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Simple Validated UV Method for Tramadol HCL in Bulk and Its Capsules

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A B S T R A C T

Tramadol (TMD), is a widely prescribed drug for moderate to severe, acute and chronic pain. The present research work discusses the development and validation of a UV spectrophotometric method for tramadol. Simple, accurate, precise and cost efficient spectrophotometric method has been developed for the estimation of TMD in bulk and its capsules dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 271 nm in water. The mean percentage recovery of TMD was found to be in range 99.29-100.88 %. Beers law was obeyed in the concentration range of 10-200 µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y = 0.0043x + 0.0758 with R^2 = 0.9997 was obtained. Validation was performed as ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The proposed method may be suitable for the analysis of TMD in bulk and capsules formulation for routine quality control purposes.

Keywords: Tramadol, chronic pain, UV spectrophotometric, beers law, capsules

A R T I C L E I N F O

CONTENTS

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1. Introduction

Tramadol [(±) Trans-2- (dimethyl lamino methyl)-1- (3-methoxy- phenyl) - cyclo hexanol hydrochloride] 1 (Figure 1) is a centrally acting opioid analgesic. It is structurally related to codeine and morphine, consists of two

enantiomers. Both enantiomers and their metabolites contribute to analgesic activity by different mechanisms. (+)-Tramadol and its metabolite (+)-Odesmethyl-tramadol (M1) are weak agonists of the μ opioid receptor. (+)-Tramadol inhibits serotonin reuptake and (–)-tramadol inhibits norepinephrine reuptake 2. Tramadol is rapidly absorbed after oral administration with a bioavailability of 65-70%. The peak plasma concentration is reached in 1-3 hours after oral administration of capsules; the therapeutic plasma concentration is in the range of 100–300 ng/mL and approximately 10–30% of the parent drug is excreted unchanged in the urine2-4. Various methods have been reported for the determination of tramadol in bulk, pharmaceutical preparations, biological fluids and hair including spectrophotometry 5-11, high performance liquid chromatography (HPLC) with different detectors3, 4, 12-17, gas chromatography (GC) with flam ionization detector18, gas chromatography- mass spectrometry (GC-MS)19 capillary electrophoresis20-21, voltammetry22 ,23 potentiometry24-26 and conductometry24. The aim of this work is to develop and validate a simple, rapid and quantitative spectrophotometric method that can be used for routine analysis and screening of tramadol in bulk and its capsule dosage forms. The method will be fully validated according to the ICH guidelines [30].

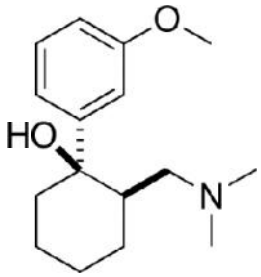

Figure 1: Chemical structure of Tramadol

2. Materials and Methods

Materials:

A PG Instruments T60 UV-Visible Double beam spectrophotometer with 1cm matched quartz cells were used for recording spectra and absorbance measurements. TMD was supplied by Hetero Labs, Hyderabad as a gift sample. The capsules of TMD were purchased from local market. The water was prepared in house by using Milli-Q apparatus. All the chemicals used were of analytical grade.

Methods:

Preparation of standard stock solution:

Accurately weighed 100 mg of TMD and transferred into 100 ml volumetric flask, dissolved in distilled water and volume was made up to mark with distilled water to give 1000μg/ml solution.

Determination of λ max:

A series of standard solutions ranging from 10-100μg/ml were prepared from the stock solution and scanned between 200-400 nm using water as a blank. The spectrum shows the maximum absorbance at 355 nm and 100μg/ml was selected as 100% concentration.

Preparation of test solution:

Twenty capsule contents of TMD were weighed and mixed. The quantity of the powder equivalent to 100 mg of TMD was transferred in to 100 ml volumetric flask. 50 ml distilled water was added and mixed for 5-10 min, filtered the solution and first few ml was discarded (1000μg/ml). 1 ml of above solution was transferred into 10 ml volumetric flask and volume was made up to mark with distilled water to give 100μg/ml.

Method development and validation:

Accuracy:

Accuracy was confirmed by recovery study as per ICH guidelines of three different concentration levels 50%, 100%, 150% by replicate analysis. The % Recovery was calculated.

Precision:

The precision of the method was determined by repeatability and intermediate precision (intra-day and inter-day).

Linearity:

For linearity a series of standard solutions ranging from 10-200μg/ml were prepared solutions was prepared and analyzed. The correlation coefficient was calculated.

Limit of detection and Limit of quantification:

Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope.

3. Results and Discussion

The present study describes a sensitive, accurate, precise, economic and reproducible method for determination of tramadol in bulk and its pharmaceutical formulation. TMD has the zero order absorbance spectra maxima (figure 2 and 3) at 354.0 nm ±1 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 10-200 μg/ml with correlation coefficient (r2) was found to be higher than 0.999 and the linearity curve was shown in figure 4. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analysed capsule powder sample. Percentage recovery for tramadol HCl was determined by all the methods and they were found to be under acceptance criteria which are 98% to 102 % according to ICH guidelines [30]. The results of accuracy were in table 1. The percentage recovery value indicates noninterference from excipients used in formulation. The precision was carried out as described in method and the results were presented in table 2. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision values; hence the developed method can be used to analyze the TMD in capsules formulation. The mean assay of the precision value is 100%. The LOD determined as the amount drug was found to be 3.49 μg/mL and the LOQ was

determined as the lowest concentration was found to be 11.63 µg/mL in formulation.

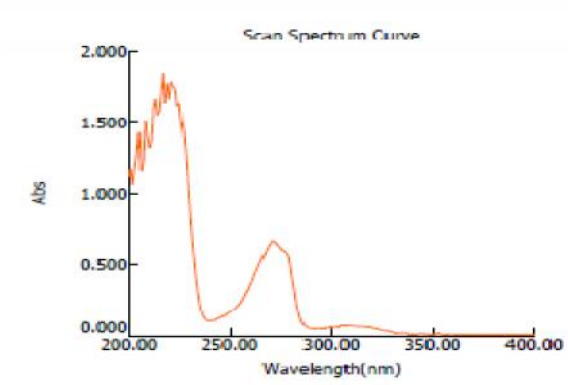

Figure 2: λmax of Tramadol pure drug

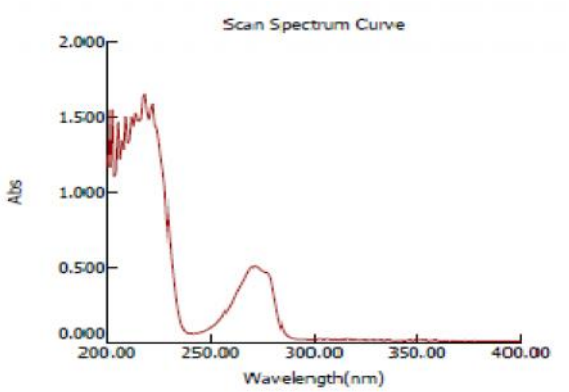

Figure 3: λmax of Tramadol capsule powder

Accuracy:

Accuracy of the developed method was determined by the recovery study at 3 concentration levels by replicate analysis (n=3). Standard drug solutions were added to a pre-analysed sample solution and percentage of total drug content was calculated. The results of accuracy studies were reported in Table 1.

Table 1: Results of Accuracy

S. No	Conc. Level	Sample Weight (mg)	Absorbance	Amount added	Amount found	% Recovery	Mean % Recovery
1	50%	23.8	0.259	50.00	50.10	100.19	99.29
2			0.253	50.00	48.94	97.87	
3			0.258	50.00	49.90	99.81	
4	100%	47.6	0.516	100.00	99.81	99.81	100.00
5			0.516	100.00	99.81	99.81	
6			0.519	100.00	100.39	100.39	
7	150%	71.4	0.777	150.00	150.29	100.19	100.88
8			0.769	150.00	148.74	99.16	
9			0.801	150.00	154.93	103.29	

Precision

The intra & inter-day precision was evaluated by analyzing six sample solutions (n = 6), at the final concentration of analyses (100 µg/ml) of TMD. The TMD concentrations were determined and the relative standard deviations (RSD) were calculated. Table 2 shows the results of precision .

Table 2: Results of Precision

S. No	Interday		Intraday	
	λ Max	Absorbance	λ Max	Absorbance
1	355	0.522	354	0.520
2	356	0.509	355	0.518
3	355	0.516	355	0.514
4	354	0.516	356	0.523
5	356	0.521	354	0.508
6	355	0.519	355	0.510
Average	355	0.517	355	0.516
SD	0.753	0.005	0.753	0.006
% RSD	0.212	0.910	0.212	1.136

Linearity and range

Calibration standards of TMD, covering the range 10-200 µg/mL were prepared with the suitable dilution made from TMD stock solution. The calibration curves were obtained by plotting the intensity of absorbance against of concentration of TMD. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

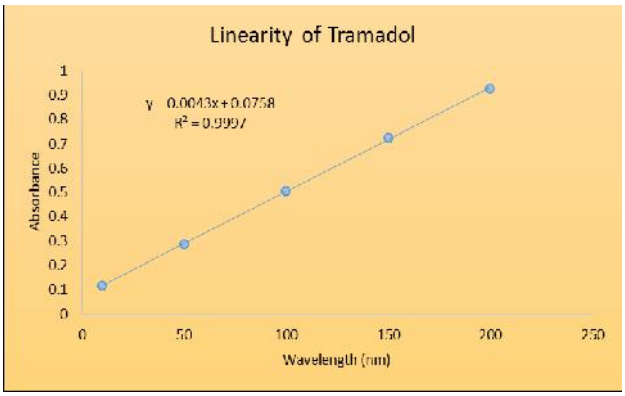

Figure 4: Linearity of Tramadol

Specificity and selectivity

The interference from endogenous compounds was investigated by the analysis of capsules of various concentrations.

Detection and Quantification limit:

The limit of quantification (LOQ) is the lowest concentration of TMD on the calibration curve that can be quantified with acceptable precision and accuracy. The LOQ was found as 3.49 µg/ml for proposed method. And the limit of detection was found to be 11.63 µg/ml.

Assay of marketed formulation:

The proposed method was applied to analyze commercially available TMD capsules having content equivalent to 100 mg (476.25 mg). Ten capsules contents were weighed and mixed equivalent to 100 mg (476.25 mg) transferred in 100 ml volumetric flask and dissolved in distilled finally volume was made up to mark with the same. The solution was then filtered through whattman filter paper # 41. This filtrate was diluted suitably with solvent to get the solution of 100 µg/ml. The absorbance was measured against distilled water as blank. The readings were taken in triplicate by performing the same experimentation in three times. The % purity and content of the drug in capsule dosage form was calculated. The mean assays of six replicate samples were found to be 100%. The results of Assay (% purity) was shown in table 3.

Table 3: % Assay results

S. No	Absorbance	% Assay
1	0.522	100.97
2	0.509	98.45
3	0.516	99.81
4	0.516	99.81
5	0.521	100.77
6	0.519	100.39
Average	0.517	100
SD	0.005	0.911
% RSD	0.910	0.910

4. Conclusion

The developed UV spectrophotometric method for the estimation of tramadol was found to be simple and useful with high accuracy, precision, and reproducible. Sample recoveries in all formulations using the above method were in good agreement with their respective label claim or theoretical drug content, this suggesting the validity of the method and non-interference of formulation excipients in the estimation. The developed method was applied for routine quality control analysis of tramadol capsules

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