UNDER THE HALO®

SMALL MOLECULE

A Modern Take on USP Gradient Methods by Employing Fused-Core® Columns

What does it mean for laboratories now that the USP's chapter <621> guidelines allow changes to modernize gradient methods? The answer is time and solvent savings without having to revalidate. Many people may be reluctant to make changes to a method that has been working without any issues. However, it could mean significant cost savings to be able to reduce run time and increase throughput by changing the original method column to one of smaller particle size and/or in smaller dimensions. Adopting an SPP column, such as HALO® the benefits are even greater. This report will examine two case studies showing time and solvent savings when modernizing the USP methods for rivaroxaban and itraconazole.

The allowable changes according to USP <621> include the ability to change the column particle type, size, and column dimension while keeping within the same USP category, such as L1 for C18. The column dimension may be changed according to -25 to +50% of the column length/particle size ratio (L/dp). Once the new column is selected, the flow rate is scaled according to the particle size and type. Then the gradient program is modified and finally the injection volume is scaled to the new column dimension.

Rivaroxaban is the active ingredient in Xarelto®, which is used to treat and prevent blood clots. The original USP monograph method specifies a fully porous particle (FPP) C18, 3.5 µm, 3.0 x 150 mm column. Calculating the L/dp ratio gives 42,857 so -25 to +50% of the L/dp gives a range of 32,143 to 64,286. Since the goal is to reduce run time and solvent consumption, a HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm column is selected. This has a L/dp ratio of 37,037 so it meets the criteria. The other criteria that must be met is that the new column is in the same L category as the monograph column. Both are C18 columns in the L1 category. An easy way to check this is to look up the L1 columns in the Chromatographic Database available from the USP (https://www.usp.org/resources/chromatographic-columns).

Figure 1 shows the comparison between the two methods for the organic impurities analysis with the original conditions shown in black and the results on the HALO® column shown in blue. The concentration of the analytes has been increased in order to easily identify them. The results using the HALO® column are 1.7 times faster and consumes 3.3 times less mobile phase. Both columns pass the system suitability criteria of a resolution of no less than (NLT) 8.0 between rivaroxaban related compound G and rivaroxaban (peaks 3 and 4) as well as no more than (NMT) 5.0% relative standard deviation (RSD). For additional examples of how the rivaroxaban monograph may be optimized see the Technical Report - Modernizing the USP Method for Rivaroxaban for Time and Solvent Savings with HALO® Columns .

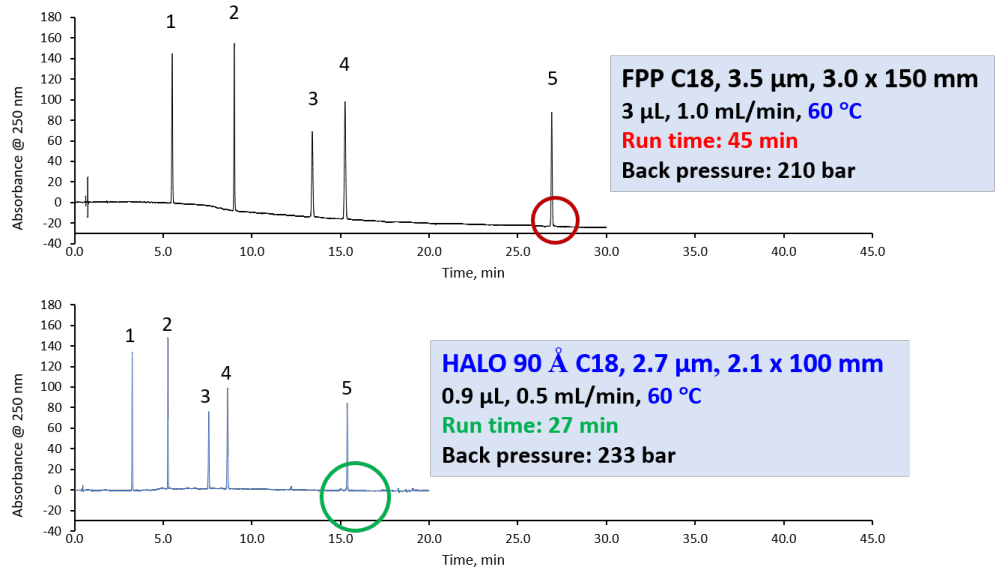

Figure 1. Comparison of the original monograph method conditions to the modernized conditions using a HALO® column for time and solvent savings.

PEAK IDENTITIES:

1. Rivaroxaban related compound B
2. Rivaroxaban related compound D
3. Rivaroxaban related compound G
4. Rivaroxaban
5. Rivaroxaban related compound J

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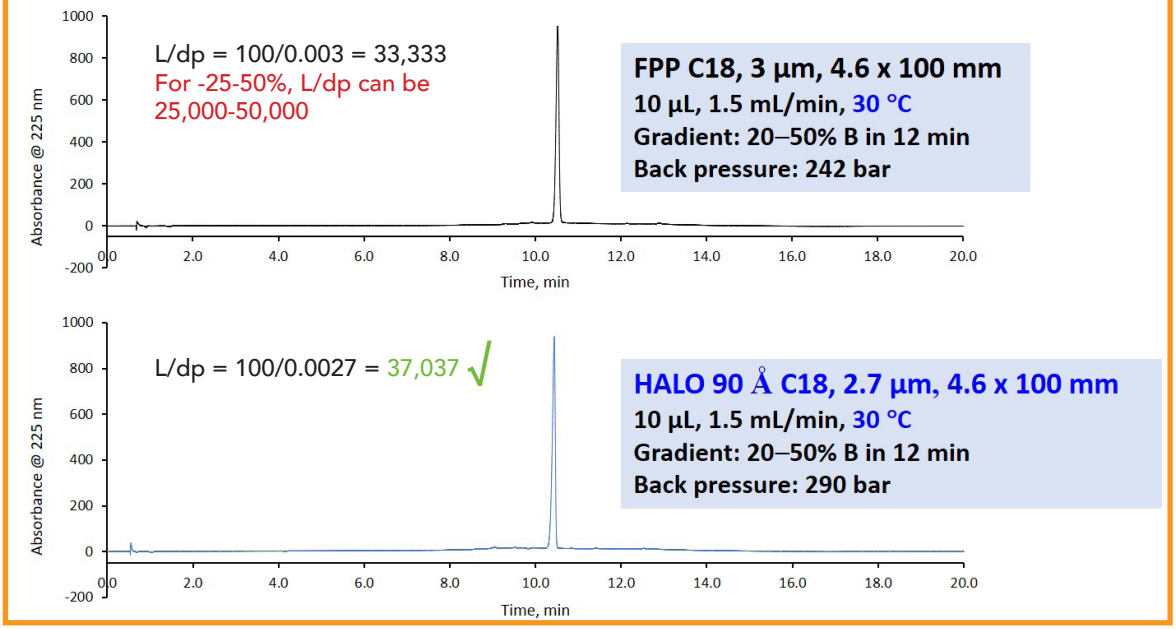
Itraconazole is used to treat fungal infections of the mouth, throat, lungs, or nails. It is sold under the brand names Onmel™ and Sporanox®. The USP monograph method uses a FPP C18, 3 µm, 4.6 x 100 mm column. Calculating the (L/dp) ratio gives 33,333 so -25 to +50% of the L/dp gives a range of 25,000 to 50,000. Two different HALO® columns were chosen to illustrate different scenarios in which the column might be changed. In the case where the legacy monograph column is unavailable and only the particle size is changed, a HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm was chosen. This column has an L/dp of 37,037. For the case where the goal is to maximize the time and solvent savings, a HALO 90 Å C18, 2 µm, 2.1 x 50 mm was selected, which has an L/dp of 25,000. Similar to the method for rivaroxaban, both columns are C18 which meets the USP <621> guidelines.

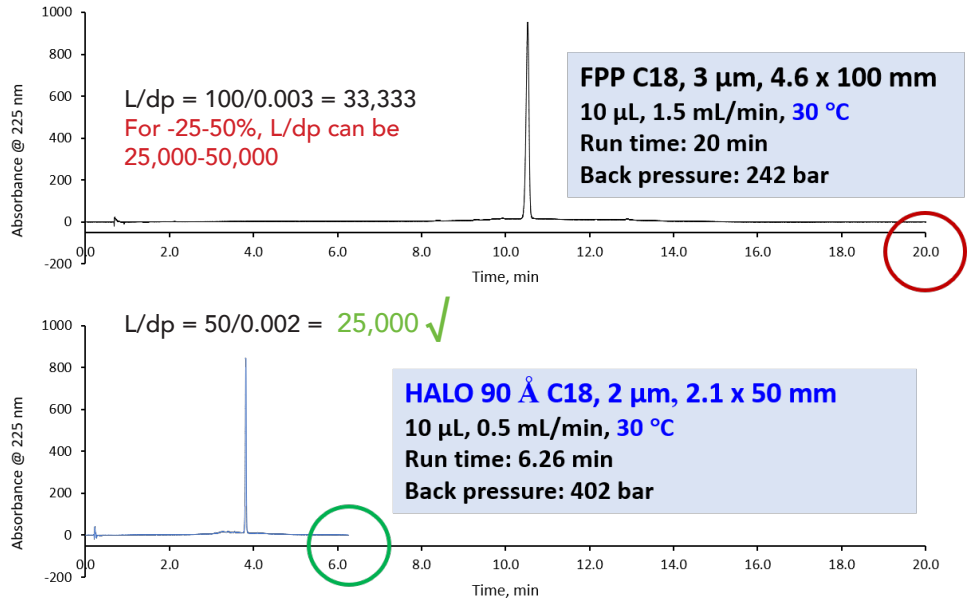
The first example in Figure 2 shows the results where only the column particle size and type are changed and all of the other method conditions are kept constant. A 2.7 µm SPP column is run instead of a 3 µm FPP column. The results pass the criteria of tailing factor NMT 2.0 and RSD NMT 2.0%. The back pressure is only ~ 20% higher with the HALO® column due to the smaller particle size. This switch would be an excellent substitute if the legacy column became unavailable.

Figure 2. Assay method for itraconazole comparing the original monograph column to a HALO® column in the same column dimension, but slightly smaller particle size showing equivalent results.

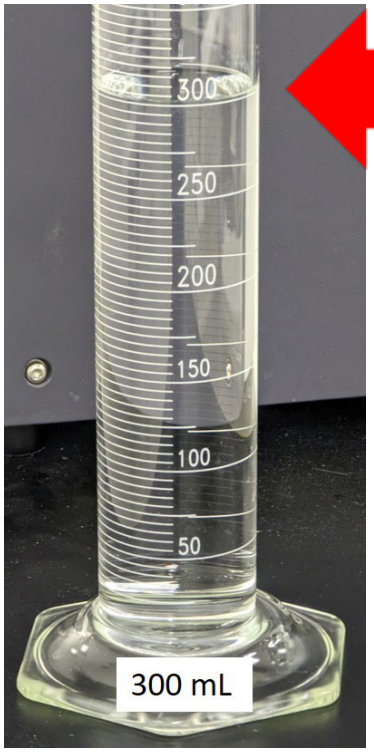


Next, examine the improvements when the assay is run using the 2 µm HALO® column in shorter length and smaller ID. Again, the system suitability criteria are met with the added advantages of more than a 3 time decrease in run time and significant solvent savings. See Figure 3 for the chromatograms and Figure 4 for the impact of mobile phase savings over the course of 10 injections. Since a smaller particle size column is run, the back pressure is slightly more than 400 bar, which does not require a UHPLC unlike sub-2-µm FPP columns, but instrument type should be considered when making column changes.

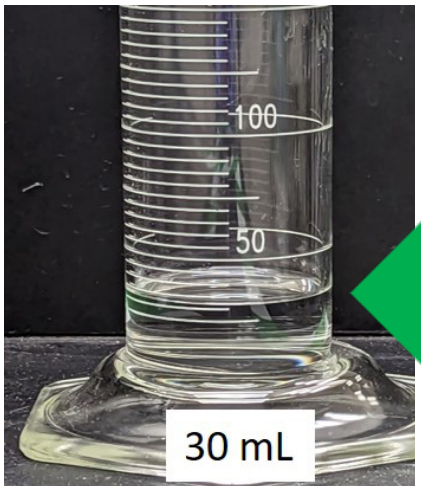
Figure 3. Assay method for itraconazole comparing the original monograph column to a HALO® column with smaller particle size, shorter length, and reduced column ID showing >3 times faster results.



For 10 Injections:



compared to

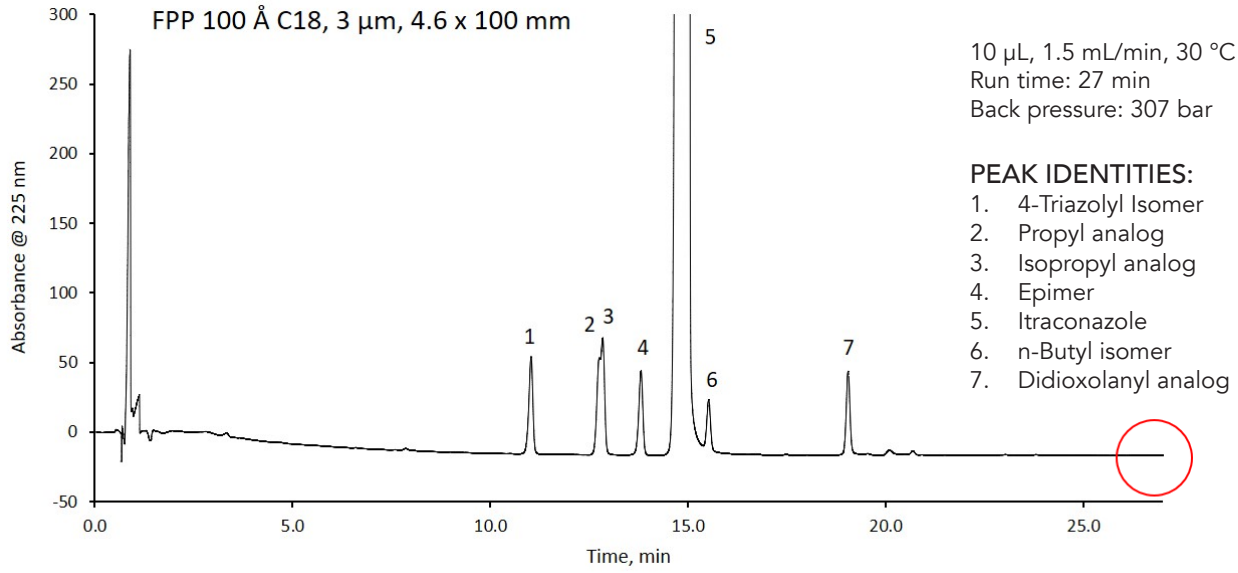


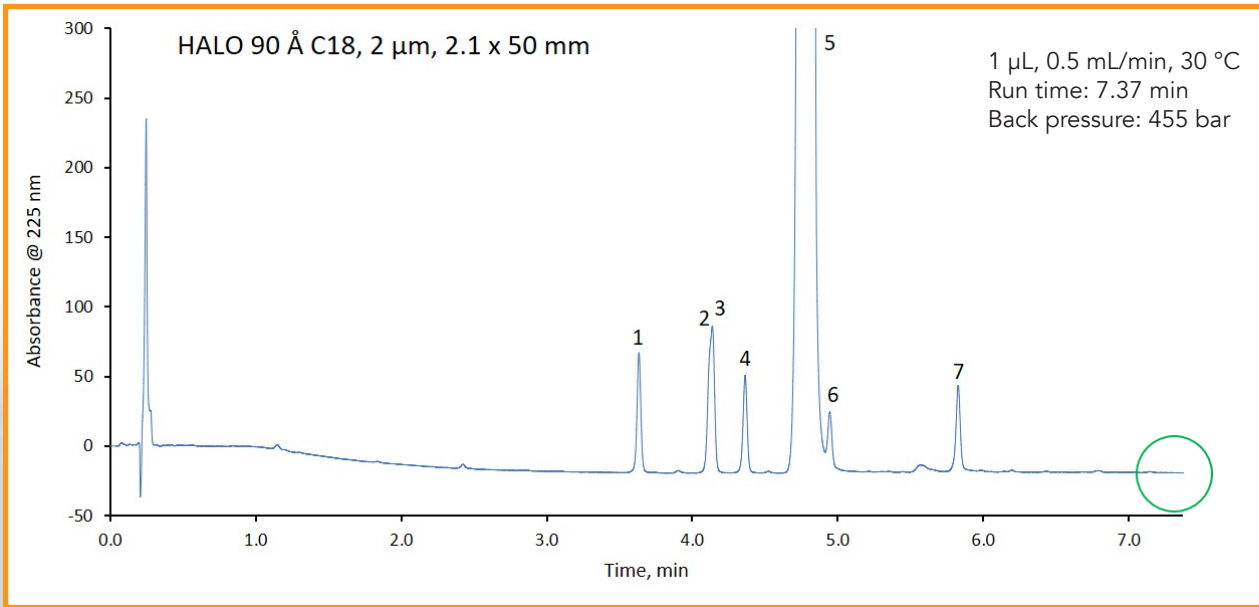
10X reduction in mobile phase used

Figure 4. 10 times reduction in solvent used for 10 injections using the modified method on the 2 µm HALO® column.

For the itraconazole system suitability mixture, the HALO 90 Å C18, 2 µm, 2.1 x 50 mm was selected. See Figure 5 for the original monograph column results compared to the modernized method results. In this example, the suitability requirements for the peak-to-valley ratio must be NLT 1.5 and the HALO® column passes by more than 2 this minimum. Additionally, the total run time is reduced by more than 3 times and solvent consumption is reduced by 11 times.

Figure 5. Comparison of the itraconazole system suitability method run on the monograph column and on a HALO® column at a speed 3 times faster while saving solvent by a factor of 11.





CONCLUSIONS

With the recent updates to the USP <621> guidelines for gradient methods, the possibilities for method modernization are quite exciting. The ability to change the column particle type, size, ID, and length to smaller dimensions without revalidation represents significant time and solvent savings as well as the ability to move methods written on legacy column technology to contemporary columns before facing a supply issue. These savings can combine for cost savings and are a step toward more sustainable methods. The use and adoption of UHPLC systems in laboratories also provides the ability to make further improvements by using even smaller particle sizes or column ID's, such as the new HALO® 1.5 mm ID that reduces the solvent consumption by 50% in comparison to a 2.1 mm ID column and 89% in comparison to a 4.6 mm ID column. More information can be found on this technology at Introducing the New Halo 1.5 | HALO® Columns for Chromatography Separations (halocolumns.com).