

Supercritical Fluid Chromatography (SFC) as a Green Chromatographic Technique for Support in Rapid Development of Pharmaceutical Candidates.



Jimmy O. DaSilva, Henry Shiuhan Yip, Vinod Hegde PhD, Alex Zaks PhD
Chemical & Physical Sciences, Separations Technology Group,
ChemDev, Schering-Plough, Union, NJ



Overview

- SFC Screening
- Analytical SFC
- Semi-preparative SFC
- Preparative SFC
- Lessons Learned
- Conclusions

Analytical SFC Screening



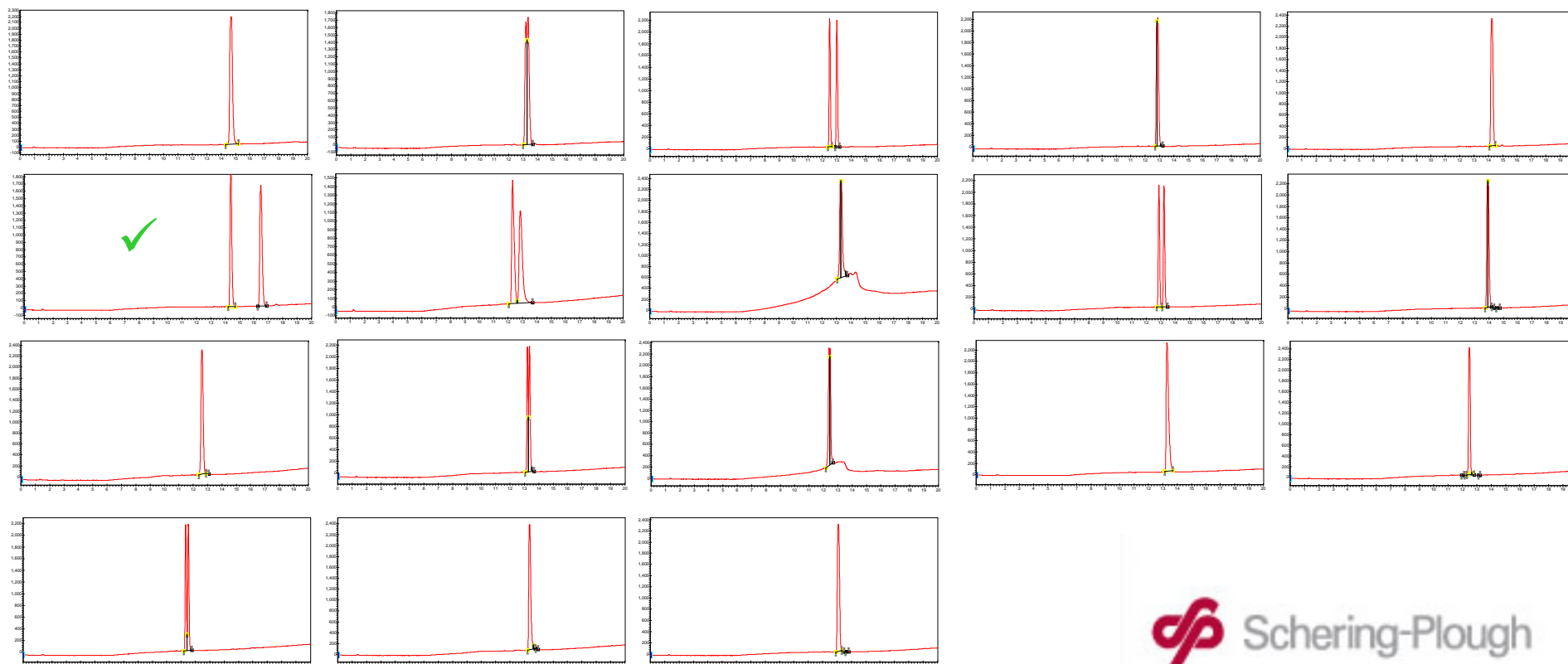
4-60% MeOH gradient
20 Minute Run

Chiral Columns

Chiralpak AD-H	S,S Whelk-O
Chiralpak AS-H	Chirobiotic V
Chiralcel OD-H	Chirobiotic T
Chiralcel OB-H	Chiris AX-QD
Chiralcel OJ-H	Chiralpak IA
Chiralcel OF	Chiralpak IB
	etc.

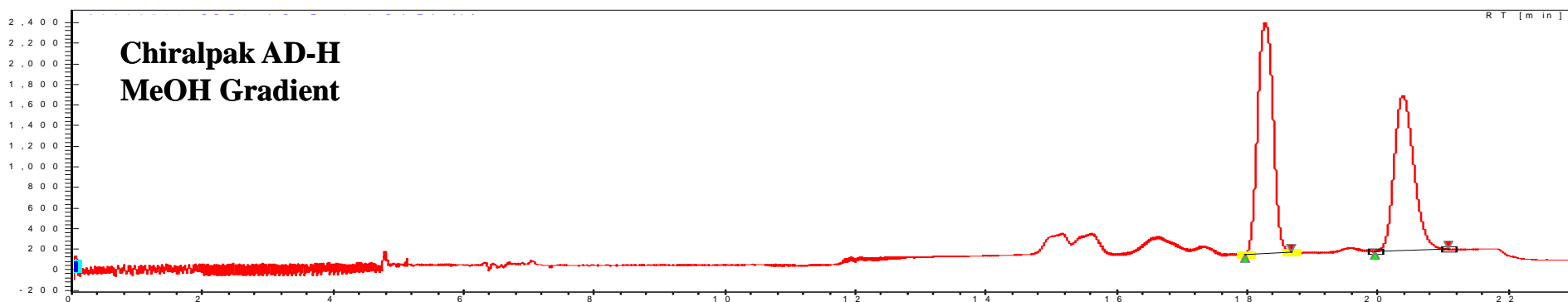
Non-Chiral Columns

Hypercarb	Perfluorophenyl
Diol	Perfluorooctyl
Amine	Cyano
NPI	Pyridine
Nitro	etc.



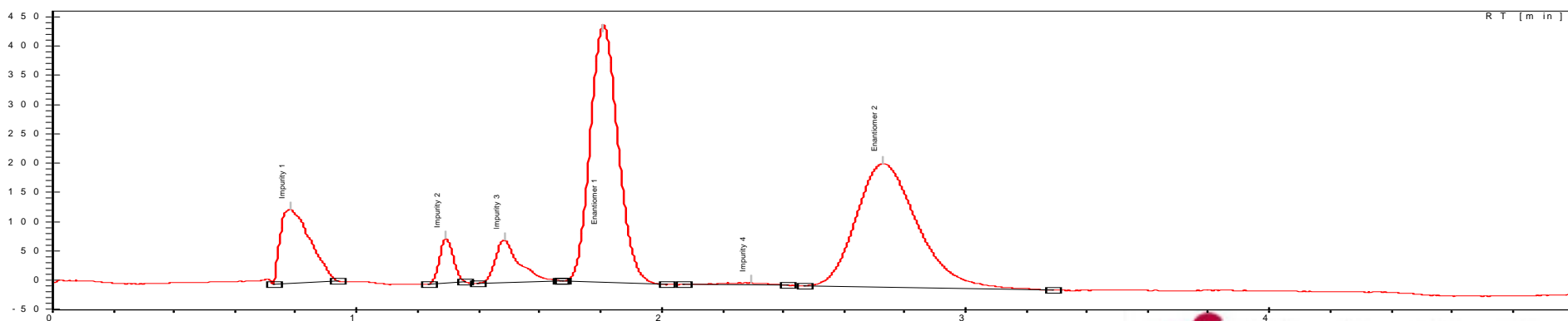
Chiral Method Development

Assay development to determine the enantiomeric ratio of key reactions on the Minigram SFC using analytical conditions. A sample at ~1 mg/mL was injected on a Chiralpak AD-H (250 x 4.6 mm) column using 4% MeOH/CO₂ for 4 mins then ramp to 50% MeOH/CO₂ at a rate of 2% MeOH/min with a 3 min hold, 2.5 mL/min, 100 bar, 35 C, 210 nm, 10 µL injection, run time 25 mins.



An isocratic method:

40% MeOH/CO₂, 5.0 mL/min, 100 bar, 35 C, 210 nm, 10 µL injection, run time 5 mins.



Analytical Solvent Reduction Calculation

Analytical HPLC Solvent Usage

$$\begin{array}{rcccccc} \text{Flow Rate} & \text{Time (8 hrs/day)} & \text{Yearly} & \text{Yearly Solvent Usage} & \text{\# of Instruments} & \\ 1.0 \text{ mL/min} & \times 480 \text{ min/day} & \times 250 \text{ days/year} & = 120 \text{ L/year} & \times & 1000 \end{array}$$

$$\begin{array}{l} = \text{Total Yearly Solvent Usage} \\ \mathbf{120000 \text{ L/year}} \end{array}$$

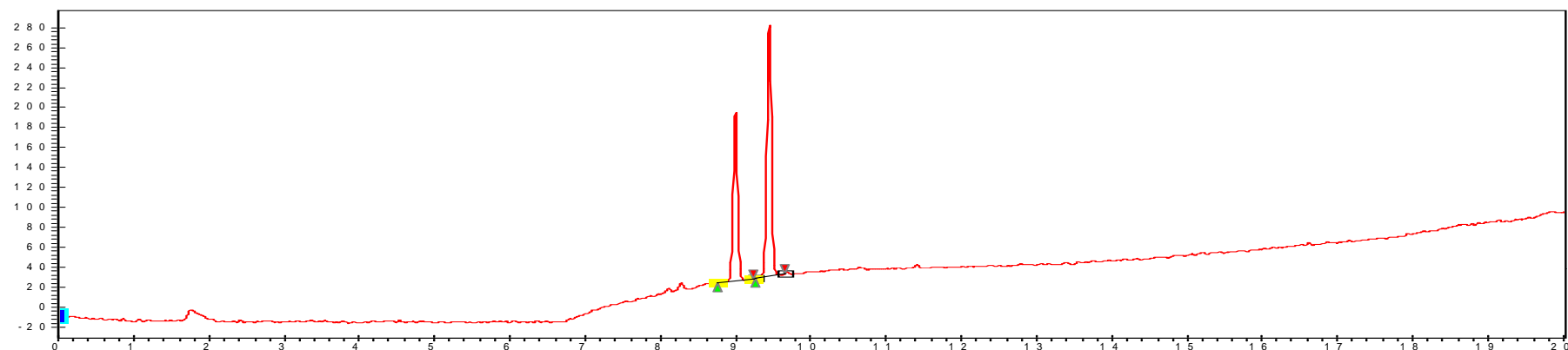
Analytical SFC Solvent Usage

$$\begin{array}{rcccccc} \text{Flow Rate} & \% \text{ Modifier} & \text{Time (8 hrs/day)} & \text{Yearly} & \text{Yearly Solvent Usage} & \text{\# of Instruments} \\ 1.0 \text{ mL/min} & \times 10\text{-}40\% & \times 480 \text{ min/day} & \times 250 \text{ days/year} & = 12\text{-}48 \text{ L/year} & \times 1000 \end{array}$$

$$\begin{array}{l} = \text{Total Yearly Solvent Usage} \\ \mathbf{12000\text{-}48000 \text{ L/year}} \\ \mathbf{60\text{-}90\% \text{ Reduction in Solvent Waste}} \end{array}$$

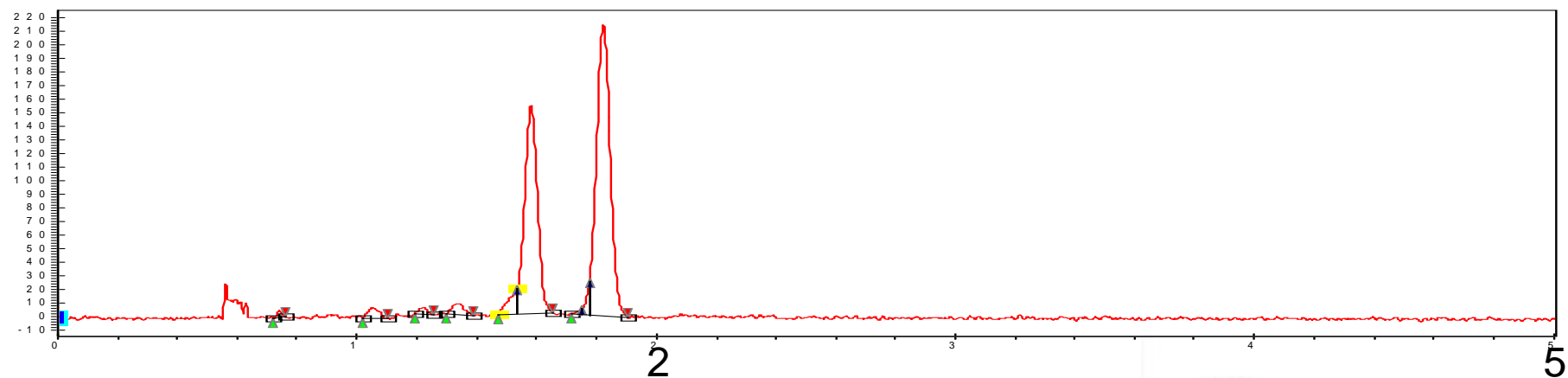
Ultra Fast Assay Development

Assay development to determine the diastereomeric ratio of key reactions on the Minigram SFC using analytical conditions. A crude sample was injected on a Princeton Chromatography's Cyano (250 x 4.6 mm) column using the standard gradient method.



Conversion to Isocratic:

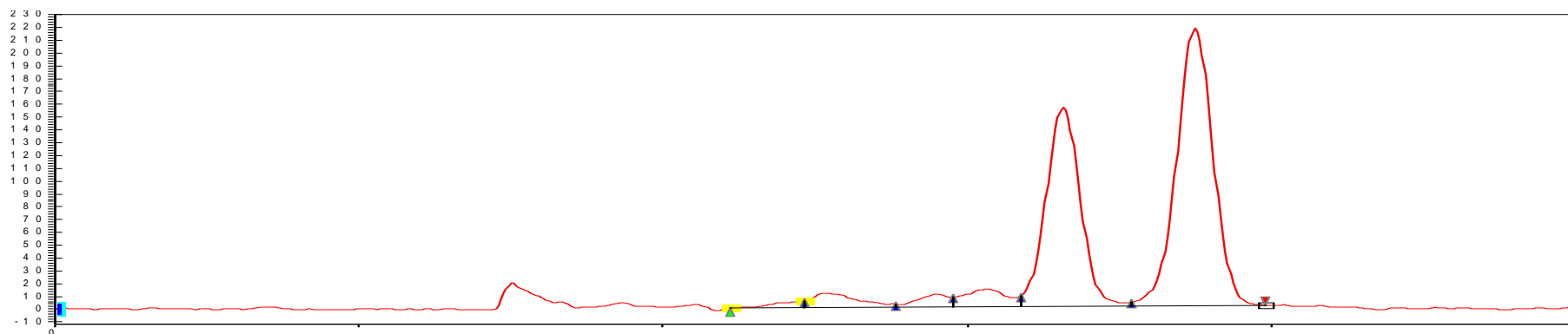
Princeton Chromatography's Cyano (250 x 4.6 mm), 15% MeOH/CO₂, 5.0 mL/min, 100 bar, 35 C, 215 nm, 10 μL injection, run time 5 mins.



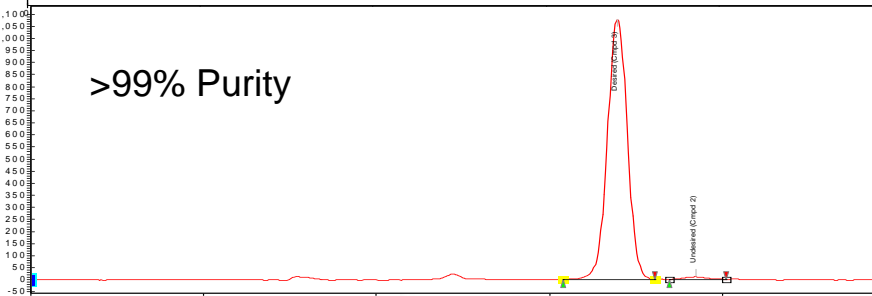
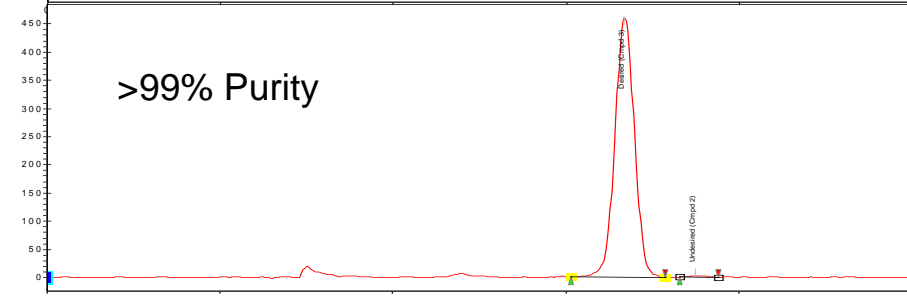
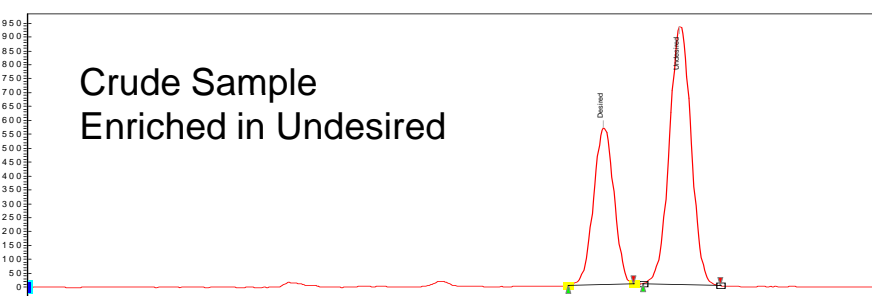
Ultra Fast Assay (1.0 min)

Ultra Fast Isocratic Method:

Princeton Chromatography's Cyano (250 x 4.6 mm), 15% MeOH/CO₂, 10.0 mL/min, 100 bar, 35 C, 215 nm, 10 μL injection, run time 5 mins.



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Analytical Solvent Reduction Calculation per Sample

Analytical HPLC Solvent Usage

$$\begin{array}{rcll} \text{Samples} & \text{Yearly} & \text{Yearly Solvent Usage} & \text{\# of Instruments} \\ 16 \text{ samples/day} & \times 250 \text{ days/year} & = 4\text{k samples/year} & \times 1000 \end{array}$$

**= Total Yearly Solvent Usage
120000 L/year for 4 Million samples**

Analytical SFC Solvent Usage

$$\begin{array}{rcll} \text{Flow Rate} & \text{\% Modifier} & \text{Time (8 hrs/day)} & \text{Yearly} & \text{Yearly Solvent Usage} & \text{\# of Instruments} \\ 10.0 \text{ mL/min} & \times 10\text{-}40\% & \times 480 \text{ min/day} & \times 250 \text{ days/year} & = 120\text{-}480 \text{ L/year} & \times 1000 \end{array}$$

$$\begin{array}{rcll} \text{Samples} & \text{Yearly} & \text{Yearly Solvent Usage} & \text{\# of Instruments} \\ 160\text{-}480 \text{ samples/day} & \times 250 \text{ days/year} & = 4\text{k samples/year} & \times 1000 \end{array}$$

**Total Yearly Solvent Usage
= 120000-480000 L/year for
40 – 120 Million samples
60-96% Reduction in Solvent Waste**

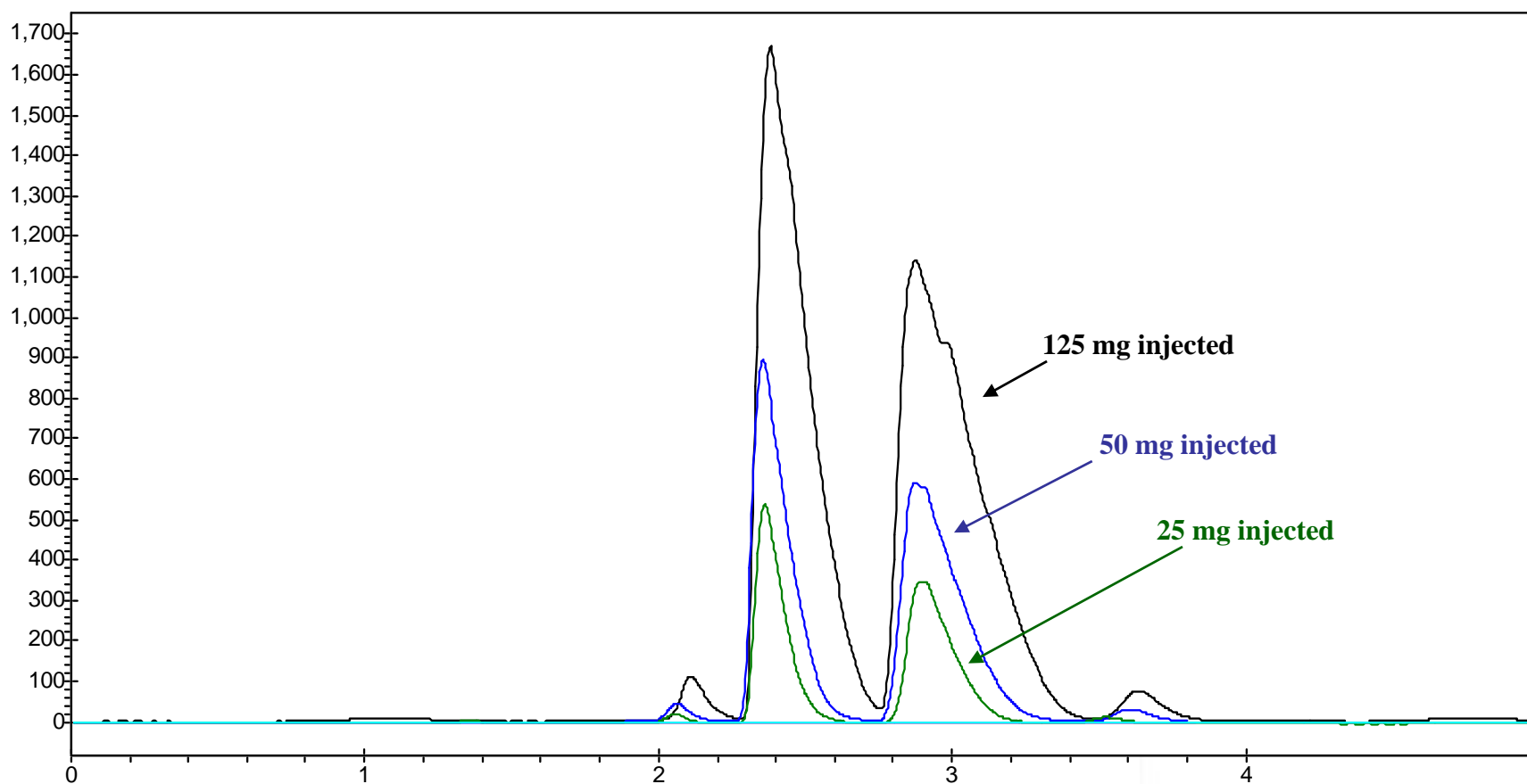
Semi-Preparative SFC System



- 50-400 mL/min
- 2-5 cm columns
- Suitable for 1-1000 grams
- Immediate prep method based on SFC screening
- Automated sample injection
- Automated fraction collection
- Convenient sample recovery

Semi-Prep SFC Loading Study

Chiralcel OF 25% 2-Propanol/75% CO₂, 70 mL/min, 100 Bar, 35°C, 290 nm



Chiral Separation of a Racemic Mixture

425 grams submitted for Separation by SFC.

42 g processed by HPLC

HPLC Method:

Column: Chiralcel OJ (20 μ m, 50 x 500 mm)

Mobile Phase: 45% 2-Propanol/Hexane

Flow Rate: 45 mL/min

Injection: 600 mg/inj

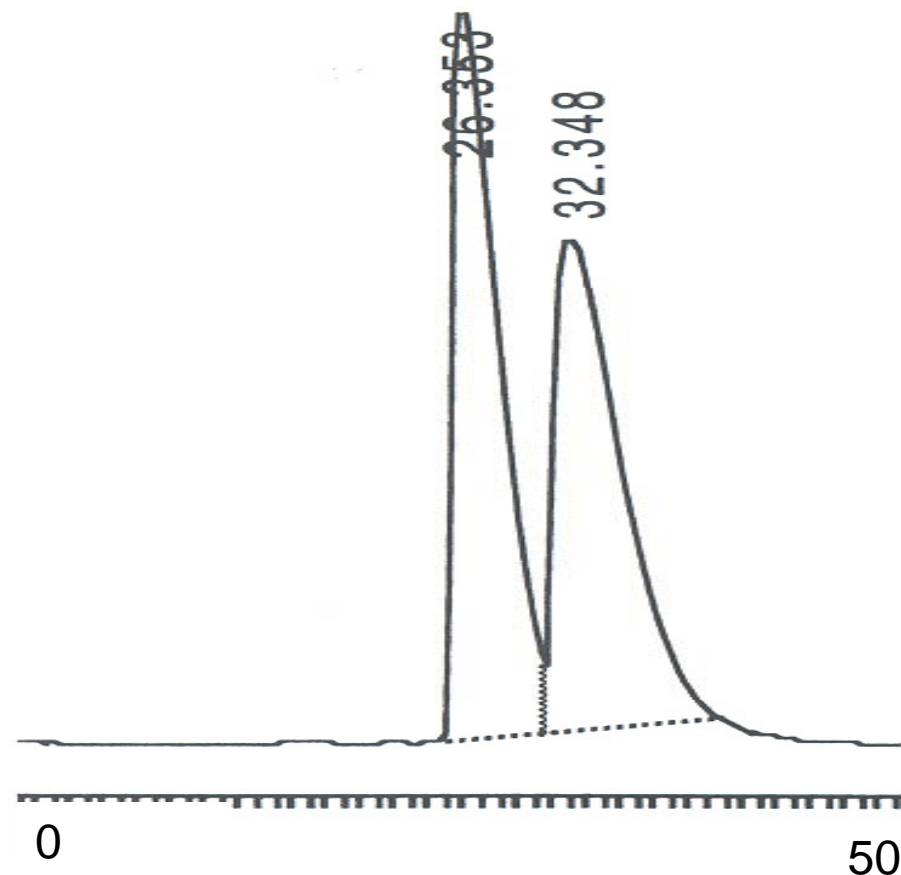
Temperature: Room Temperature

Wavelength: 254 nm

Run Time: 50 minutes

Purity: >99%

Recover: >90%

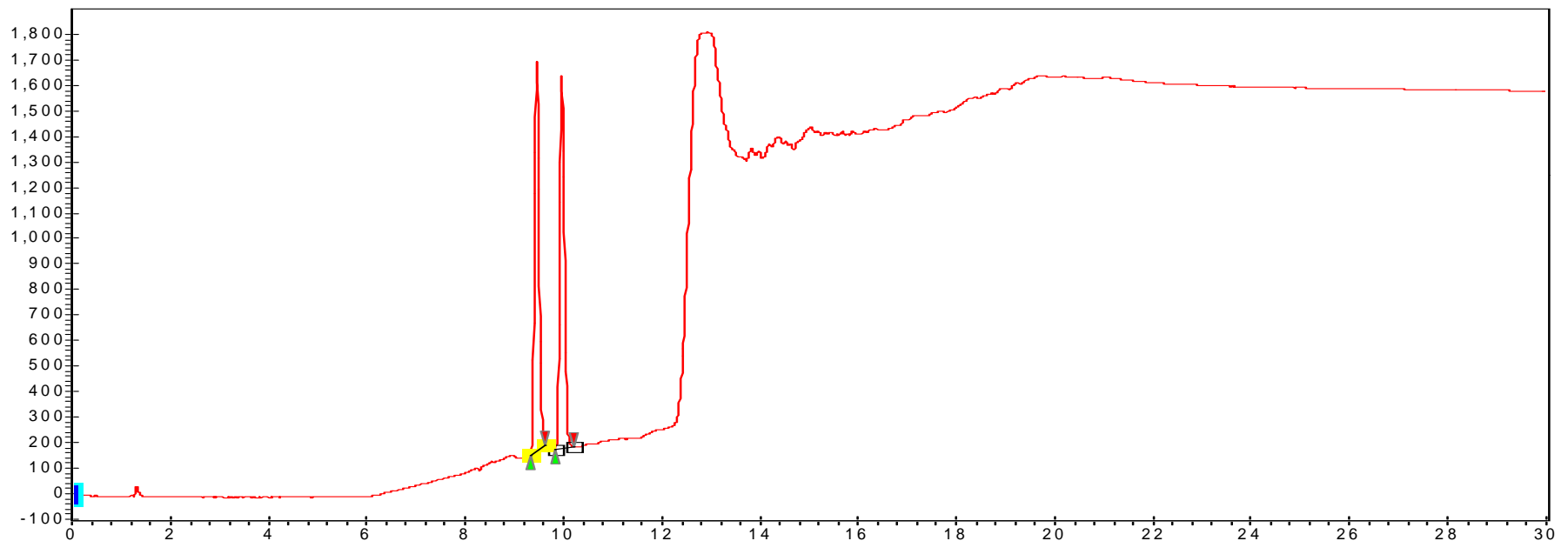


82 hours to process the 42 g sample by HPLC. A total of 220 liter of solvent was used.

Analytical SFC Screening

SFC screening and loading studies were performed on the sample of racemic material. A resolution was found on several columns. The best chromatographic resolution was found using a Chiralpak AD-H column.

Analytical Screening Gradient Chromatogram:



The results were directly translated to semi-prep SFC. The isocratic method development for the Chiralpak AD-H column was performed on the semi-prep SFC.

425 grams separated by SFC

SFC Method:

Column: Chiralpak AD-H (5 μm , 50 x 250 mm)

Flow Rate: 200 mL/min

Temperature: 35°C

Cycle Time: 1.80 minutes

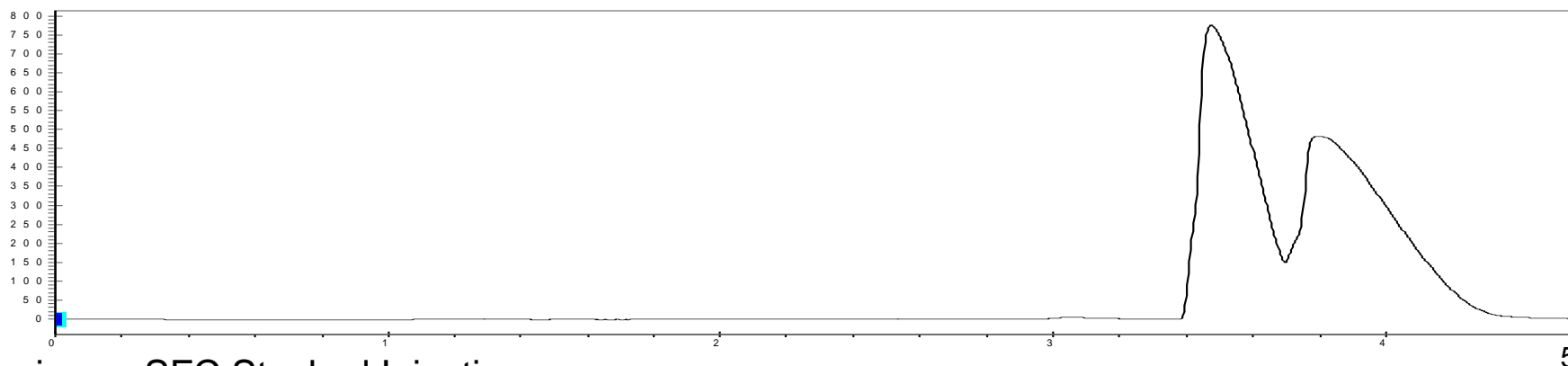
Mobile Phase: 30% Methanol/ CO_2

Injection: 330 mg/inj

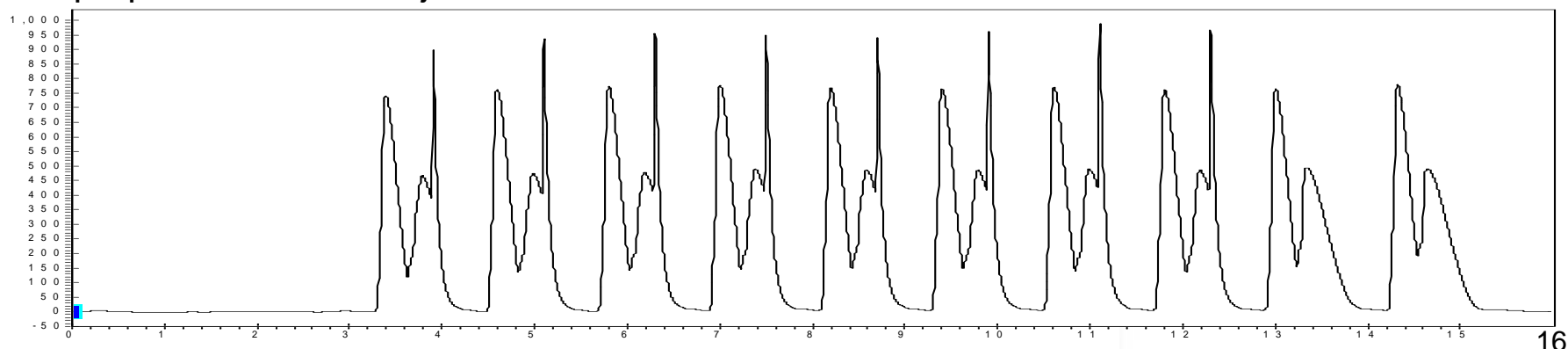
Wavelength: 280 nm

Run Time: 5.00 minutes

Semi-prep SFC Single Injection:



Semi-prep SFC Stacked Injections:



170 g of the desired enantiomer of was recovered.

Purity: >99% Recovery: ~88%

Savings Calculation

Purification Technique	Laboratory	Laboratory	Plant
	SFC	HPLC	HPLC
Productivity (kkd)	0.43	0.01	0.01
Separation Run Time (hrs)	39	825	90
Product Isolation Time (hrs)	7	115	29
Solvent Consumed (L)	140	2230	2230
Solvent Evaporated (L)	70	574	574

93.7% reduction in solvent utilization

87.8% reduction in solvent evaporation

95.2%(Lab)/56.7%(Plant) reduction in system usage

Chiral Separation of a Racemic Mixture

235 grams submitted for Separation by SFC.

8 g processed by HPLC

HPLC Method:

Column: Chiralpak AD-H (5 μ m, 50 x 250 mm)

Mobile Phase: 20% Ethanol/Hexane

Flow Rate: 100 mL/min

Injection: 210 mg/inj

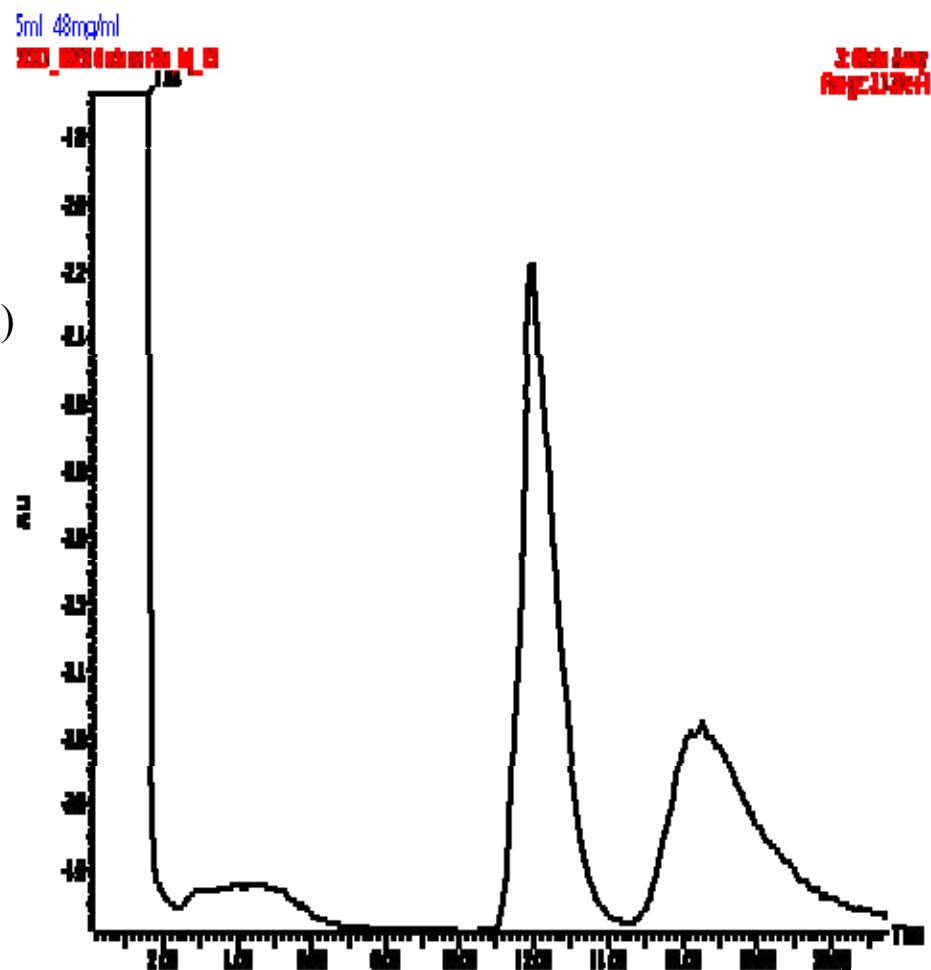
Temperature: Room Temperature

Wavelength: 240 nm

Run Time: 25 minutes

Purity: >97%

Recover: >90%

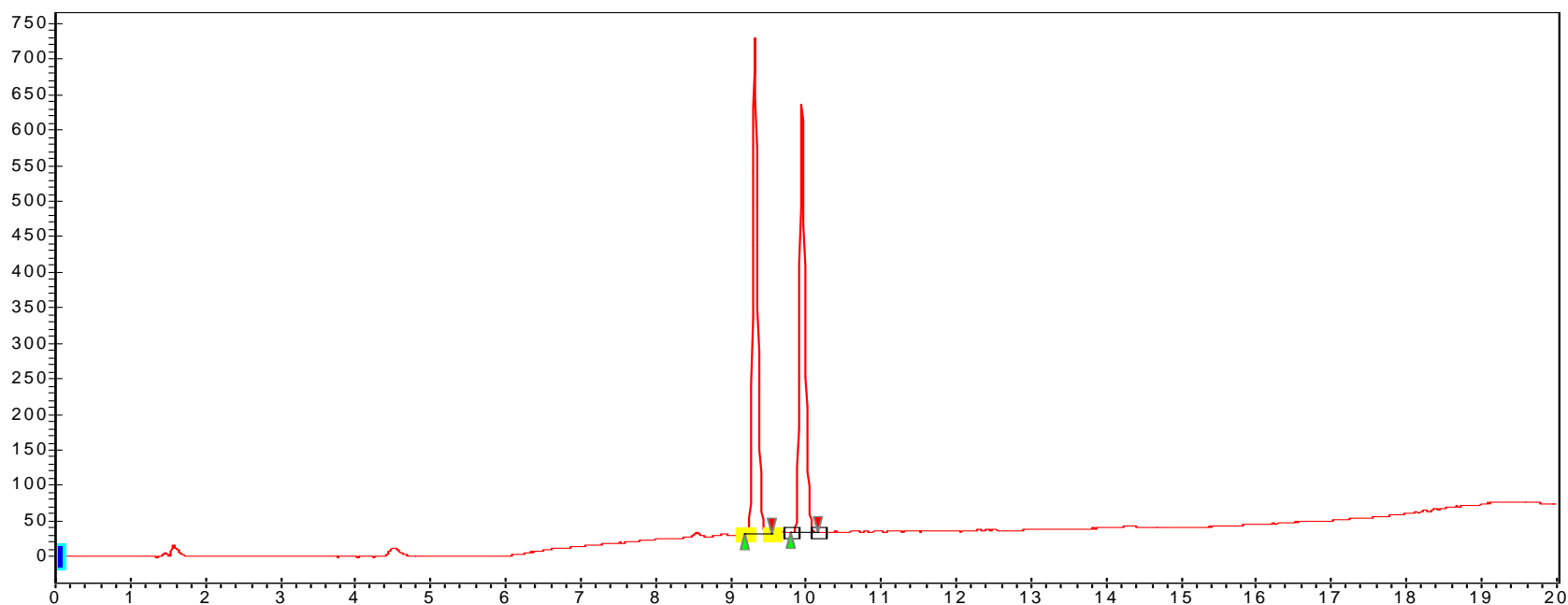


16 hours to process the 8 g sample by HPLC. A total of 95 liter of solvent was used.

Chiral Analytical SFC Screening

SFC screening and loading studies were performed on the sample of racemic material. A resolution was found on several columns. The best chromatographic resolution was found using a Chiralcel OJ-H column.

Analytical Screening Gradient Chromatogram:



The results were directly translated to semi-prep SFC. the isocratic method development for the Chiralcel OJ-H column was performed on the semi-prep SFC.

235 grams separated by SFC

SFC Method:

Column: Chiralcel OJ-H (5 μ m, 20 x 250 mm)

Flow Rate: 100 mL/min

Temperature: 35°C

Cycle Time: 0.53 minutes (Cycle time Limited due to injection speed)

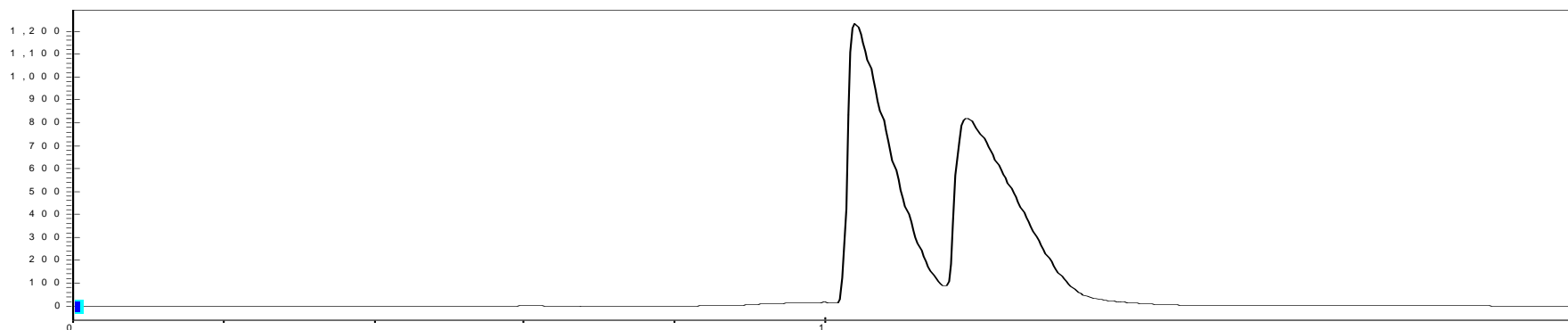
Mobile Phase: 25% Methanol/CO₂

Injection: 63 mg/inj

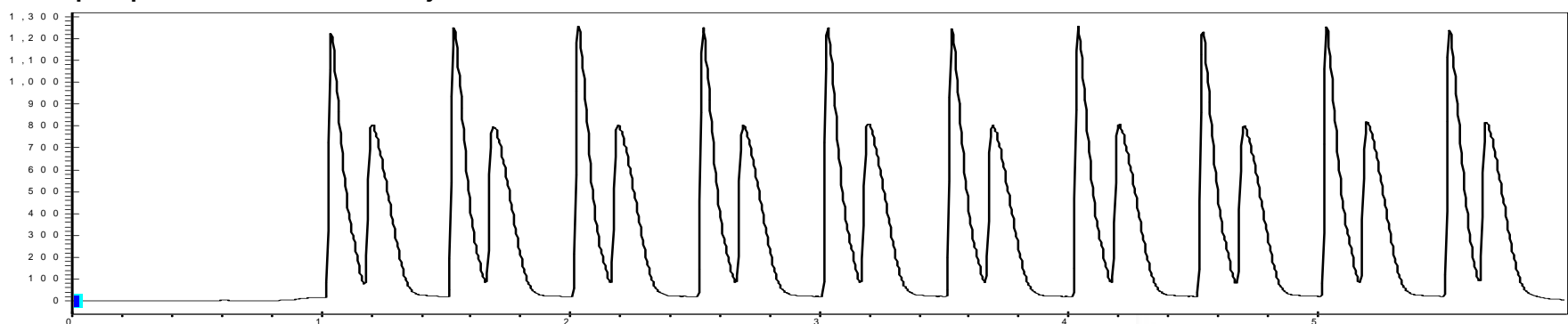
Wavelength: 270 nm

Run Time: 2.00 minutes

Semi-prep SFC Single Injection:



Semi-prep SFC Stacked Injections:



120 g of the desired enantiomer of was recovered.

Purity: >99% Recovery: ~92%

Savings Calculation

Purification Technique	Laboratory	Laboratory	Plant
	SFC	HPLC	HPLC
Productivity (kkd)	1.56	0.02	0.02
Separation Run Time (hrs)	33	466	52
Product Isolation Time (hrs)	2	112	28
Solvent Consumed (L)	50	2800	2800
Solvent Evaporated (L)	13	560	560

98.2% reduction in solvent utilization

97.7% reduction in solvent evaporation

92.9%(Lab)/36.5%(Plant) reduction in system usage

Achiral Separation of a Pharmaceutical Intermediate

13.7 Kilograms submitted for Separation by SFC.

90 g processed by HPLC

HPLC Method:

Column: Prigent Chromatography's Cyano
(5 μm , 50 x 280 mm)

Mobile Phase: 10% Ethanol/Hexane

Flow Rate: 118 mL/min

Injection: 4.5 g/inj

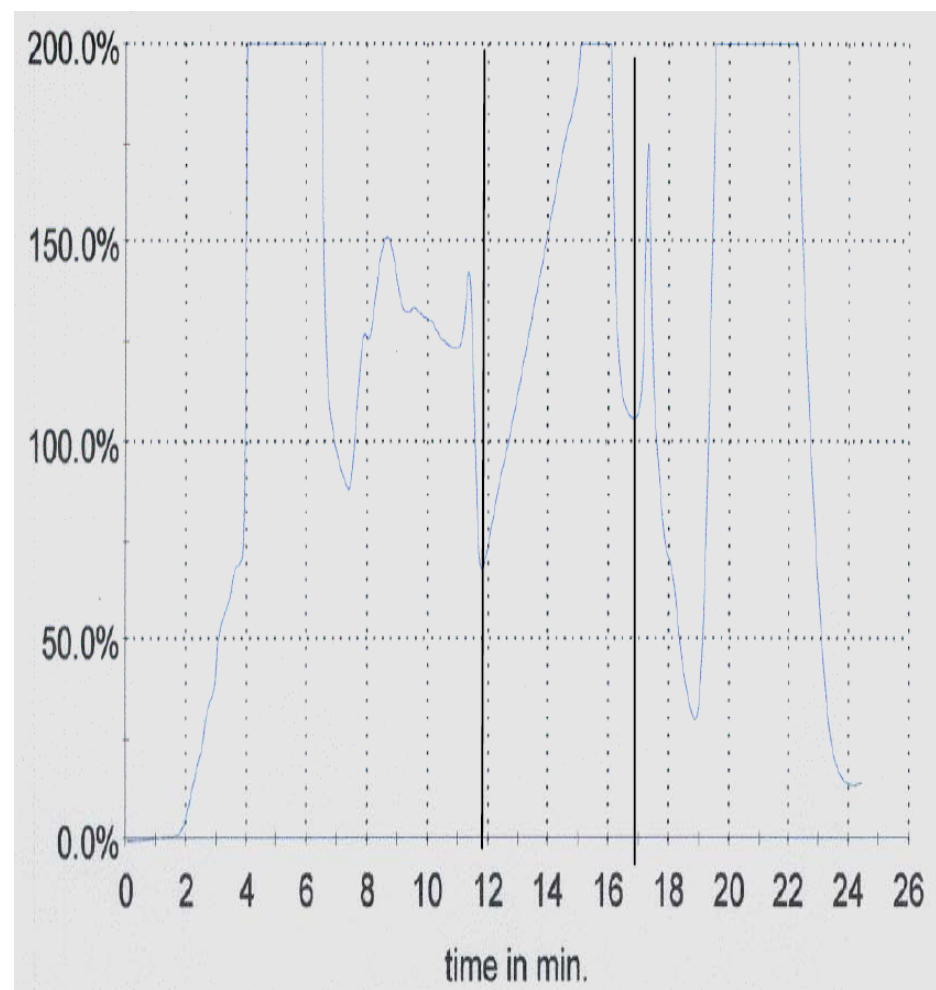
Temperature: Room Temperature

Wavelength: 293 nm

Run Time: 25 minutes

Purity: >95%

Recover: >90%

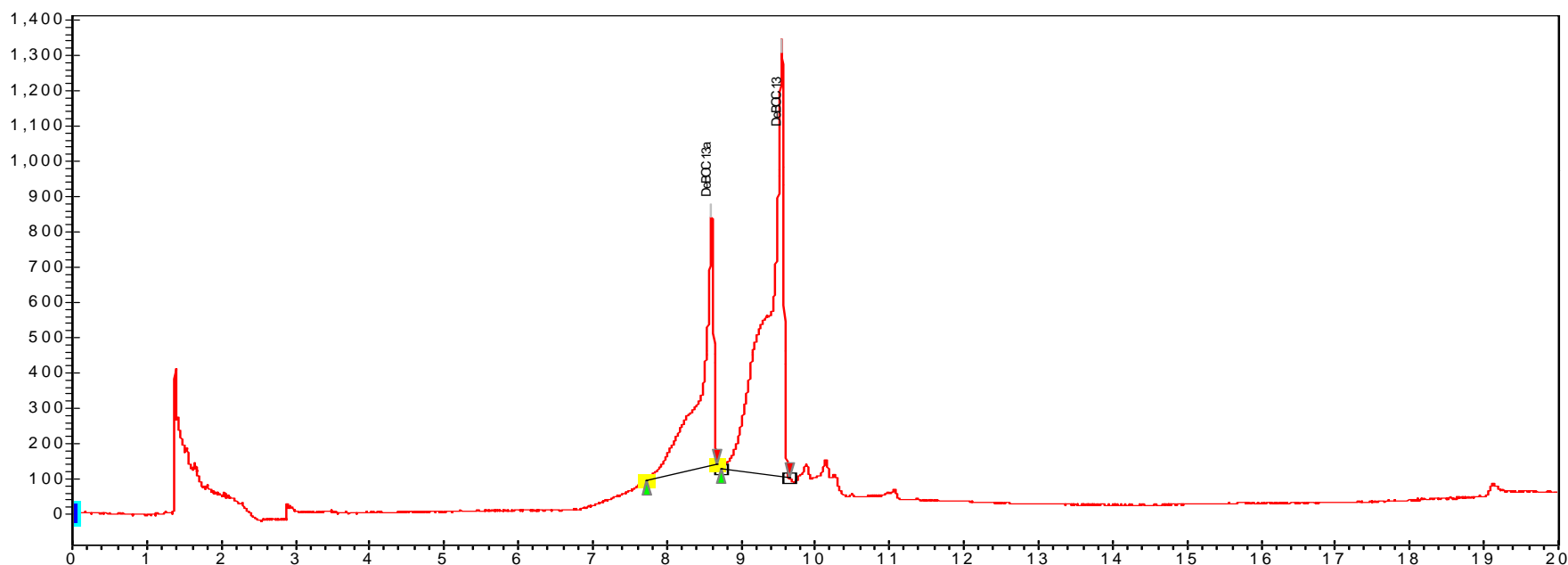


9 hours to process the 90 g sample by HPLC. A total of 65 liter of solvent was used.

Analytical SFC Screening

SFC screening was performed using both chiral and achiral columns. A resolution was found on several columns. The best chromatographic resolution was found using a Princeton Chromatography's (PC) Cyano. Other PC Cyano columns were tested but this one demonstrated the most robustness.

Analytical Screening Gradient Chromatogram:



Isocratic method development for the separation was performed on the Minigran SFC using the PC Cyano column. The isocratic method was directly translated to semi-prep SFC.

425 grams separated by SFC

SFC Method:

Column: PC Cyano (5 μm , 50 x 250 mm)

Flow Rate: 250 mL/min

Temperature: 35°C

Cycle Time: 1.35 minutes

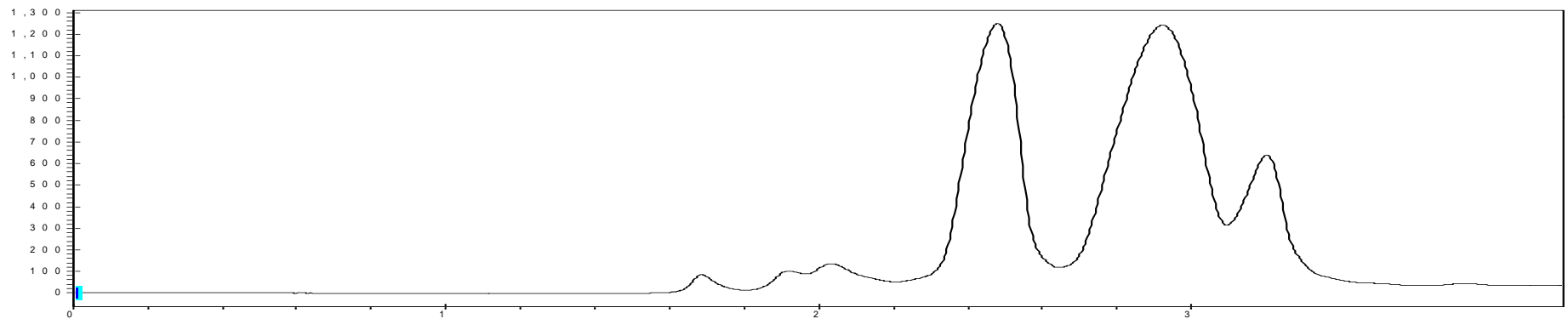
Mobile Phase: 25% Methanol/CO₂

Injection: 2.15 g/inj

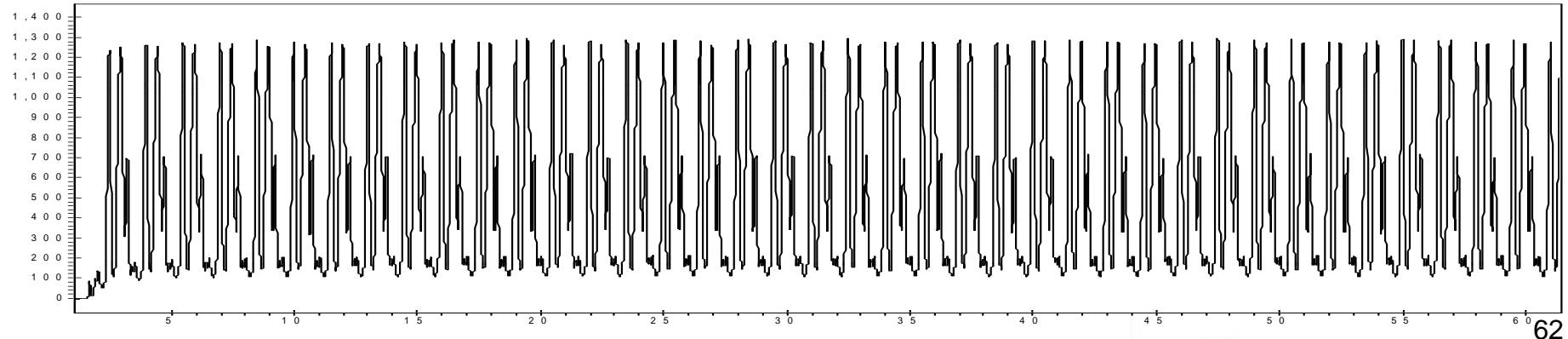
Wavelength: 280 nm

Run Time: 3.50 minutes

Semi-prep SFC Single Injection:



Semi-prep SFC Stacked Injections:



4.44 kg of the desired intermediate of was recovered.

Purity: 98% Recovery: ~92%

Savings Calculation

Purification Technique	Laboratory	Laboratory	Plant
	SFC	HPLC	HPLC
Productivity (kkd)	2.71	0.41	0.41
Separation Run Time (hrs)	144	1320	147
Product Isolation Time (hrs)	25	360	89.8
Solvent Consumed (L)	540	9350	9350
Solvent Evaporated (L)	216	1796	1796

94.2% reduction in solvent utilization
87.9% reduction in solvent evaporation
89.1%(Lab) reduction in system usage

Prep SFC Lessons Learned

• Not just Normal Phase

SFC Method:

Column: C18 (21.2x250mm)

Flow Rate: 100 mL/min

Temperature: 35°C

Cycle Time: 1.00 minute

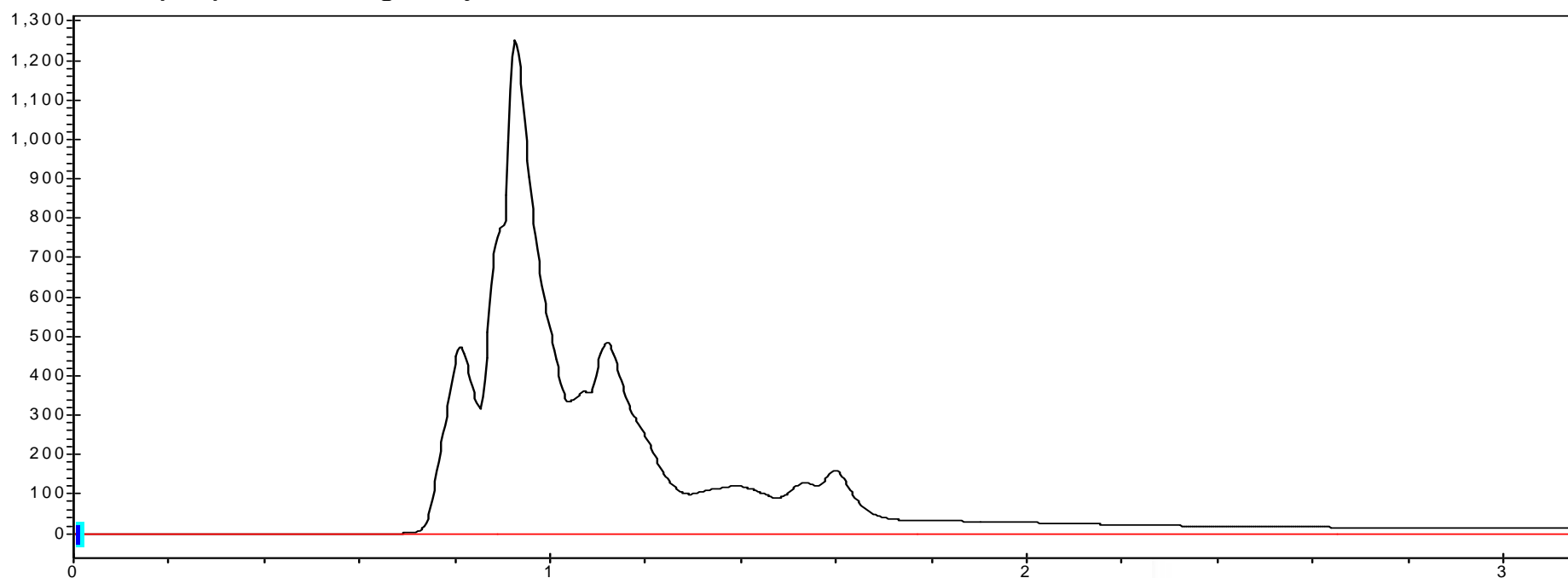
Mobile Phase: 10% Methanol/CO₂

Injection: 35 mg/inj

Wavelength: 215 nm

Run Time: 3.00 minutes

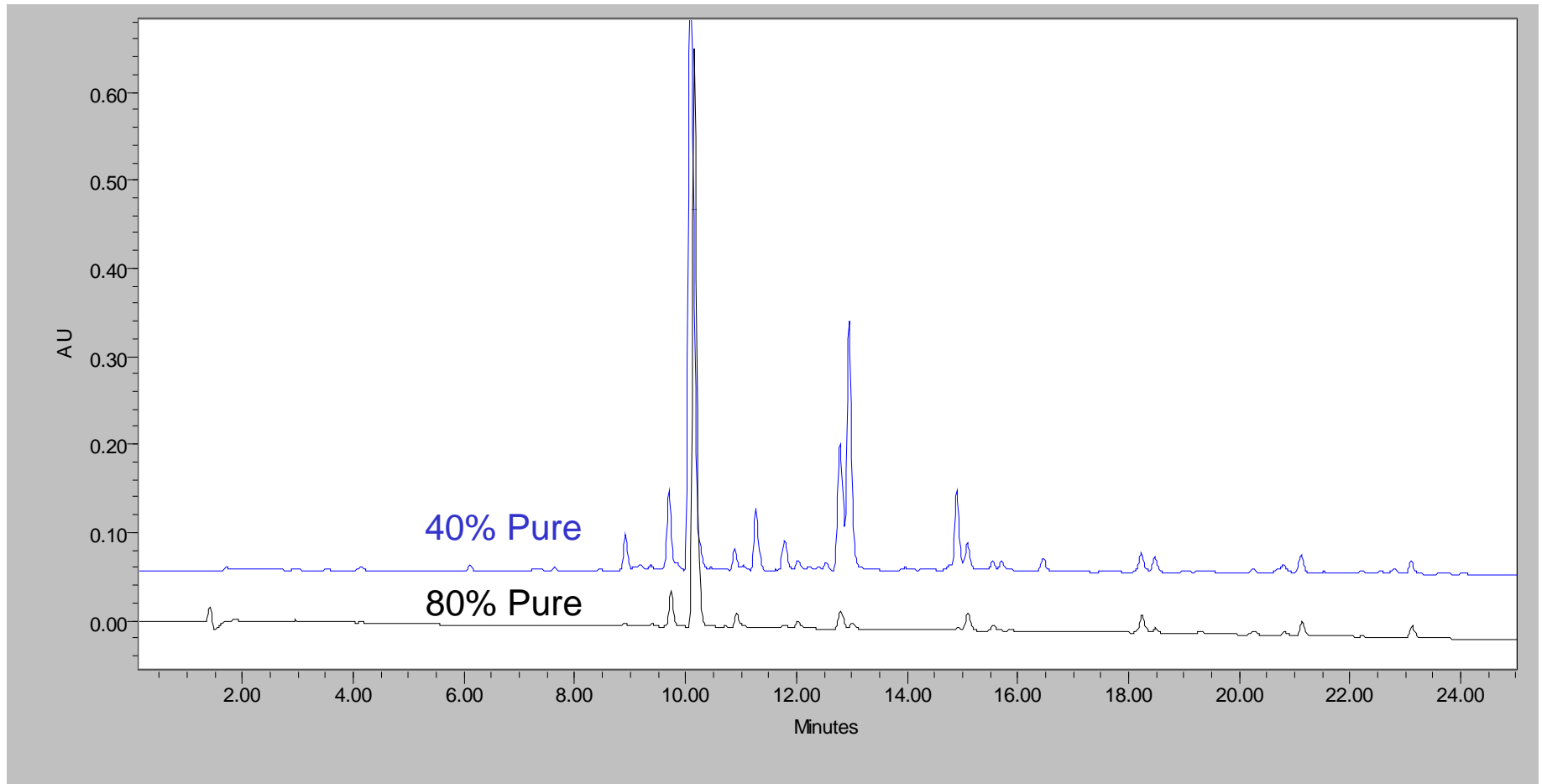
Semi-prep SFC Single Injection:



Prep SFC Lessons Learned

- Not just Normal Phase

HPLC of Results:



Prep SFC Lessons Learned

- Bigger is Not always better

SFC Method:

Column: Kromasil 60-5SIL (50 x 250 mm)

Mobile Phase: 10% MeOH/CO₂ for 4 mins ramp to 40% MeOH/CO₂ @ 4% MeOH/min

Flow Rate: 200 mL/min

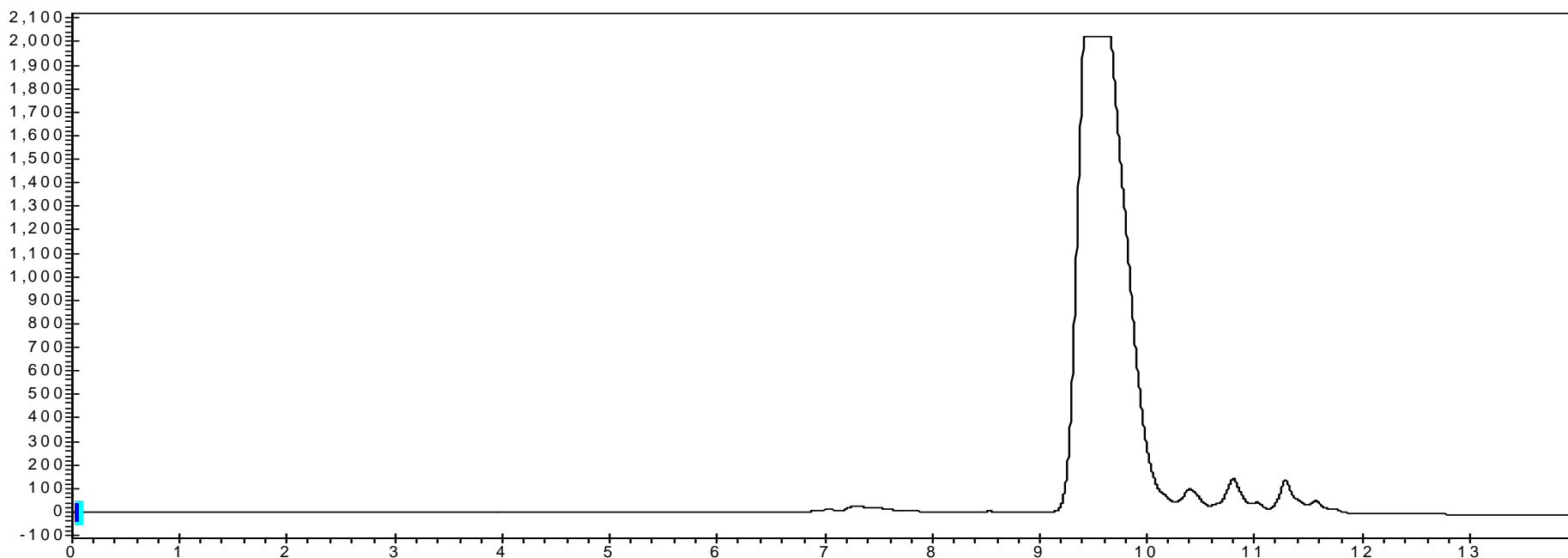
Injection: 700 mg/inj

Temperature: 35°C

Wavelength: 215 nm

Run Time: 14.00 minutes

Semi-prep SFC Single Injection:



Prep SFC Lessons Learned

- Bigger is Not always better

SFC Method:

Column: Kromasil 60-5SIL (20 x 250 mm)

Mobile Phase: 10% MeOH/CO₂ for 4 mins ramp to 18% MeOH/CO₂ @ 4% MeOH/min step to 40% MeOH/CO₂

Flow Rate: 100 mL/min

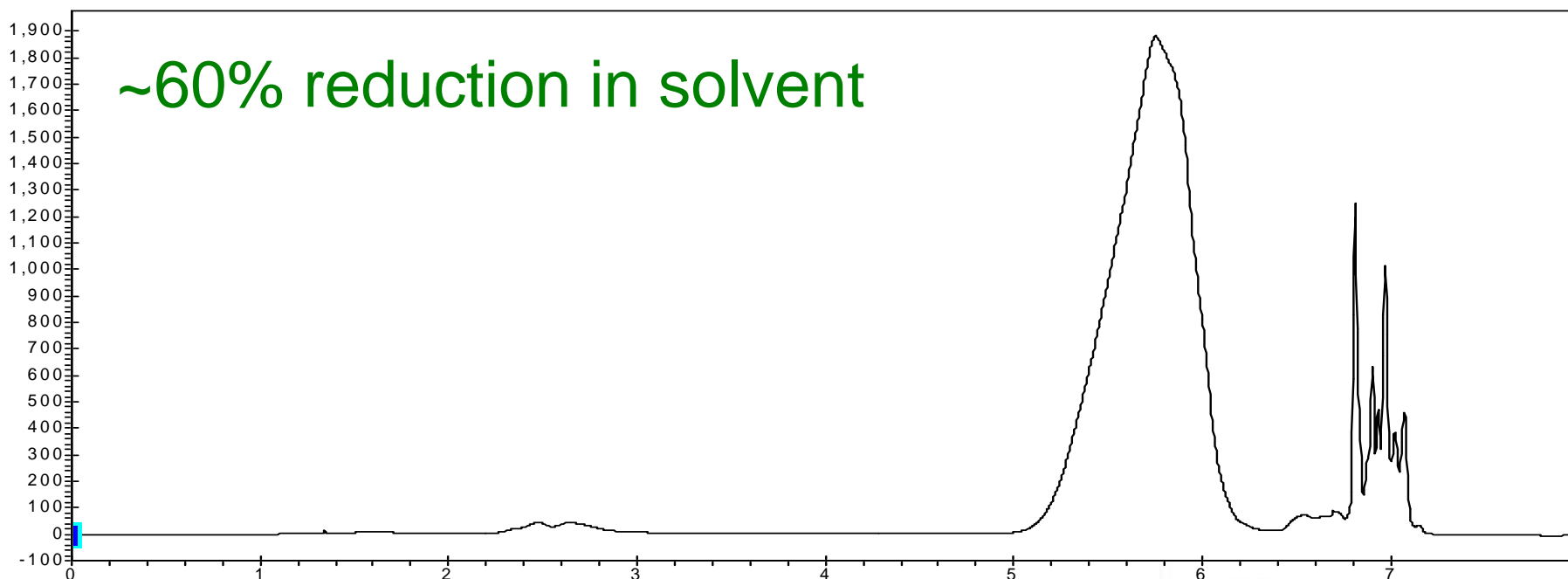
Injection: 350 mg/inj

Temperature: 35°C

Wavelength: 215 nm

Run Time: 8.00 minutes

Semi-prep SFC Single Injection:



Conclusions

- Preparative SFC can help to speed the process development effort.
- SFC is becoming the preferred technique for chiral method development, chiral analysis and purifications in support of pharmaceutical process research.
- Moving towards replacement of HPLC with SFC, owing to improved performance and solvent savings
- SFC at the analytical scale can afford faster development times for both SFC and HPLC from the rapid column scouting methods.
- SFC at the analytical scale can also afford great solvent reduction due to its fast run times.
- Preparative SFC at kilogram scale can afford greatly improved productivity and significant savings in solvent utilization and waste generation.
- Preparative SFC at even larger scale shows promise for the future.

Looking to the Future

- What will the future hold for SFC?
 - As technology get smaller?
 - Microfluidic SFC
 - Micro-prep SFC
 - As demand gets greater?
 - Super Prep SFC?
 - SMB-SFC

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