Chapter-4

Results and Discussion of Degradation Kinetics

Studies of Degradation kinetics were conducted in 0.1N NaOH on ramosetron HCl and 2.0N NaOH on ondnasetron HCl, granisetron HCl and tropisetron HCl at 60°C, 70°C and 80°C allowing 1 hour, 2 hours, 3 hours, 4 hours and 5 hours sample treatment interval. Results are discussed below:

4.1 Degradation kinetics of ramosetron hydrochloride

Degradation kinetics of ramosetron hydrochloride was conducted in 0.1N NaOH at 60°C, 70°C and 80°C for 1, 2, 3, 4 and 5 hours. All HPLC chromatograms relevant to degraded samples are presented in figures 3.4, 3.5 and 3.7.The potency remained after each degradation was calculated with the help of Microsoft Excel. These results are summarized in the table 4.1. 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 4.1-4.3. Kinetic parameters are summarized in table 4.2. Reaction activation energy (E_a) calculated with the help of Arrhenius equation was 10.05 kcal/mol.The linearity graph required to calculate reaction activation energy (E_a) was devised from reaction rate constant (k) vs inverse temperature in Kelvin (1/T). This linearity graph is shown in figure 4.4. 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 summary table 4.3.

Table 4.1: 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

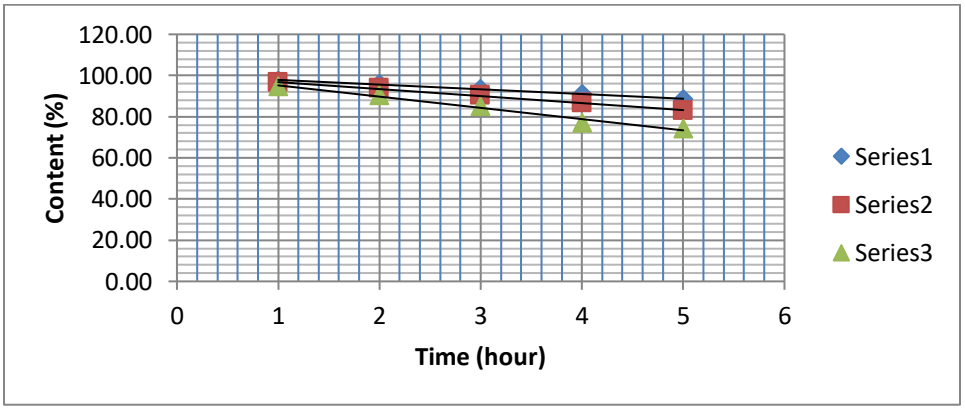

Figure 4.1: Zero order plot for the degradation of ramosetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 0.1N NaOH.

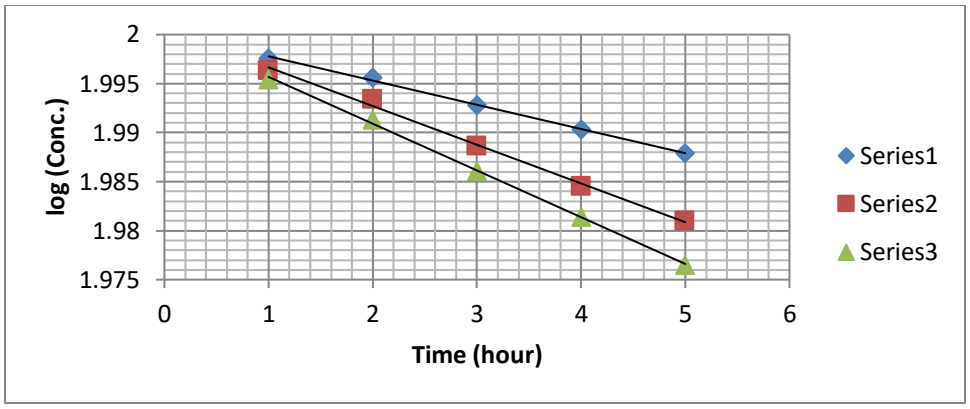

Figure 4.2: First order plot for the degradation of ramosetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 0.1N NaOH.

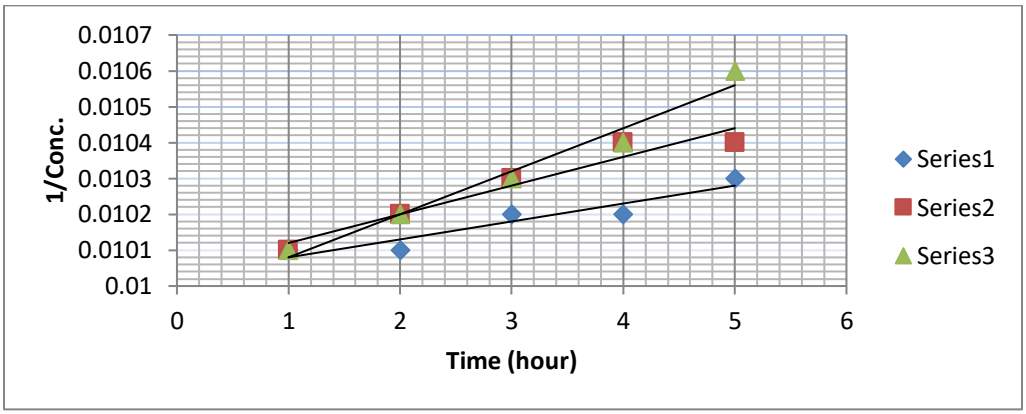

Figure 4.3: Second order plot for the degradation of ramosetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 0.1N NaOH.

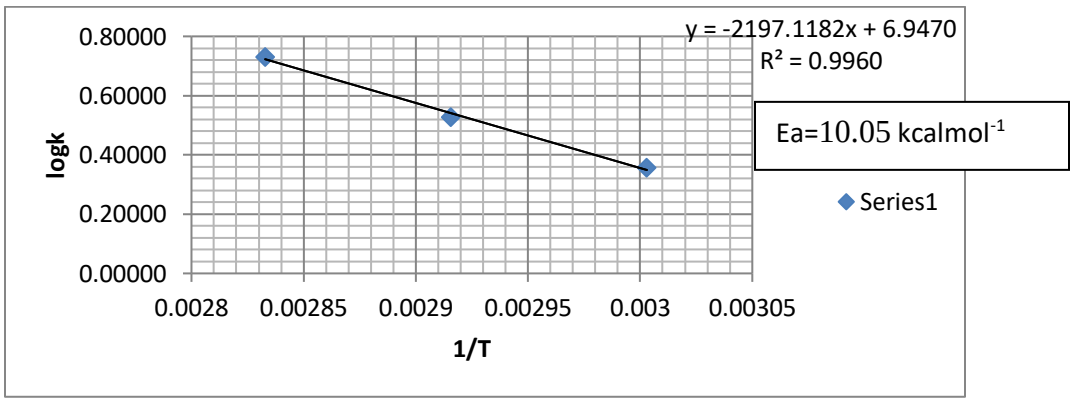

Figure 4.4: Relationship between temperature and rate constant used to calculate activation energy for ramosetron HCl.

Table 4.2: 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

Table 4.3: Summary of degradation kinetics of ramosetron HCl at 60°C, 70°C and 80°C in 0.1N NaOH.

Order	Temperature		Equation	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

4.2 Degradation kinetics of ondansetron hydrochloride

To investigate kinetics information of ondansetron hydrochloride, test samples were prepared in 2.0N NaOH at 60°C, 70°C and 80°C for 1, 2, 3, 4 and 5 hours. All HPLC chromatograms relevant to degraded samples have been presented in figures 3.13-3.15. The potency remained after each degradation was calculated with the help of Microsoft Excel. These results are summarized in the table 4.4. 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

4.5-4.7. Kinetic parameters are summarized in table 4.5. Reaction activation energy (E_a) calculated with the help of Arrhenius equation was 7.57 kcal/mol. The linearity graph required to calculate reaction activation energy (E_a) was devised from reaction rate constant (k) vs inverse temperature in Kelvin (1/T). This linearity graph is shown in figure 4.8. 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 summary table 4.6.

Table 4.4: Peak parameters and assays of degraded samples of ondansetron HCl at different temperature in 2.0N NaOH.

Temp.	Time interval	Area	Theoretical plate #	Tailing factor	Peak purity index	Assay (%)	
						Initial concentration	Potency
60°C	1 hour	2884.4	5468	0.954	1.0001	100.0	99.45
	2 hours	2882.0	5315	0.987	1.0203		98.99
	3 hours	2870.3	5211	0.968	0.9998		98.35
	4 hours	2865.0	5123	0.994	0.9999		97.80
	5 hours	2846.4	5027	0.989	0.9996		97.25
70°C	1 hour	2869.8	5687	0.998	1.0000	100.0	99.15
	2 hours	2850.0	5564	0.997	1.0023		98.50
	3 hours	2845.2	5497	1.000	1.0540		97.40
	4 hours	2825.1	5389	0.995	0.9999		96.50
	5 hours	2802.7	5212	0.999	0.9989		95.71
80°C	1 hour	11814.2	6958	0.998	0.9999	100.0	99.15
	2 hours	10440.4	6879	0.997	0.9998		98.36
	3 hours	10346.8	6584	0.999	1.0023		97.48
	4 hours	10298.3	6485	0.967	1.0054		97.03
	5 hours	8934.8	6257	0.948	0.9999		84.18

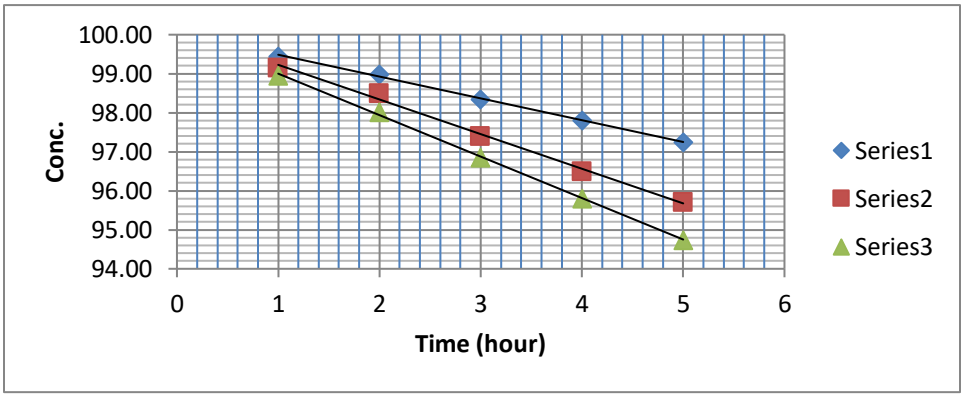

Figure 4.5: Zero order plot for the degradation of ondansetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

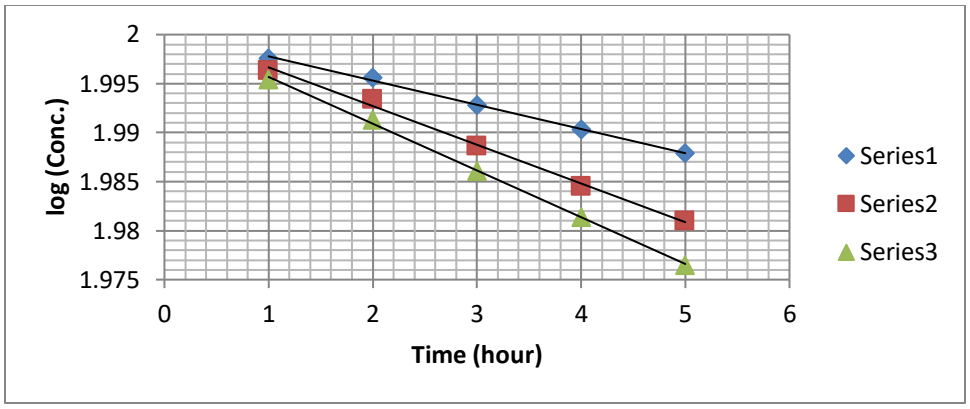

Figure 4.6: First order plot for the degradation of ondansetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

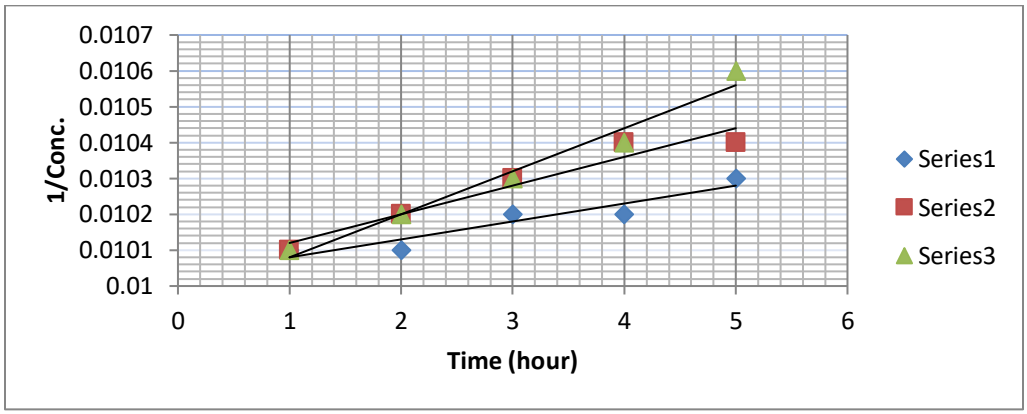

Figure 4.7: Second order plot for the degradation of ondansetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

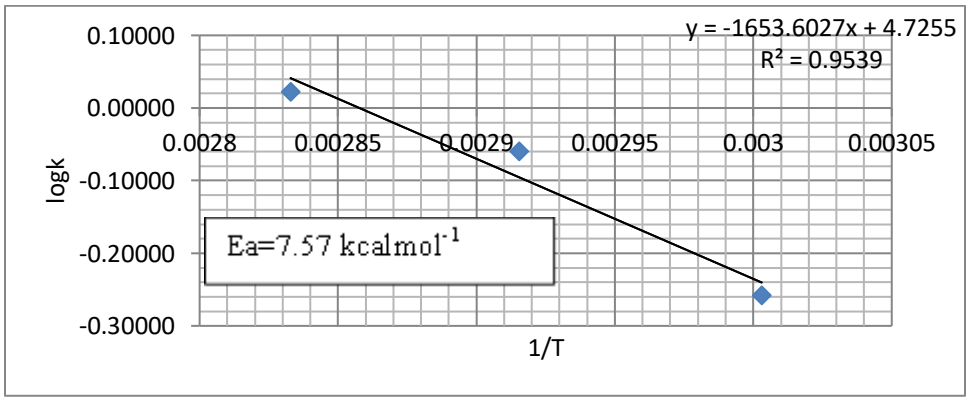

Figure 4.8: Relationship between temperature and rate constant used to calculate activation energy for ondansetron HCl.

Table 4.5: Kinetic parameters of degradation of ondansetron HCl at different temperature in 2.0N NaOH.

Temp.	Parameters	Orders		
		Zero (C vs time)	First (logC vs time)	Second (1/C vs time)
60°C	R^2 (linear correlation coefficient)	0.9988	0.9986	0.9984
	k (rate constant)	-0.5526	-0.0055	0.0001
70°C	R^2 (linear correlation coefficient)	0.9965	0.9963	0.9959
	k (rate constant)	-0.8714	-0.0090	0.0001
80°C	R^2 (linear correlation coefficient)	0.9994	0.9991	0.9987
	k (rate constant)	-1.0537	-0.0108	0.0001

Table 4.6: Summary of degradation kinetics of ondansetron HCl at 60°C, 70°C and 80°C in 2.0N NaOH.

Orders	Temperature		Equation	R^2 value	k	$t_{1\backslash 2}$ (days)	t_{90} (days)
	°C	°K					
Zero	60	333	y = -0.5526x + 100.0214	0.9988	0.05526	37.70	7.54
	70	343	y = -0.0024x + 2.0001	0.9986	0.8714	2.39	0.48
	80	353	y = 0.0001x + 0.0100	0.9984	1.0537	1.98	0.40
First	60	333	y = -0.8714x + 100.0552	0.9965	0.0055	5.25	0.72
	70	343	y = -0.0039x + 2.0003	0.9963	0.0090	3.21	0.44
	80	353	y = 0.0001x + 0.0100	0.9959	0.0108	2.67	0.37
Second	60	333	y = -1.0537x + 100.0310	0.9994	0.0001	4.17	0.42
	70	343	y = -0.0047x + 2.0002	0.9991	0.0001	4.17	0.42
	80	353	y = 0.0001x + 0.0100	0.9987	0.0001	4.17	0.42

4.3 Degradation kinetics of granisetron hydrochloride

Appropriate aliquot of granisetron hydrochloride solution in 2.0N NaOH was kept at 60°C, 70°C and 80°C for 1, 2, 3, 4 and 5 hours to prepare test samples followed by HPLC analysis. All HPLC chromatograms relevant to degraded samples are presented in figures 3.23-3.25. The potency remained after each degradation was calculated with the help of Microsoft Excel. These results are summarized in the table 4.7. 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

4.9-4.11. Kinetic parameters are summarized in table 4.8. Reaction activation energy (E_a) calculated with the help of Arrhenius equation was 16.98 kcal/mol. The linearity graph required to calculate reaction activation energy (E_a) was devised from reaction rate constant (k) vs inverse temperature in Kelvin (1/T). This linearity graph is shown in figure 4.12. 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 summary table 4.9.

Table 4.7: Peak parameters and assays of degraded samples of granisetron HCl at different temperature in 2.0N NaOH.

Temp.	Time interval	Area	Theoretical plate #	Tailing factor	Peak purity index	Assay (%)	
						Initial concentration	Potency
60°C	1 hour	3300.4	4897	0.958	0.9998	100.0	98.84
	2 hours	3282.5	4791	0.988	0.9998		97.68
	3 hours	3260.6	4991	0.972	0.9997		96.52
	4 hours	3214.4	4798	0.996	0.9995		95.36
	5 hours	2962.1	4712	0.979	0.9994		94.20
70°C	1 hour	3255.1	4851	0.992	0.9997	100.0	97.49
	2 hours	3124.6	4967	0.995	0.9979		94.98
	3 hours	3099.5	4991	0.994	0.9988		92.47
	4 hours	3054.4	4896	0.994	0.9989		89.96
	5 hours	2970.6	4857	0.997	0.9984		87.45
80°C	1 hour	10464	4458	0.993	0.9995	100.0	95.04
	2 hours	10303	4682	0.991	0.9987		90.08
	3 hours	10176	4897	0.989	0.9981		85.12
	4 hours	9547.1	4738	0.986	0.9987		80.16
	5 hours	9149.7	4825	0.947	0.9977		75.20

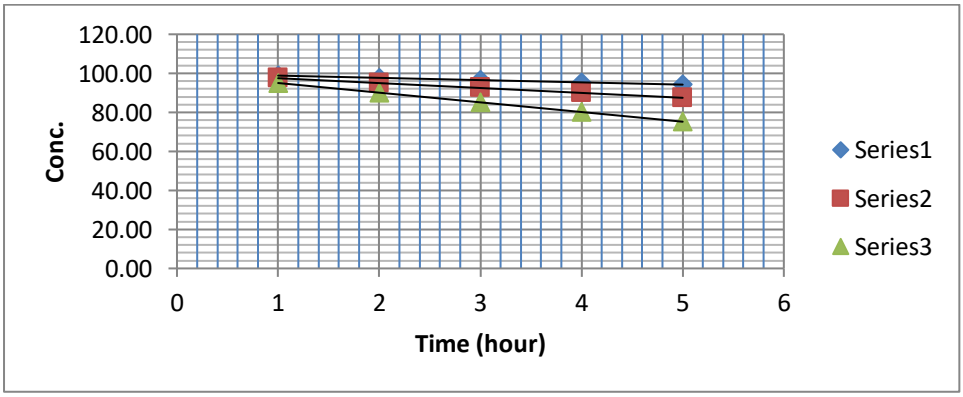

Figure 4.9: Zero order plot for the degradation of granisetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

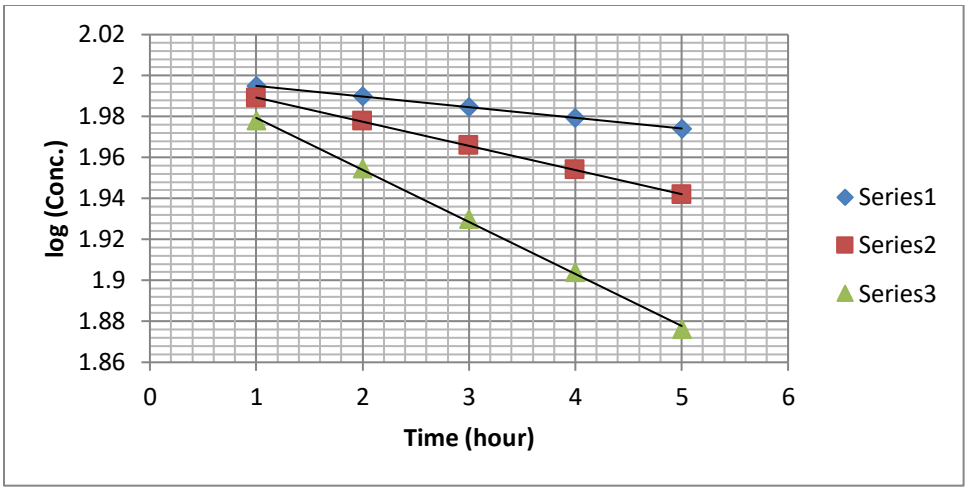

Figure 4.10: First order plot for the degradation of granisetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

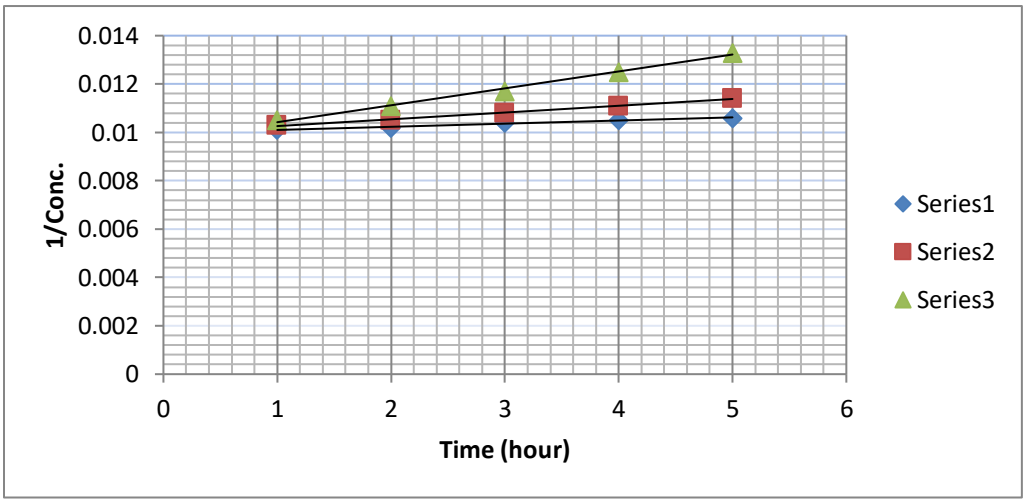

Figure 4.11: Second order plot for the degradation of granisetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

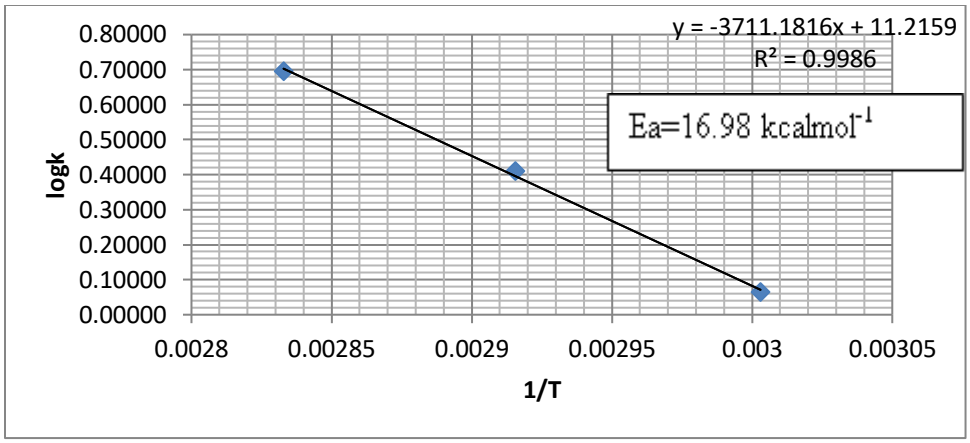

Figure 4.12: Relationship between temperature and rate constant used to calculate activation energy for granisetron HCl.

Table 4.8: Kinetic parameters of degradation of granisetron HCl at different temperature in 2.0N NaOH.

Temp.	Parameters	Orders		
		Zero (C vs time)	First (logC vs time)	Second (1/C vs time)
60°C	R^2 (linear correlation coefficient)	1.000	0.9999	0.9997
	k (rate constant)	1.1600	0.0052	0.0001
70°C	R^2 (linear correlation coefficient)	1.000	0.9996	0.9985
	k (rate constant)	2.5100	0.0116	0.0003
80°C	R^2 (linear correlation coefficient)	1.000	0.9983	0.9931
	k (rate constant)	4.9600	0.0247	0.0007

Table 4.9: Summary of degradation kinetics of granisetron HCl at 60°C, 70°C and 80°C in 2.0N NaOH.

Order	Temperature		Equation	R^2 value	k	$t_{1/2}$ (days)	t_{90} (days)
	°C	°K					
Zero	60	333	y = -1.1600x + 100.0000	1.000	1.1600	1.80	0.36
	70	343	y = -2.5100x + 100.0000	1.000	2.5100	0.81	0.16
	80	353	y = -4.9600x + 100.0000	1.000	4.9600	0.42	0.08
First	60	333	y = -0.0052x + 2.0001	0.9999	0.0052	5.55	0.76
	70	343	y = -0.0116x + 2.0005	0.9996	0.0116	2.49	0.34
	80	353	y = -0.0247x + 2.0023	0.9983	0.0247	1.17	0.16
Second	60	333	y = 0.0001x + 0.0100	0.9997	0.0001	4.17	0.42
	70	343	y = 0.0003x + 0.0100	0.9985	0.0003	1.39	0.14
	80	353	y = 0.0007x + 0.0099	0.9931	0.0007	0.60	0.06

4.4 Degradation kinetics of tropisetron hydrochloride

Analytical samples of tropisetron hydrochloride for degradation kinetics study was prepared in 2.0N NaOH at 60°C, 70°C and 80°C for 1, 2, 3, 4 and 5 hours. All HPLC chromatograms relevant to degraded samples are presented in figures 3.30-3.32. The potency remained after each degradation was calculated with the help of Microsoft Excel. These results are summarized in the table 4.10. 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 4.13-4.15. Kinetic parameters are summarized in table 4.11. Reaction activation energy (E_a) calculated with the help of Arrhenius equation was 16.86 kcal/mol. 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 4.16. 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 summary table 4.12.

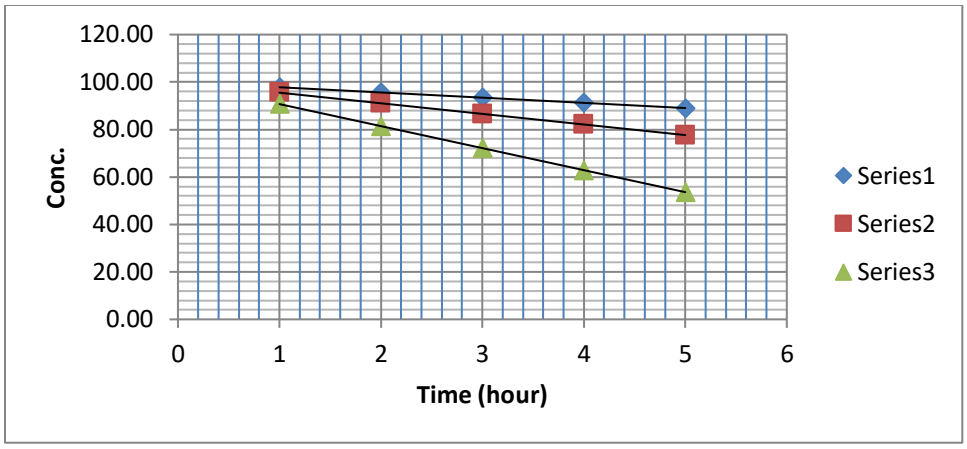

Figure 4.13: Zero order plot for the degradation of tropisetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

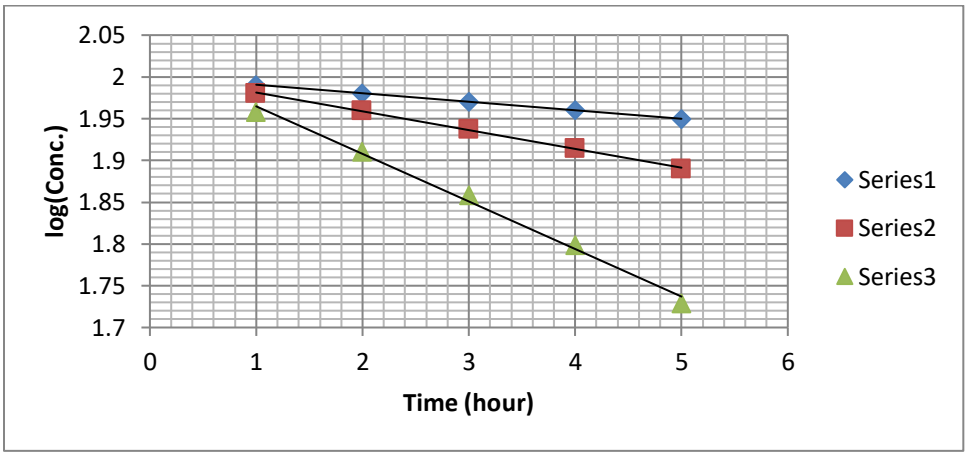

Figure 4.14: First order plot for the degradation of tropisetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

Table 4.10: Peak parameters and assays of degraded samples of tropisetron HCl at different temperature in 2.0N NaOH.

Temp.	Time interval	Area	Theoretical plate #	Tailing factor	Peak purity index	Assay (%)	
						Initial concentration	Potency
60°C	1 hour	3482.5	3584	0.951	0.9991	100.0	97.81
	2 hours	3378.7	3647	0.982	0.9988		95.61
	3 hours	3328.8	3841	0.965	0.9984		93.43
	4 hours	3099.3	3624	0.987	0.9985		91.24
	5 hours	2816.4	3921	0.981	0.9974		89.05
70°C	1 hour	3584.7	3647	0.987	0.9990	100.0	95.53
	2 hours	3014.3	3784	0.964	0.9986		91.06
	3 hours	2758.2	3582	0.954	0.9980		86.59
	4 hours	2436.7	3948	0.974	0.9982		82.12
	5 hours	2205.2	3748	0.967	0.9971		77.65
80°C	1 hour	10048	3854	0.981	0.9989	100.0	90.72
	2 hours	8253.3	3794	0.987	0.9981		81.44
	3 hours	6829.8	3824	0.964	0.9984		72.16
	4 hours	5370.3	3692	0.962	0.9981		62.88
	5 hours	4165.1	3741	0.945	0.9974		53.60

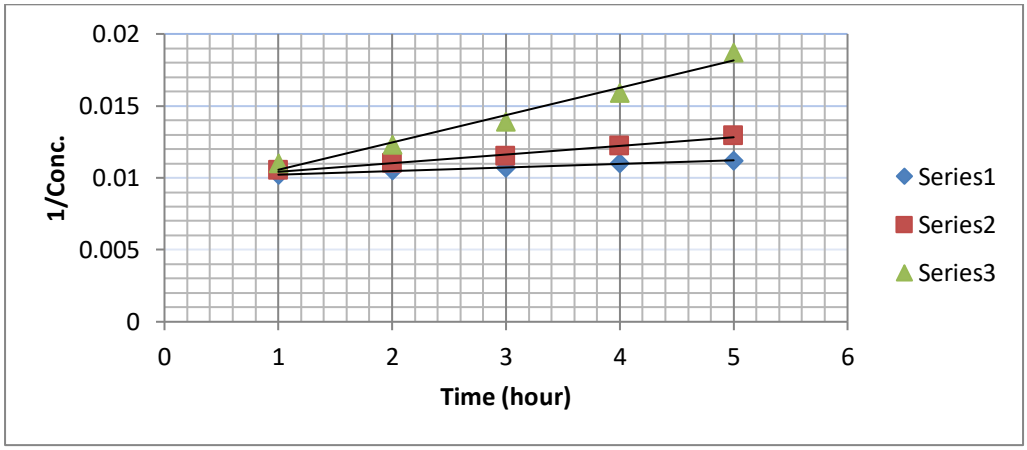

Figure 4.15: Second order plot for the degradation of tropisetron HCl at 60°C (series 1), 70°C (series 2) and 80°C (series 3) in 2.0N NaOH.

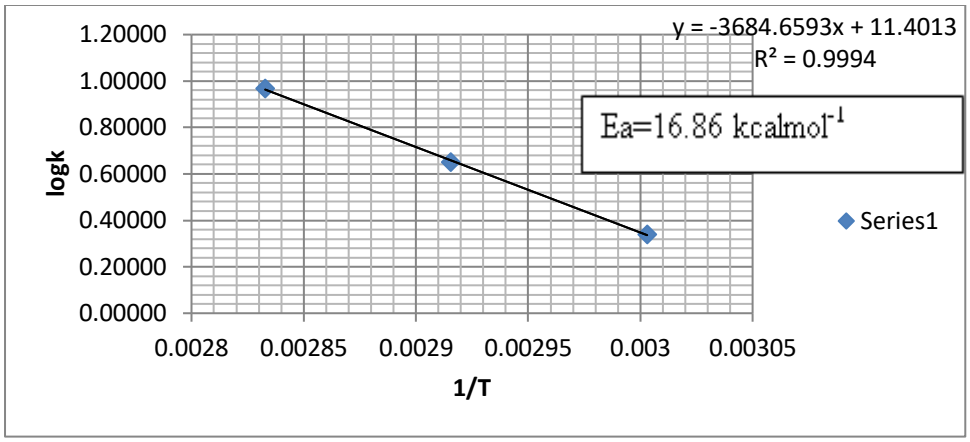

Figure 4.16: Relationship between temperature and rate constant used to calculate activation energy for tropisetron HCl.

Table 4.11: Kinetic parameters of degradation of tropisetron HCl at different temperature in 2.0N NaOH.

Temp.	Parameters	Orders		
		Zero (C vs time)	First (logC vs time)	Second (1/C vs time)
60°C	R^2 (linear correlation coefficient)	1.000	0.9997	0.9989
	k (rate constant)	2.1897	0.0101	0.0002
70°C	R^2 (linear correlation coefficient)	1.000	0.9986	0.9946
	k (rate constant)	4.4700	0.0219	0.0006
80°C	R^2 (linear correlation coefficient)	1.000	0.9918	0.9674
	k (rate constant)	9.2800	0.0538	0.0017

Table 4.12: Summary of degradation kinetics of tropisetron HCl at 60°C, 70°C and 80°C in 2.0N NaOH.

Order	Temperature		Equation	R^2 value	k	$t_{1/2}$ (days)	t_{90} (days)
	°C	°K					
Zero	60	333	y = -2.1897x + 99.9976	1.0000	2.1897	0.95	0.19
	70	343	y = -4.4700x + 100.0000	1.0000	4.4700	0.47	0.09
	80	353	y = -9.2800x + 100.0000	1.0000	9.2800	0.22	0.04
First	60	333	y = -0.0101x + 2.0004	0.9997	0.0101	2.86	0.39
	70	343	y = -0.0219x + 2.0018	0.9986	0.0219	1.32	0.18
	80	353	y = -0.0538x + 2.0103	0.9918	0.0538	0.54	0.07
Second	60	333	y = 0.0002x + 0.0100	0.9989	0.0002	2.08	0.21
	70	343	y = 0.0006x + 0.0099	0.9946	0.0006	0.69	0.07
	80	353	y = 0.0017x + 0.0094	0.9674	0.0017	0.25	0.02

4.5 Calculation

4.5.1 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

Chapter-5

Results and Discussion of Impurity Profiling

For investigation of degradants and growing impurities, all chromatograms were evaluated thoroughly. The relative retention time of any degradant or growing impurity observed from HPLC chromatogram of the stressed samples was compared with known relative retention time(RRT) to that of the degradant or growing impurity. Similarly, molecular weight of any degradant or impurity observed from MS spectrum of the stressed samples was compared with known molecular weight to that of the degradant or growing impurity. Molecular weight, intensity in CPS, peak area and RRT are considered together to identify known or unknown growing impurities or degradants.

Necessary information of established impurities is summarized in tables 5.1-5.4 and figures 5.1-5.3.

Table 5.1: Information of established impurities of ondansetron HCl.

Chemical names	Monograph name	Molecular formula	Molecular weights
1H-imidazole	Impurity-E	$C_3H_4N_2$	68.08
2-Methyl-1H-imidazole	Impurity-F	$C_4H_6N_2$	82.10
9-Methyl-1,2,3,9-tetrahydro-4H-carbazol-4-one	Impurity-C	$C_{13}H_{13}NO$	199.25
6,6¢-Methylenebis[(3*RS*)-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-1,2, 3,9-tetrahydro-4H-carbazol-4-one]	Impurity-B	$C_{37}H_{32}D_6N_6O_2$	604.77
9-Methyl-3-methylene-1,2,3,9-tetrahydro-4H-carbazol-4-one	Impurity-D	$C_{14}H_{13}NO$	211.26
(3*RS*)-3-[(dimethylamino)methyl]-9-methyl-1,2,3,9-tetrahydro-4H-carbazol-4- one,	Impurity-A	$C_{16}H_{20}N_2O$	256.34
(3*RS*)-3-[(1H-imidazol-1-yl)methyl]-9-methyl-1,2,3,9-tetrahydro-4H-carbazol- 4-one (C-desmethylondansetron)	Impurity-G	$C_{17}H_{17}N_2O$	279.34

Table 5.2: Information of established impurities of granisetron HCl.

Chemical names	Monograph name	Molecular formula	Molecular weights
1-Methyl-1H-indazole-3-carboxylic acid	Impurity-D	$C_{10}H_9NO_2$	176.18
N-[(1R,3r,5S)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1H-indazole-3-carboxamide	Impurity-B	$C_{17}H_{22}N_4O$	298.38
2-Methyl-N-[(1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2H-indazole-3-carboxamide	Impurity-A	$C_{18}H_{24}N_4O$	312.41
N-[(1R,3r,5S)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1H-indazole-3-carboxamide	Impurity-C	$C_{17}H_{22}N_4O{\cdot}HCl$	334.33

Table 5.3: Information of established impurities of tropisetron HCl.

Chemical names	Monograph name	Molecular formula	Molecular weights
1H-indole-3-carboxylic acid	Impurity-B	$C_9H_7NO_2$	161.16
(1*R*,3r,5*S*)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol (tropine)	Impurity-A	$C_8H_{15}NO$	141.21

Table 5.4: Information of established impurities of palonosetron HCl.

Chemical names	Monograph name	Molecular formula	Molecular weights
(3a*S*)-1-Oxo-2-[(3*S*)-quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinoline 1-oxide	Impurity-A	$C_{19}H_{24}N_2O_2$	312.41
(3*S*)-3-(1-Oxo-5,6-dihydro-1*H*-benzo[*de*]isoquinolin- 2(4*H*)-yl)quinuclidine 1-oxide	Impurity-B	$C_{19}H_{22}N_2O_2$	310.39
(3a*S*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro- 1*H*-benzo[*de*]isoquinolin-1-one hydrochloride	Impurity-C	$C_{19}H_{24}N_2O$	332.87
2-[(3*S*)-Quinuclidin-3-yl]-2,4,5,6-tetrahydro-1*H*-benzo [*de*]isoquinolin-1-one hydrochloride	Impurity-E	$C_{19}H_{22}N_2O$	330.85

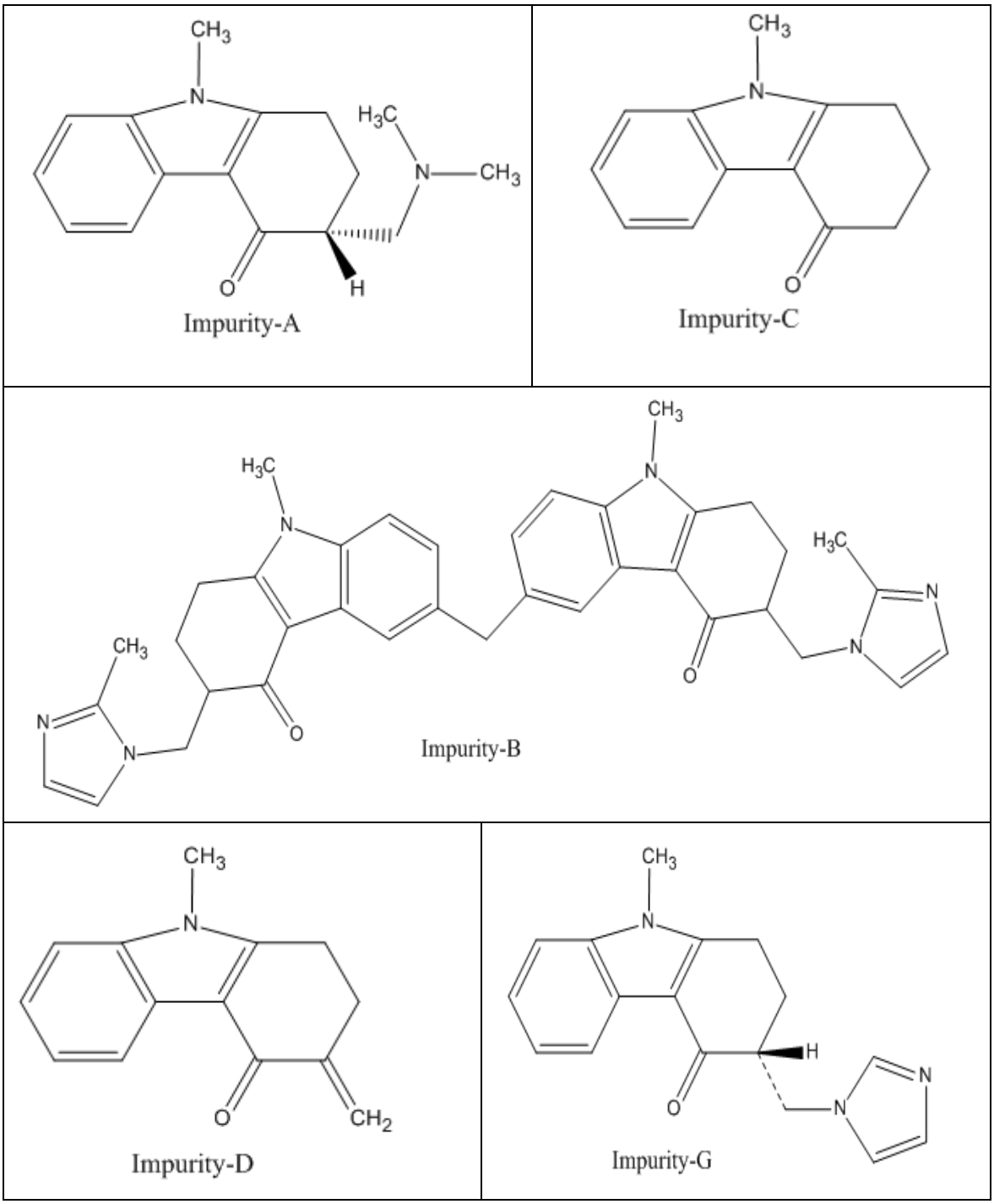

Figure 5.1: Structure of known impurities of ondansetron hydrochloride.

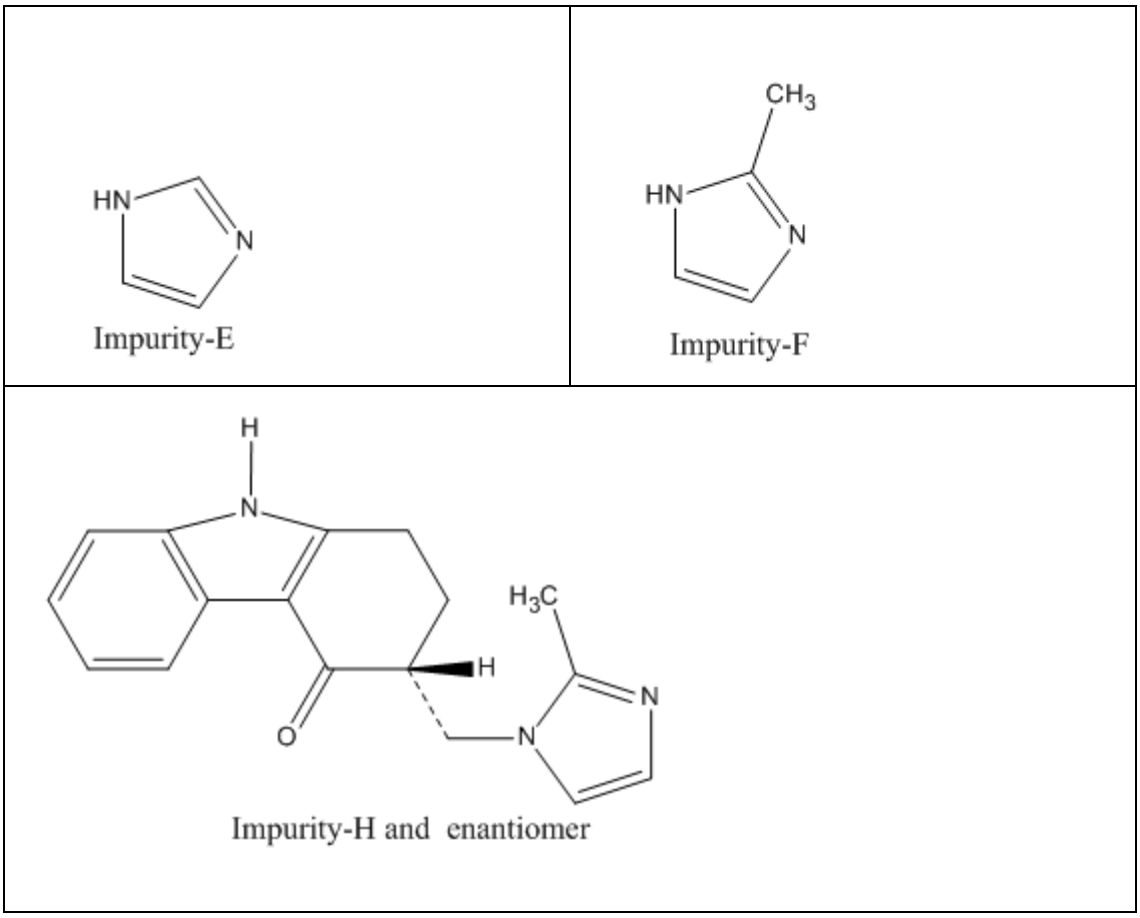

Figure 5.2: Structure of known impurities of ondansetron hydrochloride.

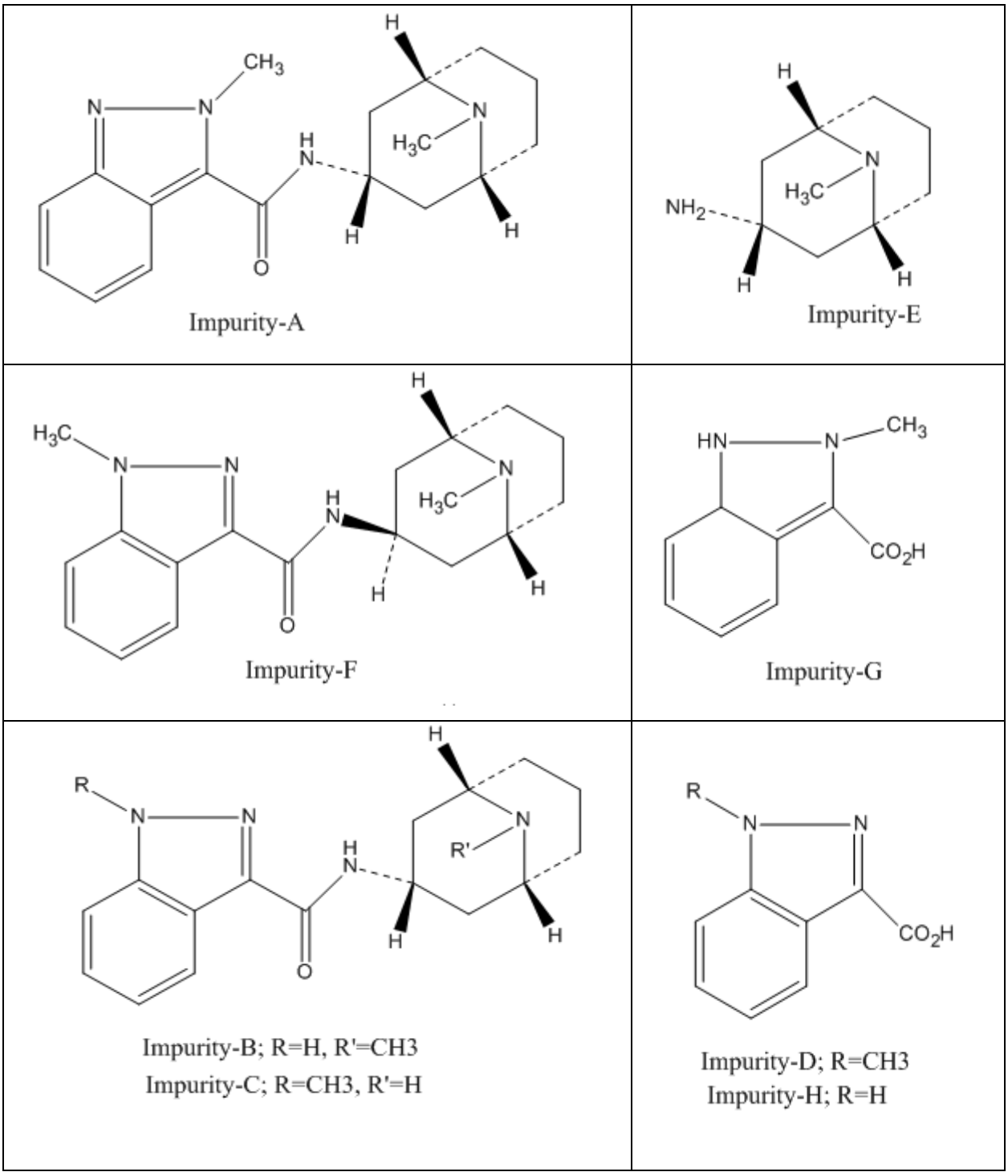

Figure 5.3: Structure of known impurities of granisetron hydrochloride.

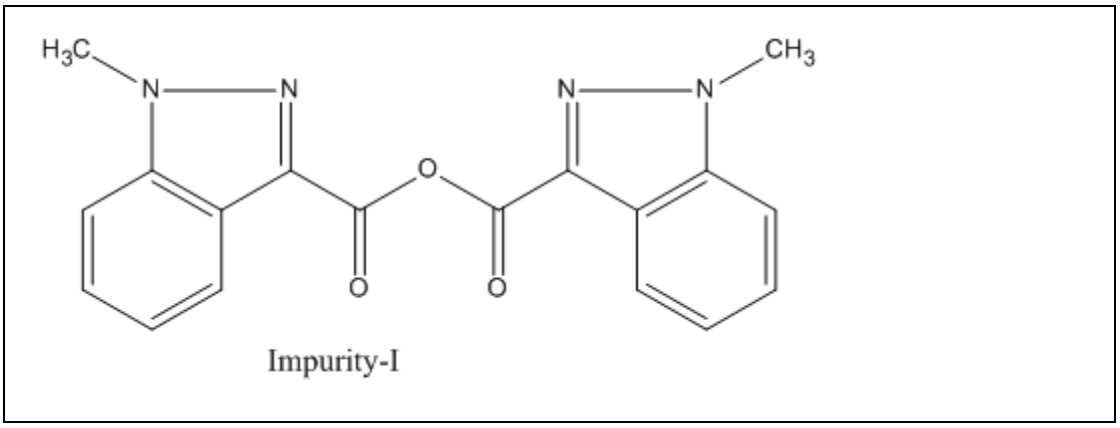

Figure 5.4: Structure of known impurities of granisetron hydrochloride.

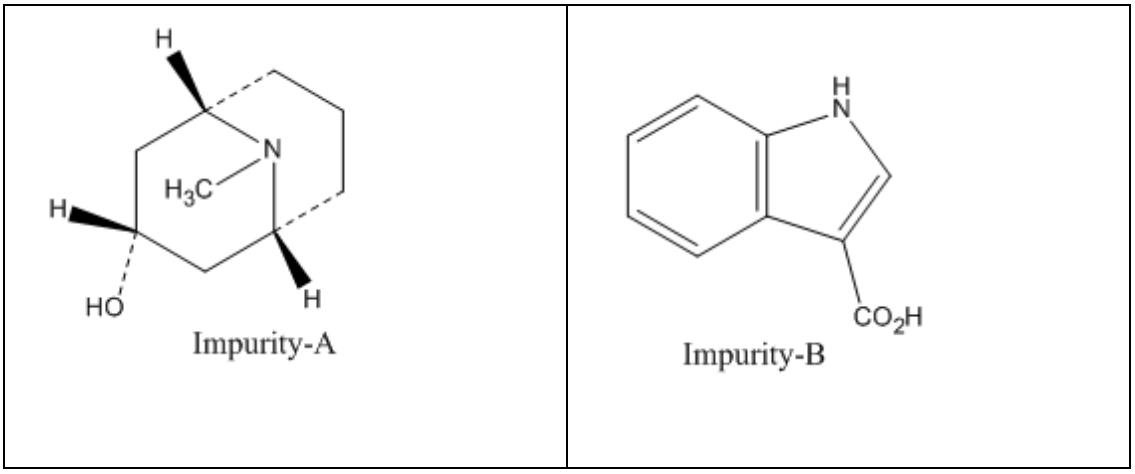

Figure 5.5: Structure of known impurities of tropisetron hydrochloride.

5.1 Impurity profiling of ramosetron hydrochloride

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

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

given in table 3.3.The relative retention time (RRT), area and % degradants or impurities derived from acidic conditions are summarized in the table 5.5.

Table 5.5: HPLC results for the impurities or degradants of ramosetron HCl in 0.5N, 1.0N and 2.0N HCl at 60°C.

Conditions	Peak identity	Retention time (min)	RRT	Area	Content (%)
0.5N HCl	Impurity-1	7.11	0.79	6.86	0.18
	Impurity-2	21.08	2.33	27.20	0.80
1.0N HCl	Impurity-3	20.90	2.32	25.82	4.7
	Impurity-4	31.53	3.50	12.85	2.3
2.0N HCl	Impurity-5	8.10	0.89	241.20	8.3
	Impurity-6	19.81	2.18	17.62	0.59
	Impurity-7	20.90	2.30	10.18	0.34

After evaluation of the degradants or impurities information provided in table 5.5, it was observed that five potential degradants or impurities were produced in acidic conditions with RRT of 0.79, 0.89, 2.18, 2.33 and 3.50.

Base degraded samples in 0.1N NaOH at 60°C, 70°C and 80°C for 1 hour, 2 hours, 3 hours, 4 hours and 5 hours were analyzed to investigate degradants or impurities. The chromatograms are shown in figures 3.4-3.7 and the results are summarized in tables 3.5-3.7.The relative retention time (RRT), area and % degradants or impurities derived from the stressed samples in basic conditions are summarized in the tables 5.6-5.8.

Table 5.6: HPLC results for the impurities or degradants of ramosetron HCl in 0.1N NaOH at 60°C.

Time interval	Peak identity	Retention time (min)	RRT	Area	Content (%)
1 hour	Impurity-8	4.85	0.38	81.5	1.8
2 hours	Impurity-9	4.92	0.38	124.7	2.2
3 hours	Impurity-10	4.95	0.38	200.1	4.9
4 hours	Impurity-11	4.96	0.38	307.4	7.9
5 hours	Impurity-12	4.95	0.38	375.5	7.1
	Impurity-13	8.92	0.67	151.4	2.9

Table 5.7: HPLC result for the impurities or degradants of ramosetron HCl in 0.1N NaOH at 70°C.

Time interval	Peak identity	Retention time (min)	RRT	Area	Content (%)
1 hour	Impurity-14	4.87	0.51	4.98	0.4
	Impurity-15	6.10	0.64	16.5	1.6
2 hours	Impurity-16	4.88	0.51	7.94	1.1
	Impurity-17	6.09	0.64	25.6	3.9
3 hours	Impurity-18	4.86	0.51	6.18	1.5
	Impurity-19	6.09	0.64	22.9	5.5
4 hours	Impurity-20	4.87	0.51	6.92	1.7
	Impurity-21	5.39	0.56	5.29	1.4
	Impurity-22	6.08	0.64	29.29	8.0
5 hours	Impurity-23	4.85	0.51	7.20	2.2
	Impurity-24	5.39	0.56	5.51	1.6
	Impurity-25	6.09	0.64	35.6	11.2

Table 5.8: HPLC results for the impurities or degradants of ramosetron HCl in 0.1N NaOH at 80°C.

Time interval	Peak identity	Retention time (min)	RRT	Area	Content (%)
1 hour	Impurity-26	4.90	0.38	209.2	3.0
2 hours	Impurity-27	5.09	0.39	403.4	0.89
	Impurity-28	9.25	0.70	2784.0	6.1
3 hours	Impurity-29	4.99	0.39	461.0	8.6
	Impurity-30	9.03	0.70	185.4	4.8
4 hours	Impurity-31	4.98	0.38	1431.6	15.8
	Impurity-32	9.08	0.70	468.8	5.2
5 hours	Impurity-33	5.0	0.39	1081.3	16.1
	Impurity-34	9.0	0.69	328.0	4.9

Upon evaluation of the results of potential degradants or impurity summarized in tables 5.6-5.8, it was observed that six different potential degradants or impurities were found in basic conditions with RRT of 0.38, 0.51, 0.56, 0.64, 0.67 and 0.70.

Oxidative stressed samples in 3.0% hydrogen peroxide at dark place for 1, 2 and 3 hours were analyzed to investigate potential degradants or impurities. HPLC chromatograms and MS spectra are evaluated together to investigate degradants or impurities. The HPLC chromatograms are shown in figures 3.8-3.9 and the results are summarized in table 5.9. On the other hand, MS spectra are shown in figures 5.6-5.8 and the relevant information is summarized in the tables 5.10-5.11.

Table 5.9: HPLC results for the impurities or degradants of ramosetron HCl in 3.0% hydrogen peroxide.

Time interval	Peak identity	Retention time (min)	RRT	Area	Content (%)
1 hour	Impurity-35	6.21	0.65	18.95	1.90
	Impurity-36	7.44	0.77	3.03	0.31
	Impurity-37	7.09	0.95	56.03	5.80
2 hours	Impurity-38	6.20	0.65	43.70	4.2
	Impurity-39	7.20	0.76	6.01	0.58
	Impurity-40	9.06	0.94	85.20	8.20
3 hours	Impurity-41	6.03	0.63	6.16	0.54
	Impurity-42	7.41	0.77	16.33	1.40
	Impurity-43	9.07	0.95	144.66	13.0

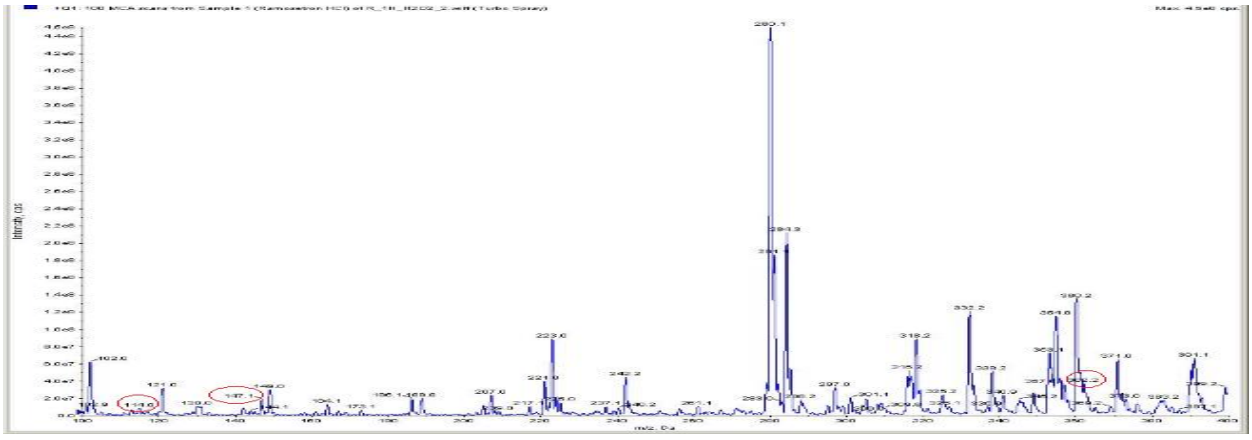

Figure 5.6: Mass spectrum of ramosetron HCl in 3.0% H_2O_2 after 1 hour.

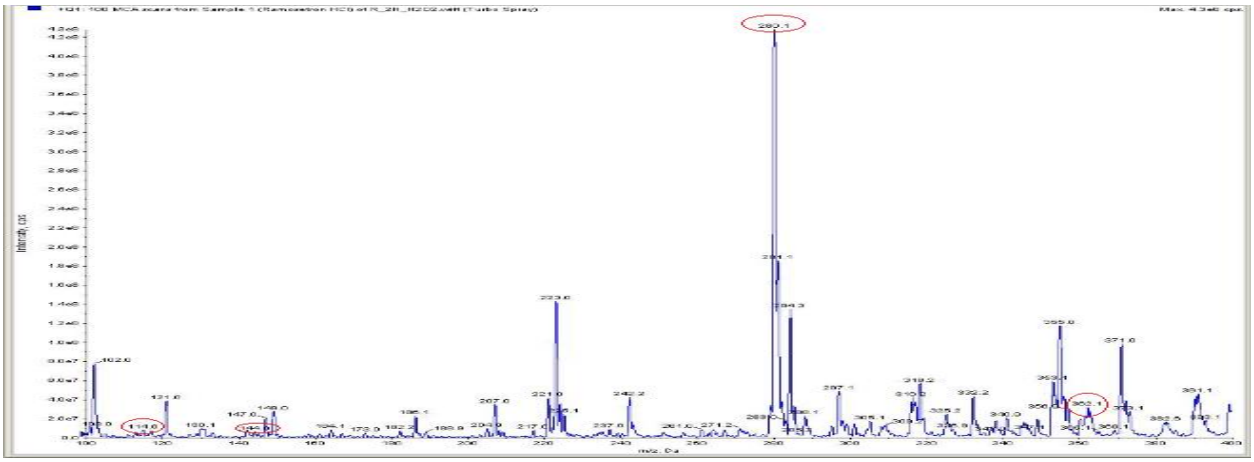

Figure 5.7: Mass spectrum of ramosetron HCl in 3.0% H_2O_2 after 2 hours.

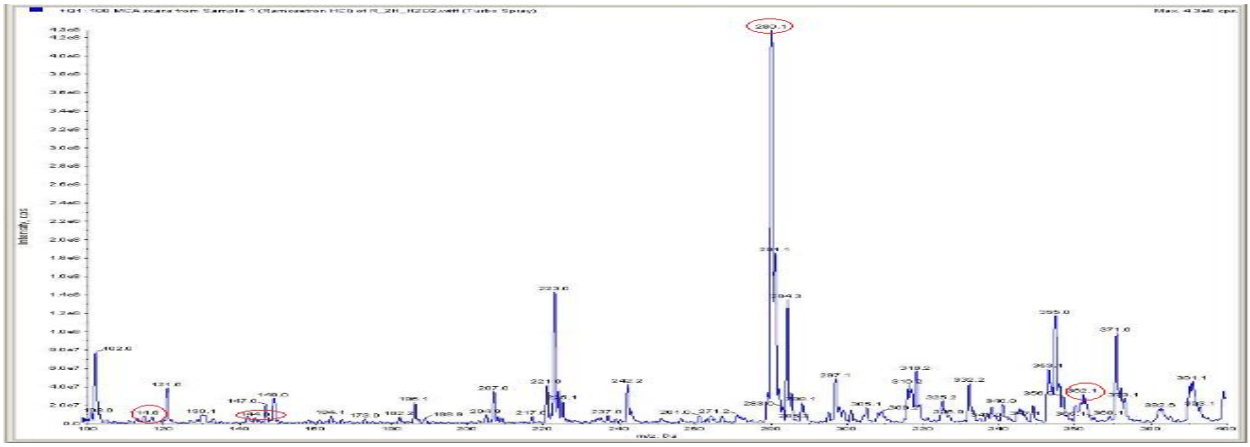

Figure 5.8: Mass spectrum of ramosetron HCl in 3.0% H_2O_2 after 3 hours.

Table 5.10: MS result for the impurities or degradants of ramosetron HCl in 3.0% hydrogen peroxide.

Time interval	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
1 hour	114.9	$1.0e^7$	113.9	Unknown
	142.1	$2.0e^7$	141.1	Unknown
	362.2	$4.0e^7$	361.2	Unknown
2 hours	114.9	$1.5e^7$	113.9	Unknown
	142.1	$2.5e^7$	141.1	Unknown
	362.2	$4.5e^7$	361.2	Unknown
3 hours	114.9	$0.8e^7$	113.9	Unknown
	142.1	$2.8e^7$	141.1	Unknown
	362.2	$4.9e^7$	361.2	Unknown

Table 5.11: HPLC and MS results for the impurities or degradants of ramosetron HCl in 3.0% hydrogen peroxide.

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Time interval	Peak identity	RRT	Area	Intensity (cps)	Molecular weight	Nominal molecular weight	Molecular formula	Comments
1hour	Impurity-35	0.65	18.95	$1.0e^7$	114.9	113.9	Unknown	Unknown
	Impurity-36	0.77	3.03	$2.0e^7$	142.1	141.1	Unknown	Unknown
	Impurity-37	0.95	56.03	$4.0e^7$	362.2	361.2	Unknown	Unknown
2 hours	Impurity-38	0.65	43.70	$1.5e^7$	114.9	113.9	Unknown	Unknown
	Impurity-39	0.76	6.01	$2.5e^7$	142.1	141.1	Unknown	Unknown
	Impurity-40	0.94	85.20	$4.5e^7$	362.2	361.2	Unknown	Unknown
3 hours	Impurity-38	0.65	43.70	$1.5e^7$	114.9	113.9	Unknown	Unknown
	Impurity-39	0.76	6.01	$2.5e^7$	142.1	141.1	Unknown	Unknown
	Impurity-40	0.94	85.20	$4.5e^7$	362.2	361.2	Unknown	Unknown

After evaluation of the results of potential degradantsor impurities summarized in tables 5.10-5.11, it was observed that three potential degradants or impurities were produced in oxidative conditions with RRT of 0.65, 0.76, 0.94 and molecular weight of 113.9, 141.1 and 361.2 Da.

Photo degradation study was conducted in 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. Again, the sample was directly exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light. HPLC chromatograms and MS spectra are considered together to find out potential degradants or impurities. The HPLC chromatograms are shown in figure 3.10 and the results are summarized in table 5.12. On the other hand, MS spectra are shown in figure 5.9 and the relevant information is summarized in the tables 5.13-5.14.

Table 5.12: HPLC results for the impurities or degradants of ramosetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Peak names	Retention time (min)	RRT	Area	Content (%)
Photo degradation	Impurity-44	5.58	0.59	141.62	2.2
	Impurity-45	5.79	0.61	137.27	2.1
	Impurity-46	6.78	0.72	3.92	0.05
	Impurity-47	7.98	0.84	11.73	0.17

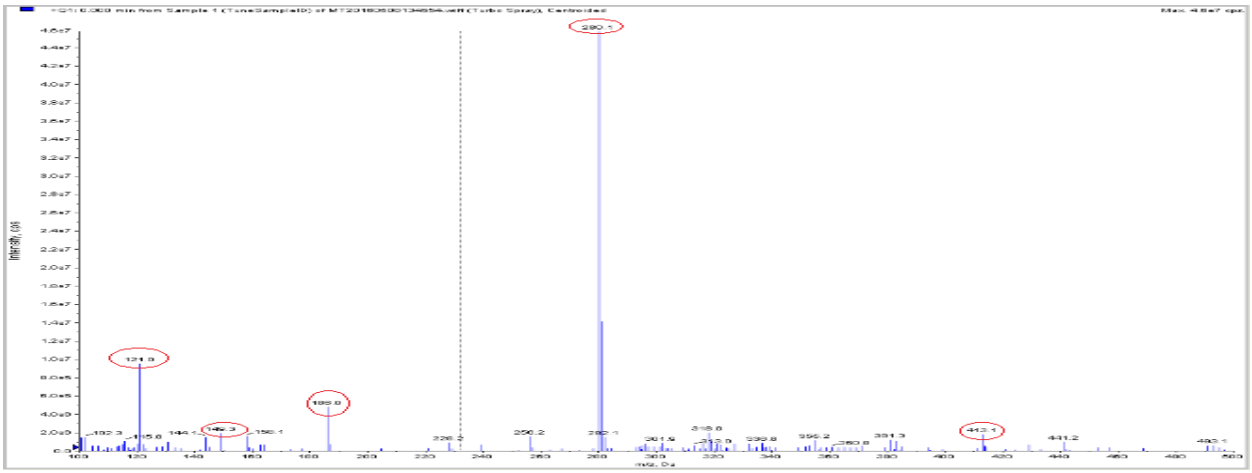

Figure 5.9: Mass spectrum of ramosetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Table 5.13: MS results for the impurities or degradants of ramosetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
Photo degradation	121.3	$1.4e^6$	120.3	Unknown
	148.9	$1.5e^5$	147.9	Unknown
	186.1	$5.2e^5$	185.1	Unknown
	413.3	$1.5e^5$	412.3	Unknown

Table 5.14: HPLC and MS results for the impurities or degradants of ramosetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Peak identity	RRT	Area	Intensity (cps)	Molecular weight	Nominal molecular weight	Molecular formula	Comments
Photo degradation	Impurity-44	0.59	141.62	$1.4e^6$	121.3	120.3	Unknown	Unknown
	Impurity-45	0.61	137.27	$1.5e^5$	148.9	147.9	Unknown	Unknown
	Impurity-46	0.72	3.92	$5.2e^5$	186.1	185.1	Unknown	Unknown
	Impurity-47	0.84	11.73	$1.5e^5$	413.3	412.3	Unknown	Unknown

Upon evaluation of the results of potential degradants or impurities summarized in tables 5.13-5.14, it was observed that four potential degradants or impurities were produced in photo-degradation exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light with RRT of 0.59, 0.61, 0.72, 0.84 and molecular weight of 120.3, 147.9, 185.1and 412.3 Da.

5.2 Impurity profiling of ondansetron hydrochloride

Aqueous degradation study was conducted with purified water at 60°C for 7 and 21 days. Degradation was observed after 21 days. The chromatogram is shown in figure 3.11 and the result is summarized in table 3.13. It was observed that one potential degradant or impurity was found in aqueous condition after 21 days with RRT of 0.72.

Acid degradation study was conducted in 0.1N, 0.5N, 1.0N and 2.0N HCl at 60°C for 7 days.
No degradation was observed in acidic conditions.

Base degraded samples in 0.1N, 0.5N and 1.0N NaOH were brought under degradant or impurity investigation. HPLC chromatograms and MS spectra are considered together to evaluate degradants or impurities. The HPLC chromatograms are shown in figure 3.12 and the results are summarized in table 5.15. On the other hand, MS spectra are shown in figures 5.10-5.12 and the relevant information is summarized in the tables 5.16-5.17.

Table 5.15: HPLC results for the impurities or degradants of ondansetron HCl in basic conditions at 60°C for 3 days.

Conditions	Peak identity	Retention time (min)	RRT	Area	Content (%)
0.1N NaOH	Impurity-2	6.54	0.65	162.0	4.1
0.5N NaOH	Impurity-3	6.51	0.65	449.6	8.1
1.0N NaOH	Impurity-4	5.18	0.51	19.85	0.39
	Impurity-5	6.52	0.65	445.4	12.7

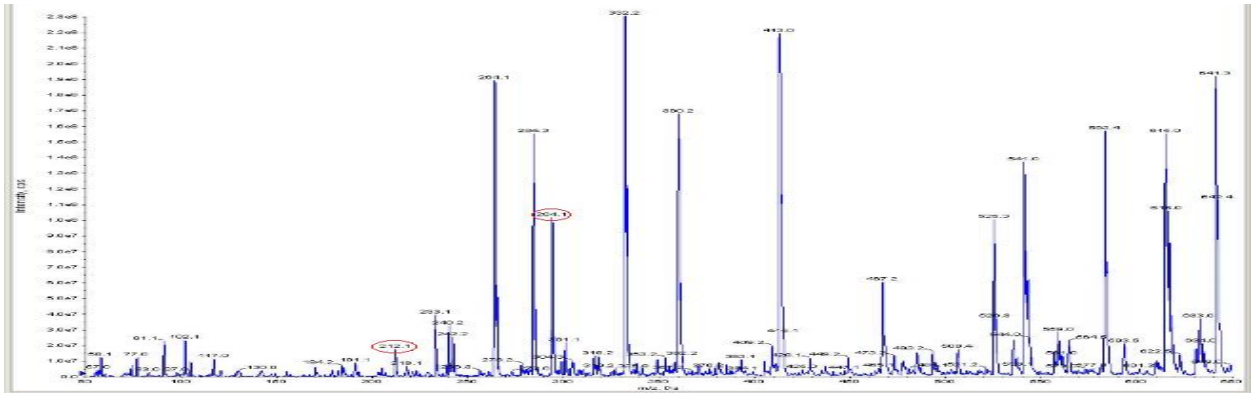

Figure 5.10: Mass spectrum of ondansetron HCl stressed with 0.1N NaOH at 60°C for 3 days.

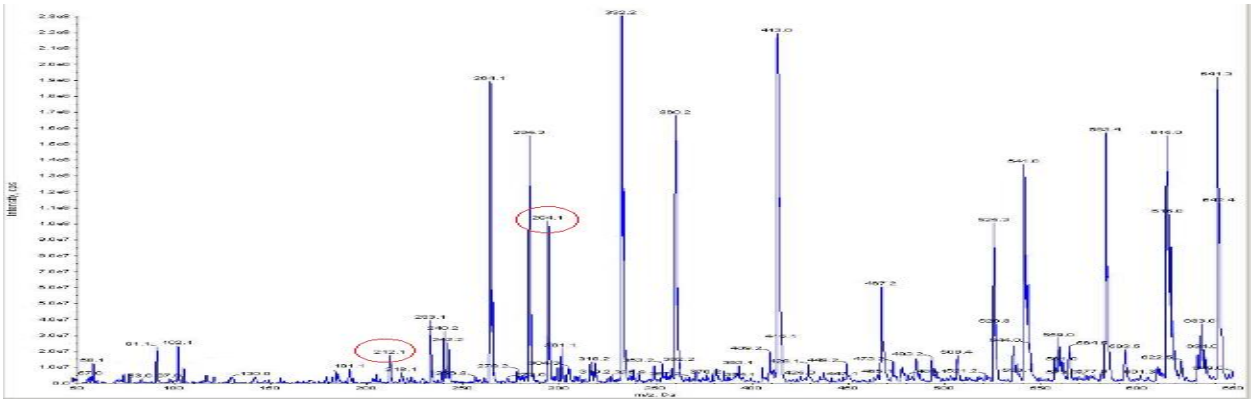

Figure 5.11: Mass spectrum of ondansetron HCl stressed with 0.5N NaOH at 60°C for 3 days.

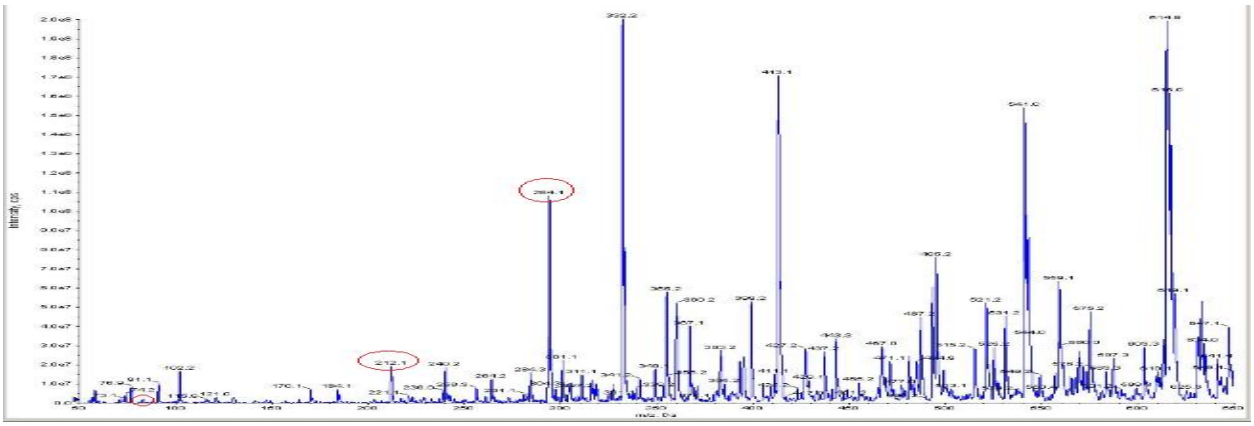

Figure 5.12: Mass spectrum of ondansetron HCl stressed with 1.0N NaOH at 60°C for 3 days.

Table 5.16: MS result for the impurities or degradants of ondansetron HCl in basic conditions at 60°C for 3 days.

Conditions	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
0.1N NaOH	212.1	$1.5e^7$	211.26	Impurity-D
0.5N NaOH	212.1	$2.0e^7$	211.26	Impurity-D
1.0N NaOH	212.1	$2.5e^7$	211.26	Impurity-D
	83.1	$0.25e^7$	82.10	Impurity-F

Table 5.17: HPLC and MS results for the impurities or degradants of ondansetron HCl in basic conditions at 60°C for 3 days.

Conditions	Peak identity	RRT	Intensity (cps)	Molecular weight	Nominal molecular weight	Molecular formula	Comments
0.1N NaOH	Impurity-2	0.65	$1.5e^7$	212.1	211.26	$C_{14}H_{13}NO$	Impurity-D
0.5N NaOH	Impurity-3	0.65	$2.0e^7$	212.1	211.26	$C_{14}H_{13}NO$	Impurity-D
1.0N NaOH	Impurity-4	0.51	$0.25e^7$	83.1	82.1	$C_4H_6N_2$	Impurity-F
	Impurity-5	0.65	$2.5e^7$	212.1	211.26	$C_{14}H_{13}NO$	Impurity-D

After evaluation of the results of potential degradants or impurities summarized in tables 5.1-5.2 and 5.16-5.17, it was observed that two known impurities, impurity-D and impurity-F, were

produced in basic conditions with RRT of 0.51, 0.65 and molecular weight of 82.1 and 211.26 Da.

Oxidative degraded samples in 3.0% hydrogen peroxide at dark place for 1, 2 and 3 hours were analyzed for degradant or impurity investigation. HPLC chromatograms and MS spectra were evaluated together to find out degradants or impurities. The HPLC chromatograms are shown in figure 3.16 and the results are summarized in table 5.18. On the other hand, MS spectra are shown in figures 5.13-5.15 and the relevant information is summarized in the tables 5.19-5.20.

Table 5.18: HPLC result for the impurities or degradants of ondansetron HCl in 3.0% hydrogen peroxide.

Time interval	Peak identity	Retention time (min)	RRT	Area	Content (%)
1 hour	Impurity-8	5.27	0.44	72	1.4
	Impurity-9	5.62	0.47	41.87	0.80
	Impurity-10	5.83	0.48	33.42	0.64
	Impurity-11	16.67	1.39	51.86	0.99
2 hours	Impurity-12	5.28	0.44	67.0	1.28
	Impurity-13	5.64	0.47	38.10	0.73
	Impurity-14	5.84	0.49	24.15	0.46
	Impurity-15	6.17	0.51	10.20	0.19
	Impurity-16	6.75	0.56	35.95	0.68
	Impurity-17	16.65	1.38	38.24	0.73
3 hours	Impurity-18	5.28	0.44	88.34	1.76
	Impurity-19	5.63	0.47	48.82	0.97
	Impurity-20	5.84	0.49	31.12	0.62
	Impurity-21	6.73	0.56	21.65	0.43
	Impurity-22	7.83	0.65	11.91	0.24
	Impurity-23	16.67	1.38	67.87	1.38

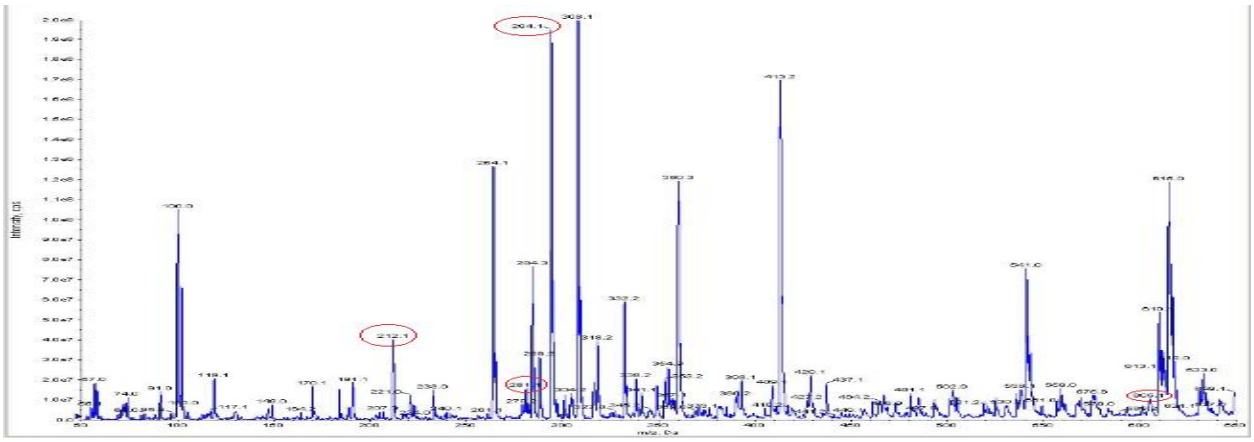

Figure 5.13: Mass spectrum of ondansetron HCl in 3.0% H_2O_2 after 1 hour.

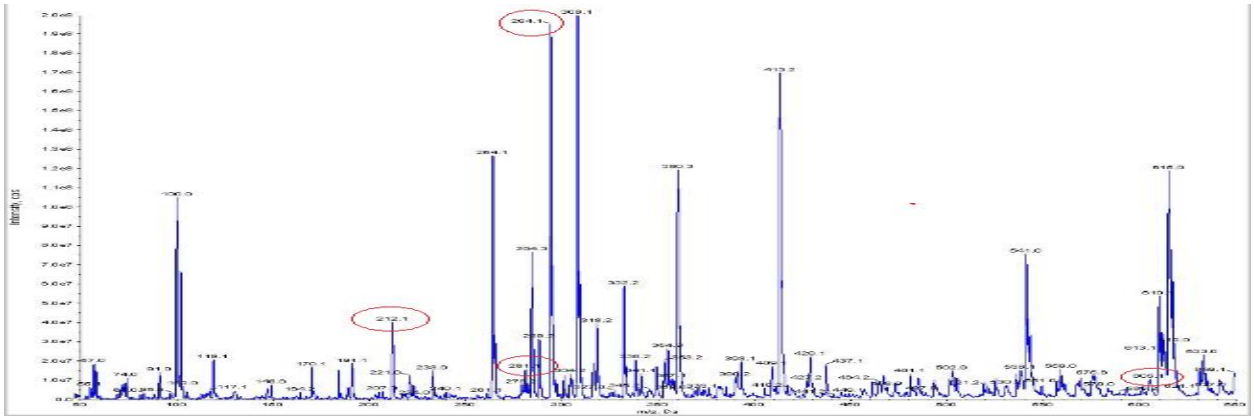

Figure 5.14: Mass spectrum of ondansetron HCl in 3.0% H_2O_2 after 2 hours.

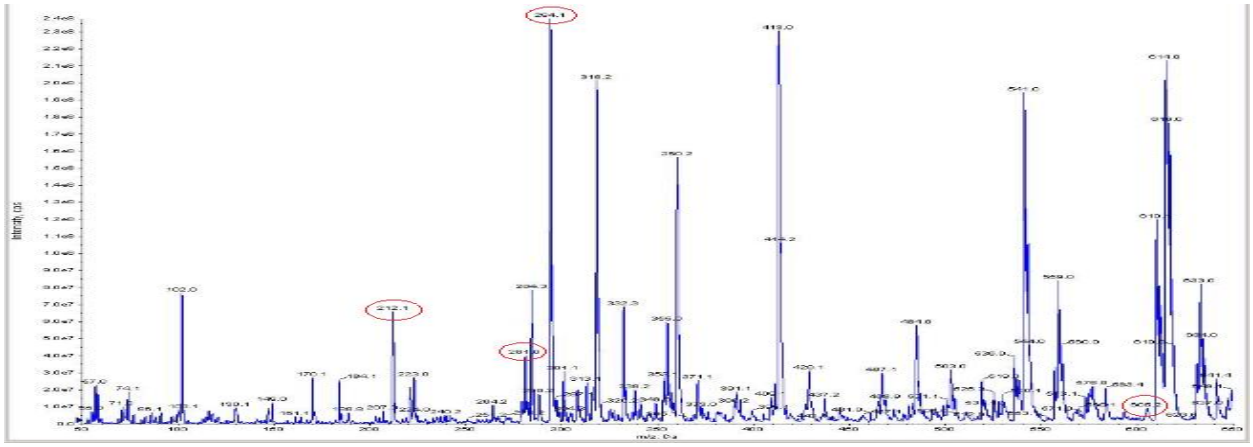

Figure 5.15: Mass spectrum of ondansetron HCl in 3.0% H_2O_2 after 3 hours.

Table 5.19: MS results for the impurities or degradants of ondansetron HCl in 3.0% hydrogen peroxide.

Time interval	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
1 hour	212.1	$4.0e^7$	211.26	Impurity-D
	281.1	$1.5e^7$	279.34	Impurity-G
	605.1	$1.0e^7$	604.77	Impurity-B
2 hours	212.1	$5.0e^7$	211.26	Impurity-D
	281.0	$2.5e^7$	279.34	Impurity-G
	605.1	$0.5e^7$	604.77	Impurity-B
3 hours	212.1	$6.0e^7$	211.26	Impurity-D
	281.0	$3.0e^7$	279.34	Impurity-G
	605.1	$1.0e^7$	604.77	Impurity-B

Table 5.20: HPLC and MS results for the impurities or degradants of ondansetron HCl in 3.0% hydrogen peroxide.

Time interval	Peak identity	RRT	Molecular weight	Nominal molecular weight	Molecular formula	Comments
1hour	Impurity-10	0.48	605.1	604.77	$C_{37}H_{32}D_6N_6O_2$	Impurity-B
	Impurity-9	0.47	281.1	279.34	$C_{17}H_{17}N_2O$	Impurity-G
	Impurity-11	1.39	-	169.1	-	Unknown-1
	Impurity-8	0.44	212.1	211.26	$C_{14}H_{13}NO$	Impurity-D
2 hours	Impurity-15	0.51	114.9	-	-	Unknown-2
	Impurity-14	0.49	142.1	604.77	$C_{37}H_{32}D_6N_6O_2$	Impurity-B
	Impurity-16	0.56	-	-	-	Unknown-3
	Impurity-13	0.47	-	279.34	$C_{17}H_{17}N_2O$	Impurity-G
	Impurity-17	1.38	-	-	-	Unknown-1
	Impurity-12	0.44	362.2	211.26	$C_{14}H_{13}NO$	Impurity-D
3 hours	Impurity-22	0.65	-	-	-	Unknown-4
	Impurity-21	0.56	605.1	604.77	$C_{37}H_{32}D_6N_6O_2$	Impurity-B
	Impurity-20	0.49	-	-	-	Unknown-3
	Impurity-19	0.47	281.0	279.34	$C_{17}H_{17}N_2O$	Impurity-G
	Impurity-23	1.38	-	-	-	Unknown-1
	Impurity-18	0.44	212.1	211.26	$C_{14}H_{13}NO$	Impurity-D

Upon evaluation of the results of potential degradants or impurities summarized in tables5.1-5.2and 5.19-5.20, it was observed that three known impurities impurity-B, impurity-D and impurity-G with relative retention time (RRT) of 0.44, 0.47, 0.49 and molecular weight of 604.77, 211.26 and 279.34 Da were produced in oxidative conditions. Four unknown degradants or impurities with RRT of 0.51, 0.56, 0.65, and 1.39 were also produced in oxidative conditions.

Photo degradation study was conducted in 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. Again, the sample was directly exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light. HPLC chromatograms and MS spectra are evaluated altogether to find out potential degradants or impurities. The HPLC chromatograms

are shown in figures 3.17-3.18 and the results are summarized in table 5.21. On the other hand, MS spectrum is shown in figure 5.16 and the relevant information is summarized in the tables 5.22-5.23.

Table 5.21: HPLC results for the impurities or degradants of ondansetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Peak name	Retention time (min)	RRT	Area	Content (%)
Photo degradation	Impurity-56	2.88	0.25	9.21	0.14

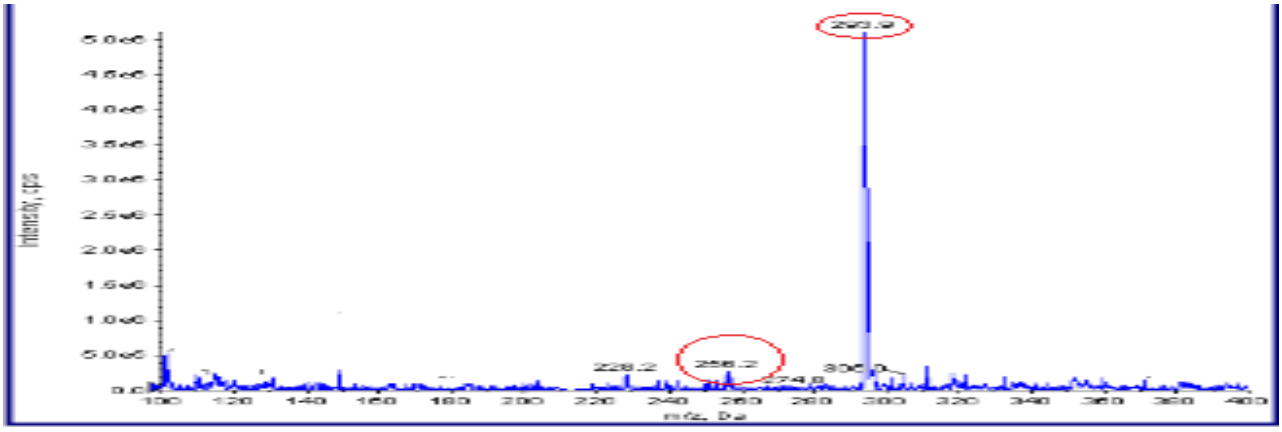

Figure 5.16: Mass spectrum of ondansetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Table 5.22: MS results for the impurities or degradants of ondansetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
Photo degradation	256.2	$0.7e^5$	255.2	Impurity-A

Table 5.23: HPLC and MS results for the impurities or degradants of ondansetron HCl exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Condition	Peak identity	RRT	Intensity (cps)	Molecular weight	Nominal molecular weight	Molecular formula	Comments
Photo degradation	Impurity-56	0.25	$0.7e^5$	256.2	255.2	$C_{16}H_{20}N_2O$	Impurity-A

After evaluation of the results of potential degradants or impurities summarized in tables 5.1-5.2and 5.22-5.23, it was observed that one known impurity, impurity-A, was produced in photo-degradation exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light with RRT of 0.25 and molecular weight of 256.2 Da.

5.3 Impurity profiling of granisetron hydrochloride

Aqueous degradation study was conducted with purified water at 60°C for 7 and 21 days. Degradation was observed after 21 days. The chromatogram is shown in figures 3.19-3.20 and the result is summarized in table 3.27. It was observed that one potential degradant or impurity was produced in aqueous condition after 21 days with RRT of 0.85.

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Degradations were observed in 0.1N, 0.5N, 1.0N and 2.0N HCl. The chromatograms are shown in figure 3.21 and the results are given in table 3.28. The RRT, area and % degradants or impurities derived from acidic conditions are summarized in the table 5.24.

Table 5.24: HPLC results for the impurities or degradants of granisetron HCl in acidic conditions at 60°C for 7 days.

Conditions	Peak identity	Retention time (min)	RRT	Area	Content (%)
0.1N HCl	Impurity-2	4.59	0.24	6.31	0.5
	Impurity-3	5.84	0.30	12.93	1.0
0.5N HCl	Impurity-4	4.66	0.24	11.48	0.62
	Impurity-5	5.84	0.30	60.63	3.4
1.0N HCl	Impurity-6	4.71	0.24	25.62	1.7
	Impurity-7	5.83	0.30	105.90	7.3
2.0N HCl	Impurity-8	4.74	0.24	43.93	1.7
	Impurity-9	5.83	0.30	246.82	9.7
	Impurity-10	11.27	0.58	45.32	1.8
	Impurity-11	23.71	1.22	47.92	1.9

Upon evaluation of the degradants or impurities information provided in table 5.24, it was observed that four different potential degradants or impurities were found in acidic conditions with RRT of 0.24, 0.30, 0.58, and 1.22.

Base degraded samples in 2.0N NaOH at 70°C and 80°C after 5 hours were brought under degradant or impurity investigation. HPLC chromatograms and MS spectra are considered together to evaluate degradants or impurities. The HPLC chromatograms are shown in figures 3.23-3.24 and the results are summarized in tables 3.31-3.32. On the other hand, MS spectra are shown in figures 5.17-5.18 and the relevant information is summarized in the tables 5.25-5.26.

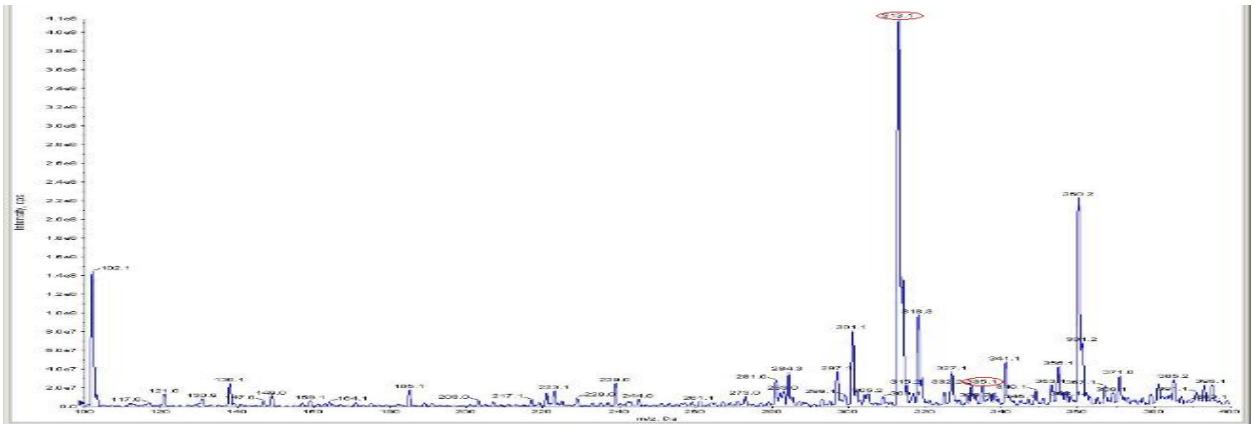

Figure 5.17: Mass spectrum of granisetron HCl in 2.0N NaOH at 70°C for 5 hours.

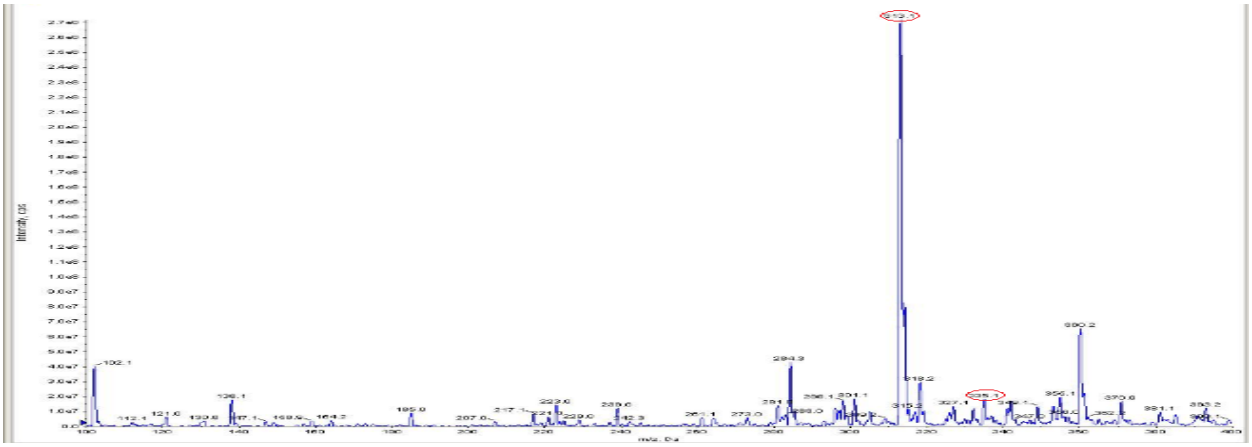

Figure 5.18: Mass spectrum of granisetron HCl in 2.0N NaOH at 80°C for 5 hours.

Table 5.25: MS results for the impurities or degradants of granisetron HCl in 2.0N NaOH for 5 hours.

Temperature	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
70°C	335.1	$2.0e^7$	334.33	Impurity-C
80°C	335.1	$2.5e^7$	334.44	Impurity-C

Table 5.26: HPLC and MS results for the impurities or degradants of granisetron HCl in 2.0N NaOH for 5 hours.

Temperature	Peak identity	RRT	Intensity (cps)	Molecular weight	Nominal molecular weight	Molecular formula	Comments
70°C	Impurity-19	0.21	$2.5e^7$	335.1	334.33	$C_{18}H_{14}N_4O$	Impurity-C
80°C	Impurity-19	0.21	$2.5e^7$	335.1	334.33	$C_{18}H_{14}N_4O$	Impurity-C

After evaluation of the results of potential degradants or impurities summarized in tables 5.3-5.4 and 5.25-5.26, it was observed that one known impurity, impurity-C, with molecular weight of 335.1 Da and formula $C_{18}H_{14}N_4O$ was found.

5.4 Impurity profiling of tropisetron hydrochloride

Aqueous degradation study was conducted with purified water at 60°C for 7 and 21 days. Degradation was observed after 21 days. The chromatogram is shown in figures 3.26-3.27 and the result is summarized in table 3.38. It is found that two potential degradants or impurities were produced in aqueous condition after 21 days with RRT of 0.35 and 0.40.

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Degradations were observed at 0.1N, 0.5N, 1.0N and 2.0N HCl. The chromatograms are shown in figure 3.28 and results are given in table 3.39. The RRT, area and % degradants or impurities derived from acidic conditions are summarized in the table 5.27.

Table 5.27: HPLC results for the impurities or degradants of tropisetron HCl in acidic conditions at 60°C for 7 days.

Conditions	Peak identity	Retention time (min)	RRT	Area	Content (%)
0.1N HCl	Impurity-3	7.2	0.24	20.3	0.30
0.5N HCl	Impurity-4	5.2	0.17	12.4	0.17
	Impurity-5	6.5	0.21	11.7	0.16
	Impurity-6	7.14	0.24	22.0	0.31
	Impurity-7	12.1	0.40	72.2	1.01
1.0N HCl	Impurity-8	5.2	0.17	7.65	0.55
	Impurity-9	6.5	0.21	9.10	0.70
	Impurity-10	7.2	0.24	7.19	0.54
	Impurity-11	12.2	0.40	9.18	0.71
2.0N HCl	Impurity-12	6.5	0.21	10.5	0.20
	Impurity-13	7.1	0.24	376.1	7.7
	Impurity -14	12.2	0.40	231.2	4.7
	Impurity-15	17.9	0.59	18.4	0.37

Upon evaluation of the degradants or impurities information provided in table 5.27, it was found that five potential degradants were observed in acidic conditions with RRT of 0.17, 0.21, 0.24, 0.40 and 0.59.

Base degraded samples in 2.0N NaOH at 60°C, 70°C and 80°C after 5 hours were analyzed to investigate degradants or impurities. HPLC chromatograms and MS spectra are considered together to find out degradants or impurities. The HPLC chromatograms are shown in figures 3.30-3.32 and the results are summarized in tables 3.41-3.43. On the other hand, MS spectra are shown in figures 3.19-3.21and the relevant information is summarized in the tables 5.28-5.29.

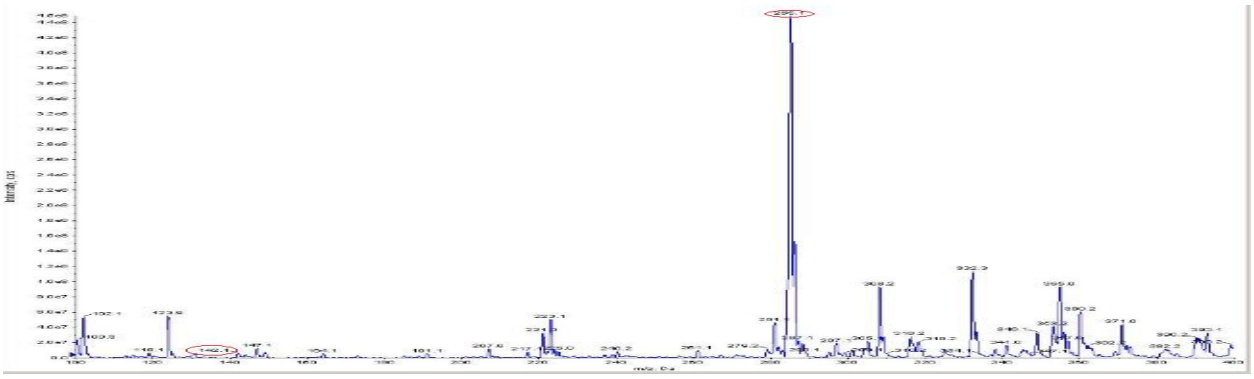

Figure 5.19: Mass spectrum of tropisetron HCl in 2.0N NaOH at 60°C for 5 hours.

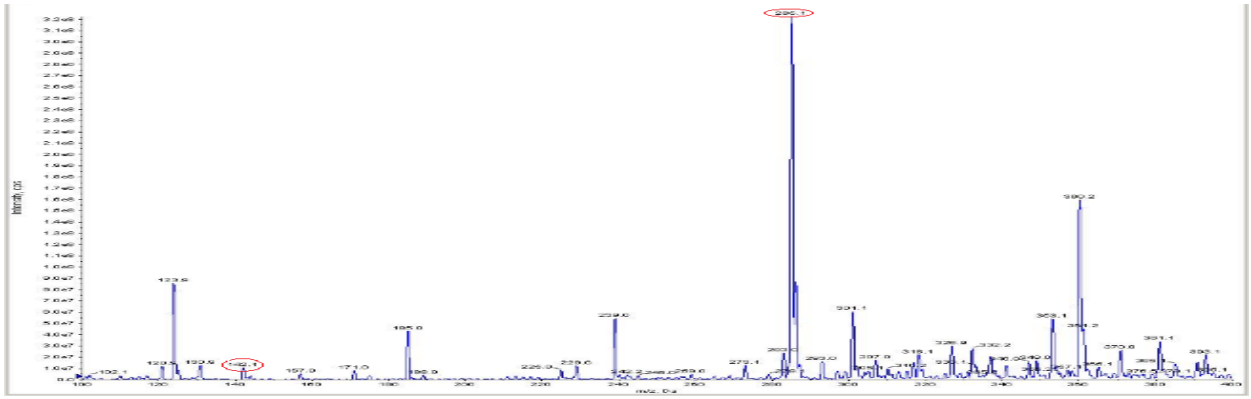

Figure 5.20: Mass spectrum of tropisetron HCl in 2.0N NaOH at 70°C for 5 hours.

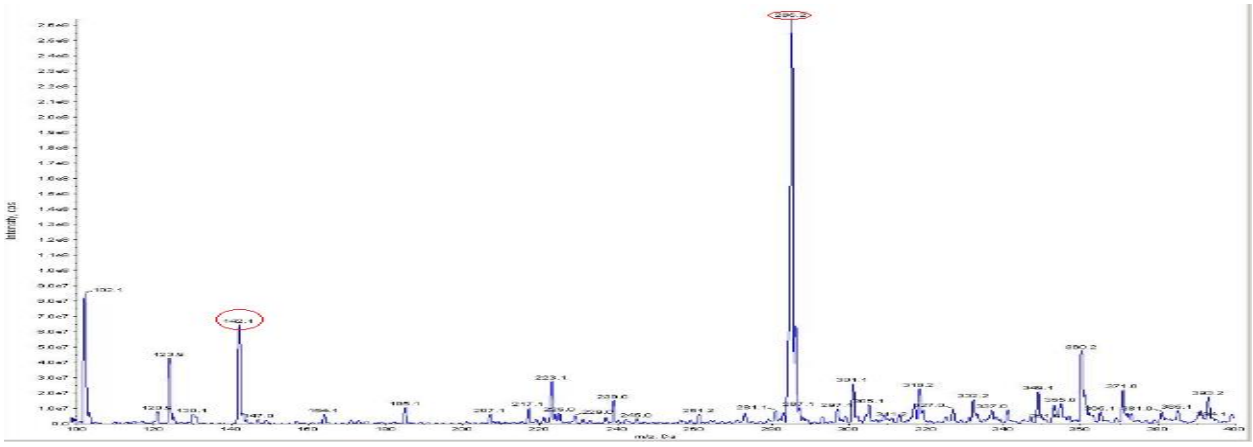

Figure 5.21: Mass spectrum of tropisetron HCl in 2.0N NaOH at 80°C for 5 hours.

Table 5.28: MS results for the impurities or degradants of tropisetron HCl in 2.0N NaOH for 5 hours.

Temperature	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
60°C	142.1	$1.5e^7$	141.2	Impurity-A
70°C	142.1	$1.8e^7$	141.2	Impurity-A
80°C	142.1	$7.0e^7$	141.2	Impurity-A

Table 5.29: HPLC and MS results for the impurities or degradants of tropisetron HCl in 2.0N NaOH for 5 hours.

Temperature	Peak identity	RRT	Area	Intensity (cps)	Molecular weight	Nominal molecular weight	Molecular formula	Comments
60°C	Impurity-27	0.10	$1.5e^7$	142.1	141.2	$C_8H_{15}NO$	Impurity-A	Impurity-27
70°C	Impurity-32	0.10	$1.8e^7$	142.1	141.2	$C_8H_{15}NO$	Impurity-A	Impurity-32
80°C	Impurity-27	0.10	$1.5e^7$	142.1	141.2	$C_8H_{15}NO$	Impurity-A	Impurity-27

After evaluation of the results of potential degradants or impurities summarized in table 5.5 and 5.28-5.29, it was observed that two known impurities were growing in basic conditions with RRT of 0.51, 0.65 and molecular weight of 82.1 and 211.26 Da.

Oxidative degradation in 3.0% hydrogen peroxide at dark place for 1, 2 and 3 hours were considered to investigate potential degradants or impurities. HPLC chromatograms and MS spectra are considered together to find out degradants or impurities. The HPLC chromatograms are shown in figures 3.33-3.34 and the results are summarized in table 5.30.

Table 5.30: HPLC results for the impurities or degradants of tropisetron HCl in 3.0% hydrogen peroxide.

Time interval	Peak identity	Retention time (min)	RRT	Area	Content (%)
1 hour	Impurity-48	6.7	0.24	20.83	0.16
	Impurity-49	10.43	0.38	847.1	6.4
	Impurity-50	11.7	0.43	63.5	0.48
2 hours	Impurity-51	6.7	0.24	7.73	0.10
	Impurity-52	10.5	0.38	580.1	7.4
	Impurity-53	11.8	0.43	42.4	0.54
3 hours	Impurity-54	6.3	0.23	23.3	0.31
	Impurity-55	6.7	0.24	21.1	0.28
	Impurity-56	10.4	0.38	761.8	10.2
	Impurity-57	11.7	0.43	55.6	0.74

Upon evaluation of the results of potential degradants or impurities summarized in table 5.5 and 5.30, it was observed that four different potential degradants or impurities were derived in oxidative conditions with RRT of 0.23, 0.24, 0.38 and 0.43.

5.5 Impurity profiling of palonosetron hydrochloride

Aqueous degradation study was conducted with purified water at 60°C for 7 and 21 days. Under these stressed conditions, no degradation was found. The results are summarized in tables 3.48-3.49.

Acid degradation study was conducted with four different strengths of hydrochloric acid at 60°C for 7 days. Degradations were found at 0.1N, 0.5N, 1.0N and 2.0N HCl. The chromatograms are shown in figure 3.36 and results are given in table 3.50. The RRT, area and % degradants or impurities derived from acidic conditions are summarized in the table 5.31.

Table 5.31: HPLC results for the impurities or degradants of palonosetron HCl in acidic conditions at 60°C for 7 days.

Conditions	Peak identity	Retention time (min)	RRT	Area	Content (%)
0.1N HCl	Impurity-1	4.6	0.13	9.23	0.16
0.5N HCl	Impurity-2	4.7	0.13	22.02	0.38
1.0N HCl	Impurity-3	4.7	0.13	30.25	0.52
2.0N HCl	Impurity-4	4.8	0.13	58.8	1.01

After evaluation of the degradants or impurities information summarized in table 5.31. It was observed that a potential degradant was evident in each acidic condition with RRT of 0.13.

Base degradation study was conducted with four different strengths of sodium hydroxide at 60°C for 7 days. The chromatograms are shown in figure 3.30 and results are given in table 3.51. The RRT, area and % degradants or impurities derived from basic conditions are summarized in the table 5.32.

Table 5.32: HPLC results for the impurities or degradants of palonosetron HCl in basic conditions at 60°C for 7 days.

Conditions	Peak identity	Retention time (min)	RRT	Area	Content (%)
0.1N NaOH	Impurity-5	4.7	0.13	22.4	0.33
0.5N NaOH	Impurity-6	4.9	0.13	169.7	2.5
1.0N NaOH	Impurity-7	4.7	0.13	264.7	3.9
2.0N NaOH	Impurity-8	4.8	0.13	665.2	9.8

Upon evaluation of the degradants or impurities information provided in table 5.32, it was observed that a potential degradant was evident in each basic condition with RRT of 0.13.

Oxidative degradation samples in 3.0% hydrogen peroxide at dark place for 1 and 2 hours were brought under degradant or impurity investigation. MS spectra were evaluated to find out

degradants or impurities. MS spectra are shown in figures 5.22-5.23 and the relevant information is summarized in the table 5.33.

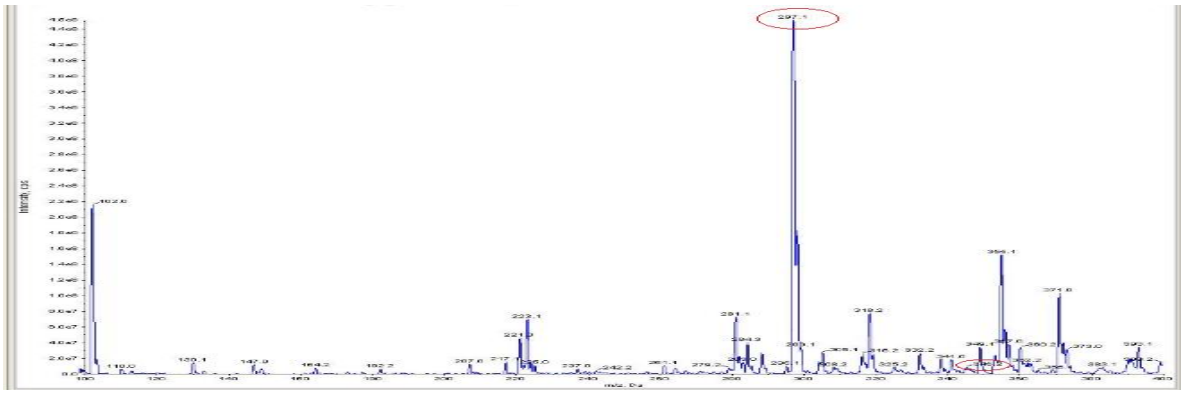

Figure 5.22: Mass spectrum of palonosetron HCl in 3.0% H_2O_2 after 1 hour.

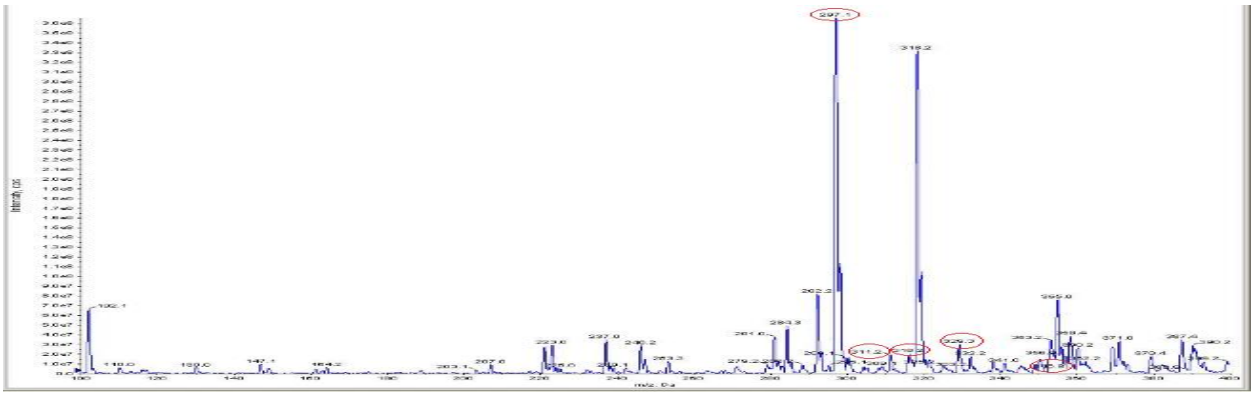

Figure 5.23: Mass spectrum of palonosetron HCl in 3.0% H_2O_2 after 2 hours.

Table 5.33: MS results for the impurities or degradants of palonosetron HCl in 3.0% hydrogen peroxide.

Time interval	Molecular weight	Intensity (cps)	Nominal molecular weight	Monograph name
1 hour	345.2	$1.5e^7$	344.4	Impurity-3
2 hours	311.2	$2.5e^7$	310.4	Impurity-B
	316.2	$2.8e^7$	314.4	Unknown
	329.3	$4.0e^7$	328.4	Unknown

After evaluation of the results of potential degradants or impurities summarized in table 5.33. It was evident that one known degradant with molecular weight of 310.4 Da and three unknown degradants or impurities with molecular weight of 314.4, 328.4 and 344.4 Da were found in oxidative conditions.

Chapter-6

Summary and Conclusion

The requirements of a robust pharmaceutical formulation are fulfilled by the complete information of chemical instability of an active pharmaceutical API. Information of 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 are also responsible to produce known and unknown impurities. So precautions should be taken to develop a robust formulation of ramosetron hydrochloride considering the derived information.

Ondansetron hydrochloride produced degradants with aqueous hydrolysis, base hydrolysis, oxidation and photolysis. Among these stressed conditions, base hydrolysis and oxidative degradation were able to degrade ondansetron hydrochloride more drastically. So, base hydrolysis and oxidative degradation are the most sensitive degradation pathways for ondansetron hydrochloride.

Granisetron hydrochloride is susceptible to the stressed condition of aqueous for long time exposure, acidic, basic and oxidative. It is not light sensitive. Among these stressed conditions, base hydrolysis and oxidative degradation were able to degrade granisetron hydrochloride more drastically. So, base hydrolysis and oxidative degradation are the most sensitive degradation pathways for granisetron hydrochloride.

Tropisetron hydrochloride was evident to produce degradants in aqueous for long time exposure, acidic, basic and oxidation conditions. It is not light sensitive. Among these stressed conditions, base hydrolysis and oxidative degradation were able to degrade tropisetron hydrochloride more drastically. So, base hydrolysis and oxidative degradation are the most sensitive degradation pathways for tropisetron hydrochloride.

Palonosetron hydrochloride is more stable than ramosetron, ondansetron, granisetron and tropisetron. Palonosetron produced significant potential degradants with acid degradation, base degradation, oxidation and photolysis. Among these stressed conditions, base and oxidative degradation were able to degrade palonosetron hydrochloride more drastically.

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, ondansetron, granisetron, tropisetron and palonosetron hydrochloride will provide all prerequisite information from studies of stress degradation, degradation kinetics and impurity profiling. However, we could not identify the impurities produced from the stressed conditions on ramosetron, ondansetron, granisetron, tropisetron and palonosetron hydrochloride. Further extensive studies are underway to isolate and characterize the degradants.

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 an expiration date and stability condition over the period of shelf-life. The activation energy of ramosetron HCl, ondansetron HCl, granisetron HCl and tropisetron HCl were calculated as 10.05 $kcalmol^{-1}$, 7.57 $kcalmol^{-1}$,16.98 $kcalmol^{-1}$ and 16.86 $kcalmol^{-1}$, respectively.

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 will help to set retest period of ramosetron, ondansetron, granisetron and tropisetron hydrochloride and also to predict shelf-life period of different dosage form of these APIs, specially lyophilized dosage form.

Two issues of fundamental importance in drug therapy are efficacy and safety. The safety of drug therapy is determined by two main factors. One is the pharmacological-toxicological profile of the drug substance, i.e. the relation of the beneficial and adverse effects of the drug materials to the human. The second is adverse effects caused by the impurities in the bulk drug material and its dosage forms. After explicit evaluation of HPLC chromatograms and mass spectra of

different stressed samples, it was observed that both known and unknown impurities and degradants have growing tendency to develop at shelf-life storage condition.

In acidic conditions, ramosetron HCl produced five potential degradants and six potentials degradants in basic conditions. Potent degradants of ramosetron HCl with molecular weight of 113.9, 141.1 and 361.2 Da were evident in oxidative conditions. ramosetron hydrochloride was found to be light sensitive and produced potent degradants with molecular weight of 120.3, 147.9, 185.1 and 412.3 Da when exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Ondansetron hydrochloride was observed to produce potential degradant in aqueous condition. However, ondansetron HCl was stable in acidic condition. In basic conditions, ondansetron HCl increased the content of two growing impurities with molecular weight of 82.1 and 211.26 Da. Three growing impurities of ondansetron HCl with molecular weight of 604.77, 211.26 and 279.34 Da were evident in oxidative conditions. On the other hand, four potent degradants of ondansetron HCl are also observed in oxidative conditions. Ondansetron hydrochloride is light sensitive and was evident of one growing impurity with molecular weight of 256.2 Da when exposed to 3.6 million lux fluorescence light and 600 watts hour/m^2 UV light.

Granisetron hydrochloride was evident to produce one degradant in aqueous condition. In acidic conditions, granisetron HCl produced four potent degradants. In basic conditions, granisetron hydrochloride was able to produce one growing impurity with molecular weight of 335.1 Da. Granisetron hydrochloride showed no photosensitivity.

Tropisetron hydrochloride produced two degradants in aqueous condition. In acidic conditions, tropisetron HCl produced five potent degradants. In basic conditions, two growing impurities molecular weight of 82.1 and 211.26 Da were seen for tropisetron HCl. Four potent degradants of tropisetron HCl were produced in oxidative conditions.

Palonosetron hydrochloride was comparatively more stable in aqueous conditions than ramosetron, ondansetron, granisetron and tropisetron HCl. In acidic conditions, palonosetron

HCl produced one potent degradant with relative retention time (RRT) of 0.13. In basic conditions, palonosetron HCl also produced similar potent degradants produced in acidic condition with relative retention time (RRT) of 0.13. One known growing impurity with molecular weight of 310.4 Da and three unknown potent degradats with molecular weight of 314.4, 328.4 and 344.4 Da were evident for palonosetron HCl in oxidative conditions.

During formulation development, process validation and stability sample testing, more attention should be deserved to control these growing impurities or potential degradants. However, it was possible to identify the potential degradants produced from the stressed conditions applied on ramosetron, ondansetron, granisetron, tropisetron and palonosetron hydrochloride. Because of this limited availability which prevented structural characterization by spectroscopic studies notable high field NMR. Further extensive studies are underway to isolate and characterize the degradants to find out the level of toxicity and genotoxicity for the sake of safety, purity and efficacy of the drug substance and drug product.

REFERENCES

Steven, W.B., Kaen, M. A. and Robert, A. R. 2011. Pharmaceutical Stress Testing: Predicting Drug Degradation. 2nd ed., Informa Healthcare, UK.

John, P. 2002. Kinetics and Product Stability. Harcourt, p.101.

Keith, G., and Rolland, I. P. 2002. Chemical Kinetics and Drug Stability. Marcel Dekker, New York, p.146.

Patrick, J.S., and Yashveer, S. 2010. Chemical Kinetics and Stability. Wolters Kluwer, New Delhi, p.318.

Luis, A., Sebastiao, F., and Hugh, B. 2007. Chemical Kinetics. Amsterdam, Boston.

Richard, J.S., and Michael, L.W. 2007. Analysis of Drug Impurities, 1st ed., Blackwell, UK.

Harry, G.B. 2002. Analytical Profiles of Drug Substances and Excipients, Volume 29. Academic Press, New Jersey.

Birajdar, L.B., and Damle, M.C. 2015. Base degradation monitoring of dolasetronmesylate by UV spectrophotometric method. *Int. J. Pharm. Res. Scholar*. **4**, 462-468.

Mushabbar, B., Praveena, B., Srinidhi, M., and Rahaman, S. K. A. 2013. Method development and validation of ondansetron in bulk and pharmaceutical dosage form by stability-indicating RP-HPLC method. *Int. J. Pharm. Tech. Res.* **5**, 86-98.

Patel, P.J., Shah, D.A., Mehta, F.A., and Chhalotiya, U.K. 2015. Development of liquid chromatographic method for estimation of ondansetron and ranitidine in combined dosage form. *Austin Chromatogr.* **2**, 1-6.

Singh, P.K., and Subas, C.D. 2013. Development and validation of a stability indicating RP-HPLC method for determination of ondansetron in orally disintegrating films. *Int. J. Res. Pharm. Sci.* **3**, 57-66.

Effat, S., Zahra, K., Shahrooz, S., Nazanin, S.R., Farhad, A., and Massoud, A. 2011. Development and validation of a stability indicating HPLC method for determination of granisetron. *Chinese Chem. Soc.* **58**, 443-449.

Bhalerao, A.V., Shirolkar, S.V., and Chitlange, S.S. 2013. Analysis of stability of granisetron hydrochloride in nasal formulations by stability-indicating RP-HPLC method. *Res. J. Pharm. Biol. Chem. Sci*. **4**, 653-663.

Mokhtar, M., Hamed, E.F., Ismail, H., and Ehab, E. 2013. Stability-indicating HPLC-DAD method for the determination of granisetron hydrochloride in its pharmaceutical preparations. *J. Appl. Pharm. Sci.* **3**, 189-202.

Vishnu, M. M., Krishnaiah, C., Kodithyala, J., Katkam, S., Mukkanti, K., Ramesh, K., and Gautam, S. 2013. Enantioseparation of palonosetron hydrochloride and its related enantiomeric impurities by computer simulation and validation. *Am. J. Anal. Chem.* **2**, 437-446.

Benjamin, T.R., and Rambabu, C. 2013. Stress degradation studies and validation method for quantification of aprepitent in formulations by using RP-HPLC. *Int. J. Chemtech Res.* **5**, 1462-1468.

Fujii, Y., Saitoh, Y., Tanaka, H. and Toyooka, *H. 2000.* Ramosetron for preventing postoperative nausea and vomiting in women undergoing gynecological surgery. *Anesth. Analg.* **90**, 472-475.

Ashwini, S. S., Sachin, A. P., and Harinath, N. M. 2012. Forced degradation study of strontium ranelate (anti-osteoporetic drug). *Int. J. Pharm. Sci. Rev. Res.* **12**, 22-26.

Zarana, M. P., Darshil, B. S., and Dilip, G. M. 2014. Development and validation of stability indicating HPLC method for estimation of ramosetronHCl. *World. J. Pharm. Res.* **3**, 4527-4535.

Marcus, B., Ian, R. B., Steven, V. L., and Nikzad, N. 2011. An overview of the key routes to the best selling 5-membered ring heterocyclic pharmaceuticals. *J. Org. Chem.* **7**, 442-495.

Elsadig, H. K. and Adam, S. 2014. Stress degradation studies on lisinoprildihydrate using modified reverse phase high performance liquid chromatography. *Am. J. Anal. Chem.* **5**, 316-322.

Srinivas, P. and Sneha, Y. 2014. Stability indicating forced degradation RP-HPLC method development and validation of olmesartanmedoxomil. *Int. J. Pharm. Sci. Res.* **5**, 2848-2855.

Brendan, A., and Whelan, J. 1994. Synthesis, structural and biological studies of potential 5-HT_3 receptor antagonists. *Dublin City University.* **1**,1-260.

Effat, S., Tannaz, N., Farnaz, R. L. and Parinaz, K. 2014. Validating a stability indicating HPLC method for kinetic study of ondansetron degradation in acidic, basic and oxidative conditions. *Res. J. Pharm. Biol. Chem. Sci.* **5**, 52-62.

Jiang, H. Y., Rex, P. and Rebecca, S. 2001. Ondansetron: A selective 5-HT_3 receptor antagonist and its applications in CNS-related disorders. *CNS Drug Reviews.* **7**, 199-213.

Corina, A., Andreea, V. and Crina, M. M. 2011. Development and validation of a new capillary zone electrophoresis method for the assay of ondansetron. *Farmacia*. **59**, 34-43.

Gary, R., Morrow, M. S., Jane, T., Hickok, M. D., Rosenthal, M. D. and Susan, N. 1995. Progress in reducing nausea and emesis. *University of Rochester Cancer Center, Rochester*. **76**, 343-357.

Brigas, F., Sautou, M. V., Normand, B., Geneve, S. and Chopineau, J. 1998. Compatibility of tropisetron with glass and plastics. Stability under different storage conditions. *J. Pharm. Pharmacol.* **50**, 407-411.

Robert, V. H. 2004. Organic Chemistry: An Intermediate Text, 2nd ed., John Wiley & Sons, New Mexico.

Fumio, T., and Roger, B. 2004. Separations and Reactions in Organic Supramolecular Chemistry: Perspectives in Supramolecular, Volume 8, John Wiley & Sons, USA.

Chung, C. C., Herman, L., Lee, Y. C., and Xue, M. Z. 2004. Analytical method validation and instrument performance verification, 2nd ed., John Wiley & Sons, New Jersey.

David, M.B. 2006. Validating Chromatographic Methods: A Practical Guide. 2nd ed., John Wiley & Sons, New Jersey.

Campo, V.L., and Carvalho, I. 2007. Hypolipemic statins and new therapeutically trends. Quim, **30**, p.425.

Christopher, J., and Bates. 1999. Analysisof Fat and Water soluble vitamins: Encyclopedia of Analytical Chemistry. **50**, 1027-1033.

Skoog, D.A., West, D.M., and Holler, F. J. 1998. Fundamentals of Analytical Chemistry.7th ed., Saunders College Publishing.

Anderson, D., Phillips, B.J., Eduards, A.J., Ayesh, R., and Butterworth, K.R. 1997. The effect of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with low or high cholesterol levels. Environ Mol Mutagens, **30**, 161-174.

Johnson, C. S. 1999. Biomarkers for establishing a tolerable upper intake level for vitamin C. *Nutr. Rev*. **57**, 71-77.

Gomes, F.P. 1995. Validation of methods for analysis of statins drugs- M.Sc. dissertation, graduate program in pharmaceuticals and drugs. University of São Paulo, São Paulo, Brazil.

Gyeong, N.S., and Taek, S.Y. 2009. Crystal forms of atorvastatin. *Arch. Pharm. Res.* **32**, 933.

Moser, U., and Bendich, A. 1990. Vitamin C: Handbook of Vitamins edited byMachlin, L.J., Marcel, New York, chapter 5.

Bratu, M.M., Birghilã, S., Bucur, L.A., Magearu, V., Dobrina, S., andMatei, N. 2003. The 1st International Symposium: New resources in Pharmaceutical Industry. Romania, Constantza, p.74.

Moffat, A.C., Osselton, M.D., and Widdop, B. 2004. Clarke's analysis of drugs and poisons- In Pharmaceuticals, Body Fluids and Postmortem Material, 3rd ed., Pharmaceutical press, London, UK.

Matei, N., Birghilã, S., Dobrina, S., and Capota, P. 2003. The 13th International Symposium: Spectroscopy in Theory and Practice, Nova Gorica, Slovenia, p.33.

Oliveira, M.A., Yoshida, M.I., Gomes, E.C.L., Mussel, W.N., Soares, C.D.V., and Pianetti, G.A. 2011. Thermal analysis applied to the drug and pharmaceutical formulations in pharmaceutical industry, Quim, **34**, 1224-1230.

Oliveira, M.A., Yoshida, M.I., Gomes, E.C.L., and Soares, C.D.V. 2011. Thermal behavior study and decomposition kinetics of amiodarone hydrochloride under isothermal conditions. *Drug Dev. Ind. Pharm.* **37**, 638-647.

Shete, G., Puri, V., Kumar, L., and Bansal, A.K. 2010. Solid state characterization of commercial crystalline and amorphous atorvastatin calcium samples. *Am. Assoc. Pharm. Sci.* **11**, 598.

Silva, K.E.R., Alves, L.D.S., Soares, M.F.R., Passos, R.C.S., Faria, A.R., and Rolim, N.P.J. 2009. Modelos de avaliação da estabilidade de fármacos e medicamentosparaa indústriafarmacêutica. *J. Basic Appl. Pharm. Sci.* **30**, 1-8.

Bakshi, M., and Singh, S. 2002. Development of validated stability-indicating assay methods—critical reviews. *J. Pharm. Biomed. Anal.* **28**, 1011-1034.

Capacio, D.R., Byers, C.E., Jackson, T.K., and Matthews, R.L. 1993. HPLC method for the determination of granisetron in guinea pig plasma. *J. Anal. Toxicol.* **17**, 151-155.

Carmichael, J., Cantwell, B.M., Edwards, C.M., Zussman, B.D., Thompson, S.R., and Harris, A.L. 1989. *Cancer Chemother. Pharmacol*. **24**, 45-49.

Cupissol, D.R., Serrou, B., and Coubel, M. 1990. A double-blind, randomised, crossover comparison of granisetron and ondansetron in 5-day fractionated chemotherapy: Assessment of efficacy, safety and patient preference. *Eur. J. Cancer.* **26,** 23.

Dewani, A.P., Barik, B.B., Chipade, V.D., Bakal, R.L., Chandewar, A.V., and Kanungo, S.K. 2012. RP-HPLC-DAD method for the determination of phenylepherine, paracetamol, caffeine and chlorpheniramine in bulk and marketed formulation. *Ara. J. Chem.* **31**, 301.

Dipal, P., and Hasumati, R. 2012. Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by spectroscopic method. *Int. J. Pham. Sci. Res.* **3(10)**, 3766-3776.

Dragica, Z., and Trajce, S. 2003. Development of an HPLC method for the determination of ranitidine and cimetidine in human plasma following SPE. *J. Pharm. Biomed. Anal.* **33**, 165-173.

Gillespie, T.A., Eckstein, J.A., and Nardella, P. 1993. Determination of dolasetron and its reduced metabolite in human plasma by GC-MS and LC. *J. Pharm. Biomed. Anal.* **11**, 955-962.

Gopala, S.T., Nagaraju, K., and Lakshmana, R.A. 2011. RP-HPLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in pharmaceutical dosage form. *Int. J. Drug Dev. Res.* **3(4)**, 362-368.

Haque, A., Shahriar, M., Parvin, N., and Islam, S.M.A. 2011. Validated RP-HPLC method for estimation of ranitidine hydrochloride, domperidone and naproxen in solid dosage form. *Asian J. Pharm. Anal.* **1**, 59-63.

Hewala, I., Bedair, M., and Shousha, S. 2012. New concept for HPTLC peak purity assessment and identification of drugs in multi-component mixtures. *Talanta.* **88**, 623-627.

Hewala, I., El-Fatatry, H., Emam, E., and Mabrouk, M. 2011. Development and application of a validated stability-indicating high performance liquid chromatographic method using photodiode array detection for simultaneous determination of granisetron, methylparaben, propylparaben, sodium benzoate and their main degradation products in oral pharmaceutical preparations. *J. AOAC. Int.* **14**, 1447-1453.

Hewala, I., El-Fatatry, H., Emam, E., and Mabrouk, M. 2010. Development and application of a validated stability-indicating HPLC method for simultaneous determination of granisetron hydrochloride, benzyl alcohol and their main degradation products in parenteral dosage forms. *Talanta.* **82**, 184-189.

Hourcade, F., Sautou, M.V., Normand, B., Laugier, M., Picq, F., and Chopineau, J. 1997. Compatibility of granisetron towards glass and plastics and its stability under various storage conditions. *Int. J. Pharm.* **154**, 95-102.

Hu, Y., Chen, S., Chen, J., Liu, G., Chen, B., and Yao, S. 2012. Optimization of sample pretreatment methods for simultaneous determination of dolasetron and hydrodolasetron in human plasma by HPLC-ESI-MS. *J. Chem. Sci.* **50**, 785-91.

Huang, C.T., Chen, C.F., and Tsai, T.H. 1999. Biliary excretion of granisetron by microdialysis coupled to HPLC with fluorescence detection. *J. Liq. Chromatogr. Relat. Technol.* **22**, 2815-2823.

Huang, C. T., Chen, K.C., Chen, C. F., and Tsai, T. H. 1998. Simultaneous measurement of blood and brain microdialysates of granisetron in rat by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B.* **716**, 251-255.

Huebert, N.D., and Schwartz, J.J. 1996. Simultaneous measurement of dolasetron and its major metabolite, MDL 74,156, in human plasma/urine. *J . Chromatogr. B.* **685(2)**, 291-297.

Jiang, Y., Lin, M., Fan, G., Chen, Y., Li, Z., Zhao, W., Wu, Y., and Hu, J. 2006. Rapid determination of granisetron in human plasma by liquid chromatography coupled to tandem mass spectrometry and its application to bioequivalence study. *J. Pharm. Biomed. Anal.* **42**, 464-466.

Johnson, C.E., Wagner, D.S., and Bussard, W.E. 2003. Stability of dolasetronin two oral liquid vehicles. *Am. J. Health-Syst. Pharm.* **60**, 2242-2244.

Kirchner, V., Aapro, M., Terrey, J. P., and Alberto, P. 1997. A double-blind crossover study comparing prophylactic intravenous granisetron alone or in combination with dexamethasone as antiemetic treatment in controlling nausea and vomiting associated with chemotherapy. *Eur. J. Cancer.* **33**, 1605-1610.

Kudoh, S., Sato, T., Okada, H., Kumakura, H., and Nakamura, H. 1994. Simultaneous determination of granisetron and 7-hydroxygranisetron in human plasma by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B.* **660**, 205-210.

Lerman, K., and Sikich, N. 1996. Pharmacokinetics of the active metabolite (MDL 74,156) of dolasetronmesylate after oral or intravenous administration to anesthetized children. *Int. J. Clin. Pharmacol. Ther.* **60**, 485-92.

Lokhande, S.R., Mhetre, S.M., Pekamwar, S.S., and Kalyankar, T.M. 2012. Development and validation of reverse phase HPLC method for simultaneous estimation of dicyclomine hydrochloride, mefenamic acid and paracetamol in tablet dosage form. *World J. Pharm. Pharmaceut. Sci.* **1(3)**, 968-980.

Mcelvain, J., Vandiver, V.J., and Eichmeier, L.S. 1997. Validation of a reversed-phase HPLC method for directly quantifying the enantiomers of MDL 74, 156, the primary metabolite of dolasetronmesylate in human plasma. *J. Pharm. Biomed. Anal.* **15(4)**, 513-21.

McElvain, J.S., Vandiver, V.J., and Eichmeier, L.S. 1997. Validation of a reversed-phase HPLC method for directly quantifying the enantiomers of MDL 74 156, the primary metabolite of dolasetronmesylate, in human plasma. *J. Pharm. Biomed. Anal.* **15**, 513-521.

McEvoy, G.K. 2003. AHFS drug information. Bethesda, MD: American Society of Health-System Pharmacists, p.2763-2764.

Meyyanathan, M.N., Venkatesh, D.N., Krishnaveni, N., Babu, B., Jeyaprakash, M.R., and Raja, R.B. 2012. RP-HPLC method for simultaneous estimation of ondansetron and ranitidine in Pharmaceutical formulation**.** *Int. J. Health Allied. Sci.* **1**, 129-132.

Miller, R.C., Galvan, M., Gittos, M.W., Giersbergen, P., Moser, P.C., and Fozard, J.R. 1993. Pharmacological properties of dolasetron, a potent and selective antagonist at 5-HT3 receptors. *Drug Dev. Res.* **28**, 84-93.

Mishra, A.N., and Rana, A.C. 2009. UV Spectrophotometric determination of ranitidine hydrochloride in pure and pharmaceutical formulation. *Int. J. Chem. Sci.* **3**, 2208-2210.

Mujtaba, A., Kohli, K., Ali, J., and Baboota, S. 2011. Development of HPTLC method for the estimation of ondansetronhydrochloride in bulk drug and sublingual tablets. *Drug Testing and Analysis.* **5**, 122-125.

Naresh, M., Ganesh, T., Biswal, A., and Reddy, N. 2013. RP-HPLC method development and validation studies of ranitidine HCl and domperidone. *Int. J. Chem. Natural Sci.* **3**, 25-28.

Panchal, V.U., and Chauhan, P.S. 2013. Simultaneous development of ondansetronHCl and pantoprazole sodium in their bulk dosage form by ratio derivative spectroscopy. *Unique J. Pharm. Bio. Sci.* **1**, 37-41.

Panjagala, S., Shanmuga, S.P., Sudarshan, R.P., and Chandra, M.G. Validated HPLC method for simultaneous estimation of zinc carnosine and aceclofenac in bulk and tablet dosage form. *Int. J. Pharm. Ind. Res.* **1(2)**, 109-114.

Patel, N.M., Fursule, R.A., Shirkhedkar, A.A., and Talele, G.S. 2006. Simultaneous estimation of ranitidine hydrochloride and ondansetron hydrochloride by reverse phase high performance liquid chromatography. *Asian J. Chem.* **18**, 2691-2694.

Patel, S. 2014. Derivative spectrophotometric method for simultaneous determination of ondansetron and omeprazole in combined dosage form. *Int. J. Chem. Technol. Appl.* **3**, 9-17.

Pattan, S.R., Jamdar, S.G., Godge, R.K., Dighe, N.S., Daithankar, A.V., Nirmal, S.A., and Pai, M.G. 2009. RP- HPLC method for simultaneous estimation of paracetamol and etoricoxib from bulk and tablets. *J. Chem. Pharm. Res.* **1(1)**, 329-335.

Pezikoglo, H., Fileli, A., Vasilakos, D., Karastergiou, P., Tanidis, A., and Halvatzoulis, E. 2004. The combination of ondansetron and ranitidine in the prevention of nausea and vomiting, intra operatively for patients under spinal anesthesia. *Regional Anesthesia & Pain Medicine.* **29**, p.51.

Pinguet, F., Bressolle, F., Martel, P., Salabert, D., and Astre, C. 1996. High-performance liquid chromatographic determination of granisetron in human plasma. *J. Chromatogr. Biomd.* **675**, 99-105.

Putta, R.K., Udaya, K.T., Jitendra, K.,and Salahyddin. 2012. HPLC analytical method development and validation studies of ondansetron and rabeprazole. *J. Biomed. Pharm. Res.* **1**, 134-143.

Ranjith, A., Sarin, K.K., and Arumughan, C. 2008. Simultaneous estimation of phenolic acids in sea buckthorn using RP-HPLC with DAD. *J. Pharm. Biomed. Anal.* **47**, 31-38.

Rao, K., Jayachandran, E., Usha, M., Gopinath, G., Venkata, R.D., and Srinivasa, R.D. 2012. UV Spectrophotometric method for determination of ondansetron hydrochloride in pure and formulation. *Int. J. Pharm. Sci.* **4**, 125-129.

RaviKumar, P., MuraliKrishna, M., Bhanuprakash, P., AnilKumar, B., and Madhusudhan, P. 2006. Derivative spectrophotometric estimation ofondansetron and paracetamol. *Eurassian J. Chem.* **3**, 134-136.

Salve, P., Gharge, D., Kirtawade, R., Dhabale, P., and Burade, K. 2010. Simple validated spectroscopic method for estimation of ranitidine from tablet formulation. *Int. J. Pharm. Technol. Res.* **2**, 2071-2074.

Sanwald, P., and Huebert, N.D. 1994. Simultaneous measurement of the major metabolites of dolasetronmesylate in human urine using solid-phase extraction and high-performance liquid chromatography. *J. Chromatogr. Biomed. Sci. Appl*. **661(1)**, 101-107.

Shaikh, K.A., Patil, S.D., and Devkhile, A.B. 2008. Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form. *J. Pharm. Biomed. Anal.* **48**, 1481-1484.

Sharma, H.L., and Sharma, K.K. 2007. Principles of pharmacology, 2nd ed., Paras medical publisher, p.368.

Simonovska, B., Prosek, M., Vovk, I., and Jelen, Z.A.1998. High-performance thin-layer chromatographic separation of ranitidine hydrochloride and two related compounds. *J. Chrom. Biomed. Sci. App.* **715**, 425-430.

Singhvi, I., and Pillali, S. 2007. Spectrophotometric simultaneous estimation of ranitidine hydrochloride and ondansetron hydrochloride from tablet formulation. *Ind. J. Pharm. Sci.* **69**, 601-604.

Tripathi, K.D. 2008. Pharmacology, 6th ed., Jaypee Brothers Medical Publishers Pvt. Ltd., p.632-647.

Trissel, L.A., and Flora, K.P. 1988. Stability studies: five years later. *Am. J. Hosp. Pharm.* **45**, 1569-1571.

Trissel, L.A. 1983. Avoiding common flaws in stability and compatibility studies of injectable drugs. *Am. J. Hosp. Pharm.* **40**, 1159-60.

Upward, J.W., Arnold, B.D., Link, C., Pierce, D.M., Allen, A., and Tasker, T.C. 1990. *Eur. J. Cancer*. **26**, S12-S15.

Wada, I., Satoh, M., Takeda, T., Nakabayashi, T., Honma, T., Saitoh, H., Takada, M., and Hirano, K. 1998. A rapid assay of granisetron in biological fluids from cancer patients. *Biol. Pharm. Bull.* **21**, 535-537.

Wahab, S.U., Mohamed, P.U., Halith, M.S., and Chandran, M. 2011. Development of RP-HPLC method for the simultaneous determination of mefenamic acid and drotaverine HCl combined tablet dosage form. *Int. J. Pharm. Sci.* **3(1)**, 115-117.

Wang, C., Fan, G., Lin, M., Chen, Y., Zhao, W., and Wu, Y. 2007. Development of a liquid chromatography/tandem mass spectrometry assay for the determination of bestatin in rat plasma and its application to a pharmacokinetic study. *J. Chromatogr. Biomd.* **850**, 101-105.

William, F.1995. Principles of medicinal chemistry, 4th ed., B.I. Waverly Pvt Ltd., p.566.

Wilson, and Gisvolds. 1998. Text book of organic medicinal and pharmaceutical chemistry, 10th ed., Lippincott-Raven publishers, p.536.

Xia, B.Y., Wu, Q., and He, G.W. 2000. Determination of granisetron hydrochloride in tablets by HPLC. *YaowuFenziZazhi.* **20**, 257-259.

Xiang, S., Wang, W., Xiang, B., Deng, H., and Xie, H. 2007. Periodic modulation-based stochastic resonance algorithm applied to quantitative analysis for weak liquid chromatography–mass spectrometry signal of granisetron in plasma. *Int. Mass Spectrom.* **262**, 174-178.

Xu, Q.A., andTrissel, L.A. 2008. Stability-indicating HPLC methods for drug analysis. *Am. Pharma. Assoc.* Washington, D.C. USA and Pharmaceutical Press: London, UK.

Zarna, D., Ronak, D.,Vaishali, K. Vidya, S., and Meeta, B. 2009. RP-HPLC method for simultaneous estimation of omeprazole and ondansetron in combined dosage forms. *Asian J. Res. Chem.* **2(2)**, 108-111.

Zhang, H.L., Ye, L., and Stewart, J.T. 1998. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP. *J. Liq. Chromatogr. Relat. Technol.* **2**, 2375-2378.

Ainley, W., and Weller, P.J. 1994. Handbook of Pharmaceutical Excipients, 2nd ed., The Pharmaceutical Press.

Albertini, S. 1995. Genotoxicity of 17 gyrase and four mammalian topoisomerase II-poisons in prokaryotic and eukaryotic test systems. *Mutagenesis*. **10**, 343-351.

Allen, J.M. 1999. Basic principles of drug photostability testing.Presented at the Photostability 1999 Conference, Wsishington, DC.

Alvarez, F.J., and Slade, R.T. 1992. Kinetics and mechanism of degradation of zileuton, a potent 5-lipoxygenase inhibitor. *Pharm. Res.* **9**, 1465-1473.

Anderson, G., and Scott, M. 1991. Determination of product shelf life and activation energy for five drugs of abuse. *Clin. Chem.* **37**, 398–429.

Arabshahi, A., Lund, D.B. 1985. Considerations in calculating kinetic parameters from experimental data. *J. Food Pro. Eng.* **7**, 239-251.

Bauer, S., Stomer, E., Kaiser, R., Tremblay, P.B., Brockmoller, J., and Roots, I. 2002. *Biomed. Chromatogr.* **16**, 187-190.

Benson, S.W. 1960. The Foundations of Chemical Kinetics. Mcgraw-Hill Book Co., Inc., New York. p.75–93.

Blythe , R.H. 1954. Shelf life and stability tests in drug packaging. Glass Packer, August.

Borham, B.M., and Olson, F.A. 1973. Estimation of activation energies from differential thermal analysis curves. *Thermochimica Acta.* **6.4**, 345-351.

Bosso, J.A., Prince, R.A., Fox, J.L. 1992. Stability of ondansetron hydrochloride in injectable solutions at -20, 5 and 25°C. *Am. J. Hosp. Pharm.* **49**, 2223-2225.

Brodersen, R. 1947 . Stability of penicillin G in aqueous solution as a function of hydrogen ion concentration and temperature. *Acta. Pharmacologicaet Toxicologica.* **3**, 345.

Brown, M.E., Dollimore, D., and Galwey, A.K. 1980. Reactions in the solid state: in Comprehensive Chemical Kinetics. Bamford, C.H., and Tipper, C.F.H. Elsevier, Amsterdam, p.340.

Brunton, L.L., Lazo, J.S., and Parker, K. L. 2005. Goodman and Gilman's, 11th ed., McGraw-Hill Medical Publishing Division, New York.

Budzikiewicz, H., Djerassi, C., and WiUiams, D.H. 1967. Mass Spectrometry of Organic Compounds. Holden-Day, San Francisco.

Byrn, S.R., Pfeiffer, R.R., and Stowell, J.G. 1999. Solid-State Chemistry of Drugs, 2nd ed., SSCI, Inc., West Layfayette, Indiana. p.259-260.

Carstensen, J.T., and Rhodes, C.T. 2000. Drug Stability- Principles and Practices, 3rd ed., Marcel Dekker, New York, NY, p.154-160.

Casto, D.T. 1994. Stability of ondansetron stored in polypropylene syringes. *Ann. Pharmacother.* **28**, 712-714.

Chung, K.C., Chin, A., and Gill, M.A. 1995. Stability of granisetron hydrochloride in a disposable elastomeric infusion device. *Am. J. Health-syst. Pharm.* **52**, 1541-1543.

Cohen, M.D., and Green, B.S. 1973. Organic chemistry in the solid state. *Chemistry in Britain*. **9**, 490-497.

Connors, K.A. 1986. Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists, 2nd ed., John Wiley & Sons, New York, p.696.

Reynolds, D.W. 2002. Available guidance and best practices for conducting forced degradation studies. *Pharm. Technol.* **26 (2)**, 48–54.

Davies, O.L., and Budgett, D.A. 1980. Accelerated storage tests on pharmaceutical products: effect of error structure of assay and errors in recorded temperature. *J. Pharm. Pharmaco.* **32**, 155-159.

Leon, D.A. 2006. Palonosetron (Aloxi): a second-generation 5-HT(3) receptor antagonist for chemotherapy-induced nausea and vomiting. *Baylor University Medical Center.* **19 (4)**, 413-416.

Dotsikas,Y., Kousoulos, C., and Tsatsou, G. 2006. Development and validation of a rapid 96-well format based liquid–liquid extraction and liquid chromatography–tandem mass spectrometry analysis method for ondansetron in human plasma. *J. Chromatogr.* **836**, 79-82.

Duddu, S.P., Monte, P.R.D. 1997. Effect of glass transition temperature on the stability of lyophilized formulations containing a chimeric therapeutic monoclonal antibody. *Pharm. Res.* **14**, 591-595.

Edwards, L.J. 1950. The hydrolysis of aspirin: A determination of the thermodynamic dissociation constant and a study of the reaction kinetics by ultra-violet spectrophotometry. *Trans. Faraday Soc.* **46**, 723-735.

Eison-Perchonok, M. H., and Downes, T. W.1982. Kinetics of ascorbic acid autoxidation as a function of dissolved oxygen concentration and temperature. *J. Food Sci.* **47**, 765-773.

Erofeev, B.V. 1946. Generalized equation of chemical kinetics and its application in reactions involving solids. *Compt. Rend. Acad. Sci.* **52**, 511-514.

Espenson, J.H. 1995. Chemical Kinetics and Reaction Mechanisms, 2nd ed., New York, McGraw-Hill, Inc.

International Pharmaceutical Excipients Council. 1998. Excipients in Pharmaceutical Dosage Forms: The Challenge of the 21st Century, IPEC, Nice.

Eyring, H. 1935. The activated complex and the absolute rate of chemical reactions. *Chem. Rev.* **17**, 65-77.

Falkson, C.I., Falkson, H.C.1995. Antiemetic efficacy of tropisetron in patients failing previous antiemetic therapy. *Oncology*. **52**, 427-431.

Florey, K. 1991. Analytical Profiles of Drug Substances. Academic Press, New York, p.588.

Fung, H.L. 1990. In: Banker, G.S., Rhodes, C.T. Modern Pharmaceutics, 2nd ed., New York, Dekker, Chapter 6.

Volans, G.N., Sims, J., Sullivan, F.M., and Turner, P. 1990. Basic Science in Toxicology, Taylor & Francis, London.

Gan, and Lei. 2012. Continuous cooling crystallization kinetics of a molten blast furnace slag. *J. Non-Crys. Solids.* **358.1**, 20-24.

Garrett, E.R. 1962. Prediction of stability of drugs and pharmaceutical preparations. *J. Pharm. Sci*. **51**, 811-833.

Garrett, E.R. 1967. In: Bean, H.S. Advances in Pharmaceutical Sciences, vol. 2. New York, Academic Press, Chapter 2.

Genton, D., and Kesselring, U.W. 1977. Effect of temperature and relative humidity on nitrazepam stability in solid state. *J. Pharm. Sci*. **66**, 676-680.

Ginstling, A.M., and Brounshtein, B.I. 1950. The diffusion kinetics of reactions in spherical particles. *J. Appl. Chem.* **23**, 1327-1338.

Goodman Brunton, L., Parler, K., Blumenthal, D., and Buxton, I. 2008. Goodman and Gillman's Manual of Pharmacology and Therapeutics. McGraw-Hill Medical Publishing Division, USA, p.647-649.

Graham, C.L., Dukes, G.E., Kao, C.F., Bertch, J.M., and Hak, L.J. 1992. Stability of ondansetron in large-volume parenteral solutions. *Ann. Pharmacother.* **26**, 768-771.

Gravallese, D.A., Musson, D.G., Pauliukonis, L.T., and Boyne, W.F. 1984. Determination of imipenem (N-formimidoylthienamycin) in human plasma and urine by high-erformance liquid chromatography. Comparison with microbiological methodology and stability. *J. Chromatogr*. **310**, 71-84.

Harmon, P.A., Kosuda, K., Nelson, E., Mowery, M., and Reed , R. A. 2006. A novel peroxy radical based oxidative stressing system for ranking the oxidizability of drug substances. *J. Pharm. Sci.* **95**, 2014-2028.

Higuchi, T. 1950. The kinetics of the hydrolysis of procaine. *J. APhA. Sci.* **39**, 405-410.

Hou, J.P., and Poole, J.W. 1969. Kinetics and mechanism of degradation of ampicillin in solution. *J. Pharm. Sci.* **58**, 447-454.

House, J.E. 2007. Principles of Chemical Kinetics. London, Elsevier.

Houston, P.L. 2001. Chemical Kinetics and Reaction Dynamics. Boston, McGraw-Hill, Inc.

Hudlicky, M. 1990. Oxidations in Organic Chemistry.ACS Monograph 186, American Chemical Society, Washington, DC.

Hussain, A., Schurman, P., Peter, V., and Milosovich, G. 1968. Kinetics and mechanism of degradation of echothiophate iodide in aqueous solution. *J. Pharm. Sci.* **57**, 411-418.

Jhee, S.S., Jeong, E.W.S., Chin, A., Inagaki, K., Fox, J. L., and Gill, M. A. 1993. Stability of ondansetron hydrochloride stored in disposable elastomeric infusion device at 4°C. *Am. J. Hosp. Pharm.* **50**, 1918-1920.

Johnson, W.A., and Mehl, R.F. 1939. Reaction kinetics in processes of nucleation and growth. *Trans. Am. Inst. Min. Metall. Eng.* **135**, 416-442 .

Jung, M.Y., Kim, S.K., and Kim, S.Y. 1995. Riboflavin-sensitized photooxidation of ascorbic acid: kinetics and amino acid. *Food Chem.* **53**, 397-403.

Huynh, K.B. 2008. Handbook of Stability Testing in Pharmaceutical Development: Regulations, Methodologies and Best Practices, 1st ed., Springer, New York.

Conners, K.A., Amidon, G.L., and Stella, V.L. 1986. Chemical Stability of Pharmaceuticals. Wiley and Sons, New York, New York, 2nd ed., p.19.

Kelly, J.W. 1993. High-performance liquid chromatographic separation of ondansetron enantiomers in serum using a cellulose-derivatized stationary phase and solid-phase extraction. *J. Chromatogr.* **622**, 291-295.

Keuleers, R.R., Janssens, J.F., and Desseyn, H.O. 2002. Comparison of some methods for activation energy determination of thermal decomposition reactions by thermogravimetry. *Thermochimicaacta*. **385.1**, 127-142.

King, S.Y.P., Kung, M.S., and Fung , H.L. 1984 . Statistical prediction of drug stability based on nonlinear parameter estimation. *J. Pharm. Sci.* **73**, 657-662.

King , S.Y., Kung , M.S., and Fung, H.L. 1984. Statistical prediction of drug stability based on nonlinear parameter estimation. *J. Pharm. Sci.* **73**, 2332-2344.

Kissinger, H.E. 1957. Reaction kinetics in differential thermal analysis. *Anal. Chem.* **29**, 1702-1706.

Kondritzer, A.A., and Zvirblis, P. 1957. Stability of atropine in aqueous solution. *J. APhA. Sci.* **46**, 531-535.

Staveley, L.A.K. 1971. The Characterisation of the Chemical Purity.Organic Compounds. Butterworths, London.

Lachman, L. 1986. The Theory and Practice of Industrial Pharmacy, 3rd ed., Philadelphia, Lea & Febiger, Chapter 26.

Lee, C.R., Plosker, G.L., and MacTavish, D. 1993. Tropisetron: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential as an antiemetic. *Drugs.* **46**, 925-943.

Leeson, L.J., and Mattocks, A.M. 1958. Decomposition of aspirin in the solid state. *J. Am. Pharm. Assoc.* **47**, 329-333.

Lieberman, H.A., Lachman, L., and Schwartz, J.B. 1989. Pharmaceutical Dosage Forms: Tablets, 2nd ed., Vol. 2, Marcel Dekker, New York .

Lingxia, P., Qing, W., and Youpei, W. 2012. Development and validation of LC-MS/MS method for determination of ondansetron in rat plasma and its application. *Latin Am. J. Pharm.* **31(2)**, 305-309.

Lowry, T.H., and Richardson, K.S. 1987. Mechanism and Theory in Organic Chemistry, 3rd ed., Harper & Row, New York.

Lund, W., and Waaler, T. 1968. The kinetics of atropine and apoatropine in aqueous solutions. *ActachemicaScandinavica.* **22**, 3085-3097.

Nakano, M., Inotsume, N., Kohri, N., and Arita, T. 1979. Re-versiblering-opening reaction of diazepam in acid media around body temperature. *Int. J. Pharm.* **3**, 195-204.

McBride, W.R., and Villars, D.S.1954. An application of statistics to reaction kinetics. *Anal. Chem.* **26**, 901-904.

McLafferty, F.W., and Venkataraghavan, R. 1982. Mass Spectral Correlations, 2nd ed., Advances in Chemistry Series 40. American Chemical Society, Washington, DC.

Moynihan, C.T. 1996. Estimation of activation energies for structural relaxation and viscous flow from DTA and DSC experiments. *Thermochimicaacta*. **280**, 153-162.

Inotsume, N., and Nakano, M. 1983. Hydrolytic behavior of dantrolene in acidic media at body temperature. *Int. J. Pharm.* **17**, 357-360.

Nelson, W. 1990. Accelerated testing: Statistical Models, Test Plans and Data Analysis. John Wiley & Sons, New York.

Olsen, B.A., Perry, F.M., Snorek, S.V., and Lewellen, P.L. 1997. Accelerated conditions for stability assessment of bulk and formulated cefaclormonohydrate. *Pharm. Dev. Technol.* **2**, 303-312.

Raval, P.B. ,Wadher, S.J., and Yeole, P.G. 2008. A validated HPTLC method for determination of ondansetron in combination with omeprazole or rabeprazole in solid dosage form. *Ind. J. Pharm. Sci.* **70(3)**, 386-390.

Porter, Q.N. 1985. Mass Spectrometry of Heterocyclic Compounds, 2nd ed., John Wiley &c Sons, New York.

Schirmer, R.E. 1982. Modern Methods of Pharmaceutical Analysis. CRC Press, Boca Raton, FL, p.157-169.

Robinson, J.W., Skelly, F. E.M., and Frame, G.M. 2005. In Undergraduate Instrumental Analysis, 6th Ed., Marcel Dekker, p.806.

Rudnic, E., and Schwartz, J.B.1990. Solid Oral Dosage Forms .Remington's Pharmaceutical Sciences, 18th ed., Mark Publishing, Easton, PA, p.1633-1647.

Sawamura, M., Takemoto, K., Matsuzaki, Y., Ukeda, H., and Kusunose, H.1994. Identication of two degradation products from aqueous dehydroascorbic acid. *J. Agri. Food Chem.* **42**, 1200-1203.

Sharp, J.H., Brindley, G.W., and Achar, B.N.N. 1966. Numerical data for some commonly used solid-state reaction equations. *J. Am. Cera. Soc.* **49**, 379-382.

Shirish, R., Patel, J., Patel, and Yogeshvar, P. 2010. Development and validation of analytical method for the determination of rabeprazole and ondansetron in pharmaceutical dosage form by reversed-phase HPLC. *Int. J. ChemTech. Res.* **2(3)**, 1531-1536.

Siluveru, M. 1997. Enantioselective determination of S-(+) - and R-(-)-ondansetron in human serum using derivatizedcyclodextrins - modified capillary electrophoresis and solid-phase extraction. *J. Chromatogr.* **691,** 217-222.

Starink, M. J. 2007. Activation energy determination for linear heating experiments: deviations due to neglecting the low temperature end of the temperature integral. *J. Mate. Sci.* **42.2**, 483-489.

Stiles, L.M., Allen, L.V., and Fox, J.L. 1992. Stability of ondansetron hydrochloride in portable infusion-pump reservoirs. *Am. J. Hosp. Pharm.* **49**, 1471-1473.

The Merck Index. 2006. In An encyclopaedia of chemicals, drugs and biological; 14th Ed., published by Merck Research Laboratories Division of Merck and co. Inc. USA, p.1395.

Tootill, J.P.R. 1961. Slope-ratio design for accelerated storage tests. *J. Pharm. Pharmac.* **13**, 75T-86T.

Trissel, L.A. 1994. Handbook on Injectable Drugs, 8th ed., American Society of Hospital Pharmacists, Bethesda.

Trivedi, J.S., Porter, W.R., and Fort, J.J. 1996. Solubility and stability characterization of zileuton in a ternary solvent system. *Eur. J. Pharm. Sci.* **4**, 109-116.

Turro, N.J. 1991. Modern MolecularPhotochemisty. University Science Books, Sausalito, CA .

Vyazovkin, S. 1997. Evaluation of activation energy of thermally stimulated solid-state reactions under arbitrary variation of temperature . *J. Compu. Chem.* **18**, 393-402.

Waterman, K.C., Carella, A.J., Gumkowski, M.J., Lukulay, P., MacDonald, B.C., Roy, M.C., and Shamblin, S.L. 2007. Improved protocol and data analysis for accelerated shelf-life estimation of solid dosage forms. *Pharm. Res.* **24**, 780-790.

Windheuser, J.J., and Higuchi, T. 1962. Kinetics of thiamine hydrolysis. *J. Pharm. Sci.* **51**, 354-364.

Yamana, T., and Tsuji, A. 1976. Comparative stability of cephalosporins in aqueous solution: kinetics and mechanisms of degradation . *J. Pharm. Sci.* **65**, 1563-1574.

Zapata, S., and Dufour, J. 1992. Ascorbic dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *J. Food Sci.* **57(2)**, 506-511.

Zhang, Lunyong, Dawei, X., and Jianfei, S. 2010. Calculating activation energy of amorphous phase with the Lambert W function. *J. Ther. Anal. Calori.* **100.1**, 3-10.

Zhou, D., Schmitt, E.A., Zhang, G.G., Law, D., Vyazovkin, S., Wight, C.A., and Grant, D.J.W. 2003 . Crystallization kinetics of amorphous nifedipine studied by model-fitting and model-free approaches. *J. Pharm. Sci.* **92**, 1779-1792.

Zvirblis, P., Socholitsky, I., and Kondritzer, A.A. 1956. The kinetics of the hydrolysis of atropine . *J. Am. Pharm. Assoc.* **45**, 450-454.

Soliman, A. 2007. Derivation of the Kissinger equation for non-isothermalglass transition peaks. *J. Therm. Anal. Cal.* **89(2)**, 389-392.

Singh, R., and Rehman, Z. 2012. Current trends inforced degradationstudy for pharmaceutical product development. *J. Pharm. Educ. Res.* **3(1)**, 54-63.

Bichsel, V.E., Curcio, V., and Gassmann, R. 1996. Requirements for the quality control of chemically synthesized peptides and biotechnologically produced proteins. *Pharm. Acta Helv.* **71**, 439-446.

Ali, N.W., Abbas, S.S., and Zaazaa, H.E. 2012. Validated stability indicating methods for determination of nitazoxanide in presence of its degradation products. *J. Pharm. Anal.* **2(2)**, 105-116.

Riddhiben, M.P., Piyushbhai, M.P., and Natubhai, M.P. 2011. Stability indicating HPLC method development—a review. *Int. Res. J. Pharm.* ***2*****(5)**, 79-87.

Synder, L.R., Glajch, J.L., Kirkland, J.J. 1997. Practical HPLC method development, 2nd ed., Wiley, New York.

Annapurna, M.M., Mohapatro, C., and Narendra, A. 2012. Stability-indicating liquid chromatographic method for the determination of letrozole in pharmaceutical formulation. *J. Pharm. Anal.* **2(4)**, 298-305.

Smela, J.W. 2005. Regulatory considerations for stability indicating analytical methods in drug substance and drug product testing. *Am. Pharm. Rev.* **8(3)**, 51-54.

Dolan, J.W. 2002. Stability-indicating assays: LC troubleshooting, LC-GC. *N. Am.* **20(4)**, 346-349.

Trabelsi, H., Hassen, I.E., and Bouabdallah, S. 2005. Stability-indicating LC method for determination of pipamperone. *J. Pharm. Biomed. Anal.* **39**, 914-919.

Qiu, F., and Norwood, D.L. 2007. Identification of pharmaceutical impurities. *J. Liq. Chromatogr. Relat. Technol.* **30**, 877-935.

Ahuja, S., and Scypinski, S. 2001. Handbook of Modern Pharmaceutical Analysis, 1st ed., Academic Press, New York.

Allwood, M., and Plane, J. 1986. The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation, *Int. J. Pharm.* **31**, 1-7.

Baertschi, S.W., and Thatcher, S.R. 2006. Sample presentation for photostability studies: problems and solutions. in: J. Piechocki (ed.), Pharmaceutical Photostability and Stabilization Technology, Taylor & Francis, New York, p.445.

Alsante, K.M., Hatajik, T.D., and Lohr, L.L. 2003. Solving impurity/ degradation problems: case studies, in: Ahuja, S., and Alsante, K.M. (Eds.), Handbook of Isolation and Characterization of Impurities in Pharmaceutical. Academics Press, New York, p.380.

Boccardi, G. 2005. Oxidative susceptibility testing, in: Baertschi, S.W. (Ed.), Pharmaceutical Stress Testing-Predicting Drug Degradation, Taylor and Francis, New York, p.220.

Gupta, A., Yadav, J.S., and Rawat, S. 2011. Method development and hydrolytic degradation study of doxofylline by RPHPLC and LC–MS/MS. *Asian J. Pharm. Anal.* **1**, 14-18.

Alsante, K.M., Ando, A., and Brown, R. 2007. The role of degradant profiling in active pharmaceutical ingredients and drug products. *Adv. Drug Deliv. Rev.* **59(1)**, 29-37.

Larsen, C., and Bundgaard, H. 1978. Polymerization of Penicillins V: Separation, identification and quantitative determination of Antigenic Poly merization products in Ampicillin Sodium preparations by high- performance liquid chromatography. *J. Chromatogr.* **A147**, 143-150.

Singh, S., and Bakshi, M. 2000. Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharm. Technol.* **24**, 1-14.

Banker, G.S., and Rhodes, C.T. 2002. Modern Pharmaceutics, 4th ed., Marcel Dekker, Inc., New York.

International Conference on Harmonisation (ICH), validation of analytical procedures: Text and Methodology Q2 (R1) in November 2005.

Ngwa, G. 2010. Forced degradation studies as an integral part of HPLC stability indicating method development. *Drug Deliv. Technol.* **10(5)**, 56-59.

Klick, S., Pim, G.M., and Waterval, J. 2005. Toward a generic approach for stress testing of drug substances and drug products. *Pharm. Technol.* **29 (2)**, 48-66.

Maheswaran, R. 2012. FDA perspectives: scientific considerations of forced degradation studiesin ANDA submissions. *Pharm. Technol.* **36(5)**, 73-80.

Jenke, D.R. 1996. Chromatographic method validation: a review of common practices and procedures II. *J. Liq. Chromatogr.* **19**, 737-757.

Carr, G.P., and Wahlich, J.C. 1996. A practical approach to method validation in pharmaceutical analysis. *J. Pharm. Biomed. Anal.* **86**, 613-618.

Szepesi, G. 1989. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis-III method validation. *J. Chromatogr.* **464**, 265-278.

Kats, M. 2005. Forced degradation studies: regulatory considerations and implementation. *Bio. Pharm. Int.* **18**, 1-7.

Brummer, H. 2011. How to approach a forced degradation study. *Life Sci. Technol. Bull.* **31**, 1-4.

International Conference on Harmonisation (ICH), Impurities in new drug substances Q3A (R2) in October 2006.

International Conference on Harmonisation (ICH), Impurities in new drug products Q3A (R2) in June 2006.

International Conference on Harmonisation (ICH), Specifications: Test procedures and acceptance criteria for new drug substances and new drug products Q6A in October 1999.

Hou, J.P., and Jungang, D.Y. 2010. Peroxide-based route assisted with inverse microemulsion process to well-dispersed Bi4Ti3O12 nanocrystals. *J. Nanoparticle. Res.* **12.5**, 1797-1805.

Matei, N.B. 2008. Kinetic studyof vitaminC degradation from pharmaceutical products. *J. Phys.* **53**, 343-351.

Bai, J.W., Gao, Z.J., Xiao, H.W., Wang, X.T., and Zhang, Q. 2013. Polyphenol oxidase inactivation and vitamin C degradation offuji apple quartesbyhigh humidity air impingement blanching. *Int. J. Food Sci. Technol.* **48**, 1135-1141.

Mercali, G.J., Jaeschke, D.P., Tessaro, I.C., Damasceno, L., and Marczak, F. 2012. Study of vitamin C degradation in acerolapulp during ohmic and conventional heat treatment. *Food Sci. Technol.* **47**, 91-95.

Abbasi, A., and Niakousari, M. 2008. Kinetics of ascorbic acid degradation in un-pasteurized Iranian lemon juice during regular storage conditions. *Pak. J. Biol. Sci.* **11**,1365-1369.

Karim, O.A. 2009. A dynamic method for kinetic model of ascorbic acid degradation during air dehydration of pretreated pineapple slices. *Int. Food Res. J.* **16**, 555-560.

Lee, S., and Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Posthar. Biotechnol.* **20**, 207-220.

Hsieh, Y., and Harris, N.D. 1987. Oxidation of ascorbic acid in copper catalysed sucrose solutions. *J. Food Sci.* **52**, 1384-1386.

Yang, J., Liu, J., and Parry, J. 2009. Physiological and biochemical functions of vitamin C. Handbook of Vitamin C Research: Daily Requirements, Dietary Sources and Adverse Effects. Kucharski, H.,and Zajac, J. editors. Nova Biomedical Books. New York.

Kadam, D.K. 2012. Evaluation of guava products quality. *Int. J. Food Sci. Nutr. Eng.* **2**, 7-11.

Ranganna, S. 2001. Handbook of Analysis and Quality Control for Fruits and Vegetable Products.New Delhi, Tata McGraw-Hill Publications.

Tiwari, B.O., O'Donnell, C.P, Muthukumarappan, K., Cullen, P.J. 2009. Ascorbic acid degradation kinetics of sonicatedorange juice during storage and comparison with thermally pasteurised juice. *Food Sci. Technol.* **42**, 700-704.

Kun, L.Z. 2011. Degradation kinetics of vitamin C in Chinese jujube during drying process. *IEEE*. **17**, 784-788.

Viera, M.C., Teixeira, A.A, and Silva, C.L.M. 2000. Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuacu (Theobromagrandiorum) nectar. *J. Food Eng.* **25**, 42-48.

Demir, V., Gunhan, T., Yagcioglu, A.K., and Degirmencioglu, A. 2004. Mathematical modeling and the determination of some quality parameters of air-dried bay leaves. *Biosys. Eng.* **88**, 325-335.

Bradbury, J. H., and Singh, U.1986. Ascorbic acid and dehydroascorbic acid content of tropical root crops from the South Pacific. *J. Food Sci.* **51 (4)**, 975-978.

Gregory, J. F.1996. Ascorbic acid. In O. Fennema, Principles of food science. Part I. Food chemistry, 3rd ed., New York, Marcel Dekker, Chapter 8.

Hsieh, Y. P., and Harris, N. D. 1993. Effect of sucrose on oxygen uptake of ascorbic acid in a closed aqueous system. *J. Agric. Food Chem.* **41**, 259-262.

Johnson, J. R., Braddock, R. J., and Chen, C. S. 1995. Kinetics of ascorbic acid loss and nonenzymatic browning in orange juiceserum: experimental rate constants. *J. Food Sci.* **60 (3)**, 502-505.

Levenspiel, O. 1972. Interpretation of batch reactor data. In Chemical reaction engineering, 2nd ed., New York, Wiley, International Edition, Chapter 3.

Krause, T., Gerbershagen, M. U., Fiege, M., Weißhorn, R., and Wappler, F. 2004. Dantrolene—A review of its pharmacology, therapeutic use and new developments. *Anaesthesia*. **59**, 364-373.

Snyder, H.R., Davis, C.S., Bickerton, R.K., and Hal-liday, R. P. 1967. 1-[5Arylfurfurylidene)amino] hydantoins: A new class of muscle relaxants. *J. Medic. Chem.***10**, 807-810.

Ellis, K.O., Castellion, A.W., Honkomp, L.J., Wes-sels, F.L., Carpenter, J.F., and Halliday,R.P. 1973. Dantrolene, a directacting skeletal muscle relaxant. *J. Pharm. Sci.* **62**, 948- 951.

Nakano, M., Inotsume, N., Kohri, N., andArita, T. 1979. Reversible ring-opening reaction of diazepam in acid media around body temperature. *Int. J. Pharm.* **3**, 195-204.

Connors, K.A., Amidon, V.J., and Stella, V.J. 1986. Chemical Stability of Pharmaceuticals- A Handbook for Pharmacists. John Wiley & Sons, New York.

Cox, P.L., Heotis, J.P., Polin, D., and Rose, G.M. 1969. Quantitative d etermination of dantrolenesodium and its metabolites by differential pulse polarography. *J. Pharm. Sci.* **58**, 987-989.

Conklin, J.D., and Sobers, R.J. 1973. Qualitative method for dantrolene and a related metabolite in urine. *J. Pharm. Sci.* **62**, 1024-1025.

Hollifield, R. D. and Conklin, J. D. 1973. Determination of dantrolene in biological specimens containing drug related metabolites. *J. Pharm. Sci.* **62**, 271-274.

Tawakkul, M.A., Faustino, P.J., Sayeed, V.A., Khan, M.A., and Khan, S.R. 2010. Development and application of a validated stability-indicating ultra-performance liquid chromatography (UPLC) method for the determination of dantrolene and its related impurities. *Clin. Res. Regul. Aff.* **27**, 21-29.

Bentley, D.L. 1973. Statistical techniques in predicting thermal stability. *J . Pharm. Sci.* **70**, 459-464.

Kennon, L. 1964. Use of models in determining chemical pharmaceutical stability. *J. Pharm. Sci.* **53**, 815.

Allen, L.V. 2008. Dosage form design and development. *Clin. Therap.* **30(11)**, 2102–2111.
Higuchi, T., and Lachman, L. 1955. Inhibition of hydrolysis of esters in solution by formation of complexes- I stabilization of benzocainewith caffeine. *J. APhA. Sci.* **44**, 521-526.

Fersht, A.R., and Kirby, A.J. 1967. The hydrolysis of aspirin-Intramolecular general base catalysis of ester hydrolysis. *J. Am. Chem. Soc.* **89**, 4857-4863.

Luo, D., Smith, S.W., and Anderson, B.D. 2005. Kinetics and mechanism of the reaction of cysteine and hydrogen peroxide in aqueous solution. *J. Pham. Sci.* **94(2)**, 304-316.

Constantino, H.R., Carrasquillo, K.G. , Cordero, R.A., Mumenthaler, M., Hsu, C.C. , Gribenow, K. 1998. Effect of excipients on the stability and structure of lyophilized recombinant human growth hormone. *J. Pharm. Sci.* **87(11)**, 412-420.

Lai, M.C., Topp, E.M. 1999. Solid-state chemical stability of proteins and peptides. *J. Pharm. Sci.* **88(5)**, 489-500.

Zahn, M. 2006. A risk-based approach to establish stability testing conditions for tropical countries. *J. Pharm. Sci.* **95**, 946-965.

Haynes, J.D. 1971. Worldwide virtual temperatures for product stability testing. *J. Pharm. Sci.* **60**, 927-929.

Kopp, S. 2006. Stability testing of pharmaceutical products in a global environment. *Regul. Aff. J.* **16(5)**, 291-294.

Creasy, K.E. 2003. Process analytical chemistry: smarter, faster, stronger. *J. Process. Anal. Chem.* **7(1)**, 203-207.

Gold, T.B., Buice, R.G., Lodder, R.A., and Digenis, G.A. 1997. Determination of extent of formaldehydeinduced crosslinking in hard gelatin capsules by near-infrared spectrophotometry. *Pharm. Res.* **14(8)**, 1046-1050.

Tsong, Y., Chen, W.J., Lin, T.Y., and Chen, C.W. 2003. Shelf life determination based on equivalence assessment. *J. Biopharm. Stat.* **13**, 431-449.

Tydeman, M.S., and Kirkwood, T.B.L. 1984. Design and analysis of accelerated degradation tests for the stability of biological standards. *J. Biol. Stand.* **12**,195-206.

Some, I.T., Bogaerts, P., Hanus, R., Hanocq, M., and Dubois, J. 2001. Stability parameter estimation at ambient temperature from studies at elevated temperatures. *J. Pharm. Sci.* **90**, 1759-1766.

O'Neil, A.J., Jee, R.D., and Moffat, A.C. 1998. The application of multiple linear regression to the measurement of the median particle size of drugs and pharmaceutical excipients by near-infrared spectroscopy. *Analyst.* **123(11)**, 2297-2302.

Bancroft, T. A. 1964. Analysis and inference for incompletely specified models involving the use of preliminary test(s) of significance. *Biometrics*. **20**, 427-442.

Chen, Ying, Q., Annpey, P., and Biao, X. 2003. Rank regression in stability analysis. *J. Biopharm. Stat.* **13**, 463-479.

Chow, Shein, C., and Annpey, P. 1995. Current issues in regulatory requirements of drug stability. *J. Food and Drug Anal.* **3(2)**, 75-85.

Chow, Shein, C., and Jun, S. 1991. Estimating drug shelf-life with random batches. *Biometrics*. **47**, 1071-1079.

Davison, A.C. and Diego, K. Topics in statistical computing: An introduction to the Bootstrap with applications in *R. Stat. Comp. Stat. Grap. Newslette* .**13**, 6-11.

Jung, Sin, H., and Zhiliang, Y. 2003. Rank-based regression with repeated measurements data. *Biometrika*. **90**, 732-740.

Rashid, M., and Mushfiqur. 2003. Rank-based tests for non-inferiority and equivalence hypotheses in multi-centre clinical trials using mixed models. *Stat. Medic.* **22**, 291-311.

Tsong, Y., Wen, Y.C., and Chi, W.C. 2003. ANCOVA approach for shelf life analysis of stability study of multiple factor design. *J. Biopharm. Stat.* **13**, 375-393.

Craft, N.E., and Scares, J.H. 1992. Relative solubility, stability, and absorptivity of lutein and β-carotene inorganic solvents. *J. Agric. Food Chem.* **40**, 431-434.

Gannu, R., Vamshi, Y.V., Kishan, V., and Rao, Y.M. 2007. Development of nitrendipine transdermal patches: *In vitro*and *Ex vivo c*haracterization. *Current Drug Delivery*. **4**, 69-76.

Robinson, J.R., and Lee, H.L., 1987. Controlled drug delivery fundamentals and applications. 2nd ed., Marcel Decker, New York, p. 524-552.

Lee, Y.L., Ching, C.H., and Chen, J.L. 1994. In vitro and in-vivo percutaneous absorption studies of ketotifen patches. *Drug Dev. Ind. Pharm.* **20(19)**, 2965-2976.

Kulschreshtha, H.K. 1976. Use of kinetic methods in storage stability studies on drugs and pharmaceuticals. *Defence Sci. J.* **26(4)**, 189-204.

Witthaus, G., Breimer, D.D., Speiser, P. 1981. Accelerated Storage Tests: Predictive value, Topics in Pharmaceutical Sciences. Elsevier, North-Holland Biomedical Press, 275-290.

Carstensen, J.T., Swarbrick, J.T. 1995. Drug Stability.Principles and Practices. 2nd ed., New York, Marcel Dekker.

Schou, S.A. 1960. Decomposition of pharmaceutical preparations due to chemical changes. *Am. J. Hosp. Pharm.* **17**, 153-161.

Stewart, P.J., Tucker, I.G. 1984. Prediction of drug stability. Part 1. Mechanism of drug degradation and basic rate laws. *Aust. J. Hosp. Pharm.* **14**,165-170.

Stewart, P.J., Tucker, I.G. 1985. Prediction of drug stability.Part 2. Hydrolysis. *Aust. J. Hosp. Pharm.* **15(1)**, 11-16.

Stewart, P.J., Tucker, I.G. 1985. Prediction of drug stability.Part 3. Oxidation and photolytic degradation. *Aust. J. Hosp. Pharm.* **15(2)**, 111-117.

Davis, J.S. 1991. Criteria for Accelerated Stability Testing, presented at the FDA/ASQC Seminar, March 11, Chicago, IL.

Carstensen, J.T. 1995. Drug Stability: Principles and Practices 2nd. ed., Swarbrick JT New York, Marcel Dekker, 3-4.

Florey, K., Brittain, H.G. 1998. Analytical Profiles of Drug Substances. New York, Academic Press, **1-25**, 1972-1998.

Singh, S., Bakshi, M. 2000. Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharm. Technol.* **22**, 1-14.

Bridle, J.H., Brimble, M.T. 1993. A stability indicating method for dipyridamole. *Drug Dev. Ind. Pharm.* **19**, 371-381.

Padmanabhan, G., Becue, I., Smith, J.B. 1989. Analytical Profiles of Drug Substances. New York, Academic Press, **18**, 76-77.

Groningsson, K., Lindgren, J.E., Lundbeg, E., Sandberg, R., Wahle´n, A. 1985. Lidocaine base and hydrochloride. In: Florey K, ed. Analytical Profiles of Drug Substances. New York, Academic Press, **14**, 226-227.

Muhtadi, F.J. 1988. Analytical profile of morphine. In: Florey K, ed. Analytical Profiles of Drug Substances. New York, Academic Press, **17**, 309.

Nassar, M.N., Chen, T., Reff, M.J., Agharkar, S.N. 1993. Didanosine. In: Brittain, H.G., ed., Analytical Profiles of Drug Substances and Excipients. New York, Academic Press, **22**, 216-219.

Johnson, B.M., Chang, P.T.L. 1996. Sertraline hydrochloride. In: Brittain, H.G., ed., Analytical Profiles of Drug Substances and Excipients. New York, Academic Press, **24**, 484.

Singh, S., Bakshi, M. 2000. Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharm. Technol.* online, 1-14.

Anderson, N.H., Johnston, D., McLelland, M.A., Munden, P. 1991. Photostability testing of drug substances and drug products in UK pharmaceutical laboratories. *J. Pharm. Biomed. Anal.* **9(6)**, 443.

Thoma, K. Survey of twenty German manufacturers (1995). 1996. In: Tønnesen, H., ed. Photostability of Drugs and Drug Formulations. London, Taylor & Francis, 136-137.

Thatcher, S.R., Mansfield, R.K., Miller, R.B., Davis, C.W., Baertschi, S.W.2001. Pharmaceutical photostability: a technical and practical interpretation of the ICH guideline and its application to pharmaceutical stability: Part I, *Pharm. Technol.* **25(3)**, 98-110.

Thatcher, S.R., Mansfield, R.K., Miller, R.B., Davis, C.W., Baertschi, S.W. 2001. Pharmaceutical photostability: a technical and practical interpretation of the ICH guideline and its application to pharmaceutical stability: Part II, *Pharm. Technol.* **25(4)**, 50- 62.

Baertschi, S.W. 1996. The Role of Stress Testing in Pharmaceutical Product Development, presented at the American Association of Pharmaceutical Scientists Midwest Regional Meeting, Chicago, IL.

Weiser, W.E. 1998. Developing analytical methods for stability testing. *Pharm. Technol.* **32**, 20-29.

Alsante, K.M., Friedmann, R.C., Hatajik, T.D., Lohr, L.L., Sharp, T.R., Snyder, K.D., Szczesny, E.J. 2001. Degradation and impurity analysis for pharmaceutical drug candidates. In: Ahuja, S., Scypinski, S., eds. Handbook of Modern Pharmaceutical Analysis. *Sep. Sci. Technol.* Academic Press, **3**, 85-172.

Reynolds, D.W., Facchine, K.L., Mullaney, J.F., Alsante, K.M., Hatajik, T.D., Motto, M.G. 2002. Available guidance and best practices for conducting forced degradation studies. *Pharm. Technol.* **26**, 26(2).

Trabelsi, H., Bouabdallah, S., Bouzouita, K., Safta, F. 2002. Determination and degradation study of haloperidol by high performance liquid chromatography. *Pharm. Biomed. Anal.* **29**, 649-657.

Caviglioli, G., Valeria, P., Brunella, P., Sergio, C., Attilia, A., Gaetano, B. 2002. Identification of degradation products of Ibuprofen arising from oxidative and thermal treatments. *J. Pharm. Biomed. Anal.* **30**, 499-509.

Breil, S., Martino, R., Gilard, V. Malet-Martino, M., Niemeyer. U. 2001. Identification of new aqueous chemical degradation products of isophosphoramide mustard. *J. Pharm. Biomed. Anal.* **25**, 669-678.

Xu, X., Bartlett, M.G., Stewart, J.T. 2001. Determination of degradation products of sumatriptan. *J. Pharm. Biomed. Anal.* **26**, 367-377.

Feng, W., Liu, H., Chen, G., Malchow, R., Bennett, F., Lin, E., Pramanik, B., Chan, T.M. 2001. Structural characterization of the oxidative degradation products of an antifungal agent SCH56592 by LC-NMR and LC-MS. *J. Pharm. Biomed. Anal.* **25**, 545-557.

Bakshi, M., Singh, B., Singh, A., Singh, S. 2001. The ICH guidance in practice: stress degradation studies on ornidazole and development of a validated stability indicating assay. *J. Pharm. Biomed. Anal.* **26**, 891-897.

Ojha, T., Bakshi, M., Chakraborti, A.K., Singh. S. 2003. The ICH guidance in practice: stress decomposition studies on three piperazinylquinazoline adrenergic receptor-blocking agents and comparison of their degradation behavior. *J. Pharm. Biomed. Anal.* **31**, 775-783.

Singh, S., Bakshi, M. 2000. Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharm. Technol.* Online, 1-14.

Olsen, B.A., Baertschi, S.W. 2003. Strategies for investigation and control of processrelated and degradation-related impurities in Pharmaceuticals. In: Ahuja, S., Alsante, K.M., eds., Handbook of Isolation and Characterization of Impurities in Pharmaceuticals. *Sep. Sci. Technol.*, San Diego, Academic Press, **5.**

Anderson, N.H. 1996. Photostability testing: design and interpretation of tests on drug substances and dosage forms. In: Tønnesen, H.H. ed. Photostability of Drugs and Drug Formulations. Great Britain, Taylor & Francis, 307.

Dressman, J.B. 1986. Comparison of Canine and human gastointestinal physiology. *Pharm. Res.* **3(3)**, 123-131.

Harmon, P.A. 2004. Development of a peroxy radical mediated oxidative stressing system for predicting general oxidative sensitivity in solid dosage forms. Forced Degradation Studies: Best Practices for the Pharmaceutical Industry. Institute for International Research, Princeton, NJ, Feb 24-25,

Richter, S., Fabris, D., Binaschi, M., Gatto, B., Capranico, G., Palumbo, M. 2004. Effects of common buffer systems on drug activity in the case of Clerocidin. *Chem. Res. Toxicol.* **17(4)**, 492-501.

List of publications

Hossain, M., Jalil, R., and Rashid, M. A. 2018. Studies of stress degradation of ramosetron hydrochloride, a 5-HT_3 antagonist. *Dhaka Univ. J. Pharm. Sci.* **17(1)**, 9-17.

Hossain, M., Jalil, R., and Rashid, M. A. 2018. Studies of degradation kinetics of a 5-HT_3 antagonist, ramosetron hydrochloride: Effect of temperature. *Dhaka Univ. J. Pharm. Sci.* **17(2)**, 167-173.

APPENDIX-I

Summary results of ramosetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Aqueous degradation at 60°C			
7 days	100	99.9	-
21 days	100	99.5	-
Acid degradation at 60°C after 7 days			
0.1N HCl	100	99.9	-
0.5N HCl	100	95.0	5.0
1.0N HCl	100	92.0	8.0
2.0N HCl	100	88.9	11.1
Base degradation at 60°C after 2 days			
0.1N NaOH	100	79.1	20.9
0.5N NaOH	100	0	100
1.0N NaOH	100	0	100
2.0N NaOH	100	0	100
Base degradation in 0.1N NaOH at 60°C after:			
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 degradation in 0.1N NaOH at 70°C after:			
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

APPENDIX-I

Summary results of ramosetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Base degradation in 0.1N NaOH at 80°C after:			
1 hour	100	94.7	5.8
2 hours	100	90.4	9.6
3 hours	100	85.2	14.8
4 hours	100	76.9	20.1
5 hours	100	74.2	25.2
Oxidation in 3.0% hydrogen peroxide at dark place after:			
1 hour	100	90.0	10
2 hours	100	84.8	15.2
3 hours	100	79.2	20.8
Oxidation in 5.0% hydrogen peroxide at dark place after:			
1 hour	100	42.1	57.9
2 hours	100	38.3	61.7
3 hours	100	26.7	73.3
Oxidation in 10.0% hydrogen peroxide at dark place after:			
1 hour	100	24.0	76.0
2 hours	100	13.9	86.1
3 hours	100	7.0	93.0
Photo degradation exposed to 1.2 million lux for:			
7 days	100	99.8	-
Photo degradation exposed to 3.6 million lux for:			
21 days	100	93.4	6.6

APPENDIX-II

Summary results of ondansetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Aqueous degradation at 60°C after:			
7 days	100	99.8	-
21 days	100	96.4	3.6
Acid degradation at 60°C after 7 days in:			
0.1N HCl	100	99.8	-
0.5N HCl	100	99.9	-
1.0N HCl	100	100.1	-
2.0N HCl	100	99.8	-
Base degradation at 60°C after 3 days in:			
0.1N NaOH	100	95.9	4.1
0.5N NaOH	100	91.9	8.1
1.0N NaOH	100	82.7	17.3
2.0N NaOH	100	67.8	32.2
Base degradation in 2.0N NaOH at 60°C after:			
1 hour	100	97.5	2.6
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 degradation in 2.0N NaOH at 70°C after:			
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

APPENDIX-II

Summary results of ondansetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Base degradation in 2.0N NaOH at 80°C after:			
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 in 3.0% hydrogen peroxide at dark place after:			
1 hour	100	88.1	11.9
2 hours	100	86.3	13.7
3 hours	100	81.4	18.6
Oxidation in 5.0% hydrogen peroxide at dark place after:			
1 hour	100	73.9	26.1
2 hours	100	70.0	30.0
3 hours	100	62.0	38.0
Oxidation in 10.0% hydrogen peroxide at dark place after:			
1 hour	100	62.0	38.0
2 hours	100	58.9	41.1
3 hours	100	55.0	45.0
Photo degradation exposed to 1.2 million lux for:			
7 days	100	99.6	-
Photo degradation exposed to 3.6 million lux for:			
21 days	100	97.8	2.3

APPENDIX-III

Summary results of granisetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Aqueous hydrolysis at 60°C after:			
7 days	100	99.9	0
21 days	100	99.7	0.3
Acid degradation at 60°C after 7 days in:			
0.1N HCl	100	97.7	2.3
0.5N HCl	100	95.0	5.0
1.0N HCl	100	89.2	10.8
2.0N HCl	100	82.9	17.1
Base degradation at 60°C after 3 days in:			
0.1N NaOH	100	98.6	1.4
0.5N NaOH	100	79.2	20.8
1.0N NaOH	100	75.3	24.7
2.0N NaOH	100	53.7	46.3
Base degradation in 2.0N NaOH at 60°C after:			
1 hour	100	98.8	1.2
2 hours	100	97.7	2.3
3 hours	100	96.5	3.5
4 hours	100	95.4	4.6
5 hours	100	94.2	5.8
Base degradation in 2.0N NaOH at 70°C after:			
1 hour	100	97.5	2.5
2 hours	100	95.0	5.0
3 hours	100	92.5	7.5
4 hours	100	90.0	10.0
5 hours	100	87.5	12.6

APPENDIX-III

Summary results of granisetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Base degradation in 2.0N NaOH at 80°C after:			
1 hour	100	95.0	5.0
2 hours	100	90.1	9.9
3 hours	100	85.1	14.9
4 hours	100	80.2	19.8
5 hours	100	75.2	24.8
Oxidation in 3.0% hydrogen peroxide at dark place after:			
1 hour	100	75.6	24.4
2 hours	100	70.4	29.6
3 hours	100	68.8	31.2
Oxidation in 5.0% hydrogen peroxide at dark place after:			
1 hour	100	65.2	34.8
2 hours	100	62.4	37.6
3 hours	100	58.6	41.4
Oxidation in 10.0% hydrogen peroxide at dark place after:			
1 hour	100	35.4	64.6
2 hours	100	30.7	69.4
3 hours	100	28.7	71.3
Photo degradation exposed to 1.2 million lux for:			
7 days	100	99.8	0
Photo degradation exposed to 3.6 million lux for:			
21 days	100	99.9	0

APPENDIX-IV

Summary results of tropisetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Aqueous degradation at 60°C after:			
7 days	100	99.8	-
21 days	100	98.8	1.2
Acid degradation at 60°C after 7 days in:			
0.1N HCl	100	99.0	0.96
0.5N HCl	100	97.5	2.6
1.0N HCl	100	97.0	3.0
2.0N HCl	100	84.3	15.7
Base degradation at 60°C after 3 days in:			
0.1N NaOH	100	81.1	18.9
0.5N NaOH	100	45.3	54.7
1.0N NaOH	100	23.5	76.5
2.0N NaOH	100	6.5	93.5
Base degradation in 2.0N NaOH at 60°C after:			
1 hour	100	97.8	2.1
2 hours	100	95.6	4.4
3 hours	100	93.4	6.6
4 hours	100	91.2	8.8
5 hours	100	89.0	11.0
Base degradation in 2.0N NaOH at 70°C after:			
1 hour	100	95.5	4.5
2 hours	100	91.1	8.9
3 hours	100	86.6	13.4
4 hours	100	82.1	17.9
5 hours	100	77.7	22.4

APPENDIX-IV

Summary results of tropisetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Base degradation in 2.0N NaOH at 80°C after:			
1 hour	100	90.7	9.3
2 hours	100	81.4	18.6
3 hours	100	72.2	27.8
4 hours	100	62.9	37.1
5 hours	100	53.6	46.4
Oxidation in 3.0% hydrogen peroxide at dark place after:			
1 hour	100	91.4	8.6
2 hours	100	90.5	9.5
3 hours	100	87.5	12.7
Oxidation in 5.0% hydrogen peroxide at dark place after:			
1 hour	100	78.4	21.6
2 hours	100	78.3	21.7
3 hours	100	76.7	23.3
Oxidation in 10.0% hydrogen peroxide at dark place after:			
1 hour	100	75.2	24.8
2 hours	100	65.2	34.8
3 hours	100	62.5	37.5
Photo degradation exposed to 1.2 million lux for:			
7 days	100	99.9	-
Photo degradation exposed to 3.6 million lux for:			
21 days	100	100.6	-

APPENDIX-V

Summary results of palonosetron hydrochloride in different stressed conditions.			
Conditions	Content (%)		Degradation (%)
	Standard	Sample	
Aqueous degradation at 60°C after:			
7 days	100	99.3	-
21 days	100	99.9	-
Acid degradation at 60°C after 7 days:			
0.1N HCl	100	99.2	0.80
0.5N HCl	100	98.1	1.9
1.0N HCl	100	97.6	2.4
2.0N HCl	100	96.9	3.1
Base degradation at 60°C after 7 days:			
0.1N NaOH	100	99.3	0.7
0.5N NaOH	100	95.2	4.8
1.0N NaOH	100	94.7	5.3
2.0N NaOH	100	87.4	12.6
Oxidationin 3.0% hydrogen peroxide at dark place after:			
1 hour	100	5.0	95.0
2 hours	100	3.0	97.0
3 hours	100	1.0	99.0
Oxidation in 5.0% hydrogen peroxide at dark place after:			
1 hour	100	-	100
2 hours	100	-	100
3 hours	100	-	100
Oxidation in 10.0% hydrogen peroxide at dark place after:			
1 hour	100	-	100
2 hours	100	-	100
3 hours	100	-	100
Photo degradation exposed to 1.2 million lux for:			
7 days	100	99.7	-
Photo degradation exposed to 3.6 million lux for:			
21 days	100	99.9	-