Environment Conservation Journal 11(3) 139-142, 2010
(ISSN 0972-3099) Abstracted and Indexed

Microbiological method for determination of cyanocobalamin in pharmaceutical formulation

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Received: 02-03-2010 **Revised: 05-06-2010** **Accepted: 20-08-2010**

Abstract

A simple and precise microbiological method has been developed for the quantitative estimation of Cyanocobalamin in pharmaceutical formulations. After employing different microorganisms for achieving best results, *Escherichia coli* 113-3 ATCC No. 11105 was found to be the most suitable. In the method zone of exhibition achieved by using the aforesaid organism were measured from which content of Cyanocobalamin in pharmaceutical formulation was determined. The results were good in most of the formulations available in the market. Cyanocobalamin is added in 100% overages in the pharmaceutical formulations which were shown during its estimation. Moreover there is no interference of other vitamins present in the formulations by using this culture and method. The method employed is economical and simple and can be used in the quality control of bulk manufacturing and also in pharmaceutical formulations

Key words: *Microbiological*, *vitamin*, *pharmaceutical*, *capsule, syrup*

Introduction

Vitamin B_{12} is the name generally used for a group of related cobalt containing compounds, also known as cobalamins, of which Cyanocobalamin and Hydroxocobalamin are the principal forms in clinical use. Cyanocobalamin is an especially common vitamer of the B_{12} vitamin family. It is the most famous vitamer of the family because it is chemically the most air stable, and it is the easiest to crystallize and therefore easiest to purify after it is produced by bacterial fermentation. The cyanide is added to the molecule by activated charcoal columns in purification. Thus the use of this form of B_{12} is the most wide spread.

Vitamin B_{12} preparations are used in the treatment and prevention of Vitamin B_{12} deficiency. Deficiency of Vitamin B_{12} leads to development of megaloblastic anaemias, demyelination and other neurological damage. A specific anaemia known as perinious anaemia develops in patients with an absence of intrinsic factor necessary for good absorption of the vitamin from dietary sources. Cyanocobalamin and Hydroxocobalamin are generally administered by intramuscular route, although Cyanocobalamin may be given orally or intranasally.

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Preparations: B-complex capsules/tablets/ syrup

The objective of this work is to develop analytical microbiological procedure which would serve as a rapid and reliable method for Cyanocobalamin in B-complex capsules/ tablets/syrup.

B-complex capsules/tablets/syrup contains many Vitamins and excipients which may interfere with microbiological analysis but this method gives good zone of exhibition of Cyanocobalamin in most of the available formulations in the market.

Experimental Organism used: *Escherichia coli* 113-3 ATCC No. 11105

Maintenance medium: Following ingredients were dissolved in 1000ml of distilled water:

Ingredients	Grams/litre
Casein enzymic hydrolysate	5.00
Yeast extract	5.00
Monopotassium phosphate	0.50
Magnesium sulphate	0.20
Sodium chloride	0.10
Sucrose	12.0
Liver extract	0.05
Ferrous sulphate	0.001
Agar	15.0

Final pH was 7.0 ± 0.2

The medium was completely dissolved by boiling on a steam bath. Approximately 10 ml portions of hot solution in test tubes were placed and plugged with cotton. Sterilized by autoclaving at 15 lbs pressure (121˚ C) for 15 minutes. Cooled and make slants were made.

Maintenance of the culture:

Three or more of the slants were inoculated by pure culture of *Escherichia coli* 113-3 ATCC No.11105. Incubated for 16-24 hrs at temperature of 35 ± 2˚C and finally stored in a refrigerator.

Assay medium: Following ingredients were dissolved in 1000ml of distilled water:

Ingredients	Grams/litre
Potassium dihydrogen phosphate	6.00
Di-potassium hydrogen phosphate	14.00
Sodium citrate hydrated	1.00
Magnesium sulphate hydrated	0.20
DL-Asparagine hydrated	8.00
L-Arginine hydrochloride	0.20
L-Glutamic acid	0.20
Glycine	0.20
L-Histidine	0.20
L-Tryptophan	0.20
L-Proline	0.20
Ammonium sulphate	10.00
Glucose (added aseptically)	10.00
Agar	15.00

Final pH was 7.0 ± 0.2

Boiled to dissolve the medium completely on a steam bath. Sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

Preparation of standard solution:

The dilutions of standard in distilled water were prepared so as to make a final dilution containing 0.1 mcg/ml & 0.05mcg/ml of Cyanocobalamin.

Preparation of test solution:

The dilutions of test sample were prepared in distilled water so as to make a final dilution containing 0.1 mcg/ml & 0.05mcg/ml of Cyanocobalamin.

Assay

The assay of Cyanocobalamin is done by using cylinder plate method or cup plate method. To the assay medium at 45˚ C 0.1 ml of culture suspension obtained from fresh slants was added. Mix it properly and poured it into sterile petri plates. Ensure that the layers of the medium are uniform in thickness, by placing the plate on a levelled surface. After solidification holes 5 to 8 mm in diameter were bored in the medium with a sterile borer. Now 0.1 ml of standard and test solutions into different cups on the plate was added. The plates were incubated at 37˚ C for 24 hrs to measure the zones of exhibition.

The assay was calculated according to the following formula

$$\%\text{Potency} = \text{antilog}\,(2 + a \times \log I)$$

Where ,

$$a = \frac{(T_2 + T_1) - (S_2 + S_2)}{(T_2 - T_1) - (S_2 - S_2)}$$

T_1 and T_2 zone readings of test solutions

S_1 and S_2 are zone readings of standard solutions.

a may have a positive or negative value and should be used alzebrically.

I = is the ratio of dilutions.

$$\text{Potency of sample} = \frac{\%\text{Potency} \times \text{assumed potency of sample}}{100}$$

The values for the assay are given in Table.1

Method validation

Accuracy and Specificity: Spiking of Cyanocobalamin in pre analysed test preparation was done and analysed. Recovery studies showed accuracy and reproducibility (Table-2). The % RSD of replicate standard solution area was less than 1%.

Linearity: Linearity was checked by preparing standard solution at five different concentration levels ranging from 0.025 to 0.40 mcg/ml.

Limit of Detection and quantification: The detection limit for Cyanocobalamin was around 0.005 mcg/ml. and the limit of quantification was 0.0125 mcg/ml.

Application: The developed method is applied for the analysis of Cyanocobalamin in real drugs samples obtained from market (Table.1).

Recovery Studies

To study the accuracy, reproducibility and precision of the proposed method, recovery experiments were carried out. A fixed amount of pre analysed sample was taken and standard drug was added at four different levels to confirm the absence of positive and negative interference from excipients.

Results and Discussion

To develop a suitable and robust microbiological method for determination of Cyanocobalamin, different microbial cultures and mediums were employed to achieve the best estimation. British Pharmacopoeia (2009), *Bergey's Manual of Systemic Bacteriology*, (1984), Indian Pharmacopoeia (2007), *The United States Pharmacopoeia* (2009). The culture organism of *Escherichia coli*113-3 ATCC No. 11105 was found to be an appropriate culture allowing adequate estimation of Cyanocobalamin without any interference from other vitamins present in the pharmaceutical formulations. To ascertain the effectiveness of the method recovery studies and accuracy were carried out.

Linearty

The plot of zone of exhibition of *Escherichia coli* Vs Concentration of Cyanocobalamin was found to be almost linear in the range of 0.0125 to 0.40 mcg/ml.The mean recovery of Cyanocobalamin by the proposed method is 98.75%, as shown in Table.2.

Table-1 Results of analysis of Cyanocobalamin in real drugs samples (Capsules/Syrup) obtained from market and results of Microbiological Assay

Formulation	Label Claim Cyanocobalamin	Quantity found mcg/capsule or 5 ml of syrup	Quantity % of label Claim (overages to the tune of 100% are present in pharmaceutical formulations)
A (Capsule)	15 mcg	28.3 mcg	188.67
	15 mcg	28.6 mcg	190.67
	15 mcg	28.0 mcg	186.67
	15 mcg	28.5 mcg	190.00
	15 mcg	28.8 mcg	192.00
B (Syrup)	15 mcg	22.5 mcg	150.00
	15 mcg	22.9 mcg	152.67
	15 mcg	22.1 mcg	147.33
	15 mcg	22.7 mcg	151.33
	15 mcg	22.5 mcg	150.00

Table.2 Results of Recovery Studies

S.No.	Component Recovery	Conc. of added drug in Pre analysed formulation (mcg/ml)	Recovery of added drug (mcg/ml)	% Found
1.	**Cyanocobalamin**	0.5	0.497	99.40
2.		1.0	0.988	98.80
3.		1.5	1.480	98.66
4.		2.0	1.960	98.15
				Mean : 98.75%

Conclusion

The developed method is fast, simple and precise. The mean amount of Cyanocobalamin found on analysis by the above proposed method was 22.54 mcg/5 ml of syrup & 28.44 mcg/cap. The low value of RSD (1.2 %) indicates that the method is precise and accurate. The mean recovery value is 98.75 % which shows that the method is almost free from interference of the other vitamins and excipients used in the formulation. The developed method for quantitative assay of Cyanocobalamin showed good results.

References

British Pharmacopoeia 2009, *The Stationary Office*, London, A334-A339.

Martindale – *The complete Drug Reference* – 33rd edition,1388-1389.

The United States Pharmacopoeia 2009, United Book Press, Baltimore, Maryland.

Indian Pharmacopoeia 2007, *Ministry of Health & Family Welfare*, Govt. of India, 23-64.

Bergey's Manual of Systemic Bacteriology, 1984 Edited by Noel R Krieg, Williams & Wilkine, Baltimore, USAVol.1,