The High-Resolution Reversed-Phase HPLC Separation of Licorice Root Extracts Using Long Rapid Resolution HT 1.8-µm Columns

Application

Food Additive, Natural Products, and Pharmaceuticals

Author

Bernard Permar and Ronald E. Majors
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

High-resolution reversed-phase HPLC analytical studies using licorice, a licorice hydrolysis product, and commercial licorice samples, showed that resolution and throughput using a ZORBAX 1.8-µm column greatly exceeded that obtained using the conventional 5.0-µm column.

Introduction

Licorice is derived from the root of the *Glycyrrhiza glabra* plant, a 4- to 5-foot woody shrub that grows in Europe, the Middle East and Western Asia. The root of the plant is known to contain about 4% glycyrrhizin, the potassium or calcium salt of glycyrrhizinic acid. The latter is a glycoside of a pentacyclic triterpine carboxylic acid (18-ß-glycyrrhetic acid) with two molecules of glucuronic acid (Figure 1).

Glycyrrhizin is about 50 times sweeter than sucrose (cane sugar) but at high dosage is known to have toxicity. Upon hydrolysis, the glycoside loses its sweet taste and is converted to the aglycone glycyrrhetinic acid plus two molecules of glucuronic acid.

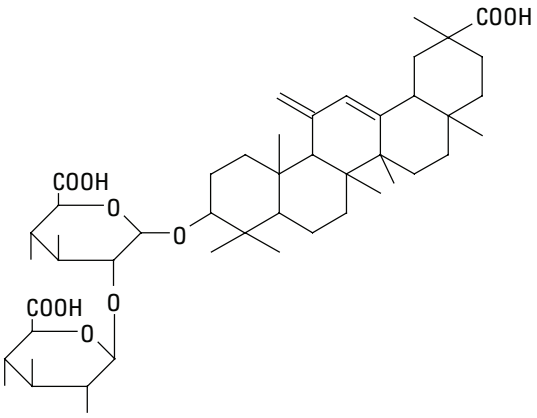


Figure 1. Structure of glycyrrhizinic acid.

Extractions from any of the many species of this plant will yield a complex mixture containing more than 100 compounds. Several of these compounds are used as additives in candy as sweeteners, in cough syrup as flavoring agents, and in drugs to mask a bitter taste, or for their therapeutic qualities, mainly in Traditional Chinese Medicines (TCMs). The medicinal properties of licorice have been known for several centuries in China, as well as India, Egypt, Greece, and Rome. Uses included cough suppressant, laxative, and treatments for gastric ulcer, early Addison disease, and liver disease. Most recently, glycyrrhizin has been shown to have antiviral activity against DNA and RNA viruses (influenza A and B, HIV, VZV, Hepatitis B and C) [1]. Licorice has also been used in topical cosmetic applications.

The abundance of certain compounds of interest will vary greatly according to the species of the plant, the time of harvest, and the method of extraction. Thus, analytical methods to follow the active ingredients are required. Gradient elution reversed-phase HPLC has been found to be an effective method for separating some of the important compounds in licorice [2]. This application note compares the traditional HPLC methodology and the newer Rapid Resolution high-throughput (RRHT) columns. We will apply these HPLC techniques to investigate the differences between two commercially available licorice root extracts.

Experimental

Two reversed-phase (RP) columns were used in this study:

- Conventional ZORBAX StableBond (SB)-C18, 4.6 mm × 250 mm, 5 µm
- ZORBAX SB-C18 RRHT, 4.6 mm × 150 mm, 1.8 µm

The smaller particle size of the RRHT column allows use of a shorter column to achieve the same resolution as the longer conventional column, and also allows more rapid separations.

HPLC conditions

Instrument:	Agilent 1200 Series Rapid Resolution System
Detector:	Multiple wavelength detector (MWD), 254 nm/100 BW, 450 nm reference
Mobile phase:	A = 1% Acetic acid in water B = 1% Acetic acid in acetonitrile

Gradient conditions for ZORBAX SB-C18 columns:

Conventional:	4.6 mm × 250 mm, 5 µm 5% to 100% B in 50 minutes
RRHT:	4.6 mm × 150 mm, 1.8 µm 5% to 100% B in 30 minutes
Flow:	1.0 mL/min
Temperature:	Ambient

Standards: Purchased from Sigma Aldrich

- (G) 0.1-mg glycyrrhizic acid ammonium salt, ~75 %, dissolved in 0.5-mL mobile phase B, then brought to 1.0 mL by adding 0.5-mL mobile phase A
- (GA) 0.1-mg 18-beta-glycyrrhetinic acid, 97%, dissolved in 0.5-mL mobile phase B, then brought to 1.0 mL by adding 0.5-mL mobile phase A

Samples:

- Licorice root extract A (HERB FARM brand)
- Licorice root extract B (Newark Natural Foods)

Both extracts should be vortexed, then filtered (0.2 micron) prior to injection.

Injection volume: 5 µL of extract

Results

The most important compound found in a typical licorice extract is G and to a lesser extent, its hydrolysis product, GA. These substances can be purchased commercially. Although some of the other components of licorice have been identified and are available commercially, they are quite expensive. Since our main objective was to demonstrate the advantage of using shorter, high-resolution HPLC columns, we used only two standards (G and GA) to develop the initial method. Figure 2a shows the gradient separation of G and GA on the conventional column (ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm) using gradient elution. Since the licorice extract to be examined was quite complex, isocratic conditions were not usable to separate all of the components. The G being more polar by virtue of the additional sugar moieties eluted first while the GA came off the column much later. Using this gradient, the GA eluted in just under 42 minutes. By switching to the shorter ZORBAX SB-C18 RRHT column (4.6 × 150 mm, 1.8 µm), the separation was virtually the same, as can be seen in Figure 2b. However, the separation time was now just over 25 min, a savings of about 40% in time.

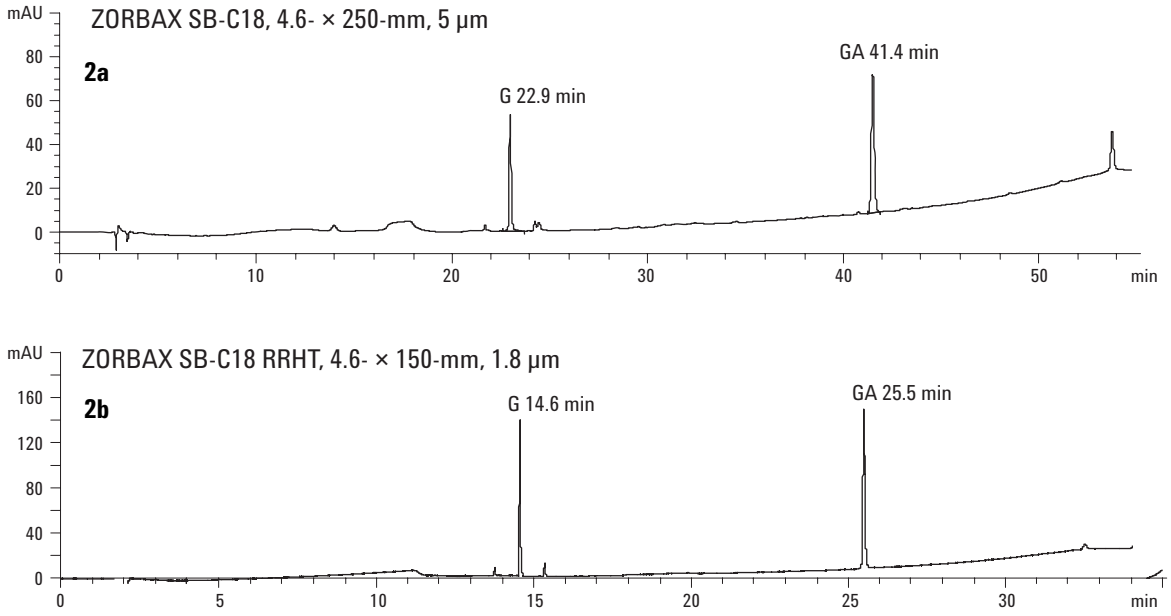


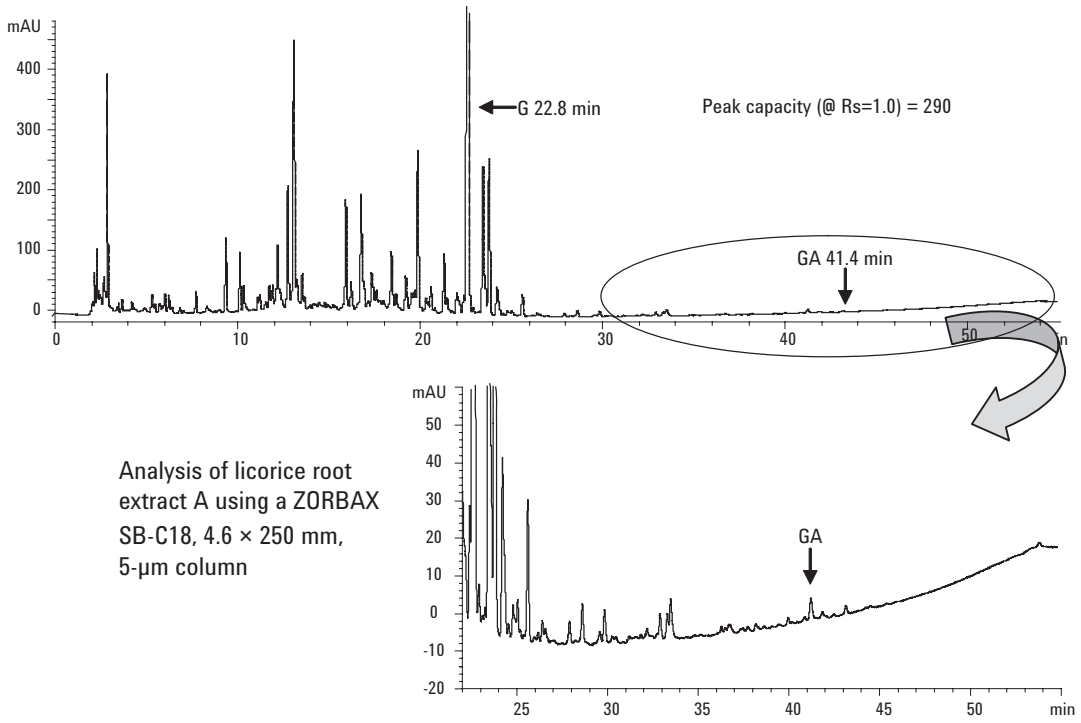
Figure 2. Gradient reversed-phase separation of G and GA on the test 5.0- and 1.8-µm columns.

To investigate the use of these columns for the separation of actual licorice root extracts, Figures 3 and 4 depict the complex chromatograms observed by injection of filtered extracts, identified in the Experimental section. Figure 3a shows the complex chromatogram obtained using the 5-µm 250-mm column. The cut-away shows the small amount of GA that was present in the extract. Since GA is a hydrolysis product of G, it should be at a much lower concentration in a licorice extract, unless the extract was treated to enhance the concentration of the hydrolyzed product. Based on the area count, the GA concentration was less than 0.5% of the G concentration in extract A.

Although the actual peaks were not counted, the calculated peak capacity (3) for the 5-µm column was determined to be 290 (resolution: 1.0). Running the same extract A on the 1.8-µm 150-mm column, one can see the finer structured (that is, higher resolution) chromatogram that results (Figure 3b). The calculated peak capacity for this higher efficiency column was determined to be 442, over 50% higher than by using the longer 5-µm column. Thus, it would be easier to determine minor components on this shorter rapid resolution column. The peaks per unit time (Resolution = 1.0) was calculated to be 17.7 peaks/min for the 1.8-µm column versus 7.1 for the 5-µm column.

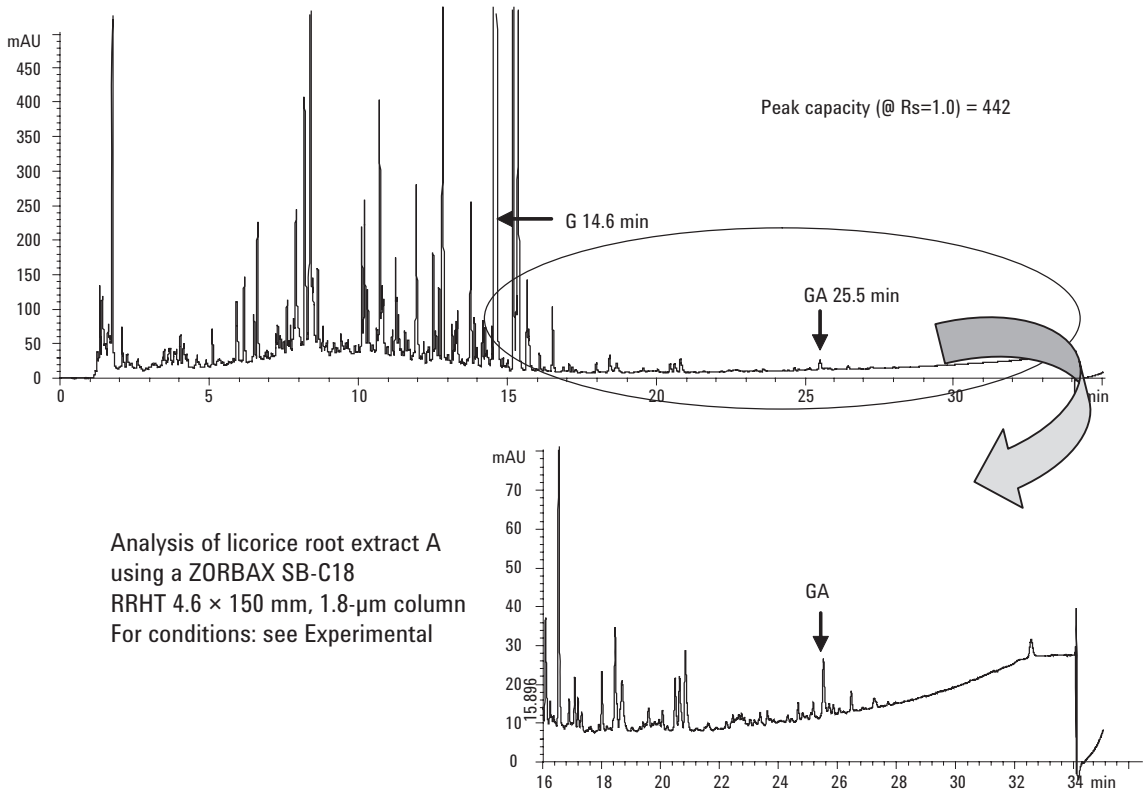
Figures 4a and 4b show similar runs using extract B. This extract was even more complex than extract A which is borne out by comparing the high resolution chromatograms of Figure 3b versus Figure 4b. Again, the calculated peaks per minute for the 1.8-µm column greatly exceeded that of the 5-µm column (17 versus 7.5 respectively). Based on the peak area counts for GA, it was roughly 1% of the concentration of G in extract B.

(A)



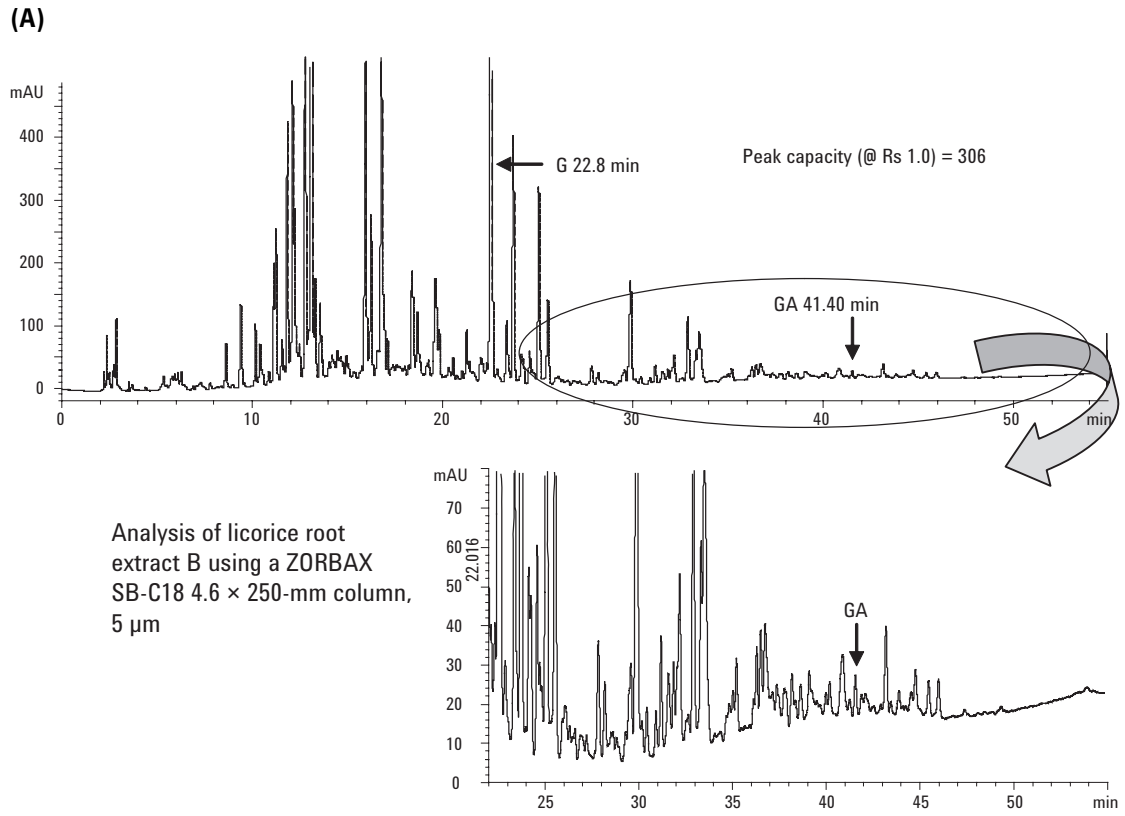
Analysis of licorice root extract A using a ZORBAX SB-C18, 4.6 × 250 mm, 5-µm column

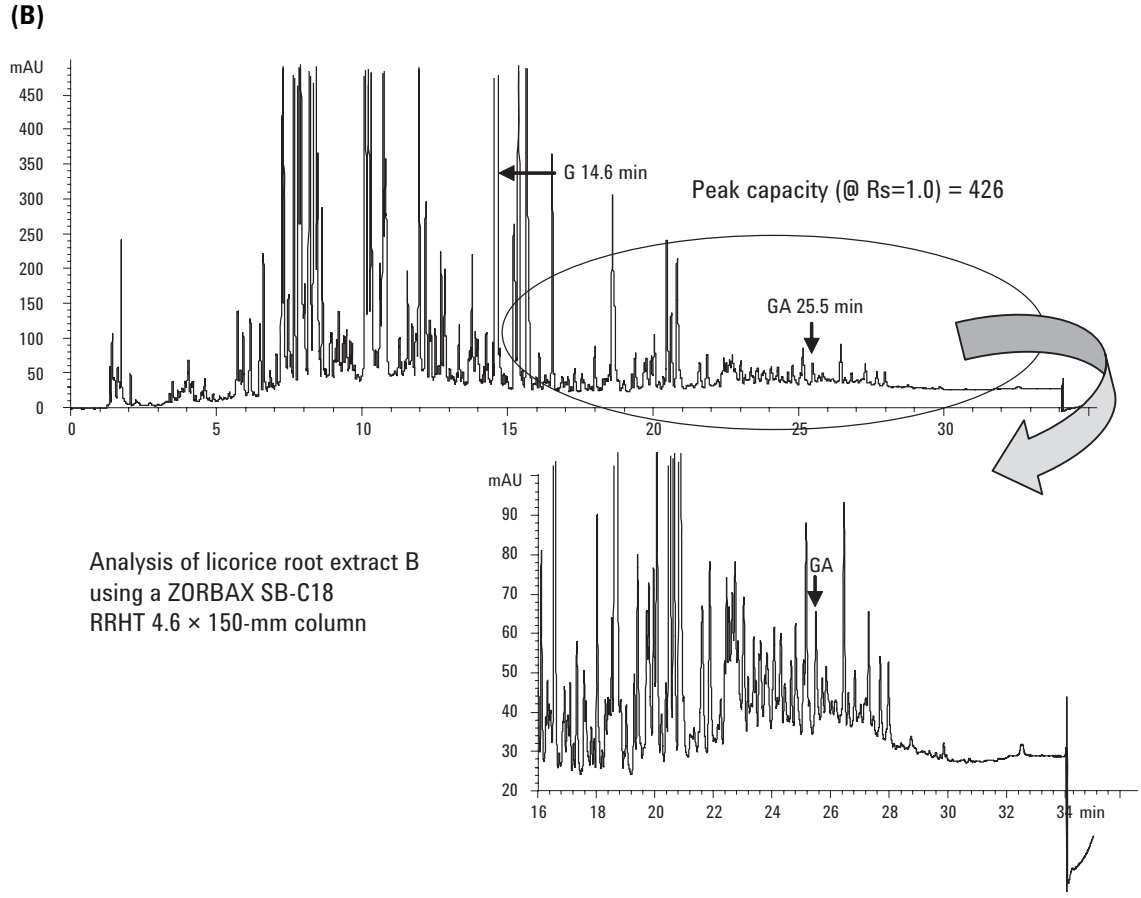
(B)



Analysis of licorice root extract A using a ZORBAX SB-C18 RRHT 4.6 × 150 mm, 1.8-µm column For conditions: see Experimental

Figure 3a and 3b. The gradient reversed-phase separation of licorice root extract A on the 5.0-µm column (A) and on the 1.8-µm column (B).





Figures 4a and 4b. Gradient reversed-phase separation of licorice root extract B on the 5.0-µm column (A) and on the 1.8-µm column (B).

Conclusions

No attempt was made to perform quantitative analysis on the components of the licorice extracts. From our studies, it was obvious that resolution and throughput using the 1.8-µm column greatly exceeded that obtained using the 5.0-µm conventional column. As more complex samples of natural products are encountered and researchers require more detailed component analyses, the use of high resolution, small particle columns should grow. In the investigation of licorice root, other natural products, and TCMs, it is necessary to have gradient capability and sensitive detection.

References

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2. I. Kitagawa, (2002) *Pure Appl. Chem.* **74** (7), 1189–1198.

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