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Nota de aplicación

Achieving Method Modernization with the New Liquid Chromatographic Gradient Allowances Provided by USP General Chapter <621> Chromatography and the Alliance™ iS HPLC System

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Este es un resumen de la aplicación y no contiene una sección experimental detallada.

Abstract

The extent to which the various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopeial analytical procedures is defined in U.S. Pharmacopeia (USP) General Chapter <621> Chromatography. In this application note, we combine the gradient method adjustments described in this chapter with the Alliance iS HPLC System to achieve both column dimension and system modernization for the USP monograph separation of antiviral drug, abacavir sulfate.

Benefits

- When paired with the Alliance iS HPLC System, U.S. Pharmacopeia General Chapter <621> Chromatography gradient method allowances generate quality data that meet regulatory requirements

- The Alliance iS HPLC System's extended chromatographic backpressure limits provide high-efficiency separations using an array of modern column dimensions resulting in run time, injection volume, and solvent savings

Introduction

The U.S. Pharmacopeia (USP) portfolio of solutions addresses quality assurance, enhances regulatory predictability, and helps manufacturers distribute quality medicines, dietary supplements, and foods. On December 1, 2022 a harmonized standard for General Chapter <621> Chromatography was released. This standard incorporates the USP with 2.2.46. Chromatographic Separation Techniques European Pharmacopoeia (EuPh) and 2.01 Liquid Chromatography Japanese Pharmacopeia (JP) texts. Harmonization of these regulatory guidance provides increased method flexibility through the employment of modernized of chromatographic tools without the need for full monograph re-validation.

Chromatographic separations are affected by both column hardware and system hardware. These parameters are critical to method performance and limitations can restrict flexibility after monographs are validated. For example, modern HPLC column hardware is commonly offered in 4.6 mm diameter for an array of new stationary phase substituents, while 5 µm HPLC particle sizes have been substituted with ≤3.5 µm particle sizes for comparable separations in less time, and with less solvent. A modern HPLC system, such as the Alliance iS HPLC System (Figure 1), which features high chromatographic separation efficiency, an intuitive touchscreen interface, tool-free column fittings, and extended HPLC operating backpressure limits, provides dependable flexibility when adjusting monograph methods to suit modern column hardware dimensions.

Figure 1. Alliance iS HPLC System with Tunable UV Detector.

In this application note, we employ the gradient method allowances described in General Chapter <621> Chromatography to achieve both column and system modernization for a representative USP monograph (Table 1).

Parameter	Allowed Adjustments in Gradient Elution
Column: Length (L) Particle size (d_p)	Per constant L/d_p between -25% to +50%.
Column: Internal diameter	The internal diameter may be adjusted.
Injection volume	Adjust when a change column diameter. A change can be made without column dimension given SST criteria are met.
Flow rate	With change in particle size and/or column dimension, calculate flow rate accordingly. ±50% with no column dimension change.
Gradient profile	Gradient adjustments based on particle size, column dimensions and flow rate
Stationary phase	No change in the identity of the substituent, physiochemical characteristics of the stationary phase and extent of chemical modification must be similar.
Porosity	A change from porous particle (TPP) to superficially porous is allowed provided SST requirements are met, and selectivity, elution order of the specified impurities to be controlled are demonstrated to be equivalent. Other combinations of L and d_p can be used provided that the ratio $(t_R/W_h)^2$ is within -25% to +50%.
Column temperature	± 10 °C isocratic, ± 5 °C gradient
Mobile phase	± 0.2 pH units, See details for binary and tertiary mixtures
Detector wavelength	No changes allowed

Table 1. The extent to which the various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopeial analytical procedures are listed in the table. The red box indicates the parameters of focus for this application note.

The impurities separation of antiviral compound, abacavir sulfate, was selected for this exercise because the validated gradient method generates a challenging, partially resolved, peak critical pair from which the chromatographic system suitability criteria are based. After performing several of the gradient system adjustments provided in USP <621> that facilitate the use of modernized hardware, resulting chromatograms were examined for the ability to meet original monograph system suitability requirements.

Materials and Methods

USP monograph:	Abacavir Sulfate, Organic Impurities
LC system:	Alliance iS HPLC System with Tunable UV (TUV) Detector
Column(s):	Symmetry™ C_{18} Column, 3.9 x 150 mm, 5 µm, p/n: WAT046980

	Symmetry C_{18} Column, 4.6 x 150 mm, 5 µm, p/n: WAT045905
	Symmetry C_{18} Column, 4.6 x 100 mm, 3.5 µm, p/n: WAT066220
	XBridge™ C_{18} Column, 4.6 x 100 mm, 3.5 µm, p/n: 1860033
	XSelect™ HSS™ T3 Column, 4.6 x 150 mm, 3.5 µm, p/n: 186004786
Software:	Empower™ 4, FR 5, Chromatography Data System (CDS)
Sample:	Abacavir Sulfate System Suitability Mixture (p/n: 1000500, USP)

Results and Discussion

A systematic approach was employed for modernization of the USP, abacavir sulfate monograph separation. First, the monograph column was identified as an L1 stationary phase substituent with a 5 µm particle size and 3.9 x 150 mm column hardware. Modern 4.6 mm diameter column hardware, equipped with L1 stationary phase substituent was selected in lengths (L) of 100 mm and 150 mm to perform method modernization. The stationary phase substituent for the columns was of 5 µm or 3.5 µm particle size (d_p). In all instances, the USP <621> guidance L/d_p ratio allowance was met at -25% to +50% of the monograph ratio.

The flow rate, injection volume and gradient start times were mathematically adjusted for the modern target columns according to the equations provided in the USP <621> guidance. First, Equation 1 maintained the linear velocity of the monograph separation by adjusting the flow rate. Second, the injection volume was adjusted according to Equation 2 to maintain the ratio of the analyte to column volume. Finally, gradient start times were adjusted in Equation 3, Table 2 according to the calculated target column flow rate, length, and particle size. The

start time adjustment preserved the gradient slope to column volume ratio reported in the monograph separation. The USP <621> guidance provided Equation 4 to allow adjustment for the system dwell volume, if specified during the monograph validation. System dwell volume was not reported in the abacavir sulfate monograph, and the separation does not include an initial isocratic hold time. As a result, a dwell volume adjustment was not applied to the calculated gradient start times during method modernization.

$$F_2 = F_1 \text{ x } \left[\frac{(dc_2^2 \times dp_1)}{(dc_1^2 \times dp_2)}\right] = \frac{1.00 \text{ mL}}{\text{min}} \text{ x } \left[\frac{(4.6 \text{ mm}^2 \text{ x } 5 \text{ µm})}{(3.9 \text{ mm}^2 \text{ x } 3.5 \text{ µm})}\right] = 1.987 \text{ mL/min}$$

F_1 = Monograph flow rate (mL/min)
F_2 = Adjusted flow rate (mL/min)
dc_1= Internal diameter monograph column (mm)
dc_2 = Internal diameter target column (mm)
dp_1 = Particle size monograph column (µm)
dp_2 = Particle size target column (µm)

Equation 1. Flow rate adjustment for the monograph column and a 4.6 x 100 mm, 3.5 µm column.

$$V_{inj2} = V_{inj1} \text{ x } \left[\frac{(L_2 \, dc_2^2)}{(L_1 \, dc_1^2)}\right] = 20 \text{ µL x } \left[\frac{(100 \text{ mm x } 4.6 \text{ mm}^2)}{(150 \text{ mm x } 3.9 \text{ µm}^2)}\right] = 18 \text{ µL}$$

V_{inj1} = Monograph injection volume (µL)
V_{inj2} = Adjusted injection volume (µL)
L_1= Length monograph column (mm)
L_2 = Length target column (mm)
dc_1 = Internal diameter monograph column (mm)
dc_2 = Internal diameter target column (mm)

Equation 2. Injection volume adjustment for the monograph column and 4.6 x 100 mm, 3.5 µm column.

$$t_{G2} = t_{G1}\, x\, \left(\frac{F_1}{F_2}\right)\left[\frac{(L_2\, x\, dc_2^2)}{(L_1\, x\, dc_1^2)}\right] = t_{G1}\, x\, \left(\frac{1.000\ mL/min}{1.987\ mL/min}\right)\left[\frac{(100\ mm\, x\, 4.6\ mm^2)}{(150\ mm\, x\, 3.9\ mm^2)}\right] = 0.467$$

(multiplier applied to monograph gradient times)

t_{G1} = Time monograph gradient (min)
t_{G2} = Time adjusted gradient (min)
F_1 = Monograph flow rate (mL/min)
F_2 = Adjusted flow rate (mL/min)
dc_1 = Diameter monograph column (mm)
dc_2 = Diameter target column (mm)

Equation 3. Gradient adjustment for the monograph column and 4.6 x 100 mm, 3.5 µm column.

Time (min) monograph	% B
0 min	5
20 min	30
35 min	90
35.1 min	5
50 min	5

→

Target column adjusted time	% B
0 min	5
0 min + (20 min*0.467) = 9.3 min	30
9.3 min + (15 min*0.467) = 16.3 min	90
16.3 min + (0.1 min*0.467) = 16.4 min	5
16.4 min + (14.9 min*0.467) = 23.4 min	5

Table 2. Gradient time adjustment with multiplier.

$$t_c = t - \left[\frac{(D - D_0)}{F}\right]$$

t = Time setting (min) given in gradient table of monograph (if available)
t_c = Corrected gradient time (min)
D = Dwell volume target instrument (mL)
D_0 = Dwell volume listed monograph (mL)
F = Flow rate (mL/min)

Equation 4. Adjustment for monograph instrument dwell volume, if available.

Manual gradient calculations for the target columns were confirmed using both the Waters Preparative OBD Column Calculator, and the Columns Calculator 2.0 (Figure 2). Online calculators were especially important because they provided an estimated maximum gradient backpressure for the adjusted gradients. This estimation, although computed for 100% organic mobile phase composition, rather than 85% organic composition in the monograph, added confidence that the adjusted methods would not exceed the 10,000-psi backpressure limit of the Alliance iS HPLC System.

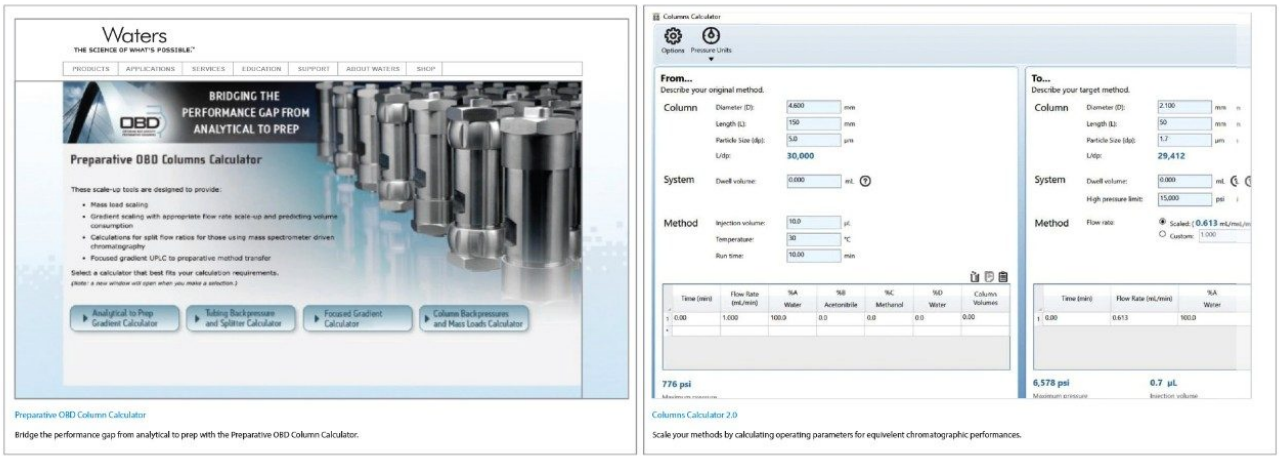

Figure 2. Waters Preparative OBD Column Calculator and the Columns Calculator 2.0 online method adjustment calculators (www.waters.com).

The monograph system suitability impurities mixture (SST) was analyzed with the adjusted gradient and modernized chromatographic hardware. For all modern column dimensions, the monograph system suitability resolution requirement of NLT 1.5 for the unresolved, abacavir critical pair was successfully achieved (Figure 3). When the SST relative retention times (RRTs) were compared to those generated with the stationary phase utilized for the initial monograph separation, they were most similar for columns of the same L1 stationary phase substituents, while RRTs varied with different L1 stationary phase substituents (Figure 4). As noted in USP <621>, peak deletions and/or inversions, may be observed using various substituents, therefore chromatographic peak identity was confirmed after method adjustment by PDA spectral analysis on an alternate system.

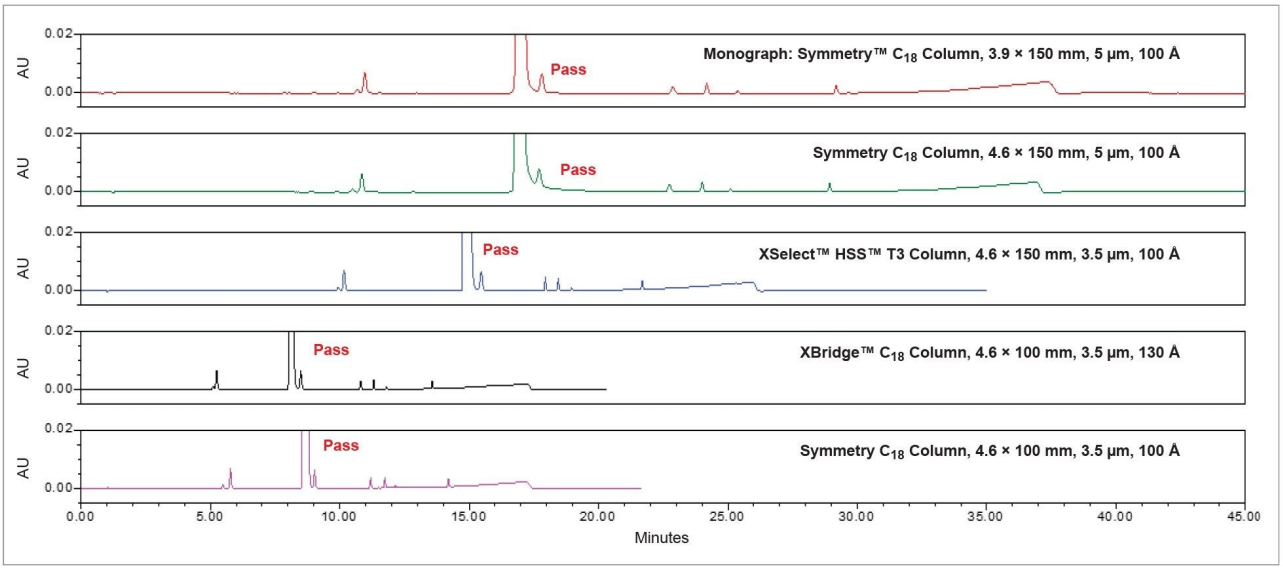

Figure 3: Overlay of monograph and adjusted column chromatograms

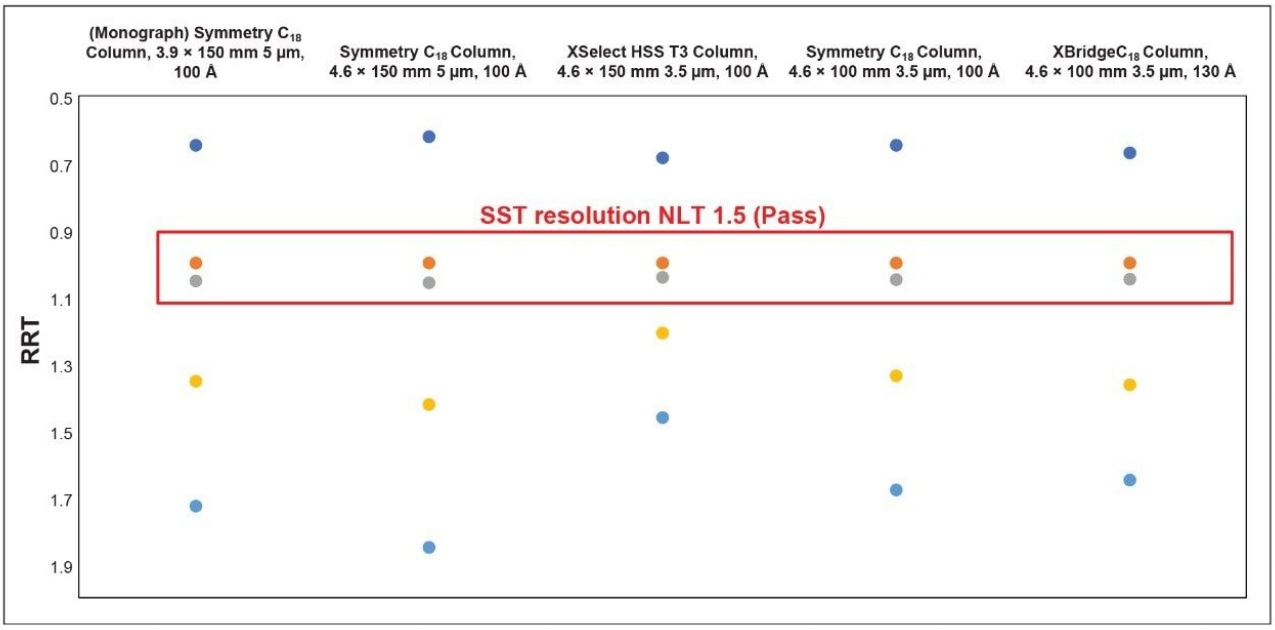

Figure 4: Comparison of the relative retention time (RRT) for system suitability mixture impurities.

The Alliance iS HPLC System, in combination with modernized column hardware dimensions, provided unique benefits for the validated monograph separation. The LC system's modernized tubing diameters facilitated the use of column hardware generating separation backpressures up to 10,000-psi, compared to conventional HPLC systems with backpressure limits of only 5,000-psi. Additionally, the monograph method particle size of 5 µm was successfully reduced to 3.5 µm while retaining USP <621> guidance L/d_p ratio requirements. These hardware combinations resulted in significantly reduced run time, injection volume, and solvent consumption for the validated monograph separation (Table 3).

HPLC column				
	(Monograph) 3.9 × 150 mm, 5 µm, 100 Å	**4.6 × 150 mm, 5 µm, 100 Å**	**4.6 × 150 mm, 3.5 µm, 100 Å**	**4.6 × 100 mm, 3.5µm, 100 Å, 130 Å**
L/dp	30,000	30,000	42,900	28,600
Run time	50 min	50 min	35 min	23 min
Runs per hour	1.2	1.2	1.7	2.6
Total mobile phase	50 mL	71 mL	71 mL	47 mL
Injection volume	20 µL	27.8 µL	27.8µL	18.5 µL
Potential benefit(s)	—	Modern diameter	Modern diameter / Decreased run time / Decreased injection volume	Modern diameter / Decreased run time / Decreased mobile phase usage / Decreased injection volume

Table 3. Modernized column hardware chromatographic benefits when paired with the Alliance iS HPLC System.

Conclusion

USP monograph gradient separations can be successfully adjusted to maintain system suitability acceptance criteria on the Alliance iS HPLC System, as per the General Chapter <621> Chromatography (December 1, 2022). The simple-to-use Alliance iS HPLC System, with intuitive touchscreen interface, enables column modernization without compromising the validated monograph system suitability. The hardware provided an operating

backpressure limit that supported a variety of modern HPLC column diameters, from which unique chromatographic benefits such as run time, injection volume and solvent savings were observed.

References

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