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Application News

No. LC-28-ADI-051

HPLC Columns –USP Method Transfer

High Speed Analysis of Cyanocobalamin in compliance with Chapter 621 of USP40

❑ Introduction

Most of the monographs in United States Pharmacopeia (USP) were developed using HPLC with traditional 5 µm column wherein high flow rates and long runtimes are commonly needed. Such methods require high running cost, longer analysis time and solvent consumption.

Advances in ultra-high-performance LC and microparticle column packing materials, provide a solution to improve productivity and reduce the running cost incurred in USP analysis.

USP General Chapter 621[1] 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 ranges and fulfill the system suitability requirements. Here, we introduced an example of isocratic analysis of cyanocobalamin[2] in compliance with General Chapter 621. Cyanocobalamin is a vitamin and responsible for many body functions. Shim-pack VP-ODS which is categorised as L1 analytical column, Prominence-i LC2030C Plus system and newer coreshell technology column were used 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 Cyanocobalamin was performed under isocratic conditions.

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 ID (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

Equation 1: Flow rate calculation for high speed analysis of Cyanocobalamin.

USP requirement of Chromatography <621>

$$F_2 = F_1 \times \frac{dc_2^{\,2} \times dp_1}{dc_1^{\,2} \times dp_2}$$

L : Column length
dp : Particle size
F : Flow rate
dc : Internal diameter of the column

F1 and F2 represent flow rates of the original and modified conditions, respectively; dc1 and dc2 are the respective column diameters; dp1 and dp2 are the particle sizes

❑ Speed enhancement for USP method

USP monograph method can be modified to shorten analysis time and reduce operational cost. Application News L464[3] 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 resolution of separation.

The assay of cyanocobalamin with 5 µm fully-porous ODS column described in the USP monograph is transferred to the new method with Shim-pack Velox C18, 2.7 µm column, within USP allowable adjustment. Analytical time and solvent consumption can be saved with transferred methods while meeting the requirements of system suitability. Original USP method employed a column with 4.6 mmI.D.X150 mmL.,5 µm particle size as shown in Table 3. The 4.6 mmI.D. X100 mmL., 2.7 µm particle size column was selected for the fast USP method while keeping the L/dp ratio constant. Flow rates 0.5 mL/min and 0.93 mL/min were selected for the analysis. The flow calculations are used as stated in USP to estimate the flow rates for the analysis as shown in Equation 1. The methods with different flow rates were tried to get better results. Details of analytical conditions are stated in Table 3.

Table 3. HPLC analysis conditions

System	(1) LC-2030C Plus
Column	(1) Shim-pack VP-ODS (4.6 X 150 mm, 5 µm)
	(2) Shim-pack Velox C18 (4.6 X 100 mm, 2.7 µm)
Mobile Phase	A) Water
	B) Methanol
	A/B = 13:7 (v/v)
Flow rate	(1) 0.50 mL/min
	(2) 0.93 mL/min
Column Temp	25°C
Injection Vol.	(1) 100 µL
	(2) 67 µL
UV Detection	LC-2030C Plus at 361 nm

❑ Shimadzu Consumables for Analysis

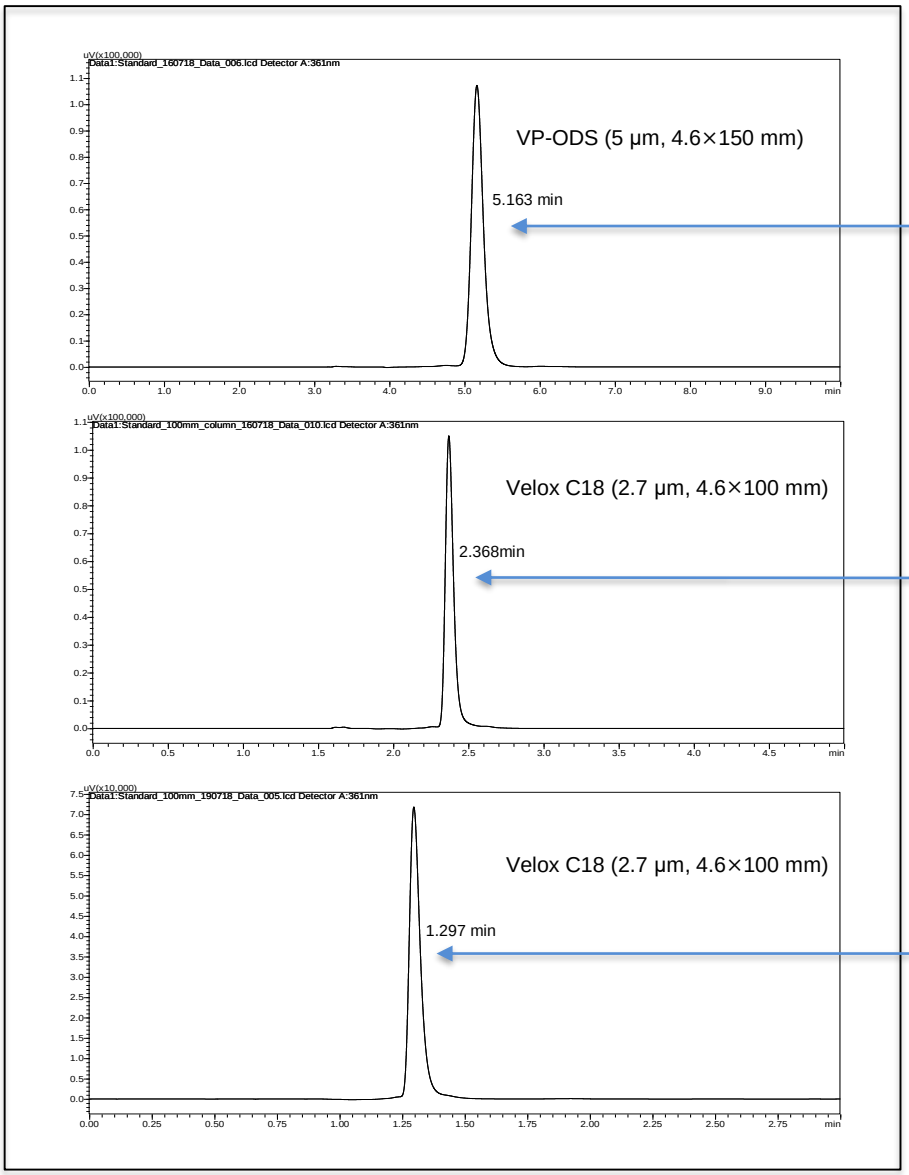
1. 1.5 ml Screw- thread amber vial (P/N : 226-54111-11)
2. Caps with PTFE/white silicone septa (P/N : 226-54113-01)
3. Solvent bottle, 1L (P/N : 226-88583-02)
4. Solvent safety caps kit 2 ports (P/N : 226-50319-01)
5. LC Solvent waste kit (P/N : 226-50330-00)
6. Very high pressure PEEK fitting (P/N : 226-50106-02)
7. Syringe filter, 25 mm, Nylon, 0.45 um (P/N : 226-52125-45)

❑ Results

Retention time of cyanocobalamin are shorter in the fast methods with flow rate of 0.93 and 0.5 mL/min eluting at 1.297 mins and 2.368 mins respectively, as compared to 5.163 mins in the original USP method (Figure 1, Table 4). Result also showed that analysis of cyanocobalamin using fast method is achievable on Prominence-i system. First, all parameters were kept same and acquired data with Velox column with 67 uL injection volume. This gave faster elution but *N* were + 81% which is more than USP-621 limit. The method was further modified to get faster elution and *N* in specified limits. The flow rate of 0.93 ml/min gives faster analysis and *N* in specified limits which improves productivity of HPLC system.

Table 4. Results

Column	*L/dp*	Flow rate (mL/min)	*N*	System suitability test result (Requirement: %RSD < 2.0 %)
VP-ODS (5 µm, 4.6×150 mm)	30,000	0.50	5,244	tR: 0.025 % Area: 0.175 % (n=6)
Velox C18 (2.7 µm, 4.6×100 mm)	37,037 (+23 %)	0.50	9,497 (+81 %)	tR: 0.035 % Area: 0.103 % (n=6)
		0.93	4,466 (-15 %)	tR: 0.084 % Area: 0.220 % (n=6)



Original Method
Shim-pack VP-ODS (5µm, 4.6×150 mm)
Flow rate: 0.5 mL/min
Injection Volume: 100 µL
Column temperature: 25 ℃
Detection: UV 361 nm

Faster Method
Shim-pack Velox C18 (2.7 µm, 4.6×100 mm)
Flow rate: 0.50 mL/min
Injection Volume: 67 µL
Column temperature: 25 ℃
Detection: UV 361 nm

Optimized Method
Shim-pack Velox C18 (2.7 µm, 4.6×100 mm)
Flow rate: 0.93 mL/min
Injection Volume: 67 µL
Column temperature: 25 ℃
Detection: UV 361 nm

❑ Conclusion

This study demonstrated the ability of Prominence-i system, and Shim-pack Velox column in analysis of cyanocobalamin, in conformity with the USP General Chapter 621.

The traditional USP analysis was improved with the fast method, where the analysis time was shortened considerably, and solvent consumption was reduced with fulfilling the allowance of method transfer in USP-621

❑ Reference

1. USP General Chapter 621, USP 40 – NF 35, First Supplement.
2. USP Monograph, Cyanocobalamin, USP 40 – NF35, First Supplement.
3. Application News No. L464, Shimadzu

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