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Development and Validation of Ninhydrin Based Colorimetric Spectrophotometric Assay for Determination of Pregabalin in Different Dissolution Mediums

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Abstract

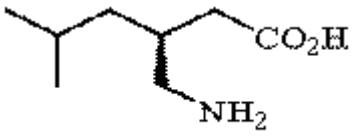
A simple, accurate, cheap and quick spectrophotometric method was developed for the estimation of pregabalin in pharmaceutical pure and dosage forms. Three different mediums (Distilled water, 0.1 N HCl with pH 1.2, buffer with pH 6.8) were taken. The method was developed based on the reaction of ninhydrin with the primary amine present in pregabalin. In all the three cases, reaction produces Rheumann's Purple colour and absorbs at 571, 555 and 569 nm in distilled water, 0.1 N HCl and pH 6.8 respectively. Beer's law was followed in the concentration range of 100-200 µg mL^{-1} in phosphate buffer with pH 6.8, 50-100 µg mL^{-1} in pH 1.2 and 10-100 µg mL^{-1} in distilled water. The method developed was successfully applied to pharmaceutical dosage forms with good efficacy and recovery was found from 98.41-99.53%. The method seems to be simple, less time consuming, cost effective and accurate for estimation of pregabalin in pharmaceutical pure and dosage forms.

Keywords:

Pregabalin, Ninhydrin, Spectrophotometry, Pharmaceutical dosage forms

1. Introduction

Pregabalin is an analogue of neurotransmitter γ-aminobutyric acid (GABA). Chemically it is described as (S)-3-(aminomethyl)-5-methylhexanoic acid. Its molecular weight is 159.23 with an empirical formula $C_8H_{17}NO_2$. Its molecular structure is



Pregabalin

It is freely soluble in water as well as in acidic and basic media. It is used in the initial therapy of diabetes related peripheral neuropathic pain in dosage range from 50-300 mg daily. Pregabalin seems to be effective in central pain. It is also used for epilepsy, fibromylagia, postherapetic neuralgia.

It is reported that pregabalin binds to an auxillary subunit (α2-δ-protein) of voltage gated calcium channels in the central nervous system & potentially displacing [^{3}H]-gabapentin. This drug is absorbed rapidly and shows linear pharmacokinetics after oral

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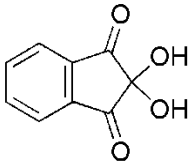
administration. It lacks hepatic metabolism & interaction with cytochrome P-450 isoenzymes. It also explains the absence of drug interactions & has oral bioavailability of 90% [1].

Very few reports are available in literature for the analysis of pregabalin. Official monographs also do not exist in any Pharmacopoeia for this particular drug. At present, in literature the reported methods for pregabalin estimation quantitatively are based on LC-MS-MS [2] & to best of our knowledge estimation in human plasma by HPLC method is based on precolumn derivatization using picrylsulfonic acid [3]. LC-MS-MS method is accurate and sensitive but also associate with few disadvantages like costly, time consuming and availability of instrument. For routine quality control tests a simple, less time consuming and reliable spectrophotometric method is needed. No spectrophotometric method is available for the estimation of pregabalin due to its poor absorbtivity in UV region and absence of a chromophore group [4]. In order to quantify pregabalin in pharmaceutical formulation and bulk, a colorimetric based spectrophotometric assay was developed and validated in the present study. This method is based on the interaction of the primary amine group present in pregabalin with ninhydrin. Ninhydrin is a reagent which is widely used to characterize and analyze amino acid, peptides and proteins. Reaction product of ninhydrin with primary amino groups produces the coloured chromophore called as Rheumann's purple. Previously, pregabalin ninhydrin complex formation and its absorbtivity in the visible region (λmax 570 nm) was reported by Friedman [5]. In the present study attempt was made for development and validation of the colorimetric based spectrophotometric assay for estimation of pregabalin.



Ninhydrin

In the present study, the mechanism of ninhydrin pregabalin reaction and colour formation was also reviewed and optimized [6]. Distilled water, 0.1 N HCl with pH 1.2 and Phosphate buffer saline (PBS pH 6.8) are commonly used media for pregabalin analysis in bulk or dissolution study of dosage forms. So attempt was also made for the method development and validation in these three media.

2. Experimental Section

2.1. Chemicals & Reagents

Pregabalin was kindly provided by Bioplus Life Science Pvt. Ltd., Hosur, in its pure form. For validation pregabalin capsule (Selaqui, Intas Pharmaceuticals, India) were purchased from a local drug store. Other chemicals & reagents include Ninhydrin (Qualigens), Disodium hydrogenphosphate and Dimethyl sulfoxide [DMSO] (Merck Specialities Private Limited, Mumbai), Potassium dihydrogenphosphate (Qualigens), Hydrochloric acid and Pyridine (s.d. fine –CHEM Ltd, Mumbai) were purchased. All these were used of AR grade. Distilled water was obtained by Mili Q apparatus by Millipore (Milliford, USA) for whole experimental work.

2.2. Apparatus

Spectrophotometric measurements were carried out using Beckman DU-600 spectrophotometer with 1 cm glass cells.

2.3. Working solutions

Stock solution of pregabalin (of pure form) was prepared in three different mediums. First, stock solution was prepared in distilled water of concentration 100 μg mL^{-1}. In second

and third flask stock solution of pregabalin was prepared in different buffers with pH 1.2 (using 0.1 N HCl) and 6.8 respectively. Further dilutions were made from the stock solution of the drug. Ninhydrin solution (3% w/v) was prepared in DMSO [7].

2.4. General procedure

Different aliquots of drug solutions were taken from stock solution of each medium and diluted with their respective mediums. The final concentration made in case of distilled water and buffer with pH 1.2 (0.1 N HCl) was 10-100 μg mL^{-1}. In case of buffer with pH 6.8, it was 100-200 μg mL^{-1}. Additionally, 0.1 mL pyridine was added only in those flasks having buffer with pH 1.2 as medium. After that 1 mL of ninhydrin reagent in DMSO was added in each flask. The flasks were heated in water bath at around 100 ˚C for 2-5 minutes. After cooling at room temperature the volume was made upto the mark with Distilled water in either case. The absorbance was measured against a reagent blank, in case of distilled water, buffer (pH 6.8) and in buffer pH 1.2) at 571, 569 and 555 nm, respectively. The standard plot was prepared by plotting absorbance vs. concentration of pregabalin.

2.5. Assay procedure for capsules

Pregabalin capsules (claiming 75 mg of pregabalin) were accurately weighted and finely powdered. The quantity of the powder equivalent to 5 mg of the drug was taken from capsule content. The drug was extracted by shaking with 20 mL of distilled water followed by other two extractions with 10 mL of distilled water. After passing through 0.45 μm Millipore filter, the solution was diluted with sufficient amount of distilled water to have concentration of 100 μg mL^{-1}. It was further diluted according to need and analyzed by proposed procedure. The nominal content of capsule was calculated from either the previously plotted calibration graphs or the corresponding regression equation.

2.6. Statistical analysis

Data are expressed as the mean ± standard deviation (SD) of obtained results. The statistical analysis of data was performed using analysis of variance (ANOVA) (Graphpad, Version 2.01, San Diego, CA).

3. Result

UV absorption of pregabalin is in lower range and exhibits λ_{max} at 210 nm and therefore, is less sensitive to UV spectrophotometric methods (Fig. 1). In this study, an attempt has been made for the development of colorimetric estimation of the drug. Since different mediums were taken, sensitivity of the drug in these mediums is noted and validated. Ninhydrin was selected as colouring agent because it has been reported to form coloured complex with pregabalin[5]. The λ_{max} of this colored complex was determined by taking wavelength scan in different mediums (Table 1). From these wavelength scans, it was seen that depending on the reaction conditions, the reaction product measured between 550-580 nm. The difference in λ_{max} was probably resulted due to difference in pH of the medium. The absorption spectrum of the ninhydrin-pregabalin colored complex in different mediums is shown in the Fig. 2a-c.

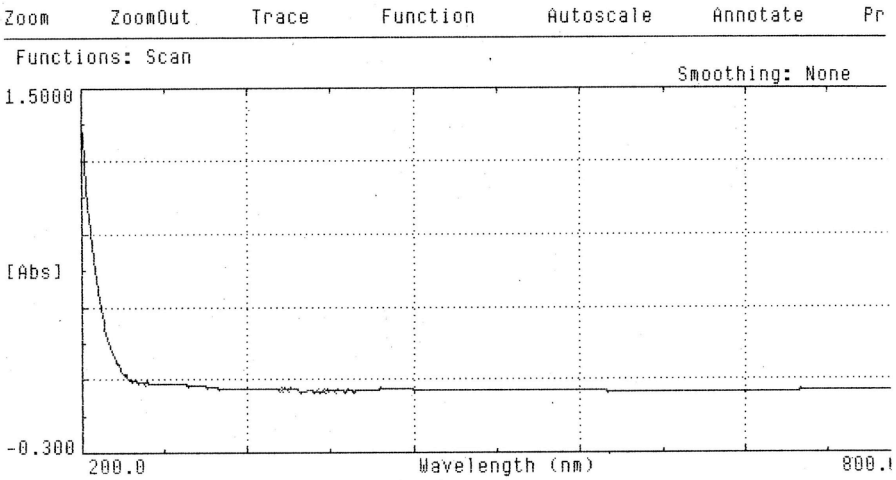


Fig. 1. UV scan spectra of pregabalin in distilled water

Under the specified optimum reaction conditions, the calibration curves for pregabalin in different mediums were constructed and shown in Fig. 3a-c. The regression equations for the results were derived. In all cases, Beer's law plots were linear with very small intercepts and good correlation coefficients were observed.

Table 2 summarizes the values of Beer's law limits, regression equation, correlation coefficient, molar absorbtivity, limit of detection (LOD) and limit of quantification (LOQ). The linear relationship was observed in the concentration range of 100-200 μg mL^{-1} in case of buffer with pH 6.8, 50-100 μg mL^{-1} in the buffer with pH 1.2 and 20-100 μg mL^{-1} in distilled water. LOD was calculated using formula 3.3σ/s, where 3.3 is a factor for LOD, σ is the standard deviation for the intercept, and s is the slope. LOQ is calculated by 10σ/s, where 10 is a factor for LOQ and rest parameters are same that of LOD.

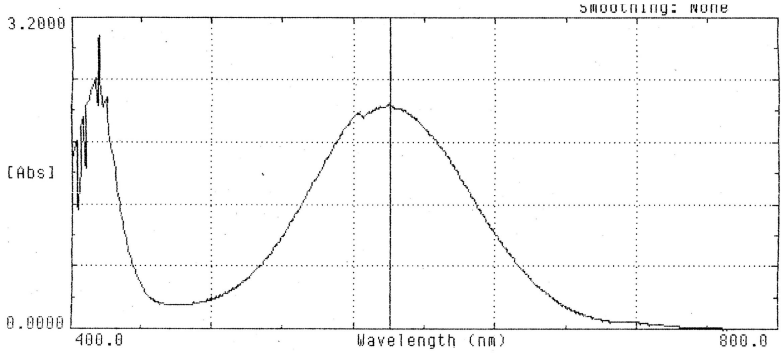
Table 1. Effect of pH of different mediums on λ_{max}

Medium taken	pH of the medium	Max Wavelength observed (nm)
Distilled water	7.4	571
Phosphate buffer	6.8	569
0.1 N HCl	1.2	555

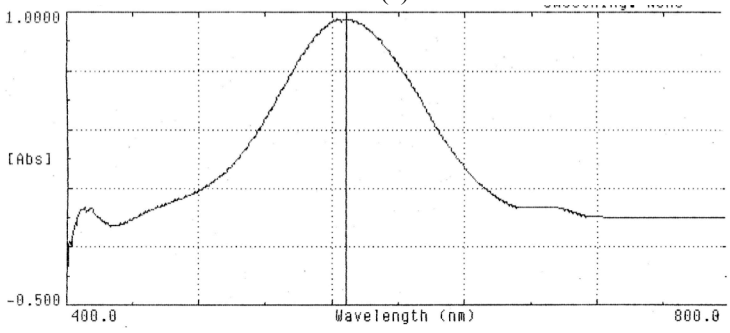
Table 2. Results of pregabalin estimation in three mediums

S.No.	Parameter	Distilled water	Buffer with pH 1.2	Buffer with pH 6.8
1	Linearity range (μg/mL^{-1})	20-100	50-100	100-200
2	Molar Absorptivity (L/mol cm^{-1})	4.561×10^3	3.522×10^3	3.023×10^3
3	Regression equation (Y = mx + c)	0.0127x – 0.273	0.009x – 0.295	0.011x – 1.261
4	Intercept ±SD	-0.275±0.002	-0.289±0.006	-1.267±0.006
5	Slope	0.013	0.009	0.011
6	Correlation coefficient r^2	0.991	0.995	0.997
7	*LOD (μg mL^{-1})	0.5196	2.4042	1.9671
8	LOD (μg mL^{-1})	1.5748	7.2855	5.9609

Values represented as mean ± SD (n=3)
*LOD is Limit of detection. **LOQ is Limit of quantification.



(a)



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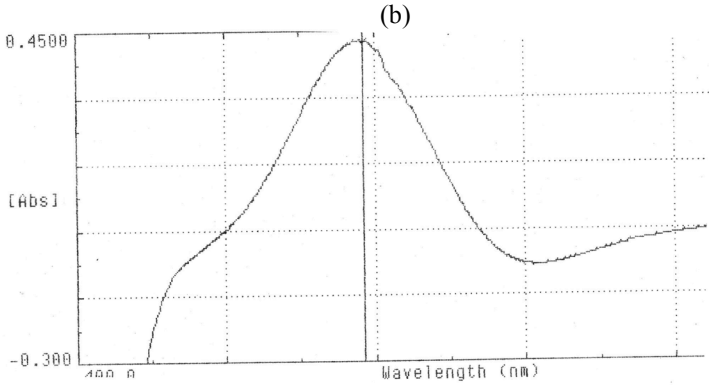
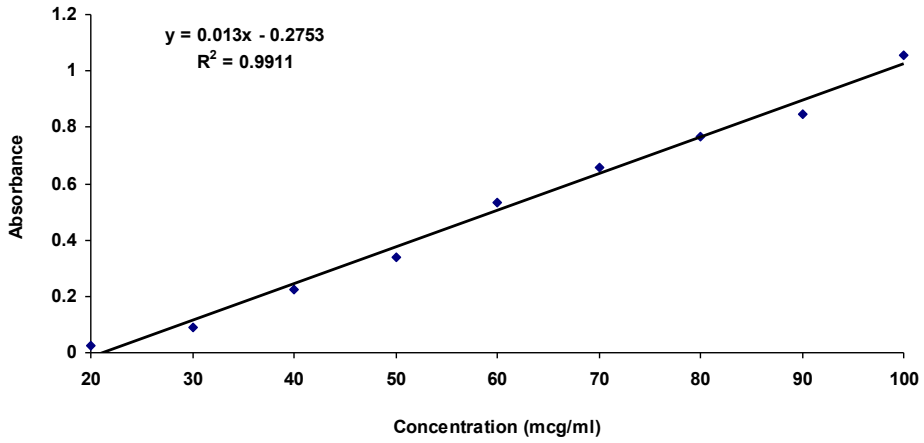


(c)

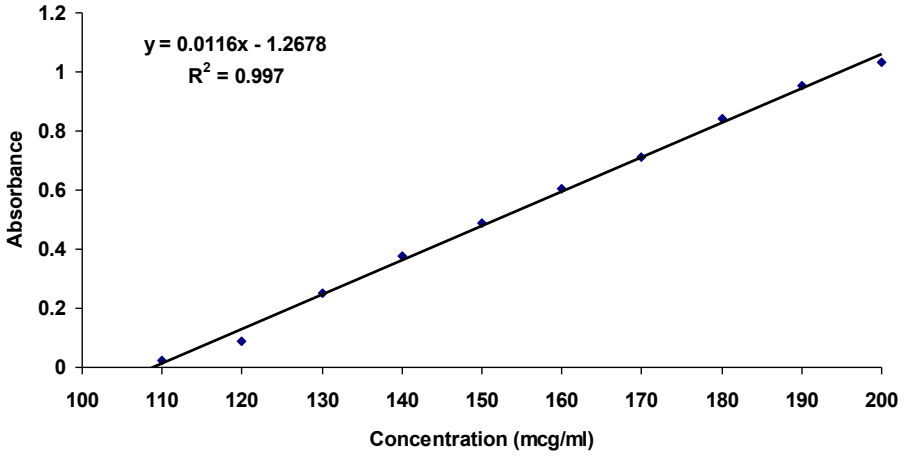
Fig. 2a-c Wavelength scan spectra of pregabalin in distilled water (a), Phosphate buffer pH 6.8 (b) and in 0.1 N HCl (c).

3.1 Method validation

The accuracy of the developed method was verified by adding known quantity of pure drug sample and assayed. The percentage recovery was calculated. Pregabalin recoveries from commercial capsules were determined for checking the accuracy of the method. Wavelength scan of marketed formulation was taken (Fig. 3) and it was found to similar with distilled water scan (Fig. 2a). This shows a good accuracy of extraction of drug from dosages form. It was very clear from the experiments that there was no interference from the excipients. Results of the analysis are shown in Table 3.



(a)



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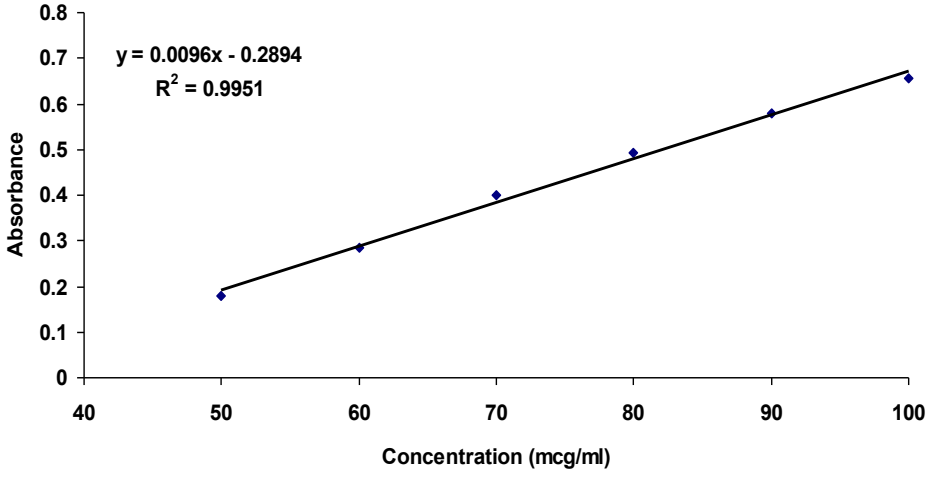

(c)

Fig. 3a-c. Calibration curve of pregabalin in distilled water (a), Phosphate buffer pH 6.8 (b) and in 0.1 N HCl.

Table 3. Results of % recovery from pure drug and pharmaceutical dosage form

S.No.	Medium taken	Amount added (µg/mL)	Total amount found (µg/mL)	% Recovery (Commercial formulation)	Total amount found in pure drug(µg/mL)	% Recovery (Pure drug)
1	Distilled water	70	68.89±0.386	98.41	68.67 ± 0.146	98.1
2		80	79.62±0.260	99.53	78.7 ± 0.349	98.5
3		90	88.74±0.311	98.60	88.38 ± 0.238	98.2

Values represented as mean ± SD (n=3)

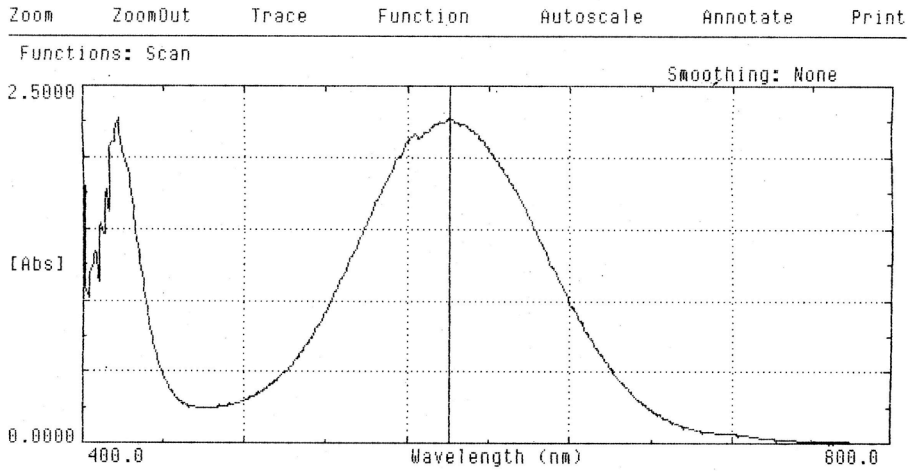

Fig. 4. Wavelength scan of extracted solution of marketed drug in distilled water.

4. Discussion

Pregabalin is a drug which has a primary aliphatic amine group and this group is known to react with many reagents to produce colored reaction products. In the present study reagent chosen is ninhydrin because it reacts with amino acid group or primary aliphatic amines very well and produces the Rheumann's purple colour complex (Fig. 5). The actual color of the product varies in light. When it is observed in artificial light it changes from blue to purple and in day light it is very much similar to the colour of potassium permanganate.[7] The λ_{max} in distilled water and buffer having pH 6.8 is around 570 nm. This may be due to polar and stearic effects of reactant. [5] The amino group in α- amino acids react with ninhydrin as a function of basicities and stearic hindrance at the temperature around 100 ˚C. The nonprotonated amino group displaces the –OH group of ninhydrin and this displacement reaction is nucleophillic type & is the rate determining step. [5]

The mechanism of reaction of pregabalin with ninhydrin in DMSO involves the reduction of ninhydrin and oxidative deamination of the primary amino group of the drug and finally their condensation to form colored reaction. For ninhydrin reaction, faint alkaline solution or alkaline conditions are required to be maintained. . [7, 8] So when we use the buffer with pH 1.2 (In 0.1 N HCl) the reaction conditions change and become acidic. The concentration of H^+ ions increases and the equilibrium is shifted. Once this type of equilibrium is achieved the colour yields become no longer kinetically controlled. In the present study, we tried to nullify this effect by using 0.1 N NaOH but the desired colour was not produced. Even the excess of 0.1 N NaOH was used to make the conditions alkaline. The reason for this may be the microprecipitation. So to overcome this problem a non aq. aprotic solvent pyridine is chosen. Pyridine was found to produce desired colour, which shows λ_{max} at 555 nm. This solvent is also capable of undergoing acid base equilibria to produce satisfactory

results. [5] One more advantage of using pyridine is that it provides desired alkalinity to reaction mixture and maintains reaction conditions.

In validation studies, during analysis of dosage forms and pure drug quite satisfactory results were obtained. The proposed method was tested for its applicability to determine pregabalin in capsules and pure form. The results obtained were indicated the excellent recovery.

5. Conclusion

The results of present study demonstrated the developed colorimetric assay can be successfully applied for routine analysis of pregabalin in bulk and pharmaceutical dosages form. The method was validated in three different mediums distilled water, 0.1 N HCl and PBS 6.8. These media are commonly used for routine quality control analysis of pregabalin in bulk and dosages form. This method is simple, selective, cost effective and less time consuming can be successfully applied to pharmaceutical formulations and pure drug sample

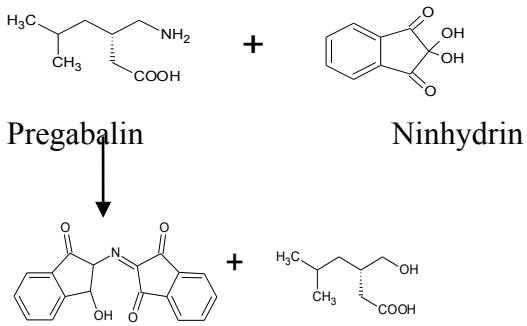

Fig. 5. Scheme of pregabalin, ninhydrin complex formation

6. Acknowledgements

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