

Parallel SFC Method Development Screening for Enhanced Speed and Quality

SFC 2011

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Global Analytical Chemistry

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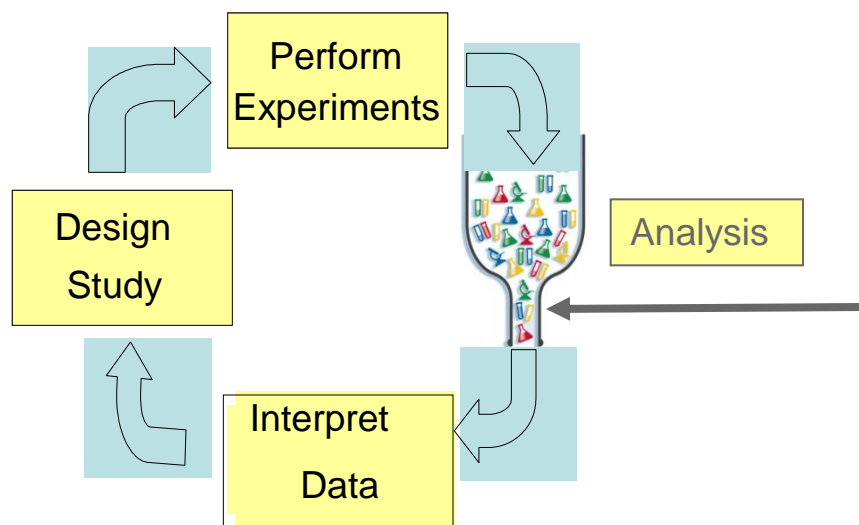
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Outline

- ▶ Background
- ▶ Sepiatec 8 channel parallel SFC Evaluation
- ▶ Real world examples in analysis and prep work
- ▶ Conclusion
- ▶ Acknowledgement

The Method Development Bottleneck ...

High Throughput Experimentation in Drug Development



- Analysis can become a major bottleneck with increasing # of samples in 96/384 well-plate format
- Analytical method development is often a rate limiting factor, especially for chiral samples

“We need fast methods for analysis ...and we need them fast.”

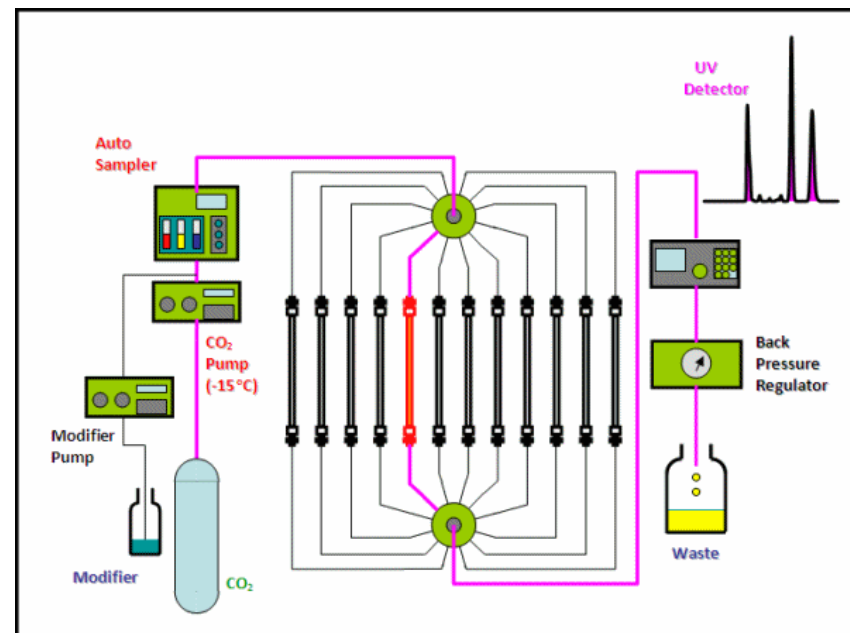
Automated Method Screening Approach to Method Development

▶ Why screen?

- High success rate
(Best selectivity often difficult to predict esp. chiral and unknown components)
- Enhanced Productivity by automation
- Systematic approach to column-mobile phase combination
- Centralized resources-
Stereochemical Center of Excellence

▶ How to screen?

- One at a time, sequential screen
- Try all the possibilities overnight, sort it out in the next morning



Overnight Screening with Standard Methods

...The Night is Long...

- slow gradients to ensure that nothing is missed.

...But Not That Long...

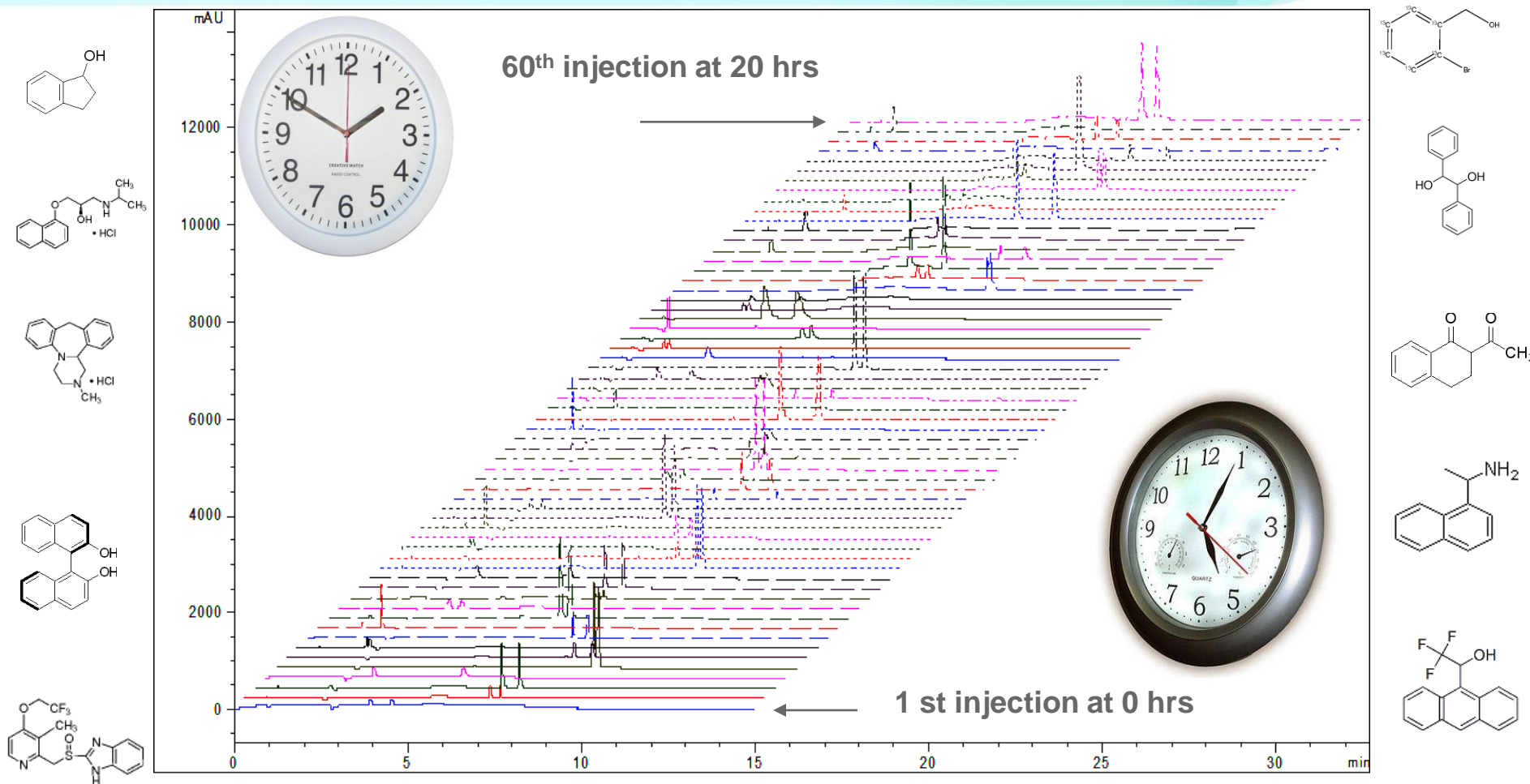
- faster gradients to get through the samples and conditions by morning, but sometimes miss marginal separations

- Doesn't Support Same Day Method Development



Wide variety of columns, mobile phases, additives
re-equilibration needed

Overnight Sequential Chiral SFC Screen for 10 samples 6 columns 1 modifier- 20 hrs



Multiparallel Method Development Screen

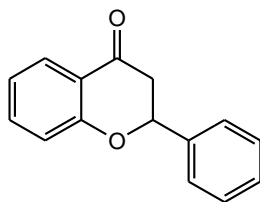


Simultaneous evaluation of several column/eluent combinations

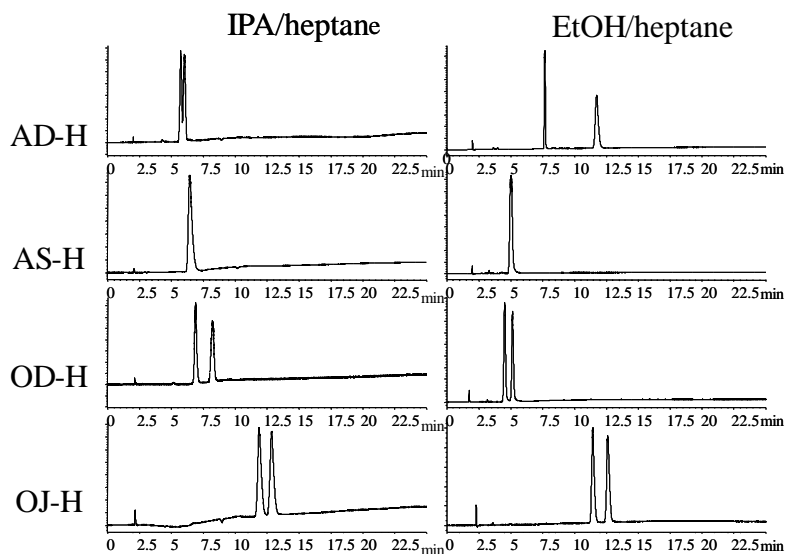
Multiparallel Chiral HPLC Method Development

Multiparallel Chiral Method Development Screening Using an 8-channel Microfluidic HPLC System

Peter Sajonz*, Xiaoyi Gong; William R. Leonard Jr., Mirlinda Biba, and Christopher J. Welch*
Separation and Analysis Technologies, Merck and Co., Inc., Rahway, NJ 07065,

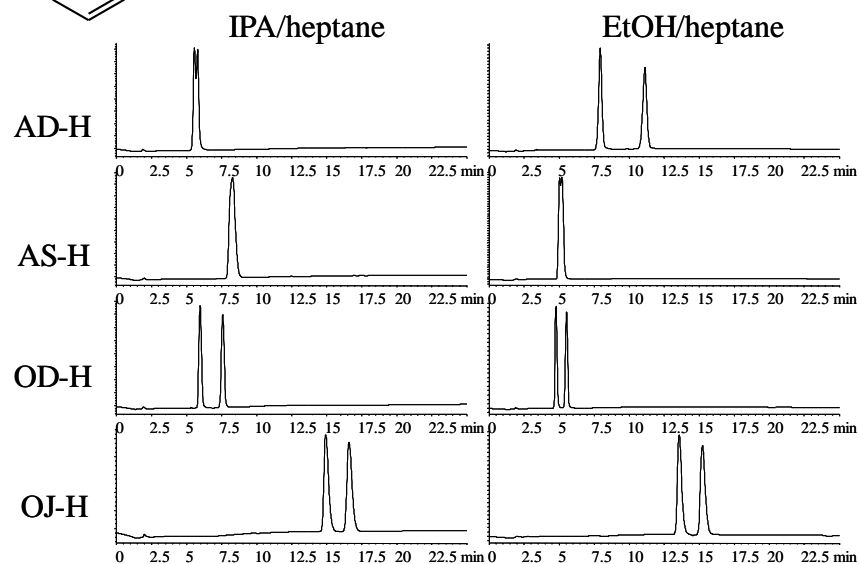


Eksigent Multiparallel Screening



30 minutes

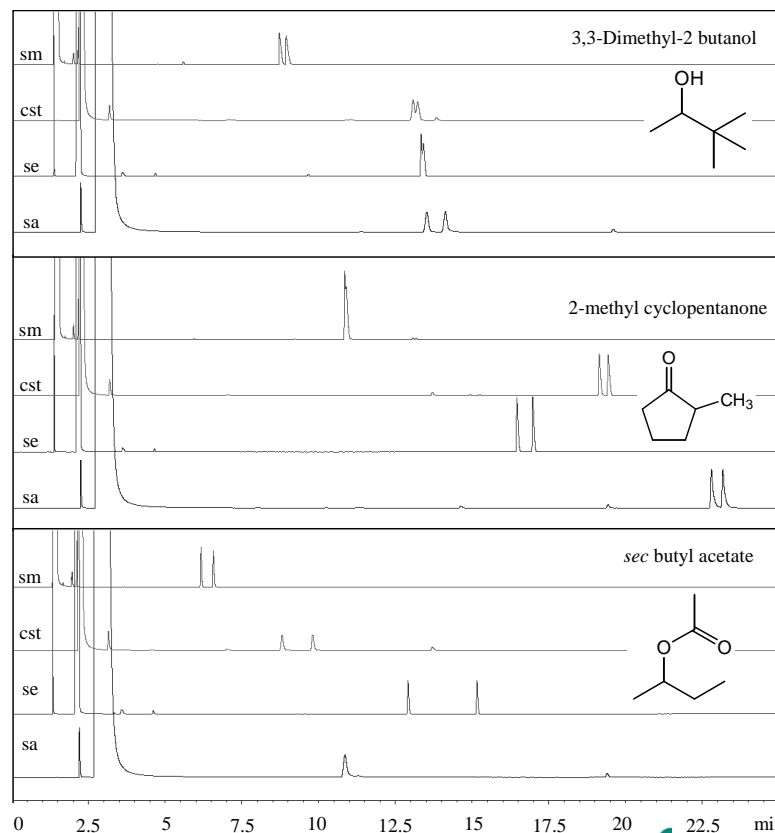
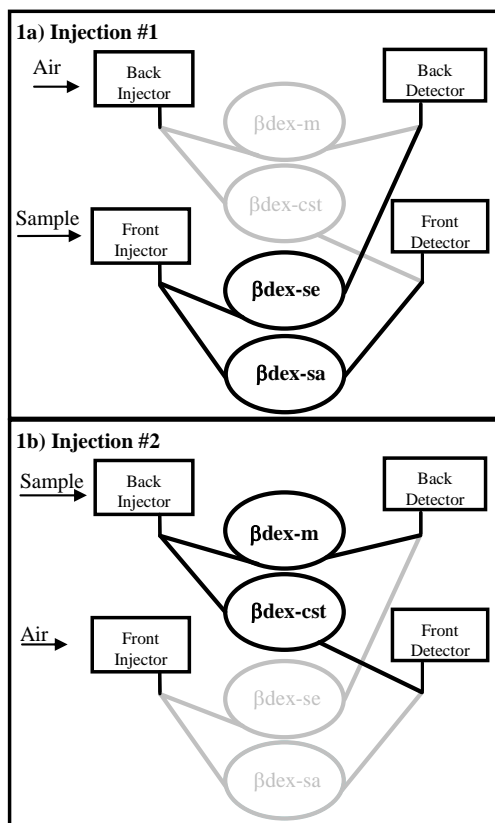
Agilent 1100 with Column Switcher



5 hours

Parallel GC Screening

- 2 x 4-columns in 2 hours with no manual column installations
- Cyclodextrin based (orthogonal selectivity to polysaccharide phases)
- Volatile Compounds without Chromophores



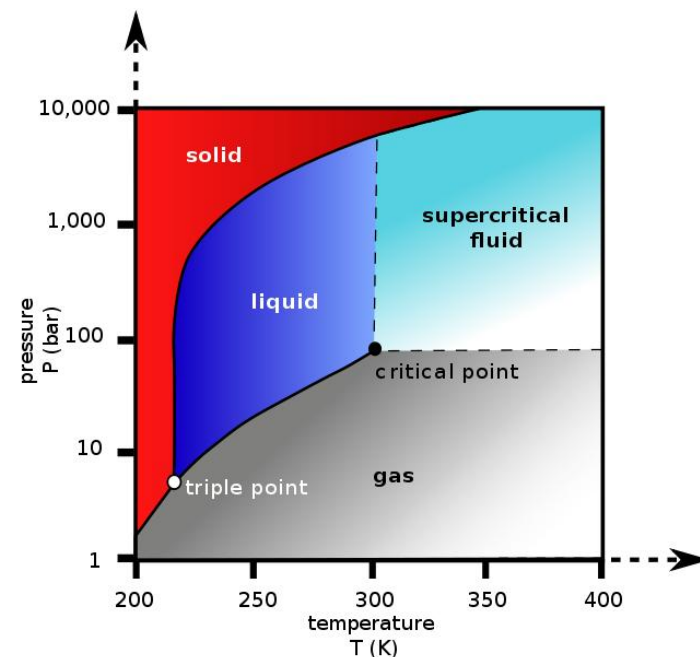
SFC Is Our Preferred Chiral Method Screening Technique

► Advantages

- Fast speed of analysis and equilibrium
- Reduced solvent consumption and disposal cost
- Prep friendly
- Orthogonal technique/selectivity to RPLC
- Sharp peaks compare to LC

► Room to improve

- Sensitivity
- Sequential screen

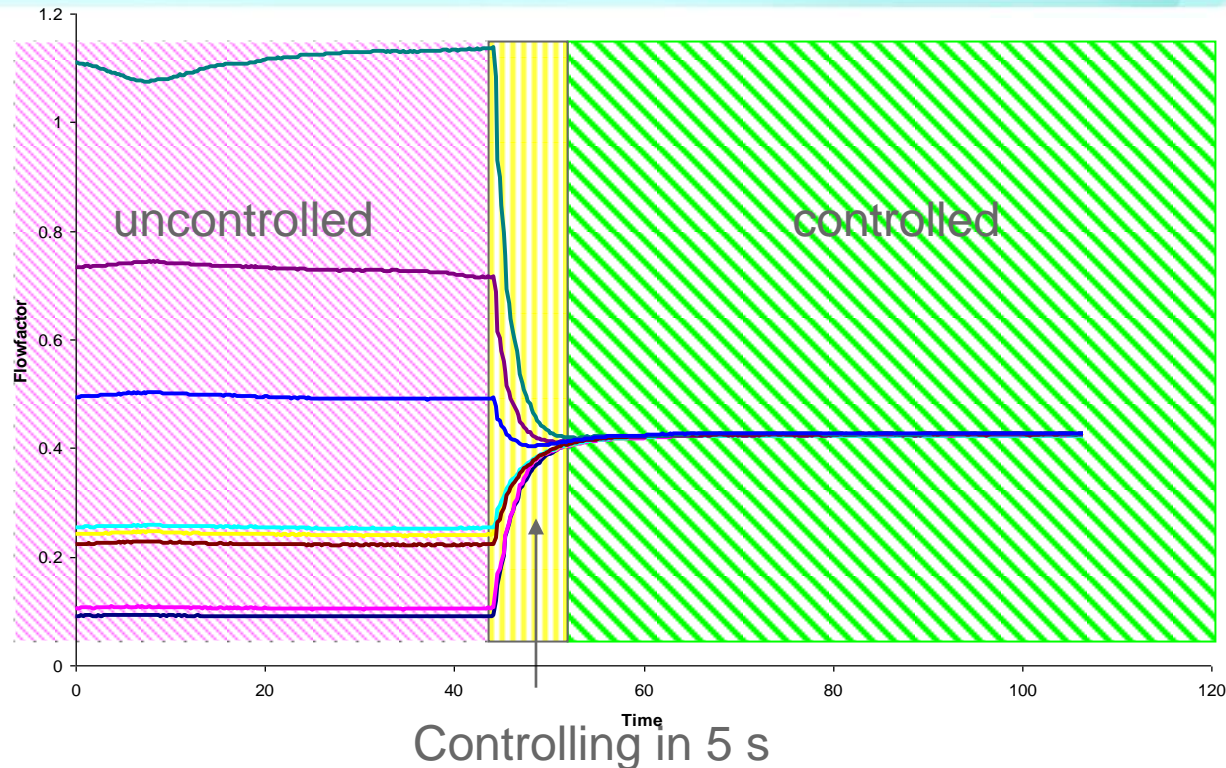


Seplatec 8 Column Parallel SFC



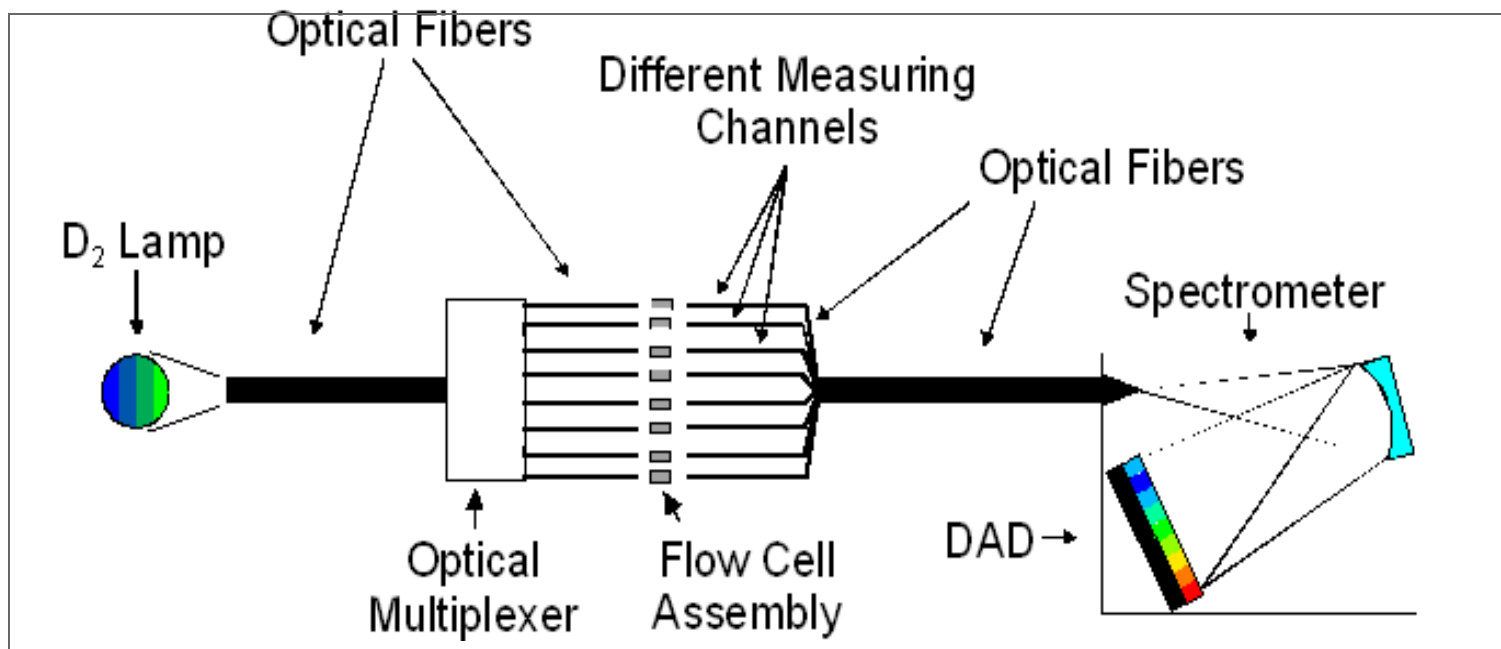
- 8 columns simultaneously (150mm, 3 micron)
- Ternary gradient system 3 x 6 =18 solvents and ability to mix 3 solvents
- Split flows, single injection to multiple columns
- Flow Control and BPR by PID feedback controller
- 8 flow cells with multiplexed single Diode Array Detector

Sepiatec 8 Channel Split Flow Control



- Flow is controlled by PID feedback controller as flow factor that is independent to viscosity of modifier
- Takes ~ 5s to adjust flows to the same for all columns of different backpressures

Sepiatec 8 Channel Multiplexed Diode Array Detector



Share one detector for 8 columns by a rotary shutter controlled by PID feedback controller

Seplatec Parallel SFC Evaluation

▶ **Desired features**

- Reproducibility (injection, and with other instruments)
- Ability to deliver and mix multiple solvents
- Chromatographic performance
- System delay volume
- Sensitivity

▶ **Evaluate and compare Seplatec parallel SFC with Berger and Aurora SFC**

- RT
- Resolution
- Sensitivity
- peak shape
- Isocratic
- Gradient

Reproducibility of Isocratic Runs

AD-H
150x5

AD-H
50x5

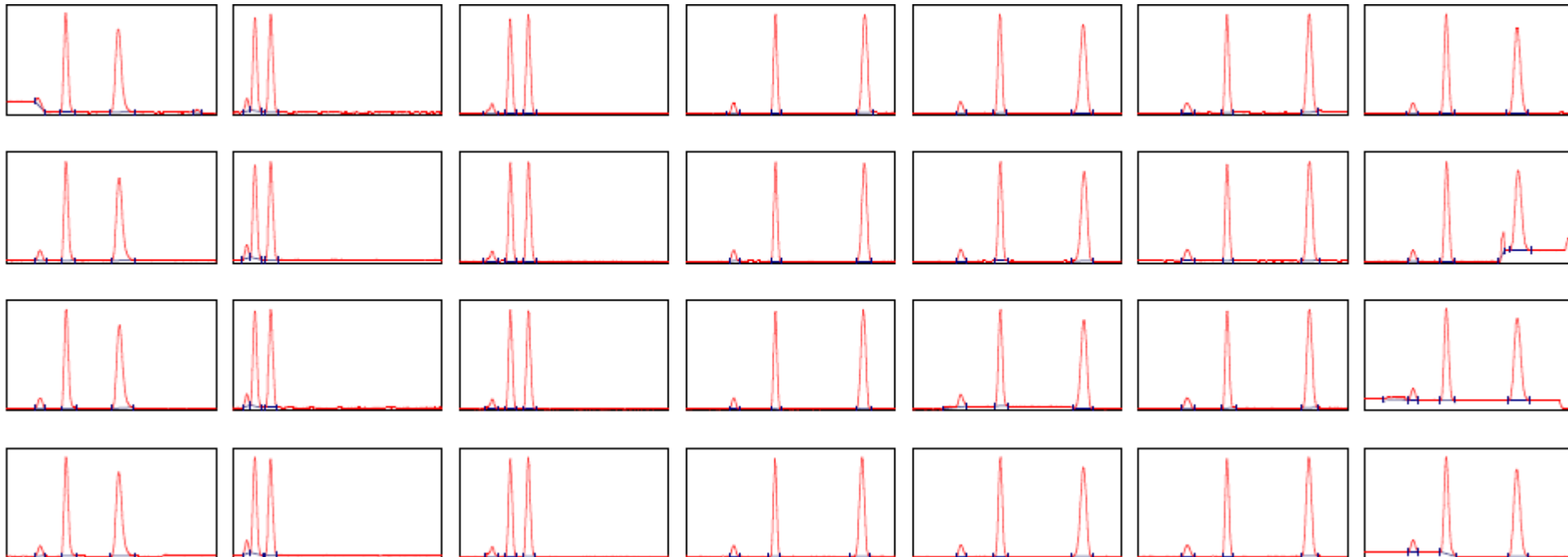
Cel Coat
150x3

AD-H
250x5

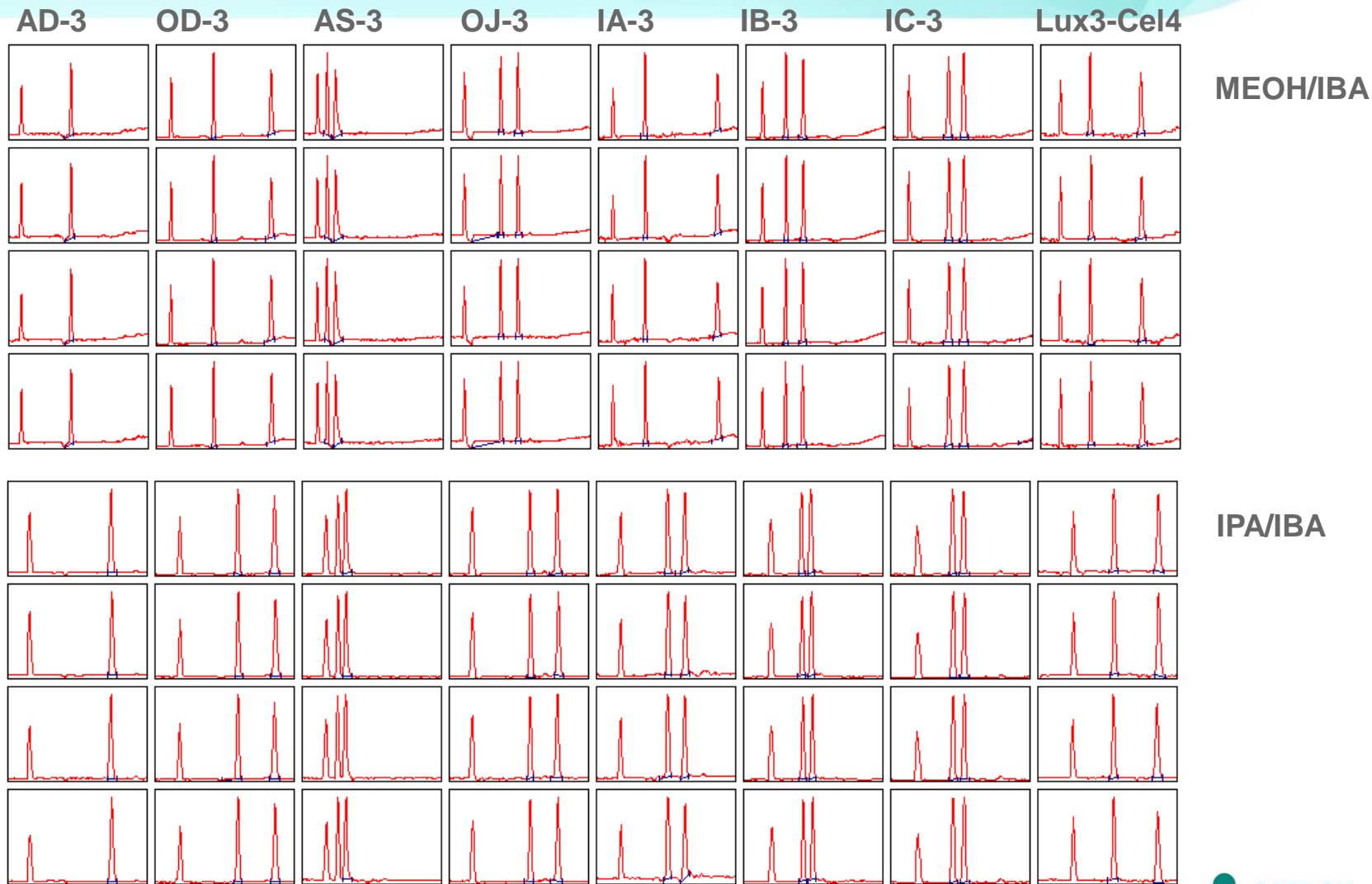
AD-H
250x5

AD-H
250x5

AD-H
250x10

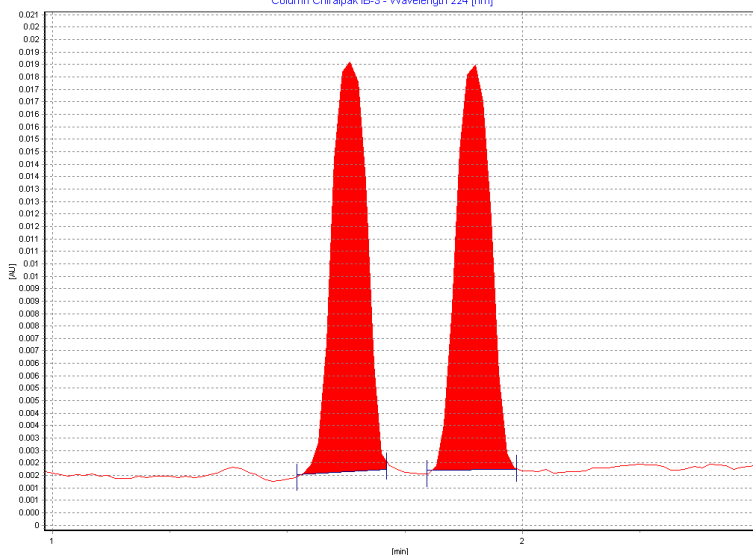


Reproducibility of Gradient Run 4% to 40%



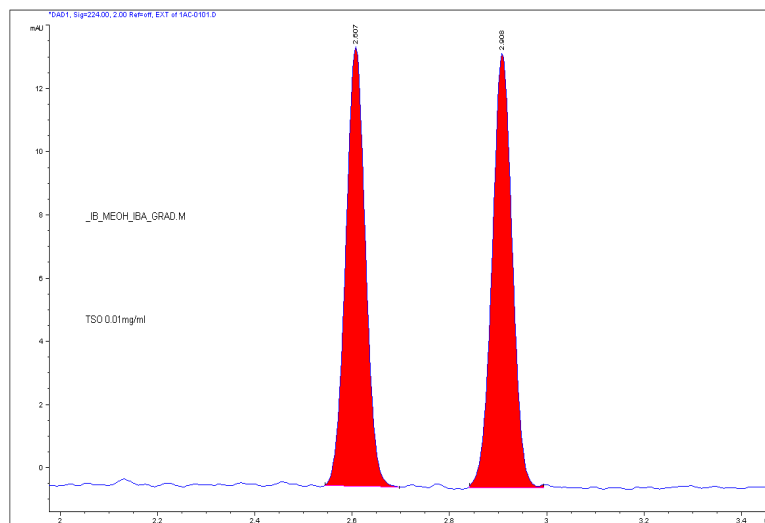
Signal to Noise of Sepiatec SFC

Sampletable 2011-07-05-signal to noise - Copy #05.07.2011 18:23:50 - Analyzed 7/5/2011 4:49:44 PM
Method_SHA-grad 4-40% MeOH w/ IBA - Samplename TSO
Column Chiralpak IB-3 - Wavelength 224 [nm]



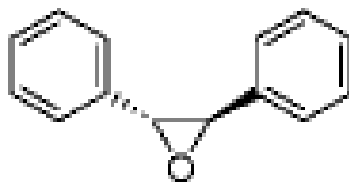
S/N=36

Sepiatec SFC



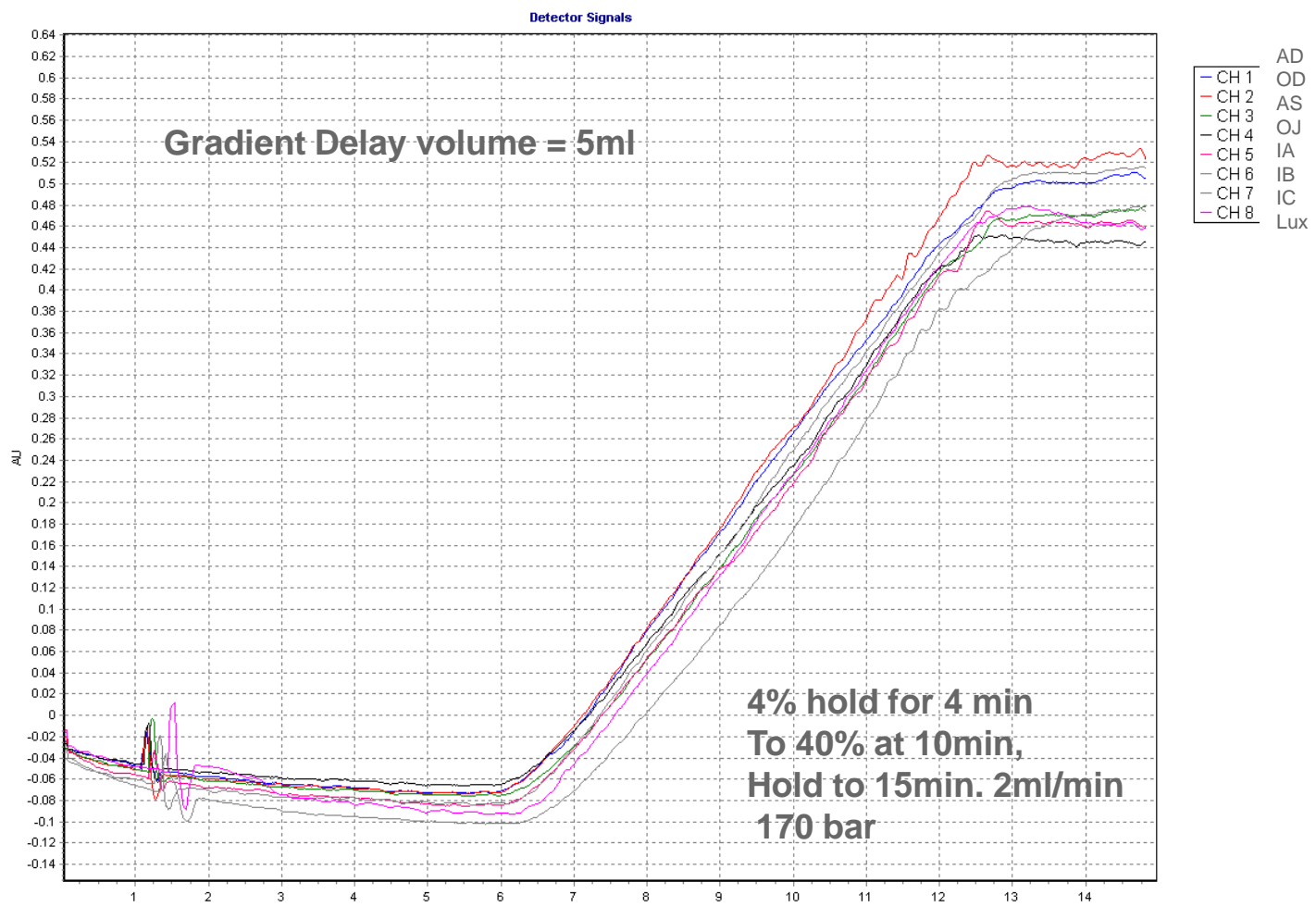
S/N=37

Aurora SFC



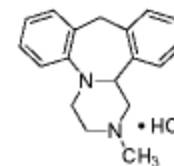
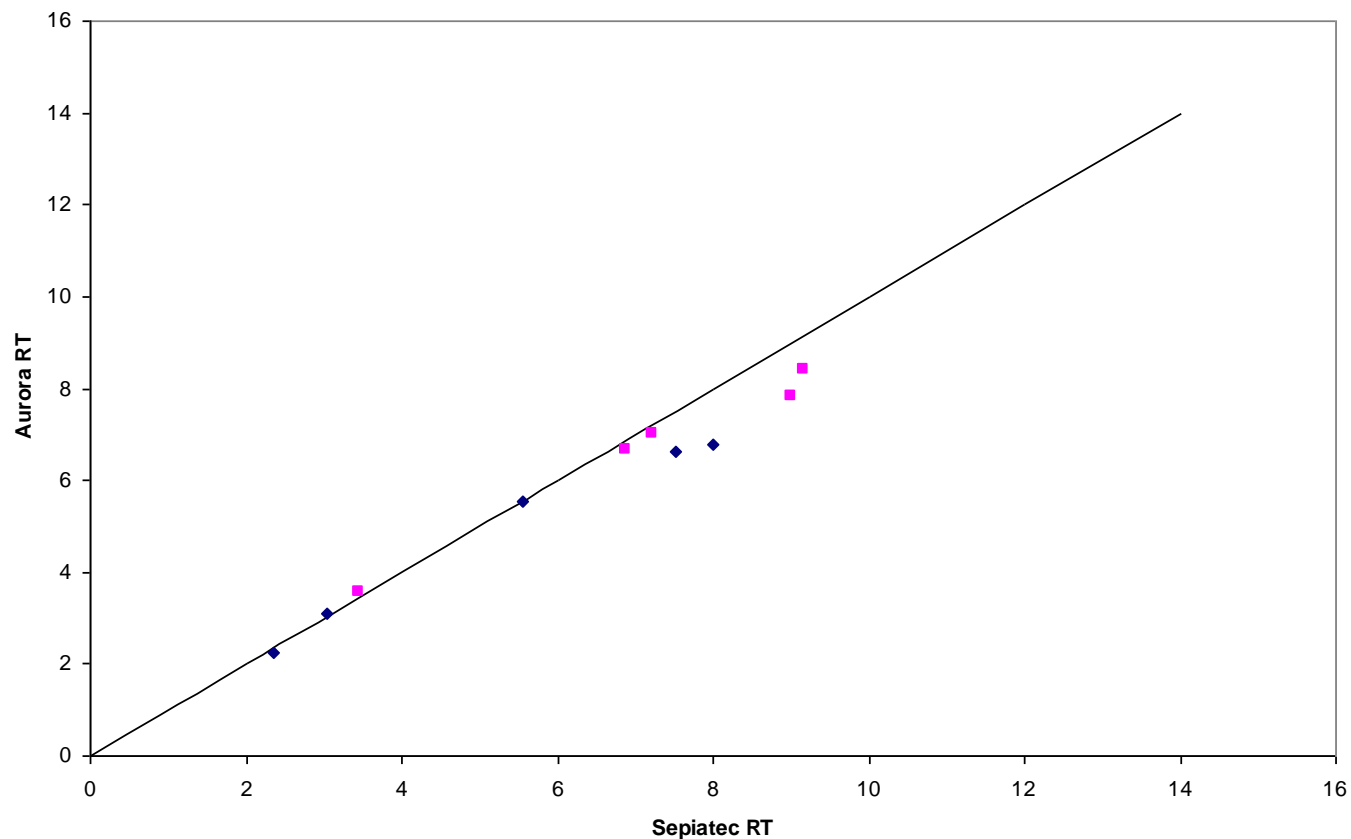
TSO 0.01mg/ml

Sepiatec SFC Gradient Delay Volume

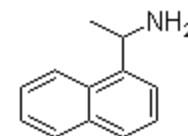


Comparison of RT Between Sepiatec and Aurora

Comparison of RTs



- ◆ Mainserin
- NapthylEthylamine
- Trendline

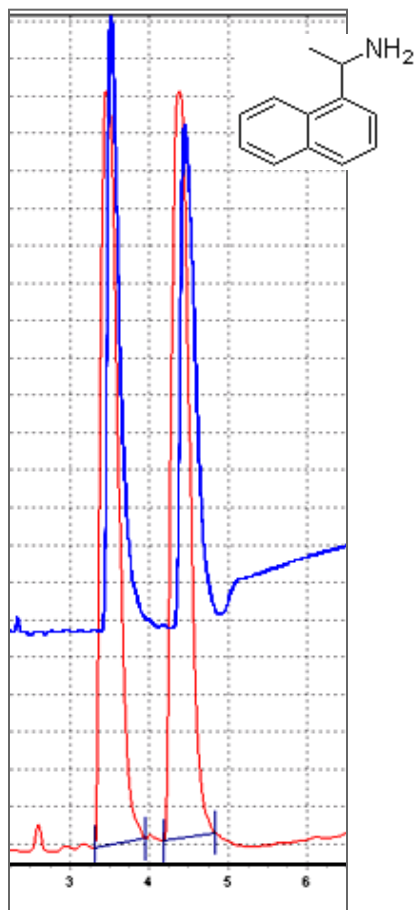


Chromatographic Comparison Sepiatec vs Aurora

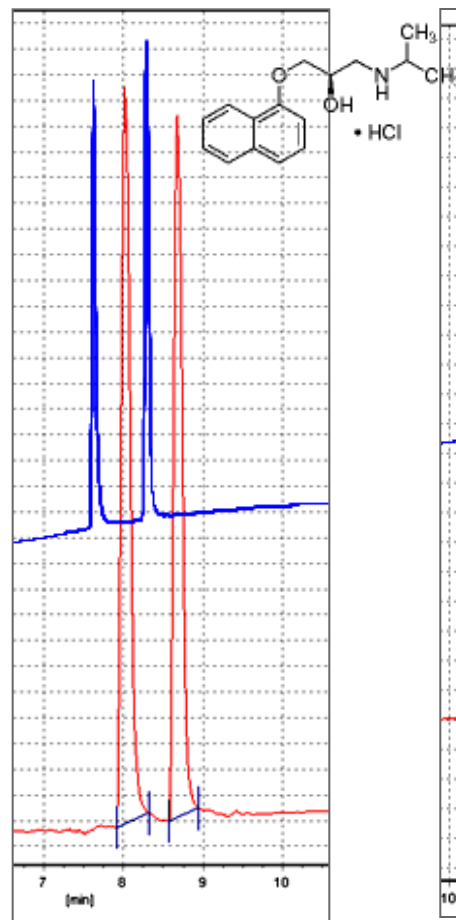
Sepiatec
R = 2.38
N = 894 / 1418

Aurora
R = 2.89
N = 2521 / 2726

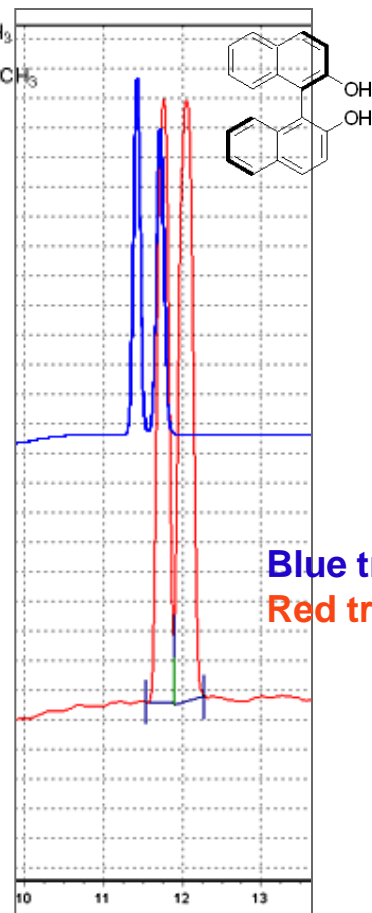
200bar, 40C.
Gradient: 4%
for 4 min to
40% at 10 min
Hold for 5min
3ml/min



Naphthylethylamine
OJ-H MeOH / IBA



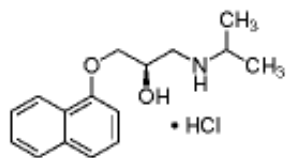
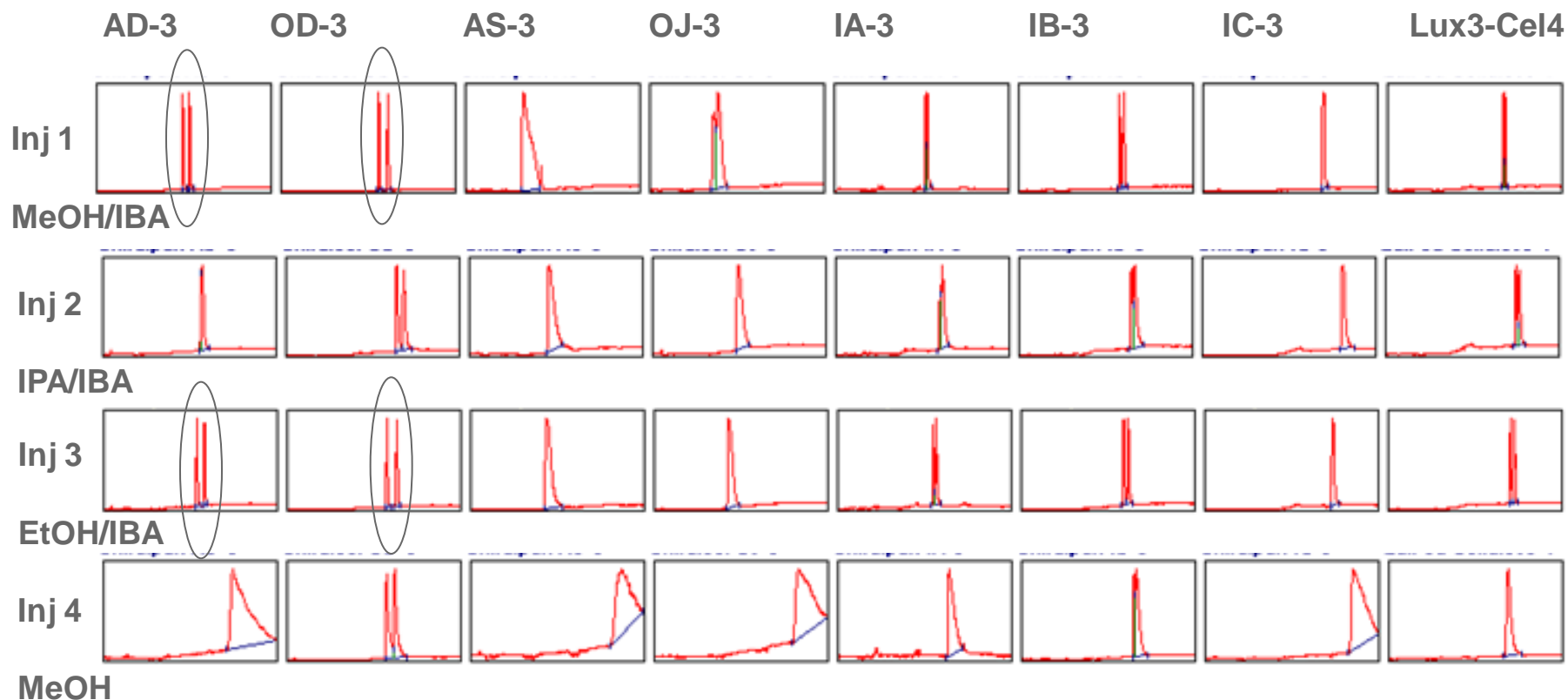
Propanolol
AD-H MeOH / IBA



Binaphthol
AD-H IPA / IBA

Blue trace: Aurora
Red trace: Sepiatec

4 Modifiers x 8 Columns Screening in 80 min



Propranolol

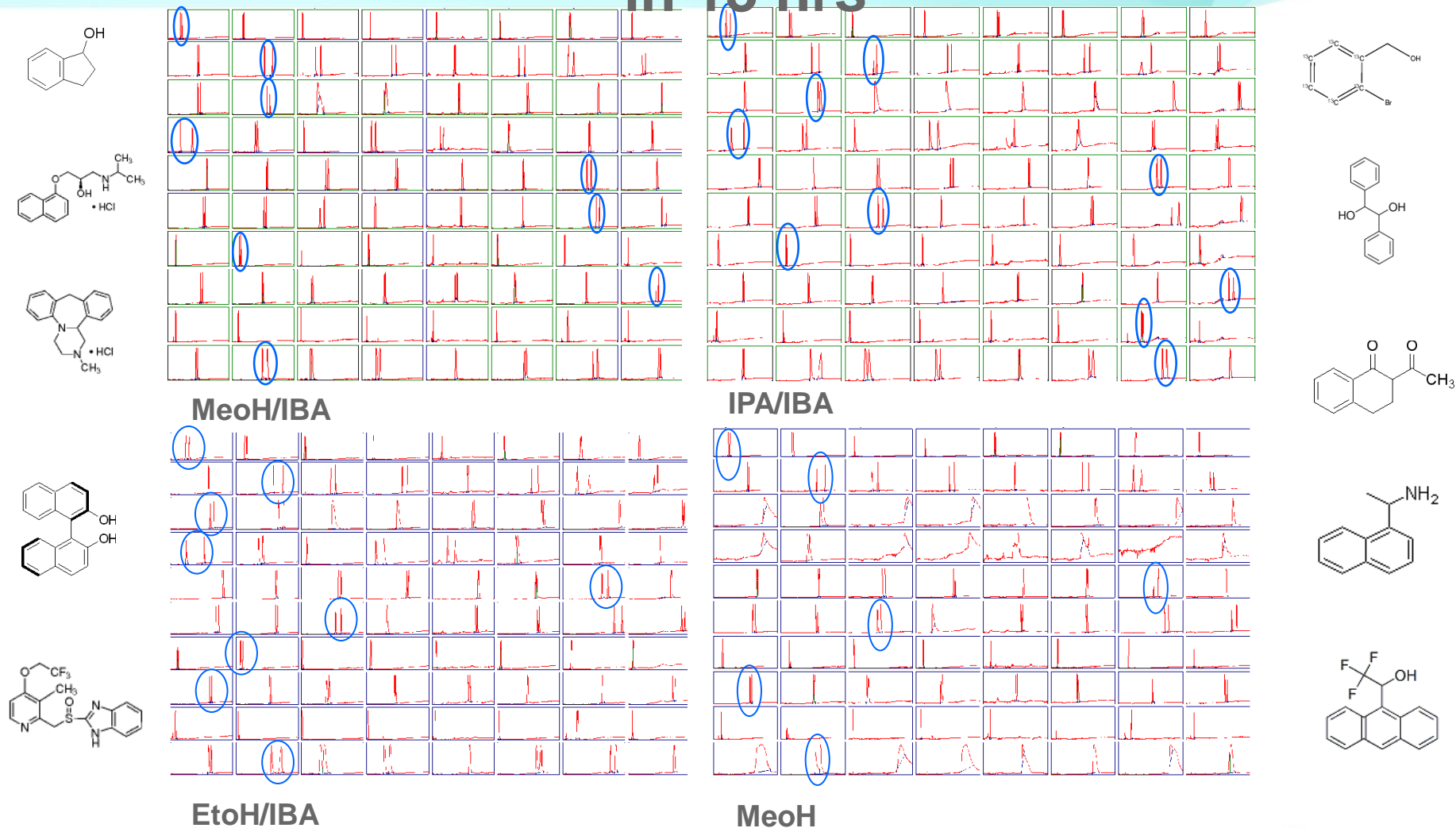
200bar, 40C. 210mm Gradient: 4% for 4 min to 40% at 10 min Hold for 5min 3ml/min

Parallel screen : 80 min ~ "lunch break"
Sequential screen : 640 min ~ overnight

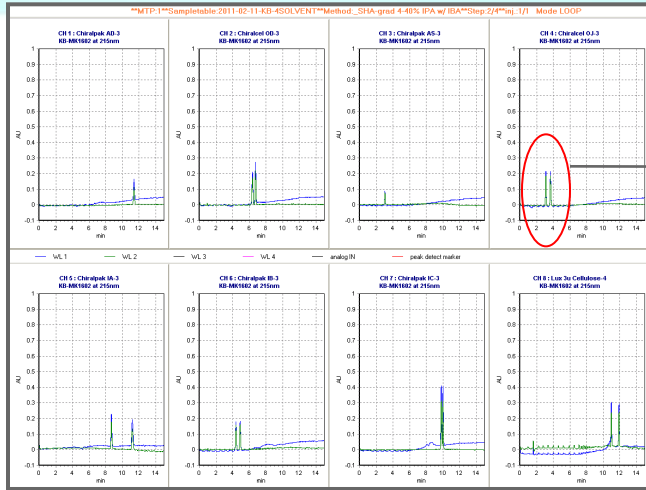
Parallel Screening Map

10 samples x 8 Columns x 4 Modifiers = 320 Chroms

in 13 hrs

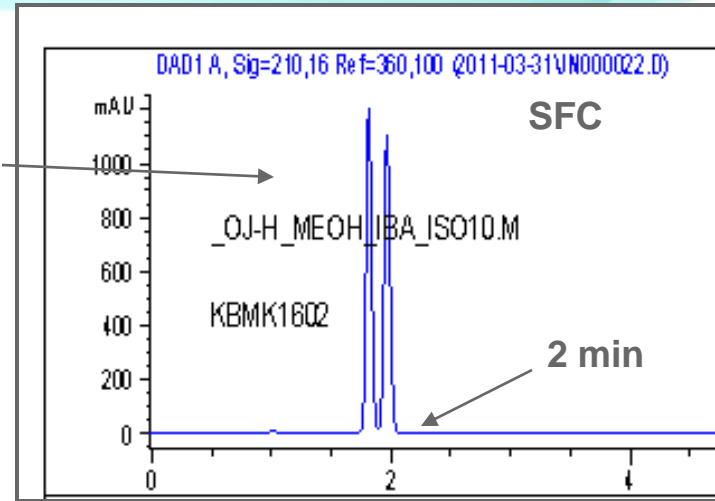


Real world Example 1: Concurrent Parallel Screens of Orthogonal Methods

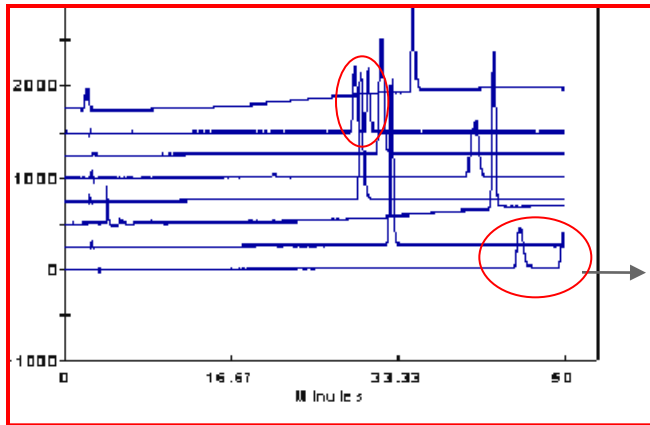


Sepiatec Parallel SFC Screens

OJH

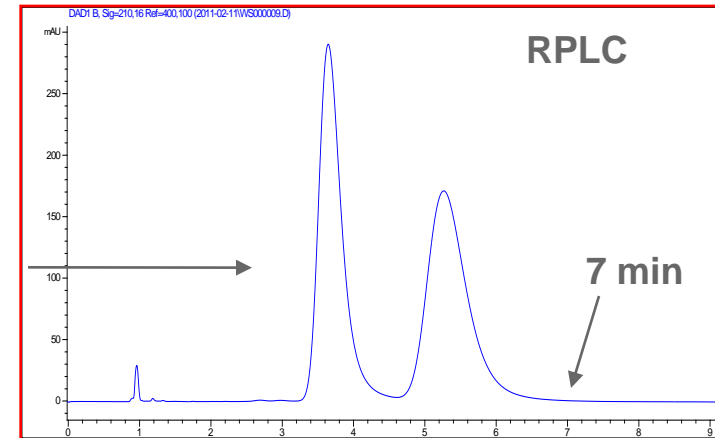


Optimization



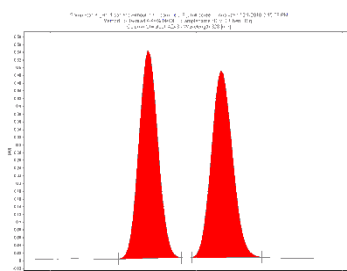
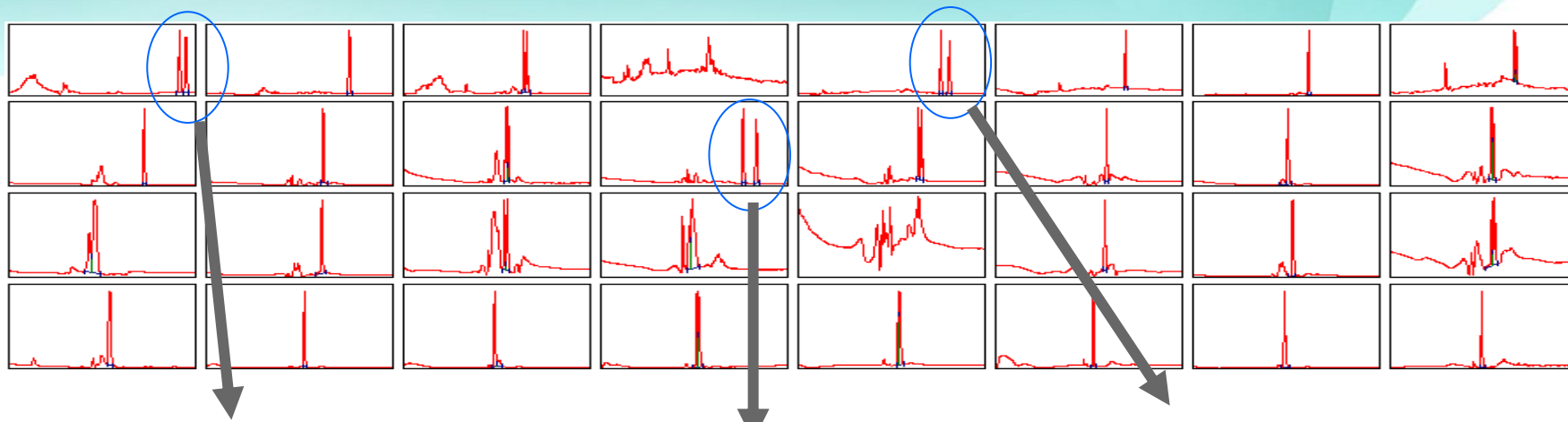
AD-RH

Eksigent Parallel RPLC

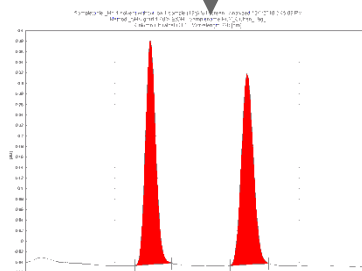


AD-RH 150x4.6, 75%ACN,25%(0.02% HClO₄,150 mM NaClO₄ (aq)), 2ml/min,

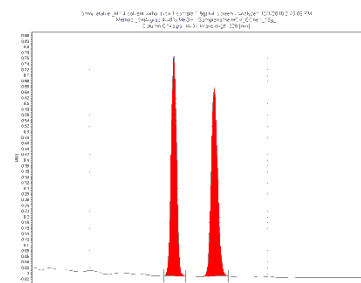
Real World Example 2 : From Screens to Prep



AD-MeOH

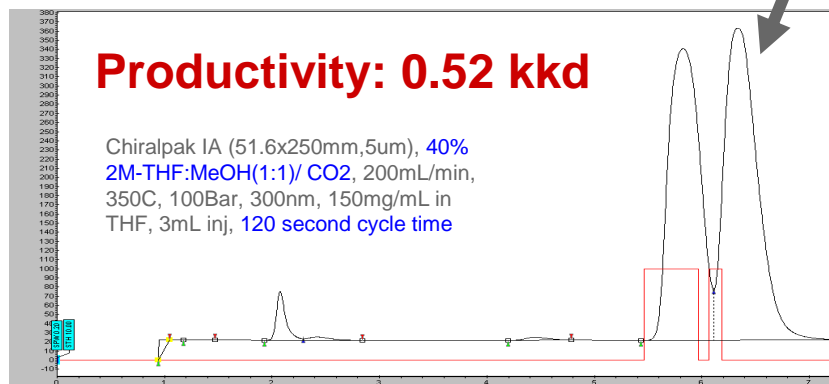


OJ-EtOH



IA-MeOH

2 KG product ←



Conclusion

- ▶ **Initial studies show Parallel SFC enhances screening speed and quality, allows more screening conditions to obtain more and better hits in 1/8 of conventional sequential screening time**
- ▶ **Evaluation is still in progress**
- ▶ **Further work**
 - needed to assess reliability / robustness
 - tertiary mp studies (three-component mixed modifiers)
 - HT parallel analysis (8 different samples using same stationary phase in parallel)

Acknowledgements

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