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STRUCTURE OF CHEMICAL COMPOUNDS, METHODS OF ANALYSIS AND PROCESS CONTROL

DEVELOPMENT AND VALIDATION OF A METHOD FOR DETERMINING LIDOCAINE HYDROCHLORIDE AND MIRAMISTIN IN AN INNOVATIVE WOUND-HEALING GEL

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An HPLC-UV method for quantitative determination of lidocaine and miramistin in a combination wound-healing gel is reported. The method was validated for specificity, linearity, accuracy, and precision. The linear range for lidocaine hydrochloride was 0.08 – 0.12 mg/mL; for miramistin, 0.04 – 0.06 mg/mL. The linear correlation coefficients were 0.9992 and 0.9996 for lidocaine hydrochloride and miramistin, respectively. The method could be used for quantitative evaluation of lidocaine and miramistin in combination medicines.

Keywords: lidocaine hydrochloride, miramistin, wound-healing gel, chitosan, HPLC.

The wound-healing gel CCCML (complex chitosan – chymopsin – miramistin – lidocaine) was developed at Sechenov University and is a combination medicine based on the complex chitosan – chymopsin with the broad spectrum antimicrobial miramistin and the anesthetic lidocaine as the active ingredients [1 – 6].

An example of quantitative determination of lidocaine in pharmaceuticals is nitrite titration with tropeolin OO indicator mixed with methylene blue [7, 8]. The pharmacopoeial method for determining lidocaine uses potentiometric titration [9]. Lidocaine hydrochloride in the dosage form Diklizol was determined by spectrophotometry at 230 and 280 nm with relative uncertainty of ± 1.5% [10, 11]. Lidocaine in Septalena tablets was analyzed (uncertainty ± 0.8%) by recording the second-derivative absorption spectrum of a solution in HCl (0.1 M) at 200 – 300 nm [10]. Lidocaine was determined by an HPLC method with diode-array detection using 17 samples of anesthetizing drugs and model mixtures [12 – 19]. Miramistin in aqueous pharmaceuticals was determined by spectrophotometry [20, 21] although the main quantitative analytical method is HPLC with UV detection [22, 23].

Existing procedures had to be adapted to quantitative determination of lidocaine and miramistin in a wound-healing gel because the dosage form includes two active pharmaceutical ingredients that can affect the analytical results.

The goal of the present work was to develop and validate a quantitative HPLC method for determining miramistin and lidocaine in a wound-healing gel.

EXPERIMENTAL PART

Model gels prepared from drug substances chitosan (NPO Bioprogress, Russia), chymopsin (MPM 42-0179-5944-04, Samson-Med, Russia), lidocaine hydrochloride (G. Amphray Laboratories, India; 99.85% active ingredient), miramistin (MPM 42-0414-2768-02, LLC INFAMED, Russia; 99.93% active ingredient), glacial HOAc (Fisher Chemi-

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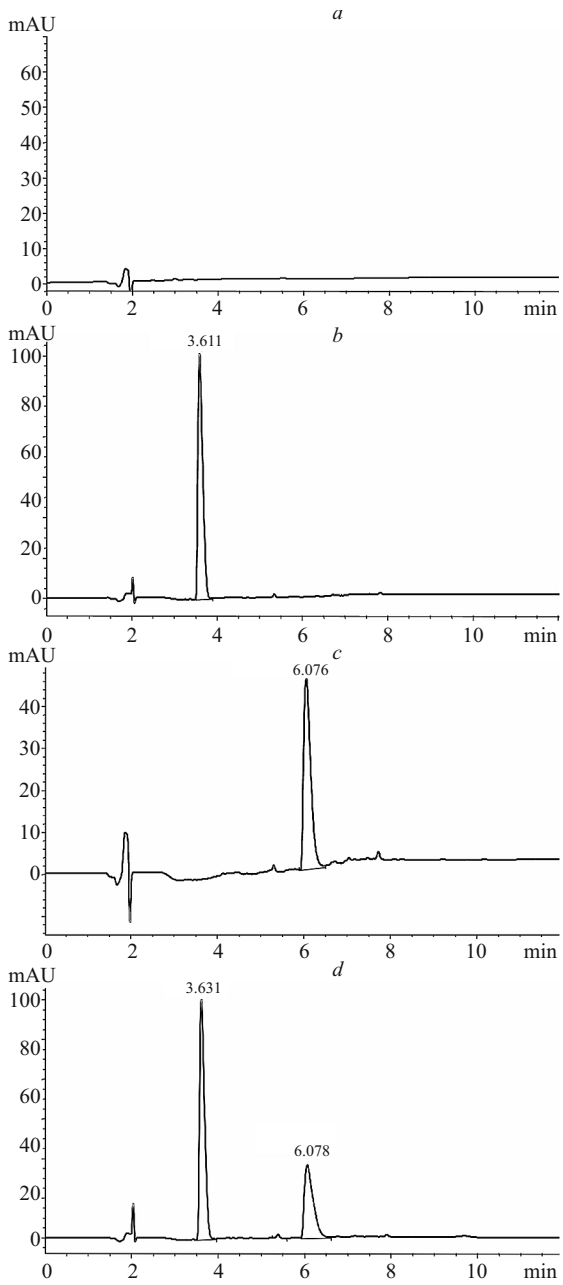

Fig. 1. Chromatograms obtained during specificity determination of the quantitative determination method: placebo solution (*a*), lidocaine standard solution (*b*), miramistin standard solution (*c*), test solution (*d*).

cal, USA), hydroxypropyl methylcellulose (Sigma-Aldrich, Japan), and distilled glycerin (Aist ZAO, Russia). Eluents were prepared using MeCN (HPLC grade, Fisher Chemical, USA), formic acid (PanReac AppliChem, USA), deionized H_2O (Millipore), and Millipore 0.45-μm nylon membrane filters (HNWP). All other reagents were domestic products of at least chemically pure grade unless otherwise noted.

Chromatographic separation used an Agilent 1200 chromatograph (Agilent Technologies, USA) equipped with a four-channel gradient pump, autosampler, column thermostat, and diode-array detector. A Zorbax Eclipse XDB-C18 chromatography column (Agilent Technologies, USA; 150 × 4.6 mm × 5 μm) was used for the separation. Results were processed using Agilent ChemStation B.04.03 software (Agilent Technologies, USA) and Excel. Samples were prepared using a GR-200 analytical balance (A&D, Japan) and pH 330i pH-meter (WTW, Germany).

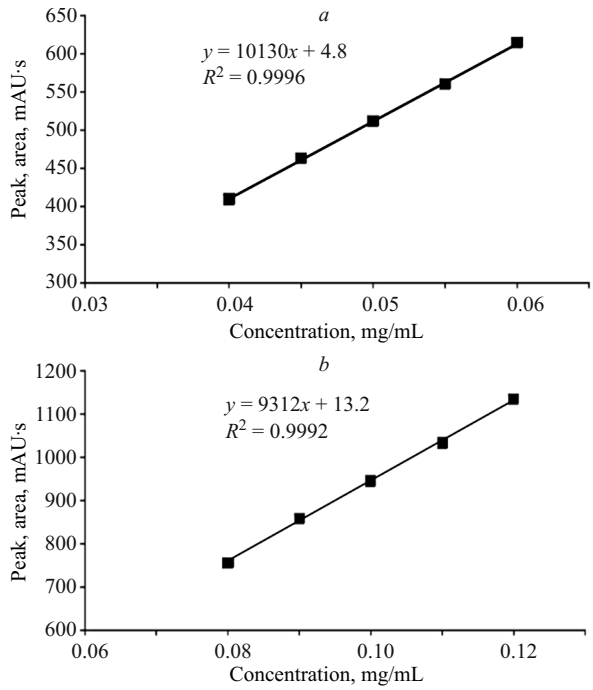

Fig. 2. Calibration curves for peak area as a function of lidocaine hydrochloride (*a*) and miramistin concentration (*b*).

Sample preparation for quantitative analysis

Preparation of formic acid solution (0.1%) (mobile phase A). Formic acid (1.0 mL) was dissolved in deionized H_2O (1000 mL).

Preparation of lidocaine hydrochloride standard solution. Lidocaine hydrochloride standard (100 mg) was placed into a 100-mL volumetric flask, treated with MeOH (10 mL), stirred, and adjusted to the mark using mobile phase A. The resulting solution (10 mL) was placed into a 100-mL volumetric flask and adjusted to the mark using mobile phase A.

TABLE 1. Chromatographic Separation Gradient for Quantitative Analysis of Lidocaine and Miramistin

Time, min	Flow, mL/min	Mobile phase, %	
		A	B
0	1	10	90
5	1	90	10
10	1	90	10
11	1	10	90
15	1	10	90

TABLE 2. Dependence of Peak Area on Concentration of Lidocaine Hydrochloride Standard Solution

Lidocaine hydrochloride concentration, mg/mL	Lidocaine hydrochloride peak area
0.08	755.4
0.09	856.8
0.1	944.2
0.11	1032.6
0.12	1133.0

Preparation of miramistin standard solution. Miramistin standard (50 mg) was placed into a 100-mL volumetric flask, stirred, and adjusted to the mark with mobile phase A. The resulting solution (10 mL) was placed into a 100-mL volumetric flask and adjusted to the mark with mobile phase A.

Preparation of test solution. The wound-healing gel being analyzed (10 g) was placed into a 100-mL volumetric flask, treated with mobile phase A (50 mL), dissolved using ultrasound at room temperature, and adjusted to the mark using mobile phase A. The resulting solution was filtered through a 0.45-ìm Millipore nylon membrane filter (HNWP).

Preparation of placebo solution. The placebo solution corresponded to the wound-healing gel composition without lidocaine hydrochloride and miramistin. Starting solution was prepared by weighing chymopsin (0.2 g), chitosan (1.0 g), glacial HOAc (0.3 mL), hydroxypropyl methylcellulose (0.1 g), and glycerin (0.9 g) in a 100-mL flask and dissolving in distilled H_2O. Then, the starting solution (10 mL) was diluted to 100 mL using mobile phase A.

Chromatography parameters for quantitative analysis of lidocaine and miramistin. The analysis was performed in gradient mode (Table 1). Mobile phase A, formic acid solution (0.1%) in H_2O; mobile phase B, MeCN; flow rate, 1 mL/min. A Zorbax Eclipse XDB-C18 chromatography column (Agilent Technologies, USA; 150 × 4.6 mm × 5 μm), sample volume 10 μL, and column temperature 30°C were used. The analysis time was 15 min. A diode-array detector was used at wavelength 262 nm.

The chromatographic system was considered suitable if the following conditions were fulfilled:

TABLE 3. Dependence of Peak Area on Concentration of Miramistin Standard Solution

Miramistin concentration, mg/mL	Miramistin peak area
0.04	409.2
0.045	462.35
0.05	511.5
0.055	559.65
0.06	613.8

TABLE 4. Accuracy of Method for Lidocaine Hydrochloride

Concentration, %	Lidocaine hydrochloride concentration, mg/mL		Response factor (recovery), %
	added	found	
80 – 1	0.080	0.080	99.9
80 – 2	0.080	0.080	100.0
80 – 3	0.081	0.081	100.0
100 – 1	0.100	0.101	101.0
100 – 2	0.101	0.101	100.0
100 – 3	0.101	0.100	99.0
120 – 1	0.120	0.120	100.0
120 – 2	0.121	0.121	100.0
120 – 3	0.120	0.121	100.8

the efficiency for the lidocaine peak in chromatograms of the standard solution should be at least 2,000 theoretical plates;

the efficiency for the miramistin peak in chromatograms of the standard solution should be at least 2,000 theoretical plates;

the resolution of the lidocaine and miramistin peaks should be at least 2;

the relative root-mean-square deviation of lidocaine peak areas in chromatograms of the standard solution should be less than 2%;

the relative root-mean-square deviation of miramistin peak areas in chromatograms of the standard solution should be less than 2%.

RESULTS AND DISCUSSION

Lidocaine hydrochloride (0.1 g per 100 g of gel) and miramistin (0.05 g per 100 g of gel) were the main active

TABLE 5. Accuracy of Method for Miramistin

Concentration, %	Miramistin concentration, mg/mL		Response factor (recovery), %
	added	found	
80 – 1	0.0400	0.0403	100.8
80 – 2	0.0398	0.0400	100.5
80 – 3	0.0402	0.0399	99.3
100 – 1	0.0500	0.0503	100.6
100 – 2	0.0500	0.0499	99.8
100 – 3	0.0501	0.0501	100.0
120 – 1	0.0601	0.0603	100.3
120 – 2	0.0598	0.0601	100.5
120 – 3	0.0600	0.0598	99.7

pharmaceutical ingredients of the wound-healing gel. Gradient elution was more successful because of the complicated composition. It could simultaneously quantify miramistin and lidocaine in a single chromatographic analysis.

Validation was conducted according to GPM.1.1.0012.15 "Validation of analytical methods" [9]. The procedure was validated for specificity, analytical range, linearity, accuracy, and precision.

Specificity of the method was indicative of the capability of the procedure to determine selectively lidocaine and miramistin in the presence of matrix components and was confirmed by chromatograms of the placebo solution, standard solutions, and test solution. The procedure was specific because the placebo chromatogram did not have peaks with retention times of lidocaine and miramistin (Fig. 1).

Analytical range and linearity. According to the regulations, the lidocaine hydrochloride content in the wound-healing gel is 0.1 g per 100 g of gel; of miramistin, 0.05 g per 100 g of gel. The lidocaine concentration would be 0.1 mg/mL; miramistin, 0.05 mg/mL if the proposed sample preparation method is used.

The analytical range of the procedure was found by preparing a series of solutions in the concentration range 0.08 – 0.12 mg/mL (for lidocaine hydrochloride) and 0.04 – 0.06 mg/mL (for miramistin).

Table 2 presents the lidocaine peak areas as functions of concentration; Table 3, miramistin.

Linearity with sample preparation for quantitative determination of lidocaine hydrochloride was demonstrated in the concentration range 0.08 – 0.12 mg/mL; of miramistin, 0.04 – 0.06 mg/mL. Each sample was analyzed six times. The obtained peak areas were used to construct calibration curves and calculate the linear regression coefficients (Figs. 2). The correlation coefficients (r) for lidocaine and miramistin were 0.9992 and 0.9996, respectively.

Accuracy and precision. Samples were analyzed at three lidocaine hydrochloride and miramistin concentrations in the test solution at 80 – 120% of the nominal concentration taken as 100%. Model solutions were prepared using accurately weighed lidocaine hydrochloride and miramistin. The procedure accuracy was evaluated from the recovery R. Tables 4 and 5 present the recovery values obtained by analyzing lidocaine hydrochloride and miramistin samples, respectively.

The recovery values should fall in the range $99\% < R < 101\%$. Tables 4 and 5 show that the procedure met these requirements.

The precision (convergence) and intralaboratory precision were confirmed during development of the new procedure. The relative standard deviation (ε_{sd}) should be <2%. For lidocaine, $\varepsilon_{sd} = 1.04\%$; for miramistin, 1.22%. A comparison of scatters of results obtained on different days by different analysts using the Fisher criterion (lidocaine hydrochloride 0.35; miramistin 0.51) showed that the difference between the scatters and between statistical averages were insignificant, which confirmed that the intralaboratory precision was satisfactory.

Thus, the procedure was reliable, accurate, and precise and could be recommended for quantitative determination of miramistin and lidocaine hydrochloride in the wound-healing gel.

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