A Sensitive UV Spectrophotometric Method for the Determination of Gabapentin

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Abstract: An accurate and validated spectrophotometric method was developed for the determination of gabapentin. This is simple, sensitive and low cost UV spectrophotometric method. The method is based on the direct measurement of the native absorbance of the drug. The detection was done at 210 nm. The method was linear in the range of 0.25 - 3.5 µg/mL with correlation coefficient of 0.9999. It is validated according to the ICH guidelines with respect to linearity, selectivity, accuracy and precision, limit of quantitation and limit of detection. The method has been applied to assess gabapentin in pharmaceutical formulations with good accuracy and precision and relatively free of interference from coexisting substances.

Keywords: Gabapentin, UV, Bulk drug, Pharmaceutical formulation, Validation.

Introduction

Gabapentin (GBP), [1 - (aminomethyl) cyclohexane acetic acid [CAS NO: 60142 - 96 - 3]; is a γ - amino butyric acid (GABA) (Figure 1) analog used for treatment of partial seizures in adults and children¹. It is a valuable adjunct to other anti - epileptic drugs in adult patients who have not achieved adequate drugs control of partial seizures with these agents used alone or combination. It has a simple pharmacokinetic profile and is not protein bound. It is particularly useful in controlling secondarily generalized tonic - clonic seizures.

Figure 1. Structure of gabapentin.

It has been shown that the action of GBP is possibly due to its high binding to $\alpha_2 - \delta$ protein, an auxiliary subunit of voltage-gated calcium channels. Potent binding at this reduces the release of several neurotransmitters which would reduce neuronal excitability and seizures².

The therapeutic importance of GBP was behind the development of analytical methods for its determination. Several HPLC methods for determination of gabapentin in human plasma have been published using derivatizing reagents such as 2, 4, 6-trinitrobenzene sulphonic acid^{3,4}, phenylisothiocyanate⁵, *o*-phthaldehyde⁶⁻¹², 4-chloro-7-nitrobenzofurazan¹³, 9-fluorenyl methyl chloroformate¹⁴ and 1,2-naphthoquinone-4-sulphonic acid sodium salt¹⁵. A HPLC method¹⁶ was developed for the determination of GBP, which samples require dissolution and complex system. A spectrophotometric method¹⁷ was developed, which reaction time, wave length and LOD value is more than the proposed method. The authors had earlier reported a novel method for the determination of GBP in bulk, pharmaceutical formulation and human urine samples but it requires derivatization¹⁸.

A direct HPLC method at 215 nm was reported for analysis of GBP in bulk drug and dosage forms in the 2008 USP¹⁹. In one of the methods, spectrofluorimetric determination of GBP was reported after derivatization with fluorecamine²⁰. In another study, colorimetric determination of gabapentin was studied based on the reaction with vanillin and ninhydrin²¹. A capillary electrophoresis method was also reported for determination of GBP in bulk drug and capsules²². In addition, these methods require long and tedious pretreatment of the samples and laborious clean up procedures prior to analysis. Therefore, it is necessary to develop a simple and suitable analytical method for the determination of GBP in bulk and pharmaceutical formulations. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity.

The aim of the present study is to develop a new method of analysis that enhances the selectivity and simplicity of the spectrophotometric methodology in UV for GBP determination in bulk and pharmaceutical formulation.

Experimental

Spectral runs were made on UV 3000⁺ UV - VIS Spectrophotometer (LABINDIA[®], Mumbai, India) [Serial Number 17 - 1885 - 01 - 0016] with 1 cm matched glass cell.

- Gabapentin (Vardhman Chemtech Ltd, Mohali, Punjab, India) used as a working standard (0.9923 mg/mg) [Standardized with USP Gabapentin (0.999 mg/mg) Lot No G0E005].
- Pharmaceutical formulations of gabapentin such as Gabapin - 300 (Intas Pharmaceuticals, Dehradun, India), Neucobal G (Sun Pharmaceutical Industries, Jammu, India), Nurokind[™] G (Mankind Pharma Ltd, New Delhi, India) and Gabaneuron (Aristo Pharmaceuticals Pvt Ltd, Mumbai, India) were purchased from local markets.

Standard GBP solution

A stock solution of GBP (100 $\mu\text{g/mL}$) was prepared by dissolving 10 g GBP in 100 mL volumetric flasks with double distilled water. The stock solution (100 $\mu\text{g/mL}$) was used to prepare the working solutions by suitable dilutions with distilled water. The solutions were stable at least 20 days in room temperature.

Procedure for determination of GBP

Aliquots of stock solution (100 $\mu\text{g/mL}$) were transferred into a set of 50 mL volumetric flasks and volumes were completed to the mark with distilled water to produce solutions in the concentration range 0.25-3.50 $\mu\text{g/mL}$. Absorbance was measured at 210 nm against the reagent blank. Calibration graphs were constructed by plotting absorbance against the final concentration of GBP.

Procedure for determination of GBP in pharmaceutical formulations

One capsule (claiming 300 g of Gabapentin) was accurately weighed and finely powdered. A quantity of the powder equivalent to 50 g of GBP was extracted by shaking with 20 mL of distilled water, followed by another two extractions each with 10 mL distilled water. After passing through a 0.45 μm millipore filter, the solution was diluted with distilled water to obtain a concentration of about 100 $\mu\text{g/mL}$. It was further diluted according to the need and then analyzed following the proposed procedures. The nominal content of the capsule was calculated either from the previously plotted calibration graphs or using regression equation.

Evaluation of bias

The bias has been evaluated by means of point and interval hypothesis tests²³⁻²⁴. In interval hypothesis the proposed method is accepted when the true mean is within $\pm 2\%$ of that of the reference method, *i.e.* $0.98 < \mu_2 / \mu_1 < 1.02$ Which can be generalized to $\theta_L < \mu_2 / \mu_1 < \theta_U$ where θ_L and θ_U are lower and upper acceptance limits, respectively.

Results and Discussion

Linearity

Aqueous solution of gabapentin was measured at 210 nm against the reagent blank Figure 2. Based on its inherent absorbance property, direct determination of GBP was achieved in pharmaceutical formulations. A calibration curve was constructed by plotting the absorbance versus final concentration of GBP, which showed a linear response showing the linear dynamic range over the concentration range 0.25-3.5 $\mu\text{g/mL}$ Figure 3. Linear regression analysis of calibration data gave the regression equations cited in Table 1 with correlation coefficients close to unity.

Figure 2. Absorption spectra of gabapentin (2.5 $\mu\text{g/mL}$).

Figure 3. Linearity of absorbance corresponding the concentration of the proposed method.

Table 1. Summary of optical and regression characteristics of the proposed method.

Parameters	Gabapentin
Linear dynamic range, µg/mL	0.25 - 3.50
Regression equation ^a	$Y = 1.005 \times 10^{-1}x - 2.0 \times 10^{-4}$
S_a	9.05×10^{-4}
S_b	4.32×10^{-4}
Correlation coefficient (r)	0.9999
LOD, µg/mL	4.40×10^{-2}
LOQ, µg/mL	1.33×10^{-1}
Variance (S_o^2) of calibration line	1.80×10^{-6}

^aWith respect to $Y = a + bX$, where X is the concentration in µg/mL, Y is Absorbance; ^bConfidence interval of the intercept and slope at 95% confidence level and ten degrees of freedom ($t = 2.228$)

Specificity and selectivity

The working standard and pharmaceutical formulation sample solutions were stressed by light and heat (up to 50 °C) for 3 hours. It was observed that stress by such conditions did not cause significant degradation. There is no change in the absorption spectra. All stressed samples (light and heat) and unstressed sample solutions were analyzed for the active content of GBP, which gave acceptable recoveries of the drug. The influence of frequently encountered excipients in pharmaceutical formulations of GBP on the proposed method was studied by adding different amounts of possible interferents to the sample. It was observed that glucose, fructose, sucrose, cellulose, starch, lactose, magnesium stearate, sodium stearyl fumarate, red oxide of iron and Ponceau 4R & Titanium dioxide did not interfere the proposed method.

Accuracy and precision

The accuracy and precision of the proposed method was established by performing intraday and interday assays by determining the content of GBP in bulk and pharmaceutical formulation samples at three different concentration levels (low, medium and high). These assays were investigated by measuring five independent analyses at 1, 2 & 3 µg/mL concentration levels within 1 day and on 5 consecutive days, respectively Table 2 & 3. The standard deviation, relative standard deviation and standard analytical error obtained by both methods are acceptable *i.e.* within the permissible bias range and therefore can be considered to be satisfactory.

Table 2. Summary of accuracy and precision results of the proposed method in pure form.

Proposed methods	Amount, $\mu\text{g/mL}$		RSD, %	REC, %	SAE ^b	C.L. ^c
	Taken	Found $\pm$ SD ^a				
Intraday assay	1.00	0.9992 $\pm$ 0.002	0.204	99.920	9.1 $\times$ 10 ⁻⁴	2.5 $\times$ 10 ⁻³
	2.00	1.9999 $\pm$ 0.003	0.136	99.950	1.2 $\times$ 10 ⁻³	3.4 $\times$ 10 ⁻³
	3.00	2.9999 $\pm$ 0.005	0.165	99.954	2.2 $\times$ 10 ⁻³	6.2 $\times$ 10 ⁻³
Interday assay	1.00	0.9998 $\pm$ 0.004	0.362	99.920	1.6 $\times$ 10 ⁻³	4.5 $\times$ 10 ⁻³
	2.00	1.9998 $\pm$ 0.003	0.147	99.990	1.3 $\times$ 10 ⁻³	3.6 $\times$ 10 ⁻³
	3.00	2.9988 $\pm$ 0.007	0.229	99.960	3.1 $\times$ 10 ⁻³	8.5 $\times$ 10 ⁻³

^a Mean for 5 independent analyses. ^bSAE, standard analytical error. ^cC.L., confidence limit at 95 % confidence level and 4 degrees of freedom ($t = 2.776$).

Table 3. Summary of accuracy and precision results of the proposed method in pharmaceutical formulations.

Proposed methods	Amount, $\mu\text{g/mL}$		RSD, %	REC, %	SAE ^b	C.L. ^c
	Taken	Found $\pm$ SD ^a				
Intraday assay						
Gabapin	1.00	0.999 $\pm$ 0.006	0.579	99.940	0.0026	0.0072
	2.00	1.999 $\pm$ 0.002	0.119	99.990	0.0011	0.0030
	3.00	2.999 $\pm$ 0.003	0.101	99.987	0.0014	0.0038
Neucobal G	1.00	0.999 $\pm$ 0.002	0.181	99.940	0.0008	0.0022
	2.00	2.000 $\pm$ 0.004	0.188	100.020	0.0017	0.0047
	3.00	3.002 $\pm$ 0.004	0.127	100.007	0.0017	0.0047
Nurokind™ G	1.00	0.999 $\pm$ 0.003	0.294	99.980	0.0013	0.0036
	2.00	2.000 $\pm$ 0.005	0.232	100.020	0.0021	0.0058
	3.00	2.999 $\pm$ 0.002	0.055	99.993	0.0007	0.0020
Gabaneuron	1.00	1.000 $\pm$ 0.002	0.244	100.00	0.0011	0.0030
	2.00	1.999 $\pm$ 0.003	0.138	99.990	0.0012	0.0034
	3.00	2.999 $\pm$ 0.003	0.103	99.993	0.0014	0.0038
Interday assay						
Gabapin	1.00	0.999 $\pm$ 0.004	0.413	99.980	0.0019	0.0053
	2.00	1.999 $\pm$ 0.004	0.210	99.980	0.0019	0.0053
	3.00	2.999 $\pm$ 0.004	0.148	99.987	0.0020	0.0056
Neucobal G	1.00	0.999 $\pm$ 0.005	0.461	99.999	0.0021	0.0058
	2.00	1.999 $\pm$ 0.005	0.237	99.990	0.0021	0.0058
	3.00	2.999 $\pm$ 0.004	0.140	99.993	0.0019	0.0053
Nurokind™ G	1.00	1.000 $\pm$ 0.003	0.340	100.020	0.0015	0.0042
	2.00	1.999 $\pm$ 0.005	0.237	99.990	0.0021	0.0058
	3.00	3.000 $\pm$ 0.004	0.116	100.013	0.0016	0.0043
Gabaneuron	1.00	0.999 $\pm$ 0.005	0.460	99.940	0.0021	0.0058
	2.00	1.999 $\pm$ 0.005	0.267	99.990	0.0024	0.0067
	3.00	2.999 $\pm$ 0.006	0.211	99.987	0.0028	0.0078

^a Mean for 5 independent analyses. ^bSAE, standard analytical error. ^cC.L., confidence limit at 95 % confidence level and 4 degrees of freedom ($t = 2.776$).

Standard addition method was employed to check the validity of the proposed procedures. In this method, a known amount of pure GBP was added to its formulated capsules at different concentration levels and the nominal value of the drug was calculated following the proposed procedure. The results are summarized in Table 4. As can be seen from the Table 4 that recovery obtained by the proposed procedure is quite satisfactory with low RSD.

Table 4. Summary of data for the determination of gabapentin in pharmaceutical preparations by standard addition method.

Formulations	Amount, $\mu\text{g/mL}$			Recovery %	RSD %	SAE ^b
	Taken	Added	Found $\pm$ SD ^a			
Gabapin	0.50	0.25	0.750 ± 0.002	100.007	0.255	0.0009
	0.50	1.00	1.500 ± 0.003	100.007	0.218	0.0015
	0.50	2.00	2.499 ± 0.003	99.996	0.134	0.0015
	0.50	3.00	3.500 ± 0.003	100.003	0.069	0.0011
Neucobal G	0.50	0.25	0.750 ± 0.004	100.007	0.518	0.0017
	0.50	1.00	1.500 ± 0.005	100.007	0.320	0.0022
	0.50	2.00	2.499 ± 0.004	99.996	0.168	0.0019
	0.50	3.00	3.500 ± 0.003	100.003	0.091	0.0014
Nurokind TM G	0.50	0.25	0.750 ± 0.002	100.007	0.197	0.0007
	0.50	1.00	1.500 ± 0.003	100.007	0.173	0.0012
	0.50	2.00	2.499 ± 0.004	99.988	0.172	0.0019
	0.50	3.00	3.499 ± 0.005	99.997	0.146	0.0023
Gabaneuron	0.50	0.25	0.750 ± 0.004	100.060	0.542	0.0018
	0.50	1.00	1.499 ± 0.006	99.993	0.371	0.0025
	0.50	2.00	2.500 ± 0.003	100.004	0.134	0.0015
	0.50	3.00	3.500 ± 0.005	100.003	0.128	0.0020

^aMean for 5 independent analyses. ^bSAE, standard analytical error.

Applicability of the proposed method

The applicability of the proposed method for the assay of GBP in drug formulations has been tested on commercially available capsules. The results of the proposed method were statistically compared with those of the developed reference method using point and interval hypothesis tests. Table 5 shows that the calculated (*t*-paired) and *F*-values at 95 % confidence level are less than the theoretical ones, confirming no significant difference between the methods compared. It can also be seen from Table 5 that the bias evaluated by interval hypothesis test is within the acceptable range of $\theta_L = 0.98$ and $\theta_U = 1.02$.

The performance of the proposed method was compared with other method Table 6. It can be seen from Table 6 that HPLC methods^{25,26} were time consuming and tedious but precision are also somewhat poor. UV method¹⁷ is sensitive but RSD values are higher. The present method is simple, fast and accurate because it does not involve any pretreatment step prior to analysis.

Table 5. Assay results of GBP in commercial capsules using the proposed method and reference method¹⁹

Formulations	Proposed method	Reference method ¹⁹
Gabapin 300		
Recovery, %	99.997	99.908
RSD	0.104	0.567
t	0.48	
F	3.35	
θ_L	0.993	
θ_U	1.004	

Table 6. Comparisons of the proposed method with other existing methods for the assay of gabapentin in pharmaceutical formulations.

Reagents	$\lambda_{\max}$, nm	Linear Dynamic Range, $\mu\text{g/mL}$	RSD	LOD	LOQ	R ²	Reference
FDNB	360	10 - 500	1.11	3.21	9.73	0.9998	25
Fluorescamine	-	0.2 - 10	4.51	0.11	0.35	0.9954	26
TCNQ	842	8 - 24	1.40	0.48	1.60	0.9997	17
DDQ	456	12- 36	1.70	1.20	3.99	0.9993	17
pCA	535	60 - 200	0.94	7.59	25.30	0.9991	17
Chloranil	521	40 - 120	0.84	3.33	11.10	0.9995	17
Iodine	360	6 - 30	0.93	0.39	1.31	0.9998	17
TCNE	412	40 - 140	1.10	3.54	11.80	0.9994	17
-	210	0.25 - 3.50	0.136	0.044	0.133	0.9999	This work

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