UDC: 615.07.242.454
https://www.doi.org/10.34907/JPQAI.2020.90.55.004

STANDARDIZATION OF THE REMINERALIZING SPRAY

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In the article the results of standardization of the remineralizing spray on parameters of "Authenticity" and "Quantitation of active ingredients", "pH level", "Viscosity" and "Microbiological purity". In the work the chemical and physicochemical methods, modified with account of specificity of dosage forms are used. All techniques, tested on lab series of spray, showed good reproducible results and are included into the normative documentation regulating technology and quality control of the remineralizing spray.

Keywords: standardization, remineralization, spray, calcium chloride, potassium phosphate disubstituted, sodium fluoride

Promising for use in remineralizing therapy are polymer-based applicative dosage forms (DF) modeled using the phosphorus-calcium coefficient and the degree of supersaturation of Ca and P, developed taking into account the basic requirements of remineralizing therapy [9].

Currently, the Russian dental market is mainly represented by sprays for moistening the oral cavity in hyposialy and xerostomia. A small range of sprays based on high-molecular compounds for the prevention and treatment of caries indicates the feasibility of their development and wider implementation in dental practice [1].

Spray, having the advantages of aerosol packaging, does not have the disadvantages associated with the use of vials under high pressure and the use of propellants as a carrier gas, such as: relatively high cost, complexity, danger, the possibility of explosion of the cylinder on impact or storage in the improper temperature conditions, high flammability, fire and explosion hazard, inconvenience during transportation, the negative impact of refrigerants on the earth's ozone layer [6,7,10,12].

Features of control tests of the developed spray are associated with combination of active components that tend to interact with each other, and with the difficulty of separation due to their identical solubility in the media used in the analysis [2,3,11].

The study purpose was standardization of remineralizing spray.

MATERIALS AND METHODS

As active pharmaceutical ingredients the follows are used: calcium chloride (FS 42-006-5675-04 P.003964.01, Karpov Chemical Plant

JSC, Mendeleevsk, Republic of Tatarstan, 200916, shelf life – 3 years), potassium phosphate disubstituted (FS 42-4297-79, Lenreactive JSC, Saint Petersburg, 101016, shelf life – 3 years), sodium fluoride (FS 2.2.0013.15, Reachim, Moscow, 201117, shelf life – 3 years); gel-forming agent: methylcellulose-35 (TU 2231-107-57684455-2003, UZPH JSC, Magnitogorsk, 221216, shelf life – 3 years); plasticizers: glycerine (FS 2.2.0006.15, Kupavna Reactive JSC, the town of Staraya Kupavna 082016, shelf life – 3 years), polyethylene oxide-400 (TU 2483-167-05757587-2000, VitaChim LLC, Kazan, 141116); purified water (FS 2.2.0020.18).

The study were concerned with five production samples of spray. The development of new medicinal products requires simple, selective, high-precision and objective methods of study of medicinal products, which allow to standardize them both at the time of receipt and during storage. Chemical and physico-chemical methods of analysis, modified to take into account the specifics of a dosage form were used in the work.

The research to prove the identity and quantify the active components in the spray, including the study of validation characteristics of the methods, was carried out at RTC "Farmtest" of the Ministry of Health of Russia.

Before the identity test, a sample was prepared by preparing an aqueous spray solution (solution A): 2 ml of the spray was dissolved in 10 ml of purified water.

The identity test was carried out according to the OFS.1.2.2.0001.15 "General identity reactions" [4,5].

To confirm the *calcium cation,* the reaction of ammonium oxalate precipitation with 4% solution was used, the resulting calcium oxalate is insoluble in acetic acid diluted to 30% and 10% ammonia solution, but it is soluble in dilute mineral acids. Determination of the *chloride ion* was carried out by the reaction of silver nitrate precipitation with 2% solution, the resulting low-dissociable silver chloride is soluble in 10% ammonia solution. To confirm the *potassium cation* in the spray, the reaction of tartaric acid precipitation with 20% solution was used, the resulting potassium hydrotartrate is soluble in dilute mineral acids and solutions of alkali metal hydroxides. Determination of the *phosphate ion* in the spray was carried out by the reaction of silver nitrate precipitation with 2% solution in a neutral medium, the resulting silver phosphate of yellow color is soluble in nitric acid diluted up to 16% and 10% ammonia solution. To confirm the *sodium cation* in the spray, a microcrystalloscopic reaction with potassium pyroantimonate solution was used, forming crystals of sodium pyroantimonate in the form of prisms. Determination of the *fluoride ion* was carried out by reaction with a zirconyl-alizarin complex based on its destruction due to the binding of the zirconium cation to form a stronger complex with fluoride ions, change in the color of the solution from red-purple to yellow was observed [2–5,11].

The quantitative determination of calcium chloride was carried out by complexometry, and 0.05 M sodium edetate solution was used as the titrant. To create the required pH value, an ammonia buffer solution was used. When testing the direct titration method, after adding an ammonia buffer solution to the analyzed solution, the abundant precipitation of calcium phosphate falls out, to avoid this reaction, a reverse titration method was proposed. A control experiment was conducted in parallel [3–5,11].

1 ml of 0.05 M sodium edetate solution corresponds to 0,01095 g $CaCl_2 \times 6H_2O$. Content of calcium chloride for 100 ml of spray is calculated by formula:

$$X = \frac{T \times (V_{K.O.} - V) \times K \times P}{a},$$

where X – content of calcium chloride in the dosage form, g; T – sodium edetate titre of 0,05 M solution for calcium chloride, g/ml; P – volume of the dosage form, ml; V – the volume of the

titrated solution used for titration, ml; $V_{к.о.}$ – the volume of the titrated solution used for titration of the control test, ml; K – coefficient of correction to the molarity of the titrated solution; a – weighted amount of the dosage form, ml.

For the quantitative determination of potassium phosphate disubstituted in the spray the acidimetric method, a variant of displacement, was used [11].

1 ml of 0,5 M hydrochloric acid solution corresponds to 0,1141 g $K_2HPO_4 \times 3H_2O$. The content of potassium phosphate disubstituted per 100 ml of spray is calculated by the formula:

$$X = \frac{T \times V \times K \times P}{a},$$

where X – content of potassium phosphate disubstituted in the dosage form, g; T – titre of 0,5 M hydrochloric acid solution for potassium phosphate disubstituted, g/ml; P – volume of the dosage form, ml; V – the volume of the titrated solution used for titration, ml; K – coefficient of correction to the molarity of the titrated solution; a – weighted amount of the dosage form, ml.

For the quantitative determination of sodium fluoride, a photoelectrocolorimetric method was used (KFC-3 photometer, ZOMZ, Russia). The method is based on reaction of destruction of the zirconyl alizarine complex with fluoride ion. The intensity of staining of the solution as a result of the reaction decreases, so in this case, the inverse dependence of the optical density on the concentration of fluoride ions is observed. The optical density of the test solution is measured at a wavelength of 520 nm, in a cuvette with layer thickness of 10 mm, the reference solution is purified water. In parallel, the optical density of the work standard solution is measured [2].

The content of sodium fluoride in the spray is calculated using the formula:

$$X = \frac{(1 - A_{AH}) \times a_{CT} \times V_{AHMK} \times P}{(1 - A_{CT}) \times a_{AH} \times V_{CTMK}},$$

where X – content of sodium fluoride in the dosage form, g; A_{AH} – optical density of the test solution; A_{CT} – optical density of the work standard solution; P – volume of the dosage form, ml; a_{AH} – weighted amount of the dosage form, ml; a_{CT} – weighted amount of the work standard (content of sodium fluoride in 2 ml of 0.02% standard solution), g; V_{AHMK} – volume of the measuring flask for dilution of the spray weighted amount, ml; V_{CTMK} – volume of the measuring flask for dilution of the work standard weighted amount, ml.

The viscosity of the remineralizing spray was determined using Ostwald capillary viscometer by the method of SP XIV ed. OFS.1.2.1.0015.15 "Viscosity" [4,5].

The pH of the spray was determined using a potentiometric method according to the OFS.1.2.1.0004.15 "Ionometry" with I-500 ionometric transducer (Aquilon, Russia) [4,5].

RESULTS AND DISCUSSION

In appearance the spray is an opalescent homogeneous viscoplastic solution and has the following composition:

Calcium chloride	0.546 (Ca^{2+} – 0.1 g/ion)
Potassium phosphate disubstituted	0.951 (HPO_4^{2-} – 0.4 g/ion)
Sodium fluoride	0.014 (F^- – 0.006 g/ion)
Methylcellulose	(35) 2.5
Glycerine	3.0
Polyethylene oxide	(400) 2.5
Purified water	up to 100.0

The spray was standardized according to the following parameters: "Identity", "Quantitative determination of active ingredients" – including validation of the proposed methods, "pH Level", "Viscosity" and "Microbiological purity" [4,5,8].

Validation of identity testing methods and quantitative determination of mineralizing

drugs was performed in accordance with OFS. 1. 1. 0012. 15 "Validation of analytical methods" [8].

The specificity of the methods was studied on model mixtures with alternating components from the declared composition.

It is found that a linear relationship is observed between the values of analytical signals and the content of the determined components in the spray in the range from 70 to 130% of the declared amount (the analytical area of the method). The correlation coefficient of the regression graph R was 0.999 for sodium fluoride, calcium chloride, and potassium phosphate disubstituted.

The correctness of the methods is confirmed by setting the "found:entered" (Zi) relationship. It is in the range of 97 to 101% for calcium chloride, 93% to 111% for potassium phosphate disubstituted, and 98% to 102% for sodium fluoride. The deviation of Z^- from 100% does not exceed the confidence interval $\delta\% = |-0.48095| \leq 0.541053$, the systematic error is statistically indistinguishable from zero, which shows the satisfactory correctness of the methods.

Intra-laboratory (intermediate) precision of the methods was proved by calculating confidence intervals, which were 0.526±0.005 g (0.95%) for calcium chloride, 0.936±0.017 g (1.86%) for potassium phosphate, and 0.0139±0.0002 g (1.44%) for sodium fluoride. Based on the obtained value of the standard deviation and confidence interval, we can conclude that the precision of the methods under study is influenced by intra-laboratory variations.

Thus, the validated methods are specific, have satisfactory precision, linearity, correctness, and are used in the standardization of the spray to determine the quantitative content of calcium chloride, potassium phosphate disubstituted, and sodium fluoride. The results of quantitative determination of active ingredients are shown in Table 1.

Table 1

THE RESULTS OF QUANTITATIVE DETERMINATION OF ACTIVE INGREDIENTS IN SPRAY

Active ingredient	Series	Metrological characteristics					
		X, r	$\bar{X}$, r	S, r	$\Delta\bar{X}$	ε, %	$\bar{\varepsilon}$, %
Calcium chloride	1	0.550	0.546	0.0035	0.00435	1.661	0.80
	2	0.548				1.657	
	3	0.541				1.650	
	4	0.544				1.645	
	5	0.546				1.637	
Potassium phosphate disubstituted	1	0.981	0.967	0.0101	0.0126	2.86	1.30
	2	0.967				2.90	
	3	0.953				2.95	
	4	0.971				2.89	
	5	0.965				2.91	
Sodium fluoride	1	0.0141	0.0141	0.00016	0.0002	3.15	1.42
	2	0.0142				3.13	
	3	0.0139				3.20	
	4	0.0143				3.11	
	5	0.0140				3.18	

Table 2

RESULTS OF STANDARDIZATION OF THE REMINERALIZING SPRAY

Series №	Description	Identity						Quantitative determination			pH potentiometric method	Capillary viscosity according to Ostwald viscometer under the SP method	Microbial purity
		Ca^{2+} with 4% ammonium oxalate solution	Cl^- with 2% silver nitrate solution in the presence of 16% diluted nitric acid.	K^+ with 20% tartaric acid solution	HPO_4^{2-} with 2% silver nitrate solution at pH=7.0	Na^+ with potassium pyroantimonate solution	F^- with zirconyl-alizarin complex	$CaCl_2$ complexometry	K_2HPO_4 Acidimetric method	NaF photoelectrocolorimetry method			
Standard requirements													
	opalescent homogeneous viscoplastic solution	white precipitate, HP in 30% acetic acid, diluted and 10% ammonia solution, P in diluted mineral acids	white cheesy precipitate, HP in 16% diluted nitric acid, and P in 10% ammonia solution	white crystalline precipitate P in diluted mineral acids and alkali metal hydroxides solutions	yellow precipitate, P in 16% diluted nitric acid and 10% ammonia solution	crystals intheform of prisms	changing the color of the solution from red-purple to yellow	0.526±0.037	0.933±0.056	0.0139±0.0028	6.5–7.5	0.67–0.71	Not higher than 10^2 GFU per 1,0 g
1	conforming	conforming	conforming	conforming	conforming	conforming	conforming	0.5260±0.0071	0.933±0.018	0.0139±0.0003	6.75±0.05	0.7030±0.020	<100
2	conforming	conforming	conforming	conforming	conforming	conforming	conforming	0.5410±0.0066	0.961±0.015	0.0126±0.0002	6.86±0.05	0.7000±0.020	<100
3	conforming	conforming	conforming	conforming	conforming	conforming	conforming	0.5460±0.0073	0.956±0.016	0.0140±0.0003	6.90±0.05	0.7012±0.020	<100
4	conforming	conforming	conforming	conforming	conforming	conforming	conforming	0.5600±0.0088	0.951±0.018	0.0154±0.0003	6.80±0.05	0.7032±0.020	<100
5	conforming	conforming	conforming	conforming	conforming	conforming	conforming	0.5650±0.0088	0.905±0.02	0.0165±0.0003	6.80±0.05	0.7030±0.020	<100

The relative error of the average result of determining the content of calcium chloride in the spray by the complexometric method is 0.80%, potassium phosphate disubstituted in the spray by the acidimetric method – 1.30%, sodium fluoride in the spray by the photoelectrocolorimetric method – 1.42%, which indicates a good reproducibility of the proposed methods.

The pH of the developed spray was 6.75–6.90±0.05, which is within the acceptable limits for dental sprays (6.5–7.5).

The viscosity value was 0.6998–0.7032±0.02.

As of parameter "Microbiological purity" the spray meets the requirements of the OFS.1.2.4.0002.15 and can be used as a local medication.

The results of evaluating the quality of the spray in five series for appearance, qualitative and quantitative analysis, pH, viscosity and microbiological purity are presented in table. 2. The data obtained indicate that spray meets the standard requirements.

CONCLUSION

A comprehensive standardization of the spray on 5 series in the study group was carried out according to the parameters "Identity", "Quantitative determination of active ingredients", "pH Level", "Viscosity" and "Microbiological purity", including the study of validation characteristics of testing methods for identity and quantitative determination of active ingredients.

The established parameters can be the criteria for evaluating the quality of the spray during stage-by-stage control in the production process and quality control of the final product and are the basis for the developed draft pharmacopoeial monograph for the spray.

The developed remineralizing spray can be recommended for professional oral hygiene for individual and medical use in cases of high intensity of caries, the presence of general and local cariesogenic factors (in particular, in orthodontic patients and patients with xerostomia who are subject to radiation therapy), the presence of foci of enamel demineralization and dental hyperesthesia.

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