Investigating the Use of Achiral SFC as an Evolving Tool in the Support of Discovery Chemistry at Novartis

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Outline of Presentation

- Backgound of SFC at NIBR
  - Cambridge support
- Normal Phase LC support
  - Single and paralell techniques
- Achiral SFC support
  - Why SFC?
  - SFC vs. NPLC
- SFC for OA Rxn Monitoring

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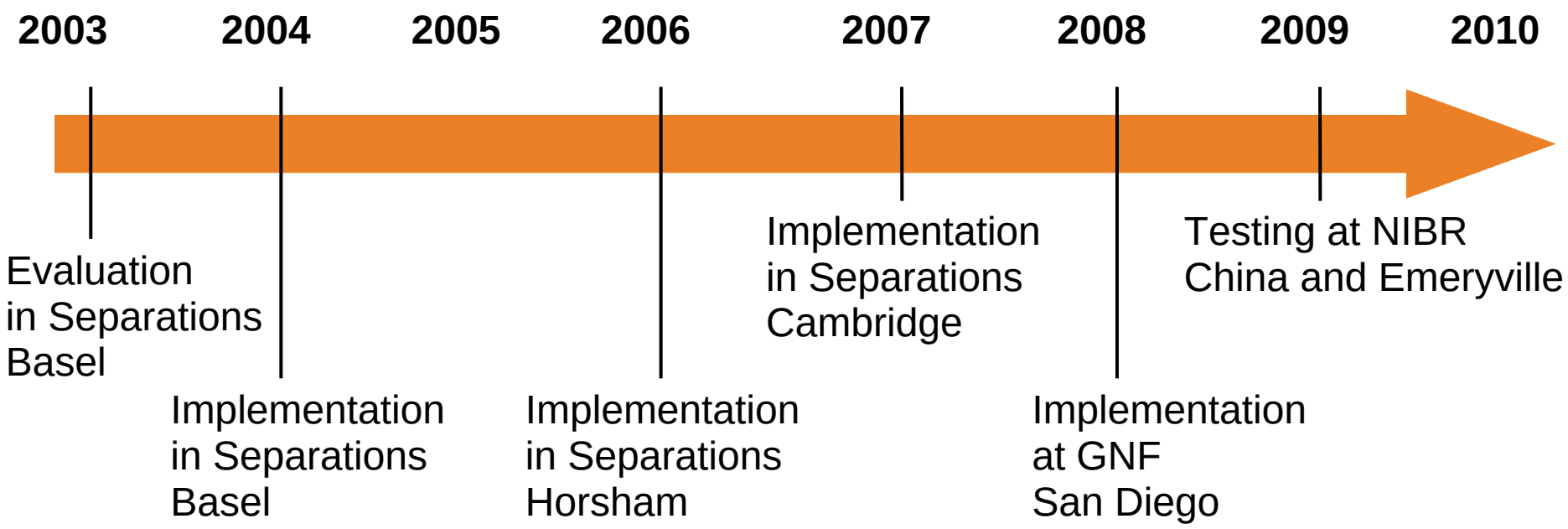
SFC at NIBR Global (Chiral Separations)



- 2 Analytical systems: 1 Berger and 1 Thar Investigator
- 1 SFC-200 Prep System

- 1 Analytical /semi-prep system Investigator

- 2 Analytical systems: Investigator
- 2 Bench top SFC 70 Prep System

- 1 Analytical /semi- prep system

- Testing

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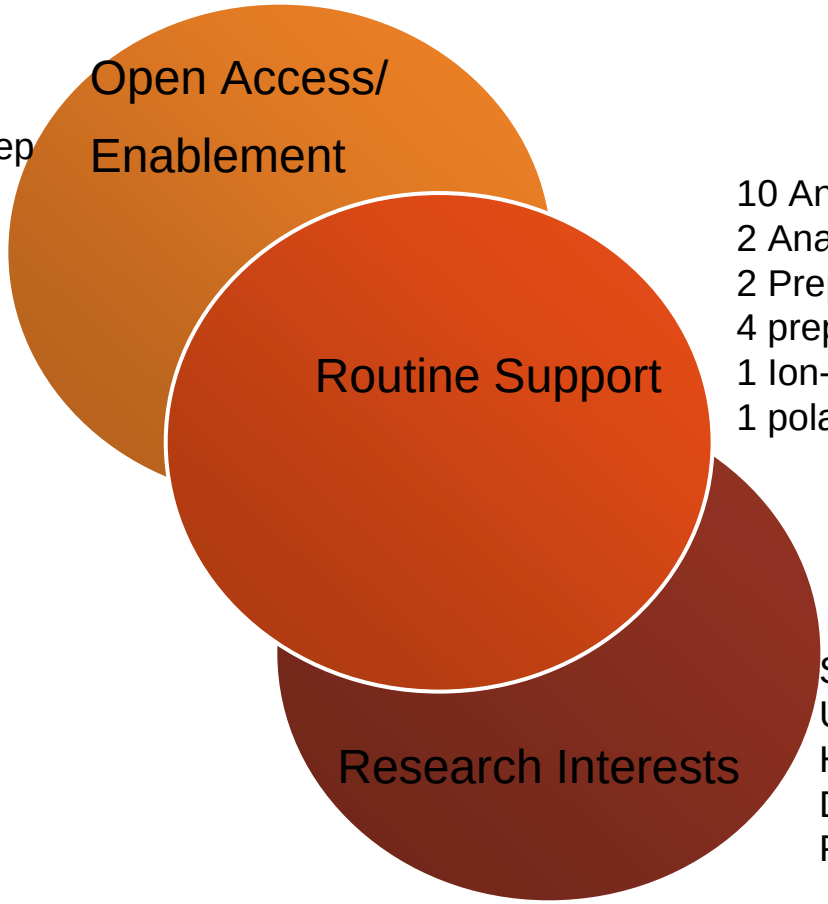
GDC Separations Lab -Our Role

Chiral and Normal Phase Separations AND GDC PROJECT SUPPORT



1 LC/MS Prep
2 LC/UV
1 UPLC-MS

10 Analytical HPLC
2 Analytical SFC
2 Prep SFC
4 prep HPLC
1 Ion-chromatography system
1 polarimeter

SFC/MS
UHPLC/UV or MS
HPLC/SPE/NMR
Data Tracking and Delivery (AWM)
Parallel chromatography

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Separations Group NIBRI Cambridge

- Supports the discovery chemistry groups in Cambridge
  - 170 Chemists working in 2 sites
  - An imbeded part of the chemistry department
- 3 members split across 2 buildings
- Service based group
  - Take on difficult separations
  - Develop enabling technologies
- Predominately chiral support via SFC and LC
  - Chemistry mandate to use more NPLC internaly

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Normal Phase Separations Support

- Increasing number of requests for analytical and preparative separations
- SFC separations 2010
  - Analytical separations: 41
  - Preprative separations: 17 (50 - 2g+)
- Shifting from LC to SFC methods in progress
  - All current submisions are being run SFC first, traditionaly run NPLC
- SFC provides many advantages
  - Faster runs and turn around times
  - Lower solvent usage
  - Integrated into Chiral screens

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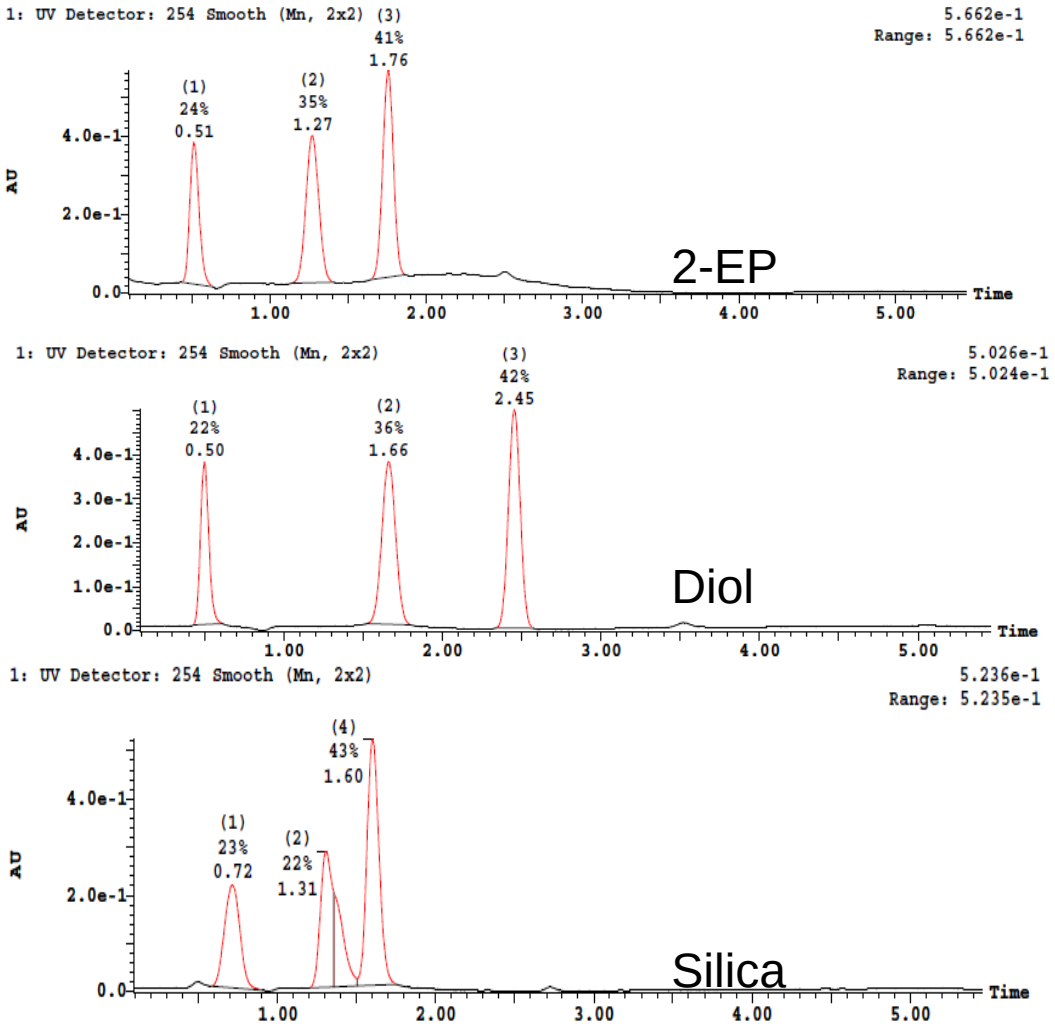
Single column NPLC systems



- Open access chemistry support tool
- Silica, Cyano, Diol, 2-Ethylpyridine, Amino, Phenyl columns
- 4.6 x 100mm, 5um
- 1-3 ml/min
  - Hep/EtOH
  - 0.2% DEA
  - 5-60% Gradients
- 5-10 min runs

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Parallel NPLC System

- 8 columns run in parallel (1.25min / col. / inj.)
- 4.6 x 100mm, 5um, 10 min run times
  - 1.25ml/min flow rates
- Silica, Cyano, Diol, 2-EP, Amino, Phenyl, PVA-Sil, other
  - Same systems as Chiral LC screen, automatic switching of columns via contact closures
- Gradients with Hep/(EtOH/IPA), +/- 0.2% DEA
  - After development in parallel final methods established via single column systems
- Prep systems available are aslo used for chiral work

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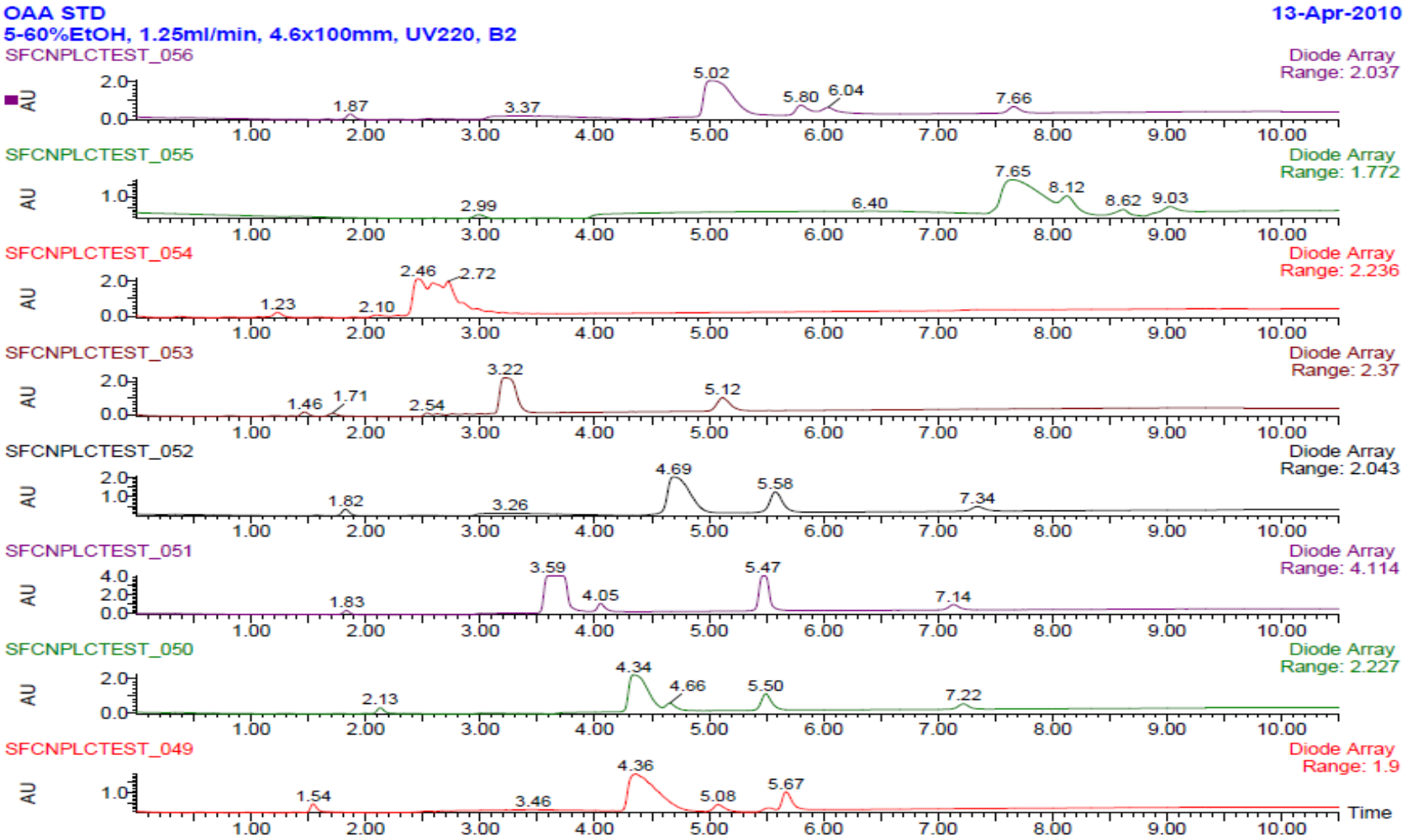
Parallel NPLC Output



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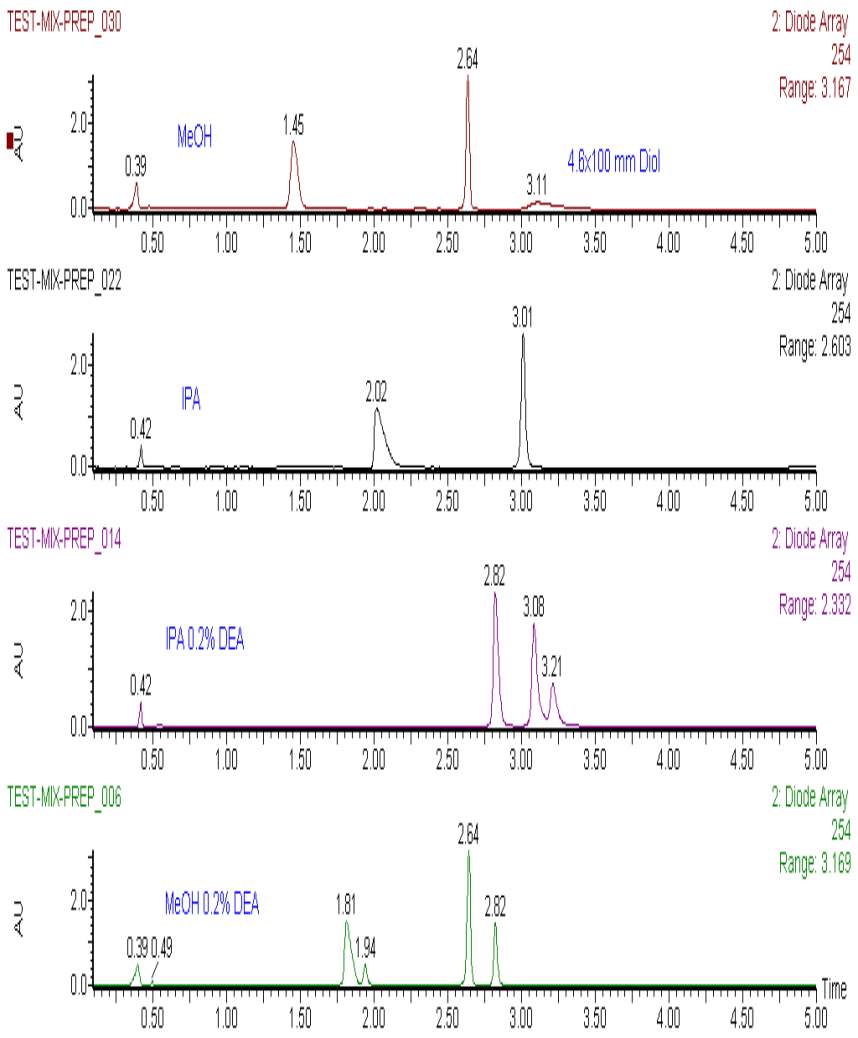
Why use SFC for Prep of Achiral samples?

- SFC is on average 3 times faster than HPLC
  - *1 SFC Instrument does what 3 HPLC instruments do*
- Solvent costs reduced by 60-70 %
  - *1 Liter CO2 = ~ 1 $*
  - *1 Liter Acetonitrile= ~ 20-30 $ (around 50‘000 L/year)*
  - *1 Liter Heptane = ~ 20 $ (around 10‘000 L/year)*
- Solvent removal reduced by about 70 % (CO2 evaporates)
- Less risk of degradation of purified compound
  - SFC does not need acidic additive in the mobile phase
- Reduced organic solvent consumption: Green chromatography
  - *Critical VOC situation can be improved*
  - *Solvent restriction issue, especially in Boston area*
- Safety: CO2, main component of the mobile phase (60-90%), is non flammable (fire extinguisher) and is much less toxicity

SFC Screening Process



- Premade sample lists
- Gradients 5-55% Modifer
- MeOH and IPA
  - 0.2% DEA or no modifier
- Princeton Chromatography Columns
  - 4.6 x 100 mm
  - Silica, CN, Diol, 2-EP
- 5ml/min, 6min runs
- 4 columns x 4 solvent systems

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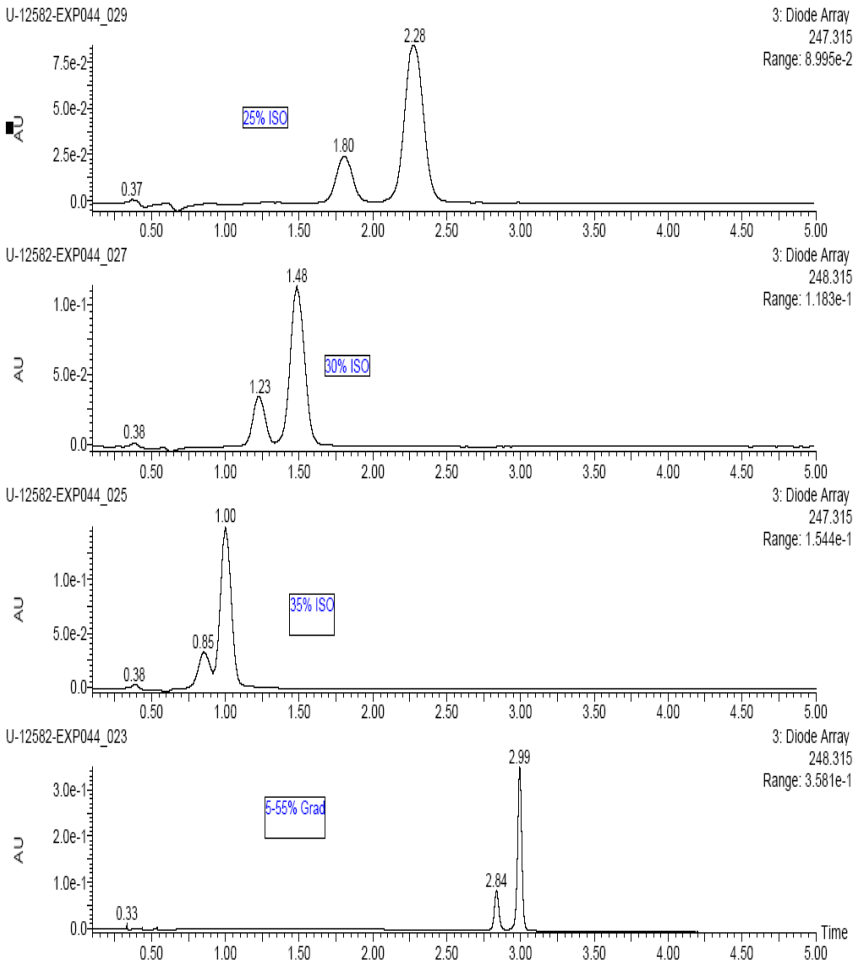
SFC Screening Output



- Screening allows for rapid selection of:
  - Columns
  - Solvents
- Isocratic methods quickly selected and tested
- Integrated into Chiral workflow and screens
  - Achiral part of Chiral screening process
  - Avoids suprises

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Prep SFC at NIBRI

- Thar 80 systems (2)
  - Single channel analytical system with 10 column oven (2)
- BDS CO2 delivery system
- Princeton Chromatography Columns
  - 20 x 150mm, 5um
  - Silica, Cyano, Diol, 2-EP
- Find we can run 5-10% less solvent than analytical runs and get similar separations
  - May result in longer run times but better separations
- Potential to stack injections or run Prep gradients

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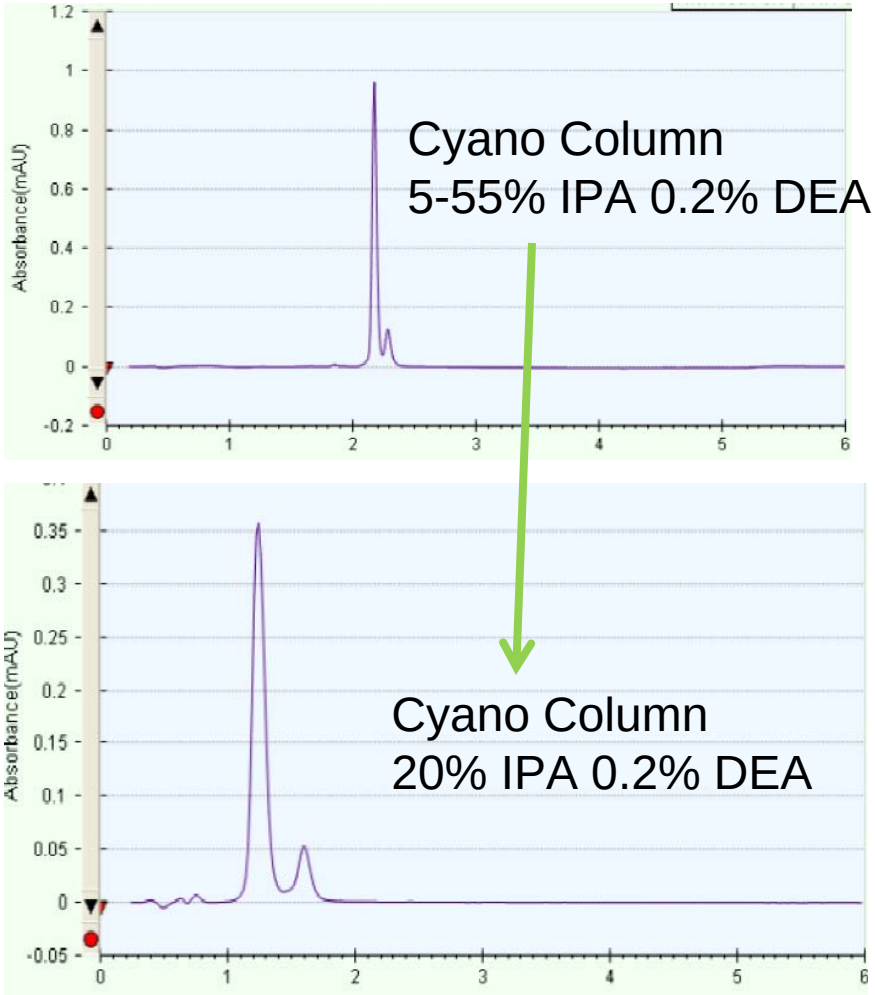
SFC vs NPLC Prep Separation Comparision

- How does it compare using a real sample?
- 2.8 g of sample submited
- Small impurity to remove
- Split into 2 batches
- MD was run on both SFC and NPLC simultaneously
- Princeton Cyano column used in SFC
  - 20 x 150mm 5um



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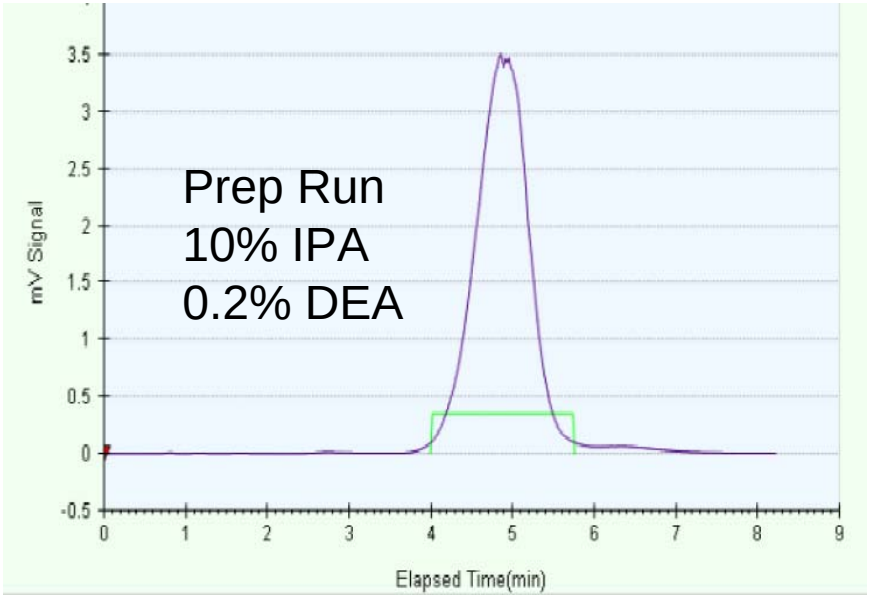
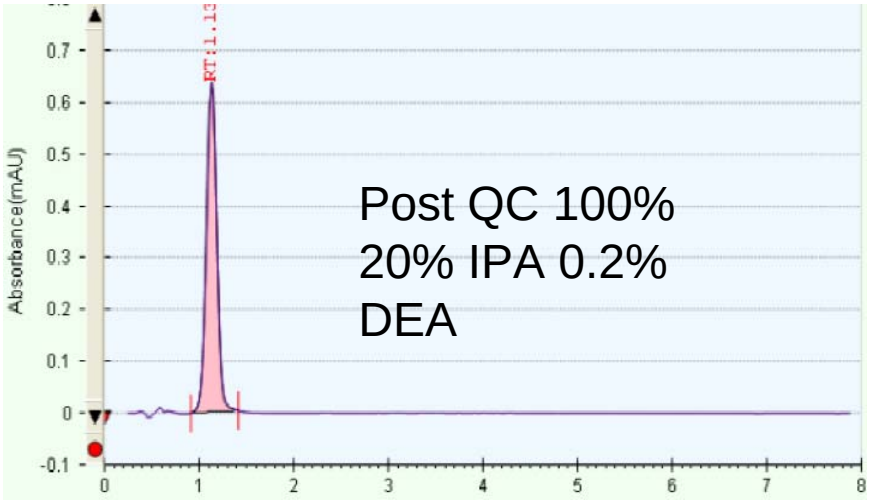
SFC Prep Separation of Sample





- 78 inj. of 2ml of MeOH
  - ~16mg/inj.
- Run time 8 min. / inj.
  - 10.4 hrs total run time
- 70g/min flow rate
- 10% IPA 0.2% DEA
- 4.4L Solvent Used
  - ~1100ml of fraction collected
- 100% pure, 1.45g recovery

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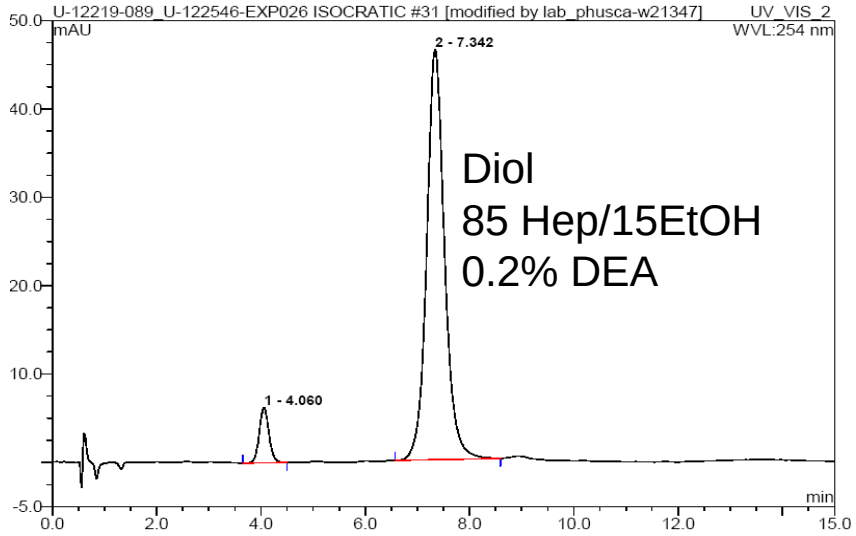
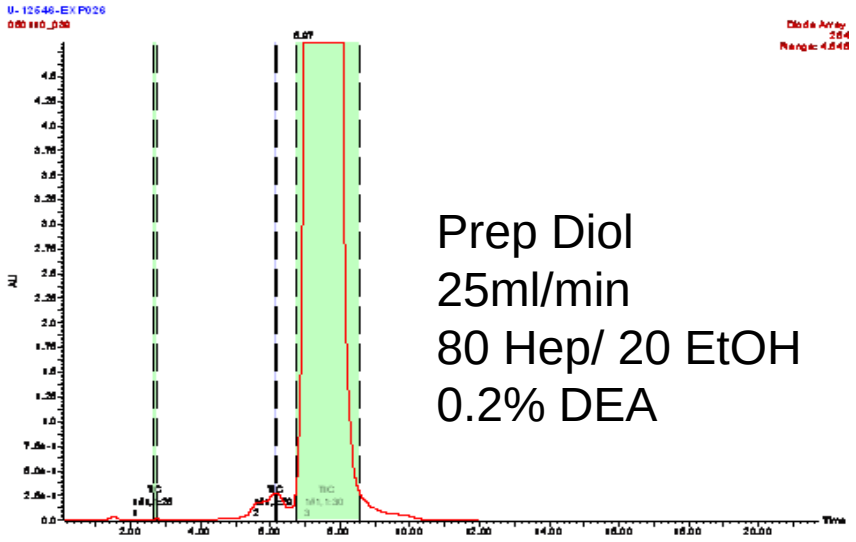
NPLC Separation of Sample





- Princeton Diol column
- 82 inj of 1ml EtOH
  - ~15mg/inj.
- Run time 12min
  - 16.4 hours of run time
- 25ml/min flow rate
- 24.6L solvent used
  - 4.1L of fractions
- 1.14g recovery, 98%+

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Highlights of SFC vs NPLC

- SFC was 32% faster in total run time
- Both had roughly the same loading and number of injections made
  - NPLC was run over 3 days, SFC completed in 1 day plus PM run
- SFC collected 75% less solvent
- **SFC used 18% of the solvent of NPLC**
- Recovery effected by division of sample possibly not being equal
- Both resulted in high purity samples

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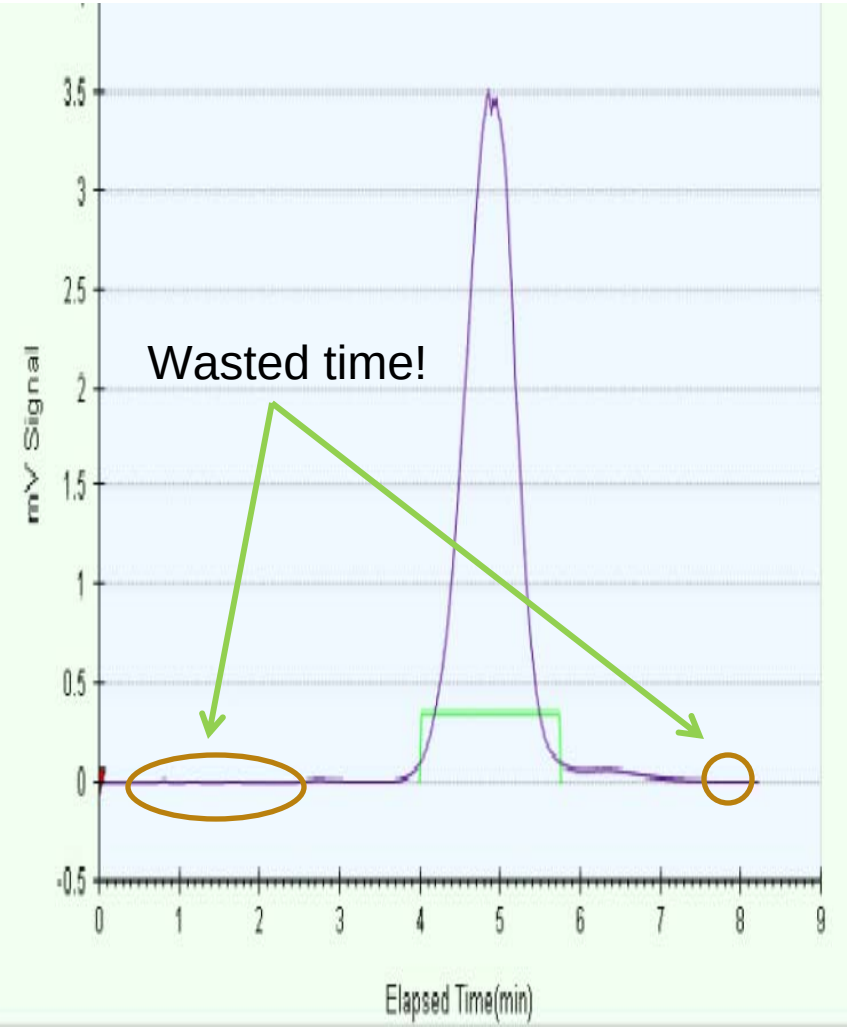
Could the SFC have been faster?



- If this sample had been stacked how much faster would it have been
- 3 minutes blank time
  - ~30% faster if stacked
- Stacking has the potential to save
  - ~3hrs total run time
  - ~1.2L of solvent
- Stacking sets up the same as in chiral separations

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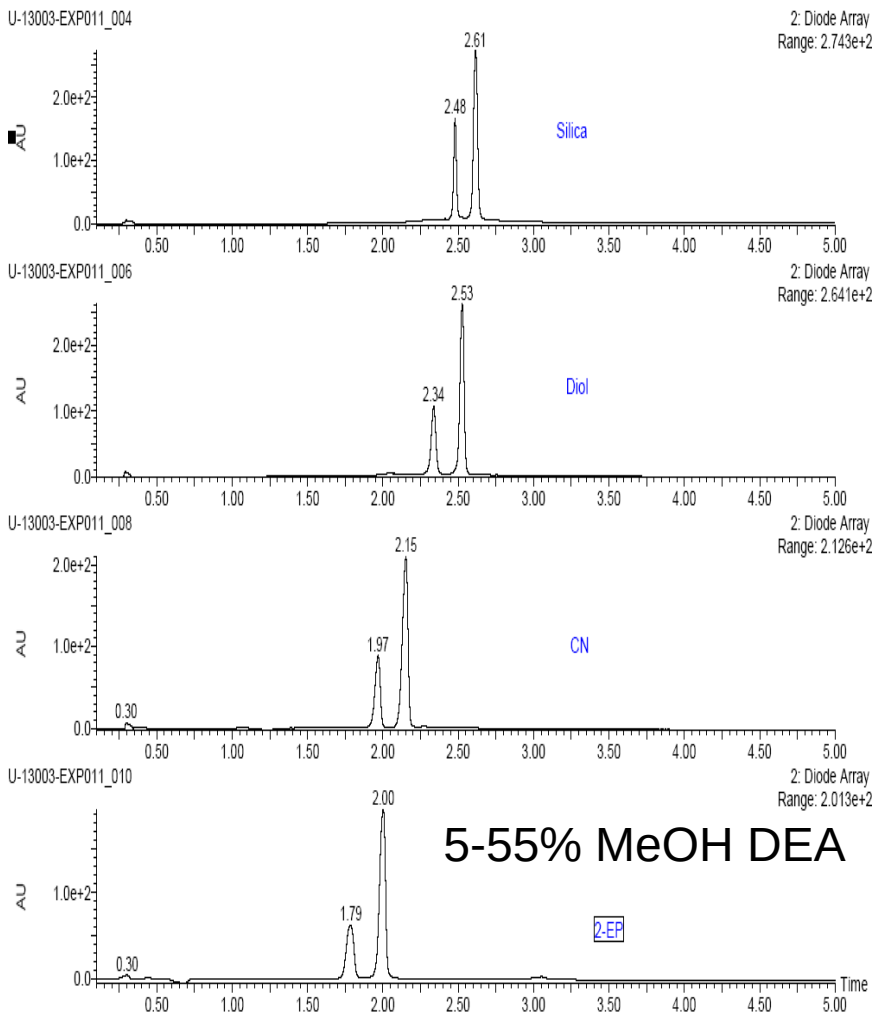
SFC Prep Separation Example 2



- 140mg submited
- Chemist had made several attempts to work up
- Run through intial screen
- Succesfully separated on 13/16 combinations using gradients of 5-55%
- 2-EP column selected for Prep runs
  - MeOH without modifier

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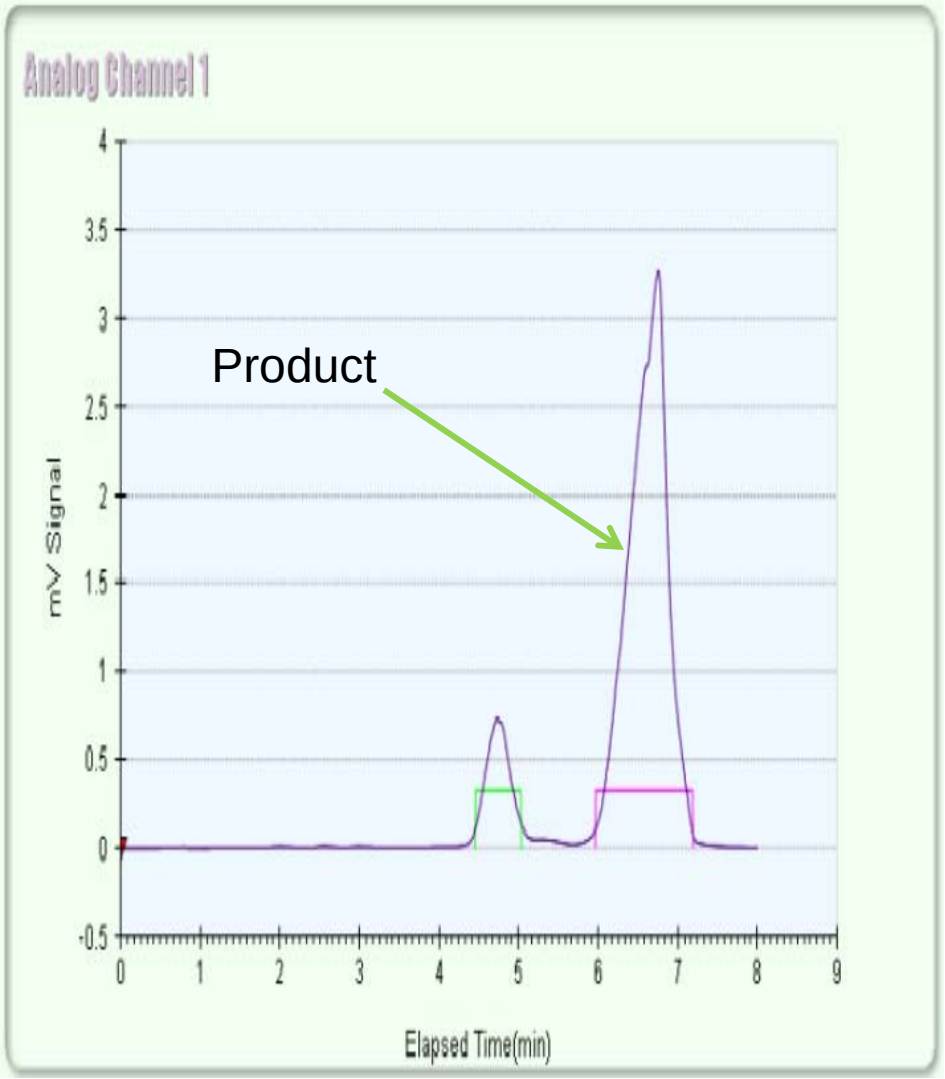
SFC Prep Separation Example 2 Cont.



- 10 injections
  - 14mg / inj.
- 65g/min flow rate
  - 10% MeOH
- 80 min total run time
  - 8 min cycle time
- 600ml of solvent used
  - F1 ~40ml collected
  - F2 ~80ml collected
- 100% pure product

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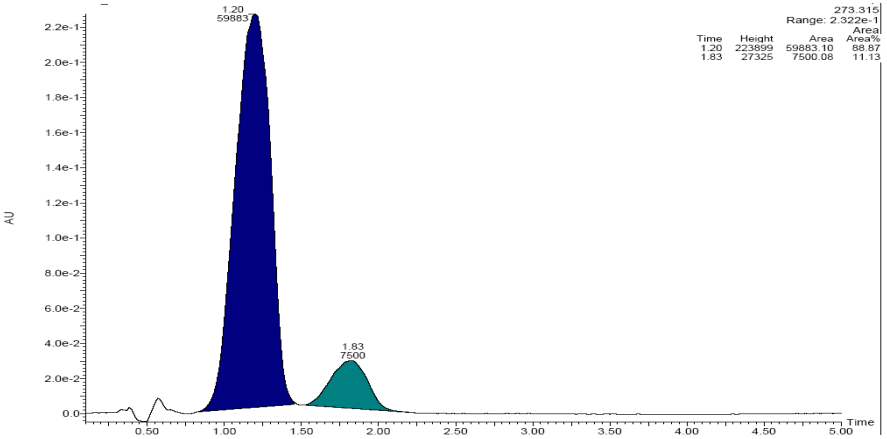
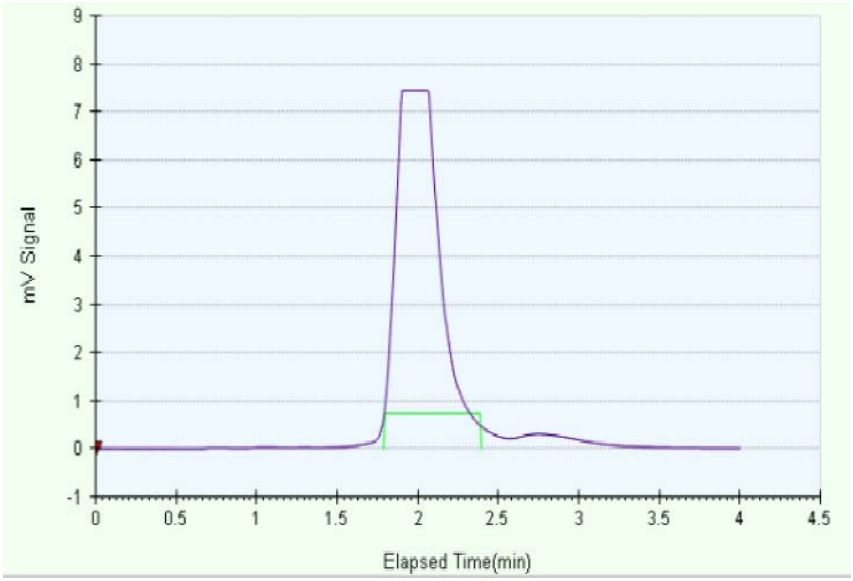
SFC Prep Separation Example 3





- 180mg submitted
  - 17 inj of 10-11mg
- Analytical Method
  - Diol 4.6 x 100mm
  - 40% IPA 0.2% DEA
- Prep Method
  - Diol 20 x 150mm
  - 35% IPA 0.2% DEA
- 4 min. run, 70g/min
- Totals
  - Time 68min
  - ~1.6L solvent
  - ~400 ml fraction

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OA-SFC as a complimentary approach to Reaction monitoring

- Is there an optimum mobile phase?
- Is there a best column chemistry / manufacturer
- Will the data be as rich as reversed phase?
- Are we more likely to precipitate?
- Can we make the interface as friendly as LC-MS
  - Mass lynx OA software on Thar system
- How do we interpret the data when we see differences

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SFC OA conditions

- Column 1: 3mmx 50 mm ES Industries 2-EP 5 micron
- Column 2: 3mmx 50 mm ES Industries Chromegabond NPI 5 micron
- 10mM ammonium formate in MeOH
- 10% to 40% in 1.5 minutes hold for 0.5 minutes.
- Total flow-3 ml/ min, ~1mL of MeOH per injection.

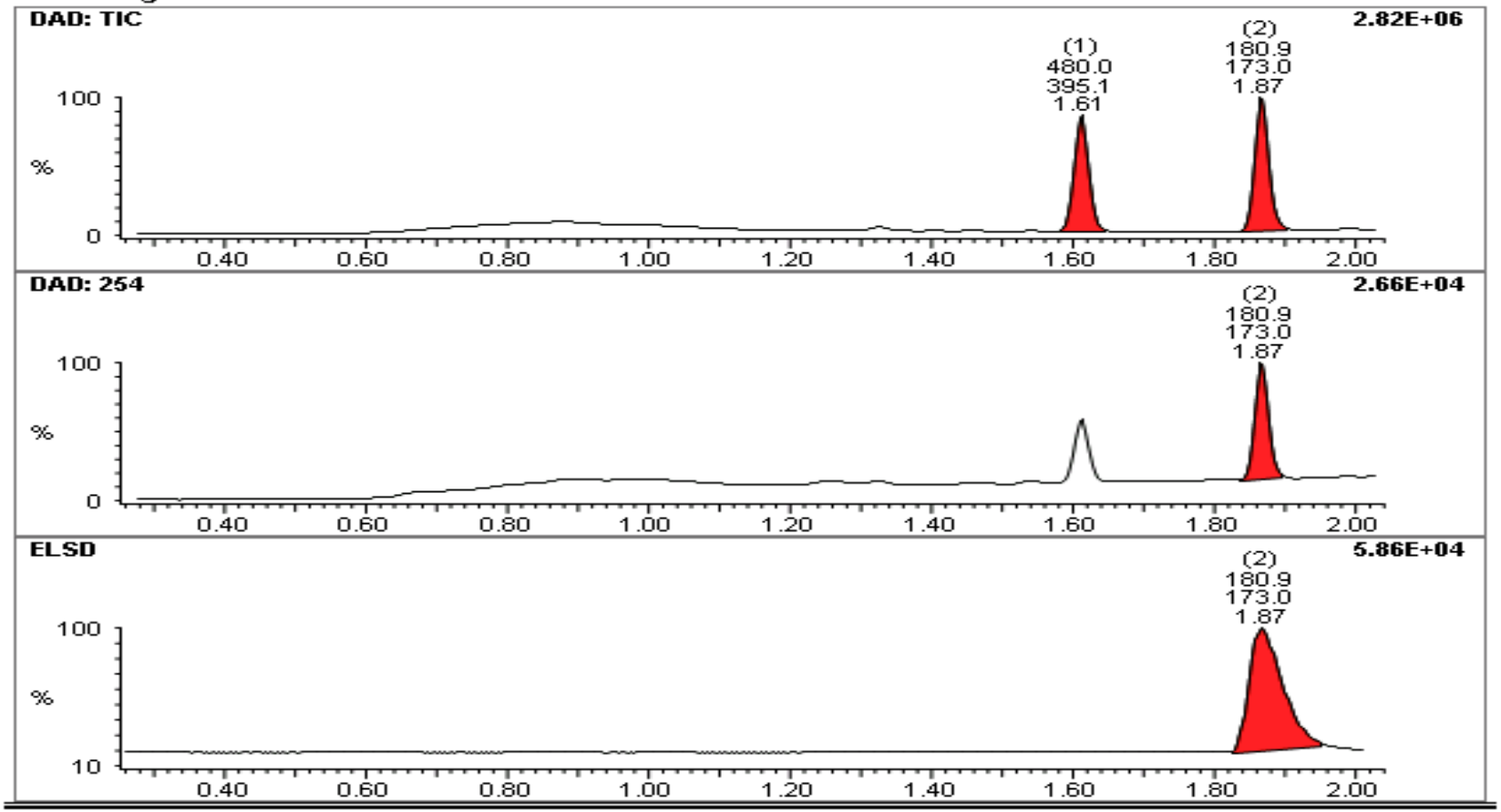
RP OA conditions

- Column 1: 3mmx 33 mm GL Sciences Inertsil C8 3 micron
- Column 2: 3mmx 33 mm GL Sciences Inertsil C18 3 micron
- 5mM ammonium formate and MeOH:ACN
- 5% to 95% in 1.6 minutes hold for 0.4 minutes.
- Total flow-2 ml/ min, ~5mL of solvent per injection.

RP analysis on C8 2mL/min

Method: **INERTSIL333_C8_NPH_2ml** Date: **13-Apr-2010** Time: **15:25:50**
Notes:

Chromatograms:



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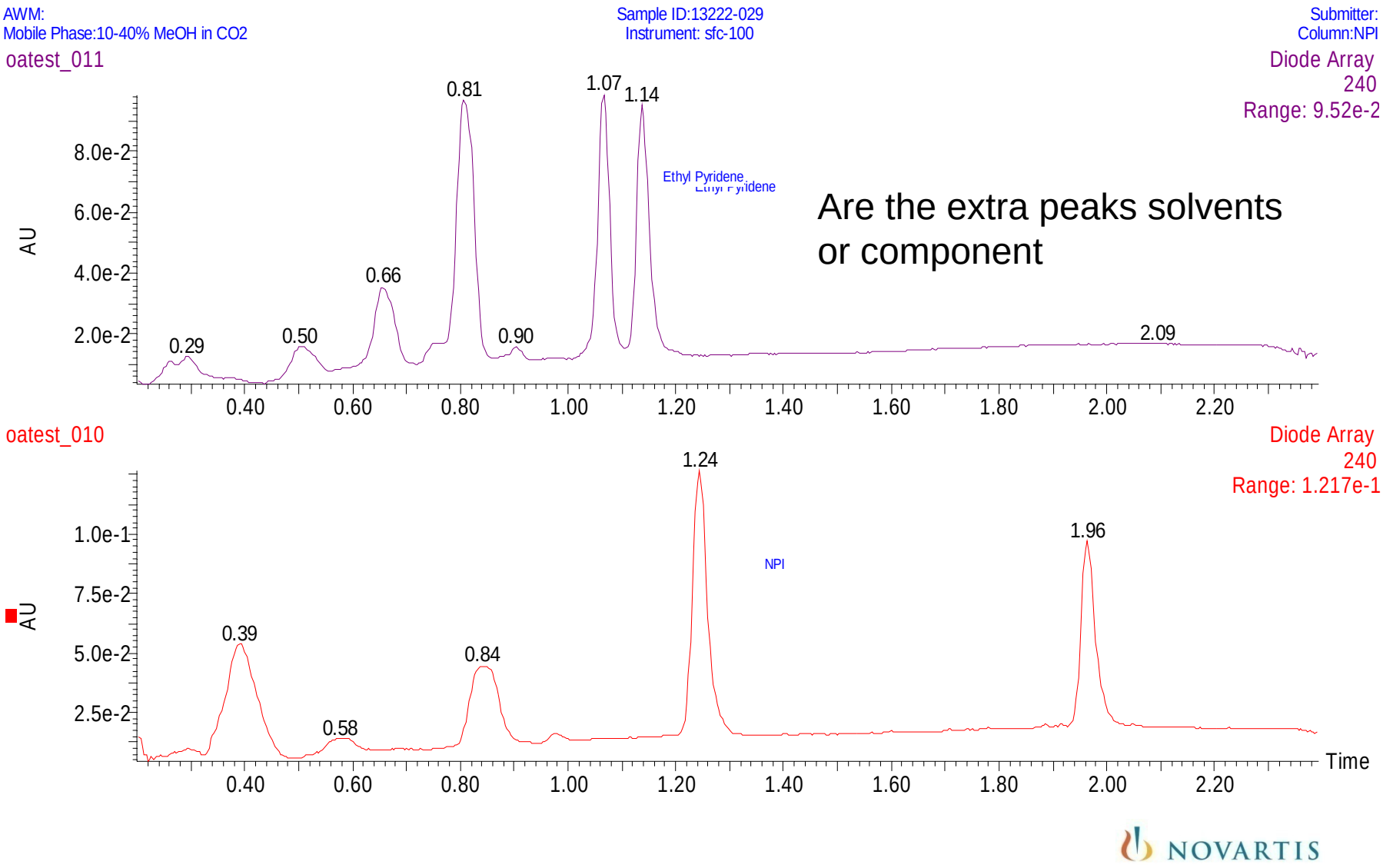
SFC-UV Analysis of previous sample



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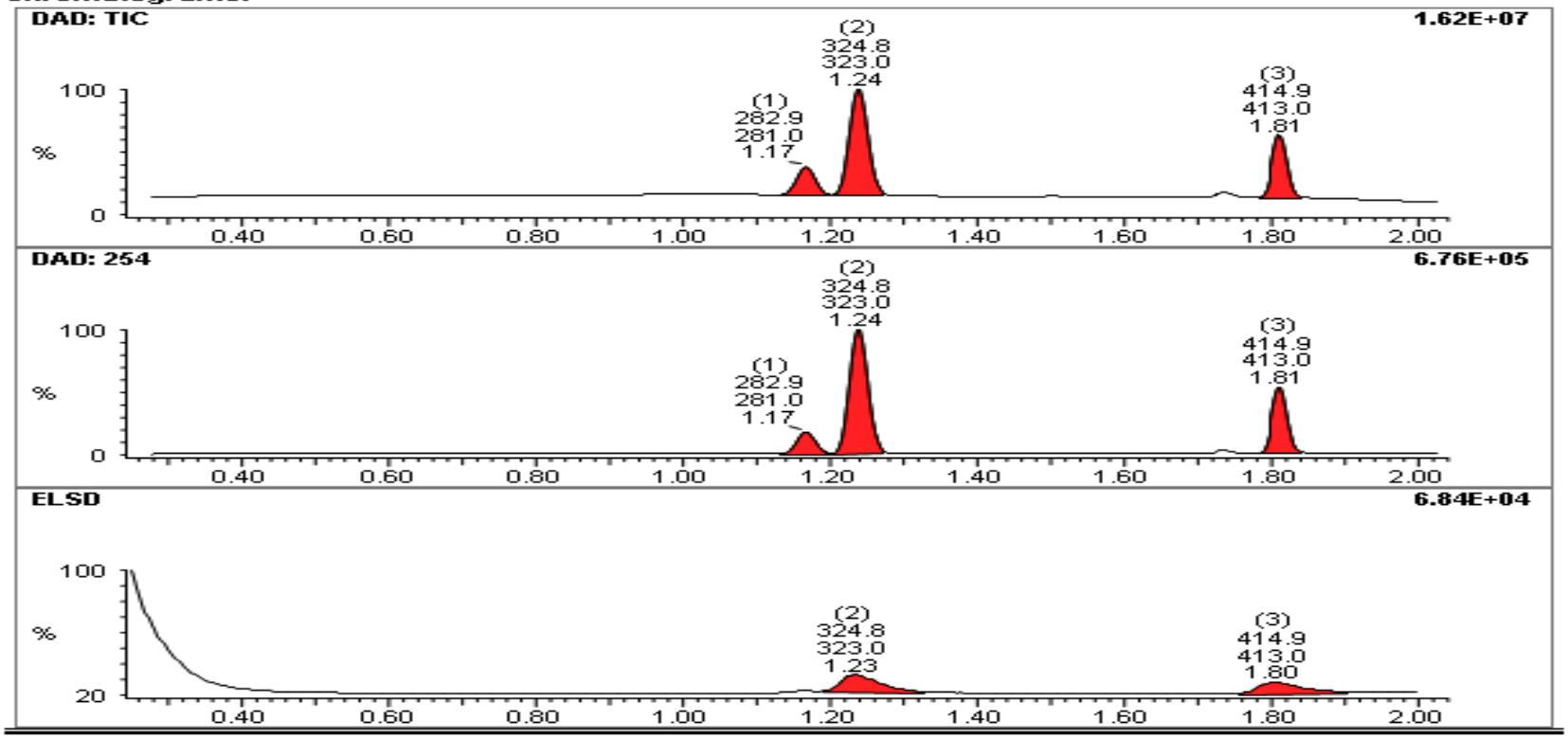
RP Analysis on C18 polar gradient

Method: **333_C18_APH_POLAR_2ml** Date: **14-Apr-2010** Time: **12:01:09**
Notes:

Chromatograms:



Peak Table:

#	Time	BPM+	BPM-	UV	ELSD	Target
1	1.17	282.87	280.96	10.8	0.0	No
2	1.24	324.83	322.96	61.7	60.7	No

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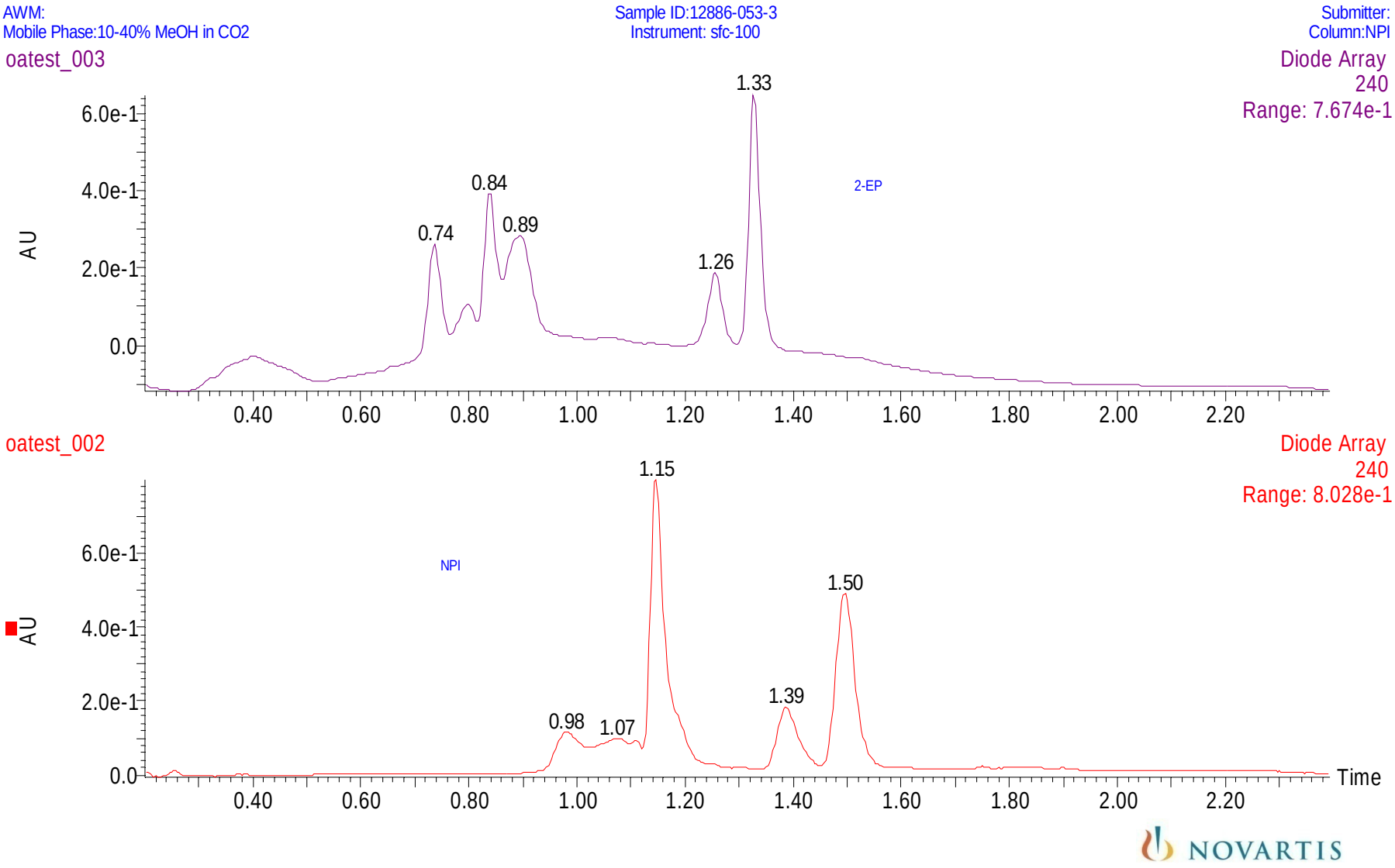
SFC-UV Analysis of previous sample



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SFC for OA Reaction Monitoring Summary

- Potential to add valuable information
- ~20% of solvent used compared to RP
- No single column chemistry is best.
- Instrumentation Robust enough?
- Instrumentation is large
  - Space available for OA instruments is limited
- BPR stability could be an issue
- We will move forward with our partners in A.S.

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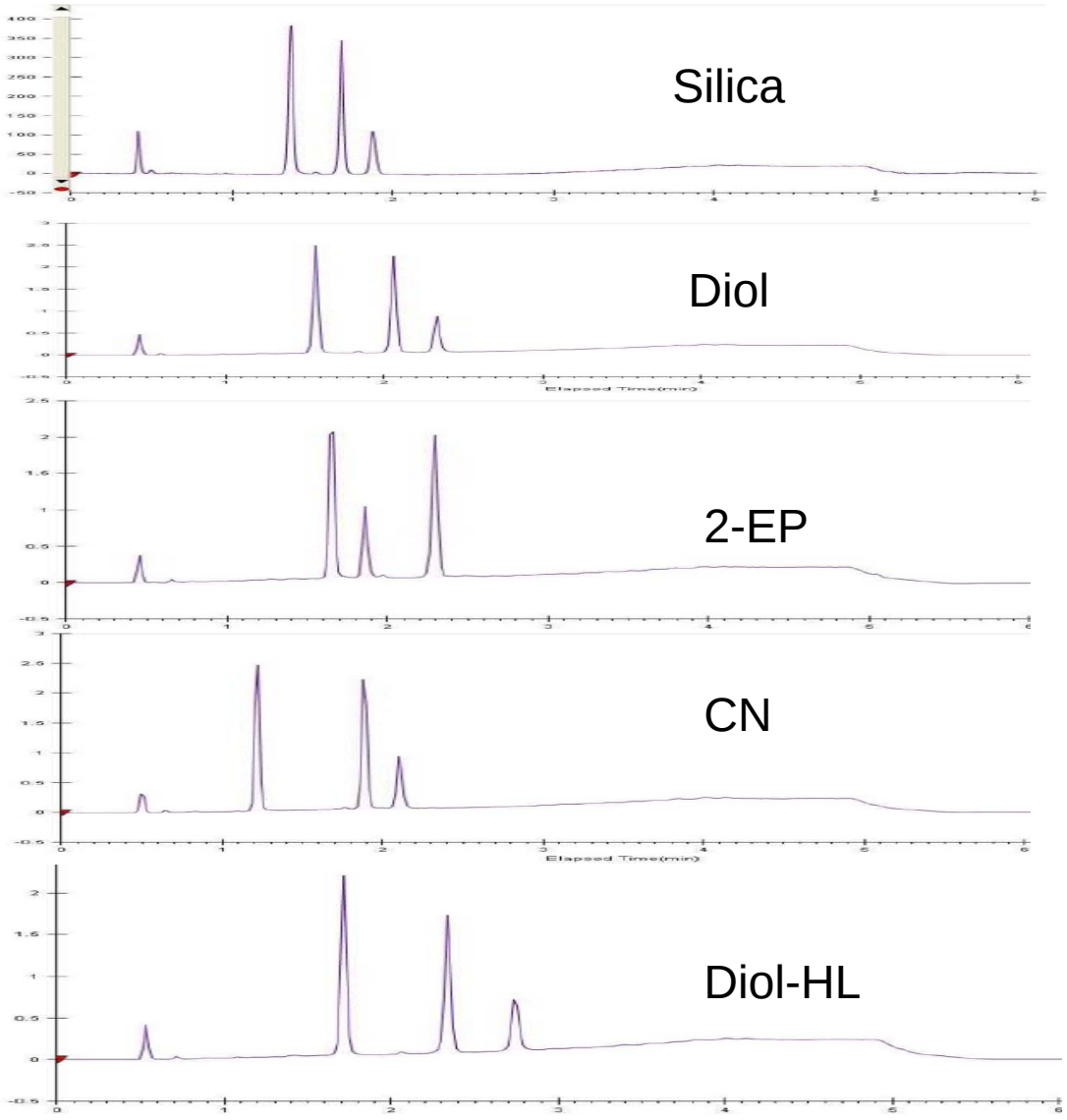
Thar 5x SFC System Evaluation

- Achiral columns
  - Silica, Diol, Cyano, 2-Ethylpyridine, Diol-HL
- MeOH 0.2% DEA
  - 5-55% Gradients
- OA-LCMS samples
- 6 minuites for 5 columns
  - 1.25 min. / col. / inj.
- System is over 7’ tall on bench



Where are we going next?

- Evaluating system suitablitiy for use in OA SFC-MS
  - Possible replacement for some older LC-MS systems
  - Use in core labs for faster analysis times and solvent issues
  - Looking for universal conditions
- Prep SFC-MS
  - Can we set up a simple assisted use systems for chemist use
- Parallel SFC
  - Demo of Thar X5 systems under way
  - Can we make our 8 Column system work via SFC
- CO2 supply issues, will this equipment need to be centralized to supply it?

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Acknolwedgements

- Thierry Mann
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- John Reilly
- Jay Larrow
- Scale Up / Separations Team

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