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Spectrophotometric Determination of Pregabalin Using Gibb's and MBTH reagent in Pharmaceutical Dosage Form

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ABSTRACT

Two simple, extractions free spectrophotometric methods (Method 1 and 2) for the quantitative estimation of Pregabalin (PGB) in bulk drugs and pharmaceutical formulations (capsules) have been developed. Method 1 is based on the formation of a colored oxidative coupling product between 2, 6-dichloroquinone chlorimide and the drug. Colored product at its λmax 400 nm shows linearity in the concentration range of 50-350µg/ml. Second method is based on reaction of oxidative coupling of Pregabalin with 3-methyl-2-benzthiazolinone hydrazone (MBTH) to form green colored product. Colored product of λmax 668 nm shows linearity in the concentration range of 50-350 µg/ml.The relative standard deviations for first method is 0.36% and for second method is 0.35% were obtained. Linear relationships with good correlation coefficients (0.998-0.999) were found between absorbance and the corresponding concentrations of the drug. The reliability and performance of the proposed methods was validated statistically. The percentage recovery ranged from 100.42% and 99.69% respectively.

Keywords: Spectrophotometric, Pregabalin, oxidative coupling, 2, 6-Dichloroquinone-4-chloroimide (gibb's), 3-methyl-2-benzthiazolinone hydrazone (MBTH).

INTRODUCTION

Pregabalin (PGB) [S-[+]-3-isobutyl GABA or (S)-3-(amino methyl)-5-methylhexanoic acid, is an anticonvulsant and analgesic medication that is both structurally and pharmacologically related to gabapentin. It was recently approved for adjunctive treatment of partial seizures in adults [1-3] in both the United States and Europe and for the treatment of neuropathic pain from post therapeutic neuralgia and diabetic neuropathy. The compound was originally synthesized with the hope of modulating brain GABA receptors or GABA synthetic enzymes. These compounds are inactive at $GABA_A$ and $GABA_B$ receptors. The mechanism of action of

pregabalin has been characterized only partially, and in particular, the cellular and molecular details of its action to reduce neurotransmitter release are incompletely known.

Literature survey reveals several methods for quantitative analysis of PGB. The methods adapted to the analysis of PGB include high–performance liquid chromatography (HPLC) [4-7], spectrofluorimetry [8], LC-MS-MS [9-11]. The determination in biological fluids normally requires the use of trace analysis techniques such as HPLC, LC, capillary electrophoresis (CE), cyclic voltametry, LC-MS, gas chromatography– mass spectrophotometry (GC-MS), inductively coupled plasma-mass spectrophotometry. These methods require long and tedious pre-treatment of the samples and laborious clean up procedures prior to analysis. An official monograph of PGB does not exist in any pharmacopoeia and determination of PGB in bulk and pharmaceutical formulations has not been yet described. A through literature search has revealed that only few spectrophotometric methods [12-14] available for determination of pregabalin in bulk drugs and pharmaceutical formulations. So there is a lot of scope for development of simple and suitable analytical spectrophotometric method for the determination of PGB in bulk and pharmaceutical formulations. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity.

2,6-Dichloroquinone-chlorimide and MBTH have been used as chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines (15-18). However, the reaction of Gibb's reagent and MBTH with PGB has not been investigated so far. The present study describes the evaluation of Gibbs and MBTH as chromogenic reagents in the development of simple and rapid spectrophotometric method for the determination of PGB in its pharmaceutical dosage forms

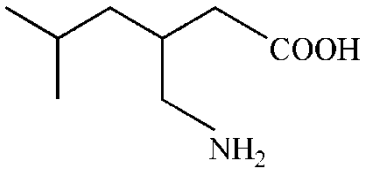


Fig 1: Chemical structure of Pregabalin

MATERIALS AND METHODS

Equipment

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

Reagents and solutions

All employed chemicals were of analytical grade and high-purified water was used throughout. Pregabalin pure sample was obtained as a gift sample from Kanvista formulations, Hyderabad, India.

2, 6-Dichloroquinone-chlorimide 0.5 %(w/v):

0.5 g of Gibb's reagent was accurately weighed, transferred into a 100 ml calibrated volumetric flask, dissolved in 10 ml of methanol, and made up the volume to the mark with

methanol to obtain a solution of 0.5% (w/v). The solution was freshly prepared and protected from light.

3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) 0.5 %(w/v)
0.5g of MBTH reagent was accurately weighed transferred into a 100 ml calibrated volumetric flask, dissolved in distilled water, and made up the volume to the mark to obtain a solution of 0.5% (w/v).

Ferric chloride (1%)
It was prepared by dissolving 1g of ferric chloride in 100 ml of distilled water.

Standard solutions:
In this method pregabalin stock solution (1000µg/ml) was prepared by dissolving 100 mg of drug in 100 ml of methanol. Working solutions of the drug were prepared by dilution of the stock solution. The marketed capsule form of PGB used in the determination was pregeb 75 with a labelled strength of 75 mg and manufactured by Torrent Pharmaceuticals Limited, Mehsana, India.

Analytical Method Development
Method 1
Standard solutions of PGB in methanol having final concentrations in the range of 50-350µg/ml were transferred into a series of 10 ml volumetric flasks. To each flask, 1.5ml of 0.5% Gibbs reagent was added. The mixture was then kept aside for 5minutes and heated for 10 minutes. The contents were diluted up to 10 ml with methanol. The absorbance of each solution was measured at 400 nm against the reagent blank. Fig. 2 and Fig. 3 indicate the calibration curve and absorption spectra.

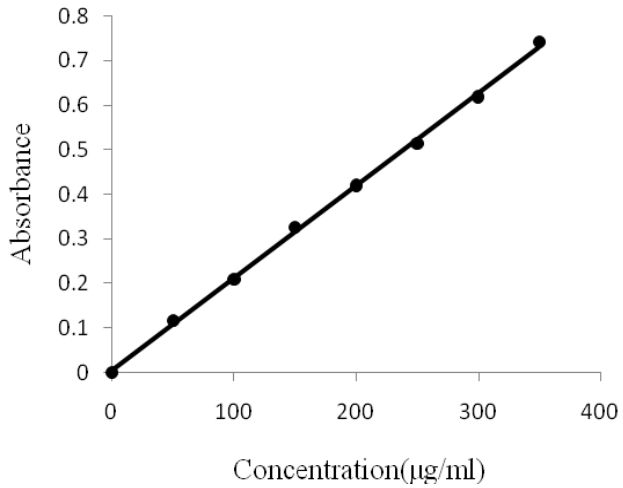


Fig .2. Calibration graph of PGB

Method 2
Standard solutions of PGB in methanol, having final concentrations in the range of 50-350 µg/ml were transferred into a series of 10 ml volumetric flasks. To each 2 ml of MBTH, 2 ml of ferric chloride was added and the volume was made up to mark with distilled water and allowed to stand for 20 minutes. The absorbance of each solution was measured at 668 nm

against the reagent blank. The colored species was stable for 2 hours and the amount of drug in the sample was computed from its calibration curve represented in Fig. 4. Absorption spectrum was represented in Fig. 5.

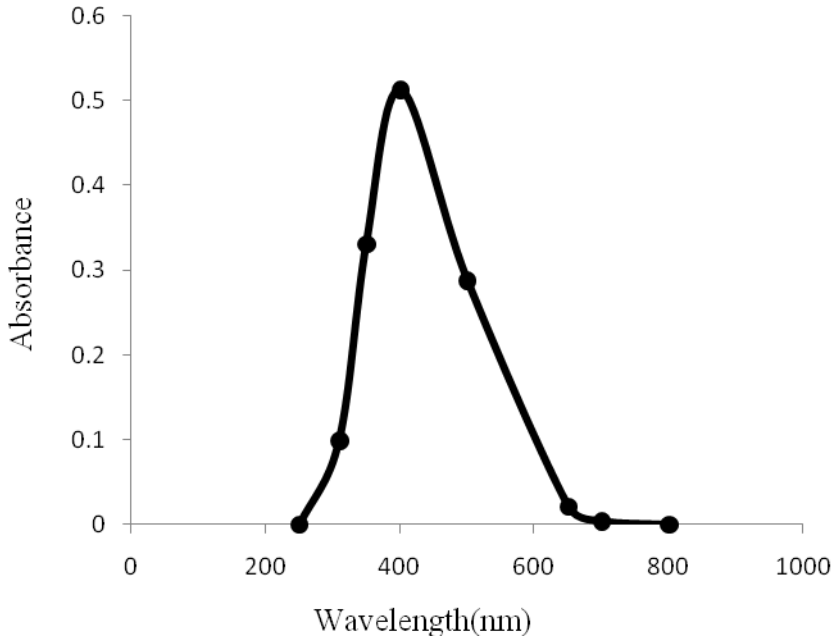


Fig .3. Absorption spectra of Gibb's with PGB against the reagent blank

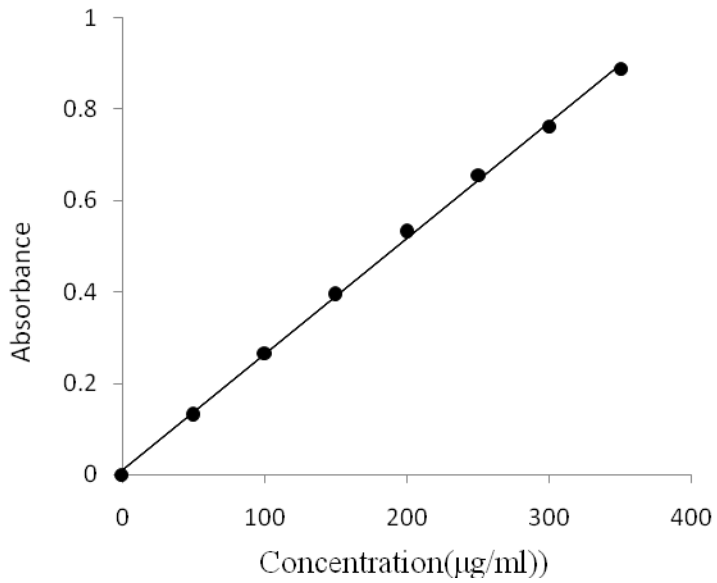


Fig .4. Calibration graph of PGB

Analysis of commercial pharmaceutical preparations

Method 1

Five capsules were weighed and their contents were mixed thoroughly. An accurately weighed portion of powder equivalent to 100 mg of PGB was weighed into a 100 ml

volumetric flask containing about 75 ml of methanol. It was shaken thoroughly for about 5-10 minutes, filtered through a whatman filter paper to remove insoluble matter and diluted to the mark with methanol to prepare 1000µg/ml solution. An aliquot of this solution was diluted with methanol to obtain a concentration of 50µg/ml. Then to that solution, 1 ml of gibb's reagent was added and gently shaken. The contents were diluted up to 10 ml with methanol.

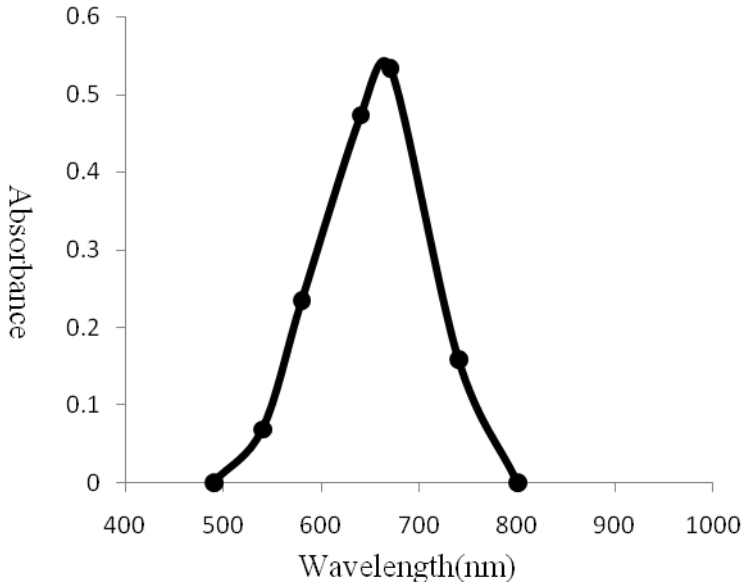


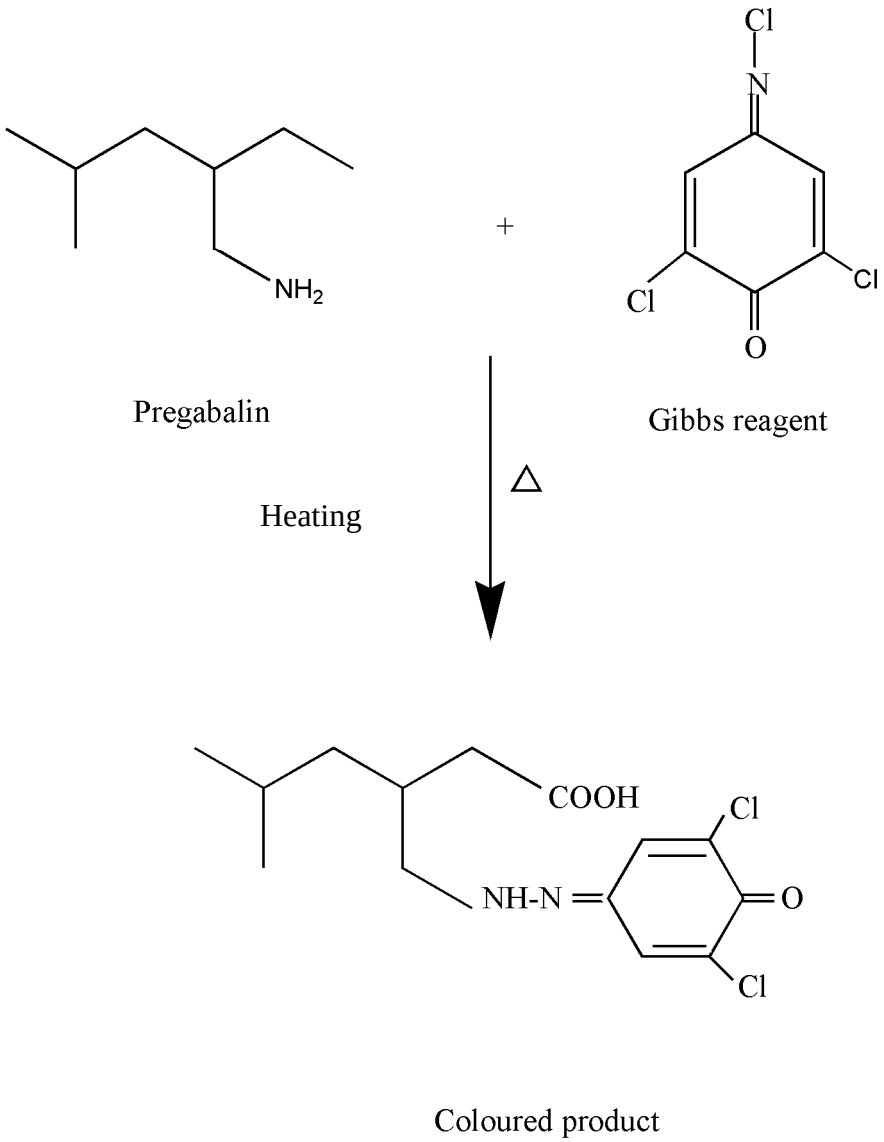
Fig .5. Absorption spectra of MBTH with PGB against the reagent blank

Method 2

Five capsules were weighed and their contents were mixed thoroughly. An accurately weighed portion of powder equivalent to the 100 mg of PGB was weighed into a 100 ml volumetric flask containing about 50 ml of methanol. It was shaken thoroughly for about 5-10 minutes, filter through a whatman filter paper to remove insoluble matter and diluted to the mark with methanol to prepare 1000µg/ml solution. An aliquot of this solution was diluted with water to obtain a concentration of 100µg/ml. Then to that solution 2 ml of MBTH and 2 ml of $FeCl_3$ is added. The mixture was then gently shaken and the appearance of green color occurs. The contents were diluted up to 10 ml with distilled water.

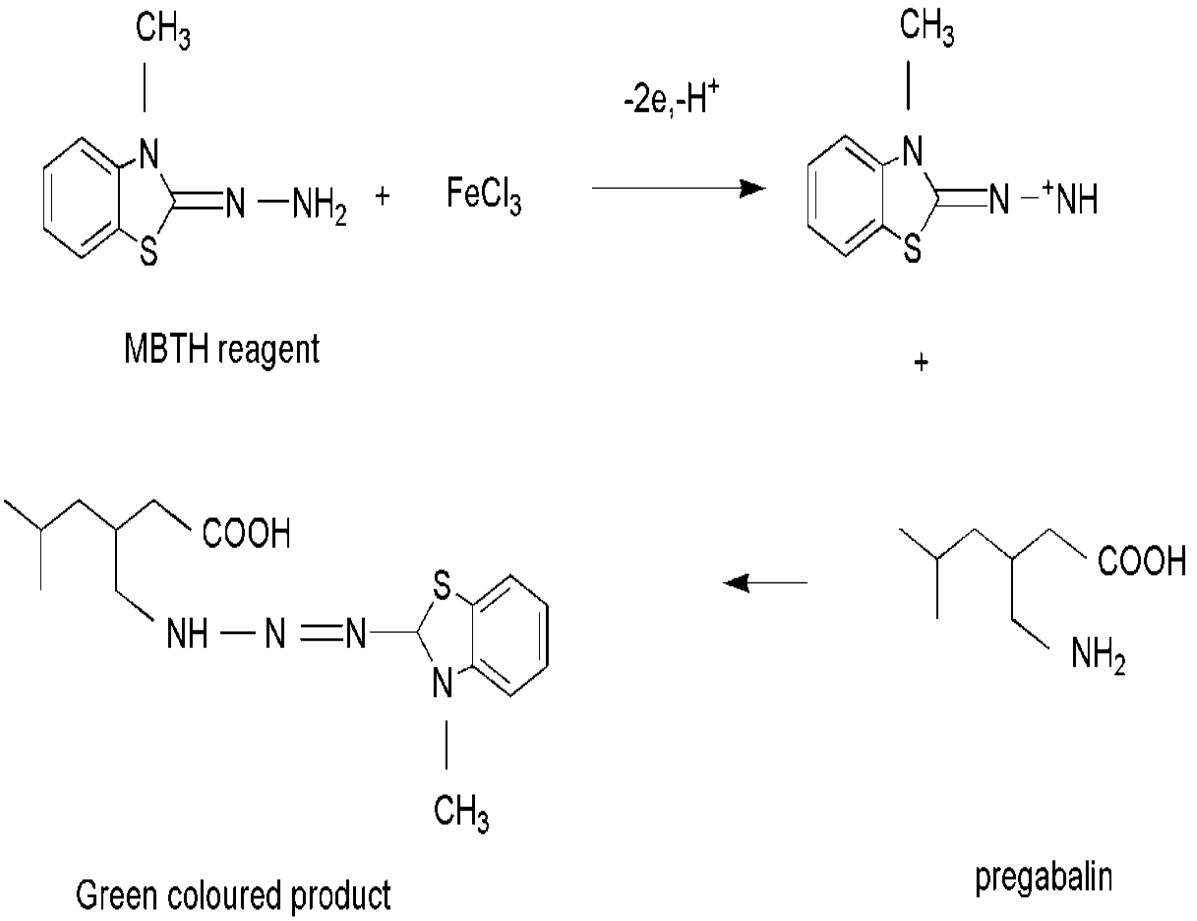
RESULT AND DISCUSSION

Oxidation of PGB was attempted in the present study for the development of spectrophotometric method for its determination. The first method is based on the reaction between the Gibb's reagent and PGB. The Gibb's reagent reacts with PGB and results in the formation of colored complex. The reaction between the gibbs reagent and pregabalin was represented in scheme 1. The reagent blank has negligible absorbance in the range used for detection of the PGB. Beer's law is obeyed in the range of 50–350 µg/ml (PGB).



Scheme 1: Reaction of Gibbs and pregabalin

In second method, the drug reacts with MBTH in the presence of $FeCl_3$ to give a green colored product. This is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH forms an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The reaction between the MBTH reagent and pregabalin was represented in scheme 2. Reproducible results were obtained in the temperature range of 20–40 °C. The reagent blank has negligible absorbance in the range used for detection of the PGB. Beer's law is obeyed in the range of 50-350µg/ml for PGB.



Scheme 2: Reaction of reagent and pregabalin

Optimisation of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimised via a number of preliminary experiments.

For method 1: The effect of the Gibb's reagent concentration was studied and found that 0.5% gave good absorbance values. So further experiments were carried out using 0.5 % of Gibb's reagent.

For method 2: The optimum conditions for the reaction were carefully studied. Maximum absorption at 668 nm was obtained immediately upon using 2 ml of 1% $FeCl_3$ and 2ml of 0.5% MBTH at ambient temperature and the product remained stable for 35 minutes.

Stability of the Chromogen:
Method 1:
Under the optimum conditions, the reaction between PGB and Gibb's reagent was completed within 10 minutes and the absorbance no longer changed after standing for up to 40 minutes. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remains stable for at least 4 hours. This allowed the processing of large batches of samples and their comfortable measurements with

convenience. This increased the convenience of the methods as well as made it applicable for large number of samples.

Method 2:
The reaction between PGB and MBTH completed within 20 minutes. The green color developed was found to be stable for long period and showed no change in the color intensity with time. This allowed the method to be followed for the intra-day studies.

Optimization of Parameter
The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. Different concentrations and volumes were tried for all the reagents, by varying the parameters at a time. For method 1 it was found that optimum concentration of Gibb's reagent was 0.5% w/v. The optimum volume was found to be 1.5 ml for Gibbs reagent. For method 2 it was found that optimum concentration of MBTH reagent was 0.5%w/v and optimum concentration of $FeCl_3$ was 1% w/v. The optimum volume was found to be 2 ml for MBTH and that of $FeCl_3$ was 2 ml.

Quantification
The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated and are given in Table 1. Regression analyses of the Beer's law plots at their respective λmax values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept and are described by the regression equation, $y = bx + c$ (where y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and x is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 1.

Table 1: Optical characteristics and validation data of pregabalin

Parameter	Values	
	Method 1:	**Method 2:**
λ_{max}/ nm	400 nm	668 nm
Beers law limits (µg/ml)	50-350	50-350
Molar absorptivity (1 /mol/cm)	3.693×10^{-4}	4.202×10^{-4}
Correlation coefficient (R)	0.999	0.998
Sandell's sensitivity(ng cm^{-2})	0.4310	0.378
Regression equation (y)	y = 0.0021x + 0.005	y = 0.0025x + 0.0096
Slope, *b*	0.0021	0.0025
Intercept, *c*	0.005	0.0096
Relative standard deviation%	0.36	0.35
Limit of detection (µg/ml)	2.457	2.3665
Limit of quantification(µg/ml)	7.448	7.1714
Interday RSD	0.38	0.78
Intraday RSD	0.32	0.5

$y = bx + c$, where x is the concentration of drug in µg/ml; Average of six determinations

Analytical Validation
The validity of the methods for the assay of PGB was examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard addition method. For this, known quantities of pure PGB were mixed

with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table 2 and 3. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

Linearity

To establish linearity of the proposed methods, a series of solutions of PGB for method 1 and 2 are (50-350µg/ml) were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

Precision

The precision of the method was determined by replicate analysis of six separate solutions of the working standards at two concentration levels of each drug. At two concentrations intraday and inter day precision studies were performed for two consecutive days. Relative standard deviation was calculated and was found to be 0.36 and 0.35 for method 1 and 2 respectively which indicates good precision of the proposed method.

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations with three serial dilutions were prepared from independent stock solutions which are having different concentrations. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug to a known preanalyzed formulation sample and the total concentration was determined using the proposed methods (Table 2 and 3)

The % recovery of the added pure drug was calculated as % recovery = [(*Ct–Cs*)/*Ca*] x 100,

Where,

Ct is the total drug concentration measured after standard addition;
Cs drug concentration in the formulation sample.
Ca, drug concentration added to formulation.

Table 2: Results of recovery study by standard addition method (method 1 and method 2)

Sl. No.	Standard Pregabalin (ml)	Standard Pregabalin (µg)	Sample Pregabalin (ml)	Sample Pregabalin (µg)	Absorbance at 403nm	Amount of Pregabalin from std. graph	Recovery of std (mg)	%Recovery
1	0.5	50	0.5	50	0.301	100.51	50.51	101.02%
2	1.0	100	0.5	50	0.430	201.23	101.23	101.23%
3	1.5	150	0.5	50	0.650	300.63	150.63	100.42%
1	0.5	50	0.5	50	0.203	99.6	24.5	99.2%
2	1.0	100	0.5	50	0.234	200.01	100.01	100.01%
3	1.5	150	0.5	50	0.321	299.8	149.8	99.86%

Robustness and ruggedness

Robustness was examined by evaluating the influence of a small variation of the method variables including the concentration of analytical reagent. It was found that small variations in these variables did not affect the method significantly. This was an indication of the

reliability of the proposed method during its routine application. The ruggedness was tested by applying the proposed method of analysis using the same operational conditions. Results obtained from inter-day RSD and intra-day RSD variations were found to be reproducible and are represented in the Table 1.

Table 3: Evaluation of accuracy and precision-(Method 1 and 2)

Drug	S.no	Label Claim (mg)	Amount found	% Purity	Average (%)	S.D	R.S. D[a]	RSD[b]	S.E.M
Pregeb 75 (Pregabalin)	1	75	73.254	97.672	98.821	0.0018	0.38	0.78	0.0018
	2		73.54	98.05					
	3		74.98	98.453					
	4		74.18	98.906					
	5		74.91	99.88					
	6		73.84	99.97					
Pregeb 75 (Pregabalin)	1	75	74.82	99.76	98.963	0.0032	0.32	0.50	0.0013
	2		73.66	98.21					
	3		74.16	98.8					
	4		74.34	99.12					
	5		73.98	98.64					
	6		74.44	99.25					

SD. Standard deviation; SEM. Standard error of mean; RSD.relative standard deviation;
a: intraday precision, b: interday precision.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for PGB by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, Where S is the slope of the calibration curve and σ is the standard deviation of *y*-intercept of regression equation. Results are represented in Table 1.

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of Pregabalin in pure form and in pharmaceutical preparations.

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