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Method Development and Validation of Baclofen Mouth Dissolving Tablets by UV Spectroscopy

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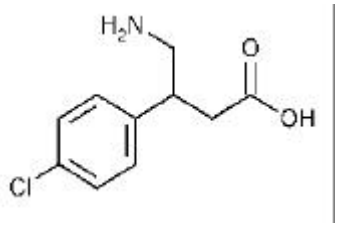
Abstract: The UV spectroscopic method of analysis is widely used for routine analytical procedure for determination of chemical compounds. The aim of this investigation was to develop and validate a simple, easy and sensitive UV spectrophotometric method for the analytical evaluation of the formulated Baclofen 5 mg experimental tablet. Baclofen shows maximum absorbance at a wavelength of 220 nm, which has been used in this study. The method provides a linear response from a quantitation range of 4µg/ml to 28 µg/ml in 0.1N HCl. The method gave satisfactory results in terms of repeatability (RSD 0.09%). The method was validated and proved to be robust and rugged. Our results demonstrate that UV spectrophometric method can be used for rapid determination of Baclofen in bulk and experimental formulation.

Key words: Baclofen % Spectrophotometric Method % Mouth Dissolving Tablet % Validation

INTRODUCTION

Mouth dissolving tablets are indicated for people who have swallowing difficulties and also ideal for active busy people [1-4]. The advantage of mouth dissolving dosage forms are increasingly being recognized and perceived by manufacturers and prescribers [5, 6]. Their growing importance has also been underlined in European Pharmacopoeia by introducing the alternate term “Orodispersible Tablet” that to be placed in the mouth where it disperses rapidly before swallowing [1, 4, 7].



According to European Pharmacopoeia, the orodispersible tablet should disperse/disintegrate in less than three minutes. The basic approach in development of mouth dissolving tablets is the use of superdisintegrants like cross-linked carboxymethylcellulose (croscarmellose), sodium starch glycolate, povidone like crospovidone etc, which ensure instantaneous disintegration of tablet after putting on tongue, thereby releasing the drug in saliva [3, 4]. The technologies used for manufacturing mouth dissolving tablets include freeze-drying, spray-drying, tablet molding, sublimation, tablet compression etc [4, 8, 9]. To the best of our knowledge, there is no compendial UV spectrophometric method available for Baclofen mouth dissolving tablets. Therefore, a UV spectrphotometric method and validation has been attempted for the analytical evaluation of Baclofen mouth dissolving tablets in our study [10-12]. In this study, the experimental batches formulated by direct compression method were subjected to analytical evaluation [13-15]. The current study demonstrates that our UV spectrophotometric method is simple and reproducible, therefore transformable to the industry for regular quality control tests.

Experimental: Baclofen, the active ingredient for the above formulation was received as a gift from Eskayef Bangladesh Limited. Other ingredients, such as distilled water, 0.1 N Hcl, whatman filter paper were provided by Popular Pharmaceutical Limited, Bangladesh. UV Spectrophotometer was used with a pair of 1 cm matched quartz cells. All other equipments used were routinely validated and of analytical grades.

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Method Development: 0.1 N HCl was evaluated as possible solvent for the assay of Baclofen from bulk and experimental formulation because it provided superior recovery and precision for the analyte of interest. The analytical method was also evaluated for the ruggedness and robustness along with its prospective utilization for the assay of analyte in the marketed formulation.

Preparation of Baclofen Standard Solution: 25 mg of the Baclofen standard (WS, Working Standard) was weighed and taken in a 100 ml volumetric flask. 60 ml diluting solution of 0.1N HCL (DS) was added and shaken well. The volume was adjusted up to 100 ml and shaken again and sonicated for 5 min. Then 5 ml of this solution was taken into a 100 ml volumetric flask and the volume was adjusted up to 100 ml. This diluted sample was scanned against 0.1N HCl as blank using Shimadzu UV-spectrophotometer and the absorption maxima of the solution was found at 220 nm.

Preparation of Baclofen Sample Solution: 10 tablets were weighed and the average weight was determined. Then 5 tablets were taken into a 100 ml volumetric flask. 60 ml DS was added and shaken for 15 min. in the shaker. The volume was adjusted up to 100 ml and sonicated for 10 min. and then filtered to achieve a clear solution. Then 5 ml solution was taken out into a 100 ml volumetric flask and the volume was adjusted up to 100 ml. The diluted sample was scanned against 0.1N HCl as blank using Shimadzu UV-spectrophotometer. The above solution showed absorption maxima at 220 nm.

Method Validation: We performed the following validation tests:

- Linearity
- Repeatability
- Reproducibility
- Selectivity/Specificity
- Robustness/Ruggedness
- System precision
- Method Precision

Preparation of Primary Stock Solution: 20 mg of Baclofen standard was accurately weighed and dissolved in 20 ml 0.1 N HCl to make the stock solution. 10 ml of this solution was diluted up to 100 ml using 0.1 N HCl. Then again 4 ml, 10 ml, 16 ml, 22 ml and 28 ml were separately taken into different flasks and diluted up to100 ml. Absorbance at 220 nm was taken using UV/Visible spectrophotometer and the values were plotted against concentration.

Linearity: The Linearity of an analytical method [10-12, 15] is its ability to elicit test results that are proportional to the concentration of the analyte with a given range. Beer's law states that absorbance is proportional to the concentration of the absorbing species [10, 12]. A calibration curve is prepared by plotting a dependent variable (absorbance Y) as a function of an independent variable (concentration X). This relation if found with a series of measurements in practice is often linear one.

Preparation of Standard Solutions and Calibration: The primary stock solution was prepared by dissolving 20 mg of Baclofen in 20 ml of 0.1 N HCl solution to obtain concentration of 1 mg/ml. 10 ml from this solution was diluted up to 100 ml using 0.1 N HCl to obtain a final concentration of 0.1 mg/ml. Then 4 ml, 10 ml, 16 ml, 22 ml and 28 ml were separately taken into different flasks and diluted up to100 ml. The absorbance was measured at 220 nm. The scanning range was finalized for study and solutions were scanned on spectrophotometer in the UV range of 200-400 nm. The absorbance was measured at the fixed wavelength of 220 nm and the values were plotted against the concentrations (Table 1). The following linearity equation was used for calculation -

$$y = 03206x-0.1232$$

Linearity of the analytical method for Baclofen in 0.1N HCl was established by the regression coefficient, R.

$$R^2 = 1.0\% \text{ (Figure 1)}$$

Repeatability: Repeatability is the precision obtained under the best possible circumstances (i.e. within the same laboratory, by the same analyst, by the same instrument and within one day when possible) and measures the size of the random error included in the method results [12]. We examined the repeatability of the above proposed method and results obtained (Table 2) indicated that the method is valid.

Ruggedness: Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions [12]. Results are shown in (Table 3).

Table 1: Observation of absorbance at different concentration of Baclofen

Concentration (µg/ml)	Absorbance (220 nm)
4	0.197
10	0.518
16	0.839
22	1.160
28	1.480
34	1.800

Table 2: Observation of Repeatability

Label claimed	Amount taken (mg/ml)	Amount found %	% RSD
5 mg	100.56	97.05±0.09	0.09

Table 3: Results of Ruggedness study

Label claimed	Analyst 1	%RSD	Analyst 2	% RSD
5 mg	97.05±0.08	0.08	97.08±0.09	0.09

Table 4: Data for Robustness Test

Variable parameter	Assay result
Analyst-1	97.08
Analyst-2	97.05
Equipment-1 (UV Spectrophotometer Model-UV-1700)	97.32
Equipment-2 (UV Spectrophotometer Model-UV-1601)	97.19
Day-1	97.67
Day-2	97.13

Reproducibility or Robustness: Reproducibility or Robustness of the method was determined by analyzing the Baclofen experimental tablets in different equipment on different day and by different analyst [11, 12]. It was observed that the method is robust enough to analyze Baclofen tablets (Table 4).

Unpaired *t* Test Results of Robustness

P Value and Statistical Significance: The two-tailed P value at 95% confidence interval equals 0.2556. By conventional criteria, this difference is considered not to be statistically significant.

Intermediate values used in calculations:

$$t = 1.3255 \quad df = 4$$

Standard error of difference = 0.176

Selectivity/Specificity: Selectivity is the ability of the method to provide a signal that is selective with respect to the property of interest (e.g. the concentration of the analyte of interest) [10, 12]. If the method is selective, the influence of potential interferences (matrix) on results is negligible. When the influence of interferences is significant, then the method cannot be considered selective.

Table 5: Data for System precision

Sample concentration (µg/ml)	Number of measurement	Absorbance	%RSD
	01	0.485	
	02	0.486	
	03	0.488	
15	04	0.485	0.26
	05	0.487	
	06	0.485	

Table 6: Data for Method precision

Sample no.	Sample weight (mg)	Assay (mg)	% of Label claimed	%RSD
01	508.90	4.78	95.02	
02	507.20	4.72	94.91	
03	507.80	4.75	94.96	0.04
04	508.70	4.74	95.00	
05	508.69	4.73	94.98	
06	508.72	4.76	95.01	

Placebo Interference: Placebo solution was prepared in the same manner as standard and sample preparation. No interference of placebo was found. The placebo showed highest absorbance at 200 nm wavelength spectrophotometrically. No interference of placebo was found.

System Precision / System Suitability: System suitability testing is an integral part of many analytical procedures. System suitability test parameters depend on the type of procedure being validated. System precision is determined by measuring the absorbance of standard solution containing 100% working concentration for six times and calculates the %RSD [9, 10]. The %RSD should be less than 2.0%. The relative standard deviation of six replicate measurements of standard solution was found to be 0.26% (limit NMT 2.0%), which indicates that the system is precise to analyze the sample (Table 5).

Method Precision: Method precision was established by analyzing six separate samples at 100% of the working concentration. Percent of result was calculated against claimed label. The %RSD of assay result of six separate samples from a single batch was found to be 0.04% (limit NMT 2.0%) which indicates that the method is precise to analyze the tablet (Table 6).

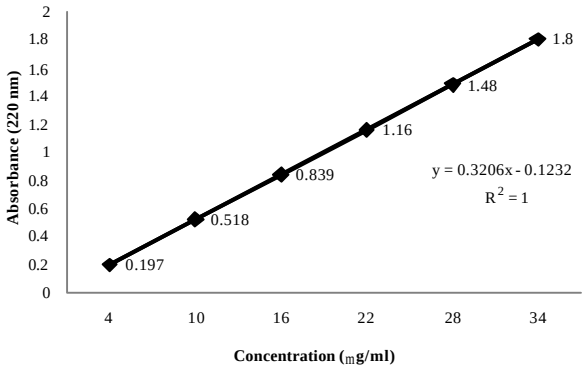

Fig: 1 Standard calibration curve of Baclofen

RESULTS AND DISCUSSIONS

It is evident from the standard calibration curve that there exists an excellent linearity characteristic with a R^2 value of 1.0% (Figure 1) [12]. The repeatability (Table 2) again shows the closeness of the observed results that enhance the reliability of the above method. The result of robustness (Table 4) has proved that there is no statistically significant difference exists within the values thus the method is robust enough to analyze [11, 12]. Again the Method precision and System precision showed that both the method (Table 5) and system (Table 6) is precise enough to analyze the formulated Baclofen tablets. Lastly the specificity as well as selectivity ensures that the observed data are totally free of any interference as the placebo interference is believed to be negligible (shows highest absorbance at 200 nm). So we can assure that the proposed method for the analytical evaluation of Baclofen is validated and can be transferred to the industry for further justification.

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