IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 3 Ver. II (May - Jun. 2015), PP 43-48
www.iosrjournals.org

Degradation Study of Methylcobalamin Injection and Change in pH by Thermal Stress

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Abstract: *Trigeminal neuralgia, diabetic neuropathy, facial paralysis and megaloblastic anemia can be treated by the use of Methylcobalamin injection. An accurate, simple, economical, precise and reproducible UV Spectrophotometric method has been developed for the assessment of Methylcobalamin in injection dosage form and validated by ICH guidelines. The standard solution (10µg/ml) was scanned between 200-400 nm and maximum absorption was recorded at 353 nm. The assay results originated98.94%. The percent recovery was calculated as 99.05% to 100.50 %. The linearity range of 10-50 µg/ml proved that it obeyed Beer's Law. Correlation coefficient (r^2) was found to be 0.99 at 353 nm with an intercept of 0.0105 and a slope of 0.0121 with RSD 1.33 complied ICH. In forced degradation study (thermal stress) we observed that Methylcobalamin assay become decreased due to thermal stress and there is slightly change in pH. The proposed method was accurate, precise, and reproducible. The commonly used excipients in formulation were not interfering. The drug was found to be unstable in heat conditions.*

Keywords: *Spectrophotometer, Methylcobalamin, Degradation, Paralysis, Neuralgia.*

I. Introduction

Methylcobalamin is used for the treatment of megaloblastic anemia[1], facial paralysis in Bell's syndrome[2], diabetic neuropathy and trigeminal neuralgia[3]. Chemically it is known as 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 and have molecular formula $C_{63}H_{91}CoN_{13}O_{14}P$[4]. Its chemical structure is presented Figure 1. It is a dark red crystalline powder soluble in water and ethanol[5]. In Japanese Pharmacopoeia (XIV) it is present officially[6].Only two UV Spectrophotometric method of analysis have been reported for the assay of Methylcobalamin raveled as a result of study. In order to conduct pharmaceutical analysis, UV-Visible spectrophotometry is one of the most commonly used methods[7, 8].But thermal forced degradation studies have not been reported by any one. Therefore in the present investigation, an attempt has been made to develop an accurate, simple and an economic UV Spectrophotometic method for the estimation of Methylcobalamin in injection formulation after degradation of material and validated for accuracy, linearity and stability[9] to forced degradation studies according to the prescribed procedures mentioned in ICH guidelines[10].

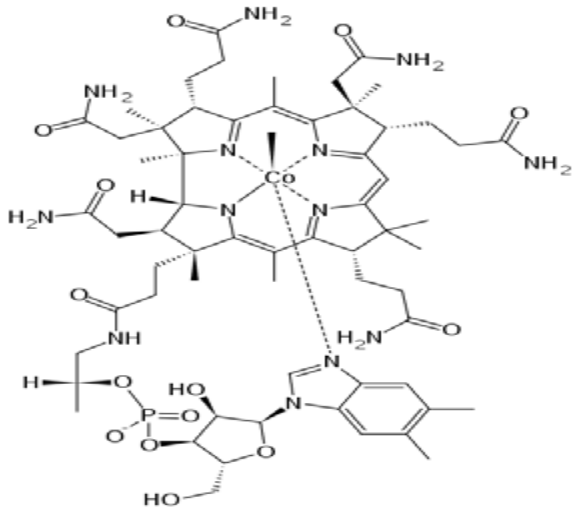

Figure1: Chemical Structure of Methylcobalamin

II. Materials And Methods

Instrumentation

In this study following instruments were used: UV-Vis Spectrophotometer Shimadzu model 1800, Ultrasonicator, pH meter, electric water bath, digital weighing balance (Shimadzu Japan, 0.001mg sensitivity) and autoclave.

Standards and chemicals

The pure drug of Methylcobalamin was gifted by Ameer Adnan Pharma Lahore. The ampoules (500 µg/ml), of different brand were purchased from local pharmacy. All the chemicals used in the experiment were of Merck Analytical Grade.

Selection of wavelength

The solution of the strength 10µg/ml was prepared and scanned for calculating the wavelength which was selected between 200-400nm by spectrometer. The results proved that the maximum absorption was at 353nm for this reason it was selected as the λmax.

Preparation of standard solution

50 mg precisely weighed quantity of Methylcobalamin was taken and dissolve by sonication for 15 minutes with 10ml of water than the volume was made up to 100ml using distilled water and concentration of 500µg/ml was obtained. Absorbance of different dilutions in water i.e. 10, 20, 30, 40 and 50µg/ml were checked at 353nm using spectrophotometer.

Assay of Methylcobalamin injection

Twenty ampoules of Methylcobalamin injections form different brands (injection, 500µg/ml) were randomly selected and contents were transferred to a 100 ml beaker, sonicated for 15 minutes. From this stock solution, taken10 ml, poured into 100ml 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 10µg/ml. The amount of drug present in injection was determined by using the absorbance ratio method.

Method Validation

Linearity studies [11]: By scanning 10, 20, 30, 40 and 50µg/ml strength, linearity of standard Methylcobalamine powder was determined with the help of UV spectrophotometer at 353 nm and recorded their absorbance. Then by taking drug concentration on x-axis and absorbance on y-axis standard graph was plotted.

Accuracy studies [12]

Recovery experiment method was used for accuracy studies. The recovery experiment was resolute at levels of 80%, 100% and 120% in prepared concentrations. The solutions were injected in triplicates for each spike and the assay was performed as per the test method. Then from these results the values calculated were, percentage recovery and quantity present (mcg).

Degradation studies

Method no 1: For the study of thermal degradation 10 ampoules were selected, closed in container and autoclaved these ampoules at different temperature and pressure for 30 minutes, then prepared dilution by using the previously mentioned method and then checked on spectrophotometer.

Method no 2: For the studies involving thermal degradation, Methylcobalamin injection solution of strength 10µg/ml in distilled water was prepared and then the resultant solutions were heated separately at 100ºC, 110ºC and 121ºC for period of 30 minutes, stored for 1 hour in dark room at room temperature and then their absorbance values were calculated or observed.

III. Results And Discussion

Parameters	UV method
Assay	99.29 -100.5 %
Linearity range	10-50 µg/ml
λ Max (nm)	353 nm
Correlation coefficient (r2)	0.9995
Standard deviation	0.00342
Intercept (c)	0.0105
Slope (m)	0.0121
Repeatability (% RSD)	1.33

Table1: The Assay Parameters

In assay, the %age content was found to be 99.29 to 100.5 % for Methylcobalamin which 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. In linearity study, the correlation coefficient was found to be 0.9995 at 353 nm with an intercept of 0.0105 and slope of 0.0121 which complied with the ICH requirement (NLT 0.999). In repeatability studies, % RSD was found to be 1.33.

Drug	**Condition**	**Assay %**	**remarks**
Methylcobalamin	Standard	100	No degradation
Mec + heat 100ºC	Thermal stress	88.25	degradation
Mec + heat 110ºC	Thermal stress	70.47	degradation
Mec + heat 121ºC	Thermal stress	54.38	degradation

Table2: Summary of degradation study for Methylcobalamin

In degradation studies for thermal Stress at 100º, 110º and 121ºC, the percent recovery was found to be 88.25, 70.47 and 54.38%. This proved that there was degradation of Methylcobalamin under heat conditions. The proposed method possessed good reproducibility, accuracy and revealed that the commonly used excipients and additives in formulation were not interfering. The method can be adopted for routine quality control.

Drug	**Condition**	**pH**	**remarks**
Methylcobalamin	Standard	6.8	No change
Mec + heat 100ºC	Thermal stress	5.3	Change
Mec + heat 110ºC	Thermal stress	4.9	Change
Mec + heat 121ºC	Thermal stress	4.1	Change

Table3: Summary of pH change study for Methylcobalamin

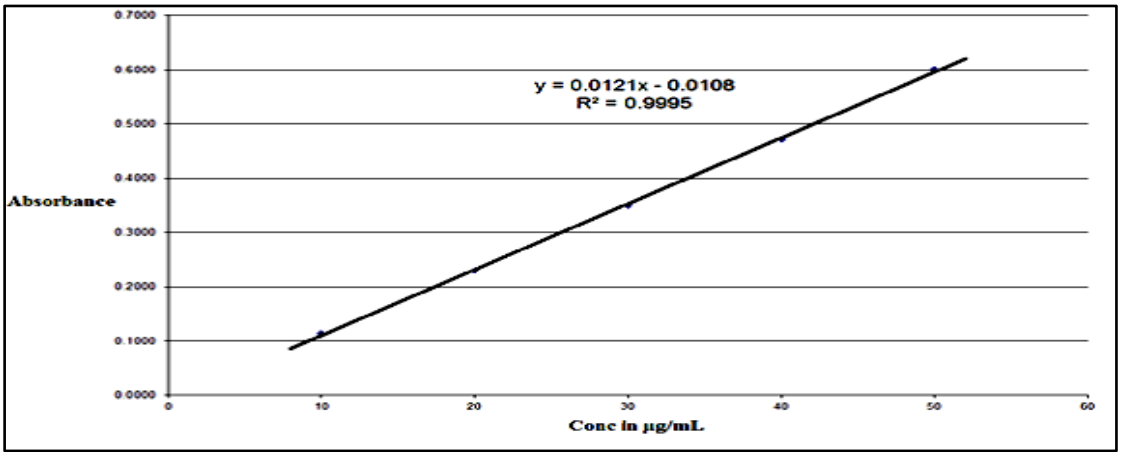

Fig 2: Linearity Plot for Methylcobalamin injection

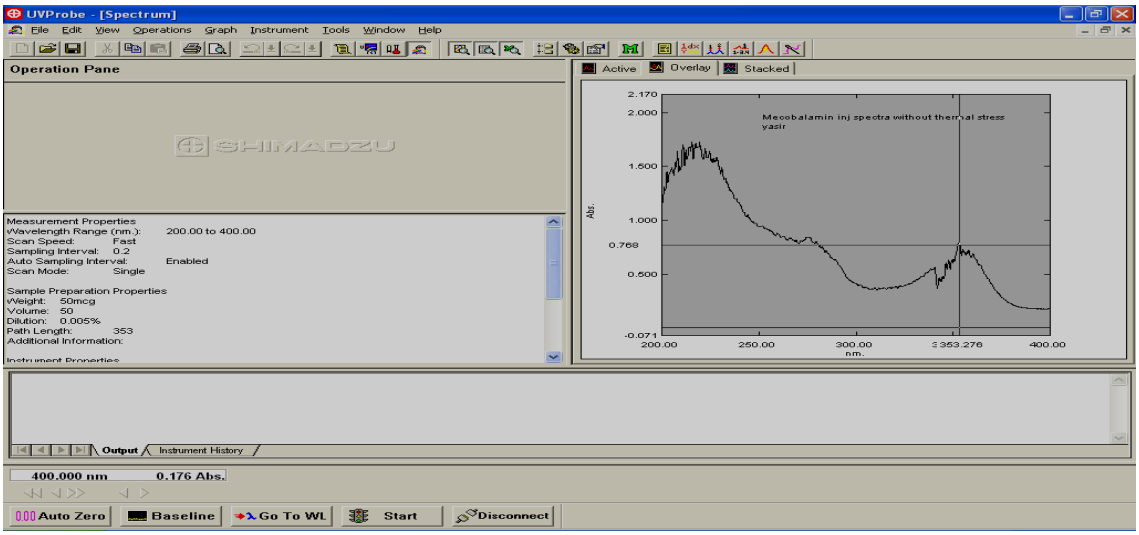

Fig 3: Spectra of Methylcobalamin injection without thermal stress

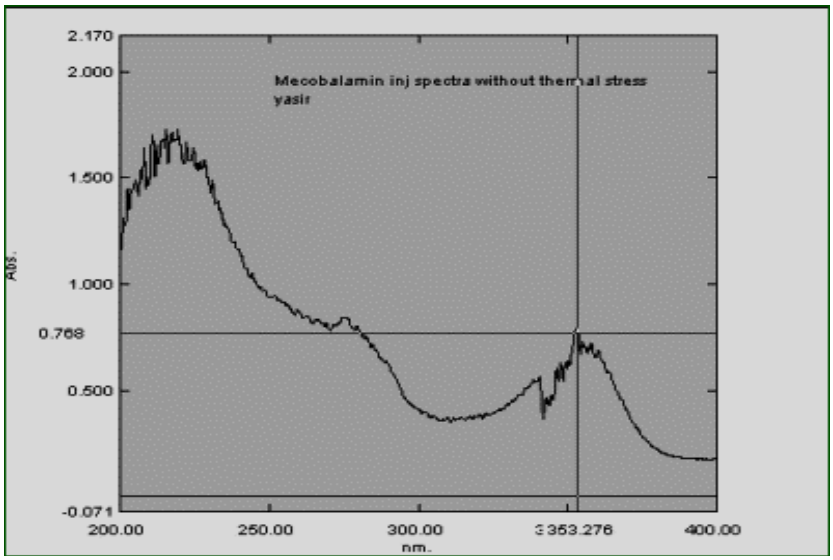

Fig 3.1: Spectra of Methylcobalamin injection without thermal stress

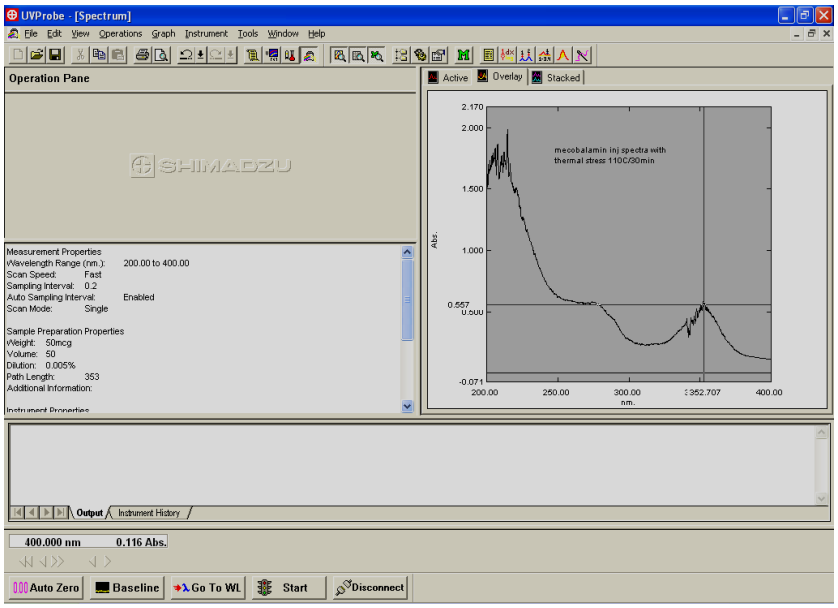

Fig 4: Spectra of Methylcobalamin injection with thermal stress (110℃/30min)

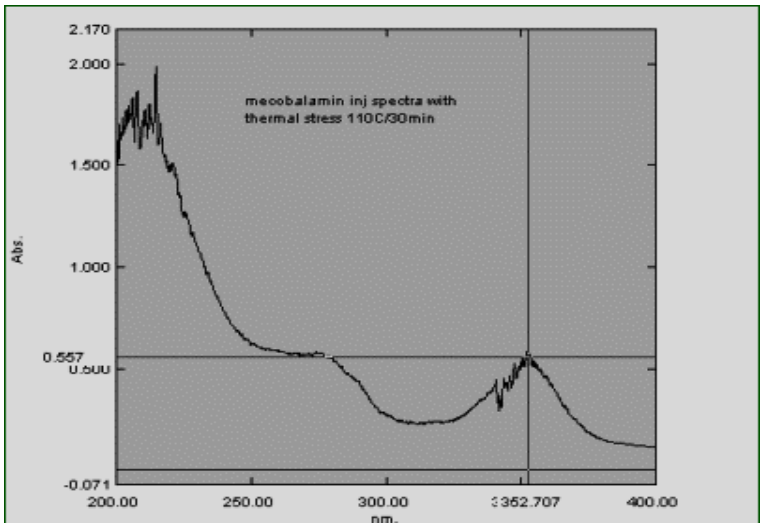

Figure 4.1: spectra of Methylcobalamin injection with thermal stress (110℃/30min)

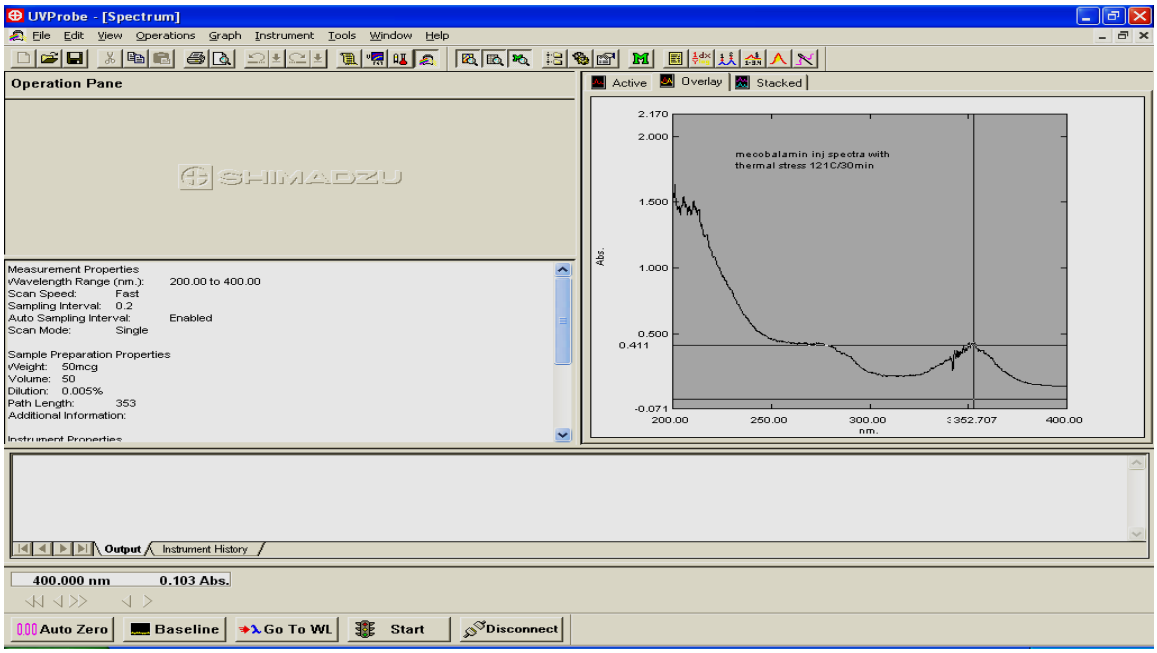

Fig 5: Spectra of Methylcobalamin injection with thermal stress (121℃/30min)

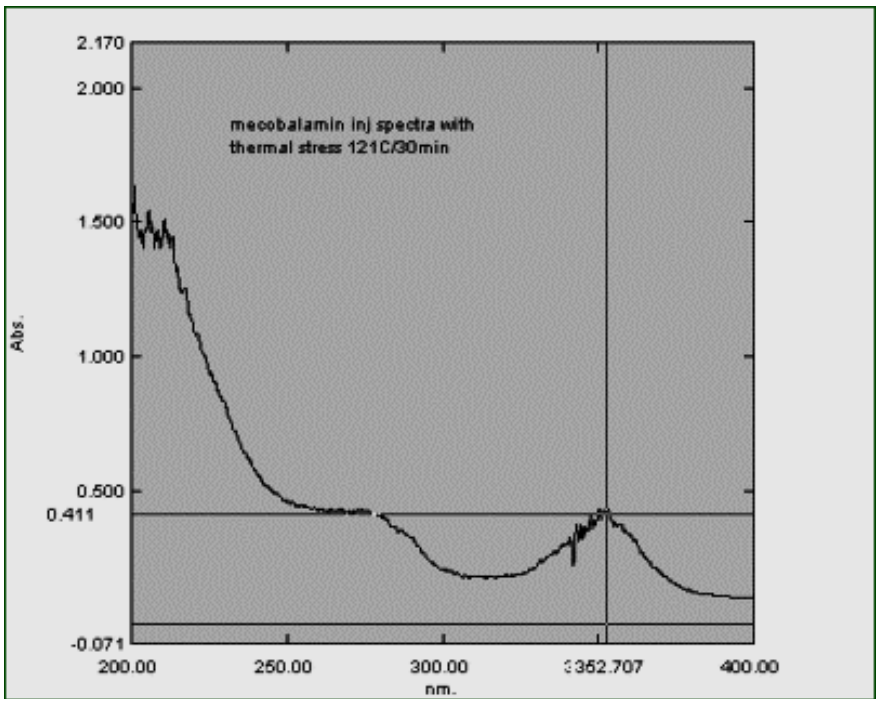

Fig 5.1: spectra of Methylcobalamin injection with thermal stress (121℃/30min)

IV. Conclusion

In UV spectrophotometry estimation of Methylcobalamin, Beer's law was found to be obeyed in the concentration range of 10-50 µg/ml. Percentage recovery was proved to be in par with ICH guidelines and the proposed method was accurate and simple. It was revealed that the commonly used excipients and additives in formulation were not interfering with analysis. Drug was found to be unstable under thermal stress. Therefore this method can be recommended for routine quality control test of injection formulations. In this study we estimated that due to excess heat and pressure Methylcobalamin injection solution degraded and its pH was also changed.

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