

Application News

No. L558

High Performance Liquid Chromatography

USP-Compliant Analysis of Vitamin in Dietary Supplement : Analysis of Cyanocobalamin by Nexera™ XR

The United States Pharmacopeia (USP) is used as one standard in quality control of dietary supplements.

Vitamin B₁₂ is a general name for vitamins which contain cobalt. Although vitamin B₁₂ is involved in blood formation and metabolism, it cannot be synthesized in the body and is rarely found in foods of plant origin. Because vitamin B₁₂ deficiency occurs easily, vitamin B₁₂ is included in many dietary supplements⁽¹⁾. Generally, the amount of vitamin B₁₂ is expressed as the cyanocobalamin equivalent value.

In USP40-NF35, "Oil and Water Soluble Vitamins with Mineral Tablets Cyanocobalamin" describes two analytical methods for cyanocobalamin, an analytical method by HPLC and a microbial assay⁽²⁾.

Here, a USP40-NF35-compliant system suitability test for cyanocobalamin, representing vitamin B₁₂, was conducted using a Shimadzu Nexera XR. An analysis of a commercial multivitamin tablet was also carried out, and the compatibility of Nexera XR and Prominence™ was verified.

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Analysis Example: System Suitability Test

Table 1 shows the analytical conditions of the system suitability test for cyanocobalamin. Table 2 shows the reference value of the test. Fig. 1 shows the chromatogram of the cyanocobalamin standard solution, and Table 3 shows the results of repeatability for cyanocobalamin.

The relative standard deviation (%RSD) of the peak area satisfied the reference value, confirming that the Nexera XR possesses system suitability.

Table 1 Analytical Conditions for Cyanocobalamin

System	: Nexera XR
Column	: Shim-pack™ GIS-C18 (150 mm × 4.6 mm I.D., 5 μm; USP code L1)
Mobile Phase	: Methanol / water =35:65
Flow Rate	: 0.5 mL/min
Column Temp.	: 20 °C
Injection Vol.	: 200 μL*
Detection	: PDA 550 nm (190 - 800 nm)

*: With additional 500 μL sample loop

Table 2 Reference Value of System Suitability Test for Cyanocobalamin

Relative standard deviation (%RSD) (n = 6)	≤3
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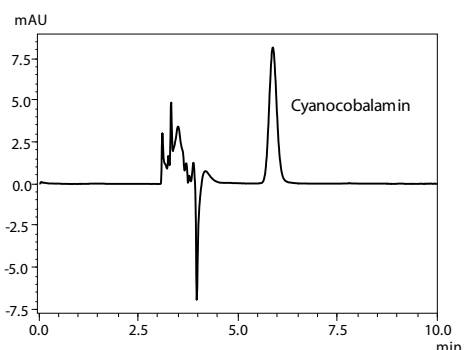


Fig. 1 Chromatogram of Cyanocobalamin (1 mg/L)

Table 3 Repeatability for Cyanocobalamin (n = 6)

	%RSD	
	Retention time	Peak area*
Cyanocobalamin	0.122	0.867

*: USP40-NF35 standard: 3% or less

Analysis of Commercial Supplement

Commercial supplement "Multivitamin Tablet" was analyzed. Fig. 4 shows the pretreatment workflow, and Fig. 2 shows the chromatogram of the commercial supplement.

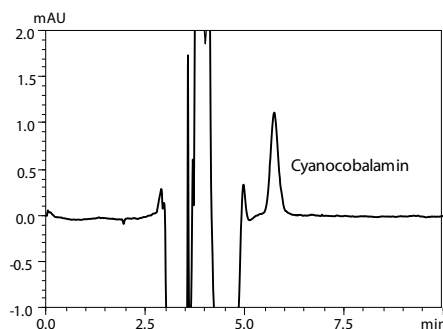


Fig. 2 Chromatogram of the Commercial Supplement

Absorption Spectrum of Cyanocobalamin

The retention time of the peak obtained from the analysis of the commercial supplement and the spectrum obtained from the peak were compared with those of the standard solution (Fig. 1) and identified.

Fig. 3 shows the spectra of cyanocobalamin in the standard solution and in the commercial supplement. The spectra have been normalized for easy comparison.

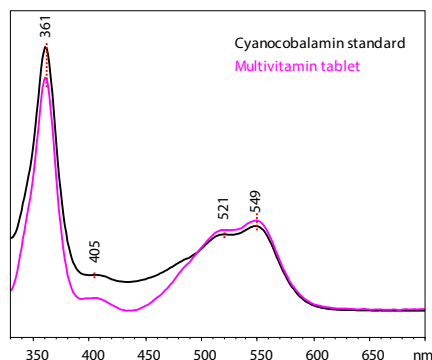


Fig. 3 Spectra of Cyanocobalamin

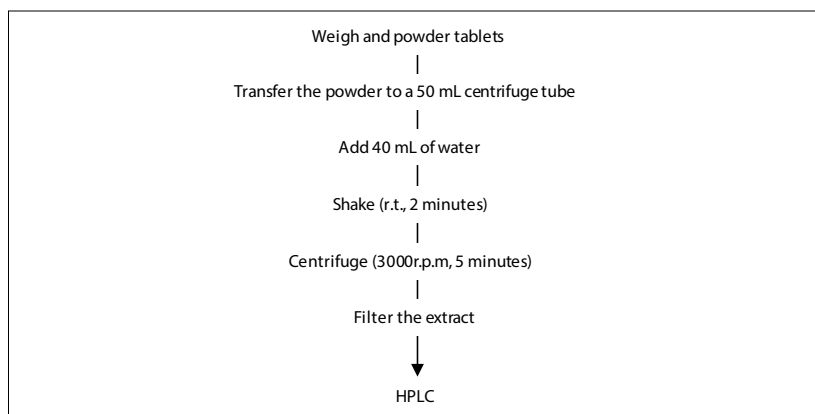


Fig. 4 Pretreatment Workflow in Cyanocobalamin Analysis

Compatibility with Prominence Series

The system suitability test and analysis of the commercial supplement described above were also carried out in the same manner with a Shimadzu Prominence series instrument. The analytical conditions and pretreatment workflow were the same as those in Table 1 and Fig. 4, respectively.

Fig. 5 shows the chromatograms of the cyanocobalamin standard solution, and Fig. 6 shows the chromatograms of the commercial supplement. Table 4 shows the quantitation values of cyanocobalamin in the commercial supplement by the two systems.

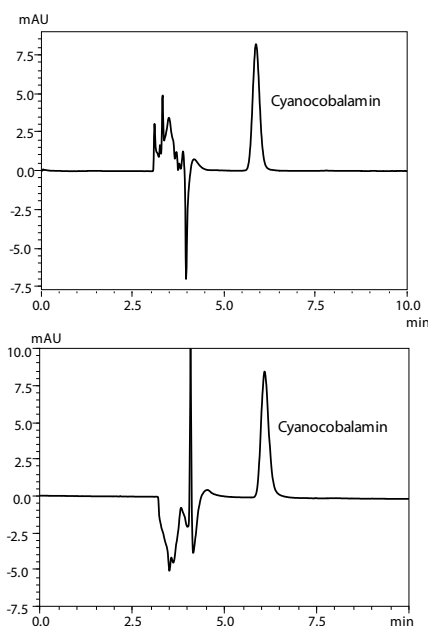


Fig. 5 Chromatograms of Cyanocobalamin (1 mg/L) (Top: Nexera XR, Bottom: Prominence)

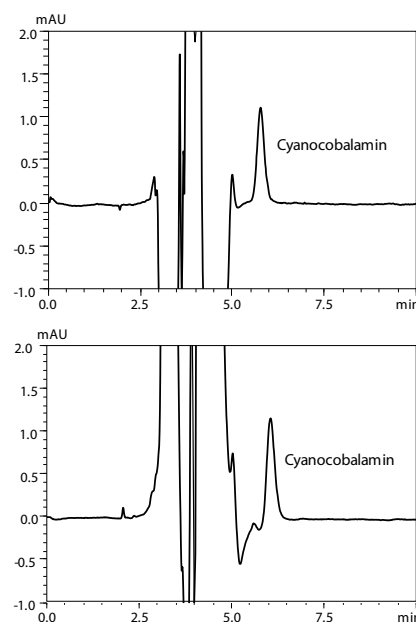


Fig. 6 Chromatograms of the Commercial Supplement (Top: Nexera XR, Bottom: Prominence)

Table 4 Quantitation Values of Cyanocobalamin in the Commercial Supplement

Calculated amount/tablet	Nexera XR	0.44 μg
	Prominence	0.49 μg
Labeled amount/tablet*	0.45-1.25 μg	

* : USP40-NF35 standard: 90% to 150% of labeled amount

Conclusion

The Nexera XR met the system suitability requirement through cyanocobalamin analysis, a typical type of vitamin B₁₂. The obtained results were compatible to those from the existing Prominence.

<References>

- (1) National Institutes of Biomedical Innovation, Health and Nutrition (<https://hfnet.nibiohn.go.jp/contents/detail177.html>)
- (2) United States Pharmacopeia 40: 7384 "Oil and Water Soluble Vitamins with Mineral Tablets Cyanocobalamin"

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