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DEVELOPMENT AND VALIDATION OF RAPID AND SIMPLE HIGH PERFORMANCE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TRAMADOL HYDROCHLORIDE FROM BULK AND FORMULATION

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ABSTRACT

The objective of the study is to develop simple, precise, selective and stability indicating high-performance liquid chromatography (HPLC) method for determination of Tramadol hydrochloride (TH) in bulk and from formulation. The chromatographic conditions comprised a reverse-phase C_{18} column, mobile phase consisting of a mixture of Orthophosphoric acid, in 2000 ml of milli-Q water (pH 6.5 adjusted with Tri ethyl amine) and Acetonitrile in the ratio of 60:40. The flow rate was adjusted to 0.8 ml/min and the detection was carried out at 273 nm. The retention time of TH was 3.1 min. The linear regression analysis data for calibration plots showed good linear relationship with coefficient of correlation value, r = 0.996 for TH in the concentration ranges of 30-50 µg/ml. The method was validated for precision, accuracy and robustness. The developed method was found to be suitable for routine quality control analysis of TH from bulk as well as formulation without interference of excipients.

KEYWORDS: Tramadol hydrochloride • stability indicating method • precision • accuracy • robustness.

INTRODUCTION

Tramadol hydrochloride (TH) (Molecular Formula: $C_{16}H_{25}NO_2$.HCl, Molecular Weight: 263.38 g/mol) an opioid analgesic is used for management of moderate to severe pain. TH is

chemically (±)-Trans-2 dimethylaminomethyl-1-(3-methoxyphenyl) cyclohexanol hydrochloride.[1] It acts via two mechanisms, by binding to the μ-opioid receptor and inhibiting the uptake of serotonin and norepinephrine.[2] The individual determination of TH has been carried out in tablets and different solid dosage forms by HPLC, in human plasma and with its active metabolite in human plasma by HPLC and by TLC densitometric method.[3] As per United States Pharmacopoeia (U.S.P) the assay method for TH is HPLC.[4] As per British Pharmacopoeia the assay method for TH is potentiometric titration.[5] The objective of present work is to develop and validate new analytical method for rapid estimation of TH in bulk drug by high-performance liquid chromatography (HPLC).

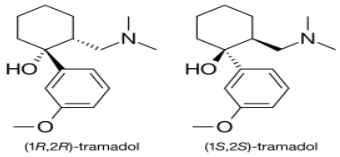


Fig. 1: Structure of Tramadol hydrochloride

MATERIALS AND METHODS

MATERIALS

Tramadol hydrochloride was procured as ~~a~~ gift sample from Indeus Pharmaceuticals Pvt. Ltd. (Mumbai, India). All other chemicals and solvents used were of Analytical Reagent/ HPLC grade.

Instrumentation

RP-HPLC analysis was carried out using Thermo finnigan software. To select the appropriate column for good elution, well shaped symmetrical peaks and high resolution, one column (Hiq Sil C_{18} column 250mm × 4.6 mm × 5μm particle size) was tested primarily. The studies revealed that the Hiq Sil symmetry C_{18} column was the best one, because it produced good elution of the drug, well shaped symmetrical peaks with high resolution, limited with the injector loop of 20 μl in isocratic mode. The pump used was P4000 plus HPLC pump and the detector UV 2230 plus detector.

Method Development

Preparation of Mobile Phase

In HPLC grade water, 0.1 ml Orthophosphoric acid was added and sonicated for 5 minutes. The pH was adjusted to 6 with Triethylamine (0.5 ml) and sonication continued for 5 minutes and then volume was made up with acetonitrile to 100 ml and again sonicated for 5 minutes.

Preparation of Standard Solution

Preparation of Stock Solution of TH: Twenty five mg of TH was weighed accurately and transferred into 25 ml volumetric flask mobile phase was added to get stock solution of 1000 µg/ml.

Preparation of Working Standard Solution of TH

Stock solution was serially diluted with mobile phase to get the concentration of 30, 35, 40, 45, 50 µg/ml of TH.

METHOD VALIDATION

Validation is a process which provides high degree of assurance that activity will consistently produce a desired result to meet its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of detection (LOD) and Limit of Quantification (LOQ) as per ICH guideline.[6]

Linearity

Various aliquots were prepared from the stock solution (1000 µg/ml) ranging from 30-50 µg/ml. Peak areas and concentrations were subjected to square least regression analysis to calculate the regression equation.

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations i.e. 50%, 100% and 150% and was analyzed in triplicate in each level of addition. The % RSD and % Recovery were within the acceptable limits in all cases.

Precision

Precision studies were carried out in terms of repeatability. Repeatability of the standard application was carried out by injecting six replicates of the standard at concentration of 40µg/ml.

Robustness

Robustness studies were carried out by making small deliberate changes in the flow rate ±0.2ml/min and wavelength of detection ±1nm and changes in parameters such as number of theoretical plates and tailing factor.

System Suitability Parameters

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. System suitability studies were carried out by injecting 5 doses of different concentrations i.e. 30, 35, 40, 45, 50 (µg/ml). The % RSD values for system suitability test parameters like Retention time (R_t), Number of theoretical plates (NTP) and tailing factor were determined.

Limit of Detection (LOD)

LOD value for the drug was calculated employing the average slope and standard deviation values from the calibration curves.

$$LOD = \frac{3.3 * SD}{S}$$

Where, SD is the standard deviation of y-intercept and S is the slope of calibration curve.

Limit of quantification (LOQ)

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve.

$$LOQ = \frac{10 * SD}{S}$$

Where, SD is the standard deviation of y-intercept and S is the slope of calibration curve.

ASSAY METHOD

Tablets were crushed in mortar and 50 mg of powder was transferred to volumetric flask. To the flask, ethanol (10 mL) was added and sonicated for 15 minutes. Then volume was made upto 100ml with 0.1N HCl (stock solution). The stock solution was diluted suitably to get a sample having concentration of 50µg/mL. Percent assay of the formulation was calculated as an average of three determinations.

RESULTS AND DISCUSSION

For the selection of mobile phase, various mobile phase systems were tried for the chromatographic separation. For the selection of analytical wavelength, the spectra of TH was scanned from 400 to 200 nm and showed peak at 273 nm. The calibration curve showed good linearity that obeys Beer- Lambert's law. The Beer- Lambert's concentration range was found to be 30-50 µg/ml at 273 nm and coefficient of correlation was found to be 0.996 and 1 (Table 1) for proposed method.

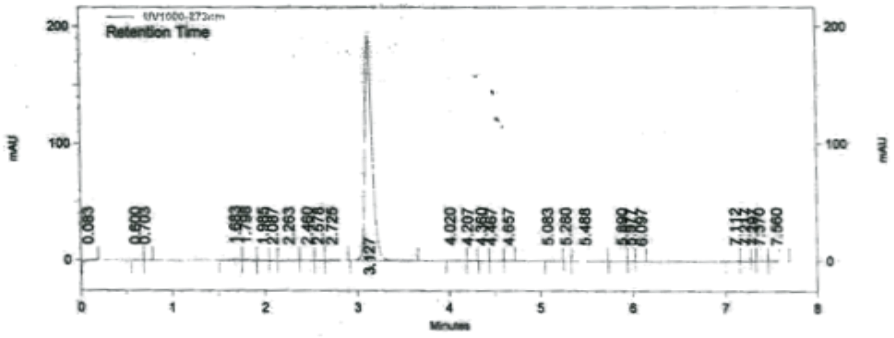


Figure2: Chromatogram of Tramadol Hydrochloride

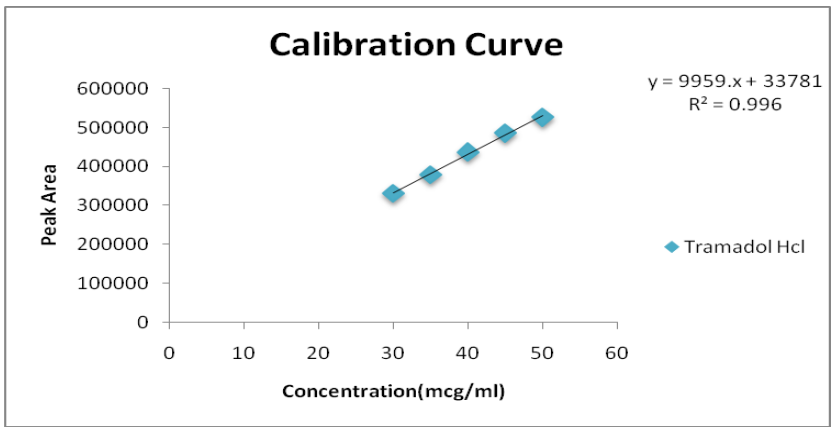


Fig.3: Calibration Curve of Tramadol Hydrochloride.

Calibration curve were prepared by taking appropriate aliquots of TH Standard solutions were injected through 10 µL loop system and chromatograms were obtained using 0.8 ml/min flow rate. The effluent was monitored at 273 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

METHOD VALIDATION

The developed method was found to be precise as the % RSD values for inter-day and intra-day were found to be less than 2% which indicates good intermediate precision (Table 3 and 4). Good recoveries of the drug were obtained at each added concentration, which indicates the method was accurate. The LOD and LOQ were found to be in microgram level, which indicates that the method is highly sensitive Finally, the system containing, orthophosphoric acid, in 2000ml of milli-Q water, 6.5 pH adjusted with Tri ethyl amine & Acetonitrile in the ratio of 60:40 %v/v gave well resolved peaks. The average retention time of TH is 3.1 min. The method was found to be robust and rugged as indicated by % RSD values which are less than 2%.

Table 1: It shows optical characteristics of Tramadol HCl

Parameters	Tramadol HCl
λmax (nm)	273 nm
Beer's range (µg/ml)	30-50 µg/ml
Correlation Coefficient (r^2)	0.996
Regression equation	y = 9959x+33781
Intercept (a)	33781
Slope (b)	9959
Flow Rate (ml/min)	0.8

TABLE 2: It Shows Results of System Suitability

Conc (µg/ml)	R_t	NTP	Assymetry Factor
30	2.967	1.189	7426
35	2.988	1.186	7644
40	2.98	1.201	7606
45	2.975	1.228	7525
50	2.983	1.231	7641
Average	2.9786	1.207	7568.4
SD	0.0080	0.0213	92.953
% RSD	0.0060	0.018	74.32

R_t: Retention Time, NTP: Number of Theoretical Plates, SD: Standard Deviation, %RSD: Relative Standard Deviation

Linearity

It was found that the drug showed linearity from 30-50 µg/ml.

TABLE 3: It Shows Results of Linearity

Conc (µg/ml)	Average Peak Area± SD
30	331220.33 ± 110.23
35	379290.67± 65.51
40	437401.33± 96.32
45	486231.33±46.33
50	526748.67±59.84

Results are mean of three readings and expressed as mean, standard deviation

Accuracy

TABLE 4: It Shows Results of Accuracy

Conc (µg/ml)	Average	%Recovery	%RSD	Mean %Recovery
50%	263109.7	50.12	2.22	100.49
100%	463681.3	101.19	2.11	
150%	871504.7	150.18	1.88	

Results are mean of three readings and expressed as mean, standard deviation

%RSD: Relative Standard Deviation

Precision

TABLE 5: It Shows Results of Precision

Conc (µg/ml)	Peak Area	R_t
30	432981	3.1
35	432983	3.1
40	432982	3.1
45	432980	3.1
50	432985	3.1
Average	432986.8	3.1
SD	2.3166	4.864
%RSD	1.8333	4.440

Results are mean of six readings and expressed as mean, standard deviation and %COV: coefficient of variance.

Robustness

TABLE 6: It Shows Results of Robustness

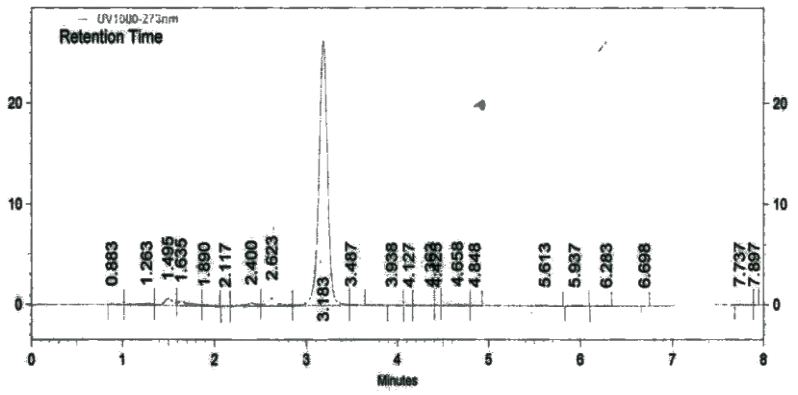
Chromatographic Parameters	Retention Time (min)	Peak Area
Flow Rate (ml/min)		
0.6(-0.2%)	4.13	555850
0.8	3.13	426209
1.0(+0.2%)	2.51	351793
Wavelength		
270(-nm)	3.13	426046
273(nm)	3.13	426209
274(+nm)	3.13	421197

Robustness of the method was carried out by deliberately made small changes in the flow rate and wavelength and therefore changes in parameters like Retention Time and Peak area. Small changes in operational parameters do not affect the results.

LOQ and LOD

The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1). The LOD was found to be 0.0007 µg/ml, and the value LOQ was found to be 0.002 µg/ml.

Assay



Chromatogram of results for assay

TABLE 7: It Shows Results of Assay

Sample	Lable claim (mg)	Percentage Recovery	
Tramadol Hydrochloride	100	Amount Found	% Assay
		98.29±0.9	98.29

The percent assay was about 98.29%. These results indicate that the present HPLC method can be successfully used for the analysis of tramadol hydrochloride in bulk and dosage forms.

CONCLUSION

All the above factors conclude that the method is accurate, precise, simple, sensitive and economic and can be adapted for routine analysis of drugs in tablet dosage form successfully. The proposed method can be used for the estimation of Tramadol hydrochloride in bulk and pharmaceutical formulation without interference from excipients.

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