Method Development and Validation for the Simultaneous Estimation of Pregabalin and Methylcobalamin In Bulk and Tablet Dosage Form Using UV-Spectrophotometry

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ABSTRACT

To method develop and validate simple, rapid, cost effective, linear, accurate, precise and economical for the simultaneous estimation of Pregabalin and Methylcobalamin in bulk and tablet dosage form by using UV-Spectrophotometry. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV-Spectroscopic method was developed for estimation of Pregabalin and Methylcobalamin in bulk and tablet dosage form and also validated as per ICH guidelines. The method based on measurement of absorbance at two wavelengths 222 nm (λmax of Pregabalin) and 219 nm (λmax of Methylcobalamin) in Ethanol and distilled water. Linearity graph of Pregabalin and Methylcobalamin were found to be linear in the concentration ranges of 30-150 μg/ml and 0.6-3 μg/ml, respectively, with their correlation coefficient values (R^2) 0.999. The low %RSD values indicate method to be accurate and precise. The % recovery of tablets found to be in range of 97-102% and other validation parameter were found to be within the limits as per ICH guidelines.

Keywords: Pregabalin, Methylcobalamin, Simultaneous equation method, UV Spectrophotometer, ICH guideline, Validation.

INTRODUCTION

Pregabalin is chemically (3S)-3-(amino methyl)-5-methylhexanoic acid. It comes under the class of anticonvulsant. Pregabalin is structurally almost like to gamma aminobutyric acid (GABA) an inhibitory neurotransmitter.

Methylcobalamin is chemically Carbanide; cobalt (2+); [(2R,3S,4R,5S)-5-(5,6-dimethyl benzimidazol-1-yl)4-hydroxy-2-(hydroxymethyl)oxolan-3-yl]1-[3-[(1R,2R,3R,5Z,7S,10Z, 12S,13S,15Z,17S,18S,19R)-2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-oxo propyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1H-corrin-24-id-3-yl]

Propanoylaminopropan-2-yl hydrogen Phosphate. Methylcobalamin is used to treat vitamin B12 deficiency.
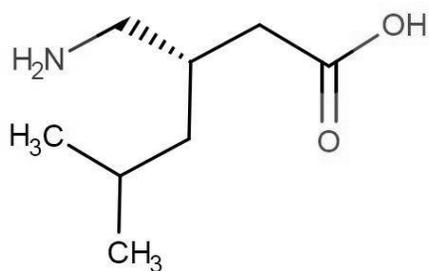
**Figure 1: Structure of PGB**   **Figure 2: Structure of MEC**
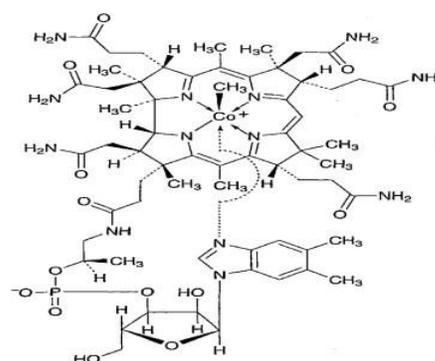
Pregabalin and Methylcobalamin is a prescription medicine used for the treatment of neuropathic pain. It reduces the pain by modulating calcium channel activity of the nerve cells. This medicine also protects nerve fibers and heals damaged nerve cells.

Combination of medicines used to treat long-lasting (chronic) pain caused by nerve damage due to diabetes, spinal cord injury. Extensive literature survey revealed that several methods for Pregabalin and Methylcobalamin in single and in combined dosage form with other drug using UV, HPLC, FTIR, UPLC and HPTLC determination have been reported.

So, an attempt was made to develop simple, rapid, cost effective, specific, accurate, precise and economical, Simultaneous equation method for determination of Pregabalin and Methylcobalamin in bulk and tablet formulations and method was validated as per ICH guidelines.

MATERIALS AND METHOD

Instrumentation

UV Spectrophotometry

Double beam UV-visible spectrophotometer (Shimadzu, model-1650PC, Japan) having two matched quartz cells with 1 cm light path was used for spectral measurements UV probe 2.42 software was loaded on to UV-visible spectrophotometer.

Chemicals and Reagents

Pregabalin and Methylcobalamin standard gift samples were provided by Vasudha Pharma Chem Ltd., Hyderabad, India. Ethanol was purchased from Changshu Hongsheng Chemical Co., Ltd. The Whatman filter paper No. 41 was obtained from Modern Science Lab. All other reagents used were analytical grade. All the glassware used was borosilicate glass. Tablet formulations (Pregeb M OD 75 mg) were obtained from local market.

Preparation of solutions

Preparation of stock solutions

 10 mg of Pregabalin and 10 mg of Methylcobalamin working standard was taken and transferred into 10ml volumetric flasks respectively and volume was made up 10 ml with ethanol. Then mixture was sonicated for 15 min to dissolve it completely. Final concentration of Pregabalin and Methylcobalamin were 1000 µg/ml and 1000 µg/ml respectively.

Construction of calibration curve:

Aliquots 5ml of Pregabalin and 0.1ml Methylcobalamin from the stock solution respectively were transferred into 10 mL volumetric flasks and volume was made up to 10ml with distilled water. From above solution aliquots of 0.6, 1.2, 1.8, 2.4, 3.0 ml was taken and transferred into a 10 ml volumetric flask and volume was made up to mark with distilled water to get a series of final concentration of Pregabalin (30-150µg/ml) and Methylcobalamin (0.6-3 µg/ml).

Preparation of sample solution

Ten tablets of formulations (Pregeb M OD 75 mg containing 75 mg of Pregabalin and 1.5 mg of Methylcobalamin was accurately weighed to find out average weight and finely powdered. The tablet Powder equivalent to about 75 mg of Pregabalin and 1.5 mg of Methylcobalamin was weighed and transferred into 100mL volumetric flask and 50 ml of ethanol was added.

The mixture was sonicated for 15 min and diluted up to the mark with distilled water and filtered through a whatman filter paper no.41. From this solution 1 ml aliquot was withdrawn into a 10 ml volumetric flask and diluted up to the mark with distilled water. Solution contains 75 µg/ml of Pregabalin, 1.5 µg/ml of Methylcobalamin.

RESULTS AND DISCUSSION:

The main objective of this study was to develop a new spectrophotometric method for

simultaneous analysis of Pregabalin and Methylcobalamin in bulk and tablet dosage form and validate it as per ICH guidelines.

Simultaneous equation method:

This method of analysis was based upon the absorption of drugs at wavelength maximum. Two wavelengths of 222nm and 219nm were selected as the λmax of two drugs for the development of the simultaneous equations. The absorbance of Pregabalin and Methylcobalamin were measured and absorptivity values were determined at all two wavelengths. The concentration of two drugs in the mixture can be calculated using the equations.

$$C_X = A_2 a y_1 - A_1 a y_2 / a x_2 a y_1 - a x_1 a y_2$$

$$C_Y = A_1 a x_2 - A_2 a x_1 / a x_2 a y_1 - a x_1 a y_2$$

Where,

A_1 and A_2 are absorbances of formulation at 222 and 219nm respectively,

Cx and Cy are the concentration of Pregabalin and Methylcobalamin respectively,

$a x_1$ and $a x_2$ are absorptivities of Pregabalin at 222 and 219nm respectively,

$a y_1$ and $a y_2$ are absorptivities of Methylcobalamin at 222 and 219nm respectively.

Determination of absorptivity value:

The absorptivity value of PGB and MEC from each solution was calculated using following formula and the results were presented in Table 1 and 2.

$$\text{Absorptivity} = \text{Absorbance} / \text{Concentration}$$

**METHOD VALIDATION**

Linearity

The linearity was evaluated by analyzing different concentrations of the standard solutions of PGB and MEC. It was found to be linear in the range of 30-150 μg/ml for PGB and 0.6-3 μg/ml for MEC. The visual characteristics such as linearity range, standard deviation on slope and intercept, correlation coefficient and regression linear equation were calculated and have been summarized in Table 1 and 2 and figure.

Table 1. Absorptivity valve for Pregabalin

Concentration (μg/ml)	λ1 – 222nm		λ2 – 219nm	
	Absorbance	Absorptivity	Absorbance	Absorptivity
30	0.137	0.00456	0.131	0.00436
60	0.273	0.00455	0.262	0.00436
90	0.410	0.00455	0.401	0.00445
120	0.540	0.0045	0.515	0.00429
150	0.670	0.00446	0.637	0.00424
	ax1 = 0.004524		ax2 = 0.00434	

Table 2. Absorptivity valve for Methylcobalamin

Concentration (µg/ml)	$\lambda_1 - 222nm$		$\lambda_2 - 219nm$	
	Absorbance	Absorptivity	Absorbance	Absorptivity
0.6	0.021	0.035	0.032	0.05333
1.2	0.042	0.035	0.061	0.05083
1.8	0.065	0.03611	0.090	0.05
2.4	0.083	0.03458	0.121	0.05041
3.0	0.106	0.03533	0.154	0.05133
	ay1 = 0.03504		ay2 = 0.05118	
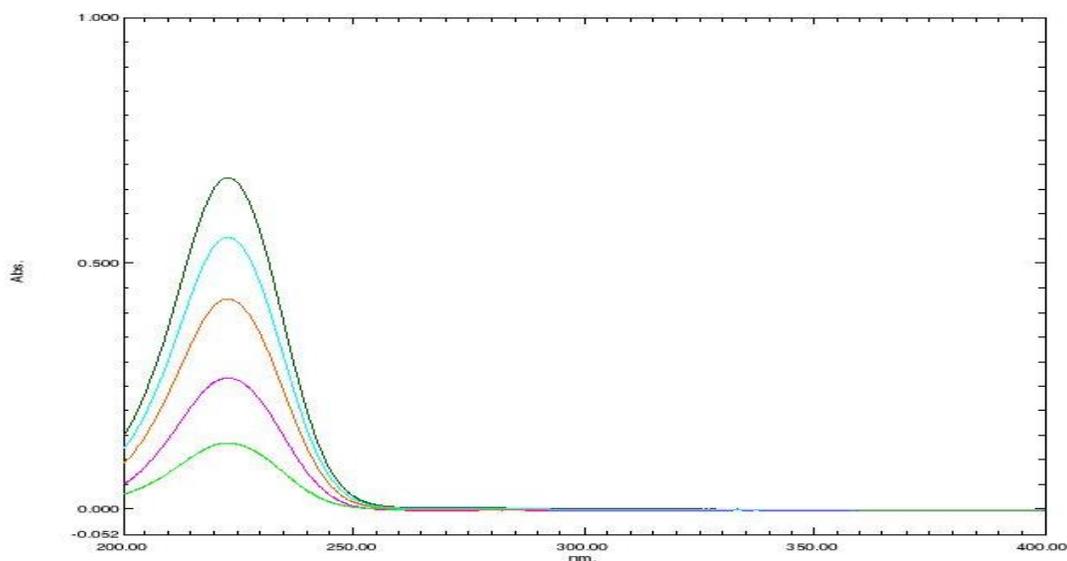
Figure 3. Absorption Spectra of Five Concentrations (30-150 µg/ml) of PGB
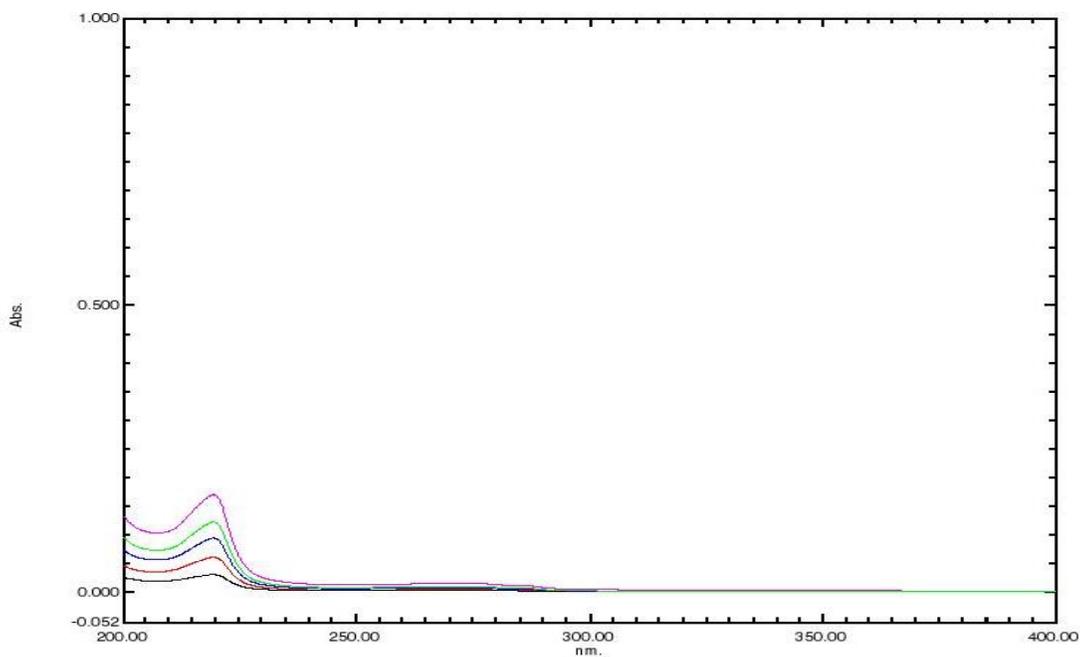
Figure 4. Absorption Spectra of Five Concentrations (0.6-3 µg/ml) of MEC
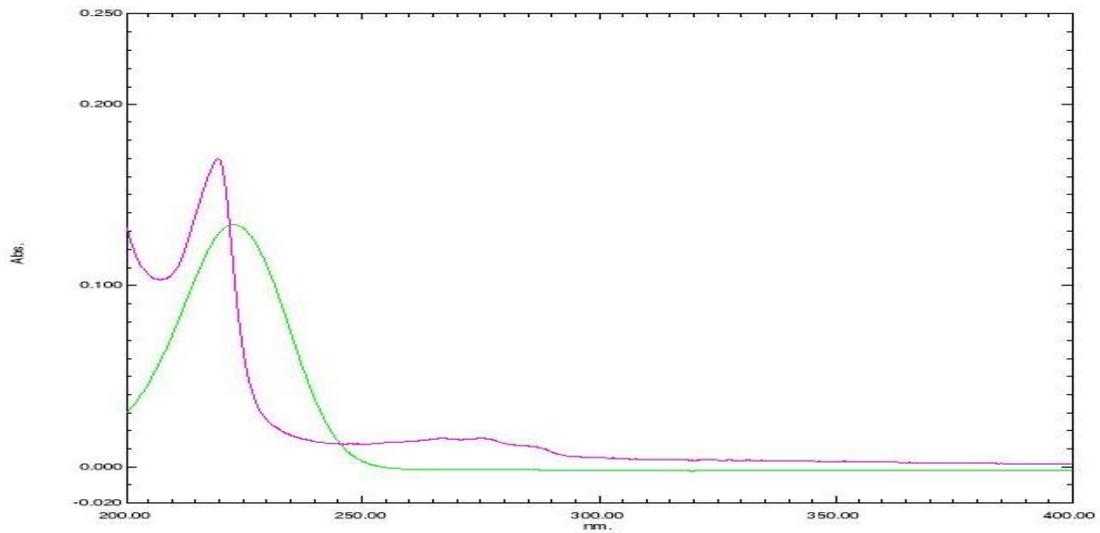
Figure 5. Overlain Spectra of Standard Solution of PGB and MEC
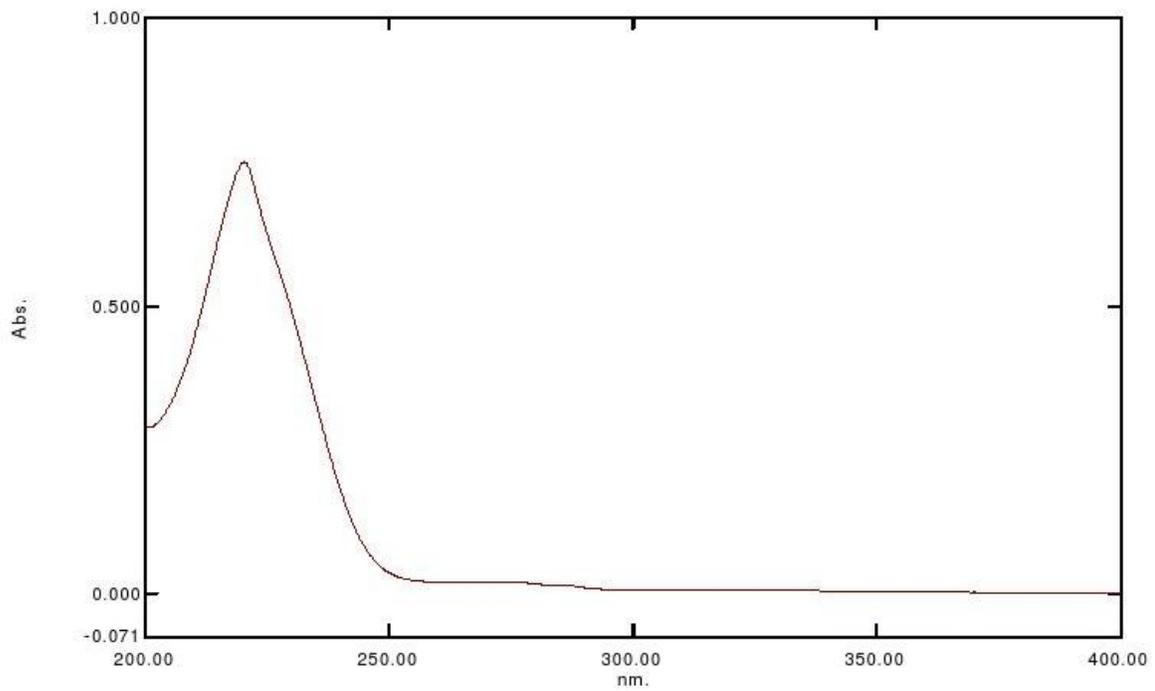
Figure 6. Overlain Spectra of Formulation

y = 0.00447238 x + 0.00290476
Correlation Coefficient r2 = 0.99985
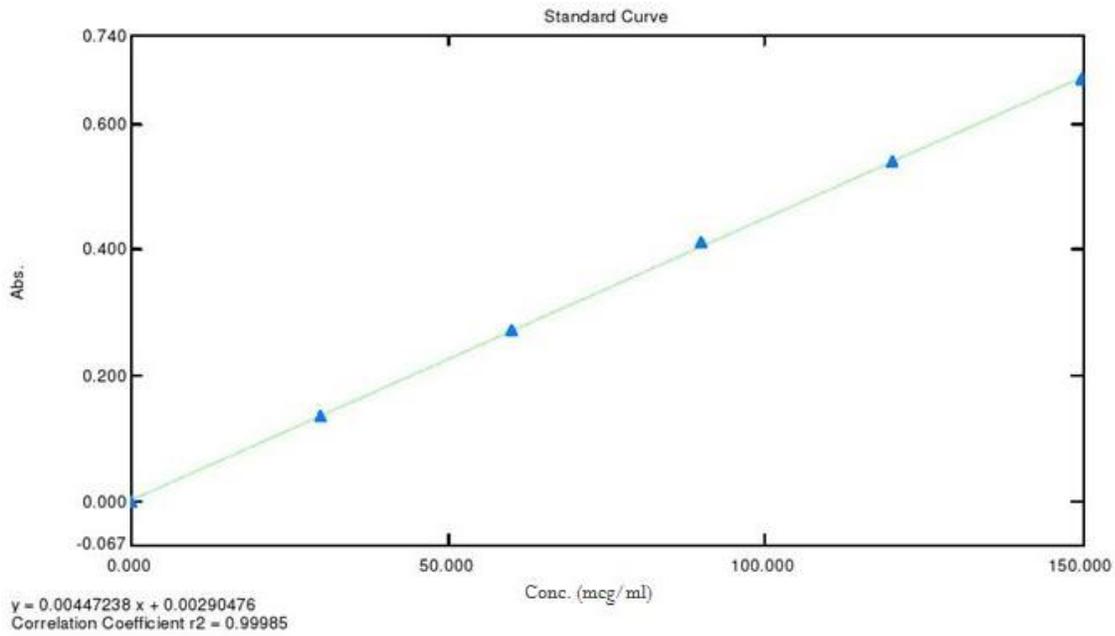
Figure 7. Linearity Graph of Pregabalin at 222nm

y = 0.00426286 x + 0.00461905
Correlation Coefficient r2 = 0.99917
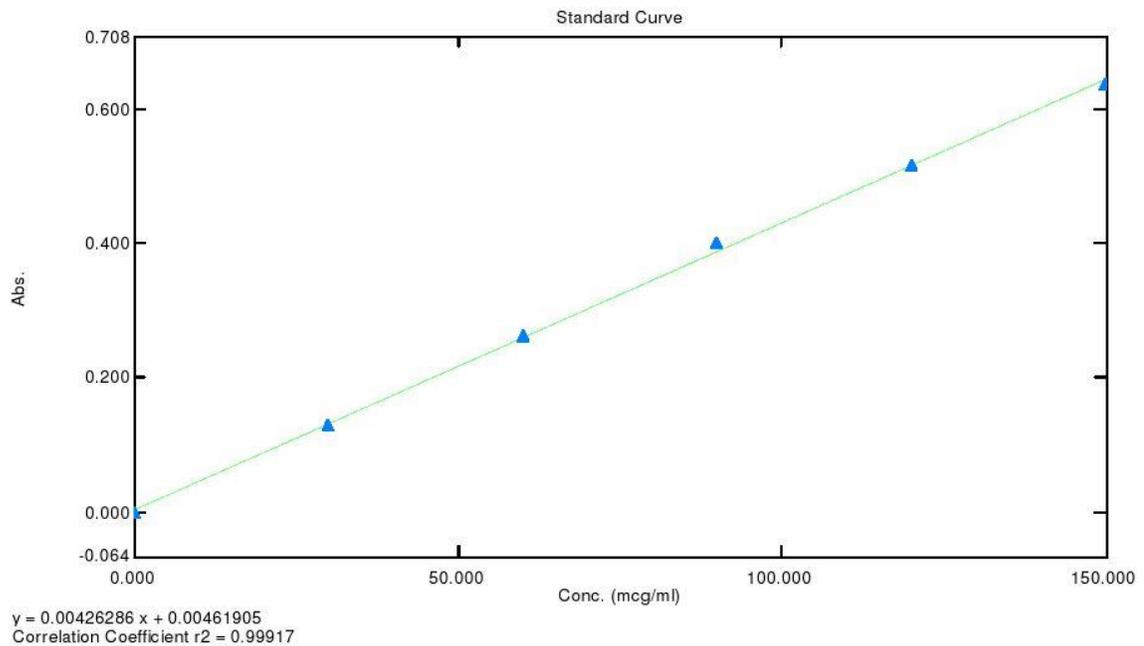
Figure 8. Linearity graph of Pregabalin at 219nm

y = 0.0351905 x + 4.76190e-005
Correlation Coefficient r2 = 0.99935
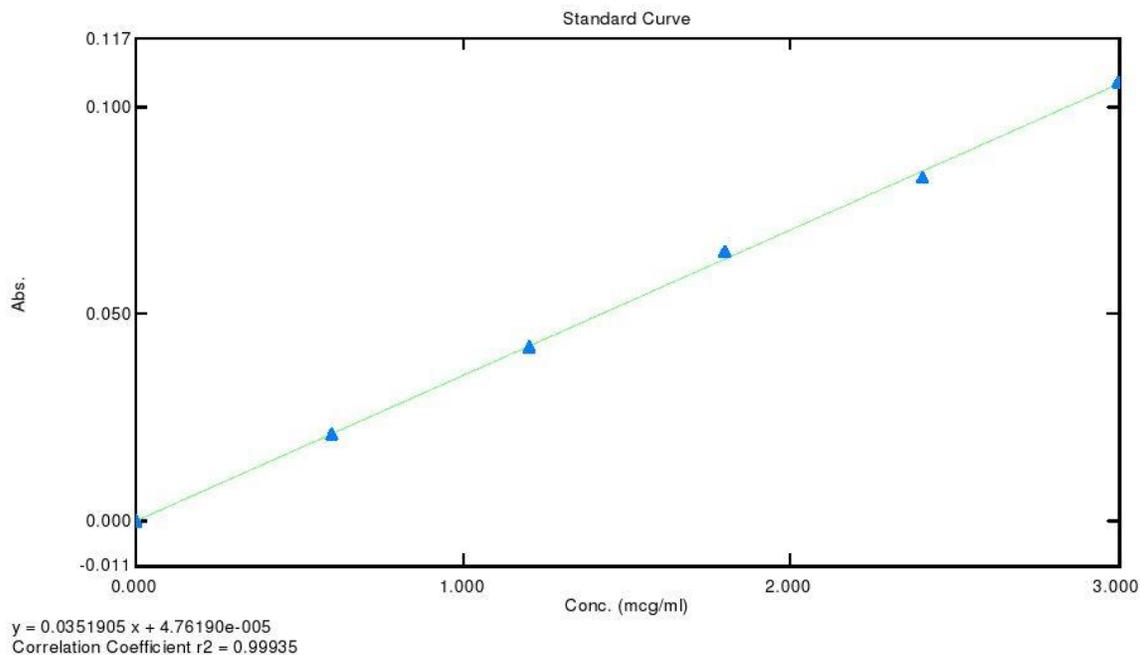
Figure 9. Linearity graph of Methylcobalamin at 222nm

y = 0.0507619 x + 1.90476e-004
Correlation Coefficient r2 = 0.99953
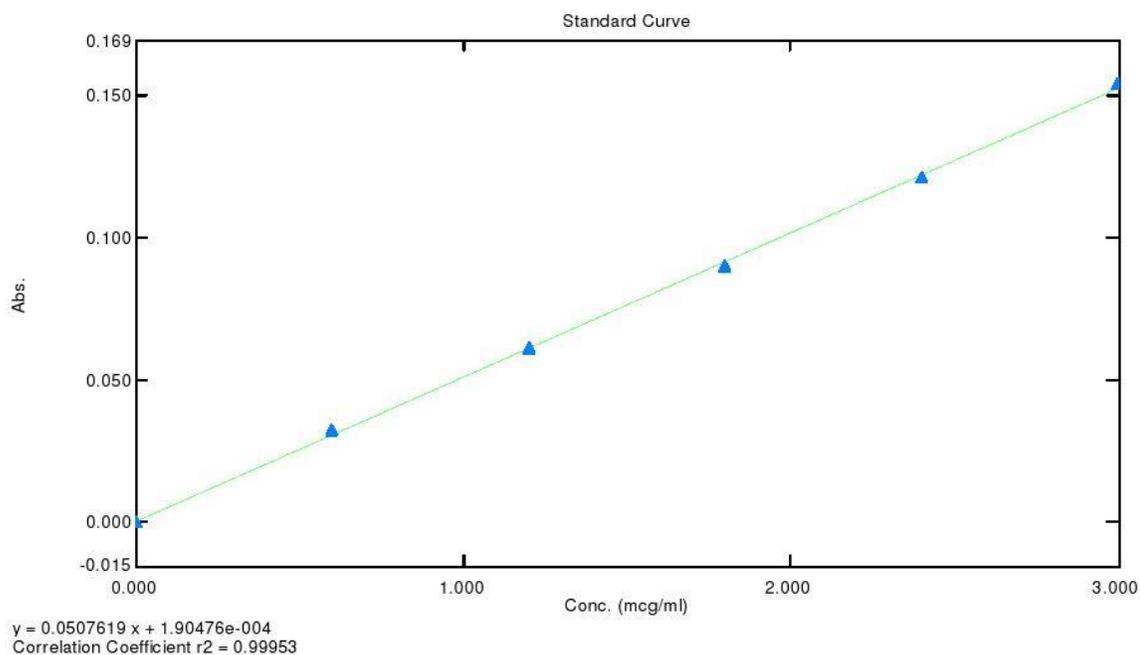
Figure 10. Linearity graph of Methylcobalamin at 219nm

Table 3. Accuracy Studies of Pregabalin and Methylcobalamin

Drug	Spiked level (%)	Amount taken (µg/ml)	Amount added (µg/ml)	%Recovery
Pregabalin	80	60	40	97.6%
	100	60	50	98.5%
	120	60	60	101.7%
Methylcobalamin	80	1.2	0.8	97.1%
	100	1.2	1	98.3%
	120	1.2	1.2	100.3%

Table 4. Intraday Precision data for analysis of PGB and MEC at 222 nm and 219 nm

Level No.	Concentration (µg/ml)		Absorbance				% RSD			
	PGB	MEC	PGB (222nm)	MEC (222nm)	PGB (219nm)	MEC (219nm)	PG B	MEC	PGB	MEC
1	60	1.2	0.273	0.042	0.262	0.061	0.21	1.38	0.22	0.95
			0.272	0.042	0.263	0.060				
			0.273	0.041	0.262	0.061				
2	90	1.8	0.410	0.065	0.401	0.090	0.17	1.09	0.17	1.55
			0.411	0.064	0.402	0.092				
			0.410	0.065	0.401	0.091				
3	120	2.4	0.540	0.083	0.515	0.121	0.26	0.85	0.13	0.58
			0.542	0.082	0.516	0.120				
			0.541	0.083	0.514	0.122				

Table 5. Interday Precision data for analysis of PGB and MEC at 222 nm and 219 nm

Level No.	Concentration (µg/ml)		Absorbancew3				% RSD			
	PGB	MEC	PGB (222nm)	MEC (222nm)	PGB (219nm)	MEC (219nm)	PGB	MEC	PGB	MEC
1	60	1.2	0.272	0.041	0.263	0.061	0.36	1.39	0.38	0.94
			0.274	0.042	0.262	0.062				
			0.273	0.041	0.264	0.061				
2	90	1.8	0.412	0.063	0.402	0.090	0.24	0.90	0.24	1.66
			0.410	0.064	0.401	0.093				
			0.411	0.064	0.403	0.092				
3	120	2.4	0.543	0.084	0.514	0.120	0.28	1.20	0.11	0.82
			0.542	0.082	0.515	0.122				
			0.540	0.083	0.514	0.121				

Table 6. Repeatability Data for Pregabalin

90µg/ml	Absorbance	
	At 222 nm	At 219 nm
	0.411	0.401
	0.410	0.404
	0.414	0.403
	0.411	0.401
	0.414	0.403
	0.414	0.404
Mean	0.412	0.402
SD	0.00204	0.00134
% RSD	0.49	0.33

Table 7. Repeatability Data for Methylcobalamin

Concentration	Absorbance	
1.8µg/ml	At 222 nm	At 219 nm
	0.065	0.090
	0.064	0.091
	0.065	0.092

	0.064	0.091
	0.065	0.092
	0.064	0.090
Mean	0.0645	0.091
SD	0.000548	0.000894
% RSD	0.84	0.98

Table 8. LOD and LOQ of Pregabalin and Methylcobalamin

Drugs	Parameters			
	LOD		LOQ	
	222 nm	219 nm	222 nm	219 nm
PGB	0.55	0.42	1.67	1.29
MEC	0.05	0.08	0.15	0.26

Table 9. Summary of Method Validation Parameters for PGB and MEC

Parameters		Pregabalin		Methylcobalamin	
		222 nm	219 nm	222nm	219 nm
Calibration range (µg/ml)		30-150	30-150	0.6-3	0.6-3
Molar Absorptivity (1 mole^{-1}cm^{-1})		0.004524	0.00434	0.03504	0.05118
Slope		0.00447	0.00426	0.03519	0.05076
Intercept		0.00290	0.00461	4.76190	1.90476
Correlation coefficient		0.99985	0.99917	0.99935	0.99953
Precision (%RSD)	Intraday	0.17-0.26	0.13-0.22	0.85-1.38	0.58-1.55
	Interday	0.24-0.36	0.11-0.38	0.90-1.39	0.82-1.66
	Repeatability	0.49	0.33	0.84	0.98
LOD (µg/ml)		0.55	0.42	0.05	0.08
LOQ (µg/ml)		1.67	1.29	0.15	0.26

Table 10. Analysis of Marketed Formulation

S.NO	Drug	Amount		% Amount Found
		Labelled	Found	
1.	Pregabalin	75	74.75	99%
2.	Methylcobalamin	1.5	1.47	98%

Accuracy (%Recovery)

The accuracy of the proposed method can be determined by using % recovery. Standard addition was used for accuracy determinations at three levels (80%, 100% and 120%) of concentrations; involving analysis of formulation of the samples containing 1.2 µg/ml of Methylcobalamin and 60 µg/ml of Pregabalin to which certain amounts of authentic drugs were added. The recovering range for Pregabalin and Methylcobalamin were found to be 97% to 102% as shown in Table 3.

Precision

Intermediate precision (Reproducibility)

Intraday and interday variations were determined by three solutions (60-120 µg/ml) of PGB and (1.2-2.4 µg/ml) of MEC within same day and three different days over a period of week. The corresponding results are shown in Table 4 and 5. The % RSD was found to be not more than 2.0%.

Method precision (Repeatability)

Repeatability was studied by calculating the RSD for five determinations of the concentration of about 90µg/ml and 1.8µg/ml for PGB and MEC respectively, performed on the same day and under same experimental conditions. The corresponding results are shown in Table 6   and 7. The %RSD was found to be not more than 1.0%.

Limit of Detection and Limit of Quantification

The limit of detection and Limit of quantification for both drugs were calculated theoretically using following equation as per ICH guidelines. These data shown that the method is sensitive Table 8.

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

$$LOQ = 10 \times \sigma/S$$

Where

σ= Standard deviation, S=Slope of the calibration curve

CONCULSION:

The Simultaneous equation method for simultaneous estimation of Pregabalin and Methylcobalamin in their bulk and tablet dosage form was developed and validated as per ICH guidelines. The Linearity was observed in the range of (30-150 µg/ml) for PGB and (0.6-3 µg/ml) for MEC with correlation coefficient ($r^2 = 0.999$). The % recoveries of PGB and MEC were in the range of 97-102% which was within the acceptance criteria. The % RSD was not more than 2% which proved the precision for the developed method. By studying all the validation parameters (Accuracy, Precision, Linearity, LOD and LOQ) we have concluded that the methods were simple, rapid, cost effective, precise, accurate and economical for the determination of Pregabalin and Methylcobalamin in their bulk and tablet dosage form. The assay results showed that the method can be successfully applied for routine analysis of PGB and MEC in bulk and tablet dosage form by using UV Spectrophotometry.

ACKNOWLEDGEMENT:

REFERENCES

1. Karchaliya CV, Patel PB. Development and validation of analytical methods for simultaneous estimation of amitriptyline hydrochloride and Methylcobalamin in their tablet dosage form by UV spectrophotometric method. PharmaTutor. 2015 Jul 1; 3 (7):46-50.

2. Mohan AJ, Raj Kumar B, Bhavya T, Ashok Kumar A. RP-HPLC Method Development and validation for the simultaneous quantitative estimation of Pregabalin, mecobalamin and alpha lipoic Acid in capsules. Int J Pharm Pharmsci. 2014; 6:270.

3. Potluri H, Battula SR, Yeturu S. Validated stability indicating RP-HPLC method for simultaneous determination of Nortriptyline and Pregabalin in bulk and combined dosage formulations. Journal of the Chilean Chemical Society.2017 Jun; 62(2):3490-5.

4. Chaudhari KL, Maheshwari DG. RP-HPLC Method for the Estimation of Epalrestat and Methylcobalamin in Their Combined Dosage Form. Ind. Amer. J. Pharma. Res. 2014; 5(3):2697-705.

5. Narmada P, Nalini G, Gowtham Y, Suhasini B, Jogi KV. RP-HPLC method development and validation for the determination of Methylcobalamin and Pregabalin in combined capsule dosage form. Int J Res Pharma Sci 2013 Jan 25; 4(1):25-9.

6. Shah RA, Shah RA. Stability indicating RP-HPLC method for simultaneous estimation of dosulepin hydrochloride and Methylcobalamin in tablet dosage form. Int. J. Appl. Pharm. 2017; 9:69-75.

7. Sravanthi T, Madhavi N. Stability Indicating UPLC Method for Simultaneous Estimation of Epalrestat and Pregabalin in Tablet Dosage Form. Research Journal of Pharmacy and Technology. 2019; 12(2):489-94.

8. Banerjee J, Padmalatha H, Chanda R. Method Development and Validation for the Simultaneous Estimation of Eplarestat and Pragabalin in Bulk and Pharmaceutical Formulation by using RP-HPLC. Asian J Pharma Analysis. 2018; 8(3):174-80.

9. Vyas AJ, Nathwani GP, Patel AI, Patel NK, Patel AB. Validated Stability Indicating RP-HPLC DAD Method for Simultaneous Determination of Amitriptyline Hydrochloride and Pregabalin in Presence of Stress degradation products in tablet dosage form. Chemical Methodologies. 2019 Jul 1; 3(4):494-508.

10. Sowjanya P. RP HPLC Method Development of Pregabalin in Bulk, Dosage Form and Validation Parameters. Journal of Hospital and Clinical Pharmacy. 2016; 2(4):61-75.

11. Singh SD, Mehta FA, Shah DA, Chhalotiya UK. Analytical RP-HPLC Method for Development and Validation of Citicoline Sodium and Methylcobalamin in Combined Tablet Formulation. Int. J. Pharm Anal. 2014; 2:432-8.

12. Shrirang DS, Mohanrao KT, Satish A, Kowthalam A. A validated method for quantitation of Pregabalin and Methylcobalamin using diff use reflectance FTIR spectroscopy in bulk and combined tablet dosage form. Abstracted by. 2020 Mar; 57 (03):55.

13. Kumari JN. Development and validation of new RP-HPLC method for simultaneous estimation of drug Duloxetine and Mecobalamin in tablet dosage form. Indian Journal of Research in Pharmacy and Biotechnology. 2016 Jul 1; 4(4):169.

14. Kavitha MP, Rajasekhar A. A validated HPLC method for the analysis of Pregabalin and Methylcobalamin in bulk and pharmaceutical formulation. Pharmacie Globale. 2013 Jul 1;4(7):1.

15. Oommen MK, Eapen SC, Velayudhankutty S, Haribabu Y. UV-Vis spectrophotometric method for estimation of Pregabalin and Methylcobalamin in bulk and capsule dosage form. Int J Pharma Research and Life Sciences. 2014 Feb 28; 2(1):6-15.

16. Singh SD, Mehta FA, Shah DA, Chhalotiya UK. Analytical RP-HPLC Method for Development And Validation Of Citicoline Sodium And Methylcobalamin In Combined Tablet Formulation. Int. J. Pharm Anal. 2014; 2:432-8.

17. Shah DA, Patelia EM, Mori A. Simultaneous Estimation of Pregabalin and Methylcobalamine in Pharmaceutical Formulation by HPTLC-Densitometry Method. Journal of Chromatography and separation technique. 2013; 4:145-52.

18. Chaudhari KL, Maheshwari DG. RP-HPLC Method for the Estimation of Epalrestat and Methylcobalamin in Their Combined Dosage Form. Ind. Amer. J. Pharma. Res. 2014; 5(3):2697-705.

19. Banerjee J, Padmalatha H, Chanda R. Method Development and Validation for the Simultaneous Estimation of Eplarestat and Pragabalin in Bulk and Pharmaceutical Formulation by using RP-HPLC. Asian J Pharm Analysis. 2018; 8(3):174-80.

20. Sravanthi T, Madhavi N. Stability Indicating UPLC Method for Simultaneous Estimation of Epalrestat and Pregabalin in Tablet Dosage Form. Research Journal of Pharmacy and Technology. 2019; 12(2):489-94.

21. Atta AM, Salama I, Gomaa MS, Mostafa S. A new HPLC method for simultaneous determination of atenolol and Pregabalin in dosage forms and in human urine. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2016 Jan 1; 6(1).

22. Patel ND, Rajyaguru H, Patel PB. Development and validation of first order derivative spectrophotometric method for simultaneous estimation of Pregabalin, methycobamin, and alpha lipoic acid in multicomponent dosage form. Int J Pharma Sci Res 2016 Jun 1;7(6):2458.

23. Kumari JN. Development and validation of new RP-HPLC method for simultaneous estimation of drug Duloxetine and Mecobalamin in tablet dosage form. Indian Journal of Research in Pharmacy and Biotechnology. 2016 Jul 1:4(4):169

24. https://www.drugbank.ca/drugs

25. https://www.pubchem.ncbi.nlm.nih.gov