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A Submicellar Liquid Chromatographic Method for Quantitative Determination of Muscle Relaxant Drug Baclofen in Solubilized System

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A high performance liquid chromatography method, using micellar solution above the critical micellar concentration (CMC) has been developed and validated to quantify baclofen (a muscle relaxant drug) in active pharmaceutical ingredients and pharmaceutical formulations. Process optimization was done at various conditions such as different surfactants, surfactant concentration, pH of buffer solution, solvent composition, solvent selection and different HPLC columns. An isocratic HPLC mode, column temperature 35 °C, Intersil ODS-3 V 250 × 4.6, 5 µm column, 0.8 mL/min mobile phase flow rate and UV detection wavelength at 220 nm was used for the purpose. The method was validated as per International Conference on Harmonization (ICH) guidelines *viz.*, specificity, linearity, range, inter and intra-day precision, accuracy (recovery) and robustness. Forced degradation conditions such as basic, acidic, oxidative, thermal degradation and photolytic are applied to prove the stability indicating nature of the proposed method.

Keywords: Critical micelle concentration, Micellar liquid chromatography, Sodium dodecyl suphate, Baclofen.

INTRODUCTION

A high submicellar liquid chromatographic method, where organic solvent content is high and surfactants are presents in the form of small cluster in the mobile phase was developed. In this submicellar liquid chromatography micelles exist in the form of monomers. These monomers associated with the stationary phase and give hither retention time of pharmaceutical drugs. Several reports reveled that the high organic solvent content in the mobile phase decreases the thickness of the surface coating [1-3]. A systematic perusal of literature reveled that *hitherto* methods [4,5] using high submicellar liquid chromatography to optimize chromatographic conditions are developed and validated.

An high submicellar liquid chromatographic method using micellar mobile phase containing surfactant above its critical micellar concentration [6,7] was developed and validated for the determination of baclofen in active pharmaceutical ingredient (API) and pharmaceutical formulations. Baclofen *i.e.*, 4-amino-3-(4-chlorophenyl)butanoic acid (Fig. 1), γ-amino butyric acid (GABA), introduced as race mate in the therapy of muscle spasticity, is still the classical prototype of the selective GABABR agonists [8]. It is primarily used to treat spasticity and is in the early research stages for the treatment of alcoholism. It is also used by compounding pharmacies in topical pain creams as a muscle relaxant. The most predominant pharmacological effects of baclofen are its muscle relaxant, anti-nociceptive and anti-drug craving properties [9,10] as well as the reduction in cognitive behaviour [11]. Baclofen is used clinically for the treatment of spasticity associated with brain and spinal cord injuries [12], drug addiction and alcoholism [13,14], gastroesophageal reflux disease (GERD) [15], cancer pain [16] and overactive bladder [17-19].

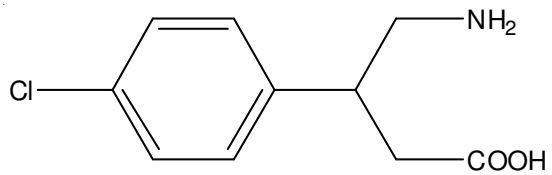

Fig. 1. Baclofen [4-amino-3-(4-chlorophenyl)butanoic acid]

A systematic perusal of literature reveled that *hitherto* a few methods [20-24] are available for the analysis of baclofen. However, most of them suffer from various bottle-necks such as long analysis time, inaccurate analysis and tedious workup procedures. Hence keeping in view to overcome the shortcomings of existing methods, it was thought to develop a novel, MLC quantitation strategy for the determination of baclofen in solubilized systems.

EXPERIMENTAL

The analytical studies were performed on an Agilent Technologies Series 1260 Infinity (Palo Alto, USA) and Waters Separation Module 2696 model (Waters Corporation, MA, USA) chromatographic system equipped with a quaternary pump, auto sampler tray and column compartments with thermostat temperature, photo diode-array detector (range 190-800 nm) and UV detector. The column was an Intersil ODS-3V 250 mm × 4.6 mm, 5 µm particle size. The pH of solutions was measured on Five Easy™ FE20 (Metteler-Toledo, Switzerland) pH meter equipment with a combined Ag/AgCl/glass electrode. A micro balance MSA3 6p 000DM (Sartorius, AG, Germany) was used for accurate weighing of sample and reference standard. A vortex shaker and sonication units were employed for sample pretreatment. 20 µL of sample was injected into the HPLC for every analysis. Peak purity was determined out, over a wavelength range 200-300 nm. The photo stability chamber Model TP000020G (Thermo Scientific Equipment Pvt. Ltd., Thane, India) utilized during forced degradation study. All the measurements were carried out at 25 ± 2 °C temperature.

Baclofen reference standard (USP) Lot No. JOH297 was obtained from LCGC with a purity of 99.40 %. Baclofen active pharmaceutical ingredients sample was kindly gifted by Medilux Laboratories Pvt. Ltd. and its formulation tablet (Baclof-10 mg, Intas Pharmaceutical Ltd) was purchased from commercial source. Ultrapure water was prepared by purification of deionized water using an ultrapure water generator device (Millipore S.A.S., Mosheim, France). Sodium dodecyl sulphate, orthophosphoric acid and acetonitrile were of Analytical, HPLC grade purity and purchased from Spectrochem, Merck (Germany) and Rankem (India).

Preparation of standard and sample solutions: Standard stock solution of 0.2 mg/mL of baclofen was prepared by dissolving the compound in mobile phase, with the aid of an ultrasonic bath and this solution was injected directly into the chromatographic system. All the solutions were filtered through filter paper of 0.45 µm Ultipor® N_{66}® nylon membranes (Pall Corporation, Mumbai, India) before analysis.

A sample solution containing baclofen at a concentration of 0.2 mg/mL was used for the accurate and precise quantification. This sample solution was prepared from the composite powder of 10 mg tablets. Mobile phase was added to the flask and shaken vigorously until the product clumps were dissolved, sonicated for 5 min and further diluted with mobile phase to make up the required volume. Approximately 15 mL of sample solution was transferred to a centrifuge tube and centrifuged at 3000 rpm for 10 min. A portion of the supernatant liquid was transferred to a HPLC vial. Baclofen working standards and test solutions were prepared directly without centrifuging and were utilized for quantification.

Optimization of chromatographic conditions: In order to optimize the chromatographic conditions, studies were performed at various mobile phase compositions and different concentrations of sodium dodecyl sulphate ranging from 0.005 to 0.3 M. The optimized mobile phase composition and sodium dodecyl sulphate concentration was 0.05 M SDS buffer (pH 7.0 with diluted orthophosphoric acid solution) and acetonitrile in the ratio 40:60 v/v. Analysis was performed in a reversed phase mode of chromatography on an Intersil ODS-3V, 250 × 4.6, 5 µm column with a mobile phase flow rate of 0.8 mL/min which is filtered through 0.45 µm nylon membranes filter paper. The column compartment temperature was thermostatted at 35 °C. Elevated column temperature increases efficiency in MLC owing to a shift in the equilibrium from micelle to the bulk solvent as well as due to a decrease in the amount of adsorbed surfactant on the stationary phase [25]. Under these optimized conditions peak efficiency, asymmetry and retention time less than 3 min for baclofen peak was obtained.

RESULTS AND DISCUSSION

Different chromatographic conditions such as column selection, solvent selection, pH variance, solubilized system and concentration of surfactants which affect the chromatographic performance of baclofen peak were carefully optimized to achieve the best chromatographic system for regular analysis. These optimized chromatographic conditions were used for quantitation of baclofen in active pharmaceutical ingredient (API) and pharmaceutical formulations. The developed method offers lower limit of detection up to 1.5 mg/mL for baclofen. The results of the optimized conditions are as follows:

Selection of appropriate detection wavelength: Analyses of 0.2 mg/mL solutions of reference and sample solutions were performed over a wavelength range 200-300 nm using the 0.05M SLS solution and acetonitrile in the ratio of 40:60 as mobile phase. The obtained peak purity spectra shows that 218.5 nm presented in Fig. 2; is the optimal wavelength to maximize the sensitivity of determination of the baclofen peak in mobile phase.

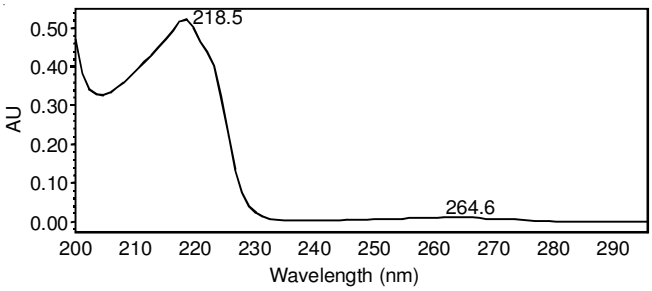

Fig. 2. Absorption spectrum of baclofen in mobile phase

Column selection: The column selectivity is the very crucial and compound specific task. A variety of HPLC columns are commercially available, however, each stationary phase provides different levels of selectivity for baclofen. Such a variation is attributed to the column modifications *viz.*, end capping, mixed-modes, amide bond linkages and phenyl and cyano bonding. Therefore, with a goal to achieve improvement in sensitivity of baclofen peak in MLC, different stationary phases that possess large differences in polarity, wet stability and retention ability were chosen. The increased selectivity is greatly influenced by various properties of baclofen *viz.*, ionic status, molecular size, hydrophobicity and hydrophilicity. Hence four different columns *viz.*, Waters Spherisorb ODS, Oyster ODS-3, Thermo Hypersil ODS and Intersil ODS-3V were screened for the desired selection using a fixed mobile

TABLE-1
OPTIMIZATION OF BUFFER pH FOR THE DETERMINATION OF BACLOFEN

Parameters	Observation at different pH						
	2.0	2.5	3.0	3.5	5.0	7.0	9.5
Peak response	600	630	740	960	750	1500	1500
Retention time	6.8	6.8	7.7	6.2	3.4	2.9	2.9
Peak tailing	1.5	1.8	1.0	1.1	2.0	1.3	1.2
Peak area	117694500	123483496	118837097	123075119	137992231	141648315	140917171
Peak height	9962872	11167430	12514504	15004324	13219728	25566083	25849709
Theoretical plate	7046	8627	14587	13099	2221	6529	6386

phase which is mentioned in above experiment of "Selection of appropriate detection of wavelength". However, out of all the columns, studies on the Intersil ODS-3V, 250 × 4.6 mm, 5 µm column gave an appreciable selectivity, peak area and retention time for baclofen detection (Fig. 3).

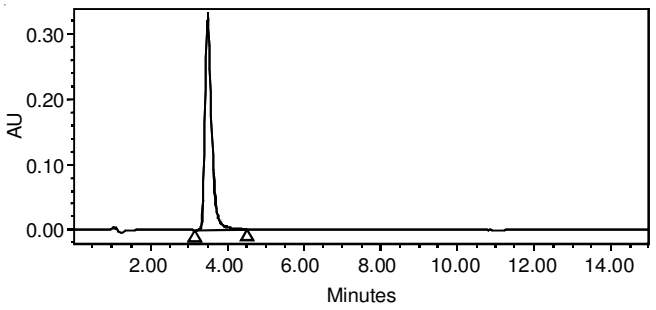

Fig. 3. Typical HPLC chromatogram of baclofen using Intersil ODS-3V column

Selection of mobile phase composition: In order to achieve best chromatographic conditions for analysis of baclofen, different mobile phase composition was optimized. A comparative evaluation of various compositions of mobile phase (acetonitrile + buffer) indicated that the addition of organic solvent decreased the thickness of the surfactant coating. However adsorption of the surfactant on the stationary phase resulted in enhanced retention. Different mobile phase composition shows variation in retention time of the baclofen peak is presented in Fig. 4.

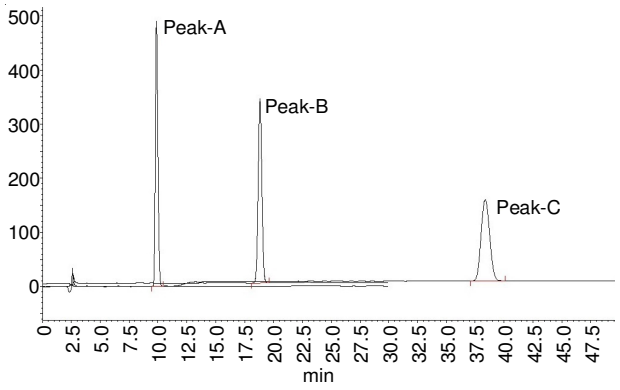

Fig. 4. Typical HPLC chromatogram shows retention time of baclofen in different mobile phase composition (Peak-A, Mobile phase Composition 40:60 :: Buffer:Acetonitrile v/v; Peak-B, Mobile phase Composition 50:50 :: Buffer:Acetonitrile v/v; * Peak-C, Mobile phase Composition 60:40 :: Buffer:Acetonitrile v/v

Effect of the pH variation: In a view to study the effect of pH variation on chromatographic behaviour of baclofen injections were made using 0.05 M sodium dodecyl sulphate solution of various pH in the range 2.0-9.5. The study showed that variation in buffer pH shows remarkable change in the area, retention time, efficiency and sensitivity of baclofen peak. The best peak height of baclofen was achieved at pH 7.0 that indicate higher sensitivity of the mobile phase. Also the mobile phase at this pH value is susceptible to the column. Hence, the mobile phase buffer of pH 7.0 was chosen for the further study. The results of optimization of the buffer pH are presented in Table-1.

Selection of solubilized system: A number of different surfactants were used in the analysis of baclofen. Surfactants play a significant role in HPLC analysis not only in solubilizing material but also by providing a specific orientation of the molecule to interact with the stationary phase of column. In our study we used different types of surfactants *i.e.* cationic, anionic and amphoteric. The increased selectivity is achieved in sodium dodecy sulphate, which is represented in Table-2 and then optimized the method with the same.

TABLE-2
COMPARATIVE DATA OF DIFFERENT SURFACTANTS

Parameters	Name of the surfactants		
	SDS	CTAB	Tween-20
Peak response	775	200	No peak observed in 70 min
Retention time	7.72	2.42	
Peak tailing	1.0	1.25	
Peak area	125673578	21799147	
Peak height	13896760	3803639	
Theoretical plate	16243	4090	

SDS = Sodium dodecyl sulphate; CTAB = Cetyl trimethyl ammonium bromide

Surfactant concentration: The effect of sodium dodecyl sulphate (SDS) solution concentration on asymmetry, retention time and efficiency of baclofen was studied using mobile phase containing sodium dodecyl sulphate in the concentration range 0.005 to 0.3 M. It was observed that concentration of 0.05 M sodium dodecyl sulphate is suitable for the preparation of mobile phase. This shows well-resolved peak, highest number of theoretical plates and peak response presented in Table-3.

Method validation: The validation of the proposed method was performed according to ICH and pharmacopeial guidelines [26-28] pertaining to the selectivity (Identification), system suitability, linearity, range, LOD, LOQ, accuracy (recovery), precision, selectivity (force degradation), sample solution stability, mobile phase stability and robustness. All the measurements for validation were performed using baclofen standard solution in mobile phase.

TABLE-3
OPTIMIZATION OF SURFACTANT CONCENTRATION

Parameters	Different concentration of sodium dodecyl sulphate surfactant (M)							
	0.3	0.25	0.20	0.15	0.10	0.05	0.01	0.005
Peak response	560	580	590	480	630	775	630	450
Retention time	11.8	11.2	10.7	9.9	9.2	7.7	6.1	5.6
Peak tailing	1.1	1.1	1.1	1.3	1.0	1.0	1.9	1.6
Peak area	123929818	124403416	124979890	127439431	126039635	125673578	126030209	126759654
Peak height	9434359	9528839	9375134	8234362	11792914	13896760	11406584	8048121
Theoretical plate	16211	16350	14361	8901	15858	16243	7227	3428

Specificity: In order to assess the specificity of the method, reference solution and sample solution of baclofen were analyzed. The clean and a clear identification without interference were achieved. Emergence of prominent peak at 2.9 min is attributed to the presence of baclofen. Forced degradation studies were also performed on baclofen to provide an indication of the stability and specificity of the proposed method. Peak purity of stressed samples of baclofen was checked using a 2996 photo diode array (PDA) detector. The degradation studies and peak purity test results derived from PDA detector, confirmed that the spectral purity of baclofen was homogenous and shows its stability indicating nature. The stress conditions employed for degradation study and observed degradation results are presented in the Table-4.

Linearity: Calibration curves were constructed using the peak area of the chromatographic peak at several different concentrations between 1.0-400 µg/mL of baclofen in mobile phase. The slope and intercept were obtained by a correlation plot between the peak areas of baclofen from duplicate injections *versus* the concentration using least square linear regression method. LOD was set as the 3.3 s criteria (3.3 times the standard deviation obtained for the solution with the lowest concentration included in the calibration divided by the slope of the calibration curve). For baclofen LOD, LOQ were the lowest studied concentration in which acceptable precision and accuracy level are obtained. These studies were performed intra and inter day separately and results were in agreement with ICH guidelines, which establish a maximum range of 80-120 % for accuracy and 15 % RSD for precision.

Leaching study: An investigation was conducted to determine the sonication time required for complete leaching of the baclofen from the sample matrix. Eight samples of 10.0 mg portions of the composite baclofen tablets were prepared. For each strength studies, two samples were also prepared using a control procedure known to fully leach the baclofen. The remaining samples were tested for various sonication times of 10, 30, 60 min. The assay results of sample solutions for 10 mg baclofen tablets (Baclof-10) were prepared using different sonication times during sample leaching and obtained results

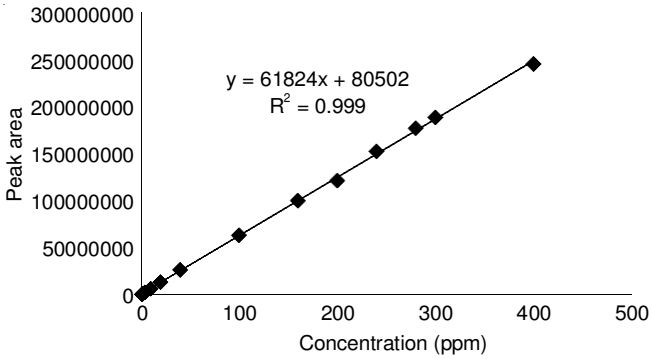

Fig. 5. Linearity of baclofen at different concentration *vs.* peak area

are presented in Table-5. Based on the results obtained, a 10 min sonication time at 40 °C was considered in the proposed method.

TABLE-5
EFFECT OF SONICATION TIME ON BACLOFEN SAMPLE (BACLOF TAB-10 mg)

Sample No.	Sonication time (min)	Baclofen label claim (%)
1*	10	99.48
2*	10	99.64
3	10	99.86
4	10	99.71
5	30	100.08
6	30	100.25
7	60	101.15
8	60	102.05

*Control procedure known to fully leach Baclofen from tablet.

Accuracy (recovery): The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. In order to prove the accuracy of the proposed method, a known quantity of the drug containing solution at five levels (LOQ, 50, 80, 100 and 150 % levels with reference to the concentration of preanalyzed sample solution) were spiked into the preanalyzed sample and the final solution was injected into the chromatographic system.

TABLE-4
FORCE DEGRADATION CONDITION AND OBSERVED RESULTS

Parameters	Conditions	Observed % degradation	Peak purity
Thermal degradation	80 °C 1 week (5-7 days)	0.09	Pass
Ultraviolet degradation	200 Wh/m^2	0.10	Pass
White light degradation	1.2 million lux hours	0.06	Pass
Acid degradation	0.1 N HCl Room temperature, 24 h	1.51	Pass
Base degradation	0.1 N NaOH Room temperature, 24 h	0.13	Pass
Oxidation (H_2O_2) degradation	3 % H_2O_2 Room temperature, 24 h	0.38	Pass

The results expressed as recovery of baclofen in ppm and variation of coefficients values are presented in Table-6.

TABLE-6
ACCURACY (RECOVERY) RESULTS OF BACLOFEN

Spike level	Added amount of baclofen (mg/mL)	Recovered of baclofen (mg/mL)	Recovery (%)
LOQ	1.486	1.545	103.95
	1.499	1.551	103.49
	1.503	1.572	104.58
		Mean recovery (%)	104.0
50 %	5.216	5.212	99.93
	5.227	5.238	100.21
	5.236	5.230	99.89
		Mean recovery (%)	100.0
80 %	8.458	8.417	99.52
	8.357	8.295	99.25
	8.289	8.284	99.94
		Mean recovery (%)	99.6
100 %	10.325	10.317	99.92
	10.338	10.285	99.49
	10.243	10.174	99.33
		Mean recovery (%)	99.6
150 %	12.084	12.079	99.96
	12.134	12.028	99.13
	12.434	12.410	99.81
		Mean recovery (%)	99.6
		Mean recovery (%)	99.7

Robustness: In order to assess the robustness of the proposed MLC strategy, it was examined by introducing six replicate injections of standard solution at 0.2 mg/mL under variable conditions *viz.*, different concentration of organic modifier and sodium dodecyl sulphate concentration in the mobile phase, column temperature, flow rate, different column lot, different wavelength and different pH of buffer solution. It was observed that the variation in these parameters does not significantly alter the system suitability parameters of the studied compound. As expected, the flow rate is the chief parameter that modifies retention time to the greatest extent. On the basis of the data obtained in the robustness test study, it was observed that the peak asymmetry of Baclofen was less than 1.5. Similarly the relative standard deviation (% RSD) in six replicate injections was found to be less than 1.0 %.

Precision: The intra-day analysis was determined by injecting test solutions six times on the same day, while the inter-day analyses correspond to the average of six measurements of the intra-day values. The results, expressed as relative standard deviations, for intra-day and inter-day values are given in Table-7. The data provides adequate precision for both analytes, which shows that the developed methodology is suitable for routine analysis.

TABLE-7
PRECISION RESULTS OF BACLOFEN
(INTRA-DAY AND INTER DAY)

Parameter	Inter-day (Precision)	Intra-day (Ruggedness)
Precision/ruggedness	99.88	99.71
Precision/ruggedness	99.45	99.92
Precision/ruggedness	99.60	99.53
Precision/ruggedness	99.77	99.80
Precision/ruggedness	99.42	99.72
Precision/ruggedness	99.62	99.56
Mean assay (%)	99.62	99.71
SD	0.18	0.15
RSD (%)	0.18	0.15
Mean assay (%)	99.67 (Precision and ruggedness)	
SD	0.16 (Precision and ruggedness)	
RSD (%)	0.30 (Precision and ruggedness)	

Conclusion

The submicellar liquid chromatographic method describe in this paper for the determination of baclofen in bulk and pharmaceutical formulation was found to be simple, sensitive, accurate, precise, rapid, robust and economical. Validation was performed as per the ICH guidelines with satisfactory results. However in the pharmacopeia, liquid chromatographic methods have reported already, were less sensitive, longer run time, more tailing in the analyte peak and using gradient program and some methods utilized phosphate buffers, ion pairing reagent both were consider to reduce the life time of column which leads increase the cost of the method too.

Here we reported a simple and sensitive method using micellar mobile phase. Furthermore it is quite worthy to mention that proposed methodology meets the fundamental requirements of "green chemistry". Also, the strategy is relatively inexpensive as compared to other methods, thereby making it more attractive and highly useful detection technique for many pharmaceutical analysis studies.

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