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Article

Precise and Sensitive Ambient Temperature Based Analytical Colorimetric Method for Pregabalin

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Pregabalin, a GABA analogue is used to treat epilepsy and neuropathic pain. The drug poses problems in analytical quantification when estimated at a shorter UV wavelength. The expensive and non-repetitive reported analytical methods necessitate the utility and development of an accurate, precise, repetitive, simple and highly sensitive colorimetric method for pregabalin in solution as well as sustained release mini matrices. Pregabalin (having primary amino group) was derivatized at alkaline pH of mixture with optimized ninhydrin solution at ambient temperature (25°C). The ninhydrin-pregabalin derivatized complex (Ruhemann's Purple) was analyzed for drug concentration at absorption maximum (λ_{max}) of 570nm. The linearity was observed in the concentration range of 5-150 µg/mL with coefficient of correlation, 0.998. The developed analytical method was validated according to ICH guidelines and proved to be highly sensitive (LOD 0.917µg/mL, LOQ 3.055µg/mL), with good inter-day as well as intra-day accuracy and precision as 4.65% and 3.75%, respectively. The proposed method was proved to be a simple, sensitive, precise and accurate for the estimation of the minute concentrations of pregabalin in pure form and the developed formulations. Results verified that the proposed method could determine pregabalin at the ambient temperature without requiring high temperatures used in the existing methods. It was concluded that developed method was easier and more suitable for analysis of pregabalin in quality control of commercial preparations.

Keywords: Pregabalin. Primary amine. Ninhydrin. Ruhemann's Purple. Mini matrices.

INTRODUCTION

Pregabalin (PGB), an analogue of GABA, is an anticonvulsant drug recommended for diabetic neuropathy and partial onset seizures in epilepsy. More than 14 million people in United States are diagnosed with diabetes mellitus, among them twenty five percent are suffering from diabetic neuropathy and seventy percent from nervous system damage respectively (Ben-Menachem, 2004). In 2004, FDA approved PGB for the treatment of diabetes-associated neuropathic pain while back in 1999, it was among the first FDA-approved antiepileptic drugs (Blommel, Blommel, 2007). PGB is also the first drug approved in Europe and United States for the treatment of postherpetic neuralgia and neuropathy (Gajraj, 2007). It works by reducing the signals of pain generated from the damaged nerve. Pregabalin belongs to BCS Class-I, and has higher aqueous solubility, greater than 30mg/mL over a wide pH range of 1 to 13. Its molecular formula is $C_8H_{17}NO_2$ (Figure 1).

As to the best of our knowledge, there is no official reported UV-Visible method available for the analysis of PGB in pharmacopeia. UV-Visible methods are always considered as cost effective, suitable and easy to handle in a limited facility of laboratory. Already available official methods for PGB analysis are based on HPLC, GC-MS and fluorescent spectrophotometer (Berry, Millington, 2005; Vermeij, Edelbroek, 2004). Of

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course, all these officially reported techniques are very sensitive and highly accurate but are also expensive as well as time consuming. The major limitation of UV-Visible methods includes, the absorption maximum for PGB that occurs at λ 210 nm, which is pondered the least sensitive for the dilute solutions and samples containing impurities, to be detected at shorter wavelength. Secondly the measurement accuracy of the minute concentrations present in pharmaceutical preparations is compromised (Gajraj, 2007) that requires a method to be developed for PGB dilutions, analyzed at greater detection wavelength than that of its original (Shep, Lahoti, 2013). Literature reports few UV-Visible methods of analysis for PGB (Bali, Gaur, 2011; Najam, Shah, Andrabi, 2013; Önal, Sagirli, 2009; Patil, Patil, Wani, 2016), but these are expensive due to either the use of expensive solvents or requirements of extensive heating and time. The already reported methods for PGB analysis, in which the colored reaction product was obtained by PGB reaction with ninhydrin require temperatures of 70ºC, 75ºC and 100ºC for different time durations (Bali, Gaur, 2011; Gupta *et al*., 2012). The limitation of these methods is to maintain the reaction temperature for such duration during the analysis of each sample which is a hectic and laborious.

Therefore, there is a need to develop a method of PGB analysis that should be highly economical, simple, equally sensitive, precise and accurate as that of the official methods. In this paper an effort was initiated to develop UV colorimetric method of PGB analysis at the ambient temperature, maintaining the alkaline pH of solutions without consuming the expensive chemicals, utilizing heat, and wasting the precious time.

MATERIAL AND METHODS

Instruments

Spectrophotometer measurements were carried out by using double beam OPTIZIN ALPHA UV spectrophotometer (AS0202-140717-04) with quartz 1 cm glass cell. The pH of solution was measured by using pH meter (Microprocessor pH/mv PHS-25CW).

Chemicals and reagents

Pregabalin was received as gift from Highnoon Laboratories (Pvt.), Pakistan. Ninhydrin of Sigma Aldrich, Germany, acetone, fuming hydrochloric acid (37% w/v), sodium hydroxide, and sodium chloride of Merck, Pakistan, were provided by University College of Pharmacy, University of the Punjab, Lahore. All the chemicals and reagents used were of analytical grade. Distilled water was used during whole study.

Derivatization of Pregabalin for Colorimetric Analysis

Preparation of buffer solutions, pH 1.2 and 6.8

The buffers, pH 1.2 and 6.8 were prepared according to the reported methods (Li *et al*., 2002).

Preparation of pregabalin stock and standard solutions

For the preparation of stock solution, 200mg of PGB was dissolved in 20mL of water and volume was made up to 100mL with the same solvent for obtaining the final drug concentration of 2mg/mL. The stock solution (2mg/mL) was used to prepare various concentrations of diluted standard solutions by selecting appropriate volumes of stock solution (2mg/mL) in 100mL volumetric flask and their volume was adjusted up to 100mL with buffer, pH 1.2 to prepare the working dilutions of 5, 10, 20, 30, 50, 75, 100, 150 and 200µg/mL.

Preparation of pregabalin dilutions for calibration curve

Pregabalin dilutions of concentration range 100, 200, 300, 400, 500, 1000, 1500 to 2000µg/mL were prepared in buffer pH 1.2 and pH 6.8 separately by using the stock solution of drug prepared in distilled water having concentration 2mg/mL. Absorbance of these concentrations were taken without derivatization against blank (buffer, pH 1.2 and pH 6.8, separately) according to study of Li *et al*., (2002) at maximum wavelength of 210nm. All concentrations were scanned to detect the maximum wavelength and calibration curve was constructed by taking the absorbance of these concentrations.

Preparation of ninhydrin solution

Ninhydrin solution was prepared in three concentrations, i.e., 1% w/v, 0.5 w/v, 0.25% w/v using ethanol-water, acetone-water as solvents separately. For the preparation of 1% w/v, 0.5% w/v and 0.25% w/v ninhydrin solutions, accurately weighed quantities, 0.5 and 0.25g of ninhydrin crystals respectively were taken separately in three sets of 100mL volumetric flasks. In one set, 50mL of ethanol was added to dissolve ninhydrin and volume of each dilution was adjusted with help of water. Similarly, in other set, all three ninhydrin concentrations (1, 0.5 and 0.25g) were dissolved separately in different volumes (5, 10 and 15mL) of acetone and volume was made up to 100mL with water.

Effect of pH on reaction mixture

For all dilutions, the pH was adjusted from 7 to 14, separately to analyze the effect of pH change on the color of reaction mixture. The pH was adjusted by adding the small volumes of 0.1N NaOH (1mL) and continued till alkaline pH 10 was obtained. The optimized reaction was noted at the pH 10.

Derivatization procedure

Different dilutions of concentrations 5, 10, 20, 30, 50, 75, 100, 150 and 200µg/mL were selected for derivatization reaction under optimized conditions. For derivatization reaction, 2mL from each dilution was taken separately in test tubes and 1mL of 0.1NNaOH was added to each dilution to adjust the pH. Different volumes (1-5mL) of 0.25% w/v, 0.5%w/v and 1%w/v ninhydrin solutions (acetone water and ethanol water) were added separately in each test tube and these set of test tubes were sonicated for 10 minutes at room temperature in a bath sonicator. Then all dilutions containing test tubes were kept at room temperature for purple color (Ruhemann's purple) development and for further analysis.

Characterization

Effect of different volumes of ninhydrin solution

Different volumes starting from 1 mL to maximum 5mL of ninhydrin solutions (0.25% w/v, 0.5% w/v and 1% w/v) were incorporated in each test tube separately, to assess the effect of each volume of ninhydrin solution on all dilution range of PGB solution for attaining optimized reaction conditions.

Selection of optimum maximum wavelength (λ_{max})

The derivatized dilutions of concentration 5, 10, 20, 30, 50, 75, 100, 150 and 200µg/mL after development of Ruhemann's purple (RP) were scanned in the range of wavelength from 400nm to 700nm. All dilutions showed maximum absorbance at 575nm (λ_{max}) and this wavelength was used to measure the absorbance in the remaining dilutions.

Construction of calibration curve after derivatization of pregabalin

The optimized derivatized PGB standard solution dilution (200µg/mL) was further used for construction of calibration curve. Ruhemann's purple formed from optimized dilution was further diluted using reagent blank to a range of concentrations, i.e., 5, 10, 20, 30, 50, 75, 100 and 150 µg/mL. A calibration curve was constructed from these dilutions by plotting the concentration against absorbance at selected λmax (575nm). All the readings were taken three times and average was drawn.

Application of Method to Gastroretentive Sustained Release Pregabalin *Mini Matrices*

Accurately weighed mini matrices containing 100mg of PGB were crushed to obtain the powder and passed through sieve no 60. The powder was then transferred to the 100mL volumetric flask and volume was made up to mark with buffer, pH 1.2. The drug in formulation was extracted after sonication at room temperature till 1 h and then filtered. The 10mL of filtrate was added to volumetric

flask and volume was made up to 100mL with water to obtain the drug content, 100µg/mL. The resulted solution was further diluted to obtain a dilution of concentration 50µg/mL. The solutions of concentration 50µg/mL and 100µg/mL were reacted under the same conditions as mentioned above with ninhydrin to obtain the Ruhemann's purple in alkaline medium. The derivatized reaction mixtures were analyzed at λmax (575nm) using dual beam UV-Visible spectrophotometer and concentration of drug content in sustained release mini matrices of pregabalin was calculated by using the regression equation of calibration curve obtained in derivatization method.

Validation of method

For the validation of method, precision, linearity, robustness and accuracy were determined according to ICH (Q2(R1)-ICH) guidelines (Guideline, 2005).

Linearity

Linearity of the method was determined from the dilution of range 05 to 150µg/mL from the derivatized primary stock solution of concentration 200µg/mL. Least square regression analysis was determined for all the dilutions.

Accuracy

To determine the accuracy three individual replicates of concentrations 5µg/mL (lowest), 50 µg/mL (intermediate) and 150µg/mL (higher, prepared from analytic derivatized stock solution) were tested. For each sample mean assay value, percent recovery, standard deviation, RSD was calculated and recorded.

Precision

To determine the precision of the method, repeatability (intraday) and reproducibility (inter-day) of the three different dilutions were determined**.** Six different times intervals of single day were selected for intraday studies and various concentrations of PGB were analyzed in triplicate (n = 6). Similarly, inter-day variations were studied by analyzing different concentrations of PGB for six consecutive days (n = 6). The results were designated in terms of standard deviation (SD), percent error (%E), percent relative standard deviation (%RSD), and percent recoveries. Same precision analysis was performed to assess the applicability of the developed analytical method for the matrix tablet formulation.

Sensitivity

The sensitivity of the proposed method was calculated in terms of LOD and LOQ defined according to ICH guidelines, where three times of noise of the response signal was taken as LOD and LOQ was ten times the noise of the signal. Many parameters such as SE and SD of the intercept, calibration curve and slope of this curve and intercept values were determined.

$$LOD = 3.3(\sigma/S) \quad \text{Equation 1}$$

$$LOQ = 10(\sigma/S) \quad \text{Equation 2}$$

Where σ represents the standard deviation of the response and S, the slope of the calibration curve.

Robustness

Robustness is usually evaluated by inducing small deliberate changes in the parameters of derivatization and calculation of effects produced on the analytical effectiveness of drug determination. The optimized parameters for derivatization were 3mL of 0.5% w/v solution of ninhydrin and 2 mL of PGB solution (120µg/mL). These optimized conditions were replaced to insert change at 2 mL volume of 50µg/mL PGB solution and 3mL of 0.4% w/v ninhydrin solution. The maximum absorption was also transferred to a smaller wavelength of 560 nm. For determining the robustness of the proposed method, the comparative standard deviation and present recovery were calculated at respective time.

RESULTS AND DISCUSSION

There is always a concern regarding a great deal of expenses and time consumption when an analyst considers

opting for a derivatization-based reaction. If a method developed is simple that proceeds at ambient temperature and progress with cheaper, commonly utilized solvents in short period of time, then it could be equally beneficial. PGB (Figure 1) contains the primary amino acids, under optimized conditions, it reacts with ninhydrin and develops a derivative of pregabalin consuming its amine to form Ruhemann's Purple (RP), which made its detection possible at higher wavelength. In this study, an effort was initiated to optimize the derivatization conditions of simple colorimetric method for PGB determination in dilutions of pure drug and sustained release mini matrices formulation. The factors which effected the derivatization were ninhydrin concentration, solvents used, temperature and pH of solutions. The novelty of this method is use of economical and readily available chemicals without requirement of higher temperature for the derivatization, contrary to the already reported methods. Furthermore, few of the existing methods do not reproduced the results as mentioned.

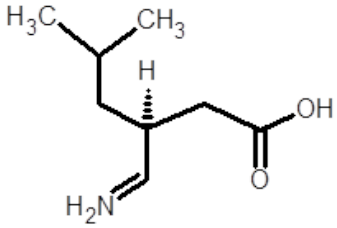

FIGURE 1 - Structure of pregabalin.

Calibration curve for pregabalin dilutions

The shorter ultraviolet wavelength always produces varying results and it was found difficult to analyze minute quantities of PGB, i.e., the concentrations lesser than 100µg/mL. The curve was not linear and with each repeated analysis, there was vast difference among the analytic values so the standard curve was drawn for dilution range 100-2000µg/mL in buffers of pH 1.2 and 6.8 as shown in Figure 2 and Figure 3, respectively. The PGB, without derivatization could be detected only at the upper limit of the above range, while the PGB concentration at the lower value of range was undetectable. It is noteworthy that from a sustained release formulation, like we studied, the PGB is released in small amounts, those are undetectable without derivatization using UV-Visible methods.

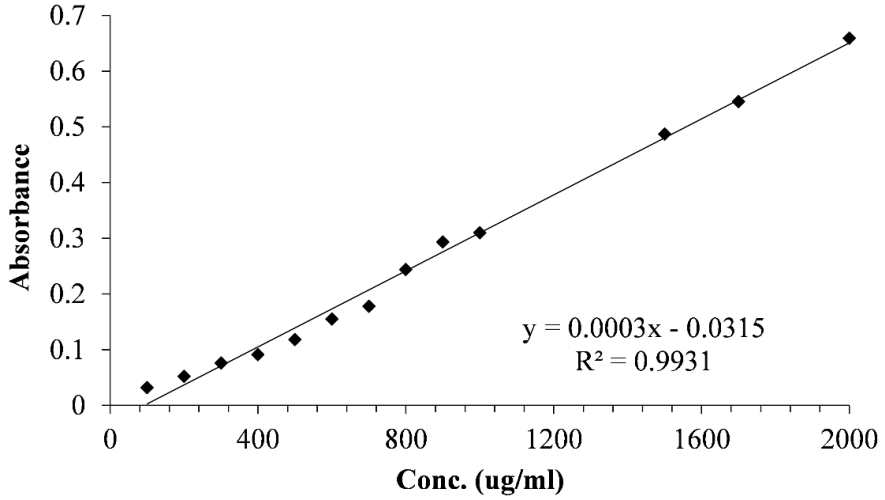

FIGURE 2 - Calibration curve without derivatization at pH 1.2 at 210nm.

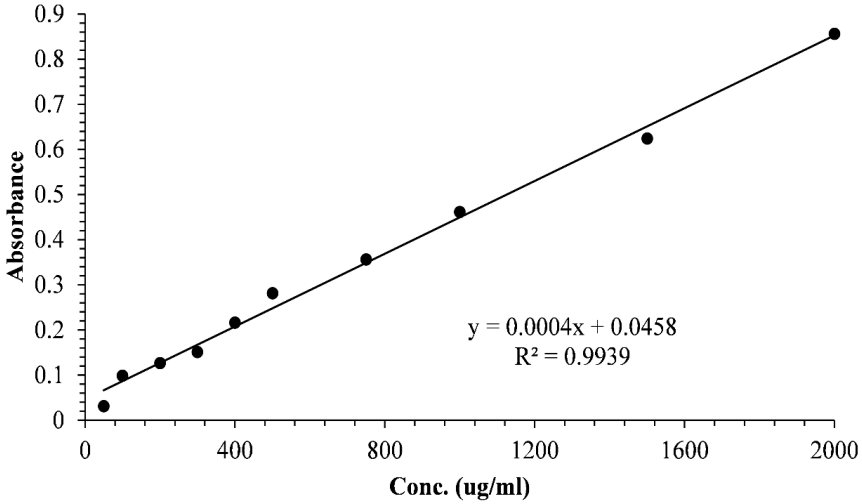

FIGURE 3 - Pregabalin calibration curve without derivatization at pH 6.8 at 210nm

Reaction of ninhydrin solution with pregabalin dilutions.

PGB contained a primary amino group that reacted with the ninhydrin in the alkaline medium and condensed to form a chromophore which gave a colored product to form a Schiff base. This reaction is only shown by the compounds which contain primary amines and ammonia. The reaction mixture in this study developed the colored product at the ambient temperature by optimizing the ninhydrin solution concentration and maintaining the alkaline pH of the reaction mixture. The addition of ninhydrin solution in PGB dilution was resulted in the development of yellow orange color within 5 min. The color darkened as the time proceeded and purple color appeared in the test tubes after 10 min. The proposed reaction is elaborated in proceeding section on effect of pH on reaction mixture and schematically represented in Figure 4.

To obtain the maximum and optimized color of reaction product, different parameters effecting the intensity of color were analyzed.

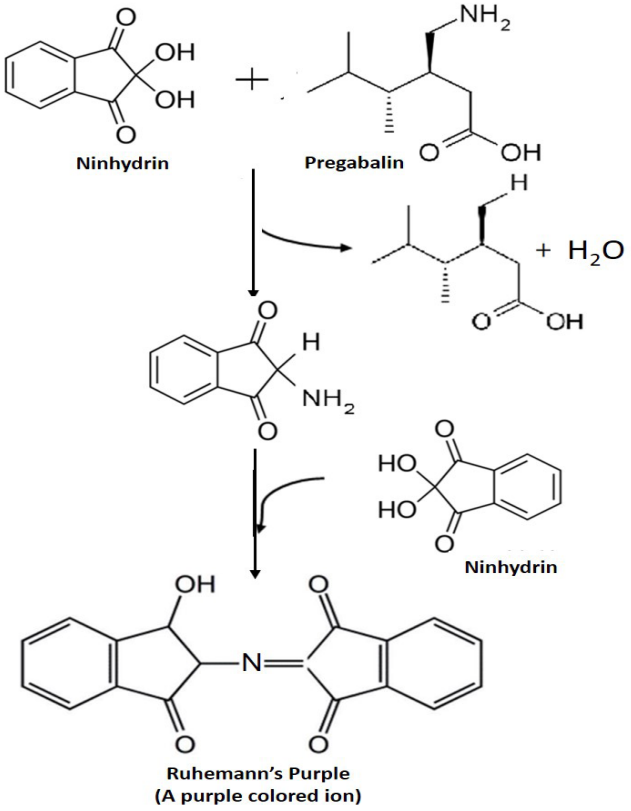

FIGURE 4 - Proposed reaction of pregabalin with ninhydrin at optimized conditions.

Calibration curve for derivatized pregabalin dilutions

The different working standard solutions of pregabalin (5-200µg/mL) were derivatized with optimized conditions, i.e., 0.5% w/v ninhydrin solution and acetone as solvent. The calibration curve drawn after development of Ruhemann's purple color is shown in Figure 5.

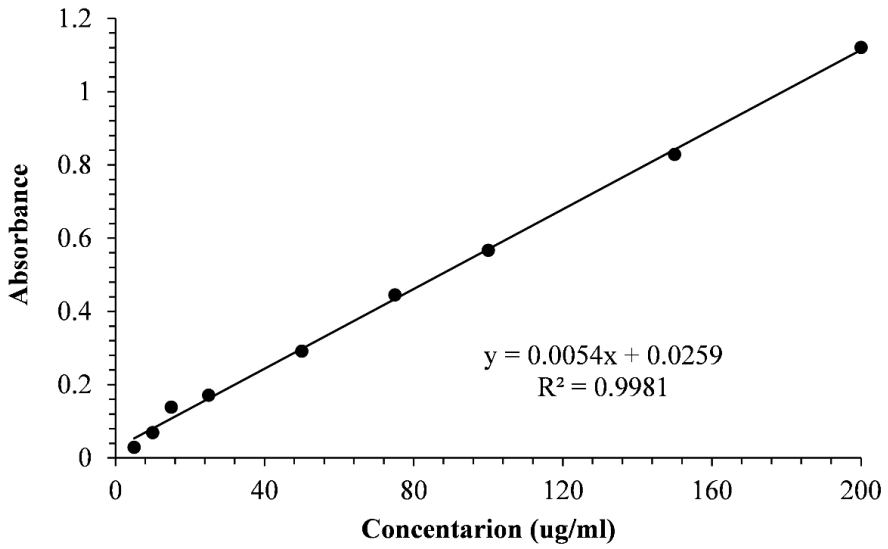

FIGURE 5 - Calibration curve for the reaction mixture containing ninhydrin acetone solution.

Effect of pH on development of color

The effect of pH of solution, volume of ninhydrin solution and solvents on color development were studied. The pH of solution effected the reaction significantly, e.g., at the acidic pH, there was no reaction occurred with the addition of ninhydrin solution, demonstrating a prominent effect of H^+ ion concentration in the reaction mixture on the reactivity of ninhydrin solution. The rate of reaction (color development) was found greatly retarded by the presence of H^+ ions in reaction mixture (Meyer, 1957). Color of reaction mixture only appeared when ninhydrin solution was added in PGB solution at basic pH (>7). The appearance of color started from basic pH <10, and got stable after some time at pH 10 because the amino acid containing un-protonated amino group showed the excellent nucleophilic properties at the alkaline pH in comparison to the acidic pH. Furthermore, ninhydrin also required alkaline condition for the preparation of colored complex (Gorumutchu, Ratnakaram, Malladi, 2019). Ninhydrin reduction took place in alkaline conditions to 2- hydroxyl indan-1,3- iodine and on condensation of $–NH_2$ group of PGB, it formed diketohydrindyllndene diketohydrindamine also purple in color (Friedman, 1971; Friedman, 2004; Rahman, Singh, Hoda, 2005). Sensitivity of reaction was evaluated to be greater in the presence of organic solvents at alkaline pH of the solution (Lamothe, McCormick, 1972). Similarly, the intensity of color was found higher when the reaction mixture contained the higher volumes of ninhydrin solution prepared in organic solvent (Friedman, 1971).

Effect of temperature on development and stability of color

The optimized reaction conditions on the basis of pH and organic solvent were further studied for the effect of temperature. For this purpose, the reaction mixture was maintained at the alkaline pH 10 with ninhydrin reagent solution for a period of 1 h at the ambient temperature preferably 30ºC. The color and intensity of the color was

found stable till the maximum time of storage. Literature suggests that ninhydrin reacts with primary amine between 30ºC to 100ºC depending on the basicity of environment. At 30ºC, -OH group from the ninhydrin is displaced and -NH_2 group from primary amino acid is attached with it (Hayashi et al., 1978; Gupta et al., 2012). Already reported methods available to detect the PGB by using the ninhydrin require to maintain higher temperature conditions, which is more tedious and time consuming. On further increase of temperature, reaction product remained stable up to 30 min of reaction. After 30 min, the color of reaction mixture started to change from blue to brown color as the temperature was increased from 30ºC to 100ºC. At basic pH, color and absorbance of solution was found stable at lower temperature in comparison to high temperature.

Effect of different concentration of ninhydrin reagent solution on color

Different concentrations of ninhydrin solutions (0.25% w/v, 0.5% w/v and 1% w/v) were added to 2mL of each PGB dilutions taken in separate test tubes. Maximum volume of ninhydrin solutions was determined by investigating the absorbance of pregabalin dilutions. Ninhydrin regent solution was added gradually in different volumes i.e. 1mL, 2 mL, 3 mL, 4 mL, 5 mL and 7 mL in each dilution and a light-yellow color appeared immediately on the addition of ninhydrin solution. Light yellow colored product turned to purple colored product after 5 min storage at ambient temperature. From all the above-mentioned test tubes, after addition of the optimized volume (5 mL) of 0.5% w/v ninhydrin acetone solution, there was no further change in the absorbance of dilutions, while in the other dilutions containing 0.25% w/v and 1% w/v ninhydrin solution, stable color and reproducible absorbance were not obtained. Therefore, only results and relationship between different volumes of 0.5% w/v ninhydrin acetone solution and absorbance of the dilutions of concentrations 10, 20, 50 and 75µg/mL of PGB are shown in Figure 6.

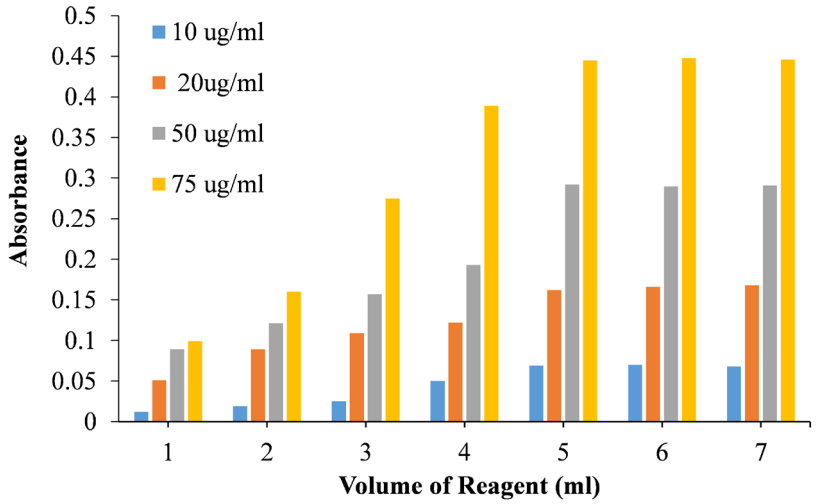

FIGURE 6 - Relationship between different volumes of acetone ninhydrin solution (0.5%w/v) and absorbance of dilutions of pregabalin.

Effect of pregabalin concentration on reaction

Intensity of color was found to be dependent on the amount of drug in different dilutions. The dilutions of higher drug concentrations showed higher color intensity as compared to dilutions of lower drug concentrations. In 50µg/mL, 75µg/mL, 100µg/mL, 120µg/mL dilutions of PGB, purple color was appeared after 5min on the addition of 5mL of acetone ninhydrin solution. In lower

concentrations (05 to 30µg/mL), intensity of purple color was low at ambient temperature.

The same sets of test tubes were also kept in sonicator and water bath to investigate the effect of heating at different temperatures (starting from 30ºC to 100ºC) and agitation for different time intervals. But after agitation, no color product was appeared in all these test tubes, color was appeared in test tube kept at the ambient temperature after 5min.

Effect of reaction time on the intensity of reaction

To determine the reaction time of PGB with 0.5%w/v ninhydrin solution, PGB's dilutions of 150, 100, 75µg/mL were reacted with ninhydrin solution and effect of reaction time on intensity of color as well as on the absorbance was studied at 575nm wavelength for maximum of 2h. The dilutions 150, 100 and 75µg/mL produced purple color showing absorbance of 0.829, 0.567, and 0.445 at 575nm, respectively. Intensity of color was increased with passage of time. It took almost 10 min to develop an intense purple color when reaction mixture containing PGB were reacted with 0.5% ninhydrin solution maintained at alkaline pH. The evaluations were made at three-time intervals i.e., 30, 60 and 120 min of reaction. For the reaction of ninhydrin with amino acid, alkaline pH is ideal because protonated amino group of the primary amino acids reacts excellently as a nucleophile at alkaline pH. Whereas protonation is less nucleophilic at the acidic pH.

Estimation of drug content from Gastroretentive mini matrices

For drug content analysis, two optimized formulations of mini matrices (under publication) prepared by melt granulation method using blends of hydrophilic and hydrophobic polymers (three tablets each) were selected and weights equivalent to 100mg PGB were analyzed for PGB estimation. Good results of assay showed the efficiency of the method. The results of drug content determination of mini matrices are given in Table I.

TABLE I - Assay of Pregabalin in sustained release mini matrices

Formulations	Extracted drug conc. from sustained release mini matrices (µg/mL)	Conc. ± SD (µg/mL)	Recovery (%age)	RSD (%age)	Error (%age)
1	100	97.89 ± 0.664	98.81	0.672	-1.185
2	50	48.54 ± 0.778	96.5	1.612	-3.5

Validation of the Developed Method

To determine the validity of method following parameters were studied.

Linearity

Calibration curve was constructed from the PGB concentration range 05 to 150µg/mL, diluted from the acetone ninhydrin derivatized PGB concentration of 200µg/mL (Ruhemann's Purple). The best fit linear regression equation (coefficient of correlation 0.998) was y = 0.0053x +0.025 (Figure 4). The result showed maximum linearity in all concentration range of 5 to 150µg/mL. The absorbance range was within 0.029 to 0.829 for concentration of 5 to 150µg/mL and 1.121 for maximum concentration of 200µg/mL. Linearity was equally preserved in calculation of concentrated dilution in calibration curve.

Sensitivity

LOD and LOQ values were determined to assess the sensitivity of the method and were found as 0.9165µg/

mL and 3.055µg/mL for LOD and LOQ, respectively. The highly sensitive values depicted that the method proved to be efficient in determining and quantification of least quantities of pregabalin in solutions as well as in sustained release mini matrices with good sensitivity. It could also be implied from the above findings, that the current method could also be applicable for drug determination from the PGB's sustained as well as the immediate release dosage forms. This method is so much sensitive in comparison to the other reported UV detection methods.

Accuracy

The parameters estimated to establish accuracy of the method were, analyzing the error of the derivatized three different dilutions (5, 10, 20 µg/mL) of PGB, percentage drug recovery, variation as standard deviation, and the relative standard deviation of all these derivatized solutions. The results are summarized in Table II. The results of % recovery and error were found within limits.

TABLE II - Inter-day accuracy and precision analysis (n = 6)

Sr. No.	Standard dilutions (µg/mL)	Conc. ± SD (µg/mL)	Recovery (%age)	RSD (%age)	Error (%age)
1	5	5.19± 0.0024	102	7.040555	1.0
2	10	9.76± 0.0039	97. 6	5.605731	-2.3
3	20	20.25± 0.0017	101.2	1.334966	1.2

Precision

To determine the repeatability and reproducibility, the derivatized concentrations of pregabalin in triplicates were analyzed at six different time interval on same day and freshly prepared derivatized concentrations in triplicate over the six different days.

The results of inter-day precision analysis (Table II) and intra-day precision analysis (Table III) computed on basis of percent recovery, standard deviation, and percent error in terms of percent RSD. The proposed method is more sensitive for the lower as well as higher concentrations of derivatized PGB as specified by results of precision analysis.

TABLE III - Intraday accuracy and precision analysis (n = 6)

Sr. No.	Standard dilutions (µg/mL)	Conc. ± SD (µg/mL)	Recovery (%age)	RSD (%age)	Error (%age)
1	5	5.05 ± 0.0012	101	3.745	1
2	10	9.75± 0.0045	97.5	6.370	-2.5
3	20	20.41 ± 0.0016	102	1.221	2.05

Robustness

The diluted concentration of PGB used caused a bit lowering of the intensity of derivatized color and delayed the color development due to the use of the lesser ninhydrin. Nevertheless, the overall analytical results were similar to that of the derivatized optimum conditions indicating least effects of deliberate changes in derivatization conditions. The standard deviation and percentage recovery were found to be 99.41% ± 0.096, respectively. The percent RSD was perceived to be 3.15%. The findings indicated that the analytical performance might be effected by the induced minor changes, but there were no significant reportable adverse events happened and analytical procedure proved to be excellent in terms of sensitivity analysis with good robustness properties of the proposed method (Hussain *et al*., 2016). The comprehensive summary representing optical and regression characteristics of validation parameters are expressed in Table IV.

TABLE IV - Summary and comparison of regression characteristics of proposed method

Parameters	Values	
	Colorimetric	**Without derivatization**
Maximum wavelength, nm	575	210
Beer's law limit, µg/mL	5-150	50-2000
Regression equation	y = 0.005x + 0.025	y = 0.0004x + 0.0458
Slope ± SD	0.025±0.00874	0.0004±0.000095
Intercept ± SD	0.0054±0.012186	0.0458±0.01274
Correlation coefficient (R^2)	0.998	0.993
Limit of detection (LOD), µg/mL	0.917	178.551
Limit of quantification (LOQ), µg/mL	3.055	541.065
Intraday precision (%RSD)	3.755	-*
Inter-day precision (%RSD)	4.65	-*

*Not calculated

CONCLUSION

The method developed for pregabalin in this study was more sensitive, easy and economical without requiring the high temperatures for samples. The method was capable of analyzing the pregabalin concentrations of 5 to 150µg/mL in the samples, just by maintaining the alkaline pH and using the optimized ninhydrin concentration (0.5%w/v) at the ambient temperature. The method could be suitable for quality control analysis of pregabalin research and commercial formulations in pharmaceutical industry, where there is a continuous and high demand for production of large number of batches.

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