Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF MECOBALAMIN IN INJECTIONS

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Received: 03 Apr 2012, Revised and Accepted: 07 Jun 2012

ABSTRACT

Mecobalamin is used in the treatment of trigeminal neuralgia, megaloplastic anemia, diabetic neuropathy and facial paralysis in Bell's palsy syndrome. A simple, an accurate and economic, precise and reproducible UV Spectrophotometry method has been developed for the estimation of mecobalamin in injection dosage form and validated by ICH guidelines. The standard (10 µg/ml) was scanned between 200-400 nm and maximum absorption was recorded at 353 nm. The assay results are found to be 98.94%. The percent recovery calculated as 99.05-100.50 %. The linearity range of 10-50 µg/ml proved that it obeyed Beer's Law and the correlation coefficient (r^2) was found to be 0.9995 at 353 nm with an intercept of 0.0105 and a slope of 0.0121 with RSD 1.33 complied ICH. In 8 hour forced degradation study in oxidation, acid, alkali and thermal stress, degradation was observed in acid and thermal stress conditions. The proposed method was accurate, precise, and reproducible. The commonly used excipients and additives in formulation were not interfering. The drug was stable on alkali and oxidative treatment and found unstable in acid and heat conditions.

Keywords: Mecobalamin, Spectrophotometry, Assay, Degradation, Chemical, Thermal.

INTRODUCTION

Mecobalamin (MeB_{12}) is a form of vitamin B_{12} used in the treatment of trigeminal neuralgia, megaloplastic anemia, diabetic neuropathy and facial paralysis in Bell's palsy syndrome.

It is chemically carbanide-cobalt(3+);[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] 1-[3-[(4Z, 9Z,14 Z)-2,13,18-tris (2-amino-2-oxoethyl)-7,12,17-tris (3-amino-3-oxopropyl)-3, 5, 8, 8, 13, 15, 18, 19-octamethyl-2, 7, 12, 17-tetrahydro-1H-corrin-21-id-3-yl] propanoylamino] propan-2-yl phosphate having molecular formula $C_{63}H_{91}CoN_{13}O_{14}P$. The chemical structure of mecobalamin is presented below (Figure 1)

It is a dark red crystalline powder soluble in water and ethanol. It is official in Japanese Pharmacopoeia (XIV).[1] Literature survey revealed that only two UV Spectrophotometric methods have been reported for its assay.[2-5] but none of the methods reported the forced degradation studies of mecobalamin in injections.

Therefore in the present investigation, an attempt has been made to develop an accurate, simple and an economic UV method for the estimation of mecobalamin in injection formulation and validated for accuracy, linearity and stability to forced degradation studies according to the prescribed procedures mentioned in ICH guidelines.

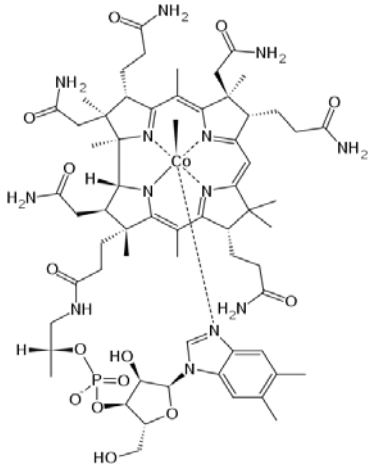

Fig. 1: Chemical Structure of Mecobalamin

MATERIALS AND METHODS

Instrumentation

Shimadzu UV-Vis Spectrophotometer model 1800, Lab India-Ultrasonicator, pH tutor Eutech- Japan, Digital electronic balance-(Shimadzu Japan, 0.001 sensitivity) and electric water bath were used for the study.

Standards and chemicals

The pure drug of mecobalamin was gifted by Blue Cross Laboratories, Goa. The injection ampoules (500 µg/ml), BIOCOBAL injection (Ordain Health Care, Chennai) were purchased from local pharmacy. All the chemicals used in the experiment were of Merck Analytical Grade. The chemicals and instruments used were, 0.1N NaOH, 0.1N HCl, hydrogen peroxide.

Selection of wavelength[6]

The wavelength was selected by scanning the 10 µg/mL of mecobalamin solution between 200 to 400 nm in a spectrophotometer. The scanned results proved maximum absorption in 353 nm, therefore 353 nm was selected as the λmax for estimation.

Preparation of standard solution[7]

To 50 mg of mecobalamin, 10 mL of distilled water added, dissolved by sonication for 10 minutes and made up to 100 mL with distilled water to get the concentration of 500 µg/mL. Appropriate dilutions were made in distilled water to get concentration of 10, 20, 30, 40 and 50 µg/mL and their absorbance measured at 353 nm in a spectrophotometer.

Assay of mecobalamin injection

Twenty ampoules of mecobalamin injections (Biocobal injection, 500 µg/mL)) were randomly selected and contents transferred to a 100 mL beaker, sonicated for 10 minutes. From this, 10 mL pipetted in to a 100 mL volumetric flask and diluted to 100 mL with distilled water (50µg/mL). From the above solution, 10 mL diluted to 50 mL with water to get the concentration of 10 µg/mL. The amount of drug present in injection was determined by using the absorbance ratio method. 8-9

Method Validation

Linearity studies

Linearity of standard mecobalamine powder was determined by scanning 10, 20, 30, 40 and 50 µg/mL solutions in a UV

spectrophotometer at 353 nm and their absorbance recorded. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis.

Accuracy studies

The accuracy was studied by recovery experiments. The recovery experiment was determined at three levels of 80%, 100% and 120% in the selected concentrations. The solutions were injected in triplicates for each spike and the assay was performed as per the test method. From this % recovery and the quantity present (mg) or recovered were calculated [7,8]

Degradation studies

In hydrogen peroxide degradation study, 10 µg/ml solution of mecobalamin injection was prepared in distilled water, from this 2 ml pipetted in to a 10 mL volumetric flask and made up to the volume with 5% H_2O_2 prepared in water. The resultant solution was allowed to stand for 8 hrs in a dark room to facilitate oxidation of the drug.

In acid degradation studies, 10 µg/ml solution of mecobalamin injection was prepared in distilled water, from this 2 ml pipetted in to a 10 mL volumetric flask, added 8 mL of 0.1N HCl and stored in dark room for 8 hours.

In alkali degradation studies, 10 µg/ml solution of mecobalamin injection was prepared in distilled water, from this 2 ml pipetted in to a 10 mL volumetric flask, added 8 mL of 0.1N NaOH and stored in dark room for 8 hours.

In thermal degradation studies, 10 µg/ml solution of mecobalamin injection was prepared in distilled water, 3 x 2 ml of the stock solutions were made up to 3 x 10 ml by distilled water and the resultant solutions were separately heated at 50º, 60ºand 80ºC for 30 minutes, stored in dark room for 1 hour, brought to room temperature and their corresponding absorbance values were recorded.[10]

RESULTS AND DISCUSSION

The assay parameters are given in table 1.

Table 1: Assay parameters

Parameters	UV Method
Assay	99.29 -100.5 %
Linearity range	10-50 µg/ml
λ Max (nm)	353 nm
Correlation coefficient (r^2)	0.9995
Standard deviation	0.00342
Intercept (c)	0.0105
Slope (m)	0.0121
Repeatability (% RSD)	1.33

In assay, the % content was found to be 99.29 to 100.5 % for mecobalamin complied with ICH guidelines limit (98-103%). In accuracy study, the % recovery was found to be 99.29, 100.50 and 99.05% for 80, 100 and 120% respectively. This was found to be within the acceptance limit of ICH guidelines (98-102%). In linearity study (Fig.2.1-2.2), the correlation coefficient was found to be 0.9995 at 353 nm with an intercept of 0.0105 and a slope of 0.0121 and it is complied with the ICH requirement (NLT 0.999). In repeatability studies, % RSD was found to be 1.33.

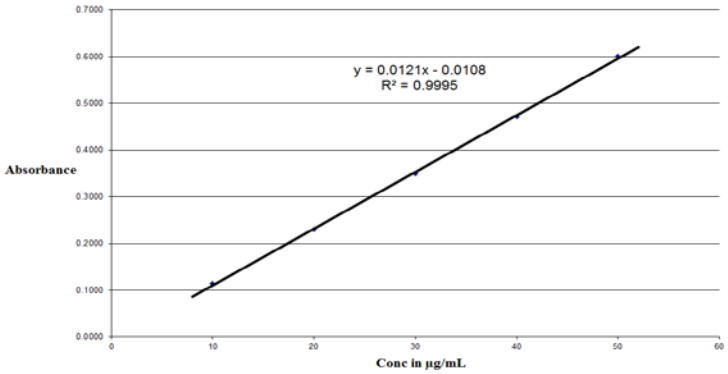

Fig. 2: Linearity Plot for Mecobalamin

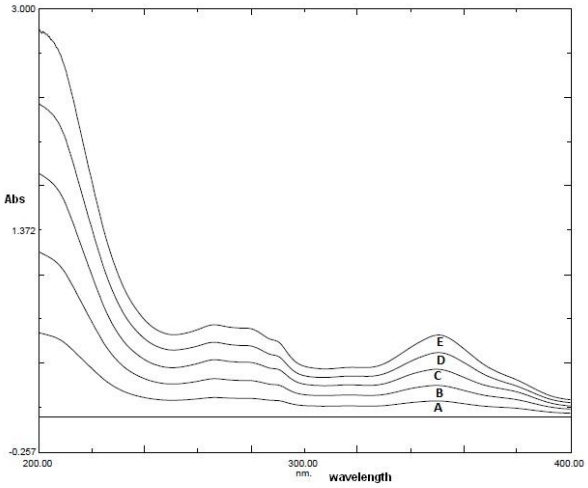

Fig. 3: Linearity curve for Mecobalamin at 10-50 µg/ml

Hint: A= 10; B=20; C=30; D=40; E=50µg/ml

In degradation studies (Fig. 3-4; table 2), percent recovery was found to be 117.77, 97.71 and 119.77% for oxidation, acid degradation and alkali degradation but for thermal Stress studies at 50º, 60º and 80ºC, the percent recovery was found to be 86.25, 64.47 and 32.38%.

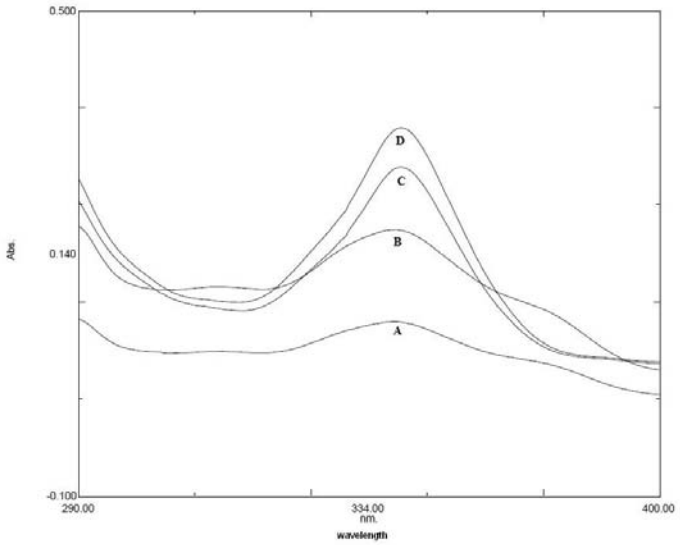

Fig. 3: Effect of thermal stress on Mecobalamin stabilty

Hint: A= 80 °C; B=60 °C; C=50 °C; D=untreated pure drug

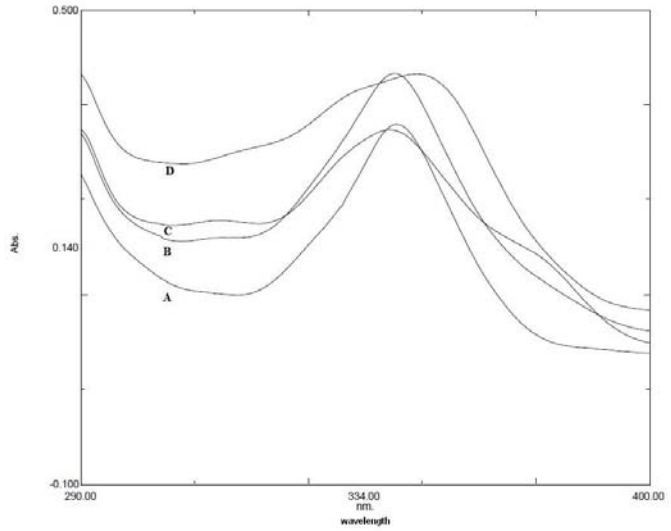

Fig. 4: Effect of acid, alkali and hydrogen peroxide on Mecobalamin stability

Hint: A= Untreated pure drug; B= 5% H_2O_2; C=0.1 N HCl; D= 0.1 N NaOH

Table 2: Summary of forced degradation study for Mecobalamin

Drug	Stress Conditions	Assay %	Remarks
Mecobalamin	Standard	100	No degradation
Mec + 5 % H_2O_2	Oxidation	101.70	No degradation
Mec + 0.1 N HCl	Acid stress	97.71	degradation
Mec + 0.1 N NaOH	Alkali stress	101.97	No degradation
Mec + heat 50ºC	Thermal stress	86.25	degradation
Mec + heat 60ºC	Thermal stress	64.47	degradation
Mec + heat 80ºC	Thermal stress	32.38	degradation

This proved that there is degradation of mecobalamin under heat conditions. The proposed method has good reproducibility, accuracy and revealed that the commonly used excipients and additives in formulation were not interfering and the drug is stable to acid, alkali and oxidative treatments. The method can be adopted for routine quality control.

CONCLUSION

In the UV spectrophotometry estimation of mecobalamin, Beer's law obeyed in the concentration range of 10-50 µg/ml. Percentage recovery proved to be in par with ICH guidelines and the proposed method is accurate, simple, and revealed that the commonly used

excipients and additives in formulation were not interfering with analysis and the drug is stable to oxidation and alkali treatments, but unstable to thermal stress and acid treatments. Therefore the method can be recommended for routine quality control test of injection formulations after further stability studies.

ACKNOWLEDGEMENT

The authors are thankful to the management of SB College of Pharmacy, Sivakasi for providing facilities to carry out this work.

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