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Spectrofluorimetric and spectrophotometric methods for the determination of vigabatrin in tablets

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Abstract

Two sensitive and selective spectrofluorimetric and spectrophotometric methods have been developed for the determination of vigabatrin in tablets. The methods are based on derivatization with 7-chloro-4-nitrobenzofurazan (NBD-Cl). The product showed an absorption maximum at 460 nm and a fluorescence emission peak at 520 nm in ethyl acetate. The color was found to be stable for at least 48 h in this solvent. The optimum conditions of the reaction were investigated and it was found that the reaction proceeds quantitatively at pH 10.0, 70 °C in 50 min when the mole ratio of the reagent to drug was 30. The reaction obeys Beer's law over the ranges of 2–10 and 0.05–1.00 μg ml^{-1} for the spectrophotometric and spectrofluorimetric measurements, respectively. The detection limits were found to be 0.50 and 0.01 μg ml^{-1} for the spectrophotometric and spectrofluorimetric methods, respectively. The proposed methods were applied to the assay of vigabatrin in tablets. The results were compared statistically with those obtained by the modified spectrofluorimetric method reported in the literature.

Keywords: Vigabatrin; 7-Chloro-4-nitrobenzofurazan; Spectrophotometry; Spectrofluorimetry

1. Introduction

Vigabatrin (γ-vinyl-γ-aminobutyric acid, γ-vinyl-GABA) is a catalytic inhibitor of brain GABA transaminase, the enzyme responsible for the degradation of the neurotransmitter, GABA. Consequently it produces an increase in brain GABA concentrations. This effect is presumed to be responsible for its antiepileptic action [1,2]. Vigabatrin has been determined in biological samples by amino acid analyzer [3], gas chromatography [4,5] and high-performance liquid chromatography (HPLC) with absorbance [6] and fluorescence [7,8] detection. Only one HPLC method with UV detection [9] has been reported for vigabatrin and its degradation product in pharmaceutical formulation.

Among the various methods available for the determination of drugs, spectrophotometry and spectrofluorimetry continue to be very popular, because of their simplicity, specificity and low cost. This study presents a new spectrophotomet-

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ric and spectrofluorimetric methods for the assay of vigabatrin in tablets. The methods are based on derivatization with 7-chloro-4-nitrobenzofurazan (NBD-Cl) which is highly sensitive and selective reagent for primary and secondary aliphatic amines. NBD derivatives are usually determined fluorimetrically [10–12], however, photometric detection has also occasionally been used [13,14].

The aim of this study was to optimize the reaction between vigabatrin and NBD-Cl. The applicability of the developed methods was evaluated through the determination of vigabatrin in bulk form or tablets.

2. Experimental

2.1. Apparatus

Spectrophotometric measurements were carried out using a Shimadzu UV-160 A spectrophotometer with 1-cm glass cells. Absorbance values were measured at 460 nm.

Fluorescence spectra and measurements were taken on a Shimadzu spectrofluorimeter Model RF-1501 equipped with xenon lamp and 1-cm quartz cells. Excitation and emission wavelengths were set at 460 and 520 nm, respectively. The measuring system of the instrument was calibrated daily by using sodium fluorescein (reference standard) solution in 0.1 N NaOH in an appropriate concentration.

2.2. Materials and reagents

Vigabatrin and its pharmaceutical preparation (Sabril®) containing 500 mg of drug per tablet were kindly supplied by Hoechst Marion Roussel (Istanbul, Turkey). NBD-Cl and other chemicals were purchased from Merck (Darmstadt, Germany). All solvents used were of analytical-reagent grade.

2.3. Solutions

Standard solutions: About 50 mg of vigabatrin, accurately weighed, was dissolved in 10 ml of water. Standard solutions (200, 400, 600, 800 and 1000 μg ml^{-1} in spectrophotometric method and 5, 10, 15, 20, 40, 60, 80 and 100 μg ml^{-1} in spectrofluorimetric method) were prepared from this solution by appropriate dilutions with water.

Sample solution: tablet powder, equivalent to about 50 mg of vigabatrin was accurately weighed and transferred into a 100-ml volumetric flask, 50 ml of water was added and shaken mechanically for 15 min. The volume was diluted with water, mixed and filtered (solution A). A 10 ml volume of solution A was adjusted to 100 ml with water in a volumetric flask (solution B). Solution A and B were used in spectrophotometric and spectrofluorimetric methods, respectively.

Reagent solution: NBD-Cl solutions in methanol (4.5 and 0.45%) were prepared freshly.

Buffer solution: 0.620 g boric acid and 0.750 g potassium chloride were dissolved with 100 ml of water. The pH was adjusted to 10.0 with 0.1 N sodium hydroxide solution and the volume was made up to 200 ml with water.

2.4. Assay procedure

An aliquot of 0.1 ml of standard or sample solution was mixed with 0.1 ml of buffer solution in a glass, stoppered tube. After addition of 0.1 ml of NBD-Cl solution, the mixture was heated at 70 °C for 50 min in a thermostatted water bath. The mixture was cooled and acidified with 0.1 ml of 0.1 N HCl solution. The derivative was extracted three times with 2 ml of ethyl acetate on a vortex mixer. After the phases had been separated by centrifugation, the combined extracts were adjusted to 10 ml with the same solvent.

2.4.1. Spectrophotometric measurements

Absorbance values of the derivative were measured at 460 nm against the blank prepared similarly.

2.4.2. Spectrofluorimetric measurements

The fluorescence intensities of the derivative were measured at 520 nm while exciting at 460 nm against a blank prepared similarly. The fluorescence intensity of the reference standard solution, with appropriate concentration, was also measured at the same wavelength combination. The

relative fluorescence intensity (I_F) was then calculated by the following equation: $I_F = x/y \times 100$. x and y represent the fluorescence intensities of the sample and reference standard solutions, respectively.

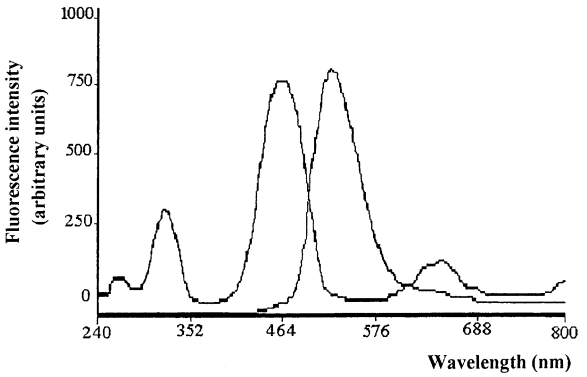

Fig. 1. Excitation and emission spectra of the reaction product of vigabatrin (1000 ng ml^{-1}) with NBD-Cl in ethyl acetate.

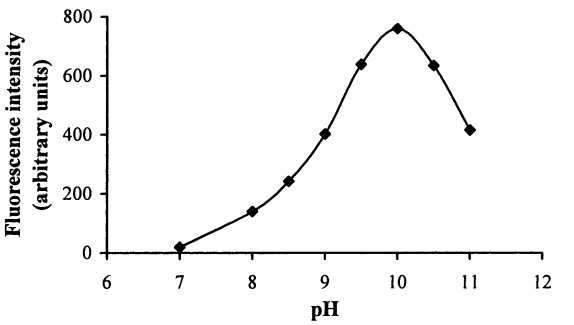

Fig. 2. Effect of the pH on the reaction of vigabatrin with NBD-Cl.

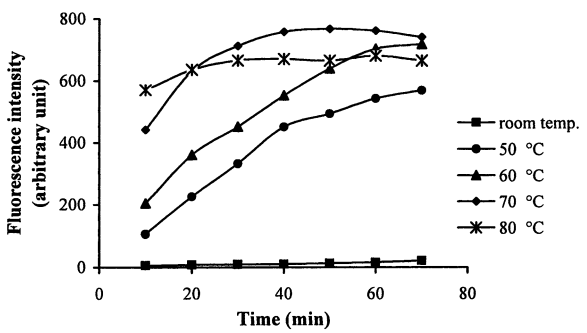

Fig. 3. Effect of the heating time on the reaction of vigabatrin with NBD-Cl.

Table 1
Analytical features of vigabatrin-NBD derivative

Parameter	Spectrophotometry	Spectrofluorimetry
Linear range (μg ml^{-1})	2–10	0.05–1.00
Detection limit (μg ml^{-1})	0.50	0.01
Slope ± S.D.	0.089 (±0.0172)	0.1617 (±0.0003)
Intercept ± S.D.	−0.071 (±0.0364)	2.6207 (±0.5352)
Correlation coefficient (r)	0.9999	0.9997
%RSD[a]	1.18	1.33
Molar absorbtivity (l mol^{-1} cm^{-1})	1.2×10^4	
Sandell's sensitivity (μg cm^{-2})	1.08×10^{-2}	

[a] Six replicate analyses.

2.5. Comparison method

For comparison of the proposed methods a spectrofluorimetric method was modified from the previously described reports [3,7]. The analysis was performed as described below:

An aliquot of 0.1 ml of standard (20–100 μg ml^{-1}) or sample (50 μg ml^{-1}) solution was mixed with 1 ml of pH 9.5 borate buffer solution and 0.125 ml of *o*-phthalaldehyde (OPA) solution (it was prepared daily by mixing 10 mg of OPA, 1 ml of methanol, 50 μl of mercaptoethanol and 9 ml of pH 9.5 borate buffer solution). The volume was diluted to 10 ml with methanol–water (1:2) and the fluorescence intensity of the solutions was measured at λ_{em}:452 nm (λ_{ex}:329 nm) within 5 min. Quinine sulfate solution in 0.1 N H_2SO_4 was used to calibrate the measuring system.

3. Results and discussion

Vigabatrin reacted with NBD-Cl to form a yellowish green colored product with light absorption at 460 nm and fluorescence emission at 520 nm (Fig. 1). The reaction was proceeded in alkaline medium. The pH dependence of the system

Table 2
Statistical evaluations of the results obtained by proposed and comparison methods for the assay of vigabatrin in tablets (each tablet contains 500 mg of vigabatrin)

Statistical value	Spectrophotometric method	Spectrofluorimetric method	Comparison method
Mean	526.77	530.52	521.34
Recovery (%)	105.35	106.10	104.27
RSD (%)	1.02	1.16	1.55
Confidence limits	521.14–532.4	524.37–536.67	513.25–529.42
t-test of significance*	$t = 1.39$	$t = 2.21$	
F-test of significance*	$F = 2.26$	$F = 1.73$	

*$n = 6$, $P = 0.05$, $t = 2.23$, $F = 5.05$.

was studied in the range of 7.0–11.0 using buffer solutions at different pH. The results indicated that maximum fluorescence was obtained at pH 10.0 (Fig. 2).

Preliminary studies reported that the reaction rate was very slow at room temperature. In this study, the derivatizaton reaction was performed at different temperatures and at various periods. As it is seen in Fig. 3, the reaction was completed at 70 °C within 50 min. Increasing the temperature to 80 °C resulted in an apparent decrease in the reaction rate.

The reagent amount required was examined by changing the mole ratio of NBD-Cl to vigabatrin. A 30-fold molar excess of reagent was found to be necessary to complete the reaction. A greater excess showed no further improvement.

The absorbance or fluorescence intensities were influenced by the solvent used. In the hydroalcoholic reaction medium, the derivative showed the lowest measurements. Some solvents such as chloroform, dichlormethane, methylisobutylketone and ethyl acetate were examined. The derivative has maximum intensity in ethyl acetate and it was stable in this solvent for at least 48 h at 4 °C in the dark.

Under the optimum reaction conditions the absorbance and relative fluorescence intensity were found to be linearly correlated to vigabatrin concentration over the range of 2–10 μg ml^{-1} and 0.05–1.00 μg ml^{-1}, respectively. Data recorded in Table 1 summarizes the characteristics of the calibration curves.

The proposed methods were applied to the analysis of commercially available tablets and the amount of vigabatrin was calculated from the regression equation of the calibration curves. The results were statistically compared with those obtained by the modified spectrofluorimetric method. Statistical comparisons in terms of Student's t-test and variance ratio F-test for these methods were given in Table 2. The calculated t- and F-values did not exceed the tabulated values, indicating that there is no significant difference between the methods in the respect of mean values and the standard deviations at 95% confidence level.

Excipients presented in the tablet do not interfere with the analysis.

4. Conclusion

The proposed spectrophotometric and spectrofluorimetric methods provided sensitive, specific and inexpensive analytical procedures for vigabatrin. They can readily be applied for routine quality control testing and drug stability monitoring. Moreover, the spectrofluorimetric method is sensitive enough for the analysis of biological samples.

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