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The utility of acetylbutyrolactone for spectrofluorimetric determination of two gamma-aminobutyric acid analogues

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Abstract

Acetylbutyrolactone was applied as a fluorogenic reagent for developing a simple, selective and economic method for determination of Two Gamma-Aminobutyric Acid (GABA) analogues containing primary aliphatic amine group (gabapentin and pregabalin). The reaction depends on the formation of Schiff bases which have strong fluorescence activities at 440 nm after excitation at 380 nm. The different reaction parameters that affect the stability of the formed Schiff base have been deeply investigated and optimized. The suggested reaction mechanism has been explained. The fluorescence-concentration plots were rectilinear over the range of 20-100 µg ml^{-1} for gabapentin and 10-100 µg ml^{-1} for pregabalin with excellent correlation coefficients (0.9998 for Gabapentin and 0.9999 for pregabalin). The lower quantification limits were 2.9 and 1.9 µg ml^{-1} for gabapentin and pregabalin, respectively. According to ICH guidelines, the method was validated and the results were in agreement with other reported methods in respect to accuracy and precision. Determination of gabapentin and pregabalin in the commercial capsules were achieved using the proposed method with excellent recoveries (100.22% for Gaptin® capsules and 100.28% for Lyrolin® capsules) and high degree of accuracy.

Key words

Gabapentin , pregabalin , Acetylbutyrolacton , Spectrofluorimetry

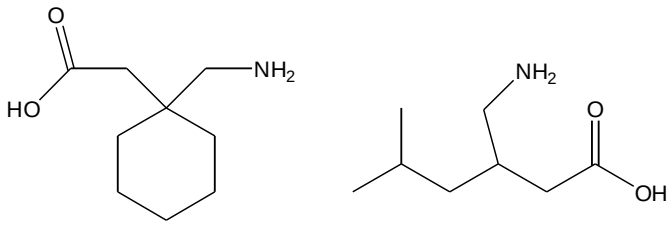
1. Introduction

Gabapentin [1-(amino methyl)cyclohexane acetic acid], and Pregabalin [3-(aminomethyl)-5-methylhexanoic acid] are gamma-amino butyric acid (GABA) analogues [1]. Gabapentin has an anticonvulsant and antiepileptic efficacy. Recently, it could be used in relieving of pain and partial seizures [2, 3] . pregabalin could be used for relieving the neuropathic pain and in treatment of epilepsy and fibromyalgia [4].The chemical structures of drugs under investigation are shown in (**figure 1**).

Several methods of analysis were reported for gabapentin and pregabalin determination in their pure forms and pharmaceutical dosage forms. Most of them are either spectrophotometry [5-14], spectrofluorimetry [15-21] high-performance liquid chromatography (HPLC) [22-25], gas chromatography–mass spectrometry (GC–MS) [26, 27],capillary electrophoresis [28, 29] and electrochemical techniques [30, 31]

Acetylbutyrolactone (4,5-dihydro-3-acetylfuran-2(3H)-one) is an analytical reagent that had been recently used for determination of some aliphatic and aromatic primary amines as sulfamethoxazole and ampicillin [32] through Schiff base formation.

The aim of the present study was to use acetylbutyrolactone in the development of a simple, selective and economic method for quantitative determination of gabapentin and pregabalin. Intensive fluorescent products were obtained by condensation of investigated drugs amino group's with acetylbutyrolactone. The proposed method was applied for determination of gabapentin and pregabalin in their commercial capsules without any interference from the excipients.



Gabapentin Pregabalin

Figure 1: Chemical structural formula of gabapentin and pregabalin

2. Experimental

2.1. Apparatus

- Spectrofluorimetric measurements were performed by using Perkin-Elmer UK model LS 45 Luminescence spectrometer equipped with a 150W Xenon arc lamp, gratting excitation and emission monochromators and a 1-cm quartz cell. Both monochromators slit width were adjusted at 10 nm. The Luminescence spectrometer was coupled with an IBM PC computer loaded with the FL WINLAB™ software.
- UV/VIS spectrophotometric measurements were carried out by using Spectronic™ Genesys™ 2PC Ultraviolet/Visible spectrophotometer (Milton Roy Co, USA).
- Milwakee SM 101 pH meter (Portugal), digital analytical balance (AG 29, Meltter Toledo, Glattbrugg, Switzerland)

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and MLW type thermostatically controlled water bath (Memmert GmbH, Schwabach, Germany) were used.

2.2. Chemicals and Reagents

All the reagents used were of analytical grade. Pharmaceutical grade Gabapentin of purity 99.5 % was kindly supplied from Delta Pharma Company (10th of Ramadan City, El Sharkeya, Egypt). Pregabalin of purity 98.0 % was kindly provided by Al Debeiky Pharmaceutical Industries (Obour City, Cairo, Egypt). These chemicals were used without further purification.

Acetylbutyrolactone (Sigma-Aldrich -Germany) was freshly prepared as 2% (*v/v*) in dimethylformamide (DMF) by dissolving 2 ml of acetylbutyrolactone in 50 ml of DMF.

Ethanol, methanol, boric acid, phosphoric acid, acetic acid, Dioxan and DMF were purchased from El-Nasr Chemical Co. (Cairo, Egypt).

In order to prepare Britton–Robinson buffer solutions (pH 2.0 – 12.0), the appropriate volumes of 0.04 M boric acid, 0.04 M phosphoric acid, 0.4 M acetic acid were mixed. The required pH was adjusted by adding 0.2 M sodium hydroxide.

2.3. Pharmaceutical Formulations

The following commercial pharmaceutical preparations were analyzed; Gaptin® capsules (Delta Pharma S.A.E, 10th of Ramadan City, El Sharkeya, Egypt) labeled to contain 100 mg gabapentin per capsule and Lyrolin® capsules (Hikma Pharma, Giza, Egypt) labeled to contain 50 mg pregabalin per capsule.

2.4. Preparation of stock and working standard solutions

An accurately weighed 20.0 mg of any of the investigated drugs was dissolved individually in 100 ml of DMF to prepare stock solutions. Further dilution with DMF was carried out to prepare the required working standard concentrations (200 µg ml^{-1}). The standard solutions of gabapentin and pregabalin were stable for at least seven days, when put in refrigerator.

2.5. General analytical procedure

Into a set of glass tubes, aliquots of standard working solutions of investigated drugs (20-100 µg ml^{-1} for gabapentin and 10-100 µg ml^{-1} for pregabalin) as final concentrations were mixed with 0.5 ml of acetylbutyrolactone solution (2% v/v). The mixed solutions were boiled in boiling water bath for 30 min (for gabapentin) and 35 min (for pregabalin). After cooling the test tubes to room temperature, test tube content's was quantitatively transferred into 10 ml volumetric flask and diluted to the mark with Britton–Robinson buffer (pH 10.5). The reaction products fluorescence's emissions were measured at 440 nm after excitation at 380 nm against reagent blank prepared similarly. The relative fluorescence intensities (RFI) obtained were plotted against the final concentration of investigated drugs (µg ml^{-1}) to construct the calibration curves.

2.6. Analysis of Capsules

The content of twenty capsules of investigated drugs were accurately weighed and mixed well. Then, an accurately weight of powder equivalent to 20 mg of the drug was dissolved in 100 ml DMF. The solution was further diluted with the Britton–Robinson buffer and a portion of the resulting solution was subjected for drug analysis using the previously described general analytical procedure. The regression equation for each investigated drug was used to estimate the nominal content of the capsules.

3. Results and discussions

Compounds containing aliphatic amino group such as ampicillin and aromatic amino group such as sulfamethoxazole[32] has been previously determined spectrofluorimetrically through their condensation with Acetylbutyrolactone. Because gabapentin and pregabalin contain a primary aliphatic amino group, both could interreact with Acetylbutyrolactone in the presence of DMF as a solvent to form fluorescent condensation products. (**Figure 2**) represents the reaction possible pathway of investigated drugs with acetylbutyrolactone. Primary amino group of gabapentin as a representive example reacts the reactive carbonyl group of acetylbutyrolactone to form the corresponding Schiff base, of the enamine tautomer type. The enamine tautomer structural rearrangement can be explained by electron delocalization and migration of the active labile proton (at the α-carbon). Upon heating, carbanion formation was facilitated with DMF slightly basic character.

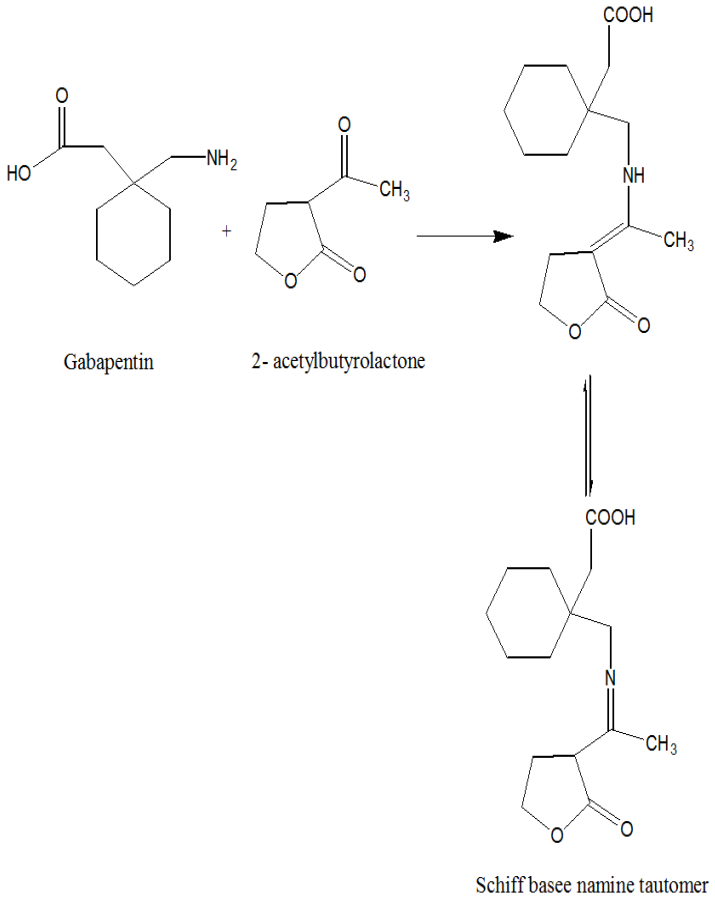

Figure 2: Suggested reaction pathway between the primary amine group of the gabapentin as a representative example with 2-acetylbutyrolactone.

After optimization of the reaction conditions, the relative fluorescence intensities of the formed product could be measured at 440 nm after excitation at 380 nm. (**Figure 3**).

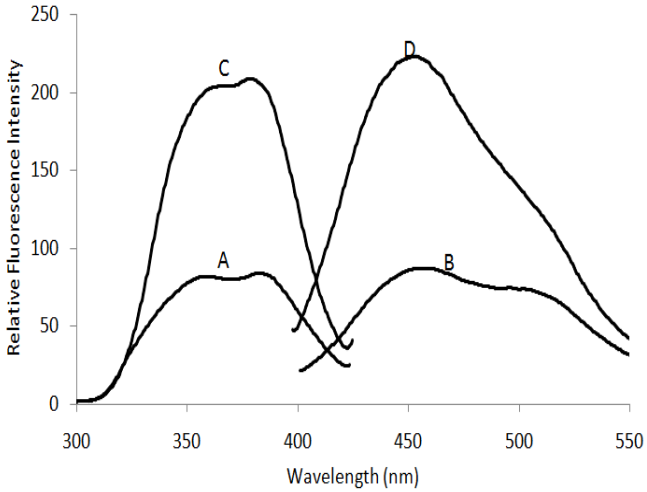

Figure 3: The fluorescence spectra of pregabalin (60 µg ml-1) as a representative example: (A, B) are excitation and emission spectra of blank respectively. (C, D) are excitation and emission spectra of formed product respectively.

3.1. Optimization of the reaction condition

In order to enhance the sensitivity and selectivity of the propose method, different factors that affect the reaction conditions such as; volume of acetylbutyrolactone, temperature, heating time, diluting solvents, and pH of diluting buffer were carefully studied.

3.1.1. Effect of Acetylbutyrolactone Volume

The effect of different volume of acetylbutyrolactone (0.2 – 1.2 ml) on RFI of the reaction product was carefully studied. Increasing the acetylbutyrolactone volume resulted in subsequent increase in the fluorescence intensity. The maximum fluorescence intensities were obtained when using 0.4 – 0.6 ml of the reagent for both drugs (**Figure 4**). It was concluded that 0.5 ml of acetylbutyrolactone solution was optimum for further investigations.

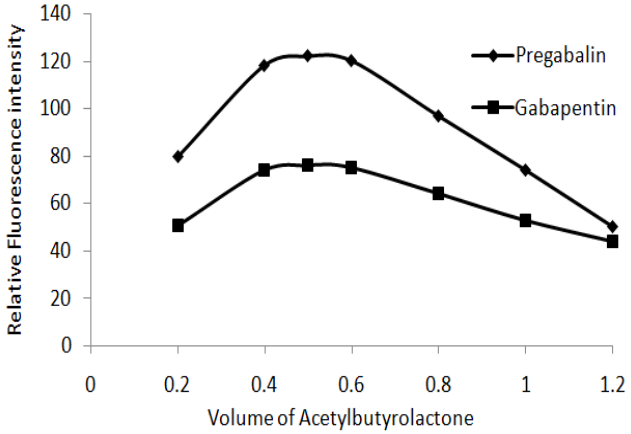

Figure 4: Effect of acetylbutyrolactone volume on relative fluorescence intensity of the formed product for gabapentin and pregabalin (60 µg ml^{-1}).

3.1.2. Effect of Temperature

After mixing the investigated drugs with acetylbutyrolactone, the solution was heated on a water bath at temperatures ranging from 60 °C to 100 °C. By increasing the temperature of the water bath, the RFI were subsequently increased for both investigated drugs up to 95 °C (for gabapentin) and kept constant to 100 °C (for pregabalin). Therefore, heating in boiling water bath for gabapentin and for pregabalin were chosen for further investigation procedure (**Figure 5**).

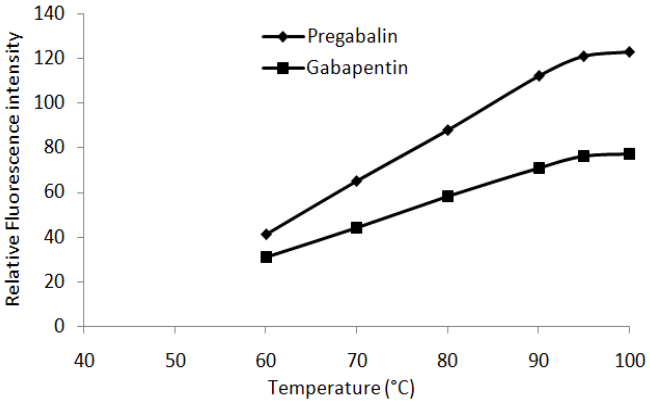

Figure 5: Effect of temperature on the intensity of the fluorescent productsof gabapentin and pregabalin(60 µg ml^{-1}) individually.

3.1.3. Effect of Heating Time

It was observed that increasing the heating time led to subsequent increase in the fluorescence intensities of the formed products. Maximum fluorescence intensities have been obtained after 25 to 35 min for gabapentin and after 30 to 40 min for pregabalin. Heating time above these range resulted in gradual decrease in the observed fluorescence intensity (**Figure 6**). Consequently, the maximum fluorescence intensities for both investigated drugs were obtained by heating the reaction mixture to 30 and 35 min for gabapentin and pregabalin, respectively.

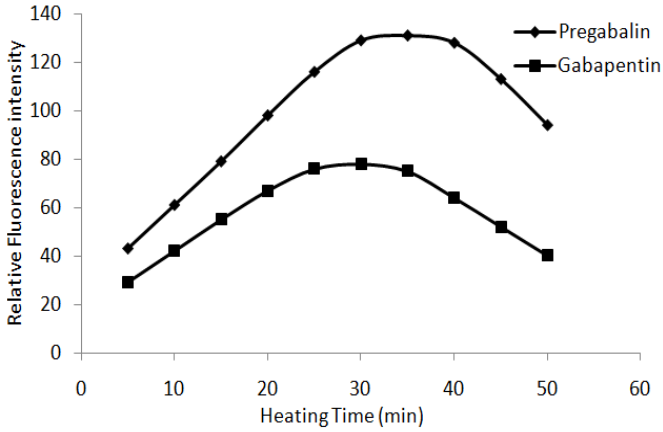

Figure 6: Effect of different heating time (in boiling water bath) on fluorescence intensity of formed product (Drug concentration is 60 µg ml^{-1}).

3.1.4. Effect of Diluting Solvent

The formed fluorophore were diluted with different solvents (Britton–Robinson buffer, Ethanol, methanol, dioxan). Of all the studied solvents, Britton–Robinson buffer give the highest fluorescence intensity, and therefore it was selected as a diluting solvent (**Figure 7**). Also, we used different types of buffers as borate buffer and phosphate buffer. We could observe that the Britton–Robinson buffer gave the highest RFI and maximum

sensitivity while phosphate and borate buffer gave very small fluorescence intensities values. Therefore, Britton–Robinson buffer was used during further investigation.

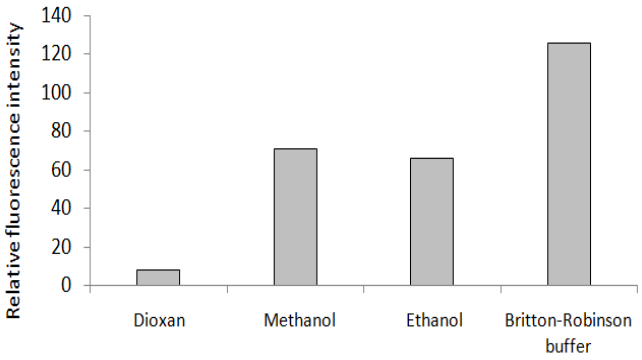

Figure 7: Effect of different diluting solvents on fluorescence intensity of formed product between acetylbutyrolactone and pregabalin(60 µg ml^{-1}) as a representative example.

3.1.5. Effect of pH of Diluting Solvent

Britton–Robinson buffer with different pH values (2–12) were used. The fluorescence intensity increased with increasing the pH value up to 10 and kept constant up to 11 for both drugs. Solutions with higher pH values led to a marked decrease in the relative fluorescence intensity (**Figure 8**). Therefore, Britton–Robinson buffer of pH 10.5 was optimal for both investigated drugs.

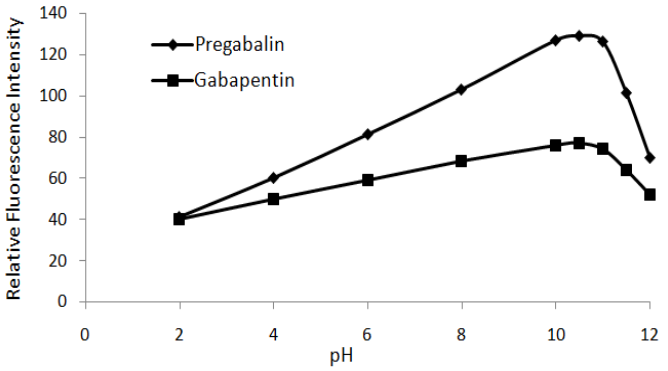

Figure 8: Effect the pH of Britton–Robinson buffer on the relative fluorescence intensity of the reaction product(Drug concentration is 60 µg ml^{-1}).

3.2. Validation of the proposed method

Validation parameters including; linearity, sensitivity, accuracy, and precision were tested according to ICH Q2B recommendations guidelines [33].

Linearity, range and sensitivity: In order to establish the standard curves, the final concentrations of the investigated drug (µg ml^{-1}) were plotted against the corrected relative fluorescence intensities (RFI of product – RFI of blank). Linear regression analysis of the data was performed and the calculated analytical parameters including slope, intercept, standard deviation of slope and intercept and correlation coefficient are summarized in Table 1. From the calibration curves, it was found that the RFI values and the drug concentrations was linear dependent within the range of 20 – 100 µg ml^{-1} for gabapentin and 10- 100 µg ml^{-1} for pregabalin. The excellent linearity of the proposed method was indicated by the high correlation coefficients. In addition, the scattering of the points around the standard curves were low as indicated by the standard deviations of the intercept (S_a) and slope (S_b) small values.

The proposed method sensitivity was evaluated by calculating the limit of detection (LOD) and limit of quantitation (LOQ).The LOD and LOQ were mathematically obtained using the following formula (LOD = 3 σ / S and LOQ = 10 σ / S), where (σ) is standard deviation of the intercept and (S) is slope of calibration curve. The obtained detection limits were 0.97 and 0.64 µg ml^{-1}, and the quantitation limit were 2.93 and 1.93 µg ml^{-1}, for gabapentin and pregabalin, respectively (**Table 1**). These values indicate the high sensitivity of the proposed method.

Accuracy: In general, the accuracy could be defined as the closeness or agreement of the obtained analytical value to the true value and measured by calculating the percentage (%) recoveries. For the proposed method, the accuracy was checked by preparation of three standard solutions containing different concentrations of the studied drugs covering the whole specified linear range. The concentration of each standard solution was measured by the proposed method in triplicate manner. All % recoveries values were from 98% to 102%, indicating the good accuracy of the proposed method (**Table 2**).

Precision: The precision is closeness or agreement of obtained analytical values to each other and is measured by calculating the relative standard deviation (RSD). The precisions were evaluated by applying the general analytical procedure for the analysis of standard drug solutions having three different concentrations within the specified linear range. The intra-day precisions were estimated by carrying out the analysis three times in the same day. However by performing the analysis on three successive days, we could estimate the inter-day precisions. The calculated relative standard deviations values were all less than 2 % which indicate good precision of the proposed method (**Table 3**).

Robustness: Robustness of the proposed method was evaluated by introducing minor changes in the experimental parameters such as volumes of Acetylbutyrolactone, pH of diluting solvent and the heating time (**Table 4**). These minor changes have no effect on the method performance as the obtained fluorescence intensities were nearly the same. As a result, the proposed methods could be considered as robust.

3.3 Application to Capsules

The quantitative determinations of commercial capsules containing investigated drugs were performed by the proposed method. The obtained results were statistically compared with those of the reported methods[6, 14], using t- and F- tests. There was no significant difference at 95% confidence level between the theoretical and calculated values, indicating good accuracy and precision of the proposed method (**Table 5**). It is clear from the analysis of the dosage forms that the presence of excipients has no significant interference in the results of the analysis. As a result, the proposed method could be successfully used for determination of commercial capsules containing investigated drugs with good accuracy, precision and selectivity.

Table 1: Analytical parameters for determination of the studied drugs using the proposed method.
*** LOD is the limit of detection and LOQ is the limit of quantitation.**

Parameters	Gabapentin	Pregabalin
Linear range (μg mL^{-1})	20 – 100	10 – 100
Intercept (a)	0.0169	1.377
Standard deviation of the intercept (S_a)	0.3918	0.4015
Slope (b)	1.336	2.079
Standard deviation of the slope (S_b)	0.0060	0.0064
Correlation coefficient (r)	0.9998	0.9999
LOD (μg mL^{-1}) *	0.97	0.64
LOQ (μg mL^{-1}) *	2.93	1.93

Table 2: Evaluation of accuracy for the proposed analytical method.
*** The value is the average of three determinations**
 # SD is the standard deviation and RSD is the relative standard deviation.

	Gabapentin			Pregabalin	
	Taken (μg mL^{-1})	Found* (μg mL^{-1})	% Recovery	Found* (μg mL^{-1})	% Recovery
1	20	20.12	100.62	20.14	100.70
2	60	59.94	99.90	60.10	100.17
3	100	100.50	100.50	100.51	100.51
Mean			100.34		100.46
SD #			0.38		0.27
RSD #			0.37		0.26

Table 3: Evaluation for precision of proposed method used for determination of the studied drugs.
RSD: Relative standard deviation

Concentration level (μg mL^{-1})	%Recovery ± RSD	
	Gabapentin	Pregabalin
Intra-day precision		
20	101.41 ± 0.76	101.14 ± 1.23
60	98.65 ± 1.27	99.10 ± 0.81
100	101.25 ± 0.74	100.19 ± 1.21
Inter-day precision		
20	99.26 ± 1.71	99.09 ± 1.26
60	101.15 ± 1.24	101.51 ± 0.79
100	99.00 ± 0.76	100.03 ± 0.73

Table 4: Robustness of the proposed method
*** Average of three determinations.**

Parameter	Gabapentin*		Pregabalin*	
	Change	% Recovery	Change	% Recovery
Acetylbutyrolactone volume	0.4 mL	99.11	0.4 mL	99.22
	0.6 mL	101.22	0.6 mL	101.24
pH of Diluting Solvent	10	99.42	10	99.13
	11	101.15	11	101.27
Heating Time	25 min	99.73	30 min	99.96
	35 min	101.73	40 min	101.63

Table 5: Statistical analysis of the obtained results using the proposed spectrofluorimetric and reported methods for analysis of the investigated drugs in commercial capsules.
*** Number of determination is five for both methods**
 # Tabulated values at 95 % confidence limit; t = 2.306 and F = 6.338.

Dosage forms	% Recovery * ± SD		t- value#	F value#
	Proposed Method	Reported Method		
Gaptin® capsules	100.22 ± 0.31	100.09 ± 0.67	0.403	4.740
Lyrolin® capsules	100.28 ± 0.47	99.79 ± 0.92	1.071	3.844

4. Conclusion

A new, simple, cheap, robust, and non-extractive spectrofluorimetric method has been developed and fully validated for gabapentin and pregabalin determination. The proposed method simply depends on condensation with -acetylbutyrolactone. The proposed method is ideally suited for determination of gabapentin and pregabalin in their commercial pharmaceutical dosage forms without any interference from common excipients.In comparison with other reported methods, our proposed method is simple, does not require elaborate treatment for the sample or tedious extraction steps or sophisticated instruments. In addition, our proposed method is economic with high degree of accuracy, precision and selectivity, making it the method of choice for quality control and quality assurance analysis.

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