Tailoring Speed, Sensitivity, and Resolution in an RRHT Analysis of Cardiac Drugs

Application

Pharmaceutical

Authors

John W. Henderson Jr. and William J. Long
Agilent Technologies
2850 Centerville Road
Wilmington, DE 19808
USA

Abstract

A variety of nitrogen-containing drugs used to treat heart disease were separated on ZORBAX Rapid Resolution (RR), 3.5-µm, and Rapid Resolution High Throughput (RRHT), 1.8-µm, columns of differing dimensions. All three column configurations produce satisfactory results, creating the luxury of deciding which column configuration to choose for finalizing a method. Speed, resolution, and sensitivity are primary goals in method development and column selection. When using RR or RRHT technology, these three factors can be quickly achieved using more than one column configuration. In this application we compare three RR and RRHT columns, considering speed, sensitivity, and resolution to make a column choice. Additionally, with the primary goals met, we demonstrate how high temperature can be used to alter selectivity and further improve resolution of critical pairs in the chromatogram.

Introduction

The Trade-Off Triangle

The relationship between speed, resolution, and sensitivity in high-performance liquid chromatography (HPLC) is often described as a triangle (Figure 1) where any two objectives can be improved, but only by compromising the third. When developing methods on traditional sized columns (4.6 × 150 or 250 mm, 5 to 10 µm), determining which column dimensions to choose is uncomplicated. Typically, if the 150-mm long columns don't provide sufficient separation, then the 250-mm lengths are chosen. Unfortunately the desired resolution is acquired at the expense of time and sensitivity.

The same holds true for rapid resolution (RR) and rapid resolution high throughput (RRHT) columns. However, with the advent of RRHT technology, the triangle has become much smaller. In effect, with the smaller triangle, when two of the variables are optimized, the third is still maintained, that is, all three variables are attainable. Secondary considerations such as pressure, temperature, and solvent

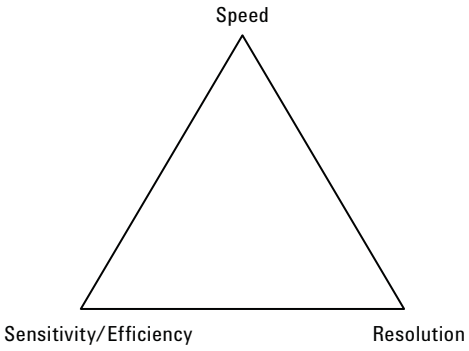

Figure 1. HPLC trade-off triangle.

consumption can now have a larger role in column selection and method development.

In this application we demonstrate some of the chromatographic tailoring that is possible by various RR and RRHT columns. A complex mixture of cardiac drugs (see Figure 2 for structures and pKa values) is separated using three different column configurations, showing the relationship between speed, sensitivity, and resolution, and that these are attainable with RRHT technology.

Experimental

Method parameters for all figures consist of an Agilent 1200 Rapid Resolution Liquid Chromatograph (LC), 1.8 or 3.5 µm ZORBAX StableBond-C18 stationary phase in 4.6-mm i.d columns of various lengths, 60 °C, diode array detector (DAD) used at 230 nm. Mobile phase channel A is 0.1% trifluoroacetic acid (TFA), 5% acetonitrile (ACN) (v/v). Mobile phase B is 0.08% TFA in 95% ACN (v/v). The smaller amount of TFA in channel B mitigates the rising baseline typically encountered by this mobile phase and low wavelength conditions. The flow rate is 2 mL/min. The mobile phase gradient slope is changed proportionally to match the column length to keep solvent-strength selectivity (k*) the same:

Table 1. Gradients for Equivalent k*

% B	50 mm	150 mm	250 mm
12.5	0 min	0 min	0 min
60	3.5	10.5	17.5
60	4	12	20
12.5	4.01	12.01	20.01

Results and Discussion

Comparing Resolution Between Three Different Column Configurations

The result in Figure 3 is a similar elution pattern for all three column configurations. The uniform ZORBAX particles, bonding, and proprietary manufacturing technology allow straightforward method scalability from longer columns packed with 5- or 3.5-µm particles, to shorter columns packed with 1.8-µm particles.

If resolution is the overriding factor determining column selection, then the 4.6 x 250 mm, 3.5-µm RR column (top chromatogram), with the most resolution between the critical pair, is the best choice, even though the shorter columns provide ample resolution in less analysis time.

Comparing Speed Between Three Different Column Configurations

Figure 4 displays the same three chromatograms viewed with the same time scale. This clearly demonstrates the time savings easily gained by simply substituting traditional analytical columns with RRHT columns. When high throughput is the primary factor determining column choice, then the 4.6 x 50 mm RRHT column (bottom chromatogram) is the best choice. It provides time savings of over a factor of four and sufficient resolution of 2.0 between peaks 3 and 4.

Comparing Sensitivity/Efficiency Between Three Different Column Configurations

In Figures 3 and 4, injection volumes were scaled proportionally to column length, just as gradient conditions were, therefore peak heights cannot be directly examined to compare sensitivity. Indirectly, however, sensitivity can still be compared if the amount injected is normalized. Table 2 compares the peak areas and heights of propranolol (peak 7). As expected, the ratio of the area to injection amount is the same when normalized. However when the ratio between height and injection amount is compared, there is a significant difference. In an isocratic analysis, some of the gain in

Table 2. Comparing the Sensitivity of the Three Column Configurations

Column	Normalized injection amount	Area	% area difference	Height	% height difference	% gain in sensitivity
4.6 × 250 mm, 3.5 µm	100	817		226		
4.6 × 150 mm, 1.8 µm	60	510	62	201	89	27
4.6 × 50 mm, 1.8 µm	20	178	22	112	50	28

1. Procainamide, pKa = 9.2
2. Procaine, pKa = 9.0
3. Nadolol, pKa = 9.7
4. Pindolol, pKa = 8.8
5. Lidocaine, pKa = 7.8
6. Disopyramide, pKa = 10.4
7. Propranolol, pKa = 9.5
8. Nifedipine
9. Nimodipine
10. Nisoldipine

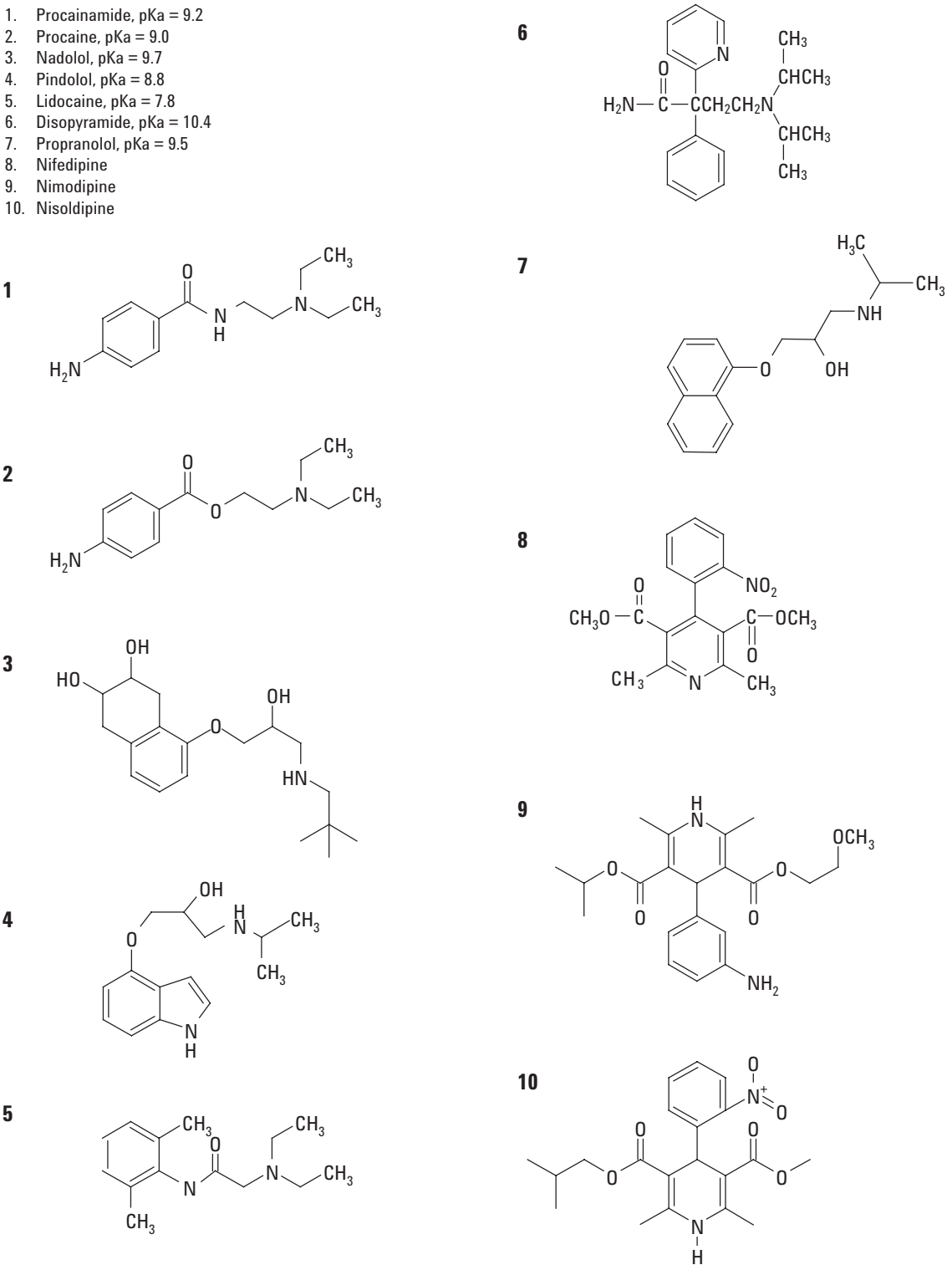

Figure 2. Heart disease drug structures and approximate pKa values listed by elution order.

sensitivity would be due to peaks eluting faster from the shorter columns and having less time to diffuse or widen. In these gradient analyses k* is maintained; the 30% gain (approximately) in height (sensitivity) was due to the higher efficiency of the smaller 1.8-µm particle compared to the larger 3.5-µm particle. Notice there is no increase in sensitivity of the 50 mm 1.8-µm compared to the 150 mm 1.8-µm column. An increase in sensitivity would be expected in an isocratic run. If sensitivity is the main factor determining column selection, either of the RRHT (1.8-µm) columns, with 30% taller peaks, are the best choice. The middle chromatogram, using a RRHT 4.6 x 150 mm, is a

good option. It has better resolution than the 50-mm and better speed than the 250-mm column.

Considering System Pressure in High Throughput Analysis

High system back pressure, or more specifically its perceived wear on instrumentation, including the column itself, is often considered a price to pay for high throughput analyses involving sub-two-micron particles. ZORBAX silica, however, is an extremely strong particle. The proprietary particle size chemistry produces back pressures typically less than other sub-two-micron columns. It has been demonstrated to last for thousands of injections and elevated temperatures (80 °C) (Figure 5), using the Agilent 1200 Series Rapid Resolution LC.

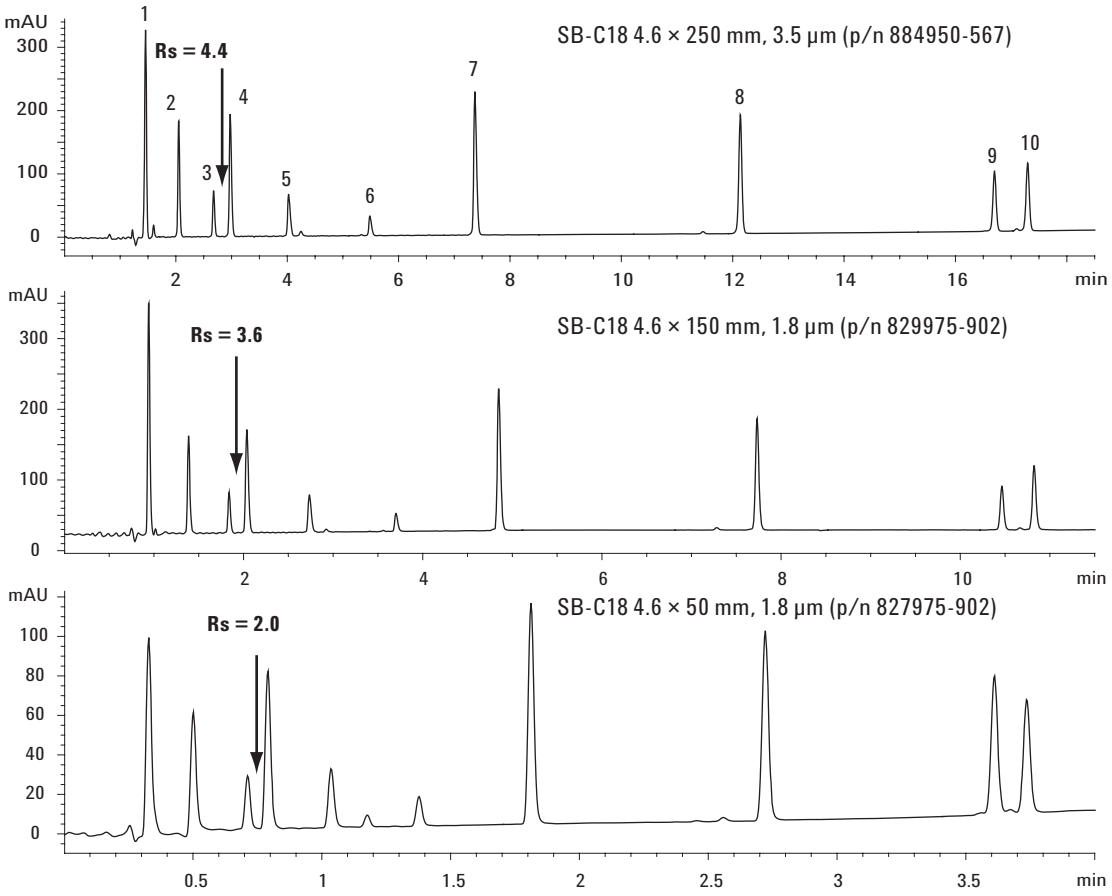

Figure 3. Scalability of SB-C18 column dimensions and its effect on resolution of the critical pair.

Figure 4 indicates the system pressures (P), in bar, of the three methods. The middle chromatogram, noted in the above paragraph is a good choice for speed, resolution, and sensitivity. It has higher pressure than the other two column configurations and is well within the operating range of the Agilent 1200 RRLC (max. 600 bar). The other two columns have lower pressure, and would be preferred for use on the standard Agilent 1200 or on previous-generation LCs, such as the Agilent 1100.

The Luxury of Column Options

The separation results achieved with the different configurations are tabulated in Table 3.

There are many other column configurations available, consisting of more than 90 columns between 30 mm and 250 mm in length, with 1.8-, 3.5-, and 5-µm particle sizes in diameters of 1.0, 2.1, 3.0 and 4.6 mm. As Table 3 indicates, each column configuration has its specific separation attributes, such as resolution, speed, and pressure, for a particular method. In general, if keeping the method conditions constant (for gradients, keeping k* constant by *changing* the gradient time), better resolution is obtained by longer columns. Longer columns, however, mean longer analysis times. Substituting a shorter column with smaller particles can significantly reduce the analysis time while still maintaining sufficient resolution.

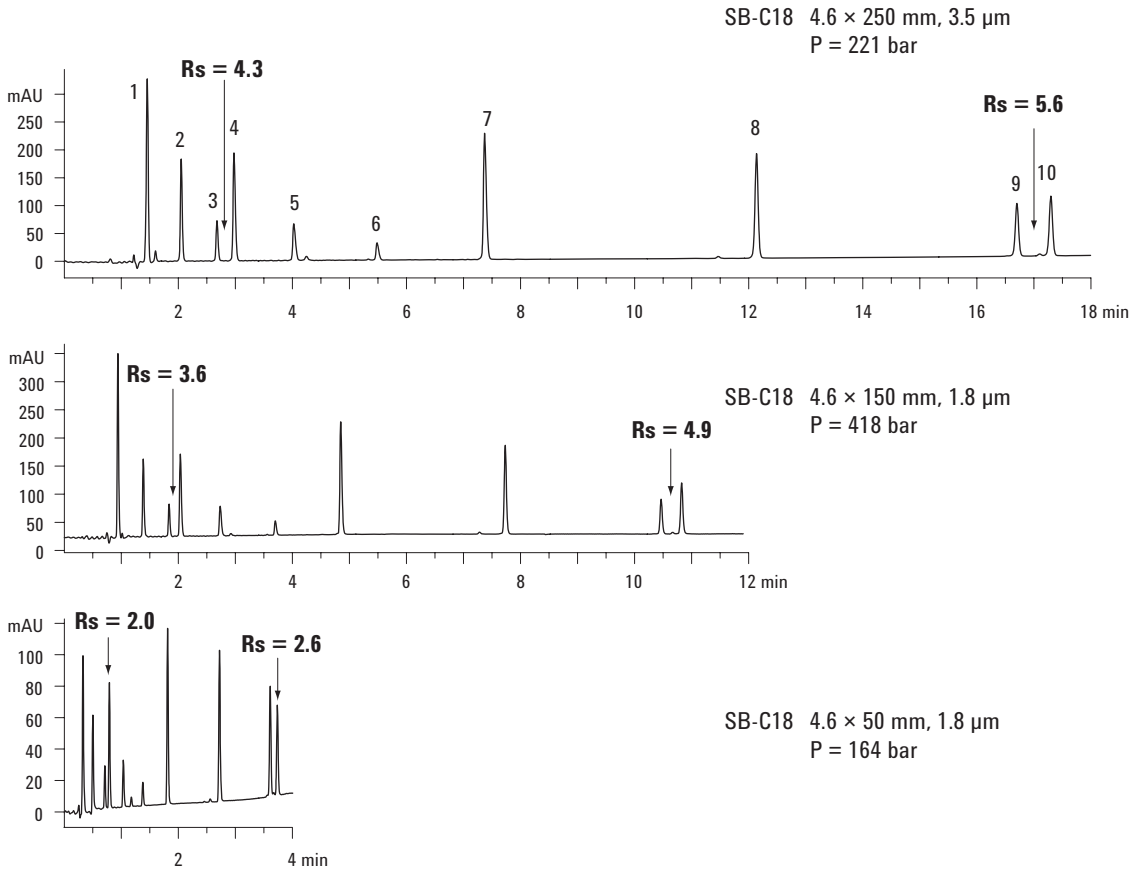

Figure 4. Scalability of SB-C18 column dimensions and its effect on analysis time and pressure.

Table 3 Gradient Separation Results of the Column Configurations, Holding K* Constant

Column Configuration			Separation Attributes		
Length (mm)	Particle (µm)	Flow (mL/min)	Resolution of critical pair	Gradient time (min)	Pressure (bar)
250	3.5	2	4.4	20	221
150	1.8	2	3.6	12	418
50	1.8	2	2	4	164

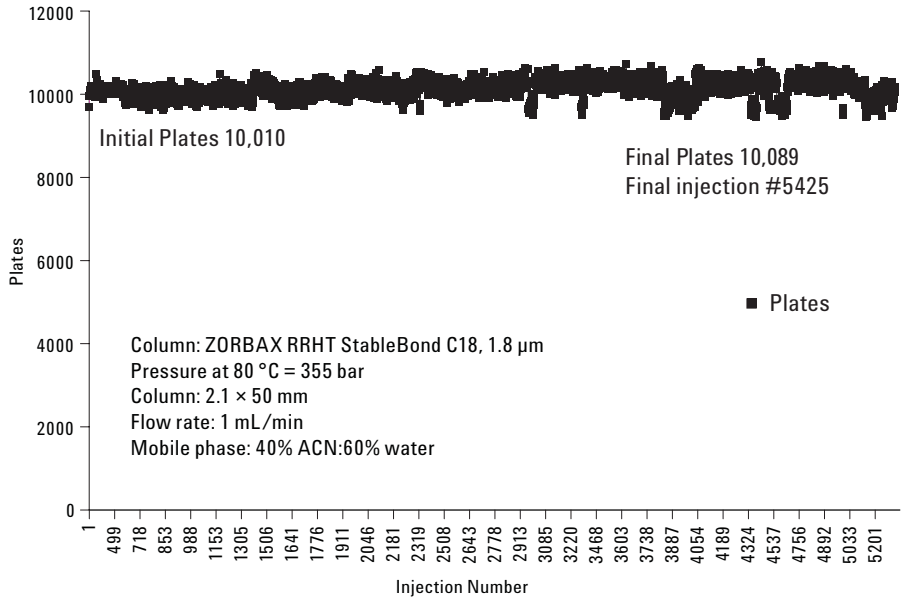

Figure 5. SB-C18 RRHT longevity at elevated temperature.

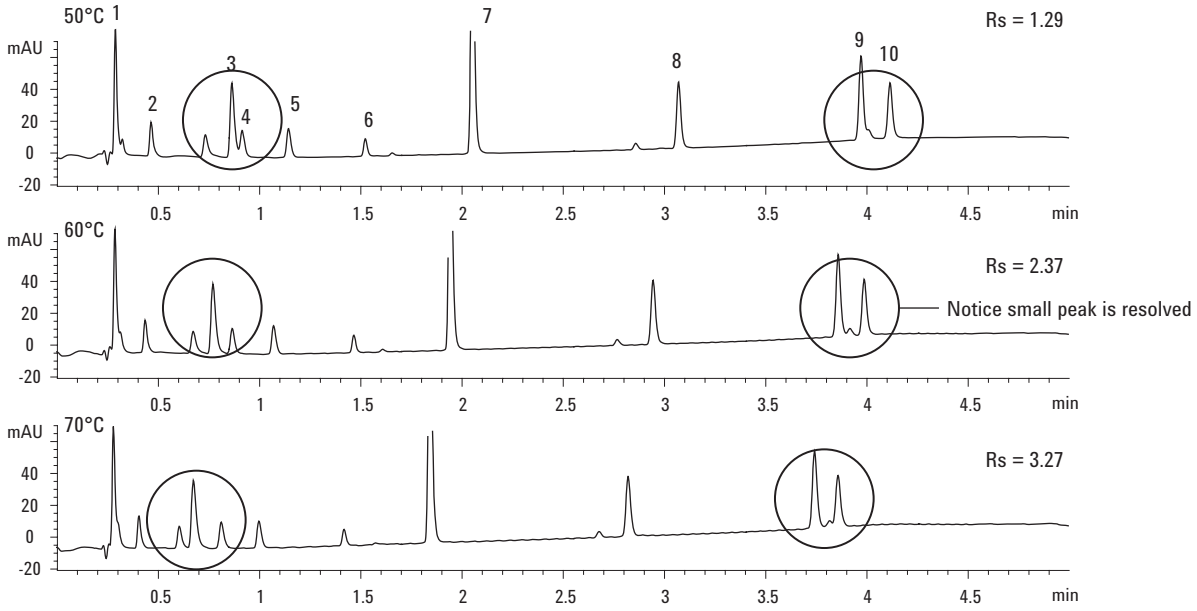

Figure 6. Use elevated temperature to maximize resolution.

Shorter columns used for gradient methods also provide a bonus of quicker column re-equilibration times. In this cardiac drug example, column re-equilibration time for the 250 mm was 4 minutes, for the 50 mm column, it was fivefold less, or 0.8 minutes.

Effect of Elevated Temperature on Selectivity

These examples demonstrate that ZORBAX RRHT columns easily provide high throughput, resolution, and sensitivity. SB-C18 RRHT columns have another powerful feature: column stability at temperatures up to 90 °C, as indicated in Figure 6. Elevated temperature is an easy but useful way to change selectivity, improve resolution, and reduce system back pressure. Figure 6 demonstrates how different temperatures influenced the heart drug method. Two critical pairs, consisting of one of the heart drugs and an unknown, are circled. Changing the temperature influenced resolution. In this example, 60 °C appears to be the most useful for the method. Resolution of the two critical pairs is largest. At a lower temperature (50 °C), resolution is lower. At a higher temperature (70 °C), the selectivity of the later eluting critical pair changes so that the unknown begins to co-elute with a third peak.

Conclusions

Speed, resolution, and sensitivity/efficiency are dynamically related to one another. One of these factors ends up being compromised to enhance the other two. RRHT technology mitigates the trade-off, offering maximum separation flexibility. In this study, we demonstrated how all three of these goals can be achieved when transferring methods from longer columns to shorter RRHT columns. Elevated temperature is another tool to improve separations. Increasing the temperature can change retention and selectivity. SB-C18 is ideal for elevated temperatures. A broad selection of ZORBAX RR and RRHT columns is available to customize methods based on the chromatographer's preference for speed, sensitivity, or resolution.

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