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A novel RP-HPLC method for quantification of tapentadol HCl in pharmaceutical formulations

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ABSTRACT

A rapid and highly sensitive reversed phase high performance liquid chromatographic method was developed for quantitative estimation of tapentadol hydrochloride in pharmaceutical preparations. The method was validated according to ICH, FDA and USP guidelines with respect to accuracy, precision, specificity, linearity, robustness, sensitivity and system suitability. The method was developed by using an isocratic condition of mobile phase comprising with 60% buffer [potassium di-hydrogen phosphate (0.03 molar) and n-heptane sulphonic acid- Na salt (0.002 molar)] and 40% acetonitrile at a flow rate of 1.0 mL/min over C-18 (ODS, 250 x 4.6 mm) column at ambient temperature. The retention time was found to be at 3.7± 0.1 min. The recovery was found as >90% which demonstrated the accuracy of the protocol. Intraday and inter-day precision studies of the new method were less than the maximum allowable limit (RSD%≤ 2.0 according to FDA). The method showed linear response with correlation coefficient (r^2) value of 0.999. Therefore, it was found to be accurate, reproducible, sensitive and less time consuming and can be successfully applied for the assay of tapentadol hydrochloride in any pharmaceutical formulations.

Keywords: HPLC, tapentadol hydrochloride, method development, isocratic elution, validation.

INTRODUCTION

Tapentadol hydrochloride chemically is 3-[(1R,2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol hydrochloride [1]. It is an analgesic and acts as an agonist of the μ-opioid receptor and as a norepinephrine reuptake inhibitor [2]. Tapentadol shows more tolerable side effects with respect to other opoids.

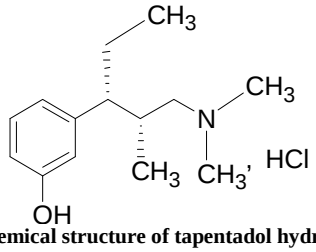


Fig.1: Chemical structure of tapentadol hydrochloride

Although tapentadol has two chiral centers and consequently four enantiomers namely S, S; R, R; S, R and R, S, only the R, R stereoisomer is commercially available as drug [3, 4].

Like other drugs tapentadol also requires some absolute parameters like quality, potency etc. to serve its best activities. It is obvious that change in the formulation or variations in the manufacturing process or use of low quality materials can affect the stability and efficacy of the product. Therefore, quality and quantity assessment ensure their safety and efficacy which can be ensured by analyzing the products during and after manufacturing and at various intervals during the shelf life of the product. Effective process validation contributes significantly to assuring drug quality. The basic principle of quality assurance is that a drug should be produced that is fit for its intended use and does not expose the consumers to risks [5].

Although several methods have been reported previously for determination of tapentadol in the pharmaceutical formulations, herein we developed a completely new RP-HPLC method with better resolution, minimum peak tailing, less time consuming and improved accuracy, precision and specificity.

MATERIALS AND METHODS

Working standard of tapentadol HCl (potency 99.21%) was a kind gift of Drug International Ltd., Dhaka, Bangladesh. HPLC grade acetonitrile was obtained from Active Fine Chemicals Ltd., Bangladesh.

Apparatus

HPLC system

High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model- SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded using LC-solutions software.

Column

Analytical reversed phase C-18 column [Luna C-18(2), 5µ, 250 x 4.6 mm, Phenomenex, Inc.] was used to analyze the standard and samples.

Preparation of mobile phase

2.72 g of potassium di-hydrogen phosphate and 0.5 g of n-heptane sulphonic acid- Na salt were dissolved in 900 mL of nano pure water. pH was adjusted to 4.2± 0.1 with dilute potassium hydroxide or dilute Ortho-phosphoric acid. Then volume up to 1000 mL with water of same quality. Then this buffer and HPLC grade acetonitrile were mixed together at a ratio of 60:40 v/v, filtered through a 0.22µm millipore filter and degassed.

Preparation of standard solutions

Stock solution of the standard drug was prepared by dissolving 25.2 mg of tapentadol HCl powder (equivalent to 25 mg tapentadol HCl) in a 50 mL volumetric flask with distilled water to get a concentration of 0.5 mg/mL. Then 5 mL of this solution was taken in another 50 mL volumetric flask and adjusted the volume to get nominal concentration of 0.05 mg/mL. Appropriate volume from stock solution was further diluted to get 80%~120% of nominal concentration (40, 45, 50, 55, 60 µg/mL).

Chromatographic conditions

All analyses were done at ambient temperature under isocratic condition. The mobile phase was run at a flow rate of 1.0 mL/min for 7 minutes. The injection volume was 10 µL for standard and samples. Before analysis, every standard and sample were filtered through 0.2 µm filter tips. The column eluate was monitored with UV detection at 215nm.

Method validation [6-10]

Specificity

The specificity of the LC method was evaluated to ensure that there was no interference from the degradation products, excipients present or other impurities in the pharmaceutical formulation. The specificity was studied by injecting the unstressed and stressed standard solutions, excipients and pharmaceutical preparations of tapentadol HCl.

Specificity-stability indication

Solution stability

Stability of tapentadol HCl in solution was checked by rendering the test solutions in tightly capped vials at room temperature and in refrigerator at 5^{o}C for 48 hr. The solutions were analyzed by HPLC at 0 hr, 24 hr and 48 hr.

Forced degradation
In this stage, forced degradation studies were undertaken to degrade the sample (e.g., drug product or API) deliberately. These studies are used to evaluate an analytical method's ability to measure an active ingredient and its degradation products without interference. Samples or drug product (spiked placebo) and drug substance were exposed to heat (105°C for 3 hr), acid (1N HCl up to 24 hr), base (1N NaOH up to 24 hr), oxidizing agent (10% H_2O_2 solution up to 24 hr), reducing agent (10% Na bisulphite solution up to 24 hr) and water (up to 24 hr) to produce 10%-30% degradation of the active. The degraded samples are then analyzed using the method to determine if there are interferences with the active or related compound(s).

Linearity
Five different concentration levels (40 µg/mL, 45 µg/mL, 50 µg/mL, 55µg/mL and 60 µg/mL) were prepared from stock solution. Then 10 µL from each solution was injected into HPLC using auto-injector and the analyses were monitored at 215 nm and repeated three times. The average peak areas were plotted against concentrations. The linearity of the proposed method was evaluated by using calibration curve to calculate coefficient of correlation, slope and intercept values.

Accuracy
The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is expressed by calculating the percent recovery (%R) of analyte recovered. In this case, to evaluate the accuracy of the proposed method, successive analysis (n = 3) for three different concentrations (40 µg/mL, 50 µg/mL and 60 µg/mL) of standard tapentadol HCl solution were carried out using the proposed method. The data of the experiment were statistically analyzed using the formula [% Recovery = (Recovered conc. /Injected conc.) x 100] to study the recovery and validity of the proposed method.

Precision
Precision of the assay was assessed with respect to both repeatability and reproducibility. The precision of an analytical method is the degree of agreement among individuals test results where the method is applied repeatedly to multiple samplings. The precision of the proposed method was checked by intra- and inter-day repeatability of responses after replicate injections and expressed as RSD% amongst responses using the formula [RSD% = (Standard deviation/Mean) x 100 %]. In the current method development and validation protocol, the precision was determined by five replicate analyses at the concentration of 40 µg/mL of standard tapentadol HCl solutions using the proposed method.

Robustness
Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in the parameters of the method. Robustness provides some indication of reliability of the analytical method during normal usage [9].The effect of the following changes in chromatographic conditions will be determined as flow rate ± 50%, solvent ratio ± 30%, pH of buffer solution ± 0.2, temperature ± 10 ^oC and detector wavelength ± 3. If these changes are within the limits that produce acceptable chromatography, they will be incorporated in the method procedure.

System suitability
System suitability tests will be performed on both HPLC systems to determine the accuracy and precision of the system by injecting six injections of a solution containing analyte at 100% of test concentration. The following parameters will be determined: theoretical plate count, tailing factors and reproducibility (percent RSD of retention time, peak area, and tailing factor for six injections).

Limit of detection (LOD)
It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection limit is usually experimental conditions and usually expressed as the concentration of analyte [e.g. µg/mL, %, ppm or ppb] in the sample. The ICH references that signal-to-noise ratio should be 3:1.

Limit of quantitation (LOQ)
It is the lowest amount of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. The limit of quantitation for chromatographic methods has been described as the concentration that gives a signal- to-noise ratio (a peak with height at least ten times as high as the baseline noise level) of 10:1. In many cases limit of quantitation is approximately two or three times of the limit of detection.

RESULTS AND DISCUSSION

Method development and validation

To develop a HPLC method, firstly, solubility profile and pKa of tapentadol HCl was investigated. Tapentadol HCl was found to be water soluble which indicated that buffer would be comprised major portion of mobile phase. Again pKa of tapentadol HCl was found 9.2 which suggested a medium having a pH less than 7. Upon this assumption initially a phosphate buffer (pH 6) was tried with methanol and acetonitrile in different ratio on a C-18 (250 x 4.6 mm, 5µ, Phenomenex) column. Satisfactory peak was not found. C-18 and C-8 columns of other brand (Prontosil, Zorbax Eclipsed etc.) were also tried but resolution of peak and tailing factor were found to be unacceptable. Then pH of buffer solution and the ratio of acetonitrile were gradually changed. Finally at pH of 4.2 of the buffer and at 60:40 of the ratio of buffer and acetonitrile, better resolution of the peak was observed. To reduce the tailing of the peak, a small amount of n-heptane sulphonic acid sodium salt (0.002 M) was added in the buffer and well-resolved and absolutely symmetry peak was found. No peak was detected close to the retention time of tapentadol HCl. The developed method was described in Table 1.

Table 1. HPLC method of tapentadol HCl

Method	Buffer (0.02M KH_2PO_4 + 0.002M n-heptane sulphonic acid Na salt) and acetonitrile in a ratio of 60:40 under isocratic condition
Column	C18, 5µm , 250 x 4.6 mm, Phenomenex
Diluting solution	Distilled water
Temperature	Ambient
Flow rate	1.0 mL/min.
Injection volume	10 µL
Monitoring wave length	215 nm
Retention time	3.7 ±0.1 min.
Tailing factor (USP)	1.02
Peak purity	1.0000

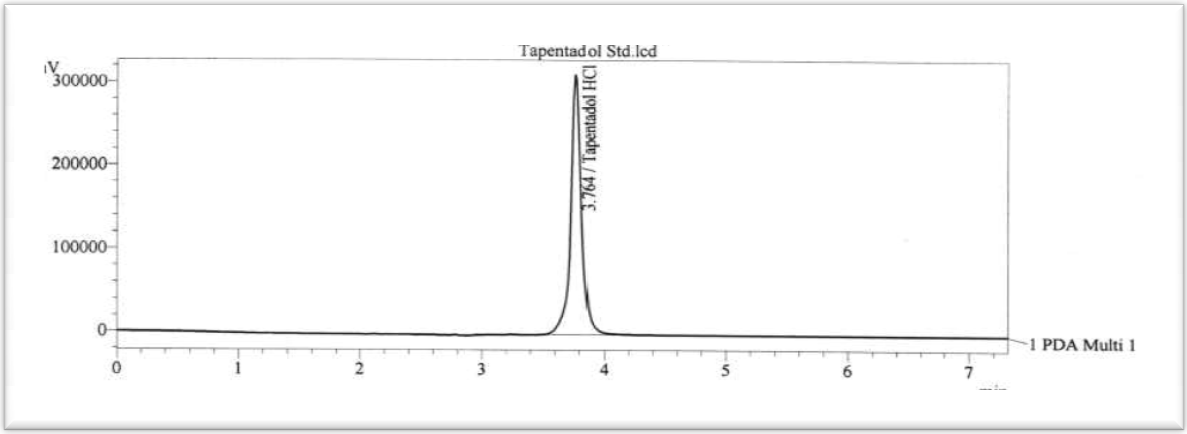


Fig. 2. HPLC chromatogram of tapentadol HCl standard

Specificity

The specificity was studied by injecting the unstressed and stressed standard solution, excipients and pharmaceutical preparation of tapentadol HCl several times on several days. No interference of placebo was observed.

Specificity-stability indication

Solution stability

Two vials were prepared by dissolving the drug in distilled water. One vial was kept in room temperature and another in refrigerator at 5°C. Area change was investigated and it was deduced from the result that tapentadol HCl was fairly stable in diluting solvent. % RSD of area changes were found <1.0 in both cases.

Forced Degradation

Solid state degradation (elevated temperature, at 105°C for 3 hr)

To establish specificity, practical tests were made under stressed and unstressed condition employing - placebo (all the ingredients except active tapentadol HCl), sample (tapentadol 75 mg tablet) and active tapentadol HCl. It was revealed that there was no interference of peak in the tapentadol region for the stressed sample, placebo and active. Hence the method was considered specific for the product.

Liquid state degradation

In this study it was found that tapentadol HCl was degraded significantly in acidic environment. Similar degradation was observed in oxidation with 10% H_2O_2 solution and in reduction with 10% Na-bisulphite solution. There was no significant degradation in basic condition and the drug was found most stable in water (Table 2).

Table 2. Summary of liquid state degradation

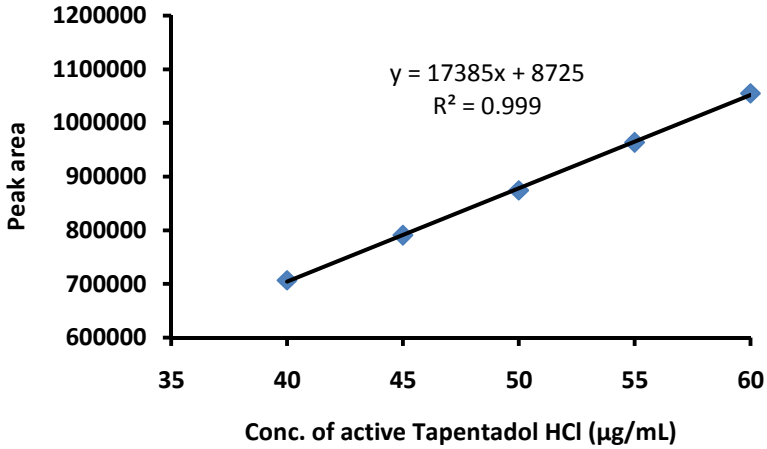
Concentration of fresh sample	Degradation Reaction	Recovered concentration after forced degradation	% Loss	Remarks
50 µg/mL	Acid hyrolysis	44.85 µg/mL	10.3	Most sensitive
50 µg/mL	Base hydrolysis	47.59 µg/mL	4.81	Less sensitive
50 µg/mL	Oxidation	46.26 µg/mL	7.48	More sensitive
50 µg/mL	Reduction	45.64 µg/mL	8.72	More sensitive
50 µg/mL	Water hydrolysis	49.64 µg/mL	0.72	Least sensitive

Linearity

Linearity of tapentadol HCl was examined on test solution 80 ~ 120% of nominal concentration. The range covered was 40 to 60 µg/mL to analyze by the proposed method. The correlation coefficient was found as 0.999 indicating good linear of calibration curve.

Table 3. Result of linearity

Concentration of tapentadol (µg/mL)	% of Nominal concentration	Response (Peak area)			Average Peak area
40	80	706058	710501	703654	706738
45	90	789577	793292	788720	790530
50	100	874889	872031	875665	874195
55	110	965499	970199	954715	963471
60	120	1053468	1054560	1056632	1054887



Correlation Coefficient: R^2 = 0.999 (Lt. ≥ 0.999)
Y intercept: 0.998% (Lt.: Area response of Y intercept<0 to 5% of nominal concentration)

Fig.3. Linearity of range of proposed method

Accuracy

Percent recovery was found between the acceptance limit (98.5% ~101.5%) demonstrated the accuracy of the method. A linear graph was obtained for amount of Tapentadol HCL added vs. recovered (Table 4).

Table 4. Accuracy of the method for Tapentadol HCl

Sample added (µg/ml)	Peak area	Average Peak area	Sample Recovered (µg/mL)	% Recovery
40	706058	706738	40	100.0
	710501			
	703654			
50	874889	874195	49.7	99.40
	872031			
	875665			
60	1053468	1054887	60	100.0
	1054560			
	1056632			

Precision (Repeatability and raggedness)

The repeatability and raggedness were measured in terms of %RSD of recovered concentration. The %RSD values depicted in Table 5 showed that the proposed method provided acceptable intra-day and inter-day variation for tapentadol HCl.

Table 5. Determination of precision

Intra-day precision								
Day	**Injected conc. (µg/mL)**	**Area**	**Slope (m)**	**Intercept (c)**	**Recovered (µg/mL)**	**Mean (µg/mL)**	**SD**	**%RSD (Intraday)**
Day-1	40	706058	17385	8725	40.11	40.10	0.15	0.37
		710501			40.35			
		703654			39.97			
		705044			40.05			
		704894			40.04			
Day-2	40	701210	17385	8725	39.83	40.09	0.33	0.83
		713510			40.54			
		711050			40.34			
		700974			39.82			
		702607			39.91			
Inter-day precision								
	Injected conc. (µg/mL)	**Inter-day mean recovered (µg/mL)**			**Mean**		**SD**	**%RSD (Inter-day)**
Day-1	40	40.104			40.10		0.01	0.02
Day-2	40	40.088						

System suitability

%RSD of system suitability parameters including peak area and retention time of tapentadol HCl were calculated as 1.046 and 0.213, respectively. Average theoretical plate and tailing factor were found as 2427 and 1.02, respectively. All parameters were found well within acceptance limit set by FDA or ICH (% RSD of peak area ≤ 2.0%; % RSD of retention time ≤ 2.0%; theoretical plates N≥1000 plates; tailing factor T≤2).

Robustness

Predetermined variations were performed under the experimental conditions to assess its robustness. Results are shown in Table 6.

Table 6. Summarization of robustness

Parameter	**Theoretical variance**	**Actual variance**	**Retention time (min)**	**Tailing factor**	**Peak purity**	**Peak area**
Flow rate	±50%	0.5 mL/min	6.707	1.391	1.00	2076149
		1.5 mL/min	1.948	0.901	1.00	695529
Solvent ratio	±30%	Buffer: ACN=70:30	6.544	1.457	1.00	1027743
		Buffer: ACN = 50:50	2.849	1.326	0.99998	1056822
pH of Buffer Solution	±0.2	pH=4.4	4.888	1.342	0.9999	1076449
		PH=4.0	4.236	1.323	0.99999	1075073
Detector Wave length	±3nm	218nm	4.386	1.325	1.00	981511
		212nm	4.353	1.311	1.00	1056165
Temperature	±10°C	20°C	4.382	1.324	1.00	1052187
		40°C	4.357	1.308	1.00	1055826

LOD and LOQ

The LOD and LOQ results were obtained as 0.0107 µg/mL and 0.0321 µg/mL, respectively.

CONCLUSION

As a part of new analytical method development, a rapid and sensitive reversed phase high performance liquid chromatographic method was developed and validated according to the guidelines of FDA, ICH and USP. The sample recoveries were in good agreement with their respective label claims suggested non-interference in the estimation. The newly developed method was found to be simple, accurate, reproducible, efficient and less time consuming. Hence, the method can be easily and conveniently adopted for routine analysis of tapentadol HCl in bulk and pharmaceutical formulations.

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