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DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC (HPTLC) METHOD FOR ESTIMATION OF TAPENTADOL HYDROCHLORIDE IN BULK AND ITS TABLET DOSAGE FORM

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High performance thin layer chromatography (HPTLC), Tapentadol Hydrochloride, validation, Estimation, Densitometric evaluation.

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ABSTRACT: A simple, accurate, rapid and sensitive high performance thin layer chromatographic method has been developed for the estimation of Tapentadol Hydrochloride in bulk and its tablet dosage form. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of methanol: toluene (4:1v/v). Densitometric evaluation of the separated zones was performed by UV detector at 272 nm. The drug was satisfactorily resolved with R_f value 0.5 ± 0.02.The method was linear over concentration range of 1500-2000 (1.5-2.0 µL) ng/spot. The analytical percent recovery was found to be 99.92%.The intra and interday precision with percent relative standard deviation (%RSD) values in the range of 0.0895 to 0.0892. The validation of the method was carried as per ICH guidelines. The proposed HPTLC method was successfully employed for routine analysis of bulk drug and its commercial tablet dosage form.

INTRODUCTION: Tapentadol is a novel centrally acting opioid analgesic drug with dual mode of action as an agonist to the µ-opioid receptor and as a norepinephrine reuptake inhibitor. It is used for the treatment of moderate to severe acute or chronic pain in adults [1, 2]. The Tapentadol is chemically 3[3-(Dimethylamino)-1-ethyl-2-methylpropyl] phenol hydrochloride (**Figure 1)**.

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The empirical formula is $C_{14}H_{23}NO.HCl$ and molecular weight 257.799 g/mol [3]. It is having potency between morphine and tramadol [4]. It is not official in any Pharmacopoeia. Literature survey revealed that very few methods are developed for determination of Tapentadol Hydrochloride such as UV- Spectrophotometric method [5], Stability Indicating RP-HPLC method and liquid Chromatography-Mass Spectrometry (LC/MS) [6, 7, 8]. So far not a single High Performance Thin Layer Chromatographic method is reported for quantitative determination of Tapentadol Hydrochloride. Hence an attempt has been made to develop simple chromatographic HPTLC method for quantitative estimation of Tapentadol Hydrochloride in bulk and its tablet dosage form which is an accurate, sensitive and precise.

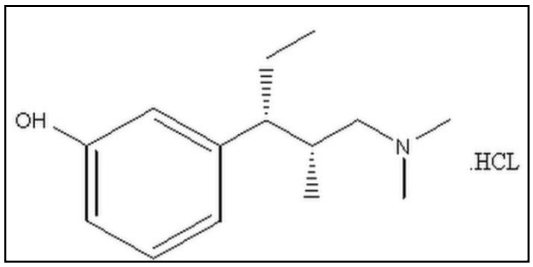

FIGURE 1: STRUCTURE OF TAPENTADOL HYDROCHLORIDE

MATERIAL AND METHODS:

Reagents and chemicals: Pure analytical sample of Tapentadol Hydrochloride was procured from MSN Laboratories Ltd. Hyderabad, (India) as a gift sample. The drug Tapentadol Hydrochloride was used without further purification and certified to contain 99.85% (w/w) on dry weight basis. The pharmaceutical tablet dosage form used in the study was Tydol-50 (Ranbaxy Laboratories Ltd.) labeled to contain 50 mg of Tapentadol Hydrochloride purchased from local pharmacy. All solvents and chemicals used in the study were Merck analytical grade.

Instrumentation: Micro-syringe (Linomat syringe Hamilton-Bonadzu Schweiz, Camag, Switzerland), pre-coated aluminum plates with silica gel G $60F_{254}$ with 250 µm thickness (Merck Germany), Linomat 5 applicator, twin tough chamber (20×10 cm; Camag), UV chamber (Camag Switzerland), TLC scanner 4 (Camag Switzerland), win CATS version 1.4.2 software were used for study.

Selection of Mobile Phase and Chromatographic Conditions: For optimization of mobile phase sample was the spotted (100 ng/spot) in form of band, 8 mm from bottom and 15 mm from side edges and was developed by linear ascending development using solvents such as ethyl acetate, toluene, methanol, acetonitrile. After several trials, binary composition of methanol: toluene (4:1, v/v) was chosen as the mobile phase for analysis with chamber saturation time of 20 min at room temperature. The length of chromatogram run was 7 cm and development time was about 15 minutes. Plates were scan by using TLC scanner 4 in absorbance mode with scanning speed of 10 mm/sec. The radiation source utilized was deuterium lamp (D2) with spectrum wavelength emitted between 200-400 nm.

Preparation of standard stock solution: Standard stock solution of Tapentadol Hydrochloride was prepared by dissolving 10 mg of drug in 10 ml of methanol to obtained final concentration of 1.0 mg/ml.

Preparation of sample stock solution: Twenty tablets (Tydol-50) were weighed accurately and powdered. Powder equivalent to 100 mg of Tapentadol Hydrochloride was weighed and transferred to 100 ml volumetric flask, dissolved in 30 ml methanol by ultra-sonication of the flask for 15 minutes and solution was filtered through Whatmann paper no. 41.The final volume was made up to the mark to obtained tablet stock solution of 1.0 mg/ml.

Selection of detection wavelength: After chromatographic development, from standard stock solution bands of different concentration were scanned over range of 200-400 nm. It was observed that drug showed considerable absorbance at 272 nm.

Method Validation: The method was validated as per ICH Q2 guidelines. The following parameters were used for validation of proposed method.

Linearity: From standard stock solution 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 µl of Tapentadol Hydrochloride spotted on the TLC plate to obtain final concentration of 1500, 1600, 1700, 1800, 1900, and 2000 ng/spot of Tapentadol Hydrochloride. The plates were developed in 20×10 cm twin through chamber using freshly prepared developing phase (5 ml) i.e. methanol: toluene (4:1 v/v) and scanned at 272 nm. Peaks were integrated. The Response area was plotted against the corresponding concentrations to obtain the calibration curve.

Precision: Precision of proposed method was determined by repeatability and intermediate precision (Intraday and Interday). Intermediate precision i.e. intraday precision was determined by analyzing three replicates of different concentration of standard Tapentadol Hydrochloride (1500 ng/spot, 1700 ng/spot, and 1900 ng/spot) for three the times in same day and for interday precision same concentrations were used for three consecutive days.

Accuracy: Accuracy of proposed method was ascertained on the basis of recovery study. Recovery studies were carried out by addition of standard working solution to pre-analyzed tablet solution at three different levels, 50 %, 100 % and 150 %. At each levels of the amount, three determinations were performed.

Robustness: In the robustness study, the influence of small, deliberate variations of the analytical parameters such plate activation time, chamber saturation time and concentration of mobile phase was checked. The effect of change in these parameters on Response factor (R_f) values and peak areas were evaluated by calculating relative standard deviations (%RSD).

Specificity: Specificity of proposed method was checked by analyzing the interference of commonly used excipients in the formulation. Pure Tapentadol Hydrochloride was spiked with common excipients such as talc, magnesium stearate and starch and then assayed by proposed method.

RESULT AND DISCUSSION: A number of experimental parameters, such as activation time, saturation time, mobile phase composition, scan modes, detection wavelength were optimized during method development. Promising results were obtained by using mobile phase methanol and toluene in the ratio of 4:1 v/v and maximum resolution with R_f value found to be 0.52 ± 0.02 **Figure 2**. Optimum wavelength for detection was 272 nm at which good detection for Tapentadol Hydrochloride was obtained **Figure 3**.

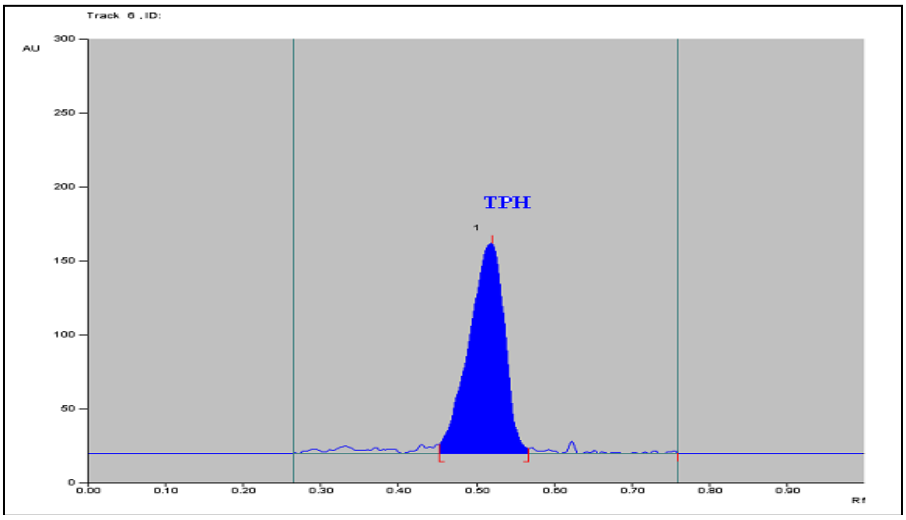

FIGURE 2: HPTLC DENSITOGRAM OF TAPENTADOL HYDROCHLORIDE

Mobile phase: Methanol: Toluene (4: 1: v/v)
R_f =0.52±0.02, Concentration: 1000 µg/mL for Tapentadol Hydrochloride
Application volume: 1.5 µL; Wavelength: 272 nm

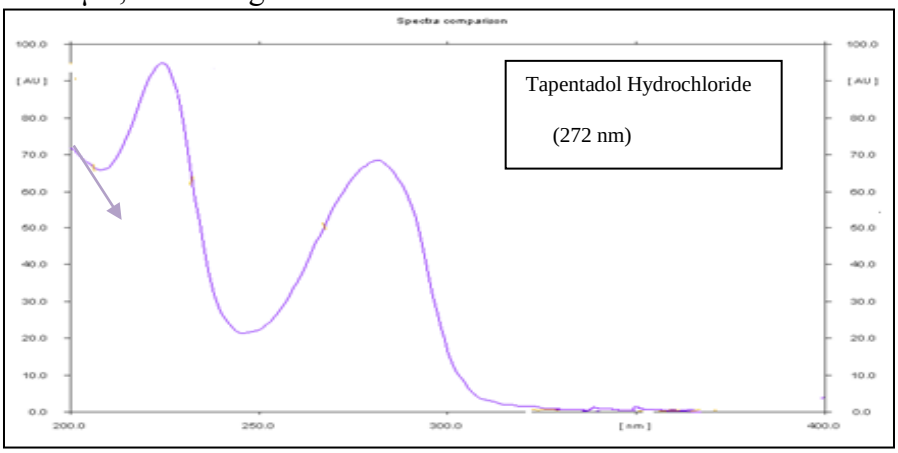

FIGURE 3: SPECTRODENSITOGRAM OF TAPENTADOL HYDROCHLORIDE (WAVELENGTH 272 nm)

Linearity of calibration curve: Linear correlation was obtained between peak area and concentration of Tapentadol Hydrochloride in the range 1500-2000 ng/spot with correlation coefficient r=0.9987. The linear regression equation was found to be y = 2.3199x+830.36 **Figure 4, Table 1**. The peak areas of Tapentadol Hydrochloride were reproducible by low coefficient variation.

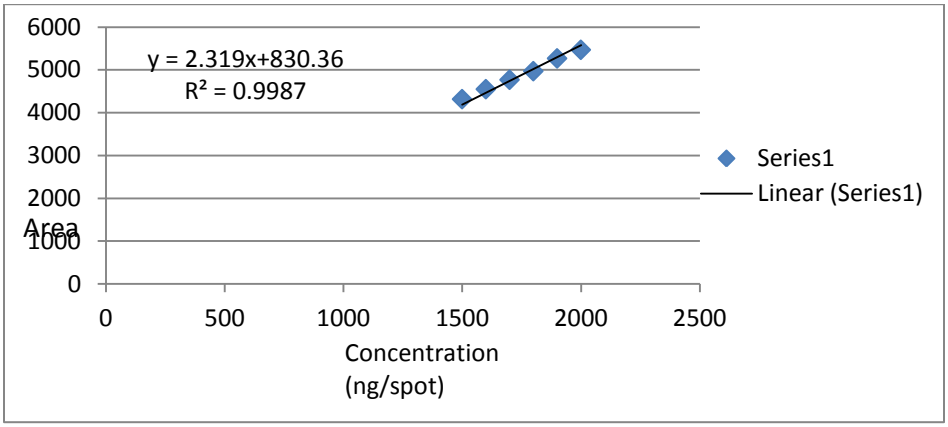

FIGURE 4: CALIBRATION CURVE FOR TAPENTADOL HYDROCHLORIDE (1500-2000 ng/spot)

TABLE 1: OPTICAL CHARACTERISTICS OF PROPOSED METHOD

Parameter	Values
Detection wavelength (nm)	272.00 nm
Beer's law limit (ng/spot)	1500-2000 (ng/spot)
Régression Equation (y = mx + c)	y = 2.3199x+830.36
Slope (m)	2.3199
Intercept (c)	830.36
Corrélation coefficient (r)	0.9987
Limit of detection (LOD)	1.116
Limit of quantitation (LOQ)	1.129

Precision: Precision of proposed method was determined by intermediate precision. The % RSD value proposed method was found less than 2 %.These low values of RSD indicate good precision of developed method **Table 2, 3.**

TABLE 2: PRECISION OF PROPOSED METHOD (INTRADAY)

Sr. No.	Concentration (ng/spot)	Interday Area			Average % RSD
		Morning	Afternoon	Evening	
1	1500	4314.2	4412.1	4408.2	
2	1500	4269.3	4342.1	4506.1	
3	1500	4298.5	4298.3	4404.8	
4	1700	4769.4	4725.1	4768.5	0.0895
5	1700	4783.2	4680.2	4750.1	
6	1700	4766.2	4698.8	4772.1	
% RSD		0983	0.0873	0.0892	

TABLE 3: PRECISION OF PROPOSED METHOD (INTERDAY)

Sr. No.	Concentration (ng/spot)	Interday Area			Average % RSD
		Day1	Day2	Day 3	
1	1500	4313.1	4314.1	4298.2	
2	1500	4314.3	4443.1	4318.1	
3	1500	4401.3	4308.3	4314.5	
4	1700	4698.2	4762.1	4768.1	0.0892
5	1700	4696.3	4769.2	4768.1	
6	1700	4612.1	4698.1	4730.1	
% RSD		0.0980	0.0856	0.0882	

Analysis of marketed formulation: The proposed method was successfully applied for determination of Tapentadol Hydrochloride in commercial tablet dosage form with label claim 50 mg/tablet. The amount of Tapentadol Hydrochloride estimated was found to 49.99 mg/tablet with % label claim of 99.98 % **Table 4** and the R_f value was in the same range i.e. 0.52 ± 0.02.

TABLE 4: RESULT OF ANALYSIS OF MARKETED FORMULATION (ASSAY)

Drug	Amt. of drug in formulation(mg/tablet)	Amt. of drug applied (ng/spot)	Amt. of drug found	% label claim	± SD	± RSD
TPH	50	1500	1495	99.98	0.568	0.002
Mean,± SD n= 6 replicate						

Accuracy: The accuracy the proposed method was ascertained by performing recovery study at three concentration levels i.e. 50%, 100% and 150%. The mean recovery of added drug at each level was found to be 99.92 ± 0.65% with standard deviation of 0.292- 0.659 **Table 5.**

TABLE 5: RECOVERIES STUDIES FOR TAPENTADOL HYDROCHLORIDE

Level of recovery	Amount taken (ng/spot)	Amount added (ng/spot)	Total amount obtained (ng/spot)	% recovery	± SD
50%	1600	800	2399	99.95	0.259
100%	1600	1600	3196	99.87	0.438
150%	1600	2400	3998	99.95	0.292

Robustness: Results of robustness study indicate that the selected factors such as chamber saturation time and plate activation time remained unaffected by small variations with percent relative standard in the range of 0.673 -0.838 **Table 6**.

TABLE 6: ROBUSTNESS DATA FOR TAPENTADOL HYDROCHLORIDE IN TEARMS OF PEAK ARE AND %RSD

Parameter	Conc. of drug applied (ng/spot)	Peak area obtained	%RSD
Duration of chamber saturation (22 mins.)	1500	4316. 9	0.673
Duration of chamber saturation	1500	4319.2	0.679
Change in plate activation Time (120ºC for 15 mins.)	1500	4318.2	0.837
Change in plate activation time (120ºC for 25 mins.)	1500	4316.1	0.838

CONCLUSION: The results of percent recovery obtained using proposed HPTLC method indicates non-interference from the excipients used in the formulation. The result of analysis of marketed tablet formulation are highly reproducible with good percent label claim. So the proposed HPTLC method is found to be rapid, sensitive reproducible and precise. It can be used for the routine analysis of formulation containing this drug without any modification in assay procedure.

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