Original Research Article

DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHODS AND VALIDATION FOR ESTIMATION OF TRAMADOL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM BY ABSORBANCE MAXIMA AND AREA UNDER THE CURVE METHOD

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ABSTRACT

We aimed this study to develop simple and economic UV spectrophotometric method for estimation of Tramadol Hydrochloride in bulk and tablet dosage form and validate as per ICH guidelines. Method involved Absorbance maxima method which based on the measurement of absorbance of Tramadol Hydrochloride in methanol: water (60:40 % v/v) atλmax of 271 nm. The developed method was validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines. Theproposed method was found to be linear within the conc. range of 30-150µg/ml for Tramadol Hydrochloride. The present methods were found to be simple, linear, precise, accurate and sensitive and can be used for routine quality control analysis for the estimation of Tramadol Hydrochloridein bulk and tablet dosage form.

Keywords: Tamadol Hydrochloride, UV spectrophotometry Absorbance maxima method and ICH guidelines.

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INTRODUCTION

Tramadol hydrochloride is a centrally acting synthetic opioid analgesic binding to specific opioid receptors. It is a non-selective, pure opioid agonist at µ, delta and kappa opioid receptors with a higher affinity for µ receptors. Other mechanisms which contribute to its analgesic effects are inhibition of neuronal reuptake of noradrenaline and enhancement of serotonin release[1, 2 & 3].It is chemically known as(±)cis-2-[(dimethylamino)methyl]-1-(3methoxyphenyl)cyclohexanol hydrochloride (Fig.1). Tramadol Hydrochloride can be estimated by UV spectrophotometry [4-8], RP-UPLC [9-12]and GC-MS [12]alone or in combination with other drugs.

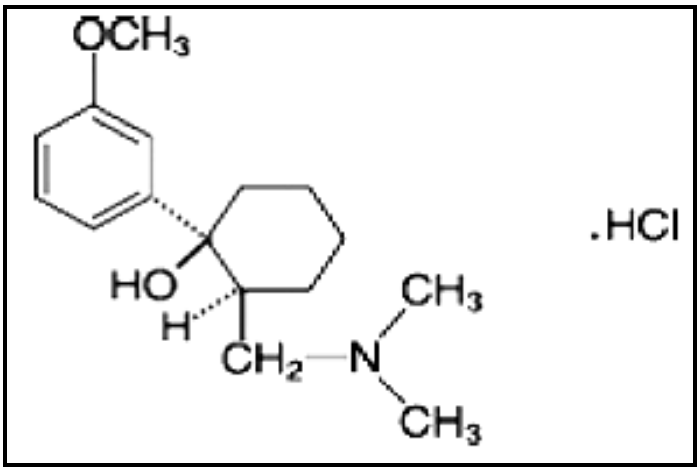

Figure 1. Chemical structure of Tramadol Hydrochloride

Because of cost-effective and minimal maintenance, UV spectrophotometry is always preferred at small scale industries. Literature survey revealsthat so far many UV spectrophotometric methods have been reported forthe estimation of Tramadol Hydrochloride in alone or in combination with other drugs. But out of them only few methods included single estimation of Tramadol Hydrochloride. Therefore the main objective of the proposed methods were to develop simple, new and economic UV spectrophotometric methods for the estimation of Tramadol Hydrochloridein bulk and tablet dosage form and validate as per ICH guidelines [13].

MATERIALS AND METHODS

Chemicals and Reagents

The pure API sample of Tramadol Hydrochloride was obtained as free gift samplefrom NulifePharmaceutical Ltd; Pune while solvent such as methanol used were of spectroscopy grade (E. Merck India) and double distilled water used for whole experiment. The marketed combined pharmaceutical dosage form ofTramadol Hydrochloride(50 mg) i.e. [Ultram]was purchased from local market.

Instrumentation

A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (2nm) and 1-cm quartz cell was used for Spectral and absorbance measurements.

Preliminary solubility studies of drug

1 gm of Tramadol Hydrochloride was weighed and solubility was checked in 10 ml water, methanol, 0.1N NaOH and 0.1 N HCl. The drug was found to be freely soluble in methanol and practically poorly soluble in water, 0.1NNaOH and 0.1 HCl. Therefore methanol and water (60:40 % v/v) was selected as diluent and drug was also found to be stable in methanol for 48 hours in stability studies.

Preparation of standard stock solutions

Transfer 25 mg of pure Tramadol Hydrochloride in separate 25 ml of volumetric flask containing sufficient quantity of methanol: water (60:40 % v/v) as diluent and then sonicated for 15 minutes and final volume made upto mark with same diluent to form 1000µg/ml std. stock solution of Tramadol Hydrochloride.. From it, transfer aliquot of 10 ml in 100 ml of volumetric flask and diluted upto mark with diluent to form 100 µg/ml working std. solution of Tramadol Hydrochloride.

Preparation of calibration curve

From above working std. stock solution of Tramadol Hydrochloride (100 µg/ml), pippete out aliquotsof 3 to 15 ml and transferred to series of 10 ml volumetric flasks and final volume made upto mark with diluent to form solutions of 30 to 150µg/ml of Tramadol Hydrochloride. These solutions were then scanned in the range of 200-400 nm against diluent as blank. The absorbance maxima (λmax) was found to be 271 nm for Tramadol Hydrochloride and then calibration curve was plotted as absorbance vs. concentration.

Sample preparation for analysis of Tablet formulation

Twenty tablets (Ultram) containing 50 mg of Tramadol Hydrochloride weighed, average weight calculated and triturated to fine powder and then weight equivalent 50mgof Tramadol hydrochloride transferred to 100 ml of volumetric flask containing proposed diluent, then sonicated for 15 minutes and filtered through Whatman filter paper no. 42 to form 500µg/ml of Tramadol hydrochloride std. stock solution and final volume made upto mark with diluent. From this, 1 ml of aliquot transferred in 10 ml of volumetric flask containing diluent to form 50µg/ml of Tramadol hydrochloride stock solution and

scanned in the range of 200-400 nm against methanol as blank at 271 nm and then drug content of solution was calculated by using standard calibration curve.

Absorbance maxima method

For the selection of analytical wavelength, working standard solution of Tramadol Hydrochloride was scanned in the spectrum mode from 200 nm to 400 nm separately. From the spectra of drug, λmax of Tramadol Hydrochloride, 271 nm was selected for the analysis (Fig. 2). Aliquots of standard stock solution were made and calibration curve was plotted.

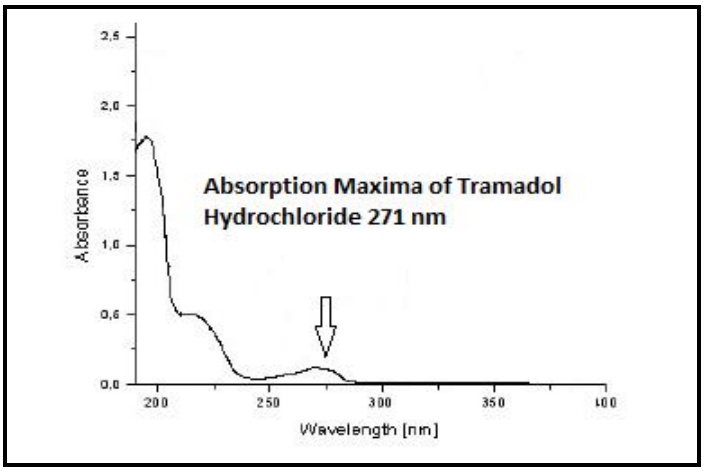

Figure 2.Absorption maxima of Tramadol hydrochloride

Validation

The present UV spectrophotometric methods was validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines for estimation of Tramadol hydrochloride in bulk and tablet dosage form.

Linearity

From std. stock solutions of Tramadol Hydrochloride (100 µg/ml), pippete out aliquots of 3 to 15 ml of Tramadol Hydrochloride transferred to series of 10 ml volumetric flasks and final volume made upto mark with methanol as diluent to form solutions of 30 to 150µg/ml of Tramadol Hydrochloride. These solutions were then scanned in the range of 200-400 nm against diluent as blank at λmax of Tramadol Hydrochloride and then calibration curve was plotted as absorbance vs. concentration to check the linear relationship between absorbance and concentration of Tramadol Hydrochloride.

Precision

Precision study expressed by carrying out Repeatability (intraday precision) and interday precision. The intraday (Repeatability) and interday precision study were carried out by estimating corresponding responses three times on the same day and on the three different days for the concentration for (90µg/ml) for Tramadol Hydrochloride. The results of precision study were reported in terms of % relative standard deviation

Accuracy

The accuracy of developed method was carried out by calculating the % recovery of Tramadol Hydrochloride by standard addition method at three different levels i.e. 50 %, 100 % and 150 %. Known amount of standard solutions of Tramadol Hydrochloride (30, 60and 90µg/ml) were added to prequantitated sample solutions of 60µg/ml of SMV).

LOD and LOQ

Limit of detection (LOD) is defined as lowest concentration of analyte that can be detected while limit of quantitation is defined as lowest concentration of analyte that can be quantitated. With suitable precision and linearity. LOD and LOQ can be calculated from the following formulas

$$LOD = 3.3 * r / S \quad \text{and} \quad LOQ = 10 * r / S$$

Where r is the Standard deviation of y-intercept of the regression line and S is slope of the calibration curve.

RESULTS AND DISCUSSION

Method development and optimization

The present study describesdevelopment and validation of simple and economic UV spectrophotometric method for the estimation ofTramadol Hydrochloride in bulk and tablet dosage form using absorbance maxima method. Solubility studies indicated that a Tramadol Hydrochloride shows better solubility in proposed diluent i.e. methanol: water (60:40 % v/v)solution as compared to solubility in distilled water and the λmax ofTramadol Hydrochloride was found to be 271 nm. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries as compared to other reported methods.

Validation

Linearity

Linearity was evaluated by analysis of Std. Tramadol Hydrochloride at five different concentrations.Tramadol Hydrochloride found to be linear within conc. range of 30-150µg/ml with regression coefficient of 0.999. The results of regression analysis are summarized in (Table 1). Results shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration. (Fig. 3)

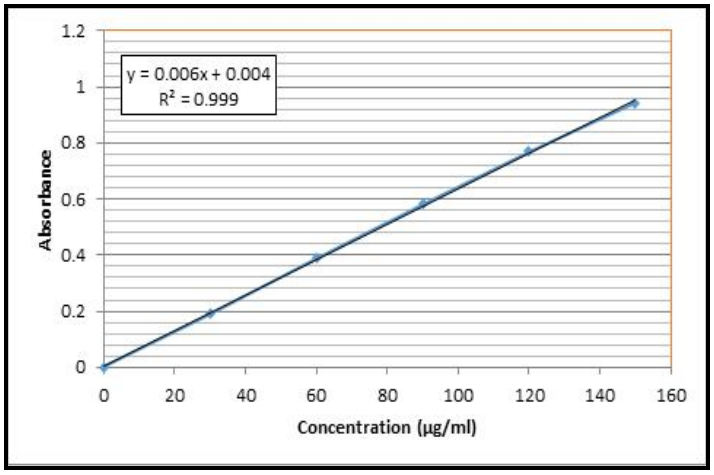

Figure 3. Calibration Curve of Tramadol hydrochloride

Precision

The repeatability (intra-days precision) is expressed as percentage relative standard deviations (% RSD). The average % RSD value of intra-days precision for Tramadol Hydrochloride at the concentration of 90 µg/ml were 0.0086 while 0.0061 for inter-days precision, The % RSD levels of intra-

day and inter-day precision were less than 2 in all cases, which indicated that there were no significant variations in the analysis ofTramadol Hydrochloride at the concentrations and the proposed method was precise which are shown in (Table 2 & 3).

Table 1.Results of regression analysis of Tramadol hydrochloride

Tramadol Hydrochloride	Beer's Range (µg/ml)	Regression equation	Regression coefficient (r^2)
Absorbance maxima method	30-150	y = 0.006x-0.004	0.999

Table 2.Results of Intraday Precision Study

Concentration (µg/ml)	Absorbance			Average % RSD
	Morning	Afternoon	Evening	
90	0.575	0.570	0.573	
90	0.580	0.573	0.588	
90	0.581	0.587	0.584	
90	0.580	0.580	0.590	
90	0.573	0.575	0.578	
90	0.579	0.580	0.588	
%RSD	**0.005**	**0.010**	**0.011**	**0.0086**

R.S.D. - Relative Standard Deviation

Table 3.Results of Interday Precision Study

Concentration (µg/ml)	Absorbance			Average % RSD
	Day 1	Day 2	Day 3	
90	0.580	0.578	0.579	
90	0.591	0.580	0.578	
90	0.582	0.581	0.580	
90	0.567	0.579	0.581	
90	0.574	0.578	0.579	
90	0.584	0.582	0.579	
%RSD	**0.0138**	**0.0028**	**0.0017**	**0.0061**

Accuracy (Recovery Study)

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing30, 60 and 90µg/ml of Tramadol Hydrochloride. The average % recoveries for three different concentrations was found to be 99.56. SMV using proposed UV spectrophotometric method. The higher values indicated that the proposed UV spectrophotometric method was accurate for the determination ofTramadol Hydrochloride in pharmaceutical dosage form. Results of recovery studies are summarized in (Table 4).

Table 4.Results of Accuracy (Recovery) Studies.

Concentration of		% drug added	Amount found	% recovery	Mean	SD	%RSD
Tablet (µg/ml)	Pure drug (µg/ml)						
60	30	50	29.38	97.93			
60	30	50	29.82	99.40	98.58	0.7474	0.0075
60	30	50	29.53	98.43			
60	60	100	59.27	98.78			
60	60	100	59.61	99.35	99.08	0.2874	0.0029
60	60	100	59.48	99.13			
60	90	150	89.66	99.62			
60	90	150	89.43	99.36	99.55	0.1664	0.0016
60	90	150	89.71	99.67			

***Average of three estimations. SD: - Standard Deviation**

LOD and LOQ

The limit of detection was found to be 0.12µg/mland the limit of quantification was found to be 0.36. Low values of LOD and LOQ indicates that the developed method was sensitive for the estimation Tramadol Hydrochloride in bulk and tablet dosage form. Results of LOD and LOQ are summarized in (Table 5).

Table 5. Results of LOD and LOQ

Drugs	LOD (µg/ml)	LOQ (ng/band)
Tramadol Hydrochloride	0.12	0.36

Assay

Analysis of sample of marketed tablet containing 10 mg Tramadol Hydrochloride was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recovery ofTramadol Hydrochloride was99.48.Results of tablet assay are summarized in (Table 6).

Table 6. Results of tablet Assay

Drug	Label Claim (mg/tab)	Amount of Drug* Estimated (mg/tab)	% Assay
Tramadol Hydrochloride	50 mg	49.80	99.48

*** Average of Six estimations**

CONCLUSION

A simple UV spectrophotometric method have been developed and validated for the determination of Tramadol Hydrochloride in bulk and tablet dosage form. The results of the validation parameters show that the UV spectrophotometric methods were found to be accurate, precise and sensitive. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industriesand successfully applied and suggested for the quantitative analysis of

Tramadol Hydrochloride in pharmaceutical formulations for QC, where economy and time areessential and to assure therapeutic efficacy.

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