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BIOANALYTICAL METHOD PROCESS OF CHROMATOGRAPHIC ANALYSIS OF TIZANIDINE IN THE FORMULATION AND HUMAN PLASMA

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ABSTRACT

An optimized, simple, accurate method is developed to quantify Tizanidine in bulk and pharmaceutical formulation using the RP – HPLC method using a silica ODS C18 column with the aid of MeOH: H_2O:0.01M orthophosphoric acid (95:4:1 v/v/v) as a mobile phase and deduced at 320nm. The RT value was found to be 2.274 with a resolution of 1.167 respectively. The spiking studies of Tizanidine in human plasma were also carried out. The linearity studies of TZA (10 -50 mcg/mL) were found to be R2 =0.99979 (API) and R2 = 0.8155 respectively. The suggested study was validated as per the criteria of the ICH guidelines. The proposed study could be easily adopted in the pharmaceutical industry for performing quantitative analyses.

Keywords: Tizanidine, HPLC, HumanPlasma, MobilePhase, ICH guidelines

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INTRODUCTION

Tizanidine (Fig.-1) is a benzothiadiazole derivative (5-chloro -N-(4,5-dihydro-1H-imidazole-2yl)-2,1,3 -benzothiadiazole-4-amine(C9H8ClN5S; MW:253.17g/mol) used to release the contraction and increased muscle tone caused by multiple sclerosis, spinal injuries, and stroke.[1] Tizanidine was approved for medical use in 1996 by USFDA. Tizanidine reduces the spasms by creating a presynaptic inhibition of motor neurons which produces neuronal firing, causing muscle spasms.[2] Chromatographic data of different elution techniques were adopted from the literature retrieval[3,4]. However, existing studies were not dependable. Several analytical techniques were used for the estimation of Tizanidine, either as a single entity or a combined entity in spectrometric and HPLC data. In the current study, an effort has been made to create a unique optimized method for Tizanidine (TZA) and validate the suggested method as per the ICH guidelines. Further, a study has been made to determine *in vitro* evaluation of Tizanidine (TZA) plasma spiking studies by constructing a linear regression analysis between the concentration of drug spiked in plasma versus drug concentration bound to plasma.

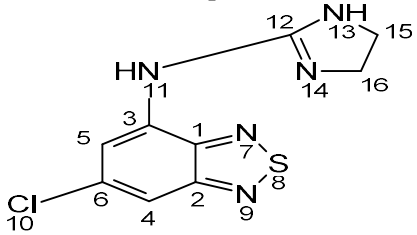

Fig -1: Structure of Tizanidine

EXPERIMENTAL

Experimental Requirements

Tizanidine (TZA) with 97.2% pure drug was procured from Yarrow Chemicals Limited, Mumbai, India. Acetonitrile, Carbinol, and water (HPLC-grade) were procured from UV chemicals, INDIA. All the

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analytical grades (AR) reagents were acquired from UV scientific, Hyderabad, India. They can be utilized directly for analysis and have no requirement for purification. Tizan (2mg) tablets belonging to sun pharmaceutical industries Limited were obtained from the local market and stored below 30º C.

Chromatographic Conditions

Shimadzu LC-2010 AD HPLC system comprises the pump and a detector of UV 20 AD was utilized to chromatograph Tizanidine (TZA) utilizing an ODS C 18 Column (250 mmX4.6 mm,5µm). An ambient temperature was maintained for a column for better elution performance. The mobile phase constitution was Methanol: Water: Orthophosphoric acid (95:4:1 v/v) in a conditional flow rate of 1.0 mL/minute. 20µL of the Tizanidine drug was injected into the chromatographic system and the eluted sample was deduced at 320nm for excitation respectively.

Tizanidine Standard Solution

The stock solution was formulated by dispersing 100mg of Tizanidine (TZA) in a 100mL water, to obtain a 1.0mg/mL (100µg/mL) solution. The resultant solution was degassed for 15 minutes. The resultant mixture was further diluted as per the requirement. The standard stock solution was stored at 2-8° C.

Tizanidine Test Solution

The average weight of twenty tablets was determined. Thus obtained tablets were pulverized to get a fine powder. About 100mg of Tizanidine (TZA) equivalent powder was taken and shifted to a standard flask to obtain the desired 100mcg/mL concentration with the a of Adiluent. Thus obtained mixture was filtered with a 0.45µm membrane filter and degassed.

Optimized Chromatographic Conditions

Mode of operation: Isocratic; Stationery Phase: ODS C_{18} (250mm X 4.6mm, 5µm); Mobile Phase : Methanol:Water: 0.02M orthophosphoric acid; Ratio: 95:4:1v/v/v; Diluent: Methanol: Water; Detection wavelength: 320 nm;Temperature: 25° C; Sample Volume: 20µL

Method Development

The optimized chromatographic parameters were arranged with an equilibrate between the stationary phase and mobile phase. The standard and sample (TZA) were injected separately in order to yield their corresponding chromatograms.

System Suitability Parameters

The chromatographic system was placed in an optimized condition and the placebo was injected in order to obtain a prominent baseline. Six (6) replicate injections of the standard solution of Tizanidine were administered into the chromatographic system and the corresponding results were exhibited in Table-1. The average area and RT value were calculated with the help of inbuilt software.

Validation of the Current Developed Method

The developed method was authenticated as per the ICH guidelines Q_2 R1.[5] Common validation criteria like linearity studies, robustness, and assay were associated with the chromatographic system.

Linearity

Aliquots of Tizanidine (TZA) varying in the range of 1.0mL -5.0mL was diluted from the standard stock solution to get the desired final concentration of 1-5µg/mL. Five (5) point linearity was determined.

Recovery Studies

The current method was subjected to recovery studies by standard addition method in various levels of the labeled amount (i.e 2-5% of labeled claim).

Precision (Intra –day and Inter –day)

Six (6) times of the standard drug (TZA) possessing the concentration of 100µg/mL were injected into the chromatographic system by selecting three varied concentrations within the linearity range starting from the first day ends on the following day.

LOD and LOQ

The Limit of detection and Limit of quantification was determined by the standard equation[6]

$$LOD = 3\ O/S \quad (1)$$

$$LOQ = 10\ O/S \quad (2)$$

Robustness

Conditions such as variation in the rate of flow and the composition of the mobile phase were adopted for examining the robustness of the developed method.[7]

Assay of the Prepared Formulations

Twenty (20) tablets were weighed accurately and pulverized. 10mg equivalent of TZA powder was taken in a 100mL standard flask and diluted to the desired concentration with diluent. Thus obtained mixture was degassed and the resultant solution was adjusted to obtain a concentration of 50µg/mL. The sample was filtered through a 0.45µM syringe filter. The final filtered solution is injected into the LC system.

Preparation of Calibration Standards in the Human Plasma[8]

Pure plasma was obtained from human volunteers and utilized for the formulation of calibration standards in the ratio 1:1. A series of concentrations (10-50µg/mL) of Tizanidine (TZA) has been spiked in pooled human plasma and mixed in a vortex mixer to ensure uniform mixing. The drug–plasma mixture was incubated at 37 º C for 48 hrs. Methanol (10.0mL) was added to the resultant mixture to precipitate the plasma protein and centrifuged at 1000 rpm. The obtained supernatant liquid was injected into the chromatographic system to obtain the peak area. A linearity graph has been plotted against the concentration of Tizanidine (TZA) spiked in the plasma versus the amount of drug bound to plasma.

RESULTS AND DISCUSSION

Tizanidine (TZA) an FDA–approved drug is a benzothiadiazole derivative used in the reduction of spasms that are caused due to presynaptic inhibition of motor neurons by exciting α -2 adrenergic receptors. Tizanidine (TZA) was separated with an optimum resolution and prominent peak. The average peak area was found to be 2417523. The retention time was 2.274min respectively (Fig.-2) (Table-1).

Table-1: System Suitability Parameters of the Tizanidine

Parameters	Tizanidine
Peak Area	2417523
Retention time	2.274
Tailing factor	0.000
USP – Theoretical Plate	2070.003
Resolution	1.167

Attempts were made under optimal experimental conditions, calibration studies were subjected to linear – regression analysis (r^2 = 0.9979) which exhibits a linear relationship between the concentrations (10-50µg/mL) and peak area (Fig.-3).

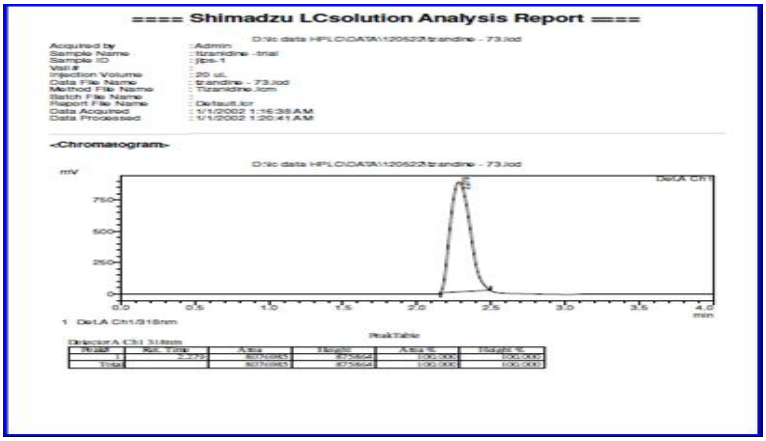

Fig.-2: Optimized Chromatogram of Tizanidine

The recovery study (Table-2) obtained was 100% and there were no interference peaks were observed. Inter and Intra –day precision studies revealed that % RSD 0.35355 and 0.56568. The values of the Limit of detection and Limit of quantification were 0.902µg/mL and 2.733µg/mL. There was no significant change in the retention time was observed when a deliberate change in the flow rate and organic phase. Hence the proposed method was robust (Table-3).

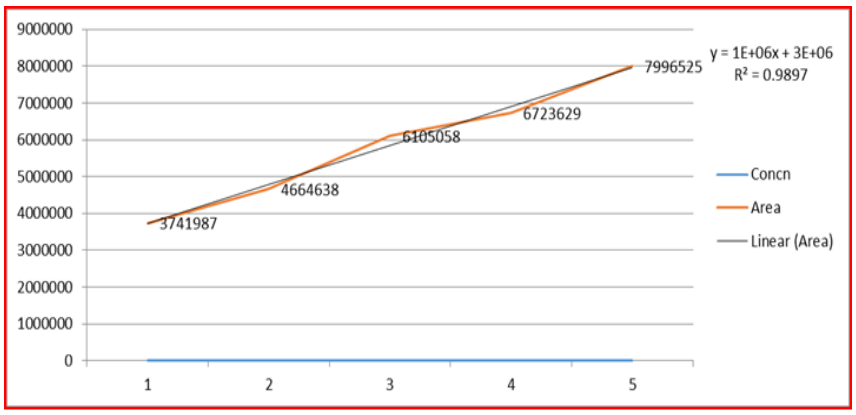

Fig.-3: Linearity Graph of Tizanidine

Table-2: Different Robustness value of Tizanidine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
The actual Flow rate of 1.0 mL/min	1568359	2.758	3363.411	1.390
Less Flow rate of 0.9 mL/min	154826	3.077	3575.5	1.397
More Flow rate of 1.1 mL/min	3072143	2.501	3244.5	1.242
More organic phase (About 5 % Increase in Methanol)	5856307	2.752	3139.0	1.431
Less organic phase (About 5 % decrease in Methanol)	3385315	2.755	3261.1	1.268

Table-3: Recovery Studies of Tizanidine

Amount (%) of drug added to the analyte	Weight Taken (mg)	RT value	Sample added (mg)	Peak area	Average Peak area	Sample recovery	Recovery		
							% of recovery	Standard deviation	% RSD
50	25	2.249	25	2740905	6427261	2.344941178	100	0.01	0.50118004
	25	2.26	25	2764767		2.324702588			
	25	2.26	25	2764767		2.324702588			
100	50	2.262	50	4792896	11222598.67	2.341506819	100.024	0.04	1.892373743
	50	2.268	50	4780755		2.347453209			
	50	2.271	50	4946843		2.268638537			
150	75	2.279	75	8076985	17619899.67	2.181494663	100.456	0.20	8.43742183
	75	2.277	75	6920263		2.546131508			
	75	2.271	75	7867955		2.239450997			

The suggested method can be adapted for the analysis in marketed formulation since it has 98.2 % w/w. Fig.-4 and 5 show the blank plasma spiked with Tizanidine (TZA) in the series of concentrations of 10-50 µg/mL. The retention time of Tizanidine (TZA) was 2.286 min. The chromatogram was very prominent obeying all the criteria. Linearity was found to be accurate for Tizanidine with the regression equation: Y=0.023x+0.0073 ($r^2 \geq 0.8155$) respectively.

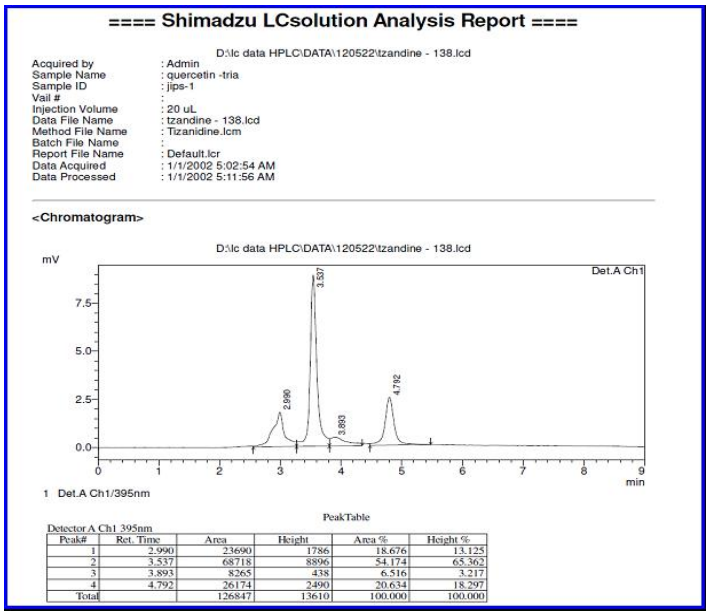

Fig.-4: Chromatogram of Metabolites of Tizanidine in Human Plasma

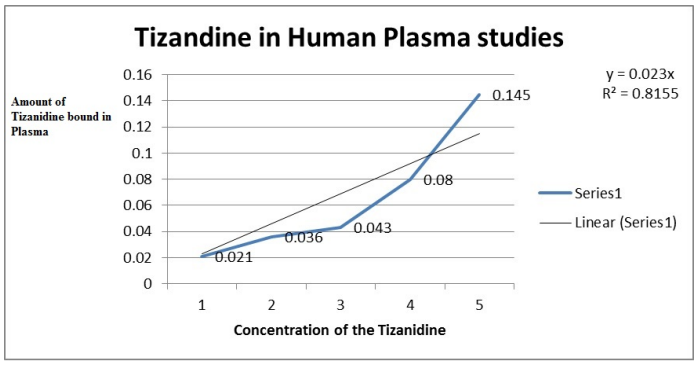

Fig.-5 Linearity Studies of Tizanidine in Human Plasma

CONCLUSION

A simplified RP- HPLC method was formulated under laboratory conditions utilizing a simplified mobile phase (Methanol: water: Ortho phosphoric acid – 95:4:1 v/v/v) system. The developed method was authenticated as per the ICH guidelines criteria. A human plasma chromatographic analysis of the Tizanidine reveals that there is an exponential increase in the drug-binding plasma versus the concentration which would be more suitable for a pharmacokinetics study.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to preparing this manuscript and participated in the reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCIDs id's given below :

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