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Research Article

Quantitative Determination of Total Anthracene Derivaties in Rubia Syrup Preparation

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ABSTRACT
A quantitative analysis method for total anthracene derivatives in *Rubia* Syrup preparation with the using of direct spectrophotometry at analytical wavelength 520 nm there was developed. The technology for producing of *Rubia* syrup from the decoction of rhizomata et radices of *Rubia tinctorum* L. in the ratio "the raw material: extract" 1:8 was elaborated. The relative degree of the determination of the total anthracene derivatives in *Rubia* Syrup in developed method with confidence probability 0,95 is no more than ±4,17%. The content of total anthracene derivatvies in *Rubia* syrup varied from 0,02±0,001% to 0,025±0,001% (calculated on ruberythrinic acid).

Key worlds: *Rubia tinctorum* L., rhizomata et radices, syrup, anthracene derivatives, ruberythrinic acid, spectrophotometry, standardization.

INTRODUCTION

Rubia tinctorum L. is perennial herb having diuretic, antispasmodic and kidney stones destroyer properties. The pharmacopoeial raw material are rhizomata et radices containing significant quantities of anthracene derivatives[1] (2.5-3,0%), which are responsible for the pharmacological action of the preparations[2]. Preparations of *Rubia tinctorum* L. have diuretic properties[3] that increase increase the motility of the muscles of the renal pelvis and ureter, promotes stones[4]. Rhizomata et radices of *Rubia tinctorum* L. have the ability to loosen and destroy kidney stones and bladder, so the funds on the basis of raw materials used in urolithiasis[5]. At the moment there are many drugs basis on rhizomata et radices of *Rubia tinctorum* L., such as tablets of «Marelin», «Cistenal»[6], but such a form as syrup does not exist[7]. In this connection these researches are actual. Rhizomata et radices of *Rubia tinctorum* L. containing anthracene derivatives from alizarin group, among which is the main glycoside ruberythrinic acid[5]. During previous studies we isolated the active ingredients, set dominant components ruberythrinic acid and elaborated methodical approaches to the standardization of *Rubia tinctorum* L. rhizomata et radices for the anthracene derivatives. These approaches to standardization were used to develop a quantitative analytical procedure for total anthracene derivatives in *Rubia tinctorum* L. rhizomata et radices syrup that combined analyses in the order raw material – preparation. The purpose of the present research - to develop methods of quantitative analysis of syrup on the basis of *Rubia tinctorum* L. rhizomata et radices.

RESULTS AND DISCUSSION

Objective. Materials: raw materials of *Rubia tinctorum* L. rhizomata et radices, made in March 2015, in the Krasnodar region. Electronic spectra were measured on the UV-spectrophotometers "Specord 40" and "UNICO". Methodology**.** Production of syrup in the laboratory began to produce a decoction of *Rubia tinctorum* L. rhizomata et radices using ratios of "raw material - finished product"(1:7, 1:8, 1:9). The volume of extractant to produce a given volume of the finished product was determined taking into account the water absorption coefficient, which is 1 ml/g. Most of decoctions prepared pharmacopoeial method: a known amount of a certain amount of raw material filled with purified water at room temperature, heated in a boiling water bath for 30 minutes, cooled for 10 min, filtered and adjusted if necessary until the desired amount of the resulting ratio "raw material - the finished product"[8]. Water extract of *Rubia tinctorum* L. rhizomata et radices was used

Table 1: Metrological characteristics of the methods of quantitative determination of the total of anthracene derivatives in preparation "Rubia syrup":

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,025	0,000 47	95	2,23	± 0,001	± 4,17 %

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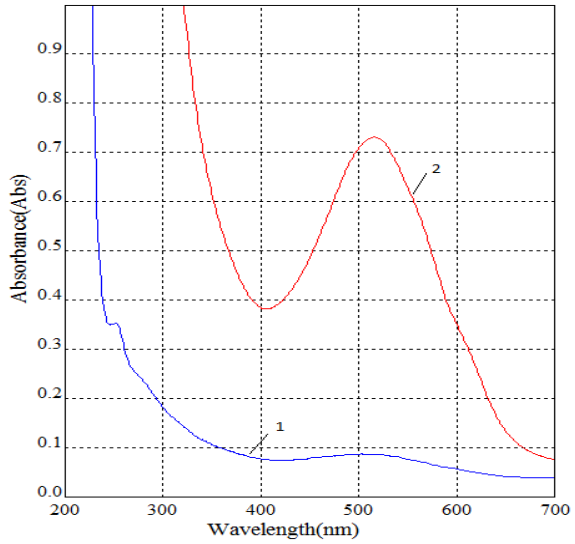

Figure 1: Electronic spectra of alkaline ammonia solution Syrup of *Rubia tinctorum* L. (1) and alkaline ammonia solution aqueous alcoholic extract from the rhizomata et radices of *Rubia tinctorum* L. (2).

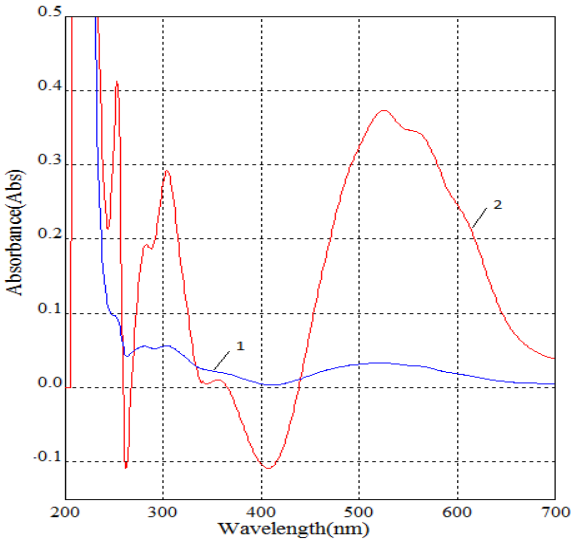

Figure 2: Electronic spectrum of alkaline ammonia solution Syrup *Rubia tinctorum* L. and alkaline ammonia solution aqueous alcoholic extract from the rhizomata et radices of *Rubia tinctorum* L. (2). (differential version).

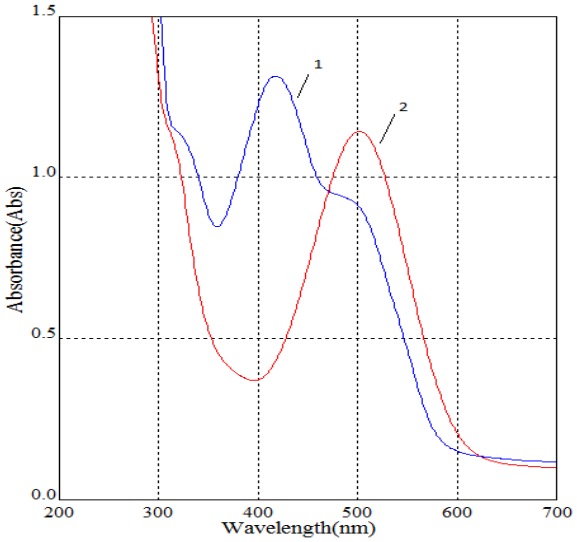

Figure 3: Electronic spectrum of solution of ruberythrinic acid (1) and alkaline ammonia solution of ruberythrinic acid (2).

instead of purified water to obtain sugar syrups by means of pharmacopoeia method. To 36 g of this aqueous extracts were mixed with 64 g of refined sugar, and the mixture was heated until complete dissolution of sugars was adjusted to boiling twice, each time with removing the resulting foam. Syrups filtered through cheesecloth into a hot, and adjusted to the initial weight of purified water[8]. In order to investigate the UV spectra obtained syrups alkaline ammoniacal medium and quantifying anthracene derivatives, accurate sample of syrup (2.0 g) was placed in a volumetric flask of 25 ml, adjusted to the mark with water and stirred (stock solution). 2 ml of this solution was placed in a volumetric flask of 25 mL and was adjusted to the mark with alkaline ammoniacal solution (test solution). The test solution was heated for 15 minutes in a boiling water bath. After cooling, the electronic shooting range of the test solution in the range of 190-700 nm. For differential in the electron spectra used as a reference solution, a solution obtained as follows: 2.0 ml of the stock solution was placed in a volumetric flask of 25 ml volume was adjusted with purified water to the mark. To quantify anthracene derivatives samples is used *Rubia tinctorum* L. rhizomata et radices procedure developed earlier (extractant - 80% ethyl alcohol, the ratio "raw material - extractant" - 1:30, extraction time - 90 min). Consequently, as analytical wavelength may be used a value of 520 nm, and as the standard sample can be serve the dominant anthtraglycoside - ruberythrinic acid. In the case of the absence of this standard in the calculation formula can be used the theoretical value of the specific absorption index (= 520) . A study of the electronic spectra showed that the electron spectra of *Rubia* syrup coincides with the spectrum extract from the rhizomata et radices of *Rubia tinctorum* L., and the maximum absorption is at 520 ± 2 nm, which is typical for alkaline ammoniacal aqueous alcoholic extract from the rhizomata et radices of *Rubia tinctorum* L. (Fig. 1). It should be noted that evaluation indicative authentication solutions are differential version of spectra (spectra of the test solutions on a background of reference solution) (Fig. 2). In the long-wavelength region of the electron spectrum alkaline ammonia solution ruberythrinic acid also there is a distinct absorption maximum at 520 ± 2 nm (Fig. 3). Consequently, as the analytical wavelength may be used value 520 nm, and the standard model can serve as dominant anthracene derivatives - ruberythrinic acid, and in the absence of a standard in the calculation formula can

Table 2: The content of total anthracene derivatives in the samples of *Rubia* rhizomata et radices decoctions

№ п/п	Decoction, from which a syrup there was prepared	Contents of total anthracene derivatives in the samples of syrups (calculated on ruberythrinic acid), %	Yield anthracene derivatives respect to their content in the fruits of *Rubia*, %
1	Decoction 1:7	0,022±0,001%	20,2±0,3%
2	Decoction 1:8	0,025±0,001%	46,3±0,4%
4	Decoction 1:9	0,023±0,001%	45,7±0,5%

be used in the theoretical value of the specific absorption index (= 520). Method for the quantitative determination of the total anthracene derivatives in *Rubia* syrup. Syrup 2.0 g (accurately weighed) was placed into a volumetric flask of 25 ml volume of purified water adjusted to the mark and mixed (solution A). 2 ml of solution A to make a volumetric flask of 25 ml volume of solution was adjusted to the mark with alkaline ammoniacal solution prepared, and heated in a boiling water bath for 15 min. After cooling, optical density is measured of the test solution in a spectrophotometer at the wavelength of 520 nm in a cuvette with a layer thickness of 10 mm. As a reference solution using purified water.

Note 1: Preparation of working solution of the standard sample of ruberythrinic acid. About 0.02 g (accurately weighed) of ruberythrinic acid is placed into a volumetric flask of 50 ml, dissolved in 30 ml of 70% ethanol by heating in a water bath. After cooling the contents of the flask to room temperature, the solution volume was adjusted to 70% ethanol to the mark (solution A of ruberythrinic acid). 1 ml solution A of ruberythrinic acid placed in a volumetric flask of 25 ml and the solution volume was adjusted to the mark with alkaline ammoniacal solution (test solution B). Solution B was placed in a 50 ml flask and heated for 15 minutes in a boiling water bath under reflux. After cooling, optical density is measured of the test solution B a spectrophotometer at a wavelength of 520 nm. As a reference solution using purified water.

Contents of total anthracene derivatives (X) based on a percentage ruberythrinic acid calculated by the formula:

$$X = \frac{D * m_0 * 25 * 25 * 1 * 100}{D_0 * m * 2 * 50 * 25},$$

Where D is optical density of the test solution; D_o - optical density of the solution of ruberythrinic acid; m - the mass of syrup, g; m_o - the mass of the standard sample of ruberythrinic acid, g;

A simplified calculation formula as an alternative:

$$X = \frac{D \times 25 \times 25}{m \times 2 \times 520},$$

where D - optical density of the test solution; m - the mass of syrup, g; 520 - specific absorption of the standard sample of ruberythrinic acid.

The content of total anthracene derivatives in syrup of *Rubia* varies from 0,02±0,001% to 0,025±0,001% (calculated on ruberythrinic acid).

The results of statistical processing of the experiments show that the error of a single determination of the total anthracene derivatives *Rubia* syrup with a confidence level of 95% is ± 4,17%.

The metrological characteristics of a method for quantitative determination of the total anthracene derivatives in syrup of *Rubia* are presented in Table 1. Using this technique, the samples were analyzed *Rubia* syrups from the frangible broths in the ratio 1: 7, 1: 8, 1: 9 (boiling on a hot plate).Was also evaluated yield anthracene derivatives in the finished product in relation to their total content in the samples of the rhizomata et radices of the studied medicinal plants. The results are shown in Table 2.

As can be seen from Table 3, similar in content to anthracene derivatives syrup is a syrup broth 1:8. However, the latter sample has an advantage anthracene derivatives extraction efficiency of feedstock and quantity of finished product obtained from the same weight of medicinal plants (3 times). Stability assessment anthracene derivatives in syrup of decoction, using the methods of thin-layer chromatography, spectroscopy in the UV and visible spectrum showed no significant differences in the qualitative composition of the other samples.

In the course of the study there was developed the method of quantitative determination of total anthracene derivatives in *Rubia* syrup and was justified the use of decoction from the the rhizomata et radices of *Rubia tinctorum* L. at a ratio of 1:8 as the substance to produce of syrup.

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