Improve Peak Shape and Productivity in HPLC Analysis of Pharmaceutical Compounds with Eclipse Plus C8 Columns

Application

Pharmaceuticals

Authors

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Abstract

Seven examples of pharmaceutical analyses employing ZORBAX Eclipse Plus C8 columns are presented. Eclipse Plus C8 is an excellent starting point for method development because of its excellent peak shape for bases, acids, and neutrals. Excellent peak shape contributes to better peak resolution. Eclipse Plus C8 exhibits different selectivity compared to Eclipse XDB C8 and Eclipse Plus C18. The shorter aliphatic C8 chain is less retentive than a C18 chain and can significantly cut analysis time compared to C18. Additionally, there is a wide variety of column configurations available for all analytical, rapid-resolution (RR), and high-throughput analyses. Several of these examples are scaled down from analytical methods to RR methods by substituting the longer column with a RR column. This combination of excellent peak shape and scalability to high-throughput methods make Eclipse Plus C8 a valuable tool to increase productivity wherever HPLC is used.

Introduction

New technology in HPLC columns is continuously being sought because no single column works for all analyses. Scientists are seeking better and faster ways to help them innovate, improve productivity, and obtain good results faster. For example, in the pharmaceutical industry, drug discovery and development require higher performance columns to increase the number of analyses that can be done accurately in a fixed time period. Evaluating potential drugs more quickly is advantageous. Additionally, government and industry regulations are increasingly applying more burden on robust instrumentation and technology. Everything from air and water to food is scrutinized for quality or safety. High-quality HPLC columns are necessary as are robust instrumentation and methodologies, all leading to reliable results.

The ZORBAX Eclipse Plus C8 columns are available in several particle sizes and column dimensions, and are an excellent tool for improving productivity. They offer excellent peak shape and efficiency for acids, neutrals, and bases. They are an ideal first choice for method development.

Selectivity of Eclipse Plus C8

Column selection is based on several factors. Selectivity is usually one of the most important factors in choosing a column. Selectivity (α) is the relative retention of two peaks:

$$\alpha = k_2 / k_1$$

Where k_2 and k_1 are the retention or capacity factors of the second eluting peak and first eluting peak, respectively. If two peaks co-elute, α is 1; for closely spaced peaks, selectivity is a value of near 1. Selectivity, or peak spacing, is altered by

changing the temperature, mobile phase, or stationary phase (column). Because selectivity is an interactive phenomenon between analyte and phases, it is often poorly understood, difficult to predict, and usually involves experimentation to optimize. Having a variety of columns available is a powerful and easy way to change α, especially if it is undesirable to change the mobile phase due to lability, solubility, pH, or other constraints.
Figure 1 demonstrates the differences in selectivity among Eclipse Plus C8, Eclipse Plus C18, and Eclipse XDB C8 columns under identical chromatographic conditions. Resolution doubles, from a resolution factor of 1.23 to 2.42, comparing Eclipse Plus C8 to Eclipse XDB-C8, even though α changes only 6% for a separation of three very different analytes.

These columns have similarities, such as the same ZORBAX base silica and Agilent controlled manufacturing technology, so one would expect similar elution patterns as shown. There are however, appreciative differences, such as proprietary silica treatment and bonding, between Eclipse Plus and Eclipse XDB, and the C18 and C8 functionality. These differences are responsible for differences in selectivity and ultimately the improvement in resolution. For closely spaced peaks, where α is close

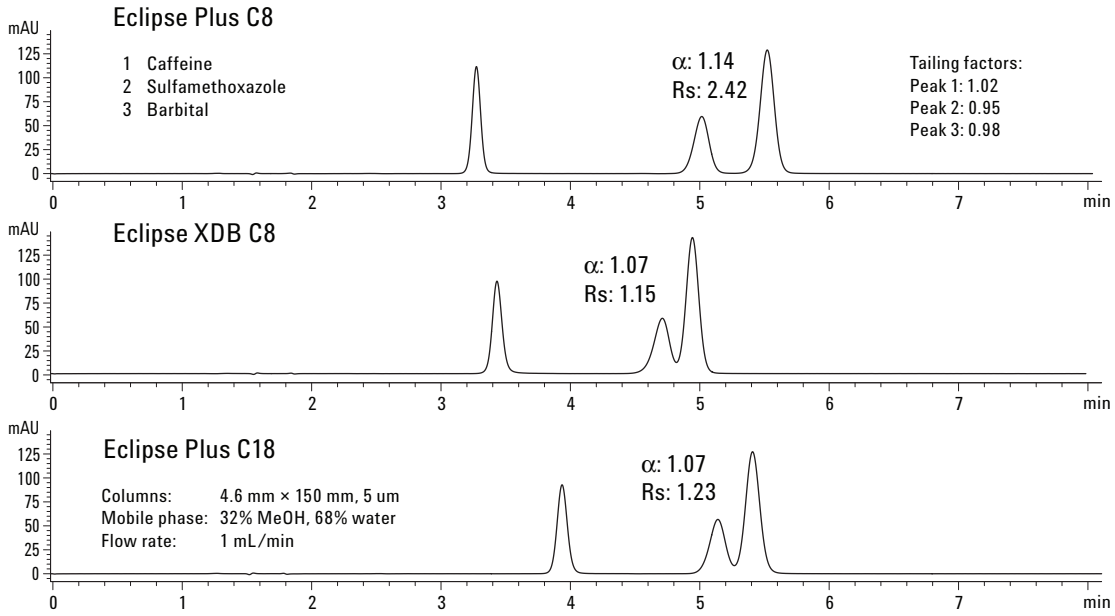

Figure 1. Differences in selectivity between Eclipse Plus C8 and other stationary phases.

to 1, small changes in α have a large effect on resolution, such as in here, where *a* went from 1.07 to 1.14, while resolution went from 1.15 to 2.42.

C8 columns are often less retentive than the longer alkyl chain C18 columns and can be used to reduce analysis time and still provide excellent selectivity. Figure 2 shows that an Eclipse Plus C8 can cut analysis time in half compared to the Eclipse Plus C18 column. The shorter aliphatic chain of the Eclipse C8 has less hydrophobic interaction with the analytes; therefore, they elute from the column more quickly. Many methods designed with C18 columns and with well-resolved peaks might easily be completed in less time by switching to Eclipse Plus C8.

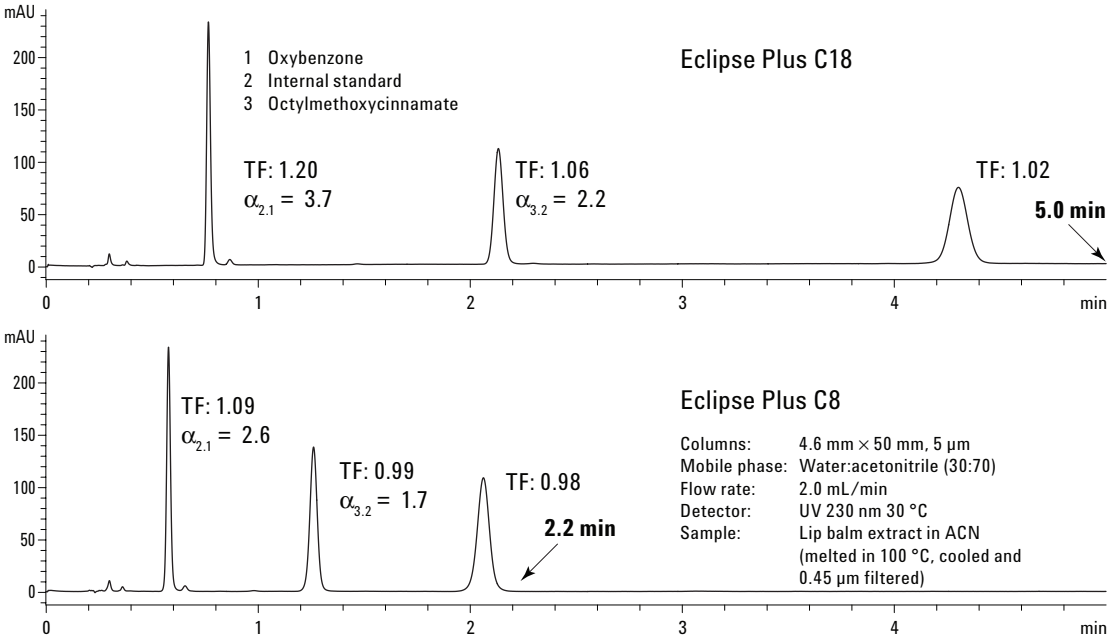

Figure 2. Different selectivity of C18 and C8 cuts time of lip balm analysis in half.

Rapid Resolution (RR)

The benefit of smaller particles is their higher efficiency when compared to larger particles. This can be exploited to create significant productivity gains. Smaller particles packed in shorter columns result in significantly faster analyses while maintaining sufficient resolution compared to the longer columns. Figures 3, 4, and 5 are examples of substituting analytical-sized (4.6 x 150 mm, 5 µm) Eclipse Plus C8 columns with shorter rapid resolution (RR) (4.6 x 50 mm, 3.5 µm) columns. The straightforward or "plug & play" substitution directly provides significant reduction in analysis time, proportional to column length, while still maintaining sufficient resolution. Sensitivity is also significantly improved by choosing the smaller particle size. Table 1 lists the peak heights, areas, and percent differences of the last eluting peak

Table 1. Sensitivity Gains with "Plug & Play" RR Columns

	Height (mAU) of Last Eluting Peak		
	Analytical Column	RR Column	% Gain of RR
Barbitals	18.9	41.1	54
Mouthwash	47.4	90.8	48
Parabens	43.5	97	55
	Area of Last Eluting Peak		
	Analytical Column	**RR Column**	**% Change in Area**
Barbitals	155.4	155.6	0
Mouthwash	1083.8	1089.8	1
Parabens	322.7	327	1

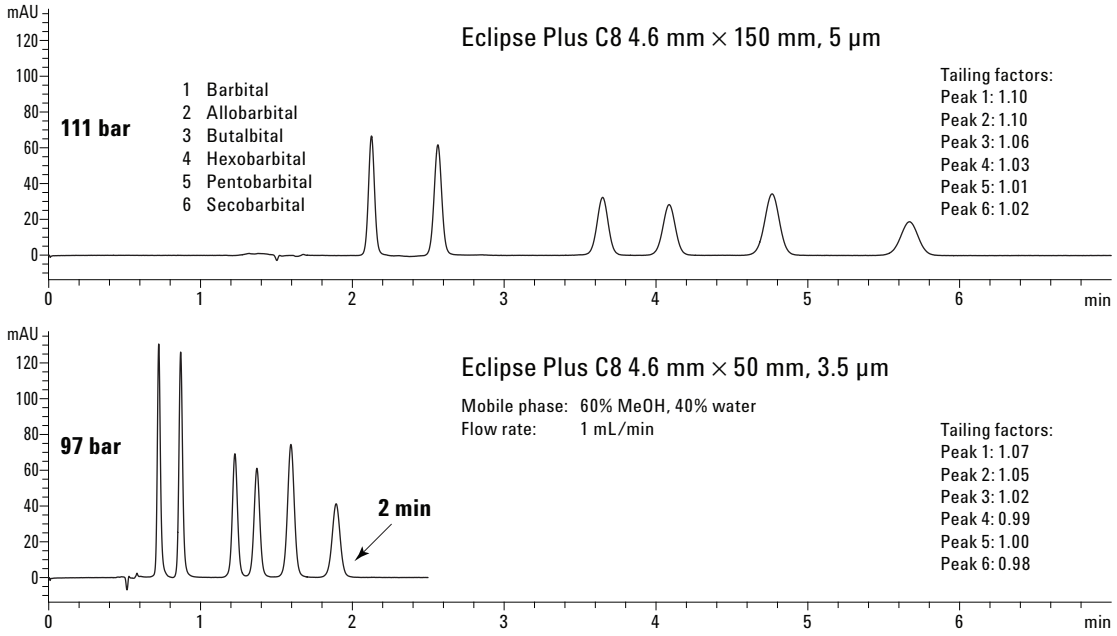

Figure 3. Plug & play RR of barbiturates.

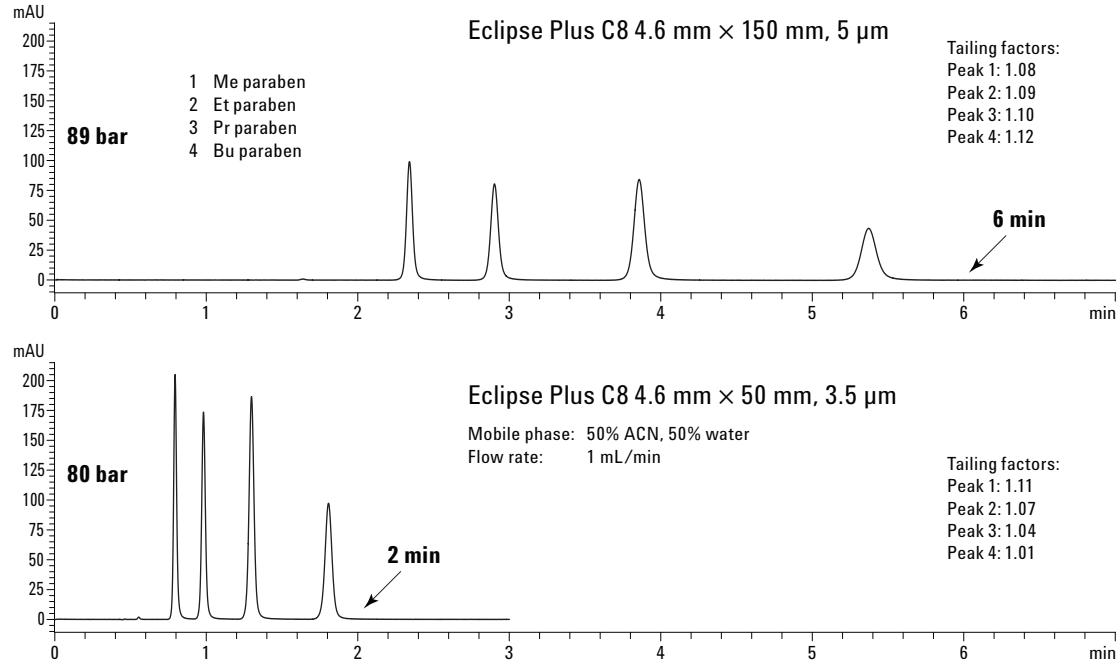

Figure 4. Plug & play RR of parabens.

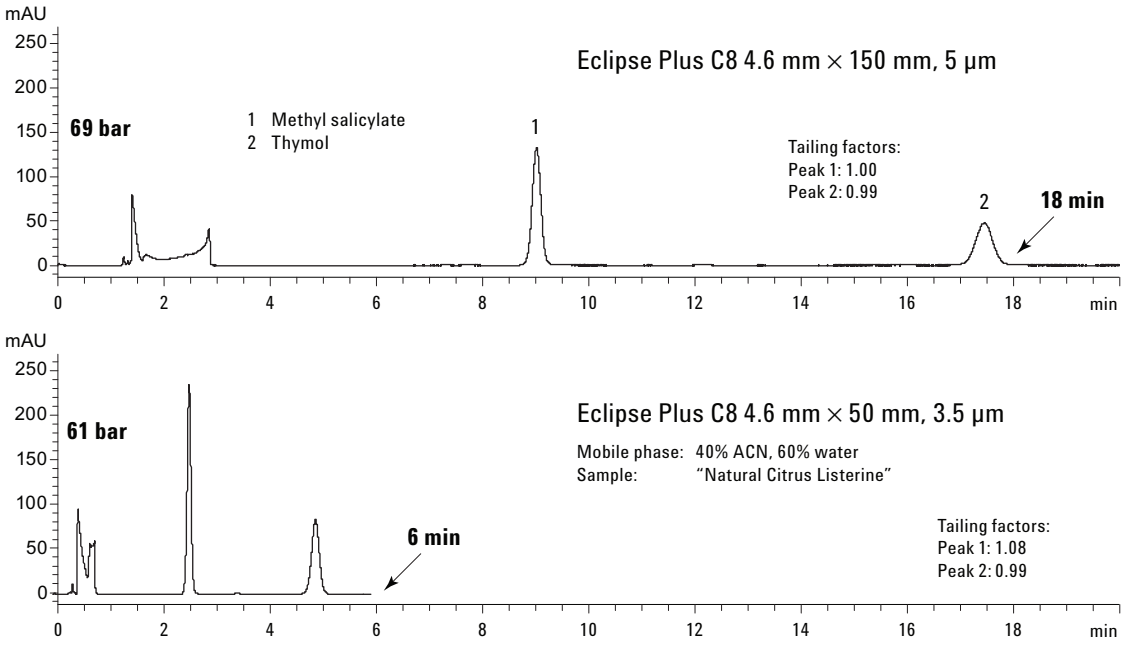

Figure 5. Plug & play RR of active ingredients in mouthwash.

between the larger column and the RR column in the three examples. Areas are the same for both columns because the injection amounts are equal; however, the peak height (sensitivity measurement [signal to noise ratio]) is about 50% taller for the RR column. System back pressures for the examples are also given in the three figures. Pressures are about equal for all three examples. The higher pressure caused by smaller particles (3.5 vs. 5 µ) found in the RR columns is offset by the shorter column length (50 vs. 150 mm). The equal pressures of analytical-sized (150 mm) columns compared to 50-mm columns support the plug & play capability of Agilent RR columns. All pressures are well within the operating range of both the Agilent 1100 and 1200 Series LCs. This provides the option of higher flow rates and even faster analyses, if desired.

Eclipse Plus C8 is available in 1.8, 3.5 and 5 µm particle sizes for all analytical, high-resolution, and fast LC analyses. Table 2 lists configurations available.

Excellent Peak Shape of Bases

The most compelling feature of Eclipse Plus columns (C8 and C18) is the excellent peak shape with all sample types: acids, bases, and neutrals. In the above examples, peak shape measured by USP tailing factor is listed within the figures. In every instance, it is close to ideal (1.0). The high level of performance — peak shape, efficiency, and resolution — is due to improved, patented silica manufacturing and bonding with start-to-finish product control.

Bases (amine-containing molecules in particular) are quite often a challenge for high-performance reversed phase chromatography. At low pH (pH < 3), bases are protonated (BH+), therefore highly water soluble and not well retained. At pH 7, some amines may still be protonated, while others have lost their proton (B). Unreacted silanols on the silica are ionized (SiO-) and interact with the protonated ion species. This type of ion-exchange secondary interaction causes peak tailing. At low pH the silanols are un-ionized (SiOH), and interaction with the basic analyte does not occur.

Figure 6 shows an example of two bases analyzed under the same conditions at pH 7. These two compounds, amitriptyline and dextromethorphan, are known to tail excessively at intermediate pH on other columns due to their partial ionization, interconverting between B and BH+. Peak shape is excellent, however, when Eclipse Plus C8 is used. Eclipse Plus has unique ZORBAX silica treatment, endcapping, and bonding that reduce interactions with ionized silanols and improve peak shape dramatically.

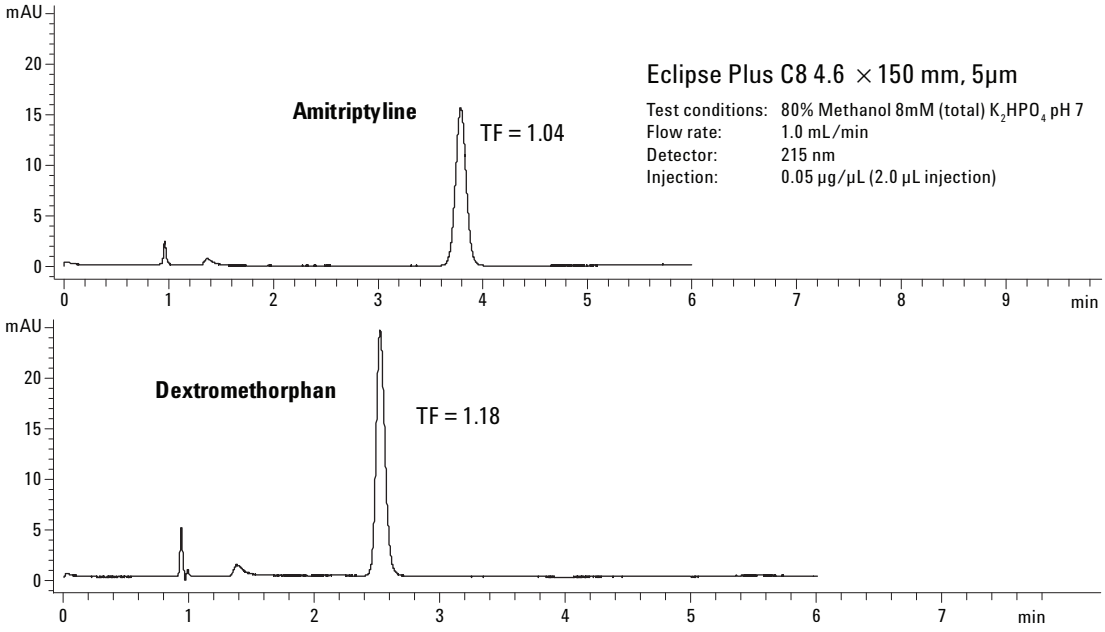

Figure 6. Excellent peak shape of basic drugs on Eclipse Plus C8.

Conclusions

Eclipse Plus C8 columns can be used to improve many HPLC applications. The symmetrical peak shape of acids, bases, and neutrals provided by Eclipse Plus C8 makes it an ideal first choice in method development. Peak symmetry influences resolution and efficiency, improving separations of closely spaced peaks. Eclipse Plus C8 also has slightly different selectivity compared to other C8 and C18 phases, making it a good choice for method development. Small differences in selectivity of closely spaced peaks can cause large differences in resolution. The inherent exceptional peak shape provided by Eclipse Plus C8 coupled with the popular column configurations in three particle sizes enable existing longer analyses to be easily scaled to high-throughput methods.

Table 2. Eclipse Plus C8 Column Configurations

		Eclipse Plus Ordering Details	
Column Description	**Size (mm)**	**Particle Size (µm)**	**Eclipse Plus C8 USP L7**
Analytical	4.6 x 250	5	959990-906
Analytical	4.6 x 150	5	959993-906
Analytical	4.6 x 100	5	959996-906
Analytical	4.6 x 50	5	959946-906
Rapid Resolution	4.6 x 150	3.5	959963-906
Rapid Resolution	4.6 x 100	3.5	959961-906
Rapid Resolution	4.6 x 75	3.5	959933-906
Rapid Resolution	4.6 x 50	3.5	959943-906
Rapid Resolution	4.6 x 30	3.5	959936-906
Rapid Resolution HT	4.6 x 100	1.8	959964-906
Rapid Resolution HT	4.6 x 50	1.8	959941-906
Rapid Resolution HT	4.6 x 30	1.8	959931-906
Solvent Saver	3.0 x 150	5	959993-306
Solvent Saver Plus	3.0 x 150	3.5	959963-306
Solvent Saver Plus	3.0 x 100	3.5	959961-306
Solvent Saver HT	3.0 x 100	1.8	959964-306
Solvent Saver HT	3.0 x 50	1.8	959941-306
Narrow Bore	2.1 x 150	5	959701-906
Narrow Bore	2.1 x 50	5	959746-906
Narrow Bore RR	2.1 x 150	3.5	959763-906
Narrow Bore RR	2.1 x 100	3.5	959793-906
Narrow Bore RR	2.1 x 50	3.5	959743-906
Narrow Bore RR	2.1 x 30	3.5	959733-906
Narrow Bore RRHT	2.1 x 100	1.8	959764-906
Narrow Bore RRHT	2.1 x 50	1.8	959741-906
Narrow Bore RRHT	2.1 x 30	1.8	959731-906
Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-937
Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-937
Guard Hardware Kit			820888-901

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