SPECTROPHOTOMETRIC METHODS FOR DETEVELOPMENT OF TOLTRAZURIL IN PRESENCE OF ITS ALKALI-INDUCED DEGRADATION PRODUCT

Amr M. Abdelfatah*1, Ahmed W. Madkour2, Ahmed A Almrasy2, and Khalid A.M. Attia2

1Department of Pharmaceutical Analytical Chemistry Faculty of Pharmacy, Badr University in Cairo

2Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.

*Corresponding author: E-mail: Amr.Mohamed93@buc.edu.eg

ABSTRACT

Toltrazuril is a veterinary antiprotozoal drug. In this work, four different simple, accurate, and sensitive spectrophotometric methods have been developed to determine toltrazuril in the presence of its alkali-induced degradation product. Two of these methods depend on selection of two wavelengths at zero order spectrum these methods include (A) dual wavelength method at 239.60 and 257.40 nm, (B) simultaneous equation method at 242.4 nm (λmax of toltrazuril) and 249.8 nm (λmax of toltrazuril degradation product), while the another two methods are (C) area under the curve method at (235-245) nm and (247-257) nm of toltrazuril and its degradation product and (D) ratio derivative method the absorption spectra of toltrazuril were divided by a suitable absorption spectrum of its degradation product (divisor) and the amplitudes of the first derivative of the ratio spectra at 219 nm were recorded. These methods have been validated for linearity, accuracy and precision and found to be rapid, precise to applied for determination of toltrazuril in Yoserzoril® suspension.

Keywords: Toltrazuril; Dual wavelength method; Simultaneous equation method; Ratio derivative method.
Introduction

Toltrazuril is a triazinetrione derivative that chemically known as: \[\text{1-methyl-3-[3-methyl-4-(trifluoromethylsulfanyl)phenoxy]phenyl}-1,3,5-triazinane-2,4,6-trione.\]

Its chemical structure is shown in figure 1. The molecular weight of toltrazuril is 425.38 gmol\(^{-1}\), toltrazuril is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), the solubility of toltrazuril in ethanol is approximately 1 mgml\(^{-1}\) and approximately 25 mgml\(^{-1}\) in DMSO and DMF.

It is an antiprotozoal drug that is used in veterinary medicine as an anticoccidial agent (Anadón and Martínez-Larrañaga 2014).

Toltrazuril is also effective against all intestinal stages of susceptible coccidia to help in the development of natural immunity to coccidiosis in layer replacer stock and breeder that are exposed to the subsequent challenge of coccidia virulent strains (Foster 2016).

Many assay methods were reported for determination of toltrazuril and its metabolites including Surface-Enhanced Raman Spectroscopy (SERS) using silver nanoparticles (AgNPs) as substrate (Shao et al. 2020), solid phase extraction- ultra high performance liquid chromatography with UV detection (SPE–UPLC–UV) (Zhao Ling et al. 2014), Fast liquid chromatography/tandem mass spectrometry (Martínez-Villalba et al. 2010), reversed-phase sequential injection chromatography (SIC) (Björklund et al. 2011), Simultaneous determination of toltrazuril and its metabolites in chicken and pig skin + fat by UPLC-UV method (Zheng et al. 2014), determination of toltrazuril and its two metabolites in surface water, soil and animal manure using LC/MS (Olsen et al. 2012), determination of diclazuril, toltrazuril and its two metabolites by gel permeation chromatography–liquid chromatography–tandem mass spectrometry (Ai et al. 2011), HPLC method for determination of toltrazuril with diclazuril (Jeong et al. 2017), and determination of toltrazuril and its residual solvents using HPLC and GC (Bawazeer 2020).

To the best of our knowledge, there are no previous analytical methods found on literature for determination of toltrazuril in the presence of its complete degradation product.

This work was carried out to develop and validate simple, selective, and sensitive spectrophotometric methods for the determination of toltrazuril concurrently with its alkali-induced degradation product. All selected methods may be used successfully for ordinary analysis in laboratories and proved to be simple and rapid.
Figure 1. Structural formula of toltrazuril.

**Experimental**

**Instruments**

Shimadzu®UV-Vis. 1800 Spectrophotometer, (Japan) equipped with 10 mm matched quartz cells, UV Probe 2.43 software. Hot plate (Medline MS300 Hot Plate Stirrer. UK). pH meter (Jenway®, 3510, USA). Sonicator (FALC®, Italy).

**Materials**

**Pure standard**

Pure toltrazuril, certified to contain 99.3%, was kindly supplied by Arabco-Med® for Pharmaceutical Industries, Obour City - Industrial area Cairo.

**Pharmaceutical preparation**

Yoserzoril® suspension, the product of Waki Pharma® for Pharmaceutical Industries, 10th Of Ramadan City, Cairo, Egypt. (Batch No.0030319), which contains 50 mg/ml of toltrazuril.

**Reagents and solvents**

Hydrochloric acid, (Piochem® Co., Egypt), prepared 1M aqueous solution. Sodium hydroxide, (Topchem® Co., Egypt), prepared as 1M aqueous solution. Absolute ethanol 99.90% (Piochem Co., Egypt).

**Preparation of alkaline degraded sample**

100 mg of toltrazuril was dissolved in 20 ml of absolute ethanol and sonicated for 5 minutes and finally 50 ml of 1 M NaOH solution was added. The flask was permitted to be heated until boiling under reflux for 4hrs, then the solution was neutralized with 1M HCl. After that, filtration of the solution was carried out and the degradation product was obtained on the filter paper and allow to dry of the filter paper.
Standard solutions

(a) **Standard stock solution of toltrazuril (1mg/ml)** was prepared by dissolving 100 mg of toltrazuril in 20 ml absolute ethanol in volumetric flask 100ml and complete the volume to the mark by absolute ethanol.

(b) **Working solution of toltrazuril (100 μg/ml)**: 10 ml of toltrazuril accurately taken from its standard stock solution and added into 100 ml volumetric flask, finally the volume was completed with absolute ethanol.

(c) **Stock solution of toltrazuril degradation product (1mg/ml)** was prepared by dissolving 50 mg of 1-methyl-3-(3-methyl-4-(4-((trifluoromethyl)thio)phenoxy)phenyl)urea. in 20 ml absolute ethanol and complete to 50 ml with absolute ethanol.

(d) **Working solution of toltrazuril degradation product (100 μg/ml)**: 10 ml of toltrazuril degradation product accurately taken from its stock solution and added into 100 ml volumetric flask, then the volume was completed with absolute ethanol.

Procedure

**Construction of calibration curves**

Different aliquots equivalent to (2 – 24 μg) of toltrazuril standard and different aliquots equivalent to (2-14μg) of its degradation product were transferred from their standard working solutions (100 μg/ml) into two separate series of 10-ml volumetric flasks and completed to volume with absolute ethanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using absolute ethanol as a blank and stored in computer.

**Method A: Dual wavelength method**

Wavelengths of 239.60 and 257.40 nm were selected to measure the difference in the absorbance between them at the zero order absorption spectra of toltrazuril, the calibration curve was constructed by plotting the differences in absorbance versus the drug concentrations in (μg/ml) and the straight line equation was obtained.

**Method B: Simultaneous equation method**

The absorbance values of toltrazuril and its degradation product at wavelength of 242.4 nm (λmax of toltrazuril) and 249.8 nm (λmax of toltrazuril degradation product) were obtained, the calibration curves were constructed by plotting the measured absorbance values versus the concentrations in μg/ml and the straight line equations were obtained. The concentration of toltrazuril in laboratory prepared mixtures and pharmaceutical
formulation was determined by substituting the absorbance and absorptivity coefficient in the following equation:

\[ C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \]

Where: \( C_x \) is the concentration of toltrazuril, \( A_1 \) and \( A_2 \) are absorbance of sample at 242.4 nm and 249.8 nm respectively, \( a_{x1} \) and \( a_{x2} \) are absorptivity of toltrazuril at 242.4 nm and 249.8 nm respectively, \( a_{y1} \) and \( a_{y2} \) are absorptivity of its degradation product at 242.4 nm and 249.8 nm respectively.

**Method C: Area under the curves method**

Area under the curves obtained from the scanned spectra over the ranges of wavelengths (235 - 245) nm and (247 - 257) nm were recorded and then the corresponding regression equations were computed form the calibration curve by plotting the area under the curve versus the drug concentrations in µgml\(^{-1}\). By applying “Cramer’s Rule” and “Matrix Method”, the concentration of toltrazuril and degradation product can be determined as follows:

\[ C_x = \frac{(a_{x1} - a_{x2}) AUC_{x3-44} - (a_{y3} - a_{y4}) AUC_{y1-22}}{(a_{x1} - a_{x2}) a_{x3-44} - (a_{y3} - a_{y4}) a_{y1-22}} \]

\[ C_y = \frac{(a_{x1} - a_{x2}) AUC_{y3-44} - (a_{y3} - a_{y4}) AUC_{y1-22}}{(a_{x1} - a_{x2}) a_{y3-44} - (a_{y3} - a_{y4}) a_{y1-22}} \]

- \( AUC_{x1-22} \): area under curve for toltrazuril \( X \) at the wavelength range (235 - 245) nm.
- \( AUC_{x3-44} \): area under curve for toltrazuril \( X \) at the wavelength range (247 - 257) nm.
- \( AUC_{y1-22} \): area under curve for degradation product at the wavelength range (235 - 245) nm.
- \( AUC_{y3-44} \): area under curve for degradation product at the wavelength range (247 - 257) nm.

**Method D: Ratio derivative method**

Toltrazuril concentrations were divided by a suitable absorption spectrum of its degradation product divisor (14 µgml\(^{-1}\)) and the amplitudes of the first derivative of the ratio spectra at 219 nm are proportional to the concentrations of the drug without interference from its degradation product (divisor).
Application to laboratory prepared mixtures

Aliquots of toltrazuril and its degradation product were mixed to prepare different mixtures containing different ratios of both. The procedures mentioned under construction of calibration curves were followed for each method and the concentrations of toltrazuril were calculated.

Application to pharmaceutical formulation

1ml of Yoserzoril® suspension (50 µgml⁻¹ toltrazuril) was accurately taken and dissolved in 20ml absolute ethanol in volumetric flask 50 ml, finally the volume was completed with absolute ethanol, sonicated for 30 minutes, and then filtered. 5ml of the filtrate were accurately transferred into 50ml volumetric flask and volume was completed with absolute ethanol to obtain a solution labeled to contain (100 µgml⁻¹) of toltrazuril. The solution was analyzed using the procedure described previously.

Results and discussion

Degradation of toltrazuril

It is found that complete alkaline degradation of toltrazuril was obtained after refluxing the drug with 1M sodium hydroxide at 100 °C for 4 hrs., where a suggested degradation pathway is shown as follows:

![Scheme (1): Suggested degradation pathway of toltrazuril.](image)

Identification of the degradation product:

Degradation product elucidation was established using different spectral methods. The FT-IR spectrum showed the appearance of two peaks at 3379 cm⁻¹ and 3325 cm⁻¹ as shown in Figure 3, instead of a sharp peak at 3295 cm⁻¹ in the spectrum of toltrazuril as shown in Figure 2. This can be assigned to the two secondary amides in the degradation
product after the hydrolysis of the triazinane ring. The disappearance of the peak at 1720 cm\(^{-1}\) of the carbonyl group also took place.

The H\(^1\)NMR presented a duplet at 2.88 ppm, corresponding to the N-methyl protons within the degradation product as shown in Figure 5, instead of a singlet that appeared at 3.42 ppm as shown in Figure 4, indicating the presence of an adjacent proton.

Mass spectroscopy showed that the compound has a molar mass of 356.36 indicating the presence of the degradation product as shown in Figure 7.

In conclusion, all the above evidences indicate that the degradation product could be 1-methyl-3-(3-methyl-4-(4-((trifluoromethyl)thio)phenoxy)phenyl)urea. As shown in (Scheme 1)

![IR spectrum of intact tolrazuril](image1)

**Figure 2. IR spectrum of intact tolrazuril**
Figure 3. IR spectrum of toltrazuril degradation product.

Figure 4. H\textsuperscript{1}NMR spectrum of toltrazuril.
Figure 5. $^1$H NMR spectrum of toltrazuril degradation product

![H$^1$NMR spectrum of toltrazuril degradation product]

Figure 6. Mass spectrum of toltrazuril.

![Mass spectrum of toltrazuril](image)

Figure 7. Mass spectrum of toltrazuril degradation product.

Spectral Characteristics

The zero-order absorption spectra of toltrazuril and its alkali-induced degradation product show overlap as shown in Figure 8. This overlap does not permit direct
determination of toltrazuril in the presence of its degradation product. To overcome this problem, different spectrophotometric methods were developed and validated to allow the determination of toltrazuril in the presence of its alkali-induced degradation product without previous separation.

![Absorption spectra of toltrazuril (20 µg ml⁻¹) and its degradation product (14 µg ml⁻¹).](image)

**Figure 8.** Absorption spectra of toltrazuril (20 µg ml⁻¹) and its degradation product (14 µg ml⁻¹).

**Method 1: Dual wavelength method.** (Chaudhari 2012; Patel and Maheshwari 2014; Sanket and Satish 2011; Fernandes et al. 2008; Oza et al. 2012)

In this method, the interference from degradation product can be removed by measuring the difference in absorbance between 239.60 and 257.40 nm; this difference is zero for degradation product, while it is directly proportional to the concentration of intact toltrazuril.

**Method 2: Area under the curve method.** (Chauhan and Patel 2012; Alexandar et al. 2017)

Area under curve method utilizes two wavelength ranges (235 - 245) and (247 - 257) nm. From the overlain spectra of toltrazuril the area under curve is determined at both the selected analytical wavelength ranges.

Within the above selected wavelength ranges, the area under curve was determined for the toltrazuril and analysis was performed using —Cramer’s Rule’’ and —Matrix Method.
The area under the curves for toltrazuril and its degradation product were recorded over the ranges of (235-245) and (247-257) nm. The calibration graphs that relate the measured areas under the curve to the concentration of each component in μg/ml were constructed and the regression equations were computed. The absorptivity values and areas under the curve for each component at the selected wavelength ranges were used to calculate the concentration of toltrazuril by the following equations:

\[
C_X = \left( \frac{A_{X1-2} - A_{X3-4} A_{X1-2} A_{X3-4}}{a_{X1-2} a_{X3-4}} \right) - \left( \frac{A_{X1-2} A_{X3-4} - A_{X1-2} a_{X3-4}}{a_{X1-2} a_{X3-4}} \right)
\]

\[
C_Y = \left( \frac{A_{Y1-2} A_{Y3-4} - A_{Y1-2} A_{Y3-4}}{a_{Y1-2} a_{Y3-4}} \right) - \left( \frac{A_{Y1-2} A_{Y3-4} - A_{Y1-2} a_{Y3-4}}{a_{Y1-2} a_{Y3-4}} \right)
\]


In this method, absorbance values were measured at 242.4 nm (λ max of toltrazuril) and 249.8 nm (λ max of toltrazuril degradation product). We have four calibration graphs that give two slopes for toltrazuril at both wavelengths 242.4 nm and 249.8 nm, and also two slopes for its degradation product at both wavelengths 242.4 nm and 249.8 nm were determined by dividing each absorbance over each corresponding concentration. The concentration of toltrazuril in laboratory prepared mixtures and pharmaceutical formulation was determined by substituting the absorbance and absorptivity coefficient in the following equation:

\[
C_X = \left( \frac{a_{Y1} - a_{Y2}}{A_2 a_{Y1} a_{Y2}} \right)
\]

Where: \( C_X \) is the concentration of Toltrazuril, \( A_1 \) and \( A_2 \) are absorbance of sample at 242.4 nm and 249.8 nm respectively, \( a_{X1} \) and \( a_{X2} \) are absorptivity of toltrazuril at 242.4 nm and 249.8 nm respectively, \( a_{Y1} \) and \( a_{Y2} \) are absorptivity of its degradation product at 242.4 nm and 249.8 nm respectively.

Method 4: Ratio derivative method. (Gülfen, Canbaz, and Özdemir 2020; Thakur and Daharwal 2016; Dinç, Yücesoy, and Onur 2002; Kul et al. 2010; AL-GHANI and AM THABET 2021)

Absorption spectra of Toltrazuril were divided by a suitable absorption spectrum of its degradation product divisor (14 μg/ml) and the amplitudes of the first derivative of the ratio spectra at 219 nm were recorded as shown in Figures (9–11).
Figure 9. Absorption spectra of toltrazuril at different concentrations (4, 8, 12, 16, 20 and 22 µg/ml⁻¹).

Figure 10. The ratio spectra of toltrazuril at different concentrations (4, 8, 12, 16, 20 and 22 µg/ml⁻¹).
Figure 11. The first derivative of the ratio spectra of toltrazuril at different concentrations (4, 8, 12, 16, 20 and 22 µg/ml).

Methods validation:

Validations of the proposed methods were assessed as per the ICH guidelines (Guy 2014). The linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity, intra-day and inter-day, precision, and repeatability of measurement.

Linearity:

Determined by building Calibration curves were constructed by analysis of different concentrations of toltrazuril. The calibration curves were generated by plotting the drug concentrations against corresponding response (absorbance). The linearity plot was constructed, and the data were treated using linear regression analysis.

- Limits of detection and quantitation:

The sensitivity of measurement of toltrazuril by use of the proposed method was assessed in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). LOQ and LOD were calculated according to ICH guidelines from the following equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantitation (LOQ) values were mentioned in Table 1.
• **Accuracy:**
  Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at different concentrations of toltrazuril within their linearity range and the concentrations were calculated each from their corresponding regression equations. The accuracy of the proposed methods was calculated and RSD% was obtained. The accepted limits of recovery are 98%-102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the methods developed. Good results were obtained as shown in Table 1.

• **Precision:**
  Precision was estimated by calculating repeatability (intra-day precision), and intermediate precision (inter day precision), precision after repeating measuring of the three different concentrations three times in the same day and assessing the sample in triplicate on three successive days using the proposed methods. The calculated RSD% values were listed as shown in Table 1., indicating satisfying precision of the proposed methods.

**Table1. Assay validation sheet of the proposed methods.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dual wavelength</th>
<th>AUC</th>
<th>Simultaneous equation</th>
<th>Ratio derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (mean ± RSD)%</td>
<td>99.51±0.853</td>
<td>99.83 ±0.632</td>
<td>99.32 ±0.704</td>
<td>99.95 ± 0.806</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability (RSD) b</td>
<td>0.553</td>
<td>0.986</td>
<td>0.768</td>
<td>0.697</td>
</tr>
<tr>
<td>Intermediate precision (RSD) c</td>
<td>0.740</td>
<td>0.989</td>
<td>0.794</td>
<td>0.554</td>
</tr>
<tr>
<td>Wavelength</td>
<td>239.60&amp;257.40 nm</td>
<td>(235-245) nm</td>
<td>(247-257)nm</td>
<td>242.4nm</td>
</tr>
<tr>
<td>Linearity range</td>
<td>(4-24µg/ml)</td>
<td>(4-24µg/ml)</td>
<td>(4-24µg/ml)</td>
<td>(4-22µg/ml)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0251</td>
<td>0.4349</td>
<td>0.3011</td>
<td>0.045</td>
</tr>
<tr>
<td>Intercept</td>
<td>+ 0.0036</td>
<td>+ 0.119</td>
<td>+ 0.1407</td>
<td>+ 0.0161</td>
</tr>
<tr>
<td>coefficient of determination (r²)</td>
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<td>0.9996</td>
<td>0.9996</td>
<td>0.9997</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>1.063</td>
<td>1.260</td>
<td>1.254</td>
<td>1.280</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>3.221</td>
<td>3.821</td>
<td>3.801</td>
<td>3.879</td>
</tr>
</tbody>
</table>

• **Application of the proposed methods for assaying of Yoserzoril® suspension.**

The proposed spectrophotometric methods were successfully applied for the determination of toltrazuril in Yoserzoril® suspension. Toltrazuril was selectively and quantified determination without interference from the excipients. Moreover, a standard addition technique was applied to check the validity of the proposed methods. The satisfying values obtained, confirmed no interference from the excipients and additives. The results obtained were shown in Table 3.

Good results were obtained as illustrated in Table 1. Table 2 shows the specificity; recovery percentages of the laboratory prepared mixture of the drug with its alkali-induced...
degradation product. The validity of the proposed procedures is further assessed by applying the standard addition technique showing no interference from excipients. The results obtained were shown in Table 3.

a Average of three determinations for three concentrations (4, 10 and 18 µgml⁻¹) for dual wavelength, (6, 14 and 22 µgml⁻¹) for AUC, (6, 10 and 14 µgml⁻¹) for simultaneous equation and (12, 16 and 20 µgml⁻¹) for ratio derivative method, for toltrazuril repeated three times.

b The intraday (n=3), average of three concentrations (4, 10 and 18 µgml⁻¹) for dual wavelength, (6, 14 and 22 µgml⁻¹) for AUC, (6, 10 and 14 µgml⁻¹) for simultaneous equation and (12, 16 and 20 µgml⁻¹) for ratio derivative method, for toltrazuril repeated three times within the day.

c The interday (n=3), average of three concentrations (4, 10 and 18 µgml⁻¹) for dual wavelength, (6, 14 and 22 µgml⁻¹) for AUC, (6, 10 and 14 µgml⁻¹) for simultaneous equation and (12, 16 and 20 µgml⁻¹) for ratio derivative method, for toltrazuril repeated three times in three days.

Table 2: Determination of toltrazuril in presence of its alkali-degradation product in laboratory prepared mixtures by the proposed methods:

<table>
<thead>
<tr>
<th>Method</th>
<th>Intact in (µgml⁻¹)</th>
<th>Degradate in (µgml⁻¹)</th>
<th>Percent of degrade</th>
<th>Intact found in (µgml⁻¹)</th>
<th>Recovery % of intact</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dual wavelength</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>17</td>
<td>19.86</td>
<td>99.28</td>
<td></td>
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<tr>
<td>18</td>
<td>6</td>
<td>25</td>
<td>17.71</td>
<td>98.36</td>
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<tr>
<td>16</td>
<td>8</td>
<td>33</td>
<td>15.71</td>
<td>98.21</td>
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<tr>
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<tr>
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<td>99.53</td>
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<td>Mean % ± SD</td>
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<td>99.83 ± 0.631</td>
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<td>Mean % ± SD</td>
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<td>99.32 ± 0.699</td>
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<td><strong>Ratio derivative</strong></td>
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<td>42</td>
<td>13.95</td>
<td>99.68</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>58</td>
<td>9.86</td>
<td>98.64</td>
<td></td>
</tr>
<tr>
<td>Mean % ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.29 ± 0.708</td>
</tr>
</tbody>
</table>
Table 3: Application of standard addition technique for the analysis of toltrazuril in Yoserzoril® suspension by applying the proposed methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pharmaceutical Taken (µgml(^{-1}))</th>
<th>Pharmaceutical found (µgml(^{-1}))</th>
<th>Pure added (µgml(^{-1}))</th>
<th>Pure found (µgml(^{-1}))</th>
<th>Recovery %</th>
<th>Mean% ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual wavelength</td>
<td>8</td>
<td>7.95</td>
<td>6</td>
<td>5.93</td>
<td>98.84</td>
<td>98.94 ± 0.233</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>10</td>
<td>9.87</td>
<td>98.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>12</td>
<td>11.87</td>
<td>98.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>14</td>
<td>13.90</td>
<td>99.28</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>8</td>
<td>7.95</td>
<td>8</td>
<td>7.92</td>
<td>99.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>10</td>
<td>9.81</td>
<td>98.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>12</td>
<td>11.87</td>
<td>98.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>14</td>
<td>13.90</td>
<td>99.29</td>
<td></td>
</tr>
<tr>
<td>Simultaneous equation</td>
<td>8</td>
<td>7.95</td>
<td>6</td>
<td>5.99</td>
<td>99.76</td>
<td>99.62 ± 0.674</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>10</td>
<td>9.99</td>
<td>99.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>12</td>
<td>11.84</td>
<td>98.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>14</td>
<td>14.03</td>
<td>100.18</td>
<td></td>
</tr>
<tr>
<td>Ratio derivative</td>
<td>8</td>
<td>7.95</td>
<td>6</td>
<td>6.00</td>
<td>100.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>10</td>
<td>9.87</td>
<td>98.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>12</td>
<td>11.91</td>
<td>99.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.95</td>
<td>14</td>
<td>13.96</td>
<td>99.71</td>
<td>99.44 ± 0.599</td>
</tr>
</tbody>
</table>
Table 4: Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of toltrazuril in yoserzoril® suspension:

<table>
<thead>
<tr>
<th></th>
<th>Dual wavelength</th>
<th>AUC</th>
<th>Simultaneous equation</th>
<th>Ratio derivative</th>
<th>Reported method (Lù 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N^*)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>(\bar{X}^*)</td>
<td>99.51</td>
<td>99.83</td>
<td>99.32</td>
<td>99.95</td>
<td>100.31</td>
</tr>
<tr>
<td>(SD)</td>
<td>0.849</td>
<td>0.631</td>
<td>0.699</td>
<td>0.805</td>
<td>1.503</td>
</tr>
<tr>
<td>(RSD^%)</td>
<td>0.853</td>
<td>0.632</td>
<td>0.704</td>
<td>0.806</td>
<td>1.498</td>
</tr>
<tr>
<td>(t^{**})</td>
<td>1.037 (2.306)</td>
<td>0.504 (2.306)</td>
<td>1.389 (2.306)</td>
<td>0.468 (2.306)</td>
<td>——</td>
</tr>
<tr>
<td>(F^{**})</td>
<td>3.130 (6.388)</td>
<td>5.879 (6.388)</td>
<td>2.428 (6.388)</td>
<td>3.481 (6.388)</td>
<td>——</td>
</tr>
</tbody>
</table>

* No. of experimental.

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

Conclusion

In this research simple, rapid, accurate, reproducible, precise and sensitive methods namely, dual wavelength, area under curve , simultaneous equation and ratio derivative were described and applied for quantitative determination of toltrazuril in pure form or in the presence of its alkali-induced degradation product without any preliminary separation step. The proposed methods do not need any sophisticated apparatus or a special program and could be easily applied in quality control laboratories. Moreover, the proposed methods were successfully applied to Yoserzoril® suspension and no interference from pharmaceutical formulation excipients was found.

REFERENCES:


طرق التحليل الطيفي لتعين التولترازوريل في وجود ناتج التكسير القلوى له

عمرو محمد عبدالفتاح*، أحمد ومية عبدالمسعود مبارك، أحمد على إبراهيم المراسي، خالد عبد السلام محمود

قسم الكيمياء التحليلية الصيدلية، جامعة بدر بالقاهرة، القاهرة - مصر

قسم الكيمياء التحليلية الصيدلية، جامعة الأزهر، القاهرة - مصر

البريد الإلكتروني للباحث الرئيسي: Amr.Mohamed93@buc.edu.eg

تولترازوريل هو دواء يستخدم في الطب البيطري، ويمكن تحليل هذا الدواء المضاد للبروتوزوال بأربع طرق مختلفة بسيطة ودقيقة ومحسسة لتحديد التولترازوريل في وجود ناتج التكسير القلوى له. طريقتان تعتمدان على اختيار طولين موجيين في طيف ترتيب صمّر، أو طريقة باستخدام الطول الموجي المذكور في 235.6، 257.4، و 242 ن.م. والطويل الموجي للاختيار للتحمل القلوى للفارس و 249.8، 258.2، بينما الطريقتين الأخريتين تحددان حسب أي المنطقتين تحت قياس المنحنى عند 235-245 ن.م. وقياس المنحنى عند 247-252 ن.م. للتلزوريل وللحمية للتحمل القلوى، وطريقة مشتقة النسبة، والتي يتم تحديدها بواسطة أطيف الامتصاص في التلزوريل واختيار طريقة اتصال مناسب من نوع التكسير (المقصوم عليه)، ثم تسجيل سعة المشتقة الأولى من أطيف النسبة عند 219 ن.م.

هذا وقد تم تطبيق هذه الطرق في تحليل مستحضر البزرزوريل وتبين أنها سريعة ودقيقة ودقيقة لتطبيقها في تحديد التلزوريل في المستحضرات الدوائية

الكلمات المفتاحية: تلزوريل؛ طريقة الطول الموجي المذكور؛ أسلوب المعادلة المتزامنة؛ طريقة مشتقة النسبة.