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RP-HPLC METHOD QUANTITATIVE ESTIMATION OF TAPENTADOL HYDROCHLORIDE AND ITS TABLETS

Mamta Bishnoi [1], Yash Paul Singla [2], Ankit Jain [3, * 4] and Birendra Shrivastava [1]

Department of Pharmaceutics [1], School of Pharmaceutical Sciences, Jaipur National University, Jaipur - 302017, Rajasthan, India.
Department of Pharmaceutical Sciences [2], Guru Jambheshwar University of Science and Technology, Hisar - 125001, Haryana, India.
Pharmaceutics Research Projects Laboratory [3], Department of Pharmaceutical Sciences, Dr. Harisingh Gour Central University, Sagar - 470003, Madhya Pradesh, India.
Department of Materials Engineering [4], Indian Institute of Science, Bangalore - 560012, Karnataka, India.

Keywords:

Tapentadol, Pharmaceutical dosage forms, Analytical validation, ICH Guidelines, HPLC

Correspondence to Author:
Dr. Ankit Jain

DBT-Postdoctoral Research Associate, Department of Materials Engineering, Indian Institute of Science, Bangalore - 560012, Karnataka, India.

E-mail: ankitjainsagar@gmail.com

ABSTRACT: The objective was to develop and validate an easy, economical, fast, reliable, reproducible, precise and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of tapentadol hydrochloride (TAP, a mu opioid-receptor agonist, and noradrenaline reuptake inhibitor) in the bulk and pharmaceutical dosage form. The chromatographic separation was achieved by using the HPLC system equipped with a C-18(2) column (250 × 4.6 mm, Particle size 5µm) at wavelength 217 nm. The mobile phase consisting of Potassium phosphate buffer: Acetonitrile (50:50 v/v) was used in isocratic mode. The flow rate was fixed at 1.0 mL/min with a continuous run up to 5 min, while the retention time was located near about 2.5 min. In the concentration range of 60-160 µgmL^{-1}, the detector response was found linear with linear regressed equation Y=22791X+120122. In the assay of TAP, 99.65% of the drug was recovered. This method proved a satisfactory validation for all the parameters such as accuracy, linearity, specificity, precision, range, ruggedness, robustness, reproducibility, and peak purity assessment as per ICH guidelines. The results of the study evidenced that it is useful for the routine determination of tapentadol in the bulk and pharmaceutical dosage forms like a tablet.

INTRODUCTION: Tapentadol, 3-[(1R,2R)-3-(dimethylamino)-1-ethyl-2-methyl]-propylphenol hydrochloride (TAP), the first US FDA-approved centrally acting analgesic for the treatment of moderate to severe acute pain [1, 2].

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It is a schedule-II controlled substance, which has a dual mechanism of action, µ-opioid receptor agonist and noradrenaline (norepinephrine) reuptake inhibitor with little serotonin reuptake inhibition [3]. Traditional opioids react through a single mechanism (µ opioid receptor binding), while TAP has a non-opioid mechanism also. Relative to a classical µ-opioid at equal analgesia doses, TAP has ≤ a 40%µ load, which contributes to its lower adverse effect profile, *i.e.*, greater gastrointestinal tolerability [4]. Tapentadol HCl has molecular formula $C_{14}H_{23}NO.HCl$ with molecular weight 257.80 g.

The partition coefficient log P value n-octanol: water is 2.87 [5]. TAP has low bioavailability (32%) due to first-pass metabolism and less plasma protein-bound (only 2%) with a half-life of 4.9 h [6]. After oral administration, > 95% of the dose is excreted in the urine as glucuronide and sulfate conjugated metabolites within 24 h [7]. So far, various methods are reported for the estimation of TAP in bulk and in pharmaceutical with different mobile phases, retention time, linearity range conc., and absorption maxima. In the present study, a trial has been made to develop a rapid, convenient and simple reverse phase HPLC method for the determination of TAP in the pharmaceutical dosage form *i.e.*, Lucynta tablet 50 mg (Lupin Laboratories Ltd.).

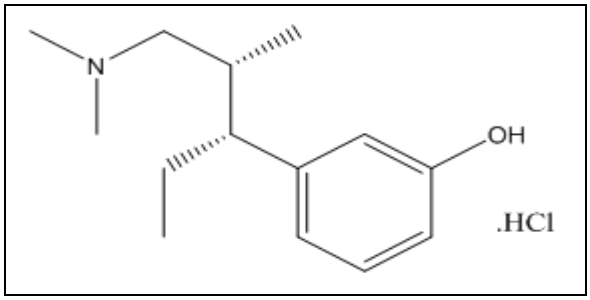

FIG. 1: CHEMICAL STRUCTURE OF TAPENTADOL HYDROCHLORIDE

EXPERIMENTAL SECTION:

Chemicals and Reagents: Tapentadol hydrochloride (TAP) was procured as a gift sample from Innova Captab, Baddi (H.P.). Lucynta tablet (Lupin Laboratories Ltd.) was obtained from the market. Cellulose nitrate membrane filters (0.45 mm and 0.22 mm), acetonitrile, and Dipotassium Phosphate buffer of HPLC grade were brought from Sigma Aldrich, New Delhi, India. All analytical grade chemicals were used unless otherwise stated. All solutions were prepared in purified HPLC grade water produced by the Milli-Q Millipore Water System (Milford, MA, USA).

Preparation of Standard Stock Solutions: A stock solution of TAP (50mg/50mL) was prepared in the volumetric flask by adding a mobile phase with the help of bath sonication (up to 15 min), and the final volume was adjusted. Appropriate aliquots (60, 80, 100, 120, 140, and 160 µg/mL) of drug solutions were prepared from 100 µg/mL solution with the mobile phase. For validation of various parameters, other dilution samples (*i.e.*, 1, 10, and 50 µg/mL) were also prepared in appropriate concentrations and stored at -20 °C [8]. All the resultant solutions before use were filtered through Whatman filter paper number 41 [9].

Preparation of Sample Solution: A working solution of the tablet was prepared with twenty tablets of TAP. All the tablets were crushed to a fine powder in the mortar and pestle. On the basis of the average weight of the tablets, fine powder equivalent to one tablet was accurately weighed and transferred to 25 mL volumetric flask. After the addition of 10 mL of the mobile phase, the volumetric flask was sonicated for 20 min in the bath sonicator to obtain a clear solution. The final volume was adjusted with the mobile phase, and the whole solution was filtered through a 0.45 µm nylon filter. Suitable aliquots of TAP solutions were prepared in the range of linearity, as previously described in the standard solution.

Instrumentation and Chromatographic Conditions: The chromatographic separation was carried out by using the HPLC system included a Shimadzu model LC-10 ADVP(Shimadzu Co., Kyoto, Japan), having a Phenomenex Luna C-18(2) column (250 × 4.6 mm, dp = 5 mm), SPD-M20A Prominence Diode array detector, (holding the deuterium lamp with a sensitivity of 0.005 AUFs), a Shimadzu model C-R5A chromatograph integrator module (chart speed at 10 mm/min), a Shimadzu model SIL-6A auto-injector, and a Shimadzu module SCL-6A system. The mobile phase used was a mixture of 0.1 M potassium dihydrogen phosphate buffer (pH adjusted to 7 with triethanolamine) and acetonitrile in the ratio of 50:50% v/v, degassed and filtered through the 0.45µm cellulose acetate membrane filter. Injection volume was fixed 20 µL with a flow rate of 1 mL/min under the isocratic mode of the mobile phase. The column was maintained at 25 °C temperature, and Photodiode array detector wavelength was fixed at 217 nm. Lab Solutions software was used for the acquisition and processing of chromatographic data.

Method Validation: Various preliminary trials were conducted by changing various chromatographic parameters to develop a new method for TAP analysis in bulk and finished pharmaceutical product *i.e.*, tablet. The developed method was validated as per ICH guidelines for

linearity, accuracy, precision, robustness, the limit of detection (LOD), the limit of quantification (LOQ), and stability of the stock solution, using the serial dilution method [2].

Peak Purity Assessment: To judge peak purity, the evaluation was carried out after the development and validation of the appropriate analytical conditions in the TAP analysis. Based on the degree of similarity of UV spectra (Peak range 190-800 nm), peak purity was assessed by class VP software (Shimadzu HPLC system). If the peak purity index was greater than the single point (*i.e.*, a positive value of the minimum peak purity index), then it was classified as pure. To evidence accuracy, six replicate analyses of a TAP at a concentration of 60 µg/mL were performed. The acceptance limit was set at ±2% for the percent coefficient of variation (% CV) to the peak area, plate number, tailing factor, the peak asymmetry of samples, and the retention time of TAP, as shown in **Table 1**.

Linearity: Linearity was evaluated by calculating the correlation coefficient [10]. A calibration curve of TAP with six different concentrations from 60 to 160 µg/mL range was plotted with peak area versus concentrations, while the samples were injected into the HPLC column in triplicate. Linear regression analysis was carried out for slope, intercept, and linear correlation coefficient (r^2) calculation [11]. The data was given in **Table 1**.

TABLE I: LINEARITY DATA OF TAPENTADOL HCl

Sample name	Sample ID	Ret. Time (min.)	Area	Theoretical Plate	Tailing Factor
TAP	60 PPM	2.500	1576187	1924.156	1.643
	60 PPM	2.507	1572020	1932.659	1.604
	60 PPM	2.508	1572629	1861.918	1.600
(Average)		2.505	1573612	1906.244	1.615
	80 PPM	2.519	2036280	1954.655	1.635
	80 PPM	2.527	2040222	1887.808	1.616
	80 PPM	2.533	2043011	1936.561	1.661
(Average)		2.526	2039838	1926.341	1.637
	100 PPM	2.538	2447755	1945.700	1.641
	100 PPM	2.539	2450911	1943.186	1.633
	100 PPM	2.539	2448901	1947.336	1.637
(Average)		2.539	2448925	1945.407	1.637
	120 PPM	2.544	2879409	1859.908	1.645
	120 PPM	2.549	2879775	1913.709	1.681
	120 PPM	2.557	2879996	1935.066	1.662
(Average)		2.549	2879727	1902.894	1.663
	140 PPM	2.561	3262297	1840.661	1.671
	140 PPM	2.565	3263778	1896.287	1.696
	140 PPM	2.579	3262569	1867.982	1.687
(Average)		2.568	3262881	1867.310	1.685
	160 PPM	2.575	3678424	1898.627	1.685
	160 PPM	2.574	3679069	1897.496	1.690
	160 PPM	2.576	3679967	1824.393	1.684
(Average)		2.575	3679153	1873.505	1.687

Accuracy and Precision: The accuracy and precision of this HPLC method were determined by analyzing three different concentrations within the calibration range in triplicate (n=3) [12].

Accuracy: The accuracy of this method was determined by calculating recoveries of TAP from the standard solutions of known concentrations at 3 different levels, *i.e.*, 70, 90, and 110 µg/mL. The average recovery of TAP was calculated from each concentration. The detailed results were given in **Table 2**.

Precision: The precision (% RSD) of the analytical procedure was evaluated by determining the intra- and inter-day coefficients of variation. Standards solutions were prepared, which were different from those used in the preparation of calibration curves.

These samples were evaluated 6 times by assaying, *i.e.*, 3 times in a day and 3 more times on the next consecutive days. The results were given in **Table 2**.

TABLE 2: ACCURACY AND PRECISION STUDIES FOR TAP

Parameter	Inter-day (Drug Conc. µg/mL)			Intra-day (Drug Conc. µg/mL)		
	110	90	70	110	90	70
Precision (% RSD)	1.29	1.22	1.98	1.06	0.98	0.99
Accuracy (% recovery)	99.81 ± 0.35	98.99 ± 0.82	100.23 ± 1.06	99.68 ± 1.71	99.79 ± 186	100.46 ± 1.00

Robustness: Robustness can be evaluated by varying the various developed TAP analysis parameters *e.g.,* the composition of the mobile phase, mobile phase flow rate, detector wavelength, changing the retention time, relative retention time (RRT), resolution and number of plates, and temperature. The flow rate of the mobile phase was changed to ± 20%, and the temperature was changed to ± 5 °C. The results are disclosed in **Table 3**.

TABLE 3: ROBUSTNESS TESTING FOR THE METHOD

Parameter	Modification	TAP (% Recovery)
Mobile phase composition ACN: 0.1 M potassium dihydrogen phosphate buffer (pH adjusted to 7 with Triethanolamine)	40:60	99.6 ± 0.34
	50:50	98.9 ± 0.27
	60:40	99.7 ± 0.32
Flow Rate (mL/min.)	1.08	96.6 ± 0.43
	1.20	99.7 ± 0.29
	1.32	99.7 ± 0.26
Injection Volume (mL)	18	98.8 ± 0.31
	20	99.7 ± 0.18
	22	98.7 ± 0.19
Column Temp. (°C)	23	98.9 ± 0.27
	25	98.5 ± 0.29
	27	99.3 ± 0.10
Detector Wavelength (nm)	215	98.6 ± 0.43
	217	99.1 ± 0.23
	220	98.7 ± 0.45

Quantification Limits (LOD and LOQ): LOD (Lowest detectable concentration of the analyte) and LOQ (Lowest amount of the analyte) were used to assess the sensitivity of the method by the standard deviation of the response and slope. LOD and LOQ were quantitatively determined by the formula 3.3×(σ/s) and 10×(σ/s) respectively where σ is the standard deviation of intercept and s is the mean of the slope. Slope S was calculated from the calibration curve of the analyte, and the standard deviation was estimated by running six blank samples.

LOQ limits were expressed as the concentration of the analyte (parts per million). After LOQ determination, LOD was taken as the one-third of LOQ for their simultaneous analysis.

Determination of the TAP in Marketed Tablet: Three working solutions of TAP was prepared from the formulation (Tablet) and analyzed with the similar HPLC parameter settings and which revealed the drug content within the specified limits. The results were shown in **Table 4**.

TABLE 4: DETERMINATION OF DRUG TAP IN TABLET

Actual concentration (µg/mL)	Calculated concentration (µg/mL)	% Recovery
80	79.85 ± 2.620 (1.27)	99.9421 ± 0.22 (0.65)
100	100.56 ± 2.398 (0.98)	100.0601 ± 0.16 (0.17)
120	119.69 ± 0.664 (0.19)	99.9236 ± 0.12 (0.26)

Values represent mean ± SD (n = 6)

Stability of Stock Solutions: A stock solution of TAP was evaluated for the stability and extent of degradation, if any, on storage. From the standard stock solution (1mg/mL), three working solutions with different concentrations (80, 100, and 120 µg/mL) were prepared and filtered through a 0.22µm filter and kept at room temperature.

HPLC run was fixed for each sample in triplicate at regular time intervals (*i.e.,* after 6 h, 3 days and on 7th day) for a period of 7 days. During the period of 7 days, the stability was judged by comparing the peak areas of the drug at different time points. **Table 5** represents stability data of the stock solution containing the drug.

TABLE 5: STABILITY OF THE DRUG'S STOCK SOLUTION

Drug Concentration (µg/mL)	% Recovery		
	6 h	Day 3	Day 7
120	99.9867 ± 0.05 (0.57)	99.379 ± 0.23 (0.32)	99.7654 ± 0.21 (0.18)
100	100.018 ± 0.02 (0.12)	99.126 ± 0.42 (0.11)	100.1298 ± 0.11 (0.14)
80	99.7672 ± 0.14 (0.46)	100.0238 ± 0.21 (0.18)	99.8667 ± 0.30 (0.22)

Values represent mean ± SD (%RSD, n = 6)

Short Term Stock Stability: A Stock solution of TAP was kept at room temperature for 6 h and chromatograms obtained by running three concentrations on the same day after 6 h.

Long Term Stock Stability: The chromatogram was developed on the 3rd and the 7th days from the preparation of the stock solution and compared with those obtained initially. Values were denoted as the peak area ± SD (%RSD) and compared with which obtained initially.

RESULTS:

Chromatographic Separation: The isocratic mode was employed for the elution of the drug, and TAP showed a retention time of 2.508 min. The run was further continued up to 5 min for the removal of traces of drugs and to re-equilibrate the system to the initial conditions. **Fig. 2** illustrates the complete chromatogram produced over 5 min, which was showing a peak at the retention times. The linearly regressed standard curve of the TAP was obtained in the range 60-160 µg/mL, shown in **Fig. 3**.

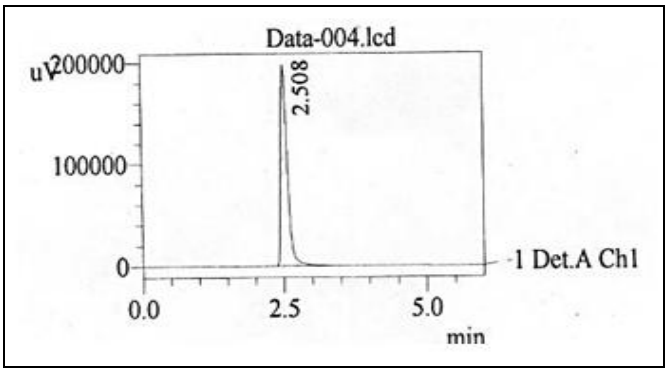

FIG. 2: CHROMATOGRAMS OF TAP (RT = 2.508 min)

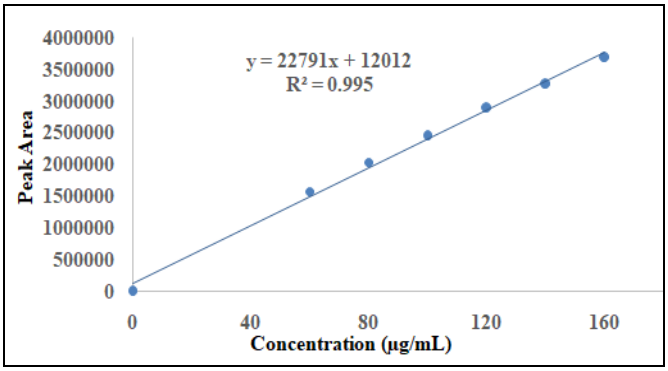

FIG. 3: LINEARLY REGRESSED CALIBRATION CURVE OF TAP IN DISTILLED WATER (λ_{max} = 217 nm)

Validation of the Method: Validation parameters have been highlighted in **Table 6** of TAP analysis. The method was validated with respect to parameters including linearity, precision and accuracy, the limit of quantitation (LOQ), and limit of detection (LOD), and stability of the solution. A standard curve was found linear. Linear regression analysis confirmed that the r^2 value was found to be 0.9951, confirming the linear relationship between the concentration of the drug and the area under the curve. Accuracy and precision of recovery data ranged from 99 to 101%.

The results of robustness showed no significant statistical differences between various altered parameters with respect to those which were received initially, and finally, the method was found to be robust. Both intra- and inter-day precision (% RSD) of working standards were less than 2% over the selected range of the drug. The calculated LOD and LOQ concentrations proved the sensitivity of the method.

The stability of the drug solution was assured by comparing the percentage recovery of concentration of TAP from the sample after 7 days with the same day percentage recovery analysis, which had shown the negligible difference. Hence, the method validated and proved for quantitative analysis of TAP in pharmaceutical formulations.

TABLE 6: VALIDATION PARAMETERS OF THE HPLC METHOD FOR TAP

Parameters	Value
Analytical wavelength (nm)	217
Linearity (µg/mL)	60-160
Slope	Y=22791X+120122
% RSD of slope (%)	1.37
Intercept	120122
Correlation coefficient (r^2)	0.9951
LOD (µg/mL)	3.33
LOQ (µg/mL)	10

Values represent mean ± SD (% RSD, n = 6)

DISCUSSION: Many mobile phase solvents/ combinations have been reported in various ratios, in literature for the separation of TAP. Here, TAP was separated using the mobile phase consisting of ACN: Potassium phosphate buffer containing triethanolamine for pH adjustments in the ratio 50:50. The rapidity of the method was proved as under the isocratic mode, the drug's retention time was observed at 2.5 min. There was no interference of the impurities as well as additives of the tablet because method selectivity was proved by

comparing the obtained chromatogram with the standard tapentadol drug chromatogram.

Linearity was confirmed over the TAP concentration range of 60-160 µg/mL with a correlation coefficient of 0.9951, as shown in **Fig. 3**. The accuracy and precision were studied in the drug concentration range of 70, 90, and 110 µg/mL, and TAP recovery was found in the range of 99.68-100.46%, as presented in **Table 2**.

For the robustness various chromatography parameters were varied *viz*. mobile phase ratio (40:60, 50:50, 60:40), flow rate (1.08, 1.20, 1.32 mL/min.), injection volume (18, 20, 22 µL), column temp (23 °C, 25 °C, and 27 °C) and the detector wavelength (215, 217, and 220 nm).

All the results were summarized in table III proved the method robust. LOD and LOQ were calculated at 3.33 and 10 µg/mL. Stability was calculated by taking percent ratio of the determined 80, 100, and 120 µg/mL concentrations after 6 h, 3 days, and 7days period which was found satisfactory as shown in **Table 5** [2, 13].

CONCLUSION: The HPLC method was developed and validated for the estimation of TAP in bulk, pharmaceutical formulations as well as for the detection of impurities and other related substances in stored formulations of TAP. The developed method was found simple, reproducible, rapid, and reliable for analysis of the drug. The validation report confirmed this method with good linearity, accuracy, precision, and adequate specificity.

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CONFLICTS OF INTEREST: All authors report no conflict of interest directly or indirectly in the publication of this manuscript.

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