

Caveat About Directly Replacing Acetonitrile with Methanol in HPLC Mobile Phases

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email?

As you might be aware, there is currently a global shortage of acetonitrile that 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. With the uncertain supply of acetonitrile, and other reasons, many laboratories have or are looking into replacing it with methanol. There is a general rule of thumb 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.

We want to point out to our readers that switching solvents not only affects retention time, but selectivity as well. The accompanying figures demonstrate this point quite dramatically. On both C18 and RP-Amide phases, a mixture of representative compounds with various functional groups shows different selectivity in the two systems. The selectivity differences even result in peak

order reversal, as is the case of the DHQ/Q pair on the RP-Amide. The neutral component naphthalene is relatively stable (isoeuotropic).

A full description of the mechanism is beyond the scope of this short report. Suffice it to say that the selectivity differences are based on the different solvation properties of acetonitrile and methanol, and are especially noticeable with polar compounds. In addition to selectivity, the type of solvent used may also influence efficiency and symmetry. This is again due to solvation effects and the solvent's ability to influence hydrogen bonding between analytes and polar groups on the sorbent.

If you would like help reformulating an established method that employs acetonitrile, we would be glad to help you. Please contact our technical services at techservice@sial.com

*Sigma-Aldrich has been able to maintain a stable supply of acetonitrile for our established customer base. We have a reliable supply chain based on multiple-sourcing from a number of our business partners producing the raw materials.

Figure 1. CH₃CN vs. CH₃OH on C18 HPLC Column (Ascentis C18)

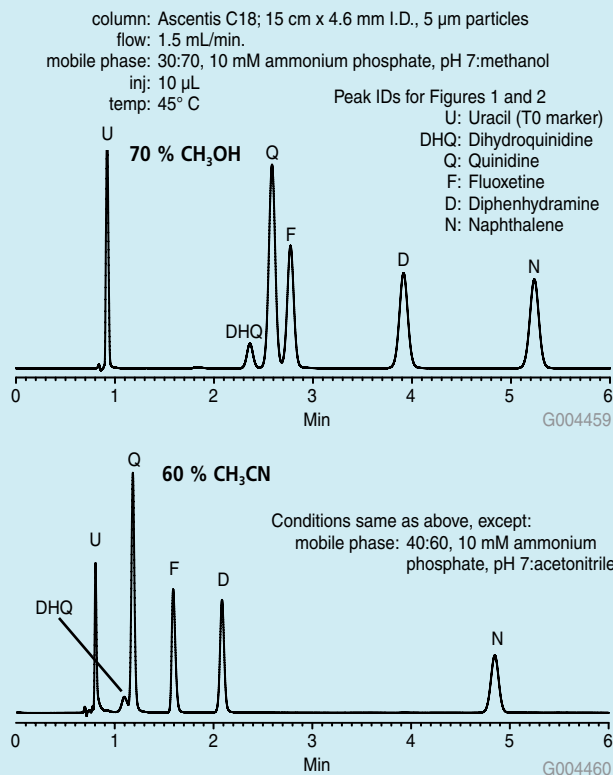


Figure 2. CH₃CN vs. CH₃OH on Amide-Based HPLC Column (Ascentis RP-Amide)

