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Estimation and Evaluation of Gabapentin and Pregabaline Anti-Epileptic Drugs in Bulk and Pharmaceutical Preparations by Eco-Friendly Bromate-Bromide Reagent

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Abstract: Tow simple and sensitive spectrophotometric methods are described for the determination of the anti-epileptic drugs (Gabapentin and Pregabaline) as apure drug andin commercial pharmaceutical preparations. These methods involve the bromination of the anti-epileptic drugs (Gabapentin and Pregabalin) with a well-known excess amount of bromate-bromide mixture in acid medium as an eco friendly brominating agent followed by the determination of unreacted bromine. The ideal conditions for the experimental work have been studied and optimized The remaining amount of bromine is estimated by the reaction with an excess amount of potassium iodide KI and the form edtriiodide (I^{-3}) is either measured directly at 350 nm and 355nmfor Gabapentin and Pregabalin (method A), or reacted with starch solutionand the measurement of the colored starch-iodine complex at 520nm and 555 nm for Gabapentin and Pregabal in respectively (method B).Absorbanceversus concentration plotswere drawn and it were linear to a certain degree, indicating that the results adheredto Beer's law over the ranges of0.30-15.0, 0.25-10.0 and2.0-10.0,0.30-6.00µg /mlfor Gabapentin and Pregabalin inboth methods, A and B. The molar absorptivities were found to be 1.489×10^4, 2.853×10^4 and 1.524×10^4,2.765×10^4 L/molcm for of Gabapentin and Pregabalin in method A and B respectively. Sandells sensitivity indexes were 0.964×10^{-3},0.102×10^{-3} and 0.164×10^{-3},0.068×10^{-3} µg/cm^2 for Gabapentin and Pregabalin in both methods respectively. The proposed method has been applied successfully for the quantitative analysis of Gabapentin and Pregabalin in pure form and in commercial pharmaceutical preparations (capsules). There wasno interference observed from the excipients. The results showeda good precision and accuracy using a standard additional method and it were statistically compared with a reference method by using the Student's t- and F-test,showing agood agreement with the standard

Keywords: Gabapentin, Pregabalin, Bromate-bromide Mixture, Brominating Agent, Spectrophotometric, Estimation.

INTRODUCTION

Gabapentin (GAB),1-(aminomethyl)-cyclohexaneacetic acid (Fig 1) and Pregabaline (PRG) (S)-3-(aminomethyl)-5-methylhexanoic acid (Fig 2) are antiepileptic drug, commonly used for treatment of epilepsy. Epilepsy, is a chronic disease that causes unexpected, recurrent seizures. A seizure may be defined as a sudden spread of an electrical activity centrally, in the brain. The main treatment for the epileptic people is the Anti-epileptic drugs (AED) (1). GAB increases GABA levels centrally in the brain. However, the exact mechanism of action is not clear, but it is thought that GAB inhibits calcium influx by blocking the calcium channels presynaptically (2).

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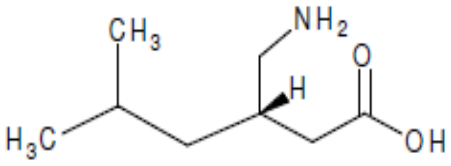
Several methods are available for quantitative determination of GAB in a pure form and in pharmaceutical preparations like fluorimetry (3), spectrofluorimetry (4), high performance liquid chromatography (HPLC) (5), capillary electrophoresis (6), potentiometric sensor (7), voltammetry (8), UV spectrophotometry (9), and automated spectrophotometry (10). According to the literature survey, there is a few reports have been observed for the use of visible spectrophotometric analysis for the determination of GAB in pharmaceutical formulations. Abdellatef et al (11) desigened a three methods depending on different reactions with the using of vanillin at pH 7.5 in McIlvain buffer, ninhydrin in DMF, and 4-benzoquinone in ethanol.

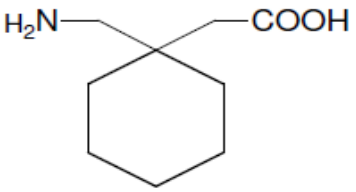
Al-Zehouri et al reported a method based on the condensation reaction of GAB with acetylacetone and with formaldehyde in Hantzsch reaction (12). The charge transfer complexes formed by the reactions of GAB (as n-electron donor) with different acceptors like iodine, chloranil,and chloranilic acid, were studied by Salem(13).

PRG is an AED structurally related to GAB and there is no spectrophotometric techniques were established in the major references like USP and BP for evaluation of PRG. Literature survey revealed few analytical procedures for assessment of PRG (14)

Several reports are there in literature for PRG determination based on chromatographic methods, i.e., gas chromatography-mass spectrophotometry (GC-MS), LC-MS-MS (3,4), HPLC (5-7) coupled with varying detect ion techniques like tandem mass spectrometry (8), f luorometry (9) and enantiospecific analysis(10).These methods may involve procedural variations including pre and post column derivatization (10). Recently, capillary electrophoresis and nuclear magnetic resonance technique was reported for PRG i nvolving complexation with cyclodextrins (11). All these are complex trace analysis techniques most of which have been employed for PRG determination in biological fluid samples. However, routine analysis of the drug in bulk powder and pharmaceutical preparations in research laboratories and pharmaceutical industry requires a relatively uncomplicated and a more cost effective method like UV/visible spectrophotometry or spectrofluorometry. Pregabalin, as such, has a poor UV/ visible absorbance profile (Figure 2) and very few reported methods have relied on generation of a chromo-phoric product by reaction of the drug with some suita blereagent. Considering the limited literature reports available in this area(12-14), we found it very pertinent to investigate and develop a novel spectrophotomet ric method for determination of pregabal in in bulk powder and pharmaceutical preparations. Ninhydrin has been used as a chromogenic agent in spectrophotometric Several reports are there in literature for PRG determination based on chromatographic methods, i.e., gas chromatography-mass spectrophotometry (GC-MS), LC-MS-MS (3,4), HPLC (5-7) coupled with varying detect ion techniques like tandem mass spectrometry (8), f luorometry (9) and enantio specific analysis(10). These methods may involve procedural variations including pre- a nd post- column derivatization (10). Recently, capillary electrophoresis and nuclear magnetic resonance technique was reported for PRG involving complexation with cyclodextrins (11). All these are complex tra ce analysis techniques most of which have been employed for PRG determination in biological fluid samples. However, routine analysis of the drug in bulk powder and pharmaceutical preparations in research laboratories and pharmaceutical industry requires a relatively uncomplicated and a more cost effective method like UV/visible spectrophotometry or spectrofluorometry. Pregabalin, as such, has a poor UV/visible absorbance profile (Figure 2) and very few reported methods have relied on generation of a chromophoric product by reaction of the drug with some suitable reagent. Considering the limited literature reports available in this area(12-14), we found it very pertinent to investigate and develop a novel spectrophotomet ric method for determination of pregabal in bulk powder and pharmaceutical preparations. Ninhydrin has been used as a chromogenic agent in spectrophotometric

Some of these methods based on chromatographic techniques (15), high performance LC (HPLC) (16.), fluorometry (17,18) and enantiospecific analysis (19). Lately, using of capillary electrophoresis and the nuclear magnetic resonance NMR technique were reported for estimation of PRG involving complexation reaction with cyclodextrins (20). The previously described methods of analysis have been suffered from many disadvantage like insensitivity, short wavelengths measurements, needing for heating or cooling step, using of expensive materials and complicated experimental conditions. Concerning the limited data available in this aspect (21), we found it is important to examine and develop a novel method for determination of PRG in bulk powder and pharmaceutical preparations. Moreover, the proposed methods are the first spectrophotometric methods for the determination of these drugs in presence of their degradation products. The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, less expensive, and less time-consuming than other published LC methods.





Scheme (1) Chemical structure of Gabapentin Scheme (2) Chemical structure of Pregabalin

EXPERIMENTAL

Apparatus

A Jena Model 1100, UV-Visible spectrophotometer (Germany) equipped with 10 mm quartz cells was used for all absorbance measurements. The measurements were done in Pharmaceutical Chemistry Department, College of Pharmacy, University of Basra, Iraq.

Reagents and Materials

All chemicals used and reagents were all of analytical grade,and double distilled water has been used to prepare the solutions used.

Bromate-Bromide Solution (KBrO3–KBr) 300 µg/mL

It was prepared by dissolving 30 mg of $KBrO_3$and0.3 g of KBr in 75 ml distilled water and completing the volume to 100 ml in volumetric flask.

This solutionhas been diluted as needed to get the working solutions containing 20 µg/ ml and 35 µg/mL for use in methods A and B.

Hydrochloric acid Solution (3M HCl)

3M HCl solution was prepared from concentrated HCl standardized with standard solution of 1 M sodium carbonate solution. It was prepared by diluting an appropriate volume of HClto 1 liter withdistilled water in a volumetric flask.

Potassium iodide Solution (KI 2%)

Tow grams of Potassium iodide KIwas weighed and dissolved in 75 ml distilled water, then completing the volume to the mark in a volumetric flask. The solution should be prepared daily as needed.

Sodium Acetate(3M CH_3COONa)

The solution of sodium acetate **(CH_3COONa)** was prepared by weighing a suitable amount from sodium acetate and dissolve it in distilled water to prepared 3M aqueous solution. This solution was only used in method A.

1% Starch Solution

The starch solution 1% was prepared by weighing of 1g of starch and dissolving it in 80 ml of boiling distilled water and completing the volume with stirring to 100ml and boil for 5minutes after which the solution was cooled to room temperature. This solution should be freshly prepared as needed and used only for B method.

Standard solutions of Gabapentin (GAB) and Pregabalin (PRG)100 µg/ml:

Pure GAB and PRG (Pharmaceutical grade) samples were provided from Cipla Ltd, India. Standard stock solutions for both GAB and PRG were prepared by dissolving the appropriate weight of the material in distilled water and completing the volume the desired.

The stock solutions GAB and PRGwere diluted appropriately with water to get required working concentrations which areused in method A and method B. The standard solutions should be kept and stored in refrigerator.

Solutions of the Commercial (GAB) and (PRG)100 µg/ml

Pharmaceutical formulations subjected for the analysis were bought from the local pharmacies in Basrah. The chosen dosage forms were Gabtin® capsules-100 mg (Al-Debeiky pharmaceutical products for Delta Parma, Egypt), Gabin® capsules 200 mg (PharmEvo Pharmaceutical Company (Pvt.) Ltd.,

Karachi, Pakistan),)Pregeb®capsules 75 mg (Torrent Pharmaceuticals Limited, Mehsana, India), and Lyrica® capsules75mg (Pfizer Co., Egypt).

20 capsules of each pharmaceutical products weighed and milled in powder form.From this, a weight equivalent to 10 mg of pure drugs were taken dissolved in 100ml distilled water and the solution swirled well and then filtered through a filter paper. First 10 ml portion of the passed solution was taken and analyzed by the working methods described later with appropriate dilution with distilled water to get30 and 25µg/ml(GAB and PRG) 20 and 15µg/ml(GAB and PRG) for method A and B respectively.

Method A (Depending on the Direct Measurement of Tri-iodide ion Concentration)

Different volumes (0.1-5.0ml) of 30 µg/mlGAB and (0.1-5.0ml) of 20 µg/mlPRG solutions were transferred into a 10 mlvolumetric flasks using a micro pipette with adjustment of the volume to 4ml with distilled water. To each volumetric flask,1ml of 3M hydrochloric acid HCl was added followed by addition 1ml of 30 µg/mL in KBrO3 mixture of bromate–bromide solution. The mixture was swirled thoroughly and the content was set aside for 15 min with shaking occasionally. For each volumetric flask 3ml of3M sodium acetate solution was added then 1ml of potassium iodide with concentration of 2%. The volume then was completed with distilled water and the absorbance of colored products was measured in 5minutes intervals at 350nm and 355nm for each GAB and PRG respectively against a blank reagent.

Method B (depending on the measurement of starch- iodine complex) volumes range from (0.1-4.0 mL) of GAB standard solution (25 µg/ml)and (0.2-4.0 ml) of PRG standard solution (15 µg/ml)were accurately measured using a micro pipette were transferred into a 10 mL volumetric flasks and the volume was brought to 4 mL by adding distilled water.

Added to each flask 1ml of 3MHCl, then1 ml of $KBrO_3$- KBr solution (15 µg/ml $KBrO_3$) was. Put the flasks aside for 15 min, then1ml of 3M HCl was added to each flask followed by 1 ml of $KBrO_3$-KBr solution (15 µg/ml $KBrO_3$).

The content was mixed with occasional shaking then, 1ml of 2%potassium iodide KI solution was added to each flask and mixed.

Lastly, to each flask, add 1ml of starch solution with 1% concentration and leave it to stand for 5min and then complete the volume with water and mixed well. The absorbance was measured for the colored product at 520nm and 555nn for each GAB and PRG respectively against a blank.

RESULTS

Bromate-bromide mixture is a valuable oxidizing reagent, used extensively in the determination of many pharmaceutical formulations by spectrophotometric methods(23-24). The present studies deal with the spectrophotometric determination of GAB and PRG Anti-epileptic drugs using this mixture as a brominating reagent. At acidic media, it produces the bromine (Br2) solution in situ, which acts a green or eco friendly brominating agent. This brominating reagent possess the advantage of avoiding the use of the a highly toxic liquid bromine solution without formation of toxic products (25).

The remaining bromine Br2 will oxidizes the iodide ion I^- producing iodine I_2 which will make a complex with the of excess iodide to form tri-iodide ion (I^{-3}). The quantity of liberated iodine I_2, resulted from the reaction of bromine Br2 with potassium iodide KI, was estimated either directly at 360 nm (method A) or it was reacted with starch solution producing the characteristic blue color of the starch-iodine complex which is measured at 570 nm (method B) (23).

Optimization the Reaction Conditions

The ideal conditions for obtaining the highest absorption value were adjusted by changing different factors such as absorption spectra, concentration of acid, time of reaction, color stability and effect of sodium acetate concentration.

Absorption Spectra

The amount of iodine I_2expressed by the un reacted bromine Br_2 with potassium iodide KI, was measured at 350 nm and 355nm for GAB and PRG solutions respectively in method A or is measured at 520nm and 555 nm for GAB and PRG solutions respectively in method B for colored chromogen of starch-iodine complex.

The absorbance of all solutions against the reagent blank was measured.(Figs. 1and2). Figure 3 Show the reaction scheme of the proposed methods.

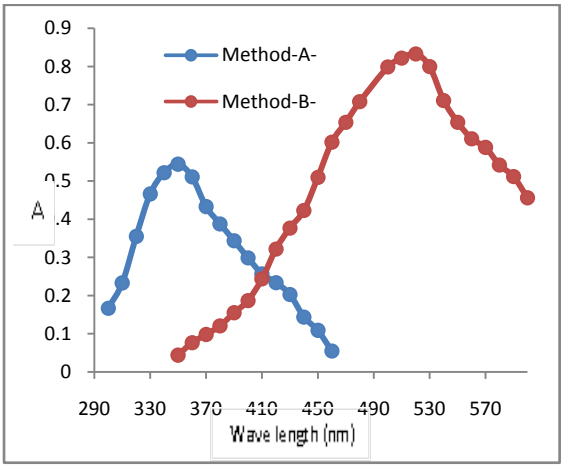


Figure 1: Absorption spectra of GAB methods A(6 µg/ml) and method B(5 µg/ml)

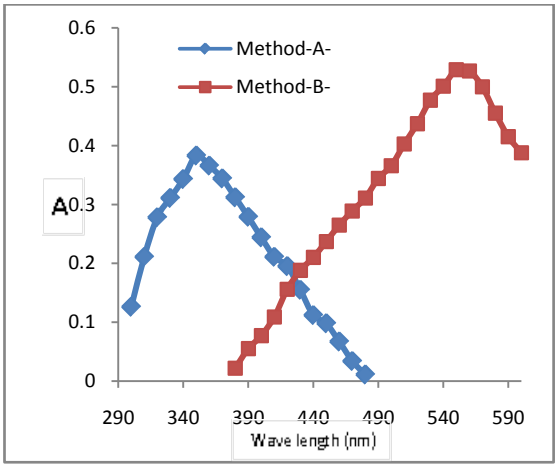


Figure 2: Absorption spectra of PRG method A (4ppm)and method B(3ppm)

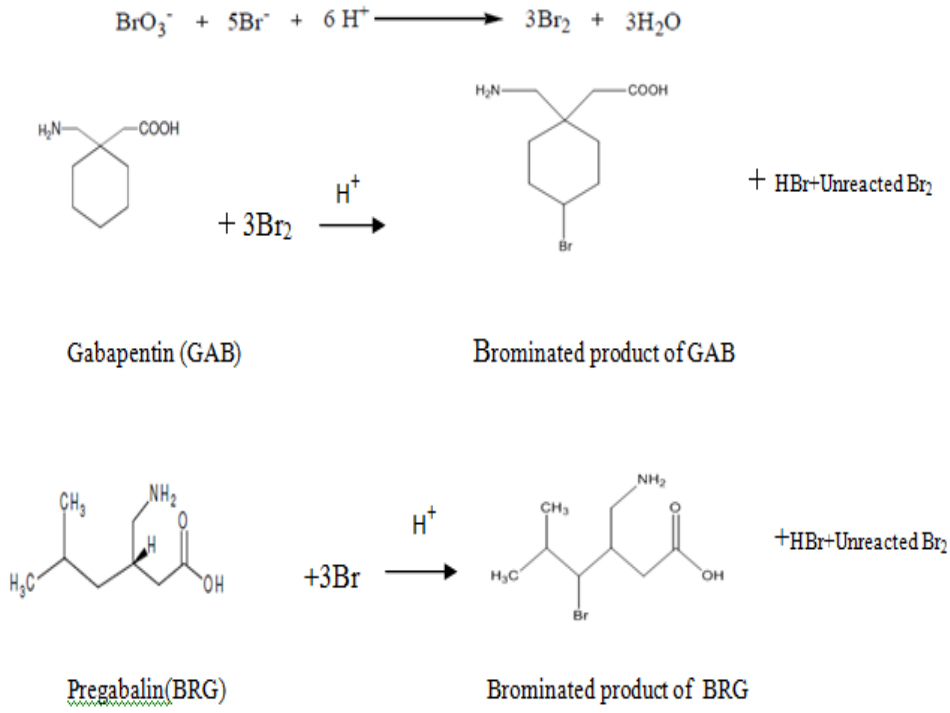
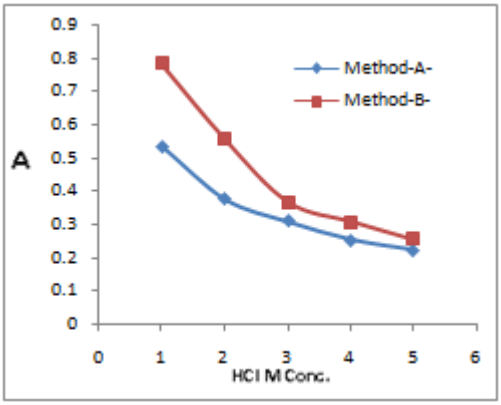


Figure 3: Mechanical interaction potential of the methods

The reaction of the unreacted bromine with Excess of KI was expected to form a yellow colored product (25) owing to formation tri-iodide ion (I^{3-}), which will be measured at 350 and 355 nm in method A for GAB and PRG drugs respectively. I method B, the un reacted bromine Br_2 reacting with excess of KI producing Iodine, will complexes with starch indicator to give a blue colored starchiodine complex GAB and PRG drugs respectively(Fig.1,2).

Effect of acid Concentration

(Figure4). The effect of acid concentration has been measured by using the proposed method work. It is studied by taking 1ml of the different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 M) of HCl and the absorbance was measured at the predetermined wavelength. At this procedure, a constant concentrations 6.0 and 4.0 µg/ml for GAB and PRG in method A, and 5 and 3 µg/mL for GAB and BEG in method B respectively were used. The effect of HCl was studied and the results showed that1.0mL of 3M HCl was the most appropriate for the bromination reaction of the drugs (Fig.4and 5).



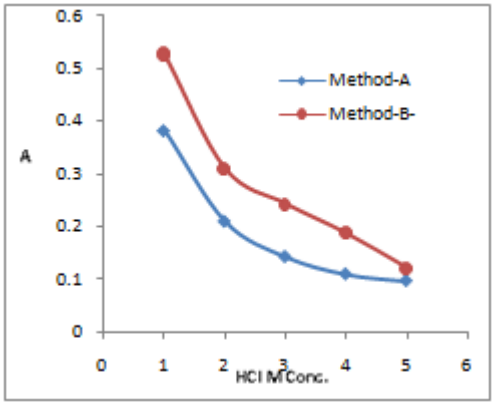


Figure 4: Effect of HCl concentration (GAB) Figure 5: Effect of HCl concentration (PRG)

Time of the Reaction and the Color Stability

Under the conditions described earlier, the reaction between the antiepileptic drugs and the in situ bromine formed was found to be 15 min in method A and 12min in method B. In method A it was found that the absorbance of the yellow solution of tri-iodide ion was stable to a limit of 50 min while absorbance of the blue color in method B lasts for 55min and 50min for GAB and PRG respectively.

Effect of sodium acetate

The effect of sodium acetate concentration has been studied on the absorbance. The Iodine liberated under the specified acidic conditions of the reaction continued for more than 30 min. Upon the addition of sodium acetate into the reaction, the liberation of Iodine stopped immediately Deferent volumes of sodium acetate (0.5-3.0ml) with a concentration of 3M were accurately transported to a series of 10ml volumetric flask and completing the work according to the methods of work described above. The results show that 1ml of sodium acetate is the right suitable volume for the study(Fig.6).

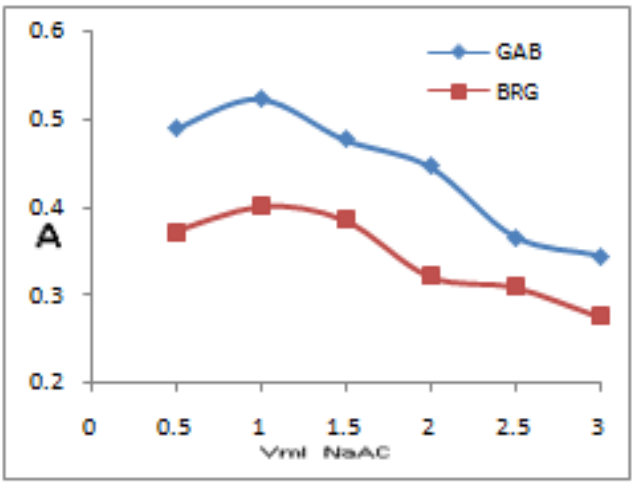


Figure 6: Effect of 3M sodium acetate volume method A for GAD and PRG

VALIDATION OF METHODS

Linearity

The calibration curves of the drugs used were draw drawn under the ideal conditions studied. They were constructed by drawing the absorbance (A) against concentration (Conc.) (Fig.7). The results were in a good agreement with Beers law in concentration range of 0.3 -16 and 0.2-10 mg/ml for GAB and PRG method A respectively and 0.25-10and 0.3-6 mg/ml for GAB and PRG method B respectively (Table 1), The regression parameters like slope, intercept, and correlation coefficient listed in Table 1. The regression parameters were calculated from the calibration graphs data along with molar absorptivity, Sandell's sensitivity are also presented in Table1.The proposed methods were validated in accordance with current ICH guidelines (26).Table 1 show that both methods have high sensitivity values through high molar absorptivity(ε), low values of Sandell's sensitivity, LOD and LOQ values (27) (Table 1).

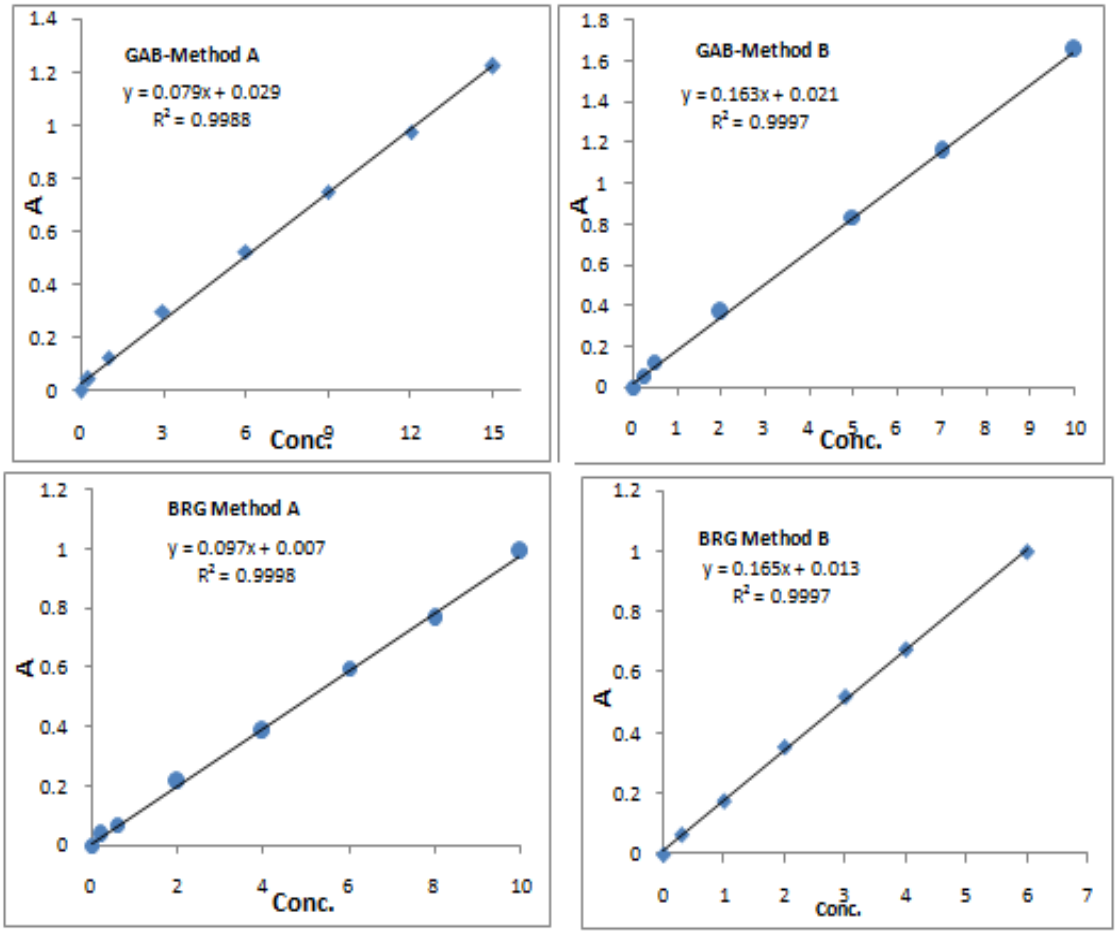


Figure 7: Calibration curve

Table 1: Analytical parameters

Parameter	GAB drug		PRG drug	
	Method A	Method B	Method A	Method B
λ max / nm	350nm	520	355	555
Beer's law limits (µg/mL)	0.30-0.15	0.25-10.0	0.20-10.0	0.30-6.0
Molar absorptivity (L/mol cm-1)	$1.489x10^4$	$2.853x10^4$	$1.5246x10^4$	$2.765x10^4$
Sandell sensitivitya (µg/cm2)	$0.964x10^{-3}$	$0.102x10^{-3}$	$0.164x10^{-3}$	$0.068x10^{-3}$
Detection limit (µg/mL)	0.095	0.075	0.070	0.100
Quantification limit (µg/ml)	0.188	0.156	0.145	0.195
Correlation coefficient (R^2)	0.9988	0.9997	0.9998	0.9997
Intercept of Correlation coefficient (R)	0.029	0.021	0.007	0.013
Slope (b)	0.079	0.163	0.097	0.165

* Mean value of five determinations; ** Relative Standard Deviation (%); *** Relative Error (%)

Precision and Accuracy

The accuracy and precision of the two methods have been evaluated by estimating three different standard solutions of the drugs and each measure has been repeatedly done for seven times, table 2.The two present methods have a high precision and a good accuracy which are indicated from the low values of the relative standard deviation percentage and relative error percentage that were recorded in the same day (intraday) to evaluate repeatability or that recorded in five days(inter day) to evaluate the intermediate precision(28).The percentage relative standard deviation (%RSD) was ≤2.534% (intraday) and ≤2.532% (inter day)for GAB, and ≤2.234% (intraday) and ≤2.548% (inter-day)for PRG. The accuracy measured by percentage relative error and appears between0.400-1.833 % and 0.250% - 2.20 for GAB and PRG respectively.

Selectivity

The effect of common excipients often accompanying the studied drugs in pharmaceutical dosage form were studied for possible interference in the assay such as talc, starch, lactose, glucose, sodium alginate, calcium gluconate and magnesium stearate. The results indicated that no profound effect was found by these excipients with 5 folds of common additives, an amount far in excess of their normal occurrence in the dosage form.

Table 2: Precision and accuracy for methods

Method	Taken (µg/mL)	Intraday (n = 7)			Interday (n = 5)		
		*Found (µg/mL)	%RSD **	***%RE	Found* (µg/mL)	**%RSD	***%RE
GAB Method A	3	3.02	2.990	o.666	2.98	2.687	0.666
	6	6.11	2.551	1.833	6.06	3.733	1.000
	10	10.09	2.546	0.900	10.12	2.532	1.200
GAB Method B	3	3.05	2.985	1.666	3.002	2.811	0.066
	5	4.98	2.534	0.400	4.88	2.760	2.400
	8	8.04	3.921	0.500	7.97	3.115	0.375
PRG Method A	2	2.02	2.234	1.000	1.99	3.876	0.500
	4	4.05	2.987	1.250	4.02	2.655	0.500
	8	8.12	2.765	1.500	8.06	2.548	0.750
PRG Method B	2	2.005	2.717	0.250	1.99	2.948	0.500
	3	3.01	3.161	0.333	3.004	2.654	0.133
	5	5.11	2.861	2.200	5.055	3.223	1.100

Recovery

A standard addition techniques were used for studying the reliability of the present methods A pure GAB and PRG standard solutions were added at three concentration levels to a fixed and known amount of GAB and PRG in capsules powder (pre-analyzed), the total amounts of GAB and PRG were measured by the descried methods. The amount sat each concentration used were repeated several times (three times), the percent of recovery were calculated. Table 4indicated that the accuracy of the methods (A and B) were unaffected by the excipients present in the formulations, and the suggested methods have a good recovery percentage values ranging around 99.98 - 105.58% with method A for GAB and PRG and between 100.99 -103.96 with method B for GAB and PRG.

Table 3: Recovery studies

Formulation studied	Method A				Method B			
	Tablet extract, (µg/ml)	Added, (µg/ml)	Found, (µg/ml)	Recovered % ± SD*	Tablet extract,(µg/ml)	Added, (µg/ml)	found, (µg/ml)	Recovered % ± SD*
Gabtin capsules-100mg	1.0	0.5	1.509	99.98±0.95	1.5	0.5	2.033	101.98±1.45
	1.0	1.0	2.052	102.99±0.78	1.5	1.0	2.528	103.35±0.69
	1.0	1.5	2.606	104.66±0.55	1.5	1.5	3.104	103.89±0.51
pregeb capsules-75mg	1.0	0.5	1.511	101.27±1.09	1.5	1.0	2.507	101.207±0.88
	1.0	1.0	2.109	105.58±0.57	1.5	1.0	2.511	100.99±0.63
	1.0	1.5	2.599	104.09±0.66	1.5	1.5	3.098	103.96±0.50

*Mean value of three determinations

Application

The present methods were successfully applied to the determination of GAB and PRG in different formula (capsule). The results were statistically studied and compared to those observed in the references (29,30).Table5 shows that there is a great convergence between current and the reference methods, statistically by calculating the Student's t- test for accuracy and variance ratio F-test for precision at 95% confidence level.

Table 5: Application of the methods

Drug brand name	Nominal value/mg	Found* (Percent of label claim ± SD)		
		Reference method	Proposed methods	
			Method A	Method B
Gabtin capsules-	100	100.52± 1.26[a]	99.89±0.73 t = 0.30,F= 1.41	99.94±0.52 t = 0.29 F = 1.39
Gabin® capsules	200	101.34± 1.05[a]	99.97±0.54 t = 1.20 ,F= 2.78	99.99±0.72 t = 1.06 F = 3.55
pregeb capsules	75	100.19±0.30[b]	100.33±0.55 t = 0.15, F =1.48	101.15±0.79 t = 0.26, F = 1.16
Lyrica capsules	75	102.26±1.98[b]	101.85±0.55 t = 0.93, F =2.55	99.87.15±0.35 t = 1.54, F = 2.32

*Average of five determinations; Calculated t value at 95 % confidence level was 2.77; Calculated F value at 95 % confidence level was 6.39.a(29),b(30)

CONCLUSION

The current two methods are sensitive and simple spectrophotometric methods. With respect to the optimum studied conditions, they do not involve complicated experimental conditions. The current methods are based on the use of bromine formed in the reaction between bromate-bromide mixture and these methods are possible to be considered as environmentally friendly methods (Green Method).The primary benefit of these methods is the avoiding of the highly toxic compounds. Also the measurements are made at a relatively high wavelength where the possible interferences that may occur from the accompanying substances are far less than that at shorter wavelengths employed in most reported methods. In addition to that, these methods are found to be high accurate and it doesn't require the use of expensive instruments, making them suitable for routine measurement methods in laboratories.

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