A VALIDATED METHOD FOR THE DETERMINATION OF TAPENTADOL HYDROCHLORIDE IN BULK AND ITS PHARMACEUTICAL FORMULATION BY DENSITOMETRIC ANALYSIS

ABSTRACT

Described in this manuscript is the first reported new, simple high performance thin layer chromatographic method for the determination of tapentadol hydrochloride in bulk and its tablet dosage form. The drug was separated on aluminum plates precoated with silica gel 60 F_{254} with butanol: water: glacial acetic acid in the ratio of 6:2:2 (V/V/V) as mobile phase. Quantitative analysis was performed by densitometric scanning at 254 nm. The method was validated for linearity, accuracy, precision and robustness. The calibration plot was linear over the range of 200-600 ng band $^{-1}$ for tapentadol hydrochloride. The method was successfully applied to the analysis of drug in a pharmaceutical dosage form.

Keywords: Tapentadol Hydrochloride, Accuracy, Tablet Dosage Form

INTRODUCTION

Chemically tapentadol hydrochloride is 3-[(1R, 2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl] phenol hydrochloride (Fig. 1). It is a centrally acting analgesic with dual mode of action as an agonist at the μ-opioid receptor site and as a norepinephrine reuptake inhibitor.[1] It is used in the treatment of moderate to severe pain. It was reported that tapentadol dosed at 50 mg 3 times daily for 7 days[2] .

Analysis plays an important role in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of drugs in bulk, in drug delivery systems, in dissolution studies (in vitro), and in biological samples (in vivo). If such a suitable method for a specific need is not available, then it becomes essential to develop a simple, sensitive, accurate, precise and reproducible method for the estimation of drug samples. The literature survey reveals that tapentadol was analyzed in urine sample by the Ultra Performance Liquid Chromatography method[3] and the availability of few HPLC methods in pharmaceutical dosage forms were also found through an extensive literature survey[4-9].

Till now no HPTLC method for quantitative analysis of tapentadol in bulk and its formulation or for stability studies was found by computer assisted literature survey either in chemical abstracts or by use of the CAMAG bibliographic service. Hence, the main objective of the work discussed in this paper was, therefore, to develop a rapid and reliable method for analysis of tapentadol in accordance with ICH guidelines[10]. Here we describe the investigation in detail.

The usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step ahead when compared with other tools of chromatography. HPTLC is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. The use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, and more effective elution techniques, high-resolution sorbents with selected particle size or chemically modified surface, the possibility of combining with other instrumental methods, and development of computer programs for method optimization all make HPTLC an important alternative method to HPLC or gas chromatography. Specifically, HPTLC is one of the ideal TLC techniques for the analytical purposes because of its increased accuracy, reproducibility, and ability to document the results, compared with standard TLC. Because of this, HPTLC technologies are also most appropriate TLC technique for conformity with GMPs. Today

the comprehensive use of TLC in Pharmaceutical analysis is demonstrated by the great number of articles published in this field. So the ultimate aim of the present study is to develop and validate HPTLC method for the determination of tapentadol in bulk drug and its dosage form. The optimization of the method separation, validation parameters and quantification of tapentadol in bulk and its formulation are reported in the following sections.

EXPERIMENTAL

Materials and Reagents

The authentic sample of tapentadol was procured from Janssen & Janssen Pharmaceuticals, Raritan. The pure drug obtained was having 99.9% w/w assay value, and was used without further purification. All chemicals and reagents used were of analytical grade. Tapentadol is available as commercial tablets under the brand name Nucynta from Ortho-McNeil-Janssen Pharmaceuticals, containing 100 mg of tapentadol and procured from the local pharmacy.

Preparation of the standard stock solution

Analyte (20, 30, 40, 50 and 60 mg) was accurately weighed and separately dissolved in distilled water in 100 mL volumetric flasks to furnish solutions in the concentration range 200-600 ng μL^{-1}. These solutions were used for the working range.

HPTLC Instrumentation

Chromatography was performed on 10 cm x 10 cm aluminum plates pre-coated with 250-µm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Before use the plates were prewashed with methanol and activated at 110° for 5 min. Samples were applied to the plates as bands of 6 mm wide and 10 mm apart by means of a Camag (Switzerland). Linomat V sample applicator equipped with a 100 µl syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was performed in a 10 cm x 10 cm twin trough glass chamber (Camag), with butanol: water: glacial acetic acid 6:2:2 (V/V/V) as mobile phase and the chamber was pre saturated with mobile phase vapour for 10 min. The development distance was 8.5 cm with a development time of approximately 60 min. After chromatography the plates were dried in a current of air by using air blowing drier. Densitometric scanning was performed with a Camag TLC Scanner 3 at 254 nm for all measurements. The scanner was operated by Wincats software Version 1.2.3. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimensions were 5 mm x 0.45 mm and the scanning speed was 20 mm s^{-1}. After chromatographic development, bands were scanned over the range 200–400 nm (spectrum scan speed 20 nm s^{-1}) so that the drug could be estimated at 254nm, which is ascertained by taking the spectrum at different concentrations between 200 –600 ng with 100 ng increment. Further it is also observed that spectra are similar in their behavior.

Standard Procedure

The standard stock solution of tapentadol was applied on a TLC plate, in the range 1µL, by use of the Linomat V sample applicator and 100µL syringe. The plate was developed and scanned under the conditions described above. Each amount was analyzed five times and peak areas were recorded. A calibration plot of peak area against respective amount was established for tapentadol hydrochloride.

Sample Procedure

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg tapentadol was weighed and transferred to a 100

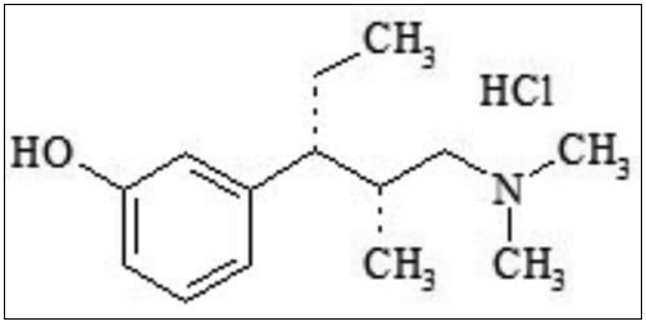

Fig.1: Structure of tapentadol hydrochloride

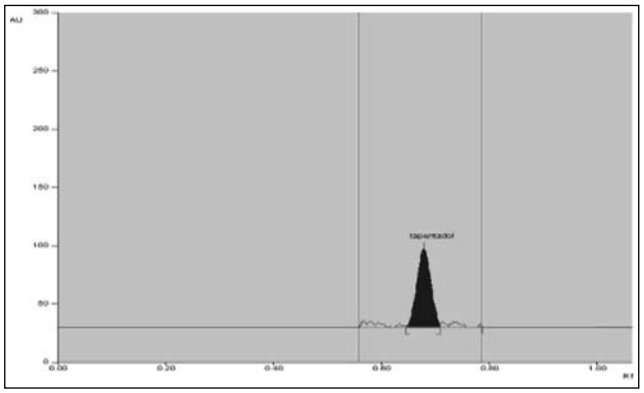

Fig. 2: A typical densitogram of tapentadol standard R_F (0.68±0.02) at 254 nm using butanol: water: glacial acetic acid 6:2:2 (V/V/V)

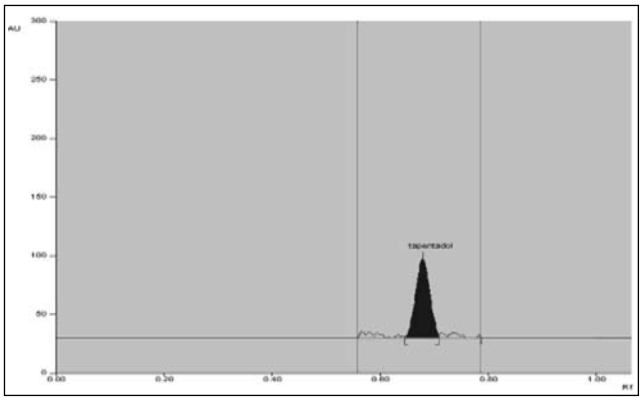

Fig. 3: A typical densitogram of tapentadol extracted from tablet formulation R_F 0.68±0.02 at 254 nm using butanol: water: glacial acetic acid 6:2:2 (V/V/V)

Table I: Results from recovery studies of tapentadol

Sample	Excess of drug added to the analyte (%)	Theoretical content (ng)	% recovery*	%RSD
Tapentadol	50	300	99.26	0.29
	100	400	99.81	0.49
	150	500	100.50	0.72

** Average of three determinations*

Table II: Analysis of Tablet formulation (n=6)

Brand name	Label claim	Amount found	% Assay	% RSD
Nucynta	100 mg	98.73	98.73	0.45

Table III: Method validation parameters of tapentadol

Parameters	Results of tapentadol hydrochloride
Linearity (ng band^{-1})	200-600
Correlation coefficient	0.9982
LOD (ng band^{-1})	37.33
LOQ (ng band^{-1})	123.99
Precision	
Interday (%RSD)	0.44
Intraday (%RSD)	0.29
Specificity	Specific

mL volumetric flask containing approximately 50 mL distilled water. The mixture was ultra sonicated for 5 min then the final dilution was made with distilled water. The solution was filtered using Whatman 41 paper and 4 µl of the filtrate was applied to a TLC plate. After the development of chromatogram the peak area of the bands was measured at 254 nm and the amount of drug in each tablet was determined from the calibration plot. The analytical procedure was repeated six times for the homogenous powder sample.

Method Validation

The limit of detection and limit of quantitation for tapentadol was calculated from the linearity data using relative standard deviation of the response and

slope of the calibration curve for tapentadol. The limit of detection of a compound is defined as the lowest concentration that can be detected. LOD value was found to be 37.33 ng/band for tapentadol. The limit of quantitation is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ value was found to be 123.99 ng/band for tapentadol respectively. To study intra-day and inter-day precision, three different concentrations of sample solutions were prepared and applied to the TLC plates. All the solutions were analyzed in triplicate on the same day and on three different days to record intra-day and inter-day variations in the results respectively. To check the accuracy of the method, recovery measurements were performed by the addition of standard drug solution at three different levels (50, 100 and 150%) to pre-analyzed sample solution (200 ng/band for tapentadol hydrochloride so that after addition of standards, samples would be in the linear range). Three replicate estimations were carried out for each concentration level. By introducing small changes in the mobile phase composition, the effects on the results were examined for robustness study. Mobile phase having different composition like butanol-water-glacial acetic acid were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. Robustness of the method was done at three different concentration levels: 200, 400 and 600 ng/band, respectively.

RESULTS AND DISCUSSION

Different mobile phases containing toluene, methanol, acetic acid, propanol, acetone, ethyl acetate and dichloro methane in different proportions were examined, of these the mixture of butanol: water: glacial acetic acid 6:2:2 (V/V/V) was found to be most suitable for the studies. The R_F value of standard tapentadol hydrochloride was 0.68±0.02 (Fig. 2). The densitogram obtained from a sample solution of tapentadol hydrochloride is depicted in Fig. 3. The calibration plot was found to be linear over the range 200–600 ng/band for tapentadol hydrochloride, with correlation coefficient of 0.9982 ± 0.0102. The LOD and LOQ were 37.33 and 123.99 ng/band respectively. The proposed HPTLC methods were validated for intra and inter day precision studies. The values of percent relative standard deviations were found to be 0.29, and 0.44 respectively which indicate that the method is precise. The method was also evaluated by assay of commercially available tablets containing tapentadol hydrochloride. Six replicate analyses were performed on accurately weighed amount of the tablets. The percentage assay was found to be 98.73 ± 0.45 for tapentadol hydrochloride (Table II). To study the accuracy of the method, recovery studies were performed. For tapentadol hydrochloride, recovery ranged from 99.26 to 100.5%, with values of percent RSD ranging from 0.29 to 0.72 indicating that the proposed HPTLC method is highly accurate (Table I). Study of the robustness of the method revealed that the peak areas were unaffected (RSD < 2%) by small changes of the operating conditions and can be inferred to be more robust. The method validation parameters are presented in Table III, respectively.

The specificity of the method was determined by analyzing drug standard and test samples. The peak for tapentadol HCl in the test sample was confirmed by comparing its R_F and spectrum with those of the standard. The peak purity of tapentadol HCl was determined by comparing spectra acquired at three different positions on the spot, i.e. peak start (S), peak apex (M), and peak end (E).

CONCLUSION

The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of tapentadol hydrochloride as bulk drug and its formulation without interference from its excipients. It may be extended for quantitative estimation of said drug in plasma and other biological fluids.

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