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Sensitive and selective spectrophotometric assay of gabapentin in capsules using sodium 1, 2-naphthoquinone-4-sulfonate

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A simple, sensitive, and selective spectrophotometric method has been developed for the determination of gabapentin (GBP) in capsules. The method is based on the reaction of GBP and sodium 1,2-naphthoquinone-4-sulfonate (NQS) in the presence of Clark and Lubs buffer of pH 11 to form an orange-coloured product which was measured at 495 nm. The parameters that affect the reaction were carefully optimized and under the optimized conditions, linear relationship was obtained in the concentration range of 7.5–75 μg ml^{-1} GBP. The molar absorptivity, limits of detection (LOD) and quantification (LOQ) and Sandell sensitivity are also reported. The proposed method was successfully applied to the determination of GBP in capsules with good accuracy and precision and without detectable interference from common excipients. The reliability of the proposed method was further established by parallel determination by the reference method and also by recovery studies. The reaction mechanism is proposed and discussed.

Keywords: gabapentin; sodium 1,2-naphthoquinone-4-sulfonate; spectrophotometry; capsules

Introduction

Gabapentin (GBP), chemically known as 1-(aminomethyl) cyclohexaneacetic acid,[1] is a new anti-epileptic drug which is a structural analogue of neurotransmitter γ-aminobutyric acid (GABA). GBP, unlike GABA, has a cyclohexane molecule system and is able to penetrate through blood-brain barrier. GBP is used for the treatment of partial onset seizures with or without secondary generalized tonic-clonic convulsions in clinical practice.[2] High performance liquid chromatography (HPLC),[3–8] capillary electrophoresis,[9,10] chemiluminometry,[11] potentiometric sensor,[12] voltammetry,[13] spectrofluorimetry,[14,15] spectrophotometry,[16–21] and automated spectrophotometry using piezoelectric pumping[22] have been employed for determining GBP in pharmaceutical preparations.

To the best of our knowledge, there are five reports on the use of visible spectrophotometry for the determination of GBP in pharmaceuticals. The method reported by Al-Zehouri *et al*.[16] was based on the condensation of GBP with acetylacetone and formaldehyde according to Hantzsch reaction. Hisham *et al*.[17] have reported three methods based on three different reactions involving the use of vanillin in the presence of McIlvain buffer pH 7.5, ninhydrin reagent in DMF medium and p-benzoquinone in ethanol medium. There are two reports[18,19] based on the charge transfer complexation reactions of GBP as n-electron donor and various pi-acceptors such as iodine, chloranil, chloranilic acid, DDQ, TCNQ, TCNE; and reaction of GBP with ninhydrin in methanol medium. The reaction of GBP with ninhydrine in DMF medium has served as the basis of spectrophotometric assay reported by Galande *et al*.[20]

However, many of the above methods suffered from one or other disadvantage like poor sensitivity, narrow linear dynamic range, measurements done at shorter wavelengths, heating or cooling step, use of expensive chemical and/or complicated experimental set-up as can be seen from Tables 1 and 2.

The reagent sodium 1, 2-naphthoquinone-4-sulfonate (NQS) has been widely used for the determination of many pharmaceutical compounds[23–26] containing primary amino group. Recently, Marta *et al*.[22] have described an automated spectrophotometric assay of GBP using a flow injection assembly with piezoelectric pumping. The method employs 1,2-naphthoquinone-4-sulfonate in alkaline medium as the reagent, but seems to be less sensitive besides employing a complicated experimental set-up.

The aim of the present work is to develop a simple, sensitive, and selective spectrophotometric method for the determination of GBP in pharmaceuticals using NQS. The method is based on the reaction of GBP through its amino group with NQS in the presence of Clark and Lubs buffer of pH 11.0. As can be seen from the linear ranges of the proposed and previously reported methods in Tables 1 and 2, it is clear that the proposed method is more sensitive than all the chromatographic methods,[3–8] the automated method[22] and many spectrophotometric methods.[16,17,20,22]

Experimental

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped with 1 cm matched quartz cells was used for all absorbance measurements.

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Table 1. Chromatographic methods reported for the determination of GBP in pharmaceuticals

Technique	Chromatographic conditions: Mobile phase	Flow rate, ml min^{-1}	Detection, UV, nm	LOD, mg ml^{-1}	Range, μg ml^{-1}	Ref.
HPLC	Ammonium dihydrogen orthophosphate buffer and methanol in 60 : 40(v/v)	1.0	200	NR	2500-7500	[3]
HPLC	Methanol-acetonitrile-potassium dihydrogen phosphate (pH 5.2; 0.028 M) (25 : 10:65, v/v)	1.0	210	NR	100-3800	[4]
HPLC	Acetonitrile-sodium dihydrogenphosphate (pH 2.5; 0.05 M) (70 : 30, v/v)	1.5	360	NR	10-500	[5]
HPLC	Methanol-potassium dihydrogen orthophosphate solution (20 : 80, v/v) containing 10% NaOH	1.0	275	NR	940-1060	[6]
HPLC	Acetonitrile – 10 mM KH_2PO_4/10 mM K_2HPO_4 (pH 6.2) (8 : 92, v/v)	1.0	210	0.005	500-5000	[7]
HPLC	Methanol – acetonitrile-20 mM KH_2PO_4 (pH 2.2) (5 : 5 : 90, v/v/v)	1.25	210	0.015	50-650	[8]

NR: Not Reported.

Materials

Pharmaceutical grade gabapentin (GBP) was received from Sun Pharmaceuticals, Mumbai, India. The following pharmaceutical preparations were purchased from commercial sources in the local market and subjected to analysis: Gabantin-100 (100 mg GBP per capsule) from Sun Pharma Sikkim, Ranipool, East Sikkim, India, and Gabapin-300 (300 mg GBP per capsule) from Intas Pharmaceuticals, Dehradun, India.

Reagents and chemicals

All the reagents and solvents used were of analytical-reagent grade and distilled water was used throughout the investigation.

Stock standard solution of gabapentin (300 μg ml^{-1})

A stock standard solution equivalent to 1000 μg ml^{-1} of GBP was prepared by dissolving accurately weighed 100 mg of pure drug in water and diluted to the mark in a 100-ml calibrated flask. The stock standard solution was diluted appropriately with the same solvent to get a working concentration of 300 μg ml^{-1} GBP before being used. The solution was stable for two days if kept in the refrigerator.

Sodium 1,2-naphthoquinone-4-sulfonate solution (NQS, 0.2%, w/v)

Accurately weighed amount (100 mg) of NQS (S. D. Fine-Chem., Mumbai, India) was dissolved in water, transferred into a 50-ml calibrated flask and diluted to the mark with water. The solution was freshly prepared and protected from light when not in use.

Clark-Lubs buffer solution (pH 11.0, H_3BO_3/KCl/NaOH)

A buffer solution of pH 11.0 was prepared by mixing 50 ml of 0.2 M aqueous solution of boric acid and potassium chloride (1.2366 g of boric acid (Sisco-Chem. Industries, Mumbai. India) and 1.4912 g of potassium chloride (Qualigens Fine Chemicals, Mumbai. India) in 100 ml water) with 50 ml of 0.2 M sodium hydroxide (Merck, Mumbai, India), and adjusted the pH with a pH meter.

Sodium lauryl sulphate solution (SLS, 5% w/v)

Five grams of SLS (Loba Chimie Ltd, Mumbai, India) was dissolved in water, transferred into a 100-ml standard flask, diluted to volume with water, and mixed well.

Construction of calibration graph

Different aliquots (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 ml) of a standard GBP (300 μg ml^{-1}) solution were accurately transferred into a series of 10-ml standard flasks and the total volume was adjusted to 2.5 ml by adding a suitable volume of water. To each flask, 2.0 ml of Clark and Lubs buffer solution of pH 11.0 and 0.5 ml of (5% *w*/*v*) SLS were added followed by 1.5 ml of 0.2% NQS solution. The reaction was allowed to proceed at room temperature (27 ± 2 °C) for 20 min, and the volume was completed to 10 ml with methanol. The resulting coloured solution was measured at 495 nm against a reagent blank prepared in the same manner without drug solution.

Assay procedure for capsules

The content of ten capsules each containing 100 or 300 mg of GBP was weighed. An accurately weighed quantity equivalent to 30 mg of GBP was transferred into a 100-ml calibrated flask and dissolved in 50 ml water. The content of the flask was shaken for 15 min; the volume was diluted to the mark with the same solvent, mixed well, and filtered using Whatman No. 42 filter paper. The first 10-ml portion of the filtrate was discarded and a suitable aliquot of the filtrate (300 μg ml^{-1} GBP) was subjected to analysis by the proposed procedure.

Results and Discussion

Absorption spectrum

The reaction of GBP with NQS in the presence of buffer of pH 11.0 results in the formation of an intense orange-coloured product which exhibits absorption maximum at 495 nm (line 1, Figure 1) whereas the maximum absorption wavelength of the reagent

Table 2. Comparison of the existing visible spectrophotometric methods and the proposed method

Reagent/s used	Methodology	λ_{max} (nm)	Linear Range, $\mu g\ ml^{-1}$ and ε, $l\ mol^{-1}\ cm^{-1}$	LOD, $\mu g\ ml^{-1}$	Reaction time, min	Remarks	Ref.
Acetylacetone and formaldehyde	Condensation product measured	415	20-140 ($\varepsilon = 1.66 \times 10^3$)	NR	20	Heating required, less sensitive	[16]
a) Vanillin	Condensation product measured	376	80-360 ($\varepsilon = 4.57 \times 10^2$)	NR	30	Less sensitive, measurements at shorter wavelengths for (a) and (c), heating required for (b) and (c).	[17]
b) Ninhydrin	Condensation product measured	569	40-280 ($\varepsilon = 5.16 \times 10^2$)	NR	5		
c) p-benzoquinone	Condensation product measured	369	80-320 ($\varepsilon = 4.63 \times 10^2$)	NR	5		
a) Iodine	Tri-iodide ion measured	360	6-30 ($\varepsilon = 6.19 \times 10^3$)	0.39	–	Shorter wavelength	[18]
b) 7,7,8,8-tetracyano-quinodimethane	Radical anions measured	842	8-24 ($\varepsilon = 7.22 \times 10^3$)	0.48	20		
c) DDQ	-do-	456	12-36 ($\varepsilon = 9.34 \times 10^3$)	1.20	–		
d) chloranilic acid	-do-	535	60-200 ($\varepsilon = 7.19 \times 10^3$)	7.59	–	Less sensitive, use of expensive organic solvent	
e) tetracyanoethylene	-do-	412	40-140 ($\varepsilon = 1.10 \times 10^3$)	3.54	15		
f) Chloranil	-do-	521	40-120 ($\varepsilon = 1.23 \times 10^3$)	3.33	20		
a) Ninhydrin	Condensation product measured	568	2-30 ($\varepsilon = 1.25 \times 10^4$)	0.15	20	Heating required	[19]
b) 7,7,8,8tetracyano-quinodimethane	Charge transfer complex measured	439	4-30 ($\varepsilon = 6.77 \times 10^4$)	0.04	15	Use of expensive organic solvent	
Ninhydrin	Coloured product measured	405	50-300	NR	5	Heating required, less sensitive	[20]
Sodium 1,2-naphthoquinone-4-sulfonate (NQS)	Automated flow injection using piezoelectric pumping	480	Up to 150	11.0 and 9.8	–	Less sensitive and complicated experimental setup	[22]
Sodium 1,2-naphthoquinone-4-sulfonate	**Coloured product of GBP with NQS measured**	**495**	**7.5–75 ($\varepsilon = 2.06 \times 10^{[3]}$)**	**2.46**	**20**	**More sensitive, wide linear dynamic range, no heating step.**	**Present method**

NR: Not Reported.

blank is in ultraviolet region (line 2, Figure 1, λ_{max} not shown). As the reagent blank absorbs little at the maximum absorption wavelength, this interference can be eliminated when the measurement is carried out at 495 nm against the reagent blank. In addition, the GBP solution is colourless, and does not absorb in the 400–600 nm range. Therefore, GBP can be determined conveniently at 495 nm against the reagent blank.

Reaction mechanism

The fact that o-quinones reacted with primary amines to give coloured products prompted Folin and Wu to search for a reagent for the quantitative colourimetric determination of amino acids and they found that the reagent sodium 1,2-naphthoquinone-4-sulfonate was the best suited.[27] The amino group of GBP displays nucleophilicity due to the fact that its lone pairs of electrons on the nitrogen atom can attack the electron deficiency center. The 4-C of sodium 1,2-naphthoquinone-4-sulfonate becomes an electron deficient center because 3,4-C=C bond conjugates with 2-C=O. So GBP can react with NQS in a nucleophilic substitution reaction.[26] The possible reaction mechanism is proposed and illustrated in Figure 2.

Optimization of the reaction conditions

Effect of pH

The effect of pH on the absorbance of reaction product was investigated by carrying out the reaction in buffer solution of different pHs. As can be seen from Figure 3, in buffers of pHs 1.0–6.0, the absorbance of the reaction product is close to zero, indicating that under high acidic conditions, GBP has difficulty to react with NQS. The possible reason may be that the amino

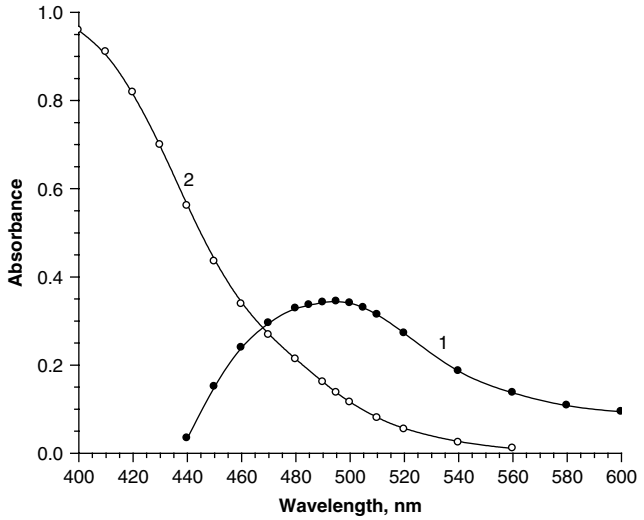

Figure 1. Absorption spectra of (1) reaction product of GBP (30 μg ml^{-1}) and NQS against reagent blank; and (2) reagent blank of NQS against water.

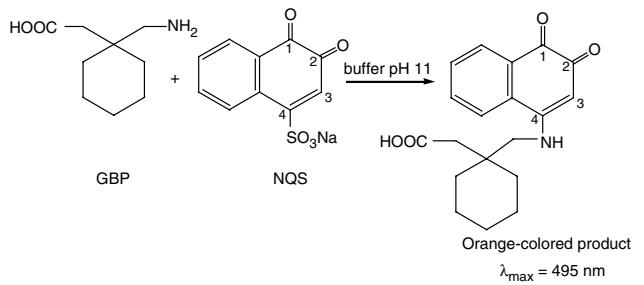

Figure 2. The possible reaction mechanism of GBP with NQS to form orange-coloured product.

group (–NH_2) of GBP is protonized and turned into protonated amine salt (–NH_3^+). So it loses the nucleophilic capability for 4-sodium sulfonate of NQS, and the nucleophilic substitution reaction can't take place easily.[26] When buffers of pHs ≥ 7.0 were used, the absorbance of the reaction product increased rapidly with increasing pH of the buffer up to 11.0. This can be attributed to the fact that at higher pH, the protonated amino group loses the proton from the amine salt (–NH_3^+) of GBP to give (–NH_2) and the nucleophilic substitution reaction occurs easily. In buffer of pH 11.0, the absorbance reaches its maximum, and at pH > 11.0, the absorbance of the reaction product decreases sharply again. Presumably, increase of hydroxide ion may hold back the nucleophilic substitution reaction between GBP and NQS and consequently, the absorbance of the solution reduces.[26] In order to achieve high sensitivity for determination of GBP, buffer of pH 11.0 was selected as the optimal experimental condition. The use of buffer of pH 11.0 and not sodium hydroxide alone was necessary to stabilize the absorbance of the reagent blank.

Effect of type of buffer solution

From Figure 3, it is clear that the absorbance of reaction product reaches its maximum by using buffer of pH 11.0. So, the effect of different buffers of pH 11.0 such as Na_2HPO_4/NaOH, Borax/NaOH, Clark and Lubs (H_3BO_3/KCl/NaOH), Na_2CO_3/HCl, KH_2PO_4/NaOH

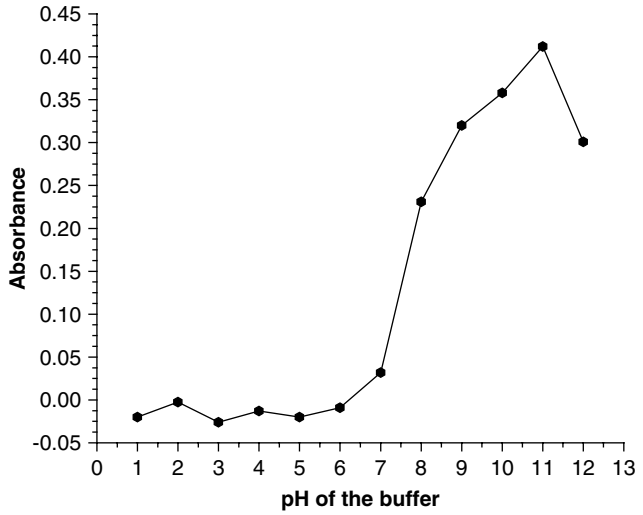

Figure 3. Effect of pH of the buffer on absorbance of the product (GBP, 30 μg ml^{-1}).

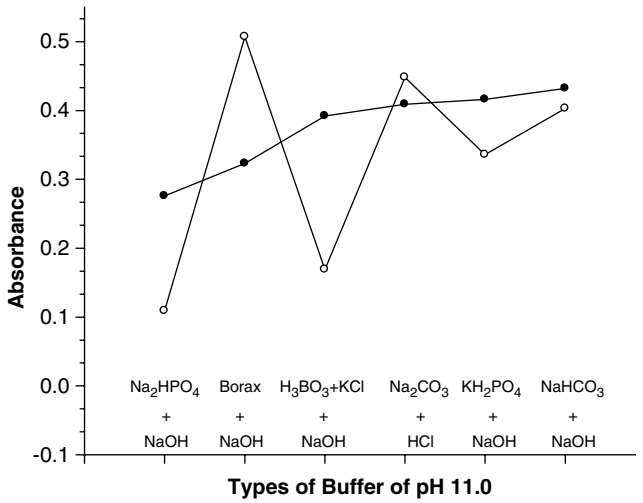

Figure 4. Effect of different buffers of pH 11.0: absorbance of the reaction product against the corresponding blank (—●—), absorbance of the blank against water (—O—).

and $NaHCO_3$/NaOH was studied (Figure 4). From Figure 4 it is obvious that Clark and Lubs buffer (H_3BO_3/KCl/NaOH) is the ideal choice since the difference between the absorbance of the measured species against the corresponding blank and the absorbance of the blank against water is maximum.

Effect of amount of buffer solution

The effect of amount of buffer solution on the absorbance of reaction product was studied using (H_3BO_3/KCl/NaOH) buffer of pH 11.0. We found that the absorbance of the reaction product decreased with increasing the volume of buffer and the colour of the measured species was less stable when the volume of buffer less than 2.0 ml. Also, a large decrease in the absorbance was noticed besides the appearance of small particles after some time when the volume of buffer was ≥ 3.0 ml. So, 2.0 ml of buffer of pH 11.0 was fixed and used throughout the assay.

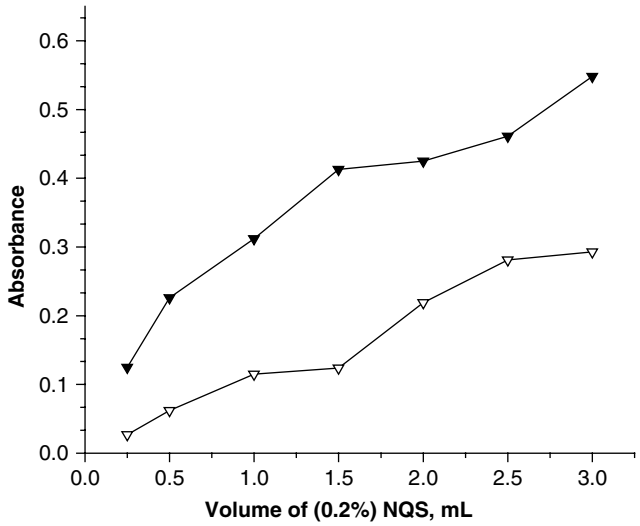

Figure 5. Effect of NQS concentration: absorbance of the reaction product against the corresponding blank (—▶—), absorbance of the blank against water (—▽—).

Effect of concentration of NQS

Following the assay procedure, the absorbance of the reaction product of a fixed concentration of GBP (30 μg ml^{-1}) with different volumes of NQS was measured against the corresponding blank and at the same time the absorbance of the reagent blank was measured against water (Figure 5). It is clear from Figure 5 that the absorbance of the reaction product remains nearly constant for 1.5–2.5 ml of the reagent but the absorbance of the reagent blank sharply increases in the same range. Therefore, 1.5 ml of 0.2% NQS was selected as the optimum concentration (0.03% *v*/*v*).

Effect of diluting solvent

In order to select the most suitable solvent for diluting the reaction mixture, different solvents such as water, methanol, ethanol, isopropanol, acetone, and 1,4-dioxane were tested. Though the absorbance of the reaction product decreases somewhat while using methanol as a diluting solvent, the increased stability of the measured species and low reagent blank absorbance necessitated the use of methanol as a diluting solvent in preference to other studied solvents except acetone. The use of acetone as a diluting solvent yielded more stability but it resulted in the least absorbance of the measured species compared with all solvents under study making the method less sensitive. Therefore, methanol was selected as a diluting solvent.

Effect of surfactant

The use of a surfactant such as sodium lauryl sulphate (SLS) was found necessary to increase the stability of the measured species as well as the reagent blank. A 0.5 ml of 5% aqueous solution in a total volume of 10 ml was found optimum.

Reaction time and colour stability

Keeping all reaction conditions unchanged, the absorbance of the reaction product was measured after standing for different time periods at room temperature. The results showed that GBP reacted immediately with NQS at room temperature and the absorbance showed increasing trend up to 20 min and remained stable for the next 40 min.

Method validation

Analytical parameters

Under optimum experimental conditions for GBP determination, the standard calibration curve was constructed by plotting the absorbance *versus* concentration. Beer's Law was obeyed over the concentration range of 7.5–75 μg ml^{-1} GBP and the equation of the line being:

$$Y = 0.0142\,X - 0.0475 \quad (1)$$

where Y is the absorbance and X is the concentration in μg ml^{-1}. The correlation coefficient (r) of the calibration plot is calculated to be (0.9998) confirming a linear increase in the absorbance with increasing the concentration of GBP. The molar absorptivity is calculated to be 2.06 × 10^3 l mol^{-1} cm^{-1} and Sandell sensitivity being 0.0829 μg cm^{-2}.

Sensitivity

The limit of detection (LOD) for the proposed method was calculated using Eqn 2,[28]

$$LOD = \frac{3.3 \times \sigma}{S} \quad (2)$$

where σ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and S is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limit was found to be 2.46 μg ml^{-1}.

The limit of quantitation (LOQ) defined as[28]

$$LOQ = \frac{10 \times \sigma}{S} \quad (3)$$

According to Eqn 3, the quantitation limit was found to be 7.46 μg ml^{-1}.

Accuracy and precision

In order to determine the precision of the proposed method, solutions containing three different concentrations of GBP were prepared and analyzed in seven replicates and the analytical results were summarized in Table 3. The low values of the percentage relative standard deviation (% RSD) and percentage relative error (% RE) indicate the high precision and the good accuracy of the proposed method. RSD (%) and RE (%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five days to evaluate intermediate precision (inter-day precision).

Selectivity

The selectivity of the proposed method was tested by placebo blank and synthetic mixture analyses. From the placebo blank analysis, it was confirmed that the change in the absorbance with respect to the reagent blank was caused only by the analyte. To study the interference from common excipients present in the capsules, a synthetic mixture with the composition of GBP, talc, starch, calcium gluconate, sodium alginate, and magnesium stearate was prepared and subjected to analysis by the proposed method after preparation of the solution using the procedure described for capsules. The percent recovery of GBP was 101.4 ± 2.07 (n = 5), suggesting no significant interference from the excipients in the assay of GBP under the described optimum conditions.

Table 3. Evaluation of intra-day and inter-day accuracy and precision

GBP[a] taken µg ml^{-1}	Intra-day accuracy and precision (n = 7)			Inter-day accuracy and precision (n = 5)		
	GBP found[a], µg ml^{-1}	% RE[b]	% RSD[c]	GBP Found[a], µg ml^{-1}	% RE[b]	% RSD[c]
15	14.66	2.27	2.65	14.43	3.80	2.93
30	29.14	2.87	1.21	28.97	3.43	2.07
45	45.49	1.09	2.40	45.67	1.49	1.96

[a.] Mean value of n determinations, [b.] Relative Error (%), [c.] Relative standard deviation (%).

Table 4. Assay of capsules and statistical evaluation of results

Capsule brand name	Found (% of nominal amount ± SD)*	
	Reference method	Proposed method
Gabantin-100	99.32 ± 1.04	97.33 ± 1.77 **t** = 2.17 **F** = 2.90
Gabapin-300	98.07 ± 1.37	96.13 ± 1.58 **t** = 2.07 **F** = 1.33

* Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.78.
Tabulated F-value at the 95% confidence level is 6.39.

Table 5. Results of spike-recovery studies from pre-analyzed capsules

Formulation studied	GBP in capsules, µg ml^{-1}	Pure GBP added, µg ml^{-1}	Total found, µg ml^{-1}	Pure GBP recovered*, Percent ± SD
Gabantin-100	29.20	15	44.30	100.7 ± 2.05
	29.20	30	60.28	103.6 ± 1.99
	29.20	45	75.14	102.1 ± 2.14
Gabapin-300	28.84	15	44.22	102.5 ± 1.03
	28.84	30	58.32	98.27 ± 2.24
	28.84	45	74.06	100.5 ± 2.66

* Mean value of three determinations.

Application to the assay of capsules

The proposed method was successfully applied to the determination of GBP in capsules (Table 4). The results obtained were statistically compared with those of the reference method[21] by applying the Student's *t*-test for accuracy and F-test for precision. The reference method consisted of the measurement of the absorbance of the aqueous extract of the capsules at 210 nm. From Table 4, it is clear that the calculated *t*-value and F-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees of freedom. The results indicated that there is no difference between the proposed method and the reference method with respect to accuracy and precision.

Recovery study

Accuracy and validity of the proposed method were further ascertained by performing recovery experiments *via* the standard addition procedure. When the capsule powder (pre-analyzed) spiked with known amounts of pure GBP at three different concentration levels (50, 100, and 150% of the quantity present in the capsule powder) was analyzed by the proposed method, the recoveries of pure drug added were quantitative (Table 5).

Conclusions

The present study proposes a simple and sensitive spectrophotometric method for the determination of gabapentin in capsules based on its reaction with NQS reagent in the presence of buffer of pH 11.0. The proposed method is superior to many spectrophotometric methods and all chromatographic methods reported so far for analysis of GBP in terms of its sensitivity. The linear range of the proposed method is 7.5–75 µg ml^{-1} GBP which is much less than many previously reported methods as can be seen from Tables 1 and 2. The proposed method is free from the usual analytical complications like heating or extraction steps. The proposed method relies on the use of simple, cheap, and easily accessible technique but provides a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, it can be used as alternative for rapid and routine determination of bulk sample and formulations in quality control laboratories.

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