Int. J. Chem. Sci.: 11(1), 2013, 390-398
ISSN 0972-768X
www.sadgurupublications.com

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BACLOFEN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

SAROJ KUMAR RAUL[*], B. V. V. RAVI KUMAR[a], AJAYA KUMAR PATTNAIK[b] and PRABHAT KUMAR SAHOO[c]

Department of Pharmaceutical Chemistry, M. R. College of Pharmacy, Phool-Baugh, VIZIANAGARAM – 2 (A.P.) INDIA
[a]Roland Institute of Pharmaceutical Sciences, BERHAMPUR (Orissa) INDIA
[b]Departments of Chemistry, Ravenshaw University, CUTTACK (Orissa) INDIA
[c]Institute of Pharmacy and Technology, Salipur, CUTTACK (Orissa) INDIA

ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Baclofen in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL min^{-1} was employed on a Waters X-bridge C_{18} column at 30°C. The mobile phase consisted of phosphate buffer: acetonitrile 80 : 20 (v/v) and the detection wavelength was at 219 nm. Linearity was observed in concentration range of 10-150 µg/mL. The retention time for Baclofen was 3.8 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Baclofen in pharmaceutical dosage forms.

Key words: Estimation, Method development, Validation, Baclofen, RP-HPLC.

INTRODUCTION

Baclofen (Figure 1) is chemically (*RS*)-4-amino-3-(4-chlorophenyl)butanoic acid and it is used as antispastic agent or muscle relaxant[1]. It reduces spasticity in many neurological disorders like multiple sclerosis, amyotrophic lateral sclerosis, spinal injuries and flexor spasms but is relatively ineffective in stroke, cerebral palsy, rheumatic and traumatic muscle spasms and Parkinsonism[2]. It acts as an agonist at GABA-B receptors[3]. In addition, research has shown baclofen to be effective in the treatment of alcohol dependence and withdrawal, by inhibiting both withdrawal symptoms and cravings.

Literature survey reveals that few spectrophotometric methods[4], HPLC methods[5-7]

[*]Author for correspondence; E-mail: saroj.raul@rediffmail.com; Mo.: 09392725971

and colorimetric method[8] has been reported for the estimation of Baclofen. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Baclofen in pharmaceutical dosage form as per ICH guidelines.

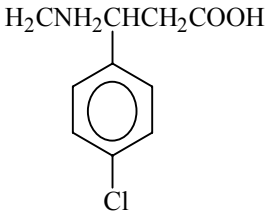

Fig. 1: Chemical structure of Baclofen

EXPERIMENTAL

Materials and method

Instrumental and analytical conditions

The HPLC analysis was carried out on Waters HPLC system (2695 module**)** equipped with PDA detector (2996 module) with auto Sampler and running on Waters Empower software. The column used is Waters X-bridge C_{18} (150 × 4.6 mm, packed with 5 µm) and detection was performed at 219 nm. The injection volume of sample was 10 µL and the run time was 10 minutes. An isocratic mobile phase containing 0.01 M phosphate buffer and acetonitrile at 80 : 20 (v/v) at the pH 2.5 was carried with the flow rate at 1.0 mL min^{-1}. The mobile phase was filtered through 0.45 µm membrane filter and degassed before use.

Reagents and chemicals

Baclofen working standard was kindly gifted by Sun Pharmaceuticals, Ahmadabad. Tablets were purchased from local pharmacy manufactured by Sun Pharmaceuticals (Liofen). Ultra pure water was obtained from a millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade

Preparation of mobile phase

1.36 g of potassium di hydrogen orthophosphate dissolved in 1000 mL of water and mixed, pH adjusted to 2.5 with ortho phosphoric acid, sonicated to degas the buffer. Transferred 200 volumes of acetonitrile and 800 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45 µm filter under vacuum. Acetonitrile and buffer in the ratio of 50 : 50 (v/v) used as diluent.

Preparation of standard solution

Accurately weighed about 10 mg of Baclofen and transferred into a 10 mL volumetric flask and 5 mL of diluent was added and sonicate to dissolve it completely and the volume was adjusted with the diluent to get stock solution of 1000 µg/mL. Then 1 mL of stock solution is transferred into 10 mL volumetric flask and make up to volume with diluent and filter through 0.45 µm filters, which gives a solution of strength 100 µg/mL.

Preparation of sample solution

Weigh 20 Baclofen tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Baclofen into a 50 mL volumetric flask. Add about 35 mL of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 1 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Method validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of Baclofen in the concentration of 10, 25, 50, 75, 100 and 150 µg/mL were prepared. The solutions were injected using 10 µL injection volumes in to the chromatographic system at the flow rate of 1.0 mL min^{-1} and the effluents were monitored at 219 nm, and chromatograms were recorded. Calibration curve of Baclofen was obtained by plotting the peak area ratio versus the applied concentrations of Baclofen, given in Table 1. The linear correlation coefficient was found to be 0.999, shown in Fig. 2.

Table 1: Linearity of Baclofen

Concentration (µg/mL)	Average area
10	188147
25	452315
50	944052

Cont…

Concentration (µg/mL)	Average area
75	1410469
100	1835207
150	2679528

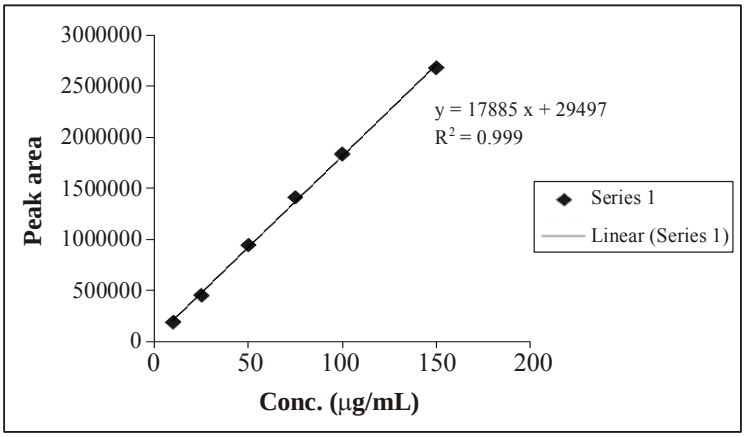

Fig. 2: Linearity curve of Baclofen

Precision

Repeatability of the method was checked by injecting replicate injections of 100 µg/mL of the solution for six times on the same day as intraday precision study of Baclofen and the % RSD was found to be 0.59, given in Table 2.

Table 2: Precision of Baclofen

Injections	Area
1	1685451
2	1673530
3	1666811
4	1664368
5	1660949
6	1658870
Mean	1668330
SD	9819.819
% RSD	0.5886

Accuracy

Baclofen reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50%, 100% and 150%). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in Table 3.

Table 3: Accuracy of Baclofen

% Conc.	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.98	99.6 %	
100%	10.0	9.96	99.6%	99.6%
150%	15.0	14.92	99.5 %	

Specificity

Spectral purities of Baclofen chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness

To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in Table 4.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Table 4: Robustness of Baclofen

Parameters	Adjusted to	Average area	R_t	SD	% RSD
Flow rate as per method 1.0 mL/min	0.8 mL/min	1699985	3.881	9618.91	0.56
	As it is	1678874	3.808	8593.6	0.51
	1.2 mL/min	1729823	3.842	10897.52	0.62
Mobile phase composition Buffer : Acetonitrile (80 : 20)	Buffer : Acetonitrile (82 : 18)	1741221	3.796	7972.48	0.46
	As it is	1678685	3.821	5987.5	0.36
	Buffer : Acetonitrile (78 : 22)	1745231	3.902	4875.45	0.28

Detection and quantitation limits

According to the determined signal-to-noise ratio, Baclofen presented limits of detection of 0.09 µg/mL and limits of quantitation of 0.25 µg/mL, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Baclofen so that the values obtained should be considered as the limit of method sensitivity.

System suitability

System suitability tests were carried out on freshly prepared standard stock solutions of Baclofen and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in Table 5.

Assay of baclofen tablet

Three different batches of Liofen were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50 mg of Baclofen was transferred to a 50 mL volumetric flask followed by the addition of 35 mL of diluent. The solution was sonicated for 10 minutes and volume adjusted with the diluents then filtered through 0.45 µm membrane filter. Further dilutions were made to get the final concentration equivalent to 100 µg/mL of Baclofen. The mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in Table 6.

Table 5: System Suitability of Baclofen

Concentration	Injection	Area	R_t
100 µg/mL	Inj-1	1695423	3.856
	Inj-2	1683552	3.865
	Inj-3	1676815	3.852
	Inj-4	1668968	3.866
	Inj-5	1658949	3.854
	Inj-6	1662870	3.798
Statistical analysis	Mean	1674430	3.8485
	SD	13658.21	0.025407
	% RSD	0.81	0.66
	Tailing factor	1.33	
	Plate count	9545	

Table 6: Contents of Baclofen in tablets (n = 6)

Sample tablet	Batch	Labelled amount (mg)	Amount found (mg) ± SD	%Amount found
Liofen (10 mg)	1	10	9.96 ± 0.11	99.6
	2	10	9.99 ± 0.14	99.9
	3	10	9.98 ± 0.12	99.8

S.D. = Standard Deviation

All the analyzed batches presented Baclofen were very close to the labeled amount. The Baclofen content in the tablets samples varied from 99.6 to 99.9%.

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Baclofen was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the phosphate buffer to acetonitrile was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of buffer : acetonitrile at the ratio of

80 : 20 (v/v). The retention time of Baclofen was found to be 3.8 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Baclofen in tablet formulation, given in Table 7. A typical chromatogram showing the separation of Baclofen is shown in Fig. 3.

Table 7: Developed chromatographic conditions

Parameters	Method
Stationary phase (column)	Waters X-bridge (150 × 4.6 mm, packed with 5 µm)
Mobile Phase	80 : 20 (Phosphate buffer : Acetonitrile)
pH	2.5 ± 0.02
Flow rate (mL/min)	1.0
Run time (minutes)	10
Column temperature (°C)	30
Volume of injection loop (µL)	10
Detection wavelength (nm)	219
Drugs Rt (min)	3.8

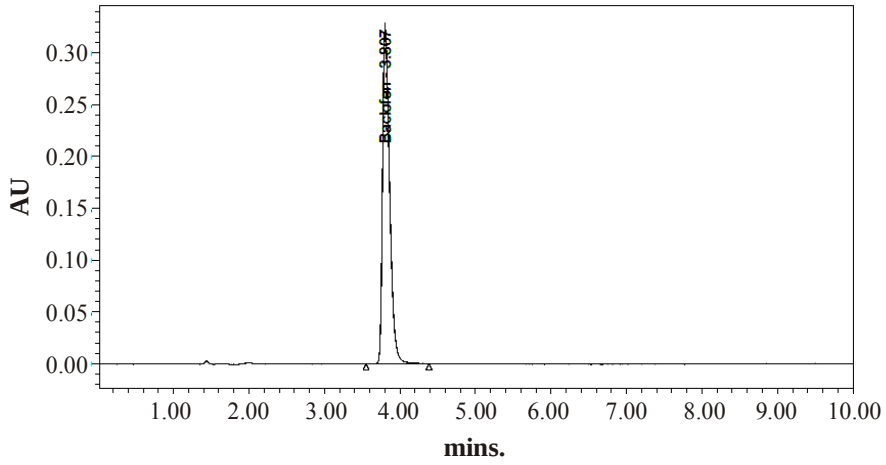

Fig. 3: Standard Chromatogram of Baclofen

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Baclofen in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Baclofen in pharmaceutical dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Sun Pharmaceuticals, Ahmadabad for providing gift sample of Baclofen and Spectrum Pharma Research Solution, Hyderabad for providing necessary facilities to carry out the research work.

REFERENCES

1. M. J. O. Neil, The Merk Index- An Encyclopedia of Chemicals, Drugs and Biologicals, 9th Ed., Merk and Co, NJ (2001) pp. 125.
2. K. D. Tripathi, Essentials of Medical Pharmacology, 5th Ed., Jaypee Brother's Medical Publishers, New Delhi (2003) pp. 316-318.
3. R. Ghosh, Text Book of Modern Concepts on Pharmacology and Therapeutics, 24th Ed., Hilton and Co, Calcutta (1991) p. 206.
4. M. Ahmed, M. Rajesh, A. S. K. Shetty and B. N. Maanasa Rajan, Int. J. Chem. Tech. Res., **3(2)**, 933-937 (2011).
5. B. N. Nalluri, K. Sushmitha, B. Sunandana and D. Prasad Babu, J. Appl. Pharmaceut. Sci., **2(7)**, 111-116 (2012).
6. M. M. Hefnawy and H. Y. Aboul-Enein, Talanta, **61**, 667-673 (2003).
7. M. Alain, M. Laurette, B. Murielle and G. Virginie, J. Chromatogr. A, **729(1-2)**, 309-314 (1996).
8. S. J. Hosseinimehr, F. Pourmorad, E. Moshtaghi and M Amini, Asian J. Chem., **22(1)**, 522-526 (2010).

Accepted : 23.12.2012