

Development and Validation of Stability Indicating HPLC Assay Method for Determination of Tapentadol in Tablet Formulation

Gaurang P. Pandya^{*}, Hitendra S. Joshi[†]

Abstract - The purpose of the research described herein was to develop simple, precise and accurate isocratic stability indicating reversed phase HPLC assay method for determination of Tapentadol solid dosage forms. Isocratic RP-HPLC method was developed on Phenomenex Luna C8 150 × 4.6mm, 5µm column using mobile phase as methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (60: 40 v/v) at a flow rate of 1.0 ml/min and the detection was carried out at 272 nm using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The validation element investigated showed that the method has acceptable specificity, accuracy, linearity, solution stability, precision and robustness.

Index Terms - Tapentadol, Stability indicating assay, Method development, Method validation

1. Introduction

Tapentadol is chemically 3-((2R,3R)-1-(dimethylamino)-2-methylpentan-3-yl)phenol (Fig.1). Its molecular formula is C₁₄H₂₃NO having molecular weight 221.34 gm/mol. Tapentadol is centrally acting analgesic with broad analgesic efficacy that was approved by Food and Drug Administration in 2008. The drug has dual mode of action in single molecule as an agonist at µ-opioid receptor and as a norepinephrine reuptake inhibitor [1], [2]. With this mode of action Tapentadol provides analgesia at comparable levels of more potent narcotic analgesics such as morphine, oxycodone and hydrocodone [3],[4] but its µ-sparing effect reduces the frequency and/or severity of side effects. Its other action on noradrenaline re-uptake gives a high analgesic potency [5]. Major sites of action of this drug are the spinal µ-opioid receptors and that the block of the noradrenaline lead to potentially synergistic activation of the spinal α₂ adrenoceptors [6].

According to ICH guidelines stress testing is an integral part of developmental strategy and is carried out under more severe condition than that of accelerated conditions. These studies provide information of drug's intrinsic stability. Stress testing is useful in developing and validating suitable analytical methods [7],[8],[9]. It is suggested in the ICH guidelines that stress testing should be done including the effect of temperature, light, oxidizing agent and susceptibility to hydrolysis across a wide range of pH values. It is also needed that analysis of stability sample should be carried out with the validated stability testing methods.

Few bioanalytical methods have been reported for the determination of Tapentadol in blood plasma by using LC-MS-MS [10],[11],[12]. Besides, some methods have been

reported for determination of Tapentadol in urine including LC-MS-MS and UPLC-MS.

To the best of our literature survey, so far there is no published report describing validated stability indicating HPLC method for determination of Tapentadol available in literature. This paper deals with forced degradation of Tapentadol under acidic hydrolysis, alkali hydrolysis and oxidation, thermal and photolytic stress condition and the validation of developed method for assay of Tapentadol from its dosage form (tablets).

2. Experimental

2.1 Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photo-diode array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu).

2.2 Reagents and Reference substance

Tapentadol standard was provided by Ami Life sciences Laboratories Ltd., Baroda (India). Tapentadol tablets containing 50 mg Tapentadol and the inactive ingredient used in drug matrix were obtained from market. HPLC grade methanol was purchased from Spectrochem Pvt. Ltd., Mumbai (India). HPLC grade water was produced in-house by Milli Q (Millipore, Milliford, USA) system. Membrane filters of 0.45µm (Millipore) were used. Analytical grade ortho-phosphoric acid, hydrochloric acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide

solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

2.3 Chromatographic conditions

Chromatographic analysis was performed on Phenomenex Luna C8 (150mm × 4.6mm i.d., 5µm particle size) column applying an isocratic elution using methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (60: 40 v/v) as a mobile phase. The mobile phase was filtered through 0.45µm membrane filter and degassed for 30 minute in an ultrasonic bath prior to its use. Flow rate of mobile phase was adjusted to 1.00 ml/min and injection volume was 20 µL. Detection was performed at 272 nm.

2.4. Standard preparation

Tapentadol standard stock solution containing 500µg/ml was prepared in a 100 ml volumetric flask by dissolving 50.00mg of Tapentadol and then diluted to volume with water as a diluent. Further take 10 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 100µg/ml).

2.5. Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml of diluent was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. Further take 10 ml of this stock solution in 50 ml of volumetric flask and make up to mark with diluent. The concentration obtained was 100 µg/ml of Tapentadol.

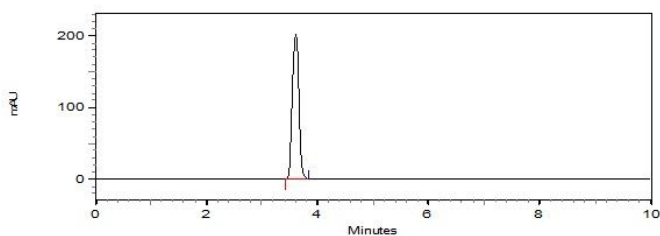


Fig. 2 Chromatogram of standard preparation

The degradation samples were prepared by transferring powdered tablets, equivalent to 50 mg of Tapentadol into a 250 ml round bottom flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After completing the

degradation treatments, the stress content solutions were allowed to equilibrate to room temperature and diluted with mobile phase to attain 100 µg/ml concentrations of Tapentadol. Specific conditions were described as follows.

2.6.1. Acidic degradation condition

Acidic degradation study was performed by taking the drug content in 0.1 N HCl at room temperature for 2.0 hours and mixture was neutralized.

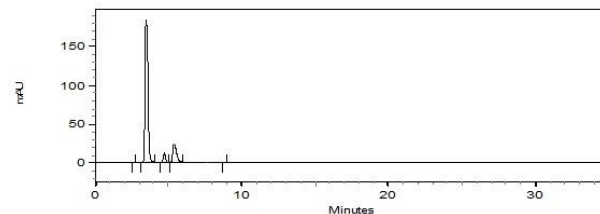


Fig. 3 Chromatogram of Acid degradation

2.6.2. Alkali degradation condition

Alkaline degradation study was performed by taking the drug content in 0.05 N NaOH at room temperature for 2.0 hours and mixture was neutralized.

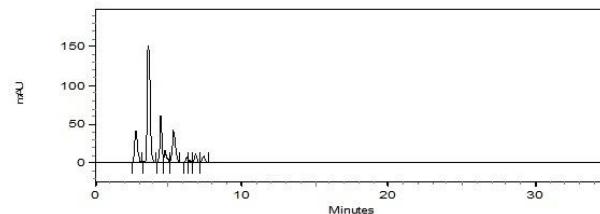


Fig. 4 Chromatogram of Alkali degradation

2.6.3. Oxidative degradation condition

Oxidative degradation study was performed by taking the drug content in 30% v/v H₂O₂ at room temperature for 2 hours.

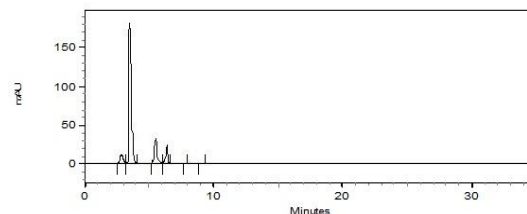


Fig. 5 Chromatogram of Oxidative degradation

2.6.4. Thermal degradation condition

Thermal degradation was performed by exposing solid drug at 80°C for 72 hours.

2.6.5. Photolytic degradation condition

Photolytic degradation study was performed by exposing the drug content in UV-light for 72 hours.

2.7. Method validation

2.7.1 Specificity study

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

2.7.2 Linearity

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration (40, 60, 80, 100, 120, 140 and 160 µg/ml). The peak areas versus concentration data were evaluated by linear regression analysis.

2.7.3 Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of Tapentadol test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition.

Table I: Evaluation data of precision study

	Tapentadol % assay	
	Interday	Intraday
	(n=6)	(n=6)
1	100.25	100.22
2	100.12	99.95
3	101.28	100.10
4	99.58	100.85
5	98.44	101.17
6	100.17	100.87
Mean	99.97	100.50
Standard deviation	0.93	0.50
%RSD	0.93	0.50

2.7.4 Accuracy

An accuracy study was performed by adding known amounts of Tapentadol to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in triplicate.

Table II: Evaluation data of Accuracy Study

Level (%)	Theoretical Concentration (mg/ml)	Observed Concentration (mg/ml)	% Recovery	% RSD
50	0.04993	0.05001	100.15	0.429
100	0.10053	0.10059	100.06	0.614
150	0.15053	0.15004	99.67	0.662

2.7.5. Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate (± 0.1 ml/min), mobile phase composition methanol - 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (62: 38 and 58: 42 v/v) and using different lot of LC column.

Table IV: Evaluation data of robustness study

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.9 ml/min	100.56	4235	1.66
Flow 1.1 ml/min	100.84	4430	1.41
Acetonitrile:water:0.1% acetic acid(62:38:0.1)	100.22	4316	1.28
Acetonitrile:water:0.1% acetic acid(58:42:0.1)	100.20	4615	1.36
Column change	99.82	4416	1.12

2.7.6. Solution stability

The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2-5°C and tested at interval of 12, 24, 36 and 48 hours. The responses for the aged solution were evaluated against a freshly prepared standard solution.

Table I: Evaluation data of solution stability study

Intervals	% Assay for test solution stored at 2 -8° C	% Assay for test solution stored at ambient temperature
Initial	100.28	100.03
12 h	100.23	99.74
24 h	100.04	99.45
36 h	99.80	99.35
48 h	99.62	99.22

3. Result and discussion

To develop a rugged and suitable HPLC method for the quantitative determination of Tapentadol, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By keeping mobile phase composition as methanol – 0.002 M potassium dihydrogen phosphate buffer pH 3.0 (60: 40 v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, methanol was chosen and to attain good peak shape. Chromatogram of standard preparation is represented in

(Fig. 2). A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. For all system suitability injections, asymmetry was less than 2.0, theoretical plate was greater than 4000 and % RSD of peak area was found less than 2.0. The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of Tapentadol during the force degradation study. There was no interference of any peak of degradation product with drug peak. Major degradation was found in alkaline condition in which product was degraded up to 22%. The major impurity peak was found at 4.263 min. (Fig. 4). In oxidative degradation, it was found that around 12 % of the drug degraded and impurity peak was found at 5.374 min. (fig. 5) and in acidic condition around 7 % of the drug degraded and impurity peak was found at 5.602 min. (fig. 3). Tapentadol was found to be slightly degraded in thermal degradation while it was stable under the photolytic condition. Seven points calibration curve were obtained in a concentration range from 40-160 µg/ml for Tapentadol. The response of the drug was found to be linear in the investigation concentration range and the linear with correlation coefficient 0.999. The result of repeatability and intermediate precision study is shown in Table 1. The developed method was found to be precise as the % RSD values for the repeatability and intermediate precision studies were <0.51 % and <0.94 %, respectively, which confirm that method was precise. The HPLC area responses for accuracy determination are depicted in Table 2. The results show that best recoveries (99.67-100.15%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. The result of robustness study of the developed assay method was established in Table 3. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Table 4 shows the results obtained in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 hours at 2 - 5° C and ambient temperature as during this time the result was not decreased below the minimum percentage. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

4. Conclusion

A new analytical method has been developed to be routinely applied to determine Tapentadol in pharmaceutical dosage form. In this study, stability of Tapentadol in present dosage form was established

through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also for stability sample analysis.

Acknowledgements

The authors are thankful for facilities & grants given under UGC- Special Assistance Programme Department Research Support (DRS) and Department of Science & Technology (DST) New Delhi, Fund For Improvement of Science & Technology (FIST), National Facility for Drug Discovery (NFDD) and Department of Chemistry, Saurashtra University, Rajkot – 360 005 (INDIA) for providing analytical facilities.

References

- [1] C. Kneip, R. Terlinden, H. Beier and G. Chen; Investigations Into the Drug-Drug Interaction Potential of Tapentadol in Human Liver Microsomes and Fresh Human Hepatocytes; *Drug Metabolism Letters*, (2008), 2: 67-75.
- [2] T. Tzschentke, T. Christoph, K. Babette, K. Schiene, H. Hennies, W. Englberger, H. Haurand; (-)-(1R,2R)-3-(3-Dimethylamino-1-ethyl-2-methyl-propyl)-phenol Hydrochloride (Tapentadol HCl): a Novel μ -Opioid Receptor Agonist/Norepinephrine Reuptake Inhibitor with Broad-Spectrum Analgesic Properties; *The Journal of Pharmacology and Experimental Therapeutics*, (2007), 323: 265-276.
- [3] R. Rob, F. Carol, H. Julie, W. Gill, M. Kate, S. Michael; Systematic review of tapentadol in chronic severe pain; *Current medical research and opinion*, (2011), 27(10), 1907-1930.
- [4] K. Regina, L. Claudia, S. Achim, B. Peter, G. Jutta, and D. Paul.; Pain Medicine: Single Dose Analgesic Efficacy of Tapentadol in Postsurgical Dental Pain: The Results of a Randomized, Double-Blind, Placebo-Controlled Study; *Anesthesia & Analgesia*, (2008), 107(6): 2048-2055.
- [5] M. Schäfer; Novel concepts for analgesia in severe pain – current strategies and future innovations; *European Journal of Pain Supplements*, (2009), 3: 6-10.
- [6] L. Bee, K. Bannister, W. Rahman and A. Dickenson; μ -opioid and noradrenergic α 2-adrenoceptor contributions to the effects of tapentadol on spinal electrophysiological measures of nociception in nerve-injured rats; *Pain*, (2011), 152: 131-139
- [7] S. Singh and M. Bakshi.; Guidance on conduct of stress tests to determine inherent stability of drugs; *Pharmaceutical Technology On-line* (2000); 24: 1-14
- [8] S. Klick, V. Muijselaar, J. Waterval, T. Eichinger, C. Korn, T. Gerding; Toward a Generic Approach for Stress Testing of Drug Substances and Drug Products; *Pharmaceutical Technology*, (2005); February: 48-66
- [9] S. Baerstichi, Pharmaceutical Stress Testing Predicting Drug Degradation, 1st edition, Chapter 6 Informa Healthcare, London, U.K., (2005), pp. 459–469.
- [10] Y. Jin, X. Jiang, N., Zhai, P. Xu, B. Yuan, and H. Xu.; LC-MS/MS method for the determination of tapentadol in rats; *Shenyang Yaoke Daxue Xuebao*,; (2012), 29(2): 143-146.
- [11] Y. Liu, C. Uboh, L. Soma, X. Li, F. Guan, and J. Chen.; Efficient Use of Retention Time for the Analysis of 302 Drugs in Equine Plasma by Liquid Chromatography-MS/MS with Scheduled Multiple Reaction Monitoring and Instant Library Searching for Doping Control; *Analytical Chemistry*, (2011), 83(17): 6834-6841.
- [12] R. Terlinden, B. Koegel, W. Englberger and T. Tzschentke; In vitro and in vivo characterization of tapentadol metabolites; *Methods and Findings in Experimental and Clinical Pharmacology*, (2010), 32(1): 31-38.
- [13] X. Jiang, Y. Jin, H. Xu, P. Xu, N. Zhai and B. Yuan.; Analysis of metabolites of tapentadol in rat urine by liquid chromatography tandem mass spectrometry; *Shenyang Yaoke Daxue Xuebao*, (2012), 29(1): 64-69.
- [14] C. Coulter, M. Taruc, J. Tuyay and C. Moore; Determination of tapentadol and its metabolite N-desmethyltapentadol in urine and oral fluid using liquid chromatography with tandem mass spectral detection; *Journal of Analytical Toxicology*, (2010), 34(8): 458-463.
- [15] R. Terlinden, J. Ossig, F. Fliegert, C. Lange, and K. Goehler; Absorption, metabolism, and excretion of ^{14}C -labeled Tapentadol HCl in healthy male subjects; *European Journal of Drug Metabolism and Pharmacokinetics*; (2007), 32(3), 163-169.
- [16] J. Bourland, A. Collins, S. Chester, S. Ramachandran and R. Backer; Determination of tapentadol (Nucynta®) and N-desmethyltapentadol in authentic urine specimens by ultraperformance liquid chromatography-tandem mass spectrometry; *Journal of analytical toxicology* (2010), 34(8): 450-457.
- [17] J. Tuyay, C. Coulter, W. Rodrigues and C. Moore, Disposition of opioids in oral fluid: Importance of chromatography and mass spectral transitions in LC-MS/MS. *Drug Test Analysis; Drug Testing and Analysis*, doi: 10.1002/dta.1324

* Gaurang P. Pandya is currently pursuing Ph.D. Degree program in Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India. E-mail: gaurangpandya@live.com

† Hitendra S. Joshi is professor in Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India. E-mail: drhsjoshi49@gmail.com