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QUANTITATIVE DETERMINATION OF GLYCYRRHIZIC ACID IN SUPPOSITORIES FOR CHILDREN

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The existing methods for quantitative determination of glycyrrhizic acid have been studied; the research on development of the method for its determination in rectal suppositories for children by extraction spectrophotometry calculated as glycyram has been conducted. According to the research results the relevant sections of the project of methods of quality control for the medicine have been developed.

According to the statistic data of the World Health Organization, today nearly a third of the world's population of children suffer from diseases of the immune system. According to reports of the Ukrainian regional children immunologists in 2012 under their supervision there were more than 630 children with primary immunodeficiency, the vast majority of them with antibody deficiency [4, 7].

According to the data of the United States (2011), only selective IgA immunodeficiency occurs with the frequency of 1:700, and in Europe – 1:250. Based on the population of Ukraine, the number of patients with this type of immunodeficiency in this country is not less than 20 000 people. The second-rate according to various authors [12] is total variable immunodeficiency; in Ukraine the number of this immunodeficiency can reach 1000 cases.

Unfortunately, today in Ukraine there are many types of immunodeficiencies, which have not been revealed yet. This is due to the fact that pediatricians, family doctors and specialists of various specialties have insufficient knowledge about this problem [4].

In addition, the range of existing children's medicines for the treatment of immunodeficiencies is too limited, which, in turn, makes it extremely important to sovle the question of their development and subsequent introduction into medical practice. In this regard, on the grounds of the complex of experimental studies rectal suppositories for children with the immunomodulating action based on natural compounds have been developed [6].

The purpose of this study is to develop a method for quantitative determination of glycyrrhizic acid (GA) in the composition of the given medicine.

Materials and methods

As an object we used rectal suppositories for children containing licorice extract with GA as the main active ingredient [1, 8, 14].

After reviewing the existing methods of quantitative determination of GA, we have found that they can be divided into several groups: determination by GA, determination by the carbohydrate part of GA, determination by glycyrrhetinic acid [3, 5].

Determination by GA includes different variations of gravimetric and titrimetric methods, including potentiometric ones. Determination by the carbohydrate part of GA is based on oxidation of the carbohydrate part of GA with Fehling reagent or the reaction with naphthoresorcinol. But pentoses, which exceed greatly the results of the quantitative determination in extracts, prevent this quantitative determination [9, 10, 13].

There is also a method of determining GA by glycyrrhetinic acid with 2, 6-diquaternary butyl-n-cresol, but this method also has its drawbacks. Generally, all methods of analysis of GA in licorice roots and in its extracts are compatible modifications of titrimetric and spectrophotometric methods [2, 11].

For quantitative determination of GA in children's rectal suppositories we proposed the method of extraction spectrophotometry in the UV region of the spectrum. As the standard for determining GA by the method mentioned above glycyram was chosen.

Results

With the purpose of elaboration of the method for quantitative determination and confirmation of its reproducibility, first the glycyram substance has been analyzed according to the method given in the existing analytical normative documentation for glycyram (the GA content should be at least 87%). The absorption spectrum of the glycyram solution and the graph of dependence of absorbance on the concentration of glycyram are shown in Fig. 1, 2.

As seen in Fig. 1, the presence of peak absorption of glycyram solution with the maximum absorption at the wavelength of (258±2) nm is observed; it fully conforms to the AND requirements for glycyram.

In Fig. 2 the dependence of absorbance on the concentration of glycyram, which has the linear character, is shown.

Metrological characteristics of the method of quantitative determination of GA in the substance of glycyram are presented in Table 1.

As seen from Table 1, the content of GA in the glycyram substance is over 90%; and it meets the AND requirements and confirms the reproducibility of the existing method.

To determine the quantitative content of GA in suppositories we used the same method – one-wave component analysis by the standard method. The composi-

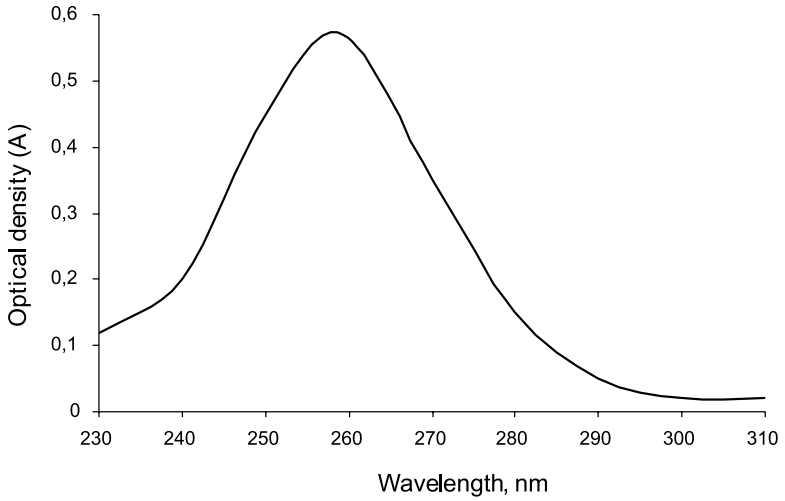

Fig. 1. The absorption spectrum of glycyram solution ($5 \cdot 10^{-5}$ g/ml).

Table 1

Metrological characteristics of the method of quantitative determination of GA in the substance of glycyram

X	$\bar{X}$	f	S	t (P, f)	ΔX, %
90.74 91.80 91.51 91.85 91.55 91.78	91.49	5	0.198	2.57	0.56

Notes: n = 6; P = 95%

tion of suppositories with the extract of licorice roots contains also the hydrophobic base and excipients. To remove the base as a solvent the possibility of using ethanol in different concentrations – 50%, 70% and 96%, and for extraction of GA – 3% acetone solution of nitric acid and 3% acetone solution of trichloroacetic acid has been studied. As a result of the experimental research 96% ethanol for removing the hydrophobic base and 3% acetone solution of nitric acid as an extractant of GA have been chosen.

In Fig. 3 the absorption spectrum of the suppository extract with the maximum absorption at the wavelength of (258±2) nm, which coincides with the peak of the absorption of glycyram solution, is shown. Optical densities of both solutions are close enough, and it confirms the correctness of the chosen method for quantitative determination of GA – the standard method. Spectral studies have also revealed that the excipients (placebo of a suppository) do not significantly affect the results of the analysis.

These studies have been accepted as the basis for of the method for quantitative determination of GA in suppositories.

The method for determination is as follows: place 1 suppository in a 250 ml conical flask, add 20 ml of 96% alcohol and mix intensively for 20 min. Filter the resulting solution through a «blue ribbon» filter previously washed with 96% alcohol in a 150 ml flask. Then add 20 ml of 96% alcohol thrice in the flask, shake for 15 min and filter in the same flask. Transfer quantitatively the precipitate from the filter into a 150 ml flask, add 25 ml of 3% acetone solution of nitric acid, mix for 10 min.

Filter the resulting solution through a «blue ribbon» filter, wash the flask with 10 ml of 3% acetone solution of nitric acid, filtering through the same filter (solution A).

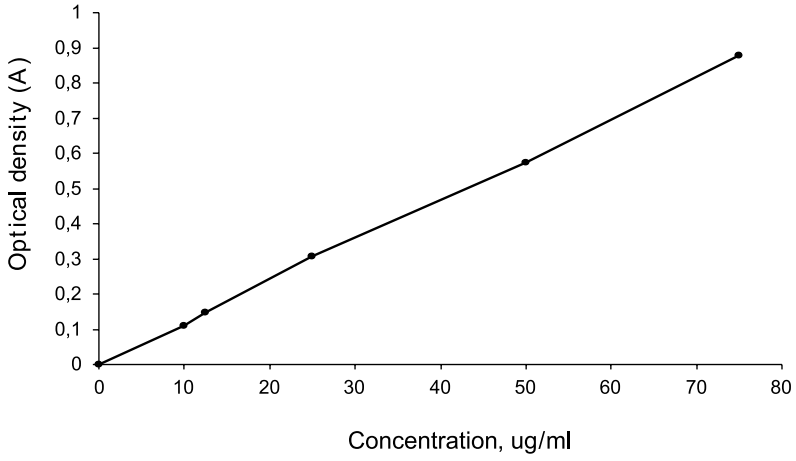

Fig. 2. The graph of dependence of absorbance on the concentration of glycyram.

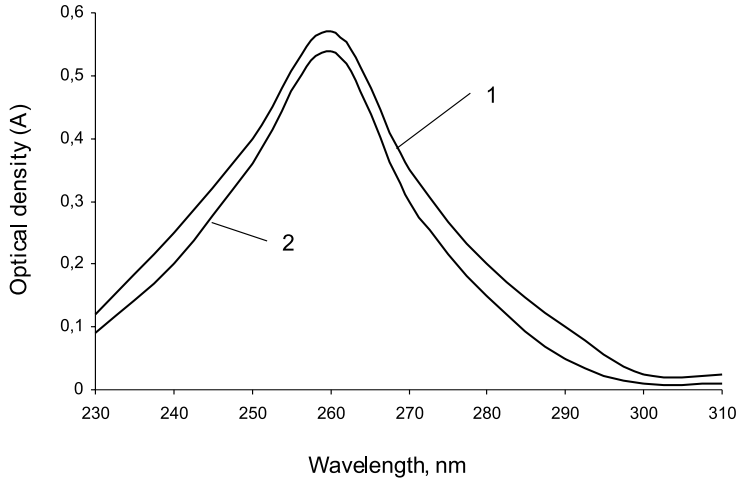

Fig. 3. The absorption spectrum of: glycyram solution – 1 and the extract of a suppository with licorice extract – 2.

Table 2

The results of determination of the quantitative content of GA in one suppository

Batch number of the drug	The quantitative content of GA in one suppository calculated as glycyram, g
110211	0.0410
same	0.0430
-//-	0.0441
-//-	0.0420
-//-	0.0450
-//-	0.0420

Notes: n =6; P = 95%

Table 3

Metrological characteristics of the method of determination of the quantitative content of GA in a suppository calculated as glycyram

X	$\overline{X}$	f	S	t (P, f)	ΔX, %
0.0410 0.0430 0.0441 0.0420 0.0450 0.0420	0.0428	5	$2.17 \cdot 10^{-6}$	2.57	3.60

Notes: n = 6; P = 95%

To solution A add concentrated ammonia solution dropwise to pH from 8.3 to 8.8 by the universal indicator (the solution with the precipitate of ammonium salt of GA).

Transfer the solution of salt to a glass filter №4, pump the liquid with the help of vacuum. Wash the precipitate on the filter with 10 ml of acetone in two steps. Using water transfer quantitatively the precipitate into a 10 ml volumetric flask, dilute the volume with water and mix (solution B).

The optical density of the test solution B is measured using a spectrophotometer at the wavelength of (258±2) nm in a cuvette with the layer thickness of 10 mm, water is used as a reference solution.

Simultaneously the optical density of solution B of glycyram is measured under the same conditions. The GA content (X) in a suppository, in grams, is calculated by the formula:

$$X = \frac{A_0 \times 10}{1\text{sup} \times 135.4},$$

where A_0 – is the optical density of the test solution; *135.4* – is the specific absorption rate of glycyram at the wavelength of 258 nm.

The GA content in a suppository should be at least 0.035 g calculated as glycyram. According to the method developed we have analyzed 6 samples of suppositories; the results are summarized in Tab. 2.

Metrological characteristics of the method are given in Tab. 3.

As seen from Tables 2 and 3, the experimental studies performed have shown that the GA content in 1 suppository is not less than 0.0350 g calculated as glycyram.

CONCLUSIONS

1. The existing methods for quantitative determination of GA have been studied and the research in developing of the method for its determination in children's suppositories has been conducted.

2. The method for quantitative determination of GA in children's suppositories with the immunomodulating action has been developed by the extraction spectrophotometry method calculated as glycyram.

3. According to the research the relevant sections of the project the quality control methods for the medicine have been developed.

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КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ ГЛИЦИРРИЗИНОВОЙ КИСЛОТЫ В СОСТАВЕ СУППОЗИТОРИЕВ ДЛЯ ДЕТЕЙ

Т.Г.Ярных, Г.Н.Мельник, О.А.Рухмакова, В.Н.Чушенко

Изучены существующие методики количественного определения глицирризиновой кислоты и проведены исследования по разработке методики ее определения в детских ректальных суппозиториях методом экстракционной спектрофотометрии в пересчете на глицирам. По результатам исследований разработаны соответствующие разделы проекта методов контроля качества на препарат.

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КІЛЬКІСНЕ ВИЗНАЧЕННЯ ГЛІЦИРИЗИНОВОЇ КИСЛОТИ У СКЛАДІ СУПОЗИТОРІЇВ ДЛЯ ДІТЕЙ

Т.Г.Ярних, Г.М.Мельник, О.А.Рухмакова, В.М.Чушенко

Вивчені існуючі методики кількісного визначення гліциризинової кислоти та проведено дослідження з розробки методики її визначення у дитячих ректальних супозиторіях методом екстракційної спектрофотометрії у перерахунку на гліцирам. За результатами досліджень розроблені відповідні розділи проекту методів контролю якості на препарат.