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Estimation of a New Gabapentin Derivative in Capsule Dosage Form by New Validated UV Spectrophotometric Method

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Abstract

A new simple, sensitive, and economic spectrophotometric method has been developed and validated for the determination of a new gabapentin derivative in pure form and pharmaceutical preparations. The method is based on the reaction between the amino group of gabapentin with benzene sulphonyl chloride to form the gabapentin derivative (sulphonamide) via addition-elimination mechanism. The new gabapentin derivative was prepared by two different methods using sodium carbonate (method 1) and sodium hydroxide (method 2). The colourless product obtained was analysed by TLC. In UV-spectrophotometry, its absorption maximum was found to be 275nm in ethanol. The linearity range for the new gabapentin derivative was found to be 2-10 µg/ml. Accuracy was performed at three concentration levels of 50%, 100% and 150% and the respective percentage recoveries were found to be 1.34,1.06 and 0.68. Precision results were found to be within the limits of acceptance criteria and the method was found to be robust with % RSD less than 2.0. The LOD and LOQ values were found to be 0.28µg/mL and 0.86µg/mL respectively.

Keywords

Derivative, Gabapentin, Spectrophotometry

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INTRODUCTION

A cyclic analogue of GABA [gamma-amino butyric acid] is Gabapentin [1-(amino methyl)-cyclohexane acetic acid] **(Fig. 1)**. It is frequently utilized to treat pain, particularly neuropathic pain, and nystagmus **[1-3].** Most patients tolerate it well, it has a modest side effect profile, and it is not metabolized as it leaves the body. It is believed to bind to the voltage-dependent calcium channel's subunit 2 in the central nervous system.

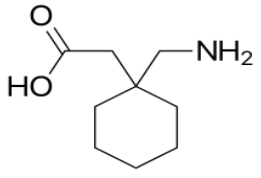


Fig. 1: Chemical structure of Gabapentin

Gabapentin has been estimated by various spectrophotometric and spectrofluorimetric methods **[4-9],** thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) **[10-14],** high performance thin layer chromatography (HPTLC) **[15, 16],** Gas chromatography (GC) **[17],** capillary electrophoresis **[18]** and potentiometric methods **[19]**.

Gabapentin has very low UV absorptivity and hence it is difficult to accurately estimate it by UV spectroscopy. Chemical derivatization is adopted by few of them to convert the non-UV absorbing gabapentin into its derivatives which can be easily detected with high sensitivity **[20]**. However, the number of such reports are very few and they suffer with some disadvantages associated with stability,

time, cost etc. Hence there is a need to develop more simpler, inexpensive, stable derivatives of gabapentin which can be easily and accurately analysed by UV spectroscopy. In the present work, Hinsberg reagent (benzene sulphonyl chloride) has been employed as a chromophoric reagent to derivatize Gabapentin and enhance its UV absorptivity.

MATERIALS AND METHODS

Instrument:

Lab India-T60 and SHIMADZU-1800 Double Beam UV-Visible Spectrophotometer with pair of 10mm matched quartz cells, BRUKER ALPHA- FTIR Spectrophotometer and. BRUKER AVANCE III 700 MHz NMR spectrometer (CSIR-IICT, Hyderabad) were used for the study.

Chemicals and reagents:

Gabapentin (API) was procured as a gift sample from Mylan Laboratories Limited, Hyderabad. Gabapentin capsules (GABANTIN-300) were purchased from Sun Pharmaceutical Industries Ltd, Mumbai, India. All the reagents and solvents used were of analytical grade.

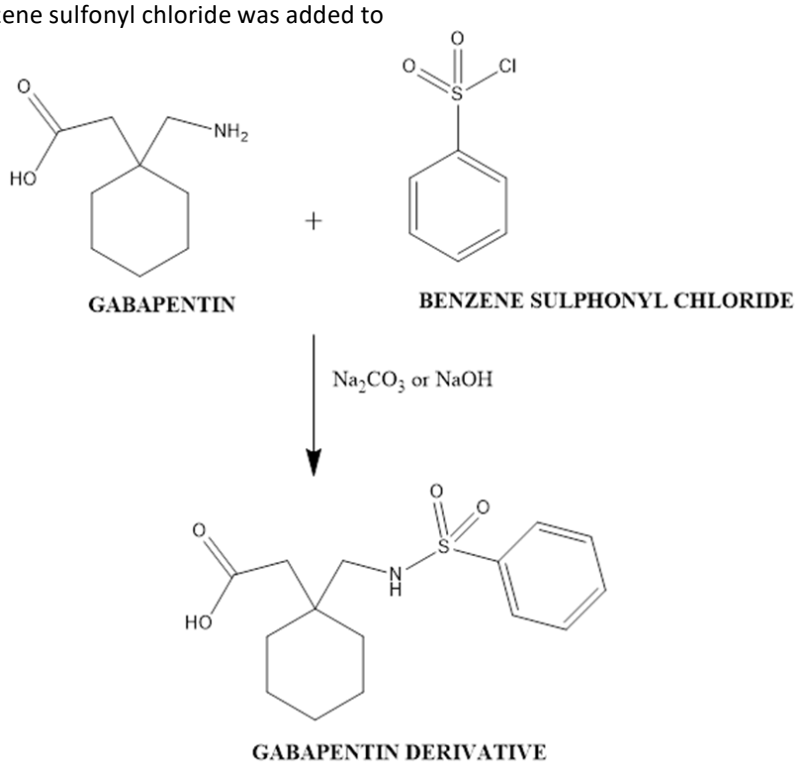
Chemical derivatization of Gabapentin:

Method 1:

In a beaker, Gabapentin (2 g, 12.7 mmol) was added and mixed with 15–20 mL of distilled water. Drop by drop, sodium carbonate solution (2–3%) was added until the gabapentin completely dissolved, and the pH was controlled between 8 and 9. Then, 1.904 mL of equimolar benzene sulfonyl chloride was added to the mixture. At room temperature, it was agitated using a magnetic stirrer, maintaining the pH of the reaction mixture between 8 to 9 with the addition of a few drops of Na_2CO_3 solution. The reaction was stirred continuously until it was finished wherein the pH of the solution ceases to fall and remains constant. Then, 2-3 M HCl was added. The product thus obtained was filtered, dried and recrystallized from aqueous ethanol.

Method 2:

2 g of gabapentin was mixed with 40 ml of 10% sodium hydroxide solution. To it, 4g (3 ml) of benzene sulphonyl chloride was added in small portions and warmed on a water bath until the completion of the reaction. Then the solution was acidified with dilute hydrochloric acid when the sulphonamides of the primary and secondary amines are precipitated. Filter off the solid and wash it with a little cold water; the tertiary amine will be present in the filtrate. To convert any disulphonamide that may have been formed from the primary amine into the sulphonamide, boil the solid under reflux with 2.0 g of sodium dissolved in 40 ml of absolute ethanol for 30 minutes. Dilute with a little water and distil off the alcohol: filter off the precipitate of the sulphonamide of the secondary amine. Acidify the filtrate with dilute hydrochloric acid to precipitate the derivative of the primary amine. It was filtered, dried and recrystallized from aqueous ethanol. **(Scheme-1).**



Scheme 1: Preparation of the new Gabapentin derivative

Method Development:

Preparation of Stock and Standard solutions:

Preparation of Stock Solution:

Accurately weighed 100 mg of Gabapentin derivative and dissolved in 10 ml of ethanol in a 100 ml volumetric flask and diluted up to the mark with ethanol to obtain the stock solution having a final concentration of 1000 µg/ml. Standard solutions of different concentrations were prepared by diluting this stock solution.

Preparation of Primary Standard Solution:

The primary standard solution was prepared by pipetting 1 ml of stock solution into a 10 ml volumetric flask and making up the volume with ethanol to produce 100 µg/ml.

Preparation of Secondary Standard Solution:

Pipetted out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml from the primary standard solution and diluted with ethanol to obtain 2µg/ml, 4µg/ml. 6µg/ml, 8µg/ml and 10µg/ml respectively.

Selection of detection wavelength for UV region:

Drug solution of 10µg/mL was scanned over the range of 200-400nm in UV region. It was observed that the drug showed the maximum absorbance at 275nm.

Preparation of sample solution (Assay):

Twenty capsules of Gabapentin were taken, and the contents were carefully collected. The capsules powder equivalent to 200 mg of Gabapentin was accurately weighed and transferred into 250ml beaker. It was mixed with 0.19 ml of benzene sulphonyl chloride in 10-15 ml of distilled water and proceeded as described in the derivatization procedure. The entire product obtained was dissolved in ethanol and then further diluted to obtain concentration equivalent to 10 µg/ml. The absorbance of this sample solution was measured at 275 nm **[21].**

Method Validation: The suggested method for the new gabapentin derivative was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness in accordance with the recommended criteria.

RESULTS

Physical data of Gabapentin derivative

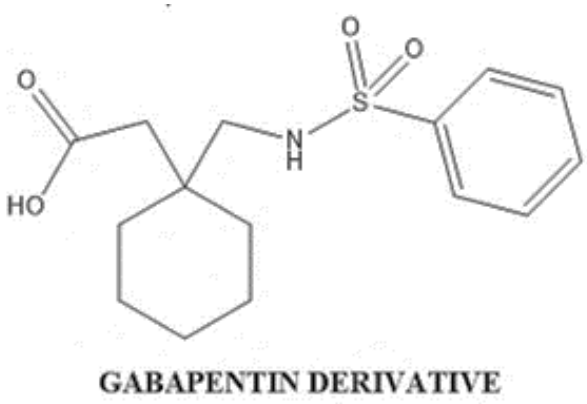


Fig. 2: Chemical structure of gabapentin derivative

IUPAC Name:

2-[1-(Benzene sulfonamido methyl) cyclohexyl] acetic acid **(Fig. 2)**

Molecular Formula : $C_{15}H_{21}NO_4S$

Molecular Weight : 311.18

Description: White solid

Solubility: Methanol

R_f value: 0.5 (Chloroform: Methanol- 4.8:0.2)

m.p (°C): 102-106 (Method-1), 103-105 (Method-2)

% Yield: 78 (Method-1), 85 (Method-2)

Spectral (IR, ^{1}H NMR) data of Gabapentin derivative:

The IR spectrum **(Fig. 3)** of gabapentin derivative revealed the sulfonamide SO_2NH- stretch at 3747.94 cm^{-1}. The carboxylic -OH stretch was observed at 3522.29 cm^{-1} and the -CO stretch was seen at 1739.11 cm^{-1}. The C-H stretch was found at 2929.87 cm^{-1}. The sulphonamide -SO_2NH stretch was observed at 995.98 cm^{-1}.

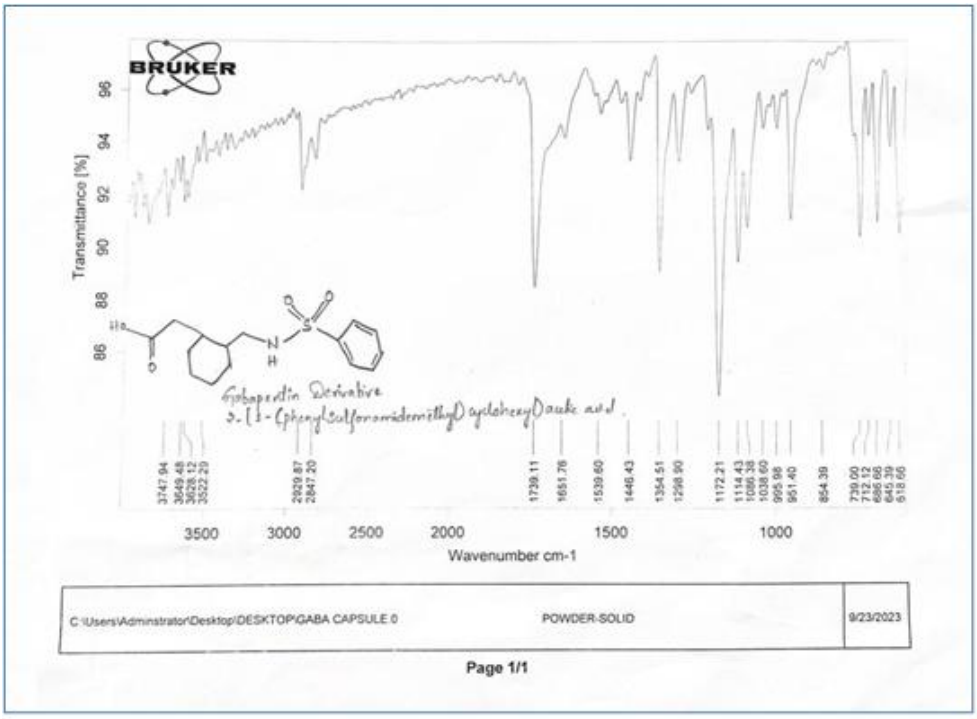


Fig. 3: IR Spectrum of gabapentin derivative derived from capsule powder.

The proton NMR spectrum of the Gabapentin derivative in deuterated methanol **(Fig. 4)** showed peaks for aliphatic protons and aromatic protons. The ten protons pertaining to cyclohexyl methylene groups were recorded as a multiplet from 1.31-1.62 ppm. A singlet for the methylene (-C*H*$_2$-CO) protons were detected as a singlet at 2.32 ppm. The -N*H* proton was observed as a singlet at 3.35 ppm while the methylene (-C*H*$_2$-NH) protons were detected as another singlet at 3.71 ppm. The aromatic protons H_3 & H_5 were observed as a multiplet from 7.55-7.62 ppm, H_4 was seen as another multiplet from 7.69-7.75 ppm and lastly H_2 & H_6 were observed as a multiplet from 7.95-8.12 ppm. The carboxylic proton which usually come downfield above 10 ppm couldn't be recorded in the present spectrum.

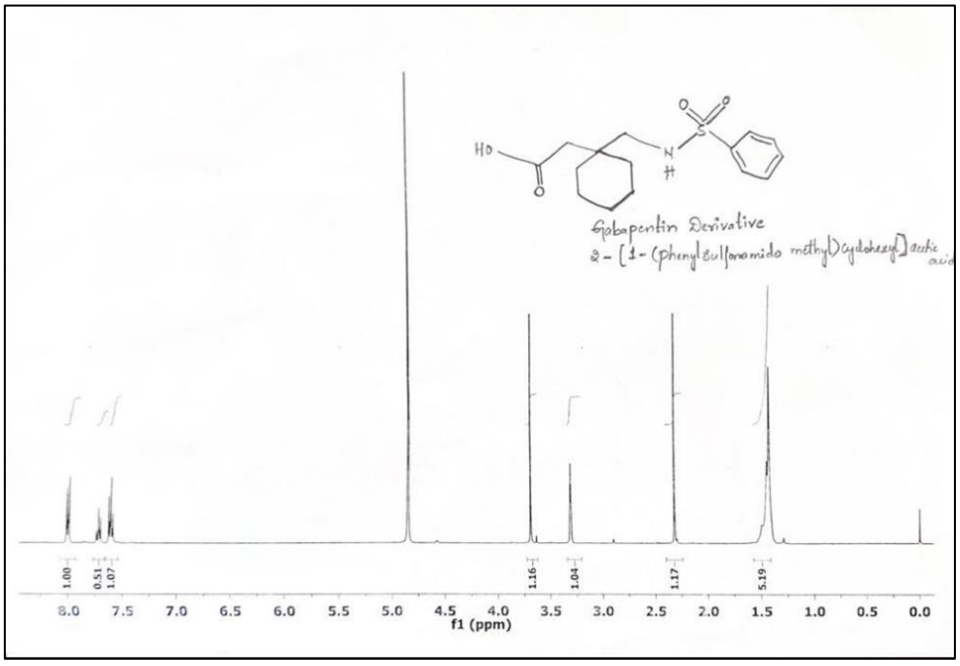


Fig. 4: ^{1}H NMR spectrum of Gabapentin derivative in methanol

Method Validation

Linearity:

The gabapentin derivative concentration ranged from 2 to 10 µg/mL during the trial. The concentration versus absorbance graphs were found to be linear **(Table 1, Fig. 5).** The correlation coefficient R^2 was found to be 0.9992.

Accuracy:

With three repetitions at each level and a constant sample amount of 15 µg/ml in UV, the accuracy test was carried out at three distinct concentration levels of 50%, 100%, and 150%, or 2, 4 and 6 µg/ml solutions for UV. In accordance with ICH norms, all nine readings percentage recovery, means, standard deviations, and percent RSD were determined. The %RSD values ranged from 0.68 to 1.34 at the three spike levels **(Table 2)** indicating that the method is accurate.

Precision:

An intermediate and intraday variation analysis was used to determine the method's precision. For the investigation, solutions containing 6, 8, 10 µg/mL of UV were used, and their absorbances were measured. Pooled % RSD was found to be less than 2 for both intermediate and intraday precision **(Tables 3 and 4).**

Limit Of Detection (LOD) and Limit of Quantification (LOQ): According to ICH guidelines, the procedure can determine the LOD and LOQ. This project's methodology is based on the calibration curve's slope and standard deviation of response.

Limit of Detection (LOD): 3.3 σ/S where σ- Standard Deviation, S-slope

Limit of Quantification (LOQ): 10 σ/S

It was discovered that the detection threshold was 0.28µg/ml and the quantitative limit was 0.86(µg/ml).

Table 1: Linearity data of gabapentin derivative

Concentration (µg/ml)	Absorbance at 275 nm
2	0.572
4	0.788
6	1.029
8	1.28
10	1.491

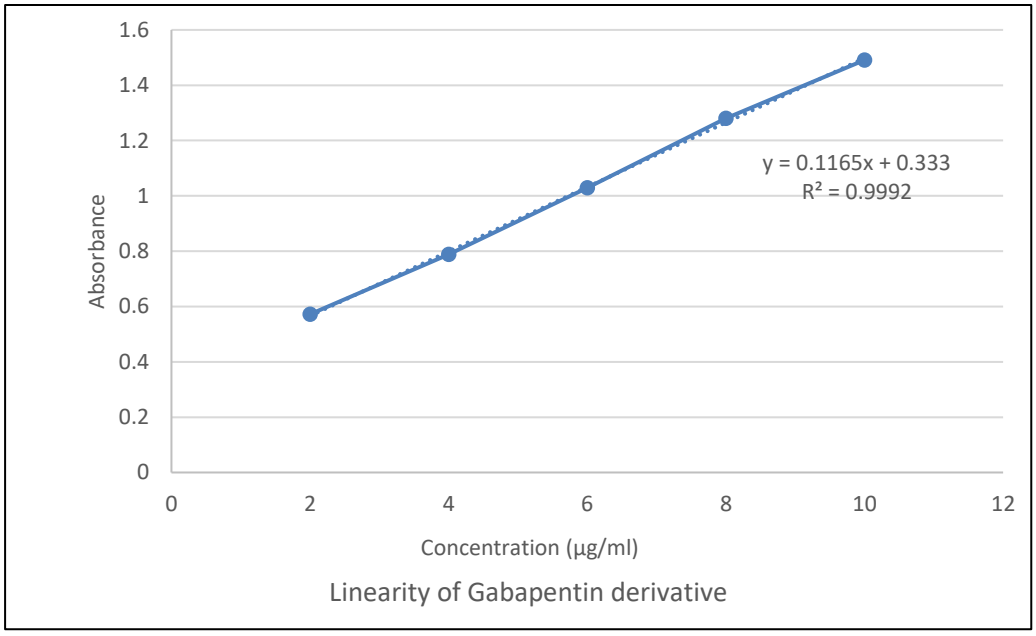


Fig. 5: Graphical representation of linearity of gabapentin derivative

Table 2: Accuracy data of gabapentin derivative

Spike level (%)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean	S.D.	%RSD
50	2	1.97 1.93 1.98	98.5 96.5 99	98	1.32	1.34
100	4	3.87 3.95 3.89	96.75 98.75 97.25	97.58	1.04	1.06
150	6	5.94 5.91 5.86	99 98.5 97.66	98.38	0.67	0.68

Table 3: Intermediate Precision data of gabapentin derivative

Day	Conc. (µg/ml)	Absorbance at 275 nm						Mean	S.D.	%RSD	Pooled %RSD
1	6	0.312	0.313	0.309	0.310	0.312	0.314	0.311	0.001	0.598	
	8	0.858	0.856	0.857	0.855	0.859	0.860	0.857	0.001	0.218	
	10	1.007	1.003	1.001	1.004	1.006	1.007	1.004	0.002	0.241	
2	6	0.541	0.544	0.545	0.544	0.546	0.547	0.544	0.002	0.380	
	8	0.474	0.476	0.477	0.476	0.478	0.477	0.476	0.001	0.285	0.394
	10	0.627	0.628	0.612	0.613	0.615	0.616	0.618	0.007	1.152	
3	6	0.587	0.589	0.590	0.591	0.592	0.593	0.590	0.002	0.366	
	8	0.873	0.874	0.875	0.876	0.877	0.878	0.875	0.001	0.213	
	10	0.951	0.952	0.953	0.951	0.952	0.953	0.952	0.000	0.093	

Table 4: Intraday Precision data of gabapentin derivative

Time	Conc. (µg/ml)	Absorbance at 275 nm						Mean	S.D.	%RSD	Pooled %RSD
11am	6	0.587	0.589	0.590	0.591	0.592	0.593	0.590	0.002	0.365	
	8	0.873	0.874	0.875	0.876	0.877	0.878	0.875	0.001	0.213	
	10	0.951	0.952	0.953	0.951	0.952	0.953	0.952	0.000	0.093	
2pm	6	0.574	0.575	0.576	0.575	0.576	0.577	0.575	0.001	0.182	
	8	0.658	0.663	0.664	0.665	0.668	0.669	0.664	0.003	0.592	0.383
	10	0.739	0.740	0.739	0.735	0.737	0.736	0.737	0.001	0.266	
5pm	6	0.567	0.566	0.567	0.568	0.569	0.570	0.567	0.001	0.259	
	8	0.651	0.662	0.661	0.660	0.662	0.667	0.660	0.005	0.793	
	10	0.732	0.733	0.734	0.741	0.742	0.743	0.737	0.005	0.679	

Table 5: Robustness data of gabapentin derivative

	Conc. (µg/ml)	Wavelength						
Change in wavelength (±2nm)		**273nm**	**274nm**	**275nm**	**276nm**	**277nm**	**Mean±S.D.**	**%RSD**
	2	0.160	0.161	0.161	0.162	0.163	0.161±0.001	0.621
	4	0.315	0.316	0.316	0.316	0.317	0.316±0.000	0.158
	6	0.554	0.554	0.555	0.555	0.558	0.555±0.001	0.180
	Absorbance at Room Temperature						**Mean±S.D.**	**% RSD**
	2	0.168	0.167	0.169			0.168±0.000	0.119
	4	0.281	0.283	0.282			0.282±0.000	0.106
	6	0.572	0.575	0.571			0.572±0.001	0.174
Change in temperature (°C)	**at Refrigerated Temperature**							
	2	0.180	0.181	0.180			0.180±0.000	0.222
	4	0.314	0.315	0.314			0.314±0.000	0.159
	6	0.567	0.568	0.569			0.568±0.001	0.176
	at Sunlight Temperature							
	2	0.173	0.174	0.174			0.173±0.000	0.115
	4	0.360	0.359	0.569			0.429±0.001	0.233
	6	0.568	0.569	0.570			0.569±0.001	0.175

Table 6: Ruggedness data of gabapentin derivative

Variation	**Concentration (µg/ml)**		
	2	**4**	**6**
Actual	0.161	0.316	0.555
	0.155	0.325	0.551
	0.164	0.334	0.549
Mean±S.D.	**0.16±0.002**	**0.325±0.005**	**0.551±0.003**
%RSD	**1.25**	**1.53**	**0.54**
Analyst to Analyst	0.146	0.287	0.540
	0.155	0.296	0.563
	0.162	0.317	0.552
Mean±S.D.	**0.154±0.002**	**0.3±0.004**	**0.551±0.011**
%RSD	**1.29**	**1.33**	**1.99**
Instrument to Instrument			
Lab India	0.268	0.365	0.569
	0.267	0.365	0.569
	0.268	0.366	0.570
Mean±S.D.	**0.267±0.000**	**0.365±0.000**	**0.569±0.000**
% RSD	**0.33**	**0.16**	**0.158**
Shimadzu	0.168	0.281	0.572
	0.167	0.283	0.575
	0.169	0.282	0.571
Mean±S.D.	**0.168±0.001**	**0.282±0.001**	**0.572±0.002**
% RSD	**0.59**	**0.35**	**0.34**

Robustness:

Robustness of the method was analysed by measuring the effect of absorbance with change in wavelength (±2 nm) and change in temperature at 2, 4 and 6 µg/mL and the findings were displayed as % RSD, which was found to < 2 **(Table 5)**. Hence the method is robust.

Ruggedness:

Analyst to analyst and instrument to instrument variations were studied at 2, 4 and 6 µg/mL in triplicate to access the ruggedness of the method **(Table 6)**. The results agree with the ICH guidelines (%RSD < 2) indicating that the method is rugged.

Assay:

The appropriate dilutions of capsule powder derivative were prepared from its stock solution and estimated for the gabapentin content according to the developed method and it was found to contain 98±1.8% of gabapentin.

CONCLUSION

Gabapentin is one of the most widely used drugs to treat epilepsy and nerve pains. Literature reveals that several spectrophotometric and HPLC methods have been developed and validated for its estimation. However, due to the low UV absorptivity of gabapentin, estimating it accurately in a cost-effective way is a challenge. To overcome this problem, chemical derivatization was adopted in spectrophotometric method for the gabapentin API and capsule powder which produced a new, stable, colourless sulphonamide derivative of gabapentin. All the validation parameters are in accordance with the ICH guidelines. The new UV-spectrophotometric method was found to be linear, accurate, precise, sensitive, simple, and economic. Hence this method can be used for the routine analysis of the gabapentin in bulk and formulations.

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