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Systematic Studies on Column Ruggedness when Separating Weak Acids Using Reversed-Phase Liquid Chromatography

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ABSTRACT

Ruggedness of three reversed-phase C_{18} columns of different brands and with different sizes was investigated for the separation of an acidic sample containing 4-hydroxybenzoic acid, acetylsalicylic acid, benzoic acid, 4-methoxyphenyl acetic acid, 3,5-dinitrobenzoic acid, o-toluic acid and phenylpropionic acid. These columns included Alltech Altima C_{18} (25×0.46 cm), Burdick & Jackson C_{18} (25×0.46 cm) and Alltech Nucleosil C_{18} (15×0.46 cm).

The optimum separation conditions were found to be under mobile phase conditions of 30% acetonitrile buffered with 15 mM of both sodium phosphate monobasic and sodium acetate at pH 3.01 when using Alltech Altima C_{18}. Identical separations with subsequent improvement in column efficiency were observed with Burdick & Jackson C_{18} and Alltech Nucleosil C_{18} columns when sodium acetate buffer concentration increased to 70 mM with re-adjusting solvent strength to 20% acetonitrile. This improvement in peak shape and column ruggedness resulted from deactivating the secondary interactions between acids studied and free silanols of silica base of the stationary phases.

INTRODUCTION

Column ruggedness measures the degree of reproducibility of the test results obtained by the analysis of *same* sample under a variety of columns. It plays an important role in the robustness of the developed high performance liquid chromatographic (HPLC) method when columns from different venders are used or when a method is transferred from one lab to another. For example, an HPLC method for the quantitation of an active ingredient in a pharmaceutical product is developed in United States and transferred to another lab in Japan. The major setback in the reproducibility of the developed method is the use of columns from different venders (Heyden, et al., 1996).

The last 10 years have shown remarkable progress in the quality and reproducibility of HPLC columns. In spite of the progress and safeguards put into place, column ruggedness is still a major concern in the field of high performance liquid chromatography.

Concerns still exist in the heavily regulated industries (pharmaceutical, environmental analysis) when columns from different venders and even from the same vender but different batches may have an adverse effect on method ruggedness (Kateman, et al., 1993 and Snyder, et al., 1997). For this reason, a developed method requires testing multiple columns from different venders when the method is tested for validation. The objective of this study is to establish a common ground to improve ruggedness of reverse-phase columns. This study is limited to a sample of weak acids and columns of C_{18} type.

MATERIALS AND METHODS

Instrumentation

The HPLC system used consisted of a Spectra Physics SP 8800 ternary pump with a Spectra Physics 100 variable wavelength detector, a Hewlett Packard 3390A integrator and a Rheodyne sample injector (model 7715i, Cotati, CA). The Alltech Altima C_{18} (25 x 0.46 cm, 5 µm) and Alltech Nucleosil C_{18} (15 x 0.46 cm, 5 µm) columns were purchased from Alltech Associates, Deerfield, Illinois, (USA). Burdick and Jackson C_{18} column (25 x 0.46 cm, 5 µm) was purchased from Burdick & Jackson, Muskegon, MI (USA). All pH measurements were performed with ATI Orion model 310 pH meter. The flow rate was maintained at 1.0mL/ min and the detector wavelength was set at 254 nm. All separations were conducted under ambient temperature.

Chemicals and Reagents

Acetonitrile and sodium acetate were obtained from Fisher Scientific Company. Benzoic acid, 4-hydroxybenzoic acid, 3,5-dinitrobenzoic Acid, 4-methoxyphenyl acetic acid, phenylpropanoic acid and sodium phosphate were obtained from Aldrich Chemical Company, Inc. Acetylsalicylic acid was obtained from Acros Organics while o-toluic acid was obtained from Eastman Kodak Co. All organic solvents used were of HPLC grade while other reagents were of ACS grade.

The mobile phase used consisted of 15 mM sodium acetate ($NaCH_3COO$) and 15 mM sodium phosphate monobasic (NaH_2PO_4) . It was adjusted to the desired pH with acetic acid and phosphoric acid and filtered through a 0.45 µm membrane filter (Millipore Corporation, Massachusetts, USA). All samples used were prepared in 50% acetonitrile (ACN).

RESULTS AND DISCUSSION

Different studies were conducted to optimize mobile phase strength and separation conditions using Alltech Altima C_{18} column. At the optimum conditions of 30% ACN, separation of sample components was tested at different pH values between 2.90 and 4.50. Poor resolution was observed between 4-methoxyphenyl acetic acid and 3,5-dinitrobenzoic acid at pH 3.50, between acetylsalicylic acid and 4-methoxyphenyl acetic acid at pH 4.52, and between benzoic acid and 4-methoxyphenyl acetic acid at pH 2.90 (Table 1). DryLab© software simulated pH 3.01 as the optimum mobile phase acidity. The simulated retention times were found in a good agreement with the experimental results at pH 3.01 with the exception of 3,5-Dinitrobenzoic acid (Table 2).

When the optimum conditions for the Alltech Altima C_{18} column were tested on Alltech Nucleosil C_{18} and Burdick and Jackson C_{18} columns, poor resolutions were observed with much shorter run times (Figure 1). These results could be related to the differences in column efficiencies as well as the significance of secondary interactions between the sample components and the silanols on the silica base of the three stationary phases. To deactivate these interactions, concentration of acetate buffer salt in the mobile phase was increased from 15 mM to 30 mM. A significant improvement in the resolution with much narrower peaks was obtained with 30% ACN containing 30 mM $NaCH_3COO$ and 15 mM of NaH_2PO_4 at pH 3.01. The differences in the resolution between the three columns studied minimized, but the differences in run times still existed. The latter was related to the differences in carbon loading of the three columns studied. This was confirmed when the solvent composition of the mobile phase was reduced to 20% ACN while maintaining pH 3.01. Separations on Alltech Nucleosil C_{18} and Burdick and Jackson C_{18} columns were found identical with the exception of some tailing in the late eluting peaks. To overcome this problem, concentration of acetate buffer was increased gradually while maintaining solvent strength and pH of the mobile phase unchanged. A significant improvement in the peak shape was obtained when 70mM sodium acetate was used (Figures 2 and 3).

These observations concluded that column ruggedness could be significantly improved when separating weak acids by simply deactivating interactions of the acids with silanols on the silica base of the stationary phase and by re-adjusting mobile phase solvent strength. In this study, this was accomplished by using a mobile phase of 20% ACN buffered with 70mM of $NaCH_3COO$ and 15mM NaH_2PO_4 at pH 3.01 for columns Alltech Nucleosil C_{18} and Burdick and Jackson C_{18} columns, while 30% ACN buffered with 15mM of both NaH_2PO_4 and $NaCH_3COO$ at similar pH for Alltech Altima C_{18} column.

LITERTURE CITED

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Table 1. pH effect on the separation the acidic sample on Alltech Altima C_{18} column.

	Name of acids	Retention Time (min)			
		pH 2.90	pH 3.50	pH 4.02	pH 4.52
1	4-Hydroxybenzoic Acid	4.82	4.82	4.77	4.66
2	Acetylsalicylic Acid	8.76	8.53	7.63	6.12
3	Benzoic Acid	10.22	10.17	9.74	8.79
4	4-Methoxyphenyl Acetic Acid	10.78	10.63	10.55	6.12
5	3,5-Dinitrobenzoic Acid	14.53	10.63	7.31	10.00
6	o-Toluic Acid	15.91	15.82	14.96	13.15
7	Phenylpropanoic Acid	16.72	16.69	16.68	15.96

Table 2. Comparison of simulated DryLab© with experimental results at pH 3.01 on Alltech Altima C_{18} Column.

	Name of acids	Retention Time (min)		
		Simulated	Experimental	% Agreement
1	4-Hydroxybenzoic Acid	4.82	4.75	98.5
2	Acetylsalicylic Acid	8.76	8.74	99.8
3	Benzoic Acid	10.22	10.18	99.6
4	4-Methoxyphenyl Acetic Acid	10.82	10.77	99.5
5	3,5-Dinitrobenzoic Acid	14.60	13.28	91.0
6	o-Toluic Acid	15.91	15.93	100.1
7	Phenylpropanoic Acid	16.72	16.79	100.4

Figure 1. Separation of the mixture of weak acids on three different column types using a mobile phase of 30% ACN buffered with 15mM of both $NaCH_3COO$ and NaH_2PO_4 at pH 3.01: (a) 25 cm Alltech Altima C_{18}Column, (b) 25 cm Burdick & Jackson C_{18} Column, (c) 15 cm Alltech Nucleosil C_{18} Column.

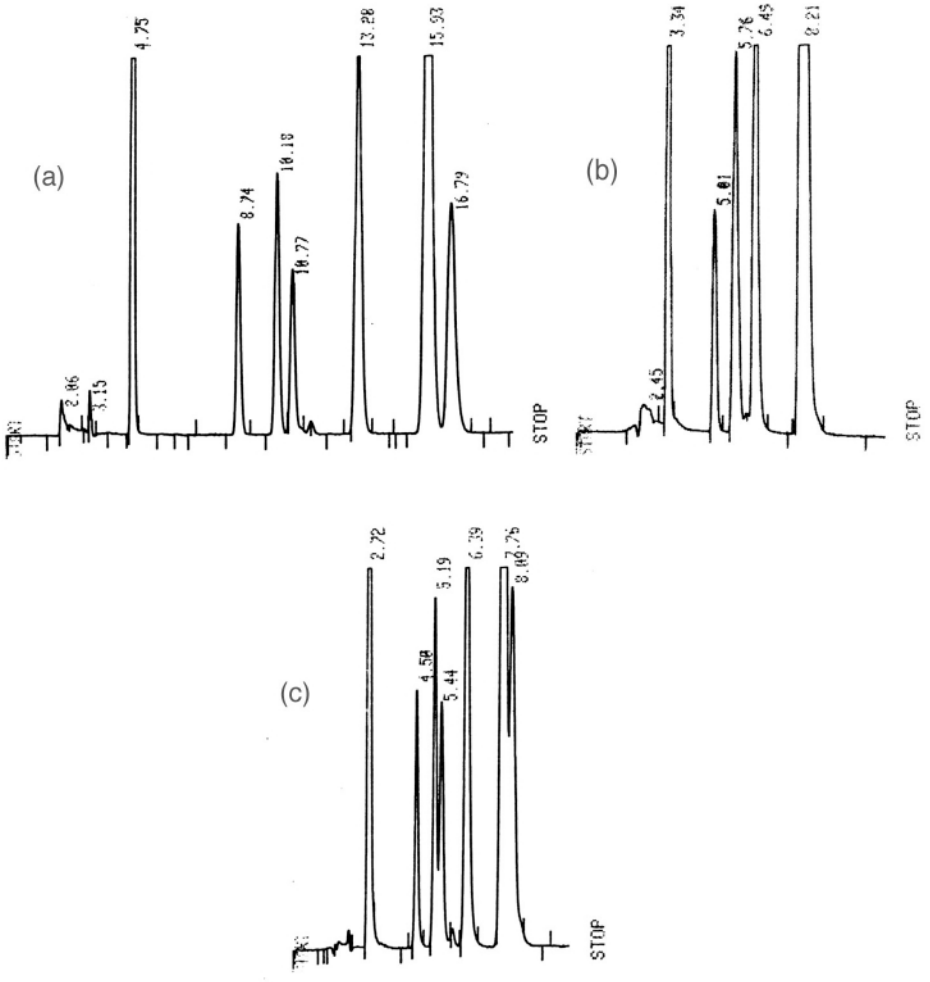

Figure 2. Separation of the mixture of weak acid on 25 cm Burdick & Jackson C_{18} Column using a mobile phase of 20% ACN buffered with 70mM $NaCH_3COO$ and 15mM NaH_2PO_4 at pH 3.01.

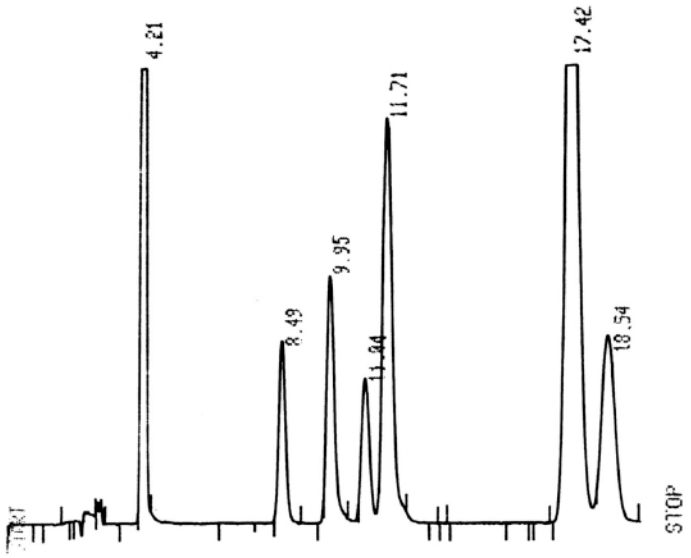

Figure 3. Separation of the mixture of weak acids on 15 cm Alltech Nucleosil C_{18} Column using a mobile phase of 20% ACN buffered with 70mM $NaCH_3COO$ and 15mM of NaH_2PO_4 at pH 3.01.