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Selective determination of tolterodine tartrate in presence of its oxidative degradation product

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ABSTRACT

Four stability-indicating methods were developed for determination of tolterodine tartrate in the presence of its oxidative degradation product (the metabolite). The degradation product was prepared *via* oxidation with hydrogen peroxide. The degradation product was characterized and structurally elucidated. The first method was the first derivative ^1D by measuring the peak amplitude at 292nm. The second method was a second derivative by measuring the peak amplitude at 236, 287, 296 nm. The third method was a high performance liquid chromatographic using a reversed phase column and a mobile phase of phosphate buffer: methanol: triethyl amine (60: 40; 0.2 by volume). The forth method was a thin layer chromatography coupled with densitometric detection.

Selective quantification of tolterodine in pure form, pharmaceutical formulation and/or in the presence of its degradant was demonstrated. The indication of stability was done under condition likely to be expected at normal storage condition.

KEYWORDS

HPLC;
TLC;
Tolterodine tartrate;
Spectroscopy;
Stability.

INTRODUCTION

Tolterodine Tartrate is an anti-muscarinic drug used for the treatment of urinary incompetance. It is(R)-2-[3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methyl phenol[R-(R*,R*)]-2,3-dihydroxybutanedioate(1:1)salt;(+)-(R) -2-[α-[2-(Diisopropylamino)ethyl]benzyl-P-cresol-tartrate(1:1)(salt) its structure is shown in the following Figure 1.

There are few analytical methods for quantitative estimation of tolterodine tartrate in pharmaceutical formulation and in biological fluids. These methods include high performance liquid chromatography[2,3] and a potentiometric method[4]. Searching the literature, there is no published data concerning the stability indication of tolterodine tartrate.

In modern analytical laboratory there is always a need for significant and simple stability-indicating method of analysis. The present work aimed to develop simple spectrophotometric and chromatographic methods for the quantification of TOL in pure

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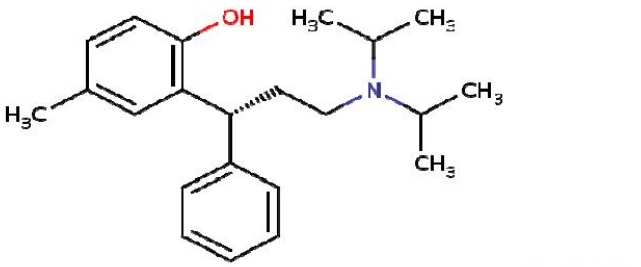

Figure 1 : Structural formula of Tolterodine tartrate

form, in pharmaceutical formulation or even in the presence of its degradant.

EXPERIMENTAL

Instruments

- Spectrophometrer Shimadzu, 1601PC dual-beam UV-Vis spectrophotometer (Japan) with matched 1-cm quartz cell, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2 nm with wavelength-scanning speed of 2800 nm min^{-1}
- pH meter Jenway Model 3510(UK), Jenway Dunmow essex CM63LB.
- A liquid chromatograph is consisted of Thermo separation products (California) liquid chromatography consisted of quaternary gradient pumping system model P4000, an ultra- violet single programmable wave length using detector model UV 1000, simple Rheodyne injector equipped with 20 µL injector loop. The separation was performed on ACE C_{18} (250 x 4.6mm, 5µm).
- TLC- Scanner was CS-9301 PC, Dual wavelength flying spot scanning densitometer, Shimadzu (Kyoto-Japan).
- Sample applicator for TLC Linomat 5 with100 µl syring (Camcge, Switzerland).
- Precoated TLC-plates, silica gel 60 F254 (20 x 20 cm, 0.25 mm), E. Merck, (Darmstadt-Germany).

Materials and reagents

All chemicals and reagents were of HPLC grade and water was bi-distilled.

Materials

Pure TOL standard powder was kindly donated by El Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt. Its potency was found to be 99.54 ± 1.24% (n=5) according to a reference HPLC method[6].

Pharmaceutical Formulation

Niltolidine Tablet®, B.N.190911, manufactured by El Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt. It was claimed to contain 2 mg TOL per tablet.

Standard solutions

TOL standard solution 1 mg mL^{-1}

It was prepared by transferring 100 mg of ToL in a 100-mL volumetric flask containing 70 mL methanol stir till complete dissolution then complete with methanol.

Drug degradant solution 1 mg mL^{-1}

Transfer 25 mg of the degradant to 25-mL volumetric flask containing 15 mL methanol stir to dissolve then complete to volume with methanol

All solution were freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 h.

Reagents

All solvents used were HPLC and spectroscopic grade.

Methanol was HPLC grade; Fisher Scientific Limited Bishop Meadow, UK.

- Hydrochloric acid and potassium dihydrogen phosphate; Adwic, El-Nasr Pharm. Co. Egypt.
- Triethyl amine was obtained from Sisco Research Laboratories PVT, LTD, India.

De-ionized water is bi-distilled from Aquatron automatic water still A4000, Bibby Sterling Ltd., UK.

Procedure

Preparation of tolterodine oxidative degradant

A mass of 50 mg TOL was transferred into 100-mL round flask and dissolving in 50-mL of water, a volume of 3 mL of 30% hydrogen peroxide was

added. The solution was heated under reflux for 7 hours (to achieve complete degradation) the obtained solution was cooled, then the solvent was evaporated using rotary evaporator and the obtained residue was characterized and structurally elucidated

Spectrophotometric method

Spectral characteristics

Serial dilutions from both intact TOL and its degradation product stock solutions were prepared and the spectra were scanned from 200-400 nm.

Linearity

Serial dilutions from both intact TOL and its degradation product standard solutions, each 1mg mL^{-1}, were prepared and the spectra were scanned from 200- 400 nm. Aliquots 5-22 mL of the drug were separately transferred from its standard stock solution (1 mg mL^{-1}) into a series of 100-mL volumetric flask the volume was completed to the mark with methanol so as to obtain a concentration in the range of 50-220 μg mL^{-1}.

First derivative spectral characteristics

The obtained spectra of different concentration were transformed to first derivative using delta λ = 4 and scaling factor of 10 for certain concentration of standard and also of the degradant. The peak amplitude was recorded at 292 nm for each concentration.

Second derivative spectrophotometric

The spectra of drug of different concentration were subjected to transformation to second derivative using " λ = 8 and scaling factor of 100 for certain concentration of standard and also of the degradant. For each concentration the peak amplitude was recorded at 236, 287, 296 nm.

Linearity

Aliquots 5-22 mL of the standard drug (1 mg mL^{-1}) were separately transferred into a series of 100- mL volumetric flask. The volume was completed to the mark with methanol so as to obtain a concentration in the range of 50-220μg mL^{-1}.

The spectra of each concentration were recorded, the peak amplitude was plotted against the corresponding concentration and the regression equations were computed. The amplitude of first derivatives peaks of TOL was measured. Calibration graph was constructed relating the peak amplitude to the corresponding concentration. The regression equation was then computed for the studied drug at the suggested wavelength and it was used for the determination of unknown samples containing TOL. For the second derivative method the previously described method was applied on the same concentrations and the second derivative was obtained and peak amplitudes were measured at 236, 287, 296 nm. Calibration graphs were constructed relating the peak amplitudes to the corresponding concentration. The regression equations were then computed for TOL at the suggested wavelengths.

Chromatography methods

High performance liquid chromatography

Chromatographic condition

Isocratic chromatographic separation was done at ambient temperature using C_{18} ACE (250 x 4.6 mm, 5 μm) analytical column. The mobile phase used was a mixture of methanol: buffer (0.02M KH_2PO_4): triethyl amine then adjusted to pH adjusted to 6. The mobile phase was filtered through 0.45 μm Millipore membrane filter and degassed for about 15 min. in an ultrasonic bath prior to use. The mobile phase was run for 30 minutes at a flow rate of 1 mL min^{-1} to reach a good equilibrium. The analysis was usually performed just after conditioning and pre washing of the stationary phase. The detection was done at 210 nm. The relative peak area ratios were plotted against the corresponding concentration giving a calibration graph and the corresponding regression equation. Concentration of unknown samples was determined using the obtained regression equation.

Linearity

Aliquots of TOL stock solution (1mg mL^{-1}) equivalent to (0.5-3 mL) were transferred to into a series of 25-ml volumetric flask and then complete to volume to the mark with solvent mixture to a final concentration of 20-120 μg mL^{-1}. Each sample was chromatographed using the previous condition. The peak area at 210 nm was recorded and plotted against

the corresponding concentration and the regression equation was computed.

Thin layer chromatography

Thin layer Chromatography has made a great progress and attained a wide acceptance as a major analytical tool for both quantitative and qualitative analysis and become a well established method for the assay of drugs in mixtures. A thin layer chromatography coupled with densitometeric detection was used for the determination of TOL in presence of its oxidative degradant. The method depends on the difference in R_f values of the drug and its degradant. The Samples were applied on TLC Linomat 5-with sample applicator which is more advantageous than spotting by uniform distribution of sample. Calibration curve was constructed represent the relation between the peak area and the corresponding concentration in the range of 3-19 μg $band^{-1}$.

Evaluation of the chromatographic condition

Mobile phase

Different developing system of different composition and proportion were tried for separation as (chloroform: methanol: acetic) and (butanol: chloroform: water), the best mobile phase was (methanol: chloroform: ammonia) in a ratio (87:13:0.9, v/v/v). The selected mobile phase allows the determination of TOL without tailing of the separated band or interference provides better precision.

Scanning wavelength

Different scanning wavelength were tried of which 210 nm proved to be the suitable wavelength for TOL and its degradation product.

Slit dimension of scanning light beam

The slit dimension should ensure good coverage of band dimensions without interference with adjacent peak. The best slit dimension was found to be (6 mm x 0.3 mm) of high sensitivity.

Analysis of laboratory prepared mixtures using the suggested methods

Aliquots of intact drug and the degraded drug were mixed to prepare different mixtures containing 10-90% (w/w) of the degradation product, and proceed as mentioned under each method. The concentration of the intact TOL was calculated from the corresponding regression equation.

Assay of pharmaceutical formulation tablets

Ten tablets were weighted to determine the average weight per tablet. They were grinded very well and a mass of 4 mg TOL was transferred into 50-mL volumetric flask containing 30 mL methanol and then placed in an ultra sonic bath for 30 min. then completed to the volume with methanol then filtered. Serial dilutions were made to prepare different solutions. The concentration was calculated from the regression equation as under each method, the mean recovery percentage with reference of the claimed amount was then obtained.

Method validation

The developed analytical methods were validated according to ICH guidelines. Comparison of the results obtained by the proposed methods and the reference ones and statistical analysis of data was done.

RESULTS AND DISCUSSION

Degradation of TOL

TOL is an active anti-muscarinic drug which can be oxidizes via metabolism or degradation. Complete degradation was achieved under oxidative condition by refluxing with 30%hydrogen peroxide for 7 hours. The main degradation product of TOL was the oxide derivative as is the Nitrogen donor of the electron while oxygen is the acceptor. The degradation product was characterized and confirmed by MS and IR spectroscopy.

Spectroscopic method

A hypochromic effect was observed during degradation of TOL. Once complete degradation occur as the characteristic peak at 280 nm was depressed.

The absorption spectra of TOL and its degradation product show sever overlap that makes the use of direct measurement of TOL in the presence of its degradant is not applicable, Figure 2.

Derivatives spectroscopy is a useful tool in

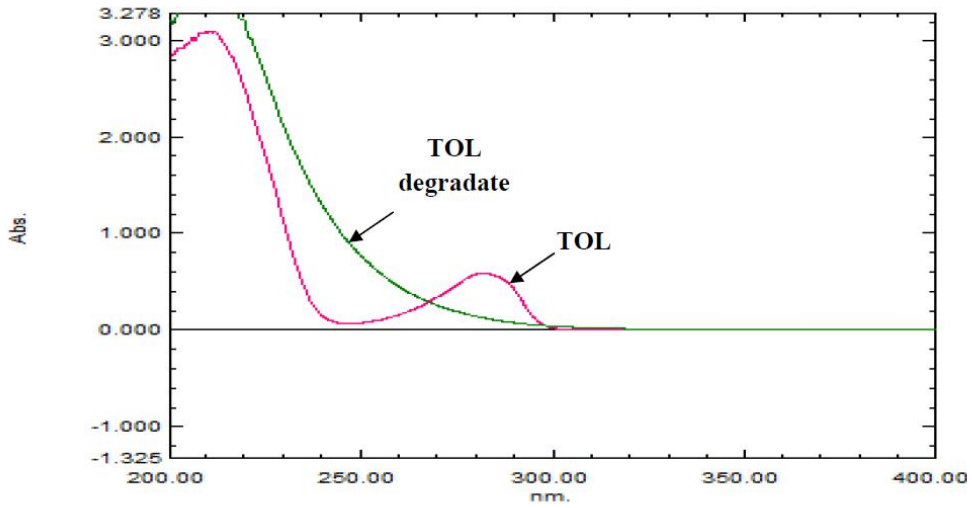

Figure 2 : Zero-order absorption spectra of intact TOL and its degradate, each, 100 µg mL^{-1}.

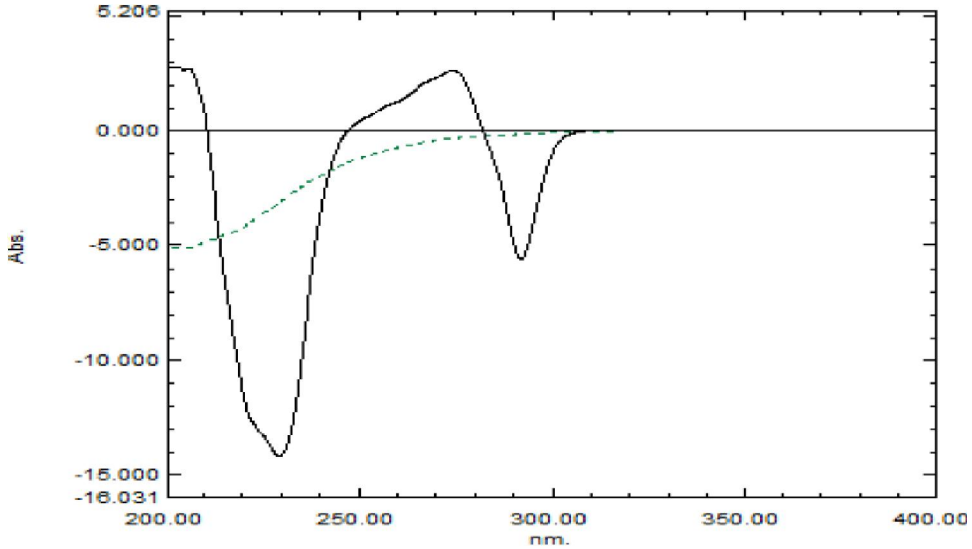

Figure 3 : The first derivative absorption spectra of intact TOL () and its oxidative degradate (…….), each 100 µg mL^{-1}

quantification of the intact TOL in the presence of its degradate. TOL could be determined by 1D at 292 nm without interference from its degradate, Figure 3.

Linear calibration graph were obtained for TOL in concentration rang 50-220 µg mL^{-1} by recording the peak amplitude and 292 nm in the first derivative method.

The regression equation was computed and found to be

Y=0.005C - 0.012, r=0.999, for at 292 nm.

For the second derivative method, TOL could be determined by at 236 nm, 287 nm and 296 nm without interference from its degradate, Figure 4. Linearity was tested at these wavelengths and the regression equations were computed and found to be

Y =0.012C + 0. 013 r=0.999 at 236 nm.

Y =0.006C - 0. 023 r=0.999 at 287 nm.

Y=0.006C + 0. 005 r=0.999 at 296 nm.

Where, Y is the peak amplitude of the spectra, C is the concentration of TOL in µg mL^{-1} and r is the correlation coefficient.

The precision of the proposed method was checked by the analysis of pure TOL samples in triplicates with good percentage recoveries.

High performance liquid chromatographic method

A simple isocratic high performance liquid chromatographic method was developed for the deter-

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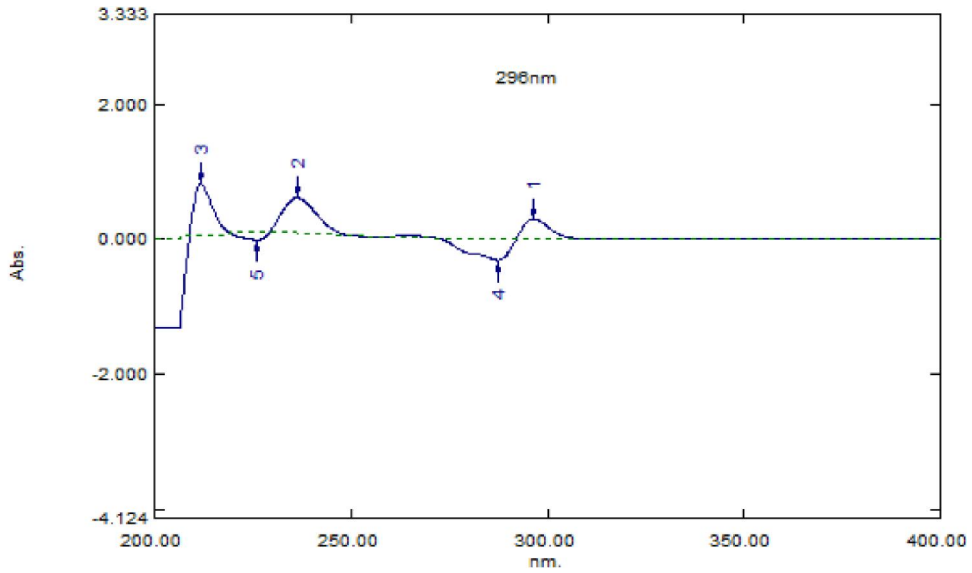

Figure 4 : The second derivative absorption spectra of intact TOL () and its oxidative degradate (…….), each 100 µg mL^{-1}

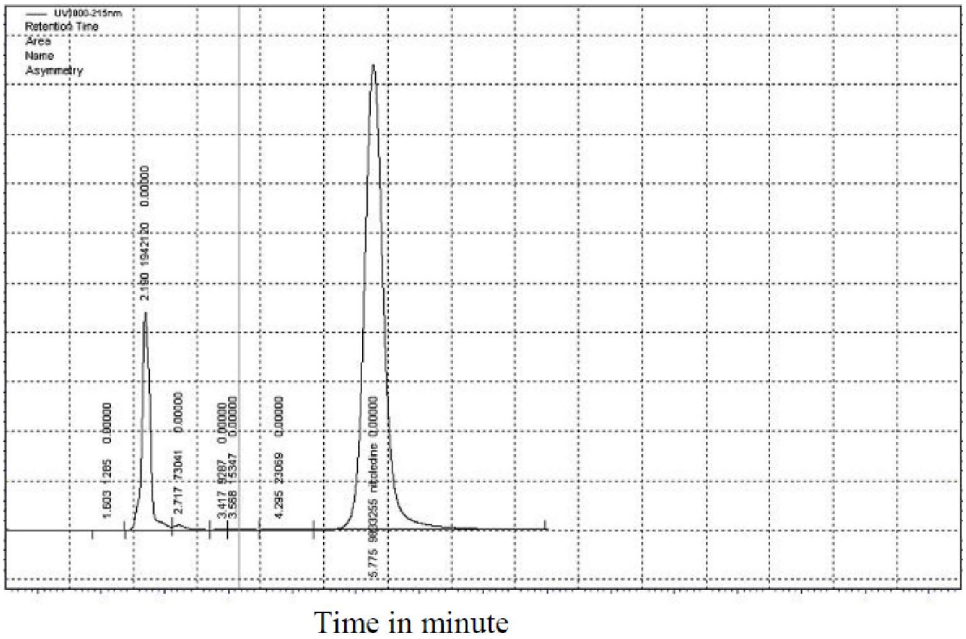

Figure 5: Liquid chromatographic separation of TOL (5.7 min) from its degradant (2.1 min), see experimental conditions

mination of TOL in pure form and in pharmaceutical preparation using ACE C_{18} (250 x 4.6mm, 5µm) analytical column. The mobile phase was chosen after several trials to reach the optimum stationary and mobile phase matching. Using the described chromatographic conditions, TOL and its degradant were well separated with average retention times of 2.1 min. and 5.7 min., for degradant and TOL, respectively, one sample can be chromatographic in 8 minutes, Figure 5.

The chromatographic system described in this work allows complete base line separation of TOL from its degradation product. Peak purity was confirmed for the HPLC peaks of both intact TOL by a pilot run using a photodiode array detector.

By plotting the peak area ratio against concentration of TOL, linearity was found to be 20-120 µg mL^{-1} using the following regression equation

Y =0.008C +0.012

Where, Y is peak area ratio, C is the concentration

of TOL and r is the correlation coefficient.

System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor and resolution. The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions such as small changes in the pH (6.0-6.2), small changes in the ratio of the mobile phase and changing the column using a C_{18} (250 x 4.6mm, 5µm) Zorbax column.

The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved.

Thin layer chromatography

Thin layer Chromatography has made a great progress and attained a wide acceptance as a major analytical tool for both quantitative and qualitative analysis and become a well established method for the assay of drugs in mixtures.

For the proposed method, densitometry scanning was done and the scanning profile is shown in Figure 6. Linear regression equation was tried, but low correlation coefficient was obtained. So, polynomial correlation was tried which gave a high correlation coefficient which insures the precision of the predicted result. Calibration curve was constructed represent the relation between the peak area and the corresponding concentration in the range of 3-19 µg band^{-1}.

The mean percentage recoveries and relative standard deviation was calculated and found to be 100.24±1.057 as shown in the table. Linear correlation coefficient was obtained. The regression equation was computed and found to be

Y=1.095 C + 7.531

Analysis of pharmaceutical formulation

The suggested methods were successfully applied for the determination of TOL in its pharmaceutical formulation, showing good percentage recoveries, TABLE 1.

The validity of the suggested methods was further assessed by applying the standard addition technique, and the precision was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis results.

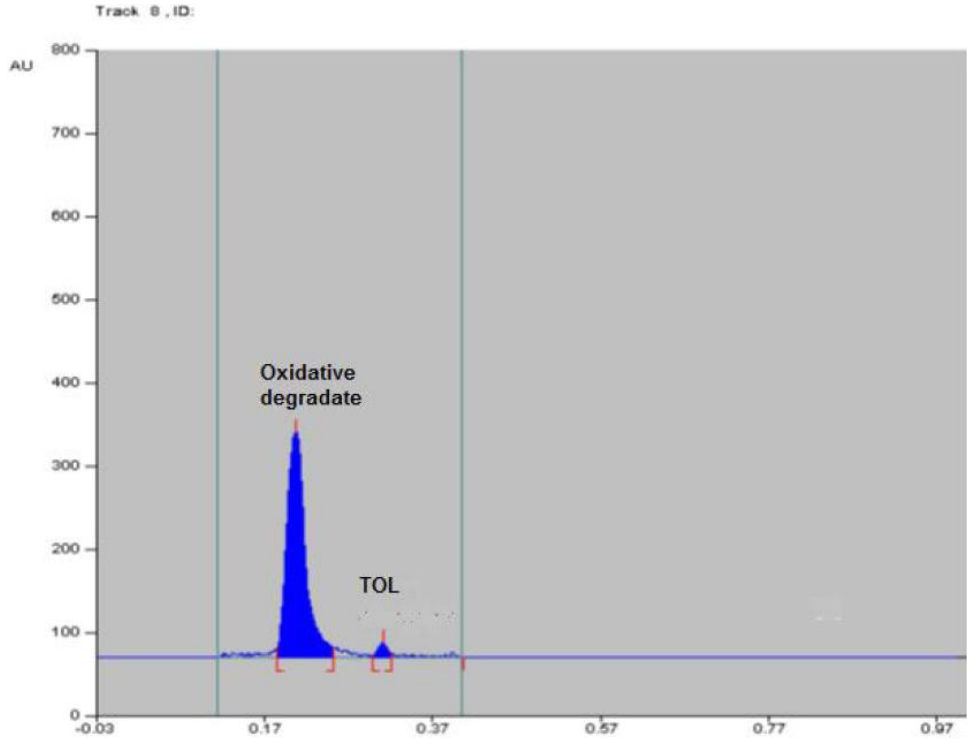

Figure 6 : Two dimensional thin layer chromatogram showing separation of TOL from its degradate

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TABLE 1 : Quantitative determination of TOL in Niltolidine(R) tablet by the proposed methods

Pharmaceutical Formulation Niltolidine tablet BN 190911	Recovery %
^{1}D method at 292 nm	98.62 ± 1.455
^{2}D method at 236nm at 287nm at 296nm	 99.90±0.552 99.66±0.687 99.55±0.502
HPLC method	99.68±0.469
TLC method	100.10±0.390

The selectivity of the proposed methods was examined by analysis of different mixtures of the intact and degraded TOL. The prepared mixtures were analyzed by the proposed methods and good recoveries were obtained.

Results of the suggested methods for determination of TOL were statistically compared with those obtained by applying the reference method[6]. The calculated t- and F values were less than the tabulated ones[5].

CONCLUSION

The suggested methods of analysis are found to be simple, accurate, selective, cheap and equally sensitive with no significant difference of the precision compared with the reference method of analysis but being also stability indicating can detect degradation impurities in the intact molecule. Application of the proposed methods for the analysis of TOL in its pharmaceutical formulation shows that neither the excipient nor the degradation product interferes with its determination.

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