ejpmr, 2016,3(1), 385-389

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF TRAMADOL HCL IN BULK DOSAGE FORM

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Article Received on 19/11/2015 **Article Revised on 10/12/2015** **Article Accepted on 01/01/2016**

ABSTRACT

A simple and reproducible method was developed for Tramadol HCl by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Tramadol HCL was separated on Greece C18 (4.6ID x 250mm, Particle size: 5 micron, using ortho phosphoric acid buffer pH 3.0 at wavelength of 225 nm using UV detector. Isocratic elution of methanol and water was used as a mobile phase with various ratios and flow rates, eventually 80:20 v/v methanol and water was being set with the flow rate of 1mL/min. The statistical validation parameters such as linearity, accuracy, precision,LOD, LOQ, Robustness, system suitability were checked, further the limit of detection and limit of quantification of Tramadol HCL concentrations were found to be 55.55ng/mL and 168.33ng/mL.

KEYWORDS: RP HPLC, Tramadol HCl, methanol, validation

INTRODUCTION

Tramadol (marketed as Ultram and as generics) is anopioidpain medication used to treat moderate to moderately severe pain. When taken as an immediate-release oral formulation, the onset of pain relief usually occurs within about an hour.[1] It has two different mechanisms. First, it binds to the μ-opioid receptor. Second, it inhibits the reuptake of serotonin and norepinephrine.[2] Its use in pregnancy is generally advised against as it may cause some reversible withdrawal effects in the newborn.[3] A small prospective study in France found that, while there was an increased risk of miscarriages, there were no major malformations reported in the newborn. There is an increased risk of opioid-related adverse effects such as respiratory depression, falls, cognitive impairment and sedation.Tramadol acts as a μ-opioid receptoragonist.[4][5] serotonin reuptake inhibitor and releasing agent.[6] norepinephrine reuptake inhibitor, NMDA receptorantagonist.[7] 5-HT2C receptor antagonist.[8] (α7)5nicotinic acetylcholine receptor antagonist.[9] TRPV1 receptor agonist.[10] and M1 and M3muscarinic acetylcholine receptor antagonist. In literature several analytical techniques like colorimetric4, spectroflurimetric5 methods have been reported on assay of Tramadol in combination with other drugs. In the present study, novel method was developed with acetonitrile (ACN) as solvent in HPLC; it is a simple method to study, detect and separate the Tramadol from mixture of com-pounds and can be adopted for regular quality assessment in pharmaceutical industry and scientific laboratories.

MATERIALS AND METHODS

All reagents used were of analytical-reagent grade. Water purification systems, reverse osmosis andultra pure water (Nanopure Human Corporation, Korea), sonicator (Digital citizen ultra sonic cleaner) for degassing of HPLC grade Methanol and ortho phosphoric acid 88% (S.D. FineChem Limited, Mumbai, India) and pure Tramadol drug.

The RP-HPLC system composed of HPLC Binary Gradient System of HPLC 3000 series instant pilot software: HPLC Workstation and certified for pharmaceutical QA/QC. It constitutes Detector: UV-3000-M (Single Wavelength) Pump: P-3000-M Reciprocating (40MPa) Column: Greece C18 (4.6ID x 250mm, Particle size: 5 micron). It is most flexible configuration for the maximum in gradient and low flow rate accuracy and precision, high-speed, multi-wavelength and full spectral UV-visible detection for peak purity analysis and spectral confirmation.Thechromatographic and integrated data were recorded in computer system.Two solvents were used, solvent-A containing methanol filtered through 0.22μm filter paper and solvent-B containing ultra pure water filtered through 0.22μm Borosil (1 liter), 0.45micron cellulose membrane filter [pH adjusted to 3.0 with 88% ortho phosphoric acid], de-gassed with sonication. Various flow rates and sol-vent compositions were provided at roomtemperature [28°C] to develop a method. The detection was performed at 225nm using UV detector 3000-M (Single Wavelength).Solution 10mg/mL of pure Tramadol was pre-pared in the mobile

phase. The solution was adequately diluted and it was taken to develop a method by various solvent ratios and flow rates.

VALIDATION OF THE METHOD
Validation of the optimized HPLC method was carried out with the following parameters.

LINEARITY
Tramadol standard stock solution of 10mg/mL was used for preparation of subsequent aliquots; aliquots of 25, 50, 75,100,125µg/mL concentrations were prepared. All measurements were repeated three times for each concentration. The calibration curves of the area under curve versus concentration were recorded.

ACCURACY
Tramadol standard stock solution of 10mg/mL was used to prepare 25,75,125µg/mL concentrations and injected for the accuracy studies. The area under curve obtained was checked and analyzed for the recovery percentage.

PRECISION
The precision of method was checked and verified by repeatability .Repeatability was checked by injecting 25,50,75mg/ml concentration of Tramadol for 3 times on the same day was injected and analyzed at different time intervals on the same day.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)
The LOD and LOQ of Tramadol were separately determined on the basis of signal (S) and noise (N) ratio, LOD and LOQ concentrations of Tramadol.

ROBUSTNESS OF THE METHOD
To determine the robustness of the developed method, minute changes were made in the flow rate, percentage of Methanol and the pH of the mobile phase and is studied for the deviations from optimized method.

SYSTEM SUITABILITY PARAMETERS
To perform the system suitability tests the standard solution was freshly prepared and injected under the condition of optimized method to study the following parameters.

RESULTS

Table no.1: Parameters of experiment.

Concentration of solvent	Flow Rate (ml/min)	Retention Time	Area Under Curve	% Recovery
25	1	2.401	295502	52.00425
50	1	2.148	763077	79.98054
75	1	2.187	1238383	90.70041
100	1	2.401	1638378	91.61826
125	1	2.206	1987153	89.75208

LINEARITY
The method gave a linear response to Tramadol drug within the concentration range of 25-125µg/mL with R2= 0.9962 as shown in figure 2.

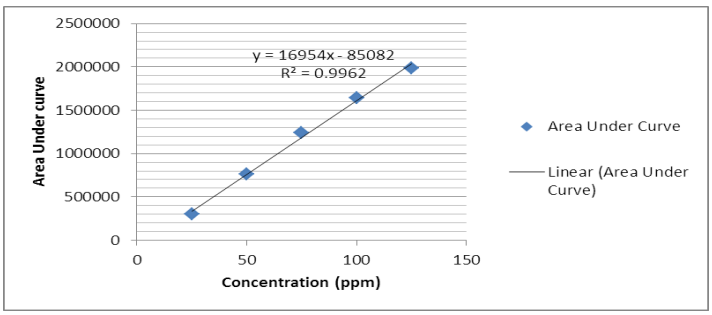

Table 2: Accuracy studies of Tramadol HCL.

	PEAK AREA		
CONCENTRATIONµg/mL	25µg/mL	75µg/mL	125µg/mL
Replicate 1	295502	1238383	1987153
Replicate 2	290759	1205147	1987404
Replicate 3	295635	1246203	1986257
AVERAGE	293965.3	1229911	1986938
SD	2777.562	21799.77	602.9685

The value of Standard Deviation is calculated for accuracy studies.

ACCURACY: The Tramadol was used for various concentrations and standard deviation is calculated for same as shown in table 2.

PRECISION

Repeatability: The repeatability, The RSD values were below 2%, indicating a good precision. The t-test value for inter-day precision was less than 0.1%, indicating the significant precision.

Table no3: Precision study of Tramadol HCL.

Concentration (µg/mL)	Peak area		
	25µg/mL	75µg/mL	125µg/mL
Replicate1	295502	1238383	1987153
Replicate 2	290759	1205147	1987404
Replicate 3	295635	1246203	1986257
AVERAGE	293965.3	1229911	1986938
SD	2777.562	21799.77	602.9685
%RSD	0.94486	1.772467	0.030347

Relative Standard Deviation = Less than 2 %

LOD AND LOQ: The LOD and LOQconcentrations of Tramadol were found to be 55.55ng/mL and 168.33ng/mL.

ROBUSTNESS OF THE METHOD

Table no 4: Robustness studies

Concentration µg/mL	Peak Area
25	293250
50	751887
75	1243326
100	1330659
125	1988268

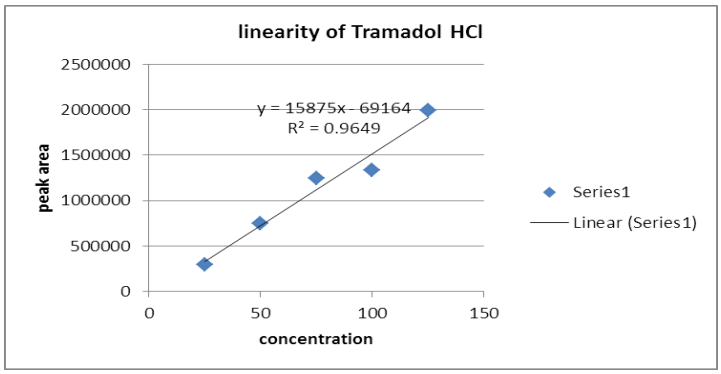

Fig 3. Robustness study using linearity curve

The regression coefficient of the curve is 0.9649

SYSTEM SUITABILITY STUDIES

The system suitability parameters such as retention time, theoretical plate number,Asymmetry factor were associated with confined values as shown in the table 5.

Table no.5: System suitability studies.

Concentration	Retention time	Theoretical plate no.	Asymmetry factor
25	2.132	3828	1.23
50	2.148	3248	1.03
75	2.187	2611	0.85
100	2.401	2318	0.85
125	2.206	2611	0.85

Number of theoretical plates is more than 2000

Asymmetry factor is less than 2.

CHROMATOGRAPHS OF TRAMADOL

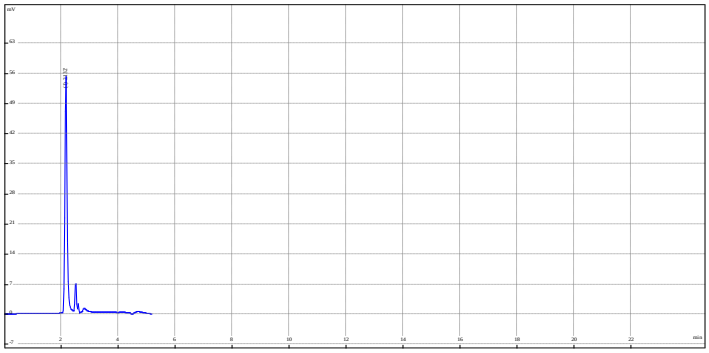

Fig4.chromatogram of 25 µg/mL .

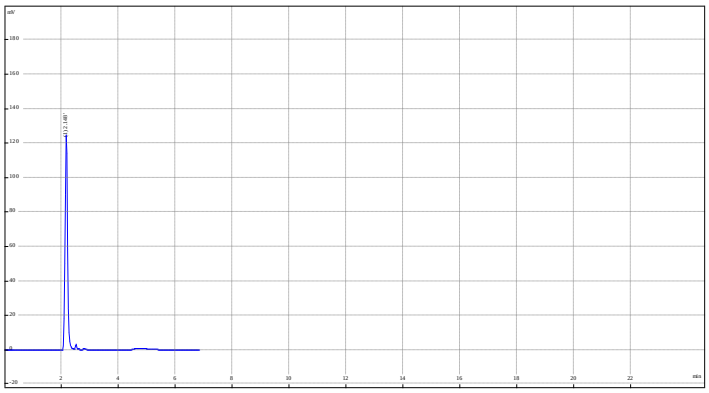

Fig5: 50µg/mL

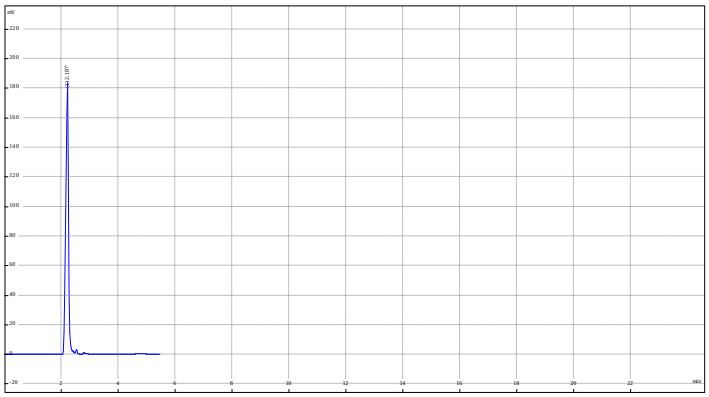

Fig6: 75 µg/mL

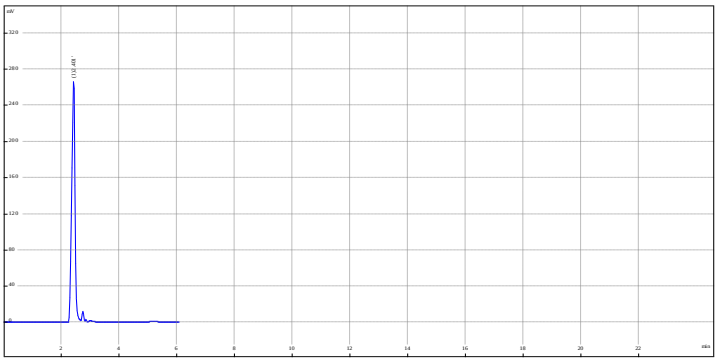

Fig.7: 100 µg/mL

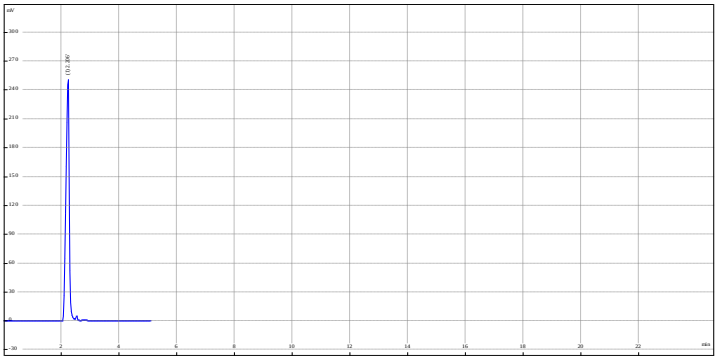

Fig.8: 125 µg/mL

DISCUSSION

The developed and validated method of Tramadol was aimed to establish chromatographic conditions, capable of qualitative and quantitative de-termination of Tramadol in pharmaceutical preparations. Tramadol was completely separated on C18 column by RP-HPLC using the isocratic elution of Methanol and water as mobile phase. When the Methanol percentage was increased starting from 50% by a decrement of every 5%, sharp peaks were observed. As a result of higher concentrations of Methanol in mobile phase and increase in the percentage of Methanol the peak was sharp pointed and well separated. The unusual peaks could be the result of improper dissolution of Tramadol in lower concentration of Methanol, therefore the chromatographic column was not able to resolve the Tramadol properly and even there was decrease in the recovery percentage of Tramadol. As Methanol concentration gradually decreases the peak broadening, fronting and tailing were remarkably reduced. It is evident that the flow rate of mobile phase in chromatography plays an important role in resolving the Tramadol, as the flow rate in-creases from 0.75 mL/min to 1. mL/min the retention time also decreased with fluctuation in Tramadol recovery (Table 1), eventually proper resolution was achieved at flow rate of 1mL/min and retention time of 2.1 minutes.

CONCLUSION

The optimized reverse phase HPLC method for Tramadol is linear, accurate, precise, robust, simple, rapid and selective. It can be adopted apparently for routine quality control analysis of raw materials, formulations and testing. The analysis time can be reduced due to less retention time and also cost effective mobile phase is useful for analysis.

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