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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PREGABALIN USING NINHYDRIN AND ASCORBIC ACID

Regina Andayani*, Supina and Friardi

Faculty of Pharmacy, Andalas University, Limau Manis Campus, Padang 25163, West Sumatera, Indonesia.

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***Corresponding Author**
Regina Andayani
Faculty of Pharmacy, Andalas University, Limau Manis Campus, Padang 25163, West Sumatera, Indonesia.

ABSTRACT

Pregabalin is an antiepileptic drug used for neuropathic pain. This study aimed to develop and validate the UV-visible spectrophotometric method for the determination of pregabalin in capsules. The analytical method was determined using the condensation reaction between pregabalin and ninhydrin-ascorbic acid in a citrate buffer pH 5. The pregabalin-ninhydrin complex was dark blue with a maximum wavelength of 568 nm. This product was assayed to evaluate the drug content in the formulation quantitatively. The calibration curve showed to be linear in the concentration range of 4-12 µg/mL with a detection limit of 0.35µg/mL and a quantitation limit of 1.19 µg/mL. The precision was assessed by determining the intra-day and inter-day precision which ranged between 0.28 – 0.82% and 0.71 – 1.13% respectively. The recovery values ranged from 99.31 ± 0.14%. These results showed that the newly developed method was simple, sensitive, accurate, and effective in determining pregabalin in capsules.

KEYWORDS: validation method, UV-visible Spectrophotometry, Pregabalin, Ninhydrin, Ascorbic acid.

INTRODUCTION

Pregabalin is a 3-isobutyl derivative of gamma-aminobutyric acid (GABA) used for therapy of painful neuropathy, anti-convulsant, anti-epileptic, and anxiolytic.[1] Pregabalin selectively binds to alpha2delta (A2D) subunits of presynaptic voltage-dependent calcium channels (VDCCs) located in the central nervous system (CNS). Binding of pregabalin to VDCC A2D

subunits prevents calcium influx and the subsequent calcium-dependent release of various neurotransmitters, including glutamate, norepinephrine, serotonin, dopamine, and substance P, from the presynaptic nerve terminals of hyperexcited neurons; synaptic transmission is inhibited and neuronal excitability is diminished.[2] The chemical name is (3S)-3-(aminomethyl)-5-methyl hexanoic acid, the molecular formula is C8H17NO2 and the molecular weight is 159.23. The structure of pregabalin can be seen in Figure 1. Pregabalin is crystal powder, white to off-white crystalline solid with solubility freely soluble in water and both basic and acidic solutions. The specific optical rotation = $+10.52^0$ (in water).[2,3,4]

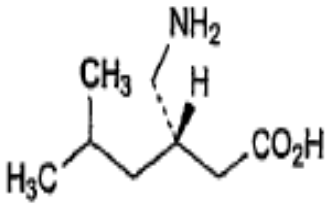

Fig. 1: Chemical structure of pregabalin.[3]

The literature study states that there are several methods for quantitative analysis of pregabalin including Thin Layer Chromatography (TLC),[5] high performance liquid chromatography (HPLC),[6] UV-visible spectrophotometry,[7] Liquid Chromatography-Mass Spectrometry (LC-MS). -MS),[8] gas chromatography-mass spectrometry (GC-MS),[9] and spectrofluorometry or fluorometry.[10]

Pregabalin determination in pharmaceutical products has also been performed by employing methods based on the derivatization of the drug with chromogenic reagents.[7,11,12,13] The UV-visible spectrophotometric method is considered a method that has a low level of difficulty, is fast, selective, sensitive, and inexpensive. Therefore, the development and validation of colorimetric methods were carried out for the determination of pregabalin in capsules.

MATERIALS AND METHODS

Reagents and materials

Standard pregabalin was obtained from PT. Kalbe Farma, ninhydrin (MILLIPORE Brand), ascorbic acid (Emsure®), sodium hydroxide (Emsure®), citric acid (Emsure®), ethanol (Emsure®), distilled water, two locally available generic and branded pregabalin tablets coded as P1 and P2 have been purchased from a local pharmacy to conduct the assay and recovery studies.

Instrument

UV- spectrophotometer (Shimadzu 1700 Pharmaspec), with a spectral bandwidth of 2 nm and 10 mm matched quartz shells were used for the developed analytical method over the range of 200-800 nm.

Preparation of 1% (w/v) ninhydrin solution

The 1% solution of ninhydrin was prepared by dissolving 1 gram of ninhydrin in 100 mL of ethanol and was kept in an amber-colored bottle.[14]

Preparation of 0.1% (w/v) ascorbic acid solution

The 0.1% solution of ascorbic acid was prepared by dissolving 100 mg of ascorbic acid in 100 mL of distilled water.[15]

Preparation of buffer solution (pH 5)

4.95 g of citric acid and 2.0 g of sodium hydroxide were dissolved in 100 mL distilled water and its pH was then adjusted to 5.0 with sodium hydroxide.[16]

Preparation of standard solution of pregabalin 1000 µg/ml

A standard solution of pregabalin was prepared by dissolving 100 mg of pregabalin in 100 ml of distilled water. The concentration of the drug was 1000 µg/ml.

The aliquot portion of the standard stock solution of pregabalin was diluted with water to obtain a concentration of 100 µg/ml.

Selection of analytical wavelengths (λ max)

For a selection of analytical wavelength range, 4 µg/ml of the drug was scanned in the UV range of 800 to 200 nm against 4.0 mL buffer solution (pH 5.0), 1.0 mL of 1%w/v ninhydrin solution, and 0.5 ml of 0.1%w/v ascorbic acid solution as blank.

Analysis of pharmaceutical formulation

Preparation of capsule sample solution

The contents of ten pregabalin capsules (claiming 75 mg of pregabalin) were mixed and weighed accurately. Then the powder equivalent to 100 mg of pregabalin was transferred into a 100 mL volumetric flask, dissolved in distilled water and sonicated for 5 minutes, filtered into a dry flask. The filtrate is added with distilled water up to the limit mark. After filtration, suitable amounts of the filtrate were then taken and the same procedure was applied as described for the calibration curve. The content of each label claim was verified by

comparing the concentrations obtained from the validated curves with the actual concentrations of the drug taken. Standard deviations were also calculated for each brand.

Validation of analysis method

Linearity

From the stock solution of pregabalin (100 µg/mL), 0,4; 0,6; 0,8; 1,0 dan 1,2 mL were quantitatively transferred to separate 10 mL calibrated screw-cap test-tubes. 4.0 mL of the buffer solution (pH 5.0), 1.0 mL of 1%w/v ninhydrin solution, and 0.5 ml of 0.1%w/v ascorbic acid solution were added and shaken for three minutes. The volume in each tube was adjusted to 9.0 mL with distilled water and kept in a boiling water bath at a temperature of 60^{o}C for 15 minutes; the tubes were then removed and cooled to room temperature. The solution in each tube was finally made up to 10.0 mL with distilled water. The blank solution was prepared similarly but without the drug. The absorption maximum was determined using the UV Vis-spectrophotometer. The solutions were scanned in the range of 800 - 200 nm against blank. The calibration graph was obtained by plotting the absorbance values at the λmax of the drug (568 nm) against corresponding concentration values and compliance with Beer Lambert's law was assessed.

Limit of Detection (LOD) and Limit of Quantity (LOQ)

LOD and LOQ of the method were established using calibration standards. LOD and LOQ were calculated as 3.3 σ/s and 10 σ/s, respectively, as per ICH definitions, where, s is the mean standard deviation of replicate determination values under the same conditions as the sample analysis in the absence of the analyte (blank determination) and s is the sensitivity, namely the slope of the calibration graphs.

Precision

Precision was investigated by analyzing three concentrations of pregabalin (4, 8, and 12 µg/mL) in three independent replicates on the same day (intra-day precision) and three consecutive days (inter-day precision). The data is represented as relative standard deviation (RSD). Low relative standard deviation (RSD) values for intra- day and interday analysis indicate good precision of the method.

Accuracy

The accuracy of the method signifies the closeness of the measured value to the true value for the sample. Accuracy is expressed as the recovery of pregabalin from the test solution using

the standard addition method at 40%, 80%, and 120% of the concentration of the test solution. The recoveries meet the method validation requirements if the range of values is 85% – 115%.[17]

The percentage recovery of the added pure drug was calculated as:

% recovery = [(Ct − Ci)/Ca]× 100

Where

Ct is the total drug concentration measured after standard addition;

Ci drug concentration in the formulation sample;

Ca, drug concentration added.

RESULTS AND DISCUSSION

The development and validation of the UV-visible spectrophotometric method for the determination of pregabalin levels with ninhydrin and ascorbic acid reagents can be well analyzed. This method was chosen because the UV-visible spectrophotometric method is a simple, accurate, and efficient analytical method. The proposed method mechanism is based on a condensation/coupling reaction between pregabalin and ninhydrin-ascorbic acid at pH 5. Pregabalin has a primary amine group that can react with ninhydrin-ascorbic acid to form a condensation reaction under acidic conditions made using citric acid buffer (pH 5) to produce a dark blue-purple color (Ruhemman's purple) (Figure 2). In this study, the color formed in the pregabalin stock solution was dark blue.

The solvent used in this study was distilled water. This is because pregabalin is polar, so it is easily soluble in distilled water. The chromophore agent used in this study was ninhydrin with the addition of ascorbic acid. According to the literature, ninhydrin is one of the chromogenic agents used for amino acid analysis. Ninhydrin can react with compounds containing amino acid groups and will form a dark blue-purple complex.[18] In this study, pregabalin was able to form a dark blue-purple complex with ninhydrin. While ascorbic acid acts as a catalyst, ascorbic acid does not react but helps accelerate the reduction of ninhydrin to hydridantine. According to Subbayamma, A.V et al, 2008, ascorbic acid can reduce ninhydrin to hydridantine.[15] The addition of a buffer solution with a pH of 5 serves to stabilize the pH of the solution in the mixture. According to Moore and Stain in Ajima (2015), the reaction of ninhydrin with the amine group must be maintained at pH 5 so that the reaction remains stable and the color of the product is optimal.[19]

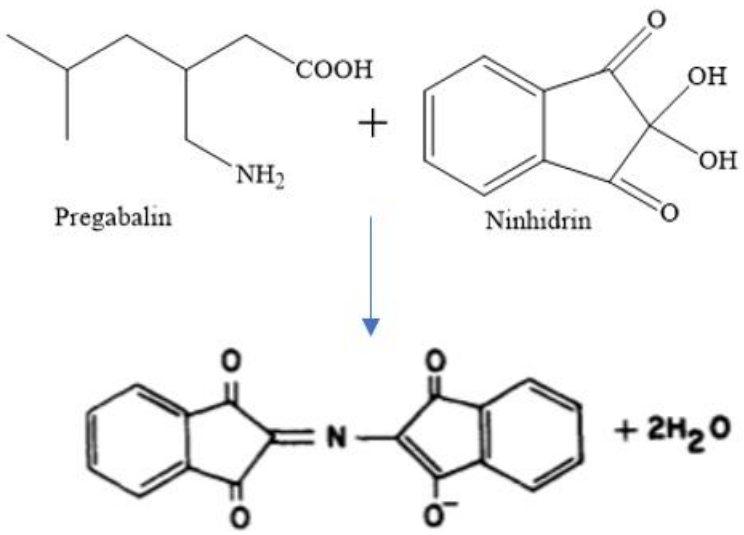

Fig. 2: Proposed reaction pathway between pregabalin and ninhydrin-ascorbic acid.

The maximum wavelength was obtained at 568 nm with an absorbance of 0.217 at a concentration of 4 g/mL. The UV-visible absorption spectrum of pregabalin can be seen in Figure 3.

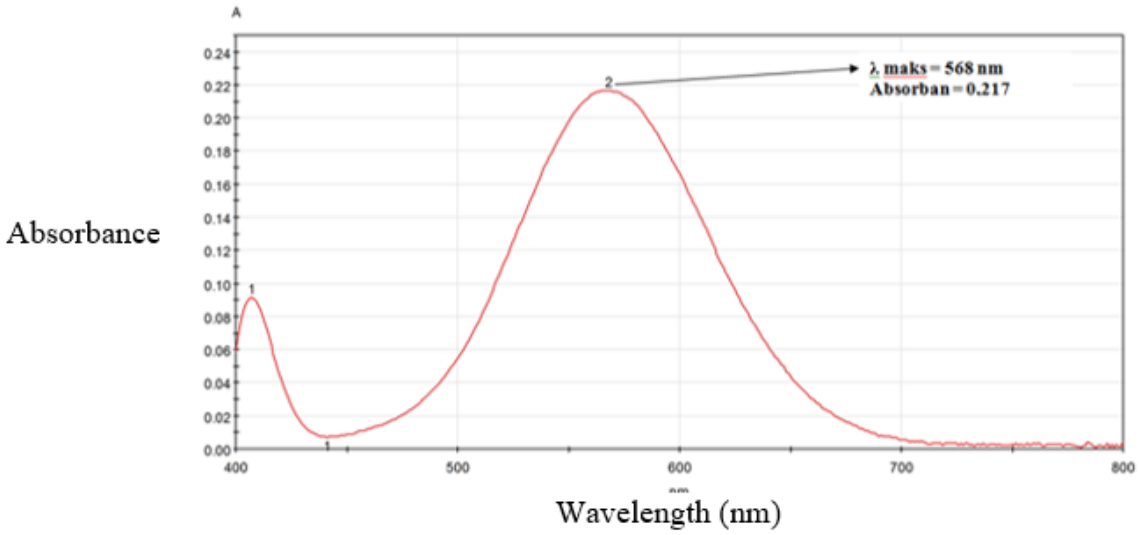

Fig. 3: Absorption spectrum for pregabalin-ninhydrin-ascorbic acid condensation product.

Validation of the analytical method aims to ensure that the analytical method is accurate, specific, reproducible, and resistant to the range of analytes to be analyzed. The validation parameters of the analytical method tested are linearity, sensitivity which includes detection limit and quantization limit, precision, and accuracy.[20,21]

The first validation step is linearity. Linearity refers to the linear relationship between concentration and absorbance. The linearity test was carried out at a concentration of 4-12 µg/mL and the regression equation y=0.0491x + 0.0454 with r=0.9989 was obtained (Figure 4). The correlation coefficient gives linear results because it meets the acceptance criteria and these indicate good linearity over the working concentration ranges [21]

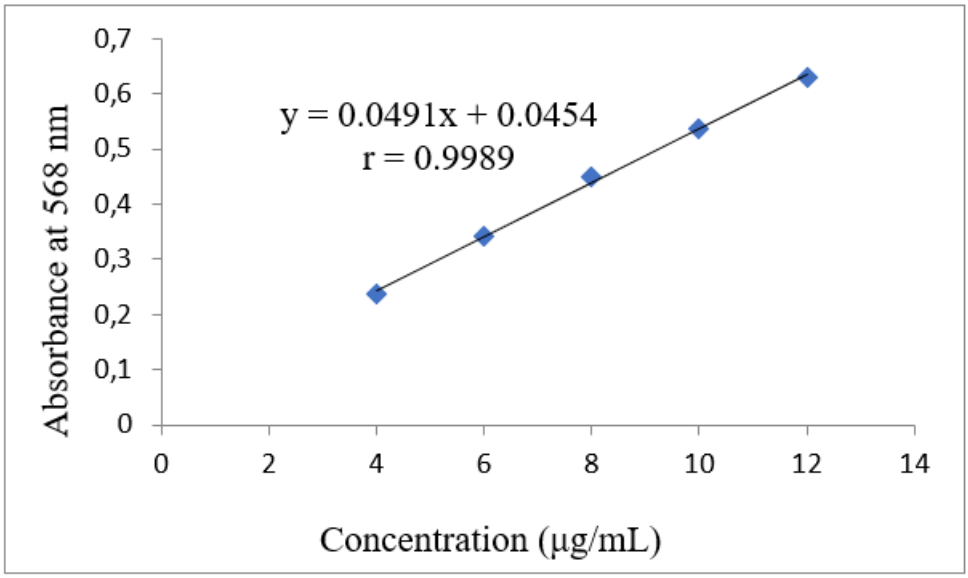

Fig. 4: Calibration curve of pregabalin.

Determination of the sensitivity of the analytical method including the limit of detection and limit of quantitation is one of the requirements for validating the analytical method.[20] The detection limit value of pregabalin is 0.35 µg/mL (Table 1), meaning that the UV-visible spectrophotometer used can detect pregabalin with the smallest concentration of 0.35 µg/mL. Pregabalin quantitation limit value of 1.19 µg/mL (Table 1) means that the analytical method provides accurate and precise results with the smallest concentration of 1.19 µg/mL.

In the study of Gupta C et al in 2013, the detection limits and quantitation limits were 0.5196 µg/mL and 1.5748 µg/mL. Meanwhile, in this study, the detection and quantitation limits were 0.3590 µg/mL and 1.1948 µg/mL. This shows that the addition of ascorbic acid can increase the sensitivity of the analytical method.

Table 1: Validation data for determination of pregabalin by the proposed method.

Validation parameter	Results
Absorption maximum	568 nm

Regression equation	y=0.0491x + 0.0454
Slope	0.0491
Intercept	0.0454
Beer's law limit (µg/mL^{-1})	4-12
Coefficient of correlation(r)	0.9989
Limit of detection (LOD)	0.3590 µg/mL
Limit of quantification (LOQ)	1.1948 µg/mL
Accuracy	99.31 ± 0.14%
Intra-day Precision	0.28-0.82%
Inter-day Precision	0.71-1.13%
% Purity	97.47%

Precision is the degree of closeness between individual test results when the procedure is applied repeatedly to multiple sampling or homogeneous samples. This precision test aims to see the selectivity, reproducibility, and repeatability of the data as well as the stability of the compounds used in the analysis, and the requirement for acceptance of intra-day and inter-day precision tests are <2%.[20] Intra-day and inter-day precision tests were performed at a concentration of 4; 8; 12 µg/mL and the relative standard deviation values obtained can be seen in Table 2. The test results show that the precision test results are < 2%, which means that the precision test has met the validation criteria.

Table 2: Precision of the proposed methods for the analysis of pregabalin.

Pregabalin (µg/mL)	**Within day, n=3**		**Between day, n=3**	
	Mean±SD	RSD (%)	Mean±SD	RSD (%)
4	3.79 ± 0.03	0.82	3.77 ± 0.04	1.13
8	7.61 ± 0.04	0.53	7.53 ± 0.05	0.71
12	11.00 ± 0.03	0.28	10.81 ± 0.10	0.93

The accuracy test expressed as recovery values at standard additions of 40%, 80%, 120% on branded pregabalin capsules were 97.32%, 100.16%, and 100.45%. While the generic pregabalin capsules were 97.08%, 99.96%, and 99.56%. The results of the accuracy tests conducted by this method are shown in Table 3.

The recovery test has acceptance criteria in the range of 85–115%.[21] The results of the validation test of the analytical method can be concluded that the newly developed analytical method is sensitive, precise, accurate, and effective for the determination of pregabalin levels in capsule preparations.

Table 3: Results of recovery study using the standard addition method.

No	**Sample**	**Endogenous**	**Pure**	**Amount**	**% Recovery**

		value	pregabalin	found	± SD*
		(µg/mL)	added (µg/mL)	(µg/mL)	
1	Generic	6	2.4	8.02	97.08 ± 0.08
			4.8	10.39	99.96 ± 0.07
			7.2	12.67	99.56 ± 0.02
2	Branded	6	2.4	8.12	97.32 ± 0.02
			4.8	10.53	100.16 ± 0.01
			7.2	12.90	100.45 ± 0.38

*Average of three determinations

Analysis of marketed formulation (Pregabalin capsules)

Table 4 gives the results of the assay for pregabalin carried out on marketed formulation by the proposed method and revealed that there is close agreement between the results obtained by the proposed methods and the label claim. The recovered drug content of pregabalin in branded and generic pregabalin capsules were 97.47± 0.56 % and 96.23 ± 2.04%, respectively.

Table 4: Assay results for pregabalin determination in capsule formulation.

Formulation	Label claim (mg)	Mean Recovery (mg) ± SD*	Mean Recovery* (%) ± SD*
Generic	75	72.17 ± 1.53	96.23 ± 2.04
Branded	75	73.10 ± 0.42	97.47 ± 0.56

*Average of three determinations

The results with the proposed method for the determination of pregabalin in its pharmaceutical formulation (Pregabalin 75 mg) suggest satisfactory recovery. Further, standard addition techniques followed to check the validity of the method has given good recoveries of the drug in presence of formulation suggesting a non-interference from formulation excipients. Hence, this method can be recommended for adoption in routine analysis of pregabalin in quality control laboratories.

CONCLUSION

The UV-visible spectrophotometric method for the determination of pregabalin using ninhydrin and ascorbic acid reagents met the validation criteria of the analytical method which included linearity, detection limit, quantitation limit, precision, and accuracy. The newly developed analytical method is simple, sensitive, accurate, and effective for the determination of pregabalin in capsules.

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