Quantitative Determination of Lincomycin in Dosage Forms by Iodometric Titration through Oxidation Reaction with Peroxomonosulfate

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The kinetics of the oxidation reaction of Lincomycin hydrochloride (Link) with potassium hydrogen peroxomonosulfate ($KHSO_5$) was studied depending on the pH of the medium. It has been established that the reaction kinetics obeys the general laws of specific acid-base catalysis. $KHSO_5$ reacts quantitatively with Link in an alkaline medium to form the corresponding sulfone-N-oxide: 1 mole of Link requires 3 moles of $KHSO_5$. Methods for the oxidimetric determination of Link were developed using $KHSO_5$ as an analytical reagent: a known excess of the reagent is added and after a specified time, its residual amount is determined by iodometry. The possibility of quantitative determination of the main substance in the substance of Link hydrochloride, as well as the determination of Link hydrochloride solution for injection "Lincomycin-Zdorovye" 300 mg/ml in ampoules of 1 ml and capsules of 0.25 g were shown. RSD does not exceed 1.7%.

Introduction

Lincomycin dihydrochloride (Link, $C_{18}H_{34}N_2O_6S{\cdot}HCl{\cdot}H_2O$, (2S,4R)-1-Methyl-4-propyl-N-[1,6,8-trideoxy-1-(methylsulfonyl)-D-erythro-α-D-galacto-octopyranose-6-yl]pyrrolidine-2-carboxamide hydrochloride monohydrate) is a mixture of antibiotics produced by strains of *Streptomyces lincolnensis* var. *lincolnensis* or otherwise obtained [1]. The structural formula of Link hydrochloride is shown in **Figure 1**. Mol. mass is 461.0 g/mol (anhydrous is 443.0 g/mol).

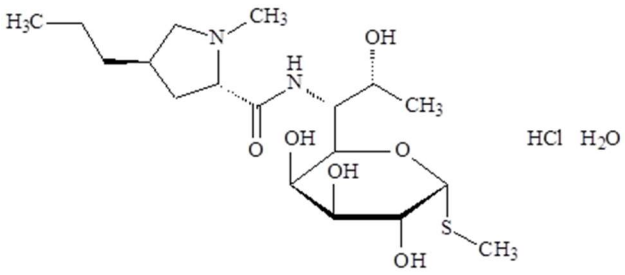

Figure 1. The structural formula of Link hydrochloride

It contains not less than 89.5% and not more than 102.0% of lincomycin hydrochloride $C_{18}H_{34}N_2O_6S{\cdot}HCl$ in terms of an anhydrous and free from residual organic solvents substance. White or almost white crystalline powder. Very

easily soluble in water, slightly soluble in alcohol 96%, and very slightly soluble in acetone.

The official standardized analytical methods for the quantitative determination of Link in pharmaceutical preparations are gas chromatography (GC) [2] and high-performance liquid chromatography (HPLC) [3].

The activity of the antibiotic Link can be determined by the microbiological method. [4]. However, due to its duration, the microbiological method makes it difficult to control the production of an antibiotic on an operational basis.

Literature sources describe methods for the quantitative determination of Link, such as electrochemical [5], iodometric titration of methanethiol formed as a result of the destruction of Link in 16-23% H_2SO_4 [6]; colourimetry [7], atomic absorption spectroscopy [8], as well as other types of spectroscopy [9-12], chromatography [13-21], Lateral Flow Immunoassay [22].

At the same time, the analysis of this series showed that the method [6] is difficult due to the difficulty of extracting CH_3SH, which must be carried out at low temperatures [23]. And the well-known Pharmacopoeial method [24] involves the use of toxic salts of mercury and glacial acetic acid as a medium, which violates the principle of "Green Chemistry".

HPLC is efficient and fast, but its sensitivity is limited by the absorption of Link UV radiation upon detection. LC-MS methods are quite sensitive, but expensive equipment must be maintained by trained personnel, which limits application to small-scale plants or field trials. TLC methods have separation capability and require only a tiny sample but suffer from poor reproducibility.

Based on the literature review, there is a need to develop sensitive and fast analytical methods for the quantitative determination of Link.

Redox titrimetry can be a useful alternative to many of the above rather complicated methods due to its cost-effectiveness, ease of implementation, sensitivity, reasonable accuracy, and broad relevancy. The iodometric determination of medicines using Caro's acid was also proposed in our previous works [25-26].

The present study aims to develop simple, sensitive and economical methods for determining Link in substances, capsules and injections using the iodometry method. The method involves the use of $KHSO_5$ in the form of a stable potassium triple salt – oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) as a titrant. A known excess of reagent is added, and after a predetermined time, the residual reagent is determined iodometrically.

Experimental part

Material and methods

Lincomycin hydrochloride substance manufactured by Fengchen Group CO., LTD, China. Data Certificate: 99.6 % (HPLC)

Lincomycin hydrochloride monohydrate (CAS Number 7179-49-9) manufactured by

FUJIFILM Wako Pure Chemical. Data Certificate: 98.0 % (HPLC); *Lincomycin hydrochloride monohydrate* (CAS Number 7179-49-9) manufactured by FUJIFILM Wako Pure Chemical. Data Certificate: 98.0 % (HPLC).

Pure analytical grade lincomycin hydrochloride monohydrate – Vetranal™ analytical standard (Sigma-Aldrich Co, St Louis, MO, USA) – was obtained commercially.

Lincomycin 10 ampoules injection solution 30%

Lincomycin hydrochloride – "Lincomycin Zdorovye" injection solution 300 mg/ml in 1 ml ampoules; manufactured by Pharmaceutical Company "ZDOROVYE", Series No. 10221.

Lincomycin hydrochloride – "Lincomycin-Darnitsa" injection solution 300 mg/ml in 2 ml ampoules; manufactured by Pharmaceutical firm "DARNITSA", Series UU20920.

Composition: active substance Link (1 ml of the injection solution contains 300 mg of Link), excipients: sodium edetate, sodium hydroxide, water for injection.

Lincomycin hydrochloride capsules 250 mg

Lincomycin hydrochloride – "Lincomycin" capsules of 250 mg in terms of Link; "AT "Kyivmedpreparat (Ukraine)", Series 0018613.

Composition: active ingredient Link hydrochloride; 1 capsule contains Link hydrochloride in terms of 100 % Link – 250 mg; excipients: pregelatinized starch, calcium stearate; composition of gelatine capsule No. 1: cap – sunset yellow (E 110), quinoline yellow (E 104), titanium dioxide (E 171), gelatine; body – titanium dioxide (E 171), gelatine.

The active oxidizing agent of Link is the salt of Caro's acid ($KHSO_5$ – potassium hydrogen peroxomonosulfate). It is triple salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (trade name Oxone) that is a form with higher stability.

Solution of Lincomycin hydrochloride, 0.01 mol/l. The accurate weight of the powder of the substance Link hydrochloride monohydrate with a known content of the main substance, equivalent to 0.4430 g of Link hydrochloride anhydrous $C_{18}H_{34}N_2O_6S \cdot HCl$, was dissolved in 70 mL of double-distilled water in a 100 mL volumetric flask, brought to the mark at +20 °C with water and carefully mixed.

The preparation of pH buffer solutions

To prepare a buffer solution with a pH = 7.0, 22.679 g of dipotassium hydrogen phosphate (K_2HPO_4) and 9.499 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved by 1 L double distilled water in 1 L volumetric flask.

For preparing 0.2 M solution of potassium pyrophosphate, 66.067 g potassium diphosphate was dissolved by double distilled water in 1 L volumetric flask.

Preparation procedure of pH buffers with 7.9 and 9.2 included: dropping of diluted hydrochloric acid into an aqueous solution of 0.2 M solution potassium pyrophosphate while measuring the pH by a pH-meter.

Initial solution of borax: 19.1 g of $Na_2B_4O_7 \times 10\ H_2O$ was dissolved by 1 L double distilled water in 1 L volumetric flask.

To prepare a buffer solution with a pH of 10.5, 47.5 mL of a 0.1 mol/L NaOH solution was added to 52 mL of the initial borax solution, which was prepared by the appropriate dilution of the saturated solution after a 24-hour exposure (50 g of borax + 50 g of double distilled water).

The pH value of the solutions was controlled using a glass electrode ESL-43-07 on an ionometer "Ionomer laboratory I-160M" (Belarus) paired with an argentum chloride electrode EVL-1M3.1 saturated with potassium chloride.

Microburette Class' A' 5 mL-10 mL PTFE Key Stopcock LABGLASS USA.

Micropipette tips, polypropylene, 1-5 mL Standard Line.

Solution of potassium hydrogen peroxomonosulfate ($KHSO_5$), 0.04 mol/L. About 1.4 g of $KHSO_5$ powder was dissolved in 70 mL of double-distilled water in a 100 mL volumetric flask, made up to the mark with water and mixed thoroughly. The exact concentration was determined by iodometric titration.

10 mL of the solution was taken with a pipette and transferred into a 100 mL volumetric flask. The volume was brought to the mark with double-distilled distilled water. Then 10.00 mL of the resulting solution was taken and transferred to a 100 mL conical Erlenmeyer flask, 1 mL of a 0.01 mol/L H_2SO_4 solution was added and, with vigorous stirring, 2 ml of a 5% KI solution. The released free iodine was immediately titrated with 0.01 mol/L sodium thiosulfate solution. Based on the results of three repeated experiments, the molar concentration of $KHSO_5$ was calculated using the formula:

$$c(KHSO_5)=V(Na_2S_2O_3)\times 0.0100\times 100.00/10.00\times 10.00\times 2.$$

Procedure for studying the kinetics of the Lincomycin oxidation reaction with $KHSO_5$. 10.00 mL of 0.01 mol/L Link hydrochloride solution, 60 ml of buffer solution and 10.00 mL of 0.04 M $KHSO_5$ solution were added to a 100 mL volumetric flask; the volume was finally diluted with water to mark and mixed thoroughly, recording the beginning of the reaction with a stopwatch. After a certain period (30 sec, 1 min, 3 min, 5 min, 10 min, 20 min), 10.00 mL of the test mixture was quickly added to the Erlenmeyer flask, in which 1 mL of 1 mol/L H_2SO_4 was already found. Immediately after that, 1 mL of a 5 % potassium iodide solution was added with shaking. The released iodine was titrated with 0.02 M thiosulfate by adding 1 mL of 1 % starch solution near the endpoint. Similarly, blank determinations are performed with water instead of the Link solution (responsible for the disclosure of $KHSO_5$ at the initial moment of the reaction).

Fourier Transform Infrared (FT-IR) Spectral Analysis. Samples were scanned at the functional group region (4000–650 cm^{-1}) using a Shimadzu FT-IR spectrometer (Shimadzu,

Kyoto, Japan). Tablets were prepared by mixing 200 mg of potassium bromide and 2 mg of the test compound (1% concentration) followed by compression in the standard manner.

Oxidation of lincomycin and isolation of its derivatives. Using (0.02 mol) of $KHSO_5$ (6.14 mg Oxone; 10 mL 2 mM) and Lincomycin HCl (4.7 mg; 10 mL 1 mM), reaction in 20 mL of aqueous solution at 25°C after 30 min gave Lincomycin sulfone. The reaction mixture after neutralization to pH 6 with 2 M NaOH was cooled, filtered and the solvent distilled off under reduced pressure. To the residue was added 20 mL of methanol and thoroughly mixed, the solution was separated from the insoluble part of the residue by filtration and the solvent was distilled of under reduced pressure. The raw product was vacuum dried over P_4O_{10} at 25°C and 13.3 Pa. Final product was as white amorphous material with m.p. 76-80°C (dec.) (Lit. 76-79; 76-78; 78-80°C [27]. FTIR-ATR Spectrum shows characteristic principal peaks at wavenumbers, cm^{-1} (KBr disk): 1146 ν_s (SO_2 group), 1295 ν_{as} (SO_2 group), 1524 (amid 2 group), 1654 (amid 1 group).

Method preparation of Lincomycin N-oxide sulfone. To an aqueous solution (0.5 mL) of lincomycin hydrochloride ($C_{18}H_{34}N_2O_6S$ HCl, Sigma-Aldrich Co, 100 mg, 0.22 mmol) was added an aqueous solution of Oxone (1.5 mL, 0.7 mmol $KHSO_5$), stirred for 30 min at room temperature after which the mixture was neutralized to pH 8.5 using 2 M NaOH solution. The mixture was shaken for 1.5 h at room temperature. After acidification to pH 6 with 2 M HCl, the reaction mixture was cooled then all procedures were performed as previously described in the above described procedure for the preparation of the sulfone derivative of lincomycin. The final product was a white amorphous material, m.p.128-131°C. According to [28] the product is a mixture of *R*- and *S*-isomers of Lincomycin *N*-oxide sulfones.

FTIR-ATR Spectrum shows characteristic peaks at 1146 and 1295 cm^{-1} corresponding to sulfone group and at 926 cm^{-1} corresponding to the *N*-oxide group which is absent in the spectrum of pure Lincomycin. Other bands were observed at 1524 (amid 2 group), 1654 (amid 1 group).

The strength of this band near 926 cm^{-1} have caused us to assign this band to the N-O stretching frequency in this compound despite the previous assignments. This might not necessarily indicate an inconsistency, as it is widely recognized that the heterocyclic N-oxides exhibit significant differences in physical and chemical properties compared to the trialkyl amine oxides. The pyrimidine and pyridine N-oxides also show no consistent absorption in the 950 cm^{-1} region previously stated to be characteristic of the N-O stretching vibration.

The melting point was determined by the open capillary method on a PTP-M instrument (instrument for determining the melting point of solids).

Procedure for determination of Lincomycin hydrochloride in model (reference) solutions. 10.00 mL of a solution containing 1-5 mg of Link hydrochloride was pipetted into a 100 mL Erlenmeyer flask. After this, 5.00 mL of 0.008 mol/L $KHSO_5$, 5.0 ml of 0.2 mol/L buffer solution (pH 9.2), and 1 mL of 0.02 mol/L NaOH were added successively with stirring and left for a particular time (6 min) at room temperature to complete the oxidation of the drug. To lower the pH, 2.0 mL of 1.0 mol/L H_2SO_4 solution and then 1 mL of 10 % potassium iodide were added while stirring the flask contents. This mixture was left for about 10 seconds. The released iodine was titrated with 0.01 mol/L thiosulfate, while 1 mL of 1 % starch solution was added near the endpoint. The whole procedure was also applied in a blank determination on water.

Procedure for the quantitative determination of the Lincomycin hydrochloride content in the substance. About 0.443 g (precise weight) of the substance powder was dissolved in 70 mL of distilled water in a 100 mL volumetric flask, brought to the mark with double-distilled water and mixed thoroughly. 10.00 mL of the resulting solution was transferred into a 100 mL volumetric flask, 70 mL of a buffer solution with pH 9.0 and 10.00 mL of $KHSO_5$ (0.04 M) was added; the volume of water was necessarily diluted to 100 mL and mixed thoroughly. After 6 minutes, 10.00 mL of the reaction mixture was quickly added to the Erlenmeyer flask with 2 mL of 1 mol/L H_2SO_4 solution. Immediately after that, 2 mL of 5% potassium iodide solution was added with shaking. The released iodine was directly titrated with 0.01 M thiosulfate by adding 1 mL of 1 % starch near the endpoint. The whole procedure was also applied in a blank determination on water.

The Link hydrochloride content in the substance in terms of the anhydrous $C_{18}H_{34}N_2O_6S{\cdot}HCl$ (w, %) was found by the formula:

$$w = ((V_0–V)\times T\times 100\times 100\times 100\%\times 100\%)/ 10\times 10\times a\times(100– w(H_2O)),$$

where V_0 – a volume of 0.0100 mol/L standard sodium thiosulfate solution used for titration in the control experiment, mL;

V – a volume of 0.0100 mol/l standard sodium thiosulfate solution used for titration in the work experiment, mL;

100 and 100 – volumetric flasks volumes, mL;

10 – a volume of dosage form solution taken for analysis, mL;

a – a weight of the substance sample, g;

$w(H_2O)$ – water content in the substance (according to the results of semi-micro determination according to Fisher), % (from 3.1 to 4.6%);

10 – a volume of the reaction mixture taken for titration, mL;

T – the amount of Link hydrochloride corresponds to 1 mL of a standard 0.0100 mol/L thiosulfate solution, g/mL.

1.00 mL of a 0.0100 mol/l standard sodium thiosulfate solution corresponds to 0.00073833

g/ml Link hydrochloride ($C_{18}H_{34}N_2O_6S$ HCl), which should be 89.5-102.0% in the preparation in terms of anhydrous substance

Procedure for the quantitative determination of Lincomycin content in a 30 % solution for injection. 0.75 mL of the test solution of the dosage form is taken using a micropipette and transferred to a 100 mL volumetric flask; the volume is brought to the mark with double-distilled water and mixed thoroughly. 20 mL of the solution is taken with a pipette. Then the analysis is performed by the "Procedure for the quantitative determination of the Link hydrochloride content in the substance".

The content of Link hydrochloride in solution for injection 30% in mg in terms of Link hydrochloride anhydrous substance in mL, X, was found by the formula:

$$X = (V_0 - V) \times 0.73833 \times 50/0.75$$

where V_0 – a volume of 0.0100 mol/L standard sodium thiosulfate solution used for titration in the control experiment, mL;

V – a volume of 0.0100 mol/L standard sodium thiosulfate solution used for titration in the work experiment, mL;

50 – dilution factor;

0.75 – a volume of injection solution taken for analysis, mL;

0.73833 – the amount of mg Link hydrochloride ($C_{18}H_{34}N_2O_6S \cdot HCl$), corresponding to 1.00 mL of a standard 0.0100 mol/L sodium thiosulfate solution, g/mL.

Link hydrochloride ($C_{18}H_{34}N_2O_6S \cdot HCl$) in the preparation should contain 285-315 mg of anhydrous substance in 1 mL of solution.

Procedure for the quantitative determination of the Lincomycin content in capsules "Lincomycin" 250 mg (AT "Kyivmedpreparat", Ukraine). About 0.335 mg (accurately weighed) of the 20 capsules content was dissolved in a 100 mL volumetric flask in 70 mL of double-distilled water, diluted with water to the mark and mixed thoroughly. 20 mL of the solution is taken with a pipette. Then the analysis is performed by the "Procedure for the quantitative determination of the Link hydrochloride content in the substance".

Link hydrochloride content in the substance in terms of the average weight of the capsule content was found by the formula:

$$X = ((V_0 - V) \times T \times 100 \times 100 \times \bar{m})/20 \times 10 \times a,$$

where V_0 – a volume of 0.0100 mol/L standard sodium thiosulfate solution used for titration in the control experiment, mL;

V – a volume of 0.0100 mol/L standard sodium thiosulfate solution used for titration in the work experiment, mL;

100 and 100 – volumes of volumetric flasks, mL;

20 –a volume of dosage form solution taken for analysis, mL;

a – sample weight of capsules content of the, mg;

$\bar{m}$ – the average weight of the capsule content, mg;

10 – a volume of the reaction mixture taken for titration, mL;

T – the amount of Link hydrochloride corresponds to 1 mL of a 0.0100 mol/L standard thiosulfate solution, mg/mL.

1.0 mL ml of a standard 0.0100 mol/L sodium thiosulfate solution corresponds to 0.73833 mg/mL of Link hydrochloride ($C_{18}H_{34}N_2O_6S \cdot HCl$), which should be 238.0-262.0 mg in the preparation, calculated on the average weight of the capsule content (anhydrous substance).

Results and discussion

The results of studying the kinetics of the reaction of *S,N*-oxidation of Link with $KHSO_5$ in an aqueous medium depending on the pH medium by the iodometric titration method (according to the consumption of the oxidizing agent) are presented in **Figure 2.**

As can be seen from **Figure 2**, at pH 8-9, after 20 minutes, the completion of the Link oxidation is achieved (stoichiometric consumption of the oxidizing agent: 3 mol of the oxidizing agent is consumed per 1 mol of Link). The relationship between the titration end points obtained by the proposed method and the amount of Link was investigated. Linearity was observed between the amount of Link and the endpoint of the titration from a correlation coefficient of 0.999, indicating that the reaction between $KHSO_5$ and the investigated Link proceeds stoichiometrically in a molar ratio of 3:1.

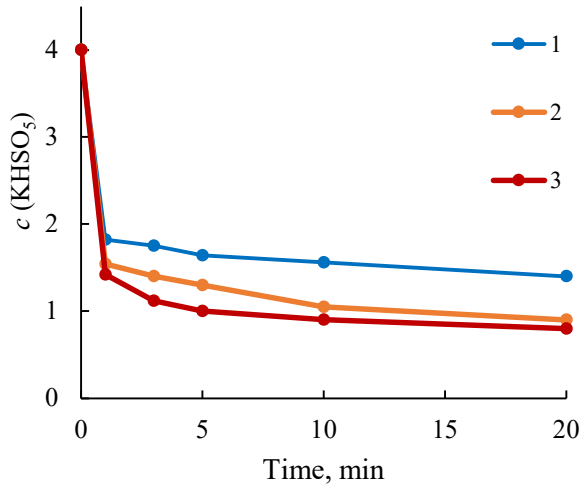

Figure 2. Kinetic curves of the oxidation reaction of Link with $KHSO_5$ depending on pH.
$c(KHSO_5) = 4.0\ 10^{-3}$ mol/L; c(Link) = $1.0\ 10^{-3}$ mol/L;
pH: *1* – 7.0; *2* – 7.9; *3* – 9.0

Kinetic curves semilogarithmic anamorphoses of the Link with $KHSO_5$ oxidation reaction depending on the pH (**Figure 3**) are linear, which indicates a second order reaction.

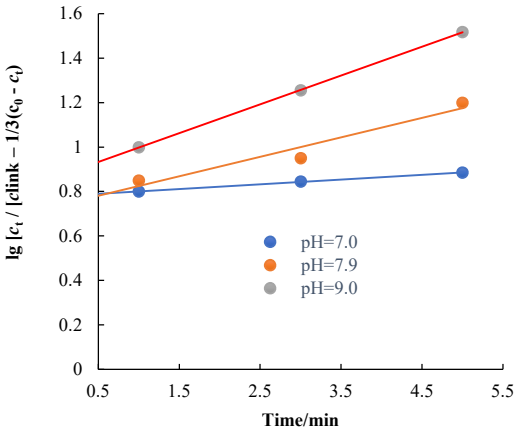

Figure 3. Semilogarithmic anamorphosis of the kinetic curves of the Link with $KHSO_5$ oxidation reaction.
c_{Link} =1.0×10^{-3} mol/L; $c(KHSO_5) = 4.0\times10^{-3}$ mol/L

The slopes of these dependencies are the k_{obs} of the Link *S,N*-oxidation reaction. The highest reaction rate was observed at pH 9. At the

same pH value, the maximum product of the Link base and the HSO_5^- monoanion and partially the SO_5^{2-} dianion mole fractions were observed.

The formula expresses the product of reacting particles mole fractions:

$$\alpha(Link^0) \times [\alpha(HSO_5^-) + (\beta \times \alpha(SO_5^{2-})].$$

where, $\alpha(Link^0) = 10^{-7.8} / (10^{-7.8} + 10^{-pH})$;

$\alpha(HSO_5^-) = (10^{-0.4} \times 10^{-pH}) / [(10^{-pH})^2 + (10^{-0.4} \times 10^{-pH}) + (10^{-0.4} \times 10^{-9.4})$;

$\alpha(SO_5^{2-}) = (10^{-0.4} \times 10^{-9.4}) / [(10^{-pH})^2 + (10^{-0.4} \times 10^{-pH}) + (10^{-0.4} \times 10^{-9.4})$.

The ratio of the oxidation of Link by the dianion and the monoanion rate constants was taken by us as $\beta = 0.2$.

It can be assumed that the non-protonated form of the tertiary Nitrogen base Link ($Link^0$), hydrogen peroxomonosulfate monoanion (HSO_5^-), and partially hydrogen peroxomonosulfate dianion (SO_5^{2-}) are involved in the reaction. Based on this assumption, we derived the kinetic equation for the reaction:

$$Rate = k_{obs} \times c(Link^0) \times c(KHSO_5)$$

$$k_{obs} = K\,\alpha(Link^0) \times [\alpha(HSO_5^-) + 0.2\,\alpha(SO_5^{2-})],$$

where, $\alpha(Link^0)$ – mole fraction of Link base;

$\alpha(HSO_5^-)$ – mole fraction of monoanion;

$\alpha(SO_5^{2-})$ – mole fraction of dianion,

which are respectively equal to:

$\alpha(Link^0) = K_a / (K_a + [H^+]) = \alpha(Link) = 10^{-7.8} / (10^{-7.8} + 10^{-pH})$;

$\alpha(HSO_5^-) = (10^{-0.4} \times 10^{-pH}) / [(10^{-pH})^2 + (10^{-0.4} \times 10^{-pH}) + (10^{-0.4} \times 10^{-9.4})$;

$\alpha(SO_5^{2-}) = (10^{-0.4} \times 10^{-9.4}) / [(10^{-pH})^2 + (10^{-0.4} \times 10^{-pH}) + (10^{-0.4} \times 10^{-9.4})$,

where K_a – dissociation constant of the Link acidic form.

It should be taken into account that

$pH = -\lg [H^+]$, and $[H^+] = 10^{-pH}$,

$pK_a = -\lg K_a$, and $K_a = 10^{-pKa}$,

$pK_a(Link) = 7.8$.

$KHSO_5$ has two dissociation constants, expressed by the following pK_a values: $pK_{a1} = 0.4$ and $pK_{a2} = 9.4$.

The linear dependence k_{obs} on the production of the Link base mole fraction by the sum of the HSO_5^- mole fractions and partially the SO_5^{2-} (**Figure 4**) indicates the correct assumption that it is the Link base and mainly the HSO_5^- that take part in the reaction (correlation coefficient R = 0.9, which is close to 1).

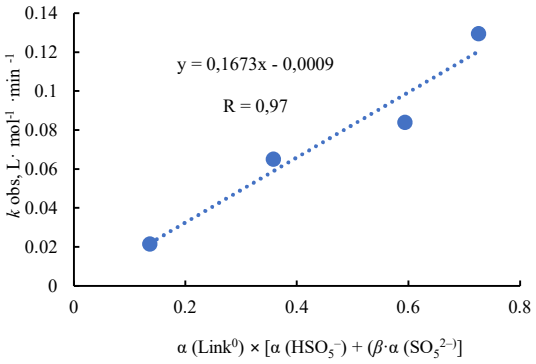

Figure 4. Dependence of the observed second order constant of the Link oxidation reaction on the product of the Link base molar fractions and the sum of the peroxomonosulfate monoanion and partially the peroxomonosulfate dianion molar fractions

There are two possible sites for oxidative attack in lincomycin: the thiomethyl group and the pyrrolidine nitrogen. Reaction products were isolated and identified by FT-IR spectroscopy as

lincomycin sulfone and lincomycin sulfone *N*-oxide.

Comparison of the spectroscopic data of isolated reaction products shows that the intermediate product is Lincomycin sulfone, and the final product formed in an alkaline medium is Lincomycin *N*-oxide sulfone.

It has been shown that both compounds are also formed by chemical oxidation with hydrogen peroxide in an acidic medium: this reaction represents a new efficient way to obtain lincomycin sulfoxide and lincomycin sulfone. The reaction products were isolated and identified by MS and NMR spectroscopy as lincomycin sulfoxide and lincomycin sulfone [29]. Oxidation of lincomycin with H_2O_2 in alkaline media leads to N-oxides, besides the conversion of thiomethyl group into sulfoxides and sulfones. NH_4OH favors formation of the S-isomer; both *R*- and *S*-isomers of the *N*-oxide are formed in the presence of NaOH [28].

Based on the data obtained, the process of Link oxidation with hydrogenperoxomonosulfate can be represented by the scheme (**Figure 5**).

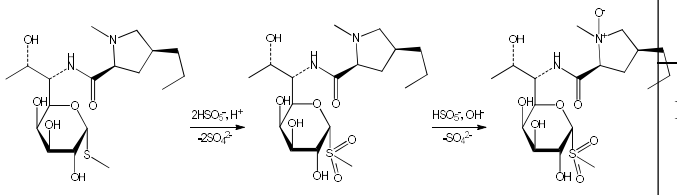

Figure 5. Scheme of the oxidation process of Link with hydrogen peroxomonosulfate

We used the obtained results of studying the reaction kinetics as the basis for developing a new method for the quantitative determination of Link by the reaction of *S*,*N*-oxidation using $KHSO_5$ as an analytical reagent.

The results of titrimetric determination of the Link content in model Link hydrochloride solutions using Oxone are given in **Table. 1**. For 1.11 mg and 4.42 mg contents, RSD was 2.85 and 0.90%, respectively.

Table 1. Titrimetric determination results of Link hydrochloride content in model solutions using oxone (n = 5; P = 0.95)

Taken, mg	**Found $\bar{x}\pm\Delta\bar{x}$, mg**	**RSD, %**	$\delta = \frac{\bar{x}-\mu}{\mu^*}100\%$, %
1.11	1.13±0.04	2.85	+1.8
2.21	2.23±0.05	1.80	+0.9
4.42	4.45±0.05	0.90	+0.7

*μ – known Link hydrochloride content in the model solution, mg.

The quantitative determination results of Link hydrochloride content in a substance are given in **Table 2.** RSD < 1.06%.

Table 2. The iodometric determination results of the Link hydrochloride content in the substance using $KHSO_5$ (n = 5; P = 0.95)

Analyzed substance	Declared content, w, %	Found $\bar{x}\pm\Delta\bar{x}$, mg	RSD, %	$\delta = \frac{\bar{x}-\mu}{\mu^*}100\%$, %
Lincomycin Hydrochloride (FUJIFILM Wako Pure Chemical Corporation)	98*	99.0±1.3	1.06	+1.0
Lincomycin hydrochloride (Fengchen Group CO., LTD, China)	99.6*	100.1±1.1	0.89	+0.50

* Data from the official HPLC method [3], µ Quantification (should be): 89.5-102.0% (based on dry matter, free of residual solvents)

Table 3. The quantitative determination results of the Link content in a solution for injection and capsules by the proposed method (n = 5; P = 0,95)

Analyzed substance, dosage form	Found $\bar{x}\pm\Delta\bar{x}$, mg/ml Recovery, %	RSD, %	μ*, mg/ml	$\delta = \frac{\bar{x}-\mu}{\mu^*}100\%$, %
Lincomycin hydrochloride, "Lincomycin Zdorovye" injection 300 mg/ml in 1 ml ampoules; pharmaceutical company "ZDOROVYE" (Ukraine), Series No. 10221	314.3±6.6 Re=105±2.2	1,70	311,4[1]	+0.9
Lincomycin hydrochloride, "Lincomycin-Darnitsa" injection solution 300 mg/ml in 2 ml amp.; Pharmaceutical firm 'DARNITSA', Series UU20920	292.3±6.0 Re=97.4±2.0	1.65	290.6[2]	+0.6
Lincomycin hydrochloride, "Lincomycin" capsules of 250 mg in terms of Link; AT "Kyivmedpreparat (Ukraine)", Series 0018613	250.6±3.4 Re=100.2±2.0	1.70	246[3]	+1.9

* Quality certificate data (HPLC); should be [1]95%.....105% (from 285 mg to 315 mg); [2]90%.....120% (from 270 mg to 360 mg); [3]95%.....105% (from 238 mg to 262 mg) Link hydrochloride in terms of Link base.

The quantitative determination results of the Link content in a solution of 30 % for injection and capsules "Lincomycin" 250 mg are given in **Table 3**. RSD = 1.65-1.7%.

Conclusions

1. The kinetics of the oxidation reaction of Lincomycin hydrochloride with $KHSO_5$ was studied depending on the pH of the medium. It has been established that the reaction kinetics obeys the general laws of specific acid-base catalysis.
2. Peroxomonosulfate reacts quantitatively with Lincomycin in an alkaline medium to form the corresponding sulfone-*N*-oxide: 1 mole of Link requires 3 moles of $KHSO_5$.
3. Methods for the oxidimetric determination of Lincomycin using $KHSO_5$ ($KHSO_5$) as a stable potassium triple salt – oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) as an analytical reagent have been developed. A known excess of the reagent is added and after a specified time, its residual amount is determined by iodometry.
4. The possibility of Lincomycin hydrochloride quantitative determination in the substance, as well as the determination of Link hydrochloride solution for injection 300 mg/ mL in 1 mL ampoules and capsules of 0.25 g, was shown. RSD does not exceed 1.7%.

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