Sensitive Spectrofluorimetric Method of Analysis for Gabapentin in Pure and Pharmaceutical Preparations

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Highly sensitive spectrofluorimetric method was developed and validated for the determination of gabapentin in pure and pharmaceutical preparations. The method was based on nucleophilic substitution reaction of gabapentin with 4-fluoro-7-nitrobenzofurazan (NBD-F) in an alkaline medium (pH 9.5) to form a highly fluorescent derivative that was measured at 521 nm after excitation at 458 nm. The factors affecting the reaction was carefully studied and optimized. The method was successfully validated for linearity, limit of detection, limit of quantification, accuracy, precision, robustness and specificity. Under the optimized conditions, linear relationship with good correlation coefficient (0.9998) was found between the relative fluorescence intensity and gabapentin concentration in the range of 10—100 ng•mL^{-1}. The limit of detection and limit of quantification were 0.43 and 1.30 ng•mL^{-1}, respectively. The proposed method was successfully applied to the determination of gabapentin in its pharmaceutical capsules. The results obtained by the proposed method were comparable with those obtained by the official method. Statistical comparison by *t*- and *F*- tests revealed that there was no significant difference between the results of the two methods with respect to mean values and standard deviations at the 95% confidence level.

Keywords gabapentin, NBD-F, spectrofluorimetric method, derivatization, determination

Introduction

The new anti-convulsant drug gabapentin (GB) [1-(aminomethyl)cyclo-hexaneacetic acid] is a structural analogue of γ-aminobutyric acid (GABA) and its action is attributed to the irreversible inhibition of the enzyme GABA-transaminase, thus preventing the physiological degradation of GABA in the brain; a secondary mechanism of a blockade for GABA uptake is also suggested.[1]

The several analytical methods have been developed for the determination of GB in biological fluids. Among the methods are gas chromatography (GC),[2,3] gas chromatography-mass spectrometry (GC-MS),[4-6] high-performance liquid chromatography (HPLC),[7-20] liquid chromatography-mass spectrometry (LC-MS),[21-24] capillary electrophoresis (CE)[25] and spectrofluorimetry.[26]

A few analytical methods have been reported for the determination of GB in pharmaceutical preparations in the literature including spectrofluorimery and spectrophotometry,[27,28] flow analysis,[29] CE[30] and HPLC.[31,32]

In this study, a highly sensitive spectrofluorimetric method with high reproducibility has been developed for the assay of GB in pharmaceutical preparations by means of the derivative formed with NBD-F, which is a specific reagent in the analysis of primary and secondary aliphatic amines.

Experimental

Apparatus

All fluorescence measurements were made on a Shimadzu RF-1501 spectrofluorimeter (Kyoto, Japan) equipped with a xenon lamp and using 1.0 cm quartz cells.

Chemicals and materials

GB was kindly supplied from Pfizer (Istanbul, Turkey). Neurontin® 100 mg capsules was obtained from commercial sources in the local pharmacy. NBD-F was purchased from Fluka (Buchs, Switzerland). Other chemicals were purchased from Merck (Darmstadt, Germany). All solvents were of analytical grade.

Solutions

The stock solution of GB was dissolved in water (1.0 mg•mL^{-1}). The working standard solutions were prepared from stock solutions by dilution (10 μg•mL^{-1}).

NBD-F was freshly prepared by dissolving 20 mg in 100 mL methanol. This reagent solution was further diluted with methanol to obtain working reagent solution (64.2 μg•mL^{-1}). Solutions were stored at 4 ℃.

Reference standard solution was prepared by diluting a stock solution of fluorescein sodium containing 1.0 μg•mL^{-1} in 0.1 mol•L^{-1} NaOH.

A borate buffer (0.1 mol•L^{-1}) was prepared by dis-

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solving 0.620 g of boric acid and 0.750 g of potassium chloride in 100 mL of water. The pH was adjusted to 9.5 with 0.1 mol•L^{-1} sodium hydroxide solution and the volume was made up to 200 mL with water.

Capsule solution

Ten capsules were weighed, finely powdered, and then a quantity of the powder equivalent to 100 mg of GB was transferred into a 100 mL volumetric flask, dissolved in 50 mL water, sonicated for 30 min, completed to volume with the water, shaken well for 5 min, and filtered into a 100 mL calibrated flask and then diluted to volume with water. An aliquot of 1 mL of the filtrate was diluted to 100 mL to prepare working sample solutions (10 μg•mL^{-1})

General procedure

An appropriate volume of 10 μg•mL^{-1} working solution (10—100 μL of GB) was transferred into 12-mL stoppered tubes. To each flask 100 μL of borate buffer (pH 9.5) followed by 100 μL of NBD-F reagent (64.2 μg•mL^{-1}) were added and mixed well. The solutions were heated in a thermostated water bath at 70 ℃ for 30 min, then left to cool and acidified with 100 μL of 0.1 mol•L^{-1} HCI. The derivatives were extracted three times with 3.0 mL of ethyl acetate. The combined organic phases were adjusted to 10 mL with the ethyl acetate. The fluorescence intensity was measured spectrofluorimetrically at λ_{ex} 458 nm and λ_{em} 521 nm against blank prepared similarly.

Method validation

Linearity

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: $I_F = x/y \times 100$, in which x and y represent the fluorescence intensities of the sample and reference standard solutions, respectively. The calibration curves were constructed by measuring relative fluorescence intensities of the analyte against concentrations of the calibration standards.

Limit of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of drugs by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of intercept of regression equation.[33]

Accuracy and precision

The accuracy and precision were also assessed by determining GB samples at three concentration levels on three different validation days. The accuracy was expressed by relative mean error (RME) and the precision by relative standard deviation (RSD).

Recovery

The recovery of the added pure drug was calculated as,

$$\%\text{Recovery} = [(C_t - C_s)/C_a] \times 100$$

where C_t is the total drug concentration measured after standard addition; C_s, drug concentration in the formulation sample; C_a, drug concentration added to the formulation.

Robustness

The robustness of the method was evaluated during the development by deliberate changes to the method parameters. The factors chosen for this study were the reaction time and volume of NBD-F.

Specificity

The specificity of the method was determined by adding deliberately some common capsule excipients, such as talc, starch, glucose, magnesium stearate and titanium dioxide to the standard drug preparation and then observing effect on the spectra.

Results and discussion

Derivatization

NBD-F is an activated halide derivative first introduced as a fluorigenic reagent for the determination of secondary and primary amines.[34] Thus, it is possible to detect in the low amount by the use of pharmaceutical-NBD-F adduct.

GB contains a primary amino group that was found to react with NBD-F. These typical nucleophilic reactions have not been reported yet for GB. Under the recommended conditions, the derivatized GB was found to be fluorescent product exhibiting highest fluorescence intensity at λ_{ex} 458 nm and λ_{em} 521 nm. Figure 1 shows the derivatization reaction of the GB with NBD-F. The excitation and emission spectra for the reaction product of the GB with NBD-F are given in Figure 2.

Optimization of reaction conditions

The factors affecting the reaction conditions (pH, the concentrations of NBD-F reagent, reaction time, temperature, and the diluting solvent) were studied.

Effect of pH In order to generate the nucleophile from GB, the reaction should be carried out in an alkaline medium. The dependence of the reaction on the pH of the reaction medium was studied using buffer solu-

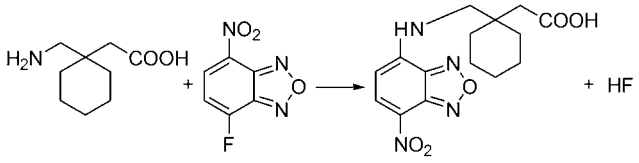

Figure 1 The reaction between GB and NBD-F.

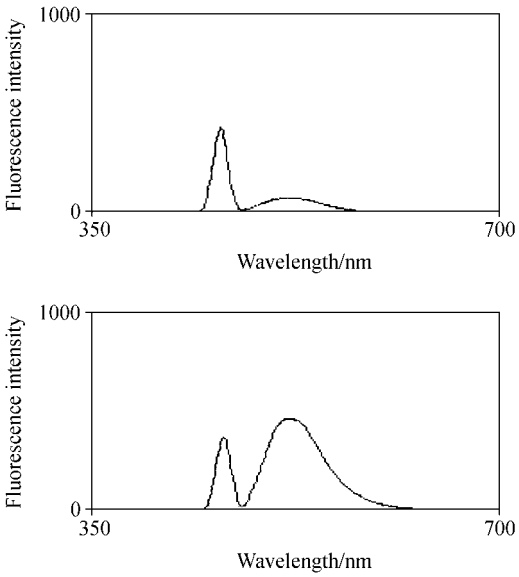

Figure 2 Blank and emission spectra of the reaction product of GB (40 ng•mL^{-1}) with NBD-F.

tions in the range of 7.0—10.0. The influence of pH on the fluorescence intensity of the reaction product was evaluated. Maximum fluorescence intensity was obtained at pH 9.5 (Figure 3). Other buffers having the same pH value such as phosphate and carbonate were tried and compared with 0.1 mol•L^{-1} borate buffer. Borate buffer was found to be superior to other buffers having the same pH value since the net fluorescence intensity was highest in case of borate buffer. This is probably, because the rate of hydrolysis of NBD-Cl to NBD-OH was much slower. This result is in agreement with that of reported method.[34,35]

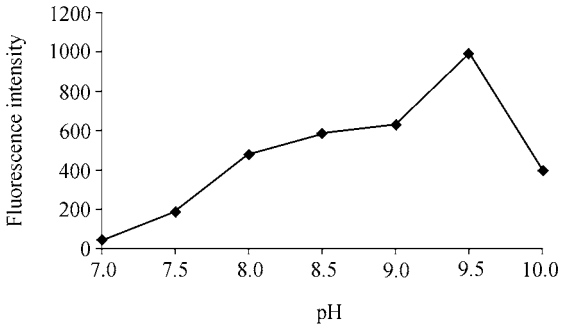

Figure 3 Effect of pH on the derivatization reaction of GB (100 ng•mL^{-1}) with NBD-F.

Effect of temperature and time The effect of temperature on the color intensity was studied in the range from room temperature, 50 to 80 ℃ for different periods of time. The reaction does not proceed at room temperature. The results indicated that the reaction was dependent on temperature, and the optimum condition was achieved by heating at 70 ℃ for 30 min (Figure 4).

Effect of concentration of NBD-F The reaction was studied as a function of NBD-F reagent concentration. The results revealed that the fluorescence intensity

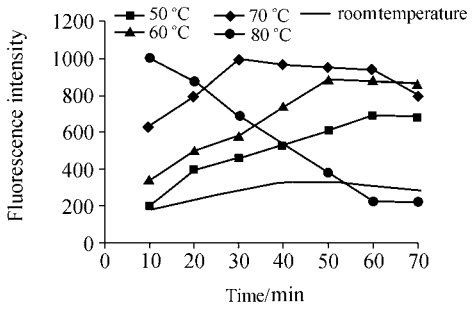

Figure 4 Effect of temperature and time on the derivatization reaction of GB (100 ng•mL^{-1}) with NBD-F.

was dependent on NBD-F concentration (Figure 5). The reagent amount required was examined by changing the mole ratio of NBD-F to GB. A 6 fold molar excess of reagent was found to be necessary to complete the reaction.

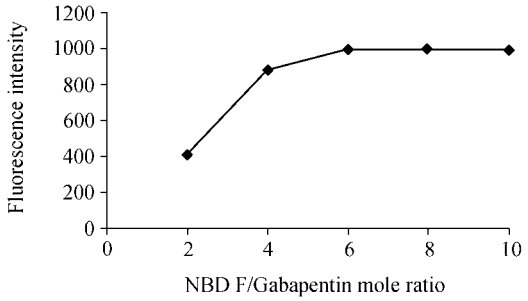

Figure 5 Effect of reagent amount on the derivatization reaction.

Effect of diluting solvent In order to select the most appropriate organic solvent for diluting the reaction solution, different solvents were tested: methanol, 1,4-dioxane, methyl isobutyl ketone, dichloromethane, ethyl acetate and chloroform. The highest readings were obtained when ethyl acetate was used for extracting solvent (Table 1).

Table 1 Effect of different polar solvents on fluorescence intensity of GB with NBD-F (100 ng•mL^{-1})

Solvent	λ_{ex} /λ_{em} (nm)	Fluorescence intensity
Methanol	462/594	178
1,4-Dioxane	461/532	293
Methyl isobutyl ketone	465/610	411
Dichloromethane	462/530	172
Ethyl acetate	458/521	994
Chloroform	461/532	425

Effect of the hydrochloric acid The fluorescence of the hydrolysis product of NBD-F, namely, 4-hydroxy-7-nitrobenzo-2-oxa-1,3-diazole (NBD-OH), is quenched by decreasing the pH of the reaction medium to less than 1. It is known that the maximum association between NBD-F and aimed compound is realized at the

basic medium. But, NBD-F is also hydrolyzed in alkaline solution. Thus, the system was stabilized by acidifying the reaction mixture to pH 2 (by adding 100 μL 0.1 mol•L^{-1} HCl) before measurement.[35]

Stability of the derivative The derivative formed from GB with NBD-F was stable in ethyl acetate at 4 ℃ in the dark for 48 h (Table 2).

Table 2 Stability of the GB with NBD-F (Initial concentration 100 ng•mL^{-1})

	Initial	After 6 h	After 24 h	After 48 h	After 72 h
Room temperature (in the dark)	991	983	960	842	751
Room temperature (in the daylight)	991	910	632	456	228
4 ℃ (in the dark)	991	990	990	990	986

Method validation

Linearity The regression plots showed that there was a linear dependence of the relative fluorescence intensity value on the concentration of the drug over the range of 10—100 ng•mL^{-1}. The equations of the calibration curves were obtained by the least-squares linear regression analysis and calculated as: $I_F = 0.1004C - 6.2\times10^{-2}$, where I_F and C are the relative fluorescence intensity and GB concentration in ng•mL^{-1}.

LOD and LOQ The LOD and LOQ were 0.43 and 1.30 ng•mL^{-1}, respectively. These values are much lower than those obtained by many other methods[25-29] (Table 3).

Table 3 Performance data of the proposed procedure (n=5)

Parameter	GB
Linear range/(ng•mL^{-1})	10—100
LOD/(ng•mL^{-1})	0.43
LOQ/(ng•mL^{-1})	1.30
Slope (b)	0.1004
SD of slope (Sb)	2.77×10^{-4}
Intercept (a)	6.2×10^{-2}
SD of intercept (Sa)	1.31×10^{-2}
Correlation coefficients (r)	0.9998

Precision and accuracy The method gave satisfactory results; mean RSD was 1.15%, indicating its good reproducibility. In accuracy study, RME values were below 5.20%. RSD and RME values were within the acceptable range, indicating that these methods have good precision and accuracy (Table 4).

Table 4 Intra-day and inter-day precision and accuracy of GB with NBD-F (n=3)

Added/(ng•mL^{-1})	Found (mean)/(ng•mL^{-1})	RSD/%	RME/%
	Intra-day		
10	10.41	2.11	4.10
40	40.45	0.74	1.13
100	100.58	0.20	0.58
	Inter-day		
10	10.62	2.92	5.20
40	40.45	0.47	1.40
100	100.71	0.47	0.71

Recovery Recovery studies were carried out by the standard addition method. In all cases, the recovery percentage values ranged between 100.51% and 100.68 %.

Robustness The robustness of the method adopted is demonstrated by the constancy of the fluorescence intensity with the deliberated minor change in the experimental parameters such as the change in the volume of NBD-F (100±5.0 μL) and the change in the reaction time (30±1 min). These minor changes that may take place during the experimental operation did not affect the fluorescence intensity of the reaction product (Table 5).

Application The proposed method has been successfully applied to the determination of the studied drug in commercial capsules. The results obtained are shown in Table 6. According to the t- and F-tests, no significant difference was found between the calculated and theoretical values of both the proposed and the official methods (HPLC)[36] at 95% confidence level. This indicates good level of precision and accuracy.

Conclusions

The proposed method involved simple derivatization of GB with NBD-F reagent, and subsequent measuring the fluorescence intensity of the fluorescent reaction product. The proposed method is highly sensitive, ac-

Table 5 Robustness of the proposed method (GB 40 ng•mL^{-1})

	NBD-F volume (100 μL+5 μL)	NBD-F volume (100 μL−5 μL)	Reaction time (30 min+1 min)	Reaction time (30 min−1 min)
Found concentration (mean)/(ng•mL^{-1})	40.06	40.06	40.08	40.05
SD	1.2×10^{-2}	2.1×10^{-2}	1.7×10^{-2}	2.2×10^{-2}
RSD	0.03	0.05	0.04	0.05
Recovery%	100.15	100.15	100.20	100.12

Table 6 Determination of GB in its pharmaceutical preparation by the proposed spectrofluorimetric and the official methods

Pharmaceutical preparation	Mean±SD		*t*	*F*
	Proposed method	Official method[36]		
Neurontin® 100 mg[a]	100.66±0.76	101.02±0.91	0.68	1.43

[a] $n=6$, $P=0.05$, $t=2.23$, $F=5.05$.

curate and reproducible to be applied to the analysis of capsules. The proposed method is suitable for routine analysis of GB in quality control and clinical laboratories.

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