**Introduction**

The gradient slope is the chief parameter modified for optimizing RP gradient separations of complex mixtures such as natural product extracts. Other method parameters that maximize resolution in gradient methods such as column length, particle size, and flow rate are sometimes ignored due to limitations of the LC system including the column. Licorice Root extract and other natural products will be separated using UHPLC instruments which provide the flexibility to better use a wider range of LC parameters including column length, temperature, and flow rate for optimum resolution. The UHPLC instruments will be used with 1.8 µm columns to evaluate column length (efficiency), flow rate, and other method parameters in addition to gradient slope, can be optimized to produce narrower peaks, higher peak capacity, and the best resolution for complex samples requiring gradients.

**Experimental**

**The Mobile and Stationary Phase**

**Stationary Phase:** ZORBAX RRHD Eclipse Plus C18

- 2.1 x 50mm, 1.8 µm PN 959757-902
- 2.1 x 100 mm 1.8 µm PN 959758-902
- 2.1 x 150 mm 1.8 µm PN 959759-902

**Gradient:**

- **Fixed Gradient Time:**
  - Licorice Root: 10 min
- **Variable Gradient Time:**
  - Licorice Root: 10 min

**Effect of Column Length on Peak Capacity**

- **50 mm column:**
  - Peak width = 0.088 min
- **100 mm column:**
  - Peak width = 0.069 min
- **150 mm column:**
  - Peak width = 0.055 min

**Effect of Flow Rate on Peak Capacity**

- **F=0.2 ml/min:**
  - Peak width = 0.068 min
- **F=0.4 ml/min:**
  - Peak width = 0.088 min

**Conclusion**

Four variables that can improve peak capacity and resolution in 1.8 µm gradient methods are:

- **Longer Columns**
- **Longer Gradient Times**
- **Higher Flow Rates**
- **Temperature**

Agilent RRHD columns and the 1290 Infinity UHPLC are designed for higher system pressure, so these four variables can be better utilized for gradient method development of complex samples.