Acetonitrile/Methanol Substitution in C18 Reverse Phase

on Combi*Flash* Systems

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Chromatography Application Note AN57

Abstract

Due to cost or solvent availability it is sometimes necessary to use a solvent different from that originally used for a method in reverse phase MPLC. Many methods use acetonitrile, but other solvents may be preferred when the purification is scaled up. A solvent substitution may be desired to improve the purification. This application note describes a method to easily estimate the concentration of a substitute solvent that yields similar results for the solvent originally used in a method.

Discussion

The ability to change solvents in reverse phase chromatography allows for a conversion from analytical to prep scale, or substitution of solvents to save money. Solvent substitution is also useful when the desired solvent is unavailable.

The nomogram in Figure 1 lists equivalent concentrations of various organic solvents commonly used for reverse phase. The concentration of the new solvent is read by using a vertical line to connect the current solvent concentration with the concentration of the new solvent. A solvent of 40% acetonitrile (MeCN) can be replaced with 50% methanol (MeOH) or 30% tetrahydrofuran (THF) to yield the same purification. In practice, depending on the compound, this can vary by ±5%, but the use of the nomogram will provide a close starting point.

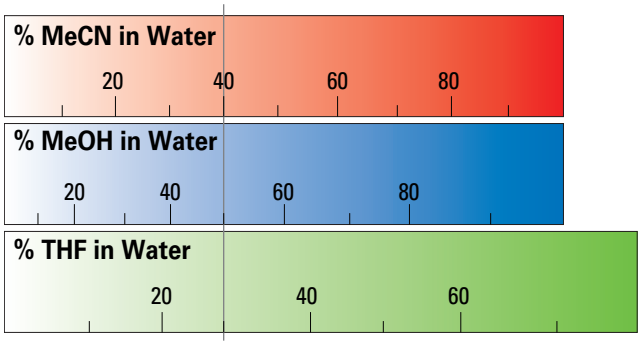

Figure 1: Nomogram to determine organic solvent composition when converting between solvents

Isocratic

All experiments were done with methyl paraben and propyl paraben adsorbed onto celite using a solid load cartridge. The experiments are compared to a run using 40% acetonitrile.

Isocratic runs are very sensitive to solvent concentration. Using the numbers from the nomogram based on 40% acetonitrile provides usable runs in Figure 2. Adjusting the concentration of methanol and tetrahydrofuran by 5% concentration (Figure 3) provides purifications closer to the original acetonitrile run.

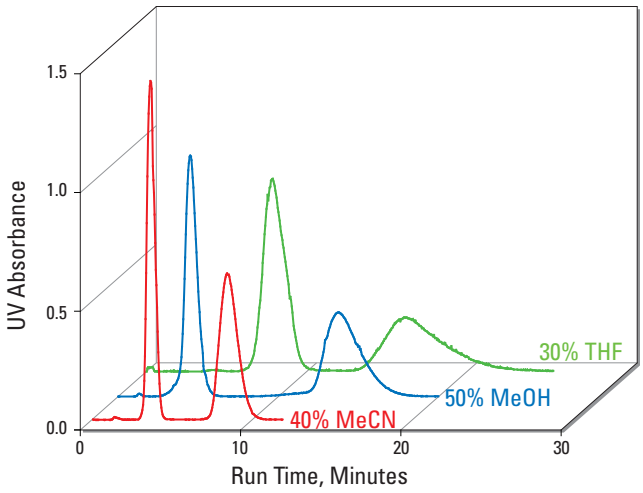

Figure 2: Results using nomogram-derived, isocratic methods to purify methyl paraben and propyl paraben

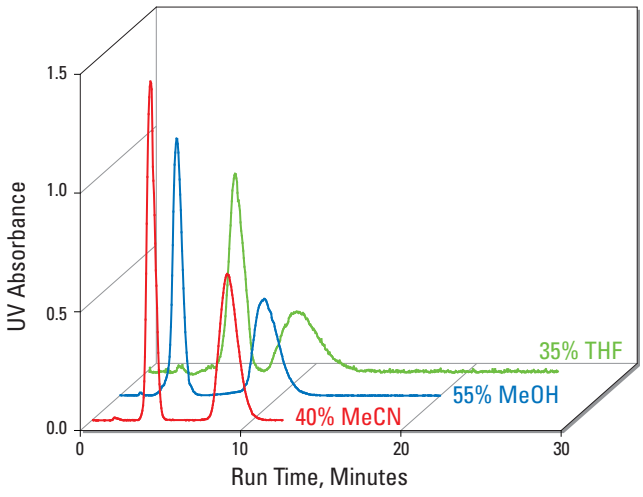

Figure 3: Results using adjusted isocratic methods to purify methyl paraben and propyl paraben

Due to selectivity differences between the solvents and various compound classes, it is difficult to exactly match chromatograms run in different solvents. This can be used to advantage to change the spacing between eluted compounds. In this case, THF shows significant differences compared to methanol and acetonitrile.

Gradient

Most MPLC purification is run with gradients. To determine the concentration of solvent at the various gradient segments, just use the nomogram as described for the isocratic method. For the example in Figure 4, the gradient ran from 5 to 95% acetonitrile. Using the nomogram provided a gradient of 5% to 95% methanol and 5% to 69% THF. All runs were methyl paraben and propyl paraben adsorbed onto celite in a solid load cartridge.

All three solvents showed purification, but with somewhat different selectivity. The selectivity difference is because the conversion between solvents is non-linear. This can be used to advantage to purify compounds by simply changing the organic solvent in a C18 run.

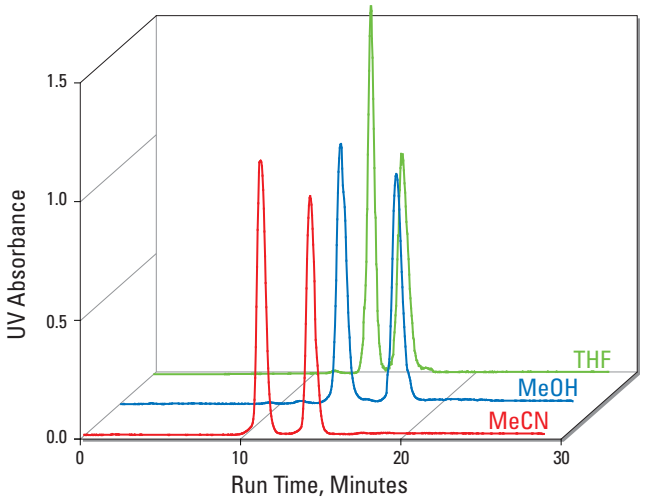

Figure 4: Chromatogram of gradient purification of methyl paraben and propyl paraben using acetonitrile, methanol, and THF.

Conclusions

C18 methods are easily converted to alternative solvents as needed based on solvent availability or cost. In addition, the solvent substitution chart provides a means of choosing a method that allows differing solvent selectivity.

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