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RESEARCH ARTICLE

A Novel Quantitative Spectrophotometric Method for the Analysis of Vigabatrin in Pure Form and in Pharmaceutical Formulation

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ARTICLE HISTORY

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Abstract: ***Background*:** Vigabatrin is a variant of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). It reduces seizure activities by inhibiting irreversibly GABA transaminase and increasing GABA concentrations.

***Objective*:** This study presents simple, sensitive, extraction-free, and cost-efficient estimation of vigabatrin using a spectrophotometric method in pharmaceutical formulation.

***Method*:** The method relies on the development of water-soluble, blue-violet colored complexes of vigabatrin and ninhydrin in a phosphate buffer of pH 7.4 with λ_{max} at 565 nm. Linearity, accuracy, precision, and specificity were performed as validation parameters for this method.

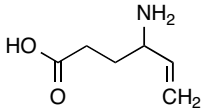
***Results*:** Assessment of different analytical factors was carried out, and statistical analysis was performed to validate the results. The findings showed a linear progression in absorbance when the concentration of vigabatrin was increased. The correlation coefficient value (r^2) of 0.9981 was observed. The operations followed the Beer's law in the span of 40-200 μgmL^{-1} with a molar absorptivity of 5.16×10^3 $Lmol^{-1}cm^{-1}$. Recovery values in the marketed formulation of the assay method indicated no interference from the common additives and excipients. The limits of detection and quantification were found to be 6.0 and 40 μgmL^{-1}, respectively.

***Conclusion*:** The presented method has demonstrated to be delicate, reliable, explicit, and intuitive that can prove beneficial for regular laboratory examination of vigabatrin in pharmaceutical dosage forms.

Keywords: Vigabatrin, ninhydrin, spectrophotomertic method, color complex, γ-aminobutyric acid, validation.

1. INTRODUCTION

Vigabatrin (Structure **1**) is a variant of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). It reduces seizure activities by inhibiting irreversibly GABA transaminase and increasing GABA concentrations [1]. Numerous analytical approaches have been published illustrating the analysis of vigabatrin using spectrophotometric [1, 2], spectrofluorimetric [3] and high-performance liquid chromatographic (HPLC) techniques [4-11].



Vigabatrin

The development of the analytical method of vigabatrin is not straightforward since this molecule does not have significant ultraviolet and visible absorption (Fig. **1**) [12]. Also, most of the reported analytical methods utilized different reagent to form colored complexes with vigabatrin [1-3]. In the work of literature, a process for the examination of gabapentin and vigabatrin has been reported through

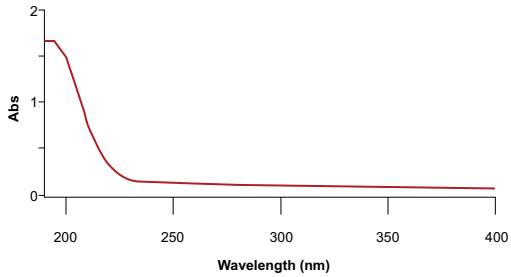


Fig. (1). UV spectra of vigabatrin.

Hantzsch reaction. The method involves condensing the amino groups in vigabatrin with formaldehyde and acetylacetone. The reaction resulted in the formation of yellow-orange fluorescent derivatives. It was then measured in the visible range spectrophotometerically [1]. Similarly, determination of vigabatrin in tablets was carried out spectrophotometrically, using derivatization reaction with NBD-Cl (7-chloro-4-nitrobenzofurazan). The reaction was performed at 70 °C in pH 10.0 having time interval of 50 min. The complex prepared in these conditions was found to possess maximum absorption at 460 nm [2]. Another highly fluorescent derivative showing absorbance at 472 nm for the determination of vigabatrin and gabapentin was prepared using stability-indicating spectrofluorimetric method. The reaction with fluorescamine proceeded in borate buffer at pH 8.2 [3].

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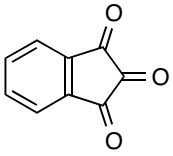
In one paper, pregabalin, gabapentin and vigabatrin were determined through fluorescence detection by derivatization with fluorescamine using a microplate reader for 5 min at room temperature [12]. Simple HPLC design for concurrent estimation of pregabalin, vigabatrin, and gabapentin in human serum was carried out using pre-column derivatization with 3-mercaptopropionic acid and OPA (*o*-phtaldialdehyde) [13]. Quantification of vigabatrin and gabapentin using chromatographic technique proceeded by using fluorescamine pre-column reaction. Later on, separation of derivatives was achieved by reverse phase HPLC [14].

Since, majority of the methods reported in literature involved pre- and post-column derivatization for analysis of vigabatrin [5, 8-10], it was pertinent to look for a method for the routine analysis of the drug in active form and formulations which would be simple, reliable, cost effective and uncomplicated having adequate sensitivity like UV spectrophotometry. Moreover, owing to the poor absorption profile of vigabatrin in the UV range and few reported work on reaction of the drug with reagents to form a chromophoric product, it was required to develop a novel and less complicated spectrophotometric method for the determination of vigabatrin in active and dosage forms for routine analysis.

Chromogenic agent ninhydrin (Structure **2**) is extensively used spectrophotometrically in different studies for the quantification of amino acid compounds, peptides, and amines. Ninhydrin gives Ruhemann's purple colored complex on reaction with many primary amines [15]. The application of ninhydrin is evident in different and diverse reactions such as decarboxylation, deamination, and transamination [16, 17]. The present work extends the chromogenic application of ninhydrin through the evaluation of vigabatrin which is a γ-amino acid derivative, quantitatively in dosage and pharmaceutical forms by employing simple and affordable spectrophotometric method.



Ninhydrin

1.1. Experiment

1.1.1. Apparatus

Cary 60 UV–visible spectrophotometer of Agilent Technologies was used to record the absorption spectra. Cary WinUV version 5.0.0.999 software (Agilent Technologies) was utilized for data acquisition. An S220 SevenCompact™, Mettler-Toledo, LLC pH meter was employed for maintaining the buffer solutions' pH.

1.1.2. Materials and Reagents

Vigabatrin was purchased from Sigma Chemicals, Saint Louis. Sabril (vigabatrin 500 mg) of Sanofi Aventis was bought from a local pharmacy. Ninhydrin was procured from Loba India. Double distilled water was used for solution preparation.

1.1.3. Preparation of Phosphate Buffer with pH 7.4

250 mL was withdrawn from 0.2 M potassium dihydrogen phosphate solution. The chemical was taken and transferred to 1000 mL flask. To this flask 195.5 mL of 0.2 M NaOH was added, and volume was made up to the mark using distilled water [18] and drops of NaOH utilized for the adjustment of the pH to 7.4.

1.1.4. Preparation of Stock Solutions as per Standard

Stock solution of vigabatrin (1000 μgmL^{-1}) was developed through the transfer of 100 mg of vigabatrin in 100 mL of water.

1.1.5. Ninhydrin Solution Preparation

1g of ninhydrin was immersed in 100 mL of distilled water to make 1% solution. The solution prepared was placed in amber-colored bottles.

2. METHODS

2 mL of phosphate buffer pH 7.4 was combined with 1.0 mL of ninhydrin solution (1% w/v). The solution was mixed with different aliquots of stock solution of vigabatrin in stopper tubes. These tubes were subjected to heating for 5 min at 70-75°C in a water bath. The solutions were diluted with distilled water after cooling to make the concentration levels from 40 to 200 μgmL^{-1}. Absorbance rates were computed in triplicate at 565 nm with reagent blank (Fig. **2**). Blank used the same amount of reagents without vigabatrin solution. The assessment for the Beer Lambert's law was obtained by plotting the absorbance values against corresponding concentration values.

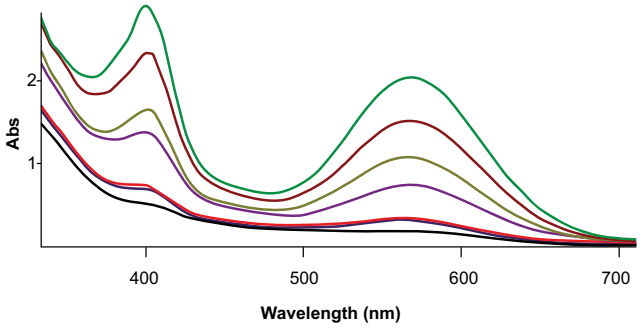
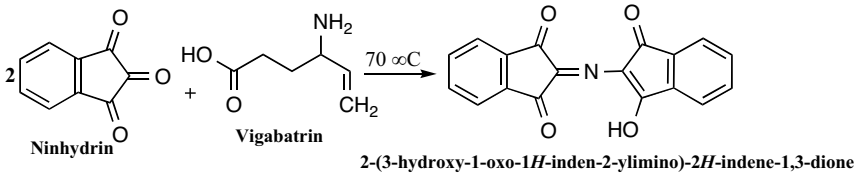


Fig. (2). UV-visible spectra of linearity study of vigabatrin-ninhydrin complex.



Scheme 1. Suggested reaction pathway between vigabatrin and ninhydrin.

2.1. Analysis of Pharmaceutical Formulation

Average weight equivalent to one tablet from 20 powdered tablets was dissolved in water to 100 mL in volumetric flasks and sonicated for 60 mins. Now the procedure described above "in methods" was applied and concentration of active ingredient in the sample was determined by calibration curve.

3. RESULTS

3.1. Effects of Buffer

The complex formation took place in phosphate buffer at pH of 7.4 among the several buffer systems tested i.e. acetate, boric acid, and phosphate buffer. The optimum pH for the development of color was found to be in pH 7.4. Insufficient color development was found by increasing the pH values.

3.2. Effects of Temperature and Heating Time

The temperature and heating time effects were studied on the development of the colored complex. It was observed that no reaction took place at room temperature in the presence of ninhydrin even at higher concentration. Hence, the mixture was heated, and it was observed that the intensity of the color increased with temperature increase. So, reaction proceeded in water bath for 5 minutes at 70-75°C to attain maximum absorption. No appreciable effect on the reaction was observed by increasing the duration of the reaction. The obtained complex was observed to be stable for more than 24 h.

3.3. Effect of Reagent Concentration

1% (w/v) solution of ninhydrin was found sufficient to complete the reaction with reproducible results. Decreasing its quantity resulted in incomplete reaction and increasing the quantity did not affect the reaction.

3.4. Validation

Validation of the method for the standard drug and marketed formulation was carried out with respect to different parameters as mentioned in ICH guidelines for method validation [19]. These include accuracy, linearity, precision, limit of quantification (LOQ) and detection (LOD), and specificity.

3.5. Linearity and Range

The regression plot (Fig. **3**) in the concentration range of 40 to 200 μgmL^{-1} were in compliance with Beer Lambert's law having correlation coefficient (r^2) of 0.9981 with a molar absorptivity value of 5.16×10^3 $Lmol^{-1}cm^{-1}$. The synopsis of the statistical parameters is shown in Table **2** for the recommended process that includes concentration ranges, the correlation coefficient, and linear regression equation. The performance data clearly depict the real picture of linear working of the ranges of concentration.

3.6. Precision

Precision was tested by calculating the relative standard deviation (%RSD) of different concentrations of vigabatrin in three independent replicates. The results of %RSD are shown in Table **2** that are very low and illustrate that the method possesses excellent precision values.

3.7. Accuracy

Accuracy was evaluated as the mean % recovery (Table **3**) by analyzing different concentration of drugs from independent stock solutions.

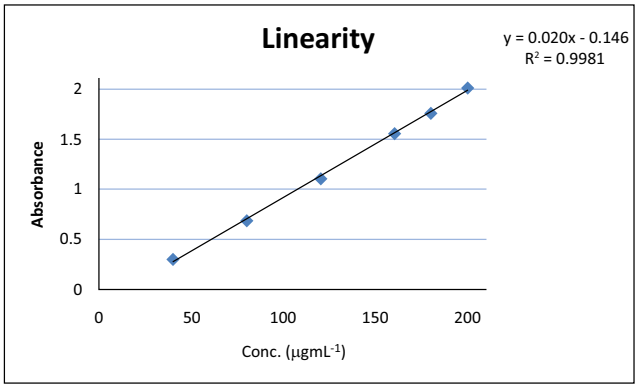


Fig. (3). Linearity plot of the method .

Table 1. Comparison of Beer's law limit and precision of proposed method with other spectrophotometric methods for the determination of vigabatrin.

Reagents	λ_{max}	Beer's law limit (μgmL^{-1})	%RSD	Reference
AAF reagent (Acetylacetone-formaldehyde)	410 nm	10-70	0.935	[1]
NBD-Cl(7-chloro-4-nitrobenzofurazan)	460 nm	2-10	1.18	[2]
Fluorescamine	472 nm	0.20–4.00	0.79/0.25	[3]
Ninhydrin	565 nm	40-200	0.194	Present method

Table 2. Linear regression functions and their statistical parameters.

Drug	Vigabatrin
λ_{max} (nm)	565
Regression parameters	
Intercept (mean±SD)	0.146±0.07
Slope (mean±SD)	0.0201±0.005
Calibration range (μgmL^{-1})	40-200
r^2	0.9981
LOD (μgmL^{-1})	6
LOQ (μgmL^{-1})	40
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	5.16×10^3
Precision (%RSD)	0.194

Table 3. Accuracy of method.

Analyte	Measuring Concentration	Measured Concentration (μgmL^{-1}) Mean±SD	Accuracy %
Vigabatrin	50	48.96±0.72	97.92
	75	74.65±0.45	99.53
	100	99.74±0.14	99.74

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

LOD and LOQ were corresponding to ICH definitions [19]. They were assessed using 3.3 σ/s and 10 σ/s, respectively. Here σ represents mean standard deviation of replicate determination values, and s is the sensitivity, namely the slope of the calibration graphs (Table **2**).

3.9. Interference

Table **4** shows the mean recovery values and %RSD of the recovery values in drug formulation that are found to be satisfactory. No potential interferences of excipients were observed in the proposed method. The absence of interference may be because all excipients lack an aliphatic primary amino group that is essential for the reaction to proceed.

3.10. Stability

The stability of the method was analyzed by calculating the absorbance of the final sample resolutions. The responses of the sample solutions showed the stability of the complex at room temperature for a minimum of 24 hours.

3.11. Analysis of Marketed Formulation

The satisfactory recovery values of the drug in the formulation (Sabril 500 mg) prove the validity of the application of the designed process in quality control laboratories and routine analysis.

4. DISCUSSION

UV-visible chromatic spectrum of the chromophoric derivative is shown in Fig. (**4**). Ninhydrin on reaction with amines, peptides, alpha amino acids, and proteins produces an aldehyde, ammonia, carbon dioxide, hydrindantin and Ruhemann's Purple (2-(3-hydroxy-1-oxo-1H-inden-2-ylimino)-2H-indene-1,3-dione) compound that is chromophoric. This chromophore group helps in detection and quantification of alpha-amino acids [20-22] and shifts the absorption maxima of vigabatrin from 190 to 565 nm after complexation. There are several basic rules for which systematic

Table 4. Determination of the vegabatrin in commercial formulation by the proposed method.

Analyte	Measuring Concentration (μgmL^{-1})	Mean recovered Concentration %	RSD %
Vigabatrin	75	99.87	0.289
	100	99.88	0.420
	125	98.60	1.037

approaches have to validate for the identification of drug in the formulation or routine analysis. Ideally it requires relatively simple methods for the preparation of samples and should follow the validation protocols set by ICH guidelines [19]. Already developed methods for the determination of vigabatrin used time-consuming steps in derivatization [1-3, 5, 8].

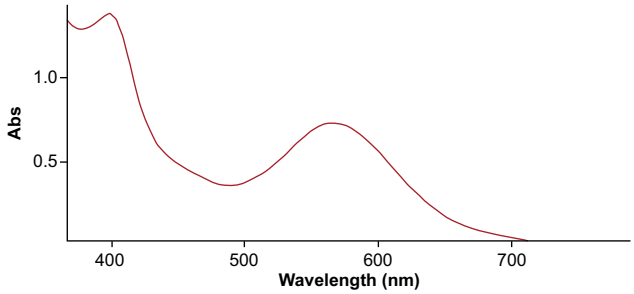


Fig. (4). UV-visible spectra of vigabatrin-ninhydrin complex.

Non-Derivatization methods employ simple preparation steps, involving only sample dilution procedures. However, capillary electrophoresis (CE) methods with UV detection may sustain inadequate sensitivity. Vigabatrin has no significant ultraviolet and visible absorption (Fig. **1**), so non-derivatization method development is complicated. Most of the developed HPLC methods do not allow high-throughput analysis due to longer run times of more than 4 to 5 min. High-throughput analysis using spectrophotometric methods with classical measurements in cuvette was also not possible. Microplate fluorescence reader [12] analysis is less available for routine quality control analysis and hence is rarely used. The present method is based on only 5 min derivatization step without requiring any extensive extraction steps. Since extraction steps are omitted in the present work, so the use of any organic solvent is also not applicable. All the procedures were carried out directly at pH 7.4 using phosphate buffer as the medium for the analysis of the drug in active and dosage forms. It makes it ideal for the routine analysis and adheres to green chemistry.

The derivatization of ninhydrin with drug is unique for primary amino groups. In the structures of all the tested excipients in the pharmaceutical preparation, amino groups were lacking. Hence, no interference of any of the excipients was observed when the process was implemented to the formulation. The validation of the process proceeded according to the guidelines of International Conference on Harmonization (ICH) [19]. Several trials were conducted in order to develop and optimize the proposed method. For this purpose, different parameters such as the effect of buffers pH, concentration of ninhydrin, temperature and optimum time period for heating were studied. Optimum conditions for the derivatization reaction between vigabatrin and ninhydrin were obtained at 70-75 °C using phosphate buffer of pH 7.4 in 5 minutes of the heating period. Table **1** describes the Beer's law limit and percent relative standard deviations (%RSD) of the reported spectrophotometric methods in comparison with the proposed method.

5. CONCLUSION

The objective to present a simplistic, rapid, inexpensive, and sensitive method for the identification of vigabatrin in bulk and formulation was achieved. It is comparable to the existing processes. The application of the proposed technique can be evaluated by the fact that it does not use any sophisticated instrumentation. It excludes extensive extraction step and omits the use of organic solvents. The validation of the process with regard to precision, accuracy, specificity, and stability further suggests its suitability for the routine analysis of vigabatrin in pharmaceutical formulations and pure form without interference from excipients. The system was used for the examination of vigabatrin in pharmaceutical tablets after satisfactory optimization and validation.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflict of interest.

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