

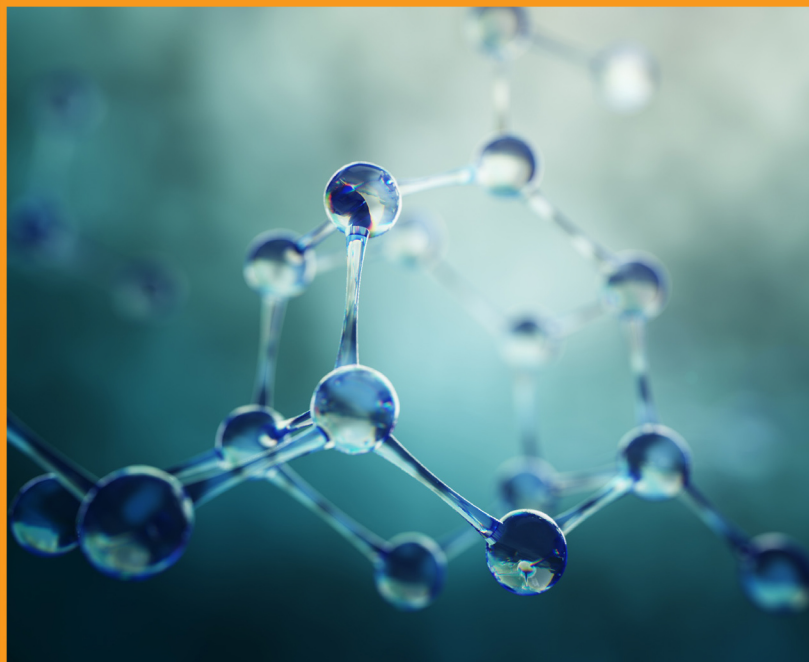
TECHNICAL REPORT: AMT_TR_PHARM_05_21

TITLE: MODERNIZATION OF 5 μ M CHINESE PHARMACOPEIA (CP) HPLC METHODS FOR HIGH THROUGHPUT LABORATORIES

MARKET SEGMENT: PHARMACEUTICAL

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ABSTRACT

A high throughput environment constantly tests chromatographers to improve and optimize separations, while under increasing output demands. This optimization often manifests in faster run times, less solvent consumption, and improved resolution. The development of sub-2-micron particle packed column technology for improved chromatographic performance goes hand in hand with the advent of UHPLC instrumentation designed to withstand higher back pressures and reduced extra-column volume contributions. UHPLC enabled labs to reduce their run times significantly in comparison to <400 bar approaches. Superficially porous particles (SPP) are an alternative column technology that are excellent for high throughput laboratories, with an added advantage of being compatible with both UHPLC and existing HPLC systems.

INTRODUCTION

Using a validated HPLC method is a very popular and great way to get your analysis started without exhausting many resources involved with method development. However, many of these methods incorporate outdated column technology (long column dimensions and large particle sizes). Such methods can be improved significantly with modern column technologies, while satisfying system suitability and the analytical method's validation specifications outlined by regulatory bodies. Better results (if not comparable) can be achieved on a smaller particle size and shorter column length hence, saving mobile phase consumption and time with superior resolution (R_s).

Three different 5 μ m Chinese Pharmacopeia methods were ran including a mixture of ginsenosides, vitamin B12, and cefotaxime. These methods (both gradient and isocratic) were transferred to a smaller particle size in order to show chromatographic improvements such as faster run times and more resolution between peaks of interest.

KEY WORDS:

HPLC, HALO®, Chinese Pharmacopeia

RESULTS:

An official Chinese Pharmacopeia (CP) method for the separation of a mixture of ginsenosides is shown in Figure 1 using a HALO 90 Å C18, 5 µm 4.6x250 mm column. The separation as specified by the CP requires a 100-minute gradient (not inclusive of a column wash of 100% organic, and re-equilibration before the next injection). Hence, the time between one injection to the next may easily take over two hours. The separation in Figure 1 meets the CP requirements with a resolution value of 2.25 ($R_s > 2.0$ between peaks 1 and 2 required). The performance of utilizing the modernized column technology is clearly highlighted by Ginsenoside Rg1 with a plate count of 19,107 - a three times higher improvement compared to the specification value of 6,000.

Transferring this separation to a smaller particle size and shorter column dimension will require method validation. However, once the method becomes validated a significant amount of time is saved and shown in Figure 2. The gradient was completed in 21.6 min compared to 100 min shown in Figure 1, saving time by almost 80 minutes for the method's gradient. It is highly recommended to keep the stationary phase fixed along with the wavelength and temperature.

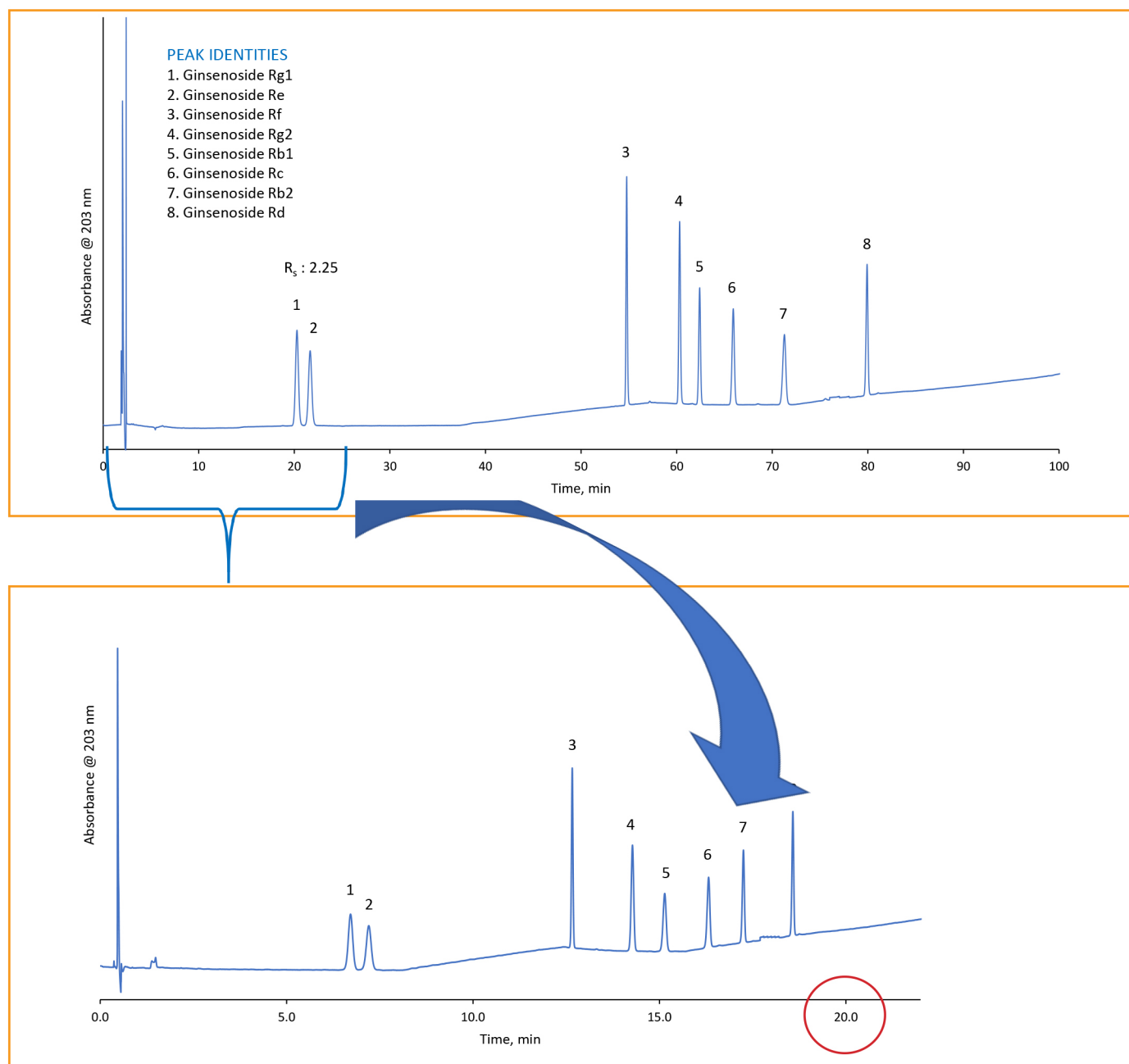
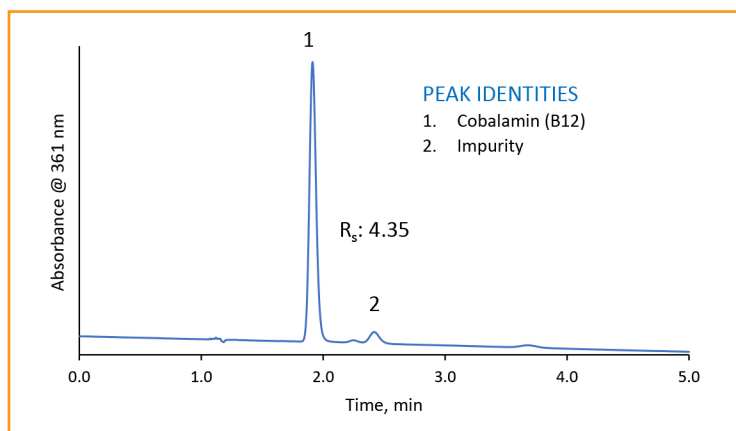


Figure 1: A separation of ginsenosides on a HALO 90 Å C18, 5 µm 4.6x250 mm column following the Chinese Pharmacopeia (CP) method.

Converting the method conditions to a 2.7 μm , 4.6 x 100 mm column resulted in a separation run time that is five times faster compared to the previous method. System suitability specifications are still met with a resolution value greater than 2.0 (2.1) and peak 1 plates (14,328) being greater than 6000. Not only does this save a significant amount of time but mobile phase consumption becomes reduced as well, by about 60 mL per injection.

Looking at another CP method involving the separation of vitamin B12 and an impurity peak, we have an isocratic separation using a mixture of methanol and sodium phosphate buffer. The separation is performed on a HALO 90 Å C18, 5 μm 4.6 x 150 mm column and can be seen in Figure 3. The column shows excellent resolution (4.35) between the two peaks meeting all of the system suitability requirements including a resolution value above 2.5. System suitability tests are very important to make sure that the HPLC system is working properly and should be tested before every sample analysis.

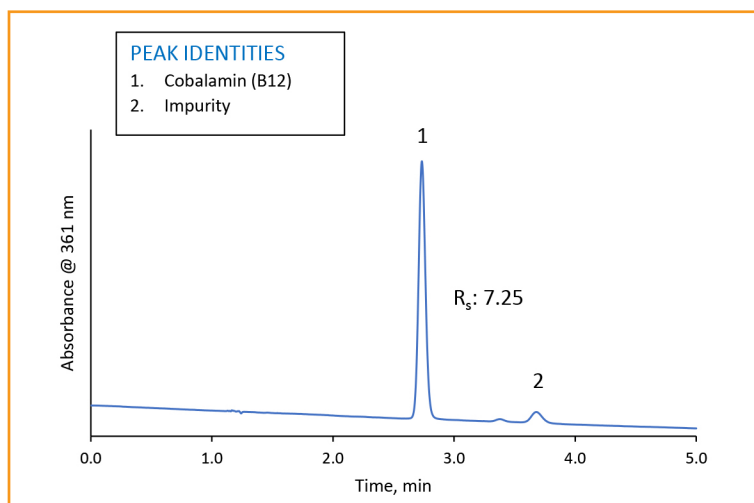


TEST CONDITIONS

Column: HALO 90 Å C18, 5 μm , 4.6 x 150mm
 Part Number: 95814-702
 Isocratic: 26/74 MeOH/ 28 mM Na₂HPO₄ pH: 3.5
 Flow Rate: 1.0 mL
 Back Pressure: 209 bar
 Temperature: 30 °C
 Detection: UV 361 nm, PDA
 Injection Volume: 10 μL System Suitability Solution
 Sample Solvent: mobile phase
 Data Rate: 100 Hz
 Response Time: 0.025 sec
 Flow Cell: 1 μL
 LC System: Shimadzu Nexera X2

Figure 3: A separation of Vitamin B12 and an impurity is separated on a HALO 90 Å C18, 5 μm 4.6 x 150 mm column following the Chinese Pharmacopeia (CP) method.

The same separation is performed on a smaller particle size while keeping the column dimension the same. One thing to keep in mind when doing this is that your back pressure will be higher because of the smaller particle size. (209 bar to 425 bar) It is a good idea to know your instrument back pressure limitations before reducing your particle size. Since the smaller particle size separation can still be ran on a standard HPLC no instrument changes have been made. Under the same conditions, the 2.7 μm column shows a significant improvement in resolution between peaks of interest with a slightly longer run time. This can be an advantage especially if there are other impurities interfering with the main peak. The separation can be seen in Figure 4.

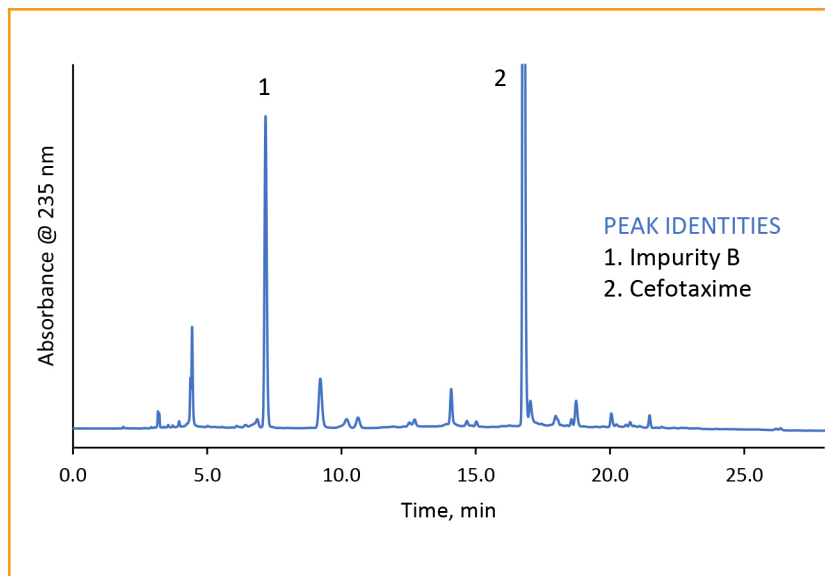


TEST CONDITIONS

Column: HALO 90 Å C18, 2.7 μm , 4.6 x 150mm
 Part Number: 92814-702
 Isocratic: 26/74 MeOH/ 28 mM Na₂HPO₄ pH: 3.5
 Flow Rate: 1.0 mL
 Pressure: 425 bar
 Temperature: 30 °C
 Detection: UV 361 nm, PDA
 Injection Volume: 10 μL System Suitability Solution
 Sample Solvent: mobile phase
 Data Rate: 100 Hz
 Response Time: 0.025 sec
 Flow Cell: 1 μL
 LC System: Shimadzu Nexera X2

Figure 4: A separation of Vitamin B12 and an impurity is separated on a HALO 90 Å C18, 2.7 μm 4.6 x 150 mm column using a modified Chinese Pharmacopeia (CP) method.

The final CP method is a separation of cefotaxime, a drug used to treat several types of bacterial infections. The method uses a gradient on a HALO 90 Å C18, 5 µm, 4.6 x 250 mm column, an excellent dimension choice since the separation has many impurities that need to be monitored. This separation can be seen in Figure 5.



TEST CONDITIONS

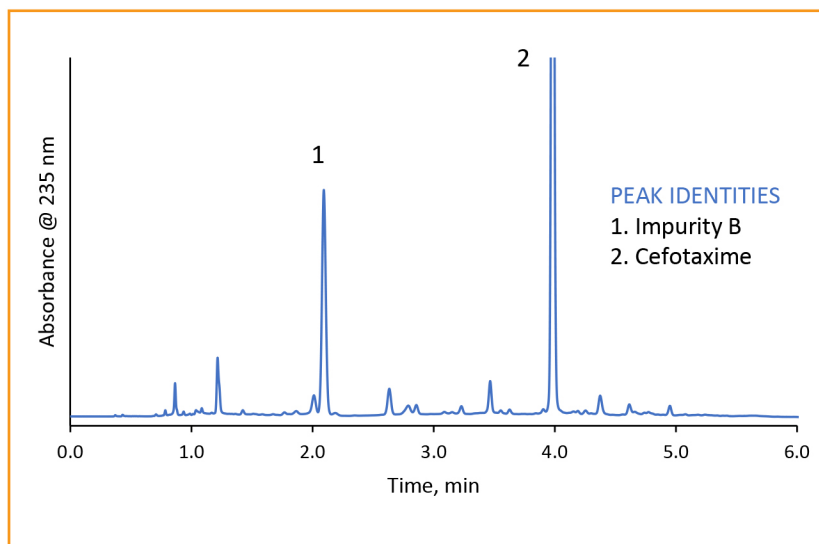
Column: HALO 90 Å C18, 5 µm, 4.6 x 250 mm
 Part Number: 95814-902
 Mobile Phase A: 86/14: 0.05 M Phosphate Buffer pH 6.25/ MeOH
 (7.1g anhydrous disodium hydrogen) phosphate to 1000mL)
 Mobile Phase B: 60/40: 0.05 M Phosphate Buffer pH 6.25/ MeOH
 (7.1g anhydrous disodium hydrogen) phosphate to 1000mL)
 Gradient:

Time	%B
0.0	5
2.0	25
8.0	25
23.0	100
28.0	100
33.0	5
43.0	5

Flow Rate: 1.0 mL/min
 Back Pressure: 189 bar
 Temperature: 30 °C
 Detection: UV: 235 nm
 Injection Volume: 10 µL
 Sample Solvent: mobile phase A
 Data Rate: 100 Hz
 Response Time: 0.025 sec.
 Flow Cell: 1 µL
 LC System: Shimadzu Nexera X2

Figure 5: A separation of cefotaxime and its impurities is separated on a HALO 90 Å C18, 5 µm 4.6x250 mm column using the Chinese Pharmacopeia (CP) method.

Again, after reducing the particle size and column dimension to a 2.7 µm, 4.6 x 100 mm column, a similar chromatogram is observed showing improvements of 4.5 times faster run times with ~27 mL of reduced solvent usage per injection. Impurity peaks are still resolved and can be monitored while satisfying all separation specifications (seen in Figure 6).



TEST CONDITIONS

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
 Part Number: 92814-602
 Mobile Phase A: 86/14: 0.05 M Phosphate Buffer pH 6.25/ MeOH
 (7.1g anhydrous disodium hydrogen) phosphate to 1000mL)
 Mobile Phase B: 60/40: 0.05 M Phosphate Buffer pH 6.25/ MeOH
 (7.1g anhydrous disodium hydrogen) phosphate to 1000mL)
 Gradient:

Time	%B
0.0	5
0.5	25
1.7	25
5.0	100
6.0	100
7.0	5
9.0	5

Flow Rate: 1.8 mL/min
 Back Pressure: 377 bar
 Temperature: 30 °C
 Detection: UV: 235 nm
 Injection Volume: 10 µL
 Sample Solvent: mobile phase A
 Data Rate: 100 Hz
 Response Time: 0.025 sec.
 Flow Cell: 1 µL
 LC System: Shimadzu Nexera X2

Figure 6: Cefotaxime and its impurities are separated on a HALO 90 Å C18, 2.7 µm 4.6x100 mm column using a modified Chinese Pharmacopeia (CP) method.

CONCLUSION:

HPLC chromatographic methods can be greatly improved and modernized by simply reducing the column's length and particle size. This not only saves time, but increases column throughput along with less mobile phase consumption and waste generation. By using modern HALO® Fused-Core® column technology, methods can be shortened thereby increasing laboratory efficiency while keeping back pressures under instrument pressure limitations. This allows the user to use the same instrument while performing method development. While method validation is a laborious process, the time spent initially may be worth the effort to realize the benefits associated with incorporating modernized column technologies such as increased sample throughput and productivity.

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