International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 1 Jan-Feb 2021, pp: 1033-1038 www.ijprajournal.com ISSN: 2249-7781

Development and Validation of a Stability Indicating RP-HPLC Method for Quantitation of Tapentadol Hydrochloride

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Date Of Submission: 10-02-2021 Date Of Acceptance: 24-02-2021

ABSTRACT: A rapid, simple and sensitive RP-HPLC method was developed for quantitation of Tapentadol Hydrochloride in bulk and pharmaceutical dosage form. The method was validated as per ICH Q2(R1) guideline. The method was developed by using 10 mM ammonium acetate buffer (pH adjusted to 3.5 by using 10% v/v glacial acetic acid): acetonitrile: methanol (60:30:10 v/v) as mobile phase. Chromatographic separation was achieved on X bridge C18 stainless steel column (250×4.6 mm, 5 µm) with flow rate of 0.6 ml/min. The retention time of drug was found to be 7.2 ± 0.5 mins.The developed method was validated for precision, recovery studies, limit of detection and limit of quantitation. Tapentadol hydrochloride showed a good correlation coefficient in the concentration range of 100-350 µg/ml (R^2 =0.9986). The drug was exposed to acidic, alkaline, oxidative, photolytic hydrolysis and dry heat degradation conditions as per ICH guidelines. Tapentadol Hydrochloride was found to be susceptible towards acidic, basic, oxidative and photolytic degradation conditions.

KEYWORDS: Tapentadol Hydrochloride, Forced degradation, Degradation products, Validation.

I. INTRODUCTION

Tapentadol HCl (TAP) is chemically 3-((2R,3R)-1-(dimethylamino)-2-methylpentan-3-yl)phenol (Figure No.1). Its molecular formula is $C_{14}H_{23}NO$ and molecular weight is 222.21gm/mol. Tapentadol Hydrochloride is a narcotic pain relieving agent and it is a centrally acting analgesic with dual mode of action as an agonist at the μ-opioid receptor and as a norepinephrine reuptake inhibitor. Its principle use is to get rid the moderate serious intense pain. Its pain relieving impact is twice than as that of morphine.[1]

Extensive literature survey reveals that few UV[2-6], HPLC[7-13], UPLC-MS[14,15] and capillary electrophoresis[16] methods are available for the estimation of Tapentadol Hydrochloride alone or in combination with other drugs. Stability indicating HPLC methods are reported using use of phosphate buffer which is not compatible with the mass spectrometer. Therefore, the present study deals with the development of stability indicating RP-HPLC method for quantification of TAP in bulk drug using a MS compatible mobile phase.

II. MATERIALS AND METHODS

Tapentadol Hydrochloride was provided as a gift sample by Wochardt Pharma Pvt Ltd, Aurangabad. Methanol and acetonitrile were of HPLC grade. Ammonium acetate, hydrogen peroxide, Sodium hydroxide, hydrochloric acid and glacial acetic acid were of AR grade and were purchased from Loba Chemie Pvt Ltd, Mumbai. Chromatographic separation was performed using a HPLC instrument (LC-2010 Shimadzu, Japan) equipped with a UV detector. LC solution software was employed for data collection and processing. Chromatographic separation was performed on X bridge C18 stainless steel column (250×4.6 mm, 5 µm).

III. EXPERIMENTAL

Preparation of mobile phase

0.77 gm of ammonium acetate was dissolved in 1000 mL of water and pH of this solution was adjusted to 3.5 using glacial acetic acid. The mobile phase was prepared by mixing ammonium acetate buffer: acetonitrile:methanol in the ratio of 60:30:10v/v/v.The mobile phase was filtered through 0.45 µm nylon membrane filter before use.

Preparation of standard stock solution

The standard stock solution was prepared by dissolving 100 mg of Tapentadol Hydrochoride in 100 ml of Methanol to yield solution containing 1000 µg/ml of Tapentadol Hydrochoride.

Preparation of marketed tablet formulation

For the present research work "Tydol tablet (50 mg) was selected. 20 tablets were accurately weighed and triturated. A quantity of powder equivalent to 50 mg of label claim was accurately

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Volume 6, Issue 1 Jan-Feb 2021, pp: 1033-1038 www.ijprajournal.com ISSN: 2249-7781

weighed and transferred into 50 ml volumetric flask containing 40 ml methanol. It was sonicated for 30 min. and volume was made up to the mark with methanol. The above solution was filtered using whatmann filter paper (No 41) to yield solution containing 1000 µg/ml of Tapentadol Hydrochoride

Forced Degradation Studies

All stress decomposition studies were performed at an initial drug concentration of 1000 µg/ml. Degradation under acidic condition was carried out in 0.1 M HCl at 80° C for about 5 hrs. Basic degradation carried out in 1M NaOH at 80° C for about 1 hr. The solution degraded under acidic condition was neutralised with 1M NaOH and vise versa. Neutral hydrolysis was performed in water at 80º for 5 hrs. Oxidative stress was carried out in 3% H_2O_2 at 80º for 5 hrs. For photolytic degradation drug solution was exposed to direct sunlight for 2 hrs. Solid drug was exposed to dry heat in oven at 80º for 17 hrs. for dry heat degradation. All the reaction solutions were suitably diluted with mobile phase before HPLC analysis.

Method Validation

The developed method was validated as per ICH Q2(R1) guideline for the following parameters. The standard solutions were prepared by dilution of the stock solution with methanol in the range of 50-350 µg/ml. The responses were measured and plotted against the corresponding concentrations to obtain the calibration Curve.The accuracy of the analytical method is the percentage of relativeness between the conventional true value and the value obtained by that method. Recovery studies of Tapentadol Hydrochloride were performed at 75%, 100% and 125% levels on the solutions prepared from the marketed tablet formulation. Solutions were injected in triplicate and peak areas were measured. Repeatability studieswere carried out using nine replicates and intermediate (inter-day) precision was carried out with three concentrations of TapentadolHydrochoride using three replicates. The results of precision studies were expressed in % RSD for both the parameters. Five sets of concentrations were prepared between 50 -350 µg/ml and the corresponding areas of these sets were measured. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae asLOD= 3.3 (SD)/S and LOQ= 10 (SD)/S, where SD is the standard deviation of the responses and S is the average of the slopes. The robustness of the optimized method was studied by changing column oven temperature (±1°C), wavelength (±1 nm) and mobile phase composition (±1%) during analysis The sample was injected in triplicate for every condition and % RSD was calculated.

IV. RESULTS AND DISSCUTION

Optimized chromatographic conditions

Following chromatographic conditions were optimised: Column: XBridge™ C18 (4.6 × 250 mm, particle size 5 µm), Mobile Phase: Acetonitrile: 10 mM ammonium acetate buffer (pH 3.5 adjusted using glacial acetic acid): Methanol in ratio of 60:30:10 v/v/v, Detection Wavelength: 273 nm, Flow rate: 0.6 mL/min, Temperature: 25° C and Injection volume: 20µL.A 20µL standard solution was injected onto column under optimized RP-HPLC conditions and chromatogram was recorded using final mobile phase. A chromatogram for TAP standard is shown in Figure No. 2.

Stress Degradation Studies:

Tapentadol Hydrochloride was subjected to various stress conditions such as acid, base and oxidative degradation, photolytic, thermal and neutral degradation. Chromatogram obtained by acid hydrolysis suggested 38.32 % degradation of TAP when refluxed at 80º C for 5 hrs in 0.1M HCl. The major degradation products formed was Tap-Deg-1 at retention time value of 2.412 min. Chromatogram obtained under alkaline hydrolysis condition showed 39.09 % degradation of TAP when refluxed at 80º C for 1 hr in 1M NaOH. The degradation product formed under this condition is indicated as Tap-Deg-2 at retention time value 9.585 min. Chromatogram obtained by Neutral degradation suggested no degradation of TAP when refluxed in HPLC water at 80º C for 2 hrs. No additional peaks from degradation products were observed in the chromatogram of TAP. Chromatogram obtained under oxidative degradation (exposed to 3% hydrogen peroxide at 80º C for 1hr) produced two degradation products named Tap-Deg-3 and Tap-Deg-4 at retention time values of 5.733 and 6.030 min. respectively. Chromatogram obtained by photolytic degradation suggested 43.06% of degradation of TAP when subjected to sunlight for 5 hrs. The major degradation product formed was Tap-Deg-5 at retention time value of 6.50 min. Chromatogram obtained by dry heat degradation suggested no degradation of Tapentadol Hydrochloride when exposed to 80º C for 7 hrs. No additional peaks from degradation products were observed in the chromatogram Tapentadol Hydrochloride under this condition. Degradation studies indicated that TAP

was susceptible to acidic, alkaline, oxidative and photolytic degradation while it was stable towards dry heat degradation. The summary of degradation studies is given in Table No 1.

Validation of developed RP-HPLC method

The developed chromatographic method was validated for linearity, range, accuracy, precision, and Limit of detection and Limit of quantification parameters as per ICH guideline Q2 (R1).Tapentadol hydrochloride showed a good correlation coefficient in the concentration range of 100-350 µg/ml (R^2 =0.9986). The linearity of calibration graphs and adherence of the system to Beer's law was validated by the high value of correlation coefficient and the acceptable value of RSD was less than 2%. Intraday precision studies were performed by repeated injections of standard drug solutions at three concentrations (100, 200 and 300 µg/ml). Inter-day precision studies were performed using the same concentrations on three different days. The method was found to be precise as the % RSD values were found within an acceptable limit. As the % RSD values for the three levels were found to be less than 2, the method was said to be accurate. To determine the robustness of the method few factors like composition of mobile phase, analytical wavelength and column oven temperature were deliberately varied. Each factor selected was changed at three levels (-1, 0 and +1). One factor was change at one time to estimate the effect. The robustness of the method was evaluated at a concentration level of 200 µg/ ml for Tapentadol Hydrochloride. Under all conditions insignificant differences in peak areas and less variability in retention time values were observed. The summary of validation parameters in given in Table No 2.

V. CONCLUSION:

A simple reproducible and specific stability indicating RP-HPLC method was developed for quantification Tapentadol Hydrochloride in a bulk drug. The method showed linear results in the concentration range of 100 to 350 µg/mL and was validated as per ICH Q2(R1) guideline. The developed method was found to be precise, accurate and robust for quantitation of Tapentadol Hydrochloride in the presence of their degradation products.

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Volume 6, Issue 1 Jan-Feb 2021, pp: 1033-1038 www.ijprajournal.com ISSN: 2249-7781

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Table No. 1– Summary of degradation products formed under various degradation conditions

Sr. No.	Degradation Condition	Total % of Degradation	% Recovery of drug	Degradation formed	t_R & RRT of Degradant in (min.)	
1	Acid	38.32	61.67	Tap-Deg-1	2.412	0.33
2	Base	39.09	60.90	Tap-Deg-2	9.585	1.31
3	Neutral	Nil	98.22	Nil	Nil	Nil
4	Oxidative	86.16	19.84	Tap-Deg-3	6.030	0.79
				Tap-Deg-4	5.733	0.80
5	Dry heat	Nil	98.97	Nil	Nil	Nil
6	Photolytic	64.00	36	Tap-Deg-5	6.520	0.79

t_R: Retention time, RRT: Relative retention time.

Table No. 2 Summary of Validation Parameters for Tapentadol Hydrochloride

Parameters		Observations	Acceptance criteria
Retention Time (min)		7.231	-
LOD (µg/ml)		8.5	-
LOQ (µg/ml)		25.84	-
Linearity (µg/ml)		50-350	-
Correlation coefficient (r^2)		0.9981	-
Accuracy (% Recovery)		99.18-100.18%	97-103%
Assay		100.87	99-101 %
Precision (%RSD)	Intraday	0.84	% RSD should be < 2%
	Interday	0.95	
Robustness: Mobile phase ratio	61:29:10	0.48	% RSD should be < 2%
	60:30:10		
	59:31:10		
Robustness:	24° C		

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Volume 6, Issue 1 Jan-Feb 2021, pp: 1033-1038 www.ijprajournal.com ISSN: 2249-7781

Column oven temp	25° C	0.51	
	26℃		
Robustness: Change in wavelength	272nm	0.50	
	273nm		
	274nm		

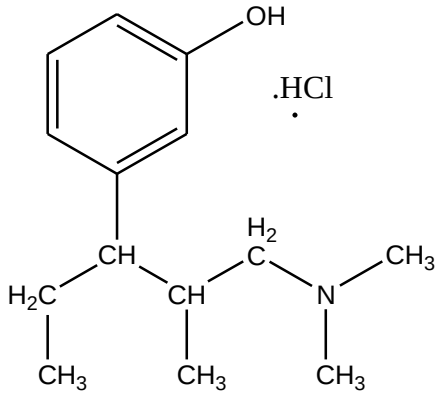

Figure No 1: Chemical Structure of Tapentadol Hydrochloride

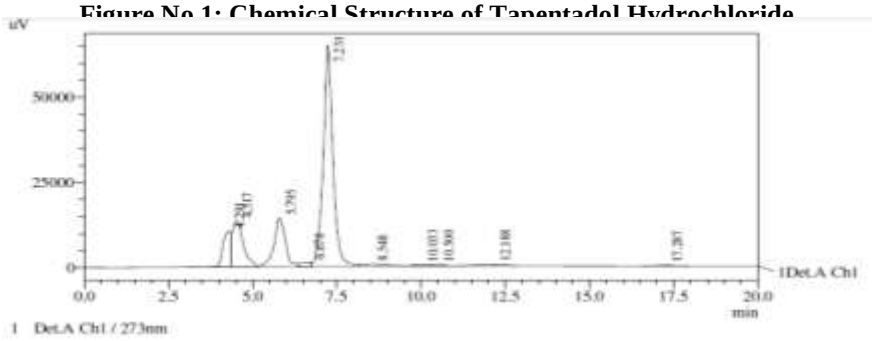

Figure No. 2 Chromatogram of Standard TAP

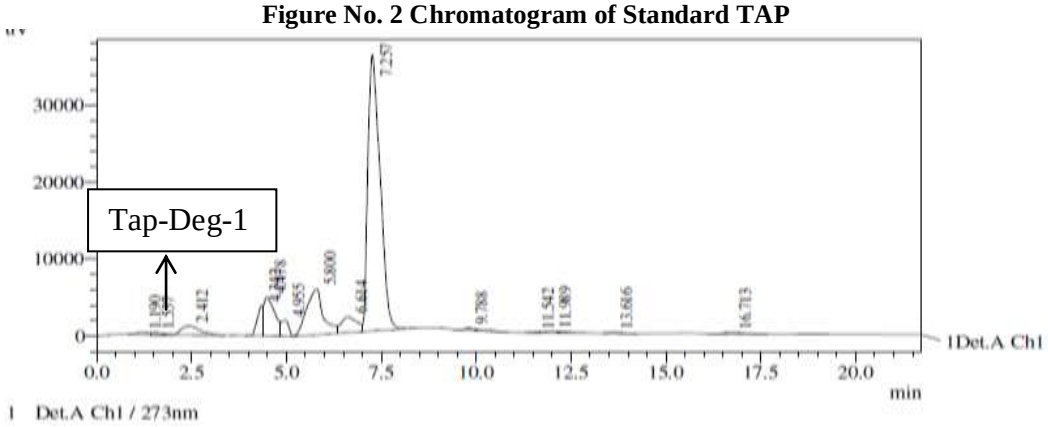

Figure No.3 Chromatograph of TAP under acidic hydrolysis

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Volume 6, Issue 1 Jan-Feb 2021, pp: 1033-1038 www.ijprajournal.com ISSN: 2249-7781

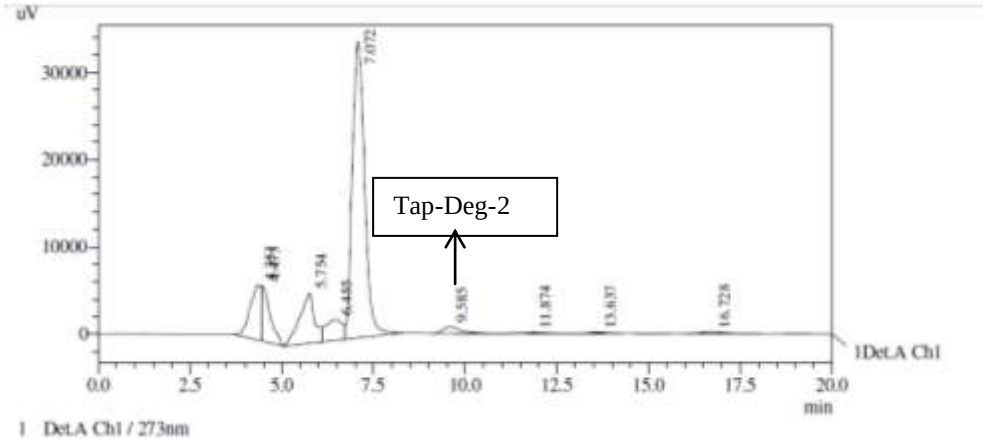

Figure No.4 Chromatograph of TAP under alkaline hydrolysis