Liquid Chromatographic Gradient Method Allowances Provided by General Chapter, USP <621> 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. On Dec 1, 2022, a harmonized standard for General Chapter <621> Chromatography was released. This standard incorporates <621> Chromatography (USP), 2.2.46. Chromatographic Separation Techniques (EuPh) and 2.01 Liquid Chromatography (JP) texts. Additions provide limits of flexibility for liquid chromatographic gradient method separation parameters such particle size, flow rate, gradient slope, and injection volume. In this poster, we implement the gradient method adjustments described in U.S. Pharmacopeia (USP) General Chapter <621> to apply method modernization for an antiviral drug impurities monograph.

Methods

The System Suitability Test Mixture (SST) was prepared according to the USP monograph for antiviral compound, abacavir sulfate. Specifications for column size and flow rate were calculated **(Formulas A-E)** for the LC instrumentation shown in **Figure 1**.

Figure 1. *LC instrumentation employed when implementing the USP <621> gradient method adjustment guidance.*

(A) $F_2 = F_1 \times \left[\frac{(dc_1^2 \times dp_1)}{(dc_2^2 \times dp_2)}\right] = \frac{1.000\ mL}{min} \times \left[\frac{(2.1\ mm^2 \times 5\ \mu m)}{(3.9\ mm^2 \times 2.5\ \mu m)}\right] = 0.580\ mL/min$

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) $V_{inj2} = V_{inj1} \times \left[\frac{(L_2 \times dc_2^2)}{(L_1 \times dc_1^2)}\right] = 20\ \mu L \times \left[\frac{(75\ mm \times 2.1\ mm^2)}{(150\ mm \times 3.9\ mm^2)}\right] = 2.9\ \mu L$

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

(C) $t_{G2} = t_{G1} \times \left(\frac{F_1}{F_2}\right) \left[\frac{(L_2 \times dc_2^2)}{(L_1 \times dc_1^2)}\right] = t_{G1} \times \left(\frac{1.000\ mL/min}{0.580\ mL/min}\right) \left[\frac{(75 \times 2.1\ mm^2)}{(150 \times 3.9\ mm^2)}\right] = 0.250$

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

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

Vd = Dwell Volume
V0 = Column void volume = 0.66 x V
Where $V = L\pi\left(\frac{D}{2}\right)^2$

V = Empty volume (mL)
L = Column length (cm)
D = Column diameter (cm)

Offset = 0.073 mL– {1.145 mL x $\left(\frac{0.171mL}{1.182mL}\right)$} = **0.0937 mL**

Option 1:

Set Empower™ Software to hold 93.7 µL after injection (Waters' Columns Calculator)

0.0937 mL offset = 93.7 µL

Option 2:

Add 0.17 mins to all steps in the gradient
(as shown in Waters Preparative OBD Column Calculator)

0.0937 mL offset / 0.580 mL/min = 0.17 mins

(E)

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 (**OBD™ Column Calculator**)
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	

Formulas (A) *Flow rate* ***(B)*** *injection volume* ***(C)*** *gradient time, and* ***(D)*** *dwell volume offset (FIO) and* ***(E)*** *final gradient table using the dwell volume offset is demonstrated for adjusting the gradient from the monograph, 3.9 x 150 mm, 5 µm HPLC platform with dwell volume 1.145 mL, to a 2.1 x 75 mm, 2.5 µm UHPLC separation platform with dwell volume 0.073 mL.*

USP MONOGRAPH Abacavir Sulfate	Adjustment Savings	
Alliance™ HPLC System	Alliance iS HPLC System	ACQUITY UPLC I-Class PLUS System
3.9 x 150 mm, 5 µm, 100Å	4.6 X 100 mm, 3.5 µm, 100Å	2.1 x 75 mm, 2.5 µm 100Å
2,500 psi gradient	5,000 psi gradient	10,000 psi gradient
50 min run time	2x less run time	12x less run time
1 runs per hour	2x more runs / hr	5x more runs / hr
50 mL mobile phase used	Same volume mobile phase	6x less volume mobile phase
20 µL injection volume used	1.5 µL less injection volume	7x less injection volume
HPLC Platform	Modern HPLC Platform	UHPLC Platform
Monograph parameters	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 2. *Example of chromatographic savings provided by gradient method adjustments allowed by the USP <621> Chapter guidance.*

Discussion

The SST relative retention times (*RRT*)s were compared to the monograph separation after injection on each instrument. Values were most similar for columns of the same L1 stationary phase substituents. The resolution of the critical pair, in all cases, passed monograph SST method criteria, therefore all instruments, and column dimensions achieved the original, validated monograph separation.

Conclusion

In this study, recently official USP <621> gradient method adjustments provide flexibility for implementation of modern LC platforms (column dimensions and instrumentation) to result in reduced run times, reduced mobile phase and sample usage, and an overall increase in the number of sample runs per hour **(Table 2)**.

References

- Achieving Method Modernization with the New Liquid Chromatographic Gradient Allowances Provided by USP General Chapter <621> Chromatography and the Alliance HPLC System, Catharine E., Layton, Paul D. Rainville, 2023, *www.waters.com*
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