ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Development of new Analytical Methods and their Validation for the Determination of Tizanidine Hydrochloride in Bulk and Marketed Formulations

Sumit Saparia[1]*,Padmavathi P. Prabhu[1],Dr. E.V.S Subrahmanyam[1],Jignesh Kaneria[1],Sagar Bechara[1],Dr A.R.Shabaraya[2]

Department of Quality Assurance, Srinivas College of Pharmacy, Valachil, Mangalore.

Department of Pharmaceutices, Srinivas College of Pharmacy, Valachil, Mangalore.

Received:
27th Mar 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

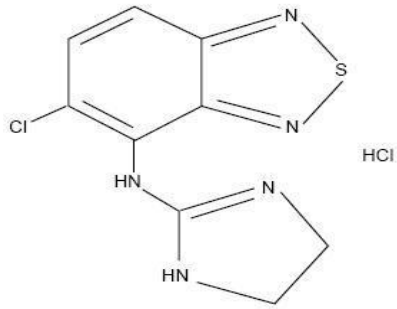
ABSTRACT

Tizanidine Hydrochloride in presence of acidic medium reacts with excess amount of potassium bromide-bromate and unreacted potassium bromide-bromate react with malachite green to produce green colour.The final stock solution was made to produce 100µg/ml with distilled water. The $\lambda_{max}$was found to be 617nm for assay. The linearity was found in concentration range of 10-30µg/ml. The correlation coefficient was found 0.999. The regression equation was found as Y=0.009x + 0.002. The method was validated according to ICH Guidelines.

Keywords: Tizanidine Hydrochloride, malachite green, potassium bromide-bromate, HCl.

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]. Tizanidine is an agonist at α2-adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. In animal models, Tizanidine has no direct effect on skeletal muscle fibers or the neuromuscular junction, and no major effect on monosynaptic spinal reflexes. The effects of Tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons[2].



5-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine, hydrochloride.

It is soluble in freely soluble in water, soluble in methanol up to now there is HPLC and spectrophotometric method developed on Tizanidine Hydrochloride[3-9].

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.

*Corresponding author: Sumit Saparia | Department of Quality Assurance, Srinivas College of Pharmacy, Valachil, Mangalore

2. MATERIALS AND METHODS

EXPERIMENTAL:

Tizanidine Hydrochloride was determined spectrophotometrically in bulk and marketed formulation by using Malachite green indicator and potassium bromide-bromate as a strong oxidizing agent.

Reagent and chemicals:

(1) Working standard stock solution (100µg/ml)
(2) 550µg/ml potassium bromide-bromate solution
(3) 1M HCl
(4) 0.05% Malachite green

Preparation of standard stock solution of Tizanidine Hydrochloride:

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

Preparation of working standard solution of Tizanidine Hydrochloride:

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

Preparation of 550µg/ml Potassium bromide-bromate solution:

Accurately 1 gm. of potassium bromide and 0.1gm of potassium bromate was weighed and transferred to a 100ml volumetric flask. The volume was made up with distilled water dissolving the contents. Further 5ml of the above solution was pipetted out into a 100ml volumetric flask and the volume was made up to the mark**.**

Preparation of 1M HCl:

8.5 ml of concentrated HCl was dissolved in100ml of distilled water.

Preparation of Malachite Green:

50mg of Malachite green was weighed accurately and diluted up to 100 ml with distilled water.

Preparation of Standard curve:

Standard curve was prepared by using pure Tizanidine Hydrochloride in the concentration range of 10-50µg/ml by this method and selecting absorbance maximum at 617nm.

Procedure:

From the working standard drug solution 1, 2, 3, 4, and 5 ml (which gives 10-50µg/ml) drug solution were placed in 6 different 10 ml volumetric flasks. To this 0.6ml of 550 µg/ml and 0.7ml of 1M HClwere added. Then after 0.5ml of 0.05% Malachite green was added and volume was made up to 10 ml with Water. The blank was also prepared in the same way omitting the drug. The absorbance of the resulting solutions was measured at 617nm against reagent blank. The result was recorded in Table 1. and graph is given in (fig. 2).

Sr.no.	Volume of working standard of drug (ml)	Concentration of drug (µg/ml)	Absorbance At 617nm
1	1.0	10	0.0987
2	2.0	20	0.1798
3	3.0	30	0.2787
4	4.0	40	0.3665
5	5.0	50	0.4578

Table 1: Absorbance of different concentration of Tizanidine Hydrochloride at 617 nm

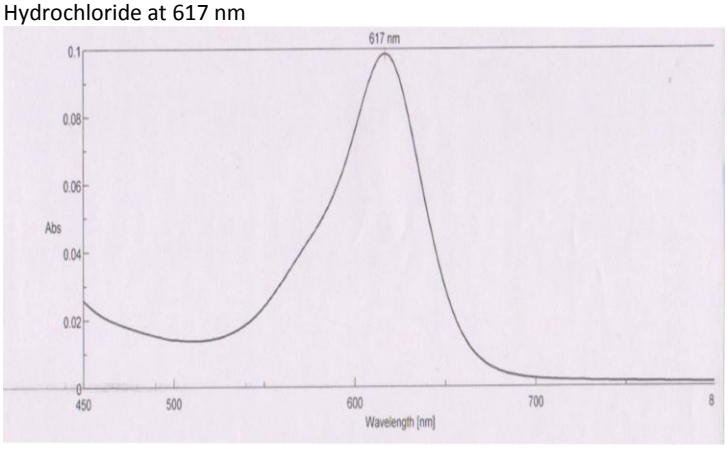


Figure 1: λ_{max}forTizanidine Hydrochloride

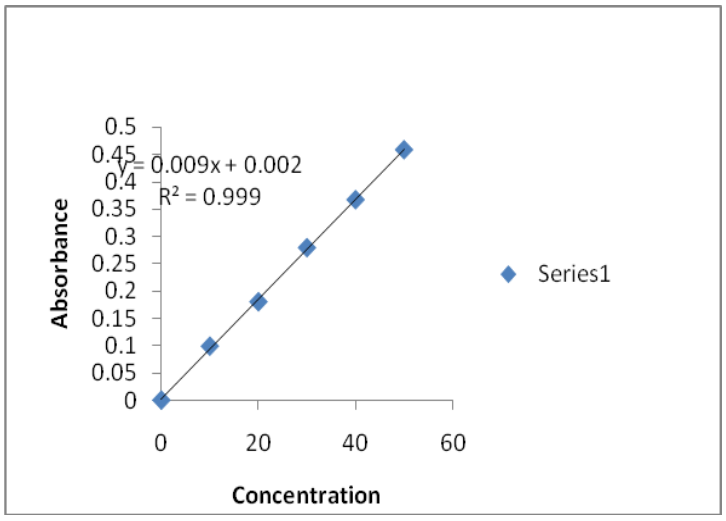


Figure 2: Calibrated Curve for Tizanidine Hydrochloride at 617 nm

METHOD VALIDATION:

Linearity:

Linearity was determined over the range of 10 to 50µg/ml. 5 different 10ml volumetric flasks were taken. To these flasks 1.0, 2.0, 3.0, 4.0 and 5.0ml of working standard of Tizanidine Hydrochloride were added. . To this 0.6ml of 550 µg/ml and 0.7ml of 1M HClwere added. Then after 0.5ml of 0.05% Malachite green was added and volume was made up to 10 ml with Water. The blank was also prepared in the same way omitting the drug. The

absorbance of the resulting solutions was measured at 617nm against reagent blank.

%Recovery (Accuracy):

The accuracy of the methods was determined by calculating % recovery of Tizanidine Hydrochloride by standard addition method. Known volumes of standard solutions of Tizanidine 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 6 µg/ml without changing the parameters for the method.The results were reported in Table 3.

Intermediate precision (Reproducibility):

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 Tizanidine Hydrochloride (10, 20, 305 µg/ml). 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 2.

Formulation	Actual concentration of Tizanidine hydrochloride(µg/ml)	Amount obtained of Tizanidine hydrochloride (µg/ml)	% Tizanidine hydrochloride
Tablet	10 µg/ml	9.89 µg/ml	98.9%

Table 2: Assay Results of Marketed Formulation

Analysis of marketed formulation:

Tizanidine Hydrochloride is marketed as Tizan of 2mg tablet manufactured by Sun Pharma Ltd. was 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 water to get 1000µg/ml concentration (Stock solution- A). This solution was further diluted to get concentration of 100µg/ml (Stock solution-B).

Recovery experiments:

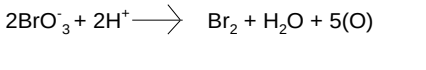
Procedure: From Stock solution B 1.0ml was pipetted out into a 10ml volumetric flask.To this 0.6ml of 550µg/ml potassium bromide-bromate and 0.7ml of 1M HCl were added. Then after 0.5ml of 0.05% Malachite green was added and volume was made up to 10 ml with water. The blank was also prepared in the same way omitting the drug. The absorbance of the resulting solution was measured at 617nm against reagent blank. The result was recorded in Table 3.

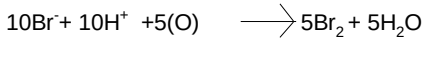
Parameter	Tizanidine Hydrochloride 617nm
Linear Range (µg/ml)	10-50
Regression Equation* (y)	y=bx+a: 0.009x + 0.002
Slope (b)	0.009
Intercept (a)	0.002
Correlation coefficient (R^2)	0.999
Limit of Detection (µg/ml)	0.03804
Limit of Quantition(µg/ml)	0.1152
%Recovery	1) At Level-1 (80%)=99.5 2) At Level-2 (100%)=100.5 3) At Level-3 (120%)=99.41
Precision(n=3)	
Intraday precision (%RSD)	0.04-0.10
Inter day Precision (%RSD)	0.04-0.10

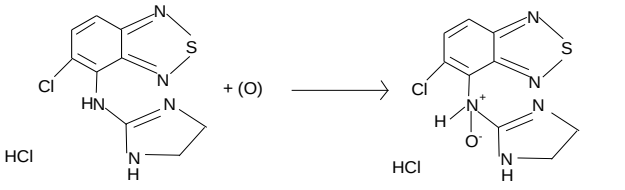
Table 3: Statistical data for Tizanidine hydrochloride by colorimetric method

3. RESULTS

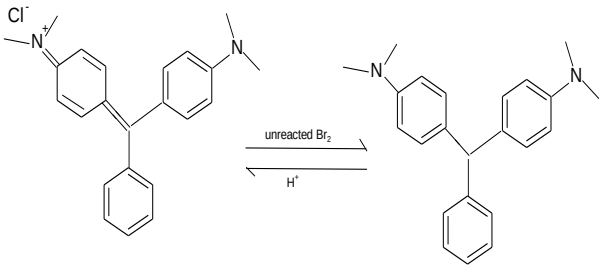
Potassium bromide-bromate strong oxidizing agent. It reacts with Tizanidine Hydrochloride in present of acidic medium. When Potassium bromide-bromate added in excess amount it produces colorless complex of Tizanidine Hydrochloride. Unreacted Potassium bromide-bromate now readily reacts with malachite green & malachite green. Remaining molecules of malachite green gives color. So, color of the final solution indirectly indicates the amount of drug present.







Oxidation Reaction



Malachite Green Malachite Green (leuco form)

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 Tizanidine 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 Tizanidine 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.ShamaRao Foundation, Mangalore for providing the facilities to carry out the present work.

7. REFERENCES

1. Chemistry 111 Lab: Intro to Spectrophotometry. Spectrophotometry 2005;E1:8.
2. http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=5153
3. International Conference on Harmonization, Guidance for industry in; Q2B Validation on Analytical Procedures: Methodology. Switzerland 1996:1-8.
4. S. Gondane, M. Deshpande, M. Mahajan and S. Sawant, Spectrophotometric method development and validation for estimation of Tizanidine and Aceclofenac in Bulk Drug & Tablet formulation. IJCRGG:620-24.
5. R. Kumar, P. Nallasivan and P. Vijayanand, spectrophotometric methods for simultaneous estimation of Aceclofenac and Tizanidine. IPJRIF:945-9.
6. M. Walash, F. Belal, M. Eid and S. Mohamed, Spectrophotometric determination of Tizanidine and Orphenadrine via ion pair complex formation using eosin Y. CCJ: 1-9.
7. D. Chakravarty, R. Charde, M.Chardeand M. Tajne, have developed Estimation of Tizanidine HCl by UV-Spectrophotometric Method. JPR; 2010:2107-09.
8. A. Kumar, M. Shankar, S. Prabhsimran and G. Shah, simultaneous spectrophotometric estimation of Diclofenac Potassium and Tizanidine Hydrochloride in bilk and solid dosage forms. IJIPLS, 2011:316-27.
9. K. Dashora and S. Saraf, spectrophotometric methods for the determination of Tizanidine Hydrochloride in solid dosage forms. Peer Reviewed ISI, FAORJ: 38687.

Conflict of Interest: None Declared

Cite this article as:
Sumit Saparia, Padmavathi P. Prabhu, E.V.S Subrahmanyam, Jignesh Kaneria, Sagar Bechara, A. R. Shabaraya. Development of new Analytical Methods and their Validation for the Determination of Tizanidine Hydrochloride in Bulk and Marketed Formulations. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (23), 57-60.

