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Research Article

Validated High-performance Liquid Chromatography Method for Quantitative Determination of Anthracene Derivatives in Decoction, Syrup and Water-Alcohol Extract of *Rhamnus cathartica* L. Fruits.

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ABSTRACT
A wide range of chromatographic methods for the analysis of anthracenderivatives in medicinal plants has been published over the years. However, no high performance liquid chromatographic methods using for the analysis of anthracenderivatives in decoction, syrup and water-alcohol extract of *Rhamnus cathartica* L. The method of high-performance liquid chromatography (HPLC) is the most sensitive and effective for identification and quantitative determination of anthracenderivatives in medical plants. Using HPLC analysis we obtained the results of the quantitative determination of anthracenderivatives in decoction, syrup and water-alcohol extract of *Rhamnus cathartica* L. fruits. The content of total anthracenderivatives in decoction amounted to 7,58 µg/20 µl, in syrup amounted to 3,99 µg/20 µl, in water-alcohol extract amounted to 8,21/20 µl µg.

Keyworlds: High-performance liquid chromatography, HPLC, *Rhamnus cathartica* L., fruits, anthracenderivatives, frangulin A, decoction, syrup, water-alcohol extract.

INTRODUCTION

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)][1], and elaborated methodical approaches to the standardization of *Rhamnus cathartica* L. fruits for the anthracene derivatives[2]. These approaches to standardization were used to develop a quantitative analytical procedure for total anthracenederivatives in *Rhamnus cathartica* L. fruits syrup that combined analyses in the order raw material – preparation[3-5]. The purpose of the present research - to develop qualitative and quantitative methods of analysis anthracenderivatives in the decoction, syrup and water-alcohol extract of *Rhamnus cathartica* L. fruits.

RESULTS AND DISCUSSION

Materials

raw materials of fruits of *Rhamnus cathartica* L., made in August 2015, in the Orenburg region (Busuluk, near the river Sakmara), after full maturation and dried in a dryer at a temperature of 50-60 °C. HPLC - "Knauer SmartLine" (Germany), column - ReproSil-Pur C_{18} 300 ODS-3, 4.0 × 250 mm ("Dr. A. Marsch Ammerbuch-Entringen", Germany).

Methodology.

Decoction

Production of syrup in the laboratory began to produce a decoction of fruits of *Rhamnus cathartica* L. using ratios of "raw material - finished product" 1:3. 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 for fruits of *Rhamnus cathartica* L. 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"[6].

Syrup

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[6].

Water-alcohol extract

Analytical sample species is crushed to the size of the particles passing through a sieve with apertures in diameter of about 1 mm. 1 g chopped species (precise linkage) is placed in a flask with a grinding capacity of 100 ml, add 50 ml of 40% ethyl alcohol. Closed the flask and weigh on

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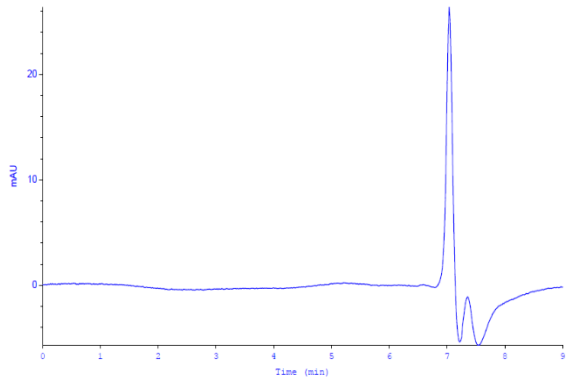

Figure 1: HPLC of Rhamnus decoction. Solvent system: 20 % buffer B. Absorbance at 420 nm.

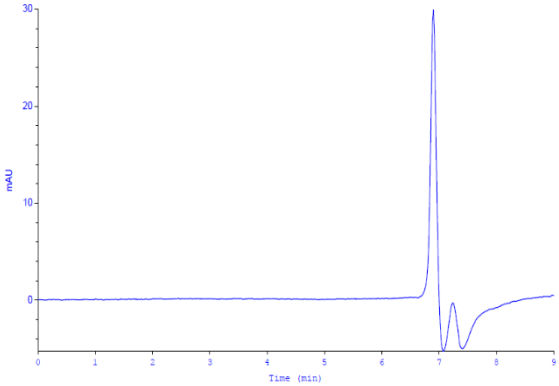

Figure 2: HPLC of Rhamnus water-alcohol extract. Solvent system: 20 % buffer B. Absorbance at 420 nm

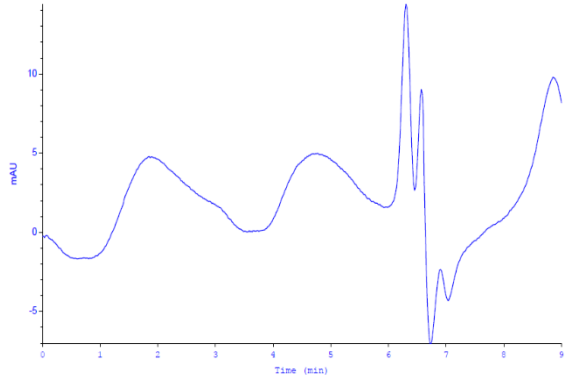

Figure 3: HPLC of Rhamnus syrup. Solvent system: 20 % buffer B. Absorbance at 420 nm.

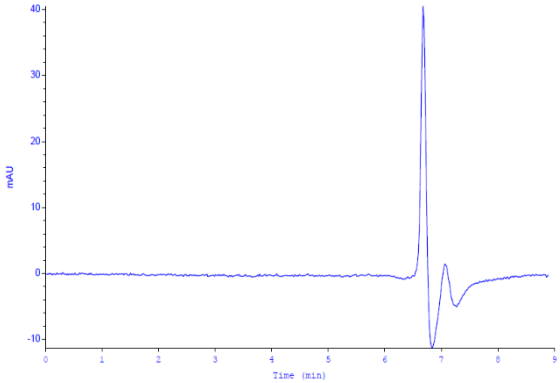

Figure 4: HPLC of frangulin A (standard sample). Solvent system: 20 % buffer B. Absorbance at 420 nm

Table 1: Chromatographic Parameters of investigated samples:

Samples	Peak area (mAU*min)	Peak height (mAU)	Concentration, µg/20 µl
Frangulin A	5,21	41,89	10
Rhamnus decoction	3,95	29,63	7,58
Rhamnus water-alcohol extract	4,28	33,22	8,21
Rhamnus syrup	2,08	14,20	3,99

calibrated scale accurate to ±0,01 g. Flask attached to reverse refrigerator and heated on a boiling water bath (moderate boiling) within 90 minutes. Then the flask close with the same tube, weighed again and fill in the missing extragent to the original mass. Removing filtered through paper filter («red» band) and cool for 30 minutes[1,2]. The studied samples were diluted 20 % buffer B (80 % acetonitrile in 0.1 % aqueous solution of trifluoroacetic acid) in a ratio of 1:50 (20 µl of the samples in 1 ml of 20 % buffer B). Then brought in a volume of 20 µl on a column ReproSil-Pur C18 300 ODS-3, 4.0 × 250 mm ("Dr. A. Marsch Ammerbuch-Entringen", Germany) integrated HPLC system "SmartLine Knauer" (Germany). Pre-column was balanced 20 % buffer B. Elution was performed gradient mode: 20-90 % (v/v) for 25 min at a flow rate of 0.5 ml/min. Absorbance at 420 nm. The study showed that the dominant component of samples is frangulin A (anthracenderivatives) (Fig. 1, 2, 3), the retention time of which coincides with that in the chromatogram of standard sample (Fig. 4). According to the results of HPLC studies are chosen the optimal conditions of chromatography, particularly, the composition of the mobile phase, presented-hydrated acetonitrile and water in the ratio 2:8 the addition of 1% acetic acid. At analytical wavelength took λ_{max} = 420 nm. The anthracenderivatives content in percentage (X) calculated by the formula:

$$X = \frac{H * m_0 * 50}{H_0 * m}$$

where H is the height of the peak of the frangulin A (Fig. 4) standard sample; H_0 – the peak height of the frangulin A (standard sample); m – the volume of aliquots, g; m_0 – mass of the frangulin A (standard sample).

The preparation of standard sample - frangulin A. About 0.02 g (precise linkage) is placed frangulin A volumetric flask with a capacity of 50 ml, dissolved in 20-30 ml of 95% ethyl alcohol, the volume was adjusted solution of 95% ethyl alcohol to the mark and mix. The content of total anthracenderivatives in decoction, syrup and water-alcohol extract of *Rhamnus cathartica* L. is varied from 0,31 % to 0,37%. The metrological characteristics of the developed method of HPLC analysis showed that a single error determination of anthracenderivatives in *Rhamnus*

Table 2: Metrological characteristics of the methods of quantitative determination of the total anthracenderivatives in *Rhamnus cathartica* decoction:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,37	0,0069	95	2,23	±0,015	±4,15

Table 3: Metrological characteristics of the methods of quantitative determination of the total anthracenderivatives in *Rhamnus cathartica* syrup:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,40	0,0086	95	2,23	±0,019	±4,78

Table 4: Metrological characteristics of the methods of quantitative determination of the total anthracenderivatives in water-alcohol extract of *Rhamnus cathartica*:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,19	0,0034	95	2,23	±0,0075	±3,97

cathartica decoction with a confidence level of 95% is ± 4,15% (Table. 2). The metrological characteristics of the developed method of HPLC analysis showed that a single error determination of total anthracenderivatives in *Rhamnus cathartica* syrup with a confidence level of 95% is ± 4,78% (Table. 3). The metrological characteristics of the developed method of HPLC analysis showed that a single error determination of anthracenderivatives in water-alcohol extract of *Rhamnus cathartica* with a confidence level of 95% is ± 3,97% (Table. 4).

CONCLUSION

Developed methodological approaches to the standardization of decoction, syrup and water-alcohol extract of *Rhamnus cathartica L*. fruits, consisting in the determination of anthracenderivatives and the using of techniques of the analysis of standard sample of Frangulin A. The method of quantitative determination of the total anthracenderivatives in terms of Frangulin A using HTLC at the analytical wavelength 420 nm. The content of total anthracenderivatives in decoction amounted to 7,58 μg/20 μl, in syrup amounted to 3,99 μg/20 μl, in water-alcohol extract amounted to 8,21/20 μl μg.

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