

# VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TRAMADOL HYDROCHLORIDE IN TABLETS AND ITS STRESS DEGRADATION STUDIES

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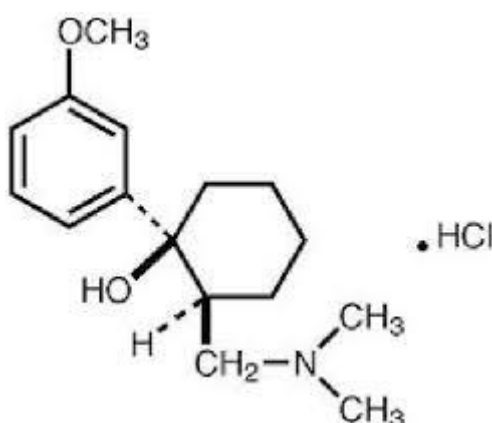
## **ABSTRACT**

*It is aimed to develop simple and economic spectrophotometric method for the estimation of Tramadol hydrochloride in bulk and tablet dosage form and validate as per ICH guidelines. Method involved absorbance maxima method which based on the measurement of absorbance of Tramadol hydrochloride in water at 265 nm. The present method was validated for parameters like linearity, precision, accuracy, Limit of Detection and Limit of Quantitation as per ICH guidelines. The proposed method was found to be linear within the concentration range of 10-160 µg/ml for Tramadol hydrochloride. It was subjected to different stress conditions as per ICH guidelines. A stability indicating spectrophotometric technique was created to analyse the drug while it was being degraded. It involved a 4 hour study, in which distilled water was used as a solvent. The amount of degraded drug was calculated by taking absorbance at 265 nm. Degradation of Tramadol hydrochloride was found to be more in alkaline and less in neutral condition.*

**Key words:** *Tramadol hydrochloride, UV spectrophotometry, Method development, Validation, Stress degradation studies.*

## 1. INTRODUCTION

Tramadol hydrochloride is an analgesic which binds to opioid receptors. It is a non-selective, pure opioid agonist at  $\mu$ , delta and kappa opioid receptors with a higher affinity for  $\mu$  receptors<sup>[1,6]</sup>. Other mechanisms which contribute to its analgesic effects are inhibition of neuronal reuptake of noradrenaline and enhancement of serotonin release. It is chemically known as ( $\pm$ )cis-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride<sup>[2,7]</sup>. (Fig.1). Tramadol hydrochloride can be estimated by UV spectrophotometry, RP-UPLC and GC-MS alone or in combination with other drugs. The use of stability testing is to give knowledge on how the quality of an active pharmaceutical ingredient



**Fig No.1 Chemical Structure of Tramadol hydrochloride**

changes with time under the influence of various environmental factors such as temperature, humidity, and light, and to provide a re-test period for the active pharmaceutical ingredient or a shelf life for the medicinal product as well as recommended storage conditions. Already UV spectrophotometric methods, UPLC and HPLC methods were done for the estimation of Tramadol hydrochloride<sup>[3,4]</sup>. Stress degradation studies of Tramadol hydrochloride was performed by HPLC but not by UV spectrophotometric method<sup>[15]</sup>. So present work was done to perform stress degradation studies by using UV spectrophotometric method.

## 2. MATERIALS METHODS

### Instrumentation and chemicals:

Spectral runs were made on a UV-Visible double beam spectrophotometer Labindia 3000 with wavelength band width of 1 nm. Tramadol hydrochloride was kindly provided by Hetero Labs (Hyderabad) as gift sample. Double distilled water was produced from distillation unit.

### Method development:

#### *Solvent selection*

Solubility studies were conducted to the Tramadol hydrochloride with various solvents. It was found that Tramadol HCl was freely soluble in distilled water and methanol. Finally distilled water was selected as it is cheap<sup>[6]</sup>.

### ***Preparation of standard stock solution***

Standard Tramadol hydrochloride of 100 mg was weighed and transformed to a 100ml volumetric flask and dissolved in 25ml of distilled water. The flask was shaken and volume was made up to the mark with distilled water to give a solution containing 1000 $\mu$ g/ml.

### ***Determination of maximum absorbance wavelength of 10 $\mu$ g/ml solution***

Stock solutions were further diluted with distilled water to get working standard concentrations of 10-160 $\mu$ g/ml and the working standards were scanned between 200-400nm for maximum absorbance that showed at 265nm<sub>[13]</sub>.

### ***Selection of analytical concentration range***

From standard stock solution of Tramadol hydrochloride, appropriate aliquots were pipette out in 10ml volumetric flasks and make up the volume with distilled water to obtain working standard solutions of concentrations from 10-160 $\mu$ g/ml. Absorbance for these solutions were measured at 265nm. These concentrations were showing linear values.

### ***Construction of Calibration Curve***

Appropriate volumes of aliquots from standard Tramadol hydrochloride stock solution were transferred to different volumetric flasks of 10ml capacity. The volume was adjusted to the mark with distilled water to obtain concentrations of 10,20,30,40,50,60  $\mu$ g/ml respectively. Absorbance value of each solution against distilled water as a blank were measured at 265nm. From that absorbance value, Regression equation and correlation coefficient ( $R^2$ ) are determined<sub>[12]</sub>.

### ***Assay***

20 tablets were weighed and finely powdered and an accurately weighed sample of powdered tablets of Tramadol hydrochloride was dissolved in 25ml of distilled water and the solution was filtered then the solution was transferred into 100ml of volumetric flask and volume was made up to the mark with distilled water. From this solution 1.6ml was taken into 10ml of volumetric flask and volume was made up to the mark with distilled water to obtain 160 $\mu$ g/ml. From this solution 1ml was taken into cuvette and the absorbance was measured at 265nm<sub>[13]</sub>.

### **Method Validation:**

#### ***Validation Parameters***

The method validation was performed in terms of specificity, linearity, LOQ, LOD, Precision, accuracy, robustness and ruggedness<sub>[8]</sub>.

#### ***Linearity***

From standard stock solution of Tramadol hydrochloride, appropriate aliquots 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 ml respectively were pipetted out in 10ml volumetric flasks and make up with distilled water to obtain working standard solutions of

concentrations from 10-160µg/ml. Absorbance for these solutions were measured at 265nm. These concentrations were showing linear values. From that absorbance value, Regression equation and correlation coefficient ( $R^2$ ) are determined.

### ***Precision***

Precision of methods was studied as intraday and inter day. Intra-day study was performed by analysing, the three different concentrations of drug for three times in the same day. Inter-day Precision was performed by analysing three different concentration of drugs for three days in a week. Three different concentration were LQC, MQC, HQC.

### ***Accuracy***

The accuracy of the proposed methods were assessed by recovery studies at three different levels i.e. 80%, 100%, 120%.The recovery studies were carried out by adding known amount of standard solution of the drug to the pre analysed tablet solutions, the resulting solutions were then reanalysed by proposed methods.

### ***Limit of detection (LOD)***

The limit of Detection was found by formula method. The formula is **LOD=3.3 standard deviation/slope**

### ***Limit of Quantification (LOQ)***

The limit of quantification was found by formula method. The formula is **LOQ=10 standard deviation/slope**

### ***Robustness***

Absorbance values were found by changing the experimental conditions slightly. Maximum absorbance wavelength was changed slightly and the values were recorded in the table.

### ***Ruggedness***

Absorbance values were taken by two analysts with the same instrument.

### **Stress degradation studies<sup>[10]</sup>:**

#### ***Acid hydrolysis***

To 15ml of stock solution (60µg/ml) of Tramadol hydrochloride, 5ml of 0.5N HCl and 5ml distilled water was added and initial absorbance was checked out then, it was kept for one hour and the absorbance was checked. This procedure was repeated for 2, 3 and 4 hours and absorbance was noted. For the blank, 5ml of 0.5N HCl was added to 20ml of distilled water.

#### ***Base hydrolysis***

To the 15ml of stock solution (60µg/ml) of Tramadol hydrochloride, 5ml of 0.5N NaOH and 5ml of distilled water was added and initial absorbance was checked. Then it was left aside

after 1, 2, 3 and 4 hours. The absorbance was checked. For the blank, 5ml of 0.5N NaoH was added to 20ml of distilled water.

### ***Neutral hydrolysis***

To the 15ml of stock solution(60 $\mu$ g/ml) of Tramadol hydrochloride, 5ml of methanol and 5ml of distilled water were added and initial absorbance and after 1,2,3,4 hours the absorbances were checked and for the blank, 5 ml of methanol was added to 20ml of distilled water.

### ***Thermal hydrolysis***

Tramadol hydrochloride sample was taken in a Petri plate and exposed to a temperature of 70 degree c for 48 hours in an oven. After 48 hours, 10mg of the sample was diluted with distilled water in order to make the volume upto 10ml. From this solution, dilutions were carried out to achieve the appropriate concentration (10 $\mu$ g/ml) and the solution was taken in cuvette for the UV-VIS Analysis.

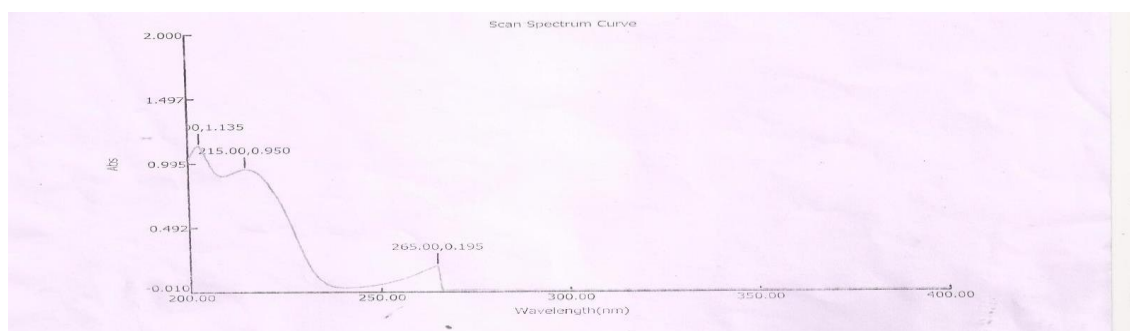
### ***Oxidative degradation***

To the 15ml of stock solution (60 $\mu$ g/ml) of Tramadol hydrochloride, 5ml of 3% hydrogen peroxide and 5ml of distilled water was added. For the blank, 5ml of 3% Hydrogen peroxide were added to the 20 ml of distilled water. These solutions were kept at room temperature for one hour then the solution was taken in a cuvette and analysed in UV. The absorbances were collected after 1 hr, 2 hr, 3 hr, 4 hr.

### ***Photolytic degradation***

Sample of Tramadol hydrochloride was exposed to sunlight for one hour then, 10mg was dissolved in distilled water make up to 10ml and from this solution appropriate dilution (10 $\mu$ g/ml) was made using distilled water and taken in cuvette for the UV analysis. The absorbances were collected after 1 hr, 2 hr, 3 hr, 4 hr.

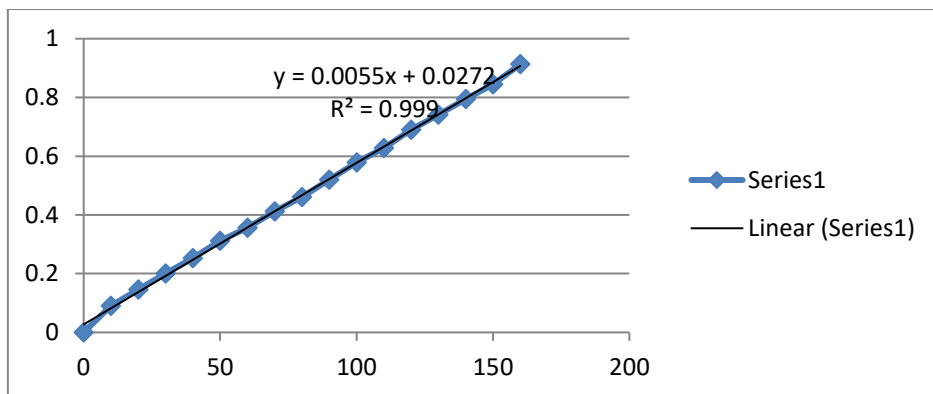
## **3. RESULTS**



**Fig. 1 Absorption Spectrum of Tramadol hydrochloride**

**Table No.1 Linearity of Tramadol hydrochloride**

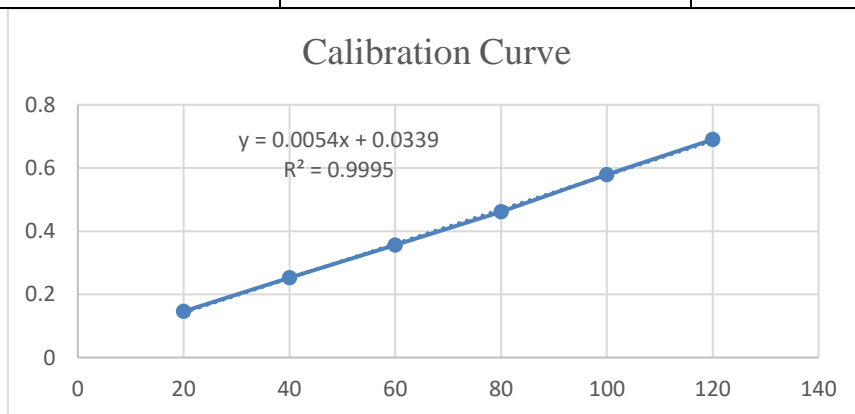
S.No	Conc( $\mu\text{g/ml}$ )	Absorbance $\pm$ SD	%RSD
1	10	0.195 $\pm$ 0.0052	1.90
2	20	0.203 $\pm$ 0.0041	1.97
3	30	0.241 $\pm$ 0.0058	0.87
4	40	0.267 $\pm$ 0.005	1.82
5	50	0.311 $\pm$ 0.0065	1.34
6	60	0.34 $\pm$ 0.0082	0.895
7	70	0.375 $\pm$ 0.0075	0.98
8	80	0.41 $\pm$ 0.0049	0.096
9	90	0.452 $\pm$ 0.0072	1.53
10	100	0.579 $\pm$ 0.0041	1.97
11	110	0.628 $\pm$ 0.0058	0.87
12	120	0.69 $\pm$ 0.0041	1.97
13	130	0.741 $\pm$ 0.0058	0.87
14	140	0.795 $\pm$ 0.005	1.82
15	150	0.845 $\pm$ 0.0082	0.895
16	160	0.913 $\pm$ 0.0075	0.98



**Fig. 2 Linearity curve of Tramadol hydrochloride (analytical range)**

**Table No. 2 Calibration curve data of Tramadol hydrochloride**

S.No	Conc( $\mu\text{g/ml}$ )	Absorbance $\pm$ SD	%RSD
1	20	0.146 $\pm$ 0.0052	1.90
2	40	0.253 $\pm$ 0.0043	1.97
3	60	0.356 $\pm$ 0.0053	0.87
4	80	0.461 $\pm$ 0.0042	0.895
5	100	0.579 $\pm$ 0.0049	1.34



**Fig.3 Calibration curve of Tramadol hydrochloride**

**Table No.3 Regression and Analytical parameters**

S.No	Parameter	Result
1	Lamda max, nm	265
2	Molar absorptivity(mol/l)	0.0059
3	Range (µg/ml)	10-160
4	Sandell's sensitivity(µg/cm <sup>2</sup> )	0.168
5	Limit of detection (µg/ml)	1.31
6	Limit of quantification (µg/ml)	3.98
7	Regression equation	Y=0.005x+0.033
8	Slope	0.005
9	Intercept	0.033
10	Correlation coefficient	0.999

**Table No.4 Precision studies of Tramadol hydrochloride**

S.No	Sample	Intra day (%RSD)	Inter day (%RSD)
	Tramadol HCl		
1	LQC	1.9	1.86
2	MQC	1.72	1.989
3	HQC	0.87	0.957



**Table No. 5 Recovery Studies**

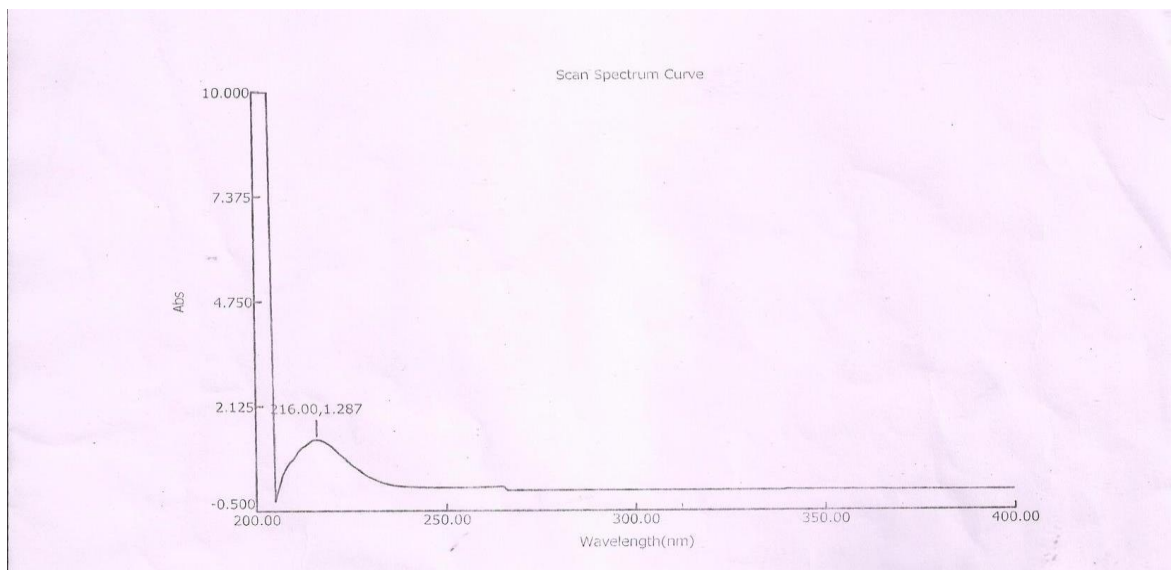
S.No	Name of the drug	Amount of Sample ( $\mu\text{g/ml}$ )	Recovery level	Amount of drug added ( $\mu\text{g/ml}$ )	Total amount found ( $\mu\text{g/ml}$ ) $\pm$ SD	%Recovery	%RSD
1	Tramadol hydrochloride	120	80%	216	239.6 $\pm$ 0.9	101	1.04
			100%	240	256 $\pm$ 0.7	100	1.81
			120%	264	272 $\pm$ 0.51	99	1.55

**Table No.6 Assay Studies**

Drug	Lable claim	Amount found	%Recovery	%RSD
Tramadol hydrochloride	50mg	49.5 mg	98.9	1.46

**Table No.7 Alkali Degradation (0.5N of NaOH) of Tramadol hydrochloride**

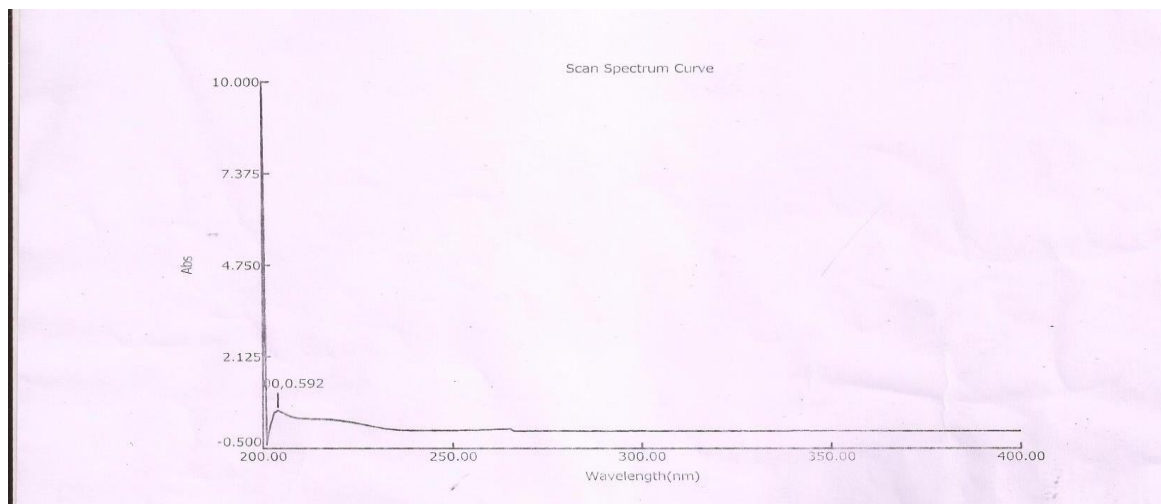
Name	Absorbance	Conc( $\mu\text{g/ml}$ )	%Degradation
Analyte at 0 h	0.114	60	0
Analyte at 1 h	0.099	52	13
Analyte at 2 h	0.005	2.6	95
Analyte at 3 h	0.004	2.1	96
Analyte at 4 h	0.004	2.1	96



**Fig.4 Alkali Degradation (0.5N of NaoH) spectrum of Tramadol hydrochloride**

**Table No.8 Acid Degradation of Tramadol hydrochloride**

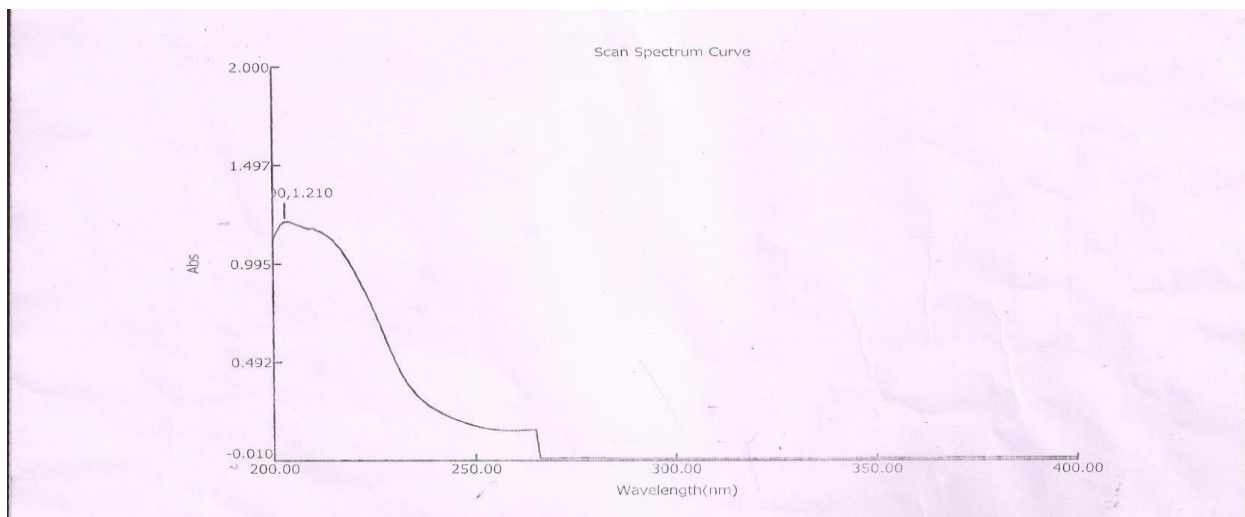
<b>Name</b>	<b>Absorbance</b>	<b>Conc(µg/ml)</b>	<b>%Degradation</b>
Analyte at 0 h	0.135	60	0
Analyte at 1 h	0.123	55	8.3
Analyte at 2 h	0.022	9.7	83
Analyte at 3 h	0.009	4	93
Analyte at 4 h	0.008	3.5	94



**Fig.5 Acid Degradation (0.5N of NaoH) spectrum of Tramadol hydrochloride**

**Table No.9 Thermal Degradation of Tramadol hydrochloride**

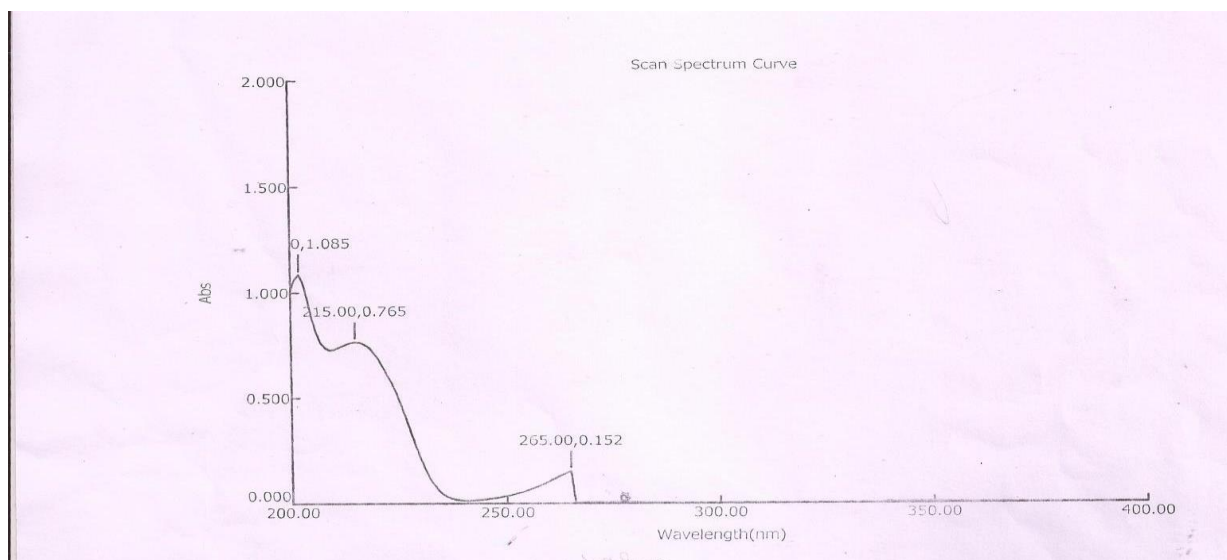
Name	Absorbance	Conc(µg/ml)	%Degradation
Analyte at 0 h	0.166	60	0
Analyte at 1 h	0.155	56	6.6
Analyte at 2 h	0.035	13	78
Analyte at 3 h	0.027	10	83
Analyte at 4 h	0.024	9	85



**Fig.6 Thermal Degradation spectrum of Tramadol hydrochloride**

**Table No.10 Photolytic Degradation of Tramadol hydrochloride**

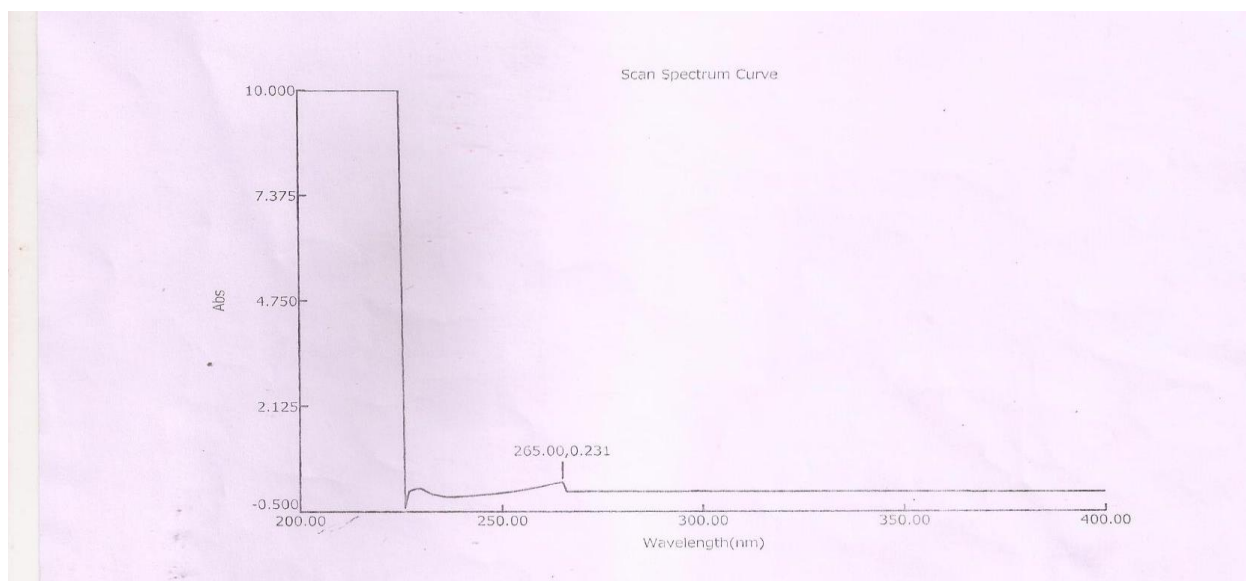
Name	Absorbance	Conc(µg/ml)	%Degradation
Analyte at 0 h	0.188	60	0
Analyte at 1 h	0.178	57	5
Analyte at 2 h	0.047	15	75
Analyte at 3 h	0.043	14	76
Analyte at 4 h	0.040	13	78



**Fig.7 Photolytic Degradation spectrum of Tramadol hydrochloride**

**Table No.11 Oxidative Degradation of Tramadol hydrochloride**

Name	Absorbance	Conc( $\mu\text{g/ml}$ )	%Degradation
Analyte at 0 h	0.200	60	0
Analyte at 1 h	0.193	58	3.3
Analyte at 2 h	0.060	18	70
Analyte at 3 h	0.046	14	76
Analyte at 4 h	0.033	10	83



**Fig.8 Oxidative Degradation spectrum of Tramadol hydrochloride**

## DISCUSSION

The selected Tramadol hydrochloride was estimated by UV spectrometry. The method was validated for all validation parameters as per ICH guidelines. The linearity range for Tramadol hydrochloride was 10-160 $\mu$ g/ml with  $R^2$  value of 0.999. The %RSD for intraday and interday was <2%. The assay of tablet dosage forms was performed. The accuracy of the method was validated by recovery studies and found to be significant and under specification limits with %Recovery (99-101) (within acceptable range 98-102%). The assay results were found to be (98.9%) (i.e. within 95-105).

## CONCLUSION

The proposed method is simple, rapid, accurate and precise and Cost effective. It can be used for the determination of Tramadol hydrochloride in pure form and in pharmaceutical dosage form as well as its percentage degradation.

It was found that Tramadol hydrochloride was highly degraded in alkali and low in neutral condition (methanol). The decreasing order of degradation was with alkali, acid, thermal, photolytic, oxidative and neutral conditions. It is used to determine storage conditions for the drug. Hence it can be successfully used to study stress degradation behavior of Tramadol hydrochloride in small scale industries where high end instruments are not available.

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