Turk J. Pharm. Sci. 9(2), 113-126, 2012

Original article

HIGHLY SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF GABAPENTIN IN CAPSULES USING SODIUM HYPOCHLORIDE

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Abstract

A simple, selective and highly sensitive spectrophotometric method is described for the determination of gabapentin (GBP) in pure form and in capsules. The method is based on the reaction of GBP with hypochloride in the presence of Kolthoff buffer of pH 7.0 to form the chloro derivative of GBP; followed by the destruction of the excess hypochloride by nitrite ion. The color was developed by the oxidation of iodide with the chloro derivative of GBP to iodine in the presence of starch and forming the blue colored product which was measured at 590 nm. The optimum conditions that affect the reaction were ascertained and under these conditions, linear relationship was obtained in the concentration range of 0.2-5.0 µg/mL GBP. The calculated molar absorptivity and Sandell sensitivity values are 3.07×10^4 L/(mol.cm) and 0.0056 µg/cm^2, respectively. The limits of detection and quantification are 0.04 and 0.12 µg/mL, respectively. The proposed method has been applied successfully to the analysis of GBP in pure form and in capsules and no interference was observed from excipients present in the capsules. 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.

Key words: *Spectrophotometry, Gabapentin, Sodium hypochloride, Capsule.*

Sodyum Hipoklorit Kullanılarak Gabapentin'in Kapsüllerde Miktar Tayini İçin Yüksek Duyarlı Bir Spektrofotometrik Yöntem

Gabapentin'in (GBP) saf halinde ve kapsüllerde miktar tayini için basit, seçici ve yüksek duyarlıklı bir spektrofotometrik yöntem önerilmektedir. Yöntem, Kolthoff tamponu (pH:7.0) içerisinde GBP nin hipoklorit ile reaksiyonuna dayanmaktadır ve ortamdaki aşırı hipoklorür nitrit ile parçalanmaktadır. GBP nin klorürlü türevinin iyodür ile oksidasyonu sonucunda meydana gelen iyot ortamdaki nişasta ile mavi renkli bir ürün meydana getirmekte ve oluşan rengin absorbansı 590 nm de ölçülmektedir. Yöntemi etkileyen şartlar belirlenmiş ve bu şartlarda 0.2-5.0 µg/mL GBP konsantrasyon aralığında doğrusal bir ilişkinin olduğu saptanmıştır. Molar absorptivite ve Sandell hassaslık değeri sırasıyla $3.07x10^4$ L/(mol.cm) ve 0.0056 µg/cm^3 olarak hesaplanmıştır. Yakalama ve tayin sınırları ise sırasıyla 0.04 ve 0.12 µg/mL olarak bulunmuştur. Önerilen yöntem kapsüldeki yardımcı maddelerin girişimi olmaksızın kapsüllerdeki GBP nin miktar tayini için başarıyla uygulanmıştır. Yöntemin güvenilirliği bir referans yöntemle paralel tayin yapılarak ve geri kazanım değerleri bulunarak ortaya konulmuştur. Reaksiyon mekanizması önerilmiş ve tartışılmıştır.

Anahtar kelimeler: *Spektrofotometri, Gabapentin, Sodyum hipoklorit, Kapsül.*

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INTRODUCTION

Gabapentin (GBP), chemically known as 1-(aminomethyl)cyclohexaneacetic acid (1), is a new antiepileptic drug which is a structural analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). GBP was originally developed for the treatment of epilepsy; it is currently also used against neuropathic pain (2). Several analytical methods have been reported for the determination of GBP in pharmaceutical preparations such as fluorimetry using sequential injection (2), high performance liquid chromatography (HPLC) (3-8), capillary electrophoresis (9,10), chemiluminometry (11), potentiometric sensor (12), voltammetry (13), spectrofluorimetry (14,15), visible spectrophotometry (16-20), UV-spectrophotometry (20,21) and automated spectrophotometry using piezoelectric pumping (22).

Only six reports are found in the literature concerning the determination of GBP in pharmaceuticals by HPLC. Rao et al. (3) have reported an isocratic HPLC method using a strong cation exchange column bonded with phenyl sulphonic acid with a mobile phase consisting of ammonium dihydrogen orthophosphate buffer and methanol in 60:40 (v/v) at a flow rate of 1.0 mL/min and UV detection at 200 nm. Gujral and Haque (4) have developed a HPLC method using a Waters C_{18} 5 μm column (150 mm × 4.6 mm) and a mixture of methanol: acetonitrile: potassium dihydrogen orthophosphate (pH5.2; 0.028 M) (25:10:65) as a mobile phase with UV detection at 210 nm. HPLC quantification of GBP in capsules after precolumn derivatization with 1-fluoro-2,4-dinitrobenzene and using amlodipine as an internal standard was reported by Souri et al. (5). In their method, the separation was carried out on a Nova-Pak C_{18} column using a mixture of acetonitrile–sodium dihydrogenphosphate (pH 2.5; 0.05 M) (70:30, v/v) as mobile phase with UV detection at 360 nm. Gujral and Haque (6) have determined the GBP in tablets by HPLC on a C_{18} 5 μm Waters column (150 mm × 4.6 mm) using a mobile phase of methanol: potassium dihydrogen orthophosphate solution (20:80, v/v) containing 10% NaOH to adjust the pH to 6.2 at a flow rate of 1.0 mL/min. Ciavarella et al. (7) have reported an isocratic reversed-phase HPLC method for the determination of GBP and its major degradation impurity. The separation was achieved on a Brownlee Spheri-5 cyano column using an acetonitrile–10 mM KH_2PO_4/10 mM K_2HPO_4 (pH 6.2) (8:92, v/v) as mobile phase. Gupta at al. (8) have developed an isocratic reversed-phase HPLC method for the determination of GBP using a Phenomenex Luna cyano column and a mobile phase consisted of methanol–acetonitrile–20 mM KH_2PO_4 (pH 2.2) (5:5:90, v/v/v) at a flow rate of 1.25 mL/min and UV detection at 210 nm.

To the best of our knowledge, there are five reports on the use of visible spectrophotometry for the determination of GBP in pharmaceuticals. Abdellatef et al.(16) 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. The condensation reaction of GBP with acetylacetone and formaldehyde according to Hantzsch reaction was reported by Al-Zehouri et al.(17). The charge transfer complexation reactions of GBP as n-electron donor and various acceptors such as iodine, chloranil, chloranilic acid, DDQ, TCNQ and TCNE were reported by Salem (18). Galande et al.(19) have reported spectrophotometric assay based on the reaction of GBP with ninhydrine in DMF medium. Siddiqui et al. (20) have reported two different reactions involving ninhydrin in methanol medium and TCNQ in acetonitrile. The reference method (21) is based on the direct measurement of the absorbance of the aqueous extract of GBP capsules at 210 nm.

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 organic solvents, use of expensive chemical and/or complicated experimental setup as can be seen from Tables 1 and 2.

Sodium hypochloride has been used for the determination of many compounds (23-27) but the reaction between hypochloride and GBP has not been investigated yet. The aim of the present work is to develop a simple, selective and highly sensitive spectrophotometric method

for the determination of GBP in bulk drug as well as in capsules using sodium hypochloride. The method is based on the chlorination reaction of GBP through its amino group with hypochloride in the presence of Kolthoff buffer (phosphate-borate) of pH 7.0, destruction of the excess hypochloride by nitrite ion and reacting of the chloro derivative with excess of KI and measuring the blue chromogen formed with starch at 590 nm. From the linear ranges and molar absorptivity values of the proposed and previously reported methods in Tables 1 and 2, it is clear that the proposed method is more sensitive than all chromatographic methods (3-8), all visible spectrophotometric methods (16-20) and the automated spectrophotometric method (22) reported so far.

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

Technique	Chromatographic conditions				LOD, µg/mL	Range, µg/mL	Ref.
	Mobile phase	Stationary phase	Flow rate, mL/ min	Detection, UV, nm			
1. HPLC	Ammonium dihydrogen orthophosphate buffer and methanol in 60:40(v/v)	strong cation exchange column bonded with phenyl sulphonic acid	1.0	200	NR	2500-7500	3
2. HPLC	Methanol- acetonitrile-potassium dihydrogen phosphate (pH 5.2; 0.028 M) (25:10:65, v/v)	Waters C_{18} 5 µm column (150 mm × 4.6 mm)	1.0	210	NR	100-3800	4
3. HPLC	Acetonitrile-sodium dihydrogenphosphate (pH 2.5; 0.05 M) (70 : 30, v/v)	Nova-Pak C_{18} column	1.5	360	NR	10-500	5
4. HPLC	Methanol-potassium dihydrogen orthophosphate solution (20:80, v/v) containing 10% NaOH	C_{18} 5 µm Waters column (150 mm × 4.6 mm)	1.0	275	NR	940-1060	6
5. HPLC	Acetonitrile–10mM KH_2PO_4/10mM K_2HPO_4 (pH 6.2) (8:92, v/v)	Brownlee Spheri-5 cyano column	1.0	210	5.0	500-5000	7
6. HPLC	Methanol–acetonitrile-20mM KH_2PO_4 (pH 2.2) (5:5:90, v/v/v)	Phenomenex Luna cyano column	1.25	210	15.0	50-650	8

NR: Not reported

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.

Materials

Pharmaceutical grade gabapentin (GBP) which is certified to be 99.5% pure 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.

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

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear Range, µg/mL and ε, L/(mol.cm)	LOD, µg/mL	Reaction time, min	Remarks	Ref.
1.	a) Vanillin	Condensation product measured	376	80-360 ($\varepsilon=4.57\times10^2$)	NR	30	Less sensitive, measurements at shorter wavelengths for (a) and (c), heating required for (b) and (c).	16
	b) Ninhydrin	Condensation product measured	569	40-280 ($\varepsilon=5.16\times10^2$)	NR	5		
	c) p-benzoquinone	Condensation product measured	369	80-320 ($\varepsilon=4.63\times10^2$)	NR	5		
2.	Acetylacetone and formaldehyde	Condensation product measured	415	20-140 ($\varepsilon=1.66\times10^3$)	NR	20	Heating required, less sensitive, measurements at shorter wavelength	17
3.	a) Iodine	Tri-iodide ion measured	360	6-30 ($\varepsilon=6.19\times10^3$)	0.39	-	Shorter wavelength	18
	b) 7,7,8,8-tetracyano-quinodimethane	Radical anion measured	842	8-24 ($\varepsilon=7.22\times10^3$)	0.48	20	Less sensitive, use of expensive organic solvent and reagents	
	c) DDQ	Radical anion measured	456	12-36 ($\varepsilon=9.34\times10^3$)	1.20	-		
	d) Chloranilic acid	Radical anion measured	535	60-200 ($\varepsilon=7.19\times10^3$)	7.59	-		
	e) Tetracyanoethy-lene	Radical anion measured	412	40-140 ($\varepsilon=1.10\times10^3$)	3.54	15		
	f) Chloranil	Radical anion measured	521	40-120 ($\varepsilon=1.23\times10^3$)	3.33	20		
4.	Ninhydrin	Colored product measured	405	50-300	NR	5	Heating required, less sensitive	19
5.	a) Ninhydrin	Condensation product measured	568	2-30 ($\varepsilon=1.25\times10^4$)	0.15	20	Heating required Use of expensive organic solvent and reagents	20
	b) 7,7,8,8-tetracyano-quinodimethane	Charge transfer complex measured	439	4-30 ($\varepsilon=6.77\times10^4$)	0.04	15		
6.	NQS	Automated flow injection using piezoelectric pumping	480	Up to 150	11.0 and 9.8	-	Less sensitive and complicated experimental setup	22
7.	**Hypochloride + buffer of pH 7.0 + nitrite + starch-KI**	**Triiodide-starch complex measured**	**590**	**0.2-5.0 ($\varepsilon=3.07\times10^4$)**	**0.04**	**1**	**Highly sensitive, wide linear dynamic range, no heating step, no use of organic solvents and uses inexpensive chemicals**	**This work**

DDQ: 2,3-dicloro-5,6-dicyano-1,4-benzoquinone, NQS: Sodium 1,2-naphthoquinone-4-sulfonate, NR: Not reported.

Reagents and Chemicals

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

Stock standard solution of gabapentin (200 μg/mL):

A stock standard solution equivalent to 200 μg/mL of GBP was prepared by dissolving accurately weighed 20 mg of pure drug in water and diluted to the mark with water in a 100 mL calibrated flask. The stock standard solution was diluted appropriately with the same solvent to get a working concentration of 20 μg/mL GBP before being used. The solution was stable for two days when kept in the refrigerator.

Sodium hypochloride solution (0.012 M):

A 2.0 mL of NaOCl (Merck, Mumbai, India, available chlorine ~ 4% w/v) was diluted with water to the mark in a 100 mL calibrated flask and standardized iodometrically (28).

Kolthoff buffer of pH 7.0:

A buffer solution of pH 7.0 was prepared by mixing 62.3 mL of 0.1 M potassium dihydrogen phosphate (S. D. Fine Chem. Ltd., Mumbai, India) and 37.3 mL of 0.05 M borax (S. D. Fine Chem. Ltd., Mumbai, India) and the pH was adjusted with a pH meter using these solutions.

Sodium nitrite (0.04 M):

An amount (276 mg) of $NaNO_2$ (Merck, Mumbai, India) was dissolved in water, transferred into a 100 mL calibrated flask and diluted to the mark with water.

Starch-potassium iodide reagent:

A 750 mg of starch soluble (Loba Chemie PVT Ltd., Mumbai, India) is dissolved in 100 mL boiling water and after 5 min 1.0 g of potassium iodide (Merck, Mumbai, India) was added slowly to the same solution and the boiling was continued an additional 5 min. This solution, when protected from the light and stored at 10° C when not in use, remained colorless for several weeks (23).

Construction of Calibration Graph

Different aliquots (0.10, 0.50, 1.00, 1.50, 2.00, 2.25 and 2.50 mL) of a standard GBP (20 μg/mL) 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, 1.0 mL of Kolthoff buffer (phosphate-borate) of pH 7.0, 1.0 mL of 0.012 M hypochloride solution and 1.0 mL 0.04 M sodium nitrite solution were added. One minute later, 1.0 mL of starch-iodide solution was added and the volume was completed to 10 mL with water. The absorbance of the blue colored solution was measured at 590 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 10 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 a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and the filtrate (100 μg/mL GBP) was diluted appropriately with water to get a concentration of 20 μg/mL GBP and suitable aliquots were subjected to analysis by the proposed procedure.

RESULTS AND DISCUSSION

Absorption Spectrum

The reaction between GBP and hypochloride, in the presence of Kolthoff buffer (phosphate-borate) of pH 7.0 followed by destruction of the excess hypochloride by nitrite ion and oxidation of iodide by chloro derivative of GBP in the presence of starch, results in the formation of an intense blue-colored product (tri iodide-starch complex). The absorption spectrum of the blue colored product recorded at 400-740 nm against the blank solution exhibited an absorption maximum at 590 nm (Figure 1). The reagent blank was completely colorless and the measurements for bulk and capsules samples were thus made at 590 nm against the reagent blank.

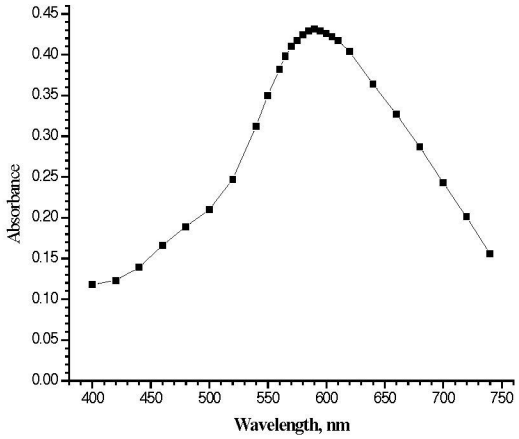


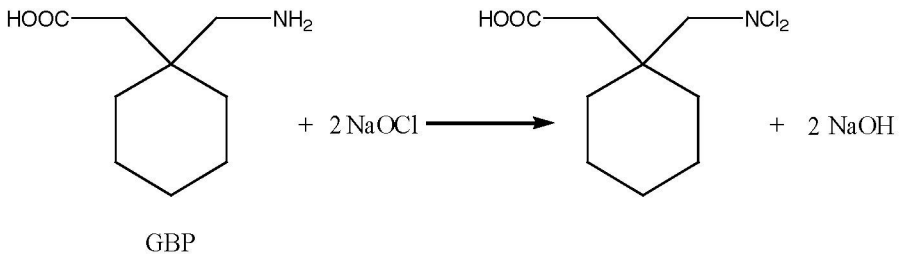
Figure 1. Absorption spectrum of reaction product of GBP (2.5 μg/mL) and hypochloride.

Reaction Mechanism

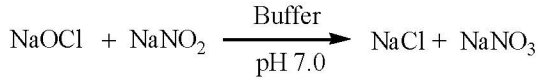
The treatment of primary amines with sodium hypochloride converts the amines into N-chloro- or N,N-dichloro-amines (29) according to the general type of reaction in which XCl is NaOCl or HOCl: $XCl + R\text{-}NH_2 \rightarrow RNHCl\ (RNCl_2) + H^+ + X^-$. Sandford et al. (30) have reported that this reaction may be viewed either as an electrophilic displacement of H^+ from the nitrogen by the chlorinating agent or as a nucleophilic displacement of X^- by the nitrogenous compound. Also, the oxidation of nitrite to nitrate by hypochloride ions and reacting of the chloro-amine with starch-KI reagent to produce the blue color was reported by the same authors (30).

The reaction mechanism involved in the proposed method can be explained in three steps. The first step is the chlorination reaction of the basic nitrogen of GBP with hypochloride in the presence of Kolthoff buffer (phosphate-borate) of pH 7.0 to form the chloro derivative of GBP. The second step is the destruction of the excess hypochloride by nitrite ion (the chloro derivative of drug is unaffected under the optimized conditions) through reducing unreacted NaOCl with $NaNO_2$. The third step is oxidation of the iodide to iodine by the chloro derivative of GBP and produce of the blue color in the presence of starch due to the formation of triiodide-starch complex. The possible reaction mechanism of the three steps is proposed and illustrated in Figure 2.

Step 1:



Step 2:



Step 3:

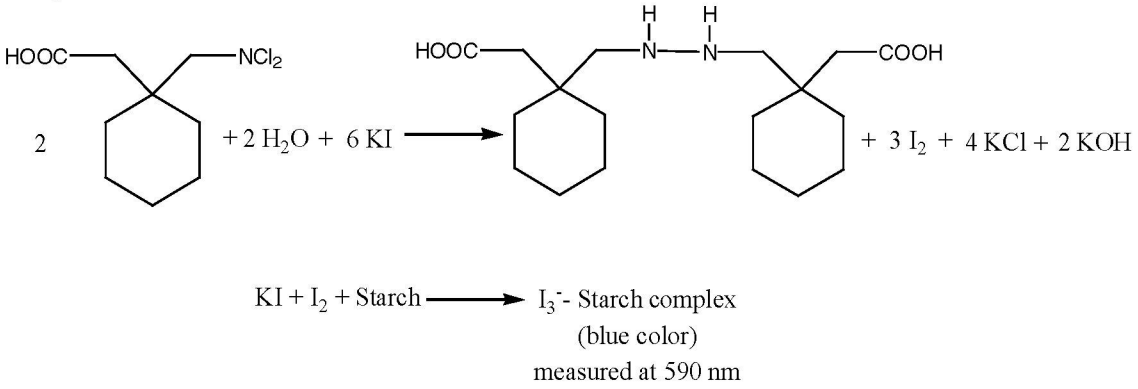


Figure 2. The proposed reaction mechanism.

Optimization of the Reaction Conditions

Effect of pH

The effect of pH on the absorbance of blue colored product was investigated by carrying out the reaction in buffer solution of different pHs. When the pH of the buffer used was ≤ 5, the blue chromogen was found to be unstable with continuous decrease in the absorbance and the blank solutions changed to dark blue color after some time. The blank solution was completely colorless while using a buffer of pH 6-9 and the absorbance of the blue colored product was stable. Among the buffer solutions of pH 6-9 used, the buffer solution of pH 7.0 exhibited maximum absorbance and the color of the reaction product was more stable compared with others. So, in order to achieve high sensitivity for determination of GBP, buffer of pH 7.0 was selected as the optimal experimental condition.

Effect of type of buffer solution

The effect of different buffers of pH 7.0 such as Na_2HPO_4/citric acid, KH_2PO_4/NaOH, Kolthoff buffer (KH_2PO_4/borax) and boric acid/borax was studied. We found that the boric acid/borax buffer is not suitable as it resulted in least absorbance of the measured species compared with all buffers tested making the method less sensitive. The remaining buffers used were found suitable for the assay but the color stability of the measured species was more with Kolthoff buffer (KH_2PO_4/borax). Therefore, Kolthoff buffer (KH_2PO_4/borax) of pH 7.0 was selected and used in the assay.

Effect of volume of buffer solution

The effect of amount of buffer solution on the absorbance of the measured species (2.0 μg/mL GBP) was studied using (0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 2.00 mL) of Kolthoff buffer (KH_2PO_4/borax) of pH 7.0. We found that in the absence of buffer the dark blue color developed in both sample and blank and the absorbance for both showing error (A > 2). So, the addition of the buffer is necessary to get a colorless blank. Also, the absorbance of the blue colored product increased with increasing the volume of buffer from 0.25-1.00 mL (Figure 3) and any extra volume of the buffer (1.00 mL < volume < 2.00 mL) had no effect on the absorbance of the measured species. So, 1.0 mL of Kolthoff buffer of pH 7.0 was fixed and used throughout the assay.

Effect of concentration of sodium hypochloride

Following the assay procedure, the absorbance of the reaction product of a fixed concentration of GBP (4.5 μg/mL) with different volumes (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mL) of 0.012 M NaOCl was measured against the corresponding blank (Figure 4). It is clear from Figure 4 that the absorbance of the colored reaction product showed maximum using (0.8-1.0 mL) of 0.012 M sodium hypochloride solution. Therefore, 1.0 mL of 0.012 M NaOCl was selected as the optimum concentration.

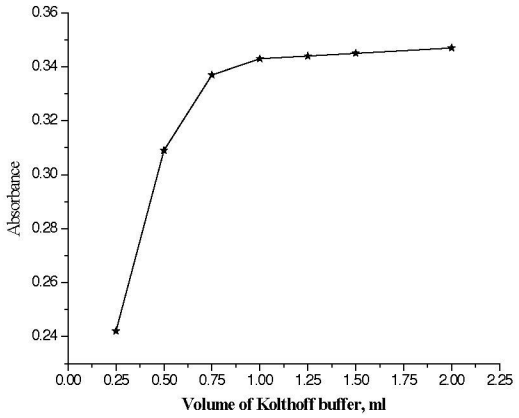


Figure 3. Effect of volume of Kolthoff buffer of pH 7.0, (GBP, 2.0 μg/mL).

Effect of concentration of sodium nitrite

The use of sodium nitrite was found necessary to destroy the excess hypochloride and avoiding the interference from it and allow the oxidation of iodide to iodine only by the chloro derivative of the drug which is the basis of the assay. For this purpose, the absorbance of the reaction product of a fixed concentration of GBP (2.5 μg/mL) with hypochloride in the presence of Kolthoff buffer of pH 7.0, different volumes (0.0-2.0 mL) of 0.04 M $NaNO_2$ and starch-iodide reagent was measured against the corresponding blank. We found that the minimum concentration of $NaNO_2$ required to get a colorless blank is 0.5 mL of 0.04 M $NaNO_2$ and maximum absorbance of the blue colored product was noticed by using 1.0 mL of 0.04 M $NaNO_2$. The absorbance of the measured species remains constant using (1.0-2.0 mL) of 0.04 M sodium nitrite. Hence, 1.0 mL of 0.04 M $NaNO_2$ was selected as the optimum concentration.

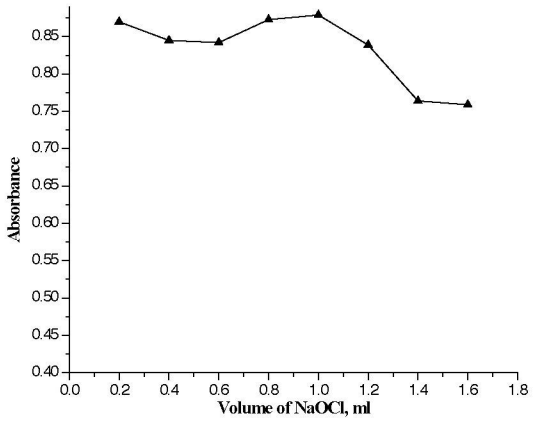


Figure 4. Effect of volume of 0.012 M sodium hypochloride, (GBP, 4.5 μg/mL).

Effect of volume of starch-iodide solution

The effect of volume (0.25, 0.50, 0.75, 1.00, 1.50 and 2.00 mL) of starch-iodide solution (details of the concentration given under the experimental section) was studied (Figure 5). From Figure 5 it is clear that the absorbance of the reaction product increases with increasing the volume of starch-iodide solution and remains constant after 1.0 mL of the reagent. Hence, 1.0 mL of starch-iodide solution was fixed and used in the assay.

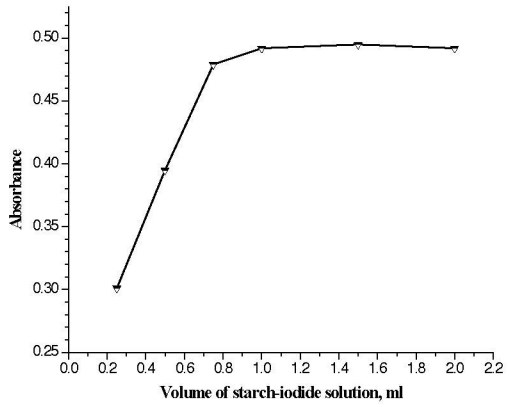


Figure 5. Effect of volume of starch-iodide solution, (GBP, 2.5 μg/mL).

Reaction time and color stability

The effect of reaction time between GBP and hypochloride in the presence of Kolthoff buffer of pH 7.0 was studied keeping all other reaction conditions unchanged. The absorbance of the reaction product was measured after different reaction times between GBP and hypochloride (0.0-3.0 min) and showed that the reaction was instantaneous and any delay in the destruction of excess hypochloride would affect the chloro derivative of drug and the absorbance of the blue colored product would decrease. Hence, the sodium nitrite solution should be added after the addition of hypochloride to the drug solution directly. Similarly, the effect of reaction time for destruction of excess hypochloride with sodium nitrite was studied keeping all other reaction conditions constant. One minute time was found to be necessary for

complete destruction of excess hypochloride with the nitrite under the optimized conditions. The absorbance of the blue colored product remains stable for at least 1 hr.

Method Validation

Analytical parameters

A linear relation is found between the absorbance and concentration in the range of 0.2-5.0 μg/mL GBP. Beer's law is obeyed and the equation of the line being:

$$Y = -0.0034 + 0.1840\ X$$

where Y is the absorbance and X is concentration in μg/mL. The correlation coefficient (r) of the calibration plot is calculated to be (0.9999) confirming a linear increase in the absorbance with increasing the concentration of GBP. The molar absorptivity is calculated to be 3.07×10^4 l $mol^{-1}cm^{-1}$, and Sandell sensitivity being 0.0056 μg cm^{-2}. The limits of detection (LOD) and quantification (LOQ) calculated as per ICH (31) guidelines are 0.04 and 0.12 μg/mL, respectively. The standard deviation of the slope (S_b) and intercept (S_a) are calculated to be 0.0012 and 0.0041, respectively.

Accuracy and precision

The precision of the proposed method was calculated in terms of intermediate precision (intra-day and inter-day) (32). Solutions containing three different concentrations of GBP were prepared and analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision) and the analytical results were summarized in Table 3. The percentage relative standard deviation (RSD %) values were ≤ 1.99 % (intra-day) and ≤ 2.21 % (inter-day) indicating high precision of the proposed method. Also, the accuracy of the method was evaluated as percentage relative error (RE %) and from the results shown in Table 3, it is clear that the accuracy is satisfactory (RE ≤ 3.00 %).

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

GBP[a] taken μg/mL	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
	GBP found[a], μg/mL	RE (%)[b]	RSD (%)[c]	GBP Found[a], μg/mL	RE (%)[b]	RSD (%)[c]
1.00	0.98	2.00	1.92	0.97	3.00	2.06
2.00	2.03	1.50	1.70	2.05	2.50	1.94
3.00	2.95	1.67	1.99	2.93	2.33	2.21

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

Selectivity

In order to evaluate the selectivity of the proposed method for the analysis of GBP in capsules, placebo blank and synthetic mixture analyses were performed. 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 formulations, 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 102.1±1.89 (n = 5), suggesting no significant interference from the excipients in the assay of GBP under the described optimum conditions.

Robustness and ruggedness

To evaluate the robustness of the method, two experimental variables, viz., the volume of buffer and the volume of sodium nitrite, were slightly varied, and the capacity of the method was found to remain unaffected by small deliberate variations. The results of thios study are presented in Table 4 and indicate that the proposed method is robust (RSD ≤ 1.76%). Method ruggedness was demonstrated having the analysis done by three analysts, and also by a single analyst performing analysis on three different instruments in the same laboratory. The inter-analysts' and inter-instruments' RSD values were ≤ 3.03% indicating ruggedness of the proposed method. The results of this study are presented in Table 4.

Table 4. Method robustness and ruggedness.

GBP taken ($\mu g\ mL^{-1}$)	Robustness (RSD %)		Ruggedness (RSD %)	
	Volume of buffer[a]	Volume of sodium nitrite[b]	Inter-analysts (n=3)	Inter-instruments (n=3)
1.0	1.13	1.76	2.07	3.03
2.0	0.65	0.94	1.02	1.98
3.0	0.87	1.06	1.46	2.64

[a]Volume of buffer was 0.9, 1.0 and 1.1 mL.
[b]Volume of sodium nitrite was 0.9, 1.0 and 1.1 mL.

Application to the assay of capsules

The proposed method has been applied successfully to the determination of GBP in capsules (Table 5). 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. The average results obtained by the proposed method and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level with respect to accuracy and precision.

Table 5. Assay of capsules and statistical evaluation of results.

Capsule brand name	Found (% of nominal amount ± SE)*	
	Reference method	Proposed method
Gabantin-100	99.32 ± 0.46	101.5 ± 0.82 **t** = 2.31 **F**= 3.10
Gabapin-300	98.07 ± 0.61	99.46 ± 0.95 **t** = 1.23 **F**= 2.39

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

Recovery study

To ascertain the accuracy and validity of the proposed method, recovery experiment was performed *via* the standard addition procedure. To a fixed and known amount of GBP in capsules powder (pre-analyzed), pure drug was added at three levels (50, 100 and 150 % of the quantity present in the capsules powder) and the total was found by the proposed method. Results of this study presented in Table 6 indicate that the commonly excipients present in the formulations did not interfere in the assay.

Table. 6. Results of recovery study by standard-addition method

Formulation studied	Capsules GBP taken, µg/mL	Pure GBP added, µg/mL	Total found of GBP, µg/mL	Bias (%)	Pure GBP recovered*, (Percent ± SE)
Gabantin-100	1.62	0.80	2.44	2.50	102.50 ± 1.07
	1.62	1.60	3.24	1.25	101.25 ± 1.20
	1.62	2.40	4.00	0.83	99.17 ± 1.17
Gabapin-300	1.99	1.00	2.97	2.00	98.00 ± 1.22
	1.99	2.00	4.02	1.50	101.50 ± 1.14
	1.99	3.00	4.92	2.33	97.67 ± 1.58

* Mean value of three determinations.

CONCLUSION

The proposed method is simple, accurate, rapid, selective and highly sensitive for the routine analysis of GBP in bulk drug as well as in capsules. The assay results demonstrate that it is possible to use sodium hypochloride in the presence of Kolthoff buffer (phosphate-borate) of pH 7.0, sodium nitrite and starch-iodide reagent for the determination of GBP in pharmaceutical formulations. The proposed method is superior to all chromatographic methods (3-8), all visible spectrophotometric methods (16-20) and the automated spectrophotometric method (22) reported so far for analysis of GBP in terms of its sensitivity. The proposed method is free from organic solvents and 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 better than that achieved by sophisticated and expensive technique like HPLC. The proposed method can be readily adopted for routine analysis in quality control laboratories.

ACKNOWLEDGEMENT

The first author (S.A.M.A.) thanks Al-Bayda' University, Republic of Yemen for awarding fellowship and University of Mysore for the permission and facilities to do the research work.

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Received: 06.01.2011
Accepted: 24.03.2011