ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Development of New Analytical Method and Its Validation for the Determination of Tramadol Hydrochloride in Bulk and Marketed Formulations

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Received:
17th July 2013
Received in revised form:
15th Aug 2013
Accepted:
25th Aug 2013
Available online:
15th Sept 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

Tramadol hydrochloride in presence of acidic medium reacts with excess amount of Chloramine-T and remaining Chloramine-T react with Crystal violet to produce blue colour. The final stock solution was made to produce 100µg/ml with methanol. The λ_{max} was found to be 591 nm for assay. The linearity was found in concentration range of 5-30µg/ml. The correlation coefficient was found 0.999. The regression equation was found as Y= 0.025X + 0.001.The method was validated according to ICH Guidelines.

Keywords: Tramadol Hydrochloride, Crystal violet, Chloramine T, H_2SO_4,

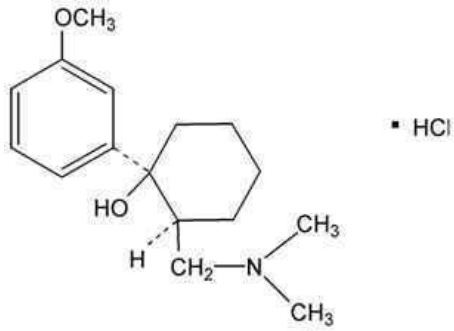
1. INTRODUCTION:

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.[1]

Tramadol hydrochloride is a centrally acting synthetic analgesic used to treat moderate to moderately severe pain. The drug has a wide range of applications, including treatment of rheumatoid arthritis, restless legs syndrome, motor neurone disease and fibromyalgia[2] .

Tramadol hydrochloride is a weak µ-opioid receptor agonist and is a reuptake inhibitor of serotonin and of norepinephrine. Tramadol is metabolized to *O*-desmethyltramadol, a significantly more potent µ-opioid agonist. Tramadol and its major metabolite(s) are distinguished from other more potent opioid agonists by relative selectivity for µ-opioid receptors[2].

Tramadol hydrochloride is a centrally-acting synthetic opioid analgesic binding to specific opioid receptors. It is a non-selective, pure agonist at mu, delta and kappa opioid receptors with a higher affinity for the mu receptor. Other mechanisms, which may contribute to its analgesic effect, are inhibition of neuronal re-uptake of noradrenaline and enhancement of serotonin release[3].



(±)-cis-2[(Dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride.

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It is soluble in freely soluble in water, methanol untill now there are HPLC and spectrophotometric method developed on Tramadol Hydrochloride.[4-13]

The validation defines eight steps and that are Accuracy, Precision, Specificity, Limit of detection, which are described Limit of quantitation, Linearity and range, Ruggedness, Robustness and that are describe in validation part.

2. MATERIALS AND METHODS

EXPERIMENTAL:

Tramadol Hydrochloride was determined spectrophotometrically in bulk and marketed formulation by using Crystal violet indicator and Chloramine T as a strong oxidizing agent.

Reagent and chemicals:

(1) Working standard stock solution (100µg/ml)
(2) 0.01M Chloramine T solution
(3) 2M H_2SO_4
(4) 0.02% Crystal violet

Preparation of standard stock solution of Tramadol Hydrochloride:

Standard stock solution prepared by accurately weighing 100 mg of Tramadol Hydrochloride in 100 ml calibrated volumetric flask and made up the volume with Methanol up to 100 ml.

Preparation of working standard solution of Tramadol Hydrochloride:

Working standard was prepared by transferring of 10ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with Methanol for getting concentration of 100µg/ml.

Preparation of 0.01 M Chloramine T solution:

chloramine-T was weighed accurately 280 mg and transferred into 100ml volumetric flask and made up the volume with distilled water.

Preparation of 2M H_2SO_4:

108 ml of concentrated H_2SO_4 was accurately transferred into 1000 ml volumetric flask and made up the volume with distilled water..

Preparation of 0.02% Crystal violet:

200 mg crystal violet was weighed accurately and diluted up to 100 ml with distilled water. From above solution transferred 10ml into 100ml volumetric flask and made up the volume with distilled water.

Preparation of Standard curve:

Standard curve was prepared by using pure Tramadol Hydrochloride in the concentration range of 5-30µg/ml by this method and selecting absorbance maximum at 591nm.

Procedure:

Different seven 10 ml volumetric flask was taken and 0.5 ml of 2M H_2SO_4 and 0.5 ml of 0.01M Chloramine-T were added in all volumetric flasks. Kept it aside for 20 minutes. Then 0.5, 1, 1.5, 2, 2.5, and 3 ml of working standard of tramadol hydrochloride were added in each volumetric flask and waits for 10 minutes. Then 0.8 ml 0.02% of crystal violet solution was added and made up the volume with methanol.The absorbance of the resulting solutions was measured at 591nm against reagent blank. The result was recorded in table 1. and graph is given in figure 2.

Sr no:	Volume of working standard of drug (ml)	Concentration in µg/ml	Absorbance at 591nm
1	0.5ml	5µg/ml	0.128
2	1.0ml	10µg/ml	0.236
3	1.5ml	15µg/ml	0.401
4	2.0ml	20µg/ml	0.512
5	2.5ml	25µg/ml	0.644
6	3.0ml	30µg/ml	0.781

Table 1 : Absorbance of different concentration of Tramadol Hydrochloride at 591 nm

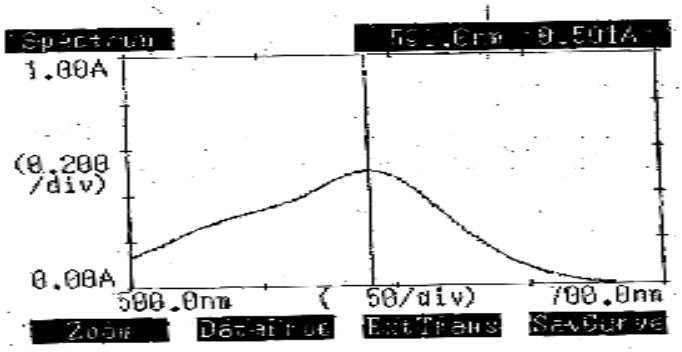

Figure 1: λ_{max} for Tramadol Hydrochloride

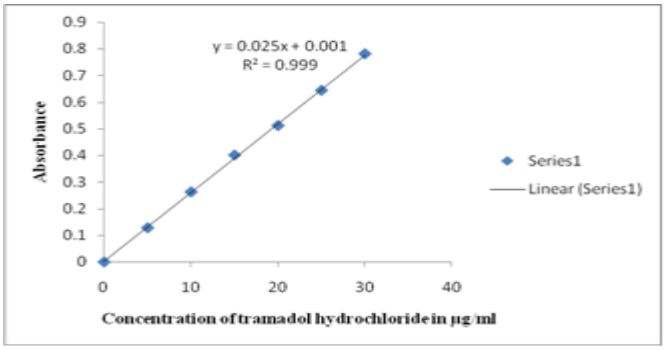

Figure 2: Calibrated Curve for Tramadol Hydrochloride at 591 nm

METHOD VALIDATION:

Linearity:

Linearity was determined over the range of 5 to 30µg/ml. 7 different 10 ml volumetric flask was taken and 0.5 ml of 2M H2SO4 and 0.5 ml of 0.01M Chloramine-T was added in all volumetric flasks. Kept it aside for 20minutes. Then 0.5, 1, 1.5, 2, 2.5, and 3 ml of working standard of tramadol hydrochloride were added in each volumetric

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flask and waits for 10 minutes. Then add 0.8ml 0.02% of crystal violet solution and made up the volume with methanol. Absorbance was measured against corresponding reagent blank at 591nm.

%Recovery (Accuracy):

The accuracy of the methods was determined by calculating % recovery of Tramadol hydrochloride by standard addition method. Known volumes of standard solutions of Tramadol hydrochloride were taken for recovery studies in 3 different levels 80, 100, 120% and recovery study was carried out. The results were reported in terms of % Recovery in table 3.

Method precision (% Repeatability):

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 5,10….30 µg/ml without changing the parameters for the method. The results were reported in Table 3.

Intermediate precision:

The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Tramadol hydrochloride (5, 10, 15 µg/ml). The results were reported in Table 3.

Reproducibility:

The absorbance readings of 10µg/ ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their reproducibility. The results were reported in Table 3.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline**:**

LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

Result is displayed in table 3

Analysis of marketed formulation:

Tramadol hydrochloride is marketed as Tramazac of 100 mg tablet manufactured by Cadila healthcare were taken for analysis

Preparation of sample solution:

Tablet powder equivalent to 100mg was weighed accurately and transferred into 100ml volumetric flask and made up the volume with Methanol to get 1000µg/ml concentration (Stock solution- A). This solution was further diluted to get concentration of 100µg/ml (Stock solution-B).

Recovery experiments:

Procedure: 0.5ml of 2M H_2SO_4 and 0.5 ml of 0.01M Chloramine-T were added in 10ml volumetric flasks. Kept it aside for 20minutes. Then 1 ml of working standard of Tramadol hydrochloride was added in 10ml volumetric flask and waits for 10 minutes. Then 0.8 ml 0.02% of Crystal violet solution was added and made up the volume with methanol. The blank was also prepared in the same way omitting the drug. The absorbance of the resulting solution was measured at 591nm against reagent blank. The result was recorded in Table 2.

Formulation	Actual concentration of Tramadol hydrochloride(µg/ml)	Amount obtained of Tramadol hydrochloride (µg/ml)	% Tramadol hydrochloride
tablet	10µg/ml	9.893 µg/ml	98.93%

Table 2 : Assay results of Marketed Formulation

Parameter	Tramadol Hydrochloride 591nm
Linear Range (µg/ml)	5-30
Regression Equation* (y)	y=bx+a: 0.025x+0.001
Slope (b)	0.025
Intercept (a)	0.001
Correlation coefficient (R^2)	0.999
Limit of Detection (µg/ml)	0.1598
Limit of Quantition(µg/ml)	0.4844
Molar Absorptivity (1/mol.cm)	2.36×10^5
%Recovery	1) At Level-1 (80%)=98.02 2) At Level-2 (100%)=99.60 3) At Level-3 (120%)=99.58
Repeatability Data(%RSD)	0.21-0.94
Reproducibility:-	
Instrument 1 (%RSD)	0.17
Instrument 2 (%RSD)	0.15
Precision(n=3)	
Intraday precision(%RSD)	0.38-0.80
Inter day Precision(%RSD)	0.14-1.23

Table 3: Statistical data for Tramadol hydrochloride by colorimetric method

3. RESULTS AND DISCUSSION

Chloramine – T is strong oxidizing agent. Its react with Tramadol hydrochloride in present of acidic medium. When Chloramine–T added in excess amount it produces colorless complex of Tramadol hydrochloride. Remaining chloramine–T now readily reacts with Crystal violet & oxidizes Crystal violet. Remaining molecules of Crystal violet gives color. So, color of the final solution indicates the amount of drug present.

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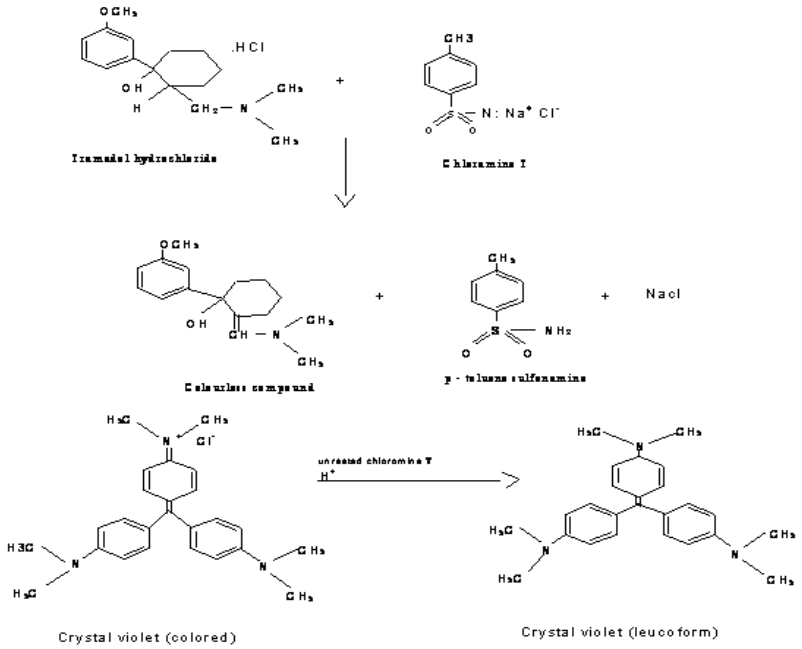

5. CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. A very few analytical method appeared in the literature for the determination of Tramadol Hydrochloride. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Tramadol Hydrochloride in bulk drug and pharmaceutical formulations has been developed.

6. ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Srinivas College of Pharmacy and A. Shama Rao Foundation, Mangalore for providing the facilities to carry out the present work.

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Conflict of Interest: None Declared

Cite this article as:
Sagarbechara, Padmavathi P. Prabhu, Subrahmanyam E.V.S , Jignesh J. kaneria, Sumit saparia,Shabaraya A.R. Development of New Analytical Method and Its Validation for the Determination of Tramadol Hydrochloride in Bulk and Marketed Formulations . Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (23), 53-56.

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