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Research Article

RP-HPLC method development and validation for the determination of methylcobalamin and pregabalin in combined capsule dosage form

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ABSTRACT

A simple, precise and accurate RP- HPLC method was developed and validated for the estimation of pregabalin and methylcobalamin in capsule formulation. The HPLC instrument used was Shimadzu Prominence LC20AT with UV detection. Inertsil ODS 3 C-18 column with dimensions of 250 mm length, particle size of 3 microns and internal diameter of 4.0 mm was used for separation. The mobile phase consisted of 60 volumes of buffer 0.01 M potassium dihydrogen and 0.01M dipotassium hydrogen phosphate and 40 volumes of solvent methanol at a flow rate of 0.6 ml/min. The wavelength of 210 nm was used for the detection. The method was validated for linearity, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines. The response was found to be linear for the concentration range of 75µg/ml to 1125µg/ml for pregabalin and 0.75µg/ml to 11.25µg/ml for methylcobalamin. The limit of detection and limit of quantification was 5µg/ml and 15µg/ml for pregabalin and 0.05µg/ml and 0.15µg/ml for methylcobalamin. This validated method was applied for the estimation of pregabalin and methylcobalamin in commercial capsule formulation.

Keywords: Combined capsule dosage form; Pregabalin; Methylcobalamin; RP-HPLC; Validation

INTRODUCTION

Pregabalin, (S) - 3 - amino methyl hexanoic acid is a structural analogue of γ-amino butyric acid It is a white crystalline solid. It is soluble in water and in both basic and acidic aqueous solutions. It is a new anticonvulsant and analgesic medication that was recently approved for adjunctive treatment of partial seizures in adults and for the treatment of neuropathic pain from postherpetic neuralgia and diabetic neuropathy. The chemical structure of prebalin is shown in fig.1.

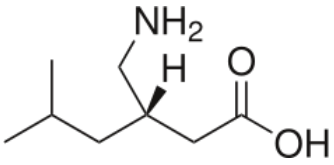

Figure 1: Chemical structure of Pregabalin

Methylcobalamin (MeB12) is a form of vitamin B12 used in the treatment of trigeminal neuralgia, megaloplastic anemia, diabetic neuropathy and facial paralysis in Bell's palsy syndrome. It is chemically carbanidecobalt(3+);[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] 1-[3-[(4Z, 9Z,14Z)-2,13,18-tris (2-amino-2-oxoethyl)-7,12,17-tris (3-amino-3-oxopropyl)-3, 5, 8, 8, 13, 15, 18, 19-octamethyl-2, 7, 12, 17-tetrahydro-1H-corrin-21-id-3-yl] propanoylamino] propan-2-yl phosphate having molecular formula C63H91CoN13O14P. It is a dark red crystalline powder soluble in water and ethanol. The chemical structure of methylcobalamin is represented in the Fig.2.

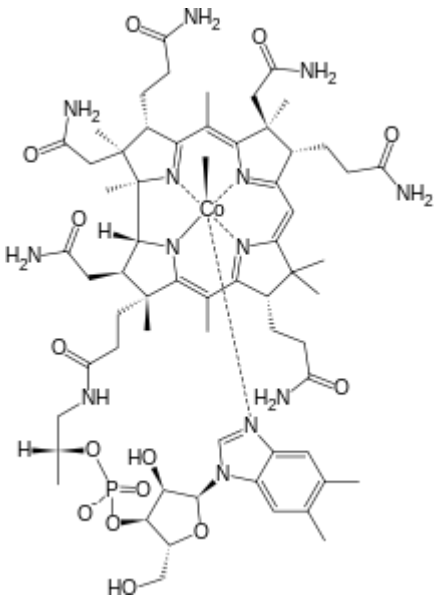

Figure 2: Chemical structure of methylcobalamin

From the literature survey it was found that HPLC, LCMS, HPTLC and spectrophotometric methods are available for the determination of pregabalin (Kasawar

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GB, 2010; Rajinder Singh et al., 2009, Ashu M et al., 2011; Onal A, 2009; Vikas V et al., 2007; Oertel R et al., 2008; Alka Bali, 2011) and methylcobalamin (Saravanan J, et al., 2010; Ganesan M et al., 2012) alone and for combined dosage form of methylcobalamin with other drugs (Varsha R, 2010; Baheti KG, 2011). But there are no methods available for the simultaneous estimation of pregabalinand methylcobalamin in combined dosage forms. Therefore in the present work a RP-HPLC method was developed and validated as per ICH guidelines for the determination of pregabalin and methylcobalamin in capsule dosage form. This method is simple, precise and accurate and can be used for routine quantitative analysis of pregabalin and methylcobalamin combined dosage forms by HPLC.

MATERIALS AND METHODS

Pregabalin and methylcobalamin were obtained from Natco Pharma Limited, Hyderabad, India. Pregabalin (75mg) and methylcobalamin (0.75mg) capsule formulation was purchased from local pharmacy. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, HPLC grade methanol all were purchased from Merck and water used was of Milli Q grade. All the solvents were filtered through 0.45 u filter and degassed.

Experimental

The HPLC instrument was of Shimadzu Prominence LC20AT with a UV detector SPD20A, SIL20AC autosampler with LC solutions software. A Inertsil ODS 3 C-18 column of dimensions 250x4.0mm x3 u with a run time of 20 min, absorption at wavelength 210 nm, injection volume of 20 µl, column temperature of 40°C and autosampler temperature of 5°C was used. Analytical balance of Mettler Toledo for weighing, Labindia make pH meter for adjusting the pH, Crest ultra sonicator for mixing and Millipore filtration unit for filtration of the solvents were used in the study.

Preparation of Mobile phase

The buffer was prepared by weighing 1.36 grams of potassium dihydrogen phosphate and 1.74 grams of

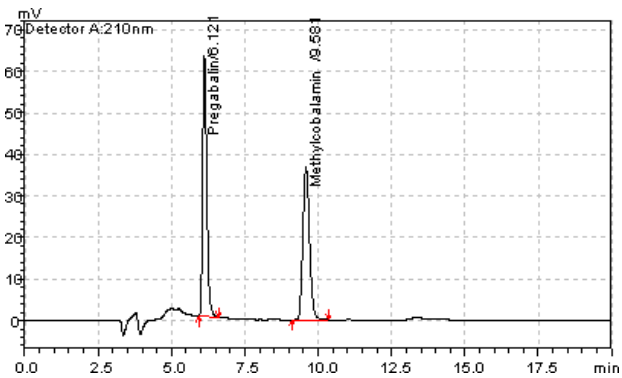

Figure 3: Representative chromatogram of pregabalin and methylcobalamin

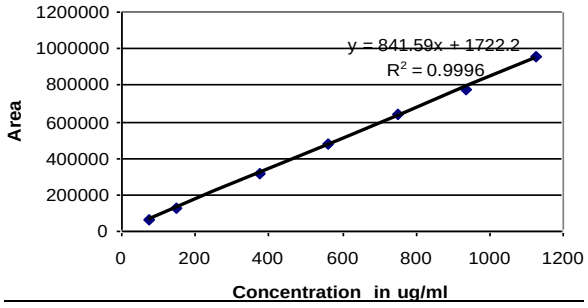

Figure 4: Calibration curve of pregabalin

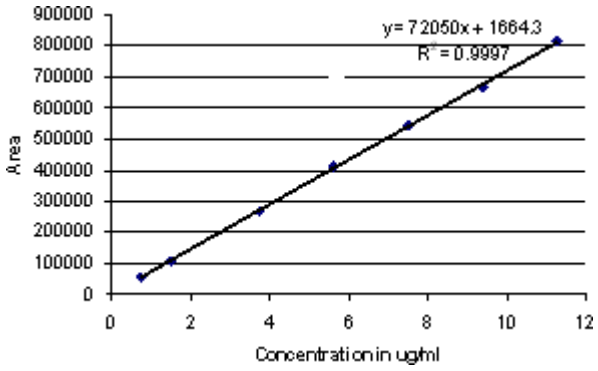

Figure 5: Calibration curve of methylcobalamin

dipotassium hydrogen phosphate in 1000 ml water. The mobile phase was prepared by mixing 60 volumes of buffer and 40 volumes of methanol. The mobile phase was filtered through 0.45 microns membrane filter paper with Millipore filtration unit and degassed using Crest ultra sonicator.

Preparation of stock solution and calibration standards

A stock solution of 7.5 mg/ml of pregabalin and 0.075 mg/ml of methylcobalamin was prepared. The calibration standards were prepared by further diluting the stock solution.

Pregabalin stock solution

187.5 mg of preabalin was weighed and dissolved in 25 ml of water to prepare 7.5 mg/ml of pregabalin.

Methylcobalamin stock solution

18.75 mg of methylcobalamin was weighed and dissolved in 100 ml of water into amber coloured volumetric flask. From this solution 10 ml was transferred into 25 ml amber coloured volumetric flask and made up to the mark to prepare 0.075 mg/ml of methylcobalamin. During the method development it was observed that methylcobalamin was unstable at ambient temperature. So the stock solution was stored in the refrigerator at about 5°C immediately after the preparation.

Reference solution preparation

The reference solution of a mixture of pregabalin (750µg/ml) and methylcobalamin (7.5µg/ml) was prepared by appropriate dilution and mixing of the stock solutions.

Sample preparation

20 capsules were selected randomly for analysis. 336 mg of the sample was taken into 100 ml amber coloured volumetric flask and made up to the mark with water. The solution was filtered using 0.45 microns filter paper.

All the calibration standards, the test and reference solutions prepared are transferred into amber coloured autosampler vials and kept in the autosampler tray maintained at 5°C until injected.

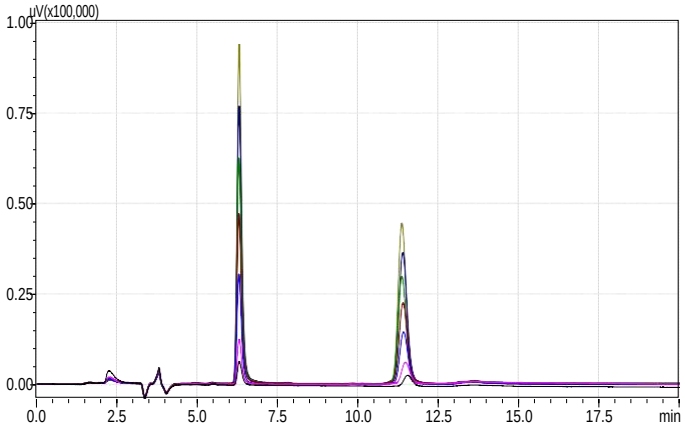

Figure 6: Overlay chromatogram

Table 1: Accuracy

Amount added (%)	pregabalin		methylcobalamin	
	%Recovery	%RSD	%Recovery	%RSD
80%	100.6	1.56	100.3	0.63
100%	99.5	1.2	101.9	0.60
120%	98.8	0.98	100.0	0.54

Table 2: Intra-day Precision

	Peak area of pregabalin	Peak area of methylcobalamin
1	650912	532808
2	651392	533137
3	651550	533223
4	651603	532614
5	652422	533349
6	651819	532894
Mean	651617	533017
SD	498	257
%RSD	0.076	0.048

RESULTS AND DISCUSSION

Method Validation

The proposed method was validated as per ICH guidelines (6). The method was optimized prior to the validation by changing the column, solvent and mobile phase pH. After performing many trials the proposed method was finalized. A resolution of 10.27 and tailing factor of 1.36 for pregabalin, 1.18 for methylcobalamin were obtained. Pregabalin was eluted at about 6.1 min and methylcobalamin at about 9.5 min. A representative chromatogram is shown in Fig.3. The validation parameters studied are linearity, LOD, LOQ, accuracy, precision and robustness.

LOD and LOQ

The limit of detection represents the concentration of the analyte that would yield signal-to-noise ratios of 3 and the limit of quantification represents concentration of the analyte that would yield signal-to-noise ratios of 10. A limit of detection of 5µg/ml, 0.05µg/ml and limit of quantification of 15µg/ml, 0.15µg/ml was established for pregabalin and methylcobalamin respectively. The relative standard deviation percentage results of the LOQ studies were 1.28 for pregabalin and 2.39 for methylcobalamin.

Accuracy

Accuracy of the developed method was confirmed by recovery study at three different concentration levels of 80 %, 100 %, and 120 % by replicate analysis. The results of accuracy are reported in table.1. The recovery study indicates that the method is accurate for quantitative estimation of pregabalin and methylcobalamin in dosage form as the statistical results are within the acceptance range (R.S.D. < 2.0%).

Table 3: Inter-day Precision

	Peak area of pregabalin		Peak area of methylcobalamin	
	Day1	Day2	Day1	Day2
1	650912	651204	532808	540104
2	651392	650463	533137	540550
3	651550	652051	533223	546144
4	651603	651558	532614	547649
5	652422	650087	533349	544971
6	651819	648845	532894	541577
Mean	651617	650701	533017	543499
SD	498	1157	257	3172
%RSD	0.076	0.17	0.048	0.584

Table 4: Robustness of the method

Parameter	Variation	Pregabalin (RT)	methylcobalamin (RT)
Flow rate	0.6	6.4	9.9
	0.7	6.2	9.4
	0.5	7.4	12.7
Column temperature	40°C	6.4	9.9
	35°C	6.9	11.2
	45°C	5.9	8.2

Table 5: Assay of capsule formulation

Drug	Labeled amount (mg)	Amount found (mg)	% Amount found
Pregabalin	75	75.6	100.8
Methylcobalamin	0.75	0.752	100.3

Linearity

Linearity was determined by injecting seven different concentrations of pregabalin and methylcobalamin of 10, 20, 50, 75, 100, 125 and 150% of the target analyte concentration. The calibration graphs were obtained by plotting the peak area verses the concentration data by least-square linear regression analysis. The method was found to be linear in the range of 75µg/ml to 1125µg/ml for pregabalin and 0.75µg/ml to 11.25µg/ml for methylcobalamin. The correlation coefficients were found to be greater than 0.999. The linearity curves of both the drugs are shown in the Fig.4 and Fig.5 and the overlay chromatograms of the calibration curve are shown in the Fig.6.

Precision (repeatability)

The precision of the method was determined by intra-day and inter-day precision. The precision was expressed as % R.S.D. The values obtained from the experiments for the repeatability of pregabalin and methylcobalamin is represented in the table.2 and 3. From the result it was found that the relative standard deviation is < 2% that indicates good repeatability.

Robustness

Robustness was evaluated by varying different parameters. The parameters include change in the flow rate and column temperature. The results of these variations are given in the table.4.

Assay of commercial combined capsule formulation of pregabalin and methylcobalamin:

The proposed validated method was used to evaluate the assay of commercial capsule containing pregabalin (75 mg) and methylcobalamin (0.75 mg). The experimental results of the amount of pregabalin and methylcobalamin are in good agreement with the label claim. The drug content was found to be 100.8% for pregabalin and 100.3% for methylcobalamin. The results of the two different lots analyzed using the proposed method is shown in the table.5.

CONCLUSION

A reverse phase HPLC method was developed and validated for the simultaneous estimation of pregabalin and methylcobalamin. The proposed method is simple, accurate and precise for routine estimation of these two drugs in capsule formulations.

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