Quantitative Determination of Decamethoxine in Pharmaceutical Preparations by its Inhibitory Effect on the Enzyme Cholinesterase

M. Ye. Blazheyevskiy†, O. V. Koval'ska†*, V. V. Diadchenko‡

† National University of Pharmacy, Ministry of Health of Ukraine, Kharkiv, Ukraine;
‡ Military Institute of Armored Forces of National Technical University "Kharkiv Polytechnic Institute", Kharkiv, Ukraine;
*e-mail: lena05021985@ukr.net

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A simple and sensitive kinetic-spectrophotometric method was developed for quantifying decamethoxine (DM) in ear/eye drops and antiseptic solutions. The assay is based on inhibition of acetylcholinesterase (AChE) by DM, followed by kinetic measurement of the residual acetylcholine via peracetic acid formation and its reaction with p-phenetidine, monitored at 358 nm (logε = 4.2). Reaction rate constants were determined from kinetic curves in the presence and absence of the inhibitor, and inhibition degree (U, %) was calculated and used for calibration. A linear relationship was observed in the range 1–6 ng/mL: U % = (11.1 ± 1.3) × C + (7.5 ± 4.5) (r = 0.996). The LOQ, corresponding to 20 % inhibition, was 1.0 ng/mL. The method was validated for selectivity, precision (RSD ≤ 2.5 %), and accuracy, and demonstrated suitability for routine analysis of pharmaceutical formulations.

Keywords: decamethoxine, ophthalmodec, auridexan, decasan, cholinesterase, acetylcholine

Кількісне визначення декаметоксину у фармацевтичних препаратах за його інгібіторною дією на фермент холінестеразу

М. Є. Блажеєвський†, О. В. Ковальська*†, В. В. Дядченко‡

† Національний фармацевтичний університет, Міністерство охорони здоров'я, Харків, Україна;
‡ Військовий інститут танкових військ Національного технічного університету "Харківський політехнічний інститут", Харків, Україна; *e-mail: lena05021985@ukr.net

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Розроблений простий і чутливий ферментний кінетико-спектрофотометричний метод кількісного визначення декаметоксину (ДМ) у вушних та очних краплях і антисептичному препараті. Інгібування ацетилхолінестерази (АХЕ) ДМ змінює кількість непрореагованого ацетилхоліну (АХ), який кількісно визначають через утворення надоцтової кислоти та її реакцію з п-фенетидином, реєструючи поглинання при 358 нм. Швидкості реакцій обчислювали з кінетичних кривих, за ними визначали ступінь інгібування (U, %), що використовували для калібрування. Отримано лінійну залежність у діапазоні 1–6 нг/мл (U, % = (11,1 ± 1,3) × C + (7,5 ± 4,5); r = 0,996), межа кількісного визначення — 1,0 нг/мл. Метод валідовано на селективність, точність (RSD ≤ 2,5 %) та правильність, підтверджено його придатність для аналізу лікарських форм.

Ключові слова: декаметоксин, офтальмодек, аурідексан, декасан, холінестераза, ацетилхолін

Introduction

Decamethoxine (DM), (1,10-decanediamine, N,N,N',N'-tetramethyl-N,N-bis((2-methyl-5-(1-methylethyl)cyclohexyl)methyl)-, dichloride) is a synthetic antiseptic and antifungal drug for local use. It is a bisquaternary ammonium compound. In terms of chemical structure and antimicrobial action, it is close to etonium (Fig. 1).

Decamethoxine is used for purulent and fungal lesions of the skin (abscesses, purulent wounds, candidiasis, etc.), for proctitis, gingivitis, periodontitis, tonsillitis, otitis, and other purulent processes. It is used endobronchially for lung diseases. In ophthalmology, in the form of eye drops, it is used for conjunctivitis and blepharoconjunctivitis and for treating contact lenses. Decamethoxine is also used for sterilization of medical instruments, devices, suture material, rubber gloves and for chemical sterilization and preservation

of bone-tendon grafts [1-3].

Decamethoxine is produced in the form of tablets to dissolve in the mouth, 0.2 mg.; 0.2% solution; eye drops of an aqueous solution and ear drops of a 0.05% alcohol solution of 5 and 10 mL each [4-5].

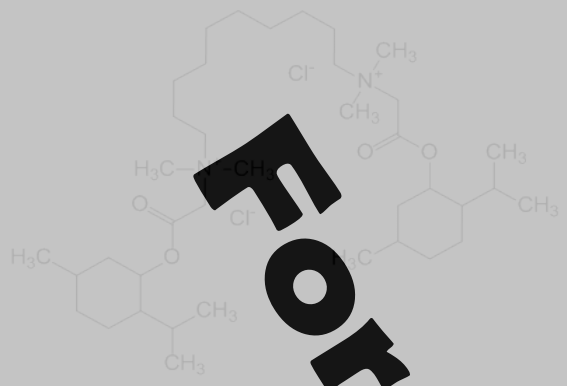

Fig.1. Structural formula of Decamethoxine.

"Dekasan"® - 0.02% solution of decamethoxine for inhalation. It has a powerful antibacterial, antiviral and fungicidal action. It also additionally has an antispasmodic, anti-inflammatory and desensitizing effect. Potentiates the action of antibacterial drugs. Does not have a toxic effect on the human body. An indispensable component of the complex treatment of infectious diseases of the respiratory tract in patients of all age groups.

Decamethoxine (at a concentration of 0.2 mg/ml.) — the active substance of the drug «Dekasan» of the Ukrainian company «Yuria-Pharm» — exerts a pronounced virucidal effect on the SARS-CoV-2 virus (coronavirus), which causes the coronavirus disease COVID-19, with prolonged exposure in 60 s, which is expressed in a decrease in the infectious titer of the virus. These data were recently included in the instructions for medical use of the drug Dekasan. Thus, decamethoxine, the active substance of inhaled «Decasan», reduces the viral load on the surfaces of the mucous membrane of the respiratory tract [6].

In medicinal preparations, DM is usually contained in relatively small quantities and is part of multicomponent medicinal forms [7]. This creates certain difficulties during its quantitative determination. At present, the method of acid-base titration in a non-aqueous environment is used for the quantitative analysis of the DM substance [8-9].

The absence of selective absorption in the UV part of the spectrum of Non-aqueous titration is the most common titrimetric procedure used in the pharmaceutical assays of many drugs [7, 10-12].

Non-aqueous titrations are widely used in Volumes I and II of the British Pharmacopeia for the assay of drug substances. A large number of drugs are either weakly acidic or weak base. The weak acids are usually titrated with tetrabutylammonium hydroxide (Bu_4NOH) or potassium methoxide (CH_3OK) in dimethyl formamide (DMF) as a solvent. Weak bases are dissolved in glacial acetic acid and titrated with perchloric acid ($HClO_4$). For weak bases, the titration medium usually used for non-aqueous titration of bases is perchloric acid in acetic acid. However, perchloric acid is not a primary standard substance, so it must be standardized using potassium hydrogen phthalate ($KHC_8H_4O_4$) in a glacial acetic acid, and acetous crystal violet as an indicator.

The photocolorimetry method is used for the analysis of medicinal forms of DM [13], extraction photometry [14], as well as spectrophotometry in the form of a DM complex with eosin at 540 nm [9, 10, 15-18]. However, the known method of photometric determination of DM using eosin H does not allow obtaining correct results, since δ > RSD [19].

A film decamethoxine selective electrode is described in the literature [20], as well as solid-contact ISE for ionometric determination of decamethoxine [21].

The method of detection and quantitative determination of dental components is described gel with decamethoxine and lidocaine hydrochloride by HPLC [22].

Previously, a review article was published on the choice of an analytical method for the quantitative determination of decamethoxine in biomedical research. In a comparative aspect, the advantages and disadvantages of known analytical methods for the quantitative determination of quaternary ammonium compounds, in particular decamethoxine in medicines, are discussed [19]. It is shown that a very promising method for the pharmaceutical analysis of dosage forms of quaternary ammonium compounds is the kinetic-spectrophotometric method based on the inhibition of the biochemical reaction of acetylcholine decomposition by the enzyme cholinesterase. Previously, optimal conditions were found in our laboratory [23] and the possibility of quantitative determination of quaternary ammonium compounds, in particular decamethoxine, in model solutions [24] and biological material [25] was shown by this method using the oxidation reaction of p-phenetidine as an indicator.

In the current article, we present the results of the development of the methodology for the quantitative determination of decamethoxine in pharmaceutical preparations of factory production in the presence of various excipients

Experimental part

Materials and methods

Materials

The chemical name of the antiseptic drug «Dekamethoxine®»: 1,10-Decamethylene-bis (N,N-dimethylmenthoxycarbonylmethyl) - ammonium dichloride. CAS: 61192-62-9. Molecular weight 693.9112 g/mol. Research substance DM® «Research production of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine» (Ukraine).

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White fine crystalline powder with a weak specific smell, easily soluble in water and 96% alcohol. The pH of the studied sample of DM ® was determined in the range from 5.5 to 7.5. It was established that this sample has a specific optical rotation from 48 to 51°, which corresponds to the standard of this medicinal product according to the State Pharmacopoeia of Ukraine. The melting temperature of DM ® was in the range of 163–168 °C with decomposition at the temperature of the above interval.

The study of mass loss during drying was carried out in accordance with the requirements of the Federal State of Ukraine. The substance in the amount of 0.5000 g was dried at a temperature from 100 to 105 °C to a constant mass [26].

Quantitative determination was carried out according to the following method. 0.25 g (exact weight) of the substance was dissolved in 10 ml of glacial acetic acid, 10 mL of acetic anhydride was added and titrated potentiometrically with a 0.1 M solution of perchloric acid, using a glass indicator electrode, as a reference electrode – saturated silver chloride electrode. During the quantitative determination, it was established that DM ® ($C_{38}H_{74}Cl_2N_2O_2$) was at least 99.0% based on the dry matter of the substance under study.

For research reagents were used: *p*-Phenetidine (4 – ethoxyaniline, *w*= 98%) (SIGMA - ALDRICH); A0281408 series, New Jersey, USA; *p* - phenetidine hydrochloride (Ph), extracted from the base by hydrogen chloride precipitation in the chloroform solution.

Pharmacopoeial acetylcholine chloride medicine - 0.2 g per amp/5 mL (manufactured by "VECTOR" – State Science Center of virology and biotechnology in Russian Federation" (Russia).

Dry protein drug of cholinesterase from horse serum - 80 mg / fL (VI class), 27 AU / mg (manufactured by SMU "Biomed", Russia).

"Stabilized Hydrogen Peroxide 30-40%" (LLC "Inter - Synthes", Boryslav, Ukraine); The content of hydrogen peroxide was determined by SPU according to the monograph "High-test hydrogen peroxide solution 27.5-31.0% [27].

«Auridexan» ear drops 0.5 mg/ml in 5 mL bottle. with krish.-drop Storage:

active substance: decamethoxine; 1 mL of solution contains 0.5 mg of decamethoxine in 100%; excipient: ethanol (70%). Series No. 20222. Manufacturer: «Experimental Plant State Scientific Center for Medicines» Kharkiv, Ukraine. Limited Liability Company «PHARMAKS GROUP» (Kyiv, Ukraine). Data of Quality Certificate No. 8: «quantification» indicator of decamethoxine (from 0.45 mg to 0.55 mg decamethoxine in 1 ml of the drug) 0.540 mg/mL.

«Oftalmodek», eye drops 0.2 mg/mL, 5 mL in a bottle. active substance: decamethoxine; Composition: 1 mL of solution contains: Decamethoxine (calculated as 100% substance) - 0.2 mg, excipients: sodium chloride, purified water. Series No. 0030222. Manufacturer: Limited Liability Company «Research Plant «State Scientific Center for Medicinal Products» (Kharkiv, Ukraine). Limited Liability Company «PHARMAKS GROUP» (Kyiv, Ukraine). Data of the Quality Certificate of the medicinal product series: «Quantitative determination» indicator of decamethoxine (tolerances: from 0.18 mg to 0.22 mg in 1 mL of the drug) 0.204 mg/mL; sodium chloride (from 8.7 mg to 9.3 mg in 1 mL of the drug) 9.2 mg/mL.

«Dekasan»® (Decasanum) Composition: active substance: decamethoxine; 1 mL of solution contains 0.2 mg of decamethoxine; excipients: sodium chloride, water for injections. Producer «Yuriya-Pharm» (Ukraine). Pharmacotherapeutic group. Antiseptic and disinfectants. ATX code D08A. 2 mL each in a single-dose container; 12 containers in a pack. Series No.: API 501/1. Quantitative determination (from 0.190 mg/mL to 0.210 mg/mL) 0.195 mg/mL.

The enzyme reaction substrate of acetylcholine chloride solution (ACh) preparation. The ampoule's content of pharmacopoeia drug acetylcholine chloride 0.2 g is in 200 mL of double-distilled water dissolved. For that end, open an ampoule, add 4.0 mL of water with pipette, and shake until acetylcholine is completely dissolved. Then pour the acetylcholine solution quantitatively transferred into 200 mL capacity measuring bottle and dilute double-distilled water to the volume.

Cholinesterase solution preparation (ChE). Add 10.0 mL double - distilled water in a flask, containing 30 mg of dry cholinesterase drug, shake up and thermostat for 10 minutes at +38 °C.

Phosphate buffer solution preparation (pH 8.35). Solution 35.75 g of disodium hydrogen phosphate in 500 ml flask and 300 ml double-distilled water, dissolve it, add 12 ml of 0.1 mol/L solution of hydrochloric acid, stir and dilute double distilled water to 500.0 ml. The ready solution pH is potentiometrically controlled.

Hydrogen peroxide solution 10%. It is prepared by the appropriate high-test hydrogen peroxide dilution with double-distilled water. The concentration in the solution was controlled according to the recommended method by permanganatometry [27].

p-phenetidine hydrochloride solution preparation 0.5%. Dissolve 0.50 g of phenetidine hydrochloride in 80 ml of double-distilled water into 100 mL capacity measuring bottle and dilute it to volume.

To measure light absorption we used a single-beam spectrophotometer (SF-26 LOMO) and 1 cm quartz cuvettes.

The solutions were thermostated using an air thermostat TS-80M-2.

pH value was measured at Ionomer I - 160M laboratory (Belarus) by using EGL 43-07 pH glass laboratory electrode together with auxiliary chloride silver electrode of EAL-1M3.1 type, saturated with potassium chloride.

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Analysis procedure

All solutions before the analysis are thermostated for 20 minutes in an air thermostat at +38ºC.

Graduation graphics

Preparation of a solution of a working standard sample (WSS) *of* DM $1 \cdot 10^{-7}$ mol/L. 0.0694 g of Decamethoxin powder is dissolved in 1000.00 mL of double distilled water. Take an aliquot of 10 mL of the resulting solution using a pipette and transfer it to a 1-liter volumetric flask, bring the volume up to the mark and mix thoroughly. Then, using a pipette, take 10 mL of this solution and transfer it to a 100 mL flask, bring the volume up to the mark and mix thoroughly.

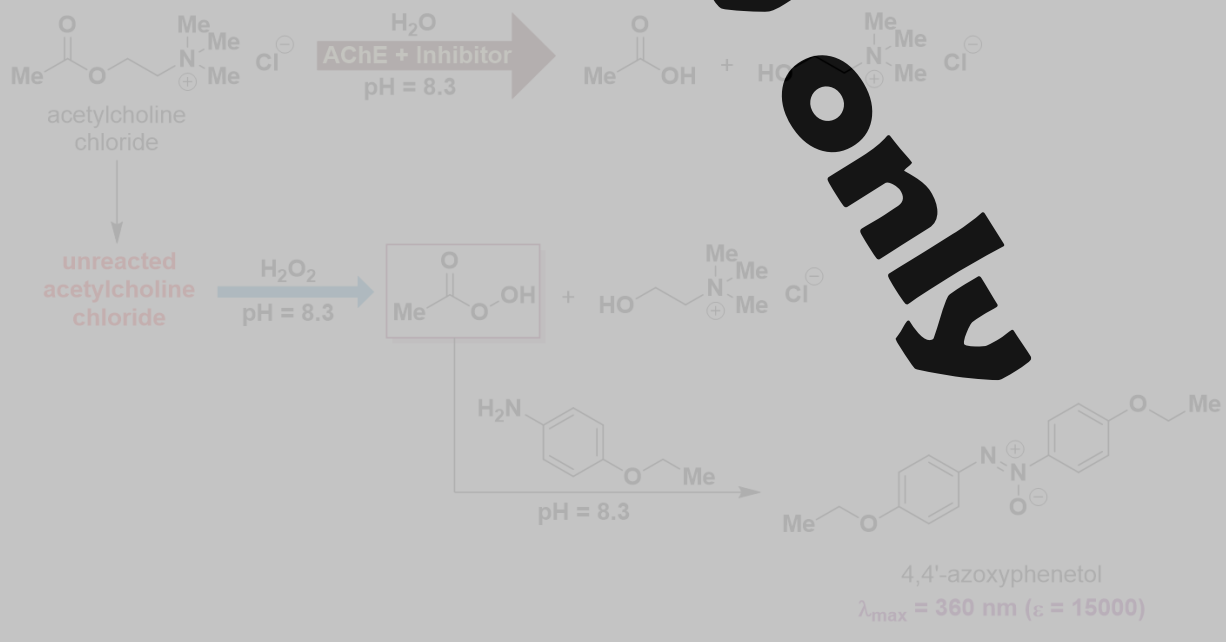
1) 10.00 mL of a buffer solution with a pH of 8.35, 0.50...1.00...1.50 mL of a solution of the work standard sample of decamethoxine (WSS) and 0.50 mL of an acetylcholinesterase solution are successively added to each graduated test tube, shaken thoroughly and kept at +38ºC for 10 min. After that, add 1.00 mL of acetylcholine solution, shake thoroughly and incubate again for 10 minutes at +38ºC, then add 1.60 mL of hydrogen peroxide solution and incubate again for 10 minutes at +38ºC, after that add 1.00 mL of n-Phenetidin's solution and 2.00...1.50...1.50... mL of distilled water, stopper each test tube, shake the solution thoroughly, and record the increase in the optical density of the solution at 358 nm in a 1 cm cuvette on a spectrophotometer for 15 min, the tangent of the linear section tg α (X) in min^{-1} was found according to the plot of the dependence of the optical density on time (that is, the kinetic curve) (Working experiments «Ach+ChE+Inh»).

2) In parallel, two more experiments are carried out: 10.00 mL of buffer solution with a pH of 8.35, 1.00 mL of distilled water, 1.00 mL of acetylcholine solution, 1.60 mL of hydrogen peroxide solution are successively added to a graduated test tube with a polished cork and shaken thoroughly, and then thermostated for 10 min at +38ºC, after which 1.00 mL of p-phenetidine solution, 2.00 mL of distilled water are added, the test tube is stoppered, the solution is thoroughly shaken and the increase in the optical density of the solution is measured at 358 nm in a 1 cm cuvette on a spectrophotometer for 15 minutes. According to the plotted graph of the dependence of the optical density on time (kinetic curve), the tangent of the slope of the linear section tg α (1) is found, in min^{-1} (*control experiment 1 «Ach»)*.

Add 10.00 mL of a buffer solution with a pH of 8.35, 0.50...1.00...1.50 ml of distilled water, 0.50 mL of acetylcholinesterase solution and 1.00 mL of acetylcholine solution to another test tube with a polished cork, and stopper the test tube , shake thoroughly and keep at +38ºC for 10 minutes. Then add 1.60 mL of hydrogen peroxide solution and shake thoroughly, then thermostat for 10 minutes at +38ºC, after which add 1.00 mL of p-phenetidine solution and 2.0 mL of distilled water, stopper the test tube, shake the solution and immediately measure the growth optical density of the solution for 15 min (control 2). According to the plot of the dependence of the optical density on time (kinetic curve), find the tangent of the linear section tg α (2), in min^{-1} (*control experiment* 2 «Ach+ChE»).

Results and discussion

DM is a quaternary ammonium compound, which is capable of inhibiting the enzyme acetylcholinesterase (AChE). In turn, a change in activity of AChE causes a change in the amount of unreacted acetylcholine (ACh) in the enzymatic hydrolysis reaction (Scheme 1).



Scheme 1. Method of the quantitative determination of quaternary ammonium compounds.

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Addition of hydrogen peroxide to the unreacted ACh leads to the formation of equivalent amount of peracetic acid. The latter can react with *p*-phenetidine giving a coloured product which makes it possible to estimate the quantitative content of peracetic acid and subsequently amount of ACh. The method involves photometric detection of the coloured product at λ_{max} = 358 нм ($\log_{10}$ ε =4.2) [21].

In Fig. 2 shows the kinetic curves of the conjugated oxidation of *p*-phenetidine with hydrogen peroxide in the presence of: Ach (1), mixtures Ach+ChE (2) and Ach+ChE+Inh (3-5).

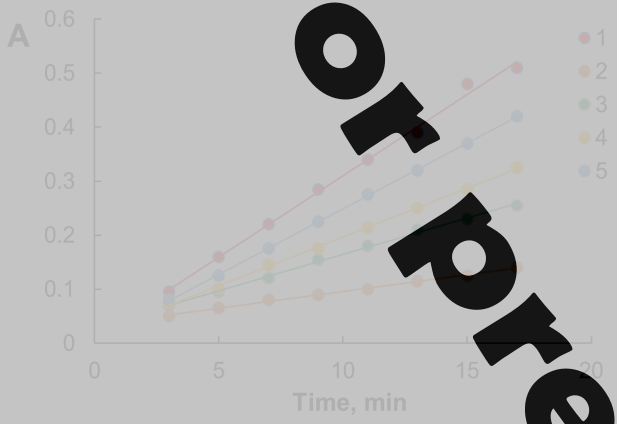

Fig. 2. Kinetic curves of the conjugated oxidation of *p*-phenetidine with hydrogen peroxide in the presence of: Ach (1), mixtures Ach+ChE (2) and Ach+ChE+Inh (3-5). *c* (Ach)=3.3·10^{-4} mol l^{-1}); *w*(H_2O_2)=1%; *w*(AchE)= 0.24 mg/mL; *C*(DM): 2.0 ng/mL (3); 4.0 ng/mL (4); 6.0 ng/mL (5); *w* (*n*-phen) = 0.03%.

Based on the linear sections of the obtained kinetic curves, the tangents of the slope angles were calculated - the conditional values of the reaction speed values, and thus a graded dependence of the degree of inhibition on the final concentration of decamethosine (an inhibitor of the cholinesterase enzyme in the biochemical reaction of the decomposition of acetylcholine) was constructed (Fig. 3). The degree of inhibition was calculated according to the formula:

$$U = \frac{[tg\alpha\,(\text{X}-\text{Inh}) - tg\alpha\,(\text{«Ach+ChE»})]}{[tg\alpha\,(\text{«Ach»}) - tg\alpha\,(\text{«Ach+ChE»})]} \times 100\%$$

where: *tg*α (X-*Inh*) is conditional reaction rate in the working experiment in the Ach– (ChE+Inh) – H_2O_2 – *p*-Phen system at *C*i concentration of the inhibitor (DM), min^{-1};

*tg*α («Ach») is conditional reaction rate in the absence of an inhibitor (DM) and cholinesterase enzyme in system Ach – H_2O_2 – *p*-Phen (*control experiment* 1), min^{-1};

*tg*α («Ach+ChE») is conditional reaction rate in the absence of an inhibitor (DM) in the system Ach – ChE –H_2O_2 – p-Phen (*control experiment 2*), min^{-1};

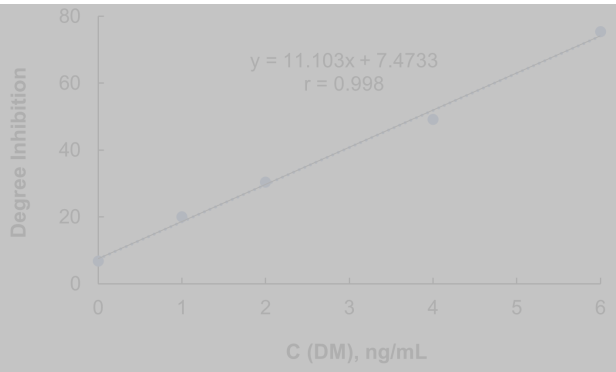

Fig. 3. Dependence of the degree of inhibition on the inhibitor concentration (DM) in the ACh-(ChE+Inh) system.

As can be seen from Fig. 3, the linear dependence of the degree of inhibition on the concentration of the inhibitor in the solution is achieved in the range of 1-6 ng/mL.

The characteristics of the calibration curve of the linear regression equation were as follows: *U*,%= (11.103±1.325)× *C* (DM) + (7.47±4.47) (Tabl. 1).

Table 1. Characteristics of linear regression equation curve calibration graph.

Characteristics	Parameters
Y= bx+a	y = 11.103x + 7.47
Correlation coefficient (r)	0.996
Linear regression eq.	Inh = 11.103 C + 7.47
Slope (b±Δb)	11.103±1.325
Intercept (a±Δa)	7.47±4.47
S.D. of slope (Sb)	0.4164
S.D. of intercept (Sa)	1.406
LOD (3 Sa/b), ng/mL	0.380
LOQ = 10 Sa/b	1.266

Note: S_a- standard deviation of the intercept of regression line; S_b, standard deviation of the slope of regression line.

The LOQ, defined as the concentration corresponding to 20% inhibition, is 1.0 ng/mL.

The obtained research results are the basis for the quantitative determination of decamethoxine in pharmaceutical preparations using the standard method.

The method of quantitative determination of decamethoxine in the tested preparation.

When analyzing «Auridexan» ear drops 0.5 mg/mL using a pipette, 1.00 mL of the drug solution is taken into a 1 liter volumetric flask and diluted with double distilled water to the mark at +20 ºC and thoroughly mixed. The resulting solution is precisely diluted 10 times with twice-distilled water. This solution is used for further research.

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Preparation of a solution of a working standard sample (WSS) *of* Decamethoxine 50 ng/mL.

0.0500 g of Decamethoxine powder is dissolved in 1000 mL of double distilled water. Take an aliquot of 10 mL of the resulting solution using a pipette and transfer it to a 1-liter volumetric flask, bring the volume up to the mark and mix thoroughly. Then, using a pipette, take 10 mL of this solution and transfer it to a 100 mL flask, bring the volume up to the mark and mix thoroughly.

When analyzing «Oftalmodek», eye drops 0.2 mg/mL, as well as «Dekasan»® solution, 1.00 mL of the drug solution is taken with a pipette, put into a 1-liter volumetric flask and diluted with twice-distilled water to the mark at +20 °C and carefully stir. The resulting solution is precisely diluted 4 times with twice-distilled water. This solution is used for further research.

Preparation of a solution of a working standard sample (WSS) *of* Decamethoxine 20 ng/mL.

0.0400 g of Decamethoxin powder is dissolved in 2000.00 mL of double distilled water. Take an aliquot of 10 mL of the resulting solution using a pipette and transfer it to a 1-liter volumetric flask, bring the volume up to the mark and mix thoroughly. Then, using a pipette, take 10 mL of this solution and transfer it to a 100 mL flask, bring the volume up to the mark and mix thoroughly.

1) 10.00 mL of a buffer solution with a pH of 8.35, 2.00 mL of a decamethoxine sample solution and 0.50 mL of an acetylcholinesterase solution are successively added to a graduated test tube with a polished cork, thoroughly shaken and kept at +38ºC for 10 minutes. After that, add 1.00 mL of acetylcholine solution, shake thoroughly and incubate again for 10 minutes at +38ºC; then add 1.60 mL of hydrogen peroxide solution and incubate again for 10 min at +38ºC, after which add 1.00 mL of n-Ph solution and 2.00 mL of distilled water, stopper the test tube, shake the solution thoroughly and record the increase in the optical density of the solution at 358 nm in a cuvette by 1 cm on a spectrophotometer for 15 min. According to the plot of the dependence of optical density on time (kinetic curve), the tangent of the linear section tg α (X) was found, in min^{-1}.

Add 10.00 mL of a buffer solution with a pH of 8.35, 0.50 ml of distilled water, 0.50 mL of acetylcholinesterase solution and 1.00 mL of acetylcholine solution to another test tube with a polished cork, stopper the test tube, shake thoroughly and keep at +38ºC within 10 min. Next, add 1.60 mL of hydrogen peroxide solution and shake thoroughly, and then thermostat for 10 minutes at +38ºC, after which add 1.00 mL of p-phenetidine solution and 2.0 mL of distilled water, stopper the test tube, shake the solution and immediately measure the growth optical density of the solution for 15 min (control 2). According to the plot of the dependence of the optical density on time (kinetic curve), the tangent of the linear section tg α (2) was found, in min^{-1}.

In parallel, an experiment with a solution of a working standard sample (WSS) of decamethoxine is performed in the same way as an experiment with a tested solution of a decamethoxine sample.

The tangent of the angle of inclination of the linear section was found according to the constructed graph of the dependence of the optical density on time (kinetic curve) tg α (st), y min^{-1} (Working experience with WSS) «Ach+ChE+Inh-Standard»).

The content of decamethoxine, *X*, mg/mL, calculated according to the formula:

$$X = \frac{Cst\,[tg\alpha\,(X) - tg\alpha\,(2)]}{tg\alpha\,(St) - tg\alpha\,(2)\}} \times k,$$

C_{st} is concentration of decamethoxine WSS solution, ng/ml.

k is dilution factor (for «Auridexan» ear drops 0.5 mg/ml k=10000; for «Oftalmodek», eye drops 0.2 mg/ml, and «Dekasan»® solution k=4000).

The results of the analysis of dosage forms of Decamethoxine according to the proposed method by the kinetic-spectrophotometric enzyme method are given in Table 2. As you can see, the relative standard deviation does not exceed 2.5% when correct (δ) – 1.1…+1.6%. $\delta = |(\bar{x} - \mu)100\% / \mu| < t_{\alpha} \times RSD/\sqrt{n}$. ($n = 5$; $P = 0.95$).

In terms of sensitivity and selectivity, the developed method surpasses all the photometric methods considered above [18]. The relatively high sensitivity of the developed method was accompanied by fairly good reproducibility. At the same time, the developed method does not require expensive equipment, specially trained personnel and complies with the principles of «green chemistry».

Conclusions

Highly sensitive methods have been developed and the possibility of quantitative determination of decamethoxine in ear and eye drops, as well as in an antiseptic preparation with a relative standard deviation not exceeding 2.5%, has been shown. The LOQ, evaluated as the concentration corresponding to 20% inhibition, is 20 ng/mL. The possibility of quantitative determination of DM in factory-produced pharmaceuticals in the presence of various excipients was demonstrated. The correctness of the obtained results was assessed by comparing the average found (with that of the independent recommended method (μ). $|(\bar{x} - \mu)100\% / \mu| < t\alpha \times RSD/\sqrt{n}$. (n = 5; P = 0,95).

In terms of sensitivity and selectivity, the developed method surpasses all the photometric methods considered above. At the same time, the developed method does not require expensive equipment, specially trained personnel and complies with the principles of “green chemistry”.

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Table 2. The results of the analysis of dosage forms of Decamethoxine according to the proposed method by the kinetic-spectrophotometric enzyme method (n = 5; P = 0.95).

Detected substance/ - analyzed drug	Found ($\bar{x} \pm \Delta\bar{x}$), mg/mL	RSD, %	Data of Quality Certificate: «quantification» (μ*)	$((\bar{x} - \mu))/(\mu^*)$ 100 (%)
Decamethoxine / – «Auridexan» Auridexan ear drops 0.5 mg/mL bottle 5 ml, No. 1; Experimental Plant State Scientific Center for Medicines/ Harkiv, Ukraine	0.521±0.014	2.17	0.540	– 1.10
Decamethoxine / «Oftalmodek», eye drops 0.2 mg/mL, 5 ml in a bottle. Limited Liability Company "Research Plant" «State Scientific Center for Medicines/Harkiv, Ukraine	0.202±0.006	2.39	0.204	– 0.98
Decamethoxine / «Dekasan»® 0.2 mg/mL (Producer «Yuriya-Pharm» (Ukraine).	0.198±0.006	2.52	0.195	+1.59

* Data of the official method specified in the Certificate, *μ*

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