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DEGRADATION AND KINETIC STUDY OF TRAMADOL HCL BY RP-HPLC

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ABSTRACT

A simple, precise and accurate isocratic RP–HPLC method was developed. The method has shown adequate separation for Tramadol HCl and degradation products. Separation was achieved on a ACE C18 (150 mm×4.6 mm i.d., 5µm particle size) column using mobile phase consisting of Acetonitrile – Water (70:30v/v) at flow rate of 1mL/min and UV detection at 272nm. This drug was subjected to acid hydrolysis and alkali hydrolysis by applying stress conditions. The linearity was investigated in the range of 40–120µg/mL (r^2 = 0.9996) for Tramadol HCl. The intraday and interday % RSD values were less than 0.9%. The LOD and LOQ were 0.24µg/mL and 0.72µg/mL Tramadol HCl was degraded in acid (1N HCl by Microwave) and alkali (1N NaOH) in different temperature conditions. Tramadol HCl was more degraded in alkaline condition compared to acidic. For the degradation kinetic in acidic and alkaline conditions the best fit was obtained for first-order reaction rate. The rate constant (k) is in the alkaline medium, 0.0034min^{-1} for R.T. NaOH, 0.0149min^{-1} for 60°C NaOH, 0.4849min^{-1} for 80°C NaOH and in acid medium 0.0653min^{-1} by Microwave. The $t_{1/2}$ was found to be less in alkaline condition compared to acidic conditions which shows, the drug has lower stability in alkaline medium. The $t_{1/2}$ is 1.18mins for alkaline condition and 6.39mins for acidic condition. The activation energy of Tramadol HCl in alkaline medium was found to be 3.771KJ/mole.

KEYWORDS: Tramadol HCl, Rate constant, Microwave, Alkali hydrolysis, Acid hydrolysis, Half life, Shelf life, RP – HPLC, Retention time, Chromatogram, Linearity,

Correlation coefficient, Arrhenius plot, Calibration curve, Interday and intraday precision, LOD, LOQ.

INTRODUCTION

Tramadol HCl (TR) is an opioid analgesic. Tramadol hydrochloride is a centrally acting opioid analgesic, used for treating moderate to severe pain. It is used in treatment of **Rheumatoid arthritis, restless legs syndrome, Parkinson's disease** and **fibromyalgia**. It also has noradrenergic and serotonergic properties that may contribute to its analgesic activity and is used for moderate to severe pain. In March **1995 ULTRAM** (JANSSEN PHARMS.) gained FDA approval in the US for treating severe pain. IUPAC name of Tramadol Hydrochloride is (1RS, 2RS)2-[(dimethylamino)methyl]-1-(3 methoxyphenyl cyclohexanol hydrochloride. Tramadol HCl is an official drug in Indian Pharmacopoeia 2010, British Pharmacopoeia 2009and United State Pharmacopoeia.[1-4]

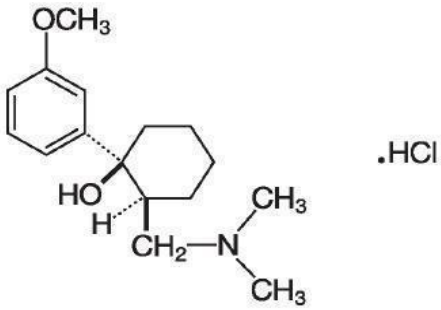

Figure-1: Chemical Structure of Tramadol HCl

EXPERIMENTAL

Materials

Tramadol HCl pure powder was obtained as a gift sample from Nascent Life Science Pvt. Ltd (Ahmedabad, Gujarat, India). Methanol (HPLC grade, Merck Specialties Private Ltd, Mumbai, India), Acetonitrile (HPLC grade, Merck Specialties Private Ltd, Mumbai, India), Water (HPLC grade, RFCL Limited, New Delhi, India).

Instrumentation

Double beam UV-Visible spectrophotometer– Shimadzu 1700, Kyoto, Japan.

- Electronic balance– Acculab, Model ALC 210, 4.
- pH meter– Chemline CL 180.
- HPLC– LC 2010CHT, Shimadzu 1700, Kyoto Japan.
- Ultra Sonicator– Enertech Fast Clean, EN 30 US, Mumbai, India.
- Hot air oven TO-90S, Thermo lab.

Preparation of standard stock solution (1000µg/mL)

Accurately weighed 10mg quantity of Tramadol HCl reference standard was transferred into 10mL volumetric flask and dissolved in 10mL methanol and sonicated for about 5min with intermittent shaking and diluted up to the mark with methanol to give a stock solution having strength 1mg/mL (1000µg/mL).

Preparation of working standard solution (100µg/mL)

100µg/mL of Tramadol HCl was prepared by diluting 1mL of stock solution to 10mL with Acetonitrile: Water (70: 30v/v).

Optimization of chromatographic conditions

The drug solution of Tramadol HCl (100µg/mL) was injected into HPLC system and allowed to run in different mobile phases like water:methanol in different ratio, water-acetonitrile were tried in order to find the optimum conditions for Tramadol HCl. It was found that mobile phase containing water:acetonitrile (30:70v/v) at a flow rate of 1.0mL/min with detection wavelength 240nm gave satisfactory results with sharp, well defined and resolved peak with minimum tailing as compared to other mobile phases. Under this condition the retention time of Tramadol HCl typically 6.54min (Figure-3) and optimized chromatographic conditions described in **Table-1.**[5-7]

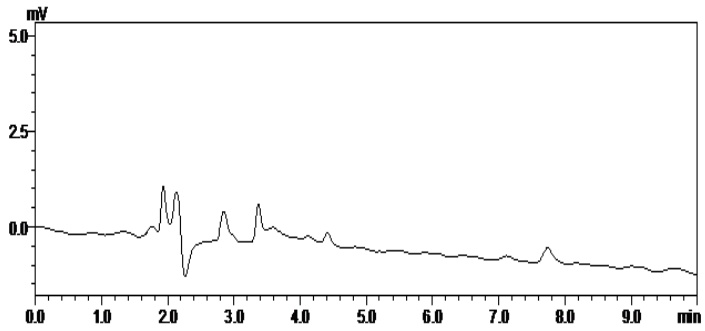

Figure-2: Chromatogram of blank

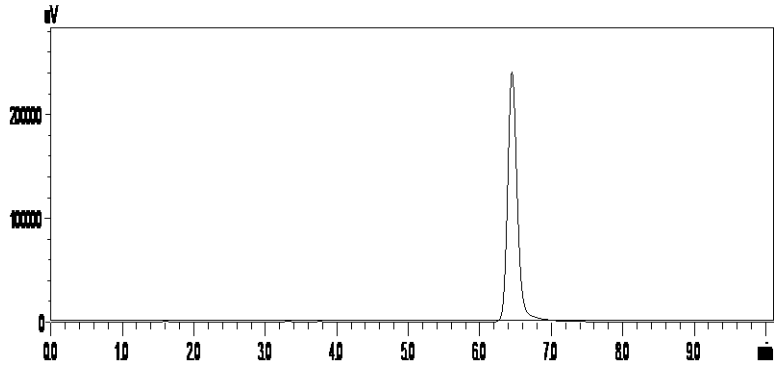

Figure-3: Chromatogram of Tramadol HCl 100µg/mL

Table-1: Optimized Chromatographic Condition for Tramadol HCl

Mobile Phase	**ACE C18 (150mm ×4.6 mm, 5µm particle size)**
Stationary Phase	ACN : Water (70:30)
Flow Rate	1mL/min
Detection Wavelength	272nm
Column Temperature	40°C
Run Time	10min
Injection Volume	20µL
Diluent	All Final Solution of Test and Standard were done with ACN : Water (70:30v/v)
Retention time (min)	6.54

a. HPLC Method Validation

The developed HPLC method was validated as per ICH guidelines for following parameters: Linearity, Limit of Detection, Limit of Quantification, Precision.

b. Degradation kinetic study[8,9]

i) Alkali hydrolysis (R.T., 60°C, 80°C)

Accurately weighed 20mg of Tramadol HCl was taken in 50mL volumetric flask, dissolved in 20mL methanol and added 10mL of 1N NaOH. The solution was kept in constant temperature water bath maintained at 60°C and 80°C temperature. From this solution,1.5mL was taken, transferred into 10mL volumetric flask at 0, 20, 40, 60, 80, 100, 120minutes for 40°C; 0, 10, 20, 30, 40, 50minutes for 60°C and 0, 3, 6, 9, 12, 15minutes for 80°C. Neutralized with 1N HCl and diluted up to mark with diluent ACN-Water (80:20v/v) (100µg/mL).

ACE C18 (150mm×4.6 mm, 5µm particle size).

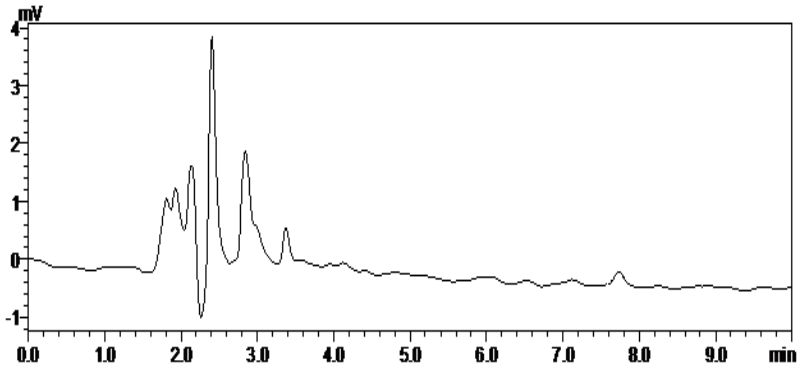

Figure-4: Chromatogram of 1N NaOH blank

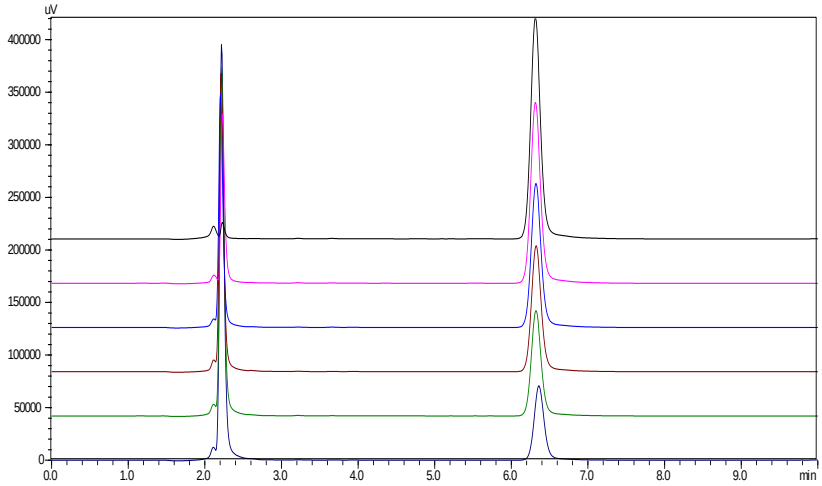

Figure-5: Overlain Chromatogram for alkali hydrolysis at Room temperature

Table-2: Calculation of degradation kinetic under alkali hydrolysis at Room Temperature

Time (hr)	Area	% Conc.	Conc. (µg/mL)	Log Conc.	1/Conc.	Rate Constant K(min^{-1})	% Degradation
0	2213671	100	100	2	0.01	-	-
1	1922351	86.84	86.84	1.93	0.0115	0.0024	13.16
2	1511494	68.28	68.28	1.83	0.0146	0.0032	31.72
3	1189848	53.85	53.85	1.73	0.0186	0.0035	46.15
4	927306	41.89	41.89	1.62	0.0238	0.0036	58.11
5	645727	29.17	29.17	1.46	0.0343	0.0042	70.83
Average						0.0034	

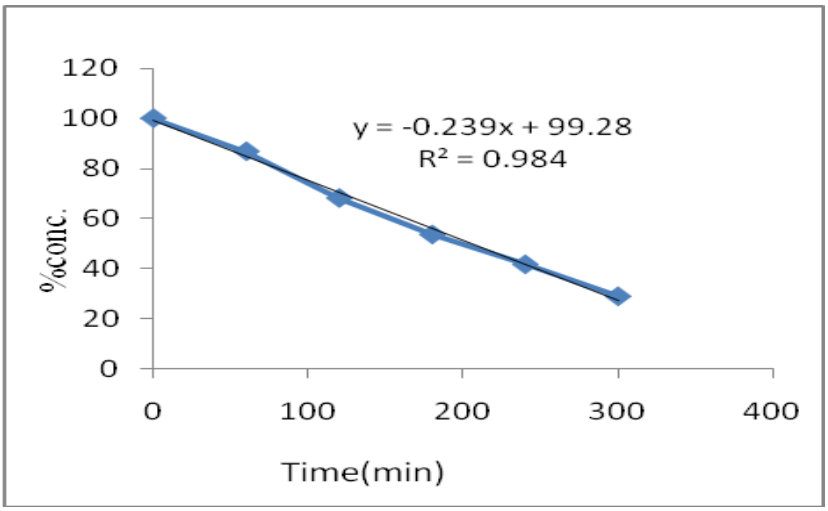

Figure-6: Zero order reaction

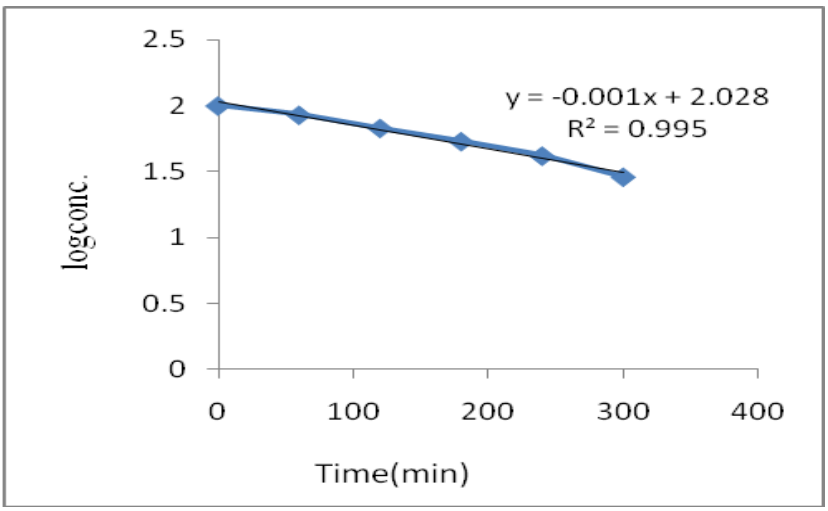

Figure-7: First order reaction

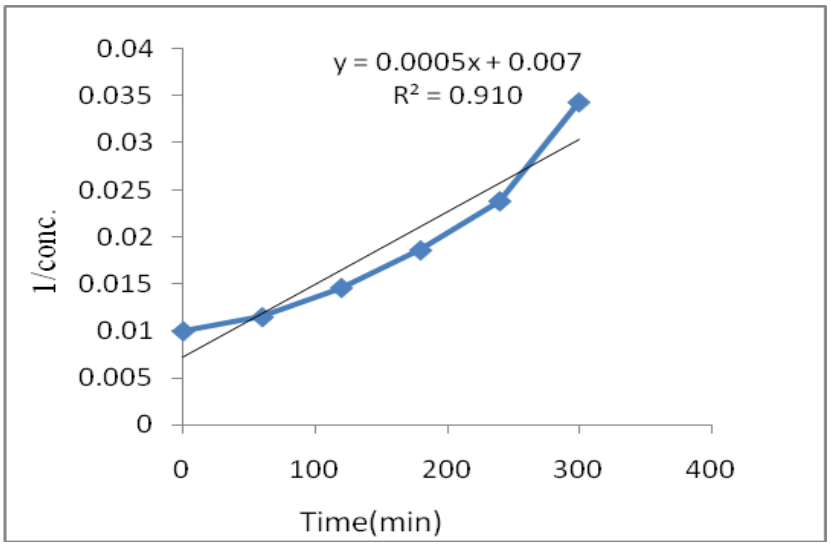

Figure-8: Second order reaction

Table-3: Order for degradation of Tramadol HCl at Room Temp. in 1N NaOH

Parameter	Zero order	First order	Second order
Correlation coefficient (r^2)	0.984	0.995	0.910

Rate constant = 0.0034min^{-1}

Half-life $t_{½}$ = 0.693/K

= 0.693/0.0034

= 203min

Shelf life t_{90} = 0.104/K

= 0.104/0.0034

= 30.58min

ii) Alkali hydrolysis (60°C)

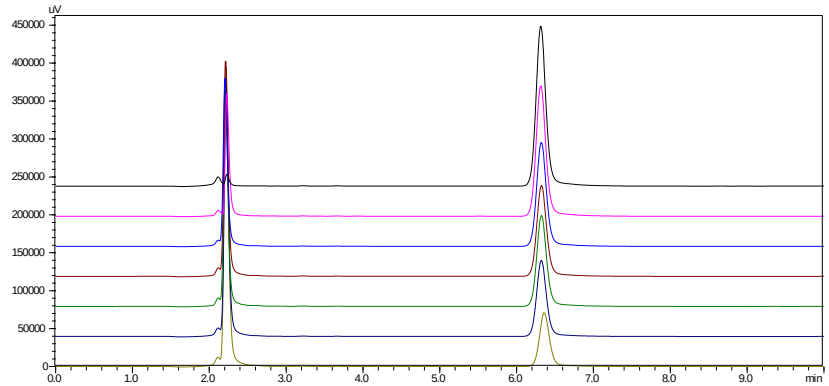

Figure-9: Overlain Chromatogram for alkali hydrolysis at 60°C temperature

Table-4: Calculation of degradation kinetic under alkali hydrolysis at 60°C temperature

Time (min)	Area	% Conc.	Conc. (µg/mL)	Log Conc.	1/Conc.	Rate Constant K(min^{-1})	% Degradation
0	2213671	100	100	2	0.01	-	-
20	1815874	82.03	82.03	1.91	0.0121	0.0009	17.97
40	1267548	57.26	57.26	1.75	0.0174	0.0139	42.74
60	732282	33.08	33.08	11	0.0302	0.0184	66.92
80	552975	24.98	24.98	1.31	0.0403	0.0173	75.02
100	302387	13.66	13.66	1.12	0.0732	0.0138	86.34
120	106477	4.81	4.81	0.68	0.2079	0.0253	95.19
Average						0.0149	

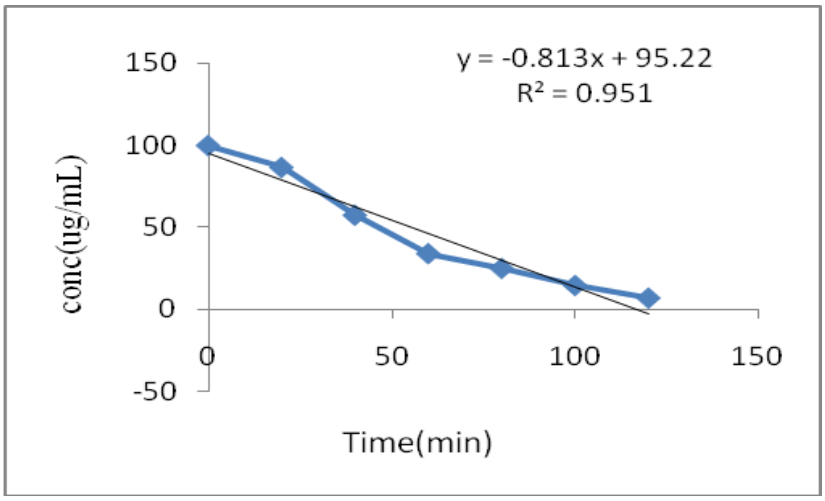

Figure-10: Zero order reaction

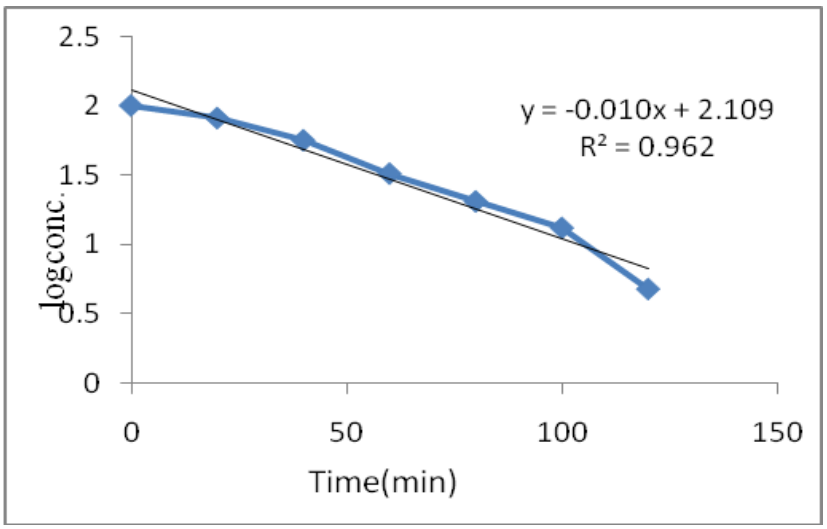

Figure-11: First order reaction

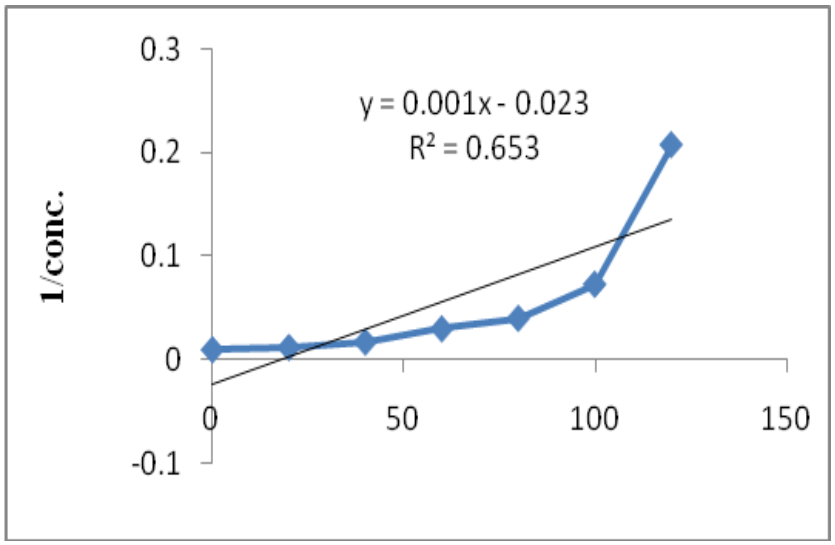

Figure-12: Second order reaction

Table-5: Order for Degradation of Tramadol HCl at 60ºC in 1.0N NaOH

Parameter	Zero order	First order	Second order
Correlation coefficient(r^2)	0.951	0.962	0.653

Rate constant = 0.0149 min^{-1}

Half-life $t_{½}$ = 0.693/K

= 0.693/0.0149

= 46.51min

Shelf life t_{90} = 0.104/K

= 0.104/0.0149

= 6.97min

iii) Alkali hydrolysis (80°C)

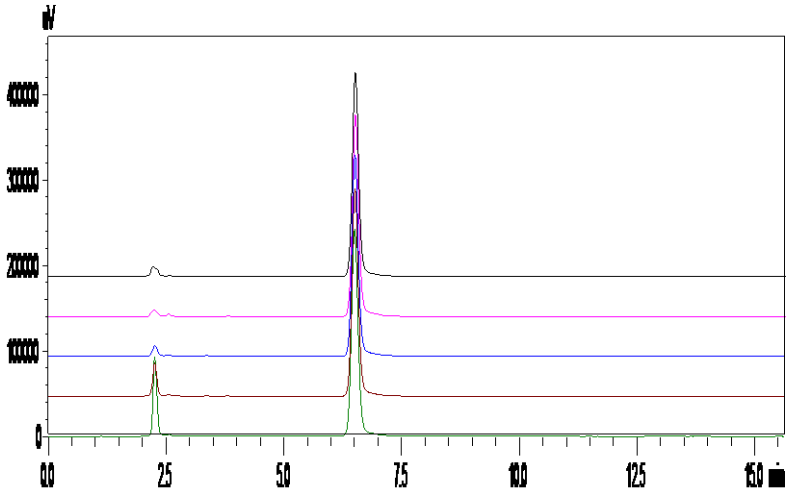

Figure-13: Overlain Chromatogram for alkali hydrolysis at 80ºC temperature

Table-6: Calculation of degradation kinetic under alkali hydrolysis at 80ºCtemperature

Time (min)	Area	% Conc.	Conc. (µg/mL)	Log Conc.	1/Conc.	Rate Constant K (min^{-1})	% Degradation
0	2213671	100	100	2	0.01	-	-
10	1754998	79.28	79.28	1.89	0.0136	0.2322	20.72
20	1279723	57.81	57.81	1.76	0.0172	0.2749	42.19
30	524197	23.68	23.68	1.37	0.0422	0.4802	76.32
40	240183	10.85	10.85	1.03	0.0920	0.5553	89.15
50	26785	1.21	1.21	0.08	0.826	0.8831	98.79
Average						0.4849	

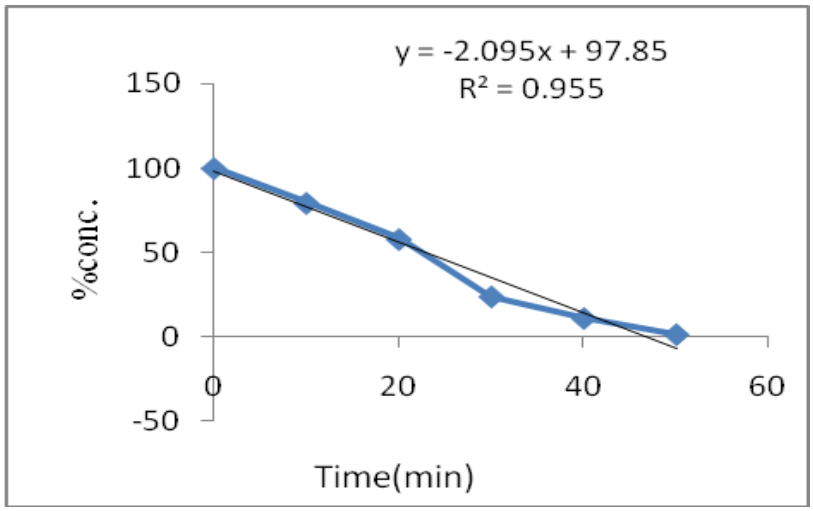

Figure-14: Zero order reaction

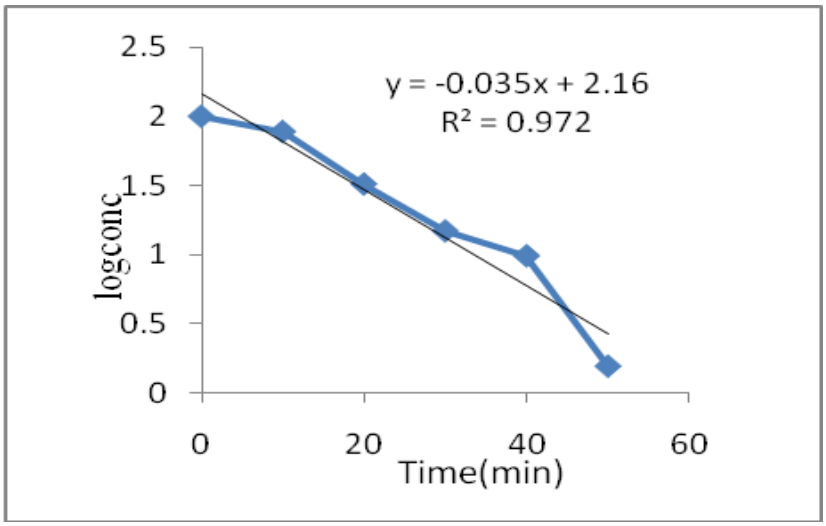

Figure-15: First order reaction

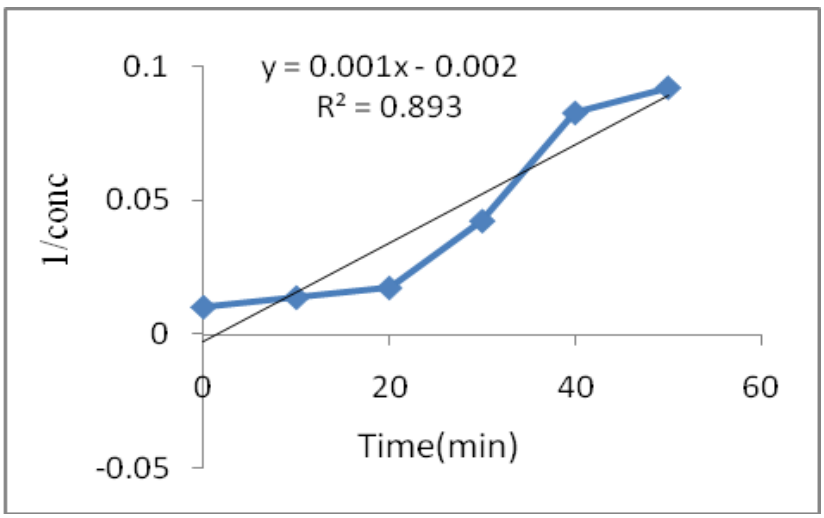

Figure-16: Second order reaction

Table-7: Order for degradation of Tramadol HCl at 80ºC temperature

Parameter	Zero order	First order	Second order
Correlation coefficient (r^2)	0.955	**0.972**	0.893

Rate constant = 0.4849min^{-1}

Half-life $t_{½}$ = 0.693/K

= 0.693/0.4849

= 1.42min

Shelf life t_{90} = 0.104/K

= 0.104/0.4849

= 13.2sec

Calculation of Energy of activation

Table-8: Data for Arrhenius plot

Temp. (ºC)	Temp. (ºK)	1/Temp ×1000	K (min^{-1})	log K
Room Temp. (±25)	298	3.3557	0.0034	-2.4685
60	333	3.0030	0.0149	-1.8268
80	353	2.8328	0.4849	-0.3143

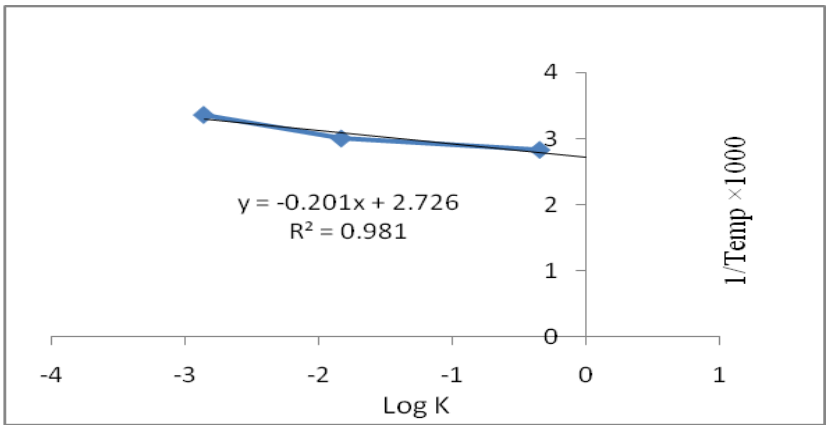

Figure-17: Arrhenius plot

Arrhenius plot

Slope = -0.201

Intercept (log A) = 2.726

Rate constant = 0.4849min^{-1}

Ea = -2.303×R×Slope

= -2.303×1.987×4.1×-0.201

= **3.771KJ/mol**

d) Acid hydrolysis by Microwave

Accurately weighed 20mg of Tramadol HCl was taken in 50mL volumetric flask, dissolved in 20mL methanol and 10mL of 1N HCl. From this solution, 1.5mL was taken, transferred into 10mL volumetric flask at 420watts for 0, 1, 2, 3, 4, 5 minutes and neutralized with 1N NaOH and diluted up to mark with diluent ACN-Water (80:20 v/v) (100µg/mL).

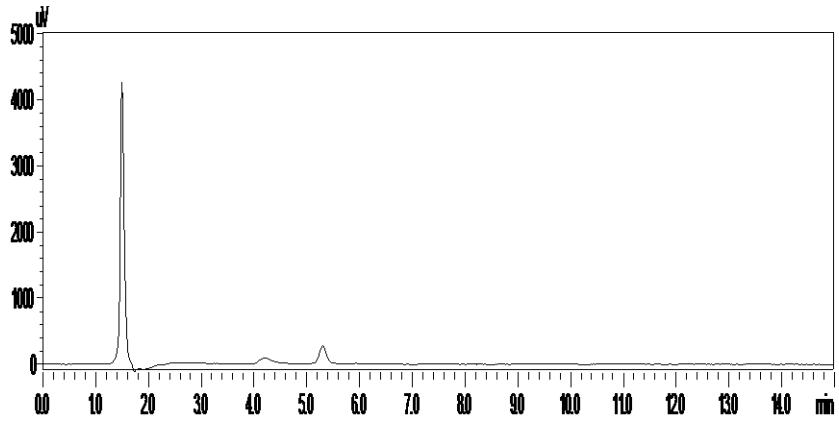

Figure-18: Chromatogram of 1N HCl blank

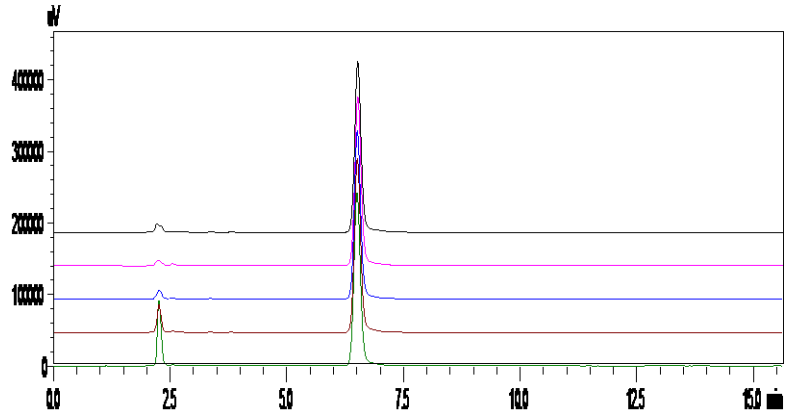

Figure-19: Overlain Chromatogram acid hydrolysis (1N HCl) by microwave

Table-9: Calculation of degradation kinetic under acid hydrolysis (1N HCl) by microwave

Time (min)	Area	% Conc.	Conc. (µg/mL)	Log Conc.	1/Conc.	Rate Constant K(min^{-1})
0	2213673	100	100 1	2	0.01	-
1	2120219	95.77	95.77	1.98	0.0104	0.0432
2	1821389	82.28	82.28	.91	0.0121	0.0847
3	1438726	64.99	64.99	1.81	0.0153	0.4310
4	1263467	57.07	57.07	1.75	0.0175	0.5609
Avg.					0.0653	

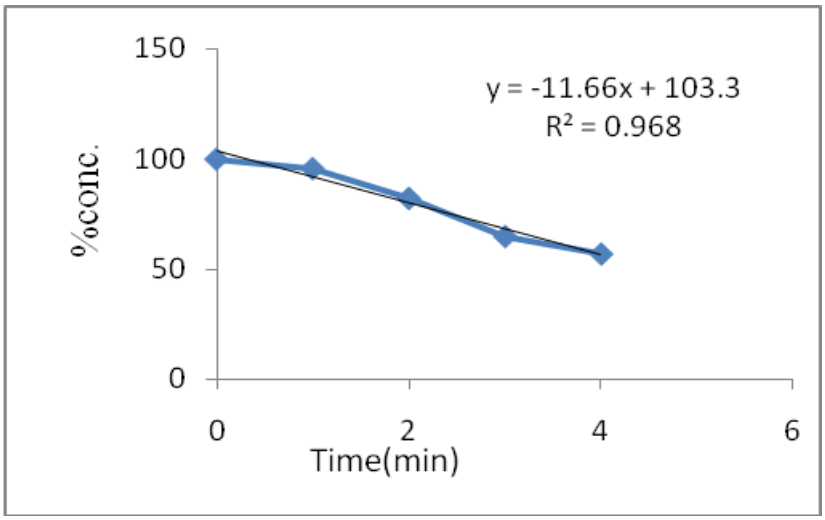

Figure-20: Zero order reaction

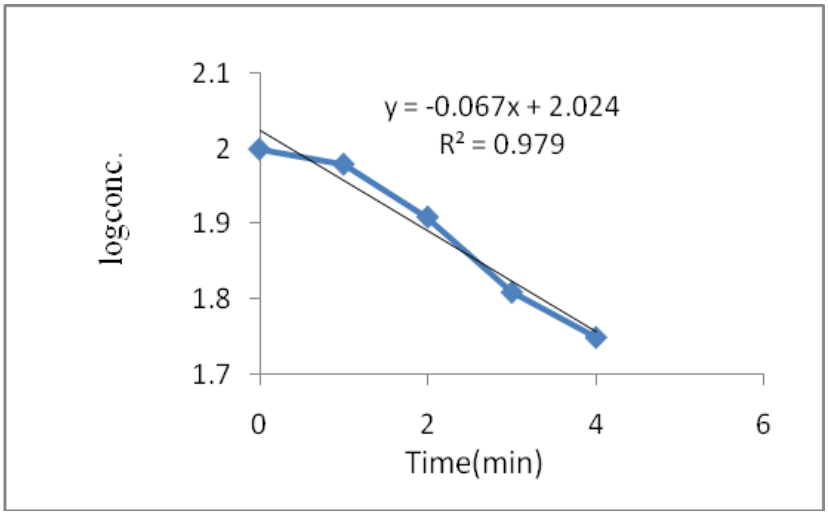

Figure-21: First order reaction

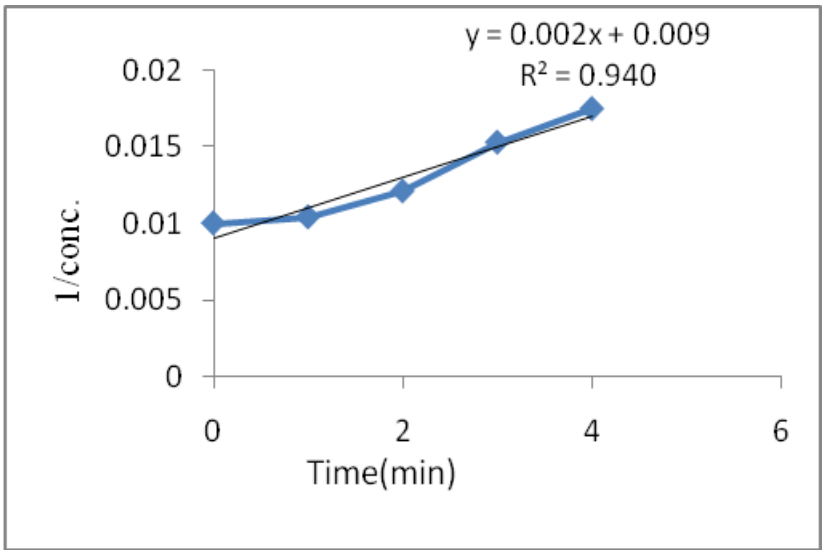

Figure-22: Second order reaction

Table-10: Order for Degradation of Tramadol HCl under acid hydrolysis (1N HCl) by microwave

Parameter	Zero order	First order	Second order
Correlation coefficient (r^2)	0.968	0.979	0.940

Rate constant = 0.0653min^{-1}

Half-life $t_{½}$ = 0.693/K

= 0.693/0.0653

= 10.61min

Shelf life t_{90} = 0.104/K

= 0.104/0.0653

= 1.59min

RESULTS AND DISCUSSION

Linearity

Overlain chromatogram of Tramadol HCl was shown in Figure-23. The linearity of Tramadol HCl was found to be in the range of 40-120µg/mL with correlation coefficient 0.999 as shown in Table-12.

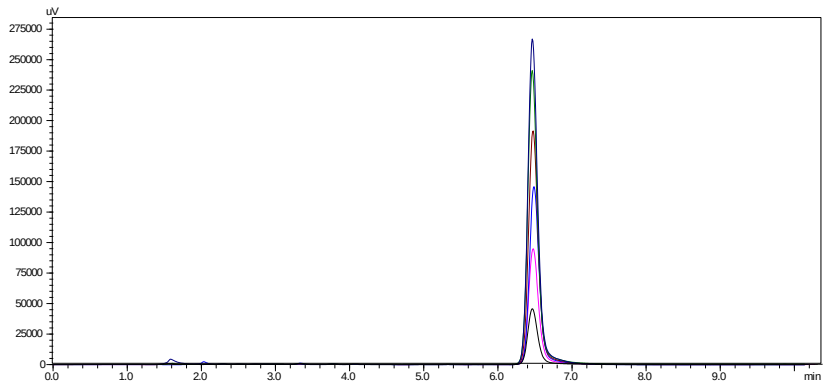

Figure-23: Overlain of Linearity Chromatogram of Tramadol HCl

Table-11: Linearity Data of Tramadol HCl

Tramadol Hydrochloride			
Concentration (µg/mL)	Area (n = 3)	SD	% RSD
40	937734	1586.813	0.17
60	1267871	2055.931	0.15
80	1891463	3654.993	0.21
100	2240932	10375.28	0.46
120	2677697	12127.09	0.45

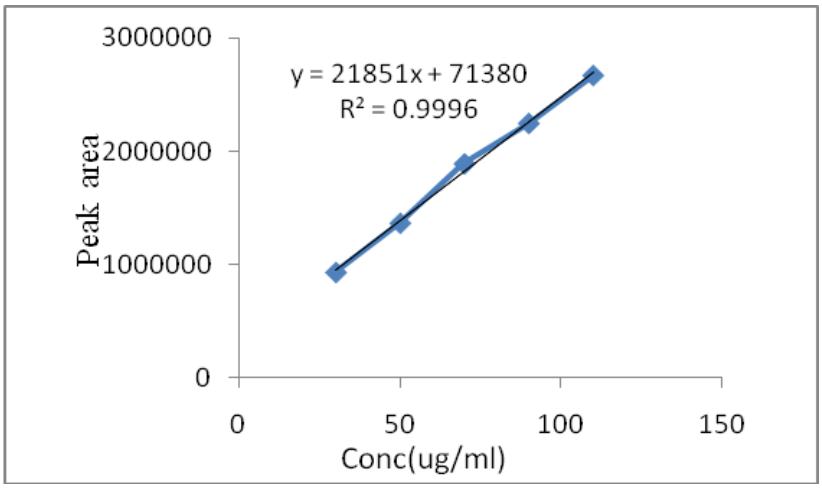

Figure-24: Calibration Curve of Tramadol HCl

Table-12: Linearity result of Tramadol HCl

Regression Analysis of Tramadol HCl	
Regression equation	Y=21851x+71380
correlation co –efficient	0.9996
Slope	21851
Intercept	71380

Precision

- **Intraday precision**

Table-13: Intraday precision

Time (hrs)	**Conc. (µg/mL)**	**Area**	**Mean**	**SD**	**% RSD**
1	**40**	935711	932473	2988.21	0.32
2		929821			
3		931889			
1	**80**	1891468	1889075	2568.588	0.14
2		1889396			
3		1886361			
1	**120**	2679807	2681167	5179.264	0.19
2		2686891			
3		2676804			

- **Interday precision**

Table-14: Interday precision

Time (day)	**Conc. (µg/mL)**	**Area**	**Mean**	**SD**	**% RSD**
1	**40**	935111	931933	2776.952	0.29
2		937921			
3		936889			
1	**80**	1894841	1891173	3295.832	0.17
2		1890216			
3		1888461			

1	**120**	2699867	2694467	6749.519	0.25
2		2686881			
3		2696714			

Limit of Detection and Limit of Quantitation

Table-15: LOD and LOQ

Parameters	Tramadol HCl
LOD	0.24µg/mL
LOQ	0.72µg/mL

Table-16: Summary of Degradation Kinetic of Tramadol HCl

Method	Degradation Condition	K (min^{-1})	$t_{(1/2)}$ (min)	t_{90} (min)	% Degradation
HPLC	R.T(±25) (NaOH)	0.0034	203	30.58	70.83
	60^0 C (NaOH)	0.0149	46.51	6.97	95.19
	80^0C (1N NaOH)	0.4849	1.42	13.2(sec)	98.79
	1N HCl By Microwave	0.0653	10.61	1.59	42.93

Table-17: Summary of validation parameters of Tramadol HCl

Sr. No.	Parameter		Tramadol HCl
1	Linearity Range		40–120µg/mL
2	Regression equation		y = 21851x + 71380
3	Correlation co-efficient		0.9996
4	Precision (% RSD)	Interday	0.14-0.32
		Intraday	0.17-0.29
5	Limit of Detection		0.24µg/mL
6	Limit of Quantification		0.72µg/mL

CONCLUSION

The method is found to be specific as there was no interference of any co-eluting degradation product after degradation study. The degraded products are well resolved with satisfactory peak purity index, indicating the methods can also be useful for determination of degraded products. The proposed method is found to be simple, accurate and precise. Tramadol HCl was highly degraded in alkaline condition. It degraded significantly in acidic condition. The alkaline and acidic degradation of Tramadol HCl were found to follow a first order reaction rate. It was found that as the temperature increased the rate of degradation increased with decrease in the $t_{1/2}$. The activation energy of Tramadol HCl in alkaline medium was found to be 3.771KJ/mole. The RP - HPLC method was found to be simple, accurate and reproducible.

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