Computational Chemistry, 2014, 2, 22-30
Published Online April 2014 in SciRes. http://www.scirp.org/journal/cc
http://dx.doi.org/10.4236/cc.2014.22004

Scientific Research

A Novel Spectrophotometric Method for Determination of Gabapentin in Pharmaceutical Formulations Using 2,5-Dihydroxybenzaldehyde

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Received 22 February 2014; revised 30 March 2014; accepted 8 April 2014

Abstract

A highly simple, rapid, sensitive and selective method is developed for spectrophotometric determination of gabapentin in pure form as well as in pharmaceutical formulations. The method is based on the formation of a yellow Schiff base derived from the condensation of gabapentin drug (1-amino methyl) cyclo hexane acetic acid and 2,5-dihydroxybenzaldehyde (DHBA) exhibiting a maximum absorbance at 445 nm. The composition, molar absorptivity and effect of different excipient have been determined spectrophotometrically. Under optimized experimental conditions, Beer's law is obeyed in the concentration range 2.57 - 37.25 μg/ml. The method is validated with respect to accuracy, precision, limit of detection and limit of quantification. The Sandell sensitivity, correlation coefficient and regression equation are calculated. The equilibrium constant and free energy change using Benesi-Hildebrand plot are also determined. The Schiff base derived from condensation of gabapentin with DHBA is also synthesized and characterized. The condensation reaction mechanism has been proposed.

Keywords

Gabapentin, 2,5-Dihydroxybenzaldehyde, Pharmaceutical Formulations, Spectrophotometric Analysis

1. Introduction

Gabapentin (GBP) (1-(aminomethyl) cyclohexane acetic acid) is a new antiepileptic drug which is a structural

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How to cite this paper: Saleh, M.S., Youssef, A.K., Hashem, E.Y. and Abdel-Kader, D.A. (2014) A Novel Spectrophotometric Method for Determination of Gabapentin in Pharmaceutical Formulations Using 2,5-Dihydroxybenzaldehyde. *Computational Chemistry*, **2**, 22-30. http://dx.doi.org/10.4236/cc.2014.22004

analogue of neurotransmitter γ-aminobutyric acid (GABA). GBP, unlike GABA, has a cyclohexane molecule system and is able to penetrate through blood-brain barrier. GBP is used for the treatment of partial onset seizures with or without secondary generalized tonic-clonic convulsions in clinical practice [1] [2]. The reported analytical methods for GBP determination include high performance liquid chromatography (HPLC) [3]-[14], spectrofluorometry [15] [16], gas chromatography [17]-[19]. Despite that the spectrophotometric techniques had been commonly applied for the determination of some other drugs [20], only few spectrophotometric methods were reported in literature for determination of GBP, based on the reaction of the primary amino group of GBP with ninhydrine reagent in organic solvent medium [21]-[23]. And other spectrophotometric methods involve the determination of GBP via formation of charge-transfer complexes with π-acceptors in pharmaceutical formulations [24]-[26].

There is no spectrophotometric method till now for determination of gabapentin in pharmaceutical formulations through formation of Schiff base with DHBA. Our present study was simple, accurate and sensitive spectrophotometric procedure for determination of GBP in pharmaceutical formulations. The method was based on the reaction of primary amino group of GBP drug with DHBA at elevated temperature to produce Schiff base. No interference was observed in the assay of GBP from common excipients. The reaction conditions and application of the method for determination of GBP in pharmaceutical formulation have been established. In addition, the stoichiometric ratio of reactants, the equilibrium constant (Kc) and free energy change (ΔG) were determined.

2. Experimental Work

2.1. Materials

All solutions were prepared from analytical grade materials with pure ethanol 99.8% (Riedel-de Haen Germany). Gabapentin (GBP) pure was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 2,5-dihydroxybenzaldehyde (DHBA) reagent was obtained from Alfa Aesor GmbH & Co KG (Zeppelinstra Be Karl Sruche, Germany). Pharmaceutical formulations of GBP such as Gabtin capsules-100 mg (Al-Debeiky pharmaceutical products for Delta pharma, Egypt), and Conventin capsules 100 mg (EVA PHARMA-Egypt) were purchased from local pharmacy.

A stock solution of 5.8×10^{-3} M GBP was prepared by dissolving the accurately weighed amount of the pure GBP in ethanol. Dilute solutions were obtained by accurate dilution. A 5.8×10^{-3} M solution of DHBA was prepared by dissolving the required amount of the reagent (DHBA) in ethanol.

2.2. Instrumentation

An evolution 300 UV-Vis. Spectrophotometer with 1.0 cm matched cells fitted with vision pro software of Thermo Election corporation (Cambridge, U.K.) was used for electronic spectral measurements. To obtain pH readings throughout the experimentation, a microprocessor pH meter (HANNA HI 211) was used.

2.3. General Procedure and Construction of Calibration Graphs

Different aliquots of GBP solution were transferred into test tubes. To each test tube 3 ml of DHBA (2×10^{-3} M) reagent in ethanol and 2 ml ethanol were added, then test tubes were heated on a water-bath at 75 ± 0.15˚C for 15 min. These solutions were transferred to volumetric flasks after cooling and the volume was made up to the mark with ethanol to provide final concentration range of 2 - 50 $\mu g \cdot ml^{-1}$.

The absorbance of the solution was measured against a reagent blank at 445 nm. The calibration graph was prepared by plotting absorbance versus concentration of gabapentin.

2.4. Analysis of Pharmaceutical Formulations

Twenty capsules of each formulation were accurately weighed and powdered in glass mortar. The quantity of the powder equivalent 10 mg of gabapentin was dissolved in 100 ml ethyl alcohol.

The procedure was continued as described in the general procedure. The amount of GBP present in capsule sample solution was determined by fitting the responses into the regression equation.

2.5. Interference from Excipients

Samples were prepared by mixing 10 mg of gabapentin with various amounts of common excipients such as cellulose, lactose, glucose, vitamin C, starch and purified talc. The procedure was continued as mentioned above in the general procedure.

3. Results and Discution

3.1. Spectral Characteristics

Gabapentin (GBP) showed weak absorption band in UV range [21]. UV-spectrophotometric methods is not enough for the determination of GBP in pharmaceutical formulations. Attachment of chromophoric group to GBP increases the sensitivity of its detection. For this reason, DHBA was chosen as a chromagenic reagent. Ninhydrin reagent was used for the determination of an aliphatic primary amine [27]-[29].

The reaction is usually carried out by heating for a short time in an organic solvent and the reaction product is measured in the visible region depending on the reaction conditions [30].

3.2. Reaction Mechanism

Shiff bases derived from the condensation of aromatic aldehyde derivatives and aromatic primary amine were synthesized and characterized [31].

The reaction of GBP with DHBA was studied in ethanol media in the temperature range 45˚C - 80˚C. The visible absorption spectra of solutions were recorded in presence of excess reagent and spectra reflect the formation of yellow product with λ_{max} = 445 nm at T = 75 ± 0.15˚C. Gabapentin interacts with DHBA reagent in ethanol medium at elevated temperature via oxidative deamination of the primary amino group followed by the condensation of the reduced DHBA to form the yellow colored Schiff base as represented by the **Scheme 1**.

The absorption spectra of gabapentin, DHBA reagent and their reaction product are shown in **Figure 1**.

3.3. Optimization of Reaction Conditions

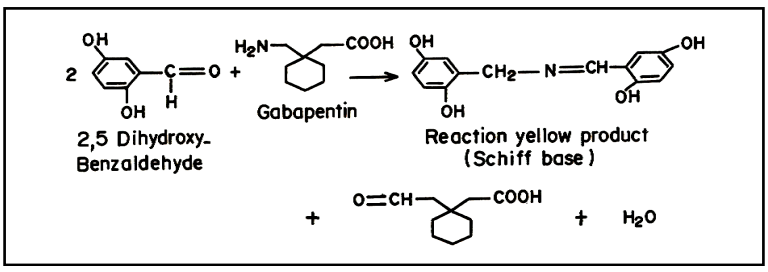
The reaction conditions were optimized. The number of parameters such as temperature, time, reagent concentration and solvent were investigated. The optimum conditions were established by changing one variable and observing its effect on the absorbance of the colored product.

3.4. Effect of Solvents

Different solvents such as water, ethanol, methanol, isopropanol, dioxane and acetonitrile have been tested, but the best results were obtained with ethanol.

3.5. The Effect of Concentration of DHBA

The effect of the volume of DHBA (80.04 µg/ml) on the absorbance of the colored product was studied in ethanol medium in the range of 1.0 - 7.0 ml at 70˚C. The absorbance increases with the increase in the volume of DHBA became constant at 6.0 ml. Further addition of DHBA did not cause change in absorbance and therefore 48.024 µg/ml DHBA was chosen as an optimum value (**Figure 2(a)**).



Scheme 1. Suggested reaction pathway between 2,5-Dihydroxy benzaldehyde (DHBA) and Gabapentin.

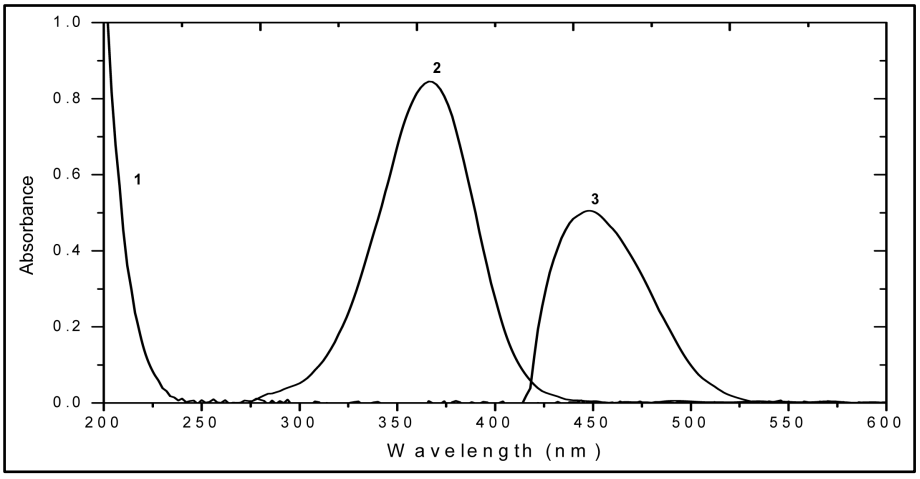


Figure 1. Absorption spectra of (1) 2.9×10^{-4} M GBP in ethanol, (2) 6.0×10^{-4} M DHBA reagent in ethanol and (3) GBP-DHBA reaction product, ($C_{GBP} = 2.9 \times 10^{-4}$, $C_{DHBA} = 6.0 \times 10^{-4}$ M). In ethanol at T = 75 ± 0.15˚C.

3.6. The Effect of Temperature

The effect of temperature on the absorbance of the reaction product was studied in ethanol medium in the range (45˚C - 80˚C), keeping the constant concentration of GBP (14.898 µg/ml) and (24.012 µg/ml) DHBA. The maximum absorbance was obtained at 75˚C ± 0.15˚C (**Figure 2(b)**).

3.7. The Influence of Time

The reaction time was determined by following the absorbance of the developed Schiff base in ethanol medium at 75˚C at different time intervals. Complete color development was attained after 15 min (**Figure 2(c)**).

3.8. Stoichiometry of the Reaction

The Stoichiometry of the GBP-DHBA Schiff base was further verified by the method of continuous variation [32]. In solution with $C_T = C_G + C_{DHBA} = 3.48 \times 10^{-4}$ mol·L^{-1} at 75˚C, the maximum of the Jop's plot corresponds to a component ratio of 1:2 (GBP:DHBA).

3.9. Equilibrium Constant and Free Energy Change

The equilibrium Constant was determined for the interaction of gabapentin drug with DHBA reagent using Benesi-Hildebrand equation [33].

$$C_a/A = 1/\varepsilon + 1/\varepsilon K_c.1/C_g$$

where C_a and C_g are the concentration of the aldehyde reagent and GBP drug respectively. A is the absorbance, ε is the molar absorptivity and K_c is the equilibrium Constant of GBP-DHBA Schiff base. The free energy change of reaction product (ΔG) was calculated from the equilibrium constant by the following equation [34].

$$\Delta G = -2.303RT \times \log K_c$$

where ΔG is the free energy change of the Schiff base (K·cal·mol^{-1}), R is the gas constant (0.001987 K·cal·mol^{-1}·deg^{-1}), T the temperature in Kelvin (273 + C^0) and K_c is the equilibrium Constant of drug-DHBA reaction product.

The calculated values of equilibrium Constant K_c and free energy change of GBP-DHBA Schiff base were found to be 4.593×10^3 and −5.832 K·cal·mol^{-1} respectively.

3.10. Construction of the Calibration Curve and Statistical Analysis

Under the optimum experimental conditions, the standard calibration curve (**Figure 3**) for the proposed method

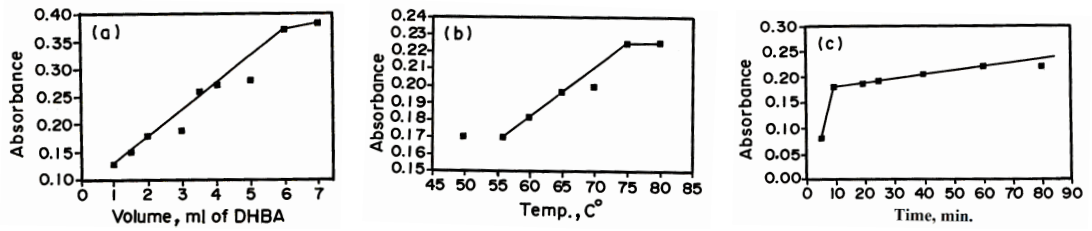


Figure 2. Reaction condition of the color formation of GBP-DHBA reaction product. (a) Effect of DHBA (by volume), GBP: 29.796 μg·ml^{-1}, T = 70˚C; (b) Effect of temperature, GBP: 14.898 μg·ml^{-1}, DHBA: 24.012 g·ml^{-1}; (c) Effect of time, GBP: 14.898 μg·ml^{-1}, DHBA: 24.012 μg·ml^{-1}, T = 75˚C.

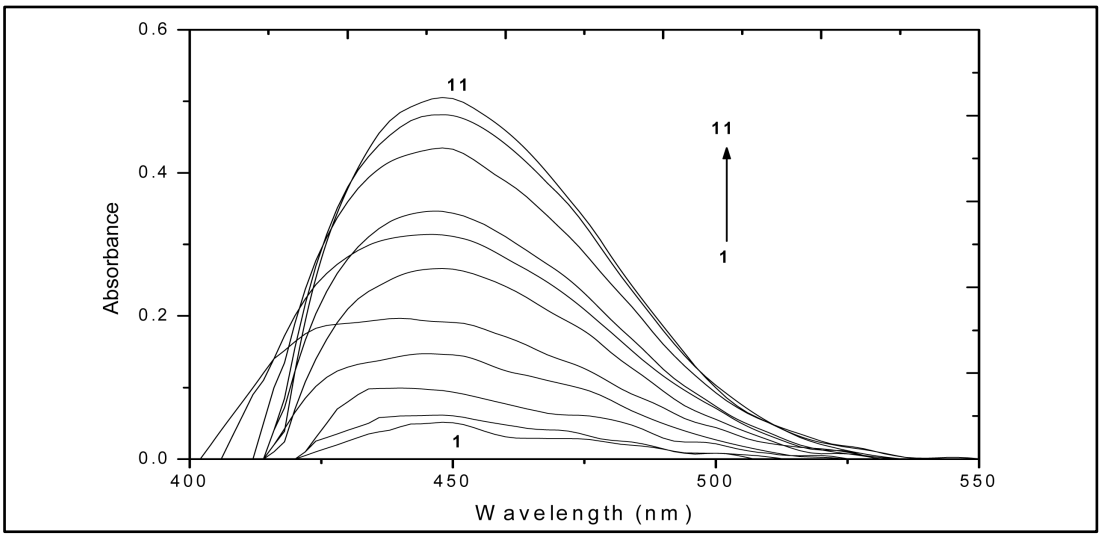


Figure 3. Absorption spectra of GBP-DHBA reaction product, GBP concentration range from 2.483 (1) to 49.66 μg·ml^{-1} (11) with regular successive additions in presence of 6.0 × 10^{-4} M DHBA reagent in ethanol at T = 75 ± 15˚C.

obeyed Beer's law over the concentration range of 2.57 - 37.25 μg/ml at λ_{max} = 445 nm. It was found that the absorbance was stable for at least two days at room temperature. Linear regression analysis of calibration data gave the regression equation cited in **Table 1**, with correlation coefficient close to unity.

The within day precision assay was carried out through replicate analysis (n = 3) of gabapentin corresponding to 15, 20, 30 μg/ml.

The interday precision was also evaluated through replicate analysis of the pure sample for three consecutive days at the same concentration levels as used in within day precision. The results of these assays are reported in **Table 2**.

As can be seen from **Table 2** that recovery values for intraday and interday precision were in the range of 99.25% to 101.53% and RSD values for intraday and interday precision were in the range of 0.0057% to 0.018%.

3.11. Specificity

The specificity of the method was investigated by observing any interference encountered from the excipients generally presented in pharmaceutical formulations. The good percentage recoveries were summarized in **Table 3** revealed that no interference was observed from any of these excipients in the proposed method.

3.12. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated for GBP-DHBA reaction product. The theoretically determined values of LOD and LOQ were cross checked by actual analysis of these concentrations using proposed method (**Table 1**).

Table 1. Regression Analysis Data and summary of validation parameters for the proposed method.

Parameters	Gapabentin
λ_{max}, nm	445 nm
Beer's law limits (μg·ml^{-1})	2.57 - 37.25
Ringbom limits (μg·ml^{-1})	3.98 - 35.48
Molar absorptivity (L mol^{-1}·cm^{-1})	2.1022×10^3
Sandell's sensitivity (μg·cm^{-1})	0.08145
Regression equation A= a + b X	A = 0.0025 + 0.01227X
Slope (b)	0.01227
Intercept (a)	0.0025
Correlation coefficient	0.989
Limit of detection (LOD), μg·ml^{-1}	0.33
Limit of quantification (LOQ), μg·ml^{-1}	0.996

Table 2. Summary of accuracy and precision results of the proposed method in pure form.

Proposed method	Amount (μg·ml^{-1})		RSD %	Recovery %	SAE[b]	C.L[c]
	Taken	Found ± SD[a]				
Intraday assay	15	15.23 ± 0.0027	0.018	101.53	1.53	$\pm 3.35 \times 10^{-3}$
	20	19.85 ± 0.0028	0.011	99.25	0.75	$\pm 3.48 \times 10^{-3}$
	30	30.09 ± 0.0023	0.0077	100.3	0.3	$\pm 2.86 \times 10^{-3}$
Interday assay	15	15.08 ± 0.0021	0.014	100.53	0.53	$\pm 2.57 \times 10^{-3}$
	20	19.98 ± 0.0072	0.0086	99.9	0.1	$\pm 2.14 \times 10^{-3}$
	30	29.97 ± 0.0072	0.0057	99.9	0.1	$\pm 2.14 \times 10^{-3}$

[a]Mean for 3 independent analysis; [b]SAE, standard analytical error; [c]C.L., confidence limit at 95% confidence level and 4 Degrees of Freedom (t = 2.776).

3.13. Applications

The proposed spectrophotometric method was applied to the determination of gabapentin in pharmaceutical formulations (Gaptin and Conventin).

The results obtained from **Table 3** suggested that the proposed method could be applied for the determination of gabapentin in its dosage forms without interference observed at concentration levels examined.

The excellent linearity of the calibration graphs was clearly evident by the values of the correlation coefficients and standard deviations as shown in **Table 4**.

3.14. Infrared Spectra

The I. R. spectra of gabapentin showed the expected doublet of primary NH_2 group at 2857 and 2931 cm^{-1}, C-N stretch at 1165 cm^{-1} and the carbonyl stretch of COOH group at 1615 cm^{-1}. 2,5-dihydroxybenzaldehyde exhibited broad band at 3277.4 - 3250 cm^{-1} owing to two OH groups and C=O stretch of aldehyde group at 1652 cm^{-1}.

The formation of the Schiff base was evidenced by comparing the I. R. spectra of GBP-DHBA reaction product with that of GBP and DHBA. It was found that the twin peaks of NH_2 in GBP disappeared indicating that the primary amine has been changed to tertiary. Also the I.R. spectra of GBP-DHBA reaction product exhibits a strong band at 1622 cm^{-1} which is characteristic of the azomethin HC=N-group.

4. Conclusion

The proposed spectrophotometric method was found to be simple, selective, rapid and sensitive compared with other established spectrophotometric methods. The reagent utilized in the proposed method was cheep, readily available and the procedure did not involve any critical reaction conditions or tedious sample preparation. Moreover, the method was free from interference by common additives and excipients. Also this method required less time for analysis, provided better RSD and LOD and had a wide concentration range over the pre-

Table 3. Recovery of gabapentin in presence of different excipients.

Excipients	Recovery ± RSD
Cellulose	99.83 ± 0.71
Lactose	99.97 ± 0.82
Carboxymethyl cellulose	98.57 ± 0.33
Glucose	99.45 ± 0.29
Vitamin C	98.97 ± 0.35
Starch	99.55 ± 0.88
Purified talc	99.85 ± 0.27

Table 4. Application of the proposed method to the determination of gabapentin drug in dosage forms.

Marketed formulation	Certified value ($\mu g \cdot ml^{-1}$)	Found value ($\mu g \cdot ml^{-1}$)	Relative error %	recovery	RSD
Gaptin[a]	377.41	375.01	0.64	99.36	0.0055
Conventin[b]	248.29	252.75	1.79	100.016	0.0082

[a]Product of Al-Debeiky Pharmaceutical products for Delta Pharma-Egypt; [b]product of EVA Pharma-Egypt.

viously published methods [35] [36]. Hence, it could be concluded that the developed spectrophotometric method was accurate, precise, and selective and could be employed successfully for the estimation of gabapentin in pharmaceutical formulations. The excellent recoveries obtained showed no interference from the common excipients.

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