International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-2, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Development and Validation of Stability-Indicating RP-HPLC Method for Tramadol in Tablet Formulation

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Manuscript No: IJPRS/V4/I2/00124, Received On: 11/06/2015, Accepted On: 15/11/2015

ABSTRACT

The present paper describes development of stability-indicating RP-HPLC method for the determination of Tramadol in presence of its degradation products, generated from forced degradation studies. Tramadol was subjected to forced degradation under acidic, basic, hydrolytic, photolytic and oxidative conditions. Successful separation of drug from degradation products formed under forced degradation conditions was achieved on a C18 column using methanol: 0.02 M potassium phosphate buffer (pH 6.8) (80:20 *v/v*) as a mobile phase at a flow rate of 1ml/ min. The detection was carried out at 272nm. The method was validated for linearity, range, accuracy, precision and selectivity.

KEYWORDS

Forced Degradation Studies, RP-HPLC, Tramadol, Validation

INTRODUCTION

A Stability – indicating assay method can be defined as "validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug products, and that are specific so that the content of active ingredients and degradation products can be accurately measured without interference".[1]

Tramadol is ((+)-trans-2-(dimethylaminomethyl)-1-(m-methoxyphenyl)-cyclohexanol hydrochloride)[2], is a narcotic analgesic and its o-desmethyl metabolite (M_1) are selective, weak OP3- receptor agonists. Opiate receptors are coupled with G- protein receptors are both positive and negative regulators of synaptic transmission via G- proteins that activate effector proteins.

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As the effector system is adenylate cycle located at the inner surface of the plasma membrane. Opioids decrease the intracellular cAMP by inhibiting adenylate cyclase.

Subsequently the release of neurotransmitters such as GABA, dopamine, acetylcholine and nonadrenalline is inhibited[3].

Literature survey revealed quantitative analytical methods for tramadol includes, simultaneous estimation of tramadol in tablet dosage form by RP-HPLC, tramadol hydrochloride in infusion form by UPLC, Chemical stabilities, few stability-indicating UPLC methods has been reported.[4,5,6,7]

The aim of the present work was to develop an accurate, specific, repeatable stability- indicating HPLC method for the determination of tramadol from tablets in presence of possible degradation products.

The proposed method was validated as per ICH guidelines.

MATERIAL AND METHODS

Materials Instrumentation

The HPLC system used consisted of a pump PU-2080 plus (JASCO Corporation, Japan) fitted with 20µL Rheodyne loop injector (7725*i*). Detection was carried out on UV-2075 detector (JASCO Corporation, Japan). The data acquisition was done on BORWIN chromatography software (Version 1.5).

Pharmaceutical grade Tramadol was kindly supplied as a gift sample by Wintech Pharmaceuticals, Nashik, India. Methanol used in analysis was of HPLC grade and all other chemicals were of analytical reagent grade, purchased from SD Fine Chemicals, Mumbai.

Chromatographic Conditions

The chromatographic separation was performed on a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm, with a mobile phase flow rate of 1.0 ml/min and the column eluent was monitored at 272 nm at an ambient temperature. The injection volume was 20 µL.

Mobile Phase

A mixture of methanol mixed with 0.02 M Potassium phosphate buffer (pH 6.8) in the ratio of 80:20 % v/v. The mixture was filtered through 0.45 µ membrane filter, degassed and used.

Degradation Studies

All forced degradation study experiments were performed at an initial drug concentration of 1 mg/mL. Degradation under acid and alkali condition was performed by heating the drug under reflux at 80°C in varying strengths of 1 N HCl for 2 hrs and 1 N NaOH for 2 hrs. Further, degradation under wet heat condition was studied by boiling an aqueous suspension of the drug under reflux for 30 min. Oxidative studies were carried out at room temperature in 30% hydrogen peroxide for 24 hrs and then heated in water bath for 10 min to remove the excess of hydrogen peroxide. Photo degradation studies were performed by exposing the drug to direct sunlight for 48 hrs. Additionally, the drug powder was exposed to dry heat in an oven at 70ºc for 48 hrs.

At the end of exposure, the drug samples were quenched diluted with mobile phase to get a final concentration of 10 µg/mL with respect to the initial concentration of the drug and subjected to chromatographic analysis. In each case, suitable blanks and controls were used to preclude errors. Appearance of secondary standard peaks and reduction in the area of drug peak concentration was taken as an indication of degradation.

Calibration Curve

Standard stock solution of Tramadol was prepared in mobile phase and mixed to obtain final concentration of about 1 mg/mL. From the above stock solution, dilutions were made with mobile phase to get seven calibration standards having concentrations of 10, 20, 30, 40, 50, 60, and 70µg/mL. The calibration standards were analyzed in three replicates and the peak areas were plotted against corresponding drug concentrations and subjected to least square linear regression to get an equation for line of best fit. The slope and intercept of the least square line were noted.

Estimation of Tramadol from Tablets

Tablets containing 200 mg of tramadol, each were prepared in- house. Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to average weight of the tablet was transferred to a 100 ml volumetric flask and shaken with 70 ml of methanol for 10 min. The excipients were separated by filtration and volume was made up to the mark with the same solvent. 0.1 ml of this solution was diluted with the mobile phase to 100 ml and subjected to chromatographic analysis using conditions mentioned above. The amount of tramadol per tablet was obtained from the regression equation of the calibration curve.

Validation of the Method

Accuracy and Precision

The accuracy and precision were evaluated by fortifying a powder mixture of blank tablets with amounts of drug corresponding to 80, 100, 120 % of label claimed and analyzing the resulting mixtures in three replicates over three days. The % recovery of added drug and % relative

standard deviation (% RSD) were taken as measures of accuracy and precision, respectively. Also, the results obtained were subjected to one way ANOVA and the between – day mean square compared to the within- day mean square by F- test.

Specificity

The specificity of the proposed method was established by the complete separation of tramadol in presence of its degradation products. Blank tablets of tramadol were chromatographed before and after subjecting to stress conditions. Absence of peaks in the blank runs at the retention time of drug was taken as indication of specificity.

Linearity and Range

The mean amounts of drug found during accuracy and precision studies were plotted against amounts added. The data pairs were subjected to regression and the slope and intercept of the resulting line were determined.

RESULTS AND DISCUSSION

Different mobile phases were tried, and a mobile phase composition of methanol and 0.02 M Potassium phosphate buffer (pH 6.8) (80:20 % v/v) was found to separate tramadol from its degradation products. The optimum wavelength for detection was 272nm at which better detector response was obtained. The average retention time for tramadol was 4.458 min.

Tramadol was found to degrade under acidic, wet heat, oxidative, dry heat conditions, while it was stable under alkaline and photolytic conditions.

The degradation behaviour of tramadol and the retention times of degradation products obtained under tested conditions are summarized in Table 1. The representative chromatograms of stressed samples of tramadol (Fig 1) showing well resolved peaks of degradation products under various stress conditions.

In the calibration studies it was found that the calibration line was linear in the range of 10-70 µg/ml with a slope of 14335, intercept of- 87760 and r^2 value of 0.9604. When tablets prepared on lab scale were analyzed using the developed method, the results obtained were in good agreement with the nominal amount of the drug. The drug content was found to be 104.6 % ± 0.506 of the added amount indicating that the method can be used for analysis of tramadol from tablet formulation.

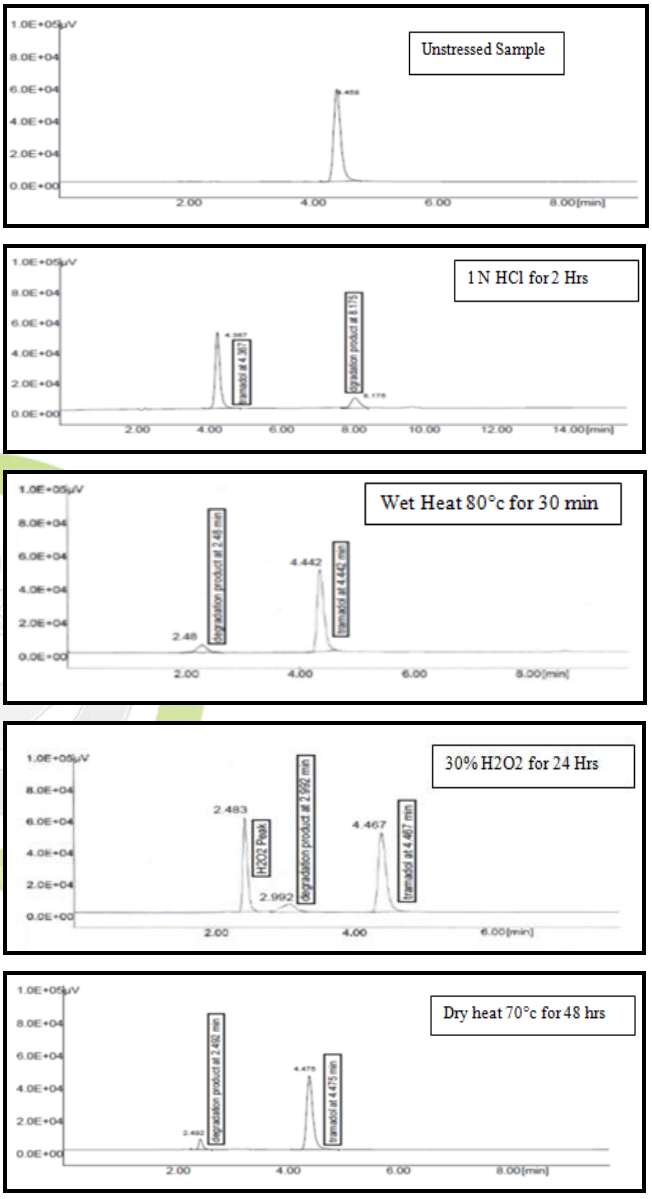

Figure 1: Typical HPLC Chromatograms of Tramadol under different stress conditions

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Table 1: Summary of forced degradation studies on Tramadol

Sr. No.	Stress Condition	Drug peak area at zero time sample (mcV.sec)	Drug peak area of stressed sample (mc.V.sec)	Retention time(s) of degradation products (min)	% Degradation
1	Acid 1N HCl (Refluxed for 2hrs)	453424.006	394802.925	8.175	12.92 %
2	Alkali 1N NaOH (Refluxed for 2hrs)	451543.56	448573.3	No degradation	No degradation
3	Wet heat 80°C for 30min	449270.248	404919.377	2.48	9.87%
4	Oxidative 30%v/v H_2O_2(in direct room temperature)	456704.02	388214.511	2.992	19.37%
5	Dry heat 70°C(kept in oven for 48hrs)	454205.02	420583	2.492	7.40%
6	Exposed to direct sunlight for 48hrs	451290.12	440810.203	No degradation	No degradation

Table: 2 Accuracy and Precision studies

Amount Added	Amount Found (mg)			Within mean square	Between mean square	F Value
	Day 1	Day 2	Day 3			
80% 160 mg	160.72	159.72	160.38	1.000	0.635	0.63
	159.58	159.47	159.59			
	161.83	160.46	161.97			
Mean	160.71	159.88	160.64			
S.D.	11.28	5.14	12.12			
%R.S.D	0.18	0.28	0.10			
100% 200mg	200.99	201.71	202.92	0.631	0.721	1.14
	201.61	200.91	201.09			
	200.06	200.58	201.43			
Mean	200.88	201.06	201.81			
S.D.	7.77	5.80	9.69			
%R.S.D	0.38	0.28	0.48			
120% 240mg	240.14	240.56	241.11	0.821	0.239	0.29
	240.55	241.49	240.14			
	241.60	240.69	242.68			
Mean						
S.D.	7.51	5.04	12.82			
%R.S.D	0.31	0.20	0.53			

The drug content was found to be 104.6 % ± 0.506 of the added amount indicating that the method can be used for analysis of tramadol from tablet formulation. The data obtained from precision and accuracy experiments are summarized in table 2. Mean values of amount found were very close to the amounts added and intraday % RSD values were very low indicating acceptable accuracy and precision of the method. The intra and inter day results at each level were subjected to one way ANOVA and F values at each level were obtained as a ratio of between mean square and within mean square. The F values were found to be lower than the tabulated $F_{2,6}$ values at $\alpha = 0.05$. These indicated that there was no significant difference between intra and inter- day variability, suggesting good intermediate precision of the method.

The least square line obtained by plotting the values of amount found versus values of amount added from table 2, had a slope of 1.005x- 1.065 and intercept of 0.033 encompassed 1 and value of y-intercept encompassed 0, indicating linearity of the method in the range of 80 to 120% of label claim.

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability-indicating. Validation of the method proved that the method is suitable for the analysis of tramadol in tablet formulation without any interference from common excipients or potential degradation products of tramadol and excipients. The developed method can be use for routine analysis of tramadol tablets or for assay of tramadol tablets from stability batches.

ACKNOWLEDGEMENT

Authors are thankful to the trustees and management of MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik for providing necessary analytical facilities and Wintech Pharmaceuticals, Nashik for provide gift sample of tramadol.

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