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Original Research Paper

QUANTITATIVE ESTIMATION OF TAPENTADOL HYDROCHLORIDE IN HUMAN PLASMA BY HPLC

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ABSTRACT

A simple, rapid, selective, sensitive, accurate and precise High Performance Liquid Chromatography (HPLC) with UV detection method has been developed and validated for determination of Tapentadol hydrochloride in human plasma. The chromatographic separation of Tapentadol hydrochloride was achieved with Chromasil C18 column using a mobile phase Methanol: water (60:40 v/v) at flow rate 1.0 ml/min. Plasma samples were processed using acetonitrile as precipitating agent to extract drug. The linearity for Tapentadol hydrochloride was found to be 100-1000 ng/ml with regression coefficient (r^2) 0.9980. The limit of quantification in plasma for Tapentadol hydrochloride was found to be 100 ng/ml. The mean recovery was obtained at 85.20 %. The method has been used to perform pharmacokinetic and bioequivalence studies in human plasma.

Keywords: Tapentadol hydrochloride, Human plasma, Bioanalytical validation, FDA guidelines, HPLC-UV detection.

INTRODUCTION

Tapentadol hydrochloride (TAP) is Opioid analgesic for treatment of moderate to severe pain and centrally acting synthetic analgesic.^{1,2} Chemically it is 3-[(2R,3R)-1-(dimethylamino)-2-methylpentan-3-yl]phenol.^{1,2} Its molecular formula and molecular weight are $C_{14}H_{23}NO.HCl$ and 257.80 respectively. Tapentadol hydrochloride is extremely water soluble. It is more stable in acidic medium. Tapentadol hydrochloride is official in Indian Pharmacopoeia.¹ Literature survey reveals that many analytical methods such as UV spectrophotometric²⁻³ and HPLC methods³⁻⁷ methods are reported for determination of Tapentadol hydrochloride individually from pharmaceutical dosage form and HPLC⁸ methods are reported for determination of TAP with other drugs in combined dosage form. HPLC is a powerful tool for analyzing small analytes such as drugs in biological samples. However, analysis of analytes present in matrices such as plasma

requires well designed sample preparation procedures such as protein precipitation, centrifugation, extraction and filtration that can introduce experimental errors and ultimately reduce precision and accuracy of measurement. The aim of this work was to develop a simple, accurate, reproducible and sensitive method for the determination of Tapentadol hydrochloride in human plasma using rapid, convenient and simple reverse phase HPLC method.

MATERIALS AND METHODS

Chemicals and Reagents

Pure sample of Tapentadol hydrochloride was procured from Emcure Pharmaceuticals Ltd., Pune. Methanol (HPLC grade) and Acetonitrile (HPLC grade) were obtained from Qualigen Laboratories Pvt. Ltd., Mumbai.

Instrumentation and Chromatographic Condition

The analysis of Tapentadol hydrochloride was carried out on an Agilent 1120 series, which comprised a degasser, binary gradient pump and UV detector. The system was controlled through Ezchrome software using Chromasil C18 (4.6 x 250 mm, 5 µm; Advanced Chromatography Systems, Johns Island, SC) column maintained at 30°C temperature and flow rate of mobile phase 1.0 ml/min. The mobile phase was composed Methanol: water (60:40 v/v). The mobile phase was kept in ultrasonicator for 30 min. and filtered through a 0.22 µm nylon membrane filter. The measurements were made with injection volume 20 µl and UV detection 272.0 nm.

Preparation of Standard Stock Solutions

A Standard stock solution of Tapentadol hydrochloride was prepared by dissolving 10 mg drug in HPLC grade methanol in 100 ml volumetric flask to get concentration 100 µg/ml. To prepare working standard solution of TOL was diluted to 1, 2, 4, 6, 8 and 10 µg/ml with methanol.

Protein Precipitation

The blank plasma sample was prepared by adding 1ml of plasma and 1ml acetonitrile and vortex for 2.0 min. Then the solution was centrifuged at 3000 RPM for 10 min. The supernatant liquid was taken and transferred to HPLC vials.

Preparation of Calibration Curve (Cc) Standards and Quality Control Samples

The working standard solutions of TOL with concentrations of 1, 2, 4, 6, 8 and 10 µg/ml was prepared by diluting with methanol. Aliquots of 0.90 ml of blank human plasma were spiked with 100 µl of working standard solutions of 1, 2, 4, 6, 8 and 10 µg/ml to get CC standards containing 100, 200, 400, 600, 800 and 1000 ng/ml of Tapentadol hydrochloride. Similarly the QC samples were prepared to contain three concentrations [100 ng/ml low quality control (LQC), 500 ng/ml middle quality control (MQC) and 1000 ng/ml high quality control (HQC)].

Preparation of Plasma Sample Solution

To 0.90 ml of plasma, 100 µl of Tapentadol hydrochloride standard solutions of 1, 2, 4, 6, 8

and 10 µg/ml, 1.0 ml acetonitrile were added to a glass tubes. Each sample was vortex mixed for 2.0 min and centrifuged (3000 rpm for 10 min). After centrifugation 50 µl aliquots of supernatant of each concentration were injected into the HPLC system.

METHOD VALIDATION⁹⁻¹¹

The method was validated in accordance with FDA guidelines

Calibration Plot

The calibration plot for the HPLC method was constructed by analysis of six solutions containing different concentrations of Tapentadol hydrochloride (100, 200, 400, 600, 800 and 1000 ng/ml). In the range 100-1000 ng/ml the data were best fitted by a linear equation $y = mx + b$, the coefficient of determination (R^2) was 0.9980.

Evaluation of Selectivity

This parameter was evaluated at the Lower Limit of Quantification (LLOQ) i.e. 50 ng/ml per ml of plasma. The LLOQ sample was mixed with 100 µl of methanol, analyzed and the drug peak area was noted. Similarly, 1 ml blank plasma was mixed with 100 µl of methanol, analyzed and the detector response for the blank was noted at the retention time of the drug. The blank response was compared with the peak area of the LLOQ sample.

Precision and Accuracy

The intra and inter-day variation of the assay were determined by replicate analysis ($n = 6$) of three concentrations of TOL [100 ng/ml low quality control (LQC), 500 ng/ml middle quality control (MQC) and 1000 ng/ml high quality control (HQC)] on the same day and at different days, respectively, using the same stock solutions and plasma batches. The percent of relative standard deviations of results of the assay were determined.

Repeatability

The repeatability (intraday precision) of the method was evaluated in triplicate on the same day for three different concentrations performing of Tapentadol hydrochloride.

Recovery

Recovery from human plasma samples was evaluated in triplicate for each of three concentrations of Tapentadol hydrochloride. The response for each level being compared with that from the corresponding standard solution. The results of recovery was given in (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The parameters LOD and LOQ were determined using the signal-to-noise ratio by comparing results of the test of samples with known concentrations of analyte to blank samples. The analyte concentration that produced a signal-to-noise ratio of 3:1 was accepted as the LOD. The LOQ was identified as the lowest plasma concentration of the standard curve that could be quantified with acceptable accuracy, precision and variability.

Stability

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.

Freeze thaw stability

The stability of low and high quality control samples were determined after three freeze thaw cycles.

RESULTS AND DISCUSSION

The method was validated in terms of limit of quantification, Recovery, Selectivity, Precision, accuracy and stability.

Linearity

The linearity was found to be 100-1000 ng/ml.

Sensitivity

In the plasma, limit of quantification was found to be 100 ng/ml for Tapentadol hydrochloride.

Precision and Accuracy

The accuracy, precision and intraday precision were carried out by preparing 6 individual LQC (100 ng/ml), MQC (500 ng/ml), HQC (1000 ng/ml) samples and the percentage of mean and C.V. was calculated (Table 1).

Recovery

The percentage recovery of the drug was found to be 85.20 %. (Table 2)

Stability

Stability of method was carried out by performing short term and long-term stock stability and freeze thaw stability. (Table 3)

CONCLUSION

This HPLC method for quantification of Tapentadol hydrochloride in human plasma is simple, accurate, precise, rapid, reproducible and selective. It is a simple, rapid, practical and economical alternative for studies of the bioavailability, bioequivalence and pharmacokinetics of this drug in human plasma.

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Table 1: Intraday, Interday precision and accuracy of Tapentadol hydrochloride in human plasma QC samples (*Average of six readings)

Concentration of QC samples (ng/ml)	Observed Concentration* (mean ng/ml)	Precision (% RSD)	Accuracy* (% mean)
Intraday Precision			
100	97.90±0.5346	0.5460	97.90
500	492.00±1.062	0.2158	98.40
1000	984.70±1.254	0.1273	98.47
Interday Precision			
100	94.52±0.1962	0.2075	94.52
500	488.40±2.865	0.5866	97.68
1000	982.40±1.535	0.1562	98.24

Table 2: Recovery of Tapentadol hydrochloride QC samples in human plasma (*Average of six readings)

Concentration of QC samples (ng/ml)	Observed Concentration* (mean ng/ml)	Recovery* (% mean)	% RSD
100	83.83	83.83	0.2863
500	446.25	89.25	1.024
1000	825.20	82.52	1.257
Mean		85.20	

Table 3: Stability of Tapentadol hydrochloride QC samples in human plasma (*Average of six readings)

Stability	Concentration of QC samples (ng/ml)	Concentration found* (mean ng/ml)	% Mean*	% RSD
Short term stability	100	97.48±0.4326	97.48	0.8421
	1000	976.60±8.635	97.66	0.9232
Long term stability	100	94.78±0.6358	94.78	0.8714
	1000	951.29±9.462	95.13	0.7382
Freeze thaw stability	100	96.31±0.6524	96.31	0.7121
	1000	968.67±10.632	96.86	0.6520

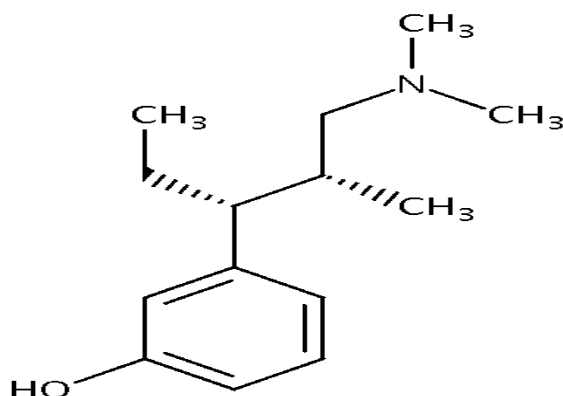


Figure 1: Chemical structure of Tapentadol hydrochloride

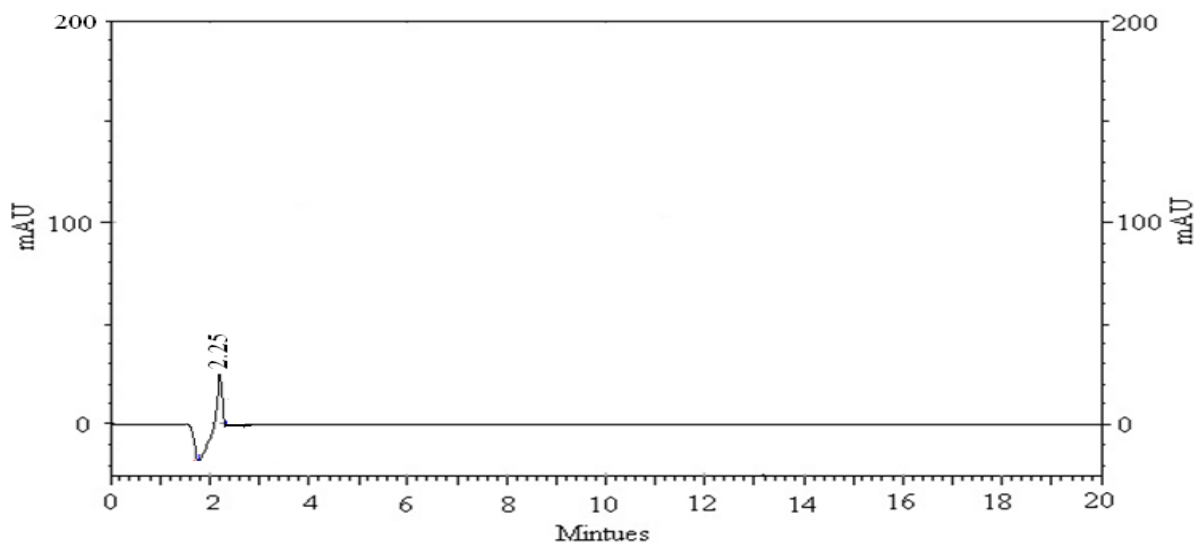


Figure 2: Typical chromatogram of Blank Human Plasma

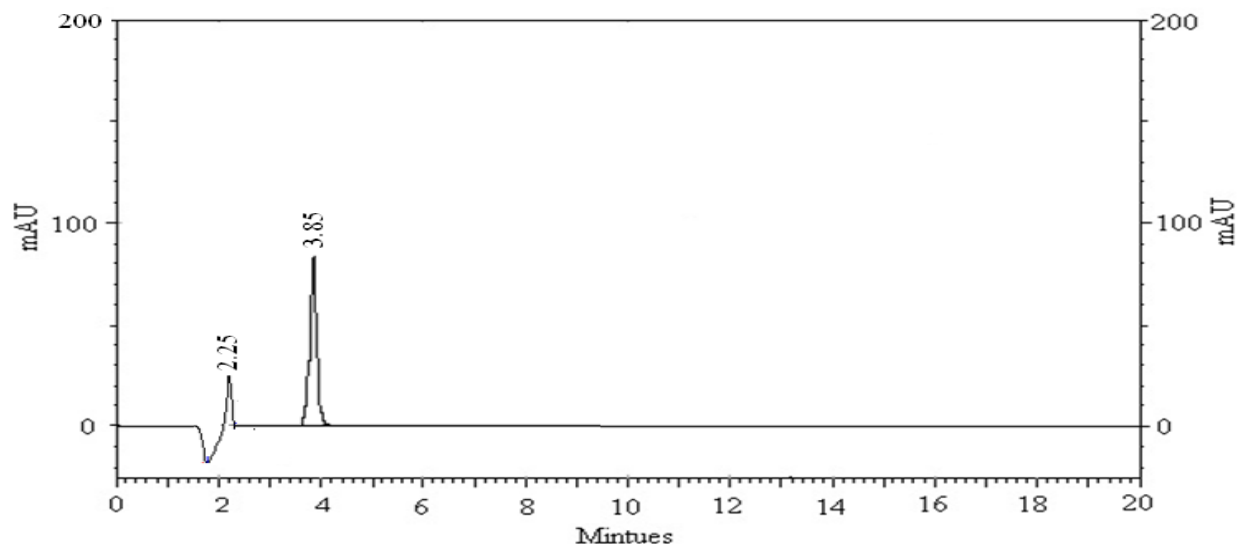


Figure 3: Typical chromatogram of Human Plasma spiked with HQC sample of TAP

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