HPLC Column Comparison Screening Study for Reversed Phase Columns

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Introduction

The selection of the proper HPLC column for any given analysis can be an intimidating task for today's analyst. The majority of chemists will begin with some type of C18 chemistry. They will try to develop the separation on that phase by using their knowledge of the analytes and the chromatographic parameters available, such as percent organic or pH. Often times this approach gives adequate retention and resolution in a reasonable amount of time.

At times, however, the above approach does not give the desired separation. The analyst may consider changing to another vendor's C18 chemistry. If this does not give the desired result, a change in the stationary phase might be needed . This will usually yield a change in selectivity and retention of the analytes and give an adequate separation. Choosing this alternative phase may also be a difficult task. In the work to be presented, we will explain the systematic approach we undertook to observe selectivity and retention differences in a variety of 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

- Mix 1 - Simple bases
- Mix 2 - Pharmaceutical bases

Table of Compounds Used (Partial List Only)*

Compound Name	% Organic	pH	k' C18	k' RP-Amide C16	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 Re-Discover Method Development Guide for entire list, T402075A.

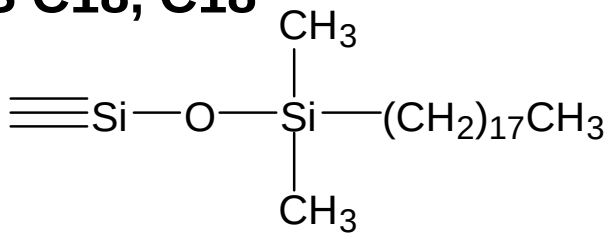
HPLC Conditions

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

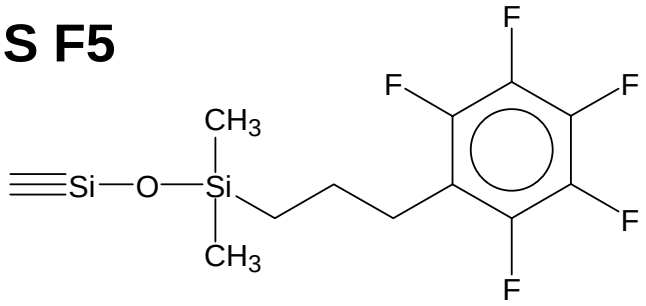
- **Non-ionic compounds: acetonitrile/water**
- **Ionizable compounds: 25mM phosphate buffers at pH 2 and pH 7**
- **The concentration of acetonitrile was varied to give a k' between 1 and 5 for most compounds.**
- **Columns run using automated switching valve.**

Phases Used in This Study

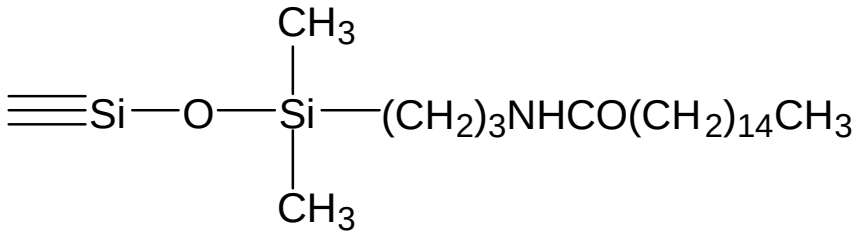
HS C18, C18



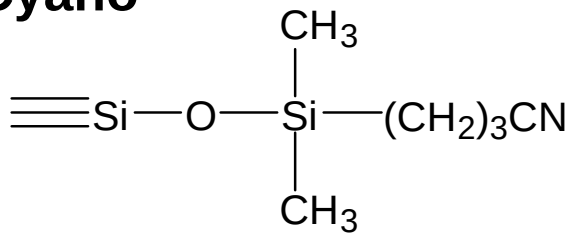
HS F5



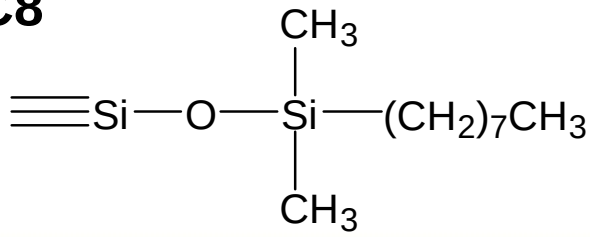
RP-AmideC16



Cyano



C8



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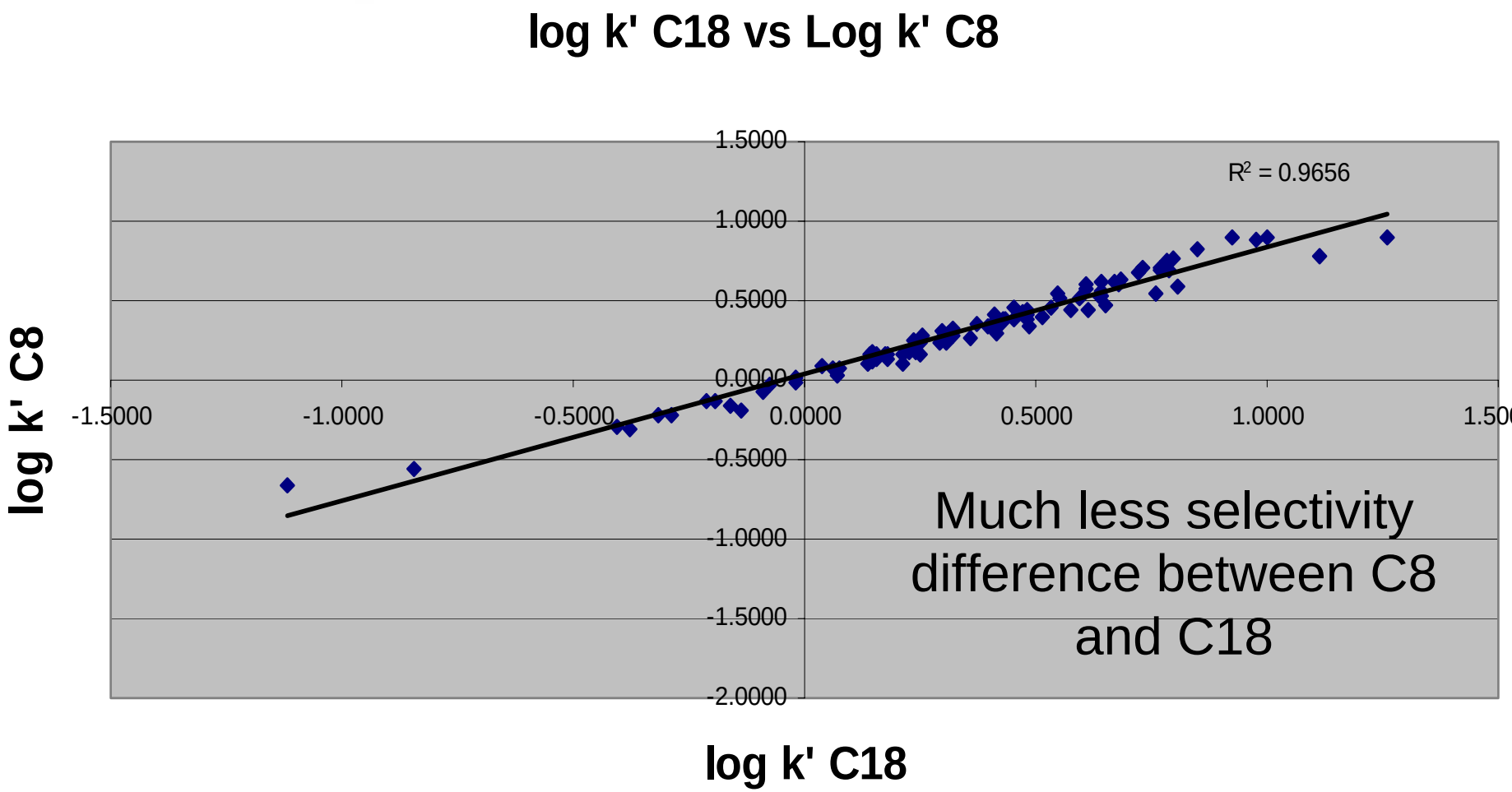
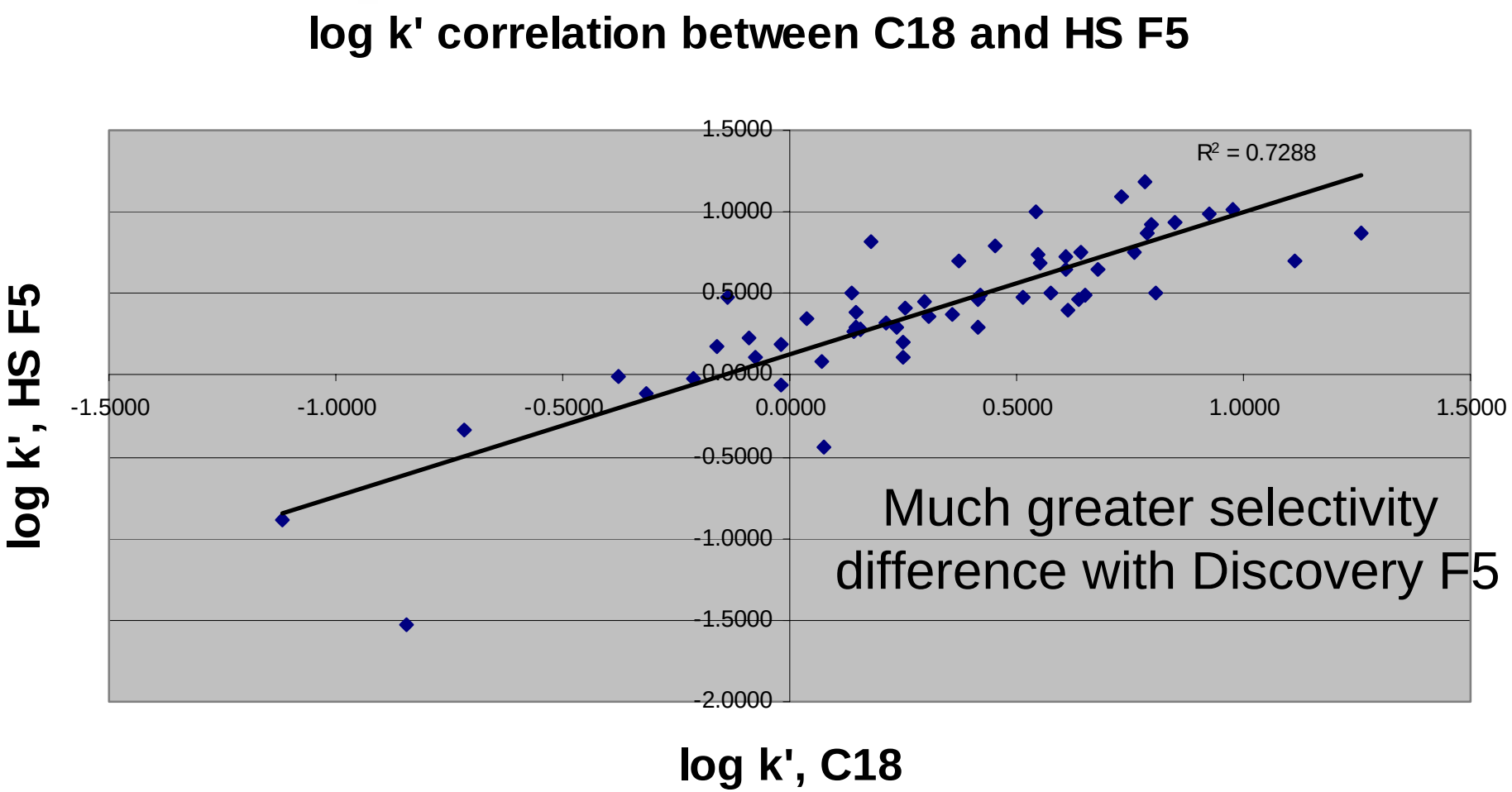
Examples of Data Obtained in Study

- **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



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How Can I Use This in My Work?

- **Look up your compound in table. (see Supelco Re-Discover Method Development Guide) 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.

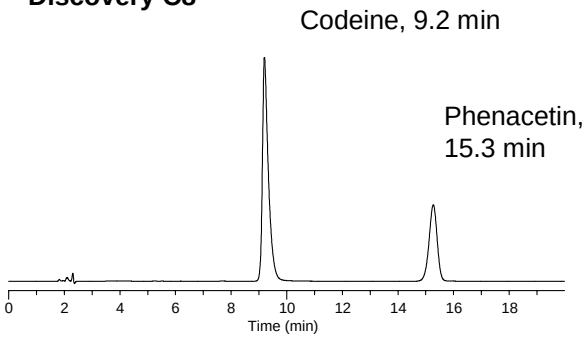
Column: The pH 7 screening data shows the compounds have the preferred elution order (codeine then phenacetin) on all but the Discovery HS F5 column, however, if the preferred elution order was reversed, the HS F5 would be the best choice.

Applying Column Screening Study Results

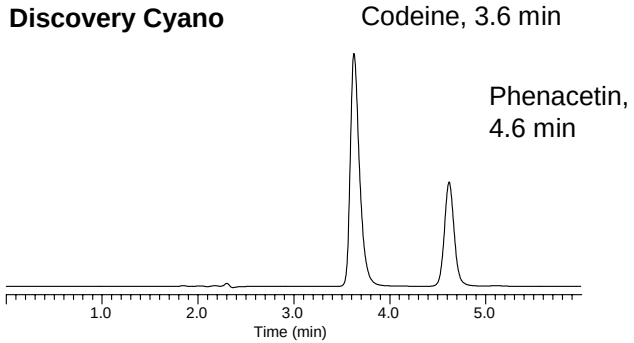
Column	k' Codeine@ 15% Acetonitrile	Est. k' Phenacetin@ 15% Acetonitrile	Alpha
C18	4.4	4.7 x 2 = 9.4	2.1
RP-AmideC16	3.3	4.8 x 2 = 9.6	2.9
C18	3.6	4.1 x 2 = 8.2	2.3
Cyano	1.1	1.3 x 2 = 2.6	2.4

Applying Column Screening Study Results

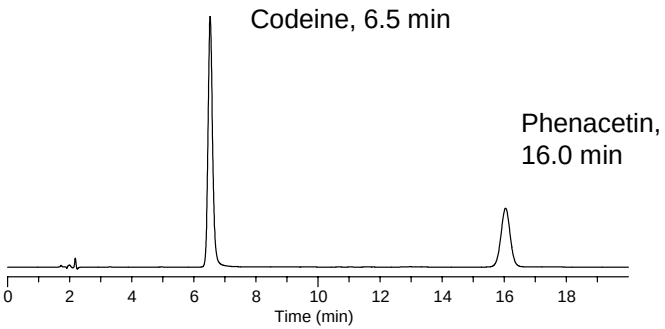
Discovery C8



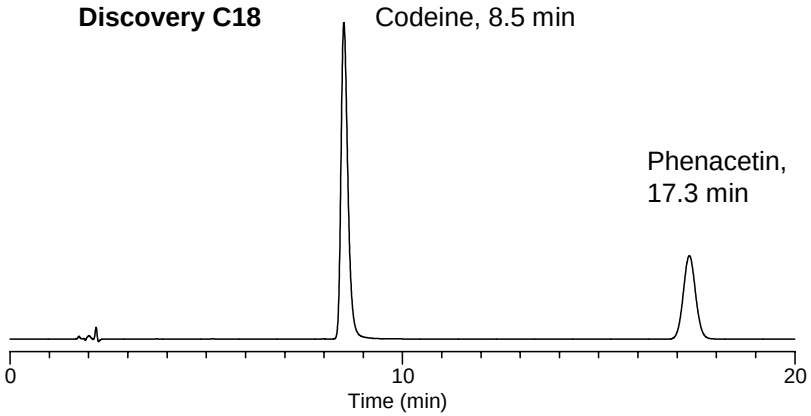
Discovery Cyano



Discovery 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: Predicted vs Actual

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.**

Column	Analyte	Predicted k'	Actual k'	Alpha Predicted	Alpha Actual
Discovery C8	Codeine	3.6	3.6	2.27	1.86
	Phenacetin	8.2	6.7		
Discovery Cyano	Codeine	1.1	1	2.36	1.6
	Phenacetin	2.6	1.6		
Discovery RP-AmideC16	Codeine	3.3	2.8	2.9	3
	Phenacetin	9.6	8.4		
Discovery C18	Codeine	4.4	3.3	2.13	2.33
	Phenacetin	9.4	7.7		

Conclusion

1. A systematic method has been developed to determine selectivity and retention differences in stationary phases.

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

3. Comparisons can be made between phases to show which phases are most alike and which are most different.

4. 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.