Increasing Sample Throughput Using ZORBAX Eclipse XDB C18 Rapid Resolution HT Columns

Application

Authors

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Abstract

Using Zorbax Rapid Resolution HT columns with 1.8 µm particles, it is possible to reduce analysis time without compromising needed resolution. The use of a short, high efficiency column permits easy scaling of existing methods from longer columns, resulting in increased sample throughput for liquid chromatography and liquid chromatography/mass spectrometry (LC and LC/MS) methods while reducing the cost of analysis.

Introduction

As the use of LC has increased, so have the demands on silica-based LC columns. The expectation for LC columns today requires the columns to be available in short lengths with high efficiency. High purity silica is also required for good peak shape of basic compounds. To meet these challenges, improvements have been made in the preparation of very high purity silica and the stability of the bonded phase chemistry. These improvements now permit the analysis of polar and non-polar compounds over a wide pH range that in the past has been reserved for polymeric supports [1].

One of the main goals in the preparation of silica particles has been to reduce the size of the particles while maintaining a very narrow size distribution. Reduction in particle size results in higher column efficiencies. Therefore, LC columns made with small particle-size silica can be made shorter and have efficiencies similar to longer columns made with larger particles. It has been demonstrated that using short columns with small particles reduces analysis times [2, 3]. As a result, typical particle size has decreased from 10 to 3.5 µm as these smaller sizes have become commercially available.

Theoretical and experimental studies [4, 5] have shown that the optimum particle size for LC separations is about 2 µm. Columns prepared with 1.8 µm totally-porous particles are the optimum size and should have about twice the efficiency of the 3.5-µm particles available today, further permitting even shorter (15 mm–50 mm) columns to be used for very fast separations. Until now, totally porous particles of 2 µm or less have been difficult to manufacture reproducibly.

We report here the preparation of short (15 mm–50 mm), efficient columns packed with 1.8-µm particles bonded with Eclipse XDB-C18. These Rapid Resolution HT (RRHT) columns are used to demonstrate faster analysis times

compared with traditional length columns (100 mm–250 mm) containing larger particles without compromising component resolution. The added benefit when using RRHT columns is the reduction in mobile phase usage, which can significantly reduce the cost of analysis.

Experimental Conditions

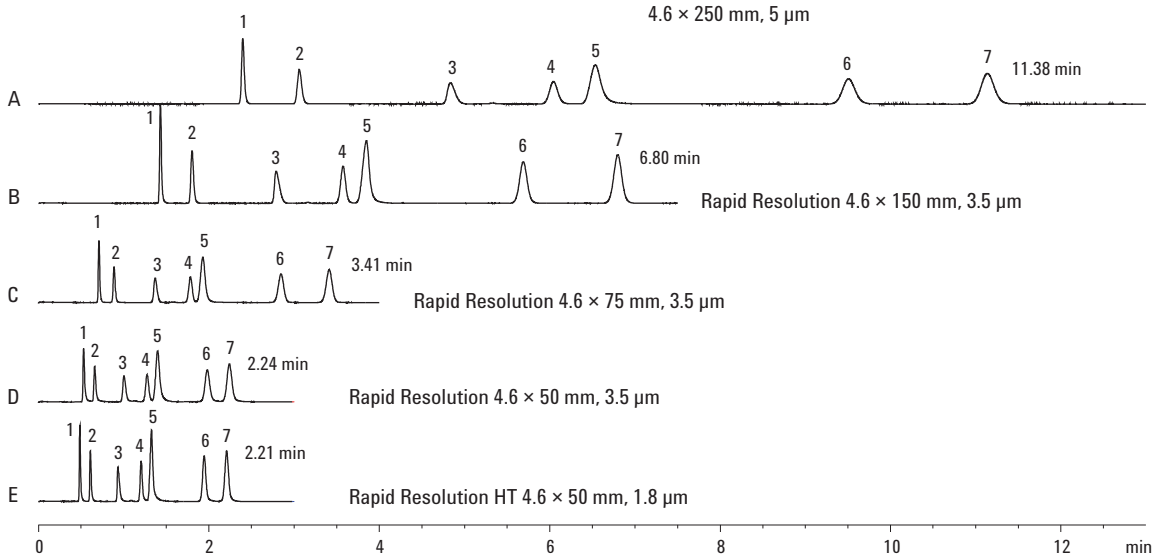
All experiments were performed using an Agilent 1100 LC equipped with a solvent degasser, binary pump, autosampler, heated column compartment, and a diode array detector. All of the modules were connected with standard lengths of 0.12-mm inner diameter (id tubing). The diode array detector was equipped with a semi-micro flow cell (Agilent part number G1314-60083) and data was collected at a rate of 0.01 second [6]. Data acquistion and instrument parameters were controlled using the Agilent ChemStation version 9.03.

The organic modifier used was methanol and was HPLC grade solvent (Burdick and Jackson). Monobasic and dibasic sodium phosphate salts (J.T. Baker) were combined and dissolved in HPLC grade deionized water to give a concentration of 20 mM and a pH of 7.0.

A sample of acids, bases, and neutrals (Sigma) was prepared consisting of naproxen (1.5 mg/mL), mefenamic acid (4.0 mg/mL), butyl paraben (0.5 mg/mL), propranolol (1.3 mg/mL), naphthalene (3.1 mg/mL), dipropyl phthalate (4.0 mg/mL), and uracil (0.4 mg/mL). The samples were dissolved in 60% methanol, 40% water.

Results

A standard sample containing acids, bases, and neutrals was used to validate the performance of the new RRHT columns for low silanol activity, high efficiency, and identical selectivity to standard 3.5 and 5-µm products. For this test, a series of Eclipse XDB-C18 phases were prepared with different particle sizes and different column dimensions. Figure 1 shows the resulting chromatograms on each of the columns tested.



Columns:	ZORBAX Eclipse XDB-C18	Mobile phase:	73% MeOH, 27% 20 mM Phosphate buffer, pH 7.0
Part numbers:	A) 990967-902	Flow rate:	1 mL/min
	B) 963967-902	Temperature:	Ambient
	C) 966967-902	Detection:	UV 254 nm
	D) 935967-902	Sample:	1) Uracil, 2) Naproxen,
	E) 925975-902		3) Mefanamic acid, 4) Butyl paraben,
			5) Propranolol, 6) Naphthalene, 7) Dipropyl phthalate

Figure 1. Reduce analysis time by 80% using RRHT columns.

Chromatogram A shows the test sample on an Eclipse XDB-C18 column with a standard dimension of 4.6 × 250 mm containing 5-µm particles. This column has high efficiency, as can be seen by the separation of butyl paraben and propranolol. The peak shape of the polar components is very symmetrical because of the highly endcapped nature of the Eclipse XDB stationary phase.

Analysis time can be reduced by increasing flow rate, but the resulting decrease in efficiency can compromise component resolution. Increasing the organic modifier content of the mobile phase reduces analysis time but may result in unwanted selectivity changes.

Another approach to reduce analysis time is to reduce column dimension and particle size. Doing this in a controlled fashion results in predictable analysis results. Chromatograms B, C, and D in Figure 1 illustrate the effect of decreasing column dimensions. Chromatogram B shows the same sample on a shorter Eclipse XDB-C18 column packed with smaller 3.5-µm particles. The expectation is that retention times would be reduced by 40% (due to a 40% decrease in length). Efficiency would be reduced only slightly, since the particle size was also reduced from 5 to 3.5 µm. The actual results show a decrease in retention of 40% and a slight decrease in resolution that still permits separation of all components.

Chromatograms C and D show the test sample on even shorter Eclipse XDB-C18 columns with the 3.5-µm particle size. Here again, retention times are very close to predicted values and resolution decreases as the columns become shorter due to efficiency decreases. Unfortunately, baseline resolution of butyl paraben and propranolol on the 50-mm column has been compromised in Chromatogram D.

Zorbax RRHT columns are available in a variety of column dimensions; their high efficiencies increase resolution, while their short lengths decrease analysis times. In this example, Chromatogram E shows the test sample on an Eclipse XDB-C18 RRHT column. Retention times are as expected, but now resolution of the key components has increased for more accurate quantitation. The sample analysis is now complete in just over 2 minutes on the RRHT column compared with just over 11 minutes with the standard column, for an 80% decrease in analysis time. It is possible to anlyze five samples on the RRHT column for every one sample on the standard column with the same amount of solvent consumption.

Table 1 summarizes some of the important chromatographic performance measures for this series of columns. Notice that the data confirms the utility of the short, highly efficient RRHT columns for maximum sample throughput, as evidenced by the significant decrease in analysis time, while maintaining sufficient resolution. Methods can be easily and predictably scaled from longer columns with larger particles to RRHT columns without changing selectivity, as is seen by actual and predicted retention times being the same. This, coupled with the availability of the highly endcapped Eclipse XDB-C18 phase, provides a stationary phase that is ideal for the analysis of polar components over a wide pH range.

Table 1. Column Performance Measures

	Retention Time*		
Column	**Actual (calc.)**	**Efficiency***	**Resolution****
4.6 × 250 mm, 5 µm	11.38	22,700	2.1
4.6 × 150 mm, 3.5 µm	6.8 (6.83)	22,000	1.8
4.6 × 75 mm, 3.5 µm	3.41 (3.40)	11,000	1.4
4.6 × 50 mm, 3.5 µm	2.24 (2.27)	6,400	1.2
4.6 × 50 mm, 1.8 µm	2.21 (2.27)	12,000	1.6

*Determined for dipropyl phthalate

** Resolution between butyl paraben and propranolol

Table 2 summarizes an estimate of the costs associated with each analysis on the Eclipse XDB-C18 standard 4.6 × 250 mm and the 4.6 × 50 mm RRHT columns. Several assumptions were made in calculating cost per analysis based on estimated costs for labor, methanol usage, and waste disposal. A cost reduction of over 75% was realized when using the RRHT column versus the standard column.

Table 2. Cost Summary (Cost/Analysis)

	Eclipse XDB-C18	**Eclipse XDB-C18 RRHT**
Column size:	4.6 × 250 mm, 5 µm	4.6 × 50, 1.8 µm
Column cost:	$500	$200
Labor ($30/h):	$6.00 (12 min)	$1.50 (2.5 min)
Solvent ($27/L):	$0.24 (8.8 mL)	$0.03 (1.1 mL)
Disposal ($2/L):	$0.02	< $0.01
Total:	$6.26	$1.54

Conclusions

In this note, we present a series of chromatograms showing several advantages of the Zorbax RRHT columns. The high efficiency of these very small particle columns maximizes component resolution. The short column lengths allow fast analysis times for increased sample throughput. Uniform silica manufacturing and stationary phase coverage allow direct scaling of methods to smaller particles for predictable and reproducible results. The Eclipse XDB-C18 stationary phase is ideal for the analysis of a wide range of polar and non-polar compounds because of its highly endcapped nature and its stability over a wide pH range.

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