Recommended by Doctor of Pharmacy, professor V.A.Georgiyants

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DEVELOPMENT AND VALIDATION OF THE METHODS FOR QUANTITATIVE DETERMINATION OF NITROFURAL BY UV SPECTROPHOTOMETRY

K.I.Proskurina

National University of Pharmacy

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Harmonization of the national system of quality assurance of medicines to the European requirements raises the analytical quality control to the high level. The validation procedure allows to evaluate the analytical procedure objectively. The results of validation studies characterize the degree of precision of the method and show systematic, random errors. The scientific work is devoted to development of quantitative determination methods of nitrofural in dosage forms of 0.02% nitrofural solution and 0.20% nitrofural ointment by spectrophotometry and their validation. The method consists in measuring the optical absorption of water test solution with the concentration of nirofural of 6×10^{-6} g/ml at the wavelength of 375 nm. The optimization of sample preparation for modified spectrophotometric methods for quantitative determination of nitrofural in dosage forms has been conducted based on estimation of total uncertainty. The stability of the optical density of water solutions of nitrofural ($\Delta t_{solution} \leq max\delta$ (0.82% ≤ 1.53%), $\Delta t_{ointment} \leq max\delta$ (0.84% ≤ 1.23%)) at the wavelength of 375 nm for one hour and at pH fluctuation ±10% (from 4.95 to 6.05) has been proven. The methods are characterized by acceptable specificity $\delta_{noise} = 0.21\% \leq max\delta = 1.53\%$. The validation parameters of the methods have been determined. The results satisfy the requirements of the State Pharmacopoeia of Ukraine and allow recommending the methods developed for quality control of nitrofural in the dosage forms chosen.

Development of new rational analytical methods of analysis based on modern principles of standardization is necessary for ensuring adequate quality control of medicines according to the requirements of the State Pharmacopoeia of Ukraine (SPhU).

In medicinal forms the quantitative determination of nitrofural is carried out by HPLC [10], differential pulse polarography [11], electron-beam stripping voltammetry [9], etc.

Among physical and chemical methods modern pharmacopoeias recommend UV spectrophotometry by the standard method for quantitative determination of nitrofural in the substance. It is in measurement of the optical density of the solutions prepared from the nitrofural substance and the standard substance (in the concentration of 6×10^{-6} g/ml [3-5, 7, 8] and 8×10^{-6} g/ml [12]) at the maximum absorption at the wavelength of 375 nm [3-5, 7, 8]. Therefore, the aim of our work is development and validation of methods for quantitative determination of nitrofural in extemporaneous dosage forms by UV spectrophotometry according to the SPhU.

Experimental Part

Formulas, which most pharmacies use to prepare dosage forms (DF) with nitrofural [1]: 0.02% nitrofural solution (its composition: 0.2 g of nitrofural, water for injections to 1 litre) and 0.20% nitrofural ointment (its composition: 0.2 g of nitrofural, 0.6 g of vaseline oil, 99.2 g of vaseline) have been chosen as objects for the experiment.

When conducting the research the substance of nitrofural manufactured by Menadiona S.A. No. 20080904, as well as such analytical equipment as a "SPECORD 200" spectrophotometer, AV 204 S/A METTLER TOLEDO analytical balance, "Sartorius AG" pH meter, reagents, volumetric glassware of class A and excipients meeting the requirements of SPhU were used for the work.

For the experiment test solutions and test ointments were prepared according to the range of the method application [6] in the following concentrations: 70.00%, 85.00%, 100.00%, 115.00%, 130.00% of the concentration specified.

The method for quantitative determination of nitrofural in DF of 0.02% solution. Dilute 3.00 ml of 0.02% nitrofural solution to 100.00 ml with water *R*. Measure the absorbance of the solution at the absorption maximum of 375 nm. The blank solution is water *R*.

The method for quantitative determination of nitrofural in DF of 0.20% ointment. To 3 g of the ointment add 10 ml of water *R* and heat on a water bath until the base melts. After cooling transfer the aqueous extract into a 100.00 ml volumetric flask. Extract nitrofural with 10 ml water *R*, repeat three times pouring the water extraction into the same flask, and dilute with water *R* to the volume of 100.00 ml. Dilute 10.00 ml of this solution to 100.00 ml with water *R*. Measure the absorbance of the solution at the absorption maximum of 375 nm. The blank solution is water *R*.

Reference solution of nitrofural. Weigh accurately 0.06 g of nitrofural in a 100.00 ml volumetric flask and suspend in 5 ml of water *R*. When the substance is completely soaked, add 70 ml of water *R* and stir until complete dissolution when heating, making it boil. Then, after complete cooling to 20ºC±2ºC, dilute with water *R* to 100.00 ml. Dilute 1.00 ml of this solution to 100.00 ml

Table 1

Calculation of the total uncertainty of methods for quantitative determination of nitrofural in dosage forms by spectrophotometry

Operation of sample preparation	Parameter	Uncertainty, %
Reference solution		
Weighing on an analytical balance, g	m_{st}	0.0002/0.06 × 100 = 0.33
Volumetric dilution, ml	100.00	0.12
Taking an aliquot, ml	1.00	0.60
Volumetric dilution, ml	100.00	0.12
Uncertainty of sample preparation	$\Delta_{sp} = \sqrt{(0.33^2 + 0.12^2 + 0.6^2 + 0.12^2)} = 0.71$	
Test solution for DF of 0.02% nitrofural solution		
Weighing on an analytical balance, g	m_{st}	0.0002/0.2 × 100 = 0.10
Volumetric dilution, ml	1000.00	0.05
Taking aliquots by pipette (5.0 ml), ml	3.00	(0.03/3) × 100 = 1.00
Volumetric dilution, ml	100.00	0.12
Uncertainty of sample preparation	$\Delta_{sp} = \sqrt{(0.1^2 + 0.05^2 + 1.0^2 + 0.12^2 + 0.71^2)} = 1.23$	
Test solution for DF of 0.20% nitrofural ointment		
Weighing on an analytical balance, g	m_{st}	0.0002/0.2 × 100 = 0.10
Weighing on an analytical balance, g	3.0	0.0002/3 × 100 = 0.006
Volumetric dilution, ml	100.00	0,12
Taking an aliquot, ml	10.00	0,50
Volumetric dilution, ml	100.00	0.12
Uncertainty of sample preparation	$\Delta_{sp} = \sqrt{(0.1^2 + 0.006^2 + 0.12^2 + 0.5^2 + 0.12^2 + 0.71^2)} = 0.87$	

with water. Measure the absorbance of the solution at the absorption maximum of 375 nm. The blank solution is water *R*.

Perform the measurements with a 1-cm cell at (20±1)°C. Calculate the content of nitrofural from the absorbance measured and the concentrations of the solutions.

Results and Discussion

According to the pharmacopoeial method of quantitative determination of nitrofural in the substance the method of absorption spectrophotometry is used; the absorption spectra of nitrofural is measured in dimethylformamide-water solution. According to this method the analytical solution concentration is 6×10^{-6} g/ml. For the analysis of DF of nitrofural we also used the concentration 6×10^{-6} g/ml.

The aliquot volume for the solution, the sample weight mass for the ointment and the volume of a volumetric flask for dilution conditioned the minimum error were chosen. The prognosis of uncertainty methods was performed taking into account the uncertainty of sample preparation (the stages of weighing and dilution) and the uncertainty of the final analytical operation [3-5]. Appropriate sample weight masses and volumetric vessels were selected theoretically (Table 1). The uncertainty of sample preparation predicted for the dosage form of the solution is 1.23%, for the ointment – 0.87%, and it is insignificant compared to the total uncertainty of the analysis results, that is the inequation $\Delta_{SP} \leq 0.32 \times \Delta_{As} = 1.54\%$ is performed. The total uncertainty of methods satisfies the following requirements: for 0.02% nitrofural solution – $\Delta_{As} = \sqrt{(1.23^2 + 0.70^2)} = 1.42\% \leq \max \Delta_{As} = 4.8\%$; for 0.20% nitrofural ointment – $\Delta_{As} = \sqrt{(0.87^2 + 0.70^2)} = 1.1\% \leq \max \Delta_{As} = 3.84\%$. Thus, the methods will give correct results in other laboratories.

Specificity was investigated by studying the relative systematic error (δ_{noise},%), which could be made by excipients or decomposition products when determining the substance. The average values of the placebo solution absorbance: $A_{blank} = 0.0010$; and the reference solution $A_{st} = 0.4873$ were found. The relative systematic error introduced by excipients was calculated by the formula: $\delta_{noise}(\%) = 100 \times A_{blank}/A_{st} = 100 \times 0.0010/0.4873 = 0.21\%$ [2]. According to the data obtained inequation $\delta_{noise} = 0.21\% \leq \max\delta = 1.53\%$ is performed, i.e. the background absorption is insignificant, and the method is characterized by acceptable specificity.

To study robustness, the validation parameter of the method, the experimental study of stability of solutions over time and the impact of pH fluctuations was conducted. Stability of analytical solutions of nitrofural in comparison with the solution made according to the SPhU method in time for 1 hour was studied [2]. The results presented in Table 2 confirm that changes in optical absorption satisfy the requirements: $\delta \leq 0.1 \times B = 0.1 \times 15 = 1.5\%$. The dimethylformamide-water solution of nitrofural is characterized by stability for 1 h. The water solution of nitrofural also has a sufficient stability, but the slightest fluctuations in absorbance are detected for 20 min. after preparation of the solution. Therefore, in further experiments all measurements were performed immediately after preparation of the solution for analysis.

The impact of small controlled changes of pH: ±10% from 4.95 to 6.05 when performing the method was investigated. The results obtained (Table 3) show that the

Table 2

Stability of the test solutions and the reference solution (by spectrophotometry)

Test solution	The term of stability study nt, min. (A_i*)					Mean	RSDt,%	Δt,%	maxδ,%
	0	15	30	45	60				
Solution (SPhU)	0.447	0.448	0.448	0.449	0.448	0.448	0.10	0.22	0.32
0.02% solution	0.440	0.440	0.439	0.438	0.436	0.440	0.38	0.82	1.53
0.20% ointment	0.447	0.447	0.446	0.445	0.443	0.447	0.39	0.84	1.23

* The values of the optical density is the average of three measurements of the solution.

Table 3

Results of the robustness research of quantitative determination method for nitrofural in DF by spectrophotometry

Test solution, %	Optical density (A_i*)			Mean	SrpH	RSDpH,%	ΔpH,%	maxδ,%
	+ 1 drop of 0.01 M HCl	without changing the conditions	+ 1 drop of 0.01 M NaOH					
Dimethylformamide-water solution of nitrofural (according to SPhU)								
85.00	0.337	0.335	0.337	0.336	0.0009	0.09	0.26	0.32
100.00	0.444	0.443	0.444	0.444	0.0005	0.07	0.15	
115.00	0.512	0.511	0.513	0.512	0.0008	0.08	0.22	
Water solution of nitrofural (DF of 0.02% nitrofural solution)								
85.00	0.382	0.379	0.380	0.380	0.0015	0.15	0.45	1.53
100.00	0.489	0.485	0.485	0.486	0.0021	0.21	0.63	
115.00	0.519	0.516	0.514	0.516	0.0022	0.22	0.63	
Water solution of nitrofural (DF of 0.20% nitrofural ointment)								
85.00	0.382	0.380	0.378	0.380	0.0017	0.17	0.49	1.23
100.00	0.450	0.447	0.446	0.448	0.0017	0.17	0.50	
115.00	0.518	0.516	0.515	0.517	0.0015	0.15	0.45	

* The values of the optical density is the average of three measurements of the solution.

Table 4

The validation characteristics of the methods for quantitative determination of nitrofural in DF of 0.02% nitrofural solution and 0. 02% nitrofural ointment

Validation characteristics	Values	Research of the solution		Research of the ointment	
		results, %	acceptance criteria, %	results, %	acceptance criteria, %
Linearity	a	1.78	$a \le 5.12$	2.7	$a \le 4.09$
	S_0	0.29	$S_0 \le RSD_0 = 2.71$	0.53	$S_0 \le RSD_0 = 2.17$
	R_c	0.9999	$R_c \ge 0.9924$	0.9997	$R_c \ge 0.9951$
Convergence	Δ_{As}	0.89	$\Delta_{As} \le max\Delta_{As} = 4.80$	1.62	$\Delta_{As} \le max\Delta_{As} = 3.84$
Accuracy	δ_Z	0.50	$\delta_Z \le 1.24$	0.68	$\delta_Z \le 0.99$
Repeatability	Δ_{intra}	1.09	$\Delta_{intra} \le max\Delta_{As} = 4.80$	1.48	$\Delta_{intra} \le max\Delta_{As} = 3.84$

optical absorption is independent of the solution's pH: ΔpH for all the solutions tested does not exceed maxδ = = 1.53%.

Such validation characteristics of the spectrophotometric methods for quantitative determination of the nitrofural solution and ointment as linearity, accuracy convergence and repeatability were studied within the application range of the method (70.00-130.00%). The results obtained are shown in Table 4. The calculated statistical values *a* (the constant term of the linear dependence), S_0 (the residual standard deviation) and *r* (the correlation coefficient) meet the acceptance criteria. Thus, the method is characterized by linearity and allows to control correctly the content of nitrofural within the range of application.

Metrological characteristics of the method do not exceed the critical value of the error (4.8%) and are characterized by qualitative analytical indicators. This method can be correctly reproduced in the laboratory (Table 4).

CONCLUSIONS

The optimization of sample preparation for modified spectrophotometric methods for quantitative deter-

mination of nitrofural in dosage forms by the standard method at the concentration of 6×10^{-6} g/ml with the absorption maximum at 375 nm has been conducted based on estimation of total uncertainty.

The stability of the optical density of water solutions of nitrofural at the wavelength of 375 nm for one hour and at pH fluctuation ±10% (from 4.95 to 6.05) has been proven.

The validation characteristics of the spectrophotometric methods (linearity, accuracy, convergence and repeatability) for quantitative determination of nitrofural in such dosage forms as 0.02% nitrofural solution and 0.02% nitrofural ointment have been investigated. The results satisfy the requirements of the State Pharmacopoeia of Ukraine and allow recommending the methods developed for quality control of nitrofural in the dosage forms chosen.

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РОЗРОБКА ТА ВАЛІДАЦІЯ МЕТОДИК КІЛЬКІСНОГО ВИЗНАЧЕННЯ НІТРОФУРАЛУ МЕТОДОМ УФ-СПЕКТРОФОТОМЕТРІЇ

К.І.Проскуріна

Ключові слова: *кількісне визначення; спектрофотометрія; валідація, нітрофурал*

Гармонізація національної системи забезпечення якості лікарських засобів до європейських вимог підіймає на високий рівень аналітичний контроль якості. Об'єктивно оцінити аналітичну методику дозволяє валідаційна процедура. Результати валідаційних досліджень характеризують ступінь точності методу та виявляють систематичні, випадкові грубі помилки. Наукова робота присвячена розробці методик кількісного визначення нітрофуралу у лікарських формах розчину нітрофуралу 0,02% та мазі нітрофуралу 0,20% методом спектрофотометрії та її валідації. Методики полягають у вимірюванні оптичного поглинання водного аналітичного розчину з концентрацією 6×10^{-6} г/мл нітрофуралу при довжині хвилі 375 нм. На основі прогнозу невизначеності проведено оптимізацію пробопідготовки для методик. Прогнозована невизначеність пробопідготовки методик для лікарської форми розчину становить 1,23%, для мазі – 0,87% і є незначимою у порівнянні з повною невизначеністю результатів аналізу 1,53% та 1,23% відповідно. При дослідженні робасності визначено, що водний розчин нітрофуралу характеризується достатньою стабільністю впродовж 1 години $\Delta t_{розчину} \le max\delta$ (0,82% ≤ 1,53%), $\Delta t_{мазі} \le max\delta$ (0,84% ≤ 1,23%). Доведено, що незначне коливання pH середовища (pH ±10% від 4.95 до 6.05) аналітичного розчину не чинить суттєвого впливу на оптичне поглинання. Методики характеризуються припустимою специфічністю δ_{noise} = 0,21% ≤ $max\delta$ = 1,53%. Визначені валідаційні характеристики методик: лінійність, збіжність, правильність та відтворюваність. Результати задовольняють вимогам Державної фармакопеї України та дозволяють рекомендувати розроблені методики для контролю якості обраних лікарських форм.

РАЗРАБОТКА И ВАЛИДАЦИЯ МЕТОДИК КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ НИТРОФУРАЛА МЕТОДОМ УФ-СПЕКТРОФОТОМЕТРИИ

К.И.Проскурина

Ключевые слова: *количественное определение; спектрофотометрия; валидация; нитрофурал*

Гармонизация национальной системы обеспечения качества лекарственных средств с европейскими требованиями поднимает на высокий уровень аналитический контроль каче-

ства. Объективно оценить аналитическую методику позволяет валидационная процедура. Результаты валидационных исследований характеризуют степень точности метода и обнаруживают систематические, случайные, грубые ошибки. Научная работа посвящена разработке методик количественного определения нитрофурала в лекарственных формах раствора нитрофурала 0,02% и мази нитрофурала 0,20% методом спектрофотометрии и их валидации. Методики заключаются в измерении оптического поглощения водного аналитического раствора с концентрацией 6×10^{-6} г/мл нитрофурала при длине волны 375 нм. На основе прогноза неопределенности проведена оптимизация пробоподготовки для методик. Прогнозируемая неопределенность пробоподготовки методик для лекарственной формы раствора составляет 1,23%, для мази – 0,87% и является незначимой по сравнению с полной неопределенностью результатов анализа 1,53% и 1,23% соответственно. При исследовании робасности определено, что водный раствор нитрофурала характеризуется достаточной стабильностью в течение 1 часа: $\Delta t_{раствора} \leq max\delta$ (0,82% ≤ 1,53%), $\Delta t_{мази} \leq max\delta$ (0,84% ≤ 1,23%). Доказано, что незначительное колебание pH среды (pH ±10% от 4.95 до 6.05) аналитического раствора не создает существенного влияния на оптическое поглощение. Методики характеризуются допустимой специфичностью $\delta_{noise} = 0,21\% \leq max\delta = 1,53\%$. Определены валидационные характеристики методик: линейность, сходимость, правильность и воспроизводимость. Результаты удовлетворяют требованиям Государственной фармакопеи Украины и позволяют рекомендовать разработанные методики для контроля качества выбранных лекарственных форм.