DEVELOPMENT OF SPECTROPHOTOMETRIC METHODFOR THE DETERMINATION OF PREGABALIN IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, fast and sensitive spectrophotometric method for the determination of pregabalin (PRG) in bulk and pharmaceutical dosage forms based on reaction with ninhydrin is developed, optimized and validated. The purple color (Ruhemenn's purple) that resulted from the reaction was stabilized and measured at 557 nm. Beer's law was obeyed in the concentration range of 4-20 µg/ml with a limit of detection (LOD) of 0.29 µg/mL.Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. All variables including the reagent concentration, heating time, reaction temperature, color stability period, and pregabalin/ninhydrin ratio were studied in order to optimize the reaction conditions. The developed method is easy to use, accurate and highly cost-effective for routine studies relative to HPLC and other techniques.

Key Words: Spectrophotometric, Pregabalin, Ninhydrin, Pharmaceutical formulations.

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INTRODUCTION

Pregabalin (PRG) is chemically (S)-3-(amino methyl)-5-methyl hexanoic acid and it as shown in (Fig.1).A literature survey reveals that some of analytical methods were developed for PRG using extractive spectrophotometricand spectrofluorometric [1-2], LC method with precolumn derivatization with marfeys reagent [3], HPLC analysis of PRG in human serum [4], Liquid chromatography - mass spectrophotometric (LC-MS-Ms) [5-7]. Hence, it is important to develop an accurate, rapid and specific stability indicating analytical method, which is suitable for regular quality control analysis of PRG in bulk and pharmaceutical formulations. The present study describes visible spectrophotometric method for the determination of PRG in bulk and pharmaceutical formulations by using ninhydrin as the dye reagent

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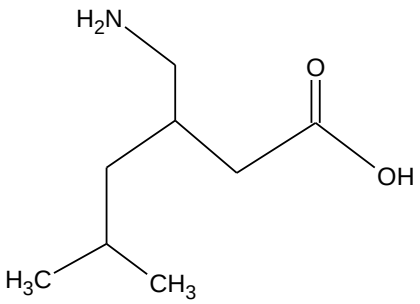

Figure 1. Structure of Pregabalin

EXPERIMENTAL

Instrument:

A Shimadzu Model No-1800, UV-visible spectrophotometer with 10 mm matched quartz cells were used for absorbance measurements. pH Measurements were made with a Digital pH- meter, Toshniwal instrument manufacture Pvt. Ltd (Mumbai, India).

Reagents and chemicals:

All chemicals and reagents were of analytical grade. PRG was obtained from Sun Pharmaceutical Industries ltd. Mumbai. Neugaba (Sun PharmaceuticalsIndustries Ltd., Mumbai.) and Gabafit-75 (Glenmark Pharmaceutical Ltd., Mumbai, India) capsules were brought from local pharmacy shops. The reagent ninhydrin was purchased from E-Merck, Mumbai, India. Distilled water was used to prepare all solutions.

Preparation of Standard solutions and reagent:

The standard solution of PRG (100 µg/mL) was prepared in water. The reagent of ninhydrin dye was prepared in ethanol[8].1 M phosphate buffer solution of pH 8 was prepared by dissolving 13.61 g of potassium dihydrogen phosphate in a 100 mL measuring flask and made volume with distilled water and pH was adjusted with 1 M NaOH.

Assay Procedure for pure drug:

For this method, accurate aliquots containing 4-20 µg/mL of PRG were transferred into series of 10 mL volumetric flask. 1mL phosphate bufferand 2 mL of ninhydrin reagent was added and the mixture was heated in a water bath at 80±5°C for 15 min. The flasks were cooled and the volume was made up to the mark with distilled water. The absorbance measurements were measured at 557 nm against a reagent blank.

Assay Procedure for capsules:

PRG formulations were purchased from local market, 20 capsules were weighed without shells and powdered. An accurate amount of the powder equivalent to 25 mg of PRG was transferred into a 25 mL calibrated flask and 15 mL of water was added. Suitable aliquots were subjected to the analysis following the procedure described above. The concentration of PRG was calculated from the corresponding regression equation. The absorbances were measured at 557 nm against a reagent blank.

Recovery studies and validation of the methods according to ICH Guidelines[9]

Recovery study for the method was done by addition of known quantity of standard drug solution to preanalysed sample at three different concentration levels. Results for recovery study are reported in the Table-2.

Ninhydrin is a well-established reagent for the determination of certain amines, amino acids and thiophenes. It has been extensively used in the determination of the compounds of pharmaceutical importance[10-11].

Reaction involved and optimization of reaction conditions

It was suggested that the reactions of ninhydrin with amine, amino acids and imino acids all proceed by the same mechanism to give diketohydrindylidene-diketohydrindamine or the Ruhemenn's purple[12].This compound would further react with amino group of PRG to give the product, which absorb maximally at 557nm as shown in Fig.2. The optimum conditions for determination of PRG were established.

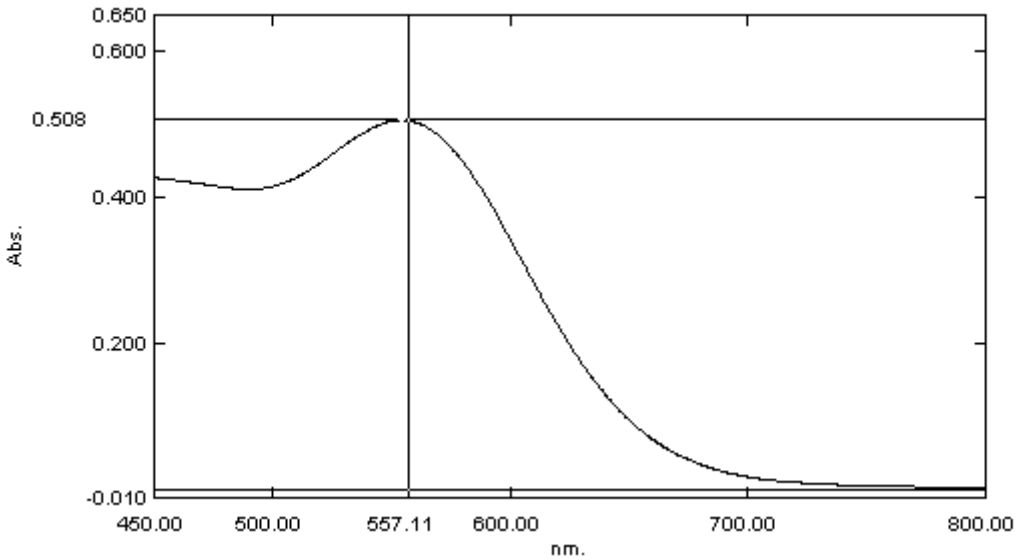

Figure2. Wavelength maximum of PRG and ninhydrin complex

PRG reacts with ninhydrin reagent in presence of phosphate buffer *via* oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored reaction product Ruhemenn's purple with λmax at 557 nm (Scheme-1). To optimize the reaction conditions, we have investigated a number of parameters such as heating time, reagent concentration, temperature, pH, stability of color. Varying one variable and observing its effect on the absorbance of the colored product established the optimum reaction conditions.

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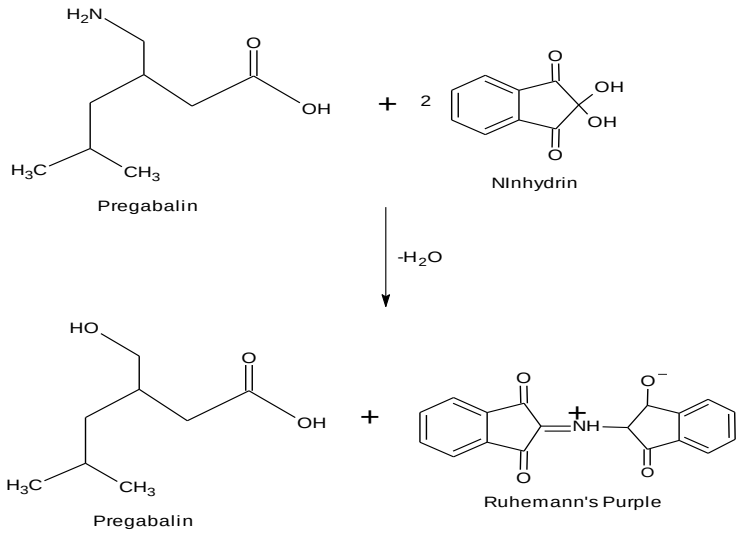

Scheme 1. Proposed reaction between pregabalin and ninhydrin

Effect of heating time

A 0.12 mL aliquot of a 100 µg/mLPRG solution was mixed with1mL phosphate buffer and 2 mL of ninhydrin solution. The reaction mixture was heated on a water bath at 80±5°C. A colored product was obtained and the color intensity reached its maximum value after 10 mins of heating. After reaching the ambient temperature, the reaction mixture was transferred to a 10 mL volumetric flask and diluted to the mark with distilled water. Hence, the absorbance was measured after 10 mins of heating. The results are shown in Fig.3.

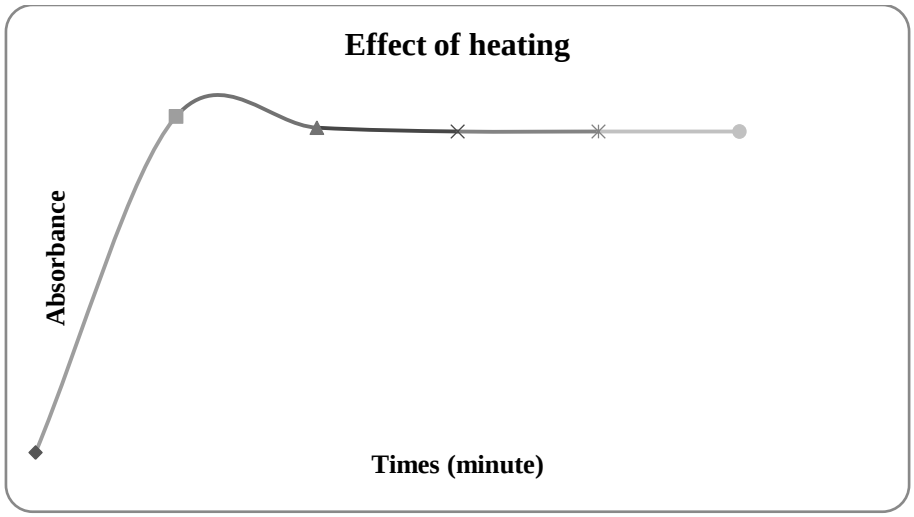

Figure3. Effect of heating time on color development

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Effect of ninhydrin concentration

To 0.12 mL of 100 µg/mLPRG solution, different volumes (0.5-3.5 ml) of ninhydrin were added. The reaction mixtures were heated for 10 mins on a water bath at 80±5°C. The colored product was diluted to 10 mL with distilled water and the absorbance was measured against a reagent blank at 557 nm. The results showed that the highest absorbance was obtained with 2 mL of ninhydrin solution that remained unaffected with higher amounts (Fig.4).

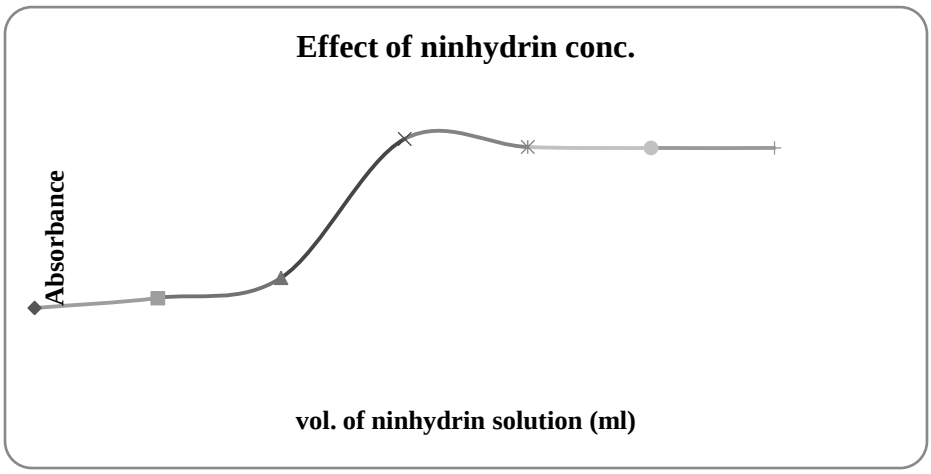

Figure4. Effect of reagent concentration on color development

Effect of temperature

To 0.12 mL of 100 µg/mLPRG solution, 2 mLof ninhydrin and 1mL of phosphate buffer solution were added. The reaction mixtures were heated for 10 mins on a water bath at 20-100°C. The colored product was diluted to 10 mL with distilled water and the absorbance was measured against a reagent blank at 557 nm. The results showed that the highest absorbance was obtained at 80±5°C (Fig. 5). The developed color was stable for 4 hrs.

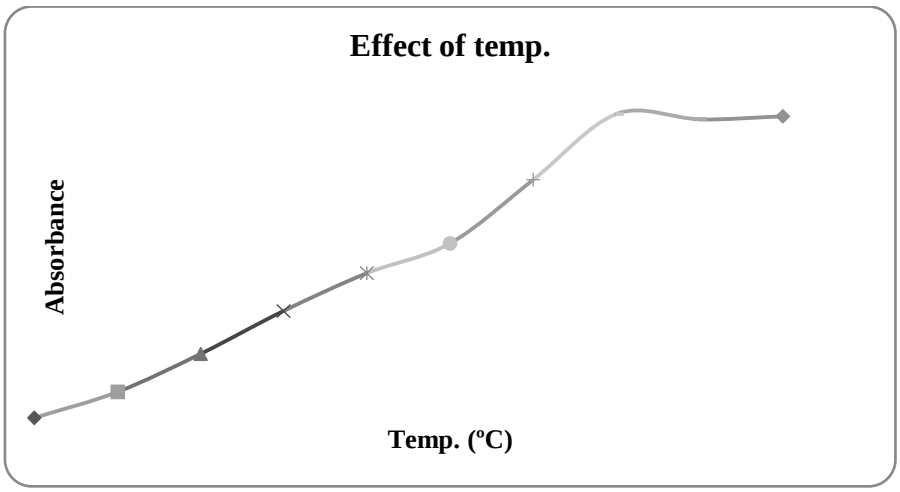

Figure 5. Effect of temperature on color development

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Effect of pH on color development

The ninhydrin reacts with PRG in basic medium. It is reported that pH effect under certain conditions on the ninhydrin reaction may have a fluctuating effect[13]. In our study, sample absorbance increased with an increase of the pH of the sample solution from 7.0 to 8.0, thus, phosphate buffer with pH 8.0 was selected for further studies.

RESULT AND DISSCUSSION

The present studies describe method for the assay of PRG in bulk and pharmaceutical formulations using ninhydrin reagent. PRG as a positively charged amino compound in basic medium formed Ruhemenn's purple colour. The absorption curves of the complexes showed maxima at 557 nm (Fig.2).Beer's law limit, molar absorptivity, detection limit and correlation coefficient were obtained by least square treatment of results. The linear relationship was found between absorbance at λmax 557 nmand concentration of drug in the range 4-20 µg/mL(Fig. 6). High values of Correlation coefficient (r=0.999) and small value of intercept validated the linearity of calibration curve and obedience to Beer's law.

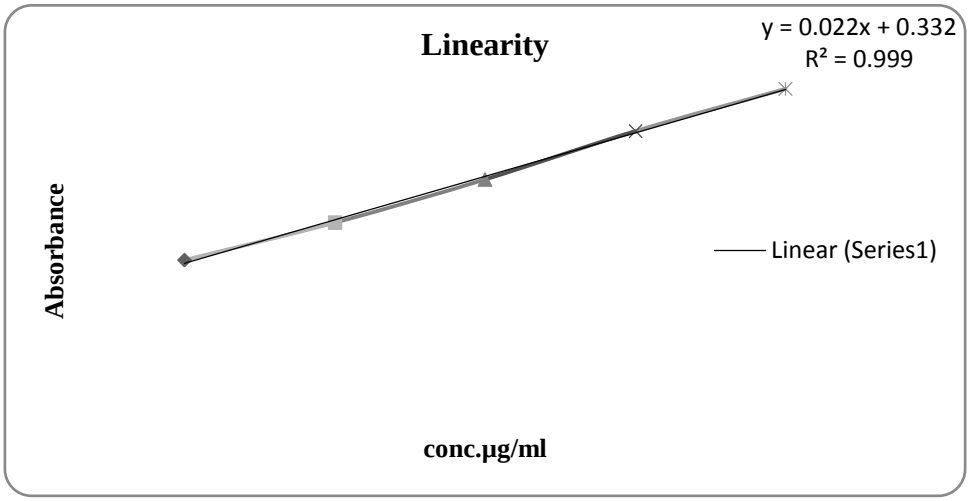

Figure 6. Beer's law range of PRG with ninhydrin

The apparent molar absorptivity and Sandell's sensitivity values together with the limits of detection (LOD) and quantification (LOQ) compiled in Table-1 are indicative of the high sensitivity of the proposed method. The LOD and LOQ were calculated using LOD = 3.3 s/S and LOQ = 10 s/S, where s is the standard deviation of seven blank determinations and S is the slope of the calibration curve. The proposed two methods were applied to the determination of PRG in its commercial capsules and satisfactory results were obtained (Table 1).

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Table 1. Optical characteristics and statistical data.

Parameters	Values
λ max(nm)	557
Beer's law range (µg/mL)	4-20
Molar Absorptivity (Lit/mol crn^{-1})	7.92x10^{3}
Sandell's sensitivity (mg/mL/0.001 abs units)	0.020
Detection limit (µg/mL)	0.29
Quantification limit (µg/mL)	0.881
Regression equation	
Slope (a)	0.022
Intercept (b)	0.332
Correlation coefficient (r)	0.999

Y[a]=a + bX Where Y is the absorbance in a cell of 10 mm path length and X is concentration in µg/mL

Table 2. Analysis of PRG in capsules (each capsule contains 75 mg of PRG).

Brand	Labeled Amount (mg)	Amount found [a](mg)±SD	% RSD	Recovery (%)
Neugaba-75	75	74.99±0.231	0.308	99.99
Gabafit-75	75	74.86±0.179	0.239	99.82

a: average of four readings.

CONCLUSION

The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The results are in good agreement with labeled value. The percentage recovery obtained indicates non interference from the common excipients used in the formulations. The proposed extractive spectrophotometric methods are rapid, sensitive, accurate and economic. Therefore they can be recommended in routine analysis of PRG in bulk and pharmaceutical formulations in quality control laboratories .

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