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Application News

No.LC-32-ADI-063

HPLC

Analysis of Cyanocobalamin tablets, assay procedure 2 as per USP41-NF36 monograph, using Shimadzu Nexera X2 Fast LC system

Introduction

Cyanocobalamin tablets (1000mcg) is a prescription and over-the-counter (OTC) synthetic form of vitamin B12 used to prevent and treat low blood levels of vitamin B12 (shown in Figure 1.).Vitamin B12 is the "generic descriptor" name for any vitamers of vitamin B12.Since, humans and animals can convert cyanocobalamin to any one of the active vitamin B12 compounds, by definition this makes cyanocobalamin itself a form(or vitamer) of B12.

USP41-NF36 monograph for cyanocobalamin tablet defines a UHPLC method for high throughput fast analysis demanding a competent UHPLC system.

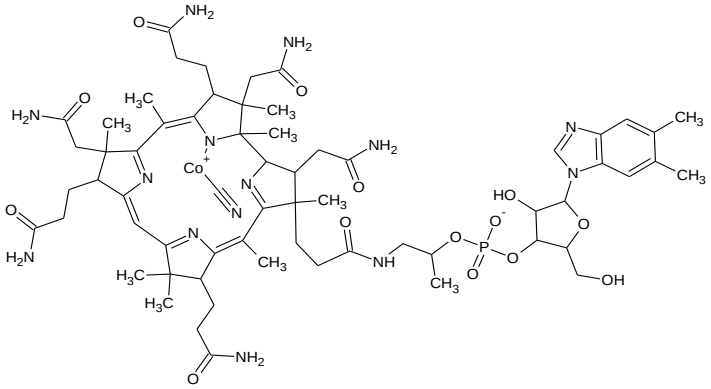

Figure 1. Structure of Cyanocobalamin

Here, we demonstrate the analysis of cyanocobalamin tablets as per USP41-NF36 monograph 1114, procedure 2[1] performed on Shimadzu Nexera X2 fast LC system in compliance with system suitability requirements of USP monograph within the permissible limits of USP chapter 621[2].

Shimadzu Nexera X2 system increases the potential of UHPLC analysis by,

Maximizing Analytical Reliability

Micro-volume plungers providing precise solvent delivery and low injection volumes to achieve excellent injection reproducibility and extremely low carryover.

Maximizing Throughput

With a pressure range up to 130 MPa, high-speed injection, overlapping injection and highly efficient gradient mixing, Nexera enables ultra-high speed and ultra-high resolution analysis.

Maximizing Expandability

Column oven and autosampler, along with the modular flexibility of the system, expand the application ranging from high-temperature analysis, green LC, auto-sample pretreatment to multidimensional LC separation.

Experimental

Chromatographic conditions, mobile phase preparations, standard and sample preparations were done in accordance to USP monograph for cyanocobalamin tablets. System suitability parameters checked as per requirements of USP monograph(refer Table 1.).

Buffer preparation: Dissolve 470.7mg of low UV hexane sulfonic acid sodium salt in water, add 1mL of phosphoric acid, dilute with water to 1000mL, and mix. Adjust pH with 50% potassium hydroxide to 3.5.

Standard preparation: 1 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in water.

Sample preparation: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 25µg of cyanocobalamin, to a suitable Erlenmeyer flask with a stopper, pipet 25ml of water, sonicate for 5min,shake vigorously for 2min and pass through a membrane filter of 0.22-µm pore size.

Table 1. Analytical Conditions

Column	:2.1mm X 10cm;1.7µm packing L1
Column Temperature	:35°C
Mobile Phase A	:Buffer
Mobile Phase B	:Acetonitrile
Gradient program (B %)	0.0-0.5min → 1.0 (%); 0.5-1.2min → 1.0-2.3(%); 1.2-1.4min → 2.3-5.0(%); 1.4-2.5min → 5.0-7.0(%); 2.5-5.0min → 7.0-18(%); 5.0-5.5min → 18-25(%); 5.5-6.5min → 25(%); 6.5-7.0min → 25.0-1.0(%); 7.0-8.0min → 1.0 (%);
Flow Rate	:0.5 mL/min
Total Run Time	:8.0 min
Injection Volume	:15 µL
Detector	:UV
Wavelength	: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)

Results

The Retention time of cyanocobalamin in standard and sample solutions is 6.38 minutes in USP method (refer Figure 2.)

The Peak for cyanocobalamin showed tailing factor of 1.51 and theoretical plates count of 80,833 which complies with USP chapter 621.

The % RSD for retention time and peak area for six replicates of standards and samples are well within the USP system suitability criteria of relative standard deviation NMT 2.0%.(refer Table 2. and Table 3.)

Overlay of six replicates of standards and samples is shown in Figure 3. and Figure 4. respectively.

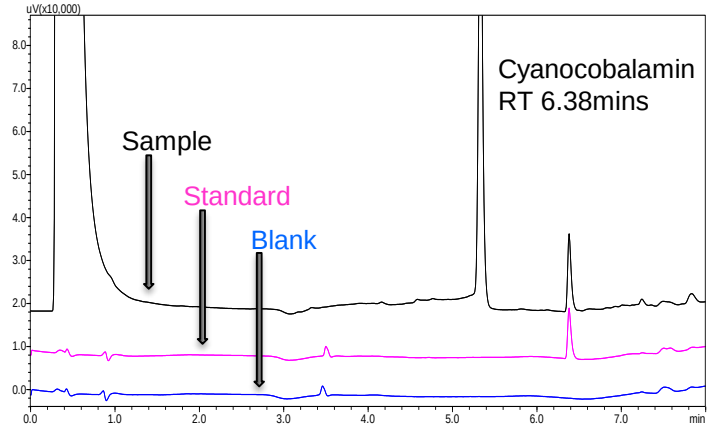

Figure 2. Overlay of blank, standard and sample

Table 2. Cyanocobalamin standard

Parameter	Observed	USP Criteria
%RSD Retention Time	0.09	NMT 2.0
%RSD Area	0.26	NMT 2.0
USP Tailing	1.51	NMT 2.0

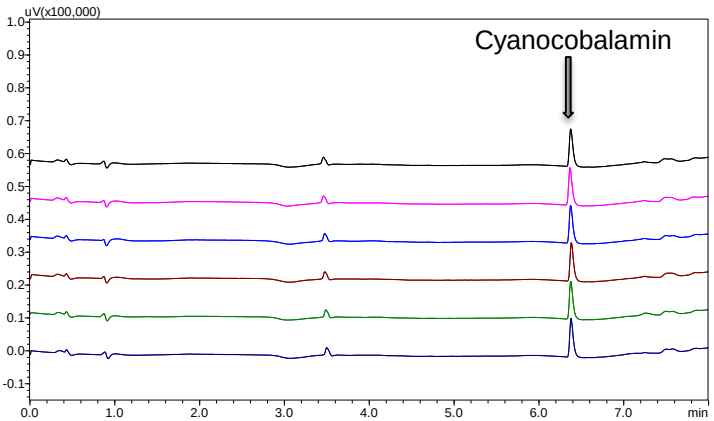

Figure 3. Overlay chromatograms of standards

Table 3. Cyanocobalamin sample

Parameter	Observed	USP Criteria
%RSD Retention Time	0.19	NMT 2.0
%RSD Area	0.30	NMT 2.0
USP Tailing	1.43	NMT 2.0

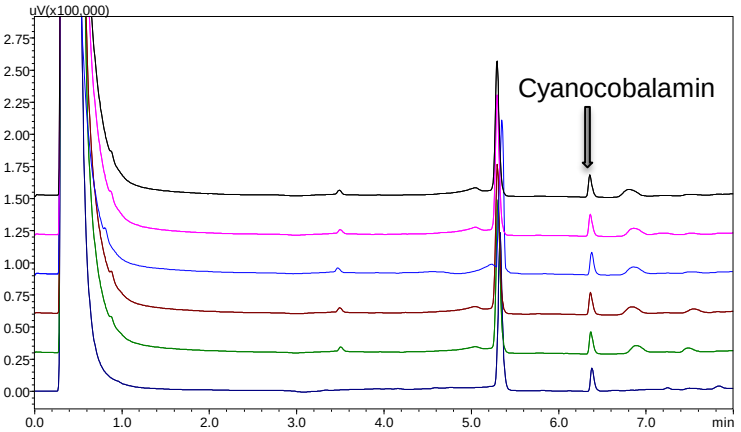

Figure 4. Overlay chromatograms of samples

Conclusion

This study successfully demonstrated the ability of Shimadzu Nexera X2 Fast LC system to analyse cyanocobalamin tablets in conformity with the USP41 Monograph 1114 Procedure 2 fulfilling all the system suitability requirements. The relative standard deviation of retention time and area for standard and sample are well below the required USP system suitability criteria.

Reference:

[1]USP Monograph, Cyanocobalamin Tablets, USP 41 – NF36, monograph 1114,Procedure 2.

[2]USP General Chapter 621, USP41–NF36, First Supplement.

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