Iranian Journal of Pharmaceutical Sciences
2013: 9 (2): 23-29
www.ijps.ir

Original Article

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

Nurmaya Effendi[*] [a], Kosman Rachmat[a], Passuke Akbar[a], Naid Tadjuddin[b]

[a]*Department of Chemical Pharmacy, Moslem University of Indonesia, Indonesia*
[b]*Department of Chemical Pharmacy, Hasanuddin University, Indonesia*

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 molesacetyl acetone and 1 mole formaldehyde in basic condition (in boric buffer pH 9.5) at 55^{o}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 (r^2) 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 children[1]. Its action mechanism is still not clear. It has been suggested that gabapentin may bind to an undefined receptor or bind site in the brain[2].

Gabapentin is a white to off-white crystalline solid with a p$K_{a1}$of 3.7 and a pK_{a2} of 10.7. It is freely soluble in water and both in basic and acidic aqueous solutions[3].

Corresponding Author: Nurmaya Effendi, Department of Chemical Pharmacy, Moslem University of Indonesia, Indonesia.

Tel: (+62)411-425 619
Email: nurmaya82@gmail.com

Cite this article as: Effendi N, Rachmat K, Akbar P, Tadjuddin N. Validated UV-Vis Spectrophotometric Method for Determination of Gabapentin using Acetyl Acetone and Formaldehyde Reagents. *Iranian Journal of Pharmaceutical Sciences* , 2013, 9 (2): 23-29.

Several analysis methods of gabapentin either in dosage forms or in human body fluids have been developed including spectrophotometric[4], spectrofluorometric[5], colorimetric[6], capillaryelectrophoresis[7], LC-MS-MS[8-9], and GC-MS[10] methods.

Gabapentin derivationhas also have been analyzed with HPLC-UV[11] and HPLC–fluorescence[12-13].

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.

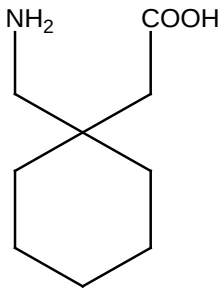

Figure 1. Structure of gabapentin.

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 molesacetyl 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^{o}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[4]. 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

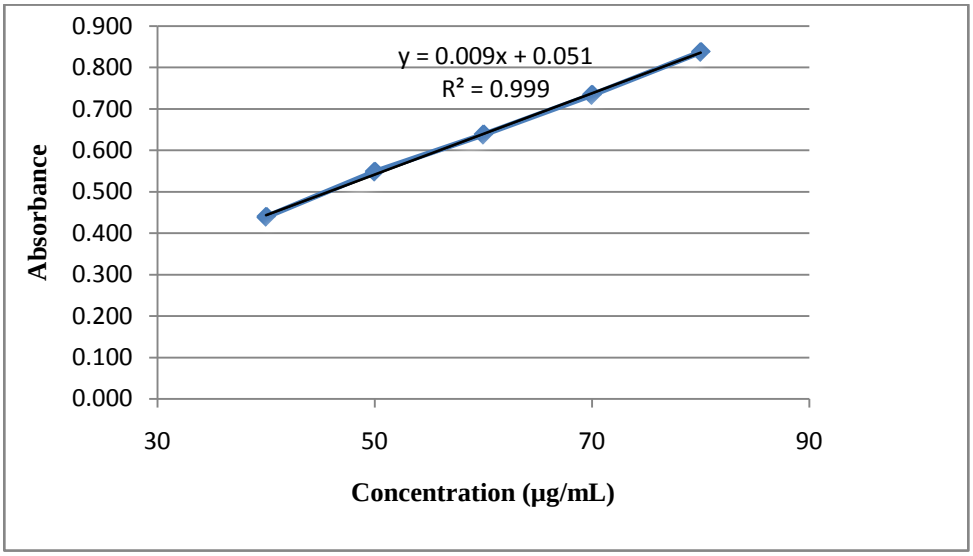

Figure 2. Calibration curve for gabapentin.

Table 1. Precision assay results of gabapentin (repeatability).

Sample (µg/mL)	Absorbance at 340 nm						RSD
	I	II	III	IV	V	VI	
40	0.443	0.446	0.437	0.433	0.445	0.441	1.27
80	0.839	0.832	0.843	0.837	0.836	0.839	0.49

Table 2. Accuracy assay results of gabapentin (60µg/mL).

Weight (mg)		theoretical gabapentin (mg/capsule)	Absorbance at 340 nm (average)	Recovery	RSD
Sample	Reference				
123.3	10.2	110.25	0.642	100.02	0.824
123.1	10.1	110.07	0.640	98.74	
123.3	10.4	110.25	0.642	98.09	
123.2	10.1	110.16	0.641	99.87	
123.1	10.3	110.07	0.641	98.80	

acetyl acetone and 1mole of formaldehyde [14] 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^{o}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).

Table 3. Accuracy assay results of gabapentin (65µg/mL).

Weight (mg)		theoretical gabapentin (mg/capsule)	Absorbance at 340 nm (average)	Recovery	RSD
Sample	Reference				
123.2	20.3	110.16	0.692	101.25	0.987
123.1	20.2	110.07	0.690	100.17	
123.4	20.2	110.34	0.693	101.20	
123.1	20.1	110.07	0.689	99.32	
123.3	20.3	110.25	0.693	101.81	

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 intra-day 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.

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.

Acknowledgments

The authors are thankful to Head of Instrumental Laboratory of Pharmacy Faculty, Moslem University of Indonesia, Indonesia for providing technical facilities to conduct this research. The authors are also thankful to Head of Chemical Pharmacy Laboratory of Pharmacy Faculty, Moslem University of Indonesia, Indonesia for the fully support to design this research.

References

[1] Walker M C, Patsalos P N. Clinical pharmacokinetics of new antiepileptic drugs.*Pharmacol. Ther.*(1995) (67): 351-384.

[2] Suman-Chauhan N, Webdale L, Hill D R, Woodruff G N.Characterization of [3H]-gabapentin binding to a novel site in rat brain: homogenate binding studies*Eur. Pharmacol.* (1993) (244):293-301.

[3] Moffat A C, Osselton M D. *Clarke's Analysis of Drugs and Poisons.* Vol. 2, 3rd ed., Widdop B Eds.Pharmaceutical Press: London p. 1069-1070 (2004).

[4] Gujral R S, Haque S M, Shanker P A. Sensitive UV Spectrophotometric Method for the Determination of Gabapentin.*J. Chem*. (2009) (6)S1: S163-S170.

[5] Belal F, Abdine H, Al-Majed A, Khalil N Y. Spectrofluorimetric determination of vigabatrin and gabapentin in urine and dosage forms through derivatization with fluorescamine. *J. Pharm. Biomed. Anal.* (2002) (27): 253- 260.

[6] Abdellatef H E, Khalil H M. Colorimetric determination of gabapentin in *pharmaceutical* formulation. *J. Pharm. Biomed. Anal.* (2003) (31): 209-214.

[7] Garcia L L, Shihabi Z K, Oles K. Determination of gabapentin in serum by capillary electrophoresis. *J. Chromatogr. B.*, (1995) (669): 157–162.

[8] Ramakrishna N V S, Vishwottam K N, Koteshwara M, Manoj S, Santosh M, Chidambara J, Sumatha B, Varma D P. Rapid quantification of gabapentin in human plasma by liquid chromatography/tandem mass spectrometry.*J. Pharm. Biomed. Anal.* (2006) (40): 360–368.

[9] Carlsson K C, Reubsaet J L E. Sample Preparation and Determination of Gabapentin in Venous and Capillary Blood using Liquid Chromatography–Tandem Mass Spectrometry.*J. Pharm. Biomed. Anal.* (2004) (34): 415–423.

[10] Hooper W D, Kavanagh M C, Dickinson R G. Determination of Gabapentin in Plasma and Urine by Capillary Column Gas Chromatography.*J. Chromatogr*. (1990) (529): 167–174.

[11] Jalalizadeh H, Souri E, Tehrani M B, Jahangiri A.Validated HPLC Method for the Determination of Gabapentin in Human Plasma using Pre-Column Derivatization with 1-Fluoro-2,4-Dinitrobenzene and its Application to a Pharmacokinetic Study. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* (2007) (854) 1-2: 43-47.

[12] Abdulrahman A, Al-Majed. Derivatization Reagent for Vigabatrin and Gabapentin in HPLC with Fluorescence Detection. *J. Liq. Chrom. Related Techno.* (2005) (28): 3119–3129.

[13] Mercolini L, Mandrioli R, Amore M, Raggi M A. Simultaneous HPLC-F Analysis of Three Recent Antiepileptic Drugs in Human Plasma.*J. Pharm. Biomed. Anal.* (2010) (21)53(1): 62-67.

[14] Susidarti R A, Rianti A, Martono S. Visible spectrophotometric determination of Sefadroxil using ethyl acetoacetate and formaldehyde reagents.*Majalah Farmasi Indonesia* (2008) (19) 1: 41-47.

ONLINE SUBMISSION

www.ijps.ir