



REGIS®
TECHNOLOGIES, INC.

Challenges and Solutions to scaling up with SFC

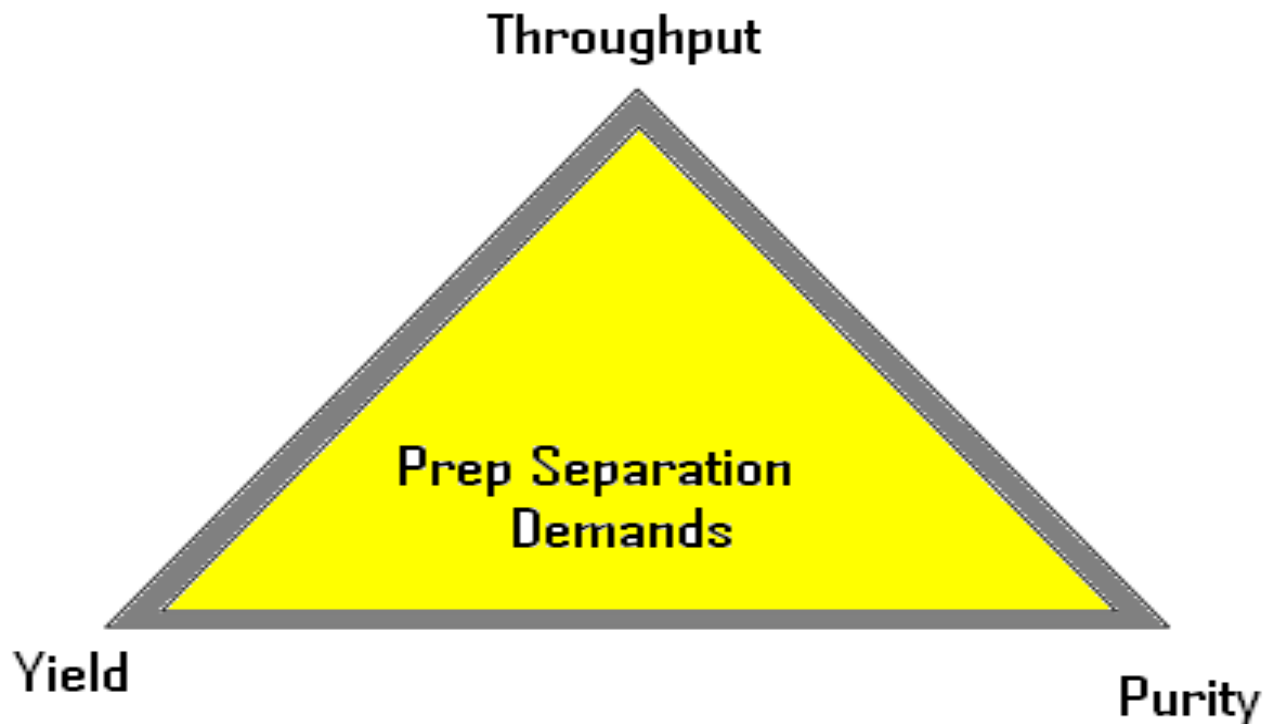
Zahid Ali

Senior Separation Scientist, Regis Technologies, Inc.

Introduction

- Regis Technologies receives more than 125 diverse chiral and achiral sample each year for screening.
- 85% of these compounds are potential candidates for purification on the preparative scale.
- Regis uses its expertise in purification technology to provide high quality, cost-effective purifications of target molecules.

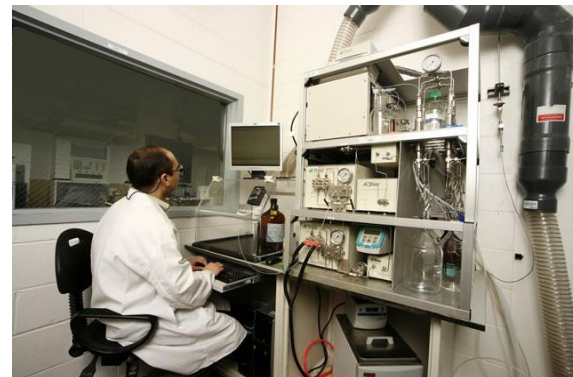
Basic Principles of Preparative SFC



In prep SFC yield, purity of the final product and cost-effectiveness of the method are emphasized

Analytical to Preparative Scale Separations

- Regis currently operates 2 analytical units for:
 - Method development
 - Free chiral/achiral screening
 - Solubility and loading studies
 - Milligram scale purification
- An intermediate scale SFC 80 unit for:
 - 2g-10g scale purification
- Preparative scale SFC 350 unit for:
 - 10g to Multi-kilogram separations
 - GMP and non GMP conditions



The Chromatographers Triangle

- Purity, Yield and Productivity are the three main challenges that face preparative chromatographers.
- It is widely known that scaling up from an analytical method is not always linear as predicted—even with the best modeling.
- In order to successfully complete a separation, it is sometimes necessary to prioritize and possibly compromise purity, yield and/or productivity.

Scale up Challenges

- Compounds with very low solubility in alcohols
- Machine and column pressure limitations
- Working without the use of chlorinated solvents or DMSO, even for sample preparation
- Working with other limitations imposed by the customer.

Solubility Effect on Scalability

- The solubility of a compound is often the limiting factor of productivity.
- This can increase the cost of a separation both in terms of time and money.
- Ultimately in some cases, it can make a preparative separation economically unfeasible.

Flow Rate Effect on Scalability

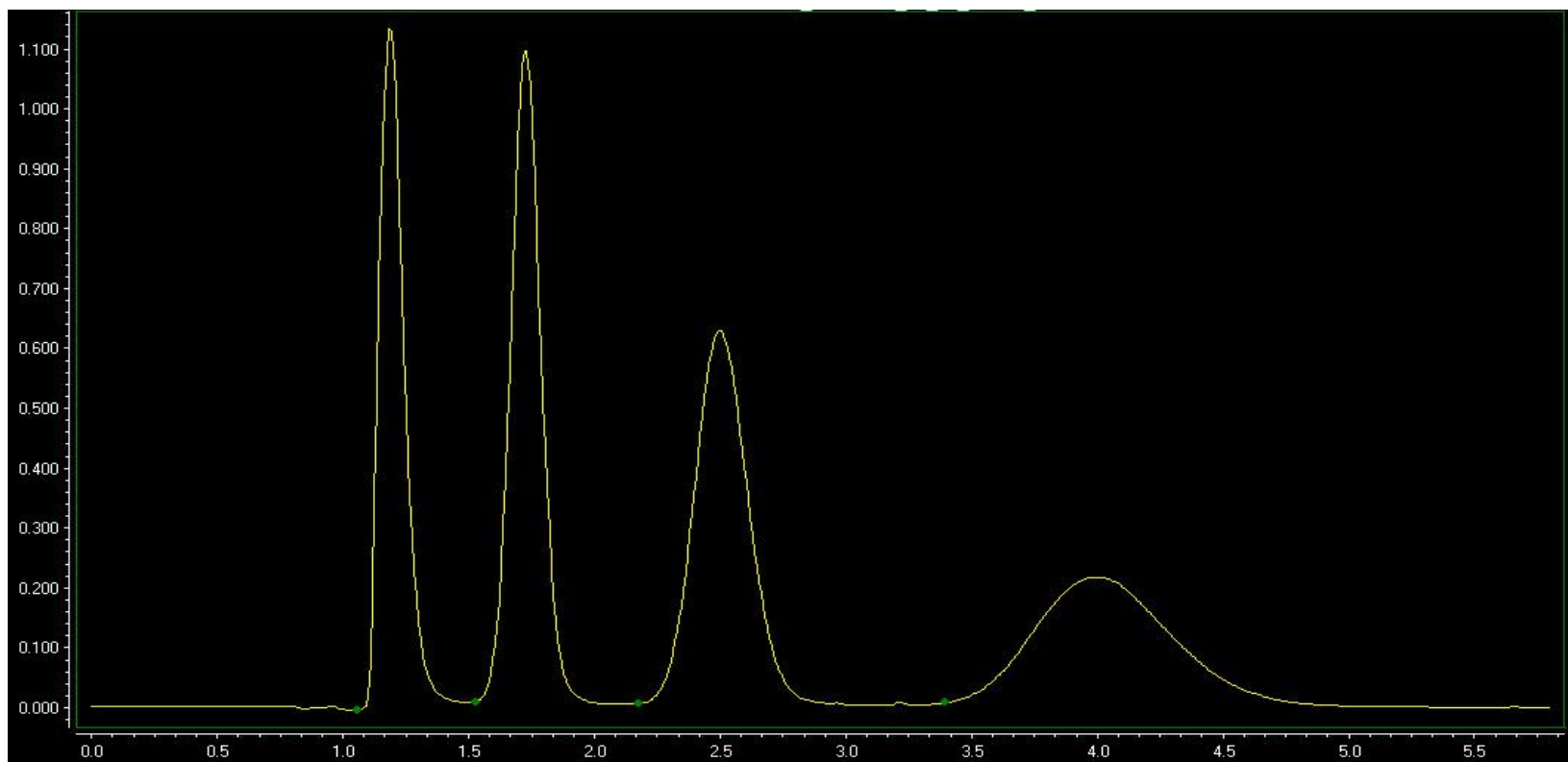
- For preparative scale purifications, pressure can also become a limiting factor.
- This is due to the limits both of the SFC system and the packed SFC column.
- We have observed that decreases in the viscosity of the mobile phase can have a direct impact on productivity because of the ability to run at higher flow rates.

Case Studies

- The following case studies were actual projects performed at Regis where the scale-up from analytical to preparative was not linear.
- Novel techniques were used to successfully complete the projects to meet the specifications set forth by our customers.
- All three projects were scaled-up to the non-GMP kilogram level.

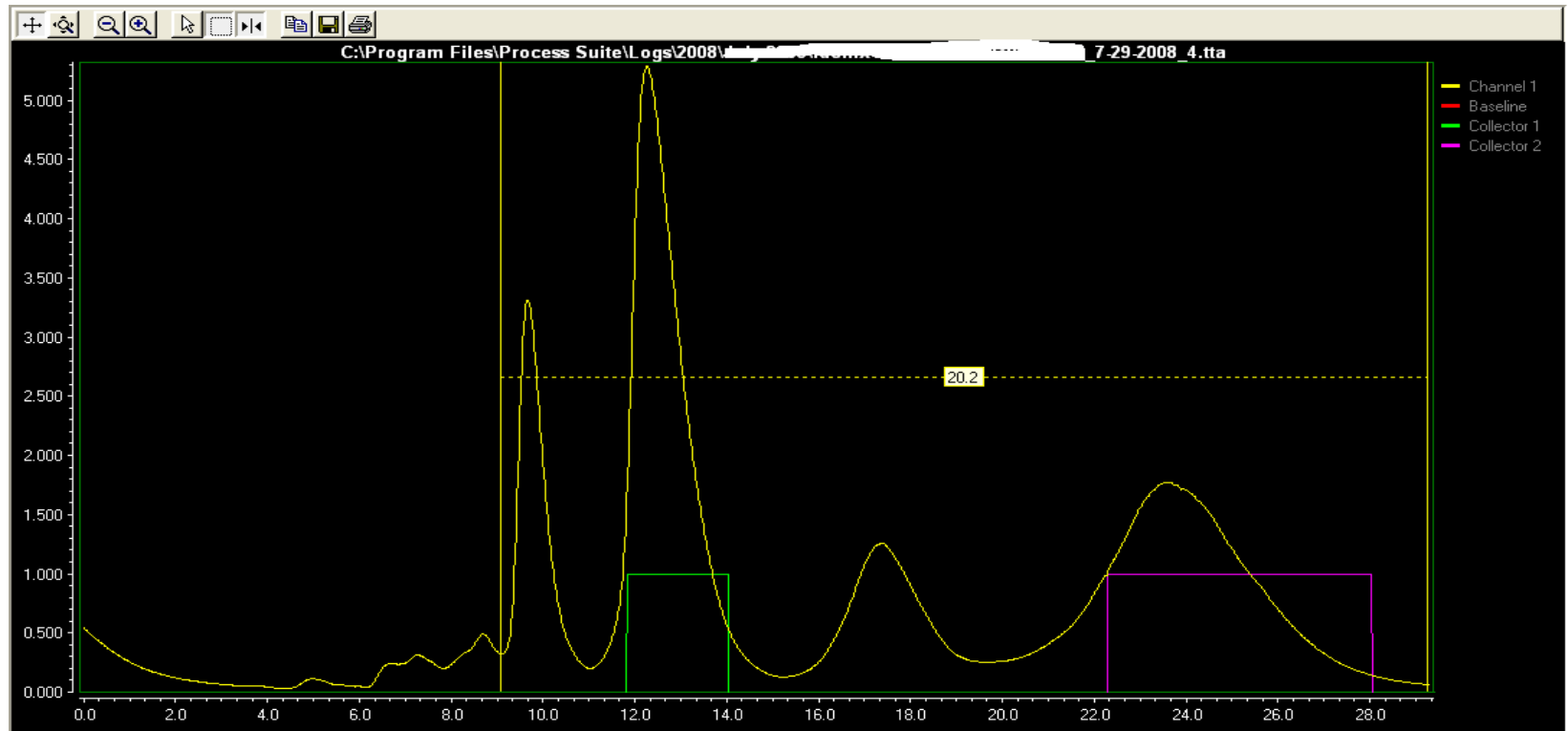
Case Study #1 - Analytical Separation

- Column: RegisPack®, 250mm x 4.6mm
- Co-Solvent : 28%IPA in + 0.5% DEA
- Flow Rate: 4ml/min
- Desired collection of Peak #2 and #4



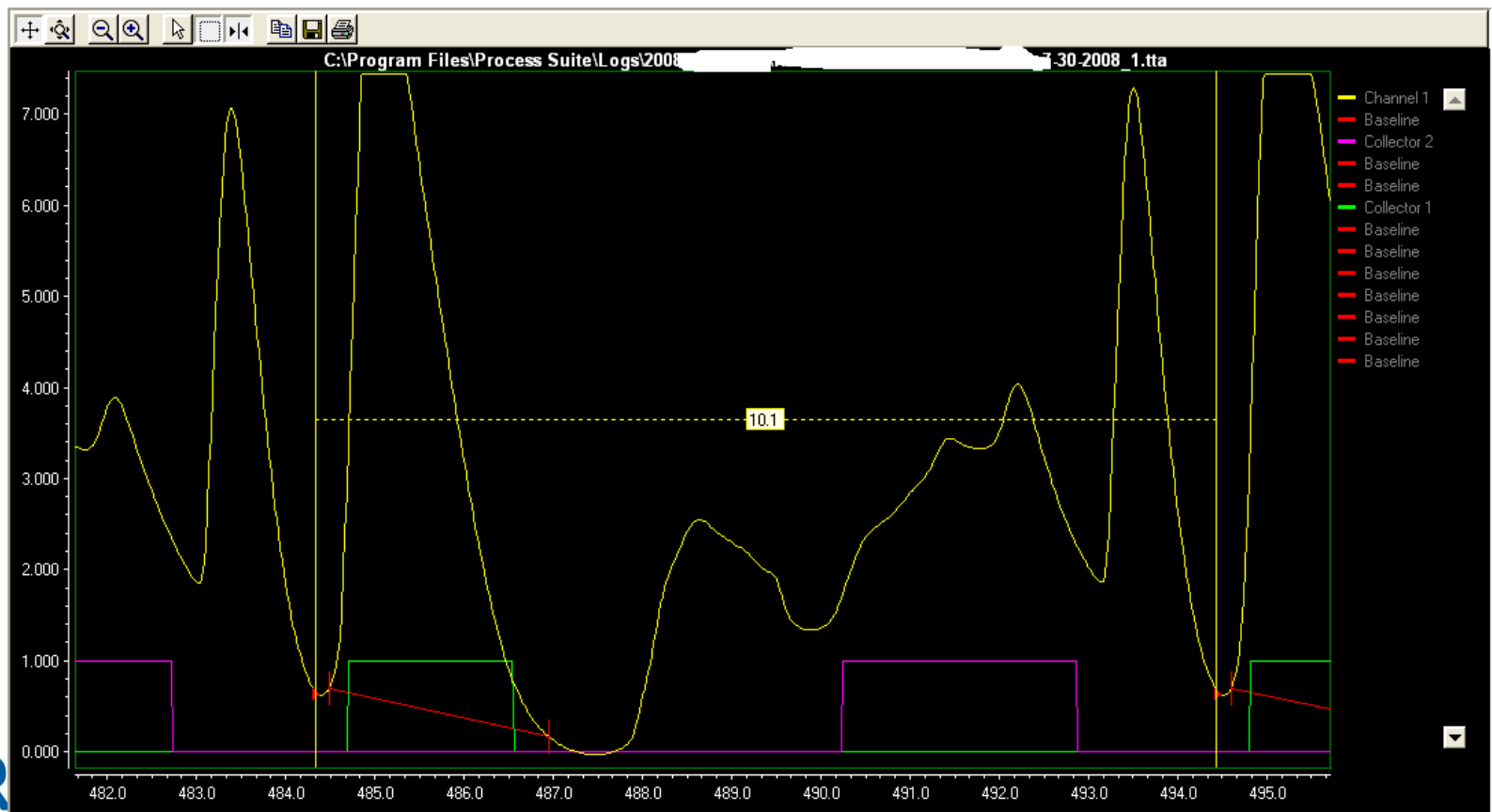
Case Study #1: Preparative Scale Up Isocratic Prep injection

- Column: RegisPack® 250mm x 50mm, 5 micron
- Co-Solvent : 28% (50:50) IPA/EtOH)+0.5% DEA
- Cycle time is 20.2 minutes
- Flow rate: 275g/min



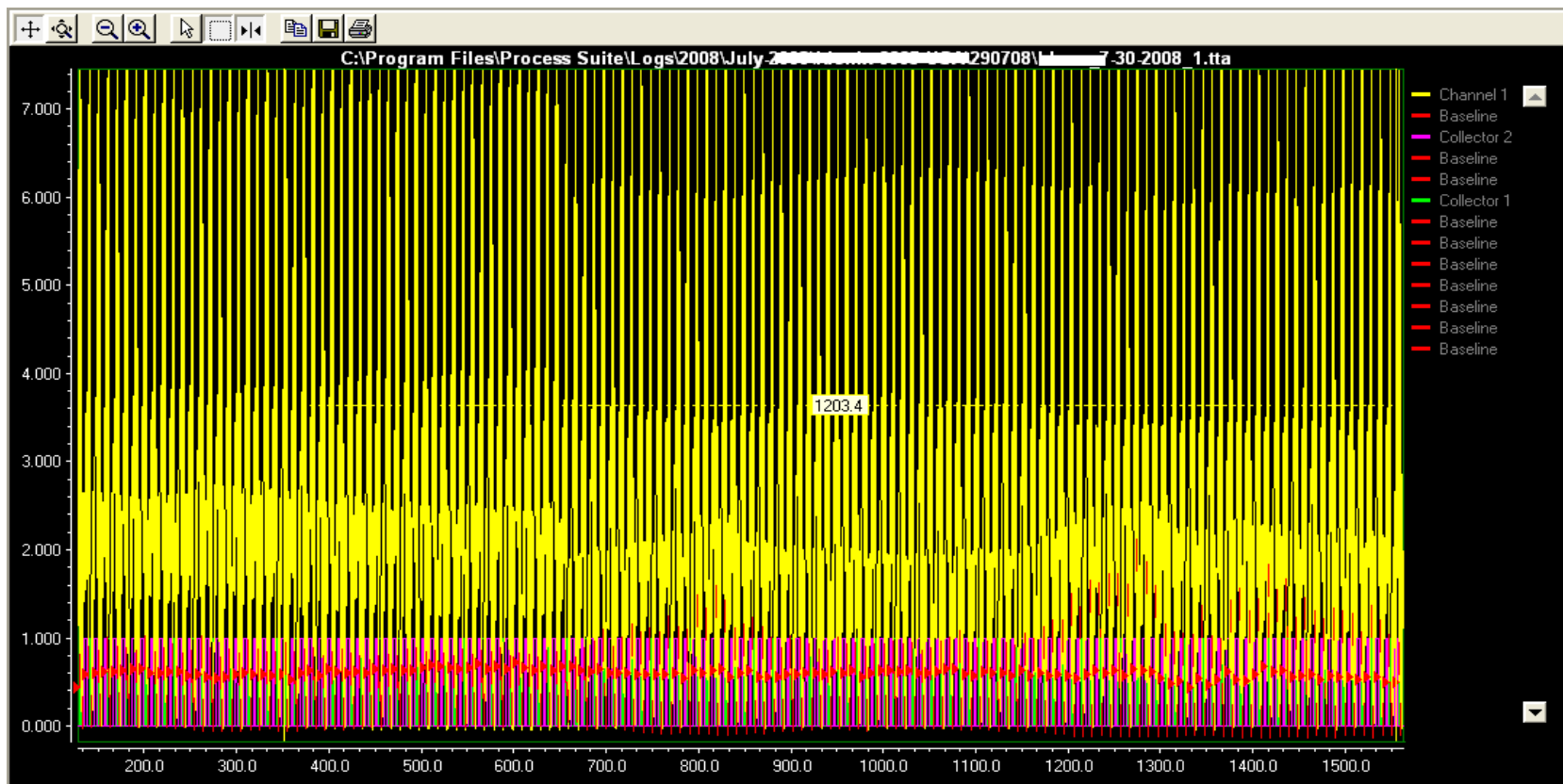
Case Study #1 : Scale Up Gradient Prep Injection

- Column: RegisPak[®]250mm x 50.0mm, 5 micron
- Co-Solvent: 23-45% of (50:50) (IPA/EtOH)+0.5% DEA
- Gradient cycle time: 10.1 minutes
- Production rate increases from 1.8g/hr to 4.3g/hr

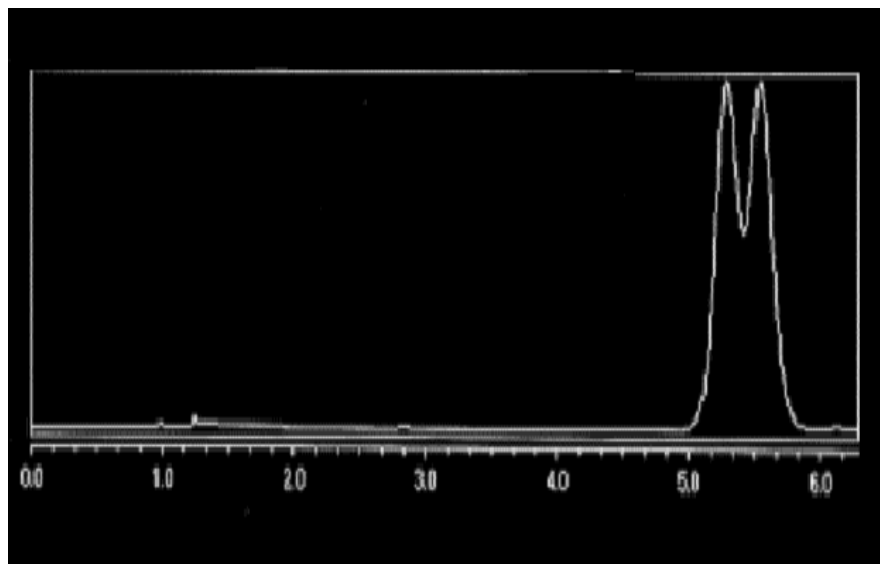


Case Study #1 : Stacked Injections (Method type gradient)

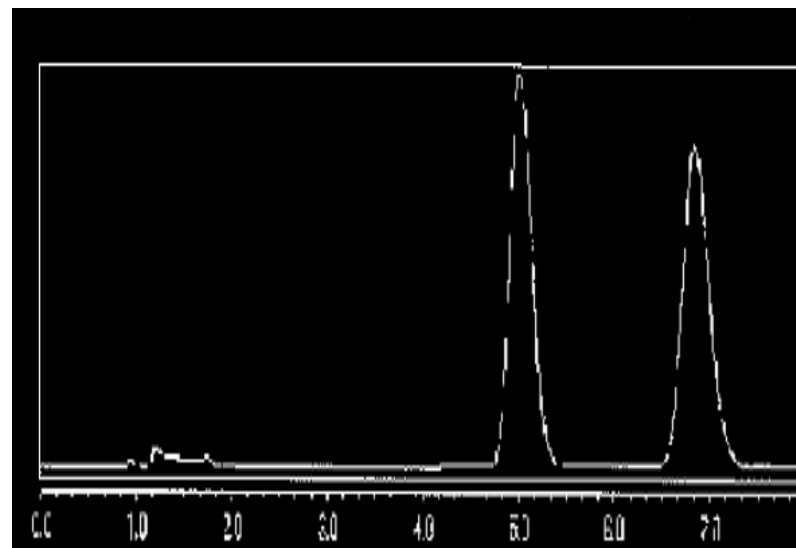
- Column: RegisPak[®]250mm x 50.0mm, 5 micron
- Co-Solvent: 23-45% of (50:50) (IPA/EtOH)+0.5% DEA
- Gradient cycle time: 10.1 minutes



Case Study #2 : Analytical Separation



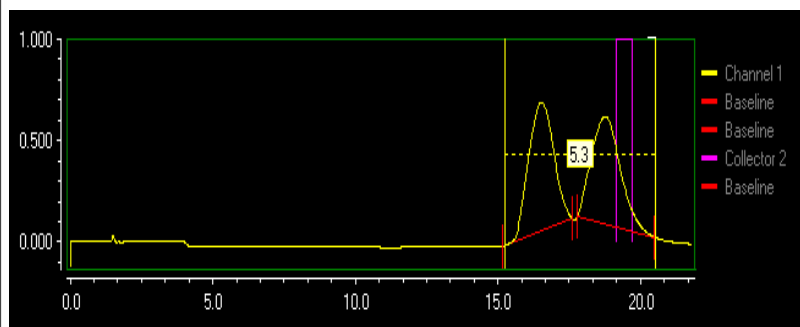
(S,S) Whelk-O1[®] 250mm x 4.6mm,
(10% EtOH co-solvent)
Flow: 4ml/min



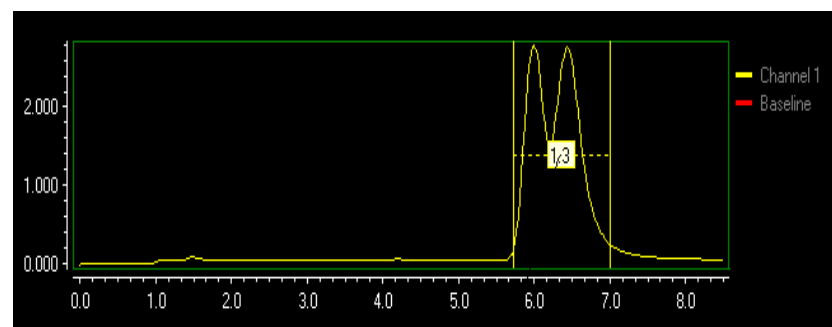
RegisCell[®] 250mm x 4.6mm,
(10% EtOH co-solvent)
Flow: 4ml/min

After routine screening on available chiral columns, the analytical SFC screening data shows partial resolution on (S,S)Whelk-O1[®] and sufficient baseline resolution on RegisCell[®]. At this point, the analytical data is starting to indicate that this compound might be a candidate for scale-up

Case Study #2 : Scale-up Using a Single Column System



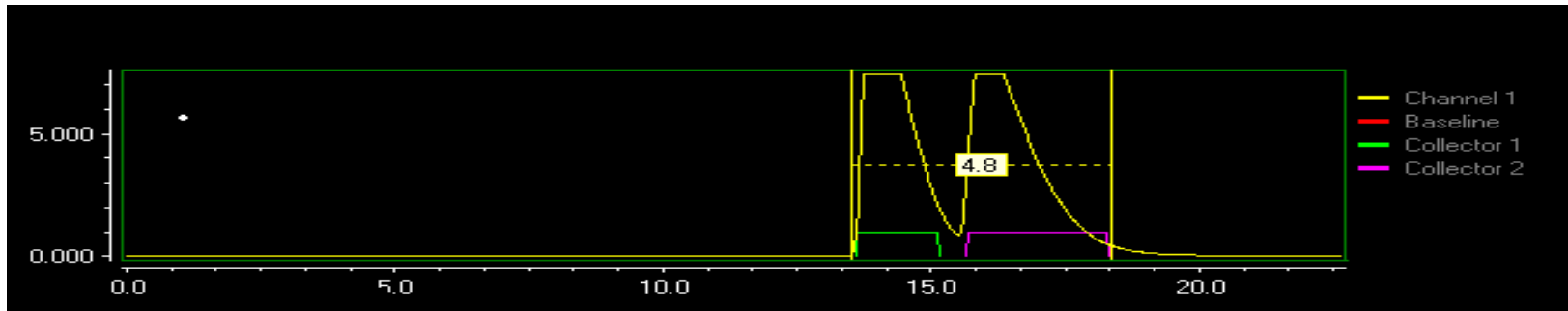
RegisCell® 250mm x 30mm (8%
EtOH Co-solvent) Flow: 275 g/min



RegisCell® 250mm x 30mm (30%
EtOH Co-solvent) Flow: 275 g/min

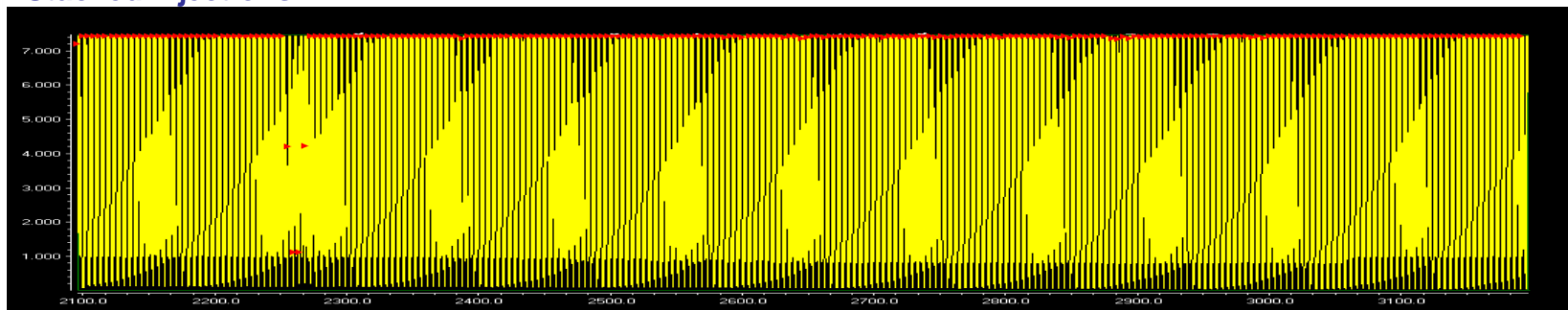
Routine preparative scale optimization techniques are employed. This compound is not scaling up linearly as predicted. Based on the optimal productivity rate of 1.5g/hr obtained, this would not be feasible candidate for a multi-kilo separation project

Case Study #2 : Scale-up *Using Two Columns in Series*



(S,S) Whelk-O1® 250mm x 50mm -Regis Cell® 250mm x 30mm (24% EtOH Co-solvent), Flow: 200 g/min
Production rate: ~ 6 g/hr

Stacked injections

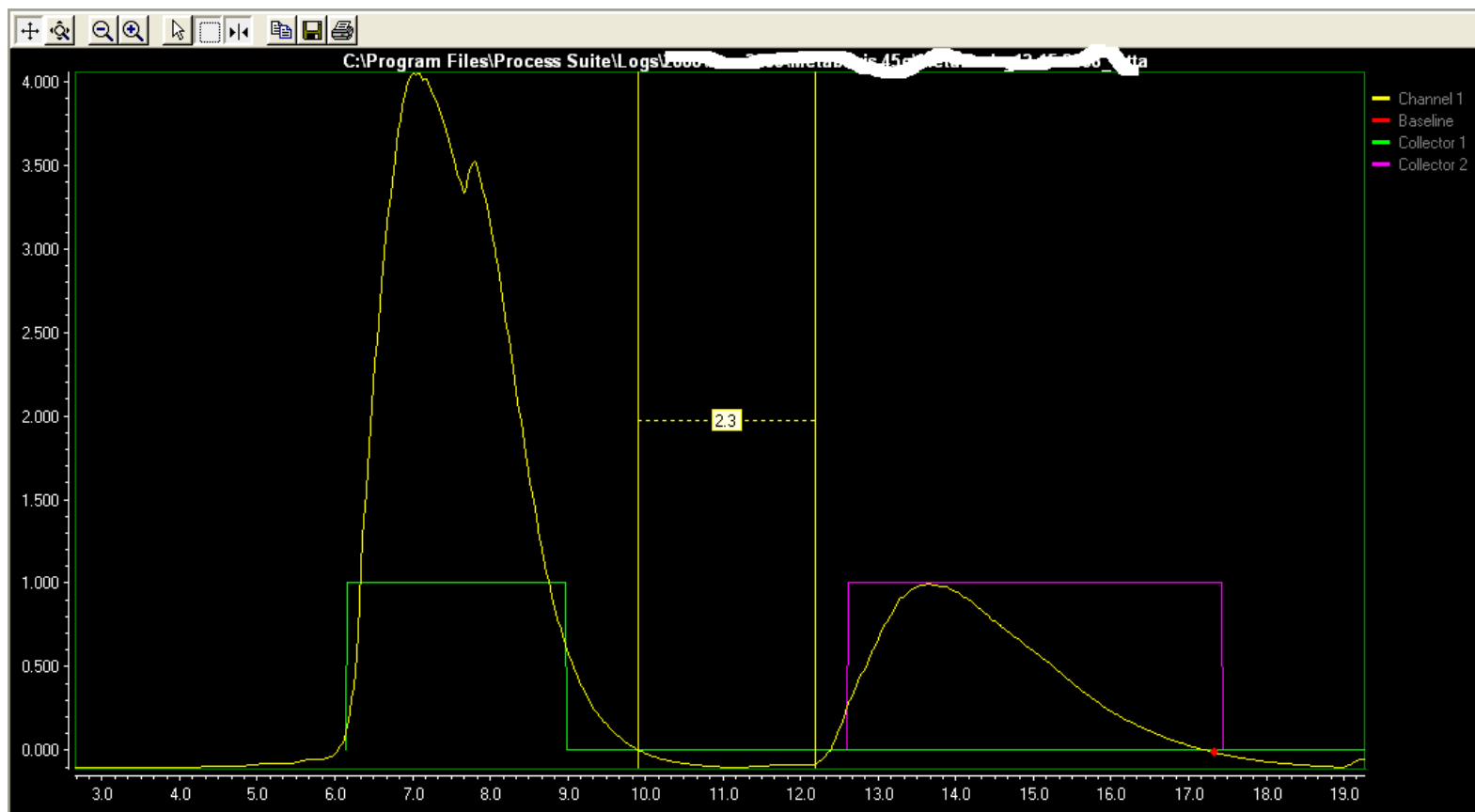


Method optimization using a multi-column pSFC system resulted in an increased production rate from 1.5g/hr to 6g/hr, making this project feasible for a multi-kilo separation

Case Study #3

Single Injection Preparative

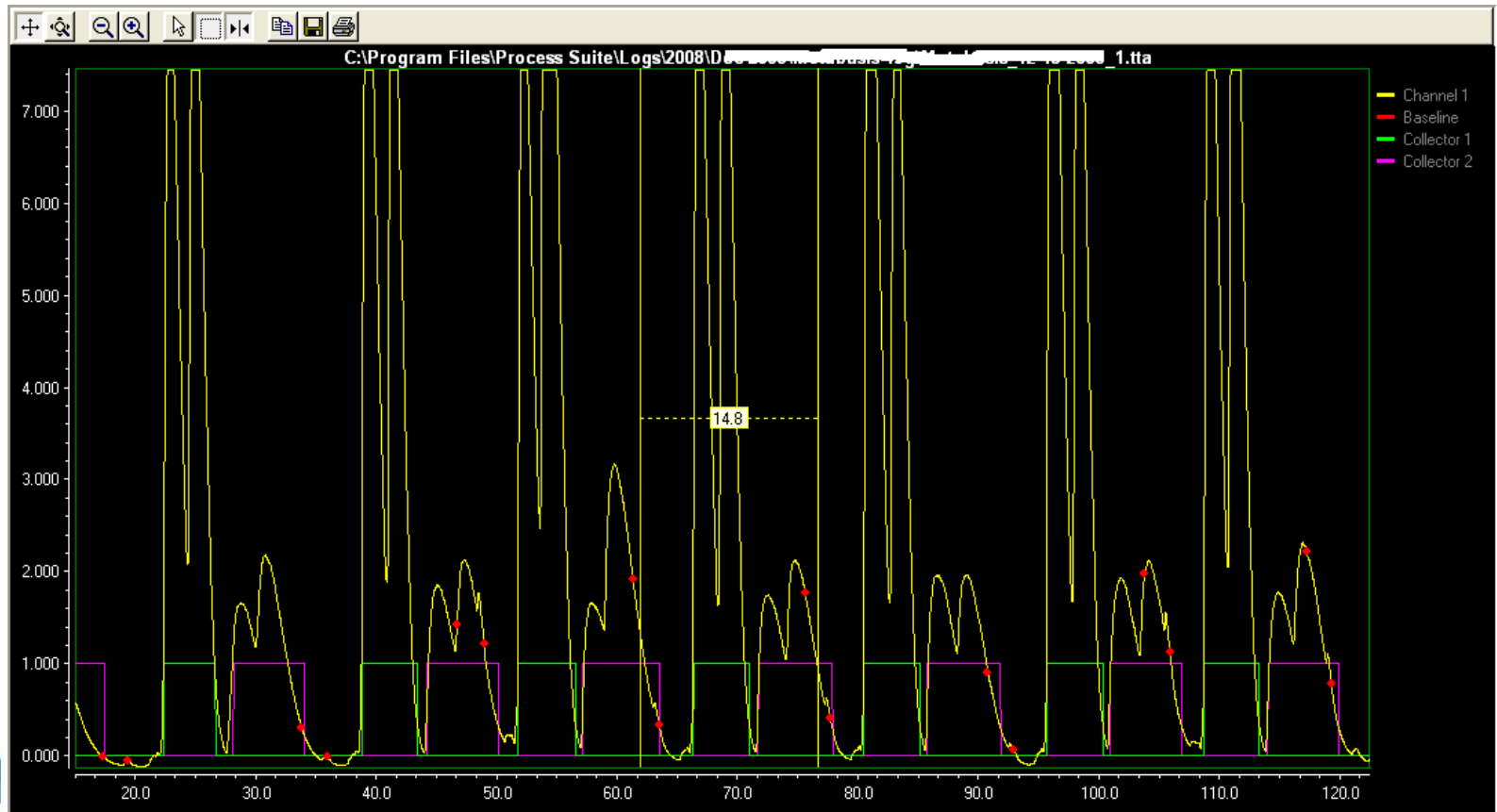
- Column: (S,S) Whelk-01[®] 250mm x 50mm, 5 micron
- Co-solvent: 65% MeOH
- Flow rate: 250g/min



Case Study #3

Stacking Injections of different volumes

- Column: (S,S) Whelk-01® 250mmX50mm, 5 micron
- Co-solvent: 65% MeOH, Flow rate: 250g/min
- Injection protocol: 25ml injection with a 2 minute delay
20ml injection with a 13 minute delay
- **Production rate increased from 2g/hr to 3.8 g/hr**



Conclusion

- SFC is a powerful technique for chiral and achiral purification on both analytical and preparative scale.
- Most of the time an analytical method is directly scaleable to the prep scale.
- Solubility issue can be solved by using chlorinated solvent as diluents, decreasing the mobile phase viscosity or by injecting small amounts of sample in a shorter cycle time to increase the productivity.
- At Regis Technologies our aim is to develop preparative methods that are robust, reproducible and can run 24/7.