IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2017 Vol.:10, Issue:3

Method Development and Validation of Tramadol Hydrochloride by RP-HPLC Method

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Khushbu Singh*

Department of pharmaceutical technology, RGPV, Patel collage of pharmacy, Bhopal.

Submission: 27 September 2017
Accepted: 5 October 2017
Published: 30 October 2017

Keywords: RP-HPLC estimation, Method development, Validation, Tramadol HCl.

ABSTRACT

A simple, selective, accurate and precise high-performance liquid chromatographic (HPLC) method for estimation of Tramadol HCl in bulk form was developed & validated. The estimation was carried out on X-Bridge C-18 (50x4.6 mm, 3.5µ) column using a mobile phase consisting of 5mM Ammonium acetate: ACN (50:50 v/v) of pH 6.5, at a flow rate 1 ml/min. The UV detection was carried out at 215nm. Method validation was performed as per the ICH guidelines. The method was validated for the precision, intermediate precision, accuracy, linearity, robustness, solution stability study, specificity, filter paper study.

INTRODUCTION

Tramadol is used to treat moderate to moderately severe pain. It has two different mechanisms. First, it binds to the mu opioid receptor. Second, it inhibits the reuptake of serotonin and norepinephrine.[1] IUPAC name of Tramadol Hydrochloride is (l RS, 2 RS)-2 [(dimethylamino)methyl]- 1-(3 methoxyphenyl) cyclohexanol hydrochloride. Tramadol is a synthetic codeine analog that is a weak μ-opioid receptor agonist. It is used as an oral non-steroidal anti-inflammatory drug with good analgesic and tolerability profile in various painful conditions.[2]

Tramadol HCl is an official drug in Indian Pharmacopoeia 2010-15, British Pharmacopoeia 2009-16 and United State Pharmacopoeia Tramadol is a synthetic analog of the phenanthrene alkaloid codeine. Tramadol is converted to O-desmethyltramadol, Opioids are chemical compounds which act upon one or more of the human opiate receptors. O-desmethyl tramadol is significantly more potent μ-opioid agonist then tramadol. The euphoria and respiratory depression are mainly caused by the μ1 and μ2 receptors; the addictive nature of opioids, is due to these effects, but tramadol's serotonergic and noradrenergic effects may contribute to possible dependence as well.[3]

MATERIAL AND METHODS:

Reagents and Chemicals: HPLC grade Methanol, Triple distilled water, Ammonium acetate were used in the study.

Chromatographic condition: A WATERS High performance liquid chromatograph equipped with SPD-20A UV detector, the purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecylsilane chemically bonded to porous silica particles of 3.5μm diameter reverse phase C18 column (Waters Symmetry RP C18, 4.6 x50mm, 3.5 μm particle size) The mobile phase consisting of 5 Mm Ammonium acetate: ACN (50:50).

Preparation of standard solution of TMH: 100 μg/ml solution of TRA was prepared by diluting 1ml stock solution to 10 ml with methanol and further diluted with methanol to get the concentration range of 10, 20, 30, 40, 50 μg/ml of TRA.

Preparation of stock solution of TMH: Weigh accurately 10 mg of TRH and transferred into 10 ml volumetric flask add 5ml of methanol and sonicated for 5 min and diluted up to mark with methanol to get a stock solution having strength 1000 µg/ml.

Method development: Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the process of demonstrating that analytical procedures are suitable for their intended use.

Validation parameter: The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to the identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

Typical validation characteristics, which should be considered, are listed below

Accuracy

Precision

Repeatability

Intermediate Precision

Specificity

Detection Limit

Quantitation Limit

Linearity

Range

Selectivity: It is the analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For selectivity, analysis of blank samples of the appropriate biological matrix should be obtained from at least six sources. Each blank sample should be tested for interference and selectivity, should be ensured at the lower limit of quantification.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products, and

excipients. Specificity measures only the desired component without interference from other species that might be present; separation is not necessarily required.

Linearity: Linearity is the ability of the analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to the concentration of an analyte in samples within a given range at which the instrumental response is proportional to the analyte concentration.

Accuracy: Accuracy is the nearness of a measured value to the true or accepted value. Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analyzed against the standard and blank solutions to ensure that no interference exists.

Precision: The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method.[7]

Robustness: robustness is defined as masseur of the ability of analytical method an analytical procedure to remain unaffected by small but deliberate variation in method parameter (pH, mobile phase composition, temperature and instrumental setting) and provides an indication of its reliability during normal usages.

System suitability parameter: system suitability parameter is the evaluation of a composition of an analytical system to show that the performance of the system meets the standard required by the method. This parameter can be calculated experimentally to provide a quantities system suitability test report number of theoretical plates (efficacy) capacity factor, separation (relative retention), resolution, telling factor relative standard deviation (precision).

RESULTS AND DISCUSSION

Specificity: The specificity of the method was determined by checking the interference of placebo with the analyte and the proposed method were eluted by checking the peak purity of tramadol hydrochloride during the forced degradation study.

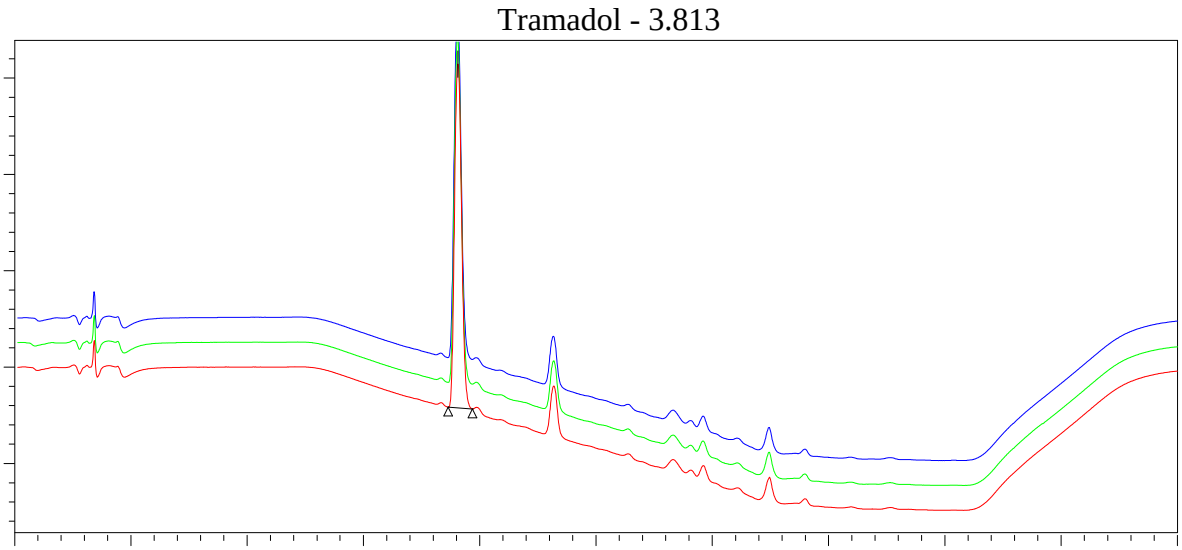

Figure 1: Chromatogram of standard preparation (Tramadol HCl)

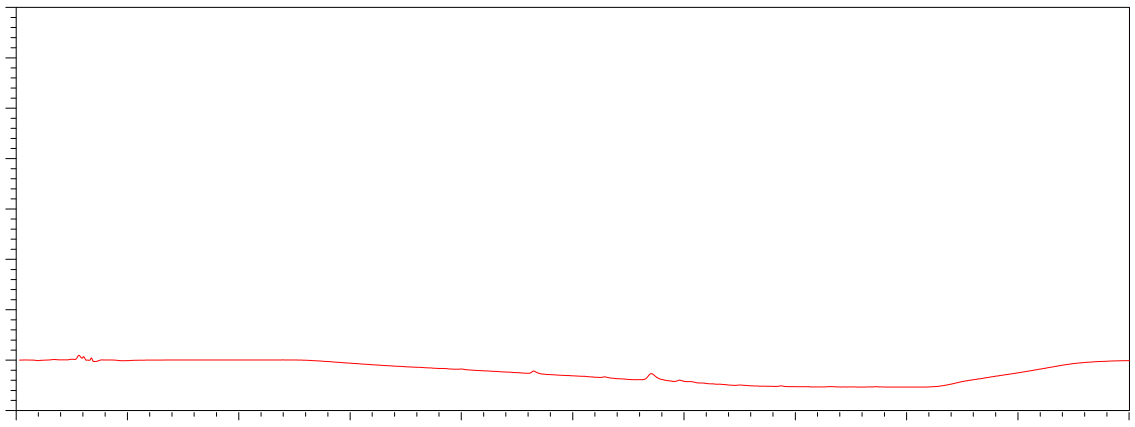

Figure 2: Chromatogram of placebo preparation (excipients)

Linearity: For linearity, even points calibration curve were obtained in a concentration range from 0.025-0.200 mg/ml for tramadol hydrochloride. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for tramadol hydrochloride was Y= 13385x+12864y with correlation coefficient 0.994 (Figure

3). Where x is the concentration of mg/ml and y is the peak area in absorbance unit. Chromatogram obtains during linearity study.

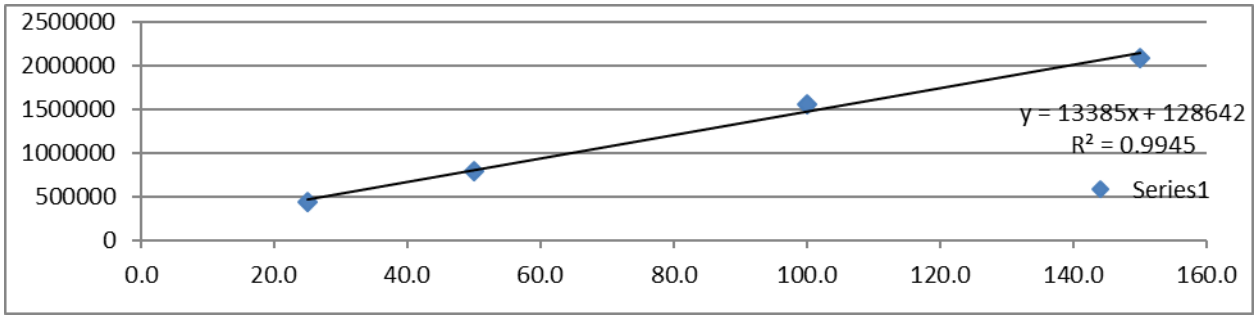

Figure 3: Linearity plot of tramadol HCl

Table I: Results of precision study of Tramadol Hydrochloride Intraday (n = 6)

Sr. No	Area	Mean area
Sample 1	1595289.1	1587758
	1580226.8	
Sample 2	1566001.8	1575667
	1585331.9	
Sample 3	1587305.1	1580535
	1573765.0	
Sample 4	1564413.0	1567462
	1570510.9	
Sample 5	1562013.7	1557127
	1552240.2	
Sample 6	1566967.5	1566966
	1566965.2	
Mean		1572585.84
Std Dev		10951.60
% RSD		0.70

Accuracy: Recovery of tramadol hydrochloride was determined at three different concentration levels. The mean recovery for tramadol hydrochloride was 97.00-98.22% (Table II).The result indicating that the method was accurate.

Table II: Results of accuracy study

Level	Concentration ppm	Area	Mean Area	Amount Added (mg)	Amount Added (ug)
50%	50	756879.58	756941.3367	24.8	49.60
		757097.12			
		756847.31			
100%	100	1556536.99	1555178	50.32	100.64
		1554162.7			
		1554835.67			
150%	150	2326588.68	2322664	75.58	151.16
		2318213.6			
		2323190.02			

Amount recovered (ug)	% recovery	mean % recovery	STD dev	% RSD
48.11	97.00	97.00	0.02	0.02
48.12	97.02			
48.11	96.99			
98.94	98.31	98.22	0.08	0.08
98.79	98.16			
98.83	98.20			
147.89	97.83	97.67	0.18	0.18
147.35	97.48			
147.67	97.69			

Solution stability study: Table III shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 18 h at 2 - 5 0C and ambient temperature with the consideration of < 2.0 % in the % assay value difference of interval value against initial value.

Table III: Evaluation data of solution stability study

% Assay for test solution stored at ambient temperature Tramadol Hydrochloride Initial

Sr. No.		Area		Mean area
STD Initial		1604632.39		1612925.255
		1621218.12		
Sample Initial		1596497.91		1585237
		1573976.19		
Primary dilution		**Secondary dilution**		
Volume(ml)	Sample wt(mg)	Diluted of (ml)	Diluted to (ml)	% Assay
50.00	245.10	1.00	10.00	99.12
50.00	245.10	1.00	10.00	97.42

% Assay for test solution Intervals (12 h) at RT

Sr. No.	Area	Mean area
STD RT	1626426.87	162426.87
	1626426.87	
Sample RT	1571287.67	1576228
	1581167.51	

Primary dilution		Secondary dilution		
Volume (ml)	Sample wt (mg)	Diluted of (ml)	Diluted to (ml)	% Assay
50.00	245.10	1.00	10.00	99.95
50.00	245.10	1.00	10.00	96.87

(12 h) at freeze

Sr. No.		Area		Mean area
STD freeze		1614216.23		1613343
		1612470.52		
Sample freeze		1583503.72		1583930
		1584355.72		
Primary dilution		Secondary dilution		
Volume (ml)	Sample wt (mg)	Diluted of (ml)	Diluted to (ml)	% Assay
50.00	245.10	1.00	10.00	99.15
50.00	245.10	1.00	10.00	97.34

% Assay for test solution Intervals (18 h) at RT

Sr. No.	Area	Mean area
STD RT	1641638.99	1641571.635
	1641504.28	
Sample RT	1596086.34	1590362
	1584636.67	

Primary dilution		Secondary dilution		
Volume(ml)	Sample wt(mg)	Diluted of (ml)	Diluted to (ml)	% Assay
50.00	245.10	1.00	10.00	100.88
50.00	245.10	1.00	10.00	97.74

(18 h) at freeze

Sr. No.		Area		Mean area
STD freeze		1609545.84		1611441
		1613335.68		
Sample freeze		1584200.01		1585260
		1586319.09		
Primary dilution		**Secondary dilution**		
Volume(ml)	Sample wt (mg)	Diluted of (ml)	Diluted to (ml)	% Assay
50.00	245.10	1.00	10.00	99.03
50.00	245.10	1.00	10.00	97.42

Robustness: The result of robustness study of the developed assay method was established in **Table IV**. The result showed that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual.

Table IV: Evaluation data of robustness study of tramadol hydrochloride

Robust Conditions	% Assay	System Suitability Parameters			
		Area	Mean area	STD Dev	% RSD
Flow 0.9ml/min	97.65	1484042	1493997.20	14078.70	0.94
		1503952			
Flow 1.1ml/min	96.73	1499131	1501541.51	3409.44	0.23
		1503952			
Low column temp	100.95	1476544	1486024.54	13407.69	0.90
		1495505			
High column temp	100.24	1503952	1475589.11	40111.67	1.72
		1447226			

CONCLUSION

It can be concluded from the entire work that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

Analytical method development followed by method validation is an important process in the drug discovery. Although the drug shows good potency, lack of validated analytical method will not allow the drug to enter the market. This is to ensure the quality and safety of the drug. The analytical methodology provides to an analyst the required data for a given analytical problem, sensitivity, accuracy, range of analysis, precision i.e. the minimum

requirements, which essentially are the specifications of the method for the intended purpose to be able to analyze the desired analyte in different matrices with surety and certainty. The main objective of this work is to give an idea about the old and novel techniques available for the analysis of drugs in their raw material and formulated forms, check the stability of the drugs in the presence of the excipients and other stress conditions experienced during their shelf life period.

Analytical methods need to be validated before their introduction into routine use; whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and whenever the method is changed, the change is outside the original scope of the method. The stability indicating assays have been developed for a large number of drugs but most of them fail to meet current regulatory requirements for separation and analysis of individual degradation products. So the discussion provided would be general and of wide use. Nowadays, it is a mandatory requirement in various pharmacopeias to know the impurities present in API's

The knowledge of the pKa, pH, and solubility of the primary compound is of utmost importance prior to the HPLC method development. Knowledge of pH can help to discern the ionizable nature of the other impurities (i.e., synthetic byproducts, metabolites, degradation products, etc.) in the mixture. Selection of buffer and mobile phase composition (organic and pH) plays a dramatic role on the separation selectivity. Final optimization can be performed by changing the temperature, gradient slope, and flow rate as well as the type and concentration of mobile-phase modifiers. The optimized method is validated with various parameters (e.g. accuracy, precision, specificity, linearity, detection limit etc.) as per ICH guidelines. The use of the C18 column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor. So the C18 column can be used to achieve high specificity in the shorter time of analysis of Tramadol HCl as per ICH Q2 (R2) guidelines. The proposed method was found to be simple, precise, accurate, linear, robust and rapid determination and quantification of Tramadol HCl. The sample recoveries were in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Tramadol HCl in capsule dosage forms. This developed and validated the method for analysis of TMD in pharmaceutical preparations is very rapid, accurate, and precise. The method was successfully applied for Parameter.

Range 0.25 µg/mL, 50 µg/ml, 100 µg/mL

Retention time (min) 3.813

Accuracy (% RSD) 0.02, 0.08, 0.18

Precision (%RSD) Intra-day (n=3) 0.70

Results from the robustness study of method.

Robust Conditions: Flow0.9mL/min, Flow 1.1mL/min, Low column temperature, High column temperature 0.94, 0.23, 0.90 respectively.

Determination of TMD in its pharmaceutical capsule formulations. Moreover, it has advantages of short runtime and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence, this method can be conveniently used for routine quality control analysis of TMD in its pharmaceutical formulations.

REFERENCES:

1. Goodman and Gilman's S. The pharmacological basis of therapeutics. 10th ed. Delhi, McGraw Hill; 2001
2. Manikandan, K.; Kathiravan, S.; Varatharajan, P.; Sabarikumar, K.; Karuppasam, C. and Radhakrishna, K. Development and validation of tramadol hydrochloride in bulk and solid dosage form by RP-HPLC method. International Journal of Biological & Pharmaceutical Research (IJBPR). 2012: 3(2): 245-251.
3. Singh Ranji. HPLC method development and validation- an overview. J Pharm Educ Res. June2013. 2013 Vol. 4: Issue No. 1: Received, May 14, 2013, Accepted, May 22, 2013.
4. ICH guidelines. Validation of Analytical Procedures: Text and Methodology Q2 (R1), in Proceedings of International Conference on Harmonization. 2005.
5. Sudha T., Kanth, Krishana V., Nukala Poorana Chandra Sainath, Mishal, Saloman Raja T. and Ganesan V. Method Development and Validation- A Review. Journal of Advanced Pharmacy Education & Research. 2012. 2 (3): 146-176. ISSN 2249-3379:146
6. Gupta V., Jain A. D. K., Gill N. S. and Gupta K. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, Int. resch. 2012. App Sci., 2(4): 17-25.
7. Sabir Md A., Moloy M. and Singh B. P. Development and validation of HPLC method - a review. international research of pharmacy. 2013; 4 (4): ISSN 2230 – 8407.
8. Anonymous 1. www.wikipedia.org/wiki/Tramadol Hydrochloride.