Validated UV spectrophotometric method for quantitative analysis of tramadol in bulk and pharmaceutical dosage form

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ABSTRACT

A precise, accurate, economical and simple UV spectrophotometric method has been developed for the determination of Tramadol hydrochloride in bulk and pharmaceutical dosage form. The Tramadol hydrochloride shows maximum absorbance at 273.5 nm in water and obeys Beer's law in the concentration range of 10-50 µg /mL with a correlation coefficient (r^2 = 0.9999). The results of analysis were validated by recovery studies. The percentage recovery method was found to be 99.53-100.41%. The relative standard deviation was found to be < 2.0 % in all cases. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The method was successfully feasible to pharmaceutical formulation because no chromatographic interferences from the tablet excipients were found. The proposed method was found to be simple, accurate and reliable for routine quantification of Tramadol hydrochloride in bulk form and pharmaceutical dosage forms.

KEY WORDS: Tramadol hydrochloride, UV spectrophotometry, Validation, tablet dosage forms.

1. INTRODUCTION

Tramadol generally available as hydrochloride salt is an opioid pain medication utilized for the treatment of moderate to moderately severe pain and also used for the management of pain in fibromyalgia. Tramadol hydrochloride[1] has got two different mechanisms. Firstly, it binds to the µ-opioid receptor. Secondly it inhibits the reuptake of serotonin and nor epinephrine. Different analytical methods for the determination of Tramadol hydrochloride are available based on UV spectrophotometry[2-6]; RP-HPLC[7,8]; GC-MS[9] and UPLC[7] in bulk and formulations alone and in combination with other drugs. Tramadol hydrochloride is chemically known as (1S, 2S)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride.

Because of its versatility UV spectrophotometry is always preferred at small scale industries. Literature survey includes very few methods of UV spectrophotometric methods for the estimation of Tramadol Hydrochloride alone or in combination with other drugs in bulk and pharmaceutical dosage form. It was planned to determine Tramadol hydrochloride by a different UV method to improve the analytical profile. Hence the main objective of present work was to develop and validate simple, precise, accurate, robust and economical UV spectrophotometric method for the estimation of Tramadol hydrochloride in bulk and pharmaceutical dosage form as per ICH guidelines. The chemical structure of Tramadol hydrochloride is shown in Fig. 1.

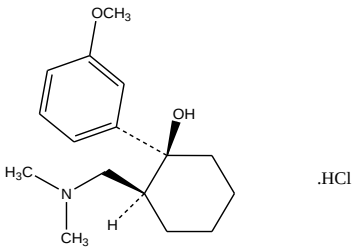

Figure.1. Chemical structure of Tramadol Hydrochloride

2. EXPERIMENTAL

2.1. Selection of solvent: A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and acetonitrile were tried based on the solubility of the drug. Maximum absorption of the drug was found to be 273.5 nm in double distilled water. So distilled water was selected as optimized solvent in this spectrophotometric method.

2.2. Instruments used: Elico Double beam SL 210 UV VIS spectrophotometer was used to record the absorption spectra. Spectrophotometer with 1 cm matched quartz cells were used for the estimation of Tramadol hydrochloride.

2.3. Reagents and Materials: Tramadol hydrochloride standard obtained as a gift sample from Hetero Drugs Ltd., Hyderabad, Telangana, India. Tramazac Tablets containing 100 mg of Tramadol are obtained from local pharmacy. Analytical grade double distilled water was

utilized throughout the experiment which was obtained by Vignan Pharmacy College, Vadlamudi, Guntur Dist, A.P, India.

2.4. Selection of detection wavelength: In UV absorption maxima method, a solution containing 10 µg/mL was scanned in UV range of 200 - 400 nm utilizing Elico Double beam SL 210 UV VIS spectrophotometer utilizing distilled water as blank. It was observed that the drug showed maximum absorbance at 273.5 nm and was selected as the detection wavelength for the determination of Tramadol hydrochloride. The UV absorption spectrum of Tramadol hydrochloride is shown in Figure 2.

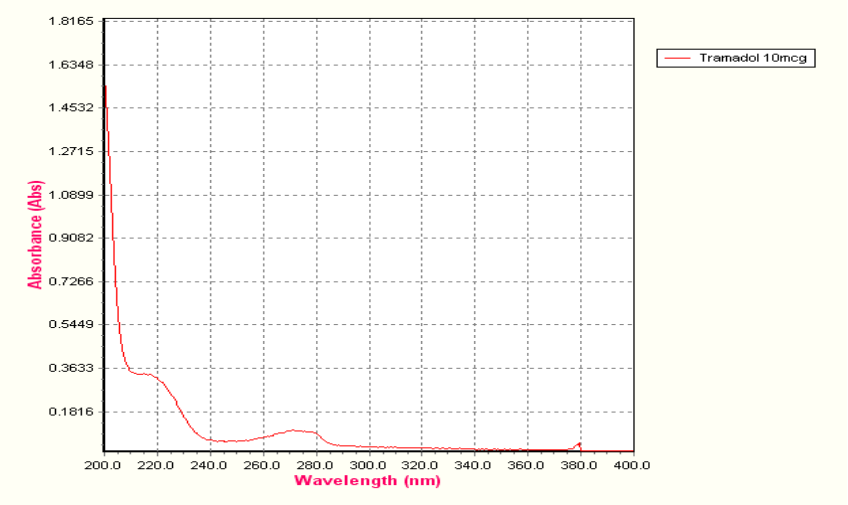

Figure.2. UV Spectrum of Tramadol hydrochloride

2.5. Preparation of standard drug solutions: A precisely weighed 10 mg of Tramadol hydrochloride pure drug was dissolved in 5 mL double distilled water and sonicated well. Then the volume was made up to the mark with double distilled water to obtain the stock solution of 1000 µg/mL. Aliquots of 1.0 to 5.0 mL portions of standard solutions were transferred to a series of 10 mL volumetric flasks and volume in each flask was adjusted to 10 mL with double distilled water to obtain the concentration range of 100-500 µg/mL to get the working standard solutions. The same dilution procedure was repeated to obtain a solutions of concentration range 10 - 50 µg/mL.

2.6. Preparation of Calibration curve: A calibration curve was constructed with the above working standard solutions (10 - 50 µg/mL) at 273.5 nm. Calibration data is presented in Table 1. Calibration curve was prepared by plotting concentration of Tramadol hydrochloride on X-axis and their respective absorbance's on Y-axis. The calibration curve is shown in Figure 3. The optical characteristics and regression data of the developed method are presented in Table 2.

Table.1. Linearity data for Tramadol hydrochloride

Concentration(µg/mL)	Absorbance
0	0
10	0.0703
20	0.1378
30	0.2053
40	0.2756
50	0.3418

Table.2. Optical characteristics, regression data of the proposed method

Parameter	Result
λ_{max} (nm)	273.5
Beer's law limits (µg / mL)	10-50
Molar absorptivity (L.mole^{-1} cm $^{-1}$)	4257.6
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.1459
Regression equation (Y= a+bc) ; Slope is	0.0068
Intercept (a)	0.000914
Standard deviation of intercept (Sa)	0.00791
Standard error of estimation (Se)	0.001094
Correlation coefficient (r^2)	0.998

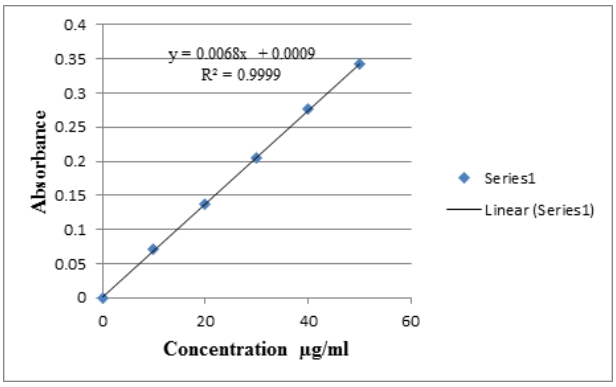

Figure.3. Calibration curve of Tramadol hydrochloride

2.7. Validation of the developed method: The proposed UV method of analysis was validated in pursuance of ICH Q2 (R1) for the parameters like system suitability, specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ)[11].

2.8. Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (% RSD) was calculated. The results obtained are given in Table 3.

2.9. Accuracy (Recovery studies): The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. The recovery studies were carried out by adding known amount of pure drug Tramadol hydrochloride at 50 %, 100 % and 150 % of preanalyzed sample and the proposed method was followed. From the amount of Tramadol hydrochloride found and % recovery was estimated. The results obtained are given in Table 4.

2.10. Ruggedness: Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. The results obtained are shown in Table 5.

2.11. LOD and LOQ: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Limit of Detection and Limit of Quantitation were calculated using following formula LOD= 3.3(SD) / S and LOQ= 10 (SD) / S, where SD=standard deviation of response (absorbance) and S= slope of the calibration. The results of LOD and LOQ are shown in Table 6.

2.12. Procedure for assay of pharmaceutical formulations: Twenty tablets of Tramazac marketed formulations were weighed and powdered in glass mortar. A quantity of tablet powder equivalent to 100 mg of Tramadol hydrochloride was transferred to 100 mL volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with distilled water. The solution was then filtered through a Whatman filter paper No 41. The filtrate was appropriately diluted further to obtain concentration in between linearity range. The absorbance of the resulting solution was measured at 273.5 nm and the amount of Tramadol hydrochloride was determined by referring to the calibration plot. Assay results are presented in Table 7.

Table. 3. Results of precision study

Precision	Intra-day	Inter-day		
		Day -1	Day -2	Day -3
Mean % recovery	100.44	100.32	99.82	99.96
SD	0.257	0.330	0.243	0.532
% RSD*	0.255	0.328	0.246	0.533

***average of 6 determinations. SD= standard deviation, % RSD= relative standard deviation.**

Table.4. Results of accuracy study

Level of recovery	Amount added (µg/mL)		Mean Percent recovery ±SD $	% RSD*
	Amount of added (µg/mL)	Amount taken (µg/mL)		
50%	8	10	99.80±1.42	0.11
100%	10	10	99.5± 0.43	
150%	12	10	100.2± 1.45	

$ = Standard deviation; * = Average of six determinations

Table.5. Ruggedness results

Parameter	Instrument-1** (Systronics model 2203)	Instrument-2** (Elico SL 159)	Analyst -1*	Analyst -2*
SD*	99.97±0.315	99.89±0.571	100.23±0.215	99.89±0.217
% RSD#	0.315	0.572	0.214	0.218

*** = Mean of three observations, ** = mean of six observations**

Table.6. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Parameter	Results
Limit of Detection (LOD)	0.296 µg/mL
Limit of Quantitation (LOQ)	0.888 µg/mL

Table.7. Assay results

Formulation	Labeled amount	Amount found *(mg) (mean ± SD)	% Assay	% RSD*
Tramazac tablets	100 mg	99.98 ± 0.3	99.98	0.322
Tramazac capsules	100 mg	99.97± 0.2	99.7	0.201

* Average of six determinations.

3. RESULTS AND DISCUSSION

For the selection analytical wavelength, Tramadol hydrochloride solution were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 - 400 nm by Elico Double beam SL 210 UV VIS spectrophotometer. The λ_{max} of 273.5 nm was selected for the determination of Tramadol hydrochloride and the absorption maxima curve was shown in Figure 2. The calibration curve for Tramadol hydrochloride were prepared in the concentration range of 10-50 µg/mL. The proposed method obeyed Beer's law in the concentration range of 10-50 µg/mL with good correlation coefficient of $r^2 = 0.9999$. Calibration data is presented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of Tramadol hydrochloride is shown in Figure 3**.** The optical characteristics and the data concerning to the proposed method is represented in Table 2. The solutions of recovery study was further determined on same day at three different times and on three different days for intra-day and inter-day Precision study. The precision of the methods was found to be good with % RSD less than 2 indicates that the method was precise and the results are presented in Table 3**.** Accuracy studies were carried out by recovery study using standard addition method at three different concentration levels (80, 100 and 120 %). The known amount of standard drug solution of Tramadol hydrochloride to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery study results was found to be in the range of 99.80 to 100.2 percentages with percentage RSD less than 2 (Table 4). Ruggedness was performed by changing two different analysts and two instruments and the results are tabulated in Table 5. It reveals that the proposed method was found to be rugged. The LOD and LOQ were found to be 0.296 µg/mL and 0.888 µg/mL respectively which shows that this method was very sensitive as they were within the permitted levels. The LOD and LOQ results are shown in Table 6. The developed method was eventually utilized in analysis of tablet and capsules formulation and were found to be within the proposed limits and also the mean % assay value was found to be 99.97 ±0.3 for tablets and 99.98± 0.2 for capsules. The assay results are given in Table 7. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

4. CONCLUSION

The developed and validated UV spectrophotometric method was found to be economical due to the use of double distilled water as a solvent throughout the experiment. None of the usual excipients employed in the formulation of Tramadol hydrochloride dosage forms interfered in the analysis of Tramadol hydrochloride by the proposed method. The system suitability parameters and system precision are determined and found within the limits. The plot is drawn between the concentration and absorbance which

is found to be linear in the concentration range of 10-50 µ/mL with good correlation coefficient greater than r^2= 0.9999. Low % Relative standard deviation and high percent of recovery indicates that the method is highly precise and accurate. Thus, the developed method for Tramadol hydrochloride was found to be simple, precise, accurate and cost effective and it can be effectively suitable for routine sample analysis of Tramadol hydrochloride in commercial tablets.

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