Studies of Degradation Kinetics of a 5-HT_3 Antagonist, Ramosetron Hydrochloride: Effects of Temperature

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ABSTRACT: Ramosetron hydrochloride is the hydrochloride salt of ramosetron, a selective serotonin (5-HT_3) receptor antagonist with potential antiemetic activity. Upon administration, ramosetron selectively binds to and blocks the activity of 5-HT subtype 3 (5-HT_3) receptors located in the vagus nerve terminal and the vomiting center of central nervous system (CNS), suppressing chemotherapy-induced nausea and vomiting. Degradation of Ramosetron HCl was conducted with 0.1N NaOH at 60°C, 70°C and 80°C to study the reaction kinetics. The reaction rate constants (k) for degradation at 60°C, 70°C and 80°C were -2.2680 $molL^{-1}s^{-1}$, -3.3714 $molL^{-1}s^{-1}$ and -5.3686 $molL^{-1}s^{-1}$ for zero order and -1.05 x $10^{-2}s^{-1}$, -1.60 x $10^{-2}s^{-1}$ and -2.70 x $10^{-2}s^{-1}$ for first order kinetics, respectively. The activation energy of Ramosetron HCl was found as 10.05 $kcalmol^{-1}$ by using Arrhenius equation.

Key words: Degradation kinetics, reaction rate constant, activation energy, half-life, shelf-life

INTRODUCTION

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental stress factors, such as temperature, humidity, and light. Stability testing is also important to establish a retest period for the drug substance during the shelf life for the drug product and recommended storage conditions.[1,2] The rate and mechanisms of reactions with particular emphasis on decomposition and stabilization of drug products are essential for formulation scientists to study, understand, and interpret conditions of instability of pharmaceuticals products as well as to be able to offer solutions for the stabilization of these products.[3] Knowing the rate at which a drug deteriorates at various hydrogen ion concentrations allows one to choose a vehicle that will retard or prevent the degradation.[4] Application of degradation kinetics in formulation results in the production of more stable drug preparations, the dosage form and rationale of which may be established on sound scientific principles.[5] Degradation and kinetics studies of some members of 5-HT_3 receptor antagonists, such as, Dalosetron mesylate,[6] Ondansetron HCl[7-9], Granisetron HCl[10-12], and Palonosetron HCl[13] influence the research intuition to take Ramosetron HCl under kinetics studies.

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MATERIALS AND METHODS

Drug substance and reagents. Pure Ramosetron hydrochloride (SMS Pharmaceutical Ltd., India), was obtained from Incepta Pharmaceuticals Ltd. (Dhaka, Bangladesh). Methanol (HPLC grade), acetonitrile (HPLC grade), triethylamine (reagent grade), hydrogen peroxide, dipotassium hydrogen phosphate anhydrous (reagent grade) and sodium hydroxide (reagent grade) were purchased from Scharlau (Scharlau S.L., Spain). HPLC grade water was prepared by PALL purification system (PALL, Cascada AN, USA). Hydrochloric acid (37% commercial grade) and orthophosphoric acid (reagent

grade) were purchased from Labscan (ACI Labscan, Thailand).

Equipments. An Agilent Technologies 1260 series HPLC system (Agilent, Infinity 1260, Germany) equipped with integral autosampler (model 1260 HiP ALS) and quaternary gradient pump (model Quat Pump VL) with an on-line degasser were used. The column compartment (model 1260 TCC) having temperature control and a diode array detector (model 1260 DAD VL+) were employed throughout the analysis. Chromatographic data was acquired using Agilent OpenLAB software.

A hot air oven (Memmert, Mumbai, India) was used to maintain constant temperature. An ultrasonicator from Power Sonic-405 (Hwashin Technology, Seoul, Korea) and pH meter from pH tutor (Eutech Instruments, Singapore) were used.

Chromatographic conditions. Chromatographic separation was achieved at a temperature of 40°C on a cyano bonded silica column (250 x 4.6 mm; CN; Kromasil) using a mobile phase comprising of a mixture of acetonitrile – methanol - buffer (50 mM dipotassium hydrogen phosphate anhydrous containing 1 ml of triethylamine per liter with pH 7.0 adjusted by diluted orthophophoric acid) in the ratio (3 : 1 : 6). The mobile phase so prepared was filtered through 0.45 µm membrane filter and degassed by sonication. Flow rate was maintained at 1.0 ml/min was maintained. The injection volume was 20 µL for all the analyses. The detection was carried out at 210 nm.

Procedure for degradation kinetics studies. Degradation kinetics of the drug substance was conducted in basic condition (0.1N NaOH) at 60°C, 70°C and 80°C for 1, 2, 3, 4 and 5 hours. The concentration of the solution kept for degradation under different time intervals was 1.0 mg/ml. The final concentration of the degraded samples was 0.2 mg/ml in mobile phase.

To explain kinetics of degradation and to find out the best fitness of the regression coefficient, the relationship between the concentration vs time was drawn for zero, first and second order reaction following Eq. 1, 2, and 3 respectively. Eq. 4 was used to calculate activation energy where, C_t and C_o are the concentrations at time t and zero, respectively. The reaction rate constant for zero, first and second order reactions were k_o, k_1 and k_2, respectively. E_a is the reaction activation energy, A is the frequency factor, R is the molar gas constant and T is the temperature in Kelvin.

$$C_t = C_0 - tk_0 \quad \text{Eq. 1}$$

$$\log C_t = \log C_0 - tk_1/2.303 \quad \text{Eq. 2}$$

$$1/C_t = 1/C_0 + tk_2 \quad \text{Eq. 3}$$

$$\log k = \log A - E_a/2.303R \; .1/T \quad \text{Eq. 4}$$

Standard solution preparation. The first dilution of the standard solution of Ramosetron hydrochloride was prepared in HPLC grade methanol to get a concentration of 5.0 mg/ml. The second dilution was done by mobile phase to get a final concentration of 0.2 mg/ml. The standard solution was prepared freshly.

Stock sample preparation for degradation kinetics study. The stock solution of Ramosetron hydrochloride was prepared in HPLC grade methanol to get a concentration of 5.0 mg/ml.

Preparation of analytical sample for degradation kinetics. An aliquot of stock sample prepared for degradation kinetics study was diluted to 5 ml with 0.1N NaOH in five different volumetric flasks to get a concentration of 1.0 mg/ml. These solutions were kept in a dry oven at 60°C, 70°C and 80°C for 1, 2, 3, 4, and 5 hours. These degraded samples were neutralized with equimolar strength and volume of hydrochloric acid respectively before further dilution with mobile phase to get a final concentration of 0.2 mg/ml.

RESULTS AND DISCUSSION

Degradation kinetics of Ramosetron hydrochloride was studied in 0.1N NaOH at 60°C, 70°C and 80°C for 1, 2, 3, 4 and 5 hours. The potency remained after each degradation was calculated with the help of Microsoft Excel. These results are summarized in the table 1. Linearity graphs of each reaction order was derived using the

relationship between concentration vs time for zero order, log concentration vs time for first order and inverse concentration vs time for second order. These graphs are presented in figures 4-6. Kinetics parameters are summarized in table 2. Half-life and shelf-life were calculated using the slope values of each regression equation. Half-life, shelf-life and regression line information are summarized in Table 3. Reaction activation energy (E_a) calculated with the help of Arrhenius equation was 10.05 kcal $K^{-1}mol^{-1}$. The linearity graph required to calculate reaction activation energy (E_a) was derived from reaction rate constant (k) vs inverse temperature in Kelvin (1/T). This linearity graph is shown in figure 7. All HPLC chromatograms relevant to degraded samples are presented in figures 1-3.

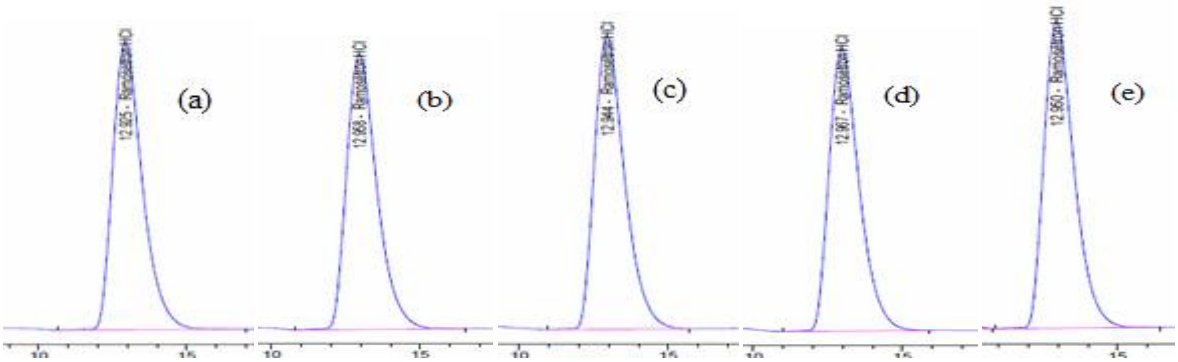

Figure 1. Chromatograms of Ramosetron HCl in 0.1N NaOH at 60°C for (a) 1 hour (b) 2 hours (c) 3 hours (d) 4 hours and (e) 5 hours.

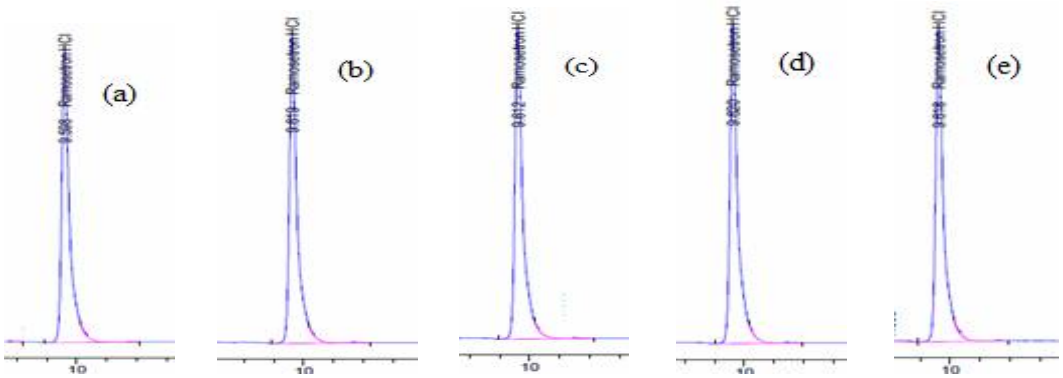

Figure 2. Chromatograms of Ramosetron HCl in 0.1N NaOH at 70°C for (a) 1 hour (b) 2 hours (c) 3 hours (d) 4 hours and (e) 5 hours.

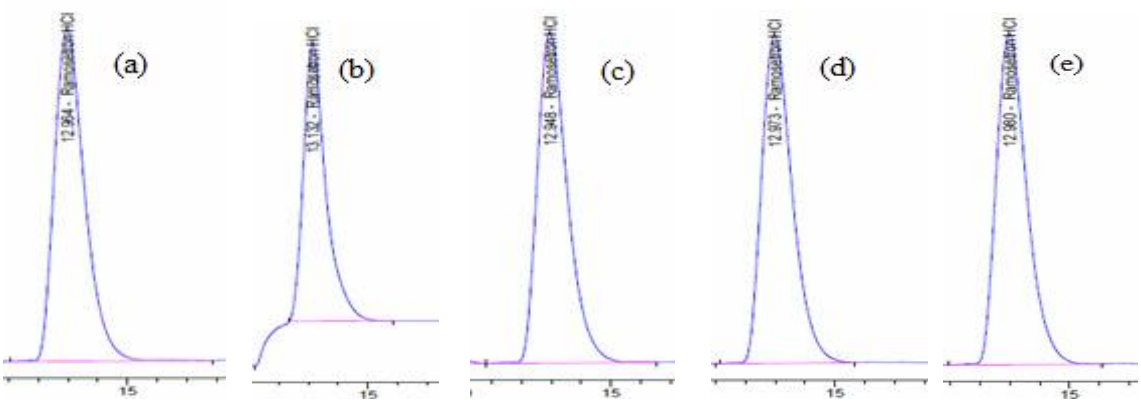

Figure 3. Chromatograms of Ramosetron HCl in 0.1N NaOH at 80°C for (a) 1 hour (b) 2 hours (c) 3 hours (d) 4 hours and (e) 5 hours.

Table 1. Peak parameters and assays of degraded samples of Ramosetron HCl at different temperature in 0.1N NaOH.

Temp.	Time interval	Area	Theoretical plate #	Tailing factor	Peak purity index	Assay (%)	
						Initial concentration	Potency
60°C	1 hour	10436	5424	0.987	0.9999	100.0	97.46
	2 hours	10159	5354	0.984	0.9997		96.0
	3 hours	9932.3	5451	0.989	0.9996		93.5
	4 hours	9802.3	5361	0.986	0.9997		91.0
	5 hours	9649.0	5248	0.982	0.9995		88.50
70°C	1 hour	2984.0	5451	0.991	0.9995	100.0	96.4
	2 hours	2859.1	5387	0.993	0.9998		93.8
	3 hours	2843.0	5451	0.992	0.9997		90.2
	4 hours	2826.0	5621	0.997	0.9996		86.6
	5 hours	2984.0	5451	0.991	0.9995		96.4
80°C	1 hour	10143	5587	0.991	0.9998	100.0	94.72
	2 hours	9936.3	5674	0.993	0.9999		90.44
	3 hours	9641.4	5910	0.997	0.9996		85.16
	4 hours	8706.9	5436	0.974	0.9997		76.88
	5 hours	8608.3	5581	0.943	0.9995		74.18

Table 2. Kinetic parameters of degradation of Ramosetron HCl at different temperature in 0.1N NaOH.

Temp.	Parameters	Orders		
		Zero (C vs time)	First (logC vs time)	Second (1/C vs time)
60°C	R^2 (linear correlation coefficient)	0.9951	0.9932	0.9908
	k (rate constant)	2.2680	0.0105	0.0003
70°C	R^2 (linear correlation coefficient)	0.9979	0.9955	0.9917
	k (rate constant)	3.3714	0.0160	0.0004
80°C	R^2 (linear correlation coefficient)	0.9880	0.9835	0.9761
	k (rate constant)	5.3686	0.0270	0.0007

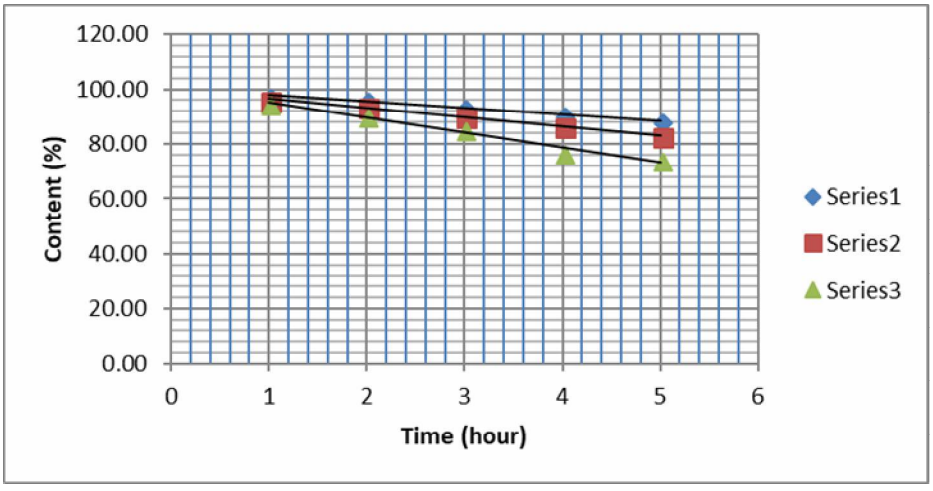

Figure 4. Zero order plot for the degradation of Ramosetron HCl in 0.1N NaOH at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 0.1N NaOH.

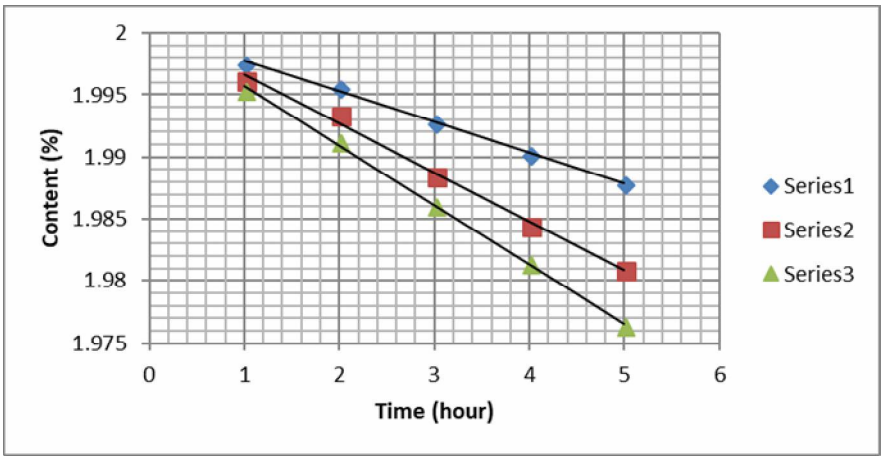

Figure 5. First order plot for the degradation of Ramosetron HCl in 0.1N NaOH at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 0.1N NaOH.

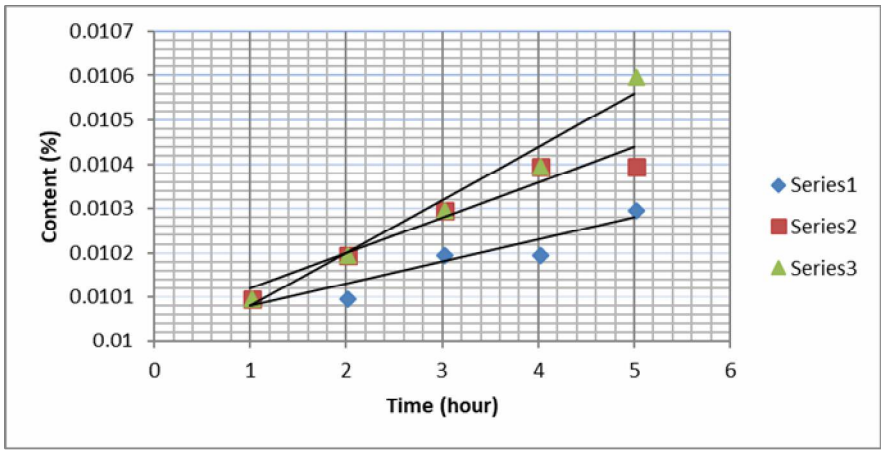

Figure 6. Second order plot for the degradation of Ramosetron HCl in 0.1N NaOH at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 0.1N NaOH.

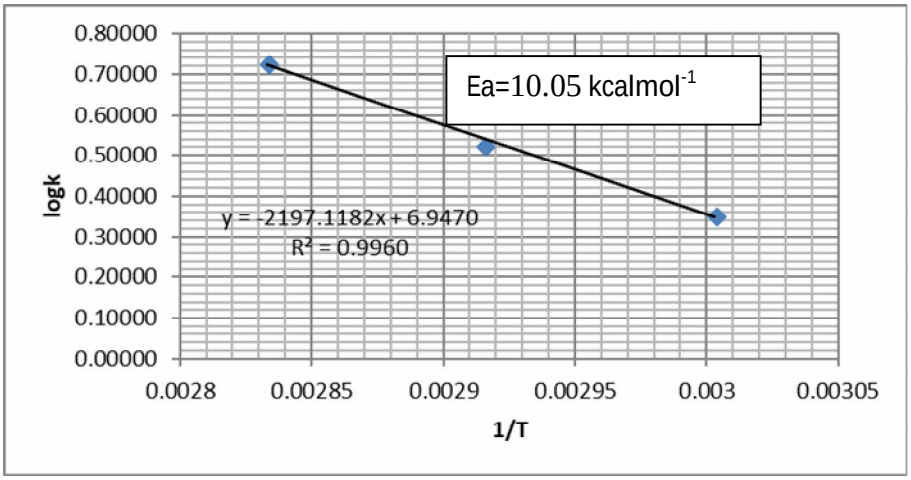

Figure 7. Relationship between temperature and rate constant used to calculate activation energy of Ramosetron HCl.

Table 3. Summary of degradation kinetics of Ramosetron HCl at 60°C, 70°C and 80°C in 0.1N NaOH.

Orders	Temperature		Regression equations	R^2 value	k	$t_{1/2}$ (days)	t_{90} (days)
	°C	°K					
Zero	60	333	y = -2.2680x + 100.0800	0.9951	2.2680	0.92	0.18
	70	343	y = -3.3714x + 100.0952	0.9979	3.3714	0.62	0.12
	80	353	y = -5.3686x + 100.3181	0.9880	5.3686	0.39	0.08
First	60	333	y = -0.0105x + 2.0008	0.9932	0.0105	2.75	0.38
	70	343	y = -0.0160x + 2.0014	0.9955	0.0160	1.80	0.25
	80	353	y = -0.0270x + 2.0041	0.9835	0.0270	1.07	0.15
Second	60	333	y = 0.0003x + 0.0100	0.9908	0.0003	1.39	0.14
	70	343	y = 0.0004x + 0.0099	0.9917	0.0004	1.04	0.10
	80	353	y = 0.0007x + 0.0098	0.9761	0.0007	0.60	0.06

CONCLUSION

Kinetic parameters such as activation energy, Ea, reactant half-life, $t_{1/2}$, reactant shelf-life, t_{90}, and reaction rate constant, k, are used extensively to calculate the retest period of a drug substance and to set expiration date and stability condition over the period of shelf-life. Degradation kinetic study revealed that the activation energy of Ramosetron hydrochloride is 10.05 $kcalmol^{-1}$. Considering the best fit regression coefficient (R^2) value, one can easily calculate reaction order to find out reaction rate constant (k) used to calculate half-life and shelf-life. Calculated half-life and shelf-life information help to set retest period of Ramosetron hydrochloride and also to predict shelf-life period of different dosage form of Ramosetron hydrochloride, specially lyophilized dosage form.

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