The Role of Methanol and Acetonitrile as Organic Modifiers in Reversed-phase Liquid Chromatography

Acetonitrile (MeCN) and methanol (MeOH) are the most commonly used organic modifiers in reversed-phase chromatography. Although both solvents offer certain advantages and disadvantages, one of their key strengths, from a chromatographic perspective, is that they offer substantially different selectivity, and as such, are valuable for method development purposes. This article will compare various characteristics of the two different solvents, such as UV cut-off, pressure and elution strength and discuss how organic eluent selection can be used as a method development tool.

Introduction

Reversed-phase mobile phases typically contain water and a less polar organic solvent (the organic modifier), together with additives such as buffers, acids or bases. In reversed-phase, the aqueous component of the mobile phase has weak analyte elution strength, whilst the organic component has a higher elution strength. The relative proportions of aqueous and organic can therefore be adjusted to control analyte retention.

A range of organic solvents are suitable for use as the organic modifier in reversed-phase liquid chromatography, although in practice, only a few have been used routinely. When selecting an organic solvent, properties such as miscibility with water, polarity, UV cut-off, viscosity and safety are important to consider. Each organic modifier has advantages and disadvantages that should be considered before their use. For example, Tetrahydrofuran (THF) and isopropanol (IPA) can be useful as both provide high elution strength. However, IPA use is limited due to its high viscosity (leading to low performance and high back pressures), whilst THF can degrade pump seals, along with PEEK tubing and fittings and requires the use of stabilising agents such as BHT to prevent peroxide formation. Acetone is an inexpensive solvent, with similar elution properties to acetonitrile, although its high UV absorbance limits its applicability for some applications.

Over the years, acetonitrile and methanol have become the two organic modifiers of choice for many reversed-phase applications. Both solvents are fully miscible with water and are compatible with common mobile phase additives and buffers (although care should be taken when using buffer salts at high organic compositions). Both solvents have advantages and disadvantages that should be considered, with final modifier choice often application dependant. For example, the cost of acetonitrile is significantly higher than that of methanol and methanol is also less toxic. However, acetonitrile has a lower UV cut-off than methanol (190 nm vs 205 nm), making it more suitable for use in applications requiring low UV detection wavelengths (note however, that it is important to use HPLC grade or better solvents for LC analyses). In addition, acetonitrile/water mixes have lower viscosity than methanol/water mixes and therefore generate substantially lower back pressures across the LC column (Figure 1). This lower backpressure is often seen as advantageous as it puts less strain on the LC system components and column, and provides scope to increase flow rate and reduce run times.

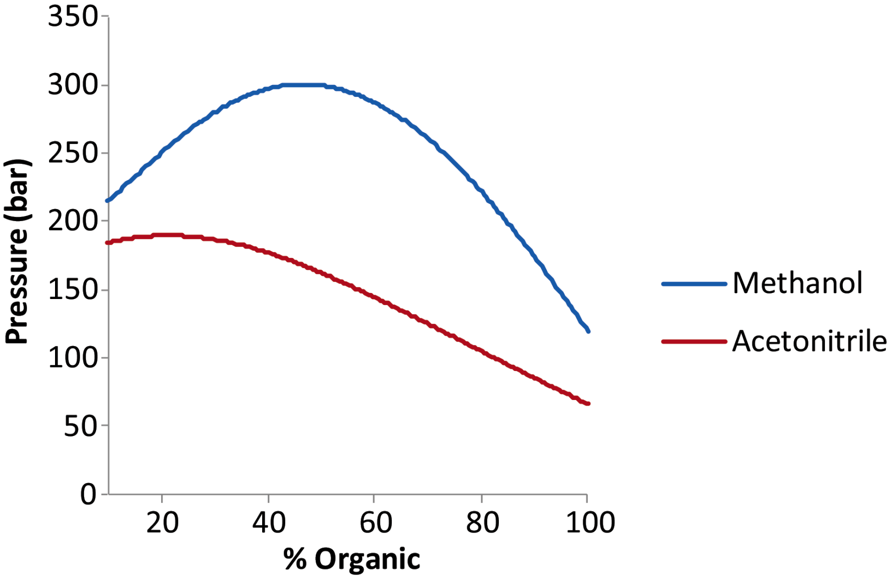

Figure 1: Experimentally determined backpressures for different compositions of methanol and acetonitrile with water on a C18 column, 100 x 3.0 mm (flow rate: 0.43 mL/min, temperature: 30°C).

Eluotropic strength

Acetonitrile has a higher elution strength than methanol for reversed-phase chromatography, therefore shorter analyte retention can be expected for equal proportions of organic to water (Figure 2). In this example, for the separation of these neutral analytes, approximately 1.7 x longer retention is obtained using methanol/water. In principle, it could be possible to increase the proportion of methanol in B to obtain a similar separation to that shown in Figure 2A (i.e. matching the eluotropic strength of the acetonitrile/water mix). Indeed, during the recent acetonitrile shortage in 2008/9, some laboratories attempted to replace acetonitrile with methanol in the mobile phase. For some applications this approach may be successful; although caution should be exercised as large changes in selectivity and elution order may occur.

Selectivity

One of the most useful aspects of the availability of both acetonitrile and methanol is that they have differing solvent properties. Methanol is a polar-protic solvent, whereas acetonitrile is a polar-aprotic solvent and possesses a stronger dipole moment. This means that the organic modifier used in the mobile phase can have a powerful effect on chromatographic selectivity. Varying the organic component of the mobile phase can therefore be a powerful method development tool. Figure 3 shows the same gradient separation run using methanol and acetonitrile as the organic modifier. In this example several co-elutions are observed when acetonitrile is used. When methanol is used different selectivity is produced, and all sample components are fully resolved. For some analytes, the change in relative retention is large and several complete reversals in elution order are observed (e.g. peak pairs 9/10 and 16/17). It is therefore highly recommended that both acetonitrile and methanol are assessed during method development to determine the most suitable solvent and to help optimise the separation.

As methanol and acetonitrile are fully miscible with one another, they can also be blended to fine-tune a separation. Figure 4 shows an example gradient separation of basic analytes where neither methanol or acetonitrile as the organic modifier provides a full separation. When methanol is used as the organic modifier, peaks 5 and 6 co-elute, whereas with acetonitrile, peaks 3 and 4 are not resolved. In contrast, by blending the two solvents together with water as a ternary mixture, it is possible to obtain intermediate selectivity and separate all seven components.

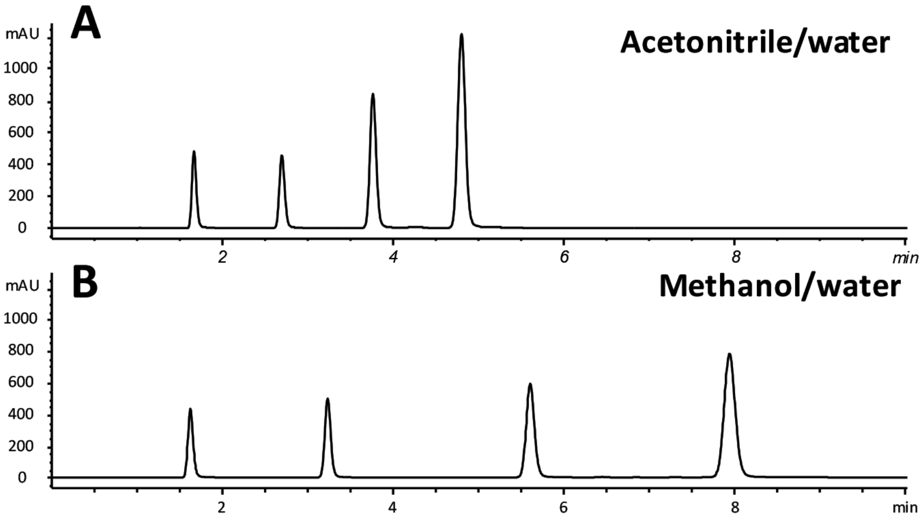

Figure 2: Comparison of the separation of neutral analytes using a mobile phase containing A) 75:25 v/v acetonitrile/water and B) 75:25 v/v methanol/water on a C18 column, 100 x 3.0 mm (flow rate: 0.43 mL/min, temperature: 30°C, injection volume: 0.5 µL, detection: UV 254 nm).

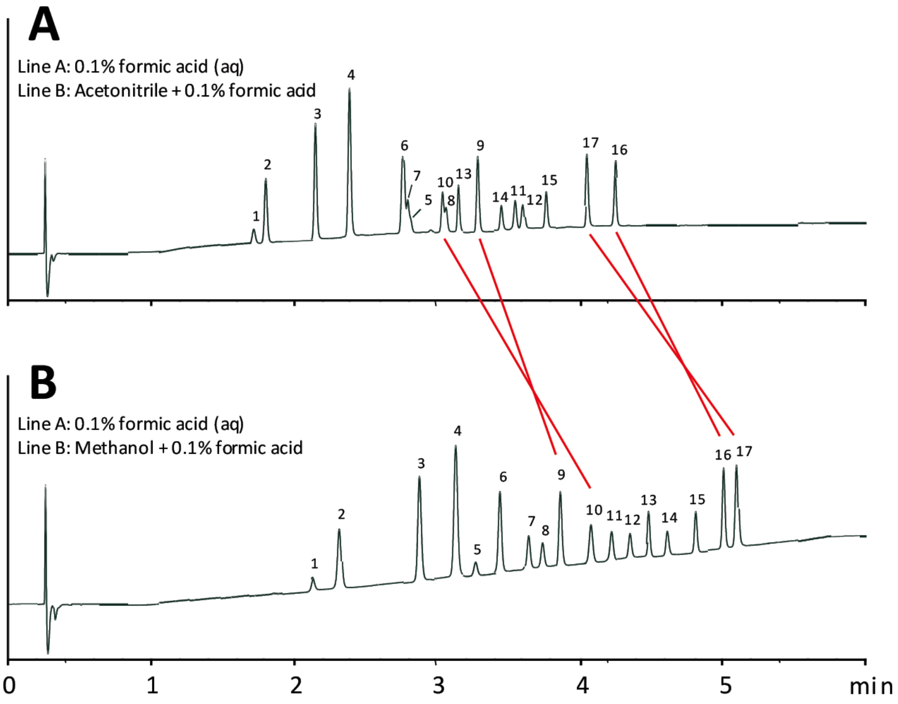

Figure 3: Gradient separation of a 17-component test mix using A) acetonitrile and B) methanol as the organic modifier. Column: ACE 3 C18-AR 50 x 2.1 mm, flow rate: 0.6 mL/min, temperature: 40°C, gradient: 3-100% B in 6.5 mins, detection: 214 nm. Sample: 1. 3-Hydroxybenzoic acid, 2. Methyl phenyl sulfoxide, 3. Quinoxaline, 4. Salicylic acid, 5. Benzylcyanide, 6. 1,2-Dimethoxybenzene, 7. Ethylparaben, 8. 1,4-Dimethoxybenzene, 9. Bendroflumethiazide, 10. Piroxicam, 11. Benzylchloride, 12. Thioanisole, 13. Sulindac, 14. Chrysin, 15. Ibuprofen, 16. 1,2,3-Trichlorobenzene, 17. Meclofenamic acid.

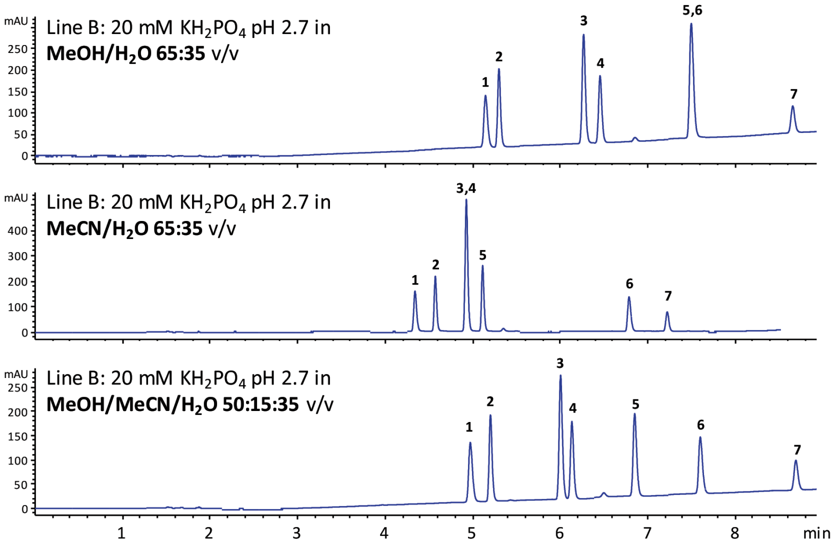

Figure 4: Blending organic solvents to achieve a gradient separation of seven basic analytes. 20 mM KH_2PO_4 pH 2.7 (aq) was used as the aqueous mobile phase component (line A) and various aqueous/organic mixes were used on line B (see figure captions). Column: ACE 3 C18, 150 x 4.6 mm, flow rate: 1.0 mL/min, temperature: 35°C, gradient: 5-70% B in 11 mins, detection: 205 nm. Sample: 1. Benzylamine, 2. Procainamide, 3. Terbutaline, 4. Salbutamol, 5. Amiloride, 6. Trimethylbenzylamine, 7. Pindolol.

Conclusions

Acetonitrile and methanol both offer advantages and disadvantages for use as the organic modifier in reversed-phase liquid chromatography, with the optimum choice being application driven. Perhaps the most important aspect of these two solvents is that they offer substantially different selectivity to one another. Investigating both methanol and acetonitrile is therefore highly recommended as a valuable approach when developing a new reversed-phase method.