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Research Article

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

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ABSTRACT

A simple, linear, precise, accurate and selective RP-HPLC method has been developed and validated for estimation of Tramadol HCl from pharmaceutical dosage form. Gradient elution at a flow rate of 0.7 ml/min tried onKromasil C18 column using a mobile phase consisting mixture of Methanol: Water (60:40v/v) pH-3 adjusted with ortho phosphoric acid. The retention time of Tramadol HCl was 3.27mins. The eluent was detected at 219 nm. Linearity was observed in the concentration range of 30-80 µg/ml for Tramadol HCl. The method is validated as per ICH guidelines. The proposed method can be successfully applied for estimation of tramadol hydrochloride in Pharmaceutical dosage forms.

***KEYWORDS**: Tramadol HCl, RP-HPLC Method, Mobile Phase, Validation.*

INTRODUCTION [1]

Tramadol is a synthetic centrally acting analgesic with a weak opioid agonistic activity. Chemically it is (1R,2R)-2-(dimethylamino)methyl))-1-(3-methoxyphenyl)cyclohexanol. It has been used in the treatment of post surgical pain, obstetric pain, cancer pain, and chronic pain of mechanical and neurological origin. Tramadol has half life of 6.3hrs and hence required frequent dosing.

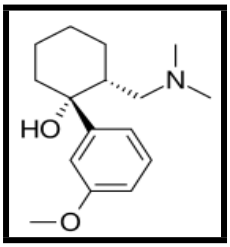

Fig. 1: Structure of Tramadol

Few analytical methods such as UV-visible spectroscopy, Potentiometry, HPLC are available for estimation of tramadol in API and Dosage forms [2-8] but they are suffering from one or other problem. Hence the present work is designed to develope and validates the simple, reliable and economic RP-HPLC method for routine analysis of tramadol.

MATERIAL AND METHOD

Instruments and analytical condition:

The HPLC analysis is carried out on Waters HPLC system equipped with UV-visible detector with Auto sampler running on DATA ACE software. The column used is Kromasil C18 (250 × 4.6 mm, 5µm) and detection was performed at 219 nm. The injection volume was 20µL and run time was 8 min. The mobile phase was used methanol and water in the ratio of 60:40 (v/v) with flow rate of 0.7ml/min. The mobile phase was filter with 0.45µm membrane filter and degassed before use.

Chemicals and Solvents:

Tramadol Hydrochloride (API) is obtained as gift sample Mecloid pharma Ltd (Mumbai). The tablets are purchased from local pharmacy Painadol SR (FDC Ltd).HPLC Grade solvents Methanol, Water (Merck), OPA (Finar) are used for study.

Selection of mobile phase:

After various trial ideal mobile phase selected is combination of Methanol and Water in the ratio of 60: 40 (v/v).

Preparation of Standard solution:

The 10 mg of standard drug is transferred to 10 ml vol. flask and volume is adjusted to 10 ml by using mobile phase. So it gives a conc. of 1000µg/ml. The given stock solution was filtered with 0.45 um membrane filter and sonicated for 10 min. of 3 cycles. Solution was further diluted to get a solution having a concentration of 100µg/mL of Tramadol Hydrochloride

Preparation of Sample solution:

Twenty tablets were weighed accurately and finely powdered. The powder (0.348g) equivalent to 50 mg of Tramadol was weighed accurately and dissolved in 50 mL Mobile phase to get conc. of 1000ug/ml. The solution was ultra sonicated for 15-20 min. and stirred magnetically for few minutes. Then it was filtered through 0.45µm membrane filter paper. Solution was further diluted to get a solution having a concentration of 100µg/mL of Tramadol Hydrochloride

Method Validation: [9-11]

Objective of method validation is demonstrating that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated in terms of linearity, range, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitaion (LOQ).

Linearity and Range:

Six different concentrations (30, 40, 50, 60, 70 and 80µg/ml) of Tramadol Hydrochloride were prepared for linearity studies. The responses were measured as peak area. The calibration curves obtained by plotting peak area against concentration showed linearity in the concentration range of 30-80ppm

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Table No. 1: Linearity study

Tramadol Hydrochloride	
Conc. (ppm)	**Peak area**
30	1270232
40	1689410
50	2193603
60	2526482
70	2944116
80	3387451

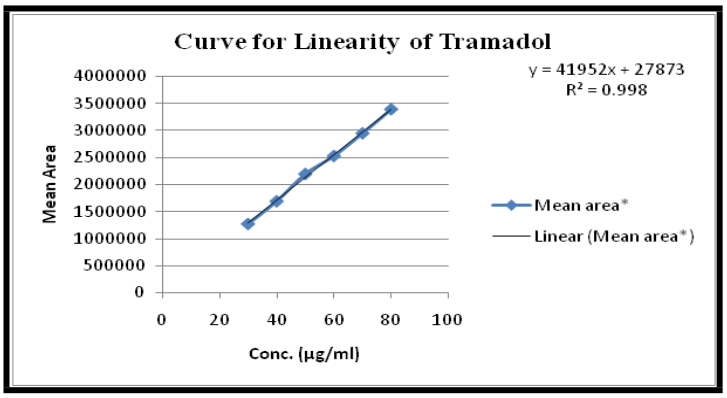

Fig. 2: Linearity study curve of tramadol hydrochloride

Selectivity and Specificity:

The selectivity of the method was checked by injecting 30ug/ml standard and sample solutions, it was observed that peaks for Tramadol Hydrochloride was obtained at retention times 3.27 min. Retention times of standard drugs and drugs from the sample solutions were same also sample peak is not interfered by other peaks.

Precision:

From the standard stock solutions, three conc. 35µg/ml, 55µg/ml, 75µg/ml Standard solutions (n=6) were injected using injector with injection volume of 20µL. The intraday and interday precisions were determined. % RSD value shown is less than 2.0 shows that method is precise. Result is given in Table 2

Table No. 2: Result for Precision data

Sr. No.	Conc. (µg/ml)	Intraday precision		Interday precision	
		Mean area	% RSD	Mean area	% RSD
01	**35**	**1486530.33**	**0.68**	**1490072.22**	**0.63**
02	**55**	**2289851.33**	**0.702285031.67**	**0.25**	
03	**75**	**3122328.00**	**1.803121993.67**	**1.20**	

Accuracy:

Recovery studies were carried out by applying the standard addition method. A known amount of standard TRD corresponding to 80%, 100%, and 120% of the label claim was added to preanalyse sample of tablet dosage form separately. The recovery studies were carried out six times, at each level of recovery. The Result is within limit as shown in table No.3

Table No. 3: Accuracy and Recovery data

Drug	Recovery level	Amount Added (µg)	Amount Found (µg)	% Recovery	Limit (98-102%)
TRD	**80**	**24**	**24.07**	**100.30**	**passed**
TRD	**100**	**30**	**29.79**	**99.30**	**passed**
TRD	**120**	**36**	**35.53**	**98.57**	**passed**

Robustness and Robustness:

Robustness is a reliability of analysis with respect to intentional change in method parameter. Robustness testing is done by slight change in mobile phase composition and varying flow rate. Ruggedness is the degree of reproducibility of the result on same sample under variety of test condition such as Different instrument analyst, days etc. It is carried out by two different analysts on two different instruments on two different days. The method is found robust and rugged. The result for robustness and ruggedness is shown in table 4 and 5 respectively.

Table No. 4: Result for Robustness study

Sr. No.	Conc.	Parameter	Variation	Mean area	% Assay Limit (98-102%)
1	**30**	Mobile phase	62:38	1310314101.49	Passed
2	**30**	Mobile phase	58:42	126723498.47	Passed
3	**30**	Flow rate	0.8 ml/min	127835498.03	Passed
4	**30**	Flow rate	0.6 ml/min	1313734101.96	Passed

Table No. 5: Result for Ruggedness study

Sr. No	Conc.	Analyst	Day	Instrument	Mean Area	% Assay	Limi (98-102%)
1	**30**	**A**	1	1	1275478	101.49	Passed
2	**30**	**B**	2	2	1318793	98.47	Passed

LOD and LOQ:

The lowest concentration which can be detected by HPLC and LOQ is the lowest concentration which can be quantified with precision and accuracy both of these can be determined by regression line. The result is given in table no. 6.

Table No. 6: Result for LOQ and LOD

Drug	LOD (µg/ml)	LOQ (µg/ml)
Tramadol HCl	0.44	1.32

Assay of Marketed preparation:

Twenty tablets (Painadol SR, 50 mg Tramadol, FDC Ltd) were weighed accurately and finely powdered. The powder (0.348g) equivalent to 50 mg of Tramadol was weighed accurately and dissolved in 50 mL Mobile phase for HPLC. The solution was ultrasonicated for 15-20 min. and stirred magnetically for few minutes. Then it was filtered through 0.45µm membrane filter paper. Solution was further diluted to get a solution having a concentration of 30µg/mL of Tramadol Hydrochloride.20µL of this solution was injected in triplicate under the specified conditions. From the peak area of Tramadol the amount of drugs in samples was computed.

System suitability testing:

System suitability is defined as examination of system previous to or during analysis to ensure system concert.

Chromatographic Conditions:

The following optimized parameters were used as a final method for the estimation of tramadol hydrochloride in bulk and dosage form.

Table No. 7: Assay of marketed product

Drug	Mean area	Label claim (mg)	Amount found (mg)	%Amount found < 2%	S.D.	%R.S.D.
Tramadol	1313789	50	49.52	99.04	12345	1.20

Table No. 8: System Suitability Parameter

Parameter	Mean study	Limits	Inference
Retention time (tR)	3.27	NLT 2.0 min	Passed
Peak Area	3387938	NLT 2000	Passed
Theoretical plates (N)	2749	NLT 2000	Passed
Tailing factor (Tf)	1.24	NMT 2.0	Passed
% RSD of RT	0.44	NMT 5%	Passed

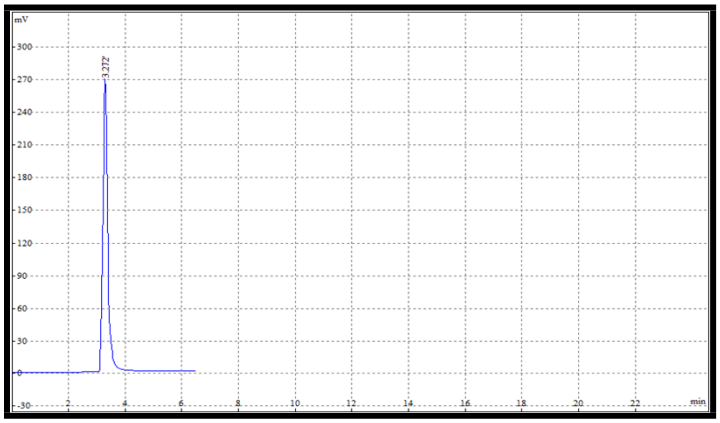

Fig. 3: Typical Chromatogram for system suitability testing

Table No. 9: Chromatographic Conditions

Instrument	Conditions
Column	KromasilC18(250x4.6mm,5µm)
Mobile phase	Methanol :Water (60:40)
Flow rate	0.7mL/ min
Wavelength	219 nm
Injection volume	20µl
Run time	8 min
Temperature	Ambient
Mode of Operation	Gradient elution

RESULTS AND DISCUSSION

Several mobile phase compositions were tried to enhance the peaks of Tramadol. The optimum mobile phase containing Methanol: Water (60:40v/v) was selected because it gives sharp peak. The pH was adjusted to 3.0 with orthophosphoric acid. Quantification was achieved with UV detection at 219 nm on the basis of peak area. A linearity study show good linear co relation exists between conc. and absorbance between conc. range 30-80ug/ml. The limit of detection (LOD) and limit of quantitaion (LOQ) were found to

be 0.44ug/ml and 1.32ug/ml. The values indicate that the method is sensitive. The intra-day and inter-day precisions were assessed by analyzing standard solutions. The % RSD was found to be below 1%. Also accuracy study is carried out .The lower values of % RSD indicate that the method is precise and accurate.

Analysis of marketed tablets was carried out using optimized mobile phase. The % drug content of tablets obtained by the proposed method was found to be between 99.04 which showed that the estimation of dosage forms were accurate within the acceptance level of 98% to 102%.

Table No. 10: summary of results of validation parameters

Parameters	Results
Linearity Range (µg/ml)	30-80 µg/ml
Correlation coefficient	0.998
LOD	0.44µg/ml
LOQ	1.32µg/ml
Precision (% RSD)	< 2.0
Accuracy	99.39%
Robustness	Robust
Assay	99.04%

CONCLUSION

The method has short analysis time. Percentage recovery shows that the method is free from interference of the excipient used in the formulation. Proposed study describes an HPLC method for the estimation of Tramadol. The validated isocratic reversed phase method employed here proved to be simple, sensitive, fast, accurate, precise and robust. Therefore, the proposed method can be used for routine analysis of Tramadol in tablet dosage form.

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