HALO®

TECHNICAL REPORT

TITLE: MODERNIZING THE USP METHOD FOR RIVAROXABAN FOR TIME AND SOLVENT SAVINGS WITH HALO® COLUMNS

MARKET SEGMENT: PHARMACEUTICAL

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ABSTRACT

As of December 1, 2022, the United Stated Pharmacopeia (USP) has updated their guidance to allow changes to gradient methods. This means both time and solvent savings can be attained by switching to Fused-Core® HALO® columns without the need for revalidation. Using the monograph method for rivaroxaban, multiple examples are presented to show implementation of method modernization to save time and solvent.

INTRODUCTION

Many USP methods for impurity analysis use 4.6 mm ID, 150 mm column length or greater, and 3 or 5 micron particle size columns. Now that the guidance in USP <621> has been updated to allow changes to the column particle, size and dimension for gradient methods, analysts have the ability to modernize their methods in order to save time and solvent and ultimately reduce the cost of the experiments. Additionally, for monograph methods using legacy columns, the method can be future proofed before legacy column supply could potentially become an issue. The steps to change columns/methods are listed in Table 1.

Provided the criteria are met and there are no changes to the elution order of the compounds, there is no need for method revalidation. Rivaroxaban is the active ingredient in Xarelto®, which is used for the treatment and prevention of blood clots. For the monograph method of rivaroxaban, the L/dp = 150/0.0035 = 42,857 so -25 to 50% of L/dp is 32,143-64,286. Table 2 lists various combinations of HALO® column lengths and particle sizes.

KEY WORDS:

USP <621>, gradient methods, Fused-Core®, modernization, solvent savings, 1.5 mm, rivaroxaban

Step Number	Action for Confirming Allowable Changes
1	Calculate Length/particle diameter (L/dp) for the column specified in the USP monograph
2	Calculate -25 to 50% of the L/dp
3	Select a smaller particle size, smaller ID, and reduced length column dimension that is in the same L category as the monograph column and is compatible with the pressure capability of the instrument that will be used
4	Adjust the flow rate according to this equation $F_2 = F_1 \times [(dc_2^2 \times dp_1) \div (dc_1^2 \times dp_2)]$
5	Adjust the gradient time according to this equation $t_{G2} = t_{G1} \times (F_1/F_2)[(L_2 \times d_{C2}^2)/(L_1 \times d_{C1}^2)]$
6	Adjust the method according to dwell volume if dwell volume is stated in monograph according to this equation $t_c = t - \frac{(D - D_0)}{F}$
7	*Adjust the injection volume according to this equation* $V_{inj2} = V_{inj1} \times (L_2 d_{C2}^2)/(L_1 d_{C1}^2)$

Table 1. Steps to change USP gradient methods.

Column Length (L) (mm)	Particle Size (dp) (mm)	L/dp
100	0.0027	37037
75	0.0027	27778*
100	0.002	50000
75	0.002	37500
50	0.002	25000*

Table 2. HALO® column lengths and particle sizes and their corresponding L/dp ratios.

The two asterisked values are below the lower limit for L/dp. However, when changing from fully porous particles (FPPs) to superficially porous particles (SPPs), other combinations of L and dp can be used provided that the ratio $(t_R/W_h)^2$ is within −25% to +50%, relative to the prescribed column for all the peaks used to determine the system suitability parameters. One point to keep in mind is that smaller volume columns are more susceptible to extracolumn volume. So the HPLC system should be optimized before switching to smaller particle size, shorter length, and smaller ID columns or a low dispersion UHPLC system should be used. However, switching from a 3 µm FPP column to a 2.7 µm HALO® column would not require major optimization. The changes required are small compared to the advantages that are possible by modernizing the method. The monograph method of rivaroxaban was translated to 3 different HALO® columns: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm, HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm, and HALO 90 Å C18, 2 µm, 2.1 x 50 mm.

EXPERIMENTAL:

The examples in this report will demonstrate time and solvent savings for the rivaroxaban system suitability mixture. A low dispersion 1 µL flow cell was used in place of a standard 1 µL flow cell for the results using the 1.5 mm ID column. All solvents used were HPLC grade. Acetonitrile and mobile phase additives were obtained from MilliporeSigma (St. Louis, MO). Rivaroxaban standards were obtained from LGC Standards (Manchester, NH).

TEST CONDITIONS:

Column: FPP C18, 3.5 µm, 3.0 x 150 mm
Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Column: HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm
Part Number: 9281X-602
Column: HALO 90 Å C18, 2 µm, 2.1 x 50 mm
Part Number: 91812-402
Mobile Phase A: 5/95 Methanol/Solution A
Mobile Phase B: Acetonitrile
Solution A: Dissolve 1.36 g of potassium dihydrogenphosphate, 1 g sodium hexane sulfonate, and 200 µL of phosphoric acid in water. Dilute with water to 1 L.
Solution B: Dissolve 1.36 g of potassium dihydrogenphosphate and 200 µL of phosphoric acid in water. Dilute with water to 1 L.
Flow Rate: 1.0 mL/min (3.0 mm)
0.5 mL/min (2.1 x 100 mm and 2.1 x 50 mm)
0.25 mL/min (1.5 x 100 mm)
Pressure: 210 bar (3.0 mm)
233 bar (2.1 x 100 mm)
197 bar (1.5 x 100 mm)
249 bar (2.1 x 50 mm)
Temperature: 60 °C
Detection: UV 250 nm, PDA
Injection Volume: 3 µL (3.0 mm)
0.9 µL (2.1 mm)
0.5 µL (1.5 mm and 2 µm, 2.1 x 50 mm)
Sample Solvent: 40/60 Acetonitrile/ Solution B
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
Instrument: Shimadzu Nexera X2

Gradients:

FPP C18, 3.5 µm, 3.0 x 150 mm

Time:	%B
0.00	2
2.00	2
8.00	16
25.00	36
37.00	80
38.00	2
45.00	2

HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm *and*
HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm

Time:	%B
0.00	2
1.14	2
4.56	16
14.26	36
21.10	80
22.00	2
27.00	2

HALO 90 Å C18, 2 µm, 2.1 x 50 mm

Time:	%B
0.00	2
0.57	2
2.28	16
7.13	36
10.55	80
12.00	2
15.00	2

PEAK IDENTITIES:

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

RESULTS:

The first translated example uses a HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm compared to the column specified by the USP monograph for rivaroxaban. See Figure 1.

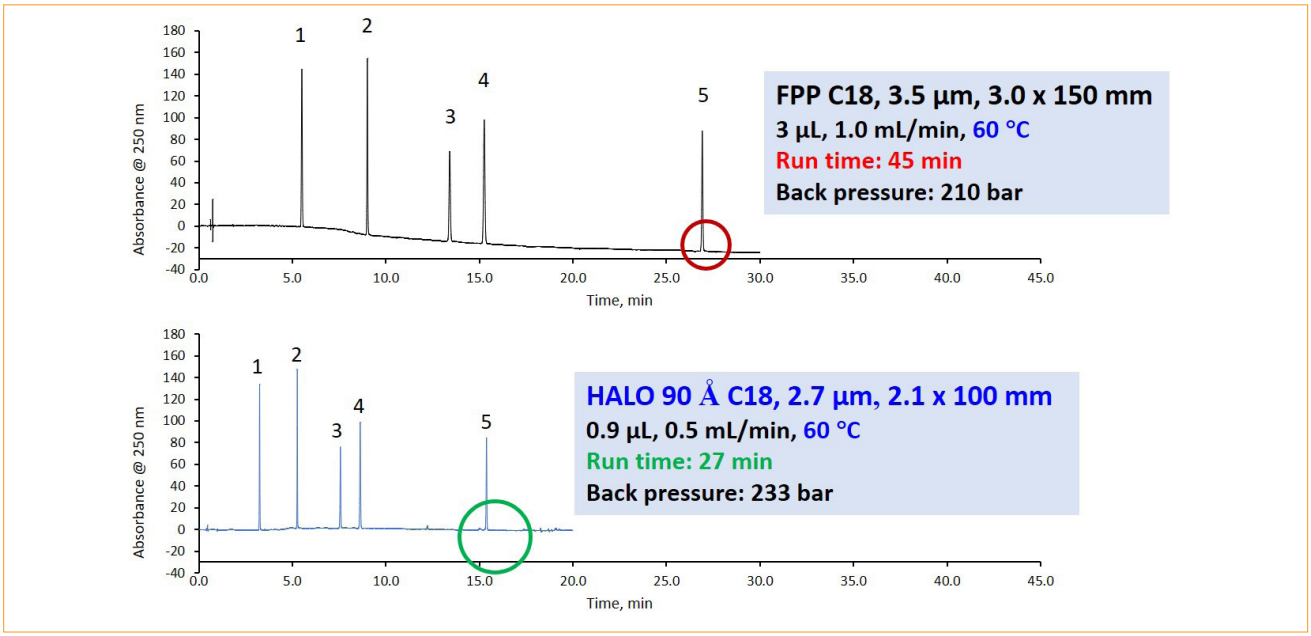

Figure 1. Comparison of monograph method for rivaroxaban to a smaller particle size and smaller dimension HALO® column running nearly twice as fast and using 3.3 times less mobile phase.

The method translated to the HALO® column saves 18 minutes/run and uses 3.3 times less mobile phase. The injection volume was reduced from 3 µL to 0.9 µL, which is factor of 3.3 less sample used. The system suitability criteria are met since peaks 3 and 4 (rivaroxaban related compound G and rivaroxaban) are resolved by NLT 8.0.

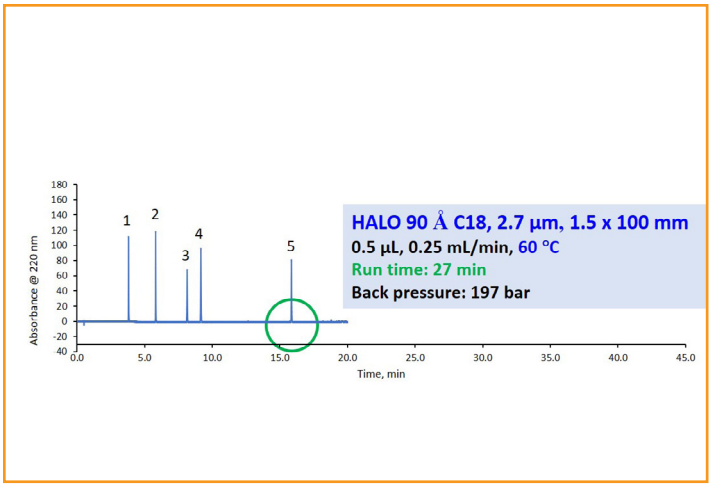

Figure 2. HALO® 1.5 x 100 mm column showing double the solvent savings of a HALO® 2.1 x 100 mm column.

The next example also uses a 100 mm length HALO® column, but this time in 1.5 mm ID. See Figure 2.

The time savings are the same as the 2.1 x 100 mm HALO® column, but now the solvent savings are doubled to 6.7 times less solvent consumed. This is because the optimum linear velocity of a 1.5 mm ID HALO® column is half that of a 2.1 x 100 mm HALO® column. For labs looking to reduce solvent consumption, the 1.5 mm ID columns could be a smart option.

The final example is using a 2 µm, 2.1 x 50 mm HALO® column. This column does not meet the L/dp ratio criteria, but it does meet the criteria for $(t_R/W_h)^2$ to be within−25% to +50% of the monograph method. By moving the method to this column, the method is 3 times faster and saves 6 times the solvent of the monograph method. The back pressure has only increased ~19% over the monograph column to 249 bar, which is still within the operating parameters of an HPLC system (< 400 bar).

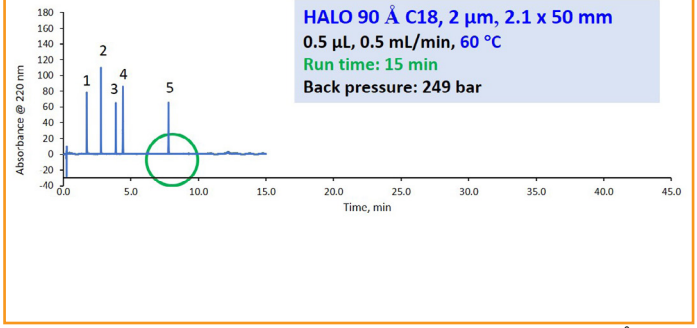

Figure 3. For the ultimate time and solvent savings, a HALO 90 Å C18, 2 µm, 2.1 x 50 mm column is 3 times faster and saves 6 times the solvent of the monograph method.

CONCLUSION:

For laboratories who want to increase their throughput and use less mobile phase, modernizing their legacy USP gradient methods is now possible with the updated USP <621> guidance. By switching to smaller particle size, smaller dimension HALO® columns, significant time and solvent may be saved all without the need for revalidation. The 1.5 mm ID columns are an excellent option for those that have adopted UHPLC systems and seek the best performance in their instrumentation and column technology.

Overall, the time and solvent savings combine for cost savings, which are advantages for all labs in today's competitive market.