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Application News

No. AD-0171

HPLC Columns – USP Analysis

High Speed Analysis of Budesonide 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 were commonly needed. Such methods require high solvent consumption and high running cost for instrument and analysis time. 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 ranges within which HPLC and GC parameters may be changed. Analysis method can be modified so long as the values are within the permissible range and fulfilled the system suitability requirements. Here, we introduced an example of isocratic analysis of Budesonide monography in compliance with General Chapter 621. Budesonide is a glucocorticoid used in the management of asthma, the treatment of various skin disorders and allergic rhinitis. Shim-pack GIST C18 analytical column, Prominence-i LC2030C Plus system were employed in this application.

❑ Permissible adjustment to HPLC Parameters

Table 1 listed the allowable adjustment to HPLC parameters according to General Chapter 621. Adjustable parameters include particle size, flow rate, column length and diameter. Analysis of Budesonide was performed under isocratic conditions.

❑ High-Speed Analysis Conditions

USP analysis can be modified to shorten analysis time and reduce running 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 while maintaining the linear velocity.

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 column with 4.6 mmI.D. × 150 mmL., 5 µm particle size (Table 2). 2 columns, (2) 3.0 mmI.D. × 100 mmL., 3 µm particle size, and (3) 3.0 mmI.D. × 50 mmL., 2 µm particle size (Table 2) were selected for the fast USP method while keeping the L/dp ratio constant. Flow rates 0.8 mL/min and 1.0 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 Budesonide.

USP method	Column dimension	L/dp	Ratio
Original	4.6 x 150 mm, 5 µm	30000	1 (100%)
Fast	3.0 x 100 mm, 3 µm	33333	1.11 (+11%)
	3.0 x 50 mm, 2 µm	25000	0.8 (-17%)

❑ Results & Discussion

Retention time of Budesonide was shorter in the fast method as compared to the original USP method (Figure 1,Table 4). Result also showed that analysis

of Budesonide using fast method was achievable on Prominence-i system. This suggests the robustness and efficiency of Prominence-i system and the Shim-pack column in performing fast analysis where analysis time is shortened and solvent consumption is reduced.

Table 3: Analytical conditions for USP analysis of Budesonide.

System	LC-2030C Plus
Column	(1) Shim-pack GIST C18 (4.6 x 150 mm, 5 µm) (2) Shim-pack GIST C18 (3.0 x 100 mm, 3 µm) (3) Shim-pack GIST C18 (3.0 x 50 mm, 2 µm)
Mobile phase	A) 3.17 mg/mL monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid (pH 3.2 ± 0.1) B) Acetonitrile A/B = 68/32 (v/v)
Flow rate	(1) 1.50 mL/min (2) 1.00 mL/min (3) 0.78 mL/min
Column temp.	25°C
Injection vol.	(1) 20 µL (2) 5 µL (3) 1 µL
Detection	LC-2030C Plus at 254 nm
Flow cell	Standard cell (for LC-2030C Plus)

Table 4: Analysis results of Budesonide.

System Suitability	Column		
	1	2	3
Resolution (≥ 1.5)	2.3	2.2	2.1
USP plate count for Epimer B (≥ 5500)	8274	7533	7406
Relative retention time (Epimer A = 1.1 x Epimer B)	1.1	1.1	1.1

❑ Conclusion

This study demonstrated the ability of Prominence-i system and Shim-pack GIST C18 column in analysis of Budesonide 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 Prominence-i system.

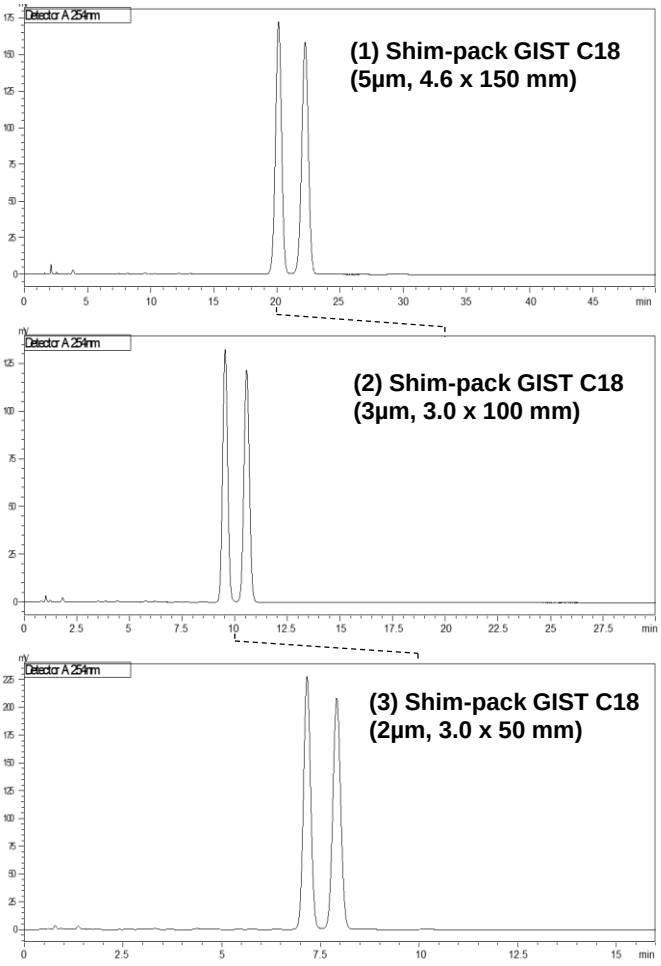

Figure 1: Chromatograms of Budesonide (0.5 mg/mL) following USP with different columns.

❑ References

1.Budesonide, https://www.ncbi.nlm.nih.gov/mesh/68019819
2.Application News No. L464, Shimadzu https://solutions.shimadzu.co.jp/an/n/en/hplc/jpl214028.pdf
3.USP General Chapter 621, USP 40 – NF 35, First Supplement
4.USP Monograph, Budesonide, USP 34 – NF29, First Supplement

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