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Simple and Validated Ultraviolet Spectrophotometric Method for the Estimation of Baclofen in Bulk Form.

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ABSTACT

A simple, sensitive, precise, accurate and economical spectrophotometric method of analysis for Baclofen in bulk form was developed and validated. The method employed water as solvent and the drug shows maximum absorbance at 220 nm. The absorbance was found to increase linearly with increasing concentration of baclofen, which is corroborated by the calculated correlation coefficient value (r^2 = 0.999). The linear regression analysis data for the calibration plot showed good linear relationship with in the concentration range of 10 – 100 µg/ml. The limit of detection and limit of quantitation were found to be 1.25316 µg/ ml and 3.797468 µg /ml respectively. This method was tested and validated for various parameters according to ICH guidelines. The results demonstrated that the procedure is accurate, precise and reproducible (R.S.D. < 2 %).

Keywords: Baclofen, Validation, Estimation, UV spectrophotometry.

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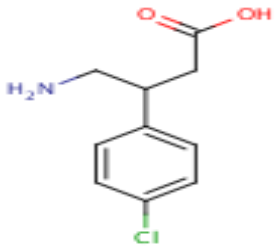
INTRODUCTION

Baclofen is chemically β-(amino methyl)-4-chlorobenzene propanoic acid and it is used as antispastic agent or muscle relaxant. The molecular formula of Baclofen is $C_{10}H_{12}ClNO_2$. 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 Indian Pharmacopoeia 2007.Physico-chemical properties of Baclofen are valuable tool and provide valuable clues for the choice of various feasible and possible conditions for HPLC separation. Baclofen is a compound of medium polarity and is amphoteric in nature. It occurs as zwitterions at pH 7 having pKa value of 3.89 (for strongest acid) and 9.79 (for strongest base) and a melting point of 192-193^0 and log P value 1.3. Literature survey reveals that few methods have been developed for Baclofen determination in bulk marketed preparation and in biological fluids with HPLC [1-7]. These methods though highly specific and sensitive for Baclofen, it is felt worthwhile to develop a simple and rapid UV method which will be accurate, precise and economical for determination of Baclofen in bulk forms by conducting systematic trials.

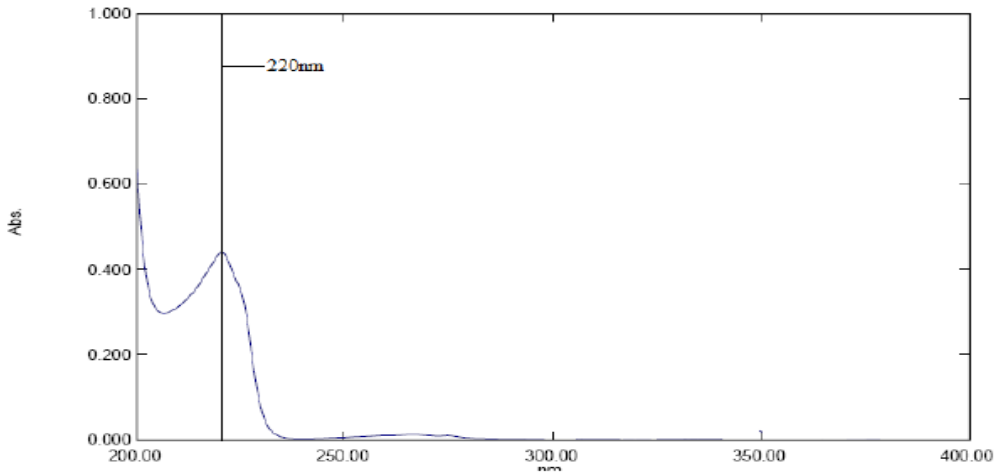
Figure 1: Baclofen Structure



MATERIALS AND METHODS

The spectrophotometric measurements were carried out using a double beam Schimadzu UV – Visible spectrophotometer (UV – 1800) connected to computer and loaded with UV-Probe software. The instrument has an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm (1.0 cm) cell path length. Shimadzu AUX-220 balance was used for weighing the samples. All the reagents used were of analytical grade.
In order to ascertain the wavelength of maximum absorption (λ max) of the drug, qualitative solution of the drug was prepared in water and scanned by using UV spectrophotometer within the wavelength region of 200– 400 nm against water as blank. The calibration curve was constructed for absorbance versus concentration of baclofen. The resulting spectrum was shown in Fig. 2, and the absorption curve showed characteristic absorption maxima at 220 nm for baclofen.

Figure 2: Scanning and determination of maximum wavelength (λ max)



Scanning spectra of Baclofen over scanning range 400-200 nm using water as blank

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Preparation of Standard Stock Solutions

Standard stock solution (primary) was prepared by dissolving 10 mg of baclofen in 10 ml of water to get concentration of 1mg/ml (1000µg/ml) and was stored at room temperature during the study. Secondary stock solution was prepared daily by diluting 1ml of the primary stock solution to final volume of 10 ml using water to get concentration of 0.1mg/ml (100µg/ml).

Preparation of calibration standard solutions

Suitable aliquots of the secondary standard solution of baclofen (10 – 60 ml) were transferred to a series of calibrated 100 ml standard volumetric flasks and the volume was made up to the mark with water.

RESULTS AND DISCUSSIONS

Method Validation

Validation is one of the most important steps in method development for analytical determinations. The main validation parameters such as linearity and range, accuracy and precision, recovery, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated in developed method

Linearity and range

Table 1: Calibration values of Baclofen

Concentration of Baclofen (µg/ml)	Abs. at 220 nm*
10	0.023
20	0.046
30	0.070
40	0.099
50	0.115
60	0.139
70	0.162
80	0.185
90	0.208
100	0.234

*Average of three determinations

Table 2: Summary of optical and regression parameters

Sr.no.	Parameter	Observations
Optical characteristics		
1	Apparent molar absorptivity (l/mol.cm)	8455
2	Sandell's sensitivity (µg/cm^2/A)	0.062
Regression Analysis		
1	Slope	0.002
2	Intercept	0.003
3	Regression Coefficient (r)	0.999
Validation Parameters		
1	λ max(nm)	220
2	Specificity	No interference at analyte wavelength
3	Beer's law limit (Linearity,µg/mL)	10-100 µg/ml
4	Limit of detection (µg/mL)	1.25
5	Limit of quantitation (µg/mL)	3.79

The absorbance values for different standard solutions (10, 20, 30, 40, 50, 60,70,80,90 and 100 µg/ml) of baclofen were measured at λ max 220 nm, against water as blank. Each point of the calibration graph corresponded to the mean value obtained from three independent measurements (Table 1). The calibration graph (Fig. 3) was constructed by plotting absorbance versus concentration of baclofen. The calibration graph of the absorbance versus concentration was found to be linear over the range of 10 – 100 µg/ml for the

proposed method. The linear regression equation obtained was y = 0.002x + 0.003, where y is the absorbance and x is the concentration in µg/ml of pure drug solution. The summary of optical and regression parameters was shown in Table 2.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of bulk samples of baclofen to 20 µg/ml so that overall concentration will be within the linearity range. The accuracy was expressed in terms of percent recovery. The mean of percentage recovery values was 93.48 – 100.88. The results were given in Table 3. The statistical analysis of data obtained for the estimation of baclofen indicates a high level of accuracy for the proposed method as evidenced by the low values of standard deviation and relative standard deviation.

Figure 3: Calibration Graph of Baclofen

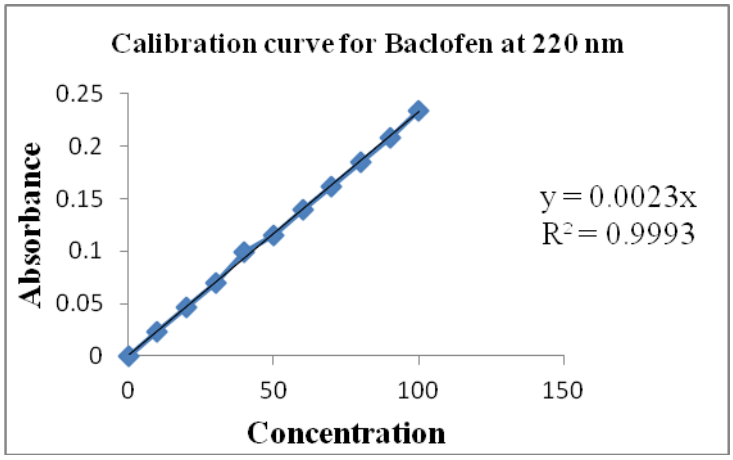

Table 3: Statistical analysis of data obtained for the estimation of baclofen

Level of recovery (%)	Nominal concentration used (µg/ml) (a)	Amount of drug spiked (µg/ml) (b)	Total amount of drug µg/ml (a+b) (µg/ml) (Therotical)	Amount of drug recovered (µg/ml) (Practical)	Statistical evaluation	% recovery = Practical/Therotical x 100
50	20	9.80	29.80	27.79		93.27
				28.30		94.97
				27.48		92.21
					Mean	93.48
					SD	1.39
					RSD	1.49
100	20	20.10	40.10	39.95		99.21
				40.20		100.25
				41.21		102.77
					Mean	100.88
					SD	1.67
					RSD	1.66
150	20	30.20	50.20	47.73		95.08
				47.29		94.20
				47.60		94.83
					Mean	94.70
					SD	0.45
					RSD	0.48
					Grand Mean	95.95

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Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Two different concentrations of baclofen in the linear range (20 and 30 µg/ml) were analyzed in six independent series in the same day (intra-day precision) and in six consecutive days (inter-day precision) and results were given in Table 4.

Table 4: Intraday and Inter-day precision readings of the proposed method.

Concentration of Baclofen (µg/ml)	Concentrations (µg/ml)*			
	Intraday (Mean ±SD) n = 6	% RSD	Inter-day (Mean ± SD) n = 6	% RSD
30	28.56 ± 0.376	1.318	30.63 ± 0.007	1.142
40	28.59 ± 0.322	1.126	40.44 ± 0.545	1.357

The RSD values of intra-day studies varied from 1.126 to 1.318 and inter-day studies varied from 1.142 to 1.357 showed that the precision of the method was satisfactory.

Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedure for assay of 20 µg/ml and 30 µg/ml of Baclofen using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to be reproducible, since there was no significant difference between analysts. Thus, the proposed methods could be considered rugged (Table 5).

Table 5

Sr.no	Test Concentration (µg/ml)	Concentration (µg/ml)			
		Analyst 1		Analyst 2	
1	20		20.578		20.515
			20.832		20.832
			20.642		20.642
			20.452		20.452
			20.578		20.578
			20.389		20.389
		Mean	20.580	Mean	20.570
		SD	0.160	SD	0.160
		% RSD	0.753	% RSD	0.764
2	30		28.933		28.870
			28.047		28.300
			28.933		28.616
			28.806		28.553
			28.363		28.363
			28.300		28.616
			28.560		28.550
			0.376		2.204
			1.318		0.715

Detection of LOD and LOQ

For determination of sensitivity of the proposed method, LOD and LOQ were calculated. Based on the signal to noise ratio they were quantified. The lowest detectable concentration of the analyte by the method is LOD where as the minimum quantifiable concentration is LOQ. LOD and LOQ for baclofen were calculated according to the ICH guidelines by using S (relative standard deviation of the response) and σ (slope of the calibration curve) and the results indicate that the proposed method is sensitive to detect and quantify.
LOD = 3.3 × σ /S = 1.25316 µg /ml and LOQ = 10 × σ /S = 3.797468 µg /ml

CONCLUSIONS

In this study a simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of baclofen in bulk form. The proposed method can be used for the routine quality control analysis of baclofen in bulk form. This method has the lowest LOD value and is more sensitive method. From the results obtained, we concluded that the suggested method showed high sensitivity, accuracy and precision.

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