Validated UV-Vis Spectrophotometric Method for Determination of Gabapentin using Acetyl Acetone and Formaldehyde Reagents

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Abstract

Gabapentin is an anticonvulsant widely used in the treatment of epilepsy. An accurate and validated spectrophotometric method was developed as an alternative method to determine gabapentin in pharmaceutical products. This is a simple, accurate, precise, selective and visible spectrophotometric method. The method is based on the measurement of drug absorbance as a result of yellow solution formation reaction between gabapentin and condensation products both from 2 moles acetyl acetone and 1 mole formaldehyde in basic condition (in boric buffer pH 9.5) at 55°C for 15 minutes. The detection was performed at 340 nm. This method is linear in the range of 10.8–80.0 µg/mL with correlation coefficient (r²) of 0.99954. The method was validated according to USP Category I requirements for gabapentin. The validation characteristics include accuracy and precision, linearity, range, limit of detection and limit of quantitation. The acceptance validation criteria were found in all cases.

Key words: acetyl acetone, formaldehyde, gabapentin, method, spectrophotometry, validation, visible.

1. Introduction

Gabapentin [1-(amino methyl) cyclohexane acetic acid; structure I] is a γ-amino butyric acid (GABA) analog used for partial seizure treatment in adults and children1. Its action mechanism is still not clear. It has been suggested that gabapentin may bind to an undefined receptor or bind site in the brain2.

Gabapentin is a white to off-white crystalline solid with a pKₐ₁ of 3.7 and a pKₐ₂ of 10.7. It is freely soluble in water and both in basic and acidic aqueous solutions3.
Several analysis methods of gabapentin either in dosage forms or in human body fluids have been developed including spectrophotometric, spectrofluorometric, colorimetric, capillary electrophoresis, LC-MS-MS, and GC-MS methods. Gabapentin derivation has also been analyzed with HPLC–UV and HPLC–fluorescence.

The aim of the present study is to develop a new analysis method of gabapentin that enhances the selectivity and simplicity of the visible spectrophotometric method using acetyl acetone and formaldehyde to form its new chromophore.

![Figure 1. Structure of gabapentin.](image)

2. Materials and Methods

2.1. Materials

Gabapentin certified reference standard was purchased from Jiangxi Synergy Pharmaceutical Co., LTD, China. Gabapentin drug substance was purchased from Kimia Farma, Indonesia, acetyl acetone, formaldehyde (E. Merck), boric acid (E. Merck), and potassium chloride (E. Merck). Spectral runs were made on PD-303UV spectrophotometer (Apel) with 1 cm matched glass cell.

2.2. Standard Gabapentin Solution

A stock solution of gabapentin (1000 µg/mL) was prepared by dissolving 100 mg gabapentin in 100 mL volumetric flasks with double distilled water. It was used to prepare the working solutions by suitable dilutions with distilled water.

2.3. Preparation of Boric Acid and 0.25 M Potassium Chloride Solutions

Boric acid was prepared by dissolving 7.729 grams boric acid and 9.319 grams potassium chloride in 500.0 mL volumetric flasks with double distilled water.

2.4. Preparation of Boric Acidic Buffer

Boric acidic buffer was prepared by dissolving 25.0 mL boric acid and 0.25 M potassium chloride solutions as well as 0.2 N sodium hydroxide until reaches pH 8.0 in 100.0 mL volumetric flasks with double distilled water. It was prepared in pH 8.5; 9.0; 9.5; 10.0; 10.5 and 11.0 respectively.

2.5. Preparation of Gabapentin Standards

Standard solutions were prepared by diluting stock solution for the final concentrations of 40.0; 50.0; 60.0; 70.0 and 80.0 respectively in optimum boric acidic buffer.
2.6. Determination of Optimum Condition
Gabapentin and Acetyl Acetone and Formaldehyde

Gabapentin working solution (60 µg/mL) (6 mL) was mixed with reagent solution (2.0 moles acetyl acetone and 1.0 mole formaldehyde freshly) in 100.0 volumetric flasks. To reach the optimum working condition, the working solution was treated in some pH that were 8.5; 9.0; 9.5; 10.0; 10.5 and 11.0 respectively. The pH optimum condition is the highest absorbance between them. The temperature optimum condition of working solution was treated in some temperatures that are 45; 55; 65 and 75°C respectively. The optimum condition is the highest absorbance between them. Another optimum condition in this study is incubation time which treated in some time reaction between gabapentin and reagent solution that are 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes respectively and the optimum condition based on the highest absorbance of these solutions.

2.7. Method Validation

The method was validated according to the United States Pharmacopeia Category I requirements. The following validation characteristics addressed: linearity, range, accuracy, precision, limitation of detection and quantization.

2.7.1. Linearity and Range

Standard calibration curves were prepared with fifth calibrators over a concentration range of 40-80 µg/mL (40.0; 50.0; 60.0; 70.0; and 80.0 µg/mL) for gabapentin. The absorbance data versus gabapentin concentration were treated by linear correlation coefficient. The standard curves were evaluated for intraday linearity. The range was internal between the highest and lowest analyzed concentration of which are its obtained acceptable linearity, accuracy, and precision.

2.7.2. Accuracy and Precision

The accuracy and precision of the proposed method was established by intraday assay by determining the content of gabapentin in the first capsule of pharmaceutical product at two different concentration levels (low and high). The precision method was established by measuring absorbance of two working solution concentrations.

Precision was expressed by the %R.S.D of the absorbance analyses. The accuracy was established by evaluating the amount determined from the standard solution and comparing to the respective nominal value expressed as percent recovery.

The method accuracy was also tested on all drug products at two concentrations with two respective samples. The standard addition method was utilized. For capsule drug product, the 20 capsules were weighed then the contents emptied into a glass mortar. The vacant capsule
shells were weighed to determine the average fill weight in each capsule. The fill material was gently ground using a glass pestle for 1 min to break any aggregated or cemented material. Drug product was then spiked with gabapentin stock solution up to the target concentration. The target concentrations were 60 µg/mL and 65 µg/mL. All samples were sonicated for 25 min. The flasks were adjusted to volume and mixed well. The recovery presentation was calculated by comparing the absorbent of known spiked amount of gabapentin to the detected absorbent in the spiked sample after subtracting the amount detected absorbent in the unspiked sample.

2.7.3. Limit of Detection and Quantitation

The limit of detection of gabapentin is the lowest concentration where it was acceptable to detect. In addition, for an estimate of the limit of detection of gabapentin was calculated from two to three times to noise value.

The limit of quantitation of gabapentin is the lowest concentration where its acceptable accuracy and precision were obtained. In addition, an estimate of the limit of quantitation of gabapentin was calculated from ten times to noise value.

3. Results and Discussion

3.1. Selection and Optimization of Analytical Method

Gabapentin has weak chromophore and absorbs a short UV wavelength that is 210 nm. When direct UV detection is not feasible for dissolution analysis due to potential interferences by solvent, visible spectrophotometry is often used. The primary aromatic amine group of gabapentin will react with the condensation product of 2 moles of

![Figure 2. Calibration curve for gabapentin.](image-url)
acetyl acetone and 1mole of formaldehyde in basic condition to form the new chromophore groups that will give absorption at a wavelength of 340 nm.

Optimum product formation reaction is strongly influenced by pH, incubation temperature and incubation time. Based on the obtained observations, the pH 9.5, 55°C and 15 minutes are optimum condition. All these results are obtained by the maximum absorbance value in each test.

3.2. Method Validation

The following method validation characteristics were addressed for gabapentin: accuracy, precision, limit of detection, limit of quantization, linearity, and range.

3.2.1. Linearity and Range

Linearity of the method was confirmed by preparing gabapentin standard curves for the analytical range of 10.81-80.0 µg/mL with correlation coefficient \( r^2 \) 0.99954. A calibration curve was constructed by plotting the absorbance versus final concentration of gabapentin, which indicates a linear response that reveals the linear dynamic range over the concentration range 40.0-80.0µg/mL (Figure 2).
3.2.2. Accuracy and Precision

Accuracy and precision were established across the analytical range for gabapentin. The accuracy and intraday precision were calculated from gabapentin samples. The results for intraday precision are summarized in Table 1. The accuracy results for gabapentin in drug product indicate good recovery and summarized in Table 2 and Table 3. The results for gabapentin accuracy are tested in drug product at two concentration levels by standard addition technique which ranged from 98.09-100.02% with R.S.D 0.82% (60 µg/mL), 99.21-100.49%, and R.S.D 0.85% (65 µg/mL).

3.2.3. Limit of Detection and Quantization

An estimated of detection limit of gabapentin based on 3 x S/N is 3.24 µg/mL. An estimated of limit of quantitation of gabapentin is based on 10 x S/N is 10.81 µg/mL.

<table>
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<tr>
<th>Sample</th>
<th>Reference</th>
<th>theoretical gabapentin (mg/capsule)</th>
<th>Absorbance at 340 nm (average)</th>
<th>Recovery</th>
<th>RSD</th>
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4. Conclusion

The proposed method was validated according to USP Category I requirements for gabapentin. The standard deviation and mean calculated standard error for the method are low that indicate high degree of precision method. Hence, it can be concluded that the developed UV-Vis spectrophotometric method using acetyl acetone and formaldehyde is accurate, precise and selective.

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References


