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Assay of Yohimbine Chloride in Bulk Samples and Pharmaceutical Formulations by Extractive Spectrophotometry

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Three simple and sensitive spectrophotometric methods (A–C) for the assay of yohimbine chloride in pure and dosage forms based on the formation of chloroform soluble ion-associates under specified experimental conditions are described. Three acidic dyes, namely, wool fast blue BL (WFB BL, Method A), tropaeolin-000 (Tp000, Method B) and naphthol blue 12BR (NB 12BR, Method C) are utilized. The extracts of the ion-associates exhibit absorption maxima at 580, 480 and 595 nm for methods A, B and C, respectively. Beer's law and the precision and accuracy of the methods are checked by the UV reference method. The results are reproducible with an accuracy of ±1.0%. The methods are found to be suitable for the determination of yohimbine chloride in the presence of the other ingredients that are usually present in dosage forms.

Key Words: Assay, Yohimbine, Ion-associates, Spectrophotometry.

INTRODUCTION

Yohimbine chloride[1] (YHB) is a principal indole alkaloid derived from the bark of the yohimbine tree (*Pusinystalia yohimbe* and *Corynanthe yohimbi*). It is also found in the Rauwolffia root and the dried bark of *Aspidosperma quebracho*. It is an α-adrenergic blocker and has been used for the treatment of impotency. It is chemically known as methyl-17 α-hydroxy-yohimban-16 α-carboxylate hydrochloride. Literature mentions few methods such as spectrophotometry[2–4], high performance liquid chromatography[5–10], mass spectroscopy[11], gas chromatography[12] and fluorimetry[13, 14] for its determination in biological fluids and dosage forms. Although spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, no colorimetric method has been reported so far for the determination of yohimbine. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing yohimbine chloride. The

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present paper describes three simple and sensitive extraction spectrophotometric methods for the determination of Yohimbine chloride, based on its tendency to form chloroform extractable ion-association complexes with acidic dyes WFB BL, Tp000 and NB 12BR under specified experimental conditions by exploiting the basic nature of the drug molecule.

EXPERIMENTAL

A Systronics 166 digital UV spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements. A Systronics 361 digital pH-meter was used for pH measurements.

All reagents and chemicals used were of analytical grade and doubly distilled water was used throughout. Aqueous solutions of WFB BL (BDH, Mumbai, India, 0.2%), TP000 (BDH, Mumbai, India, 0.2%) and NB 12BR (BDH, Mumbai, India, 0.2%) were prepared by dissolving the required amount in doubly distilled water. The solutions were washed with chloroform to remove the chloroform-soluble impurities and the residual solvent was removed by bubbling with nitrogen.

The glycine-HQ buffer[16] solutions (pH 1.3 for Method C and pH 1.5 for Method A) were prepared.

Preparation of standard drug solution

A 1 mg/mL solution was prepared by dissolving 100 mg of pure YHB in 100 mL of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations 100 μg/mL for M_1, M_2 and M_3, respectively.

Recommended procedures

In to a series of 125 mL separating funnels containing aliquots of standard YHB solution [0.5–2.5 mL, 100 μg/mL (M_1, M_2 or M_3)], 6.0 mL of buffer solution pH 1.5 (M_1 or M_3) or 0.1 M HCl (M_2) 2.0 mL of dye solution [WFB BL (M_1), TP000 (M_2) and NBB (M_3)] were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 mL with distilled water and 10 mL of chloroform was added. The contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 590 nm (M_1), 480 nm (M_2) and 620 nm (M_3) against the reagent blank. The amount of YHB was calculated from the calibration plot.

RESULTS AND DISCUSSION

The optimum conditions for the colour development in each method were established by varying the parameters one at a time[14], keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

Optimum conditions fixation

Conditions under which the reaction of YHB with each dye fulfills the essential

analytical requirements were investigated. All the experimental conditions studied were optimized at room temperature (25 ± 1°C) and were established by varying one parameter at a time (10) and observing its effect on the absorbance of the coloured species.

Different organic solvents such as benzene, toluene, nitrobenzene, carbon tetrachloride, 1,2-dichloromethane, chloroform, ethyl acetate and isobutyl ketone were tested for the extraction of the ion-association complex formed between YHB and each dye. Chloroform was suggested as the solvent of choice for the extraction of the coloured complex with respect to maximum stability.

In order to establish the optimum pH range (for M_1–M_3), YHB was allowed to react with the respective dye in aqueous solution buffered between pH 1.0–10.0 and the complex formed was extracted into chloroform for absorbance measurement. The result shows that a quantitative extraction was produced between pH 1.1–1.5 (for M_1, M_3) or 0.1 M HCl (for M_2). All subsequent studies were carried out at pH 1.5 (for M_1, M_3) or 0.1 M HCl (for M_2). The volume of this buffer added (4–10 mL) had no effect in methods for M_1, M_2 (pH 1.5) and M_3 (0.1 M HCl), respectively. A 6.0 mL portion of buffer was found to be optimal in methods M_1, M_2 and M_3. The minimum shaking time was determined by varying the shaking time from 1–10 min, although 1 min was sufficient, prolonged shaking had no adverse effect on the extraction and 2 min was selected for this study. A ratio of 2 : 3 of organic to aqueous phases was required for efficient extraction of the coloured species and lower reagent blank reading. It was found that better reproducibility and lower reagent blank were achieved if the dye was purified by extraction with chloroform initially. The colour products were stable up to 30 min. The stoichiometric ratio of the YHB to dye was found as 1 : 1 with WFB BL or TP000 and 2 : 1 with NB 12BR through slope analysis method.

Chemistry of the coloured species

YHB possesses tertiary nitrogen, involves in ion-association complex formation with an acid dye (WFB BL, M_1; Tp000, M_2; NB 12BR, M_3), which is extractable into chloroform. The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing. The possible structure of the ion-association complex in each instance was established based on the analogy reports for similar types of molecules with acidic dyes and was further confirmed by slope-ratio studies. The protonated nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behaves as a single unit being held together by electrostatic attraction.

Interference studies

The interference studies in the determination of YHB in pharmaceutical formulation revealed that the normally existing excipients and additives like

starch, lactose, gelatin, talc, magnesium stearate, aluminum hydroxide, sorbitol, calcium silicate and glycerine do not interfere even when present in excess of the anticipated amount.

However, a preliminary clean up procedure with chloroform is necessary to avoid interference due to the presence of reducing sugars like lactose, if present, prior to the estimation of YHB in formulations for Methods A, B and C, respectively.

Analytical data

The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity for the method are given in Table-1. The precision of the method was found by measuring absorbances of six replicate samples containing known amounts of drug. Regression analysis using the method of least squares was made to evaluate the parameters. The accuracy of the methods was ascertained by comparing the results by the reference method (Table-2). This comparison shows that there is no significant difference between the results of studied methods and those of the reference one.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR YOHIMBINE

Parameters	Method M_1	Method M_2	Method M_3
	WFB BL	TP000	NB 12 BR
λ_{max} (nm)	590	480	620
Beer's Law limits (µg/mL)	5–25	5–25	2–10
Molar absorptivity (L mol^{-1} cm^{-1})	1.250×10^4	8.440×10^3	2.439×10^4
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0317	0.0360	0.0160
Regression equation (y = a + bc): Slope (b)	0.0312	0.0270	0.0610
Standard deviation on slope (S_b)	129×10^{-4}	2.50×10^{-4}	5.80×10^{-4}
Intercept (a)	0.0069	0.0013	0.0009
Standard deviation on intercept (S_a)	1.98×10^{-3}	3.3×10^{-3}	3.82×10^{-3}
Standard error of estimation (S_e)	1.89×10^{-3}	3.2×10^{-3}	3.65×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9998
Relative standard deviation*	0.4230	0.2380	0.3560
% error in bulk sample (95% confidence limit)†	0.3760	0.0690	0.2320

*Average of six determinations considered.
†Average of three determinations.

TABLE-2
ASSAY OF YHB IN PHARMACEUTICAL FORMULATIONS

Pharmaceutical sample (Labelled amount)	% Recovery (mg)			
	Proposed method			Ref. method
	M_1	M_2	M_3	
T_1 (5 mg)[a]	99.97 ± 0.49	99.89 ± 0.37	99.20 ± 0.88	99.51 ± 0.70
	t = 11	t = 1.54	t = 0.62	
	F = 1.27	F = 1.35	F = 1.58	
T_2 (5 mg)[b]	99.63 ± 0.21	99.81 ± 0.17	99.28 ± 0.54	98.93 ± 0.77
	t = 1.71	t = 0.98	t = 1.31	
	F = 2.67	F = 2.01	F = 2.03	
T_3 (5 mg)[a]	100.13 ± 0.63	99.88 ± 0.54	99.25 ± 0.65	99.48 ± 0.88
	t = 1.16	t = 0.46	t = 0.75	
	F = 2.06	F = 1.54	F = 1.83	
T_4 (5 mg)[b]	99.27 ± 0.21	99.86 ± 0.24	99.56 ± 0.69	98.90 ± 0.35
	t = 0.22	t = 0.99	t = 0.15	
	F = 2.57	F = 3.72	F = 1.88	

Average (± RSD) of six determinations; the t and F values refer to comparison of the proposed method with the reference method; theoretical values at 95% confidence limits, t = 2.57, F = 5.05.

Conclusion

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi-component mixture. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibilities in the assay of a particular component in a complex dosage formulation. In the present study, YHB was determined successfully as a pure compound as well as a component in representative dosage formulation. The proposed methods are simple, selective and can be used in the routine determination of YHB in bulk samples and formulations with reasonable precision and accuracy.

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