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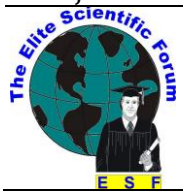

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RESEARCH ARTICLE

Method Development and Validation of Mecobalamin Injection By UV-Spectrophotometer

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ABSTRACT

An efficient, accurate, simple, fast, precise and reproducible UV-spectrophotometric method was developed using pH-7.0 buffer (monobasic potassium dihydrogen phosphate) for the validation of mecobalamin injection. The standard 20 µg/mL solution was scanned between the range of 200 – 600 nm and λ_{max} was found to be 522 nm. Validation parameters were checked for the developed method as per ICH guidelines. The linearity concentration range 16 – 24 µg/mL showed that it obeyed Lambert's law and the developed method was linear with a good correlation coefficient. Standard addition method was performed for the proposed method and percent recovery studies revealed that the developed method was accurate. Percent relative standard deviation (RSD) value was calculated for precision studies which showed that the developed method was highly precise. The established method was validated in terms of linearity, precision, intermediate precision, accuracy, robustness and ruggedness. The results of all these parameters significantly showed that the developed method can be used for the routine validation of mecobalamin injection in pharmaceutical laboratories.

INTRODUCTION

Mecobalamin commonly termed as methylcobalamin that occurs naturally in pure form as vitamin B_{12} efficiently improves the conduction of nerves (Sawangjit et al., 2020). Mecobalamin has been indicated to treat megaloblastic anemia, diabetic neuropathy, trigeminal neuralgia and facial paralysis in Bell's palsy syndrome (Mehmood et al., 2015). Cobalamins contain cobalt atom in the center surrounded by four ligands of coplanar nitrogen which resemble corrin ring. Alkylcobalamins contain an alkyl group that is present near the cobalt atom in the top axial position. The bottom axial position is attributed to the nitrogen atom of dimethyl benzimidazole nucleotide (Motwani et al., 2011). In 1962, the very first time biologically active compound alkylcobalamin was discovered as mecobalamin (Guest et al., 1962). This discovery has been made several years after the first description of another vitamin B_{12} co-enzyme called adenosylcobalamin (Barker et al., 1972). Chemically mecobalamin is Carbanide;cobalt(3+);[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] 1-[3-[(4Z,9Z,14Z)-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 $C_{63}H_{91}CoN_{13}O_{14}P$ (Fig. 1). As other derivatives of vitamin B_{12} mecobalamin is also a tetrahedral organometallic compound in which the central cobalt atom is coordinated by pyrrole of the corrin ring having four equatorial nitrogen ligands (Banerjee and Ragsdale, 2003). It is also bounded with an axial ligand appended from the margin of ring D of the corrin. The methyl group is attached as a ligand from the upper axial in mecobalamin (Fig. 1). Mecobalamin is an important cofactor form of the B_{12} enzymes, used in the catalytic reactions of methyltransferases which take part in metabolismic

reactions of amino acids in many organisms and one-carbon metabolism and CO_2 fixation in anaerobic microbes.

Among all these B_{12}-depended enzymes methionine synthase is a comprehensively studied enzyme in human beings present in the chromosome (Shao et al., 2018). As a cofactor in methionine synthase enzyme, mecobalamin catalyses the transfer of the methyl group from methylenetetrahydrofolate to homocysteine (Hcy) to form methionine and tetrahydrofolate, obtained as other products of this reaction (Banerjee and Ragsdale, 2003; Barker et al., 1972). Only two UV spectrophotometric methods have been reported for its assay in the literature survey (Mounnissamy et al., 2004; Maybodi and Darzi, 2008; Galande et al., 2010; Saravanan et al., 2010). The present study was aimed to develop a simple, accurate and economical UV spectrophotometric method for the estimation of mecobalamin in injection formulation followed by its validation for accuracy, linearity and precision according to the prescribed procedures as per guidelines of the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use.

MATERIALS AND METHODS

Chemicals and reagents

All the solvents, reagents and chemicals were of analytical grade. These chemicals were obtained from Merck (KGaA 64271, Darmstadt-Germany) and Penta Scientific Companies and used without any further purification.

Instrument

In this study, micropipette of capacity 10-100 µL (Labtech, USA), *UV-VIS* double beam spectrophotometer (UV - 1800 Series, Shimadzu, USA) having the wavelength range of 200nm - 800 nm with two matched quartz cells of 1 cm, Ultrasonic Cleaner (Delmer, India), electronic balance (Electric Mettler Toledo balance, model AL 204) and portable pH meter (Hanna HI-8424) were used.

Sample injection

This experimental work was done on sample Injection Mecobalamin (Hilton Pharma) comprising of active ingredient mecobalamin. Each injection consists of 500µg/mL mecobalamin.

Preparation of standard stock solutions

Mecobalamin (100mg) was dissolved in 100mL of buffer solution (pH 7.0) with sonication for 15 minutes. The standard stock solution of concentration (1000µg/mL) was prepared. Two mL of that solution was transferred into an amber volumetric flask and diluted up to the 1:100 volume with pH-7.0 buffer. The concentration of the working standard solution was adjusted to 20µg/mL.

Selection of wavelength

For the validation of mecobalamin, the selection of wavelength was done by scanning the working standard stock solution (20µg/mL) in the range of 200 - 600 nm. The stable λ_{max} was found to be 522nm against pH-7.0 buffer (Monobasic potassium dihydrogen phosphate).

Sample solution

A total of 20 ampules (each containing 500µg/mL) of mecobalamin injection were transferred into 100mL of amber volumetric flask and diluted with buffer solution (pH-7.0) and the final concentration of sample solution was adjusted as 100µg/mL. Twenty 20 mL of that solution was taken into amber volumetric flask and diluted up to the total volume of 100 mL withbuffer solution (pH-7.0). The final concentration of working sample preparation was adjusted as 20 µg/mL.

Preparation of working standard solutions for linearity

Appropriate aliquots from100 µg mL^{-1} (sample stock solution) were taken in amber volumetric flask and diluted up to the volume of 100 mL with buffer solution (pH-7.0) to obtain the concentration range of 16, 18, 20, 22 & 24 µg mL^{-1}. The absorbance was checked at 522 nm.

Method development

The standard stock solution of 1000 µg mL^{-1} of mecobalamin was prepared by weighing accurately 100 mg of standard mecobalamin, taken in 100mL of the amber flask and diluted with distilled water, ethanol and pH 7.0 buffer solution, separately. The sample stock solution of mecobalamin injection was prepared by accurately taking 20 ampules (each ampoule containing 500µg/mL) taken in 100mL of amber flask and diluted with distilled water, ethanol and pH 7.0 buffer, separately. By appropriate dilution of standard and sample stock solutions, different concentrations (16 µg mL^{-1}, 18 µg mL^{-1} and 20 µg mL^{-1}) of standard solution and sample solution with diluents were prepared (Table 1). These different solutions of standard and sample mecobalamin were scanned in the range of 200 to 600 nm to determine the wavelength of maximum absorbance, using buffer (pH-7.0) as a blank. Mecobalamin showed maximum absorption at 522nm. Thus, method validation for mecobalamin was carried out using buffer (pH 7.0). Amount of mecobalamin in each injection was calculated by using following formula as follows:

Absorbance of sample and standard preparations was observed at 522 nm using pH-7.0 buffer as blank. The percentage of mecobalamin was calculated by using the following formula:

Mecobalamin contents (%assay) = (Absorbance of sample / Absorbance of standard) × 100

= (0.126/0.127) × 100 = 99.21%

Method validation

The analytical method was developed and validated as per the ICH guidelines for validation of analytical

procedures to explain linearity, precision, intermediate precision, accuracy, robustness and ruggedness for the mecobalamin.

Linearity

The linearity was determined by analyzing the given concentrations of the working sample solutions of mecobalamin. The concentration range was found to be 16 - 24 µg mL^{-1} for mecobalamin according to the Beer Lambert's Law. The calibration curve to determine the linearity for mecobalamin was plotted between absorbance and concentration (Table 1 and Fig. 2).

Accuracy (Percent Recovery)

The standard addition method (Bhinge and Malipatil, 2016) was performed to determine the accuracy. Nine sample solutions were prepared by adding an appropriate amount (10 - 30 µg) of active drug to study the % recovery and absorbance was measured at 522 nm for each sample to calculate the accuracy of the developed method (Table 2).

System precision

The precision study of analytical procedure shows the closeness of the results, obtained by analyzing five samples of same concentration 20µg/mL at 522nm (Table 3). The percent RSD of these samples should be within 2% as per ICH guidelines.

Intra-day precision (repeatability) and inter-day precision (intermediate precision)

Repeatability was calculated by analyzing five different sample solutions of mecobalamin containing the same concentration (20 µg/mL) and percent RSD was calculated. The repeatability of the method was carried out by analyzing these various samples of mecobalamin. Intermediate precision was carried out by performing analyst to analyst variation. Mean SD and RSD (% values) were calculated and provided in Table 4.

Robustness

The robustness of the analytical method is its ability to remain unchanged by small but intended variation in method parameters for validation of mecobalamin in injection form. For this purpose, standard and sample solutions were analyzed at the standard λ_{max} 522nm and also above and below that value. With slight variation in wavelength, % assay was calculated that should be in the range of 98 to 110% (Table 5).

Ruggedness

To determine the ruggedness of the proposed method, the sample solutions were analyzed by two different analysts. The RSD values (%) were calculated and given in table 4, which should be less than 3% as per ICH guidelines.

RESULTS AND DISCUSSION

Method development

The method described in this research work provides a favorable and efficient way for the validation of mecobalamin injection by UV spectrophotometric method (Mehmood et al., 2015). The 522 nm wavelength was selected for validation of mecobalamin (Fig. 3).

Linearity of the developed method was observed in the concentration range of 16 - 20µg mL^{-1}. The absorbance of mecobalamin in the given range of concentrations was found to be 0.1026 to 0.1556 (Table 1). In this analytical method, the absorbance of the drug was observed at 522 nm for each concentration as shown in Fig. 2.

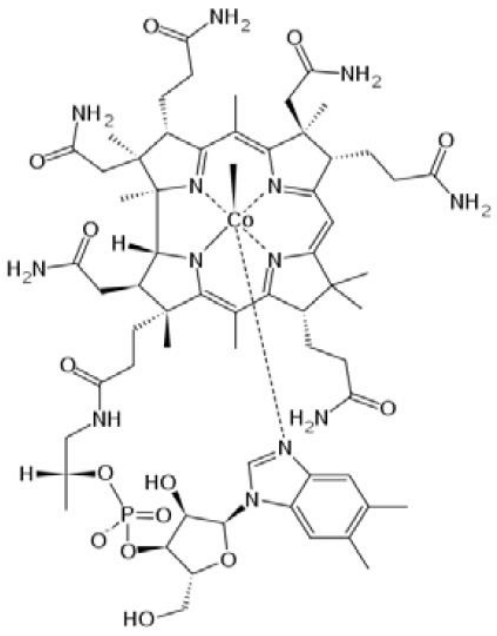

Fig. 1: Chemical structure of mecobalamin.

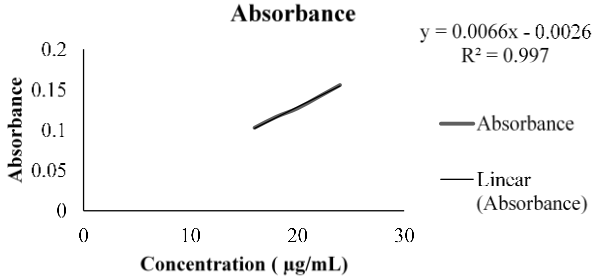

Fig. 2: Calibration curve of mecobalamin at 522 nm.

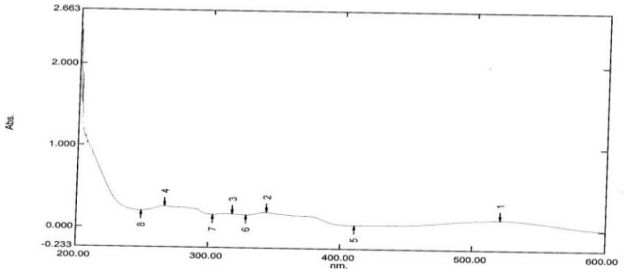

Fig. 3: UV spectrum of mecobalamin.

Table 1: Calibration data for linearity at 522nm

Linearity (Working standard)				
Standard concentration (µg/mL)	No. of samples	Absorbance	Mean	% Assay
20µg/mL	1	0.127	0.1266	99.65
	2	0.127		
	3	0.126		
Linearity (Sample solution)				
Sample Concentrations (µg/mL)	No of samples	Absorbance	Mean	% Assay
16	1	0.103	0.1026	80.78
	2	0.102		
	3	0.103		
18	1	0.116	0.116	91.33
	2	0.116		
	3	0.116		
20	1	0.127	0.1266	100.05
	2	0.127		
	3	0.126		
22	1	0.141	0.141	111.37
	2	0.141		
	3	0.141		
24	1	0.155	0.1556	122.95
	2	0.156		
	3	0.156		

Table 2: Accuracy study of method

Amount of mecobalamin in sample (µg)	Amount of mecobalamin added in sample (µg)	Total amount of mecobalamin (µg)	Amount (µg) Recovered at 522nm	% Recovery (n = 3)	Mean % Recovery
500	10.00	510	508.95	99.79	99.90
500	10.00	510	509.47	99.98	
500	10.00	510	509.75	99.95	
500	20.00	520	520.09	100.01	100.00
500	20.00	520	520.08	100.01	
500	20.00	520	520.00	100.00	
500	30.00	530	529.50	99.90	99.96
500	30.00	530	529.96	99.99	
500	30.00	530	529.97	99.99	

Table 3: Precision data analytical method.

No. of samples	Absorbance	%Assay
1	0.127	100
2	0.126	99.21
3	0.126	99.21
4	0.127	100
5	0.127	100
Mean	0.1266	99.68
Relative Standard Deviation	0.004326%	

Table 4: Intra-day and inter-day precision of mecobalamin sample solution.

Intraday and intermediate precision			Intraday assay	(n = 5)		Analyst-to-Analyst (n = 10)		
	Preparations	Absorbance	Mean	%SD	%RSD	Mean	%SD	%RSD
Analyst 1	1	0.127	0.1266	0.000548	0.004326	0.1265	0.000548	0.004329
Mecobalamin	2	0.126						
	3	0.126						
	4	0.127						
	5	0.127						
Analyst 2	1	0.127	0.1264	0.000548	0.004333			
Mecobalamin	2	0.126						
	3	0.127						
	4	0.126						
	5	0.126						

SD = Standard deviation; RSD = Relative Standard deviation.

Table 5: Robustness of the analytical method.

Samples	Concentration	λ_{max} (λ_{522})		λ_{max+1} (λ_{523})		λ_{max-1} (λ_{521})	
	(µg mL^{-1})	Absorbance	% Assay	Absorbance	% Assay	Absorbance	% Assay
Sample	20	0.127	99.21	0.1253	99.44	0.1287	99.79
Standard	20	0.126		0.126		0.129	

Table 6: The appearance of mecobalamin injection.

Sr. No.	Appearance parameters	Injection Methycobal
1	Dosage Form	Injection
2	Colour	Red
3	Clarity	Clear from any visible contamination
4	pH	5.0 to 7.0 at 30ºC
5	Average Volume/ampoule	1mL
6	Packing	Light Proof

The consequences of various physical and analytical parameters for validation of mecobalamin are discussed as below:

Physical parameters

General tests

The appearance of sample injection Methycobal 1 mL ampoule containing mecobalamin 500µg/mL was examined by following parameters as given in Table 6.

Identification of mecobalamin in injection

The sample (20µg/mL) was prepared in buffer (pH.7.0) and the absorbance was measured at different wavelengths of 522nm, 342nm and 266nm. The ratio of absorbance of 266/A342 and A522/A342 was calculated which should be within 1.3 to 1.5 and 0.55 to 0.75, respectively. The results showed that ratio of A266/A342 was 1.35 and ratio of A522/A342 was 0.65 which confirmed the mecobalamin.

Method validation

Linearity

Linearity was observed by plotting a graph between absorbance versus concentration (Kumar et al., 2014). The calibration curve for mecobalamin expressed a linear relationship in the concentration range of 16 - 24 µg mL^{-1}. The linear data of the calibration curve was validated by the value of correlation coefficients (r^2). The results of that parameter were found to be in the given range of 98 to 110% (Table 1; Fig. 2).

Accuracy (Percent Recovery)

The standard addition method was adopted for the accuracy studies of the proposed method. The recoveries (%) for mecobalamin were found in the range of 99.79 - 100.01% and the means of recovery assay was calculated as 99.94% that was closed to standard value of mecobalamin, showed good accuracy (Table 2) and indicated that the developed method was accurate for the validation of mecobalamin. The values of the % recovery were given in Table 2, which exhibited the accuracy of the proposed method.

Precision

The precision study of that analytical method was explained by analyzing the five samples of the same concentration in the same day (same time) given in Table 3 and thus the developed method showed a high degree of precision. The developed analytical method was précised. The % assay for intraday precision (same time) studies were found to be within the range of 98 - 110 % as per ICH guidelines (Table 3).

Robustness

The % assay at different wavelengths (λ_{522} and $\lambda_{max\pm1}$) was calculated to determine the robustness of the selected method. The calculated % assay was found to be within the given range as per ICH guidelines (Table 5).

Ruggedness

Ruggedness was carried out by calculating the RSD (%) of different samples of the same concentrations by different analysts on same instrument and same day (different time). The %RSD was found to be 0.004326-0.004333% that is less than 3% indicating that the method is rugged. The analytical results were found to be within limit, data shown in Table 4. Mecobalamin or methylcobalamin occurs naturally as vitamin B_{12} and found effective against various human diseases **(**Zhang et al., 2013, Takahashi et al., 1992**)**. A number of procedures are available in literature for its assay **(**Zhang et al., 2013**)**; however, little work is reported on UV spectroscopy. Therefore, a UV spectrophotometric method has been developed and validated by accuracy, linearity, precision, intermediate precision, ruggedness and robustness. In conclusion, the maximum stable absorbance was found at λ_{max} 522nm for mecobalamin injection in pH-7.0 buffer. The concentration range 16 - 24 µg mL^{-1} obeyed the Beer Lambert law. The % standard deviation and % relative standard deviation calculated very low values represented the high degree of precision (98-110%) of the developed method. The percent recovery assay was found near the standard value used for mecobalamin. The robustness lies within the range of ICH guidelines and the ruggedness was found to be 0.004326-0.004333%. Hence the method was accurate, precise and linear It can be successfully applied for the routine validation of mecobalamin injection in pharmaceutical industries.

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Authors' contributions

MA and RM: Designed the study, Experimental design and laboratory experiments. AG and MI: Data analysis. AA, ZN and AH: Manuscript writing. SK and UA: Did experimental work. All authors read and approved the final draft of manuscript.

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