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"Estimation and Validation of Methylcobalamin in Tablet Dosage form using UV-Visible Spectrophotometric Method"

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Abstract

An ultraviolet (UV) spectrophotometric method was developed and validated for quantitavie determination of Methylcobalamin (Mecobalamin) in tablet dosage form. Methylcobalamin is a cobalamin, a form of vitamin B12 and used to prevent or treat pathology arising from a lack of vitamin B12 , such as pernicious anemia and is also used in the treatment of peripheral neuropathy, diabetic neuropathy ans as a preliminary treatment for amyotrophic latera sclerosis.

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The very simple, Fast, accurate and economical methods have been proposed for the determination of Mecobalamin. Mecobalamin was measured by using Uv spectroscopy method with the solution of methanol, the linearity was found to be 0.9981 and the accuracy showed mean % RSD of 0.791694 and with total meam % RSD 0.97923 in intermediate precision, range %RSD 0.652005 all the parameters values were within standard limit thus Analytical method was validated according to ICH guideline for the determination of Methylcobalamin. The method was found to be precise and validated as per ICH guidelines.

Keywords: UV Spectrophotometry; Methylcobalamin.

1. Introduction

Methylcobalamin (MeB12) is a form of vitamin B12, Chemically carbanide;cobalt(3+);[5-(5,6-dimethylbenzimidazole-1-1-yl)-4-hydroxy-2-hydroxymethyl)oxolan-3-yl]-1-[3-[(4Z,9Z,14Z)-2,13,18-tris(2-amino-2oxoethyl1)-7,12,17-tris(3-amino-3-oxopropyl)3,5,8,8,13,15,18,19-octamethyl2,7,12,17-tetrahydro-1H-corrin-21-id-3-yl]propanoylamino] propan-2-yl phosphate chemically having molecular formula C63H91CON13O14P and official in Japanese Pharmacopoeia (XIV). It is a dark red crystalline powder soluble in water and ethanol [1]. Chemical structure of MeCbl is represented in Figure 1. MeCbl is co enzymatically active cobalamin derivative associated to human growth, cell development and an important component of several enzymes which is involved in the metabolism of certain amino acids[2]. Methylcobalamine is important for the brain and nerves, and for the production of red blood cells, sometimes used in people with pernicious anemia, diabetes, and other conditions [3]. It is a cofactor in the enzyme methionine synthase, which functions to transfer methyl groups for the regeneration of methionine from homocysteine [4].

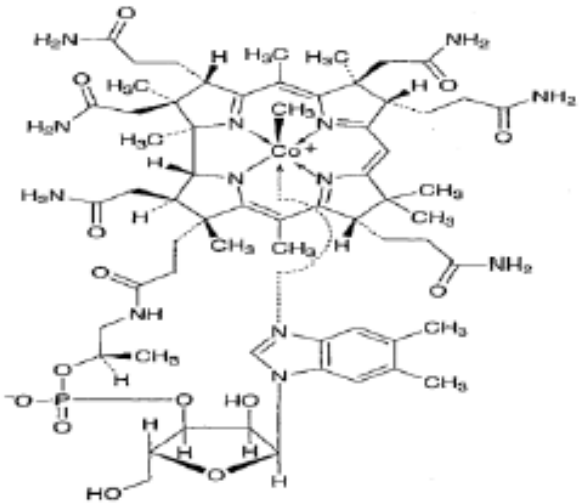

Figure 1: Structure of Methylcobalamin

A Review of literature found that RPHPLC, LCMS, HPTLC and spectrophotometric methods are available for the determination of Methylcobalamin (5,6,7) alone and for combined dosage form of methylcobalamin with

other drugs (8,9,10). Among various survey, only two UV Spectrophotometric methods have been reported for its assay (8, 10). Therefore the present work developed a simple UV spectrophotometry method and validated as per ICH guidelines for the determination of methylcobalamin in tablet dosage form. So this method will be helpful for the establishment of simple, precise, accurate and economic method for the quantitative analysis of methylcobalamin.

2. Materials and methods

2.1 Instrumentation

A PerkinElmer UV-Visible spectrophotometer (λ 365) with an even set of 10 mm quartz cuvettes were used for measurement of absorbance for the analysis. All analytical weight measurements were done on a PRECISA (XB120A) electronic balance. Ultra bath sonicator (Spectra lab, model-UCV-100D) was used for sonication process.

2.2 Reagent and materials

Reference Methylcobalamin was obtained as gift sample from Time Pharmaceuticals (Mukundapur-5, vhaisakhori) and Methanol (AR) grade was supplied by Thermo Fisher Scientific India Private Limited. Tablet dosage form was procured from market. Distilled water was used for the preparation of all solutions.

2.3 Preparation of Standard Solution

Methylcobalamin 25 mg was accurately weighed and transferred into 50 ml volumetric flask, dissolved in methanol with the helped of sonicator for 1 to 3 minutes and diluted to the mark with same solvent. From this, 1 ml of the solution was pipetted into another 10 ml volumetric flask, and diluted up to the mark with same solvent.

2.4 Sample preparation

For the estimation of the drug in tablet formulation 20 tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 1 mg methylcobalamin was weighed. The powder was transferred to 20 ml volumetric flask and add 10 ml methanol then it was sonicated for 1 to 3 minutes to dissolve the drug completely and made volume up to the mark with same diluents. The solution was filtered through whatman filter paper. The absorbance's value was measured at 522 nm using methanol as blank.

Calculation: (Content of Methylcobalamin in % w/w)

$$\frac{\textbf{Sp absorbance}}{\textbf{Std absorbance}} \times \frac{\textbf{Wstd}}{\textbf{50}} \times \frac{\textbf{1}}{\textbf{100}} \times \frac{\textbf{100}}{\boldsymbol{Wsp}} \times \frac{\textbf{50}}{\textbf{5}} \times \textbf{Potency of std}\,\% \times (\textbf{100}\% - \textbf{LOD})\% \times \textbf{100}$$

Where,

Wsp = Weight of sample Wstd = Weight of standard

LOD = Loss on drying

2.5 Preparation of Calibration curve

Working standard solution was prepared by taking 5ml of stock solution into a 50ml volumetric flask. The volume was made up to mark to produce 100µg/ml solution with methanol. From the above working solution 0.2ml, 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml were pipetted and transferred to 6 individual 10 ml volumetric flask and finally the volume was made upto the mark with diluent. Solutions were scanned from 200-600 nm UV range by using methanol as a blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.

2.6 Method validation

The proposed method was validated as per ICH guidelines such as accuracy, precision, linearity, specificity and system suitability parameters were summarized below.

2.7 System suitability

System suitability testing is performed to verify that the repeatability of the system are adequate for the analysis to be performed. System suitability is the checking of a system before or during the analysis which demonstrates that the system is operating properly and is ready to deliver results with acceptable accuracy and precision. For the system suitability testing five replicate of standard solution was prepared and samples were also analyzed and % RSD was calculated.

Table 1: System suitability Methylcobalamin WS

Sr. No.	Test solution	Peak Abs.
1	Standard 1	0.2997
2	Standard 2	0.2995
3	Standard 3	0.2998
4	Standard 4	0.2992
5	Standard 5	0.2999
6	Standard 6	0.3059
	Average:	0.300333
	% RSD:	0.946147

2.8 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present in the formulation. It was shown that excipients do not interfere with the proposed method and the recovery of the drug was above 98%.

2.9 Accuracy

Accuracy is the closeness of the agreement between the true value of reference and the value found. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (50%, 100% & 150%). The accuracy was then calculated as the percentage of analyte recovered by the assay. The present recovery study indicates good accuracy of the method. The results of the accuracy study are given in table no.:2.

Table 2: Percentage recovery data of Methylcobalamin

Percentage	% Recovered	Mean recovery (%)	% RSD
50	100.88	100.67	0.89323
	101.44		
100	99.68		
	98.81	98.46	0.6317134
150	98.21		
	99.36		
	101.02	100.06	1.1635875
	98.17		
	100.99		
Mean %RSD	0.791694		

3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision here is considered at two levels only: a) system precision (repeatability) and b) intermediate precision (ruggedness).

a) System Precision (Repeatability)

Method precision of experiment was performed by preparing the standard solution of methylcobalamin (25 mg/ml) for six times and analysed as per the proposed method.

Table 3: System Precision

Sr. No.	Test solution	Peak Abs.
1	Standard 1	0.2997
2	Standard 2	0.2995
3	Standard 3	0.2998
4	Standard 4	0.2992
5	Standard 5	0.2999
6	Standard 6	0.3059
	Average:	0.300333
	% RSD:	0.946147

b) **Intermediate precision (Ruggedness)**

a. Six replicate of standard and six individual sample solutions by three analysts was prepared on the first day keeping the other condition same.

b. According to the procedure samples were analyzed.

c. Second day again six replicate of the samples by three analysts was prepared keeping the other conditions same.

d. According to the procedure samples were analyzed.

e. The individual day and combined day area between three analyst was compared and showed in the table below. Analyst: A Analyst: B

Day: 1 Day: 1

Day: 2 Day: 2

Table 7

Analyst A: Day 1

Sr. No.	**Test Solution**	**Weight (g)**	**Peak Abs.**	**Assay (%)**
1	Standard	25.5	0.3008	
2	Sample 1	70.6	0.1671	100.88
3	Sample 2	70.3	0.1673	101.44
4	Sample 3	71.4	0.1665	99.68
5	Sample 4	141.8	0.3287	98.18
6	Sample 5	141.1	0.3251	98.21
7	Sample 6	140.1	0.3233	99.36
Average %	**99.56**			
RSD %:	**1.35691**			

Analyst B: Day 1

Sr. No.	Test Solution	Weight (g)	Peak Abs.	Assay (%)
1	Standard	25.3	0.2997	
2	Sample 1	70.4	0.166	100.80
3	Sample 2	71.2	0.1644	98.01
4	Sample 3	71.5	0.1671	99.19
5	Sample 4	141.2	0.3336	100.28
6	Sample 5	141.8	0.3329	99.65
7	Sample 6	141.5	0.3312	99.35
Average %	**99.43**			
RSD %:	**0.815786**			

Analyst A: Day 2

Sr. No.	Test Solution	Weight (g)	Peak Abs.	Assay (%)
1	Standard	25.3	0.3033	
2	Sample 1	71.8	0.1664	98.37
3	Sample 2	71.6	0.1657	98.23
4	Sample 3	72.1	0.1678	98.78
5	Sample 4	141.5	0.3295	98.84
6	Sample 5	141.9	0.3364	100.62
7	Sample 6	141.1	0.3325	100.02
Average %	**99.15**			
RSD %:	**0.969811**			

Analyst B: Day 2

Sr. No.	Test Solution	Weight (g)	Peak Abs.	Assay (%)
1	Standard	25	0.3133	
2	Sample 1	70.9	0.1659	99.32
3	Sample 2	72.5	0.1728	101.17
4	Sample 3	71.3	0.1703	101.38
5	Sample 4	141.8	0.3364	100.69
6	Sample 5	141.5	0.333	99.89
7	Sample 6	141.3	0.3344	100.45
Average %	**100.48**			
RSD %:	**0.774416**			

Table 4: RSD between three analysts on two different days

Analysts	**Day 1**	**Day 2**
A (%RSD)	1.35691	0.815786
B (%RSD)	0.969811	0.774416
Mean % RSD	**1.16336**	**0.795101**
Total Mean % RSD	**0.97923**	

4. Linearity

The linearity of any analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of the method was evaluated by constructing calibration curves at six concentration levels over a range of 80% to 120% for methylcobalamin (r^2 =0.9981). The calibration curves were developed by plotting peak versus concentration (n=5).

Table 5: Obtained Peak abs. at 522 nm

Sr. No.	Concentration of Analyte	Peak Abs. at 522 nm
1	80%	0.2412
2	90%	0.2696
3	100%	0.2969
4	110%	0.3318
5	120%	0.3624
	Correlation Coefficient (R^2)	0.9981

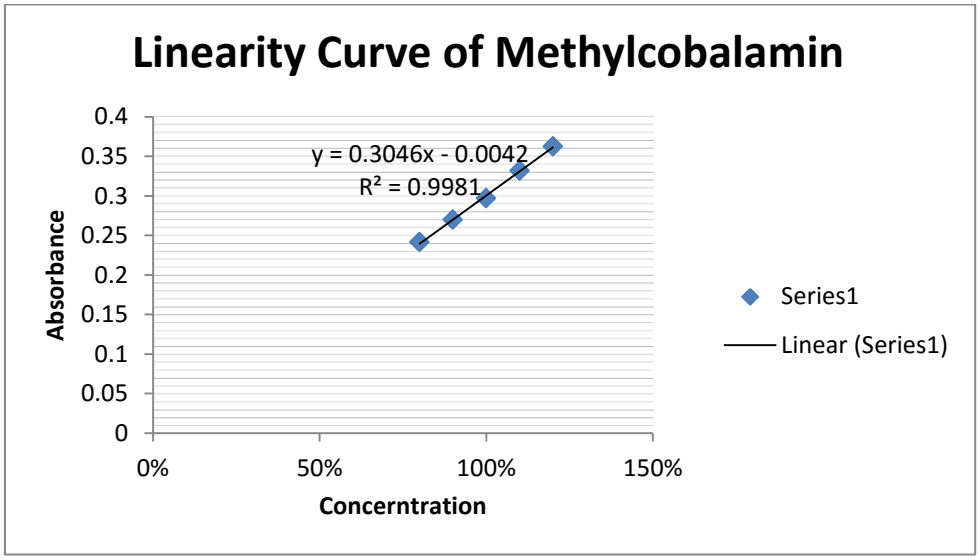

Figure 2

5. Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Table 6

Sr. No.	Concentration of Analyte	Peak Abs. at 522 nm	% Recovery
1	80%	0.2712	100.24
2	90%	0.3059	100.35
3	100%	0.3386	100.07
4	110%	0.3675	99.13
5	120%	0.3992	98.97
	Mean % Recovery	99.75	
	Correlation Coefficent (R^2)	0.999	

7. Result and Discussion

A simple and reliable method has been developed for the determination of assay of methylcobalamin in tablet dosage formulation. Beers law was followed in concentration range of 80 %-120 % for Methylcobalamin at 522 nm in methanol. Correlation coefficient (R^2) was found to be 0.9981. The precision was studied for System Precision, i.e., Repeatability (% RSD less than 1 %, i.e., 0.946147) and Intermediate precision, i.e., Ruggedness with three analyst for two different days keeping other conditions same and it is concluded that % RSD, Mean % RSD, Total Mean % RSD was found in the range of 1.35691 -0.969811, 0.815786-0.774416, 1.16336-0.795101, 0.97923 respectively which indicates that method is reliable and very likely to produce the same and predictive results. Accuracy of the method was determined by calculating the mean % recovery at 50 %, 100 % and 150 % level and found in the range of 98.46 to 100.67 %. Range was determined by calculating Mean % Recovery (i.e., 99.75) and Correlation Coefficent (i.e., R^2= 0.999) at 80-120 % concentration of Luliconazole which indicates that set method is precise, accurate and linear at 80-120 % concentration level, all that values were within standard limit showed that the method is stastically vaididated.

8. Conclusion

The observation and results obtained from the validation study clearly indicated that the developed analytical method is accurate, precise, specific and linear. Moreover it has advantage of short run time and possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Since all the result are within the limit, the above stated analytical method is validated as per the ICH guidelines and can be employed for routine analysis of Methylcobalamin solid dosage form.

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