A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF BACLOFEN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new, simple, specific, sensitive, rapid, accurate and precise RP-HPLC method was developed and validated for the estimation of Baclofen in bulk drug and pharmaceutical formulations. Baclofen was chromatographed on a reverse phase C18 column (150x4.6mm I.D., particle size 5 µm) in a mobile phase consisting of ammonium acetate buffer (pH 5.0 adjusted with orthophosphoric acid) and methanol in the ratio 40:60 v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min with detection at 220 nm. The detector response was linear in the concentration of 10-50µg/mL. The limit of detection and limit of quantitation was found to be 1.025 and 3.107µg/mL, respectively. The intra day and inter day variation was found to be less than 1%. The mean recovery of the drug from the solution was 100.1%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of Baclofen in bulk drug and pharmaceutical formulations.

Keywords: RP-HPLC, Baclofen, Estimation, Tablets.

INTRODUCTION

Baclofen is an orally administered synthetic antispastic agent or muscle relaxant**.** 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 (Tripathi, 1991). It may act as an agonist at GABA-B receptors (Ghosh, 1991).

Baclofen is chemically β-(amino methyl)-4-chlorobenzene propanoic acid (fig.1) and it is used as antispastic agent or muscle relaxant. The molecular formula of Baclofen is $C_{10}H_{12}ClNO_2$(Indian Pharmacopoeia, 2007), the molecular mass of Baclofen is 213.67g/mol. It is freely soluble in water, 0.1N HCl and 0.1N NaOH, slightly soluble in methanol, very slightly soluble in ethanol. It is official drug in I.P, B.P and U.S.P (Indian Pharmacopoeia, 2007) (British Pharmacopoea, 2005) (US Pharmacopoeia, 2004).

Literature survey reveals that, only bioanalytical methods by HPLC and few Spectrophotometric methods were found using human plasma and urine for the quantitative estimation of Baclofen in bulk drug and pharmaceutical formulations (Laurette, 1996) (Wuis, 1985) (Mohammed, 2003) (Zhu, 2003) (Pesez, 1974).

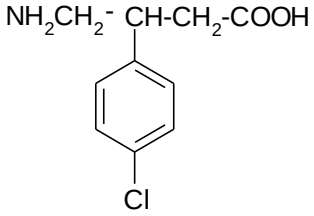

Figure.1. Chemical structure of Baclofen

MATERIALS AND METHODS

Quantitative HPLC was performed on a isocratic high pressure liquid chromatography (shimadzu HPLC class VP-Series) with one LC-10 AT VP pump, UV/VIS detector SPD-10A VP, CTO-10 AS VP column oven (shimadzu), SCL-10A VP system controller (shimadzu), a disposable guard column LC-18 (PELLIGUARD)TM, LC-18, 2 cm, supelco, inc., Bellefonte, and a Reverse Phase C-18 Column (150mm x 4.6 mm i.d.particle size 5 µm) was used . The HPLC system was equipped with the software class, N-2000 CHROMTECK (Shimadzu).

Reagents and chemicals: Ammonium acetate and orthophosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Methanol of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Baclofen was a gift sample by Unicare Pvt. Ltd., Gujarat. The commercially available Baclofen tablets were procured from the local market.

Preparation of buffer: (0.005 M) Ammonium acetate buffer was prepared by dissolving 0.3854 g of Ammonium acetate in 1000 mL of milli-Q water and the pH was adjusted to 5.0 with orthophosphoric acid.

Chromatographic conditions: The mobile phase consisting of ammonium acetate buffer (pH 5.0 adjusted with orthophosphoric acid) and methanol was in the ratio of 40:60 v/v filtered through 0.45µ membrane filter before use, degassed and pumped from the solvent reservoir into the column at a flow rate of 1.0 mL/min. The detection

was monitored at 220nm and the run time was 10 minutes. The volume of injection loop was 20 µL prior to the injection of the drug solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Procedure: Stock solution of Baclofen was prepared by dissolving 10 mg of Baclofen in 10 mL standard volumetric flask containing 2.5 mL of mobile phase and the solution was sonicated for 20 min. and then made up to the mark with mobile phase to get a concentration of 1000 µg/mL. Subsequent dilutions of this solution were made with mobile phase to get concentration of 10-50 µg/mL. The standard solutions prepared as above were injected into the 20 µL loop and the chromatogram was recorded and shown in Figure 2.

The retention time of Baclofen was found to be 5.345 min. The calibration curve was constructed by plotting concentration versus peak area ratio. The amount of Baclofen present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug versus concentration were found to be linear and the results are furnished in Table 1.

Assay: Twenty tablets each containing 10 mg were weighed accurately and powdered. A quantity equivalent to100 mg of Baclofen was weighed accurately and transferred to 100 mL volumetric flask containing 30 mL of mobile phase. The contents were sonicated for 20 min. and made up to the mark with the mobile phase. The resulting solution is filtered through a membrane filter. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of Baclofen present in tablet formulation was determined by comparing the peak area from the standard. The results were furnished in Table 2.

Validation of proposed method: Selectivity of the method was assessed on the basis of elution of Baclofen using the above mentioned chromatographic conditions. To study the linearity, limit of detection, limit of quantitation and correlation co-efficient, retention time, theoretical plates, tailing factor has been validated for the determination of Baclofen. The results are furnished in Table 3.

Linearity: The standard curve was obtained in the concentration range of 10-50 µg/mL. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

Precision: The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table 4.

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ for Baclofen were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Baclofen.

Robustness: The robustness was checked by changing the flow rate to 0.8 and 1.2 mL/min and the wavelength 218 and 222nm the method suits best.

Accuracy: The accuracy of the HPLC method was assessed by adding known amount of drug solution to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 30 µg/mL so as to give the percentage recovery. The results are furnished in Table 5.

RESULTS AND DISCUSSION

By applying the proposed method, the run time of the method was set at 10 min and Baclofen appeared on the typical chromatogram at 5.345 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 10-50 µg/mL. The regression equation of Baclofen concentration over its peak area ratio was found to be Y=33706.00+94203.00x (r = 0.9993) where Y is the peak area ratio and X is the concentration of Baclofen (µg/mL). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 1%. The tailing factor was found to be 1.285, which indicates good shape of peak. The numbers of theoretical plates were found to be 6889.163, which indicate efficient performance of the column. The limit of detection and limit of quantitation was found to be 1.025 µg/mL and 3.107 µg/mL, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of ammonium acetate buffer and methanol were tested. The

use of ammonium acetate buffer and methanol in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery of Baclofen ranging from 99.90 to 100.40 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

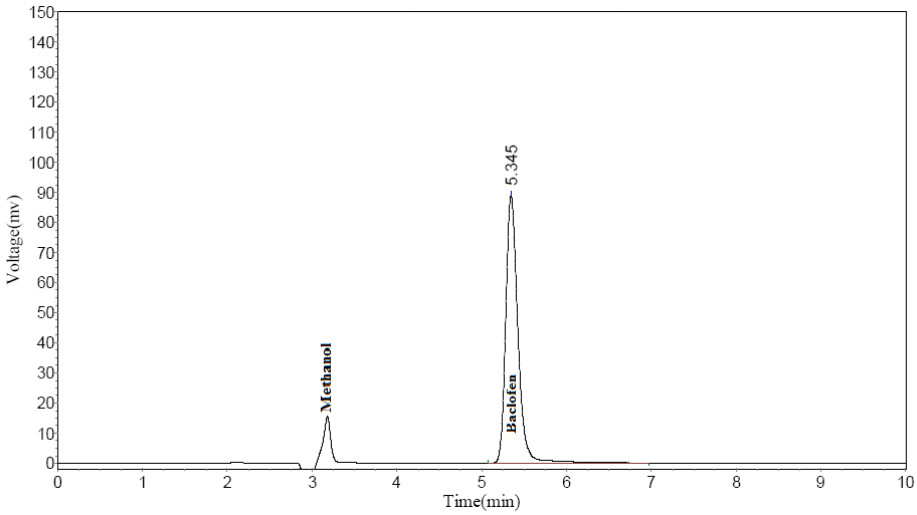

Figure.2. Typical chromatogram of Baclofen

Table.1. Calibration data of the method

Concentration,µg/mL	Peak area (n=5)
10	244882.703
20	593024
30	894065.813
40	1252336.75
50	1600501.75

Table 2. Assay of Baclofen

Formulation	Label claim(mg)	Amount found(mg)	% Amount found
Brand-1	10	10.03	100.3
Brand-2	10	10.01	100.1

Table.3. System suitability parameters

Parameter	Result
Linearity, µg/ml	10-50, µg/ml
Correlation coefficient	0.9993
Retention time, min	5.345
Theoretical plates (N)	6889.163
Tailing factor	1.285
LOD, µg/ml	1.025
LOQ, µg/ml	3.107

Table.4. Precision of the proposed HPLC method

Concentration of Baclofen µg/ml	Intra-day Precision		Inter-day Precision	
	Mean area (n=3)	%C.V	Mean area (n=3)	%C.V.
80	708423.8	0.98	706383.9	0.92
100	896317.6	0.32	897090.7	0.16

120	1072514.0	0.75	1063113.0	0.71

Table.5. Recovery studies of the proposed HPLC method.

Concentration	Amount of Baclofen Added, mg	Amount of Baclofen Found, mg	% Recovery	Mean Recovery
80%,24µg/ml	81.6	81.9	100.4%	100.1%
100%,30µg/ml	102	102.1	100.1%	
120%,36µg/ml	122.4	122.2	99.9%	

CONCLUSION

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of Baclofen in pharmaceutical formulations. Hence, this method can easily and conveniently adopt for routine quality control analysis of Baclofen in bulk drug and its pharmaceutical formulations.

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REFERENCES

British Pharmacopoeia, 2005, 1, 202.

Ghosh.R, Text book of Modern Concepts on Pharmacology and Therapeutics, Hilton and co, Calcutta, 1991, 24, 206.

Indian Pharmacopoeia, 2007, 1, 2, 144, 768-770.

Laurette M, Murielle B, Virginie G, High-Performance Liquid Chromatographic determination of baclofen in human plasma, J of Chrom A, 1996, 729(1-2), 309-314.

Mohamed MH and Hassan YA, Enantioselective high- Performance liquid chromatographic method for the determination of baclofen in Human plasma, Talanta, 2003, 61(5), 667-673.

Pesez M, Bartos J, Colorimetric and Fluorimetric analysis of Organic Compounds and Drugs, Marcel Dekker, INC, Newyork, 1974, 129, 131, 137-139, 200-201.

Tripathi K D, Essentials of Medical Pharmacology, Jaypee brother's medical publishers, 2003, 5, 316-318.

US Pharmacopoeia NF, 2004, 23, 207.

Wuis E.W, Dirks R.J, Vree T.B, et al; High-Performance Liquid Chromatographic Analysis of baclofen in plasma and urine of man after precolumn Extraction and derivatization with O- phthaldialdehyde, J. of Chrom B, 1985, 337, 341-350.

Zhu Z and Neirinck, Chiral separation and determination of R-(-)-and S-(+) - Baclofen in human plasma by high-performance liquid chromatography, J of Chrom B, 2003 March; 785(2), 277-283.