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The Development And Validation Of Spectrophotometric Assay For Determination Of Gabapentin In Capsules Using Ninhydrin And Ascorbic Acid

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Abstract:
Background*:Gabapentin, 1-(Aminomethyl)cyclohexane acetic acid; is a γ-aminobutyric acid (GABA) analog used to treat partial seizures and neuropathic pain. This study aims to develop and validate an accurate, efficient and simple UV-visible spectrophotometric method for the determination of gabapentin content in capsule preparations.*
Materials and Methods*: The method is based on the coupling reaction of the primary amino group of gabapentin with ninhydrin-ascorbic acid at pH 5 to form a purple diketohydrindamine-diketohydrindylidene (Ruhemann purple) with a maximum wavelength of 568 nm. The validation of the analytical method includes linearity, limits of detection, limits of quantitation, precision and accuracy.*
Results*: The linearity test in the concentration range of 30-70 µg/mL yielded a value of r = 0.9999 with detection and quantitation limits of 1.06 µg/mL and 3.22 µg/mL. Precision tests at concentrations of 30;50; and 70 µg/mL resulted in relative standard deviation (RSD) of 1.07%, 1.09% and 1.23% for intra-day precision and 1.56%, 1.02% and 0.70% for inter-day precision. The percentage recovery on generic drug was 96.76-100.50%, and on branded drug was 96.41-99.79%. The suggested method could be used for the determination of gabapentin in capsules. No interference could be observed from the additives in the capsules.*
Conclusion: *The developed method was successfully validated and applied to the determination of gabapentin in capsules without any interference from common excipients. Hence, this method can be potentially useful for routine laboratory analysis of gabapentin.*
Key Word*: gabapentin, ninhydrin, ascorbic acid, spectrophotometric, validation*

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I. Introduction

Gabapentin or 1-(aminomethyl) cyclohexaneacetic acid is an analogue of gamma-aminobutyric acid (GABA), it is neither a GABA agonist nor antagonist and its mechanism of action is unknown. Gabapentin is an antiepileptic used as monotherapy or adjunctive therapy in the treatment of partial seizures with or without secondary generalisation. Gabapentin is also used in the treatment of neuropathic pain[1].

Gabapentin with molecular formula $C_9H_{17}NO_2$ and molecular weight 171.24. Gabapentin is a crystal solid; white to almost white with solubility, easily soluble in water, in acidic and basic solutions[2]. A 2% solution in water has a pH of 6.5 to 8.0 [3]. The structure of gabapentin can be seen in Figure 1.

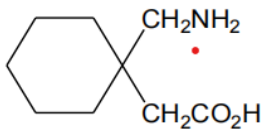

Figure 1. Chemical structure of gabapentin[2]

Determination of gabapentin levels has been carried out using various methods, especially using high performance liquid chromatography (HPLC) with various detection methods[4,5,6,7,].They are more labor intensive, time consuming, not easy to work with them, and the columns and solvents are expensive, dangerous, or unavailable. In addition, the reported gas chromatography (GC)[8] and capillary electrophoresis (CE) methods[9] for the determination of gabapentin require laborious sample pretreatment and laborious cleaning procedures prior

to analysis. They are likely to be limited by the routine availability of these techniques. High performance thin layer chromatography (HPTLC)[10], and UV-Vis spectrophotometry[11] have been used for the determination of gabapentin in pure form, pharmaceutical formulations, and/or biological samples either alone or in combination with other drugs.

Gabapentin has been analyzed by ultraviolet spectrophotometry at a wavelength of 210 nm[12]. The structural formula of gabapentin does not have a strong chromophore group so it cannot be detected properly at ultraviolet wavelengths. Therefore, measurement of gabapentin levels must be carried out at visible wavelengths using chemical reagents as chromogens. Several chromogenic agents that have been used for gabapentin analysis include ninhydrin and acceptor, salicyl aldehyde, reagents acetyl acetone and formaldehyde, 2,5-dihydroxybenzaldehyde, p-Dimethyl aminobenzaldehyde, chromotropic acid and 4-Chloro-7-Nitrobenzo- 2-Oxa- 1,3-Diazole (NBD-Cl)[13-19]

Ninhydrin and ascorbic acid have been used for drug analysis by UV-Visible spectrophotometry method. Some drugs that have used ninhydrin and ascorbic acid are lisinopril at a wavelength of 595 nm and acyclovir at a wavelength of 540 nm[20,21]. Ninhydrin is a chromogenic agent that can react with amine groups, amino acids and thiophene to produce the color diketohydrindamine-diketohydrindylidene or Ruhemann's Purple[20]. Ascorbic acid acts as a reducing agent on ninhydrin compounds to form hydridanthin[22].

The UV-Vis spectrophotometric method is widely used for quality control of pharmaceuticals because of its distinctive advantages such as lower cost, durability, simplicity, good accuracy and precision[14]. Therefore, this research developed a method for determining gabapentin levels in pharmaceutical preparations by derivatization reactions using ninhydrin and ascorbic acid to produce chromophores that can be detected by UV-Vis detectors[20].

Validation is the act of proving that any procedure, process, equipment, material, activity or system functions as expected under a certain set of conditions and also provides the required accuracy, precision, sensitivity and ruggedness[23].

II. Material And Methods

Instrument

UV-Vis spectrophotometry (Thermo Scientific). The UV-Visible spectrophotometer was equipped with a quartz cell with a 10 mm path length. E. KERN electrical balance is used for weighting the sample.

Reagents and chemicals

Gabapentin was purchased from (Jiangxi synergy pharmaceutical CO., LTD), All chemicals used were of analytical grade. Ninhydrin, sodium hydroxide, ethanol pa, citric acid, ascorbic acid, distilled water, generic and branded gabapentin capsules (labeled to contain 300 mg gabapentin) were obtained from a pharmacy in Padang, West Sumatera, Indonesia..

Methods

Preparation of ninhydrin solution (1% w/v)

A 1% of ninhydrin was prepared by dissolved by weight 1 g of ninhydrin in ethanol. Then the volume was completed to 100 ml in a volumetric flask with ethanol.

Preparation of 0.1% ascorbic acid solution

A 0.1 g amount of ascorbic acid dissolved in distilled water and the volume was completed to 100 ml in a volumetric flask.

Preparation of buffer solution (pH 5)

4.95 mg of citric acid and 2 g of NaOH were weighed and dissolved in distilled water and the volume was completed to 100 ml in a volumetric flask.Then check the pH with a pH meter

Preparation of standard stock solution of gabapentin 5 mg/mL

Standard gabapentin stock solution (5 mg/ml) was freshly prepared by dissolving 500 mg of gabapentin standard in 100 ml volumetric flask using distilled water.

Selection of the maximum absorption wavelength for chromogen

Into 25 ml volumetric flasks, 2.5 ml of gabapentin stock solution (5 mg/ml) were transferred and then 10 ml of buffer solution, 5.75 mL of 1% (w/v) ninhydrin, followed by ascorbic acid 0.1% (w/v) solution were added. The content of each flask was shaken thoroughly and heated on a water bath at 60°C for 30 minutes. The flasks were left to cool for 10 minutes, then volume was completed with distilled water to have 500 µg/ml of

gabapentin. 3 mL of this solution was transferred to a 25 mL volumetric flask and completed with distilled water to obtain a concentration of 60 µg/mL. The absorbance was measured at a wavelength of 400–800 nm and the maximum absorption wavelength (λmax) was determined.

Method validation

Analytical method validation was performed according to the ICH guidelines (2005) with respect to accuracy, precision, specificity, linearity, limit of detection (LOD), and limit of quantitation (LOQ).

Gabapentin calibration curve creation (Linearity)

The linearity of the proposed method was determined by measuring the absorbance of six concentrations (30, 40, 50, 60, and 70 µg/ml of gabapentin) covering the range (30–70 µg/ ml). Five measurements were done for each concentration. The absorbance was plotted against concentration. The regression line and correlation coefficient were evaluated as well as validity of regression line was verified by statistical analysis.

Limit Detection (LOD) and Limit Quantitation (LOQ)

LOD is done using the formula; LOD = 3.3 SD / S and LOQ is done using the formula; LOQ = 10 SD/S; where SD is the standard deviation of the residuals from the regression line and S is the slope of the regression line.

Precision

Intra-day precision was assessed by analysis of three different concentrations (30, 50 and 70 µg/ml) of gabapentin at three different time periods of the same day. While, the inter-day precision was studied by repeating the procedure for three different concentrations (30, 50 and 70 µg/ml) of gabapentin on three different days and the relative standard deviation (RSD) was calculated for each concentration.

Accuracy

Accuracy was obtained by calculating the recovery of gabapentin from the test solution using the standard addition method of 80%, 100%, and 120% of the concentration of the test solution. The recovery meets the method validation requirements if the % recovery with a value of 85%–115%.

Determination of gabapentin levels in pharmaceutical preparations (capsules)

Twenty capsules were emptied, weighed, and mixed thoroughly. An accurately weighed portion of the resulting powder equivalent to 125 mg gabapentin was taken and dissolved in 25 ml distilled water with good shaking for 5–10 minutes, then filtered with filter paper to remove insoluble matter. The filtrate solution was completed with distilled water to have 5 mg/ml of gabapentin. Into 25 ml volumetric flasks, 2.5 ml of gabapentin stock solution (5 mg/ml) were transferred and then 10 ml of buffer solution, 5.75 mL of 1% (w/v) ninhydrin, followed by ascorbic acid 0.1% (w/v) solution were added. The content of each flask was shaken thoroughly and heated on a water bath at 60°C for 30 minutes. The flasks were left to cool for 10 minutes, then volume was completed with distilled water to have 500 µg/ml of gabapentin. 8 mL of this solution was transferred to a 100 mL volumetric flask and completed with distilled water. The absorbance was measured at a maximum absorption wavelength (λmax). Triplicate measurements were done for each concentration and the percentage of recovery was calculated from the calibration curve.

III. Results and Discussion

Selection of the maximum absorption wavelength for chromogen

The method development for determining gabapentin levels by UV-Vis spectrophotometry was chosen because it is a simple, accurate and efficient analysis. This analytical method uses several reagents such as ninhydrin, ascorbic acid, buffer solution and distilled water. Ninhydrin functions as a chromogenic reagent which produces diketohydrindamine-diketohydrindylidene which gives a purple color (Ruhemann's purple) so that it can be detected at visible wavelengths (400-800 nm)[13]. Ascorbic acid acts as a reducing agent on ninhydrin compounds to form hydridanthin[20]. Buffer solution to maintain the pH of the solution to remain at pH 5 because this reaction occurs in acidic conditions[22].

The procedure consists of certain steps such as heating, grinding and filtering. The heating process is a thermal degradation reaction so that amino acids will produce amine electrons (NH_2) which can react with ninhydrin[13,22]. Grinding is done to homogenize the sample. Furthermore, filtering is carried out to obtain a filtrate that is free of insoluble compounds that can inhibit absorption in the spectrum analysis area.

The maximum wavelength of gabapentin was determined in the visible wavelength range (400-800 nm). In this study, the maximum absorption wavelength was 568 nm with an absorbance of 0.532 at a concentration of 60 µg/mL. The gabapentin absorption spectrum can be seen in Figure 1

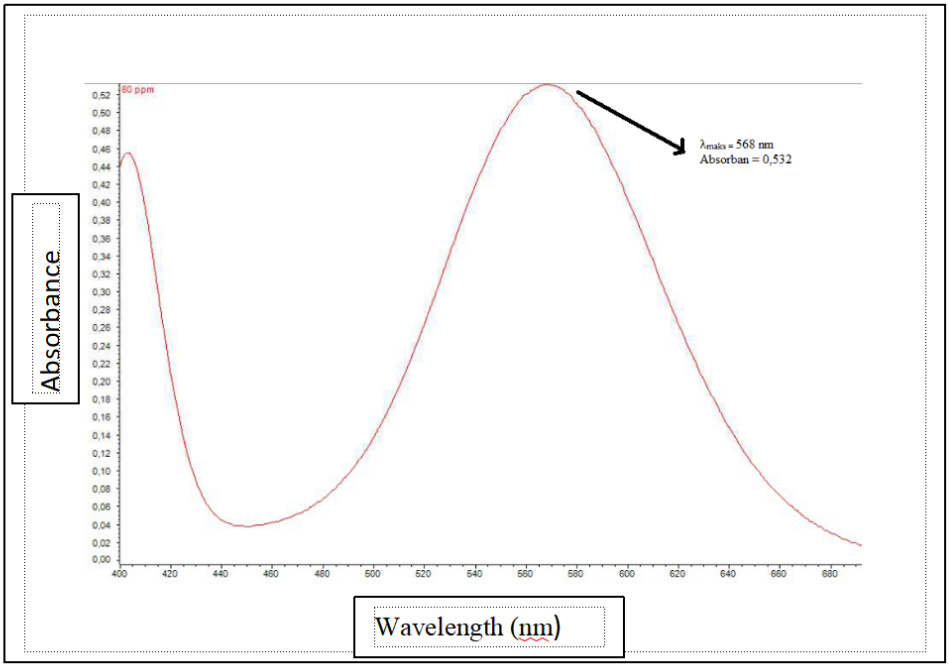

Fig.1 UV-Vis absorption spectrum of gabapentin 60 µg/mL with ninhydrin-ascorbic acid in distilled water

Ninhydrin reagent is used for the determination of an aliphatic primary amine or an amino acid group[22,24]. The presence of an aromatic ring inhibits the response; the inhibition increasing the nearer amine group is to the ring. The reaction is usually carried out by heating for a short time in an acidic-medium of ascorbic acid. Gabapentin reacts with ninhydrin reagent in ascorbic acid medium via oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored reaction product-Ruhemenn's purpre-with λ_{maks} at 568 nm (Fig. 2).

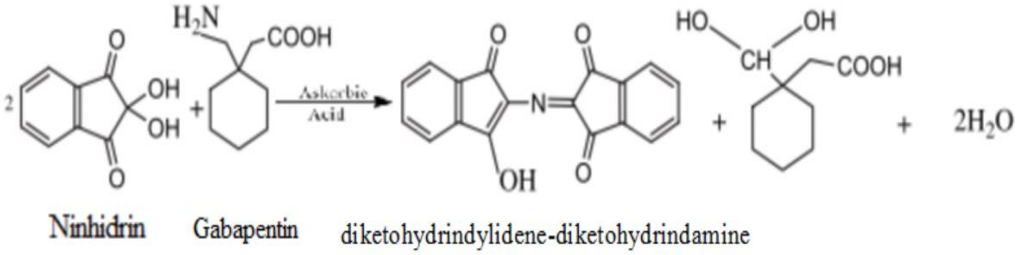

Fig 2. Proposed mechanism of the reaction between gabapentin and ninhydrin in presence of ascorbic acid

Validation

The method was validated with respect to linearity and range, accuracy and precision, limit of detection (LOD) and limit of quantification (LOQ), selectivity and robustness[25,26]. The developed method was validated for the pure drug as well as marketed formulation of gabapentin (generic) and gabapentin (branded). The various validation parameters are shown in Table no 1.

Table no1: Validation data for determination of gabapentin by proposed method

Validation parameter	Results
Absorption maximum	568 nm
Regression equation	Y =0,009x - 0,0161
Beer's law limit (µg.mL^{-1})	30-70
Coefficient of correlation (r)	0,9999
Limit od detection (LOD)	1,0637547
Limit of quantification (LOQ)	3,2234991
Accuracy	98,49-99,06

Precision (% CV)	Intraday = 1.130 Interday = 1,095
% Purity	104,40-109,55

Linearity is the ability of the analytical method to provide a direct or good mathematical response, proportional to the concentration of the analyte in the sample. The observed parameter is the value of r, where a data is linear if the value of r = 1 or -1. If the correlation coefficient value obtained is close to one then the analytical method is concluded to have a linear calibration[25,26]. The linearity test obtained a value of r = 0.9999, so it can be concluded that the results are linear. Standard plot of gabapentin by ninhydrin method can be seen in Figure 3.

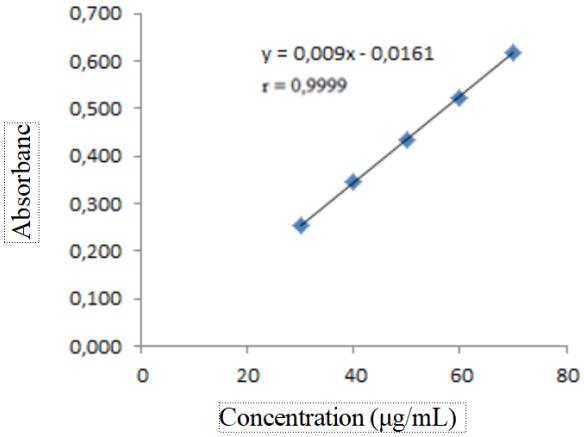

Fig 3. Standard plot of gabapentin by ninhydrin method.

Limit of detection (LOD) and limit of quantitation (LOQ.) of the method were established using calibration standards (Fig 3). LOD and LOQ were calculated as 3.3 s/s and 10 s/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, i.e. the slope of the calibration graph. The detection limit and quantization value limit can be seen in Table no. 1.

Precision is a measure of the closeness between observed values obtained independently from several homogeneous samples and expressed as the relative standard deviation (RSD) of the observed values[25,26]. In this study, the precision was determined intraday and interday on days 1, 2 and 3. The precision test was carried out at three standard concentrations of 30; 50; and 70 µg/mL. The coefficient of variation is 1.07%; 1.09%; and 1.23% on intraday testing and 1.56%; 1.02%; and 0.70% on the interday test, respectively. The coefficient of variation is ≤2%. Therefore, the results of the precision test have met the validation requirements[26]. The results of precision determination can be seen in Table no 2.

Table no 2: Precision of the proposed methods for the analysis of gabapentin

Gabapentin (µg.mL^{-1})	Intra-day, n=3		Inter-day, n=3	
	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
30	27,46±0,29	1,07	26,97± 0,42	1,56
50	44,46 ±0,48	1,09	43,97±0,45	1,02
70	60,20±0,74	1,23	59,72±0,42	0,70

In this study the intra day and interday precision values are 1,13% and 1,09%, respectively.

Analysis of gabapentin using UV-vis spectrophotometry with ninhydrin-ascorbic acid obtained a coefficient of variation of 1.11%. Meanwhile, the study by Siddiqui et al., 2010, obtained a higher precision value of 1.34%. It can be concluded that the results of gabapentin analysis using ninhydrin-ascorbic acid have a better precision value[13.]

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[25]. According to the FDA in Yusfa, the recovery test has acceptance criteria ranging from 85%–115%[25,26]. Recovery values from standard addition method for generic gabapentin capsules analysis ranged from 96.76 to 100.50% (Table no 3). Recovery studies with branded gabapentin capsules returned values ranging from 96,41 to 98,22% (Table no 4). The results in the accuracy table show that the percentage of recovery meets the criteria. The results of the validation of the analytical method show that the analytical method used is valid.

Table no 3: Results of recovery studies with generic gabapentin capsules by standard addition method

Initial Conc ((μg.mL^{-1})	Conc added ((μg.mL^{-1})	Conc recovered ((μg.mL^{-1})	Recovery(%)± SD[a]
43,83	17,53	60,79	96,76± 0,82
	35,05	78,86	99,93± 0,64
	52,58	96,68	100,50± 0,77

[a]Average of determinations carried out in triplicate.

Table no 4: Results of recovery studies with branded gabapentin capsules by standard addition method

Initial Conc ((μg.mL^{-1})	Conc added ((μg.mL^{-1})	Conc recovered ((μg.mL^{-1})	Recovery(%)± SD[a]
41,75	16,71	57,86	96,41± 0,92
	33,41	75,09	99,79± 0,65
	50,12	90,98	98,22± 0,74

[a]Average of determinations carried out in triplicate.

Analysis of marketed formulation (gabapentin capsules)

In determining the levels of gabapentin in generic and branded capsules, The recovered drug content of gabapentin in generic and branded capsules on marketed formulations were found to be 109,55 and 104,396%. The results can be seen in Table no 5.

Table no 5: Assay results for gabapentin determination in capsule formulation

Formulation	Label claim (mg)	Mean recovery (mg) ±SD[a]	Mean recovery (%) ±SD[a]
Gabapentin (generic)	300	328,564±0,62	109,55±0,77
Gabapentin (branded)	300	313,278±0,47	104,396±0,54

[a]Average of nine determinations, three each with starting powder amount equivalent to 300 mg gabapentin

The results of the proposed method for determining the concentration of gabapentin in its pharmaceutical formulation in both generic and branded gabapentin capsules showed satisfactory recovery.

IV. Conclusion

Validation of the analytical method has provided rigorous and accurate results for the analysis of gabapentin in pharmaceutical formulations using ninhydrin-ascorbic acid without any interference from the formulation excipients. Therefore, this spectrophotometric method can be recommended for routine application to the analysis of gabapentin in quality control laboratories.

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