TN-1348

Ph. Eur. Monograph 1651: Clarithromycin Assay and Related Substances

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Introduction

Clarithromycin is an antibiotic used to treat a variety of bacterial infections including strep throat, pneumonia, skin infections, and Lyme disease. This study for Clarithromycin and its related substances is based on the European Pharmacopoeia monograph where an end-capped octadecylsilyl silica gel stationary phase is used under gradient conditions. In this technical note, we report the separation of Clarithromycin and its related substances using a Luna™ 3 µm C18(2) column and a Luna Omega 3 µm C18 column compared to a ZORBAX® 3.5 µm SB-C18 column.

System suitability per Monograph 1651 (Ph. Eur. 11.0) for Clarithromycin Assay and Related Substances is a maximum symmetry factor of 1.7 for the peak due to Clarithromycin and a minimum peak-to-valley ratio of 3.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak for the peak due to Clarithromycin in the chromatogram obtained with Reference Solution (d).

According to Ph. Eur. Chapter 2.2.46, 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 100 mm, and the particle size was 3.5 µm; therefore, the Luna C18(2) column and the Luna Omega C18 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 for 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) = 1.1
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 3 µm C18(2) and the Luna Omega 3 µm C18 columns would be:

$$F_2 = 1.1 \, x \, \frac{4.6^2 \, x \, 3.5}{4.6^2 \, x \, 3}$$

$$F_2 = 1.28$$

A change in particle size, and thus in column volume, impacts the gradient volume which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume. The new gradient time for each gradient segment can be calculated 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 for the Luna 3 µm C18(2) column and the Luna Omega 3 µm C18 column would be:

$$t_{G2} = 32.0 \, x \, \left(\frac{1.1}{1.28}\right) \, x \, \left(\frac{100 \, x \, 4.6^2}{100 \, x \, 4.6^2}\right)$$

$$t_{G2} = 27.43$$

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

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

$$Gradient\ adjustment\ factor = 0.857$$

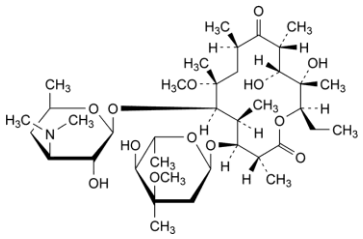
The new gradient timetable for the Luna 3 µm C18(2) column and the Luna Omega 3 µm C18 column is shown in the gradient table.

All requirements for System Suitability for Clarithromycin Related Substances were met by all columns. However, the maximum symmetry factor of 1.7 for Clarithromycin Assay was not achieved by any column tested. See Note in Conclusions.

All solutions were prepared as indicated in Ph. Eur. monograph 1651 for Clarithromycin. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0000320, Clarithromycin CRS
- Y0000321, Clarithromycin for Peak Identification CRS

Figure 1. Clarithromycin Structure



LC Conditions

Column: ZORBAX 3.5 µm SB-C18, 100 x 4.6 mm
Luna 3 µm C18(2), 100 x 4.6 mm (00D-4251-E0)
Luna Omega 3 µm C18, 100 x 4.6 mm (00D-4784-E0)

Mobile Phase: A: **Buffer**
B: Acetonitrile

Gradient:	**ZORBAX SB-C18** Time (min)	**Luna C18(2) Luna Omega C18** Adjusted Time (min)	%B
	0	0	25
	32	27.43	60
	34	29.14	60

Flow Rate: 1.1 mL/min 1.28 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detector: UV @ 205 nm
System: Waters® ACQUITY® H-Class UHPLC

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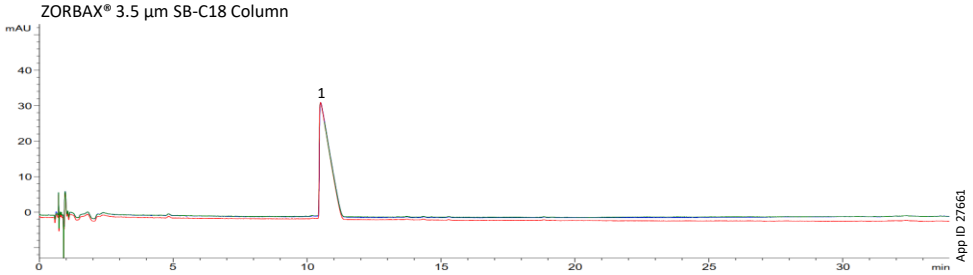
Table 1. Preparation of Solutions

Solution	Composition
Buffer	4.76 g/L solution of Potassium Dihydrogen Phosphate R, adjusted to pH 4.4 with dilute Phosphoric Acid R or a 45 g/L solution of Potassium Hydroxide R, filtered through a C18 filtration kit.
Test Solution	Dissolve 75.0 mg of the *Clarithromycin CRS* in 25 mL of Acetonitrile R1 and dilute to 50.0 mL with Water R.
Reference Solution (a)	See Test Solution
Reference Solution (b)	Dilute 5.0 mL of Reference Solution (a) to 100.0 mL with a mixture of equal volumes of Acetonitrile R1 and Water R.
Reference Solution (c)	Dilute 1.0 mL of **Reference Solution (b)** to 10.0 mL with a mixture of equal volumes of Acetonitrile R1 and Water R.
Reference Solution (d)	Dissolve 3.0 mg of *Clarithromycin for Peak Identification CRS* in 1.0 mL of Acetonitrile R1 and dilute to 2.0 mL with Water R.

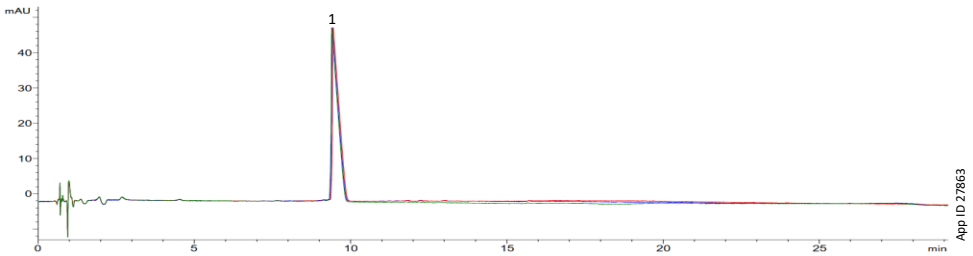
Figure 2. System Suitability Test for Assay Using Reference Solution (a).

ZORBAX® 3.5 µm SB-C18 Column



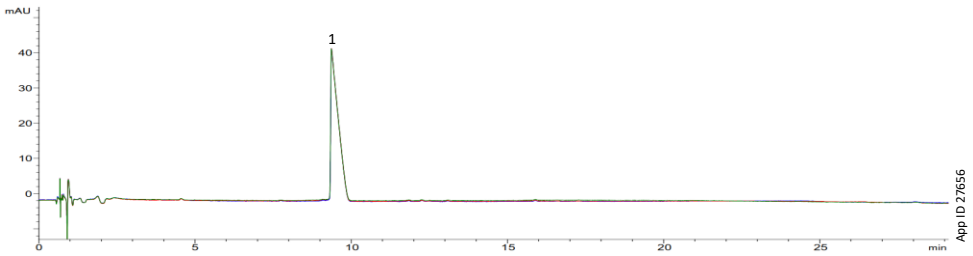
Peak No.	Analyte	Retention Time (min)	Symmetry Factor*
1	Clarithromycin	10.503	5.85
N = 3 Injections			

Luna™ 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	Symmetry Factor*
1	Clarithromycin	9.518	3.63
N = 3 Injections			

Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Symmetry Factor*
1	Clarithromycin	9.371	5.39
N = 3 Injections			

*See Note in Conclusions

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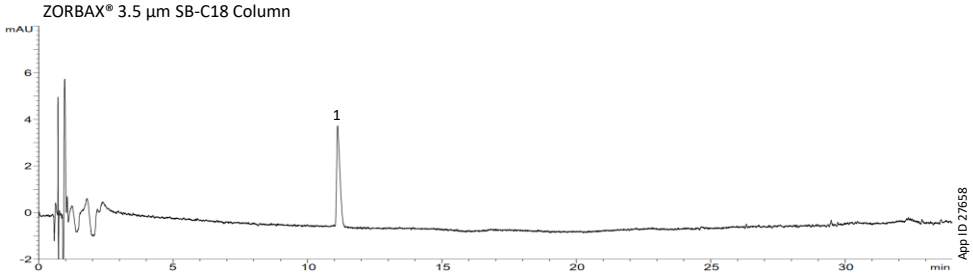
Figure 3. System Suitability Test for Related Substances Using Reference Solution (b).

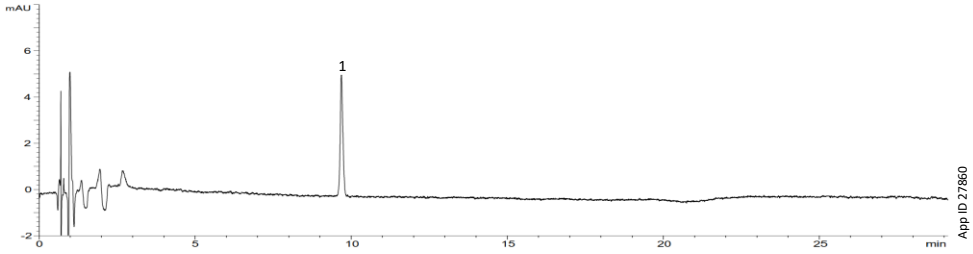
ZORBAX® 3.5 µm SB-C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Width	Symmetry Factor
1	Clarithromycin	11.123	37.1	4.3	0.1383	1.71

N = 3 Injections

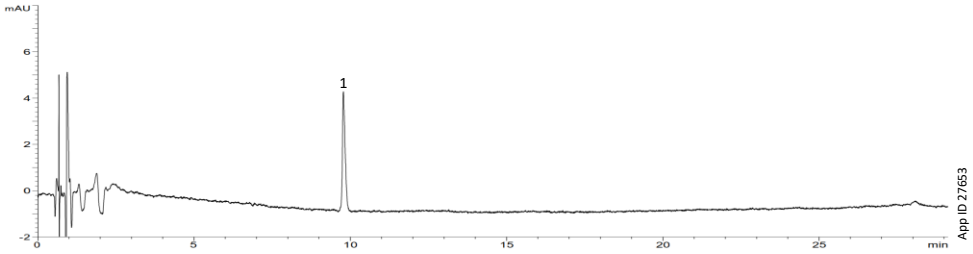
Luna™ 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Width	Symmetry Factor
1	Clarithromycin	9.685	32.8	5.3	0.0925	1.12

N = 3 Injections

Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Width	Symmetry Factor
1	Clarithromycin	9.779	32.1	5.2	0.0817	1.39

N = 3 Injections

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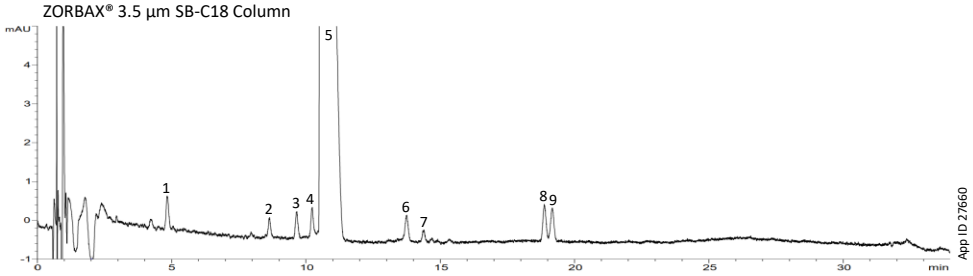
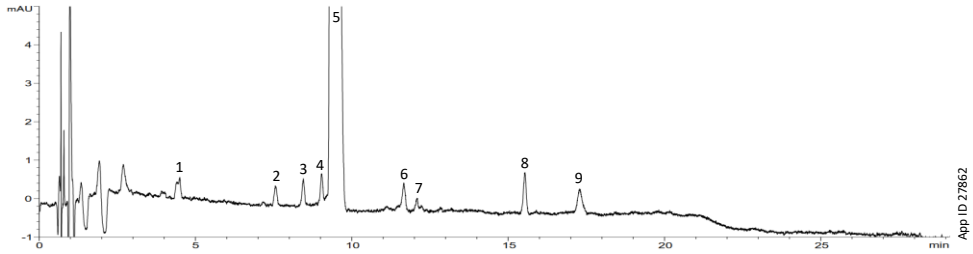
Figure 4. System Suitability Test for Related Substances Using Reference Solution (d).

ZORBAX® 3.5 µm SB-C18 Column



Peak No.	Analyte	Retention Time (min)	Area	% Area	Peak-to-Valley Ratio
1	Impurity A	4.833	6.2	0.847	—
2	Impurity B	8.640	3.9	0.523	—
3	Impurity C	9.655	4.8	0.652	—
4	Impurity D	10.230	6.2	0.847	5.67
5	Clarithromycin	10.557	685.7	93.694	
6	Impurity E	13.748	5.9	0.807	—
7	Impurity F	14.392	3.0	0.407	—
8	Impurity G	18.878	8.3	1.139	—
9	Impurity H	19.171	7.9	1.08	—
N = 3 Injections					

Luna™ 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	Area	% Area	Peak-to-Valley Ratio
1	Impurity A	4.495	5.4	0.86	—
2	Impurity B	7.558	3.8	0.599	—
3	Impurity C	8.447	4.9	0.781	—
4	Impurity D	9.038	4.5	0.720	5.65
5	Clarithromycin	9.326	584.8	93.326	
6	Impurity E	11.663	4.8	0.769	—
7	Impurity F	12.093	3.7	0.591	—
8	Impurity G	15.533	7.5	1.191	—
9	Impurity H	17.281	7.3	1.158	—
N = 3 Injections					

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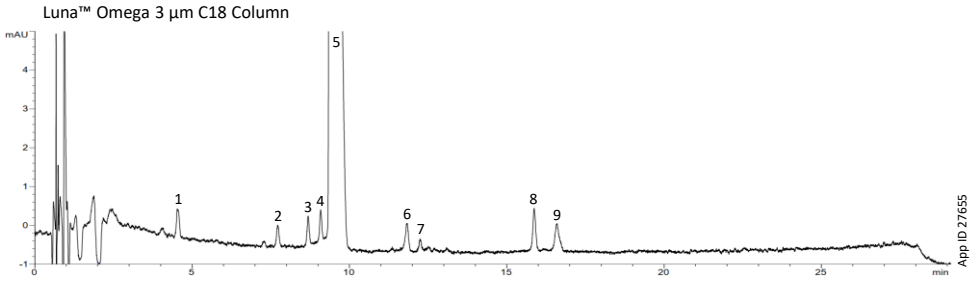
Figure 4 Cont'd. System Suitability Test for Related Substances Using Reference Solution (d).



Peak No.	Analyte	Retention Time (min)	Area	% Area	Peak-to-Valley Ratio
1	Impurity A	4.534	5.2	0.839	—
2	Impurity B	7.717	3.4	0.541	—
3	Impurity C	8.687	3.9	0.618	—
4	Impurity D	9.085	4.4	0.697	7.36
5	Clarithromycin	9.378	584.2	93.559	
6	Impurity E	11.830	5.7	0.917	—
7	Impurity F	12.245	2.3	0.364	—
8	Impurity G	15.870	7.8	1.246	—
9	Impurity H	16.595	7.6	1.218	—
N = 6 Injections					

Conclusions

The Ph. Eur. Chapter 2.2.46 now allows for adjustment of gradient methods. However, differences in column dimensions (L and dp) must be properly accounted for to ensure performance of the method per the original monograph. Further adjustments to the flow rate and gradient timetable were required when using the Luna 3 µm C18(2) column and the Luna Omega 3 µm C18 column, with the L/dp ratio for the Luna and Luna Omega columns well within the allowable adjustment per Chapter 2.2.46. Comparable results to the original column were obtained using the Luna 3 µm C18(2) column and the Luna Omega 3 µm C18 column, demonstrating that these columns are a suitable alternative for the Ph. Eur. monograph 1651 for Clarithromycin Assay and Related Substances. One notable benefit for using the Luna Omega 3 µm C18 column is the increased peak-to-valley ratio achieved between Impurity D and Clarithromycin. In this technical note we have illustrated the various adjustments that must be made to successfully adjust for system differences and column dimensions.

NOTE: The observed failure to achieve the required system suitability of the symmetry factor for Clarithromycin using Reference Solution (a), with the column indicated as acceptable for this monograph in the EDQM knowledge database, has been shared with EDQM. They have shared that this has been brought to their attention and it is planned to bring this observation to the attention of the Group of Experts in charge of this monograph.

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Luna™ Ordering Information

3 µm MidBore™ and Analytical Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	**30 x 3.0**	**50 x 3.0**	**150 x 3.0**	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**4 x 2.0***	**4 x 3.0***
									/10pk	/10pk
Silica(2)	—	00B-4162-Y0	00F-4162-Y0	00A-4162-E0	00B-4162-E0	—	00D-4162-E0	00F-4162-E0	AJ0-4347	AJ0-4348
C8(2)	00A-4248-Y0	00B-4248-Y0	00F-4248-Y0	00A-4248-E0	00B-4248-E0	00C-4248-E0	00D-4248-E0	00F-4248-E0	AJ0-4289	AJ0-4290
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJ0-4286	AJ0-4287
CN	—	00B-4254-Y0	00F-4254-Y0	00A-4254-E0	00B-4254-E0	00C-4254-E0	00D-4254-E0	00F-4254-E0	AJ0-4304	AJ0-4305
Phenyl-Hexyl	—	00B-4256-Y0	00F-4256-Y0	—	00B-4256-E0	00C-4256-E0	00D-4256-E0	00F-4256-E0	AJ0-4350	AJ0-4351
NH_2	—	00B-4377-Y0	00F-4377-Y0	—	00B-4377-E0	—	00D-4377-E0	00F-4377-E0	AJ0-4301	AJ0-4302
HILIC	—	00B-4449-Y0	00F-4449-Y0	—	—	—	00D-4449-E0	00F-4449-E0	AJ0-8328	AJ0-8329
PFP(2)	—	00B-4447-Y0	00F-4447-Y0	—	00B-4447-E0	—	00D-4447-E0	00F-4447-E0	AJ0-8326	AJ0-8327
									for ID: 2.0-3.0 mm	3.2-18.0 mm

Luna Omega Ordering Information

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0*/10pk**
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJ0-7606
C18	00B-4784-E0	00D-4784-E0	00F-4784-E0	00G-4784-E0	AJ0-7612
SUGAR	—	00D-4775-E0	00F-4775-E0	00G-4775-E0	AJ0-4495
					for ID: 3.2 – 8.0 mm

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

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