

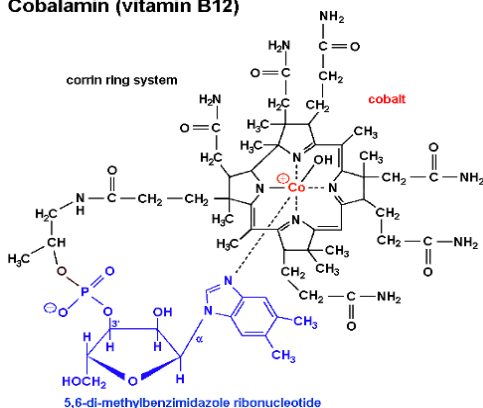
Peak Identification of Cyanocobalamin

According to USP 35-NF 30 S2

Summary

Cyanocobalamin or vitamin B12 is an essential Vitamin B. Cyanocobalamin is classified as a water-soluble vitamin. Food sources of vitamin B12 include fish, shellfish, meats, and dairy products. Vitamin B12 is necessary for growth, cell reproduction, production of red blood cells (hematopoiesis), and the production of myelin and nucleoprotein. Vitamin B12 also plays an important role in the breakdown or metabolism of fats and carbohydrates and has a role in protein synthesis. Vitamin B12 deficiency may result in anemia, gastrointestinal problems, and nerve damage. Cyanocobalamin is used to treat pernicious anemia and vitamin B12 deficiency. This application note shows how peak identification of Cyanocobalamin can be performed by using UV/VIS spectroscopy.

Cobalamin (vitamin B12)



Sample

Cyanocobalamin (CAS # 68-19-9)

Equipment

- UV7 (ME30254726) with single cell holder
- Rectangular quartz cuvettes, path length 10 mm
- Analytical balance XP205 (ME-11106027)
- Volumetric flask of 100 ml



Sample preparation

Weigh accurately 15 mg cyanocobalamin in a 50 ml volumetric flask. Fill up to the mark with deionized water and sonicate until it is completely solved. Dilute this solution 1:10 with deionized water to get the sample solution.

Fill three cuvettes with the sample solution and one with deionized water for the blank.

As Cyanocobalamin is light sensitive material use amber colored flask for solution preparation.



Measurement

Create a method using the following method parameters:

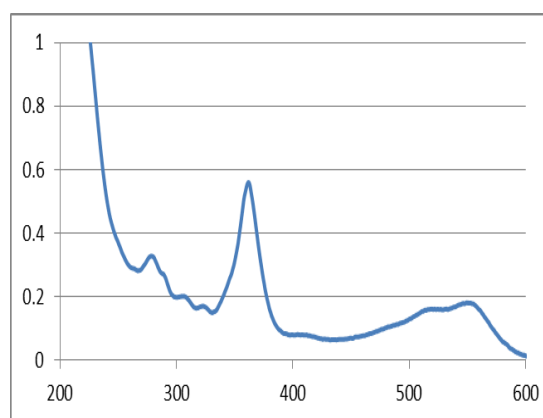
Method:	Fixed Wavelength
Path length:	1 cm
Wavelength:	278, 361, 550nm
Calculation:	Wavelength 1
Unit:	nm
Formula:	LambdaSMax(276,280)
Result limits:	Yes
Lower Limit:	277
Lower Limit:	279
Calculation:	Wavelength 2
Unit:	nm
Formula:	LambdaSMax(359,363)
Result limits:	Yes
Lower Limit:	360
Lower Limit:	362
Calculation:	Wavelength 3
Unit:	nm
Formula:	LambdaSMax(546,554)
Result limits:	Yes
Lower Limit:	548
Lower Limit:	552
Calculation:	Ratio A361/A278
Formula:	A2/A1
Result limits:	Yes
Lower Limit:	1.7
Lower Limit:	1.9
Calculation:	Ratio A361/A550
Formula:	A2/A3
Result limits:	Yes
Lower Limit:	3.15
Lower Limit:	3.4

Note: this method is available as a template on the UV7, UV5Nano and UV5Bio spectrophotometer.

Results

The absorption spectrum exhibits maxima at 278 ± 1 , 361 ± 1 , and 550 ± 2 nm. Mean of the absorbance ratio obtained for A_{361}/A_{278} is 1.751 and for A_{361}/A_{550} is 3.226.

Sample		Acceptance criteria
278.4 nm	0.293 A	278 ± 1 nm
361.0 nm	0.513 A	361 ± 1 nm
550.4 nm	0.159 A	550 ± 2 nm
Abs. Ratio A_{361}/A_{278}	1.751	1.70 - 1.90
Abs. Ratio A_{361}/A_{550}	3.226	3.15 - 3.40



Characteristic spectrum of cyanocobalamin

Conclusion

The absorbance ratio of cyanocobalamin at A_{361}/A_{278} and A_{361}/A_{550} is 1.751 respectively 3.226. The results are in agreement with USP specifications.

Further information

www.mt.com/uv-vis