

*Original research article***STABILITY INDICATING SPECTROFLUORIMETRIC QUANTIFICATION OF TAPENTADOL HCL AND APPLICATION TO *IN-VITRO* DISSOLUTION STUDIES**

Panikumar.D.Anumolu*, Haripriya A, Sirisha N, Venkat Raju Y, Sunitha G, Venkateswara Rao A.

Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Andhra Pradesh-500090

ABSTRACT

Purpose: In this present work a simple, rapid, specific and highly sensitive spectrofluorimetric method was developed and validated for the quantification of tapentadol HCl bulk drug and pharmaceutical dosage forms and proposed method was also applied to study of forced degradation and *in-vitro* dissolution studies.

Materials and Methods: The fluorescence intensity of tapentadol HCl in distilled water was measured at emission wavelength 592 nm after excitation at 272 nm by using a Shimadzu (Japan) RF-5301 PC spectrofluorophotometer. A linear relationship was found between fluorescence intensity and concentration in the range of 1-6 µg/mL with a good correlation coefficient – 0.999.

Results: The detection and quantification limits were found to be 23.01 and 76.72 ng/mL, respectively. The proposed method was applied for quantification of tapentadol HCl in tablets, with percentage recovery of 99.95–101.45% and percentage RSD values were found to be less than 2 for accuracy and precision studies. Statistical analysis of the results revealed high accuracy and good precision.

Conclusion: The suggested procedures could be used for the determination of tapentadol HCl in drug substance and drug products as well as in presence of its degradation products.

Keywords: Emission Wavelength – 592 nm, Excitation Wavelength – 272 nm, Fluorimetry, Forced degradation, tapentadol HCl.

Corresponding author: Panikumar D Anumolu Gokaraju Rangaraju College of Pharmacy, Department of Pharmaceutical Analysis, Hyderabad, Andhra Pradesh- 500090. E.: panindrpharma@yahoo.co.in; Tel.: 919010014734; Fax: 040-23041700

INTRODUCTION

Tapentadol hydrochloride (Tapentadol HCl) is centrally acting synthetic analgesic [1, 2]. It is chemically 3-[(1*R*, 2*R*)-3-(dimethylamino)-1-ethyl-2-methylpropyl] phenol hydrochloride [Figure 1]. After thorough literature survey, we came to know that there is no stability indicating method available for the estimation of tapentadol HCl either in bulk drug or in formulation by spectrofluorimetry.

Spectrofluorimetry has assumed a major role in drug analysis because of its greater sensitivity and selectivity than absorption spectrophotometry [3-9]. Hence, we aimed to develop and

validate a simple, precise, economical and accurate spectrofluorimetric method for the estimation of tapentadol HCl and application of the proposed method for forced degradation and *in-vitro* dissolution studies of tapentadol HCl bulk drug and marketed formulations.

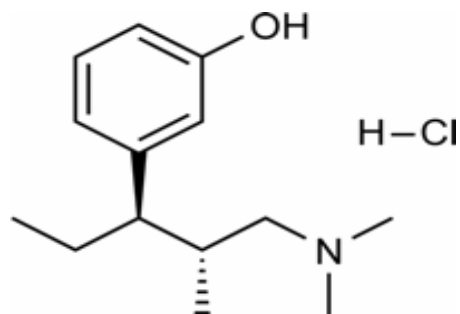


Figure 1. Structure of tapentadol HCl

MATERIALS AND METHODS

Instruments

The fluorescence spectra and measurements were recorded using a Shimadzu (Japan) RF-5301 PC Spectrofluorophotometer, equipped with 150 watt Xenon arc lamp, 1cm quartz cell was used, connected to RFPC software. The instrument was operated both at low and high sensitivity with excitation and emission slit width set at 5 nm, dissolution apparatus (Electro lab TDT-08L), analytical balance (Shimadzu AUX 220, Japan) and pH meter (Elico) were used for the study.

Materials

Tapentadol HCl active ingredient sample was gifted by MSN Laboratories Private Limited (Hyderabad, India). Tapenta-50 and Tydol-50 both commercial formulations containing 50 mg of the drug were obtained from the local pharmacies. The analytical grade solvents such as methanol, ethanol, acetonitrile, acetone, dimethyl Formamide (DMF), dimethyl sulphoxide (DMSO) were obtained from Sd Fine-Chem Ltd., Mumbai.

Preparation of standard solutions

A stock solution of tapentadol hydrochloride was prepared by dissolving 10 mg of the drug in 10 mL of distilled water to get 1mg/mL stock solution [10, 11]. Standard solutions were prepared by appropriate dilution of the stock solution with the same solvent.

Analysis of tapentadol hydrochloride in tablet dosage form

Twenty tablets of each marketed formulation (Tapenta-50 and Tydol -50) were taken and accurately weighed. An accurately weighed powder equivalent to 10 mg tapentadol HCl was transferred to volumetric flask of 10 mL capacity. The flask was shaken and volume was made up to the mark with water. The solution was filtered through whatmann filter paper (No.41). From the filtrate a final concentration of 5 µg/mL solution was prepared for the estimation of tapentadol HCl.

The amount of tapentadol HCl present in the sample solution was determined by substituting fluorescence intensity into the equation of the straight line representing the calibration curves for tapentadol HCl, with correction for dilution.

Method validation

The method was validated linearity, precision, accuracy, selectivity and sensitivity by the following procedures [12, 13].

Linearity

Aliquots of tapentadol HCl stock solutions were transferred into a series of 10 mL volumetric flasks so that the final concentration was in the range of 1.0-6.0 µg/mL. Then, the volumes were completed to the mark with distilled water and mixed well. The fluorescence intensities of the solutions were measured at 572 nm after excitation at 272 nm. The calibration curve was constructed by plotting the analyte intensity against the concentration (µg/mL).

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on standard calibration curve.

Selectivity

Selectivity is the ability of the method to accurately measure a compound in the presence of other components such as impurities, degradation products and matrix components. The selectivity of the proposed method was evaluated through the analysis of a placebo solution, which it was prepared with the common excipients (lactose, starch, microcrystalline cellulose, magnesium stearate, titanium dioxide and talc) of the pharmaceutical formulation. Thus, the mixture of component inert was prepared in their usual concentration employed in tablets (concentrations were determined based in Handbook of pharmaceutical Excipients and calculated for medium weight of content). The developed method was applied to in order to check if any component of the formulation could generate a response or a read with emission band similar to the drugs.

Precision

Precision of the method was determined by intra-day precision and inter-day precision variations as per ICH guidelines. For both intra-day precision and inter-day precision of the samples containing tapentadol HCl 2,4, and 6 µg/mL were analyzed six times on the same day (intra-day precision) and for three consecutive days (inter day precision). The % RSD was calculated.

Accuracy

The accuracy of the method was determined by calculating recovery of tapentadol HCl by the method of standard additions. Tablet powder (Tapenta and Tydol) equivalent to 10 mg of tapentadol HCl was transferred into three different 10 mL volumetric flasks and to it 80%, 100% and 120% of pure bulk drug was added respectively and made the volume up to the mark with distilled water. These solutions were further diluted with distilled water and analyzed by using water as blank. The amount of tapentadol HCl was estimated by measuring fluorescence intensity at the appropriate wavelengths. The recovery was verified by estimation of drug in triplicate preparations at each specified concentration level.

Forced degradation studies

Forced degradation studies were carried out by exposing the sample solution to stress conditions such as acidic, alkaline, oxidative, thermal and UV effect.

Stock solution was prepared by dissolving 10 mg of tapentadol HCl in 10 mL of 1N HCl. From the above stock solution 1mL was taken in 10 mL volumetric flask and to this 1mL of 1N NaOH was added to neutralize the solution and the volume was made up to the mark with distilled water. One of the concentrations in the range of linearity was prepared by appropriate dilution and scanned in spectrofluorimeter.

This procedure was repeated for every 1 hr. Similarly alkaline degradation (1N NaOH), oxidative degradation (3% H₂O₂), thermal degradation (hot air oven at 105⁰C) was performed. For UV degradation 50 mg of accurately weighed drug was placed in UV cabinet at short wavelength (254 nm) and readings for intensity were taken hourly.

Dissolution studies

Procedure for dissolution testing of Tapentadol HCl tablet formulation

Dissolution testing [14] of tapentadol HCl tablet formulations (Tapenta-50 and Tydol-50) were carried out using USP apparatus type-1 (basket), 900mL of 0.1 N HCl as dissolution medium with a speed of 75 rpm at 37±0.5⁰C temperature and sampling intervals were 10, 20, 30 , 45, 60 min as recommended by FDA guidelines [14, 15]. Sampling aliquots of 5.0 mL were withdrawn at specified intervals and replaced with an equal volume of fresh medium to maintain constant total volume. The amount of drug dissolved was sampled, pooled (n=3) and analyzed by the developed and validated spectrofluorimetric method. Amount of the dissolved drugs were calculated using their respective calibration curves. The content results were used to calculate the percentage release on each time of dissolution profile. The cumulative percentage of drug dissolved was plotted against time.

RESULTS AND DISCUSSION

Selection of optimum $\Delta\lambda$

Tapentadol HCl solution (10 µg/mL) was prepared and placed in spectrofluorimeter for the determination of excitation and emission wave lengths. Emission wavelength was found to be at 592 nm after excitation at 272nm. The emission spectrum of tapentadol HCl in water is shown in Figure 2.

Effect of pH

The influence of pH on fluorescence intensity of the tapentadol HCl was investigated using different kinds of buffers covering the whole pH range (1.0-9.0). The native fluorescence intensity of tapentadol HCl was higher in water (neutral pH) than other buffers as shown in Figure 3. Hence, for simplicity of the method no buffer was used throughout the study.

Effect of diluting solvent

Dilution with different solvents such as water, methanol, ethanol, acetonitrile, dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) was employed. The fluorescence intensity of tapentadol HCl was higher in water and methanol compared with other solvents as shown in Figure 4. Hence, in this method water was the best solvent for dilution as it gave the highest fluorescence intensity with no spectral interference, economical and eco-friendly.

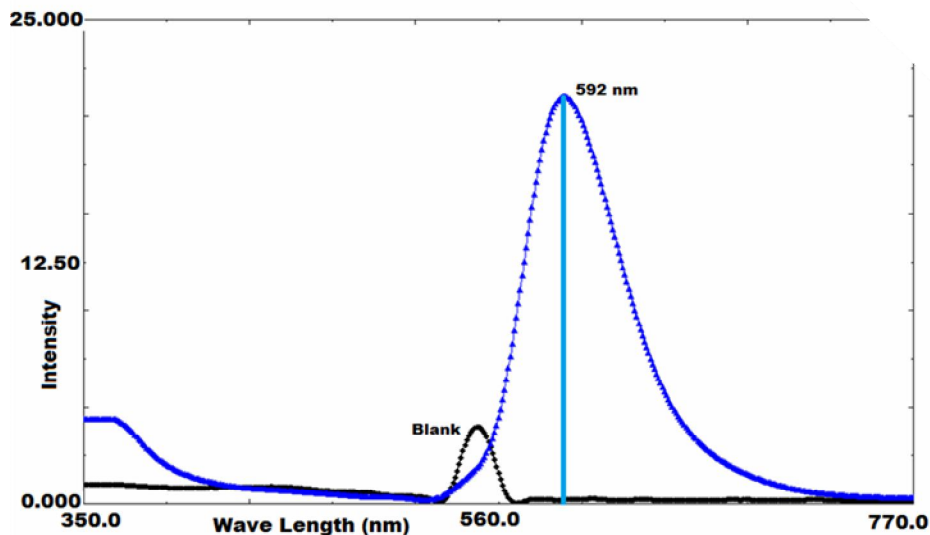


Figure 2: Emission spectrum of tapentadol HCl in distilled water at 592 nm after excitation at 272 nm

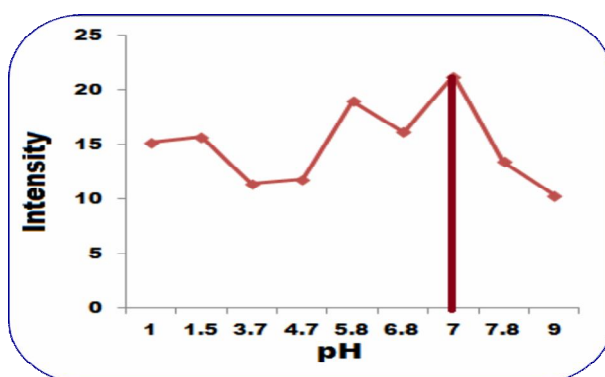


Figure 3: Effect of pH on fluorescence intensity of tapentadol HCl

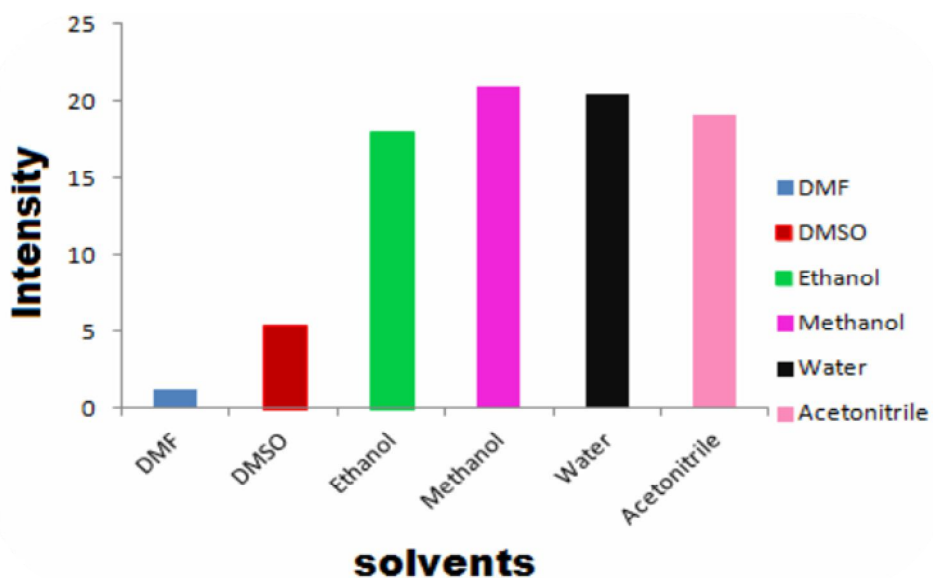


Figure 4: Effect of solvents on fluorescence of tapentadol HC

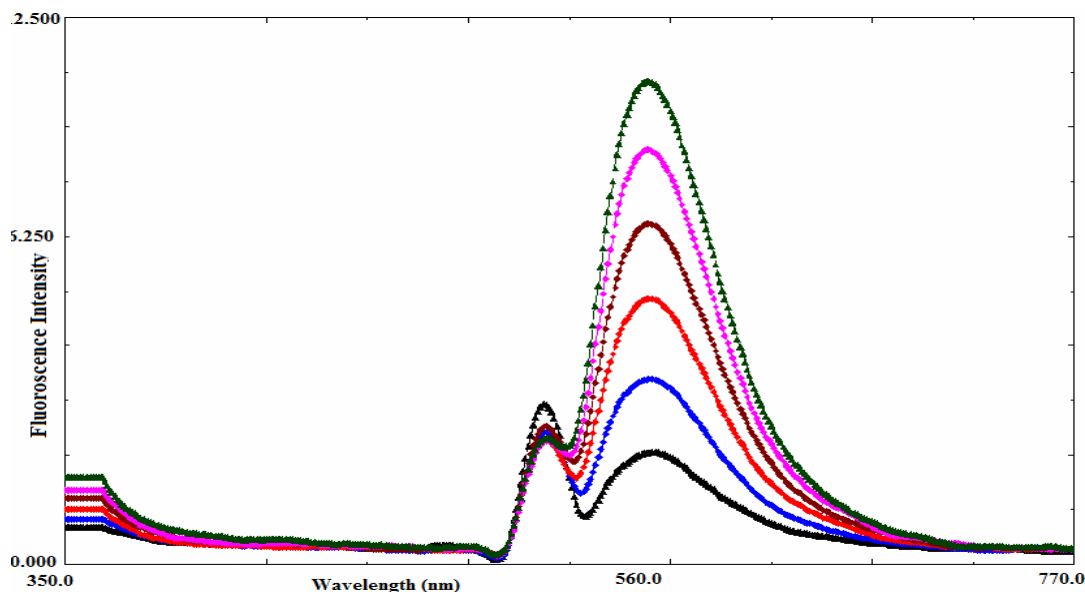


Figure 5: Spectrofluorimetric linearity range of tapentadol HCl (1-6 µg/mL)

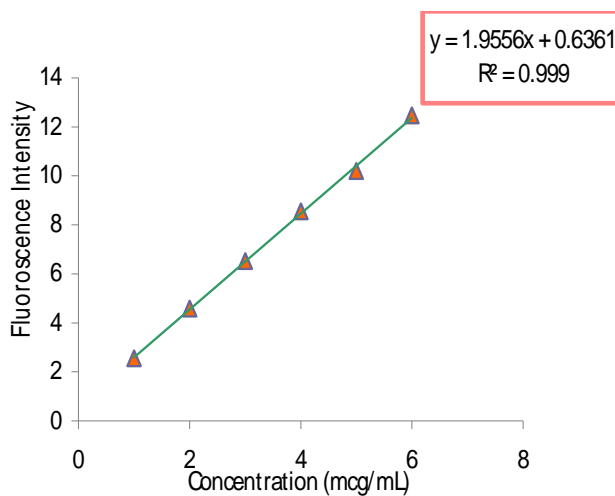


Figure 6: Standard plot of tapentadol HCl in distilled water at 592 nm (λ_{EM})

Effect of stability time

The effect of time on the development and stability of the fluorescence intensity of tapentadol HCl was also studied. It was found that the fluorescence readings remained stable for more than 24 hours.

Method validation

The calibration curve was linear in the range of 1 – 6.0 µg/mL with a correlation coefficient of 0.999 (Figure 5 & 6). Limit of detection (LOD) and Limit of quantification (LOQ) were experimentally verified to be 23.01 and 76.72 ng/mL, respectively, which indicated that the

method shows high sensitivity. Optimized conditions for proposed method are given in Table 1. The percentage recoveries were found to be in the range of 99.5-102 %. This indicates that the method is accurate (Table 2). Results from precision expressed in terms of %RSD, found to be less than 2. No significant differences between intraday and inter day precision, which indicated that the method is reproducible (Table 3).

Table 1: System suitability Parameters

Parameter	Value
Excitation Wavelength (nm)	272
Emission Wavelength (nm)	592
Range ($\mu\text{g/mL}$)	1-6
Limit of Detection (ng/mL)	23.01
Limit of Quantification (ng/mL)	76.72
Correlation Coefficient (r^2)	0.999
Slope (m)	1.9555
Intercept (c)	0.6366
Regression equation	$Y=1.955x+0.6366$

Table 2: Accuracy of the method (Recovery studies)

Formulation	Recovery Level (%)	Amount of the drug in tablet powder (mg)	Tapentadol pure drug added (mg)	Mean (mg/mL) \pm S.D. (n=3)	% Amount Recovered	% RSD
TAPENTA-50	80	10	8	17.872 \pm 0.236	99.95	1.3681
	100		10	20.667 \pm 0.153	101.35	0.738
	120		12	22.232 \pm 0.252	101.41	1.117
TYDOL-50	80	10	8	18.12 \pm 0.125	100.66	0.689
	100		10	20.09 \pm 0.245	100.45	1.219
	120		12	22.32 \pm 0.364	101.45	1.63

Applications

Assay

The proposed method was applied to the quantification of tapentadol HCl in tablet dosage forms. The assay results show that the proposed method was selective for tapentadol HCl without interference from the excipients used in the tablet dosage form. The values were shown in Table 4.

Table 3: Precision of the method

Concentration (mcg/ml)	Intra-day precision			Inter day precision		
	Mean ($\mu\text{g/mL}$) \pm S.D. (n=3)	% recovery	% RSD	Mean ($\mu\text{g/mL}$) \pm S.D. (n=3)	% recovery	% RSD
2	1.985 \pm 0.013	99.25	0.654	1.991 \pm 0.011	99.55	0.578
4	4.056 \pm 0.0645	101.45	1.55	3.996 \pm 0.055	99.9	1.397
6	6.047 \pm 0.063	100.78	1.04	5.886 \pm 0.009	99.76	0.154

Table 4: Analysis of commercial tablets (assay)

Formulation	Tapentadol HCl		
	Label claim (mg)	Amount found (mg) (AM \pm SD) (n=3)	% RSD
Tapenta	50	51.46 \pm 0.971	1.89
Tydol	50	50.09 \pm 0.462	0.922

Table 5: Cumulative % drug release data in zero order, first order, Higuchi and Hixon-Crowell cube root equations

Fitting of release data into zero order, first order, Higuchi and Hixon-Crowell cube root equations				
Formulation	Equations And r^2			
	Zero	First	Higuchi	Hixon-Crowell Cube root
Tydol -50 mg tablet	$y = 0.523x + 19.37$ $r^2 = 0.723$	$y = 0.007x + 1.292$ $r^2 = 0.659$	$y = 6.4797x + 0.7711$ $r^2 = 0.9375$	$y = 0.017x + 2.695$ $r^2 = 0.681$
Tapenta 50mg tablet	$y = 0.247x + 33.54$ $r^2 = 0.531$	$y = 0.002x + 1.520$ $r^2 = 0.511$	$y = 2.992x + 25.21$ $r^2 = 0.640$	$y = 0.007x + 3.216$ $r^2 = 0.517$

In-vitro dissolution studies

Dissolution studies of two tapentadol tablets (Tapenta 50 and Tydol 50) conducted as per FDA guidelines and analysed by proposed spectrophotometric method. The cumulative percentage of drug dissolved was plotted against time from the dissolution studies.

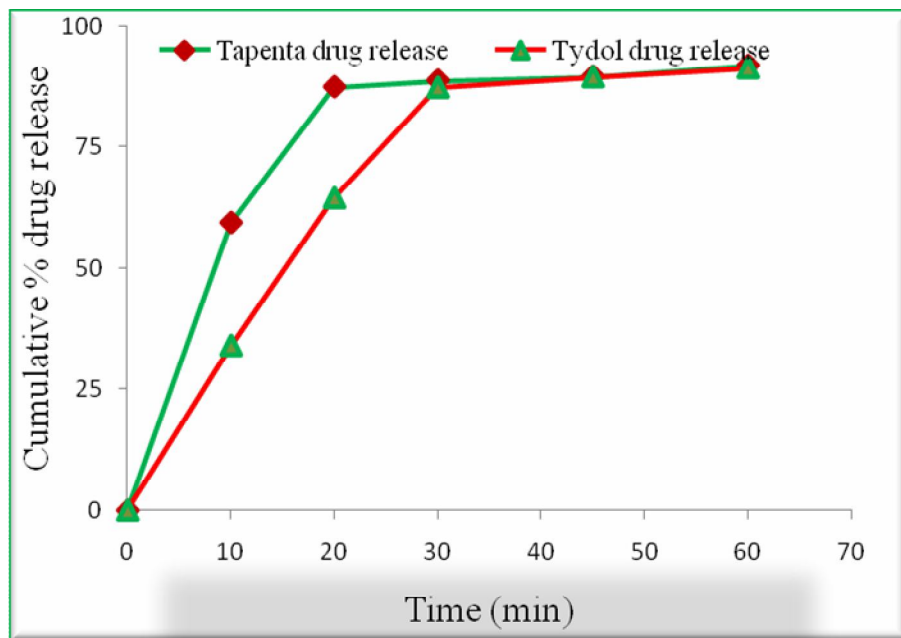


Figure 7: *In-vitro* dissolution studies of tapentadol tablet formulations

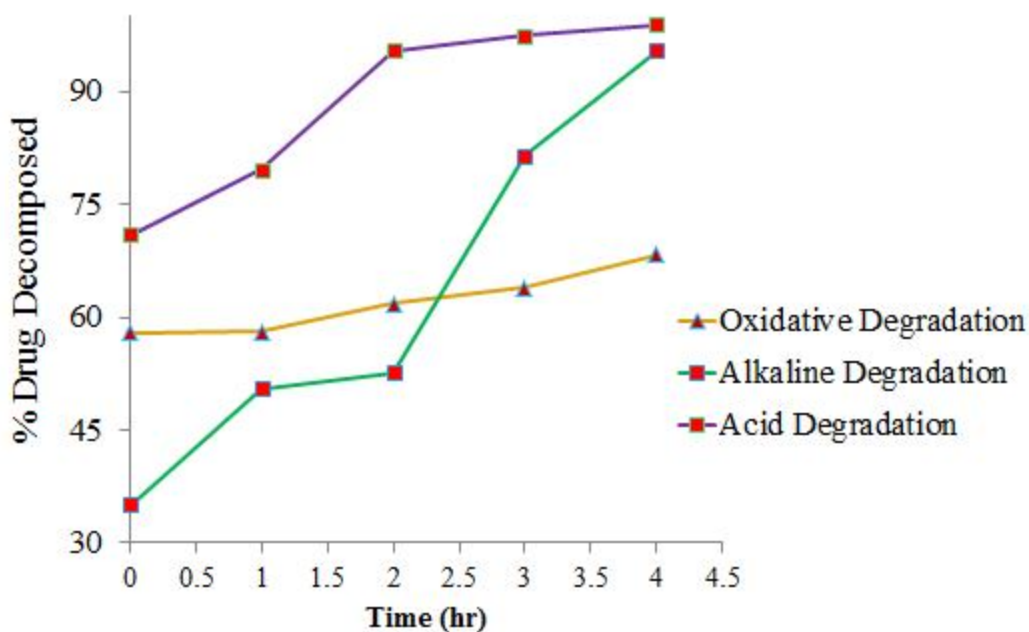


Figure 8: Stability profile of tapentadol HCl in acid (1N HCl), basic (1N NaOH) and oxidative (3% H₂O₂)

The percentage drug release was found to be more than 90% for both formulations, which is correlating with FDA guidelines [Figure 5]. Release of tapentadol formulations in 0.1N HCl during dissolution was processed for regression analysis and is it plotted into graphs to understand the linear relationship or kinetic principles (Table 5). From the data it is clear that the

dissolution phenomenon of tapentadol HCl follows zero order kinetics and it is controlled by diffusion process

Forced degradation studies

The stability of tapentadol HCl was studied in terms of fluorescence intensity in the presence of acid, alkaline, oxidative agent, photo and thermal degradation and construct the plot between amounts of drug degraded in terms of (%) Vs time interval. Considerable degradation of the drug was observed in 1N HCl (99.02% in 4 hrs), 1N NaOH (95.2% in 4 hrs), slow degradation in 3% H₂O₂ (80% in 24 hrs) [Figure 8] and no significant difference was observed in thermal and UV cabinet.

CONCLUSION

A simple, rapid, sensitive, precise and accurate new spectrofluorimetric method has been developed and validated as per ICH guidelines for the quantification of tapentadol HCl in bulk drug and formulations. The assay values were in good agreement with their respective labeled claim. This spectrofluorimetric method has been found to be better because of its high specific, sensitivity, readily available solvent, economical, eco-friendly and also utilized for forced degradation studies and *in-vitro* dissolution studies. The percentage drug release was found to be more than 90% for both formulations. Forced degradation revealed that the drug was not susceptible to thermal and UV effect and it was degraded in acidic, alkaline, and oxidative degradation methods. These advantages encourage that; the proposed method can be routinely employed in quality control for analysis of tapentadol HCl in tablet dosage forms, dissolution studies and in the presence of degradation products.

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