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A NOTE ON THE ESTIMATION OF YOHIMBINE CHLORIDE IN BULK AND PHARMACEUTICAL FORMULATIONS BY PRECIPITATION REAGENTS

K. V. S. PARASADA RAO, D. SIREESHA, C. SRINIVASULU, G. PRABHAKAR*

Department of Pharmaceutical Sciences,
Andhra University, VISAKHAPATNAM–530 003 (A.P.) INDIA

and

JAMEELUNNISA BEGUM

Department of Chemistry, St. Therrisa College for Women.

ABSTRACT

Simple spectrophotometric methods (A–C) for the assay of yohimbine chloride[1] (YHB) based on the formation of its complexes with alkaloidal precipitants are described. YHB undergoes quantitative precipitation in the form of molecular complexes with iodine (I_2, Method A), ammonium molybdate (AM, Method B) or phosphomolybdic acid (PMA, Method C), when used in excess. In addition to precipitation reactions, color reactions have also been combined to estimate YHB. These are based on the color formation with either unreacted precipitant of the filtrate (I_2) or released precipitant from the molecular complex (AM or PMA) with chromogenic reagent such as p–N–methyl aminophenol sulphate (PMAP)–sulphanilic acid (SAc) (for I_2), potassium thiocyanate (for AM), cobalt nitrate (Co (II))–disodium salt of ethylenediamine tetra acetic acid (EDTA) complex (for PMA).

Key word : Yohimbine, Precipitation reagent.

INTRODUCTION

Alkaloids are detected with the aid of group of reactions due to their chemical properties, structure and presence of functional groups. These reactions are based on the ability of the alkaloid to yield insoluble complexes mainly with AM, I_2 and PMA and hence, these reagents are named as alkaloidal precipitants[2]. The precipitate is ascribed due to the formation of a

*Address for communication: Prof: G.Prabhakar,
Professor, Pharmaceutical Food & Drug Analysis, Department of Pharmaceutical Sciences,
Andhra University, Visakhapatnam – 530 003. (A.P)

molecular complex resulting from the interaction of the unshared electron on nitrogen in amine with an unoccupied molecular orbital of the alkaloidal precipitant molecule. Yohimbine chloride (YHB) is a principal indole alkaloid derived form the bark of the yohimbine tree (Pusinystalia yohimbe, corynanthe yohimbi). It is also found in the Rauwolfia root and the dried bark of Aspidosperma quebracho. It is α–adrenergic blocker and it has been used for the treatment of impotence. It is chemically known as methyl–17–alpha–hydroxy – yohimban –16 alpha–carboxylate hydrochloride. Literature mentions a few methods such as spectrophotometry[3–6], high performance liquid chromatography[7–12], mass spectroscopy[13], GC[14] and flourimetry[15] for its determination in biological fluids and dosage forms. Very few visible spectrophotometric methods for the determination of YHB have been reported. The aim of the present work is to provide simple and sensitive visible spectrophotometric method for the estimation of YHB in bulk form and formulations. The effects in this accord resulted to develop the present methods.

EXPERIMENTAL

Instruments: Spectral and absorbance measurements were made on Systronics UV–Visible Spectrophotometer 117 with 10 mm matched quartz cells.

Reagents : All the chemicals and reagents used were of analytical grade and the solutions were freshly prepared. Aqueous solution of I_2 (0.089%) in 0.83% of potassium iodide (KI), PMAP (2%), SAc (0.4%), hydrochloric acid (HCl) (1M) for method A; AM (2%), PTC (10%), conc. HCl (used as it is) for method B; PMA (4%) Co(II) (3%), DETA (4%) for method C, 0.01 M HCl for methods B and C were prepared in triple distilled water. A one 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 50 µg/mL for method A, 400 µg/mL for method B and C, respectively.

Method A

Aliquots of working standard solution (0.5–2.5 mL, 400 µg/mL) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 mL with distilled water. Then 2.0 mL each of 1M HCl and I_2 were added successively and centrifuged for 5 min. The precipitate was collected by filtration and subsequently washed with 2 mL distilled water. The filtrate and washings were collected in 25 mL graduated test tubes. Then 3.0 mL of PMAP solution and 2.0 mL SAc solution were added successively and the volume was made up to the mark with distilled water. The absorbance was measured during next 30 min. at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and in turn drug concentration was obtained by subtracting the absorbance of the test solution from blank. The amount of drug was calculated from calibration graph.

Method B

Aliquots of working standard solution (0.5–2.5 mL, 400 µg/mL) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 mL with 0.01 M HCl. Then 1.0 mL of AM was added and centrifuged for 5 min. The precipitate was collected by filtration followed by washing with 50% alcohol until it is free from the reagent. The precipitate in each tube was dissolved in 5.0 mL of acetone and transferred into 25.0 mL graduated tube. The 5 mL of conc. HCl and 3 mL PTC solution were successively added and kept aside for 30 min and then volume in each tube was made up to the mark with distilled water. The absorbance was measured at 460 nm against a similar reagent blank. The amount of drug YHB was calculated form the calibration graph.

Method C

Aliquots of working standard solution (0.5–2.5 mL, 50 µg/mL) were delivered into a series of centrifuge tubes and volume in each tube was adjusted to 3.0 mL with 0.01m HCl. The 2.0 mL PMA was added and centrifuged for 5 min. The precipitate was collected by filtration followed by washing with distilled water until it is free from the reagent. The precipitate in each tube was dissolved in 5 mL of acetone and transferred into 25 mL graduated tubes. One mL each of Co (II) and EDTA solutions were successively added and the tubes were heated for 15 min. at 60^{o}C in water bath. The tubes were cooled and the solution in each tube was made upto the mark with distilled water. The absorbance was measured at 840 nm against a similar reagent blank. The amount of drug was calculated from its calibration graph.

RESULTS AND DISCUSSION

The optimum conditions for the color development of methods (A, B and C) were established by varying the parameters one at a time keeping the others fixed and observing the effect produced on the absorbance of the colored species.

The optical characteristics such as Beer's law limits; molar absorptivity and Sandell's sensitivity for each method (A–C) are given in Table1. The precision of each method to the drug was found by measuring the absorbance of six separate samples containing known amounts of drug and the results obtained are incorporated in Table1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and standard error of estimation (S_e) for each system and is presented in Table 1.

The accuracy of the methods was ascertained by comparing the results by proposed and reference methods (UV) statistically by t– and F–tests (Table 2). The comparison shows that

Table 1. Optical characteristics, precision and accuracy of the proposed methods for YHB

Parameters	Method A	Method B	Method C
$\lambda_{max\ (nm)}$	520	460	840
Beer's Law limits (μg/mL)	8–40	8–40	1–5
Molar absorptivity (l $mole^{-1}cm^{-1}$)	5.094×10^3	6.010×10^3	5.707×10^4
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	0.07674	0.06504	0.00685
Regression Equation y = a + bc*			
Slope (b)	0.01305	0.01536	0.1482
Intercept (a)	– 0.00040	– 0.00170	– 0.00640
Correlation coefficient (r)	0.9999	0.9999	0.9997
Relative Standard Deviation (%)**	0.453	0.343	0.258
% Range of error ** (0.05 level confidence limit)	0.379	0.287	0.216

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

Table 2. Determination of YHB in pharmaceutical formulations

Sample$^{\Delta}$ (Tablets)	Labeled method (mg)	Amount obtained (mg)				+		
		UV* Method	Proposed method			Recovery (%)		
			A	B	C	A	B	C
T_1	5	4.98± 0.022	4.99± 0.010 F = 2.57 t = 0.22	5.01± 0.026 F = 1.43 t = 0.87	4.99± 0.010 F = 2.67 t = 0.22	99.97	100.22	99.97
T_2	5	4.99± 0.006	5.01± 0.020 F = 1.36 t = 0.78	4.99± 0.024 F = 1.25 t = 1.08	5.00± 0.031 F = 2.06 t = 1.16	100.1	99.97	100.13
T_3	5	4.99± 0.009	4.98± 0.027 F = 1.54 t = 0.46	5.00± 0.032 F = 2.07 t = 1.15	4.98± 0.033 F = 2.09 t = 1.14	99.88	99.87	100.13
T_4	5	4.98± 0.034	5.00± 0.006 F = 1.18 t = 0.99	4.99± 0.027 F = 1.46 t = 0.38	5.01± 0.025 F = 1.42 t = 0.88	99.99	99.89	100.22

$^{\Delta}$Four different batches of tablets from a pharmaceutical company.

there is no significant difference between the results of studied methods and those of reference ones. The similarity of the results is obvious that during the application of these methods, the excipients that are usually present in pharmaceutical formulations do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods recovery experiments were carried out. The recovery of the added amounts of standard drug were studied at 3 different levels. Each level was repeated 6 times. From the amount of drug found, the % recovery was calculated in the usual way.

The higher λ_{max} values of all the proposed methods have a decisive advantage since the interference from the associated ingredients should be generally less at higher wavelengths than at lower wavelengths. Thus the proposed visible spectrophotometric methods are simple and sensitive with reasonable precision, accuracy and constitute better alternatives to the existing ones for the routine determination of YHB in bulk forms and pharmaceutical formulations.

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