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R E S E A R C H A R T I C L E

Validation of HPLC Test Method for Methylcobalamin 1500mcg Tablets

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A B S T R A C T

The current research paper gives method validation report for the quantification of methylcobalamin tablets. The method involved a gradiensystem of Phosphate buffer pH 3.5 and acetonitrile. The column used is Inertsil C18 column with dimentions (5µ* 4.6 mm id *150 mm). The compounds gave a good UV response at 256 nm, hence the detection wavelength. The developed method is simple, precise, robust and stands validated as per ICH guidelines.

Key words: Methylcobalamin, ICH guidelines, Phosphate buffer, Metformin Hydrochloride

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Methylcobalamin (mecobalamin, MeCbl, or MeB12) is a cobalamin, a form of vitamin B12. It differs from cyanocobalamin in that the cyano at the cobalt is replaced with a methyl group. Methylcobalamin is equivalent physiologically to vitamin B12 and can be used to prevent or treat pathology arising from a lack of vitamin B12 intake. Methylcobalamin is also used in the treatment of peripheral neuropathy, diabetic neuropathy, and as a preliminary treatment for amyotrophic lateral sclerosis. Metformin Hydrochloride is in Sustained Release dosage form and Methylcobalamin and Calcium carbonate is in conventional dosage form in the formulation. Analytical method developed is a Reverse phase HPLC method for estimation of assay and impurity of Methylcobalamin, titrimetric method for Calcium carbonate and Spectrophotometer method for Metformin Hydrochloride.

Method validation for Tablets of strength 1500mcg for methylcobalamin in combination is performed. Analytical Method validation for all the three drugs are carried out as per USP Category I & Category III data elements. Method validation for Tablets of strength 500mg for Metformin Hydrochloride SR Tablets, and 200mg for calcium carbonate Tablets in combination is performed and the research article will be published.

2. Materials and Methods

Purified Water, Potassium dihydrogen Orthophosphate AR grade, Hexane sulphonic acid sodium salt, Acetonitrile HPLC grade, Orthophosphoric acid AR grade, Methanol HPLC grade Merck millipore.

Chemical and Standard Solution Preparations

Preparation of Buffer for Mobile Phase (A): 0.02 M of KH_2PO_4, pH adjusted to 3.5 with phosphoric acid. Add 3.7gm per liter of Hexane sulphonic acid sodium salt.

Preparation of Buffer for diluent: 0.02 M of KH_2PO_4, pH adjusted to 3.5 with phosphoric acid.

Standard Resolution Solution

About 30 mg of Methylcobalamin WS was weighed and transferred in 1000 ml amber coloured volumetric flask. To this 800 ml of buffer for diluent was added and kept for sonication for about 10 minutes in ice cold water. Finally the volume is made up with acetonitrile.

Sample Solution

The contents of 20tablets were weighed and their average weight was determined. 5 intact tablets were transferred to a 250 ml amber colour volumetric flask. To this 150 ml of buffer for diluent was added, sonicated using ice cold water. Further 50 ml of additional buffer for diluent was added and sonicated. Finally the volume was made upto the mark using acetonitrile. The solution was filter through 0.45µ membrane filter.

Table 1: Instrumentation and Chromatographic Conditions

Parameter	Description
Instrument	Shimadzu UFLC Prominence System
Pump	LC – 20 AD binary pumps
Injector	Autosampler (SIL – 20 AC HT)
Injection volume	50 µl at 10 °C
Column oven	CTO – 20 AC at 40 °C
Column	Inertsil C18 (5 µ * 4.6 mm id * 150 mm)
Mobile Phase	Gradient system of Buffer : Acetonitrile
Flow Rate	1.5mL/min
Detector	UV Detector- UV-4075
Detection Wavelength	256 nm

Time (mins)	Buffer (%) (A)	Acetonitrile (%) (B)
0	95	5
10	80	20
18	70	30
18.5	95	5
20	95	5

Method Validation

System Suitability:

System suitability tests were carried out to ensure reproducibility of the equipment. The test was carried out by injecting standard solution in 5 replicates, single injection of blank solution and placebo solution.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components which may be expected to be present. The tests were carried out by injecting diluent blank, Standard Solution of calcium carbonate, Placebo Solution of Metformin HCl, Placebo solution of Methylcobalamin, Metformin HCl Standard Solution and Methylcobalamin Standard Solution, placebo solution of calcium carbonate and Sample Solution.

Accuracy

Accuracy of the analytical method will be established by carrying out assay of Tablets at 3 dose levels. i.e. 80%, 100% and 120% of strength.

Set I: 500mg of Metformin HCl, 200mg of Calcium carbonate & 1.5mg of methylcobalamin) of the Tablets. Accuracy will be done in three sets.

Set II: 1010mg (Average weight) of tablets contain 500mg of Metformin HCl, 1.5mg of Methylcobalamin and 508.5mg of placebo.

Set III: 1275mg (Average weight) of tablets contain 500mg of Metformin HCl, 200mg Calcium carbonate, 1.5mg of Methylcobalamin and 573.5mg of placebo.

Precision

Precision is the measure of either the degree of reproducibility or of repeatability of the analytical method under normal conditions. The test was carried out with 6 assay samples in replicate injections of standard solutions.

Linerity: The linearity of an analytical procedure is its ability to obtain test results which are directly proportionally to the concentration of analyte in the sample. Linearity of Methylcobalamin is carried out in the range 12mcg/ml to 42mcg/ml.

Stability:

In order to ascertain that drug solutions under analysis are stable under laboratory working conditions, it is necessary to determine effect on the analytical solution for analysis time. Stability for Standard solution of Methylcobalamin (working level) is carried out at bench top at room temperature for Methylcobalamin upto 4 hrs. Solutions were injected at 0, 1, 2, 3 & 4 hr onto the HPLC system equilibrated under the conditions mentioned as above. Peak area were determined.

3. Results and Discussion

System Suitability:

The method was found to be suitable for the proposed analysis as the relative standard deviation of average peak area of system suitability test is not more than 2.0 %

Specificity:

Retention time obtained with test sample is comparable to the retention time obtained for the standard. All peaks are well separated from each other indicating the specificity of the analytical method for Rabeprazole Sodium and Acelofenac.

Precision:

Precision measured at all level was within the acceptable criterion of NMT 2.0 % indicating the efficiency of method for the proposed analysis.

Linearity

The correlation coefficient was found to be more than 0.9990. Hence the method could be said to be linear in the given concentration range.

Stability:

The standard solution of methylcobalamin was found to be stable for more than 4 hours under bench top conditions.

4. Conclusion

The method developed for analysis of Methylcobalmin in presence of metformin hydrochloride and calcium carbonate stands to be validated as per ICH guidelines. The limits for all the parameters were met with no interference from the placebos of the tablets and hence this method can be used as quality control tool for analysis of the capsules.

5. Reference

[1] ICH Harmonized Tripartite Guideline prepared within the Third International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH), Validation of Analytical Procedures: Methodology, 1996.