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Meeting System Suitability for USP Abacavir and Lamivudine Tablets Assay

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Introduction

Abacavir and Lamivudine in combination are used with other medications to treat Human Immunodeficiency Virus (HIV) infection. In this technical note, we report the separation of Lamivudine from its related chiral and diastereomeric impurities in Abacavir and Lamivudine tablets per the USP monograph using a Luna™ Omega 3 µm C18 column and a Kinetex™ 2.6 µm EVO C18 as alternative L1 columns to a XTERRA® 3.5 µm MS 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 Abacavir and Lamivudine Tablets Assay requires resolution no less than (NLT) 1.0 between Lamivudine-S-Oxide and Lamivudine-R-Oxide and NLT 1.0 between Lamivudine diastereomer and Lamivudine, and a percent relative standard deviation (%RSD) of no more than (NMT) 1.5 % each for Abacavir and Lamivudine with five replicate injections.

According to USP General Chapter <621>, 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 C18 column and the Kinetex 2.6 µm EVO 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 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 columns would be:

Luna Omega C18

$$F_2 = 1.5 \, x \, \frac{4.6^2 \, x \, 3.5}{4.6^2 \, x \, 3.0}$$

$$F_2 = 1.75$$

Kinetex EVO C18

$$F_2 = 1.5 \, x \, \frac{4.6^2 \, x \, 3.5}{4.6^2 \, x \, 2.6}$$

$$F_2 = 2.02$$

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:

Luna Omega C18

$$t_{G2} = 4.00 \, x \, \left(\frac{1.5}{1.75}\right) \, x \, \left(\frac{150 \, x \, 4.6^2}{150 \, x \, 4.6^2}\right)$$

$$t_{G2} = 3.43$$

Kinetex EVO C18

$$t_{G2} = 4.00 \, x \, \left(\frac{1.5}{2.02}\right) \, x \, \left(\frac{100 \, x \, 4.6^2}{150 \, x \, 4.6^2}\right)$$

$$t_{G2} = 1.98$$

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

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

Luna Omega C18

$$Gradient\ adjustment\ factor = 0.858$$

Kinetex EVO C18

$$Gradient\ adjustment\ factor = 0.495$$

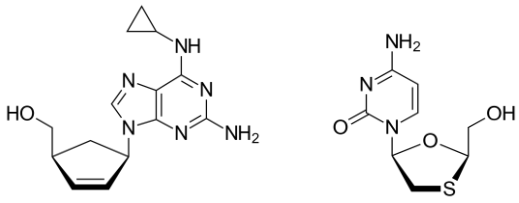
The new gradient timetables for the Luna Omega 3 µm C18 column and the Kinetex 2.6 µm EVO C18 column are shown in the gradient table of the LC Conditions on the next page.

All requirements for System Suitability for Abacavir and Lamivudine tablets Assay were met by all columns; however, the Kinetex EVO C18 column provided significantly reduced retention times and decreased resolution versus the original column. The Luna Omega 3 µm C18 column provided improved resolution between Lamivudine and its diastereomer.

All solutions were prepared as indicated in the USP Monograph for Abacavir and Lamivudine tablets. USP Abacavir Sulfate RS (Catalog No. 1000408), USP Lamivudine RS (Catalog No. 1356836), USP Lamivudine Resolution Mixture C RS (Catalog No. 1356869) were purchased from USP. USP Lamivudine Resolution Mixture C RS is a mixture of Lamivudine and the following impurities (other impurities may also be present):

- Uracil
- Lamivudine-Uracil derivative
- Cytosine
- Lamivudine-S-Sulfoxide
- Lamivudine-R-Sulfoxide
- Lamivudine Carboxylic Acid
- Lamivudine Diastereomer
- Salicylic Acid

Figure 1. Abacavir and Lamivudine Structures.



Abacavir Lamivudine

LC Conditions

Column: XTERRA® 3.5 µm MS C18, 150 x 4.6 mm
Luna™ Omega 3 µm C18, 150 x 4.6 mm (00F-4784-E0)
Kinetex™ 2.6 µm EVO C18, 100 x 4.6 mm (00D-4725-E0)

Mobile Phase: A: Water / Trifluoroacetic Acid (2000:1, v/v)
B: Acetonitrile / Methanol / Trifluoroacetic Acid (1000:1000:1, v/v/v)

Gradient:

XTERRA MS C18 Time (min)	Luna Omega C18 Adjusted Time (min)	Kinetex EVO C18 Adjusted Time (min)	%B
0	0	0	0
4	3.43	1.98	0
12	10.29	5.94	30
12.1	10.37	5.99	60
13.1	11.23	6.49	60
13.2	11.31	6.54	0
20.2	18.31	13.00	0

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

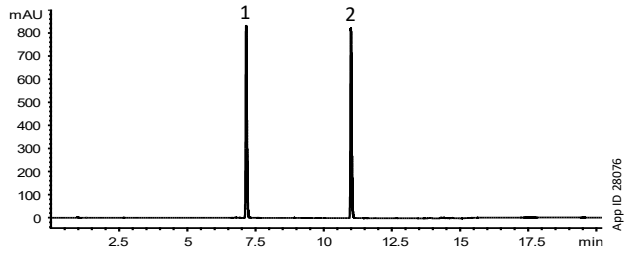
Flow Rate: 1.5 mL/min | 1.75 mL/min | 2.02 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detector: UV @ 270 nm
System: Agilent® 1260

Table 1. Preparation of Solutions

Solution	Composition
Diluent	0.1 N Hydrochloric Acid
Standard Solution	0.35 mg/mL of *USP Abacavir Sulfate RS* and 0.15 mg/mL of *USP Lamivudine RS* in Diluent. Sonicate to dissolve prior to final dilution. Pass through a nylon filter (Clarify™ Syringe Filter, Part No.: AF8-7707-12).
System Suitability Solution	Dissolve the contents of one vial of *USP Lamivudine Resolution Mixture C RS* in 2.5 mL of Diluent. *Note: One vial of USP Lamivudine Resolution Mixture C RS contains 0.8 mg of USP Lamivudine Resolution Mixture C RS.*

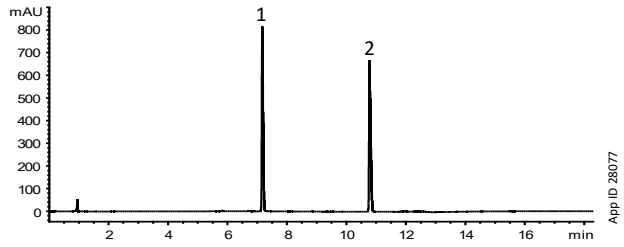
Figure 2. Standard Solution.

XTERRA 3.5 µm MS C18 Column



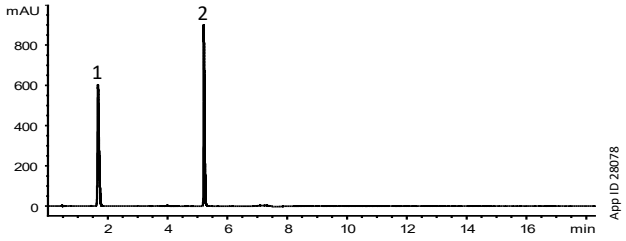
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Lamivudine	7.08	2628.84	0.23
2	Abacavir	10.80	2640.18	0.35
N=5 Injections				

Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Lamivudine	7.20	2393.20	0.16
2	Abacavir	10.78	2453.76	0.20
N=5 Injections				

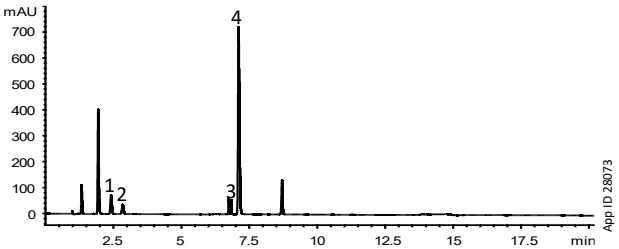
Kinetex 2.6 µm EVO C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Lamivudine	1.67	2099.52	0.15
2	Abacavir	5.21	2132.2	0.21
N=5 Injections				

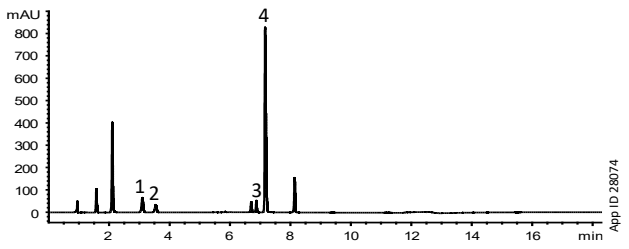
Figure 3. System Suitability Solution.

XTERRA® 3.5 µm MS C18 Column



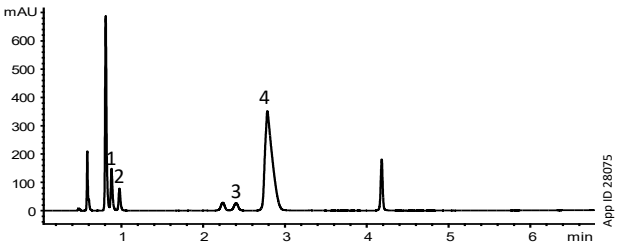
Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Lamivudine-S-Oxide	2.38	267.14	4.12
2	Lamivudine-R-Oxide	2.80	154.56	
3	Lamivudine Diastereomer	6.91	124.42	3.59
4	Lamivudine	7.18	2674.94	
N=5 Injections				

Luna™ Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Lamivudine-S-Oxide	3.06	242.40	4.33
2	Lamivudine-R-Oxide	3.51	141.00	
3	Lamivudine Diastereomer	6.86	113.96	4.30
4	Lamivudine	7.15	2447.48	
N=5 Injections				

Kinetex™ 2.6 µm EVO C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Lamivudine-S-Oxide	0.85	225.36	2.42
2	Lamivudine-R-Oxide	0.95	126.98	
3	Lamivudine Diastereomer	2.30	99.60	2.86
4	Lamivudine	2.66	2149.52	
N=5 Injections				

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

Kinetex™ Ordering Information

2.6 µm Analytical Columns (mm)							SecurityGuard ULTRA Cartridges‡
Phases	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00A-4725-E0	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	00G-4725-E0	AJ0-9296
PS C18	00A-4780-E0	00B-4780-E0	—	00D-4780-E0	00F-4780-E0	00G-4780-E0	AJ0-8949
Polar C18	00A-4759-E0	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	—	AJ0-9530
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	—	AJ0-9207
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	—	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	—	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	—	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	—	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	—	AJ0-8774
F5	00A-4723-E0	00B-4723-E0	—	00D-4723-E0	00F-4723-E0	—	AJ0-9320

for 4.6 mm ID

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

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