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Simultaneous estimation of gabapentin and methylcobalamin in bulk and pharmaceutical dosage form by RP-HPLC

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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 an Inertsil C18 column (150x4.6, 5µ particle size) using a mobile phase Buffer: Acetonitrile in the ratio of 60:40 v/v. The flow rate was 1.0 ml/min, effluents were detected at 264 nm and 10µl of sample was injected. Retention time of Gabapentin & Methylcobalamin was found to be 2.7 and 4.13 min respectively. Linearity of the method was in the concentration range of 25-150 µg for Gabapentin & 0.125-0.750 µg for 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 & Methylcobalamin tablets and was fairly good in comparison with other methods.

Keywords: Gabapentin, Methylcobalamin, HPLC.

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INTRODUCTION

Gabapentin is (Figure 1) is 1-(amino methyl) cyclo hexane acetic acid. It is an anticonvulsant drug for neuropathic pain and adjunct for seizures. It can be used in generalized anxiety disorders. Methylcobalamin (Figure 2) is α-(5, 6 dimethyl benzimidazol) cobalmid cyanide (3+). 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 therapy[1-4].
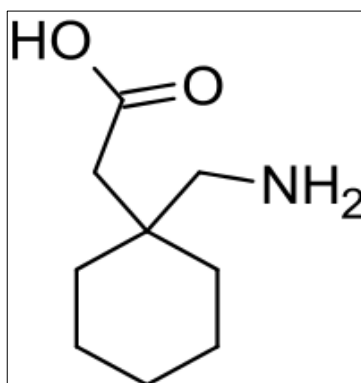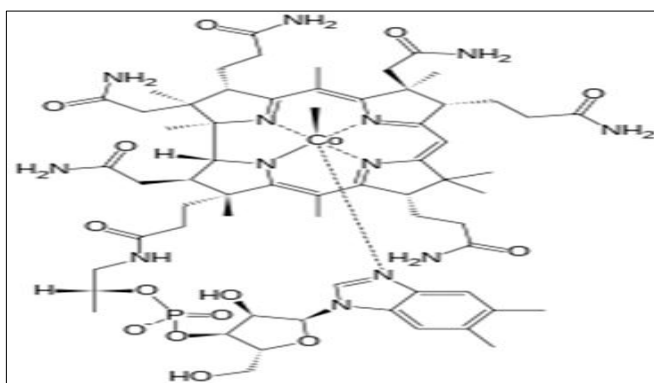

Figure 1: Gabapentin

Figure 2: Methylcobalamin

Literature survey revealed HPTLC, UV HPLC 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 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.

MATERIALS AND METHODS[5-11]

Gabapentin (GABA), Methylcobalamine (MECO) and Chemicals were obtained as a sample from Bio-Leo Analytical Labs private Ltd with the percentage purity of 99.1% and 99.7%, respectively. A commercial tablet formulation Gabantin Plus® from Sun Parma (Mumbai, Maharastra, India) containing 100 mg of GABA and 500µg of MECO was purchased from local market and used within their shelf life period. HPLC grade acetonitrile was obtained from Merck Limited. Analytical grade Buffer obtained from SD Fine (Mumbai, India). HPLC grade water was obtained by distilling deionizer water produced by a Milli-Q Millipore water system (Milford, MA, USA). All other chemicals used were of pharmaceutical or analytical grade.

Preparation of mobile phase:

Mixture of Buffer and Acetonitrile (60:40 ratios) was sonicated and the resulting solution was degassed by vacuum filtration through 0.4 micron membrane filter.

Preparation of standard stock solutions:

Gabapentin working standard 100 mg was weighed and transferred along with 0.5 of mg of Methylcobalamine working standard into 100 ml volumetric flask, diluent was added for making up volume. The resultant solution was sonicated.

Standard preparation:

One ml of standard stock solution was transferred into 10 ml volumetric flask and diluted to volume with diluent.

Preparation of sample solution:

Pre weighed 20 Tablets were finely triturated. Finely grinded sample quantitatively equivalent to 100 mg of Gabapentin and 0.5 mg of Methylcobalamine was transferred into 10 ml volumetric flask; 25 ml of diluents was added. The resultant was sonicated for 10 minutes and diluted to volume with diluent. Further the solution was filtered through filter paper. Ten ml of filtrate was diluted to 100 ml with mobile phase.

Method development:

After various trials, the following chromatographic conditions were finally optimized for the simultaneous estimation of Gabapentin & Methylcobalamine in a tablet dosage form. Mobile phase constitutes of Buffer: Acetonirile in the ratio of 60:40 v/v. Detection wave length 264 nm flow rate 1.0 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally 100 µg/ml of the standard solution of the individual samples of Gabapentin and Methylcobalamine and mixed standard solutions were injected and the chromatograms were recorded. The separation of Gabapentin and Methylcobalamine was observed with retention times of 2.7 and 4.13 min respectively. The typical chromatograms of the standard solutions were recorded for the repeatability and the respective chromatogram was given in Figure 3.
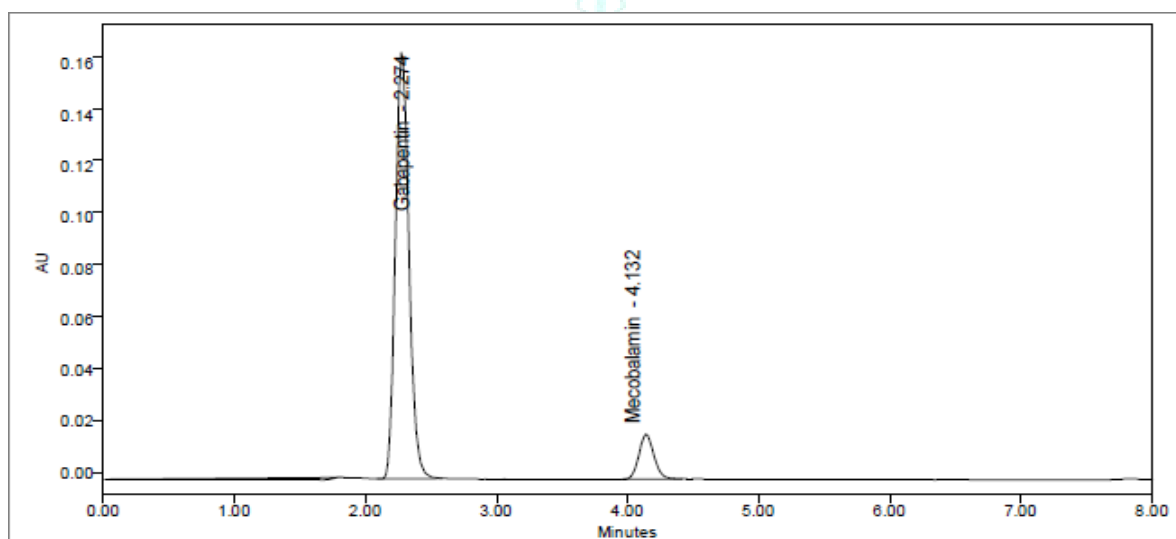
Figure 3: Typical chromatogram of Gabapentin and Methylcobalamine detection at 264nm.

Method validation:

The proposed method was validated as per ICH guidelines.

System suitability data for Gabapentin and Methylcobalamin

SR .No	Peak Name	Retention Time	Area	Plate Count	USP Tailing
1	Gabapentin	2.274	1173835	3345	1.15
2	Methylcobalamin	4.132	136983	6228	1.11

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.

Table 1: System Precision Study Results:

Name	Gabapentin	Methylcobalamin
Injection-1	1179101	136890
Injection -2	1173310	136556
Injection -3	1176860	136884
Injection -4	1177331	136415
Injection -5	1182173	136967
Injection -6	1188341	135452
Mean	1179519	136527
Standard deviation	5204.9	569.53
% RSD	0.441	0.42

Table 2: Method Precision Study Results:

Name	Gabapentin	Methylcobalamin
Injection-1	1187345	136875
Injection -2	1180165	138974
Injection -3	1189456	136888
Injection -4	1178144	138565
Injection -5	1178345	137145
Injection -6	1181122	138788
Mean	1182430	137873
Standard deviation	4804.6	1002.45
% RSD	0.406	0.73

Linearity:

The linearity range was found to be from 25 µg to 150 µg for Gabapentin and 0.125 µg to 0.75 µg for Methylcobalamin.
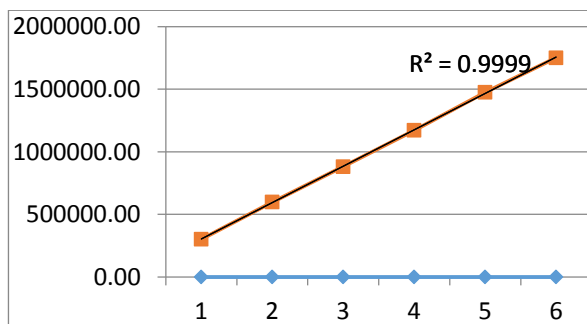
Figure 4: Linearity of Gabapentin
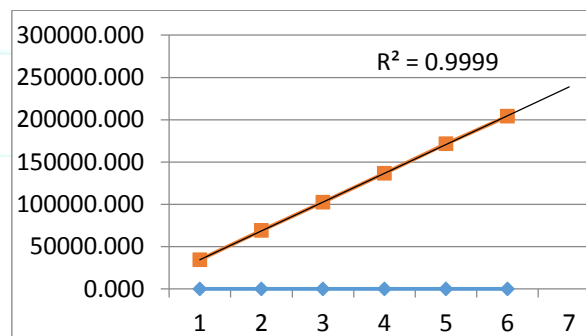
Figure 5: Linearity of Mecobalamine

Accuracy:

Accuracy of the assay method was evaluated in triplicate using three concentration levels 80, 100 and 120µg/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 (80, 100 and 120%) concentration levels. The percentages of recoveries for Gabapentin and Methylcobalamin were calculated.

Table 3: Accuracy data for Gabapentin:

% Concentration (at specification level)	Area	Amount Added (mg)	Amount found (mg)	% Recovery	Mean Recovery
80%	924479	80.00	78.76	98.45	
100%	1168610	100.00	99.55	99.55	99.09
120%	1398264	120.00	119.12	99.27	

Table 4: Accuracy data for Methylcobalamin:

% Concentration (at specification level)	Area	Amount Added (mg)	Amount found (mg)	% Recovery	Mean Recovery
80%	110498	0.40	0.40	100.83	
100%	136596	0.50	0.50	99.72	99.93
120%	163137	0.60	0.60	99.24	

Table.5: LOD of Gabapentin and Methylcobalamin:

Parameters	Gabapentin	Methylcobalamin
Slope	19494	10926
Intercept (C)	4627	487.5
LOD (µg/ml)	0.78	0.15

Table 6: LOQ of Gabapentin and Methylcobalamin:

Parameters	Gabapentin	Methylcobalamin
Slope	194943	10926
Intercept (C)	4627	487.5
LOQ (µg/ml)	2.73	0.45

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 1.0 ml/min, to study the effect of flow rate on the retention time; flow was changed by ± 0.2 units from 0.8 to 1.2 ml/min. The effect of the column temperature on retention time was studied at 30 ºC.

Table 7: Robustness data for Gabapentin and Methylcobalamin:

Parameter	Gabapentin		Methylcobalamin	
	Retention Time	Theoretical Plate no	Retention Time	Theoretical Plate no
Flow rate- 0.8 ml	2.00	3043043	3.363	2137383
Flow rate- 1.2 ml	2.368	2413944	4.813	1688920
Temperature - 25°C	2.261	2755918	4.157	1932082
Temperature- 35°C	2.273	2970931	4.323	1945900

Table 8: Comparison table:

CROMATOGRAPHIC CONDITIONS			
	Method Developed	REF 10	REF 11
HPLC system	WATERS E 2695	WATERS 515 pump	WATERS E 2695
Detector	PDA	PDA	PDA
Column	C 18 (150x4.6mmx 5µm)	C 18	C 18 (250x4.6mmx 5µm)
Temperature	30°C	Room Temp.	30°C
Elution	Isocratic	Isocratic	Isocratic
Mobile Phase	Buffer:CAN (60:40)	0.1% OPA:ACN (55:45v/v)	OPA :MEOH (50:50v/v)
Flow Rate	1ml/min	1ml/min	0.8ml/min
Injection volume	10µl	10µl	10µl
Wavelength	264nm	271nm	275nm
Run time	8 min	5 min	6 min

SYSEM SUTABILITY PARAMETERS						
	Method Developed		REF 10		REF 11	
	GABA	MCOB	GABA	MCOB	GABA	MCOB
RT(min)	2.7	4.13	2.5	3.08	2.896	4.459
LOD (µg/ml)	0.78	0.15	0.984	0.0068	2.83	2.44
LOQ (µg/ml)	2.73	0.45	3.28	0.0226	9.46	8.16
Linearity (µg/ml)	25 -150	0.125 - 0.750	600 -1800	1 - 3	50 - 150	50 – 150
Accuracy (%)	99.00 -99.55	99.24 -100.83	99.98 -100.28	99.65 - 100.38	100.0 - 100.0	100.0 - 100.0
Precision (%RSD)	0.15	0.21	0.13	0.13	0.34	0.13

CONCLUSION

The present results provide clear evidence that the proposed method can be successfully used for simultaneous determination of drug content in marketed formulations.

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