HPLC Column Comparison Screening Study for Reversed Phase Columns

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Introduction

When developing a new HPLC method, selecting the right column can be a time-consuming task. The most common approach to HPLC method development is to begin with a C18 column, make mobile phase changes (such as pH, or % organic) based on knowledge of the analytes. If this fails, often another brand of C18 is tried. In many instances, this approach produces an acceptable method.

However, this conventional C18-based approach can take an enormous amount of precious time and often does not give the desired separation. To save time and obtain the desired separation, a change in the stationary phase chemistry away from C18 is often the path to take. But which non-C18 column should one choose? In the work to be presented, we will explain the systematic approach we undertook to observe selectivity and retention differences in a variety of functionalized reversed-phases.

We describe the compound sets used in the column screening data experiments, hardware and results. An example is shown how an analyst can use this screening data in a practical separation.

Purpose of Study

-Develop systematic method to determine selectivity and retention differences in stationary phases as well as overall performance.

-Use screening data as a means to help analysts quickly select a suitable phase as a starting point for methods development.

Determination of Compound Test Mixes

Compounds chosen to represent basic structure or functional groups of small molecules encountered in various industries utilizing HPLC.

Neutrals

- Parabens
- Alkyl benzenes
- Functionalized benzenes- 3 groups

Bases

- Mix 1 - simple bases
- Mix 2 - pharmaceutical bases

Acids

- Acids Test 1 - simple acids
- Acids Test 2 - pharmaceutical acids

Table of Compounds Used (Partial List Only)*

Compound Name	% Organic	pH	k' C18	k' RP-AmideC16	k' C8	k' Cyano	k' HS F5
5% CH3CN							
aniline	5	pH 2	0.7	0.5	0.7	0.4	1.5
benzyl amine	5	pH 2	1.4	0.8	1.3	0.5	3.1
nizatidine	5	pH 2	1.6	1	1.3	0.7	2.4
o-aminobenzoic acid	5	pH 2	6.2	4.6	5.8	1	8.3
procainamide	5	pH 2	0.7	0.5	0.6	0.4	3
pyridine	5	pH 2	0.2	0.2	0.2	0.3	0.5
10% CH3CN							
codeine	10	pH 2	2	1.2	1.7	0.7	2.8
hydrochlorothiazide	10	pH 2	3	4.3	2.7	3.1	2.3
lidocaine	10	pH 2	5.9	3	5.1	1	3
phentermine	10	pH 2	4.8	2.6	4.3	0.8	3.5
quinidine	10	pH 2	2.1	1.4	1.9	1	8.7

*Refer to Discovery Brochure (T402075) for entire list.

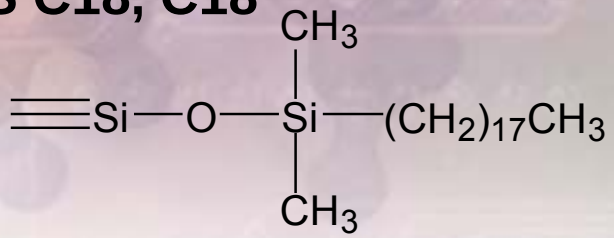
HPLC Conditions

Conditions chosen based on simplicity of mobile phase for non-ionic compounds and for ionizable compounds. Mobile phases were chosen to cover the pH range of silica based phases.

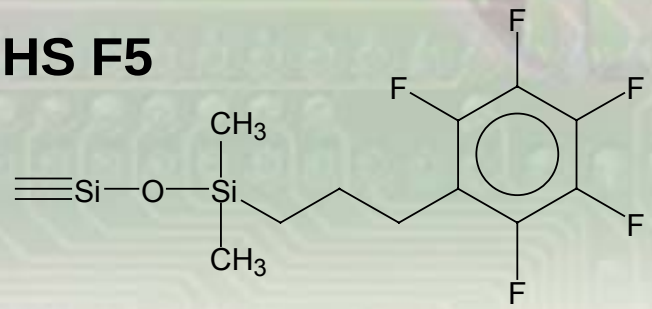
- Non-ionic compounds: acetonitrile:water
- Ionizable compounds: 25mM phosphate buffers at pH 2 and pH 7
- The concentration of acetonitrile was varied to give a k' between 1 and 5 for most compounds.
- Columns run using automated switching valve

Phases Used in This Study

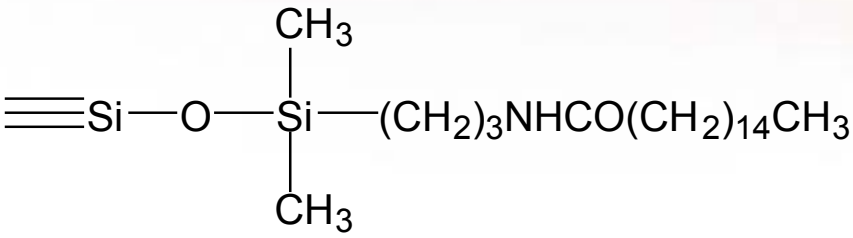
HS C18, C18



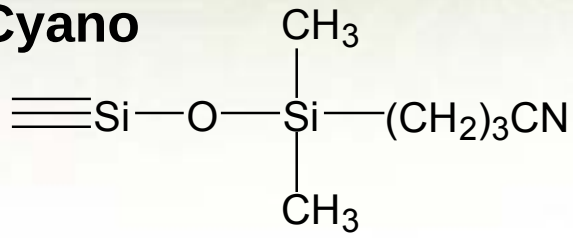
HS F5



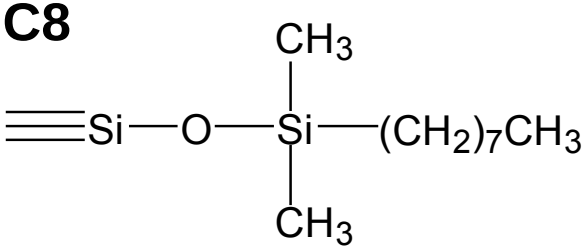
RP-AmideC16



Cyano



C8



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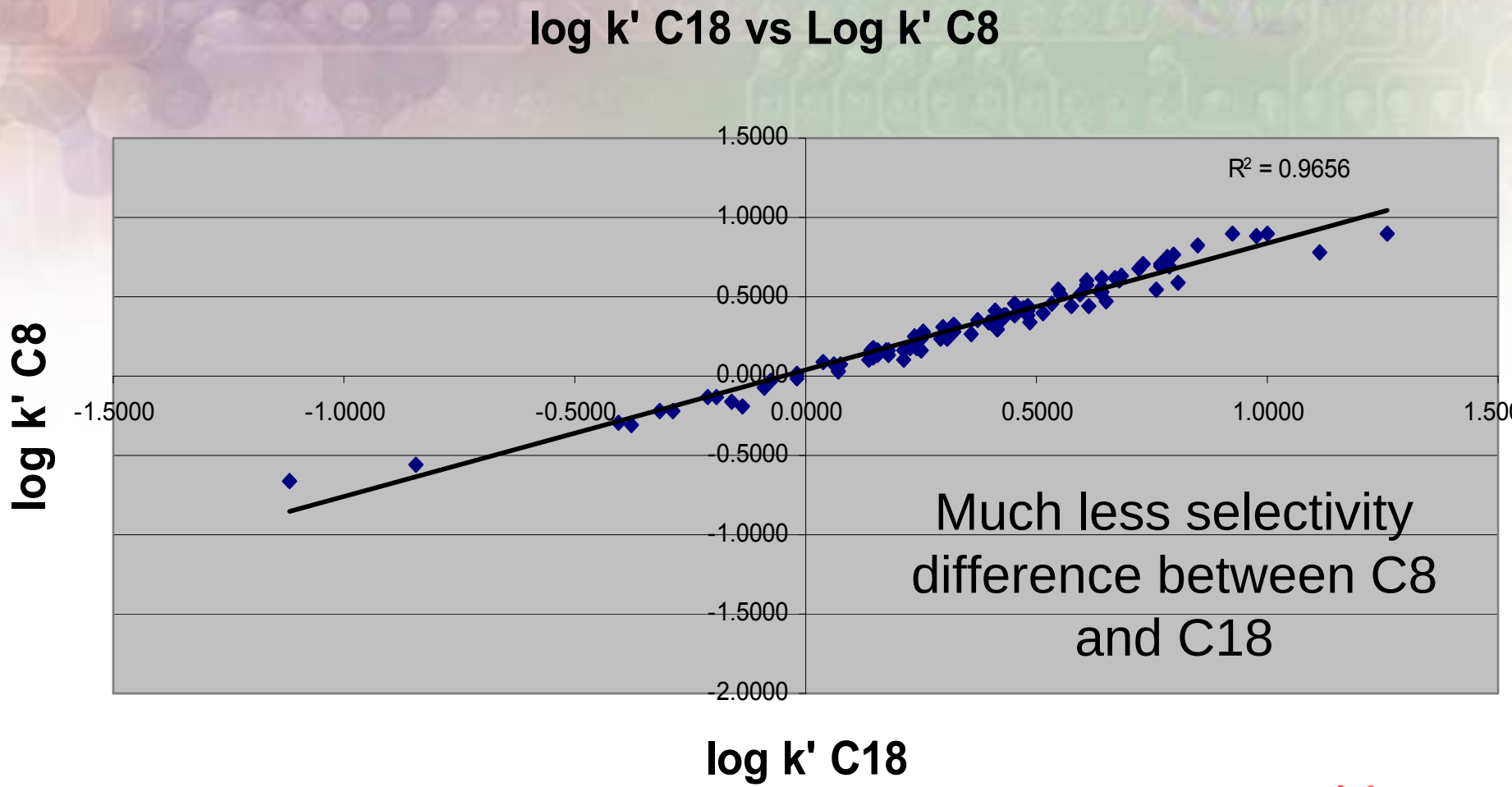
Examples of Data Obtained in Study

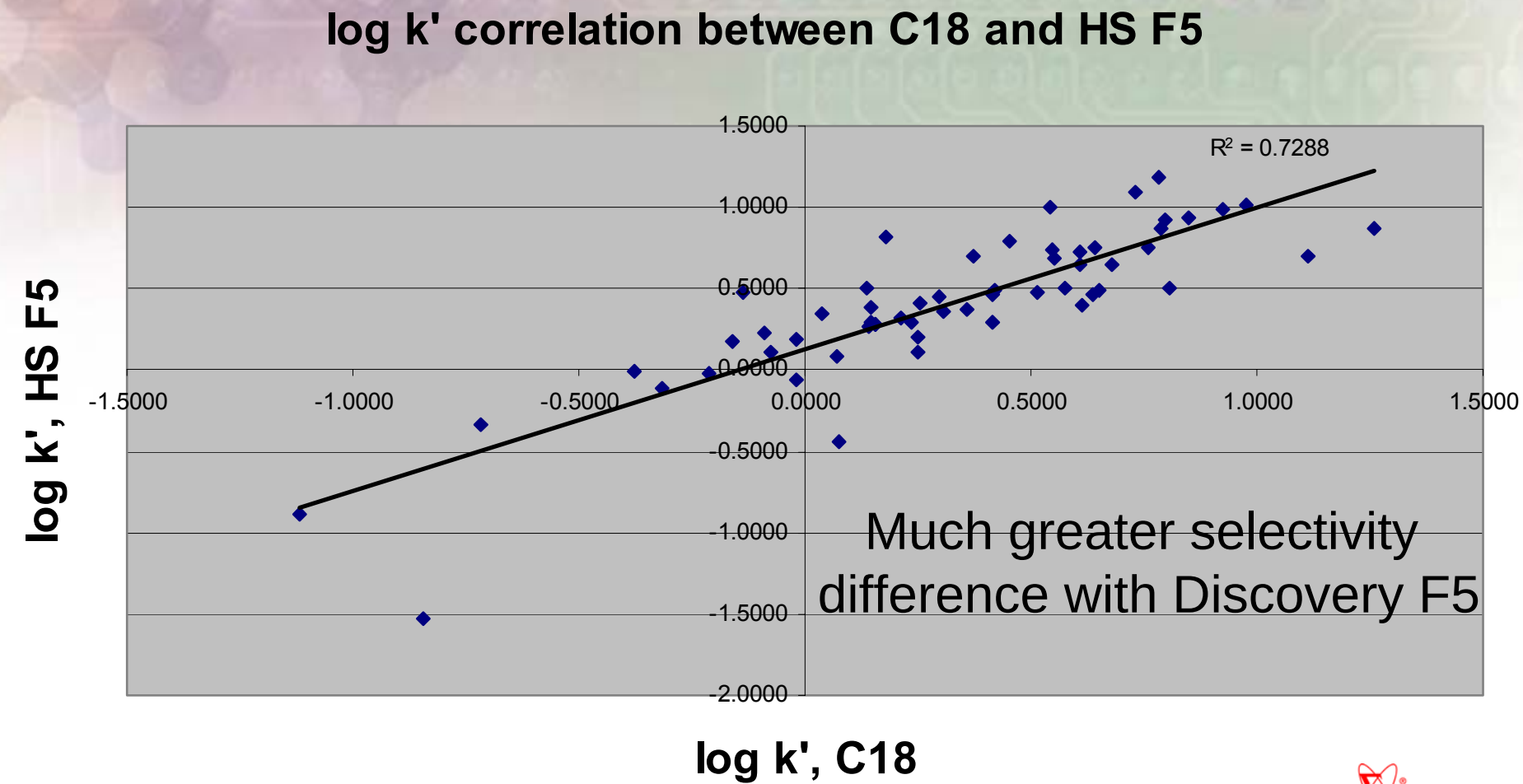
- k’ values
- USP tailing factors
- Selectivity of compounds on the different phases
- Can compare interactions of compounds between phases, for example:

  C8 vs. C18 interactions
  C18 vs. F5 interactions

Example: Column Screening Study Results



Example: Column Screening Study Results



Conclusions on Column Screening Study

- Screening quickly shows differences in retention and selectivity for different phases
- Screening allows for selection of a suitable phase as a starting point for methods development
- Comparisons can be made between phases to show which phases are most alike and which are most different

However, how does this help me in my work?

How Can I Use This in My Work?

- Look up your compound in table. (see Supelco Rediscover Method Development Guide-T402075) If exact compound does not appear in the table, look for one with similar structure or functionality.
- Considering the acetonitrile concentration: If different percentages of acetonitrile used in screening, use the very general rule-of-thumb that an increase of 5% (v/v) of the organic modifier results in a 2-fold decrease in k'.
- Choose pH 2 or pH 7
- Choose column or columns that give the right amount of retention for your compound or representative compound.
- Run the experiments.

What Am I Trying to Accomplish?

Consider the desired end result. Are you looking for :

- certain elution order
- speed
- good retention and resolution
- desire to have flexible method if formulation changes in future
- other requirements

A Practical Example

Sample: phenacetin and codeine

Assume: preferred elution order is codeine, then phenacetin

pH: on the pH 2 chart, the compounds elute at very widely different % acetonitrile (10% and 25%) making an isocratic separation potentially difficult. At pH 7, however, codeine was run at 15% acetonitrile, and phenacetin at 20% acetonitrile. Choose the pH 7 condition.

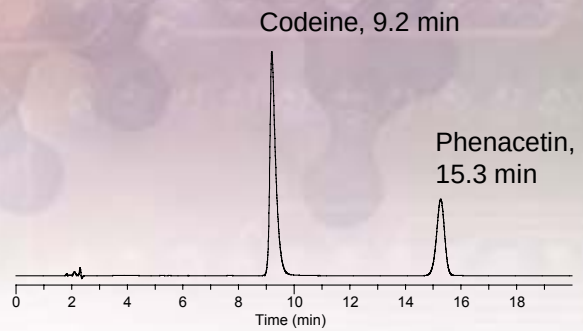
Column: The pH 7 screening data shows the compounds have the preferred elution order (codeine then phenacetin) on all but the Discovery® HS F5 column, however, if the preferred elution order was reversed, the HS F5 would be the best choice.

Applying Column Screening Study Results

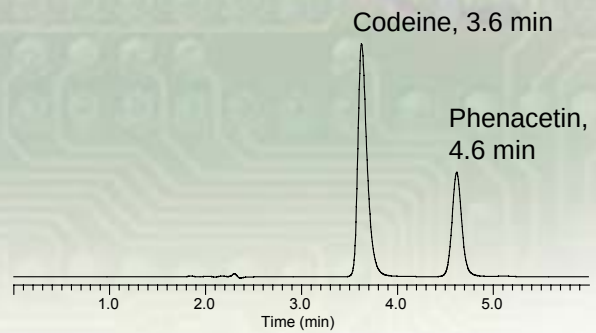
Column	k' Codeine at 15% CH_3CN	Estimated k' Phenacetin at 15% CH_3CN	Predicted alpha (k' phenacetin / k' codeine)
Discovery C18	4.4	4.7 x 2 = 9.4	2.1
Discovery RP-AmideC16	3.3	4.8 x 2 = 9.6	2.9
Discovery C8	3.6	4.1 x 2 = 8.2	2.3
Discovery Cyano	1.1	1.3 x 2 = 2.6	2.4

Applying Column Screening Study Results

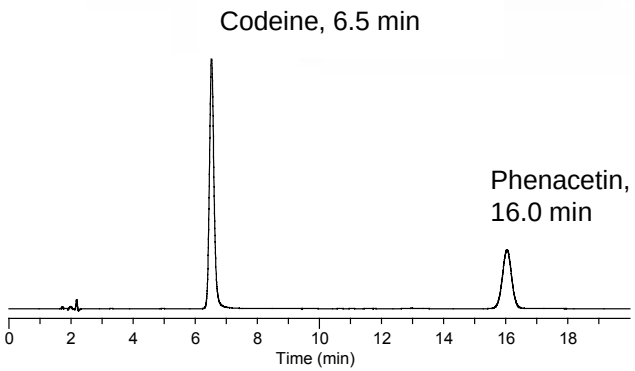
Discovery C8



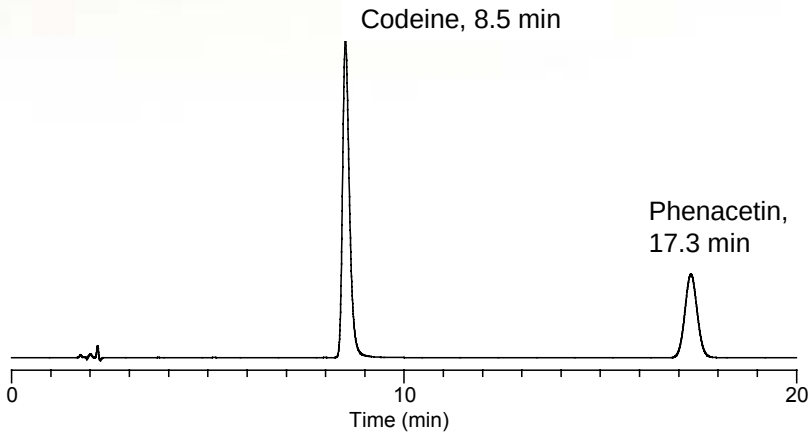
Discovery Cyano



Discovery RP-AmideC16



Discovery C18



Columns: 15cm x 4.6mm, 5µm each
Mobile Phase: 25mM H_3PO_4, pH to 7.0 w/ NH_4OH:Acetonitrile (85:15, v/v)
Flow Rate: 1mL/min
Detection: UV @ 220nm
Injection Volume: 10µL
Samples: 100µg/mL each, codeine and phenacetin in mobile phase buffer

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Analysis of Results

From the experiments:

- If speed is desired, choose Cyano.
- If formulation contains other compounds, Amide C16 or C18 is adequate due to large amount of peak space between compounds of interest.
- C8 gives adequate separation for a general method
- Overall, gain knowledge of separation so that you are better prepared to make adjustments in method, if formulation changes in future, knowing that you can change column if necessary to accommodate these changes.

Applying Column Screening Study Results: Predicted vs. Actual

Column	Analyte	Predicted k'	Actual k'	Predicted alpha (selectivity)	Actual alpha (selectivity)
Discovery C8	Codeine	3.6	3.6	2.3	1.9
	Phenacetin	8.2	6.7		
Discovery CN	Codeine	1.1	1	2.4	1.6
	Phenacetin	2.6	1.6		
Discovery RP-AmideC16	Codeine	3.3	2.8	2.9	3.0
	Phenacetin	9.6	8.4		
Discovery C18	Codeine	4.4	3.3	2.1	2.3
	Phenacetin	9.4	7.7		

Conclusion

1. We have developed a systematic method to determine selectivity and retention differences in stationary phases.

2. Column screening can help analyst quickly select a suitable phase as a starting point for methods development.

3. The presented study confirms that the predictions are correct.

4. Note that retention times are only estimated from the screening procedure as evidenced by the differences observed between predicted and obtained retention values.