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Kinetic spectrophotometric methods for determination of trimetazidine dihydrochloride

Ibrahim A. Darwish *

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

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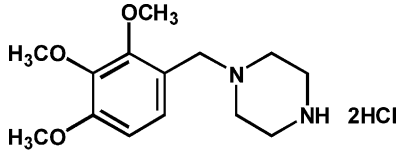
Abstract

Four simple and sensitive kinetic spectrophotometric methods (I–IV) for the determination of trimetazidine dihydrochloride (TRMZ) have been developed. Method I was based on the oxidation of the drug with alkaline $KMnO_4$ producing green manganate species. Method II was based on the formation of colored condensation product between TRMZ and 4-chloro-7-nitrobenzofurazan (NBD-Cl). Method III was based on reaction of TRMZ and with 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS) forming orange colored product. Method IV was based on the formation of a violet charge-transfer complex between trimetazidine base and *p*-chloranil (*p*CL). These reactions were followed spectrophotometrically by measuring the rate of color development at 610, 475, 485 and 560 nm for the reactions with $KMnO_4$, NBD-Cl, NQS, and *p*CL, respectively. The variables affecting the reactions were carefully investigated and the conditions were optimized. The stoichiometries of the reactions were determined, and the reactions pathways were postulated. The initial rate and fixed time methods were utilized for constructing the calibration graphs for the determination of TRMZ concentration. The assays limits of detection were 0.2–2.5 $\mu g\,ml^{-1}$. The analytical performance of the methods, in terms of accuracy and precision, were statistically validated; the results were satisfactory. The methods have been successfully applied to the determination of TRMZ in commercial pharmaceutical formulations. Statistical comparison of the results with the reference method showed excellent agreement and proved that no significant difference in the accuracy and precision.

Keywords: Trimetazidine; Initial rate method; Fixed time method; Kinetic spectrophotometry; Pharmaceutical analysis

1. Introduction

Trimetazidine (TRMZ); 1-[(2,3,4-trimethoxyphenyl)methyl]piperazine dihydrochloride is a clinically effective antianginal agent that has been used in the prophylaxis and management of angina pectoris, and in ischemia of neurosensorial tissues as in Meniere's disease [1]. The antianginal efficacy of TRMZ is comparable to propranolol but it does not reduce cardiac rate–pressure product or coronary blood flow [2]. Trimetazidine exhibits some cytoprotective effects on myocardial energy metabolism and exerts an antianginal effect in the absence of significant hemodynamic effects [3]. For these clinical successes, TRMZ has become unique among the antianginal agents, and it has been clinically used throughout many countries worldwide [4,5].



Trimetazidine dihydrochloride

Trimetazidine dihydrochloride have been determined in pharmaceutical formulations and/or biological fluids by high-performance thin-layer chromatography [6], liquid chromatography [7–9], gas chromatography–mass spectrometry [10], adsorptive stripping voltammetry [11], and chemiluminescence [12]. Spectrophotometry, because of its inherent simplicity, is considered a more convenient alternative

* Tel.: +2 088 2411251; fax: +2 088 2332776.
E-mail address: iadarwish@yahoo.com.

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technique. Since TRMZ contains a weakly absorbing chromophore in its molecule, only few spectrophotometric methods have been reported for its determination [13–16]. These methods include direct UV measurements [13], formation of ion-pair associates with bromophenol blue [14], methyl orange and tropaelin [15], formation of complex with Fe(III) chloride [16], and formation of enamine derivative with acetaldehyde–chloranil combination [14]. These methods are somewhat insensitive, time consuming and/or not simple to perform. Therefore, the development of simple and sensitive methods for the determination of TRMZ was necessary.

Kinetic spectrophotometric methods are becoming of great interest in chemical and pharmaceutical analysis [17]. The application of these methods offered some specific advantages such as improved selectivity, avoiding the interference of the colored and/or turbidity background of the samples, and possibility avoiding the interference of the other active compounds present in the commercial product if they are resisting the reaction conditions established for the proposed kinetic method. No attempts have yet been made to determine TRMZ by any kinetic spectrophotometric method.

The present work describes, for the first time, the development of four simple and sensitive kinetic spectrophotometric methods for the determination of TRMZ in its pharmaceutical formulations. These methods were based on the oxidation of TRMZ with alkaline $KMnO_4$ producing green manganate species, formation of colored condensation products between TRMZ and each of 4-chloro-7-nitrobenzofurazan (NBD-Cl), and 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS), and formation of a violet charge-transfer complex between trimetazidine base and *p*-chloranil (*p*CL). These reactions were followed spectrophotometrically by measuring the rate of color development at 610, 475, 485, and 560 nm in case of $KMnO_4$, NBD-Cl, NQS, and *p*CL, respectively. The initial rate and fixed time methods were adopted for determination of TRMZ in commercial formulations after full investigations.

2. Experimental

2.1. Apparatus

A Lambda-3 B (Perkin-Elmer Corporation, Norwalk, USA) and UV-1601 PC (Shimadzu, Kyoto, Japan) ultraviolet–visible spectrophotometers with matched 1 cm quartz cells, were used for all measurements.

2.2. Chemicals and reagents

Trimetazidine dihydrochloride (Servier Egypt Industries Limited, 6th October, Guiza, Egypt). Potassium permanganate (Merck, Schuchardt, Munich, Germany) was 1×10^{-2} M aqueous solution. 4-Chloro-7-nitrobenzofurazan (NBD-Cl; Sigma Chemical Co., St. Louis, USA) was 1×10^{-2} M, prepared in acetone fresh daily. 1,2-Naphthoquinone-4-sulphonic acid sodium salt (NQS; Aldrich Chemical Co., Milwaukee, USA) was 1×10^{-2} M aqueous solution. 2,3,5,6-Tetrachloro-1,4-benzoquinone (*p*-chloranil; Sigma Chemical Co., St. Louis, USA) was 1×10^{-2} M, prepared in acetonitrile fresh daily. Sodium hydroxide (El-Nasr Chemical Co., Cairo, Egypt) was 1 M and 0.05 M aqueous solutions for reaction of TRMZ with $KMnO_4$ and NQS, respectively. Sodium bicarbonate (El-Nasr Chemical Co., Cairo, Egypt) was 0.4 M aqueous solution for reaction with NBD-Cl. Vastarel® tablets (Servier Egypt Industries Ltd., 6th October, Guiza, Egypt; under license of Les Laboratories Servier, France) and Metacardia® tablets (Global Napi Pharmaceuticals, Egypt) are labeled to contain 20 mg of trimetazidine dihydrochloride per tablet. All solvents and other chemicals used throughout this study were of analytical grade.

2.3. Preparation of standard solutions

2.3.1. For methods I–III (reactions with $KMnO_4$, NBD-Cl, and NQS, respectively)

An accurately weighed amount (50 mg) of TRMZ was quantitatively transferred into a 50 ml calibrated flask, dissolved in 30 ml distilled water, and completed to volume with the same solvent to produce a stock solution of 1 mg ml^{-1}. This stock solution was then diluted with distilled water to obtain working standard solutions of 10, 50, and 50 μg ml^{-1} for reactions with $KMnO_4$, NBD-Cl, and NQS, respectively.

2.3.2. For method IV (reaction with pCL)

An accurately weighed amount (100 mg) of TRMZ was dissolved in 10 ml distilled water in a 25 ml beaker. The solution was transferred quantitatively into a 100 ml separating funnel, and then rendered alkaline with ammonia solution, and the excess ammonia was evaporated by heating the solution in a water bath (MLW type, Memmert GmbH, Schwa bach, Germany). The liberated trimetazidine base was extracted with four 20 ml portions of 1,2-dichloroethane. The combined extracts were passed through a small funnel containing anhydrous sodium sulphate (2 g) into a 100 ml calibrated flask. The contents of the separating funnel were washed three times with 1,2-dichloroethane. The combined extracts and washings were then diluted with acetonitrile to obtain a stock standard solution of 1 mg ml^{-1}, calculated as TRMZ. This stock solution was diluted with acetonitrile to obtain a working standard solution of 100 μg ml^{-1}.

2.4. Preparation of sample solution of pharmaceutical tablets

Twenty tablets of each formulation were weighed and finely powdered. A quantity of the mixed powder equivalent to 100 mg of the active component was transferred into a 50 ml calibrated flask and dissolved in 25 ml water. The contents of the flask were swirled, sonicated for 5 min, and then completed to the mark with water. The mixtures were mixed

well, filtered, and the first portion of the filtrate was rejected. The filtrate solution was diluted with water to yield working solutions of 50, 300, 400, and 800 μg ml^{-1} for analysis by $KMnO_4$, NBD-Cl, NQS, and *p*CL, respectively. For analysis by *p*CL reagent, a measured volume of the filtrate was transferred into a 50 ml separating funnel and rendered alkaline with ammonia solution. The procedure was completed as described under the preparation of standard solution for *p*CL method.

2.5. General recommended procedures

2.5.1. Method I (oxidation with $KMnO_4$)

Accurately measured aliquots of TRMZ solution containing 10–200 μg ml^{-1} were transferred into separate 10 ml calibrated flasks. One milliliter of sodium hydroxide solution (1 M) was added followed by 2 ml of $KMnO_4$ solution (1×10^{-2} M). The solution was diluted to volume with water and mixed well. After mixing, the reaction mixture was transferred to a thermostatically controlled water bath adjusted to 60 ± 5 °C, and allowed to react. The absorbance was recorded as a function of time of 15 min at 610 nm against reagent blank treated similarly.

2.5.2. Method II (coupling with NBD-Cl)

Accurately measured aliquots of TRMZ solution containing 50–1500 μg ml^{-1} were transferred into separate 10 ml calibrated flasks. A 0.5 ml of sodium bicarbonate solution (0.4 M) was added followed by 1 ml of NBD-Cl solution (1×10^{-2} M). The solution was diluted to volume with 50% (v/v) aqueous acetone at 25 ± 5 °C, and mixed well. After mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded as a function of time for 30 min at 475 nm against reagent blank treated similarly.

2.5.3. Method III (condensation with NQS)

Accurately measured aliquots of TRMZ solution containing 100–900 μg ml^{-1} were transferred into separate 10 ml calibrated flasks. One milliliter of sodium hydroxide solution (0.05 M) was added followed by 1 ml of NQS solution (1×10^{-2} M). The solution was diluted to volume with water at 25 ± 5 °C, and mixed well. After mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded as a function of time for 10 min at 485 nm against reagent blank treated similarly.

2.5.4. Method IV (charge-transfer complexation with pCL)

Accurately measured aliquots of trimetazidine base solution containing 100–1500 μg ml^{-1} were transferred into separate 10 ml calibrated flasks. One milliliter of *p*CL was added, the solution was diluted to volume with acetonitrile at 25 ± 5 °C, and mixed well. After mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded as a function of time for 12 min at 560 nm against reagent blank treated similarly.

2.6. Data acquisition and processing

The kinetic data recorded for each method were transformed to the Slide Write Plus software, Version 5.011 (Advanced Graphics Software, Inc., CA, USA) for curve fitting, regression analysis, and statistical calculations. The initial rate (V) of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance–time curve. The calibration curve was constructed by plotting the logarithm of the initial rate ($\log V$) of reaction versus logarithm of the concentration ($\log C$) of the TRMZ. Alternatively, the calibration curve was constructed by plotting the absorbance measured after a fixed time of 2 min for $KMnO_4$, NQS, and *p*CL, and 5 min for NBD-Cl.

3. Results and discussion

3.1. Reactions involved and optimization of the experimental conditions

In the present work, the reactions of TRMZ with each of alkaline $KMnO_4$, NBD-Cl, NQS, and *p*CL reagents were investigated. These reactions yielded colored reaction products; the absorption spectra for these products are given in Fig. 1. The formation of these colored products was utilized in the development of four kinetic spectrophotometric methods (I–IV) for determination of TRMZ in bulk and pharmaceutical formulations.

3.1.1. Method I (oxidation with $KMnO_4$)

Potassium permanganate, as a strong oxidizing agent, has been used in the oxidimetric-based analytical methods for the determination of many compounds [18–20]. During the

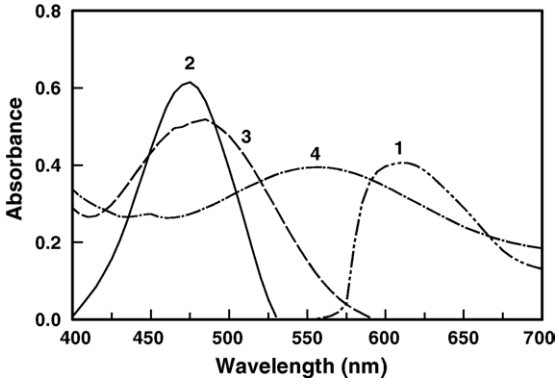

Fig. 1. Absorption spectra of the reaction products of TRMZ with alkaline $KMnO_4$ (1), NBD-Cl (2), NQS (3), and *p*CL (4). The reactions were performed according to the conditions given in Table 1. The concentrations of TRMZ were 2.95×10^{-5}, 2.95×10^{-4}, 1.48×10^{-4}, and 3.54×10^{-4} M for reaction with $KMnO_4$, NBD-Cl, NQS, and *p*CL, respectively.

course of the reaction, the valence of manganese changes and intermediate ions have been suggested as participating oxidants. The main oxidizing species and the extent of oxidation reaction depend on the nature of the substrate (organic or inorganic), and the pH of the reaction medium. In alkaline medium, the heptavalent manganese changes to the green colored Mn(VI), while in neutral and acidic medium the permanganate is further reduced to the colorless Mn(II). This behavior of permanganate was the basis of its use in the development of the spectrophotometric methods. Trimetazidine dihydrochloride was found to be susceptible for oxidation with alkaline $KMnO_4$ producing a green color peaking at 610 nm (Fig. 1). At this wavelength, the various experimental parameters affecting the development and stability of the reaction product were carefully studied and optimized. The effect of $KMnO_4$ concentration on the reaction was studied by carrying out the reaction at room temperature ($25 \pm 5\,^{\circ}C$) using 1 ml of the standard TRMZ solution ($100\,\mu g\,ml^{-1}$) and varying volumes (0.25–3 ml) of the $KMnO_4$ solution (1×10^{-2} M). The reaction increased substantially with increasing the concentration of $KMnO_4$ (Fig. 2A). Maximum absorbance was obtained when 2 ml of $KMnO_4$ solution was used (the concentration in the final assay solution was 2×10^{-3} M). Further increase in the concentration had no effect of the reaction. The effect of medium alkalinity on the reaction was investigated by carrying out the reaction using varying volumes (0.25–3 ml) of sodium hydroxide solution (1 M). The maximum absorbance was obtained when 0.5–3 ml was used; 1 ml was selected for the further experiments. The effect of temperature was studied in the range of 25–80 °C. The absorbance and reaction rate increased with increasing temperature up to 50 °C, and higher temperature had no effect (Fig. 2A). Therefore, $60 \pm 5\,^{\circ}C$ was selected as the optimum temperature.

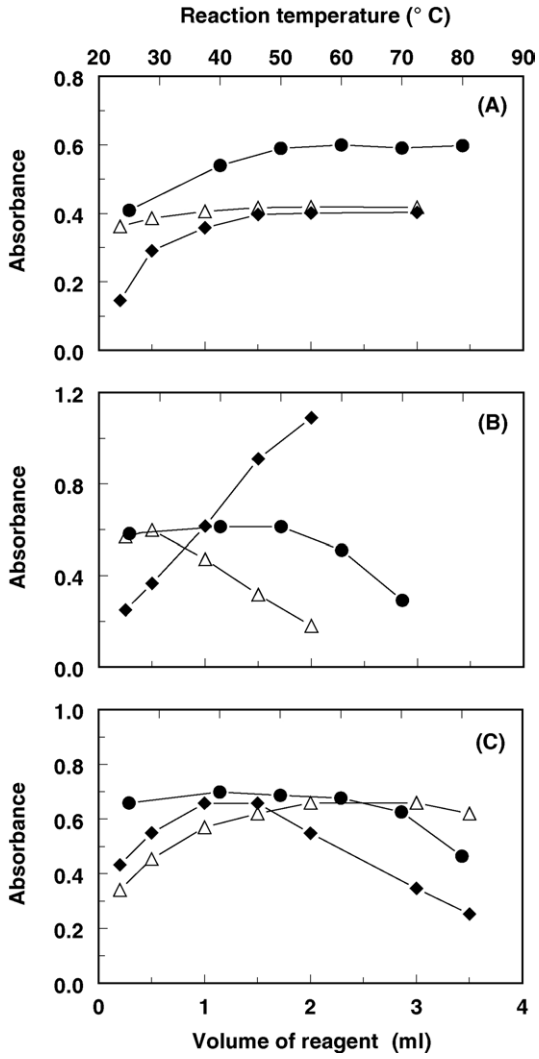

Fig. 2. Optimization of conditions for the reaction of TRMZ with $KMnO_4$ (A), NBD-Cl (B), and NQS (C). The conditions were the volume of the analytical reagent solution (♦), volume of alkaline solution (△), and the reaction temperature (●). The concentration of the analytical reagents was 1×10^{-2} M in all cases. The alkaline solutions were: 1 M NaOH, 0.4 M $NaHCO_3$, and 0.05 M NaOH for reactions with $KMnO_4$ (A), NBD-Cl (B), and NQS (C). The concentrations of TRMZ were 2.95×10^{-5} for reaction with $KMnO_4$ and 1.48×10^{-4} for reactions with NBD-Cl and NQS.

3.1.2. Method II (coupling with NBD-Cl)

4-Chloro-7-nitrobenzofurazan (NBD-Cl) is an activated halide derivative was first introduced as a fluorigenic reagent for the determination of some amines [21]. In further, it was used as a chromogenic reagent for the colorimetric determination of many pharmaceutical amines, e.g. β-blockers [22] and H_2-receptor antagonists [23]. The present study describes the conditions under which the reaction of TRMZ with NBD-Cl fulfills the requirements necessary for its spectrophotometric analysis (the use of NBD-Cl in fluorimetric analysis of TRMZ is currently investigated in our laboratory).

Owing to the presence of labile chloride in the chemical structure of NBD-Cl, a daily fresh solution is recommended. The effect of NBD-Cl concentration on the reaction was checked out at room temperature ($25 \pm 5\,^{\circ}C$) and away from direct sun or artificial daylight. As shown in Fig. 2B, the reaction of TRMZ with NBD-Cl was dependent on the concentration of NBD-Cl solution. The concentration of 1×10^{-3} M in the final assay solution (1 ml of 1×10^{-2} M reagent solution) was selected for further investigations. This concentration was selected on the expense of the sensitivity, whereas negligible absorption blank readings were observed at this concentration. To generate the nucleophile from TRMZ, different inorganic bases were tried: borax, sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solution of a concentration range of 0.1–0.5 M. Best results were obtained in case of sodium bicarbonate where with other bases either precipitation of white colloid occurred upon addition of NBD-Cl, high blank readings, non-reproducible results, and/or weak sensitivity were observed. Studies for optimization of sodium bicarbonate concentration on the reaction revealed that the optimum concentration in the final assay solution was 0.02 M (0.5 ml of 0.4 M reagent solution) was optimum (Fig. 2B). The solvents

used for NBD-Cl reagent as well as for dilution of the reaction mixture were carefully studied using different solvents of varying polarities: acetone, ethanol, methanol, acetonitrile, and water. A marked hypsochromic shift with hypochromic effect was observed in the absorption spectrum of the colored product with increasing solvent polarity. This finding was in agreement with the fact that polar solvents cause stabilization of the ground state of $n \rightarrow \pi^*$ transition peaks through a hydrogen bonding [24], and also was coincident with previous results [25]. Dilution of the reaction product with absolute acetone resulted in precipitation of white colloid owing to the separation of the sodium bicarbonate base. To avoid any precipitation, 50% (v/v) aqueous acetone was used. Under these recommended conditions, the reaction was found to be independent on the temperature in the range of 25–50 °C, however higher temperature had negative effect (Fig. 2B). Therefore, the further investigations were carried out at room temperature (25 ± 5 °C).

3.1.3. Method III (condensation with NQS)

1,2-Naphthoquinone-4-sulphonic acid sodium salt (NQS) has been used as an analytical reagent for the determination of pharmaceutical amines [26] and thiols [27] by spectrophotometry [26] and spectrofluorimetry [27], after the reduction of the drug–NQS adduct to a fluorescent 1,2-dihydroxynaphthalene-4-sulphonic acid derivative. In the present study, TRMZ was found to react with NQS and produce an orange-colored reaction product of λ_{max} at 485 nm (Fig. 1). The effect of NQS concentration on the reaction was studied by using different volumes (0.25–3.5 ml) of NQS solution (1×10^{-2} M). The absorbance and the initial reaction rate increased with increasing the concentration of NQS and maximum values were attained when the concentration in the final assay solution was 1×10^{-3} M (1 ml of 1×10^{-2} M reagent solution) was used (Fig. 2C). Maximum absorbance was obtained when 2 ml of sodium hydroxide solution (5×10^{-2} M) was used (Fig. 2C). In spite of heating the reaction mixture in the range of 40–50 °C had slight positive effect on the reaction, however to simplify the procedure, further testing was performed at room temperature (25 ± 5 °C). The solvents used for NQS reagent as well as for dilution of the reaction mixture were carefully studied using different solvents: water, methanol, ethanol, and acetonitrile. Water was found to be the optimum solvent as the highest absorbance values were obtained.

3.1.4. Method IV (charge-transfer complexation with pCL)

p-Chloranil, as a good electron-π-acceptor, has been used in the charge-transfer-based spectrophotometric methods for many electron-donating pharmaceutical amines [28]. Since free amines, rather than their corresponding acid salts have the electron-donating ability [14], therefore the free trimetazidine base should be liberated prior carrying out the reaction. The interaction of trimetazidine with *p*CL at room temperature gave colored chromogen showing absorption maxima at 580 nm (Fig. 1). The results of variations in the *p*CL reagent concentration indicated that 1 ml of the concentration of 1×10^{-2} M was optimum. In order to select the most appropriate solvent, the reactions were carried out in different solvents: acetonitrile, methanol, ethanol, acetone, propan-1-ol, propan-2-ol, butan-1-ol, diethylether, carbon tetrachloride, and 1,4-dioxane. Small shifts in the position of the maximum absorption peak were observed, and the absorption intensities were also influenced. Non-polar solvents such as dichloroethane were found to produce colored charge-transfer complex with low molar absorptivity value. In polar solvents such as acetonitrile, high molar absorptivity was attained. This was attributed to its high dielectric constant [29] that promotes maximum electron transfer from trimetazidine, as an electron donor (D), to the acceptor moiety (A) with the formation of intensely colored radical ions:

$$D + A \rightleftharpoons (D\text{–}A)\,\text{complex} \overset{\text{polar solvent}}{\rightleftharpoons} D^{\bullet+} + A^{\bullet-}$$

The dissociation of the (D–A) complex was promoted by the high ionizing power of the polar solvent and the resulting peaks in the absorption spectra of trimetazidine–acceptor reaction mixtures were similar to the maxima of the radical anions of the acceptors obtained by the iodide reduction method [30].

3.2. Stoichiometry and reaction mechanisms

The stoichiometry of the reactions of TRMZ with $KMnO_4$, NBD-Cl, NQS, and *p*CL was determined by the molar ratio method [31]. The TRMZ:reagent ratios were 1:2 with $KMnO_4$, and 1:1 with the other reagents. Based on the presence of two amino groups (secondary and tertiary) and their liability for oxidation to the corresponding N-oxide, the reaction of TRMZ with $KMnO_4$ was proposed to proceed according to the pathway given in Fig. 3A. Based on the presence of one –NH of the piprazine liable for coupling reaction with NBD-Cl and replacement reaction NQS, these reactions were postulated to proceed according to the pathways given in Fig. 3B and C. In spite of the presence of two amino groups capable for electron donation to the acceptor *p*CL, however the molar ratio was 1:1. This indicated that only one center, most probably the more basic tertiary amino group, might contributed in the formation of the charge-transfer complex with *p*CL (Fig. 3D).

3.3. Kinetics of the reactions

Under the above-described optimum conditions, summarized in Table 1, the absorbance–time curves for the reactions of TRMZ with $KMnO_4$, NBD-Cl, NQS, and *p*CL reagents were constructed (Fig. 4 for NQS, as a representative example). The initial rates of the reaction were determined from the slope tangents of the absorption–time curves. The order of the reaction with respect to the analytical reagent was deter-

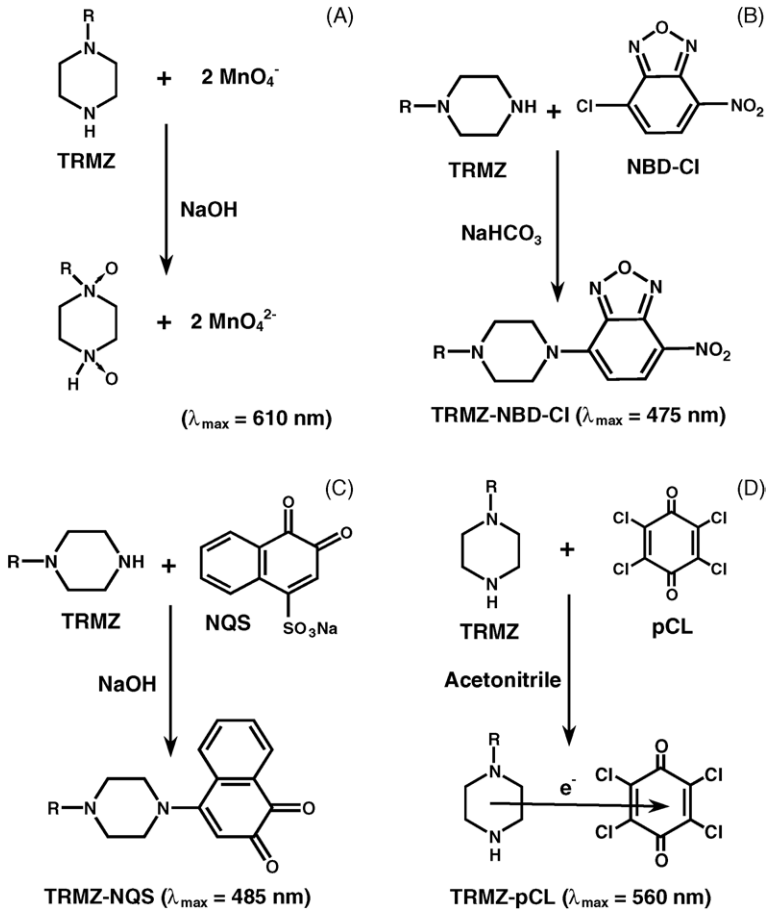

Fig. 3. The proposed pathways for the reaction of TRMZ with alkaline $KMnO_4$ (A), NBD-Cl (B), NQS (C), and *p*CL (D).

mined by studying the reaction at different concentrations of the reagent with fixed concentration of TRMZ. The plot of the initial rate, d*A*/d*t*, against the initial absorbance was linear passing through the origin indicating that the initial order of the reactions with respect to the analytical reagent was 1. The order with respect to TRMZ was evaluated from the measurement of the rates of the reaction at several concentrations of TRMZ at a fixed concentration of each reagent, which were found to be 1. However under the optimized experimental conditions, the concentration of TRMZ was determined using relative excess amounts of the analytical and other conditional reagents. Therefore, pseudo-zero order conditions were obtained with respect to their concentrations.

3.4. Quantitation methods

3.4.1. Initial rate method

The initial rates of the TRMZ reactions would follow a pseudo-first order, and were found to obey the following equation:

$$V = \frac{\Delta A}{\Delta t} = K'C^n$$

where V is the reaction rate, A the absorbance, t the measuring time, K' the pseudo-first order rate constant, C the molar concentration of TRMZ, and n is the order of the reaction. The logarithmic form of the above equation is written as

Table 1
Optimum conditions for the kinetic spectrophotometric methods for determination of TRMZ

Method	Chromogenic reagent		Conditioning reagent		Solvent	Temperature (°C)	λ_{max} (nm)
	Concentration (M)	Volume (ml)	Name	Volume (ml)			
$KMnO_4$	1×10^{-2}	2	NaOH (1)[a]	1	Water	60	610
NBD-Cl	1×10^{-2}	1	$NaHCO_3$ (0.4)	0.5	50% (v/v) aqueous acetone	25	475
NQS	1×10^{-2}	1	NaOH (0.05)	2	Water	25	485
*p*CL	1×10^{-2}	1	–[b]	–[b]	Acetonitrile	25	560

[a] Figures in parenthesis are the molar concentrations of the conditioning reagents.
[b] No conditioning reagent was used.

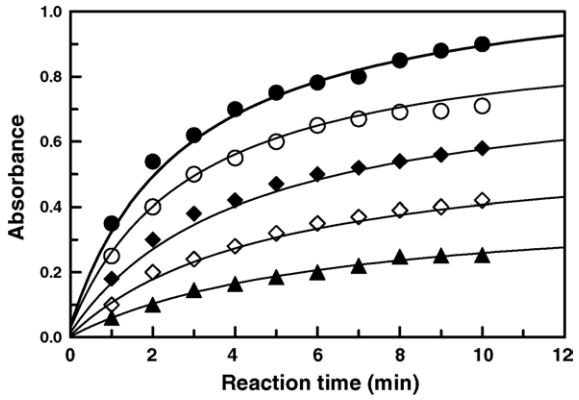

Fig. 4. Absorbance–time curve for the reaction of TRMZ with NQS. The concentrations of TRMZ were 2.95×10^{-5} M (▲), 5.9×10^{-5} M (◊), 8.85×10^{-5} M (♦), 1.18×10^{-4} M (◯), and 1.48×10^{-4} M (●).

follows:

$$\log V = \log \frac{\Delta A}{\Delta t} = \log\ K' + n \log C$$

Regression analysis using the method of least square was performed to evaluate the slopes, intercepts and correlation coefficients. The analytical parameters and results of regression analysis are given in Table 2. The values of n (≈1) in the regression equation confirmed that the reactions of TRMZ with all the reagents were first order with respect to the TRMZ concentration. The limits of detection (LOD) were calculated [32] and found to be 0.2, 1.0, 2.0, and 2.5 μg ml^{-1} with $KMnO_4$, NBD-Cl, NQS, and *p*CL reagents, respectively. These low values confirmed the good sensitivity of the methods and consequently their capabilities to determine low amounts of TRMZ.

3.4.2. Fixed time method

In this method, the absorbance of the reaction solutions containing varying amounts of TRMZ was measured at a preselected fixed time. Calibration plots of absorbance versus the concentrations of TRMZ were established at fixed periods of time for each particular reaction (Table 3). The regression equations, coefficients of correlation, and detection limits are given in Table 3. The lowest detection limits were obtained with fixed times of 10, 40, 6, and 6 min with $KMnO_4$, NBD-Cl, NQS, and *p*CL reagents, respectively. However, the fixed times of 2 min (with $KMnO_4$, NQS, and *p*CL) and 5 min (with NBD-Cl) showed wider concentration ranges for quantification. According to the ICH guidelines for validation of analytical procedures [33], the detection limit is not required to be part of the validation. Therefore, on the basis of wider concentration range and less time of analysis, the fixed time of 2 min (with $KMnO_4$, NQS, and *p*CL) and 5 min (with NBD-Cl) were recommended for determination.

3.5. Validation of the proposed methods

3.5.1. Accuracy and precision

The accuracy and precision of the proposed kinetic spectrophotometric methods were determined [34] at three concentration levels of TRMZ by analyzing five replicate samples of each concentration by both the initial rate and fixed time methods. The relative standard deviations (R.S.D.) for the results did not exceed 2% (Table 4), proving the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of TRMZ in its pharmaceutical tablets.

3.5.2. Analytical recovery and interference liabilities

The accuracy of the proposed methods was also checked by performing recovery experiments using the standard addition method [35]. Known amounts of the pure TRMZ were added to preanalyzed TRMZ-containing pharmaceutical tablets, and then determined by the recommended procedures. The obtained mean recoveries and relative standard deviations were in the range 98.2–102.8 and 0.80–1.85%, respectively (Table 5). These results prove the accuracy of the proposed methods and absence of interferences from the common excipients. It is worth noting that all the proposed kinetic spectrophotometric methods were performed in the visible region away from the UV-absorption region of the UV-absorbing interfering excipient materials that might be co-extracted from the TRMZ-containing tablets. Although the method involving potassium permanganate, being based on oxidation reaction, is not selective, but the obtained good recoveries ensured the suitability of the method for the analysis of TRMZ in its dosage forms without interference from the common reducing excipients. This was attributed to the great sensitivity of the method that necessitated the dilution

Table 2
Analytical parameters for the initial rate method of the spectrophotometric methods for determination of TRMZ

Method	Linear range, M	Least square equation ($\log V = \log K' + n \log C$)[a]		Correlation coefficient (r)	LOD (μg ml^{-1})
		Intercept ($\log K'$)	Slope (n)		
$KMnO_4$	2.95×10^{-6} to 2.95×10^{-5} (1–10)[b]	1.3695	1.0002	0.9992	0.2
NBD-Cl	1.48×10^{-5} to 1.48×10^{-4} (5–50)	2.2596	1.0005	0.9995	1.0
NQS	1.48×10^{-5} to 1.48×10^{-4} (5–50)	1.9667	0.9999	0.9998	2.0
*p*CL	2.95×10^{-5} to 4.42×10^{-4} (10–150)	2.3768	1.0005	0.9999	2.5

[a] V is the reaction rate, K' is the conditional rate constant, n is the order of reaction, and C is the molar concentration of TRMZ.
[b] Figures in parenthesis are the linear range in μg ml^{-1}.

Table 3
Analytical parameters for fixed time method of the kinetic spectrophotometric methods for determination of TRMZ

Reaction time (min)	Linear range ($\mu g\,ml^{-1}$)	Intercept (a)	Standard deviation of intercept ($\pm S_a$)	Slope (b)	Standard deviation of slope ($\pm S_b$)	Correlation coefficient (r)	LOD ($\mu g\,ml^{-1}$)
$KMnO_4$ method							
2	2–20	0.0133	0.0106	0.0427	0.0017	0.9967	0.50
4	1–14	0.0576	0.0071	0.0546	0.0012	0.9981	0.43
6	1–12	0.0786	0.0093	0.0552	0.0015	0.9994	0.33
8	1–12	0.0902	0.0085	0.0592	0.0014	0.9998	0.29
10	1–12	0.1009	0.0099	0.0599	0.0016	0.9995	0.26
NBD-Cl method							
5	5–150	0.0326	0.0067	0.0055	0.0002	0.9972	2.44
10	5–80	0.0230	0.0091	0.0113	0.0003	0.9987	1.61
20	5–50	0.0470	0.0054	0.0151	0.0003	0.9994	0.72
30	5–40	0.1006	0.0024	0.0161	0.0008	0.9997	0.35
40	5–40	0.1487	0.0027	0.0173	0.0010	0.9998	0.31
NQS method							
2	10–90	−0.0156	0.0149	0.0108	0.0005	0.9974	5.20
4	5–70	0.0202	0.0105	0.0134	0.0003	0.9992	2.50
6	5–60	0.0572	0.0069	0.0146	0.0002	0.9997	2.02
8	5–50	0.0923	0.0058	0.0151	0.0002	0.9998	4.20
10	5–50	0.0974	0.0154	0.0158	0.0005	0.9987	5.50
*p*CL method							
2	10–220	0.0012	0.0316	0.0042	0.0003	0.9993	4.05
4	10–150	0.0065	0.0067	0.0051	0.0001	0.9997	2.63
6	10–150	0.0179	0.0058	0.0054	0.0001	0.9998	2.15
8	10–150	0.0159	0.0158	0.0058	0.0002	0.9995	5.45
10	10–150	0.0177	0.0126	0.0060	0.0001	0.9993	4.20

of the sample, and consequently the excipients beyond their interference capability.

3.5.3. Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation in the experimental parameters on the analytical performance of the method [36]. In these experiments, one parameter was changed where as the others were kept unchanged, and the recovery percentage was calculated each time. It was found that none of these variables significantly affected the performance of the method; the recovery values were 98.6–102.2 ± 0.56–1.25%. This provides an indication of the reliability of the proposed method during the routine application of the proposed method. Ruggedness was tested by applying the proposed methods to the assay of TRMZ using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the relative standard deviations (R.S.D.) did not exceed 2%.

Table 4
Evaluation of the accuracy and precision of the initial rate and fixed time methods of the proposed kinetic spectrophotometric methods for determination of TRMZ

Spectrophotometric method	Amount taken ($\mu g\,ml^{-1}$)	Recovery (% ± R.S.D.)[a]	
		Initial rate method	Fixed time method
$KMnO_4$	2	100.5 ± 1.85	101.4 ± 1.84
	4	99.8 ± 1.28	100.8 ± 1.15
	8	99.4 ± 1.55	98.5 ± 1.42
NBD-Cl	10	98.4 ± 1.05	99.6 ± 1.24
	20	99.7 ± 0.75	100.8 ± 0.65
	40	101.2 ± 0.95	100.2 ± 1.25
NQS	10	102.5 ± 1.20	101.8 ± 1.05
	20	99.8 ± 0.85	98.5 ± 0.68
	40	98.5 ± 0.96	99.2 ± 1.05
*p*CL	25	100.4 ± 1.57	101.6 ± 1.04
	50	98.5 ± 1.02	99.6 ± 0.95
	100	102.4 ± 1.25	101.8 ± 1.02

[a] Recovery was calculated as the amount found/amount taken × 100. Values are mean ± R.S.D. for five determinations.

Table 5
Standard addition method for the determination of TRMZ by the initial rate and fixed time methods of the proposed kinetic spectrophotometric methods

Method	Tablet	Amount added ($\mu g\,ml^{-1}$)	Recovery (% ± S.D.)[a]	
			Initial rate method	Fixed time method
$KMnO_4$	Vastarel (5)[b]	5	100.4 ± 0.84	101.6 ± 1.24
	Metacardia (5)	5	99.8 ± 1.25	99.6 ± 1.04
NBD-Cl	Vastarel (20)	20	99.6 ± 1.42	98.4 ± 0.94
	Metacardia (20)	20	100.3 ± 0.80	100.2 ± 1.22
NQS	Vastarel (20)	20	101.3 ± 1.42	102.1 ± 1.84
	Metacardia (20)	20	98.2 ± 0.95	99.8 ± 0.95
*p*CL	Vastarel (50)	50	99.2 ± 1.04	102.8 ± 1.85
	Metacardia (50)	50	100.4 ± 1.22	99.8 ± 1.09

[a] Mean ± S.D. for five determinations.
[b] Figures in parenthesis are the amounts in $\mu g\,ml^{-1}$ taken for analysis.

Table 6
Analysis of tablets containing TRMZ by the reported and the initial rate and fixed time methods of the proposed kinetic spectrophotometric methods[a]

Spectrophotometric method	Tablet	Initial rate method			Fixed time method		
		Label claim (% ± S.D.)[a]	*t*-Value[b]	*F*-value[b]	Label claim (% ± S.D.)[a]	*t*-Value	*F*-value
$KMnO_4$	Vastarel	100.1 ± 1.25	2.74	1.5	99.8 ± 1.26	1.09	1.53
	Metacardia	100.7 ± 1.65	1.92	1.74	100.6 ± 1.95	1.34	2.43
NBD-Cl	Vastarel	99.9 ± 1.52	1.45	2.22	100.2 ± 1.65	2.73	2.62
	Metacardia	100.8 ± 1.88	1.96	2.26	100.9 ± 1.95	2.48	2.43
NQS	Vastarel	100.1 ± 1.34	2.62	1.73	100.1 ± 1.54	2.39	2.28
	Metacardia	100.5 ± 1.65	0.85	1.74	100.7 ± 1.96	1.71	2.46
*p*CL	Vastarel	100.2 ± 1.85	2.51	3.29	99.8 ± 1.46	0.99	2.05
	Metacardia	100.9 ± 1.52	2.69	1.48	100.4 ± 1.55	0.67	1.54

[a] The label claim % for Vastarel and Metacardia tablets determined by the reported method [14] were 99.6 ± 1.02, and 100.3 ± 1.25, respectively. Values are mean ± S.D. of five determinations.
[b] The tabulated values of *t* and *F* at 95% confidence limit are 2.78 and 6.39, respectively.

3.6. Application of the proposed methods

The initial rate and fixed time methods of the proposed kinetic spectrophotometric methods for determining TRMZ have been tested on commercial pharmaceutical tablets. The concentration of TRMZ was computed from its corresponding regression equations. The results of the proposed methods (initial rate and fixed time) were statistically compared with those of the reported method [14], in respect to the accuracy and precision. The obtained mean recovery values of the labeled amounts were 99.8–100.9 ± 1.25–1.96% (Table 6). In the *t*- and *F*-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated similar precision and accuracy in the analysis of TRMZ in tablets.

It is evident from these results that all the proposed methods are applicable to the analysis of TRMZ in its pharmaceutical tablets with comparable accuracy and precision. However, the critical comparative evaluation and/or recommendations of some of these methods might be based on the experimental conditions (e.g. availability of reagents, simplicity of the procedure, reaction time, and diluting solvent), and the sensitivity that determines the amount of specimen used for analysis. For example, the method involving oxidation with alkaline potassium permanganate gave the highest sensitivity, however the procedure required an extra apparatus, water bath. The other methods were performed at room temperature, however the methods involving condensation with NBD-Cl and charge-transfer complexation with *p*CL used organic solvents for dilution, which required special care in their waste. The method involving charge-transfer complexation with *p*CL did not require any conditioning reagents, however a pre-liberating the free trimetazidine base was necessary prior to the analysis. The method involving condensation with NQS gave high sensitivity, was easily performed at room temperature, and used water (green solvent) for dilution. From these considerations, NQS-based method is might be the most recommended method for routine use in quality control laboratories for analysis of TRMZ.

4. Conclusion

Four simple and sensitive kinetic spectrophotometric methods for the determination of TRMZ have been successfully developed and validated. These methods were based on the oxidation of TRMZ with alkaline $KMnO_4$, coupling reac-

tion with each of NBD-Cl and NQS, and charge-transfer complexation with *p*CL. The initial rate and fixed time methods for these spectrophotometric methods can be easily applied to the determination of TRMZ in pure and tablets. The proposed methods are sensitive enough to enable determination of lower amounts of the drug, and compared favourably with the previously reported methods in terms of accuracy and precision. The proposed methods, because they involve measurements in visible region, are more selective than the previously reported spectrophotometric method that involves measurement in ultraviolet region [13]. The proposed methods are superior to the previously reported ion-pair-based methods [14,15], as they do not require elaborate treatment of the samples and/or tedious extraction of the chromophores. Furthermore, the proposed methods use a spectrophotometer that is usually available in all quality control laboratories, rather than the previously reported methods that use expensive and not usually available instruments [7–12]. These advantages encourage the application of the proposed methods in routine analysis of TRMZ in quality control laboratories, as alternatives for the existing methods.

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