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Development of Assay Method for Miramistin in an Innovative Medication Wound Healing Gel

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Abstract:
High performance liquid chromatography (HPLC) is widely used for quantitative identification of active compounds in substances and medical forms. In the standard documentation development for a new medicinal form - wound-healing gel it was offered to use the width UV-detection method for quantitative determination and authenticity confirmation. The present work purpose was the development of chitosan and benzyldimethyl[3-(myristoilamine)propyl]ammonium chloride (miramistin) quantitative analysis technique for wound-healing gel medicine using the HPLC method. The separation was achieved on the Zorbax Eclipse XDB-C18 column 5 microns Agilent, USA (150 mm x 4,6 mm) and linear solvents gradient of 0,1 M phosphate buffer with pH 3 (A) and pure methanol (B). Methanol gradient from 10 to 80% of methanol for 10 mins at a flow rate of 0,8 ml/min was set. As a result the selective express-technique of miramistin identification in wound-healing gel by the reverse-phase high performance liquid chromatography method with spectrophotometric detection was developed. The developed technique has a good linearity with correlation rate of 0,999 and identification relative error not exceeding 2%.

Keywords: miramistin, quality control, high performance liquid chromatography, quantification, complex chitosan-miramistin (CCM), wound-healing gel.

INTRODUCTION

Polysaccharide complex of chitosan with benzyldimethyl[3-(myristoilamine)propyl]ammonium chloride provides prolonged antibacterial and antifungal effect, strengthens the immune cells functional activity through the stimulation of local nonspecific immunity [1-3].

The purpose of the study was the development of quantitative identification technique of wound-healing gel medicine in comparison with miramistin. Miramistin is an active component of wound-healing gel medicine. Miramistin was chosen as an antibacterial component due to its strong antibacterial effect against both aerobic and anaerobic bacteria, Gram-positive and Gram-negative organisms, both as the monocultures and as the associations, including hospital strains that has a multi-resistance to antibiotics. The molecules of the medicine influence an outside shell of microbial cell, which results in destruction and death of bacteria [2, 4, 5].

Quantitative and qualitative definition of medicine in comparison with miramistin was performed high performance liquid chromatography (HPLC) method. HPLC allows to combine several tests in one sample including “Authenticity” and “Quantification”.

MATERIALS AND METHODS

Chitosan which was made by Scientific and Production Association "Bioprogress" (Shchelkovo city, Moscow region, Russian Federation) (drug humidity 10%, specifications 9289-067-00472124-03, diacylation degree 80,0%; kinematic viscosity not less than 383,7 cSt (cStokes), molecular mass about 500 kDa), proteolytic complex of crab’s hepatopancreas(PC) (specifications 9154-032-11734126-10; proteolytic activity according to casein 0.9 PE/mg; activity according to BApNa 43 nmol/mg*min; activity according to azokoll 28 Units/mg, Scientific and Production Association "Bioprogress" (Shchelkovo city, Moscow region, Russian Federation); trypsin from pancreas of cattle (pharmacopoeial manufacturer’s article (PMA) 42-0179-5943-04 “SamsonMed”, Russia) (activity according to casein 8.2 PE/mg); chymopsin (pharmacopoeial manufacturer’s article (PMA) 42-0179-5944-04 “SamsonMed”, Russia) (activity according to casein9.0 PE/mg); miramistin (pharmacopoeial manufacturer’s article (PMA) 42-0414-2768-02, limited liability company (LLC) “InfaMed”, Vydnoe city, Moscow region, Russian federation); polyacrylamide (science-technical company (STC) “Atombiotech”, specifications 9398-001-059669 19-2008, ID 34680, Coloreon Limited, England); distilled glycerin (closed joint-stock company “Stork”, Saint-Petersburg, state standard 6824-96). Acetonitrile (HPLC grade) and deionized water (Millipore) was used for the analysis. Millipore type nylon membrane filter (HNWP) with pores size of 0,45 microns. All other reactants (unless specifically stated) are produced in Russia, qualification not below than “CP” (chemically pure).

Equipment

Chromatographic separation was performed on Agilent 1200 chromatograph equipped with four-channel gradient pump, autosampler, columns and samples thermostat, spectrophotometric detector with diode matrix. For results processing Agilent ChemStation (ver. B.04.03) and Excel software was used. For sample preparation analytical weigher A&D GR-200, Japan, pH/m pH 330i, (WTW, Germany) was used.

All measuring devices used in work are registered in State register of measuring means and have valid certificates of calibration.

HPLC methods

The Zorbax Eclipse XDB-C18 Agilent, USA 5 microns (150 mm x 4,6 mm) column was used for separation. Column temperature was set at 30 °C. Mobile phases used for analysis: 0.1M phosphate buffer with pH 3 (A) and pure methanol (B). The flow rate was set at 0.8 ml/min. Mobile phase gradient from 10% of B solvent (0 min) to 80% B solvent in 10 minutes after beginning of the analysis was used. The integration time: 10 min, total time of analysis: 15 min, including the column equilibration by initial mobile phase for following analysis. Injection volume: 10 µl. Detector’s wavelength: 262 nm. Chromatographic column was flushed with mix of mobile phases A:B (90:10) with the speed 0,8 ml/min within a few hours before the beginning of identification until the stable baseline is formed.

Preparation of standard miramistin solution

100 mg of the miramistin standard was placed into 100 ml dimensional flask, 10 ml of deionized water was added, mixed and brought the solution to the scale by the mobile phase A. 0.5 ml of this solution was placed into 100 ml dimensional flask and brought to the scale by the mobile phase A.

Preparation of testing solution

1 g of wound-healing gel was placed into 100 ml dimensional flask, 10 ml of deionized water was added, mixed until the dissolution brought to the scale by the mobile phase A and filtered through the Millipore type nylon membrane filter with the pore size of 0.45 microns.

Preparation of placebo solution

Placebo solution matches the composition of the wound-healing gel base without miramistin. For preparation of original solution 0.2 g of chymopsin, 1 g of chitosan, 0.3 ml of glacial

acetic acid, 0.1 g of hydroxypropyl methylcellulose and 0.9 g of glycerin was weighted in 100 ml dimensional flask and dissolved in distilled water. Then 1 ml of original solution was diluted with the mobile phase A to the 100 ml volume.

RESULTS

Chromatographic column Zorbax Eclipse XDB-C18 Agilent, USA 5 µm (150 mm x 4,6 mm) was chosen for qualitative and quantitative analysis.

Relative retention time of miramistin was about 7 min. The used column supplied a symmetric peak on chromatogram in the eluent system A – 0.1 M phosphate buffer (pH 3,0) and pure methanol. In this system miramistin eluted as a symmetric peak. It was proven by calculated value of the asymmetry rate which should not exceed 1,5. Chromatographic column efficiency on miramistin peak is not less than 2000 theoretical plates and relative standard deviation calculated for the miramistin peak's area on repeated standard solution chromatograms was less than 2,0 %.

In relation to the complex composition of the medicine, isocratic elution becomes inexpedient due to more hydrophobic components can be accumulated in the column and may affect on the results of subsequent analyses. Mobile phase gradient mode of elution using methanol gradient (from 10 to 80%) was applied in the analysis (0-10 mins), conditions were selected experimentally.

The spectrum of 0,01% miramistin water solution registration was led in the interval of wavelengths from 210 nm to 400 nm with the step of 2 nm. Miramistin spectrum is presented in Figure 1, wavelengths of miramistin maximum and minimum absorptions are presented in Table 1. Chromatograms of placebo solution, standard miramistin solution and the sample of wound-healing gel registered at wavelength of 262 nm are presented in Figure 2. The time of miramistin retention time in the system was at 7 min. Calibration curve is linear within the range of concentrations from 1 to 10 µg/ml. Selected conditions were used hereinafter for authenticity identification and quantitative analysis of miramistin in the sample of wound-healing gel. Miramistin cleared pharmaceutical substance was used as a standard comparative sample.

Miramistin concentration (%) in wound-healing gel was calculated in accordance to the formula:

$$X = \frac{S_1 \times a_0 \times 100 \times P}{S_0 \times 200 \times a_1 \times 0{,}05},$$

S_1 – Miramistin peak's area on testing solution chromatogram;
S_0 – Miramistin peak's area on standard lidocaine chromatogra
a_1 – Gel hanging used for testing solution preparation;
a_0 – Miramistin SS hanging (in mg);
P – Active substance concentration in miramistin SS (in %);

Miramistin connentration in wound-healing gel should be from 95% to 105% from declared amount.

The technique reproducibility was evaluated by various wound-healing gel samples parallel analyses. The relative standard deviations values obtained at all investigated intervals of concentrations indicates a high technique reproducibility.

Developed technique was used for miramistin identification in wound-healing gel, results are presented in Table 2. It is calculated that relative error of identification of investigated substances in accordance to developed technique does not exceed 1,23%. The validation evaluation of developed technique on different ratings is conducted: precision (convergence, reproducibility), specificity, linearity (Table 3). The results are presented in Table 3 confirming the suitability for analysis of developed technique.

Relative standard deviations for the peaks' areas and the main substance retention times in the wound-healing gel do not exceed the evaluation criteria. This technique is characterised by linearity, reproducibility and accuracy of identification. The quantity of relative systematic error does not exceed 2,0 % for the main substance identification. It is confirmed that the procedure of miramistin quantitative identification, its authenticity for the HPLC method in the wound-healing gel is executed properly and supplies a necessary degree of precision for a series of measurements which were carried out in repeated analyses of samples from series under different conditions: different analysts, different days, different chromatographs. Additional peaks on chromatograms did not appear during the analysis of samples through a longer period of time that confirms the medicie stability.

Table 1. Wavelengths of miramistin maximum and minimum absorptions.

Compound	Λ max, nm	Λ min, nm
Miramistin	262	238

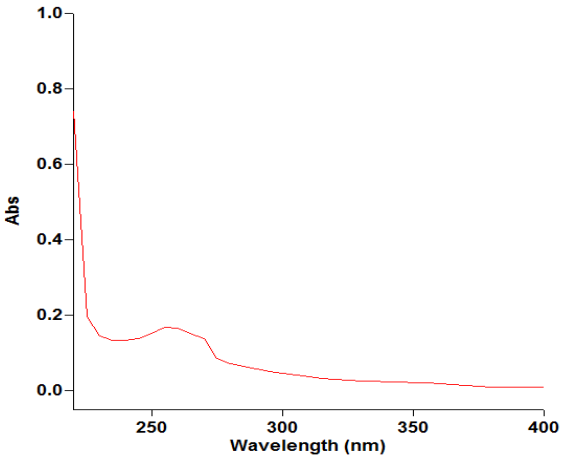
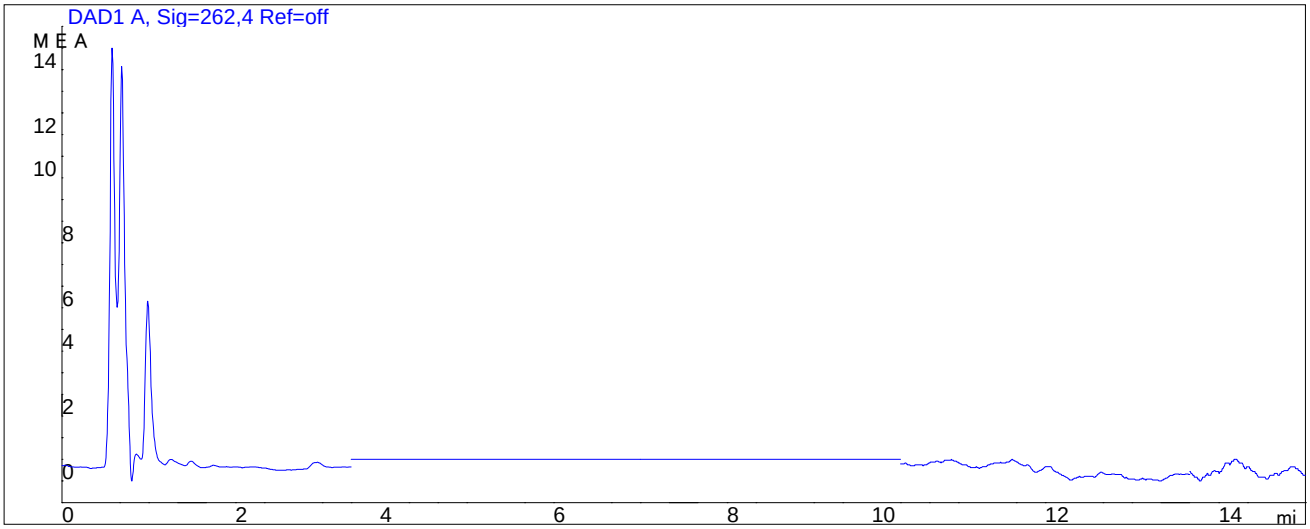


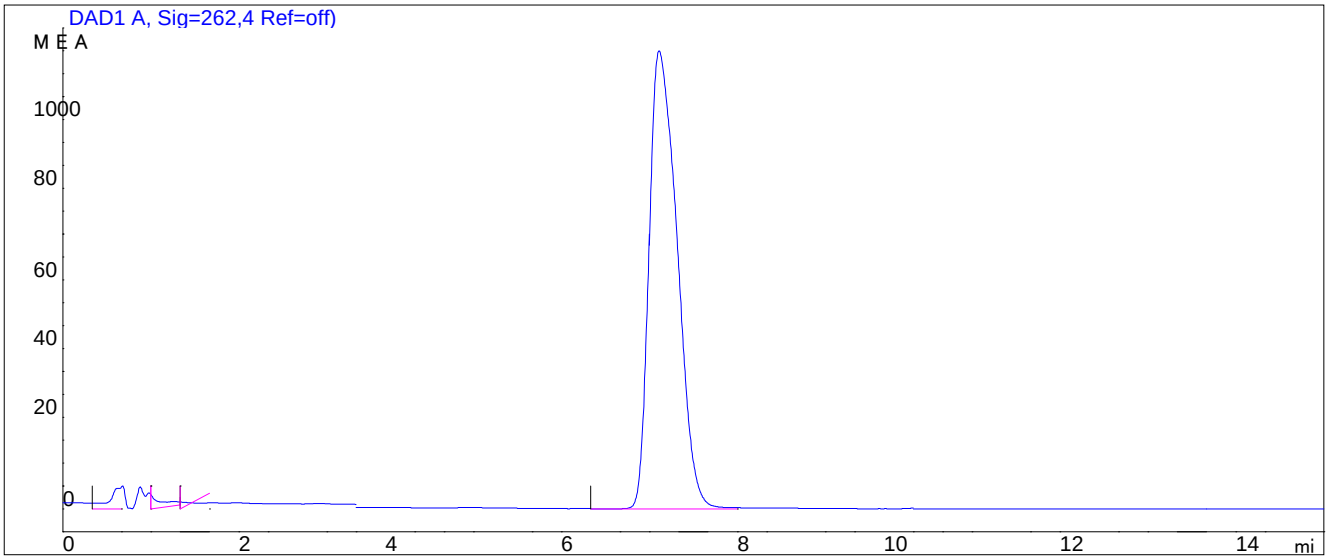
Figure 1. Miramistin spectrum

Table 2. Results of miramistin quantitative identification in the wound-healing gel by the HPLC method

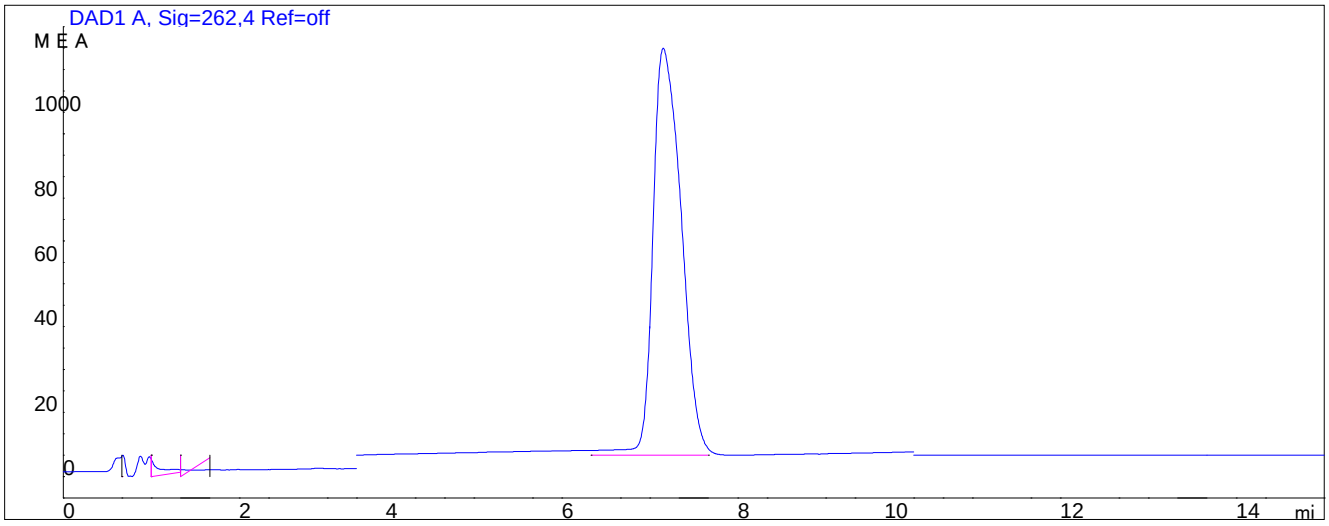
Drug dosage form	N (series number)	Metrological performance(n=9, P=95%)						
		Average value, % $\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$	Sum of squared deviations $\sum \Delta^2$	Dispersion $V = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}$	Sample standard deviation $S = \sqrt{V}$	Relative standard deviation $\frac{S}{\bar{x}} \cdot 100$, %	Half-width of a confidence interval $\bar{x} - \mu = \pm \frac{t_{P,f} \cdot S}{\sqrt{n}}$	Relative error of an average value, % $\frac{\bar{x} - \mu}{\bar{x}} \cdot 100$
Wound-healing gel	01/06032017	100,84	3,98	0,995	0,997	0,989	1,24	1,23
Wound-healing gel	02/09032017	100,34	2,32	0,579	0,761	0,758	0,944	0,941



A



B



C

Figure 2. Chromatograms of placebo solution (A), standard miramistin solution (B) and the wound-healing gel sample (C)

Table 3. Results of validation evaluation of the technique of miramistin quantitative identification in the wound-healing gel by the HPLC method

Validation characteristics	Acceptance criteria	Received result		Conformity result
		1	2	
Chromatography – System Suitability (quantification)	Chromatographic column efficiency not less than 2000	2938	2944	passed
	Relative standard deviation no more than 2 % (the peak's area)	0,35	0,40	passed
	Relative standard deviation no more than 2 % (retention time)	0,33	0,63	passed
	Asymmetry parameter of the peak not more than 2.5	1,1	1,2	passed
Specifity	There are no peaks which interfere miramistin identification in the analysis of placebo solution	No peaks which interfere the miramistin identification in the analysis of placebo solution		passed
Linearity	For the relation $Y = a + bx$ the factor of linear regression $r^2 \geq$ 0,995	0,9995		passed
	Intersection with the Y axis should be not more than 2 % of nominal concentration response	1,25 %		passed
Reproducibility	Systematic error value during the identification of proximity degree of results of single quantitative value identifications of the main substance in the wound-healing gel does not exceed 2,0 %	0,59 %		passed
Intra-laboratory accuracy	Random factors influence on the main substance quantitative identification is not significant if RSD does not exceed 2 %	1,62 %		passed
Stability of the solution	SS miramistin solution should be stable during 3 days at 5 °C	Is stable for 3 days		passed

CONCLUSION

High performance liquid chromatography is the one of the main methods of pharmaceutical medicines quality monitoring in different characteristics: authenticity, quantitative identification, presence of impurities. The technique of miramistin quantitative identification was developed. This technique is characterized with high selectivity, reproducibility and accuracy of miramistin identification in the presence of other components of wound-healing gel. Miramistin is an effective and safe antibacterial agent which is widely used both as a separate drug and as a part of complicated medicinal forms. Given technique can be used for development of recreated medicinal forms analysis methods.

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