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SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF EPALRESTAT AND METHYLCOBALAMIN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate, and reproducible UV-spectrophotometric method has been developed for simultaneous estimation of Epalrestat and Methylcobalamin in tablet dosage form. In Absorbance ratio method ,two wavelengths 238 nm and 257.80 nm were used over the concentration range of 50 - 250 µg/ml and 0.5 - 2.5 µg/ml for Epalrestat and Methylcobalamine respectively. Correlation coefficient found to be 0.9995.Accuracy for both the drugs were in the range of 98-101 %. The method was validated as per the International Conference on Harmonization (ICH) guidelines.

Keywords: Spectrophotometric analysis; Absorbance ratio method; Epalrestat and Methylcobalamin

INTRODUCTION

Epalrestat is Aldose reductase inhibitor. Epalrestat inhibited high glucose-mediated Neutrophilendo-thelial adhesion molecules not only through inhibition of a PKC-dependent pathway,but also through increased endothelial NO production.It is Used in treatment of Diabetes. Chemically it is described 2-[(5Z)-5-[(E)-3-phenil-2-methylprop-2-enylidene]-4-oxo-2-thioxo-3-thiazolidinyl]acetic acid.[1-2] Methylcobalamin is use as Vitamin supplement. The synthesis of methionine from homocysteine requires a folate coenzyme as vitamin B12-dependent enzyme. Chemically it is described Coα-[α-(5,6-Dimethylbenz-1H-imidazolyl)]-Coβ-Methylcobamid. [3-4] Methylcobalamin is official in Japan Pharmacopoeia and HPLC method is given in Japan pharmacopeia. [5] Extensive literature survey revealed that methods were reported for the estimation of Epalrestat [6-8] and Methylcobalamin [9-10] alone and in combination with other drugs are spectrophotometric methods [11], HPTLC Method [12-13], and RP-HPLC methods[14-17] for Methylcobalamin and Epalrestat.

Q-Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilution of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbance is measured at two wavelengths. One being the λmax of one of the component (λ2) and the other being a wavelength of equal absorptivities of the two components i.e. an isoabsorptive point.

Cx = (Qm – Qy / Qx – Qy) *A1/ ax1

Cy = (Qm – Qx / Qy– Qx) *A1/ ay1

Qm= Abs of sample at 400nm (A2)/ Abs of sample at 438nm (A1)

Qx= Absorptivity of EPAL at 400nm/ Absorptivity of EPAL at 438nm

Qy= Absorptivity of MCB at 400nm/ Absorptivity of MCB at 438nm
Where,
Qx and Qy are value of EPAL and MCB respectively, ax1 and ay1 are absorptivity value at isosbestic point for EPAL and MCB.

MATERIALS AND METHODS

Double beam UV-visible spectrophotometer (Simadzu-1800, Software –UV Probe, Version 2.42) having two matched quartz cells with 1 cm light path. Absorbance measurements were recorded in a pair of 10 mm matched quartz cells. The scan speed was set at medium and the band width was fixed at 2 nm.
EPAL and MCB were kindly supplied by Triveni chemicals pvt.ltd. , Vapi. Sodium hydroxide used was of analytical grade. Distilled water was used throughout the work. Alrista-Plus* tablets and labeled to contain 150 mg EPAL and 1.5 mg MCB are manufactured by Prosperity Macleods Pharmaceuticals Ltd. Both pharmaceutical preparations were purchased from the local market.

RESULTS AND DISCUSSION

For stock solutions of EPAL 1000 μg/mL and MCB 1000 μg/mL were prepared in water. Portions of both solutions were separately diluted with water to attain the concentration ranges of 200 μg/mL for EPAL and 20μg/mL for MCB specified in Table 1. The absorption spectra of the prepared standard solutions were recorded in the range of 200–400 nm against water. For the determination of EPAL and MCB, wavelengths were selected from the overlay spectra of EPAL and MCB. Wavelength of detection was 400 nm (λmax of EPAL) and 438 nm (iso-absorptivity point) for Absorbance ratio method.

Assay of commercial formulation:

The injection powder equivalent to 100 mg of EPAL and 100 mg of MCB was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with water. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 20 ml of this solution was diluted to 100 ml with water and than 2 ml of this solution was further diluted to 10 ml with water. Absorbance of the resulting solution was measured at 400 nm and 438 nm against water for Absorbance ratio method. The concentration of EPAL and MCB can be obtained by using absorbance ratio method.
Spectral characteristics and optimization of the measurements.
To determine wavelength for measurement, standard spectra of EPAL and MCB were scanned between 200 - 400 nm against water. Absorbance maxima were obtained isoabsorptive point obtained at 400 nm and 438nm. Overlay spectra of EPAL and MCB are presented in figure 3.

Validation of the proposed spectrophotometric method

Linearity and ranges:

The linearity of the proposed method was evaluated through the analysis of six serial concentrations of each drug. The produced response was plotted as a function of the corresponding concentration and the calibration equation was calculated using the least squares regression method. Table 1 summarizes the regression and statistical parameters of the studied drugs. As seen, the regression analysis verifies the good linearity of the method as indicated by the close to unity correlation coefficient (r^2) values.

Limits of detection and quantification:

Limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines. LOD was defined as $3.3S_a/b$ and LOQ was computed as $10S_a/b$, where S_a is the standard deviation of the intercept and b is the slope of the calibration curve. The sensitivity of the proposed method can be confirmed by the low LOD and LOQ values obtained (Table 1).

Precision and accuracy:

According to the ICH guidelines, the within-day repeat-ability of the proposed method was assessed through the analysis of 3 concentration levels prepared in triplicates. Correspondingly, the between-day precision was studied on the same levels over 3 consecutive days. Table 2 contains the values of percentage relative standard deviation (RSD %) which did not exceed 1.7% for both mixtures indicating the acceptable level of precision of the proposed method.

SUMMARY AND CONCLUSION

Applications of the proposed method: Analysis of commercial tablets:

The proposed method was successfully applied to the analysis of both mixtures in their pharmaceutical preparations. Results obtained were precise and in good agreement with the labelled claim as concluded from the satisfactory values of % recovery and RSD (%) gathered in Table 3. Simple, rapid and sensitive methods are proposed for the analysis of binary mixtures. The applicability of the developed method was evaluated through the determination of both drug combinations in pharmaceutical tablets with good accuracy and precision. Therefore, the presented methodology is adequate for the routine quality control analysis of these fixed-dose combinations.

Table 1. Regression and analytical parameters for the determination of the two drug combinations using the proposed spectrophotometric method.

Parameter	Absorbance ratio method			
	EPAL		MCB	
Wavelength (nm)	400	438	400	438
Concentration range (µg/mL)	50-250	50-250	0.5-2.5	0.5-
Intercept	0.1378	0.058	0.0227	0.021
Slope	0.0616	0.022	0.058	0.0062
Correlation coefficient (r^2)	0.9952	0.9996	0.9953	0.9995
Regression equations	0.0616 + 0.1378	0.022 + 0.058	0.058 + 0.0227	0.0062 + 0.021
Repeatability (%RSD,n=6)	0.308	0.787	0.659	0.758
Intraday precision (%RSD,n=3)	0.1331-0.1964	0.5154-0.7338	0.6006-0.7364	0.5137-1.0748
Interday precision (%RSD,n=3)	0.7338-0.8695	0.888 – 1.107	0.7338 -0.8695	0.3378 –1.0216
Accuracy (%recovery)	99.0-100.6	98.25 – 102	99.66-101.33	99.66 -101.33
LOD (µg/ml)	0.37		2.47	
LOQ (µg/ml)	1.13		7.5	

Table 2. Precision data for the determination of the two drugs using the proposed spectrophotometric method. (n=3)

Drug	Normal values µg/ml	Absorbance Ratio method			
		% RSD			
		Intraday		Interday	
		400 nm	438 nm	400 nm	438 nm
EPAL	100	0.1964	0.5154	0.4937	1.0800
	150	0.1674	0.6436	0.4358	1.1070
	200	0.1331	0.7338	0.3518	0.8880
MCB	1	0.6631	1.0748	0.8695	0.5208
	1.5	0.7364	0.8416	0.7338	1.0216
	2	0.6006	0.5137	0.7549	0.3378

Table 3. Accuracy for the determination of the two drugs using the proposed spectrophotometric method (n=3)

Drug	Normal values µg/ml	Drug spiked µg/ml	Absorbance Ratio method	
			% Recovery	
			400nm	438 nm
EPAL	100	80	100.60	100.90
	100	100	99.00	98.25
	100	120	99.17	102.00
MCB	1	0.8	100.55	99.66
	1	1	101.33	101.33
	1	1.2	99.66	99.89

Table 4. Analysis of EPAL and MCB in tablets by the Simultaneous equation method.

Marketed formulation	Labled claimed (mg/tab)		Amt. found (mg/tab)		% Label claimed	
	EPAL	MCB	EPAL	MCB	EPAL	MCB
ALRISTA PLUS	100	1	99.93	0.95	98.25	99.33

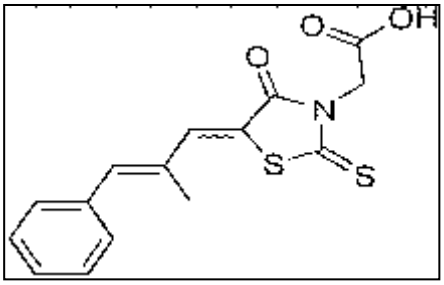

Figure.1. Structure of Epalrestat

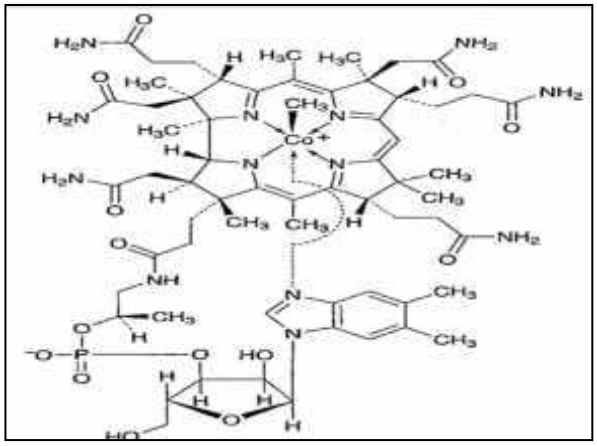

Figure.2.Structure of Methylcobalamin

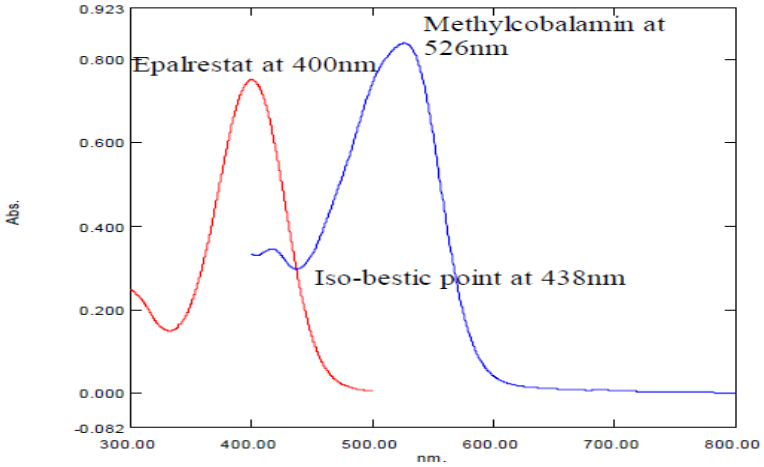

Figure. 3. Absorption spectra of EPAL and MCB shows λ max of EPAL at 400 nm and isoabsorptive point at 438 nm.

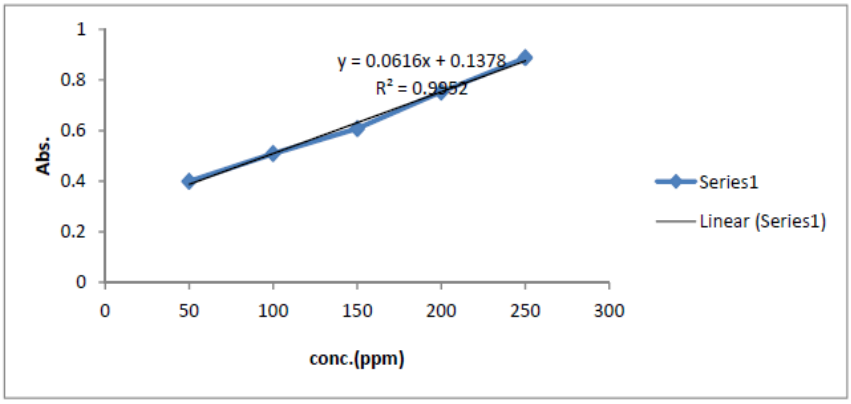

Figure 4A. Calibration curve for EPAL at 400 nm in water

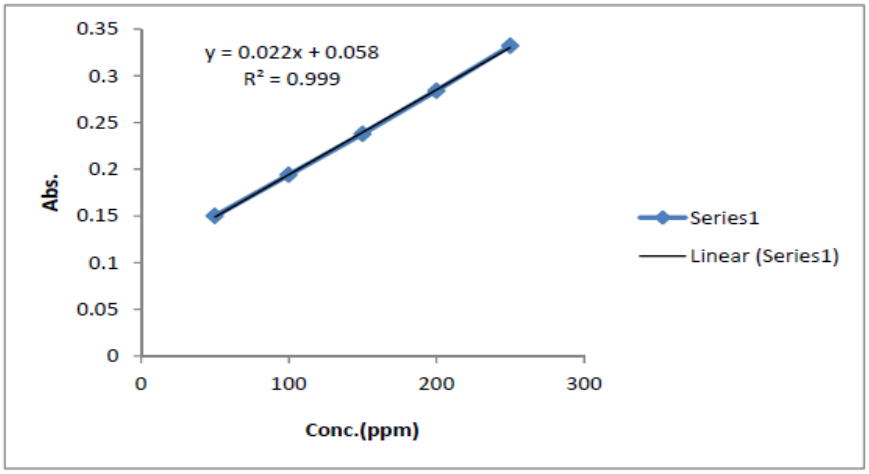

Figure 4B. Calibration curve for EPAL at 438 nm in water

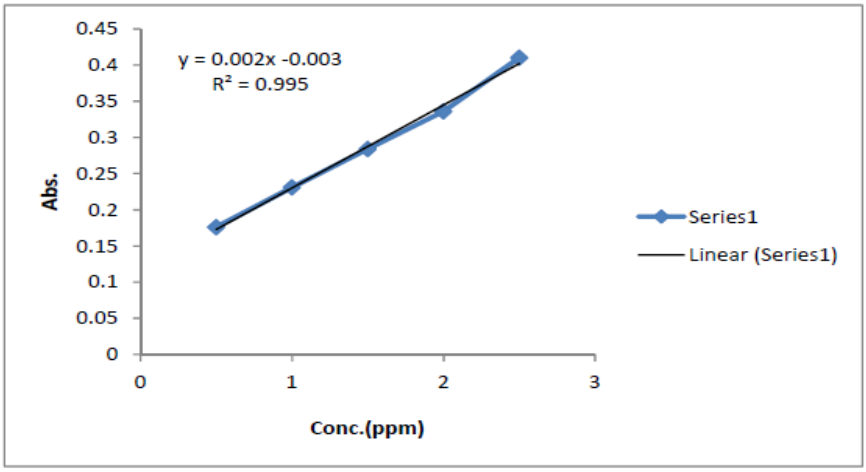

Figure 4C. Calibration curve for MCB at 400 nm in water

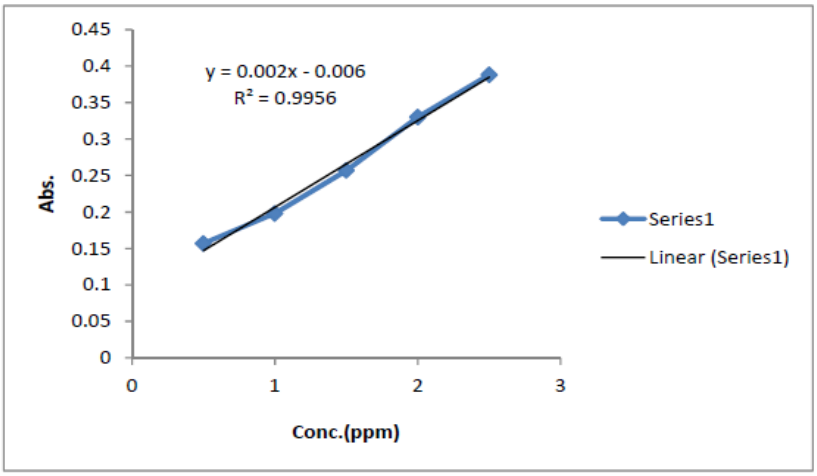

Figure 4D. Calibration curve for MCB at 438 nm in water

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