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Development and Validation of New Analytical Method for the Estimation of Tizanidine Hydrochloride in Bulk and in Formulation by UV Spectrophotometric Method

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ABSTRACT

The IUPAC Name of Tizanidine hydrochloride is 5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine. Tizanidine is a short-acting drug for the management of spasticity. Tizanidine is an agonist at α2-adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons[1].The effects of tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons.tizanidin comes in the form of a tablets and capsules. Present research work deals with UV spectrophotometric method for the estimation of tizanidin hydrochloride in pure form. For the estimation of tizanidine ,solvent system employed was distilled water and wave length of detection (λ_{max}) was 268.5 nm. The linearity was obtained in the range 5-30 µg/ml, with a regression coefficient, R^2=0.999. The LOD and LOQ were found to be 0.33µg/ml and 1.02820µg/ml respectively. The percentage recovery of Tizanidine hydrochloride sample was found with in the limit 96.48%- 105.76% Mean of SD 99.27±1.9216 (%RSD 1.9351,SE 0.7903), Mean of SD 98.51±1.8960(%RSD 1.9255,SE 0.7861). Obtained results showed that there is minimum intraday and interday variation. The developed method was validated and recovery studies were also carried out. Sample recovery using the above method was in good agreement with their respective labeled claims, thus suggesting the validity of the method and non-interference of formulation excipients in the estimation.

Keywords: spectrophotometric, tizanidine hydrochloride, α2adrenergic receptor

INTRODUCTION

Tizanidine is a short-acting drug for the management of spasticity. 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 [1]. The effects of tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. Alpha-2 adrenergic receptors mediate the catecholamine- induced inhibition of adenylate cyclase through the action of proteins [2-4]. Tizanidine hydrochloride chemical formula is $C_9H_8CLN_5S$ and molecular weight is 253.711.

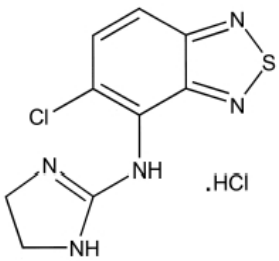

Figure 1. Structure of tizanidine hydrochloride

Pharmacokinetics, Absorption and Distribution

Following oral administration, tizanidine is essentially completely absorbed. The absolute oral bioavailability of tizanidine is approximately 40% (CV = 24%), due to extensive first-pass hepatic metabolism [5]. Tizanidine

is extensively distributed throughout the body with a mean steady state volume of distribution of 2.4 L/kg (CV = 21%) following intravenous administration in healthy adult volunteers. Tizanidine is approximately 30% bound to plasma proteins [6-8].

Metabolism and Excretion

Tizanidine has linear pharmacokinetics over a dose of 1 to 20 mg. Tizanidine has a half-life of approximately 2.5 hours (CV=33%). Approximately 95% of an administered dose is metabolized. The primary cytochrome P450 isoenzyme involved in tizanidine metabolism is CYP1A2. Tizanidine metabolites are not known to be active; their half-lives range from 20 to 40 hours. Following single and multiple oral dosing of 14C-tizanidine, an average of 60% and 20% of total radioactivity was recovered in the urine and feces, respectively.

Literature survey carried out revealed that several methods have been reported for the estimation of tizanidine hydrochloride by using various analytical methods like U.V,HPLC method for the simultaneous estimation of two component drug mixture of tizanidine and valdecoxib in combined tablet dosage form have been developed [9]. developed a simple Spectrophotometric methods for the simultaneous estimation of Ibuprofen and Tizanidine from tablet dosage form. Simultaneous equation method involves the measurement of absorbance at two wavelengths 264 nm (λmax of Ibuprofen) and 319 nm (λmax of Tizanidine) [10]. The main objective of the present work is the development of accurate, precise and reproducible methods for Tizanidine hydrochloride was tried out this is a worthwhile objective to pursue the present research work. It was planned to validate the developed method as per ICH guidelines

MATERIALS AND METHODS

Instruments

SHIMADZU S-90 D Digital Balance, ELICO SL 210 Double Beam - UV – Visible spectrophotometer with pair of 10 mm matched quartz cells, Digital P^H Meter by Globe instruments Model: 011G, Digital p^H Meter by ELICO Model L1 120,Sonicator Model SVC 320 were used for the estimation of tizanidine hydrochloride.

Selection of Solvent

The solubility of Tizanidine hydrochloride was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility test for Tizanidine hydrochloride was carried out in different polar and non-polar solvents. From the solubility studies, Distilled water was selected as suitable solvent for proposed method.

Preparation of Standard Stock Solution

100 mg of Tizadine hydrochloride(USP) raw material was accurately weighed and transferred into the 100 ml volumetric flask and dissolved in minimum quantity of distilled water and made up to 100 ml with distilled water.and the final concentration was made as 1000µg/ml

Selection of λ_{max}

The standard stock solution was further diluted with distilled water to get 10 µg/ml concentration. The solution was scanned between 200 and 400 nm range using distilled water as blank. From the UV Spectra 268.5nm was selected as λ_{max} for analysis of Tizadine hydrochloride. Stability of the Tizadine hydrochloride in water was studied by measuring the same solution at this λ_{max} in different time intervals. It was observed that Tizadine hydrochloride in water was stable for more than 2 hours.

Calibration Graph

In this aliquots of stock solution of Tizadine hydrochloride (0.5-3.0ml of 100 µg /ml) were transferred in to 10 ml volumetric flask and made up to the mark with distilled water. The absorbance of different concentration solutions were measured at 268.5 nm against blank. The samples were found to be linear from 5-30 µg /ml. The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear in the concentration range of 5-30 µg /ml.

Quantification of formulation

Contents of twenty tablets of formulation (TIZANIX) containing 2mg of Tizanidine hydrochloride was accurately weighed to find out the average weight.Tablets powder equivalent to 20 mg of Tizanidine hydrochloride was transferred in to 50 ml volumetric flask, added distilled water and made up to the volume. From the clear solution, further dilution was made to bring 10 µg /ml using distilled water. The prepared solution was measured at 268.5 nm. The amount of Tizanidine hydrochloride was determined by using slope and intercept values from calibration graph.

Recovery Studies

To the pre-analyzed formulation, a known quantity of standard solution (2,4 and 6 µg/ml solution) was added and the contents were mixed well, finally made up to the volume with distilled water. Absorbance was measured at 268.5nm. Amount present was calculated from slope and intercept. Then the % recovery was determined by using the following formula [11].

$$\% \text{ Recovery} = \frac{N\sum xy - \sum x \sum y}{N\sum x^2 - (\sum x)^2} \times 100$$

where, N = Number of observations
X = Amount Added in microgram/ml
Y = Amount recovered in microgram/ml.

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

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was

calculated by using the average value of slope(s) and standard deviation of intercept [12].

$$\textbf{Limit of detection} = \frac{3.3 \times \sigma}{S} \ \mu g/ml$$

Where: σ = The standard deviation of the response.
S = The slope of the calibration curve.

$$\textbf{Limit of quantitation} = \frac{10 \times \sigma}{S} \ \mu g/ml$$

Where: σ = The standard deviation of the response
S = The slope of the calibration curve.

Repeatability

Repeatability of the method was checked by repeating the measurement of formulation six times.

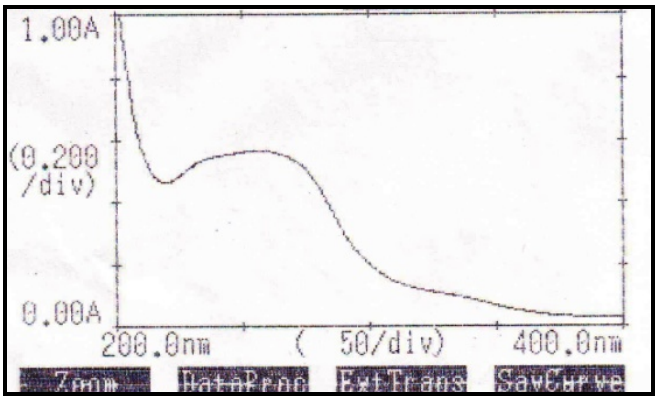

Figure 2. Ultra violet absorption spectrum of Tizanidine hydrochloride.

RESULTS AND DISCUSSION

The solubility of Tizanidine hydrochloride was determined in a variety of solvent ranging from non polar to polar using essentially a method of Schefter and Higuchi. The drug was found to be freely soluble in distilled water, acetate buffer 4.6, and very soluble in phosphate buffer 6.8 , 0.1N Hcl Solubility profile of Tizanidine hydrochloride.

100 mg of Tizanidine hydrochloride raw material was accurately weighed and transferred into the 100 ml volumetric flask and dissolved in minimum quantity of distilled water and made up to 100 ml with distilled water, resulting in 1000 mcg/ml of drug concentration. It was scanned in the range of 200-400 nm and it shows constant λ_{max} at 268.5 nm. Stability of the absorbance at their λ_{max} was also checked for up to 2 hours. The linearity of the drug Tizanidine hydrochloride was found, its calibration curve was constructed and is shown in Fig 2, the optical characteristics such as Beer's law limit (5-30µg/ml), sandell's sensitivity (0.021855), correlation coefficient (0.999), slope(0.061) and intercept(0), molar absorptivity (1.2810×10^2), were calculated and shown in Table 5.

Table 1. Calibration Curve values of Tizanidine Hydrochloride

S.No	Concentration (mcg/ml)	Absorbance
1.	5	0.3292
2.	10	0.6091
3.	15	0.9187
4.	20	1.2346
5.	25	1.5293
6.	30	1.8319

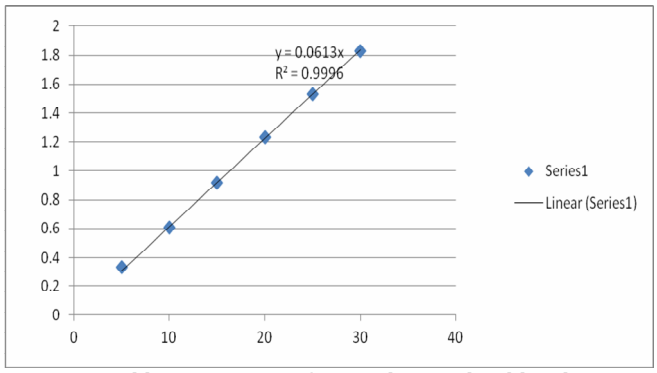

Figure 3. Calibration Curve of Tizanidine Hydrochloride

The limit of detection and limit of quantification were determined from the linearity studies. The limit of detection was found to be 0.33930µg/ml and the limit of quantification was found to be 1.02820µg/ml. It is shown in Table 2. Table 2 shows the result of formulation quantification on TIZANIX tablets repeatability also found to be within the limits 1.98-2.02 (99.5 ± 1.5), %RSD value 0.774. To evaluate the accuracy of the method, known amount of pure drug (2, 4 and 6 µg/ml solution) was added to the previously analysed solution containing pharmaceutical formulation and the mixture was analysed by the proposed method and the recoveries were calculated. The percentage recovery of Tizanidine hydrochloride sample was found with in the limit 96.48%- 105.76% Mean of SD 99.27±1.9216 (%RSD 1.9351,SE 0.7903), Mean of SD 98.51±1.8960(%RSD 1.9255,SE 0.7861), These values were given in Table 6.

Table 2. Quantification of Formulation- Tizanix by UV method

S. No	Labelled Amount (mg/tab)	Amount found (mg/tab)	%Obtained	Average %	S.D	%RSD
1	2	2.01	100.5	100	0.015492	0.774
2	2	1.99	99.5			
3	2	2.01	100.5			
4	2	1.99	99.5			
5	2	1.98	99			
6	2	2.02	101			

Table 3. Inter Day Precision Studies

S. No	Concentration	Absorbance	Average/ mean	S. D	RSD
1	10 mcg/ml	0.6664	0.6667	0.012	1.79
2	10mcg/ml	0.6567			
3	10 mcg/ml	0.6539			
4	10 mcg/ml	0.6862			
5	10 mcg/ml	0.6746			
6	10 mcg/ml	0.6629			

Table 4. Intra Day Precision Studies

S. No	Concentration	Absorbance	Average/ mean	S. D	RSD
1	10 mcg/ml	0.7535	0.656	0.057	0.087
2	10mcg/ml	0.6396			
3	10 mcg/ml	0.6465			
4	10 mcg/ml	0.6911			
5	10 mcg/ml	0.6046			
6	10 mcg/ml	0.6050			

Table 5. Optical Characteristics of Tizanidine Hydrochloride in UV Method

Parameters	Method Values
λ_{max}(nm)	268.5
Beer's law limit(µg/ml)	5-30
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.021855
Molar absorbtivity(L mol^{-1} cm^{-1})	1.2810x10^2
Correlation Co-efficient (r)	0.999
Regression equation (Y=mx+c)	Y=0.061x+0
Slope(m)	0.061
LOD(µg/ml)	0.33930µg/ml
LOQ(µg/ml)	1.02820µg/ml

Table 6. Recovery Studies for Formulation- Tizanix by UV Method. The Percentage recoveries found to be 99.92 to 100.44, which is well within the acceptance criteria of 98-102%. This indicates that the proposed method is accurate.

S. No	Recovery	Target in µg/ml	Spiked in µg/ml	Total in µg/ml	Amount in µg/ml	% Recovery
1	50%	10	5	15	14.89	99.2
2	50%	10	5	15	14.91	99.4
3	50%	10	5	15	15.06	100.4
4	100%	10	10	20	19.70	98.53
5	100%	10	10	20	19.89	99.45
6	100%	10	10	20	19.68	98.4
7	150%	10	15	25	24.98	99.92
8	150%	10	15	25	24.78	99.12
9	150%	10	15	25	24.70	98.8

Tizanidine hydrochloride is a drug used as skeletal muscle relaxant. The proposed analytical methods are simple, reliable, rapid, sensitive, reproducible and accurate for the estimation of Tizanidine hydrochloride. The method adopted for our studies are simple UV-Spectroscopic method. The drug samples were analyzed by UV spectroscopy using distilled water as solvent and the average content of drug present in the formulation was found to be 2 mg (100 %). The above method do not suffer from any interference due to common excipients. Therefore it was shown that the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Tizanidine hydrochloride. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

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