Using acetone instead of acetonitrile offers another tool for selectivity & alternate solvent for ANP

Method Development Application

Acetone in the Mobile Phase

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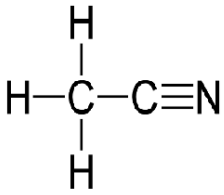
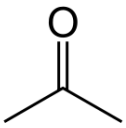
INTRODUCTION

Acetonitrile is perhaps the most widely used organic mobile phase solvent in HPLC separations. It has a number of desirable properties, including the ability to dissolve a wide variety of compounds, miscibility with water, and a low UV absorbance cutoff (~195 nm). On the other hand, it does have notable drawbacks as well. It is moderately toxic, since it is metabolized in the body to hydrogen cyanide. Furthermore, it is somewhat expensive compared to many other solvents. There was even a worldwide shortage of acetonitrile in 2008–2009 due to a variety of economic factors. See Figure 1 for structures of acetonitrile and acetone.



Acetonitrile



Acetone

Figure 1

Under suitable conditions, acetone can act as a useful solvent for an HPLC mobile phase. Like acetonitrile, it is a polar aprotic solvent. Acetone can give comparable retention in many cases, thereby acting as an acetonitrile substitute. Sometimes a change in elution order can even be observed, and therefore acetone can be used a selectivity tool as well. It is less harmful to the environment than acetonitrile in terms of disposal.

Aside from all of these benefits, acetone does have one important drawback that the chromatographer needs to consider: It absorbs strongly in much of the UV spectral range. If you use a detection wavelength of approximately 330 nm or higher, the background noise from the acetone will be manageable. Hence, acetone may be useful for analyses involving compounds that absorb in the high UV or visible range. Alternatively, its absorbance characteristics may not be relevant if you are using a non-UV based detection method. LC-MS has become increasingly popular in recent years and acetone may be a great choice in this case.

Here we present the various ways in which acetone may be used to the benefit of the modern chromatographer. We describe its potential as a substitute, as a selectivity tool, and as a superior solvent in certain aspects.

A: DI H_2O + 0.1% TFA
B1: ACN + 0.1% TFA
B2: Acetone + 0.1% TFA

Gradient:

Time (min)	%B
0	15
1	15
14	60
15	60
16	15

Flow Rate: 0.3 mL/min
Injection Volume: 1.0 µL
Detection:

Acetonitrile Method

0–10 min: 350nm
10–20 min: 525nm

Acetone Method

0–11.5 min: 350nm
11.5–20 min: 525nm

EXPERIMENTAL

Materials

Doxycycline hyclate, meclocycline sulfosalicylate, eosin Y, brilliant blue G, and crystal violet were from Sigma–Aldrich (St. Louis, MO, USA). Deionized water (DI H_2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (ACN) and acetone (HPLC grades) were obtained from GFS Chemicals, Inc. (Powell, OH, USA). TFA was from GFS Chemicals as well.

Instrumentation

A Hewlett–Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength UV detector was used. The system was interfaced with Agilent Chemstation (Santa Clara, CA, USA) software. The analytical column was 2.1 mm (i.d.) x 50 mm and packed with a Bidentate C18 2.o stationary phase (MicroSolv Tech. Corp., Eatontown, NJ, USA). The particle diameter was 2.2 µm and the pore size was 120Å. Two gradients were used. The two programs were the same except one used solvent B1 and the other used solvent B2.

Samples

1000 mg/L stock solutions were prepared for each analyte in a diluent of acetonitrile/ 0.1% TFA (v/v). Aliquots of the stock solution were mixed to obtain concentrations in the range 10–100 mg/L. Individual standard dilutions were also prepared in the range 10–100 mg/L. The diluent used was 90/10/0.1 acetonitrile/DI water/TFA.

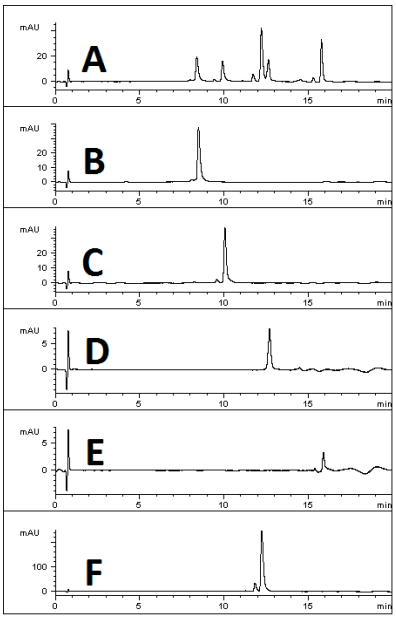


Figure 2

Chromatograms using Acetone method

A. Mix
B. Doxycycline
C. Meclocycline
D. Brilliant Blue
E. Eosin Y
F. Crystal Violet

RESULTS AND DISCUSSION

Five test solutes were chosen for the study. Each had significant absorption in the high UV and/or visible region. This allowed for the use of acetone in the mobile phase with minimal background interference. In order to obtain a direct comparison, the two methods that were used differed only by the organic solvent (*i.e.* either acetone or acetonitrile).

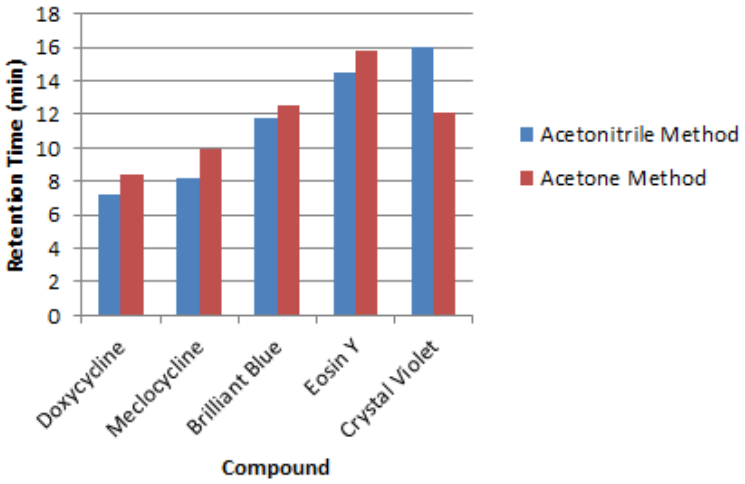


Figure 3

The results (Figures 2 and 3) showed several interesting aspects of the use of acetone. First, retention with acetone was only slightly higher for four out of five solutes. If the gradient is adjusted to account for this difference, near equivalent results could be obtained for these four analytes. Therefore, acetone could serve as a suitable substitute for acetonitrile in the case of some separations. Considering the benefits of using acetone discussed earlier, this could be a very attractive prospect for reducing costs in the laboratory.

Another interesting point is the retention behavior of the fifth analyte, crystal violet. Here we observe notable differences in retention between use of acetonitrile vs. acetone. In the context of the separation, we even observe a change in elution order. This difference can be a beneficial tool for the analytical chemist during method development.

You could even have a B solvent which consists of both acetone and acetonitrile to obtain retention characteristics somewhere between these two data sets. Hence, acetone can be used as a selectivity tool in the same manner as other common method parameters such as temperature, pH, or ionic strength.

Finally, acetone has chromatographic benefits in terms of peak shape. When the tailing factors of each analyte were compared using both methods, it was found that acetone tended to give more symmetrical peak shapes. In the case of two analytes (brilliant blue and eosin Y), peak tailing was notably improved (see Figure 4).

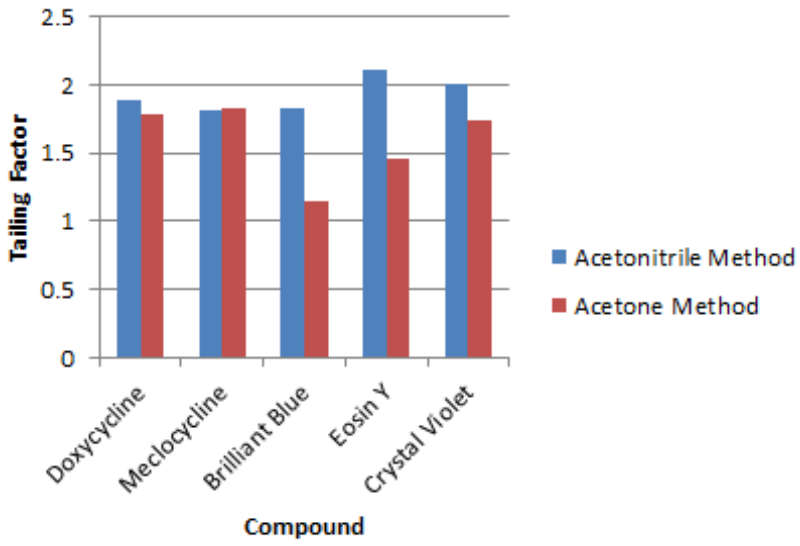
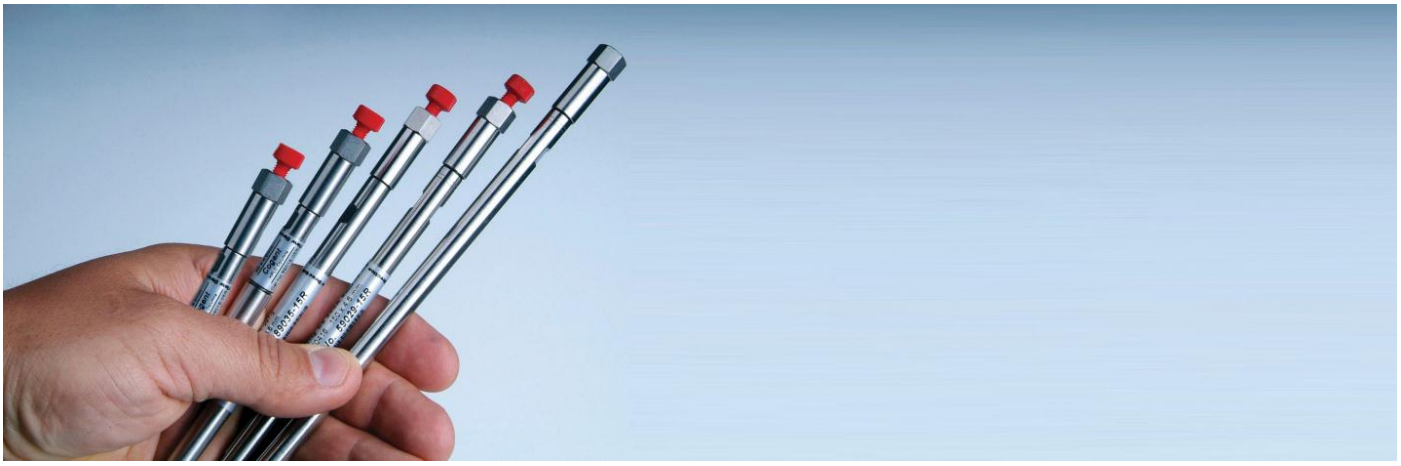


Figure 4

CONCLUSION

Acetone can provide comparable retention to acetonitrile in many cases. Given its lower cost as well as lower toxicity, there are several incentives to use acetone instead of acetonitrile if it is applicable to the separation. Some analytes may even exhibit a notable difference in retention, leading to potentially beneficial selectivity changes.



Catalog Number	Description
40218-02P-2	Cogent Bidentate C18 2.o HPLC column 120A 2.2um 20mm x 2.1mm. 1 each.
40218-03P-2	Cogent Bidentate C18 2.o HPLC column 120A 2.2um 30mm x 2.1mm. 1 each.
40218-05P-2	Cogent Bidentate C18 2.o HPLC column 120A 2.2um 50mm x 2.1mm. 1 each.
40218-10P-2	Cogent Bidentate C18 2.o HPLC column 120A 2.2um 100mm x 2.1mm. 1 each.
40218-15P-2	Cogent Bidentate C18 2.o HPLC column 120A 2.2um 150mm x 2.1mm. 1 each.
40218-75P-2	Cogent Bidentate C18 2.o HPLC column 120A 2.2um 75mm x 2.1mm. 1 each.

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