Research Article

RP-HPLC Method Development and Validation for the Estimation of Pregabalin and Methylcobalamin in Formulation

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Received: 10-03-2022; **Revised:** 20-05-2022; **Accepted:** 28-05-2022; **Published on:** 15-06-2022.

ABSTRACT

By considering the current regulatory requirement for an analytical method development, a reversed phase high performance liquid chromatographic method for routine analysis of pregabalin and Methylcobalamin has been developed . The optimized method was achieved using unisol C-18 (3 µm, 4.6 ×150 mm) column with mobile phase consisting of mixture of water and methanol (40: 60 v/v) with a flow rate of 0.6ml/min at 210 nm. The optimized method was then validated according to the ICH guidelines. The developed method was found linear over the concentration range of 50-150 µg/ml for pregabalin and 5-15 µg/ml for Methylcobalamin and the detection and quantitation limit was found to be 2.69µg/ml ,8.18 µg/ml for pregabalin and 1.74µg/ml, 5.27µg/ml for Methylcobalamin respectively. Therefore, a sensitive, robust, accurate method was developed with high degree of practical utility.

Keywords: HPLC, Pregabalin, Methylcobalamin, Estimation, Validation.

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DOI: 10.47583/ijpsrr.2022.v74i02.007

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v74i02.007

INTRODUCTION

Pregabalin ($C_8H_{17}NO_2$)is an anticonvulsant drug used for neuropathic pain, as an adjunct therapy for partial seizures, and in generalized anxiety disorder.[1]

Methyl cobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) belongs to the class of chemical entities known as cobalamin derivatives. These are organic compounds containing a corrin ring, cobalt atom, and a nucleotide moiety. Cobalamin derivatives are actually derived from vitamin- B12.[2]

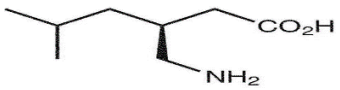

Figure1: Pregabalin

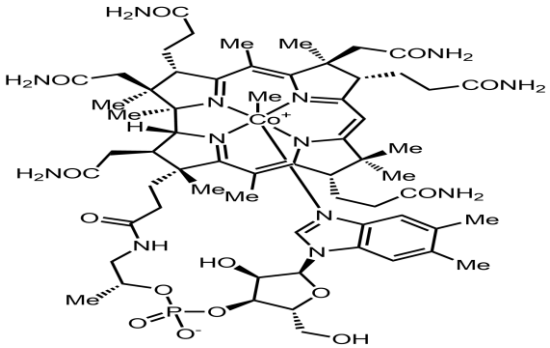

Figure 2: Methyl cobalamin

MATERIALS AND METHODS

Drug sample**:** Pregadoc M-75mg Cap. (Lupin Pharm. Pvt. Ltd.)

Mobile phase composition**:** Water and Methanol (40:60)

Instruments Used

HPLC	: Agilent technologies 1200 infinity series
Balance	: Metler Toledo ME204
pH meter	: Eutech instruments pH 700
Sonicator	: PCI Instruments

Vacuum filtration unit : Milli pore (XI 0422050)

Reason for Selection of Mobile Phase

- pH range of Pregabalin is 1 to 13, Methyl cobalamin is 3 to 7 and column is 1.5 to 9.
- So, the mobile phase with the pH range of 3 to 7 is more effective.
- pH range of water is 7, methanol is 4.5 to 6 and acetonitrile is 5.5 to 7. Hence, these three solvents can be used for the mobile phase
- Both pregabalin and methyl cobalamin are freely soluble in aqueous solutions, methanol and insoluble in organic solvents.
- The cost of water and methanol(HPLC grade) is very low when compared with the cost of organic solvents like acetonitrile, ether and acetone etc., and those are easily available in the market.

By considering all the above properties, Water: Methanol is the best choice for the mobile phase. A proper ratio of these two will elute sharp peaks which are reproducible with excellent tailing factor.

Detection of wavelength

The sensitivity of the HPLC method depends upon the proper selection of wavelength. Drug solution of 100 µg/ml was scanned over the range of 200-400 nm in UV region using different solvents like water, acetonitrile, hexane and methanol. It was observed that the drug showed maximum absorbance in methanol and water at 210 nm and hence methanol and water was used as solvent and 210 nm was used as maximum wavelength for detection of pregabalin and Methyl cobalamin for further study.

Preparation of Standard stock solutions

Accurately weighed 100 mg of Pregabalin and methyl cobalamin and transferred to 100 ml volumetric flask and 3/4 th of diluents was added to this flask and sonicated for 10 minutes. Flask was made up with diluents and labelled as Standard stock solution. (1000 µg/ml pregabalin and methyl cobalamin)

Preparation of Standard working solutions (100% solution)

10 ml from each stock solution was pipette out and taken into a 100 ml volumetric flask and made up with diluent. (100 µg/ml Pregabalin and methyl cobalamin)

Preparation of Dilutions

Dilute the working standard solution (100 µg/ml) to give of 50µg/ml to 150 µg/ml for pregabalin and 5µg/ml to 15 µg/ml for Methyl cobalamin.

RESULTS AND DISCUSSIONS

Optimized Chromatographic Conditions

Mobile phase : Water and Methanol(40:60)

Flow rate : 0.6 ml/min

Column : Agilent technologies unisol C18 (4.6 x 150mm, 3µm)

Detector wave length : 210 nm

Column temperature : 30°C

Injection volume : 10µL

Run time : 10 min

Retention time : 5.540 min for Pregabalin and 2.593 min for Methyl cobalamin.

Inference**:** Peak obtained for both Pregabalin and Methyl cobalamin was good with excellent peak characteristics and it was eluted at 5.540 min and 2.593min for pregabalin and Methyl cobalamin respectively. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

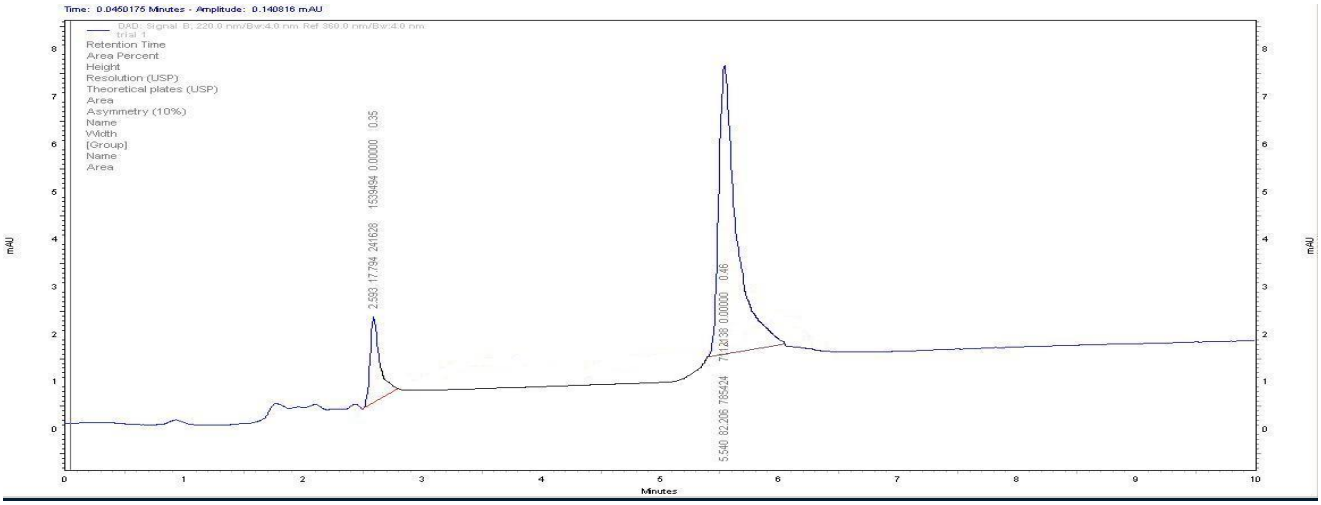

Figure 3: RP-HPLC Peak elution at 210nm

Method Validation

The optimized method which is derived from the trials can be validated and all the parameters should be checked.

The following parameters can be validated in HPLC method. They are:[3,4,5]

1) Accuracy

2) Precision

3) Linearity

4) Specificity

5) System suitability

6) Sensitivity

7) Robustness

8) Ruggedness

Accuracy

Accuracy is the degree of closeness of measurements of a quantity to that quantity's true value.

Accuracy of the method was determined by calculating the recoveries of Pregabalin and Methyl cobalamin by the standard addition method.[6]

Table 1: Accuracy table of Pregabalin

% Level	Standard amount(mcg/ml)	Spiked amount(mcg/ml)	Amount found(mcg/ml)	% Recovery	Mean %Recovery
50%	30	15	44.9	99.9	99.8
	30	15	44.6	99.6	
	30	15	45	100	
100%	30	30	60	100	99.9
	30	30	59.9	99.9	
	30	30	59.7	99.8	
150%	30	45	74.9	99.9	99.9
	30	45	74.6	99.7	
	30	45	75	100	

Table 2: Accuracy table of Methyl cobalamin

% Level	Standard amount(mcg/ml)	Spiked amount(mcg/ml)	Amount found(mcg/ml)	% Recovery	Mean %Recovery
50%	3	1.5	4.49	99.4	99.5
	3	1.5	4.45	99.1	
	3	1.5	4.5	100	
100%	3	3	6.01	100	99.5
	3	3	5.88	99	
	3	3	6.03	100	
150%	3	4.5	7.5	100	99.8
	3	4.5	7.47	99.6	
	3	4.5	7.49	99.9	

Inference

The percentage recovery was calculated for 50, 100 and 150 % spiked concentrations and they were found to be 99.8%, 99.9% and 99.9% for Pregabalin and 99.5%, 99.5% and 99.8% for Methyl cobalamin respectively.

Precision

Precision is a description of random errors, a measure of statistical variability.[7] The Precision of the instrument was checked by repeated injection and measurement of peak areas and retention time of solution. Types are:

Intraday precision

Table 3: Intraday precision table of Pregabalin and Methyl cobalamin

S. No	Retention time of Pregabalin	Area of Pregabalin	Retention time of Methyl cobalamin	Area of Methyl cobalamin
1.	5.54	785424	2.593	241628
2.	5.48	784321	2.596	241742
3.	5.55	784341	2.610	240946
4.	5.56	786521	2.594	241346
5.	5.54	785561	2.593	241564
6.	5.58	784321	2.594	241594
Mean	5.54.4	785083.17	2.597	241470
S.D	0.0342	910.86	0.0066	287.44
%RSD	0.61	0.11	0.26	0.12

Inference: The %RSD of intraday precision for retention time and peak area were found to be 0.61 and 0.11 for Pregabalin and 0.26 and 0.12 for Methyl cobalamin respectively.

Interday Precision

Table 4: Interday precision table of Pregabalin and Methyl cobalamin

S. No	Retention time of Pregabalin	Area of Pregabalin	Retention time of Methyl cobalamin	Area of Methyl cobalamin
1.	5.540	785424	2.593	241628
2.	5.542	785494	2.594	242742
3.	5.540	785424	2.593	241546
4.	5.562	785396	2.596	241694
5.	5.538	785546	2.592	240642
6.	5.554	785356	2.596	241946
Mean	5.546	785440	2.594	241699.7
S.D	0.0097	2140.17	0.0017	677.43
%RSD	0.17	0.27	0.06	0.28

Inference

The %RSD of interday precision for retention time and peak area were found to be 0.17 and 0.27 for Pregabalin and 0.06 and 0.28 Methyl cobalamin respectively

Linearity

Linearity is the property of a mathematical relationship or function which means that it can be graphically represented as a straight line.[8] Linearity was studied by analyzing five standard solutions covering the range of standard concentrations of sample solutions.

Table 5: Linearity table for Pregabalin for Methyl cobalamin

S. No	Concentration (µg/ml) for Pregabalin	Peak area for Pregabalin	Concentration (µg/ml) for Methyl cobalamin	Peak area for Methyl cobalamin
1	50	2236574	5	213457
2	75	3996947	7.5	325467
3	100	5434656	10	439456
4	125	7012347	12.5	526437
5	150	8594657	15	631274

Inference

The response was found to be linear and the correlation coefficient was found to be 0.999 for both Pregabalin and Methyl cobalamin respectively.

Specificity

Demonstration of specificity is done to confirm that the procedure is unaffected by the presence of impurities or excipients. [9]This is performed by running blank concentration.

Inference

Retention times of Pregabalin and Methyl cobalamin were found to be 5.540min and 2.593min. We did not find any interfering peaks in blank .So this method was said to be specific respectively

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.[10]

Table 6: System suitability parameters for Pregabalin & Methyl cobalamin

S. No.	Retention time		Tailing Factor		Theoretical Plates	
	Pregabalin	Methyl cobalamin	Pregabalin	Methyl cobalamin	Pregabalin	Methyl cobalamin
1	5.540	2.593	1.41	1.14	10509	6757
2	5.540	2.593	1.43	1.09	9896	6724
3	5.540	2.593	1.38	1.12	9959	6596
4	5.540	2.593	1.42	1.08	10324	6973
5	5.540	2.593	1.39	1.13	10102	6762
6	5.540	2.593	1.43	1.12	9936	6636

Inference

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2. All the system suitable parameters were passed and were within the limits.

Sensitivity

a) LOD: It is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit.[11]

LOD = (3.3 x S.D.)/ slope

b) LOQ: It is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

LOQ = (10 x S.D.)/ slope

Table 7: Sensitivity table for Pregabalin and Methyl cobalamin

Molecule	LOD	LOQ
Pregabalin	2.69	8.18
Methyl cobalamin	1.74	5.27

Inference

The LOD value was found to be 2.69µg/ml for Pregabalin and 1.74µg/ml for Methyl cobalamin at signal to noise ratio 3:1. LOQ value was found to be 8.18µg/ml for Pregabalin and 5.27µg/ml for Methyl cobalamin at signal to noise ratio 10:1.

Robustness

It is the measure of a method remain unaffected by small deliberate changes in method parameters like flow rate and mobile phase composition ratio.[12]

Table 8: Robustness data for Pregabalin and Methyl cobalamin

S. No.	Condition	%RSD	%RSD
		Pregabalin	Methyl cobalamin
1	Flow rate (-) 0.4ml/min	1.08	1.03
2	Flow rate (+) 1.2ml/min	0.58	1.01
3	Mobile phase (-) 30:70	0.72	0.23
4	Mobile phase (+) 70:30	0.72	0.26

Inference

The %RSD for flow rate was found to be 1.08 and 0.58 for pregabalin and 1.03 and 1.01 for Methyl cobalamin and %RSD for mobile phase was found to be 0.72 and 0.72 for Pregabalin and 0.26 and 0.23 for Methyl cobalamin respectively.

Ruggedness

The ruggedness of the method was studied by analyzing the sample by two or more analysts.[13,14] The % RSD value between these analysts were calculated.

Table 9: Ruggedness table for Pregabalin and Methyl cobalamin

S. No.	Condition	Retention time(min)		Area	
		Pregabalin	Methyl cobalamin	Pregabalin	Methyl cobalamin
1	Analyst I	5.540	2.593	785424	241628
2	Analyst II	5.667	2.647	792436	246574
3	Mean	5.603	2.62	788636	244101
4	SD	0.089	0.038	6018.63	3497.35
5	%RSD	1.59	1.45	1.81	1.43

Inference

The %RSD obtained by different analysts were 1.59 and 1.81 for Pregabalin and 1.45 and 1.43 for Methyl cobalamin respectively.

CONCLUSION

A simple, rapid, reliable, robust and optimized reversed phase high performance liquid chromatographic method for the simultaneous estimation of Pregabalin and Methyl cobalamin in formulation was successfully developed and validated as per International Conference on Harmonization guidelines. Accordingly, the method can be used for the routine analysis of Pregabalin and Methyl cobalamin in capsules.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.