A simple kinetic spectrophotometric method for determination of Tizanidine hydrochloride in pharmaceutical preparations

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1. Introduction

Tizanidine hydrochloride (TIZ) is 5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine hydrochloride. It is a muscle relaxant and alpha2 adrenergic agonist.1

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It is used to treat the spasms,2,3 cramping, and tightness of muscles caused by medical problems, such as multiple sclerosis, spastic diplegia, back pain, or certain other injuries to the spine or central nervous system.
Several methods have been applied for the determination of TIZ in dosage forms and in biological fluids. The different techniques published include high-performance liquid chromatography (HPLC), TLC, GC and electrochemical.

In this paper, a study of the determination of TIZ in pure form and in different pharmaceutical preparations available in the Egyptian market has been undertaken. The proposed method is based on the alkaline oxidation of TIZ with potassium permanganate at a fixed time of 25 min.

Different factors affecting these reactions have been studied. The proposed method has the advantage of being rapid, simple, accurate, economic, and sensitive.

### 2. Experimental

#### 2.1. Apparatus

A JASCO 530 V spectrophotometer that was equipped with a 10 mm quartz cell and had a kinetic accessory equipped with a temperature-controlled cell was used for all spectrophotometric measurements.

Comparative HPLC determinations have been carried out by the aid of an Agilent HPLC system (Model 1200) with auto sampler under the conditions of the standard procedure cited in Section 2.3.3.

#### 2.2. Materials and reagents

The following materials and reagents were used:

- Potassium permanganate (Merck, Germany); 1 × 10⁻² M aq. solution, freshly prepared.
- Sodium hydroxide (BDH, UK): 0.5 M aq. solution was purchased from Novartis pharma (Sawah St-Amiria-Cairo, Egypt).
- Sirdalud (4 mg), Roysan (4 mg), Tizyl (4 mg), Rekan (4 mg) and S.M.R (2 mg) tablets containing TIZ were obtained from commercial sources in the local market.
- Standard solutions: A stock solution was prepared by dissolving 20.0 mg of TIZ in 100 ml of bidistilled water and further diluted with the same solvent as appropriate.

#### 2.3. Procedures

##### 2.3.1. Recommended general procedure

Aliquots of 1.0 ml KMnO₄ solution (1 × 10⁻² M) and 1.0 ml of 0.5 M NaOH solution were placed in 10 ml volumetric flasks. Accurate volumes of the working solution over the concentration range of 2–13 μg ml⁻¹ were added, and the solutions were mixed well and diluted to volume with bidistilled water. At a fixed time of 25 min, the absorbance was measured directly at 610 nm against a reagent blank. The calibration graph was constructed by plotting the final concentration of the drug against the absorbance values, measured at a fixed time of 25 min., alternatively; the corresponding regression equation was derived.

##### 2.3.2. Procedure for the tablets

Twenty tablets were weighed and pulverized. Weighed quantity of the powder equivalent to 20 mg of TIZ was transferred into a small conical flask containing 3 x 30 ml of distilled water. The resulting extract was filtered into a 100 ml volumetric flask. The conical flask was then washed with a few milliliters of bidistilled water.
tilled water, and the washings were poured into the same volumetric flask and complete to the mark with the same solvent. Aliquots covering the working concentration range were transferred into 10 ml volumetric flasks.

The recommended procedure was then followed as described in Section 2.3.1. The nominal content of the tablets was determined by using either the calibration curve or the corresponding regression equation.

2.3.3. Procedure for reference method

Equal volumes (about 20 μL) of the standard preparation and the assay preparation were separately injected into the HPLC apparatus comprising a 230 nm detector and 4.6 mm × 15 cm column that contained packing L7; the flow rate is about 1.0 ml per minute and the column temperature is maintained at 35 °C. The relative retention times are about 0.5 for TIZ. The chromatograms were recorded, and the responses were measured for the major peaks by calculating the quantity in mg by the formula 500 (c ra/rs), where c is the concentration in mg per mL of TIZ ra/rs peak areas obtained from the assay preparation and standard preparation, respectively.

3. Results and discussion

3.1. Optimization of the parameters

TIZ was found to react with KMnO4 in alkaline medium, producing a bluish-green color due to the production of manganate ion which absorbs maximally at 610 nm (Fig. 1).

The absorbance of the reaction product remained stable for at least 60 min. The intensity of the color increased with time; therefore, a kinetically based method was developed for the determination of TIZ in its pharmaceutical dosage formulations.

The different variables that affect the formation of manganate ion were studied and optimized.

3.1.1. Effect of time

The intensity of the color produced increased gradually and reached its maximum after 25 min, where it remained stable for at least 1 hr (Fig. 2).

3.1.2. Effect of potassium permanganate concentration

The absorbance increased substantially with an increase in the concentration of potassium permanganate. It was found that 1 ± 0.1 ml of 1×10⁻² M KMnO₄ was adequate for the development of maximum absorbance (Fig. 3).

3.1.3. Effect of sodium hydroxide concentration

The influence of NaOH concentration on the reaction rate was also studied using 0.12 ml of 0.5 M NaOH. It was found that increasing the volume of the 0.5 M NaOH up to 1.0 ml also increased the absorbance of the reaction product, while further increases in volume resulted in a very slight decrease in the absorbance. Thus, 1 ± 0.1 ml of 0.5 M NaOH was found to be the most suitable concentration for the development of maximum absorbance (Fig. 4). Other alkaline reagents with the same concentration, such as KOH and NH₄OH, were also tested to identify the best alkaline medium. However, their effect on the color development was less than that of NaOH; therefore, the latter reagent was used throughout the study.

Oxidation of TIZ with KMnO₄ was carried out in the presence of NaOH. Trials were made to determine the drug

![Figure 4](image-url) Effect of volume of NaOH (0.5 M) on the absorbance intensity of Tizanidine hydrochloride (2.5×10⁻³ M), KMnO₄ = 1×10⁻³ M, λ = 610 nm.

![Figure 5](image-url) (A) Continuous variation plot for oxidation of Tizanidine hydrochloride with KMnO₄. (B) Molar ratio plot for oxidation of Tizanidine hydrochloride with KMnO₄.
3.1.4. Effect of temperature
At room temperature, the reaction rate increased substantially with time, although the heating of the solution was found to increase the rate of the reaction. However, MnO₂ was precipitated at 60 °C; therefore, room temperature was selected as the optimum temperature.

3.2. Stoichiometry and reaction mechanism

Under the optimum conditions, the stoichiometry of the reaction between KMnO₄ and TIZ was investigated by both the Job’s¹⁰ and molar ratio methods.¹¹

By the application of Job’s method of continuous variations, a series of solutions were prepared by mixing equimolar solutions of drug and KMnO₄ (1 × 10⁻³ M) while keeping the total molar concentration constant (2 × 10⁻⁴ M). The concentration of NaOH and time of the reaction were almost constant at 0.5 M and 25 min, respectively.

The plot of the absorbance of these solutions at 610 nm versus the mole fraction of the drug is presented graphically in Fig. 5A and B. The results show that the molar ratio of KMnO₄ to TIZ was 2:1.

Based on the obtained molar reactivity and given the fact that the oxidation of sulfur-containing compounds is smoother and more rapid in an alkaline medium,¹² the reaction pathway is proposed to proceed as follows:

![Reaction pathway diagram]

3.3. Analytical performance

The rate of the reaction was followed at room temperature with various concentrations of the drug in the range 2–13 μg ml⁻¹, keeping KMnO₄ and NaOH concentrations constant (Fig. 6). The rate of reaction was also found to be dependent on TIZ concentrations.

The reaction rate was found to obey the following equation:

\[ \text{Rate} = K'[\text{TIZ}]^n \]  

(1)

Here \( K' \) is the pseudo-order rate constant and \( n \) is the order of the reaction.

The rate of the reaction may be estimated by the variable-time method¹³ as \( \Delta A/\Delta t \), where \( A \) is the absorbance and \( t \) is the time in seconds.

Taking logarithms of rates and concentrations, Eq. (1) is transformed into:

\[ \log(\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log[\text{TIZ}] \]  

(2)

Regression of \( \log \) (rate) versus \( \log[\text{TIZ}] \) gave the regression equation,

\[ \log(\text{rate}) = 0.7622 + 0.908 \times \text{TIZ} \]  

(3)

Hence, \( K' = 5.78 \text{ s}^{-1} \) and the reaction is first order (\( n = 0.908–1 \)) with respect to TIZ.

3.4. Evaluation of the kinetic methods

The quantitation of TIZ under the optimized experimental conditions outlined above would result in a pseudo-first order reaction with respect to its concentration. However, the rate

![Kinetic diagram]
will be directly proportional to TIZ concentration in a pseudo-first order rate equation as follows:

\[
\text{Rate} = K' \cdot \text{TIZ}
\]

(4)

Several experiments were then carried out to obtain TIZ concentration from the rate data according to Eq. (4).

Initial rate, rate constant, fixed-absorbance and fixed-time methods\(^ {14,15} \) were tried and the most suitable analytical method was selected, taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

3.4.1. The rate constant method

A graph of log absorbance versus time for TIZ concentrations in the range \(1.0 \times 10^{-5} - 3.5 \times 10^{-3}\) M was plotted and all appeared to be rectilinear.

Pseudo-first order rate constants (K') corresponding to different TIZ concentrations (C) were calculated from the slopes multiplied by \(-2.303\).

Regression of (C) versus K' gave the following equations:

\[
K' = -0.00119 + 27.42C \quad (r = 0.8456)
\]

3.4.2. The fixed concentration method

Reaction rates were recorded for different TIZ concentrations in the same range as illustrated above. A preselected value of the absorbance (0.2212) was fixed and the time was measured in seconds.

The reciprocal of time (1/t) versus the initial concentration of TIZ was plotted, and the following equation of the calibration graph was obtained:

\[
1/t = -0.00859 + 617.96C \quad (r = 0.9979)
\]

3.4.3. The fixed-time method

Reaction rates were determined for different concentrations of TIZ. At a pre-selected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentration of TIZ were established at fixed times of 5, 10, 15, 20 and 25 min.

It is clear that the slope increases with time; the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 25 min, which was, therefore, chosen as the most suitable time interval for measurement (Table 1).

After optimizing the reaction conditions, the fixed-time method was applied to the determination of TIZ in pure form over the range 2–13 \(\mu\text{g ml}^{-1}\).

Analysis of the data gave the following equations:

\[
A = -0.00051 + 0.0505C \quad (r = 0.9997)
\]

where \(A\) is the absorbance at 610 nm and C is the concentration in \(\mu\text{g ml}^{-1}\). Ringbom\(^ {16} \) optimum concentration range value is 2.9–10 \(\mu\text{g ml}^{-1}\). The limit of detection (LOD) was found to be 0.27 \(\mu\text{g ml}^{-1}\) and the limit of quantification (LOQ) was found to be 0.90 \(\mu\text{g ml}^{-1}\) (Table 1).

3.5. Accuracy and precision of the proposed method

Once the optimum conditions of the reagents to be used have been optimized, the accuracy and precision of the fixed-time method should be established. In order to do this, four different concentration levels (5, 8, 10 and 12 \(\mu\text{g ml}^{-1}\) of pure TIZ) were selected. The short-term precision (intra-day assay) was determined by measuring five independent analyses at each concentration level within one day.

<table>
<thead>
<tr>
<th>Pharmaceutical formulation</th>
<th>Taken ((\mu\text{g ml}^{-1}))</th>
<th>Added ((\mu\text{g ml}^{-1}))</th>
<th>Found ± SD*</th>
<th>Recovery ± RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirdalud®</td>
<td>2</td>
<td>3</td>
<td>4.976 ± 0.026</td>
<td>99.52 ± 0.522</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>7.902 ± 0.0277</td>
<td>100.88 0.406</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>11.916 ± 0.0492</td>
<td>99.30 ± 0.412</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation of five determinations.

b SAE = standard analytical error.

c CL = confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

**Table 2** Tests of precision of the kinetic method on the determination of pure Tizanidine hydrochloride.

<table>
<thead>
<tr>
<th>Fixed time method</th>
<th>Tizanidine hydrochloride</th>
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<tbody>
<tr>
<td></td>
<td>Taken ((\mu\text{g ml}^{-1}))</td>
</tr>
<tr>
<td>Intra-day assay</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>8</td>
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<td></td>
<td>10</td>
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<td>Inter-day assay</td>
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</tr>
</tbody>
</table>

*Mean ± standard deviation of five determinations.

b SAE = standard analytical error.

c CL = confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)
The daily precision (inter-day assay) was evaluated by measuring the TIZ content at each level on five consecutive days by the fixed-time method. The standard deviation (SD) mean percent recoveries and relative standard deviation (RSD) obtained in intra-day and inter-day assays (Table 2) can be considered to be satisfactory. Thus, the proposed method is effective for the determination of TIZ in drug formulations.

3.6. Analytical recovery and interference liabilities

The accuracy of the proposed method was also checked by performing recovery experiments using the standard addition method. Known amounts of the pure drug were added to TIZ tablets, and then determined by the recommended procedure. The obtained mean recoveries and relative standard deviations were in the range 98.77–99.52% and 0.35–0.52%, respectively (Table 3). These results prove the accuracy of the proposed method and absence of interference from the common excipients.

3.6.1. Interferences

No interferences were observed in the determination of TIZ with alkaline oxidation with potassium permanganate at a fixed time of 25 min in the presence of additives lactose, glycerol, propylene glycol, sugar, sodium acetate and starch which are usually present as fillers and excipients in pharmaceutical preparations.

3.7. Analytical applications

The fixed-time method was applied for the determination of TIZ in pharmaceutical preparations. The results obtained for the analysis of the drug in pharmaceutical preparations (tablets) compared with those obtained with the official method (Table 4).

Student’s t-test and F-test values of 95% confidence level did not exceed the theoretical values of 3.182 and 9.12 for the t-test and F-test, respectively, indicating no significant difference between the proposed method and the official one with regard to accuracy and precision.

However, the principal advantage of the proposed method is its suitability for routine quality control of the drug alone and in its dosage forms without fear of interferences caused by excipients commonly present in tablets.

### 4. Conclusion

The kinetically based method proposed in this work for the quantitation of TIZ is a direct and satisfactorily sensitive method. Furthermore, it does not need the elaborate treatment, tedious extraction and sophisticated apparatus required in chromatographic methods. The data given above reveal that the proposed method is accurate and sensitive with good precision and accuracy. With such method, one can do analysis at low cost without losing accuracy. Also, it can be used for routine determination of TIZ in its pure form and in pharmaceutical preparations.

### References