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VALIDATION OF THE SPECTROPHOTOMETRIC METHOD OF DETERMINATION OF DRUG SUBSTANCE IN MEDICINE "CHLOROPYRAMINUM, CREAM"

The article is devoted to validation of method of quantitative determination of drug substance – chloropyramine in medicine "Chloropiraminum, cream for external application, 10 mg/g".

The research purpose was to confirm documentarily that a spectrophotometric method is suitable for quantitative determination of chloropyramine hydrochloride in medicine "Chloropyraminum, cream for external application, 10 mg/g" and allows getting results with the required metrology characteristics. The research objects were the samples of medicine "Chloropyraminum, cream for external application, 10 mg/g". The absorbance measurements were conducted at the spectrophotometer of RV 2201A.

It was developed the plan of validation, including the determination of the following validation characteristics: selectivity, trueness, application range, linearity and precision (repeatability and reproducibility within the laboratory). For every validation characteristic the criteria of acceptability were calculated. In accordance with the developed plan the experimental researches were carried out and it was found out that validation characteristics were in the accordance with the criteria of acceptability. As a result of validation which was carried out in accordance with TKP 030-2013 (02040) it was confirmed that spectrophotometric method for determination of chloropyramine hydrochloride in medicine "Chloropyraminum, cream for external application, 10 mg/g" is available.

Key words: validation, chloropyramine, cream, quantitative analysis, validation characteristics, criteria of acceptability.

Introduction. "Chloropyramine" (suprastin) is one of the most widely used sedative antihistamines. It has a significant antihistamine activity, peripheral anticholinergic and antispasmodic effect moderate. It is effective in most cases, for the treatment of seasonal and perennial allergic rhinoconjunctivitis, angioedema, urticaria, atopic dermatitis, eczema, itching of various etiologies; in parenteral form for the treatment of acute allergic conditions, requiring emergency assistance. Medicament produced in different dosage forms (tablets, injections, ointments, creams) and has a plurality of trade names ("Chloropyramine" "Suprastin", "Allergozan").

The drug "Chloropyramine-cream" has a cream white color, smooth consistency, which is composed of: Chloropyramine hydrochloride, polyethylene glycol 400, polyethylene glycol 4,000.

The active ingredient is a drug Chloropyramine hydrochloride (N-[(4-chlorophenyl)methyl]-N', N'-dimethyl-N-2-pyridinyl-ethane-1,2-diamine hydrochloride) of white or almost white, crystalline powder with low odor, readily soluble in water and chloroform, soluble in 96% alcohol.

Quantitative determination Chloropyramine hydrochloride can be carried out by two methods: spectrophotometric method and the non-aqueous titration with perchloric acid.

Nonaqueous titration method is based on the reaction of Chloropyramine hydrochloride mercury acetate (II), formed with Chloropyramine acetate solutionwichis titrated with 0.1M perchloric acid until green dye (indicator – 0.1% crystal violet solution).

The main disadvantages of the method are the use of a non-aqueous titration, the titration setup sealed and toxic volatile solvent.

Spectrophotometric method for determining the solutions is based on the ability to absorb ultraviolet radiation Chloropyramine hydrochloride, with a maximum absorptionwichis observed at a wavelength (λ_{max}), equal to 244 nm.

To confirm the suitability of analytical methods are provided for the validation procedure which includes the following stages: design validation plan, experimental studies, statistical processing of the results and the formulation of the validation report.

The purpose of this work is to document the data validation that the spectrophotometric method is suitable for the quantitative determination of Chloropyramine hydrochloride drug "Chloropyramine, cream for external use 10 mg/g" and allows to obtain results with the required metrological characteristics.

Main part. Object of research is to investigate some prototypes of PM Chloropyramine in the form of cream for external use 10 mg/g. The components of the cream is LS base: polyethylene glycol 400; polyethylene glycol 4,000.

To carry out the test solution of 1.000 g of drug was dissolved in 50.0 ml of 96% alcohol. Then itwas adjusted to 100.0 ml of 96% alcohol and stirred.

Chloropyramine hydrochloride of 0.050 g was taken as the standard sample solution (SB). Then it was dissolved in 50.0 ml of 96% alcohol; 1.0 ml of the resulting solution was adjusted to 100.0 ml of 96% alcohol and stirredwith the solution: 96% ethyl alcohol, optical density measurements was carried out with a spectrophotometer PB 2201A. Measurement conditions: wavelength 244 nm; cell with 10 mm layer thickness. The results of measurements of the optical densities of the test solution and a solution of the hydrochloride salt of SB Chloropyramine were calculated depending on considering concentration of Chloropyramine PM hydrochloride.

Results and discussion. It was selected as a validation characteristics: selectivity, accuracy, application range, linearity and precision (repeatability and within-laboratory reproducibility).

Validation criteria in the table below was establishedin accordance with the recommendations of [1/2] for each validation characteristics.

Selectivity is determined by comparing the optical "placebo" mud density and drug solution with analytical wavelengthof 244 nm. The eligibility criteria is the components of "placebo" should not affect the determination of Chloropyramine hydrochloride PM.

An analysis was performed on five concentration levels: 0.007, 0.008, 0.010, 0.012, 0.013 mg/ml, which corresponds to 70, 80, 100, 120 and 130% of the nominal content Chloropyramine hydrochloride PM respectively to determine the linearity of the method of application within range (70–130% of the nominal content Chloropyramine hydrochloride). The following equation was used according to the linear dependenceusing the normalized coordinate by the least squares:

$$A_i / A_{st} \cdot 100 = b\left(C_i / C_{st}\right) \cdot 100 + a,$$

where A_i is optical density; A_{st} is optical density of 100% for the given values of the argument; C_i is concentration, C_{st} is the concentration of 100%.

In accordance with the recommendations given in [2], in addition to the requirements for the correlation coefficient and residual standard deviation (RSD_0) it was calculated eligibility criteria for free as a member, for spectrophotometry necessarily determine whether a straight line passes through the origin.

Repeatability was assessed by the results of 6 parallel tests carried out on a series of drugs in a single day in the same conditions. The eligibility criteria is the relative standard deviation (RSD, %) for the definitions and it should not exceed 2.0%.

Within-laboratory reproducibility was assessed by the results of 6 parallel tests a series of drugs, carried out by two analysts on different days. As the results of testsit was performed by one of analysts using the results obtained by determining the frequency of occurrence. The test results were checked for homogeneity by Q-sample test. In order to detect statistically significant differences between the variances and the arithmetic mean of the results of the two series, produced under different conditions, using the criteria of Fisher and Student. Eligibility criteria are the calculated values of Fisher's exact test and Student's test shall not exceed the tabulated values for the 95% confidence level and a predetermined number of measurements; relative standard deviation of the results (RSD, %), calculated for the quantitative content Chloropyramine hydrochloride obtained in terms of repeatability They must not exceed 2.0%.

Determining the accuracy of the procedure was verified by quantitative testing of model solutions prepared with a known amount Chloropyramine hydrochloride three concentrations within the range of application methods: 8.0; 10.0; 12.0 mg/g (80; 100; 120% of the nominal content Chloropyramine hydrochloride) using 6 plays for each concentration. Validation of the data was carried out by calculating the offset $\left| \bar{x} - \mu \right|$ test the significance of differences between the random variable $\bar{x}$ from the constant μ and the mean degree of reduction of the eligibility criteria $(\overline{Z})$. The percentage of the recovery obtained in the analysis of solutions with an active substance content of 80, 100 and 120%, adjusted 100%, should be in the range of 95.0 to 105.0%. The displacement of the measurement resultis divided by the standard deviation to the absolute average value shall not exceed the value of t-test for a confidence probability P = 95% and the specified number of dimensions.

In determining the specificity technique has been found that placebo solution components have no absorption at a wavelength of 244 nm and a method of determining the quantitative content in Chloropyramine selective PM defined with respect to the component.

Statistical processing of the linear dependence of the values obtained from the hydrochloride content Chloropyramine specified content correlation coefficient of linear regression graph was 0.999 and the value of the free term – 1.63, the residual standard deviation is 0.0015. Thus, the method is linear in the range 70–130%, which corresponds to concentrations of working solutions of 0,007–0.013 mg/ml of Chloropyramine.

It was establishedthe convergence (repeatability) method tests of the quantitative determination of Chloropyramine hydrochloride. The relative standard deviation of the mean (RSD, %) was calculated for the content of Chloropyramine hydrochloride obtained in PM.

Validation results (range *D* – 70–120%, the content of tolerance *B* = ±5%, *n* = 6)

Validationcriteria	Resultingvalue	Criticalvalue
Specificity The components of "placebo" should not distort the result	Corresponded	–
Linearity: $y = bx + a$ Residualstandarddeviation $RSD_0 \le \frac{b \cdot 0.32 \cdot B}{t_{(0.95;n-2)}}$, % Freemember $\lvert a \rvert \le \Delta_a = t_{(0.95;n-2)} \cdot RSD_a$ **Correlation coefficient** $r \ge \sqrt{1 - \frac{b \cdot 0.32 \cdot B / t_{(0.95;n-2)}}{RSD_y}}$	 0.0015 1.63 0.999	 max $RSD_0 = 0.497$ max $a = 3.17$ min $r = 0.99$
Accurateness Percentage of recovery $\overline{Z}$, % Student's criterion	 $\overline{Z} = 97.99$ $t_0 = 1.75$	 $\overline{Z} = 95–105$ $t_{(0.95;5)} = 2.57$
Frequency Coefficient of variation RSD, %	0.23	max $RSD = 2$
Intermediate precision Coefficient of variation RSD, % Student's criterion $t_0 \le t_{(0.95;f)}$ Fisher's criterion $F_0 \le F_{(0.95;f_1;f_2)}$	 0.30 0.13 1.71	 max $RSD = 2\%$ $t_{(0.95;10)} = 2.23$ $F_{(0.95;5;5)} = 5.05$

The repeatable conditions is 0.23%, which meets the criterion of acceptability: not more than 2.0%.

It was confirmed within reproducibility of the method: the calculated values of the Fisher criteria and Student does not exceed the limit values; the relative standard deviation of the mean (RSD, %) results for the quantitative determination Chloropyramine hydrochloride is 0.3% in PM, which corresponds to the acceptability criteria: not more than 2.0%.

The accuracy of the procedure is confirmed by the relevant tests on simulated samples for the three concentrations within the range of application of testing methods, with 6 repetitions. The results determine the lack of emissions (homogeneous sample). The average percentage of recovery was 97.99%, which corresponds to the eligibility criteria established 95.0–105.0%. The offset measurement result was divided by the standard deviation to the absolute average value. It does not exceed the value of Student's t test for $P = 95\%$ and the specified number of dimensions.

Determination results of performance characteristics and validation accuracy quantification techniques Chloropyramine hydrochloride are presented in the table.

Conclusion. Validation results are confirmed with the suitability of the use of spectrophotometric method for the quantitative determination of Chloropyramine hydrochloride as sucndrug as "Chloropyramine, in the form of cream for external use 10 mg/g".

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