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Spectrophotometric determination of Pregabalin using 1, 2-Napthaquinone-4-sulfonic acid Sodium and 2, 4 dinitrophenyl hydrazine in pharmaceutical dosage form

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ABSTRACT

Two simple, extraction free spectrophotometric methods (Method-1 and 2) for the quantitative estimation of pregabalin (PGB) in bulk drugs and pharmaceutical formulations (Capsules) have been developed. The first method is based on the condensation of pregabalin with 1, 2-napthaquinone-4- sulfonic acid sodium (NQS) in alkaline media to yield orange colored products. Pregabalin at its λmax 485 nm shows linearity in the concentration range of 5-45µg/ml. The first method is based on the oxidation of 2, 4- dinitrophenylhydrazine (2, 4-DNP) and coupling of the oxidized product with drugs to give intensely colored chromogen. Condensed product of pregabalin at its λmax 461 nm shows linearity in the concentration range of 50-450 µg/ml. The relative standard deviations were 0.487% and 0.25% respectively for 2 methods. Linear relationships with good correlation coefficients (0.993-0.998) were found between absorbance and the corresponding concentrations of the drug. The reliability and performance of the proposed methods was validated statistically. Percentage recovery ranged from 100.5 and 99.867 respectively.

Keywords: spectrophotometric, Pregabalin (PGB), 1, 2- Napthaquinone 4-sulfonic acid sodium 2, 4-Dinitrophenyl hydrazine (2, 4-DNP).

INTRODUCTION

Pregabalin (PGB) [S-[+]-3-isobutyl GABA or (S)-3-(amino methyl)-5-methylhexanoic acid, Lyrica] 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.

PGB undergoes minimal metabolism in human with unchanged parent representing the majority (≥90 %) of drug-derived material. This contrasts with gabapentin, which is absorbed via a capacity limited L-amino acid transport system from the proximal small bowel into the blood stream.

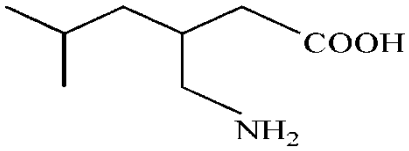


Fig1: Structure of pregabalin

Literature survey reveals that the methods adapted to the analysis of PGB include high–performance liquid chromatography (HPLC) [4-7], spectrofluorimetry [8], LC-MS-MS [9]. 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 [10, 11] 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. NQS [12-14] and 2, 4-DNP [15] have been used as chromogenic reagents for the spectrophotometric determination of many pharmaceutical amines and ketones. However, the reactions of NQS (method-1) 2, 4-DNP (method- 2) reagents with PGB have not been investigated so far. The present study describes the evaluation of NQS and 2, 4-DNP as chromogenic reagents in the development of simple and rapid spectrophotometric method for the determination PGB in its pharmaceutical dosage forms.

MATERIALS AND METHODS

Apparatus

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. PGB pure sample was obtained as a gift sample from Kanvista Formulations, Hyderabad, India.

Standard solutions

Pregabalin stock solution (1000 µg/ml) was prepared by dissolving 100mg of drug in 100 ml of distilled water. 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 labeled strength of 75 mg and manufactured by Torrent Pharmaceuticals Limited, Mehsana, India.

1, 2-Naphthoquinone-4-sulphonate (NQS) 0.5 %(w/v)
0.5 g of NQS was accurately weighed transferred into a 100 ml calibrated volumetric flask, dissolved in 5 ml distilled water, and made up the volume with distilled water to obtain a solution of 0.5% (w/v). The solution was freshly prepared and protected from light during use.

0.01 N Sodium hydroxide solutions
0.2 g of sodium hydroxide is accurately weighed and transferred into a 500 ml volumetric flask and made up to the mark with distilled water.

2, 4-Dinitrophenyl hydrazine (2, 4-DNP) 0.08 %(w/v)
A 0.08% w/v of the reagent solution was freshly prepared by dissolving 0.08 g of 2, 4-DNP in 2 ml of concentrated sulphuric acid and diluting to 100 ml with water.

10N Sodium hydroxide solution.
40 g of sodium hydroxide dissolved in 100 ml of distilled water.

Potassium iodate 4 % (w/v).
A 4% w/v potassium iodate solution was prepared by dissolving 4 g in 100 ml of distilled water.

Preparation of calibration curve
Method 1
Standard solutions of PGB in water, having final concentrations in the range of 5-50 µg/ml was transferred into a series of 10 ml volumetric flasks. To these solutions, 1 ml of 0.01N sodium hydroxide is added, 1 ml of 0.5% NQS is added. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted up to 10 ml with distilled water. The absorbance of each solution was measured at 485 nm against the reagent blank and the calibration curve and absorption spectra are represented Figure.2 and Figure.3

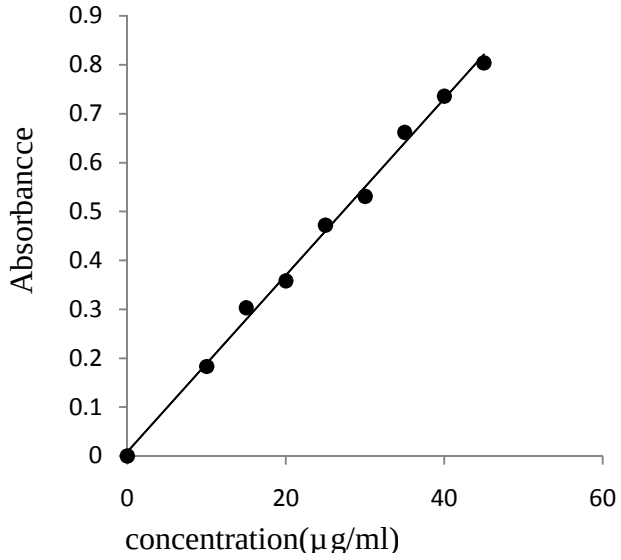


Fig 2: Calibration graph of Pregabalin (PGB)

Method 2
Standard solutions of PGB in water, having final concentrations in the range of 50-450 µg/ml was transferred into a series of 10 ml volumetric flasks, to these solutions 1.5 ml of 2, 4-DNP

(0.08%) and 1.5 ml of potassium iodate were added, which were made alkaline by adding 1 ml each of sodium hydroxide. The red colored solution hence was further diluted to the volume with water. The absorbance of each solution was measured at 461 nm against the reagent blank and the calibration curve and absorption spectra are represented figure.4 and figure.5

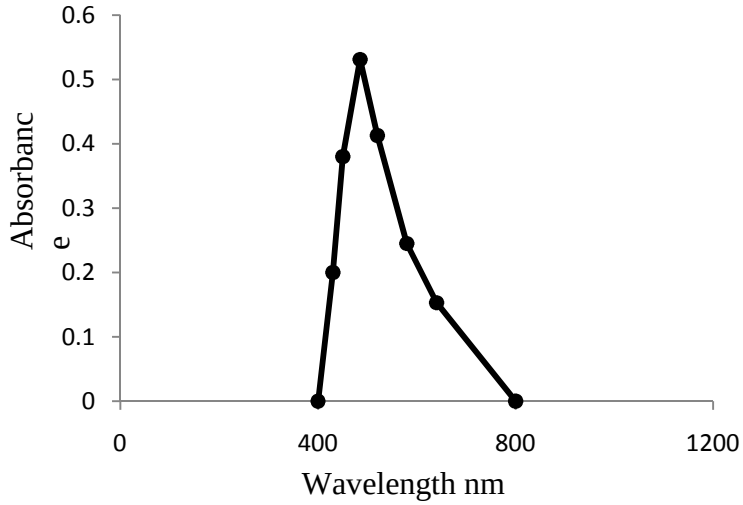


Fig3: absorption spectra of NQS with PGB against the reagent blank

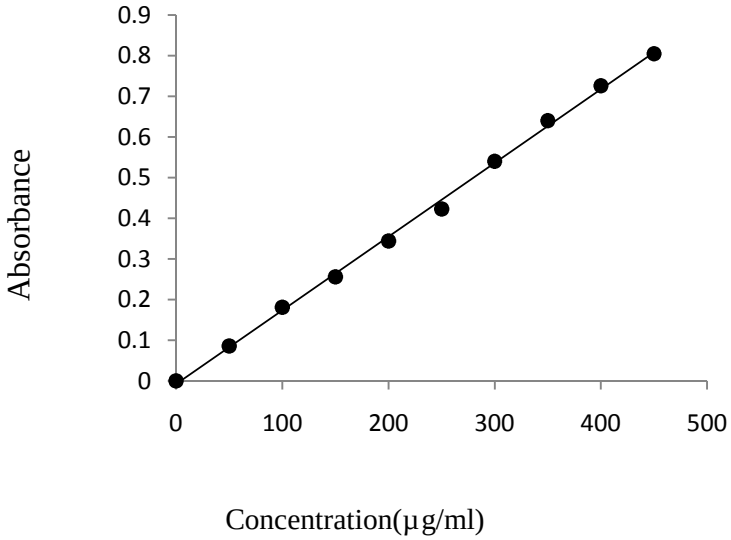


Fig 4: Calibration graph of PGB with 2, 4-DNP

Procedure for pharmaceutical formulations

Method 1

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 75 ml of distilled water. It was shaken thoroughly for about 5-10 min, filtered thorough whatman filter paper to remove insoluble matter and diluted to the mark with distilled water to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain a concentration of 40 µg/ml. Then to that solution 1 ml of 0.01N sodium hydroxide was added, 1 ml of 0.5% NQS was added. The mixture was then gently shaken until the appearance of orange color. The contents were diluted up to 10 ml with distilled water.

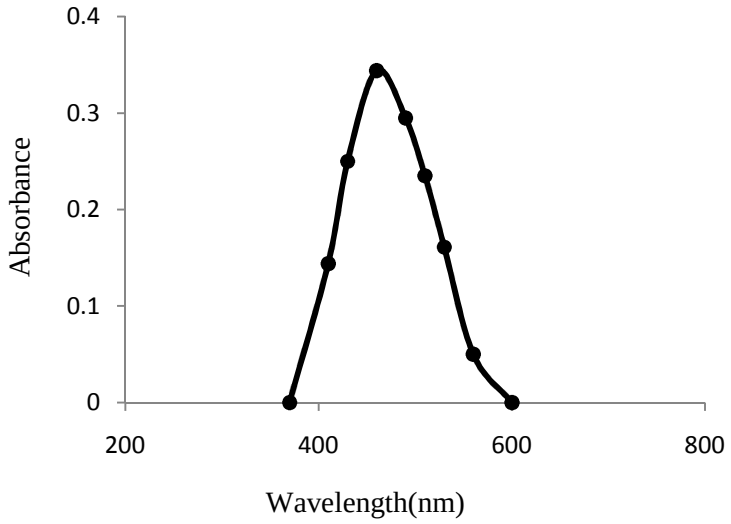


Fig5: absorption spectra of 2, 4- DNP with PGB against the reagent blank

Method 2

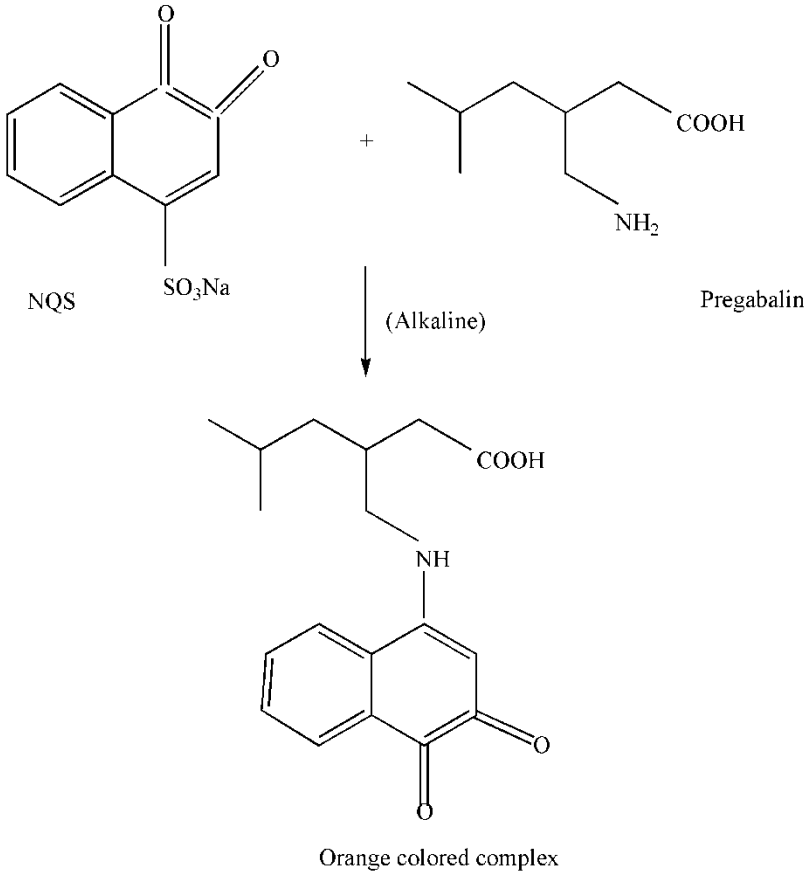
Five capsules were weighed and their contents were mixed thoroughly. An accurately weighed portion of powder equivalent to the 100 mg of PGB was weighed into a 100 ml volumetric flask containing about 75 ml of distilled water. It was shaken thoroughly for about 5-10 min, filtered thorough whatman filter paper to remove insoluble matter and diluted to the mark with distilled water to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain a concentration of 100 µg/ml. Then to that solution 1.5 ml of 2, 4-DNP was added, and 1.5 ml of Potassium iodate and 1 ml of 10N sodium hydroxide were added. The mixture was then gently shaken until the appearance of red color. The contents were diluted up to 10 ml with distilled water.

RESULTS AND DISCUSSION

The first method is based on the reaction between the NQS and pregabalin. The NQS reagent reacts with PGB at the free NH_2 group represented in scheme 1. The reagent blank has negligible absorbance in the range used for detection of the PGB. In the present investigation, NQS reagent forms colored complexes with PGB in alkaline conditions and their absorbances were measured at 485. Because of the presence of amine as chromophoric group in the PGB molecule, derivatization of the compound was attempted with NQS as a result colored complex has been formed which was estimated spectrophotometrically

The absorption spectra of the reaction product of oxidized 2, 4-DNP with drug show maximum absorption (λmax) at 461 nm and the reaction between 2, 4-DNP and PGB was represented in scheme 2 and . The blank solution was slightly red in color that had negligible absorbance at the λmax in which the drug was analysed. The formed color was stable for more than two hours. A temperature range of 20-30 ^{0}C is preferred for the reaction. 2, 4-DNP is oxidized by potassium iodate to give diazonium cation which reacts with drug by electrophilic substitution to give deep colored chromogen. Beer's law is obeyed in the range of 50-450 µg/ml for PGB.

Scheme 1: reaction between NQS and PGB



Optimization of parameter

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. The studying of NQS concentrations revealed that the reaction was dependent on NQS reagent. The absorbance of the reaction solution increased as the NQS concentration increased, and the highest absorption intensity was attained at NQS concentration of 0.25 % (w/v). Higher NQS concentrations up to 1.25 % had no effect on the absorption values. Further experiments were carried out using 0.5 %. The optimum volume of NQS was found to be 1ml and the optimium volume NaOH was found to be 1ml. For method 2 it was found that optimum concentration of potassium iodate was 4% *w/v* and optimum concentration of 2, 4-DNP was 0.08% w/v and optimum concentration of sodium hydroxide was 10N. The optimum volume was found to be 1.5 ml for potassium iodate and that of 2, 4-DNP was 1.5 ml and sodium hydroxide was 1 ml.

Stability of the Chromogen

Method 1

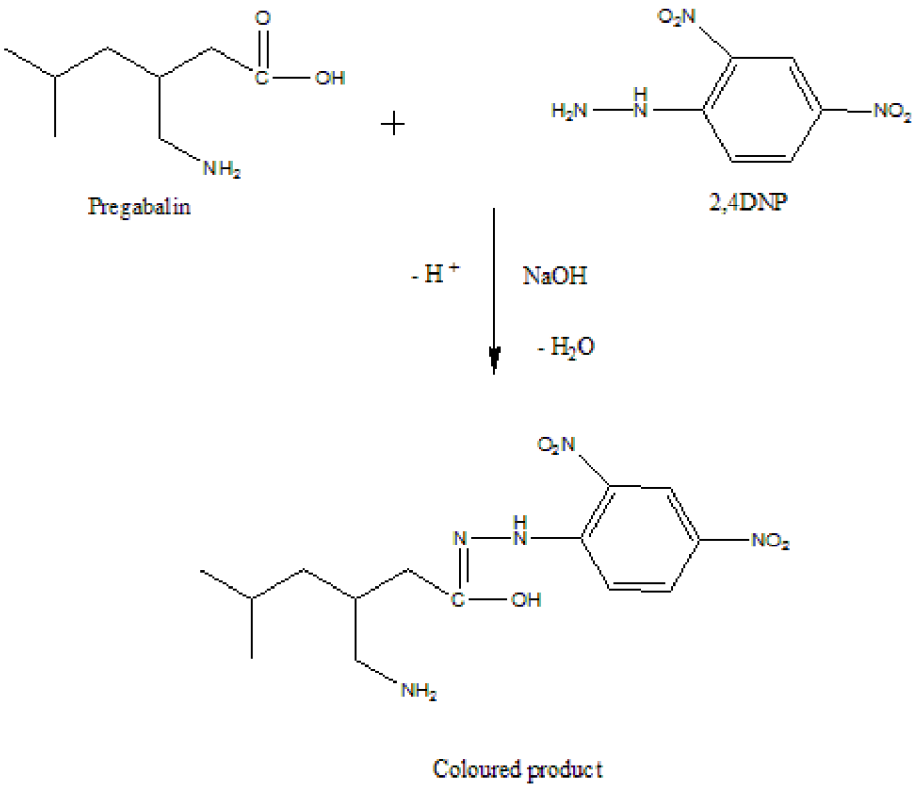
Optimum reaction time was evaluated by monitoring the colour development at room temperature. It was observed that the reaction got stabilized within 5 minutes. The developed colour remained stable at room temperature for about a further 1hr.

Method 2

Under the optimum conditions, the reaction between PGB and 2, 4-DNP was completed within 2 minutes at room temperature 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 1 hour. This increased the convenience of the methods as well as made it applicable for large number of sample.

Scheme 2: reaction between 2,4- DNP and PGB



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.The 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.

Validation of the method

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 calibration curve 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.

Table 1. Optical Characteristics and Statistical Data for the Regression Equation of the Proposed Methods

Parameter	Values	
	Method 1:	**Method 2:**
λ_{max}/ nm	485 nm	461 nm
Beers law limits (µg/ml)	5-45	50-450
Molar absorptivity (1 /mol/cm)	$0.17834x10^{-02}$	0.273824×10^{-3}
Correlation coefficient (R)	0.993	0.998
Sandell's sensitivity(ng cm^{-2})	0.0546	0.212
Regression equation (y)	y=0.018x+0.008	Y=0.001x-0.007
Slope, *b*	0.018	0.001
Intercept, *c*	0.008	0.007
Relative standard deviation%	0.487	0.25
% Range of error (95% confidence limits)	0.89	1.906
Limit of detection (µg/ml)	0.29	1.906
Limit of quantification(µg/ml)	0.88	5.77

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

Linearity

To establish linearity of the proposed methods, a series of solutions of pregabalin for method 1 (5–45 µg/ml), method 2 (50-450 µg/ml) and 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 given in table 4.

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 three serial dilutions were prepared from independent stock solutions and analyzed. Accuracy was assessed as the percentage relative error and mean % recovery. 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. Recovery values were calculated and represented in table 2 and 3.

The % recovery of the added pure drug was calculated as

$$\% \text{ recovery} = [(Ct–Cs)/Ca] \times 100,$$

Where
Ct is the total drug concentration measured after standard addition;
Cs drug concentration in the formulation sample;
Ca, drug concentration added to formulation.
Results of recovery study by standard addition method

Table 2: Method 1: Recovery studies for PGB (method 1)

S.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	1.0	10	1.0	10	0.301	20	10	100%
2	1.5	15	1.0	10	0.426	26	16	106.6%
3	2.0	20	1.0	10	0.523	29	19	95%

Table 3: Recovery studies for PGB (method 2)

S.NO	Standard pregabalin (ml)	Standard pregabalin (µg)	Sample pregabalin (ml)	Sample pregabalin (µg)	Absorbance at 461nm	Amount of PGB from std. graph	Recovery of std (mg)	%Recovery
1	0.5	50	0.5	50	0.174	99.5	49.5	99%
2	1.0	100	0.5	50	0.376	201.3	101.3	101.3%
3	1.5	150	0.5	50	0.532	299	149	99.33%

Table 4: 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) Method-I	1	75	73.254	97.672	98.821	3.545	0.238	0.487	0.0152
	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) Method-II	1	75	74.68	99.57	99.162	0.00104	0.25	0.59	0.004282
	2		74.232	98.97					
	3		73.86	98.48					
	4		74.94	99.42					
	5		74.56	99.41					
	6		73.97	98.626					

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

Ruggedness

To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drugs were carried out. The within-day RSD values were less than 1% and this indicate that the proposed method has reasonable ruggedness.

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

The LOD and LOQ for Pregabalin 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. The results of LOD and LOQ are given 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 PGB in pure form and in pharmaceutical preparations.

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