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Original Article

DETERMINATION OF HYDROLYSIS PARAMETERS OF YOHIMBINE HCl AT NEUTRAL AND SLIGHTLY ACIDIC MEDIUM

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ABSTRACT

Objectives: In the process of investigating various new drug delivery systems of yohimbine HCl (Yoh), it was necessary to study some of the physical chemical properties of the drug including its stability at neutral, acidic and slightly acidic conditions.

Methods: A validated HPLC method was developed and employed for analysis of Yoh containing solutions. The mobile phase composed of 60% menthol: 40% NaOAc (%v/v), and Gemi C18 column, 5 µm particle size was used as a stationary phase. The degradation product was found to be yohimbinic acid (YA). The retention times for Yoh and YA were 5 and 3 minutes, respectively. This study investigated the kinetics of hydrolysis of Yoh at pH 6.0 and 7.0 at temperatures from 50 °C to 80 °C.

Results: The reaction followed first order kinetics and the activation energy ΔE of the reaction at pH 6 and pH 7 was found to be 16.2 and 16.8 Kcal. $mole^{-1}$, respectively. While the values of A were found to be 41.8 and 44.1 Kcal. $mole^{-1}$ at pH 6 and 7, respectively. The pseudo first order rate constants (K) at pH 6 and 7 were calculated as 2.76 x 10^{-3} h^{-1} and 3.42 x 10^{-3} h^{-1}, respectively.

Conclusion: Such results indicate high stability of the drug at these pH values. At highly acidic medium the reaction was found to be extremely slow indicating the absence of acid catalysis on the hydrolysis of Yoh. Thus, the yohimbine ester group resists hydrolysis in highly acidic conditions.

Keywords: Yohimbine HCl, Yohimbinic acid, Stability, pH, Hydrolysis, Kinetic, Activation, Energy.

INTRODUCTION

Yohimbine is the principal indole alkaloid extracted from the stem bark of the yohimbe tree (*Pausinystalia yohimbe* Rubiaceae (K. Schumann) Pierre ex Bielle). It can be also isolated from rauwolfia roots (*Rauwolfia serpentina* Apocynaceae (Benth)). Traditionally, all forms of impotence have been treated with extracts of Yohimbe tree [1]. It is a competitive antagonist selective for α -2-andrenoceptors, which are thought to be located on nerve terminals and receptors to mediate inhibition of transmitter release [2]. The presynaptic release of noradrenaline is increased by α-2-antagonists resulting in increased sympathetic outflow. It may also interact with α-1-adrenoceptors and, in high concentrations, serotonin and dopamine receptors [3].

Yohimbine is a monoamine oxidase inhibitor that has the potential to interact with tyramine-containing foods and stimulants such as phenylephrine and phenylpropanolamine [4]. It affects the gastrointestinal, genitourinary, respiratory, cardiovascular [2, 5] and central nervous systems [6-8]. It is commonly indicated to treat impotence, orthostatic hypotension, and diabetic neuropathy in a dose of 15 mg/day. Furthermore, its use for the treatment of male erectile dysfunction has been longer than the use sildenafil for such indication [1, 8, 9].

In street use, it is a mild hallucinogenic and aphrodisiac [10]. Owen et. al proposed that yohimbine is eliminated via metabolism because its rapid plasma clearance was not due to renal elimination or sequestration by RBC [11]. Whilst, incomplete absorption of yohimbine from the gastrointestinal tract or liver first pass effect might be responsible for the low oral bioavailability [12].

Chemically, yohimbine is methyl ester of 17, α-hydroxy-yohimban-16, α-carboxylic acid (fig. 1). It is a colorless, weakly basic, tertiary indole alkaloid with a pK_a of 6–7.5. It is readily soluble in chloroform and ethanol and is sparingly soluble in diethyl ether with a characteristic ultraviolet spectrum of λ_{max} 279–282 nm [13, 14].

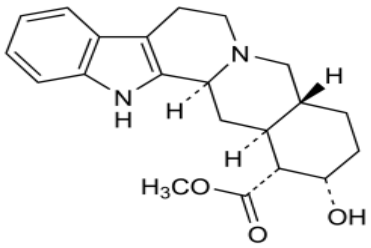

Fig. 1: Structural formula of yohimbine

Studying the kinetics of drug hydrolysis based on an Arrhenius equation to calculate the main degradation activation energy is a crucial part of the pre-formulation stage of drug formulation and development. To our knowledge, there has been no research concerning the hydrolysis kinetics of yohimbine HCl (Yoh) at neutral and slightly acidic medium. However, the mechanism and kinetics of hydrolysis of yohimbine base in the highly alkaline medium have been studied [15].

The aim of the present work is to study the kinetic of hydrolysis of Yoh at two different pH values; 6 and 7 at temperatures of 50, 60, 70 and 80 °C using a validated HPLC method. Furthermore, hydrolysis of Yoh was investigated at low pH.

MATERIALS AND METHODS

Solvents and chemicals

All chemicals used throughout this study were of analytical grade. Anhydrous NaOAc was purchased from Fisher Scientific Company, HCl from Merck. Methanol and bi-distilled water were obtained from Riedeldehaen, Sigma–Aldrich, Germany, Sodium hydroxide from

Medex. Yoh HCl 98.56% and YA were purchased from Dalian Ruishengda International Trade CO., China.

Instrumentation and chromatographic conditions

For HPLC analysis a Finnigan Surveyor (Thermo Electron Corporation, CA, USA) was employed coupled with detector UV-ViS and an auto-sampler and LC pump. A mobile phase consisting of (60% methanol: 40% 0.1M, NaOAc) with a pH of 7 (adjusted with Hydrochloric acid) was circulated through Phenomenex, Gemini column with particle size of 5 µm and dimensions of 150 x 4.6 mm. The flow rate was 1 ml/ min with an injection volume of 25 µl. Absorption was measured at 280 nm wavelengths that was optimum for Yoh and YA. Triplicate injections were analyzed for each sample. The chromatographic conditions were modified from an established method [15] then optimized and validated to analyze Yoh and YA. (table 1).

Table 1: Chromatographic conditions employed for HPLC analysis of yohimbine HCl and yohimbinic acid

HPLC conditions	Pump flow rate	Auto sampler injection volume	Auto sampler temp	Column oven temp
	1.0 ml/min	25 µl	25˚C	25˚C
Chromatography	Mobile phase	60% methanol: 40% 0.1M NaOAc (pH 7) (pH adjusted with phosphoric acid)		
	Column type	Phenomenex, Gemini C18 column (150 x 4.6 mm i. d 5µ)		
	Expected Retention times (min)	Yohimbine	Yohimbinic acid	
		5	3	
Detection Conditions	Wavelength	280 nm		

Standard solutions and calibration

Standard solutions of Yoh and YA within the concentration range of 6.5 µg/ml to 100µg/ml in methanol were freshly prepared. The calibration curve samples were analyzed using HPLC and the calibration curves were plotted.

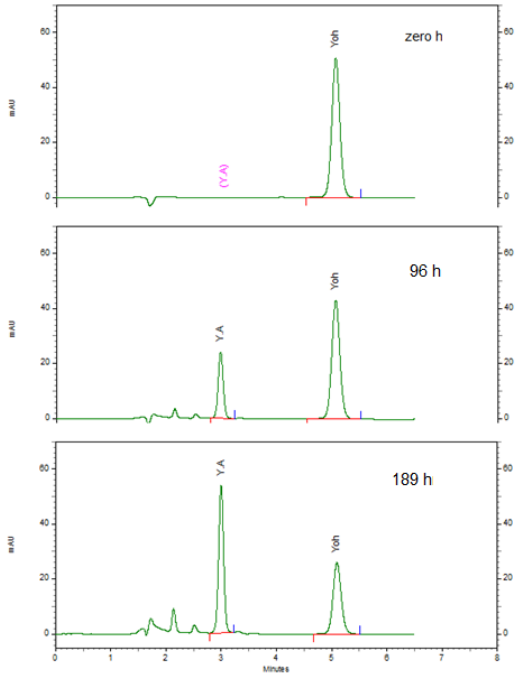

Fig. 2: Representative chromatograms of yohimbine HCl and yohimbinic acid at pH 6 at zero time, after 96 h and 189 h

Kinetic measurements

A stock solution of Yoh in methanol was prepared by weighing 50 mg (Phoenix analytical balance, USA) in 10 ml to get a concentration of 5000 µg/ml. The reaction was initiated by diluting 1 ml of stock solution in 100 ml solutions of pH 6 (adjusted by HCl) and pH 7 (adjusted by NaOH). The pH was determined by a pH meter (Bante instrument Co., China). The samples were kept in amber glass vials and immersed in a thermostatic water bath (Thermolab Industries, India) at desired temperatures of 50, 60, 70 and 80 °C. Furthermore, resistance to hydrolysis at low pH 2 (adjusted by HCl) was also studied. Samples were taken at specific time intervals and directly injected into the HPLC system. Kinetic model, rate constant, the effect of temperature and pH were extensively studied.

RESULTS

Interference and retention time

Using the validated HPLC method mentioned in table 1, YA and Yoh were completely separated with retention times of 3 and 5 min, respectively (fig. 2). The representative chromatograms illustrated Yoh and YA at pH 6 at zero time and after 96 h and 189 h, respectively.

Calibration curve

A Calibration curve was obtained analyzing six points in the range of concentration 6.5-100µg/ml. The calibration curves for Yoh and YA had good linearity within the linear range with the correlation coefficients (R^2) of more than 0.9993 and 0.998 respectively (fig. 3a, 3b).

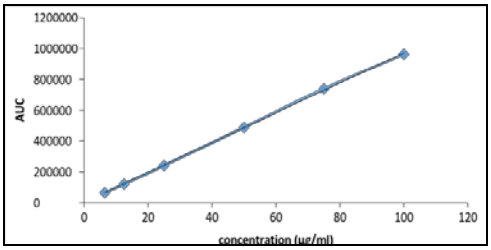

Fig. 3(a): Calibration curve of yohimbine HCl

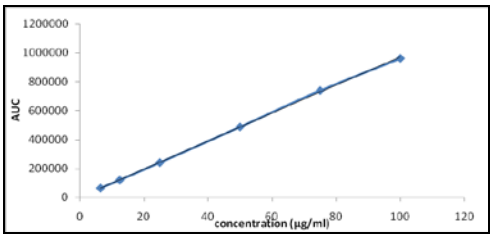

Fig. 3(b): Calibration curve of yohimbinic acid

Kinetic model

Fig. 4a demonstrates the decrease in peak area with time for Yoh and the increase of YA peak area with time.

Fig. 4b shows the same decrease & increase in concentration with time for both Yoh & YA, respectively.

The rate of appearance of YA is equal to the rate of disappearance of Yoh indicating that hydrolysis is the main route of decomposition of Yoh at pH 7.0.

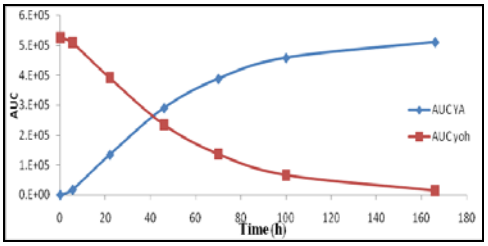

Fig. 4(a): Hydrolysis of yohimbine HCl and formation of yohimbinic acid at 80 °C and pH 7

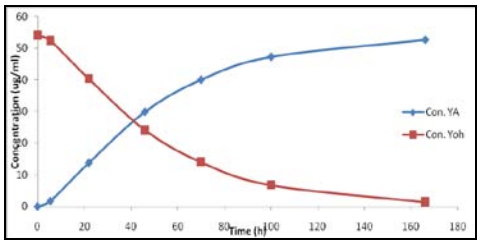

Fig. 4(b): Hydrolysis of yohimbine HCl and formation of yohimbinic acid at 80 °C and pH 7

Subsequent to showing the area under the curve (AUC) versus time after conducting hydrolysis of Yoh at 50, 60, 70 and 80 °C at pH 6 and 7, it was crucial to test and verify the kinetic model of the reaction.

Integrated form of the first order kinetic equation (1) is:

$$\ln(Co/C) = K\,t$$

Where: C equal C_t Equation (1)

Where, C_o is the initial concentration of Yoh in the reaction mixture (50 µg/ml), C is the concentration of yohimbine at time t (i. e., $C_o - C_{disappear}$); K *is* the first order rate constant. So, it could be expressed in equation (2)

$$\ln[\,(Co/Co - C\,disappear)] = K\,t$$

Equation (2)

Therefore, a plot was made between $ln\ (C_o\ /\ C_t)$ versus time t. Regression analysis was carried out to test whether the data followed a linear relationship in fig. 5a 5b, where C_o was the initial concentration of Yoh, and C_t was the concentration of Yoh at time t.

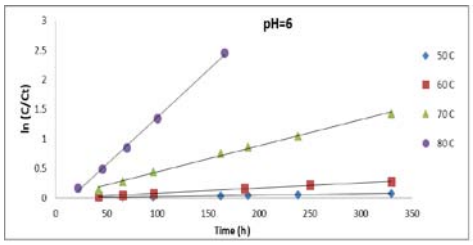

Fig. 5(a): Plot of ln C_o/C_t versus time (h) at pH 6

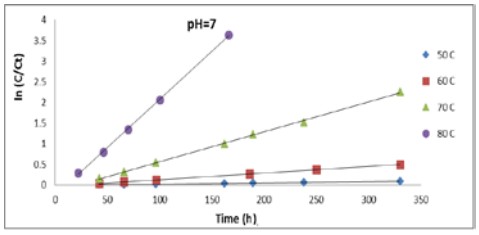

Fig. 5(b): Plot of ln C_o/C_t versus time (h) at pH 7

Rate constants from fig. 5a and. 5 b was obtained and is presented in table 2.

Table 2: Rate constants (h^{-1}) at different temperatures and pH values

Temperature (°C)		50	60	70	80
K (h^{-1}) ×10^{-3}	pH 6	0.24	0.94	4.42	15.99
	pH 7	0.29	1.53	7.25	23.34

Taken together, obtained results suggested that the hydrolysis reactions could be represented by a pseudo first-order-reaction model (fig. 4 and 5).

Effect of temperature

Throughout this study temperatures varied between 50 to 80 °C. The effect of temperature on the rate constant of the reaction was determined by constructing an Arrhenius plot at pH 6 and pH 7. Activation energy was determined from the slope of these plots.

Arrhenius equation is as shown in Equation 3

$$K = A\,e^{\Delta E/RT}$$

Equation (3)

Where K is the rate constant at temperature T, E is the activation energy, A represents pre-exponential factor, T is the temperature in K, and R is *constant coefficient*. Depending on the rate constant k in different temperatures, a chart was drawn of *ln k* versus *1/T* (fig. 6). Furthermore, activation energy *Ea* was calculated and pre-exponential factor *A* was determined by the slope and intercept of the straight line.

Data obtained from fig. 4 and fig. 5 were utilized; ΔE=16.2 KJ. $mol^{-1}$and 16.8 KJ. mol^{-1}, while the values of A were 41.8 and 44.1 KJ. $mol^{-1}$for pH 6 and pH 7, respectively. Therefore, Equation 3 is utilized to construct Equations 4 and 5.

$$K = 16.2\,e^{-41.769/8.312\,T}$$

Equation (4)

$$K = 16.8\,e^{-44.093/8.312T}$$

Equation (5)

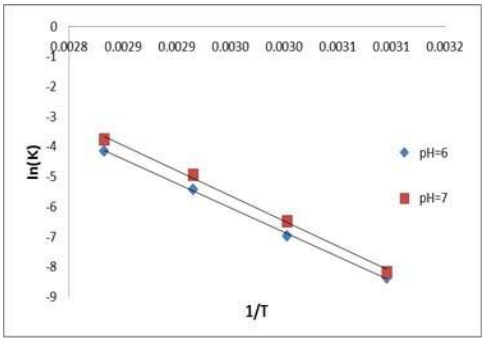

Fig. 6: Plot of ln K vs. 1/T at pH 6 and 7

From equations 4 and 5, the pseudo first order rate constants (K) at pH 6 and 7 were calculated as 2.76 x 10^{-5} h^{-1} and 3.42 x 10^{-5} h^{-1}, respectively. Overall, these findings indicate high stability of the drug at these pH media.

DISSCUSSION

The rate of appearance of YA was found to be equal to the rate of disappearance of Yoh indicating that hydrolysis is the main decomposition pathway of Yoh at pH 7.0.

The yohimbine ester group was found to be stable enough at pH 6, 7, this will allow us to proceed in developing a transdermal delivery preparation around neutral pH, which is currently under investigation in our laboratories. This was expected based on previous study of base catalysis hydrolysis of Yoh [15].

However, the hydrolysis reaction was found to be extremely slow at pH 2.0, less than 1% reduction of Yoh peak was found after two weeks at 50 °C. The apparent absence of an expected acid catalysis for the hydrolysis of the methyl ester group [16] of Yoh could be attributed to resistance of protonation of acyl oxygen due to its involvement in intramolecular hydrogen bonding with the Beta hydroxyl group forming a stable six-membered ring (fig. 7). Another factor may attribute to the resistance to acid catalysis is the full protonation of the two tertiary nitrogens, thus the excessive+ve charge may play role in inhibiting the easy access of the proton to the alkyl group. Since we did not find anything in the literature to support this explanation further investigation will be carried out on other similar esters that carry excessive+ve charges.

The hydrolysis reaction was monitored at pH 2. The pH was adjusted using HCL. The reaction was found to be very slow less than 1% reduction of Yoh, pH peak was found after two weeks at 50 °C. The apparent absence of acid catalysis may be attributed to the resistance of protonation of the acyl oxygen due to its involvement in intra-molecular hydrogen bonding with the B-hydroxyl group (fig. 7). Thus, Yoh might be resistant to acid catalysis at low pH.

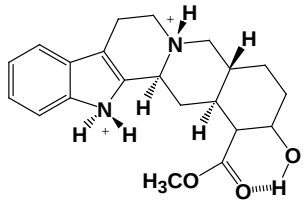

Fig. 7: Hydrogen bonding with the B-hydroxyl group

CONCLUSION

Hydrolysis is the main route of degradation of Yoh at pH 6 and 7 to form YA and it follows a first order kinetics. Yoh around neutrality was found to be stable enough to develop a transdermal preparation at this pH, which is in progress in our labs. Although Yoh is a simple methyl ester, yet it resists acid catalysis and the drug was found to be very stable at very low pH (2), possibly due to the formation intra molecular H-bonding between the acyl group of the ester and the B-hydroxyl group. The role of full protonation of the two nitrogen in the resistance of acid catalysis of hydrolysis of Yoh, is under investigation in our lab.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

Declared None

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