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Development and Validation of UV Spectrophotometric Method for Simultaneous estimation of Epalrestat and Methylcobalamin in the Pharmaceutical Dosage Form

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Abstract: A new sensitive, simple, rapid and precise spectrophotometric method has been developed for simultaneous estimation of Epalrestat (EPAL) and Methylcobalamin (MEC) in pharmaceutical dosage form. This method was based on UVspectrophotometric determination of two drugs, using simultaneous equation method. It involves measurement of absorbance's at two wavelengths 390nm(λmax of Epalrestat (EPAL)) and 354nm (λmax of Methylcobalamin (MEC)) in methanol for the simultaneous quantitative determination of Epalrestat and Methylcobalamin in the binary mixture without previous separation. The linearity was observed 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 Mthylcobalamin in combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.
Keywords: Epalrestat, Methylcobalamin, UV spectrophotometric method; Simultaneous equation method.

Introduction

- **Epalrestat**

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]

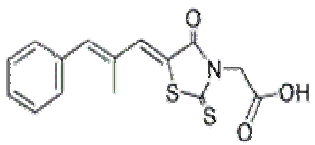

Figure:1 Structure of Epalrestat

- **Methylcobalamin**

➢ 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.

- 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[1,3,5-6]

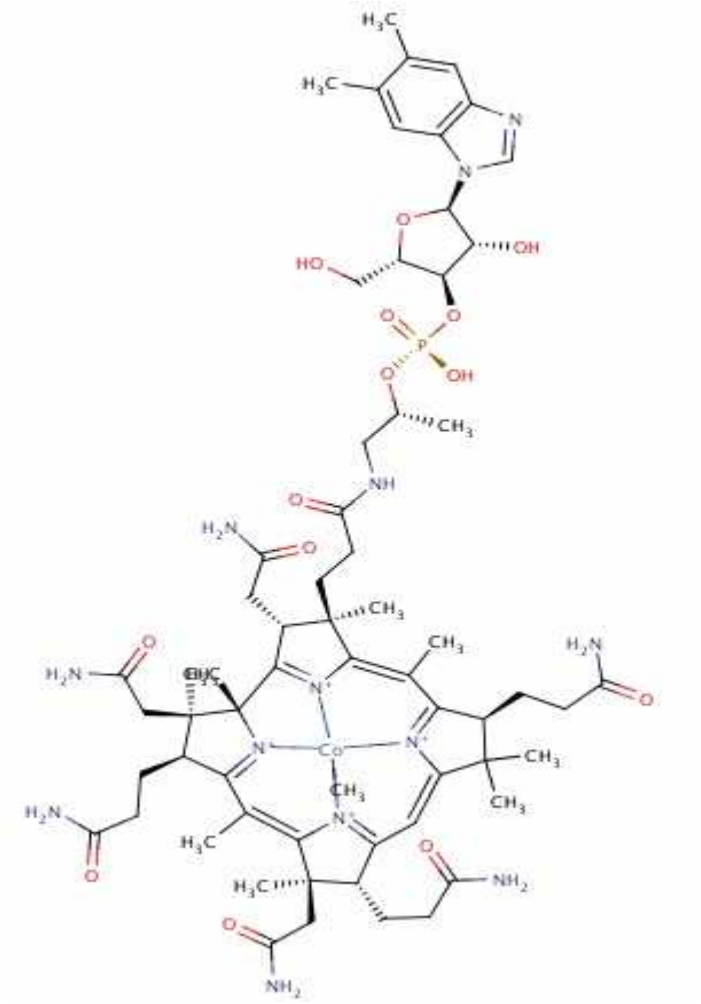

Figure:2 Structure of Methylcobalamin

Material 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 TOR (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 of each solution was measured at 390 nm and 354 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.

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 of each solution was measured at 390 nm and 354 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.

Simultaneous Equation Method

From the stock solution of 1000µg/ml, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the λmax. Epalrestat has λmax of 390 nm while methylcobalamin has λmax at 354 nm respectively. Standard solutions were prepared having concentration 3-15µg/ml for epalrestat and 15-35µg/ml for Methylcobalamin. The absorbances of these standard solutions were measured at 390 nm and 354 nm and calibration curves were plotted at these wavelengths. Two simultaneous equations (in two variables Cx and Cy) were formed using these Absorptivity coefficient values. The absorbances were measured at the selected wavelengths and absorptivity (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations

$$CX = A2ay1 - A1ay2ax1ay2 - ax2ay1 \ldots\ldots (1)$$
$$Cy = A1ax1 - A2ax1ax1ay2 - ax2ay1 \ldots\ldots (2)$$

Where, A1 and A2 are absorbance's of mixture at 390 nm and354 nm respectively, ax1 and ax2 are absorptivities of epalrestat at 2 wavelengths respectively and ay1 and ay2 are absorptivities of Methylcobalamin at 2 wavelengths respectively. Cx and Cy are concentrations of Epalrestat and Methylcobalamin respectively.

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. The powder was transferred to 100 ml volumetric flask and add MEC standard A.P.I. powder 9.9 mg as standard addition 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 15μg/ml of EPAL and 15 μg/ml MEC respectively. The absorbance's values were read at 390 and 354 nm and concentration was obtained by solving the simultaneous equation.

Method Validation [(7)]

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 (intraday) precision and reproducibility (interday precision).

1) Intraday Precision

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

2) Interday 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%.

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.

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.

Result & Discussion

Selection of wavelength for simultaneous estimation of EPAL and MEC

To determine the wavelength for measurement, EPAL (6 µg/ml) and MEC (30 µg/ml) solutions were scanned between 800-200 nm. Absorbance maxima were obtained at their λmax 390 nm and 354 nm for EPAL and MEC respectively. It is shown in Figure:3

- **Method Validation**

➢ **Linearity & Range**

➢ The linearity of EPAL was found to be in the range of 3-15 µg/ml. Linearity data for EPAL Table 1.

➢ The linearity of MEC was found to be in the range of 15-35 µg/ml. Linearity data for MEC Table 2.

Table 1: Data for Linearity of EPAL

Drug	Conc. (µg/ml)	Absorbance mean ± S.D.(n =3)		%RSD	
		390 nm	354 nm	390 nm	354 nm
EPAL	3	0.568±0.0004	0.2296±0.0004	0.08	0.20
	6	0.844±0.0066	0.338±0.0051	0.78	1.53
	9	1.167±0.0008	0.467±0.0008	0.06	0.17
	12	1.453±0.0086	0.589±0.0021	0.59	0.36
	15	1.781±0.0057	0.725±0.0040	0.32	0.55

Table 2: Linearity data for MEC

Drug	Conc. (µg/ml)	Absorbance mean ± S.D. (n = 3)		%RSD	
		354nm	390nm	354nm	390nm
MEC	**15**	0.244±0.0004	0.050±0.0004	0.19	0.93
	20	0.336±0.0012	0.067±0.0004	0.37	0.70
	25	0.416±0.0004	0.083±0.0004	0.11	0.56
	30	0.509±0.0018	0.099±0.0012	0.37	1.25
	35	0.595±0.0009	0.115±0.0004	0.15	0.40

Precision

1. Intraday Precision

The data for Intraday precision for EPAL and MEC is in range of % RSD was found to be 0.09-1.39% and 0.08-1.51 %for EPAL at 390 and 354 nm and 0.11-0.45% and 0.55-1.37% for MEC at 354 nm and 390 nm respectively.

2. Interday Precision

The data for Intraday precision for EPAL and MEC is in range of % RSD was found to be 0.10-0.67% and 0.07-1.36% for EPAL at 390 and 354 nm 0.14-1.09% and 0.56-1.35 % for MEC at 354 and 390 nm respectively.

3. Repeatability

The data for repeatability for EPAL was found to be 0.10 and 0.17% at 390 and 354nm. MEC repeatability data was found to be 0.56 and 1.09% at 354 nm and 390 nm respectively.

- **LOD and LOQ**

➢ LOD values for EPAL and MEC were found to be 0.30µg/ml and 0.21µg/ml respectively. LOQ values for EPAL and MEC were found to be 0.92µg/ml and 0.64µg/ml respectively.

- **Accuracy**

➢ Accuracy of the method was confirmed by recovery study from marketed formulation at three levels (80%, 100%, and 120%) of standard addition. Percentage recovery for EPAL and MEC were found to be in the range of 98.22-101.09% and 99.33-99.76%. Data indicating recovery studies of Epalrestat and Methylcobalamin shown in Table 3.

- **Analysis of Marketed formulation**

➢ Applicability of the proposed method was tested by analyzing the commercially available tablet formulation. (EPALRICA-M). It is shown in Table 4.

Table 3: Data indicating recovery studies of EPAL and MEC

Drug	%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	8.84±0.0012	98.22
	100	5	5	9.92±0.0009	99.20
	120	5	6	11.12±0.0049	101.09
MEC	80	15	12	26.82±0.0017	99.33
	100	15	15	29.93±0.0009	99.76
	120	15	18	32.80±0.0017	99.39

Table 4: Analysis of marketed formulation

Tablet	Drug	Label claim (mg)	Amount found (mg)(n=3)	%label claim
Epalrica-M	Epalrestat	150	147.30	98.20
	Methylcobalamin	1.5	1.48	98.80

Table 5: Regression Analysis data and Summary of Validation Parameters for the proposed method

Parameters	EPAL		MEC	
Wavelength (nm)	390	354	354	390
Beer's Law Limit (µg/ml)	3-15	3-15	15-35	15-35
Regression equation (y = mx +c)	y = 0.0101x + 0.0252	y = 0.0414x + 0.0974	y = 0.0175x - 0.0175	y = 0.0032x + 0.0018
Slope (m)	0.0101	0.0414	0.0175	0.0032
Intercept (c)	0.0252	0.0974	0.0175	0.0018
Correlation Coefficient (R^2)	0.9992	0.9987	0.9996	0.9998
Repeatability (% RSD, n=6)	0.10	0.17	1.09	0.56
Intraday (n=3) (% RSD)	0.09-1.39	0.08-1.51	0.11-0.45	0.55-1.37
Interday (n=3) (% RSD)	0.10-0.67	0.07-1.36	0.14-1.09	0.56-1.35
LOD(µg/ml)	0.30		0.21	
LOQ(µg/ml)	0.92		0.64	

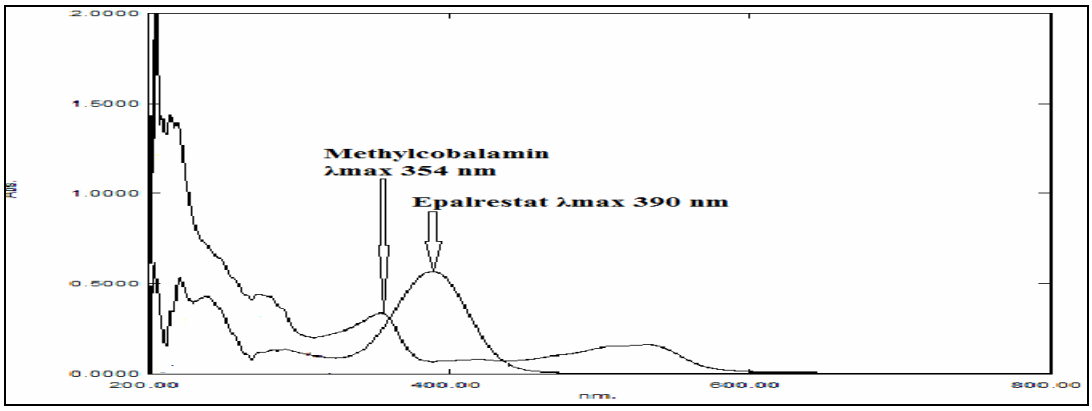

Figure: 3 Overlain Spectra of EPAL (6 µg/ml) and MEC (30 µg/ml)

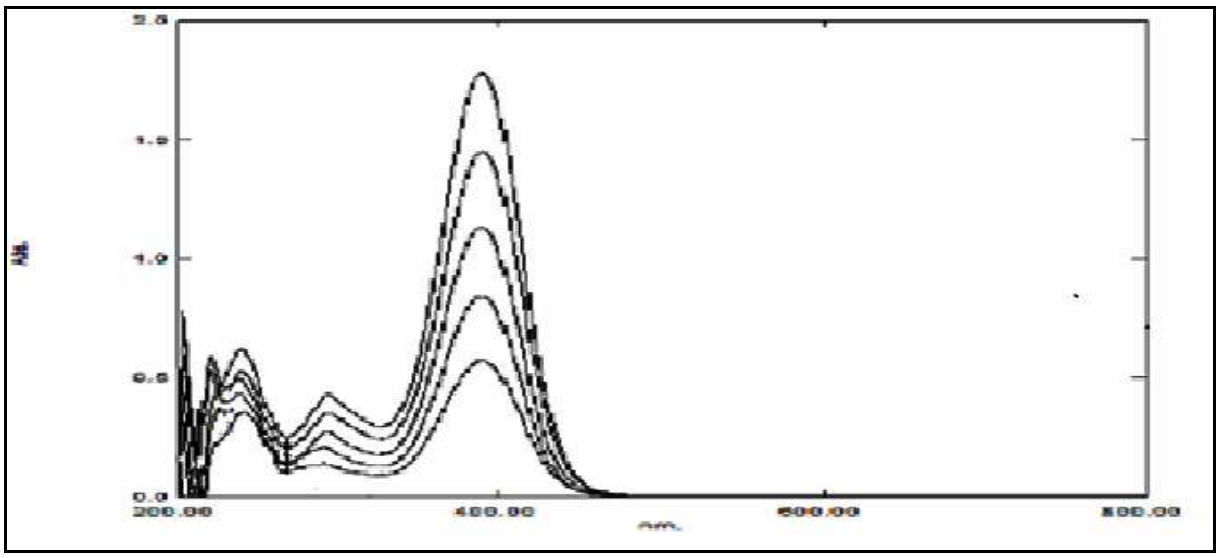

Figure: 4 Overlain Spectra of EPAL (3-15 µg/ml)

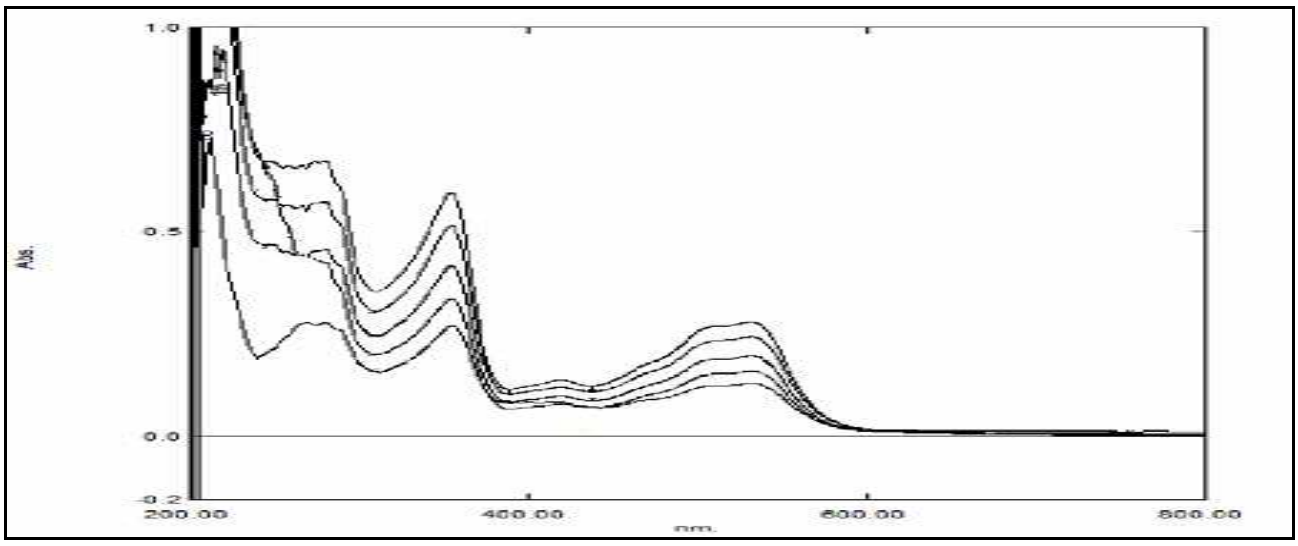

Figure: 5 Overlain Spectra of MEC (15-35 µg/ml)

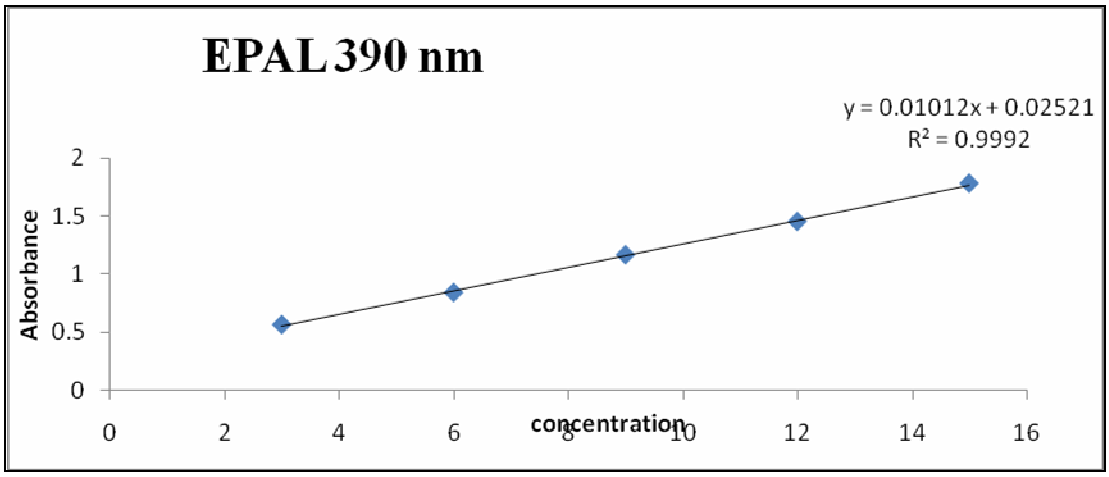

Figure: 6 Calibration curve of EPAL at 390 nm

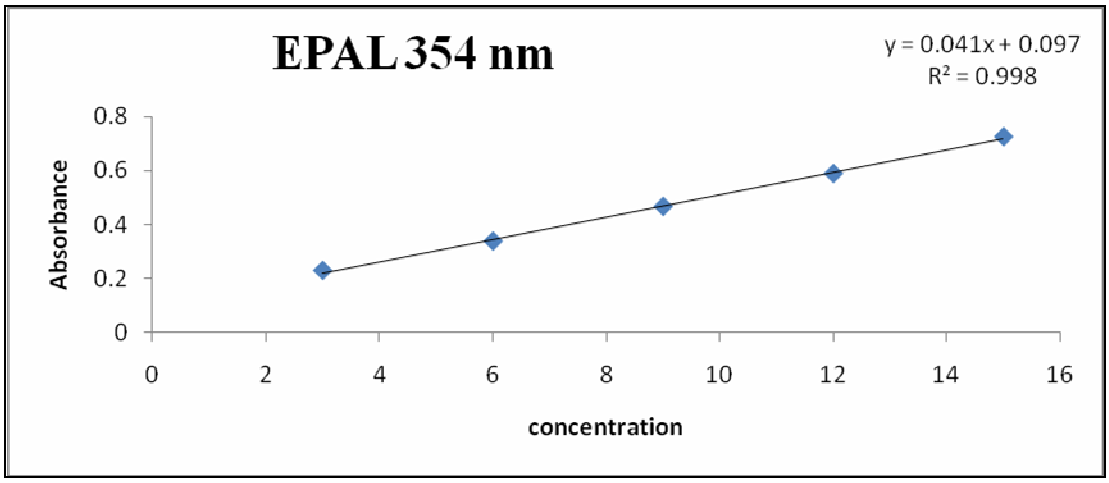

Figure: 7 Calibration curve of EPAL at 354 nm

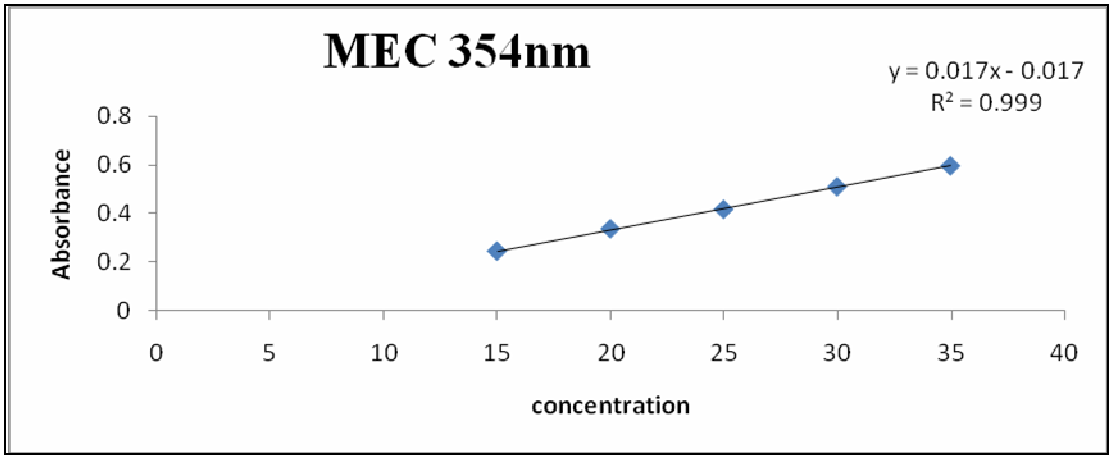

Figure: 8 Calibration curve of MEC at 354 nm

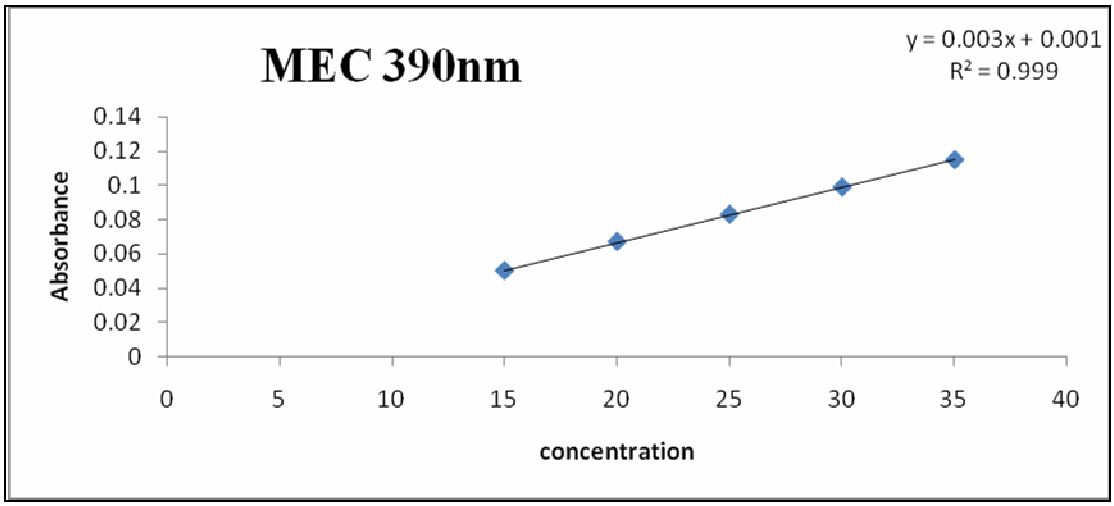

Figure: 9 Calibration curve of MEC at 390 nm

- **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 developed method is recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations.

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