

응용 자료

## Enabling Viscous Solvents with Elevated Temperature

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### Abstract

This application note explores the benefits of using elevated temperature to exploit the characteristics of organic modifiers to change selectivity and decrease chromatographic run times with the Waters ACQUITY UltraPerformance LC (UPLC) System.

## Benefits

- Isopropanol, an ideal solvent choice, has demonstrated benefits in speed, selectivity, and versatility during the method scouting process

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## Introduction

The use of elevated temperature in liquid chromatography has become an active area of research. As the column temperature is increased, the mobile phase viscosity is decreased. This means that with a constant mobile phase, flow rate, and column geometry, the operating pressures are reduced at higher temperatures. These higher temperatures also allow faster separations to be obtained with equivalent resolution due in part by flattening of the van Deemter curve and operating at higher flow rates.

Raising the column temperature in excess of 90 °C significantly reduces solvent viscosity enabling the use of solvents such as Isopropanol that have rarely been used by chromatographers due to column backpressure limitations.

This application note explores the benefits of using elevated temperature to exploit the characteristics of organic modifiers to change selectivity and decrease chromatographic run times with the Waters ACQUITY UltraPerformance LC (UPLC) System.

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## Experimental

### UPLC Conditions

Instrument: ACQUITY UPLC System

Column: ACQUITY UPLC BEH C<sub>18</sub>, 2.1 x 150 mm, 1.7 µm

|                   |   |
|-------------------|---|
| Flow rate:        | 0.45 mL/min   |
| Injection volume: | 5 µL (Full Loop injection mode)   |
| Column temp.:     | 90 °C   |
| Mobile phase:     | A: 0.1% Formic Acid in water<br>B: Isopropanol (IPA) or Methanol (MeOH) or Acetonitrile (ACN) |
| Gradient:         | 5% – 95% B over 10 min  |
| Curve:            | Linear  |

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## Results and Discussion

### Exploring Organic Modifiers

Figure 1 illustrates how the back pressure of a 2.1 x 150 mm ACQUITY UPLC BEH C<sub>18</sub> Column changes over the solvent range of a 5–95% aqueous/organic gradient for isopropanol, methanol, and acetonitrile. It is observed that the isopropanol solvent reaches a backpressure maximum of 11,000 psi at a flow rate of 0.45 mL/min at 90 °C.

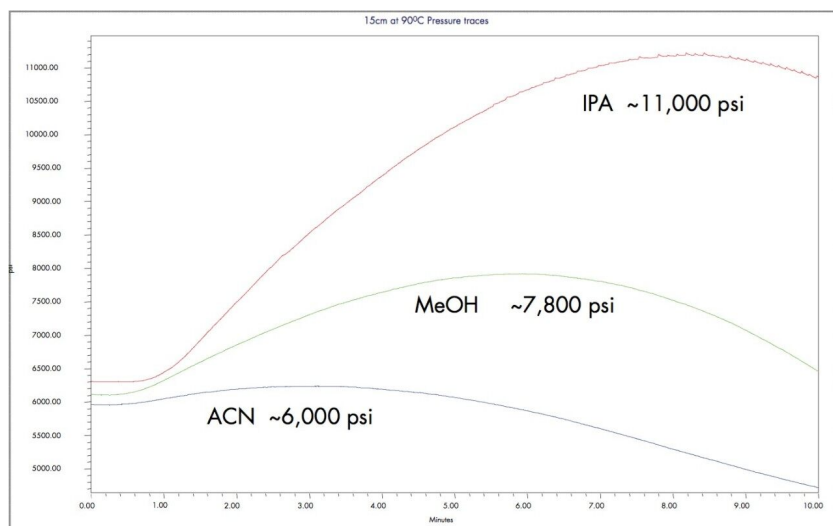


Figure 1. Solvent pressure profile.

## Identifying Isopropanol Benefits

The benefits of using mobile phase modifiers such as isopropanol are four-fold; 1.) faster analysis, 2.) sharper peaks, 3.) different analyte selectivity, and 4.) less toxicity. The effect of isopropanol on the speed of analysis can be seen in Figure 2. When compared to methanol and acetonitrile, IPA gives a significant reduction in analysis time due to its greater elutropic strength. Thus, analysis time can either be reduced and/or organic solvent concentration can be reduced to elute all of the sample components in the column, resulting in consumption savings.

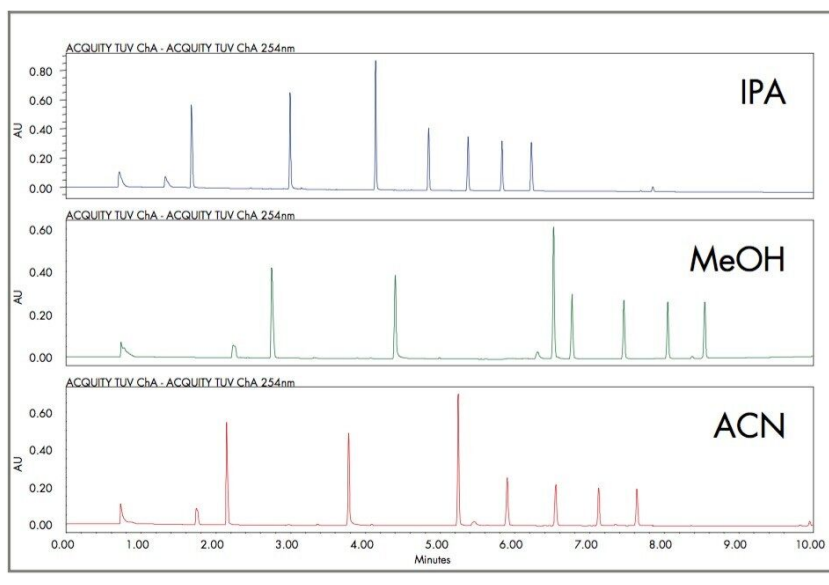


Figure 2. Separation of a standard mixture of components of varied hydrophobicity from Bromoguanosine (Peak 1) to Octanophenone (Peak 8).

## Analysis of Pharmaceutical Raw Material

The use of high temperature enables the use of these “new” solvent choices that offer great potential for method development. The selectivity advantages of IPA are illustrated in the analysis of the glucocorticosteroid Budesonide (Figures 3 and 4). Here, R-epimer and S-epimer of Budesonide were baseline resolved with IPA, whereas with MeOH they were completely unresolved. And with ACN, they were only approximately 65% baseline resolved, despite being retained to a greater extent (Figure 3). The resolution of the major impurities eluting before the API are affected, resulting in a greater number of peaks separated (vs. MeOH). In general, the peak shape and resolution of the impurities are enhanced when using IPA compared to MeOH (Figure 4).

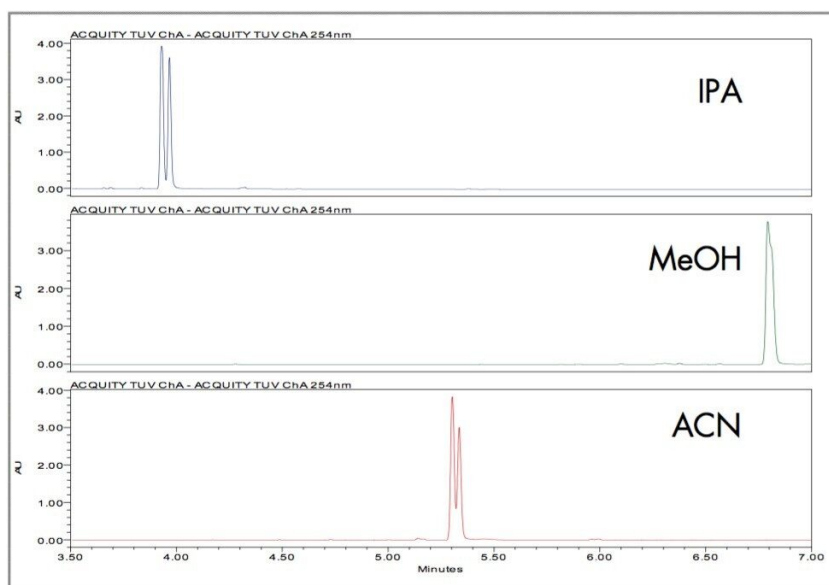
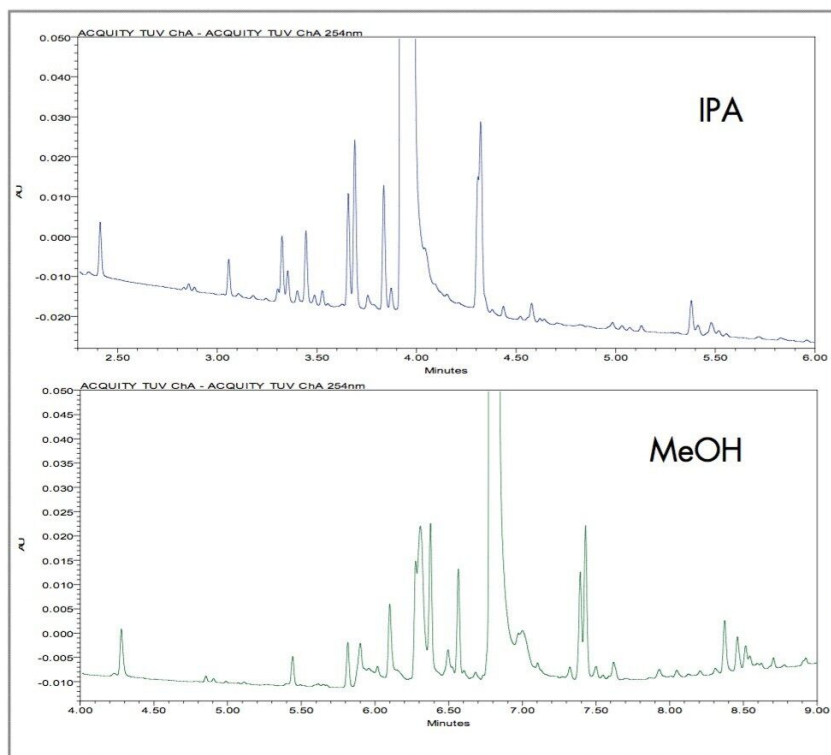


Figure 3. Resolution comparison of the chromatograms of Budesonide using varied organic modifiers. The first peak is the R-epimer, followed by the S-epimer.



*Figure 4. Zoomed view showing the Budesonide impurities resulting from 5–95% B gradient scouting experiments. Note the varying resolution and selectivity.*

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## Conclusion

The solvent pressure profiles shown demonstrate how high temperature enables the use of more viscous mobile phases such as IPA with the ACQUITY UPLC System and sub-2  $\mu\text{m}$  particle technology. Isopropanol, an ideal solvent choice, has demonstrated benefits in speed, selectivity, and versatility during the method scouting process. Collectively, the experiments in this application note show how the combination of UPLC and elevated temperature provide the method development chemist with the ability to explore more avenues for chromatographic separation success.

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## Featured Products

ACQUITY UPLC System <<https://www.waters.com/1000396>>

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