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Oxidative Coupling Reactions for the Estimation of Yohimbine

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Three simple spectrophotometric methods (A–C) for the assay of yohimbine chloride (YHB) in pure state and in formulations have been developed based on the oxidative coupling reaction of YHB, with N,N-dimethylamino-*p*-phenylenediamine (DMPD) in the presence of chloramine-T (CAT) (Method A), 4-aminophenazone (4-AP) in the presence of IO_4^- (Method B) and 3-methyl benzothiazolinone hydrazone (MBTH) in the presence of cerium(IV) [Ce(IV)] (Method C). Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 8.0–24.0, 4.0–20.0 and 10.0–50.0 µg/mL for methods A, B and C, respectively. The results of analysis have been validated statistically and by recovery studies.

Key Words: Yohimbine, Oxidative coupling, Estimation.

INTRODUCTION

Yohimbine chloride[1] (YHB) is a principal indole alkaloid derived from the bark of the yohimbine tree (*Pusinystalia yohimbe*, *Corynanthe yohimbi*). It is also found in the rauwolffia root and the dried bark of *Aspidosperma quebracho*. It is α-adrenergic blocker and has been used for the treatment of impotence. It is chemically known as methyl-17-α-hydroxy-yohimban-16-α-carboxylate hydrochloride. Literature mentions a 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. Three visible spectrophotometric methods A, B and C, based on the oxidative coupling reaction of YHB with the reagents such as DMPD-CAT (Method A), 4-AP-IO_4^- (Method B) and MBTH-Ce(IV) (Method C) have been developed. All the methods are applicable to the determination of YHB in bulk form and in formulations.

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EXPERIMENTAL

Spectral and absorbance measurements were made on Systronics UV-Visible spectrophotometer 166 with 10 mm matched quartz cells. A Systronics 365 digital pH-meter was used for pH measurements.

All the chemicals used were of analytical grade. All the solutions were prepared freshly in doubly distilled water.

Aqueous solutions of 2.39×10^{-3} M DMPD (Merck) and 7.11×10^{-4} M CAT (Loba) were prepared for method A. Aqueous solutions of 2.46×10^{-2} M 4-AP (Ferack) and 4.68×10^{-3} M IO_4^- (BDH) were prepared for Method B. Aqueous solutions of 8.58×10^{-3} M MBTH (Loba) and 1.58×10^{-2} M ceric ammonium sulphate in 0.36 N H_2SO_4 (BDH) were prepared for Method C.

Preparation of drug solutions

The stock solution (mg/mL) of yohimbine hydrochloride (YHB) was prepared by dissolving 100 mg of it in 100 mL of distilled water. A portion of this stock solution was diluted stepwise with the same solvent to obtain the working standard YHB solution of concentration of 200 μg/mL (Methods A–C). Sample solutions for formulations (tablets) were prepared exactly in the same manner as given under the standard solutions with prior filtration before making up to volume and analyzed as described for pure samples.

Procedures

Method A: Aliquots of YHB solution (1.0–3.0 mL, 200 μg/mL) were transferred into a series of 25 mL calibrated tubes. Then 1.0 mL of DMPD solution (0.05%), 1.0 mL of CAT and 4.0 mL of isopropanol were added successively to each tube. The volume was brought up to 15 mL with distilled water and kept aside for 10 min at room temperature. The volume was made up to the mark with methanol. The absorbances were measured at 520 nm against a reagent blank prepared in a similar manner during the stability period (10–45 min). The amount of YHB was calculated from the calibration graph.

Method B: Aliquots of standard YHB solution (0.5–2.5 mL, 200 μg/mL) were transferred into a series of 25 mL calibrated tubes. Then 2.0 mL of 4-AP and 5.0 mL of $NaIO_4$ solutions were added and kept aside for 3 min. The volume in each tube was made up to the mark with distilled water. The absorbance was measured at 530 nm against a similar reagent blank. The amount of drug was computed from its calibration graph.

Method C: Volumes of standard YHB (0.5–2.5 mL, 200 μg/mL) were transferred into a series of 10 mL graduated tubes. The total volume in each tube was brought to 3.0 mL with distilled water. 1 mL each of MBTH and ceric ammonium sulphate were added and the tubes were kept aside for 5 min at room temperature. The solutions in each tube were made up to the mark with distilled water and the absorbances were measured after 5 min at 620 nm against a reagent blank. The amount of YHB present was computed from its calibration graph.

RESULTS AND DISCUSSION

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

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table-1. The precision of each method was found by measuring absorbances of six replicate samples containing known amounts of drug and the results obtained are incorporated in Table-1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and are presented in Table-1. The accuracy of each method was ascertained by comparing the results by proposed and reference methods (UV) statistically (Table-2). This comparison shows that there is no significant difference between the results of proposed methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods, the additives and excipients that are usually present in tablets do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were performed by adding a fixed amount of the drug to the pre-analyzed formulations. The amount of drug found and the % recovery were calculated in the usual way.

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

Parameters	Method A	Method B	Method C
λ_{max} (nm)	520	530	620
Beer's law limits (μg/mL)	8.0–24.0	4.0–20.0	10.0–50.0
Molar absorptivity (L mol^{-1} cm^{-1})	1.84×10^4	1.24×10^3	4.45×10^4
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.0420	0.0320	0.0460
Regression equation (y = a + bc)*:			
Slope (b)	0.0240	0.0310	0.0110
Intercept (a)	–0.0040	–0.0008	0.0012
Correlation coefficient (r)	0.9999	0.9999	0.9998
Relative standard deviation(%)†	0.5930	0.3530	0.484
% range of error† (0.05 level confidence limit)	0.3760	0.0690	0.2320

*Y = a + bc, where c is the concentration in g/mL
†From six determinations.

The proposed methods are applicable for the assay of drug (YHB) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and λ_{max} among the proposed methods are C > B > A, respectively. 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.

TABLE-2
DETERMINATION OF YHB IN PHARMACEUTICAL FORMULATIONS

Sample (tablets)*	Labelled method (mg)	Amount obtained (mg)				Recovery (%)		
		UV method*	Proposed method					
			A	B	C	A	B	C
T_1	5	4.98	4.97	4.99	4.99	99.71	99.87	99.80
T_2	5	4.99	5.01	4.99	5.01	100.08	99.90	100.20
T_3	5	4.98	5.01	5.01	4.99	100.22	100.20	99.97
T_4	5	4.99	4.99	4.98	4.99	99.80	99.70	99.80

*Four different batches of tablets from a pharmaceutical company.

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