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VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF BACLOFEN

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ABSTRACT

Three simple and sensitive methods (methods A, B and C) for the determination of baclofen (BC) in bulk samples and formulations are described. Mehod A (λ_{max} 520 nm) is based on the reaction BC with safranin O (SFNO) to form an ion-association complex , which is extractable into chloroform aqueous phase; method B (λ_{max}520 nm) is based on the reaction of drug BC with brucine and sodium metaperiodate ($NaIO_4$) under acidic conditions forming coloured bruciquininone derivatives, while method C (λ_{max} 450 nm) is based on the reaction of drug with 2,3-dichloro – 5-6-dicyno-1,4-benzo-quinone (DDQ). The concentration measurements are reproducible within a relative standard deviation of 1%.

Key words: Baclofen, Spectrophotometry.

INTRODUCTION

Baclofen is a central skeletal muscle relaxant, relative beta-2-agonist. This drug is official BP[1], USP[2], EP[3], JP[4], Merck Index[5], Martindale Extra Pharmacopoeia[6], Remington[7] and PDR[8]. A survey of literature revealed that few methods based on UV[9,10]-visible spectrophotometry[11-15] (BC) have been reported. This paper describes three methods (methods A, B and C). In method A, safranin O reacts with the drug to form ion-associate complex at pH 9.8, which is extractable into chloroform layer from aqueous layer (λ_{max} 520 nm). In method B, brucine oxidises to quinine under acidic conditions, which in turn undergoes neucleophilic attack on the coupler (BC) to give coloured I-mono substituted bruciquinone derivative (λ_{max} 520 nm). Method C involves complex formation of BC with DDQ (λ_{max} 500 nm). The methods now proposed are applicable to bulk sample as well as formulations of BC.

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EXPERIMENTAL

A Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometers were used for the spectral and absorbance measurements. An Elico Li-120 digital pH meter was used for pH measurements.

Reagents

Aqueous solutions of safranine (2.857×10^{-4} M), brucine (5.067×10^{-3} M), H_2SO_4 (2.3 M) and pH 9.8 ammonia buffer were prepared.

Standard drug solution

One mg/mL stock solution of drug (BC) was prepared by dissolving 100 mg of drug in 100 mL of double distilled water for methods A and B. The working standard solutions of BC (500 μg/mL for method A and 200 μg/mL method B) were prepared.

One mg/mL stock solution of drug (BC) was prepared by dissolving 25 mg of drug in 25 mL of ethanol for method C. The working standard solution of BC (50 μg/mL) was prepared by dissolving it in distilled water.

Sample drug solution

Table equivalent to 50 mg of active ingredient (BC) was transferred to a 50 mL volumetric flask; shaken thoroughly with 1 mL of 0.1 N HCl followed by dilution to 50 mL with distilled water and filtered to remove insoluble portion, if any and further diluted as in standard solution preparation.

One-mg/mL stock solution was prepared by dissolving 25 mg of BC equivalent tablet powder in 25 mL ethanol.

Recommended procedures

Method A: Aliquots of standard drug solution (0.5-3.0 mL, 5.0 μg/mL; BC) were taken in a series of separating funnels and 5.0 mL (2.857×10^{-4} M) of safranine O and 1 mL of buffer (pH 9.8) solutions were added. The total volume of aqueous phase in each separating funnel was adjusted to 10 mL with distilled water and 10 mL of chloroform was added. The contents were shaken for 2 minutes. The two phases were allowed to separate and absorbence of the separated layers were measured at 520 nm against similarly prepared reagent blank. The amount of BC was calculated from the calibration curve.

Method B: Aliquots of the standard drug solution (0.5 – 3.0 mL, 100 μg/mL; BC) were transferred into a series of 10 mL calibrated tubes. Three mL of (5.067 x 10^{-3} M) brucine solution, 1.5 mL of (9.35 x 10^{-3} M) $NaIO_4$ and 2.0 mL of (2.3 M) H_2SO_4 were added to each tube and tota volume was made up to 10 mL with distilled water. The tubes were thoroughly shaken and placed in boiling water bath for 15 minutes. The reaction mixture was cooled to room temperature and made up to 10 mL with distilled water. The absorbence of each solution was measured at λ_{max} 520 nm against a reagent blank. The amount of BC in the sample was computed from the appropriate calibration graph.

Method C: Aliquots of drug solution (free base form) (0.5-3.0 mL, 1000 μg/mL; BC) were taken in a series of 10 mL calibrated tubes and the chloroform in each tube was evaporated in a hot water bath to dryness. The residue was dissolved in 0.5 mL of methanol and 20 mL DDQ (4.405 x 10^{-3} M) solution was added, shaken well and made up to mark with dichloromethane. Reagent blank was simultaneously prepared. The absorbance of solution was read at 500 nm against a reagent blank. The amount of the drug was computed from Beer's law plot.

RESULTS AND DISCUSSIONS

The reaction conditions were established by varying one parameter at a time and observing it's effect on the absorbance of the coloured species. In method A, the optimum conditions were found to be 4.0 – 6.0 mL of (2.85 x 10^{-4} M) safranine O and 1 mL of buffer solution was found to be necessary to maintain the pH 9.8 for maximum colour development and waiting time of 1-5 minutes was found to give maximum colour intensity.

In method B, the optimum conditions were found to be 2.3 M H_2SO_4 (1.5 – 2.5 mL), 2.5 – 3.5 mL of 5.067 x 10^{-3} M brucine and 1.2 – 1.8 mL of 9.35 x 10^{-3} M $NaIO_4$. Other oxidants such as Fe (III), Cr (VI), (IV), (V), IO_3^- and $S_2O_8^{-2}$ were tried instead of IO^-_4 and found to be inferior. In method C, 1.5 –2.8 mL of DDQ (4.405 x 10^{-3} M) solution was used and final dilution was done with dichloromethane.

The optical characteristics such as Beer's law limits, molar absorptivity,Sandell's sensitivity, correlation coefficient (r), regression equation, percent RSD range, of error (95% confidence limits) are listed in Table 1.

Commercially available formulation tablets of BC were successfully analysed by the proposed and reference methods. The values obtained by proposed methods are given in Table 2. Recovery experiments were carried out by adding known amounts of the drug (BC)

to it's pre-analysed formulation and the results are presented in Table 2. The commonly existing excipients and additives in tablets (BC) didn't interfere in the proposed methods.

Table 1: Optical and regression characteristics, precision and accuracy of the proposed methods for baclofen

Parameter	Method		
	A	**B**	**C**
	SFNO	**Brucine- IO_4^-**	**DDQ**
λ_{max} (nm)	520	520	500
Beer's Law Limits ($\mu g.mL^{-1}$)	2.5-15.0	5-30	2.5-150
Detection limit ($\mu g.mL^{-1}$)	3.693×10^{-1}	1.328×10^{-4}	9.56×10^{-2}
Molar absorptivity ($mole^{-1}\ cm^{-1}$)	5.8767×10^{3}	3.654×10^{3}	2.679×10^{4}
Sandell's sensitivity ($\mu g.cm^{-2}$/0.01 absorbance unit)	3.636×10^{-2}	5.847×10^{-2}	7.92×10^{-3}
Slope (b)	2.713×10^{-2}	1.729×10^{-2}	2.532×10^{-2}
Standard deviation on slope (S_b)	3.43×10^{-4}	7.862×10^{-5}	5.333×10^{-5}
Intercept (a)	6.0×10^{-4}	-1.533×10^{-3}	-8.0×10^{-4}
Standard deviation in intercept (S_a)	3.3399×10^{-3}	7.654×10^{-4}	8.068×10^{-4}
Standard error of estimation (S_e)	3.387×10^{-3}	8.223×10^{-4}	8.944×10^{-4}
Correlation coefficient (r)	0.99968	0.9999	0.9999
Relative standard deviation (%)*	0.6790	0.411	0.3741
0.05 level	0.7127	0.4314	0.3927
0.01 level	1.1177	0.6765	0.6159
% Error in bulk samples**	0.3636	-0.2906	0.2645

*Average of six determinations considered

** Average of three determinations

Table 2: Assay of BC in pharmaceutical formulations

Formulations*	Labelled	Amount Found by proposed methods**				% Recovery by proposed method***		
	Amount (mg)	A SFNO	B Brucine-IO_4^-	C DDQ	Reference method	A SFNO	B Brucine -IO_4^-	C DDQ
Tablets	10	9.94 ± 0.09	9.94 ± 0.05	9.91 ± 0.06	9.95 ± 0.10	99.46 ± 0.91	99.48 ± 0.59	99.16 ± 0.65
		F = 1.25	F = 0.31	F = 2.47				
		t = 0.12	t = 0.11	t = 1.09				
Tablets	25	24.96 ± 0.05	24.97 ± 0.04	24.96 ± 0.05	25.03 ± 0.05	99.87 ± 0.20	99.90 ± 0.16	99.86 ± 0.22
		F = 1.11	F = 1.73	F = 1.06				
		t = 0.99	t = 0.99	t = 0.99				
Tablets	10	9.94 ± 0.09	9.90 ± 0.12	9.94 ± 0.09	9.96 ± 0.10	99.43 ± 0.99	99.09 ± 1.24	99.47 ± 0.91
		F = 1.15	F = 1.36	F = 1.35				
		t = 0.90	t = 1.98	t = 1.34				
Tablets	25	24.92 ± 0.06	24.92 ± 0.19	24.92 ± 0.06	24.98 ± 0.11	99.71 ± 0.25	99.70 ± 0.76	99.69 ± 0.27
		F = 3.06	F = 2.92	F = 2.69				
		t = 0.95	t = 0.50	t = 1.02				

*Formulations from four different pharmaceutical companies.

**Average ± standard deviation on six determinations, the t– and F– test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57.

***Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

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