



Application of SFC in Process Analytical Chemistry for Drug Development

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Outline

- Introduction
- SFC Capabilities in Process Analytical Chemistry
- Examples of Typical Applications
- Conclusion

Introduction

Process Analytical Chemistry

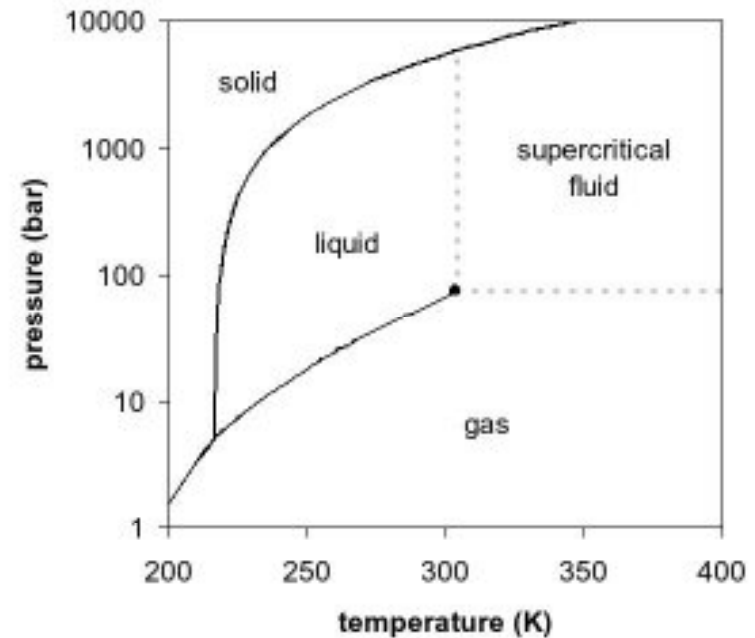
- A function of Process Research and Development
- Responsible for all small molecule API research and development supporting late Discovery through commercial launch



SFC Advantages

SFC is gaining popularity in pharmaceutical industry

- Low viscosity and high diffusivity
- Higher flow rates and lower pressure drops than in HPLC
- Faster analysis
- Less solvent consumption than LC – greener and cheaper
- Easier and quicker solvent removal for purification work



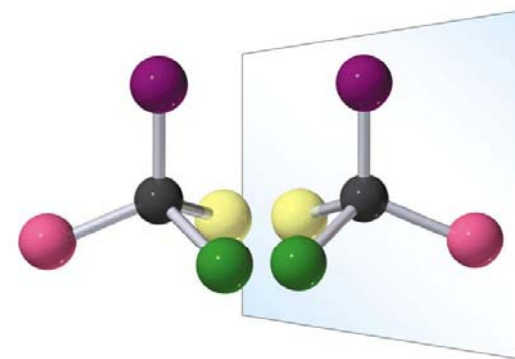
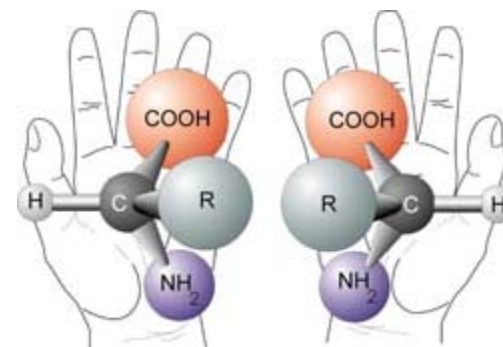
Our SFC Capabilities

Two SFC Systems:

- Berger analytical and MiniGram (two in one) (2004)
 - Column and modifier switcher
 - UV and Diode Array Detector
 - SFC ProNTto software
- Thar analytical system with Mass compatibility (2008)
 - Column and modifier switcher
 - Diode Array detector
 - Chemstation software
 - Mass Spec connected to SFC as needed (shared mass detector with LC/MS)

Our Typical Applications

- Chiral Analysis
 - Chiral purity analysis
 - Method development
 - Chiral column screening
- A-chiral separation
- Impurity isolation and purification
- SFC/MS for enantiomer/impurity confirmation/identification

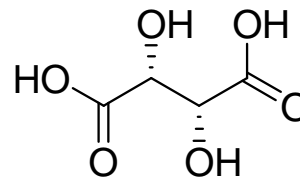


Chiral Analysis

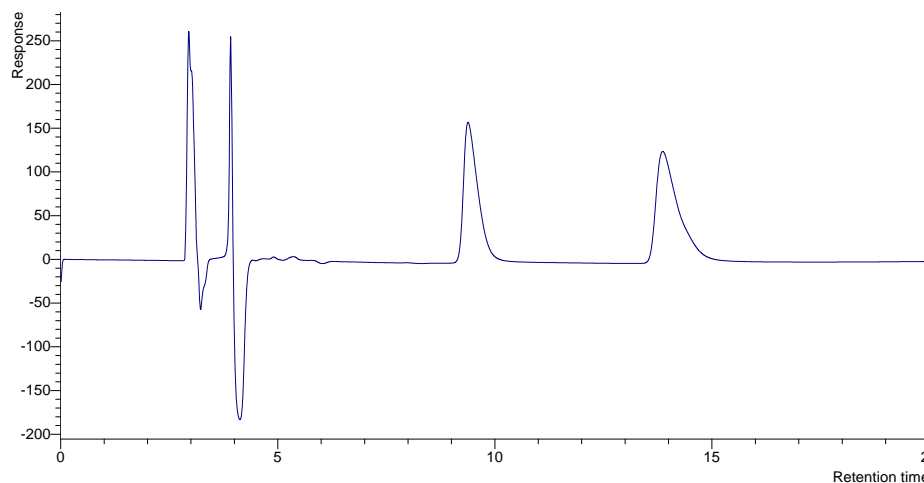
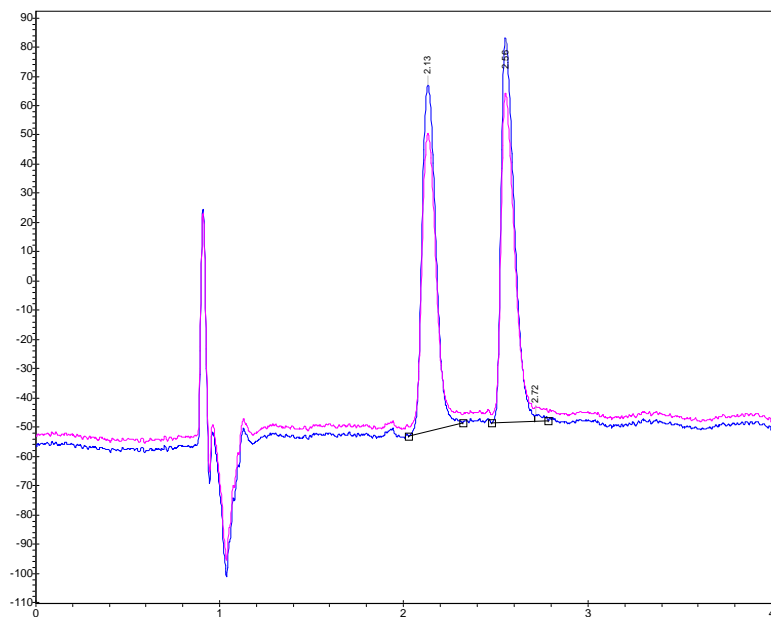
- Method developed and sample analyzed using analytical SFC
 - SFC system qualification
 - Availability of 2nd system
 - Method transfer
 - May not be as sensitive
- Method developed using SFC and then transfer the conditions to HPLC
 - Additional time needed for method conversion
 - Lose the SFC advantage in shorter analysis time
- Column and modifier screening using SFC for faster method development

Analysis of Tartaric Acid by SFC and HPLC

SFC: AD-H Column, 20% EtOH,
0.1% TFA, 3 ml/min, 210 and 214 nm
UV detection

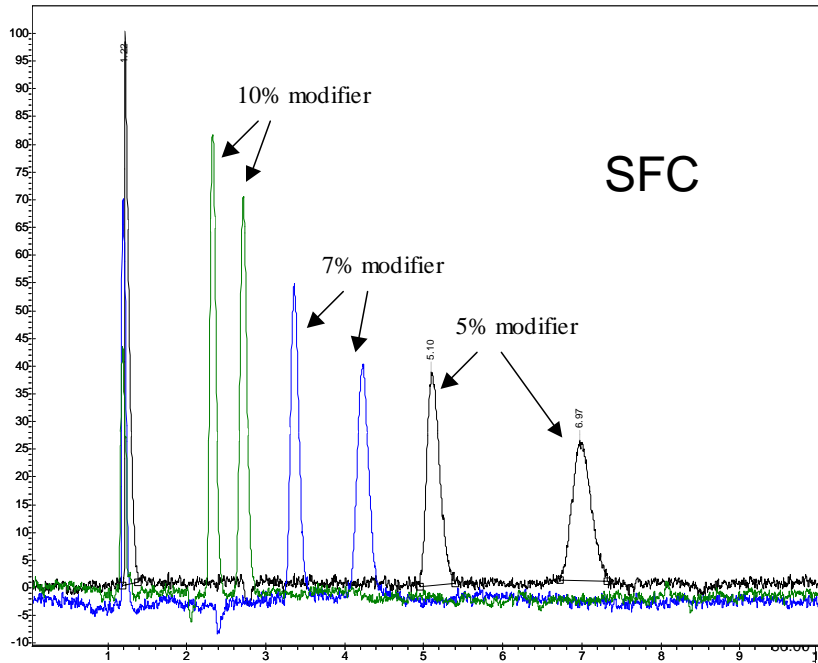


HPLC: AD-H column, Hexane: EtOH: TFA
80:20:0.1%, 1 ml/min, 210 nm UV detection

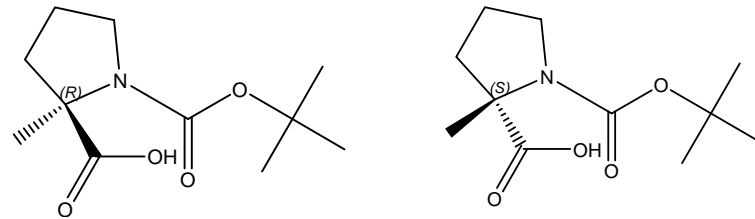


Separation achieved in 3 minutes by SFC!
Direct condition correlation between SFC and HPLC

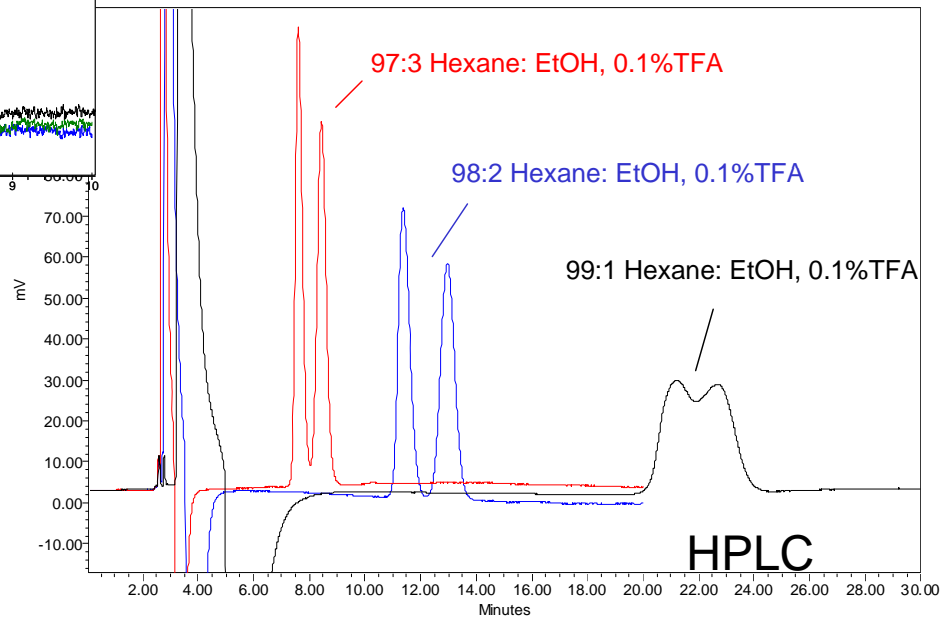
Analysis of Boc-methyl-proline by SFC and HPLC



SFC Conditions:
Modifier: ethanol with 0.1%TFA
Outlet pressure; 100 bar
Column temperature: 35 °C
Flow rate; 2.5 ml/min

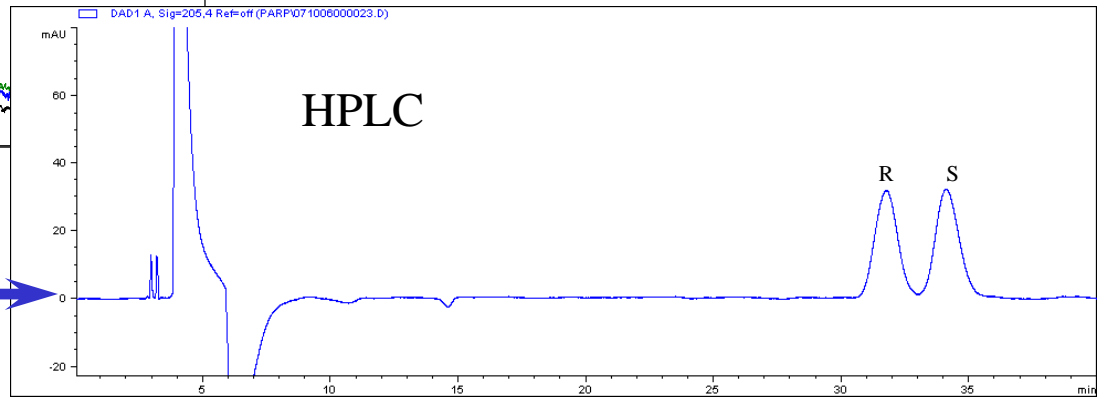
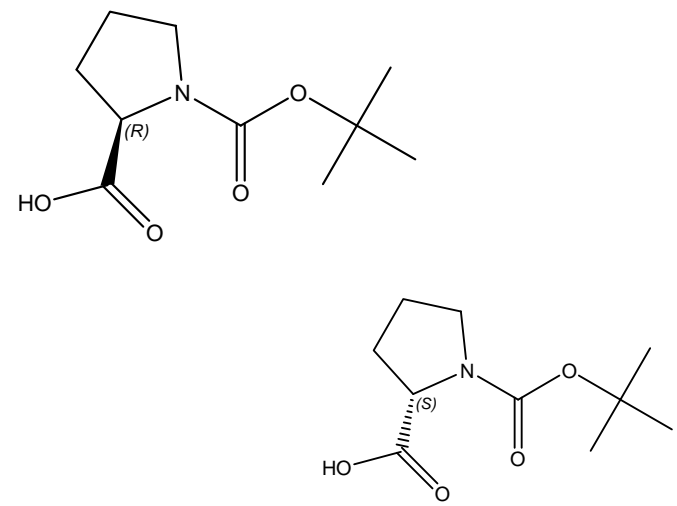
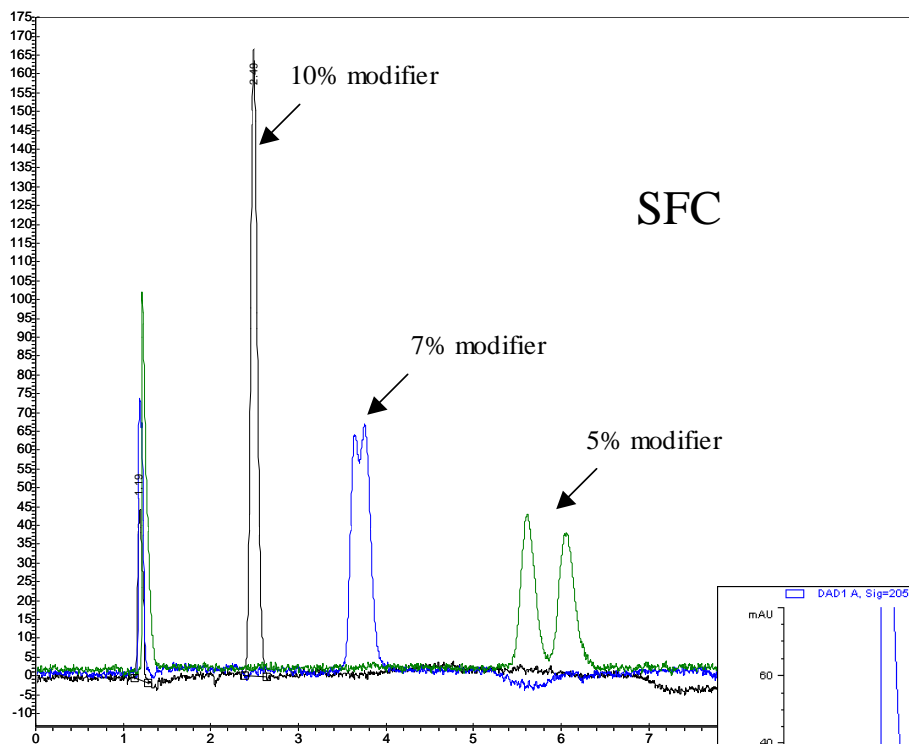


**Column: Chiralpak AD-H,
25cm x 4.6 mm, 5 μ m**



Analysis of Boc-proline by SFC and HPLC

Example chromatograms with 5%, 7% and 10% modifier for Boc-proline using SFC



Optimal HPLC conditions:
AD-H column, 99:1 Hexane: EtOH,
0.1% TFA, 1.0 ml/min, 40 °C

Screening for Even Faster Method Development

- Screening using different columns and modifiers has been widely used
- Effectiveness of the screening is of importance
- What will be the best approach for pharmaceutical compounds

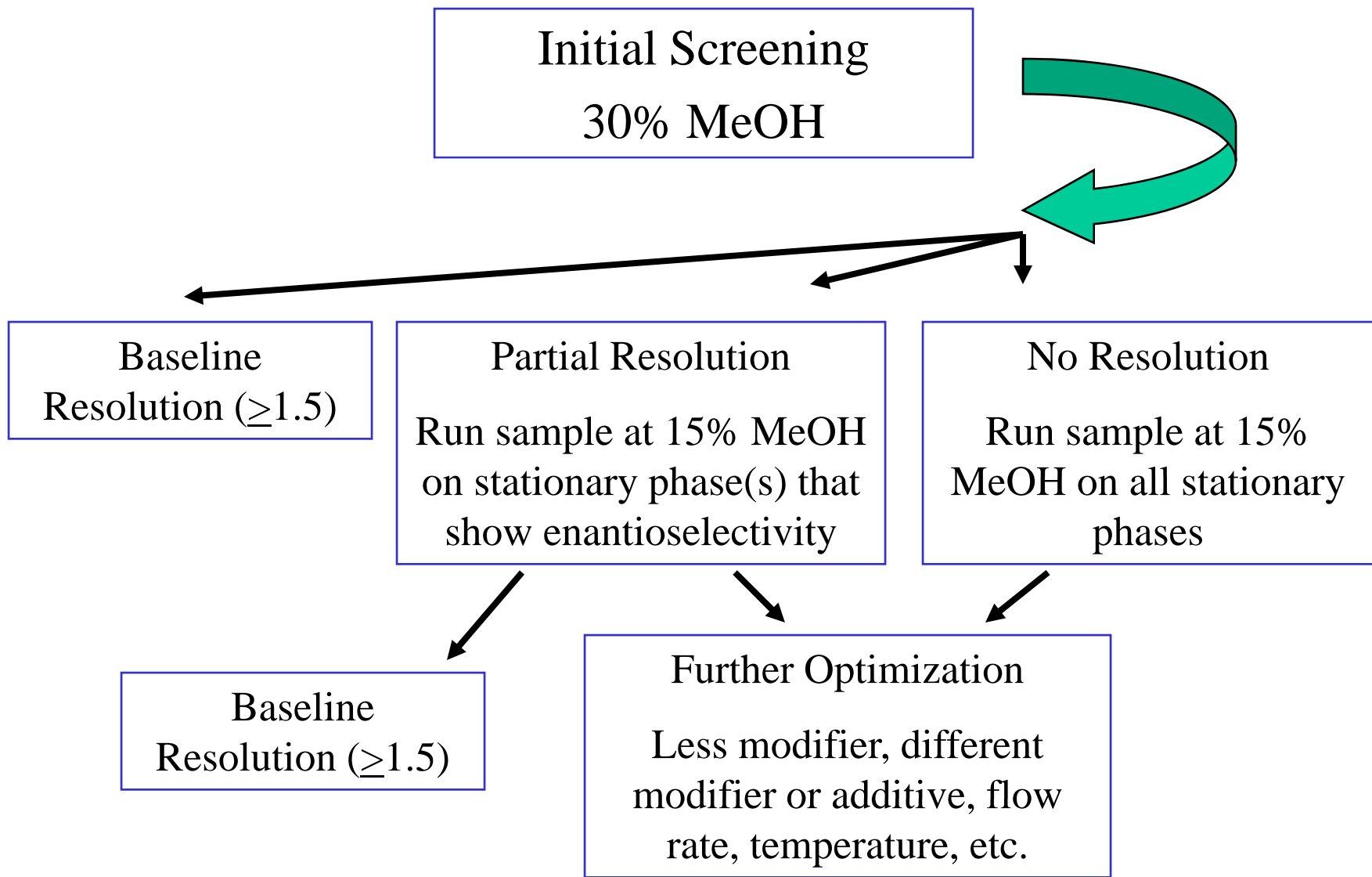
Screening Evaluation

- Comparison of isocratic versus gradient were conducted
- 12 Abbott compounds and 14 commercial compounds were used for the study
- Six columns were chosen for the study
 - Chiracel OD-H and OJ-H, Chiralpak AD-H
 - Supelco Chirobiotic TAG and P-CAP
 - DEABV polymeric column

Screening – Experimental Conditions

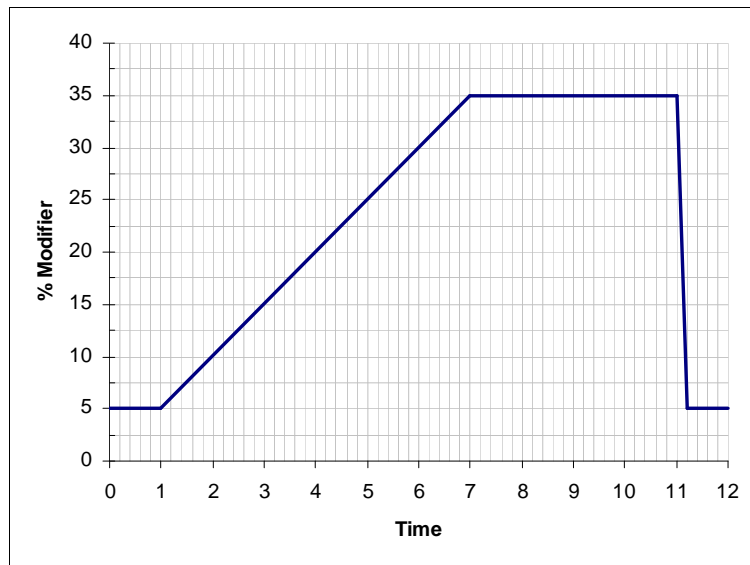
- SFC Conditions
 - Temperature: 35 °C
 - Outlet Pressure: 100 bar
 - Flow: 3 mL/min
 - 205 and 220 nm for isocratic, 210 and 250 nm for gradient runs

Isocratic Screening



Gradient Screening

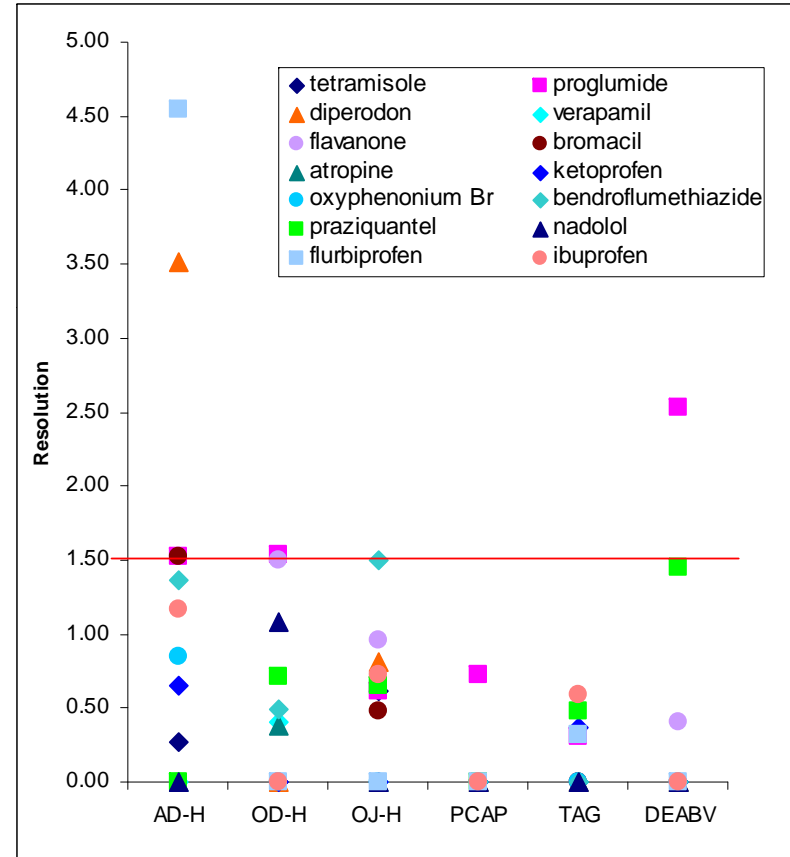
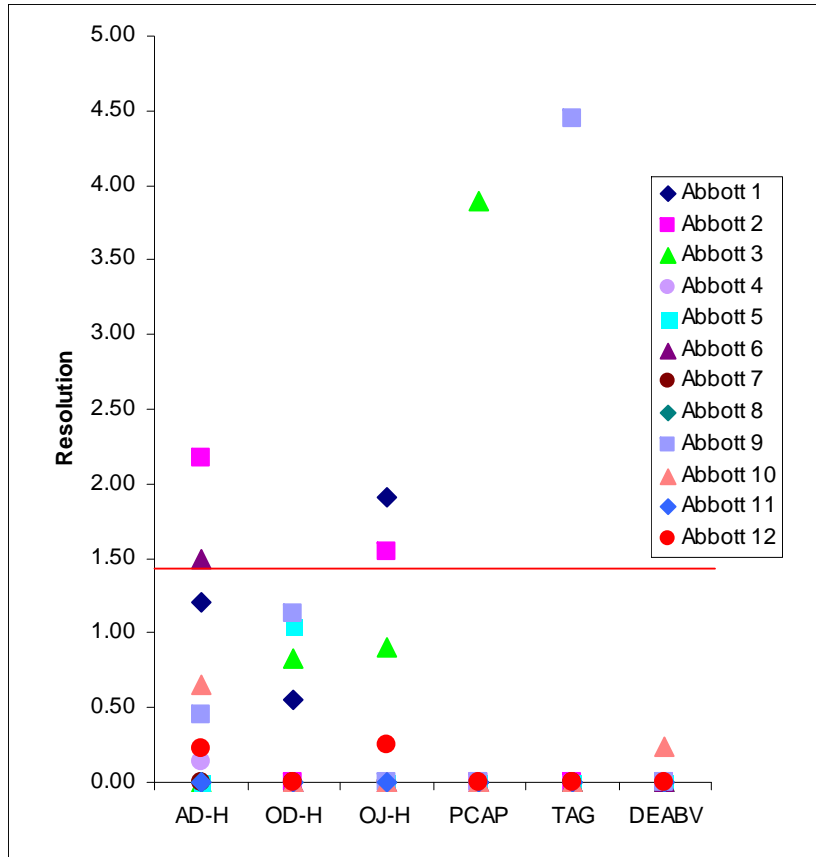
- Gradient Program: Hold at 5% modifier for 1.0 minute, increase at a rate of 5% per minute to 35%, hold at 35% for 4 minutes, then reduce to 5% at a rate of 99% per minute.



- Screening procedures:
 - Baseline resolution: Use gradient method or extract isocratic modifier % from chart for CSPs with enantioselectivity
 - Partial resolution: Choose the modifier % where the first peak elutes for CSP showing enantioselectivity
 - No separation: try different modifiers, additives, or isocratic screening at an appropriate modifier percentage

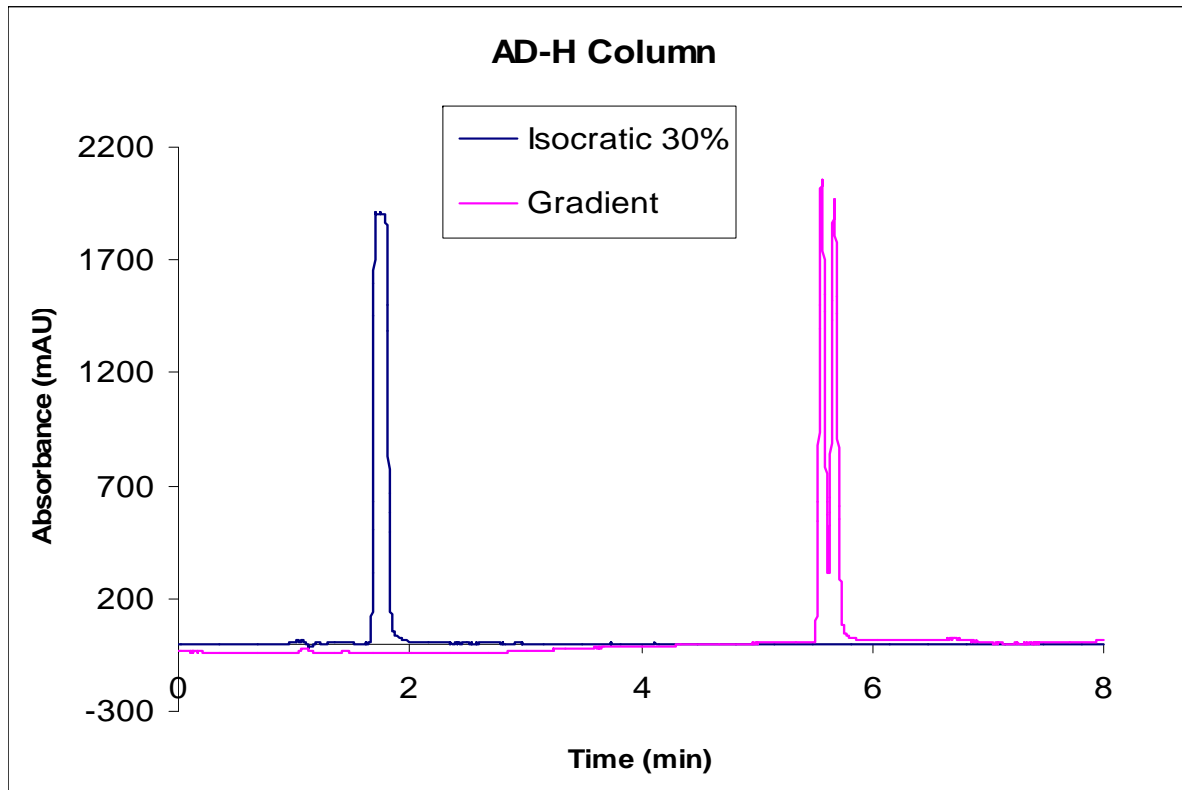
Column Screening – Example Results

Gradient flow with methanol as modifier



Column Screening – Example Chromatogram

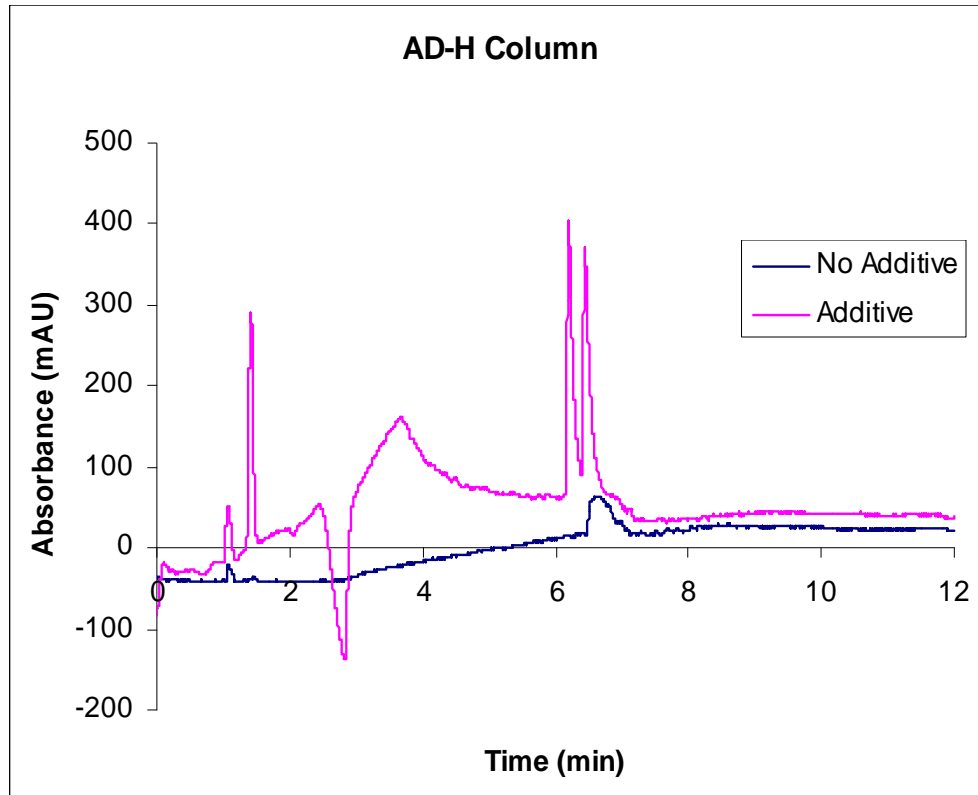
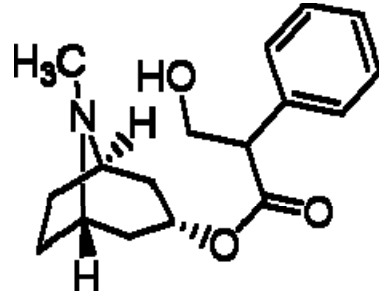
Ketoprofen



Modifier: Methanol

Column Screening – Example Chromatogram

Atropine



Modifier: Gradient with or without TEA as additives

Conclusion for screening

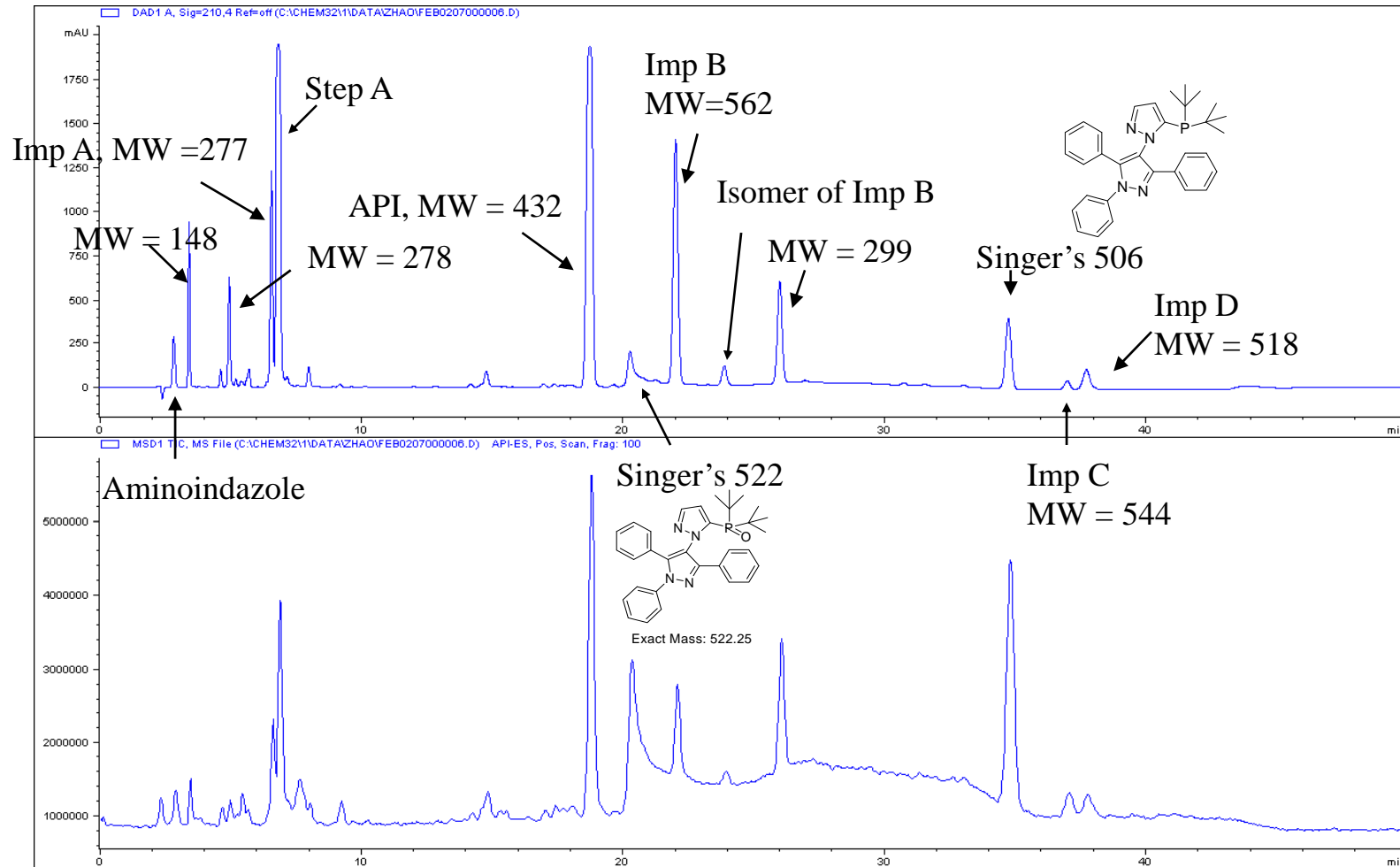
- Gradient Screening method was the most efficient
- Taking 72 minutes to complete screening.
- 24 of 26 compounds were separated with 10 baseline resolved.
- The isocratic screening was also useful, but takes 96 minutes to complete screening.
- 20 of 21 compounds were separated and 11 were baseline resolved.

A-Chiral Separation and Impurity Isolation

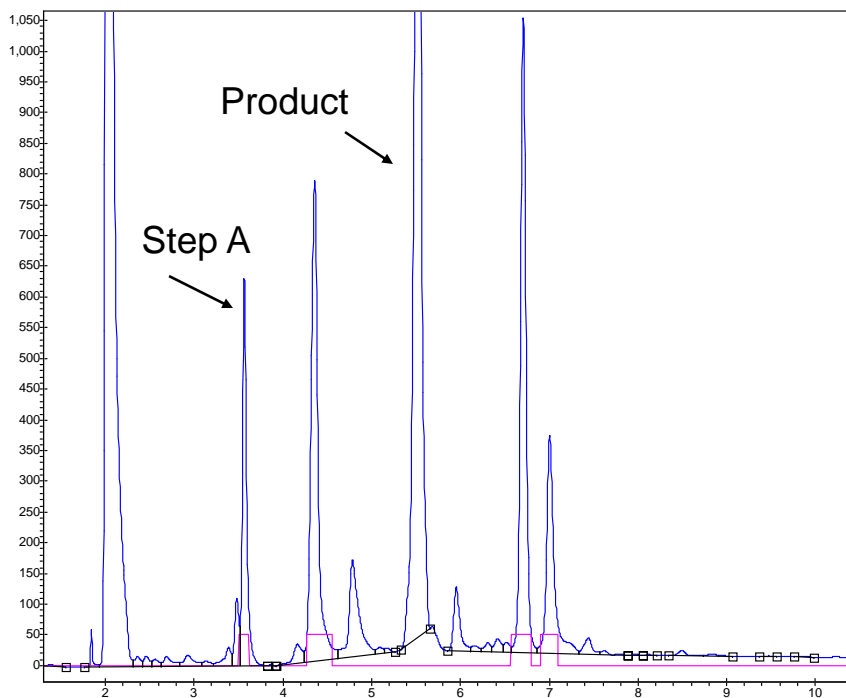
Background

- LC/MS analysis was conducted on mother liquor of an Abbott API
- Impurity structures were proposed
- To confirm the structure by NMR, it's desired to isolate the impurities

Impurity Profile of Mother Liquor



A-Chiral Separation and Impurity Isolation



Four impurities were isolated overnight and samples were dried in a couple of hours!

- SFC was used to for the impurity isolation
- 4 impurity peaks were resolved and isolated using MiniGram
- Isolated samples were submitted for NMR analysis
- Peak of interest was correlated using peak size and UV spectrum for this specific case between HPLC and SFC, and analyze the fraction by HPLC. This step can be simplified if SFC/MS is available
- Proposed impurity structures were confirmed by NMR

Conclusion

- SFC can be used very effectively for chiral column screening
 - Identify the best stationary phase
 - Continue with SFC or transfer to HPLC
- SFC has advantages for chiral separations in comparison to HPLC
 - Fast separation
 - Less solvent consumption
 - Improved resolution
- Convenient for isolation and purification
 - Quicker run time
 - Faster solvent removal

Acknowledgements

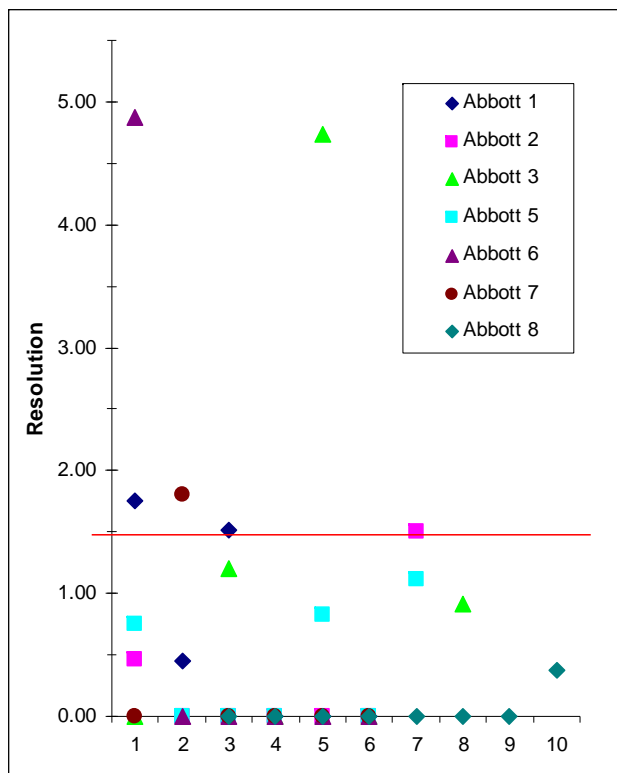
- Jie Zheng for the tartaric acid by HPLC data
- Molly Warnke (summer intern) for SFC screening work
- Department of R45T
- Dr. Daniel Armstrong for insightful screening discussion and loaning the DEABV polymeric column

Questions

Back up slides

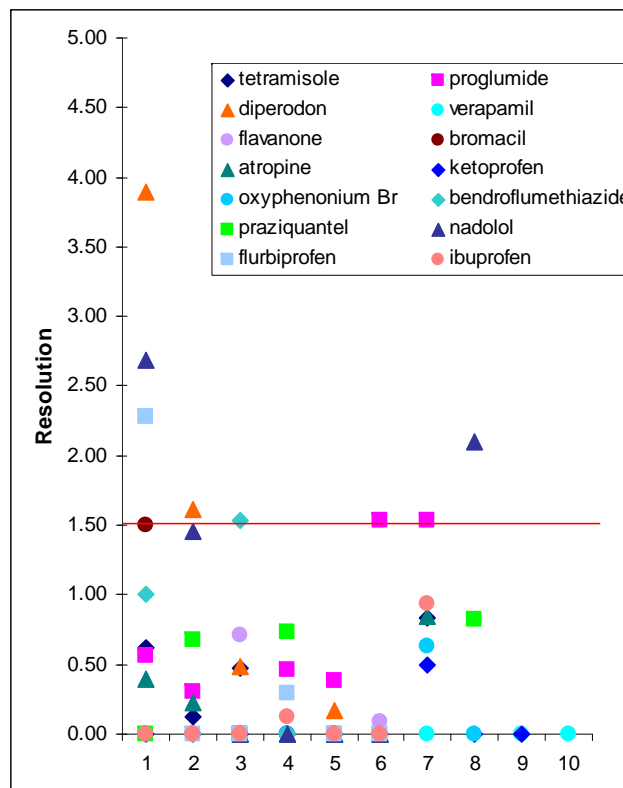
Isocratic Screening Results

Abbott Compounds



Under these conditions, all Abbott compounds were separated with 5 of 7 baseline resolved.

Commercial Compounds



- 1-AD-H 30% MeOH
- 2-OD-H 30% MeOH
- 3-OJ-H 30% MeOH
- 4-TAG 30% MeOH
- 5-PCAP 30% MeOH
- 6-DEABV 30% MeOH
- 7-AD-H 15% MeOH
- 8-OD-H 15% MeOH
- 9-OJ-H 15% MeOH
- 10-TAG 15% MeOH

All MeOH modifier contains 0.1% TEA.

Points on or above the red line represent baseline resolution.

6 of 14 commercial compounds were baseline resolved and 13 of 14 compounds were partially resolved under these conditions.

Column Screening – Results

Gradient flow with 0.1% TEA in methanol as modifier

