TN-1359

Separation of Rivaroxaban and its Organic Impurities per USP Monograph

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Introduction

Rivaroxaban is a factor Xa inhibitor, an anticoagulant, that decreases the clotting ability of the blood and helps prevent clots from forming in the blood vessels. In this technical note, we report the separation of Rivaroxaban from its organic impurities per the USP monograph using a Luna™ Omega 3 µm C18 column as an alternative L1 column to a ZORBAX® Eclipse® 3.5 µm XDB-C18 column originally used to elucidate the monograph. The allowable adjustments pertaining to gradient separations are highlighted together with the necessary calculations that are required for the adjusted method conditions to remain compliant with the original monograph.

System suitability per USP Monograph for the Rivaroxaban Assay requires a tailing factor no more than (NMT) 2.0 and a percent relative standard deviation (%RSD) of NMT 0.73 % for six replicate injections. System suitability per USP Monograph for the Rivaroxaban Related Organic Impurities requires resolution no less than (NLT) 8.0 between Rivaroxaban related Compound G and Rivaroxaban, %RSD NMT 5.0 %, and a Signal-to-Noise (S/N) ratio NLT 10

According to USP General Chapter <621> , the configuration of the equipment employed may significantly alter the resolution, retention time, and relative retentions described. Differences in system dwell volume can have an impact on the results obtained for gradient methods. Monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for analytical procedure development and that actually used for implementation. The systems used in this study had different dwell volumes (1.5 mL for the Agilent® 1260, and 0.3944 mL for the Waters® ACQUITY® H-Class) than described in the monograph (0.92 mL)*, so the time points stated in the gradient table must be replaced by the adapted time points calculated using the following equation:

$$t_c = t - \frac{(D - D_0)}{F}$$

t_c = adapted time point (min)
t = time point indicated in the monograph (min)
D = Dwell volume (mL)
D_0 = Dwell volume used for development of the method (mL)
F = flow rate (mL/min)

The adapted time points for the ZORBAX Eclipse 3.4 µm XDB-C18 column would be:

Agilent 1260

$$t_c = 2 - \frac{(1.5 - 0.92)}{1}$$

$$t_c = 1.42$$

Waters ACQUITY H-Class

$$t_c = 2 - \frac{(0.3944 - 0.92)}{1}$$

$$t_c = 2.53$$

Each time point in the gradient would be adjusted by -0.58 minutes for the Agilent 1260 system and +0.53 minutes for the Waters H-Class system and is shown in the gradient table of the LC Conditions on the next page.

Adjustments to column dimensions for gradient methods will be allowed provided that the L/dp ratio remains constant or within the range between -25 % to +50 % of the prescribed L/dp ratio indicated in the monograph. In this monograph, the indicated column length was 150 mm, and the particle size was 3.5 µm; therefore, the Luna Omega 3 µm column used here would be an allowed adjustment. When the particle size is changed, the flow rate requires adjustment because smaller-particle columns will require higher linear velocities to deliver the same performance. The flow rate is adjusted for particle size using the following equation:

$$F_2 = F_1 \, x \, \frac{dc_2^2 \, x \, dp_1}{dc_1^2 \, x \, dp_2}$$

F_1 = flow rate indicated in the monograph (mL/min)
F_2 = adjusted flow rate (mL/min)
dc_1 = internal diameter of the column indicated in the monograph (mm)
dc_2 = internal diameter of the column used (mm)
dp_1 = particle size indicated in the monograph (µm)
dp_2 = particle size of the column used (µm)

The adjusted flow rate for the Luna Omega 3 µm column would be:

$$F_2 = 1 \, x \, \frac{3^2 \, x \, 3.5}{3^2 \, x \, 3.0}$$

$$F_2 = 1.17$$

Adjustments to column dimensions, particle size, or mobile phase volumetric flow rate for gradient methods will impact the slope of the gradient, which can impact selectivity. It is therefore important to adjust the gradient times using the following equation:

$$t_{G2} = t_{G1} \, x \, \left(\frac{F_1}{F_2}\right) \, x \, \left(\frac{L_2 \, x \, dc_2^2}{L_1 \, x \, dc_1^2}\right)$$

t_{G1} = gradient time indicated in the monograph, or adjusted for dwell volume (min)
t_{G2} = adjusted gradient time (min)
F_1 = flow rate indicated in the monograph (mL/min)
F_2 = adjusted flow rate (mL/min)
L_1 = column length indicated in the monograph (mm)
L_2 = new column length (mm)
dc_1 = internal diameter of the column indicated in the monograph (mm)
dc_2 = internal diameter of the column used (mm)

For the second gradient segment, the adjusted gradient time would be:

Agilent 1260

$$t_{G2} = 1.42 \, x \, \left(\frac{1}{1.17}\right) \, x \, \left(\frac{150 \, x \, 3^2}{150 \, x \, 3^2}\right)$$

$$t_{G2} = 1.22$$

Waters ACQUITY H-Class

$$t_{G2} = 2.53 \, x \, \left(\frac{1}{1.17}\right) \, x \, \left(\frac{150 \, x \, 3^2}{150 \, x \, 3^2}\right)$$

$$t_{G2} = 2.16$$

A gradient adjustment factor can be calculated and used to determine the new gradient segment times using:

$$Gradient\ adjustment\ factor = \left(\frac{t_{G2}}{t_{G1}}\right)$$

Agilent 1260

$$Gradient\ adjustment\ factor = 0.86$$

Waters ACQUITY H-Class

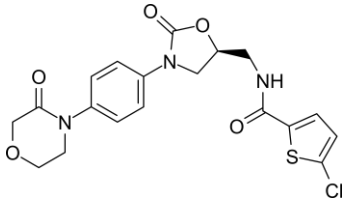
$$Gradient\ adjustment\ factor = 0.85$$

The new gradient timetable for the Luna Omega 3 µm C18 column is shown in the gradient table of the LC Conditions on the next page.

All requirements for System Suitability for Rivaroxaban Assay and Organic Impurities were met by all columns.

All solutions were prepared as indicated in the USP Monograph for Rivaroxaban. USP Rivaroxaban RS (Catalog No. 1604530), USP Rivaroxaban Related Compound B RS (Catalog No. 1604552), USP Rivaroxaban Related Compound D RS (Catalog No. 1604563), USP Rivaroxaban Related Compound G RS (Catalog No. 1604596), and USP Rivaroxaban Related Compound J RS (Catalog No. 1604574) were purchased from USP.

Figure 1. Rivaroxaban Structure



*Dwell volume noted in Ph. Eur. Rivaroxaban monograph using the same column and chromatographic conditions.

LC Conditions

Column: ZORBAX® Eclipse® 3.5 µm XDB-C18, 150 x 3.0 mm
Luna™ Omega 3 µm C18, 150 x 3.0 mm (00F-4784-Y0)

Mobile Phase: A: Methanol / **Solution A** (5:95, v/v)
B: Acetonitrile

Gradient:		ZORBAX Eclipse Agilent® 1260	Luna Omega Agilent 1260	Waters® ACQUITY® H-Class	
	Time (min)	**Adjusted Time (min)**	**Adjusted Time (min)**	**Adjusted Time (min)**	**%B**
	0	0	0	0	24
	2	1.42	1.22	2.16	24
	8	7.42	6.36	7.30	77
	25	24.42	20.93	21.87	24
	37	36.42	31.22	32.16	24
	37.1	36.52	31.30	32.25	98
	45	44	38.30	39.00	98

NLT 7 min of column equilibration with the initial mobile phase conditions is recommended between injections.

Flow Rate: 1.0 mL/min (ZORBAX Eclipse)
1.17 mL/min (Luna Omega)

Injection Volume: 5 µL

Temperature: 60 °C

Detector: UV @ 250 nm

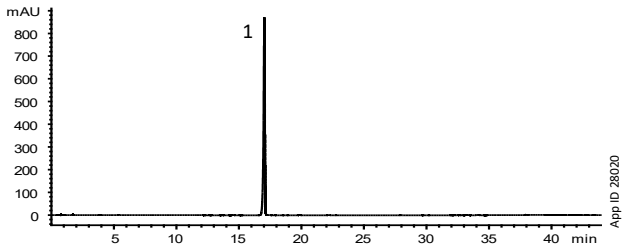
System: Agilent 1260
Waters ACQUITY H-Class UHPLC

Table 1. Preparation of Solutions

Solution	Composition
Solution A	Dissolve 1.36 g of Potassium Dihydrogen Phosphate, 1 g of 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 Dihydrogen Phosphate and 200 µL of Phosphoric Acid in Water. Dilute with Water to 1 L.
Diluent	Acetonitrile / **Solution B** (40:60, v/v).
Standard Solution (Assay)	0.5 mg /mL of *USP Rivaroxaban RS* in **Diluent**.
System Suitability Solution (Organic Impurities)	0.5 mg/mL of *USP Rivaroxaban RS* and 0.5 µg/mL each of *USP Rivaroxaban Related Compound B RS*, *USP Rivaroxaban Related Compound D RS*, *USP Rivaroxaban Related Compound G RS*, and *USP Rivaroxaban Related Compound J RS* in **Diluent**.
Standard Solution (Organic Impurities)	0.5 µg/mL of *USP Rivaroxaban RS* in **Diluent**.
Sensitivity Solution (Organic Impurities)	0.25 µg/mL of *USP Rivaroxaban RS* in **Diluent** from the **Standard Solution**.

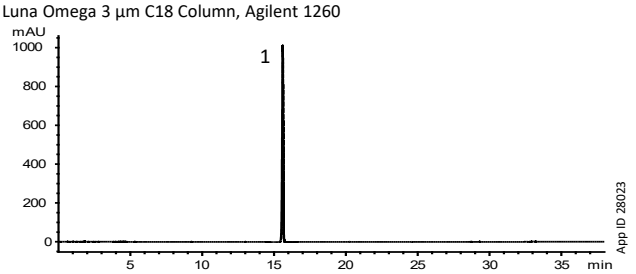
Figure 2. Standard Solution - Assay

ZORBAX Eclipse 3.5 µm XDB-C18 Column, Agilent 1260



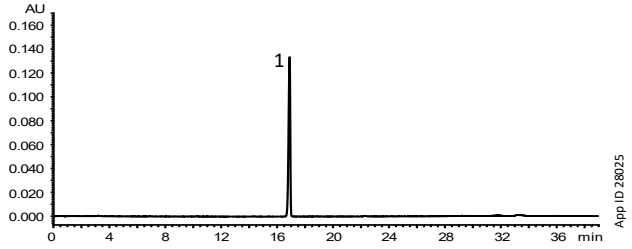
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Rivaroxaban	17.00	6387.75	0.23	0.76
N=6 Injections					

Luna Omega 3 µm C18 Column, Agilent 1260



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Rivaroxaban	15.61	5553.95	0.63	0.86
N=6 Injections					

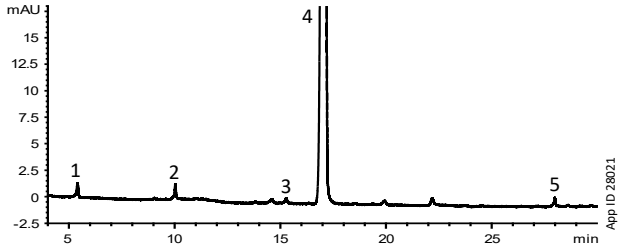
Luna Omega 3 µm C18 Column, Waters ACQUITY H-Class



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Rivaroxaban	16.90	1136825	0.60	0.77
N=6 Injections					

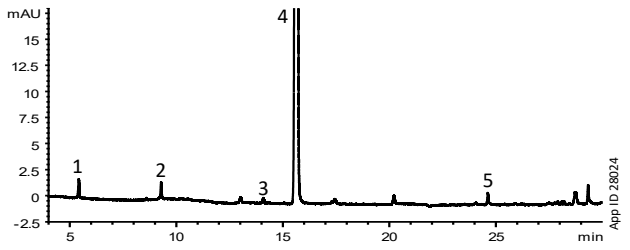
Figure 3. System Suitability Solution – Organic Impurities

ZORBAX® Eclipse® 3.5 µm XDB-C18 Column, Agilent® 1260



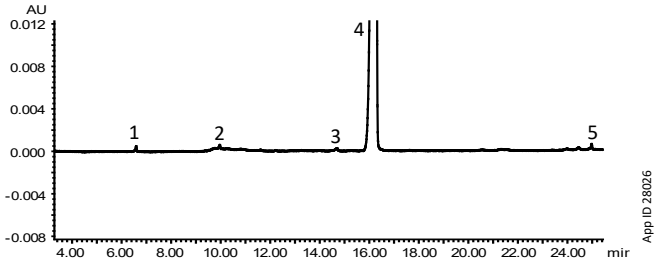
Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Related Compound B	5.40	10.2	-
2	Related Compound D	10.04	11.1	-
3	Related Compound G	15.26	4.5	10.23
4	Rivaroxaban	17.08	5559.4	
5	Related Compound J	27.97	6.7	-
N=6 Injections				

Luna™ Omega 3 µm C18 Column, Agilent 1260



Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Related Compound B	5.42	7.4	-
2	Related Compound D	9.29	8.1	-
3	Related Compound G	14.10	3.6	11.36
4	Rivaroxaban	15.66	4779.4	
5	Related Compound J	24.63	5.5	-
N=6 Injections				

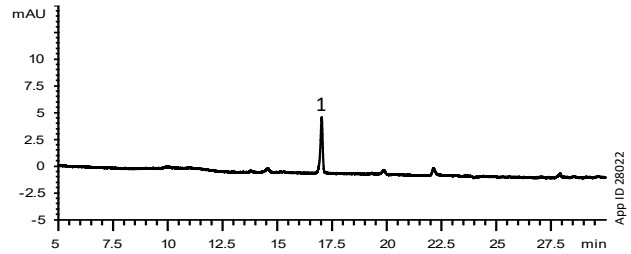
Luna Omega 3 µm C18 Column, Waters® ACQUITY® H-Class



Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Related Compound B	6.80	2102	-
2	Related Compound D	10.08	1444	-
3	Related Compound G	14.87	2011	9.22
4	Rivaroxaban	16.24	1824246	
5	Related Compound J	25.05	2373	-
N=5 Injections				

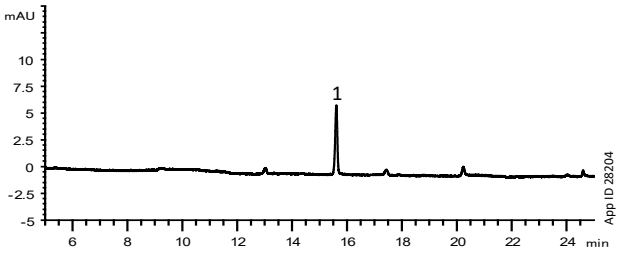
Figure 4. Standard Solution – Organic Impurities

ZORBAX® Eclipse® 3.5 µm XDB-C18 Column, Agilent® 1260



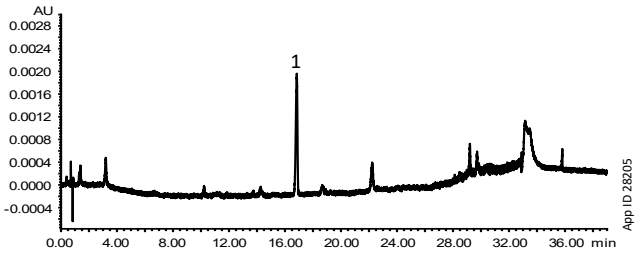
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Rivaroxaban	17.03	40.06	2.72
N=6 Injections				

Luna™ Omega 3 µm C18 Column, Agilent 1260



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Rivaroxaban	15.61	36.38	4.28

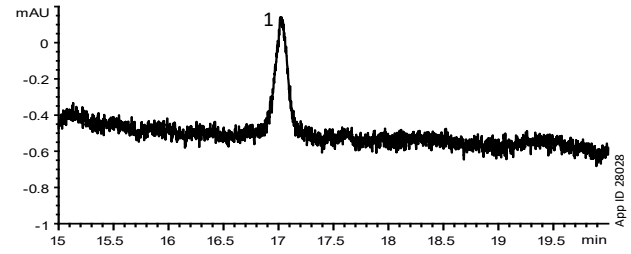
Luna Omega 3 µm C18 Column, Waters® ACQUITY® H-Class



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Rivaroxaban	16.83	17544.6	1.49
N=5 Injections				

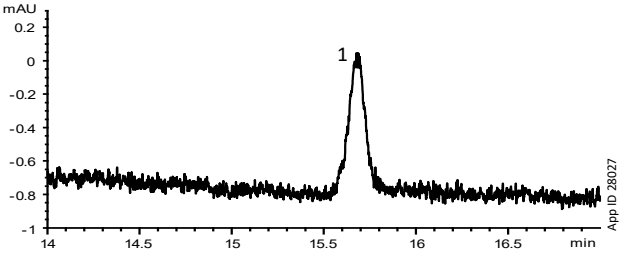
Figure 5. Sensitivity Solution – Organic Impurities

ZORBAX Eclipse 3.5 µm XDB-C18 Column, Agilent 1260



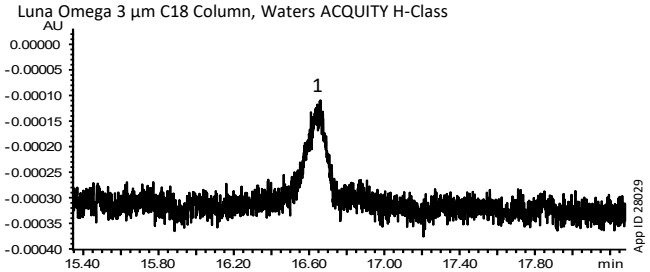
Peak No.	Analyte	Retention Time (min)	Area	S/N
1	Rivaroxaban	17.04	5.25	11.25
N=6 Injections				

Luna Omega 3 µm C18 Column, Agilent 1260



Peak No.	Analyte	Retention Time (min)	Area	S/N
1	Rivaroxaban	15.65	5.22	12.23

Luna Omega 3 µm C18 Column, Waters ACQUITY H-Class



Peak No.	Analyte	Retention Time (min)	Area	S/N
1	Rivaroxaban	16.67	1682	10.64
N=6 Injections				

Luna™ Omega Ordering Information

3 µm MidBore™ Columns (mm)				SecurityGuard™ Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0*/10pk**
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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