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RP-HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF GABAPENTIN AND METHYLCOBALAMIN IN TABLET DOSAGE FORMS

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ABSTRACT

A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Gabapentin & Methylcobalamin from bulk and formulation. Chromatographic separation was achieved isocratically on a Waters C_{18} column (250x4.6, 5µ particle size) using a mobile phase Orthophosphoric acid: methanol in the ratio of 50:50 v/v. The flow rate was 0.8 ml/min and effluent was detected at 275 nm and 10µl of sample was injected. Rt of Gabapentin & Methylcobalamin was found to be 2.9 and 4.4 min respectively. Linearity of the method was in the concentration range of 50-150 % for Gabapentin & Methylcobalamin. Percent recoveries obtained for both the drugs were 100.00%. The percentage RSD for precision of the method was found to be less than 2%. The method was validated according to the ICH guidelines. The method developed was successfully applied for the analysis of simultaneous estimation of Gabapentin & Mecobalamine tablets.

Keywords: Gabapentin, Methylcobalamin, Methanol, orthophosphoric acid.

INTRODUCTION:

Gabapentin (Figure 1) is 2-[1-(amino methyl) cyclohexyl] acetic acid[1]. It is an anticonvulsant drug for neuropathic pain and adjunct for seizures. It can be used in generalized anxiety disorders. Methylcobalamin (Figure 2) is (carbanide; cobalt; [5-5, 6-dimethyl benzimidazol-1- yl)-4-hydroxy-2-(hydroxymethyl) oxolan-3-yl] 1-[3- [2,13,18-tris (2-amino-2- oxoethyl)- 7,12,17- tris(3-amino- 3-oxopropyl)-3, 5, 8, 8, 13, 15, 18, 19- octamethy-2, 7, 12, 17 tetra hydrocorrin-3-yl] propanoyl amino] propan-2-yl hydrogen phosphate[2]. It is a form of Vit-B12.It is a water soluble vitamin with a key role in the normal functioning of brain, and nervous system. It has been shown to protect those who take it from neurological conditions and ageing in a way that it makes different from other drugs or therapies[3].

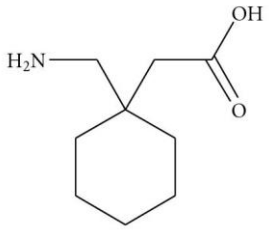

Figure 1: Chemical Structure of Gabapentin

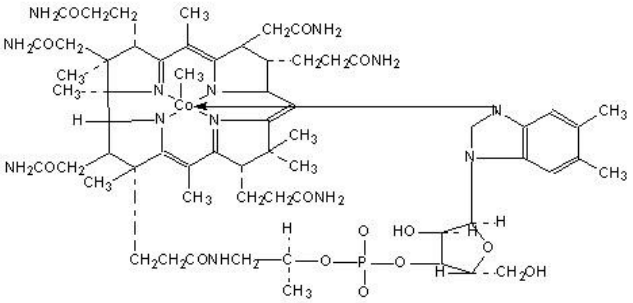

Figure 2: Chemical Structure of Methylcobalamin

Literature survey revealed HPTLC[3], UV[4-5], HPLC[6-9] methods for the estimation of Gabapentin and Methylcobalamin. The present study aims to develop simple, accurate, precise and selective RP-HPLC assay procedure for the analysis of

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Gabapentin and Methylcobalamin in bulk drug samples and in combined dosage. The method is optimized and validated as per the International conference on Harmonization (ICH) guidelines[10].

Materials and Methods:

Quantitative HPLC was performed on Agilent 1220 infinity HPLC system connected with PDA Detector 2998 and Empower-2 Software. Analytical column was Agilent Zorbin C18 (250 X 4.6 mm, 5µ) Column. Pharmaceutical grade Gabapentin and Methylcobalamin were kindly supplied as a gift sample by Newland Laboratories, Hyderabad, and Andhra Pradesh, India. Methanol was of HPLC grade and Purchased from Merck, Darmstadt, Germany. Ortho phosphoric acid and orthophosphoric acid was analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-Q-RO water purification system. Gabapentin and methylcobalamin Tablets (Nurokind G, gabapentin (300 mg), methylcobalamin (500mg) Make: Mankind Parma, New Delhi, India) was purchased from the local market.

Preparation of Ortho phosphoric acid buffer:

Accurately weighed 6.8 gms of potassium dihydrogen Ortho-phosphate in a 1000 ml standard flask and dissolved in minimum quantity milli-Q water and finally the volume was made upto the mark with the same. pH was adjusted to 2.2 with 0.1 N HCl.

Preparation of mobile phase:

600 ml of above buffer solution was transferred into a 1000 ml volumetric flask, to this 400ml of methanol was added, shaken well, filtered and degassed.

Preparation of standard solutions:

Accurately weighed 600 mg of gabapentin and 1mg of methylcobalamin were dissolved in mobile phase and sonicated for 10 min room temperature, later the volume made upto 50 ml in volumetric flasks. For simultaneous quantitative studies of both drugs, a series of standard working solutions containing both the drugs were prepared by an appropriate dilution of a mixture of stock solutions.

Preparation of sample solution:

A powder equivalent to 809 mg was accurately weighed, transferred into a 50 ml volumetric flask containing mobile phase. The above mixture was sonicated for about 10 min for complete mixing. This solution was filtered through Whatman No.1 filter paper. From the filtrate different aliquots were taken in separate 10 ml volumetric flasks and diluted with mobile phase up to the mark so as to get a concentration ranging from 50-150 µg/ml each of gabapentin and mecobalamin. Each of these solutions (10 µl) was then injected three times into the column. The mean peak areas of each drug were calculated and the drug contents in the tablets were quantified using the respective regression equations.

Method validation

The proposed method was validated as per ICH guidelines[11].

Specificity (Forced decomposition studies)

Specificity is the ability of the method to measure the analyte response in the presence of its excipients. The specificity of the developed LC method for gabapentin and Methylcobalamin was carried out in the presence of its degradants. Stress studies were performed for on tablets to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted with a stress condition of UV light (254 nm), acid (0.5N HCl), base (0.5N NaOH) and oxidation (3.0% H_2O_2) to evaluate the ability of the proposed method to separate analytes from its degradation product. For light studies, study period was 10 days whereas for hydrolytic, acid, base and oxidation, it was 24 h. Peak purity test was carried out for the gabapentin and Methylcobalamin peak by using PDA detector in stress samples.

Precision

The precision of the method verified by repeatability and by intermediate precision. Repeatability was checked by injecting six individual preparations of gabapentin and Methylcobalamin real sample (tablets). The intermediate precision of the method was also evaluated using different analyst and performing the analysis on different days. Precision of assay method was evaluated by carrying out six independent assays of real sample of gabapentin and Methylcobalamin at 100 µg/ml level against qualified reference standard. The intermediate precision of the assay method was evaluated by different analysts by making use of different columns and different lot of the sample.

Linearity

Linearity test solutions for the assay method were prepared from gabapentin and Methylcobalamin stock solution at six concentration levels from 50 to 150% of assay analyte concentration (50, 75, 100, 125 and 150 µg/ml). The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the method were prepared by diluting stock solution to the required concentrations.

Accuracy

Accuracy of the assay method was evaluated in triplicate using three concentration levels 50, 100 and 150µg/ml on real sample (tablets). Standard addition and recovery experiments were conducted on real sample to determine accuracy the method. Study was carried out in triplicate using three (50, 100 and 150%) concentration levels. The percentages of recoveries for gabapentin and Methylcobalamin were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the system suitability parameters were evaluated. Tailing factor for gabapentin and Methylcobalamin was recorded. The flow rate of the mobile phase was 0.8 ml/min, to study the effect of flow rate on the retention time, flow was changed by ± 0.2 units from 0.6 to 1.0 ml/min. The effect of the column temperature on retention time was studied at 25 and 35 ºC.

RESULTS AND DISCUSSION:

The absorption wavelength for Gabapentin and Methylcobalamin is determined after several trials. The absorbance spectra of the diluted standard and working solutions of Gabapentin and Methylcobalamin in methanol are recorded on a UV spectrophotometer. They are scanned in the wavelength 200 nm - 400 nm range using quartz cuvettes with 10 mm path length. The maximum absorption wavelength was observed at 275 nm for the two drugs. This is in good agreement with the reported wavelengths for these drugs combination.

Specificity (Forced decomposition studies)

Gabapentin and Methylcobalamin was found to degrade significantly in acid hydrolysis and in base hydrolysis and mild degradation was observed in UV and peroxide stress conditions. Figure 3a, 3b shows the representative chromatogram of standard and sample gabapentin and Methylcobalamin respectively. Photodiode array detector was employed to check and ensure the homogeneity and purity of gabapentin and Methylcobalamin peak in all the stressed sample solutions. Assay studies were carried out for stress samples against gabapentin and Methylcobalamin qualified working standard. The results are presented in Table 1. The purity and assay of gabapentin and Methylcobalamin was unaffected by the presence of its degradation products and thus confirms the stability-indicating power of the developed method.

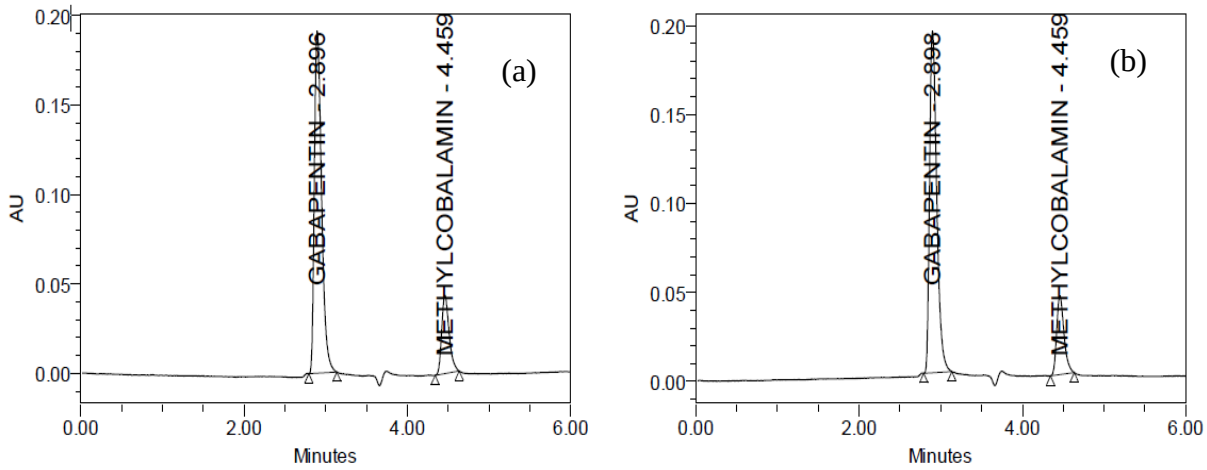

Figure 3. Representative chromatograms of a) Standards of Gabapentin and mecobalamin
b) Tablet sample of Gabapentin and mecobalamin

Table 1: Data of Forced decomposition studies

Parameter	Area of Gabapentin	% Assay of Gabapentin	% Degradation of Gabapentin	Area of mecobalamin	%Assay of mecobalamin	% Degradation of mecobalamin
Acid	2917192	67	-32	3109695	70	-30
Base	2648221	61	-38	3231239	73	-27
Peroxide	2527496	58	-41	3227403	73	-27
Sunlight	2970691	68	-31	4110524	93	-7
Heat	3968873	91	-8	4125196	93	-7

Precision

The % RSD during the method precision study of Gabapentin was 0.14 and 0.06% for retention time and Peak area respectively and 0.16 % and 0.34 % for retention time and Peak area of Methylcobalamin respectively. The % RSD for the area of Gabapentin and Methylcobalamin were well within 2 %, conforming good precision of the method. The % RSD values are presented in Table 2.

Table 2: Precision data

S.no	Gabapentin		Mecobalamin	
	RT	**Area**	**RT**	**Area**
injection1	2.902	577331	4.466	135567
injection2	2.912	577845	4.478	135737
injection3	2.905	576963	4.475	135338
injection4	2.908	577681	4.468	136090
injection5	2.902	578037	4.466	136098
Injection6	2.902	577669	4.467	134898
Mean	2.905	577587.667	4.470	135621.333
Std. Dev.	0.004	384.586	0.005	461.883
% RSD	0.142	0.067	0.116	0.341

Linearity

Linear calibration plot for above method was obtained over the calibration range 50 µg/ml to 150 µg/ml. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Table 3: Linearity data

S.no	**Conc.(µg/ml)**	**Gabapentin**	**Conc.(µg/ml)**	Mecobalamin
		Peak Area		**Peak Area**
1.	1200	587801	50	135451
2.	1800	880166	75	203858
3.	2400	1174368	100	271112
4.	3000	1468744	125	339640
5.	3600	1765133	150	397235
Slope		490.54	Slope	2637.4
Intercept		2054.4	Intercept	5719.2
Correlation coefficient (r^2)		1	Correlation coefficient (r^2)	0.999

Accuracy

Accuracy was determined by analyzing a sample of known concentration (reference standard solutions) and comparing the measured value with the true value, and using the method of standard additions. A table 3 summarizes the accuracy results, expressed as percent recovery. The method showed good recovery.

Table 3: Results of Accuracy

S. No	**Analyte**	**Accuracy Level**	**Sample weight**	**Amount added (µg/ml)**	**Amount Found (µg/ml)**	**% Recovery**	**% Mean**
1	**Gabapentin**	50%	404.60	237.840	237.87	100	
2		100 %	809.00	475.562	476.38	100	
3		150%	1213.60	713.402	712.90	100	100
4	**Mecobalamin**	50%	50	0.399	0.40	100	
5		100 %	100	0.797	0.80	100	
6		150%	150	1.196	1.20	100	100

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during normal usage. In order to perform the robustness study of the proposed method deliberate modifications in flow rate and column temperature were made. The results are shown in Table 4. It can be seen that every employed condition, the chromatographic parameters are in accordance with established value. A change of ± 0.2 unit of flow rate and column temperature had no impact on chromatographic performance (Table 4). According to the data of robustness test study proposed criteria for system suitability test (tailing factors, theoretical plates number and repeatability (R.S.D.)). It is used to verify that the repeatability of the system are adequate for the analysis intended.

Table 4: Results of Robustness

Parameter	Gabapentin			Mecobalamin		
	RT	Theoretical plates	Asymmetry	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	4.077	1664871	1.2	6.288	13848	1.1
Increased flow rate(1.2ml/min)	2.260	1667389	0.9	3.490	12780	1.0
Decreased temperature(25^0c)	4.001	1667845	0.8	6.278	11932	1.0
Increased temperature(35^0c)	4.084	1669852	0.9	6.282	11110	0.9

Detection and Quantification Limit:

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N is 10 were determined experimentally for the proposed methods and results are given in Table 5.

Table 5: LOD and LOQ

s.no	Sample name	LOD(µg/ml)	LOQ (µg/ml)
1	**Gabapentin**	2.44	8.15
2	**Mecobalamin**	5.68	1.89

Assay:

The proposed validated method was successfully applied to determine Gabapentin and Methylcobalamin in tablet dosage form. The result obtained was comparable with corresponding labeled amounts. The results were shown in table 6.

Table 6: Results of Assay

Sample no.	Gabapentin		Mecobalamin	
	Area	%Assay	Area	%Assay
1	577331	99	135567	99
2	577845	99	135737	99
3	576963	100	135338	99
4	577681	100	136090	99
5	578037	100	136098	99
6	577669	100	134898	99
Mean	2892778.50	100	4387169.67	99
Std. Dev.	3047.26	0.10	2228.45	0.05
% RSD	0.11	0.11	0.05	0.05

CONCLUSION:

The wide linearity range, Accuracy, Short retention times, & Simple Mobile phase imply that the proposed method can be successfully employed for routine quantification of Gabapentin and Methylcobalamin in combined dosage form. The method is economic too as the cost of mobile phase used is less compared to costly solvents that has to be used like acetonitrile for the quantification of Gabapentin and Mecobalamin. Also the forced degradation studies imply that this method is Stability Indicating Method development & validated according to ICH guidelines, one can adopt in an industry confidently for routine analysis.

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