

Estimation of Tramadol Hydrochloride in Bulk and Formulation by Second Order Derivative Area Under Curve UV-Spectrophotometric Methods

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Abstract

Simple, fast and reliable spectrophotometric methods were developed for determination of Tramadol Hydrochloride in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Distilled Water. The quantitative determination of the drug was carried out using the second order Derivative Area under Curve method values measured at 272-280nm. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Tramadol Hydrochloride using 2-10µg/ml ($r^2=0.9925$) for second order Derivative Area under Curve spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. The developed methods were successfully applied to estimate the amount of Tramadol Hydrochloride in pharmaceutical formulations.

Keywords: Tramadol hydrochloride, Second order Derivative, Area under Curve (AUC).

1. Introduction

Tramadol hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Chemically it is (1RS, 2RS)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride (Figure 1). 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. Chemically it is [2-(dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol]. It is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Literature survey reveals that, several spectrophotometric method [1-3] TLC- densitometries [4], UV spectrophotometric and HPLC-DAD methods [5], HPLC method [6-8] High Performance thin layer chromatography-densitometry [9], have been reported for the estimation of tramadol in pharmaceutical formulations. Few analytical methods were reported in literature for the determination of tramadol and other combination

drugs which includes spectrophotometric method [10-12], spectrophotometric and spectrofluorimetric method [13-15].

To our notice, no UV- spectrophotometric method using Second Order Derivative Area under Curve has been reported for the determination of Tramadol Hydrochloride in bulk and tablets. Hence an attempt has been made to develop new Second Order Derivative Area under Curve spectrophotometric method for estimation of Tramadol Hydrochloride in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

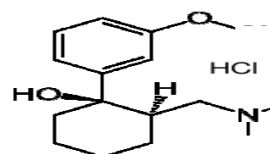


Fig. 1: Structure of Tramadol Hydrochloride.

2. Materials and Methods

2.1 Derivative Spectrophotometric Methods.

Derivative spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences or interferences due to an indistinct shoulder on side of an absorption band. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D⁰ spectrum. The absorbance of a sample is differentiated with respect to wavelength λ to generate first, second or higher order derivative.

$[A]=f(\lambda)$: zero order

$[dA/d\lambda=f(\lambda)]$: first order

$[d^2A/d\lambda^2]=f(\lambda)$: second order

The first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ max of the absorption band. The second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ max of the fundamental band [16].

2.2 Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2 representing start and end point of curve region. The area under curve between λ_1 and λ_2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 272 to 280 nm.

$$\text{Area calculation: } (\alpha+\beta) = \int_{\lambda_2}^{\lambda_1} A d\lambda$$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end

point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelength range start and end point of curve region[17].

2.3 Apparatus and instrumentation:

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.4 Materials:

Reference standard of Tramadol Hydrochloride API was supplied as gift sample by Lupin Laboratory Park, Aurangabad. Distilled water was getting from Research - Lab Fine Chem Industries, Islampur, Mumbai, Maharashtra. Tablet sample with label claim 50mg per tablet were purchased from local market Pune.

2.5 Method development:

2.5.1 Preparation of Standard and Sample Solutions:-

Stock solution of 10 μ g/ml of Tramadol Hydrochloride was prepared in Distilled Water, Second Order Derivative Area under Curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Distilled water in a concentration range of 2, 4, 6, 8, and 10 μ g/ml with Distilled water for Second Order Derivative Area under Curve spectrophotometric methods. Distilled water was used as a blank solution. [4-5]

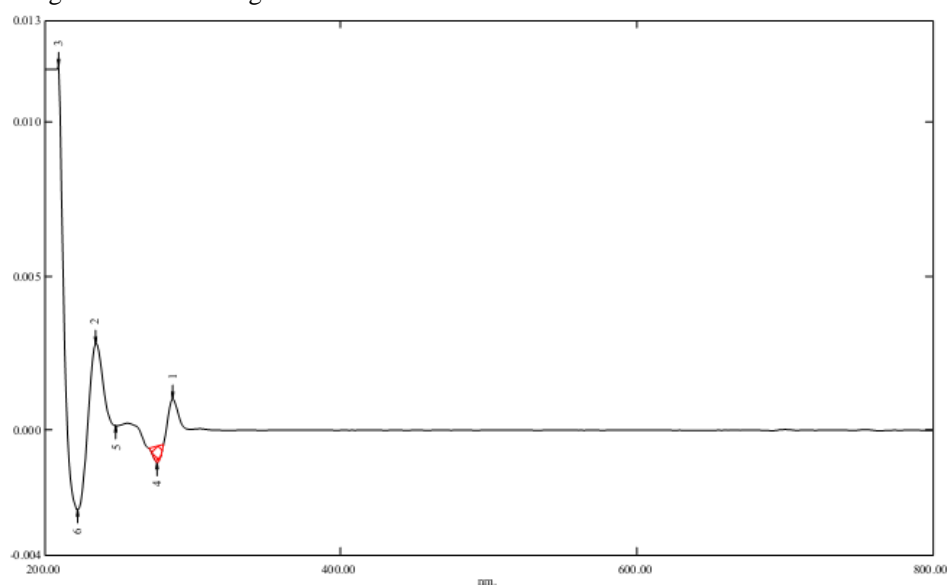


Fig. 2: Second order derivative Area under Curve spectrum of Tramadol Hydrochloride in Distilled water (10 μ g/ml).

2.5.2 Calibration curve for Tramadol Hydrochloride.

The dilutions were made from Standard Stock solution to get concentration of 2, 4, 6, 8, and 10 μ g/ml respectively. These solutions were scanned from 400

to 200 nm and Second Order Derivative Area under Curve values was integrated in the range of 272-280 nm. The calibration curve was plotted between areas under curve values against concentration.

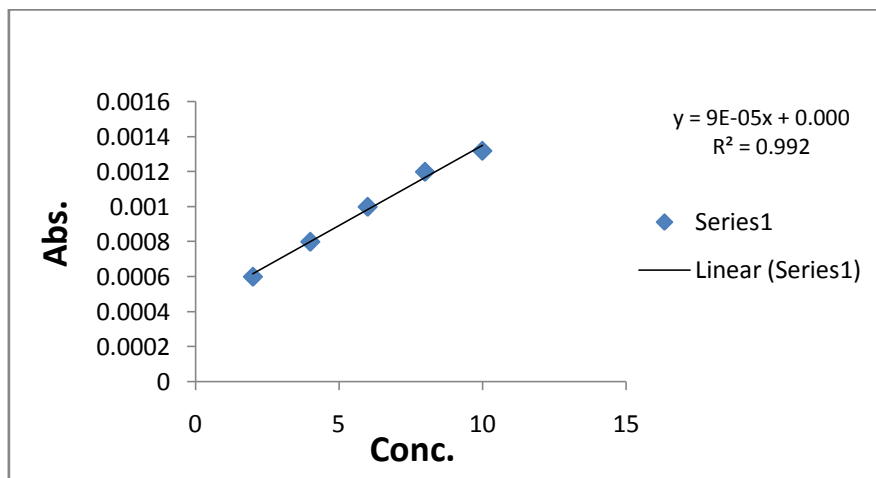


Fig. 3: Linearity of Tramadol Hydrochloride.

2.5.3 Assay of tablet formulation

Twenty tablets each containing 50mg of Tramadol Hydrochloride were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Tramadol Hydrochloride was transferred in 100 ml of volumetric flask. A 50 ml of distilled water was added and sonicated for 15

minutes. Then solution was further diluted up to the mark with distilled water. The solution was filtered using Whatmann filter paper no. 41, first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10 μ g/mL solution with water, subjected for UV analysis using distilled water as blank. This procedure was repeated three times.

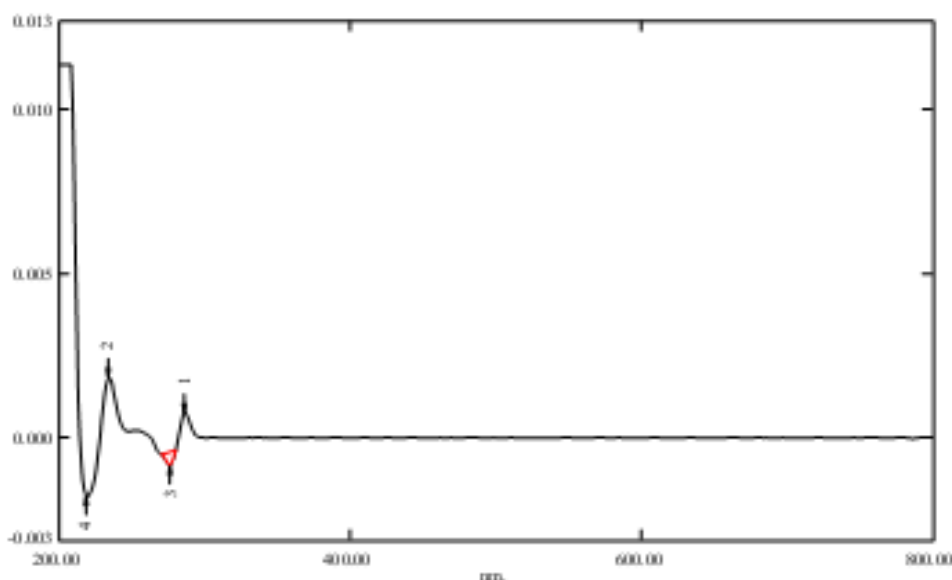


Fig. 4: Second order derivative Area under Curve spectrum of Tramadol Hydrochloride of dosage form in Distilled water (10 μ g/ml).

Table 1: Assay of tablet dosage form

Sr. No.	Sample Solution Concentration (μ g/ml)	Amount found (%)	Mean % found*	%RSD*
1	10	100.13		
2	10	100.22	100.17	0.045
3	10	100.17		

*n=3, % RSD = % Relative Standard Deviation.

2.6 Method validation

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

2.6.1 Accuracy

The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of

10µg/ml Sample solution. Second Order Derivative Area under curve (AUC) was measured in wavelength range 272-280 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 2: Accuracy results for Tramadol Hydrochloride.

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amount. Added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	10	12	22	99.88		
100	10	15	25	99.86	99.87	0.0115
120	10	18	28	98.88		

2.6.2 Precision

The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday

precision was studied by integrating area of standard solution of 10µg/ml concentration at six independent series in the same day. Interday precision studies were performed by integrating area of standard solution of 10µg/ml concentration on three consequent days. The %RSD Was calculated.

Table 3: Precision Study.

Parameter	Intra day	Inter-day
Sample sol conc. µg/ml	10	10
AUC (mean)	0.0013	0.0014
%RSD	0.8836	0.8208

2.6.3 Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$\text{LOD} = 3.3 \sigma / S$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$\text{LOQ} = 10 \sigma / S$$

Where, σ is standard deviation of the response and S is the slope of the calibration curve.

LO& LOQ of Tramadol Hydrochloride was found to be 1.04µg/ml & 3.17µg/ml respectively.

Table 4: Summary of validation parameters

Parameter	Result
λ range	272-280
Regression Equation (y=mx+c)	Y=9E-05x + 0.0004
Linearity range	2-10µg/ml
Slope	9E-05
Intercept	0.0004
Correlation coefficient (R ²)	0.9925
Limit of Detection (LOD) µg/ml	1.04
Limit of Quantitation (LOQ) µg/ml	3.17
Accuracy (Mean % Recovery)	99.87
Precision (%RSD)	0.8836

3. Results and Discussion

The UV visible spectroscopic method for the Tramadol Hydrochloride by Second order derivative Area under Curve was found to be simple, accurate, economical and reproducible. The drug concentrations were found to be linear in the range of 02-10 µg/ml and the correlation coefficient value of 0.9925 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 0.8836 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.8836 and 0.8208 respectively thus the method is observed as precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value is ≤ 2 indicates the accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 1.04µg/ml & 3.17µg/ml respectively. The result of the analysis for pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for routine quality control analysis of Tramadol Hydrochloride in bulk and pharmaceutical formulations.

4. Conclusion

The UV spectroscopic AUC method for the analysis of Tramadol Hydrochloride by Second order derivative Area under Curve 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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