

# The Incorporation of SFC into the Purification Laboratory for Chiral and Achiral Samples

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# Purification Group

- Support Lead Optimization and Lead Generation, ~55 chemists
- Primary Research area is Central Nervous System (CNS)
  - Basic compounds
  - Chiral compounds
- Purify compounds for final submission, intermediates, chiral compounds before enantiomeric separation. Additionally, purify enantiomers, diastereomers, isomers and determine %ee
- Group comprised of three full time and one half time employee
- Approximately 900 compounds submitted for purification per year, ranging in amounts mgs to >200 grams

# Drivers for the Incorporation of SFC into Lab

- Separation of enantiomers, diastereomers, and isomers
- Orthogonal method of separation for achiral compounds, increasing variety of achiral stationary phases available
- Need for faster turn around of samples, less time for dry down, goal of one day for routine samples
- In-house separation of larger batches of chiral compounds for toxicological studies

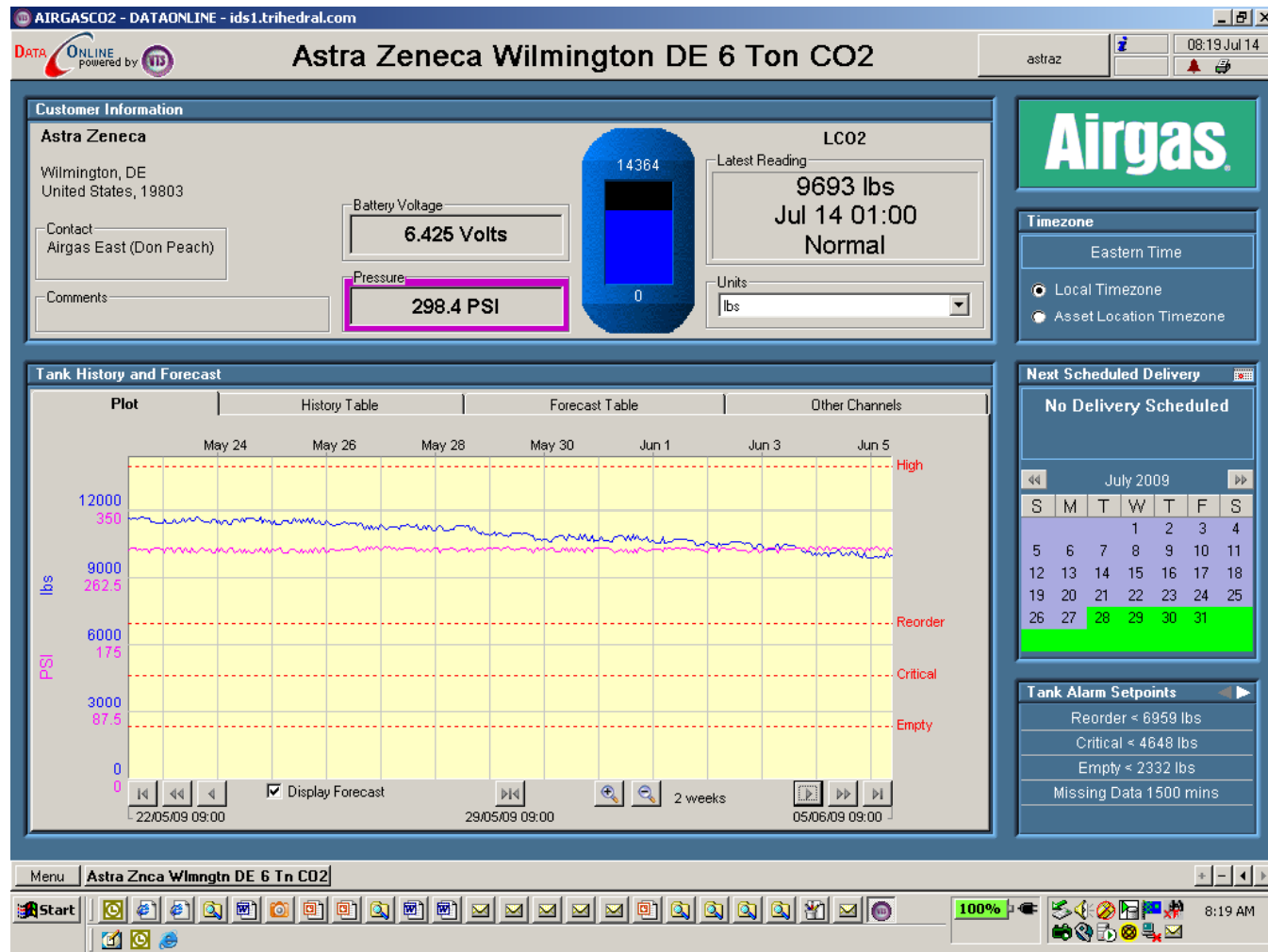
# Instrumentation

- Analytical SFC/UV/MS, Analytical SFC/UV
- MultiGram II, preparative instrument with a flow rate up to 70ml/min, UV detection
- MultiGram III, preparative instrument with a flow rate up to 400ml/min, UV detection
- SFC/MS, preparative instrument with a flow rate up to 100ml/min

# Infrastructure to support use of CO<sub>2</sub>

- Bulk tank of liquid CO<sub>2</sub>, utilizing the gaseous CO<sub>2</sub>
  - Reduces possibility of introducing contaminants into compounds
- Vatron pumps to compress the gaseous CO<sub>2</sub> to liquid CO<sub>2</sub>
  - Compressed air used to drive the Vatron pumps
- Orbitally welded stainless steel tubing delivers liquid CO<sub>2</sub> to lab
- Two stage regulators control pressure to instruments
  - Analytical vs preparative instruments
- Safety alarms to monitor the levels of CO<sub>2</sub> in the air

# Bulk Tank Monitoring



# Chiral Separations

# Columns for Screening-Chiral

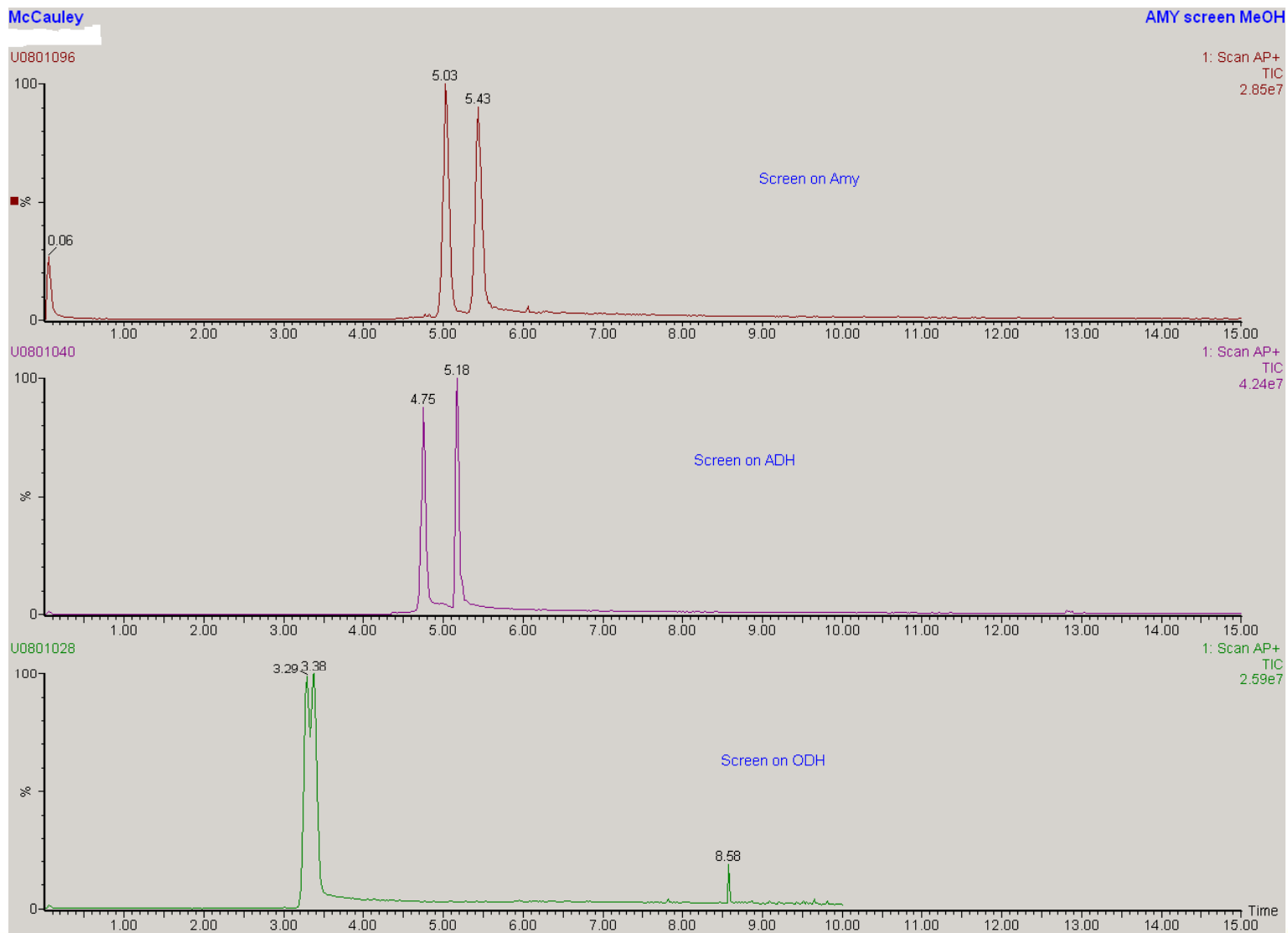
- ADH
- ODH
- OJH
- ASH
- IC
- IA
- Lux cellulose-2
- Lux cellulose-1
- Lux amylose-1
- Kromasil AmyCoat
- Kromasil CelluCoat
- Welko-1
- ULMO
- Kromasil DMB

# Scout Methodology for Chiral Samples

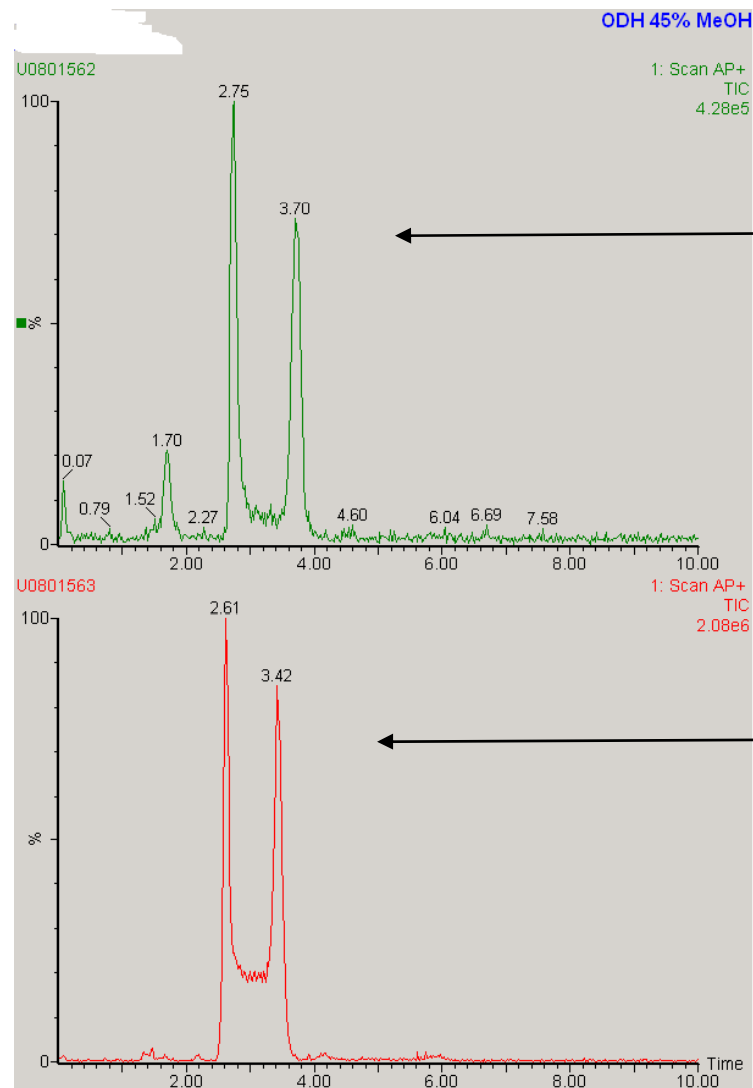
- Gradient 5 to 65% modifier over 10 minutes
- Mixing of several compounds together and use the mass spec to separate the compounds
- For complex samples, use gradient 10 to 70% modifier over 15 minutes
- Once column identified, optimization of modifier may be required



# Chiral Screen of Sample



# Atropisomers

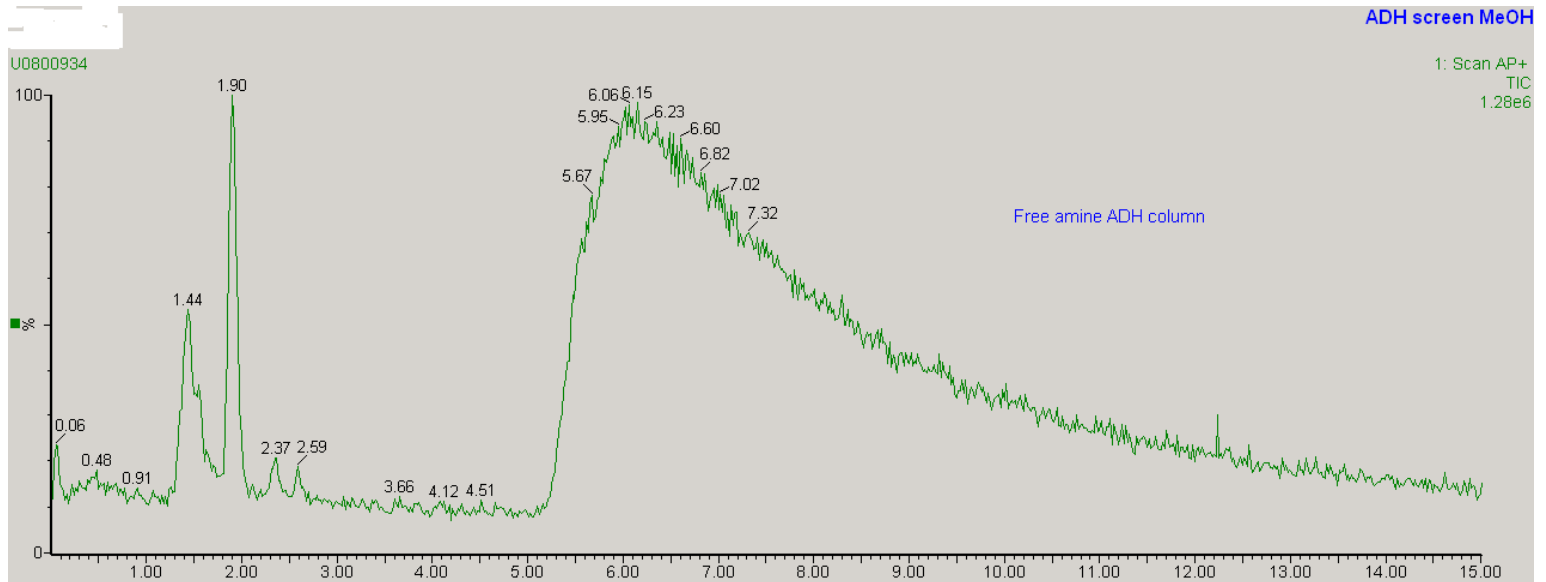


22 deg C

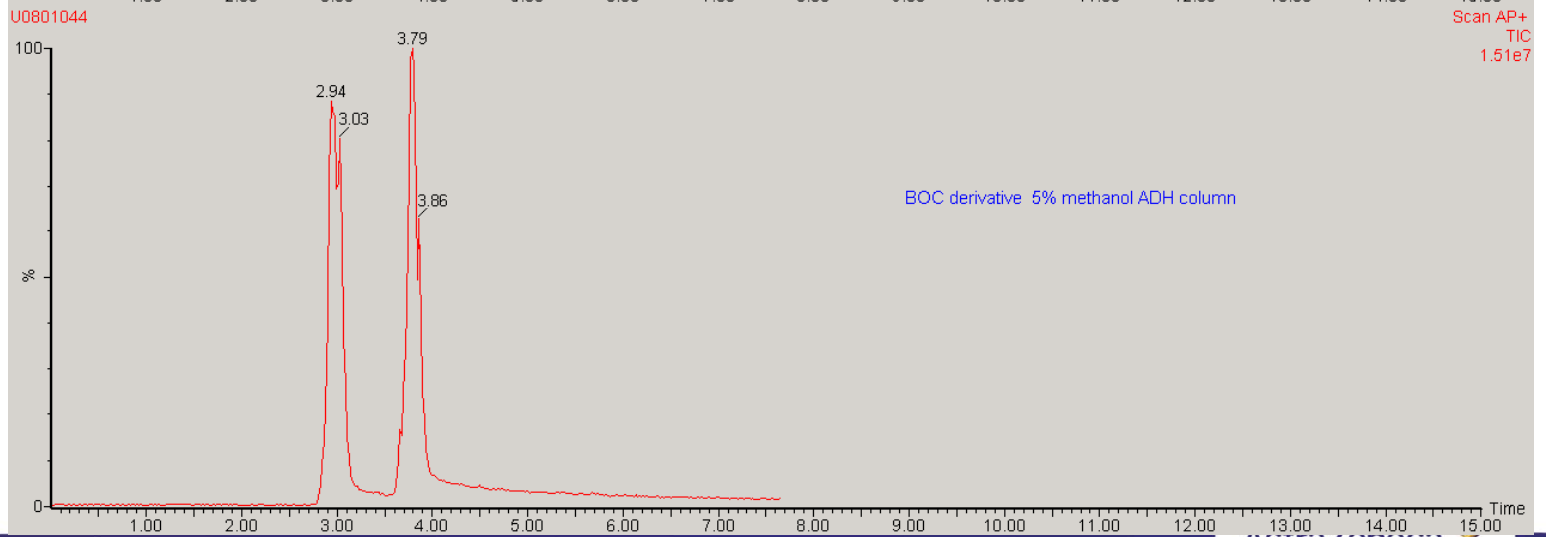
37 deg C

# Amine vs BOC Derivative

Amine



Boc



# Preparative Chiral Separations

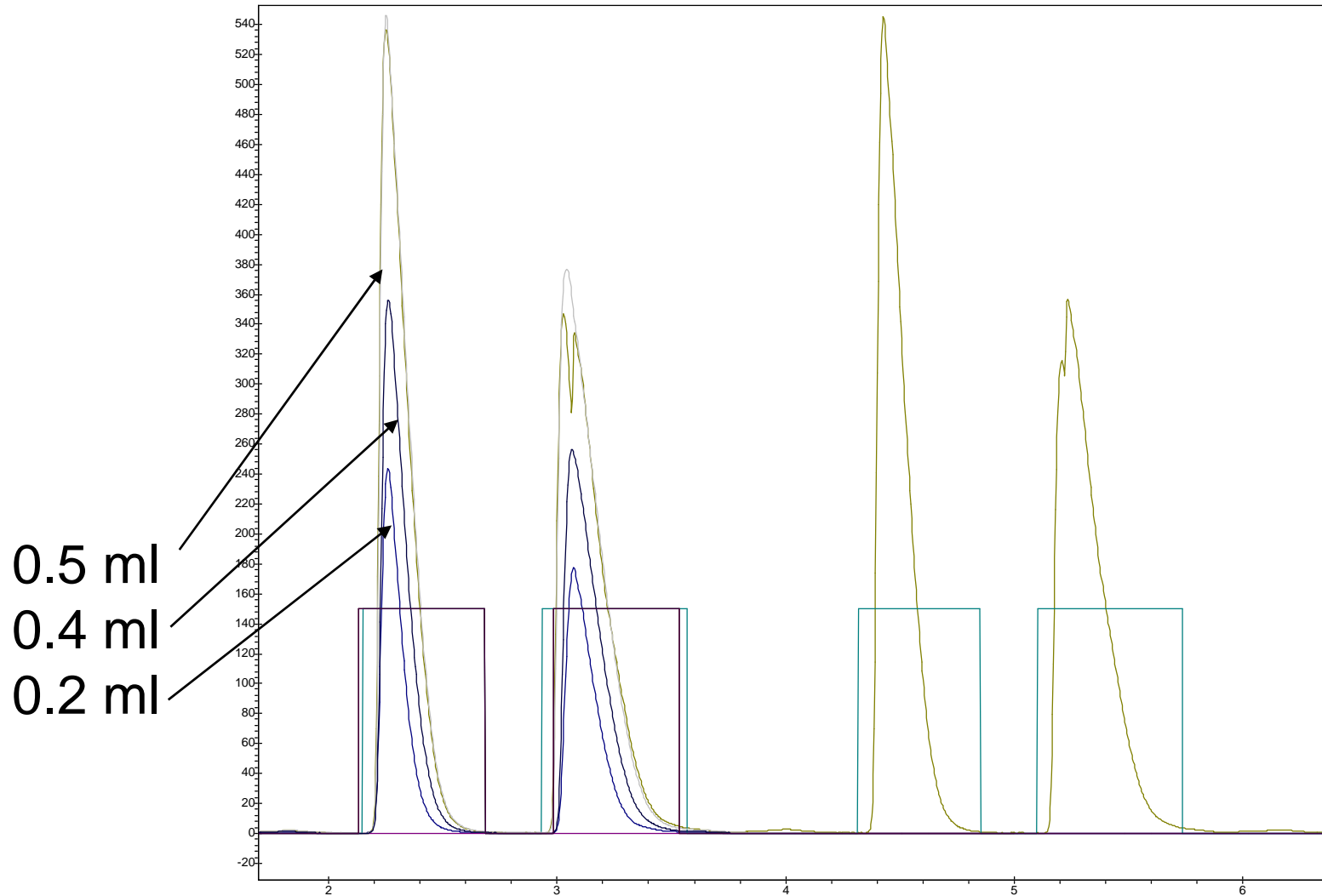
# Chiral

- Solvents used: Methanol, Isopropanol, Ethanol, mixtures such as 1:3 Isopropanol:ethanol
- Additives used: 0.5% N,N, Dimethylethylamine or Isopropylamine
- Column Dimensions: 21.2, 30 or 50 x 250 mm, 5 micron particles
- Sample size: 10 mgs to 230 grams

# Chiral Column Statistics-18 months

<b>Column</b>	<b>Usage numbers</b>
ADH	104
ODH	29
IC	35
IA	4
OJH	23
ASH	12
ULMO	1
LUX-Cellulose-2	33 (9 month record)

# Loading in Preparation to Stacking



# Use of Waters Scaling Calculator

## Column 1

I.D. (mm)	4.6
Length (mm)	250
Mass Load (mg)	6
Inj volume (uL)	10
Flow rate (mL/min)	1.95
Column vol. (ml)	3.488

## Column 2

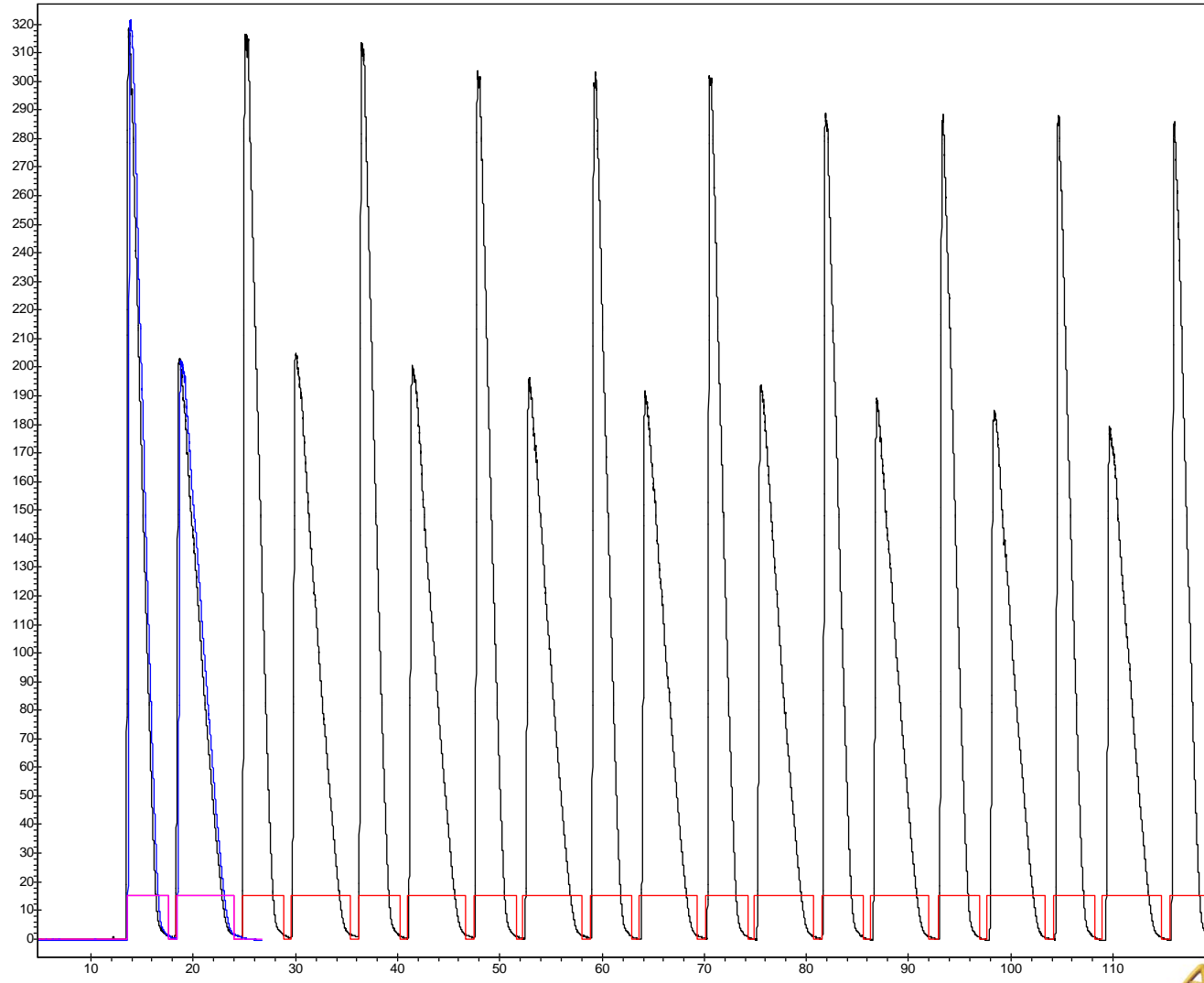
I.D. (mm)	30
Length (mm)	250
Mass Load (mg)	255.198
Inj volume (uL)	425.331
Flow rate (mL/min)	82.9
Column vol. (ml)	148.365

## Experimentally SFC:

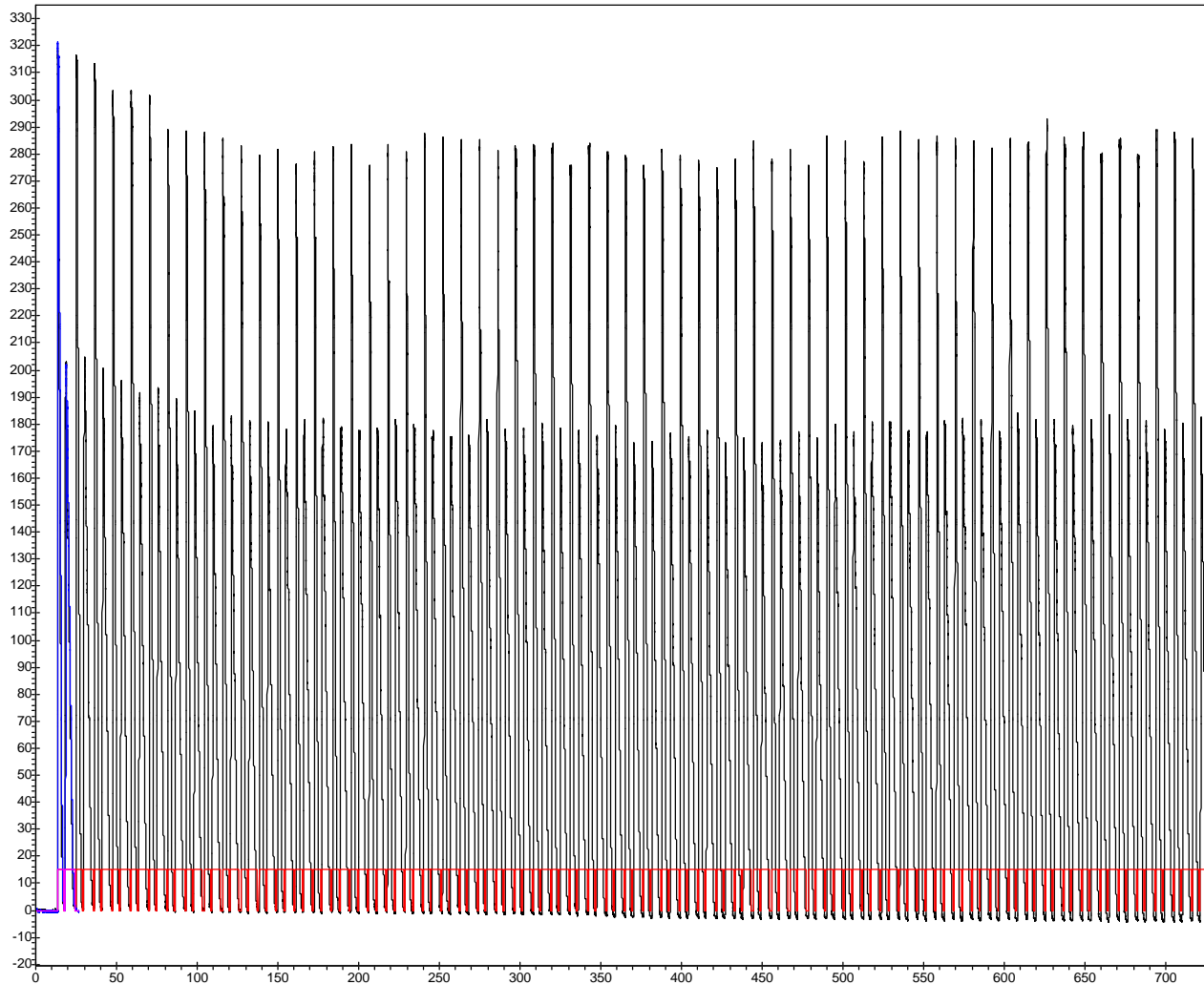
Mass Load (mg)	285
Inj volume (uL)	350
Flow rate (mL/min)	130

# Separation of Chiral Intermediate

285 mgs/inj

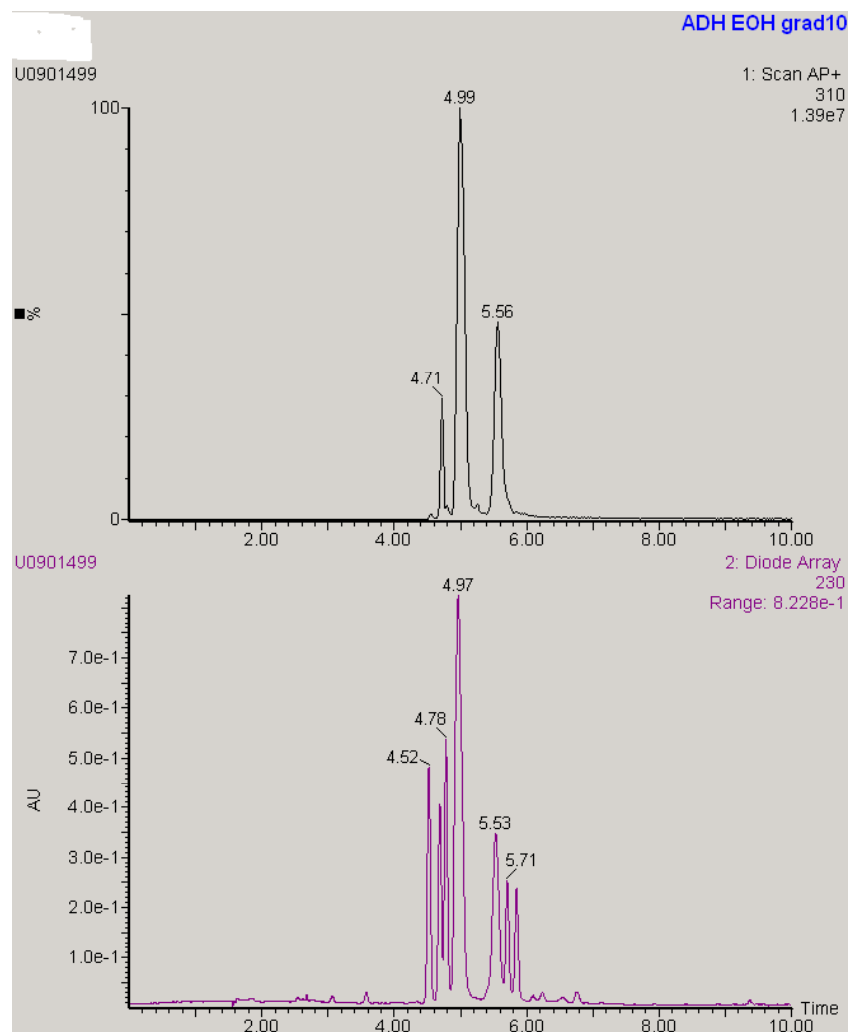


# Stacked Injections on Lux-2

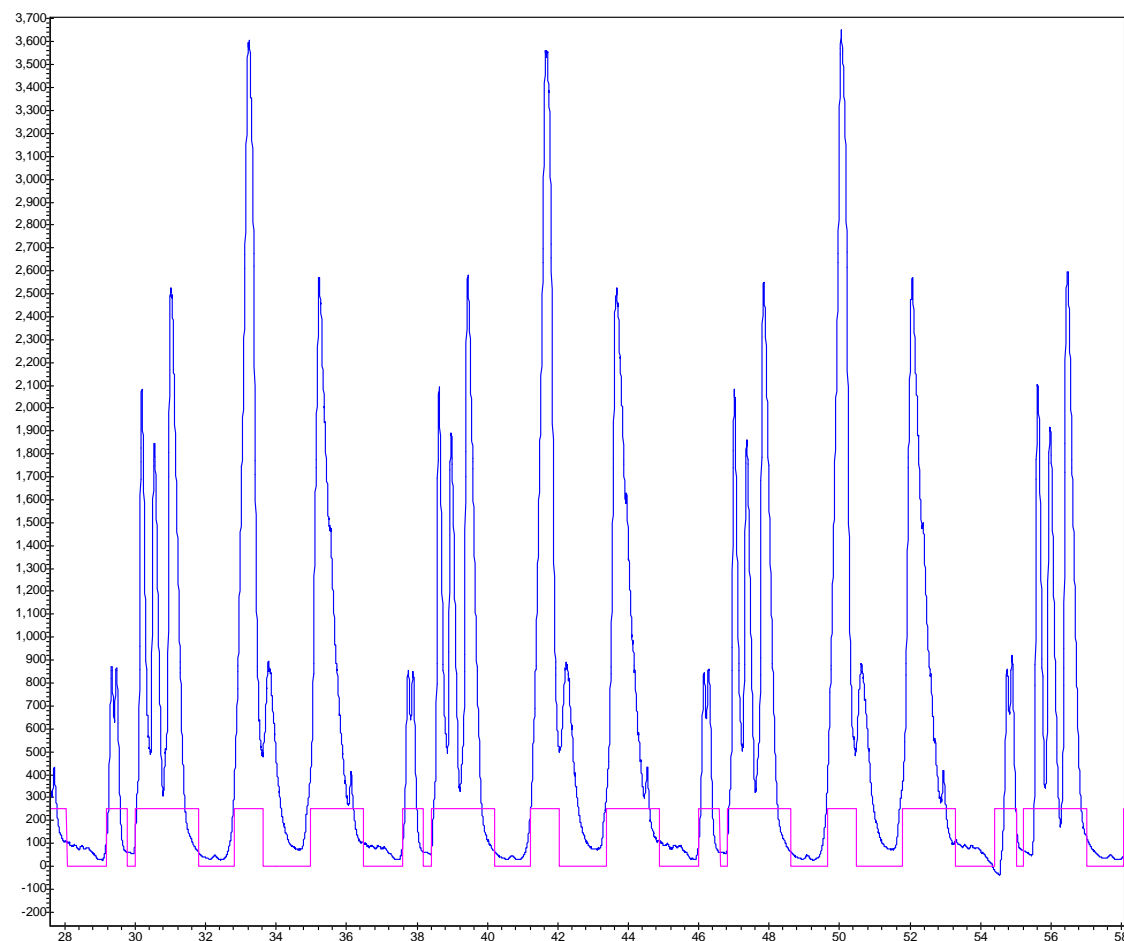


Run time=  
730 minutes

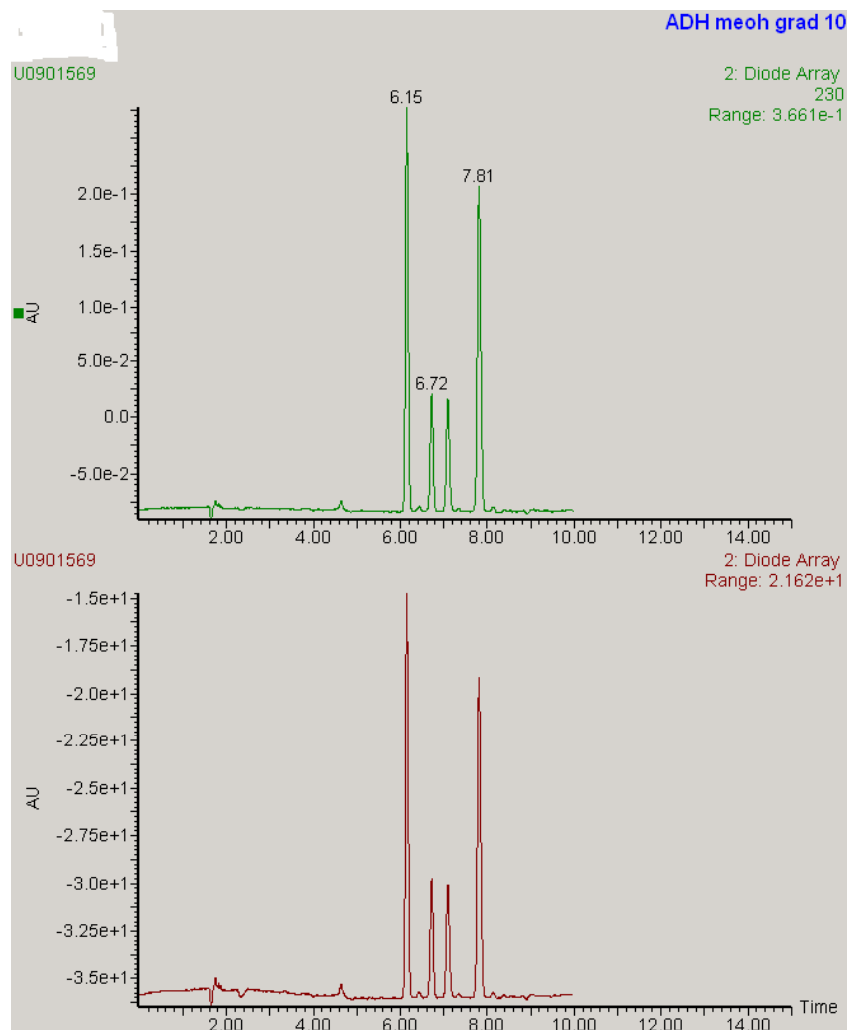
# Analytical Screen of Diastereomers



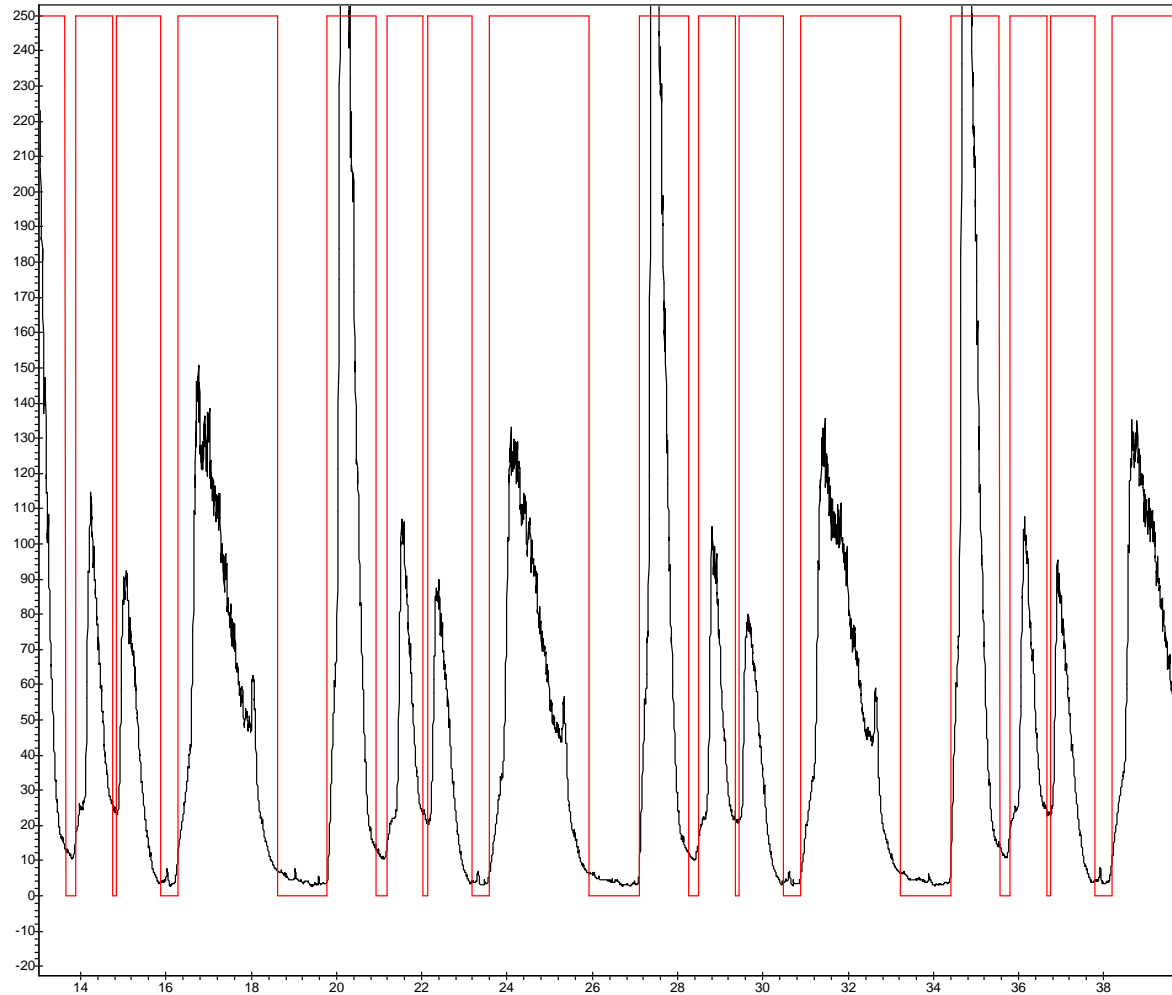
# Preparative separation of isomers from a complex mixture



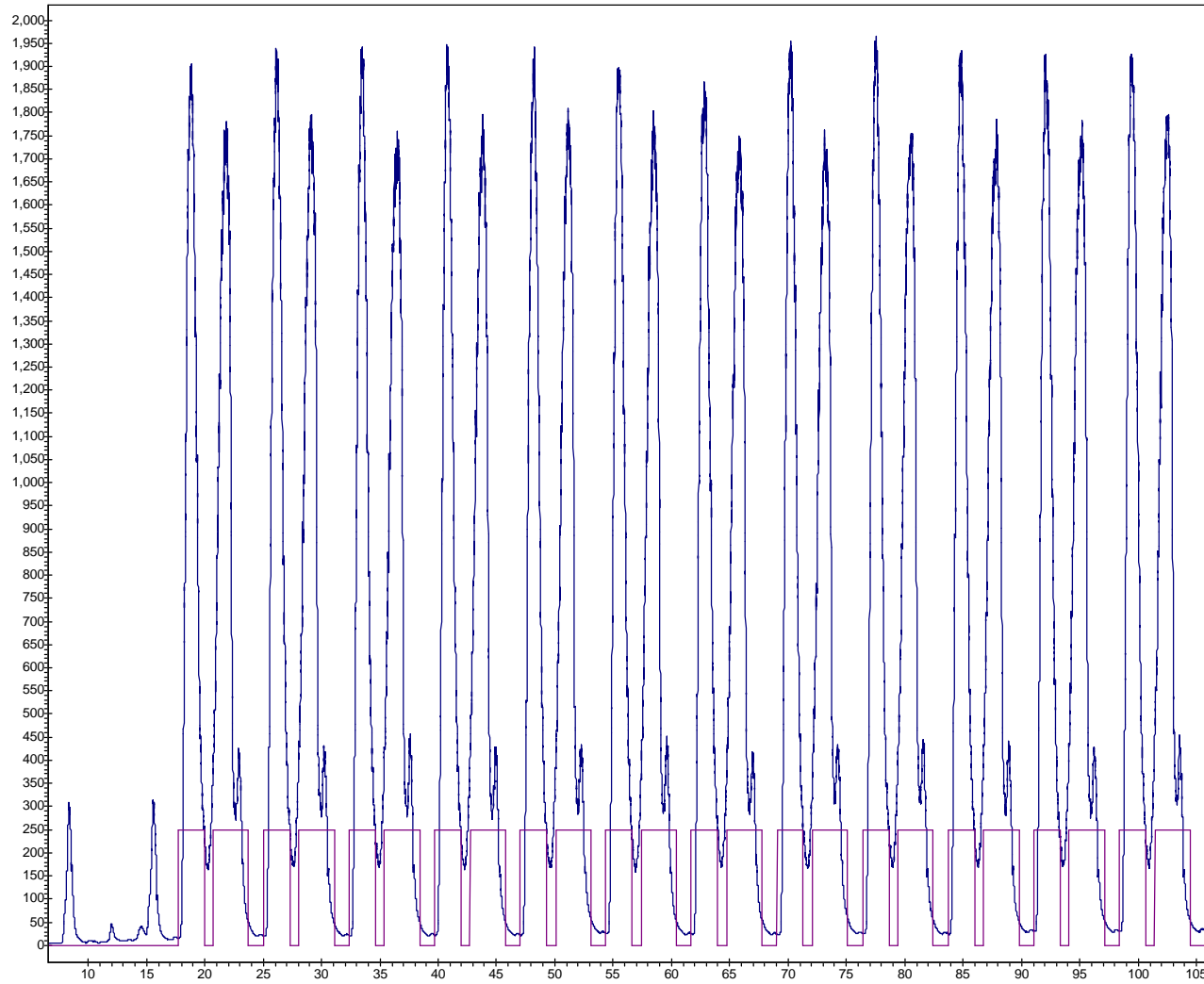
# Analytical Separation Four Diastereomers



# Preparative Separation of four Diastereomers



# Difficult Separation



# Conclusions on Chiral Separations

- Screening multiple columns and solvents yields best possible separation.
- Derivatization may be required.
- Closely eluting peaks may require shaving of the peaks and collection of the middle section for reinjection
- Familiarity with samples may speed up purification.

# Achiral Samples

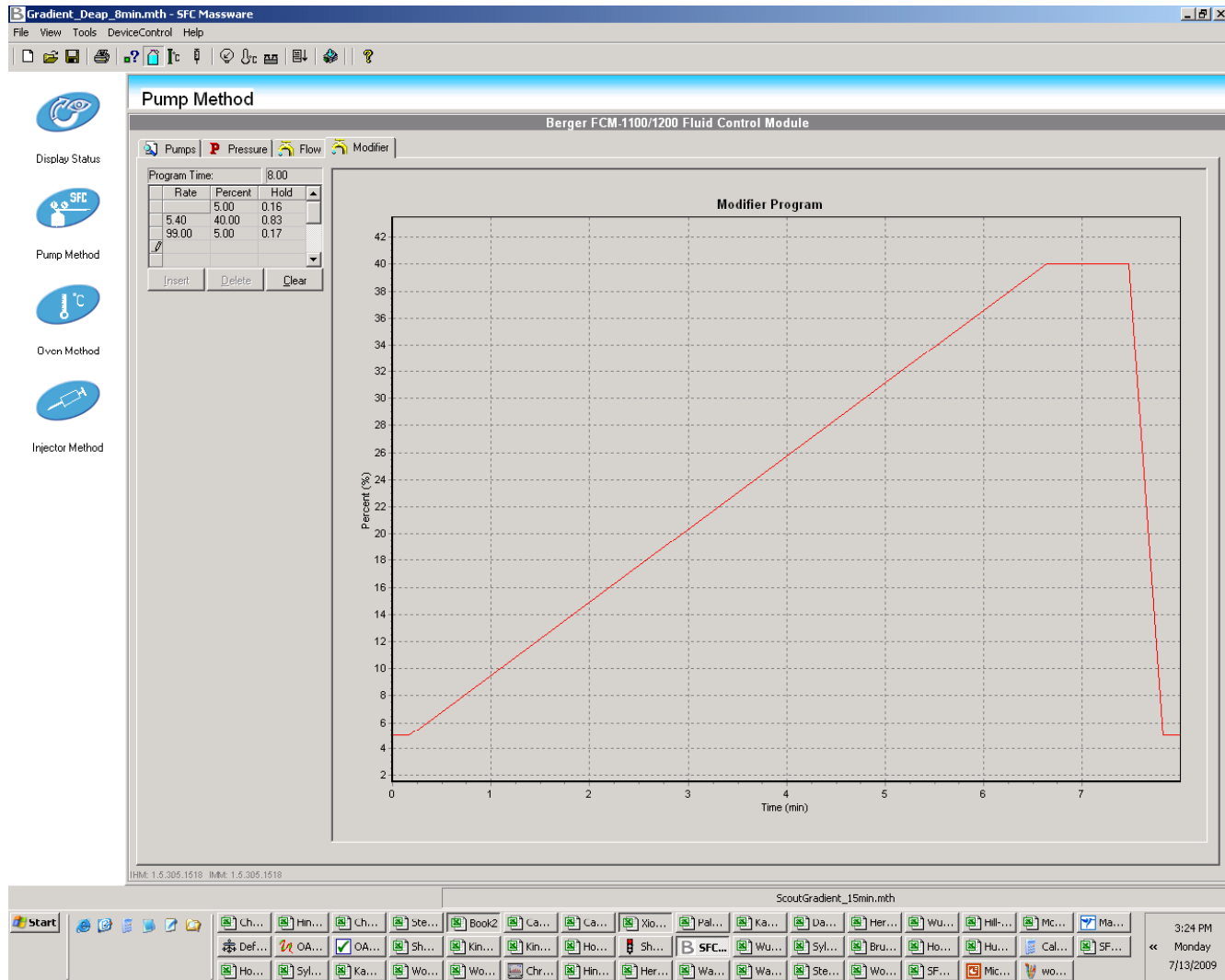
# Method Development

- Initial screen using LC/TOF with basic conditions on a Gemini NX column results in inadequate or no separation
- Screen sample on SFC with variety of achiral phases such as Cyano, DEAP, Amino, Pyridine
- Develop conditions for separation using either stacked or gradient

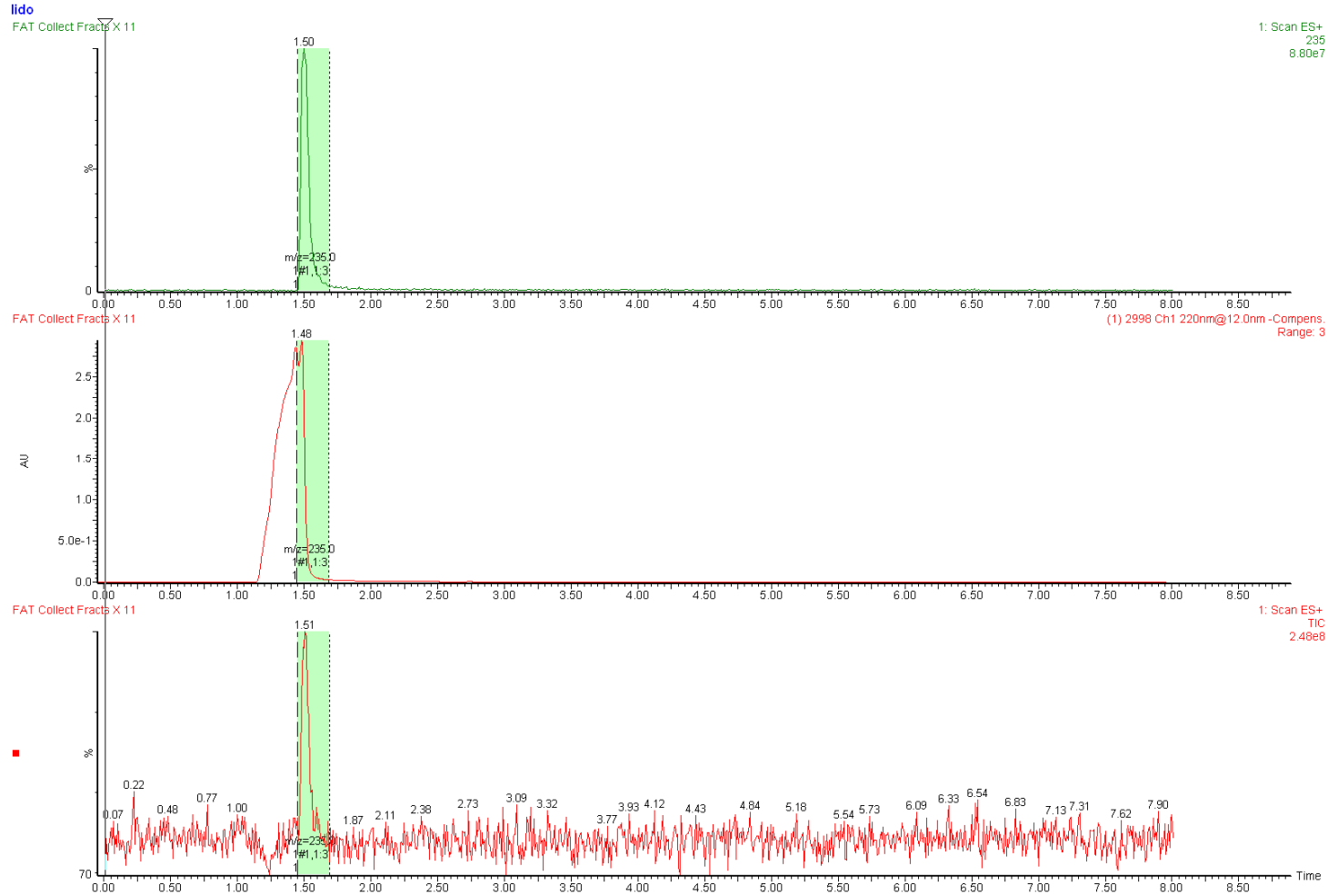
# Columns- Achiral

- DEAP (diethyl amino propyl)
- Cyano
- Diol
- Amino
- Pyridine
- Diol-HL
- PA (propyl acetimide)

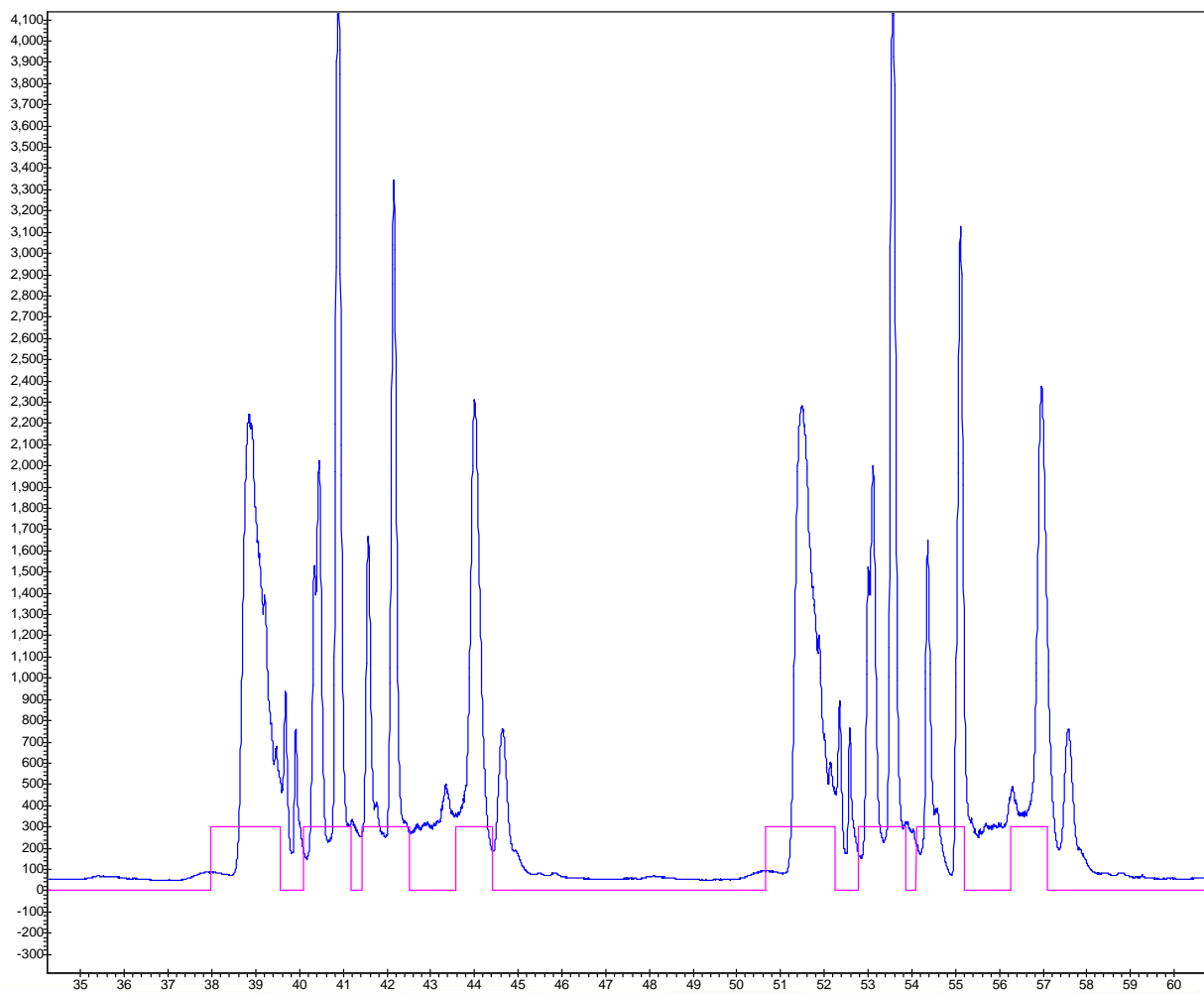
# Scout Method For Achiral



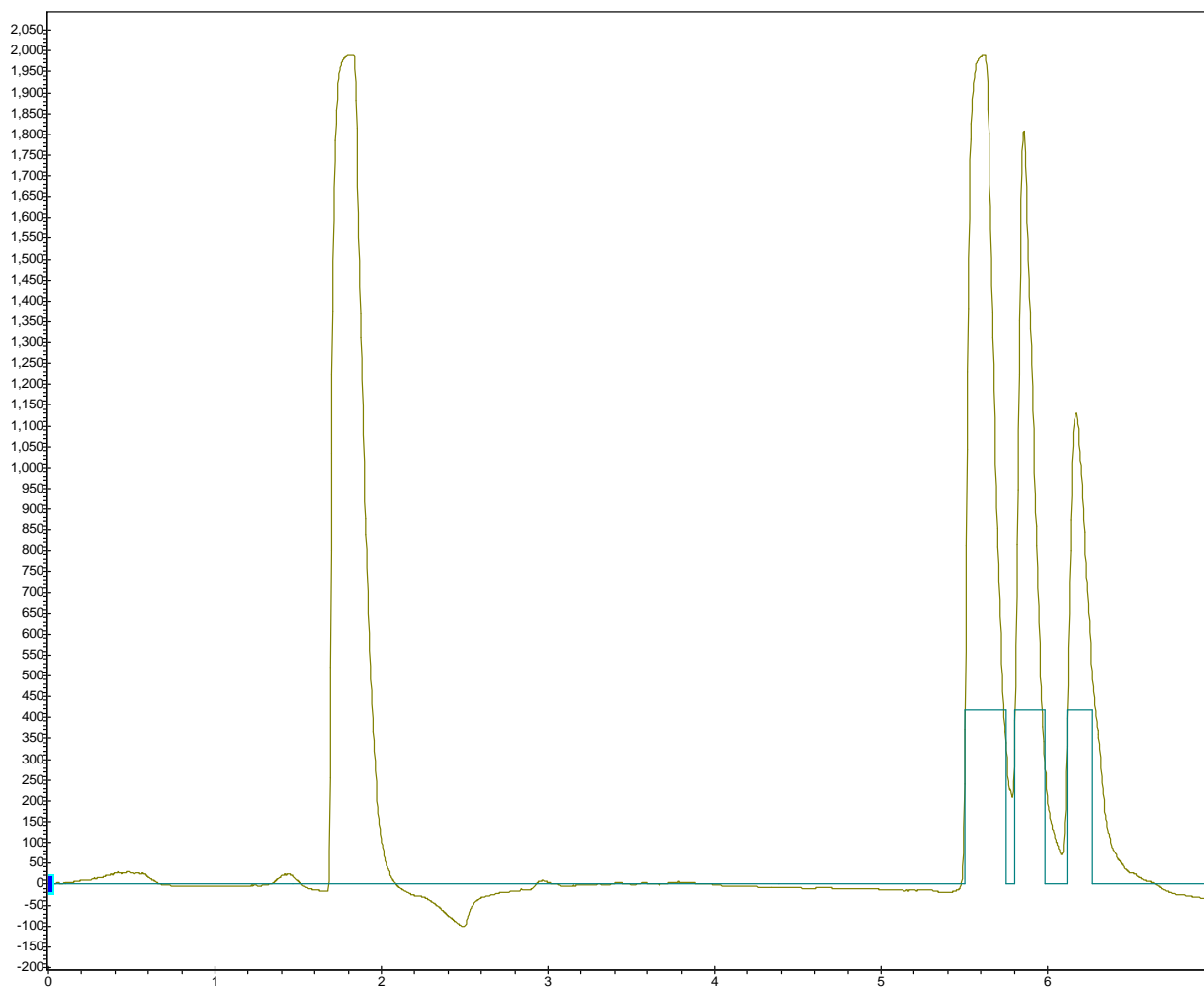
# Analysis of Standard on SFC/MS



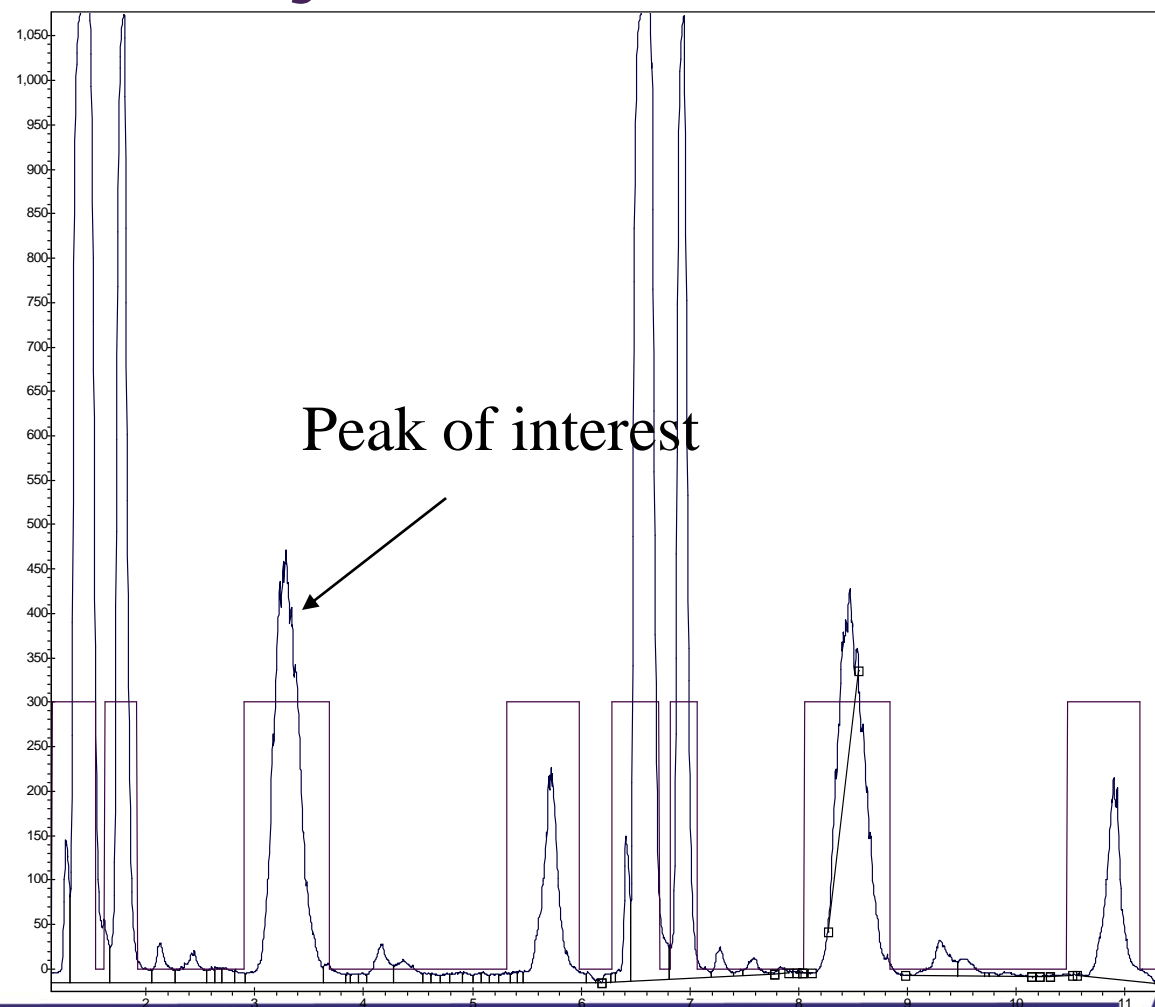
# DEAP column, diastereomers, four isomers, stacked injections



# Cyano column-separation of isomers using a gradient, 5-40% methanol



# DEAP column separation of achiral compound and impurities, stacked injections



# Conclusions

- Alternative columns
  - DEAP (diethyl amino propyl)
  - Cyano
  - Amino, Diol, Diol-HL, PA (propyl acetimide)
- Alternative conditions
  - Isocratic
  - Gradient
- Orthogonal separation compared to LC/MS

# Wilmington Purification Group

- Dave Coomber
- Frances McLaren
- Xiaomei Ye