Abstract. A novel selective, simple, sensitive, and cost effective kinetic spectrophotometric initial rate method for the determination of suxamethonium chloride has been developed. It is based on the indicator reaction of catalytic 3,3',5,5'-tetramethylbenzidine oxidation. The analytical performance of the method was validated statistically with respect to limit of detection (LOD) and limit of quantitation (LOQ), accuracy, precision, and linearity.

Keywords: suxamethonium chloride, perhydrolysis, kinetic spectrophotometry, initial rate method, 3,3',5,5'-tetramethylbenzidine, pharmaceutical analysis.

1. Introduction

Suxamethonium (SUX) chloride also known as succinylcholine (2,2'-[butanediolbis(oxy)]bis(N,N,N-trimethylethanaminium (Fig. 1) is a depolarising neuromuscular blocker used to produce muscle relaxation. It is used in surgical and other procedures in which a rapid onset and brief duration of muscle relaxation is needed, including endotracheal intubation, endoscopic examinations and electrically or pharmacologically induced convulsive therapy [1].

\[
\text{(H}_3\text{C)}_2\text{N}\\text{O}\\text{H} = \text{O} \quad \text{O} \quad \text{N(CH}_3)_2 \quad 2 \text{Cl} \quad 2 \text{H}_2\text{O}
\]

Fig. 1. Chemical structure of suxamethonium

The drug substance suxamethonium chloride is the subject of British [2] and American [3] Pharmacopoeial monographs. The USP describes a HPLC method for its determination in its bulk and in a pharmaceutical formulation and the BPh describes a back titration with bromothymol blue as indicator. SUX is chemically instable compound and in aqueous alkaline solution is rapidly hydrolized [4]. Several analytical procedures have been previously reported for the determination of SUX in its bulk, pharmaceutical formulations and/or biological fluids which include potentiometric [5], chemiluminesimetric [6], capillary electrophoresis methods [7], etc. However, most of them uses HPLC and it is noteworthy that even it does not ensure complete separation between SUX and its degradation products and, therefore, a highly selective detector like mass spectrometry [4, 8], electrochemical [9, 10] or fluorescence [11] is needed to counterbalance the low resolution of the analytical separation.

Spectrophotometry, that still belongs to the most frequently used analytical techniques in pharmaceutical analysis, provides practical and significant economic advantages over the described methods [12] but the lack of a chromophore in SUX requires other detection techniques in place of direct UV absorbance measurement. Moreover, no simple method that sufficiently separates SUX and its degradation products in a pharmaceutical formulation has been described in the literature [13]. For this reason, kinetic spectrophotometric method can be considered as a simple and inexpensive detection technique for routine analysis.

An attempt has been made to determine SUX based on the perhydrolysis reaction of analytically important ester functional group with p-phenetidine as a chromogenic substrate for peroxyacid indication [14]. Assay of SUX in the presence of its hydrolysis products and sensitivity is a distinctive advantage of this method. The opportunity of quantitative determination of low concentrations of peroxyacids in the presence of large excess of hydrogen peroxide by using 3,3',5,5'-tetramethylbenzidine was shown earlier by one of the authors of this article [15]. Therefore, in the present paper we describe another simple kinetic spectrophotometric method of SUX assay with 3,3',5,5'-tetramethylbenzidine based on the perhydrolysis reaction.
2. Experimental

2.1. Apparatus

All spectrophotometric measurements were made on SF-46 spectrophotometer (LOMO, USSR) with 1 cm matched quartz cells. Laboratory ionometer I-130 (“Analitpribor”) with glass electrode ESL 43-07 type (reference electrode – a silver/silver chloride electrode EVL-1M3.1 type) was used to control the pH.

2.2. Chemicals and Dosage Form

Succinylcholine chloride dihydrate (SUX) ≥ 98.0 %, (Sigma-Aldrich, USA), 3,3',5,5'-tetramethylbenzidine dihydrochloridohydrate (TMB), (Sigma-Aldrich, Germany), was ≥ 97 %, prepared in 50 % ethanol. All solvents and other chemicals used throughout this study were of analytical grade. Lysthenon®; ampoules 5 ml (Takeda Austria GmbH, batch number 10748637), solution for injection, labeled to contain 20 mg SUX per ml.

2.3. Preparation of SUX Working Standard (WS) Solution, 0.2965 mg/ml

Into a 50 ml calibrated flask an accurately weighed amount (0.16300 g, contains 0.14823 g of C₉H₁₈O₄N₂Cl₂, calculated on the anhydrous basis) of SUX was dissolved in 40 ml of double-distilled (DD) water. After complete dissolution of SUX, the resulting solution was completed to volume with the same solvent and shaken thoroughly. Five milliliters of the obtained solution were pipetted to a 50 ml volumetric flask, completed to the mark with DD water, and shaken thoroughly.

2.4. Preparation of Pharmaceutical Formulation Sample Solution

Contents of the ampoule Lysthenon® was quantitatively transferred into a 250 ml volumetric flask. The volume was completed to the mark with DD water to obtain sample solution of concentration 0.4 mg/ml and shaken thoroughly. All the solutions were prepared freshly.

2.5. Obtaining of Calibration Curve

From 1.00 to 5.00 ml of Sux WS solution was transferred into 25 ml calibrated flask. Ten milliliters of the phosphate buffer solution (0.2 mol/l, pH = 8.4), 6 ml of TMB (2·10⁻² mol l⁻¹) solution and 1.0 ml of hydrogen peroxide (30 %) were added. The reaction mixture was complete to volume with DD water and mixed. After mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded (at 420 nm) as a function of time vs. reagent blank treated similarly. Time was controlled from the moment of solution mixing with the stop-watch.

2.6. Procedure of Assay

Two milliliters of the sample solution were transferred into 25 ml calibrated flask. 10 ml of the phosphate buffer solution (0.2 mol/l, pH = 8.4), 6 ml of TMB (2·10⁻² mol l⁻¹) solution, and 1 ml of hydrogen peroxide (30 %) were added. The reaction mixture was completed to volume with DD water and mixed. After mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded (at 420 nm) as a function of time vs. reagent blank treated similarly. Time was controlled from the moment of solution mixing with the stop-watch.

The kinetic data that have been recorded were transformed to the Excel 2003 which is part of the Microsoft Office 2003 System for curve fitting, regression analysis and statistical calculations by the reference of State Pharmacopeia of Ukraine [16]. The content of SUX was computed by the method of standard.

3. Results and Discussion

The reaction involved in the present study is based on the interaction of SUX with excess of hydrogen peroxide in a weak alkaline medium forming peroxysuccinic acid (PSA). The formed PSA is then allowed to react with TMB to give colored 3,3',5,5'-tetrametyldiphenoquinonediimine derivative [17], which exhibits absorption maxima at 420 nm (Fig. 2).

The absorption spectrum for the reaction product is given in Fig. 3.

This color reaction has not been reported yet for SUX, therefore, the present study was devoted to the investigation of this reaction for SUX and its employment in the development of a novel kinetic spectrophotometric method for the determination of SUX in bulk and in commercially available pharmaceutical product Lysthenon®. Since this proposed reaction employed the ester group of SUX, it is anticipated that its degradation products devoid of the ester group will not interfere and ultimately a selective method will be developed.

Under known optimum conditions for the involved indicator reaction [18], the absorbance-time curves for the reaction at varying SUX concentrations were generated (Fig. 4). As the concentrations of TMB and hydrogen peroxide are much higher than the analyte concentration, the order of indicator reaction became pseudo first order with respect to SUX, therefore its perhydrolysis is the limitative stage of the process. The conditional initial rate (tgα, min⁻¹) of the reaction at different SUX concentration was evaluated from the linear site (from 3 to 7 min) of the slope of the initial tangent to the absorbance-time curve. The value of the conditional initial rate of the reaction of blank solution (without examined substance) was 4.34·10⁻³ tgα, min⁻¹. In further calculations it was subtracted from the same value of the WS solution or sample solution.
As can be seen from Fig. 4 it increases with increasing SUX concentration. The calibration curve obtained by plotting conditional initial rate of reaction vs. final concentration of SUX under the optimum conditions showed a linear relationship over the range 9 – 59 µg/ml (Fig. 5).

The method of least square was performed to estimate the regression characteristics of the obtained calibration curve (Table 1). Test of statistical significance of the slope angle $\alpha$ showed that one-sided confidence interval is 4.3 times less compared to the value of the coefficient ($b = (1.3 \pm 0.3) \times 10^{-3}$) and, therefore, for this assessment given constant is significant. One-sided confidence interval for the intercept term $a$ is 3.5 times higher compared to the value of the coefficient ($a = (3.0 \pm 10.4) \times 10^{-3}$) and, therefore, for this assessment given constant is insignificant. Preliminary evaluation indicates statistically reasonable correctness and appropriateness of transition from two-parameter regresional dependence of the form $y = b \cdot x + a$, to one-parameter regresional dependence of the form $y = b_1 \cdot x$. Homogeneity of their dispersions in the $F$-test, $\xi < F$ ($\xi \approx 1.05$, at the critical value of $F(0.95, 3, 4) = 6.5914$) allows to use the regression dependence of the form $y = b_1 \cdot x$. Test of statistical significance of the slope angle $b_1$ showed that one-sided confidence interval is less than 13 times compared with the value of the coefficient, therefore for this assessment given constant is significant. Thus the finite equation is $\tan \alpha = (b_1 \pm \Delta b_1) \cdot [\text{Sux}]$. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on standard deviation of the analytical signal $\sigma$ for samples that do not contain examined substance ($\tan \alpha = 0.0042, 0.0043, 0.0045, 0.0044, 0.0043$) and the slope of calibration curve $b$ and expressed as $\text{LOD} = 3.3 \sigma / b$, $\text{LOQ} = 10 \sigma / b$.

The low value of LOD confirms the good sensitivity of the method and consequently its capability to determine low amounts of SUX. The linearity of the proposed method was also evaluated with normalized coordinates ($r = 0.994$) [19]. The values of $a$ and $|b|^{-1}$ do not exceed the confidence limits of their uncertainty (requirement of statistical insignificance), $a \leq t(95 \%, n-2) \cdot \sigma_a(6.4 < 22.5)$ and $|b|^{-1} \leq t(95 \%, n-2) \cdot \sigma_b(2.6 \cdot 10^{-2} < 0.2)$.

Precision and accuracy of the proposed method were determined at three levels of SUX concentrations (low, medium, and high of 23.7, 35.6 and 47.4 µg/ml, respectively). Five replicates of each sample were analyzed. The relative standard deviations for the results did not exceed 2.2% (Table 2), proving the high reproducibility of the results and the precision of the method. This good level of precision was suitable for analysis of SUX in its pharmaceutical dosage form.

The proposed kinetic spectrophotometric initial rate method was applied for the analysis of SUX-containing commercial pharmaceutical formulation Lysthenon® (Table 3).
Fig. 4. Absorbance-time curves of 3,3’,5,5’-tetramethylphenoxoquinone diimine accumulation for the reaction of varying concentrations of SUX with TMB (2.4×10⁻³ mol/l) and H₂O₂ (0.2240 mol/l) at pH = 8.4.

Fig. 5. Calibration curve of SUX determination in TMB-H₂O₂-SUX system. Experimental conditions: c(TMB) = 2.4×10⁻³ mol/l; c(H₂O₂) = 0.2240 mol/l; pH = 8.4; T = 293 K.

Table 1

<table>
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<tr>
<th>Parameter</th>
<th>Data</th>
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<tbody>
<tr>
<td>Calibration range, μg/ml</td>
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<tr>
<td>b₁</td>
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<tr>
<td>Sₙ</td>
<td>3.5×10⁻⁶⁺</td>
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<tr>
<td>±σSₙ</td>
<td>0.1×10⁻⁴⁺</td>
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<tr>
<td>Regression equation</td>
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<tr>
<td>σ</td>
<td>1.14×10⁻⁶⁺</td>
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<tr>
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<td>LOQ, μg/ml</td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Amount taken, μg/ml</th>
<th>Amount found, X ± ΔX</th>
<th>Recovery ± RSD, %</th>
<th>δ*, %</th>
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<tbody>
<tr>
<td>23.7</td>
<td>23.6 ± 0.6</td>
<td>99.5 ± 2.2</td>
<td>-0.5</td>
</tr>
<tr>
<td>35.6</td>
<td>35.8 ± 0.8</td>
<td>100.6 ± 1.7</td>
<td>0.6</td>
</tr>
<tr>
<td>47.4</td>
<td>47.0 ± 1.0</td>
<td>99.2 ± 1.7</td>
<td>-0.8</td>
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Note: *δ = (X – μ) · 100% / μ

Table 3

Results of analysis of SUX in pharmaceutical formulation by the proposed method (n = 5, P = 0.95)

<table>
<thead>
<tr>
<th>Labelled amount, mg</th>
<th>Amount found, mg</th>
<th>Recovery ± RSD, %</th>
<th>δ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.103*</td>
<td>0.102 ± 2.0·10⁻³</td>
<td>99.7 ± 1.6</td>
<td>-0.3</td>
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</table>

Note: *SUX content is determined according to reference standard pharmacopoeial method relative to the anhydrous succinylcholine chloride (C₁₄H₂₉N₂O₄Cl₂) [3]
4. Conclusions

A simple and express kinetic-spectrophotometric method for the determination of SUX has been proposed. Assay of SUX in the presence of its hydrolysis products without preliminary separation is an important advantage of the described method. Moreover, the proposed method does not require expensive instruments and/or critical analytical reagents. The analytical performance of the method was validated with respect to LOD, LOQ, accuracy, precision, and linearity for SUX estimation in pure substance and the results were satisfactory. Compared to the reference method, pharmaceutical formulation Lysthenon® contains $0.102 \pm 2.0 \times 10^{-3}$ g of $\text{C}_{14}\text{H}_{30}\text{N}_{2}\text{O}_{4}\text{Cl}_{2}$, relative to the anhydrous basis (RSD = 1.6 %; $\delta = -0.3 \%$).

References
