Trade Science Inc.

Spectroscopy

An International Journal

Full Paper

SAIJ, 1(1), 2016 [028-044]

A comparative study of different approaches for stability-indicating determination of tizanidine in presence of its oxidative degradation product

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ABSTRACT

Five, simple, accurate, selective and sensitive methods were developed for the determination of tizanidine hydrochloride in presence of its oxidative degradation product without previous separation. Confirming this novel degradation way of tizanidine HCL by IR, ^{1}H NMR and mass spectroscopy techniques. The first method is the first derivative technique, measure peak amplitudes of derivatized spectra at 293 nm. 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 224 nm. The third method is the ratio difference technique which depends upon measuring peak amplitudes of ratio spectra at 231 and 320 nm 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 321 nm. The fifth method is the dual wavelength technique which depends upon measuring peak amplitudes of zero order spectra at 290 and 326 nm. The calibration graphs were linear in the range of (2.5 6 20 ug ml^{-1}) for tizanidine HCL.

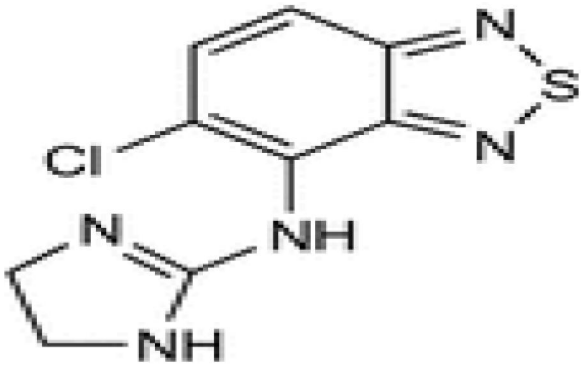
KEYWORDS

Tizanidine hydrochloride;
First derivative;
Derivative ratio;
Ratio difference;
Mean centering;
Dual wavelength.

INTRODUCTION

Tizanidine hydrochloride 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothia diazole hydrochloride (Figure 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, sen-



M. Wt = 253.711 g / mol

Figure 1 : Chemical structure of tizanidine hydrochloride

sitive, accurate and precise stability indicating methods for determination of tizanidine hydrochloride in presence of its oxidative degradate without sophisticated instruments or any separation steps.

THEORY

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].

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

The methods is based on the fact that, upon dividing the absorption spectrum of a compound by another 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 absorption spectrum of the other compound, a new spectrum (ratio spectrum) will result.

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 considered as a constants, where derivatization of the constant is equal to zero[37-41].

Ratio difference technique

The following step 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'.

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

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

Where, P1 and P2 are the amplitudes of mixture spectrum at λ1 and λ2 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].

Mean centering of ratio spectra technique

The following step 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

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

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

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].

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].

EXPERIMENTAL

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. Hot plate (Torrey Pines Scientific, USA). Rota-Vapor SCI-Logics (RE-100-PRO) with Buchi pump.

Materials

A. Tizanidine hydrochloride was kindly provided by Sigma Pharmaceutical Company, Cairo, Egypt, with purity of 99.9%.
B. Sirdalud® tablet: manufactured by Novartis Pharma; labeled to contain 4 mg of tizanidine hydrochloride per tablet.
C. Hydrogen peroxide 3%. was obtained from El-Naser Pharmaceutical Company.
D. Whatmann filter paper n° 41.

Standard solution

Stock solution of 100 ug /mL for tizanidine HCL was prepared by dissolving 10 mg 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.

Preparation of hydrogen peroxide-induced degradation product

A stock solution containing 50 mg tizanidine HCL in 50 ml distilled water was prepared. To 10 mL of this stock solution, 5 mL of hydrogen peroxide (3 %, v/v) was added. The solution was refluxed at 100° C for 4 hours. The time required for complete degradation was followed by spotting on TLC plates at 30 minutes intervals for 4 hour. The plates was 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 vaccum, the residue was dissolved by least amount of methanol,

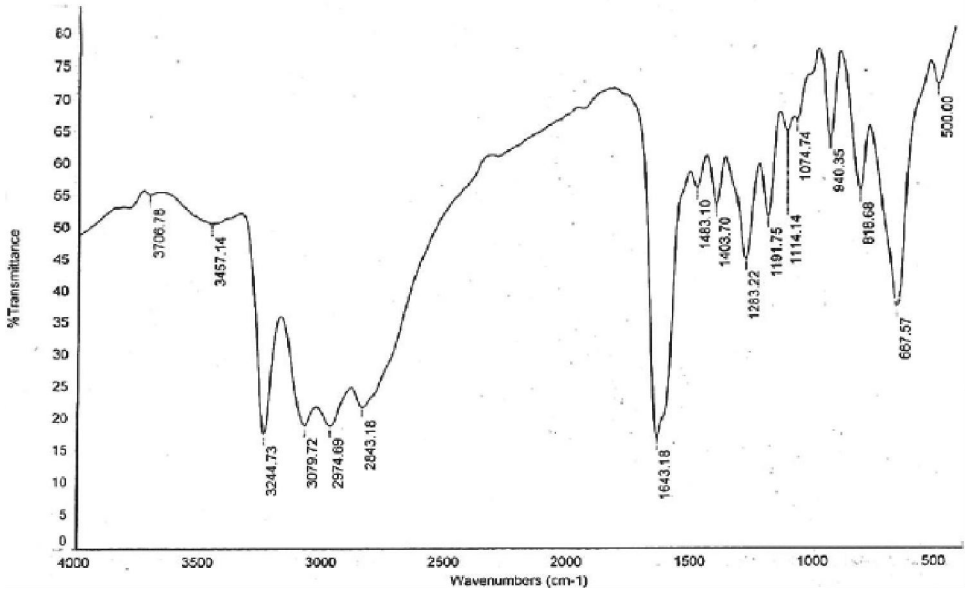

Figure 2 : IR spectrum of intact tizanidine hydrochloride

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

filtrered then filtrate was left to dry at room temperature, dissolve the residue in 100 ml distilled water to give degradate stock solution of (100 ug / mL).

IR × spectroscopy of tizanidine hydrochloride

1.0 mg of finely ground sample of intact tizanidine and its degradate were separately mixed with about 100 mg 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 / inch2 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 HCL (Figure 2).

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.

Pharmaceutical formulation

Sirdalud® tablets: Ten sirdalud tablets were accurately weighed, crushed and mixed. An amount equivalent to 10 mg of tizanidin 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.

TABLE 2 : ^{1}H NMR data of tizanidine hydrochloride

Position	1H (δ)
Aromatic (C - H) at position 6	8.18 (d, J=9.3 Hz, 1H)
Aromatic (C- H) at position 7	7.91 (d, J=9.3 Hz, 1H)
Imidazole ring (C- H_2)	3.69 (S, 4H)
Imidazole ring (N-H)	11.15 (bs, 1H)
N-H	8.45 (s)

PROCEDURES

Construction of calibration curves (linearity)

Accurately measured aliquots equivalent to (0.25 - 2 ml) of tizanidine HCL from stock solution (100 ug/ml) were, separately, transferred into a series of 10 ml volumetric flasks and the volume of each one was completed to the mark with distilled water, to reach the concentration range of (2.5 - 20 ug mL-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 293 nm in derivative mode at which degradate shows zero absorbance, the corresponding regression was computed.

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 224nm, calibration graphs relating absorbance at 224 nm to the corresponding concentration of tizanidine were constructed, and the corresponding equation was computed.

For determination of tizanidine HCL by ratio difference technique

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The zero order spectra of the prepared solutions were divided by the spectrum of 15 ug mL-1 degradate. The peak amplitude of the ratio spectra were measured at 231 and 320 nm. Calibration graphs relating the "P231-320 to the corresponding concentrations of tizanidine HCL were constructed, and the corresponding regression equation was computed.

For determination of tizanidine HCL by mean centering technique

The data of ratio spectra which previously be obtained in range (200 400 nm), 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.

For determination of tizanidine HCL by dual wavelengths technique

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

Accuracy

Accuracy was assured by carrying out the previously mentioned procedures under linearity for the determination different concentration of pure

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	224	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	0.616
LOQ (µg ml^{-1})	0.361	0.536	0.567	0.451	1.867
Regression equation*					
Slope (*b*)	0.0016	0.0056	0.3225	0.4261	0.0153
Intercept (*a*)	0.0003	0.0014	0.0357	0.0676	0.0068
Regression coefficient (r^2)	0.9999	0.9999	0.9997	0.9997	0.9998

TABLE 4 : Intraday and interday accuracy and precision for the determination of tizanidine by the proposed methods

Method	Conc µg.ml^{-1}	Intraday			Interday		
		Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
First Derivative	10	9.88±0.018	98.75	0.0182	10±0.022	98.75	0.223
	12.5	12.35±0.025	98.83	0.202	12.42±0.035	99.33	0.282
	15	15±0.033	100.01	0.220	14.90±0.037	99.31	0.248
Derivative Ratio	7.5	7.43 ± 0.049	99.05	0.659	7.49 ± 0.033	99.84	0.441
	10	9.93 ± 0.069	99.29	0.695	10.11 ± 0.039	101.07	0.386
	15	15.11± 0.079	100.71	0.523	15.11 ± 0.049	100.71	0.324
Ratio Difference	7.5	7.55±0.076	100.73	1.009	7.57±0.029	100.92	0.381
	10	10.11±0.026	101.14	0.261	10.11±0.046	101.14	0.455
	12.5	12.51±0.065	100.07	0.520	12.44±0.052	99.55	0.418
Mean Centering	7.5	7.47±0.069	99.62	0.924	7.45±0.01	99.36	0.137
	10	10.01±0.075	100.14	0.746	9.94±0.085	99.38	0.855
	12.5	12.45±0.099	99.60	0.799	12.4±0.031	99.16	0.252
Dual Wavelength	12.5	12.36±0.151	98.89	1.221	12.45±0.075	99.59	0.606
	15	14.98±0.151	99.84	1.008	15.06±0.151	100.42	1.002
	17.5	17.39±0.100	99.40	0.574	17.35±0.038	99.15	0.217

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

	Intact in (μg ml^{-1})	Degradate in (μg ml^{-1})	Percent of degradate	Intact found in (μ ml^{-1})	Recovery % of intact
First derivative	17.5	2.5	12.5	17.19	98.21
	15	5	25	14.81	98.75
	12.5	7.5	37.5	12.38	99
	10	10	50	10.06	100.63
	7.5	12.5	62.5	7.50	100
	5	15	75	5.06	101.25
	Mean ± SD%				99.64±1.18
Derivative Ratio	17.5	2.5	12.5	17.25	98.57
	15	5	25	14.75	98.33
	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
	Mean ± SD%				98.93±0.47
Ratio Difference	17.5	2.5	12.5	17.84	101.94
	15	5	25	15.19	101.30
	12.5	7.5	37.5	12.75	101.96
	10	10	50	10.25	102.49
	7.5	12.5	62.5	7.49	99.90
	Mean ± SD%				101.52±0.999
Mean Centering	17.5	2.5	12.5	17.58	100.45
	15	5	25	15.26	101.77
	12.5	7.5	37.5	12.70	101.62
	10	10	50	10.11	101.14
	7.5	12.5	62.5	7.50	100.02
	Mean ± SD%				101.00 ±0.750
Dual wavelength	17.5	2.5	12.5	17.18	98.15
	15	5	25	15.28	101.87
	12.5	7.5	37.5	12.41	99.24
	10	10	50	9.99	99.87
	7.5	12.5	62.5	7.44	99.17
	5	15	75	5.02	100.39
	Mean ± SD%				99.78±1.38

tizanidine HCL. The concentration was calculated from the corresponding regression equations.

Precision

Intra-day precision (Repeatability)

Three concentrations of tizanidine HCL were analyzed three times intraday using the previously mentioned procedures. The percentage of recoveries of each concentration of tizanidine HCL and its relative standard deviation were calculated using the suggested methods (TABLE 4).

Intermediate precision

Three concentrations of tizanidine HCL were analyzed on three successive days using the procedure stated under linearity. The percentage of recoveries of each concentration of tizanidine HCL and its relative standard deviation were calculated using the suggested methods (TABLE 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

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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).

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).

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).

RESULTS AND DISCUSSION

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

TABLE 6 : Application of standard addition technique to the analysis of Sirdalud® TABLEts by applying the proposed methods

		Sirdalud® tablets		
Method	**Taken µg ml^{-1}**	**Pure added µg ml^{-1}**	**Pure found µg ml^{-1}**	**Recovery %**
First Derivative	5	7.5	7.56	100.83
		10	10.00	100.00
		12.5	12.31	100.50
		15	14.81	100.42
Mean ± RSD%				100.42 ± 0.341
Derivative Ratio	5	5	4.98	99.64
		10	10.11	101.07
		12.5	12.61	100.86
		15	15.11	100.30
Mean ± RSD%				100.37 ± 0.633
Ratio Difference	5	2.5	2.52	100.69
		5	5.05	100.95
		12.5	12.53	100.24
		15	14.88	99.18
Mean ± RSD%				100.26 ± 0.778
Mean Centering	5	2.5	2.45	98.00
		5	4.97	99.31
		10	9.96	99.55
		15	14.79	98.58
Mean ± RSD%				98.86 ± 0.717
Dual Wavelength	5	2.5	2.5	100.13
		5	4.99	99.74
		7.5	7.54	100.48
		10	10.08	100.85
Mean ± RSD%				100.30 ± 0.474

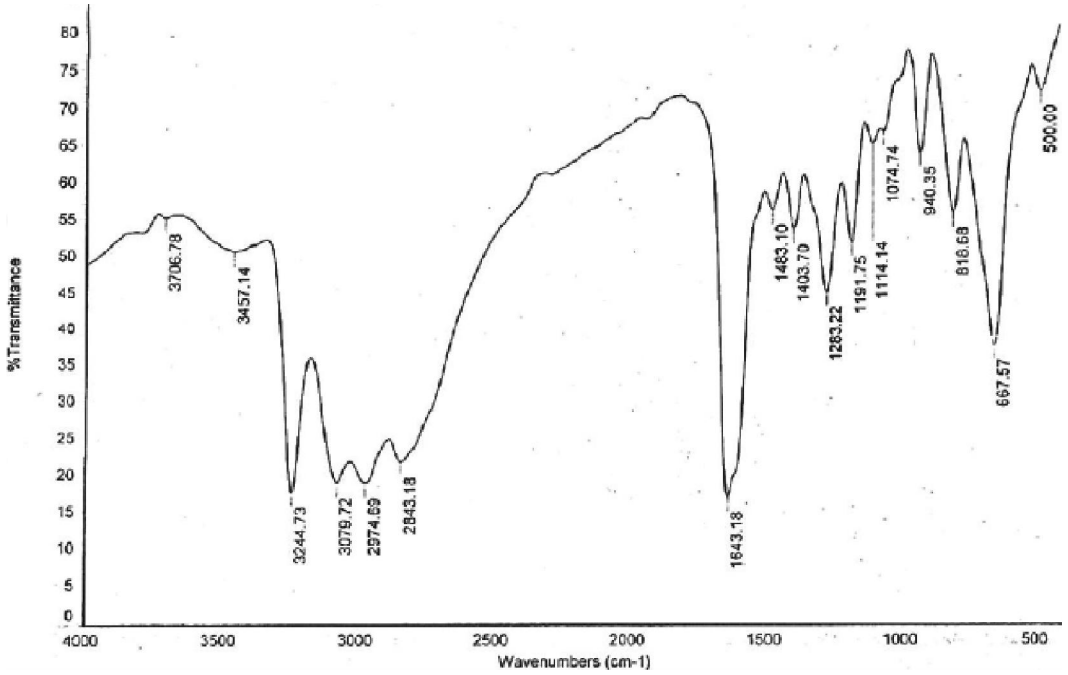

Figure 3 : IR spectrum of intact tizanidine hydrochloride

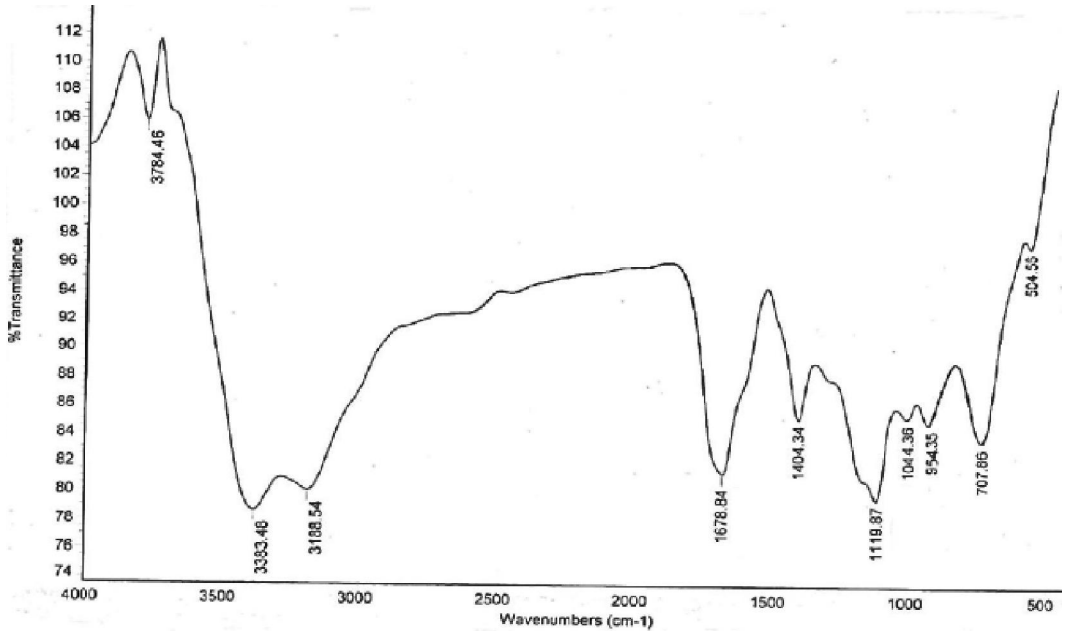

Figure 4 : IR spectrum of oxidative degradation product of tizanidine hydrochloride

previous separation.

Confirmation of degradation product

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.

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 (Figure 3), showed data as mentioned in (TABLE 1). However, IR spectrum of degradate (Figure 4), showed disappearance of band of C 6 N 6 C of imidazole ring at 1191.75 cm^{-1}, disappearance of sec-

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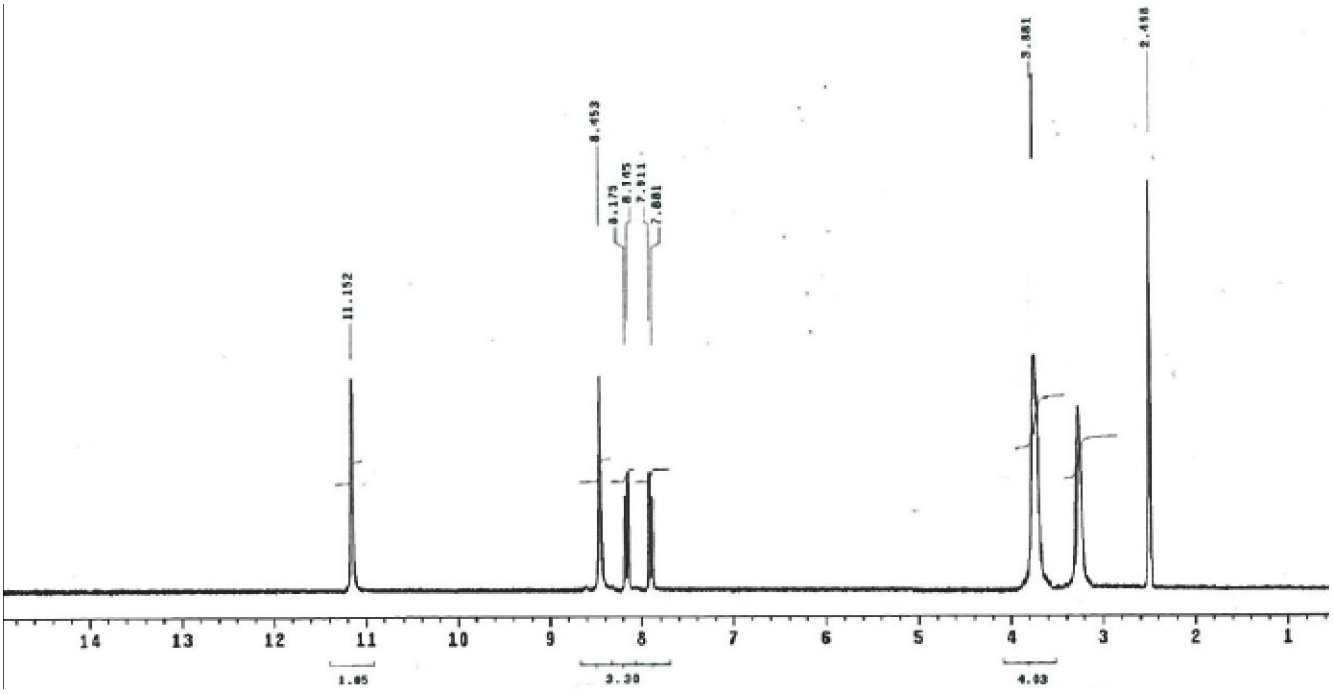


Figure 5 : ^{1}H NMR of intact tizanidine hydrochloride

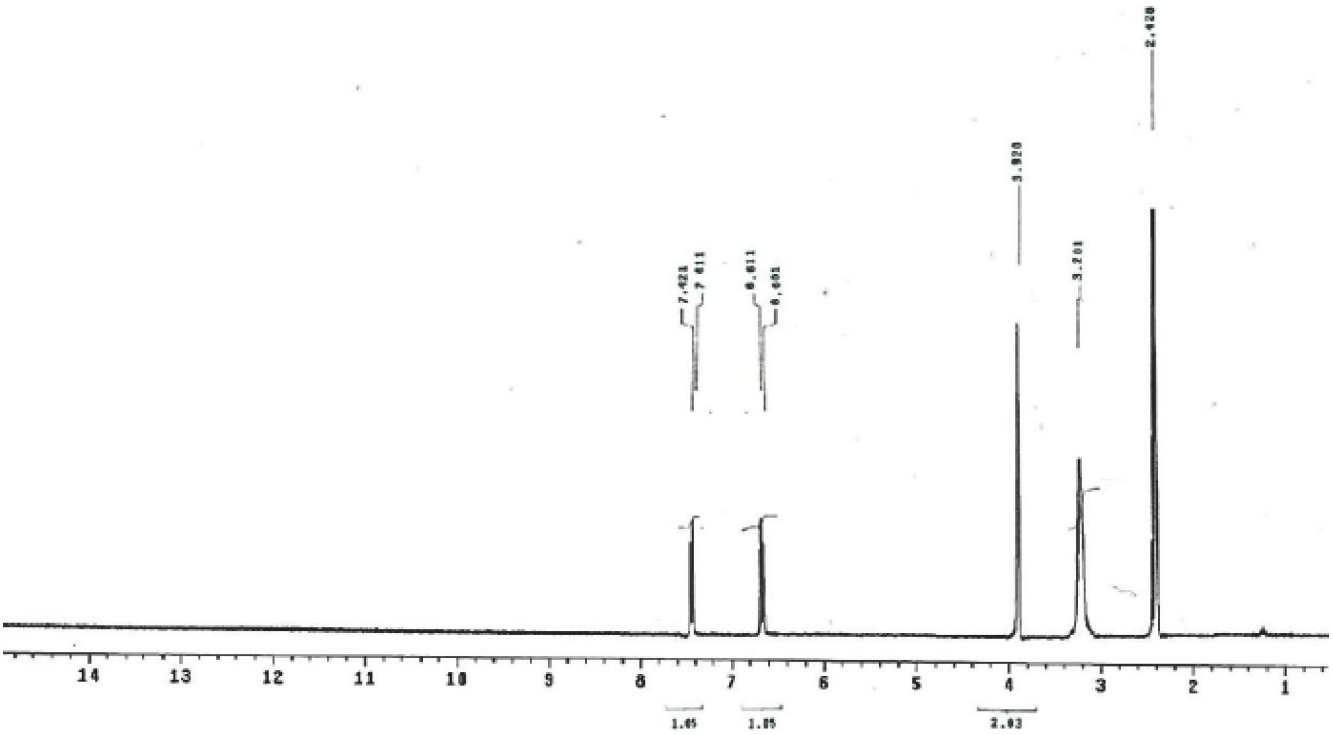


Figure 6 : ^{1}H NMR of oxidative degradation product of tizanidine hydrochloride

ondary amine (N 6 H) band at 3244.73 and 3079.72 cm^{-1}, disappearance of imidazole (C 6 H) band at 2843.18 cm^{-1} and appearance of primary amine (N 6 H_2) band at 3383.48 cm^{-1}.

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

The ^{1}H NMR of the intact tizanidine HCL (Figure 5), showed data as mentioned in (TABLE 2), While ^{1}H NMR of degradate (Figure 6), showed disappearance of CH_2 of imidazole ring, appearance of aromatic (C 6 H) peaks at 7.421 and 6.611 ppm and appearance of (N 6 H_2) peak at 3.92 ppm.

Confirmation of degradation product using mass spectroscopy techniques

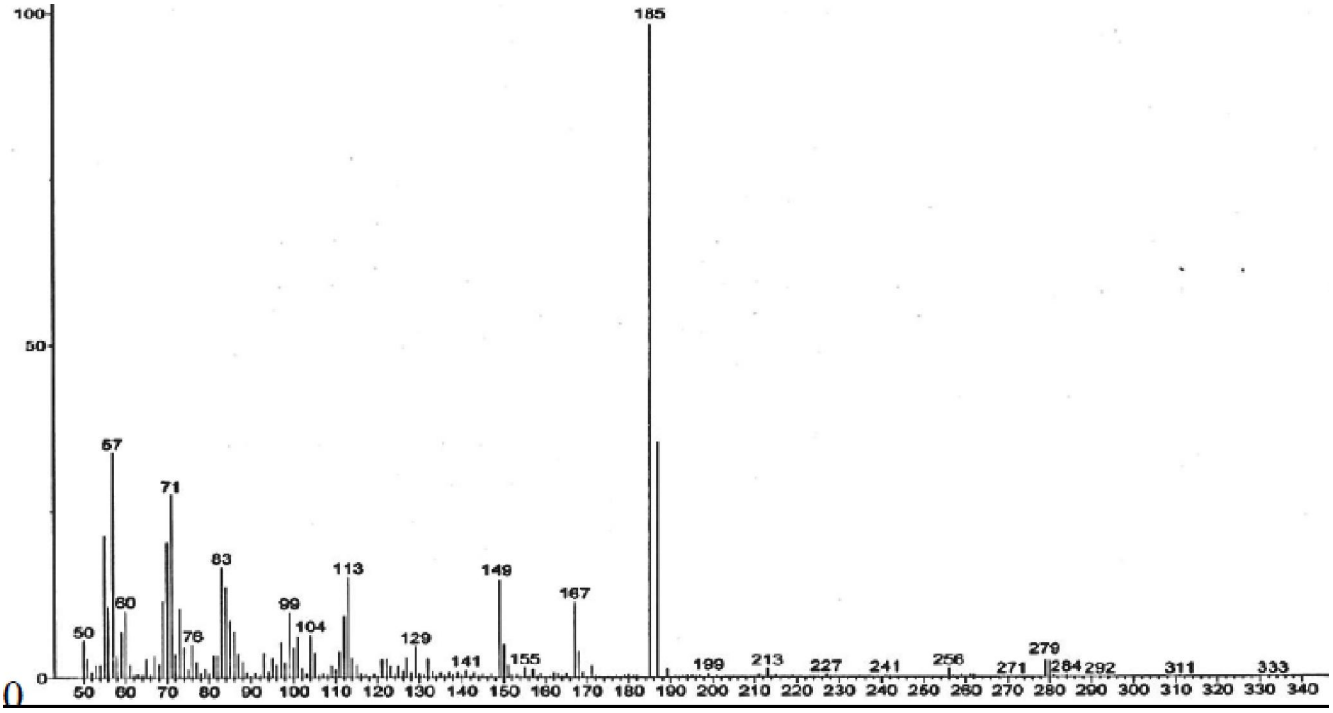
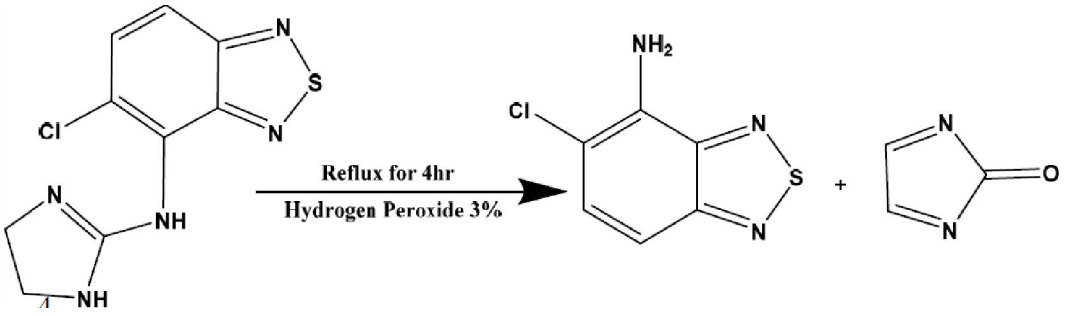


Figure 7 : Mass spectroscopy of oxidative degradation product of tizanidine hydrochloride



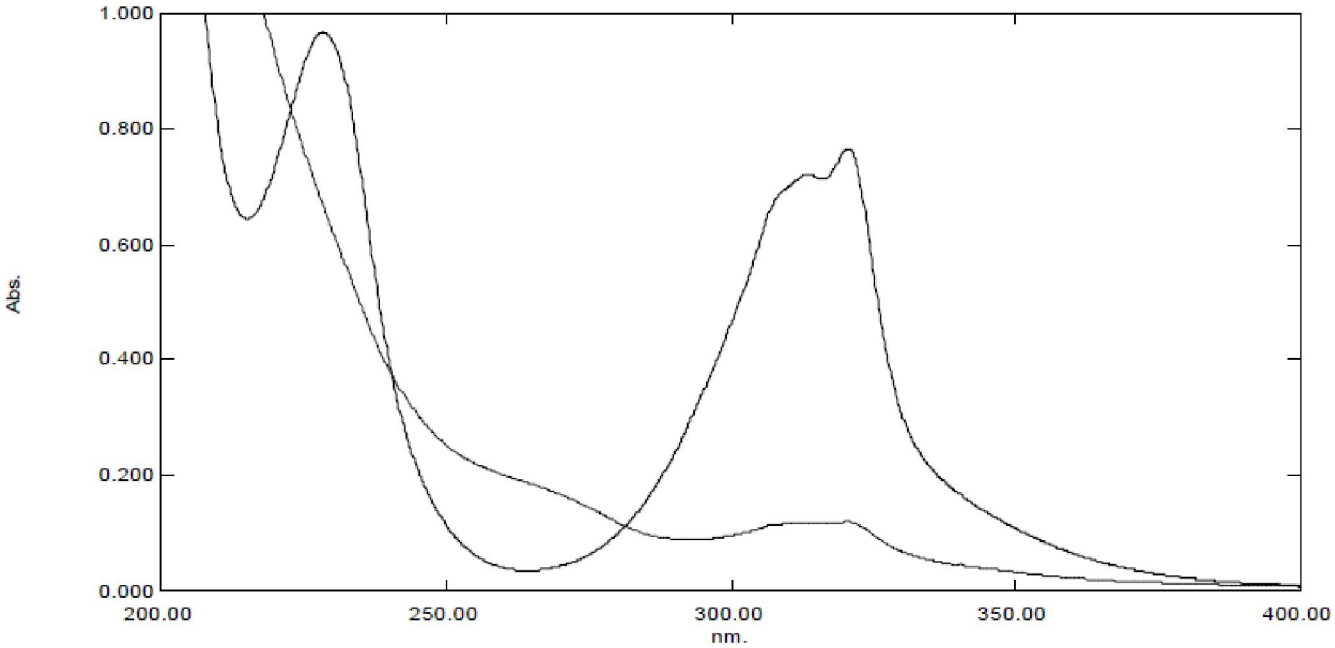


Figure 8 : Zero order absorption spectra of 15 ug mL^{-1}of tizanidine (—) and 15 ug mL^{-1} of degradate(…...)

Odd number of molecular ion peak at 185 m/z, and presence of molecular ion peaks separated by 2

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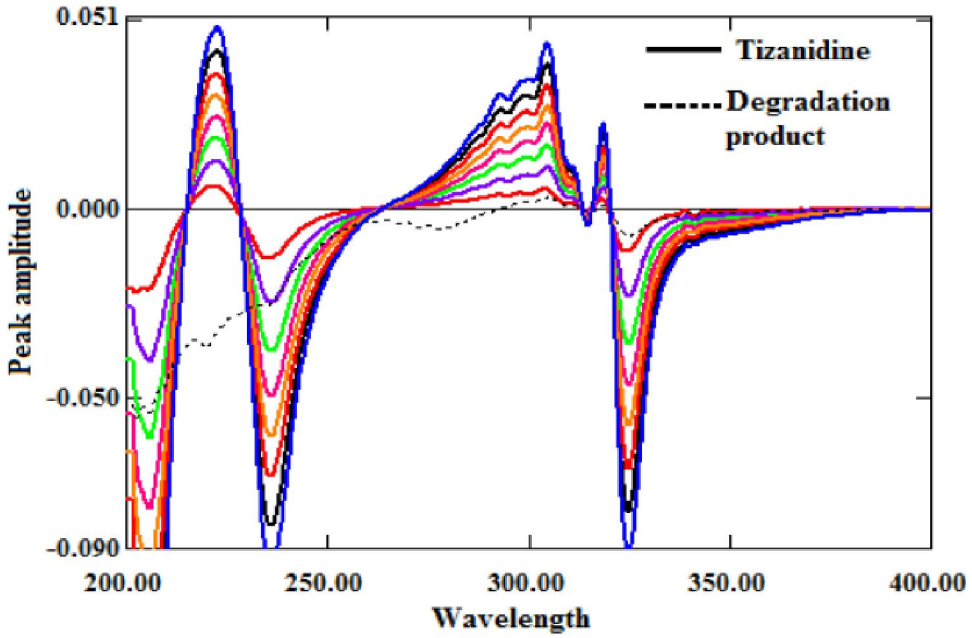

Figure 9 : First derivative of zero order spectra of tizanidine (2.5 -20ug mL^{-1}) (—), with first derivative of zero order spectrum of degradate (15 ug mL^{-1}) (…)

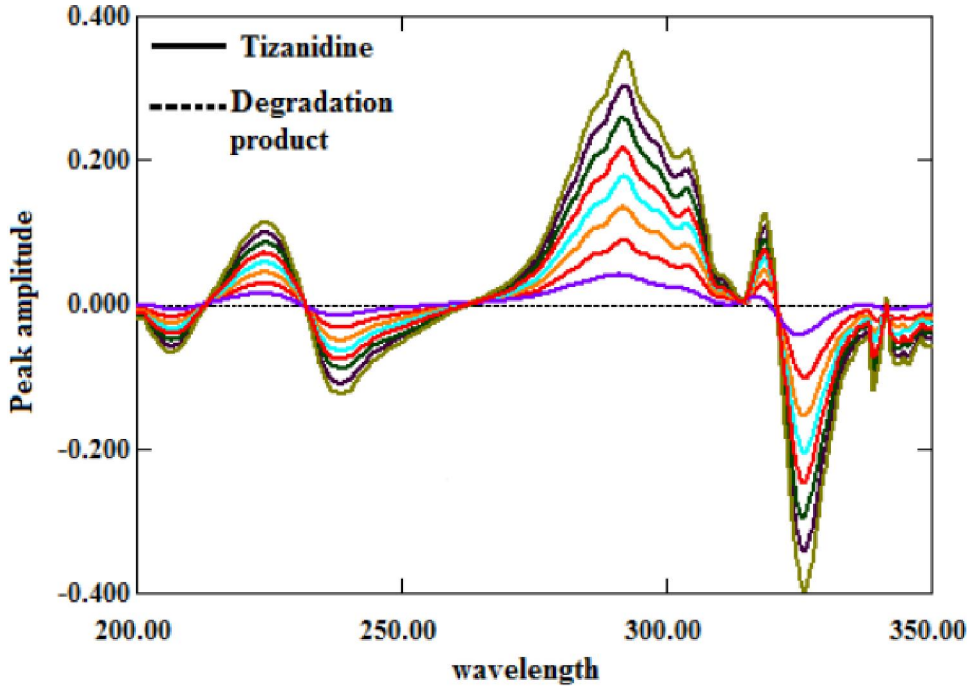

Figure 10 : First derivative of ratio spectra in the concentration range of (2.5 – 20 ug ml^{-1}) of tizanidine HCL using 15 ug mL^{-1} of degradate as divisor

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 (Figure 7).

According to the data obtained from previously study of IR, 1H 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 (Figure 8), that the application of the direct spectrophotometry failed to determine tizanidine HCL in presence of its oxidative degradate.

The suggested methods start by scanning zero

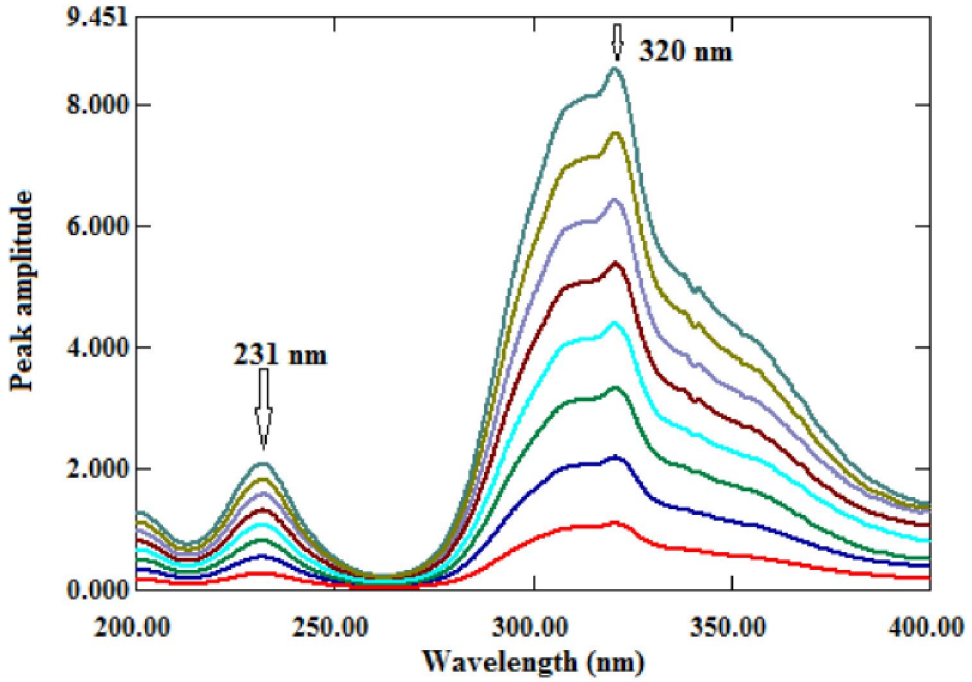

Figure 11 : Ratio spectra of tizanidine HCL using 15 ug ml^{-1} of degradate as a divisor in the concentration range of (2.5 – 20 ug ml^{-1})

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 (Figure 8). 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.

(Figure 9) showing that, the sever overlapping in first derivative of zero order spectra which can be resolved at 293 nm, at this wavelength zero cross point of degradation product showing no interference to intact tizanidine HCL.

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 293 nm against the corresponding concentration of tizanidine HCL. Good linearity is obtained in the concentration range of (2.5 - 20 ug mL^{-1}). 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 293 nm, 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 (Figure 10) 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 224 nm against the corresponding concentration of tizanidine. Good linearity is obtained in the concentration range of (2.5 - 20 ug mL^{-1}). 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 224 nm, X is the concentration in ug mL^{-1} and r^2 is the regression coefficient as shown in (TABLE 3).

In ratio difference technique, two wavelengths

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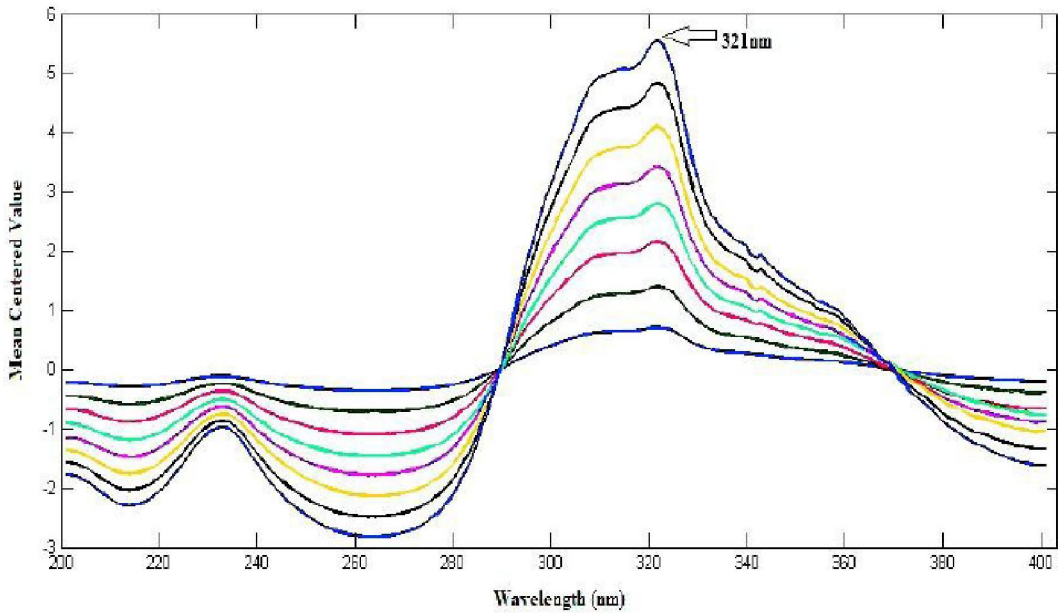

Figure 12 : Mean centered ratio spectra of tizanidine in concentration range of (2.5 – 20 µg ml^{-1}) Using degradate (15 µg ml^{-1}) as a divisor within range of (200-400nm)

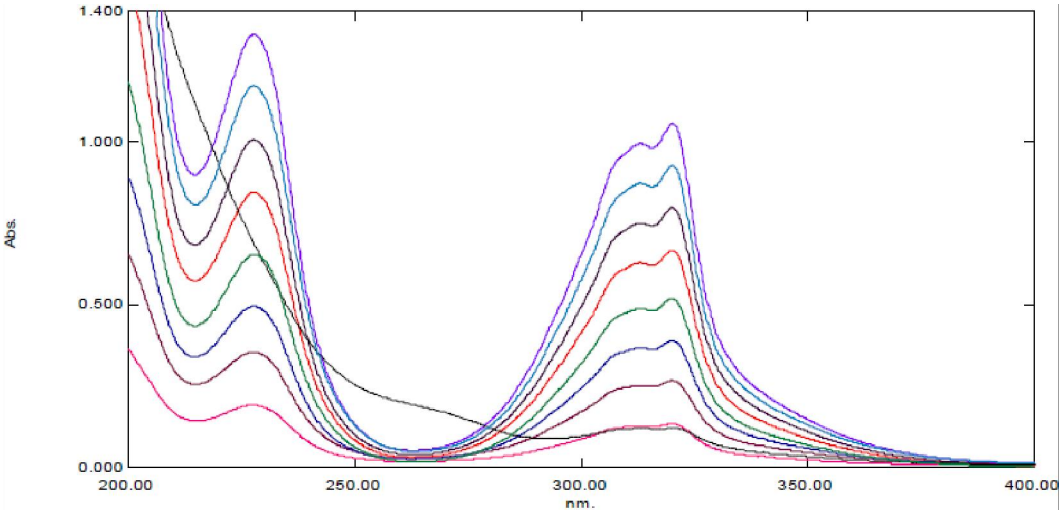

Figure 13 : Zero order absorption spectra of intact tizanidine in concentration range of (2.5 20 ug mL^{-1}) (—), with zero order absorption spectrum of degradate (15 ug mL^{-1}) (…..)

(231 and 320 nm) were chosen on the ratio spectra (Figure 11), 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^{-1} 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^{-1} and r^2 is the regression coefficient as shown in (TABLE 3).

In mean centering method, ratio spectra of tizanidine HCL using suitable divisor (15 ug mL^{-1}) were mean centered using MATLAB using the data within range 200-400 nm (Figure 12), linear correlation was obtained between mean centered values at 321 nm against the corresponding concentration of tizanidine HCL. Good linearity is obtained in the concentration range of (2.5 20 ug mL^{-1}). 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).

In dual wavelength method, the zero-spectra of

intact tizanidine HCL and its degradation product show sever overlapping as shown in (Figure 13). 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 326 nm), 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 $\Delta P_{290-326}$ was obtained in the concentration range of (2.5-20 ug mL^{-1}) and the corresponding regression equation was computed.

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

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.

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^{-1}) were used in both of ratio difference and mean centering methods, concentrations of (7.5, 10 and 15 ug mL^{-1}) were used in derivative ratio technique, concentrations of (10, 12.5 and 15 ug mL^{-1}) were used in first derivative method, and concentrations of (12.5, 15 and 17.5 ug mL^{-1}) 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).

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).

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).

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

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
$X^?$	99.86	99.75	100.45	99.33	100.70	100.12
SD	0.971	0.936	0.887	0.753	0.648	0.427
Variance	0.942	0.875	0.787	0.568	0.420	0.183
*t***	0.538 (2.306)	0.812 (2.306)	0.762 (2.306)	2.029 (2.306)	1.666 (2.306)	____
F**	5.160 (6.388)	4.790 (6.388)	4.304 (6.388)	3.107 (6.388)	2.297 (6.388)	____

*** No. of experimental, ** The values in the parenthesis are tabulated values of t and F at (p= 0.05),**

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 wave length 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). The 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.

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