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Development and Validation of Stability Indicating High Performance Liquid Chromatography Method for Determination of Baclofen

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ABSTRACT

A new, simple, specific, accurate and precise RP-HPLC method was developed for determination of Baclofen. In the present study, stress testing of Baclofen was carried out according to ICH guidelines Q1A (R2). Baclofen was subjected to stress conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Extensive degradation was found to occur in acidic, condition. Mild degradation was observed in basic and at thermal conditions. Successful separation of drug from degradation products formed under stress conditions was achieved on a Hypersil BDS C18 column (250 mm × 4.6 mm, 5.0 µ particle size) using acetonitrile: acetate buffer (pH 3.7 ± 0.05) (50:50 v/v), at a flow rate of 1.0 mL/min and column was maintained at 40˚C. Quantification and linearity was achieved at 272 nm over the concentration range of 5 - 100 µg/mL for Baclofen. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness.

Keywords: Stability-indicating, HPLC, Baclofen, Validation, Stress Testing.

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INTRODUCTION

Baclofen, (β-(Aminomethyl)-4-chlorobenzenepropanoic acid), (Figure 1), is a central nervous system depressant used as a skeletal muscle relaxant. It is primarily used to treat spasticity.[1] Baclofen is a gamma-amino-butyric acid (GABA) derivative used as a skeletal muscle relaxant. Baclofen stimulates GABA-B receptors leading to decreased frequency and amplitude of muscle spasms.[2] It is especially useful in treating muscle spasticity associated with spinal cord injury. It appears to act primarily at the spinal cord level by inhibiting spinal polysynaptic afferent pathways and, to a lesser extent, monosynaptic afferent pathways.[3]

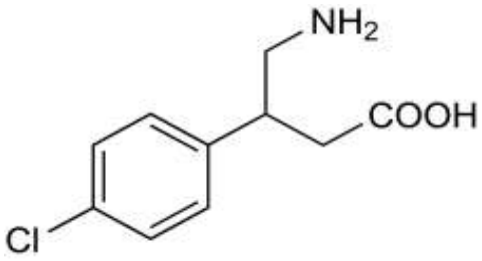

Figure 1: Structure of Baclofen

Literature survey reveals that few spectrophotometric method[4], HPLC methods[5-8] and colorimetric method[9] has been reported for the estimation of Baclofen. The aim of the present study is to develop a simple, precise and accurate stability indicating reversed-phase HPLC method for the estimation of Baclofen in pharmaceutical dosage form as per ICH guidelines.[10-11]

MATERIALS AND METHOD

Instrumentation:

Perkin Elmer (USA) HPLC system (series 200) equipped with Perkin Elmer series 200 pump system having back pressure 5000 psi, manual injector of 20 µL loop, UV-Visible detector and Hypersil BDS C18 column (250 mm x 4.6 mm i.d., 5 µm); Sartorious, analytical balance; An ultra-sonicator; A Shimadzu model 1800 double beam UV/Vis. spectrophotometer with a pair of 10 mm matched quartz cells.

Reagents and Materials:

Baclofen was kindly gifted by Intas Pharmaceuticals Ltd. Ahmedabad, India. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and HPLC grade water was used for mobile phase preparation. Nylon membrane filter 0.45µ syringe filter. Hydrogen peroxide, sodium hydroxide and hydrochloric acid AR grade.

Chromatographic Conditions:

The chromatographic separation was achieved on Hypersil BDS C_{18} column, using mobile phase

comprised of acetonitrile: acetate buffer (pH 3.7 ± 0.05) (50:50 v/v), at a flow rate of 1.0 mL/min. and column was maintained at 40˚C. The mobile phase was filtered through nylon 0.45 µm membrane filter and was degassed before use. The determination was carried out at 272 nm wavelength by UV-Visible Detector. The injection volume was 20 µL and total run time was 10 min. The analysis was performed at 25 ± 2 ˚C temperatures.

Preparation of the Mobile Phase

The mobile phase was prepared by mixing 50 mL acetonitrile and 50 mL acetate buffer (pH 3.7 ± 0.05) previously filtered through 0.45 µm nylon membrane filter. The mobile phase was degassed for 15 minutes by sonicating the solution before use.

Preparation of Diluent

Acetonitrile: Water (50:50% v/v) is used as diluent.

Preparation of standard solution

Accurately weighed Baclofen (25 mg) was transferred to a 25 mL volumetric flask, dissolved in and diluted to the mark with diluent to obtain a standard stock solution (1 mg/mL).

Preparation of working standard solution (10 µg/mL)

Standard solution (0.1 mL) was transferred in a 10 mL volumetric flask and diluted up to the mark with mobile phase.

Preparation of hydrochloric acid (0.1N)

Accurately transferred 0.85 mL concentrated hydrochloric acid (36%) to 100 mL volumetric flask and diluted up to the mark with distilled water.

Preparation of sodium hydroxide (0.1N)

Accurately weighed and transferred 0.4 gm sodium hydroxide to 100 mL volumetric flask, dissolved in 60 mL distilled water and diluted up to the mark with distilled water.

Analysis of Tablet Dosage Form

Twenty tablets (Liofen tab) were weighed and average weight was calculated. The tablets were finely powdered; a quantity of powder equivalent to 25 mg Baclofen was weighed accurately and transferred to a 25 mL volumetric flask containing 15 mL diluent, and sonicated for 15 minutes. Allowed to stand at room temperature for 5 min and the volume was made up to the mark with diluent to obtain the sample stock solution (1 mg/mL). The solution was filtered through 0.45µ membrane filter. Aliquot (1 mL) was taken and transferred to 10 mL volumetric flask and volume was made up to the mark with diluent to give a solution containing 100 µg/ml Baclofen. The solution (2 mL) was transferred to 10 mL volumetric flask and diluted up to the mark with mobile phase to give a solution containing 20 µg/mL Baclofen. An aliquot (20 µL) was injected and the

chromatogram was recorded. The peak area was noted and the amount of Baclofen was calculated from the regression equation.

Forced Degradation Study

Baclofen was subjected to various forced degradation conditions to effect partial degradation of the drug preferably in 20-80% range. The study provides information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities.

Effect of Acid, Alkaline and Neutral Hydrolysis

Accurately weighed Baclofen 25 mg was transferred to three different 100 mL volumetric flasks and dissolved in diluent (20 mL). Hydrochloric acid (0.1N, 10 mL), sodium hydroxide (0.1N, 10 mL) and water (10 mL) were added to separate flasks containing drug samples and mixed properly for acidic, alkaline and neutral degradation respectively and stored at room temperature for 72 h.

The samples were neutralized with base or acid as appropriate and diluted up to the marks with diluent to obtain stock solutions (250µg/mL). Dilutions were made with mobile phase to obtain the degraded Baclofen solutions (25µg/mL).

Effect of Oxidation

Accurately weighed Baclofen 25 mg was transferred to a 100 mL volumetric flask and dissolved in diluent 20 mL. Hydrogen peroxide solution (3%) 10 mL was added, mixed properly, and stored at room temperature for 72 h. The sample was diluted up to the mark with acetonitrile to obtain stock solution (250µg/mL). Dilution was made with mobile phase to obtain the degraded Baclofen solution (25µg/mL).

Effect of Heat

Baclofen 25 mg was distributed over a glass plate and kept in an oven at 60°C for 72 h, then Baclofen was transferred in a 100 mL volumetric flask and dilutions were made with mobile phase to obtain the degraded Baclofen solution (25 µg/mL).

Effect of Light

Baclofen solution (prepared by dissolving 25 mg Baclofen in 20 mL acetonitrile in 100 mL volumetric flask) was exposed to sun light for 48 h, while Baclofen 25 mg in powder state was exposed to UV light for 48 h. After exposure, dilutions were made to obtain the degraded Baclofen solutions (25µg/mL). Aliquots (20 µL) of the stressed samples were injected into the HPLC system as described under chromatographic conditions and the chromatograms were recorded.

METHOD VALIDATION

As per the ICH guideline Q2 (R1), the method validation parameters like specificity, linearity,

accuracy, precision, limit of detection, limit of quantitation and robustness were studied.

Solution Stability

Sample solutions were kept at 25 ± 2°C (24 hours) and 2 - 8^0C (3 days), respectively. Assay percentage of initial time period was compared with these two time periods. The change in the assay percentage was calculated. The difference between assay results should not be more than 2 % for formulation, and 0.5% for API.

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in presence of component that may be expected to be present in the sample matrix. Chromatograms of Baclofen solutions and degraded samples were studied in order to provide an indication of the stability indicating properties and specificity of the method. The stress conditions employed were acidic, alkaline, neutral, oxidative, thermal and photolytic; the degraded samples were analyzed against freshly prepared sample solutions

Linearity (Calibration Curve)

Standard solutions (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mL equivalent to 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/mL of Baclofen) were transferred in a series of 10 mL volumetric flasks and diluted to the mark with mobile phase. An aliquot (20 µL) of each solution was injected under the operating chromatographic conditions as described earlier. Calibration curve was constructed by plotting peak areas versus concentrations, and the regression equation was calculated. Each response was average of three determinations.

Accuracy (% Recovery)

Accuracy of the method was determined by calculating percentage recovery of baclofen by the standard addition method. Known amount of standard solutions of Baclofen (0, 5, 10 and 15 µg/mL) were added to a pre-analyzed sample solution of Baclofen (10 µg/mL). Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the calibration curve.

Precision

Repeatability was checked by repeatedly (n = 6) injecting Baclofen solution (10 µg/mL) and recording the chromatogram. Intra-day and inter-day precisions of the developed method was determined by measuring the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of Baclofen (10.0, 20.0 and 30.0 µg/mL). The results were reported in terms of relative standard deviation.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of response (σ) and slope (S) of the calibration curve.

LOD = 3.3 x σ/S

LOQ =10 x σ/S

Robustness

Robustness was studied by analyzing the samples of Baclofen by deliberate variation in the method parameters. The change in the response of Baclofen was noted. Robustness of the method was studied by changing the extraction time of Baclofen from tablet dosage form by ± 2 min, composition of mobile phase by ± 2% of organic solvent, wavelength by ± 2 nm, flow rate by ± 0.2 mL/min and column oven temperature by ± 2ºC. The changes in the response of Baclofen were noted and compared with the original one.

System-Suitability Test

System suitability tests were used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were retention time, tailing factor and theoretical plates of chromatographic peak as RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

Selection of Column and Mobile Phase

As per the published literature and knowledge of the molecule, reverse phase liquid chromatography (RP-HPLC) is suitable for analysis of Baclofen. In case of RP-HPLC various columns are available, but as the main aim of the method was to resolve the compound from degraded products, C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was preferred over the other columns. Resolution is the most important criteria for the method, it is imperative to achieve good resolution among the compound and degraded products. As per the value of pKa and solubility of compound various composition of mobile phase were tried.

The chromatographic conditions were optimized with a view to develop a stability indicating assay method, which can separate the drug from its degradation products with good resolution. Mobile phase consisting of acetonitrile: acetate buffer (pH 3.7 ± 0.05) (50:50 % v/v) at a flow rate of 1.0 mL/min, was found to be satisfactory to obtain well-resolved peaks with better reproducibility and repeatability for Baclofen. (Figure 2)

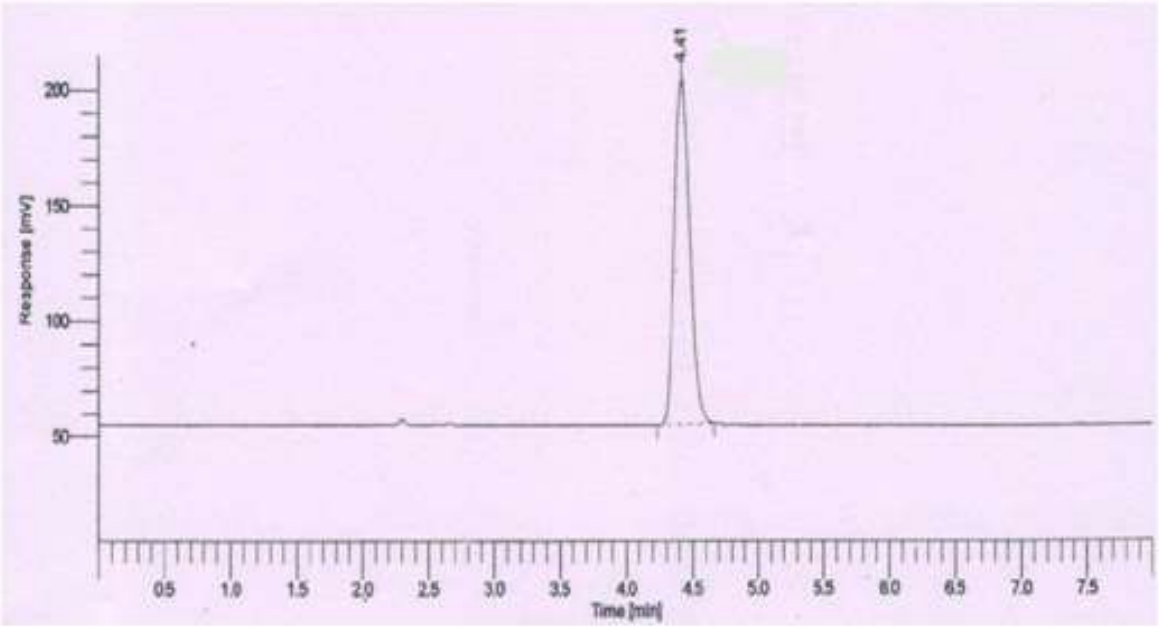

Figure 2: Chromatogram of Baclofen with retention time of 4.41 min

Method Validation:

Solution Stability

The change in assay results after storage at 25°C (24 hours) and 2-8°C (3 days) was evaluated. It was found that the difference in assay results was not more than 2 % for formulation, and 0.5% for API, indicating stability of Baclofen solution.

Specificity

The developed analytical method was found to be specific as there was no inference of any related impurities after the stress degradation study (Figure 3). It was shown that the Baclofen peaks were free from excipients and co-eluting impurities.

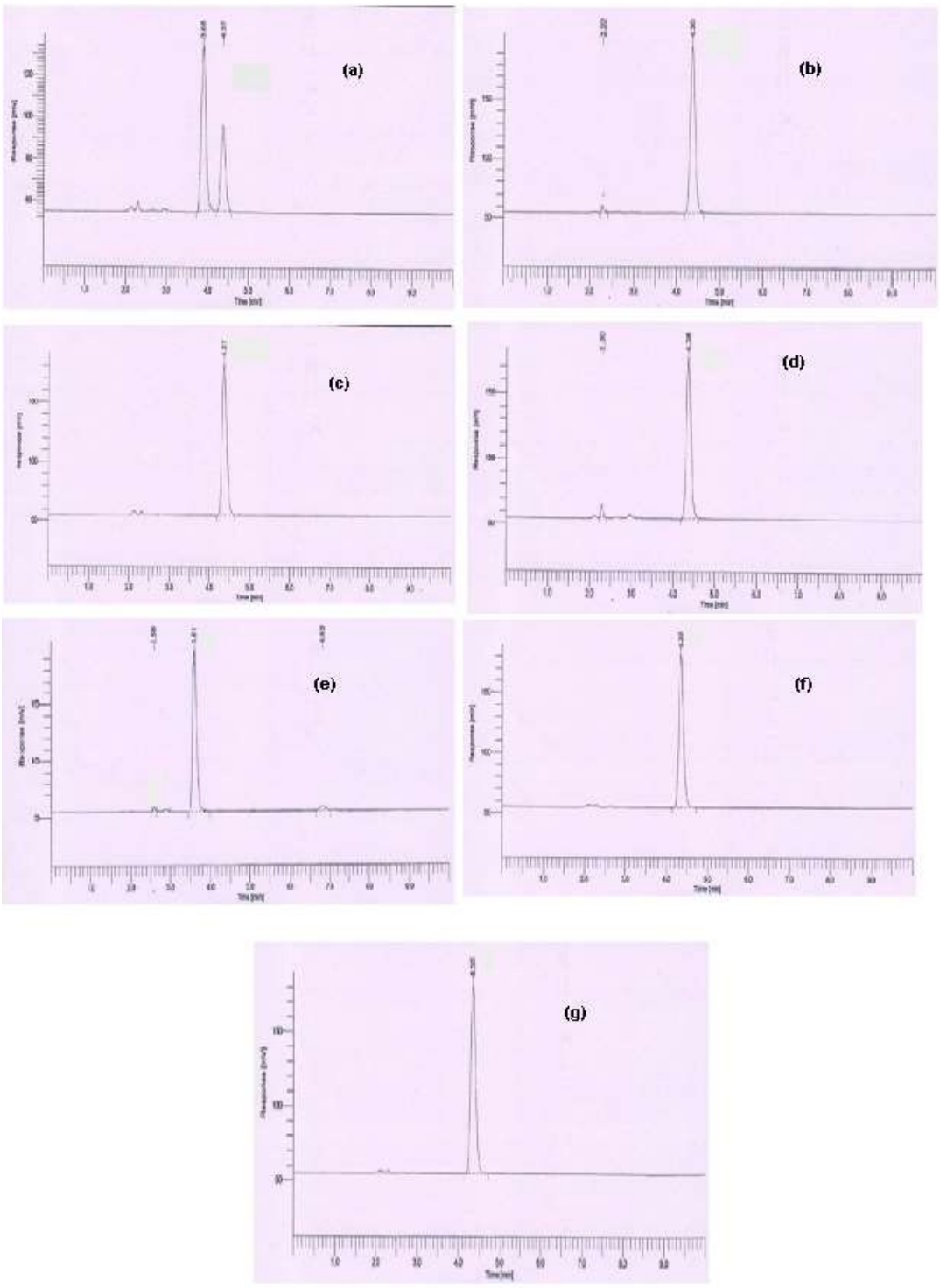

Figure 3: Chromatograms of Baclofen after (a) acidic hydrolysis; (b) basic hydrolysis; (c) neutral hydrolysis; (d) oxidative degradation; (e) thermal degradation; (f) photolytic (Sun light) degradation, (g) photolytic (UV light) degradation

Linearity

The linear correlation was obtained between peak area and concentration of Baclofen in the range of 5-30 µg/mL, the linearity of the calibration curve was validated by the value of correlation coefficient of the regression (r), the regression analysis of the calibration curves (Figure 4) is listed in Table 1.

Table 1: Optical and regression characteristics (n=3)

Parameter	Baclofen
Linearity range (µg/mL)	5-30
Linearity equation	y = 38217x + 72833
LOD (µg/mL)	0.062
LOQ (µg/mL)	0.187
Correlation coefficient (r)	0.9960

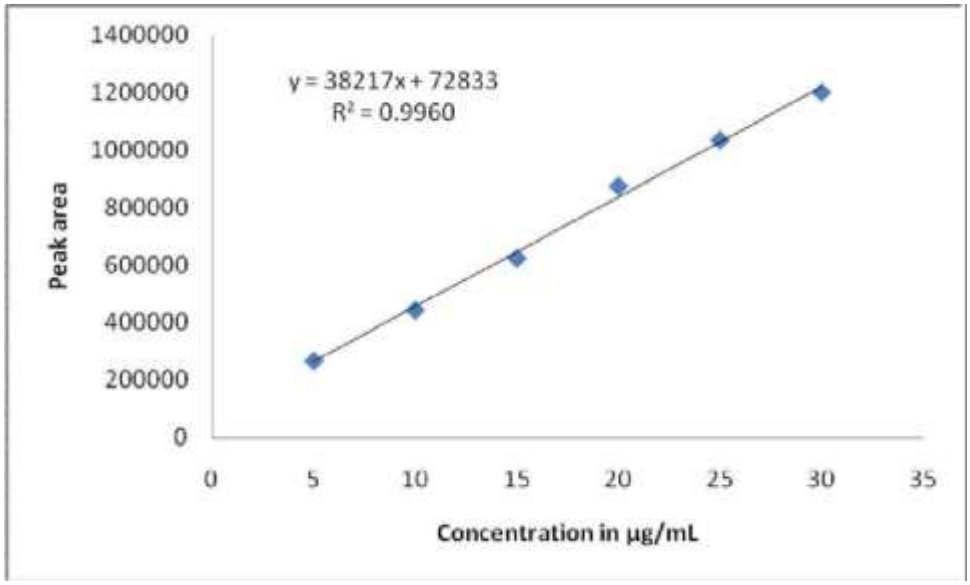

Figure 4: Calibration curve of Baclofen

Accuracy (% Recovery)

The accuracy study was carried out by the standard addition method. The percent recoveries were found in the range of 98.86-101.12 %, which indicated accuracy of the method. (Table 2)

Table 2: Results of recovery study (n=3)

Amount Taken (µg/mL)	Amount Added (µg/mL)	Amount found(µg/mL)	Recovery ± S.D, %	% RSD
10	0	10.05	100.50 ± 0.92	0.92
10	5	14.83	98.86 ± 1.65	1.65
10	10	19.90	99.50 ± 1.37	1.37
10	15	25.28	101.12 ± 0.77	0.77

Precision

The % RSD for repeatability (Table 3) of Baclofen was found to be 1.25. The value of % RSD for

intra-day precision was found to be in the range of 0.93 - 1.15% and inter-day precision was found to be in the range of 1.07 - 1.22 %, which indicated that the method was precise. (Table 4)

Table 3 Results of repeatability (n=6)

Drug	Baclofen Peak area
1	443614.0
2	454953.1
3	443275.4
4	445228.7
5	448743.8
6	438564.2
Mean	445729.9
SD	5590.19
% RSD	1.25

Table 4 Results of Intra-day and Inter-day precision (n=3)

Baclofen (µg/mL)	Intra-day precision Mean peak area ± SD	% RSD	Inter-day precision Mean peak area ± SD	% RSD
10	443614.0 ± 4152.12	0.93	448745.3 ± 4836.18	1.07
20	875777.0 ± 9638.35	1.10	878418.6 ± 9858.21	1.12
30	1200410.0 ± 13864.16	1.15	1201059.6 ± 14728.26	1.22

Limit of detection and limit of quantification

The Limit of detection (LOD) for Baclofen was found to be 0.062 µg/mL, while the Limit of quantification (LOQ) was 0.187 µg/mL.

Robustness

The method was found to be robust as the results were not significantly affected by slight variation in extraction time, composition of mobile phase, wavelength and flow rate of the mobile phase.

System-Suitability Test

The % RSD of system-suitability test parameters was found satisfactory. The results are listed in (Table 5)

Table 5: System suitability test parameters (n = 6)

No.	Retention time, Min.	Tailing factor	Theoretical plates
1	4.41	1.49	9282.12
2	4.41	1.49	9254.23
3	4.35	1.48	9237.48
4	4.27	1.49	9187.75
5	4.38	1.47	9265.58
6	4.41	1.45	9176.38
Mean	4.37	1.48	9233.92
SD	0.055	0.016	42.88
% RSD	1.26	1.08	0.46

Analysis of Tablet Dosage Form

The proposed RP-HPLC method was successfully applied for determination of Baclofen from tablet dosage form. The percentage of Baclofen was found to be satisfactory; which was comparable with the corresponding label claim. (Table 6)

Table 6: Analysis results of tablet dosage form (n=3)

Formulation	Labelled amount (mg)	Amount found (mg)	Assay ± SD, %
LIOFEN® Tab	10.00	9.96	99.64 ± 1.72

Degradation Study

Forced degradation study of Baclofen was carried out under various stress conditions and resultant chromatograms are depicted in (Figure 3.(a)-(g))

Effect of Acid, Alkaline and Neutral Hydrolysis

Baclofen was found to undergo 74.88 % decomposition under acidic stress condition with a major degradation product at retention time of about 3.88 min and minor degradation product at retention time of about 2.30 min and minute decomposition about 2.4% under basic stress condition with a degradation product at retention time of about 2.30 min (Figure.3 (a) and (b)) respectively. Under neutral degradation condition, no degradation was observed. (Figure 3 (c)). Hence, Baclofen was found to be highly degradable in basic condition, and very minute degradable in acidic condition but not degradable in neutral condition.

Effect of Oxidation

In oxidation stress condition, almost 7.5 % of Baclofen was degraded and degradation peak appeared in chromatogram at 2.30 min retention time. (Figure 3 (d)).

Effect of Heat

Under dry thermal stress condition, Baclofen was degraded about 8.3 % with degradation product at retention time of about 2.58 and 6.83 min. (Figure 3 (e)).

Effect of light

When Baclofen in solution state was exposed to sun light; and Baclofen in powder state was exposed to UV light, no degradation was observed, respectively (Figure 3 (f) and (g)).

The samples exposed to acidic, alkaline, neutral, oxidative, thermal and photolytic conditions were colorless. In Photolytic stability, Baclofen was found to be stable showing no degradation. All degradates were resolved from Baclofen peak and the percentage degradation for each condition indicated that there was no interference from degradates in determination of the Baclofen in tablet dosage form. Thus, the proposed, method was found to be "Stability Indicating".

Table 7: Results of stress degradation study

Stress conditions/duration	% Degradation
Acidic/0.1N HCl 72 h	74.88
Alkaline/ 0.1N NaOH / 72 h	2.40
Neutral/water/ 72 h	0.00
Oxidative/ 3% H_20_2/ 72 h	7.50
Photolysis/ Sun light/ 48 h	0.00
Photolysis/ UV light/48 h	0.00
Thermal 60°C / 72 h	8.30

CONCLUSION:

An isocratic stability indicating reverse phase liquid chromatographic method has been developed and validated for the estimation of Baclofen in tablet dosage form, the method was found to be specific as there was no interference of any co-eluting impurities after stress degradation study. The proposed method was found to be simple, accurate, precise, sensitive and robust. Hence, it can be used successfully for the routine analysis of Baclofen in pharmaceutical dosage forms, and for analysis of stability samples obtained during accelerated stability study

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