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Stability indicating RP-HPLC method for the determination of Tramadol Hydrochloride in Sterile Dosage form

K. Dhanalakshmi*[1], S. Sumitha[1], and S. Sivaramakrishnan[2]

[1]Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University Tirunelveli-627012, India

[2]Caplin Point Laboratories, Chennai – 601201, India

Abstract : A simple, well-organized and reproducible RP-HPLC method for determination of Tramadol Hydrochloride injection dosage form has been developed and validated. The Chromatographic Separation was carried out on Zorbax C_8 (250× 4.6 mm; 5µm) column using the mobile phase consists of buffer and Acetonitrile in the ratio 65:35. The mobile phase was flowed at the rate of 0.1 ml/min and effluent was detected at 270 nm. The retention time of Tramadol Hydrochloride was 4.273 min. The method was validated according to ICH guidelines and the acceptance criteria for specificity, linearity, accuracy, precision, robustness, and ruggedness were met in all cases. The method was linear in the range of 50µg/ml of Tramadol Hydrochloride. The percentage relative standard deviation for precision was found to be less than 2.0%.

Keywords: Tramadol Hydrochloride, RP-HPLC, Sterile dosage form, Stability indicating method.

Introduction

Tramadol Hydrochloride is a class of drug called analgesic drugs. It works by narcotic to relieve pain in the body. It is used to relieve the moderate and severe pain in the body. The analgesic effect of Tramadol Hydrochloride may result from the narcotic inhibitor. Tramadol Hydrochloride belongs to amino cyclohexanol group[(1-5)].Various analytical methods had reported in literature for the estimation of Tramadol Hydrochloride individually and in combination with other drugs by HPLC method[(6-7)], UV Spectrophotometry, Reversed phase ion pair High performance liquid chromatography, and by Mass spectrophotometry[(8-10)] in oral dosage form. In this present work, an attempt was made to develop an easy, sufficient stability indicating method for the estimation ofTramadol Hydrochloride in sterile dosage form by RP-HPLC. The proposed method was validated in accordance with International Conference on Harmonization (IHC) guidelines.

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The chemical name for tramadol hydrochloride is (±)cis-2[(dimethylamino) methyl]-1-(3methoxyphenyl) cyclohexanol hydrochloride. Its structural formula is-

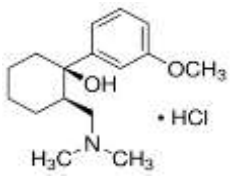

Figure No 1: Structure of Tramadol Hydrochloride

Materials and Methods

Chemicals: Pure standards of Tramadol Hydrochloride Injection 50 mg/mL were purchased from Sigma-Aldrich India. HPLC grade acetonitrile, water, and Trifluoracetic acid were purchased from Fischer scientific and other chemicals used were analytical grade.

Chromatographic Conditions:

Instrument : Agilent HPLC or equivalent
Column : Zorbax C_8, (250 × 4.6 mm), 5 µm
Pump Mode : Isocratic
Flow Rate : 1.0 mL / minute
Detector Wavelength : 270 nm
Column Oven Temperature : Ambient
Sample Cooler Temperature : Ambient
Injection volume : 15 µL
Run Time : 8 minutes

Preparation of Buffer:

Mix 2mL of Trifluoracetic Acid in 1000mL of water and filter through 0.45 membrane filter.

Preparation of Mobile Phase:

Prepare a degassed mixture of buffer and Acetonitrile in ratio of 65:35 v/v.

Preparations of standard solution:

Accurately weigh about 50 mg of Tramadol Hydrochloride working standard into a 100 mL volumetric flask, add about 60 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix. Further dilute 5 mL of the solution into 25 mL volumetric flask, make up the volume with diluent and mix.

Preparation of sample solution:

Transfer 2 mL of standard solution into 100 mL volumetric flask, add 60 mL of diluent mix and make up the volume with diluents and mix. Further dilute 5 mL of the solution to 50 mL with diluent, mix and filter through 0.45 Nylon membrane filter.

Procedure:

Inject diluent (blank) into the chromatograph and record the chromatogram. Inject standard solution in five replicate injections into the chromatograph and record the chromatograms and then Inject sample solutions in duplicate injections into the chromatograph and record the chromatograms.

Calculation:

Assay (mg/mL) of Tramadol Hydrochloride ($C_{16}H_{25}NO_2$. HCL): $= \frac{A_T}{A_S} \times \frac{D_S}{D_T} \times \frac{P}{100}$

% Labeled Amount: $= \frac{Assay\left(\frac{mg}{mL}\right)}{LC} \times 100$

Where, A_T: Average area of Tramadol peak obtained from the sample chromatogram, A_S: Average area of Tramadol peak obtained from the standard chromatogram, D_S: Dilution factor for standard preparation, D_T: Dilution actor for sample preparation, P: Percentage purity of Tramadol Hydrochloride working standard, LC: Label claim for Tramadol Hydrochloride in tramadol HCL Injection (50 mg/mL).

Results and Discussion

Specificity

The specificity of the HPLC method is illustrated in Fig.2a-b, where a complete separation of Tramadol Hydrochloride was noticed in presence of other inactive excipients used in injections. In addition, there was no interference at the retention time in the chromatogram of placebo solution. In peak purity analysis with DAD, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data of retention time and area of the analyte in standard and sample were presented in the Table no 1.

Linearity

The Linearity of this method was determined at seven levels from 50%-150% of operating concentrations for Tramadol Hydrochloride. The Plots of peak area of each sample against respective concentration of Tramadol Hydrochloride were found to be linear in the range of 50% -150% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The linear regression equations and correlation coefficient were found. It observed that correlation coefficient and regression analysis were within the limits which shown in the table no 2 and fig no 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Reproducibility examines the precision between laboratories and it is often determined in collaborative studies. Reproducibility data for Tramadol Hydrochloride is expressed in %RSD and it was less than 2%(Table no 3) which indicates that the method was highly precise.

Repeatability

Repeatability is the precision of the method under the same operating conditions over a short period. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Tramadol Hydrochloride is expressed in %RSD and it was less than 2% (Table no 4)which indicates that the method was highly precise.

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard Tramadol Hydrochloride were added to pre-analyzed samples at a level from 75% up to 125% and then subjected to the proposed HPLC method individually. It was observed that good recoveries of Tramadol Hydrochloridewere obtained at each added concentration level shown in table no 5 which demonstrated that the method was highly accurate.

Robustness

The Measure of a method capacity remains unaffected by small, but deliberate variation in method. Robustness of the Tramadol Hydrochloride was carried out by varying some chromatographic method parameters. The sample preparations were analyzed as per methodology by changing the ratio of solvents in the mobile phase and flow rate by means of +10% or -10%. it was observed and shown in the table no 6 that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The ruggedness data for Tramadol Hydrochloride were observed and shown in the table no 7 that there were no marked changes in the chromatogram, which demonstrated that, the proposed method of ruggedness.

Forced Degradation

The study was intended to ensure the effective separation of Tramadol Hydrochloride and its degradation peaks of formulation ingredients at the retention time of Tramadol Hydrochloride. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. The samples were subjected to various forced degradation conditions and percentage of degradation under various treatment conditions were calculated. The evaluation of chromatographic peak response of the analyte from every degradation method is shown in the table no 8 which is homogeneous and free of co-eluting peaks.

Stability Studies

Standard and Sample solutions were prepared as per test method, analyzed initially and at different time intervals by keeping the solutions at room temperature (~ 25°C) the results are shown in table no 9 which indicates the method was stable.

Table No. 1: Specificity for Tramadol Hydrochloride

S. No	Name of the solution	No. of Injections	Retention Time (min.)	Peak Purity
1.	Blank	1	Nil	-
2.	Placebo	1	Nil	-
3.	Standard	1	4.273	1.000000
4.	Sample	1	4.273	1.000000

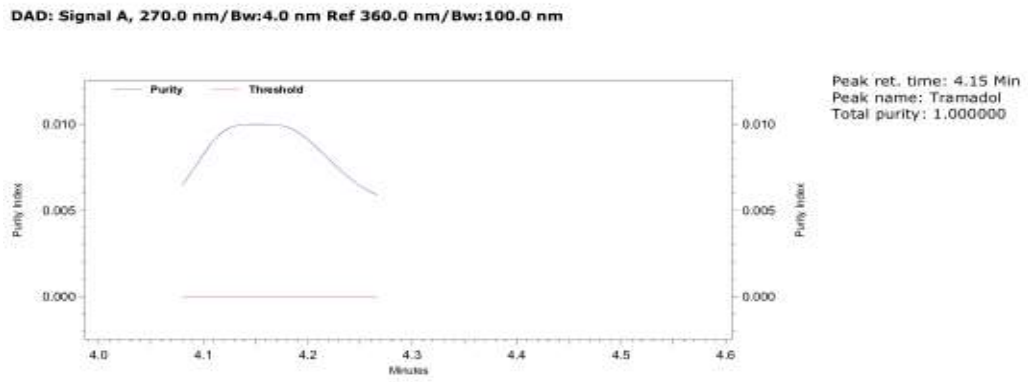

Figure No.2a: Chromatogram for the study of Standard peak purity

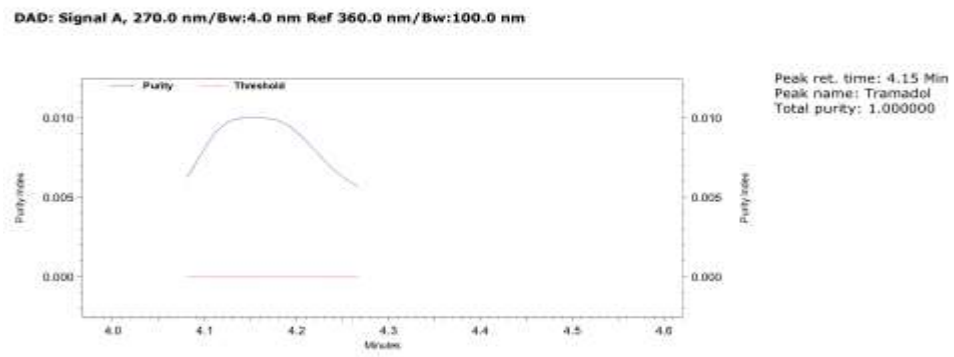

Figure No.2b: Chromatogram for the study of Sample peak purity

Table No. 2: Linearity of response for Tramadol Hydrochloride

S.No	Target level %	Concentration (µg/mL)	Area 1	Area 2	Average Area
1.	50	50.5	33173142	33185861	33179502
2.	80	80.8	53512875	53505383	53509129
3.	90	91.0	60294697	60307583	60301140
4.	100	101.1	67169933	67014936	67092435
5.	110	111.2	73793351	73854643	73823997
6.	120	121.3	81144883	81170148	81157516
7.	150	151.6	101069546	101101702	101085624
				Slope	673067
				Intercept	-863664
				% Y-Intercept	-1.3
				Residual Sum of Squares	196875
				Correlation Coefficient	0.99996

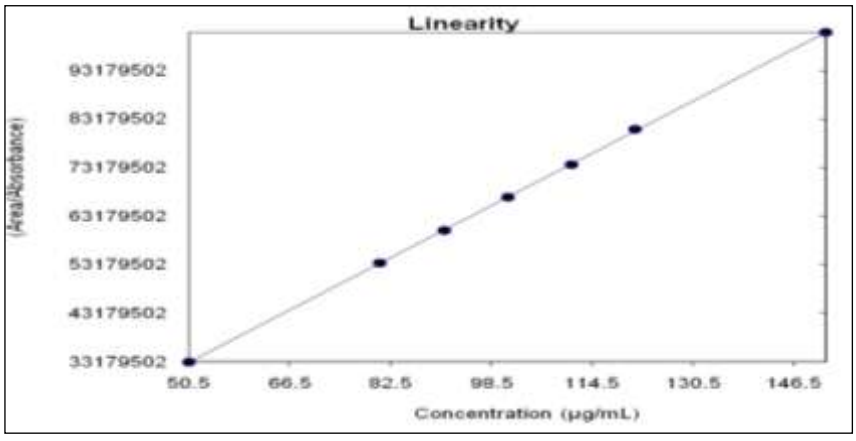

Figure No.3: Linearity curve for Tramadol Hydrochloride

Table No. 3: Precision- Reproducibility for Tramadol Hydrochloride

S.No	StdInjection	Area	Mean	SD	% RSD	95% Confidence Interval (±)
1.	1	1033207	**1033424**	**282**	**0.1**	**296**
2.	2	1033344				
3.	3	1033214				
4.	4	1033757				
5.	5	1033806				
6.	6	1033215				

Table No. 4: Method Precision-Repeatability for Tramadol Hydrochloride

S. No	Sample Name	Area	Average Area	Amount of drug present (mg/mL)	Drug Recovery (%)
1.	Sample 1-1	66158179	66138374	51.37	102.7
	Sample 1-2	66118568			
2.	Sample 2-1	65630743	65566450	50.92	101.8
	Sample 2-2	65502156			
3.	Sample 3-1	65419658	65424980	50.81	101.6
	Sample 3-2	65430302			
4.	Sample 4-1	65382543	65367372	50.77	101.5
	Sample 4-2	65352200			
5.	Sample 5-1	65541166	65458170	50.84	101.7
	Sample 5-2	65530361			
				Mean	**101.7**
				SD	**0.22**
				%r RSD	**0.4**
				95% Confidence Interval (±)	**0.2**

Table No. 5: Accuracy for Tramadol Hydrochloride

S.No	Target Level	Area	Amount of drug present (mg)	% Recovery
1.	75% Sample-1	48697834	74.01	98.7
2.	75% Sample-2	48812848	74.19	99.1
3.	75% Sample-3	48682410	73.99	99.0
4.	100% Sample-1	64934210	98.69	98.8
5.	100% Sample-2	64828265	98.53	98.7
6.	100% Sample-3	64852978	98.56	98.7
7.	125% Sample-1	81371140	123.67	98.9
8.	125% Sample-2	81289914	123.54	98.8
9.	125% Sample-3	81434938	123.76	99.2
			Mean	**98.8**
			SD	**0.3**
			% RSD	**0.3**

Table No. 6: Robustness of Tramadol Hydrochloride

S. No	Parameter	Variation	Area	Mean	SD	% RSD	RT (min)
1.	Flow Rate	- 10%	72815900	72814061	69463	0.1	4.580
		+ 10%	59574226	59524539	48608	0.1	3.747
2.	Organic in mobile phase	- 2% absolute	65488147	65481014	54360	0.1	4.520
		+2 % absolute	65850385	65800312	42239	0.1	3.793

Table No. 7: Ruggedness of Tramadol Hydrochloride

S.No	Sample Name	Area	Average Area	Content in mg/Unit	% Labeled Amount
1.	Sample 1-1	1052899	1053015	50.82	101.6
	Sample 1-2	1053130			
2.	Sample 2-1	1052565	1054913	50.91	101.8
	Sample 2-2	1057261			
3.	Sample 3-1	1054797	1055202	50.93	101.9
	Sample 3-2	1055607			
4.	Sample 4-1	1051808	1051648	50.76	101.5
	Sample 4-2	1051488			
5.	Sample 5-1	1054241	1051648	50.89	101.8
	Sample 5-2	1054477			
6.	Sample 6-1	1053446	1053562	50.85	101.7
	Sample 6-2	1053678			
Mean					**50.90**
SD					**0.16**
RSD (%)					**0.3**
95% Confidence Interval (±)					**0.1**

Table No.8: Forced Degradation of Tramadol Hydrochloride

S.No	Sample treatment condition	Area	Content in (mg / Unit)	% Labeled amount	% Degradation
1.	Acid Degradation(2NHCl) 85°C/30 minutes	65778085	50.31	100.6	1.0
2.	Acid Degradation(2NHCl) 85°C/60 minutes	66098774	50.56	101.1	0.5
3.	Base Degradation 2NNaOH 85°C/30 minutes	65666516	50.23	100.5	1.2
4.	Base Degradation 2NNaOH 85°C/60 minutes	66031676	50.51	101.0	0.6
5.	Peroxide Degradation 10% H_2O_2 85°C / 30 minutes	65701677	50.26	100.5	1.1
6.	Peroxide Degradation 10% H_2O_2 85°C / 30 minutes	65758098	50.30	100.6	1.0

Stability Studies

Standard and Sample solutions were prepared as per test method, analyzed initially and at different time intervals by keeping the solutions at room temperature (~ 25°C) the results are shown in table no 9wich indicates the method was stable.

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Table No.9:Stability studies for Tramadol Hydrochloride

Time	Standard Solution		Sample Solution	
	Area	% Difference	Area	% Difference
Initial	1028744	-	1052591	-
1 Hour	1031378	-0.3	1056409	-0.4
2 Hours	1031969	-0.3	1056055	-0.3
3 Hours	1032463	-0.4	1057170	-0.4
4 Hours	1032565	-0.4	1059101	-0.6
5 Hours	1033653	-0.5	1060425	-0.7
6 Hours	1034839	-0.6	1061564	-0.9
7 Hours	1036163	-0.7	1062662	-1.0
8 Hours	1036814	-0.8	1064900	-1.2
10 Hours	1038954	-1.0	1064712	-1.2
11 Hours	1039191	-1.0	1066850	-1.4
12 Hours	1038510	-0.9	1068902	-1.5
13 Hours	1039328	-1.0	1064764	-1.2
14 Hours	1041095	-1.2	1067743	-1.4
15 Hours	1040713	-1.2	1067060	-1.4
24 Hours	1033632	-0.5	1070313	-1.7
29 Hours	1029574	-0.1	1066120	-1.3
34 Hours	1028653	0.0	1067186	-1.4
39 Hours	1027827	0.1	1067411	-1.4
48 Hours	1029099	0.0	1068937	-1.6

Conclusion

The Proposed study describes a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for quantitative determination of Tramadol Hydrochloride in sterile dosage form. The method was validated as per ICH guidelines and found to be simple, specific, linear and precise. Therefore, the proposed method can be successfully used for the routine analysis of Tramadol Hydrochloride in pharmaceutical dosage form without interference.

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