International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.1, pp 34-41, Jan-Mar 2013

Stability Indicating RP-HPLC Method For The Determination Of Tapentadol In Bulk And In Pharmaceutical Dosage Form

Gajanan M. Marathe, Pravin O. Patil*, Dilip A. Patil,

Ganesh B. Patil, Sanjay B. Bari

Department of Quality Assurance, H.R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist: Dhule (M.S.) India 425 405.

Corres.author: rxpatilpravin@yahoo.co.in
Tel/fax : +91- 2563-257599

Abstract: This study was designed to develop and validate simple, rapid and economical stability indicating RP- HPLC method for estimation of tapentadol in bulk and in tablet dosage form. The stability of tapentadol was investigated under different stress conditions, including acidic, alkaline, photolytic and thermal, as recommended by the ICH guidelines. Reversed-phase chromatography was performed on a Princeton C8 column with acetonitrile and pH3, 10 mM KH_2PO_4 buffer containing(0.1% v/v) triethylamine (35:65 v/v), as mobile phase at a flow rate of 1.0 mL min^{-1}. Detection was performed at 217 nm and a sharp peak was obtained for tapentadol at a retention time of 3.8 min. Linear regression analysis data for the calibration plot showed a good linear relationship between response and concentration in the range 5–30 µg mL^{-1}; the regression coefficient was 0.998 and the linear regression equation was Y=60915x+91924. The detection and quantification limits were 0.448 and 1.49 µg mL^{-1} respectively. Degradation was observed for tapentadol in base. The drug was found to be stable in the other stress conditions. The method was fully validated, showing satisfactory results for all the parameters tested. The method was validated for accuracy, precision, reproducibility, specificity, robustness, detection and quantification limits, in accordance with ICH guidelines.
Keywords- Stability Indicating Method, **T**apentadol, **M**ethod validation, **R**P-HPLC.

INTRODUCTION

Tapentadol is a new centrally acting oral analgesic developed by Johnson & Johnson Pharmaceutical Research and Development that was approved by the FDA in November 2008 for the treatment of moderate and severe acute pain.[1][2] Tapentadol hydrochloride is [(-)-(1*R*,2*R*)-3-(3-dimethylamino-1-ethyl-2-methylpropyl)-phenolhydrochloride] **(Figure 1).**[3],[4] It is a novel µ opioid receptor agonist and nor-epinephrine reuptake inhibitor with broad spectrum analgesic properties.[1,5,6] A limited number of publications were found in the literature concerning determination for stereoisomer's of the novel µ-opioid receptor agonist tapentadol hydrochloride because of two chiral centers[7], as well the estimation of tapentadol (Nucynta®) and *N*-des-methyl-tapentadol in authentic urine specimens using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry.[8]

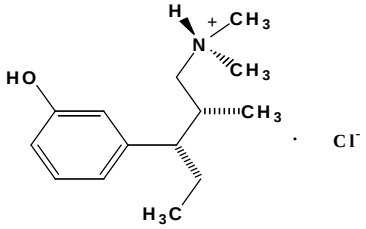

Figure 1: Chemical structure of tapentadol

Forced degradation or stress testing is undertaken to reveal specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products [1]. Stability indicating methodology provided in the United States-Food and Drug Administration (US-FDA) stability guideline of 1987 [2] and the draft guideline of 1998 [3] Stability-indicating assay methods (SIAMs) can be specific one, which evaluates the drug in the presence of its degradation products, excipients and additives, or selective one which is able to measure the drug and all the degradation products in the presence of excipients and additives [4]. Regulatory agencies recommend the use of stability-indicating methods [5] (SIMs) for the analysis of stability samples; this requires stress studies in order to generate the stressed samples, method development and method validation [6]. With the advent of the International Conference on Harmonization (ICH) guidelines [7], requirements for the establishment of SIMs have become more clearly mandated. Stress testing can help identifying degradation products and provide important information about the intrinsic stability of drug substances [8].

The aim of present work is to develop an accurate, specific, repeatable stability indicating RP-HPLC method for the determination of tapentadol. The proposed method for analysis of tapentadol in bulk and pharmaceutical preparations was validated as per ICH guidelines [9,10] and its updated international convention.[11]

EXPERIMENTAL

MATERIALS AND METHODS

Tapentadol was obtained as a gift sample from Zydus Cadila, Ahemdabad, India. The formulation Duovolt® tablet of IPCA Laboratories Limited was purchased form market. All the chemicals used like sodium hydroxide, hydrochloric acid, potassium dihydrogen phosphate, triethylamine and ortho-phosphoric acid are of analytical grade and mobile phase solvents of HPLC grade were purchased from MERCK Chem. Ltd., Mumbai.

Instruments

The Agilent 1200 HPLC system with PDA detector by Agilent Technologies Hewlett-Packard-Strasse 876337 Waldbronn, Germany, with Ezchrom Elite software and chromatographic separation were performed using Princeton C 8 column (250 mm × 4.6 mm id, 5 µm particle size). SHIMADZU AUX–220 analytical balance by Shimadzu corporation,1 Nishinokyo-Kuwabaracho, nakagyo-ku, Kyoto-shi, 604-8511 Japan; and Equitron® (Digital ultrasonic cleaner) were used during the study.

Chromatographic conditions

Chromatographic separation was achieved on a Princeton C 8 column (250 mm × 4.6 mm id, 5 µm particle size), using a mobile phase with a buffer containing a mixture of 10mM aqueous potassium dihydrogen orthophosphate (0.1% triethylamine), pH adjusted to 3.0 : Acetonitrile (65:35v/v). The mobile phase was filtered through a 0.45 µm membrane filter. The mobile phase flow rate was 1.0 mL/min. The spectrum was monitored at 217 nm. The injection volume was 20 µL. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report using above mentioned software. The similarity curve for Tapentadol peak was plotted over time during elution.[12]

Preparation of standard solutions

An accurately weighed sample (10mg) of tapentadol dissolved in 100 ml of mobile phase involumetric flask to give standard stock solution of 100µg/ml. The working standard solutions were obtained by diluting the stock solution. The 30.0µg/ml solution was obtained by dilution of the stock solution in mobile phase. All the volumetric flasks containing tapentadol solution were stored at room temperature.

Preparation of Solution of Marketed Formulation

Twenty tablets were weighed and average weight was calculated. Tablets were crushed to a fine powder. A quantity of powdered tablets equivalent to 10 mg of tapentadol, was transferred to 100mL volumetric flask. Then, 25 mL of mobile phase was added, placed for 10 min in ultrasonicator and more mobile phase was added until the solution reached 100mL. The solution was filtered through a 0.45 µm membrane filter (Milli-pore). The peak purity was checked with the PDA.

RESULTS AND DISCUSSION

Chromatographic Method Development and Optimization

The method development trials were carried out using different buffers, different ratio of buffer, and acetonitrile as well with different columns. Using Princeton C18 column 250mm x 4.6mmid, 5 µm particle size, different trials were carried out with KH_2PO_4 solution at pH 3.5, Acetonitrile: methanol (50:30:20 v/v/v) but tailing was greater than 2, also system suitability parameters is not obtained well. The mobile phase conditions were optimized to avoid interference from solvent and formulation excipients. Other criteria, for example, time required for analysis, flow rate of mobile phase, symmetry of the eluted peaks, assay sensitivity, solvent noise, and use of same solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. Different trials were carried on using Princeton C8 column 250mm x 4.6 mm internal diameter (particle size 5 µm). Detection of eluted peaks was done by PDA detector. In the mobile phase composition addition of triethylamine and an increase in the buffer content could improve peak shape, with less tailing. Finally, we used acetonitrile and 10 mM KH_2PO_4 buffer containing(0.1% v/v) triethylamine (35:65 v/v) having pH 3, as mobile phase at a flow rate of 1.0 mL min^{-1}. A sharp peak was obtained for tapentadol at retention time of 3.8 min detected at 217 nm. The chromatographic column temperature was maintained at 25°C (±2°C) with the column thermostat. This was found to be optimum to provide adequate peak separation, with a reduced amount of tailing and resulted in the best resolution among all the other combinations tested. The profile for Tapentadol peak was flat line with value nearer to 1000 **and t**he value of total peak purity was 1 in purity plot **(Figure 2)**. The value of the spectral similarity nearer to 1000 for Tapentadol shows that the peak was pure and no co-elution of impurity or any other hidden peak.

Assay of Tapentadol Tablets

Analiquot filtrate from marketed formulation was diluted with mobile phase to produce 10µg/mL solution, as the working sample solution. Samples prepared to identical conditions, but protected from light, were used as a control **(Figure 3).**

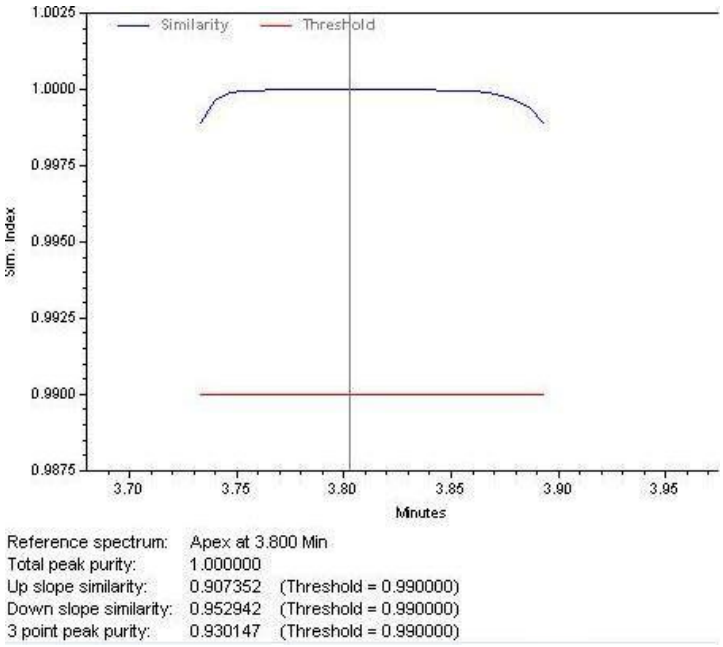

Figure 2: Peak Purity of Tapentadol

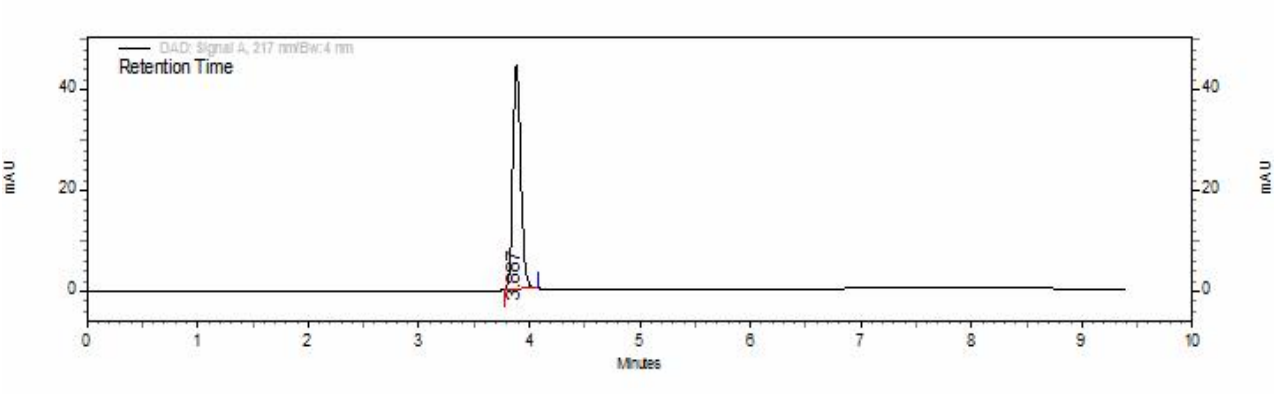

Figure3: Chromatogram of tapentadol tablet formulation

The assay results of samples from Duovolt® tablet dosage form was comparable with the value claimed on the label. The amount of tapentadol in tablet formulation was found to be 99.82±1.34 indicates the suitability of method for routine analysis of tapentadol in tablet dosage form.

METHOD VALIDATION

Linearity

Appropriate amounts of tapentadol stock solution (100µg/mL) were diluted with mobile phase to give concentrations of 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0µg/mL **(Figure 4)**. The calibration curves were drawn by plotting the peak areas against the corresponding concentration. The slope and Y-intercept of the calibration curve was calculated.

Calibration graph was found to be linear; that is adherence of the system to Beer's law was found over the concentration range 5-30µg/mL with correlation coefficient of 0.998 **(Table 1)**.

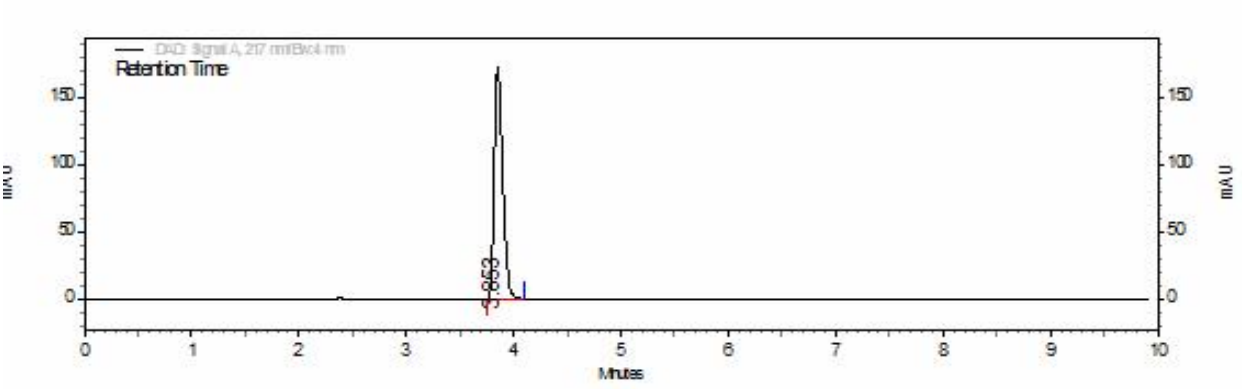

Figure 4: Chromatogram of tapentadol

Table1: Regression characteristics for analysis of tapentadol by RP-HPLC method

Parameters	
Equation	Y=60915x+91924
Slope	60915
Y-intercept	91924
Correlation Coefficient (r^2)	0.998

Precision

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability was evaluated by assaying samples, at the same concentration 15µg/mL during the same day. The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated. Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. Intra–day precision was determined by analyzing, the three different concentrations 10 µg/ml, 15 µg/ml and 20 µg/ml of tapentadol, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days, over a period of one week.

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 0.932 for six replicate determinations **(Table 2).** The % RSD for intra- and inter-day precision of tapentadol at three different concentrations such as 10, 15, 20 µg/mL were observed to be <2, **(Table 3)** which indicates that the methods to be precise. Both these methods are simple, economical, and rapid and can suitably be used for determination of tapentadol in bulk and in tablets.

Limit of Detection and Limit of Quantification

The LOD and LOQ for tapentadol were studied by injecting a series of dilute solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of tapentadol and calculated the %RSD for the areas. Limit of Detection (LOD) = 3.3(SD/S), where SD is the residual standard deviation and S is the slope of the line. The calculation method is again based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula LOQ = 10(SD/S). The LOD and LOQ[9] for tapentadol were found to be 0.448 and 1.49µg mL^{-1}respectively.

Accuracy

Accuracy of the method was assessed at three different concentration levels i.e. 80%, 100, 120%. To the pre-analyzed sample solution (10 µg/ml of tapentadol) a known quantity of standard drug was added at three different levels and pre-analyzed by proposed method.

Accuracy was evaluated by the simultaneous determination of the analytes in solutions prepared by the standard addition method. Recoveries of the added analytes were determined from their calibration curves **(Table 4)**.

Table 2: Repeatability

Concentration [«g/mL]	Peak area [n=6]	S.D	% RSD
15	976212.7	9104.361	0.9326

Table 3: Results of precision studies (Intra-day and inter-day)

Drug	Conc. [«g/ml]	Intra-day Amount Found [«g/ml]		Inter-day Amount Found [«g/ml]	
		Mean	% RSD[n= 3]	Mean	% RSD[n= 3]
TAPENTADOL	10	9.886	0.6734	10.033	0.926
	15	15.013	1.1270	14.983	1.368
	20	19.69	0.288739	19.866	0.895

SD = Standard deviation

RSD = relative standard deviation

Table 4: Results of Recovery Studies

Drug	Initial amount [«g/mL]	Amount added [«g/mL]	Amount recovered ï S.D. [«g/mL, n = 3]	% Recovery	% RSD
Tapentadol	10	8	7.87 ± 0.062	98.37	0.79
	10	10	10.09 ± 0.027	100.69	0.56
	10	12	11.98 ± 0.045	99.89	0.38

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was assessed by making variations in flow rate and proportion of mobile phase. An appropriate concentration of 10 µg/ml of tapentadol was subjected to analysis **(Table 5)**.

System suitability

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. Number of theoretical plates for tapentadol peaks should not be less than 2000, tailing factor for the analyte peaks should not be more than 2.00, the relative standard deviation for five replicate injections of standard preparation should not be more than 2.00 **(Table 6).**

Table 5: Results Robustness evaluation of the HPLC method

Chromatographic conditions	Retention Time n=3	Peak area n= 3	Theoretical plate n=3	Asymmetry (10%) n=3
A: Flow Rate (mL/min)				
0.90	4.29	753811	14582	1.22
1.00	3.79	630336	13356	1.20
1.10	3.540	625149	12683	1.154
Mean ï SD	**3.874ï 0.382**	**669765.3 ï 72831.87**	**13540.33ï 962.83**	**1.19ï 0.033**
B: Percentage ACN in mobile phase (v/v)				
10 mM KH_2PO_4 (pH 3) (0.1% triethylamine):ACN (70:30 v/v)	4.99	679105	12625.6	1.32
10 mM KH_2PO_4 (pH 3) (0.1%triethylamine):ACN (60:40 v/v)	3.27	692651	13639.67	1.11

Table 6: System Suitability Test

System Suitability Parameters	Proposed Method
Retention Time (Rt) ± SD	3.800 ± 0.0824
Capacity Factor (K)	43.70
Theoretical Plate (N)	13050
Tailing Factor (T)	1.165

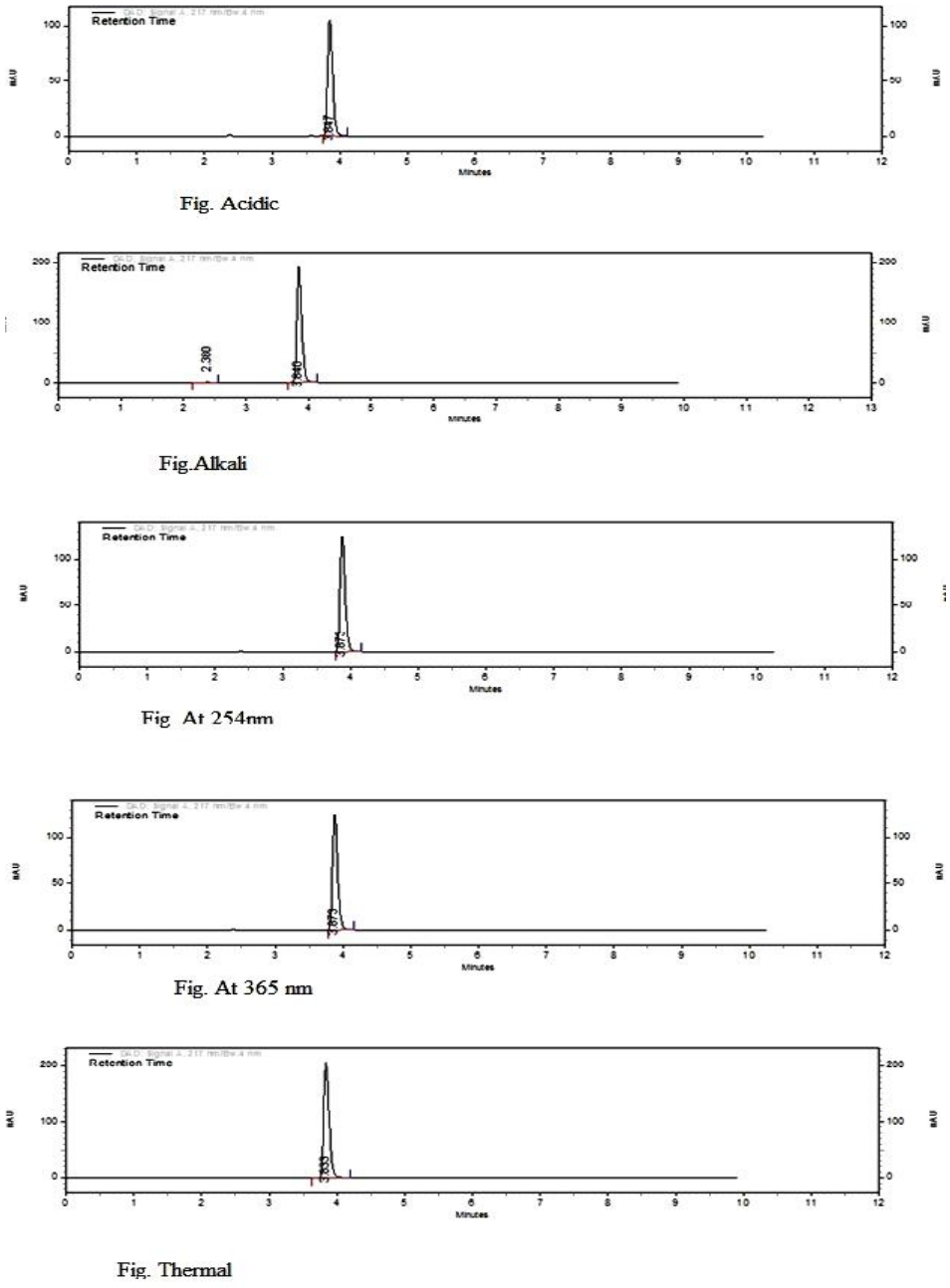

Figure 5: Typical chromatograms: (a) Acid degradation chromatogram; (b) Base degradation chromatogram; (c) Photo-degradation chromatogram; [1] Short wavelength at 254 nm, [2] Long wavelength at 365 nm; and (d) Thermal degradation

Forced degradation

The Forced degradation for tapentadol was studied by injecting dilute solutions of known concentrations treated with different forced degradation conditions. The typical forced degradation conditions used, includes acidic, basic, thermal, hydrolytic and photolytic degradation (in excess of ICH conditions). The drug samples were injected in HPLC column.

Acid degradation

Tapentadol 10 mg was taken and transferred to 50 ml flask. Add 10 ml of 0.5 N hydrochloric acid and reflux for 1hr at 70^0C. Neutralized with 0.5N sodium hydroxide and volume was made up with diluent. The 0.3 ml of solution of degraded API was taken and diluted to 10 ml in volumetric flask with diluents (**Figure 5**).

Alkali degradation:

Tapentadol 10 mg was taken and transferred to 50 ml volumetric flask; 10 ml of 0.5N sodium hydroxide was added, refluxed for 1hr at 70^0C. Above solution was neutralized with 0.5N hydrochloric acid. The 0.3 ml of solution of degraded API was taken and diluted to 10 ml in volumetric flask with diluents (**Figure 5**).

Photolytic degradation

For photo degradation, 10 mg drug was irradiated with a UV lamp at short wavelength (254nm) and long wavelength (365nm) in a cabinet for 24h. Solutions of the irradiated drug samples were prepared after quantitative transfer in volumetric flask with mobile phase. Then solution of 30 µg mL^{-1} concentration was prepared (**Figure 5**).

Thermal degradation

For thermal stress, 10 mg drug was placed in hot air oven at 70°C for 24 h. in open Petri dish. Solution of the solid drug sample was subsequently prepared after quantitative transfer and diluting with mobile phase to produce concentration of 30 µg mL^{-1}(**Figure 5**).

CONCLUSION

The simple RP- HPLC method was developed for the quantitative determination of tapentadol and its possible degradation products. Tapentadol shows minor alkali degradation. The % RSD values for precision studies of tapentadol were found to be < 2, indicates that, the developed method is precise. The method was fully validated; showing satisfactory results for all the parameters tested and can suitably be used for determination of tapentadol in bulk and in tablets.

ACKNOWLEDGEMENTS

The authors are thankful to Zydus Cadila Ahemdabad, for providing gift sample of tapentadol and H.R. Patel Institute ofPharmaceutical Education and Research, Shirpur (M.S.) for providing the required facilities to carry out this research work.

REFERENCES

[1] Richards-WL, Kraner J, New Drugs: Tapentadol. Tox Talk. 2009; 33:20.
[2] Drug Enforcement Administration, Department of Justice. Schedules of controlled substances: placement of tapentadol into schedule II. Final rule Fed Register, 2009; 74: 23790.
[3] Kleinert R, Hallmann C, Steup A, Black P, Goldberg J, Desjardins P. Presented at the 25th Annual Meeting of the American Pain Society. 2006 May 3-6; San Antonio, Tex. Abstract 6276.
[4] Weber H, Hallmann C, Steup A, Konder R, Black P, Terlinden R, *et al.* Presented at the 25th Annual Meeting of the American Pain Society. 2006 May 3-6; San Antonio, Tex. Abstract 6263.
[5] Thomas M, Tzschentke T, Christoph K, Babette K, Schiene H, Hennies W, *et al.* JPET 2007; 323:265–276.
[6] Christa K, Rolf T, Beier H, Genfu C, Drug Metab Lett. 2008; 2: 67-75.
[7] Ravikumar K, Sridhar B, Pradhan N, Khunt M, Acta Cryst C 2011; 67:71-76.
[8] Bourland J, Collins A, Chester S, Ramachandran S, Backer R. J Anal Toxicol 2010; 34:450-457.
[9] ICH, Q2 (R1) Validation of Analytical Procedure, Test and Methodology, International Conference on Harmonization. Geneva. October 1996.
[10] ICH [Stability Testing of New Drug Substances and Products (Q1AR2)], International Conference on Harmonization, Food and Drug Administration, USA, November 1996 and February 2003.
[11] Q1B Stability Testing: Photo stability Testing of New Drug Substances and Products, 1996.
[12] Manual of Agilent Chemstation for LC 3D systems, Agilent Technology Germany, 6:8, 2008, 20-39.

*****