Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN 0976-9595

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TRAMADOL IN BULK AND ITS FORMULATIONS BY UV-SPECTROSCOPY

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ABSTRACT

The present study describes a simple, accurate, precise and cost-effective UV-Spectrophotometric method for the estimation of Tramadol Hydrochloride, an analgesic, in bulk and pharmaceutical dosage form. The drug was dissolved in double distilled water (used as solvent). Because of cost-effective and minimal maintenance, UV-spectrophotometry is always preferred at small scale industries. The λ_{max} or the absorption maxima of the pure drug was found to be 271nm. A linear response was observed in the range of 30 to 150µg/ml with the regression co-efficient of 0.999. The method was validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Tramadol Hydrochloride in quality control of formulation without interference of the excipients. The developed method is simple, precise, rugged, robust, and economical. The method can be used for routine analysis of tramadol from its tablet formulation. The described methods can be readily utilized for analysis of pharmaceutical formulation.

Keywords: Tramadol Hydrochloride, UV-Spectrophotometry, Beer's-Lambert's law, ICH guidelines, accuracy, Validation.

1. INTRODUCTION

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. Spectroscopy is a general methodology that can be adapted in many ways to extract the information you need (energies of electronic, vibrational, rotational states, structure and symmetry of molecules, dynamic information) [1]. Ultraviolet-Visible Spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves the measurement of the amount of Ultraviolet (190-380nm) radiation by a substance in a solution. A compound or drug which possesses conjugated double bond absorbs UV radiation at a specific wavelength and this character of the drug is specific for a fixed solvent system. The wavelength at which maximum absorption occurs is called λ_{max}. It is independent of concentration. For a drug to be measured by the ultraviolet analytical method, it should follow the Beer's-Lambert's law. Because of cost-effective and minimal maintenance, UV-spectrophotometry is always preferred at small scale industries [2, 3].

Tramadol Hydrochloride, chemically known as (1S, 2S)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclo-hexanol hydrochloride, is a synthetic codeine analogue and has central analgesic properties with effects similar to opioids, such as morphine and codeine, acting on specific opioid receptors. The hydrochloride salt of tramadol is used as a narcotic analgesic for moderate to severe pain, it can be addictive and weakly inhibits norepine-phrine and serotonin reuptake. Tramadol is marketed as a racemic mixture of both R- and S-stereoisomers,

because the two isomers complement each other's analgesic activities. The (+)-isomer is predominantly active as an opiate with a higher affinity for the μ-opiate receptor (20 times higher affinity than the (-)-isomer) [4, 5]. Tramadol, sold under the brand name Ultram among others, is an opioid pain medication used to treat moderate to moderately severe pain. When taken by mouth in an immediate-release formulation, the onset of pain relief usually begins within an hour. It is also available in injection. It may be sold in combination with paracetamol (acetaminophen) or as longer-acting formulations. Its analgesic effects take about one hour to come into effect and 2 to 4 h to peak after oral administration with an immediate-release formulation. On a dose-by-dose basis, tramadol has about one-tenth the potency of morphine (thus 100mg is commensurate to 10mg morphine but may vary) and is practically equally potent when compared with pethidine and codeine [6]. For pain moderate in severity, its effectiveness is equivalent to that of codeine at low doses, and hydrocodone at very high doses; for severe pain it is less effective than morphine [7, 8]. Different analytical methods for the determination of tramadol hydrochloride are available based on UV spectrophotometry. 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 [9, 10]. The chemical structure of tramadol hydrochloride is shown in fig. 1.

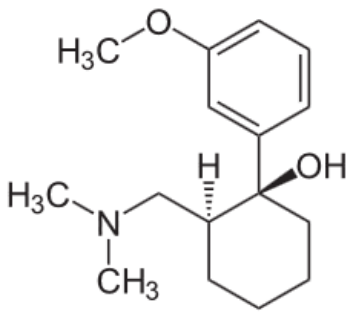

Fig. 1: Chemical structure of tramadol

The aim of this work is to develop and validate a simple, accurate and low-cost analytical method by using UV-spectrophotometry for the estimation of tramadol hydrochloride in bulk and pharmaceutical dosage forms.

2. MATERIAL AND METHODS

2.1. Reagents and chemicals used

The pure API sample of Tramadol Hydrochloride was obtained as free gift sample from KP Labs, Hyderabad, Telangana, India. The marketed combined pharmaceutical dosage form of Tramadol Hydrochloride (50mg) *i.e.,* Contramal, was purchased from local market in Hyderabad. Analytical grade double distilled water, methanol and acetonitrile were utilized throughout the experiment.

2.2. Instruments used

Shimadzu UV-1800 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. 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 271nm in double distilled water. Hence, double distilled water was selected as optimized solvent in this spectrophotometric method.

2.4. Preparation of stock solution

A precisely weighed, 25mg of tramadol hydrochloride pure drug was transferred to 25ml volumetric flask (clean and dry). Then 15ml of double distilled water was added and dissolved the drug by vigorous shaking and sonicated well. The volume was then made up to the mark with double distilled water to obtain the stock solution of 1000 μg/ml.

2.5. Preparation of working standard solution

From stock solution 10 ml was further diluted to 100 ml with double distilled water to get the solution having concentration 100μg/ml.

2.6. Determination of absorbance maxima (λ_{max})

From the above working standard solution, 1 ml was pipetted out into a 10 ml volumetric flask and the volume was made up to the mark with distilled water to prepare

a concentration of 10 µg/ml. The sample was then scanned in UV/VIS-Spectrophotometer in the range 200-400nm using distilled water as blank and the wavelength corresponding to maximum absorbance was found to be 271nm (fig.2).

2.7. Preparation of calibration curve

From the working standard solution, pipetted out 0.3 ml, 0.6 ml, 0.9 ml, 1.2 ml, and 1.5 ml and diluted to 10 ml using distilled water to produce 30µg/ml, 60µg/ml, 90µg/ml, 120µg/ml and 150µg/ml solutions, respectively. The absorbance of the solutions at the λ_{max} of 271nm using distilled water as blank was measured. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (fig. 3). The curve shows linearity in the concentration range of 30 to 150µg/ml. The correlation co-efficient (r^2) was found to be 0.999.

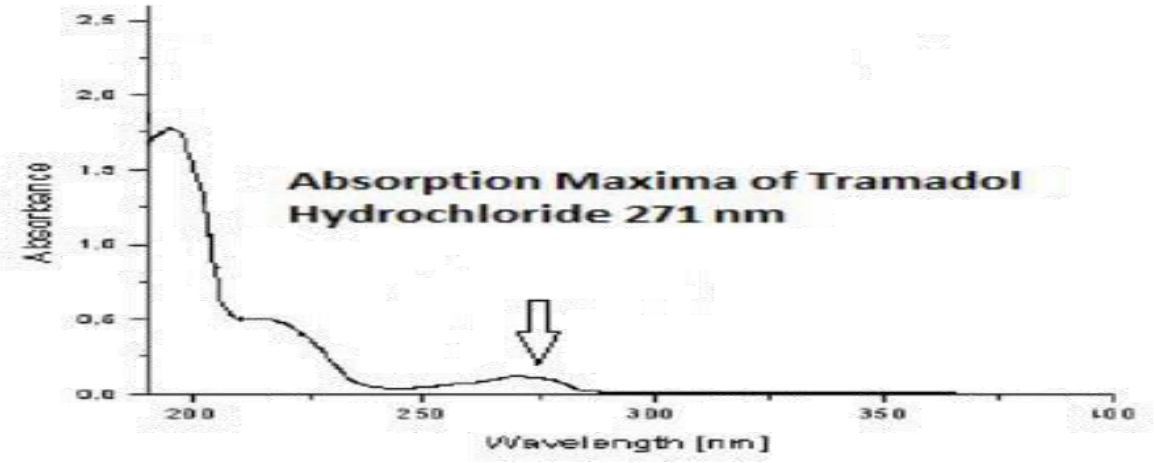

Fig. 2: Absorption maxima of tramadol hydrochloride

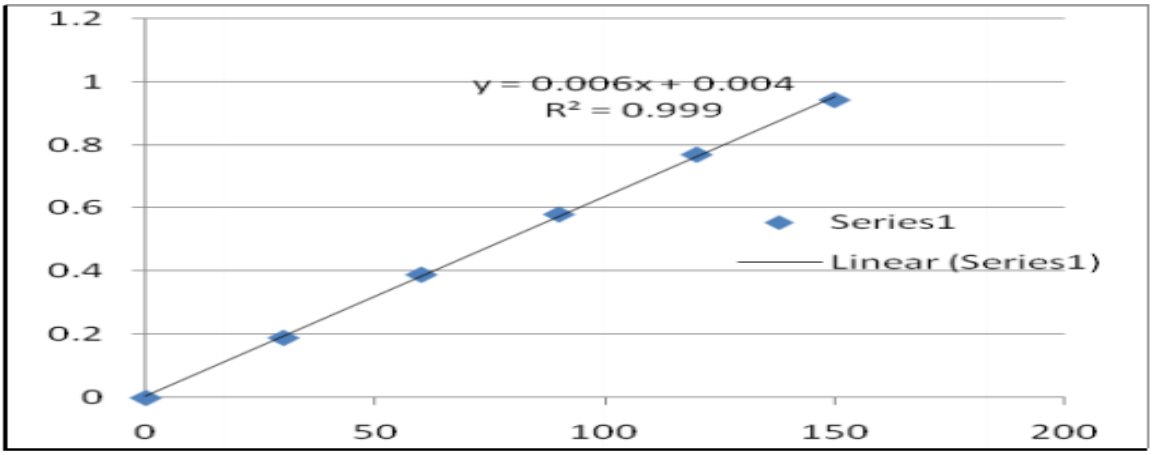

Fig. 3: Calibration curve of tramadol hydrochloride

2.8. Assay (Contramal-50mg)

A quantity of powder equivalent to 25mg of Tramadol Hydrochloride was taken in a 25ml volumetric flask and was first dissolved in 15ml of double distilled water by shaking the flask for 3 to 5 min and diluted up to the mark with distilled water. The solution was then filtered using Whatman filter paper No.40. From this filtrate, appropriate dilutions were made with distilled water to obtain the desired concentration (90, 120 and 150µg/ ml). The solutions were analysed in UV and the result was indicated by % recovery as given in table 8.

2.9. Validation of the developed method

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting 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).

2.9.1. Linearity

Various aliquots were prepared from the working standard solution (100µg/ml) ranging from 30 to 150µg /ml. The samples were scanned in UV/VIS-Spectrophotometer using distilled water as blank. It was found that the selected drug showed linearity between the 30 to 150µg/ml (table 1 and 2).

2.9.2. Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations *i.e.,* 50%, 100% and 150% in which the amount of marketed formulation (Contramal-50mg) was kept constant (60mg) and the amount of pure drug varied *i.e.*, 30mg, 60mg and 90mg for 50%, 100% and 150% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table 1 and 4).

2.9.3. Precision

Precision of the method was demonstrated by intra-day and interday variation studies. In intra-day variation study, 6 different solutions of same concentration that is 90µg/ml were prepared and analyzed three times in a day *i.e.,* morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (table 1 and 5). In the inter-day variation study, six different solutions of same concentration (90µg/ml) were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table 6).

2.9.4. Robustness

Robustness of the method was determined by carrying out the analysis at five different wavelengths (*i.e.,* 271±0.5). The respective absorbances were noted and the result was indicated by % RSD (table 1 and 7).

2.9.5. Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD (table 10).

2.9.6. Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The LOD was calculated using the formula involving standard deviation of response and slope of calibration curve (table 9).

$$\mathbf{LOD} = \frac{\mathbf{3.3\ X\ SD}}{\mathbf{S}}$$

Where,
SD is the standard deviation of Y-intercept,
S is the slop of calibration curve.

2.9.7. 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 (table 9).

$$\mathbf{LOQ} = \frac{\mathbf{10\ X\ SD}}{\mathbf{S}}$$

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

3. RESULTS AND DISCUSSION

The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.99% to 101.3%) of the drug were obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in sub-microgram level, which indicates the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%.
The assay results shows that the amount of drug was in good agreement with the labelled claim of the formulation as indicated by % recovery (101.01%). Summary of validation parameters of proposed spectro-photometric method was shown in table 1.

Table 1: Validation parameters

Parameter	Result
Linearity indicated by correlation co-efficient	0.999
Precision indicated by % RSD	0.0152
Accuracy indicated by % recovery	98.1-101.94
Limit of detection (LOD), µg/ml	0.12 µg/ml
Limit of quantitation (LOQ), µg/ml	0.36 µg/ml
Linear regression equation	y=0.22x+0.008
Robustness indicated by % RSD	0.00243
Ruggedness indicated by % RSD	0.0042
Assay indicated by % purity	99.063

Table 2: Linearity

Concentration (µg/ml)	Absorbance
30	0.190
60	0.390
90	0.581
120	0.770
150	0.942

Table 3: Optical characteristics

Optical characteristics	Result
Beer's law limit (µg/ml)	30-150
Molar extinction coefficient	18847.4
Correlation coefficient (r^2)	0.999
Regression equation	y=0.006x+0.004
Slope (a)	0.006
Intercept (b)	0.004

Table 4: Accuracy studies

Conc. (µg/ml)		% Drug added	Amount found	% Recovery	Mean	SD	% RSD
Tab.	API						
60	30	50	29.38	97.95	99.31	1.189	0.0119
		50	29.52	100			
		50	29.53	100			
		100	59.07	98.5	99.00	0.6557	0.006
		100	59.37	98.9			
		100	59.84	99.6			
		150	88.46	98.1	98.8	0.866	0.008
		150	89.07	98.98			
		150	89.38	99.32			

Table 5: Intraday precision

Conc. (µg/ml)	Absorbances			Avg. % RSD
	Forenoon	Afternoon	Evening	
90	0.575	0.570	0.573	
	0.580	0.573	0.588	
	0581	0.587	0.584	
	0.580	0.580	0.590	
	0.573	s0.575	0.578	
	0.579	0.580	0.588	
% RSD	0.005	0.105	0.011	0.0409

Table 6: Interday precision

Conc.(µg/ml)	Absorbances			Avg. % RSD
	Day 1	Day 2	Day 3	
90	0.580	0.578	0.579	
	0.591	0.580	0.578	
	0.582	0.581	0.580	
	0.567	0579	0.581	
	0.574	0.578	0579	
	0.584	0.582	0.579	
% RSD	0.0144	0.003	0.010	0.00946

Table 7: Robustness

λ_{max}	Absorbance	Mean	SD	% RSD
270.8	0.577	0.574	0.006075	0.01058
270.9	0.577			
270.7	0.576			
270.6	0.576			
270.5	0.576			
270.0	0.557			

271.1	0.578
271.2	0.577
271.3	0.577
271.4	0.577
271.5	0.576
271.6	0.575

Table 8: Assay of formulation (Contramal-50mg)

Conc. (µg/ml)	Absorbance	% Purity	% RSD
30	0.185	98.0	0.016
90	0.578	99.59	0.001
150	0.940	99.60	0.3336

Table 9: LOD and LOQ

Drug	LOD	LOQ
Tramadol Hydrochloride	2.2 µg/ml	6.66 µg/ml

Table 10: Ruggedness

Analyst	Conc. (µg/ml)	Absorbance	Mean	SD	% RSD
Analyst 1	90	0580	0.580	0.0015	0.0025
		0.579			
		0.582			
Analyst 2	90	0.567	0.575	0.0085	0.0147
		0.576			
		0.584			

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 30-150µg/ml with good correlation coefficient greater than r^2= 0.999. Low % Relative Standard Deviation and high percent of recovery indicates that the method is highly precise and accurate.

All the above factors led to a conclusion that the proposed method is accurate, precise, simple, robust and cost effective and can be applied successfully for the estimation of tramadol hydrochloride in bulk and pharmaceutical formulation. Because of cost-effective and minimal maintenance, the present UV spectro-photometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of tramadol hydrochloride in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

5. ACKNOWLEDGEMENT

The authors wish to thank the management of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Hyderabad, Telangana, India for providing necessary equipment for research, constant encouragement, praiseworthy inspiration, facilities and support.

Conflict of interest

Author declares that there is no conflict of interest to disclose.

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