



## Development and Validation of RP- HPLC, UV-Spectrometric and Spectrophotometric Method for Estimation of Tapentadol Hydrochloride in Bulk and in Laboratory Sample of Tablet Dosage Form

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### ABSTRACT

Three novel, simple, accurate and rapid methods have been developed and validated for estimation of tapentadol hydrochloride in bulk and in laboratory tablet sample. In RP- HPLC method, elution was achieved in isocratic mode using combination of 50mM phosphate buffer pH 3.62 and acetonitrile in ratio of 70:30 (% v/v) with 0.1% triethylamine and using HiQ Sil C8 column having specification, 250 x 4.6 mm and 5 $\mu$ m particle size. The flow rate was 1 ml/min and detection was done at 285 nm. UV-Spectrophotometric estimation of tapentadol was carried out at 272 nm. Third method consists of quantification of tapentadol using Folin-Ciocalteu reagent in presence of 20% sodium carbonate solution. The blue colour chromogen formed is measured at wavelength of maximum absorption 750 nm for tapentadol against reagent blank. All three developed methods were validated according to ICH guidelines. Furthermore assay results obtained by three methods were compared statistically as well.

**Keywords:** Tapentadol Hydrochloride, RP – HPLC, UV- Spectrophotometry, Folin-Ciocalteu reagent.

### INTRODUCTION

Agonists of the  $\mu$ -opioid receptor are being used conventionally for the treatment of moderate to severe pain. Tapentadol hydrochloride (TAP) is novel centrally-acting oral analgesic.

Chemically it is 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2methylpropyl] phenol monohydrochloride. It is not official in any pharmacopoeia. [1-3]

Up till now only few methods are reported for estimation of TAP which includes, estimation of tapentadol and its metabolite N-desmethyl tapentadol in urine and oral fluids by using ultra pressure liquid chromatography with tandem mass detection (LC-MS/MS).[4] Furthermore one study is reported which discusses about determination of four stereoisomers of TAP by X-ray crystal structure analysis.[5]

Literature survey revealed that no method has been reported for estimation of TAP in bulk as well as in formulation. Hence it was endeavored to develop various analytical methods like, RP-HPLC and UV- Spectrophotometric methods for estimation of TAP in bulk drug. So far marketed formulation of TAP is not available in Indian market. Hence a laboratory sample of tablet dosage form was developed to check applicability of developed method.

## EXPERIMENTAL SECTION

### Instrumentation

HPLC system (Jasco, Japan) consists of binary pumps - PU-2080, and solvent mixing module- MX-2080, equipped with Photo Diode Array (PDA) Detector, MD-2015 Plus (Jasco Japan), and Borwin software was used for the data acquisition and data collection.

UV/Vis double beam spectrophotometer, 2400 PC series (Shimadzu, Japan) with spectral width of 1 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells were used for development of UV-Visible spectrophotometric method. pH meter (111E / 101E, Analabs Scientific Instruments Ltd, India) and range of pH 0 to 14 with resolution  $\pm 0.01$  pH, accuracy  $\pm 0.01$  pH was used. All weighing was done on analytical balance (CX 220, Citizen India Ltd).

### Materials and Reagents

Working standard of TAP (99.9% pure) was used; AR grade Potassium dihydrogen phosphate, Sodium Carbonate Methanol, triethylamine and glacial acetic acid were purchased from S.D. Fine Chemicals (Mumbai, India). Folin-Ciocalteu Reagent was procured from Sisco Research Laboratories Pvt. Ltd, (India). HPLC grade methanol and acetonitrile was purchased from Rankem (Mumbai, India). Millipore water was used for RP-HPLC method and double distilled water was used for UV-VIS spectrophotometric method.

### Chromatographic Conditions for RP-HPLC Method

The separation was carried out in isocratic mode using HiQ Sil C8 column (250 x 4.6 mm, 5 $\mu$ m) at ambient temperature. Mobile phase composition was acetonitrile and 50mM phosphate buffer in the ratio of (30:70 v/v) throughout study. Phosphate buffer was prepared by dissolving 3.4 gm KH<sub>2</sub>PO<sub>4</sub> in 500 ml of millipore water. pH of this buffer was adjusted to 3.60 with glacial acetic acid. Triethylamine (0.1% v/v) was added as modifier. The mobile phase was filtered through a nylon 0.45  $\mu$ m, 47 mm membrane filter and degassed before use. The flow rate was 1 ml/min. Injection volume was 20 $\mu$ l throughout study and detection was done at 285 nm. Mixture of acetonitrile and water (50:50 v/v) was used as diluent throughout the RP-HPLC analysis.

### UV- Spectrophotometric Method

For selection of detection wavelength, 50 $\mu$ g/ml solution of TAP in methanol was scanned in the range of 200 – 400 nm using UV- visible spectrophotometer. Wavelength of maximum absorption was selected for quantification of TAP in standard as well as in sample solutions.

### Spectrophotometric Method using Folin-Ciocalteu Reagent [6,7]

Folin-Ciocalteu reagent (FCR) was diluted with distilled water in the ratio of 1:3 with distilled water in present study. To each aliquot of standard as well as tablet sample, 1 ml FCR and 3 ml 20% sodium carbonate was added and kept aside for 15 minutes at room temperature. Volume was made up to 10 ml with distilled water. Blank solution was also prepared simultaneously by omitting TAP. For selection of detection wavelength 20  $\mu$ g/ml solution of TAP previously treated with FCR was scanned in the range of 400 – 800 nm against reagent blank using UV-Vis spectrophotometer. All the prepared standard and sample solutions were analyzed at 750 nm.

### Preparation of Standard Stock Solution

Standard Stock solution of TAP having concentration of 1mg/ml was prepared in methanol. Appropriate aliquots from stock were diluted using respective diluent to get working standard concentration of 25  $\mu$ g/ml for RP-HPLC, 50  $\mu$ g/ml for UV method and 20  $\mu$ g/ml for FCR method, respectively.

### Laboratory tablet sample Preparation

Laboratory tablet sample (50 mg of TAP per tablet) was prepared using directly compressible microcrystalline cellulose as a diluent as well as binder. Each of 0.5% of Magnesium stearate and talc used as antiadherent and glident respectively. Average weight of tablet was approximately 200 mg. Placebo tablet were prepared excluding TAP. Twenty tablets of the laboratory sample were weighed, powdered and powder quantity equivalent to 100 mg of tapentadol hydrochloride was transferred to 100 ml volumetric flask. Tablet powder was dissolved in 60 ml methanol and the sample was sonicated for 20 minutes with intermittent shaking. Sample was further diluted up to mark with methanol, and filtered through 0.45 $\mu$ m membrane filter. From this filtrate appropriate aliquot was taken and diluted with respective diluent to get final concentration of 25  $\mu$ g/ml for RP-HPLC, 50  $\mu$ g/ml for UV and 20  $\mu$ g/ml for FCR method respectively. All the samples were analyzed as described in experimental conditions. Amount of TAP was calculated from response of standard and test sample as well as from linearity obtained by respective method.

Developed RP- HPLC, UV-Vis spectrometric and FCR methods were validated in terms of various validation parameters such as precision, accuracy, specificity, linearity, robustness and solution stability as per ICH guidelines.[8]

### **Validation**

#### **System suitability**

System suitability of developed RP-HPLC method was assessed by injecting six replicate injections of standard solution of 25 µg/ml of TAP. Relative standard deviation (RSD) of peak area; peak parameters such as asymmetry, theoretical plates, and retention time were calculated.

#### **Linearity**

##### **1. For RP-HPLC**

To construct calibration curve, solutions having 5, 10, 25, 50, 75 and 100 µg/ml of TAP was prepared individually from standard stock solution. An aliquot (20 µl) of each solution was analyzed as described in chromatographic conditions. Calibration curves were constructed by plotting the peak areas versus the concentrations of TAP and the regression equations were calculated.

##### **2. For UV- Spectrophotometric Method**

TAP solution containing 20, 40, 50, 60, 80 and 100 µg/ml was prepared individually from standard stock solution. The solutions were analyzed at 272 nm in quantitative mode. Calibration curve was constructed by plotting absorbance versus concentration of TAP and the regression equations were calculated.

##### **3. For FCR Method**

5 ml of aliquot from standard stock was pipetted out accurately in 50 ml volumetric flask and volume was made up to the mark with distilled water. From this solution 0.5, 1, 1.5, 2, 3, and 3.5 ml of aliquot was withdrawn in separate 10 ml volumetric flask. To each flask, 1 ml of FCR and 3 ml of 20% sodium carbonate was added and kept aside for 15 minutes at room temperature. Finally volume was made up with distilled water to get concentration of 5, 10, 15, 20, 30, and 35 µg/ml of TAP. Absorbances of colored solutions were recorded against reagent blank at absorption maxima of 750 nm. Calibration curve was constructed by plotting absorbance versus concentration and the regression equations were calculated.

For All the three methods the each response was the average of six determinations.

#### **Method Precision**

The intraday and interday precision of the proposed methods were determined by estimating the corresponding responses three times on the same day and on three different days respectively. Three different concentrations of TAP were selected to perform precision study of RP-HPLC (5, 10 and 25 µg/ml), UV- Visible (20, 40, and 50 µg/ml) and FCR (5, 10, and 15 µg/ml). Repeatability (precision) was assessed by analyzing tablet samples six times having concentration of 50 µg/ml, 20 µg/ml, and 20 µg/ml for UV-Spectrophotometry, RP-HPLC and FCR method respectively. Results of repeatability study were expressed in terms of % RSD.

#### **Accuracy**

The accuracy of the method was performed by conducting recovery studies using standard addition method. Known amount of standard TAP was added at three different levels approximately 80%, 100% and 120 % of predetermined amount respectively. Samples were analyzed by all three developed methods. The % recovery and % average recovery of TAP was calculated.

#### **Specificity**

Specificity was checked by checking the interference from placebo for both UV and FCR methods. In case of HPLC, specificity was confirmed by performing peak purity test by checking homogeneity of peak at upslope, apex and downslope of peak using Borwin PDA software.

#### **Robustness**

Robustness of method was confirmed by making small but deliberate changes in the optimized HPLC conditions like, change in detection wavelength ( $\pm 2$  nm), flow rate ( $\pm 0.2$  ml), pH of mobile phase ( $\pm 0.05$ ). The effect of these changes on system suitability parameters as well as on the estimation of TAP was determined. In case of UV-spectrophotometric method and FCR method robustness was assessed by making change in detection wavelength by  $\pm 2$  nm and results were compared.

**Solution stability**

Solution stability was checked by reanalyzing previously analyzed sample after 24 hrs and results compared with initial samples. Standard solution stability was confirmed by comparing response of old standard against freshly prepared standard solution.

**RESULTS AND DISCUSSION****RP-HPLC Method**

To optimize various RP-HPLC parameters such as peak shape, peak symmetry and run time, several trials were taken. Promising results obtained using C-8 column and mobile phase consist of acetonitrile and 50mM phosphate buffer in the ratio of (30:70 v/v). Triethylamine (0.1% v/v) was used as modifier to reduce peak tailing and to get symmetrical peaks. Optimum wavelength for detection was 285nm at which good detector response for TAP was obtained. Retention time of TAP in developed method was found to be 5.4 minutes (Fig. 1).

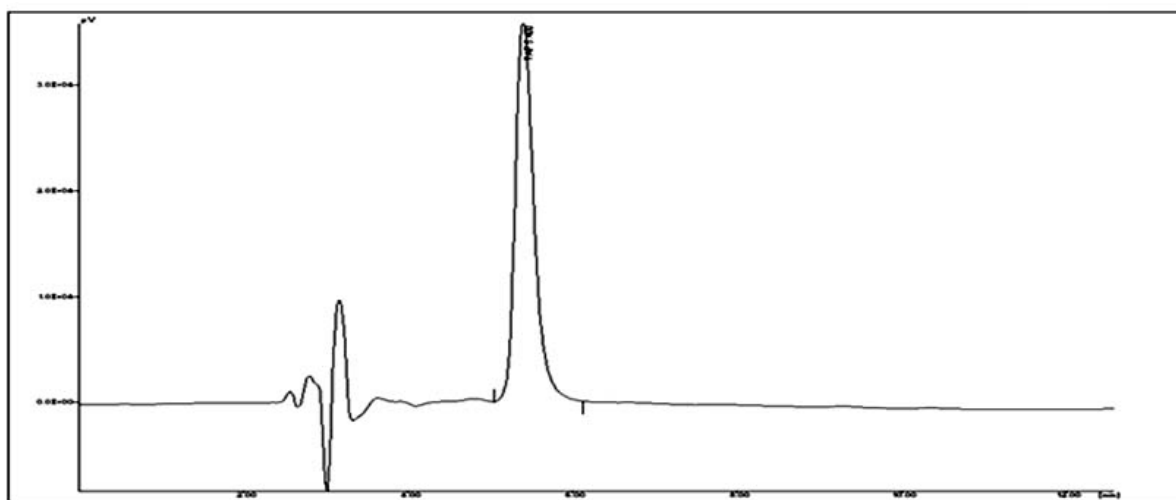


Fig.1: RP- HPLC chromatogram of TAP having concentration of 50 $\mu$ g/ml in methanol.

The proposed HPLC method showed acceptable system suitability parameter with symmetrical peak shape of TAP. The value of relative standard deviation 0.7 % of peak area was found to be within limits which indicate injection repeatability of the method. System suitability parameters such as, asymmetry, theoretical plates, and retention time were well within its acceptance criteria as depicted in table 1.

Table No 1 - System suitability Parameters for developed RP –HPLC Method

Parameter	Observation
Retention time (min)	5.40
% RSD of Peak Area (n =6)	0.7
Theoretical Plates	4396
Asymmetry	1.33

**UV-Spectrophotometric Method**

Scanned UV spectrum of 50  $\mu$ g/ml of TAP gave showed in absorption maxima at about 272 nm in methanol, which was selected for quantification of TAP. A typical UV spectrum of TAP is presented in fig. 2.

**FCR Method**

A thought was given to increase the sensitivity of method by chemical derivatization of TAP. It is reported that FCR can react with phenols and non phenolic reducing substances to form chromogen that can be detected spectrophotometrically.[6,7] TAP possess free phenolic group which reacts with the FCR in the alkaline medium, which results in blue colored product. The blue color formation by FCR with drug is almost similar to Folin phenol protein reaction. [9,10] The colored product is due to oxidation of the TAP and the reduction of FCR. The color formation is due to the reduction of 1, 2 and 3 oxygen atoms of FCP reagent and the formation of molybdenum blue or tungsten blue which shows absorption maxima at 750 nm (Fig. 3) for TAP. Colored chromogen was found to be stable for almost 3hrs at room temperature.

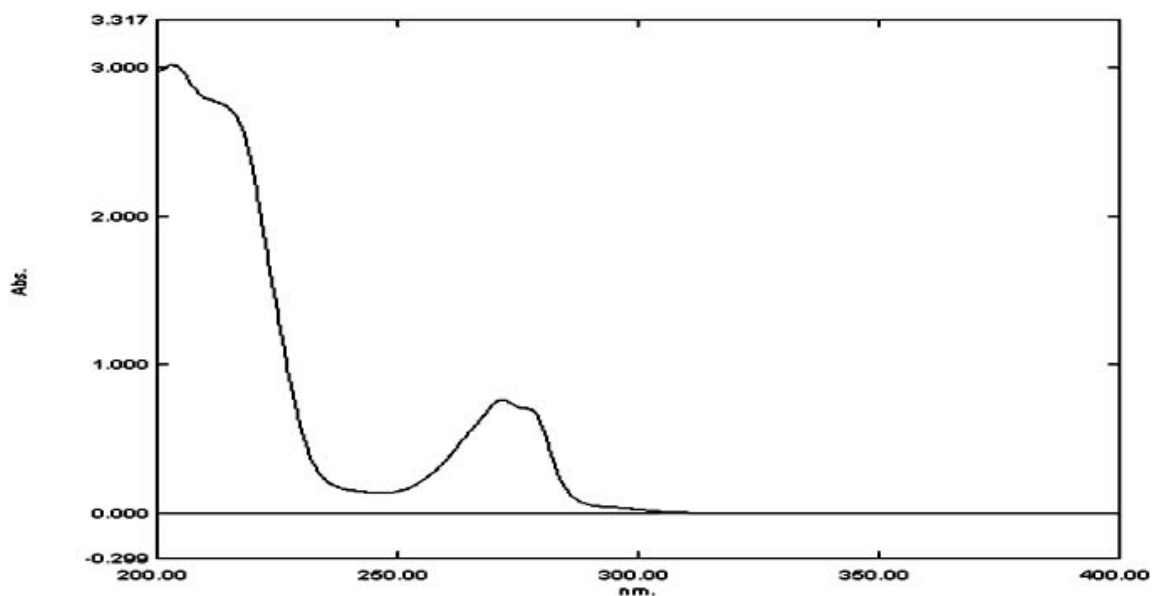


Fig. 2: UV Spectrum of TAP 50µg/ml in Methanol shows absorption maxima at 272 nm

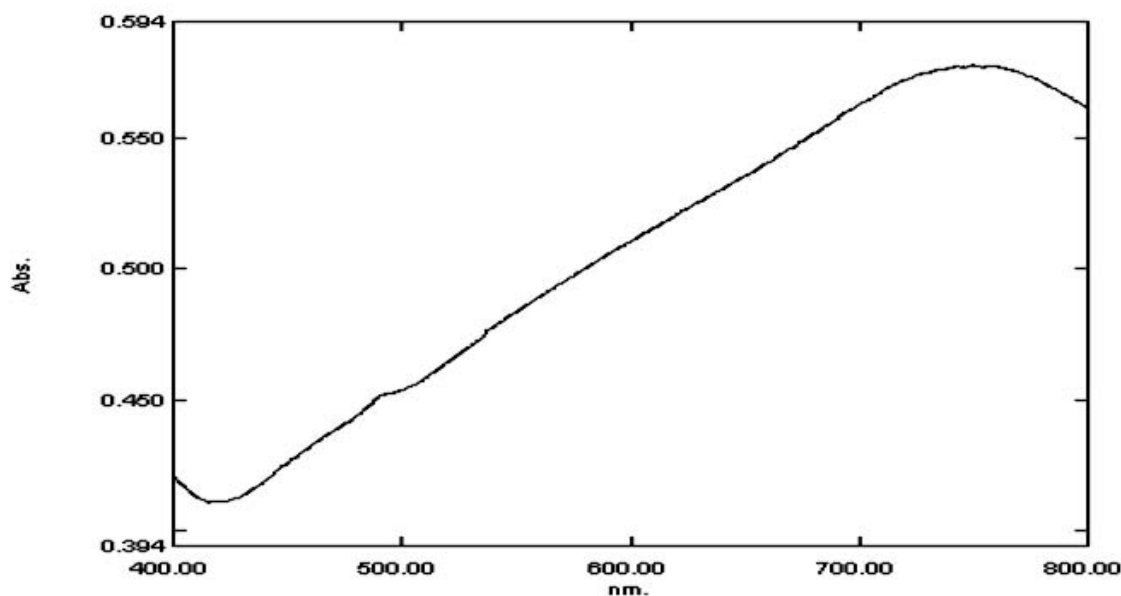


Fig. 3: UV-Visible Spectra of TAP 20µg/ml derivatized with FCR shows absorption maxima at 750nm.

### Method Validation

Developed three methods were validated as per of ICH guidelines.[8]

Table No 2 - Summary of Linearity Study

Parameter	HPLC Method	UV Method	FCR Method
Concentration range (µg/ml) <sup>a</sup>	5 - 100	20 - 100	5 - 35
Slope(m)	6838.6	0.0063	0.026
Intercept (c)	5663.9	0.0065	0.010
Correlation co-efficient (r)	0.9998	0.9995	0.9990

<sup>a</sup>(n =6)

### Linearity

For RP-HPLC method linear correlation was obtained between peak area and concentration for TAP in the range of 5-100 µg/ml. In case of UV spectrophotometric method correlation was obtained between absorbance and

concentration for TAP in the range of 20-100 µg/ml. For FCR Method linearity was obtained in the concentration range of 5- 35 µg/ml. Linearity of the three methods is summarized in table 2.

### Precision

Interday and intraday variations in estimation of TAP were studied for all the three methods (Table 3). The % RSD value for all the three methods found less than 2%. These low values of RSD indicate good precision of developed methods.

**Table No. 3 - Summary of Precision Study**

Parameter	HPLC Method	UV Method	FCR Method
Repeatability (% RSD) <sup>a</sup>	0.62	0.48	0.39
Intraday Precision (% RSD) <sup>b</sup>	0.59	0.50	0.45
Interday Precision (% RSD) <sup>c</sup>	0.70	0.65	0.55
Specificity	Specific	Specific	Specific
Solution Stability	Stable for 24 Hrs at room temperature	Stable for 24 Hrs at room temperature	Colored Chromogen stable for 3Hrs at room temperature.

<sup>a</sup> n = 6, <sup>b,c</sup> n =

### Accuracy

The accuracy study reveals the positive and negative influence of additives which usually present in dosage forms, on the quantification parameters. The recovery study data is depicted in table 4. The mean % recovery of quantification of TAP was more than 99.3 %, 99.9 and 99.6% for RP-HPLC, UV Spectrophotometric and FCR method respectively.

**Table No. 4 - Results of recovery studies of TAP by proposed methods**

Proposed method	Amount of TAP Present <sup>a</sup>	Amount of standard TAP added <sup>a</sup>	Mean % Recovery ± S.D <sup>b</sup>
RP-HPLC	25	20	99.3 ± 0.22
	25	25	99.7 ± 0.42
	25	30	99.4 ± 0.28
UV Spectrophotometric	50	40	100.0 ± 0.57
	50	50	99.93 ± 0.15
	50	60	100.1 ± 0.32
FCR Method	20	16	99.8 ± 0.42
	20	20	99.7 ± 0.47
	20	24	99.6 ± 0.57

<sup>a</sup> µg/ml for RP-HPLC, UV Spectrophotometric and FCR method.

<sup>b</sup> Average of three determinations.

### Specificity

For RP-HPLC method peak purity of TAP was assessed by comparing their respective spectra at peak start, apex and end position of peak. Peak purity value of TAP was more than 990 at upslope, apex and downslope of peak which confirms homogeneity of peak. No interference was observed at the retention of TAP in sample solution.

For UV spectrophotometric method placebo showed no absorbance near the detection wavelength of TAP which indicate the method is specific for estimation of TAP at 272nm. For FCR method placebo and reagent blank is also treated in same way like sample. Placebo and reagent blank didn't show any characteristic colour when treated with FCR method which proves specificity of FCR method for estimation of TAP.

### Robustness

The system suitability parameter and results of assay in changed condition were found within acceptance limits (with in ± 2% of optimized condition) which assure robustness of method. In case of UV spectrophotometric method and FCR method assay was calculated by estimating TAP at ± 2 nm of absorption maxima of respective method. Results of assay found within acceptable range which confirms robustness of the method.

### Solution Stability

Solution stability was checked by analyzing solutions after 24 hours. Difference in the result is less than 2% when compared with freshly prepared standard for both HPLC and UV method which indicates; prepared solution can be analyzed within 24 hrs. In case of FCR coloured chromogen found stable up to 3 hours hence it is essential to analyze samples within 3 hrs.

**Comparison of proposed Methods**

The assay results of TAP obtained using proposed RP-HPLC, UV and FCR methods (Table 5) were compared using one way ANOVA test. P value found to be 0.3985 at the 0.05 significance level. Therefore P value found greater than 0.05 indicates there is no significant difference with respect to accuracy and precision between the proposed methods.

**Table No. 5 - Assay results by developed three methods**

Parameter	HPLC Method	UV Method	FCR Method
Mean Assay (%) (n= 3)	99.18	99.0	99.33
% RSD	0.09	0.25	0.26

**CONCLUSION**

The developed analytical methods; RP-HPLC, UV and FCR found to be simple, rapid, specific, accurate and rugged for estimation of TAP. Statistical comparison shows that there is significant difference with respect to accuracy and precision of all three methods. RP-HPLC method offers wide linear range for estimation of TAP. Additionally FCR method is more sensitive than UV spectrometric method which allows estimation of TAP in lower concentrations. Developed three methods can be used for routine quality control analysis of tapentadol in bulk as well as in its finished pharmaceutical product.

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