Quantitative determination of 0.05 % chlorhexidine solution by capillary electrophoresis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of the work was to estimate the quantitative content of 0.05 % chlorhexidine solution by capillary electrophoresis.

Materials and methods. The object of the study was the drug 0.05 % solution of chlorhexidine for local and external use of pharmacopoeia quality. The study was carried out on a Kapel-104T device, equipped with an ultraviolet photometric detector and personal computer with the Multichrom software. Electrophoretic determination of the quantitative content of chlorhexidine was carried out in the UV region of the spectrum at a wavelength of 254 nm with a positive voltage on a capillary of 16 kV and a recommended capillary thermostating from 20 °C to 30 °C. The sample was introduced in pneumatical mode in the 30 mbar for 5 seconds. The analysis time was 5 minutes. As the leading electrolyte an aqueous solution consisting of 0.6 % solution of imidazole, 0.6 % solution of sodium tetraborate and 0.5 % solution of tartaric acid were used. The determination of chlorhexidine in the test solution was carried out in the UV spectral region at a wavelength of 254 nm, since at this wavelength one of the absorption maxima of the indicated drug was observed.

Results. The validation analysis showed that the technique is characterized by specificity, linearity in the concentration range from 100 μg/ml to 700 μg/ml (y = 0.3438x – 12.4789; r = 0.9913), accuracy for chlorhexidine levels of 80–120 % (R = 98.82–100.44 %), precision (S_r = 0.44–0.61 %). The relative error of the result of separate determination of chlorhexidine in 0.05 % solution using capillary electrophoresis was 1.28 %.

Conclusions. Quantitative determination of 0.05 % chlorhexidine solution by capillary electrophoresis was carried out and the parameters of the validation of the electrophoretic technique were established. The proposed electrophoretic conditions can serve as the basis for the development of methods for the quantitative determination of chlorhexidine bigluconate as part of this drug.

Key words: chlorhexidine, capillary zone electrophoresis, biguanides, amidines, antiseptics.

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Кількісне визначення 0,05 % розчину хлоргексидину методом капілярного електрофорезу

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Мета роботи – оцінювання кількісного вмісту 0,05 % розчину хлоргексидину методом капілярного електрофорезу.

Матеріали та методи. Об'єкт дослідження – лікарський препарат 0,05 % розчин хлоргексидину для місцевого та зовнішнього застосування фармакопейної якості. Дослідження виконали на приладі Капель-104Т, що обладнаний ультрафіолетовим фотометричним детектором, який працює при довжині хвилі 254 нм, а також кварцевим капіляром завдовжки 0,5 м до детектора, з внутрішнім діаметром 75 мкм, джерелом високої напруги позитивної полярності з регульованою напругою від 1 кВ до 25 кВ і персональним комп'ютером із програмним забезпеченням Мультихром. Електрофоретичне визначення кількісного вмісту хлоргексидину в 0,05 % розчині для місцевого та зовнішнього застосування виконали при позитивній напрузі на капілярі 16 кВ і рекомендованому термостатуванні капіляра від 20 °С до 30 °С. Введення проб здійснювали пневматично в режимі 30 мБар протягом 5 секунд. Час аналізу – 5 хвилин. Як провідний електроліт застосовували водний розчин, що складається з 0,6 % розчину імідазолу, 0,6 % розчину натрію тетраборату та 0,5 % розчину кислоти винної. Визначення хлоргексидину в розчині, що вивчали, виконали в УФ-області спектра при довжині хвилі 254 нм, оскільки при цій довжині хвилі спостерігали один із максимумів поглинання цього лікарського препарату.

Результати. Валідаційний аналіз показав, що методика характеризується специфічністю, лінійністю в діапазоні концентрації від 100 мкг/мл до 700 мкг/мл (y = 0,3438x – 12.4789; r = 0,9913), правильністю для вмісту хлоргексидину 80–120 % (R = 98,82–100,44 %), прецизійністю (S_r = 0,44–0,61 %). Відносна похибка результатів окремого визначення хлоргексидину в 0,05 % розчину з використанням капілярного електрофорезу становила 1,28 %.

Висновки. Виконали кількісне визначення 0,05 % розчину хлоргексидину методом капілярного електрофорезу. Запропонували електрофоретичні умови, котрі можуть бути основою для розроблення методики кількісного визначення хлоргексидину біглюконату у складі цього лікарського препарату.

Ключові слова: хлоргексидин, капілярний електрофорез, бігуанідини, амідини, антисептичний засіб.

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Количественное определение 0,05 % раствора хлоргексидина методом капиллярного электрофореза

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Цель работы – оценка количественного содержания 0,05 % раствора хлоргексидина методом капиллярного электрофореза.

Материалы и методы. Объект исследования – лекарственный препарат 0,05 % раствор хлоргексидина для местного и наружного применения фармакопейного качества. Исследование проводили на оборудовании Капель-104Т, оснащенном ультрафиолетовым фотометрическим детектором, работающим при длине волны 254 нм, а также кварцевым

Ключевые слова: хлоргексидин, капиллярный электрофорез, бигуанидины, амидины, антисептическое средство.

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капилляром длиной 0,5 м до детектора, внутренним диаметром 75 мкм, источником высокого напряжения положительной полярности с регулируемым напряжением от 1 кВ до 25 кВ и персональным компьютером с программным обеспечением Мультихром. Электрофоретическое определение количественного содержания хлоргексидина в 0,05 % растворе для местного и наружного применения проводили при положительном напряжении на капилляре 16 кВ и рекомендуемом термостатировании капилляра от 20 °С до 30 °С. Ввод пробы осуществляли пневматически в режиме 30 мБар в течение 5 секунд. Время анализа – 5 минут. В качестве ведущего электролита использовали водный раствор, состоящий из 0,6 % раствора имидазола, 0,6 % раствора натрия тетрабората и 0,5 % раствора кислоты винной. Определение хлоргексидина в изучаемом растворе проводили в УФ-области спектра при длине волны 254 нм, так как при этой длине волны наблюдали один из максимумов поглощения указанного лекарственного препарата.

Результаты. Валидационный анализ показал, что методика характеризуется специфичностью, линейностью в диапазоне концентраций от 100 мкг/мл до 700 мкг/мл (y = 0,3438x – 12,4789; r = 0,9913), правильностью для уровней содержания хлоргексидина 80–120 % (R = 98,82–100,44 %), прецизионностью (S_r = 0,44–0,61 %). Относительная ошибка результата отдельного определения хлоргексидина в 0,05 % растворе с использованием капиллярного электрофореза составила 1,28 %.

Выводы. Проведено количественное определение 0,05 % раствора хлоргексидина методом капиллярного электрофореза и установлены параметры валидации электрофоретической методики. Предложенные электрофоретические условия могут быть основой для разработки методики количественного определения хлоргексидина биглюконата в составе данного лекарственного препарата.

Introduction

Chlorhexidine is one of the leading antiseptics of a wide spectrum of action, represented on the Russian pharmaceutical market. In its chemical structure, chlorhexidine refers to the derivatives of biguanidine and amidine and is N,N‴-bis (4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide, available as acetate, dihydrochloride and di-D-gluconate [1].

Chlorhexidine, which has been used for more than half a century as a curative and therapeutic-prophylactic antiseptic and disinfectant for various infections. It is now widely used in disinfectological, surgical, obstetric-gynecological, dental, dermatologereal and urological practices [1–3].

The undoubted advantages of this drug are the high pharmacological activity against a wide range of microorganisms and the lack of resistance to it, low degree of absorption through undamaged skin, and its availability in terms of value [4,5]. It should be noted that the assortment list of drugs containing chlorhexidine as an active ingredient is quite diverse. This drug is presented in the form of liquid, solid and soft dosage forms. At the same time, the most popular and affordable for the consumer is 0.05 % solution of chlorhexidine for local and external use [6–9]. The latter is listed in the list of vital and essential medicines for medical use for 2018 and is characterized by fairly large share of the presence in the pharmacy nomenclature.

The wide prevalence of 0.05 % solution of chlorhexidine in the pharmaceutical market makes it necessary to carefully monitor its quality. In this regard, special attention of researchers is focused on the introduction of modern and rapid methods of pharmaceutical analysis of drugs containing chlorhexidine.

At present, both physical-chemical and chemical methods of pharmaceutical analysis are used to evaluate the quantitative content of chlorhexidine in solution [10–12]. In this case, UV-spectrophotometry serves as a reference method in the domestic practice of quality control of given drug [11]. At the same time, foreign sources of scientific information contain data on the possibility of analyzing chlorhexidine in various drugs by acid-base titration in protogenic solvent medium, as well as using HPLC and capillary electrophoresis [10,12–14]. Among these methods, capillary electrophoresis is the most promising in controlling the quality of drugs containing chlorhexidine, and its advantages are well known and described in numerous scientific works [15–18].

The purpose

The purpose of this work was to assess the quantitative content of 0.05 % chlorhexidine solution by capillary electrophoresis.

Materials and methods

The object of the study was the drug 0.05 % solution of chlorhexidine for local and external use of pharmacopoeia quality. The standard sample (SS) of solution of chlorhexidine (20 %, Sigma), imidazole (95 %, Sigma), sodium tetraborate (99.5 %, Sigma), tartaric acid (99.5 %, Sigma), hydrochloric acid (puriss., Vecton), sodium hydroxide (puriss., Vecton) were also used in the work. Purified water used for analysis was obtained by distillation on a DE-4 distiller.

The study was performed using capillary ion electrophoretic analyzer (capillary electrophoresis device Kapel-104T, OJSC RPC Lumex, Russia) equipped with an ultraviolet photometric detector operating at a wavelength of 254 nm, and a quartz capillary 0.5 m long to the detector, internal diameter of 75 micrometers, a source of high voltage of positive polarity with adjustable voltage from 1 kV to 25 kV and a personal computer with the software Multichrome.

To evaluate the quantitative content of 0.05 % solution of chlorhexidine by means of capillary electrophoresis, the drug was not diluted. To prepare a solution of SS of chlorhexidine, 0.5 ml of a 20 % solution of chlorhexidine was placed in a 200 ml volumetric flask, the volume of the solution was adjusted to the mark with water and mixed. The analyzed samples of the test and standard solutions with a volume of 1 nl were dosed into the device at least three times and electrophoretograms were recorded.

Electrophoretic determination of the quantitative content of chlorhexidine in 0.05 % solution for local and external use was performed with a positive voltage on 16 kV capillary and recommended capillary thermostatting from 20 °C to 30 °C. The sample was introduced in pneumatical mode in the 30 mbar for 5 seconds. The analysis time was 5 minutes. As the leading electrolyte we used an aqueous solution consisting of 0.6 % solution of imidazole, 0.6 % solution of sodium tetraborate and 0.5 % solution of tartaric acid. The prepared solution was used during the working day. Between the tests, the capillary was successively rinsed for 2 minutes with each reagent. Initially, washed with a 1 M solution of hydrochloric acid, then purified water, 1 M solution

of sodium hydroxide and again purified water, and then with a leading electrolyte. The determination of chlorhexidine in the solution was carried out in the UV region of the spectrum at a wavelength of 254 nm, since at this wavelength one of the absorption maxima of the indicated drug was observed.

The content of chlorhexidine bigluconate in 1 ml of the drug in micrograms (X) was calculated by the formula:

$$X = \frac{S_1 \cdot a_0 \cdot P}{S_0 \cdot 200},$$

S_0: is the peak area on the electrochromatogram of the solution of SS of chlorhexidine bigluconate; S_1: is the peak area of chlorhexidine bigluconate on the chromatogram of the drug; a_x: is a sample of SS of chlorhexidine bigluconate, ml; a_0: is a sample of SS of chlorhexidine bigluconate, ml; P: is a content of chlorhexidine bigluconate in SS of chlorhexidine bigluconate, %.

The content of chlorhexidine bigluconate in 1ml of 0.05 % solution of the drug should be from 450 µg to 550 µg.

To test the suitability of the electrophoretic system, at least 3 electrophoregrams of the solution of SS of chlorhexidine were obtained. Established criteria related to the reliability of determining the beginning and end of the peak of the analyte, as well as criteria that characterize the separation ability of the system and the reproducibility of measurement results (*Table 1*).

An electrophoretic system is considered suitable if, during electrophotometry of a chlorhexidine SS solution, the number of theoretical plates are not less than 10000, the tailing factor of a peak is not more than 2, the resolution between two peaks must be at least 3.0; relative standard deviation of the peak area is not more than 2 %.

Thus, according to the data reflected in table 1, it is clear that the suitability of the electrophoretic system meets the requirements of domestic and international documents.

The validation of the electrophoretic technique for the quantitative determination of chlorhexidine bigluconate in 0.05 % solution was carried out in accordance with the requirements of domestic and international standards for the following parameters: Specificity, Linearity, Accuracy and Precision [19–21].

Results

Determination of the specificity of the electrophoretic technique was carried out by comparative analysis of electrophoregrams of SS chlorhexidine (*Fig. 1*) with 0.05 % solution of chlorhexidine for local and external application (*Fig. 2*), which revealed the coincidence of chlorhexidine peaks in the test and standard solutions by migration time.

In addition, as seen in *Fig. 2*, the peak of chlorhexidine is distinctly separated from related impurities. Thus, the above electrophoretic conditions for the analysis of 0.05 % solution of chlorhexidine are useful for the quantitative determination of chlorhexidine by the method of capillary electrophoresis.

A further stage of the study was confirmation of the linearity of the electrophoretic technique (*Fig. 3*).

The linear dependence of the photometric signal on the concentration of chlorhexidine observed in the concentration range from 100 µg/ml to 700 µg/ml was approximated by the method of least squares by the linear equation: y = 0.3438x – 12.4789. The correlation coefficient was 0.9913, which meets the requirements of the State Pharmacopoeia of the Russian Federation XIII edition (r ≥ 0.99).

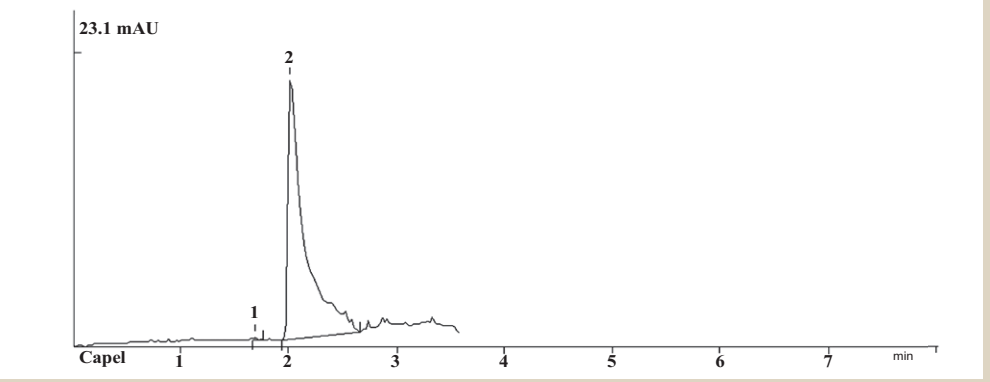

Fig. 1. Electrophoregram of the solution of SS of chlorhexidine (peak 2 – chlorhexidine).

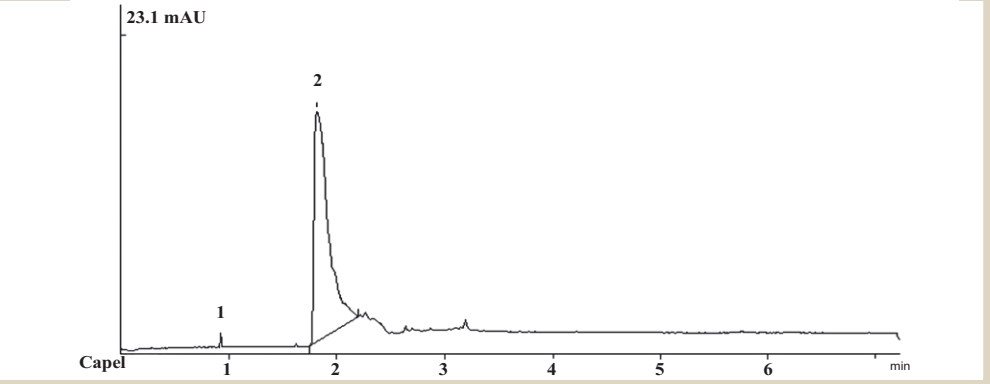

Fig. 2. Electrophoregram of 0.05% chlorhexidine solution (peak 2 – chlorhexidine).

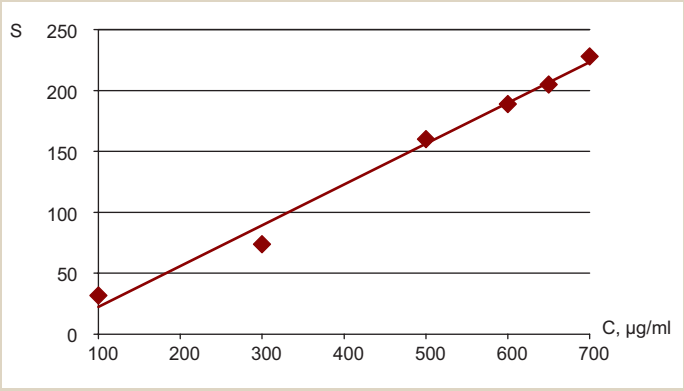

Fig. 3. Calibration graph of the peak area dependence on the chlorhexidine concentration in the solution.

Table 1. The results of determining the basic parameters of the suitability of the electrophoretic system

Suitability parameter	Installed value	Eligibility criteria [20,21]
Tailing factor (T)	1.80 ± 0.02	T ≤ 2
Number of theoretical plates (N)	11025 ± 832	N ≥ 2000
Resolution (Rs)	3.50 ± 0.18	Rs ≥ 1.5
Relative standard deviation (RSD)	1.50 ± 0.12	RSD ≤ 2.0 %

Thus, the calibration graph illustrated in *Fig. 3*, the linear regression equation and the correlation coefficient allow us to conclude that the proposed technique has a linear dependence.

Determination of the validity of the electrophoretic technique according to the parameter Accuracy was carried out using an additive method. For this purpose, model mixtures were prepared at three levels of concentrations (80 %, 100 %, 120 %) consisting of an aqueous solution of the drug with an established chlorhexidine content and a known concentration of the solution of chlorhexidine SS introduced therein (*Table 2*).

As can be seen from the data presented in *Table 2*, the recovery in the determination of content of chlorhexidine in model mixtures ranged from 98.82 % to 100.44 %, which is consistent with the recommended interval for USP (100.0 ± 2.0 %) for the methods of the first category.

The next step in the validation of the technique was the determination of the precision parameter (intermediate precision) (*Table 3*).

The results presented in *Table 3* demonstrate that when studying the intermediate precision parameter, the relative standard deviation was 0.44 % and 0.61 %. The data obtained do not exceed the recommended value (2.0 %) according to the USP.

According to the totality of the studies conducted, it can be concluded that the capillary electrophoresis tech-

Table 2. Results of quantitative determination of chlorhexidine in model mixtures

Content chlorhexidine in a 0.05 % solution, μg/ml	The concentration (μg/ml) of the introduced solution of SS of chlorhexidine	Expected content of chlorhexidine in the model mixture (μg/ml)	The sum of the peak areas of the test solution with a standard additive	Found (μg/ml)	Found (μg/ml), average value	Recovery (R), in %	Mean recovery (R), in %
533	400	933	306	925	926	99.14	99.25
533	400	933	307	930		99.68	
533	400	933	305	922		98.82	
533	500	1033	342	1032	1032	99.90	99.90
533	500	1033	343	1035		100.19	
533	500	1033	342	1030		99.71	
533	600	1133	376	1129	1134	99.64	100.09
533	600	1133	379	1138		100.44	
533	600	1133	378	1136		100.26	

Table 3. Results of validation of the electrophoretic technique in the parameter Precision (intermediate precision) in model mixtures

Sample number	The content of chlorhexidine, μg/ml (day 1)	Metrological characteristics	The content of chlorhexidine, μg/ml (day 2)	Metrological characteristics
1	534	$\overline{X}$ = 531 S = 2.3238 S_x = 0.9485 S_r^x = 0.44 $\Delta\overline{X}$ = 5.97 $\Delta\overline{X}$ = 2.43	527	$\overline{X}$ = 528 S = 3.2249 S_x = 1.3163 S_r^x = 0.61 $\Delta\overline{X}$ = 7.90 $\Delta\overline{X}$ = 3.38
2	529		531	
3	528		526	
4	532		530	
5	533		533	
6	531		525	

Table 4. Results of statistical processing of the method for quantitative determination of 0.05 % solution of chlorhexidine (n = 6, P = 95 %) in real samples

Metrological characteristics						
$\overline{X}$, μg/ml	f	S	S_x	T (P,f)	ΔX	ε
529	5	2.6458	1.0801	2.57	6.79	1.28

nique proposed to evaluate the quantitative content of 0.05 % solution of chlorhexidine showed the compliance of the validation parameters (specificity, linearity, accuracy and precision) requirements of domestic and international regulatory documents.

Subsequent tests were devoted to the quantitative determination of chlorhexidine in real samples of its 0.05 % solution (*Table 4*).

Conclusions

Quantitative determination of 0.05 % chlorhexidine solution by capillary electrophoresis was carried out and the parameters of the validation of the electrophoretic technique were established. The proposed electrophoretic conditions can serve as the basis for the development of methods for the quantitative determination of chlorhexidine bigluconate as part of this drug.

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