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Research Article

Spectrophotometric Method for Determination of Gabapentin in Pharmaceutical Formulation by Derivatization with 4-Chloro-7-Nitrobenzo-2-Oxa-1,3-Diazole (NBD-Cl)

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Abstract

Rapid, sensitive and validated spectrophotometric methods for the determination of antiepileptics gabapentin (GAB) in pure forms and in pharmaceutical formulations was developed. The method is based on the formation of complex between drug and the chromogenic reagent 4-Chloro-7-Nitrobenzo-2-Oxa-1,3-Diazole (NBD-Cl) producing complex in methanolic medium which showed an absorption maximum at 576 nm. The optimization of the reaction conditions such as: pH, the volume of buffer, and reaction time were investigated. Beer's law is obeyed in the concentration ranges 10-60 μg ml^{-1}. The molar absorptivity, detection and quantification limits are also calculated. The correlation coefficients were 0.9996 with a relative standard deviation (RSD%) of 45.87. The method successfully applied to the determination of GAB in pharmaceutical formulation.

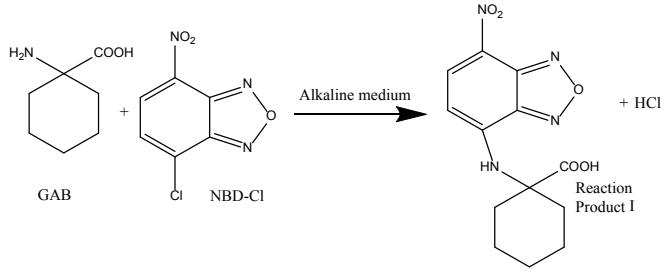
Keywords: GAB; Spectrophotometric; Pharmaceutical formulation; Method validation; NBD-CL

Introduction

Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) (GAB) (Scheme 1). It is anticonvulsant drugs used in the treatment of epilepsy and neuropathic pain, as an adjunct therapy for partial seizures in adults and children [1-4].

From literature survey there are several methods for the determination of (GAB) using High performance liquid chromatography HPLC [5-7] spectrofluorimetry [8,9], capillary electrophoresis [10] spectrophotometry [11-13]. However, many of the above methods have one or more disadvantages like poor sensitivity, high cost solvents, need tedious extraction procedures, measurements done at shorter wavelengths, heating or cooling step, use of expensive chemical. Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories.

2-Chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-CL) has been proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group [14-27]. The applications of NBD-CL for determination of pharmaceutical bearing amine group have been reviewed by Elbashir et al. [28,29].



Scheme 1: Reaction mechanism of GAB with NBD-Cl.

The reaction between NBD-Cl and GAB has not been investigated yet. Therefore, the present study was devoted to investigate the reaction between NBD-Cl and GAB, and use this color reaction in the development of simple rapid spectrophotometric method for determination of GAB in its dosage form.

Experimental

Apparatus

All absorbance measurements were made with a Double beam UV-1800 (SHIMADZU, Japan) ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells were used for the spectrophotometric measurements. pH meter model pH211 (HANNA, Italy).

Materials and reagents

The solvent (methanol) used in this work were of HPLC grade, and distilled water.

Pure drugs and pharmaceutical capsules: Pharmaceutical grade gabapentin (GAB) which is certified to be 99.4% pure was received from Azal Pharma, Khartoum, Sudan, were used as working standards.

The commercial capsules used in the present investigation were obtained from commercial sources in the local Pharmacy - Gabix capsules (Getz pharma, Karachi, Pakistan), labeled to contain 100 mg GAB per capsule.

Stock standard solutions: A standard stock solutions of the studied drug containing 1.0 mg mL^{-1} was prepared by dissolving 1.0 mg of pure drug in 2.0 mL methanol and was further diluted to 10 mL with distilled water the same to obtain the working concentration. The standard solution was kept in dark place.

Reagent: NBD-Cl 0.2% (w/v) was prepared by dissolving the appropriate weight of the reagent in approximately 25 mL of methanol, and then completed to the mark with methanol in 100 mL volumetric flask. This solution was stable for 1 week.

Procedure: An aliquot of 0.10-0.6 mL from standard solution was added to 1.5 mL buffer solution in 10 mL volumetric flask, 1.0 mL of

0.2% NBD-Cl was added to the later and the volume was brought to 10 mL with water and mixed. The absorbance of the derivative was measured after twenty five min at 476 nm against a blank prepared similarly.

Determination of GAB in dosage forms: For preparation of sample solution, ten capsules were weighed and powdered then a quantity of powder equivalent to 27 mg of GAB was transferred into a small conical flask dissolved in methanol. Filtered into 10 mL volumetric flask and completed to the mark with distilled water to obtain 1000 μgmL^{-1} concentration. Aliquot volume was transferred into 10 mL volumetric flask, and then the procedure was applied as described in calibration curve. The nominal content of the capsule was determined from regression equation.

Results and Discussion

The study and development of the method for the determination of GAB in pure and pharmaceutical formulations, was performed to optimization of the experimental conditions in order to achieve both maximum sensitivity and selectivity. This step comprised the investigation of the influence of the pH, and evaluation of the time required to complete the reaction and the buffer volume.

Absorption spectra

The absorption spectrum of GAB was recorded against water (Figure 1), it was found that GAB exhibits a maximum absorption peak (λ_{max}) at 197 nm. Because of highly blue shifted λ_{max} of GAB its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of GAB red–shifted light-absorbing derivative was necessary. The reaction between GAB and NBD-CL was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting λ_{max} at 476 nm, and the λ_{max} of NBD-CL was 342 nm. The λ_{max} of GAB- NBD-CL derivative was red-shifted, eliminating any potential interference. Therefore, the measurements were carried out at 476 nm.

Optimization of the reaction conditions

The optimum conditions for the development of method were established by varying the parameters one at a time while keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as, pH, buffer volume, and time of heating were studied.

The influence of pH on the absorbance of product I was investigated in the range of 8.0-13.0, the absorbance of the solution increases rapidly up to pH 10.0 and then decrease (Figure 2). At pH 10.0, the absorbance reaches its maximum; in other words, the degree of the nucleophilic substitution reaction is also maximal. At pH>10.0, the absorbance of solution decreases sharply. Presumably it may be that the increase of hydroxide ion holds back the nucleophilic substitution reaction between GAB and the chromogenic reagent. Consequently, the absorbance of the solution reduces. In order to keep the high sensitivity for the determination of GAB, pH 10.0 was selected for optimal experimental conditions.

By following the reaction for various lengths of time it was found that the reaction went to completion over 20 min and a longer reaction time was not necessary Figure 3.

Keeping pH at 10.0, the effect of amount of buffer solution on the absorbance of product I was also studied. It shows that the absorbance of product I enhances rapidly with the rise of amount of buffer solution, and becomes maximal when the amount of buffer solution is 1.5 mL. Therefore, the amount of 1.5 mL buffer solution was selected to ensure the highest absorbance of product I, as shown in Figure 4.

The continuous variation method of equivalent mole method was used to determine the composition of Product. The result is shown in Figure 5. As can be seen, the mole ratio of GAB and NBD-Cl of Product I is 1:1. Based on the observation molar ratio, the reaction pathway was postulated to proceed as shown in Scheme 1.

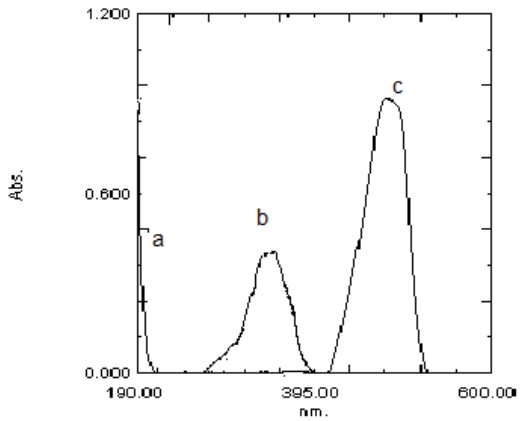


Figure 1: (a) Absorption spectra of gabapentin (100 µg/mL) against water (b) NBD-Cl (0.2% w/v) blank against water. (c) The reaction product of gabapentin (100 µg /mL) with NBD-Cl against reagent.

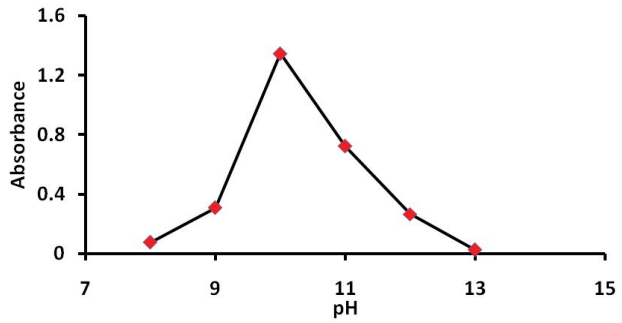


Figure 2: Effect of pH on absorbance of product I. 1.0 mL of gabapentin (100 µg/mL), 1.0 mL of borate buffer, 1.0 mL NBD-Cl (0.2%), Room temperature 25 min.

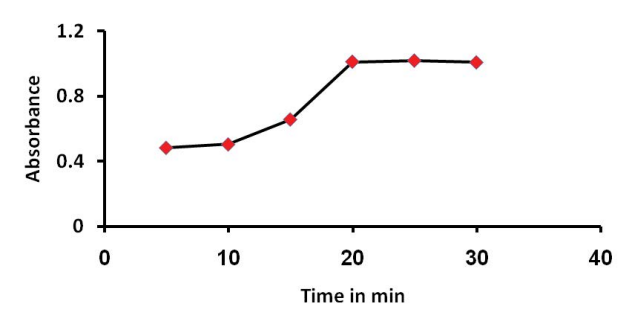


Figure 3: Effect of time on absorbance of product I. 1.0 mL of gabapentin (100 µg/mL), 1.0 mL of buffer (pH 10), 1.0 mL NBD-Cl (0.2%), at room temperature.

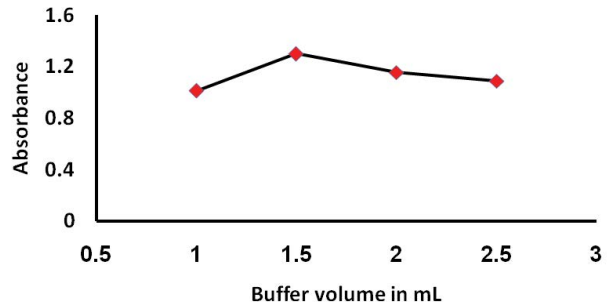


Figure 4: Effect of the volume of the buffer on absorbance of product I.0 mL of gabapentin (100 µg/mL), 1.0 mL NBD-Cl (0.2%), Buffer (pH 10) at room temperature (20 min).

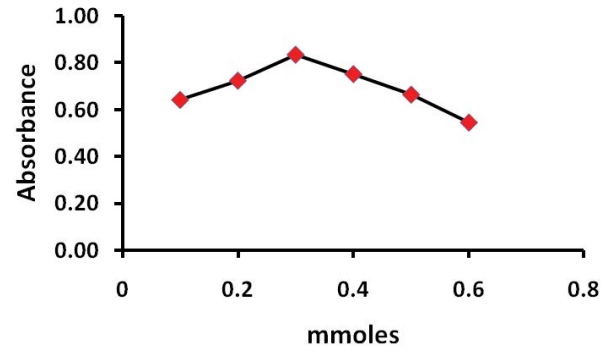


Figure 5: Determination of product formation by continuous variation method. VR: NBD-Cl (4×10^{-4} M); VD: GAB (4×10^{-4} M); VR+VD=10 mL.

Validation of the proposed method

The validity of the methods was tested regarding linearity, limit of detection (LOD) limit of quantification (LOQ), and accuracy according to International Conference on Harmonization (ICH) [30] and United States Pharmacopeia [31] guidelines.

Under the described experimental condition, linear relationship was found between the absorbance at λ_{max} 476 nm and the concentration of the drug. The regression equation was found to be as A=0.046+0.009C (r^2=0.9997, n=6) (where A is the absorption, and c is the concentration of GAB in µg mL^{-1}). The limits of detection (LOD) and limits of quantification (LOQ) where determined using the formula:

LOQ=10s/b

LOD=3:3s/b

Where s is the standard deviation of the intercept, and b is the slope, the obtained results are summarized on Table 1.

The recovery of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to a known concentration of the drug sample. The average percent recoveries obtained in range 99.3-105.1% (Table 2).

Applications of the method

The proposed method was applied to the pharmaceutical formulation of GAB; indicate the high accuracy of the proposed method for the determination of the studied drug. The proposed method has the advantage of being virtually free from interferences by excipients. The percentage was 103.3 ± 1.2% (value is means of five determinations).

Parameter	Value
Measurement wavelength (nm)	476
Linear range (µg mL^{-1})	10-60
Intercept	0.04613
Slope	0.00878
Standard deviation	0.00459
Correlation coefficient (r^2)	0.9996
Limit of detection, LOD (µg mL^{-1})	1.725
Limit of quantification, LOQ (µg mL^{-1})	5.227
Molar absorptivity, e (1 mol^{-1} cm^{-1})	6.2×10^3

Table 1: Parameters for the performance of the proposed method.

Sample No	Sample content (µg/ ml^{-1})	GAB. added (µg/ ml^{-1})	Found	Recovery% ± SD
1	10	5	14.9	99.3 ± 0.002
2	10	25	36.8	105.1 ± 0.002
3	10	45	55.3	100.5 ± 0.002

Table 2: Recovery studies for the determination of GAB, by the proposed method.

Conclusion

The present paper described the evaluation of NBD-Cl as analytical reagents in the development of simple, sensitive, and accurate spectrophotometric methods, for the determination of GAB in pharmaceutical formulation. The proposed method is simple, reliable, specific, accurate, reproducible, and highly sensitive, for the determination of GAB in commercially available dosage forms. The procedure presented here does not need necessitate any expensive apparatus; therefore the proposed method can be used advantageously as a routine method for the determination of GAB in quality control and industry.

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