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Simultaneous Estimation of Pregabalin and Methylcobalamin in a Marketed Formulation by using a Novel Gradient RP-HPLC Method

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Abstract: *The current research work describes a simple, precise and accurate gradient reverse phase-HPLC method for the estimation of Pregabalin and Methylcobalamin present in a marketed formulation. The chromatographic separation was optimized by gradient HPLC on a C_{18} Cosmosil column (25cm× 4.6mm i.d, 5µm particle size) with a mobile phase consisting of A: Potassium dihydrogen phosphate (pH 7.2 using Triethylamine) and B: Methanol (100%) with time gradient programme at a flow rate of 1ml/min. The column oven temperature was maintained at 25°C and injection volume was 30µl. The detector wavelength was 210 nm. The retention time of Pregabalin and methylcobolamin was found to be 4.792 min and 7.586 min respectively. The developed method was validated in terms of specificity, Accuracy, Precision, Linearity, robustness and forced degradation studies.*

Keywords: **Pregabalin, Methylcobalamin, Reverse phase-High performance liquid chromatography, Validation, Forced degradation.**

INTRODUCTION

Pregabalin (3S)-3-(aminomethyl)-5-methylhexanoic acid is an antiepileptic, anticonvulsant and neurotransmitter [1, 2]. This drug produces its actions by binding to the alpha2-delta (α2δ) subunit of the voltage-gated calcium channels and decreases the pain by modulating calcium channel activity of the nerve cells [5]. It is freely soluble in water both in acidic and basic aqueous solution [3]. Methylcobalamin is Coα-[α-(5,6-dimethylbenz-1H-imidazolyl)]-Coβmethylcobolamide a form of vitamin B (B12) which helps in the production of myelin, a substance that protects the nerve fibres and rejuvenates damaged nerve cells used in the treatment of trigeminal neuralgia, megaloplastic anaemia, diabetic neuropathy and facial paralysis in Bell's palsy syndrome [3, 4, 5].

The combination of Pregabalin and Methylcobalamin is prescribed for treatment of spinal cord injury-related neuropathic pain, fibromyalgia, preoperative pain, migraine, chronic pain and peripheral neuropathy [5, 6]. The literature survey reveals that only isocratic method has been developed for the estimation of Pregabalin and Methylcobalamin [3, 7]. No gradient reverse phase-high performance liquid chromatography method been reported. Thus the present work was carried out to develop a novel, accurate and precise gradient method, and to validate the method for simultaneous estimation of the two drugs.

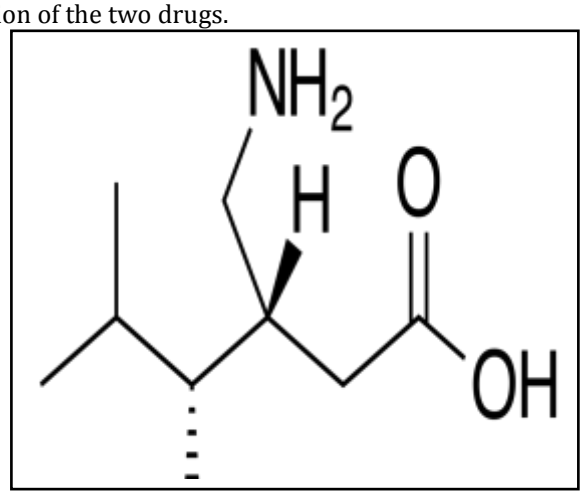

Fig. 1: Chemical structure of Pregabalin [7]

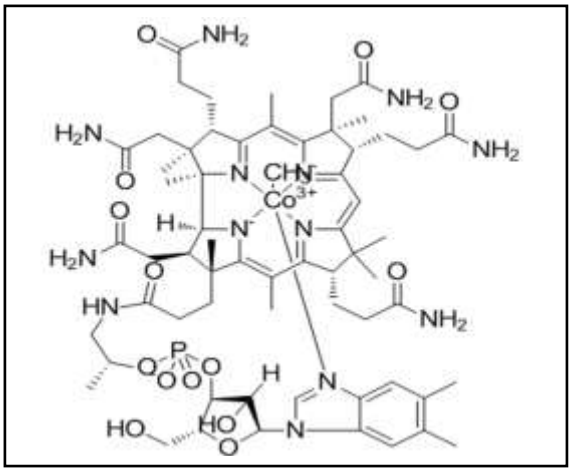

Fig. 2: Chemical structure of Methylcobalamin [7]

MATERIALS AND METHODS

Pregabalin was procured as a gift sample from Intas Pharmaceuticals Mumbai and Methylcobolamin was obtained from Piramal Enterprises Limited Mumbai. Marketed dosage form Pregeb M was purchased from a local pharmacy. Methanol (HPLC grade), Triethylamine, Potassium dihydrogen phosphate, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and Milli Q water were provided by Oriental College of Pharmacy (Navi Mumbai).

Instrumentation

RP-HPLC Shimadzu LC Prominence-i 2030 model, Lab Solution software and C_{18} Cosmosil column (250mm× 4.6mm i.d, 5µm particle size) were used for the method development and validation, Kroma Tech (KL-1.5) sonicator was used for sonication, PH meter (EQIUP-TRONICS), Digital balance (Conitech) and hot air oven EXPO HI-TECH were used.

Chromatographic conditions

The separation of Pregabalin and Methylcobalamin was carried out on RP-HPLC Shimadzu LC Prominence-i 2030 model with Cosmosil C_{18} (250mm× 4.6mm i.d, 5µm particle size) column. Mobile phase was A: potassium dihydrogen phosphate buffer (10mmol, PH 7.2 using Triethylamine) and B: Methanol in gradient manner (shown in Table 1) at a flow rate of 1ml/min, injection volume was 30µl, column temperature was maintained at 25°C and Pregabalin, Methylcobalamin were detected at 210 nm using an ultraviolet (UV) visible detector.

Table 1. Gradient programme

Time	Mobile Phase A (%)	Mobile Phase B (%)
0.01	60	40
2.00	60	40
10.00	40	60
12.00	40	60
17.00	60	40
20.00	60	40

Selection of wavelength

Wavelength selected was 210 nm after doing thorough literature survey [1, 3, and 7].

Preparation of standard solution

Standard stock solution was prepared by dissolving 100 mg of Pregabalin into a 50 ml volumetric flask about 30 ml of diluent was added, sonicated for 5 mins and volume was adjusted to the mark with diluent.

Further 25 ml of above solution was taken into a 50 ml volumetric flask.

5 mg of Methylcobalamin was dissolved into a 50 ml volumetric flask about 30 ml of diluent was added, sonicated for 5 mins and volume was adjusted to the mark with diluent.

Further 5 ml of the above solution was taken into that same 50 ml volumetric flask about 30 ml of diluent was added, sonicated for 5 mins and then volume was adjusted with the diluent.

Preparation of sample solution

10 capsules were emptied and equivalent powder weight was dissolved in a 50 ml volumetric flask about 30 ml of diluent was added and then sonicated for 10-15 mins volume was adjusted to the mark with diluent, sonicated and filtered through 0.45µ filter.

Method validation

The developed method for Pregabalin and Methylcobalamin was validated for parameters such as system suitability, specificity, linearity, precision, accuracy, robustness and solution stability as per ICH guidelines [8-11].

Forced degradation studies

Forced degradation is the degradation of drug substance and drug product [12]. It is the method of subjecting drug compounds to intense chemical and environmental conditions to find out product breakdown levels, preliminary degradation kinetics, and to identify potential degradation products [13] which consecutively helps in the development of formulation and package.

For acid and alkali stress condition, 5ml of 1N HCl and 1N NaOH were added respectively to the sample solution and kept for 2 hr, for oxidative degradation 5ml of 30% H_2O_2 was added and kept for 2 hr and thermal degradation was performed by keeping the sample solution in a petri dish at 60°C in oven for 2 hr.

RESULTS AND DISCUSSION

Method development

A series of trials were performed using different mobile phases such as potassium dihydrogen phosphate buffer (pH 4.5): methanol, potassium dihydrogen phosphate: methanol in gradient mode using different columns such as Prontosil and Shim-pack C18 to develop a RP-HPLC method for simultaneous estimation of Pregabalin and Methylcobalamin in a marketed dosage form. Finally a typical chromatogram was obtained using A: potassium dihydrogen phosphate (pH 7.2) and B: Methanol in gradient manner as mobile phase on Cosmosil C_{18} column (250mm× 4.6mm i.d, 5µm particle size) with injection volume of 30µl at flow rate of 1ml/min. The column temperature was 25°C and detection was carried out at 210 nm. The retention time was 4.792 min and 7.586 min for Pregabalin and Methylcobalamin respectively. Typical chromatograms of standard and sample solution of Pregabalin and Methylcobalamin are shown in Fig. 3 and 4. The same developed method was applied for forced degradation studies of Pregabalin and Methylcobalamin marketed dosage form. The optimized chromatographic conditions are given in Table 2.

Table 2: Optimized chromatographic conditions for Pregabalin and Methylcobalamin

Parameters	Optimized conditions
Column	Cosmosil C_{18} (250mm×4.6mm i.d, 5µm particle size)
Mobile phase	potassium dihydrogen phosphate : Methanol (in gradient manner)
Diluent	Water
Column temperature	25°C
Wavelength	210 nm
Flow rate	1ml/min
Injection volume	30µl
Run time	20 min
Retention time	4.792 min and 7.586 min

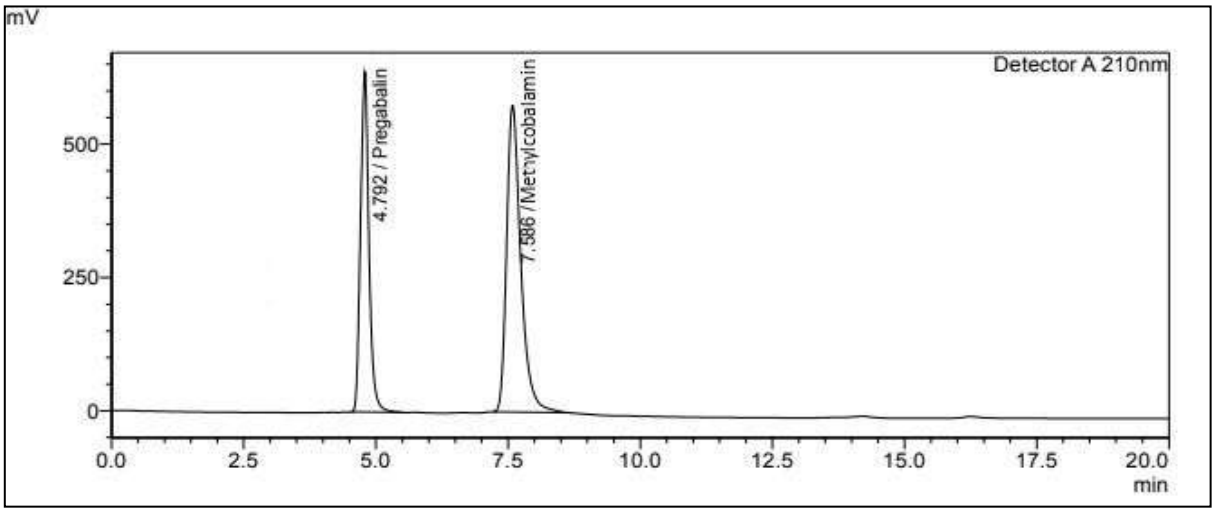

Fig. 3: Typical chromatogram of standard Mixture of Pregabalin and Methylcobalamin

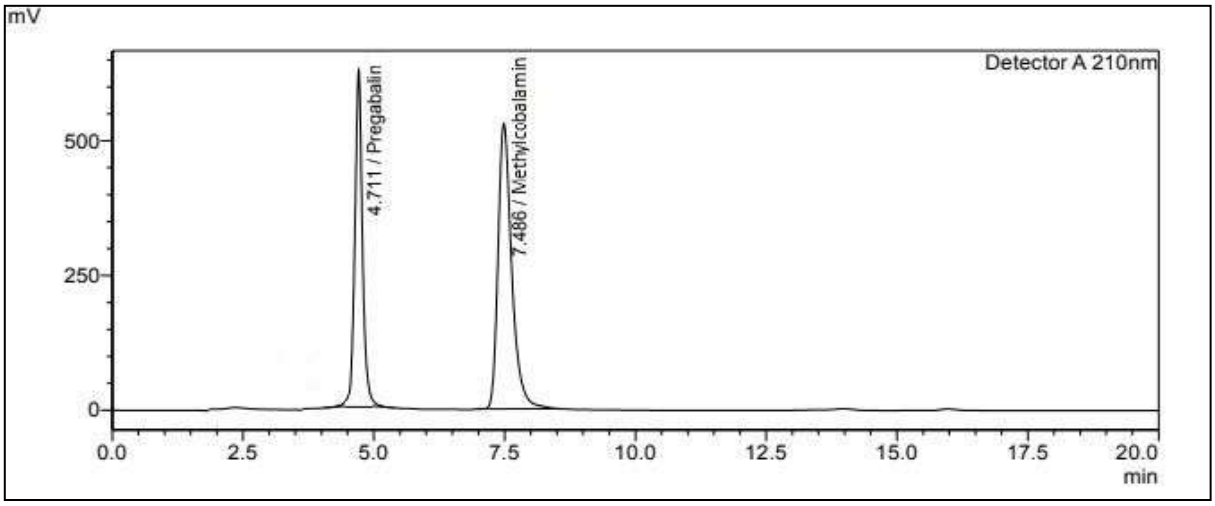

Fig. 4: Typical chromatogram of a sample of Pregabalin and Methylcobalamin

System suitability

System suitability was done by injecting six replicate injections of the standard solution and retention time, tailing factor and number of theoretical plate were evaluated. The standard solution of Pregabalin and Methylcobalamin was prepared as per the above method and injected. All the results of system suitability parameters are tabulated in Table 3 which is within the limits.

Table 3. System suitability parameters

Parameters	Pregabalin	Methylcobalamin
Retention time	4.792	7.586
Tailing factor	1.110	1.390
Number of theoretical plate	5028	4154

Precision

Precision is defined as the variability among replicate measurements, i.e., how close the values are to each other [14].

The system precision and method precision were performed by injecting six injections of Pregabalin and Methylcobalamin standard and sample of the same concentration. The percentage relative standard deviation (%RSD) was calculated from the chromatogram area and was <2%. From precision result, it was found that the method is precise. The results of system and method precision are tabulated in Table 4 & 5.

Table 4. System precision results

Sr. No.	Peak area	
	Pregabalin	Methylcobalamin
1	6989111	10994231
2	6985124	10983256
3	6965841	10826541
4	6995369	11032548
5	6987985	11015632
6	6952163	10923698
Average	6979266	10962651
SD	16621.5	76364
%RSD	0.23%	0.69%

Table 5. Method precision results

Sr. No.	% Assay	
	Pregabalin	Methylcobalamin
1	98.90	99.99
2	99.26	99.12
3	98.99	99.89
4	99.41	99.76
5	99.30	100.97
6	98.80	99.68
Average	99.10	99.67
SD	0.23	0.56
%RSD	0.23	0.56

Linearity

The linearity of a method is a check of how well a calibration plot of response vs. concentration estimates a straight line. Linearity can be determined by performing single measurements at several analyte concentrations [15].

The linearity of the developed method was determined at different concentration levels ranging from 80-120% for Pregabalin and Methylcobalamin. The linearity curve was constructed by plotting peak area versus concentration and the regression coefficient (r^2) was found to be 0.9999 for Pregabalin and 0.9991 for Methylcobalamin. From linearity results, it was found that the developed method is linear (Fig. 4 and Fig. 5). Results are shown in Table 6.

Table 6. Linearity results for Pregabalin and Methylcobalamin

Sr. No.	Concentration level (%)	Peak Areas	
		Pregabalin	Methylcobalamin
1.	80	5588548	8736542
2.	90	6289990	9839131
3.	100	6989106	10994133
4.	110	7689454	12051668
5.	120	8368106	13258250
Coefficient correlation (r^2)		0.9999	0.9991

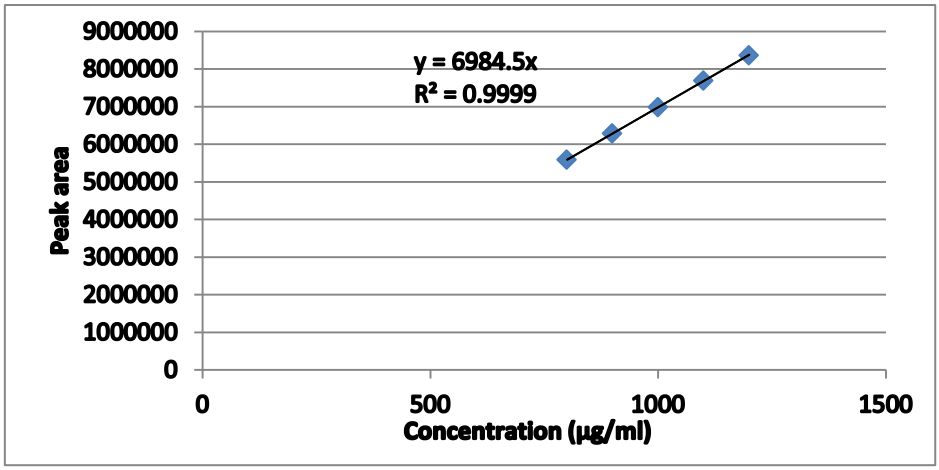

Fig. 5: Linearity graph of Pregabalin

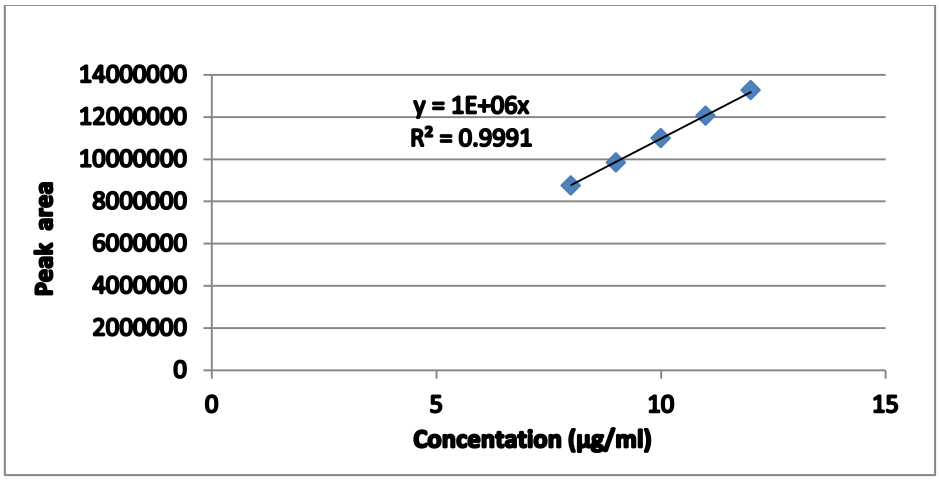

Fig. 6: Linearity graph of Methylcobalamin

Accuracy

Accuracy shows the nearness of agreement between the value which is received either as a traditional true value or an accepted reference value and the value found [16].

The accuracy of Pregabalin and Methylcobalamin was performed by calculating recovery studies of the sample at three different levels (80%, 100%, and 120%). At each level three replicates were injected into the chromatographic system and the mean percentage recovery for Pregabalin and Methylcobalamin was found within a limit of 98-102% and from this result, it was found that the developed method is accurate and results are tabulated in Table 7 and 8.

Table 7. % Recovery studies for Pregabalin

Level	% recovery	Average	SD	%RSD
80%	99.12 98.40 98.78	98.76	0.36	0.36
100%	99.26 99.02 99.12	99.15	0.12	0.12
120%	99.03 99.16 98.34	98.84	0.44	0.44

Table 8. % Recovery studies for Methylcobalamin

Level	% recovery	Average	SD	%RSD
80%	99.66 99.77 99.56	99.66	0.10	0.10
100%	99.78 99.87 99.86	99.50	0.55	0.55
120%	98.98 99.51 100.42	99.63	0.72	0.72

Robustness

It is regarded as the level of ability of an analytical technique, to remain similar by minute deliberate changes in the technique parameter. The different technique parameters which can be modified in High-Performance liquid chromatography are pH, flow rate, temperature of the column, and mobile phase composition [17].

The developed method was evaluated for robustness by small deliberate changes in optimized method parameters such as flow rate (±0.2ml), and temperature (±2 ° C). It was found that none of the parameters could cause an alteration in the peak area and retention time. The %RSD was found to be within limits, and the method was robust as shown in Table 9.

Table 9. Robustness results

Parameters	Proposed	Variation	Pregabalin	Methylcobalamin
			%Assay	
Flow rate	1.0 ml/min	0.8 ml/min	99.32	99.60
		1.2 ml/min	99.21	99.42
Temperature	25°C	23°C	99.32	99.36
		27°C	98.21	99.18

Specificity:

As per the official guideline to be applied for method validation ICH Q2 (R1), specificity is defined as:

"The ability to measure clearly the analyte in the presence of components which may be expected to be present" [18]

The method was found to be specific as all of the peaks were sharp, well separated and no other interference was found.

Assay of marketed formulation

For assay of marketed formulation (Pregeb M: 75 mg Pregabalin and 750 mcg Methylcobalamin) 10 capsules were emptied and equivalent powder weight was dissolved in a 50 ml volumetric flask about 30 ml of diluent was added and then sonicated for 10-15 mins volume was adjusted to the mark with diluent, sonicated and filtered through 0.45µ filter.

The percentage assay for the marketed formulation was found to be 100.74% and 99.42% for Pregabalin and Methylcobalamin respectively shown in Table 10.

Table 10. %Assay of marketed formulation

Tablet	Drug	%Assay
Pregeb M	Pregabalin	100.74
	Methylcobalamin	99.42

Solution stability

Sample solution of Pregabalin and Methylcobalamin was injected at different time intervals and percentage assay was calculated. It was found that solution was stable over a period of 48 hr without any degradation of the solution.

Stability results are shown in Table 11.

Table 11. Solution stability results

Time interval	%Assay	
	Pregabalin	Methylcobalamin
Initial	99.35	99.21
24 hr	99.32	99.32
48 hr	98.01	100.05

Forced degradation studies

Forced degradation studies were performed on marketed tablet formulation by treating the marketed formulation under stress conditions such as acidic, alkaline, oxidative and thermal conditions. The degradation products are shown in Fig 7-10.

The forced degradation results are tabulated in Table 12.

Acid degradation

In acid degradation condition (1N HCl), both Pregabalin and Methylcobalamin degraded completely and a peak was observed at 2.891 min in chromatogram (Fig. 7).

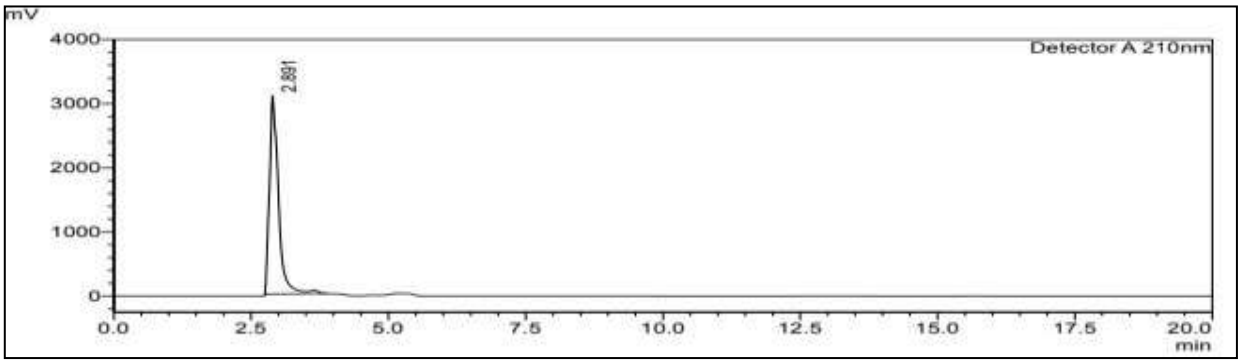

Fig. 7: Chromatogram of acid degradation

Base degradation

In alkali degradation condition (1N NaOH), Methylcobalamin completely degraded and percentage for degradation of Pregbalin was found to be 2.58%. Peaks of degradation were also seen in chromatogram (Fig. 8)

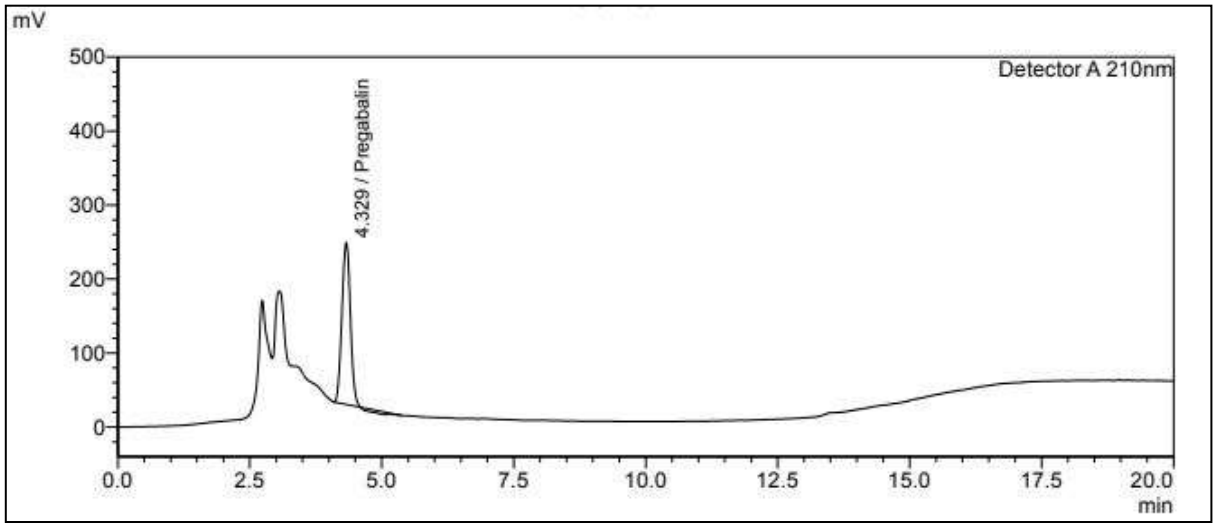

Fig. 8: Chromatogram of alkali degradation

Thermal degradation

In thermal degradation, both Pregabalin and Methylcobalamin did not degrade and percentage degradation was found to be 2.29% and 0.15% for Pregabalin and Methylcobalamin respectively. No peak of degradation was observed in the chromatogram (Fig. 9)

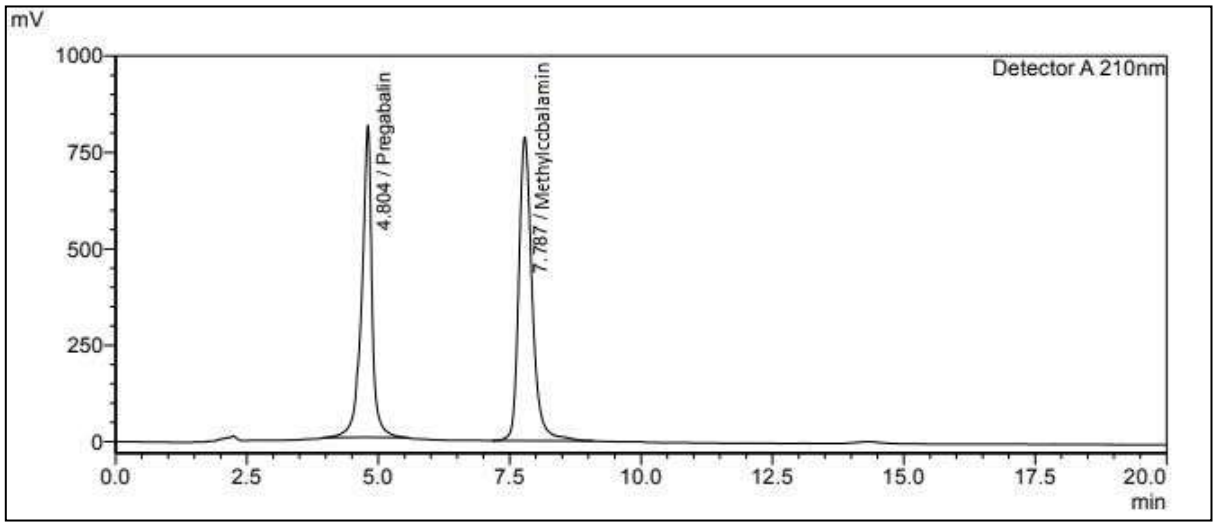

Fig. 9: Chromatogram of thermal degradation

Oxidative degradation

In oxidative degradation condition (30% H_2O_2), both Pregabalin and Methylcobalamin showed degradation. The degradant peak was observed at 2.896 min in chromatogram (Fig. 10)

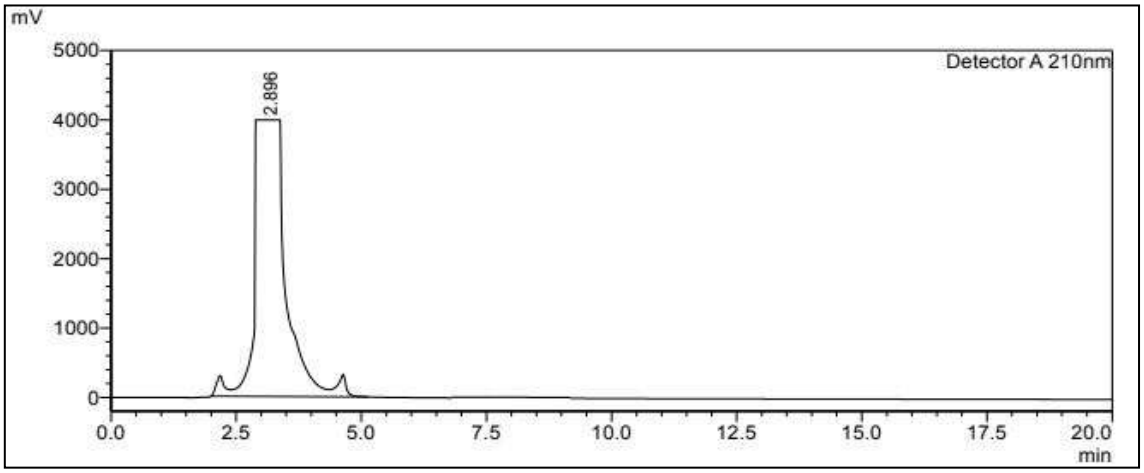

Fig. 10: Chromatogram of oxidative degradation

Table 12. Forced degradation results

Conditions	% Assay	
	Pregabalin	Methylcobalamin
Control sample	100.74	99.42
Acid treated sample	0.0	0.0
Base treated sample	98.16	0.0
Heat treated sample	98.45	99.27
Peroxide treated sample	0.0	0.0

CONCLUSION

A novel gradient RP-HPLC method for simultaneous estimation has been developed and validated for Pregabalin and Methylcobalamin in bulk and pharmaceutical dosage form. The proposed RP-HPLC method allows for precise, accurate, and reliable estimation of drugs simultaneously in combined dosage form in a gradient manner. The RSD for all

parameters was found to be within limits, the developed method can be used for routine quantitative simultaneous estimation of Pregabalin and Methylcobalamin in pharmaceutical preparation.

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AUTHOR'S CONTRIBUTION

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors claim that there are no conflicts of interest regarding the publication of this article.

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