CASE STUDY

Meeting System Suitability using <621> Allowable Adjustments 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. The USP monograph for the tablets assay describes a separation utilizing an L1 column. In this case study we will demonstrate that using allowable adjustments it is possible to successfully replace the original column, an XTERRA® 3.5 µm MS C18 column, with either another fully porous column, Luna™ Omega 3 µm C18, or alternatively with a core-shell, Kinetex™ 2.6 µm EVO C18 column. Both columns are considered as alternative L1 columns.

We also report the separation of Lamivudine from its related chiral and diastereomeric impurities in Abacavir and Lamivudine tablets. 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. In order that any alteration to the column is valid, it is necessary to refer to the allowable adjustments permitted in USP General Chapter <621>. We will explain these adjustments and how they are applied in this monograph. It should be noted that the most recent update to chapter <621> allow for adjustments to be made to gradient methods, prior to this adjustments to particle size and column dimensions were not allowed in gradient monographs.

Finally, we will look at the results of the system suitability tests and highlight the advantages for a user to adopting either the fully porous Luna Omega column or the core-shell Kinetex EVO column.

Key Concepts

- Adjustments to particle size are now allowable for gradient monographs
- Applying <621> Allowable Adjustments, new column dimensions were utilized to explore method optimizations
- Replacing the original column with a Core-Shell column resulted in significant time savings

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.
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Allowable Adjustments – Chapter <621>

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

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

$$t_{G2} = 3.43$$

Kinetex™ EVO C18

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

$$t_{G2} = 1.98$$

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

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

Luna Omega C18
Gradient adjustment factor= 0.858

Kinetex EVO C18
Gradient adjustment factor= 0.495

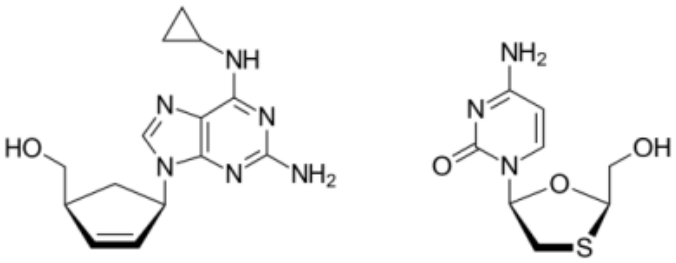
The new gradient timetables for the Luna Omega 3 µm C18 column and the Kinetex 2.6 µm EVO C18 are shown in the gradient table of the LC Conditions on the next page.

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

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

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)

Flow Rate: **Xterra:** 1.5 mL/min
Luna Omega: 1.75 mL/min
Kinetex: 2.02 mL/min

Injection Vol.: 10 µL

Temperature: 40 °C

Detector: UV@270 nm

System: Agilent® 1260

Gradient:

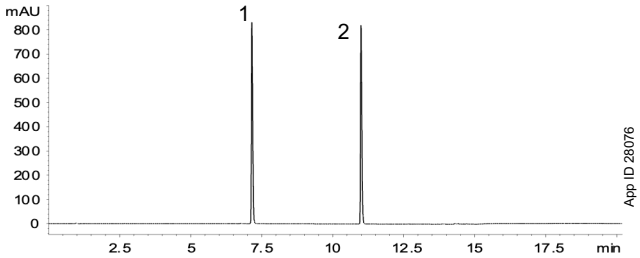
Xterra MS C18	Luna Omega C18	Kinetex EVO C18	
Time (min)	Adjusted Time (min)	Adjusted Time (min)	% B
0	0	0	0
4	3.43	1.98	0
12	10.29	5.94	30
12.1	10.37	5.99	60
13.1	11.23	6.49	60
13.2	11.31	6.54	0
20.2	18.31	13.0	0

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™, 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.

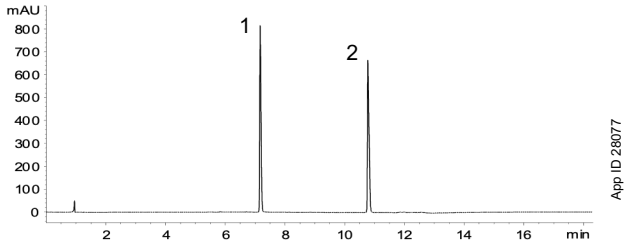
Figure 2. Standard Solution.

XTERRA 3.5 µm MS C18 Column



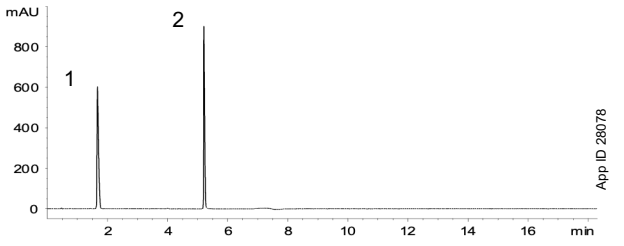
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Lamivudine	7.08	2628.84	0.23
2	Abacavir	10.80	2640.18	0.35
N=5 Injections				

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				

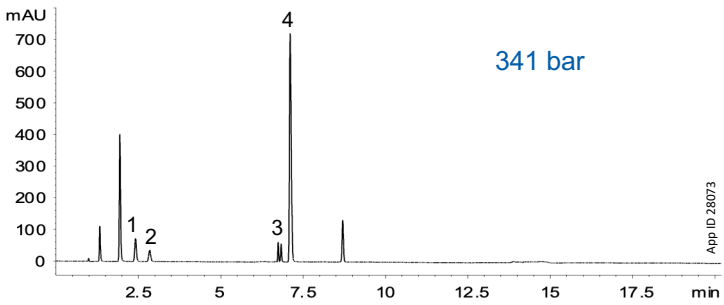
Kinetex 2.6 µm EVO C18 Column



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				

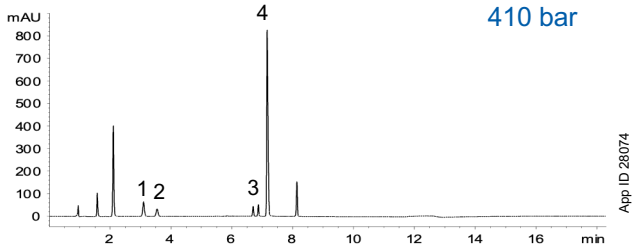
Figure 3. System Suitability Solution.

XTERRA® 3.5 µm MS C18 Column



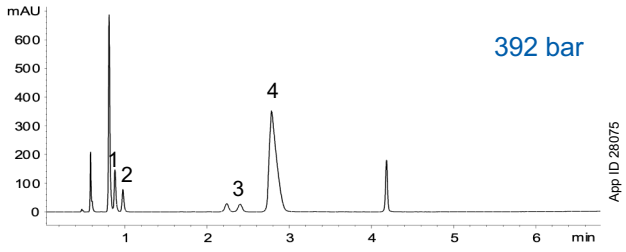
Peak No.	Analyte	Retention Time (min)	Area	Resolution
1	Lamivudine-S-Oxide	2.38	267.14	4.12
2	Lamivudine-R-Oxide	2.80	154.56	
3	Lamivudine Diastereomer	6.91	124.42	3.59
4	Lamivudine	7.18	2674.94	
N=5 Injections				

Luna™ 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				

Discussion

When considering using an alternative column to that used in the elaboration of the monograph this data shows that replacing one fully porous column with another, while maintaining the L/dp ratio within the range -25 % to +50 % for the original column, will allow system suitability to be achieved. The benefits to the enduser of selecting a different column are availability and performance (factors such as peak shape, resolution, reproducibility or lifetime) arising from the use of a newer technology column. In terms of run time however, it is necessary for a reduction of particle size to be significant if a shorter column is to be utilized while still maintaining an allowable L/dp ratio. It is this shortening of the column length while maintaining efficiency that facilitates shorter run time with the benefits of time and cost savings that this realizes. The reduction in particle size will also impact the operating backpressure, both due to the smaller particle size of the packing material (change in backpressure is inversely proportional to the inverse square of change in particle size) and due to the relatively higher linear velocity.

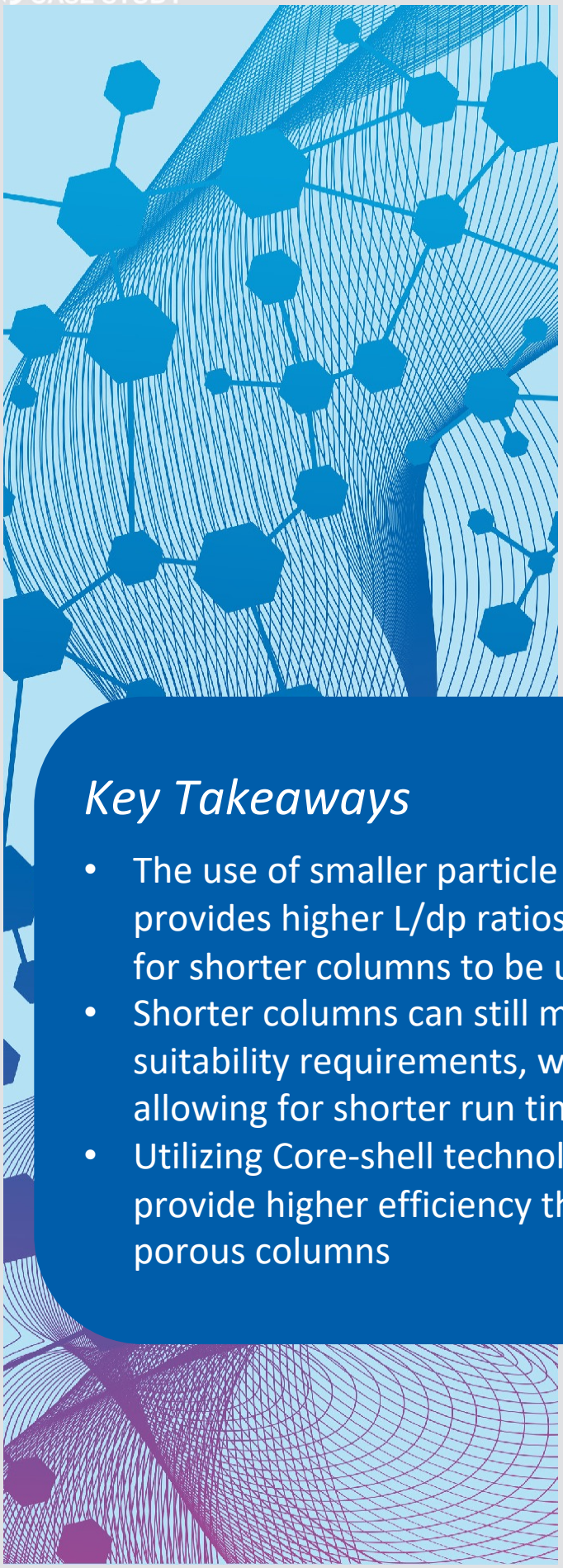
If looking to smaller particle columns, it is possible to reduce the column length in combination with the use of a smaller particle size, while maintaining the L/dp ratio. In the example here:

XTERRA 3.5 µm MS-C18 150 x 4.6 mm – L/dp ratio = 42.86
Luna Omega 3 µm C18 150 x 4.6 mm – L/dp ratio = 50
Kinetex 2.6 µm EVO C18 150 x 4.6 mm – L/dp ratio = 57.69
Kinetex 2.6 µm EVO C18 100 x 4.6 mm – L/dp ratio = 38.46

It would be possible to use a Kinetex 2.6 µm 150 x 4.6 mm column however this would not provide a significant improvement in run time. The use of the shorter 100 x 4.6 mm column, which is an allowable adjustment, provides a significantly reduced run time of 13 min compared to 20.2 min for the XTERRA column. This is a function of the fact that in order to maintain selectivity for the gradient separation the gradient time points are scaled to take into account the change in column dimension and flow rate used. A further advantage of the use of the 10 cm long column is that the operating backpressure is maintained within the limits of the instrument, despite being run at a volumetric flow rate which is 35% higher than the original flow rate described in the monograph.

Core-shell technology provides two significant benefits to the user. Firstly, the columns have lower retention, which assists in the reduction of run time. Retention is typically 10% lower than for fully porous columns in routine applications. Secondly core-shell columns provide higher plate count than fully porous columns of the same dimensions. Backpressure is a function of particle diameter and column length, not particle morphology, so the operating pressure of the columns is lower than for a fully porous column while plate count is maintained.

CASE STUDY



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Summary

From the run time data obtained in this study the use of the Kinetex 2.6 µm EVO C18 column would save 7.2 mins per injection based on the gradient times required (20.2 min for Xterra MS C18, 13 min for Kinetex EVO C18). Over 50 injections this would represent a time saving of 6 hours

In order to simplify the process of performing the necessary calculations detailed here we have developed an on-line calculator. This program allows you to enter details of the column in the monograph, your preferred choice of column dimensions and particle size, together with monograph details such as flow rate and gradient program if applicable. The web-based application will then provide feedback as to whether the chosen column represents and allowed adjustment. Additionally scaled flow rate and adjusted gradient program details are provided. Click here to try out the calculator

Key Takeaways

- The use of smaller particle columns provides higher L/dp ratios, allowing for shorter columns to be used.
- Shorter columns can still meet system suitability requirements, while allowing for shorter run times
- Utilizing Core-shell technology columns provide higher efficiency than fully porous columns

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