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

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ABSTRACT

A simple, accurate, economical and reproducible HPLC method for simultaneous estimation of two component drug mixture of Gabapentin and Methylcobalamin (MCB) in combined tablet form have been developed. The detection was performed at 271 nm. The retention time of Gabapentin and Methylcobalamin was found to be 2.5 min and 3.08 min respectively. Linearity was observed in concentration range of 600-1800mcg/ml of Gabapentin and 1-3mcg/ml of Methylcobalamin. The reverse phase chromatographic method used C18 column and 0.1% Orthophosporic acid:acetonitrile in ratio of 55:45 as mobile phase. Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness.

Keywords: Gabapentin, Methylcobalamin, HPLC, Validation, Reverse Phase.

INTRODUCTION

Gabapentin is 2-[1-(aminomethyl) cyclohexyl]acetic acid [1]. It is an anticonvulsant drug for neuropathic pain and adjunct for seizures. It can be used in generalised anxiety disorders. 1,2 Methylcobalamin (MC;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 tetrahydrocorrin-3-yl] propanoylamino] 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 [9]. Literature survey revealed UV [4-5], HPLC [6-8], HPTLC [3] methods for the estimation of Gabapentin and MCB. The present study aims to develop simple, accurate, precise and selective RP-HPLC assay procedure for the analysis of PGB and MCB 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

Chemicals and Reagents

Gabapentin and MCB were obtained from Rainbow Pharma Labs. The mobile phase consisted of orthophosphoric acid and Acetonitrile which are of HPLC grade. Water of HPLC grade was used in the preparation of mobile phase. The commercial formulation of Gabapentin and MCB was procured from local pharmacy.

Instruments

Chromatographic separation was performed on HPLC system -Water's 515 pump, PDA Detector, equipped with a solvent delivery pump, sample injector and column thermostats. Empower 2 Chromatographic system software was applied for data collecting and processing.

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Chromatographic Conditions

The mobile phase orthophosphoric acid and Acetonitrile in ratio 55:45 was found to resolve Gabapentin and Methylcobalamin. The mobile phase was filtered on a 0.45µ membrane filter and then ultrasonicated for 30 min. The flow rate was set to 1.0ml/ min. Both the drugs showed good absorbance at 271 nm, which was selected as wavelength for further analysis.

Preparation of Standard Stock Solution

Accurately weighed and transferred 600 mg of Gabapentin and 1 mg of MCB working standard into a 100mL clean dry volumetric flask and added diluents. It was sonicated to dissolve completely and made volume up to the mark with the same diluents (Stock solution). From this, 5 ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent

Preparation of Sample Solution

Accurately weighed and transferred tablet powder equivalent to 600 mg of Gabapentin and 1 mg of MCB into a 50mL clean dry volumetric flask and added diluent. It was sonicated for 20 min to dissolve the drug completely and made volume up to the mark with the same diluents. From this, 5 ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluents.

Preparation of Calibration Curves

Calibration curve was prepared by taking appropriate aliquots of standard Gabapentin and Methylcobalamin, stock solutions in different 10ml volumetric flasks and diluted up to the mark to get a concentration of 600-1800 mcg/ml for Gabapentin and 1-3 mcg/ml for MCB. The solutions were injected into the chromatographic system at the flow rate of 1.0 ml / min and the effluents were monitored at 271 nm, chromatograms were recorded. The calibration curves of Gabapentin and Methylcobalamin was constructed by plotting average peak area versus % of concentration and was presented in Figure 1 and Figure 2.

Optimized chromatographic conditions

Diluent**:** Water
Mobile phase: Orthophosphoric acid: Acetonitrile (55:45)
Flow rate: 1.0mL/min
Column: Agilent zorbax SB C_{18}, 4.6*250mm,5 microns
Detector wavelength: 271nm
Injection volume: 10 µL

Method Validation

The proposed HPLC method was validated as per ICH guidelines such as accuracy, precision, linearity and range, robustness, LOD and LOQ .System suitability parameters were summarized in Table 4.The results obtained by doing the assay of marketed formulations was summarized in Table 1. The results of recovery studies are depicted in Table 2 and Table 3.

Accuracy

Accuracy of the method is the closeness of test results obtained by method to the assay value. Accuracy must be established across the specified range of the analytical procedure. Accuracy determined over the range of 50%, 100%, and 150% of the sample concentration. The accuracy was then calculated as the percentage of analyte recovered by the assay. The present recovery study indicates good accuracy of the method. The results of the accuracy study are given in Table 2 and Table3.

Precision

Intraday precision variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of ananalytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Limit of detection (LOD)

The limit of detection in the smallest concentration can be detected and not quantified as an exact value. LOD can be calculated as

$$LOD = 3.3\ \sigma/S$$

Where σ= Standard deviation of the y-intercept,
S=Slope of calibration curve.

Limit of Quantification (LOQ)

The limit of the quantification is the lowest amount of analyte in the sample which can be determined quantitatively.

$$LOQ=10\sigma/S$$

Where σ= Standard deviation of the y-intercept,
S=Slope of calibration curve.

Robustness

Variation in the flow rate and temperature has been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by such variations. Analytical concentration at level 100% was analysed by preparation at each level (with duplicate readings) against a standard solution. The results show that percentage relative standard deviation is less than 2.0%.

RESULTS AND DISCUSSION

In this method, the conditions were optimized to obtain complete elution of Gabapentin and Methylcobalamin. Mobile phase and flow rate selection was based on peak parameters (height, tailing factor and theoretical plates), run time, resolution. The run time was set at 5 min and the retention time for Gabapentin and

Methylcobalamin was found 2.5 and 3.08 and min as shown in Figure 3. The sample solution was injected 6 times and the retention times were found to be same. The regression equation was used to estimate the amount of Gabapentin and Methylcobalamin, either in formulation or in validation study (precision and accuracy). Robustness of the proposed method was determined by analysis of sample by changes in different parameter like flow rate, and temperature using similar operational and environmental conditions.

The proposed method was validated in accordance with ICH parameters and applied for analysis of the same in marketed formulations (Table 1-4). Linear relationship (r2=0.99) was observed between the concentration of Gabapentin and Methylcobalamin and the respective peak areas in the range 600-1800 mcg/ml and 1-3 µg/ml. The linear regression coefficient of gabapentin and Methylcobalamin was found to be 0.999 and 1 respectively. To develop a simple, precise, accurate method for the simultaneous estimation of Gabapentin and Methylcobalamin, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination.

Table 1. Result of Gabapentin and Methylcobalamin in marketed formulation

Drug	Labelled amount	Assay %
Gabapentin	300mg	99.04
Methylcobalamin	0.5mg	99.86

Table 2. Recovery study of Gabapentin

Level(%)	Mean % Recovery	%RSD
50	100.28	0.19
100	100.04	0.07
150	99.98	0.06

Table 3. Recovery study of Methylcobalamin

Level(%)	Mean % Recovery	%RSD
50	99.65	0.4
100	100.38	0.44
150	99.83	0.07

Table 4. Linear regression data of Gabapentin and Methylcobalamin

Validation Parameters	Gabapentin	Methylcobalamin
Linearity	600-1800mcg/ml	1-3mcg/ml
Correlation coefficient	0.999	1
LOD	0.984	0.0068
LOQ	3.28	0.0226
Precision	0.13	0.13
Tailing	1.59	1.7

Fig 1. Linearity of Gabapentin

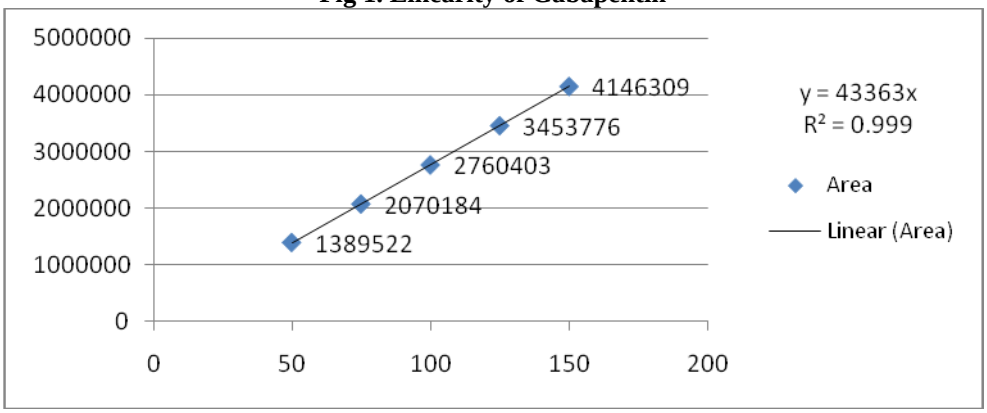


Fig 2. Linearity of Methylcobalamin

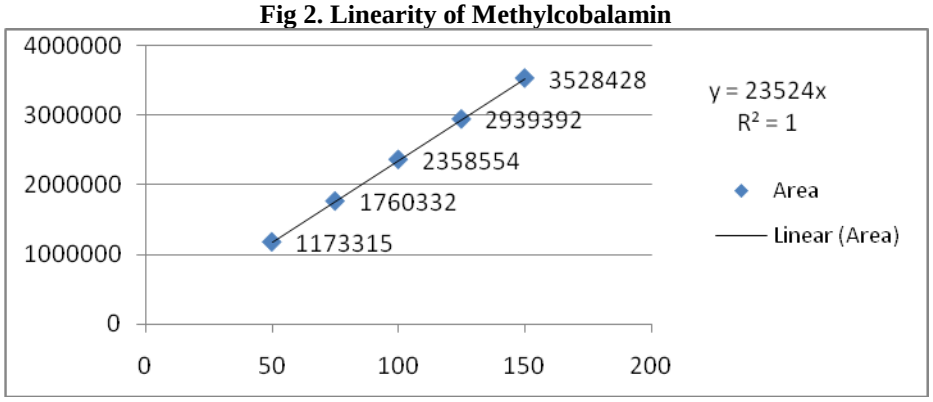
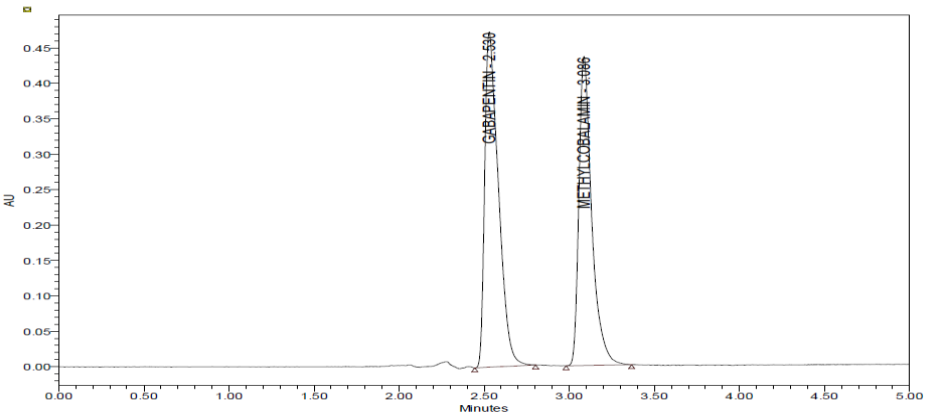


Fig 3. Typical chromatogram of Gabapentin and Methylcobalamin



CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for the simultaneous determination of Gabapentin and Methylcobalamin in bulk as well as in pharmaceutical preparations. There were no analytical methods reported so far for this estimation. The excipients of the commercial samples analysed did not interfere in the analysis, which proved the specificity of the method for these drugs. The developed method involves direct quantification of both the components. Hence, the developed RP-HPLC method can be adopted for the routine quality control analysis in the combination formulation.

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