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SIMULTANEOUS ESTIMATION OF PREGABALIN AND METHYLCOBALAMIN BY RP-HPLC IN BULK DRUG AND COMBINED TABLET DOSAGE FORM

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Abstract

The aim of this work is to develop a specific, precise, accurate, linear, simple, rapid and cost effective analytical method for the estimation of Pregabalin and Mecobalamin in combined tablet dosage form by RP-HPLC method. In the present investigation initial trials were made to develop LC conditions for the separation of Pregabalin and Mecobalamin using Pottasium dihydrogen phosphate buffer (pH6.5) as aqueous mobile phase and acetonitrile, THF as organic phase. The HPLC method was developed using Zodiac column, 250 X 4.6mm, 5 µ with Mobile phase consisting Pottasium dihydrogen phosphate buffer (pH6.5): ACN :THF(75:25:150) at 1mL/min flow rate Detection was carried out at 210nm and injection volume was 20µL.Developed method was validated in terms of linearity, range, specificity, accuracy, robustness and ruggedness. The limit of Detection was found to be 0.06ppm for pregabalin and 0.13ppm for Mecobalamin respectively.LOQ was found to be 0.41ppm for Mecobalamin and 0.20ppm for Pregabalin indicating high sensitivity method. The assay of Pregabalin and Mecobalamin tablets was performed by comparing the area of standard and sample. Assay data obtained was found to be suitable and lies well within the acceptance range of 98-102%.

Keywords: RP-HPLC, Pregabalin, Linearity, LOD, Metylcobalamin.

Introduction

This is a sophistication of the century-old technique and is the most widely used of all the analytical separation techniques[1-8]. In high performance liquid chromatography (HPLC) the liquid mobile phase is forced through the stationary phase under pressure. A simple HPLC includes a solvent reservoir to hold the mobile phase, a pump to pressurize the mobile phase, and injector to allow injection of a small volume of the sample mixture under high pressure, a column containing the bed of stationary phase, a detector to detect the presence of components as they exit the column, and a recorder to record the detector signal.

The present aim of the work is to develop a specific, precise, accurate, linear, simple, rapid and cost effective analytical method for the estimation of Pregabalin and Mecobalamin in combined tablet dosage form by RP-HPLC method. The work extends to validate the developed method as per ICH guidelines.

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The main objective of this investigation is to develop a sensitive and validated RP-HPLC method for the estimation of Pregabalin and Mecobalamin in bulk and formulations

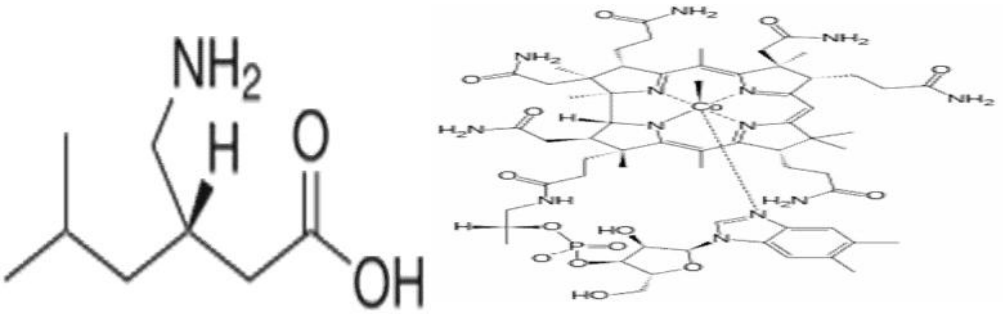

Fig. No. 01: Chemical structure of Pregabalin and Methylcobalamin

Experimental

Chemicals and materials

PRG was procured from Seemed lab limited, Hyderabadand Mehylcobalamine was obtained from Biocon limited, Bangalore. Methanol, Tetra Hydro Fluorine, all are Obtained from S.D fine chemicals, Delhi. All the reagents used were of Analytical reagent grade. Tablet formulation PREGABALIN M 75 (Torrent Pharmaceuticals Ltd) and NOVA PLUS CAP (Cipla Pharmaceuticals Ltd.) containing labeled amount of 75mg Pregabalin and 0.75 mg of Methyl - cobalamine was procured from local market.

Chromatographic conditions

A zodiac C18 (250×4.6 mm i.d) chromatographic column equilibrated with mobile phase ACN:THF:Buffer (75:25:150, v/v, pH 6.5) was used. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 210 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 6 min.

Sample preparation

Standard stock solution of Pregabalin

Accurately weigh 7.5mg of Pregabalin and transfer into a clean and dry 10ml volumetric flask, dissolve with sufficient volume of mobile phase and make up to 10ml with mobile phase to obtain the concentration of 7500µg/ml of Pregabalin.

Standard stock solution of Mecobalamin

Accurately weigh 100mg of Mecobalamin and transfer into a clean and dry 10ml volumetric flask, dissolve with sufficient volume of mobile phase and make up to 10ml with mobile phase and in that take 1.5ml of solution and transfer into a clean and dry 10ml volumetric flask, dissolve with sufficient volume of mobile phase and make up to 10ml with mobile phase to obtain the concentration of 1500µg/ml of Mecobalamin.

Working standard solution of Pregabalin and Mecobalamin

1ml of stock solution was further diluted in a 10ml volumetric flask with mobile phase to get a concentration of 7.5µg/ml of Pregabalin and 1.5µg/ml of Mecobalamin.

Method validation

The developed method was validated for linearity and range, specificity, accuracy, precision, Limit of detection, Limit of quantitation, robustness and solution stability as per ICH guidelines.

Linearity and range

The standard solutions are to be prepared at five different concentration levels ranging from 60 % to 140 % of working concentration and finding the response at each concentration level for assay. The calibration curves were developed by plotting peak area versus concentration (n=5).

Specificity

The specificity of the method was ascertained by analyzing PRG and MC in presence of excipients like talc, magnesium stearate and micro-crystalline cellulose were used for capsule formulations. The peak of PRG and MC were confirmed by comparing Rt values and respective spectra of sample with those of standards.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of PRG and MC by method of standard additions. The accuracy was performed by spiking standard into 80%, 100% and 120% working concentration samples and the samples

were injected in triplicate for each concentration level.

Method precision (Repeatability)

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of PRG and MC and the results are reported in terms of relative standard deviation.

Limits of detection (LOD) and Limits of quantitation (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines. $LOD=3.3\times\sigma/S$; $LOQ=10\times\sigma/S$; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness

The robustness of the proposed method was determined by making variations in the flow rate and wavelength which may differ but the responses were still within the limits of the assay.

Results and Discussion

Method development and optimization of chromatographic conditions

In the present investigation initial Variables were made to develop LC conditions for the separation of Pregabalin and Mecobalamin using Potassium dihydrogen phosphate buffer (pH6.5) as aqueous mobile phase and acetonitrile as organic phase. The HPLC method was developed using Zodiac column, 250 X 4.6mm, 5µ with Mobile phase consisting Pottasium dihydrogen phosphate buffer (pH6.5): ACN :THF(75:25:150) at 1mL/min flow rate Detection was carried out at 210nm and injection volume was 20µL.

Validation of the method

System suitability

System suitability was used to verify the reproducibility of the chromatographic system. System suitability parameters are evaluated by measuring the closeness of the obtained values for the parameters like tailing factor, theoretical plates which were found to be 1.414 and 2174 respectively for Mecobalamin and 1.211 and 4343 respectively for Pregabalin .

Specificity

The specificity of the method was established by injecting the standard and sample solutions to observe any interference with the drug peak. No interference at the retention time of the analyte was observed.

Linearity

Linearity studies performed at 60-140% level of selected drug concentration by constructing graph between concentrations vs. peak area. Correlation coefficient was calculated, the data was found within the acceptance criteria of 0.999. The result was found to be within limit so the method is linear over the concentration range 0.999.

Table No. 01: Linearity Preparations

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution (µg /ml)	
			Methylcobalamin	Pregabalin
Preparation 1	0.6	10	1.8	90
Preparation 2	0.8	10	2.4	120
Preparation 3	1.0	10	3	150
Preparation 4	1.2	10	3.6	180
Preparation 5	1.4	10	4.2	210

Table No. 02: Linearity Data

Levels	Pregabalin		Methylcobalamin	
	Conc. (mcg)	Peak area	Conc. (mcg)	Peak area
Level 1 (60%)	90	5220.62	1.8	133.17
Level 2 (80%)	120	6487.143	2.4	172.347
Level 3 (100%)	150	7761.64	3	213.59
Level 4 (120%)	180	9289.088	3.6	261.212
Level 5 (140%)	210	11200.238	4.2	289.655
Slope	1476.1		40.184	
Y-Intercept	3563.4		93.444	
Correlation Coefficient, R2	0.9916		0.9954	

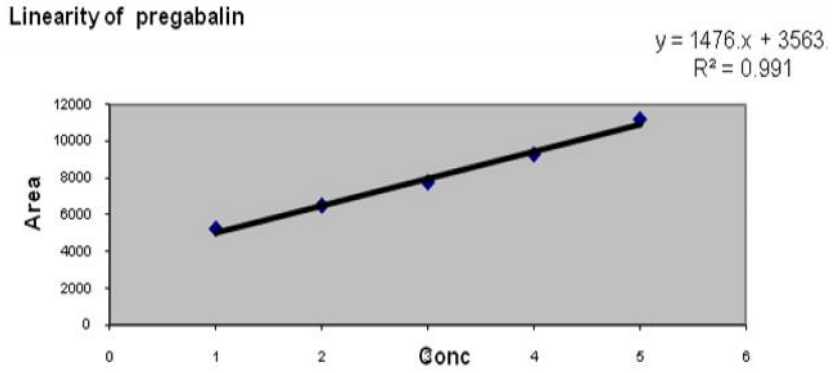

Fig. No. 02: Calibration curve of Pregabalin by HPLC

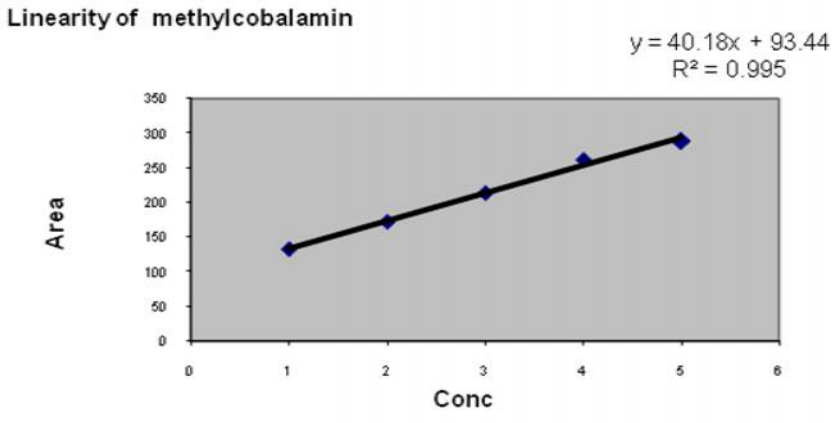

Fig. No. 03: Calibration curve of methylcobalamin by HPLC

Precision

Precision of drug was verified by repeatability. It was checked by injecting six preparations of the combination of Pregabalin and Mecobalamin sample solutions. % RSD was calculated. % RSD of area response for Pregabalin and Mecobalamin is NMT 2 which indicates the method to be precise.

Table No. 03: Precision (Repeatability) data

Injection No	Pregabalin		Methylcobalamine	
	Retention time (minutes)	Peak area	Retention time (minutes)	Peak area
1	4.180	7761.64	2.503	143.590
2	4.223	7716.294	2.517	142.454
3	4.240	7823.707	2.51	140.866
4	4.243	7876.64	2.5	140.904
5	4.297	7810.702	2.483	140.280
6	4.180	7739.849	2.503	141.688
Mean	4.227	7788.139	2.5027	141.630
SD	0.044	59.670	0.0114	1.221
%RSD	**1.05**	**0.77**	**0.46**	**0.86**

Accuracy

The accuracy was performed by spiking standard into 80%, 100% and 120% working concentration samples and the samples were injected in triplicate for each concentration level. The data was found suitable and lies well within the acceptance range of 98-102%.

The recovery of was found to be in the range of 99.01- 101.27% for Mecobalamin and 99.00-101.11% for Pregabalin which indicates a good accuracy of the method to that of the labeled claim.

Table No 04: Accuracy data for Pregabalin and Mecobalamin

Sample	Accuracy	Peak area	Mean % recovery
Mecobalamin	80%	223.59	99.14
	80%	213.59	
	80%	203.59	
	100%	271.212	100.61
	100%	261.212	
	100%	241.212	
	120%	309.655	98.99
	120%	296.655	
	120%	298.655	
Pregabalin	80%	8761.64	99.83
	80%	7761.64	
	80%	7761.64	
	100%	9189.088	99.73
	100%	9389.088	
	100%	9289.088	
	120%	11100.238	99.60
	120%	11200.238	
	120%	10080.238	

LOD &LOQ

LOD was found to be 0.13ppm for Mecobalamin and 0.06ppm for Pregabalin and LOQ was found to be 0.41ppm for Mecobalamin and 0.20ppm for Pregabalin indicating high sensitivity of the method.

Table No. 05: Linearity Data (LOD, LOQ)

Levels	Mecobalamin		Pregabalin	
	Conc. (ppm)	Peak area	Conc. (ppm)	Peak area
Level 1 (60%)	75	3762.552	7.5	585.374
Level 2 (80%)	100	4746.249	10	807.768
Level 3 (100%)	125	5630.868	12.5	998.120
Level 4 (120%)	150	6691.961	15	1188.007
Level 5 (140%)	175	7625.193	17.5	1399.190
SD(σ)	39.5	1529.6	3.95	317.6
Slope(S)	967.1		200.79	
Y-Intercept	2790.1		393.33	
LOD	0.13ppm		0.06ppm	
LOQ	0.41ppm		0.20ppm	

Robustness

As part of the robustness, deliberate changes in the flow rate, wave length was made to evaluate the impact on the method. The obtained results indicated that the minor changes in the flow rate and wave length did not affect the actual conditions.

Table No. 06: Robustness data for effect of flow rate variation

Flow rate	Pragabalin				Methylcobalamin		
(mL/min)	Injection No.	RT (min)	Efficiency(th.pl)	Asymmetry	RT (min)	Efficiency (th.pl)	Asymmetry
0.8	1	2.927	2109	1.175	5.127	3101	1.630
1.2	1	2.120	1829	1.321	3.717	2755	1.556

Table No. 07: Robustness data for effect of wavelength variation

Wavelength (nm)	Mecobalamin				Pregabalin		
	Injection No.	RT (min)	Efficiency(th.pl)	Asymmetry	RT (min)	Efficiency (th.pl)	Asymmetry
244	1.	2.450	2309	1.379	4.293	4343	1.270
	2.	2.453	2316	1.414	4.303	4364	1.263
248	1.	2.453	2192	1.448	4.300	4173	1.214
	2.	2.443	2174	1.414	4.297	4167	1.231

Ruggedness

The standard solution and sample solution were injected by different analysts and the area for injections in HPLC was measured. It was checked that the results were reproducible under different analysts. Hence the proposed method was found to be rugged.

Forced Degradation

The prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature. On comparing the forced degradation conditions with the normal condition, the drugs were found to be stable. They were not found to be affected by the above mentioned stress conditions.

Assay

The assay of Pregabalin and Mecobalamin tablets was performed by comparing the area of standard and sample. Assay data obtained was found to be suitable and lies well within the acceptance range of 98-102%.

Table No. 08: Analytical Method Validation Report for the Simultaneous Estimation of Pregabalin and Mecobalamin by RP-HPLC

S. No	Parameters	Limit	Observations
1	System suitability	%RSD - NMT 2 TF - NMT 2 TP - NLT 2000	
2	MET: TF-1.414 TP-2174 PRE: TF-1.211 TP-4343 Resolution: 7.869	No Interferences at retention time of the analyte peak.	Interference at retention time of the analyte peak not observed
3	Precision	RSD NMT 2.0%	MET: %RSD- 0.39% PRE: %RSD- 0.89%
4	Linearity of detector response	Correlation co-efficient NLT 0.999	MET: 0.9994 PRE: 0.9992
5	Accuracy	% Recovery range 98-102%	MET: 99.01- 101.27% PRE: 99.00- 101.11%
6	Robustness	Should comply with system suitability parameters	Within limits
7	Assay	%Assay 100%±2%	MET: 99.79% PRE: 100.11%

Conclusion

This developed and validated method for simultaneous analysis of PRG and MC in pharmaceutical preparations is very rapid, accurate, and precise. The method was successfully applied for determination of PRG and MC in its pharmaceutical capsule formulations. Moreover it has advantages of short run time and the possibility

of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence this method can be conveniently used for routine quality control analysis of PRG and MC in its pharmaceutical formulations.

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