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Development and validation of the procedure for the quantification of the lyophilized liposomal dosage form of the LHS-1208 indolocarbazole derivative

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Abstract:

Scope: the development and validation of the procedure for the quantitative spectrophotometric determination of the active ingredient in the lyophilized liposomal dosage form (LLF-lyo) of the LHS-1208 indolocarbazole derivative.

Materials and Methods –The "LHS-1208 liposomal, lyophilizate for the preparation of dispersion for injection 1.8 mg" drug and the LHS-1208 substance. Spectrophotometry.

Results –Maxima at 320±2, 286±2, 238±2 and 203±2 nm have been observed in the electronic absorption spectrum (EAS) of the alcohol solution of the LHS-1208 substance in the range from 200 to 800 nm. It has been established that the EAS of an alcoholic solution of liposomal LHS-1208 in the range from 230 to 500 nm is identical to the EAS of an alcohol solution of its substance by the shape of the curve and the position of the maxima. To quantify the LHS-1208 in the LLF-lyo, the most intense absorption band with a maximum of 320±2 nm has been selected. It has been shown that at a wavelength of 320 nm the optical density for the alcoholic solution of the auxiliary components is close to zero and does not affect the identification and quantification of LHS-1208 in the LLF-lyo composition, and the technique can be considered as *specific*. Based on the results of the validation assessment by the "linearity", "accuracy", and "precision" characteristics, it has been proved that the developed procedure is applicable for quantification of LHS-1208 in the LV 80-120% concentration range.

Conclusion – The procedure for the quantification of LHS-1208 in LLF-lyo by spectrophotometry has been developed and validated.

Keywords: LHS-1208, liposomes, quantitative analysis, spectrophotometry, validation.

INTRODUCTION

Currently, chemotherapy remains the basis of drug treatment of malignant tumors. This is the primary method of treatment of hemoblastoses and important component in the treatment of many solid tumors, along with surgery and radiological methods. However, there is no and, apparently, there will never appear a single, universal remedy that gives effect in all malignant tumors. The spectrum of action of a particular drug is usually limited to a few, and sometimes to only one tumor or its morphological variant [1].

Among chemical compounds of natural and synthetic origin a class of N-glycosides indolo [2] carbazole derivatives can be distinguished, which exhibit various types of biological activity, including antitumor activity. It is known that indolocarbazole derivatives have a strong inhibitory effect on cyclin-dependent kinases, protein kinase C and tyrosine kinase, and can effectively inhibit topoisomerase I, which participates in the processes of DNA transcription, replication and repair [2-4].

A promising representative of the class of indolocarbazole derivatives is the LHS-1208 compound synthesized in the laboratory of chemical synthesis of the N.N. Blokhin Russian Cancer Research Center, Federal State Budgetary Institution of the Ministry of Health of Russia. In vivo studies have shown the high anti-tumor activity of LHC-1208 against P-388 lymphocytic leukemia, L-1210 lymphoid leukemia, Ehrlich's ascites tumor, Lewis epidermoid lung carcinoma, B-16 melanoma, RSM-5 cervical cancer and the ACATOL adenocarcinoma of the large intestine. In this regard, the LHS-1208 compound has been selected for further studies on the development of a potential anticancer drug [5]. However, despite the high antitumor effect, a significant drawback of LHS-1208 is its low solubility in water. The use of biocompatible nanostructured delivery systems-liposomes - is the most physiologically acceptable way to increase the solubility of active antitumor substances and, consequently, their bioavailability. Due to this, the LHS-1208 liposomal dosage form for intravenous administration as a lyophilizate has been created in the laboratory for the development of dosage forms of the N.N. Blokhin Russian Cancer Research Center, Federal State Budgetary Institution of the Ministry of Health of Russia [6, 7].

The development of analysis techniques is one of the stages of research on the development of a drug. The quality control methods are selected at the stage of pharmaceutical development and are dictated by the tasks of control over production stages and the finished goods quality assurance. At the same time, the methods of analysis allowing to quickly and reliably detect abnormalities in the quality and properties of the drug at the earliest stages of the study are of great importance. The spectrophotometry in the visible and UV spectral range is a simple and reliable method of the LF quality control, widely used in the pharmaceutical analysis of liposomal drugs [8-10].

The purpose of this study was the development and validation of the procedure for the quantitative spectrophotometric determination of the active substance in the "LHS-1208 liposomal, lyophilizate for the preparation of dispersion for injection 1.8 mg" drug.

MATERIALS AND METHODS

Materials: "LHS-1208 liposomal, lyophilizate for the preparation of dispersion for injection 1.8 mg" (N.N. Blokhin Russian Cancer Research Center, Federal State Budgetary Institution of the Ministry of Health of Russia), H.H. LHS-1208 substance (N.N. Blokhin Russian Cancer Research Center, Federal State Budgetary Institution of the Ministry of Health of Russia); egg phosphatidylcholine (lecithin, E PC S, Lipoid, Germany), cholesterol (Chol; Sigma-Aldrich, Co., Japan) and polyethylene

glycol-2000-distearophosphatidylethanolamine (PEG-2000-DGFA, Lipoid, Germany), purified water, FS.2.2.0019.15; ethyl alcohol 95%, FS.2.1.0036.15 (OJSC "Flora Kavkaza", Russia);

Equipment: Sartorius 2405 Analytical scales (Sartorius AG, Germany); Transsonic T310 ultrasonic bath (Elma, Germany); Cary 100 spectrophotometer (Agilent Technologies, Australia).

Quantification of LHS-1208 in LLF-lyo

The quantitative content of LHS-1208 is determined by spectrophotometry in the UV and visible range (State Pharmacopeia XIII) [11] using a working standard (CO) at a wavelength of 320 nm. The optical density of the analyzed sample is measured using 95% alcohol as a compensation solution.

CO preparation. 15-20 ml of 95% alcohol is added to the accurately weighed portion of LHS-1208 1.0 mg substance and placed in a US bath for 5-10 minutes to accelerate the dissolution process. The resulting alcohol solution is quantitatively transferred to a 50 ml volumetric flask and made up to the mark with the alcohol (solution A). 5 ml of solution A is extracted in a 25 ml flask and made up to the mark with the alcohol (solution B). Solution B is used freshly prepared.

Quantitative analysis procedure. 5.6 ml of water are added to the contents of the LLF-lyo LHS-1208 vial and mixed until a homogeneous liposomal dispersion is obtained. The dispersion is quantitatively transferred to a 50 ml volumetric flask, a small amount of 95% alcohol is added and mixed and made up to the mark with the alcohol (solution A). 3 ml of solution A is extracted in a 25 ml flask and made up to the mark with the alcohol (solution B). The optical density of the obtained alcohol dilution is measured in cuvettes with an optical layer thickness of 10 mm at the absorption maximum with respect to the reference solution. In parallel, the optical density of the CO alcohol solution of the LHS-1208 substance is measured relative to the reference solution.

The content of LHS-1208 in the vial (X, mg) is calculated by the following formula:

$$X = A \times a_0 \times {}^{V}/_{A_0} \times V_0,$$

where A and A_0 are the optical densities of solutions of the LLF-lyo sample and CO of LHS-1208, respectively; a_0 is the portion of the LHC-1208 CO, in mg; V and V_0 are the the dilutions of the LLF-lyo sample and the LHS-1208 CO, respectively.

Validation of the procedure for the quantitative spectrophotometric analysis of the LHS-1208 LLF-lyo

During the validation, the quantitative spectrophotometric analysis of LHS-1208 was evaluated in the LLF-lyo framework, guided by the general pharmacopoeia article OFS.1.1.0012.15 "Validation of analytical techniques" (State Pharmacopoeia XIII) [11], by typical characteristics: specificity, range of application or analytical area, linearity, correctness, and precision.

Statistical analysis

The data obtained during the experiment were statistically processed using the Microsoft Office Excel 2010 program. The following notations of metrological characteristics have been accepted in this article: a –intercept on the ordinate axis, b –slope of line (slope coefficient), r –correlation parameter, n –sample volume, f –number of degrees of freedom, x_{av} –sample average, S –standard deviation, $x_{av}\pm\Delta x$ –confidence interval, P –confidence probability, ε –relative error, CV – coefficient of variation, $F_{tabul.}$ and $F_{calc.}$ –the tabulated and calculated value of the Fisher criterion, respectively, $t_{tabul.}$ and $t_{calc.}$. –the tabulated and calculated value of the Student's criterion, respectively. In statistical processing, the confidence probability was assumed to be 95%.

RESULTS AND DISCUSSION

Development of the procedure for the quantitative spectrophotometric analysis of LHS-1208 in the LLF-lyo composition

Maxima at 320±2, 286±2, 238±2 and 203±2 nm have been observed in the electronic absorption spectrum of the alcohol solution of the LHS-1208 substance in the range from 200 to 800 nm (Figure 1).

The most intense absorption band has been observed at a wavelength of 320 nm, therefore the peak has been chosen as an analytical one in the quantification of LHS-1208 in liposomes.

In the study of the electronic absorption spectrum of alcohol dilution of "empty" liposomes, it has been established that the auxiliary components have absorption maxima at 203±2, 269±2 and 280±2 nm (Figure 2).

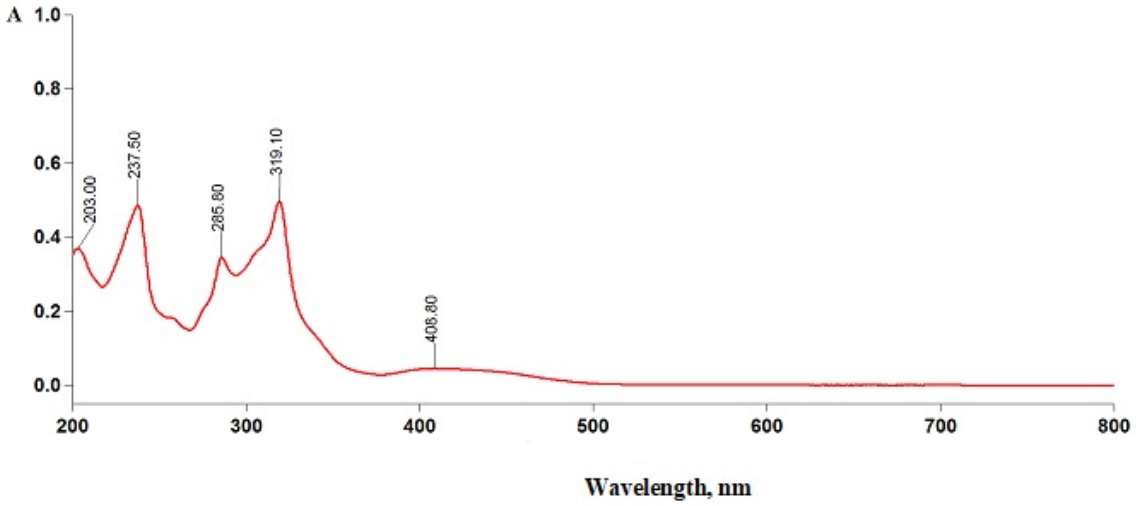

Figure 1. The electronic absorption spectrum of alcohol dilution

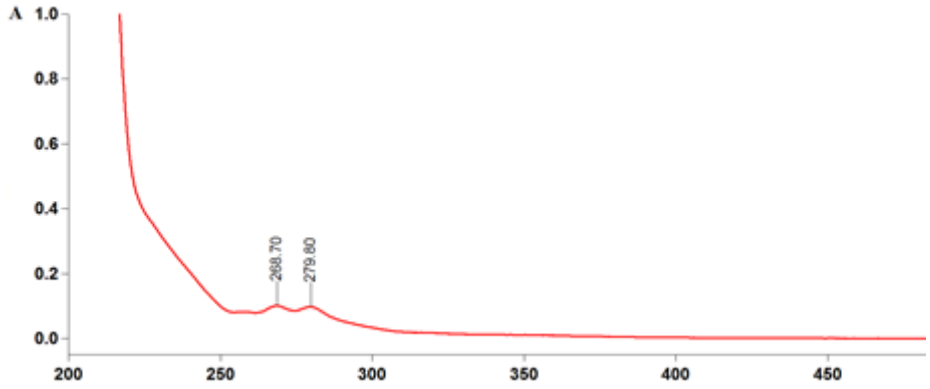

Figure 2. The electronic absorption spectrum of alcohol dilution of "empty" liposomes

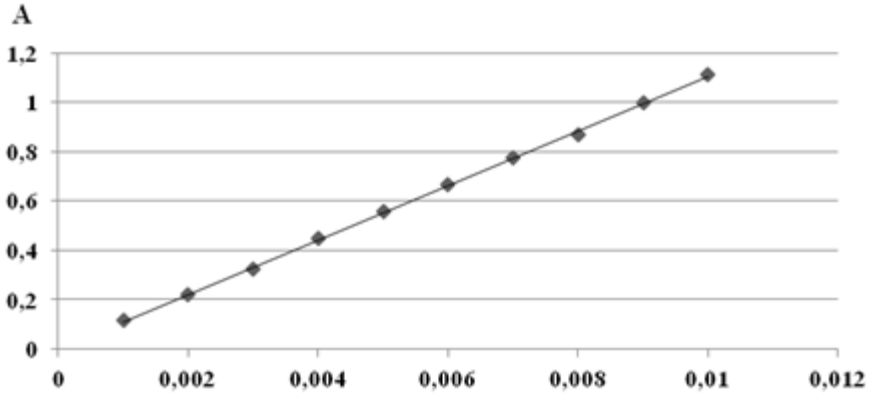

Figure 3. The evaluation of the observance of the Bouguer-Lambert-Beer law

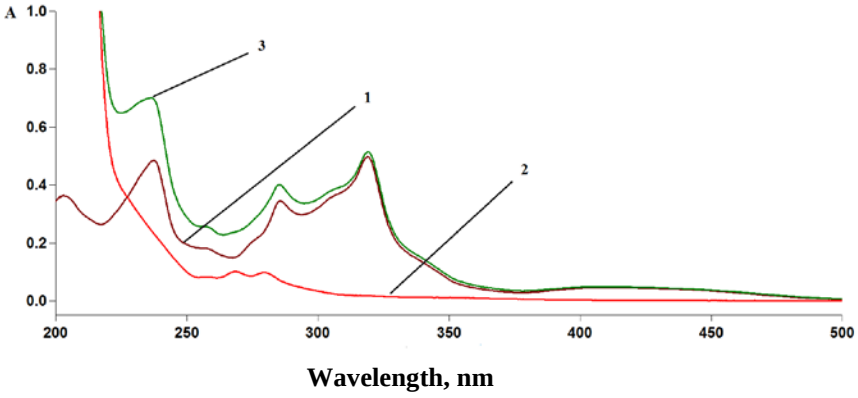

Figure 4. Electronic absorption spectra of alcohol dilutions of the LHS-1208 substance (1), auxiliary substances (2) and LHS-1208 LLF-lyo (3)

At a wavelength of 320 nm, the value of the optical density for alcohol dilution of the auxiliary components is 0.007 nm, and, therefore, it does not affect the identification and quantification of LHS-1208 in the LLF-lyo composition. Therefore, measurement of the optical density of the LF dilution was carried out with respect to 95% ethyl alcohol.

In order to select the operating concentration and verify compliance with the Bouguer-Lambert-Beer law at 320 nm wavelength, alcohol dilutions of the LHS-1208 substance were prepared in the 0.001-0.010 mg/ml concentration range. For alcohol dilutions of the LHS-1208 substance, the linear dependence of the optical density on the concentration at a wavelength of 320 nm was observed throughout the investigated range (Figure 3), which indicated the compliance with the basic law of light absorption - the Bouguer-Lambert-Beer law.

Taking into account the linear dependence of the optical density of the solution on the concentration for quantification of LHS -1208, there has been selected an alcoholic dilution of LLF-lyo with the production of the sample solution with a theoretical

concentration of active substance of 0.005 mg/ml, which corresponds to an optical density of about 0.5. The relative error in the quantification of LHS-1208 in LLF-lyo was 0.56% (Table 1).

Table 1. The results of quantification of LHS-1208 in LLF-lyo

LLF-lyo vial number LHS-1208	Optical density of test solution	Optical density of CO	Content of LHS-1208 in the vial	Statistical Characteristics
1	0.476	0.446	1.78	n = 6, f = 5, x_{av} = 1,78, S = 1.1 × 10^{-2}, x_{av}±Δx (P = 95%) = 1.78±0.01, ε = 0.56 %
2	0.475		1.78	
3	0.473		1.77	
4	0.470		1.76	
5	0.479		1.79	
6	0.477		1.78	

Validation of the procedure for the quantitative spectrophotometric analysis of LHS-1208 in the LLF-lyo composition

The main objective of the analytical procedure validation is experimental evidence that this technique is suitable for the purposes for which it is intended [12].

Specificity, or the selectivity of the technique, manifests itself in the ability of the analytical test to measure the content of the analyzed substance in the sample as part of other components, the presence of which in the preparation is very likely [12]. In order to confirm the specificity of the spectrophotometric determination of LHS-1208 in liposomes, the electronic absorption spectra of alcohol dilutions of the LHS-1208 substance and the LHS-1208 LLF-lyo auxiliary substances were compared.

As can be seen in Figure 4, the curves of the solution of the substance (1) and the LLF-lyo (3) are similar in shape and position of the maxima, and the optical density of the auxiliary substances in the 320 nm range is close to zero (curve 2) and does not significantly affect the concentration value of the active substance in the dosage form. Thus, the electronic absorption spectrum of liposomal LHS-1208 can be used to determine the authenticity of LHS-1208 in the LLF-lyo, and the technique can be considered as specific.

The *application range* serves as a measure of the maximum concentration of the analyzed substance that can be measured with an acceptable degree of accuracy and convergence. This is the upper limit of linearity determination [12]. For the procedure for the quantification of LHS-1208 in the LLF-lyo, a range of 80-120% of the desired concentration of the analytical solution (1.8 mg/bottle) has been established. Within this interval, the results obtained using the validated procedure should have an acceptable level of linearity, accuracy and precision.

Linearity lies in the potential of the control study to obtain test results (response factor) that are directly proportional to the concentration of the analyzed substance in the sample. The definition of this parameter allows refining the measurement range of a specific analytical test [12]. The linearity of the developed procedure for the quantification of LHS-1208 in the LLF-lyo was confirmed in a specified range of application by measuring the optical density of the analyzed solution at 5 levels of the substance concentration in the sample - 80, 90, 100, 110 and 120%. To carry out the study, model solutions of LLF-lyo, representing alcoholic solutions of active and auxiliary substances with different concentrations of LHS-1208, were prepared. According to the results of the study provided in Table 2, the correlation coefficient has been above 0.999, which indicates a close linear relationship between the concentration of LHS-1208 and the optical density in the specified 80-120% application range.

Table 2. Evaluation of the linearity of the LHS-1208 spectrophotometric analysis procedure

Concentration of LHS-1208		Optical density value, A	Regression
%	mg/ml		
80	0.24	0.374	0.378
90	0.27	0.430	0.428
100	0.30	0.480	0.478
110	0.33	0.528	0.528
120	0.36	0.576	0.578
Statistical Characteristics			Results
b			1.673
a			-0.024
r			0.9995

The linear regression equation applied to the results has the following form: y = 1.673x - 0.024. A graphic representation of the linearity of the procedure is shown in Figure 5.

Correctness (accuracy) expresses the degree of correspondence of the actual parameter of the preparation to its calculated value. To measure the correctness, tests are carried out under artificially created loads, and the degree of effectiveness of the tests is determined as follows: a sample, the characteristics of which are known, is mixed with excipients, and the actual parameter of the drug is compared with the result of the control study. Correctness is usually expressed in the value of a systematic error or in the percentage of error between the measured and true value (experimental value / real value × 100%) [12]. The correctness of the developed procedure was evaluated using 9 determinations at 3 concentrations of the active substance, covering a specified analytical range of 80-120%. To this end, the same as in the evaluation of "linearity", three analyzable solutions were prepared for each of the three concentrations - 80, 100, and 120%. Based on the obtained results of 9 measurements, the percentage of the difference between the specified and the true values to a specified value (response), the confidence interval and the relative error of the average result were calculated. Based on the results of the study presented in Table 3, it can be seen that the procedure for the quantification of LHS-1208 in the LFL-lyo is correct since the results obtained are close to the true value and the relative error of the mean result does not exceed 0.23%.

Table 3. Evaluation of the correctness of the LHS-1208 spectrophotometric analysis procedure

Concentration, %	Sample No.	Content of LHS-1208, mg		Response, %
		Experimental value	true value	
80	1	1.45	1.44	100.69
	2	1.45	1.45	100.00
	3	1.44	1.44	100.00
100	1	1.81	1.80	100.56
	2	1.80	1.80	100.00
	3	1.82	1.82	100.00
120	1	2.16	2.16	100.00
	2	2.18	2.17	100.46
	3	2.16	2.15	100.47
Statistical Characteristics, %				Results
x_{av}				100.24
S				0.295
x_{av}±Δx (P = 95%)				100.24±0,23
ε				0.23

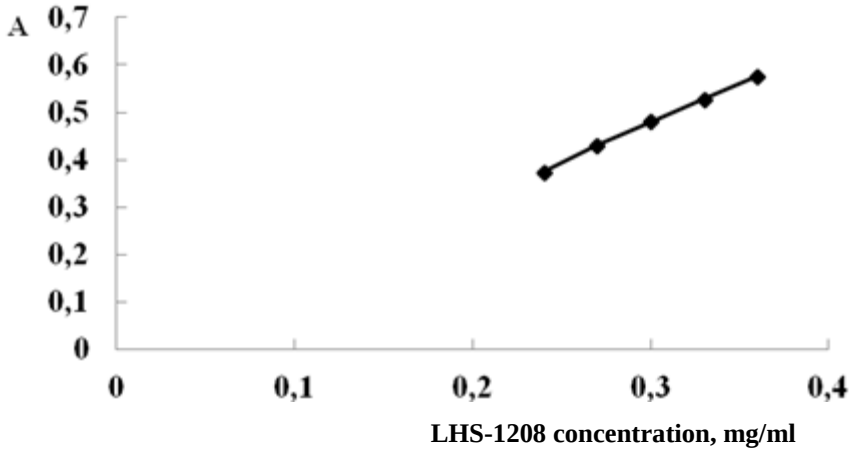

Figure 5. Graphical representation of linear regression linearity of the procedure for the quantitative analysis of LHS-1208 in LFL-lyo at 320 nm

The validation of the analytical methodology for quantification includes the evaluation of precision. Precision is manifested in the degree of correspondence between the series of measurements obtained as a result of the control study. This category is usually expressed in the coefficient of variation, which is the standard deviation of the experimental values divided by the concentration of the substance analyzed. It is customary to use several types of precision in the measurements. The precision of the developed procedure for the spectrophotometric analysis of LHS-1208 in the LLF-lyo was determined at two levels: *repeatability (convergence)* and *intralaboratory (intermediate) precision.*

Repeatability of the procedure was evaluated by independent results obtained under the same conditions in one and the same laboratory (one and the same performer and equipment) within a short period of time in 6 determinations at 100% concentration of the analytical solution (1.8 mg/vial). Based on the data obtained (Table 4), the developed quantitative analysis procedure can be considered precise under repeatability conditions, since the coefficient of variation has a value of less than 10% (0.88%), which indicates a slight variability in the variational series.

Table 4. Evaluation of the convergence of the LHS-1208 spectrophotometric analysis procedure

Concentration, %	Sample (vial) No.	LHS-1208 content in the sample, mg
100	1	1.82
	2	1.83
	3	1.80
	4	1.82
	5	1.84
	6	1.80
Statistical Characteristics		Results
x_{av}, mg/vial		1.82
S, mg/vial		1.6×10^{-2}
CV, %		0.88
$x_{av}\pm\Delta x$ (P= 95%), mg/vial		1.82±0.02
ε, %		1.1

The intralaboratory precision of the validated procedure was evaluated in different laboratory conditions (different days, different investigators) in 6 determinations at 100% concentration of the analytical solution (1.8 mg/vial). When comparing the results of quantitative determination of LHS-1208 in LLF-lyo obtained by two investigators, it has been found that $F_{calc} < F_{tabul}$ *and* $t_{calc} < t_{tabul.}$ Therefore, the differences between the mean values and the standard deviations of the results are random and the technique can be considered precise in conditions of intralaboratory precision (Table 5).

Table 5. Evaluation of the intralaboratory precision of the LHS-1208 spectrophotometric analysis procedure

Concentration, %	Sample (vial) No.	LHS-1208 content in the sample, mg	
		Researcher 1	Researcher 2
100	1	1.82	1.81
	2	1.83	1.80
	3	1.80	1.82
	4	1.82	1.82
	5	1.84	1.81
	6	1.80	1.81
Statistical Characteristics		Results	
x_{av}, mg/vial		1.82	1.81
S, mg/vial		1.6×10^{-2}	7.75×10^{-3}
CV, %		0.88	0.43
$x_{av}\pm\Delta x$ (P = 95%), mg/vial		1.82±0.02	1.81±0.01
ε, %		1.10	0.55
$F_{tabul}(5\%; 5; 5) = 5.05$		$F_{calc} = 4.33$	
$t_{tabul}(95\%; 10) = 2.23$		$t_{calc} = 1.37$	

CONCLUSION

The procedure for quantification of LHS-1208 in LLF-lyo by spectrophotometry has been developed using an operating standard sample at a wavelength of 320 nm. It has been shown that the relative error of the quantitative analysis of the LHS-1208 concentration in the lyophilizate using this technique is less than 1%. The developed technique for the following validation characteristics has been validated: specificity, analytical range, linearity, accuracy and precision. Based on the results of the study, the main validation characteristics meet the eligibility criteria.

REFERENCES

[1] Manzyuk, L.V. and L.F. Ivanova Osnovy lekarstvennogo lecheniya zlokachestvennykh opukholey [Basics of drug treatment of malignant tumors]. In the: Oncology for practicing doctors. S.S. Chistyakov, Moscow, 2009, pp: 37–49.

[2] Smirnova, Z.S., I.Yu. Kubasova, L.M. Borisova, M.P. Kiseleva, T.D. Miniker, I.L. Plikhtyak et al. Protivoopukholevaya aktivnost' N-glikozidov proizvodnykh indolo[2,3-a]karbazola [Antitumor activity of N-glycosides of indolo [2,3-a] carbazole derivatives]. Russian Biotherapeutic Journal 2006; 5 (3): 123-127.

[3] Tatarskiy, V.V. Mekhanizmy gibeli opukholevykh kletok pri deystvii novykh uglevodorodnykh proizvodnykh indolokarbazolov [Mechanisms of death of tumor cells under the action of new hydrocarbon derivatives of indolocarbazoles]: the author's thesis. Moscow, 2010. Date View April 19, 2018 https://elibrary.ru/item.asp?id=19328937

[4] Sanchez C, Zhu L, Brana AF, Salas AP, Rohr J, Mendez C et al. Combinatorial biosynthesis of indolocarbazole compounds. PNAS 2005;102(2):461–466.

[5] Melnik, S. Ya., N.K. Vlasenkova. L.D. Garayeva, I.S. Golubeva, O.V. Goryunova, V.A. Eremina et al. Sposob polucheniya N-glikozidov proizvodnykh indolo[2,3-a]pirrolo[3,4-s]karbazol-5,7-dionov, obladayushchikh tsitotoksicheskoy i protivoopukholevoy aktivnost'yu [A method for the preparation of N-glycosides of indolo [2,3-a] pyrrolo [3,4-c] carbazole-5,7-dione derivatives having cytotoxic and antitumor activity]. Patent No No.2427585 dated 10.12.2009, Date View April 19, 2018 https://elibrary.ru/item.asp?id=18735362.

[6] Gulyakin, I.D., A. Khashem, L.L. Nikolayeva, M.V. Dmitriyeva, D.A. Afanasyeva, M.A. Baryshnikova et al. Development of a new technology for the preparation of a dosage form for the intravenous administration of the LHS-1208 indolocarbazole derivative. Russian Biotherapeutic Journal 2016; 15 (2): 55-60.

[7] Khashem, A., I.D. Gulyakin, L.L.Nikolayeva, M.V. Dmitriyeva, L.A. Korol', A.P. Polozkova et al. Opredeleniye optimal'nogo soderzhaniya krioprotektora pri liofilizatsii liposomal'noy lekarstvennoy formy proizvodnogo indolokarbazola –LHS-1208 [Determination of the optimal cryoprotectant content during lyophilization of the liposomal dosage form of the indolocarbazole derivative - LHS-1208]. Materials of the IV All-Russian Scientific and Practical Conference with international participation named after A.Yu. Baryshnikov "Domestic antitumor drugs", Moscow, 2017, pp. 4–5.

[8] Chan Thi Khay Iyen, Ye.V. Ignat'yeva, A.P. Polozkova, G.V. Ramenskaya, N.A. Oborotova Kachestvennyy i kolichestvennyy analiz novoy liofilizirovannoy liposomal'noy lekarstvennoy formy fotoditazina [Qualitative and quantitative analysis of a new lyophilized liposomal dosage form of photoditazine]. Chemical-Pharmaceutical Journal 2010; 44(6):53–56.

[9] Zhang Si, E.V. Sanarova, M.V. Dmitrieva, A.P. Polozkova, N.A. Oborotova, A.V. Lantsova, etc. Razrabotka metodiki kolichestvennogo analiza liposomal'noy lekarstvennoy formy novogo analoga gipotalamicheskogo gormonasomatostatina, obladayushchego protivoopukholevoy aktivnost'yu [Development of a technique for the quantitative analysis of the liposomal dosage form of a new analogue of the hypothalamic hormone somatostatin, having antitumor activity]. Materials of the IV All-Russian Scientific and Practical Conference with international participation named after A.Yu. Baryshnikov "Domestic antitumor drugs", Moscow, 2016:100. Date View April 19, 2018 https://elibrary.ru/item.asp?id=26427807.

[10] Archinova, O.Yu., E.V. Ignatyeva, N.A. Oborotova Analiz liofilizirovannoy lekarstvennoy formy fotoditazina metodami tonkosloynoy khromatografii i spektrofotometrii [Analysis of the lyophilized dosage form of photoditazine by thin-layer chromatography and spectrophotometry]. The questions of Biological, Medical and Pharmaceutical Chemistry 2012;3:28–32.

[11] State Pharmacopoeia of the Russian Federation. 13th Edition. Moscow, 2015.

[12] Bykovskiy, S.N., I.A. Vasilenko, M.I. Kharchenko, A.B. Belov, I.E. Shokhin, E.A. Dorina Rukovodstvo po instrumental'nym metodam issledovaniy pri razrabotke i ekspertize kachestva lekarstvennykh preparatov [Manual on instrumental methods of research in the development and examination of the quality of drugs]. Moscow: Pero, 2014.