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New Validated UV-Spectrophotometric Method for the Determination of a New Pregabalin Derivative in Capsule Dosage Form

S. Deepthi, G. Divya, D. Joyce Sharon, V. Sandhya, J. Deepthi Akanksha, P. Vivek Sagar and K. Sirisha*
Department of Pharmaceutical Analysis, Sarojini Naidu Vanitha Pharmacy Maha Vidyalaya, Tarnaka, Secunderabad, Telangana-500017, India.

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***Corresponding Author Email:** ragisirisha@gmail.com

Abstract
Pregabalin, an anti-epileptic drug has very low UV absorptivity and hence normally it is difficult to analyze this drug by UV Spectroscopy. Benzene sulfonyl chloride, a derivatizing agent, was used to introduce a chromogen for the purpose of detecting pregabalin in bulk and capsules. A wavelength of 205nm was used to find the pregabalin after derivatization by UV-spectroscopy. The spectrophotometric validation parameters such as linearity, precision, accuracy, robustness, and ruggedness were studied and verified using the ICH guidelines. With a correlation coefficient of 0.999, the linearity between 20µg/ml and 100µg/ml was observed. The intermediate and intraday precision's respective RSDs were found to be 1.27% and 1.40%. The accuracy concentration range was spiked at 50%, 100%, and 150%, and the %recovery values were found to be in the range of 95.7% to 99.4%. The method was found to be rugged and robust. Without any interference from typical excipients, the devised approach was effectively verified and applied to the detection of pregabalin in bulk and pharmaceutical formulation.

Keywords
Absorbance, Derivative, Methanol, Pregabalin, UV

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INTRODUCTION
Pregabalin is chemically known as (S)-3-(aminomethyl)-5-methyl hexanoic acid, commonly used to control seizures & convulsions. Although pregabalin is a derivative of GABA (γ-aminobutyric acid), it has no impact on GABA receptors. The two primary manifestations of neuropathic pain, allodynia, and hyperalgesia are lessened by pregabalin. Additionally, it acts as an analgesic, anxiolytic, and in the management of opioid withdrawal.[1]

Pregabalin is a saturated carboxylic acid that lacks π electron density. It has very low UV absorptivity and hence it is difficult to accurately estimate it by UV spectroscopy. Chemical derivatization of this molecule increases its UV absorptivity. Earlier workers have analyzed pregabalin by UV-Visible spectrometry after carrying out its derivatization with reagents like xanthone, ninhydrin, ascorbic acid etc.[2,3] For UV-spectroscopic studies of pregabalin, mostly methanol was used as solvent.[4,5] Apart from UV-spectrophotometry,[6-10] HPLC[11-17] and hyphenated techniques like LC-MS-MS,[18,19] SPE-LC-MS/MS using PFP HPLC column[20] were used to estimate pregabalin. All of the strategies that have been described so far involve intricacy in derivatization and evaluation. Hence the present work was aimed to prepare a simple, stable,

economic derivative of pregabalin using a very basic reagent, benzene sulfonyl chloride (Hinsberg reagent) in distilled water at basic pH and to estimate it easily, quickly, and accurately by UV-spectrophotometry.

MATERIALS AND METHODS

Materials:

Pregabalin API was a gift sample from Dr Reddy's laboratories. Pregabalin-75mg capsules were purchased from Shree Medicals to conduct derivatization and recovery studies. Methanol, ethanol, n-hexane, ethyl acetate, benzene sulfonyl chloride, chloroform, sodium carbonate and dimethyl sulfoxide (DMSO) were used for this study.

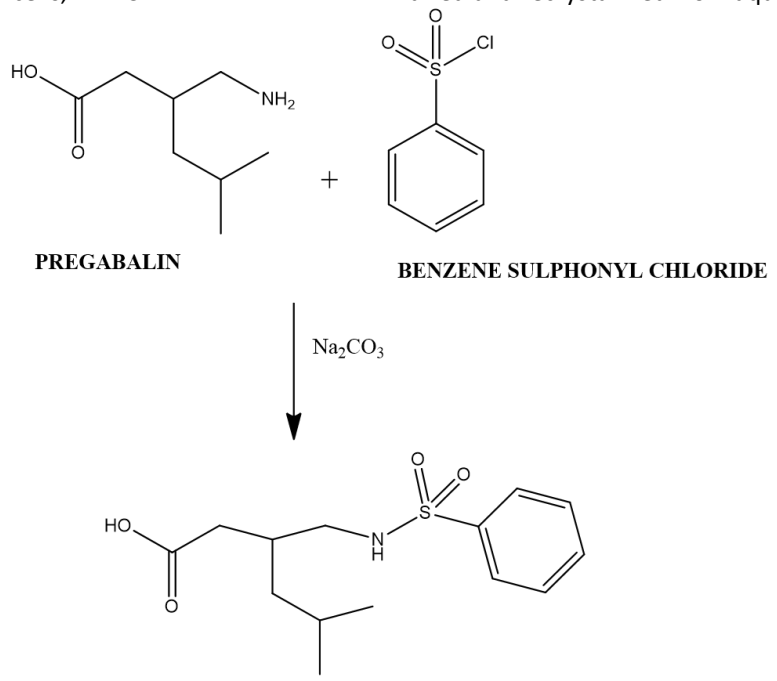
Instruments:

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.

Methods:

Chemical derivatization of pregabalin:

In a beaker, Pregabalin (2.02g, 12.7 mmol) was dissolved in 15-20ml of distilled water. Drop by drop, 3% sodium carbonate solution was added until the pregabalin completely dissolved and the pH was between 8-9. Then, benzene sulfonyl chloride (1.904 ml) in an equimolar ratio was added to the mixture **(Scheme 1)**. At room temperature, it was agitated using a magnetic stirrer. The reaction mixture's pH was maintained at 8 to 9 by periodically adding a few drops of Na_2CO_3 solution. Stirring continued till the completion of reaction. At this point, the solutions pH ceased to decrease and remained constant. Then, precipitate was filtered, washed with distilled water, dried and recrystallized from aqueous alcohol.[21]



PREGABALIN + **BENZENE SULPHONYL CHLORIDE**

PREGABILIN DERIVATIVE

5-methyl-3-(phenylsulfonamidomethyl)hexanoic acid

Scheme 1: Synthesis of Pregabalin Derivative.

Analytical method development:

Preparation of Stock Solution:

Accurately weighed 100 mg of Pregabalin derivative and dissolved in 10 ml of methanol in a 100 ml volumetric flask and diluted up to the mark with methanol to obtain the stock solution having a final concentration of 1000µg/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 diluting 1 ml of stock solution with 9 ml of methanol in a 10 ml volumetric flask to produce 100µg/ml.

Preparation of Secondary Standard Solution:

Pipetted out 0.1 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml from the primary standard solution and diluted with methanol to obtain 1µg/ml, 2µg/ml, 4µg/ml. 6µg/ml, 8µg/ml and 10µg/ml respectively.

Determination of λ_{max}:

A 10µg/ml solution of pregabalin derivative in methanol was scanned in the 190–300nm region to obtain the absorption maxima (λ_{max}).

Assay:

Twenty capsules of Pregabalin were taken and the contents were carefully collected. The capsules powder equivalent to 200 mg of Pregabalin 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 methanol and then further diluted to obtain concentration equivalent to 10 µg/ml. The absorbance of this sample solution was measured at 205 nm.[21]

Analytical method validation:

Linearity:

According to the defined range, linearity is the capacity to get findings that are directly proportional to the analyte concentration in the sample. The linearity was measured by preparing solutions of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml via serial dilution process. The linearity was determined at a wavelength of 205nm.

Precision:

When the method is used repeatedly to do multiple sampling of a homogenous sample, the precision of an analytical method is measured by the degree of agreement among individual test findings.

a) Intermediate precision:

Intermediate precision was done for three consecutive days. The triplicate absorbance values of solutions ranging from 20µg/ml to 60µg/ml were noted at a wavelength of 205nm and the %RSD was calculated.

b) Intraday precision:

Intraday precision was done by recording the absorbances of sample solutions ranging from 20 µg/ml to 60µg/ml at three different timings in same day. The %RSD was calculated for the noted absorbances.

Accuracy:

The degree to which test findings acquired using an analytical procedure are accurate in relation to the true value. The solutions ranging from 20µg/ml to 60µg/ml are spiked by adding a known concentration of analyte to the sample to 50%, 100% and 150%. The % recovery was calculated for the noted absorbances.

Robustness:

Robustness is the measurement of an analytical method's ability to produce results that are accurate even under slightly different circumstances. The absorbances of solutions ranging from 20µg/ml – 60µg/ml were recorded at various temperatures and wavelengths.

Ruggedness:

Reproducibility of test results under the range of conditions often anticipated between laboratories and analysts is measured by ruggedness. The absorbances for sample solutions 20 µg/ml – 60µg/ml are recorded when performed in different instruments i.e., SHIMADZU-1800 Double Beam UV-Visible Spectrophotometer with a pair of 10mm matched quartz cells and LAB INDIA UV-Visible Spectrophotometer and performed by different analysts.

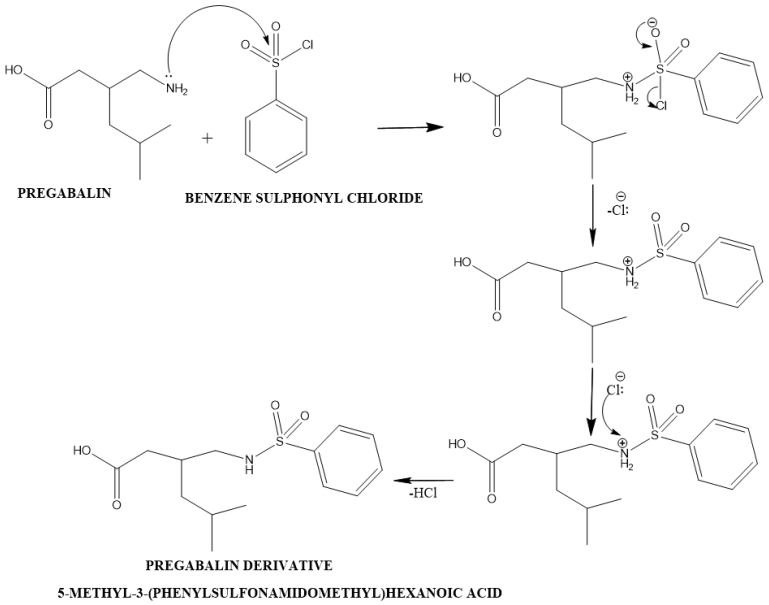


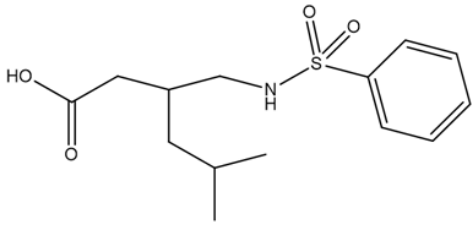
Fig. 1: Mechanism of reaction for the new Pregabalin derivative

RESULTS AND DISCUSSION

Chemical derivatization of Pregabalin derivative: Chemical derivatization of Pregabalin with benzene sulphonyl chloride (Hinsberg reagent) via addition-elimination reaction **(Fig.1)** produced the new, stable, colorless sulphonamide derivative of Pregabalin, 5-methyl-3-(phenylsulfonamidomethyl) hexanoic acid. It was purified and characterized by physical and spectral data.

Identification of derivative by TLC studies:
The progress of Pregabalin derivatization reaction was cautiously monitored by TLC in different solvent systems. The solvent system containing n-hexane: ethyl acetate (1:0.5) revealed that pregabalin derivative was formed as its R_f value differed from the Pregabalin and that the derivatives produced from API and Capsule powder were same as both of them displayed the same R_f value.

Physical data of Pregabalin derivative:



PREGABILIN DERIVATIVE

5-methyl-3-(phenylsulfonamidomethyl)hexanoic acid

Fig. 2: Chemical structure of Pregabalin Derivative

IUPAC Name: 5-methyl-3-(phenyl sulfonamido methyl) hexanoic acid **(Fig.2)**
Molecular Formula : $C_{14}H_{21}NO_4S$
Molecular Weight : 299.39
Description: White solid
Melting Point: 118-122ºC
Solubility: Methanol
R_f value: 0.6 (n-hexene: ethyl acetate-1:0.5)
% Yield: 88
Spectral (IR, 1H NMR) data of Gabapentin derivative:
IR spectrum (γ, cm^{-1}): 3749.71(SO_2NH- str), 3356.87 (-OH str), 2927.11 (C-H str), 1649.00 (-CO str), 992.27 (-SO_2 str).

^1H NMR spectrum (CD_3OD, ppm): 0.78-0.95 (m,6H,2x-CH_3), 3.15-3.52 (m,6H, 3x-CH_2+NH), 3.56-3.75 (m,1H,-CH, isopropyl), 3.77-3.87 (m,1H,-CH_2-CH-CH_2), 7.35-7.48 (t,3H,Ar-H,H_3, H_4,H_5), 7.82-7.88 (d,2H,Ar-H,H_2,H_6).

Analytical method development:
Determination of absorption maxima of derivative:
Standard Pregabalin derivative solution of concentration 10 µg/ml in methanol was scanned in UV Spectrophotometer from 190-300 nm range and the λ_{max} was found to be 205nm **(Fig. 3)**.

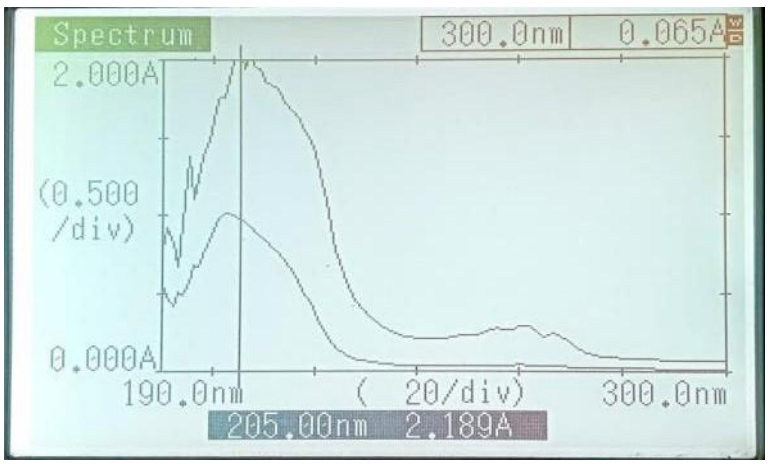


Fig. 3: Determination of λ_{max} of pregabalin derivative

Analytical method validation:

The parameters are validated according to the ICH guidelines and the results were found to be within the limits.

- **Linearity:**

The linearity was observed in the concentration range of 20µg/ml-100µg/ml **(Fig. 4, Table 1)** with the correlation coefficient r^2 = 0.999 **(Fig.5)**.

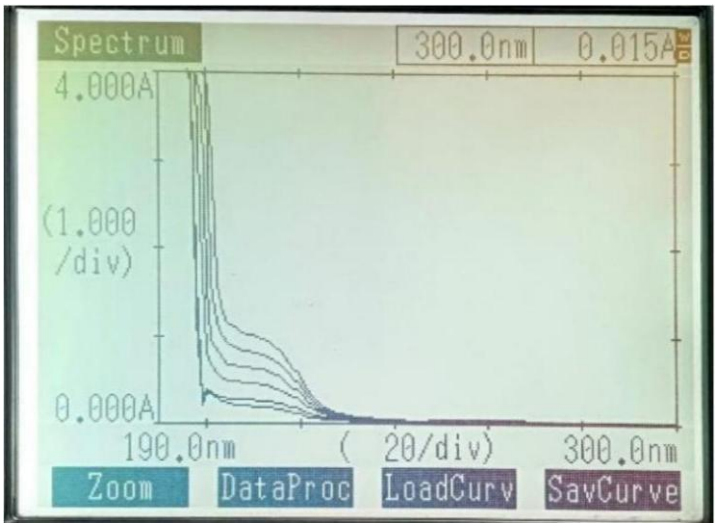


Fig. 4: Spectrum showing overlay of Pregabalin derivative at different concentrations.

Table 1: Linearity of Pregabalin derivative

Concentration (µg/ml)	Absorbance at 205nm
20	0.254
40	0.428
60	0.605
80	0.789
100	0.948

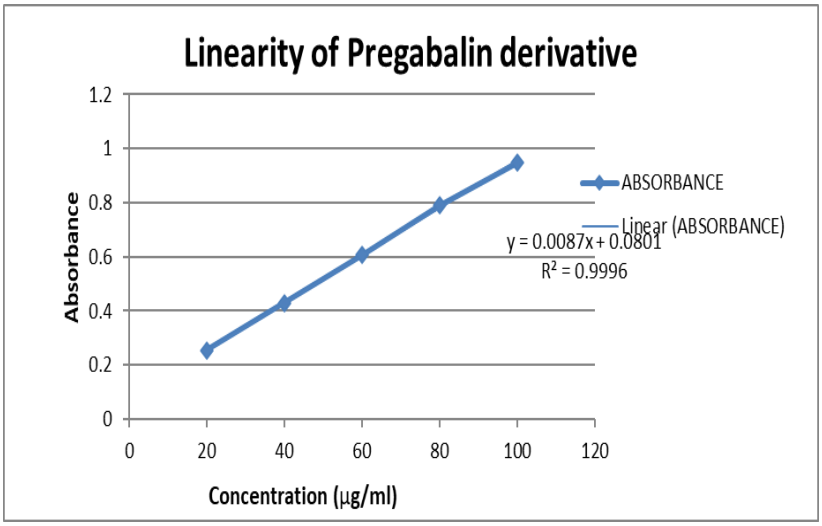


Fig. 5: Calibration curve of Pregabalin derivative at 205nm

- **Intermediate precision:**

The intermediate precision was done on three different days for the concentrations ranging 20µg/ml-60µg/ml and the pooled %RSD was found to be 1.27 **(Table 2)**.

Table 2: Intermediate precision of Pregabalin derivative

Day	Conc. (µg/ml)	Absorbance at 205nm						Mean	S. D	%RSD	Pooled %RSD
	20	0.155	0.152	0.153	0.154	0.155	0.160	0.154	0.002	1.29	
1	40	0.355	0.359	0.349	0.352	0.359	0.353	0.354	0.003	0.84	
	60	0.557	0.559	0.557	0.555	0.544	0.542	0.552	0.007	1.26	
	20	0.162	0.159	0.158	0.160	0.155	0.160	0.159	0.002	1.25	
2	40	0.359	0.360	0.359	0.356	0.362	0.350	0.357	0.004	1.12	1.27
	60	0.541	0.535	0.542	0.539	0.544	0.544	0.540	0.003	0.55	
	20	0.275	0.289	0.279	0.275	0.289	0.279	0.281	0.006	2.13	
3	40	0.354	0.359	0.348	0.352	0.355	0.349	0.352	0.004	1.13	
	60	0.520	0.529	0.518	0.216	0.500	0.520	0.571	0.009	1.92	

- **Intraday precision:**
  The intraday precision was done at three different times on the same day. The pooled %RSD of intraday precision was found to be 1.40 **(Table 3)**. This indicates that the method is precise.

Table 3: Intraday precision of Pregabalin derivative

Time	Conc(µg/ml)	Absorbance at 205nm						Mean	S.D	%RSD	Pooled %RSD
	20	0.120	0.122	0.122	0.125	0.123	0.120	0.122	0.0018	1.47	
9:30am	40	0.416	0.418	0.412	0.410	0.392	0.417	0.410	0.0067	1.63	
	60	0.541	0.535	0.542	0.520	0.539	0.535	0.535	0.008	1.49	
	20	0.225	0.224	0.219	0.221	0.227	0.220	0.222	0.003	1.35	
2:00pm	40	0.336	0.338	0.332	0.331	0.341	0.336	0.335	0.0037	1.10	1.40
	60	0.554	0.564	0.561	0.566	0.549	0.561	0.559	0.006	1.07	
	20	0.179	0.174	0.169	0.173	0.177	0.179	0.175	0.0036	2.0	
5:00pm	40	0.402	0.401	0.411	0.409	0.401	0.401	0.404	0.004	0.99	
	60	0.521	0.534	0.511	0.517	0.521	0.531	0.522	0.008	1.53	

Table 4: Accuracy results of Pregabalin derivative

Spike level (%)	Concentration (µg/ml)	Standard absorbance	Test absorbance	Spiked absorbance	%Recovery	Mean	SD	%RSD
50	20	0.228	0.254	0.479	98.6	98.9	1.03	1.04
		0.225	0.249	0.476	100.8			
		0.225	0.261	0.480	97.3			
100	40	0.302	0.401	0.693	96.6	95.7	0.5	0.52
		0.309	0.402	0.698	95.7			
		0.300	0.405	0.690	95			
150	60	0.345	0.424	0.765	98.8	99.4	1.93	1.94
		0.348	0.420	0.762	98.2			
		0.338	0.422	0.765	101.4			

- **Accuracy:**
  The accuracy results of Pregabalin derivative are presented in **Table 4**. The % recovery was calculated using the following equation:

  % Recovery = (A_{Spiked}-A_{Test}/$A_{Standard}$) x 100

  The mean % recovery was found to be in the range of 95.7 to 99.4. The % RSD values at all the three spike levels were found to be less than 2. Hence the method is accurate.

- **Robustness:**
  The robustness was calculated under two different conditions viz., change in wavelength and change in temperature and the results are shown in **Tables 5 and 6**. At three different concentrations, not much variation was found in the absorbance of the pregabalin derivative upon changing the wavelength and temperatures. The % RSD values in both the cases were found to be

less than 2 indicating the robustness of the method.

Table 5: Results for robustness after change in wavelength

Change in wavelength. (±2 nm)	Concentration (µg/ml)	Wavelength						
		203nm	204nm	205nm	206nm	207nm	Mean±SD	%RSD
	20	0.201	0.194	0.187	0.185	0.186	0.190±0.0028	1.47
	40	0.351	0.346	0.346	0.345	0.346	0.346±0.002	0.57
	60	0.466	0.466	0.463	0.464	0.465	0.464±0.002	0.43

Table 6: Results of Robustness at different temperatures

Change in temperature	Concentration(µg/ml)	Absorbance at 205nm			Mean±SD	%RSD
	At Room temperature					
	20	0.187	0.241	0.131	0.186±0.002	1.07
	40	0.346	0.448	0.359	0.384±0.004	1.04
	60	0.463	0.522	0.495	0.493±0.009	1.82
	At Re-frigerated temperature					
	20	0.246	0.233	0.202	0.227±0.004	1.76
	40	0.410	0.354	0.394	0.386±0.003	0.77
	60	0.510	0.486	0.488	0.494±0.008	1.61
	At sunlight temperature					
	20	0.289	0.119	0.216	0.208±0.004	1.92
	40	0.414	0.364	0.442	0.406±0.005	1.23
	60	0.496	0.524	0.518	0.512±0.007	1.36

- **Ruggedness:**

Ruggedness, also called as repeatability was done by different analysts and by using different instruments. The results obtained are tabulated in **table 7**. The method was found to be rugged as the results were repeatable from analyst to analyst and from instrument to instrument with %RSD values less than 2.

Table 7: Ruggedness for analyst to analyst and instrument to instrument

Variations	Concentration (µg/ml)		
	20	**40**	**60**
	0.189	0.348	0.572
Actual	0.190	0.355	0.587
	0.185	0.359	0.569
Mean±SD	**0.188±0.002**	**0.354±0.005**	**0.576±0.009**
%RSD	**1.06**	**1.41**	**1.56**
	0.169	0.431	0.526
Analyst to Analyst	0.176	0.439	0.521
	0.182	0.428	0.533
Mean±SD	**0.175±0.003**	**0.432±0.005**	**0.526±0.006**
%RSD	**1.71**	**1.15**	**1.14**
Instrument to Instrument			
	0.084	0.242	0.368
Lab India	0.085	0.251	0.368
	0.083	0.244	0.372
Mean±SD	**0.0843±0.001**	**0.245±0.004**	**0.369±0.007**
%RSD	**1.19**	**1.63**	**1.89**
	0.195	0.362	0.481
Shimadzu	0.191	0.358	0.487
	0.189	0.349	0.496
Mean±SD	**0.191±0.003**	**0.356±0.006**	**0.488±0.007**
%RSD	**1.57**	**1.68**	**1.43**

Assay:

The method was used to estimate Pregabalin in pharmaceutical formulation (capsule) and the results obtained are presented in **Table 8**, which reveal that pregabalin can be recovered well by this method.

Table 8: Assay results of Pregabalin in Capsules

Label Value (mg)	Amount Found (mg)	n	Recovery (%)	%RSD
75	73.5	10	98	1.58

CONCLUSION

A new, simple UV-spectrophotometric method was developed for the determination of Pregabalin in Capsules. It was validated for linearity, accuracy, precision, robustness, and ruggedness. According to ICH criteria, all parameter values were found to be within the acceptable ranges. It can be concluded that this new spectrophotometric method was found to be accurate, precise, robust, rugged, reliable and cost-effective. Hence, it can be employed for the routine quantitative analysis of Pregabalin in bulk and capsules using a UV spectrophotometer.

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