ESTIMATION OF TIZANIDINE PRESENT IN TABLET FORMULATION BY RP-HPLC

G. SAI KIRAN
Research scholar,
Sri JJT University, Jhunjhunu, Rajasthan
Email: saikiran.gadipalli@gmail.com

RAKESH KUMAR JAT
Director & Principal.
Sri JJT University, Jhunjhunu, Rajasthan,

ABSTRACT

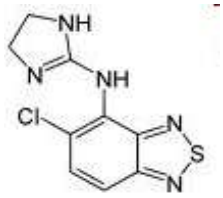
*A simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method was developed for estimation of Tizanidine in tablet dosage form on RP C-18 Column (BDS Hypersil 250*4.6 mm) using mobile phase as Buffer- Acetonitrile (60:40 v/v) The flow rate was 1.0 ml/min and effluent was monitored at 232nm. The retention time is 4.05 respectively. Proposed method was validated for Precision, Accuracy, Linearity range, Robustness and Ruggedness.*

***Key Words**: Tizanidine Reverse Phase High Performance Liquid Chromatography.*

INTRODUCTION

Tizanidine is a yellowish green 5-chloro-*N*-(4, 5-dihydro-1*H*-imidazol-2-yl)

-2, 1, 3-benzothiadiazol-4-amine ofMolecular formula $C_9H_8ClN_5$ S-HCl



,Molecular weight 290.2 *g/mol* and Melting point is 280 °*C* which is solubility water,methanol and acetonitrile Tizanidine is orally active ,In the brain (tizanidine hydrochloride) inhibit activity in the locus CeruleusIn the spinal cord primary action is on polysynaptic pathways to reduce release of excitatory neurotransmitters and reduce the sensitivity of the post-synaptic neuron to excitatory neurotransmitters.Drug that is used as a muscle relaxant. It is a centrally acting α-2 adrenergic agonist. It is used to treat the spasms, cramping, and tightness of muscles caused by medical problems such as multiple sclerosis These formulation available as tizan, tizpa .Literature survey reveals that various analytical techniques to analyze the formulation by spectroscopic methods[7,8,9],High performance low pressure method [9] ,high pressure methods[10,11] and Bioanalytical techniques[12] were available. The developed RP-HPLC method very economic, specific, accurate and précised method for analysis formulation of Tizanidine

MATERIALS AND METHODS:

Instrument:

High Performance Liquid Chromatographic system (Shimadzu) equipped with two LC 20AT liquid pumps, Rheodyne Injector (2E 7725, 20 µl loop), SPD 20A UV/Vis detector and Spinchrome software, Glass Van Hypodermic injecting syringe, an BDS Hypersil C-18 RP column (250 cm* 4.6 mm ID).

Reagents:

Acetonitrile of HPLC grade (Merck), Double Distilled Water.
Drug:Tizanidine.

Buffer preparation: Weigh accurately 6.8g of phosphate buffer and dissolve it in 1000ml of Milli-Q water. Adjust the pH to 3 with orthophosphoric acid, filter through 0.45µm Nylon membrane filter and degas.

Chromatographic condition: The mobile phase containing Buffer : Acetonitrile (60:40) was found to resolve Tizanidine. The mobile phase was filtered through 0.45-µ-membrane filter and the ultrasonicated for 30 min. the flow rate was set at 1.0 ml/min.. the drugs showed good absorbance at 232 nm, which was selected as wavelength for further analysis all determinations were performed at ambient column temperature**.**

Sample preparation

Weight accurately 20 tablet(2.1mg) and from it equivalent weight of 2.1mg was taken and dissolve in acetonitrile make upto 100ml. from it take 1ml and makeup to 100ml he final conc to 10µg/ml

Standard Preparation: Accurately Weighed and transferred Tizanidine 2mg of each into a 10 ml clean dry volumetric flask, and diluents was added, sonicated for 5 minutes, and diluted to the mark.

Diluted Standard: Pipette out 1 ml of standard stock solution dilute to 10ml with diluent.

Evaluation of System Suitability:

Inject 10µl of the diluted standard solution in five replicate injections, into the chromatograph and record the chromatograms.

The column efficiency as determined from Tizanidine peaks is not less than 3000 USP plate count and the tailing factor for Tizanidine peak is not more than 2.0.

The relative standard deviation for the peak areas of the five replicate injections is not more than 2.0%.

Procedure: Separately inject 10µl of the blank, Standard (five injections) and samples solution in duplicate into the liquid chromatography, record the chromatographs and measure the peak areas.

Calibration Curve:

Calibration curves were prepared by taking appropriate aliquots of Tizanidine stock solution in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 20, 40, 60, 80, 100 and 120 mcg/ml.. These solutions (n=6) were injected and chromatogram were taken. Flow rate was maintained at 1.0 ml/min. temperature of column kept ambient and the column effluents were monitored at 232 nm. Calibration curve was constructed by plotting peak area Vs concentration and regression equation was computed. R^2 values of tizanidine were found to be as 0.999

RESULT AND DISCUSSIONS:

To develop a simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method for estimation of, Tizanidine different mobile phases were tried and the proposed chromatographic conditions were found to

be appropriate for the quantitative determination. System suitability tests were carried as per ICH guidelines.

Method Validation:

The proposed RP-HPLC method was validated as per ICH guidelines.

Specificity:

The peak purity of Tizanidine were assessed by comparing the retention time of standard Tizanidine and sample good correlation was obtained between the retention time of standard and sample. Placebo and blank were injected and there were no peaks. There are no interferences hence method is specific.

Linearity:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range Tizanidine were found to be as 40-150mcg/ml,. The regression equation for Tizanidine were found to be as y = 7.615x-107.54. and with correlation coefficient (R^2) 0.9991 respectively.

Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise.

Accuracy (Recovery studies):

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Known amounts of standard Tizanidine were added to the pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in table.

Robustness:

The Robustness of method as carried out by changing the Chromatographic conditions such as Flow rate and Temperature variations. With the change of Flow rate of 0.8 ml, 1.0 ml and 1.2ml, change of Column temperature with of 24, 25 and 26^{o}Cmore and their tailing factor, plate count obtained within the limit

Ruggedness:

The ruggedness of method carried out by using the different HPLC system and Intraday Precision .percentage RSD are calculated for both parameters

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The lowest amount of analyze in sample that can be detected, but not necessary quantified, Tizanidine Standard with those of blank.

The lowest amount of analyze in the sample that can be determined with acceptable precision and accuracy was determined by the comparison of measured signal with 241 µg/ml and 2041 µg/ml

ASSAY

Sample preparation

Weight aacurately 20 tablet(2.1mg) and from it equivalent weight of 2.1mg was taken and dissolve in acetonitrile make upto 100ml. from it take 1ml and makeup to

100ml he final conc to 10µg/ml

Standard Preparation: Accurately Weighed and transferred Tizanidine 2mg of each into a 10 ml clean dry volumetric flask, and diluents was added, sonicated for 5 minutes, and diluted to the mark

Calculation:

Calculate the amount of each drug by using the following formula

% of Assay =A**x**C**x**E**x**G**x**(100-WATER) **x**0.99**x**P / B**x**D**x**F**x**L.A**x**100 (Mg/tablet)

Where,

A = Average peak area of sample
B = Average peak area of standard.
C = Dilution factor of standard.
D = Dilution factor of sample
E = weight of standard.
F = weight of sample.
G = Average weight of sample.
P = Potency of standard.
L.A = Labeled amount.

Tables and Figures:

System suitability:

Parameter	Tizanidine
Tailing Factor	1.05
Number of theoritical plate	5974
Resoloution	7230

Linearity

Conc in µg	Area
40	202.312
60	350.190
80	489.579
100	654.618
120	811.596

Accuracy:

S. No	Sample peak area	Standard peak area	% Recovery	Stastical analaysis
S1 80%	469.499	479.655	97.883	Avg-475.002, SD-4.766% RSD-0997
S2 80%	477.800	479.655	99.613	
S3 80%	478.173	479.655	101.850	
S4 100%	683.893	691.015	98.969	Avg-687.103, SD-2.954,% RSD-0.430
S5 100%	689.709	691.015	99.811	Avg-836.518, SD-.6812,% RSD-0.918
S6 100%	687.709	691.015	100.50	
S7 120%	841.631	847.561	99.361	
S8 120%	840.238	847.561	99.136	
S9 120%	827.685	847.561	97.655	

Precision

S.No	Area
1	639.509
2	637.537
3	639.356
4	637.893
5	640.884
Avg	638.435
SD	1.7070
%RSD	0.2673

Robust ness:

Flow rate variation:

S.No	Flow Rate (Ml)	Rt	Peak Area
1	0.8	2.387	397.220
2	1	1.947	280.131
3	1.2	1.946	280.134

Temperature variation:

S.NO	AREA	
1	633.756	25 ºC
2	635.706	27 ºC
Avg	634.31	
SD	1.3788	
%RSD	0.21723	

Ruggedness:

System-system variation:

Sample No.	Peak area.	
	Analyst-1	Analyst-2
1.	637.123	637.123

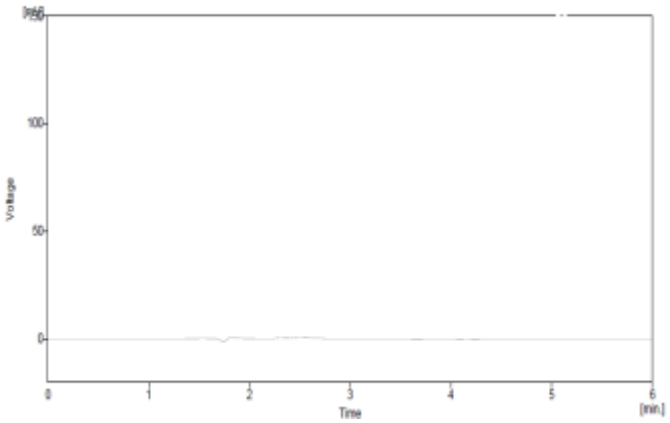
Intraday Precision:

S.No	Area
1	639.696
2	634.942
3	638.283
4	637.123
5	634.982
Avg	637.005
SD	2.075
%RSD	0.325

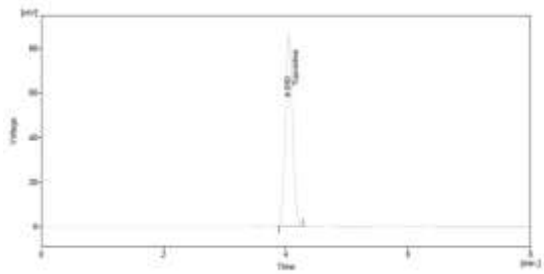
Sample	LOD	LOQ
Tizanidine	241 µg/ml	2041 µg/ml

Chromatograms:

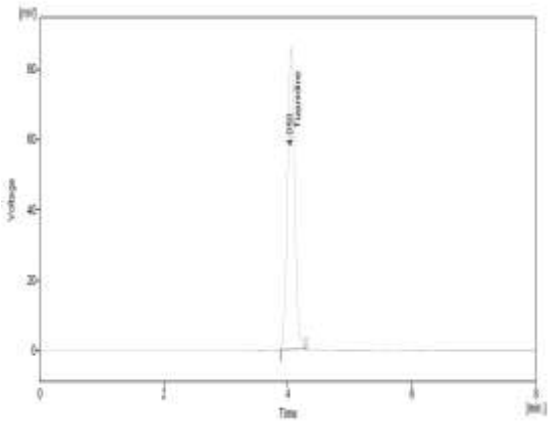
Blank



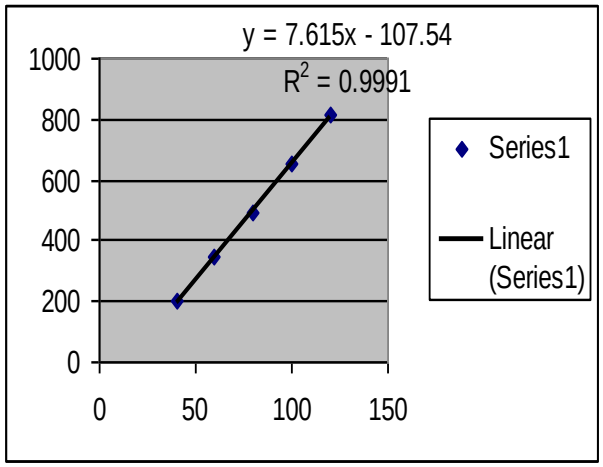
Standard:



Sample:



Linearity Graph:



CONCLUSION:

The proposed method is simple, specific, accurate and precise and hence can be used in routine for s estimation of Tizanidine in tablet dosage. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The %RSD for all parameters was found to be less than one, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative estimation of Tizanidine in tablet dosage form.

ACKNOWLEDGEMENTS:

The authors are thankful to Mr. K. Chandra Shekar, Managing Director of Chandra Labs, Balanagar, and Hyderabad for providing necessary facilities and. We are also thankful to Faculty of Roland Institute of pharmaceutical sciences for his moral support and guidance.

Reference:

1. http://en.wikipedia.org/wiki/Losartan
2. Kun Guo, Qiuxiang Yin*, Yu Yang, Meijing Zhang and Jingkang Wang, Solubility of Losartan Potassium in Different Pure Solvents from (293.15 to 343.15) K, *J. Chem. Eng. Data*, 2008, 53 (7), pp 1467–1469.
3. Goa K. L., Losartan potassium: A review of its pharmacology, clinical efficacy and tolerability in the management of hypertension, Drugs, 1996, vol. 51, n°5, pp. 820-845.
4. http://www.tapi.com/tapiteva/productPages/Hydrochlorothiazide
5. Beermann B, Groschinsky-Grind M, Rosén A. (1976). "Absorption, metabolism, and excretion of hydrochlorothiazide". Clin Pharmacol Ther 19 (5 (Pt 1)): 531–7.
6. Uniformed Services University Pharmacology Note Set #3 2010, Lectures #39 & #40, Eric Marks
7. M Gandhimathi, Simultaneous Estimation Of Losartan Potassium And Hydrochlorthiazide In Combination,Indian journal of

pharmaceutical sciences , Volume : 63 , Issue : 2 , Page : 165-166

8. Wankhede SB, Spectrophotometric and HPLC methods for simultaneous estimation of amlodipine besilate, losartan potassium and hydrochlorothiazide in tablets.Indian J Pharm Sci. 2010 Jan:72(1):136-40.
9. Obando MA ,Simultaneous determination of hydrochlorothiazide and losartan potassium in tablets by high-performance low-pressure chromatography using a multi-syringe burette coupled to a monolithic column.,Anal Bioanal Chem. 2008 Jul:391(6):2349-56. Epub 2008 May 24.
10. Erk N.,Analysis of binary mixtures of losartan potassium and hydrochlorothiazide by using high performance liquid chromatography, ratio derivative spectrophotometric and compensation technique.,J Pharm Biomed Anal. 2001 Feb:24(4):603-11.
11. Deanne L Hertzog, Development and validation of a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. J Pharm Biomed Anal. 2003 Jan 1:30 (5):1507-14
12. Salvadori MC ,Simultaneous determination of losartan and hydrochlorothiazide in human plasma by LC/MS/MS with electrospray ionization and its application to pharmacokinetics.,Clin Exp Hypertens. 2009 Jul:31(5):415-27