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Research Article

ISSN:2230-7346
Journal of Global Trends in Pharmaceutical Sciences

Vol.3, Issue 3, pp -755-762, July–September 2012

RP-HPLC METHOD FOR DETERMINATION OF TAPENTADOL IN BULK AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

The objective of the current study is to develop a Rapid, Simple, Specific, Accurate, Precise and Reproducible validated RP- HPLC method for the estimation of Tapentadol Hydrochloride in the Pharmaceutical dosage form. Tablet Formulation containing 50, 75 and 100 mg are available in the market. RP-HPLC analysis was carried out using 0.1 M potassium di hydrogen phosphate buffer pH adjusted to 3.6 with ortho phosphoric acid: Acetonitrile (50:50 v/v) as mobile phase and Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermsil) as stationary phase with detection wavelength of 243 nm utilizing Shimadzu HPLC (LC-2010HT) equipped with DAD Detector. Linearity was obtained in the concentration range of 10-50 µg/ml for Tapentadol Hydrochloride. The % recovery of the drug was found to be 99.9% – 100.00%. LOD and LOQ were found to be 0.01µg/ml and 30µg/ml at 243nm for Tapentadol Hydrochloride respectively. Method was statistically validated for Accuracy, Precision, Specificity, LOQ, Robustness and Ruggedness according to ICH guidelines and can be used for routine analysis of Pharmaceutical dosage form.

Keywords: Tapentadol Hydrochloride, Accuracy, Precision, Specificity, Symmetry.

INTRODUCTION:

Tapentadol, (–)-(1*R*, 2*R*)-3-(3-dimethylamino-1- ethyl- 2-methyl-propyl)-phenol hydrochloride (tapentadol HCl), with respect to its *in vitro* characteristics and its analgesic, antihyperalgesic, and antiallodynic properties in rat and mouse models of acute and chronic pain. Tapentadol, a centrally acting synthetic analgesic, received initial U.S. approval in 2008[1] and was then placed into the schedule II category of the Controlled Substances Act in May of 2009[2]. The drug is a novel, centrally acting oral analgesic with a dual mode of action that has demonstrated efficacy in clinical application. It is suggested that the broad analgesic profile of tapentadol and its relative resistance to tolerance development may be due to a dual mode of action consisting of both MOR activation and NE reuptake inhibition[3].

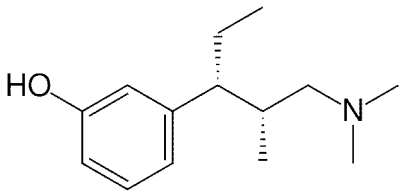

Figure 1: Structure of tapentadol

To date, only two LC–MS methods to detect TAL in biological matrices (urine [4] and urine and oral fluid[5]) have been reported in the literature; however there have been no studies on HPLC method for detection of TAL in pharmaceutical formulations. To address this shortfall, the aim of the present paper was to develop and validate a new simpler methodology to quantify TAL in tablet formulation using HPLC with diode array detection (HPLC–DAD).

Materials and methods:

Chemical and reagents:

Pure powder (>99.9% purity) of TAL was supplied by MSN Laboratories Pvt. Ltd., Hydrabad. The tablet Dosage form of TPL was purchased from Anukar Pharmacy, Hyderabad. HPLC grade acetonitrile (ACN) were purchased from Scharlau (Sentmenat, Spain). Analytical grade potassium di hydrogen phosphate buffer was obtained from SD Fine (Mumbai, India). HPLC grade water was obtained by distilling deionised water produced by a Milli-Q millipore water system (Milford, MA, USA). All the other reagents and materials were of analytical grade and supplied from commercial sources. The aqueous and organic components of the mobile phase were mixed and degassed under vacuum by the HPLC. The LC mobile phases were filtered through 0.2 µm cellulose acetate membrane filters (Sartorius Ste-dim Biotech S.A., Aubagne Cedex, France) with a solvent filtration apparatus.

Mobile phase:

A mixture of 0.1 M potassium di hydrogen phosphate buffer (pH 3.6, adjusted with ortho phosphoric acid) and acetonitrile in the ratio of 50:50 %v/v was used as mobile phase. Mixed solvents were filtered through 0.2 µm cellulose acetate membrane filters (Sartorius Ste-dim Biotech S.A., Aubagne Cedex, France) with a solvent filtration apparatus, degassed used as mobile phase. Same was used as diluents for the preparation of drug solutions.

Standard and working solutions:

Stock solution of TAL in mobile phase with a concentration of 1000 µg mL^{-1} was prepared using volumetric flask. This was stored at +4 °C. To obtain a final concentration of 200 µg mL^{-1}, an appropriate dilution of the stock standard solution was prepared by diluting 10–50 mL with mobile phase. Working solutions were prepared daily from the above mentioned stock solutions. The containers used for storage were screw-capped tubes coated externally by aluminium foil.

To carry out the sample solution (assay of pharmaceutical preparation), 20 tablets were taken and weighed individually, obtaining afterwards the average weight of these tablets, finally they were ground. An appropriate portion of this powder, equivalent to 100mg of TPL was weighed and placed in a 50ml volumetric flask, dissolving it with 25ml of mobile. This solution was sonicated for 15 min to dissolve and remove the entire active from the tablet. Once the time had elapsed, it was diluted up to 50 ml with additional mobile phase. 5 ml of aliquot was taken and transferred to volumetric flask of 25 ml capacity and volume was made up to the mark with the diluent. This solution was used for the estimation of TPL (30µg/ml).

HPLC–DAD instrumentation and chromatographic conditions:

The HPLC system was Shimadzu consisting of quaternary gradient system (600 Controller), in line degasser, Diode array detector and auto sampler. Chromatographic separation assay was performed with a Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermsil), maintained at ambient temperature. The mobile phase was pumped at a flow rate of 1.0 mL min^{-1}. The detection wavelength was 243 nm.

RESULTS AND DISCUSSION:

To determine the analytical wavelength, solutions of Tapentadol Hydrochloride was scanned in the range of 200 and 400 nm with the help of UV spectrophotometer and maximum absorbance was observed at 243 nm. Hence 243nm was selected as the analytical wavelength.

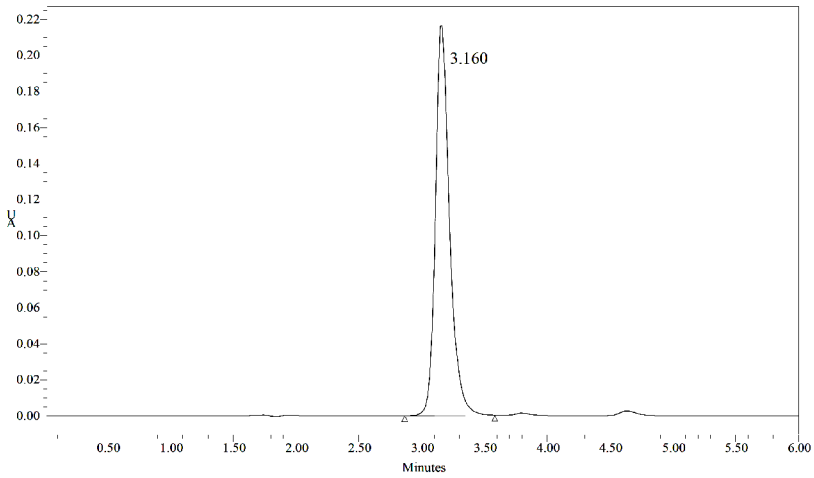

Figure 2: Chromatogram of Tapentadol Hydrochloride in Formulation at 221 nm

Validation study:

The method was validated with respect to the following parameters given below as per ICH guidelines[6,7]. Linearity was established by triplicate injections of standard solution containing Tapentadol Hydrochloride in concentration ranges of 10-50 µg/ml.

Precision was evaluated in terms of intraday and interday precision. The intraday precision was investigated using three different concentrations of standard solutions. The intraday and interday precision was investigated by analyzed. The results were reported in terms of % CV.

The accuracy of the method was determined by calculating recoveries of Tapentadol Hydrochloride by the standard addition method. For this known amount of standard solutions of Tapentadol Hydrochloride (50, 100, and 150 % level) were added to preanalyzed sample solutions. The amount of Tapentadol Hydrochloride was analyzed by using the regression equations of the calibration curve.

The specificity of the method was established through resolution factor of the drug peak from the nearest resolving peak and also among all other peaks. To assess the method specificity, powder without Tapentadol Hydrochloride (placebo) was prepared with the excipients as required for commercial preparation and compared with respective drug standard to evaluate specificity of the method. Representative chromatograms of placebo and standard were compared for retention time, resolution factor.

Method robustness was performed by applying small changes in the composition of mobile phase, detection wavelength, pH and flow rate. Robustness of the method was done at three different concentration levels of Tapentadol Hydrochloride (40, 50 and 60 ìg/ml).The results were expressed in terms of % CV. The system suitability parameters like theoretical plates (Tp), and asymmetry factor (As), resolution (Rs), retention time (RT) and tailing factor (Tf) were calculated by LC solution software. The HPLC system was equilibrated with the initial mobile phase composition, followed by six injections of the standard solutions having the same concentration. These six consecutive injections were used to evaluate the system suitability on each day of method validation. In order to establish system suitability for the instrument, six consecutive injections of Tapentadol Hydrochloride was prepared from standard solution and analyzed.

Linearity:

Linearity of the method was evaluated at five concentration levels by diluting the standard solution in the concentration range of 10-50 µg/ml of Tapentadol Hydrochloride. The results show that an excellent correlation existed between the peak area and concentration of analyte. The calibration curve was prepared by plotting the peak area versus the concentration and the regression equation was calculated. The calibration curve was repeated for six times and the average results are mentioned in table 1. The calibration curves are shown in figure 3.

S.No	Linearity Level	Concentration	Area
1	I	10µg/ml	690872
2	II	20µg/ml	1194381
3	III	30µg/ml	1677524
4	IV	40µg/ml	2105550
5	V	50µg/ml	2568454
Correlation Coefficient			0.999

Table 1: Linearity studies of Tapentadol

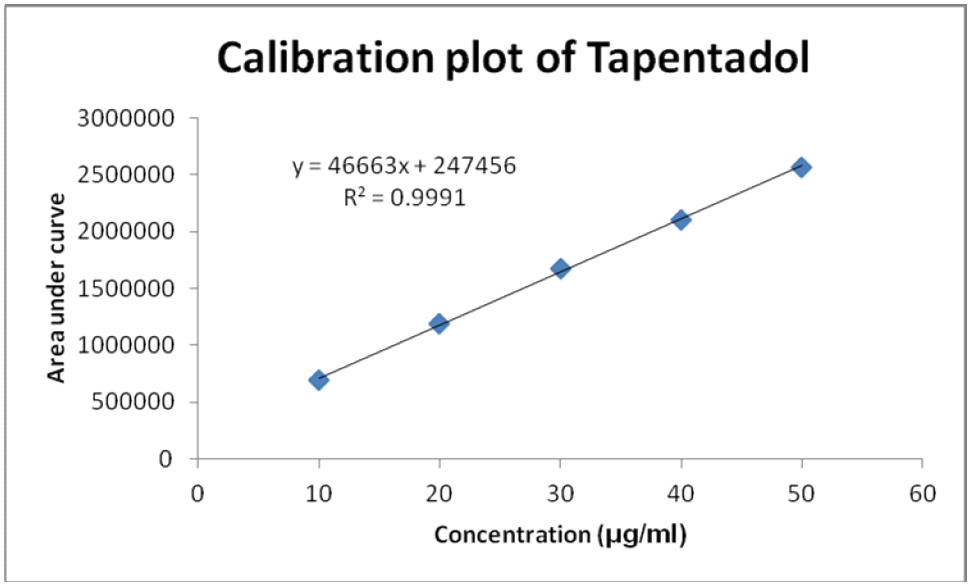

Figure 3: Linearity plot of tapentadol

Precision:

Method precision:

The results of repeatability (method precision) experiment are shown in table 2. Method precision was determined by repeatedly injecting 10-50 µg/ml concentration of Tapentadol Hydrochloride (n = 6). The developed method was found to be precise and the results are expressed in terms of % RSD.

Table 2: Precision results

Injection	Area
Injection-1	1637847
Injection-2	1641918
Injection-3	1646889
Injection-4	1651135
Injection-5	1659608
Average	1647479
Standard Deviation	8433.9
%RSD	0.51

Accuracy (% Recovery):
Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of standard drug solution at three concentration levels (50, 100, and 150 %) was added to the pre analyzed sample solution. This solution was analyzed under the chromatographic conditions mentioned in table 4. The assay was repeated over 3 consecutive days to obtain intermediate precision data. The results of accuracy are shown in table 3.

Table 3: Accuracy results

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1692637	5.0	5.0	100.0%	99.7%
100%	3353926	10.0	9.91	99.1%	
150%	5074031	15.0	14.9	99.9%	

LOD and LOQ:

These data show that the method is sensitive for the determination of Tapentadol Hydrochloride. The Limit of detection was found to be 0.01µg/ml and Limit of Quantification was found to be 30µg/ml.

Specificity and selectivity:

The resolution factor for Tapentadol Hydrochloride from the nearest resolving solvent peak was > 3 in all samples. The placebo shows no detector response near retention times of 3.16 min, while the Tapentadol Hydrochloride standard display good resolute peak [Figure 2] and no interference from excipients present in the formulation indicate specific nature of the method.

System suitability:

As system suitability test was an integral part of chromatographic methods development and were used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Tapentadol Hydrochloride was evaluated.

The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values. Tailing factor for the peak due to Tapentadol in Standard solution was not be more than 2.0. Theoretical plates for the Tapentadol peak in Standard solution were 2800. This shows that the method have been found in concordance to the acceptability criteria.

Table 4: Optimized HPLC conditions for the estimation of TPL

S. No	Parameter	Description/Value
1.	Stationary Phase	Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermsil)
2	Mobile Phase	0.1 M Potassium di hydrogen phosphate buffer pH adjusted to 3.6 with ortho phosphoric acid: Acetonitrile (50:50 v/v)
3	Flow rate	1.0 ml/min
4	Detection Wavelength	243.0 nm
5	Detector	DAD or UV detector.
6	Injection	Auto sampler
7	Injection volume	20 µl
8	Column Temperature	Ambient
9	Run time	5 mins
10	Diluent	Mobile Phase (0.1 M Potassium di hydrogen phosphate buffer pH adjusted to 3.6 with ortho phosphoric acid: Acetonitrile (50:50 v/v))

CONCLUSIONS:

A validated HPLC analytical method has been developed for the determination of TPL in API and dosage forms. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from excipients found in the tablet dosage forms. The method is suitable for the routine analysis of TPL in either bulk, API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments, such as LC–MS or GC–MS. These methods are complicated and costly rather than a simple HPLC-UV method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals.

ACKNOWLEDGEMENTS:

The authors are grateful to MSN Laboratories, Hyderabad, India for proving a gift sample of Tapentadol and to the Management of Deccan School of Pharmacy, Hyderabad, A. P. India for providing the necessary facilities and chemicals.

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