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應用手冊

USP Analysis of Glimepiride on an Alliance HPLC System: Modernization of a USP Method

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Abstract

In this application, the assay for glimepiride was performed according to the USP monograph.

Using the ACQUITY Columns Calculator, the USP assay for glimepiride was scaled to a column with a smaller particle size and a shorter length column. Additional parameters, including flow rate and injection volume, were also scaled accordingly. These modifications allowed for a reduction in the run time of over 50% and a significant reduction in the mobile phase consumed, while still meeting system suitability criteria – thereby illustrating the potential for method modernization on traditional HPLC systems.

Benefits

- Alliance system flexibility enables method modernization for USP methods
- Scalable columns allow for method adjustment
- ACQUITY UPLC Columns calculator provides tools to ensure proper method scaling, including column configuration and conditions (flow rate and injection volume)

Introduction

Many United States Pharmacopeia (USP) monographs consist of legacy methods that were developed on older and/or obsolete HPLC instrumentation. While these analyses meet the pre-defined system suitability criteria, the conditions often have long runtimes and higher flow rates, typical of HPLC column dimensions. These conditions often require significant resources such as, instrument and chemist time and solvent consumption. The USP, however, has outlined allowable method adjustments¹ thereby enabling laboratories to modernize legacy methods. These adjustments can include scaling particle size and column dimensions to maintain a constant L/d_p , where L is the length of the column and d_p is the particle size of the packing material. In this application, the assay for glimepiride was performed according to the USP monograph.² Following the allowable adjustments, the isocratic method was also scaled to a smaller particle size and column dimensions to decrease run time and improve throughput.

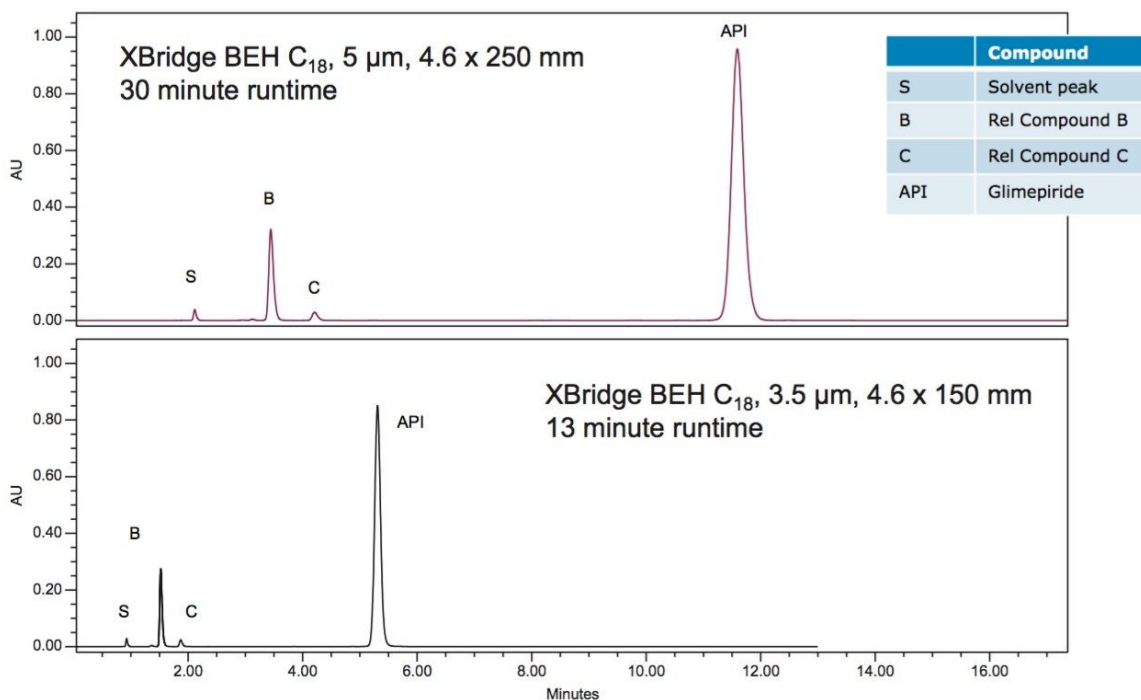


Figure 1. Comparison of the USP system suitability standard for glimepiride, using a 5 µm, 4.6 x 250 mm column (top chromatogram). The method was also scaled to a 3.5 µm, 4.6 x 150 mm (bottom chromatogram). All method adjustments were performed following USP Chapter <621> guidelines.

Experimental

All standards and samples were prepared following the USP monograph.² Glimepiride standard (p/n 1292303), related compound B (p/n 1292325), related compound C (p/n 1292336) were purchased from United States Pharmacopeia. For the glimepiride standard preparation, 20 mg was weighed and dissolved in 80/20 acetonitrile/water (v/v) and brought to volume in a 100 mL volumetric flask yielding a final concentration of a 0.2 mg/mL. Stock solutions containing related compound B and related compound C were prepared in 80/20 acetonitrile/water at 0.1 mg/mL. The system suitability standard was then prepared by combining 1 mL of related compounds stock and adding 49 mL of the 0.2 mg/mL glimepiride standard solution, yielding final concentrations of 0.196 mg/mL (glimepiride) and 2 µg/mL (related compound B and C). The glimepiride drug substance sample was prepared by transferring 20 mg of sample into a 100 mL volumetric flask and bringing to volume with 80/20 acetonitrile/water (v/v).

Common conditions

LC system:	Alliance e2695 with a 2998 PDA and HPLC Analytic mm flow cell (column preheater installed)
Mobile phase:	0.5 g of monobasic sodium phosphate in 500 mL adjusted to pH 2.1–2.7 then add to 500 mL of acetonitrile
Column temperature:	25 °C
UV wavelength:	228 nm
Wash Solvent:	80/20 acetonitrile/water
Sample:	0.2 mg/mL of glimepiride in 80/20 acetonitrile/water
Chromatography data system:	Empower 3 FR2

Varied method parameters

Method:	Official USP monograph
Flow rate:	1.2 mL/min
Column:	XBridge BEH C ₁₈ , 130Å, 5 µm, 4.6 x 250 mm (p/n 186003117) (L/d _p =50,000)
Injection volume:	20 µL
Run time :	30 min
Method:	Scaled USP monograph
Flow rate:	1.71 mL/min

Method:	Scaled USP monograph
Column:	XBridge BEH C ₁₈ , 130Å, 3.5 μm, 4.6 x 150 mm (p/n 186003034) (L/d _p =42,857)
Injection volume:	12 μL
Run time :	13 min

Results and Discussion

The assay for glimepiride was performed following the USP monograph on an Alliance HPLC System with a 2998 Photodiode Array Detector and a 30 cm column heater equipped with a passive preheater (Figure 1, top chromatogram). The samples were prepared as previously described according to the USP monograph.² Five injections of the system suitability standard and the API standard were analyzed.

The system suitability criteria for the assay states, “the resolution, R, between glimepiride related compound B and glimepiride related compound C is not less than 4.0... [and] the relative standard deviation (RSD) for replicate injections is not more than 2.0%.”¹ Based on the specified RSD requirements and USP Chapter <621>, the results generated using both methods met system suitability criteria (Table 1). In addition, the relative standard deviation for glimepiride was 0.11% for the retention time and less than 0.3% for peak area. Both of which are well within the system suitability criteria for this assay of NMT 2.0%. The USP resolution for related compound B and related compound C of 4.6 meet the system suitability criteria of not less than 4.0.

	USP System Suitability Criteria	5um, 4.6x 250 mm column	3.5 um, 4.6 x 150 mm column
Glimepiride retention time % RSD (5 injections)	NA	0.11%	0.05%
Glimepiride area % RSD (5 injections)	Not more than (NMT) 2.0%	0.25%	0.27%
USP resolution between related compound B and related compound C	Not less than (NLT) 4.0	4.6	4.0

Table 1. USP system suitability results for glimepiride under the original USP method conditions and the scaled USP method conditions.

The HPLC assay for glimepiride, while meeting system suitability criteria, requires a 4.6 x 250 mm column and a

30 minute run time. Method adjustments were desired to decrease the overall run time. The method was scaled according to the USP guidelines,¹ which permits scaling column dimensions, particle size, and flow rate for isocratic separations. Keeping the L/dp ratios within -25% to +50% of the original method, the 4.6 x 250 mm, 5 µm column was scaled to a 4.6 x 150 mm, 3.5 µm column (Figure 1, bottom chromatogram). The flow rate was adjusted based on particle size of the column as per USP Chapter <621> based on $F_2 = F_1 (d_2^2 / d_1^2)$ where F= flow rate and d= diameter of the column. The injection volume was also adjusted for the column volume. All calculations were performed using the ACQUITY UPLC Columns Calculator (Figure 2).

Analytical Factors

Isocratic Gradient

Molecular Weight: **0-100** Da

Column Temperature: **25** °C

Retention Factor (k'): **8**

Calculate

From HPLC → **To HPLC**

From HPLC CV = 2.742 mL L/dp = 50,000

Column Length (L): **250** mm

Column Diameter: **4.6** mm

Particle Diameter (dp): **5** µm

Injection Volume: **10.0** µL

To HPLC CV = 1.645 mL L/dp = 42,857

Column Length (L): **150** mm

Column Diameter: **4.6** mm

Particle Diameter (dp): **3.5** µm

Original Isocratic Flow

1944 psi

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
▶ 1	Initial	1.20	100.0	0.0	0.0	0.0	--

Figure 2. ACQUITY UPLC Columns Calculator set up for scaling the USP method.

As with the original HPLC method, the scaled method met all the system suitability requirements (Table 1). The glimepiride retention time RSD was 0.05% while the peak area was 0.27%. In addition, the USP resolution, while slightly lower, met the system suitability criteria with a value of 4.0 for all injections.

To determine the stability of the scaled method, the drug substance samples were bracketed by five replication injections of the standard, aliquoted into two separate vials. Each analysis was 13 minutes, therefore the series of injections occurred over 3.25 hours. Analysis of the standards show the results meet the USP system suitability

criteria, with a glimepiride peak area RSD of 0.31 and a retention time RSD of 0.22 (Table 2).

Name: Glimepiride

	Name	Sample name	Injection	RT	Area
1	Glimepiride	Glimepiride_Std_1	1	5.34	4100595
2	Glimepiride	Glimepiride_Std_1	2	5.34	4134751
3	Glimepiride	Glimepiride_Std_1	3	5.34	4127391
4	Glimepiride	Glimepiride_Std_1	4	5.35	4117872
5	Glimepiride	Glimepiride_Std_1	5	5.35	4127247
6	Glimepiride	Glimepiride_STD_2	1	5.36	4111610
7	Glimepiride	Glimepiride_STD_2	2	5.37	4096476
8	Glimepiride	Glimepiride_STD_2	3	5.37	4115210
9	Glimepiride	Glimepiride_STD_2	4	5.37	4119435
10	Glimepiride	Glimepiride_STD_2	5	5.37	4101000
Mean				5.36	4115159
Std. dev.				0.01	12163
% RSD				0.22	0.31

Bracket 1 (rows 1-5)
 Sample injections (5) (row 5)
 Bracket 2 (rows 6-10)

Table 2. The report table comparing the 2 sets of standard injections that bracket the five replicate injections of the sample.

As mentioned previously, the active pharmaceutical substance of glimepiride was analyzed using the scaled assay for glimepiride (Figure 3). The percentage of glimepiride was found to be 99.67%. This value was calculated by using the formula found in the USP monograph.

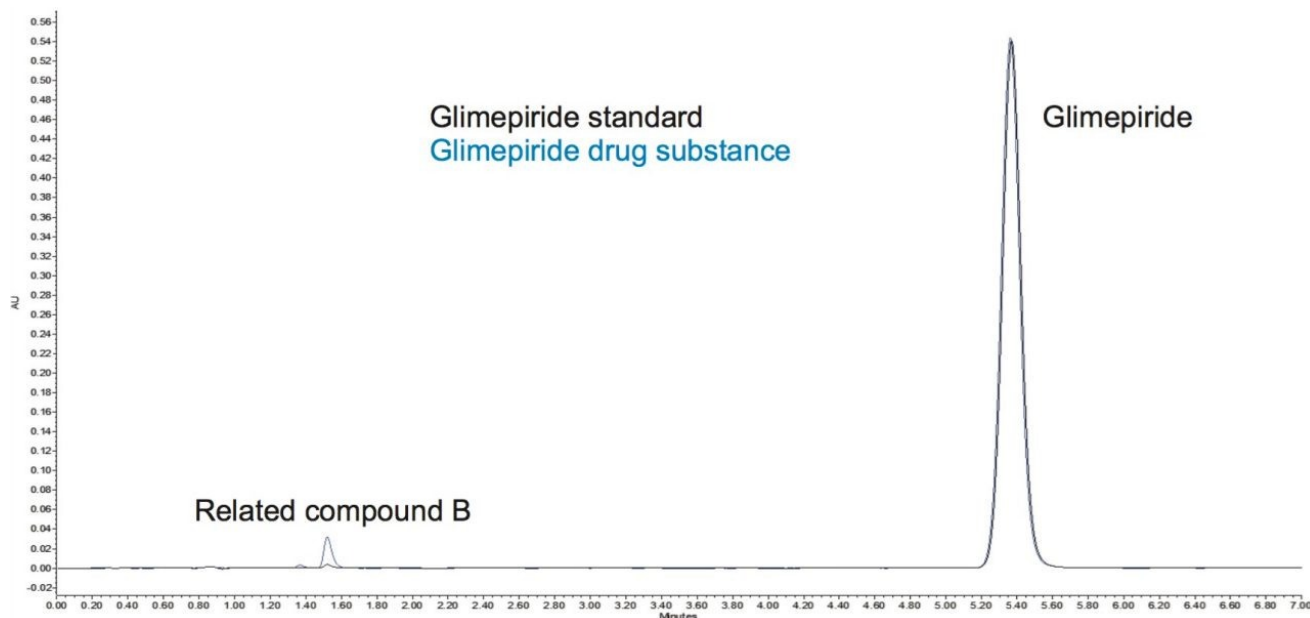


Figure 3. Overlay of standard and sample chromatograms on the Alliance HPLC system, performed using the scaled column dimensions. Blue chromatogram is the sample, black chromatogram is the standard.

Conclusion

By taking advantage of the guidelines in USP Chapter <621>, many legacy methods can be updated for higher throughput while still maintaining current system suitability criteria on legacy HPLC instrumentation. Using the ACQUITY Columns Calculator, the USP assay for glimepiride was scaled to a column with a smaller particle size and a shorter length column. Additional parameters, including flow rate and injection volume, were also scaled accordingly. These modifications allowed for a reduction in the run time of over 50% and a significant reduction in the mobile phase consumed, while still meeting system suitability criteria – thereby illustrating the potential for method modernization on traditional HPLC systems.

References

1. Chapter <621> *CHROMATOGRAPHY* United States Pharmacopeia and National Formulary (USP38-NF33 S1) Baltimore, MD: United Book Press, Inc.; 2015. p. 424.
2. Glimepiride Official Monographs, USP38–NF33. United States Pharmacopeia and National Formulary (USP 38-NF 33 S1) Baltimore, MD: United Book Press, Inc.; 2015. p. 3671.

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720005695, May 2016



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