Development of Novel Stationary Phases for Supercritical Fluid Chromatography

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Setting the stage…

- Pharmaceutical Compounds
  - Diverse compounds with wide range of polarity in same sample
    - Product, reagents, by-products, etc
  - Samples in DMSO
  - Purification needed
    - Samples stored in DMSO
    - Stability with salts, water a concern
  - Broad, rapid gradients
    - 5-50% in 2.5 min, > 5mL/min

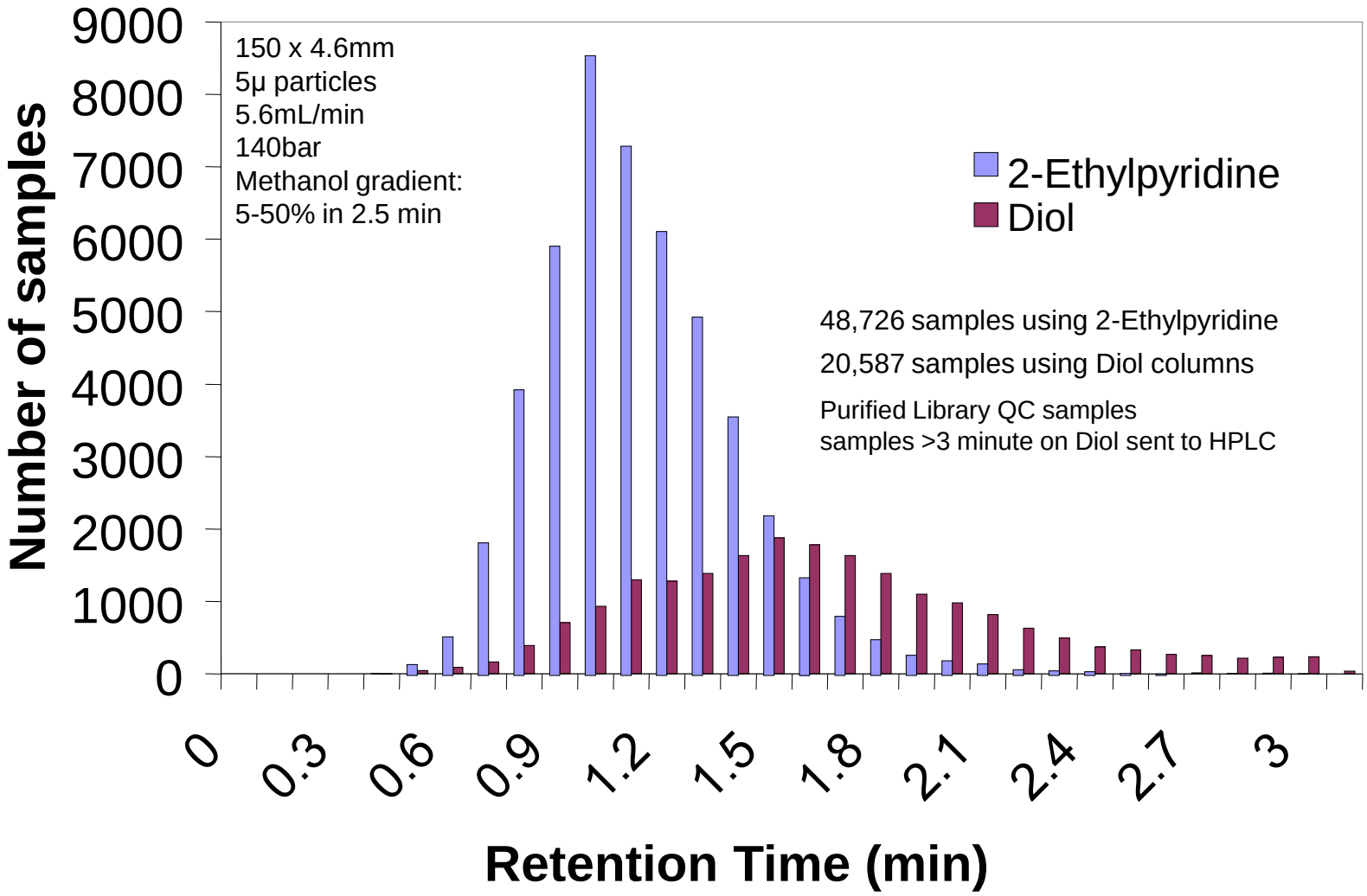
- Traditional SFC Phases – Bonded HPLC phases (Silica, Diol, Amino, CN, Phenyl)
  - Limitations – poor peak shape without additives, salts
    - Good for acidic compounds
    - Poor for basic without additives

- SFC Specific Phase – 2-Ethylpyridine Bonded phase
  - Expanded the applicability of SFC for achiral compound purification
    - Low retention
    - Good selectivity
    - No additives

2-Ethylpyridine Phase

- Advantages over Diol, CN, Phenyl, Amino, silica
  - Aromatic
    - Lipophilic
    - Selectivity to substituted aromatics
  - Basic, but not ionic
  - Mid-range pKa (used to express basicity)
  - Short aliphatic chain, base close to the silica support
    - Masking silanol activity?
    - Low density – lipophilicity?

Retention Characteristics for Combinatorial Libraries



Outline

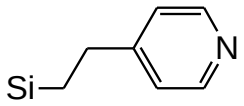
Rationale for new phases based on Pyridine data

- Change position of N
  - E.g. 2-ethyl to 4-ethyl
- Introduce non-acidic OH
- Explore similar aromaticity, different basicity
  - Combinations of phases (e.g. Pyridine/Diol)
  - Pyrimidine, piperazine, piperadine, quinoline, morpholine*
- Modify the aliphatic chain
  - Opens up huge chemical space with propyl chain
  - Polar embedded linkers
  - Retention tuning
- Additive effects

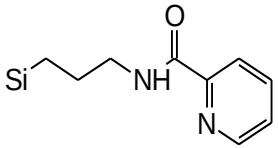
*Out of scope for this presentation

Novel Phases

Pyridinyl Phases

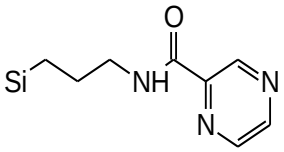


4-Ethylpyridinyl

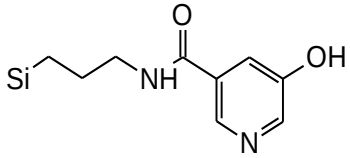


2-Ethylpyridinyl

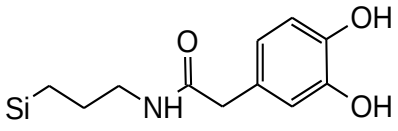
New Prototype Phases



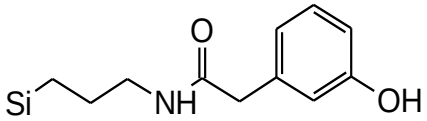
2-Ethylpyrazinyl



5-Hydroxy-3-pyridinyl



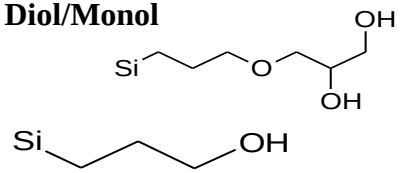
(3, 4)-Dihydroxyphenyl



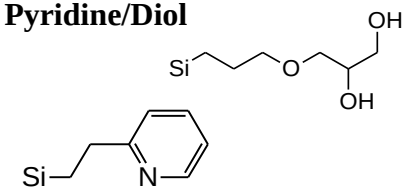
3-Hydroxyphenyl

Mixed Phases

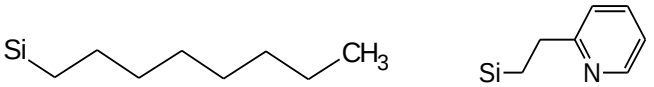
Diol/Monol



Pyridine/Diol

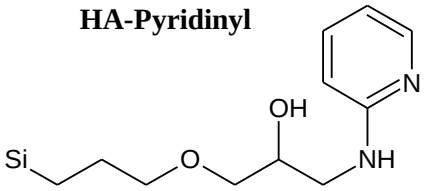


C8 / 2-ethylpyridinyl

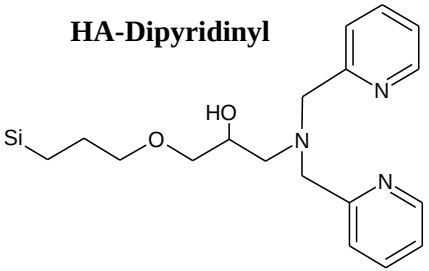


Hydroxy Amino (HA) Phases

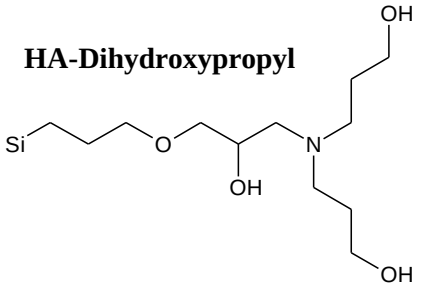
HA-Pyridinyl



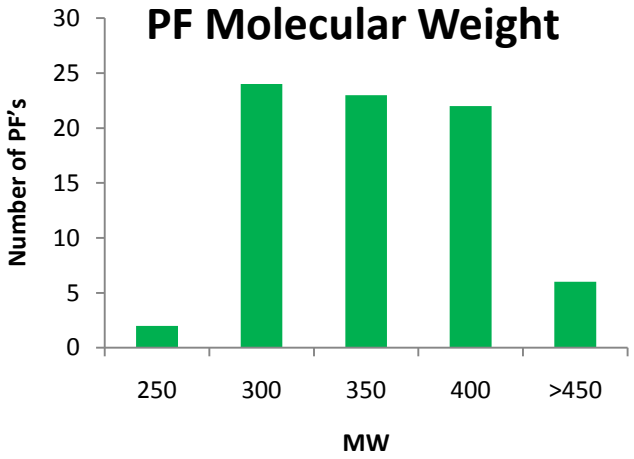
HA-Dipyridinyl

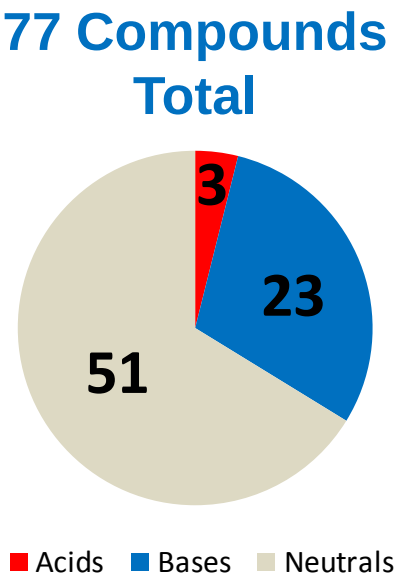


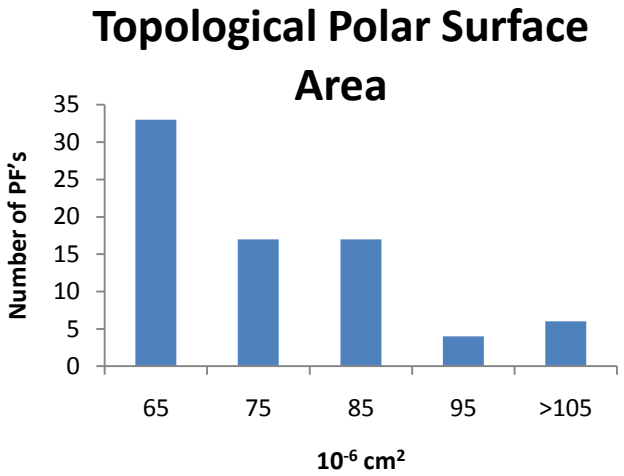
HA-Dihydroxypropyl

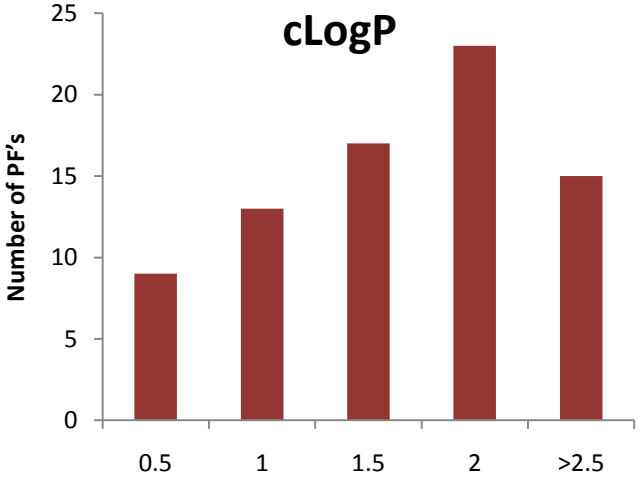


Study Compound Properties

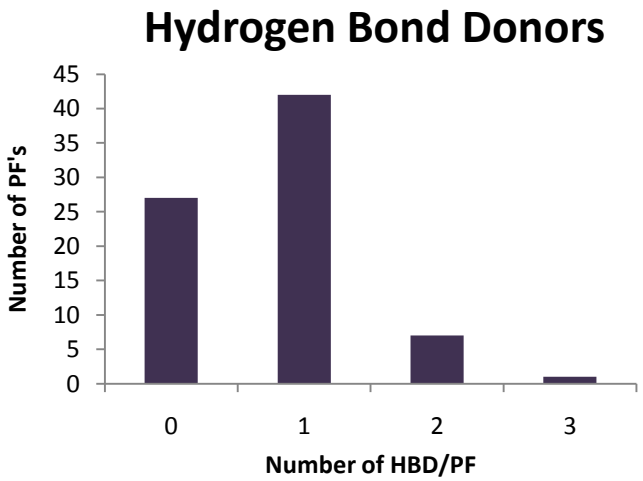








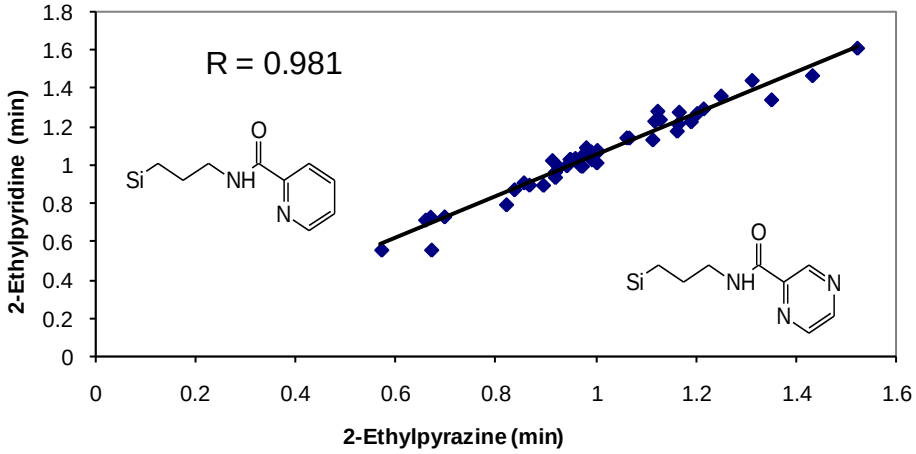
General Conditions
150 x 4.6mm
5µ particles
5.6mL/min
140bar
Methanol
Gradient 5-50% in 2.5 min



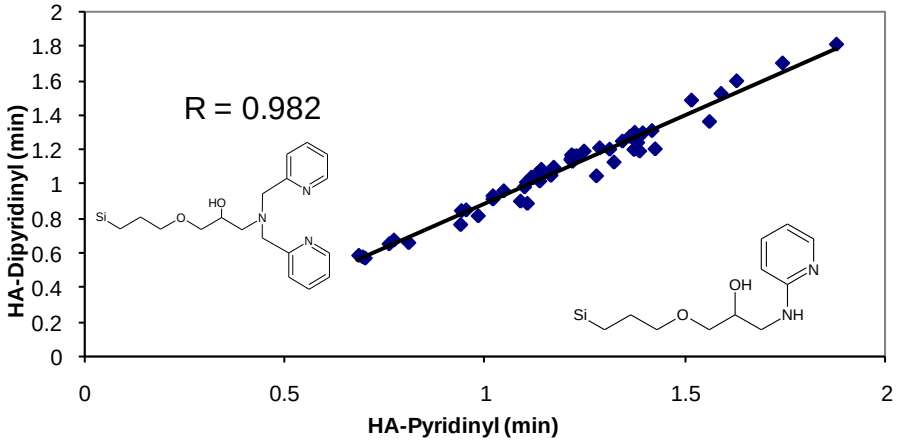
Column Selectivity – Correlating Retention

Examples of **Similar** Retention between Columns

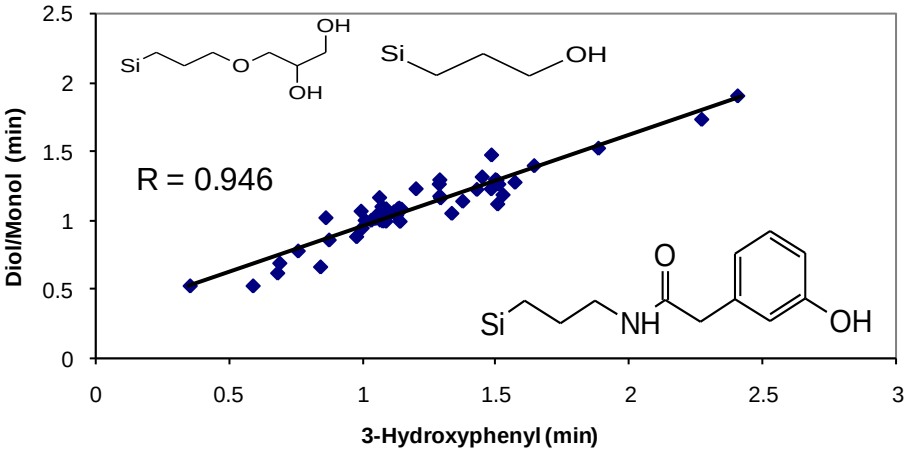
2-Ethylpyridine — 2-Ethylpyrazine



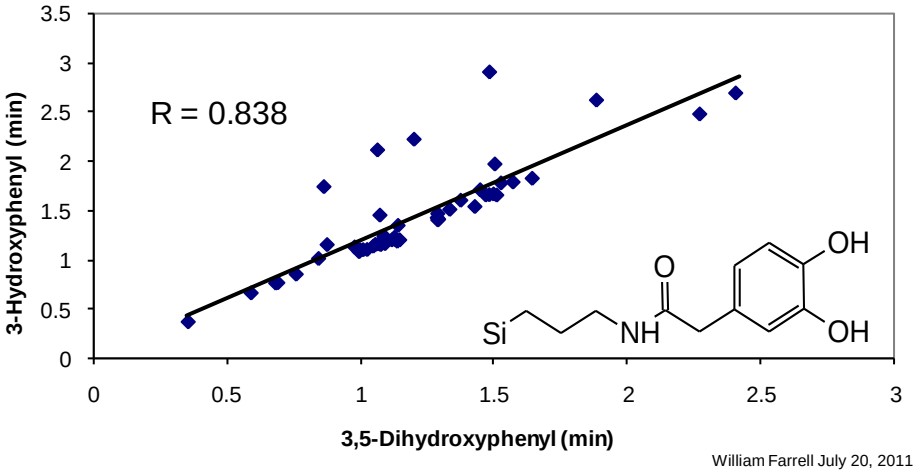
HA-Dipyridinyl — HA-Pyridinyl



Diol/Monol — 3-Hydroxyphenyl



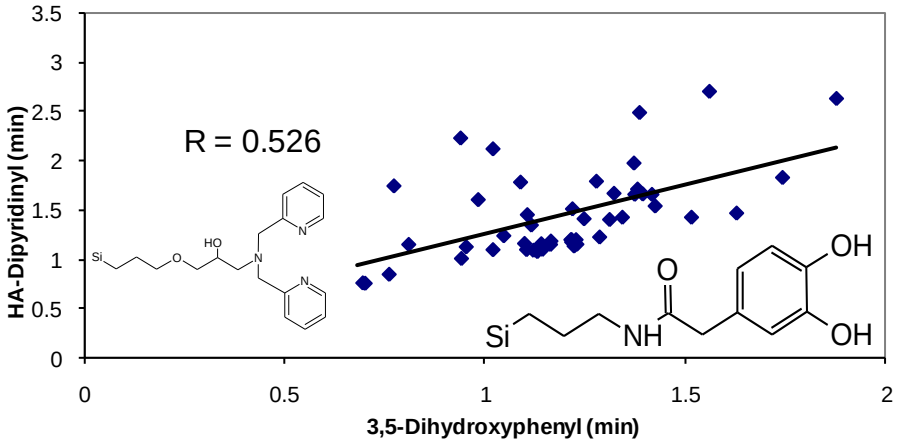
3-Hydroxyphenyl — 3,5-Dihydroxyphenyl



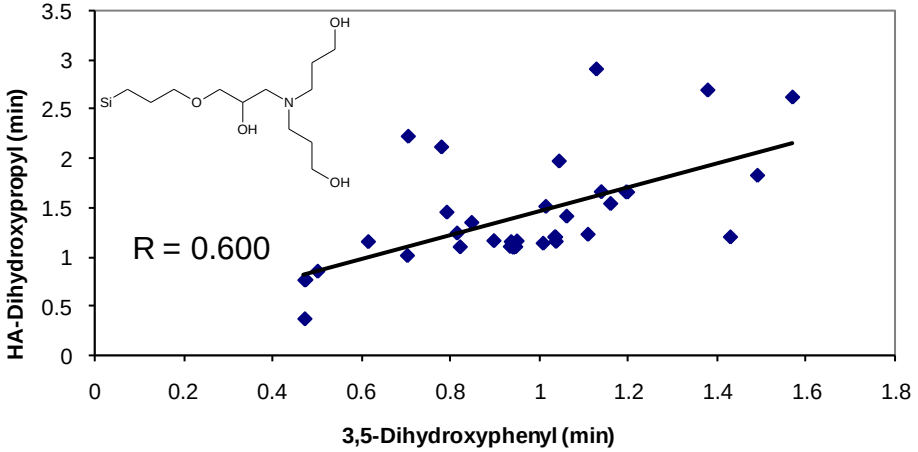
Column Selectivity – Correlating Retention

Examples of **Dissimilar** Retention between Columns

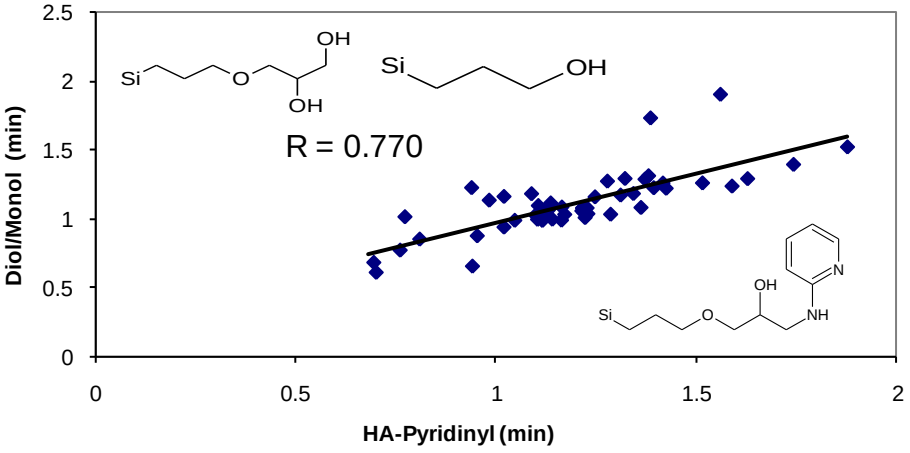
HA-Dipyridinyl — 3,5-Dihydroxyphenyl



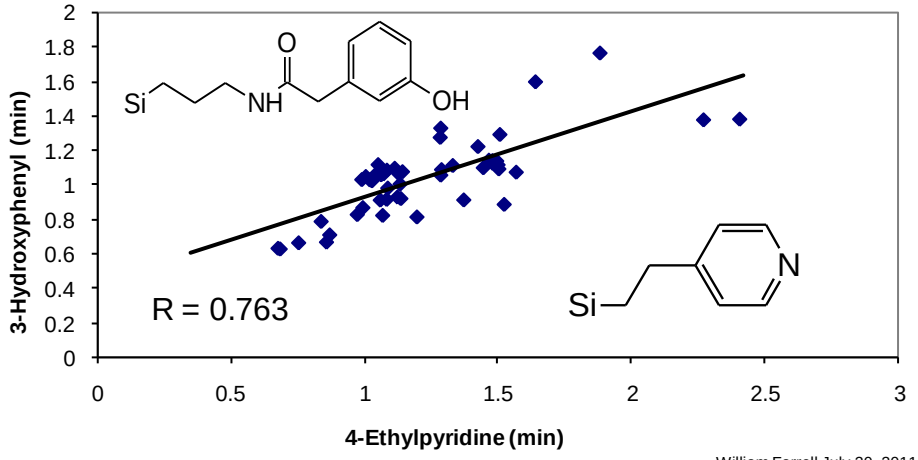
HA-Dihydroxypropyl — 3,5-Dihydroxyphenyl



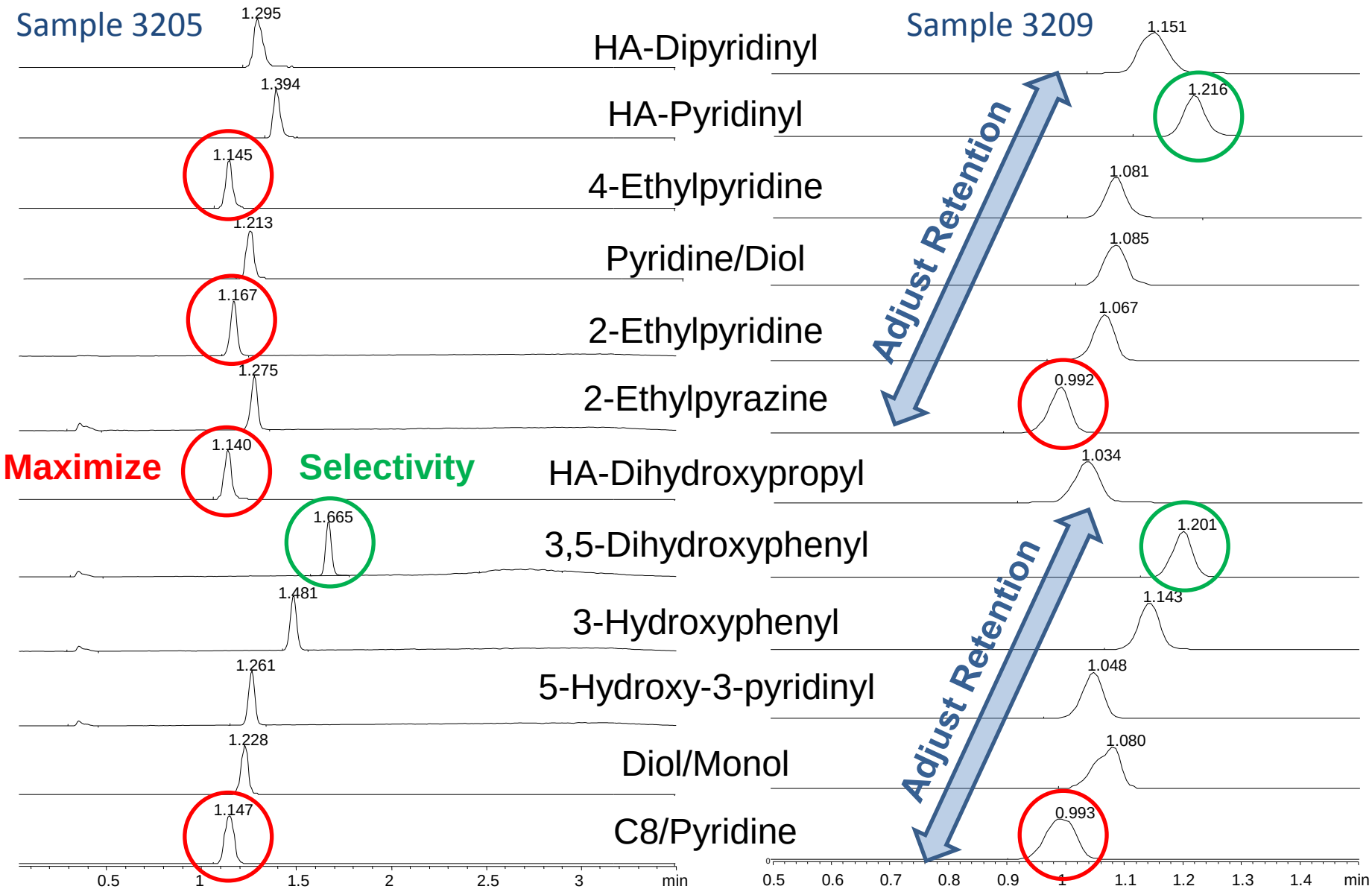
Diol/Monol — HA-Pyridinyl



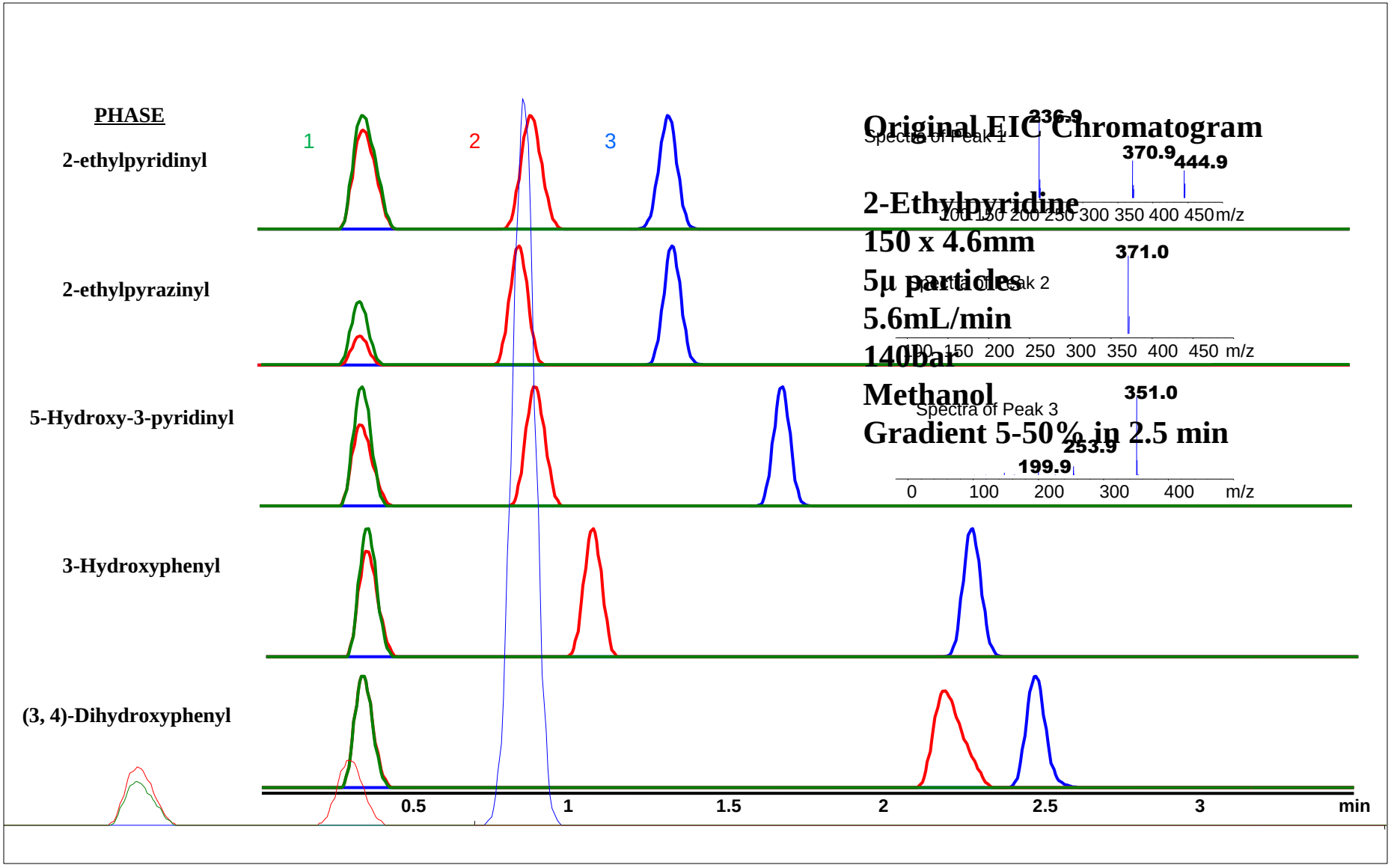
3-Hydroxyphenyl — 4-Ethylpyridine



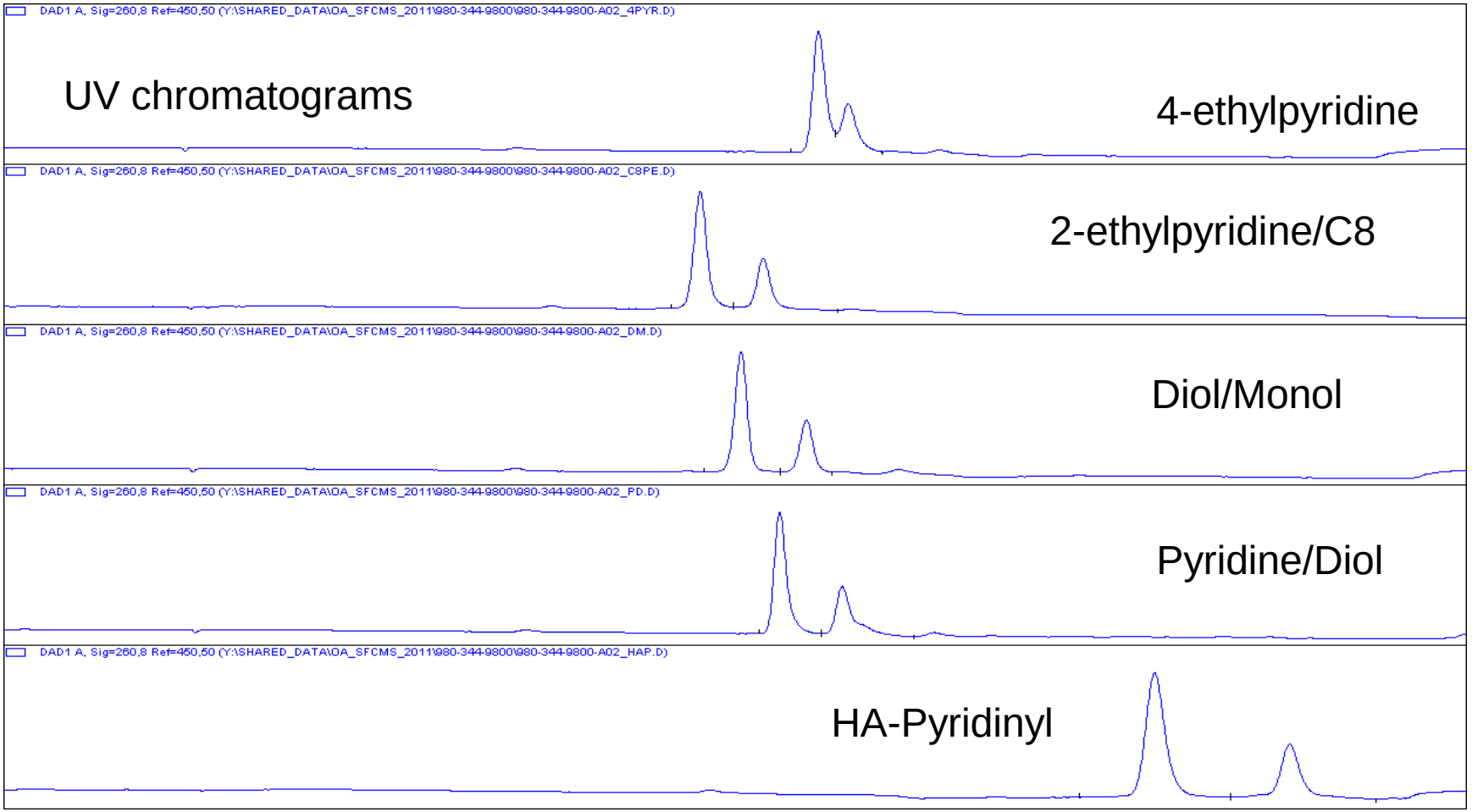
Example Chromatograms



Normalized EIC chromatogram of library PF sample on each phase.

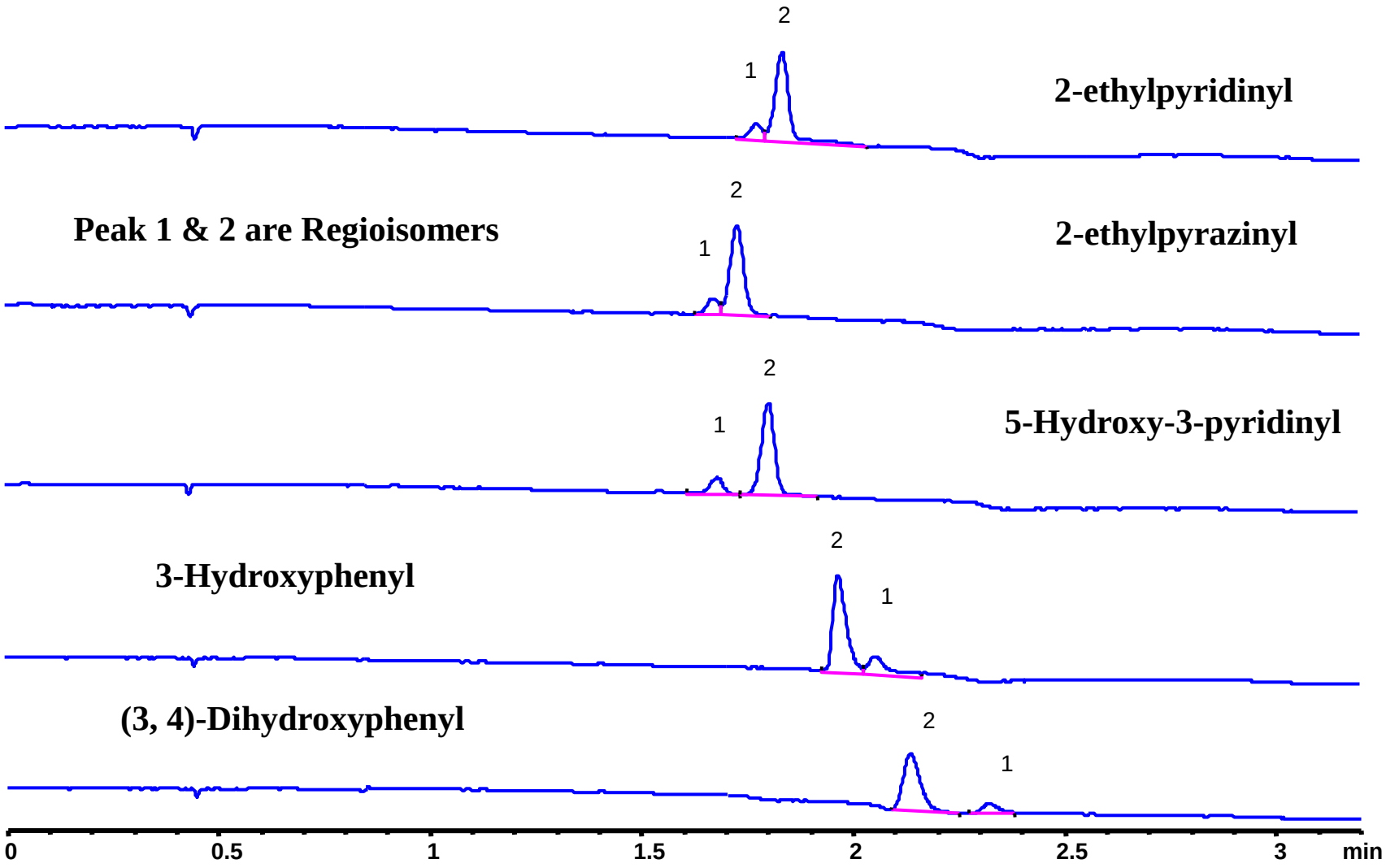


PF on custom SFC columns

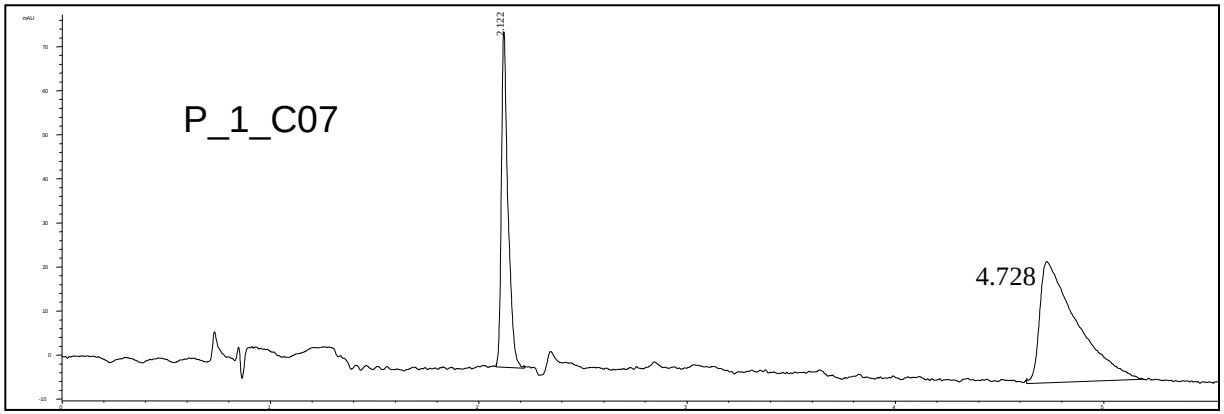


5 – 50% methanol gradient

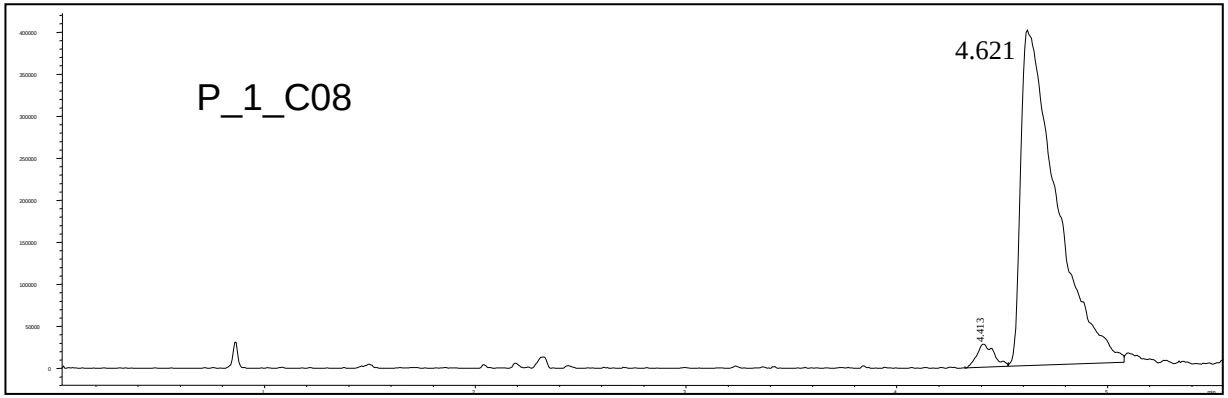
Selectivity differences between Pyridinyl- & Hydroxyl-based phases



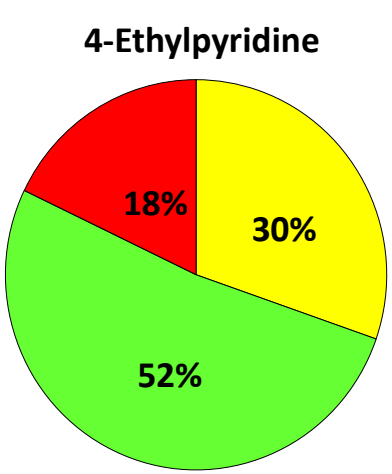
Selectivity's not the only difference

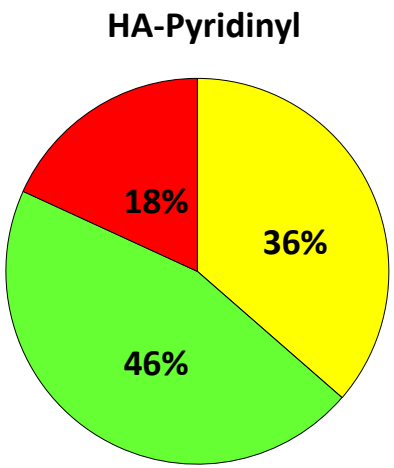


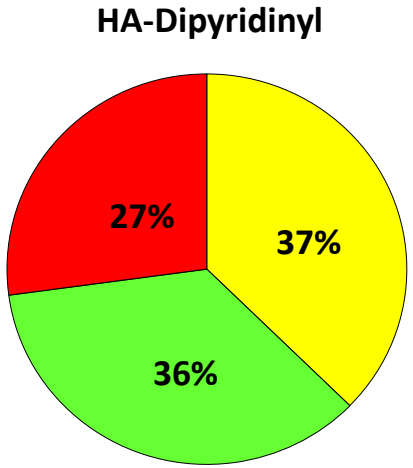
Zymorsphere Diol, 150 x 4.6mm; 5µ particles
5.6mL/min; 140bar
Methanol gradient 5-50% in 5 min



Tailing Factor – Pyridinyl Columns



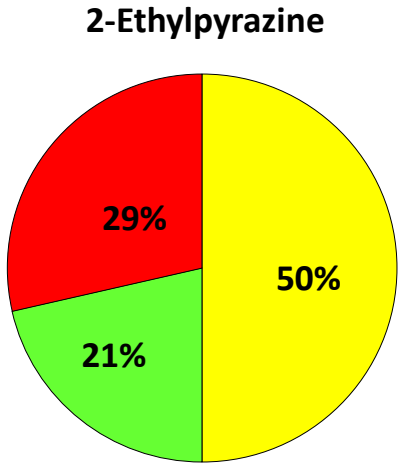


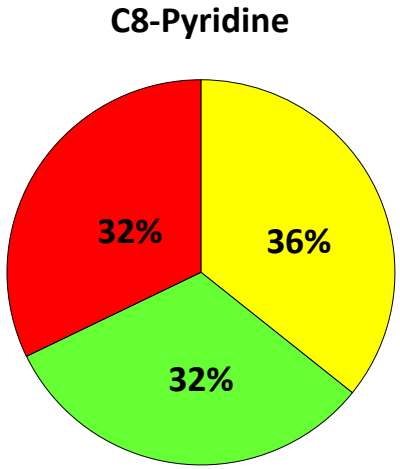


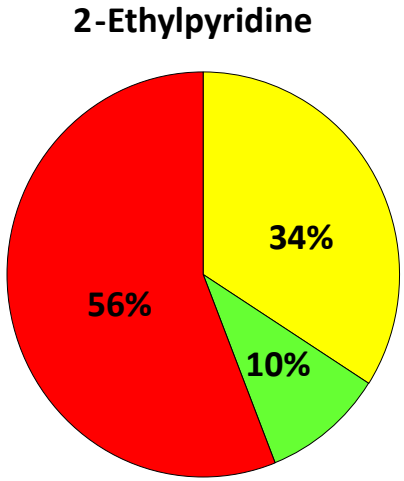
0.9 – 1.1

0.8 – 0.9
1.1 – 1.2

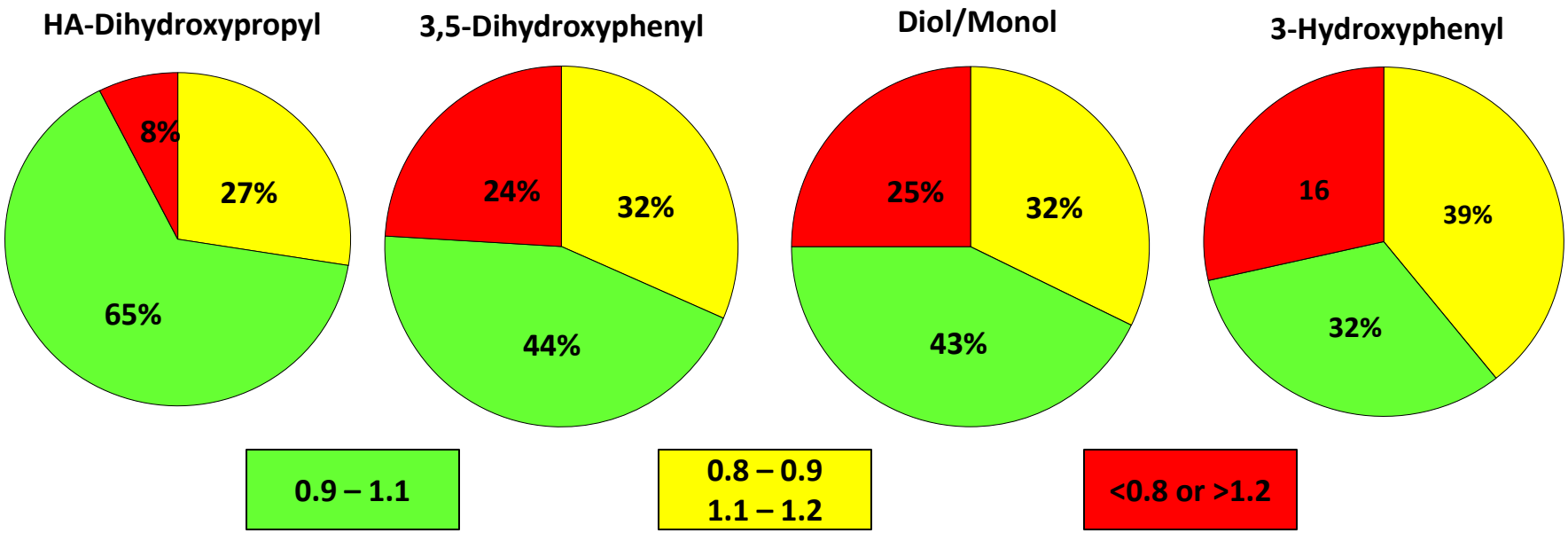
<0.8 or >1.2





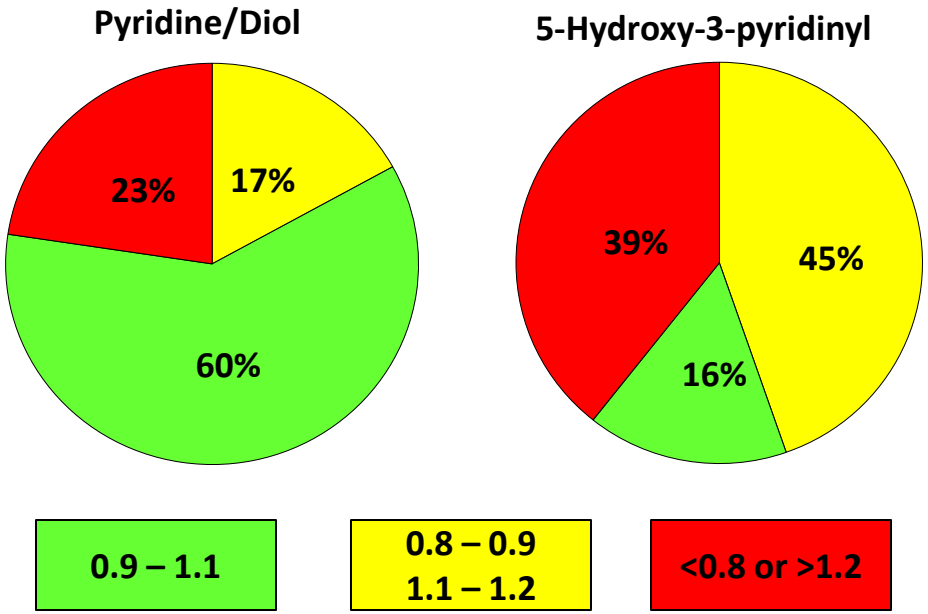


Tailing Factor – Hydroxyl Columns



Tailing Factor

Hydroxyl – Pyridinyl Columns



Results Summary for 77 PF compounds

Best

Worst

Column	Acceptable Tailing (%)
HA-Dihydroypropyl	92
3-Hydroxyphenyl	84
4-Ethylpyridine	82
HA-Pyridinyl	82
Pyridine/Diol	77
3,5-Dihydroxyphenyl	76
Diol/Monol	75
HA-Dipyridinyl	73
2-Ethylpyrazine	71
C8-Pyridine	68
5-Hydroxy-3-pyridinyl	61
2-Ethylpyridine	44

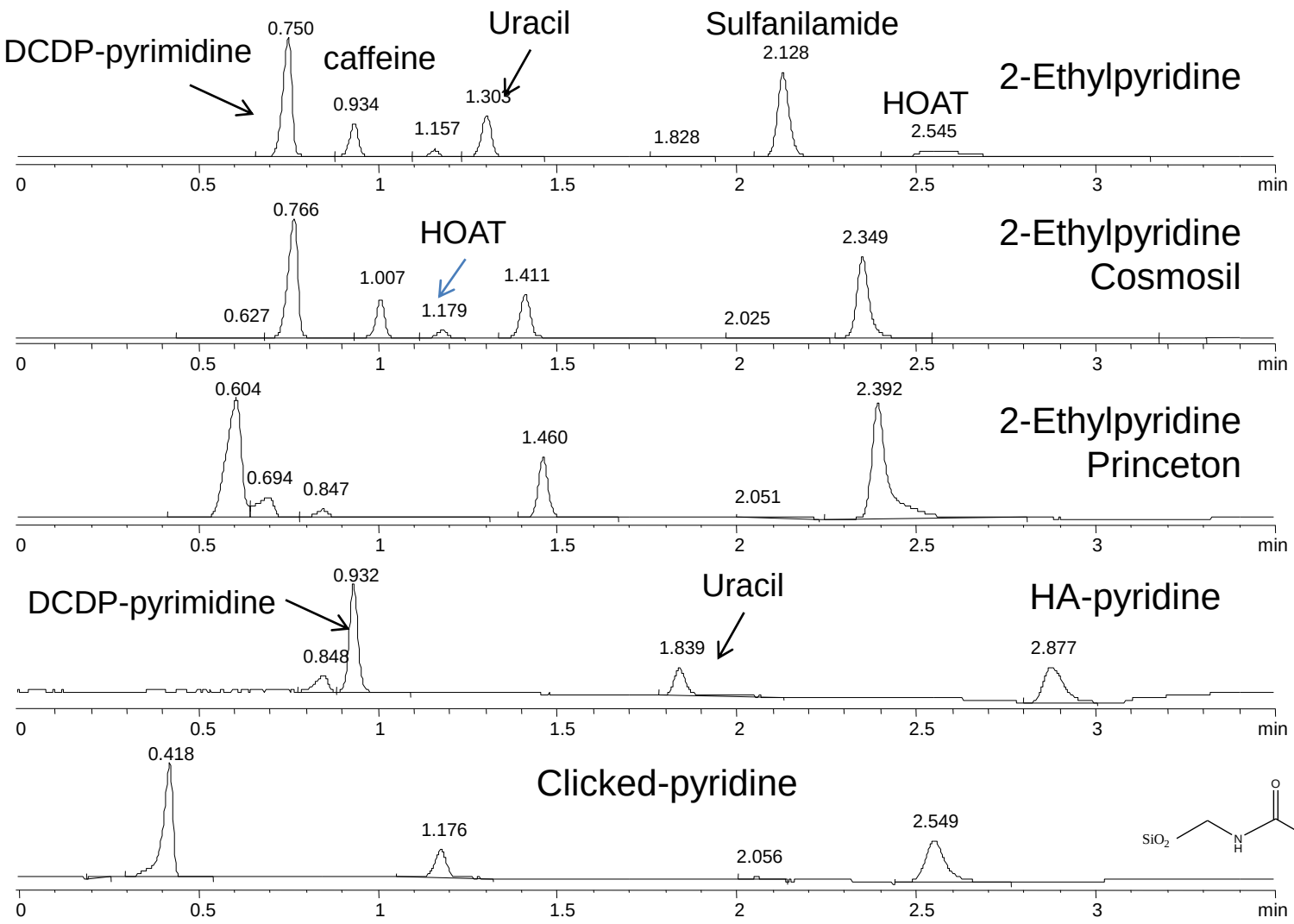
Retention Time Correlation Coefficients (R)	4-Ethyl pyridine	C8-Pyridine	Diol/Monol	HA-Dihydroxy propyl	HA-Dipyridinyl	HA-Pyridinyl	Pyridine/Diol	2-Ethyl pyrazine	2-Ethyl pyridine	5-Hydroxy-3-pyridinyl	3-Hydroxy phenyl
4-Ethylpyridine											
C8-Pyridine	0.682										
Diol/Monol	0.764	0.974									
HA-Dihydroxypropyl	0.928	0.749	0.799								
HA-Dipyridinyl	0.942	0.661	0.728	0.959							
HA-Pyridinyl	0.954	0.691	0.770	0.953	0.982						
Pyridine/Diol	0.914	0.793	0.843	0.938	0.938	0.962					
2-Ethylpyrazine	0.932	0.808	0.876	0.906	0.909	0.958	0.949				
2-Ethylpyridine	0.950	0.827	0.877	0.919	0.937	0.979	0.970	0.981			
5-Hydroxy-3-pyridinyl	0.907	0.898	0.942	0.912	0.846	0.893	0.914	0.953	0.924		
3-Hydroxyphenyl	0.763	0.917	0.946	0.818	0.692	0.744	0.797	0.880	0.860	0.961	
3,5-Dihydroxyphenyl	0.541	0.900	0.882	0.600	0.526	0.564	0.618	0.665	0.648	0.772	0.838

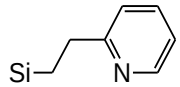
Outline

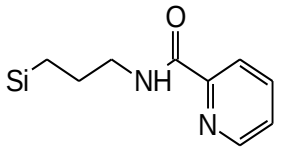
Rationale for new phases based on Pyridine data

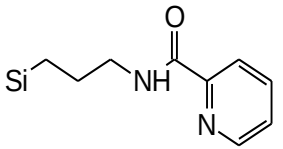
- ❑ Change position of N
  - — E.g. 2-ethyl to 4-ethyl
- ❑ Introduce non-acidic OH
- ❑ Explore similar aromaticity, different basicity
  - — Pyrimidine, piperazine, piperadine, quinoline, morpholine
- ❑ **Lengthen aliphatic chain**
  - — **Opens up huge chemical space with propyl chain**
  - — **Retention tuning**
- ❑ Additive effects

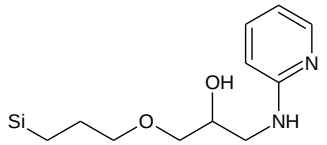
Pyridine phase with modified linker

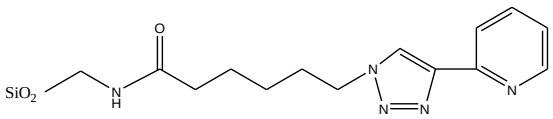












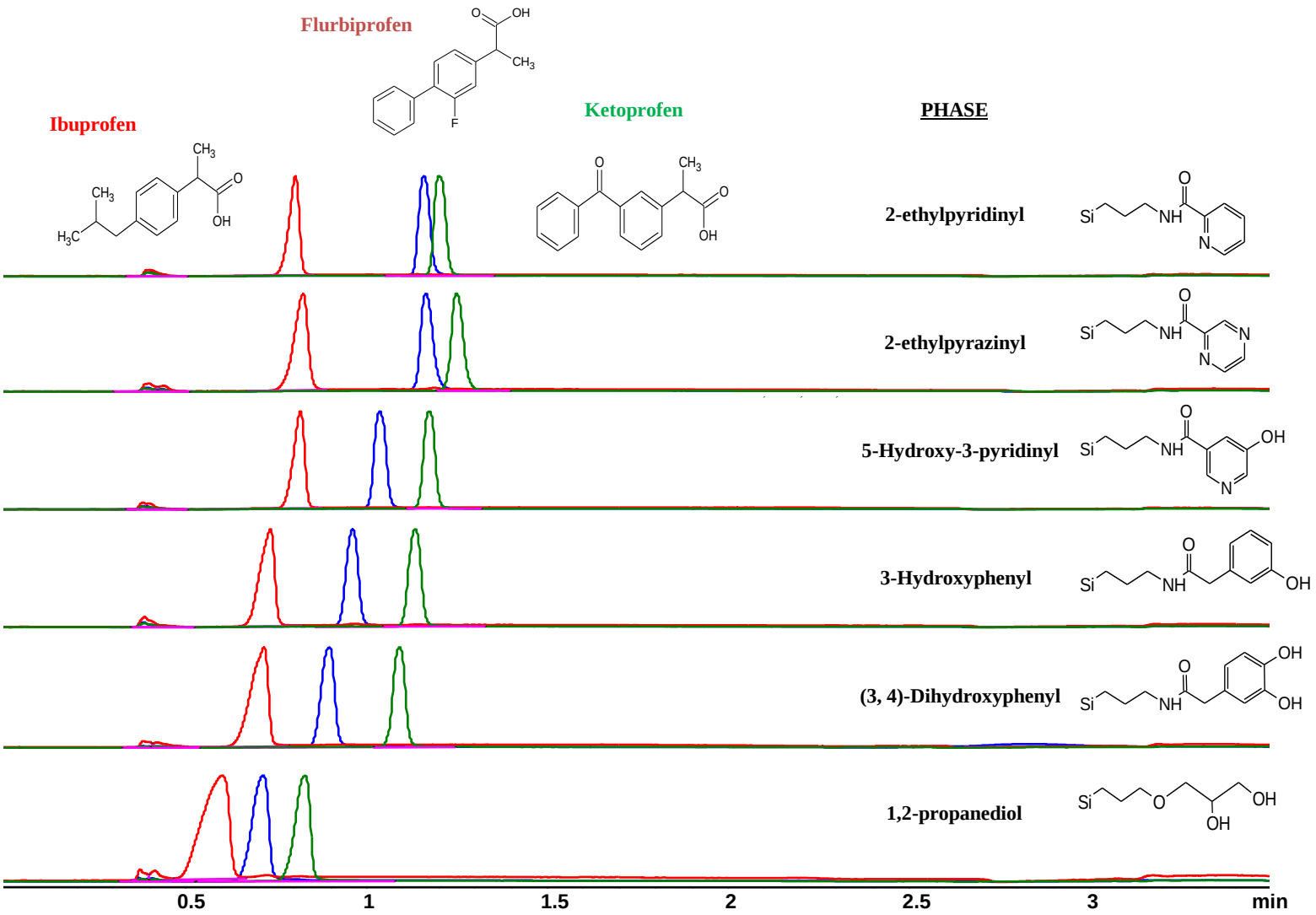
150 x 4.6 mm; 5micron; 5.6mL/min methanol gradient (5-50% @ 18%/min)

Outline

Rationale for new phases based on Pyridine data

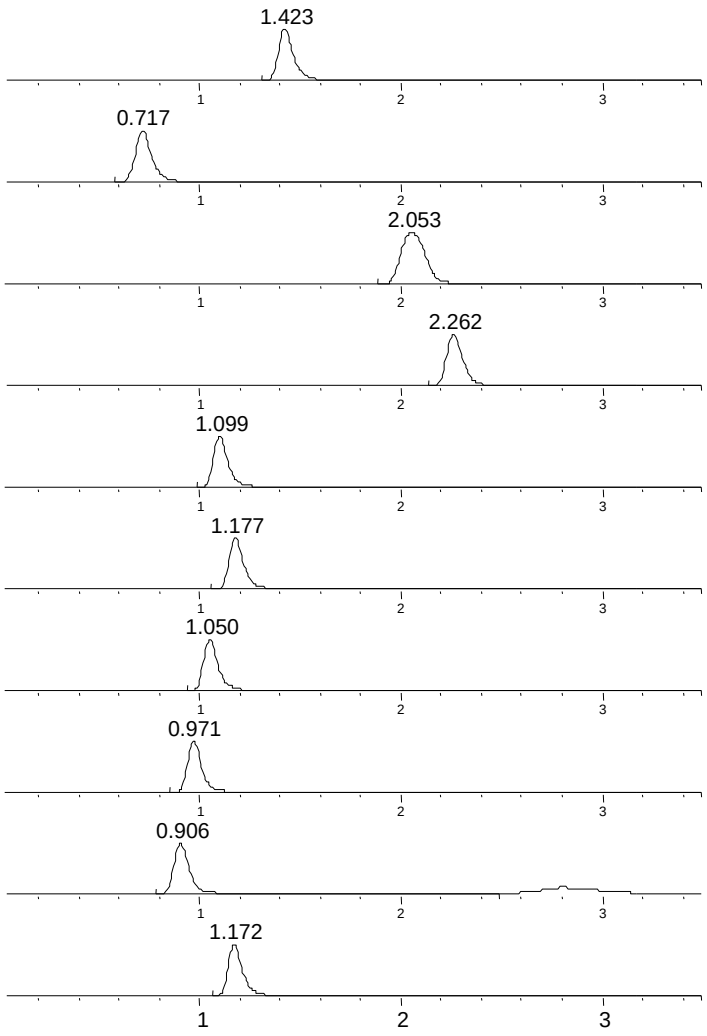
- Change position of N
  - E.g. 2-ethyl to 4-ethyl
- Explore similar aromaticity, different basicity
  - Pyrimidine, piperazine, piperadine, quinoline, morpholine
- Lengthen aliphatic chain
  - Opens up huge chemical space with propyl chain
  - Retention tuning
- Introduce non-acidic OH
- **Additive effects**

Separation of the 3 acidic components on each phase.



Effect of additive on Flurbiprofen

Methanol Only



4-Ethylpyridine

Diol/Monol

HA-Dipyridinyl

HA-Pyridinyl

Pyridine/Diol

2-Ethylpyrazine

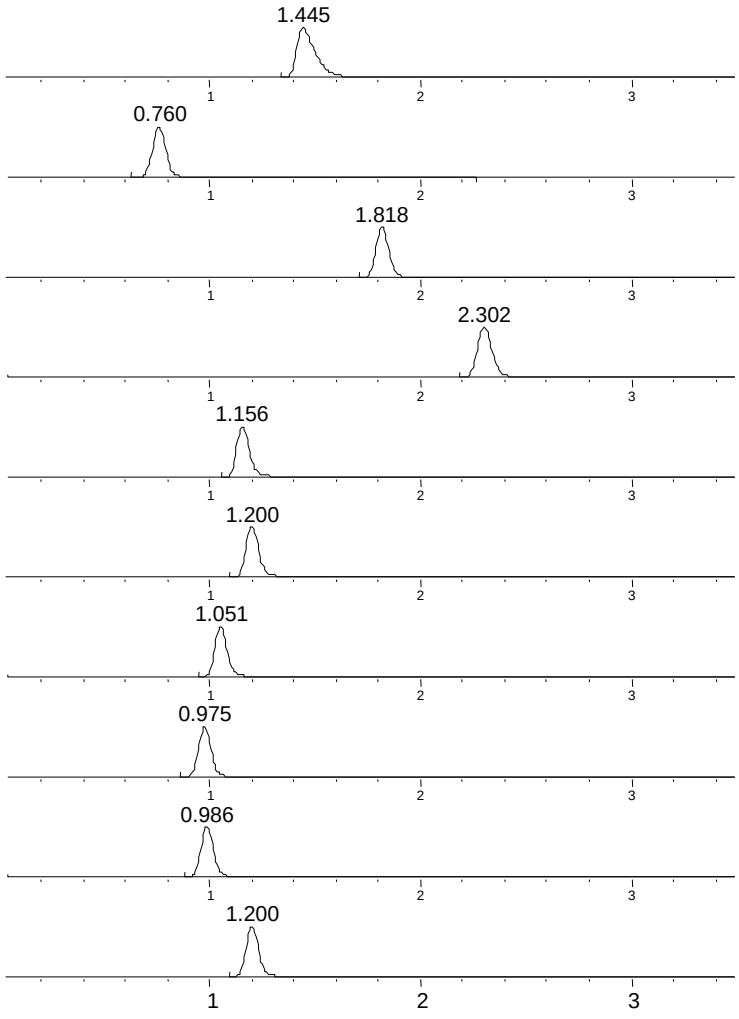
5-Hydroxy-3-pyridine

5-Hydroxyphenyl

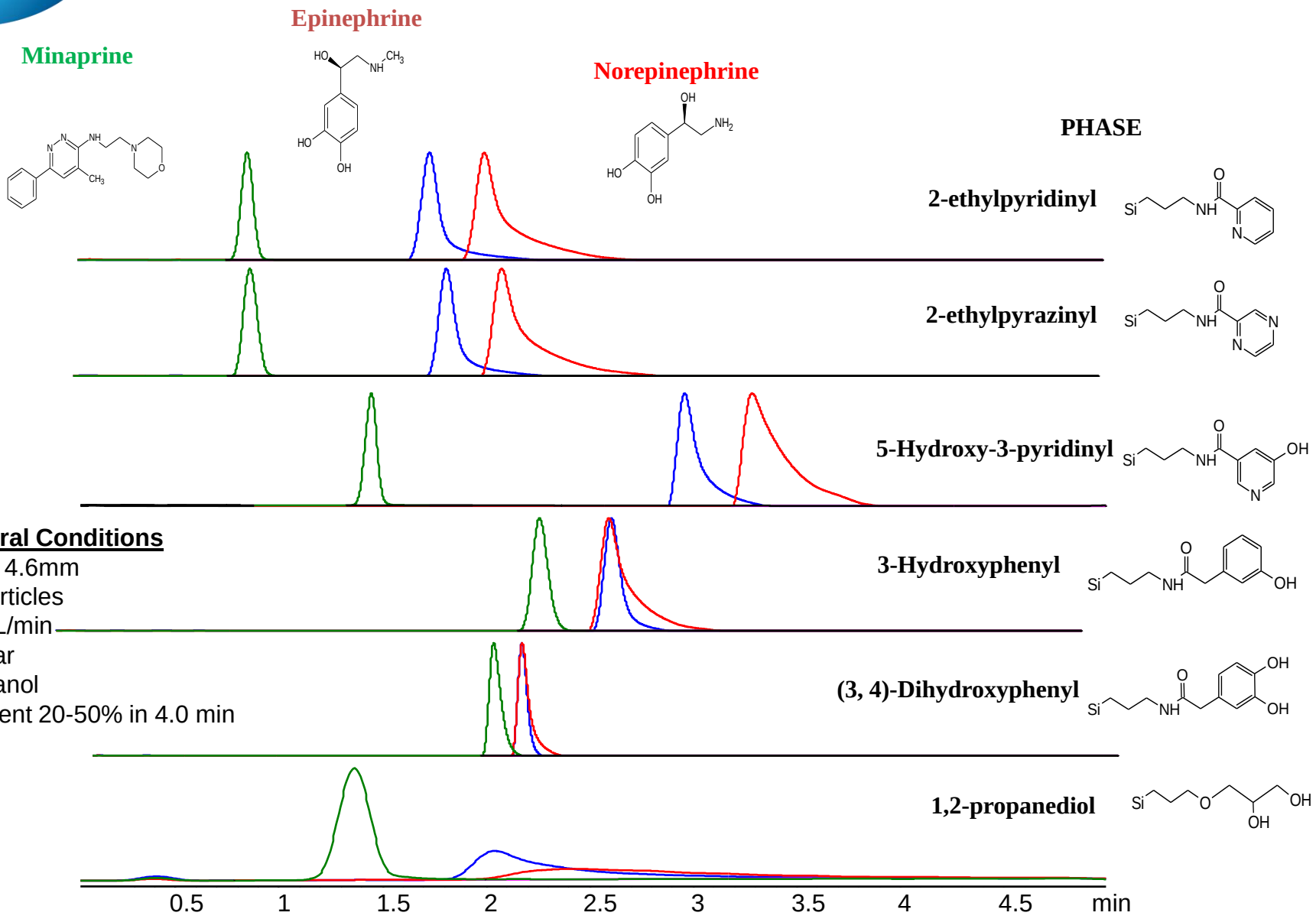
3,5-Dihydroxyphenyl

2-Ethylpyridine

20mM Ammonium Formate In Methanol



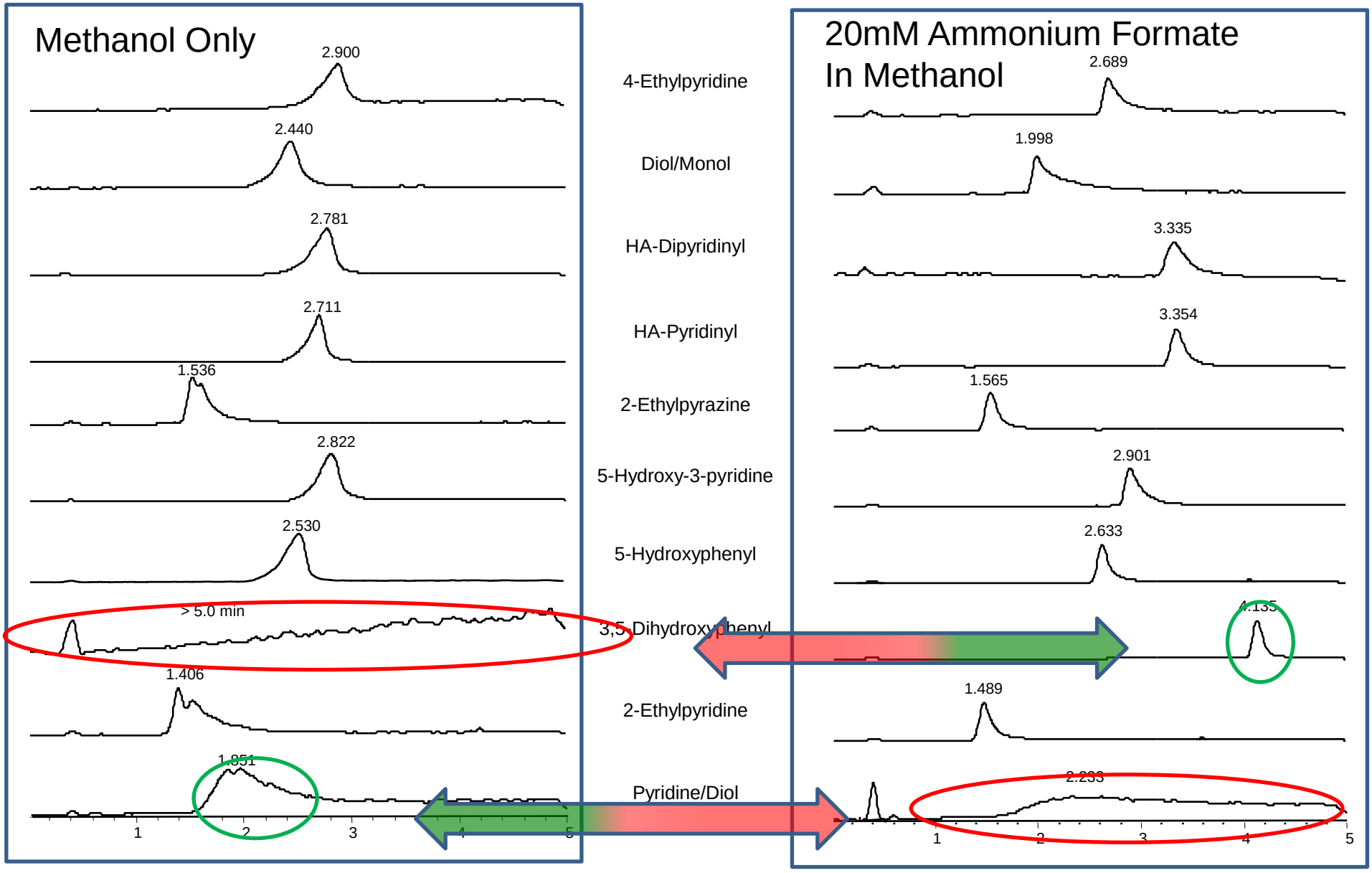
Separation of the 3 basic components on each phase



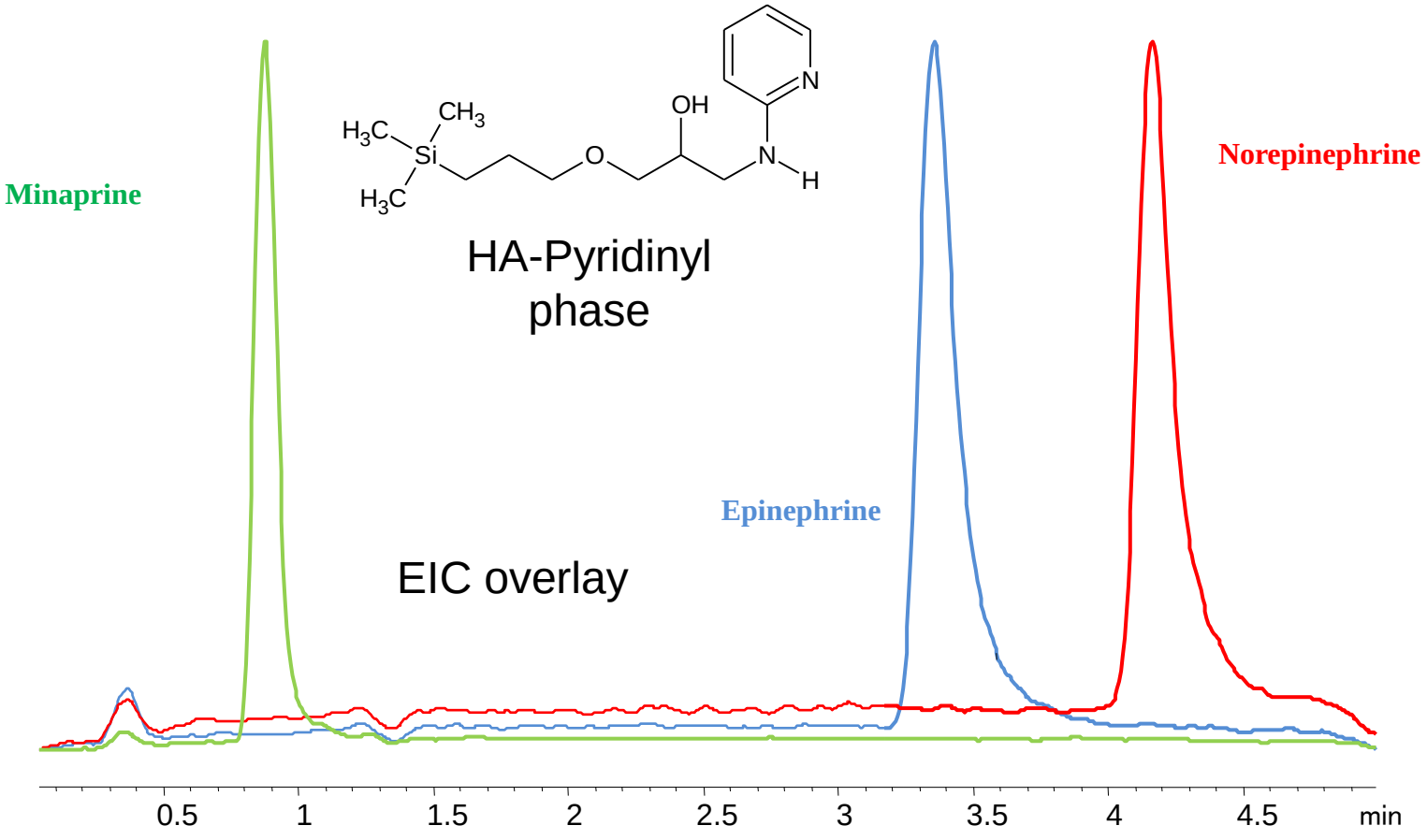
General Conditions

150 x 4.6mm
5µ particles
5.6mL/min
140bar
Methanol
Gradient 20-50% in 4.0 min

Effect of additive on Epinephrine



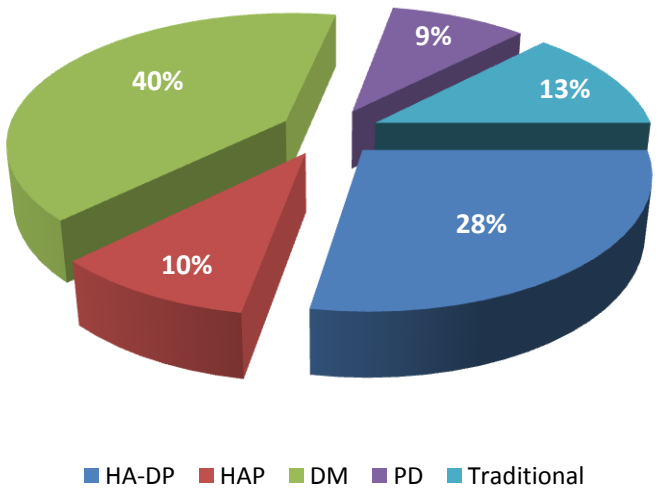
Basic columns for basic solutes

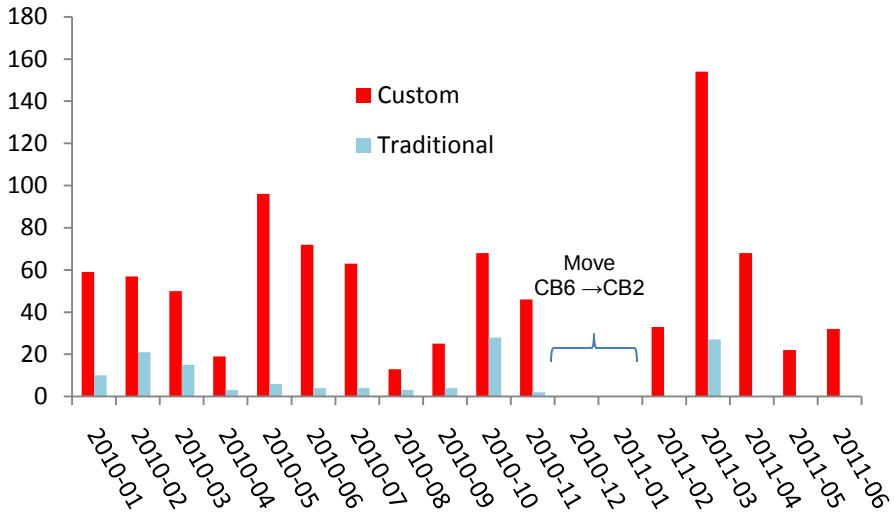


4.6 x 150mm i.d. column
20 mM Ammonium Formate in Methanol
4mL/minute flow rate @ 140 bar
20 – 50% Solvent Gradient in 5 minutes

Summary

87% of PF's purified by SFC used Custom Columns





Last 1000 PF's Since Jan 2010

Traditional
2-Ethylpyridine
4-ethylpyidine
1,2-Propanediol

★ Includes 3 libraries of structurally similar compounds

Column Classifications
Choosing a column

“Basic” Analytes

HA-Dipyridinyl

HA-Pyridinyl

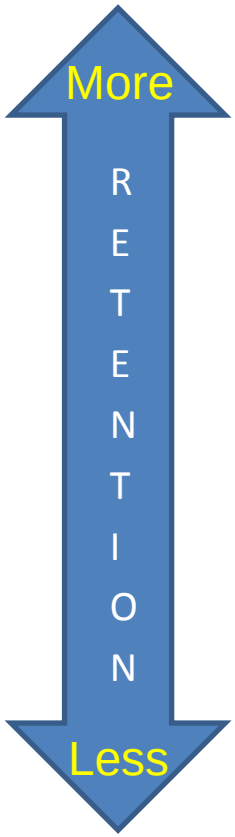
4-Ethylpyridine

Pyridine/Diol

2-Ethylpyridine

2-Ethylpyrazine

HA-Dihydroxypropyl



“Acidic” Analytes

3,5-Dihydroxyphenyl

3-Hydroxyphenyl

5-Hydroxy-3-pyridinyl

Diol/Monol

C8-Pyridine

Future Directions

- Continue to develop new phases
  - Fill in the gaps
  - Develop mechanistic model for retention
- Utilize existing chromatographic data for *in silico* prediction
  - Exploring ACD Labs Chrom Genius software
- Apply SFC to new areas
  - Molecular properties
  - Metabolism, phosphorylated sugars, biomarkers, etc

Acknowledgements

Pfizer LJ

- Chemistry
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  - Pat Sandra
  - Melissa Dunkle
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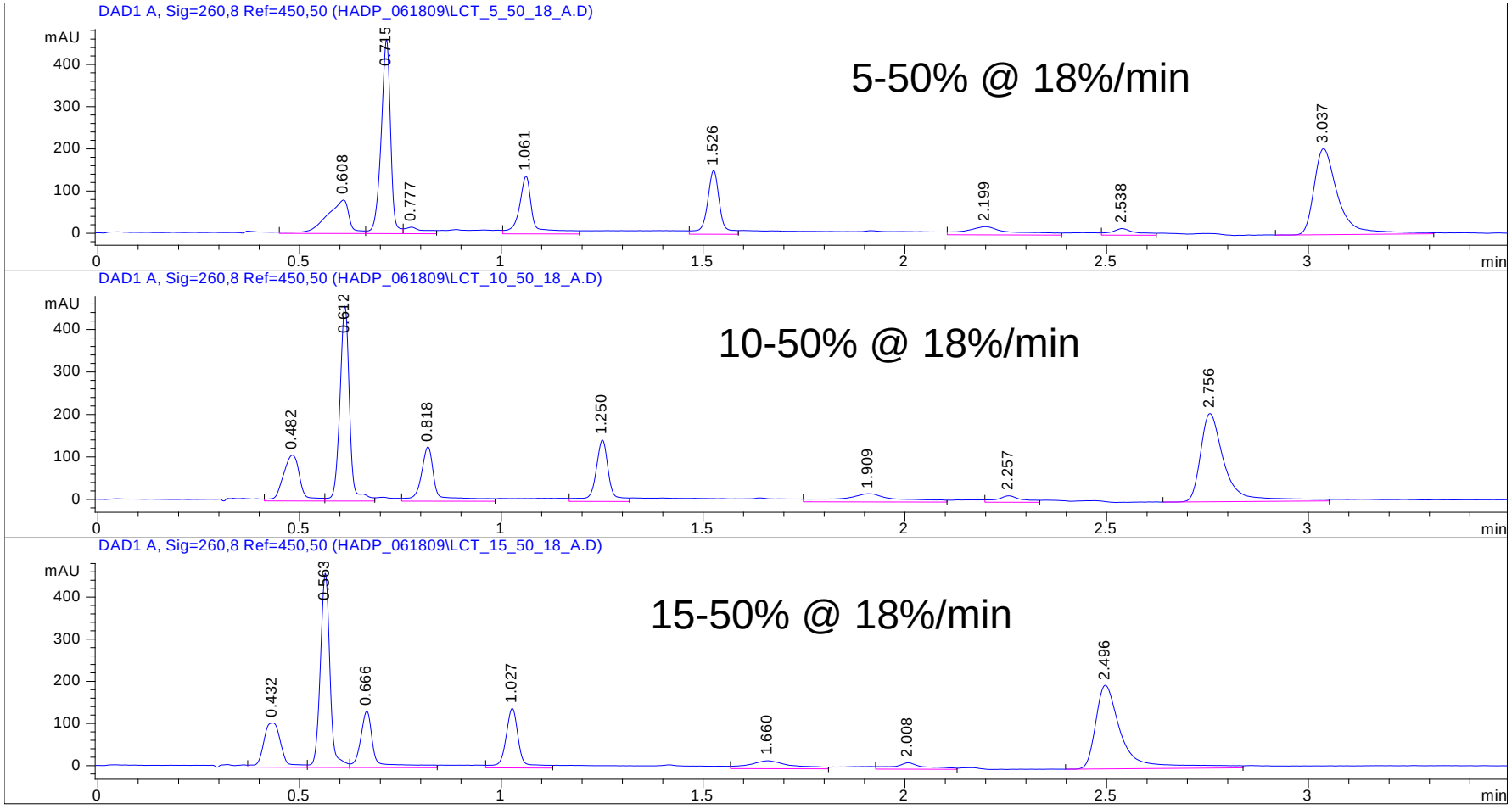
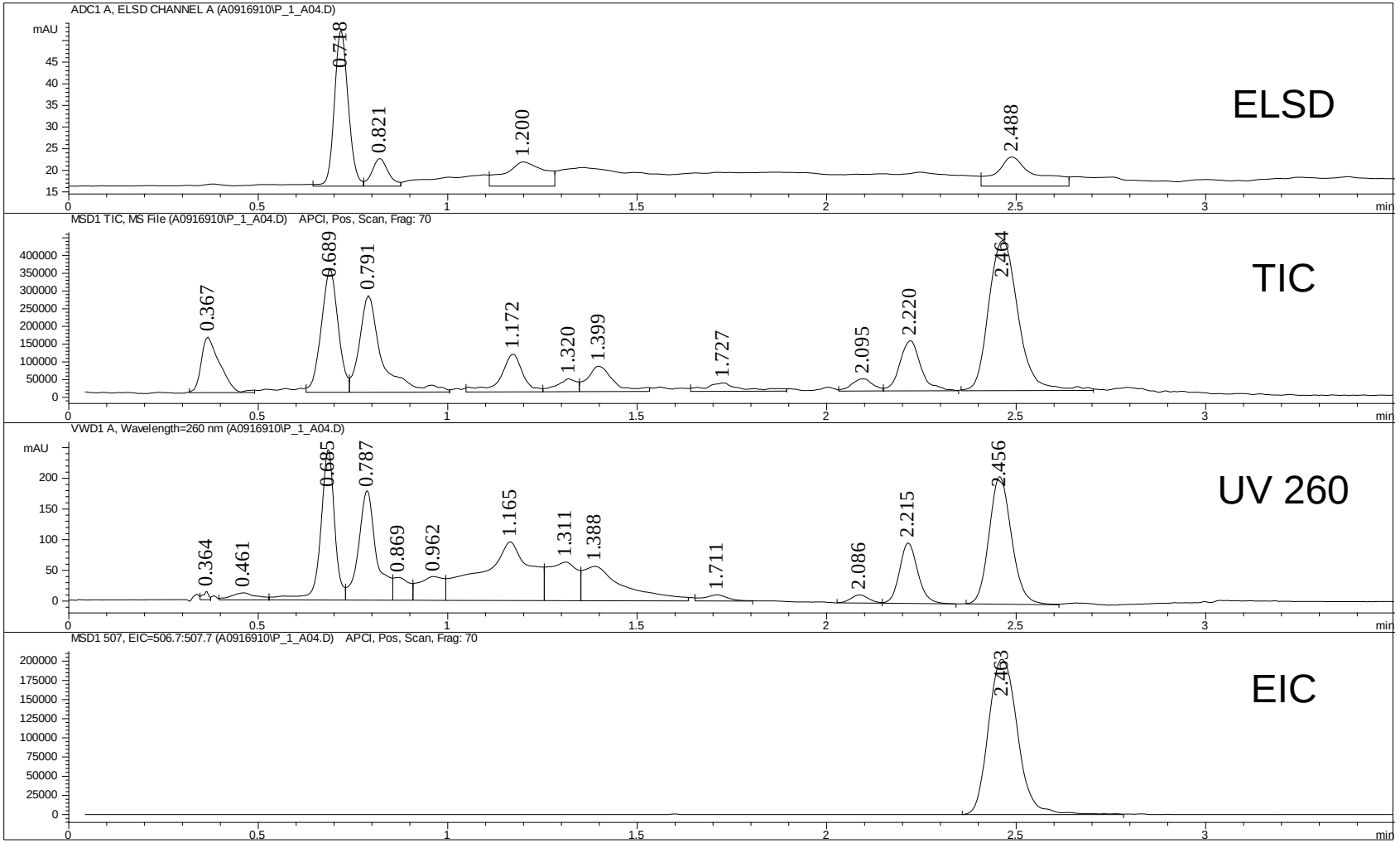
- Zymor – Emmanuel Lambridis
- Princeton Chromatography
  - Walton Caldwell
  - Jeff Caldwell
- Nacalai USA
  - Toshi Ono

Questions

HADP Gradient effects

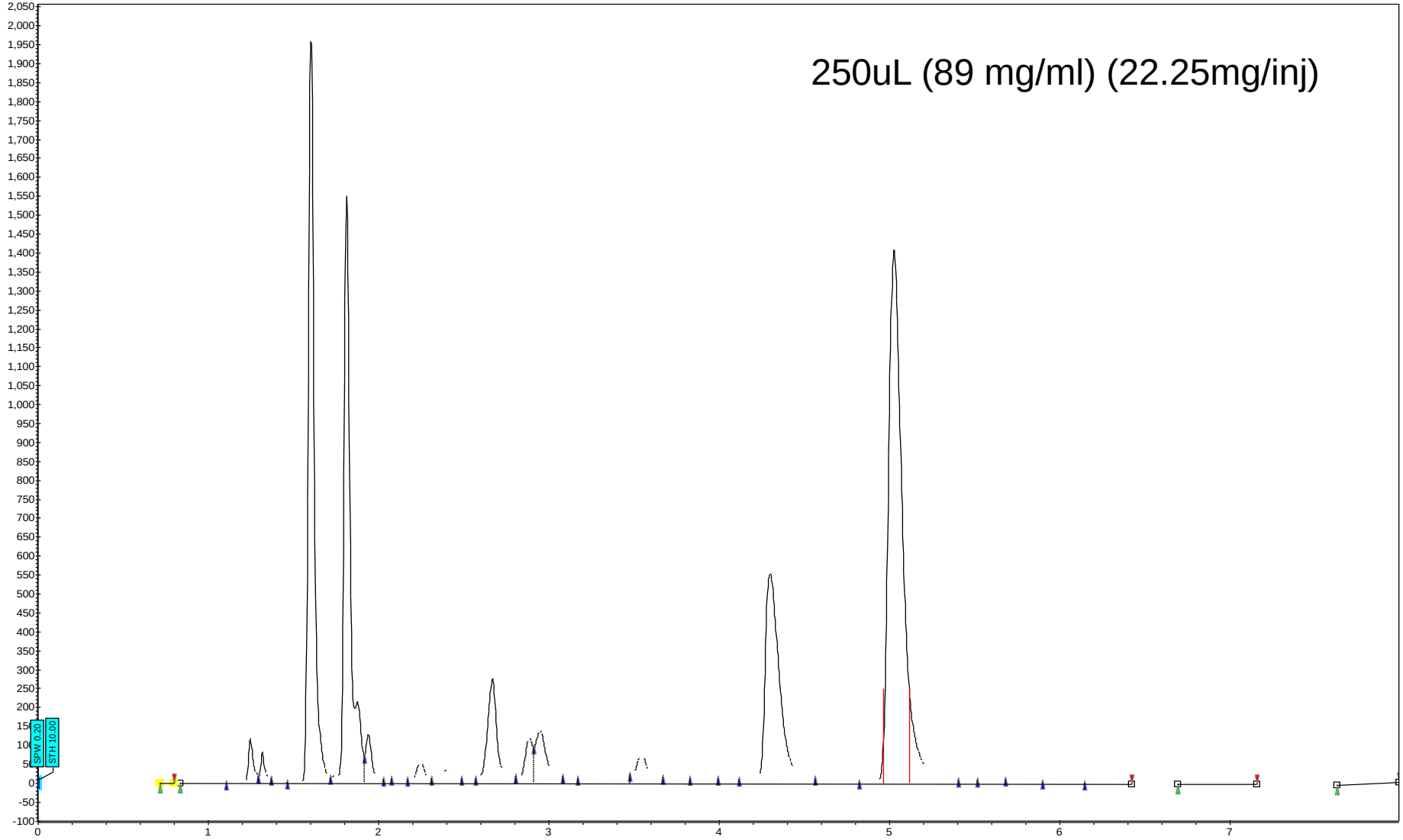


Analytical on HADP



4.6x150mm HADP;10-50% MeOH @ 18%/min; 5.6ml/min

Purification on HADP



21x150mm HADP column; 10-50% MeOH @ 9%/min; 60mL/min

Explorative phases

Collaborators…

Commercial Vendors

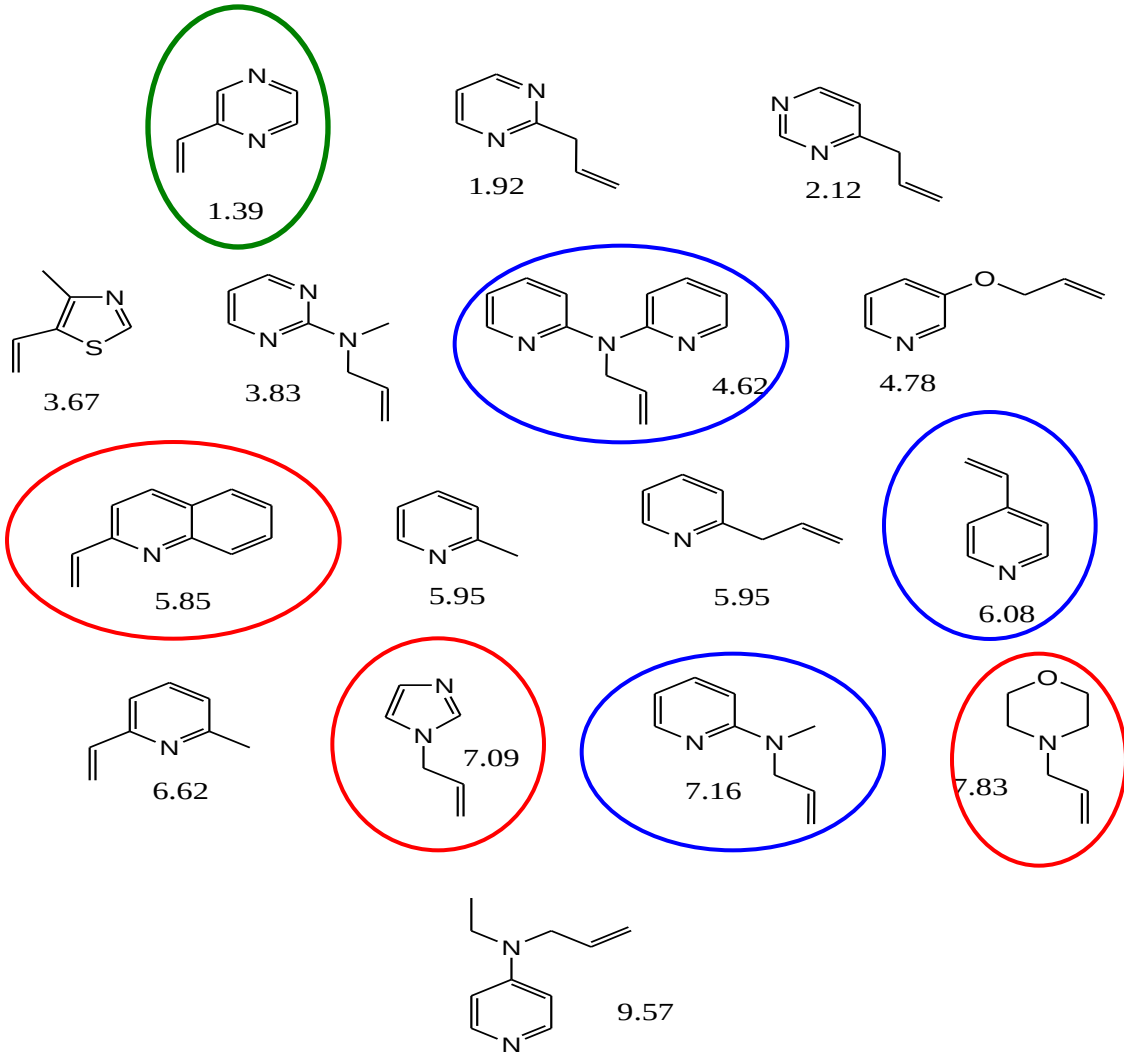
- Princeton Chromatography
- Nacalai USA

Academic – PARC

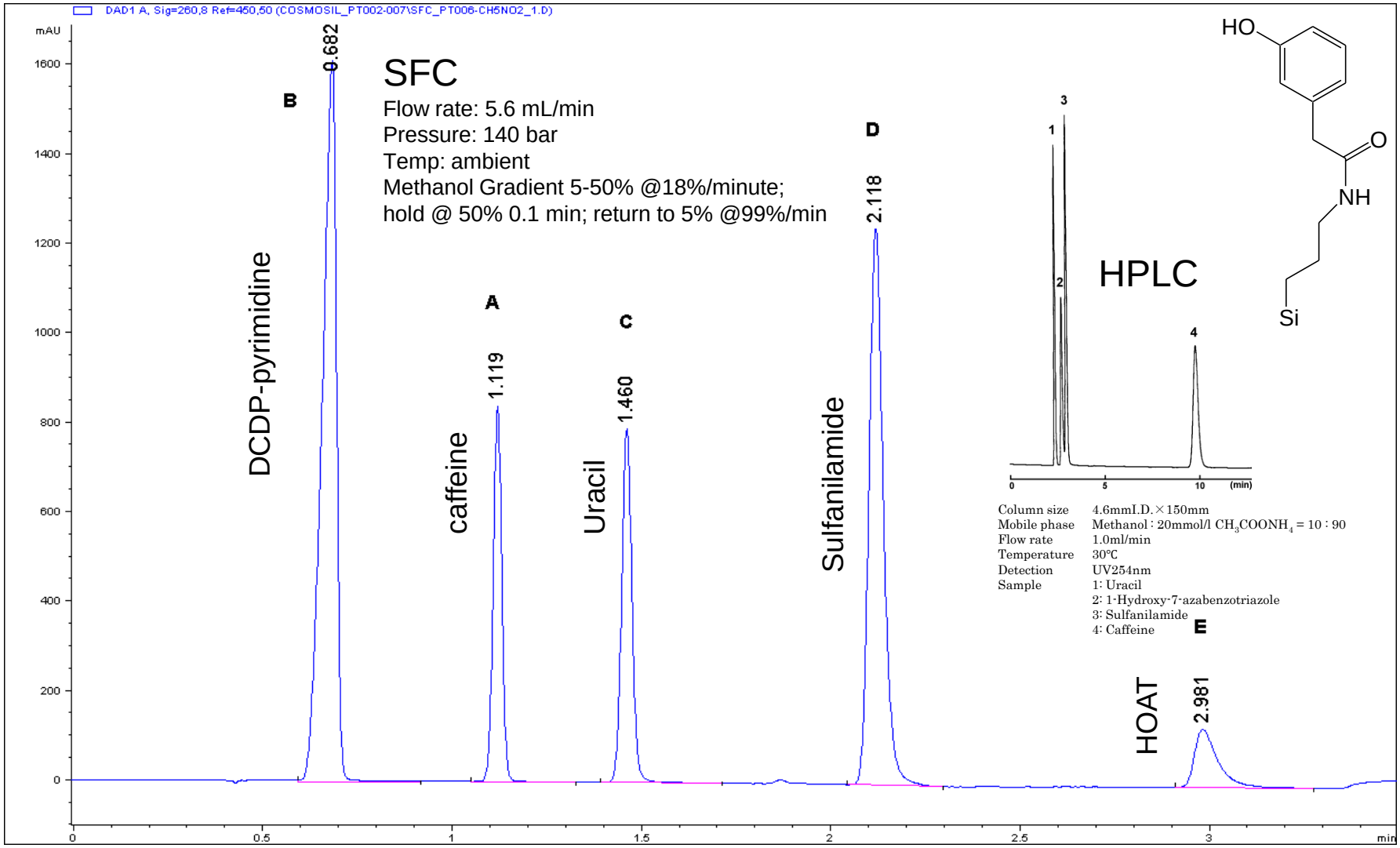
Pat Sandra & Melissa Dunkle

(2 year post-Doc project)

- Synthesize and characterize phases using Click Chemistry

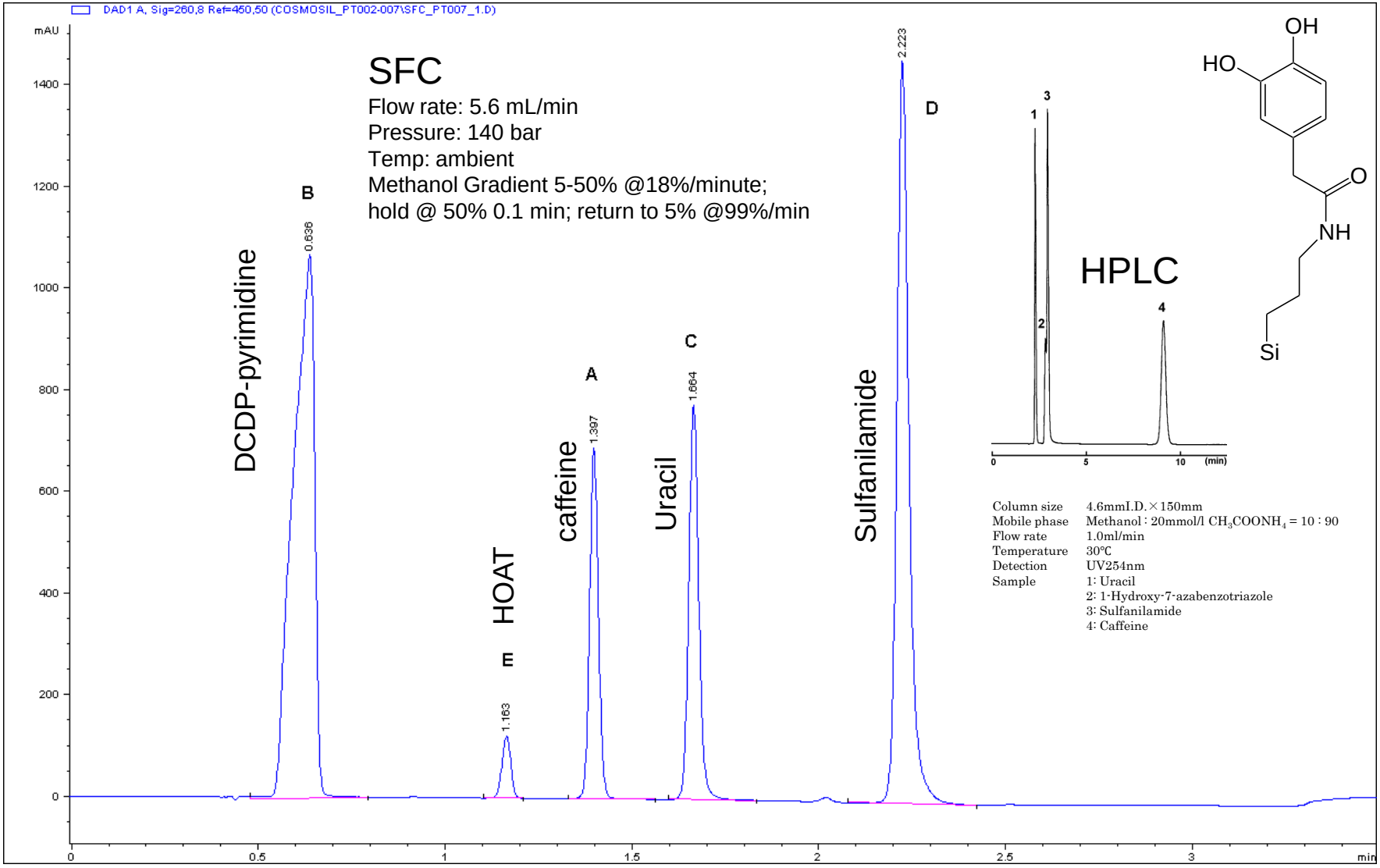


Phenol Phase

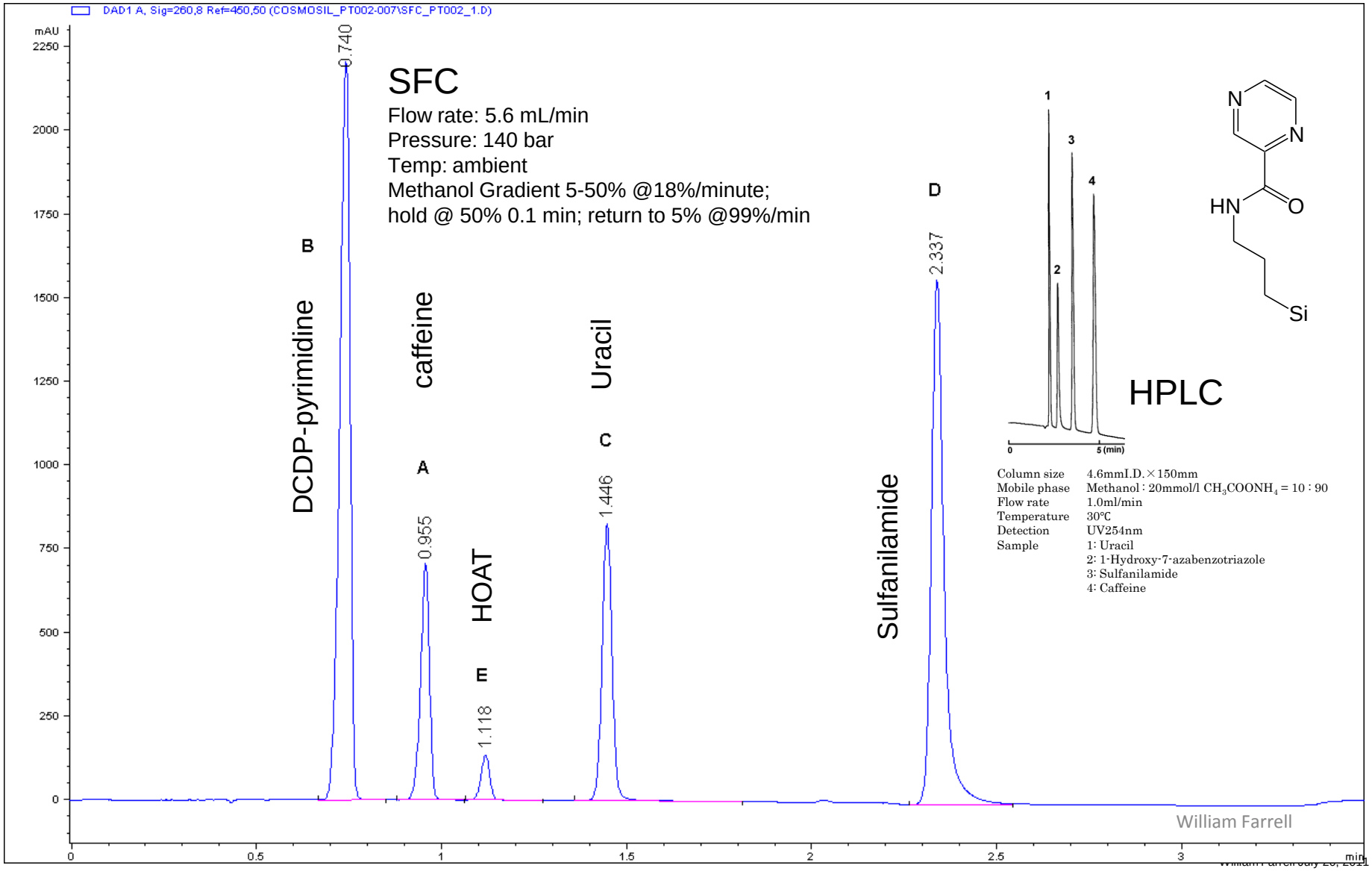


MeOH with 20mM Ammonium Formate

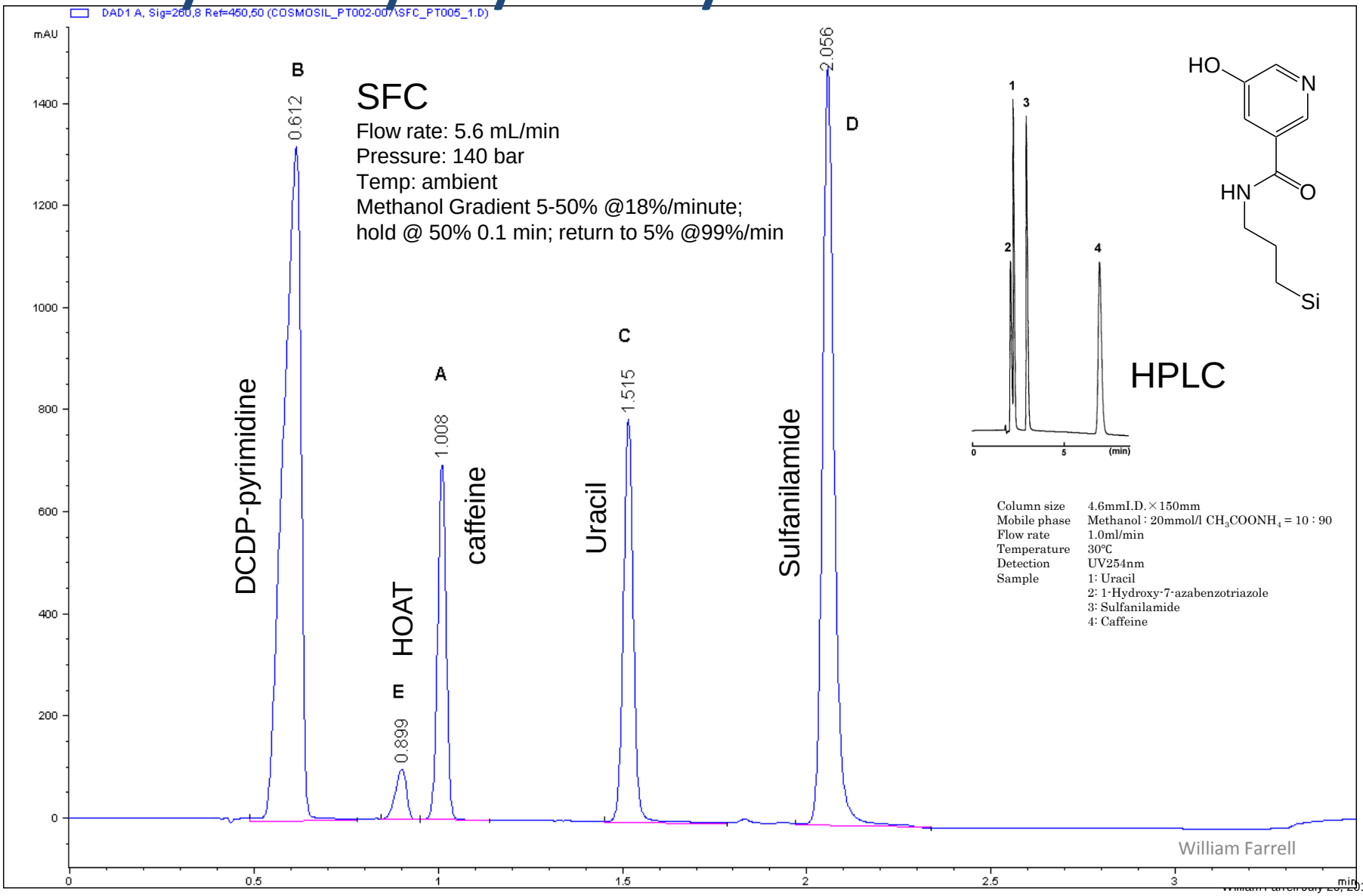
Catechol Phase



Pyrazinyl Phase



Hydroxy Pyridinyl Phase



Pyridinyl Phase