RESEARCH ARTICLE

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Development and Validation of Novel Spectrofluorimetric Method for Estimation of Tapentadol Hydrochloride in Bulk and in Laboratory Sample of Tablet Dosage Form

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Abstracts: Novel, sensitive and accurate spectrofluorimetric method has been developed and validated for estimation of tapentadol hydrochloride in bulk and in laboratory tablet dosage form. The method is based on measurement of native fluorescence of tapentadol in distilled water at 298 nm after its excitation at 273 nm. The fluorescence intensity-concentration plot was linear in the concentration range of 1- 10 µg/ml with good correlation coefficient 0.9990. The developed method was successfully applied for determination of tapentadol hydrochloride in laboratory sample of tablet dosage form. Furthermore developed method was successfully validated as per ICH guidelines in terms of, linearity (1 - 10 µg/ml), repeatability (RSD 0.39 %), precision (intra-day variation, RSD, 0.17 to 0.63 % and inter-day variation, RSD, 0.34 to 0.63 %) and accuracy (99.44 to 99.62 %). The limit of detection and quantification was found to be 0.011 and 0.034 µg/ml respectively. The developed method proved to be simple, economic and precise. Therefore proposed method can be employed for the routine quality assessment of the tapentadol hydrochloride in bulk as well as in pharmaceutical dosage forms.

INTRODUCTION

Activation of the µ-opioid receptor the important alternative, which is commonly used for the treatment of moderate to severe pain. These are very useful in case of acute pain. Tapentadol hydrochloride (TAP) is novel centrally-acting synthetic analgesic. Chemically it is 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2methylpropyl] phenol monohydrochloride. TAP is not official in any pharmacopoeia. [1-3]

Up till now only few methods have been reported for estimation of TAP which includes, estimation of TAP and its metabolite N-desmethyl tapentadol in urine and oral fluids by using ultra pressure liquid chromatography with tandem mass detection (LC-MS/MS). [4-5] In addition another reported study discusses about four stereoisomers of TAP. Furthermore the crystal and molecular structures of four stereoisomers of tapentadol hydrochloride have been determined by X-ray crystal structure analysis. [6] Recently UV-Spectrophotometric [7] and RP-HPLC [8-10] methods have been reported for estimation of TAP.

Literature survey revealed that so far no study has been reported for estimation of TAP in bulk as well as in formulation by spectrofluorimetric technique. Hence it was endeavored to develop sensitive, accurate, and precise spectrofluorimetric method by using native fluorescence of TAP for estimation of TAP in bulk drug. When study was carried out marketed formulation of TAP was not available in Indian market. Hence a laboratory sample of tablet dosage form was developed to check applicability of developed method.

MATERIALS AND METHODS

Instrumentation

Spectrofluorimeter, FP 6500 with single quartz cell of 1 cm path length (Jasco, Japan) was used for fluorescence measurement. Spectra manager software was used for the data acquisition and data collection. All weighing was done on analytical balance (Model CX 220, Citizen India Ltd).

Materials

Working standard of tapentadol hydrochloride 99.9 % pure was used. Methanol AR grade was purchased from Rankem (Mumbai, India). Double distilled water used throughout study.

Experimental

Preparation of Standard Stock Solution

An accurately weighed quantity of 50 mg TAP was transferred into 50 ml volumetric flask. About 25 ml of methanol was added and sonicated to dissolve. The solution was cooled at room temperature and made up to volume with methanol to get final concentration of 1000 µg/ml.

Preparation of Working Standard Solution

TAP working standard solution was prepared by diluting standard stock solution (5.0 ml) to 50 ml with double distilled water (diluent) to produce required concentration (100 µg /ml).

Spectrofluorimetric Detection

The standard solution of TAP was scanned over the range of 220 nm to 400 nm wavelengths for selection of excitation wavelength in excitation mode. It showed highest florescence intensity at 273 nm (Figure 1). Therefore excitation wavelength 273 nm was selected to find out emission wavelength. The standard solution of TAP was scanned over the range of 220 nm to 400 nm wavelengths for selection of emission wavelength by selecting 273 nm as an excitation wavelength in emission mode. It showed highest florescence intensity at 298 nm (Figure 2). So, emission wavelength of 298 nm was selected for measurement of fluorescence intensity of TAP. Wavelength search in software also shows excitation and emission wavelength of 273 nm and 298 nm respectively (Figure 3).

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Table 1: Assay Results by Developed Spectrofluorimetric Method

Parameter	Result
Mean Assay (%) (n= 3)	99.88
% RSD	0.80

Table 2: Summary of Linearity Study

Parameter	Result
Concentration range (µg/ml)	1-10
Slope(m)	44.18
Intercept (c)	2.984
Correlation co-efficient (r)	0.9990

(n = 6)

Table 3: Results of Recovery Studies of TAP for Developed Spectrofluorimetric Method

Amount of TAP Take (µg/ml)	Amount of Standard TAP Added in (µg/ml)	Mean Amount Recovered [a] (µg/ml)	Mean % Recovery ± SD [b]
4	3.2	7.1	99.17± 0.50
4	4	8	100 ±1.25
4	4.8	8.7	99.36 ±0.67

[a] Average of three determinations, [b] Average of three determinations

Table 4: Summary of Validation Parameter

Parameter	Result
Method Precision(% RSD)[c]	0.39
Intraday Precision(% RSD)[d]	0.17 – 0.64
Interday Precision(% RSD)[e]	0.34 – 0.62
Specificity	Specific
Solution Stability	Stable for 24 Hours at room temperature

[c] n = 6, [d] and [e] n = 3 for each concentration

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 µm membrane filter. From this filtrate appropriate aliquot was taken and diluted with diluent to get final concentration of 6 µg/ml. Amount of TAP was estimated from fluorescence intensity of standard and test sample as well as from linearity equation. Assay result of tablet is as shown in Table 1. Fluorescence Spectra of tablet sample is shown in Figure 4. Overlay spectra of standard and tablet sample is depicted in Figure 5. Developed spectrofluorimetric method was validated by assessing various validation parameters. Different parameters studied such as, precision, accuracy, specificity, linearity, robustness and solution stability as specified by International conference on Harmonization(ICH) guidelines. [11]

Validation of Proposed Method

Linearity

Calibration curve was constructed over a concentration range of 1-10 µg/ml for TAP. Accurately measured working standard solutions of TAP (0.1, 0.2, 0.4, 0.6, 0.8 and1.0 ml) were transferred separately to 10 ml volumetric flask. Volume was made up to the mark with diluent.

The fluorescence intensity of all the resulting solutions was measured at an emission wavelength of 298 nm. The calibration curve was prepared by plotting fluorescence intensity versus concentration (µg/ml) and regression equation was calculated. Each response was the average of six determinations.

Accuracy

The accuracy of the method was assessed by calculating recoveries of TAP by the standard addition method. Known amounts of standard solutions of TAP were added at 80 %, 100 % and 120 % levels to preanalysed sample solution of TAP (4 µg/ml). The amount of TAP recovered was calculated by extrapolating fluorescence intensity values to the regression equation of the calibration curve. From the amount recovered percentage recovery was calculated.

Method Precision

Repeatability (method precision) was assessed by analyzing tablet samples six times having concentration of

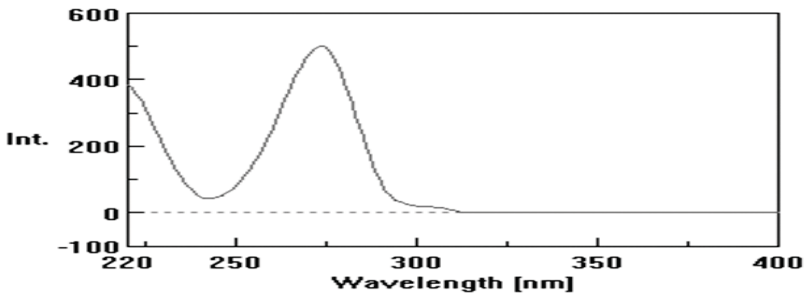

Figure 1: TAP showing excitation wavelength at 273 nm

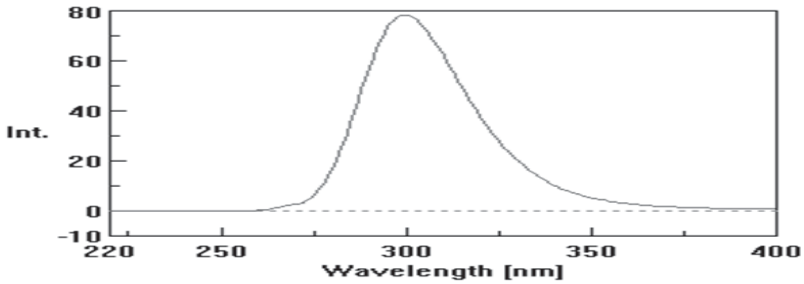

Figure 2: TAP showing emission wavelength at 298 nm

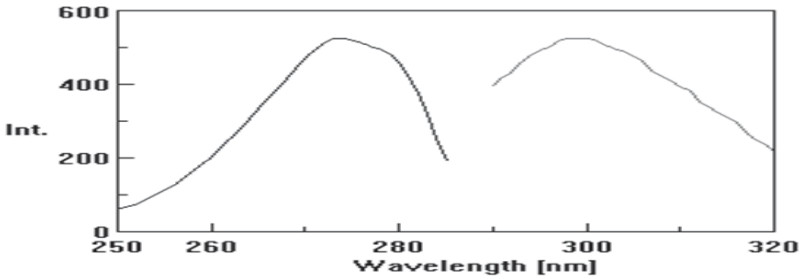

Figure3: Fluorescence spectra of tap showing excitation and emission wavelength 273 nm and 298 nm respectively

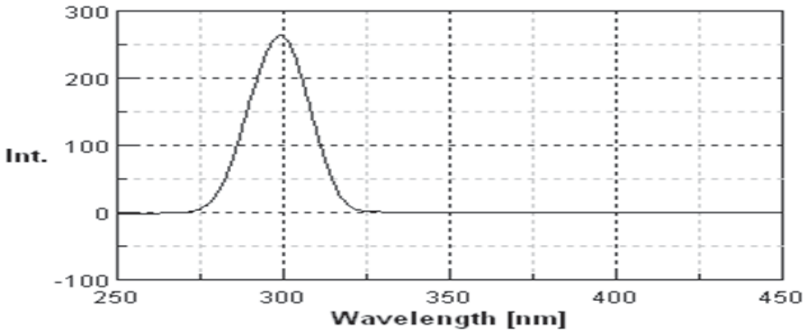

Figure 4: Fluorescence spectra laboratory tablet sample of TAP (6 µg/ml)

6 µg/ml. Results of repeatability study were evaluated in terms of percentage relative standard deviation (RSD).

The intraday and interday precision of the proposed methods were determined by measuring the corresponding fluorescence intensities of three different concentrations of TAP three times on the same day and on three consecutive days respectively. Three different concentrations of TAP (4, 6 and 8 µg/ml) were selected to perform precision study.

Specificity

Specificity was checked by checking the interference from placebo at both excitation and emission wavelength of TAP.

Solution Stability

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

Robustness

Robustness of method was confirmed by making small but deliberate changes in the developed spectrofluorimetric method. Emission wavelength was change by (± 2 nm). Fluorescence intensity was measured at 296 nm and 300 nm.

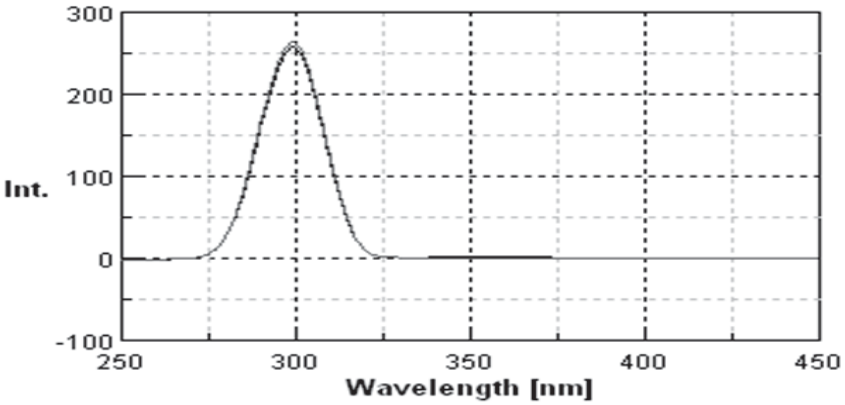

Figure 5: Overlay Fluorescence spectra of laboratory tablet sample and standard (6 µg/ml)

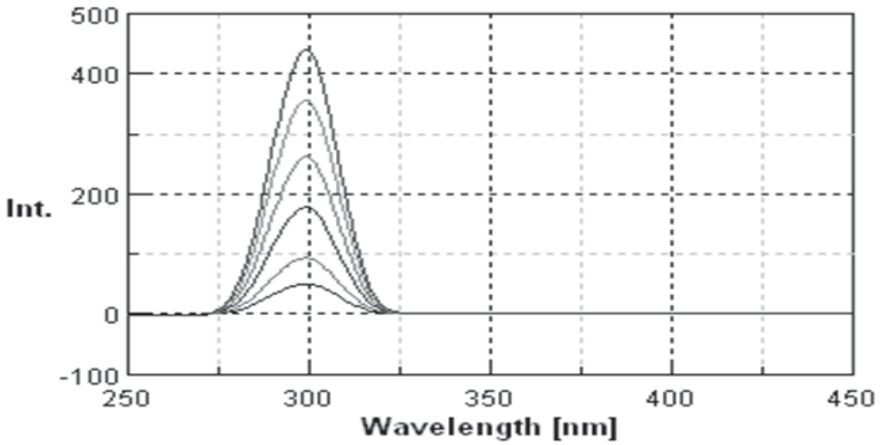

Figure 6: Overlay spectra for linearity of TAP (1 µg/ml -10 µg/ml)

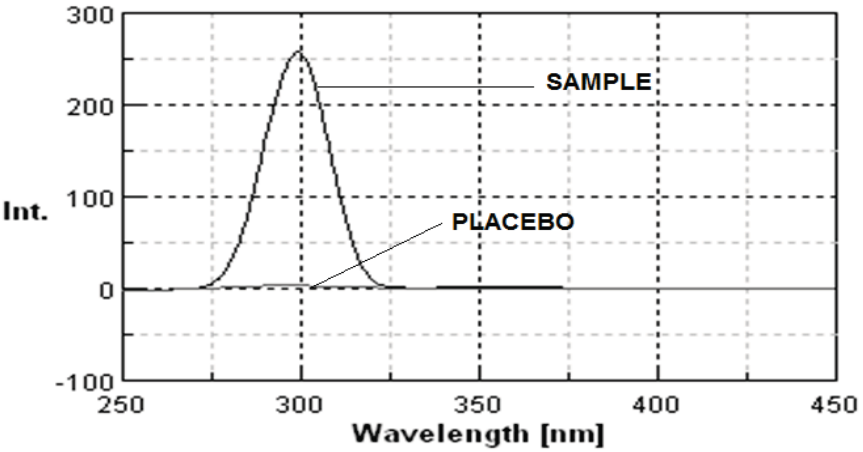

Figure 7: Overlay of placebo and laboratory tablet sample of TAP

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and the LOQ of TAP were calculated by using the equations given by ICH guidelines as depicted below. [11]

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ = Standard deviation of the response. S = Slope of calibration curve.

RESULTS AND DISCUSSION

Present spectrofluorimetric method is based on the measurement of native fluorescence of TAP. TAP exhibits emission and excitation at 273 nm and 298 nm respectively (Figure 3).

Method Validation

Developed spectrofluorimetric method was validated as per of ICH guidelines. [11]

Linearity

Linear correlation was obtained between fluorescence intensity and concentration for TAP in the range of 1-10

µg/ml. Results of linearity is summarized in Table 2. Overlay fluorescence spectrum for linearity of TAP is as shown in Figure 6.

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 furnished in Table 3. Accuracy of quantification of TAP was in the range of 99.17 – 100 %

Precision

Repeatability, Interday and intraday variations in estimation of TAP were studied for developed spectrofluorimetric method (Table 4). The Percentage RSD value found less than 2%. These low values of RSD confirm precision of developed method.

Specificity

Specificity study was performed on placebo sample. Placebo doesn't show any emission as well as excitation near estimating wavelengths of TAP. Which indicate the method is specific for estimation of TAP. Overlay spectra of placebo and tablet sample show there is no interference from placebo (Figure 7).

Solution Stability

Solution stability of standard and tablet sample was checked by analyzing preanalysed solutions after 24 hours. Difference in the result was less than 2 % when compared with freshly prepared standard and initial results of tablet sample (Table 4).

Robustness

The results of assay obtained by in changed parameter i.e. changing emission wavelength by ± 2 nm, were found within acceptance limits which suggests robustness of method.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ values for TAP by developed spectrofluorimetric method found to be 0.011 and 0.034 µg/ml respectively. These low values of LOD and LOQ confirm sensitivity of the developed method.

CONCLUSION

A novel, simple, accurate, sensitive and cost effective spectrofluorimetric method has been developed for the estimation of TAP in bulk and it laboratory tablet sample. The results of the analysis of laboratory tablet samples by proposed method are highly reproducible and reliable. The excipients present in laboratory tablet sample do not interfere in quantification of TAP which was confirmed by checking placebo interference. Consequently developed method is highly specific for estimation of TAP. Additionally low values of LOD and LOQ prove sensitivity of developed method. Therefore proposed spectrofluorimetric method can be routinely employed for routine estimation of TAP in bulk and in pharmaceutical dosage from.

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