

A Systematic Study of Achiral SFC Stationary Phases Using a Computational Calculated Molecular Diversity Model

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Objective

- The goal of this study is to provide the chromatographer with a sound starting point to match the appropriate stationary phase with the specific chemical class being analyzed.
 - This will minimize the time necessary for method development screening.
 - More importantly, this will eliminate the time consuming task of sorting through the large amounts of data generated.

Introduction

In recent years a number of achiral stationary phases have been developed specifically for achiral SFC. Not all of these columns are well-suited for all structural classes of compounds, presenting a bottleneck when vast arrays of chromatographic data must be acquired and interpreted to find a suitable match. In order to assess the selectivity of each column, a structurally diverse set of chemical building blocks were selected within four distinct functional group classes (carboxylic acids, amines, alcohols and amides) for evaluation. This selection was assisted using in-house software to identify fifteen reagents within each class with maximal Tanimoto dissimilarity (derived from ECFP4 descriptors).

Fifteen compounds for each of the four chemical classes were analyzed on eight different achiral stationary phases, in both their end-capped, and non end-capped forms. The retention times and peak shape were tracked for these compounds through use of mass spectrometry. The resulting data allows us to make recommendations for matching achiral stationary phases to chemical classes. In addition, the data provides an insight into the effect of end-capping in SFC.

Experimental

Chromatographic conditions:

Flow rate: 3 mL/min
Mobile phase: 90% CO₂:10% methanol
Back pressure: 150 bar
Column temperature: 25 degrees C
Run time: 60 minutes
Detection: UV @ 214nm coupled with positive APCI mass spectrometry for compound identification

Columns evaluated (both end-capped and non end-capped):

Silica, Ethyl pyridine (EP), 4-EP, Basic, Pyridyl amide, diethyl amino propyl (DEAP), Nitrophenol, & PFP

All phases are bonded to the same base GreenSep silica for a controlled method of eliminating retention contributions from varied stationary phase supports.

Sample compounds:

Carboxylic acids, alcohols, amides, amines
Dissolved in methanol, at approximately 1mg/mL

Discussion

Each collection of chemical classes was evaluated on the various stationary classes with compounds being identified through use of mass spectrometry. We present the chromatographic data we feel delivers the best separation for each of the four chemical classes as well as an examples of why a single stationary phase is not recommended for all structural classes.

The best separation for this study is defined as the chromatogram displaying controlled retention, controlled elution, a gaussian shaped peak lacking appreciable fronting and/or tailing, and a reasonable amount of selectivity for the diverse test probes we examine in this study. We chose 10% as our mobile phase B component as it is well documented that at low co-solvent percentages such as 2%, co-solvent will adsorb to the stationary phase and bias retention studies. We elected to not exceed 10% as we want to maximize interactions of the solute with the stationary phase.

It is important to note this study does not use mobile phase additives to control peak shape. This was intentional as low additive utilization is a major incentive for SFC to become more widely accepted. SFC has been known for decades to be heavily used in preparative chromatography labs where the ideal separations are performed additive free, when possible. The authors recognize some separations absolutely need additives and they must be used on these occasions.

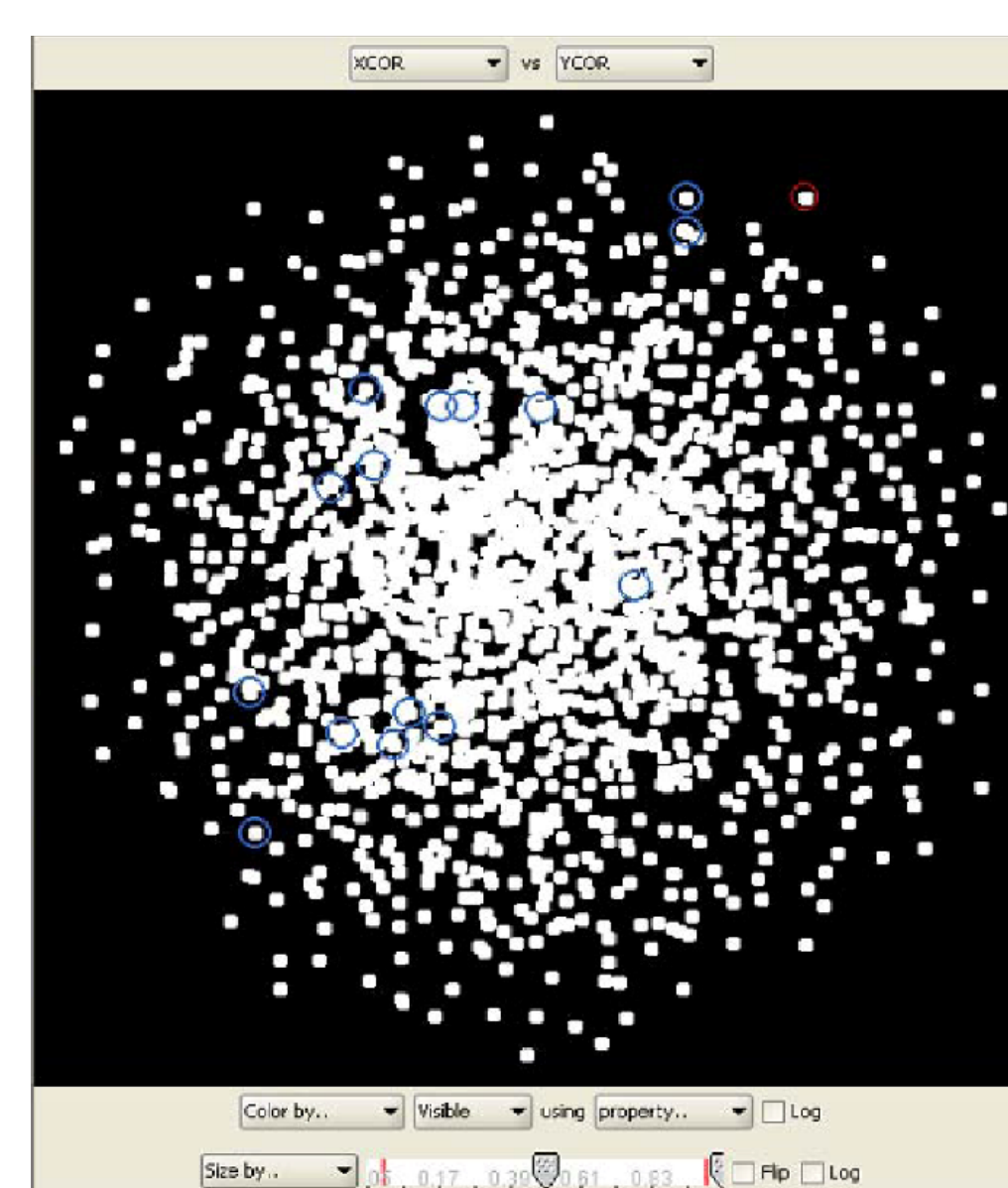


Figure 1.

Screen shot of custom written "Small Molecule Browser" used to identify structurally diverse reagents within each functional group class. This projection is a 2-dimensional non-linear map of the ECFP4-derived Tanimoto similarity matrix. In rough terms, and allowing for distortions in the projection, molecularly similar compounds end up grouped together and molecularly different compounds tend to be further apart. The red dot in the upper right quadrant was randomly selected as a starting point and the software was instructed to find the fourteen most diverse carboxylic acids, relative to the starting point. The resulting selections are highlighted in blue.

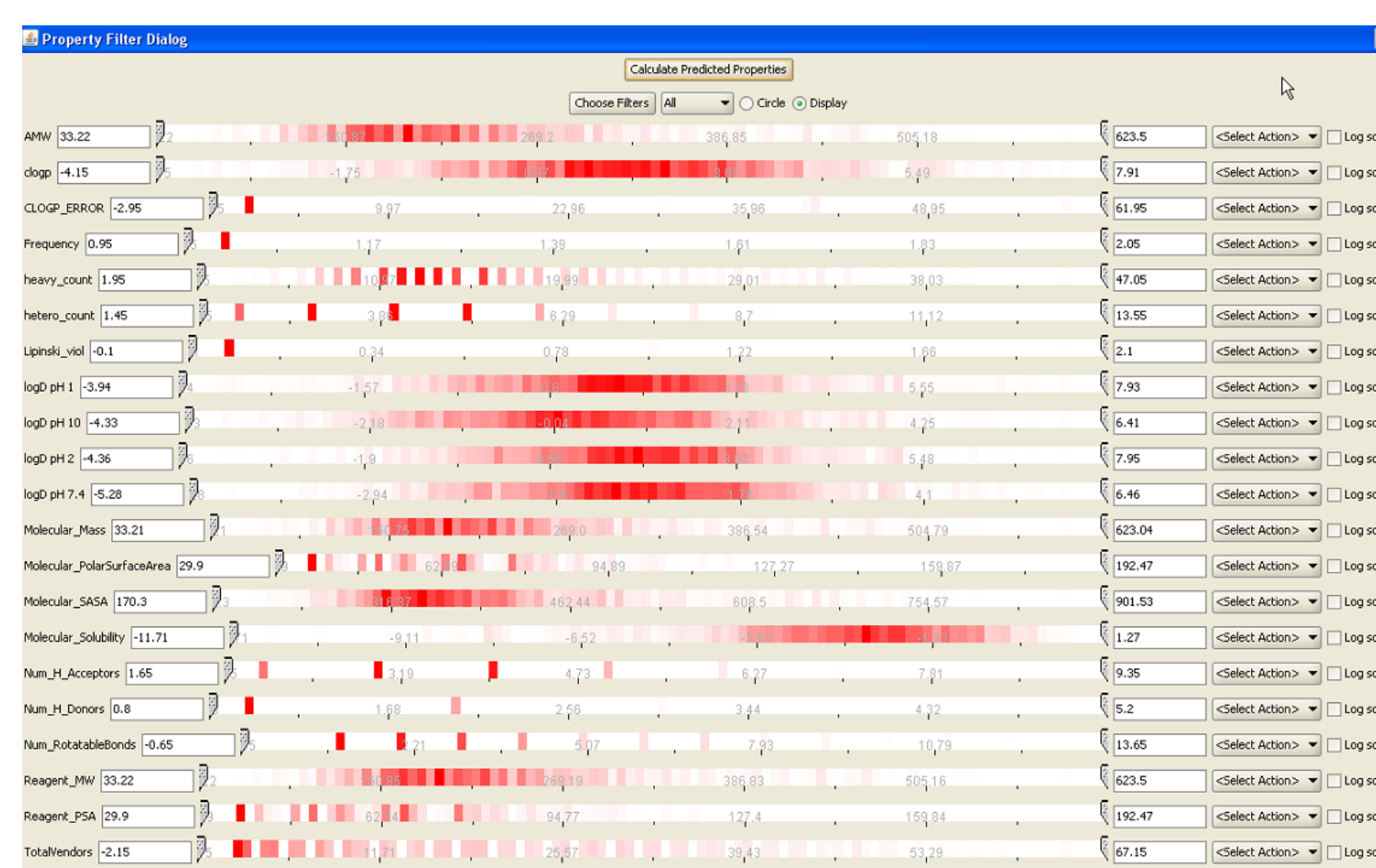
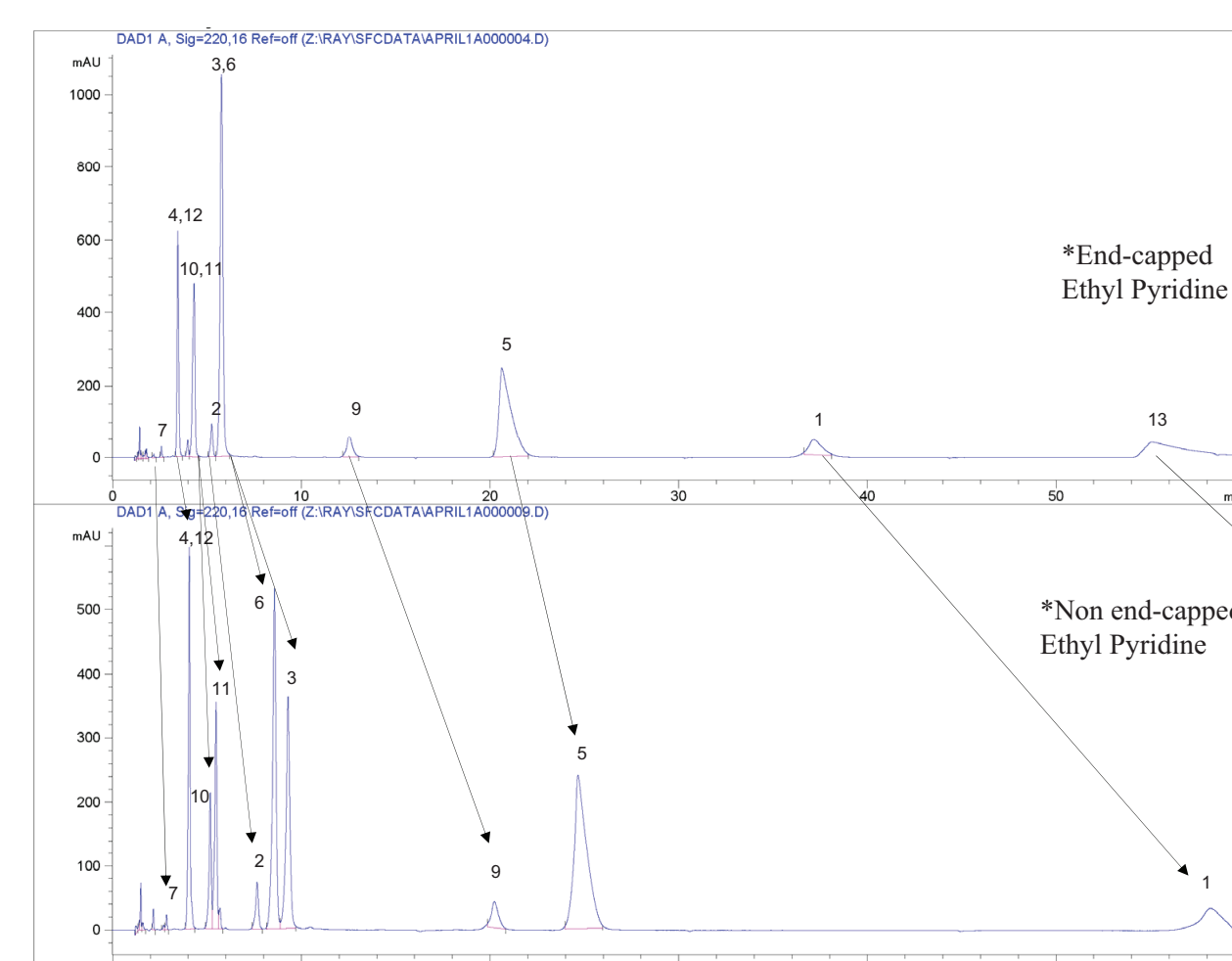


Figure 2.

Calculated parameters utilized in arranging carboxylic acid collection into its multi-dimensional chemical space. This study did not elect to use filters to ensure the most random and diverse collection as possible.

Figure 3a: Carboxylic Acid Results



- The acid test probes revealed similar, acceptable peak shape for all peaks on both the end-capped, and the non end-capped EP and Basic columns.
- The non end-capped EP column had additional selectivity as evident by critical pairs 10/11 and 3/6 as well as additional retention as evident by increased retention on all peaks other than peak 7.
- The non end-capped version of the Basic column did not demonstrate a change in selectivity when compared to its end-capped version as we experienced with the EP column.
- The non end-capped Basic column demonstrated no increase in retention as a result of having more free silanols present, suggesting that the residual silanols are not able to compete with the imidazole for interacting with the acidic test probes. This is supported by the increased pKa of imidazole compared to pyridine, 7.0 to 5.25, respectively.
- The EP column in either end-capped form is the preliminary column recommended for analysis of carboxylic acids by this study.
- Previous studies have shown excessive retention of acidic compounds on the EP column. Out test probes were largely unaffected by this phenomena, but if the user does experience this in their laboratory, the end-capped version would be the recommended solution.

Figure 4: Alcohol Results

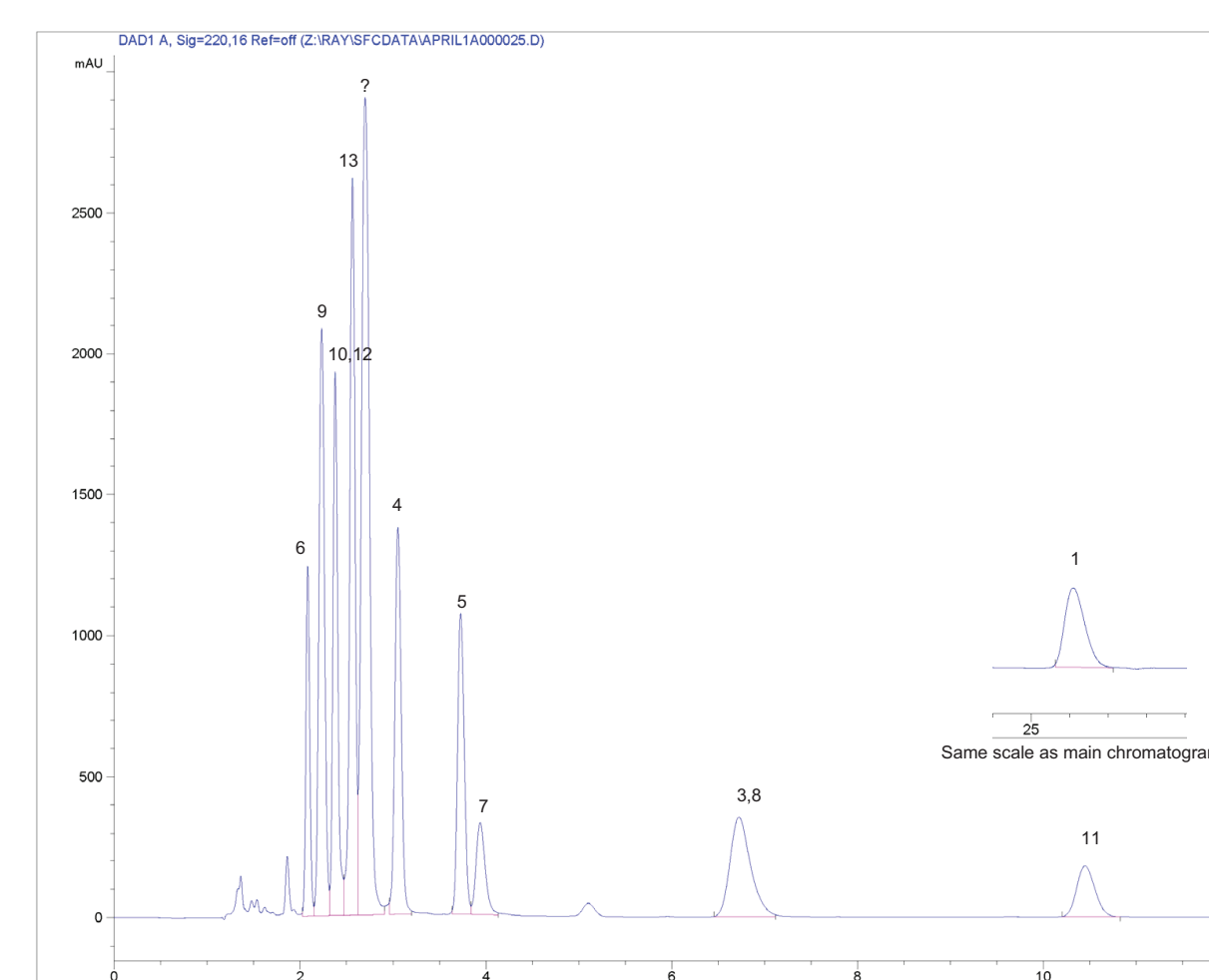
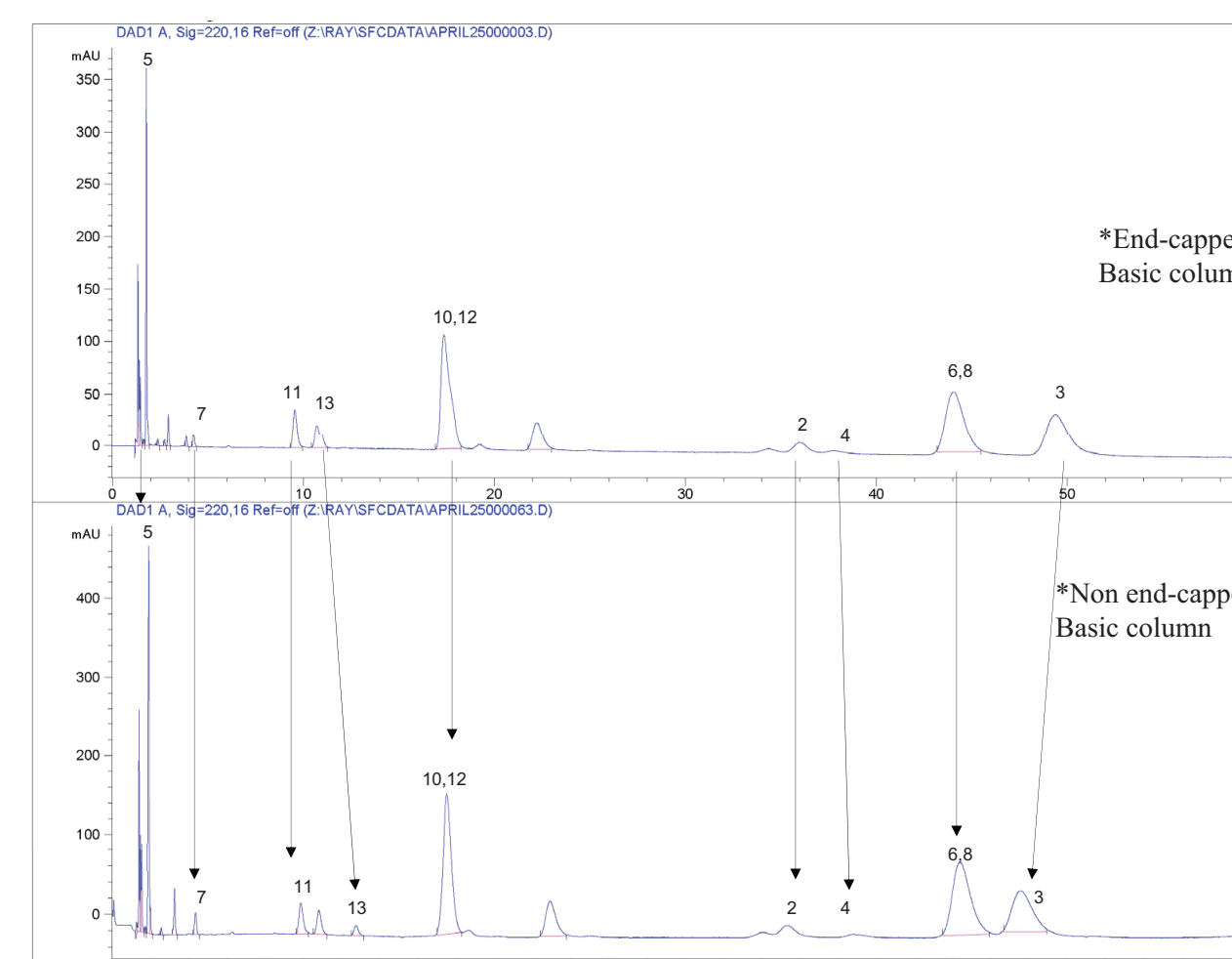


Figure 3b: Carboxylic Acid Results



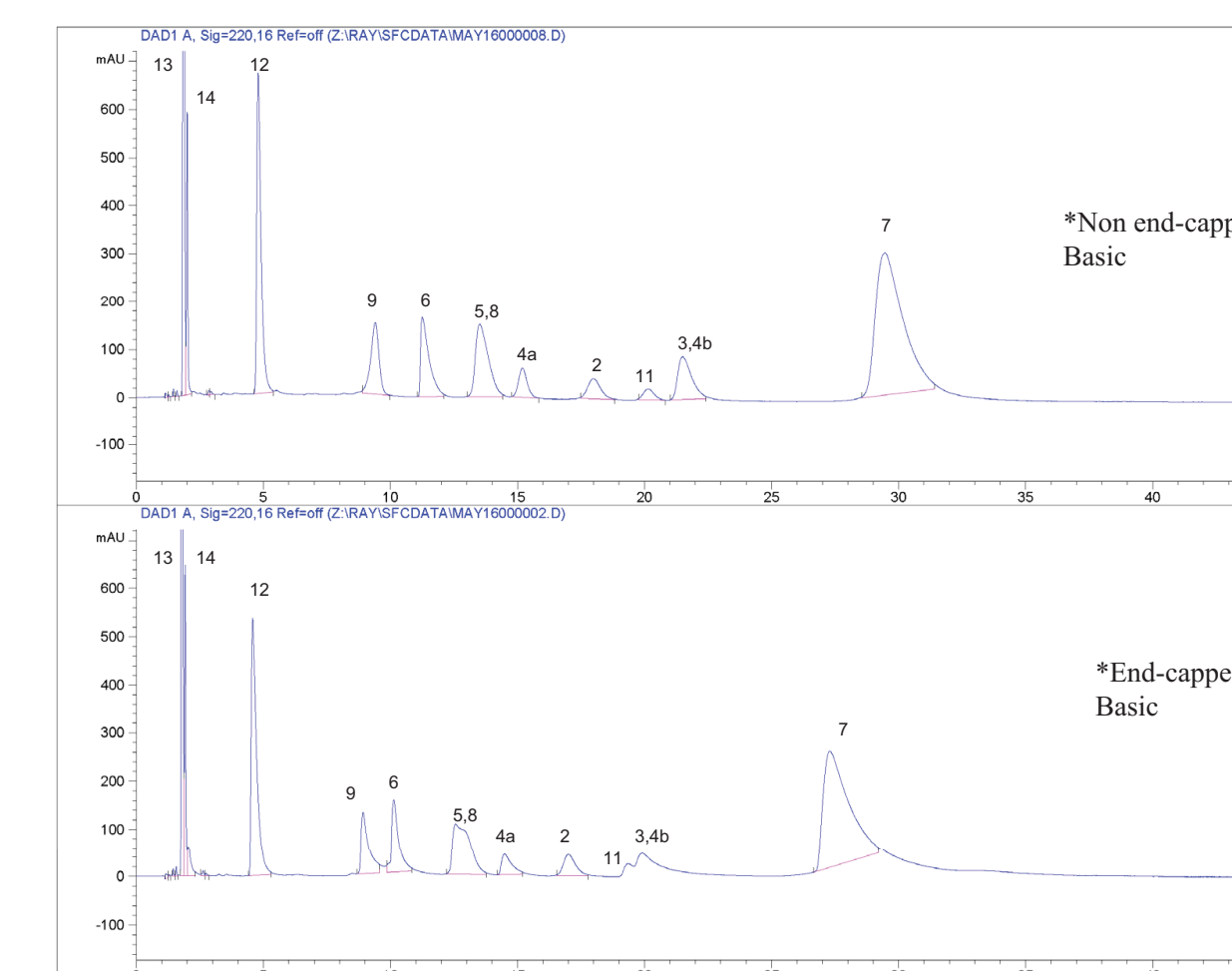
- The DEAP column provides excellent peak shape for the alcohols even for compound 1 at 25 minutes (see insert).
- The extremely efficient peaks are more than likely due to this column possessing just one retention mechanism, hydrogen bond accepting capabilities.
- This is a small sampling of alcohols but the DEAP column appears to have a vast degree of selectivity for our diverse set of alcohols.
- The ideal selectivity, controlled retention/controlled elution, and extremely efficient peaks make the DEAP column the preliminary column for alcohol analysis.

Acknowledgements

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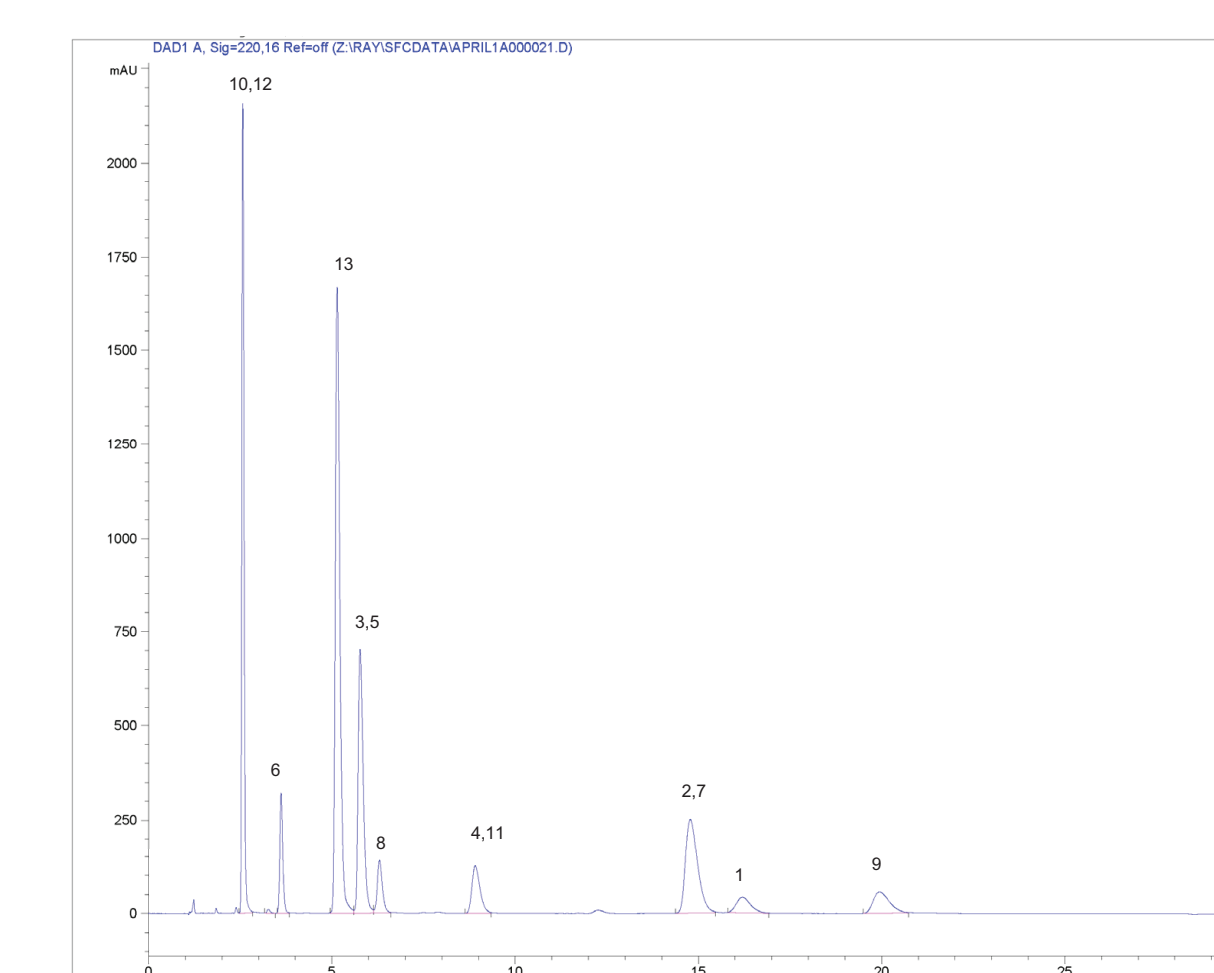
Results

Figure 5: Amine Results



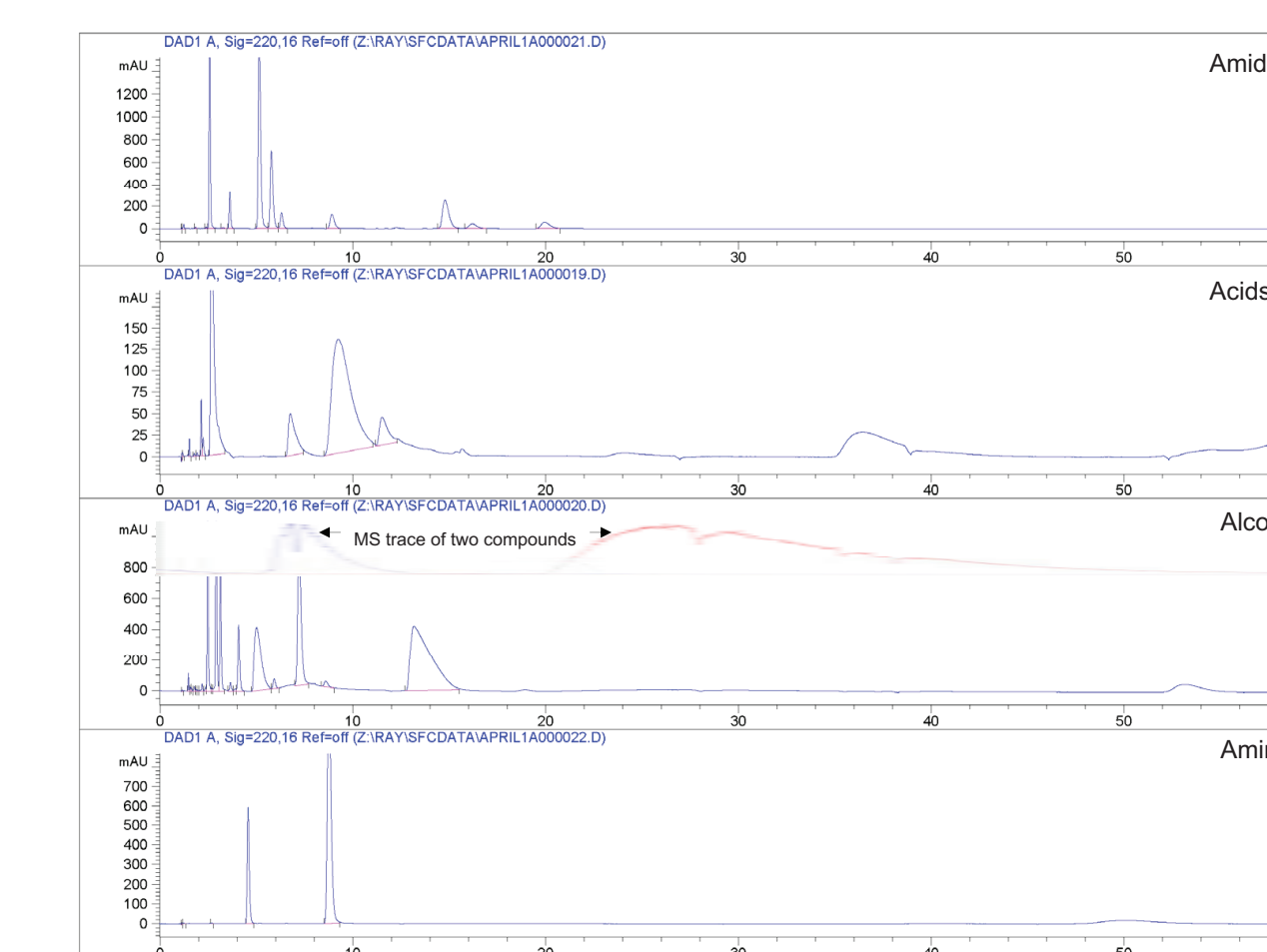
- The Basic non end-capped column has superior peak shape for most of the amines analyzed when compared to its end-capped version.
- Eight of the amines are primary amines, used as reagents in a drug discovery lab. This would suggest there would be little hydrogen bond accepting character displayed by these analytes.
- The longer retention suggests strong pi-pi interactions as all but one of the amine analytes possess aromatic systems.
- The non end-capped Basic column is the preliminary column of choice for the analysis of amines.

Figure 6: Amide Results



- The nitro (non end-capped) provided excellent peak shape for the amides analyzed.
- The peaks are very efficient with even peak 9 being nicely shaped out at 20 minutes.

Figure 7: Example of single phase not being universal



- The non end-capped Nitro column was selected to display the varied retention characteristics displayed by a single phase on independent chemical classes. The highly variable results for the four classes on this single column summarize the study nicely.
- The amide chromatogram displays controlled retention, controlled elution, efficient peaks, and a vast degree of selectivity making this phase the preferred suggestion for the analysis of amides.
- The carboxylic acid chromatogram reveals broad, inefficient peaks. This class of compounds also displays excessive retention as evident from the peaks eluting at 50-60 minutes and even several compounds that did not elute from the column.
- The alcohol chromatogram displays the worst peak shape of all four classes of compounds on this column. Several broad peaks are observed in the UV data while alcohols 12 and 13 had to be extracted from the mass spectrometer signal (see insert) to be visible.
- The amine chromatogram is self explanatory in the fact that this class of compounds are too strongly retained.

Summarize Classes on Stationary Phases

	Silica	EP ec	EP non-ec	Basic ec	Basic non-ec	PA ec	PA non-ec	DEAP non-ec	Nitro ec	Nitro non-ec	4-EP ec	4EP non-ec
Acids	M	G	BEST	G	G	B1,3	B1,3	B1	B3	B3	M	B3
Alcohols	M	M	M	G	G	G	G	BEST	M	B3	M	B3
Amides	G	B2	M	M	M	M	G	G	G	Best	M	M
Amines	B1	B2,3	M	M	BEST	M	M	M	B1	B1	B1,3	B1,3

BEST = best - B1=too retentive G = good - B2=lacks selectivity M = medium - B3=poor peak shape B = bad - B4=little retention

Conclusion

- This study identifies a preliminary column to serve as a starting point for the four chemical classes analyzed.
 - Ethyl Pyridine (non end-capped) for carboxylic acids
 - DEAP (non end-capped) for alcohols
 - Nitro (non end-capped) for amides
 - Basic (non end-capped) for amines
- Amides generally produced the most consistent peak shape across all stationary phases evaluated while amines tended to have the worst peak shape.
- Carboxylic acids were on average the most strongly retained class of compounds on most columns evaluated. Amines were slightly less retentive but had inefficient peak shape with severe tailing encountered frequently, for example in the case of silica.
- An estimated, normalized retention order would be carboxylic acids<amines<alcohols<amides with the carboxylic acids being the most strongly retained.
- From the end-capping study, it appears the silanol groups on the non-end-capped stationary phases play a role in maintaining the quality of separations. This confirms the silanols do play a significant role in the separation mechanisms in SFC.
- More chemical classes of compounds will be subjected to these stationary phases to enable matching of additional chemical classes with appropriate stationary phase.
- The approach described in this study will be in the development of new stationary phases for SFC.