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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF TRAMADOL HYDROCHLORIDE IN BULK AND FORMULATION

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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 first dissolved in N-N-dimethylformamide and final volume was made up with distilled water. The λ_{max} or the absorption maxima of the drug was found to be 271nm. A linear response was observed in the range of 30- 150µg/ml with the regression coefficient 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.

Keywords: N-N-dimethylformamide, Tramadol Hydrochloride, UV-spectrophotometry.

INTRODUCTION

Tramadol hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Tramadol hydrochloride possesses agonist actions at the µ-opioid receptor and effects reuptake at the noradrenergic and serotonergic systems. Tramadol is a compound with µ-agonist activity.

It is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Tramadol is available in the form of oral drops, tablets, capsules and injections. There are various methods available for estimation of tramadol hydrochloride like UV spectrophotometric, spectrofluorometry, HPLC, gas chromatography, GC-MS and LCMS, capillary electrophoresis, HPTLC, HPTLC-densitometry, etc.

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.

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

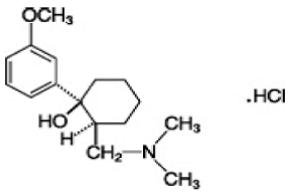

Fig. 1: Structure of Tramadol Hydrochloride

MATERIALS AND METHODS

Materials and Methods

Pharmaceutical grade Tramadol Hydrochloride was supplied by Abbott Piramal India Ltd. The N-N-dimethylformamide was purchased from Merck Milipore, Merck Specialities Private Ltd. and commercially available tablets CONTRAMAL(equivalent to 50 mg of tramadol hydrochloride) one of Abbott Piramal India Ltd. was purchased from market for analysis.

Apparatus

Shimadzu UV-1800 double beam spectrophotometer with 1 cm path length supported by Shimadzu UV-Probe software, version 2.21 was used for spectral measurements with 1 cm matched quartz cells. Shimadzu balance (BL-220H) was used for all weighing.

METHOD DEVELOPMENT

Solubility Test

Solubility test for the drug Tramadol Hydrochloride was performed by using various solvents, which includes distilled water, ethanol, methanol, chloroform, phosphate buffer (pH-4&8), N-N-dimethylformamide, acetone, 0.1M HCl and ethanol: water (1:9). However, N-N-dimethylformamide and distilled water (1:9) was chosen as solvent for developing the method.

Preparation of Stock Solution

Weigh accurately 25 mg of and transferred to 25ml volumetric flask. Then add 2.5mL of N-N-dimethylformamide and dissolve the drug by vigorous shaking for 3 to 5 minutes. Then the final volume was made up with distilled water.

Preparation of Working Standard Solution

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

Determination of λmax

From the above working standard solution, 1 ml was pipette 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. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200-400nm using distilled water as blank and the wavelength corresponding to maximum absorbance (λ_{max}) was found to be 271nm (fig. 1).

Preparation of Calibration Curve

From the working standard solution, pipette 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. Then measure the absorbance of these solutions at the λ_{max} of 271nm using distilled water as blank. Then, the calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (in fig.2). The curve shows linearity in the concentration range of 30-150µg/ml. The correlation coefficient (r^2) was found to be 0.999.

Assay of Tramadol Hydrochloride capsules (CONTRAMAL - 50mg)

A quantity of powder equivalent to 25mg of **of** Tramadol Hydrochloride was taken in a 25ml volumetric flask and it was first dissolved in 2.5ml of N-N-dimethylformamide by shaking the flask for 3 to 5 minutes and diluted up to the mark with distilled water. Then the solution was filtered using Whatmann filter paper No.40. From this filtrate, appropriate dilutions were made with distilled water to obtain the desired concentration (90, 120 and 150µg/ml). These solutions were analyzed in UV and the result was indicated by % recovery given in Table 8.

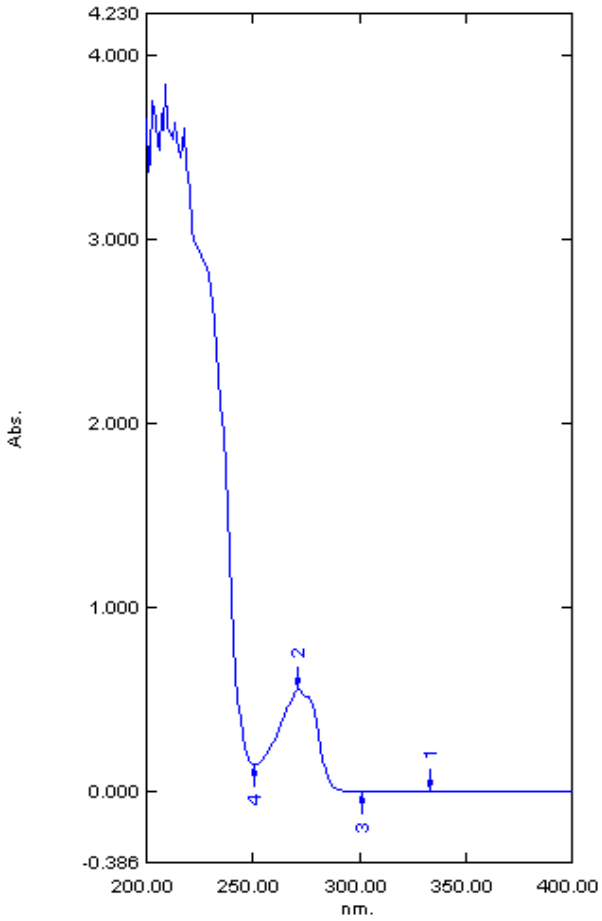

Fig. 1: UV spectrum of Tramadol Hydrochloride (10µg/ml)

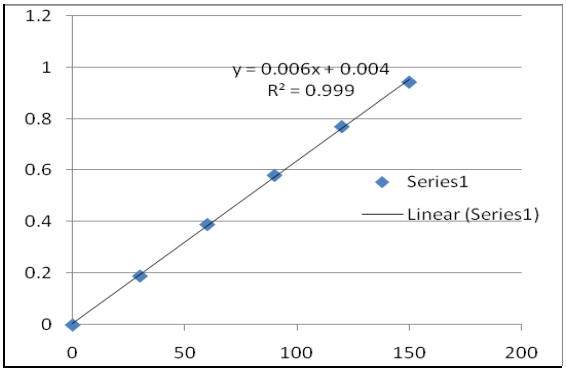

Fig. 2: Calibration curve of Tramadol Hydrochloride

Method Validation

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 pre-determined 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).

Linearity

Various aliquots were prepared from the working standard solution (100µg/ml) ranging from 30-150µg/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 30-150µg/ml (Table 1& 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 was varied i.e., 30mg, 60mg and 90mg for 50%, 100% and 150% respectively[12]. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (Table 1 & 4).

Precision

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, 6 different solutions of same concentration that is 90µg/ml were prepared and analysed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (table no.1&5). In the inter-day variation study, 6 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 No.6).

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&7).

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 No.10).

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 no.9).

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

Where, SD is the standard deviation of Y-intercept and S is the slop of calibration curve[13].

Limit of Quantification

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 No.9).

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

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

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 spectrophotometric method was shown in Table 1.

Table 1: Summary of validation

Parameter	Result
Linearity indicated by correlation coefficient	0.999
Precision indicated by %RSD	0.0152
Accuracy indicated by % recovery	98.1-101.94
Limit of detection (LOD), µg mL-1	0.12µg/ml
Limit of quantitation (LOQ), µg mL-1	0.36µg/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 table for Tramadol Hydrochloride

S. No.	Concentration	Absorbance (µg/ml)
1.	30	0.190
2.	60	0.390
3.	90	0.581
4.	120	0.770
5.	150	0.943

Table 3: Optical characteristic of Tramadol Hydrochloride

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 of Tramadol Hydrochloride

S. No.	Con. of		% drug added	Amount found	%Recovery	Mean	SD	%RSD
	tab. (µg/ml)	pure drug (µg/ml)						
1.	60	30	50	29.38	97.94			
2.	60	30	50	29.53	100	99.31	1.189	0.0119
3.	60	30	50	29.53	100			
4.	60	60	100	59.07	98.4			
5.	60	60	100	59.38	98.9	99.0	0.6557	0.006
6.	60	60	100	59.84	99.7			
7.	60	90	150	88.46	98.2			
8.	60	90	150	89.07	98.97	98.82	0.866	0.008
9.	60	90	150	89.38	99.31			

Table 5: Intra-day precision

S. No.	concentration (µg/ml)	Absorbances			Avg. % RSD
		Morning	A.noon	Evening	
1.	90	0.575	0.570	0.573	
2.	90	0.580	0.573	0.588	
3.	90	0.581	0.587	0.584	
4.	90	0.580	0.580	0.590	
5.	90	0.573	0.575	0.578	
6.	90	0.579	0.580	0.588	
%RSD		0.005	0.105	0.011	0.0409

Table 6: Inter-day precision

S. No.	Concentration (µg/ml)	Absorbances			Avg. % RSD
		Day 1	Day 2	Day 3	
1.	90	0.580	0.578	0.579	
2.	90	0.591	0.580	0.578	
3.	90	0.582	0.581	0.580	
4.	90	0.567	0.579	0.581	
5.	90	0.574	0.578	0.579	
6.	90	0.584	0.582	0.579	
%RSD		0.0144	0.003	0.010	0.00946

Table 7: Robustness method for Tramadol Hydrochloride

S. No.	λ max	Absorbance	Statistical analysis
1.	270.9	0.577	
2.	270.8	0.577	
3.	270.7	0.576	Mean =0.574
4.	270.6	0.576	SD=0.006075
5.	270.5	0.576	%RSD=0.01058
6.	270.0	0.557	
7.	271.1	0.578	
8.	271.2	0.577	
9.	271.3	0.577	
10.	271.4	0.577	
11.	271.5	0.576	
12. 271.6		0.575	

Table 8: Assay of Tramadol Hydrochloride (CONTRAMAL-50mg)

S. No.	Concentration (µg/ml)	Absorbance	% Purity	%RSD
1.	30	0.185	98.0	0.016
2.	90	0.578	99.59	0.001
3.	150	0.940	99.60	0.0336

Table 9: LOD & LOQ of Tramadol Hydrochloride

S. No.	LOD	LOQ
1.	2.2µg/ml	6.66µg/ml

Table 10: Ruggedness of method for Tramadol Hydrochloride

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

CONCLUSION

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.

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