Chapter-3

Results and Discussion of Stress Degradation Studies

Stress degradation studies were conducted in aqueous, acid, base, hydrogen peroxide and light at 60°C, 70°C and 80°C with various sample treatment interval on ramosetron HCl, ondnasetron HCl, granisetron HCl, tropisetron HCl and palonosetron HCl. Results are discussed below.

3.1 Stress degradation of ramosetron hydrochloride

Ramosetron hydrochloride was evident to degrade in acidic, basic, oxidative and photolytic conditions and no degradation was found in aqueous condition.

3.1.1 Aqueous degradation

Aqueous degradation study was conducted in purified water at 60°C for 7- and 21-days. Under these stressed conditions, no degradation was found. The results are summarized in tables 3.1-3.2.

Table 3.1: Relationship between relative retention time (RRT), area and content of ramosetron HCl in water at 60°C after 7 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
Aqueous degradation (7 days)	Ramosetron HCl	1	6650.7	0.9997	100.0	99.9

Table 3.2: Relationship between relative retention time (RRT), area and content of ramosetron HCl in water at 60°C after 21 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
Aqueous degradation (21 days)	Ramosetron HCl	1	7006.02	0.9998	100.0	99.5

3.1.2 Acid degradation

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Degradations were observed with 0.5N, 1.0N and 2.0N HCl. No degradation was evident in 0.1N HCl. The chromatograms are shown in figures 3.1-3.2 and results are given in table 3.3.

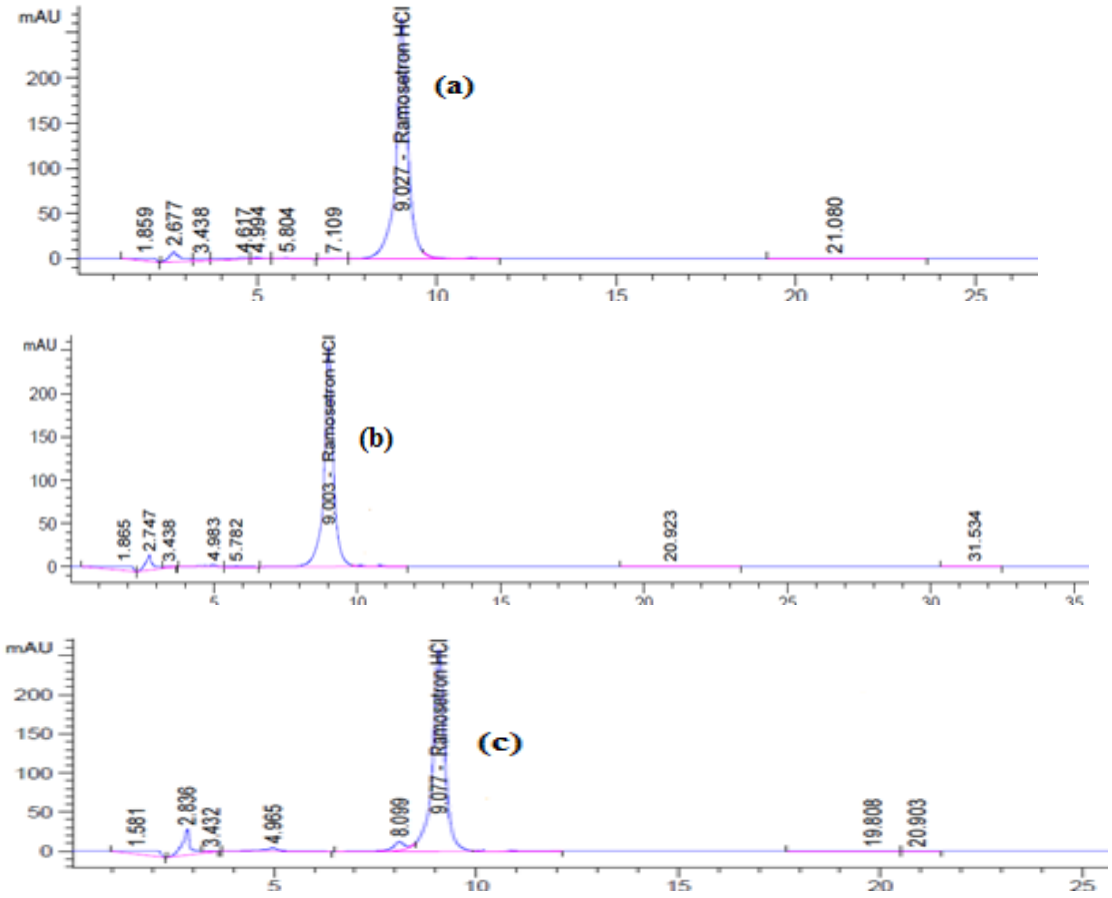

Figure 3.1: Chromatograms of ramosetron HCl at 60°C after 7 days in (a) 0.5N HCl, (b) 1.0N HCl, and (c) 2.0N HCl.

3.1.3 Base degradation

At first, base degradation was conducted in 0.1N, 0.5N, 1.0N and 2.0N NaOH at 60°C for 2 days. About 20.9% degradation was found in 0.1N NaOH and about 100.0% degradation was evident in 0.5N, 1.0N and 2.0N NaOH. The chromatogram is shown in figure 3.3 and results are given in table 3.4.

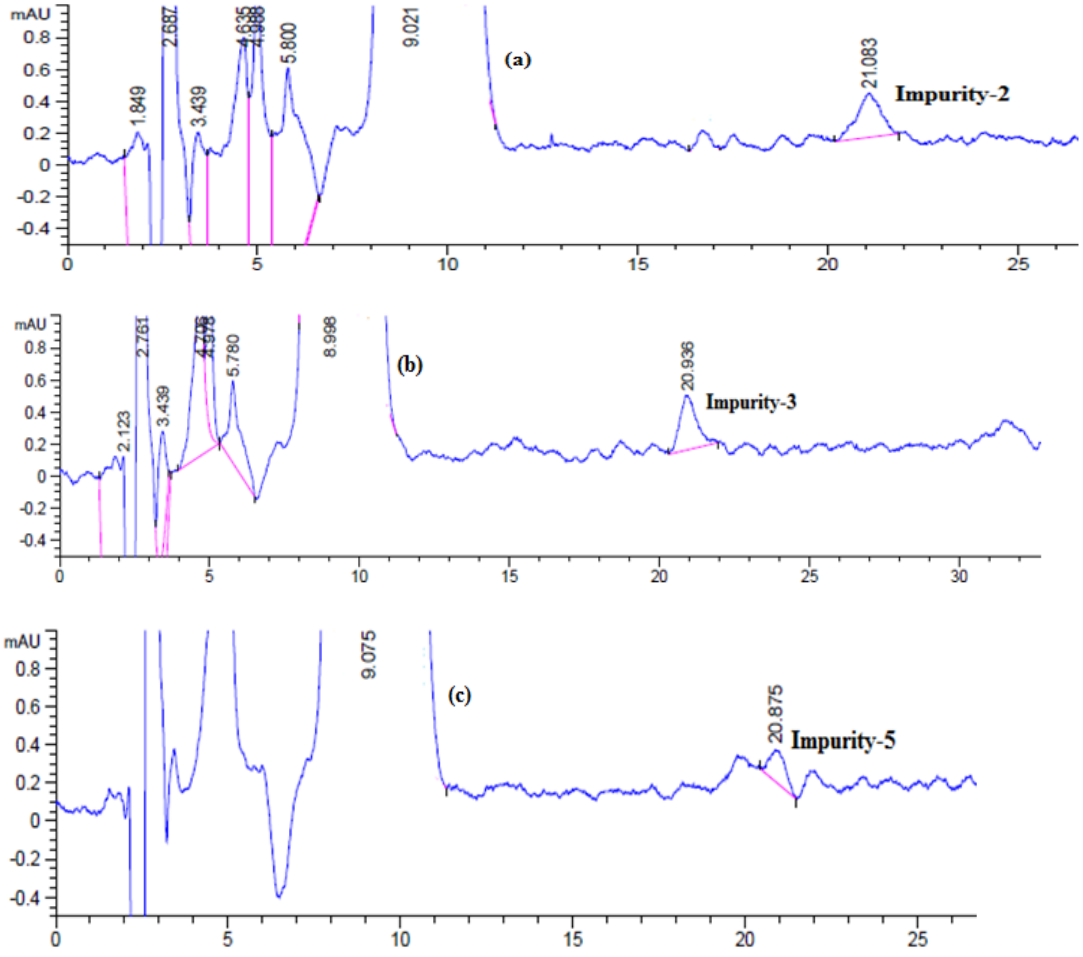

Figure 3.2: Extended chromatograms for impurities of ramosetron HCl at 60°C after 7 days in (a) 0.5N HCl, (b) 1.0N HCl, and (c) 2.0N HCl.

Secondly, base degradation was carried out in 0.1 N NaOH at 60°C, 70°C and 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours. Here, 2.54%, 4.0%, 6.5%, 9.0% &11.5% degradations were found at 60°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours respectively. However, 3.6%, 6.2%, 9.8%, 13.4% &17.0% degradation were found at 70°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours respectively. On the other hand, 5.28%, 9.56%, 14.84%, 20.12% &25.22% degradation were found at 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, respectively.

Table 3.3: Relationship between relative retention time (RRT), area and content of ramosetron HCl in acid at 60°C after 7 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N HCl	Ramosetron HCl	1	6482.2	0.9989	99.9	-	-
0.5N HCl	Ramosetron HCl	1	6194.4	0.9999	95.0	-	5.0
	Impurity-1	0.79	6.86	-	-	0.18	
	Impurity -2	2.33	27.20	-	-	0.80	
1.0N HCl	Ramosetron HCl	1	5866.6	0.9998	92.0	-	8.0
	Impurity -3	2.32	25.82	-	-	4.7	
	Impurity -4	3.50	12.85	-	-	2.3	
2.0N HCl	Ramosetron HCl	1	5651.4	0.9998	88.9	-	11.1
	Impurity -5	0.89	241.20	-	-	8.3	
	Impurity -6	2.18	17.62	-	-	0.59	
	Impurity -7	2.30	10.18	-	-	0.34	

At 60°C, one additional peak was found after 1 hour, two additional peaks were found after two hours, two additional peaks were found after three hours, two additional peaks were found after four hours and two additional peaks were found after five hours. The chromatograms are shown in the figure 3.4 and results are given in table 3.5.

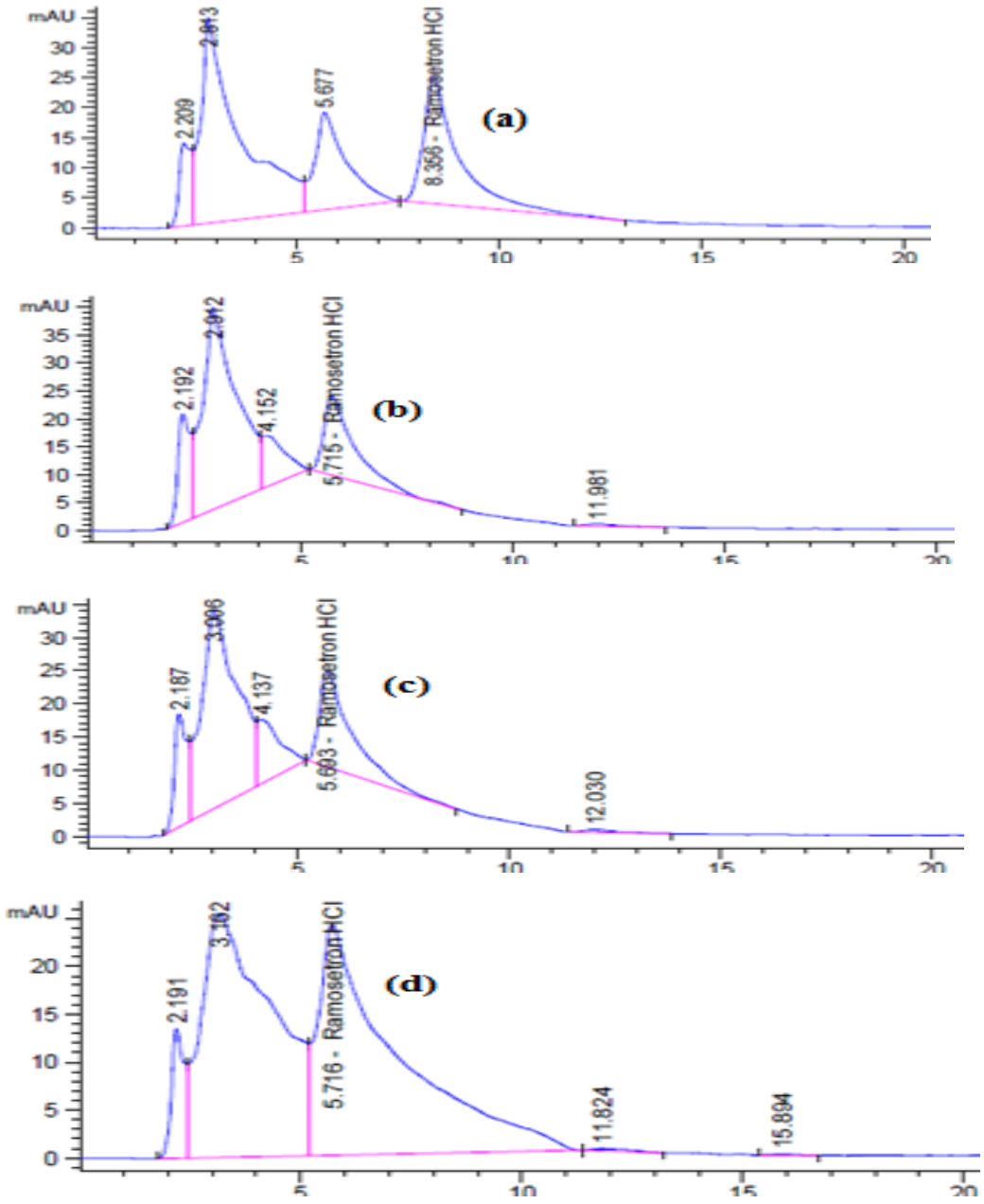

Figure 3.3: Chromatograms of ramosetron HCl at 60°C after 2 days in (a) 0.1N NaOH, (b) 0.5N NaOH, (c) 1.0N NaOH, and (d) 2.0N NaOH.

Table 3.4: Relationship between relative retention time (RRT), area and content of ramosetron HCl in base at 60°C after 2 days.

Conditions	Peak identity	RRT	Area	Content (%)		
				Sample	Impurity	Total impurities
0.1N NaOH	Ramosetron HCl	1	1262.9	79.1	-	20.9
0.5N NaOH	Ramosetron HCl		-		-	100
1.0N NaOH	Ramosetron HCl		-		-	100
2.0N NaOH	Ramosetron HCl		-		-	100

At 70°C, two additional peaks were found after 1 hour, two additional peaks were found after two hours, two additional peaks were found after three hours, and three additional peaks were found after four hours and three additional peaks were found after five hours. The chromatograms are shown in the figures 3.5-3.6 and results are given in table 3.6.

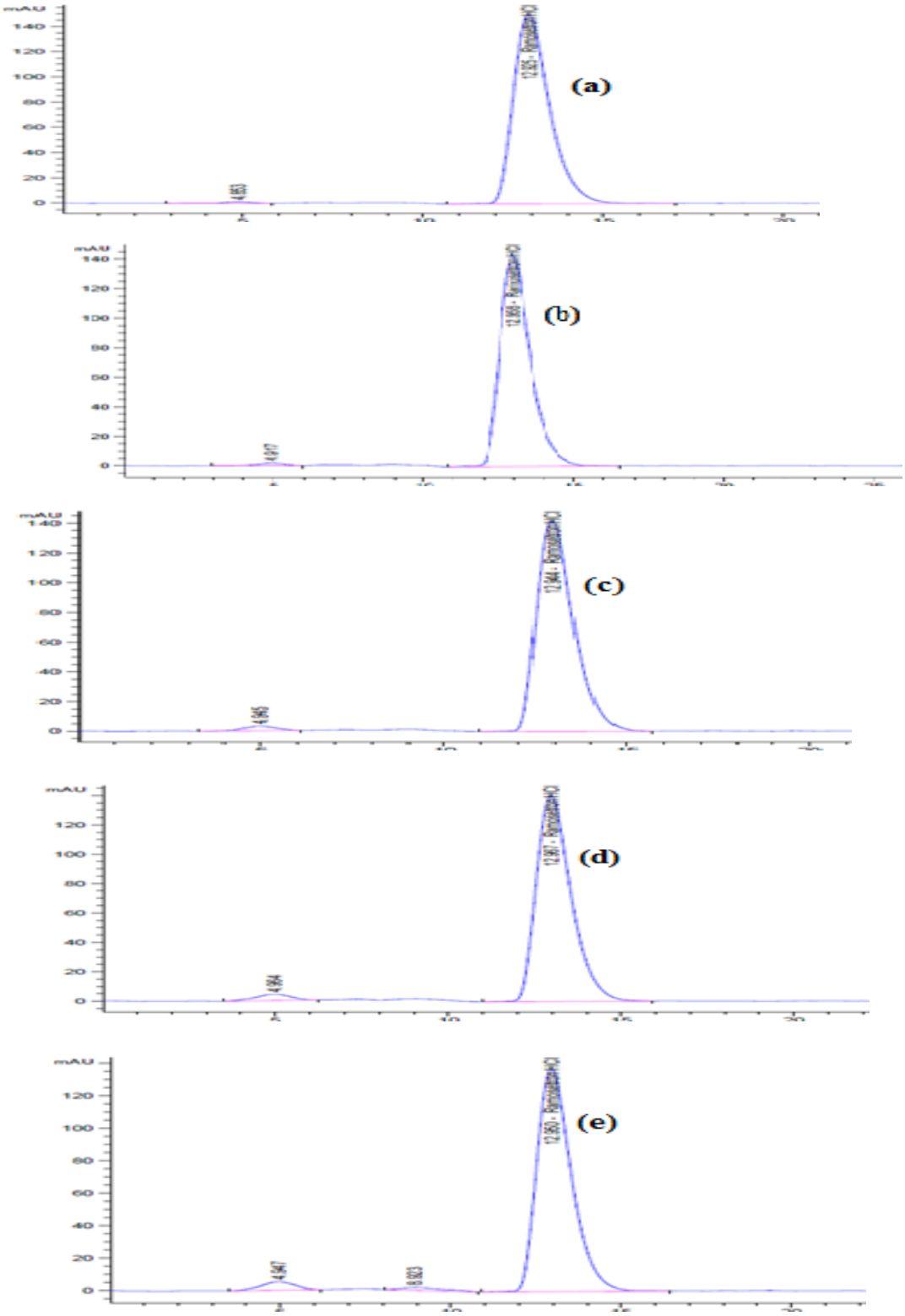

Figure 3.4: Chromatograms of ramosetron HCl in 0.1N NaOH at 60°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.5: Relationship between relative retention time (RRT), area and content of ramosetron HCl in 0.1N NaOH at 60°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ramosetron HCl	1	10436	0.9999	97.46	-	2.54
	Impurity-8	0.38	81.45	-	-	1.76	
2 hours	Ramosetron HCl	1	10159	0.9997	96.0	-	4.0
	Impurity-9	0.38	124.74	-	-	2.18	
3 hours	Ramosetron HCl	1	9932.3	0.9996	93.5	-	6.5
	Impurity-10	0.38	200.09	-	-	4.88	
4 hours	Ramosetron HCl	1	9802.3	0.9997	91.0	-	9.0
	Impurity-11	0.38	307.4	-	-	7.85	
5 hours	Ramosetron HCl	1	9649.0	0.9995	88.50	-	11.5
	Impurity-12	0.38	375.5	-	-	7.1	
	Impurity-13	0.67	151.4	-	-	2.9	

At 80°C, one additional peak was found after 1 hour, two additional peaks were found after two hours, two additional peaks were found after three hours, two additional peaks were found after four hours and two additional peaks were found after five hours. The chromatograms are shown in the figure 3.7 and results are given in table 3.7.

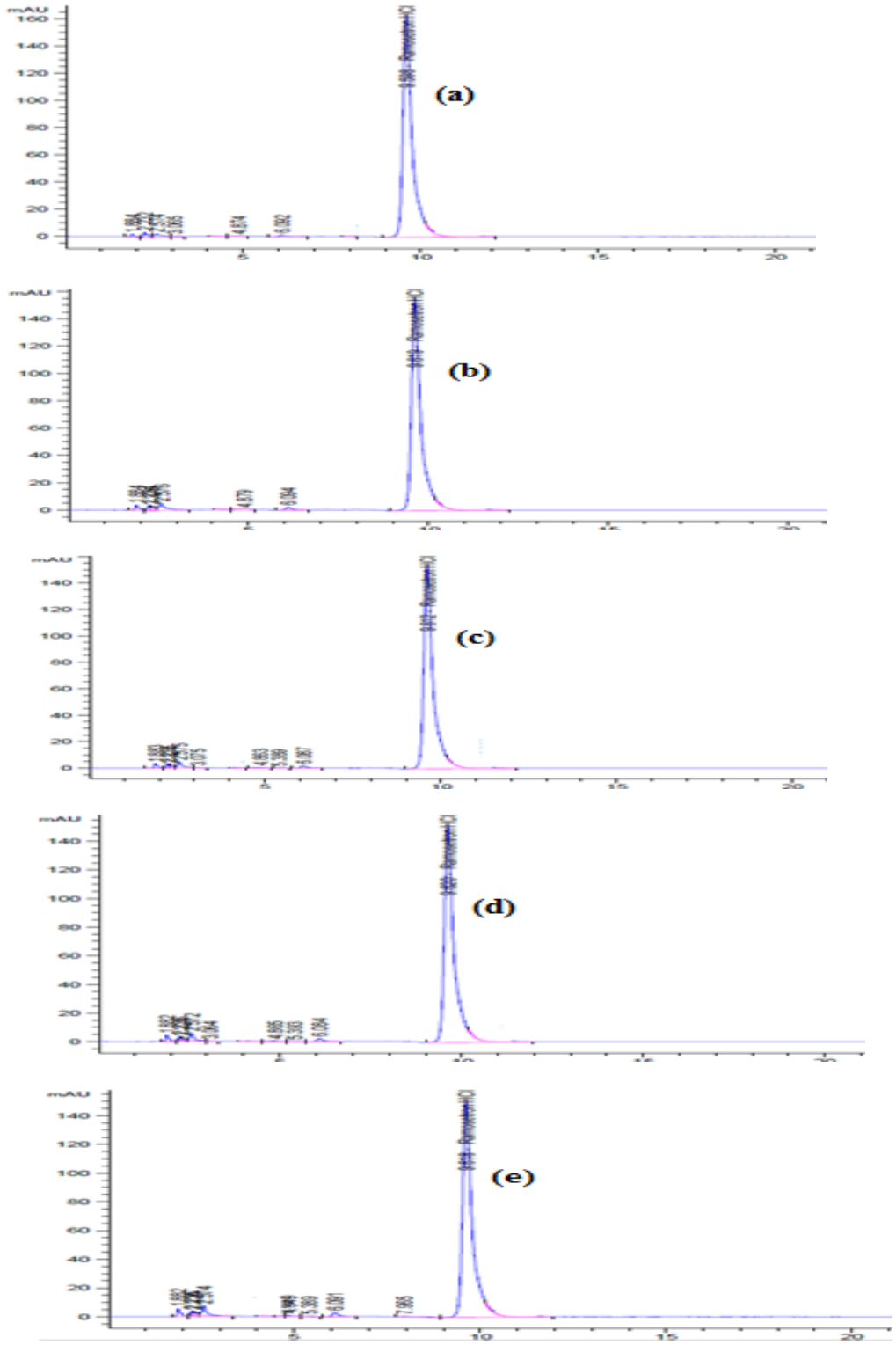

Figure 3.5: Chromatograms of ramosetron HCl in 0.1N NaOH at 70°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

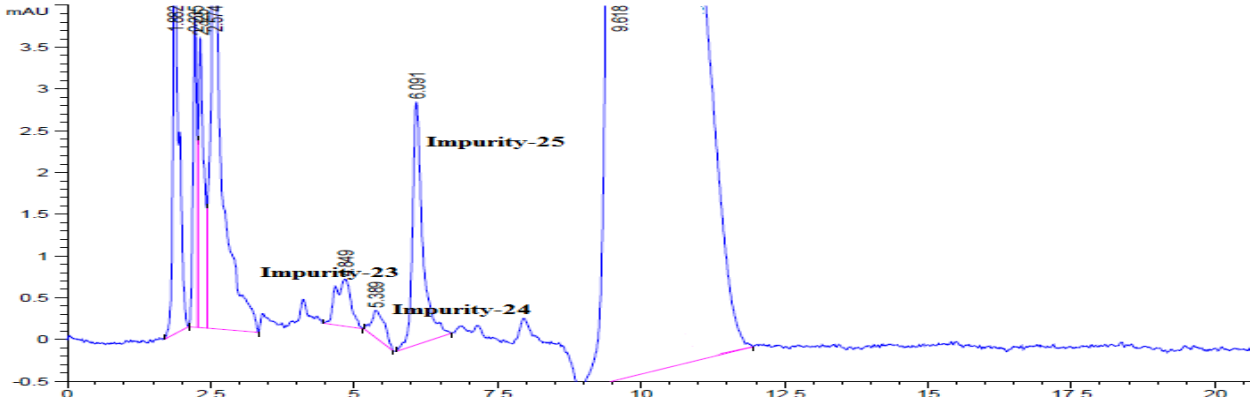

Figure 3.6: Extended chromatograms for impurity of ramosetron HCl in 0.1N NaOH at 70°C after 5 hours.

3.1.4 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 the figures 3.8-3.9 and results are given in tables 3.8-3.10.

Table 3.6: Relationship between relative retention time (RRT), area and content of ramosetron HCl in 0.1N NaOH at 70°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ramosetron HCl	1	2984.0	0.9995	96.40	-	3.6
	Impurity-14	0.51	4.98	-	-	0.4	
	Impurity-15	0.64	16.48	-	-	1.6	
2 hours	Ramosetron HCl	1	2859.1	0.9998	93.80	-	6.2
	Impurity-16	0.51	7.94	-	-	1.1	
	Impurity-17	0.64	25.57	-	-	3.9	
3 hours	Ramosetron HCl	1	2843.0	0.9997	90.20	-	9.8
	Impurity-18	0.51	6.18	-	-	1.5	
	Impurity-19	0.64	22.93	-	-	5.5	
4 hours	Ramosetron HCl	1	2826.0	0.9996	86.60	-	13.4
	Impurity-20	0.51	6.92	-	-	1.7	
	Impurity-21	0.56	5.29	-	-	1.4	
	Impurity-22	0.64	29.29	-	-	8.0	
5 hours	Ramosetron HCl	1	2788.8	0.9994	83.0	-	17.0
	Impurity-23	0.51	7.20	-	-	2.2	
	Impurity-24	0.56	5.51	-	-	1.6	
	Impurity-25	0.64	35.64	-	-	11.2	

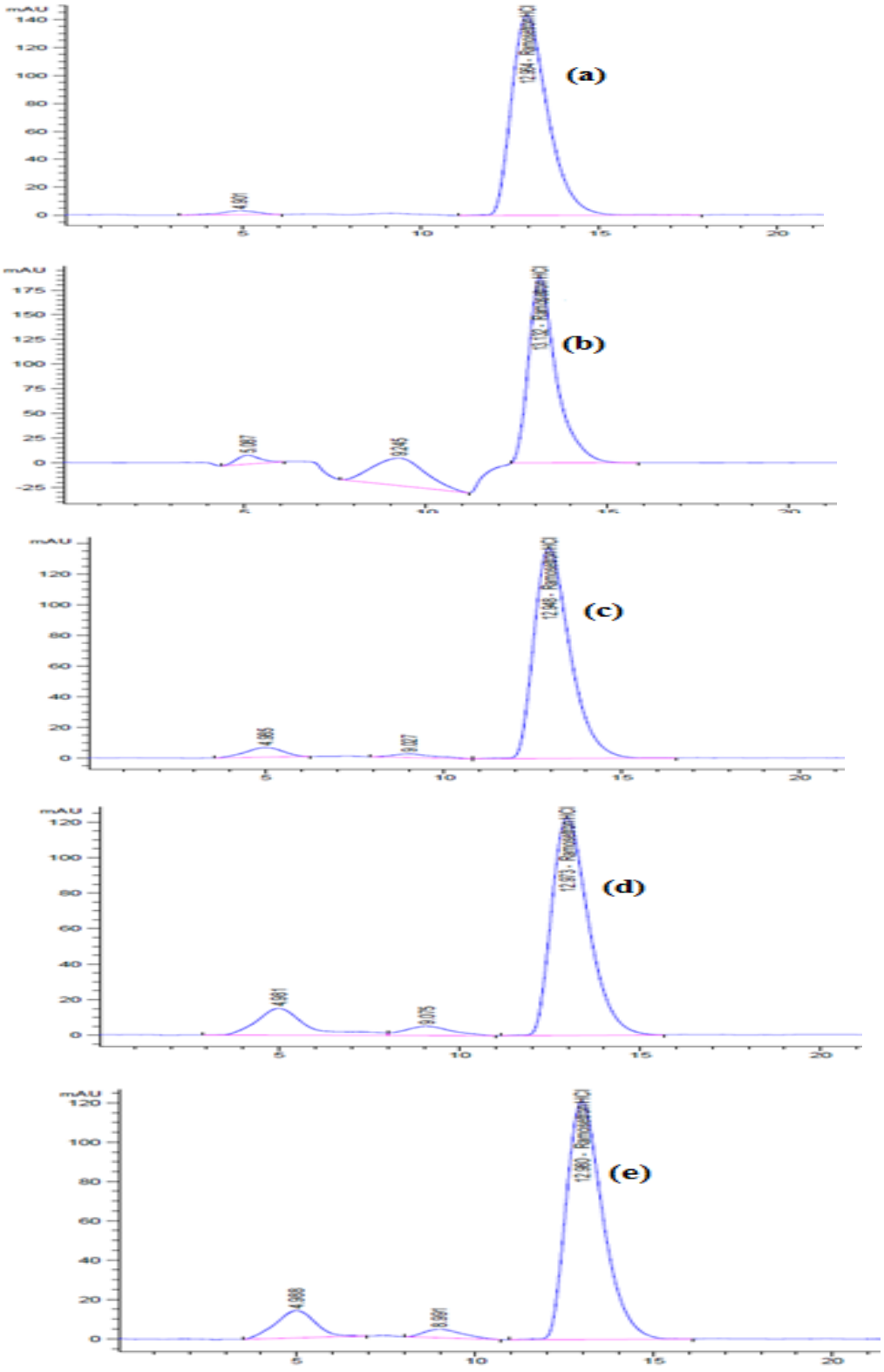

Figure 3.7: Chromatograms of ramosetron HCl in 0.1N NaOH at 80°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.7: Relationship between relative retention time (RRT), area and content of ramosetron HCl in 0.1N NaOH at 80°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ramosetron HCl	1	10143	0.9998	94.72	-	5.28
	Impurity-26	0.38	209.2	-	-	3.02	
2 hours	Ramosetron HCl	1	9936.3	0.9999	90.44	-	9.56
	Impurity-27	0.39	403.43	-	-	0.89	
	Impurity-28	0.70	2783.7	-	-	6.1	
3 hours	Ramosetron HCl	1	9641.4	0.9996	85.16	-	14.84
	Impurity-29	0.39	461.0	-	-	8.6	
	Impurity-30	0.70	185.4	-	-	4.8	
4 hours	Ramosetron HCl	1	8706.9	0.9997	76.88	-	20.12
	Impurity-31	0.38	1431.6	-	-	15.8	
	Impurity-32	0.70	468.8	-	-	5.2	
5 hours	Ramosetron HCl	1	8608.3	0.9995	74.18	-	25.22
	Impurity-33	0.39	1081.3	-	-	16.1	
	Impurity-34	0.69	328.0	-	-	4.9	

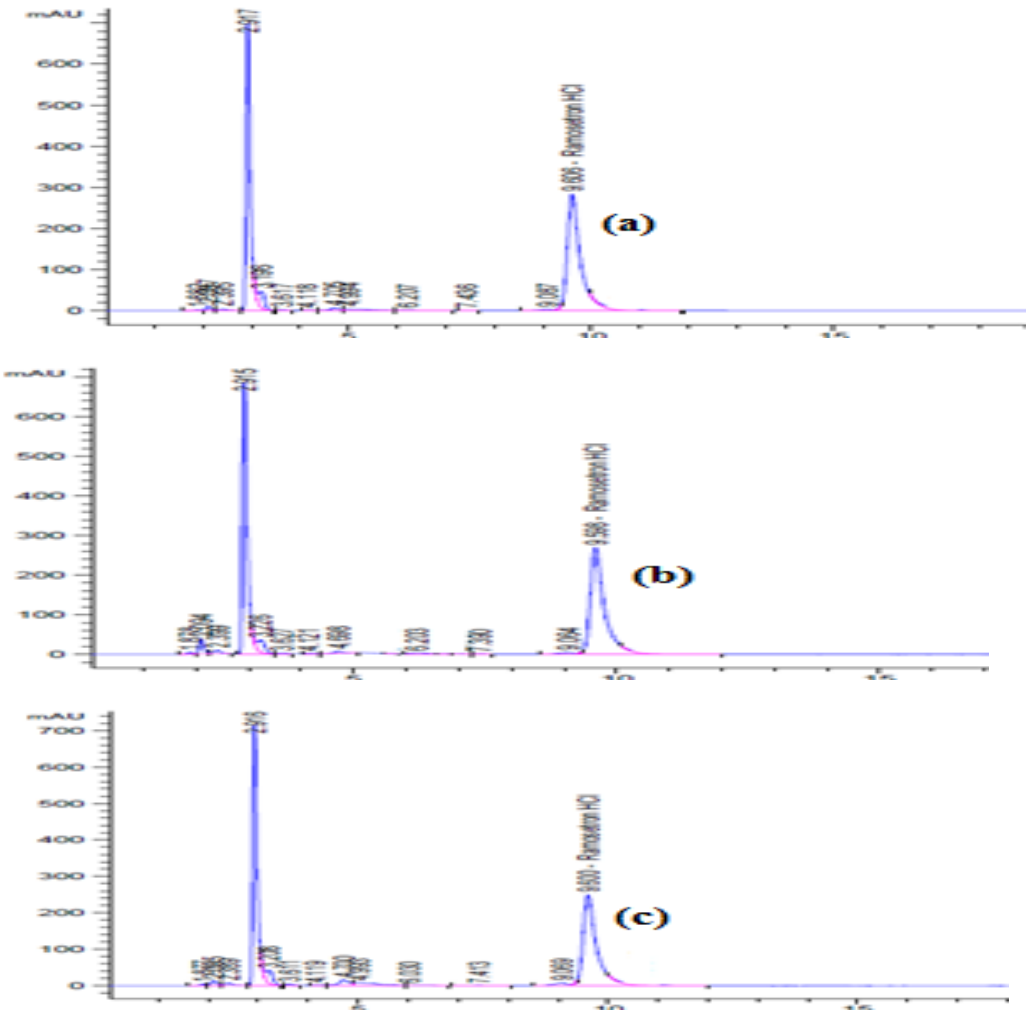

Figure 3.8: Chromatograms of ramosetron HCl in 3.0% H_2O_2 after (a) 1 hour, (b) 2 hours, and (c) 3 hours.

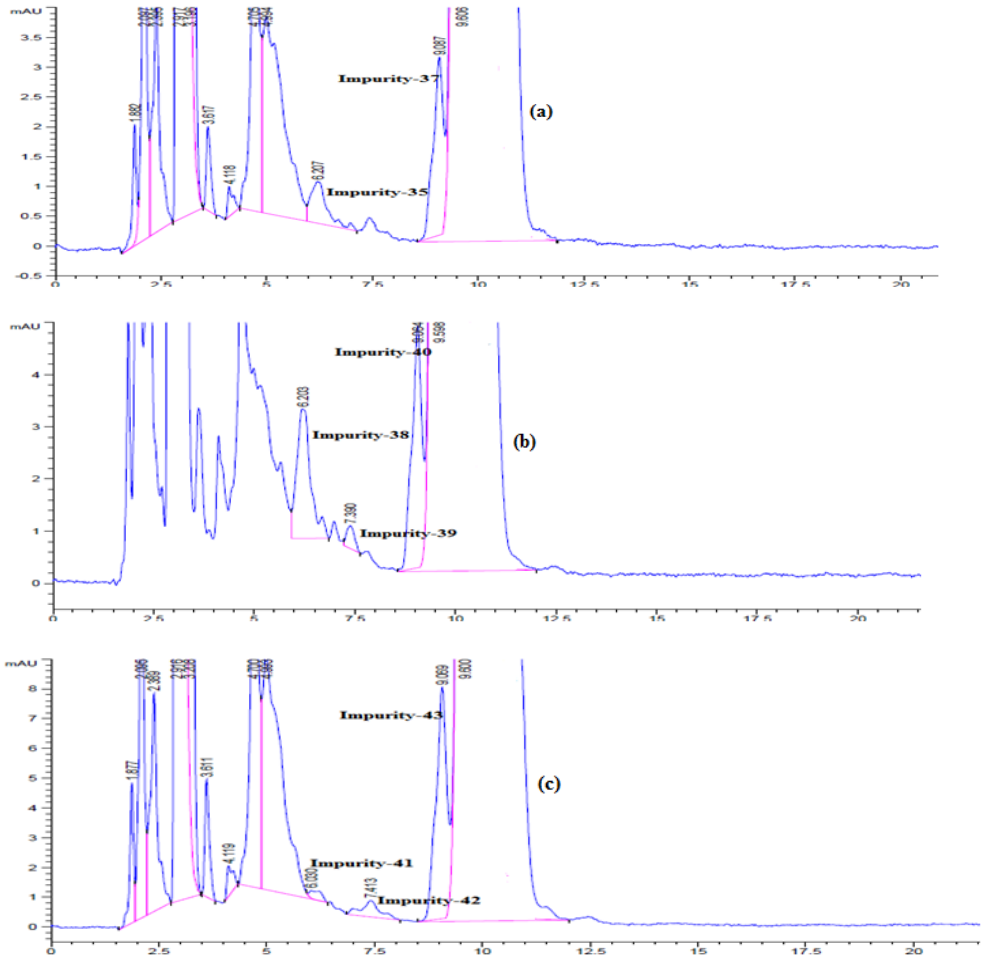

Figure 3.9: Extended chromatograms for impurities of ramosetron HCl in 3.0% H_2O_2 after (a) 1 hour, (b) 2 hours, and (c) 3 hours.

3.1.5 Photo degradation

Photo degradation study was carried out with bulk drug substance. The sample was directly exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light. Under this stress condition, no degradation was found. However, 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 chromatograms are shown in the figure 3.10 and results are given in table 3.11.

Table 3.8: Relationship between relative retention time (RRT), area and content of ramosetron HCl in 3.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ramosetron HCl	1	5377.98	0.9999	90.0	-	10.0
	Impurity-35	0.65	18.95	-	-	1.9	
	Impurity-36	0.77	3.03	-	-	0.31	
	Impurity-37	0.95	56.03	-	-	5.8	
2 hours	Ramosetron HCl	1	5061.76	0.9989	84.8	-	15.2
	Impurity-38	0.65	43.70	-	-	4.2	
	Impurity-39	0.76	6.01	-	-	0.58	
	Impurity-40	0.94	85.20	-	-	8.2	
3 hours	Ramosetron HCl	1	4727.82	0.9998	79.2	-	20.8
	Impurity-41	0.63	6.16	-	-	0.54	
	Impurity-42	0.77	16.23	-	-	1.4	
	Impurity-43	0.95	144.66	-	-	13.0	

Table 3.9: Relationship between relative retention time (RRT), area and content of ramosetron HCl in 5.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ramosetron HCl	1	3993.56	0.9988	42.1	-	57.9
2 hours	Ramosetron HCl		3633.35	0.9989	38.3	-	61.7
3 hours	Ramosetron HCl		2534.88	0.9984	26.7	-	73.3

Table 3.10: Relationship between relative retention time (RRT), area and content of ramosetron HCl in 10.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ramosetron HCl	1	2278.17	0.9978	24.0	-	76.0
2 hours	Ramosetron HCl		1318.8	0.9987	13.9	-	86.1
3 hours	Ramosetron HCl		668.66	0.9977	7.0	-	93.0

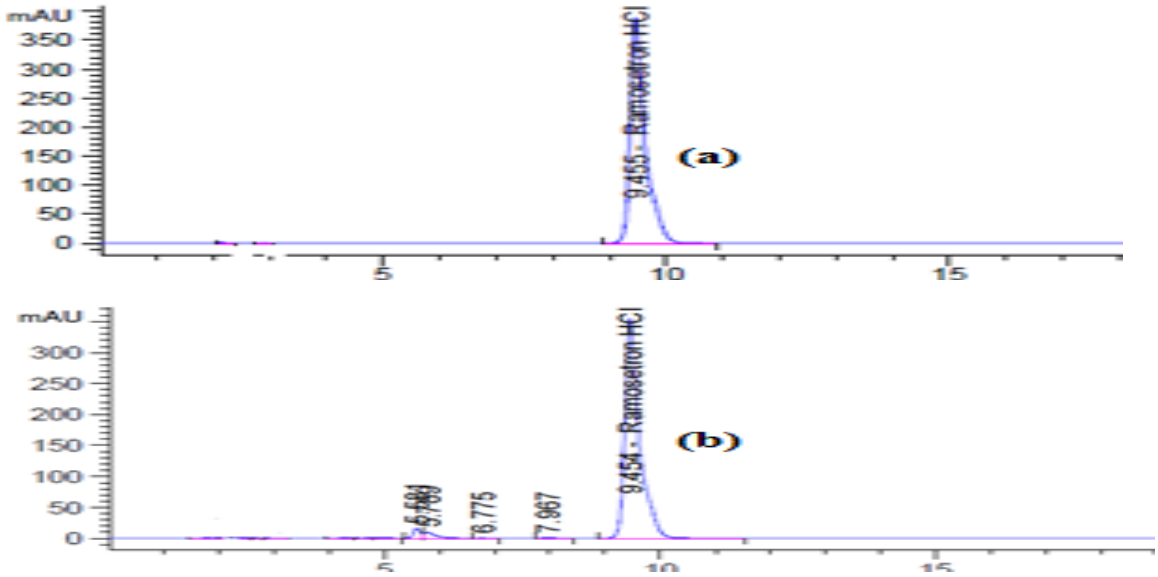

Figure 3.10: Chromatograms of ramosetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light for (a) controlled sample, and (b) stressed sample.

Table 3.11: Relationship between relative retention time (RRT), area and content of ramosetron HCl after photo degradation exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light and 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1.2 million lux, 7 days	Ramosetron HCl	1	6832.2	0.9999	99.8	-	-
3.6 million lux, 21 days	Ramosetron HCl	1	6465.4	0.9998	93.4	-	6.6
	Impurity-44	0.59	141.62	-	-	2.2	
	Impurity-45	0.61	137.27	-	-	2.1	
	Impurity-46	0.72	3.92	-	-	0.05	
	Impurity-47	0.84	11.73	-	-	0.17	

3.2 Stress degradation of ondansetron hydrochloride

It was observed that ondansetron hydrochloride degraded in aqueous, basic, oxidative and photolytic conditions and no degradation was found in acidic condition. Data and chromatograms derived from each stressed condition applied on ondansetron HCl are summarized below.

3.2.1 Aqueous degradation

In water, aqueous degradation study was conducted at 60°C for 7 days. Under this stressed condition, no degradation was observed but 3.6% degradation was found after 21 days. One additional peak apart from the principal and blank peaks was found with retention time 8.15 min and RRT 0.72. The peak area was 225.6. The chromatogram is shown in figure 3.11and the results are summarized in tables 3.12-3.13.

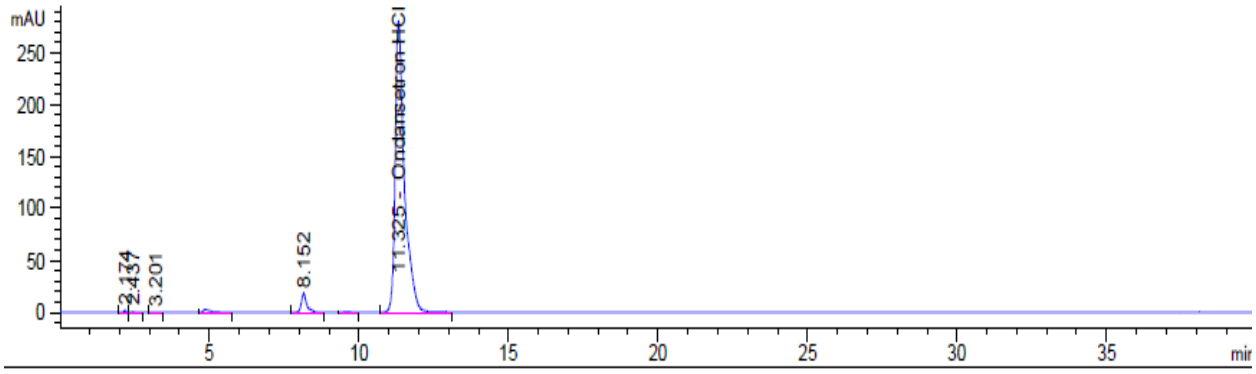

Figure 3.11: Chromatogram of ondansetron HCl in water at 60°C after 21 days.

Table 3.12: Relationship between relative retention time (RRT), area and content of ondansetron HCl in water at 60°C after 7 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
Aqueous degradation (7 days)	Ondansetron HCl	1	5992.5	0.9987	100.0	99.8

Table 3.13: Relationship between relative retention time (RRT), area and content of ondansetron HCl in water at 60°C after 21 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total Impurities
Aqueous degradation (21 days)	Ondansetron HCl	1	5967.7	0.9998	96.4	-	3.6
	Impurity-1	0.72	225.6	0.9997	--	2.6	

3.2.2 Acid degradation

Acidic degradation was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. No degradation was observed under these conditions. The results are given in table 3.14.

Table 3.14: Relationship between relative retention time (RRT), area and content of ondansetron HCl in acid at 60°C after 7 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
0.1N HCl	Ondansetron HCl	1	5788.0	0.9999	100.0	99.8
0.5N HCl	Ondansetron HCl		5893.3	0.9996		99.9
1.0N HCl	Ondansetron HCl		5890.0	0.9998		100.1
2.0N HCl	Ondansetron HCl		6047.0	0.9999		99.8

3.2.3 Base degradation

At first, base degradation was conducted in 0.1N, 0.5N, 1.0N and 2.0N NaOH at 60°C for 3 days. Here, 4.1%, 8.1%, 17.3% and 32.2% degradation were observed for 0.1N NaOH, 0.5N NaOH, 1.0N NaOH, and 2.0N NaOH, respectively. One additional peak was observed for both 0.1N NaOH and 0.5N NaOH stressed samples. On the other hand, two additional peaks were found for both 1.0N NaOH and 2.0N NaOH stressed samples. The chromatograms are shown in figure 3.12 and results are given in table 3.15.

Secondly, base degradation was carried out in 2.0N NaOH at 60°C, 70°C and 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, where 2.6%, 4.0%, 6.5%, 9.0% and 11.5% degradation were observed at 60°C, respectively. However, 3.6%, 6.2%, 9.8%, 13.4% and 17.0% degradation were observed at 70°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, respectively. On the other hand, 5.3%, 9.6%, 14.8%, 23.1% and 25.8% degradation were found at 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, respectively.

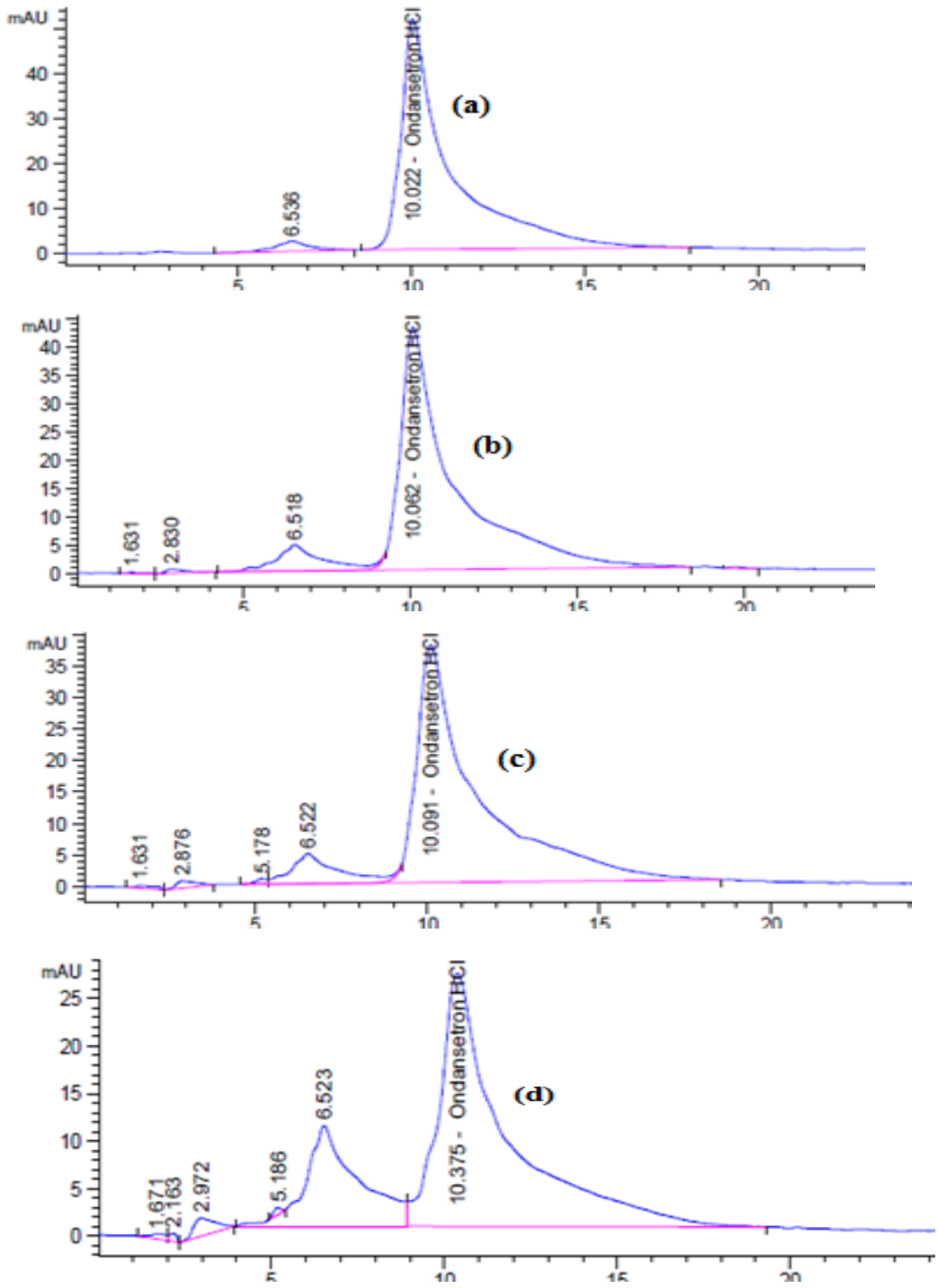

Figure 3.12: Chromatograms of ondansetron HCl at 60°C after 3 days in (a) 0.1N NaOH, (b) 0.5N NaOH, (c) 1.0N NaOH, and (d) 2.0N NaOH.

Table 3.15: Relationship between relative retention time (RRT), area and content of ondansetron HCl in base at 60°C after 3 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N NaOH	Ondansetron HCl	1	4956.9	0.9998	95.9	-	4.1
	Impurity-2	0.65	162.0	-	-	3.4	
0.5N NaOH	Ondansetron HCl	1	4718.6	0.9989	91.9	-	8.1
	Impurity-3	0.65	449.6	-	-	7.5	
1.0N NaOH	Ondansetron HCl	1	4584.4	0.9994	82.7	-	
	Impurity-4	0.51	19.85	-	-	0.39	17.3
	Impurity-5	0.65	445.4	-	-	11.7	
2.0N NaOH	Ondansetron HCl	1	3559.7	0.9993	67.8	-	
	Impurity-6	0.50	12.3	-	-	0.33	32.2
	Impurity-7	0.63	1108.2	-	-	29.7	

At 60°C, one degradant peak was found in each time point at 1 hour, 2 hours, 3 hours and 4 hours and two degradant peaks were found in 5 hours. The chromatograms are shown in the figure 3.13 and results are given in table 3.16.

At 70°C, two degradant peaks were found in each time point at 1 hour, 2 hours and 3 hours and three degradant peaks were found in each time point at 4 hours and 5 hours. The chromatograms are shown in the figure 3.14 and results are given in table 3.17.

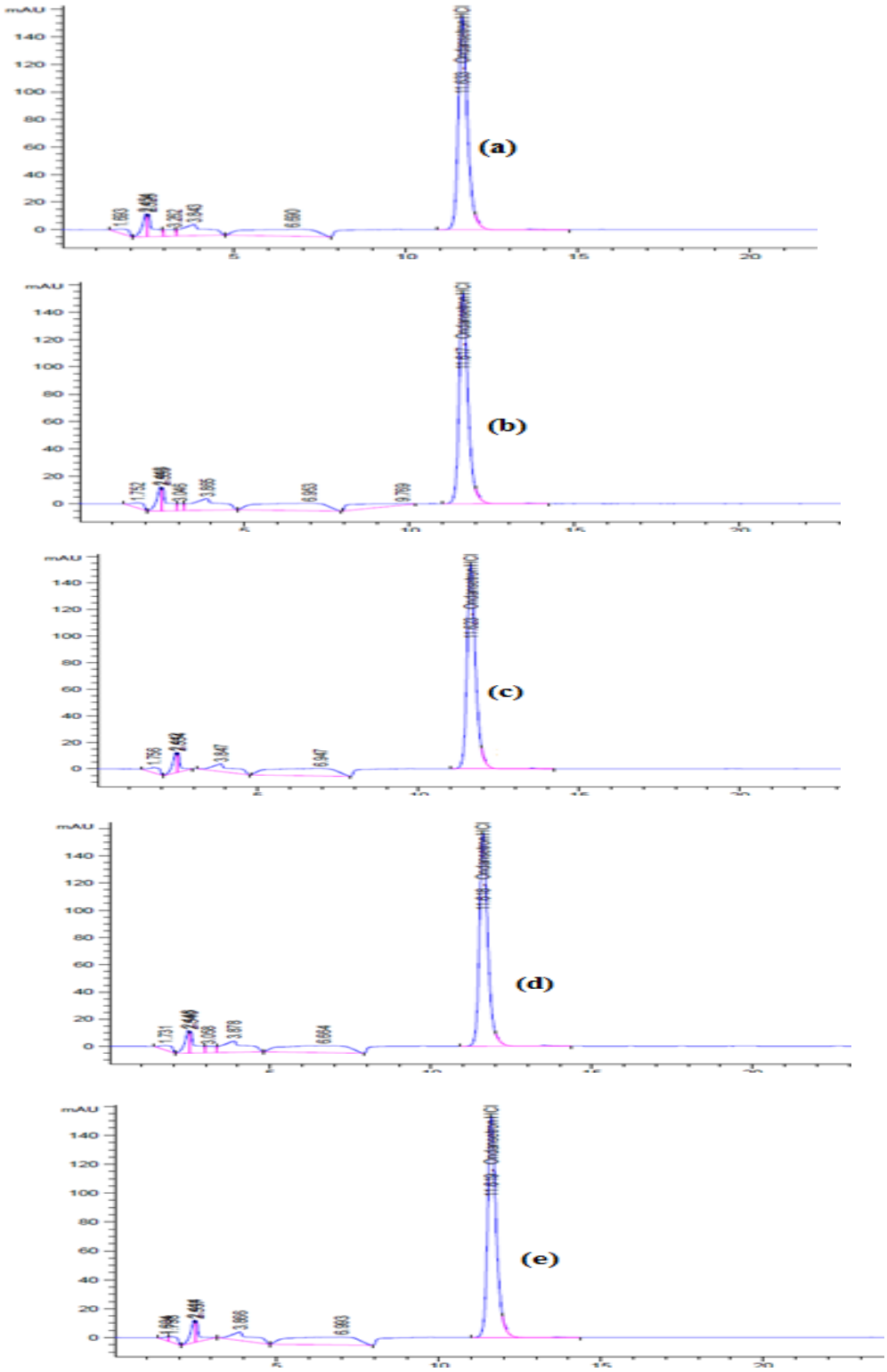

Figure 3.13: Chromatograms of ondansetron HCl in 2.0N NaOH at 60°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.16: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 2.0N NaOH at 60°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ondansetron HCl	1	10436	0.9999	97.5	-	2.6
	Impurity-8	0.38	81.45	-	-	1.8	
2 hours	Ondansetron HCl	1	10159	0.9997	96.0	-	4.0
	Impurity-9	0.38	124.74	-	-	2.7	
3 hours	Ondansetron HCl	1	9932.3	0.9996	93.5	-	6.5
	Impurity-10	0.38	200.09	-	-	4.8	
4 hours	Ondansetron HCl	1	9802.3	0.9997	91.0	-	9.0
	Impurity-11	0.38	307.4	-	-	7.8	
5 hours	Ondansetron HCl	1	9649.0	0.9995	88.5	-	11.5
	Impurity-12	0.38	375.5	-	-	7.1	
	Impurity-13	0.67	151.4	-	-	2.9	

At 80°C, one degradant peak was found after 1 hour and two degradant peaks were found in each time point at 2 hours, 3 hours, 4 hours and 5 hours. The chromatograms are shown in the figure 3.15 and results are given in table 3.18.

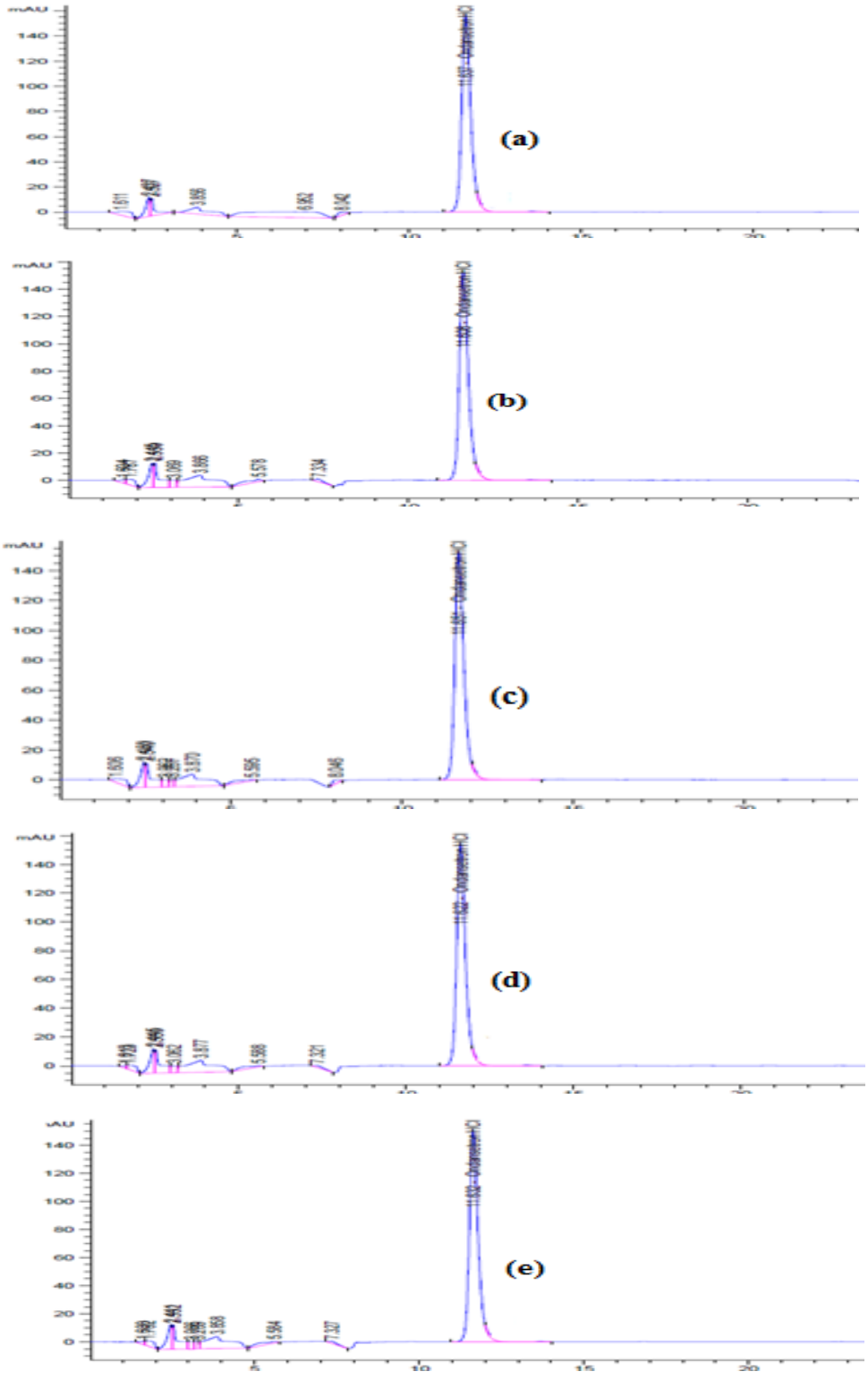

Figure 3.14: Chromatograms of ondansetron HCl in 2.0N NaOH at 70°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

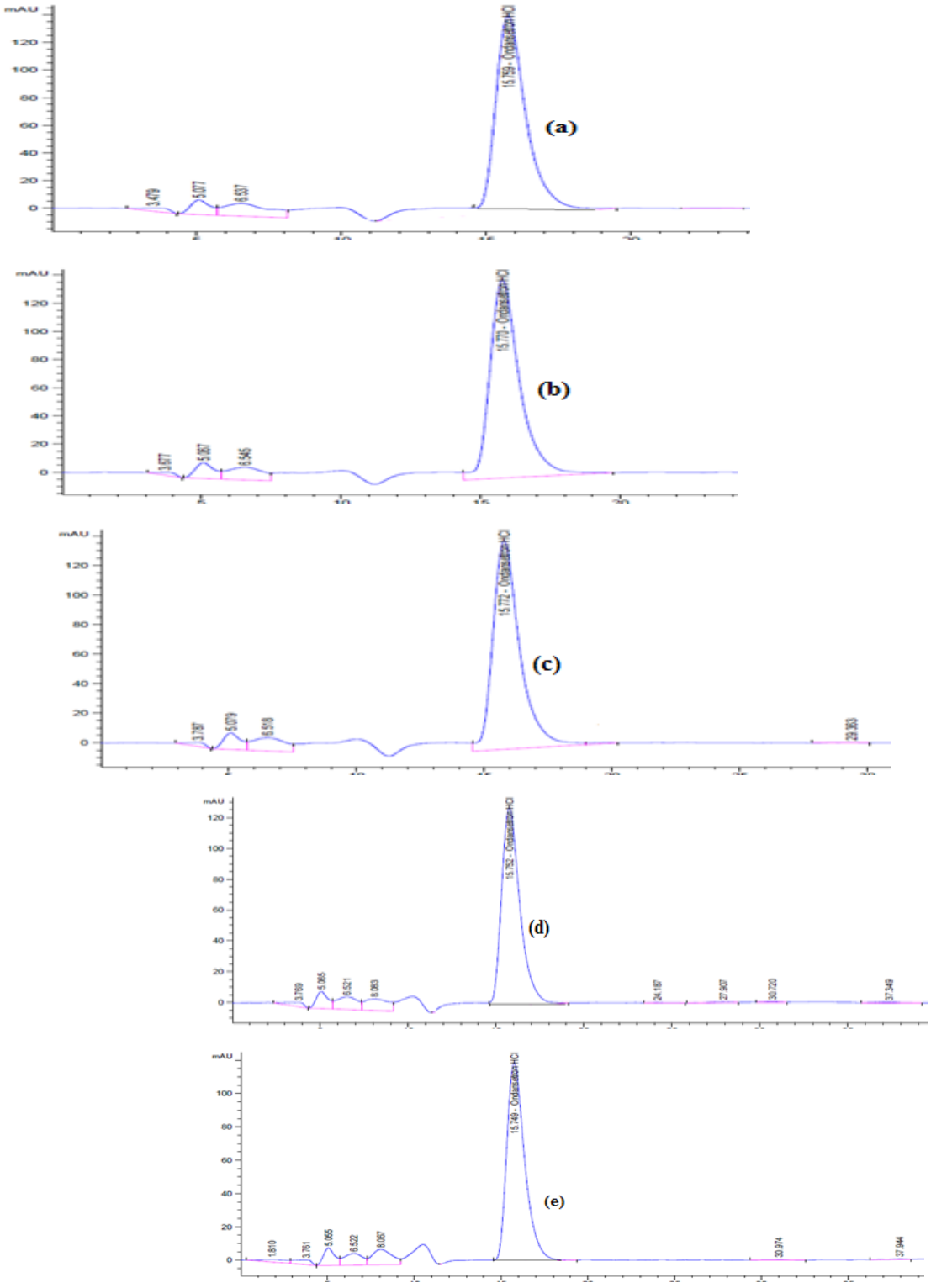

Figure 3.15: Chromatograms of ondansetron HCl in 2.0N NaOH at 80°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.17: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 2.0N NaOH at 70°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ondansetron HCl	1	2984.0	0.9995	96.4	-	3.6
	Impurity-14	0.51	4.98	-	-	0.46	
	Impurity-15	0.64	16.48	-	-	1.5	
2 hours	Ondansetron HCl	1	2859.1	0.9998	93.8	-	6.2
	Impurity-16	0.51	7.94	-	-	1.2	
	Impurity-17	0.64	25.57	-	-	3.8	
3 hours	Ondansetron HCl	1	2843.0	0.9997	90.2	-	9.8
	Impurity-18	0.51	6.18	-	-	1.7	
	Impurity-19	0.64	22.93	-	-	6.3	
4 hours	Ondansetron HCl	1	2826.0	0.9996	86.6	-	13.4
	Impurity-20	0.51	6.92	-	-	2.0	
	Impurity-21	0.56	5.29	-	-	1.5	
	Impurity-22	0.64	29.29	-	-	8.5	
5 hours	Ondansetron HCl	1	2788.8	0.9994	83.0	-	17.0
	Impurity-23	0.51	7.20	-	-	2.2	
	Impurity-24	0.56	5.51	-	-	1.7	
	Impurity-25	0.64	35.64	-	-	11.1	

Table 3.18: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 2.0N NaOH at 80°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ondansetron HCl	1	10143	0.9998	94.7	-	5.3
	Impurity-26	0.38	209.2	-	-	3.9	
2 hours	Ondansetron HCl	1	9936.3	0.9999	90.4	-	9.6
	Impurity-27	0.39	403.43	-	-	1.0	
	Impurity-28	0.70	2783.7	-	-	7.0	
3 hours	Ondansetron HCl	1	9641.4	0.9999	85.2	-	14.8
	Impuirty-29	0.39	461.0	-	-	8.6	
	Impurity-30	0.70	185.4	-	-	3.4	
4 hours	Ondansetron HCl	1	8706.9	0.9997	76.9	-	23.1
	Impurity-31	0.38	1431.6	-	-	15.1	
	Impurity-32	0.70	468.8	-	-	4.9	
5 hours	Ondansetron HCl	1	8608.3	0.9997	74.2	-	25.8
	Impurity-33	0.39	1081.3	-	-	16.9	
	Impurity-34	0.69	328.0	-	-	5.1	

3.2.4 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, 11.9%, 13.7% and 18.6% degradation were observed in 3.0% hydrogen peroxide after 1, 2 and 3 hours, respectively. However, 26.1%, 30.0% and 38.0% degradation were observed in 5% hydrogen peroxide after 1, 2 and 3 hours, respectively. On the other hand, 38.0%, 41.1% and 45.0% degradation could be seen for 10.0% 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. The chromatograms are shown in the figure 3.16 and results are given in tables 3.19-3.24.

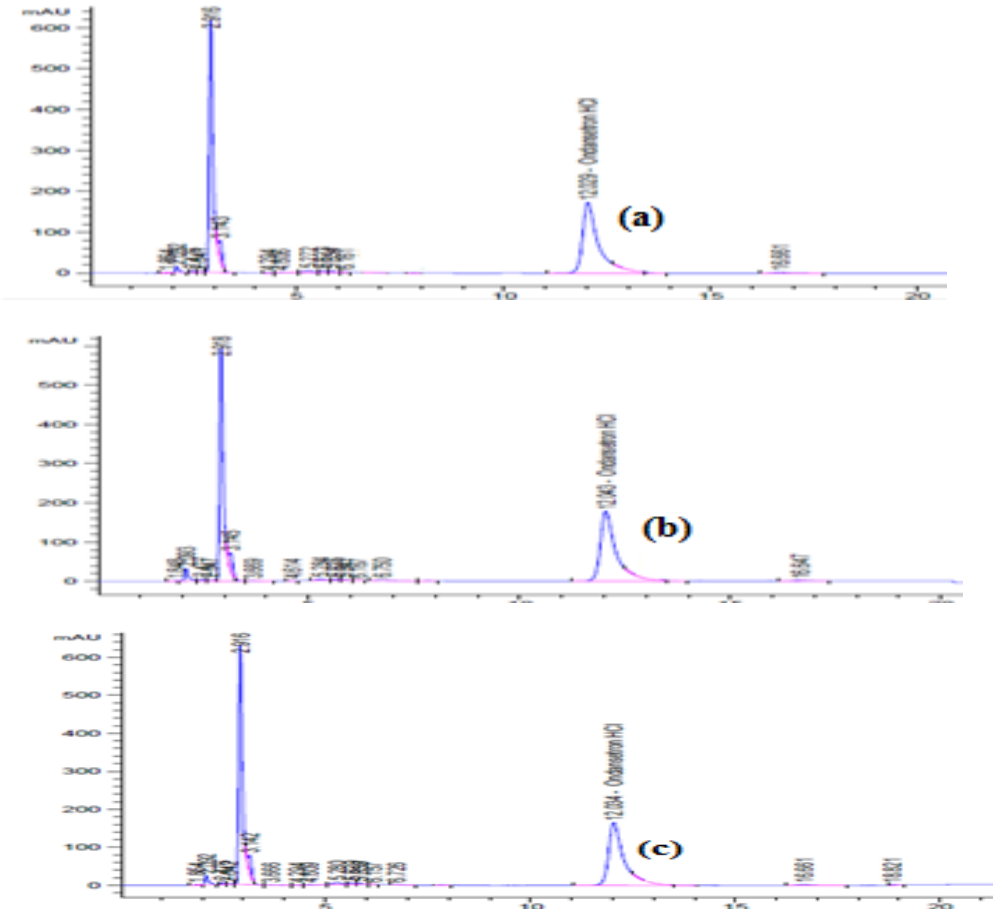

Figure 3.16: Chromatograms of ondansetron HCl in 3.0% H_2O_2 after (a) 1 hour, (b) 2 hours, and (c) 3 hours.

Table 3.19: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 3.0% hydrogen peroxide at dark place after 1, and 2 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ondansetron HCl	1	4718.17	0.9998	88.1	-	11.9
	Impurity-35	0.44	72.0	-	-	2.5	
	Impurity-36	0.47	41.87	-	-	1.5	
	Impurity-37	0.48	33.42	-	-	1.2	
	Impurity-38	1.39	51.86	-	-	1.8	
2 hours	Ondansetron HCl	1	4619.11	0.9987	86.3	-	13.7
	Impurity-39	0.44	67.00	-	-	2.8	
	Impurity-40	0.47	38.10	-	-	1.6	
	Impurity-41	0.49	24.15	-	-	1.0	
	Impurity-42	0.51	10.20	-	-	0.42	
	Impurity-43	0.56	35.95	-	-	1.5	
	Impurity-44	1.38	38.24	-	-	1.6	

Table 3.20: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 3.0% hydrogen peroxide at dark place after 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
3 hours	Ondansetron HCl	1	4359.49	0.9999	81.4	-	18.6
	Impurity-45	0.44	88.34	-	-	4.0	
	Impurity-46	0.47	48.82	-	-	2.2	
	Impurity-47	0.49	31.12	-	-	1.4	
	Impurity-48	0.56	21.65	-	-	0.95	
	Impurity-49	0.65	11.91	-	-	0.50	
	Impurity-50	1.38	67.87	-	-	3.0	

Table 3.21: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 5.0% hydrogen peroxide at dark place after 1, and 2 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ondansetron HCl	1	4344.41	0.9997	73.9	-	26.1
	Impurity-51	0.44	81.03	-	-	5.7	
	Impurity-52	0.47	54.49	-	-	3.8	
	Impurity-53	0.48	37.97	-	-	2.6	
	Impurity-54	0.56	36.49	-	-	2.5	
	Impurity-55	1.39	75.50	-	-	5.3	
2 hours	Ondansetron HCl	1	3956.58	0.9996	70.0	-	30.0
	Impurity-56	0.44	85.41	-	-	4.5	
	Impurity-57	0.47	66.74	-	-	3.5	
	Impurity-58	0.48	42.31	-	-	2.2	
	Impurity-59	0.56	78.46	-	-	4.1	
	Impurity-60	0.65	24.97	-	-	1.3	
	Impurity-61	0.68	30.72	-	-	1.6	
	Impurity-62	1.38	109.7			5.8	

Table 3.22: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 5.0% hydrogen peroxide at dark place after 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
3 hours	Ondansetron HCl	1	3930.75	0.9992	62.0	-	38.0
	Impurity-63	0.44	83.34	-	-	6.4	
	Impurity-64	0.47	79.58	-	-	6.1	
	Impurity-65	0.48	58.03	-	-	4.5	
	Impurity-66	0.56	29.65	-	-	2.2	
	Impurity-67	0.65	18.79	-	-	1.4	
	Impurity-68	1.39	124.23	-	-	9.5	

Table 3.23: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 10.0% hydrogen peroxide at dark place after 1, and 2 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Ondansetron HCl	1	3650.97	0.9999	62.0	-	38.0
	Impurity-69	0.44	101.73	-	-	4.0	
	Impurity-70	0.47	115.08	-	-	4.6	
	Impurity-71	0.48	123.08	-	-	4.9	
	Impurity-72	0.56	142.97	-	-	5.7	
	Impurity-73	0.65	13.25	-	-	0.52	
	Impurity-74	0.68	12.89	-	-	0.48	
	Impurity-75	1.39	119.29	-	-	4.8	
2 hours	Ondansetron HCl	1	3347.614	0.9993	58.9	-	41.1
	Impurity-76	0.44	78.44	-	-	3.8	
	Impurity-77	0.47	102.32	-	-	5.0	
	Impurity-78	0.48	97.15	-	-	4.8	
	Impurity-79	0.56	168.11	-	-	8.2	
	Impurity-80	0.65	15.93	-	-	0.74	
	Impurity-81	0.68	17.34	-	-	0.83	
	Impurity-82	1.39	134.83	-	-	6.6	

Table 3.24: Relationship between relative retention time (RRT), area and content of ondansetron HCl in 10.0% hydrogen peroxide at dark place after 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
3 hours	Ondansetron HCl	1	3154.83	0.9999	55.0	-	45.0
	Impurity-83	0.44	141.28	-	-	6.3	
	Impurity-84	0.47	150.5	-	-	6.7	
	Impurity-85	0.48	153.11	-	-	6.9	
	Impurity-86	0.56	95.64	-	-	4.3	
	Impurity-87	0.65	18.60	-	-	0.81	
	Impurity-88	0.68	14.47	-	-	0.63	
	Impurity-89	1.39	166.5	-	-	7.4	

3.2.5 Photo degradation

Photo degradation study was conducted with bulk drug substance. The sample was directly exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light. Under this stressed condition, no degradation was found. However, 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 2.3% degradation. One peak apart from the principal and blank peaks was found with retention time 2.88 min and RRT 0.25. The chromatograms are shown in the figures 3.17-3.18 and results are given in table 3.25.

3.3 Stress degradation of granisetron hydrochloride

It was observed that granisetron hydrochloride degraded in aqueous, basic, oxidative and photolytic conditions and no degradation was found in acidic condition. Data and chromatograms derived from the experiment in stressed conditions are summarized below.

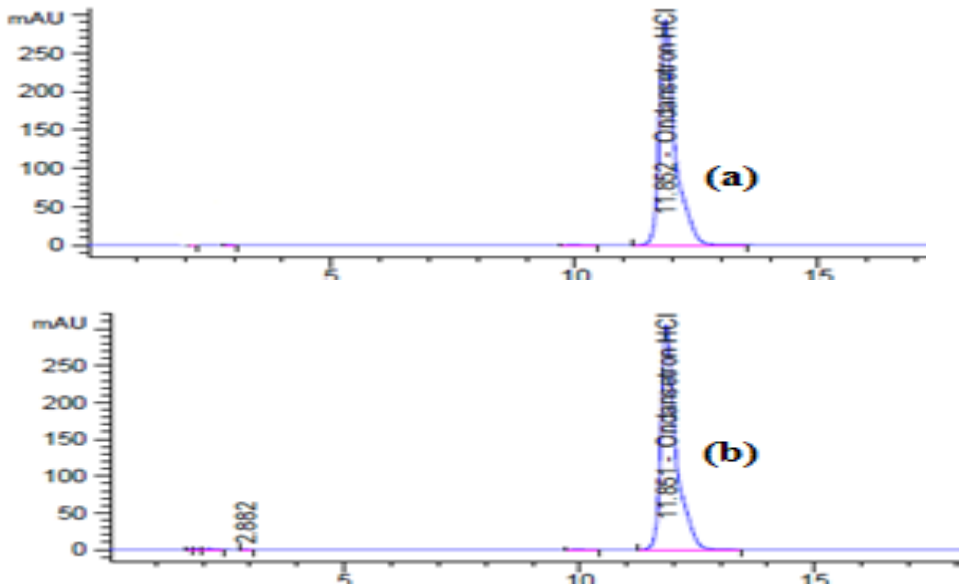

Figure 3.17: Chromatograms of ondansetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light for (a) controlled sample, and (b) stressed sample.

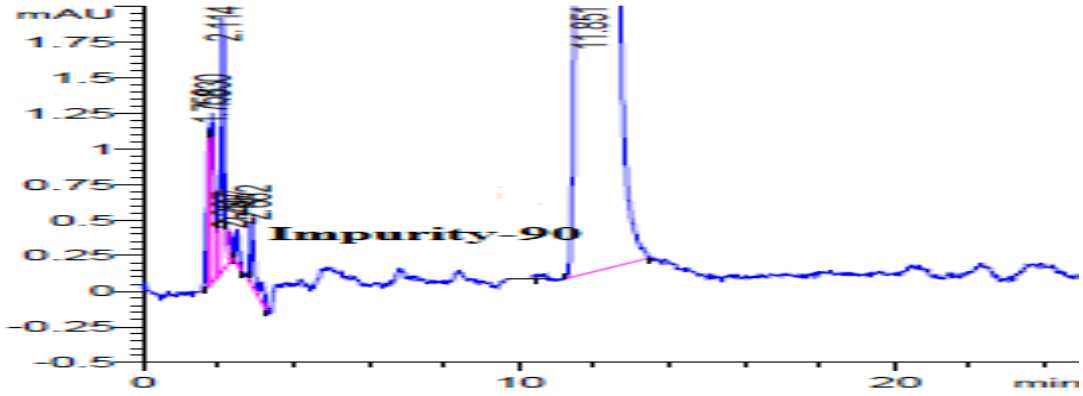

Figure 3.18: Extended chromatograms for impurity of ondansetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light for stressed sample.

Table 3.25: Relationship between relative retention time (RRT), area and content of ondansetron HCl after photo degradation exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light and 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1.2 million lux, 7 days	Ondansetron HCl	1	6998.25	0.9998	99.6	-	-
3.6 million lux, 21 days	Ondansetron HCl	1	6747.15	0.9999	97.8	-	2.2
	Impurity-90	0.25	9.21	-	-	1.14	

3.3.1 Aqueous degradation

Stressed degradation study for aqueous environment was conducted in purified water at 60°C for 7- and 21-days. Under this stress condition, no degradation was found for first 7 days but 0.3% degradation was found after 21 days. One additional peak apart from principal and blank peaks was found with retention time 13.9 min and RRT 0.85. . The chromatogram is shown in figures 3.19-3.20 and the results are summarized in table 3.26-3.27.

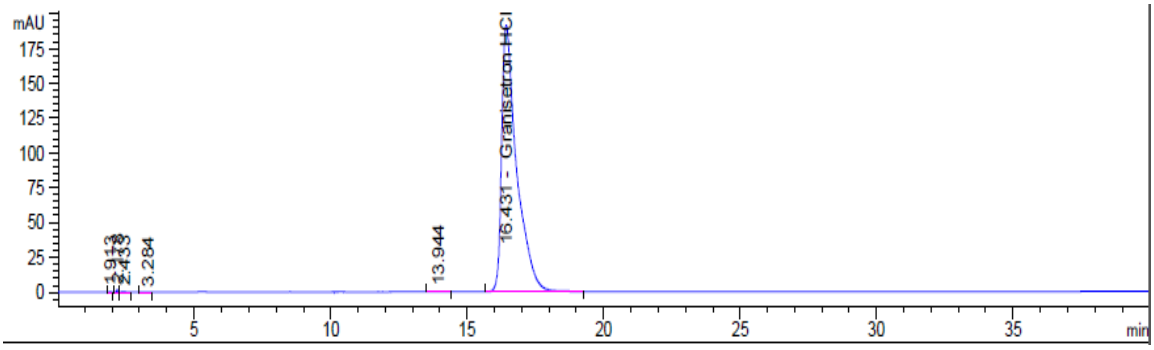

Figure 3.19: Chromatogram of granisetron HCl in water at 60°C for 21 days.

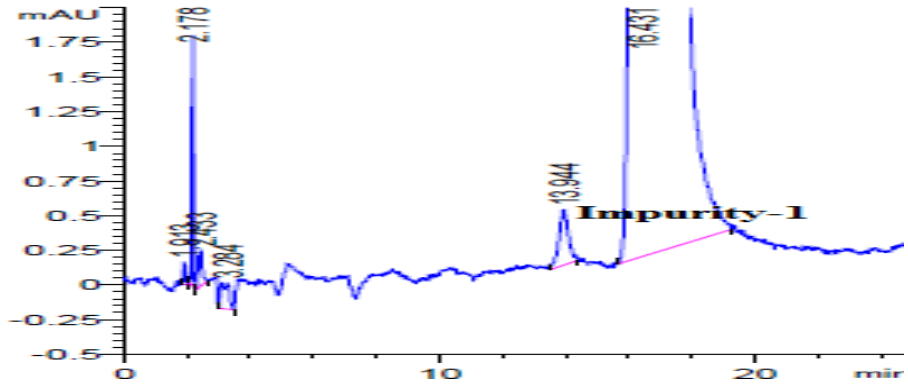

Figure 3.20: Extended chromatogram for impurity of granisetron HCl in water at 60°C for 21 days.

Table 3.26: Relationship between relative retention time (RRT), area and content of granisetron HCl in water at 60°C after 7 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
Aqueous degradation (7 days)	Granisetron HCl	1	6583.77	0.9998	100.0	99.9

Table 3.27: Relationship between relative retention time (RRT), area and content of granisetron HCl in water at 60°C after 21 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
Aqueous degradation (21 days)	Granisetron HCl	1	7303.68	0.9989	99.7	-	0.3
	Impurity-1	0.85	8.26	-	-	0.30	

3.3.2 Acid degradation

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Here, 2.31%, 5.0%, 10.8% and 17.1% degradation were observed with 0.1N, 0.5N, 1.0N and 2.0N HCl, respectively. The chromatograms are shown in figure 3.21 and results are given in table 3.28.

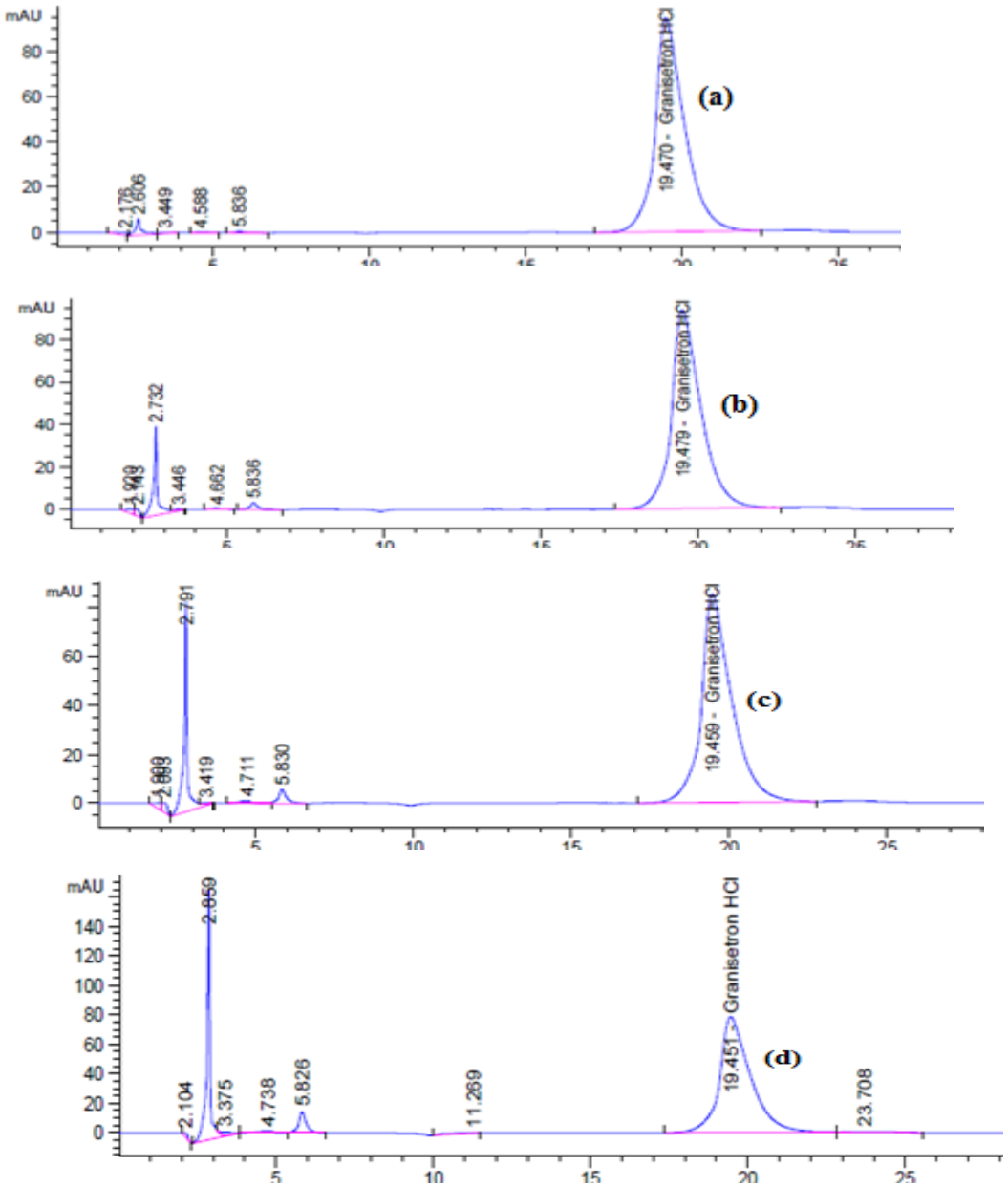

Figure 3.21: Chromatograms of granisetron HCl at 60°C after 7 days in (a) 0.1N HCl, (b) 0.5N HCl, (c) 1.0N HCl, and (d) 2.0N HCl.

Table 3.28: Relationship between relative retention time (RRT), area and content of granisetron HCl in acid at 60°C after 7 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N HCl	Granisetron HCl	1	6208.0	0.9999	97.7	-	2.3
	Impurity-2	0.24	6.31	-	-	0.5	
	Impurity-3	0.30	12.93	-	-	1.0	
0.5N HCl	Granisetron HCl	1	6151.8	0.9998	95.0	-	5.0
	Impurity-4	0.24	11.48	-	-	0.62	
	Impurity-5	0.30	60.63	-	-	3.4	
1.0N HCl	Granisetron HCl	1	5752.7	0.9997	89.2	-	10.8
	Impurity-6	0.24	25.62	-	-	1.7	
	Impurity-7	0.30	105.90	-	-	7.3	
2.0N HCl	Granisetron HCl	1	5307.5	0.9999	82.9	-	
	Impurity-8	0.24	43.93	-	-	1.7	17.1
	Impurity-9	0.30	246.82	-	-	9.7	
	Impurity-10	0.58	45.32	-	-	1.8	
	Impurity-11	1.22	47.92	-	-	1.9	

3.3.3 Base degradation

Base degradation was conducted initially in 0.1N, 0.5N, 1.0N and 2.0N NaOH at 60°C for 3 days. Here, 1.4%, 20.8%, 24.7% and 46.3% degradation were observed with 0.1N, 0.5N, 1.0N and 2.0N NaOH, respectively. The chromatogram is shown in figure 3.22 and results are given in table 3.29.

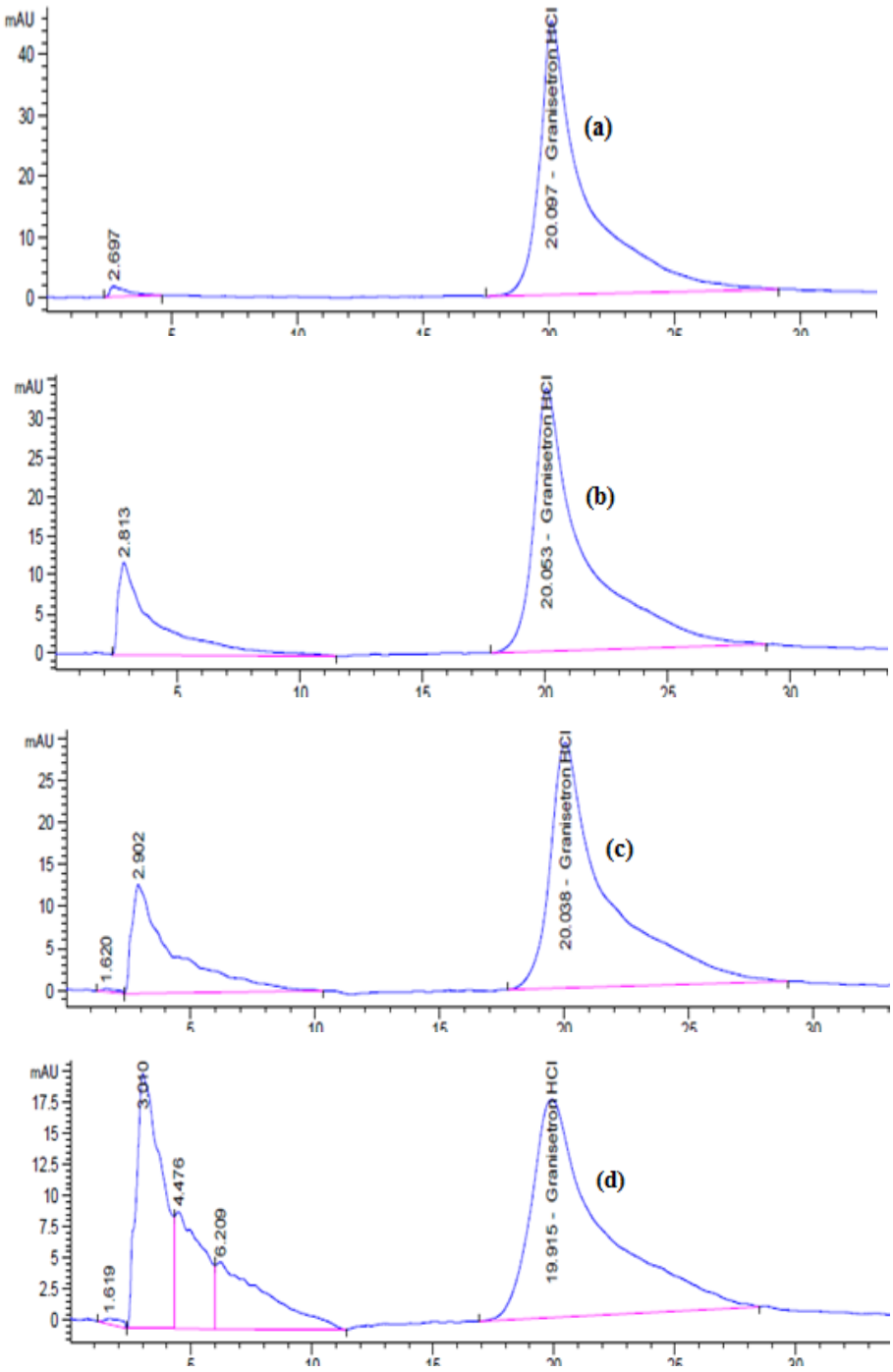

Figure 3.22: Chromatograms of granisetron HCl at 60°C after 3 days in (a) 0.1N NaOH, (b) 0.5N NaOH, (c) 1.0N NaOH, and (d) 2.0N NaOH.

Table 3.29: Relationship between relative retention time (RRT), area and content of granisetron HCl in base at 60°C after 3 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N NaOH	Granisetron HCl	1	5761.6	-	98.6	-	1.4
	Impurity-12	0.13	84.3	-	-	1.2	
0.5N NaOH	Granisetron HCl	1	4819.7	-	79.2	-	20.8
	Impurity-13	0.14	1267.6	-	-	20.82	
1.0N NaOH	Granisetron HCl	1	4561.1	-	75.3	-	24.7
	Impurity-14	0.08	15.02	-	-	0.22	
	Impurity-15	0.14	1479.8	-	-	21.8	
2.0N NaOH	Granisetron HCl	1	3521.8	-	53.7	-	46.3
	Impurity-16	0.08	25.2	-	-	0.38	
	Impurity-17	0.15	1488.0	-	-	22.8	
	Impurity-18	0.31	775.0	-	-	11.9	

Secondly, base degradation was conducted out in 2.0N NaOH at 60°C, 70°C and 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours. Here, 1.2%, 2.3%, 3.5%, 4.6% and 5.8% degradation were found at 60°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours respectively. However, 2.5%, 5.0%, 7.5%, 10.0% and 12.6% degradation were found at 70°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours respectively. On the other hand, 5.0%, 9.9%, 14.9%, 19.8% and 24.8% degradation were found at 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours respectively.

All chromatograms at 60°C are shown in figure 3.23 and results are given in table 3.30**.**

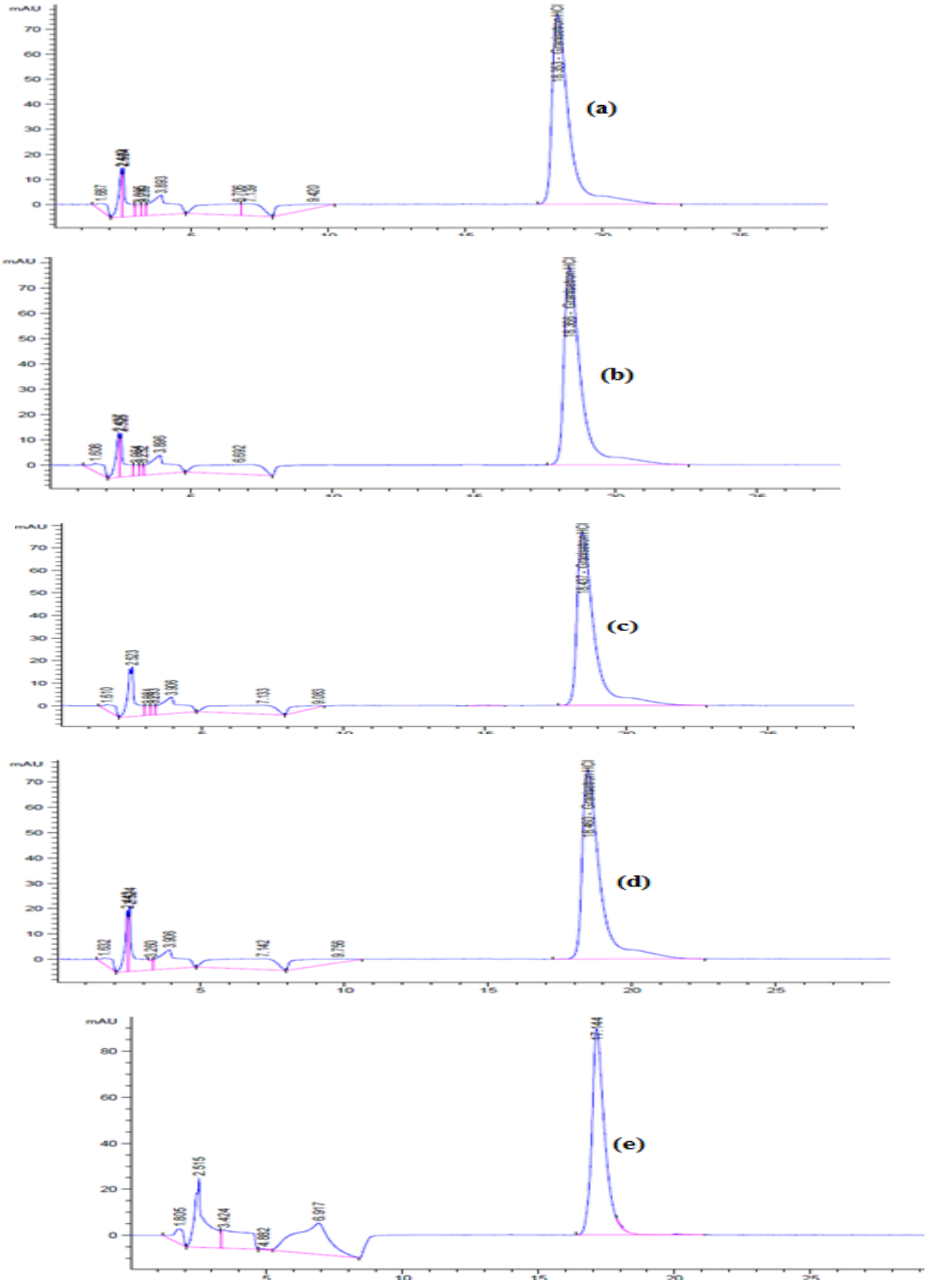

Figure 3.23: Chromatograms of granisetron HCl in 2.0N NaOH at 60°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.30: Relationship between relative retention time (RRT), area and content of granisetron HCl in 2.0N NaOH at 60°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Granisetron HCl	1	3300.4	0.9998	98.8	-	1.2
2 hours	Granisetron HCl		3282.5	0.9998	97.7	-	2.3
3 hours	Granisetron HCl		3260.6	0.9997	96.5	-	3.5
4 hours	Granisetron HCl		3214.4	0.9995	95.4	-	4.6
5 hours	Granisetron HCl		2962.1	0.9994	94.2	-	5.8

All chromatograms at 70°C are shown in figure 3.24 and results are given in table 3.31**.**

At 80°C, one degradant peak was found after 1 hour,2 hours and 3 hours. Two degradants peaks were found after 4 hours and 5 hours. The chromatograms are shown in the figure 3.25 and results are given in table 3.32.

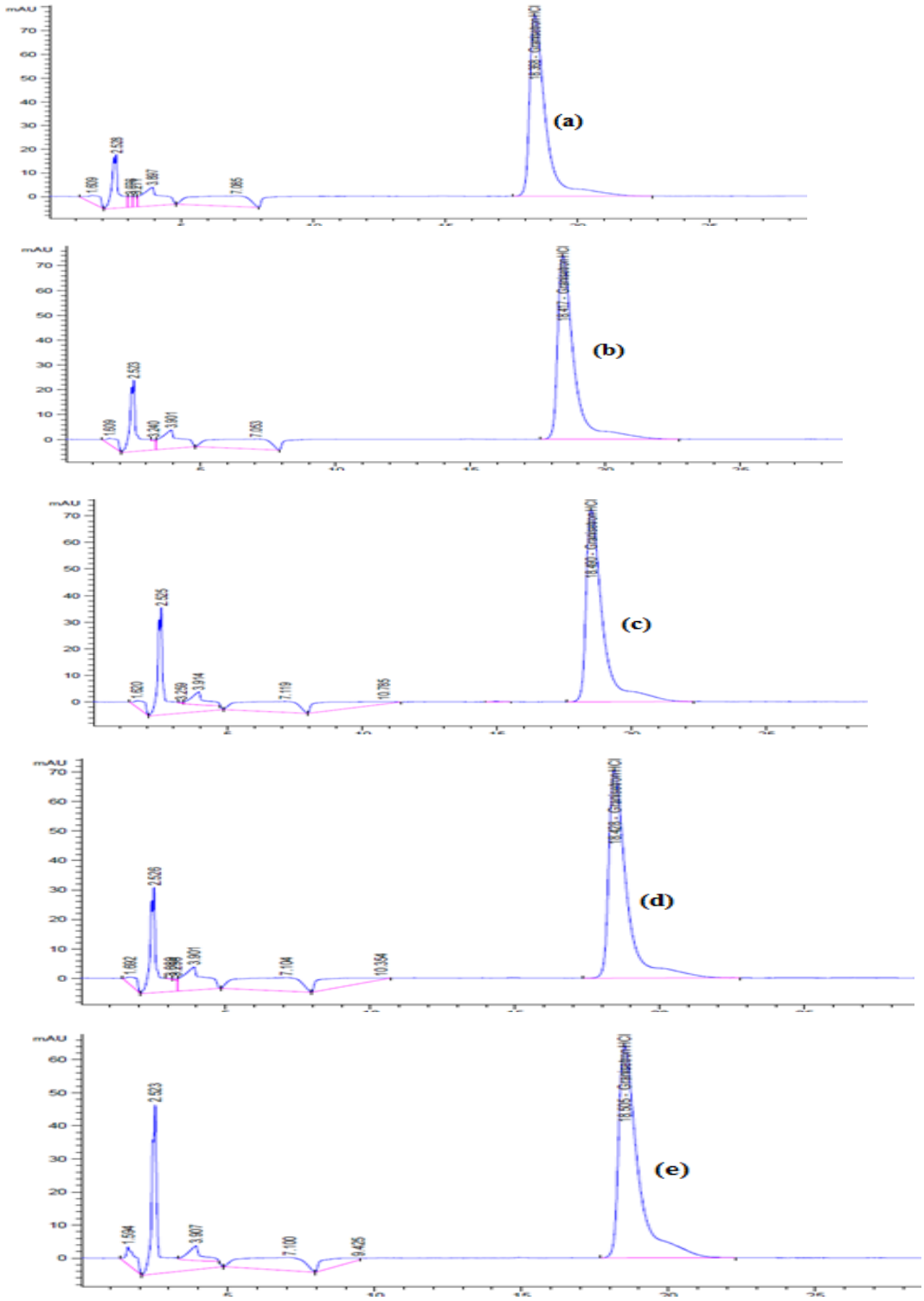

Figure 3.24: Chromatograms of granisetron HCl in 2.0N NaOH at 70°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.31: Relationship between relative retention time (RRT), area and content of granisetron HCl in 2.0N NaOH at 70°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Granisetron HCl	1	3255.1	0.9997	97.5	-	2.5
2 hours	Granisetron HCl		3124.6	0.9979	95.0	-	5.0
3 hours	Granisetron HCl		3099.5	0.9988	92.5	-	7.5
4 hours	Granisetron HCl		3054.4	0.9989	90.0	-	10.0
5 hours	Granisetron HCl		2970.6	0.9984	87.5	-	12.6

3.3.4 Oxidative degradation

Oxidative degradation was conducted with three different known strengths of hydrogen peroxide in dark place for 1, 2 and 3 hours. Different percentages of degradation were evident at different stressed conditions. Here, 24.4%, 29.6% and 31.2% degradation were found for 3% hydrogen peroxide after 1, 2 and 3 hours, respectively.

However, 34.8%, 37.6% and 41.4% degradation were observed with 5% hydrogen peroxide after 1, 2 and 3 hours, respectively. On the other hand, 64.6%, 69.4% and 71.3% 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. The results are given in tables 3.33-3.35.

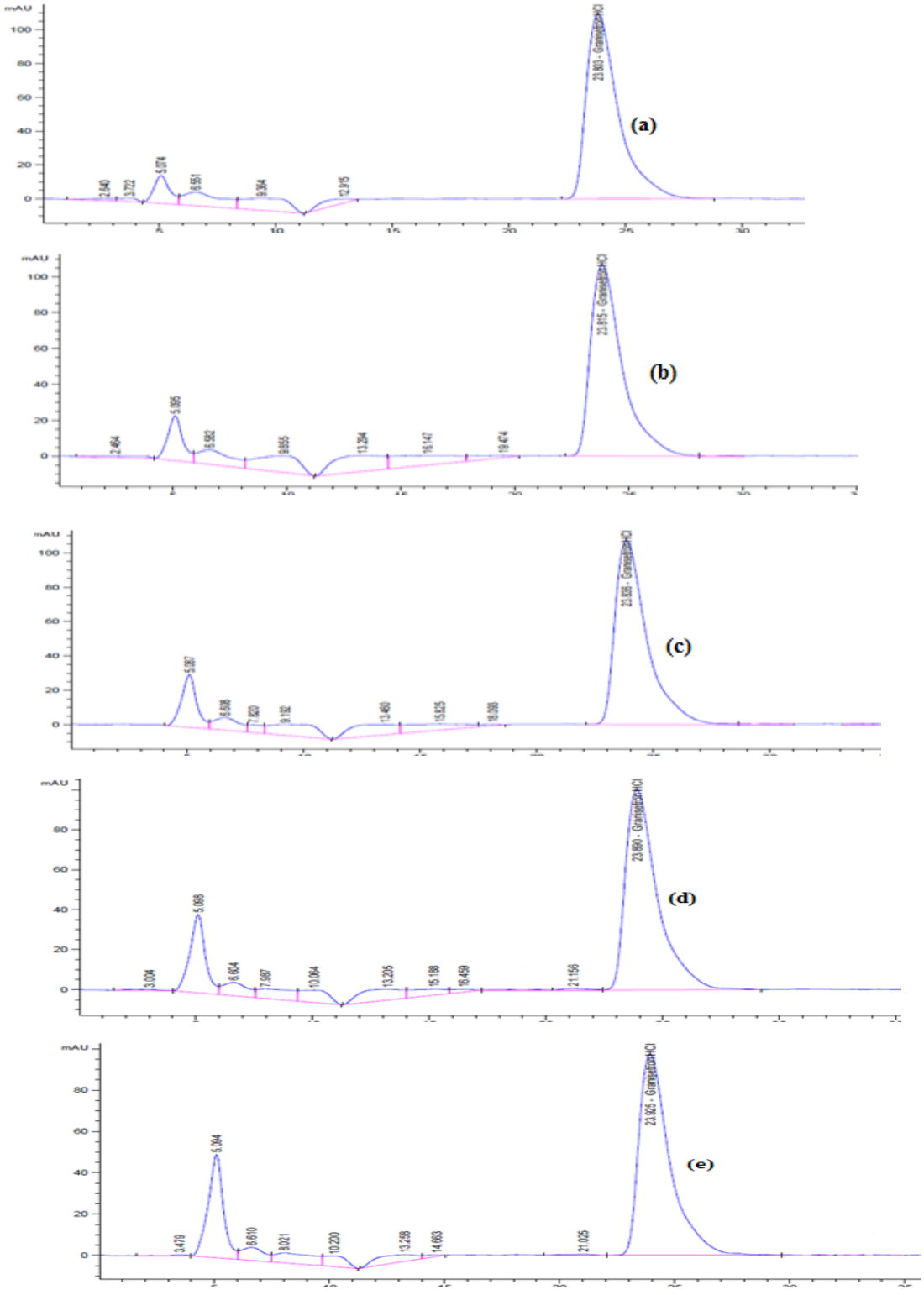

Figure 3.25: Chromatograms of granisetron HCl in 2.0N NaOH at 80°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.32: Relationship between relative retention time (RRT), area and content of granisetron HCl in 2.0N NaOH at 80°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Granisetron HCl	1	10464	0.9995	95.0	-	5.0
	Impurity-19	0.21	759.0	-	-	4.6	
2 hours	Granisetron HCl	1	10303	0.9987	90.1	-	9.9
	Impurity-20	0.21	1140.6	-	-	7.0	
3 hours	Granisetron HCl	1	10176	0.9981	85.1	-	14.9
	Impurity-21	0.21	1375.8	-	-	9.2	
4 hours	Granisetron HCl	1	9547.1	0.9987	80.16	-	19.8
	Impurity-22	0.21	1785.1	-	-	16.4	
	Impurity-23	0.89	63.91	-	-	0.6	
5 hours	Granisetron HCl	1	9149.7	0.9977	75.2	-	24.8
	Impurity-24	0.21	2296.2	-	-	19.5	
	Impurity-25	0.89	63.13	-	-	0.5	

Table 3.33: Relationship between relative retention time (RRT), area and content of granisetron HCl in 3.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Granisetron HCl	1	4734.1	-	75.6	-	24.4
2 hours	Granisetron HCl		4409.1	-	70.4	-	29.6
3 hours	Granisetron HCl		4373.9	-	68.8	-	31.2

Table 3.34: Relationship between relative retention time (RRT), area and content of granisetron HCl in 5.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak names	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Granisetron HCl	1	4113.1	-	65.2	-	34.8
2 hours	Granisetron HCl		4001.2	-	62.4	-	37.6
3 hours	Granisetron HCl		3725.5	-	58.6	-	41.4

Table 3.35: Relationship between relative retention time (RRT), area and content of granisetron HCl in 10.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Granisetron HCl	1	2247.3	-	35.4	-	64.6
2 hours	Granisetron HCl		1940.6	-	30.7	-	69.4
3 hours	Granisetron HCl		1822.0	-	28.7	-	71.3

3.3.5 Photo degradation

Bulk drug substance was used as sample to conduct photo degradation study. The sample was directly exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light. Under this stress condition, no degradation was observed. However, 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 also showed no degradation. The results are given in table 3.36.

3.4 Stress degradation of tropisetron hydrochloride

Tropisetron hydrochloride was subjected to the stressed studies in aqueous, acidic, basic, oxidative and photolytic conditions. It was evident that Tropisetron HCl goes under degradation in aqueous, basic, oxidative and photolytic conditions and no degradation was found in acidic condition.

Table 3.36: Relationship between relative retention time (RRT), area and content of granisetron HCl after photo degradation exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light and 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1.2 million lux, 7 days	Granisetron HCl	1	19834	0.9995	99.8	-	-
3.6 million lux, 21 days	Granisetron HCl		7577.9	0.9999	99.9	-	-

3.4.1 Aqueous degradation

Aqueous degradation study was conducted with purified water at 60°C for 7- and 21-days. No degradation was found after 7 days but 1.2% degradation was observed after 21 days. Two additional peaks apart from the principal and blank peaks were found with retention time 7.5 and 8.7 min and RRT 0.35 and 0.40, respectively. The sensitivity of the peaks was 24.3 and 15.2, respectively. The chromatogram is shown in figures 3.26-3.27 and results are given in tables 3.37-3.38.

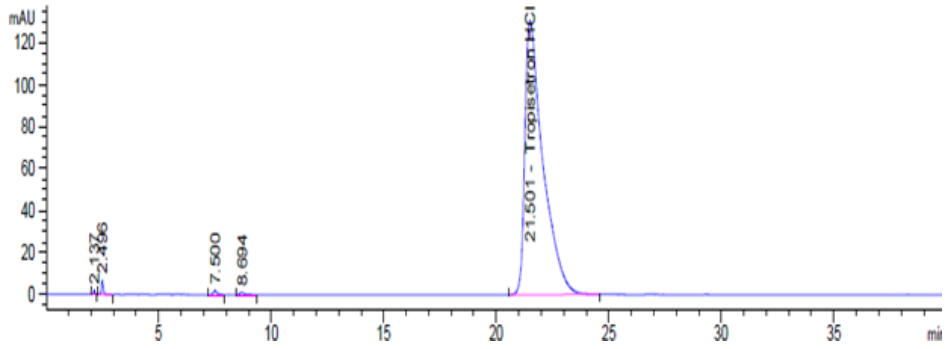

Figure 3.26: Chromatogram of tropisetron HCl in water at 60°C for 21 days.

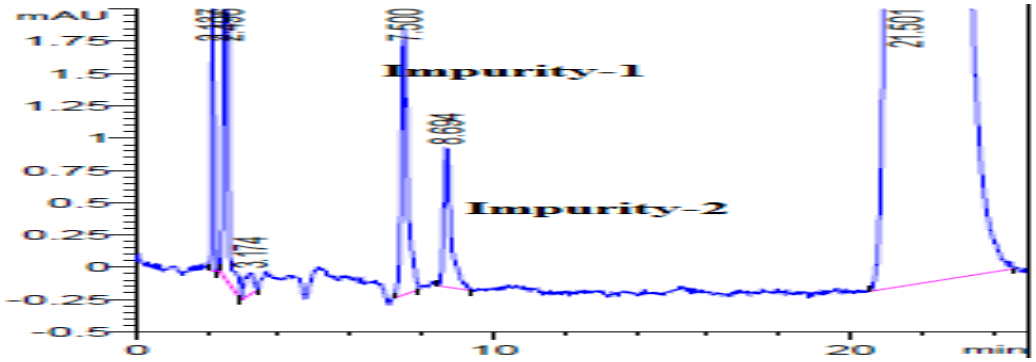

Figure 3.27: Extended chromatogram for impurities of tropisetron HCl in water at 60°C for 21 days.

Table 3.37: Relationship between relative retention time (RRT), area and content of tropisetron HCl in water at 60°C after 7 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
Aqueous degradation (7 days)	Tropisetron HCl	1	3300.4	0.9998	99.84	-	-

Table 3.38: Relationship between relative retention time (RRT), area and content of tropisetron HCl in water at 60°C for 21 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
Aqueous degradation (21 days)	Tropisetron HCl	1	7294.5	0.9999	98.82	-	1.2
	Impurity-1	0.35	24.3	-	-	0.62	
	Impurity-2	0.40	15.2	-	-	0.38	

3.4.2 Acid degradation

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Here, 0.96%, 2.6%, 3.0% and 15.7% degradations were observed in 0.1N, 0.5N, 1.0N and 2.0N HCl, respectively. The chromatograms are shown in figure 3.28 and results are given in table 3.39.

3.4.3 Base degradation

At first, base degradation was conducted in 0.1N, 0.5N, 1.0N and 2.0N NaOH at 60°C for 3 days. Here, 18.9%, 54.7%, 76.5% and 93.5% degradations were observed in 0.1N, 0.5N, 1.0N and 2.0N NaOH, respectively. The chromatogram is shown in figure 3.29 and results are given in table 3.40.

Secondly, base degradation was carried out in 2N NaOH at 60°C, 70°C and 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours. Here, 2.1%, 4.4%, 6.6%, 8.8% and 11.0% degradations were found at 60°C after 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, respectively. However, 4.5%, 8.9%, 13.4%, 17.9% and 22.4% degradation were found at 70°C after 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, respectively. On the other hand, 9.3%, 18.6%, 27.8%, 37.1% and 46.4% degradation were found at 80°C after 1 hour, 2 hours, 3 hours, 4 hours and 5 hours, respectively.

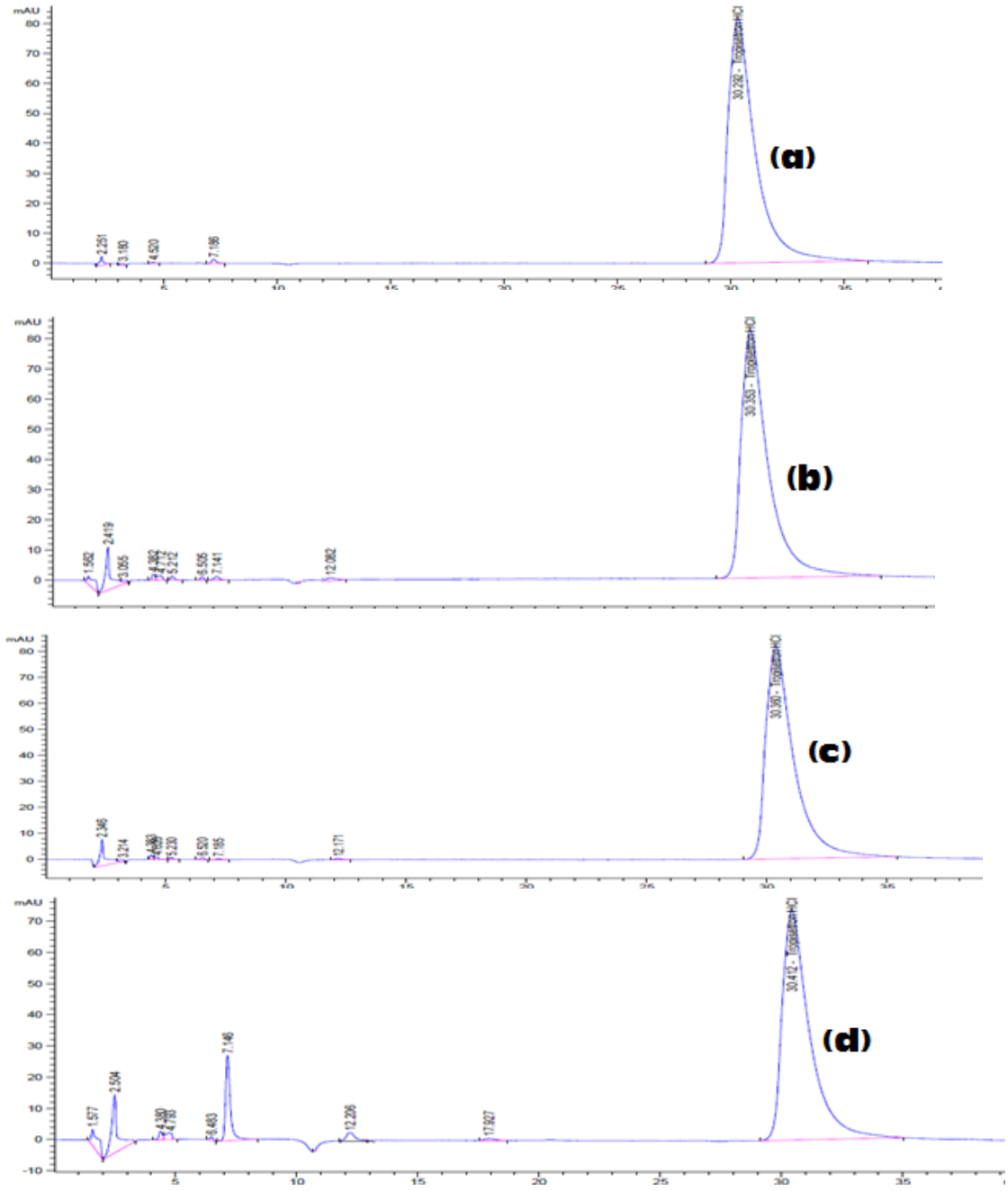

Figure 3.28: Chromatograms of tropisetron HCl at 60°C after 7 days in (a) 0.1N HCl, (b) 0.5N HCl, (c) 1.0N HCl, and (d) 2.0N HCl.

Table 3.39: Relationship between relative retention time (RRT), area and content of tropisetron HCl in acid at 60°C after 7 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N HCl	Tropisetron HCl	1	6795.4	0.9999	99.0	-	0.96
	Impurity-3	0.24	20.3	-	-	0.30	
0.5N HCl	Tropisetron HCl	1	6686.6	0.9999	97.5	-	2.6
	Impurity-4	0.17	12.4	-	-	0.17	
	Impurity-5	0.21	11.7	-	-	0.16	
	Impurity-6	0.24	22.0	-	-	0.31	
	Impurity-7	0.40	72.2	-	-	1.01	
1.0N HCl	Tropisetron HCl	1	6659.0	0.9998	97.0	-	3.0
	Impurity-8	0.17	7.65	-	-	0.55	
	Impurity-9	0.21	9.10	-	-	0.70	
	Impurity-10	0.24	7.19	-	-	0.54	
	Impurity-11	0.40	9.18	-	-	0.71	
2.0N HCl	Tropisetron HCl	1	5784.1	0.9997	84.3	-	15.7
	Impurity-12	0.21	10.5	-	-	0.20	
	Impurity-13	0.24	376.1	-	-	7.7	
	Impurity-14	0.40	231.2	-	-	4.7	
	Impurity -15	0.59	18.4	-	-	0.37	

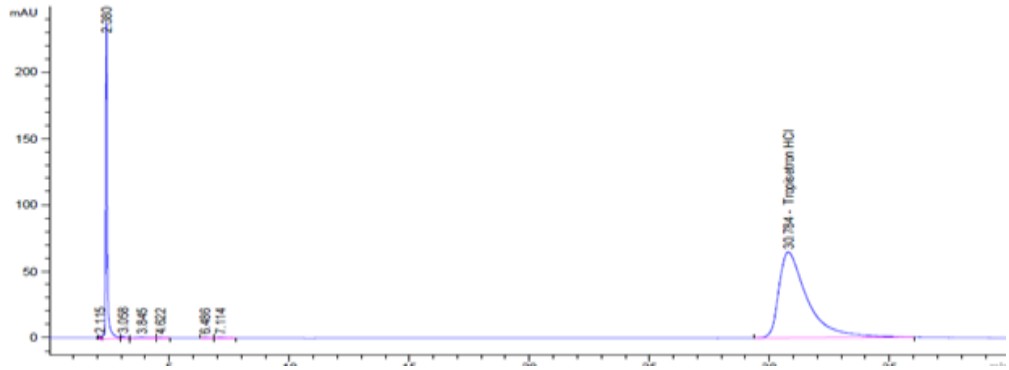

Figure 3.29: Chromatogram of tropisetron HCl in 0.1N NaOH at 60°C after 3 days.

At 60°C, one additional peak was found after each time interval. The chromatograms are shown in the figure 3.30 and results are given in table 3.41.

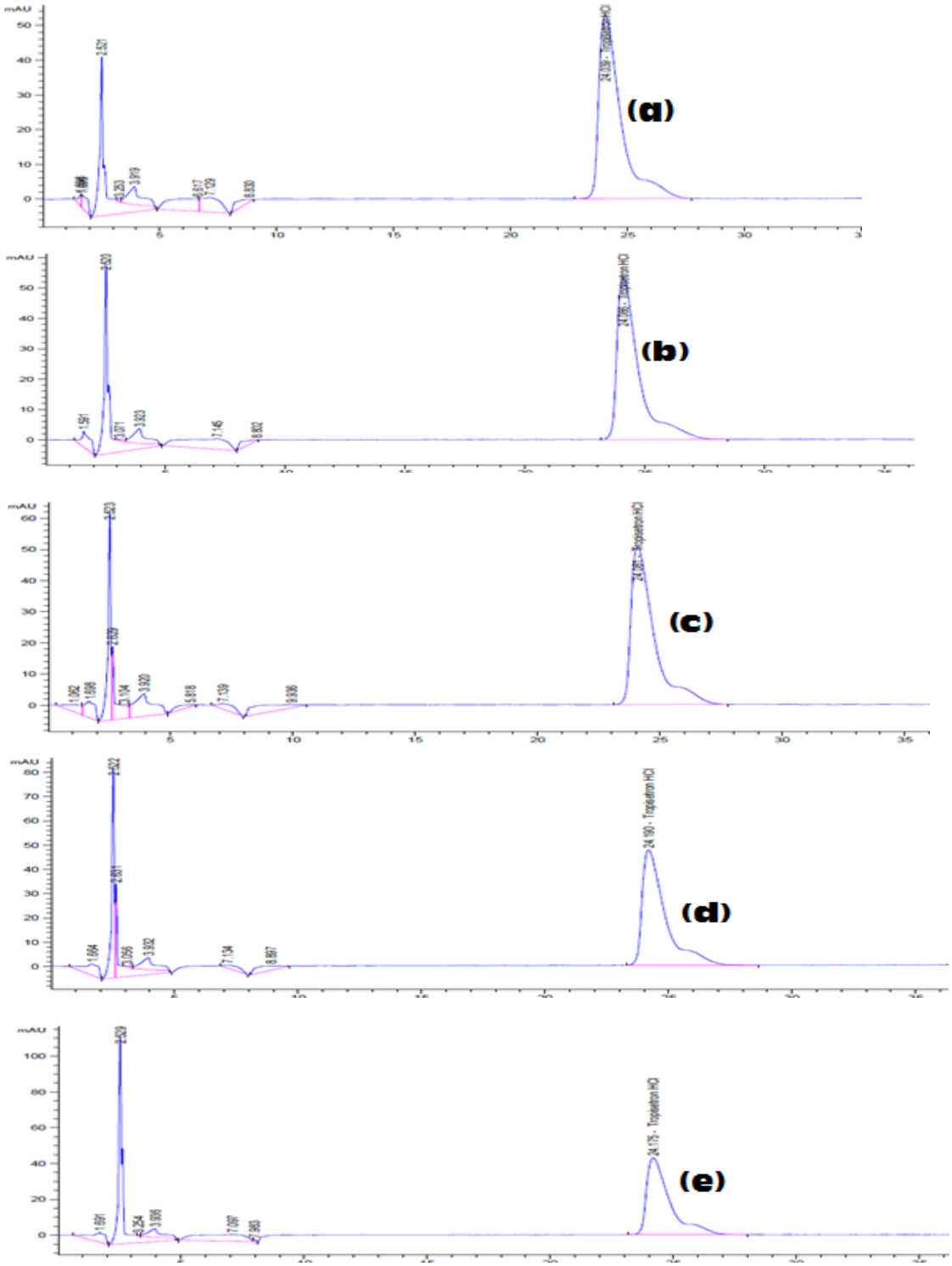

Figure 3.30: Chromatograms of tropisetron HCl in 2.0N NaOH at 60°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.40: Relationship between relative retention time (RRT), area and content of tropisetron HCl in base 60°C after 3 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N NaOH	Tropisetron HCl	1	5343.9	0.9997	81.1	-	18.9
	Impurity-16	0.08	1158.9	-	-	17.6	
	Impurity-17	0.21	6.05	-	-	0.09	
	Impurity-18	0.23	8.9	-	-	0.14	
0.5N NaOH	Tropisetron HCl	1	3064.6	-	45.3	-	54.7
	Impurity-19	0.08	3475.4	-	-	51.3	
	Impurity-20	0.21	8.91	-	-	0.13	
	Impurity-21	0.23	38.67	-	-	0.57	
1.0N NaOH	Tropisetron HCl	1	1686.7	-	23.5	-	76.5
	Impurity-22	0.08	4998.7	-	-	70.98	
	Impurity-23	0.21	7.30	-	-	0.10	
	Impurity-24	0.23	65.29	-	-	0.93	
2.0N NaOH	Tropisetron HCl	1	470.2	-	6.5	-	93.5
	Impurity-25	0.08	6294.1	-	-	86.4	
	Impurity-26	0.23	120.5	-	-	1.66	

Table 3.41: Relationship between relative retention time (RRT), area and content of tropisetron HCl in 2.0N NaOH at 60°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Tropisetron HCl	1	3482.5	0.9991	97.8	-	2.1
	Impurity-27	0.10	55.19	-	-	1.55	
2 hours	Tropisetron HCl	1	3378.7	0.9988	95.6	-	4.4
	Impurity-28	0.10	122.5	-	-	3.44	
3 hours	Tropisetron HCl	1	3328.8	0.9984	93.4	-	6.6
	Impurity-29	0.10	182.3	-	-	5.12	
4 hours	Tropisetron HCl	1	3099.3	0.9985	91.2	-	8.8
	Impurity-30	0.10	251.0	-	-	7.05	
5 hours	Tropisetron HCl	1	2816.4	0.9974	89.0	-	11.0
	Impurity-31	0.10	324.0	-	-	9.11	

At 70°C, one additional peak was found after each time interval. The chromatograms are shown in the figure 3.31and results are given in table 3.42.

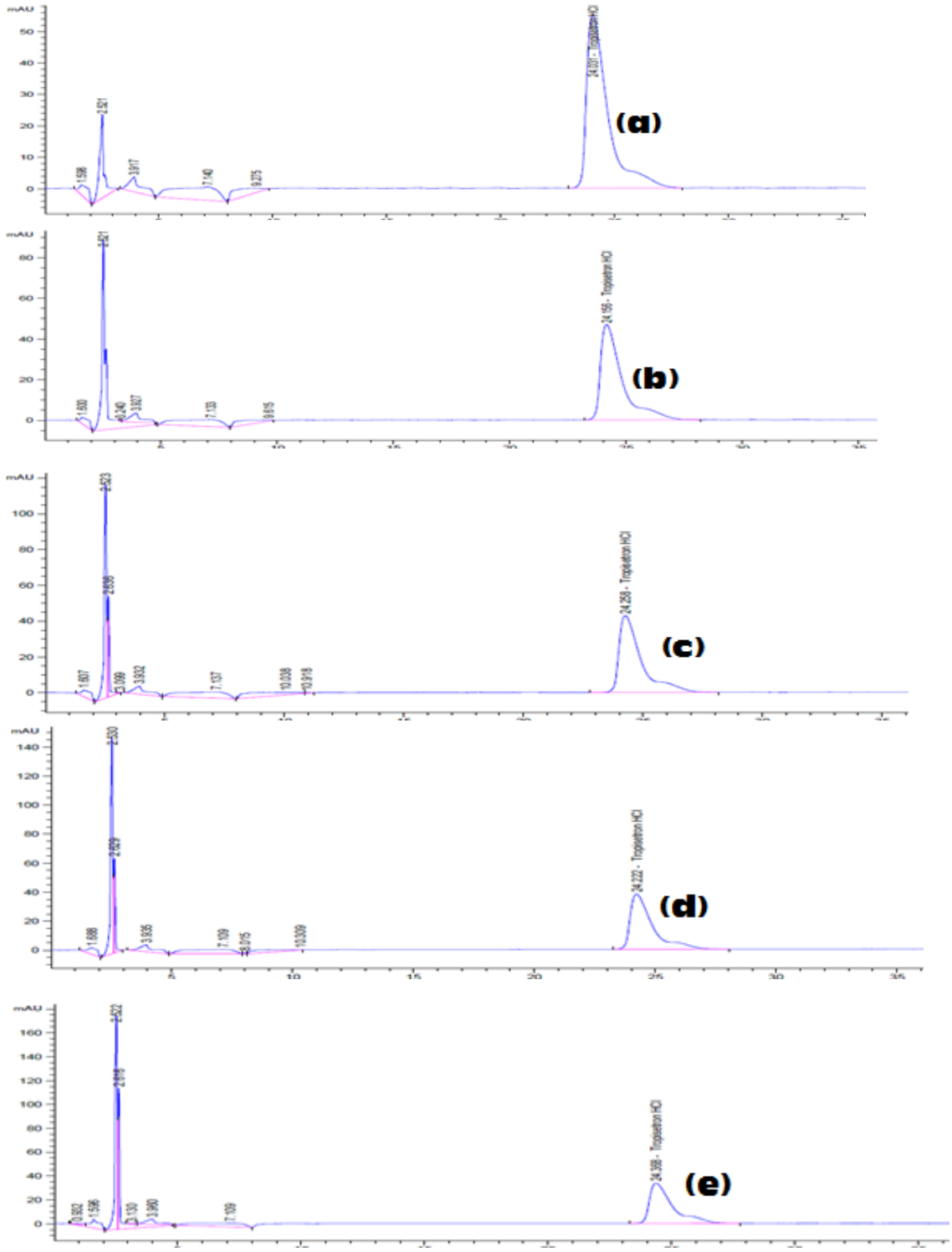

Figure 3.31: Chromatograms of tropisetron HCl in 2.0N NaOH at 70°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.42: Relationship between relative retention time (RRT), area and content of tropisetron HCl in 2.0N NaOH at 70°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Tropisetron HCl	1	3584.7	0.9990	95.5	-	4.5
	Impurity-32	0.10	120.4	-	-	3.21	
2 hours	Tropisetron HCl	1	3014.3	0.9986	91.1	-	8.9
	Impurity-33	0.10	282.9	-	-	7.54	
3 hours	Tropisetron HCl	1	2758.2	0.9980	86.6	-	13.4
	Impurity-34	0.10	454.4	-	-	12.11	
4 hours	Tropisetron HCl	1	2436.7	0.9982	82.1	-	17.9
	Impurity-35	0.10	592.1	-	-	15.78	
5 hours	Tropisetron HCl	1	2205.2	0.9971	77.7	-	22.4
	Impurity-36	0.10	763.2	-	-	20.3	

At 80°C, two additional peaks were found after 1 hour, three additional peaks were found after two hours. Two additional peaks were found after three, four and five hours. The chromatograms are shown in the figure 3.32 and results are given in table 3.43.

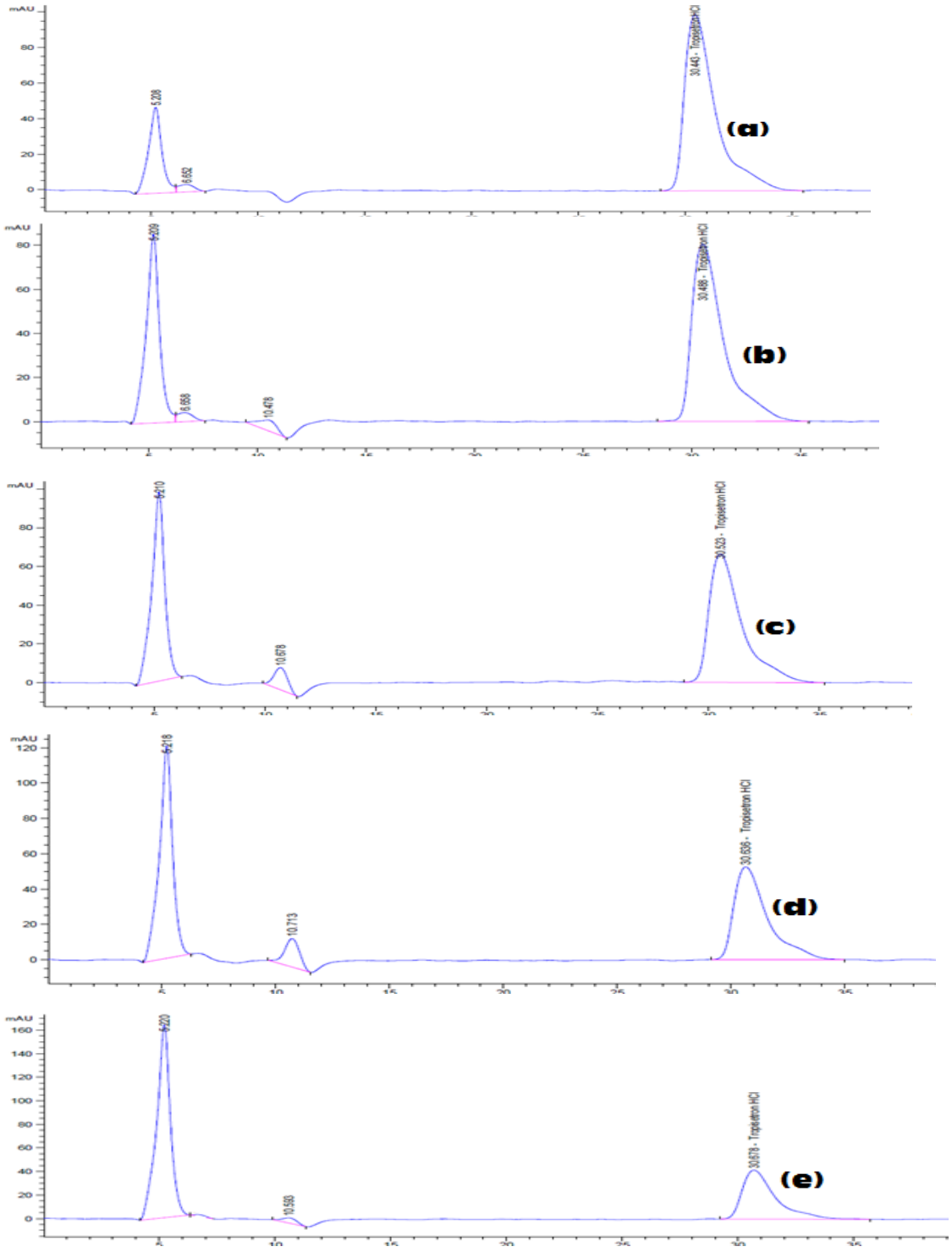

Figure 3.32: Chromatograms of tropisetron HCl in 2.0N NaOH at 80°C after (a) 1 hour, (b) 2 hours, (c) 3 hours, (d) 4 hours, and (e) 5 hours.

Table 3.43: Relationship between relative retention time (RRT), area and content of tropisetron HCl in 2N NaOH at 80°C.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Tropisetron HCl	1	10048	0.9989	90.7	-	9.3
	Impurity-37	0.17	841.8	-	-	7.6	
	Impurity-38	0.22	48.7	-	-	0.44	
2 hours	Tropisetron HCl	1	8253.3	0.9981	81.4	-	18.6
	Impurity-39	0.17	1606.0	-	-	14.5	
	Impurity-40	0.22	55.4	-	-	0.5	
	Impurity-41	0.34	110.8	-		1.0	
3 hours	Tropisetron HCl	1	6829.8	0.9984	72.2	-	27.8
	Impurity-42	0.17	2580.7	-	-	23.3	
	Impurity-43	0.34	299.1	-	-	2.7	
4 hours	Tropisetron HCl	1	5370.3	0.9981	62.9	-	37.1
	Impurity-44	0.17	3511.1	-	-	31.7	
	Impurity-45	0.34	476.3	-	-	4.3	
5 hours	Tropisetron HCl	1	4165.1	0.9974	53.6	-	46.4
	Impurity-46	0.17	4818.1	-	-	43.5	
	Impurity-47	0.34	132.9	-	-	1.2	

3.4.4 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, 8.6%, 9.5% and 12.7% degradation were found with 3% hydrogen peroxide after 1, 2 and 3 hours, respectively.

However, 21.6%, 21.7% and 23.3% degradation were observed in 5% hydrogen peroxide after 1, 2 and 3 hours, respectively. On the other hand, 24.8%, 34.8% and 37.5% 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. The chromatograms are shown in the figures 3.33-3.34 and results are given in tables 3.44-3.46.

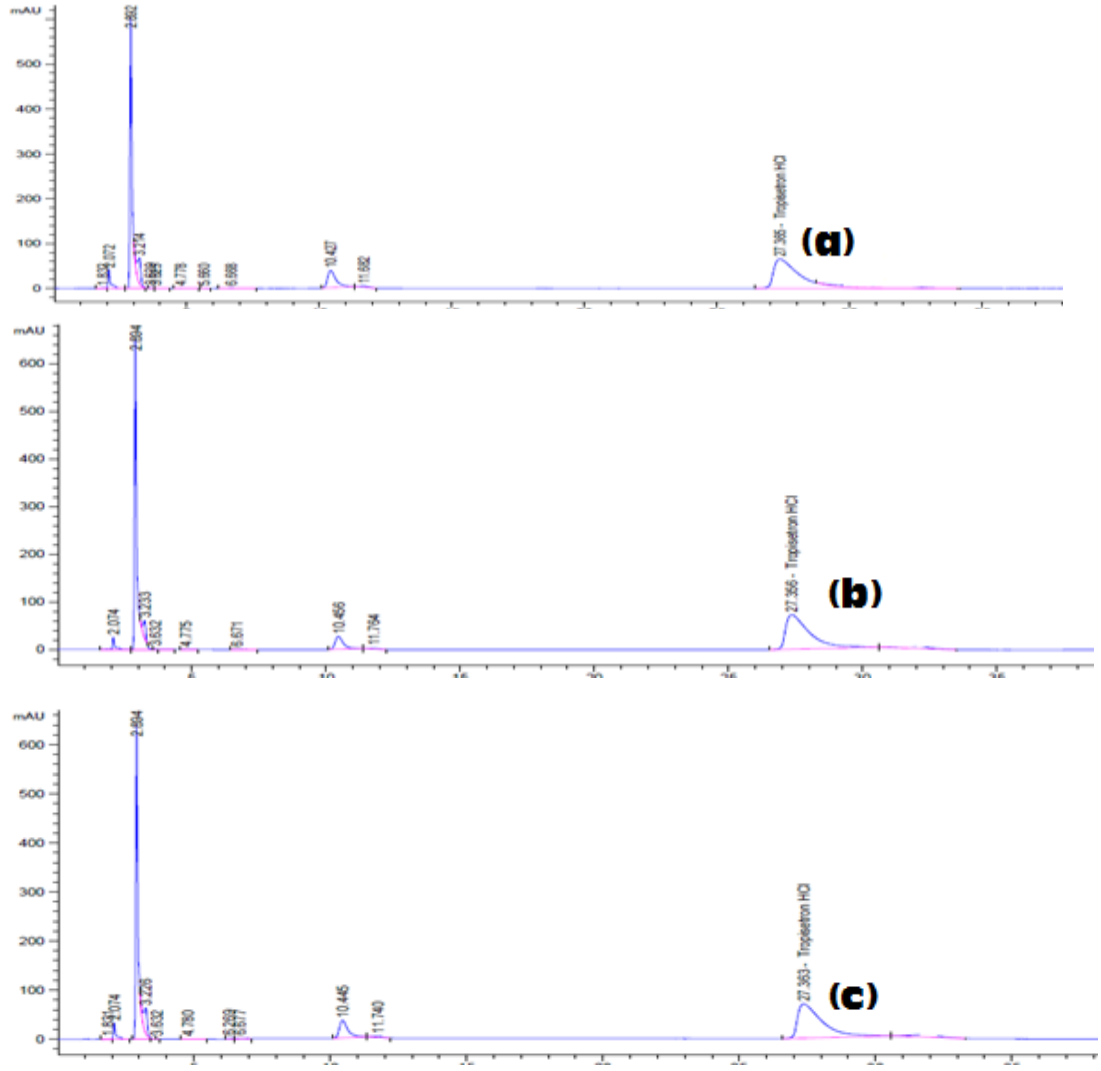

Figure 3.33: Chromatograms of tropisetron HCl in 3.0% H_2O_2 after (a) 1 hour, (b) 2 hours, and (c) 3 hours.

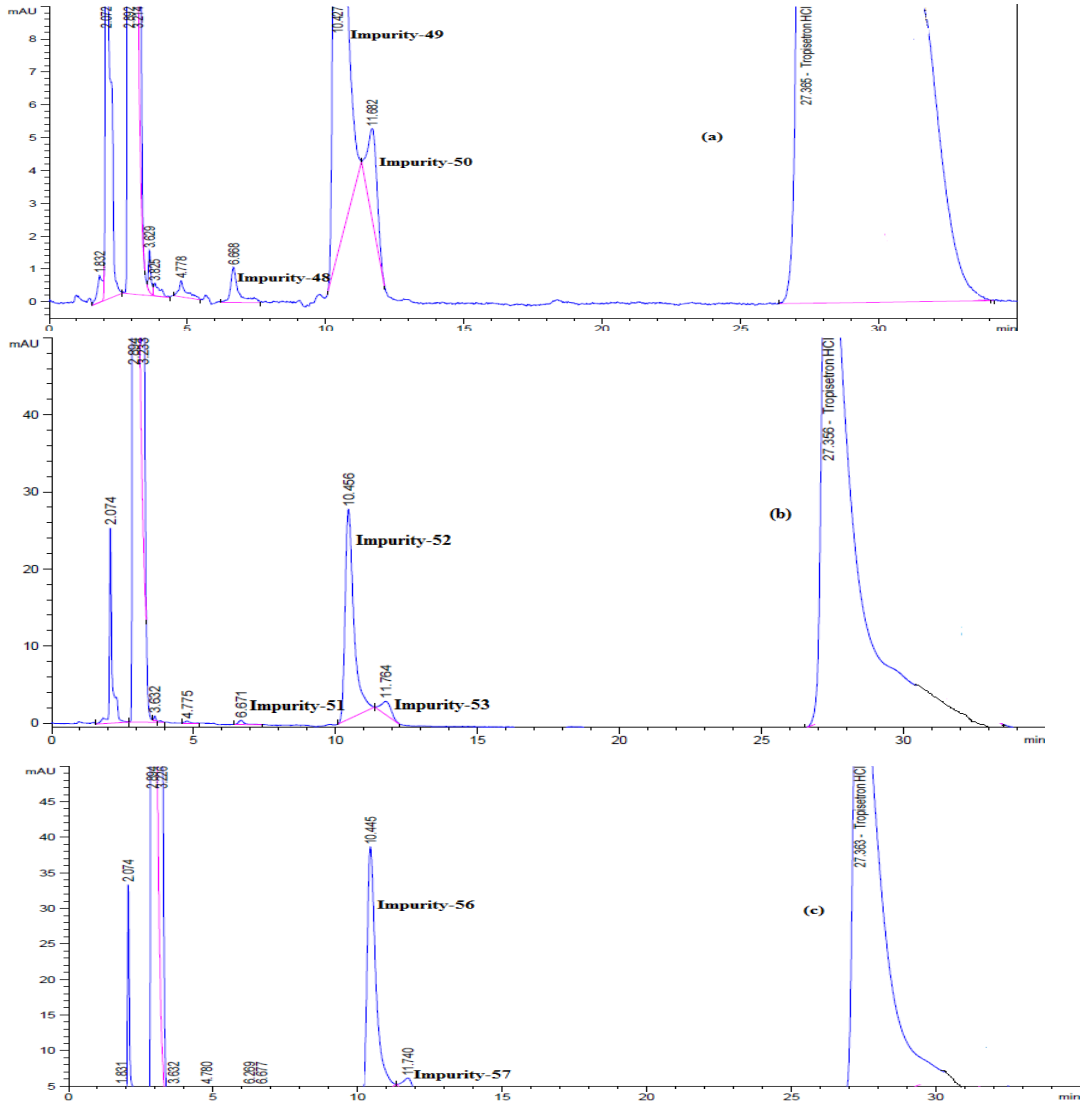

Figure 3.34: Extended chromatograms for impurities of tropisetron HCl in 3.0% H_2O_2 after (a) 1 hour, (b) 2 hours, and (c) 3 hours.

3.4.5 Photo degradation

Photo degradation study was carried out with bulk drug substance. The sample was directly exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light. Under this stress condition, no degradation was found. However, the sample was directly exposed to 3.6 million lux fluorescent light and 600 watts hour/m^2 UV light. At these conditions, no degradation was also observed. The results are given in table 3.47.

Table 3.44: Relationship between relative retention time (RRT), area and content of tropisetron HCl in 3.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Tropisetron HCl	1	4603.8	0.9997	91.4	-	8.6
	Impurity-48	0.24	20.83	-	-	0.16	
	Impurity-49	0.38	847.1	-	-	6.4	
	Impurity-50	0.43	63.5	-	-	0.48	
2 hours	Tropisetron HCl	1	4557.6	0.9987	90.5	-	9.5
	Impurity-51	0.24	7.73	-	-	0.10	
	Impurity-52	0.38	580.1	-	-	7.4	
	Impurity-53	0.43	42.4	-	-	0.54	
3 hours	Tropisetron HCl	1	4397.2	0.9984	87.3	-	12.7
	Impurity-54	0.23	23.3	-	-	0.31	
	Impurity-55	0.24	21.1	-	-	0.28	
	Impurity-56	0.38	761.8	-	-	10.2	
	Impurity-57	0.43	55.6	-	-	0.74	

Table 3.45: Relationship between relative retention time (RRT), area and content of tropisetron HCl in 5.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Tropisetron HCl	1	3950.4	0.9954	78.4	-	21.6
2 hours	Tropisetron HCl		3943.4	0.9934	78.3	-	21.7
3 hours	Tropisetron HCl		3864.7	0.9968	76.7	-	23.3

Table 3.46: Relationship between relative retention time (RRT), area and content of tropisetron HCl in 10.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Tropisetron HCl	1	3785.1	0.9928	75.2	-	24.8
2 hours	Tropisetron HCl		3286.1	0.9961	65.2	-	34.8
3 hours	Tropisetron HCl		3147.4	0.9919	62.5	-	37.5

Table 3.47: Relationship between relative retention time (RRT), area and content of tropisetron HCl after photo degradation exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light and 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1.2 milliom lux, 7 days	Tropisetron HCl	1	17336.5	0.9992	99.9	-	-
3.6 milliom lux, 21 days	Tropisetron HCl		7423.1	0.9997	100.6	-	-

3.5 Stress degradation of palonosetron hydrochloride

It was observed that palonosetron hydrochloride degraded in aqueous, basic, oxidative and photolytic conditions and no degradation was found in acidic condition. Results of each stressed condition applied on palonosetron hydrochloride are summarized below:

3.5.1 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 observed. The chromatogram is shown in figure 3.35 and the results are summarized in tables 3.48-3.49.

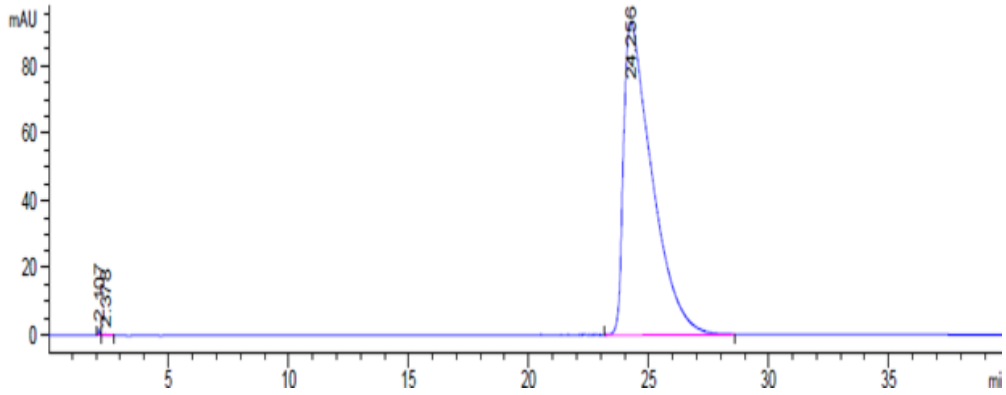

Figure 3.35: Chromatogram of palonosetron HCl in water at 60°C after 21 days.

Table 3.48: Relationship between relative retention time (RRT), area and content of palonosetron HCl in water at 60°C after 7 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
Aqueous degradation (7 days)	Palonosetron HCl	1	6756.6	0.9998	100.0	99.3

Table 3.49: Relationship between relative retention time (RRT), area and content of palonosetron HCl in water at 60°C after 21 days.

Condition	Peak identity	RRT	Area	Peak purity index	Content (%)	
					Standard	Sample
Aqueous degradation (21 days)	Palonosetron HCl	1	7616.6	0.9997	100.0	99.9

3.5.2 Acid degradation

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Here, 0.80%, 1.9%, 2.4% and 3.1% degradations were found with 0.1N, 0.5N, 1.0N and 2.0N HCl, respectively. The chromatograms are shown in figure 3.36 and the results are given in table 3.50.

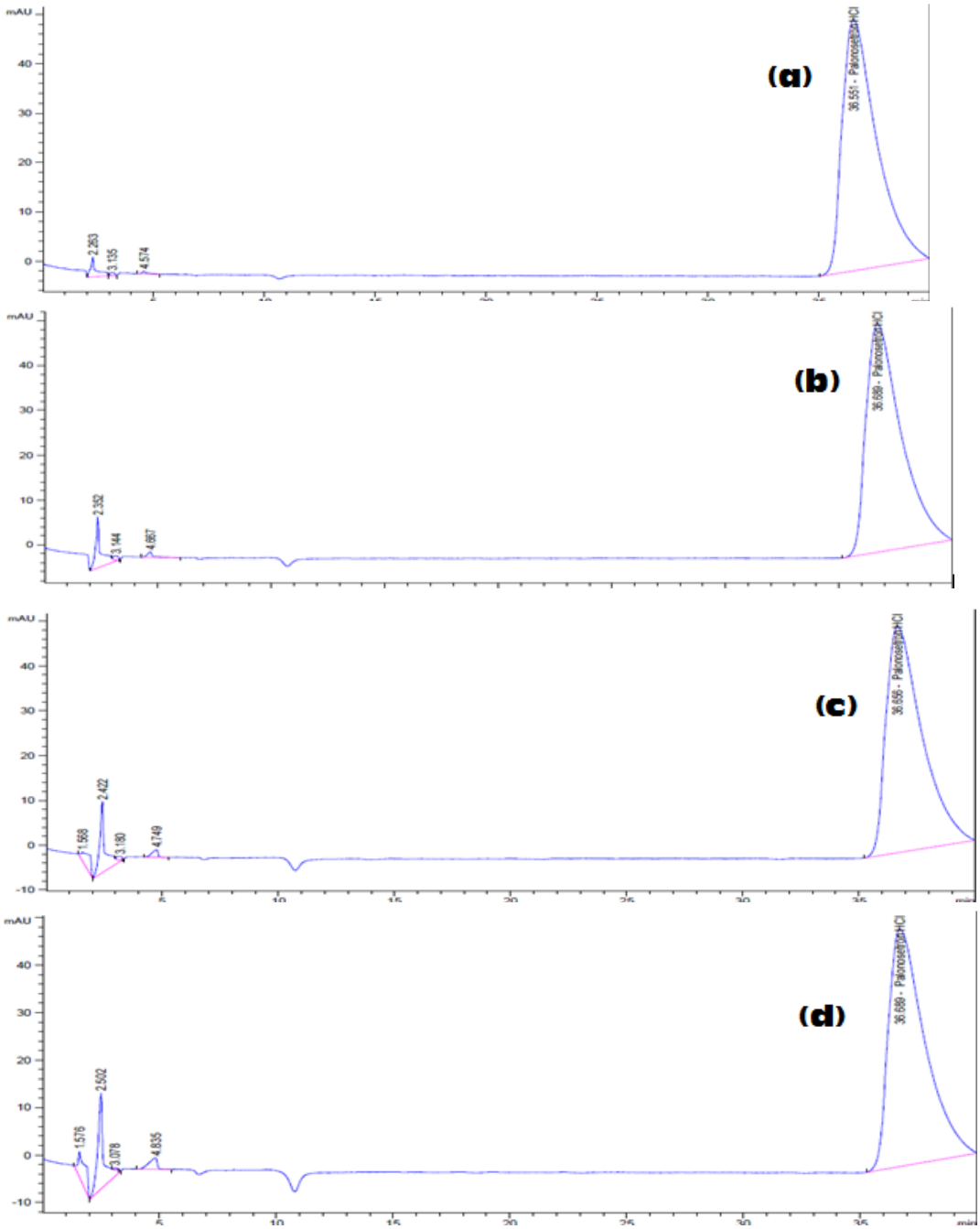

Figure 3.36: Chromatograms of palonosetron HCl at 60°C after 7 days in (a) 0.1N HCl, (b) 0.5N HCl, (c) 1.0N HCl, and (d) 2.0N HCl.

3.5.3 Base degradation

Base degradation was conducted in 0.1N, 0.5N, 1.0N and 2.0N NaOH at 60°C for 7-days. Here, 0.7%, 4.8%, 5.3% and 12.6% degradations were found in 0.1N, 0.5N, 1.0N and 2.0N NaOH,

respectively. For each condition, one additional peak apart from principal and blank peaks was observed. The chromatograms are shown in figure 3.37 and results are given in table 3.51.

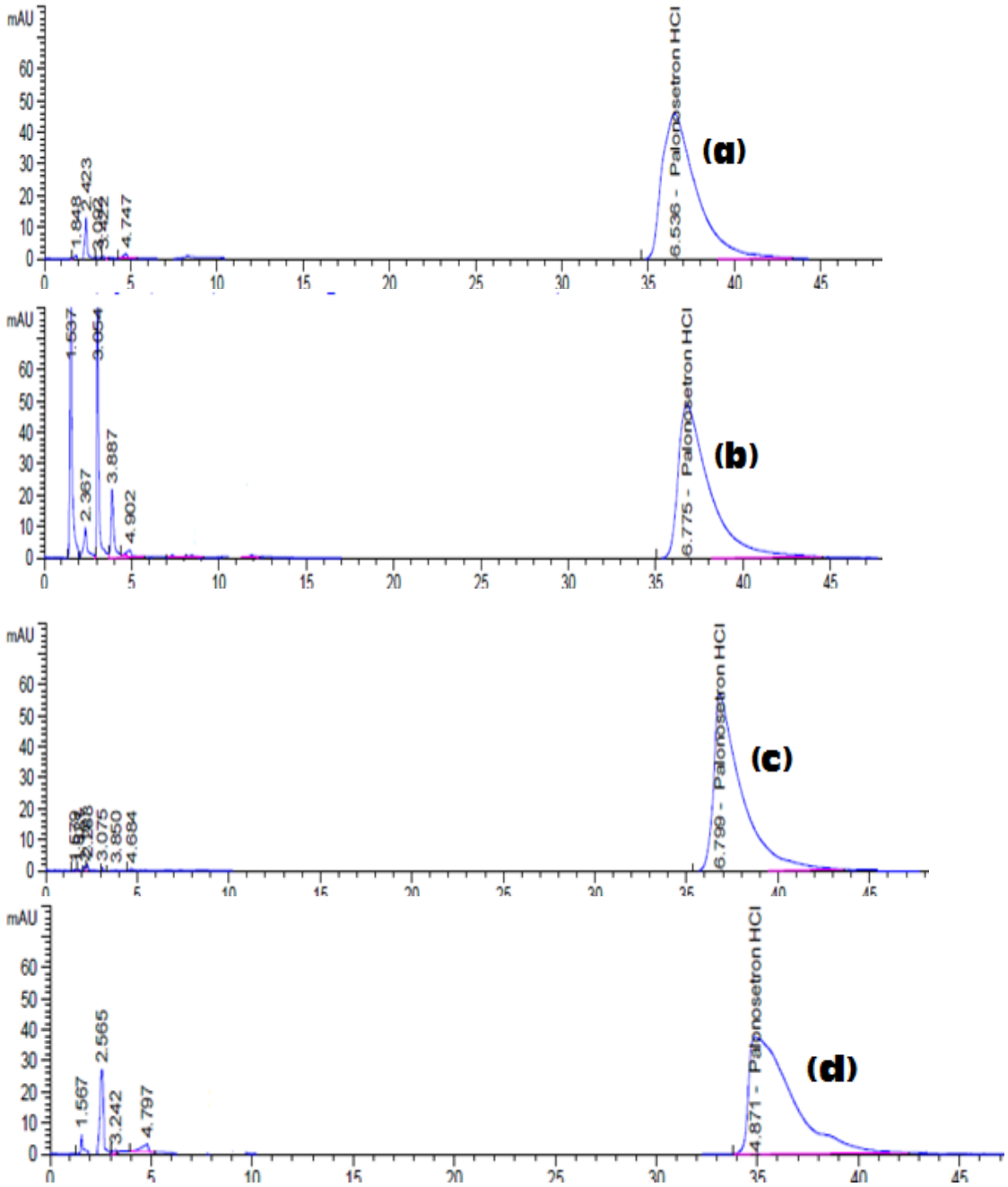

Figure 3.37: Chromatograms of palonosetron HCl at 60°C after 7 days in (a) 0.1N NaOH, (b) 0.5N NaOH, (c) 1.0N NaOH, and (d) 2.0N NaOH.

Table 3.50: Relationship between relative retention time (RRT), area and content of palonosetron HCl in acid at 60°C after 7 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N HCl	Palonosetron HCl	1	5510.7	0.9988	99.2	-	0.80
	Impurity-1	0.13	9.23	-	-	0.16	
0.5N HCl	Palonosetron HCl	1	5448.4	0.9974	98.1	-	1.9
	Impurity-2	0.13	22.02	-	-	0.38	
1.0N HCl	Palonosetron HCl	1	5422.3	0.9965	97.6	-	2.4
	Impurity-3	0.13	30.25	-	-	0.52	
2.0N HCl	Palonosetron HCl	1	5382.7	0.9946	96.9	-	3.1
	Impurity-4	0.13	58.8	-	-	1.01	

3.5.4 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, 95.0, 97.0 and 99.0% degradation were found for 3% hydrogen peroxide after 1, 2 and 3 hours, respectively.

On the other hand, almost 100.0% degradation was observed with 5% and 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 the figure 3.38 and the results are given in tables 3.52-3.54.

Table 3.51: Relationship between relative retention time (RRT), area and content of palonosetron HCl in base at 60°C after 7 days.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
0.1N NaOH	Palonosetron HCl	1	6366.0	-	99.3	-	0.7
	Impurity-5	0.13	22.4	-	-	0.33	
0.5N NaOH	Palonosetron HCl	1	6104.0	-	95.2	-	4.8
	Impurity-6	0.13	169.7	-	-	2.5	
1.0N NaOH	Palonosetron HCl	1	6074.2	-	94.7	-	5.3
	Impurity-7	0.13	264.7	-	-	3.9	
2.0N NaOH	Palonosetron HCl	1	5600.8	-	87.4	-	12.6
	Impurity-8	0.13	665.2	-	-	9.8	

3.5.5 Photo degradation

Photo degradation study was carried out with bulk drug substance. The sample was directly exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light. Under this stress condition, no degradation was found. However, the sample was directly exposed to 3.6 million lux fluorescent light and 600 watts hour/m^2 UV light. At these conditions, no degradation was also observed. The chromatograms are shown in the figure 3.39and the results are given in table 3.55.

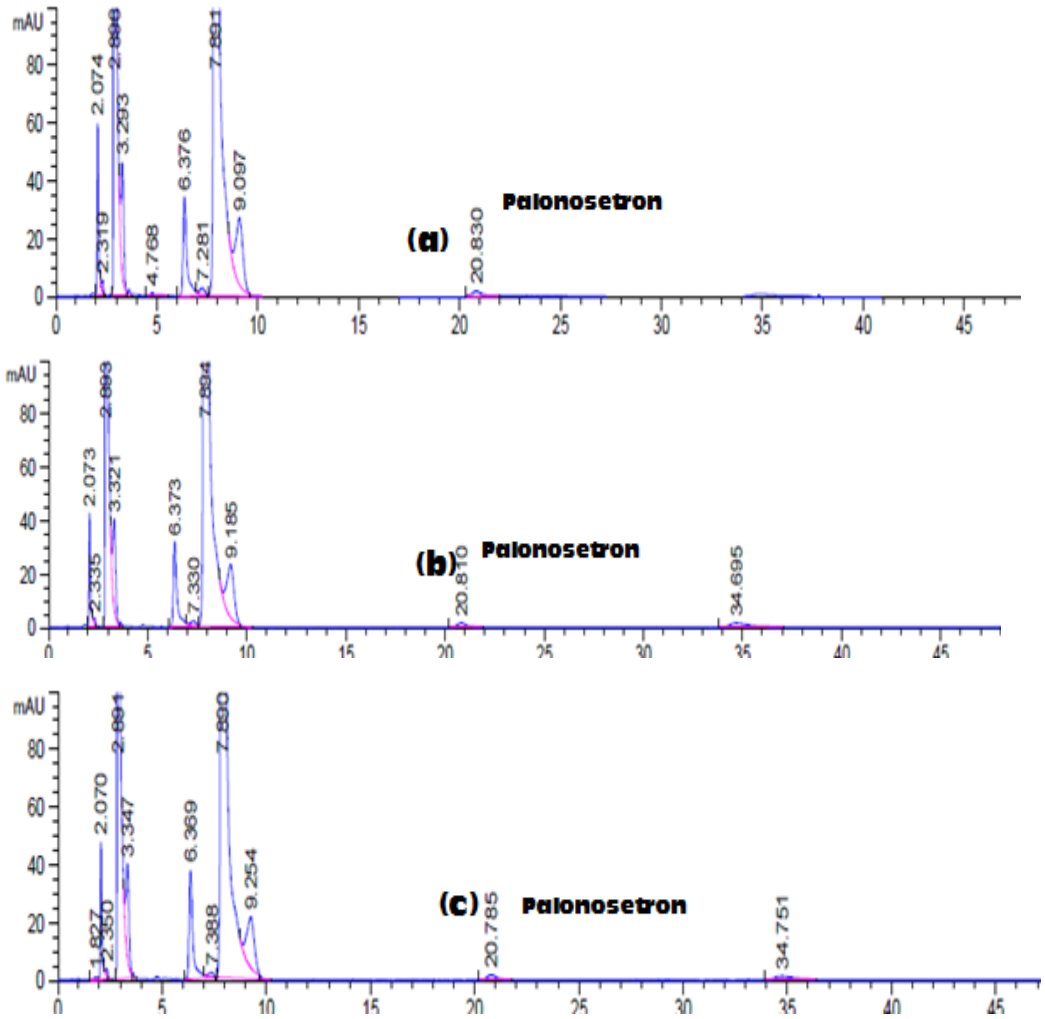

Figure 3.38: Chromatograms of palonosetron HCl in 3.0% H_2O_2 after (a) 1 hour, (b) 2 hours, and (c) 3 hours.

Table 3.52: Relationship between relative retention time (RRT), area and content of palonosetron HCl in 3.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Palonosetron HCl	1	5377.98	0.9999	5.0	-	95.0
2 hours	Palonosetron HCl		5061.76	0.9989	3.0	-	97.0
3 hours	Palonosetron HCl		4727.82	0.9998	1.0	-	99.0

Table 3.53: Relationship between relative retention time (RRT), area and content of palonosetron HCl in 5.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Palonosetron HCl	1	3993.56	0.9988	-	-	100.0
2 hours	Palonosetron HCl		3633.35	0.9989	-	-	100.0
3 hours	Palonosetron HCl		2534.88	0.9984	-	-	100.0

Table 3.54: Relationship between relative retention time (RRT), area and content of palonosetron HCl in 10.0% hydrogen peroxide at dark place after 1, 2 and 3 hours.

Time interval	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1 hour	Palonosetron HCl	1	2278.17	0.9978	-	-	100.0
2 hours	Palonosetron HCl		1318.8	0.9987	-	-	100.0
3 hours	Palonosetron HCl		668.66	0.9977	-	-	100.0

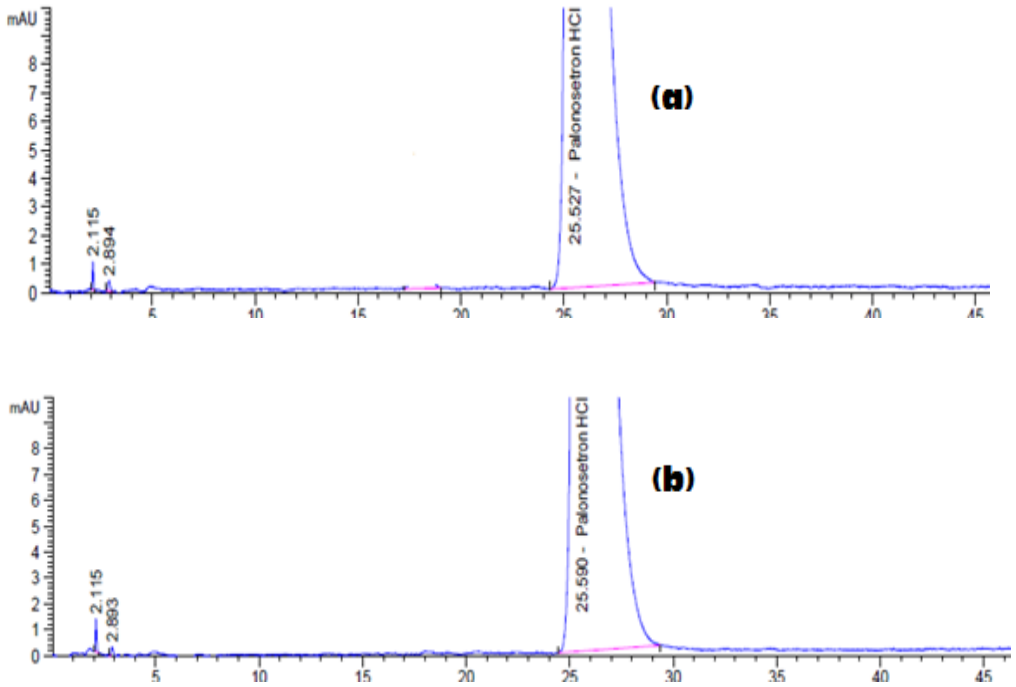

Figure 3.39: Chromatograms of palonosetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light for (a) controlled sample, and (b) stressed sample.

Table 3.55: Relationship between relative retention time (RRT), area and content of palonosetron HCl after photo degradation exposed to 1.2 million lux fluorescence light and 200 watts hour/m^2 UV light and 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Conditions	Peak identity	RRT	Area	Peak purity index	Content (%)		
					Sample	Impurity	Total impurities
1.2 million lux, 7 days	Palonosetron HCl	1	17877.3	0.9991	99.7	-	-
3.6 million lux, 21 days	Palonosetron HCl		6620.72	0.9994	99.9	-	-

3.6 Calculation

3.6.1 Impurity calculation

There are different types of related substance (impurity) analysis methods. Area percentage method, high-low method and external standard method are more frequently used. The formula of area percentage method was used to calculation individual impurity. It is mentioned below.

$$\% \text{ impurity} = \frac{A_i}{A_t} \times 100$$

Here,

A_i = peak area of any impurity

A_t = total peak area

3.6.2 Potency calculation

Potency was calculated by the following formula.

$$\% \text{ potency} = \frac{A_s}{A_{st}} \times \frac{W_{st}}{W_s} \times D \times P\ (\%) \times 100$$

Here,

A_s = peak area of sample

A_{st} = peak area of standard

W_{st} = weight of standard in mg

W_s = weight of sample in mg

D = dilution factor

P = potency of standard in %.