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Liquid Chromatographic Estimation of Epalrestat and Methylcobalamin in Pharmaceutical Formulation.

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Research Article

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ABSTRACT

A sensitive, selective and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Epalrestat and Methylcobalamin both as a bulk drug and in formulation. The method employed Luna C18 column (250 x 4.6 mm id, 5 µm particle size) as the stationary phase while acetonitrile: 0.02 M KH_2PO_4 buffer (90:10 v/v, pH 6.5) was used as mobile phase. The R_t of Epalrestat and Methylcobalamin were observed to be 5.2 and 2.1 minutes, respectively. Analysis was carried out in absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for Epalrestat and Methylcobalamin over a concentration range of 1- 40 µg/ml and 0.2-10 µg/ml respectively with correlation co-efficient of 0.9995 for epalrestat and 0.9991 for methylcobalamin. The LOQ was found to be 0.2 µg/ml for epalrestat and 0.8 µg/ml for methylcobalamin. The method was validated as per ICH guideline and it was found to be accurate, precise and robust. Marketed formulation was analyzed successfully

INTRODUCTION

EPL is chemically 2-[(5Z)-5-[(E)-3-phenil-2-methylprop-2-enylidene]-4-oxo-2-thioxo-3-thiazolidinyl] [1, 2]. Epalrestat work by inhibiting Aldose Reductase Enzyme [3]. MCA is chemically 3-[({2-[(diaminomethylidene) amino]-1, 3-thiazol-4-yl} methyl) sulfanyl]-N'-sulfamoylpropanimidamide [4, 5]. MCA is active form of vit B12. It is used for the treatment of peripheral neuropathy, diabetic neuropathy [6, 7].

Literature survey revealed there is no published chromatographic method for this combination of drug. The present paper describes a simple, accurate and precise method for reverse phase liquid chromatographic estimation of EPL and MCA in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [7]. In the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC method.

MATERIALS AND METHODS

Instruments

Instrument Perkin Elmer USA, Series 200, Phenomenex Luna C18 column (250 x 4.6 mm id, 5µm particle size) was used for analytical method development. The chromatographic data were processed by Totalchrom navigator HPLC version 6.3.1 Software.

Materials

Epineurone Tablet (50mg EPL and 0.5mg MCA) manufactured by Aristo Pharmaceutical Ltd. All chemicals and reagents used were of AR grade.

Reagents

All the chemicals used were of AR grade.

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Selection of Analytical wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. Overlay UV spectra of both the drugs showed that EPL and MCA absorbed appreciably at 254 nm, so detection was carried out at 254 nm (figure 1).

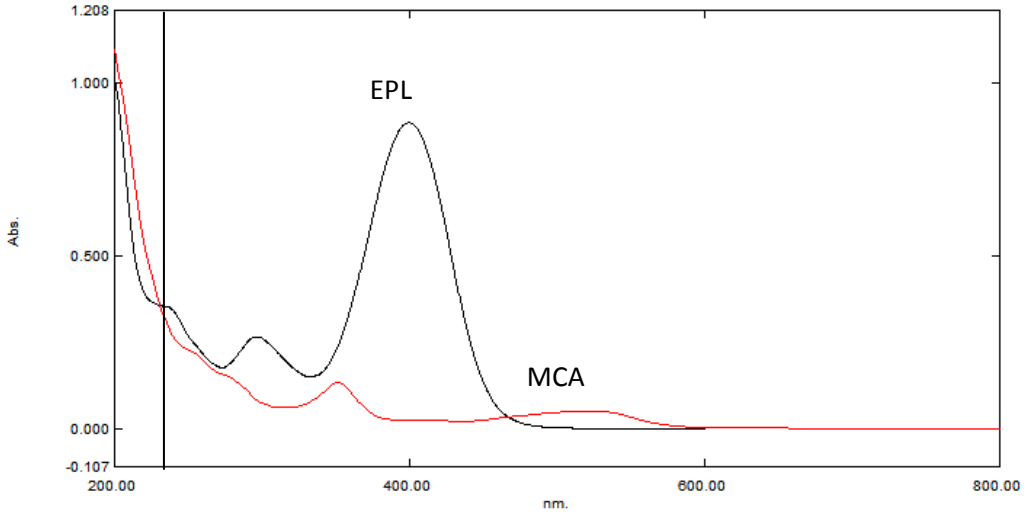

Figure 1: Overlain spectra of EPL and MCA (10µg/ml each)

Preparation of Mobile Phase

Buffer 0.02 M KH2PO4 was prepared by weighing 2.72 g of KH2PO4 and dissolving in 1000 ml of water. Mobile phase was prepared by mixing 100 ml of 0.02M KH2PO4 Buffer and 900 ml of acetonitrile. The pH was adjusted to 6 using o-phosphoric acid (1%) or triethyl amine (1%) of the mobile phase was 6. Solution was filtered through Whatman filter paper No. 41 and sonicated for 10 min and this solution was used as a mobile phase.

Preparation of Standard Stock Solutions

EPL (10 mg) and MCA (10 mg) were accurately weighed and transferred to two separate 10 ml volumetric flask and dissolved in few ml of acetonitrile. Volumes were made up to the mark with acetonitrile to yield a solution containing 1000 µg/ml of EPL and 1000 µg/ml of MCA, respectively. Appropriate aliquot from above solutions were taken and diluted with mobile phase to obtain final concentration of 100 µg/ml and 100 µg/ml of EPL and MCA respectively.

Chromatographic conditions

Phenomenex Luna C18 column (250 x 4.6 mm id, 5µm particle size) chromatographic column equilibrated with mobile phase 0.02M KH2PO4 buffer: acetonitrile (10:90, v/v) was used. Mobile phase flow rate was maintained at 1 ml min-1and effluents were monitored at 254 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 7 min.

Calibration Curve for EPL and MCA

Appropriate aliquot of stock solution of EPL and MCA was taken in same 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentration of 1, 5, 10, 20, 40 µg/ml of EPL and 0.2, 0.5, 1, 5, and 10 µg/ml of MCA, respectively .

Validation Parameter

Linearity

The calibration curve for EPL was found to be linear in the range of 1-40 µg/ml with a correlation coefficient of 0.9995. The calibration curve for MCA was found to be linear in the range of 0.2-10µg/ml with a correlation coefficient of 0.9991. The regression analysis of calibration curves is reported (Table 1, 2) (figure 3, 4).

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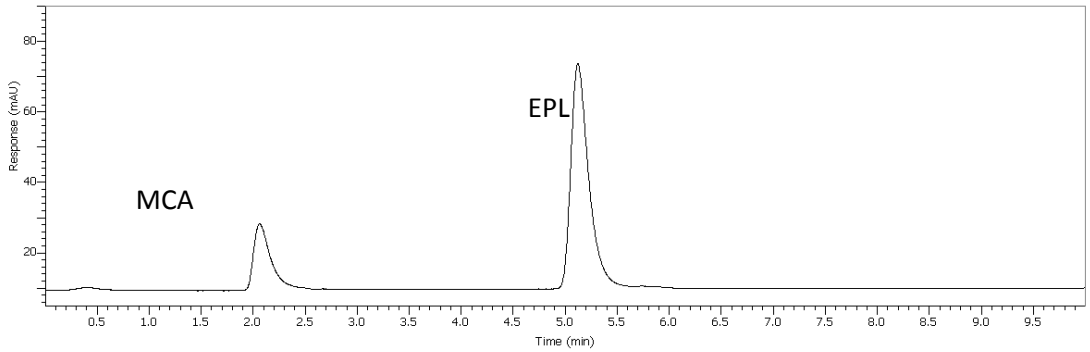

Figure 2: Acetonitrile: 0.02 M KH_2PO_4 (90:10% v/v) (pH 6.5)

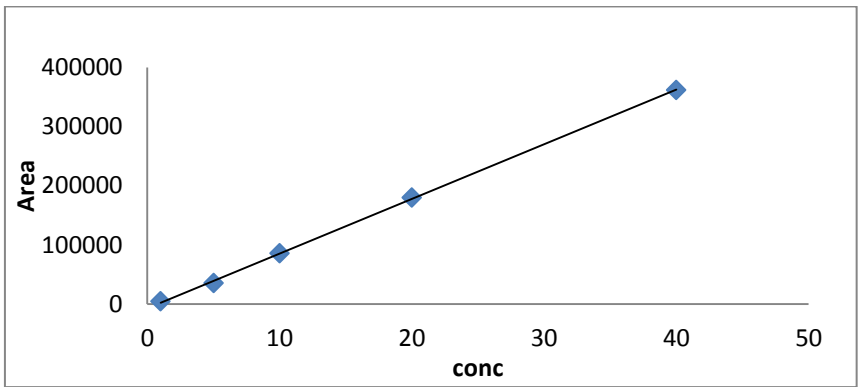

Figure 3: Calibration curve for EPL

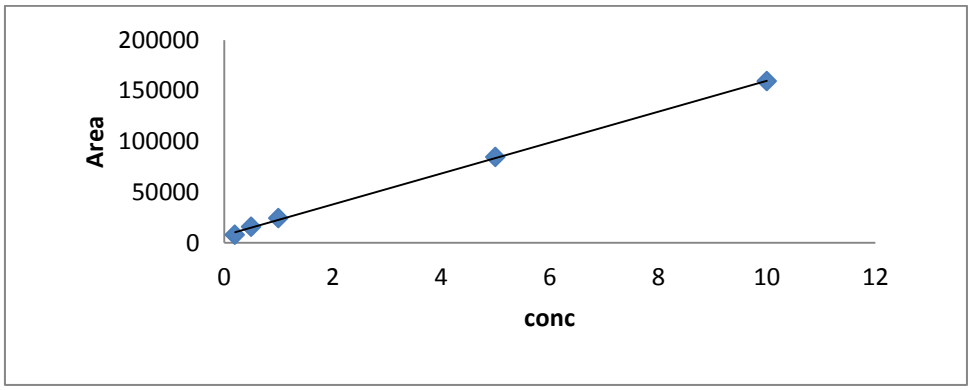

Figure 4: Calibration curve for MCA

Precision

Intraday precision

The intraday studies were carried out by measuring response for 3 concentrations for 3 times a day. The % RSD was found to be 0.74- 1.27 % for EPL and 0.77-1.52 % for MCA (Table 3, 4). These %RSD value was found to be less than ± 2.0 indicated that the method is precise.

Interday precision

The interday studies were carried out by measuring response for 3 concentrations for 3 times at 3 different days. The % RSD was found to be 0.76-2.01% for EPL and 0.78-2.14 % for MCA (Table 3, 4). These %RSD value was found to be less than ± 2.0 indicated that the method is precise.

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Repeatability study

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day. The % RSD was found to be 0.42% for EPL and 0.46% for MCA (Table 5). These %RSD value was found to be less than ± 2.0 indicated that the method is precise.

Accuracy

The accuracy of the method was determined by calculating recoveries of EPL and MCA by method of standard addition. The recoveries found to be 100.56% -101.71 % and 109.18%-111.91% for EPL and MCA respectively. The high values indicate that the method is accurate (Table 6).

Limit of detection and limit of quantification

LOD is the lowest amount of the analyte that can be detected. From the visual observation of chromatogram, the LOD for EPL was found to be 0.08 µg/ml and for MCA was found to be 0.2 µg/ml. LOQ is the lowest amount of the analyte that can be detected and quantified. LOQ of the EPL was found to be 0.2 µg/ml and LOQ of the MCA was found to be 0.8 µg/ml.

Robustness

Robustness of the method was studied by changing the mobile phase composition, pH of the mobile phase and flowrate (Table 7).

Solution stability

Stability of standard and sample solution of EPL and MCA were evaluated at room temperature for 24 hr. Both the drugs were found to be stable with a recovery of more than 98% (Table 8).

System suitability parameters

System suitability test was carried out and the results are summarized in Table 9.

Analysis of Marketed Formulation

Twenty tablets were weighed and finely powdered. Tablet powder equivalent to 10 mg EPL (and 0.1 mg MCA) was weighed and transferred in to a 25 ml volumetric flask containing few ml of mobile phase. The flask was sonicated for 5 minutes. The solution was filtered through Whatman filter paper No. 41 in another 25 ml volumetric flask and volume was made up to the mark using mobile phase(stock A). Estimation of MCA was carried out from stock A at 254 nm. From the stock A appropriate aliquot was pipette out in 10 ml volumetric flask and make up the volume with mobile phase to obtain the 40 µg/ ml of EPL (stock B). Estimation of EPL was carried out from stock B at 254 nm. The quantitation was carried out by keeping peak area in regression equation and amount of EPL and MCA were determined (Table 11).

Table 1: Regression analysis of calibration for EPL by HPLC method

Concentrations (µg/ml)	Area Mean ± S.D. (n=5)	% RSD
1	4356.854±46.52	1.06
5	35236.63± 318.55	0.90
10	85397.09±401.32	0.46
20	179748.8±297.82	0.16
40	361852.5±564.06	0.15
Slope	9241.2	
Intercept	7148.4	
R^2	0.9995	

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Table 2: Regression analysis of calibration for MCA by HPLC method

Concentrations (µg/ml)	Area Mean ± S.D. (n=5)	% RSD
0.2	7914.86±71.74	0.90
0.5	15853.46±107.09	0.67
1	24392.3±88.39	0.36
5	84431.69±184.98	0.21
10	159504.6±252.63	0.15
Slope	15253	
Intercept	7475.2	
R^2	0.9991	

Table 3: precision data for estimation of EPL (n=3)

Conc µg/ml	Intraday (n=3) (area	%RSD	Interday (n=3) (area)	%RSD
1	4767.20	1.08	4788.12	2.01
10	87896.26	1.27	86346.00	1.32
40	363668.10	0.74	364021.10	0.76

Table 4: precision data for estimation of MCA (n=3)

Conc. µg/ml	Intraday (n=3) (absorbance)	% RSD	Interday (n=3) (absorbance)	% RSD
0.2	7399.91	1.52	7369.95	2.14
1	24194.20	0.77	24485.17	0.83
10	154970.00	0.77	154172.25	0.78

Table 5: Repeatability data for EPL AND MCA

Concentration	EPL 6 µg/ml	Rt (Min)	MCA 50 µg/ml	Rt (Min)
Area	85496.51	5.21	24356.25	2.13
	85503.38	5.21	24378.94	2.14
	85400.92	5.22	24329.36	2.11
	85193.56	5.21	24468.35	2.13
	84901.98	5.23	24375.23	2.13
	85985.63	5.21	24463.65	2.14
Mean.	85413.67	5.21	24395.29	2.13
Std. Dev.	361.2412	0.0076	57.5351	0.0195
% RSD	0.42	0.14	0.23	0.46

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Table 6: Accuracy data of EPL and MCA (n=3)

Amount of drug added initially from formulation		Amount of standard drug added (µg/ml)		% recovery ± SD (n = 3)		%RSD	
EPL	MCA	EPL	MCA	EPL	MCA	EPL	MCA
10	1	0	0	100.56± 0.92	111.65± 1.70	1.00	1.70
10	1	5	0.5	101.40±0.51	109.18± 1.52	0.52	1.56
10	1	10	1	103.20± 0.49	110.72±0.62	0.50	0.66
10	1	15	1.5	101.71± 0.41	111.91±0.51	0.49	0.52

% recovery ± SD (n = 3) Amount of standard drug added

Table 7: Robustness studies of HPLC method

Parameter	Method condition	RT		% RSD of peak area	
		EPL	MCA	EPL	MCA
pH	6	5.28	2.01	0.47	0.57
	7	5.11	1.89	0.45	0.52
Mobile phase Ratio Acetonitrile: 0.02 M KH_2PO_4	85: 15	19.64	2.35	0.42	0.62
	95: 05	3.47	2.02	0.39	0.71

Table 8: Solution stability study

Time (Hrs.)	Area (n=3)		% RECOVERY	
	EPL 10 (µg/ml)	MCA 1 (µg/ml)	EPL	MCA
0	85260.22	22728.12	100	100
3	84965.42	22557.54	99.65	99.25
6	84650.86	22675.72	99.27	98.77
24	84366.75	22330.26	98.95	98.25

Table 9: System suitability study

Parameters	EPL	MCA
Asymmetric factor	1.15	1.1
Resolution	3.08	
Theoretical plates	3630.71	4672.46

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Table 10: Summary of Validation Parameters of HPLC

Parameters	EPL	MCA
Range	1 - 40µg/ml	0.2 – 10µg/ml
Retention time (min)	5.21	2.13
% RSD for Retention time	0.14	0.46
Asymmetric factor	1.15	1.1
Resolution	3.08	
Theoretical Plates	3630.71	4672.46
Detection limit (µg/ml)	0.08	0.2
Quantitation limit (µg/ml)	0.2	0.8
Accuracy (%)	100.56% -101.71 %	109.18%-111.91%
	Precision (%RSD)	
Intra-day (n=3)	0.74 - 1.27 %	0.77-1.52 %
Inter-day (n=3)	0.76 -2.01%	0.78 -2.14%
Repeatability (%RSD)	0.42%	0.46%
Robustness	Robust	Robust
Specificity	Specific	Specific

Table 11: Analysis of marketed formulation

Formulation	Labeled Amount (mg)		Amount found (mg)		% Of drug found ± SD (n=3)	
	EPL	MCA	EPL	MCA	EPL	MCA
EPINEURONE	40	4	39.8	4.5	99.50±0.8371	113.54±0.0111

CONCLUSION

The RP-HPLC method for the simultaneous estimation of EPL and MCA has been developed. The method was validated as per ICH Q2 (R1) guideline for accuracy, precision, linearity, specificity and robustness. The developed method was successfully applied to marketed formulation.

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