

Technical Report

Approaches to Lessening the Impact of the Acetonitrile Shortage on Your Reversed-Phase HPLC Separations

Background

The current global shortage of acetonitrile is likely to last into the first half of 2009. Any practicing chromatographer knows the importance of acetonitrile as a mobile phase component for reversed-phase separations. Besides this current shortage, there are other reasons to reduce the use acetonitrile, and, indeed, all organic solvents in chromatographic methods.

We want to help our customers by giving them options on how to reduce the use of solvents in general, and how to replace the acetonitrile with other solvents. In this short communication, we will describe some techniques that are commonly employed to reduce solvent consumption.

Exploring Your Options

There are varying degrees to which an HPLC method may be altered. Regulated methods and those with very closely-eluting peaks are relatively rigid. We can work with you within the constraints of your methodology to help you reduce acetonitrile consumption. Important information to gather at the outset includes:

- If the method is regulated, what aspects are you able to change and to what degree?
- Whether regulated or not, what are the critical resolution requirements that must be maintained?

Rely on our Technical Expertise

We are here to help you explore, develop and transition to alternative methods that reduce acetonitrile consumption. Our expert technical staff is at your disposal to guide you through the techniques we recommend. We also have a state-of-the-art Analytical and Purification Services laboratory that you can contract to do the work, leaving you time to tend to your other projects.

General Approaches to Reduce Solvent Consumption in HPLC Methods

Techniques to reduce acetonitrile consumption either reduce the amount of mobile phase that is used by the method, or reduce the amount of undesirable component of the mobile phase (in this case, acetonitrile). The individual methods can be combined to maximize their impact.

Step 1 – Look at the specifics of the method and related processes and evaluate where you can make reductions

The first step is to take a critical look at your method and what occurs pre- and post-separation. Some relatively simple changes could result in significant solvent savings. Some common factors to pay attention to include:

- Excessive column equilibration time. Most modern columns equilibrate within 10 column volumes.
- Excessive time after the final peak has eluted or between injections.
- Flushing and storage solvents. If pure acetonitrile is used for either one, it could likely be replaced with methanol, or solutions of acetonitrile in water. Even 20% acetonitrile solutions are adequate to prevent microbial growth and maintain column wetting.

Step 2 – Evaluate your options to reduce or replace solvents

Once the relatively non-essential use of solvents has been minimized, you can begin to explore the options at your disposal to replace or reduce the acetonitrile in the mobile phase. These options fall into two categories:

1. Those that **do not** affect the selectivity (α) of the separation
2. Those that **do** affect or are likely to affect the selectivity (α) of the separation

Obviously, the choice depends on how much flexibility you have to make changes and the overall extent to which you wish to reduce consumption. These options are summarized in the following paragraphs.

Category 1 – Options that (generally) do not affect selectivity

- a. Use Ascentis® Express columns
 - The increased efficiency of the Fused-Core™ particle in the Ascentis Express HPLC columns enables the use of shorter columns that save acetonitrile. In fact, **solvent savings as high as 75%** can be realized by converting a conventional C18, 25 cm x 4.6 mm I.D., 5 µm particle method to Ascentis Express C18, 10 cm x 4.6 mm ID., 2.7 µm Fused-Core particle.

Table 1. Effect of Column I.D. on Mobile Phase Consumption and Other Factors

I.D.*	Flow Rates	Injection Volume	Sensitivity	Mobile Phase Consumption	Typical Flow Rates	Typical Column Composition
4.6 mm	1.0	1.0	1	1.0	1 - 2 mL/min.	stainless steel
3 mm	0.42	0.42	2	0.42	0.40 - 1.0 mL/min.	stainless steel
2.1 mm	0.21	0.21	5	0.21	0.2 - 0.4 mL/min.	stainless steel
1 mm	0.047	0.047	21	0.047	50 - 100 μ L/min.	glass-lined stainless steel
0.5 mm	0.012	0.012	85	0.012	10 - 20 μ L/min.	glass-lined stainless steel
0.32 mm	0.0048	0.0048	207	0.0048	5 - 10 μ L/min.	fused silica
0.18 mm	0.001531	0.001531	653	0.001531	1 - 5 μ L/min.	fused silica
75 μ m	0.000266	0.000266	3,762	0.000266	0.2 - 1 μ L/min.	fused silica
50 μ m	0.000118	0.000118	8,464	0.000118	0.1 - 0.2 μ L/min.	fused silica
25 μ m	0.000030	0.000030	33,856	0.000030	< 0.1 μ L/min.	fused silica

* assumes columns are of the same length

b. Reduce column I.D.

- By far, 4.6 mm is the most common HPLC column I.D. in use today. By reducing column I.D., consumption of mobile phase and sample is also reduced in proportion to the square of the inverse ratio of the I.D. (Table 1). For example, a 2.1 mm I.D. column consumes $(4.6/2.1)^2$ -fold or nearly five-fold less mobile phase. By keeping constant linear velocity, retention times are not affected. One disadvantage of narrower I.D. columns is that the column efficiency in terms of plates/m generally decreases with decreasing column I.D. This may be due to more difficulty in packing, or to the higher relative influence of wall effects. One way to overcome this is to use smaller particles. For example, a 15 cm x 2.1 mm I.D. column packed with 3 μ m particles will have about the same plates as a 15 cm x 4.6 mm I.D. column packed with 5 μ m particles.

c. Use a less retentive stationary phase

- Use shorter alkyl chain stationary phases. For example, most RP-HPLC separations are performed on octadecyl (C18) bonded stationary phases. By using a shorter chain, for example octyl (C8), retention time is significantly reduced under the same mobile phase conditions. However, the same retention time can be obtained by decreasing the strength (% organic) of the mobile phase. For most compounds, selectivity will be comparable. There are even shorter alkyl phases, C5 and C4, for example. But the shorter the alkyl chain the more accessible the silica surface and the influence of surface interactions that may alter selectivity.
- Use the same stationary phase, but on lower surface area particles. Surface area of most modern HPLC particles fall between 90 and 300 m²/g. Retention is proportional to the amount of stationary phase in the column that is available to the analyte. The lower the surface area, the lower the total amount of phase per column. (It is important to note that one can have the same amount of bonded phase per unit surface area irrespective of the total surface area.) The most common method to reduce the surface area is to use larger pore diameter particles, 300 Å instead of the typical 100 Å, for example.



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The expert staff of our Analytical and Purification Services Laboratory is at your disposal to help you modify or design methods to reduce acetonitrile consumption, or to tackle any other separation challenge.

d. Use higher % of water and increase temperature

- Simply reducing the amount of organic solvent in the mobile phase will increase the retention time. This increase can be offset by concurrently increasing the analysis temperature. Generally, in RP-HPLC retention time is halved by every 10 °C increase in temperature. Temperature affects different compounds to differing extents, and may affect selectivity or stability. Also, most silica-based HPLC columns should not be operated above 70 °C.

Category 2 – Options that do affect or are likely to affect selectivity

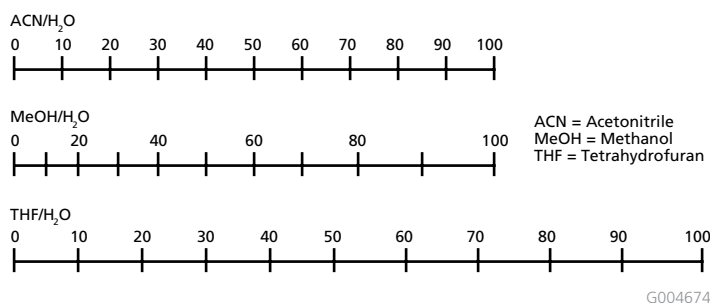
a. Replace the acetonitrile with another suitable solvent

- Methanol is the most common alternative, but others include THF, and ethanol. The general rule of thumb is to increase the organic component by ten percentage units when changing from acetonitrile to methanol in a reversed-phase method. This is based on the relative eluotropic strength of the two solvents. That is, if a method uses 40% acetonitrile, the same elution strength, and retention time, is achieved with 50% methanol. Figure 1 shows the relative reversed-phase eluotropic strengths of acetonitrile, methanol and THF.

- In addition to selectivity, the type of solvent used may also influence efficiency and symmetry. If the replacement solvent gives lower efficiency, as in the discussion of column I.D., it may be offset by using 3 μm rather than 5 μm particles.

Figure 1 provides for the interconversion of reversed-phase mobile phases having the same strength. Vertical lines in this figure intersect mobile phases having the same strength. For example, 40% Acetonitrile has the same strength as 50% Methanol or 30% THF.

Figure 1. Relative Strength for Different Solvents



Supelco technical resources are at your disposal to work with you to find the best option to reduce your consumption of acetonitrile. Please feel free to contact us.

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b. Change the pH

- If mobile phase pH is changed to decrease analyte retention, decreasing the mobile phase strength by decreasing the organic solvent can restore retention. Neutral compounds on neutral stationary phases (like C18, C8, phenyl, etc.) are not affected by mobile phase pH. However, retention of acidic compounds is reduced by increasing the pH, while the converse is true for basic compounds. The pH will also affect selectivity, especially when analytes are of mixed functionality. The particle type and bonded phase chemistry will also influence maximum and minimum pH values.

c. Investigate alternate stationary phase chemistries

- The stationary phase chemistry can affect both retention and selectivity. Retention of nonpolar compounds on classic alkyl-only phases, like C18 and C8, can be reduced significantly by using more polar stationary phases, like the RP-Amide versions of Ascentis or Ascentis Express. The amount of organic solvent in the mobile phase can then be decreased to achieve the similar retention. In addition to providing lower retention, another application for using alternate stationary phase chemistry is when there is too much or wasted resolution in the method. For most separations, an alpha (k_2/k_1) value of the critical pair greater than two is adequate, more than that is a waste of resolution.

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