Studies of Stress Degradation of Ramosetron Hydrochloride, a 5-HT_3 Antagonist

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ABSTRACT. Three issues of fundamental importance in drug therapy are safety, efficacy and stability. Extensive information derived from stress degradation studies of Ramosetron HCl will expand the scientific thought further to ensure to achieve the intended quality of Ramosetron HCl as drug substance and drug products available in the market. Stress degradation screening of Ramosetron HCl was conducted in aqueous, 0.1N, 0.5N, 1.0N and 2.0N acid-base and oxidative (3, 5 and 10 % H_2O_2) conditions and photo degradation. No degradation was found in aqueous condition at 60°C for 7 days. Acceptable degradations were found in 0.5 N, 1N and 2N HCl at 70ºC for 7 days, 0.1N NaOH at 60°C for 2 days, 3% hydrogen peroxide at room temperature for 1, 2 and 3 hours in dark and 3.6 million lux fluorescence light or 600 watts hour/m^2 UV light. Among other applied stress conditions, the base hydrolysis and oxidative methods degraded Ramosetron hydrochloride more drastically than the other stressed conditions.

Key words: Degradation pathways, stress degradation, photo degradation, oxidative degradation, acid-base degradation, efficacy

INTRODUCTION

Stress testing to elucidate the intrinsic stability of the drug substance is part of the development strategy and is normally carried out under more sever conditions than those used for accelerated testing.[5,6] Stress testing of drug substance can help identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule, and validate the stability indicating power of the analytical procedures used.[12,13] The nature of the stress testing will depend on individual drug substance and the type of the drug product involved. Aqueous hydrolysis, acid-base hydrolysis, oxidation and photolytic degradation were utilized to investigate degradation pathways and degradants.[15,16]

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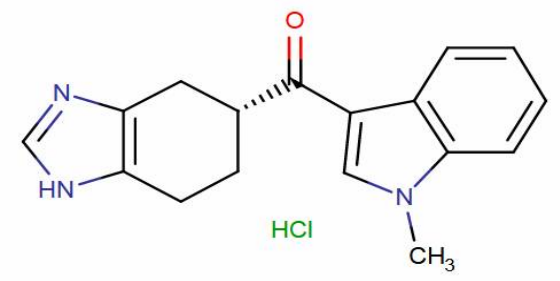
Ramosetron hydrochloride (INN) is the hydrochloride salt of ramosetron, a selective serotonin (5-HT) receptor antagonist with potential antiemetic activity.[1,2] Upon administration, Ramosetron selectively binds to and blocks the activity of 5-HT sub-type 3 (5-HT_3) receptors located in the vagus nerve terminal and in the vomiting center of central nervous system (CNS), suppressing chemotherapy-induced nausea and vomiting.[3] High performance liquid chromatography, LC-MS/MS and enantioselective LC/MS/MS methods have been used for the determination of Ramosetron in biological fluids. Few HPLCs and spectrophotometric methods were also reported for the determination of Ramosetron hydrochloride in pharmaceutical dosage forms.[8] Chemical structure of Ramosetron hydrochloride is given below:



Ramosetron HCl

MATERIALS AND METHODS

Drug substance and reagents. Pure Ramosetron hydrochloride bulk, manufactured by SMS Pharmaceutical Ltd., India, was obtained from Incepta Pharmaceutical Ltd. (Dhaka, Bangladesh), the manufacturer of finished dosage form of Ramosetron hydrochloride. 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). LC-MS grade methanol was procured from Panreac (Panreac, E.U).

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 was 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 Open LAB software. A hot air oven (Memmert, Mumbai, India) was used to maintain constant temperature. The stress photodegradation was carried out in a photostability chamber (Oswarld OPSH-G-16-GMP series, Oswarld scientific, Mumbai, India) equipped with illumination bank made of light source as described in the ICH guideline Q1B. 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 bonded phase cyano 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 dilute 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 of 1.0 ml/min was maintained. The injection volume was 20 µL for all the analyses. The detection was carried out at the wavelength of 210 nm.

Procedure for stress degradation study. Stress degradation of the drug substance was conducted under aqueous, acidic and basic hydrolysis, oxidative and photolytic conditions. Photo degradation of the drug substance was conducted in solid state. The concentration of the solution kept for degradation under different stress conditions was 1.0 mg/ml. The final concentration of the stress solution was 0.2 mg/ml in mobile phase.

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. This standard solution was prepared on the day of analysis.

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

Analytical stress degradation sample preparation for aqueous hydrolysis. An aliquot of stock sample prepared for degradation study was diluted to 5 ml with purified water to get a concentration of 1.0 mg/ml. This solution was kept in a dry oven at 60°C for 7- and 21-days. These stressed

treated samples were further diluted with mobile phase to get a final concentration of 0.2 mg/ml.

Analytical stress degradation sample preparation for acid hydrolysis. An aliquot of stock sample prepared for degradation study was diluted to 5 ml with 0.1N HCl, 0.5N HCl, 1N HCl and 2N HCl solutions separately to get a concentration of 1.0 mg/ml. These solutions were kept in a dry oven at 60°C for 7- and 21-days. These stressed samples were neutralized with equimolar strength and volume of sodium hydroxide, respectively before further dilution with mobile phase to get a final concentration of 0.2 mg/ml.

Analytical stress degradation sample preparation for basic hydrolysis. An aliquot of stock sample prepared for degradation study was diluted to 5 ml with 0.1N NaOH, 0.5N NaOH, 1N NaOH and 2N NaOH solutions separately to get 1.0 mg/ml. These solutions were kept in a dry oven at 60°C for 7 days. These stressed treated 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.

Analytical stress degradation sample preparation for oxidation. An aliquot of stock sample prepared for degradation study was diluted to 5 ml with 3% H_2O_2, 5% H_2O_2 and 10% H_2O_2 solutions separately to get a concentration of 1.0 mg/ml. These solutions were kept in a dark place for 1 hour, 2 hours and 3 hours. These stressed treated samples were further diluted with mobile phase to get a final concentration of 0.2 mg/ml.

Analytical stress degradation sample preparation for photolysis. Bulk powder of Ramosetron hydrochloride was evenly spread on aluminum foil and kept in the photostability chamber for direct exposure of fluorescence light (1.2 and 3.6 million lux) and UV light (200 and 600 watts hour/m^2) along with control sample wrapped with similar aluminium foil. A portion of the exposed and control samples were dissolved in 1.0 ml of HPLC grade methanol and diluted with mobile phase to get a final concentration of 0.2 mg/ml.

RESULTS AND DISCUSSION

Intentional degradation was attempted through various stressed conditions such as aqueous, acid, base, oxidation and photolytic treatments to achieve maximum degradation of 20%. It was observed that Ramosetron hydrochloride degrades with acidic, basic, oxidative and photolytic conditions and no degradation was found in aqueous condition.

Aqueous degradation. Aqueous degradation study was conducted with purified water at 60°C for 7- and 21-days. Under these stressed conditions, no degradation was found.

Acid degradation. Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Degradations were found with 0.5N, 1N and 2N HCl. No degradation was observed for 0.1N HCl condition. The chromatograms are shown in figure 1 and results are given in table 1.

Base degradation. Base degradation was conducted in 0.1N, 0.5N, 1N and 2N NaOH at 60°C for 2 days. About 20.9% degradation was found with 0.1N NaOH and about 100.0% degradation was evident in 0.5N, 1N and 2N NaOH. The chromatogram is shown in figure 2 and results are given in table 2.

Oxidative degradation. Oxidative degradation was conducted with three different strengths of hydrogen peroxide at dark place for 1, 2 and 3 hours. Different percentages of degradation were evident at different stressed conditions. Here, 10.0, 15.2 and 20.8% degradation were found for 3% hydrogen peroxide after 1, 2 and 3 hours, respectively. However, 57.9, 61.7 and 73.3% degradation were observed with 5% hydrogen peroxide after 1, 2 and 3 hours, respectively. On the other hand, 76.0, 86.1 and 93.6% degradation could be seen for 10% hydrogen peroxide after 1, 2 and 3 hours, respectively. The conditions that produced not more than 20% of degradants are considered as appropriate stressed conditions. Three additional peaks apart from the principal and blank peaks were found for each stressed condition of 3% H_2O_2 at 1, 2 and 3 hours.

The chromatograms are shown in figure 3 and results are given in table 3.

Photo degradation. Photo degradation study was carried out with bulk drug substance. The sample was directly exposed to 3.6 million lux fluorescent light and 600 watts hour/m^2 UV light. At these conditions, the sample showed 6.64% degradation. Four peaks apart from principal and blank peaks were found. The chromatogram is shown in figure 4 and results are given in table 4.

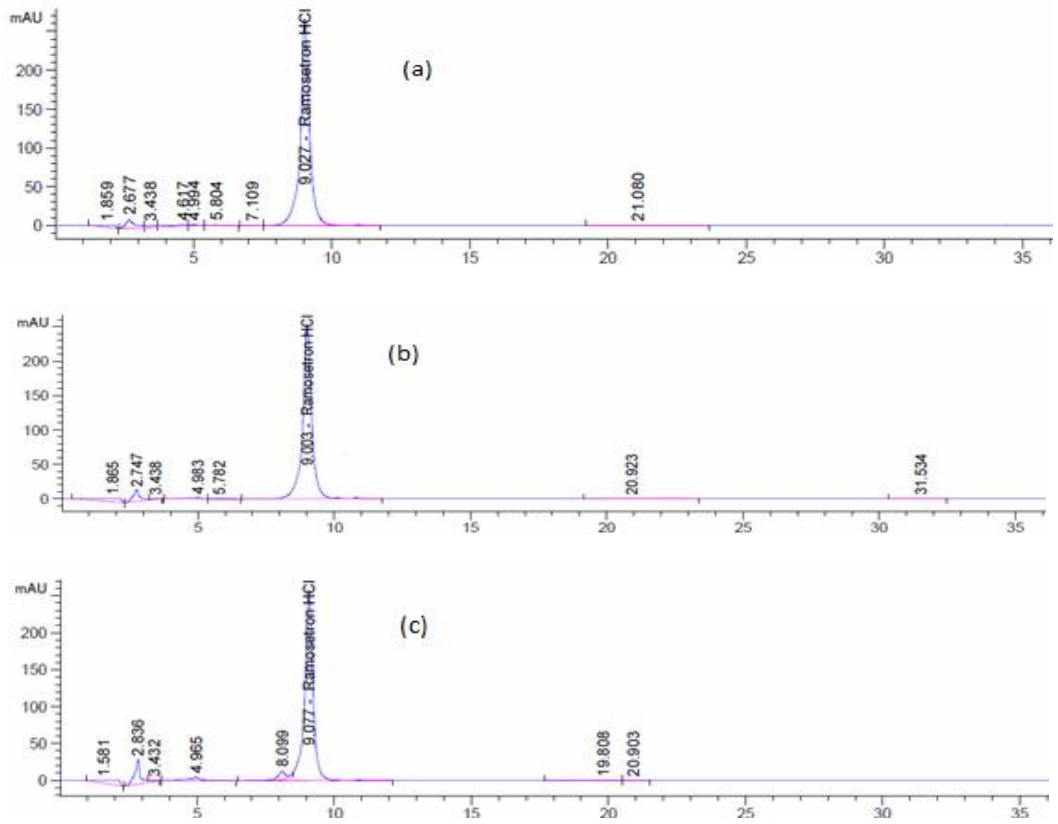


Figure 1. Chromatograms of Ramosetron HCl after 7 days stressed conditions (a) 0.5N HCl, (b) 1.0N HCl, (c) 2.0N HCl.

Table 1. Relationship between retention time, area and content of Ramosetron HCl after acid hydrolysis for 7 days at 60°C.

Condition	Peak for	Retention time (min)	RRT	Area	Peak purity index	Content (%)		
						Sample	Impurity	Total impurities
0.1N HCl	Ramosetron HCl	9.04	1	6482.2	0.9989	99.9	-	-
0.5N HCl	Ramosetron HCl	9.03	1	6194.4	0.9999	95.0	-	
	Impurity-1	7.11	0.79	6.86	-	-	0.18	5.0
	Impurity-2	21.08	2.33	27.20	-	-	0.80	
1N HCl	Ramosetron HCl	9.00	1	5866.6	0.9998	92.0	-	
	Impurity-3	20.9	2.32	25.82	-	-	4.7	8.0
	Impurity-4	31.53	3.50	12.85	-	-	2.3	
2N HCl	Ramosetron HCl	9.08	1	5651.4	0.9998	88.9	-	
	Impurity-5	8.10	0.89	241.20	-	-	8.3	11.1
	Impurity-6	19.81	2.18	17.62	-	-	0.59	
	Impurity-7	20.90	2.30	10.18	-	-	0.34	

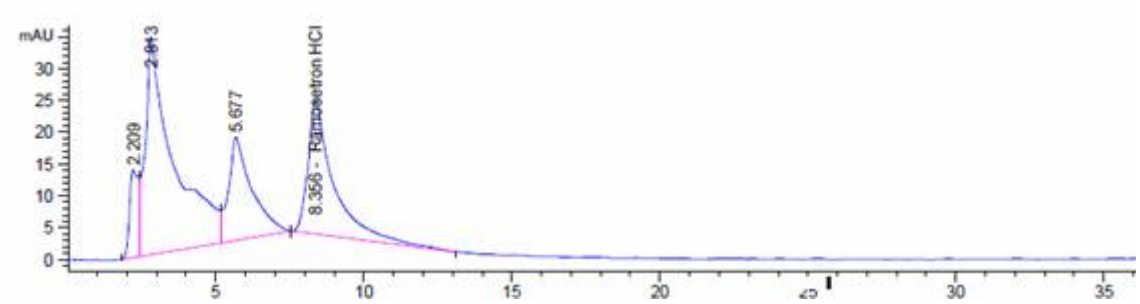


Figure 2. Chromatogram of Ramosetron HCl under stressed conditions with 0.1N NaOH for 2 days.

Table 2. Relationship between retention time, area and content of Ramosetron HCl after base hydrolysis at 60°C for 2 days.

Condition	Peak for	Retention time (min)	RRT	Area	Peak purity index	Content (%)		
						Sample	Impurity	Total impurities
0.1N NaOH	Ramosetron HCl	8.356	1	1262.9	-	79.1	-	20.9
0.5N NaOH	Ramosetron HCl	-	-	-	-	-	-	100
1N NaOH	Ramosetron HCl	-	-	-	-	-	-	100
2N NaOH	Ramosetron HCl	-	-	-	-	-	-	100

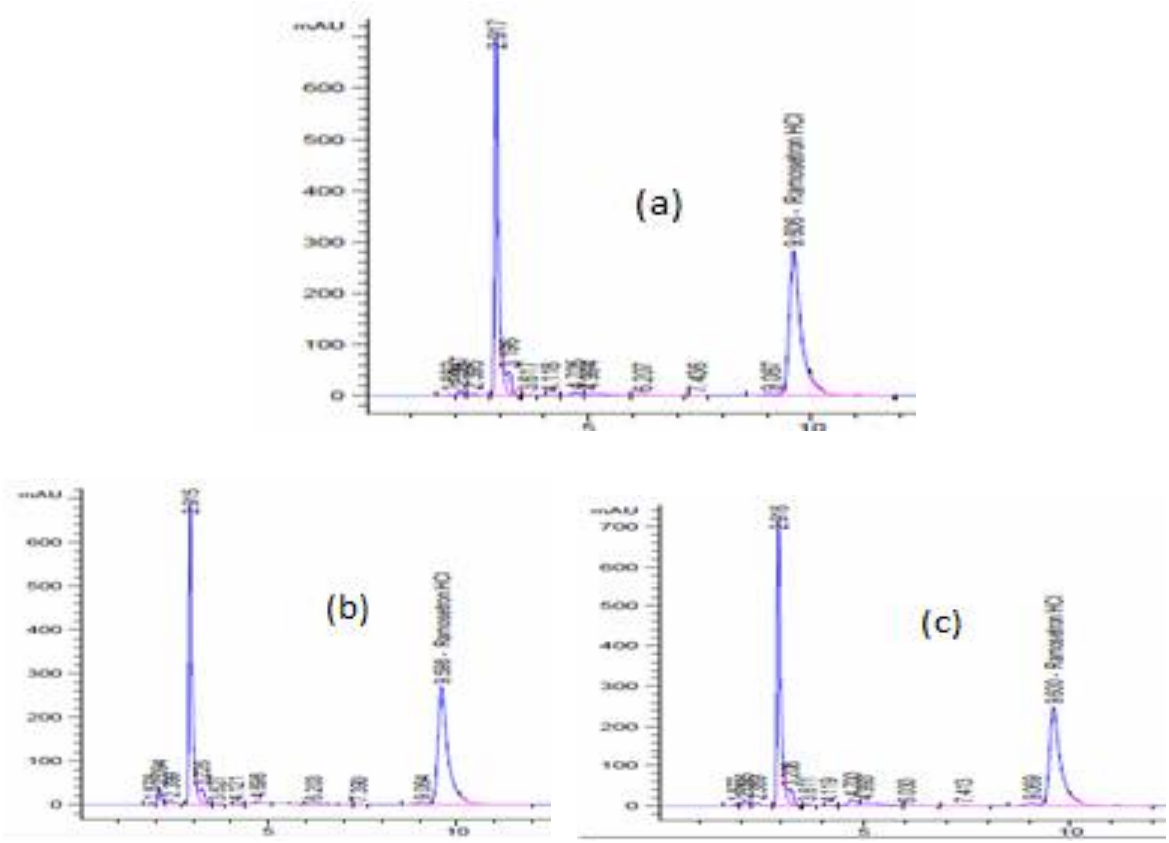


Figure 3. Chromatograms of stressed sample of Ramosetron HCl with 3% H_2O_2 for (a) 1 hour, (b) 2 hours, (c) 3 hours.

Table 3. Relationship between retention time, area and content of Ramosetron HCl after oxidation by 3% hydrogen peroxide, at 1 , 2 and 3 hours.

Condition	Sample	Retention time (min)	RRT	Area	Peak purity index	Content (%)		
						Sample	Impurity	Total impurities
3% H_2O_2, 1 hr	Ramosetron HCl	9.61	1	5377.98	0.9999	90.0	-	10.0
	Impurity-8	6.21	0.65	18.95	-	-	1.9	
	Impurity-9	7.44	0.77	3.03	-	-	0.31	
	Impurity-10	9.09	0.95	56.03	-	-	5.8	
3% H_2O_2, 2 hrs	Ramosetron HCl	9.60	1	5061.76	0.9989	84.8	-	15.2
	Impurity-11	6.20	0.65	43.70	-	-	4.2	
	Impurity-12	7.31	0.76	6.01	-	-	0.58	
	Impurity-13	9.06	0.94	85.20	-	-	8.2	
3% H_2O_2, 3 hrs	Ramosetron HCl	9.60	1	4727.82	0.9998	79.2	-	20.8
	Impurity-14	6.03	0.63	6.16	-	-	0.54	
	Impurity-15	7.41	0.77	16.23	-	-	1.4	
	Impurity-16	9.07	0.95	144.66	-	-	13.0	

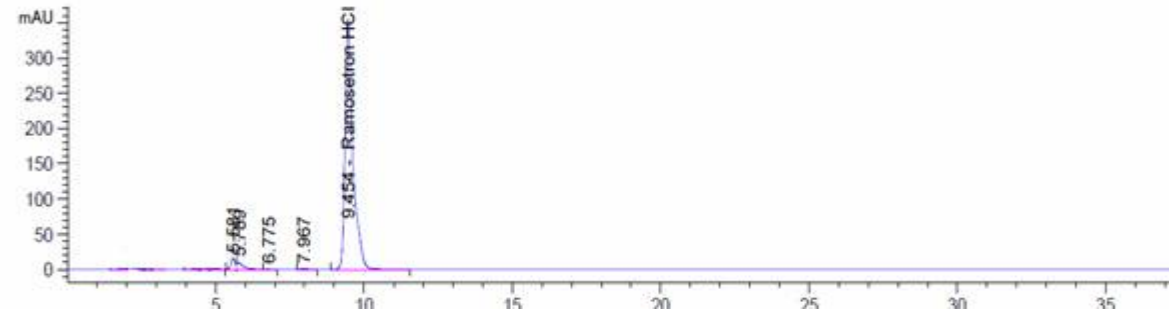


Figure 4. Chromatogram of Ramosetron HCl (Stressed sample exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light).

Table 4. Relationship between retention time, area and content of Ramosetron HCl after photo degradation exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Sample	Retention time (min)	RRT	Area	Peak purity index	Content (%)		
						Sample	Impurity	Total impurities
Photo degradation	Ramosetron HCl	9.45	1	6465.4	0.9998	93.4	-	-
	Impurity-17	5.58	0.59	141.62	-	-	2.2	6.6
	Impurity-18	5.79	0.61	137.27	-	-	2.1	
	Impurity-19	6.78	0.72	3.92	-	-	0.05	
	Impurity-20	7.98	0.84	11.73	-	-	0.17	

Table 5. Summary of results of different stressed degradations of Ramosetron HCl.

Condition	Content (%)		Degradation (%)
	Standard	Sample	
Aqueous hydrolysis			
7 days	100	99.9	-
21 days	100	99.5	-
Acid hydrolysis			
0.1N HCl	100	99.9	-
0.5N HCl	100	95.0	5.0
1N HCl	100	92.0	8.0
2N HCl	100	88.9	11.1
Base hydrolysis			
0.1N NaOH	100	20.9	20.9
0.5N NaOH	100	0	100
1N NaOH	100	0	100
2N NaOH	100	0	100
Base hydrolysis with temp. (0.1N NaOH, 60°C)			
1 hour	100	97.5	2.5
2 hours	100	96.0	4.0
3 hours	100	93.5	6.5
4 hours	100	91.0	9.0
5 hours	100	88.5	11.5
Base hydrolysis with temp. (0.1N NaOH, 70°C)			
1 hour	100	96.4	3.6
2 hours	100	93.8	6.2
3 hours	100	90.2	9.8
4 hours	100	86.6	13.4
5 hours	100	83.0	17.0

Table 6. Summary of results of different stressed degradations of Ramosetron HCl.

Condition	Content (%)		Degradation (%)
	Standard	Sample	
Base hydrolysis at elevated temperature (0.1N NaOH, 80°C)			
1 hour	100	94.7	5.3
2 hours	100	90.4	9.6
3 hours	100	85.2	14.8
4 hours	100	76.9	23.1
5 hours	100	74.2	25.8
Oxidation			
3% Hydrogen peroxide			
1 hour	100	90.0	10
2 hours	100	84.8	15.2
3 hours	100	79.2	20.8
5% Hydrogen peroxide			
1 hour	100	42.1	57.9
2 hours	100	38.3	61.7
3 hours	100	26.7	73.3
10% Hydrogen peroxide			
1 hour	100	24.0	76.0
2 hours	100	13.9	86.1
3 hours	100	7.0	93.0
Photo degradation	100	93.4	6.6

CONCLUSION

The requirements of a robust pharmaceutical formulation are fulfilled by the complete information of chemical instability of an active pharmaceutical API. Information on forced degradation studies of Ramosetron hydrochloride revealed that the API degraded with acid hydrolysis, base hydrolysis, oxidation and photolysis. Among these stressed conditions, base hydrolysis and oxidative degradation were able to degrade Ramosetron hydrochloride more drastically. So, base hydrolysis and oxidative degradation are the most sensitive degradation pathways for Ramosetron hydrochloride. Other conditions such as acid hydrolysis is also responsible to produce known and unknown impurities. So precautions should be taken to develop a robust formulation considering the derived information. The growing tendency of known and unknown impurities will give exclusive forecast for drug excipients compatibility study. Stability indicating method of any dosage form of Ramosetron hydrochloride will provide all prerequisite information from such studies. However, we could not identify the impurities produced from the stressed conditions on Ramosetron HCl. Further extensive studies are underway to isolate and characterize the degradants.

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