

Application of SFC and Hyphenated SFC Techniques in Drug Discovery

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Discovery Analytical Chemistry
Chemical Technologies
Chemical and Screening Sciences

Wyeth Research
Collegeville, Pennsylvania

Collegeville Discovery Chromatography

Who we are and what we do...

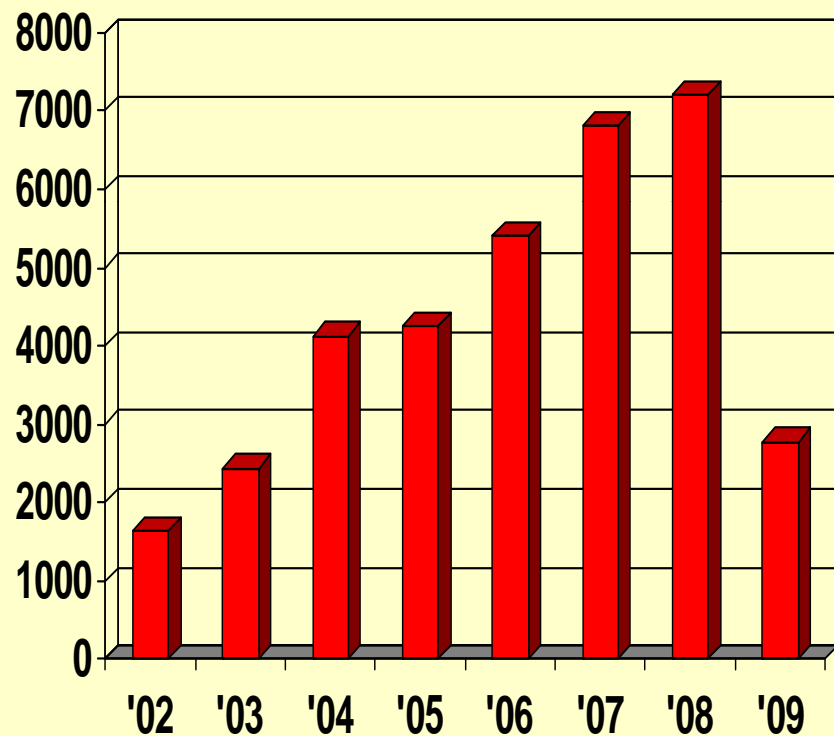
"Group" is now comprised of only 2 FTEs with 30+ years of combined expertise in Analytical Chemistry (Chromatography, MS, NMR, Profiling)

Our chromatography responsibilities include:

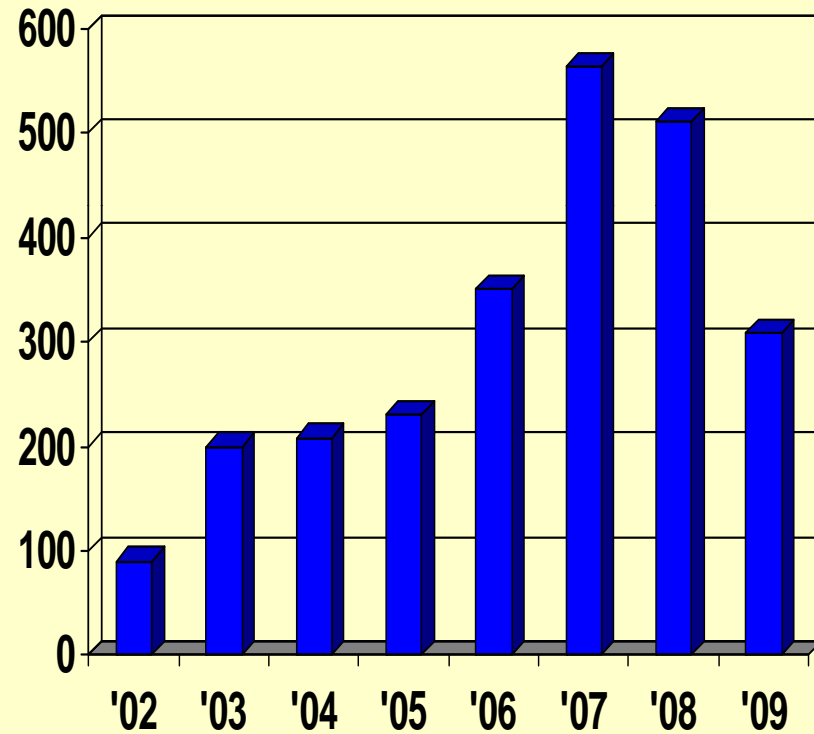
- Performing Rapid Quantitative HPLC Analyses (24hr turnaround) for every compound submitted to the Wyeth library from Collegeville Medicinal and Exploratory Chemists
- Development of Separation Methods and Achieving Preparative Purifications of Difficult Synthetic Targets, Including All Chiral Preparative Chromatographic Isolations (10+mg - 100 grams)
- Developing Methods for Enantiomeric Quantification of Chirally Synthesized Molecules

Collegeville Chromatography Submissions

■ AN HPLC



■ PREP HPLC/SFC



Despite having the same FTE support that was available in 2002, the number of submissions has increased 5-fold.

Advantages of Supercritical Fluid Chromatography

- Faster analyses
- Improved resolution
- Faster method development, gradient separations
- Wide range of useful columns and detectors
- Range of modifiers to affect separations – often no need for additives
- Safe, Green and Cost Efficient
- Easy to train non-experts in basic SFC use

to our customers, medicinal chemists, SFC means
low solvent volumes and NO WATER

SFC Instrumentation at Our Disposal

6 + 1 total SFCs in the labs:

1 Berger Analytical SFC equipped w/ 6-column and 6-solvent valves;
Waters ZQ mass spectrometer available for SFC/MS screening

1 Berger Analytical SFC equipped with two 6-column valves and one 6-solvent valve; Jasco CD detector available for SFC/CD screening

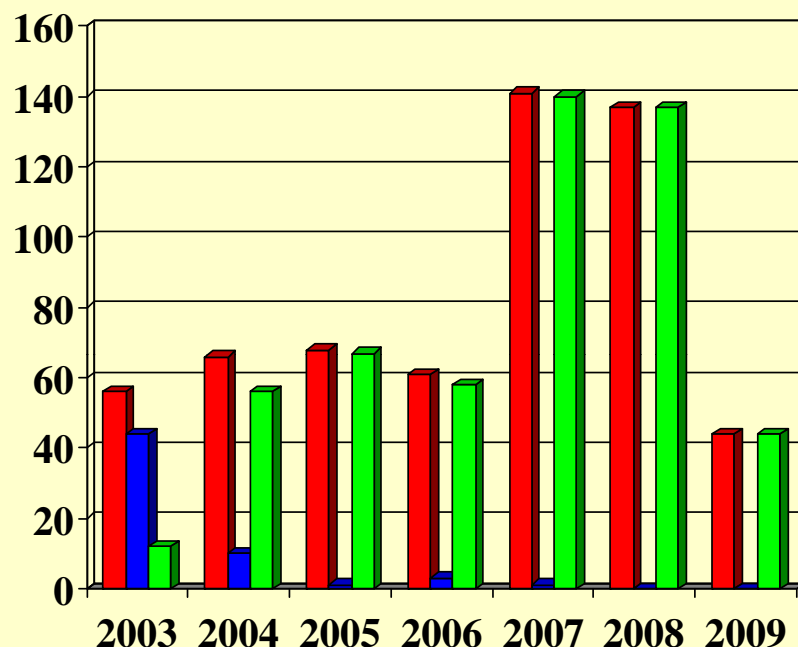
1 Berger Analytical SFC equipped with two 6-column valves and one 6-solvent valve

1 Berger Minigram SFC

2 Berger Multigram II prep SFC systems

1 Agilent 1100/MSD/Aurora Fusion with 10-column switching valve

Impact of SFC on Colleagueville Chiral Chromatographic Separations



Chiral Preparative Submissions

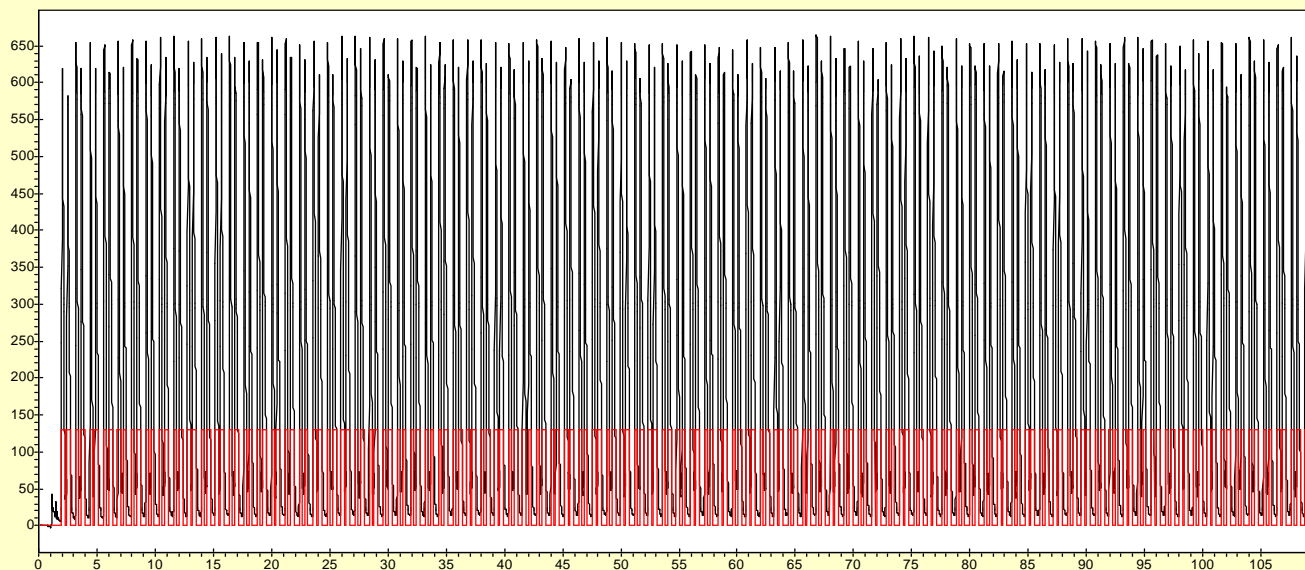
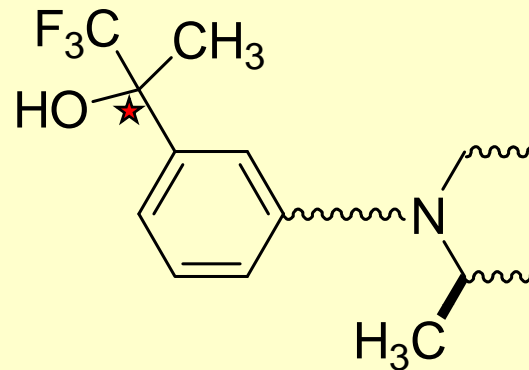
➤ Successful utilization of SFC for chiral separations has transformed our method development paradigm.....

- SFC using column and modifier screening is now performed first, with subsequent HPLC screening only when SFC is unsuccessful.

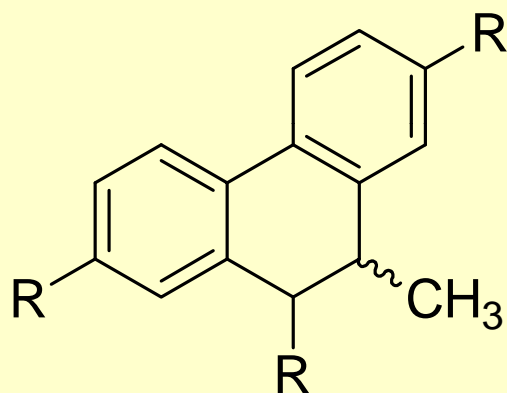
Diastereomer Purification of XXX-165

103.7 gram prep :
Chiralpak AS-H, 15% IPA
run ~20hr/day for 3 days

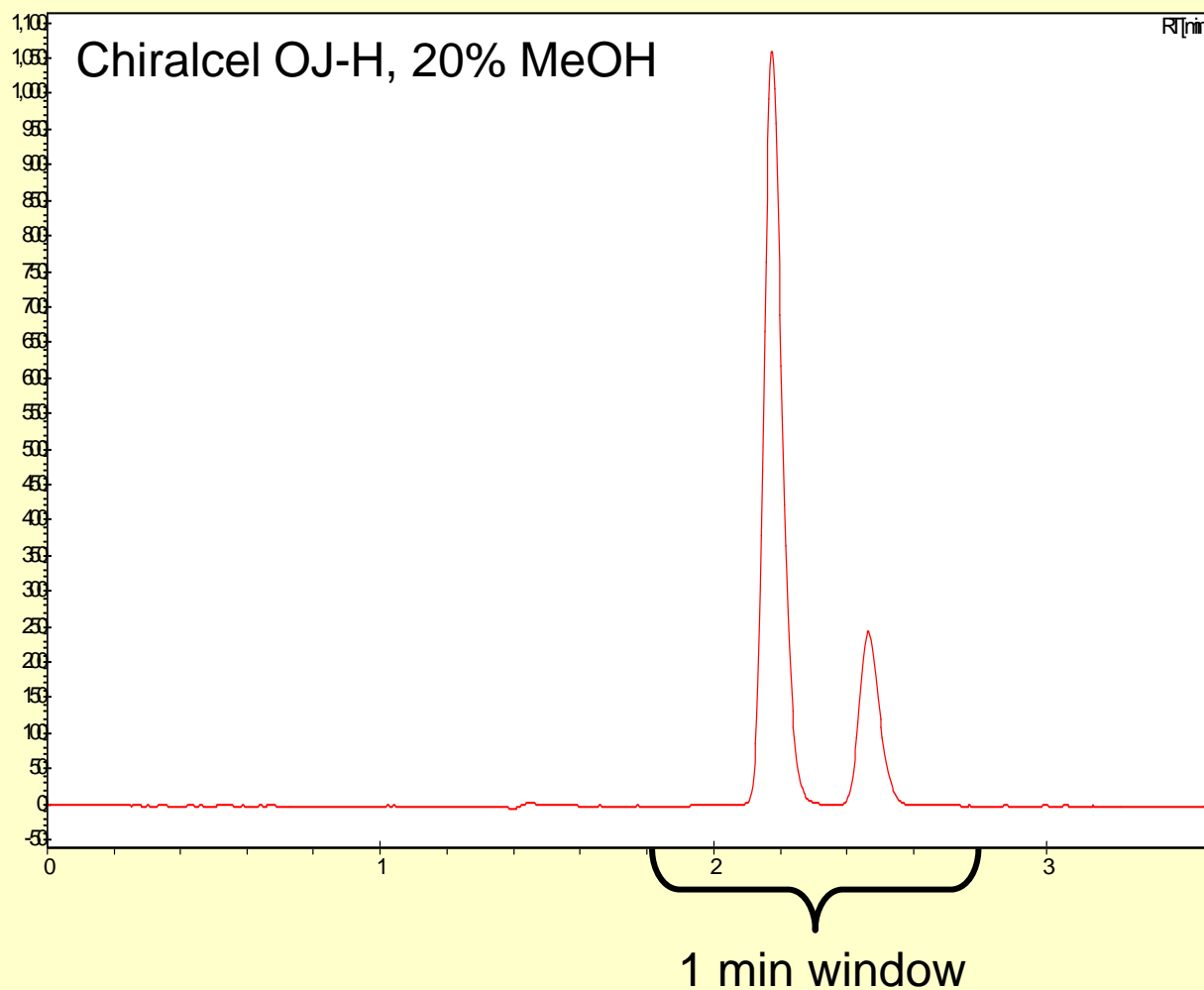
Two diastereomers were dried
and returned to DSC in 5 days



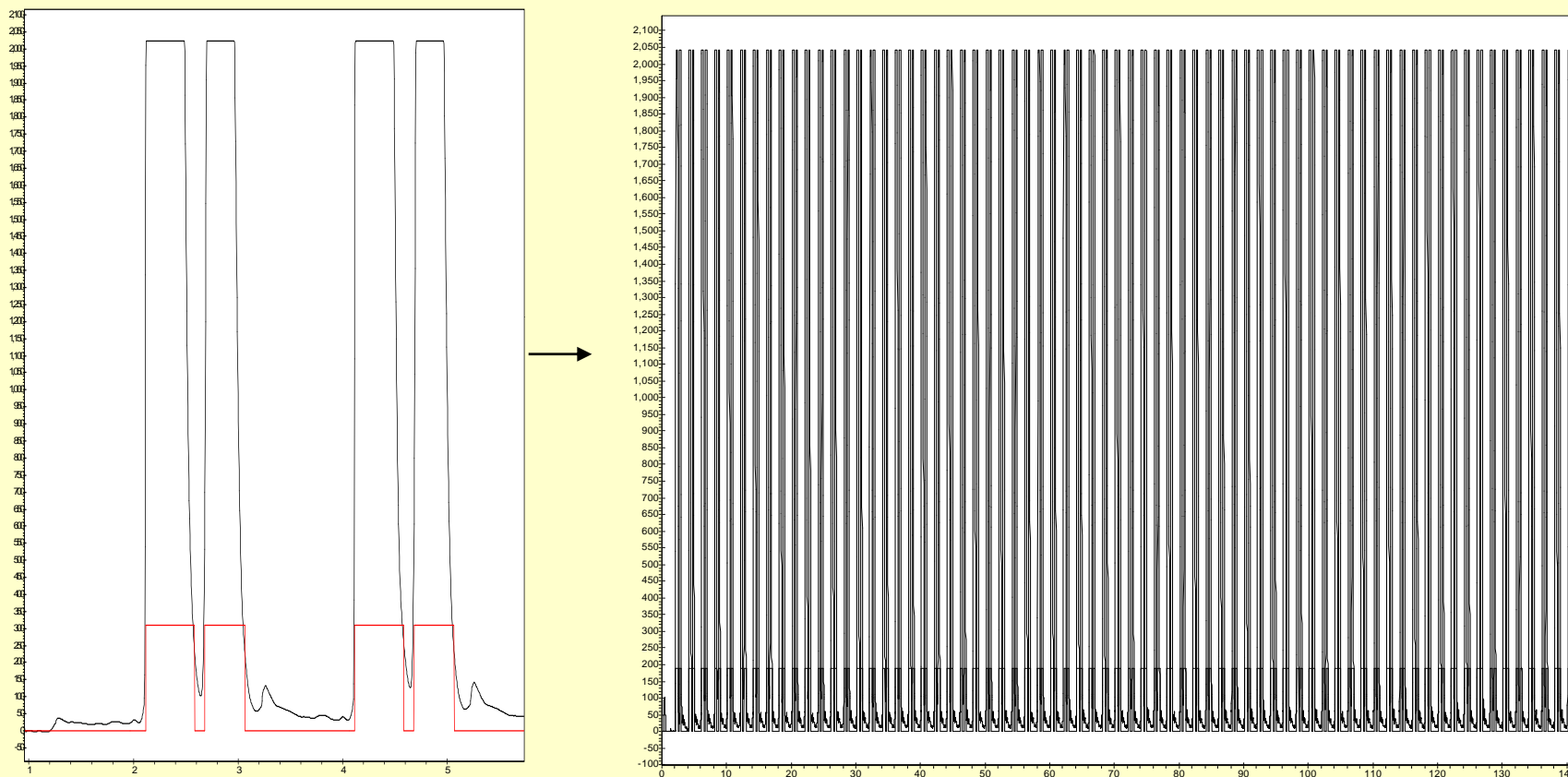
Chiral SFC Purification of Compound "A"



46 gram chiral separation
4:1 eutomer/distomer

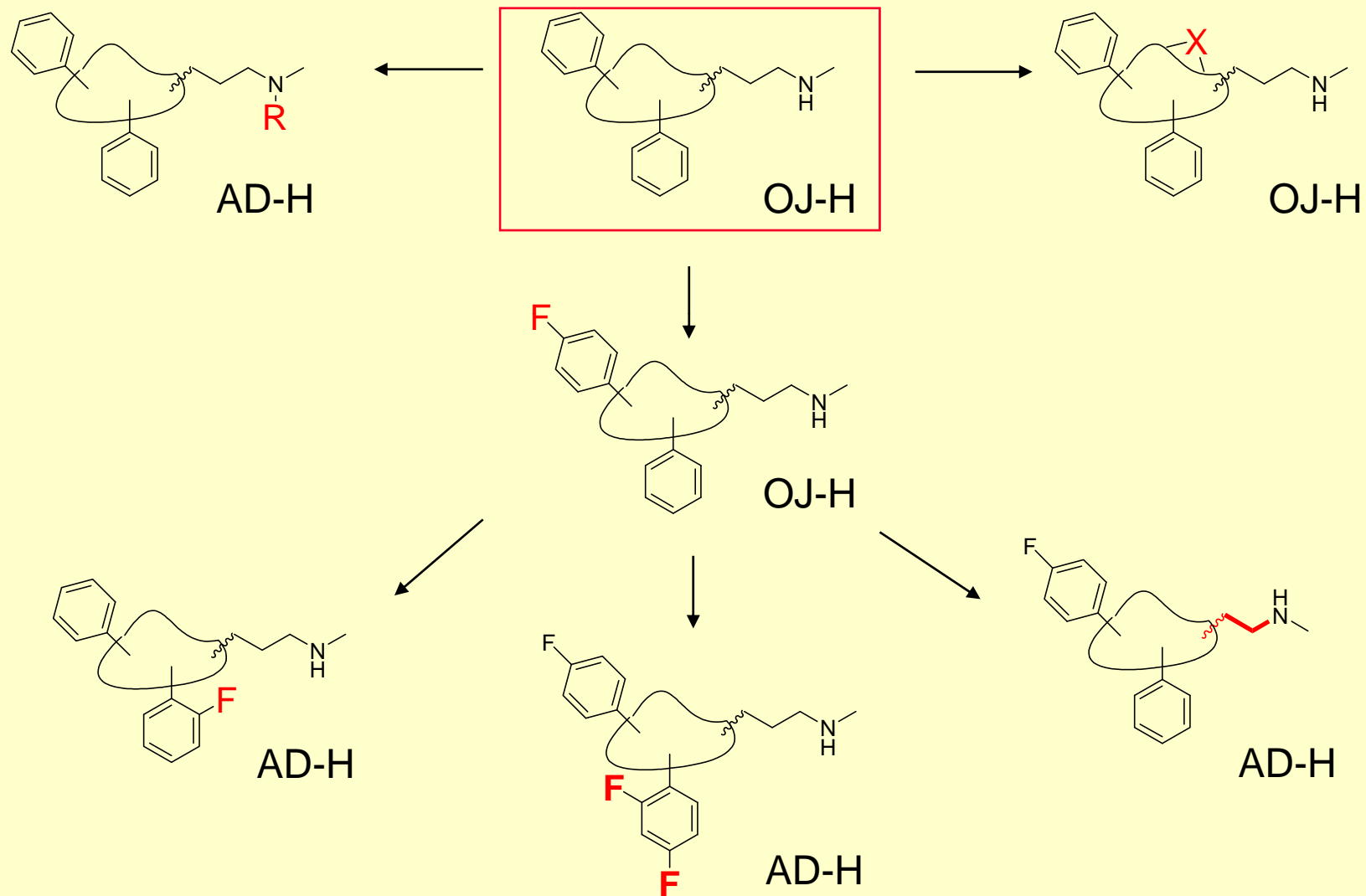


Stacked Injections of Compound A



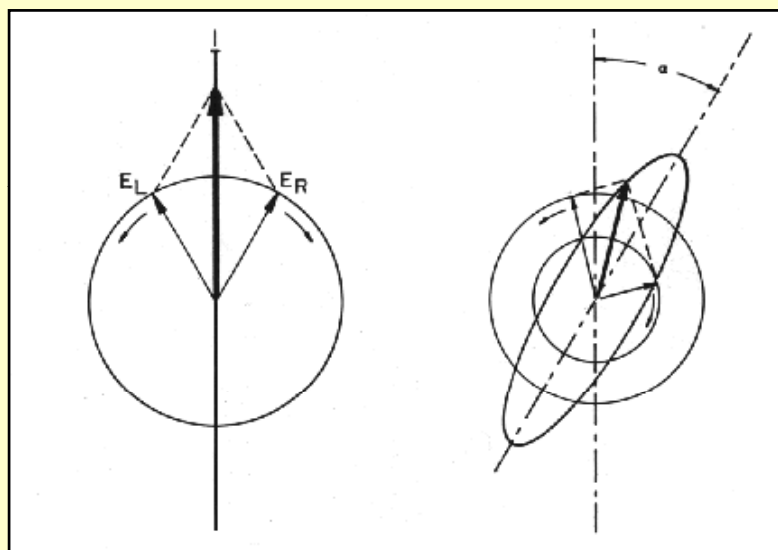
~81 mg injected every 2 min = 2.42 gr/hr = 46 grams in ~19 hr
Eutomer was returned to chemist in <2L of 100% MeOH

Chiral Purifications are still Unpredictable...



Chiral SFC w/ CD and UV

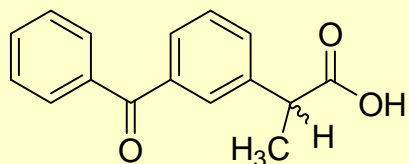
- Circular dichroism (CD) is observed when optically active matter absorbs left and right handed circularly polarized light differently



Benefits to using SFC w/ CD including distinguishing enantiomer peaks from achiral impurities and ability to follow peak of interest (eutomer) in a series of compounds in order to have it elute first from column to ensure enantiomeric purity

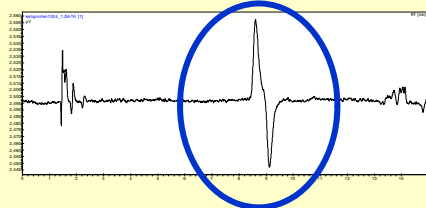
Disadvantage: only single wavelength

Monitoring the Effects of **Modifier** on Elution Order by CD

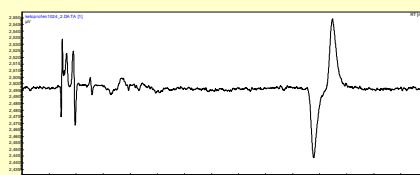


ketoprofen

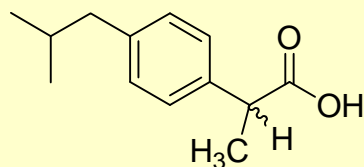
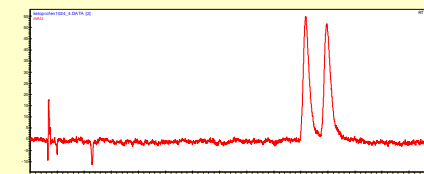
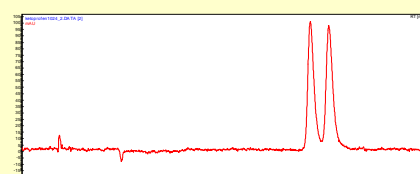
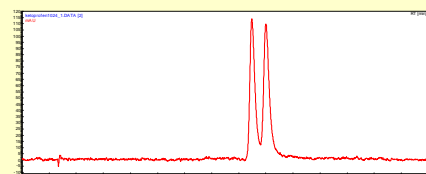
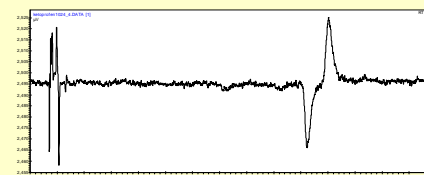
MeOH



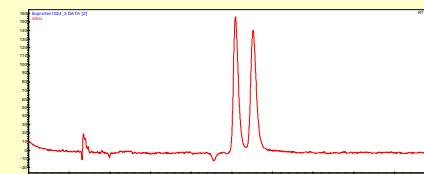
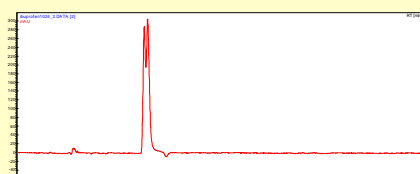
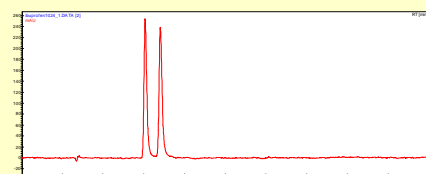
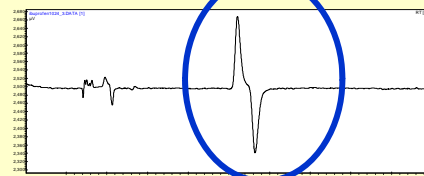
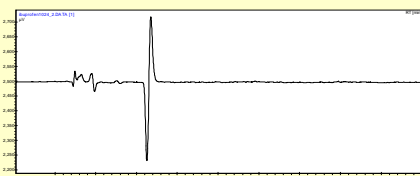
EtOH



IPA

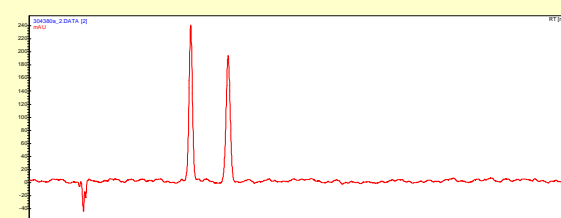
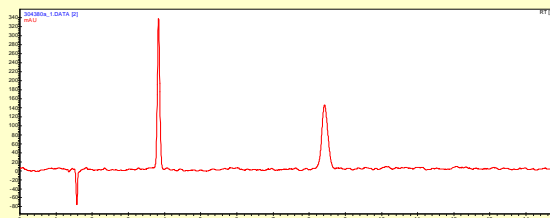
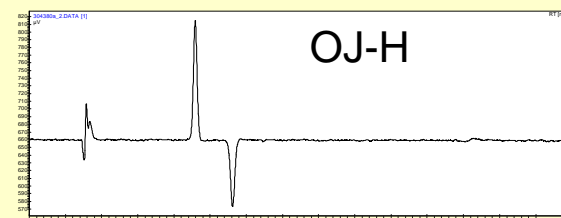
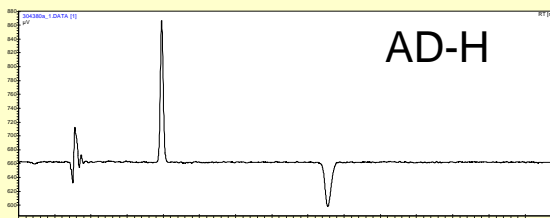
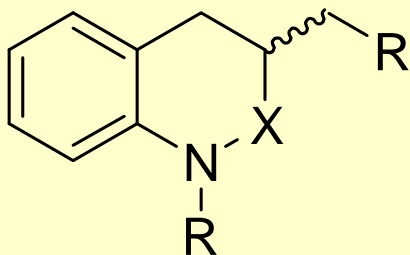


ibuprofen



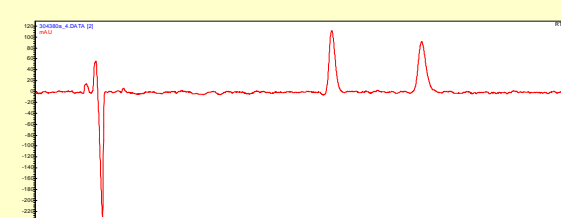
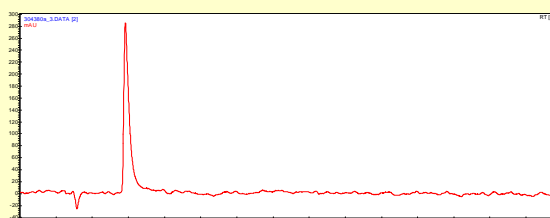
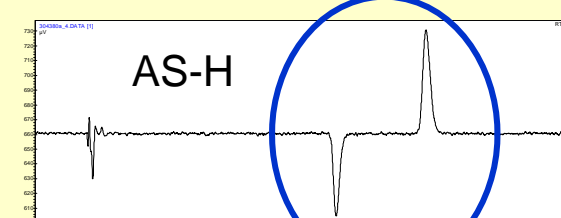
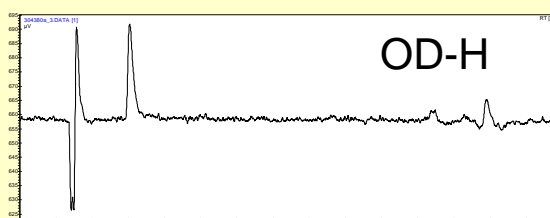
UV trace at 220 nm (red) CD trace at 260 nm (black), AD-H 4.6 x 250 mm, 2.0 mL/min, 100 bar, 35 C, 10% modifier

Monitoring the Effects of **CSP** on Elution Order by CD



Elution order varies from among the CSPs

+/- on both the AD and OJ columns, co-elution on OD and reversal of elution (-/+) on AS



Chiral SFC with **APCI/ESI-MS** Detection

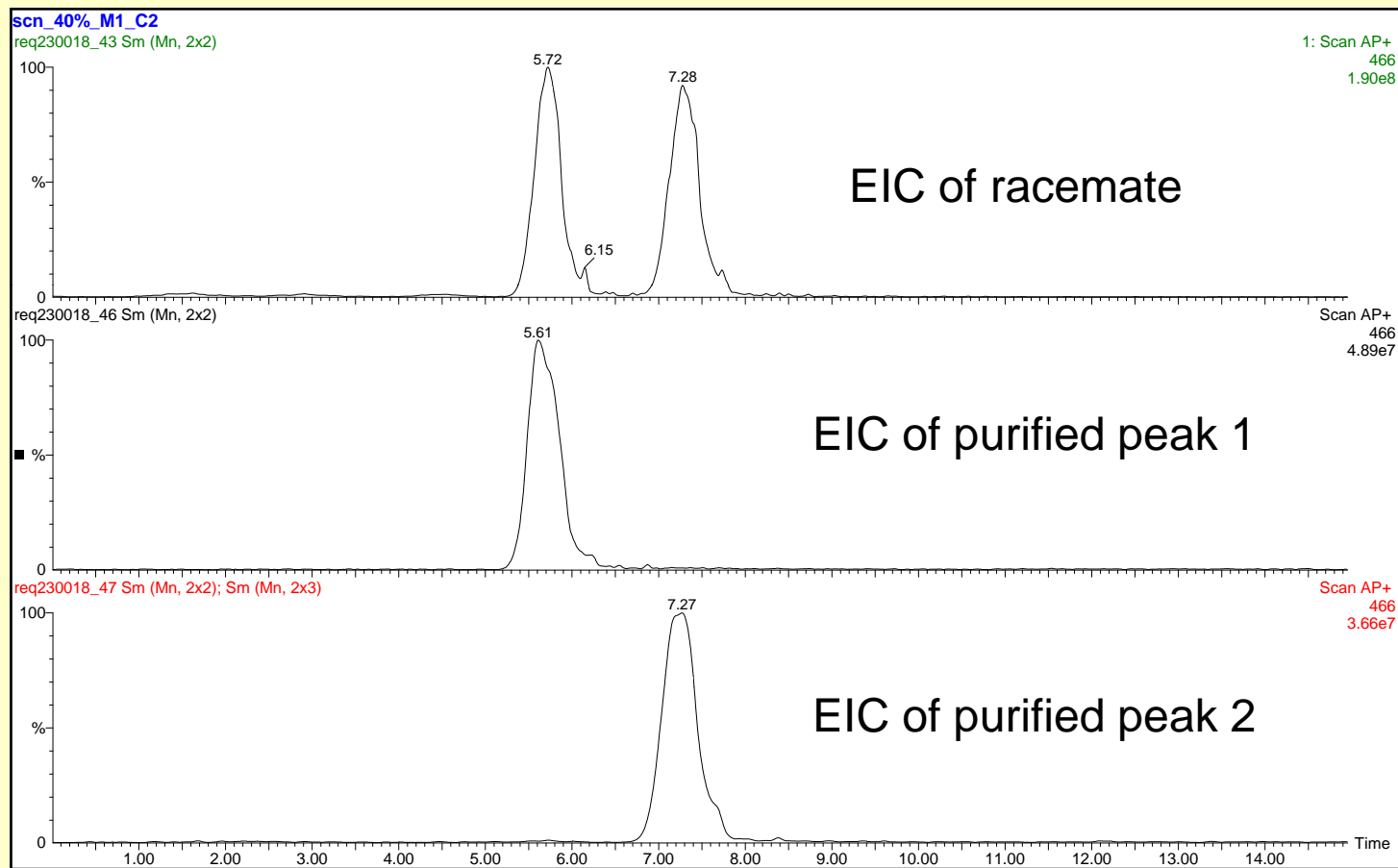
Applications

- Determination of Chiral Purity (% ee) in Addition to UV
- Determination of Chiral versus Achiral Impurities
- Chiral Separations of non-UV active constituents
- Chiral Screening with sample pooling for increased throughput

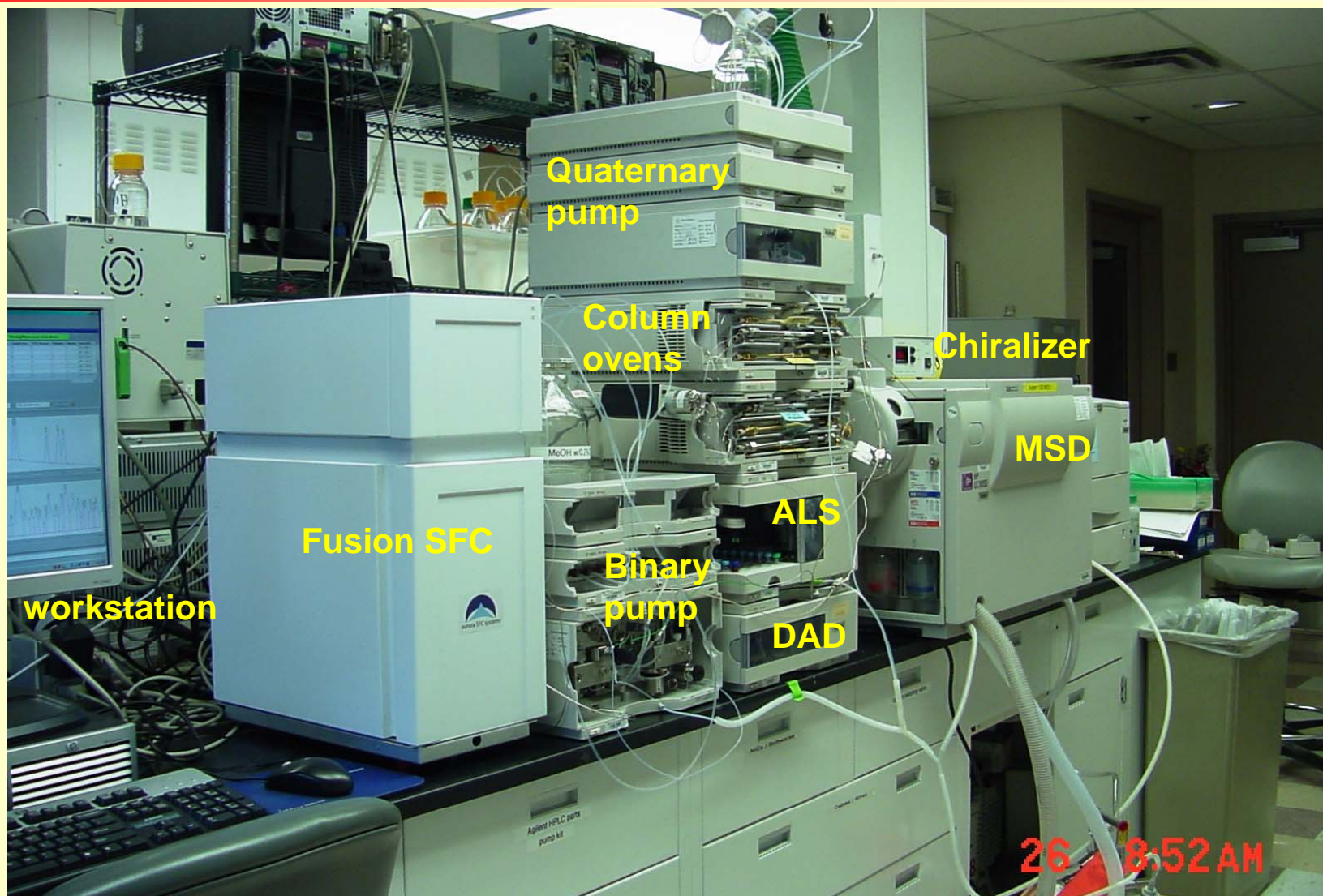
SFC/MS Screening



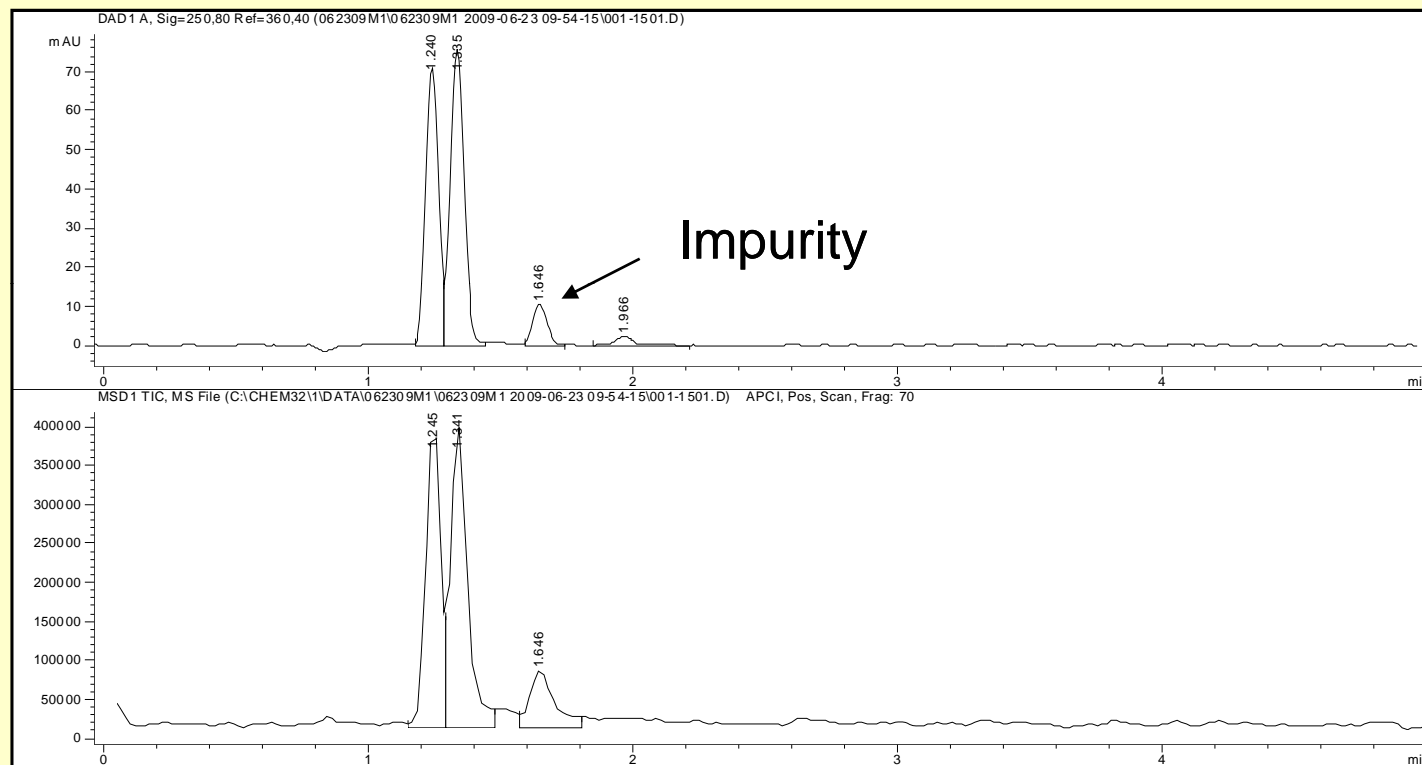
SFC/APCI-MS for Enantiomeric Excess



SFC/MS Screening

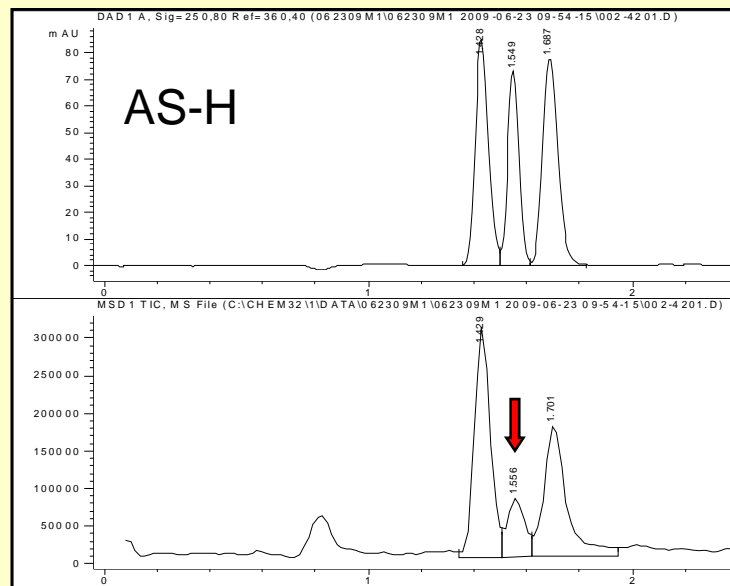
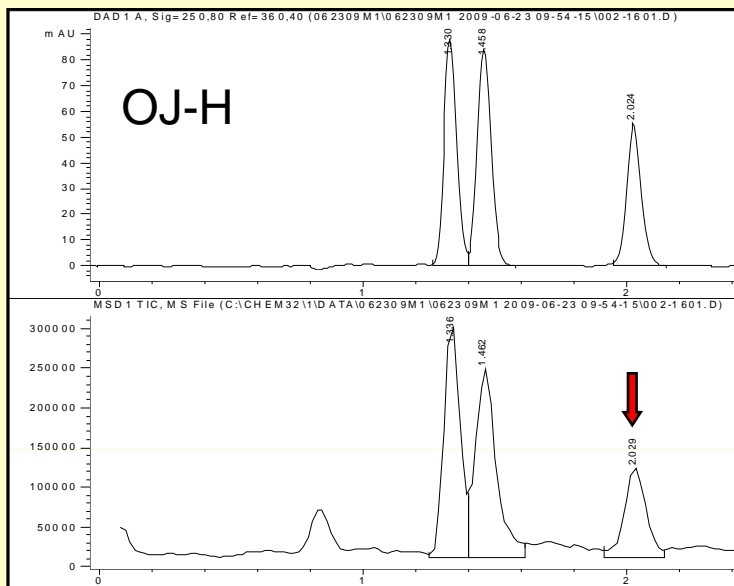
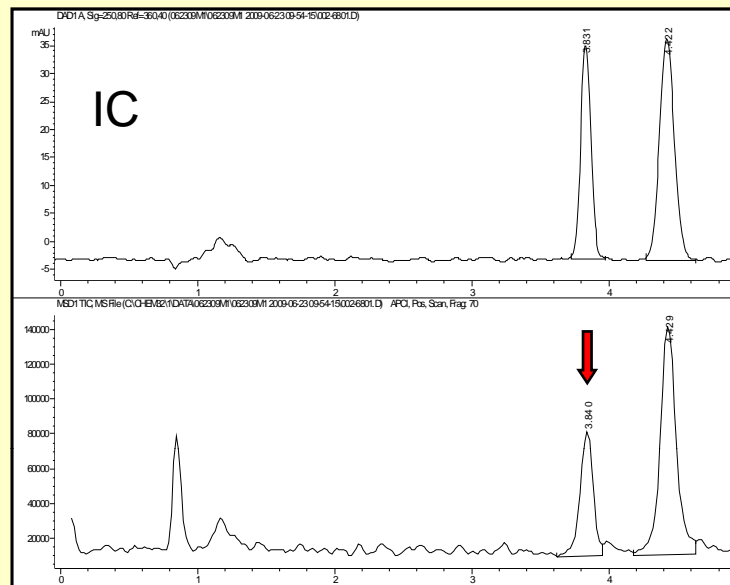
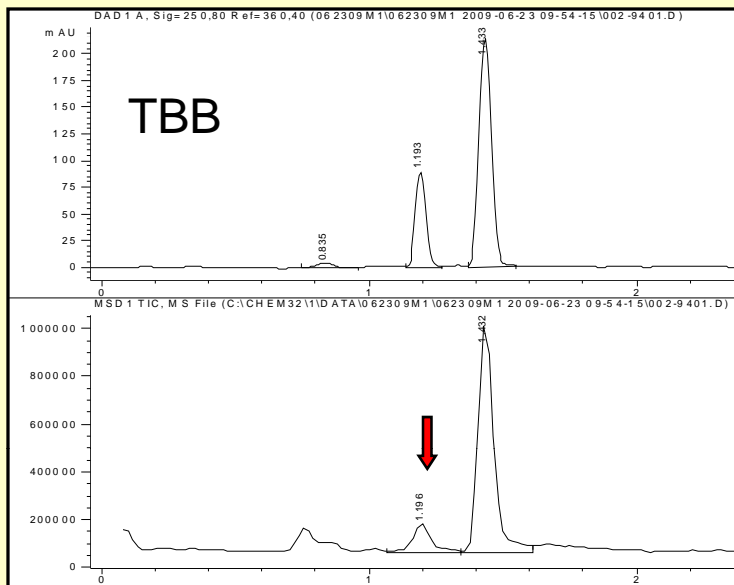


Sometimes, achiral impurities in chiral separations are obvious...



Chiralcel OJ-H

Other times, mass spectral data is invaluable...



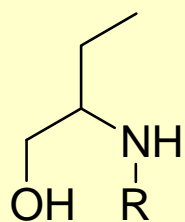
Chiral Library Screening with Sample Pooling using SFC/APCI+MS

Combine "N" racemic samples for chiral method development

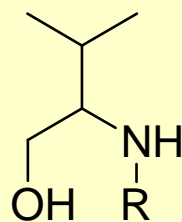
Chiral SFC to separate enantiomers of each racemate

MS to differentiate enantiomeric pairs

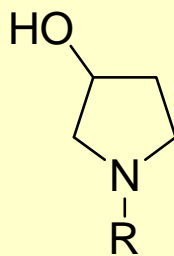
'N' x Faster chiral analysis than single sample screening



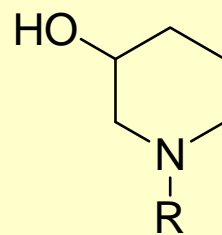
A= m/z 231



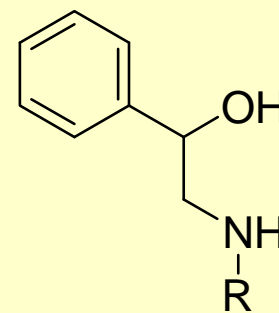
B= m/z 245



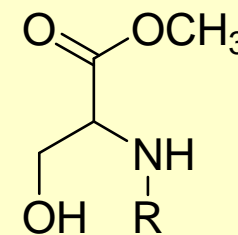
C= m/z 229



D= m/z 243

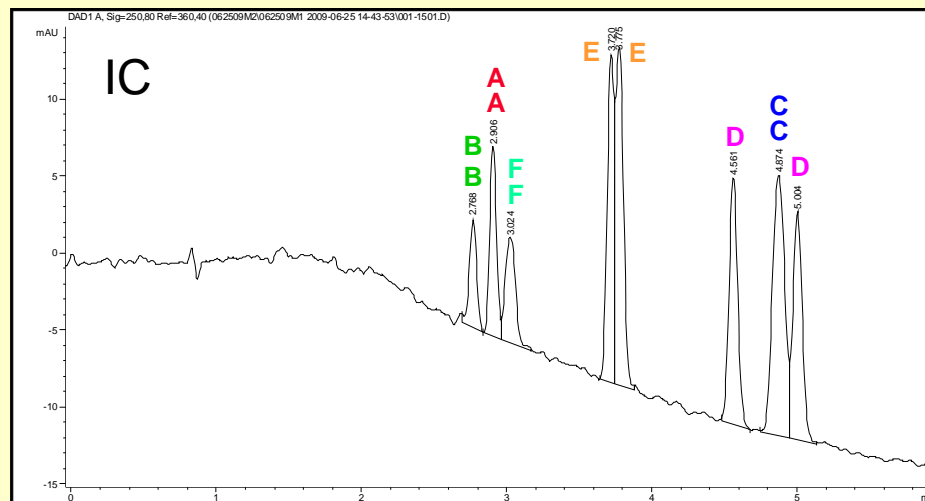
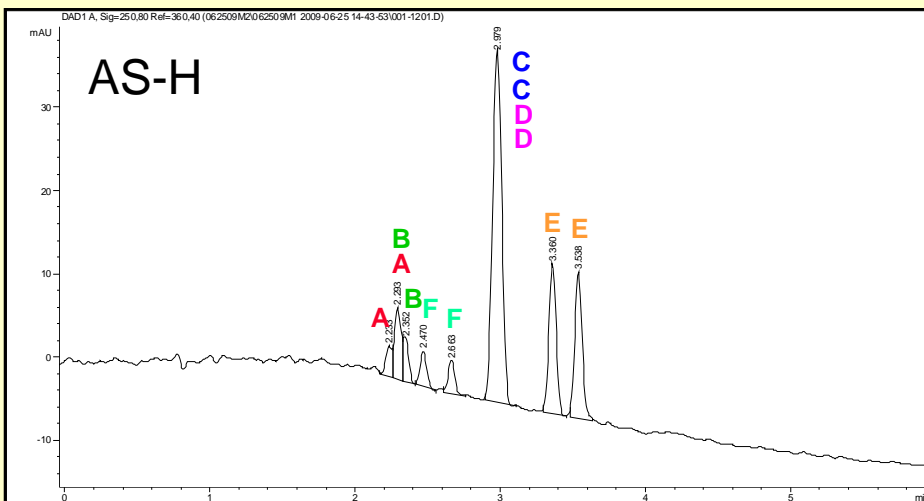
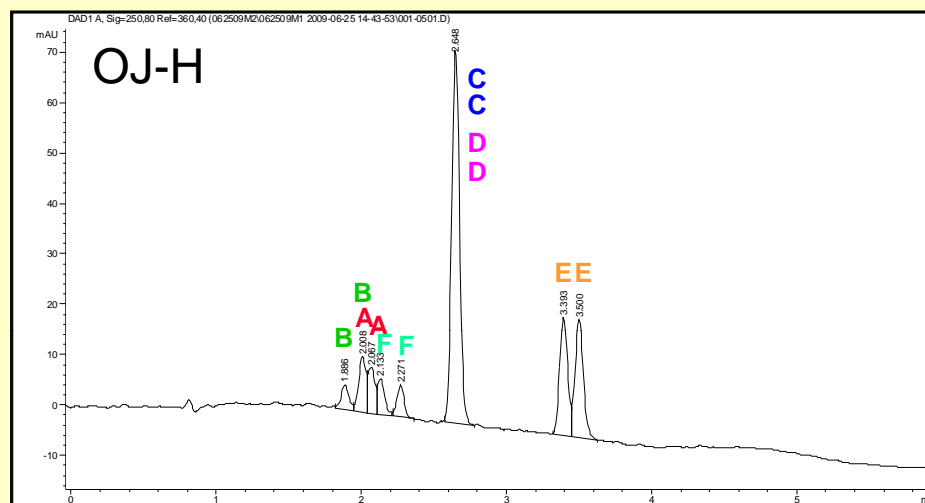
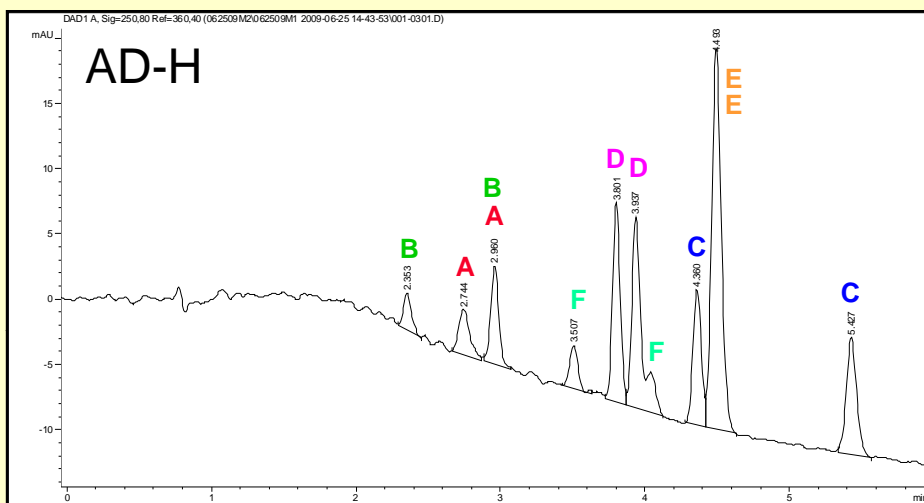


E= m/z 279



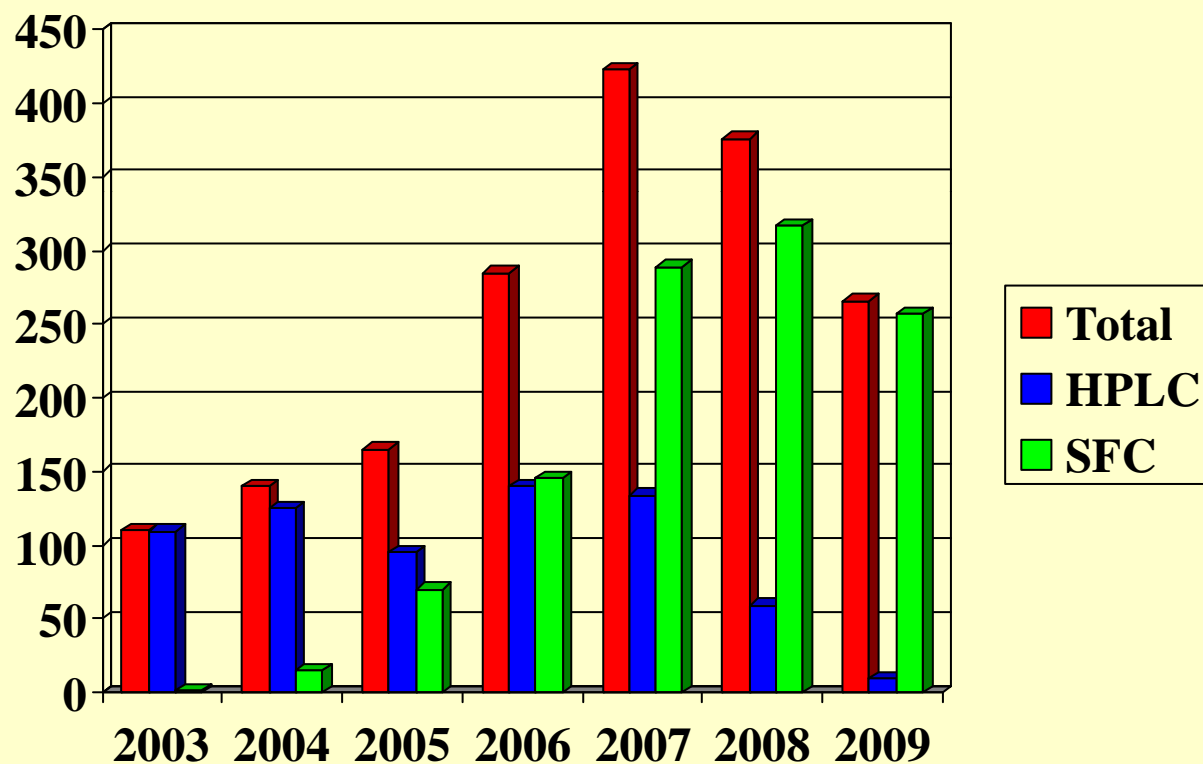
F= m/z 261

Sample Pooling Results from 6-Component Mixture



Impact of SFC on Collegeville Achiral Chromatographic Separations

Analytical HPLC analyses trend: ↑ Number of Achiral prep requests: ↑



Achiral Preparative Submissions

Achiral SFC Stationary Phases

Kromasil CN, DIOL, Phenyl, Silica, NH₂

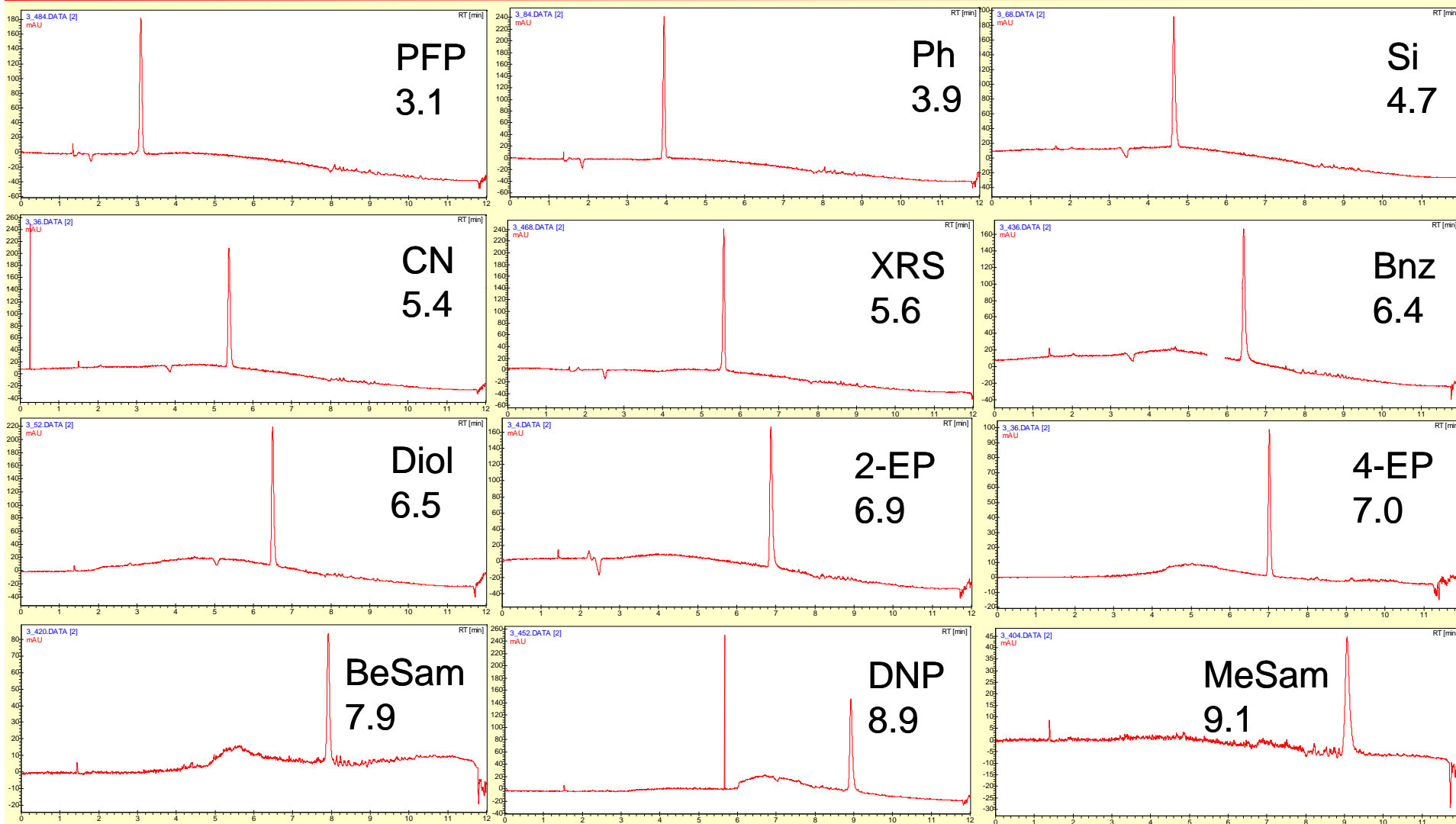
Princeton Chromatography 2-Ethylpyridine, 4-Ethylpyridine, 2CN:DIOL, Dinitrophenyl, Benzenesulfonamide, Benzamide, Methanesulfonamide

ES Chromegabond NO₂, Aminophenyl, Pyridylamide

Phenomenex DIOL, PFP

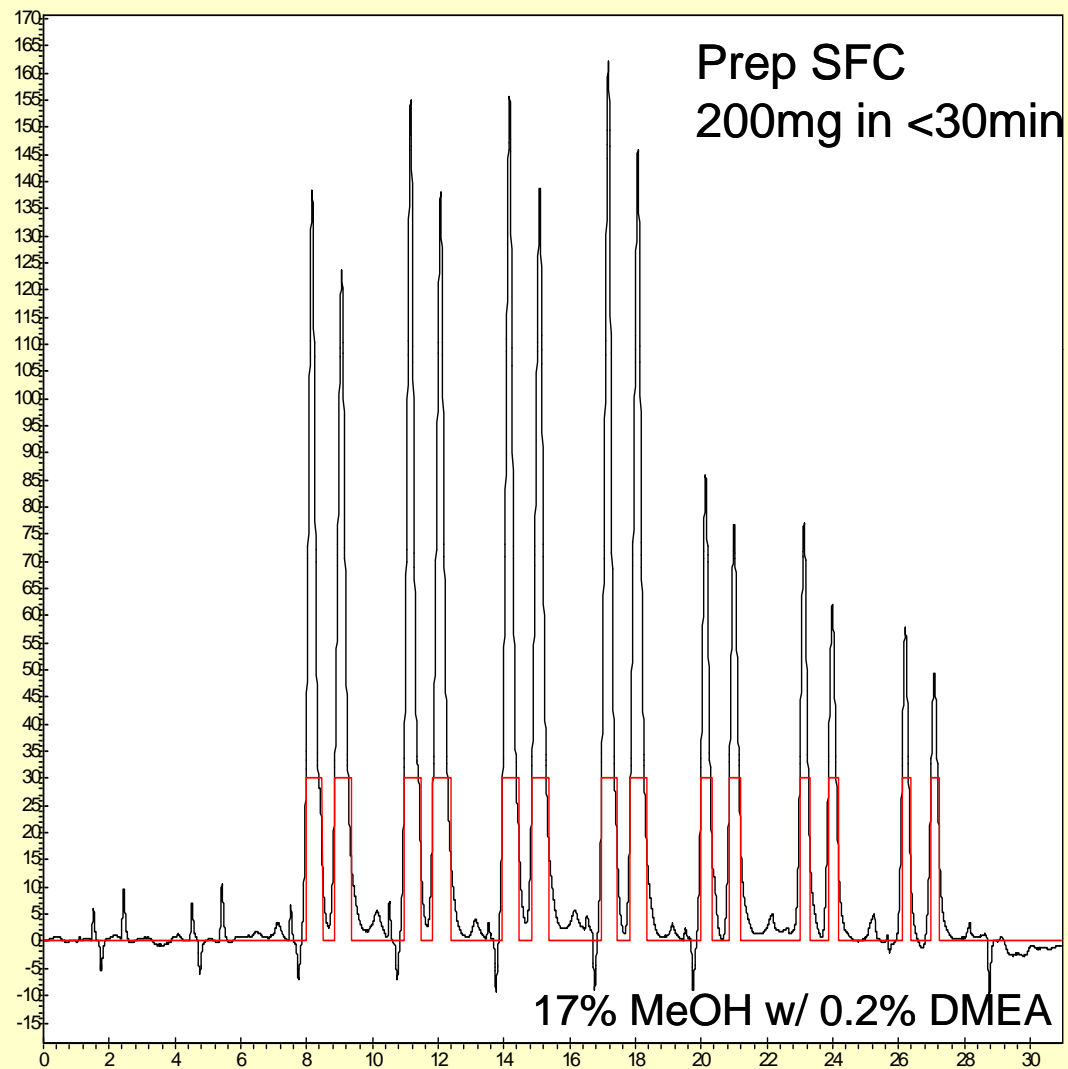
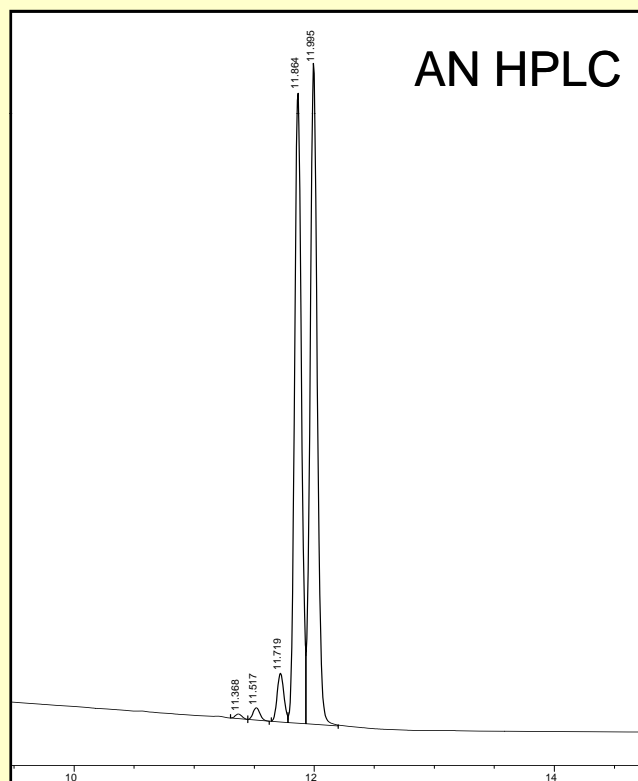
Varian Pursuit XRS diphenyl

Warfarin SFC Column Screen

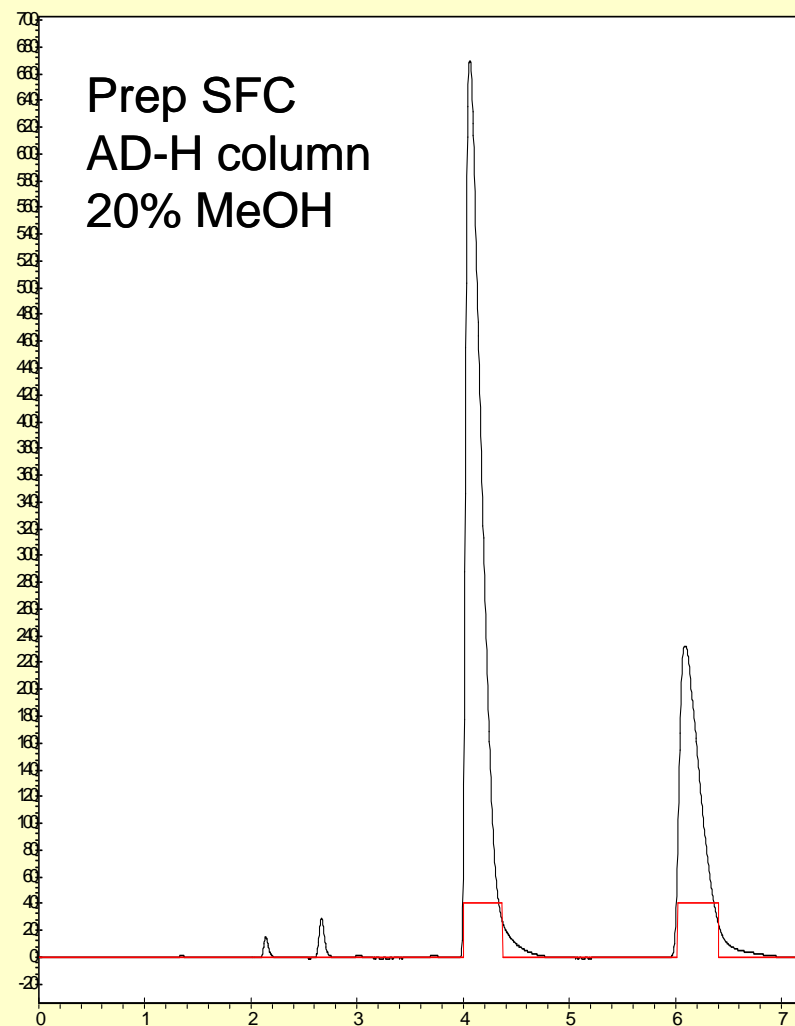
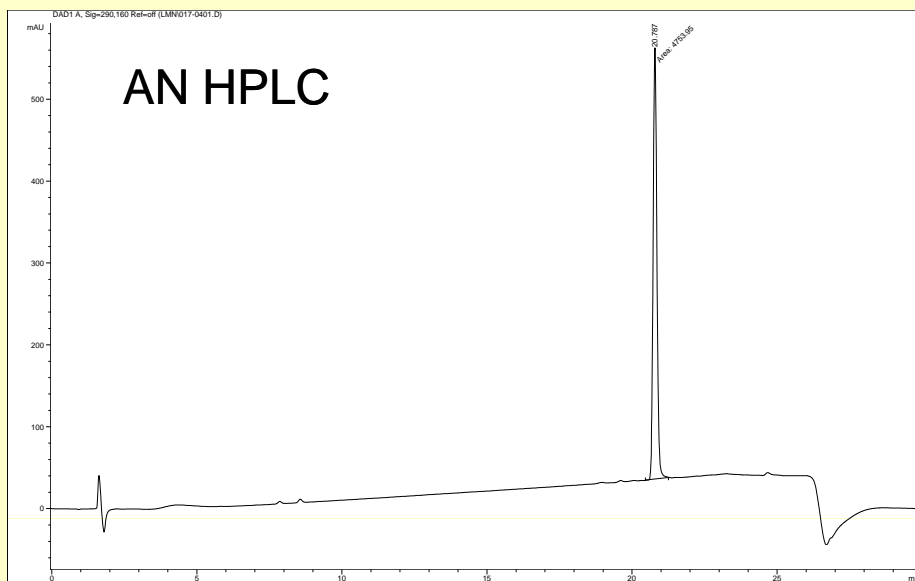
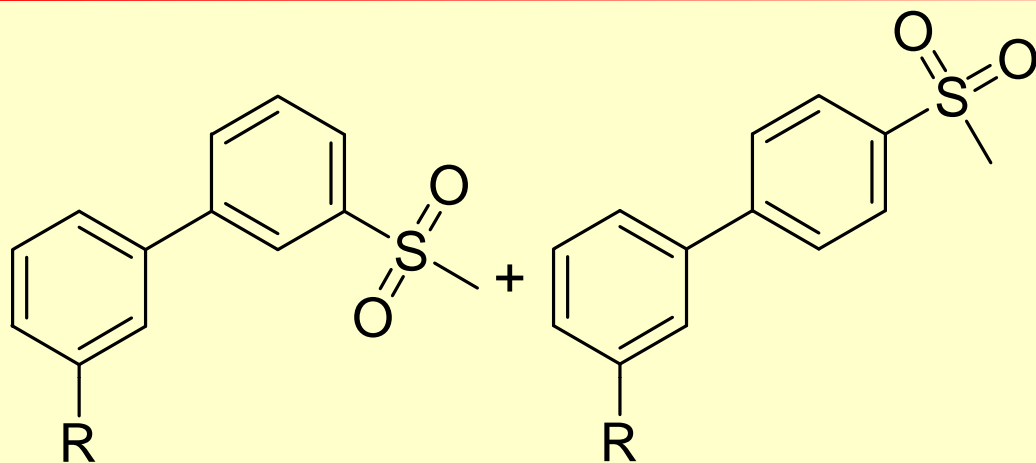


Real Life Examples of Achiral SFC in Drug Discovery

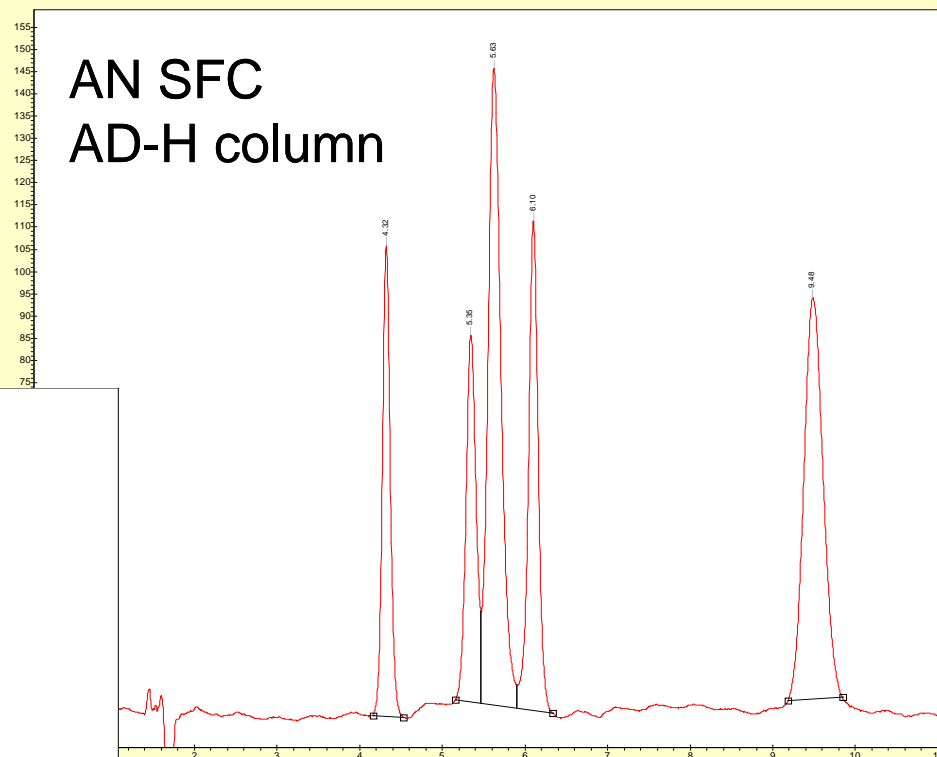
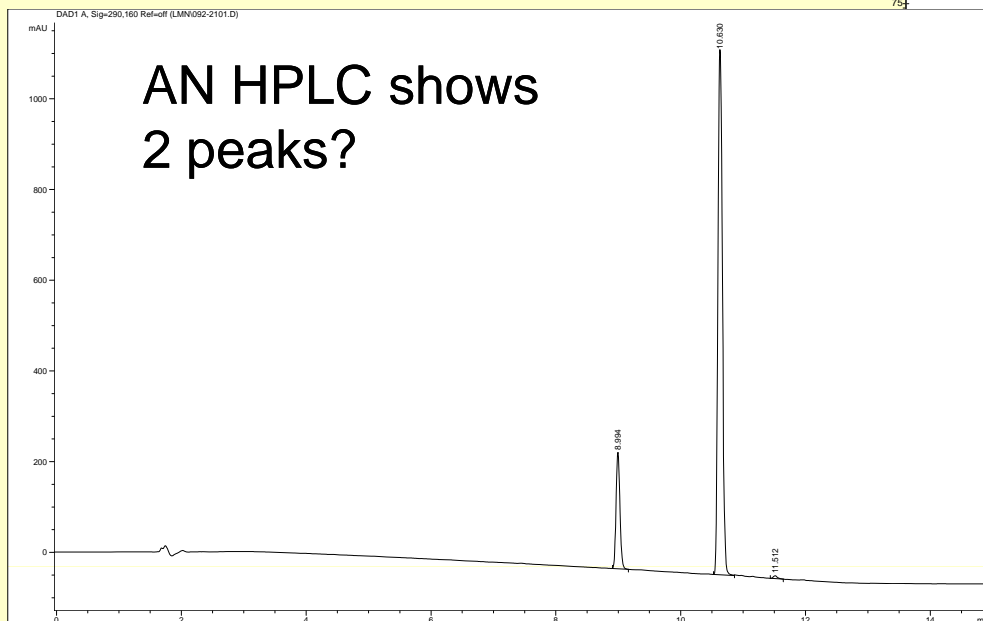
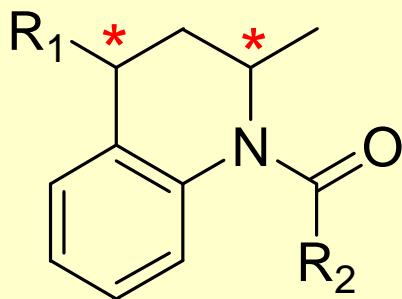
Diastereomer Separation on Ethylpyridine



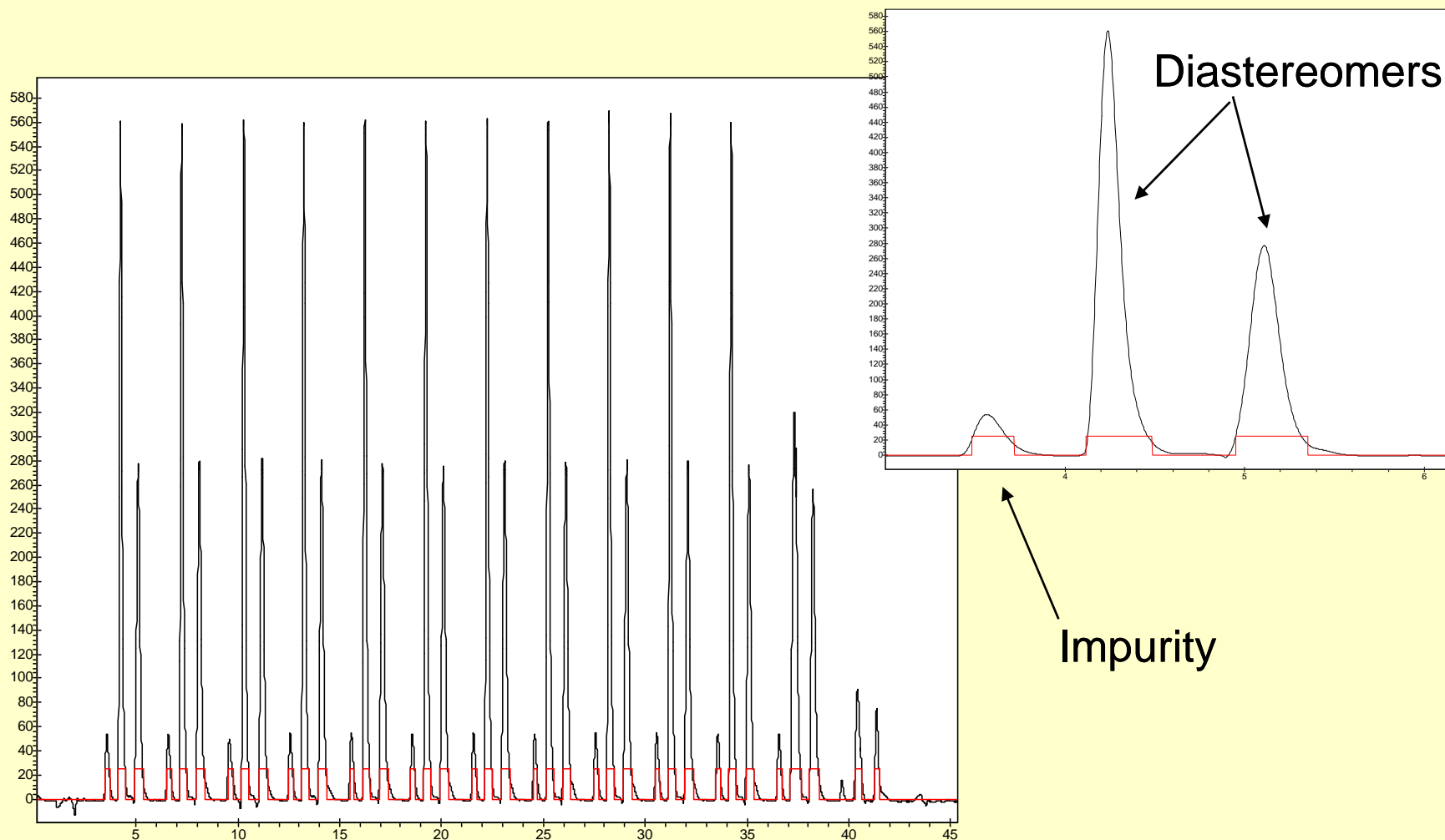
Achiral SFC Separations on Chiral Columns



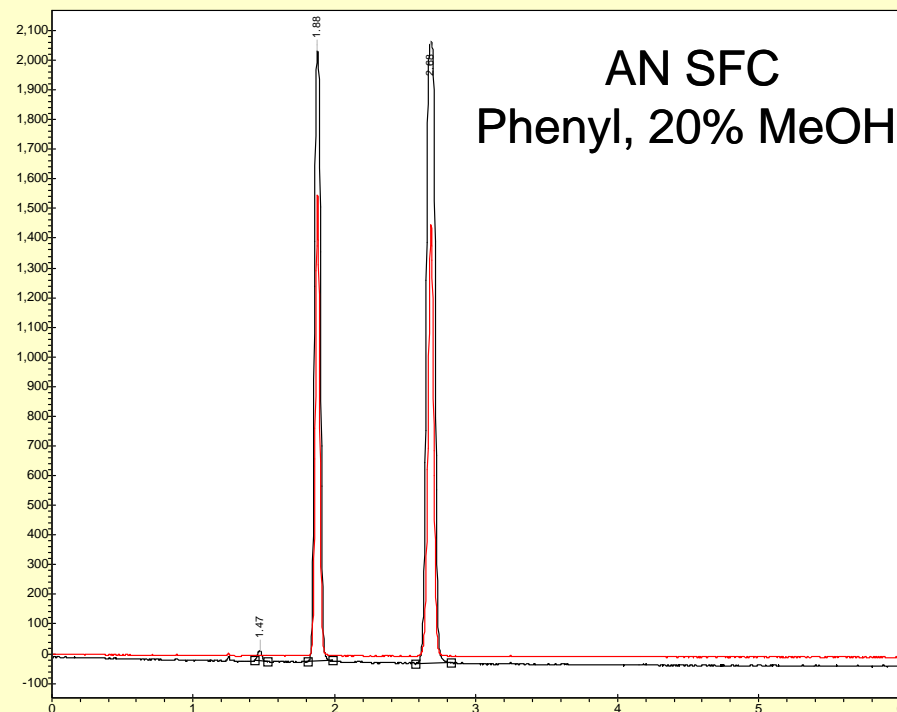
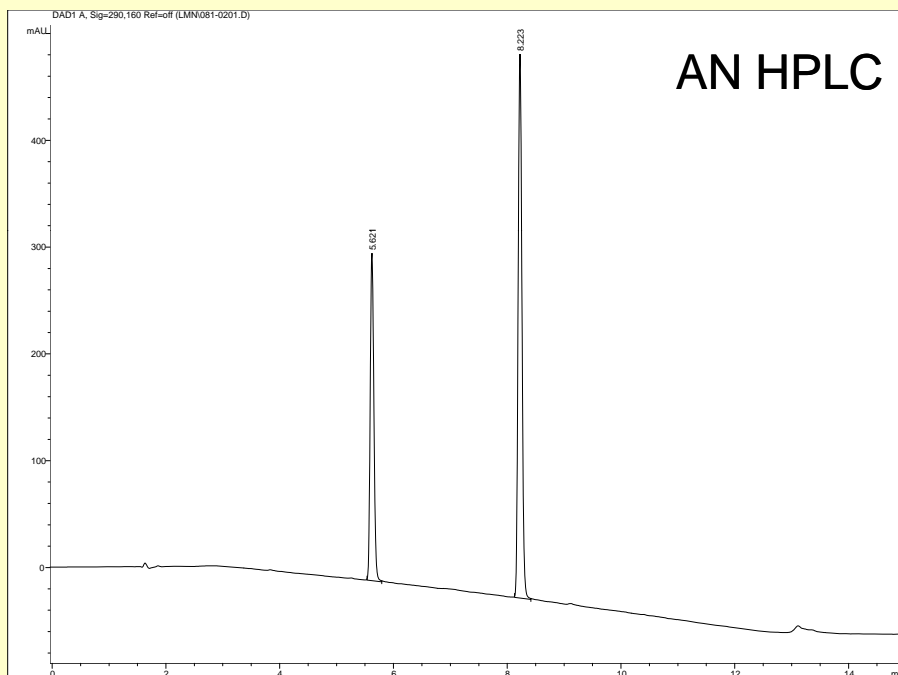
Diastereomer Separation w/o Separation of Enantiomers



Diastereomer Separation w/o Separation of Enantiomers on DIOL Column

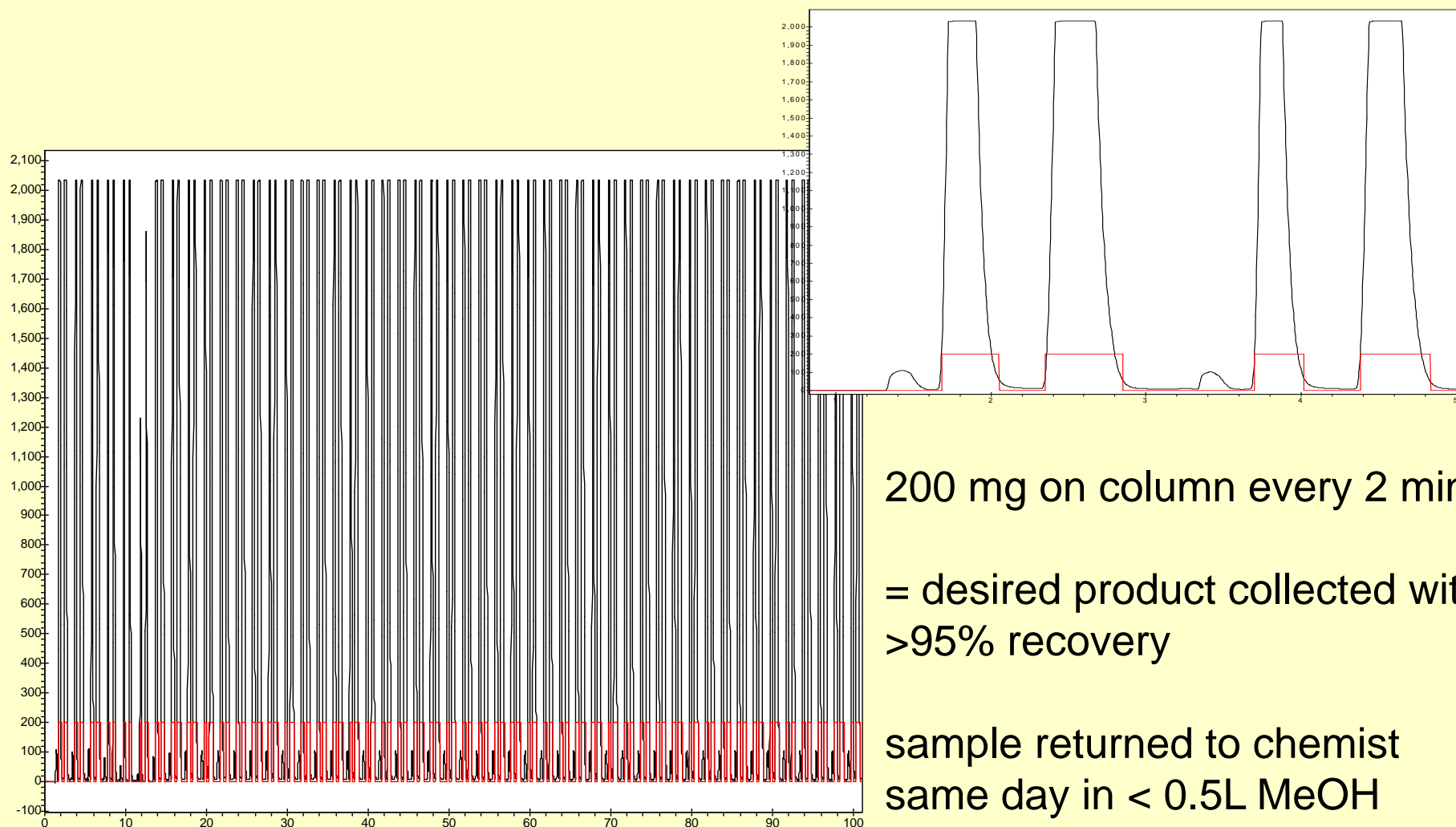


Incomplete Coupling Reaction for Purification - 10 grams and a tight deadline



While separation is readily achieved by either chromatographic method...

SFC Purification on Kromasil Phenyl - 10 grams in 105 min

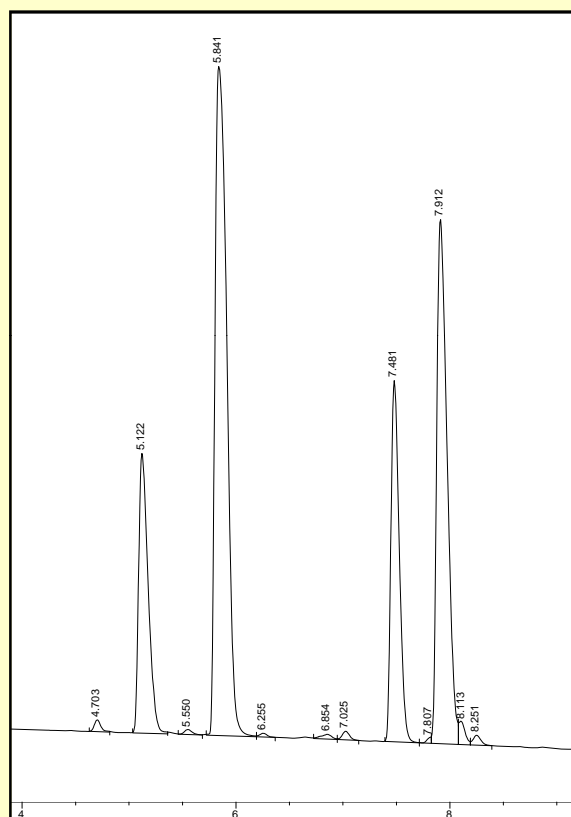


200 mg on column every 2 min

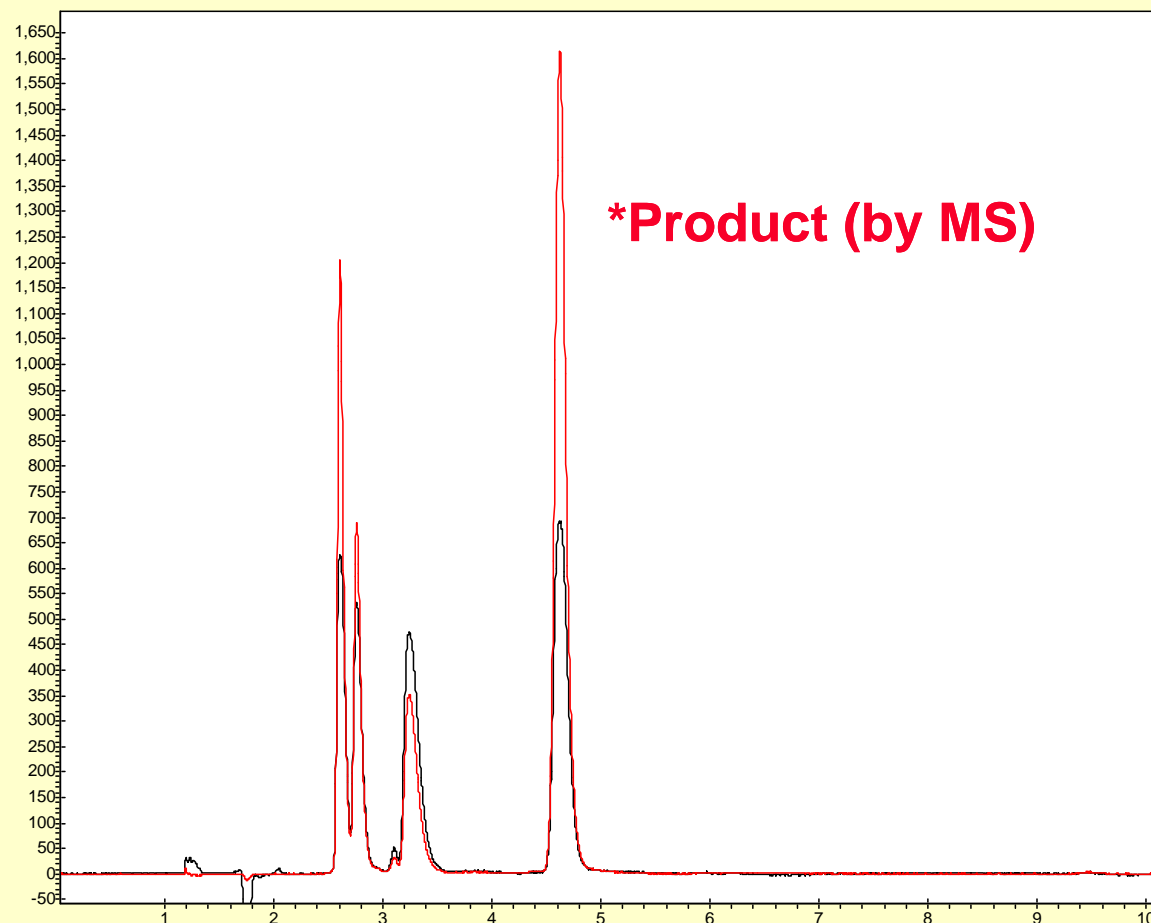
= desired product collected with
>95% recovery

sample returned to chemist
same day in < 0.5L MeOH

Synthetic Chemistry Gone Awry: SFC Purification of Crude Mixtures



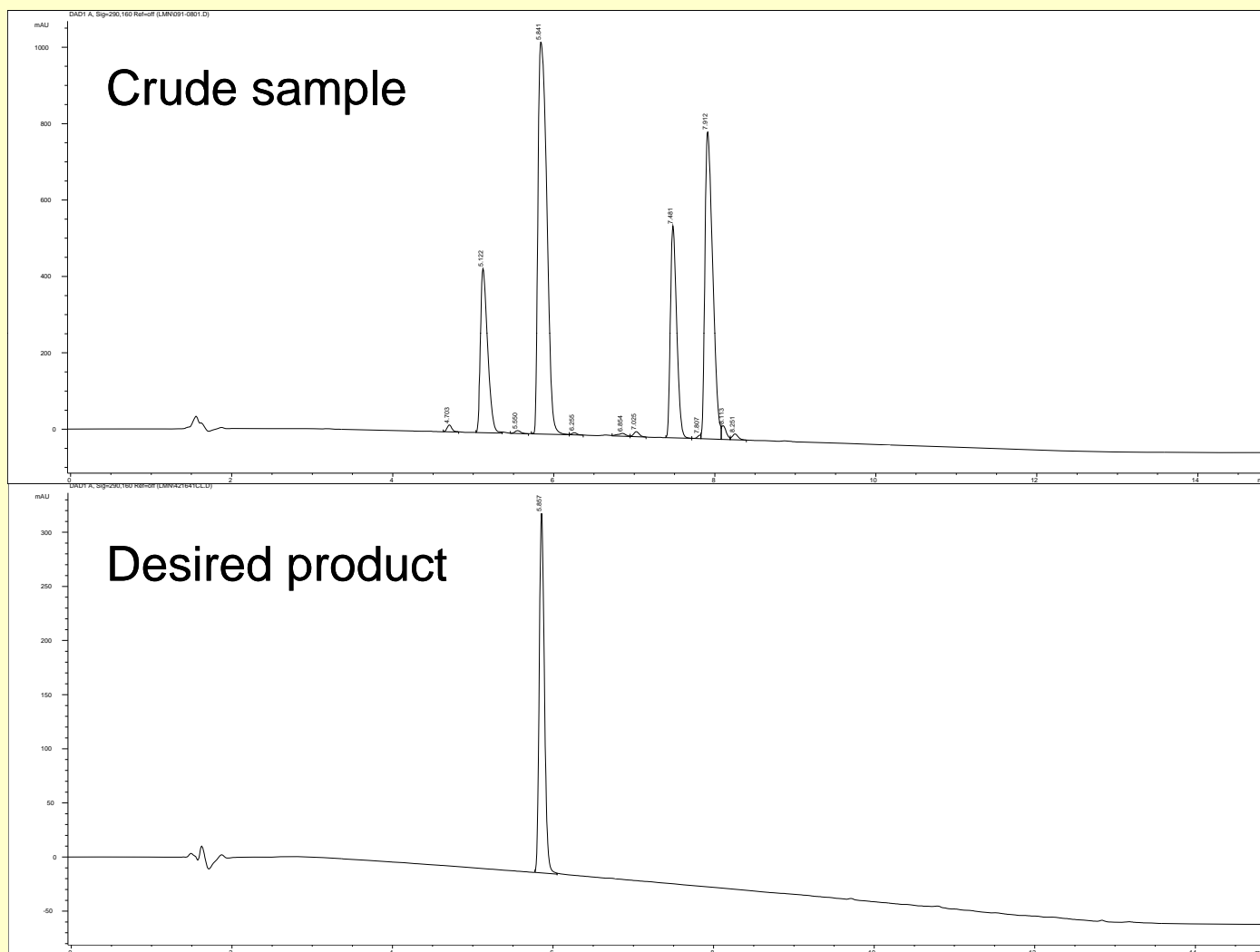
AN HPLC



***Product (by MS)**

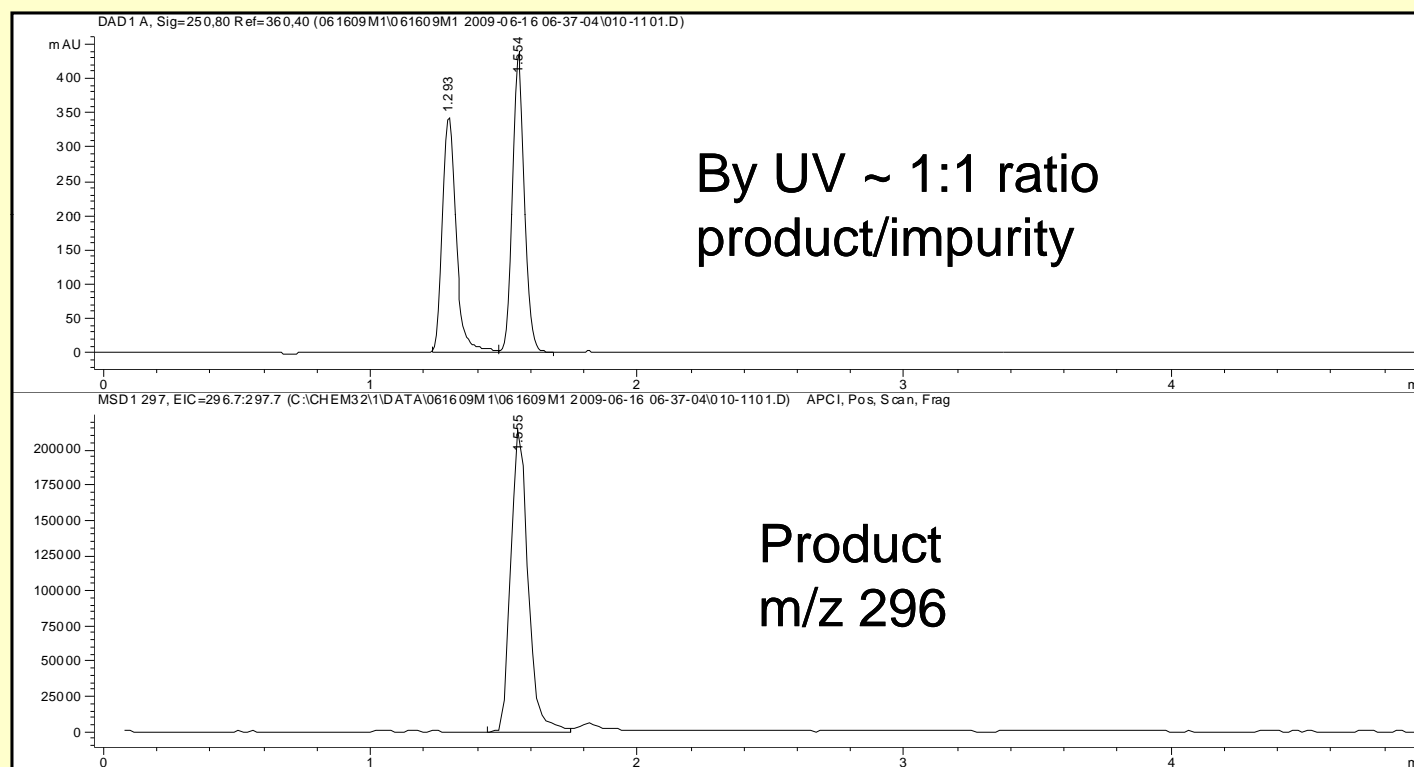
Prep SFC on 2-Ethylpyridine

AMES Purity Achieved via Prep SFC



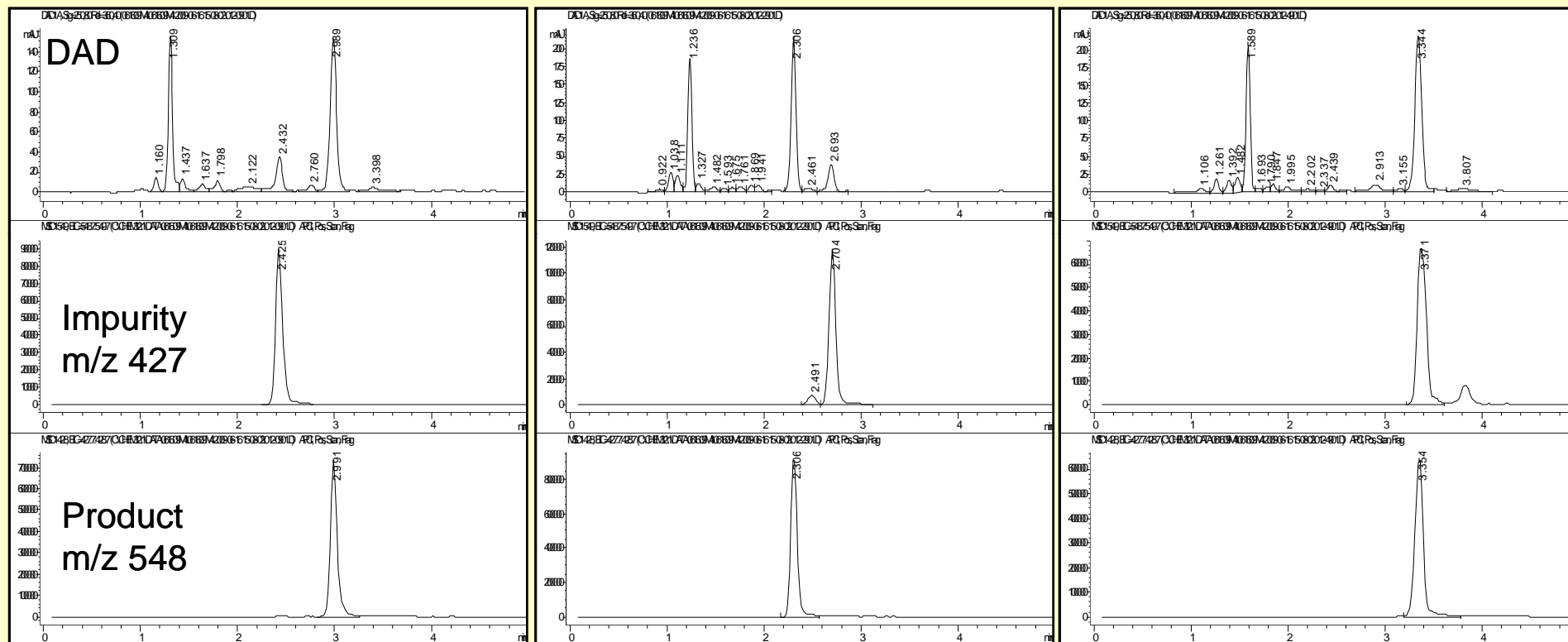
SFC/MS, because sometimes UV is not enough...

Sample xxx452



Kromasil Diol

Impurity detection by SFC/MS



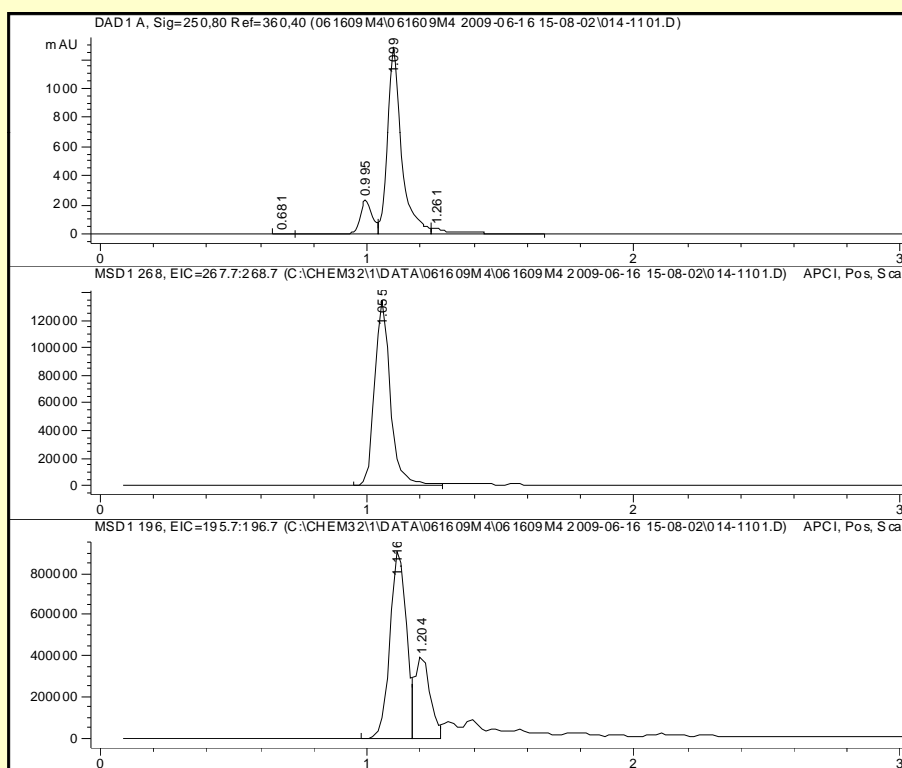
PTN-benzamide

KR-phenyl

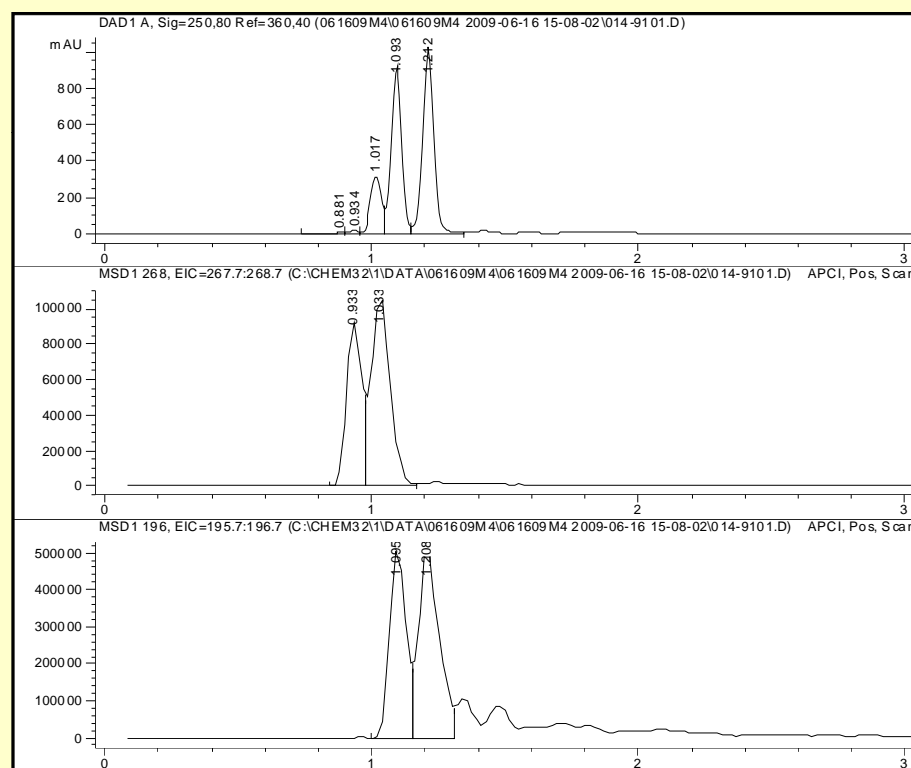
VAR-diphenyl

Isomer detection by SFC/MS

Sample xxx145 contained minor impurity but appeared to by an isomeric mixture by NMR. SFC/MS screening was used to determine presence of E/Z isomers that were able to be separated (not observed by HPLC)



PTN-benzamide



PTN-MeSAM

Conclusions

While not the “Universal” chromatographic technique, SFC has proven to be a powerful and complementary method to HPLC for performing both chiral and achiral separations.

Through screening of the many available stationary phases and implementation of hyphenated techniques such as SFC/MS and SFC/CD, SFC can be utilized as successfully as preparative HPLC in the drug discovery environment.

Acknowledgements - Support & Discussions

Wyeth Research

Senior Management: Oliver McConnell, Guy Carter and Tarek Mansour

Collegeville: Philip Garrett

Princeton: Michael Kagan, Michael Chlenov, Sergey Melnikov

Cambridge: Peter Tate, Nelson Huang

Pearl River: Mark Tischler, Mairead Young

Collaborators

Yingru Zhang (BMS)

Christina Kraml (Lotus Separations)

Bill Watts (Chiral Technologies)

Terry Berger, Dave Boulton, Kimber Fogelman, Bob Nairn, Rick Wikfors (Aurora SFC Systems)