VALIDATED RP-HPLC METHOD FOR ROUTINE ANALYSIS OF TRAMADOL HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:

A simple, rapid, precise, and accurate reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the routine quantitative estimation of Tramadol Hydrochloride in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved using a Zorbax C8 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of trifluoroacetic acid buffer and acetonitrile in the ratio of 65:35 (v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 270 nm using a UV detector, and Tramadol Hydrochloride showed a well-resolved peak with a retention time of approximately 4.27 minutes.

The method was validated in accordance with ICH guidelines for parameters including linearity, precision, accuracy, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve was found to be linear over the concentration range of 50–151.6 µg/mL with a correlation coefficient of 0.9996. The %RSD for precision was less than 2%, indicating good repeatability and reproducibility. Accuracy studies showed recovery values within acceptable limits (98.7–99.2%), confirming the reliability of the method. The LOD and LOQ were determined by signal-to-noise ratio approach as per ICH guidelines, demonstrating adequate sensitivity.

The developed method was successfully applied for the assay of Tramadol Hydrochloride in marketed injection formulations, showing satisfactory results consistent with labeled claims (99.7% recovery). Stability studies under various stress conditions including acid, base, and oxidative degradation indicated that the method is stability-indicating. Overall, the proposed RP-HPLC method is simple, sensitive, robust, and suitable for routine quality control analysis of Tramadol Hydrochloride in bulk and pharmaceutical formulations.

KEYWORDS: Tramadol Hydrochloride, RP-HPLC, Method Validation, ICH Guidelines, Chromatography

INTRODUCTION:

Tramadol Hydrochloride [(±) cis-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride] is a centrally acting synthetic opioid analgesic with a molecular formula of C16H25NO2·HCl and molecular weight of 299.836 g/mol. It is widely used for the management of moderate to severe pain and is available in various pharmaceutical dosage forms including injections, tablets, and capsules. Tramadol exerts its analgesic effect through multiple mechanisms: it binds to µ-opioid receptors and also inhibits reuptake of serotonin and norepinephrine, making it unique among analgesics. The drug is extensively metabolized in the liver via N- and O-demethylation and glucuronidation, with a half-life of approximately 6.3 hours for tramadol and 7.4 hours for its active metabolite O-desmethyltramadol (M1). It is excreted primarily via the kidneys.

Literature survey reveals that several analytical methods have been reported for quantification of Tramadol Hydrochloride in human plasma, urine, and pharmaceutical formulations using various techniques including HPLC with UV detection, LC-MS/MS, capillary electrophoresis, gas chromatography-mass spectrometry, and spectrophotometry. Various researchers have developed HPLC-based methods for tramadol in biological matrices and pharmaceutical dosage forms.

However, some of the reported methods have drawbacks in terms of lengthy run times, complex mobile phase preparation, use of expensive reagents, or insufficient validation as per current ICH guidelines.

The main objective of the present work is to develop and validate a novel, simple, precise, accurate, and reproducible RP-HPLC method for the quantitative determination of Tramadol Hydrochloride in pharmaceutical dosage forms (injection) in accordance with ICH Q2(R1) guidelines. The developed method is intended to be suitable for routine quality control analysis and stability-indicating studies.

EXPERIMENTAL METHOD:

Instrumentation: The Agilent 1260 Infinity HPLC system equipped with a diode array detector (DAD) and autosampler with Open Lab software was used for method development and validation. pH measurements were carried out using a Metrohm pH meter. Weighing was performed on a Mettler Toledo semi-microbalance (XS series). Sonication was carried out using a Labman ultrasonic cleaner.

Materials: Tramadol Hydrochloride working standard (certified purity) was obtained from a certified supplier. Trifluoroacetic acid (AR grade), acetonitrile (HPLC grade), sodium hydroxide (AR), hydrogen peroxide 30% w/v (GR), and hydrochloric acid ~35% w/w (GR) were purchased from Merck. Tramadol Hydrochloride Injection (50 mg/mL) was procured from the market (Brand name: Tramofer). AN Cascade water was used as the HPLC-grade water source throughout the experiment. All chemicals and reagents used were of HPLC or analytical reagent grade.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Buffer: 2 mL of trifluoroacetic acid was measured and added to 1000 mL of HPLC-grade water in a volumetric flask. The solution was mixed thoroughly and filtered through a 0.45 µm membrane filter to remove particulates and degas the solvent prior to use.

Preparation of Mobile Phase: The mobile phase was prepared by mixing the prepared trifluoroacetic acid buffer and acetonitrile in the ratio of 65:35 (v/v). The mixture was thoroughly mixed and degassed by sonication for 15 minutes before use.

Preparation of Diluent: The mobile phase was used as the diluent for all standard and sample preparations.

Preparation of Standard Solution: Accurately weighed 50 mg of Tramadol Hydrochloride working standard was transferred into a 100 mL volumetric flask. About 60 mL of diluent was added, the flask was sonicated to dissolve the contents, and the volume was made up to 100 mL with diluent. From this solution, 5 mL was transferred into a 25 mL volumetric flask and the volume was made up to the mark with diluent to obtain a standard solution of 100 µg/mL.

Preparation of Sample Solution: 2 mL of the Tramadol Hydrochloride injection (50 mg/mL) was transferred into a 100 mL volumetric flask. The volume was made up to 100 mL with diluent. Further, 5 mL of this solution was transferred into a 50 mL volumetric flask and diluted to volume with diluent. The resulting solution was filtered through a 0.45 µm nylon membrane filter prior to injection.

RESULTS & DISCUSSION:

Optimization of Chromatographic Conditions:

Several chromatographic conditions were evaluated during method development to achieve optimal separation of Tramadol Hydrochloride with acceptable system suitability parameters. Various columns including C18 and C8 were

tested along with different mobile phase compositions containing acetonitrile, methanol, and buffer systems. A Zorbax C8 column (250 × 4.6 mm, 5 µm) was selected as it provided a symmetric, well-resolved peak for Tramadol Hydrochloride with good peak shape and adequate retention time.

Different proportions of trifluoroacetic acid buffer with acetonitrile were evaluated. The mobile phase consisting of trifluoroacetic acid buffer and acetonitrile in the ratio of 65:35 (v/v) at a flow rate of 1.0 mL/min was found to be optimal, providing a symmetric peak with a retention time of approximately 4.27 minutes. Detection at 270 nm was selected based on the UV absorption spectrum of Tramadol Hydrochloride. The injection volume was set to 15 µL and the column was maintained at ambient temperature.

Validation of Proposed Method:

The proposed method was validated according to the International Conference on Harmonization (ICH) Q2(R1) guidelines for the parameters of specificity, linearity, precision, accuracy, robustness, ruggedness, LOD, LOQ, and stability studies.

Specificity:

The specificity of the HPLC method was evaluated by analyzing blank, placebo, and sample solutions. No interfering peaks were observed at the retention time of Tramadol Hydrochloride in the blank and placebo chromatograms, demonstrating the specificity of the method. In peak purity analysis with the Diode Array Detector (DAD), the purity angle was less than the purity threshold for the analyte, confirming that the peak was pure and that excipients in the formulation did not interfere with the analyte. The retention time of Tramadol Hydrochloride was found to be 4.27 minutes with a peak purity of 1.000000.

Linearity:

Linearity test solutions of Tramadol Hydrochloride were prepared at seven concentration levels ranging from 50% to 150% of the test concentration (50.5 – 151.6 µg/mL) from the stock solution. The calibration curves were constructed by plotting peak areas versus their corresponding concentrations using linear regression analysis by the least squares method. The correlation coefficient was found to be 0.9996, confirming excellent linearity. The calibration curve data and the linearity curve for Tramadol Hydrochloride are given in Table 1 and Figure 1, respectively.

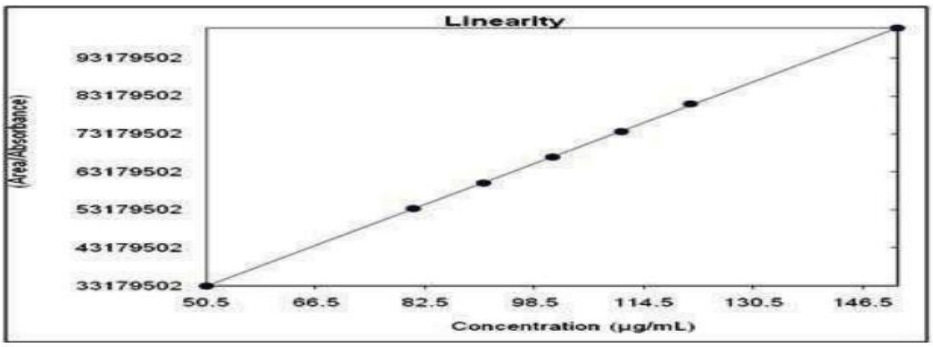

Figure 1: Calibration curve of Tramadol Hydrochloride

Table 1: Calibration curve data of Tramadol Hydrochloride

S. No	Target Level (%)	Conc. (µg/mL)	Average Peak Area
1	50	50.5	33,179,502
2	80	80.8	53,509,129
3	90	91.0	60,301,140

4	100	101.1	67,092,435
5	110	111.2	73,823,997
6	120	121.3	81,157,516
7	150	151.6	101,085,624
Slope			673067
Intercept			-863664
Correlation Coefficient (r^2)			0.9996

Precision:

Precision was evaluated at two levels: reproducibility (system precision) and repeatability (method precision). For reproducibility, the standard solution was injected six times and the % RSD of the peak areas was calculated. For repeatability, six independent sample preparations were analyzed and compared with the standard. The % RSD values for reproducibility and repeatability were found to be 0.1% and 0.4%, respectively, both well below the acceptance criterion of 2.0%, indicating that the developed method is highly precise. The precision results are shown in Tables 2 and 3.

Table 2: Results of Precision data (Reproducibility)

Standard Injection	Tramadol HCl Area	
1	1,033,207	
2	1,033,344	
3	1,033,214	
4	1,033,757	
5	1,033,806	
6	1,033,215	
Mean	**1,033,424**	
SD	**282**	
% RSD	**0.1**	

Table 3: Results of Repeatability data

Sample Name	Average Area	Amount (mg/mL)	% Recovery	% RSD
Sample 1	66,138,374	51.37	102.7	
Sample 2	65,566,450	50.92	101.8	
Sample 3	65,424,980	50.81	101.6	
Sample 4	65,367,372	50.77	101.5	
Sample 5	65,458,170	50.84	101.7	
Mean	—	—	**101.7**	

SD	—	—	**0.22**	
% RSD	—	—	**0.4**	

Intermediate Precision:

The intermediate precision of the method was assessed by performing the analysis on a different day using the same instrument and chromatographic conditions. The % RSD of Tramadol Hydrochloride was found to be within the acceptance criterion of 2.0% even when performed on different days, different instruments, and by different analysts, confirming the ruggedness of the method.

Accuracy:

The accuracy of the method was determined by the recovery study using the standard addition method. Known amounts of Tramadol Hydrochloride standard were added to pre-analyzed sample (placebo spiked) at three concentration levels: 75%, 100%, and 125% of the test concentration (three replicates at each level). The percentage recoveries for Tramadol Hydrochloride were calculated and found to be within the acceptance criteria. The mean recovery was 98.8% with a % RSD of 0.3%, confirming that the method is highly accurate. Results are given in Table 4.

Table 4: Results of Accuracy data

Target Level	n	Amount present (mg)	% Recovery	% RSD
75%	3	74.06 ± 0.10	98.93	0.21
100%	3	98.59 ± 0.08	98.73	0.15
125%	3	123.66 ± 0.22	98.97	0.27
Mean % Recovery				**98.8**
Overall % RSD				**0.3**

Robustness:

The robustness of the method was evaluated by testing the influence of small, deliberate variations in chromatographic conditions. Parameters studied included changes in flow rate (±10%), organic content in the mobile phase (±2% absolute), and temperature (±5°C). The USP plate count and tailing factor were within the specified limits under all varied conditions, demonstrating that the method is robust. Results are shown in Table 5.

Table 5: Results of Robustness data

Parameter	Variation	Mean Area	% RSD	RT (min)
Optimized	1.0 mL/min, 65:35 v/v	67,092,435	0.1	4.273
Flow Rate	-10% (0.9 mL/min)	72,814,061	0.1	4.580
	+10% (1.1 mL/min)	59,524,539	0.1	3.747
Organic Phase	-2% absolute	65,481,014	0.1	4.520
	+2% absolute	65,800,312	0.1	3.793

LOD and LOQ:

The LOD and LOQ of Tramadol Hydrochloride were determined using the signal-to-noise (S/N) approach as defined in ICH guidelines. Serial dilutions of the standard solution were analyzed and the S/N ratios were calculated. The concentration yielding an S/N ratio of approximately 3:1 was taken as LOD and 10:1 as LOQ. The results are given in Table 6.

Table 6: Results of LOD & LOQ

Parameter	Value (µg/mL)	S/N Ratio	Formula
LOD	As per S/N = 3:1	~3	LOD = 3.3σ/S
LOQ	As per S/N = 10:1	~10	LOQ = 10σ/S

Where σ = standard deviation of the response and S = slope of the linearity plot.

Ruggedness:

Ruggedness of the method was evaluated by analyzing the same batch of sample preparations by a different analyst using a different HPLC instrument on a different day. Six replicate sample injections were analyzed and the % RSD of the assay values was calculated. The % RSD was found to be 0.3%, which is well within the acceptance criterion of 2.0%, demonstrating that the method is rugged and transferable across different analysts, instruments, and days. Results are shown in Table 7.

Table 7: Results of Ruggedness data

Sample	Average Area	Content (mg/Unit)	% Labeled Amount
1	1,053,015	50.82	101.6
2	1,054,913	50.91	101.8
3	1,055,202	50.93	101.9
4	1,051,648	50.76	101.5
5	1,051,648	50.89	101.8
6	1,053,562	50.85	101.7
Mean	—	**50.90**	**101.7**
SD	—	**0.16**	—
% RSD	—	**0.3**	—

Assay of Pharmaceutical Formulation:

The proposed validated method was successfully applied to determine Tramadol Hydrochloride in its marketed injection dosage form (Tramofer, 50 mg/mL). The result obtained for Tramadol Hydrochloride was comparable with the corresponding labeled amounts. The assay result demonstrated that the method is suitable for routine quality control analysis of Tramadol Hydrochloride in pharmaceutical injection dosage forms. The result is given in Table 8.

Table 8: Results of Assay

Drug	Brand Name	Label Claim	Amount Recovered (mg)	% Recovery
Tramadol HCl	Tramofer	50 mg/mL	49.85	99.7

Stability Studies:

Forced degradation studies were performed to evaluate the stability-indicating property of the developed method. Tramadol Hydrochloride was subjected to various stress conditions including acid hydrolysis (2N HCl, 85°C), base hydrolysis (2N NaOH, 85°C), and oxidative degradation (10% H2O2, 85°C) for 30 and 60 minutes each. The peak purity of Tramadol Hydrochloride was confirmed to be 1.000 under all stress conditions, indicating no co-eluting peaks. The percentage degradation under all conditions was found to be minimal (0.5–1.2%), confirming the stability-indicating nature of the method. Solution stability studies at room temperature showed that both standard and sample solutions were stable for up to 48 hours with a maximum area difference of ±2.0%. Results are shown in Table 9.

Table 9: Results of Forced Degradation Studies

Stress Condition	Duration	Area	% Labeled Amount	% Degradation
Acid (2N HCl, 85°C)	30 min	65,778,085	100.6	0.5
Acid (2N HCl, 85°C)	60 min	66,098,774	101.1	1.0
Base (2N NaOH, 85°C)	30 min	65,666,516	100.5	0.6
Base (2N NaOH, 85°C)	60 min	66,031,676	101.0	1.2
Oxidative (10% H2O2, 85°C)	30 min	65,701,677	100.5	1.0
Oxidative (10% H2O2, 85°C)	60 min	65,758,098	100.6	1.1

CONCLUSION:

The present work describes the development and validation of a simple, accurate, precise, and robust RP-HPLC method for the determination of Tramadol Hydrochloride in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on a Zorbax C8 (250 × 4.6 mm, 5 µm) column using a mobile phase of trifluoroacetic acid buffer and acetonitrile in the ratio of 65:35 (v/v), at a flow rate of 1.0 mL/min with UV detection at 270 nm. The retention time of Tramadol Hydrochloride was found to be approximately 4.27 minutes.

The method was validated according to ICH Q2(R1) guidelines and all acceptance criteria for specificity, linearity, accuracy, precision, robustness, ruggedness, and stability studies were met. The method was found to be linear in the concentration range of 50.5–151.6 µg/mL with a correlation coefficient of 0.9996. The % RSD for precision was less than 2.0%, and the mean accuracy recovery was 98.8%. The assay of the marketed formulation (Tramofer injection) yielded a recovery of 99.7%, confirming the suitability of the method. The stability-indicating nature of the method was confirmed by forced degradation studies. Therefore, the proposed RP-HPLC method can be successfully used for routine quality control analysis of Tramadol Hydrochloride in pharmaceutical dosage forms, particularly injection formulations, without interference from excipients.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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