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Application News

No. AD-0173

HPLC Columns – USP Analysis

High Speed Analysis of Pregabalin in Accordance with Chapter 621 in USP 40

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❑ Introduction

Many United States Pharmacopeia (USP) monographs were developed using traditional 5 µm column where high flow rates and long runtimes are commonly needed. Such methods require high running cost, long analysis time, and large solvent consumptions. Advance in ultra-high-performance LC and micro-particle column packing material provides a solution to improve productivity and reduce the running cost incurred in USP analysis.

USP General Chapter <621> stated the permissible range within which HPLC and GC parameters may be changed. Analysis method can be modified so long as the values are within the permissible ranges and fulfilled the system suitability requirements. Here, we introduced an example of isocratic analysis of Pregabalin monography in compliance with General Chapter 621. Pregabalin is an antiepileptic agent used for neuropathic pain. Shim-pack™ GIST C18 analytical column, Prominence™-i LC-2030C Plus and Nexera™ X2 system were employed in this application.

❑ High-Speed Analysis Conditions

USP analysis can be modified to shorten analysis time and reduce operational cost. Application News L464 listed the details pertaining to changes allowed for fast USP-compliant analysis. Permissible changes include shortening column length, reducing column inner diameter and increasing column flow rate. Table 1 lists the allowable adjustments to HPLC parameters according to General Chapter 621. Adjustable parameters include particle size, flow rate, column length and diameter. Analysis of Pregabalin was performed under isocratic conditions.

Changes to column dimension are allowed so long as the ratio of column length (L) to column particle size (dp) are within the permissible range (-25% to +50%) (Table 1). This is to preserve the peak-resolution. Original USP method employed a (1) 4.6 x 250 mm, 5 µm particle size column (Table 2). (2) 3.0 × 150 mm, 3 µm particle size column, and (3) 2.1 × 100 mm, 2 µm particle size column, were selected for the fast USP method while keeping the L/dp ratio constant. Flow rates of 0.5 mL/min and 0.7 mL/min were selected for the 2 new columns following the calculation as stated in USP method. Details of analytical conditions are stated in Table 3.

Table 1: Allowable adjustment to HPLC parameters according to General Chapter 621.

Particle size (dp)	L/dp ratio constant or Theoretical plate number: -25 to +50%
Column length (L)	
Column I.D. (dc)	Any allowed if linear velocity is constant
Flow rate*	Combination of dp and dc: ± 50%
Injection vol.	Can be adjusted as consistent with precision and detection limits
Column temp.	± 10° C

*$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$
F_1 and F_2 represent flow rates of the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters; dp_1 and dp_2 are the respective particle sizes.

Table 2: Selection of columns for analysis of Pregabalin.

USP method	Column dimension	L/dp	Ratio
Original	4.6 x 250 mm, 5 µm	50000	1 (100%)
Fast	3.0 x 150 mm, 3 µm	50000	1 (+0%)
	2.1 x 100 mm, 2 µm	50000	1 (+0%)

Table 3: Analytical conditions for USP analysis of Pregabalin.

System	(1) LC-2030C Plus (2) Nexera X2
Column	(1) Shim-pack GIST C18 (4.6 x 250 mm, 5 µm)* (2) Shim-pack GIST C18 (3.0 x 150 mm, 3 µm)* (3) Shim-pack GIST C18 (2.1 x 100 mm, 2 µm)
Mobile phase	Water / Acetonitrile = 95/5 (v/v)
Flow rate	(1) 1.0 mL/min (2) 0.7 mL/min (3) 0.5 mL/min
Column temp.	25°C
Injection vol.	(1) 20 µL (2) 1 µL (3) 1 µL
Detection	(1) LC-2030C Plus at 205 nm (2) SPD-M30A at 205 nm
Flow cell	(1) Standard cell (for LC-2030C Plus) (2) Standard cell (for SPD-M30A)

**Prominence-i LC-2030C Plus system with standard UV cell was used for analysis with column 1 and 2.*

❑ Results & Discussion

Retention time of Pregabalin was shorter in the fast method, 1.9 min and 3.7 min respectively, as compared to 9.8 min in the original USP method (Figure 1, Table 4). Result produced with column 2 also showed that analysis of Pregabalin using fast method is achievable on Prominence-i system. This suggests the efficiency of Prominence-i system and the Shim-pack GIST C18 column in performing fast analysis where analysis time is shortened and solvent consumption is reduced.

Table 4: Analysis results of Pregabalin.

System Suitability		Column		
		1	2	3
Tailing factor	≤ 1.5	1.21	0.98	0.96
RSD	≤ 0.73%	Rt 0.07	Rt 0.17	Rt 0.23
		Area 0.20	Area 0.34	Area 0.15

❑ Conclusion

This study demonstrated the ability of Nexera X2 and Prominence-i system, and Shim-pack GIST C18 column in analysis of Pregabalin, in conformity with the USP General Chapter 621. The traditional USP analysis was improved with the fast method, where the analysis time was shortened and solvent consumption was reduced. Additionally, the fast method is applicable on both Prominence-i and Nexera X2 system.

❑ References

1.Application News No. L464, Shimadzu https://solutions.shimadzu.co.jp/an/n/en/hplc/jpl214028.pdf
2.USP General Chapter 621, USP 40 – NF 35, First Supplement
3.USP Monograph, Pregabalin, USP 40 – NF35, First Supplement

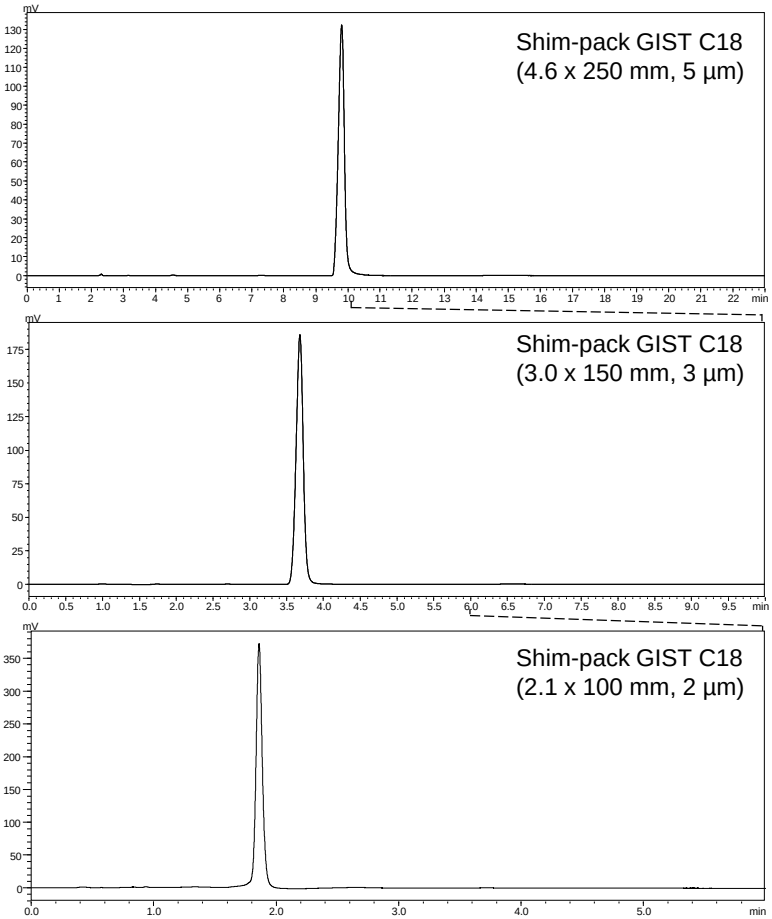

Figure 1: Chromatogram of USP analysis for Pregabalin (2 mg/mL) with column (1) Shim-pack GIST C18 (4.6 x 250 mm, 5 µm), (2) Shim-pack GIST C18 (3.0 x 150 mm , 3 µm), (3) Shim-pack GIST C18 (2.1 x 100 mm , 2 µm).

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