

Research Article**Quick and precise RP-HPLC method development for Tapentadol hydrochloride**Mamta Bishnoi¹, Ankit Jain^{2*}, Yashpaul Singla³, Birendra Shrivastava¹¹Department of Pharmaceutics, School of Pharmaceutical Sciences, Jaipur National University, Jaipur (Raj.), India-302 017²Pharmaceutics Research Projects Laboratory, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar (M.P.), India - 470 003³Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar (Haryana), India-125001

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Abstract

Background: Tapentadol hydrochloride (TAP) is a novel opioid that binds and activates opioid receptor in the central nervous system to modify the approach our body interprets pain. It has dual mechanism of action (mu opioid-receptor agonist and noradrenaline reuptake inhibitor), this feature makes it an attractive member of opioid class. **Objective:** The aim of the present study was to develop and validate a simple, rapid, selective, sensitive, accurate and precise High Performance Liquid Chromatography (HPLC) with UV detection method to quantify TAP in rat plasma. **Material and methods:** Different analytical parameters, such as linearity, accuracy, precision, specificity with intentional degradation, limit of detection and limit of quantification (LOQ), were determined according to the ICH guidelines. The chromatographic separation of tapentadol hydrochloride was achieved with LC-2010 HT column using a mobile phase, potassium phosphate buffer: acetonitrile (50:50 v/v) at flow rate 1.0 ml/min. using a UV detector set at 272 nm with a continuous run up to 5 min. Plasma samples were processed using acetonitrile as precipitating agent to extract drug. **Results and conclusion:** The linearity for tapentadol hydrochloride was found to be 100-1000 ng/ml with regression coefficient (r^2) > 0.9970. The recovery ranged from 98.9 to 100.8% for the drug with a relative standard deviation (%RSD) of <2%. Stability analysis revealed that the drugs remained stable for sufficient time. The limit of quantification in plasma for tapentadol hydrochloride was found to be 10ng/ml. The mean recovery was obtained at 98.96%. The chromatographic runs were specific with no interfering peaks at the retention times of the analytes confirmed by the experiments. The method can be used to perform pharmacokinetic and bioequivalence studies in rat blood/serum.

Keywords: Tapentadol, Rat serum, Bio-analytical validation, HPLC

Introduction

Tapentadol, 3-[(1R,2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol hydrochloride (TAP), was approved by the Food and Drug Administration in 2008, and then positioned into the schedule II category of the Controlled Substances Act in May, 2009 (Wade and Spruill, 2009). TAP characterized as centrally acting analgesic with dual mechanisms of action, namely mu opioid receptor agonist and nor-adrenaline reuptake inhibitor (MOR-NRI) (Tschentke et al., 2006) (Figure 1). It appears to be

well tolerated as an analgesic as 50, 75, and 100 mg doses and is recommended for osteoarthritis and low back pain (Fidman and Nogid, 2010; Lange et al., 2010). In mice, tapentadol was shown to be more potent than morphine against heat hyperalgesia. In humans, tapentadol is rapidly absorbed after intake and extensively metabolized via Phase 2 pathways. After oral administration, 70% of the dose is excreted in the urine as conjugated metabolites, 3% of the drug is excreted unchanged, and 13% as N-desmethyltapentadol IDMT (Singh et al., 2013). In the area of pain management, most major laboratories use plasma as the matrix of choice for therapeutic and bioavailability analysis, so a procedure for determining drug in plasma was developed. Since TAP has the potential to contribute to the analgesic arsenal for humans and animals (Haywood et al., 2018; Pierce and Shipstone,

***Address for Corresponding Author:**

Dr. Ankit Jain (M. Pharm. PhD),

Department of Pharmaceutical Sciences,

Dr. Harisingh Gour Vishwavidyalaya, Sagar (M.P.), India - 470 003

Email: ankitjainsagar@gmail.com, dr.jnankit@gmail.com

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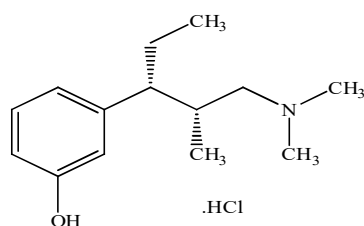


Figure 1. Chemical structure of Tapentadol hydrochloride

2012), pharmacokinetic (PK) and pharmacodynamic (PD) studies utilizing drug concentrations in plasma are essential to determine safety and efficacy of TAP.

Till now, the reported methods include UPLC (Hillewaert et al., 2015), HPLC (for Canine Plasma) (Giorgi et al., 2012) and liquid chromatography with tandem mass spectral detection (LC-MS-MS) methods (for Urine and urine and oral fluid) (Coulter et al., 2010) for the determination of the drug in various biological fluids and RP-HPLC (Sherikar and Mehta, 2012; Goud and Reddy, 2012; Jain and Basniwal, 2013) and spectrophotometric methods for determination of the drug in its pharmaceutical dosage form (Rizwana et al., 2012; Mobrouk et al., 2013; Muzib et al., 2013). In the present study, an attempt has been made to develop a simple, accurate, reproducible and sensitive method for the determination of Tapentadol hydrochloride in rat plasma using rapid, convenient and simple reverse phase HPLC method.

Materials and methods

Chemicals and Reagents

Tapentadol hydrochloride (TAP) was procured as a gift sample from Innova Captab, Baddi (H.P.). HPLC grade Acetonitrile and Dipotassium Phosphate buffer was purchased from Sigma Aldrich, New Delhi, India. All solutions were filtered through cellulose nitrate membrane filters (0.45 mm and 0.22 mm) were purchased from Himedia (Mumbai, India). All chemicals were of analytical grade unless stated otherwise and used as received. Purified HPLC grade water was obtained by distilling deionised water produced by a Milli-Q Millipore Water System (Milford, MA, USA) and was used to prepare all solutions.

Preparation of Standard Stock Solutions

A stock solution of tapentadol hydrochloride was prepared by accurately weighing 50 mg of drug, transferring to 50 ml volumetric flask, and added 20ml of mobile phase. After sonication up to 15 minutes, volume was made up with the mobile phase. Appropriate aliquots of drug solution were prepared with mobile phase to obtain final solutions of 100, 200, 400, 600, 800 and 1000 ng/ml of tapentadol hydrochloride. Resultant solutions were filtered through Whatman filter paper number 41. Samples for the determination of recovery; precision and accuracy were also prepared by spiking control in

appropriate concentrations (i.e., 1, 10 and 50 µg/mL) and stored at -20°C.

Instrumentation and chromatographic conditions

The HPLC analysis was carried out by using HPLC system (Shimadzu Co., Kyoto, Japan) consisted of a Shimadzu model LC-10 ADVP, fitted with a Phenomenex Luna C-18(2) column (4.6-250 mm, dp=5 mm; Hyderabad, India), SPD-M20A Prominence Diode array detector, (possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 280 nm), 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. Mobile phase consist of a mixture of 0.1 M potassium di hydrogen phosphate buffer (pH adjusted to 7 with triethanolamine) and acetonitrile in the ratio of 50:50 %v/v was used as mobile phase. Mixed solvents were filtered through 0.2 µm cellulose acetate membrane with a solvent filtration apparatus, degassed used as mobile phase. Same was used as diluents for the preparation of drug solutions. The mobile phase was kept in ultrasonic bath sonicator for 30 min. and filtered through a 0.22 µm nylon membrane filter. Injection volume was 20 mL with a flow rate of 1 mL/min. All experiments conducted on the HPLC were carried out in isocratic mode. The column temperature was maintained at 25° C and elution was monitored at 272 nm using a Photo diode array detector. All chromatographic data were acquired and processed with the Lab Solutions software.

Solution state stability testing

Stability testing was carried out to evaluate the stability and extent of degradation of the stock solution containing the drugs in mobile phase. Fresh stock solution containing tapentadol hydrochloride (1 mg/mL) was prepared and then working solutions at three concentration levels were made from this standard solution and kept at 4–6°C. Sampling was done at regular time intervals for a period of 7 days in triplicate. Each sample was run in HPLC after filtering through a 0.22-mm filter. The peak areas of the individual drugs were compared at different time points to determine the stability as a function of time.

Validation of the analytical method

The developed method was validated as per the ICH guidelines for linearity, accuracy and precision and specificity. Limit of detection (LOD) and limit of quantification (LOQ) were determined using the serial dilution method.

Linearity

The linearity of the method used for TAP analysis was evaluated from the standard curve of detector response

(peak area) against analyte concentration. The concentration range was chosen on the basis of anticipated drug concentration in the release study samples and 8-point calibration curves were generated on 3 successive days with standard working solutions of their combination. The solutions were injected in triplicate into the HPLC column. The linearity of the analytical procedure was evaluated by plotting detector response (the peak area) against analyte concentration. Linear regression analysis was carried out to calculate the slope, intercept and linear correlation coefficient (r^2).

Accuracy and precision

Accuracy and precision of the analytical method was determined by analyzing quality control (QC) samples at three different concentrations within the calibration range in triplicate ($n=3$). QC standards were prepared in the same media and were independent of those used for the preparation of calibration curves. The precision (%RSD) of the analytical procedure was evaluated by determining the intra- and inter-day coefficients of variation and reported as %RSD for a statistically significant number of replicate measurements. The intra-day precision of theselected method was estimated by the analysis of three different concentrations of the drug in triplicate and three times on the same day. The inter-day precision was assessed by analyzing samples in the same way as for the intra-day precision assay and was repeated for 3 consecutive days.

Specificity

Specificity is the ability of the analytical method to measure accurately and specifically the analyte of interest in the presence of other components that might be expected to be present in the sample. Specificity of the analytical method was evaluated. Assessments were based on quantification limits.

Quantification limits

LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte, whereas LOQ is the lowest amount of the analyte in a sample, which could be quantitatively determined with suitable precision and accuracy. LOQ was assessed by the standard deviation of the

response and the slope method. Slope S was calculated from the calibration curve of the analyte and the standard deviation was estimated by running five blank samples while LOD was taken as the one-third of LOQ for their simultaneous analysis, LOQ and LOD were estimated by the serial dilution method.

Application of the method

Estimation of TAP in serum from rats ($n = 3$) was performed. Blood sample of rat was collected through cardiac puncture in a centrifuge tube which contains heparin (anticoagulant) and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected, then added 2ml of 0.4% ortho – phosphoric acid and was deproteinized with equal amount of acetonitrile for half an hour to precipitate proteins. The precipitated proteins were separated by centrifugation at 5000rpm for 10min and supernatant was collected and filtered through 0.45 μ m membrane filter. From this serum 5 sets of serum samples (each having 0.9 ml.) with varying drug concentration were prepared and made up to the volume with the mobile phase by spiking drug free serum with an appropriate volume of a known amount of drug at a concentration range 100ng – 1000 ng/ml of serum and filtered. Serum was equilibrated at 37°C for 20 min. The mixture was vortex-mixed for 30 s. After centrifugation at 5500 x g for 10 min, the supernatant was separated and filtered. Filtrate was injected into the HPLC column immediately.

Results

Chromatographic separation

The TAP showed a retention time of 2.158 min. The isocratic mode was employed for the elution of the drug. Nevertheless, the drug got eluted within 2.5 min; the run was further continued upto 5 min to ensure the complete removal of traces of drugs from the column and to re-equilibrate the system to initial conditions. Figure 2 illustrates the complete chromatogram generated over 5min, which shows the peak. Figure 3 shows the graph of standard curve of TAP obtained in the range 100–1000ng/mL.

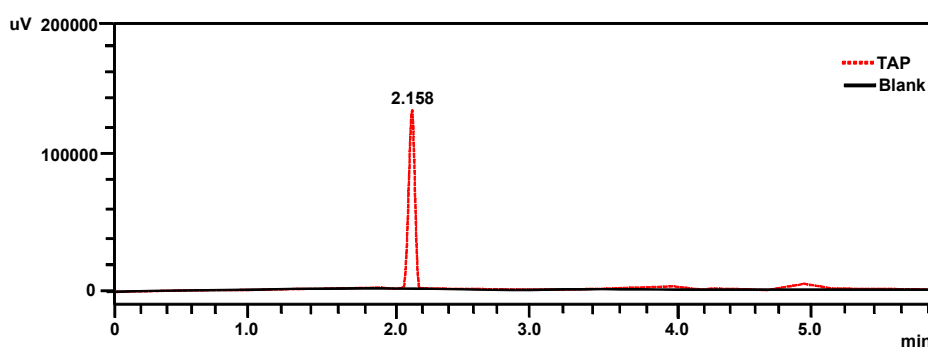


Figure 2. Chromatograms of Blank and TAP (RT = 2.158 min)

Stability of stock solutions

Table 1 represents stability data of the stock solution containing the drug.

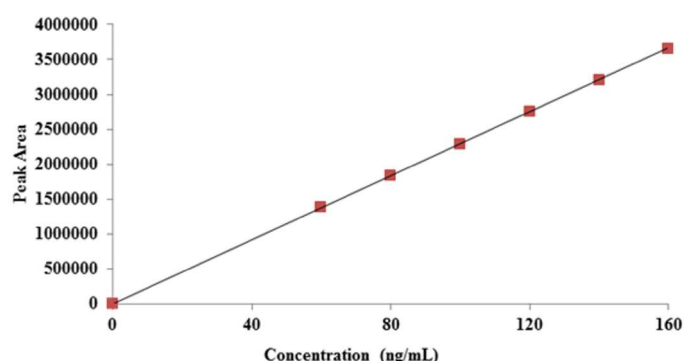


Figure 3. Linearly regressed calibration curve of TAP in serum ($\lambda_{\text{max}} = 272\text{nm}$)

Table 1. Stability of the drug's Stock Solution

Drug Concentration (ng/mL)	% Recovery		
	After 6 hr	Day 3	Day 7
800	99.9799±0.07 (0.57)	99.739±0.17 (0.65)	99.8369±0.13 (0.14)
400	100.022±0.06 (0.12)	99.106±0.31 (0.26)	100.1823±0.09 (0.09)
100	99.6885±0.11 (0.33)	100.0572±0.09 (0.13)	99.8981±0.21 (0.25)

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

Table 2. Ruggedness/robustness testing for the method

Parameter	Modification	TAP (% Recovery)
Mobile phase composition ACN: 0.1 M potassium di hydrogen phosphate buffer (pH adjusted to 7 with Triethanolamine)	40:60	98.2±0.31
	50:50	99.4±0.22
	60:40	98.5±0.35
Flow Rate (mL/min.)	1.08	95.9±0.37
	1.20	98.9±0.23
	1.32	98.1±0.37
	1.40	98.1±0.37
Injection Volume(mL)	18	98.4±0.28
	20	99.4±0.11
	22	98.2±0.23
	24	98.2±0.23
Column Temp.(°C)	23	98.7±0.33
	25	99.2±0.15
	27	98.3±0.16
	29	98.3±0.16
Detector Wavelength (nm)	270	98.9±0.29
	272	99.4±0.54
	274	97.8±0.61
	276	97.8±0.61

Values represent mean ± SD (n = 6)

Short term stock stability

A Stock solution of Tapentadol hydrochloride was kept at room temperature for 6 hours.

Long term stock stability

A Stock solution of Tapentadol hydrochloride and was kept at room temperature for 15 days.

Chromatograms obtained by running three concentrations on the same day after 6 hr., on the 3rd and the 7th days from the preparation of stock solution have been compared with those obtained initially. Values given under same day after 6 hr., day 3 and day 7 denote the peak area ± SD (%RSD) calculated with respect to the average peak area of the respective concentrations as obtained initially.

Ruggedness/robustness testing

The ruggedness/robustness of the method was checked after deliberately altering the following parameters: composition of the mobile phase, mobile phase flow rate, injection volume, column temperature and detector wavelength (Mulholland, 1988). The results showed no significant statistical differences between various altered parameters with respect to those which were received initially i.e. retention time, relative retention time (RRT), resolution and number of plates (Table 2).

Validation of the method

Validation parameters have been highlighted in table 3 of TAP analysis. The method was validated with respect to

parameters including linearity, limit of quantitation (LOQ), and limit of detection (LOD), suitability, precision and accuracy. Standard curve was generated in triplicate on 3 consecutive days distributed evenly across the linearity range. Values are reported as mean \pm SD of three calibration curves. Accuracy and precision data showed that the recoveries ranged from 99 to 101%. Both intra- and inter-day precision (%RSD) of QC standards were less than 2% over the selected range of the drug (Table 4). Accuracy and precision were determined with QC samples. Triplicate samples were analyzed on 3 consecutive days. For intra-day determinations, three standard curves were prepared on the same day. For inter-day determinations, three standard curves were generated on three consecutive days. Accuracy is represented by percent recovery (mean \pm SD) and precision by percent RSD. Recovery of the drug from solutions prepared in mobile phase with pH adjusted with triethanolamine was assessed at three concentration levels in triplicate (Table 5). Results of intentional degradation have been summarized in table 6.

Table 3. Validation Parameters of the HPLC Method for TAP

Parameters	Value
Analytical wavelength (nm)	272
Linearity ($\mu\text{g/mL}$)	100-1000
Slope	13992.8 \pm 1763.5
% RSD of slope (%)	1.37
Intercept	578
Correlation coefficient (r^2)	0.9995
LOD ($\mu\text{g/mL}$)	3.33
LOQ ($\mu\text{g/mL}$)	10

Table 4. Accuracy and Precision Studies for TAP

Parameter	Inter-day (Drug Conc. $\mu\text{g/mL}$)			Intra-day (Drug Conc. $\mu\text{g/mL}$)		
	750	650	550	750	650	550
Precision (%RSD)	1.35	1.11	0.98	1.25	0.99	0.97
Accuracy (%recovery)	100.01 \pm 0.15	98.95 \pm 1.12	100.13 \pm 0.86	99.98 \pm 1.27	99.89 \pm 1.36	99.42 \pm 1.56

Values represent mean \pm SD (n = 6)

Table 5. Results of Specificity Studies of TAP

Actual concentration (ng/mL)	Calculated concentration (ng/mL)	%Recovery
750	749.85 \pm 8.620 (1.27)	99.9499 \pm 0.02 (0.15)
550	550.56 \pm 5.398 (0.98)	100.156 \pm 0.06 (0.27)
350	349.69 \pm 0.664 (0.19)	99.95875 \pm 0.12 (0.36)

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

Discussions

Chromatographic separation

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. When the isocratic mode was employed, the drug got eluted within 2.2 min; the run was further continued for 5 min to ensure the complete removal of traces of drugs from the column and to re-equilibrate the system to initial conditions.

Stability of stock solutions

The stock solution was found to be stable for 1 week as recovery and %RSD are seen to be within statistical limits. Further, no considerable change was observed in the calculated concentration of the drug during the period. Hence, the solutions remain stable over a period of 7 days at 4–6°C.

Ruggedness/robustness testing

The ruggedness/robustness testing of the method with deliberate alterations in parameters, viz. composition of the mobile phase, mobile phase flow rate, injection volume, column temperature and detector wavelength, resulted in acceptable range, i.e., more than 96%. The parameters of chromatographic separation (retention time, RRT, resolution and number of plates) were also almost same on varying the operational parameters.

Validation of the method

The method developed was validated for analytical performance parameters such as linearity, accuracy, precision, specificity and quantification limits as per the ICH guidelines. Linear regression analysis confirmed that the r^2 value was found to be 0.9995, confirming the linear relationship between the concentration of the drug and the

area under the curve. The calculated LOD and LOQ concentrations proved the sensitivity of the method (Jain et al., 2014). Specificity evaluation was observed that the peak of the drug was well observed and not being interfered with serum contents (Jain et al., 2013). Hence, the method can be suitably employed for quantitative analysis of a drug in the biological samples.

Conclusion

The HPLC method was developed for estimation of Tapentadol hydrochloride in various samples of rat's blood/serum as well as other organs. The developed method is confirmed simple, rapid and reliable for analysis of the drug using the mobile phase, i.e., ACN and Potassium phosphate buffer (with Triethanolamine, for pH adjustments) with retention time 2.158 ± 0.009 min. Validation report confirms that the method has good linearity, accuracy, precision and adequate specificity, and it can be employed to find out the concentration of TAP in rat's biological samples.

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Conflicts of interest: Not declared.

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