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VALIDATION OF THE METHOD FOR DETERMINING THE AMOUNT OF Na+ AND K+ IONS IN THE COMBINED DRUG «REHYDRATE ZINC» AND EVALUATION OF THE STABILITY OF THE STABILITY OF THE METHOD

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Abstract. In this study, the purity and amount of sodium (Na^+) and potassium (K^+) ions contained in the drug "Rehydrate Zinc" were determined using atomic absorption flame spectrometric methods (in the emission mode). The advantages of the combined use of atomic absorption and atomic emission spectrometric methods, the exclusion of destructive factors in the analysis of substances contained in complex drugs, the correct choice of a nebulizer and temperature, as well as the stability of the method when using highly sensitive methods were studied. The developed method of analytical analysis was validated for the following parameters: specificity, linearity, precision and reliability. The emission and atomic absorption flame spectrometric method can be used in manufacturing plants and research laboratories, in quality control and standardization of rehydrating solutions containing electrolytes, infusion solutions.
Key words: atomic absorption spectrometry, Shewhart control charts, electrolyte solutions, sodium, potassium, validation parameters, specificity, linearity, precision, accuracy.

«REGIDREYD RUX» KOMBINIRLANGAN DORI VOSITASI TARKIBIDAGI Na+ VA K+ IONLARINI MIQDORINI ANIQLASH USULINI VALIDATSIYALASH VA USULNING BARQARORLIGINI BAHOLASH

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Annotatsiya. Ushbu tadqiqotda elektrolitlar saqlagan «Regidreyd Rux» dori vositasi tarkibidagi natriy (Na^+) va kaliy (K^+) ionlarining chinligi va miqdori atom-absorbsion alangali spektrometrik usullari (emissiya rejimida) yordamida aniqlandi. Atom-absorbsion va atom-emission spektrometrik usullarni birgalikda qo‘llashning afzalliklari, murakkab tarkibli dori vositalari tarkibidagi moddalarni tahlilida halaqit beruvchi omillarni bartaraf etish, atomizator va haroratni to‘g‘ri tanlash, shuningdek, sezgirligi yuqori bo‘lgan usullardan foydalanishda usulning barqarorligi kabi muammolar o‘rganildi. Ishlab chiqilgan analitik tahlil usuli quyidagi ko‘rsatkichlar bo‘yicha validatsiyalandi: hususiyligi (specificity), chiziqliligi (linearity), takrorlanuvchanligi (precision) va to‘g‘riligi (trueness). Emission va atom-absorbsion alanganli spektrometrik usuli yordamida ishlab chiqarish korxonalarida va ilmiy tadqiqot laboratoriyalarida, tarkibida elektrolitlar saqlagan regidratant eritmalar, infuzion eritmalar sifatini nazorat qilishda va standartlashda qo‘llanilishi mumkin.
Kalit so‘zlar: atom absorbsion spektroskopik usul, Shuxart nazorat kartalari, elektrolitlar, natriy, kaliy, validatsiya, hususiylik, chiziqlilik, qaytariluvchanlik, to‘g‘rilik.

INTRODUCTION.

Currently, scientific research is being carried out on a global scale to improve the technology, quality control, validation and existing methods of analytical analysis of complex-containing peroral rehydration drugs. Sodium and potassium ions are the most important electrolytes in the composition of peroral rehydration drugs. Since the amount of these ions is directly dependent on the osmolarity and efficiency of the rehydrating solutions, it requires the correct and effective application of methods for controlling and quantifying their quality. It is not possible to determine a separate amount of sodium and potassium chlorine salts in the composition of drugs with a complex composition, the properties of which are composed of drug substances close to each other. The reason is that the Har two store the ham chlorine ion. For this reason, [1, 2, 3] sodium and potassium ions in saline solutions stored by multicomponent electrolytes have been suggested to be determined in the AAS method, using the argentometric titration method of total chlorine ions. The problems that arise in the standardization of drugs with a complex composition, consisting of several drug substances, necessitate the development of further improved methods in the future.

To date, pharmaceutical practice has used the atomic absorption (emission) spectrometric method to extract complex-containing drug-containing elements (Na, K, Ca, Zn, and b.q)are widely used in quantification. With the simplicity, accuracy, speed and sensitivity of the AAS and AES methods, scientists not only in our country, but all over the world are using them in their scientific research with high interest. The accuracy of the method depends on the

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concentration of the substance, and it is 1-4%, the sensitivity is 0.001 mcg/ml, depending on the characteristics of the analytical line, the sample composition, the type of equipment [4]. Although the method is not new, but the methods developed according to the nature of the object under study are fundamentally different from each other. That is, it is important to develop a suitable methodology, taking into account the factors that affect the method of analysis of an object. The following situations can cause errors in the analysis of elements: the separation of the atoms being examined into ions under the influence of flame temperature, the formation of stagnant chemical compounds on the surface of the flame, the absorption of light without selection, the influence of organic compounds in the composition of the drug on the background of the analysis and other factors.

Each validated method testifies to the fact that the selected techniques give reproducible and accurate results. Taking into account the fact that the production of the drug" regided zinc " (composition: glucose-13.5 g, Sodium Citrate dihydrate-2.9 g, Sodium Chloride-2.6 g, potassium chloride-1.5 g, zinc sulfate-0.050 G, purified water-up to 1L) on an industrial scale has been established, it became necessary to assess the stability and validate the quality control analysis methods of this drug [5].

This study aims to obtain a valid method for the determination of the amount of sodium and potassium ions contained in the drug" Regidrate Zinc " using the flame atomic absorption spectrophotometric (emission mode) method, interpretation of the stability of the method.

METHODS AND MATERIALS.

Using the atomic-absorption flame spectrophotometric method (emission mode), a method for determining the amount of sodium (Na^+) and potassium (K^+) ions in the composition of the drug "Regidrate Zinc" was developed [6].

The detection of ions in the drug's composition used a type AA500, (PG-instruments, UK) (emission mode) atomic-absorption spectrophotometer equipment.

Analysis conditions:

Wavelength:	Na^+–588,6 nm, K^+–768 nm
Measurement time:	5 s
Signal tracking height:	5 mm
Atomizer:	Flame (air-acetylene)
Flow rate:	1000 ml/min

An emission signal was measured at the corresponding wavelength for the element being detected (Na^+-589,6nm, K^+-768nm)[5]. Standard solutions (100 mcg/ml) were prepared by dissolving potassium and sodium chloride with state standard sample (SSS) deionized water. Potassium chloride substance 99,17%; sodium chloride substance 99,77% were used as standard samples. Tris buffer 99,0% solution was applied with the aim of reducing ionization levels of disruptive factors. Flame was applied as an atomizer. Flame is considered the lowest temperature atomizer and is mainly used in the determination of alkaline and alkaline earth metals. Acetylene-air gas mixtures were used for the flame (combustion rate 266 sm/s). The sensitivity of the method is 10-2 mass%, the stability is high, and the relative standard deviation is 0,01-0,05 range. The calibration of the equipment was carried out using etalon solutions with a clear concentration of the elements being detected [7].

Sodium ion (Na^+).

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1 Solution: 20 ml of the drug solution diluted with up to 100 ml of water;
2 Solution: 10 ml of the drug solution diluted with up to 100 ml of water;
3 solution: 5 ml of the drug solution diluted with up to 100 ml of water (a solution).
Water was used as a comparable solution.
The amount of sodium ions in g/ml was determined using the following formula:

$$X = \frac{A \times m \times 23}{B \times 1000 \times 58.44}$$

here:
A-is the indicator of the solvent (solution a);
B-is the indicator of the standard solution (solution B).
1 ml of the drug contains 0,001448 g to 0,001959 g of sodium ions (Na^+).
Potassium ion (K^+).
1 Solution: 20 ml of the drug solution diluted with up to 100 ml of water;
2 Solution: 10 ml of the drug solution diluted with up to 100 ml of water;
3 solution: 10 ml of the drug solution diluted with up to 50 ml of Water (s solution).
The amount of potassium ions in g/ml was determined using the following formula:

$$X = \frac{S \times m \times 39}{D \times 1000 \times 74.55}$$

here:
S-indicator of the probable solution (S solution);
D-is the indicator of the standard solution (D Solution).
1 ml of the drug stores from 0,0006679 g to 0,009036 g of potassium ions (K^+).
Preparation of standard solutions.
Preparation of the WSS (work standard solution) solution that stored the sodium ion: A measure of 1000 ml in volume was placed in the flask, dried for 3 hours at about 105°C, 4,3294 g of sodium chlorine and 1,5000 g of potassium chloride, dissolved in water and transported to the mark with the same solvent.

The main solution of 20 ml of sodium was placed in a measuring flask with a volume of 100 ml and transported with water to the mark. From the resulting solution, 10 ml was measured and delivered to a volume of up to 100 ml of water. From this resulting solution, 5 ml was taken and transferred to a measuring flask with a volume of 50 ml and delivered to the mark with water (solution B).
Preparation of The WSS solution that stored the potassium ion: In a measuring flask with a volume of 1000 ml, dried for 3 hours at about 105°C, 1,5000 g of potassium chloride and 4,3294 g of sodium chloride were released, dissolved in water and transported to the mark with the same solvent.
The main solution of 20 ml of potassium was placed in a measuring flask with a volume of 100 ml and brought to the mark with water. From the resulting solution, 10 ml was measured and delivered to a volume of up to 100 ml of water. From this resulting solution, 10 ml was taken and transferred to a measuring flask with a volume of 50 ml and delivered to the mark with water (solution D). When determining K^+ ions, sodium ions in standard solutions are compared to sodium ions in a solution that is tested to have a ratio of 1:25.

RESULTS.

Using the Atom-absorption spectrophotometric method, reliable results were obtained that met the criteria of conformity in determining the amount of sodium and potassium ions.

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Statistical processing of the results obtained was carried out in accordance with the UzPh. The accuracy of the method was assessed using the styling criterion.
A metrological description of the results of the analysis was presented in Table 1.
Table 1.
Results of the analysis and metrological description of the style (n=5; P=95%; t(p,f)=2,78)

ND standard	Amount found, g/l	Metrological description
Sodium ion (Na^+)		
1,448 g/l to 1,959 g/l	1,8460 1,7030 1,8042 1,7321 1,8115	$X_{mid,\ g/l}$=1,7790 F=4 S^2=0,06 $\Delta\bar{x}$=0,03% $\bar{\varepsilon}_{\%}$=0,1
Potassium ion (K^+)		
0,6679 g/l to 0,9036 g/l	0,7881 0,7800 0,7865 0,7902 0,7877	$X_{mid,\ g/l}$=0,7865 F=4 S^2=0,004 $\Delta\bar{x}$=0,02% $\bar{\varepsilon}_{\%}$=0,05

According to the data presented in table 1, the content of sodium and potassium ions in the drug "Regidrate Zinc" is 1,7790 g/l and 0,7865 g/l, the analysis obtained showed that the average relative errors of the results in the statistical description of the results were 0,1% and 0,05%.
Standard rehydration solutions designed for drinking must meet clearly defined requirements. To do this, at all stages of the production of the drug, it is necessary that the quality control and supply systems work smoothly. The result of assessing the stability of the process is caused in many cases by changes in the results of the analysis in the quality control of preliminary and finished products, deviations. One of the important elements of statistical management of process stability are control cards.
With the help of Shewhart control cards, the quality of quantitative analysis processes was assessed using the flame emission and atomic absorption spectrometric method (AE-AAS) of Na^+ and K^+ ions contained in the drug "Regidrate Zinc".
Control cards-signaling, but cannot indicate the reasons for the instability of the process. To determine the causes of instability of the analysis process, it is necessary to study a number of external and internal influencing factors, as it were, to eliminate physical and chemical disruptive factors into the method in order to ensure the accuracy and rigidity of the results [8].
Based on the formulas given in GOST R ISO 7870-2-2015, CL (Central line), UCL (Up central line), LCL (Low central line) values were determined. X card Na^+ for CL=0.094, UCL=2.06, LCL=-1.96; K^+ for CL=-1.07, UCL=3.42, LCL=-5.57. R card Na^+ for CL=3.57, UCL=7.54, LCL=0; K^+ for CL=7.8, UCL=16.74, LCL=0. On the basis of the calculated center line values, as well as the values of the upper and lower limits, X (corresponding values) and R (variable

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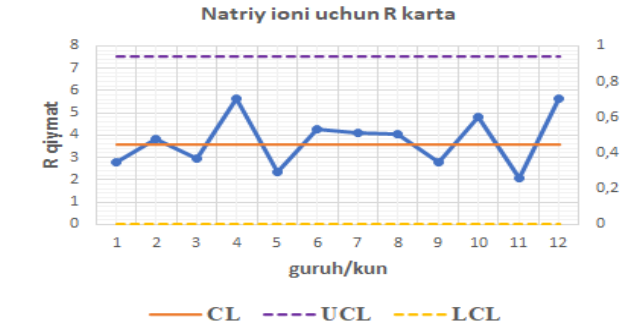
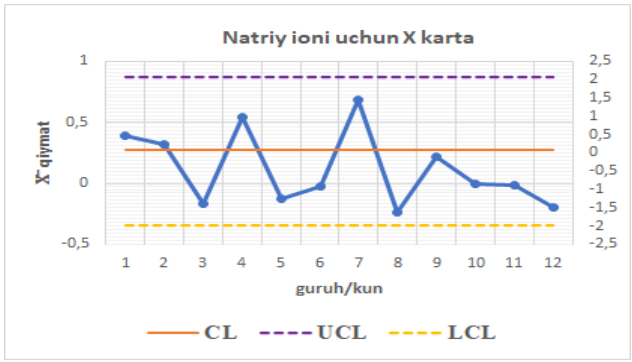
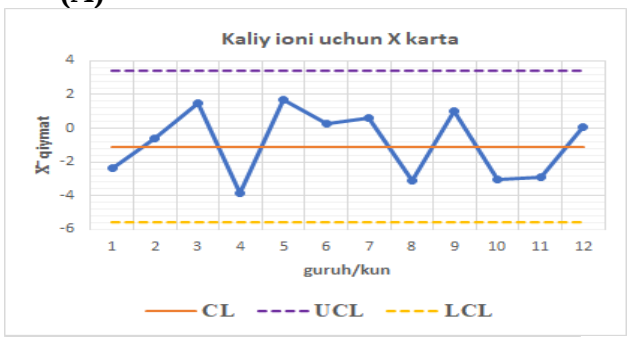
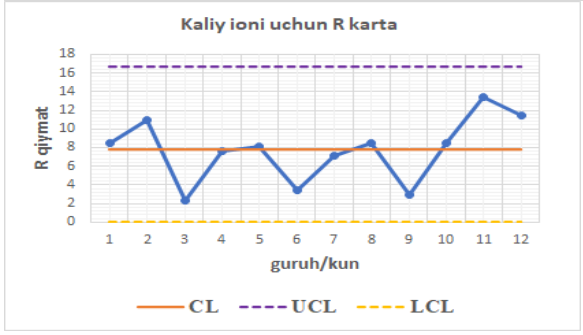
range) fuzzy control cards were created. Drawings made with respect to the Na^+ and K^+ ions contained in the rehydrating solution are shown in figure 1.





(A) **(B)**





(C) **(D)**

Figure 1. Graph drawing of X and R cards for (A) and (B) sodium ions, (C) and (D) potassium ions

As can be seen from the above drawings, the corresponding values X and the range of changes R values are located between the upper and lower limits. This has shown that it is consistent

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with all indicators, that the method of quantitative analysis is stable, and that interference factors in the analysis process do not affect the results.
Validation of the analysis method was carried out in terms of selectivity, linearity, precision and accuracy [9].
Testing the accuracy of the method is based on quantifying sodium and potassium ions by adding a specific number of standard probing solutions. The alternative criterion for the correctness of the method, the average amount of sodium and potassium ions calculated in relation to 100%, that is, it is necessary to be in the range of 100±5% [10]. The results of determining the correctness of the method are presented in tables 2.
Table 2.
The accuracy results of the AE-AAS method used to quantify the sodium ion in "Regidrate Zinc"

Given amount of sodium ion, g/l and concentration level, %		Found amount of sodium ion, g/ml	Response factor (R),%
0,001360	80%	0,001362	100,15
		0,001360	100,00
		0,001361	100,07
0,001700	100%	0,001699	99,94
		0,001720	101,18
		0,001701	100,06
0,002040	120%	0,002035	99,75
		0,002045	100,25
		0,002041	100,05
The average value of the response factor			100,16

The results of the accuracy validation index of the AE-AAS method used to quantify the potassium ion contained in "Regidrate Zinc" are shown in table 3.
Table 3.
The accuracy results of the EA-AAS method used to quantify the potassium ion in "Regidrate Zinc"

Given potassium ion, g/l and concentration level, %		Found amount of potassium ion, g/ml	Response factor (R), %
0,000629	80%	0,0006288	100,00
		0,0006286	99,97

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		0,0006290	100,03
0,000786	100%	0,0007861	100,05
		0,0007860	100,04
		0,0007855	99,97
0,000943	120%	0,0009432	100,00
		0,0009435	100,03
		0,0009430	99,98
The average value of the response factor			100,01

In the data presented in tables-2-3 above, the each for ions should be released at a level of high resolution, and this range should be in the range of 95,0-105,0%. Here, the range ranged from 99,75% to 101,18% for sodium ions, to 99,97% to 100,05% for potassium ion.

6 solutions containing 100% of the active ingredients of the drug "Regidrate Zinc" were prepared. The results are shown in table 4.

Table 4.

The results of the precision of the AE-AAS method, which was used to assess the amount of sodium ion in the composition of the drug "Regidrate Zinc"

№ samples	Given amount of sodium ions, g/ml	Analyst 1		Given amount of sodium ions, g/ml	Analyst 2	
		Found amount of sodium ions			Found amount of sodium ions	
		g/ml	%		g/ml	%
Sample №1	0,001701	0,001700	99,94	0,001700	0,001710	100,59
Sample №2	0,001701	0,001707	100,35	0,001700	0,001698	99,88
Sample №3	0,001701	0,001705	100,24	0,001700	0,001695	99,71
Sample №4	0,001701	0,001702	100,06	0,001700	0,001704	100,24
Sample №5	0,001701	0,001699	99,88	0,001700	0,001702	100,12
Sample №6	0,001701	0,001701	100,00	0,001700	0,001697	99,82
Mean		0,001702	100,08	Mean	0,001701	100,06
Standard deviation		0,000003		Standard deviation	0,000006	
Relative standard deviation, %		0,18		Relative standard deviation, %	0,32	

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Table 5
The results of the precision of the EA-AAS method, which was used to assess the amount of potassium ion in the composition of the drug "Regidrate Zinc"

№ samples	Given amount of sodium ions, g/ml	Analyst 1		Given amount of sodium ions, g/ml	Analyst 2	
		Found amount of sodium ions			Found amount of sodium ions	
		g/ml	%		g/ml	%
Sample №1	0,000786	0,000780	99,24	0,000780	0,000778	99,74
Sample №2	0,000786	0,000790	100,51	0,000780	0,000781	100,13
Sample №3	0,000786	0,000792	100,76	0,000780	0,000785	100,64
Sample №4	0,000786	0,000789	100,38	0,000780	0,000779	99,87
Sample №5	0,000786	0,000788	100,25	0,000780	0,000780	100,00
Sample №6	0,000786	0,000778	98,98	0,000780	0,000783	100,38
Mean		0,000786	100,02	Mean	0,000781	100,13
Standard deviation		0,000006		Standard deviation	0,000003	
Relative standard deviation, %		0,73		Relative standard deviation, %	0,33	

Figures 4-5 show that the relative standard deviation value of the method of determining the amount of sodium and potassium ions in a solution prepared in 6 samples of the drug "Regidrate Zinc" should not exceed 3,0%. The relative standard deviation for the sodium ion was 0,18% and for the potassium ion 0,32%.

Table 6
Emission signal results obtained after analysis processes of samples of sodium and potassium chloride at different concentrations

№ sample	Sample			
	Detected concentration, g/ml emission signal		Detected concentration, g/ml emission signal	
	Na^+	K^+	Na^+	K^+
1	0,001350	0,000630	80,0	80,0
2	0,001529	0,000707	90,0	90,0

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3	0,001702	0,000785	100,0	100,0
4	0,001870	0,000865	110,0	110,0
5	0,002037	0,000943	120,0	120,0
Linear regression: y=a+b*x				
Deviation, b			58296,61	127677,77
Ordinate axis intersection, a			1,03567	-0,354732
Correlation coefficient, r			0,9998	0,99998
Variation coefficient, r^2			0,9997	0,9999

Based on the results obtained, drawings of the dependence of the concentration of sodium and potassium ions in solution on the emission signal were compiled. Using the Microsoft Office Excel program, the trend line was pulled and the regression equation was compiled. The results are given in figures 2-3.

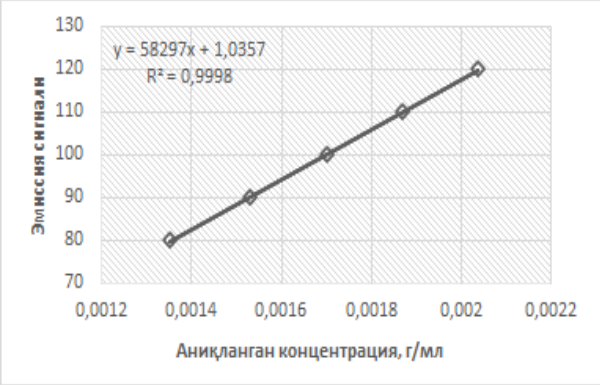

Figure 2. Dependence of the emission signal on the concentration of sodium ions

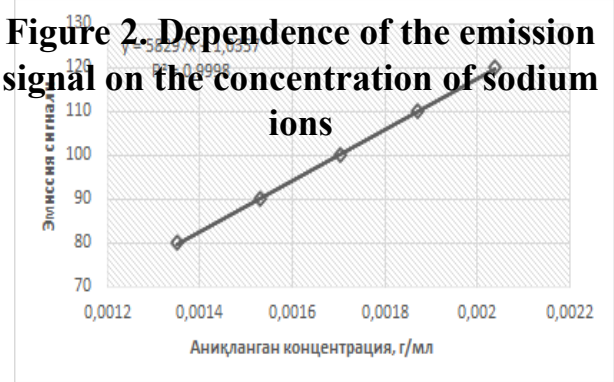

Figure 3. Dependence of the emission signal on the concentration of potassium ions

As can be seen from figures 2 and 3, almost all experience points fit over the trend line. Variation coefficient R^2=0,9998 in value, sodium ion content from 0,001350 to 0,002037 g/ml, potassium ion content from 0,000630 to 0,000943 g/ml in value R^2=0,9998 was observed linearity dependence drawing.

CONCLUSIONS.

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Using the Atom-absorption flame spectrophotometric (emission mode) method, sodium and potassium ions were quantified and 1ml of "Regidrate Zinc" contains 0,6679-0,9036 mg/ml of K^+ ions, 1,448-1,959 mg/ml of Na^+ ions.
The developed methods were evaluated on the validation indicators of analytical analysis methods. The results obtained were confirmed to meet the validation requirements of the developed styles in terms of specificity, linearity, pecision and trueness and expected results could be achieved.
On the basis of the calculated center line values, as well as the values of the upper and lower limits, X (corresponding values) and R (variable range) fuzzy control cards were created. It has been proven that the corresponding values X and the range of changes R values lie between the upper and lower limits. This has shown that it is consistent with all indicators, that the method of quantitative analysis is stable, and that interference factors in the analysis process do not affect the results.
The developed method of analysis can be applied to the determination of sodium and potassium ions in the composition of drugs of complex composition.

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