*Journal of Pharmaceutical Research International*

***33(11): 7-16, 2021; Article no.JPRI.66200***
***ISSN: 2456-9119***
*(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)*

# RP-HPLC Method Development and Validation of Tapentadol Hydrochloride in Bulk and Pharmaceutical Formulations

**G. Sangeetha[1*], M. Swamivel Manickam[2] and P. Sanil kumar[3]**

*[1]Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore-560035, Karnataka, India.*
*[2]Department of Pharmacy, Annamalai University, Chidambaram-608002, Tamil Nadu, India.*
*[3]Accenture, Bangalore-560029, Karnataka, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author GS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MSM and PSK' managed the analyses of the study. Author PSK managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JPRI/2021/v33i1131239
Editor(s):
(1) Dr. Aurora Martínez Romero, Juarez University, Mexico.
Reviewers:
(1) Olha Shapoval, Kharkiv National Medical University, Ukraine.
(2) Hussein Nasser Khalf Al-Salman, University Of Basrah, Iraq.
(3) Vijaya Sachin Vichare, PES Modern College of Pharmacy (For Ladies), India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/66200

***Original Research Article***

***Received 28 December 2020***
***Accepted 03 March 2021***
***Published 15 March 2021***

## ABSTRACT

**Objective:** Tapentadol Hydrochloride was approved (November 2008) by the United States Food and Drug Administration for the relief of moderate to severe pain. It is an opioid analgesic, acts by dual mechanism as opioid receptor agonist and norepinephrine reuptake inhibitor. The present research work was aimed to develop an accurate, precise, and rapid RP-HPLC method and subsequently validates the method according to the International Conference on Harmonization (ICH) guidelines for the determination of Tapentadol Hydrochloride.
**Methods:** Tapentadol Hydrochloride was analyzed by using High-Performance Liquid Chromatography. Better separation of the drug was achieved by using a Symmetry C18 column (150x4.6mm, 3.5µm) with the mobile phase consisted of a mixture of Orthophosphoric acid (0.1% of Orthophosphoric acid in HPLC water) and acetonitrile in the ratio of 30:70 v/v at a flow rate of 1

**Corresponding author: E-mail: sange2008@gmail.com;*

ml/min, and the detection was at the wavelength of 219nm using a PDA detector.
**Results:** The retention time of Tapentadol Hydrochloride was found to be 3.747 ± 0.127 min. The method was found to be linear in the range of 10-200 ug/ml with a correlation coefficient ($r^2$) of 0.9991. The LOD and LOQ of the method were calculated to be 0.1 and 1µg/ml respectively. The method precision and system precision was estimated and the results were calculated as % RSD values, which were found to be within the limits. Recovery of Tapentadol Hydrochloride was found to be 100.1%, which confirms the efficiency of the method.
**Conclusion:** The developed RP-HPLC method was validated using standard ICH guidelines. The developed method can be used for the analysis of both tapentadol hydrochloride bulk and formulations.

*Keywords: RP-HPLC; tapentadol hydrochloride; validation; method development; pain management.*

## 1. INTRODUCTION

Tapentadol Hydrochloride is considered under BCS (Biopharmaceutical Classification System) class I drug, which is highly soluble and permeable but it is low lipid-soluble [1]. Tapentadol Hydrochloride 3-[(1R, 2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl] phenol monohydrochloride is a centrally acting opioid analgesic. The molecular formula of Tapentadol Hydrochloride is $C_{14}H_{23}NO$ [2]. It acts by a dual mechanism as opioid receptor agonist and norepinephrine reuptake inhibitor [3]. Tapentadol Hydrochloride was approved (Nov 2008) by the USFDA (United States Food and Drug Administration) for the relief of moderate to severe pain with the recommended dose of 50 to 100 mg every 4 to 6 h [4]. Tapentadol is effective in the pain management of osteoarthritis and low back pain [5] and effective against inflammation, visceral, nociceptive, and neuropathic conditions [6]. The present study was aimed to develop and validate a simple, rapid, specific, and reliable method for the determination of Tapentadol Hydrochloride by RP-HPLC (Reverse Phase-High Performance Liquid Chromatography) as per the ICH guidelines. Also, the objective of the study was considered to be cost-effective, minimizes time consumption, minimizes the retention time without compromising the sensitivity. So it can be a desirable method for the routine analysis of Tapentadol Hydrochloride.

## 2. MATERIALS AND METHODS

### 2.1 Instrumentation

Method development and validation were performed on a Waters 2695 HPLC system, equipped with an autosampler and PDA detector. The analysis was carried out with asymmetry C18, (150mmx40mm, 5µm) dimensions at ambient temperature. The data compilation and evaluation were done with empower 2 software.

### 2.2 Chemicals and Reagents

Tapentadol Hydrochloride was provided as a gift sample from Symed labs, Hyderabad, India. Orthophosphoric acid, acetonitrile HPLC grade was procured from Hi media. Methanol and triethylamine were obtained from Fine Chemicals. All other chemicals and reagents used were of analytical grade.

### 2.3 Preparation of Solution

#### 2.3.1 Preparation of buffer (0.1% of Orthophosphoric acid)

1 ml of Orthophosphoric acid was measured accurately and added into 1000 ml of the volumetric flask; the volume was dissolved and diluted to 1000 ml with HPLC grade water (Milli-Q water). The pH was adjusted using triethylamine HPLC grade to 4.0±0.05 and the buffer was degassed in an ultrasonic water bath and filtered through a 0.45µm filter using vacuum filtration.

#### 2.3.2 Preparation of mobile phase

The mobile phase was prepared by mixing 0.1% Orthophosphoric acid and acetonitrile in the ratio of 30:70 v/v. The mobile phase was degassed in an ultrasonic water bath for 15min and filtered through a 0.45µm filter using vacuum filtration.

#### 2.3.3 Preparation of diluent

The mobile phase was used as diluent.

#### 2.3.4 Preparation tapentadol hydrochloride standard solution

100 mg of Tapentadol Hydrochloride working standard was weighed into a 100 ml volumetric flask diluted to the volume with diluent (Mobile

phase). Further 5 ml of the above solution was diluted to 50 ml using a diluent to get the final concentration of 100 µg/ml.

### 2.3.5 Preparation of tapentadol hydrochloride sample solution

Twenty tablets of Tapentadol hydrochloride (Each tablet contains 100 mg of Tapentadol Hydrochloride) were taken in a mortar and crushed into a powder. 172 mg of tapentadol Hydrochloride sample equivalent to 100 mg was transferred into a 100 ml volumetric flask diluted with diluent. It was sonicated for 30 min to dissolve the components. Further 5 ml of the above solution was diluted to 50 ml using a diluent to get the final concentration of 100 µg/ml.

### 2.3.6 Selection of wavelength for method development

A stock solution of 1000 mg/ml of Tapentadol Hydrochloride was prepared and further serial dilutions were made to get the concentration of 100 µg/ml with methanol. The wavelength was selected by scanning the above standard drug solution between 200 to 400nm. The scanned results showed the maximum absorbance at 219 nm. Therefore 219 nm was selected as the detection wavelength for the RP-HPLC investigation Fig. 2.

### 2.3.7 Method development

The chromatographic method was developed by performing the trials considering the response as United States Pharmacopoeia (USP) plate count and tailing factor. The variable considered for conducting experiments were column type, mobile phase composition. The wavelength (219 nm), flow rate (1.00 ml/min), injection volume (10µl) were kept constant throughout the trails. The experimental chromatographic conditions for method development have shown in Table 1.

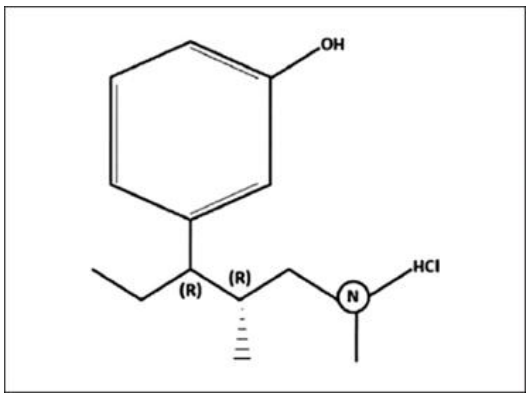


**Fig. 1. Chemical structure of Tapentadol Hydrochloride**

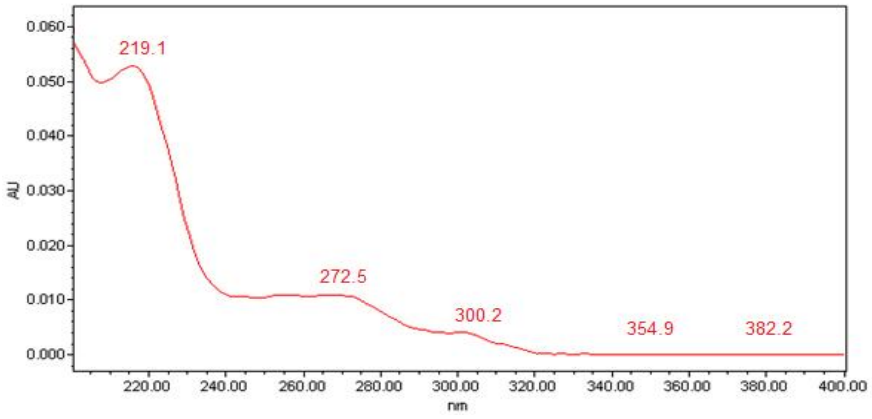


**Fig. 2. UV spectra of Tapentadol Hydrochloride**

**Table 1. Experimental conditions of method development**

| Trial No | Composition of mobile phase | Buffer | Column dimensions and pore size | Flow rate | Injection volume | Observations |
|---|---|---|---|---|---|---|
| 1 | ACN:Water (80:20) | -- | X-Bridge 150x4.6 mm,3.5µ | 1.00 ml/min | 10 µl | Plate count was not within the limit |
| 2 | ACN: Water (70:30) | - | X-Bridge C18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | The broad peak was obtained |
| 3 | ACN: Water (60:40) | - | X-Bridge C18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | The plate count was not within the limit |
| 4 | ACN: Water (50:50) | - | X-Bridge C18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | USP Tailing was not within the limit |
| 5 | ACN:Buffer (80:20) | 0.1% TFA | X-Bridge C18 150x4.6 mm,3.5µ | 1.00 ml/min | 10 µl | Less retention time |
| 6 | ACN:Buffer (70:30) | 0.1% TFA | Symmetry C18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | Plate count and tailing was not within the limit |
| 7 | ACN: Buffer (80:20) | 0.1% TFA | Luna C18 250x4.6 mm, 5µ | 1.00 ml/min | 10 µl | Peak tailing was not within the limit |
| 8 | ACN:Buffer (70:30) | 0.1% TFA | Symmetry C18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | Two peaks were observed |
| 9 | ACN:Buffer (80:20) | 0.1% OPA | Symmetry C18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | Peak shape was not proper |
| 10 | ACN:Buffer (70:30) | 0.1% OPA | Symmetry C-18 150x4.6 mm, 3.5µ | 1.00 ml/min | 10 µl | Plate count and tailing were within the limit. |

*ACN – Acetonitrile, TFA- Tri fluoro acetic acid, OPA- Ortho Phosphoric acid*

## 2.4 Construction of Calibration Curve

The standard stock solution was diluted using diluent (Mobile phase) to get the various concentrations of 10, 25, 50, 75, 100, 125, 150, 200 µg/ml. The chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linear plot was constructed using the mean peak areas of the respective concentrations.

## 2.5 Method Validation

The developed method was validated for specificity, system specificity, linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness, and system suitability parameters as described in ICH guidelines [7].

### 2.5.1 Specificity

Specificity was demonstrated by spiking a pure specific concentration of a drug with appropriate levels of impurities. A marketed formulation of Tapentadol Hydrochloride (Tapal 100) from MSN Pharma was taken and specificity was determined in 6 replicate at a concentration of 100 µg/mL and percentage RSD (Relative Standard Deviation) was calculated.

### 2.5.2 System suitability

System suitability is defined as, the checking of a system, before or during the analysis of unknowns, to ensure system performance [8]. The system suitability was assessed by six replicate analysis of Tapentadol Hydrochloride at a concentration of 100 µg/ml. The acceptance criterion was ± 2% for the percentage (% RSD) for the peak area and retention times for Tapentadol Hydrochloride.

### 2.5.3 Linearity and range

Linearity is the ability to obtain test results that are directly proportional to the concentration of the analyte. Linearity was determined by three injections of eight different Tapentadol Hydrochloride concentrations (10, 25, 50, 75, 100, 125, 150, 200 µg/ml). The average peak areas were plotted against concentrations. Linear regression analysis was used to assess the linearity using the least square regression method [7]. In general, a value of correlation coefficient (r2) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

### 2.5.4 Accuracy

The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is obtained by calculating the percentage recovery (%R) of the analyte recovered. In this case, to evaluate the accuracy of the developed method, successive analysis (n = 3) for three different concentrations (50 µg/ml, 100 µg/ml, and 150 µg/ml) of the standard Tapentadol Hydrochloride solution was performed using the developed method. The data of the experiment were statistically analyzed using the formula [% Recovery = (Recovered conc /Injected conc) x 100] to study the recovery and validity of the developed method. The mean recovery should be within 90–110% to be accepted.

### 2.5.5 Precision

The precision was determined by six to replicate analyses at a concentration of 100 µg/mL of standard Tapentadol Hydrochloride solution using the developed method and % RSD was calculated. The precision was expressed as % RSD, which was found to be less than 2% depicting satisfactory precision of the system according to USP [9].

### 2.5.6 Robustness

Robustness is an analysis of the reliability of analysis to deliberate variations in method parameters. It is the measurements of variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure [8]. In the present study, robustness was checked by allowing a small intentional change in injection flow rate (±0.2 ml/min), and organic phase buffer concentration by ±7 and % RSD was determined.

### 2.5.7 Limit of detection and limit of quantification

LOD is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy. These two parameters were calculated using the formula

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ= standard deviation of response (peak area) and S = slope of the calibration curve.

## 3. RESULTS AND DISCUSSION

### 3.1 Development of HPLC Method

Chromatogram 3A represents the blank mobile phase, and chromatogram 3B represents Tapentadol Hydrochloride with an average retention time of 3.747 ± 0.127 min and with no interfering peaks. This is an indication of the specificity of the developed HPLC method.

### 3.2 System Suitability

System suitability was assessed by six replicate analyses of Tapentadol hydrochloride at a concentration of 100µg/ml. The acceptance criterion was ±2% for the percentage relative standard deviation (% RSD) for the peak area and retention times for Tapentadol Hydrochloride as shown in Table 2.

### 3.3 Linearity

From the stock solution, 10, 25, 50, 75, 100, 125, 150, 200 µg/ml solutions were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas of their respective concentrations. The correlation coefficient ($r^2$) was found to be 0.9991. The linearity data of Tapentadol Hydrochloride has shown in Tables 3 & 4 and the calibration plot has shown in Fig. 4.

### 3.4 Accuracy

A recovery study was performed to check the accuracy of the method at three levels 50%, 100%, and 150%. A recovery study was carried out 3 times for each level and the percentage recovery and % relative standard deviation were calculated. The mean recovery of Tapentadol Hydrochloride was found to be 100.1% (Table 5).

**Table 2. System suitability analysis of Tapentadol Hydrochloride**

| Injections | Drug | RT | Area | %area | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Tapentadol Hydrochloride | 3.744 | 1891923 | 100 | 5634 | 1.10 |
| 2 | Tapentadol Hydrochloride | 3.746 | 1895508 | 100 | 5623 | 1.10 |
| 3 | Tapentadol Hydrochloride | 3.746 | 1887820 | 100 | 5580 | 1.10 |
| 4 | Tapentadol Hydrochloride | 3.746 | 1891040 | 100 | 5600 | 1.10 |
| 5 | Tapentadol Hydrochloride | 3.752 | 1888217 | 100 | 5558 | 1.09 |
| 6 | Tapentadol Hydrochloride | 3.752 | 1893460 | 100 | 5567 | 1.09 |
| MEAN | | | 1891328 | RT- Retention Time | | |
| SD | | | 2981.24 | | | |
| % RSD | | | 0.16 | | | |

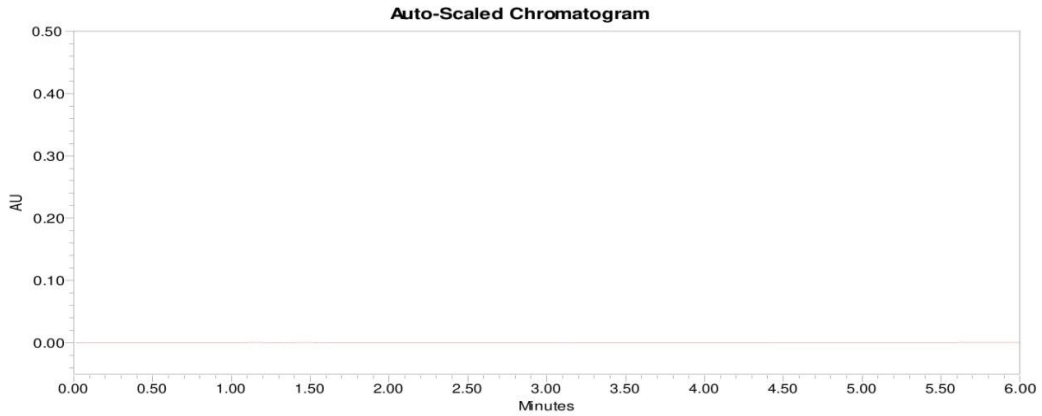


**Figure 3A. Blank chromatogram**

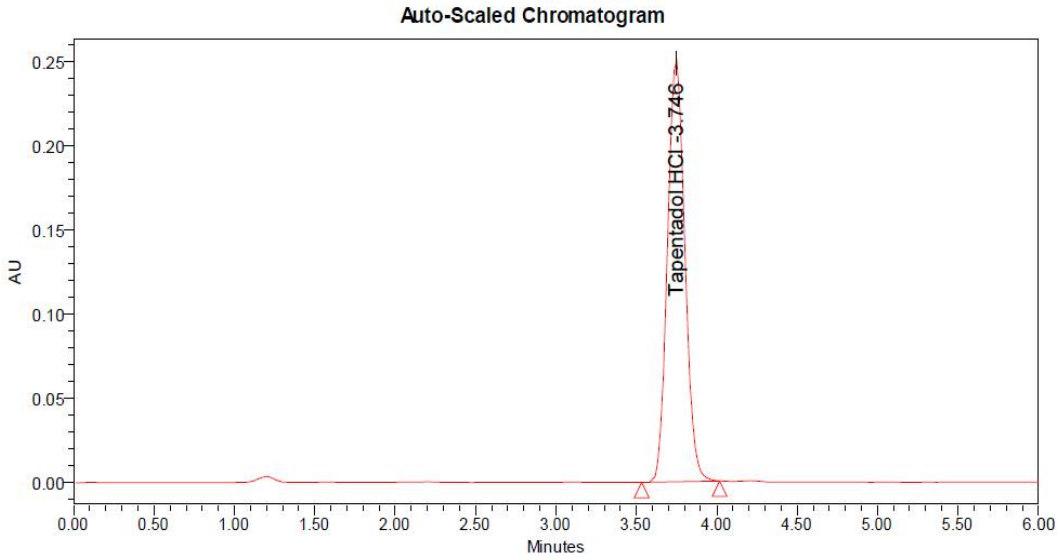


**Fig. 3B. Retention time of Tapentadol Hydrochloride**

**Table 3. linearity of Tapentadol Hydrochloride**

| Sl no | Concentration µg/ml | Area |
|---|---|---|
| 1 | 10.00 | 184880 |
| 2 | 25.00 | 459113 |
| 3 | 50.00 | 896209 |
| 4 | 75.00 | 1206347 |
| 5 | 100.00 | 1834505 |
| 6 | 125.00 | 2178517 |
| 7 | 150.00 | 2655617 |
| 8 | 200.00 | 3502114 |

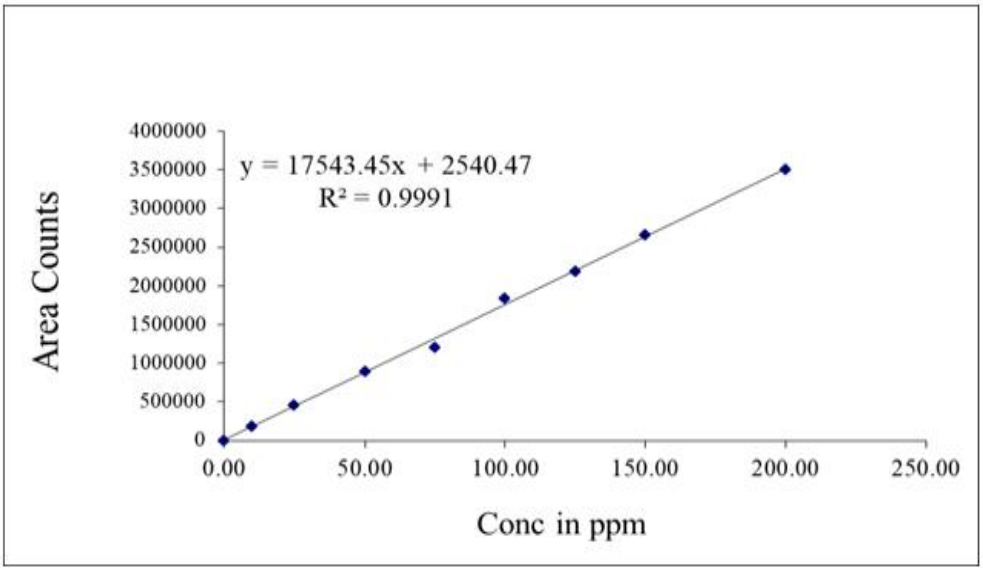


**Fig. 4. Calibration plot of Tapentadol Hydrochloride**

**Table 4. Regression characteristics of linearity of Tapentadol Hydrochloride**

| Parameters | Results |
|---|---|
| Linearity range (µg/ml) | 10- 200 |
| Regression equation (y=mx+b) | y=17543.45x+2540.47 |
| Slope (m) | 17543.45 |
| Intercept (b) | 2540.47 |
| The correlation coefficient ($r^2$) | 0.9991 |

**Table 5. Recovery study results of Tapentadol Hydrochloride**

| Sl No | Accuracy range | Amount of API added (mg) | Amount recovered (mg) | % Recovery |
|---|---|---|---|---|
| 1. | 50% Accuracy | 50 | 49.47 | 98.9 |
| 2. | | 50 | 49.52 | 99.0 |
| 3. | | 50 | 49.6 | 99. 2 |
| 4. | 100% Accuracy | 100 | 100.89 | 100.9 |
| 5. | | 100 | 100.49 | 100.5 |
| 6. | | 100 | 101 | 101.0 |
| 7. | 150% Accuracy | 150 | 151.02 | 100.0 |
| 8. | | 150 | 150.5 | 100.3 |
| 9. | | 150 | 150.4 | 100.3 |
| Mean | | | | 100.1 |
| SD | | | | 0.889 |
| % RSD | | | | 0.89 |

**Table 6. Method precision**

| SL NO | Sample weight (mg) | Area | Mean Area Counts | % Label Claim |
|---|---|---|---|---|
| 1 | 172 | 1900847 | 1900847 | 100.5 |
| 2 | 172 | 1897995 | 1897995 | 100.4 |
| 3 | 172 | 1903999 | 1903999 | 100.7 |
| 4 | 172 | 1907151 | 1907151 | 100.8 |
| 5 | 172 | 1903482 | 1903482 | 100.6 |
| 6 | 172 | 1902710 | 1902710 | 100.6 |
| MEAN | | | | 100.6 |
| SD | | | | 0.141 |
| % RSD | | | | 0.14 |

**Table 7. Intermediate precision**

| Sl NO | Sample weight (mg) | Area | Mean | % Label Claim |
|---|---|---|---|---|
| 1 | 172 | 1900654 | 1900654 | 100.5 |
| 2 | 172 | 1897351 | 1897351 | 100.3 |
| 3 | 172 | 1903262 | 1903262 | 100.7 |
| 4 | 172 | 1905471 | 1905471 | 100.8 |
| 5 | 172 | 1903031 | 1903031 | 100.6 |
| 6 | 172 | 1902356 | 1902356 | 100.6 |
| Mean | | | | 100.6 |
| SD | | | | 0.172 |
| % RSD | | | | 0.17 |

**Table 8. Robustness of the developed method for Tapentadol Hydrochloride**

| SL NO | Parameters | Sample weight | Area | % Label claim | Mean | SD | %RSD |
|---|---|---|---|---|---|---|---|
| 1 | Flow plus (1. 2 ml/min) | 172 | 1562279 | 100.6 | 100.3 | 0.306 | 0.31 |
| | | 172 | 1554826 | 100.2 | | | |
| | | 172 | 1553007 | 100.0 | | | |
| 2 | Flow minus (0.8 ml/min) | 172 | 2270105 | 99.7 | 99.9 | 0.2 | 0.2 |
| | | 172 | 2274649 | 99.9 | | | |
| | | 172 | 2277997 | 100.1 | | | |
| 3 | Organic (ACN: Buffer) plus (77: 23) | 172 | 1933129 | 100.2 | 100.1 | 0.058 | 0.06 |
| | | 172 | 1932307 | 100.1 | | | |
| | | 172 | 1931989 | 100.1 | | | |
| 4 | Organic (ACN: buffer) minus (63:37) | 172 | 1939870 | 100.2 | 100.2 | 0.058 | 0.06 |
| | | 172 | 1940360 | 100.2 | | | |
| | | 172 | 1943200 | 100.3 | | | |

### 3.5 Precision

Method precision and intermediate precision are tabulated in Tables 6 and 7. Precision was expressed as percentage relative standard deviation (% RSD), which was found to be less than 2% depicting satisfactory precision of the system.

### 3.6 Robustness

Robustness was checked by allowing the small change in injection flow rate (±0.2 ml/min) and organic phase buffer concentration by ±7 and % RSD was determined and results are reported in Table 8. 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. Considering a slight change in flow rate and organic phase concentration was found to be less than 2%.

### 3.8 Limit of Detection and Limit of Quantification

LOD and LOQ were found to be 0.1 and 1 µg/ml respectively. The LOQ expresses the sensitivity of the developed method.

## 4. CONCLUSION

The results of the developed RP-HPLC method met all the validation parameters. The % RSD of precision was found to be less than 2%. The retention time of Tapentadol Hydrochloride was found to be 3.747 ± 0.127 min. The mean recovery of Tapentadol Hydrochloride was found to be 100.1%. LOQ confirms the sensitivity of the method. So, it is concluded that the developed RP-HPLC method was simple, specific, rapid, precise and accurate. It can be used for the analysis of Tapentadol Hydrochloride bulk and formulations.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENT

The authors are grateful to Symed labs, Hyderabad, for their generous contribution to the drug.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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