Analysis of Artemisinin Using the Agilent 1200SL with Rapid Resolution High Throughput (RRHT) Columns and ChromSword Software

Application Note

Pharmaceutical

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Abstract

Development of a simple isocratic method for the analysis of artemisinin is accomplished with minimal operator interaction. After final mobile phase optimization, several additional columns are screened before final column choice. In this work, an automation program, ChromSword software, is used to aid the analysts in choosing the column suited for the analysis.

An automated method development HPLC system can help busy scientists. Even when a powerful analytical technique, such as mass spectroscopy will be used, having sharp, well-resolved peaks will improve the quality of data and simplify the analysis. In this example, fully automated, unattended method development was performed during a six-hour period when the instrument would have otherwise not been used.

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Introduction

Artemisinin is the active ingredient found in sweet wormwood (*Artemisia annua L.*). The medicinal value of the plant was first discovered more than 2,000 years ago in China, where the plant has been used for centuries to treat a variety of ailments, including fevers and malaria. The active compound was isolated first in 1971 and named Qinghaosu or Artemisinin [1]. Since then, artemisinin and its derivatives have been studied and used as potent antimalarial agents. Artemisinin itself is no longer used, being replaced by its more bioavailable derivatives artemether and artesunate. At present its use is strictly controlled under World Health Organization guidelines, as it has proven to be effective against forms of multidrug-resistant malaria [2,3].

Structures and Description of Artemisinin Compounds

Artemisinin and its derivatives are sesquiterpene lactones that contain an internal double-oxygen bridge. It is this rare peroxide bridge linkage that is thought to be responsible for the majority of its antimalarial action. The structures of these compounds are shown in Figure 1. They have weak UV absorption, and so detection is best performed at low UV wavelengths [4]. Artemisinin is reduced to dihydroartemisinin (DHA), which may then be converted to artemether or artesunate. In the body these derivatives revert to DHA, which is the active form. Analysis of DHA is complicated by the formation in solution of epimers at the hydroxyl carbon. Monitoring of synthetic reactions and the stability of artemisinin derivatives requires resolution of these forms. It is quite difficult to resolve artemisinin, artesunate, and both epimers of DHA in reasonable run time.

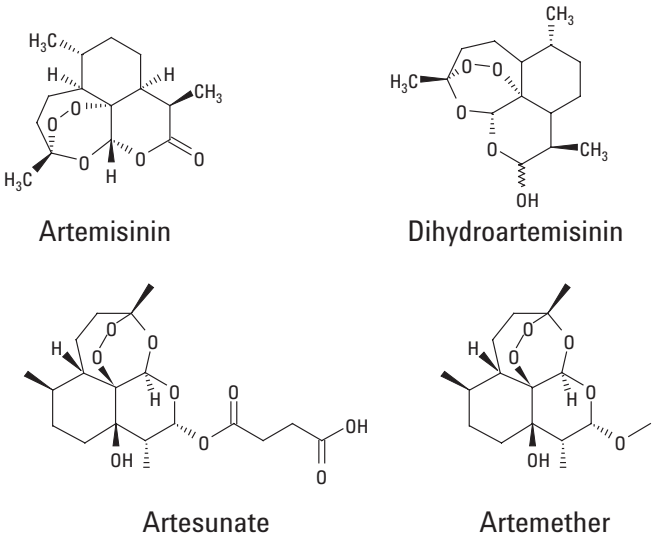

Figure 1. Structures of artemisinin derivatives.

HPLC method optimization is a time-consuming task that takes a fair amount of expertise. It is a good practice in method development to follow a standard development protocol. One such protocol is as follows: Select a high-quality C18 or C8 bonded phase first for good retention and resolution with typical acidic, basic, and neutral samples. The second step is to choose a simple mobile phase that is likely to work with many samples. The choice may depend on the detector that will be used; formic acid is a good choice as a starting mobile phase additive if a mass spectrometer will be implemented. Phosphate buffer or TFA is a good starting point if a UV detector will be used. Best results are typically obtained at low pH with acidic or basic compounds. Acids are typically protonated and good retention is obtained and basic compounds have good peak shape. The organic modifier can be acetonitrile or methanol. Typically, acetonitrile is chosen due to its low UV window and low viscosity. The percent organic component of the mobile phase is varied to optimize selectivity. Alternate bonded phases can be investigated to completely optimize a method if needed. Ideally, a resolution of greater than 2 for all peaks is desirable – and to do this successfully requires high-efficiency column choices. It is also desirable to have the first peak retain at least one column volume (k' = 1). This reduces retention variability of that peak due to mobile phase variations. Typically, a preferred analysis time of less than 20 minutes is desired [5,6].

ChromSword is a computer program that can reside on an Agilent 1100 [7] or 1200 Series HPLC system, which interactively controls the instrument. You establish the starting points for the method (for example, column solvent, etc.) much as if you were developing a method without the software. The difference is that methods are created and documented without further user interaction.

ChromSword Auto was developed by Agilent's collaboration partner ChromSword Baltic. In addition to the experiments shown in this paper, it can operate in a fully automatic mode screening columns, solvents, and temperature combinations.

Experimental

HPLC analysis was performed with the Agilent 1200 Series Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL with mobile phase A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; flow rate set to 1 mL/min
- G1376C automatic liquid sampler (ALS) SL
- G1316B thermally controlled column (TCC) compartment SL, temperature was 25 °C

- G1316C diode array detector (DAD) wavelength used was set to 220,4 nm Ref = off, with a G1315A-60024 semimicro-flow cell (5 mm path, 6 µL volume)
- Columns used include: Agilent ZORBAX Eclipse Plus C18 4.6 mm × 100 mm, 1.8 µm (p/n 959964-902); ZORBAX Extend C18 4.6 mm × 100 mm, 1.8 µm (p/n 728975-902); ZORBAX StableBond SB-CN 4.6 mm × 100 mm, 1.8 µm (p/n 828975-905)

Chemicals

18 MΩ water was produced onsite; formic acid and acetonitrile were purchased from Sigma Aldrich (Bellfonte, PA). Samples of artemisinin materials were generously supplied by the Clinton Foundation.

Results and Discussion

Automated method development using an isocratic method is carried out following several steps. The analyst chooses a column (a ZORBAX Eclipse Plus C18 4.6 mm × 100 mm, 1.8 µm) and a pair of solvents (water with 0.1% formic acid and acetonitrile with 0.1% formic acid). The choice of this mobile phase pair was made in order to accommodate both UV and MS detection. It is also recommended by many investigators to start method development with a simple acidic mobile phase in order to achieve best analyte peak shape. A first run is made at high organic concentration. In this initial experiment a column is evaluated at an 80% organic mobile phase followed by a wash for 20 column volumes at 100% organic mobile phase. If peaks are detected at the level specified by the software (using a designated wavelength; in this case, the level is set at 5,000 units, a convention of the ChromSword program), ChromSword directs the HPLC to another experiment with a lower organic mobile phase in an attempt to separate and detect additional peaks. ChromSword continues to direct the chromatograph to weaker mobile phase conditions, following each experiment with a 100% organic wash. Experiments proceed until no additional peaks are found, and then following an additional lower concentration to be certain that no additional peaks are present. ChromSword then directs the chromatograph to optimize the separation. In this case a total of 12 experiments are made over 6 unattended hours. The resultant 12 chromatograms are summarized in Figure 2. The final result is shown in Figure 3. As can be seen, the minimum resolution is 2.56. Given that

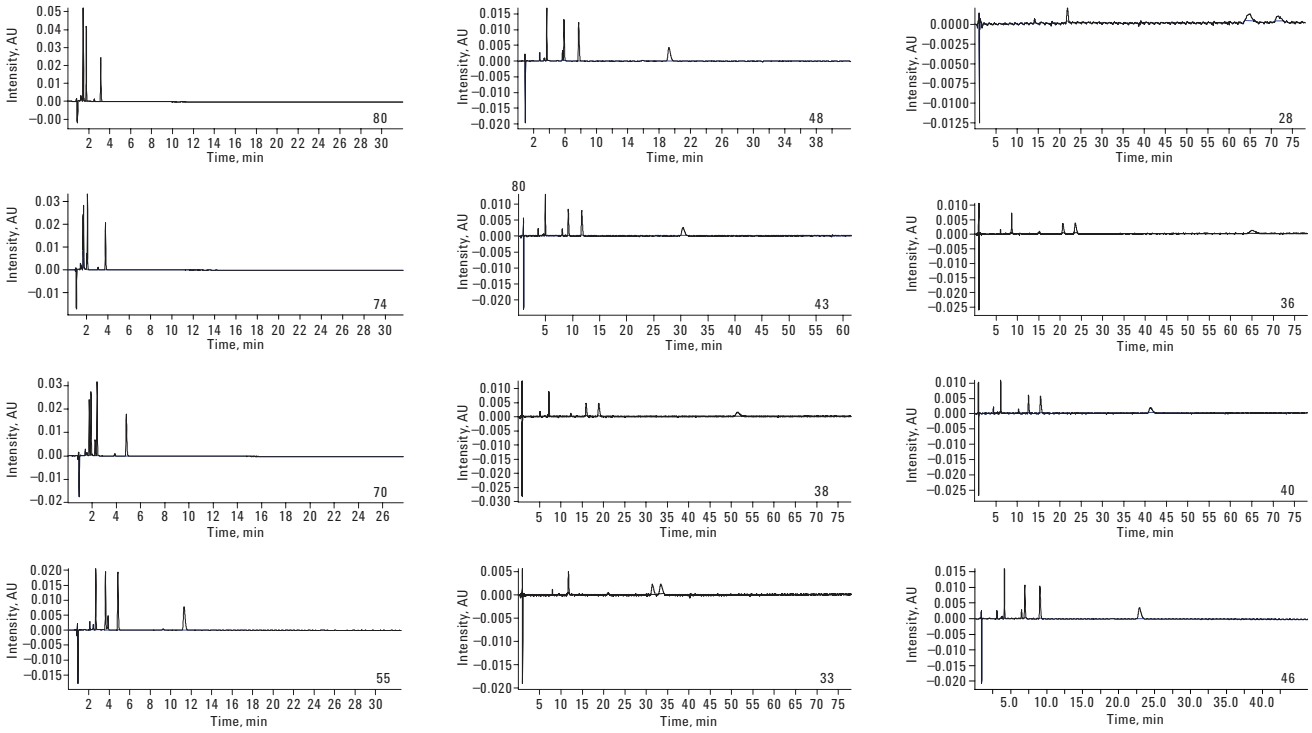

Figure 2. ChromSword Eclipse Plus C18 optimization experiment: 4.6 X 100 mm 1.8 µm (acetonitrile/water with 0.1% formic acid) mixture of artemisinin derivatives.

the experiment only required 6 hours, another column (a ZORBAX Extend C18, 4.6 mm × 100 mm, 1.8 µm column) was evaluated. Both C18 columns achieved similar separations, both leaving the pattern of well-separated early compounds followed by a significantly later eluting compound. The separation achieved on the ZORBAX Eclipse Plus C18 column takes slightly longer and the resolution on the 2,3 peak pair is better. In this case an experienced analyst recognizes an opportunity to use a cyano column and so a ZORBAX StableBond SB-CN column is evaluated using the mobile phase chosen in the final ZORBAX Eclipse Plus C18 experiment. Since the resulting chromatogram achieves all method development goals, no additional optimization experiments were performed. The ZORBAX StableBond SB-CN has been shown to possess good lifetime, even under aggressive acidic conditions where most cyano phases fail [8].

Conclusions

Three RRHT columns were used to develop chromatographic conditions for additional investigations. A mobile phase consisting of acetonitrile, water, and formic acid was optimized for the separation of several artemisinin components. The resultant chromatography using a ZORBAX StableBond SB-CN 4.6 mm × 100 mm, 1.8 micron column is accomplished in less than 5 minutes. A total of 12 unattended hours was used to develop the chromatography shown. The resulting conditions can be used for LC/MS or as a starting point for further method development.

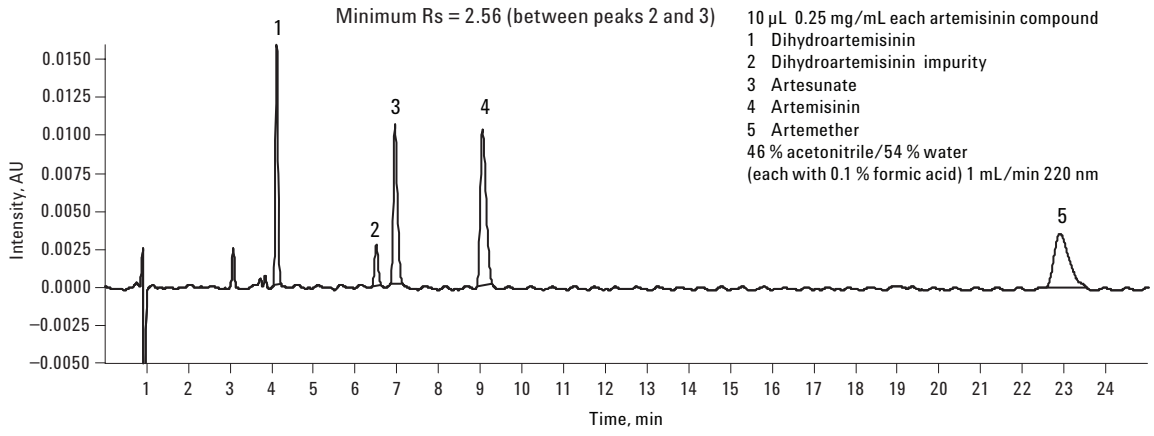

Figure 3 Final artemisinin chromatogram from ChromSword using Agilent ZORBAX Eclipse Plus C18 column.

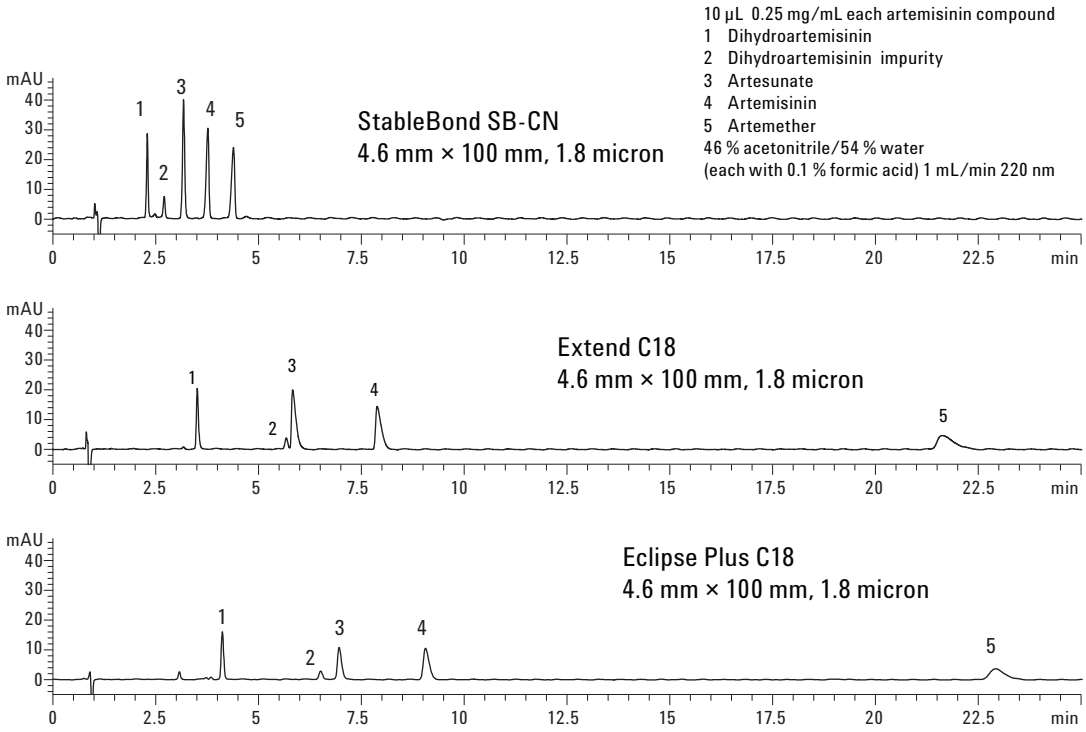

Figure 4. Comparison of three alternate chromatograms (Agilent ZORBAX Eclipse Plus C18, ZORBAX Extend C18, and ZORBAX StableBond SB-CN columns).

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