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Research Article

Dual wavelength spectrophotometric method for simultaneous estimation of epalrestat and methylcobalamin in bulk and combined dosage form

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective dual wavelength spectrophotometric method for the simultaneous determination of Epalrestat and Methylcobalamin in combined tablet dosage form. The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The principle for dual wavelength method is ―the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest. The method was based on determination of Epalrestat at the absorbance difference between 323 nm and 366 nm and Methylcobalamin at the absorbance difference between 313.50 nm and 335.50 nm. The linearity was obtained in the concentration range of 3-15 µg/ml for Epalrestat and 15-35 µg/ml for Methylcobalamin. The accuracy and precision of the method was determined and validated statically. The method showed good reproducibility and recovery with % RSD less than 2. Method was found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Epalrestat and Methylcobalamin in bulk and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Key words: Epalrestat, Dual wavelength, Methylcobalamin, Recovery

INTRODUCTION

Epalrestat occurs as yellow to orange crystal or crystalline powder. It has no odour or taste. It is chemicaly 2-[(5Z)-5-[(E)-3-phenil-2-methylprop-2-enylidene]- 4-oxo-2-thioxo-3-thiazolidinyl]acetic acid. It is an aldose reductase inhibitor. Chemical structure of Epalrestat is shown in figure 1[1-4].

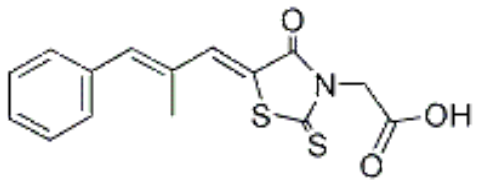

Figure:1 Structure of Epalrestat

Methylcobalamin (mecobalamin, MeCbl, or MeB_{12}) is a cobalamin, a form of vitamin B_{12}. It differs from cyanocobalamin in that the cyanide is replaced by a methyl group. Methylcobalamin features an octahedral cobalt(III) centre. Methylcobalamin can be obtained as bright red crystals. From the perspective of coordination chemistry, methylcobalamin is notable as a rare example of a compound that contains metal-alkyl bonds. Nickel-methyl intermediates have been proposed for the final step of methanogenesis[1-4]. Methylcobalamin is one of the two coenzyme forms of vitamin B12 (the other being adenosylcobalamin). It is a cofactor in the enzyme methionine synthase which functions to transfer methyl groups for the regeneration of methionine from homocysteine. Methylcobalamin is also used in the treatment of peripheral neuropathy, diabetic neuropathy, and as a preliminary treatment for amyotrophic lateral

sclerosis.Structure of Methylcobalamin is shown in Figure:2

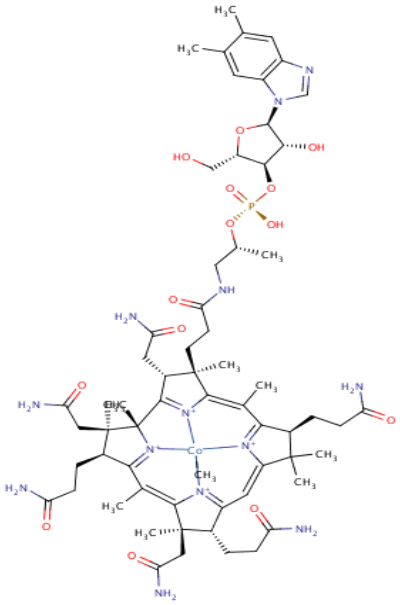

Figure:2 Structure of Methylcobalamin

MATERIALS AND METHODS

INSTRUMENTATION

A UV-visible spectrophotometer, model UV 1800 (Shimadzu) was used to measure absorbance of the resulting solutions using UV-Probe software version 2.31. A Digital analytical balance (Wensar DA13-220) and ultrasonic sonicator (Equitron) were used in the study.

REAGENTS AND MATERIALS

Pure Epalrestat (EPAL) & Methylcobalamin (MEC) kindly gifted as a gift sample by Symed Labs, Hyderabad, , India and Weast Coast Pharma, India. Tablet formulation procured from local market. All analytical grade chemicals and solvents were obtained from Merck (India). Methanol used as solvent in the study. Borosil volumetric flasks of 10, 50,100 ml capacity and pipettes – 1ml, 5ml,10ml, beakers, measuring cylinders etc.

PREPARATION OF SOLUTIONS

Preparation of Standard Stock Solutions

Epalrestat (1000 µg/ml)

Accurately weighed EPAL (100 mg) was transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (1000 µg/ml).

Methylcobalamin (1000 µg/ml)

Accurately weighed MEC (100 mg) was transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (1000 µg/ml).

Preparation of Working Standard Solutions

Epalrestat (100 µg/ml)

Standard Stock solution (5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with methanol.

Methylcobalamin (100 µg/ml)

Standard Stock solution (5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with methanol.

PREPARATION OF CALIBRATION CURVE

Calibration curve for Epalrestat

Aliquots of working standard solution of EPAL (100 µg/ml) 0.3, 0.6, 0.9, 1.2 and 1.5ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get concentrations 3, 6, 9, 12 and 25 µg/ml. Absorbance difference of each solution was measured at 323 nm and 366 nm using methanol as a blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.

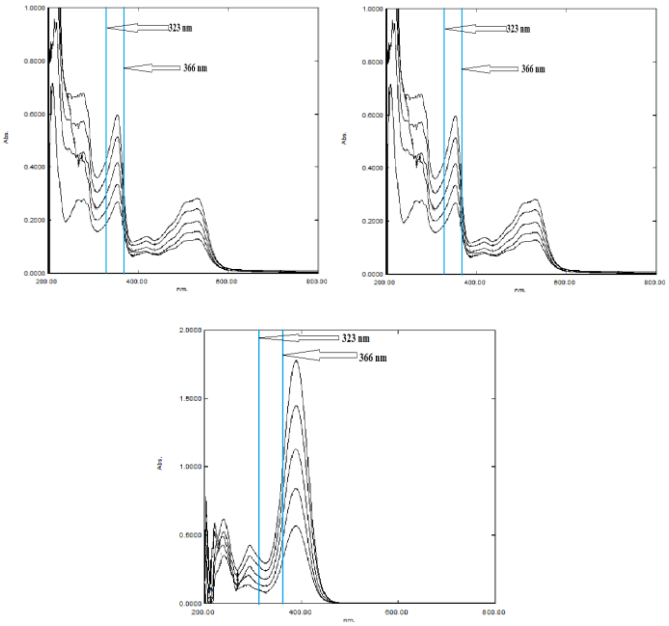

Figure 3: Spectra of EPAL and MEC for different conc. At 323nm and 366nm where MEC has same absorbance and EPAL has different absorbance

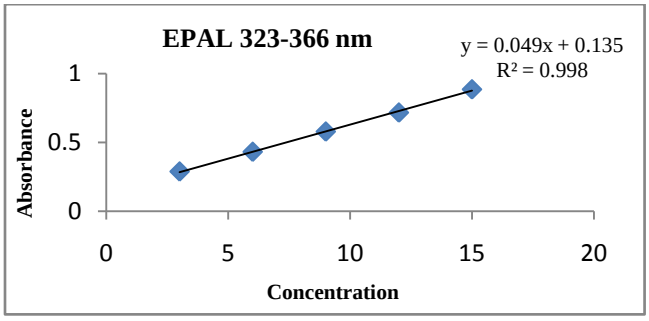

Figure 4: Calibration curve of EPAL

Calibration curve for Methylcobalamin

Aliquots of working standard solution of MEC (100 µg/ml) 1.5, 2, 2.5, 3 and 3.5ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get concentrations 15, 20, 25, 30 and 35 µg/ml. Absorbance difference of each solution was measured at 313.50 nm and 335.50 nm using methanol as a blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.

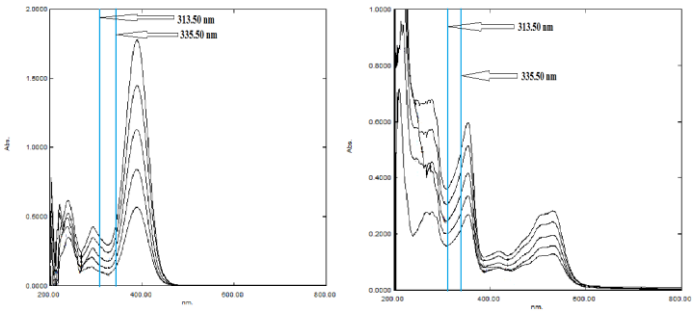

Figure 5: Spectra of EPAL and MEC for different conc. At 313.50nm and 335.50nm where EPAL has same absorbance and MEC has different absorbance

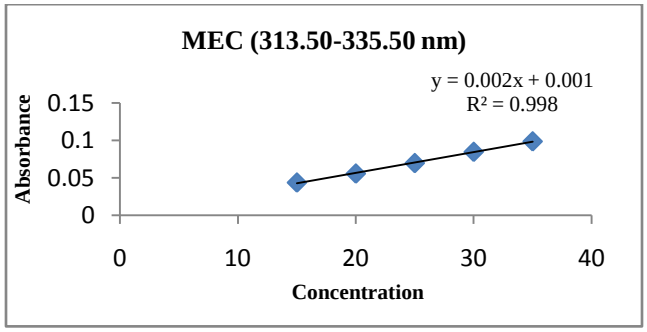

Figure 6: Calibration curve of MEC

METHOD [5-7,9-12]

The working standard solutions of EPAL and MEC were prepared separately in methanol having concentration of 15 µg/ml and 15 µg/ml respectively. They were scanned in the wavelength range of 800-200 nm. From the overlain spectra, four wavelengths 313.50 nm, 335.50 nm, 323 nm and 366 nm were selected for quantitation of the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of EPAL is carried out by measuring the absorbance difference value at between 323 nm and 366 nm where MEC have same absorbance at both the wavelength. The difference between 323 nm and 366 is directly proportional to concentration of EPAL in the mixture. The quantitative determination of MEC is carried out by measuring the absorbance difference value at 323.50 nm and 335.50 nm where EPAL has same absorbance at both the wavelength. The difference between 313.50 nm and 335.50 nm is directly proportional to concentration of MEC in the mixture.

Preparation of sample solution

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 10 mg EPAL and

0.1 mg MEC was accurately weighed and add the 9.9 mg of A.P.I. of MEC in the mixture because of the lower absorbance of MEC. The powder was transferred to 10 ml volumetric flask and shaken vigorously with methanol for 15 min and the solution was sonicated for 15 minutes and filtered through Whatman filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 15 µg/ml of EPAL and 15 µg/ml of EPAL respectively. The absorbance difference values were read at 323 nm and 366 for EPAL and 313.50 nm and 335.50 nm for MEC by thus concentration was obtained by solving the regression line equations.

METHOD VALIDATION [8]

The developed method was validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantitation (LOQ) and robustness in accordance with the ICH guideline.

Linearity and Range

Linearity was studied by preparing standard solutions at 5 different concentrations. The linearity range for EPAL and

MEC were found to be 3-15 µg/ml and 15- 35 µg/ml respectively. Linearity was assessed in the terms of slope, intercept and correlation coefficient for both the drugs.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate (intra-day) precision and reproducibility (inter-day precision).

1) Intra-day Precision

Solutions containing 6, 9, 12 µg/ml of EPAL and 20,25,30 µg/ml of MEC were analyzed three times on the same day and %R.S.D was calculated.

2) Inter-day Precision

Solutions containing 6, 9, 12 µg/ml of EMI and 20, 25, 30 µg/ml of MEC were analyzed on three different successive days and %R.S.D was calculated.

3) Repeatability

Method precision of experiment was performed by preparing the standard solution of EPAL (9µg/ml) and MEC (25 µg/ml) for six times and analysed as per the proposed method. Coefficient of variation (%CV) was not more than 2%.

Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times (N / S)$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOQ = 10 \times (N / S)$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Accuracy

Accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100%, 120%) taking into consideration percentage recovery of added bulk drug samples. The experiment was repeated three times by spiking previously analysed samples of tablet with three different concentrations of standards.

Analysis of Marketed formulation

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation. (EPALRICA-M).

RESULTS

Table1: Regression analysis data and summary of validation parameters for the proposed method

Parameters	EPAL	MEC
Wavelength (nm)	323-366	313.50-335.50
Beer's Law Limit (µg/ml)	3-15	15-35
Regression equation (y = mx +c)	y = 0.0494x + 0.1353	y = 0.0028x + 0.0013
Slope (m)	0.0494	0.0028
Intercept (c)	0.1353	0.0013
Correlation Coefficient (R^2)	0.9989	0.9986
Repeatability * (% RSD)	1.15	1.50
Intraday ** (% RSD)	0.13-1.21	0.50-1.29
Interday* (% RSD)**	0.13-9.50	0.50-1.33
LOD(µg/ml)	0.011	0.34
LOQ(µg/ml)	0.62	1.88

*Results are mean of six determination of one concentration.
** Results are mean of three determinations of three concentrations.
***Results are mean of three determinations of three concentrations

Table 2.: Data indicating recovery studies of EPAL and MEC

Drug Name	% level of recovery	Amount of drug taken (µg/ml)	Amount of drug spiked (µg/ml)	Total amount found µg/ml) ± s.d. (n=3)	% Recovery
EPAL	80	5	4	8.92±0.0004	99.11
	100	5	5	9.91±0.0009	99.10
	120	5	6	11.14±0.0060	101.27
MEC	80	15	12	26.27±0.0004	98.77
	100	15	15	29.53±0.0004	98.43
	120	15	18	32.75±0.0004	99.24

Table 3: Analysis of marketed formulation

Tablet	Drug	Label claim (mg)	Amount found (mg)(n=3times)	%label claim
Epalrica-M	Epalrestat	150	148.9±0.0016	99.26
	Methylcobalamin	1.5	1.47±0.0047	98.46

CONCLUSION

A simple, accurate and precise spectrophotometric method has been developed and validated for the routine analysis of EPAL and MEC in API and tablet dosage forms. The spectrophotometric method is suitable for the simultaneous determination of EPAL and MEC in multi-component formulations without interference of each other. The dual wavelength method is rapid, simple and sensitive. The developed method is recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations.

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