Separation of Sulfonamides Using Temperature Programming and an Environmentally Friendly "Green" Mobile Phase

Introduction

Green chemistry is the use of chemistry for pollution prevention. It includes the design of chemical products and processes that reduce or eliminate the use and generation of substances that are harmful to our health and environment. Since the Pollution Prevention Act of 1990, many organizations such as the U.S. Environmental Protection Agency (EPA) and the International Union of Pure and Applied Chemistry (IUPAC) have instituted green chemistry programs.

Because of copious amounts of hazardous chemicals generated when performing HPLC separations, this technology is a good candidate to investigate the use of green chemicals such as water and ethanol to replace some of the existing mobile phases.

In this note, we will demonstrate the feasibility of using a green solvent mixture of ethanol and water as a mobile phase in conjunction with the added benefits of temperature programmed liquid chromatography (TPLC) to replace the more traditional acetonitrile and water mixture in the separation of sulfonamides.

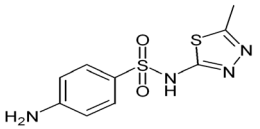
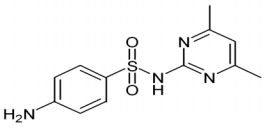
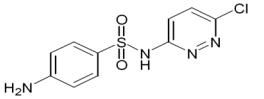
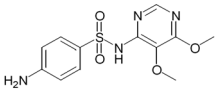
Instrumentation

Both separations were optimized using an Agilent 1100 HPLC, Zorbax StableBond C18, 150 x 3.0 mm, 3.5µm particles (Agilent Technologies) and a Polaratherm Series 9000 high temperature column compartment equipped with mobile phase preheating (Selerity Technologies Inc.).

Results

Figure 1 shows the sulfoamide analysis using acetonitrile in the mobile phase. Solvent gradients 40°C, 60°C, and 80° were performed. The analysis at 80°C was done at two different flow rates. Figure 2 shows the separation of sulfonamides using ethanol in the mobile phase. Three solvent gradients at 40°C, 60°C, and 80°C were conducted, in addition to a temperature program from 70°C to 90°C at 20°/min.

Table 1:
Structure of Sulfonamides

Peak	Name	Compound Structure
1	Sulfamethizole	
2	Sulfamethazine	
3	Sulfachlorpyridazine	
4	Sulfamethoxine	

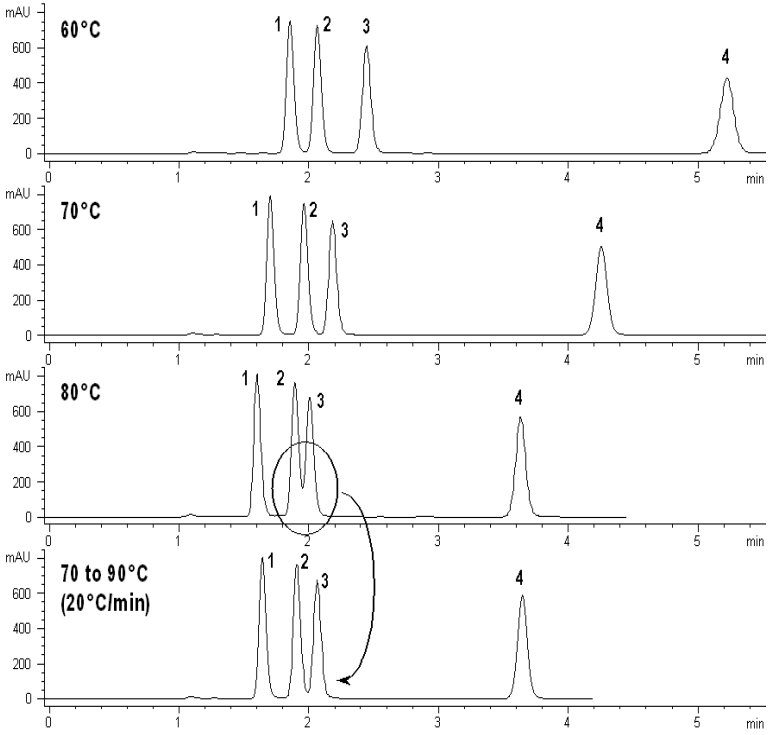
Discussion

The goal of the study was to develop a method using a green solvent mixture that is equivalent to a method using a hazardous mobile phase composition. When using acetonitrile and water as the mobile phase the chromatogram was optimized with baseline resolution between all four components in 3.9 minutes at an isothermal temperature of 60°C and a flow rate of 1.2 mL/min. The mobile phase composition was then replaced with a mixture of ethanol and water, and with the added benefits of temperature programming a baseline separation was achieved in 3.8 minutes.

***Figure 1*: StableBond C_{18} column using acetonitrile in mobile phase**

Column: Zorbax StableBond C_{18}, 150 x 3.0 mm, 3.5 µm particles
Sample: Sulfonamide test mixture (Agilent Technologies)
Injection: 2 µL
Detection: UV, 270 nm
Mobile phase: A = 0.1% acetic acid in water, B = 0.1% acetic acid in acetonitrile
Flow-rate: 0.6 or 1.2 ml/min
Gradient: 20 to 50% B in 2 min

Notice the temperature dependent selectivity changes. Temperature and flow rate were fiine-tuned and for optimal separation.



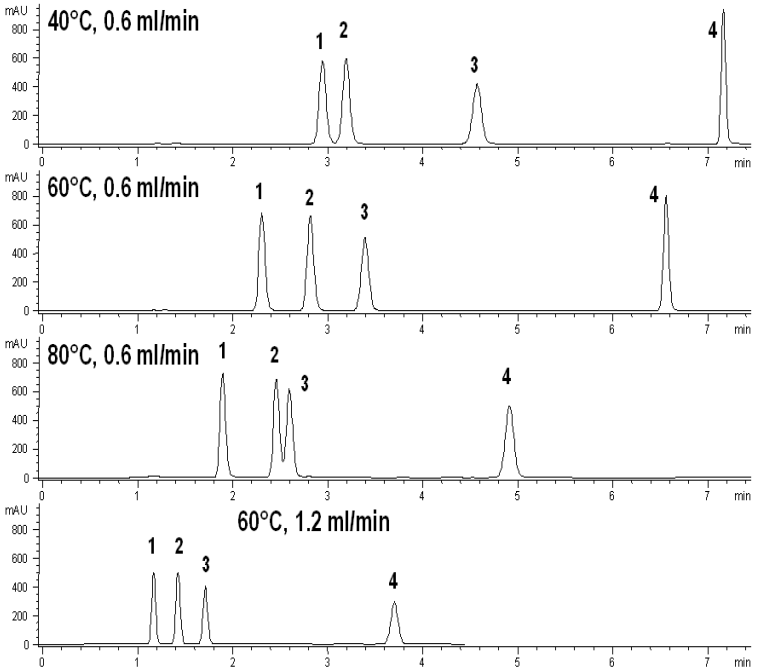


***Figure 2*: StableBond C_{18} column using ethanol in the mobile phase**

Column: Zorbax StableBond C_{18}, 150 x 3.0 mm, 3.5 µm particles
Sample : Sulfonamide test mixture (Agilent Technologies)
Injection: 2 µL
Detection: UV, 270 nm
Mobile phase: A = 0.1% acetic acid in water, B = 0.1% acetic acid in **ethanol**
Flow-rate: 0.6 ml/min
Gradient: 17 to 50% B in 3 min

Again, notice the temperature dependent selectivity changes. The temperature for the solvent gradient runs and the temperature program were fine-tuned for optimal separation and analysis time.

Acknowledgments:

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