Toward Green and Sustainable Hydrophilic Interaction Liquid Chromatography (HILIC) Workflows: Can We Replace Acetonitrile? Are There Alternative Modes?

David S. Bell*[1], Jan Pschierer[2] and Sandra Ruiz Perez[2]

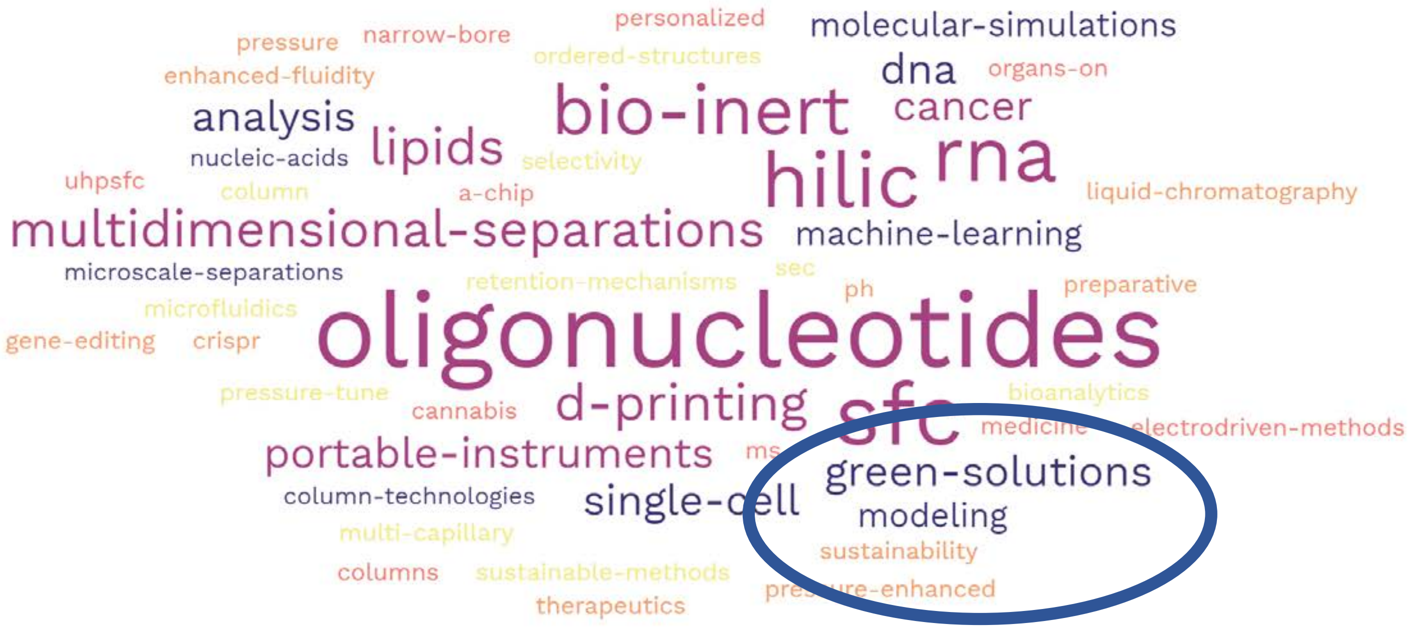
[1]Restek Corporation
110 Benner Circle, Bellefonte, PA, USA 16823
[2] Restek GmbH, Schaberweg 23, 61348 Bad Homburg v.d.H., Germany

Andrew Alpert, (1951-2023)

- Father of HILIC, inspirational
- Always game for a fundamental discussion
- A solid human
- Contributor

http://labs.wisc.edu/gelab/people.html, Ying Ge, PhD
University of Wisconsin, Madison

Green and Sustainable Analytical Chemistry



Cory Muraco, David S. Bell
LCGC North America, September 2022, Volume 40, Issue 9
Pages: 417–423
https://doi.org/10.56530/lcgc.na.ws5783s4

Green Hydrophilic Interaction Liquid Chromatography (HILIC)

HILIC is a useful technique for polar analytes that do not retain well by reversed-phase

Uses high percentages (typically >70%) acetonitrile

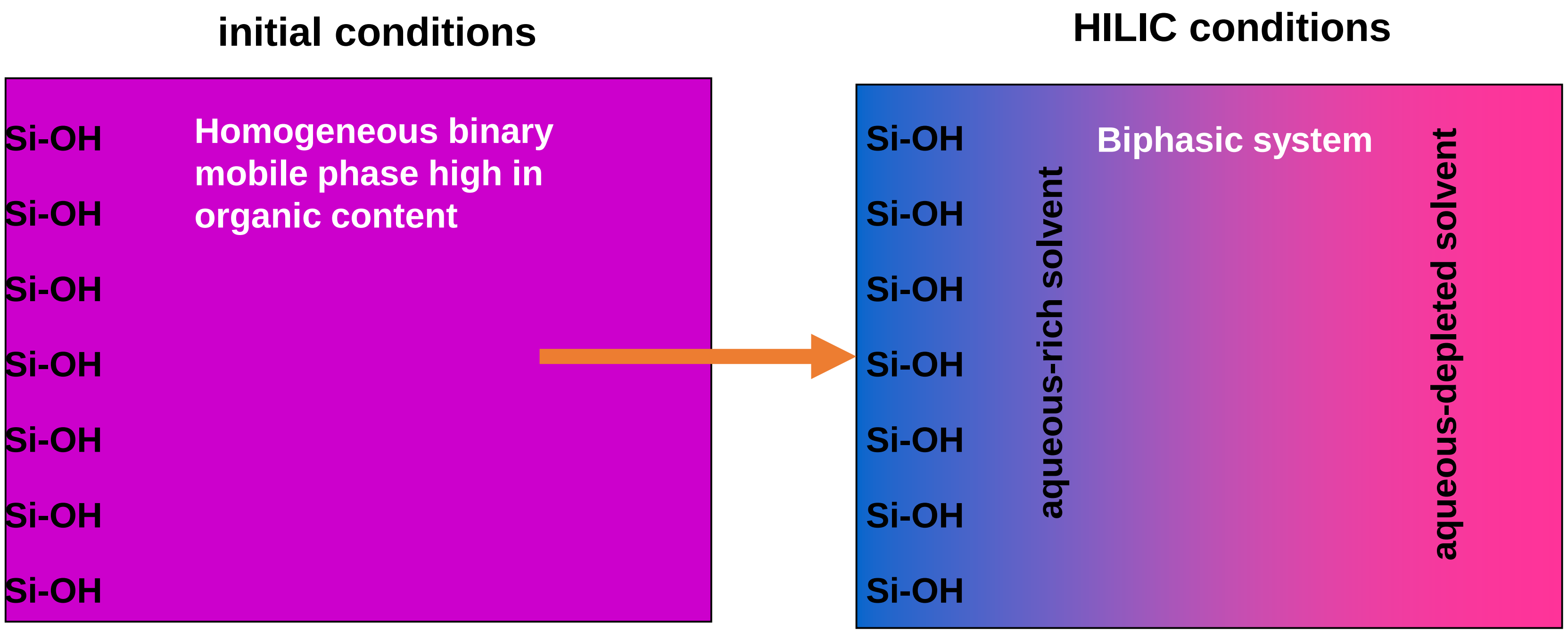
‘problematic’

‘not bio-based’

Can one obtain HILIC or HILIC-Like separations without the use of toxic or environmentally unfriendly solvents?

- Directly substitute other ‘green’ or ‘greener’ solvents for acetonitrile
- Use greener alternative modes of chromatography
- Minimize impact of current practices

Biphasic Solvent Distribution at HILIC Phase Surface



Simplistic cartoon showing the preferential solvation of water on the polar surface. Upon equilibration a biphasic system develops whereby polar solute can partition from the primarily organic mobile phase to the aqueous rich layer adsorbed on the surface

D. Bell, *LCGC North America, 33 (2)*

Organic Solvent as a Variable "In HILIC"

Acetonitrile = most common organic solvent used in HILIC

Acetonitrile + Water = 

Mountain, R. D., J. Phys. Chem. A 1999, 103, 10744-10748

Methanol is also a common additive found in the literature and in applications

Methanol + Water = 

D. Bell, The Impact of Methanol on Hydrophilic Interaction Liquid Chromatography (HILIC) Retention Mechanisms – A Systematic Approach, 50th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2022), San Diego, CA

Objective

Investigate several ‘green’ and/or ‘sustainable’ solvents as substitutes for acetonitrile in HILIC separations

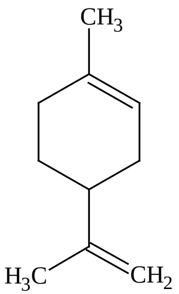
Initial set of solvents tested:

- **recommendations vary**
- **subset of ‘recommended’ solvents/not exhaustive**

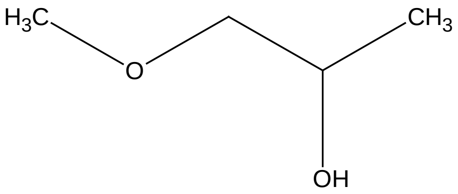
Chemical	CAS		Volume	Price	Normalized Price
Ethyl Lactate, 500 mL	97-64-3		500mL	$ 56.00	$112/L
Dimethyl Carbonate	616-38-6		500mL	$ 39.00	$78/L
Dimethyl Isosorbide	5306-85-4		1L	$ 150.00	$150/L
Cyrene	53716-82-8		1L	$ 216.00	$216/L
1-methoxy-2-propanol	107-98-2		500mL	$ 21.00	$42/L
(R)-(+)-Limonene, 97%	5989-27-5		500mL	$ 94.70	$189.40/L

Acetonitrile: $229/L

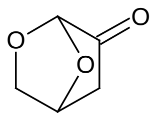
Solvent Structures



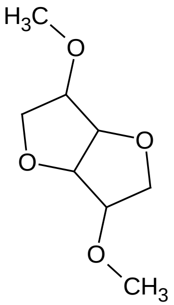
Limonene



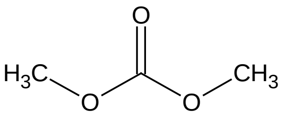
1-methoxy-2-propanol



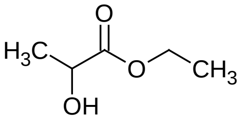
Cyrene(TM)



dimethyl isosorbide



dimethyl carbonate



Ethyl (S)-(-)-lactate

Solubility – Can I make a HILIC Mobile Phase?

As a first pass to select potential solvents, solubility of 10 mM ammonium formate in water at a 1:9 water:solvent ratio was investigated

Results

- X Cyrene and limonene both showed immiscibility with water/ammonium formate
- X Dimethyl isosorbide and dimethyl carbonate showed some miscibility, but the prospects are low for them as a major mobile phase component
  - High viscosity
  - High UV absorbance (maybe MS?)
- ✓ 1-methoxy-2-propanol showed full miscibility and what appeared to be reasonable density (and likely low uv absorption) and was deemed most likely
- ✓ Ethyl lactate also showed miscibility and is thus and additional contender

https://www.nottingham.ac.uk/news/green-chemistry-process

Mobile Phase Study

1-methyl-2-propanol (1M2P) was initially used to prepare a mobile phase consisting of 10 mM ammonium formate in 10:90 water:1M2P

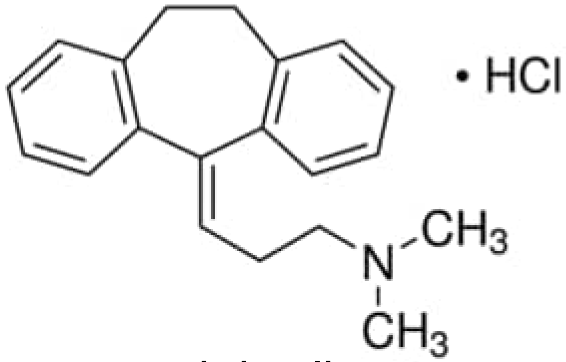
A HILIC mix consisting of amitriptyline, benzoic acid, benzylamine, cytosine, naproxen, and toluene sulfonic acid along with each individual were injected

- Column: Raptor HILIC-Si – 10 x 2.1, 2.7 µm
- Flow rate: 0.170 µL/min*
- Injection volume: 2 µL
- Column Temperature: 35℃

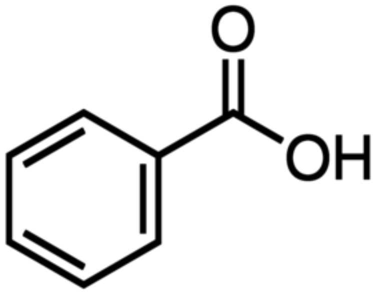
*** flow rate adjusted to be well under 4000 psi with this system (~3500 psi)**

https://www.agro-chemistry.com/news/green-chem-launched-in-ghent/

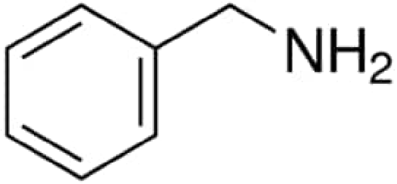
Probes



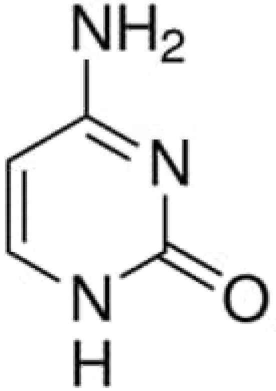
Amitriptyline
LogP 4.79, pKa 9.4



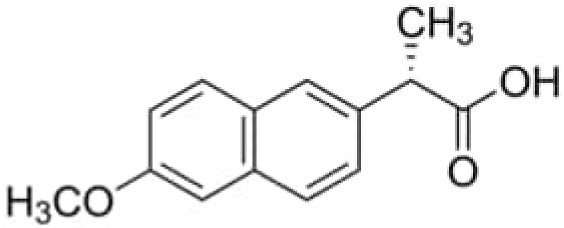
Benzoic Acid
LogP 1.87, pKa 4.2



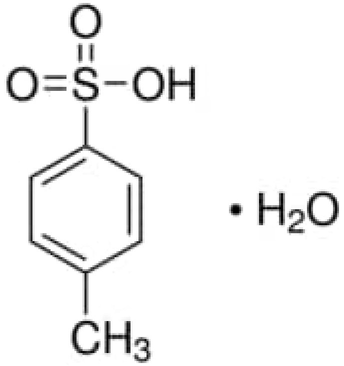
Benzylamine
LogP 1.09, pKa 9.34



Cytosine
LogP -0.469, pKa -

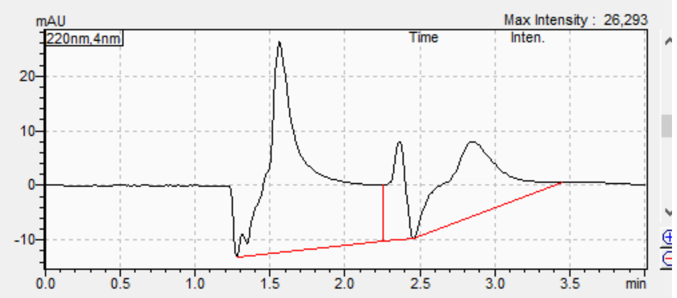


Naproxen
LogP 1.794, pKa - 5

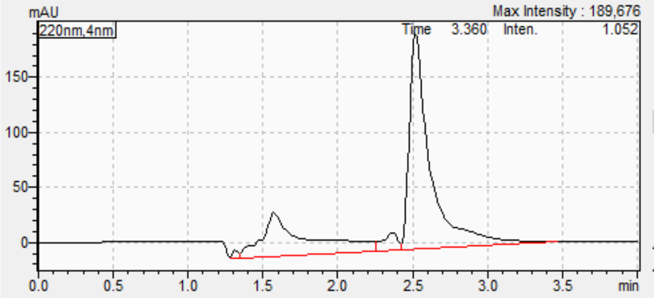


Toluene sulfonic acid
LogP -0.032, pKa -1.34

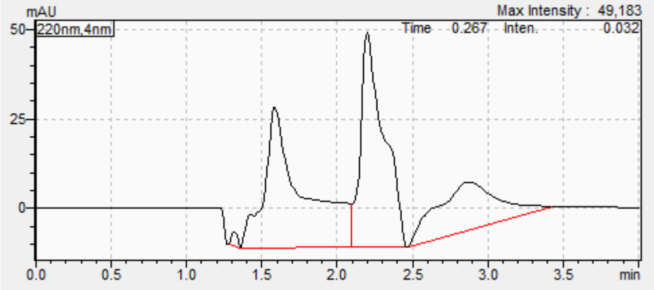
Results – 1M2P Mobile Phase



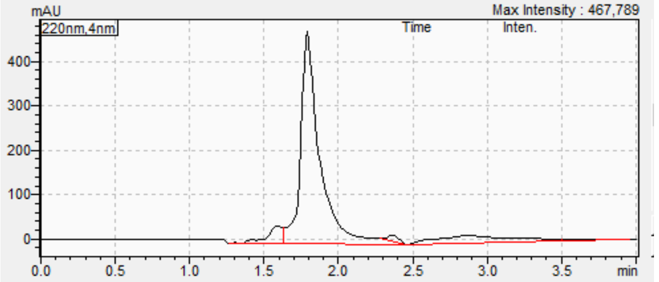
Blank



Amitriptyline



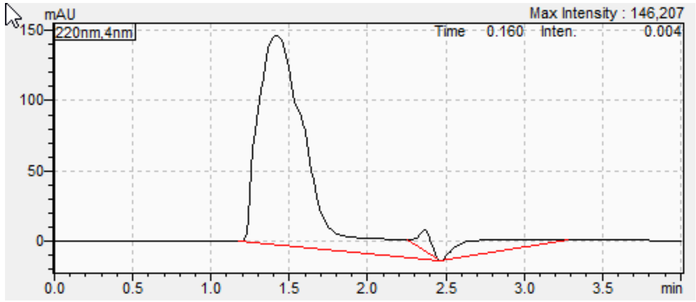
Benzylamine



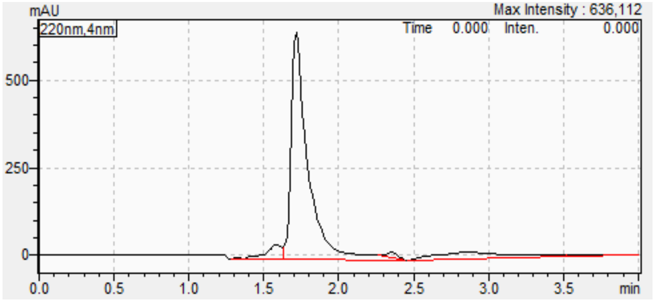
Cytosine

- Amitriptyline retained – IEX
- Cytosine only slightly retained – limited partition

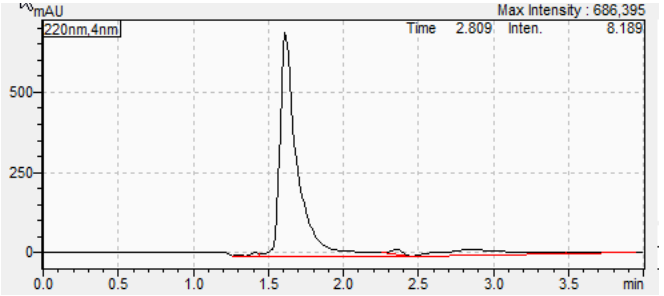
Results – 1M2P continued



Toluene sulfonic acid



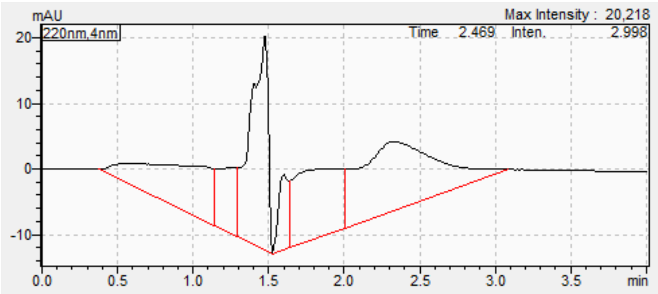
Benzoic acid



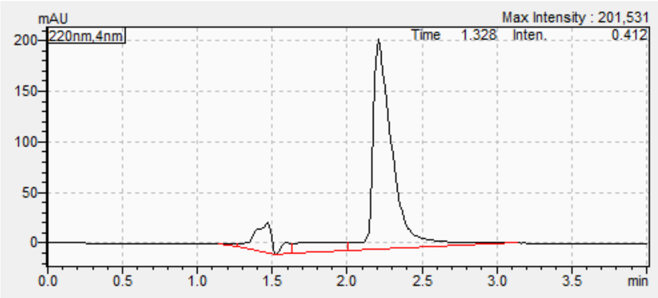
Naproxen

- Acids poorly retained
- TSA excluded
- Overall – similar to previous work using methanol

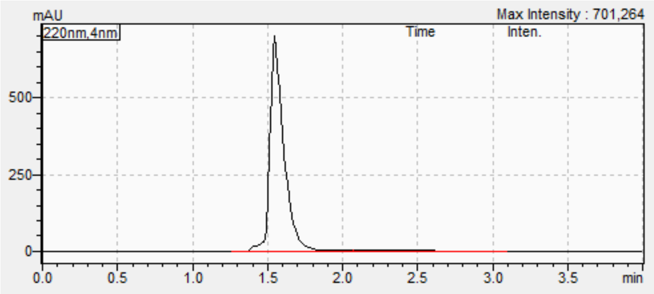
Methanol



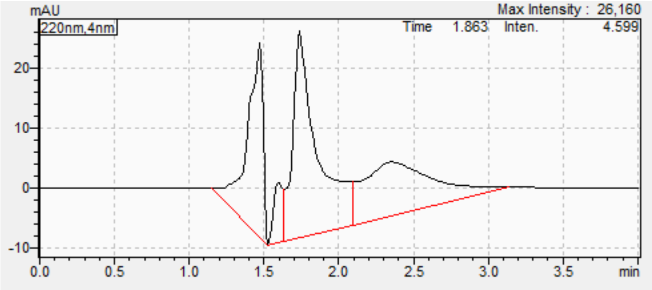
Blank



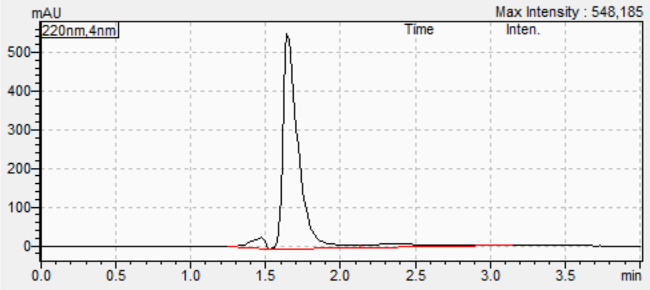
Amitriptyline



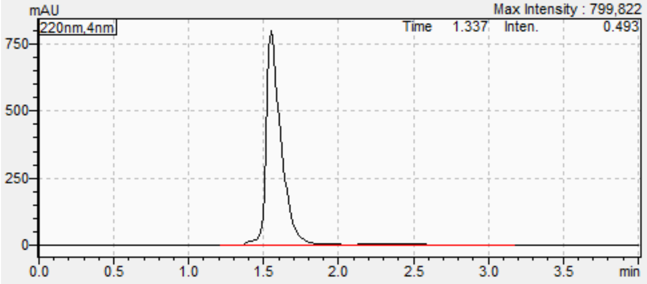
Benzoic acid



Benzylamine

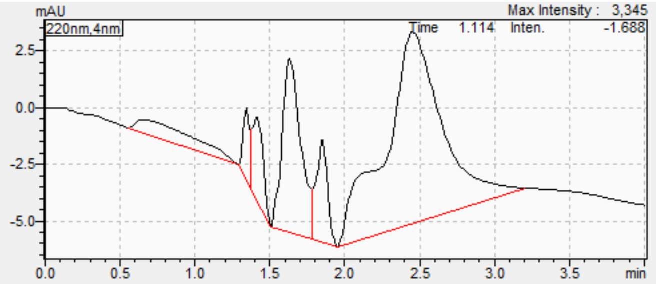


Cytosine

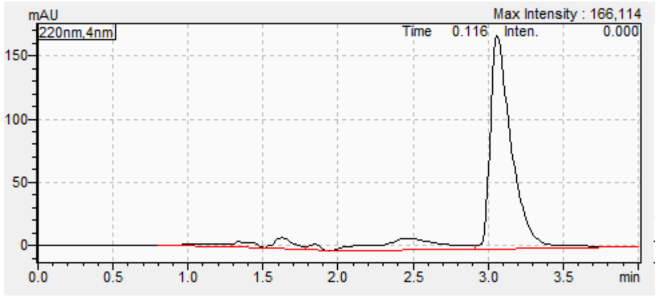


Naproxen

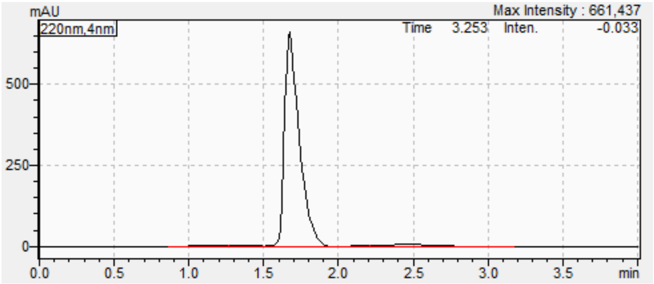
Results – Ethanol Mobile Phase



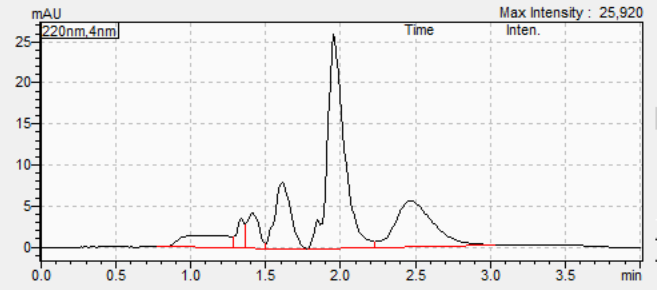
Blank



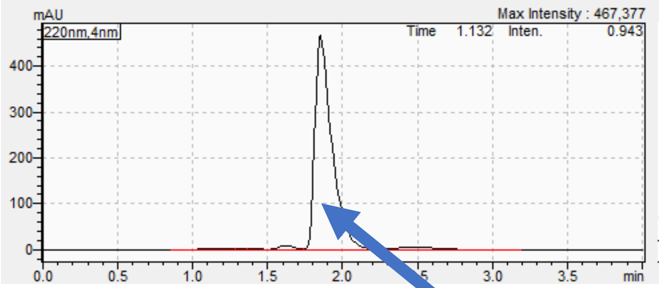
Amitriptyline



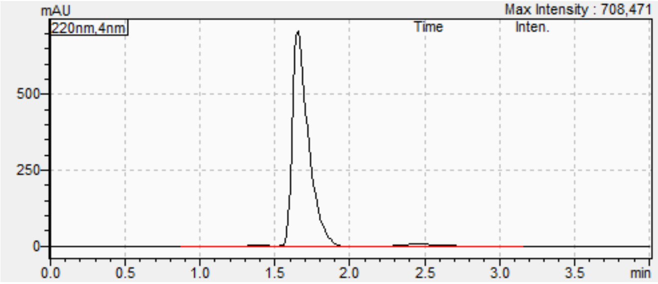
Benzoic acid



Benzylamine



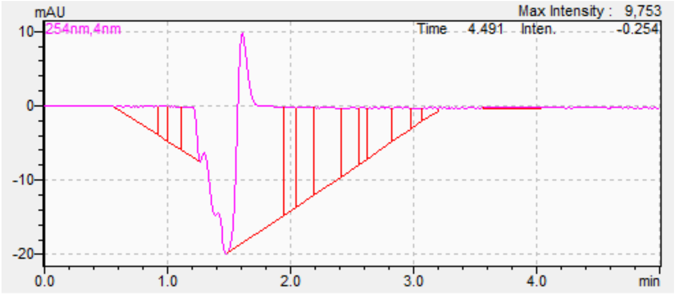
Cytosine



Naproxen

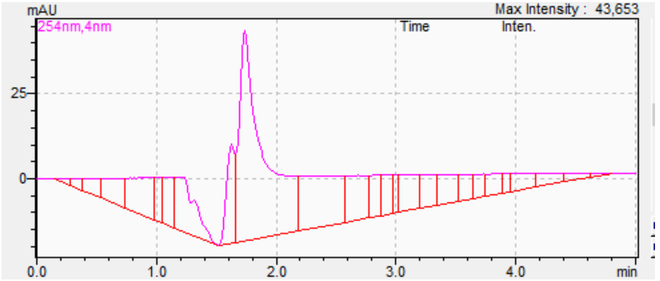
Slightly more retention than methanol

Results – Ethyl Lactate Mobile Phase (90:10 w 5mM AF)

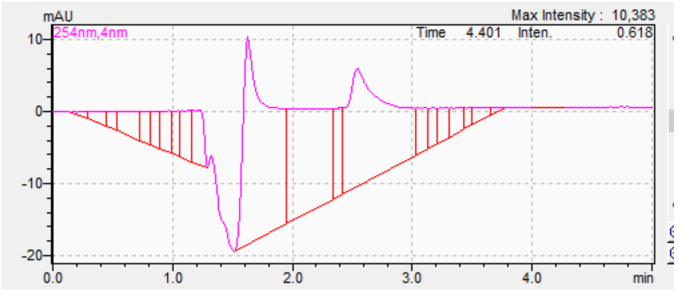


Blank

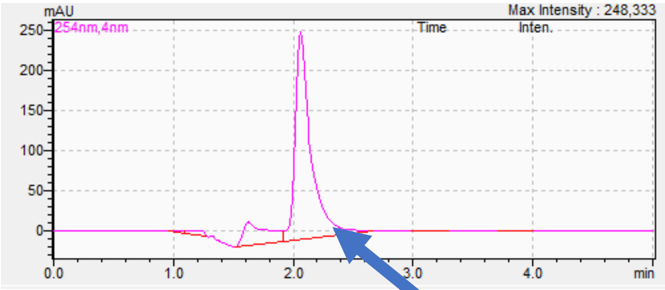
Amitriptyline



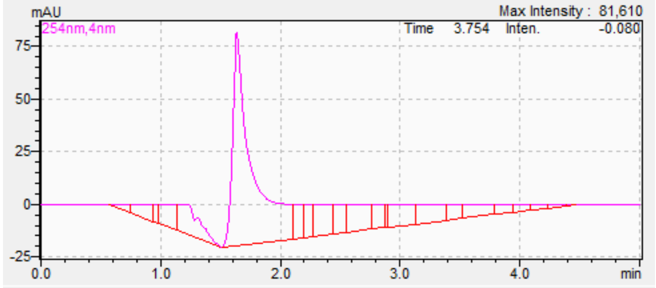
Benzoic acid



Benzylamine



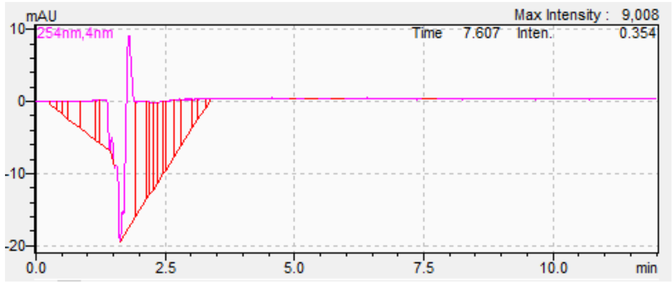
Cytosine



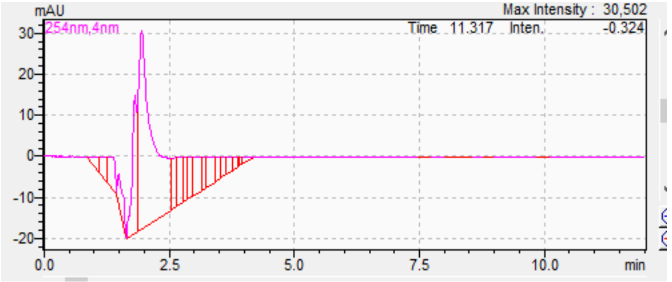
Naproxen

Slightly more retention than alcohols

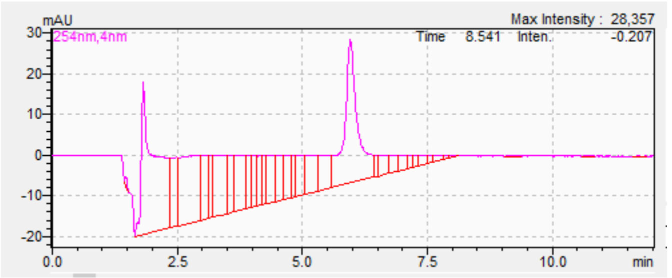
Results – Ethyl Lactate Mobile Phase – Polar X



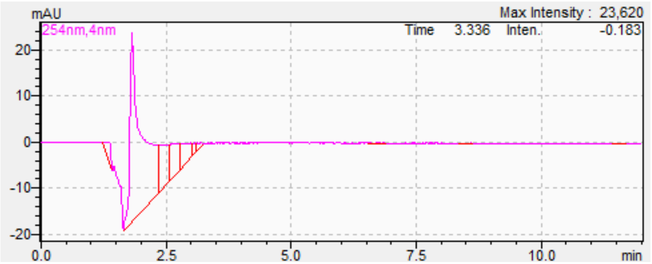
Blank



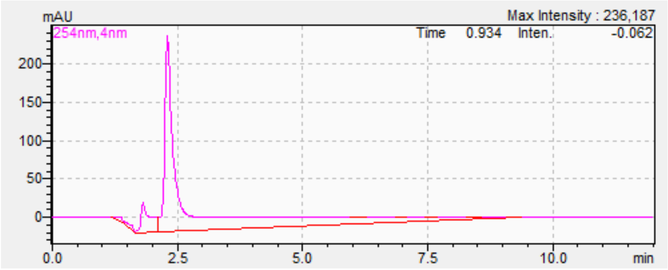
Amitriptyline



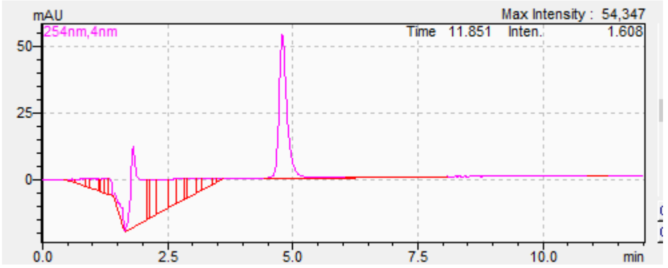
Benzoic acid



Benzylamine



Cytosine

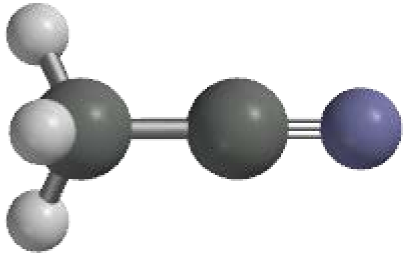


Naproxen

- Retention of the acids (IEX)
- Some partitioning observed

Conclusions – solvent substitution

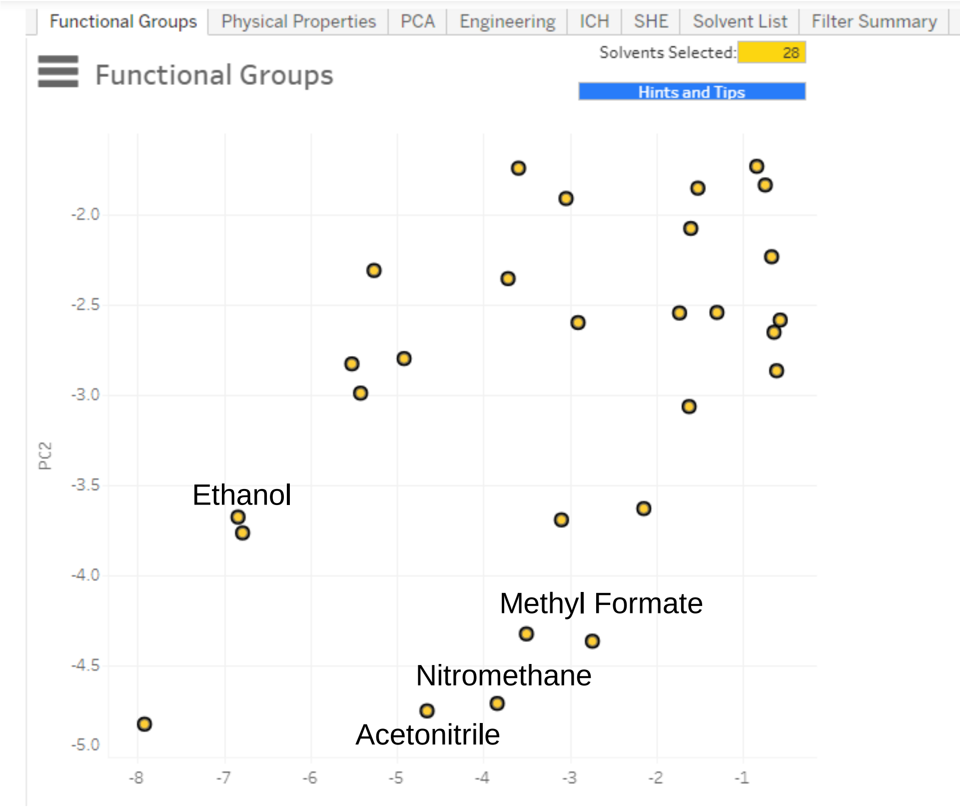
- There does not seem to be much promise for 1-methyl-2-propanol, at least not over more standard alcohols, including ethanol
- Ethanol does appear to have some merit for further development/research
- Ethyl lactate is promising as an acetonitrile replacement. Density is high and uv cutoff will negate many uv applications, but it may be suitable for higher wavelength absorbers and (potentially) MS, – still minimal partition relative to acetonitrile
- Dimethyl carbonate and dimethyl isosorbide are not completely out of the running, but they are not likely to be effective replacements
- Cyrene and limonene are very unlikely to be useful as major mobile phase components due to solubility issues (among others).



http://www.chem.ucla.edu/~harding/IGOC/A/acetonitrile.html

More systematic…
More comprehensive…

Solvent Selection Tool



https://www.acs.org/greenchemistry/research-innovation/tools-for-green-chemistry/solvent-tool.html

Other approaches?

Ion-Exchange?

per-aqueous liquid chromatography (PALC)?

Embedded Polar Group (EPG) phases in RPLC?

Enhanced-Fluidity HILIC? SFC?

Minimization – use standard conditions, just less of it –

https://www.transitionsib.com/why-the-future-of-your-business-is-critical-to-its-value/

Acknowledgements

Andrew Alpert

Restek Colleagues, Jan Pschierer and *Sandra Ruiz Perez*

HPLC Organizers

https://fut-ure.com/living-in-dusseldorf-for-expats/