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A Comparative Study of Different Approaches for Stability-Indicating Determination of Tizanidine in Presence of Its Oxidative Degradation Product

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Keywords: Tizanidine hydrochloride; first derivative; derivative ratio; ratio difference; mean centering; dual wavelength

ABSTRACT

Five, simple, accurate, selective and sensitive methods were developed for the determination of tizanidine hydrochloride in presence of its oxidative degradation product without pretreatment. Confirming this novel degradation way of tizanidine HCL by IR, ^{1}H NMR and mass spectrometer techniques. The first method is the first derivative technique, measure peak amplitudes of derivatized spectra at 293nm. The second method is the first derivative of ratio spectra (1DD) which used for the determination of tizanidine HCL in presence of its degradation product at 326nm. The third method is the ratio difference technique which depends upon measuring peak amplitudes of ratio spectra at 231 and 320nm using 15 ug /mL degradate spectrum as a divisor. The fourth method is the mean centering of ratio spectra technique which depends upon centering data of ratios at 321nm. The fifth method is the dual wavelength technique which depends upon measuring peak amplitudes of zero order spectra at 290 and 326nm. The calibration graphs were linear in the range of (2.5−20 ug/mL) for tizanidine HCL.

1. INTRODUCTION:

Tizanidine hydrochloride 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothia diazole hydrochloride (Fig. 1) is α2- adrenergic agonist and centrally active skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants [1,2]. It reduces spasticity by increasing presynaptic inhibition of motor neuron. It is also used in the symptomatic treatment of painful muscle spasm associated with musculoskeletal condition [3]. In literature, a radioimmunoassay method [4], spectrophotometry [5-19], Voltametry [20-22], Gas-chromatography [23,24], HPTLC [25-27], HPLC [12,25,28-33], LC- MS [34], have been reported for the determination of tizanidine hydrochloride.

Reviewing the literature on the determination of tizanidine hydrochloride revealed the lack of any stability indicating spectrophotometric methods for the determination of tizanidine HCL in presence of its oxidative degradation product, the aim of this work is to develop a simple, economic, rapid, sensitive, accurate and precise stability indicating methods for determination of tizanidine hydrochloride in presence of its oxidative degradate without sophisticated instruments or any separation steps.

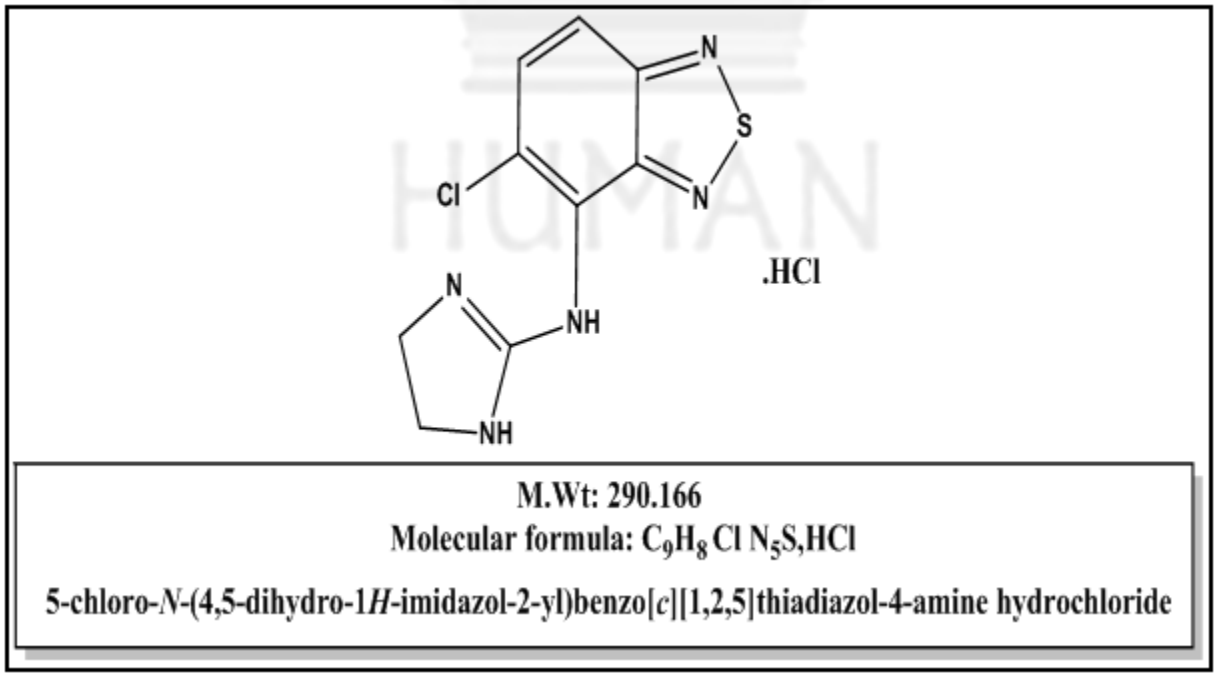


Figure (1): Chemical structure of tizanidine hydrochloride

2. Theory

2.1. Theory of first derivative technique:

It is based on first derivation of zero order absorption spectra of intact and degradate, and measure absorbance of intact derivatized spectra at specific wavelength at which degradate derivatized spectrum absorbance is equal to zero. [35-37]

2.2. Theory of first derivative of ratio spectra, ratio difference and mean centering techniques:

The methods are based on the fact that, upon dividing the absorption spectrum of a compound by standard spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. While upon dividing the absorption spectrum of a compound by the standard spectrum of the other compound, a new spectrum (ratio spectrum) will result.

2.2.1. First derivative of ratio spectra technique:

Is based on derivation of the ratio spectra, where the ratio of different concentrations of degradate which divided by a divisor spectrum considered as a constants, where derivatization of the constant is equal to zero. [37-41]

2.2.2. Ratio difference technique:

The following step after obtaining the ratio spectra will simply be calculating the difference in absorbance between selected two points in ratio spectrum.

Mathematically it can be explained as follows:

In the ratio spectrum of a mixture of tizanidine (X) and degradate (Y) divided by a divisor Y'.

$$\mathbf{P_1 = P_{1x} + K} \quad (1)$$

$$\mathbf{P_2 = P_{2x} + K} \quad (2)$$

Where, P_1 and P_2 are the amplitudes of mixture spectrum at λ_1 and λ_2 respectively, K is the constant resulting from Y /Y'.

$$\Delta P_{\lambda 1-\lambda 2} = P_1 - P_2 = (P_{1x} + K) - (P_{2x} + K) = P_{1x} - P_{2x} \quad (3)$$

So the component degradate (Y) will be completely cancelled and the difference will represent the tizanidine HCL (X) component only. [42]

2.2.3. Mean centering of ratio spectra technique:

The following step after obtaining the ratio spectra, will simply be centering or mean centering of data of ratio spectra and does not need any derivatization steps, to explain the mean centering expression let us consider a three-dimensional vector:

$$Y = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix}$$

We center or mean center (MC) this column by subtracting the mean of three numbers

$$\vec{y} = \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix},$$

$$MC(Y) = y - \vec{y} = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix} - \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix} = \begin{bmatrix} +2 \\ -2 \\ 0 \end{bmatrix}$$

But, the data of ratio spectra of degradate which divided by a divisor is considered as a constant, since mean centering of constant values is equal to zero

$$X = \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix}$$

We center or mean center (MC) this column by subtracting the mean of three numbers

$$X' = \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix},$$

$$MC(Y) = X - X' = \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix} - \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}$$

It could be proved that if the vectors Y or X are multiplied by n (a constant number), the mean center vector is also multiplied by n and also if a constant number is added to the vectors Y or X, the mean center of these vectors is not changed. [41, 43, 44]

2.3. Theory of dual wavelength method:

In this method, difference in absorbance at two selected wavelengths is calculated. The difference in absorbance at selected wavelengths was found to be zero for degradate, while difference in absorbance for the intact spectra have the different values. [44, 45]

3. MATERIALS AND METHODS:

Experimental:

3.1. Instruments

- A double beam UV-Visible spectrophotometer (Shimadzu 1800, Japan) and it is connected to IBM compatible computer. The software UV-Probe Ver. 2.43, MATLAB® version R2013b and PLS-Toolbox were used.
- Pye-Unicam SP-3-300 infrared spectrophotometer (potassium bromide disc)
- Varian Mercury VX-300 NMR spectrometer.
- Shimadzu GCMS-QP-1000EX mass spectrometer at 70 ev.
- Rotary evaporator (Scilogex, USA) with Buchi pump.
- Hot plate (Torrey pines Scientific, USA).
- pH meter 3510 (Jenway, USA).

3.2. Materials

- Pure tizanidine hydrochloride was kindly supplied by Sigma Pharmaceutical Company, Cairo, Egypt, with purity of 99.25%.
- Sirdalud® tablet: manufactured by Novartis Pharma; labeled to contain 4mg of tizanidine hydrochloride per tablet (B. No. 14274A), purchased from local market.
- Hydrogen peroxide 3% was obtained from El-Naser Pharmaceutical Company.
- Whatmann filter paper n^{o} 41.

3.3. Standard solution

Stock solution of 100 ug /mL for tizanidine HCL was prepared by dissolving 10mg of tizanidine HCL in 100 mL distilled water. Different sets of working solution at various concentrations were prepared by appropriate dilution of the stock solution.

3.4. Preparation of hydrogen peroxide-induced degradation product

A stock solution containing 100mg tizanidine hydrochloride in 10 mL distilled water was prepared. 10 mL of 3% hydrogen peroxide was added. The solution was refluxed at 100ºC for 4 hours. The time required for complete degradation was followed by spotting on TLC plates at 30 minutes intervals for 4 hours. The plates were developed using toluene: acetone: ammonia (5: 5: 0.1, by volume). Then evaporate the solution under vacuum till dryness, the residue was dissolved by distilled water and filtered (several times), evaporate the filtrate using Rota-vapor under vacuum, the residue was dissolved by least amount of methanol in 100 mL volumetric flask, filtered then filtrate was left to dry at room temperature, dissolve the residue in 50 ml distilled water, then complete to the mark with water, to obtain a stock solution labeled to contain degradate derived from 1 mg/mL of tizanidine hydrochloride. Working solution of degradate (100 µg/mL) was obtained by further dilution of the stock solution with distilled water.

3.5. IR – spectroscopy of tizanidine hydrochloride:

Very small amount of intact tizanidine hydrochloride and its degradate powder were separately mixed with about 100mg of dried potassium -bromide (KBr) powder in a small ball mill. The mixtures were pressed in a special die at 10,000 - 15,000 lb/inch^2 to yield a transparent disc. Disc was formed in vacuum to eliminate occluded air. The disc was then held in the instrument beam for spectroscopic examination. Infrared absorption spectrum of drug was recorded in the wavelength region 4000 cm^{-1} to 500 cm^{-1} using a Fourier transform IR spectrometer (Shimadzu). The recorded IR spectrum of intact was similar to the reference spectrum of tizanidine hydrochloride, (**Fig. 2).**

3.6. Laboratory prepared mixtures

Accurate aliquots equivalent to (25-175 ug) of tizanidine HCL into series of 10 ml volumetric flasks from its stock solution (100 ug/mL) and portion equivalent to (25 – 175 ug) of degradate from its stock solution (100 ug/mL) were added to the same flasks and volumes were completed to mark with distilled water and mixed well.

3.7. Pharmaceutical formulation

Ten tablets were accurately weighed, crushed and mixed. An amount equivalent to 10mg of tizanidine HCL was weighed and transferred into 100 mL volumetric flask. To ensure complete extraction of drug, it was sonicated for 15.0 min. in 10 mL methanol and filtered using Whatmann filter paper no 41, into 100 mL volumetric flask, the extract was filtered, filtration system was evaluated to ensure that filter does not adsorb any of drug, filtrate was left to dry at room temperature, then residue was dissolved in distilled water and the volume was completed to the mark with distilled water.

4. Procedures

4.1. Construction of calibration curves (linearity):

Different aliquots of tizanidine hydrochloride standard solution (100 µg/ml) ranging from (25–200) µg were transferred to 10 ml volumetric flasks and completed to the volume with distilled water. The absorption spectra (from 200 to 400nm) of these solutions were recorded using distilled water as a blank.

4.1.1. For determination of tizanidine HCL by first derivative technique:

The zero order spectra of the prepared solutions will be derivatized to the first order derivative, measure the absorbance of tizanidine at 293nm in derivative mode at which degradate shows zero absorbance, the corresponding regression was computed.

4.1.2. For determination of tizanidine HCL by the first derivative of ratio spectra technique:

The zero order spectra of the prepared solutions were divided by the spectrum of 15 ug mL^{-1} degradate (as a divisor), giving ratio spectra which will be derivatized. The peak amplitude of derivatized spectra was measured at 326nm, calibration graphs relating absorbance at 326nm to the corresponding concentration of tizanidine HCL were constructed, and the corresponding equation was computed.

4.1.3. For determination of tizanidine HCL by ratio difference technique:

The zero order spectra of the prepared solutions were divided by the spectrum of 15 ug/mL degradate. The peak amplitude of the ratio spectra was measured at 231 and 320nm. Calibration graphs relating ΔP231-320 to the corresponding concentrations of tizanidine HCL were constructed, and the corresponding regression equation was computed.

4.1.4. For determination of tizanidine HCL by mean centering technique:

The data of ratio spectra which previously be obtained in range (200–400nm), will be mean centered using MATLAB®, calibration graphs relating to the corresponding concentration of tizanidine HCL were constructed, and the corresponding regression equation was computed.

4.1.5. For determination of tizanidine HCL by dual wavelengths technique:

In zero order spectra, the difference absorbance at (290 and 326nm) was found to be zero for degradate, calibration graphs relating difference absorbance at (290 and 326nm) to the corresponding concentration of tizanidine HCL were constructed, and the corresponding equation was computed.

4.2. Accuracy

Accuracy was assured by carrying out the previously mentioned procedures under linearity for the determination different concentration of pure tizanidine HCL. The concentration was calculated from the corresponding regression equations.

4.3. Precision

4.3.1. Intra-day precision (Repeatability)

Three concentrations of tizanidine HCL were analyzed three times intraday using the previously mentioned procedures under "section 4.1." and each procedure related to each method. The percentage of recoveries of each concentration of tizanidine HCL and its relative standard deviation was calculated using the suggested methods (Table 4).

4.3.2. Intermediate precision

Three concentrations of tizanidine HCL were analyzed on three successive days using the procedure mentioned under "section "4.3.1.". The percentage of recoveries of each concentration of tizanidine HCL and its relative standard deviation was calculated using the suggested methods (Table 4).

4.4. Limit of detection (LOD) and limit of quantification (LOQ):

The LOD and LOQ parameters were determined from regression equation,

$$LOD = 3.3\ Sy\ /a$$

$$LOQ = 10\ Sy\ /\ a$$

Where (Sy) is a standard error of the calibration curve and (a) is the slope of the corresponding calibration curve (Table 3).

4.5. Application to laboratory prepared mixtures:

Laboratory prepared mixtures containing different ratios of tizanidine HCL and its oxidative degradate within their calibration ranges were prepared. The spectra of these mixtures were recorded and the procedures under construction of calibration curves were then followed but using the recorded spectra of the prepared mixtures. Recoveries were calculated as previously mentioned in accuracy, and percentages of degradate in mixtures were calculated (Table 5).

4.6. Application to pharmaceutical formulation:

Different concentrations within calibration range of each method (First derivative, derivative ratio, ratio difference, mean centering and dual wavelength methods) were prepared from the solution of the pharmaceutical preparation, the spectra of these prepared concentrations were recorded and procedures under construction of calibration curves were followed using the recorded spectra of the pharmaceutical formulation prepared solution.

The validity of the methods was assessed by applying the standard addition technique (Table 6).

5. RESULTS AND DISCUSSION:

Simple spectrophotometric methods were developed for the determination of tizanidine HCL in presence of its oxidative degradation product without previous separation.

5.1. Confirmation of degradation product:

5.1.1. Confirmation of degradation product using TLC technique:

Firstly, time required for complete degradation was exactly determined by spotting on TLC plates every 30 minutes using mobile phase system consists of toluene - acetone - ammonia (5:5:0.1 v/v/v), complete degradation of tizanidine HCL was confirmed by absence of spot in the region of the degradate corresponds to the spot of the intact drug.

5.1.2. Confirmation of degradation product using IR techniques:

Confirming degradation using IR technique for both intact tizanidine HCL and its oxidative degradate was achieved, IR spectrum of the intact tizanidine **(Fig. 2),** showed data as mentioned in (**Table 1).** However, IR spectrum of degradate **(Fig. 3),** showed disappearance of band of C−N−C of imidazole ring at 1191.75 cm^{-1}, disappearance of secondary amine (N−H) band at 3244.73 and 3079.72 cm^{-1}, disappearance of imidazole (C−H) band at 2843.18 cm^{-1} and appearance of primary amine ($N-H_2$) band at 3383.48 cm^{-1}.

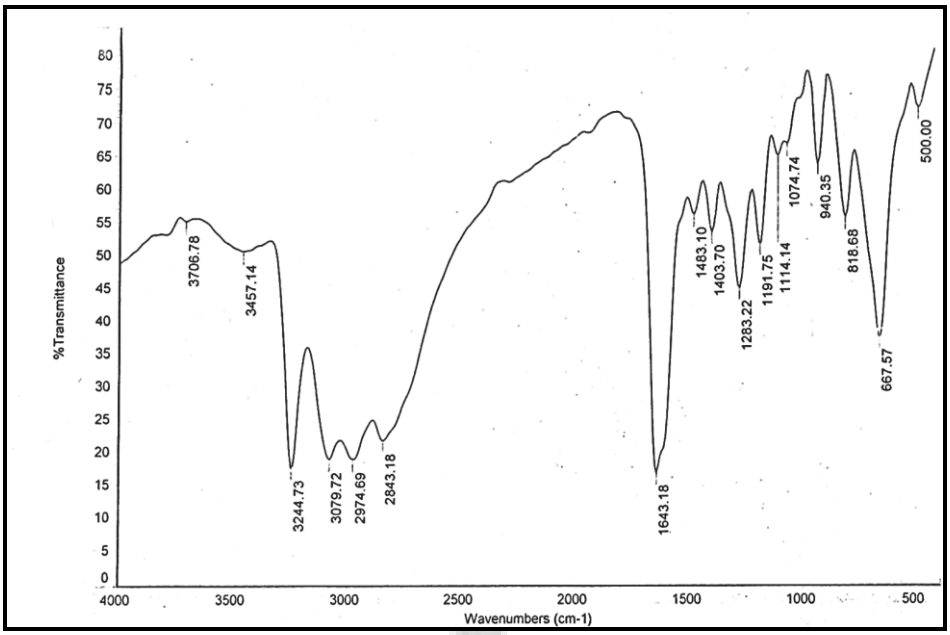


Figure (2): IR spectrum of intact tizanidine HCL.

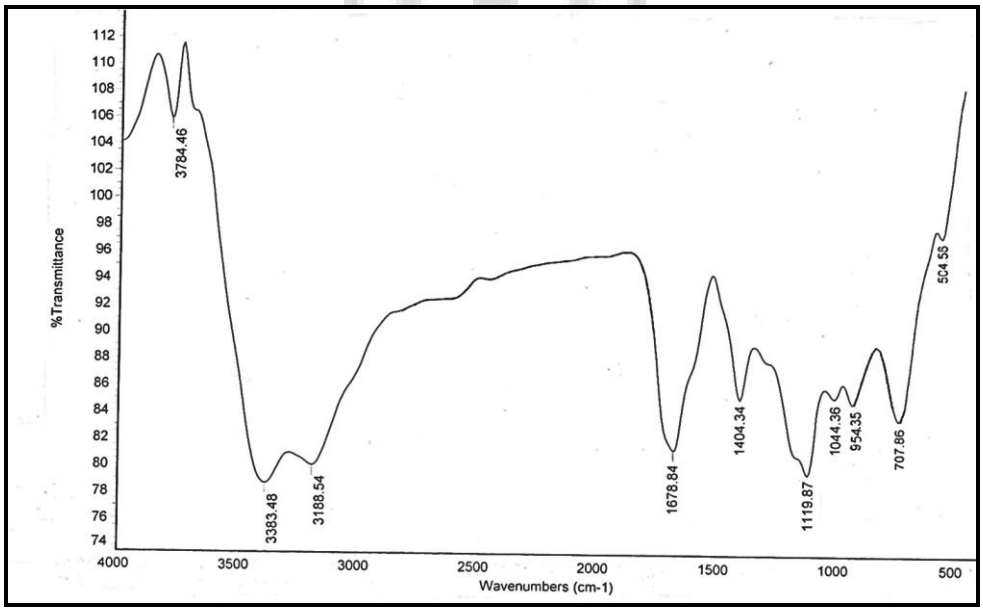


Figure (3): IR spectrum of oxidative degradation product of tizanidine HCL.

5.1.3. Confirmation of degradation product using ^{1}H NMR techniques:

The ^{1}H NMR of the intact tizanidine HCL **(Fig. 4),** showed data as mentioned in **(Table 2),** While ^{1}H NMR of degradate **(Fig. 5),** showed disappearance of CH_2 of imidazole ring, appearance of aromatic (C−H) peaks at 7.421 and 6.611 ppm and appearance of ($N-H_2$) peak at 3.92 ppm.

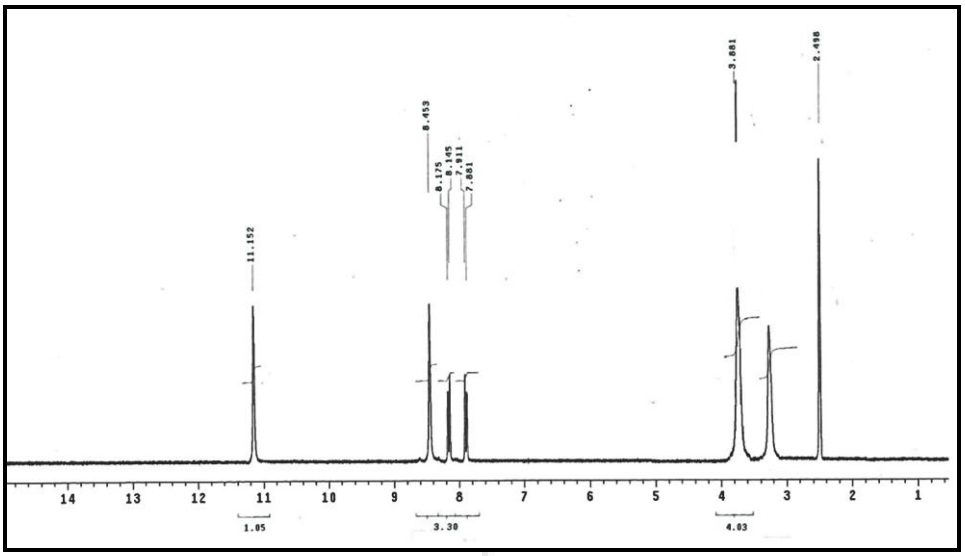


Figure (4): ^{1}H NMR of intact tizanidine HCL.

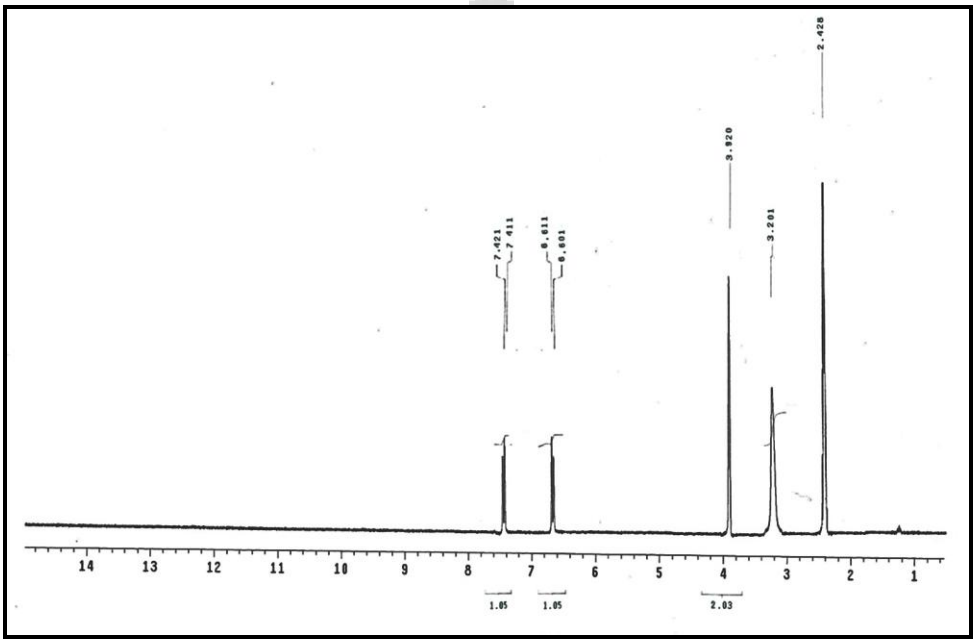


Figure (5): ^{1}H NMR of oxidative degradation product of tizanidine HCL.

5.1.4. Confirmation of degradation product using mass spectrometer techniques:

Odd number of molecular ion peak at 185 m/z, and presence of molecular ion peaks separated by 2 m/z units (M and M+2) with a ratio of 3:1 in the peak height at (185 and 187 m/z), indicate that presence of three nitrogen atoms and one chloride atom respectively in oxidative degradation product **(Fig. 6).**

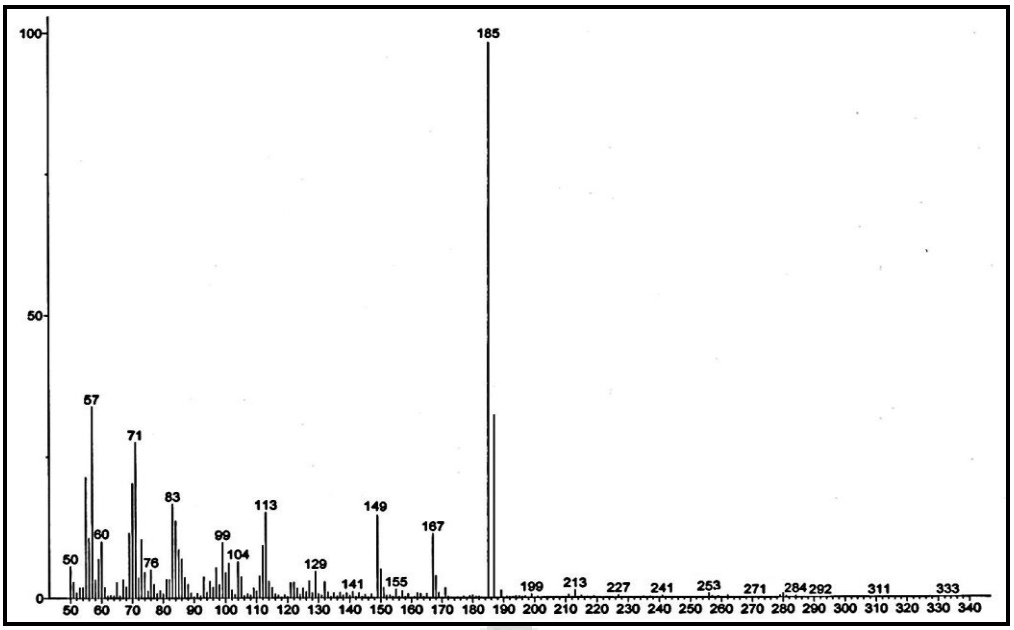
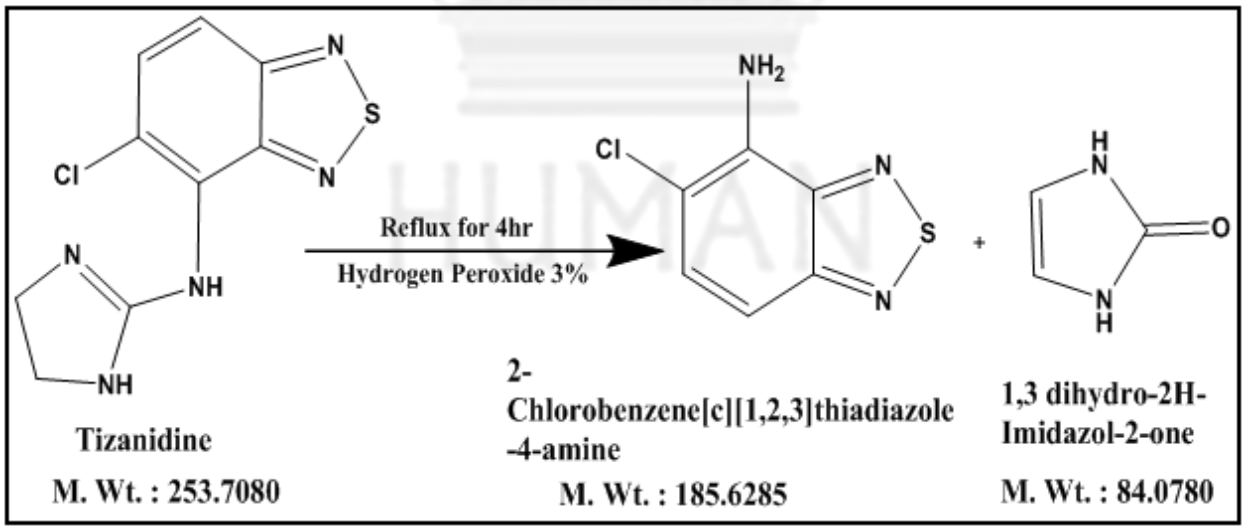


Figure (6): Mass spectroscopy of oxidative degradation product of tizanidine HCL.

According to the data obtained from previously study of IR, ^{1}H NMR and mass spectroscopy, the schematic representation of the expected mechanism of tizanidine HCL degradation is:



The zero-order absorption spectra of tizanidine HCL and its oxidative degradate show high degree of interference as shown in **(Fig. 7),** that the application of the direct spectrophotometry failed to determine tizanidine HCL in presence of its oxidative degradate.

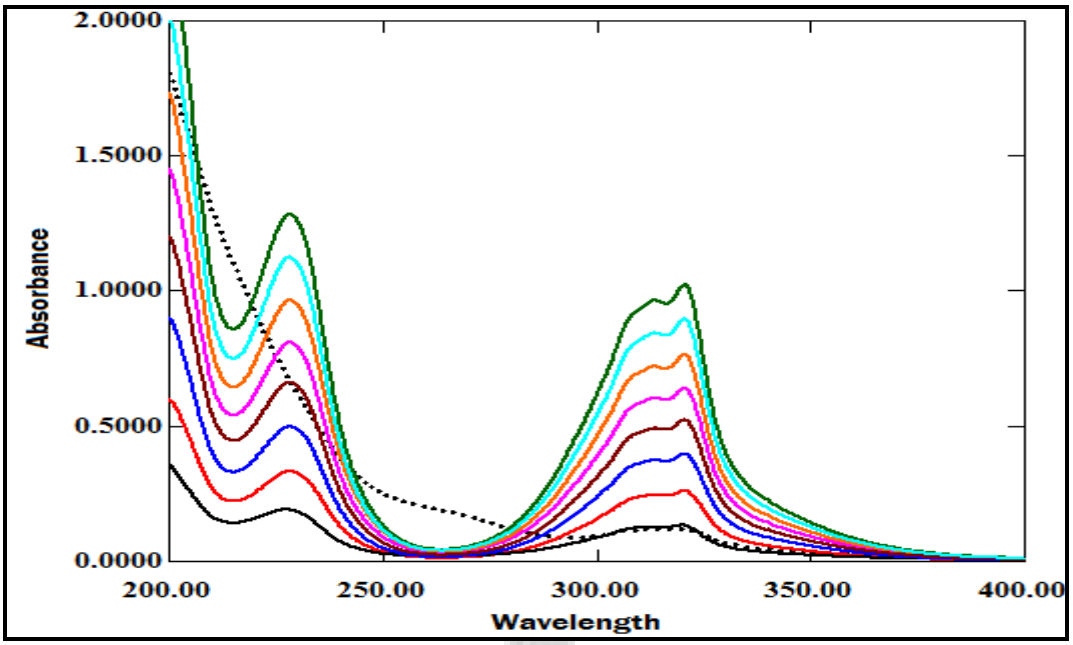


Figure (7): Zero-order absorption spectra of tizanidine hydrochloride (2.5 – 20 µg/ ml) and its oxidative degradation product (15 µg/ ml) in distilled water.

The suggested methods start by scanning zero order spectra of the prepared standard solution of tizanidine HCL and its degradate in distilled water. Different concentrations of tizanidine HCL and different divisor concentrations of degradate were tried, careful choice of the divisor is mandatory; the selected divisors should compromise between minimal noise and maximum sensitivity. The divisor concentration 15 ug/mL gave the best results regarding average recovery percent when used for the prediction of tizanidine HCL.

In first derivative method, the zero-spectra of intact tizanidine HCL and its degradation product show sever overlapping as shown in **(Fig. 7).** However, this sever overlapping in zero order spectra can be resolved by conversion of zero-order to higher first derivative spectra of tizanidine HCL and its degradation product.

(Fig. 8) showing that, the sever overlapping in first derivative of zero order spectra which can be resolved at 293nm, at this wavelength zero cross point of degradation product showing no interference to intact tizanidine HCL.

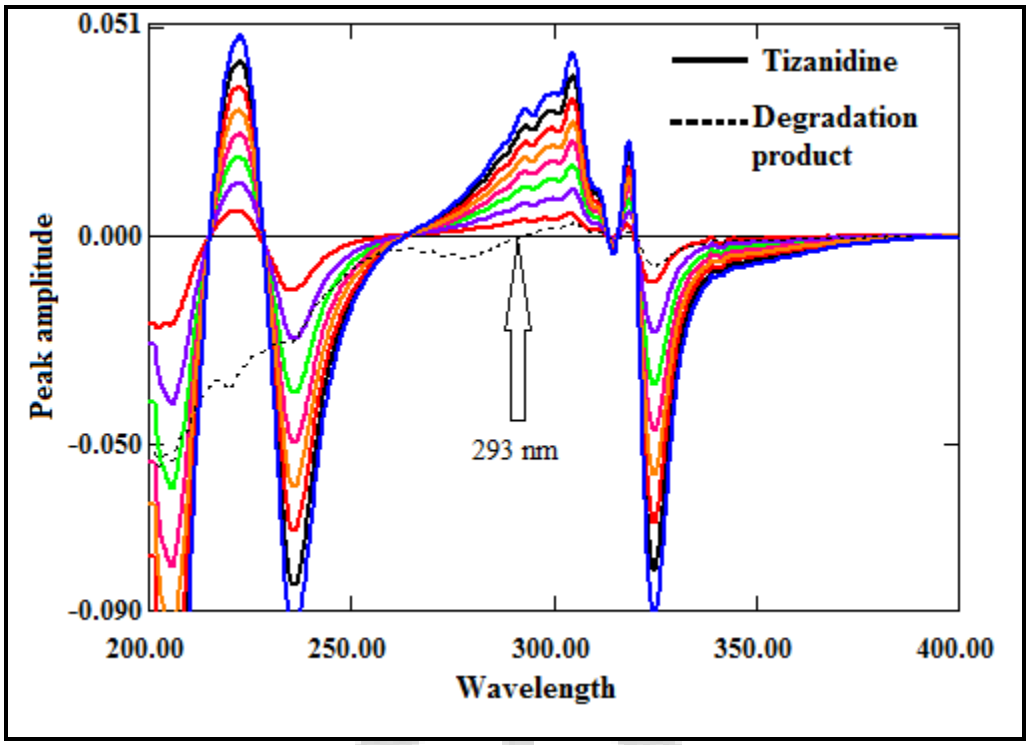


Figure (8): First derivative of zero order absorption spectra of tizanidine hydrochloride at various concentrations (2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml) and its oxidative degradation product (15 µg/ ml) in distilled water.

So that, the peak at this wavelength was chosen for selective determination of intact drug in presence of its oxidative degradate. Linear correlation was obtained between peak amplitude values at 293nm against the corresponding concentration of tizanidine HCL. Good linearity is obtained in the concentration range of (2.5–20 ug/mL). The corresponding regression equation was computed.

P_{293}= 0.0016 x – 0.0003 r^2 =0.9999

Where P is the peak amplitude of the first derivative at 293nm, X is the concentration in ug mL^{-1} and r^2 is the regression coefficient as shown in **(Table 3).**

In first derivative of ratio spectra method, the method is based on the derivation of the ratio-spectra as shown in **(Fig. 9)** for resolving interference. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing simple measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum

or a minimum). The calibration graph for the method was constructed by plotting peak amplitude at 326nm against the corresponding concentration of tizanidine.Good linearity is obtained in the concentration range of (2.5–20 ug/mL). The corresponding regression equation was computed.

Y_{224}= 0.0056 x + 0.0014 r^2 =0.9999

Where Y is the peak amplitude of the first derivative of the ratio spectra at 326nm, X is the concentration in ug mL^{-1} and r^2 is the regression coefficient as shown in **(Table 3).**

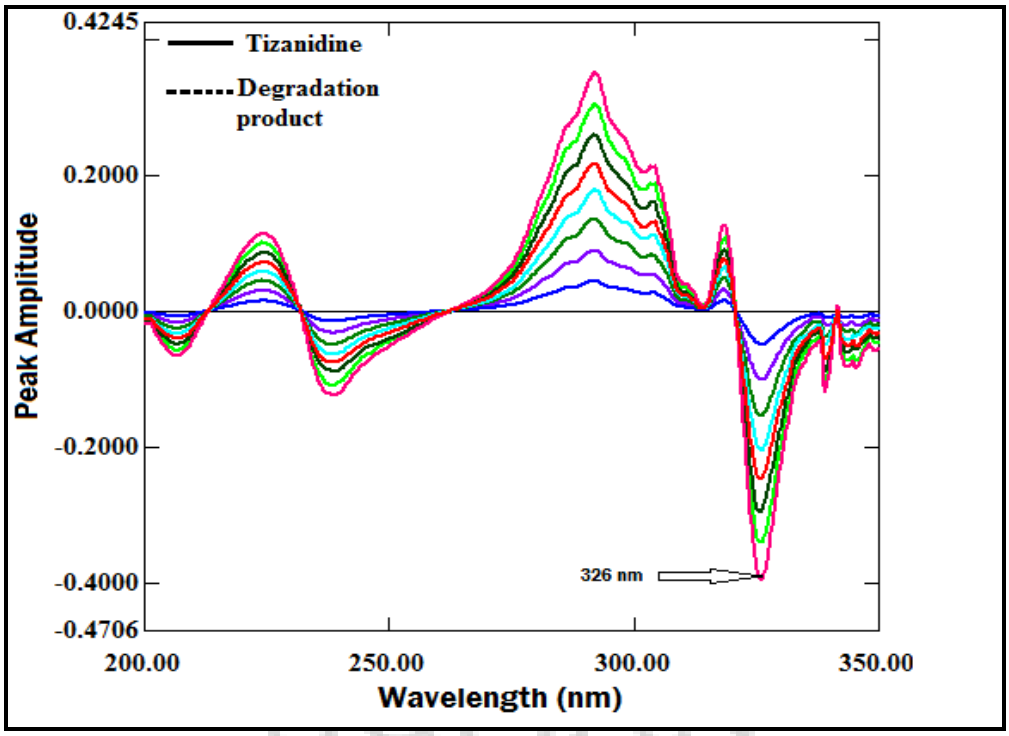


Figure (9): First derivative of the ratio spectra of tizanidine hydrochloride at various concentrations (2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml) using 15 µg/ml of degradate as a divisor.

In ratio difference technique, two wavelengths (231 and 320nm) were chosen on the ratio spectra **(Fig. 10),** difference between these two wavelengths $\Delta P_{231-320}$ was calculated, good linearity at $\Delta P_{231-320}$ was obtained in the concentration range of 2.5-20 ug/mL and the corresponding regression equation was computed.

$\Delta P_{231-320}$ = 0.3225 x +0.0357 r^2 =0.9997

Where ΔP is the amplitude difference at the selected wavelengths, X is the concentration in ug/mL and r^2 is the regression coefficient as shown in **(Table 3).**

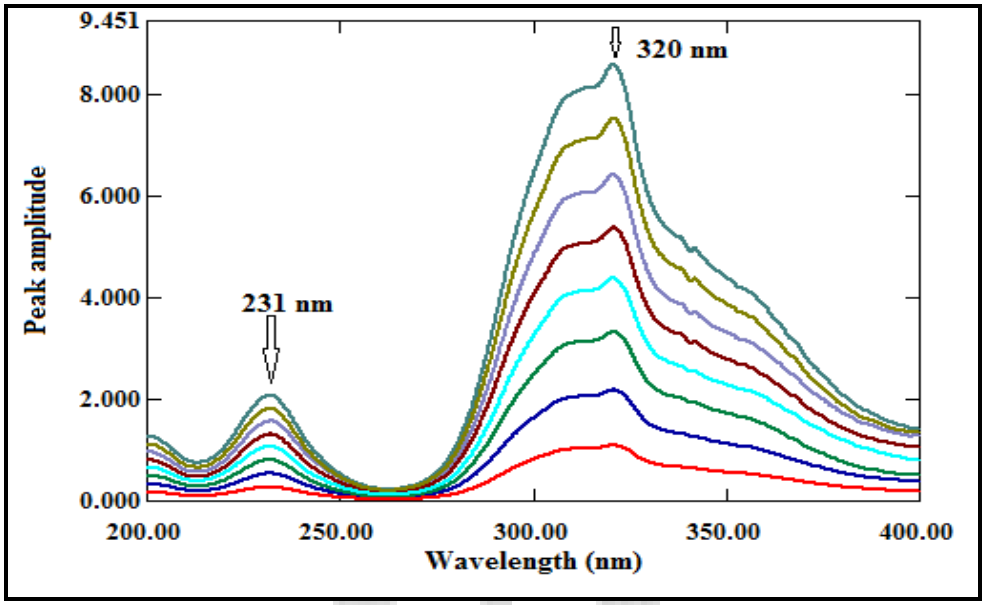


Figure (10): Ratio spectra of tizanidine hydrochloride at various concentrations (2.5, 5, 7.5, 10, 12.5, 17.5 and 20 µg/ml) using 15 µg/ml of degradate as a divisor.

In mean centering method, ratio spectra of tizanidine HCL using suitable divisor (15 ug/mL) were mean centered using MATLAB using the data within range 200-400nm **(Fig. 11),** linear correlation was obtained between mean centered values at 321nm against the corresponding concentration of tizanidine HCL. Good linearity is obtained in the concentration range of (2.5–20 ug/mL). The corresponding regression equation was computed.

MCN_{321}= 0.4261 x + 0.0676 r^2 =0.9998

Where MCN is the peak amplitude of the mean centered ratio spectra, X is the concentration in ug mL^{-1} and r^2 is the regression coefficient as shown in **(Table 3).**

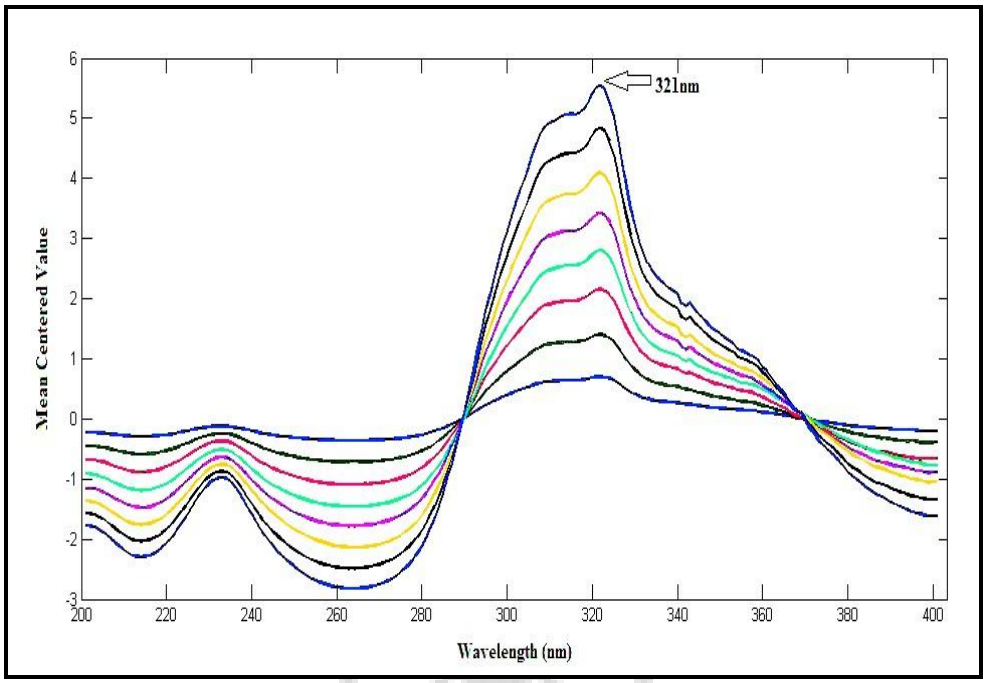


Figure (11): Mean centering of the ratio spectra of tizanidine hydrochloride at various concentrations (2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml) using 15 µg/ml of degradate as a divisor.

In dual wavelength method, the zero-spectra of intact tizanidine HCL and its degradation product show sever overlapping as shown in **(Fig. 7).** However, determination of intact tizanidine HCL in presence of its degradation product can be achieved by calculating difference in absorbance at two selected wavelengths (290 and 326nm), when the difference in absorbance at these wavelengths was found to be zero for degradate, while the intact spectra have the different absorbance values. So that, determination of tizanidine HCL at these wavelengths can be achieved without interference to its degradate. Good linearity at $\Delta P_{290\text{-}326}$ was obtained in the concentration range of (2.5-20 ug/mL) and the corresponding regression equation was computed.

$\Delta P_{290\text{-}326} = 0.0153\ x - 0.0068$ $\qquad r^2 = 0.9998$

Where ΔP is the amplitude difference at the selected wavelengths, X is the concentration in ug mL^{-1} and r^2 is the regression coefficient as shown in **(Table 3).**

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated for each method as shown in **(Table 3),** more small values of both LOD and LOQ indicate that more sensitive of method.

5.1.5 Accuracy and Precision:

According to the ICH guideline, three replicate determination of three different concentration of the studied drug in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. concentrations of (7.5, 10 and 12.5 ug/mL) were used in both of ratio difference and mean centering methods, concentrations of (7.5, 10 and 15 ug/mL) were used in derivative ratio technique, concentrations of (10, 12.5 and 15 ug/mL) were used in first derivative method, and concentrations of (12.5, 15 and 17.5 ug/mL) were used in dual wavelength method, and Accuracy as recovery percent (R%), and precision as percentage relative standard deviation (RSD%) were calculated and results were listed in **(Table 4).**

5.1.6 Specificity:

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of tizanidine HCL and its degradate. The results were listed in **(Table 5).**

5.1.7 Pharmaceutical Applications:

The proposed methods were applied to the determination of the studied drug in **Sirdalud® tablets**. The statistical comparison between the results obtained by applying the proposed methods and those obtained by applying the reported method[19] showed less calculated t and F values revealing no significant difference in accuracy and precision, **(Table 6).**

5.1.8 Statistical comparative discussion of proposed methods:

All data mentioned above related to previous tables and figures introduce a comparative discussion for five techniques which applied for manipulating tizanidine HCL and its oxidative degradation product, namely (first derivative, derivative ratio, ratio difference, mean centering and dual wavelength). It was illustrated that first derivative technique exceeded the other techniques in terms of (LOD and LOQ) and correlation coefficient (r^2=0.9999), where we know

that "more small values of LOD and LOQ, more sensitive the methods", and first derivative technique seems to be simplest one regarding manipulation of data and not need neither ratio spectra nor special software (MATLAB), in **(Table 5)** we can note that percent of degradate concentration related to intact concentration for first derivative and dual wavelength is more great than those of other methods also, first derivative technique has great benefit in cases where high degree of interference could be found among spectra.

Statistical comparison of the results obtained by the proposed methods and official method was shown in (**Table 7)**. Calculated [t and F] values were less than the theoretical ones indicating that there was no significant difference between the proposed and the official method with respect to accuracy and precision.

Finally, the proposed methods are simple without requirement for sophisticated technique or instruments, they also sensitive, selective and can be used for manipulation of tizanidine HCL in their available dosage forms.

6. Tables:

6.1. List of Tables:

Table (1): IR-Spectrum of intact tizanidine hydrochloride.
Table (2): ^{1}H NMR data of tizanidine hydrochloride.
Table (3): Spectral data for determination of tizanidine by proposed methods.
Table (4): Intraday and interday accuracy and precision for the determination of tizanidine by the proposed methods.
Table (5): Determination of tizanidine and its degradate in their laboratory mixtures by the proposed methods.
Table (6): Application of standard addition technique to the analysis of Sirdalud® tablets by applying the proposed methods.
Table (7): Statistical comparison between the results obtained by applying the proposed spectrophotometric and reported methods for determination of tizanidine HCL in sirdalud® tablets.

Table (1) IR-Spectrum of intact tizanidine hydrochloride

Type of Vibration	Observed Peak (cm^{-1})
Secondary amine N-H stretch	3244.73
Secondary amine N-H bending	3079.72
C=C aromatic ring stretch	1643.18 and 1403.70
Aromatic C-Cl stretch	1074.74
C-N Stretch	1283.22 and 1191.75
C−N−C	1191.75
Aromatic -N	818.68
(C−H) of imidazole ring	2843.18
Ring bending Strong	667.57

Table (2): ^{1}H NMR data of tizanidine hydrochloride

Position	1H (δ)
Aromatic (C−H)	7.91 and 8.18 (d, J=9.3 Hz)
Imidazole ring (C−H_2)	3.69 (S, 4H)
Imidazole ring (N−H)	11.15 (bs, 1H)
N-H	8.45 (s)

Table (3): Spectral data for determination of tizanidine by proposed methods:

Parameters	First derivative	Derivative ratio	Ratio difference	Mean centering	Dual wavelength
Wavelength (nm)	293	326	231 & 320	321	290 & 326
Linearity range (µg ml^{-1})	2.5 — 20	2.5 — 20	2.5 – 20	2.5 — 20	2.5 — 20
LOD (µg ml^{-1})	0.119	0.177	0.187	0.149	0.200
LOQ (µg ml^{-1})	0.361	0.536	0.567	0.451	0.607
Regression equation*					
Slope (*b*) Intercept (*a*)	0.0016 -0.0003	0.0056 0.0014	0.3225 0.0357	0.4261 0.0676	0.0153 -0.0068
Regression coefficient (r^2)	0.9999	0.9998	0.9997	0.9998	0.9998

* $y = a + bx$ where y is the response and x is the concentration.

Table (4): Intraday and interday accuracy and precision for the determination of tizanidine by the proposed methods:

Method	Conc µg/ml	Intraday			Interday		
		Found Conc. + SD	Accuracy (R%)	Precision (RSD%)	Found Conc. + SD	Accuracy (R%)	Precision (RSD%)
Derivative Ratio	10	9.88±0.018	99.56	0.706	9.98±0.022	100.05	0.065
	12.5	12.35±0.025	99.26	0.540	12.42±0.035	99.13	0.332
	15	15.01±0.033	99.98	0.079	14.90±0.037	99.96	0.542

	7.5	7.43 ± 0.049	99.65	0.525	7.49 ± 0.002	99.71	0.580
	10	9.93 ± 0.069	99.73	0.387	10.11±0.001	99.93	0.172
	15	15.11± 0.079	100.23	0.435	15.11±0.011	100.10	0.187
	7.5	7.53 ±0.028	100.34	0.372	7.52 ±0.028	100.28	0.374
	10	10.09 ±0.006	100.92	0.064	10.09 ±0.008	100.87	0.077
	12.5	12.41 ±0.015	99.24	0.118	12.40 ±0.009	99.22	0.076
Mean Centering	7.5	7.44±0.023	99.74	0.470	7.45±0.010	99.71	0.613
	10	10.01±0.075	100.12	0.121	10.02±0.115	100.11	0.137
	12.5	12.46±0.091	99.76	0.237	12.42±0.016	99.80	0.236
Dual Wavelength	12.5	12.47±0.065	99.31	0.435	12.45±0.075	100.20	0.723
	15	15.02±0.131	100.03	0.127	15.06±0.151	99.74	0.455
	17.5	17.39±0.100	99.76	0.361	17.35±0.038	99.66	0.495

Table (5): Determination of tizanidine and its degradate in their laboratory mixtures by the proposed methods:

	Intact in (µg/ml)	Degradate in (µg/mL)	Percent of degradate	Intact found in (µg/mL)	Recovery % of intact
First derivative	17.5	2.5	12.5	17.44	99.64
	15	5	25	14.94	99.58
	12.5	7.5	37.5	12.44	99.5
	10	10	50	10.06	100.63
	7.5	12.5	62.5	7.44	99.17
	5	15	75	5.01	100.25
	Mean ± RSD%				**99.79±0.539**
Derivative Ratio	17.5	2.5	12.5	17.43	99.59
	15	5	25	14.93	99.52
	12.5	7.5	37.5	12.43	99.43
	10	10	50	9.93	99.29
	7.5	12.5	62.5	7.43	99.05
	5	15	75	5.02	100.36
	Mean ± RSD%				**99.79±0.539**
Ratio Difference	17.5	2.5	12.50	17.58	100.45
	15	5	25.00	15.07	100.47

	12.5	7.5	37.50	12.44	99.55
	10	10	50.00	9.94	99.39
	7.5	12.5	62.50	7.49	99.90
	5	15	75.00	5.02	100.36
	Mean ± RSD%				**100.02±0.499**
Mean Centering	17.5	2.5	12.5	17.58	100.45
	15	5	25	15.01	100.05
	12.5	7.5	37.5	12.45	99.57
	10	10	50	9.97	99.73
	7.5	12.5	62.5	7.50	100.02
	Mean ± RSD%				**99.96 ±0.338**
Dual wavelength	17.5	2.5	17.37	12.50	99.27
	15	5	15.08	25.00	100.57
	12.5	7.5	12.41	37.50	99.24
	10	10	9.99	50.00	99.87
	7.5	12.5	7.44	62.50	99.17
	5	15	5.02	75.00	100.39
	2.5	17.5	2.47	87.50	98.82
	Mean ± RSD%				**99.62±0.668**

Table (6): Application of standard addition technique to the analysis of Sirdalud® tablets by applying the proposed methods.

		Sirdalud® tablets		
Method	**Taken µg/ml**	**Pure added Mg/ml**	**Pure found µg/ml**	**Recovery %**
First Derivative	5	7.5	7.56	100.83
		10	10.01	100.00
		12.5	12.56	100.50
		15	15.06	100.42
Mean ± RSD%				100.44 ± 0.341
Derivative Ratio	5	5	4.98	99.64
		10	10.07	100.71
		12.5	12.61	100.86
		15	15.11	100.71
Mean ± RSD%				100.48 ± 0.561
Ratio Difference	5	2.5	2.50	99.82
		5	5.03	100.51
		12.5	12.52	100.19

		15	14.90	99.34
Mean ± RSD%				99.97 ± 0.503
Mean Centering	5	2.5	2.48	99.2
		5	4.97	99.4
		12.5	12.51	100.08
		15	14.92	99.47
Mean ± RSD%				99.56 ± 0.463
Dual Wavelength	5	2.5	2.50	100.13
		5	4.99	99.74
		7.5	7.54	100.48
		10	10.08	100.85
Mean ± RSD%				**100.30 ± 0.474**

Table (7): Statistical comparison between the results obtained by applying the proposed spectrophotometric and reported methods for determination of tizanidine HCL in sirdalud® tablets:

	First Derivative	Derivative Ratio	Ratio Difference	Mean Centering	Dual Wavelength	Reported method[10]
N*	5	5	5	5	5	5
$\bar{X}$	100.03	99.22	100.10	99.33	100.11	100.16
SD	0.574	0.990	0.598	0.753	0.448	0.592
Variance	0.574	0.998	0.597	0.758	0.448	0.591
t**	0.329 (2.306)	1.816 (2.306)	0.160 (2.306)	1.920 (2.306)	0.129 (2.306)	____
F**	1.065 (6.388)	2.793 (6.388)	1.018 (6.388)	1.618 (6.388)	1.746 (6.388)	____

* No. of experimental.

** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

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