A Simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form

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ABSTRACT

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of methylcobalamin in capsule dosage form. A phenomenex Gemini C18 5µm column having 250 x 4.6mm id in Isocratic mode with mobile phase containing acetonitrile: 0.05M Sodium di hydrogen ortho phosphate (20:80 %v/v pH: 3.5) was used. The flow rate was 1ml/min and effluents were monitored at 376nm. The retention time of methylcobalamin was 7.8min. The concentration curves were linear in the concentration range of 10 to 50µg/ml. The developed method was validated for specificity, precision, linearity, accuracy, ruggedness, robustness and solution stability. Recovery of methylcobalamin in formulations was found to be in the range of 98.62% to 99.80%. Proposed method was successfully applied for the quantitative determination of methylcobalamin in formulations.

Keywords: RP-HPLC, Methylcobalamin, Validation and Chromatography.

1. INTRODUCTION

Methylcobalamin is a dark red crystalline powder and it has been referred for neurological illness, diabetic neuropathy, hearing loss and Alzheimer’s disease. Methylcobalamin is designated as Coα-[α-(5, 6-dimethylbenz-1H-imidazolyl)]-Coβmethylcobamide and has an empirical formula C₆₃H₉₁CO₁₃O₁₄P. [1-5]

From the literature survey various methods have been reported for the estimation of methylcobalamin in biological matrices such as plasma, which includes HPLC, UV and ICP-MS but no single reverse phase high performance liquid chromatographic method was reported for the estimation of methylcobalamin in capsule dosage form. So aim of the present work was to develop simple, rapid, precise and reproducible reverse phase high performance liquid chromatographic method for determination of drug in bulk and capsule dosage form.

This paper describes validated RP-HPLC method for estimation of methylcobalamin in bulk and pharmaceutical formulations. The proposed method was optimized and validated as per the ICH guidelines. [6-7]

2. EXPERIMENTAL

2.1. Reagents and chemicals
Methylcobalamin was obtained as a gift samples from Themis medicare Ltd., (Vapi, India), Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals Ltd.,(Mumbai, India), Sodium dihydrogen ortho phospate (AR grade) was purchased from EMerck Ltd.,( Mumbai, India). The pharmaceutical dosage forms used in the study was Reneuron capsules labeled containing 500μg of Methylcobalamin from the retail shop.

2.2. Chromatographic conditions

Analysis was carried out using Shimadzu HPLC system, Consisting of LC 10AT pump. A phenomenex Gemini C_{18} 5μm column having 250 x 4.6mm id in gradient mode with mobile phase containing acetonitrile: 0.05M Sodium di hydrogen ortho phosphate (20:80 %v/v; pH: 3.5) was used at a flow rate 1ml/min. A Rheodyne injector with 20 μl loop was used for injecting the sample. Detection was carried out using UV/VIS detector at 376 nm.

2.3. Preparation of mobile phase

800ml of (0.05M) Sodium dihydrogen ortho phospate buffer was mixed with 200ml of Acetonitrile in the ratio of 80:20 (%v/v). The pH of the mobile phase was adjusted to 3.5 with otho phosphoric acid. Then sonicated for 15min and filtered through a 0.45 μm filter.

2.4. Standard solutions

2.4.1. Stock solutions

Weighed accurately about 100mg of methylcobalamin and was dissolved in 100 ml of mobile phase in to the 100ml light resistant volumetric flask. The final solutions containing 100μg/ml of methylcobalamin.

2.4.2. Working standard

3ml of stock solution was taken in 10ml light resistant volumetric flask and diluted up to mark with mobile phase. The final solution containing 30μg/ml.

2.5. Sample preparation

Twenty capsules powder were accurately weighed. A quantity of powder equivalent to 30 mg of methylcobalamin and transferred in to 100ml light resistant flask and made up the required volume by using mobile phase. Pipetted out 5ml of resulting solution in to the 50 ml light resistant flask and made up the required volume by using mobile phase and sonicated for 15 min. Then finally filtered through 0.45μ filter.

3. RESULT AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard solution of methylcobalamin to check various parameters. System suitability results are as follows:

- Retention time: 7.8 min
- Asymmetric factor: 1.60
- Theoretical plate: 3928
- Calibration range: 10-60μg/ml

3.1. Method validation

The proposed method has been validated for the assay of methylcobalamin in formulation using following parameters:

3.2. Linearity:

Linearity was studied by preparing standard solution at different concentration levels. The linearity range was found to be 10-50μg/ml. The regression equation was found to be \( y = 19.594x + 407.18 \) with coefficient of correlation \( R^2 = 0.9996 \) (Fig.1.)
3.3. Precision

Precision was studied to find out intra and inter day variations in the proposed method of methylcobalamin at different concentration levels: 20\(\mu\)g/ml, 30\(\mu\)g/ml and 40\(\mu\)g/ml on the same day and three different days respectively. The percentage RSD was calculated for intra and inter day precision and found to be less than 2%. The results of precision are presented in the table 1.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu)g/ml</td>
<td>Concentration obtained (\mu)g/ml</td>
<td>% RSD</td>
</tr>
<tr>
<td>20</td>
<td>20.01</td>
<td>0.432</td>
</tr>
<tr>
<td>30</td>
<td>29.95</td>
<td>0.238</td>
</tr>
<tr>
<td>40</td>
<td>40.12</td>
<td>0.785</td>
</tr>
</tbody>
</table>

* Mean of three replicate determinations.
** Mean of determinations at three different days.

Table 2: Recovery study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery</th>
<th>Area obtained</th>
<th>Average area</th>
<th>Amount Recovered in mg</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>798.86</td>
<td>798.2712</td>
<td>23.6</td>
<td>98.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>999.23</td>
<td>998.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylcobalamin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>995.24</td>
<td>994.5133</td>
<td>30.01</td>
<td>100.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>989.07</td>
<td>120</td>
<td>1196.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1198.78</td>
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<td></td>
</tr>
</tbody>
</table>

Table 3: Assay of methylcobalamin from bulk and capsule dosage form

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Experiments</th>
<th>Labeled claim (mg/tab)</th>
<th>Obtained (mg/tab)</th>
<th>% assay (mg/tab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reneuron capsules</td>
<td>1</td>
<td>500</td>
<td>499.92</td>
<td>99.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>500</td>
<td>500.03</td>
<td>100.06</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>500</td>
<td>499.08</td>
<td>99.12</td>
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<tr>
<td>Bulk drug</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>99.91</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>99.23</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>99.76</td>
</tr>
</tbody>
</table>

Fig 1: Sample chromatogram
3.4. Recovery Study

The accuracy of the method was determined by calculating recovery of methylcobalamin 80%, 100% and 120% was added to a pre-quantified sample solution. The recovery studies were carried out three times over the specified concentration range and the percentage recovery of methylcobalamin was found to be in the range of 98.62% to 100.01% and the results are presented in the table 2.

3.5. Robustness

Robustness of the method was studied by changing the λ max from 376 to 370 and the mobile phase composition of organic phase changed by ± 5% and pH± 2. The results showed that the retention time and peak area of methylcobalamin is remains almost unchanged and no significant degradation was observed.

3.6. Assay

The standard and sample solutions were injected three times separately; chromatograms and the peak areas were recorded. A representive chromatograms of sample has been given in Fig 1. The amount of drug present per capsule was determined. Thus obtained results were presented in the table 3.

4. CONCLUSION

Proposed study describes new reverse phase high performance liquid chromatographic method for the estimation of methylcobalamin in formulations the method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery studies shows that, the method is free from interference of the other active ingredients and additives used in the formulation. Therfore proposed method can be used for routine analysis for the estimation of methylcobalamin in bulk and capsule dosage form.

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6. REFERENCES