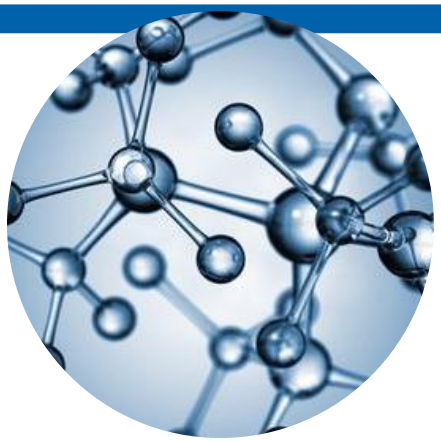

TN-1343

Ph. Eur. Monograph 2217: Lamivudine Related Substances with Ph. Eur. Method Modernization

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Introduction

Lamivudine is an antiretroviral medication that belongs to a class of drugs known as nucleoside reverse transcriptase inhibitors (NRTIs) and plays a crucial role in combination therapy for managing the human immunodeficiency virus (HIV) infection. Additionally, Lamivudine is also utilized in the treatment of chronic hepatitis B virus (HBV) infection. This study for Lamivudine and its related substances is based on the Ph. Eur. monograph where an end-capped octadecylsilyl silica gel stationary phase is used under isocratic conditions. In this technical note, we demonstrate the potential method improvements that can be achieved within the defined allowable adjustments of chromatographic conditions per the Ph. Eur. Monograph 2217 for Lamivudine using Luna™ Omega C18 and Kinetex™ C18 columns compared to the Hypersil® BDS C18 column originally used in the monograph.

The Ph. Eur. 2.2.46 chromatography chapter has been updated from July 2022. The adjustments of chromatographic conditions have suggested the extent to which the various parameters of a chromatographic analysis method may be adjusted without fundamentally modifying the pharmacopoeia analytical procedures. Based on these allowable adjustments several "Methods" were developed and listed in **Table 1**.

System suitability per Ph. Eur. Monograph 2217 for Lamivudine Related Substances is a minimum resolution between Related Impurity F and Related Impurity A of 1.5, and a minimum resolution between Related Impurity B and Lamivudine of 1.5. An adjustment is only allowed when the system suitability requirements are met.

All the reference solutions were prepared as indicated in Ph. Eur. monograph 2217 for Lamivudine. Salicylic Acid R, Cytosine R, and Uracil R were purchased from Sigma-Aldrich®. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0000425, Lamivudine CRS
- Y0000518, Lamivudine for System Suitability 1 CRS

Table 1. Different Methods Within Allowable Adjustments of Chromatographic Conditions USP <621> and Ph. Eur. 2.2.46.

Method Parameter	Allowable Adjustments	Method 1 (Monograph Method)	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7
Stationary Phase	No change of the identity of the substituent permitted.	End-capped octadecylsilyl silica gel per Ph. Eur. (As Specified)	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Column Dimension (particle size and length)	The particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range −25 % to +50 % of the prescribed L/dp ratio.	Length = 250 mm Particle Size = 5 µm L/dp = 50	Length = 150 mm Particle Size = 3 µm L/dp = 50 (Deviation = 0.0 %) (Allowed)	Length = 150 mm Particle Size = 3 µm L/dp = 50 (Deviation = 0.0 %) (Allowed)	Length = 150 mm Particle Size = 2.6 µm L/dp = 57.69 (Deviation = +15.38 %) (Allowed)	Length = 150 mm Particle Size = 2.6 µm L/dp = 57.69 (Deviation = +15.38 %) (Allowed)	Length = 100 mm Particle Size = 1.6 µm L/dp = 62.5 (Deviation = +25 %) (Allowed)	Length = 100 mm Particle Size = 1.6 µm L/dp = 62.5 (Deviation = +25 %) (Allowed)
Column Internal Diameter	In the absence of a change in particle size and/or length of the column, the internal diameter of the column may be adjusted.	ID = 4.6 mm (As specified)	ID = 4.6 mm (Allowed)	ID = 4.6 mm (Allowed)	ID = 3.0 mm (Allowed)	ID = 3.0 mm (Allowed)	ID = 2.1 mm (Allowed)	ID = 2.1 mm (Allowed)
Flow Rate	Flow rate is adjusted for changes in column diameter and particle size using the following equation: $F_2 = F_1 \times \frac{dc_2^2}{dc_1^2} \times \frac{dp_1}{dp_2}$ F_1 = flow rate indicated in the monograph, in mL/min F_2 = adjusted flow rate, in mL/min dc_1 = internal diameter of the column indicated in the monograph, in mm dc_2 = internal diameter of the column used, in mm dp_1 = particle size indicated in the monograph, in µm dp_2 = particle size of the column used, in µm After an adjustment due to a change in column dimensions, an additional change in flow rate of ± 50 percent is permitted.	1.0 mL/min (As Specified)	1.67 mL/min (Deviation from linear flow rate after adjustment = 0.0 %) (Allowed)	1.0 mL/min (Deviation from linear flow rate after adjustment = -40 %) (Allowed)	0.82 mL/min (Deviation from linear flow rate after adjustment = 0.0 %) (Allowed)	0.5 mL/min (Deviation from linear flow rate after adjustment = -39.0 %) (Allowed)	0.65 mL/min (Deviation from linear flow rate after adjustment = 0.0 %) (Allowed)	0.4 mL/min (Deviation from Linear flow rate after adjustment = -38.5 %) (Allowed)
Column Temperature	± 10 °C	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Composition of Mobile Phase	The amount of the minor solvent components may be adjusted by ± 30 % relative; no component is altered by more than 10 % absolute.	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Detector Wavelength	No adjustment permitted	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Injection Volume	When column dimensions are changed, it may be adjusted with the equation: $V_{inj2} = V_{inj1} \times \frac{L_2}{L_1} \times \frac{dc_2^2}{dc_1^2}$ V_{inj1} = injection volume indicated in the monograph, in mL V_{inj2} = adjusted injection volume, in mL dc_1 = internal diameter of the column indicated in the monograph, in mm dc_2 = internal diameter of the column used, in mm L_1 = column length indicated in the monograph, in mL L_2 = new column internal diameter, in mL	10 µL (As specified)	6 µL (Calculated injection volume as per the new column dimensions)	6 µL (Calculated injection volume as per the new column dimensions)	3 µL (Calculated injection volume as per the new column dimensions)	3 µL (Calculated injection volume as per the new column dimensions)	1 µL (Calculated injection volume as per the new column dimensions)	1 µL (Calculated injection volume as per the new column dimensions)

*Have questions or want more details on implementing this method? We would love to help! Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

LC Conditions

Column: Hypersil® BDS 5 µm C18, 250 x 4.6 mm – Method 1
Luna Omega 5 µm C18, 250 x 4.6 mm (00G-4785-E0) – Method 1
Kinetex™ 5 µm C18, 250 x 4.6 mm (00G-4601-E0) – Method 1
Luna Omega 3 µm C18, 150 x 4.6 mm (00F-4784-E0) – Method 2, 3
Kinetex 2.6 µm C18, 150 x 3.0 mm (00F-4462-Y0) – Method 4, 5
Luna Omega 1.6 µm C18, 100 x 2.1 mm (00D-4742-AN) – Method 6, 7

Mobile Phase: Mobile Phase Table 1

Flow Rate: 1.0 mL/min – Method 1, 3
1.67 mL/min – Method 2
0.82 mL/min – Method 4
0.5 mL/min – Method 5
0.65 mL/min – Method 6
0.4 mL/min – Method 7

Injection Volume: 10 µL – Method 1
6 µL – Method 2, 3
3 µL – Method 4, 5
1 µL – Method 6, 7

Temperature: 35 °C

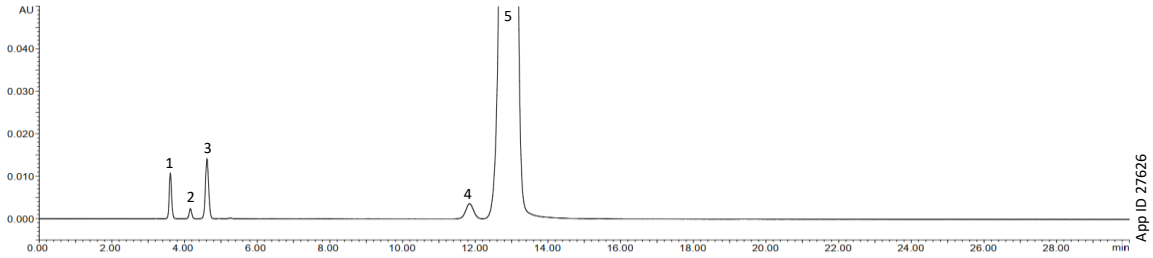
Detector: UV @ 277 nm

System: Waters® ACQUITY Arc® HPLC – Method 1, 2, 3, 4, 5
Waters ACQUITY® H-Class UHPLC – Method 6, 7

Table 2. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	Mix 5 volumes of Methanol and 95 volumes of a 1.9g/L solution of Ammonium Acetate R, previously adjusted to pH 3.8, with Glacial Acetic Acid R.
Test solution	Dissolve 50 mg of *Lamivudine CRS* in Mobile Phase and dilute to 100 mL with Mobile Phase.
Reference solution (a)	Dilute 1 mL of the test solution to 100 mL with Mobile Phase. Dilute 1 mL of this solution to 10.0 mL with Mobile Phase.
Reference solution (b)	Dissolve 5 mg of Salicylic Acid R in Mobile Phase and dilute to 100 mL with Mobile Phase. Dilute 1 mL of the solution to 100 mL of Mobile Phase.
Reference solution (c)	Dissolve 50 mg of *Lamivudine CRS* in Mobile Phase and dilute to 100 mL with Mobile Phase.
Reference solution (d)	Dissolve 5 mg of Cytosine R and 5 mg of Uracil R in Mobile Phase and dilute to 100 mL with Mobile Phase. Dilute 2 mL of the solution to 10 mL with Mobile Phase.
Reference solution (e)	Dissolve 5 mg of *Lamivudine for System Suitability 1 CRS* (containing impurities A and B) in 2 mL of Mobile Phase. Add 1 mL of reference solution (d) and dilute to 10 mL with Mobile Phase.

Figure 1. System Suitability Test for Related Substances using Reference Solution (e) for Method 1 on a Hypersil BDS 5 µm C18, 250 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	3.63	49796	10745	-	1.08
2	Impurity F	4.18	12538	2404	3.07	1.07
3	Impurity A	4.64	88036	14137		1.05
4	Impurity B	11.90	55128	3591	2.56	0.99
5	Lamivudine	12.99	12965790	758418		0.95
N=6 Injections						

Have questions or want more details on implementing this method? We would love to help!
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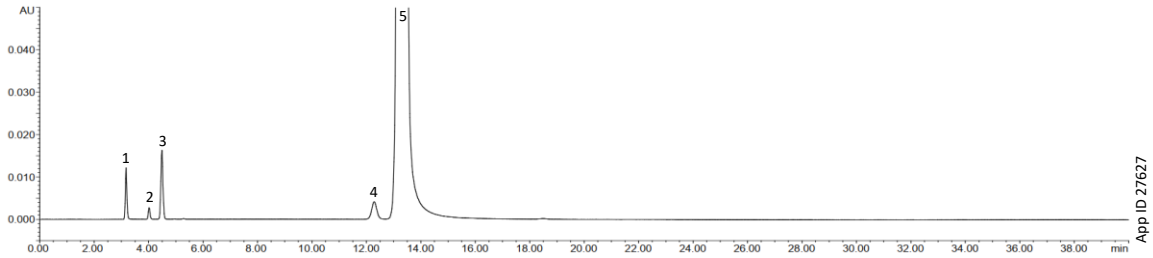
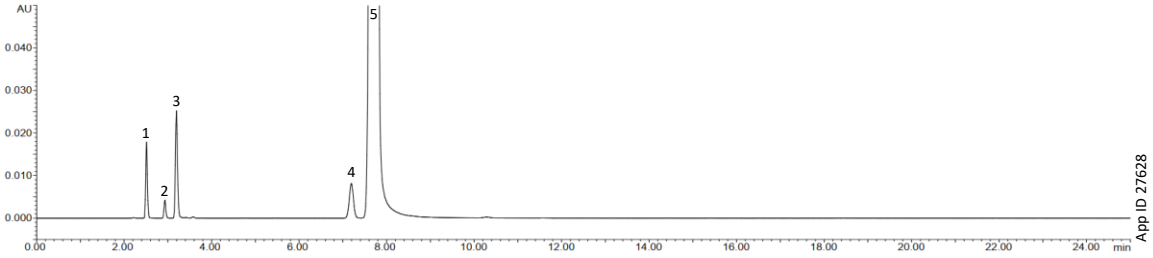
Figure 2. System Suitability Test for Related Substances using Reference Solution (e) for Method 1 on a Luna™ Omega 5 µm C18, 250 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	3.17	48970	12260	-	1.16
2	Impurity F	4.02	12416	2754	3.59	1.12
3	Impurity A	4.49	87249	16330		1.06
4	Impurity B	12.26	54297	4128	2.87	1.02
5	Lamivudine	13.29	12747943	890457		0.97
N=6 Injections						

Figure 3. System Suitability Test for Related Substances using Reference Solution (e) for Method 1 on a Kinetex™ 5 µm C18, 250 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	2.51	47992	17880	-	1.13
2	Impurity F	2.93	12391	4238	3.17	1.13
3	Impurity A	3.19	84512	25208		1.12
4	Impurity B	7.19	54082	8153	2.87	1.00
5	Lamivudine	7.72	12386627	1711247		0.87
N=6 Injections						

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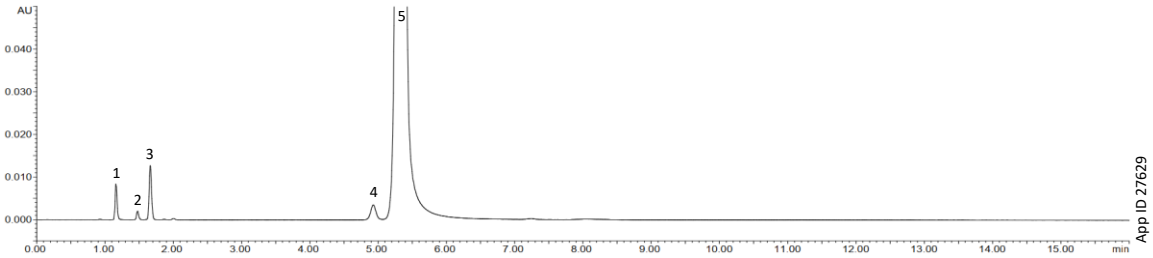
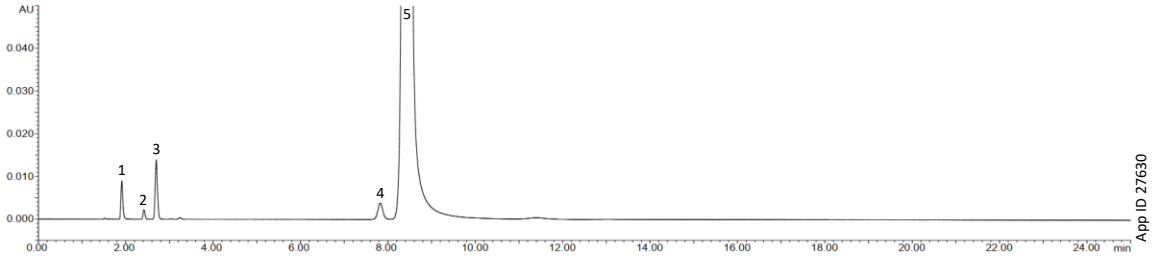
Figure 5. System Suitability Test for Related Substances using Reference Solution (e) for Method 2 on a Luna™ Omega 3 µm C18, 150 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	1.16	17318	8364	-	1.18
2	Impurity F	1.48	4435	2027	3.01	1.14
3	Impurity A	1.66	31017	12653		1.09
4	Impurity B	4.91	18402	3439	2.74	0.99
5	Lamivudine	5.32	4502183	755672		0.98
N=6 Injections						

Figure 6. System Suitability Test for Related Substances using Reference Solution (e) for Method 3 on a Luna Omega 3 µm C18, 150 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	1.91	28433	9028	-	1.25
2	Impurity F	2.42	7361	2228	3.07	1.17
3	Impurity A	2.70	51080	13961		1.13
4	Impurity B	7.84	30722	3850	2.87	1.02
5	Lamivudine	8.46	7398722	841204		0.97
N=6 Injections						

Have questions or want more details on implementing this method? We would love to help!
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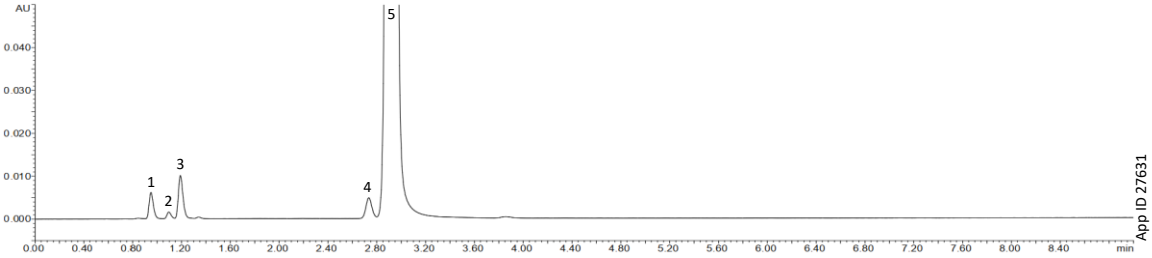
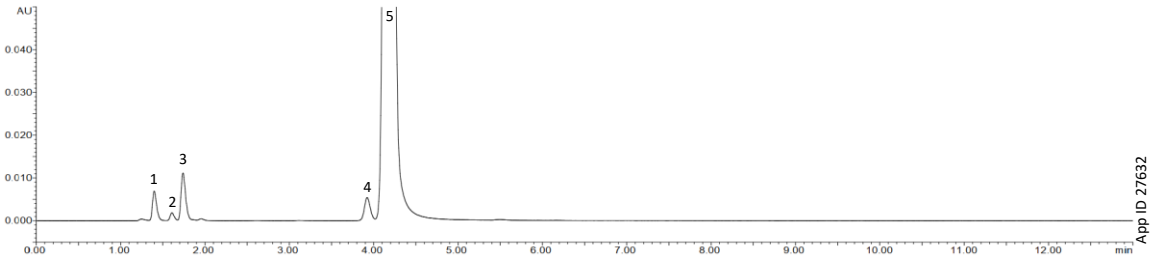
Figure 7. System Suitability Test for Related Substances using Reference Solution (e) for Method 4 on a Kinetex™ 2.6 µm C18, 150 x 3.0 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	0.93	14937	6168	-	1.27
2	Impurity F	1.07	3430	1523	1.43*	1.10
3	Impurity A	1.17	26180	10080		1.27
4	Impurity B	2.68	15011	4680	2.03	1.00
5	Lamivudine	2.87	3890542	1103271		0.99
N=6 Injections						

*Does not meet system suitability for minimum resolution of 1.5.

Figure 8. System Suitability Test for Related Substances using Reference Solution (e) for Method 5 on a Kinetex 2.6 µm C18, 150 x 3.0 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	1.40	23393	6852	-	1.34
2	Impurity F	1.60	5573	1746	1.40*	1.14
3	Impurity A	1.74	41230	11021		1.33
4	Impurity B	3.91	23954	5208	2.04	1.03
5	Lamivudine	4.17	6107149	1225570		0.98
N=6 Injections						

*Does not meet system suitability for minimum resolution of 1.5.

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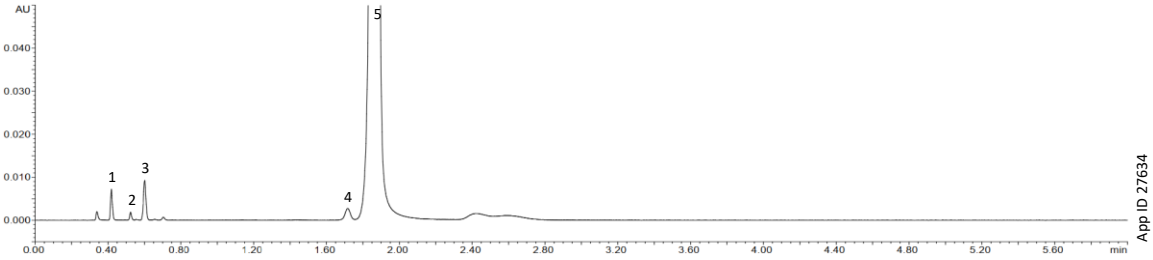
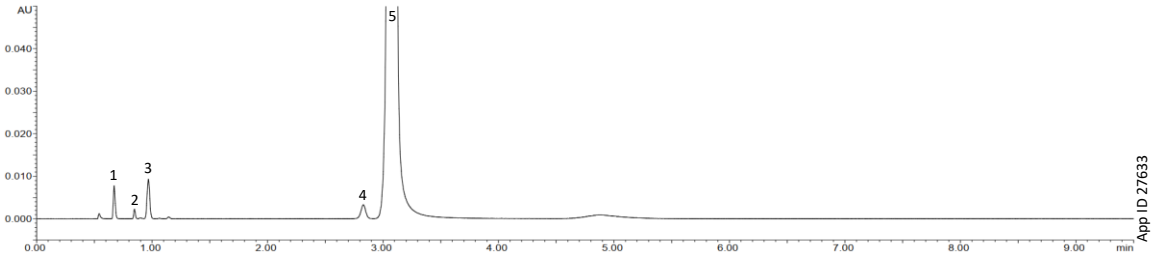
Figure 9. System Suitability Test for Related Substances using Reference Solution (e) for Method 6 on a Luna™ Omega 1.6 µm C18, 100 x 2.1 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	0.42	5167	7143	-	1.15
2	Impurity F	0.52	1288	1876	3.49	1.16
3	Impurity A	0.60	8978	9073		1.07
4	Impurity B	1.72	5205	2636	2.65	0.93
5	Lamivudine	1.86	1318839	577333		0.96
N=6 Injections						

Figure 10. System Suitability Test for Related Substances using Reference Solution (e) for Method 7 on a Luna Omega 1.6 µm C18, 100 x 2.1 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution	Symmetry Factor
1	Impurity E	0.67	8782	7734	-	1.22
2	Impurity F	0.85	2214	2306	3.43	1.15
3	Impurity A	0.97	14910	8900		1.04
4	Impurity B	2.82	9594	3295	3.14	0.98
5	Lamivudine	3.07	2258695	702159		0.93
N=6 Injections						

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Table 3. Summary of System Suitability Results per Method and Column with % Reduction in Run Time.

Method #	Column	L/dp	Resolution (Impurity F and A)	Resolution (Impurity B and Lamivudine)	Run Time* (min)	% Reduction	Column Backpressure (psi)
1	Hypersil® BDS 5 µm C18, 250 x 4.6 mm	50	3.07	2.56	40	--	2000
1	Luna™ Omega 5 µm C18, 250 x 4.6 mm	50	3.59	2.87	40	0 %	2430
1	Kinetex™ 5 µm C18, 250 x 4.6 mm	50	3.17	2.87	23	43 %	2300
2	Luna Omega 3 µm C18, 150 x 4.6 mm	50	3.01	2.74	16	60 %	5700
3	Luna Omega 3 µm C18, 150 x 4.6 mm	50	3.07	2.87	26	35 %	3440
4	Kinetex 2.6 µm C18, 150 x 3.0 mm	57.69	1.43**	2.03	9	78 %	5450
5	Kinetex 2.6 µm C18, 150 x 3.0 mm	57.69	1.40**	2.04	12	70 %	3650
6	Luna Omega 1.6 µm C18, 100 x 2.1 mm	62.5	3.49	2.65	6	85 %	14200
7	Luna Omega 1.6 µm C18, 100 x 2.1 mm	62.5	3.43	3.14	9	78 %	9300

*Run times determined as 3x retention time for Lamivudine, as indicated in Ph. Eur. monograph 2217.
**Does not meet system suitability for minimum resolution of 1.5.

Conclusions

The results show that the system suitability criteria were met by all columns except the Kinetex 2.6 µm C18, 150 x 3.0 mm column. The use of a Luna Omega 3 µm C18, 150 x 4.6 mm column and a Luna Omega 1.6 µm C18, 100 x 2.1 mm column are allowed adjustments to the original column dimension with the flow rates scaled accordingly to accommodate the adjustment to column length (L), internal diameter (ID), and particle size (dp). With the Luna Omega 1.6 µm C18, 100 x 2.1 mm column, we demonstrated a reduction in total analysis time by 60 % (from 16 minutes to 6 minutes with a 1.67 mL/min flow) over the Luna Omega 3 µm C18, 150 x 4.6 mm column, and of 85 % compared to the original column dimension (5 µm, 250 x 4.6 mm) specified in the monograph.

While we demonstrated a reduction in total analysis time by 64 % (from 25 minutes to 9 minutes with a 0.82 mL/min flow) with the Kinetex 2.6 µm C18, 150 x 3.0 mm column when compared to the original column dimension, we were not able to meet the system suitability requirement for minimum resolution of 1.5 between impurities F and A. This highlights a note of caution - adjustments which are allowed to column dimensions also must meet all system suitability requirements in order to be considered an allowed adjustment to Pharmacopoeia methods.

Although the run time was increased when decreasing the flow rate from 0.65 to 0.4 mL/min with the Luna Omega 1.6 µm C18, 100 x 2.1 mm column, it was necessary to reduce the flow rate within the allowed ±50 % in order to greatly reduce the backpressure of the column from 14200 psi to 9300 psi. However, system suitability was still maintained with this column and the overall run time was still reduced by 78 % relative to the original monograph conditions. With the use of a core shell column in the original column dimension, we demonstrated a reduction in total analysis time by 37.5 % over a fully porous Luna Omega column.

Have questions or want more details on implementing this method? We would love to help!
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Luna™ Omega Ordering Information

5 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-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
C18	00B-4785-E0	00D-4785-E0	00F-4785-E0	00G-4785-E0	AJ0-7612

for ID: 3.2 – 8.0 mm

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

1.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502

for 2.1 mm ID

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

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Kinetex™ Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
F5	00B-4724-E0	00D-4724-E0	00F-4724-E0	00G-4724-E0	AJ0-9320
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
HILIC	—	—	00F-4606-E0	00G-4606-E0	AJ0-8772

for 4.6 mm ID

2.6 µm Midbore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	**30 x 3.0**	**50 x 3.0**	**75 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
PS C18	00A-4780-Y0	00B-4780-Y0	—	00D-4780-Y0	00F-4780-Y0	AJ0-8950
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	00D-4461-Y0	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321

for 3.0 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

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