Research Article

Quantitative Estimation of Tizanidine in Pharmaceutical Formulations by Visible Spectrophotometry Using Gold (III)

A.Giri[1], B. Saritha[1], Prof.T. Sreenivasulu Reddy*[1]
[1]Department of Chemistry, S.K.University, Anantapur, Andhra Pradesh, India.
***Corresponding author's E-mail:** tsrreddychem@gmail.com

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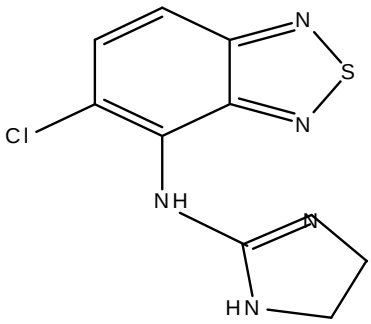
ABSTRACT

Gold (III) reacts with Tizanidine in the pH range 1.0-5.0 forming a yellow colored complex solution which has maximum absorbance at 450 nm. Studies were carried at pH 3.0. The colour intensity attains a maximum value after 30 minutes at 60° C. The straight line relation between absorbance and amount of Tizanidine obeys the equation A = 0.0133 C - 0.0023. The linear plot indicates that Beer's law is obeyed in the range 5.0-70.0 µg/ml of Tizanidine. The molar absorptivity and sandell's sensitivity are 3.355×10^3 l mol^{-1} cm^{-1} and 0.0757µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 30 µg/ml Tizanidine is 0.002. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9999. The effect of various excipients was studied. The composition of the complex is established as 1:1 [Au (III): Tizanidine]. The stability constant of the complex is 4.22×10^4. The developed method was validated according to ICH guidelines and was found to be accurate and precise. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and ruggedness are studied. The proposed method is accurate, precise, highly sensitive and selective, for the estimation of Tizanidine in its pharmaceutical formulations without interference from either Aceclofenac or paracetamol. Hence the proposed method is successfully applied for the determination of Tizanidine in pharmaceutical formulation.

Keywords: Au (III), Method validation, Tizanidine, Visible Spectrophotometry.

INTRODUCTION

Tizanidine is a centrally acting α,α,2-adrenergic agonist. Tizanidine is a white to off-white fine crystalline powder, odorless or with a faint characteristic odor. Tizanidine is soluble in water and methanol; solubility in water decreases as the pH increases. Its chemical name is 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiodiazole hydrochloride. Its molecular formula and molecular weight are $C_9H_8ClN_5S.HCl$ and 290.2 respectively. Its structural formula is;



Tizanidine is a centrally active skeletal muscle relaxant. Tizanidine is found to be effective and of at least similar antispastic efficacy to diazepam and baclofen in clinical trails. It shows relatively low oral bioavailability and is extensively metabolized in the liver via ring hydroxylation. The plasma concentration of Tizanidine after oral administration is presumed to be several nano grams.

It is a myotonolytic agent used in the treatment of spasticity in patients with cerebral or spiral injury. It is an antispastic agent with similar efficacy to that of baclofen and a more favorable tolerability profile. Therefore, Tizanidine appears to be an attractive therapeutic alternative for patients with spasticity associated with cerebral or spinal damage. Clinical trials with Tizanidine when administered alone have shown that it is safe and effective for spasticity control.

It reduces spasticity by increasing presynaptic inhibition of motor neurons. The effects of Tizanidine are greatest on polysynaptic pathways. The overall effects of these actions are thought to reduce facilitation of spinal motor neurons. It also reduces increased muscle tone associated with spasticity in patients with multiple sclerosis or spinal cord injury.

An efficient gas chromatography – mass spectrophotometry (GC – MS) method is developed and validated for the determination of Tizanidine in human plasma by Jaeick Lee *et* al.[1] A new isocratic stability indicating HPLC method is reported for the determination of Tizanidine in drug substances and formulated products. Recovery for Tizanidine from the tablets is from 99.5 to 99.8% and precision is 1% (n=9).[2] Simultaneous determination of Tizanidine and refecoxib in pharmaceutical dosage form using HPLC and HPTLC methods is carried out by Neeraj Kaul et al.[3] Extractive Spectrophotometric method for the assay of Tizanidine hydrochloride[4] has been proposed based on the formation of an ion – pair with metanil yellow in acidic medium and the subsequent extraction of the ion pair into chloroform. The yellow colored ion – pair shows absorption maxima at 410nm.

Shankar M.B. et al developed two methods for simultaneous Spectrophotometric determination of Tizanidine and diclofenac[5] in tablets. Two simple methods for simultaneous estimation of valdecoxib and Tizanidine in combined dosage form have been described. Method one involves formation of Q – absorbance equation at 239.6 (iso absorptive point) and at 241nm, while method two involves formation of simultaneous equation at 241 and 229nm, using methanol as solvent by Devarajan and Sivasubramanian Lakshmi.[6] Method proposed for the estimation of Tizanidine simultaneously either Aceclofenac or Paracetamol by Q analysis and area under curve method are reported by Sujata et al.[7] Simultanious ratio derivative method is developed by Patil Poonam.[8]

The literature survey showed no report of a direct visible Spectrophotometric method for the determination of Tizanidine. In continuation of our work in developing simple visible Spectrophotometric methods for the assay of pharmaceutical formulations using metal ions[9], now we report a simple, sensitive and precise visible Spectrophotometric procedure for the determination of Tizanidine in bulk and pharmaceutical formulation based on its color reaction with gold (III).

MATERIALS AND METHODS

All chemicals and solvents used were of analytical reagent grade.

Solutions

Gold (III) solution

1gm of chloroauric acid (Johnson Mathews, materials technology, U.K.) is dissolved in distilled water after adding few drops dilute HCl. The solution is made up to the mark in 100 ml volumetric flask. The gold content of the solution is determined by rhodamine B method.[10] The working solutions are prepared by diluting the stock solution.

Tizanidine solution

100 mg of Tizanidine is weighed accurately and transferred into a 100 ml standard flask, dissolved and made up to the mark in double distilled water. This solution is diluted as required.

Buffer solutions

Buffer solutions are prepared by adopting the standard procedures reported in the literature.[11] The solutions employed for the preparation are given below.

pH	Constituents
0.5 – 3.0	1 M Sodium acetate + 1 M Hydrochloric acid
3.0 – 6.0	0.2 M Sodium acetate + 0.2 M Acetic acid
7.0	1.0 M Sodium acetate + 0.2 M Acetic acid
8.0-12.0	2.0 M Ammonia + 2.0 M ammonium chloride

Instruments employed

a) UV-Visible recording spectrophotometer (UV – 160A)

Shimadzo Corporation Spectrophotometric Instrument Plant, Analytical Instruments Division, Kyoto, Japan developed a versatile and indigenous microprocessor based UV-Visible recording spectrophotometer (UV-160A).

b) ELICO digital pH meter

ELICO digital pH meter manufactured by M/s ELICO Private Limited, Hyderabad, India is used for measuring the pH of buffer solutions. The instrument has a temperature compensate arrangement. The reproducibility of measurements is within ± 0.01 pH.

Procedure

Preparation of pharmaceutical sample (Tablets)

A known number of tablets are weighed and ground to a fine powder. A portion of the powder containing 100 mg of the active component is accurately weighed into a 100 ml calibrated flask, 60ml of distilled water are added and shaken thoroughly for about 20 minutes to extract the drug. The contents are diluted to the mark, mixed well and filtered using quantitative filter paper to remove the insoluble residue. The filtrate is diluted to get required concentration of drug.

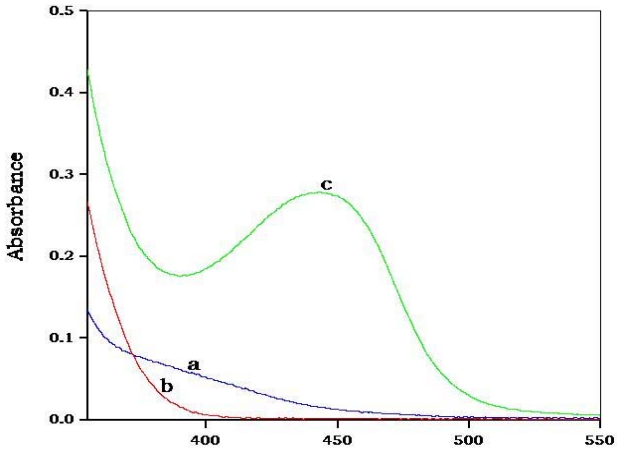


Figure 1: Absorption spectra of a) Au (III) vs. buffer blank b) TND vs. buffer blank; c) Au(III) – TND vs. buffer blank [Au(III)] = 5.0 x 10^{-3}M; [TND]= 8.0 x 10^{-5}M

Table 1: Effect of temperature on the absorbance of the Experimental solution

Temperature(^{0}C)	Absorbance
40	0.213
50	0.306
60	0.412
65	0.415
70	0.417

[tizanidine] = 1.0 x 10^{-4}M; pH = 3.0

[gold (III)] = 5 x 10^{-3}M; λ = 450 nm

Table 2: Tolerance limit of excipients

Excipient	Tolerance limit (µg/ml)
Fructose	1604
Glucose	1120
Sucrose	1748
Lactose	2173
Gelatin	2311
Starch	1817
Sodium Alginate	1690
Boric Acid	2415
Magnesium stearate	2012

Amount of TND = 25 .0 µg/ml; pH = 3.0

Table 3: Optical and regression characteristics of the proposed method for Tizanidine

Parameter	Tizanidine
Analytical Wavelength (nm)	450
Beer's law limits (µg/ml)	5 – 70
Limits of detection (µg/ml)	0.4947
Limits of quantization (µg/ml)	1.4842
Molar absorptivity ($l.mol^{-1}cm^{-1}$)	3.355×10^3
Sandell's Sensitivity ($\mu g/cm^2$)	0.0757
Regression equation (y= a + b x)	
Slope (b)	0.0133
Intercept (a)	-0.0023
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0020

Table 4: Assay of Tizanidine in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found* (mg)	Error (%)
Brand-I (Sun Pharmaceutical Industries Ltd-Tablet)	2.00	2.03	-1.5
Brand-II (SIRDALUD Novartis Pharma Ltd, – Tablet)	2.00	1.98	-1.00

*Average of Six determination

Absorbance spectrum

The absorption spectra of the gold (III) solution, Tizanidine solution and Tizanidine –gold (III) complex solution in buffer solution of pH 3.0 against the buffer blank are recorded in the wavelength range 300-650nm. The spectra are presented in fig.1 show that the complex has an absorption maximum at 450 nm. Neither gold (III) nor Tizanidine have significant absorbance at 450 nm. Hence, analytical studies were made at 450 nm.

Assay of Tizanidine in tablets

A known aliquot of pharmaceutical sample solution of Tizanidine is added to a 10ml volumetric flask containing 5 ml of buffer solution of pH 3.0 and 0.5 ml of gold (III) [5×10^{-3}M] solution. The contents are made up to the mark with distilled water. The absorbance is measured at 450 nm against the gold (III) blank after heating the experimental solution to 60^0C for 30 minutes and cooling it to room temperature. The amount of Tizanidine is computed from the pre determined calibration plot at 450 nm.

Order of addition of constituent solutions on the absorbance of the experimental solution

The order of addition of the various constituent solutions, the buffer solution, gold (III) solution and Tizanidine solution is varied and the absorbance of the experimental solution is measured in each case at 450 nm. The results reveal that the absorbance remains unchanged irrespective of the order of addition of various constituent solutions.

Effect of surfactants on the absorbance

To a series of experimental solutions containing buffer solution of pH 3.0, gold (III) solution and Tizanidine solution, an aliquot of the known percentage of different surfactant solutions are added and the absorbance is measured at 450 nm after heating the experimental solution to 60^0C for 30 minutes and cooling it to room temperature. The studies reveal that the absorbance of the solution remained the same in all cases indicating that surfactants have no effect on the absorbance.

Effect of temperature on the absorbance of experimental solution

The effect of temperature on experimental solution was studied and the results are shown in table 1**.** The results indicate that the absorbance attains maximum value at 60^0C. Hence, the absorbance is measured after heating the experimental solution at 60^0C for 30 minutes and cooling it to room temperature.

Effect of excipients

Various amounts of excipients that are generally associated with Tizanidine in its pharmaceutical formulations are added to a fixed amount of Tizanidine (25µg/ml) solution and the absorbance measurements are carried out under optimal conditions. The concentration (µg/ml) at which various excipients do not cause an error of more than ± 4% in absorbance is taken as the tolerance limit and the results are given in table - 2.The data in table 2 indicate that the excipients that are associated with Tizanidine do not interfere when present even in large quantities in the determination of Tizanidine making the method highly selective.

RESULTS AND DISCUSSION

Tizanidine reacts with gold (III) in the pH range 1.0-5.0 forming a yellow colored complex solution. The absorption spectrum of the yellow colored Au (III) – Tizanidine complex shows (Figure 1) absorption maximum at 450 nm. At this wavelength either Au (III) or Tizanidine has no significant absorbance. The color intensity of the complex is maximum at pH 3.0 Hence studies were carried at pH 3.0. The color formation attains maximum intensity after 30 minutes of heating at 60°C. There after the color of the complex remains stable for more than 24 hours. A fivefold molar excess of gold (III) is sufficient to produce maximum absorbance. The absorbance varied linearly with the concentration of Tizanidine Beer's law is obeyed in the range 5.0-70.0 µg/ml of Tizanidine. The straight line plot obeyed the equation A = 0.0133 C - 0.0023. Optical characteristics and regression data are presented in Table 3. The method was applied successfully for the assay of Tizanidine in pharmaceutical formulation. The data are presented in Table 4.

Table 5: Intra- and Inter- day precision studies of Tizanidine (n=3, p=0.05)

Conc. (µg/ml)	Mean absorbance ± SD		% RSD		Calculated value of t
	DAY-1	DAY-2	DAY-1	DAY-2	
20	0.286±0.001	0. 283±0.002	0.70	0.54	0.091
30	0.430±0.002	0.428±0.001	0.48	0.36	0.125
40	0.570±0.001	0.568±0.002	0.27	0.44	0.851

Table 6: Recovery studies for Tizanidine in tablets

Tablet	Amount of sample(µg/ml)	Amount of drug added(µg/ml)Amount	Amount Recovered(µg/ml)	%Recovery ± SD
BRAND-I (TIZPA Blue Cross Laboratories Ltd., – Tablet)	30	20	49.48	98.96 ±0.002
	30	30	60.10	100.31±0.002
	30	40	71.12	101.6±0.003
BRAND-II (ZATRU Systopic Laboratories Ltd, – Tablet)	20	20	39.20	98.0 ±0.001
	20	30	50.14	100.2±0.002
	20	40	59.20	98.6 ±0.002

Table 7: Ruggedness studies for the Tizanidine in tablets

Tablet	Analyst- I			Analyst- II	
	Label Claim (mg)	Amount found* (mg)	(%) Recovery ±SD	Amount found* (mg)	(%) Recovery ±SD
Brand-I	2.00	1.80	90.00±0.001	1.97	98.5± 0.001
Brand- II	6.00	6.18	103.00±0.002	5.98	99.66 ±0.001

*Average of Six determination

Method Validation and Statistical Analysis

The developed method was validated as per official specifications of ICH guidelines. The validation parameters show that the method is accurate and precise. Statistical results are expressed interms of mean ± SD, %RSD and student t-test and are calculated with the aid of Excel-2007. Dfferences were considered significant at the 95%confidence limit. Repeatability of the method was verified by intraday and inter day precision studies (Table-5). Accuracy of the method was studied by recovery studies and the results are summarized in Table-6, Ruggedness studies were carried out by changing the analyst and the results are shown in Table 7.

CONCLUSION

The proposed method for the determination of Tizanidine is a simple visible Spectrophotometric procedure which is not only fairly rapid, precise and sensitive but also is within the reach of an ordinary clinical laboratory. The linearity parameter and the corresponding regression data indicate excellent linear relationship (γ =0.9999). Survey of literature shows no report of a simple, sensitive visible Spectrophotometric procedure for the estimation of Tizanidine. Methods reported for its determination either use costly instrumentation or suffer from interference due to excipients.

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