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A validated stability indicating LC assay method for pregabalin in bulk drugs and pharmaceutical dosage forms

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ABSTRACT

A novel, sensitive gradient RP-LC assay method has been developed for the quantitative determination of Pregabalin in bulk drugs and Pharmaceutical dosage forms. Pregabalin is an anti-epileptic drug. Efficient chromatographic separation was achieved using C18 stationary phase with simple mobile phase combination delivered in a gradient mode and quantitation was carried out using ultraviolet detection at 210 nm. The mobile phase consists of buffer and methanol delivered at a flow rate of 1.0 mL min⁻¹. Buffer consists of 5 mM di-potassium hydrogen phosphate anhydrous, pH adjusted to 7.0 by using ortho phosphoric acid. In the developed HPLC method the resolution (R_s) between Pregabalin and its all degradation products was found to be greater than 2.0. Regression analysis shows an r value (correlation coefficient) of greater than 0.99 for Pregabalin. The inter and intraday precision values for Pregabalin was found to be within 2.0% RSD at its specification level. The method has shown good and consistent recoveries for Pregabalin (99.2-101.2%) in bulk drugs and (98.0-100.2 %) in dosage forms. The test solution was found to be stable in diluent for 48 h. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in acid hydrolysis, base hydrolysis and oxidative stress conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Column liquid chromatography;
Pregabalin;
Forced degradation;
Pharmaceutical dosage forms;
Validation;
Stability indicating.

INTRODUCTION

Pregabalin (S)-3-(amino methyl)-5-methylhexanoic acid (Figure 1) is an anti-epileptic drug. The generic name of Pregabalin is Lyrica, also called an anticonvulsant. It works by slowing down impulses in the brain that cause seizures. Pregabalin also affects chemicals in the brain that send pain signals across the nervous system. Pregabalin is used to control seizures and to treat

fibromyalgia. It is also used to treat pain caused by nerve damage in people with diabetes (diabetic neuropathy) or herpes zoster (post-herpetic neuralgia).

Few analytical methods were reported in literature for the quantification of Pregabalin in human plasma^[1,8]. As far as we are aware there is no stability-indicating LC method for quantitative estimation of Pregabalin. In this paper we described validation of an assay method for accurate quantification of Pregabalin in bulk drug

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samples along with method validation as per ICH norms. Intensive stress studies were carried out on Pregabalin, accordingly a stability-indicating method was developed, which could separate various degradation products.

The present drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH)^[9] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to separation of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Accordingly, the aim of present study was to establish inherent stability of Pregabalin through stress studies under a variety of ICH recommended test conditions and to develop a stability-indicating assay method^[10-13].

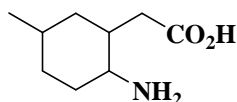
EXPERIMENTAL

Chemicals

Samples of Pregabalin were received from United States Pharmacopeia-India, Hyderabad, India (Figure 1). Commercially available Lyrica capsules were purchased from Pfizer, India. HPLC grade methanol was purchased from Merck, Darmstadt, Germany. Analytical reagent grade di-potassium hydrogen phosphate anhydrous and ortho phosphoric acid was purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples used in this study were of greater than 99.5% purity.

Equipment

The LC system used was Waters 2695 binary pump plus auto sampler and a 2996 photo diode array detector. The output signal was monitored and processed using Empower software on Pentium computer (Digital equipment Co.); water baths equipped with MV con-



(S)-3-(aminomethyl)-5-methylhexanoic acid

Figure 1 : Structure and label of Pregabalin

troller (Julabo, Seelabach, Germany) were used for hydrolytic studies. Stability studies were carried out in humidity chamber (Thermo lab humidity chamber, India) and photo stability studies were carried out in a photo stability chamber (Sanyo photo stability chamber, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

Chromatographic conditions

The chromatographic column used was Inertsil ODS, (250 × 4.6) mm with 5 μ m particles. The mobile phase A consists of 5 mM dipotassium hydrogen phosphate, pH adjusted to 7.0 using ortho phosphoric acid and mobile phase B consists of methanol. The flow rate of the mobile phase was 1.0 mL min⁻¹. The HPLC gradient program was set as: time (min)/% solution B: 0/0, 5/0, 20/50, 25/50, 27/0 and 40/0. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 210 nm. The injection volume was 10 μ L. Water was used as diluent.

Preparation of solutions

Preparation of standard solutions

A stock solution of Pregabalin (100.0mg mL⁻¹) was prepared by dissolving appropriate amount in the diluent. Working solutions were prepared from above stock solution for assay determination.

Preparation of sample solution

Twenty capsules were weighed and the content transferred into a clean and dry mortar, grinded well (each capsule contains 200 mg of pregabalin and lactose monohydrate, cornstarch, and talc as inactive ingredients). Then equivalent to 1000 mg of active pharmaceutical ingredient was transferred to 100 mL volumetric flask, 75 mL of diluent added and kept on rotary shaker for 10 min to disperse the material completely and sonicated for 10 min and diluted to 100 mL (10.0 mg mL⁻¹). The resulting solution was centrifuged at 3,000 rpm for 5 min. Supernatant solution was filtered using 0.45 μ nylon 66-membrane filter and is used for the analysis.

Specificity/Application of stress (Forced degradation study)

Specificity is the ability of the method to measure

the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the developed LC method for Pregabalin was determined in the presence of its degradation products. Forced degradation studies were also performed on Pregabalin to provide an indication of the stability indicating property and specificity of the proposed method. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60°C), acid hydrolysis (1N HCl), base hydrolysis (1N NaOH), water hydrolysis and oxidation (6% H₂O₂). For heat and light studies, study period was 10 days where as for acid, base, water hydrolysis and oxidation it was 48 h. Peak purity of stressed samples of Pregabalin was checked by using 2996 Photo diode array detector of Waters.

Analytical method validation

Assay studies were carried out for stress samples against qualified reference standard and the mass balance (% assay + % of impurities + % of degradation products) was calculated.

Precision

Precision was determined through repeatability (intra-day) and intermediate (inter-day) precision. Assay method precision was evaluated by carrying out six independent assays of test sample of Pregabalin against qualified reference standard. The %RSD of six assay values obtained was calculated. The intermediate precision of the assay method was evaluated by different analyst and by using different instrument from the same laboratory.

Linearity and range

To establish linearity of the assay method, calibration solutions were prepared from stock solution at five concentration levels from 50 to 150 % of assay analyte concentration (5000, 8000, 9000, 10000, 11000, 12000 and 15000 µg mL⁻¹). Average peak area at each concentration level was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calcu-

late the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses. The % y-intercept for assay method was calculated. Analytical range of the method was established from the analysis of sensitivity curves. Upper and lower levels of range were also established.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 5000, 10000 and 15000 µg mL⁻¹ in bulk drugs and pharmaceutical dosage forms. For each concentration, three sets were prepared and injected in duplicate. The percentages of recoveries were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution between Pregabalin and its degradation products were evaluated. The flow rate of the mobile phase was 1.0 mL min⁻¹. To study the effect of flow rate on the resolution, 0.2 units changed i.e 0.8 and 1.2 mL min⁻¹. The effect of column temperature on resolution was studied at 20°C and 30°C instead of 25°C. The effect of pH on resolution of impurities was studied by varying ± 0.1 pH units (i.e. buffer pH altered from 7.0 to 6.9 and 7.1). In the all above varied conditions, the components of the mobile phase were held constant.

Solution stability

The solution stability of Pregabalin in the assay method was carried out by leaving the test solutions of sample in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed 6 h interval up to the study period against freshly prepared standard solution. The %RSD of assay of Pregabalin was calculated for the study period during the solution stability experiments.

RESULTS AND DISCUSSION

Method development and optimization

The basic chromatographic conditions were designed to be simple and easy to use and reproduce and

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TABLE 1 : System suitability report

Compound	USP tailing factor (T)	No of theoretical plates USP tangent method(N)
Pregabalin	0.92	5185

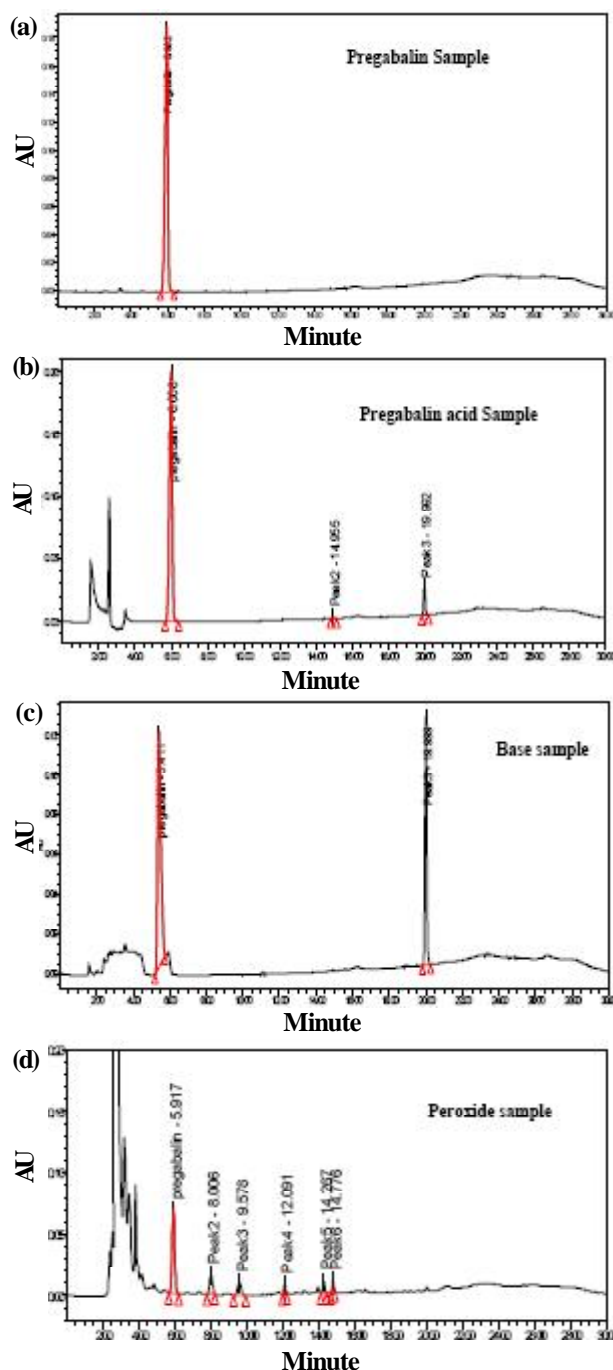


Figure 2 : Typical chromatograms of pregabalin sample and stressed samples

were selected after testing the different conditions that affect LC analysis, for example column, aqueous and organic components of the mobile phase, proportion of

mobile phase components, detection wavelength, diluent, concentration of analyte, etc. For mobile phase selection, preliminary trials using mobile phases of different composition containing Potassium dihydrogen phosphate monohydrate buffer (10 mM) with pH 4.5 and methanol was chosen for initial trial on an Inertsil C18, 25 cm length, 4.6 mm ID and 5 μ m particle size, resulted in poor peak shape. When potassium phosphate buffer was replaced by di-potassium hydrogen phosphate adjusted the pH to 7.0 by addition of ortho phosphoric acid better peak shape was obtained. Sample concentration of 1 mg mL⁻¹ was injected. As pregabalin is a amino acid it is more polar due to the presence of amino group and carboxylic acid group pregabalin was eluted early (~2 min). To increase the retention of pregabalin organic phase in the mobile phase was removed (methanol) and 100 % buffer was injected as mobile phase. The retention of pregabalin was improved to 6 minutes. But the response of pregabalin peak is very less. Hence concentration was increased to 10 mg mL⁻¹ and injected the solution results were satisfactory (TABLE 1).

The proportion of the mobile phase components was optimized to reduce retention times and enable good resolution of Pregabalin from the closely eluted degradation products obtained by hydrolysis and oxidative degradations. During the Specificity studies, degradant peak was observed at ~120 min. To improve the retention of Pregabalin degradant peak gradient programme was selected. The mobile phase A consists 5 mM of di-potassium hydrogen phosphate and methanol as mobile phase B. Different gradient programmes tried to optimize the retention time of degradant peaks and resolution (R_s) between the degradants. Satisfactory results were obtained with the gradient programme, time (min) / % solution B: 0/0, 5/0, 20/50, 25/50, 27/0 and 40/0.

The effect of the pH was studied on the retention of pregabalin. When pH was acidic (pH 2) due to the ionization of amino group the polarity of the compound increased and the retention of pregabalin was decreased (~2 min) same result was obtained when the pH of the mobile phase was 8.5 due to the ionization of carboxylic acid group, hence neutral pH was selected (7.0) for pregabalin analysis.

Results of forced degradation studies

Degradation in acidic solution

When the drug was exposed to 1 N HCl (24 h at room temperature), no degradation was observed. When more stressed conditions were applied (1 N HCl at 60°C temperature for 5h.), the drug gradually undergone degradation with time and prominent degradation was observed (~5 %) (figure 2(b)).

Degradation in basic solution

When the drug was exposed to 1 N NaOH (24 h at room temperature), no degradation was observed. When more stressed conditions were applied (1 N NaOH at 60°C temperature for 5 h.), the drug gradually undergone degradation with time and prominent degradation was observed (~30 %) (Figure 2(c)).

Oxidative conditions

The drug was exposed to 6% hydrogen peroxide. Pregabalin has shown significant sensitivity towards the treatment of hydrogen peroxide. The drug gradually undergone degradation with time in 6% hydrogen peroxide (reflux for 5 h) and prominent degradation was observed (~15 %) (Figuer 2(d)).

Degradation in neutral (Water) solution

No degradation was observed after 2 h at 60°C temperature.

Photolytic conditions

The drug was stable to the effect of photolysis. When the drug powder was exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200-watt hours/square meter (w/mhr) (in photo stability chamber), no degradation was observed.

Thermal degradation

The drug was stable to the effect of temperature. When the drug powder exposed to dry heat at 60°C for 10 days, no degradation was observed.

From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the Pregabalin peak was homogeneous and pure in all the analyzed stress samples. No degradants were observed after 30 min in the extended run time of 60 min of all the Pregabalin samples. The mass balance of stressed

TABLE 2: Summary of forced degradation results

Stress condition	Time	Mass balance	
		% Assay of active substance	(%Assay + % impurities + % Degradation products)
Acid hydrolysis (1N HCl, reflux at 60°C)	5 h	94.5	98.7
Base hydrolysis (1N NaOH reflux at 60°C)	5 h	69.5	99.5
Oxidation (6% H ₂ O ₂ reflux at 60°C)	5 h	74.2	99.2
Water hydrolysis (reflux at 60°C)	2 h	100	99.7
Thermal (60°C)	10 days	100	99.7
Light (photolytic degradation)	10 days	100	99.8

TABLE 3: Results of intermediate precision

S. no.	Parameter	Variation	%RSD for assay
1	Different system	(a) Waters 2695 Alliance system	0.8
		(b) Agilent 1100 series VWD system	0.6
2	Different Column	(a) B.No: 00102	0.8
		(b) B.No:00104	0.5
3	Different Analyst	(a) Analyst-1	0.5
		(b) Analyst-2	0.4

samples was close to 99.5% (TABLE 2). The assay of Pregabalin is unaffected in the presence of its degradation products confirm the stability indicating power of the developed method.

Method validation

Precision

The %RSD of assay of Pregabalin during assay method precision study was within 1 % confirming the good precision of the developed analytical method.

The %RSD of assay results obtained in intermediate precision study was within 1% confirming the ruggedness of the method (TABLE 3).

Linearity

Calibration curve obtained by the least square regression analysis between average peak area and concentration showed linear relationship with a regression coefficient of 0.999 over the calibration ranges tested, i.e. 5000- 15000µg mL⁻¹ for assay calculation. The best-fit linear equation obtained was $y = 8.3914x + 37.8876$. At all concentration levels, standard deviation of peak area was significantly low and RSD was

TABLE 4: Linearity results for assay method validation

Level	Con($\mu\text{g/mL}$)	Mean area	Response calculated through trend line equation	Residual	Residual square	Sensitivity
Level-1	5000	454.59	457.4561	-2.8661	8.2146	9.092
Level-2	8000	724.4	709.1972	15.2028	231.1245	9.055
Level-3	9000	783.16	793.1109	-9.9509	99.0208	8.702
Level-4	10000	876.81	877.0246	-0.2146	0.0461	8.768
Level-5	11000	962.46	960.9383	1.5217	2.3155	8.750
Level-6	12000	1037.16	1044.8520	-7.6920	59.1672	8.643
Level-7	15000	1300.59	1296.5931	3.9969	15.9750	8.671
Regression coefficient	0.9995		Residual sum of square		415.8638	
Slope	8.39137		SE of slope	0.117737		
Intercept	37.88762		SE of Intercept	12.26796		
% y-Intercept	4.32		$y = 8.39137x + 37.88762$			

TABLE 5: Results of accuracy study for drug substance and drug product

Added (μg)(n= 3)	Recovered (μg)	% Recovery	% R.S.D
Drug substance			
50	49.6	99.2	0.10
100	99.3	99.3	0.23
150	151.8	101.2	0.70
Drug product			
50	49.0	98.0	0.50
100	100.2	100.2	0.40
150	148.95	99.3	0.60

n =3, Number of determinations

TABLE 6: Results of robustness study

S. no.	Parameter	Variation	Resolution (R_s) between Pregabalin and degradants
1	Temperature ($\pm 5^\circ\text{C}$ of set temperature)	(a) At 20°C	3.2
		(b) At 30°C	3.1
2	Flow rate ($\pm 20\%$ of the set flow)	(a) At 0.8mL min^{-1}	3.3
		(b) At 1.2mL min^{-1}	3.1
3	pH (± 0.1 unit of set pH)	(a) At 6.9	3.2
		(b) At 7.1	3.3

below 1.0%. Analysis of residuals indicated that residuals were scattered within $\pm 2\%$ with respect to 100% concentration response. The % Y intercept is also within the limit ($\pm 2\%$ with respect to 100% area response). The points in the sensitivity graph were scattered within $\pm 2\%$ with respect to 100% concentration response. The results were given in TABLE 4.

Accuracy

The recovery of Pregabalin in bulk drugs ranged from 99.2-101.2% and in pharmaceutical dosage forms ranged from 98.0-100.2% (TABLE 5).

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, col-

TABLE 7: Batch analysis

Batch no: Drug substance	Assay by HPLC (%)
0803	98.9
0805	99.5
0808	99.5
Batch no: Drug product	Assay by HPLC (%)
PB80201	101.4
PB80201	99.5
PB80201	99.2

umn temperature and pH) revealed that the resolution between closely eluting degradation peaks was greater than 3.0, illustrating the robustness of the method (TABLE 6).

Solution stability

The %RSD of assay of Pregabalin during solution stability and mobile phase stability experiments was within 1.0. The solution stability experiments data confirms that sample solution used during assay determination was stable up to the study period of 48 h.

Assay analysis

Analysis was performed for different batches of Pregabalin in both bulk drug samples (n=3) and dosage forms (n=3). Results were given in TABLE 7.

CONCLUSION

The gradient RP-LC method developed for quantitative determination of Pregabalin in bulk drug is precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Pregabalin samples.

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