Investigation on Effect of Stress on Dissolution Stability of Drug Product by Applying Thermal and NonThermal Methods of Analysis

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Authors’ contributions
This work was carried out in collaboration among all authors. Author KRG designed the study and wrote the protocol. Author RPC performed actual experimentation and the statistical analysis and wrote the first draft of the manuscript. Author ARP managed the analyses of the study. Author AVG managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aim: The aim of the research work has to development and validation of dissolution test method for Tapentadol using HPLC method, investigate the effects of stress on dissolution stability by thermal and non-thermal methods. The present research work mainly focused on the evaluation and compares the influence of accelerated-aging conditions on the drug content and in vitro dissolution stability.

Place and duration of Study: Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, New Kamptee, Nagpur (MS).

Methodology and Results: Saturated solubility study of tapentadol were carried out using different dissolution media and different conditions such as type of dissolution medium, volume of dissolution medium and rotation speed of paddle were evaluated. Basis of it, dissolution testing were carried out on a suitably calibrated USP Apparatus II (TDT-06L) at 50 ± 1 rpm, under sink conditions in 900 mL of deaerated distilled water at 37±0.5°C for each test and selected most
optimized dissolution parameter which given maximum % release of drug. The drug release was evaluated by high-performance liquid chromatographic method. Also proposed method were validated as per ICH guidelines with respect to system suitability, linearity, precision, accuracy, range, robustness, ruggedness and solution stability parameters were evaluated and the obtained results were within the acceptable range. The stress on dissolution stability of standard powdered drug, tablet formulation and packed strip formulation were investigated by using thermal and non thermal methods. The results obtained in all stress conditions such as thermal, humidity, UV light and visible light were evaluated for drug content and drug release. The results were statistically evaluated by applying two-way ANOVA followed by post-hoc Bonferroni test and their results represented as a graphical plot.

**Conclusion:** In our investigation of stress dissolution of drug it was found that Tapentadol HCl std. drug was susceptible to degradation. The tablet and packaged formulation were susceptible to photolytic degradation indicated by difference in drug content while the release was more affected under UV exposed to tablet and strip packaged formulation as compared to other stress conditions.

**Keywords:** Tapentadol dissolution method; stress studies; thermal and non-thermal methods; ANOVA design.

1. **INTRODUCTION**

Tapentadol HCl, chemically it is 3- [(2R, 3R)-1- (dimethylamino) 2-methylpentane - 3-yl] Phenolhydrochloride having chemical formula C_{14}H_{33}NO and 221.3385 as molecular weight. It shows dual mode analgesic activity like agonist at the μ-opioid receptor as well as a nor-epinephrine reuptake inhibitor. U.S.A approved tapentadol as centrally acting synthetic analgesic in 2008 and were categorised into the schedule-II category drug of the Controlled Substances Act in May of 2009. It has broad analgesic profile but due its dual mode of action consisting of both MOR activation and NE reuptake inhibition developed relative resistance to tolerance. It was first designed - synthesized in USA and formulation were marketed by Ortho-McNeil-Janssen Pharmaceuticals under the trade name of Nucynta [1,2]. Tapentadol shows more tolerable side effects with respect to other opioids. Although tapentadol has two chiral centers and consequently four enantiomers namely S, S; R, R; S, R; and R, S, only the R, R stereoisomer is commercially available as drug [3,4].

The stability studies of drug formulation are related to chemical decomposition. Formulation containing different excipients may interact with API during exposure to various conditions such as high temperatures or high humidity result in reduction in in-vitro dissolution, a most important critical quality attribute of oral solid dosage formulation. The dissolution stability is considered as a critical quality control parameter because it affects bioavailability result in changes in in-vitro release profile of drug during storage. Absence of changes in dissolution studies provides assurance that the bioavailability remains constant throughout the aging protocol. The tablets exposed to various aging conditions such as pH, temperature, photolytic conditions and high or low humidity etc, may affect the release characteristics of drug. [5,6,7,8].

![Structure of Tapentadol hydrochloride](image)

**Fig. 1. Structure of Tapentadol hydrochloride**
The literature survey revealed that tapendolol HCl analyzed by various analytical methods such as UPLC-Tandem Mass Spectrometry [9], RP-HPLC [10,11,12,13], HPLC with spectrofluorimetric method [14], Stability-Indicating LC Method for the simultaneous estimation of Tapentadol and its impurities [15]. One method was based on pH stability indicating spectrofluorimetric quantification and in-vitro dissolution studies [16]. But there is no method developed till date to investigate the aging conditions effects on drug release as well as their evolution by thermal and non thermal methods.

Our present research work focused on investigation of drug dissolution released kinetics at different aging conditions. For these, standard drug, tablet formulation and packed strip exposed to accelerated-aging conditions such as humidity and temperature (40°C±2°C/75% ± 5% RH), temperature for thermal studies (60°C), pH, (UV light and visible light) and investigated their effects on the drug content, visual appearance, intra-molecular change and in-vitro dissolution stability of tapentadol HCl marketed formulation available in the Indian market. Aging conditions could affect the dissolution stability of these formulations in a different manner, playing an important role in drug bioavailability and interchangeability of the products during the shelf life.

The proposed methods were validated as per the ICH guideline for specificity, precision, linearity, accuracy and range. There are three categories of precision: repeatability, reproducibility, and intermediate precision. Repeatability is the precision of the method under the same operating conditions over a short time. Reproducibility determines the precision between laboratories. Intermediate precision is a measure of intralaboratory variance using different operators on different days, equipments and so forth and is not required in cases where reproducibility has been performed. The method robustness should be evaluated, and if measurements were affected by variation in method parameters, then these should be controlled or a statement should be included in the methods [17,18,19].

2. MATERIALS AND METHODS

2.1 Reagents and Samples

Analytical grade phosphoric acid, potassium dihydrogen orthophosphate and HPLC grade triethylamine, methanol and acetonitrile were used as mobile phase, phosphate buffer (pH 6.8) was used as the dissolution medium and HPLC grade reagents were used for chromatographic determinations. Tapentadol HCl immediate-release tablet formulations, manufactured by different pharmaceutical companies were kindly provided by local hospitals. They all contained 100 mg Tapentadol HCl but different excipient compositions. The mobile phase comprised of 0.05M potassium dihydrogen phosphate buffer: acetonitrile, pH 2.8 (65:35) at flow rate 1.0 mL/min. The mobile phase was filtered through a 0.45 μm membrane filter and sonicated for 15 min.

2.2 Apparatus

The dissolution test was performed in a six-station Electrolab TDT dissolution tester (model TDT-06L) in accordance with USP 27 general methods. Analytical Technology Limited isocratic system consisted of hyperchrome ODS 5µm C18 column (250 X 4.6 mm), UV-3000 detector and P-3000 Pump, Rheodyne injector with 20 μL capacity. Analysis was performed at ambient temperature. The detection was monitored at 217nm. A Shimadzu UV 1700 double beam UV-Visible spectrophotometer using 1.0-cm quartz cells and digital pH meter, model NIG-333 (Nainasolariss make) was used to determine the pH of all solutions. The ultrasonic bath used for deaeration (PCI, Mumbai) and Thermolab stability chamber.

2.3 Method Validation

The proposed methods were validated as per the ICH guideline for system suitability, linearity, precision, accuracy, range, robustness, ruggedness and solution stability.

2.4 Preparation of Sample Solutions

2.4.1 Preparation of selected mobile phase

The mobile phase was prepared by mixing potassium dihydrogen o-phosphate buffer (pH 2.8) and acetonitrile in ratio of 65:35 v/v. It was used as a diluent.

2.4.2 Preparation of standard solution

A 1mg/ml stock solution of Tapentadol was prepared in mobile phase.
2.4.3 Sample preparation for stress dissolution stability [17, 18, 19]

2.4.3.1 General procedure for standard exposed preparation

In thermal and humidity, the samples were exposed at 60°C, 40°C/75%RH stability condition for 2 month and photolytic condition (UV-light and Visible light) for 7th and 9th day. An accurately weighed quantity of 50mg Tapentadol HCL was transferred in 50mL volumetric flask, further diluted to make 20µg/mL as stock solution.

2.4.3.2 General procedure for samples and strip formulation preparation

In thermal and humidity studies, the samples were exposed at 60°C, 40°C/75%RH stability condition for 2 month. These thermal and humidity samples were analyzed using 900mL of phosphate buffer pH 6.8 medium at 37 ± 0.5°C on 7th, 15th, 30th, 45th, 52th and 60th day. Photolytic exposure (UV-light and Visible light) were analyzed on 7th and 9th day under earlier finalized dissolution condition respectively. The exposed samples were analyzed for area of unknown (Au) for the effect of stress on dissolution of drug.

2.4.3.3 General procedure of sample preparation of dissolution method parameter selection for tapentadol HCl analysis

Weight and drop the one tablet in each of the six dissolution vessels containing Acetate buffer pH 4.5/phosphate buffer 6.8 for drug under analysis. After specified the time point, 10 ml of the aliquot was withdraw from a zone midway between the surface of the dissolution media and top of the rotating blade, not less than rotating away from the wall of vessel. These solution were used as sample solution.

2.5 Methods

2.5.1 Dissolution Method parameters for estimation of Tapentadol HCl

The saturated solubility study of tapentadol was carried out using different dissolution media. Various dissolutions were performed to optimize the parameters like dissolution media, dissolution media volume and apparatus, using the optimized chromatographic conditions and solubility data of the drug. On this basis, the most optimized dissolution parameters which showed greater % release of drug were selected for further study. Also proposed method was validated as per ICH guidelines.

2.5.2 Studies of stress on dissolution stability of drug product

2.5.2.1 Non-thermal method

HPLC study:

i. Thermal study: Thermal exposed (60°C) samples (marketed formulation, strip formulation and standard drug) were analyzed after period of 2 month and estimated by the HPLC.

ii. Humidity study: Humidity exposed (40°C/75% RH) samples (marketed formulation, strip formulation and standard drug) were analyzed after period of 2 month and estimated by the HPLC.

iii. Photostability study: It includes photostability (UV and Visible light) samples (marketed tablet formulation, strip formulation and standard drug) were analyzed after period of 7th and 9th day respectively estimated by the HPLC. Further their assessment was done for effect of stress on drug content and drug release.

iv. pH study: In the pH study, Tapentadol tablet powder equivalent to about 66.72 mg of Tapentadol HCL drug were transferred to a series of 6 different 25 mL volumetric flask and volume make up with different pH diluent i.e. pH 1.2, pH 2.2, pH 4.6, pH 6.6, pH 7.4 and pH 8.0 up to the mark. Dilutions were prepared and stored at 60 °C for 6 hours. All the samples withdrawn at the intervals of 1h, 2h, 3h, 4h, and 5h and further diluted with mobile phase, from the chromatographic data % contents were calculated.

Microscopic study: In the optical microscopic study, samples stored at thermal exposed (60°C) and humidity exposed (40°C/75%RH) for 2 months, exposed samples were observed for visual or microscopic appearance. In the optical microscopic study, samples stored at photostability conditions (UV light and Visible light) and exposed samples for 7th and 9th day. The changes in samples were observed for visual or microscopic appearance, whiskers and colour changes appearance on the surface of the
photostability condition and exposed samples within the 7th and 9th day.

**Spectrometric study:** In the stress testing, photostability exposed samples estimated by the spectrometric method. In case of UV and visible light study, was not much difference in the percent drug content of the marketed formulation (Tablet) and strip formulation on exposure condition on 7th and 9th day.

2.5.2.2 Thermal method

**Differential scanning Colorimetry (DSC):** In these study, formulation samples were exposed to 60°C, 40°C/75% RH for 2 month and photostability (UV- light and visible light) study for 9th day and 7th day respectively.

3. RESULTS AND DISCUSSION

3.1 Finalization of Dissolution Method Parameters for Estimation of Tapentadol HCl

Various dissolution profiles were carried out in different dissolution media for drug.

3.1.1 Saturated solubility study

Saturated solubility study of tapentadol was carried out using different dissolution media such as purified water, 0.1 N HCL, acetate buffer (pH 4.5), phosphate buffer (pH 6.8) and saturated solubility were found to be 0.72, 1.02150, 0.694 and 0.8764 gm/mL respectively. Depending on their graphical plots of solubility studies, acetate buffer (pH 4.5) and phosphate buffer (pH 6.8) were selected. The drug released by using phosphate buffer (pH 5.8) was found to be greater as compared to acetate Buffer (pH 4.5). % drug released of USP apparatus I and II were calculated as 108.07 and 106.53 at 55 min respectively. Also % drug released at 50 and 75 RPM were found to be 106.53 and 107.24 at 55 min. The % released at different volumes such as 1000, 900 and 500 ML were found as 99.63, 106.53 and 102.82 at 55 min respectively. Depending on above dissolution optimization parameters like dissolution media, dissolution media volume and apparatus, using the optimized chromatographic conditions and solubility data of the drug to select a set of parameter that will give maximum % release of drug. The chromatogram of the drug recorded for trials for dissolution media.

**Conclusion**

From the above all observation, the following dissolution parameter had been finalized for the estimation of tapentadol HCl shown in Table No. 1. and chromatogram was run (Fig. 2.).

**Table 1. The finalized dissolution parameter selected for the dissolution analysis of Tapentadol**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tapentadol HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution Media</td>
<td>pH6.8 Phosphate Buffer</td>
</tr>
<tr>
<td>USP Apparatus</td>
<td>USP Apparatus II (TDT-06L), 900mL</td>
</tr>
<tr>
<td>Agitation / Rotation (RPM)</td>
<td>50</td>
</tr>
</tbody>
</table>

![Fig. 2. Chromatogram for test sample at 55 min](image-url)
From the chromatogram, the retention time, area (mV), asymmetry, theoretical plates were found to be 3.683 min, 298.202 (mV), 1.086, 3308 respectively (Fig. 2.).

3.2 Dissolution Method Validation (as per the ICH guideline)

3.2.1 System suitability data

The chromatographic conditions were set as per the optimized parameter mentioned in research articles, Kiran N. Kale and Krishna R. Gupta published a paper (2015) in AJPST “Development and validation of stability indicating RP-HPLC method for determination of Tapentadol in bulk and pharmaceutical dosage forms” [13]. The work was carried out in our own lab, hence was further accepted for the present experimentation. The study of the system suitability parameters was carried out using, six replicate injections of 20 µL standard solution were injected and chromatogram run. The chromatogram of system suitability parameter shown in Fig. 3.

From the chromatograms peak area were noted and results are shown in Table 2.

3.2.2 Linearity of test method

The linearity study was demonstrated by considering the label claim of the respective drug. The solution prepared as 10%-200% of the target concentration. All the solutions of different concentration level were prepared such as 10.90 µg/ml to 109.0 µg/ml with the use of selected mobile phase. All the test sample solutions of tapentadol injected in ascending order with different concentrations level and chromatogram run. The linearity of test method was established by plotting a graph between peak area v/s Concentration of drug in µg/mL as show in Fig. 4.

![Chromatograph of system suitability parameter](image)

**Table 2. Observation of system suitability parameter**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Std. Wt. taken (mg)</th>
<th>Area (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td>1240.757</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>1247.982</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>1239.891</td>
</tr>
<tr>
<td>4.</td>
<td>25.6</td>
<td>1244.669</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>1260.074</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>1235.500</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1244.81</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>Retention time</td>
<td></td>
<td>3.642</td>
</tr>
<tr>
<td>Asymmetry</td>
<td></td>
<td>1.066</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td></td>
<td>3346.66</td>
</tr>
</tbody>
</table>
Fig. 4. Linearity of tapentadol HCl at different concentration

From Table 4, the correlation coefficient for tapentadol was found to be 0.9995 and it was concluded that the response of AUC for the drug were found to be linear.

3.2.3 Precision of the test method: Repeatability

The test solutions were obtained by performing the dissolution of the respective drug using optimized dissolution parameters. The six replicate of the test volume 20 µL solutions of each drug so obtained were injected and chromatogram run. From the peak areas, the % dissolution of the drug was calculated using formula No. 1 given below.

\[
\%\ Drug\ release = \frac{(As \times Vdm \times Cstd \times dil.\ factor)}{(Astd \times DL) \times 100}
\]

Where,

- \(As\) = Area of sample
- \(Astd\) = Area of standard
- \(DL\) = Drug load
- \(Vdm\) = Volume of dissolution media
- \(Cstd\) = Concentration of standard

From the observations mean and % RSD of % Dissolution were found to be 105.86 and 0.90 respectively.

3.2.4 Accuracy of test method

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by spiking the drug (Tapentadol HCl 50%-150%) in dissolution medium considering the label claim of the respective drug as 100% drug target concentration and performing the dissolution test. The percent recovery of the drug was calculated using equation (2) & (3). The mean % recoveries of each drug at each spiked level were observed to be in range of 95% to 105% ascertaining the accuracy of the method. From the observation mean and %RSD were found to be 101.87 and 2.0 respectively.

\[
Total\ amount\ estimation = \frac{Au}{As} \times Cs \times \text{dil. factor} \times \text{Voll of stock}
\]

\[
\%\ Recovery = \frac{Total\ amount\ estimation - \text{lable claim}}{\text{standard added}} \times 100
\]

3.2.5 Range of test method

The test solutions for each drug prepared under accuracy study were used for the range determination. All the test solutions in the range of 80%-120% of target concentration were prepared and injected to run chromatogram. The % dissolution of each test solution for the drug was calculated. Range study of test method was established by plotting a graph between peak area v/s Concentration of drug in µg/mL (Fig. 5) and correlation coefficient was determined.

From Table 5, the mean, % RSD and correlation coefficient for Tapentadol was found to be 1.0452, 1.24 and 0.9959 respectively.
3.2.6 Robustness of test method

In these study deliberate changes in the method was done; i.e. Change in flow rate (1.0 ±0.2 mL/min), change in pH of mobile phase (2.8 ± 0.2 unit), change in detection wavelength (217 ± 5.0 nm) and change in mobile phase composition (organic portion of 35%±5%). From the result of robustness study, it concluded that the RT and Asymmetry at different flow rates, at different pH of buffer solution, at different organic portion in mobile phase and change in wavelength were comparable with that of the optimized chromatographic conditions.

3.2.7 Ruggedness

The studies were carried out for two different parameters; i.e. Day (Intraday and Interday) and Analyst to Analyst variation. In intra-day and inter-day variation, results estimation by proposed methods was found to be varying. The % RSD was found to be 1.25 and 4.83 for inter-day and intra-day studies respectively. In interday study, % drug release was found to be decrease after two day storage suggested that the Tapentadol HCl is unstable in solution form while in intra-day study, solution was found to be stable up to 3 hrs. The Chromatograph were recorded and overlapped chromatogram of interday samples of 3days are shown in Fig. 5. The results of estimation for Tapentadol HCl by different analyst were very much reproducible with % RSD 0.93. This indicates that method acceptable in the hands of different analysts.

3.2.8 Solution stability

Test solution stability was carried out for a period of 48 hours and the solutions were analyzed at an interval of 24 hours. The area under curve at an interval of 24 hours were recorded and the difference in % release of initial and the subsequent time interval for the drug was found to be 7.43% after 48 hours.

3.3 Application of Stress on Dissolution Stability

3.3.1 Non thermal method

3.3.1.1 HPLC study

3.3.1.1.1 Thermal study

Thermal exposed (60°C) samples (marketed formulation, packed strip formulation and standard drug were analyzed after period of 2 month and estimated by the HPLC. The thermal study reveals that there was not much difference in % drug release for marketed formulation and strip formulation on exposure to thermal condition for 2 months. However, the marketed formulation and strip formulation were degraded around 5.5% and 1.67% of drug content respectively. The overlain chromatograms of sample of 0 day and 2 month revealed that no additional peaks were generated in chromatogram of marketed formulation and packed strip formulation respectively. It was estimated that, degraded to the extent of 11.50%, but no additional peak observed in chromatographic condition. From the chromatographic data, the zero order plot of release showed in Figs. 7 and 8. From the dissolution curve, it was observed that not much difference in the release after 2 months.
### Table 3. Results of ruggedness test method

<table>
<thead>
<tr>
<th>Parameters which was changed</th>
<th>Mean</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1.108</td>
<td>2.89</td>
</tr>
<tr>
<td>Wavelength</td>
<td>1.022</td>
<td>0.81</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>1.022</td>
<td>0.84</td>
</tr>
<tr>
<td>pH of mobile phase buffer</td>
<td>3328.5</td>
<td>0.61</td>
</tr>
</tbody>
</table>

![Chromatogram of interday A-sample or 1st day, B-sample for 2nd day, C-sample for 3rd day](image)

**Fig. 6.** Chromatogram of interday A-sample or 1st day, B-sample for 2nd day, C-sample for 3rd day

![Zero order plot of thermal exposed samples](image)

**Fig. 7.** Zero order plot of thermal exposed samples

Zero graphical representation of thermal exposed and thermal exposed packaging samples was established by plotting a graph between % drug release v/s Time in hrs.

### 3.3.1.1.2 Humidity study

Humidity exposed (40°C/75% RH) samples (marketed formulation and packed strip formulation) were analyzed after period of 2 month and estimated by the HPLC.

From the results of humidity study reveals that there was not much difference in % drug release for marketed formulation and packaged marketed strip formulation on exposure to humidity condition for 2 month. However, the marketed formulation and strip formulation were degraded around 3.15% and 0.40% of drug content respectively. The overlain chromatograms of sample of 0 day and 2 month revealed that no additional peaks were generated in
chromatogram of marketed formulation and strip formulation. The overlain chromatograms of sample of 0 day and 2 month std. exposed sample estimated that, degraded to the extent of 15.57%, but no additional peak observed in chromatographic condition.

![Figure 8. Zero order plot of thermal exposed packaging strip samples](image)

**Fig. 8. Zero order plot of thermal exposed packaging strip samples**

![Figure 9. Zero order plot of humidity exposed samples](image)

**Fig. 9. Zero order plot of humidity exposed samples**

![Figure 10. Zero order plot of humidity exposed packaged strip samples](image)

**Fig. 10. Zero order plot of humidity exposed packaged strip samples**
From Figs. 9 and 10 the zero order plot of release showed, it was observed that not much difference in the release was observed from the dissolution curve after 2 months.

Zero order graphical representation was established of humidity exposed and humidity exposed packaged strip samples by plotting a graph between % drug release v/s Time in hrs.

3.3.1.1.3 Photostability study

1) UV light

From the observations, UV light study reveals that there was not much difference in % drug release for marketed formulation and strip formulation on exposure to UV light condition for 7th day. However, the marketed formulation and strip formulation were degraded around 5.66% and 6.44% of drug content respectively.

From Fig. 11, the zero order plot of release showed, it was observed that not much difference in the release was observed from the dissolution curve after 7th day.

2) Visible light

Visible light studies reveals that there was not much difference in % drug release for marketed formulation and packaged marketed strip formulation on exposure to visible light condition for 9th day. However, the marketed formulation and packaging marketed strip formulation were degraded around 3.78% and 8.47% of drug content respectively.
Table 4. Observation and result of exposed samples

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Exposed condition</th>
<th>Assay (Mean± sd)</th>
<th>S1 Dissolution stage</th>
<th>Max.% dissolution at 55 min (mean ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal -</td>
<td>100.01</td>
<td>Fulfill</td>
<td>100.31</td>
</tr>
<tr>
<td>1 month</td>
<td>Thermal exposed sample</td>
<td>Tablet formulation</td>
<td>105.66</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packaging formulation</td>
<td>103.30</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td>Humidity exposed sample</td>
<td>Tablet formulation</td>
<td>94.44</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packaging formulation</td>
<td>106.43</td>
<td>Fulfill</td>
</tr>
<tr>
<td>2 Month</td>
<td>Thermal exposed sample</td>
<td>Tablet formulation</td>
<td>94.54</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packaging formulation</td>
<td>98.36</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td>Standard drug</td>
<td>Table formulation</td>
<td>88.50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packaging formulation</td>
<td>96.86</td>
<td>Fulfill</td>
</tr>
<tr>
<td>7 day</td>
<td>Humidity exposed sample</td>
<td>Tablet formulation</td>
<td>100.41</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td>Standard drug</td>
<td>Table formulation (UV light)</td>
<td>84.43</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packaging formulation (UV light)</td>
<td>94.35</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td>Photostability exposed</td>
<td>Standard drug</td>
<td>93.57</td>
<td>Fulfill</td>
</tr>
<tr>
<td>9 day</td>
<td></td>
<td>Table formulation (Visible light)</td>
<td>96.23</td>
<td>Fulfill</td>
</tr>
<tr>
<td></td>
<td>Photostability exposed</td>
<td>Packaging formulation (Visible light)</td>
<td>91.54</td>
<td>Fulfill</td>
</tr>
</tbody>
</table>

Zero order graphical representation was established by plotting a graph between % drug release v/s Time in hrs and is shown in Fig.12.

The observations and results of % content of the thermal, humidity and photostability samples of exposed samples, packaged samples and standard drug were analyzed and summarized in Table 4.

3) pH study

The % drug contents at the interval of 5 hours were found to be 105.90, 100.29, 99.44, 96.64, 97.07 and 96.47% at pH 1.2, pH 2.2, pH 4.6, pH 6.6, pH 7.4 and pH 8.0 respectively. Under various pH conditions the drug content were found to be 7-8% in pH 2.2 and 4.5 (acetate buffer), except the pH 1.2, 6.6, 7.4 and 8.0 showed around 2-4% change in drug content after 5h. The study of chromatograms recorded for above pH samples showed no additional peak generated in the chromatogram even after 5 h of study.

A) Microscopic study

In the optical microscopic study, samples stored at thermal exposed (60°C) and humidity exposed (40°C/75%RH) for 2 month, no changes were observed in visual or microscopic appearance. Photographs of appearance and surface on the
Thermal exposure

0 Day | 2 Month | 0 Day | 2 Month

a) Exposed sample  
b) Packaging sample  

Fig. 13. Thermal exposed samples of marketed formulation

Humidity exposed

0 DAY | 2 MONTH | 0 DAY | 2 MONTH

a) Exposed sample  
b) Packaging sample  

Fig. 14. Humidity exposed samples of marketed formulation

Photostability study

UV light study

0 DAY | 7 DAY | 0 DAY | 7 DAY

a) Exposed sample  
b) Packaging sample  

Fig. 15. UV light exposed samples of marketed formulation

Visible light study

0 DAY | 7 DAY | 0 DAY | 7 DAY

a) Exposed sample  
b) Packaging sample  

Fig. 16. Visible light exposed samples of marketed formulation
tablet in each of the tapentadol HCl tablets before and after storage condition describe above for 2 month are shown in Figs. 13 and 14. In exposed samples, whiskers and colour changes appeared on the surface of the exposed samples within the two month.

In the optical microscopic study, samples stored at photostability condition (UV light and Visible light) for 7th and 9th day, no changes were observed in visual or microscopic appearance. Photographs of appearance and surface on the tablet in each of the tapentadol HCl tablets before and after storage condition describe above for 7th and 9th day are shown in Figs. 15 and 16. In exposed samples, whiskers and colour changes appeared on the surface of the exposed samples within the 7th and 9th day.

\[
\text{% Contents} = \frac{Au}{Astd} \times \frac{\text{Conc.std} \times \text{dil.factor} \times \text{Avg.Wt.} \times \text{Stock sol.n}}{\text{Wt.taken} \times \text{label claim}} \times 100
\]  

(5)

In the stress testing, photostability exposed samples estimated by the spectrometric method. In case of UV and visible light study, were not much difference in the % content of the marketed formulation (Tablet) and strip formulation on exposure condition in 7th and 9th day.

In UV light study, powder and standard drug (Figs. 19, 20) were degraded to the extent of 18.96% and 25.35% respectively as compared to normal sample.

In visible light study, powder and standard drug (Figs. 23, 24) the powder and standard drug were degraded to the extent of 19.54% and 12.74% respectively as compared to normal sample.

\[
\text{Fig. 17. Spectra of exposed Samples(tablet) under UV- light}
\]

\[
\text{Fig. 18. Spectra of exposed Packaged samples under UV- light}
\]

\[
\text{Fig. 19. Spectra of exposed tablet Powder samples under UV-light}
\]

\[
\text{Fig. 20. Spectra of exposed standard drug under UV- light}
\]
Conclusion: As compared to standard unexposed drug, drug sample (tablet) and tablet powdered drug showed hypochromic effect, whereas exposed packaged sample and standard drug showed hyperchromic effect under UV-light.

C) Visible light study

The samples was exposed by visible light for 7th day, the % content degradation of the drug determine by the following spectrogram.

![Fig. 21. Spectra of exposed samples under visible light](image1)

![Fig. 22. Spectra of exposed packaged samples under visible light](image2)

![Fig. 23. Spectra of exposed standard drug under visible light](image3)

![Fig. 24. Spectra of exposed powder sample under visible light](image4)

Conclusion: As compared to standard unexposed drug, exposed sample (tablet), packaged drug and standard drug showed hypochromic effect, whereas exposed tablet powder sample showed hyperchromic effect under visible light.

The % content of the photostability study (UV light and Visible light) samples are as follows
Table 5. Observation and results of photostability study

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Storage condition</th>
<th>Exposed samples</th>
<th>% content</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 day</td>
<td>UV exposed sample</td>
<td>Std. drug</td>
<td>74.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tablet</td>
<td>93.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packed tablet</td>
<td>94.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tablet Powder</td>
<td>81.04</td>
</tr>
<tr>
<td>9 day</td>
<td>Visible exposed sample</td>
<td>Std. drug</td>
<td>87.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tablet</td>
<td>96.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Packed tablet</td>
<td>92.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tablet Powder</td>
<td>80.46</td>
</tr>
</tbody>
</table>

The results obtained in all the stress conditions for drug content and drug release were statistically evaluated by applying two-way ANOVA followed by post hoc-Bonferroni test. The graphical plot for drug content and drug release are shown in Figs. 25-26.

1. Assessment of stress on Drug content

As depicted in Fig. 1, two-way ANOVA demonstrated significantly decreased in % degradation in thermal, humid, Photo UV and Photo visible (P< 0.001) groups as compare to control group in exposed tablet. Moreover, significant decrease in % degradation in Photo UV and Photo visible (P< 0.001) groups as compare to control group in exposed tablets [ F= 2.95, DFn=4, DFd=40], whereas it is non-significant for thermal and humid group compare to control group in exposed and packaged tablet formulation.

2. Assessment of stress on drug Release

As depicted in Fig. 26, two-way ANOVA demonstrated significantly decreased in % degradation in thermal, humid, Photo UV and Photo visible (P< 0.001) groups as compare to control group in exposed tablet. Moreover, significant decrease in % degradation in Photo UV and Photo visible (P< 0.001) groups as compare to control group in exposed tablets [ F= 2.95, DFn=4, DFd=40], whereas it is non-significant for thermal and humid group compare to control group in exposed and packaged tablet formulation.

3.3.2 Thermal method

A) Differential scanning Calorimetry (DSC)

First peak at 147.6°C, which indicates that it is in pure and crystalline nature, another peak at melting 186.6°C, it somewhat looking like board one, which might be due to amorphous and semi-amorphous in nature, the last peak found at 208.1°C, which is in diffuse form and it is on the basis of structure, it contains some functional groups like OH, NH₂ and CH₃ these groups may be responsible for oxidation or change in their physiochemical properties. Thermogram of exposed marketing formulation and packed marketing formulation strip was compared with unexposed thermogram.

Fig. 25. % Degradation in control, thermal, humid, Photo UV and Photo visible groups. Each bar represent mean±SEM.*P<0.001vs.control group in exposed and unexposed tablets (Two-way ANOVA followed by post hoc-Bonferroni test)
Fig. 26. % Release in control, thermal, humid, Photo UV and Photo visible groups. Each bar represent mean ±SEM. *P<0.001 vs control group in exposed and unexposed tablets (Two-way ANOVA followed by post-hoc Bonferroni test)

Fig. 27. DSC thermogram of Unexposed std(A), exposed formulation to thermal (B), humidity (C), UV (D) and visible light (E)

**Tablet exposed samples:** DSC thermogram of unexposed std. and exposed formulation to thermal, humidity, UV and visible light shown in Fig. 27 whereas peak value; i.e. melting point and enthalpy as shown in Table 6. It was compared with std. Thermogram and std. value respectively.

From the above study, it was observed that there was not much difference in the melting point of the drug in case of thermal and humidity exposed samples while in UV and visible light sample it shows a significant difference in the melting point of samples; i.e. it may be due to change in the crystalline form or oxidation reaction. In case of
visible light, it shows an additional peak at 199.4°C with enthalpy (-8.01mW) and change in melting point to the 213.1°C from 208.1°C compared with the std. sample. It can be concluded that tablet sample was found to be susceptible to UV and visible light exposure.

**Tablet packaged exposed samples:** DSC thermogram of unexposed std. and tablet packaged exposed formulation to thermal, humidity, UV and visible light shown in Fig. 28 whereas peak of packaged samples value; i.e. melting point and enthalpy as shown in Table 7. It was compared with std. thermogram and std. value respectively.

From the above study, it was examine that there was not much difference in the melting point of the drug in case of thermal and thermal exposed packaged samples but, in UV and visible light sample it shown a significant difference in the melting point of samples; i.e. it may be estimated

<table>
<thead>
<tr>
<th>Exposed tablet</th>
<th>Peak 1 (Melting pt. °C)</th>
<th>Enthalpy (mW)</th>
<th>Peak 2 (Melting pt. °C)</th>
<th>Enthalpy (mW)</th>
<th>Peak 3 (Melting pt. °C)</th>
<th>Enthalpy (mW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal</td>
<td>147.8</td>
<td>-11.23</td>
<td>186.9</td>
<td>-11.29</td>
<td>208.4</td>
<td>-6.63</td>
</tr>
<tr>
<td>Humidity</td>
<td>147.9</td>
<td>-11.01</td>
<td>185.6</td>
<td>-13.55</td>
<td>208.0</td>
<td>-9.14</td>
</tr>
<tr>
<td>UV light</td>
<td>147.2</td>
<td>-13.96</td>
<td>186.6</td>
<td>-13.97</td>
<td>207.9</td>
<td>-8.54</td>
</tr>
<tr>
<td>Visible light</td>
<td>148.2</td>
<td>-14.34</td>
<td>189.4</td>
<td>-12.75</td>
<td>213.1</td>
<td>-9.44</td>
</tr>
</tbody>
</table>

**Table 6. Differential Scanning Colorimetry (DSC) observations and results for thermal, humidity, UV-light & visible light exposed tablet samples**

![Fig. 28. DSC thermogram of Unexposed std. (A), tablet packaged exposed formulation to thermal (b), humidity (c), UV (d) and visible light (e)](image-url)
Table 7. Differential scanning Colorimetry (DSC) observations and results for thermal, humidity, UV-light & visible light exposed packaged strip tablet samples

<table>
<thead>
<tr>
<th>Exposed packaged tablet</th>
<th>Peak 1 Melting pt.(°C)</th>
<th>Enthalpy (m W)</th>
<th>Peak 2 Melting pt. (°C)</th>
<th>Enthalpy (m W)</th>
<th>Peak 3 Melting pt. (°C)</th>
<th>Enthalpy (m W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal</td>
<td>147.5</td>
<td>-13.96</td>
<td>186.5</td>
<td>-13.96</td>
<td>208.2</td>
<td>-8.4</td>
</tr>
<tr>
<td>Humidity</td>
<td>148.5</td>
<td>-11.81</td>
<td>187.8</td>
<td>-13.55</td>
<td>208.0</td>
<td>-8.14</td>
</tr>
<tr>
<td>UV light</td>
<td>147.8</td>
<td>-11.33</td>
<td>187.1</td>
<td>-11.3</td>
<td>207.7</td>
<td>-6.63</td>
</tr>
<tr>
<td>Visible light</td>
<td>148.7</td>
<td>-16.69</td>
<td>180.8</td>
<td>-9.48</td>
<td>222.2</td>
<td>-8.74</td>
</tr>
</tbody>
</table>

that change in the crystalline form or oxidation reaction. In case of visible light, it was shown an additional peak at 127.8°C at enthalpy -9.48. It was shown that packed sample susceptible to visible light in packaged formulation.

4. CONCLUSION

From our study it can be concluded that the dissolution test method for Tapentadol HCI using phosphate buffer pH 6.8 as dissolution media was successfully developed and validated. The validated method was used to study the effect of stress on drug release.

In our investigation of stress on dissolution of drug it was found that tapentadol HCI std. drug was susceptible to degradation. The tablet and packaged formulation were susceptible to photolytic degradation indicated by difference in drug content while the release was more affected under UV exposed tablet and packaged formulation as compare to other stress condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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