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Development and Validation of A Stability Indicating RP-HPLC Method for Determination of Tapentadol in Tapentadol Hydrochloride Tablets

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ABSTRACT

A simple, precise, rapid and accurate stability indicating reversed-phase high performance liquid chromatography (RP-HPLC) method is developed for the estimation of tapentadol in tapentadol hydrochloride tablets. The separation was achieved by using a Waters 2695 HPLC System consisting of analytical column Eclipse XDB-C_{18} (5µm; 150x4.6mm) and wavelength detector- Waters 2489 UV is used for analysis. The mobile phase consisting of A- phosphate buffer (pH 6.5±0.05): methanol in the ratio of 65:35 (v/v) is used. The flow rate is 1.0 mlm^{-1} and the effluents are monitored at 210 nm. The retention time is about 12.0 m. The detector response is linear in the concentration range of 50.0-150.0 μgml^{-1}. The respective linear regression equation being y = 956825 + 569233. The percentage assay of tapentadol is 99.65%. The method is validated as per ICH guideline by determining its specificity, accuracy, precision, linearity & range, ruggedness, robustness and system suitability. The results of the study show that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of Tapentadol in tablet dosage form.

KEY WORDS: Tapentadol Hydrochloride, RP-HPLC, Validation, System suitability tests.

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INTRODUCTION

Tapentadol hydrochloride (**Fig.1**) is 3-[(1*R*,2*R*)-3-(dimethylamino)-1-ethyl-2methylpropyl]phenol monohydrochloride - (m.f. $C_{14}H_{23}NO \cdot HCl$; m.w. 257.80) [1]. Tapentadol is a centrally-acting synthetic analgesic. Although its exact mechanism is unknown, analgesic efficacy is thought to be due to mu-opioid agonist activity and the inhibition of norepinephrine reuptake.

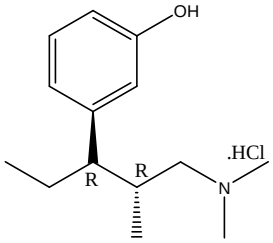

Figure-1: Chemical structure of tapentadol hydrochloride

Literature survey revealed that; very few spectrophotometric methods and HPLC methods are available for estimation of tapentadol individually as well as in combination are available[2,3,4,5,6,7,8]. An attempt has been made to develop a new stability indicating RP-HPLC method for its estimation in tablet dosage form with good accuracy and precision [9, 10]. The method is validated according to the ICH Q2 (R1) and other relevant regulatory guidelines.[11]

EXPERIMENTAL

INSTRUMENTATION

Waters 2695 HPLC System, consisting of degasser, binary pump, column oven, and variable wavelength detector Waters 2489 UV is used for analysis. The analytical column Eclipse XDB-C_{18} (5µm; 150x4.6mm) is used. The waters empowers software ran on IBM computer operated with Windows XP professional used for this method.

REAGENTS AND CHEMICALS

Methanol used was of HPLC grade from E. Merck, India. Diammonium hydrogen orthophosphate used was of AR grade from S.D. Fine Chem- Limited, India. Ortho phosphoric acid used was of AR grade from E. Merck, India. Triethylamine used was of AR grade from E. Merck, India. Hydrochloric acid used was of AR grade from E. Merck, India. HPLC grade water was obtained using millipore water purification system. Working standard of tapentadol hydrochloride with potency of 98.7 % (on as is basis) was obtained from Ami Life Sciences. All volumetric-glassware were pre-calibrated by the

manufacturer (Borosil) and were of grade A. Tablets manufactured by Glenmark Pharmaceuticals Limited; used for estimation has been procured from the market.

CHROMATOGRAPHIC CONDITIONS

The analysis was carried out with UV detection at 210 nm using a 20 µl injection volume. Assay was performed using Eclipse XDB-C_{18} reversed-phase column eluted with buffer and methanol (65:35, %v/v) at a flow rate of 1.0 mlm^{-1}. Chromatography was carried out at 35°C column temperature. The solvents were mixed, filtered through a membrane filter of 0.45 micron pore and degassed in ultrasonic bath prior to use.

STANDARD SOLUTION PREPARATION

STANDARD STOCK SOLUTION

Standard stock solutions of 1000 μgml^{-1} of tapentadol hydrochloride were prepared in diluent (0.1N methanolic HCl).

WORKING STANDARD SOLUTION

Transferred 5 ml of standard stock solution to a 50 ml volumetric flask. Diluted up to the volume with diluent and mixed. It was filtered through a 0.22 µ membrane filter.

SAMPLE PREPARATION

20 tablets of the product under study were weighed and powdered. A portion equivalent to the weight of 50.00mg was accurately weighed and transferred to a dry 50 ml volumetric flask and 20 ml of diluent was added. The volumetric flask was sonicated for 15 min with intermittent shaking. Cool to room temperature and volume made up to the mark with diluent & mixed. Suitable aliquots of solution were filtered through a 0.45 µm nylon filter. Each of standard (**Fig.2**) and sample preparation (**Fig.3**) were injected into the chromatograph and the responses were recorded.

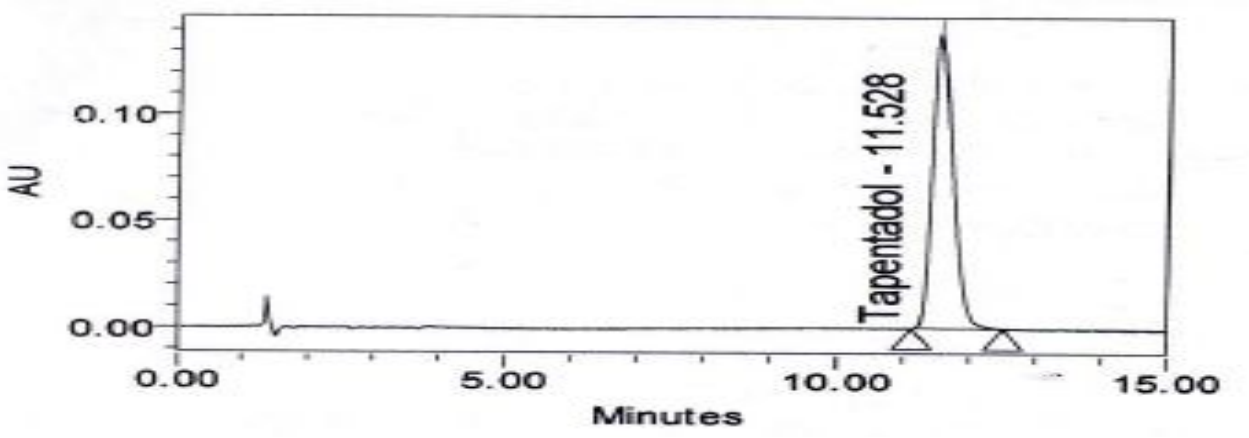

Fig.2 HPLC Chromatogram of Standard Tapentadol Hydrochloride

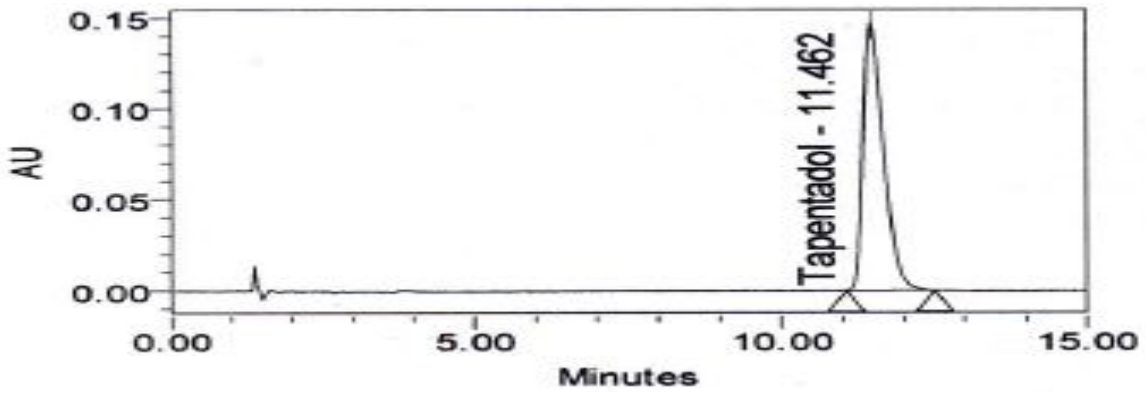

Fig. 3 HPLC Chromatogram of Tapentadol Sample

METHOD VALIDATION [11]

LINEARITY & RANGE

A series of standard curves were prepared over a concentration range of 50.0 – 150.0 μgml^{-1} by diluting the standard stock solution of tapentadol hydrochloride (1000 μgml^{-1}) in 0.1NHCl (as diluent). The data from peak area versus drug concentration plots were treated by linear least square regression analysis and r^2 was found 0.9993. The standard curves were evaluated for intra-day and inter-day reproducibility. Each experiment was repeated in triplicate.

PRECISION

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 50 μgml^{-1} concentration six times.

ACCURACY

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Placebo of tapentadol hydrochloride tablets 50 mg were spiked with tapentadol hydrochloride standard solution (50 μgml^{-1}) so as to get five different levels (50%, 80%, 100%, 120% and 150%) and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recoveries (%), RSD (%) were calculated for each concentration.

RUGGEDNESS

The ruggedness of the method was demonstrated by analysis of the samples as for precision study by a second analyst. The RSD of the two sets of data indicates the ruggedness of the method.

ROBUSTNESS

The robustness of the method was determined to assess the effect of small but deliberate changes of the chromatographic conditions on the determination of tapentadol. The different variations are in flow

rates by ± 0.1 mL/min, in wavelength by ± 2 nm, in pH by ± 0.2, and in mobile phase composition by ± 5%. The concentration of the solution analyzed was 50 $\mu g ml^{-1}$.

SYSTEM SUITABILITY TESTS

The chromatographic systems used for analyses must pass the system suitability criteria before sample analysis can commence. The injection repeatability, tailing factor (T), theoretical plate number (N) and % RSD (% relative standard deviation) for the principal peak were the parameters tested on a 50 µg/mL sample of tapentadol hydrochloride to assist the accuracy and precision of the developed HPLC system.

SPECIFICITY

The specificity of the method was determined by purity angle and purity threshold of standard and test solution.

RESULTS AND DISCUSSION

Tapentadol hydrochloride, a weak acid, is freely soluble in water. The final decision on mobile phase composition and flow rate was made on the basis of peak shape, peak area, tailing factor, baseline drift, ease of preparation, use of readily available cost-effective solvents and time required for analysis. Initial trial experiments were conducted, with a view to select a suitable solvent system for the accurate estimation of the drug. These included methanol–water, acetonitrile-water, methanol-buffer, acetonitrile: buffer, methanol–acetonitrile-water and acetonitrile–methanol in different ratio. Flow rates between 0.5 and 1.2$ml min^{-1}$ were studied. A mobile phase system comprising of phosphate buffer-methanol (65:35 % v/v) was found to be optimum and a flow rate of 1.0 $ml m^{-1}$ gave an optimal peak shape and was selected. The solvents were mixed, filtered through a membrane filter of 0.45 micron pore and degassed in ultrasonic bath prior to use. Using a reversed-phase C_{18} column, the retention times for tapentadol was observed to be 11.528 & 11.462 min. Total run time was kept 15.0 min. The maximum absorption of tapentadol was detected at 210 nm and this wavelength was chosen for the analysis. The developed method was linear showing the coefficient of correlation of 0.9999. % RSD of accuracy study for five levels (50%, 80%, 100%, 120% and 150%) showed below 2.0% and precision was found to be 0.56. The method was also found to be robust as there was no significant change in the peak area, peak shape and retention time of Tapentadol. The system suitability tests performed verified the resolution, column efficiency and repeatability of the chromatographic system.

LINEARITY

Peak area versus drug concentration was plotted to construct a standard curve for Tapentadol and linearity was shown in concentration range of 50.0 µgml^{-1} to 150.0 µgml^{-1}. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation, r = 0.9999; slope = 956825 and intercept = 569233 over the concentration range studied. **Fig.4**

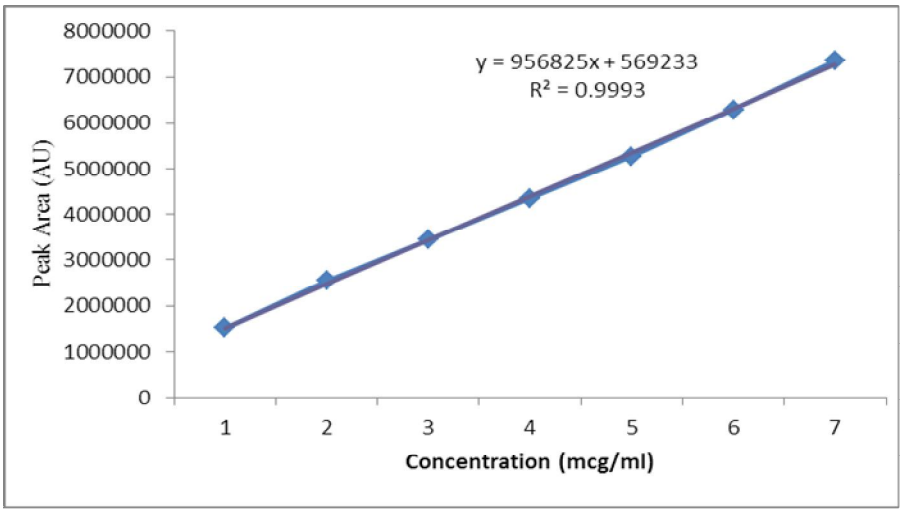

Fig. 4-Linearity graph of tapentadol hydrochloride

PRECISION

The % assay for tablet was calculated and % RSD was found to be 0.56% which proved that the method was precise, as depicted in **Table 1**.

Table No.1: Precision of developed method at working level

Sample No.	Area
1	2988224
2	3004201
3	2964916
4	2991557
5	2978963
6	2960823
Mean	2981447
SD	16556.95
% RSD	0.56

ACCURACY

The % recovery was calculated for triplicate samples and for all levels and mean recovery was calculated. The mean recovery was well within the acceptance limit hence the method was accurate, as depicted in **Table 2**.

Table No.2: Recovery studies of Tapentadol orally disintegrating films 4 mg

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 50 % -1	25.12	25.10	99.92
Accuracy 50 % -2	25.11	25.06	99.80
Accuracy 50 % -3	25.12	25.23	100.43
Accuracy 80 % -1	40.09	40.09	100.00
Accuracy 80 % -2	40.21	40.19	99.95
Accuracy 80 % -3	40.21	40.22	100.02
Accuracy 100 % -1	50.03	50.17	100.27
Accuracy 100 % -2	50.76	50.54	99.57
Accuracy 100 % -3	50.48	50.36	99.76
Accuracy 120 % -1	60.17	60.05	99.80
Accuracy 120 % -2	60.85	60.67	99.70
Accuracy 120 % -3	60.34	60.43	100.15
Accuracy 150 % -1	75.76	75.82	100.08
Accuracy 150 % -2	75.28	75.01	99.64
Accuracy 150 % -3	75.44	75.05	99.48
Mean	99.87		
SD	0.15		
%RSD	0.15		

RUGGEDNESS

The % assay and RSD for samples prepared by second analyst was calculated and found within limit. Then RSD of analyst 1 and analyst 2 was calculated and found within limit. This proved that the method is rugged, as depicted in **Table 3**.

Table No.3: Ruggedness Analysis

Analyst 1		**Analyst 2**	
Sample	**% Assay**	**Sample**	**% Assay**
1	99.01	1	98.94
2	98.95	2	98.87
3	98.61	3	98.54
4	98.39	4	98.31
5	100.85	5	100.69
6	99.70	6	99.60
Mean	99.25	Mean	99.16
SD	0.82	SD	0.88
% RSD	0.83	% RSD	0.88

ROBUSTNESS

The results of the analysis (% RSD ranged from 0.10 to 1.04 %) of the samples under the conditions of the above variations indicated the nature of robustness of the method.

SYSTEM SUITABILITY TESTS

The results of the system suitability tests assure the adequacy of the proposed HPLC method for routine analysis of Tapentadol Tablets. The RSD of six consecutive injections performed under the precision test (**Table 1**) was found to be 0.56% and thus shows good injection repeatability. The tailing factor (T) for Tapentadol peak was found to be 1.46, reflecting good peak symmetry. The theoretical plate number (N) was found to be 6315, thus demonstrating good column efficiency.

SPECIFICITY

The chromatograms obtained showed separation of the analyte from the excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis. No interference of the placebo mixtures with the peak of Tapentadol was observed.

CONCLUSION

The HPLC method developed is accurate, precise, reproducible and specific. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Tapentadol in bulk drug and in tablets. The method

developed was then subjected to validation as per ICH guidelines and showed that method is linear, precise, accurate and robust[12].

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