

Development and validation of spectrophotometric method for determination of gabapentin in bulk and pharmaceutical dosage forms based on Schiff base formation with salicylaldehyde

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ABSTRACT

A sensitive, simple, and economic spectrophotometric method was developed and validated for the determination of gabapentin in pure form and pharmaceutical preparations. The method is based on Schiff base condensation reaction of the primary amino group of gabapentin with salicylaldehyde reagent in the presence of acetate solution at 45°C for 20 minutes. The obtained yellow-colored derivative in methanolic medium showed absorption maxima at 403 nm. Under the optimum conditions, Beer's law was obeyed in the concentration range of 6 to 100 µg/ml with correlation coefficient value of 0.9961. Limit of detection and limit of quantification were found to be 1.16 and 3.48 µg/ml, respectively. The validity of the described method was assessed according to the International Conference on Harmonization guidelines. The mean percentage recoveries ± SD were 100.57 ± 1.5 by applying the standard addition technique. The method was repeatable and precise (RSD ≤ 0.61% and ≤ 1.34%, respectively) and was successfully applied for the determination of the investigated drug in its pharmaceutical dosage form without detectable interference from the additives, hence, can be suggested for routine analysis of the gabapentin.

INTRODUCTION

Gabapentin (GBP), chemically known as 1-(aminomethyl)cyclohexanecarboxylic acid (Fig. 1), has a molecular formula of C₉H₁₇NO₂ and a molecular weight of 171.24 (USP 32, 2009). GBP is a white to almost white crystalline powder that is sparingly soluble in water, slightly soluble in ethanol (96%), and practically insoluble in methylene chloride. It dissolves in dilute acids and dilute solutions of alkali hydroxides. A 2% solution of GBP in water has a pH of 6.5 to 7.5 (BP, 2015; Martindale 36, 2009).

Although GBP was originally developed as anticonvulsant in the treatment of epilepsy as a structural analogue of gamma-aminobutyric acid, the precise mechanism by which GBP produces its analgesic and antiepileptic actions is yet unknown. However, it is believed to involve voltage-gated N-type calcium

ion channels in the central nervous system, reducing calcium influx into the nerve terminals. In this way, the nerves become less excitable, reducing the release of other neurotransmitters (Davies *et al.*, 2007; Fornasari, 2017).

GBP is used for the treatment of complex partial seizures, with or without secondary generalization. It is currently used as a medication to relieve pain, especially neuropathic pain and as an

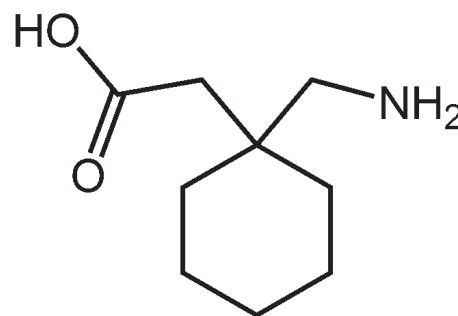


Figure 1. Chemical structure of gabapentin.

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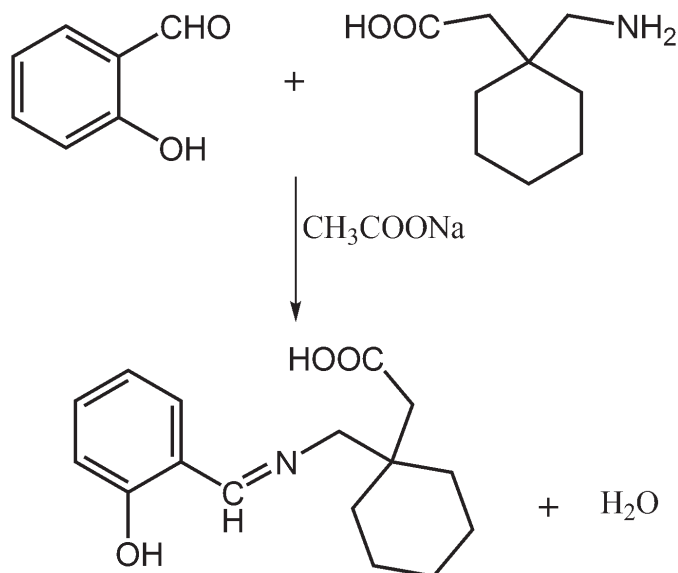


Figure 2. Schematic representation of proposed reaction of gabapentin with SA.

adjunct for the treatment of acute postsurgical pain (Behnam *et al.*, 2012; McNamara, 2011).

Reviewing the literature revealed that several direct and indirect analytical methods which based on gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), UV-Vis spectrophotometry, and spectrofluorometry have been used for the determination of GBP in pure form, pharmaceutical formulations, and/or biological samples either alone or in combination with other drugs.

Various methods that have been reported for the determination of GBP mostly rely on chromatographic techniques, especially HPLC with various detection methods (Gupta *et al.*, 2008; Ojha *et al.*, 2007; Rao *et al.*, 2009; Yagi *et al.*, 2012). They are more labor-intensive, time-consuming, not easy to work with them, and their columns and solvents are either expensive, hazardous, or not available. In addition, GC (Borrey *et al.*, 2005) and CE methods (Cao *et al.*, 2012) reported for the GBP determination require long and tedious pretreatment of the samples and laborious clean up procedures prior to analysis. They are likely to be limited by routine availability of these techniques.

Several spectroscopic methods were developed for GBP analysis in pure form and in pharmaceutical formulations either with spectrophotometric or spectrofluorimetric determination. Due to the absence of strong absorbing chromophore in GBP, its direct spectrophotometric analysis is problematic and mostly depends on the derivatization with chemical reagents (Adegbolagun *et al.*, 2018; Adegoke *et al.*, 2018; Ambala and Patel, 2011; Fonseca *et al.*, 2017; Gouda and Malah, 2013; Gujral *et al.*, 2009; Saleh *et al.*, 2014; Siddiqui *et al.*, 2010). The official methods for the identification and quantitation analysis of GBP are prescribed in BP and USP (BP, 2015; USP 32, 2009).

UV-Vis spectrophotometry is widely used for the quality control of pharmaceuticals because of its distinctive advantages

such as lower cost, robustness, simplicity, good accuracy, and precision. Therefore, a new, simple, sensitive, and accurate derivatization spectrophotometric method for the determination of GBP in pure form as well as in pharmaceutical formulations was developed in which, the use of salicylaldehyde (SA) as a derivatizing reagent for gabapentin was examined, for the first time, to produce a chromophore detectable by UV-Vis detector. The proposed method based on the formation of Schiff's bases (imine compound) as a result of condensation reaction of primary amino group of GBP with active carbonyl group of SA in the presence of acetate solution in one-step reaction (Fig. 2), which did not require vigorous conditions, extraction, or any clean-up step prior to detection in comparing with other reported procedures (Ambala and Patel, 2011).

MATERIALS AND METHODS

Instruments

Double beam scanning UV-Vis Spectrophotometer (Shimadzu 1601, Japan) with 1 cm matched quartz cell connected to a computer with UV-Probe software. Thermostatically controlled water bath (SB-24, Tokyo Aikakikai, Japan) and pH meter (Shimadzu, Japan) were used.

Reagents and chemicals

GBP standard was kindly donated by Bet Jalla Company (Ramallah, Palestine). Commercially available pharmaceutical dosage form, gabapentin capsules (300 mg/capsule), was purchased from the local market. Analytical grade Methanol was purchased from Merck (Germany). SA, glacial acetic acid, and sodium acetate supplied by (Sigma-Aldrich, Germany). Pharmaceutical grade excipients: starch, lactose monohydrate, magnesium stearate, and talc were kindly supplied by domestic pharmaceutical companies.

Preparation of reagents

SA solution 0.3% (v/v)

SA reagent was prepared by accurately transferring 0.30 ml of SA into 100-ml volumetric flask, mixed thoroughly with warm methanol, and completed the volume to 100 ml with methanol. The solution was freshly prepared daily and protected from light.

Sodium acetate solution 0.6% (w/v)

The solution was prepared by dissolving 0.60 gm of sodium acetate in 100 ml volumetric flask using distilled water.

Acetate buffer solutions

Acetate buffer solutions were prepared by weighing and transferring different amounts of sodium acetate into 1,000 ml volumetric flask, dissolved with distilled water, then different volumes of acetic acid solution 2 M were added and then the volume completed with distilled water to 1,000 ml (USP 32, 2009).

Gabapentin standard solution

Standard GBP stock solution (2 mg/ml) was freshly prepared by dissolving 100 mg of GBP standard in 50-ml volumetric flask using distilled water.

Selection of analytical wavelength for chromogen

Into two 10 ml volumetric flasks, 0.1 and 0.4 ml of GBP stock solution (2 mg/ml) were transferred and then 2 ml of SA 0.3% (v/v) solution followed by 1 ml of sodium acetate 0.6% (w/v) solution were added. The content of each flask was shaken thoroughly and heated on a water bath at 45°C for 20 minutes. The flasks were left to cool for 10 minutes, then volume was completed with methanol to have 20 and 80 µg/ml GBP, respectively. The absorption spectra and absorption maxima of the resulting solutions (chromogen) were determined against a blank prepared in the same manner without the examined drug.

Optimization of reaction conditions

Effect of temperature

The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures: RT, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, and 70°C.

Effect of solvent

The effect of solvent on the absorbance of chromogen was studied using distilled water and methanol for the dilution of final samples.

Effect of volume of SA 0.3% (v/v) solution

The effect of concentration of SA solution was studied by measuring the absorbance of GBP derivative with different added reagent volumes: 1.0, 1.5, 2.0, and 2.5 ml of SA 0.3% (v/v) solution.

Effect of volume of sodium acetate 0.6% (w/v) solution

The effect of concentration of sodium acetate solution was studied by measuring the absorbance of GBP derivative with different added volumes: 0.5, 1.0, 1.5, and 2.0 ml of sodium acetate solution 0.6% (w/v).

Effect of pH

The effect of pH was studied by measuring the absorbance of GBP derivative at different pH of acetate buffer solutions: 4, 4.5, 5, 5.5, and 7.5.

Effect of reaction time

The effect of reaction time was studied by carrying out the reaction for different time periods: 5, 10, 15, 20, 25, and 30 minutes.

Effect of order of addition

The effect of reagents addition order was studied by carrying out the reaction in different orders.

General procedure for gabapentin determination

Into 10 ml volumetric flask, 0.4 ml of GBP stock solution (2 mg/ml) was transferred followed by 2 ml of SA reagent and 1 ml of sodium acetate solution. The content of the flask was shaken thoroughly and heated on a water bath at 45°C for 20 minutes. The flask left to cool for 10 minutes and the volume was completed with the methanol. The absorbance of the resulting solution was

measured at 403 nm against a blank prepared in the same manner without the examined drug.

Construction of calibration curve for gabapentin

Accurately measured aliquots of GBP stock solution were transferred into separate 10-ml volumetric flasks and then the general procedure for gabapentin determination [Section "General procedure for gabapentin determination"] was conducted to produce standard solutions having concentrations of 6–100 µg/ml. The absorbances of these standard solutions were measured at 403 nm and calibration curve was plotted. The correlation coefficient (*r*), slope and intercept, and molar absorptivity of the drug were determined using the calibration curve.

Method validation

Analytical method validation was performed according to the ICH guidelines (2005) with respect to accuracy, precision, specificity, linearity, limit of detection (LOD), and limit of quantitation (LOQ).

Linearity and range

The linearity of the proposed method was determined by measuring the absorbance of six concentrations (6, 10, 20, 40, 80, and 100 µg/ml of GBP) covering the range (6–100 µg/ml). Five measurements were done for each concentration. The absorbance was plotted against concentration. The regression line and correlation coefficient were evaluated as well as validity of regression line was verified by statistical analysis.

Specificity

The specificity of the method was investigated by adding the common used excipients in the preparation of GBP formulations (starch, magnesium stearate, talc, and lactose) to the drug, then procedure was followed as described and the percent of the drug recovery was calculated.

Accuracy

Accuracy of the method was ascertained by performing recovery studies using standard addition method, in which standard drug aliquots were added at three different levels (20, 40, and 60 µg/ml) to a pre-analyzed sample of GBP capsule powder equivalent to 20 µg/ml. These three concentrations were measured and each of them was repeated three times to calculate the accuracy.

Precision

Intraday precision was assessed by analysis of three different concentrations (20, 80, and 100 µg/ml) of the drug at three different time periods of the same day. The relative standard deviation (RSD) was calculated for each concentration. On the other hand, inter-day precision was studied by repeating the procedure for three different concentrations (20, 80, and 100 µg/ml) of the drug on three different days and RSD was calculated for each concentration.

LOD and LOQ

The LOD is expressed using the following formula (ICH, 2005); $LOD = 3.3 \sigma/S$ and the LOQ is expressed using the

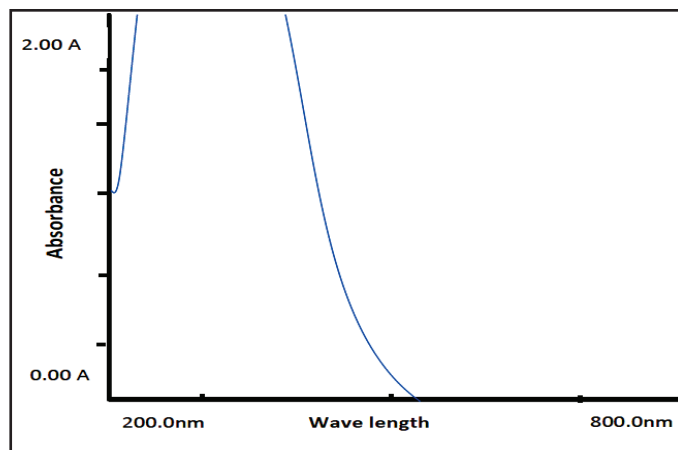


Figure 3. Absorption spectrum of blank solution of SA and sodium acetate against methanol.

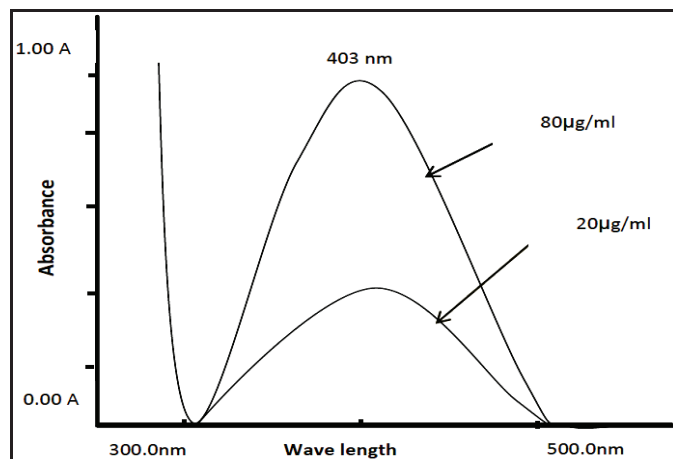


Figure 4. Absorption spectra of GBP derivative product (20 and 80 µg/ml)

following formula (ICH, 2005); $LOQ = 10 \sigma/S$; where, σ is the standard deviation of residuals of the regression line and S is the slope of the regression line.

Stability of chromogen

The effect of time on the stability of chromogen was studied by following the absorption of the resulting GBP derivative at different time intervals up to 1 hour at 403 nm after the completion of the flask's volume with methanol against the blank.

Application to gabapentin pharmaceutical dosage form (capsules)

Twenty capsules were emptied, weighed, and mixed thoroughly. An accurately weighed portion of the resulting powder equivalent to 100 mg GBP was taken, dissolved in 25 ml distilled water with good shaking for 5–10 minutes, then filtered with filter paper to remove insoluble matter. The filtrate solution was diluted to 50 ml with distilled water to prepare 2-mg/ml

Table 1. Summary of optimum conditions for reaction of GBP with SA.

Variable	Studied range	Result
Solvent	Methanol or water	Methanol
Volume of 0.3% (v/v) SA solution (mL)	1.0–2.5	2.0
Volume of 0.6% sodium acetate solution (mL)	0.5–2.0	1.0
pH	4–7.5	7.5
Temperature (°C)	25–70	45
Reaction time (min)	5–30	20
Order of addition	Different	No effect

solution. 0.2 and 0.4 ml of the resulting solution were transferred into two 10 ml volumetric flasks then the procedure was followed as described above (Section “General procedure for gabapentin determination”). Triplicate measurements were done for each concentration and the percentage of recovery was calculated from the calibration curve.

RESULTS AND DISCUSSION

The reaction of aldehydes or ketones with ammonia or 1°-amines forms imine derivatives, also known as Schiff base). Water is eliminated in the reaction, which is acid-catalyzed and reversible in the same sense as acetal formation. The pH for imine-producing reactions must be carefully controlled. The rate, at which these compounds are formed, is generally greatest near a pH of 5, and drops at higher and lower pH's (Sorrell, 2006).

Selection of analytical wavelength for chromogen

The derivatization reaction between GBP and SA was performed and the absorption spectrum of the formed yellow-colored product in methanolic medium was scanned against the blank. The blank was also scanned against methanol in the range of 200–800 nm (Fig. 3). It was found that the absorbance of the derivative product increased directly with GBP concentration and exhibiting λ_{max} at 403 nm (Fig. 4) at which selectivity enhanced through avoiding interferences from excipients in contrast to shorter λ_{max} of other reviewed methods (Siddiqui *et al.*, 2010).

Optimization of derivatization reaction conditions

To optimize the reaction variables, the effects of temperature, solvent, reaction time, pH, and concentrations of SA and sodium acetate on the absorbance of the formed product, with respect to maximum sensitivity and adherence to Beer's law, were studied through controlled experiments. The results of the optimization studies are summarized in Table 1.

Method validation

Linearity

The calibration curve was constructed by plotting the absorbance of the drug versus the drug concentration within the specified range. A linear correlation was found between concentration and absorbance in the range 6–100 µg/ml (Fig. 5)

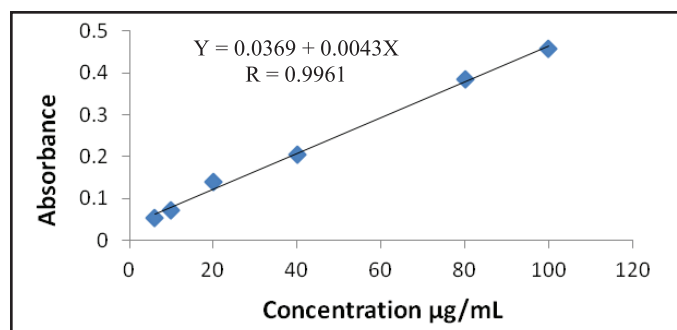


Figure 5. Calibration curve of GBP analysis (mean of five replicates).

Table 2. Results of specificity study for GBP analysis.

Concentration (µg/ml)	Amount recovered (µg/mL)	% Recovery ^a ± SD
90	90.76	99.88 ± 0.91
	89.12	
	89.80	
60	60.79	100.26 ± 1.07
	59.51	
	60.15	

^aValues are mean of three determinations.

with a correlation coefficient equal to 0.9961. Molar absorptivity of GBP chromogen was calculated ($\epsilon = 684.95 \text{ l/mol.cm}$). The LOD and LOQ for the proposed method were found to be 1.16 and 3.48 $\mu\text{g/ml}$, respectively. These obtained parameters were comparable to the parameters obtained in published spectrophotometric methods of analysis of GBP (Ambala and Patel, 2011).

Specificity

The specificity of the method was investigated by observing any interference encountered from common excipients, usually used in the pharmaceutical formulations of GBP. The degree of interference was evaluated by measuring the percentage recovery of GBP at two concentration levels (60 and 90 $\mu\text{g/ml}$) with various amounts of diverse excipients added to the drug. No interference was found in the analysis of GBP as shown in Table 2.

Accuracy

Accuracy of the method was ascertained by performing recovery studies using a standard addition method. The obtained results in Table 3 showed excellent mean recovery percent values (102.22%–99.28%) with low SD values (≤ 1.5) which indicate high accuracy of the proposed analytical method.

Precision

Intraday precision was assessed and the results are summarized in Table 4. RSD values were found to be very small ($<1.0\%$) indicating high repeatability of the proposed method. Moreover, the inter-day precision was also assessed and the results are summarized in Table 4. The obtained RSD values

Table 3. Results of recovery study for gabapentin analysis.

Base level (µg/mL)	Spiked amount (µg/mL)	Recovered amount (µg/mL)	% Recovery ^a ± SD
20	120	40.05	100.21 ± 0.085
		40.02	
		40.05	
20	40	59.89	99.28 ± 0.477
		59.51	
		59.73	
20	60	81.41	102.22 ± 0.99
		81.88	
		80.71	

^aValues are mean of three determinations.

Table 4. Evaluation of the precision of the analytical procedure of gabapentin.

	Actual amount (µg/ml)	Recovered amount (µg/ml)	Mean ^a	SD	RSD
Intraday	20	20.15	20.03	0.13	0.62
		19.90			
		20.03			
	80	80.02	80.64	0.59	0.73
		80.72			
		81.19			
100	100.1	100.1	0.47	0.47	
	99.63				
	100.57				
Inter-day	20	20.01	19.86	0.13	0.67
		19.78			
		19.78			
	80	81.41	80.47	1.07	1.34
		79.30			
		80.71			
100	99.79	99.33	0.47	0.47	
	98.86				
	99.33				

^aValues are mean of three determinations.

were less than 1.34% indicating good precision of the proposed method.

Stability of chromogen

It was found that the use of methanol as a solvent yielded good results in terms of the stability of GBP derivative which remained at least for 1 hour after dilution and this is sufficient time for the performance of the analysis for many samples.

Application to gabapentin pharmaceutical dosage form

The developed spectrophotometric method has been successfully applied to the determination of GBP in capsules. The percentage of recovery mean for GBP capsules was 101.01 ± 1.23 which is satisfactory and very close to the label claim and lie within the USP accepted range (90%–110%; USP 32, 2009).

Table 5. Determination of gabapentin in capsules by developed method and comparison with validated published method.

Dosage form	% Recovery ^a ± SD	
	Proposed method	Published method
GBP capsules 300 mg	101.01 ± 1.231 t = 0.014 ^c	99.91 ± 0.567 ^b

^aValues are mean of six determinations.

^bResults for GBP determination in capsule by published method (Gujral *et al.*, 2009).

^cTabulated $t_{0.05,10}$ value = 2.23 at 95% confidence level and 10 degrees of freedom.

The percentage of recovery with low values of standard deviation indicates the accuracy and precision of the proposed method (Table 5). The estimated drug content obtained by the proposed method was statistically comparable to the results obtained by a published method (Gujral *et al.*, 2009). No significance difference was found at 95% confidence level (p value < 0.05) as shown in Table 5.

CONCLUSION

The present study described the development and validation of a simple spectrophotometric assay for the determination of GBP. The obtained results revealed that the proposed method is time saving, precise, accurate and do not require prior extraction procedure or heating and have the advantages of simplicity and sensitivity. Hence, it could be concluded that the developed method could be successfully applied in routine analysis for GBP determination in bulk and in pharmaceutical dosage forms.

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