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Development and validation of RP-HPLC method for estimation of Tapentadol hydrochloride in its tablet dosage form.

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ABSTRACT

A reversed phase high-performance liquid chromatographic method has been developed for the quantification of Tapentadol hydrochloride in tablet dosage form. The mobile phase consisting of solvent A Methanol: Solvent B Acidic water (pH 3.8 adjusted by using triethylamine and o-phosphoric acid) in ratio of (58:42 % v/v) was delivered at the flow rate of 1.2 ml/min and UV detection was carried out at 271 nm. The separation was achieved using C_{18} reverse-phase column (250 X 4.6 mm I.D., particle size 5µm). The method was linear over the concentration range of 10-50 µg/ml. The analytical recovery obtained was 100.234%. The validation of method carried out as per ICH guidelines. As per validation data it was found that method is specific, robust and precise within the described concentration range. The described RP-HPLC method was successfully employed for the analysis of three different commercial brands of Tapentadol hydrochloride tablets.

Key Words: novel analgesic, Tapentadol hydrochloride, RP-HPLC method, formulation

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INTRODUCTION

Tapentadol 3-[(1R, 2R)-3-(3-dimethylamino)-1-ethyl-2-methylpropyl] phenol has been approved as immediate release tablets in 50 mg, 75 mg and 100 mg formulation by the United States Food and drug administration on November 20, 2008, and is available by prescription only on December 1, 2009 [1]. Tapentadol HCl immediate release dosage form got approval in India with 50, 75, 100 mg strength on 18.04.2011 [2]. It is having potency in between tramadol and morphine. As per literature survey reveals information that some of the methods like UPLC/Tandem mass spectrometry [3] for urine analysis, LC/MS/MS [4] for urine and oral fluid analysis, Chiral HPLC [5] for isomeric purity, HPLC/Fluorescence [6] for metabolite determination, HPLC/ion-pair chromatography [7] for Serum and urine analysis has been reported. But yet not a single method has been reported for its determination in bulk and solid (tablet) dosage forms by reversed phase high-performance liquid chromatographic (RP-HPLC) method. This study was designed to develop a simple and reliable method to quantitate tapentadol HCl in a relatively short time with high sensitivity. Therefore, this study focused on the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of tapentadol HCl in bulk drug and formulations. The established method was validated with respect to specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability.

MATERIALS AND METHODS

Chemicals and reagents

Water (HPLC Grade) and Methanol (HPLC Grade) of Astron chemicals pvt. ltd. ortho phosphoric acid and triethylamine of analytical grade, and double distilled water. Tapentadol Hydrochloride drug was procured from MSN laboratories Ltd. Hyderabad, (India) as a gift sample. Tablets were procured from local pharmacy.

Instruments

High performance liquid chromatography of SHIMADZU UFLC, model (LC-20AD prominance liquid chromatograph), detector (PDA Detector SPD-M20 A), Column C18 (250×4.6×5µm) of spincotech pvt. Ltd., digital pH meter of Janki Impex pvt. Ltd. and analytical balance of Acculab (Sartorius group) were used. All the chromatographs were recorded by using LC solution software.

Chromatographic condition

In binary gradient HPLC system mobile phase Methanol 58 volumes, Acidic water 42 volumes (pH 3.8 adjusted by using triethylamine and o-phosphoric acid) filtered by using 0.22 µm nylon filter under vacuum condition and then used for further processes. This mobile phase combination was delivered with 1.2 ml/min. flow rate through C_{18} column (250× 4.6 mm I.D., particle size 5µm) for separation. The analyte was monitored at 271 nm detection wavelength, and 15^0C temperature with 25 µl injection volume.

Standard stock solution preparation

50 mg of tapentadol hydrochloride was dissolved in 50 ml water then make up to 100 ml with double distilled water then it was filtered through vacuum assemble consisting 0.45 µm nylon filter and then used.

Sample solution preparation

Twenty tablets were weighed and crushed to a fine powder. The powder equivalent to 50 mg of tapentadol HCl was taken in a 100-mL volumetric flask containing 50 ml distilled water and shake well for half an hour. Then make up to mark with double distilled water. The resultant mixture was filtered through 0.45 µm nylon filter. The desired concentration for the drug was obtained by accurate dilution, and the analysis was followed up as in the general analytical procedure.

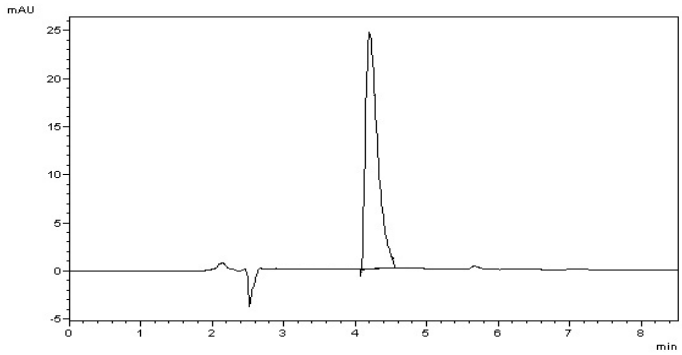

Figure 1: Chromatogram of tapentadol hydrochloride

RESULTS AND DISCUSSION

Optimized chromatographic condition and linearity

A UV scan of Tapentadol HCl showed a maximal absorbance at or near 271 nm. Initial method development was conducted on a C_{18} (250 mm × 4.6 mm I.D. particle size 5 µm) column was used for separation at 15^0C temperature. The chromatographic conditions were optimized with respect to specificity, resolution, and time of analysis. The results of the chromatogram were retention time = 4.132, USP tailing factor = 1.520, theoretical plate = 2562.212, calibration range = 10-50 µg/ml, and area = 1756748. Linearity was studied by preparing standard solution at different concentration levels. The linearity range was found to be 10-50µg/ml. The regression equation was found to be $y = 7460\ x$ with coefficient of correlation 0.999 where x is concentration and y is peak area. A mobile phase of a mixture (58 volumes) of Methanol and (42 volumes) of Acidic water pH 3.8 adjusted by using triethylamine and

ortho-phosphoric acid was found to provide a reproducible, baseline resolved peak [Figure 1]. These conditions allowed for separation of Tapentadol HCl from tablet formulation.

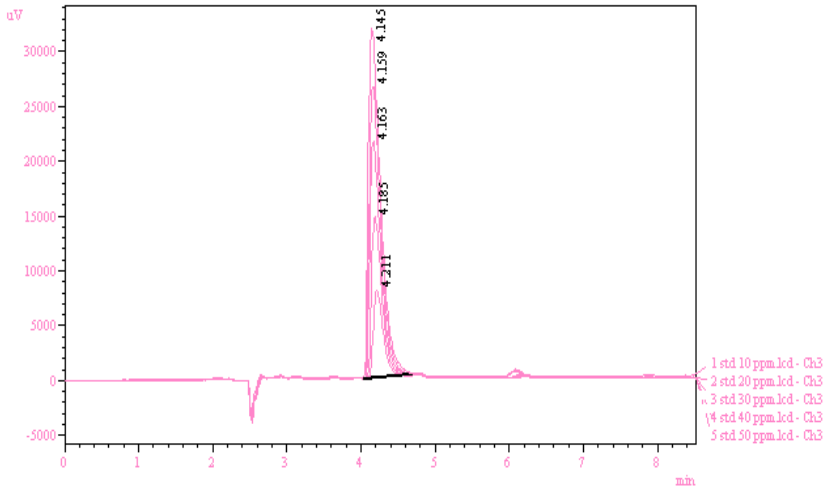

Figure 2: Linearity chromatogram for tapentadol hydrochloride

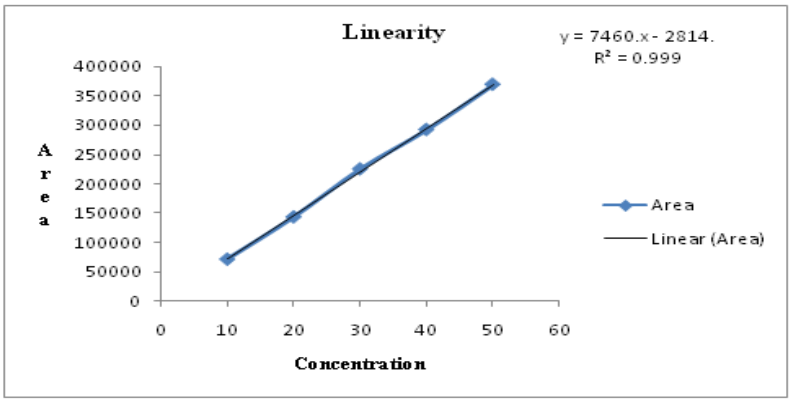

Figure 3: Calibration curve for linearity

Table 1: Observation table for Accuracy

Concentration level	Area	Total amount (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean % Recovery
80%	146270	20	10	20.154	101.544	100.771
	145265	20	10	20.085	100.846	
	143940	20	10	19.992	99.924	
100%	223957	30	20	29.828	99.139	99.95
	226370	30	20	30.042	100.208	
	227036	30	20	30.101	100.503	
120%	289590	40	30	39.636	98.786	99.982
	294531	40	30	40.141	100.471	
	295170	40	30	40.207	100.689	
Total Mean % Recovery						100.234

Method validation

As per ICH guideline (1996) and USP (2003) guideline for validation recommended parameters like accuracy, precision, robustness system suitability, limit of detection, limit of quantitaion are given as follows:

Accuracy:
Accuracy was performed by spiking the standard solution in the preanalyzed sample solution at 80%, 100% and 120% levels. Observation is shown in the [Table 1], which reveals that %Recovery is well within the given criteria of 98%-102%.

Precision
As per the guideline it is given that 6 replicate of same concentration should be taken for the observation. A 50 µg/ml concentration solution used, which was prepared from working standard solution for six replicate injections. This was analyzed for intraday precision and inter-day precision. Observation is shown in the [Table 2] which reveals that %RSD is well within the given criteria of 2%.

Limit of detection (LOD)
As per guideline detection limit can be calculated by using the formula:

LOD = 3.3×s/S

Where, 's' is the standard deviation of intercepts, and 'S' is the Slope of the calibration curve.

LOD of the method is given in the [Table 2].

Limit of Quantitation (LOQ)
As per guideline Quantitaion limit can be calculated by using the formula:

LOQ = 10×s/S

Where,'s' is the standard deviation of intercepts, and 'S' is the Slope of the calibration curve.

LOQ of the method is given in the [Table 2].

System suitability
For this take five observations of same concentration and check %RSD of theoretical plates, retention time and Area of five observations, it should not be more than 2% and observation reveals that it is less than 2%. Observation table is given in [Table 2].

Robustness
By changing the flow rate at ±0.2 ml/min, temperature at $\pm 1^0$C, and wavelength at ±2 nm, and retention time of tapentadol HCl was noted. The factors selected were flow rate, pH, and wavelength does not affect much to the retention time result. Results indicate that the selected factors remained unaffected by small variations of these parameters [Table 2].

Table 2: Observation table for summary of validation

Sr. no.	Parameter	Result
1.	Linearity range	10-50 µg/ml
2.	Regression line Equation	Y= 7460 x- 2814
3.	Correlation coefficient (R^2)	0.999
4.	Accuracy (Mean % Recovery)	99.950% -100.711 %
5.	Precision Repeatability (%RSD) Intraday Inter-day	 0.002% -0.006% 0.337%-1.098%
6.	System Suitability (%RSD)	0.293%-1.214%
7.	Limit of Detection	0.009329 µg/ml
8.	Limit of Quantitation	0.028711 µg/ml
9.	Robustness (%RSD)	0.011% - 0.351%

Applicability of method

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation TAPAL, ZYNTAP, TYDOL observation is as per [Table 3]. Observation reveals that method is applicable for estimation of drug in tablet dosage form.

Table 3: observation table for applicability of method

Brand Name	API Present mg/tablet	Assay (%Label claim)
TAPAL	50	99.98 *
ZYNTAP	50	100.2 *
TYDOL	50	98.26*

Note: Above data is Average of five determinations.

CONCLUSION

Developed RP-HPLC method is accurate, sensitive, precise, and linear within the range of 10-50µg/ml. It can now transfer to use it for routine laboratory analysis of tapentadol HCl in bulk and pharmaceutical dosage form.

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