RESEARCH ARTICLE

A Validated Reversed-phase High-performance Liquid Chromatography Analytical Method for the Analysis of Methylcobalamin in Bulk Drugs and T-Dosage Formulation

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ABSTRACT

The aim of the existing work is to develop and validate a reversed-phase high-performance liquid chromatography (RP-HPLC) technique for the quantitative determination of methylcobalamin in bulk and t formulations that is easy, rapid, precise, accurate, affordable, and sensitive.

Proceeding a princeton (C18) column with dimensions (250 x 4.6 mm, 5 µ), the isocratic elution technique was used. The mobile buffer phase comprised water (pH 6.5, adjusted with sodium chloride) and methanol in the relation (55:45) v/v. It was shown that the methylcobalamin retention time was 2.022 minutes under ideal circumstances.

The correlation coefficient (r2) for the 900–2400 mcg/mL methylcobalamin concentration range, which was used to verify the method's linearity, was 0.9994. Methylcobalamin had a recovery rate of 99.98–100.01% and RSD of 2%. The marketed t formulation test was successfully completed with 99.86%.

The proposed and validated approach underwent the accuracy, precision, specificity, linearity, and system suitability testing advised by the ICH. RP-HPLC was used in the market formulation.

Keywords: Methylcobalamin, Reversed-Phase HPLC, Validation.

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Conflict of interest: None

INTRODUCTION

Carbamide; cobalt (3+); methylcobalamin (MeCbl) [5- (5,6- dimethylbenzimidazol-1-yl) oxolan-3-yl] -4-hydroxy-2-(hydroxymethyl) -1-[3-[(4Z,9Z,14Z)-2,13,18-tris(2-amino-2oxoethyl) - 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] Propan-2-yl phosphate [propanoylamino] (Figure 1). Megaloblastic anemia, diabetes, and peripheral neuropathy are all treated with methylcobalamin.[1-3] According to the literature review, high-performance liquid chromatography (HPLC) should be considered as a technique for estimating methylcobalamin in individual or combined t dosage formulations.[4-13] However, the stated technique has the disadvantages of a long runtime and being less cost-effective due to the high proportion of organic phase. As a result, the HPLC technique for determining methylcobalamin in combined t and bulk dose forms is an accurate, precise, simple, specific, and cost-effective technology.

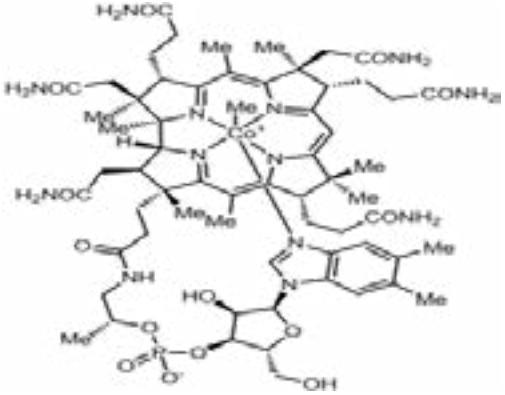

Figure 1: Methylcobalamin structure.

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Table 1: Chromatographic conditions optimization

Chromatographic condition	
Mobile phase	Water (3 pH, sodium chloride): Methanol (55:45) v/v 1.0 mL per min.
Flow rate	
Column	Princeton C-18 column (250 mm × 4.6 mm, 5 µ)
Detector wavelength	210 nm
Column temperature	30ºC
Injection volume	10 µL
Runtime	20 minutes
Diluent	Methanol
Retention time (RT)	2.022 minutes

Table 2: Results of specificity.

Parameter	*Methylcobalamin*
Theoretical Plate	3067
Retention Time	3.844
Tailing factor	1.19
%RSD	0.6

Table 3: Results of linearity.

Parameter	*Methylcobalamin*
Concentration Range (mcg/mL)	900–2400
Slope (m)	39.496
Intercept	1058.3
Coefficient correlation (r^2)	0.9994

METHODOLOGY

Reagents and Chemicals

Analytical level Methylcobalamin was acquired from Nervmax-SR 75®, a t-marketed formulation from the Ahmedabad-based pharmaceutical company Cadila Pharmaceuticals Limited.

Analytical-grade o-phosphoric acid, methanol, and acetonitrile were used.

Instrumentation

The researchers employed Lab Solution software of a Shimadzu HPLC system and a PDA detector.

Chromatographic Conditions Optimization

A buffer mobile phase is containing (6.5 pH, sodium chloride) with methanol in the relation of (55:45) v/v, flow rate 1.0 mL per minute, at 210 nm, was used to achieve chromatographic isolation on RP column Princeton C-18 with dimensions (250 mm x 4.6 mm, 5 μ). 10 µL of injection volume, pH of the solvent solution at 3.0 (1). The standard and sample methylcobalamin chromatographs of a mixture are summarized in Figures 2 and 3, respectively (Table 1).

Procedure for Preparing a Methylcobalamin Standard Solution

A 150 mcg methylcobalamin dose was weighed and placed in a volumetric flask with a capacity of 10 mL. The content dissolved into methanol and increased to the required volume level. The final product was sonicated for 15 minutes and contained 15000 mcg of methylcobalamin.

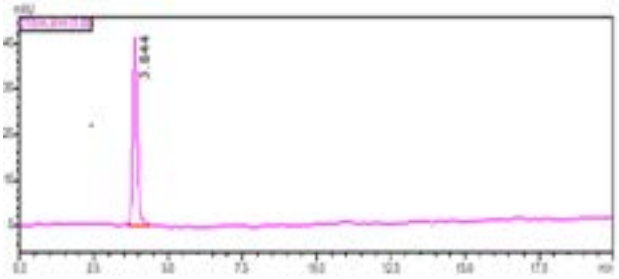

Figure 2: Methylcobalamin chromatograph (standard).

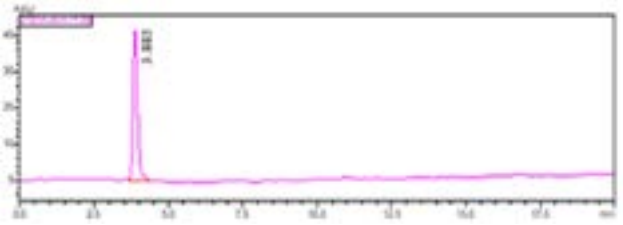

Figure 3: Methylcobalamin chromatograph (sample).

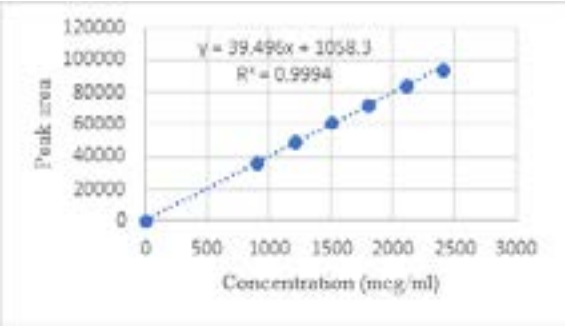

Figure 4: Linearity curve for methylcobalamin.

Procedure for Preparing a Methylcobalamin Sample Solution

Measured Twenty ts and finely ground, the correctly weighed quantity of powder containing 1500.00 mcg of methylcobalamin was put into a 10.0-milliliter volumetric flask. Methanol (5 milliliters) was poured into the powder. 1.0 milliliter shifted to a 10-milliliter flask, where it was diluted to the desired concentration with diluent to yield a sample solution containing 1500.00 mcg methylcobalamin per milliliter. Six replicate t solutions containing 1500.00 mcg methylcobalamin per milliliter were produced in the same way.

Assay Procedure

Following stationary phase equilibration, three repeat injections of individual sample solutions were produced, and chromatograms were noted. The following formula was used to compute the peak capacity of an amount of medication included in the regular mass of a t as a percentage of the labeled claim.

$$\%Assay = \frac{A_{sam} \times C_{std} \times DF \times Avg.Wt.}{A_{std} \times Wttaken \times LC}$$

Table 4: Results of recovery.

Compound	*Spiked conc. (%)*	*Amount conc. taken (mcg/mL)*	*Amount conc. found (mcg/mL)*	*Percentage recovery*
Methylcobalamin	80	1200	1200.48	100.04
	100	1500	1500.16	100.01
	120	1800	1799.68	99.98

Table 5: Results of robustness.

Parameter quantity estimated [%]		*Methylcobalamin*	
		RSD [%]	
Alteration in wavelength (210 ± 2 nm)	208 nm	100.04	0.0695
	212 nm	100.08	0.0546
Alteration in flow rate (1.0 ± 0.1 mL/min)	0.9 mL/min	99.44	0.1268
	1.1 mL/min	99.92	0.1884

*Each number represents the average of three observations.

Table 6: Summary

Parameter	*Methylcobalamin*
Calibration range (mcg/mL)	900–2400
Optimized wavelength (nm)	210
Retention Time	3.844
Regression equation (Y)	Y = 39.496x + 1058.3
Slope	39.496
Intercept	1058.3
Coefficient correlation (r2)	0.9994
Precision (% RSD)	
Intraday	0.10
Interday	0.06
%Assay	99.86
Limit of detection (µg/mL)	9.78
Limit of quantification (µg/mL)	0.61

%RSD: Percentage RSD.

Method Validation

Specificity

The specificity is defined by comparing the chromatographs of standard and sample solutions (Table 2).

Limit of Quantification and Limit of Detection

When assessed with sufficient precision and accuracy, the lowermost concentration sample and the lowermost analyte concentration serve as the limits of quantification and detection, respectively. By ICH guidelines, LoD and LoQ were considered using the formulas LoQ = 10/S and LoD = 3.3/S, respectively. S stands for the standardization plot's slope and represents the regression line's standard deviation.

Linearity

Linearity was obtained at various concentrations ranging from 900 to 2400 mcg/mL. To establish linearity, the analysis of least-squares linear regression was used. Peak regions versus linear regression analysis and associated concentrations were calculated on the provided curves (Table 3). Figure 4 depicts the methylcobalamin linear curve.

The measurement of the precision of a region of six competent operating standards for manipulating the RSD in methylcobalamin. Six different methylcobalamin test samples were assayed against authorized working standards to establish the result, and the percent RSD was calculated. Multiple analysts and different days were used to demonstrate the technique's intermediate precision.

Accuracy

The analytical accuracy operation identifies the covenant as being present in the middle of the value, which is established as a perfect, accurate value or a conventional mention value. Calculations were made at three unlike levels of the label claim. (80, 100, and 120%) (Table 4).

Robustness

The chromatographic parameters were carefully varied and the resolution for methylcobalamin was evaluated to postulate the robustness of research of the approved chromatographic approach. To investigate the impact of wavelength on the assessment, as well as wavelength variations of less than 2 nm, i.e., 208 and 212 nm from the initial 210 nm. By changing 0.1 millimeters per minute flow rate, or 0.9 and 1.1 mL per minute, from a specific flow rate, 1.0 mL per minute, to examine the impact of flow rate on the assessment (Table 5).

Stability of Solution

For 24 hours, the sample's stability was observed. 0.9 for the relative standard deviation suggests that the approach is s for 24 hours. As a result, the approach was discovered to be particular.

RESULTS AND DISCUSSION

Development and Optimization of HPLC

Initial results presented that a solvent solution with a buffer (6.5 pH, sodium chloride) and methanol combination in a relation of (55:45) v/v, the flow rate at 1.0 mL per minute, produced well-resolved drug peaks at 210 nm. The retention period lasted 2.022 minutes.

Validation of the Technique

LoQ and LoD

For methylcobalamin, the limits of quantification and detection were 9.78 and 0.61, respectively.

Linearity

The calibration data were subjected to least-squares linear regression analysis to determine linearity. Methylcobalamin calibration plots were linear in the 900–2400 mcg/mL concentration range. Concentrations were determined using the resulting curves after plotting peak regions against a linear regression analysis. The linear equation for the methylcobalamin calibration plots was Y = 39.496x + 1058.3 the correlation coefficient was 0.9994.

Precision

The values of methylcobalamin for intraday precision and interday precision were 0.06 and 0.10, respectively. Method, system, and intermediate precision findings with a %RSD of less than 2.0% indicate that the procedure was exact.

Accuracy

The values of methylcobalamin for interday precision and intraday precision were 0.10 and 0.06, respectively.

Robustness

Chromatographic circumstances were purposefully adjusted and estimated to determine the robustness of the new approach. This research aimed to see how the flow rate of wavelength affected the assessment (Table 6).

Study of Methylcobalamin from Marketed Ts

The methylcobalamin content of the commercial preparation was determined to be 99.64%.

CONCLUSION

Create RP-HPLC, an efficient, precise, accurate, and cost-effective method for estimating methylcobalamin in combination t and bulk dose form.

CONFLICT OF INTEREST

There is no conflict of interest.

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