MODERNIZATION OF AN ANTIVIRAL IMPURITIES MONOGRAPH USING THE GLOBALLY HARMONIZED PHARMACOPEIAL GUIDANCE FOR LIQUID CHROMATOGRAPHY

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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. A harmonized standard for General Chapter <621> Chromatography was recently released. This standard incorporates 621 Chromatography USP, 2.2.46. Chromatographic Separation Techniques EuPh, 2.01 Liquid Chromatography JP and Chinese Pharmacopeial ChP texts. Additions provide limits of flexibility for liquid chromatographic gradient method separation parameters such particle size, flow rate, gradient slope, and injection volume. Formulas for integration of chromatographic data have been officially updated to maximize the utility of modern chromatographic data systems CDS with implementation of globally harmonized integration formulas. In this poster, we describe the harmonized U.S. Pharmacopeia USP General Chapter <621> as it applies to method modernization using an updated HPLC System for an antiviral drug impurities monograph.

METHODS

Method Adjustment Guidance:	USP General Chapter <621>, Official Dec1, 2022
USP Monograph:	Abacavir Sulfate, Organic Impurities
Columns:	Symmetry™ C18 Column,3.9x150mm, 5 µm, 100Å, p/n:WAT046980
	Symmetry C18 Column, 4.6x150mm, 5 µm, 100Å, p/n: WAT045905
	Symmetry C18 Column, 4.6x100mm, 3.5 µm, 100Å p/n: WAT066220
	XBridge™ C18 Column, 4.6x100mm, 3.5 µm,100Å, p/n: 1860033
	XSelect™ HSS T3 Column, 4.6x150mm, 3.5 µm, 100Å, p/n: 186004786
	Symmetry C18 Column, 2.1 x 100mm, 3.5um,130Å, p/n:186005256
	XSelect HSS T3 Column, 2.1 x 75mm, 2.5um,100Å, p/n: 186005644
	XBridge BEH C18 Column, 2.1 x 75mm, 2.5um, 130Å, p/n:186006030
	Symmetry C18 Column, 3.0x150 mm, 3.5 µm, p/n:18600695
HPLC Instruments:	Alliance™ HPLC System,
	Alliance iS System **(Figure 1)**
UHPLC Instruments:	ACQUITY™Arc System, ACQUITY UPLC™ I-Class PLUS System
Software:	Empower™ 3 Chromatography Data System (CDS)
Sample: Mixture	Abacavir Sulfate System Suitability Test SST) Mixture (p/n 1000500, USP)

Figure 1. *Modernized instrumentation employed when modernizing the HPLC platform under the harmonized USP <621> gradient method adjustment guidance.*

A systematic approach was employed for harmonized implementation of the 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 and 2.1 diameter column hardware, equipped with L1 stationary phase substituent was selected in lengths (L) of 75 mm, 100 mm, and 150 mm. The column stationary phase substituent was of 5 µm, 3.5 µm, or 2.5 µm particle size (d_p). In all instances, the USP <621> requirement L/d_p ratio allowance was met at -25% to +50% of the monograph ratio for all of the particle size and column dimension combinations.

The flow rate, injection volume, and gradient start times were mathematically adjusted for the target columns, according to the formulas provided in the USP <621> guidance. First, **Formula 1**, **(A)** was used to maintain the linear velocity of the monograph separation by adjusting the flow rate. Second, the injection volume was adjusted according to **Formula 1, (B)** to maintain the ratio of the analyte to column volume. Finally, gradient start times were adjusted in **Formula 2, Table 1** according to the calculated target column flow rate, length, and particle size. This formula preserved the gradient slope to column volume ratio utilized in the monograph.

Formula 1: (A) *Adjustment for flow rate and* ***(B)*** *injection volume when moving from the 3.9 x 150 mm, 5 µm monograph column to 2.1 x 75 mm, 2.5 µm column dimensions.*

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

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)

B $V_{inj2} = V_{inj1} x \left[\frac{(L_2 x\ d_{c2}^2)}{(L_1 x\ d_{c1}^2)}\right] = 20\ \mu L\ x \left[\frac{(75\ mm\ x\ 2.1\ mm^2)}{(150\ mm\ x\ 3.9\ mm^2)}\right] = 2.9\ \mu L$

V_{inj1} = Monograph injection volume (µL)
V_{inj2} = Adjusted injection volume (µL)
L_1 = Length monograph column (mm)
L_2 = Length target column (mm)
dc1 = Internal diameter monograph column (mm)
dc2 = Internal diameter target column (mm)

Formula 2: *Adjustment of gradient time when moving from the monograph column to modern, 2.1 x 75 mm, 2.5 µm column dimensions.*

$t_{G2} = t_{G1} x \left(\frac{F_1}{F_2}\right) \left[\frac{(L_2 x\ d_{c2}^2)}{(L_1 x\ d_{c1}^2)}\right] = t_{G1} x \left(\frac{1.000\ mL/min}{0.580\ mL/min}\right) \left[\frac{(75 x\ 2.1\ mm^2)}{(150 x\ 3.9\ mm^2)}\right] = 0.250$

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)
d_{c1} = Diameter monograph column (mm)
d_{c2} = Diameter target column (mm)

Time (min)	% 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 *0.250) = 5.00 min	30
5.00 min + (15 min *0.250) = 8.75 min	90
8.75 min + (0.1 min *0.250) = 8.78 min	5
8.78 min + (14.9 min*0.250) = 12.50 min	5

Table 1: *Monograph and modified gradient account to USP Chapter <621>.*

Although not defined un USP <621>, **Formula 3** was applied to the gradient (**Table 2**) to account for the 15 fold difference in instrument dwell volume, and over 6 fold difference in column void volume, when moving from a HPLC to a UHPLC platform. The monograph did not include an isocratic step before the start of the gradient, therefore the equation for gradient dwell volume provided in USP <621> was not utilized in our study.

Manual gradient calculations for the target columns were confirmed using both the Waters Preparative OBD Column Calculator, and the Columns Calculator 2.0 found at *www.waters.com*. Online calculators were especially important because they provided an estimated maximum gradient backpressure for the adjusted gradients. Although computed for 100% organic mobile phase composition, rather than 85% organic starting composition (monograph), the estimation added confidence that the adjusted methods were in range of the upper backpressure limit for the HPLC/UHPLC Systems.

$Offset = Vd_{Target\ Instrument} - \{Vd_{Original\ Instrument} x \left[\frac{(V0_{Target\ Instrument})}{(V0_{Original\ Instrument})}\right]\}$

Where $V = L\pi\left(\frac{D}{2}\right)^2$

Vd = Dwell Volume
V0 = Column void volume = 0.66 x V
V = Empty volume
L = Column length
D = Column diameter (cm)

$Offset = 0.073\ mL - \{1.145\ mL\ x\ (\frac{0.171 mL}{1.182 mL})\} = 0.0937\ mL$

Formula 3: *Offset adjustment of gradient time when moving from the 3.9 x 150 mm, 5 µm HPLC monograph platform to the 2.1 x 75 mm, 2.5 µm UHPLC platform. This step is not defined in USP <621>, therefore it is for information only.*

Adjust HPLC platform to Target UHPLC Platform (Column + Instrument)	
ACQUITY™UPLC™ I-Class System Backpressure Max 18,000 psi. Dwell: 0.073 mL	
2.1 x 75 mm, 2.5 µm	
Gradient Option 1:	**Gradient Option 2:**
0 min with 93.7 µL Hold after injection (**Empower Software**)	0 min
5.00 min	5.00 min + 0.17 min = 5.17 min
8.75 min	8.75 min + 0.17 min = 8.92 min
8.78 min	8.78 min + 0.17 min = 8.94 min
12.5 min	12.5 min + 0.17 min = 12.67 min
Predicted Scaled Backpressure ~12,000 psi	

Table 2: *HPLC to UHPLC platform gradient method adjustment when accounting for system and column volume.*

RESULTS

For all column dimensions employed, the original monograph resolution requirement of “Not Less Than (NLT) 1.5” for the impurity critical pair was successfully achieved for the system suitability impurities mixture (SST) after applying harmonized method modernization (**Figure 2**).

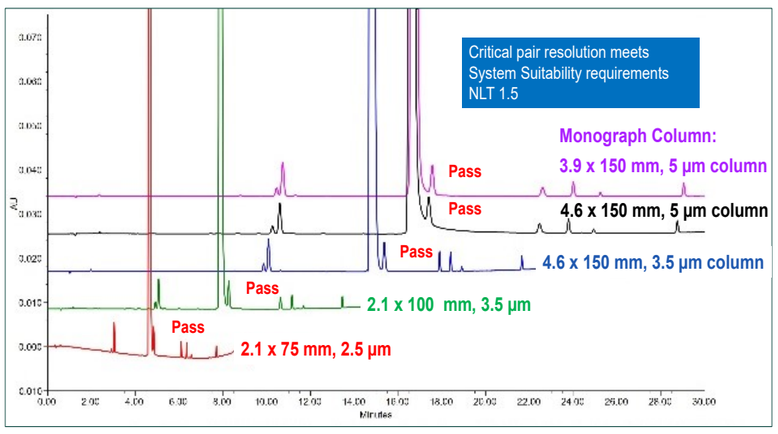

Figure 2. *Overlay of the chromatography for the various modernized column dimensions which fall within the USP guidance recommended L/d_p range.*

	HPLC Platforms				UHPLC Platforms	
System	**Alliance HPLC System / Alliance iS HPLC System**	**Alliance HPLC System / Alliance iS HPLC System**	**Alliance iS HPLC System**	**Alliance iS HPLC System**	**ACQUITY Arc™ System / ACQUITY UPLC™ I-Class PLUS System**	**ACQUITY UPLC I-Class PLUS System**
Column	**(Monograph) 3.9 x 150mm, 5 µm, 100Å**	**4.6 x 150 mm, 5 µm, 100Å**	**4.6 x 150 mm, 3.5 µm, 100Å**	**4.6 x 100 mm, 3.5 µm, 100Å, 130Å**	**2.1 x 100 mm, 3.5 µm, 130Å**	**2.1 x 75 mm, 2.5 µm 100Å**
L/D_p	30,000	30,000	42,900	28,600	28,600	30,000
Maximum Predicted Backpressure	2,500	2,500	7,500	5,000	7,000	10,000
Run time	50 min	50 min	**35 min**	**23 min**	**23 min**	**12 min**
Runs per hour	1.2	1.2	1.7	**2.6**	**2.6**	**5**
Total mobile phase	50 mL	71 mL	71 mL	**47 mL**	**11 mL**	**8 mL**
Injection Volume	20 µL	27.8 µL	27.8 µL	**18.5 µL**	**3.9 µL**	**2.9 µL**
Benefit(s) over monograph column	-	**Modern diameter**	**Modern diameter Decreased run time Decreased injection volume**	**Modern diameter Decreased run time Increased runs per hour Decreased mobile phase Decreased injection volume**	**Modern diameter Decreased run time Increased runs per hour Decreased mobile phase Decreased injection volume**	**Modern diameter Decreased run time Increased runs per hour Decreased mobile phase Decreased injection volume**

Table 3. *Chromatographic savings provided by gradient method adjustments allowed by the USP <621> Chromatography chapter guidance.*

CONCLUSIONS

- **With the harmonized USP <621> (Dec 1, 2022), methods can be adjusted mathematically using manual calculations, or the Waters.com online calculators, in order to meet the original monograph system suitability criteria.**
- **An offset formula, although not provided in the USP <621> guidance, can be used to adjust for differences in system dwell volume when moving from HPLC to UHPLC.**
- **Significantly reduced run time, injection volume, and solvent consumption was observed while maintaining the original, monograph separation criteria for both HPLC, Alliance iS System, and UHPLC, ACQUITY UPLC I-Class Plus and ACQUITY Arc System, platforms (Table 3).**
- **Modern LC platforms (column dimensions and instruments) can be implemented successfully when adjusting a USP monograph.**

REFERENCES

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