**ABSTRACT**

A quantitative analysis method for total anthracene derivatives in *Rhamnus* Syrup preparation with the using of direct spectrophotometry at analytical wavelength 524 nm there was developed. The technology for producing of *Rhamnus* syrup from the decoction of fruits of *Rhamnus cathartica* L. in the ratio “the raw material: extract” 1:3 was elaborated. The relative degree of the determination of the total anthracene derivatives in *Rhamnus* Syrup in developed method with confidence probability 0,95 is no more than ±4,17%. The content of total anthracene derivatives in *Rhamnus* syrup varied from 0,12±0,002% to 0,25±0,003% (calculated on frangulin A).

**Keywords:** *Rhamnus cathartica* L., fruits, syrup, anthracene derivatives, frangulin A, spectrophotometry, standardization.

**INTRODUCTION**

*Rhamnus cathartica* L. is one of the most popular sources of relaxants in official medicine. The pharmacopoeial raw material are fruits containing significant quantities of anthracene derivatices\(^1\) (2.5-5%), which are responsible for the pharmacological action of the preparations\(^2\). Anthracene derivatives operate only in the large intestine where they are hydrolyzed by *E. coli*. The resulting aglycones irritate the bowel wall and increases peristalsis\(^2\). Laxative action of anthracene derivatives is slow\(^4\), mild and long-term (8-10 hours)\(^2\). Though the first mention of the use of bark of *Rhamnus cathartica* L. dates back to the tenth century\(^4\), to date there is no registered drugs on the basis of *Rhamnus cathartica* L\(^7\). During previous studies we isolated the active ingredients, set dominant components [6-O-α-L-rhamnopyranoside of frangula-emodin (frangulin A) and 6-O-β-O-apiofuranoside of frangula-emodin (frangulin B)], and elaborated methodical approaches to the standardization of *Rhamnus cathartica* L. fruits for the anthracene derivatives\(^8\). These approaches to standardization were used to develop a quantitative analytical procedure for total anthracene derivatives in *Rhamnus cathartica* L. fruits that combined analyses in the order raw material – preparation\(^9\). The purpose of the present research - to develop methods of quantitative analysis of syrup on the basis of fruits of *Rhamnus cathartica* L.

**RESULTS AND DISCUSSION**

**Objective.** Materials: raw materials of fruits of *Rhamnus cathartica* L., made in August 2014, in the Orenburg region (Buzuluk, near the river Sakmara). 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 the bark of *Rhamnus cathartica* L. using ratios of "raw material - finished product" (1:1, 1:3, 1:6). 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"\(^10\).

Water extract of fruits of *Rhamnus cathartica* L. was used 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\(^10\). 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.

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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 the fruits of *Rhamnus cathartica* L. procedure developed earlier (extractant - 40% ethyl alcohol, the ratio "raw material - extractant" - 1:50, extraction time - 60 min). Consequently, as analytical wavelength may be used a value of 524 nm, and as the standard sample can be serve the dominant anthraglycoside - frangulin A. In the case of the absence of this standard in the calculation formula can be used the theoretical value of the specific absorption index (= 180).

It is also important to note that in a direct spectrophotometry (Fig. 1) and the differential
spectrophotometry (Fig. 2) were obtained with comparable absorbance values, indicating the possibility of the use of both variants in the methods of quantitative determination of the total anthracene derivatives. As a methodological decision to choose an easier option - direct spectrophotometry, as in the case of Rhamnus fruits, as well as syrup of Rhamnus (Shmygareva A.A., Kurkin V.A., 2012). In the UV spectra of alkaline ammoniacal solution of frangulin A there was observed the maximum of absorbance at wavelength of 524 nm (Fig. 3).

Method for the quantitative determination of the total anthracene derivatives in Rhamnus 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 524 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 frangulin A. About 0.02 g (accurately weighed) of frangulin A 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 frangulin A). 1 ml solution A of frangulin A was placed into 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 524 nm. As a reference solution using purified water.

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

\[ X = \frac{D \times m_0 \times 25 \times 25 \times 1 \times 100}{D_0 \times m \times 2 \times 50 \times 25} \]

Where D is optical density of the test solution; \( D_0 \) - optical density of the solution of frangulin A; m - the mass of syrup, g; \( m_0 \) - the mass of the standard sample of frangulin A, g; A simplified calculation formula as an alternative:

\[ X = \frac{D \times 25 \times 25}{m \times 2 \times 180} \]

Where D - optical density of the test solution; m - the mass of syrup, g; 180 - specific absorption of the standard sample of frangulin A.

The content of total anthracene derivatives in syrup of Rhamnus varies from 0,22±0,002% to 0,25±0,003% (calculated on frangulin A).

The results of statistical processing of the experiments show that the error of a single determination of the total anthracene derivatives Rhamnus 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 Rhamnus are presented in Table 1. Using this technique, the samples were analyzed Rhamnus syrups from the frangible broths in the ratio 1:1; 1:3; 1:5 (boiling on a hot plate) and 1:6 (indicated by the ratio of raw materials and parts by weight of the finished product). Was also evaluated yield anthracene derivatives in the finished product in relation to their total content in the samples of the fruits 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 1:3, obtained by boiling on a hot plate. 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 prepared by boiling on a hot plate, 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 *Rhamnus* syrup and was justified the use of decoction from the fruits of *Rhamnus cathartica* L. at a ratio of 1:3 as the substance to produce of syrup.

REFERENCES