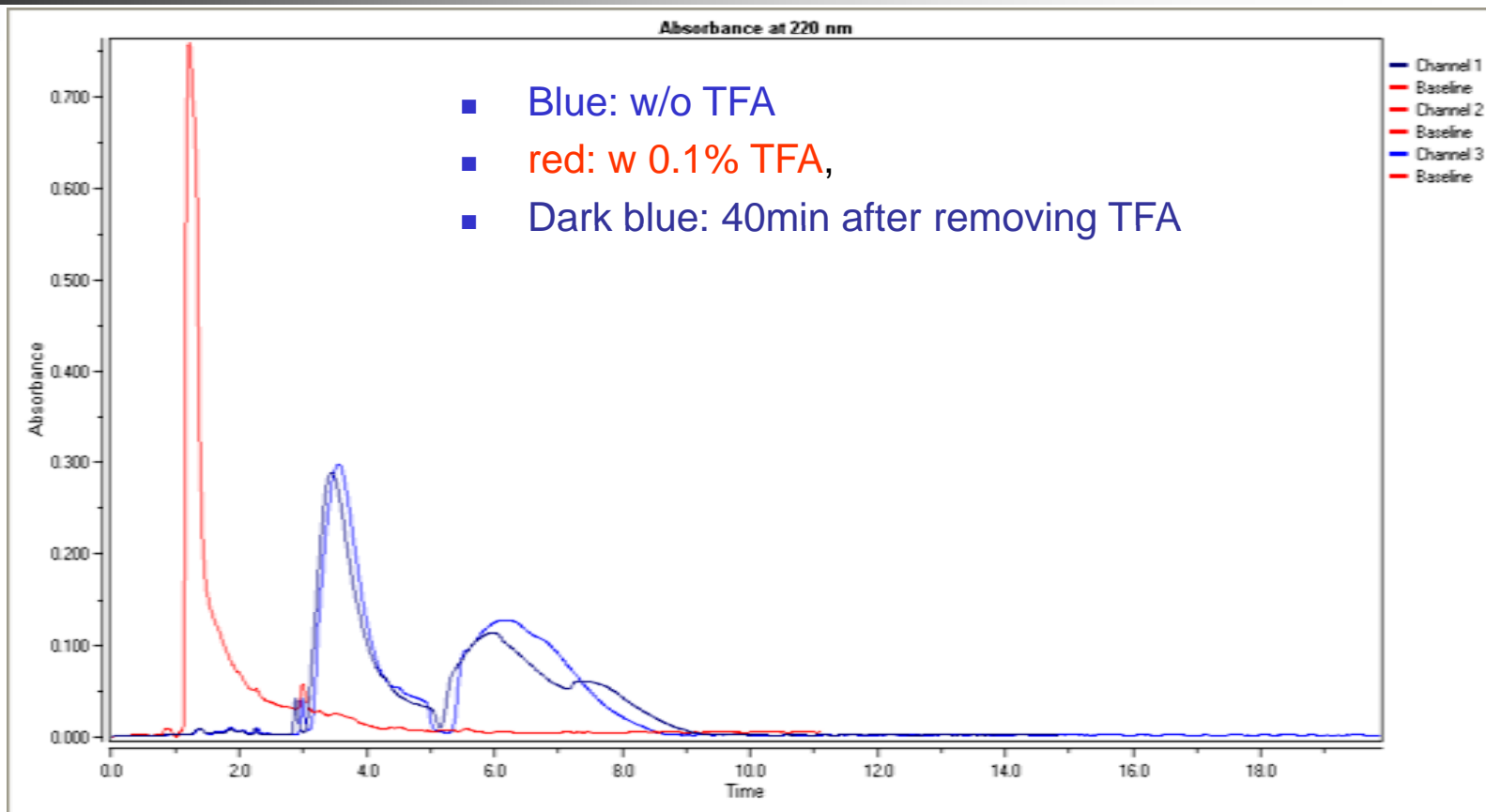
■ Mobile phase: 30%IPA with or without 0.1%TFA in CO₂

Little Memory Effect of DEA in SFC



- Column: AD-H (.46x25cm)
- Mobile phase: 15%MEOH with or without 0.1%DEA in CO₂

Little Memory Effect of TFA in SFC

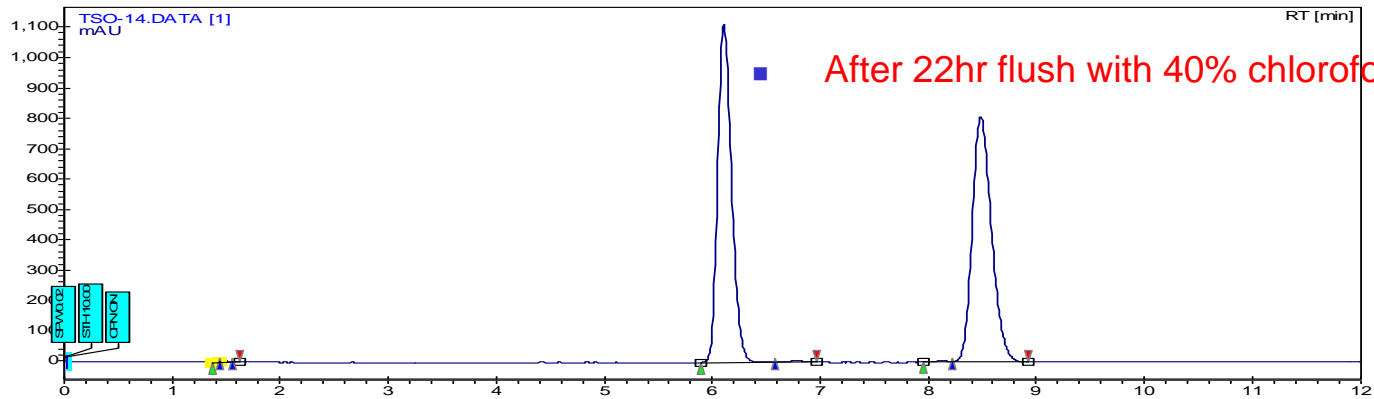
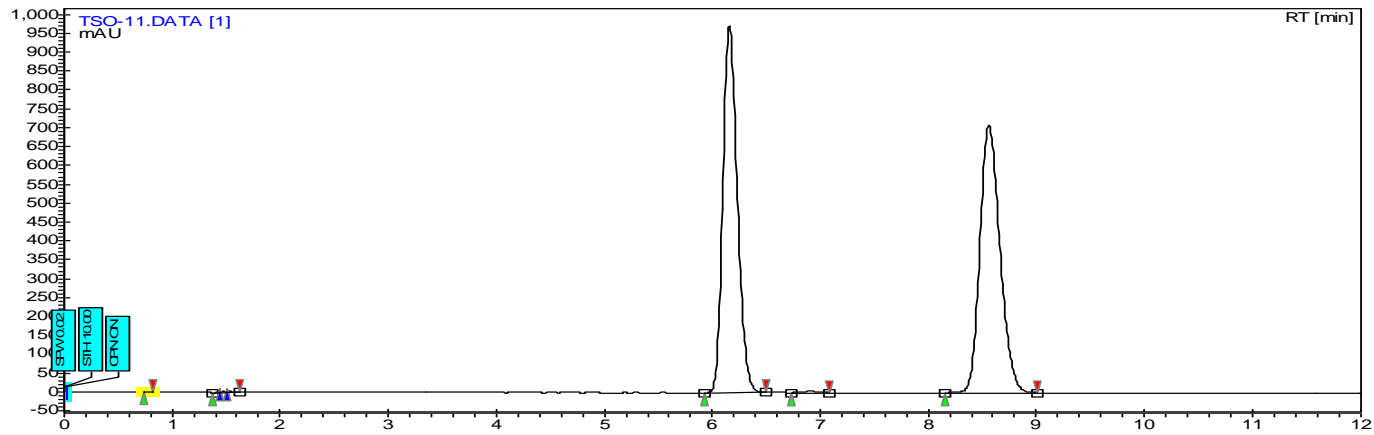


■ Column: AD-H (.46x25cm)

■ Mobile phase: 15%MEOH with or without 0.1%TFA in CO₂

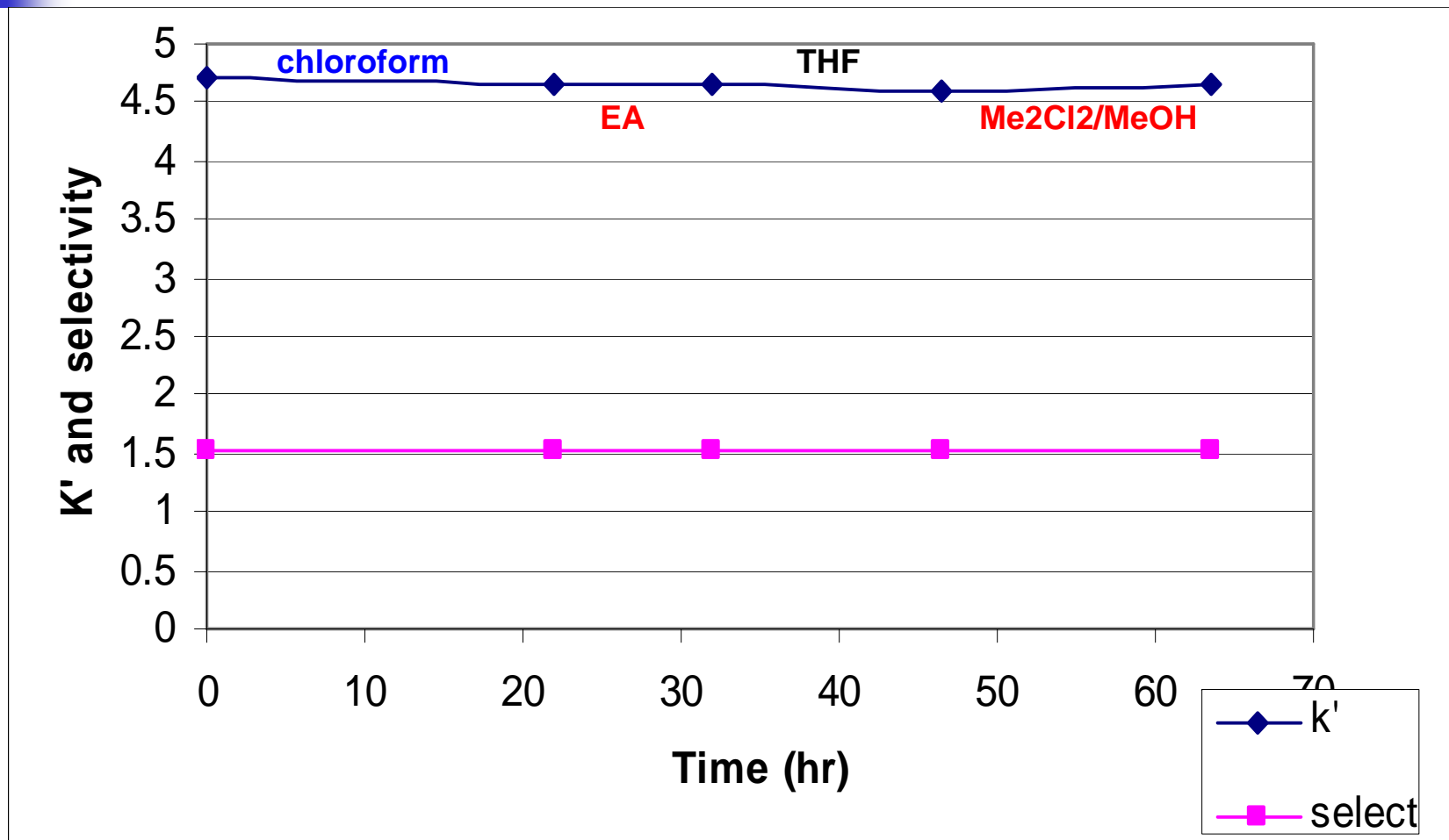
Stability of Chiralcel OJ in Chloroform

■ TSO test at time zero

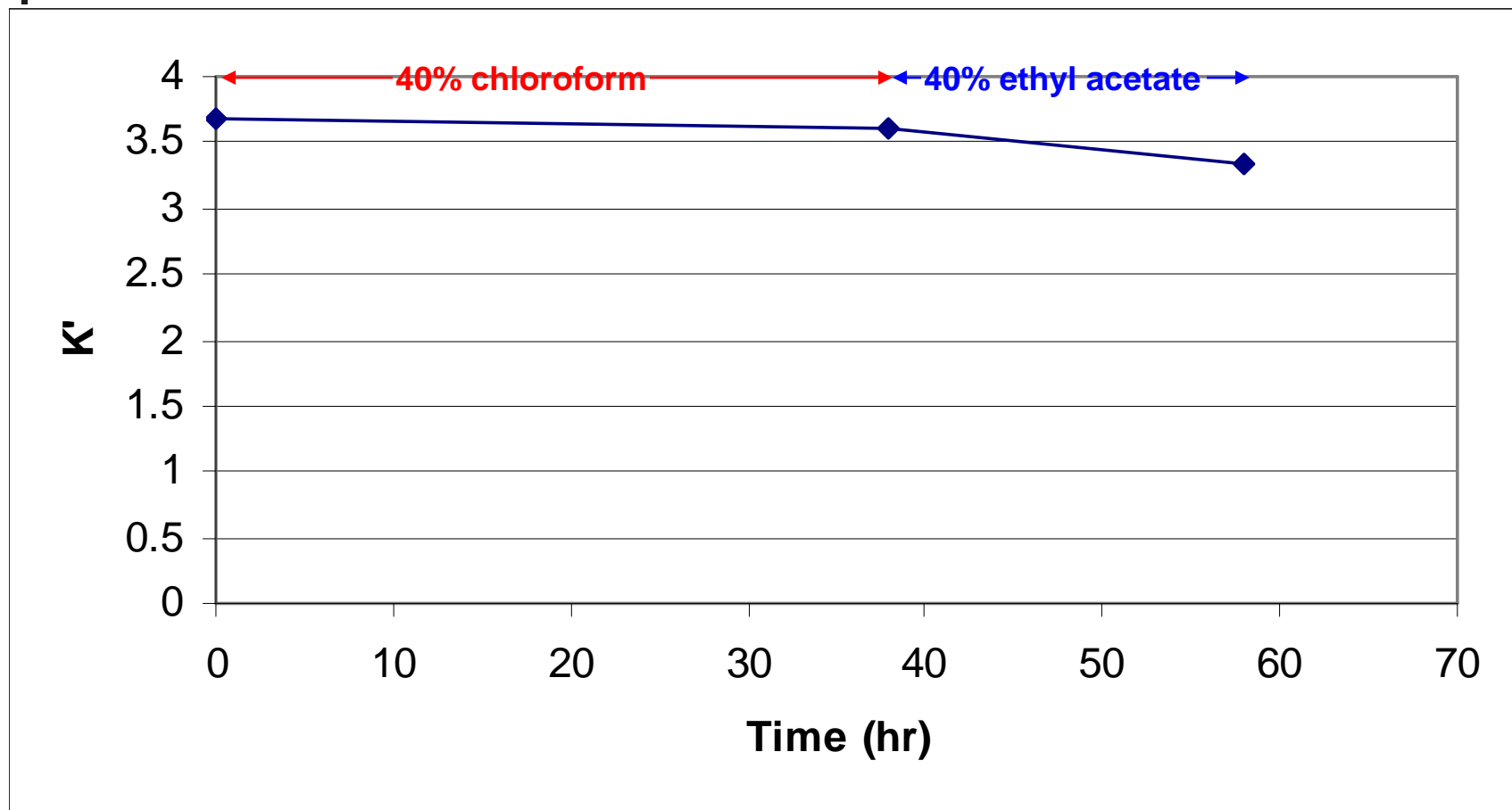


TSO conditions: 10%MeOH 2ML/MIN 100 Bar 35 C

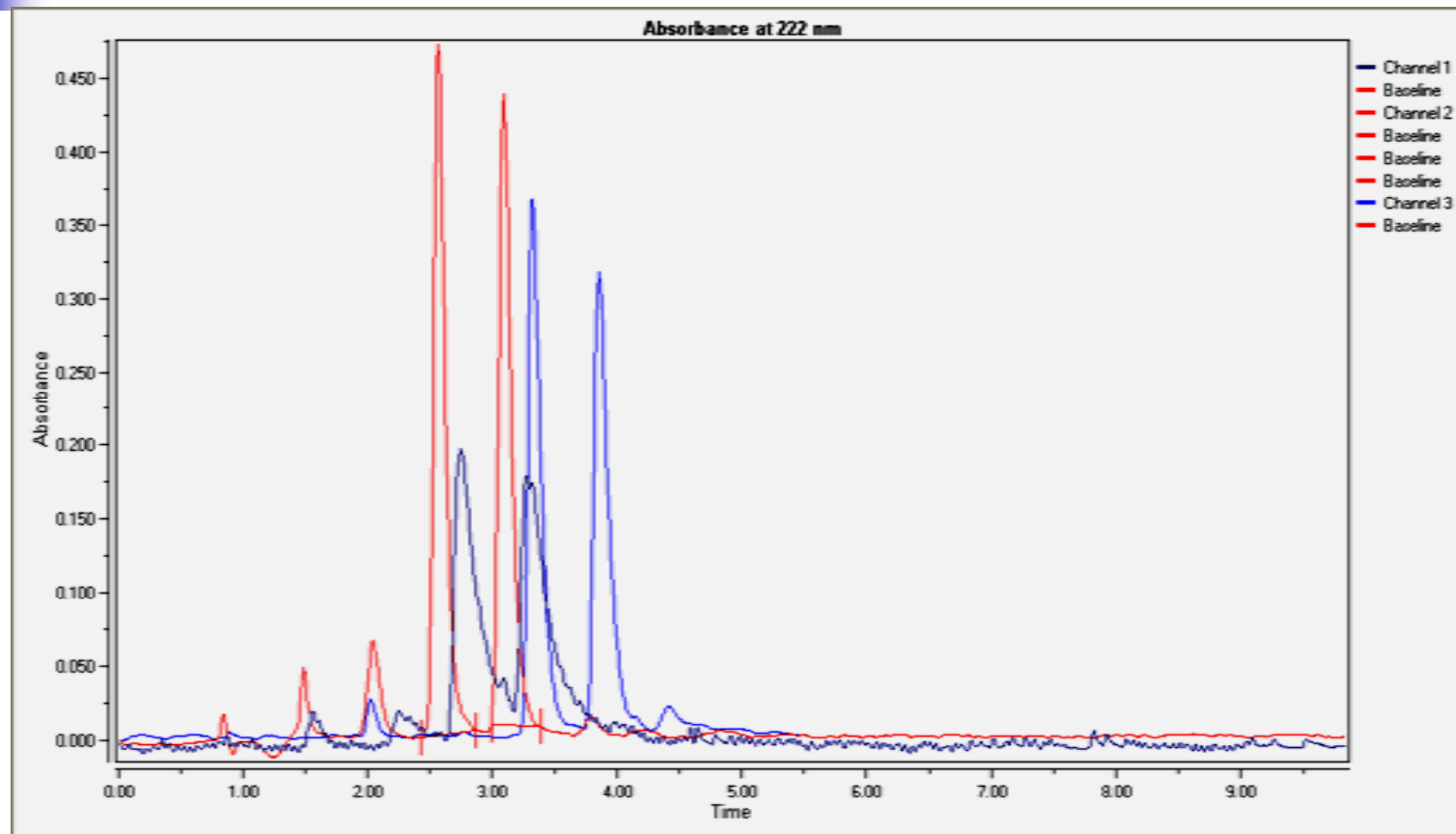
OJ in Various Exotic Solvents



Chiralpak AD in Chloroform and Ethyl Acetate



Water Effect in SFC



Dark blue: w/o additives in IPA (10%); blue: 5%water in IPA (7%); red: 0.1%TFA in IPA(10%)



Preparative Chiral SFC

- Promises of preparative SFC
- Challenges of method development and scale-up
- Case studies- *chiral SFC vs. HPLC*



Promises of Preparative SFC

- Less solvent consumption/disposal cost
- Less packaging time after separations
- Higher throughput with shorter cycle time
- Solubility issues minimized
- Higher resolution than HPLC, ideal for low α separations



Method Development

- Screening in various
 - columns and
 - co-solvents
 - in gradient mode:10% to 50% in 8min on Thar or Berger MDS
- Optimizing the isocratic conditions

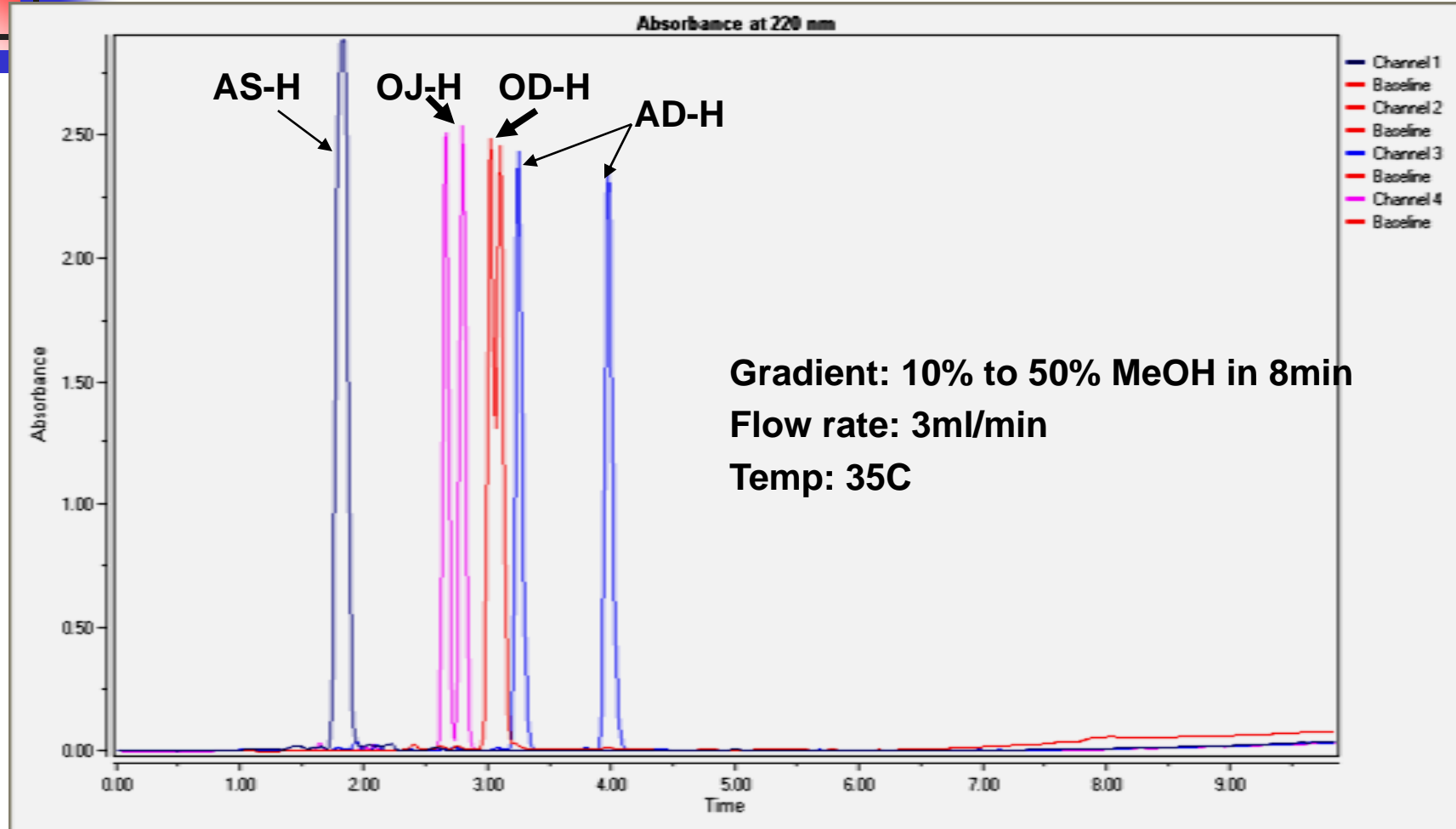


Method Development

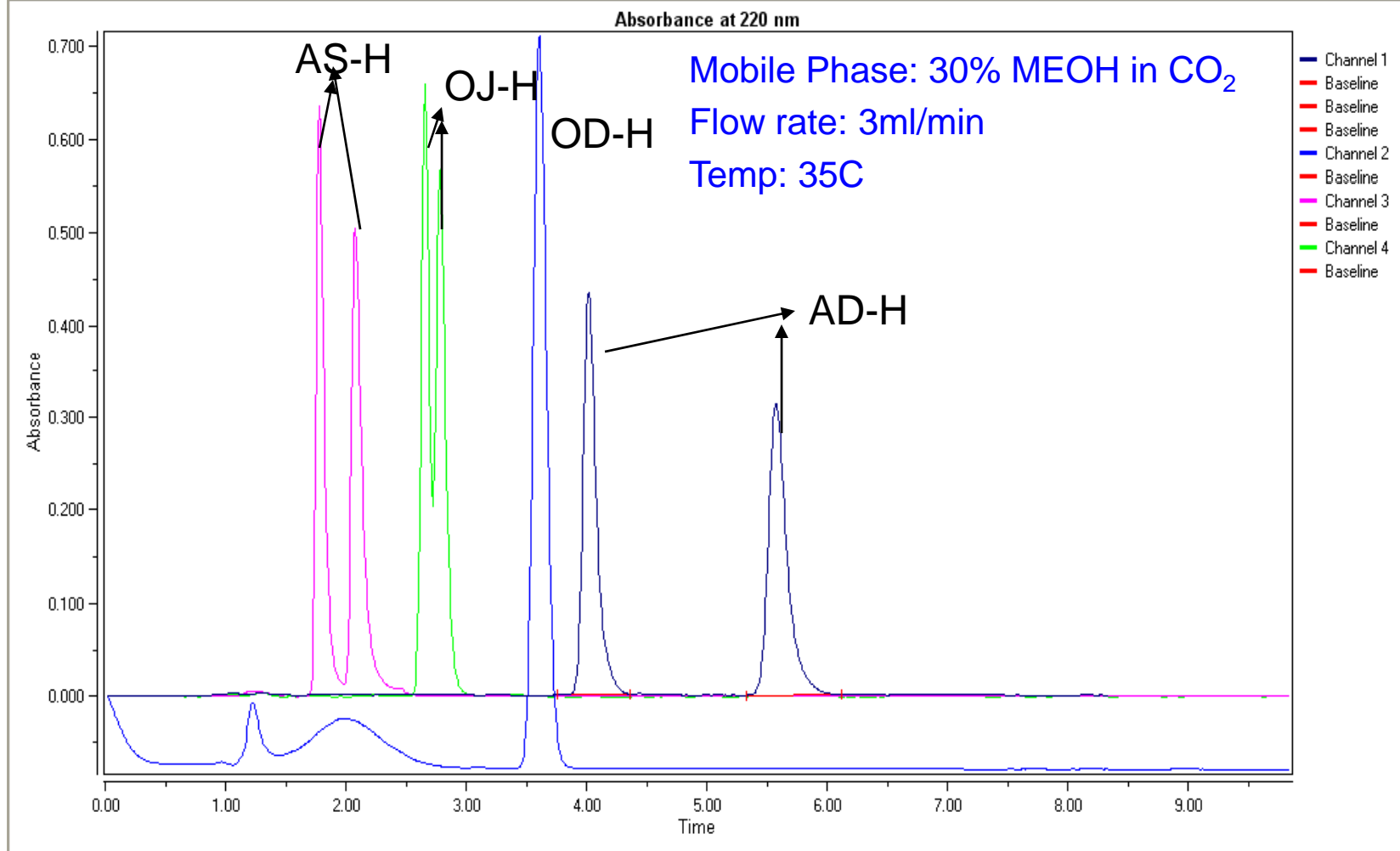
Types of Chiral Stationary Phases (CSP)

- Cellulose/Amylose Derivatives
 - ChiralCel OD, OJ etc.
 - ChiralPak AD, AS
 - ChiralPak IA, IB, IC
 - Lux-Cellulose-1, Cellulose-2, and Amylose-2
- Pirkle-type
 - Whelk-O1

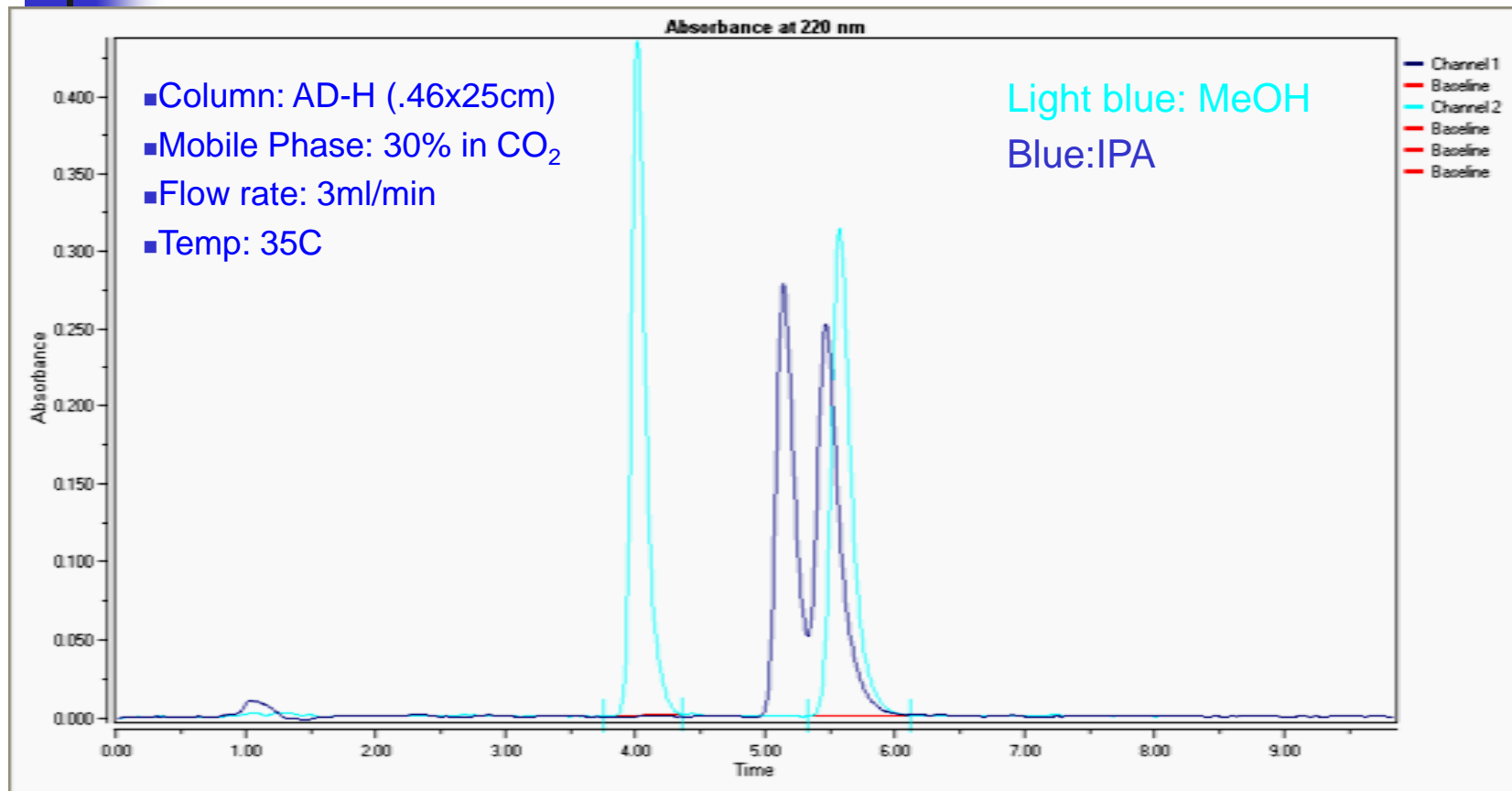
Method Development: Column Effect in Gradient



Method Development: Column Effect in Isocratic



Method Development: Co-solvent Effect





Scale-up

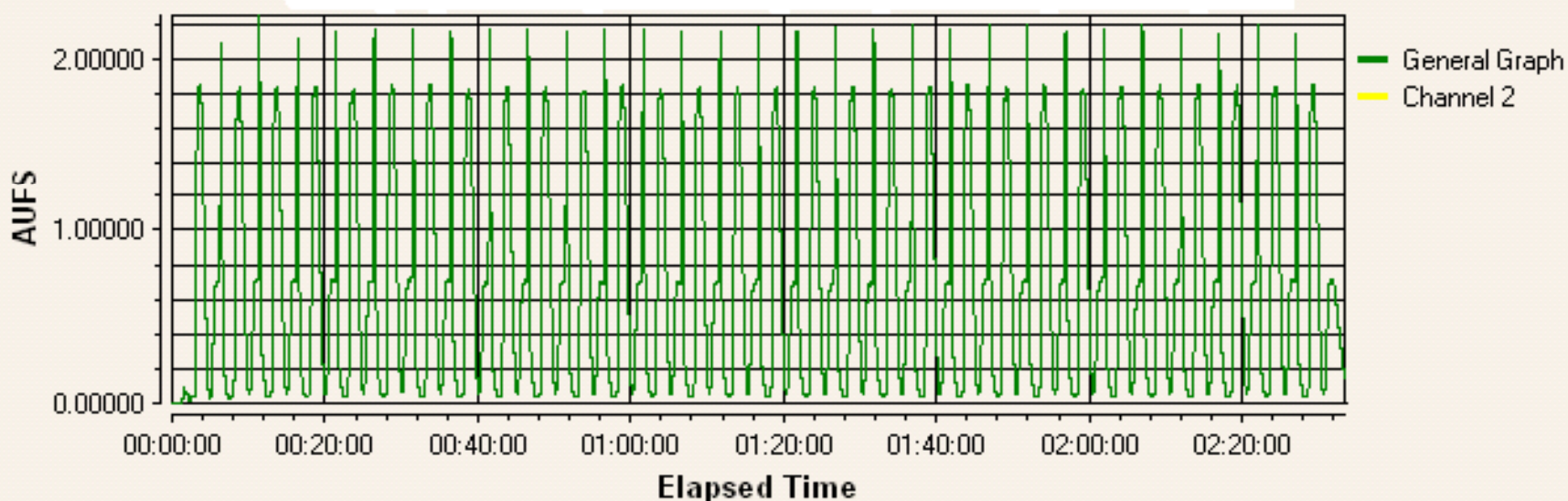
- **Sample solubility-** MeOH/chloroform, MeOH/methylene chloride, and IPA/ACN to increase solubility
- **Scale up on preparative columns**
 - 2x50cm, 3x25cm on SFC-200 or MGIII
 - 5x25cm, 5x50cm on SFC-350
 - 7.5x30cm (radial compression)
- **Sample stability could be an issue**



Scale-up: Direct Injection vs. Loop Injection in Mixed Stream

- When the organic percentage is relatively low (i.e. less than 10%),
 - direct injection can eliminate peak distortion problem,
- Direct injection can also prevent concentrated samples from precipitating out in the system

Scale-up Ex.1: Preparative Chiral SFC of a Discovery Intermediate



Direct and stacked injection

Chiralcel OD-H (3x25cm)

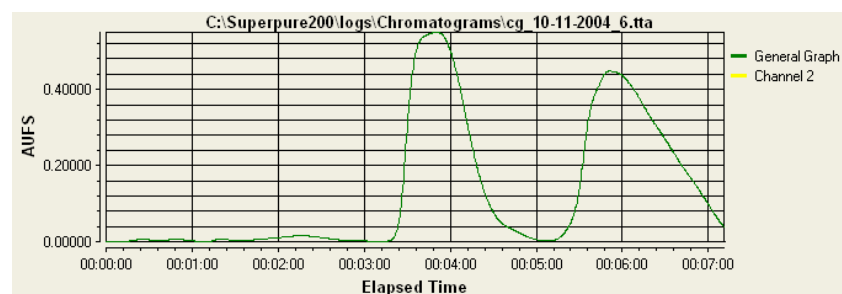
CO₂/EtOH (95/5) @100bars

120g/min @220nm, and 36°C,

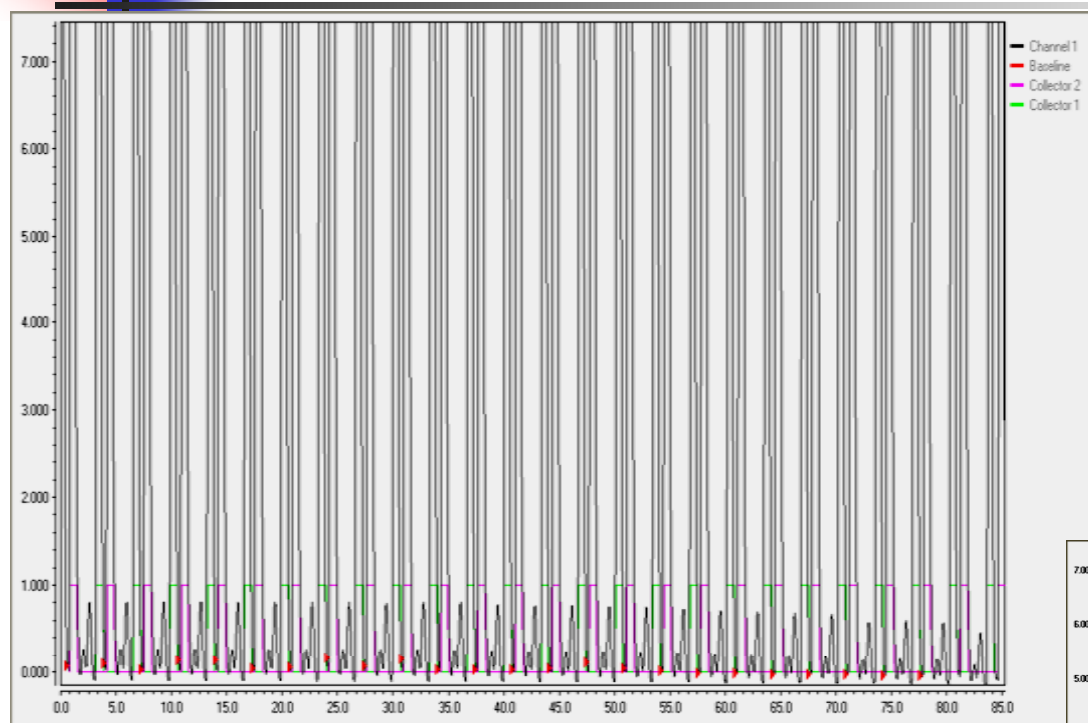
0.2g/5min

**Total ~310g
processed**

Single injection (loading study)

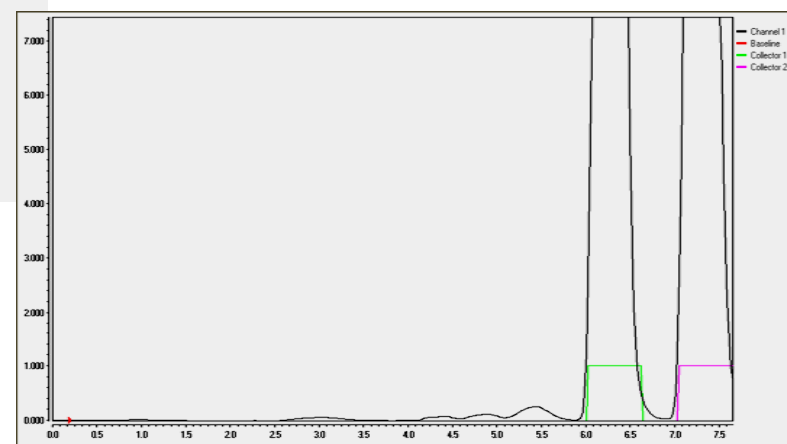


Scale-up Ex.2: Preparative Chiral SFC on a 5x50cm Column

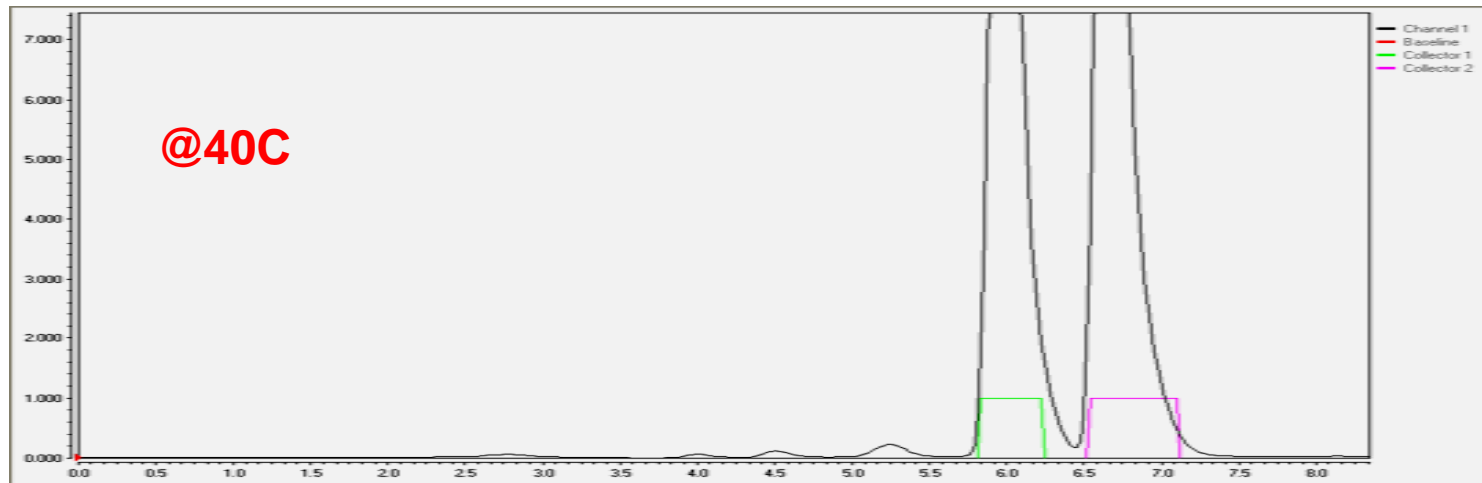
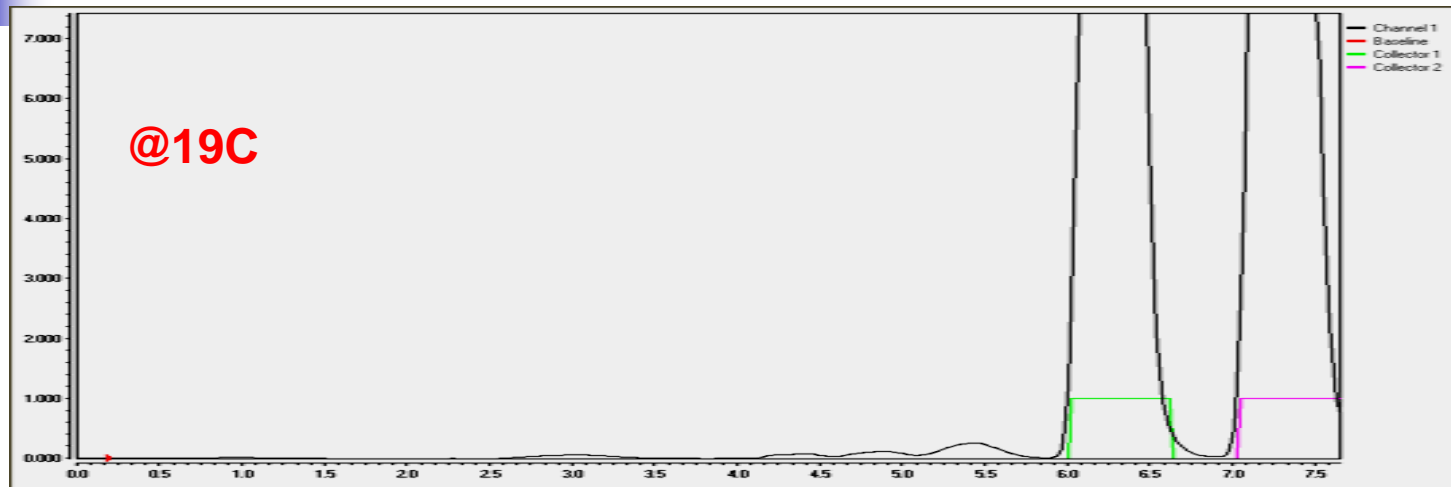


- Direct and stacked injection
- **Whelk-O1(RR, 5x50cm)**
- **CO₂/IPA (85/15)@100bars**
- **320ml/min @220nm, and 20C,**
- **0.27g/3.2min, total 100g**

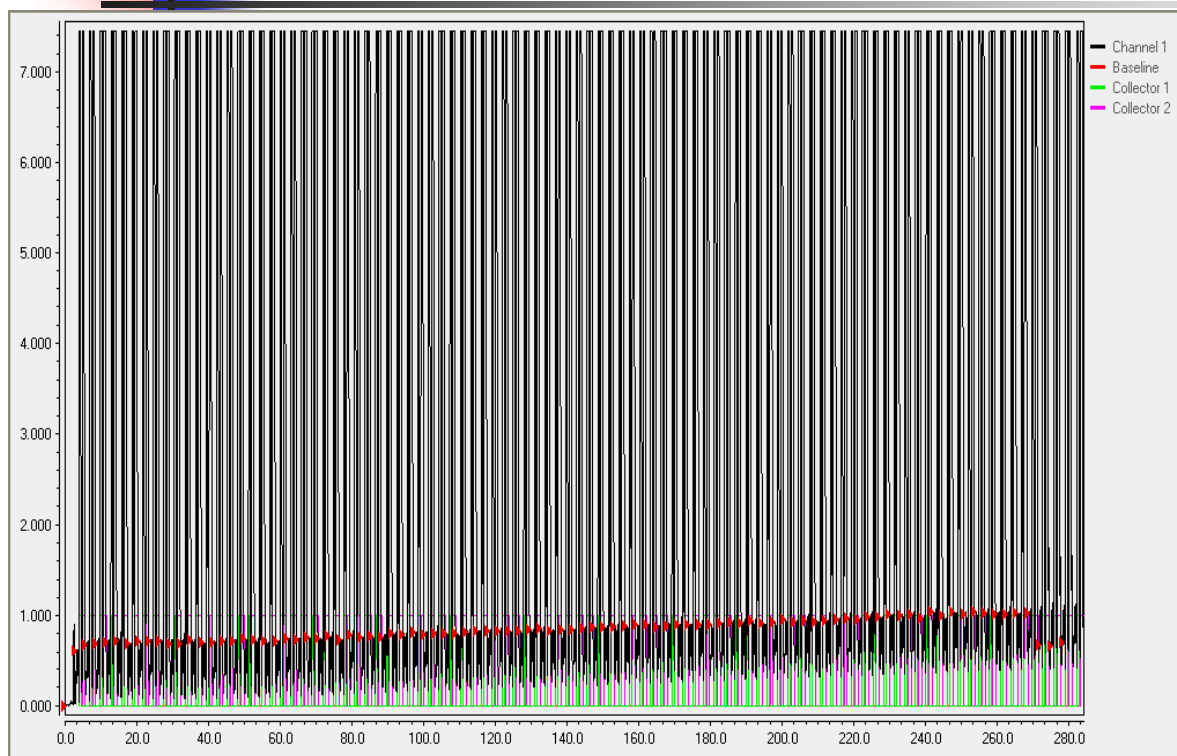
- Loading study



Scale-up Ex.2: Temperature Effect

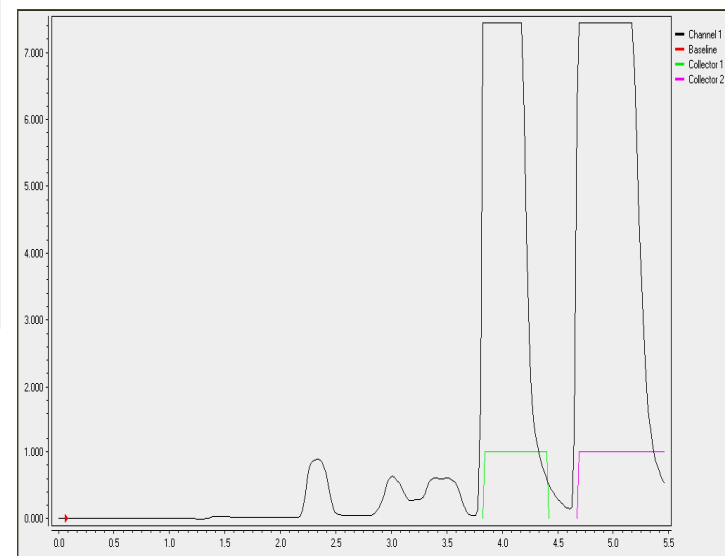


Scale-up Ex.3: Preparative Chiral SFC on a 5x25cm Column

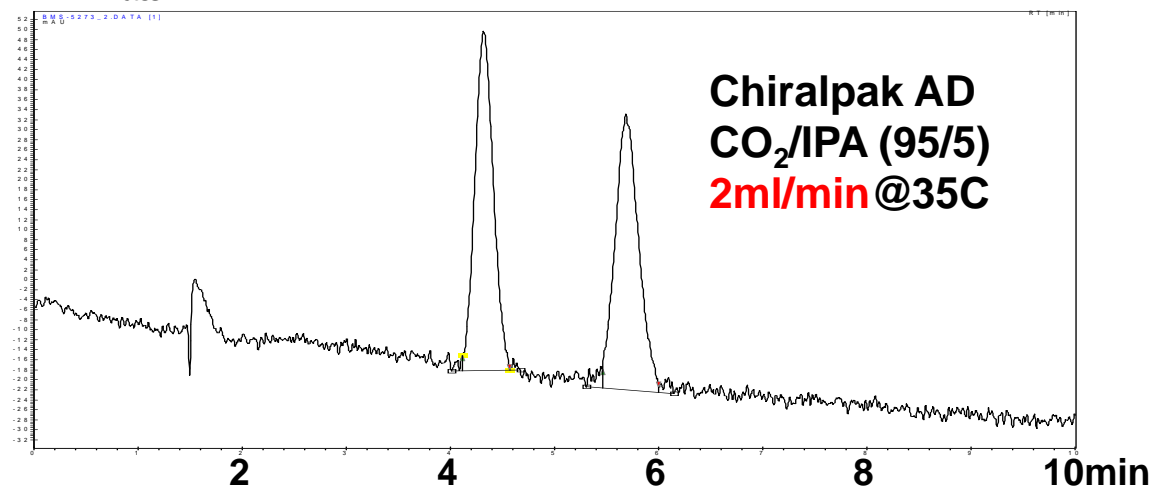
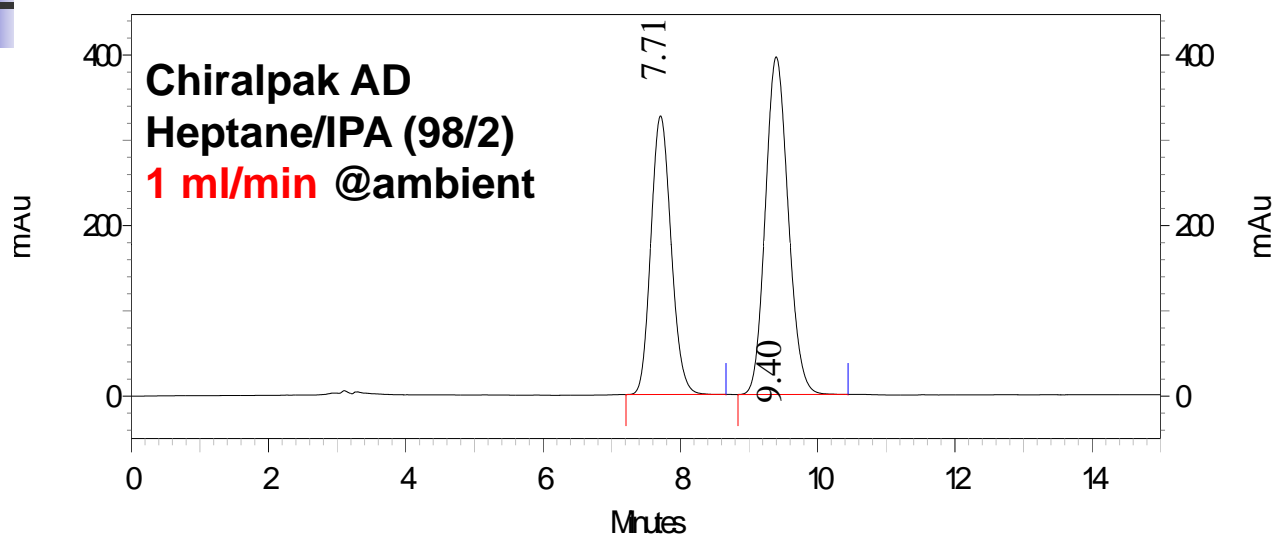


- Direct and stacked injection
- Chiralpak AD-H (5x25cm)
- CO₂/(IPA-ACN, 1-1) (80/20) @250ml/min
- 100mg/ml, 1.5cc/3min, total 250g

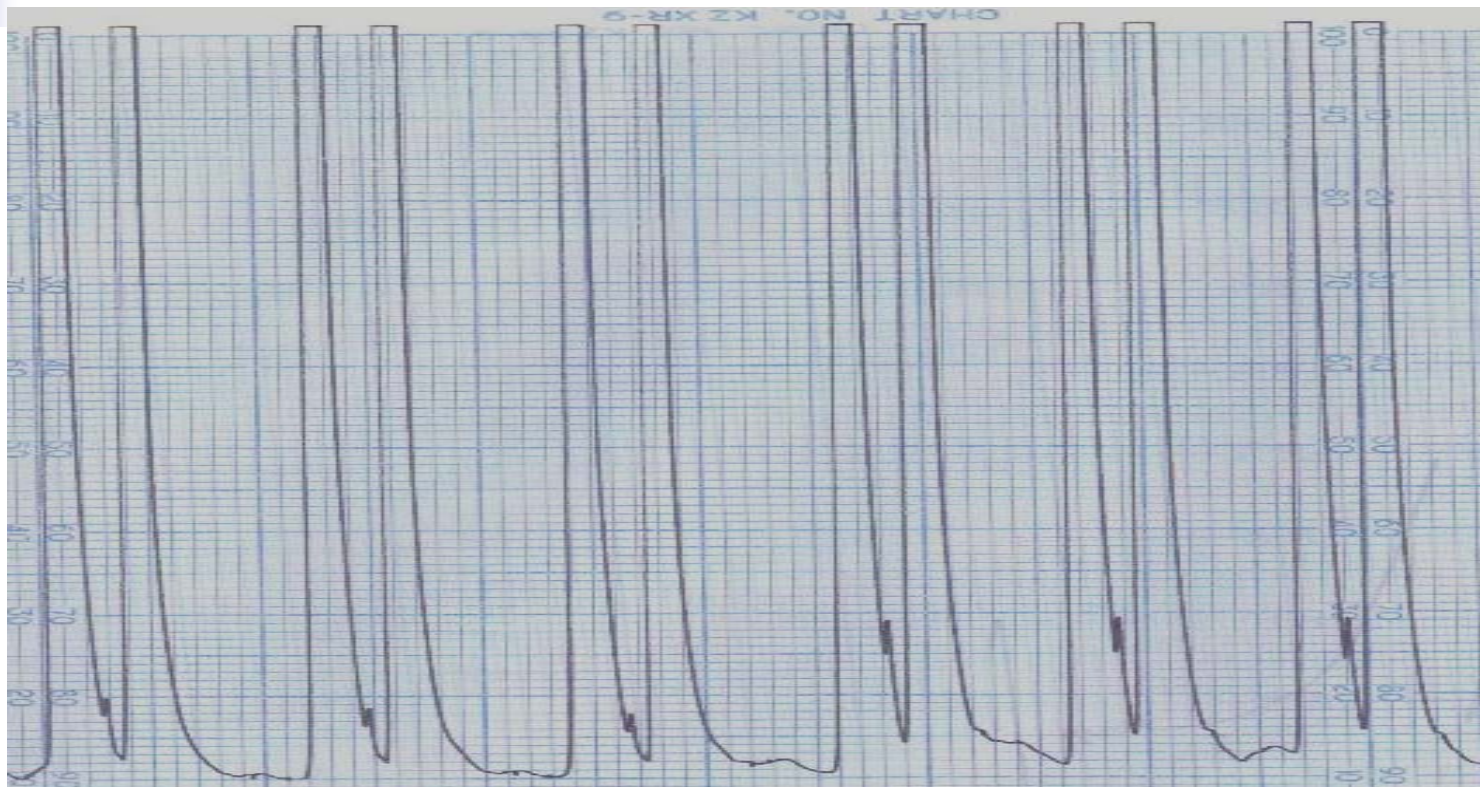
single run-loading study



Case Study #1: Analytical Chiral Separation of a Discovery Compound



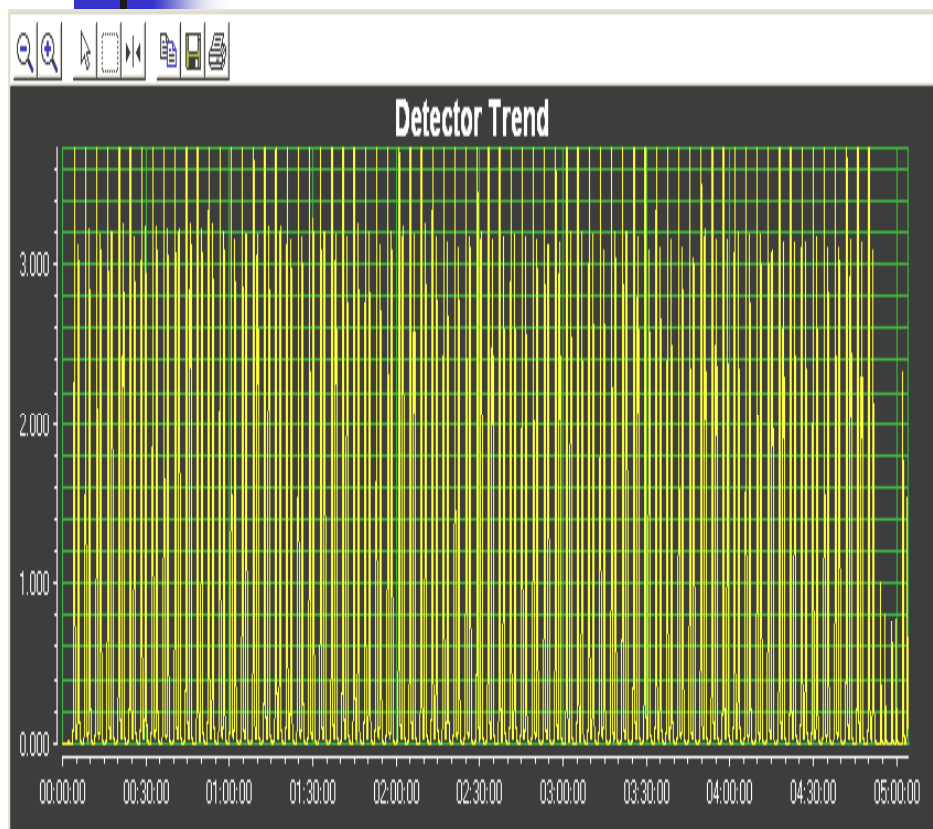
Case study #1: Preparative HPLC Chiral Purification



Chiralpak AD (5x50cm)
Hex./IPA (98/2) @ 60cc/min
8.5cc of 16mg/ml/26min

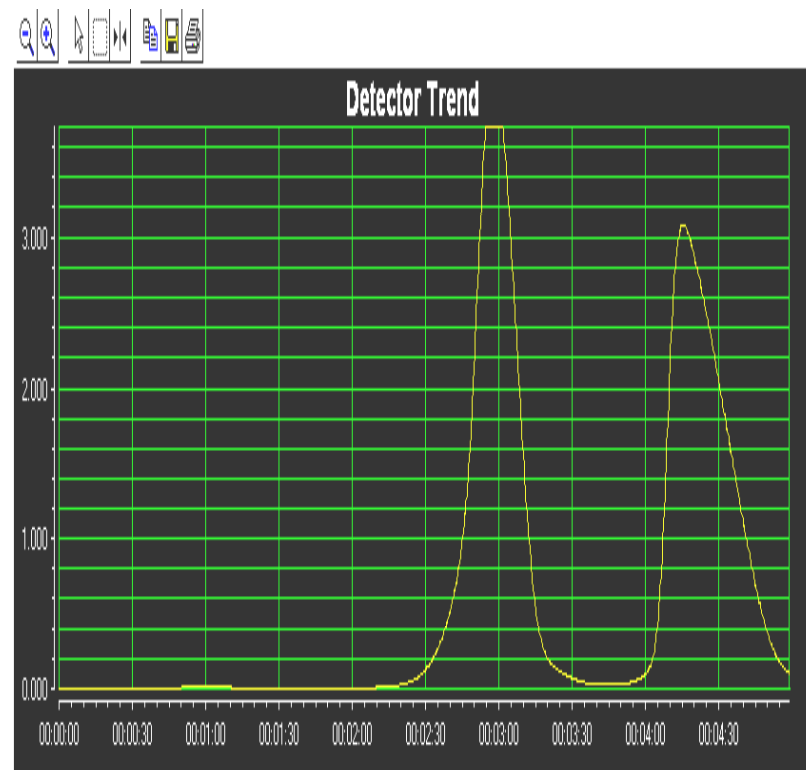
Throughput: 315mg/hr

Case study #1: Preparative SFC Chiral Purification



Cycle time: 4min

Throughput: ~1.1g/hr



ChiralPak AD-H (3x25cm,5um)
CO₂/IPA(95/5) at 100ml/min, 5cc of
15mg/ml

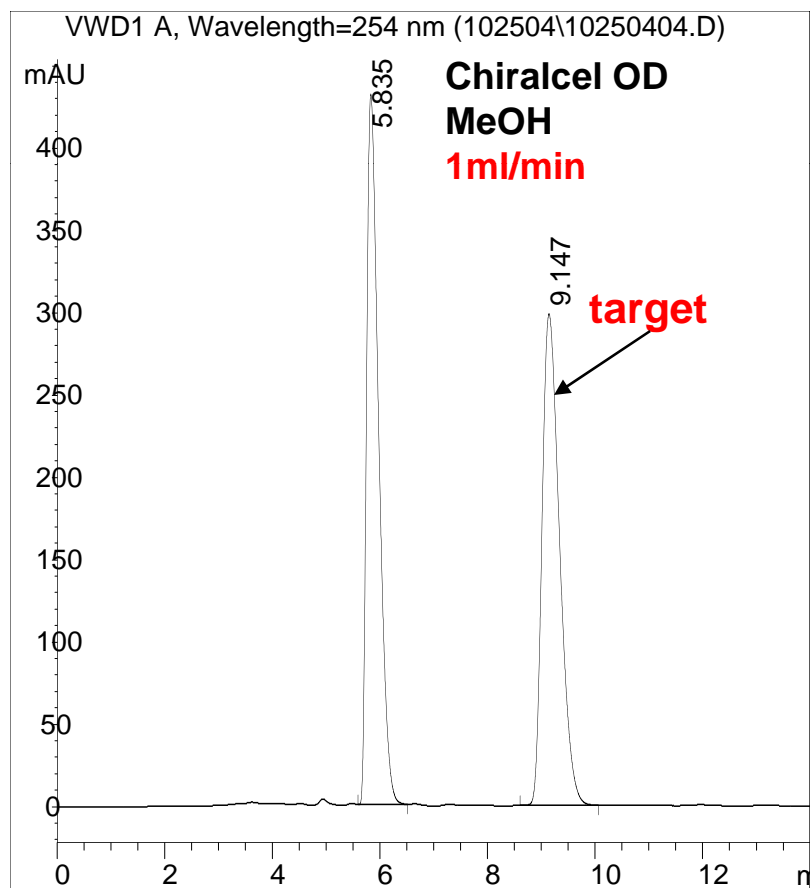
Case study #1-continued

	SFC	HPLC
Chiral stationary phase	AD	AD
Column dimensions	3x25cm	5x50cm
Mobile phase	CO ₂ /IPA (95/5)	Hex./IPA (98/2)
Flow rate	100ml/min	60ml/min
Cycle time (min)	4	26
throughput (g)/hr	1.1	0.35
Total solvent consumption (l)	5.5	206

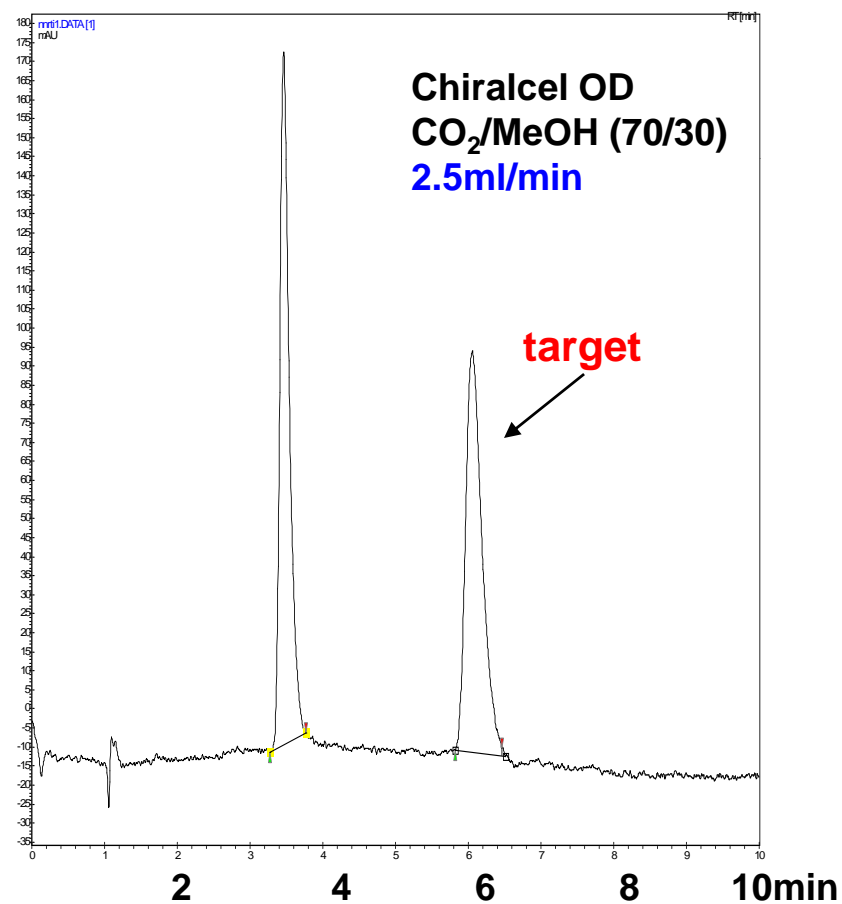
Total amount of racemic mixture processed: 20g

Case Study #2: Analytical Chiral Separation of a Discovery Compound

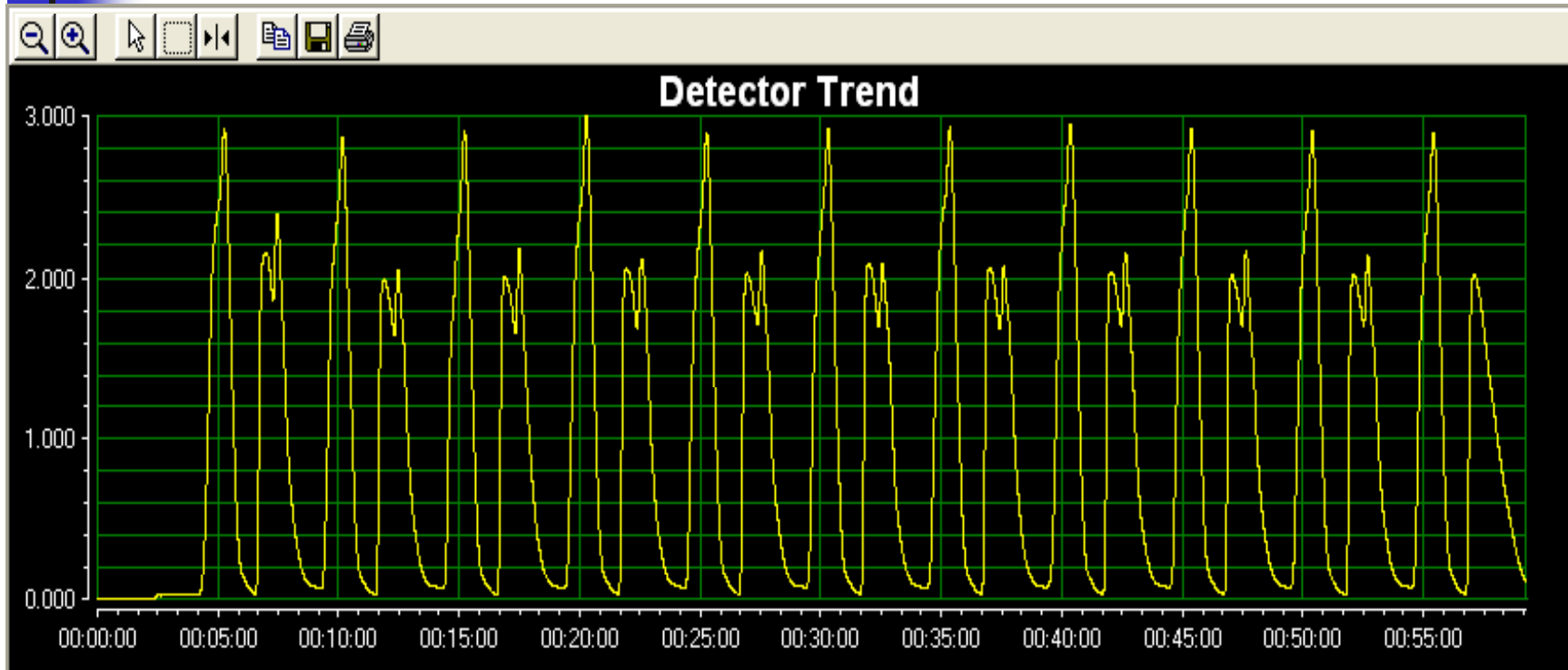
HPLC



SFC



Case study #2: Preparative SFC Chiral Purification



ChiralCel OD-H (3x25cm,5um)
CO₂/MeOH(70/30) at 90g/min and
220nm, 90bars, 35°C
10cc of 15mg/ml/5min
Stack injections

Throughput: 1.9g/hr
Total ~500g processed



Case study #2

	SFC	HPLC
Chiral stationary phase	OD	OD
Column dimensions	3x25cm	3x25cm
Mobile phase	CO ₂ /MeOH (70/30)	MeOH
Flow rate	90ml/min	30ml/min
Cycle time (min)	5	11
throughput (g)/hr	1.9	1.0
Total solvent consumption (l)	426	900

Total amount of racemic mixture processed: 500g

Case study# 3

	S F C	H P L C
Chiral stationary phase	A D	There is no suitable H P L C method for the preparative purification.
Column dimensions	7.5 x 30 cm	
Mobile phase	C O 2 / M e O H (65/35)	
Flow rate	160 g / m in	
Cycle time (m in)	100	
Sample (g) / h r	2.52	
Sample / unit vol. of solvent (g / l)	0.75	
Sample solubility (g / l)	15	
Total solvent consumption (l)	400	

**Total amount of racemic mixture processed:
~300g**



Conclusions

- SFC is efficient for larger scale chiral separations. It is a powerful technique that dovetails nicely with the fast pace of modern day pharmaceutical R&D. The key advantages of SFC include:
 - Shorter analysis time
 - Higher production rate
 - Less solvent consumption and fraction volume
 - Reduced drying time and disposal cost
- Should be the front line tool for chiral separations