LC Columns for Reducing Solvent Use and Waste

Technical Overview

Abstract

Acetonitrile is the most versatile and commonly used solvent in HPLC, but it is currently in critically short supply. Laboratories can realize a 40 to 90 percent reduction in acetonitrile usage by changing the HPLC column while keeping the same bonded phase. Possibilities include reduction of the diameter of the column, a change to a shorter column with a smaller particle size, or a simultaneous reduction of length, diameter, and particle size. These options are easy to implement, do not require redevelopment of the method, and in some cases significantly reduce the analysis time. This Technical Overview discusses these opportunities and considers how to balance reduction of solvent use with regulatory requirements and the desire to use conventional LC instrumentation.

Agilent Technologies

Introduction

Practitioners of HPLC face a worldwide shortage of acetonitrile, the most popular LC solvent. Acetonitrile is a byproduct of the production of acrylonitrile, which is used to produce plastics and acrylic fibers. The global economic slowdown has reduced the demand for acrylonitrile, and acetonitrile production has fallen sharply. As a result, labs are faced with diminishing allocations of this critical solvent, along with rapidly escalating costs. The shortage is likely to be long-lived, and many labs are taking measures to reduce usage.

A number of approaches exist to reduce acetonitrile use for HPLC. They include changing column dimensions and/or particle size, switching from acetonitrile to methanol for the complete analysis or for column rinsing and storage, recycling acetonitrile for isocratic analyses, and reducing column equilibration time. While many organizations are attempting to switch their analyses from acetonitrile to methanol, this change of mobile phase often affects the resolution and sometimes changes the elution order, requiring additional work on the method. Changes of column dimensions and/or particle size are typically easier to implement because they do not change the chemistry or require redevelopment of the method.

This Technical Overview discusses how to select new columns to achieve solvent savings of 40 to 90 percent, and describes the column choices that avoid the need to revalidate methods or modify the LC hardware. The following options are explored:

- Reducing the column diameter from 4.6 mm to 3.0 or 2.1 mm
- Shortening the column while reducing particle size from 5 µm to 3.5 or 1.8 µm
- Reducing column length, column diameter, and particle size

Method adjustments that avoid the need to revalidate

Labs can reduce their solvent usage by changing their column diameter, length, and/or particle size, and these changes can sometimes be made without requiring a complete method revalidation. Regulated labs that do not want to revalidate methods must limit changes to those that are classified as method adjustments. It is important to review the latest method adjustment criteria that are published by the United States Food and Drug Administration (FDA)[1] and the United States Pharmacopoeia (USP). The International Conference on Harmonization (ICH) guidelines and other pharmacopeial guidelines are often very similar, but LC practitioners should always check the most current documents and their company standard operating procedures (SOPs).

Table 1 shows the current USP and FDA method adjustment criteria for column dimensions. The USP and FDA allow reduction of column length by 70 percent, so one can go from a 250 mm column down to a 75 mm column, or from a 150 mm column to a 50 mm column. The guidelines allow substantial flexibility as long as the required resolution is maintained.

Table 1. USP and FDA method adjustment criteria for column dimensions and related LC parameters.

Parameter	Maximum Specifications	Comments/ Examples
Column length	± 70%	250 mm → 75 mm 150 mm → 50 mm
Column internal diameter	± 25%	
Flow rate	± 50%	
Injection volume	Reduce as much as needed – must still meet detection limits and precision	If you change to a smaller/ shorter column, make the appropriate change in injection volume.
Particle size	Reduce by up to 50%	Change column length *and* particle size to keep resolution the same. 5 µm → 3.5 µm (– 30%) 3.5 µm → 1.8 µm (– 49%)

Reduction of column diameter is not quite as straightforward. The current FDA and USP recommendations for a method adjustment are ± 25 percent change in column diameter, which does not allow much flexibility. The USP has proposed a revision that would allow adjusting the column internal diameter (ID) as much as required, provided linear velocity is constant.[2] This proposal is described in the USP 32nd supplement revisions, PF34 number 5, and is expected to be final near the end of 2009. The new guidelines will apply to methods that are final after that time.

To qualify as a method adjustment, one can reduce particle size by as much as 50 percent, which means a switch from 5 µm to 3.5 µm particles, or from 3.5 to 1.8 µm particles. A change from 5 µm to 1.8 µm particles is too large and would require method revalidation. Methods that are developed on columns with 3.5 µm particles can go up or down in particle size and stay within the recommended guidelines.

A change in column dimensions often necessitates a change in mobile phase flow rate or in the volume of sample that is injected. Per the criteria for a method adjustment, the flow rate can vary by 50 percent. One can reduce injection volume as much as is needed to maintain sensitivity and detection limits.

Method adjustments require method verification, but not a complete revalidation. They also require documentation to regulatory agencies such as FDA and USP. The details depend upon each company's standard operating procedures, as well as requirements by different agencies.

Reducing column diameter

The smaller the column diameter, the lower the LC flow rate, and the more solvent is saved. To maintain the same analysis time and resolution as the column diameter is reduced, it is important to reduce flow rate to keep the linear velocity the same. The flow rate reduction leads to a corresponding drop in the solvent used.

Saving solvent is only one benefit of columns with a smaller diameter. These columns also provide greater sensitivity because peaks are eluted in a smaller volume of solvent, so the concentration of the analyte is effectively increased. The injection volume can be decreased to maintain the same sensitivity.

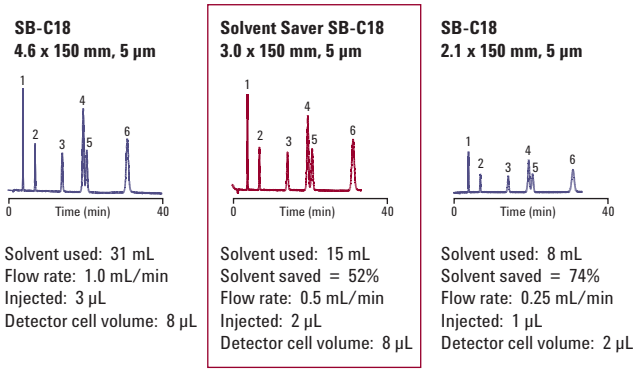
Table 2 shows the dramatic savings in solvent cost that are possible by moving to columns with smaller diameters. Labs that currently use columns with a 4.6 mm ID can save up to 60 percent of their solvent by switching to Agilent ZORBAX Solvent Saver columns with a 3.0 mm ID. Solvent Saver columns are available in all ZORBAX phases, so current methods can be immediately adjusted for substantial savings. The change from 4.6 mm ID to 3.0 mm ID falls within the future USP guidelines for a method adjustment. Labs can save even more – 80 percent – by changing from 4.6 mm columns to narrow bore columns with a 2.1 mm ID. However, the latter change does require revalidation of the method.

Table 2. HPLC columns of smaller diameter substantially reduce solvent usage and waste.

	Standard Analytical	Solvent Saver	Narrow Bore
			
Column internal diameter	4.6 mm	3.0 mm	2.1 mm
Actual solvent used	100 mL	40 mL	20 mL
Decrease in solvent use		60%	80%

Application example

Figure 1 compares a separation of antibacterial drugs on columns with three different diameters. The 4.6 mm Agilent ZORBAX SB-C18 column separates the compounds in 31 minutes, generating 31 mL of solvent waste. The shorter 3.0 mm Solvent Saver column uses only 15 mL of solvent for the same separation – a 50 percent savings. The particle size, bonded phase, and column length are the same, and the critical peaks 4 and 5 show the same resolution. The injection volume was reduced from 3 µL to 2 µL to maintain comparable sensitivity, but the same LC setup was used. This change in column ID from 4.6 to 3.0 mm currently requires method revalidation, but would be considered a method adjustment by future USP guidelines.



Column:	Agilent ZORBAX SB-C18
Mobile phase:	20:80 ACN:citrate/phosphate pH 2.6 (200/87/713 ACN/0.2M Na_2HPO_4/0.1M citric acid)
Temperature:	Ambient
Sample:	Antibacterials

1. Sulfamerazine
2. Furazolidone
3. Oxolinic acid
4. Sulfadimethoxine
5. Sulfaquinoxoline
6. Nalidixic acid

Figure 1. Separation of antibacterials on columns with smaller diameters shows how to reduce solvent requirements by 50 to 75 percent.

As shown in Figure 1, the narrow bore 2.1 mm column generates only 8 mL of solvent waste, a savings of 75 percent relative to the 4.6 mm ID column. While this column saves additional solvent, it requires more attention to the LC setup, and changing from 4.6 to 2.1 mm necessitates revalidation of the method. To use a narrow bore column effectively, the injection volume and the volume of the detector cell are reduced. These steps minimize extra-column volume and maintain the efficiency of the system.

Calculating new flow rates and injection volumes for smaller diameters

LC practitioners can use simple equations to calculate flow rates and injection volumes when they change column ID. The following equation can be used to calculate the flow rate that maintains a constant linear velocity and the same separation when the column ID is changed:

$$F_2 = F_1 \bullet (d_2)^2/(d_1)^2$$

where F_2 is the new flow rate, F_1 is the original flow rate, d_2 is the new column diameter, and d_1 is the original column diameter.

The next equation can be used to scale the injection volume for the new column ID:

$$V_2 = V_1 \bullet [(r_2^2 \bullet L_2)/(r_1^2 \bullet L_1)]$$

where V_2 is the new injection volume, V_1 is the original injection volume, r_2 is the new column radius, L_2 is the new column length, r_1 is the original column radius, and L_1 is the original column length.

When LC modifications are necessary

As the ID of the column is reduced, peak volumes are reduced, which sometimes necessitates LC changes to maintain resolution. The peak width remains the same, but the peak volume decreases with the lower flow rate. Peak volumes less than 60 µL require optimized instrumentation for maximum efficiency. This is generally not an issue for Agilent ZORBAX Solvent Saver columns with 3.0 mm ID, but it can be a concern with 2.1 mm columns.

Peak volumes also depend on peak retention. The earlier a peak elutes, the smaller the peak volume. Table 3 compares the peak volumes at three different retention factors (k values) for three different column IDs. The table assumes a constant number of theoretical plates on each column. The retention factor (k) is calculated as

$$k = (t_r - t_o)/t_o$$

where t_r is the retention time of the peak of interest and t_o is the retention time of an unretained peak.

Table 3. Based on peak volume, Agilent ZORBAX Solvent Saver columns with 3.0 mm ID can be used on most LCs without modification.

Column dimensions	Void volume (µL)	Peak Volume (µL)		
		k = 1	k = 3	k = 5
Analytical 4.6 x 150 mm 1.0 mL/min	1.50	114	229	343
Solvent Saver 3.0 x 150 mm 0.4 mL/min	0.64	46	92	137
Narrow Bore 2.1 x 150 mm 0.2 mL/min	0.28	23	46	69

N (number of theoretical plates) = 11,000 (constant)
Shaded cells indicate need for LC configuration that minimizes extra-column volume

For best results, peak volumes of less than 60 µL require an LC configuration with minimized volume. Peaks that elute at the beginning of the run on 2.1 mm ID columns have small volumes and are very susceptible to broadening. With a 2.1 mm column, peaks with k values that are less than 5 will spread unless extra-column volume is minimized. With a 3.0 mm column, once k is greater than 1, peak broadening is no longer a problem.

LC modifications to reduce extra-column volume

Extra-column volume is in any tubing or connector in the system, other than the column, where the sample peak could broaden and efficiency could be reduced. It includes the volumes of the sample loop, connecting tubing, fittings, and detector cell. All of these must be minimized when peaks elute early in the run on a 2.1 mm column. The following are recommended:

- Detector cell volume of 2 µL or less
- Reduced injection size (usually less than 5 µL)
- Capillary tubing with 0.12 mm ID
- Micro-injection system, with either an internal loop or a small 2 to 5 µL external loop
- Unions with zero dead volume

Reducing column diameter – deciding between 3.0 and 2.1 mm columns

Relative to 4.6 mm columns, both 3.0 and 2.1 columns bring significant solvent savings. The narrow bore (2.1 mm ID) columns save up to 80 percent of solvent, provided the LC has been modified for optimum performance. The modifications may be worth the effort if the method is used frequently or if the lab is accustomed to setting up LCs for these columns (for example, for LC/MS).

Agilent ZORBAX Solvent Saver columns (3.0 mm ID) are a better choice for some people. It is possible to achieve solvent savings of up to 60 percent using these columns with standard LC equipment. However, it is still important to make connections with short lengths of 0.12-mm ID tubing, and any connectors should be zero-dead-volume.

Reducing column length while reducing particle size to 3.5 µm

While the previous section considered reduction of column diameter to save solvent, this section and the next one cover reduction of column length. Column length determines analysis time, but the combination of column length and particle size determines efficiency, so the two parameters are considered together. Simultaneous decrease of column length and particle size leads to substantial reductions in analysis time and solvent waste while maintaining resolution. Agilent ZORBAX Rapid Resolution columns with a 3.5 µm particle size work well for this type of application, and are discussed in this section. Agilent ZORBAX Rapid Resolution High Throughput (HT) columns with a 1.8 µm particle size are another good choice, and they are discussed in the next section.

Simultaneous decrease of column length and particle size leads to substantial reductions in analysis time and solvent waste while maintaining resolution.

Table 4 illustrates the savings in solvent use and analysis time that are possible when reducing particle size and column length at the same time, while keeping the column ID constant. Recall that changing from a 250 mm to a 75 mm length column maximizes the allowed change in column length, per the FDA and USP criteria for a method adjustment. Changing the particle size from 5 µm to 3.5 µm is only a 30 percent reduction – well within the FDA and USP guidelines of 50 percent for a method adjustment. As shown in Table 4, switching from columns with 5 µm particles to shorter Agilent ZORBAX Rapid Resolution columns with 3.5 µm particles reduces analysis time and solvent usage by 40 to 50 percent, without compromise of efficiency.

Table 4. Relative to columns with 5 µm particles, shorter columns with 3.5 µm particles substantially reduce analysis time and solvent waste.

	5 µm		3.5 µm	5 µm		3.5 µm
Dimension	4.6 x 250 mm		4.6 x 150 mm	4.6 x 150 mm		4.6 x 75 mm
Analysis time	30 min	40% reduction	18 min	18 min	50% reduction	9 min
Solvent waste	30 mL		18 mL	18 mL		9 mL
N	20,000		20,000	12,000		12,000

N = number of theoretical plates

Application example

Figure 2 shows the results of changing from a column that is 150 mm length with 5 µm particles to one that is 50 mm length with 3.5 µm particles. The analysis time is reduced from six minutes to two minutes without loss of resolution, and 67 percent of the solvent is saved. The column ID remains the same. This change would require revalidation of the method because the change in column length is more than 70 percent, but the per-analysis savings are so large that revalidation may be worthwhile.

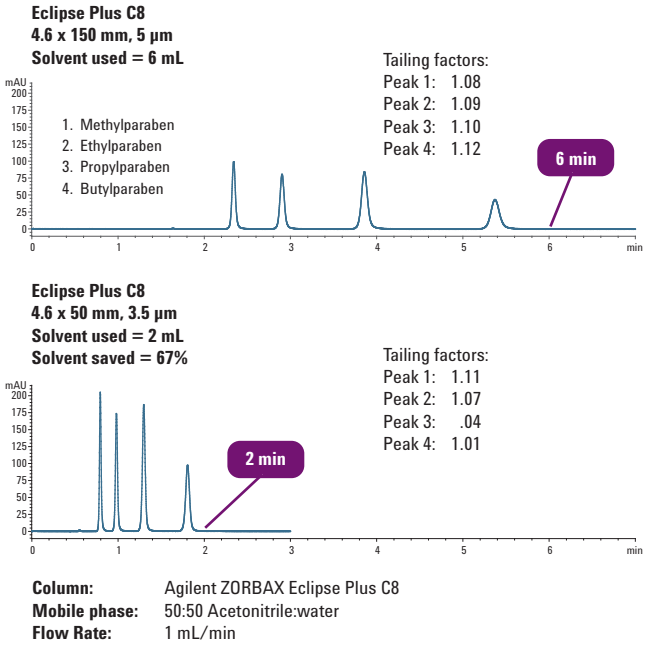


Figure 2. Changing to a shorter Agilent ZORBAX Rapid Resolution column with a 3.5 µm particle size reduces analysis time and solvent use by 2/3.

LC modifications are seldom required

A previous section discussed the effects of extra-column volume when changing to columns of smaller diameter (4.6 mm to 3.0 mm to 2.1 mm). Table 5 shows the effect of extra-column volume when reducing column length. Moving from 150 mm columns to 100 mm columns is of no concern. Going down to 75 mm columns is only a concern when k is less than 1. So extra-column volume is less of a problem with shorter columns and smaller particle sizes, especially with the 3.5 µm particle size. Therefore, it is seldom necessary to optimize the LC when making this change.

Table 5. Based on peak volume, Agilent ZORBAX Rapid Resolution columns with 3.5 µm particles can be used on most LCs without modification.

Column dimensions	Void volume	Peak Volume (µL)		
		k = 1	k = 3	k = 5
4.6 x 150 mm N = 20,000	1.5 mL	85	170	255
4.6 x 100 mm N = 15,000	1.0 mL	72	145	217
4.6 x 75 mm N = 10,000	0.75 mL	60	120	180

Flow rate: 1.0 mL/min
Shaded cell indicates need for LC configuration that minimizes extra-column volume.

Benefits of using columns with 3.5 µm particles

Agilent ZORBAX Rapid Resolution columns reduce analysis time and solvent waste by up to 50 percent over 5 µm columns, while maintaining resolution. The backpressure is typically less than 200 bar for both the 4.6 x 150 mm and 4.6 x 75 mm Rapid Resolution columns, making them acceptable for routine use. Both configurations can be used with standard HPLC equipment because the peak volumes and internal column volumes are large enough that band broadening and loss in efficiency due to extra-column volume are of minimal concern. As a result, no instrument modifications are required to use these columns for maximum efficiency. A lab can switch from 5 µm particles to 3.5 µm particles without revalidating methods, provided that the column length is decreased no more than 70 percent.

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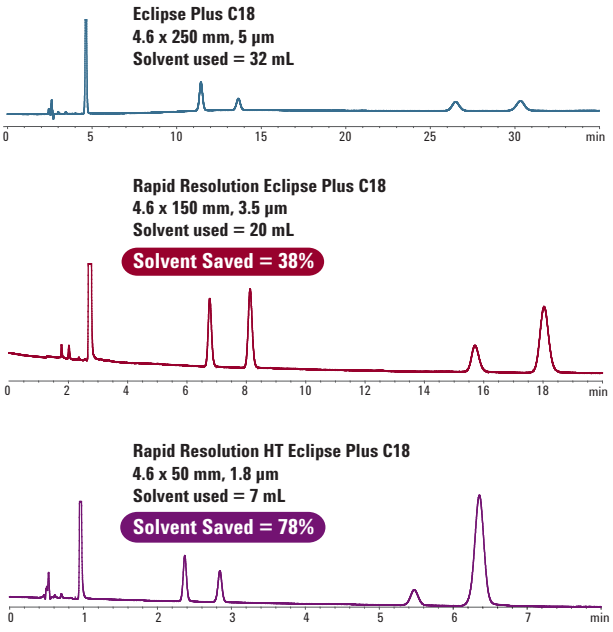
Reducing column length while reducing particle size to 1.8 µm

When reducing particle size, the next step is a change to columns with 1.8 µm particles, such as Agilent ZORBAX Rapid Resolution High Throughput (RRHT) columns. This step down in particle size means even shorter columns with the same resolution. Shorter columns translate to shorter run times, and even greater sample throughput and solvent conservation.

Application examples

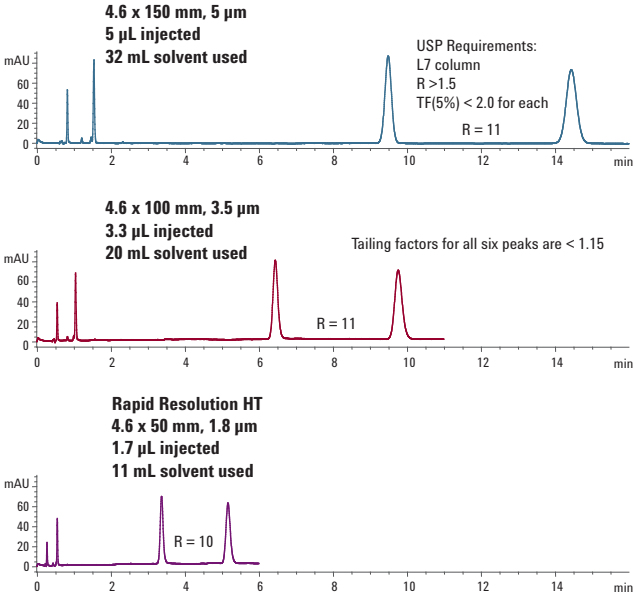
Figure 3 shows an analysis where the column length and particle size are both reduced while the column diameter remains constant. Changing from a 4.6 x 250 mm column with a 5 µm particle size to 4.6 x 150 mm column with a 3.5 µm particle size allows a savings of 38 percent of the solvent and does not require method revalidation. Taking the next step down to a 4.6 x 50 mm column with 1.8 µm particles yields a solvent savings of 78 percent. A switch from a 5 µm particle size to a 1.8 µm particle size requires revalidation of the method, but may be cost-effective if the method is used frequently.



Column: Agilent ZORBAX Eclipse Plus C18
Mobile phase: 60:40 pH 4.5 sodium acetate:methanol
Flow Rate: 1.0 mL/min
Flow cell: Micro/high pressure 6mm, 1.7 µL
Detection: UV 254 nm

Figure 3. Changing to a shorter Agilent ZORBAX Rapid Resolution HT column with 1.8 µm particle size reduces analysis time and solvent use by almost 80 percent.

Figure 4 shows a similar example of a USP assay for ibuprofen oral suspension. The USP requirements are for an L7 column with resolution greater than 1.5 and a tailing factor less than 2. Changing from a 4.6 x 150 mm, 5 µm column to a 4.6 x 100 mm, 3.5 µm column saves considerable time and solvent and meets the method requirements. No revalidation is needed. Switching to a 4.6 x 50 mm, sub-2 µm column preserves resolution and saves even more solvent, but necessitates revalidation.



Mobile phase: 63:37 water: acetonitrile + 1.8 mL H_3PO_4
Flow Rate: 1.0 mL/min
Temperature: Ambient
Sample: Children's ibuprofen oral suspension with benzophenone as internal standard, prepared as described in USP

Figure 4. Converting the USP assay for ibuprofen oral suspension from a conventional column to an Agilent ZORBAX Rapid Resolution HT column maintains resolution and uses about 2/3 less solvent.

Table 6 shows another example, where a lab started with a 5 µm column, tried a 3.5 µm column, then went to a 1.8 µm column. The solvent usage decreased from 37.5 mL to 3 mL, a savings of 92 percent. This lab worked in a process area, so there were few restrictions on method changes. However, scientists did need to revise the method and write appropriate documentation for their company. With the savings from reduced solvent use and analysis time, the cost to change the method was quickly recovered.

Table 6. Comparison of results on Agilent ZORBAX columns with different particle sizes shows a 92 percent reduction in solvent usage for a process LC method.

	5 µm	3.5 µm	1.8 µm
Resolution	4.1	1.4	1.6
Selectivity	1.08	1.06	1.05
Theoretical plates	56108	14314	23190
k′	6.362	4.485	4.12
Run time (including re-equilibration)	25 min	6.5 min	2 min
Solvent usage	37.5 mL	14.25 mL	**3 mL**

Solvent savings = 92%

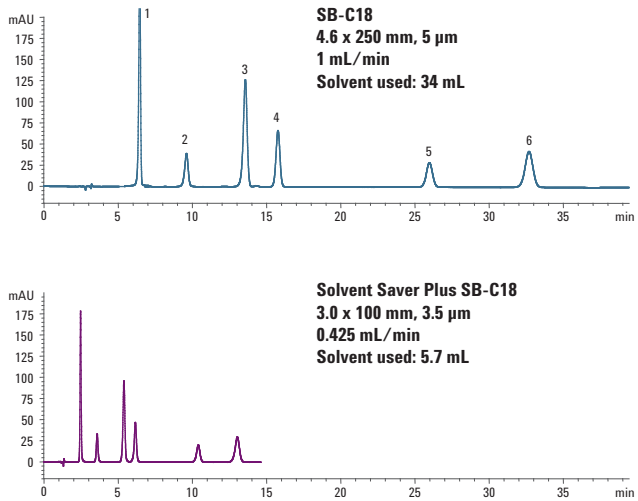
Benefits and tradeoffs when changing to columns with 1.8 µm particles

Relative to columns with 5 µm particles, Agilent ZORBAX RRHT columns with 1.8 µm particles reduce analysis time and solvent waste by 65 to 90 percent, without compromise of resolution. The 50 mm columns can be used with standard LC systems, while longer ones require an LC such as the Agilent 1200 Series Rapid Resolution LC system, which has been designed for high backpressure. While changing from 5 µm to 1.8 µm particles does require revalidation of methods, that cost can be offset over time by lower analysis costs and greater lab productivity.

Agilent ZORBAX RRHT columns with 1.8 µm particles reduce analysis time and solvent waste by 65 to 90 percent, without compromise of resolution.

Reducing column length, column diameter, and particle size

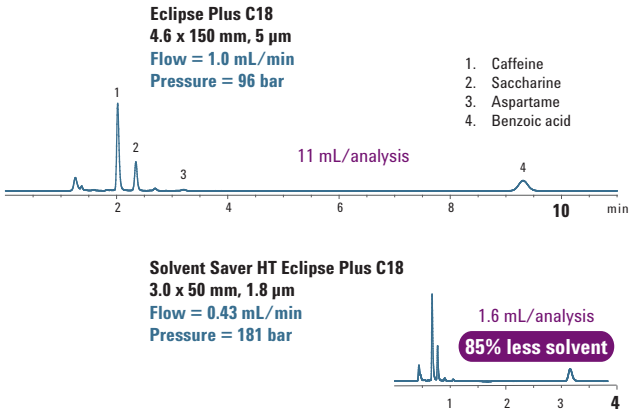
Earlier sections discussed changing either column diameter or the combination of column length and particle size. This section describes simultaneous changes of all three parameters – column length, diameter, and particle size. Figure 5 shows an example where a step down in all three yields a solvent savings of more than 80 percent. While this change does not qualify as a method adjustment (because of the changes in column ID and flow rate), the savings in solvent and analysis time are dramatic.



Column: **Agilent ZORBAX SB-C18**
Mobile phase: 25% methanol in 0.4% formic acid

Figure 5. This column change saves 83 percent of the solvent, decreases analysis time by 57 percent, and uses a conventional LC setup.

The analysis of a diet soda provides another interesting example of the solvent savings afforded by changing the column length, diameter, and particle size. The top chromatogram in Figure 6 shows a simple isocratic separation that takes about 11 minutes and uses about 11 mL of solvent per analysis. The bottom chromatogram shows that an Agilent ZORBAX Solvent Saver HT column uses only 1.6 mL of solvent per analysis – a reduction of 85 percent. The backpressure doubles from 96 to 180 bar, but that is still well below the 400-bar pressure limit on a typical LC. When columns that contain sub-2 µm particles are quite short – 50 mm in length – they can usually operate below the pressure limit on a standard LC.



Column:	**Agilent ZORBAX Eclipse Plus C18**
Mobile phase:	79:21 20 mM phosphate:acetonitrile
Temperature:	25°C
Detection:	UV 220 nm, 16, ref = 420, 20
Flow cell:	3 mm, 2 µL
Data rate:	1 s
Injection volume:	3 µL for 4.6, 1.5 µL for 3.0
Instrument:	Agilent 1200 Series Binary LC System SL
Sample:	0.2 µm filtered diet soda

Figure 6. Analysis of this diet soda sample shows how an Agilent ZORBAX Solvent Saver HT column with 1.8 µm particles maximizes solvent savings.

Conclusion

The shortage of acetonitrile is expected to continue for some time, and the price for this popular LC solvent may remain high even after supplies stabilize. Many labs need to reduce acetonitrile usage immediately, and changing to a different LC column configuration with the same bonded phase is an option that is often easy to implement and saves considerable solvent.

One approach is to reduce the column internal diameter so a lower flow rate can be used. The Agilent ZORBAX Solvent Saver columns with 3.0 mm ID reduce solvent use at least 40 percent from a standard 4.6 mm ID column, and are readily compatible with any LC system. Columns with a 2.1 mm ID save even more solvent, but may require that the LC be modified to reduce extra-column volume.

A second option is to simultaneously reduce the column length and particle size. This approach minimizes solvent use by reducing analysis time. The analysis time can be shortened by 40 to 90 percent, which drops solvent use by 40 to 90 percent while increasing sample throughput. Agilent ZORBAX Rapid Resolution (3.5 µm) and Rapid Resolution HT (1.8 µm) columns provide hundreds of choices for saving acetonitrile this way.

A third possibility is to reduce column length, column diameter, and particle size, all at the same time. Any of these approaches yields substantial solvent savings, and many choices qualify as method adjustments that require documentation to regulatory agencies but do not necessitate revalidation of the method.

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1. FDA Office of Regulatory Affairs
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2. USP 30 Second Supplement Revisions, PF34(5), in process and expected to be final Dec 2009.

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