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UDC 543.062:543.422.7:615.262.1

THE ASSESSMENT OF THE METHOD FOR QUANTITATIVE DETERMINATION OF PREDNISOLONE IN THE OINTMENT BY THE REACTION WITH PHENYLHYDRAZINE

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Key words: photocolorimetry; quantitative determination; prednisolone; validation; phenylhydrazine

Adaptation and validation of the analytical method of photocolorimetric determination of prednisolone using the reaction with phenylhydrazine by the standard method have been carried out in the scientific research laboratory according to standard procedures as described in the State Pharmacopoeia of Ukraine. The main validation characteristics have been identified using the model solution with the standard substance. It has been found that the criteria of linearity and precision are observed in the range of concentrations of 80-120% (0.08-0.12 mg/ml in the test solution or 0.4-0.6% of prednisolone in the ointment), and they are statistically and practically insignificant ($a \le S_a \times 1.86 = 4.7$, $\Delta as\% \le 3.20 = 2.86$, $\delta \le 1.0240 = 0.92$). The correct results obtained have allowed to test the method for determining prednisolone in the model extraction from "0.5% Prednisolone" ointment in the range of 80-120% of the nominal concentration. The study of robustness of the method performed has allowed to determine that it is necessary to follow a strict temperature range during the reaction to produce correct results (60°C±1°). The amount of phenylhydrazine in the reagent may vary between 0.4-1.0 g/l. Criteria of linearity, precision and convergence for the model extraction of the ointment are also observed within the whole range of determination and are statistically and practically insignificant ($a \le S_a \times 1.86 = 4.4$, $\Delta as\% \le 3.20 = 2.71$, $\delta \le 1.0240 = 1.00$). The method is acceptable for use in laboratories of the drug quality control and could be applied for determination of prednisolone in "0.5% Prednisolone" ointment with the hydrophobic base within the range of concentrations of 0.4-0.6%.

Prednisolone (Prednisolonum) – (11-β)-11,17,21-trihydroxypregna-1,4-diene-3,20-dione – is a dehydrated drug of the group of corticosteroids with an average therapeutic effectiveness that is widely used in medical practice in the form of injections, tablets, ointments, sprays, aerosols, creams, etc. However, injectable dosage forms and ointment are the most common. The latter is used to treat most of skin diseases [5].

For the quantitative determination of *prednisolone* in the substance the SPhU, EPh and a number of other pharmacopoeias recommend spectrophotometry as the variant of the absorbance method with preliminary purity control by HPLC [3, 6, 7, 10]. In soft dosage forms it is most frequently determined chromatographically (HPLC, TLC, etc.) [8]. The spectrophotometric method is uncommon because of the lower specificity, but it is much cheaper and easier to perform [9].

The aim of this research was the study and further adaptation of the method for quantitative determination of prednisolone in the ointment using the photocolorimetric method by the reaction of phenylhydrazine.

Materials and Methods

For our research a pharmacopoeial standard sample of prednisolone PSS SPhU No.11/1-2143 (the shelf-life from 01/13/2014 to 01.2015, the content of prednisolone is 99.8%) and "0.5% Prednisolone" ointment Nizhpharm, RN UA/4949/01/01 batch 80414 were used.

In the experiment the following analytical equipment was used: a CPK-2 photocolorimeter, cuvettes with the thickness of 10 mm, an AB 204 S/A METTLER TOLEDO analytical balance, a TS-80 M-2 thermostat, reagents and measuring glassware of class A meeting the requirements of the SPhU.

The method of the quantitative spectrophotometric determination of prednisolone in the ointment by the reaction with phenylhydrazine:

Test solution: To the accurately weighed quantity of the ointment equivalent to 10.0 mg of prednisolone add 25 ml of 96% alcohol *R*. Heat on a water heater to dissolution of the base, then cool in ice. Filter the resulting mixture through a paper filter previously soaked in ethanol to a 100.0 ml volumetric flask.

Standard solution: Dissolve the accurately weighed quantity of the standard powder in 96% alcohol *R* preparing the solution with the accurate concentration of prednisolone equivalent to 0.1 mg/ml.

Sulfuric acid reagent (SAR): Prepare the solution of concentrated sulfuric acid, 96% alcohol *R* and purified water in the ratio of 4: 3: 3.

Modified phenylhydrazine sulfuric acid reagent (MPSAR): dissolve 65 mg of phenylhydrazine hydrochloride in 100 ml of SAR.

Procedure: Pipet 2.0 ml of the *Test solution* into each of two 50 ml conical flasks (identified as *Test solution* and *Blank Test solution*). Add 2.0 ml of the *Standard solution* into each of two 50 ml conical flasks (identified as *Standard solution* and *Blank Standard solution*). Pipet 2.0 ml of dehydrated alcohol into a 50 ml conical flask (identified as *Blank reagent*). Add 20.0 ml of SAR to the *Blank Test solution* and the *Blank Standard solu-*

Table 1

Metrological characteristics of the photocolorimetric method for quantitative determination of prednisolone by the reaction with phenylhydrazine using a standard solution of prednisolone

Parameters	Value	Criteria (for tolerances of ± 10%)	Conclusion
b	0.9430	–	–
S_b	0.0346	–	–
a	4.7	1) statistically acceptable value: $a \le S_a \times 1.86$ 2) practically acceptable value: $a \le 5.12$	satisfied satisfied
S_a	3.4882	–	–
RSD_o	1.3399	≤1.8070	satisfied
r	0.9998	≥0.9924	satisfied
Mean, Z%	99.08	–	–
Relative standard deviation, Sz%	1.54	–	–
Relative confidence interval $\Delta as\% = t(95\%,8) * Sz = 1.8595 * Sz =$	2.86	$\Delta as\% = 10.00 \times 0.32 = 3.20$	satisfied
Systematic error δ	0.92	$\delta \le 1.0240$	satisfied

tion. Add 20.0 ml of MPSAR to the *Test solution, Standard solution* and *Blank reagent*. Place the flasks into a thermostat at 60°C ±1° for about 45 min, then cool in a chilled water cooler. Determine the absorbances of the solutions at the wavelength of the maximum absorbance at about 400 nm with a photocolorimeter against dehydrated alcohol. Calculate the amount of prednisolone in the ointment, in percents, by the formula:

$$C = \frac{(A - A_{sar} - A_{blank})}{(A_{st} - A_{sar/st} - A_{blank})} \cdot 100\%,$$

where: A – is the absorbance of the test solution; A_{sar} – is the absorbance of the blank test solution; A_{blank} – is the absorbance of the blank reagent; A_{st} – is the absorbance of the standard solution; $A_{sar/st}$ – is the absorbance of the blank standard solution.

The time of a single analysis takes approximately 1 hour 30 min.

Measurement of the optical density of the model solutions obtained was conducted three times removing the cuvette. Statistical analysis of the experimental data was carried out in accordance with Article of the SPhU "Statistical analysis of the results of chemical experiments" [2].

Because this method is validated for determination of prednisolone only in creams, and for ointments such methods are not available, it is impossible to predict accurately the effect of excipients and other conditions on the course of the reaction. Therefore, at first we investigated the basic validation characteristics of the method (linearity, precision, reproducibility) using the standard solution of prednisolone as a model.

Results and Discussion

It is known that during the interaction of prednisolone or other 17-oxysteroids with phenylhydrazine a coloured yellow compound is formed; its quantitative content can be determined by photocolorimetry [1]. This reaction is recommended by the USP for the quantitative analysis of corticosteroids in creams [11]. However, for ointments, in which other excipients are used, such methods are not available.

The assessment of *linearity* was performed by the standardized procedure within the whole range of determination (80-120% according to the SPhU). For this purpose 9 model solutions with accurate concentrations and the reference solution were used, their optical density was measured three times. The results obtained were statistically processed by least squares for the straight line Y = b·x + a as required by the SPhU. The calibration graph was plotted in the normalized coordinates [2, 4, 12]. The calculated statistical values *b*, S_b, *a*, S_a, RSD_0 and *r* are given in Tab. 1. The assessment of *convergence* and *reproducibility* was conducted in parallel with determination of linearity by measuring the optical density of 9 model solutions three times.

Since the method of photocolorimetric determination of prednisolone by Porter-Zilber reaction using the standard solution of prednisolone as a model gives correct results, it has been decided to test it to determine the quantitative content of prednisolone in the ointment.

Before studying the validation characteristics of the method three samples of the ointment (100%) were analyzed. We received somewhat understated results than expected, so the study of robustness was conducted in more detail, namely the study of the effect of temperature and the amount of the reagent on the course of the reaction.

Table 2

The effect of temperature on the course of the photocolorimetric reaction of prednisolone with phenylhydrazine

Temperature, °C	Optical density of the solution*	Optical density of the solution without excipients
20	0.13	0.05
40	0.32	0.15
60	0.46	0.25
100	0.21	0.13

*The average of 3 measurements taking into account the excipients.

Table 3

The effect of the amount of the reagent on the optical density

Amount of phenylhydrazine in 100 ml of the reagent / Optical density of the solution*				$A_{average}$	S_r	RSD, %	Δ, %	maxδ, %
40 mg	60 mg	80 mg	100 mg					
0.510	0.505	0.503	0.510	0.507	0.0036	0.3559	0.76	1.024

*The average of 3 measurements taking into account the excipients.

Table 4

The study of metrological characteristics of the method for photocolorimetric determination of prednisolone by the reaction with phenylhydrazine

Parameters	Value	Criteria (for tolerances of ± 10%)	Conclusion
b	1.0552	–	–
S_b	0.0325	–	–
a	4.4	1) statistically acceptable value: $a \le S_a \times 1.86$ 2) practically acceptable value: $a \le 5.12$	satisfied satisfied
S_a	3.2746	–	–
RSD_o	1.2578	≤ 1.8070	satisfied
r	0.9998	≥ 0.9924	satisfied
Mean, Z%	101.00	–	–
Relative standard deviation, S_z%	1.45	–	–
Relative confidence interval $\Delta as\% = t(95\%,8) * S_z = 1.8595 * S_z =$	2.71	$\Delta as\% = 10.00 \times 0.32 = 3.20$	satisfied
Systematic error δ	1.00	$\delta \le 1.0240$	satisfied

The effect of temperature on the course of the photocolorimetric reaction. It has been found that the reaction is quite exacting to temperature fluctuations and can run with the formation of different products (Tab. 2). Therefore, we recommend to heat the reaction mixture and keeping it in a thermostat at 60°±1°C in order to provide completeness of the reaction.

The effect of the amount of phenylhydrazine on the results of analysis. According to the literature to conduct the reaction studied the phenylhydrazine reagent should be used in the concentration of 0.065%. It has been found that the concentration range of phenylhydrazine within 0.04-0.1% allows to obtain the results of the required accuracy (Tab. 3).

Stability. According to the requirements of SPhU tests were carried out for an hour by measuring the optical density every 10 minutes (7 measurements). During this period of time the optical density did not change significantly and was 0.460 ($\Delta t = 0.54\% \le 1.024\% = max\delta$) for the solution of the ointment taking into account the excipients.

The study of *linearity* was carried out by the standardized procedure measuring the optical density of 9 model solutions (containing prednisolone extracted from the ointment) with accurate concentrations and the reference solution (containing prednisolone PSS) and three blank solutions. The method satisfies the requirements of linearity. The study of *reproducibility* and *convergence* also shows the correctness of the method (Tab. 4).

The values of LOD and LOQ were also calculated for the method; they were 9.31 and 31.03.

CONCLUSIONS

1. The main validation characteristics of the method of photocolorimetric determination of prednisolone by the reaction with phenylhydrazine have been determined for the model solution with the standard substance of prednisolone. It has been found that the method meets the validation criteria and can be tested to determine the ointment.

2. The parameters of linearity, reproducibility, convergence, stability and robustness for the method for quantitative determination of prednisolone in the ointment by the reaction with phenylhydrazine using the standard method have been determined. The correct results have been obtained in the range of 80-120% (corresponding to 0.4-0.6 g of prednisolone in the ointment).

3. The method can be used for quantitative determination of prednisolone in "0.5% Prednisolone" ointment in the domestic laboratories of the drug quality control.

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ОЦІНКА МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ПРЕДНІЗОЛОНУ В МАЗІ ЗА РЕАКЦІЄЮ З ФЕНІЛГІДРАЗИНОМ

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Ключові слова: *фотоколориметрія; кількісний аналіз; преднізолон; валідація; фенілгідразин*

Проведено адаптацію та валідацію аналітичної методики фотоколориметричного визначення преднізолону за реакцією з фенілгідразином методом стандарту в умовах науково-дослідної лабораторії за стандартними процедурами, описаними в Державній фармакопеї України. Визначені основні валідаційні характеристики при використанні стандартної субстанції для приготування модельного розчину та встановлено, що критерії лінійності та прецизійності виконуються в діапазоні концентрацій 80-120% (а саме 0,08-0,12 мг/мл в досліджуваному розчині або 0,4-0,6% преднізолону в мазі) та є статистично і практично незначущими ($a \le S_a \times 1,86 = 4,7$, $\Delta as\% \le 3,20 = 2,86$, $\delta \le 1,0240 = 0,92$). Отримані коректні результати дозволили апробувати методику для визначення преднізолону в модельному вилученні з мазі «Преднізолон 0,5%» в діапазоні 80-120% від номінальної концентрації. Було проведено дослідження робасності методики та встановлено, що для отримання коректних результатів необхідно дотримуватися чіткого інтервалу температури при проведенні реакції (60°С±1°). Кількість фенілгідразину в реактиві при цьому може коливатися в межах 0,4-1,0 г/л. Критерії лінійності, прецизійності та збіжності для модельного вилучення з мазі також виконуються на всьому діапазоні визначення та є статистично і практично незначущими ($a \le S_a \times 1,86 = 4,4$, $\Delta as\% \le 3,20 = 2,71$, $\delta \le 1,0240 = 1,00$). Методика є прийнятною для використання в лабораторіях контролю якості лікарських засобів і може бути запроваджена для визначення преднізолону в мазі «Преднізолон 0,5%» з гідрофобною основою в межах концентрацій 0,4-0,6%.

ОЦЕНКА МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ПРЕДНИЗОЛОНА В МАЗИ ПО РЕАКЦИИ С ФЕНИЛГИДРАЗИНОМ

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Ключевые слова: *фотоколориметрия; количественный анализ; преднизолон; валидация; фенилгидразин*

Была проведена адаптация и валидация аналитической методики фотоколориметрического определения преднизолона по реакции с фенилгидразином методом стандарта в условиях научно-исследовательской лаборатории согласно стандартных процедур, указанных в Государственной фармакопее Украины. Определены основные валидационные характеристики при использовании стандартной субстанции для приготовления модельного раствора и установлено, что критерии линейности и прецизионности соблюдаются в диапазоне концентраций 80-120% (а именно 0,08-0,12 мг/мл в исследуемом растворе или 0,4-0,6% преднизолона в мази) и являются статистически и практически незначимыми ($a \le S_a \times 1,86 = 4,7$, $\Delta as\% \le 3,20 = 2,86$, $\delta \le 1,0240 = 0,92$). Полученные корректные результаты позволили апробировать методику для определения преднизолона в модельном извлечении из мази «Преднизолон 0,5%» в диапазоне 80-120% от номинальной концентрации. Проведенные исследования робастности методики позволили установить, что для получения корректных результатов необходимо придерживаться четкого интервала температуры при проведении реакции (60°С±1°). Количество фенилгидразина в реактиве при этом может колебаться в пределах 0,4-1,0 г/л. Критерии линейности, прецизионности и правильности для модельного извлечения из мази также выполняются по всему диапазону определения и являются статистически и практически незначимыми ($a \le S_a \times 1,86 = 4,4$, $\Delta as\% \le 3,20 = 2,71$, $\delta \le 1,0240 = 1,00$). Методика является приемлемой для использования в лабораториях контроля качества лекарственных средств и может применяться для определения преднизолона в мази «Преднизолон 0,5%» с гидрофобной основой в пределах концентраций 0,4-0,6%.