International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 01 (A); January 2020; Page No.20863-20866
DOI: http://dx.doi.org/10.24327/ijcar.2020.20866.4085

Review Article

BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TAPENTADOL HYDROCHLORIDE IN RAT PLASMA BY RP-HPLC

Heera Battu, Konda Jeevitha, and Jayanti Vijaya Ratna

Department of Pharmaceutics, Au College of Pharmaceutical sciences, Vishakhapatnam, Andhra Pradesh

A R T I C L E I N F O

Article History:
Received 06th October, 2019
Received in revised form 14th November, 2019
Accepted 23rd December, 2019
Published online 28th January, 2020

Key words:

Tapentadol hydrochloride, bioanalytical, HPLC

A B S T R A C T

Tapentadol hydrochloride (TPHCL), is a centrally acting analgesic drug with a dual mechanism of action involving mu-opioid receptor agonism and nor epinephrine reuptake inhibition. A rapid, sensitive, simple and cost-effective high performance liquid chromatographic method for the determination of TPHCL by UV detection in rat plasma is to be developed and validated. Extraction process involved is protein precipitation using diethyl ether: dichloromethane (60:40 v/v). TP HCL was eluted under isocratic mode using an Altima Grace Smart C18 column. The mobile phase consists of 60:40 v/v methanol: 20mM sodium perchlorate buffer (pH 6.8) at flow rate of 1.0 ml/min with wavelength of detection at 236 nm. The injection volume was 20 µl. The runtime of the method was 10 min. The method exhibited good linearity in the range 50-250 ng/ml. The correlation coefficient (r2) value was found to be 0.998. Precision study showed % CV value less than 2% in all selected concentrations. The lower limit of quantitation (LLOQ) was found to be 50ng/mL. While, accuracy of LLOQ was found to be 94.60±7.57 % and %RSD was 8.00% while the intra- and inter-day accuracies were around accuracies were 98.28-102.99% respectively. A rapid, sensitive, simple and cost effective method for the estimation of Tapentadol in human plasma was developed and validated according ICH guidelines.

INTRODUCTION

Tapentadol hydrochloride, l, 3-[(1R, 2R)-3-(dimethylamino)-1-ethyl-2-methyl] propylphenol hydrochloride (TPHCL), generally, it is a centrally acting analgesic with a dual mode of action as an agonist of the µ-opioid receptor and a norepinephrine reuptake inhibitor, which is the frequently prescribed mostly in the treatment of moderate to severe pain for both acute (following injury, surgery, etc.) and chronic musculoskeletal pain. It is also specifically indicated for controlling the pain of diabetic neuropathy. TPHCL has a half-life of four hours. It is a BCS Class-I drug and have high solubility and high permeability. On oral administration, the usual oral dose of TPHCL in treating musculoskeletal pain is 600 mg/day. It is administered as immediate release products containing 50 mg, 75 mg, or 100 mg 4-6 times a day. It is also administered as a single oral controlled release system containing 500 mg per tablet. The aim of the current study is to produce a TPHCl micro emulsion gel that, when applied on skin, releases 100 percent of its drug content over a period of 24 hrs

MATERIALS AND METHODS

Tapentadol hydrochloride is purchased from Sigma-Aldrich, HPLC grade Methanol was procured from Fisher Scientific while others chemicals are of AR grade purchased from Loba Chemie, India.

Corresponding author:* **Heera Battu
Department of Pharmaceutics, Au College of Pharmaceutical sciences, Vishakhapatnam, Andhra Pradesh

Instrumentation

Chromatographic separation was performed on a Peak chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Preparation of stock and standard solutions

A stock solution of mg/ml (1000µg/ml) was prepared by accurately weighing 25 mg of the standard drug dissolved in 25ml of methanol. A standard concentration of 1000µg /ml was obtained. The solution was filtered and was used as standard stock solution. The solution was filtered through 0.45µg/ml nylon membrane filter paper and was used as standard stock solution. The stock solutions were preserved safely and were used when it required. Further dilutions were prepared as per the standard procedure.

Extraction of drugs from plasma

Prior to sample analysis, 100µL of each solution was extracted using 300µL of diethyl ether: dichloromethane (60:40 v/v) for protein precipitation. Further, each of the mixtures was vortex for a period of 5 min in a vortex mixer with subsequent centrifugation at 10000 rpm, for a period of 10 min at 4°C using a centrifuge. For each sample, an aliquot of a supernatant was isolated and subjected to dryness. The residue was reconstituted in 100µL of mobile phase and subsequently centrifuged at 10000 rpm for10 min at 4°C in a centrifuge. The supernatant was finally collected and directly injected for analysis. This procedure was followed for all samples of calibration curve plasma spiked dilutions and plasma spiked samples.

Method development

Optimization method

The chromatographic method was optimized as a mixture of 20mM sodium perchlorate buffer (pH 6.8) and methanol (40:60 v/v) at a flow rate of 1 mL/min with detection wavelength of 236nm by using Altima Grace Smart C-18 column by changing various parameters on trial & error basis. During the method optimization water and phosphate buffer in various strengths are tried along with methanol and acetonitrile as organic solvent. The mobile phase composition of 60:40 v/v methanol: buffer was gave good resolution, retention times of TPD with minimal tailing factor in acceptable range. The method was optimized with the mobile phase composition of methanol and buffer 60:40 (v/v).

The system suitability of the method was done by working stock standard of individual drugs were injected HPLC to determine the individual retention times of drugs. Then working standard solution was injected five times and we considered relative standard deviation (RSD) for five consecutive injections ≤2, resolution between two adjacent peaks ≥2 and tailing factor <2 acceptable values [12]. Resolution (R), relative standard deviation from five replicate injections of working standard mixture solution, tailing factor (T) and retention time drug was presented in **[Table. 1]**. System suitability test confirmed that the chromatographic system was adequate for the analysis planned to be done. Then, the method was validated for various validation parameters according to the US FDA guidelines.

Method validation

Selectivity and Specificity

The selectivity of the present method was established by checking the blank potassium ethylene diamine tetra acetic acid (K_2EDTA) rat plasma (without spiking with TPD) obtained from six different sources. As these blood samples were collected from six different rats, all possible K_2EDTA plasma profiles will be included which may contain any interfering compounds that elute along with TPD. While, also spiked at LLOQ concentration of TPD with plasma of one donor. The specificity of the present method was established by checking the interference of TPD retention time with that of blank. This was done by injecting six replicates of matrix blank. In this study there was no peak interference of TPD retention time (**Fig. 2.).** This clearly shows the specificity and selectivity of the method.

Carryover effect

The carryover effect of the present method was established by using six injections of plasma blank and an upper limit of quantification (ULOQ) of TPD. These samples were analyzed alternately to check any carryover in the blank sample. In this study there were no such effects observed.

Linearity

The linearity of this method was evaluated by linear regression analysis, using least square method. The drug concentrations were linear in a range of 50-300 ng/mL. Calibration standards were prepared by spiking required volume of working standard solution along with internal standard into different 10 mL volumetric flasks and volume made up with methanol to yield concentrations 50, 100, 150, 200, 250, and 300 ng/mL of TPD. The resultant peak area of the drug and internal standard were measured. Calibration curve was plotted between peak areas of drug against concentration of the drug. (**Fig. 3.)**, represents the linearity graph, regression coefficient (r^2) including the slope and y-intercept. The present method linearity range 50-300 ng/mL, it will cover all the strengths of TPD in rat plasma. The data was represented in **Table.2**.

Sensitivity

The standard chromatogram of AMP at LLOQ level was presented in Fig. **(4)**. The lower limit of quantitation (LLOQ) was found to be 50 ng/mL. The percent accuracy of LLOQ was 94.60±7.57 % and precision denoted by %RSD was 8.00%.

Extraction recovery

The extraction recovery was determined at three concentration levels (LQC, MQC and HQC) for AMP and IS by comparing the peak area of AMP obtained by injecting the standard drug spiked with plasma followed extraction, the peak area of AMP obtained by injection standard drug of same concentration. The extraction recoveries were calculated and found to be 84.93± 3.44 (Table.**3.**) for TPD.

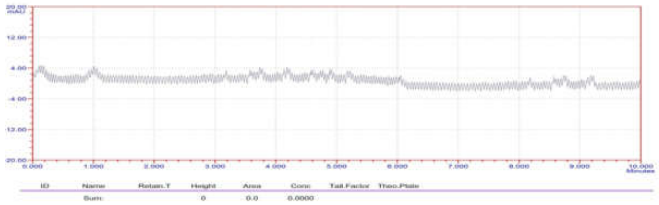

Fig 1 Chromatogram of blank plasma

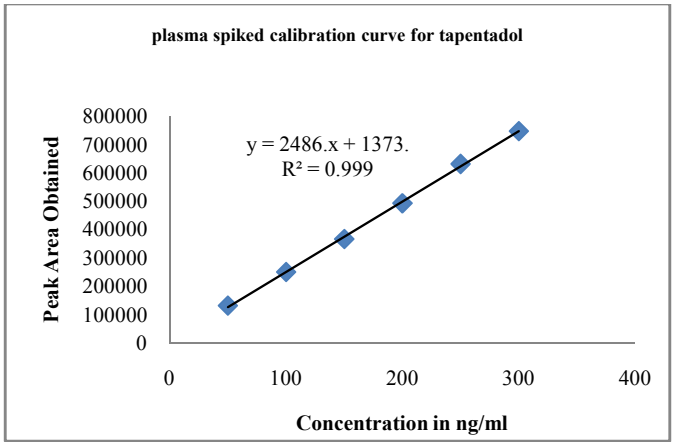

Fig 2 Linearity graph of Tapentadol

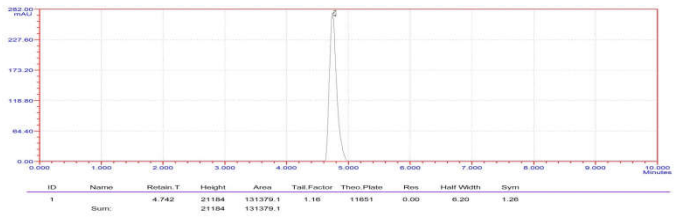

Fig 3 chromotogram of Tapentadol

Table 1 System Suitability Conditions

S.NO	Parameter	Results
1	Concentration	200ng/ml
2	Retention time (RT)	4.78min
3	Peak Area	492665
4	Theoretical Plates	5318
5	Tailing Factor	1.38

Table 2 Plasma Spiked Calibration Curve

S.NO	Concentration (ng/mL)	Peak Area
1	50	131379
2	100	249734
3	150	366263
4	200	492665
5	250	631573
6	300	747178

Table 3 Extraction recovery of TPD from plasma

Level	Concentration	Average Area of Aqueous	Extracted Area	% Recovery
50%	150	398370	323993	81.3296
	150	398370	323426	81.18727
	150	398370	320942	80.56373
100%	200	554826	491688	88.62011
	200	554826	491920	88.66192
	200	554826	496448	89.47804
150%	250	737317	623886	84.61567
	250	737317	622255	84.39446
	250	737317	631181	85.60507

Note: Number of sample (n=3);

Table 4 Precision of TPD

S.NO	HQC	MQC	LQC
1	747603	495256	134875
2	749081	493957	139107
3	747689	500528	131131
4	750453	493369	135541
5	744118	497427	135741
6	749138	499123	134439
RSD	**0.292**	**0.581**	**1.90**

Note: Values are expressed in Mean±SD,

Short Term Stability

S.NO	HQC	MQC	LQC
1	748987	498049	129793
2	742886	497417	133842
3	748630	495952	128368
4	748602	498022	133311
5	751556	500532	131597
6	749017	499110	134458
RSD	**0.383**	**0.310**	**1.836**

Long Term Stability

S.NO	HQC	MQC	LQC
1	748235	497013	132732
2	755404	502845	133842
3	747493	510000	132314
4	747250	495219	133282
5	749559	496785	132509
6	749832	494487	129266
RSD	**0.403**	**1.196**	**1.208**

Bench Top Stability

S.NO	HQC	MQC	LQC
1	747513	499008	136194
2	746225	499881	136462
3	748367	500173	138112
4	746381	493993	137840
5	744881	503876	137439
6	749396	503100	134647
RSD	**0.218**	**0.703**	**0.943**

Freez Thaw Stability

S.NO	HQC	MQC	LQC
1	750321	497255	131994
2	751631	496600	134784
3	747460	496431	131611
4	755976	500705	135493
5	755264	497594	134255
6	749867	499997	135051
RSD	**0.438**	**0.363**	**1.234**

Intra-day and Inter-day Precision and Accuracy

The intra- and inter-day precision and accuracy of this assay was determined by analyzing replicates of QC samples at three concentrations on 6 different days. The coefficients of variation for the intra- and inter-day precision were <3.03%. The intra- and inter-day accuracies were 98.28-102.99%. The low levels of coefficients of variation i.e.: 1.86%-3.03% (Table **4.)** indicate the method is accurate and precise. All these criteria were acceptable and follow US FDA guidance [9].

Robustness

Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Present method didn't show any significant change when the critical parameters were modified. The tailing factor of drug was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

Ruggedness

Ruggedness was studied along with precision and accuracy of batches where effect of column change and analyst change were observed. The observed value for column variation and results obtained for precision and accuracy were within the acceptance criteria (i.e. there were no changes in the retention time, recovery and precision of the drug) according to US-FDA and ICH.

Stability studies

The stability of drug was studied at different conditions for quality control (QC) of samples. The samples were analyzed and compared with freshly analyzed QC samples, no difference were found in accuracy and precision. There were no documented reports in literature about stability of AMP in plasma. To find any changes in stability of AMP in plasma, we carried stability studies at different conditions like freeze-thaw, wet extract, dry extract stability etc. In present method we studied stability of AMP in plasma for 24h, freeze thaw stability after three cycles and other stability studies. These studies enlighten the information regarding degradation of drug during the analysis and storage of plasma samples. From

these results stability of samples represented (Table **4**), the accuracy of all samples stability were found to be >95% indicating that there was no degradation of drug at different conditions and this also follows the acceptance criteria laid by US-FDA [9].

CONCLUSION

The developed method is rapid, sensitive, rugged and reproducible with high recovery. Each sample requires less than 5 min of analysis time. Drug and IS were extracted with the simplest protein precipitation method with less matrix effect. The developed method was successfully applied in the pharmacokinetic study to evaluate plasma concentration of TPHCL in healthy rats.

References

1. Singh DR, Nag K, Shetti AN, Krishnaveni N. Tapentadol hydrochloride: A novelanalgesic. Saudi journal of anaesthesia. 2013; 7(3):322.
2. Christoph T, De Vry J, Tzschentke TM. Tapentadol, but not morphine, selectivelyinhibits disease-related thermal hyperalgesia in a mouse model of diabetic neuropathicpain. Neuroscience letters. 2010; 470(2):91-4.
3. Gohler K, Brett M, Smit JW, Rengelshausen J, Terlinden R. Comparativepharmacokinetics and bioavailability of tapentadol following oral administration ofimmediate-and prolonged-release formulations. *Int J Clin Pharmacol Ther*. 2013;51(4):338-48.
4. Thakkar HP, Pancholi N, Patel CV. Development and evaluation of a once-dailycontrolled porosity osmotic pump of tapentadol hydrochloride. AAPS PharmSciTech.2016; 17(5):1248-60.
5. Wild JE, Grond S, Kuperwasser B, Gilbert J, McCann B, Lange B, Steup A, Häufel T,Etropolski MS, Rauschkolb C, Lange R. Long-term safety and tolerability of tapentadolextended release for the management of chronic low back pain or osteoarthritis pain. PainPractice. 2010; 10(5):416-27.

How to cite this article:

Heera Battu, Konda Jeevitha, and Jayanti Vijaya Ratna (2020) 'Bio-Analytical Method Development and Validation of Tapentadol Hydrochloride in Rat Plasma By RP-HPLC', *International Journal of Current Advanced Research,* 09(01), pp. 20863-20866. DOI: http://dx.doi.org/10.24327/ijcar.2020. 20866.4085

****